Asymmetric Synthesis and Application of α−Amino Acids
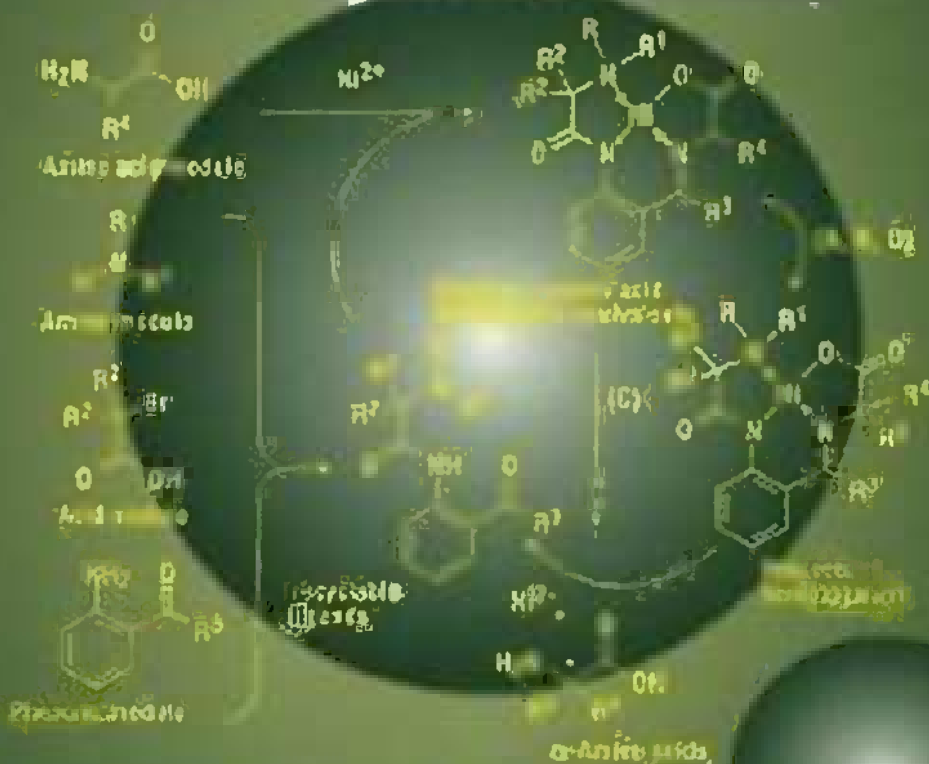
(A) Asymmetric catalytic hydrogenation
(B) Asymmetric nucleophilic homologation
(C) Deracemization of racemic α-AA
(D) Asymmetric isomerization asira biomimetry (E) Hydantoin chemistry technologies

EDITED BY

Vadim A. Soloshonok
and Kunisuke Izawa

Asymmetric Synthesis and Application of α-Amino Acids

Asymmetric Synthesis and Application of α-Amino Acids

Vadim A. Soloshonok, Editor
The University of Oklahoma

Kunisuke Izawa, Editor
Ajinomoto Company, Inc.

**Sponsored by the
Divisions of Organic Chemistry and Medicinal Chemistry**

Foreword

The ACS Symposium Series was first published in 1974 to pro-
vide a mechanism for publishing symposia quickly in book form. The
purpose of the series is to publish timely, comprehensive books devel-
oped from ACS sponsored symposia based on current scientific re-
search. Occasionally, books are developed from symposia sponsored by
other organizations when the topic is of keen interest to the chemistry
audience.

Before agreeing to publish a book, the proposed table of con-
tents is reviewed for appropriate and comprehensive coverage and for
interest to the audience. Some papers may be excluded to better focus the
book; others may be added to provide comprehensiveness. When
appropriate, overview or introductory chapters are added. Drafts of
chapters are peer-reviewed prior to final acceptance or rejection, and
manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review
papers are included in the volumes. Verbatim reproductions of previ-
ously published papers are not accepted.

ACS Books Department

Table of Contents

Enzymatic Approach

Preface

Alpha-Amino acids (α-AAs) are indispensable building blocks of life, as we know it on this planet, and one of a few classes of organic compounds with recognition in the street. Besides their primary function as structural units of peptides and proteins, they also serve countless biological functions in most living things. Thus, apart from 20 proteinogenic/coded α-AAs, hundreds of structurally varied α-AAs have been found in the peptides of cell walls and capsules of numerous bacteria and fungi as well as in various natural antibiotics. Furthermore, naturally occurring α-AAs have been continually used as a "chiral pool" for the preparation of a plethora of biologically and pharmacologically active compounds and are widely applied in the pharmaceutical, agrochemical, and food industries. Tailor-made α-AAs are increasingly employed in the preparation of new synthetic enzymes, hormones, and immunostimulants. More recently, sterically constrained α-AAs have found fundamental applications in the rational de novo design of peptides and peptidomimetics with enhanced metabolic stability and physiological functions. The structural diversity, biological activity, and application of α-AAs in organic synthesis, biochemistry, food, fragrances, and health-related sciences are far too broad to be covered in a single review. On the other hand, this brief introductory text might convince the reader that α-AAs have been and continue to be at the forefront of organic-synthesis science because of their remarkable impact on virtually every bio-related industrial and academic endeavor.

Chemistry and various applications of α-AAs have been the subject of numerous review articles over the years, following the first and only comprehensive treatment of this subject published by Professor Robert M. Williams in 1989. However, each review article typically focuses on a particular aspect of synthesis or application of α-AAs, leaving aside other

aspects of α-AAs science. This book represents, for the first time, an attempt to give the reader a snapshot of the most active and promising current research activity in the general field of α-AAs, instead of collecting comprehensive data on a particular aspect. We hope this book will convey to the reader the vibrant breadth and ingenuity of the current research in the area of asymmetric synthesis, and will prove to be both deeply instructive and stimulating.

This book is derived from the ACS Symposium *Asymmetric Synthesis of α-Amino Acids. Novel Developments and Future Directions*, as part of the 233rd American Chemical Society (ACS) National Meeting, March 25–29, 2007, Chicago, Illinois, featuring 11 speakers from 6 countries. In the design of this book, we decided to invite contributions from 17 more leading scientists in the field, to give the reader a more complete overview of current ideas and potential in α-AA research. Moreover, we decided to emphasize the current state of the art in the industrial production of enantiomerically pure α-AA, inviting contributions from several leading industry researchers. The book consists of four general sub-topics, including Stoichiometric, Catalytic and Enzymatic approaches for the preparation of enantiomerically pure α-AA, application of α-AAs in the total synthesis of complex natural products, and one chapter on the most advanced and innovative, in our opinion, approach for preparation of α-peptides. Of particular interest to us as editors is that many of the data the reader will find in this book have not been previously published, rendering this volume a prime source for new and stimulating research data.

As organizers of the symposium and editors of this book, we gratefully acknowledge the truly outstanding contributions from 11 speakers at the symposium and 28 authors of the book, all of whom have made our work both enjoyable and highly rewarding.

Finally, it is with great pleasure that we acknowledge the leadership of the ACS Division of Organic Chemistry for giving us an opportunity to organize the Symposium and for providing generous financial support. We are also grateful to the ACS Division of Medicinal Chemistry for cosponsoring the Symposium and for their generous financial support. Our particular thanks go to the Ajinomoto Company, Inc. (Tokyo, Japan) for their prominent

and encouraging support and for providing the major financial contribution to the Symposium.

Vadim A. Soloshonok
Department of Chemistry
The University of Oklahoma
620 Parrington Oval, Room 208
Norman, OK 73019–3051

Kunisuke Izawa
AminoScience Laboratories
Amino Acids Company
Ajinomoto Co., Inc.
1–1 Suzuki-cho, Kawasaki-ku
Kawasaki-shi 210–8681, Japan

Stoichiometric Approach

Chapter 1

Recent Developments in the Application of Organometallic Chemistry to Amino Acid Synthesis

Richard F. W. Jackson

The Department of Chemistry, The University of Sheffield, Dainton Building, Sheffield S3 7HF, United Kingdom

Alkylzinc iodide reagents derived from amino acids are useful intermediates for the synthesis of non-proteinogenic amino acids using metal-catalyzed coupling reactions. Dipolar aprotic solvents, specifically dimethylformamide, reduce the rate of elimination of β-amino alkylzinc reagents. These β-elimination reactions can be further suppressed by using trifluoroacetyl protection on nitrogen, which reduces intramolecular coordination to zinc, a key requirement in the elimination process. Acidic protons, specifically phenols, are tolerated in Negishi cross coupling reactions of these reagents.

Introduction

Alkylzinc iodides **1-5**, derived from readily available proteinogenic amino acids, react under metal catalysis with a wide range of electrophiles to produce enantiomerically pure protected non-proteinogenic amino acids (*1*).

Of particular interest are the three β-amino organozinc reagents **1**, **3** and **5**, since these reagents might be expected to be unstable towards β-elimination in an analagous manner to the corresponding β-oxygenated organozinc reagents **6** which eliminate spontaneously (Figure 1) (*2*), since the stabilities of the leaving groups (⁻OR and ⁻NHBoc) would be expected to be very similar. Uncovering the reasons for the differing behaviour of reagents **1**, **3** and **5** has been the focus of substantial recent effort, and this is the main topic of this chapter.
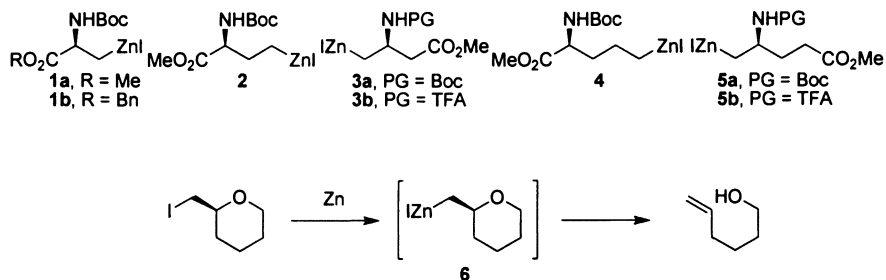
Figure 1. Elimination of a prototypical β-oxygenated organozinc reagent

The synthetic utility of amino acid derived organozinc iodides is illustrated by the Pd-catalyzed Negishi reactions of the serine-derived reagent **1** with acid chlorides to give 4-oxo amino acids **7** (*3*), with aryl iodides to give phenylalanine derivatives **8** (*4*) and with vinyl halides and triflates to give unsaturated amino acids **9** (*5*). Reaction with allylic substrates is possible using copper catalysis, leading to butenylglycine derivatives **10** (Figure 2) (*6*).
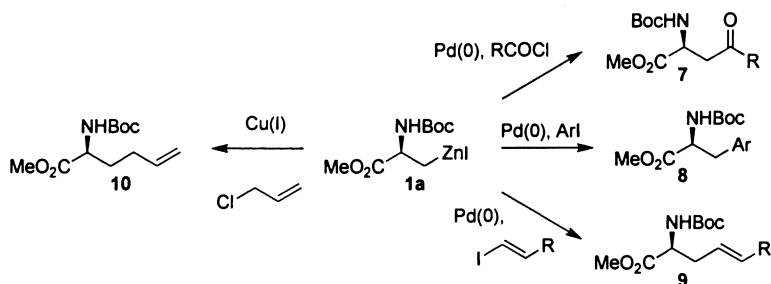
Figure 2. Reactions of serine-derived reagent 1 to give non-proteinogenic amino acids

The serine-derived reagents **1a** and **1b** have been widely used for the preparation of substituted phenylalanine derivatives, including compounds containing difluorophosphonates **11** (*7*), boronates **12** (*8*) and heterocycles **13** (*9*), **14** (*10*) and **15** (*11*). The preparation of these compounds illustrates the functional group tolerance of the reaction.

One of the most challenging examples of this general class of coupling is the intramolecular Negishi reaction employed in the synthesis of the cyclic tripeptide natural product, K13 **18**. Insertion of activated zinc into the alkyl iodide **16** in dimethylformamide (DMF), followed by treatment with a Pd(0) catalyst, gave the expected product **17** (Figure 3) (*12*). The alkylzinc reagent derived from **16** is amongst the most functionalized alkylzinc reagents that have been prepared.

Reagents and Conditions:
i) Zn*, DMF, rt, 20 min.
ii) Pd₂(dba)₃, P(o-tol)₃, 3 x 10⁻³ M THF, 60 °C, 16 h, 35 %
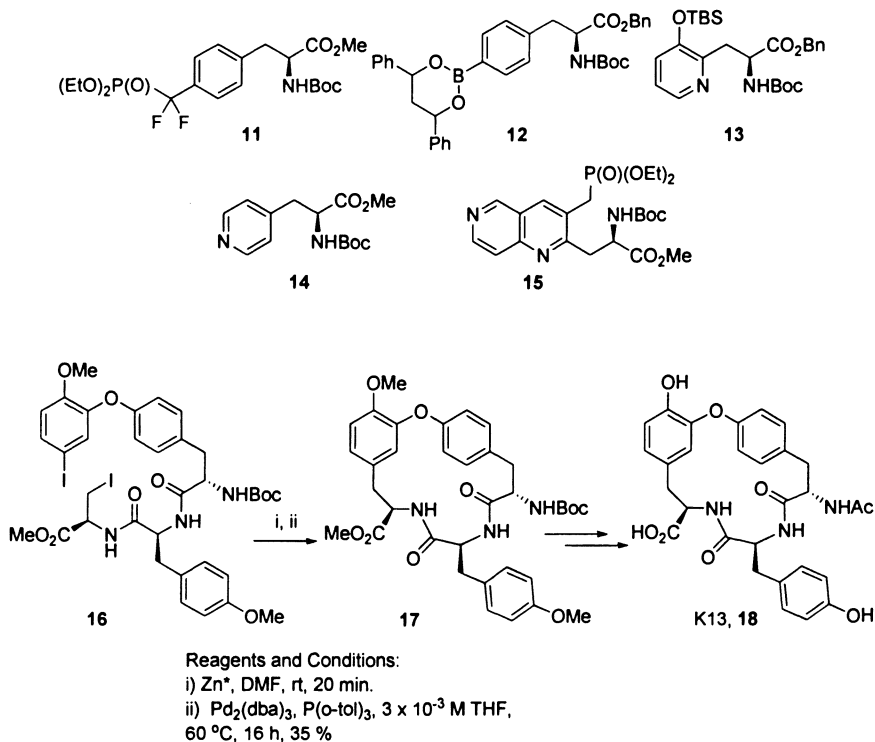
Figure 3. Synthesis of protected K13 by Intramolecular Negishi Reaction

Spectroscopic Studies on Serine-derived Alkylzinc Reagents

The nature of the solvent is important in both the reactivity and stability of β-amino organozinc reagents. Initial ^{1}H NMR studies of the alkylzinc reagent **1b** showed that it was formed efficiently in both THF and DMF, but that the signals due to the two diastereotopic protons adjacent to zinc were quite different in the two solvents (*13*). In THF, one proton appeared as a triplet, and the other a double doublet; both signals were quite broad, and there is a substantial chemical shift difference. In DMF, the signals for both protons were closer in chemical shift and, whilst clearly still inequivalent, were sharp double doublets. The most likely interpretation is that, in THF, there is internal coordination of zinc by the carbamate carbonyl group **19**, but in DMF the solvent competes with this internal coordination **20** (Figure 4). Coordination by the ester carbonyl group is of course also possible, so the true solution structure is likely to be dynamic.

In an effort to establish the (minimum) number of solvent molecules coordinated to the zinc reagent **1a** in DMF, the solution was directly analyzed
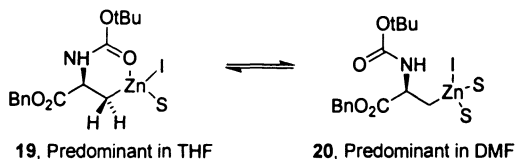
Figure 4. Possible Solution Equilibria for Alkylzinc Reagent **1b**

by positive ion ESI-MS (*14*). At a range of cone voltages, the only mass ion detected was at 339, formed by loss of iodide ion and containing one molecule of DMF. The presence of DMF in the only observed mass ion does suggest that at least one molecule of DMF is coordinated to zinc in solution (since this is where ionization takes place in the electrospray experiment), although association of more DMF molecules either through coordination to zinc, or by hydrogen bonding to the carbamate proton in solution, is possible. In the gas phase, it is likely that both the carbamate and ester carbonyl groups can co-ordinate to zinc thereby displacing DMF, forming the bicyclic cation **21** (Figure 5).
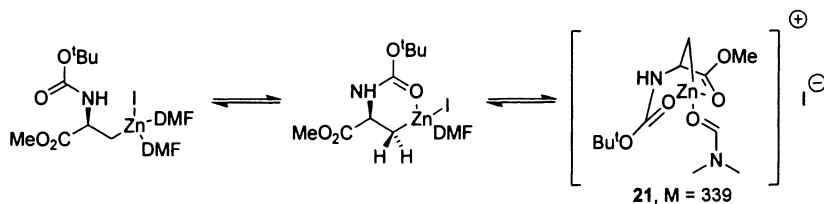
Figure 5. Formation of only observable ion derived from **1b** in ESI-MS

Spectroscopic Studies on Reagent 3a: Suppression of β-Elimination

Early efforts to use reagents **3a** and **5a** were plagued by problems of instability, specifically towards β-elimination. In THF especially, the rate of elimination of **3a** to form methyl but-3-enoate **22** was sufficiently fast that very poor yields of products arising from attempted Negishi coupling reactions were obtained. In DMF, however, the rate of elimination was much slower, and the yields in Negishi coupling reactions correspondingly higher (*15*). This observation suggested that internal coordination of the carbamate carbonyl group to zinc may promote the elimination reaction. A careful NMR study of the β-elimination reaction of **3a** showed that the reaction was first-order in both solvents, and allowed the activation parameters to be determined (Table I). Interpretation of these results is not straightforward, but the large negative entropy of activation in THF does indicate a highly ordered transition state.

Table I. Activation Parameters for Elimination of Reagent 3a

Solvent	$\Delta H^{\ddagger}\ kJ\ mol^{-1}$	$\Delta S^{\ddagger}\ J\ K^{-1}\ mol^{-1}$
THF-d$_8$	+ 70	- 85
DMF-d$_7$	+ 90	- 31
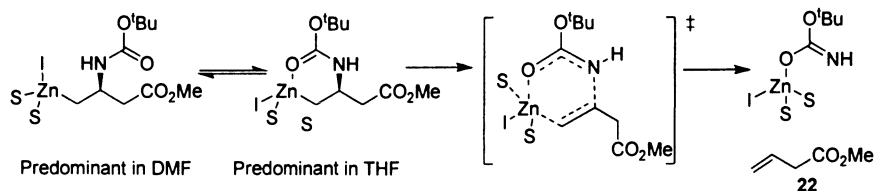
Figure 6. Proposed Mechanism for syn-Elimination of Reagent 3a

This suggests that the elimination most likely proceeds via a *syn*-process (Figure 6), in which coordination of the carbamate carbonyl group to zinc is necessary. In THF, carbamate coordination to zinc already exists, to some extent, in the ground state, so the enthalpy of activation is less than in DMF, in which loss of strongly bound DMF is required. The larger negative entropy of activation in THF can be explained by an enhanced requirement for coordination of zinc by THF as the transition state is formed. In DMF, zinc is already solvated in the ground state, so loss of DMF partially compensates entropically for the formation of the ordered transition structure. The entropy of activation is therefore less negative in DMF.

Notwithstanding these proposals, they are only significant if they lead to the design of a better reagent. Since the elimination reaction appeared to be dependent on the Lewis basicity of the carbonyl function, not the stability of the whole leaving group, it seemed reasonable to choose a nitrogen protecting group in which Lewis basicity was minimized. The trifluoroacetamide **3b** was therefore identified as a potentially improved reagent. This reagent contained a rather acidic proton, which was a concern. In the event, the reagent **3b** could be prepared (*16*), and in DMF the ^{1}H NMR spectrum showed that the two diastereotopic protons adjacent to zinc were now coincident (Figure 7).

The trifluoroacetamide **3b** proved to be more stable towards β-elimination than the original reagent **3a** (by a factor of more than 3-fold) when followed by ^{1}H NMR (*16*). Careful analysis of the kinetic data established, however, that elimination of **3b** was in fact a second order process (*17*). This was confirmed by studying the decomposition at a range of different initial concentrations of reagent **3b**, a classical method for discriminating between first and second order processes (Table II). The kinetic studies were carried out at initial concentrations very close to 1 *M*, allowing direct comparison of the 1st and 2nd order rate constants.
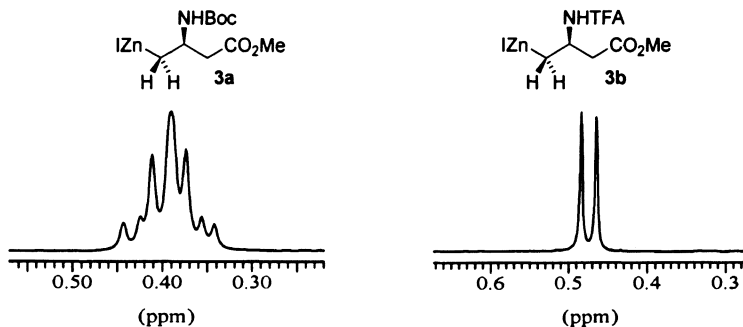
Figure 7. *^{1}H NMR Signals for Indicated Protons of 3a and 3b in DMF-d$_7$. (Reproduced with permission from reference 16. Copyright 2003 The Royal Society of Chemistry.)*

Table II. Rate Constants for Elimination of 3b

Initial Conc. of Reagent 3b	Apparent 1st Order Rate Constant/10^{-6} s^{-1}	2nd Order Rate Constant/10^{-6} M^{-1}s^{-1}
0.2 M	0.8	3.0
0.4 M	1.5	2.7
0.8 M	3.0	2.7

The observation that the change in protecting group had not only influenced the rate of the elimination reaction, but had even changed the mechanism of the process, was testament to the critical nature of the nitrogen protecting group. A possible explanation for this behaviour is that, rather then the alkylzinc halide, **3b**, undergoing elimination, it is in fact the corresponding dialkylzinc species **23** (the concentration of which is related to the square of the concentration of the alkylzinc halide, **3b**) formed through Schlenk pre-equilibrium, that undergoes the elimination reaction (Figure 8). This is reasonable on the basis that the carbonyl oxygen of the trifluoroacetamide group is not able to coordinate to zinc, and therefore promote the *syn*-elimination reaction, which occurs in the case of the corresponding Boc-derivative **3a**. The alternative elimination pathway, namely a straightforward *anti*-elimination, cannot occur in the alkylzinc iodide due to the electron-withdrawing character of the iodide ligand (which reduces the electron density at the carbon atom bound to zinc). However, the dialkylzinc species, without this influence, can undergo the elimination reaction.
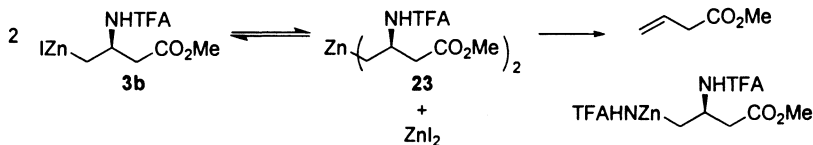
Figure 8. Proposed Elimination Mechanism for Reagent 3b

When the stability of the two glutamic acid-derived alkylzinc reagents **5a** and **5b** (determined by ^{1}H NMR) was compared, it was found that the introduction of the trifluoroacetamido group had an even larger stabilizing effect in this series. The rate constant for the first order elimination of **5a** was found to be much larger than that observed for the corresponding aspartic acid derivative **3a**, while the second order rate constant for the elimination of the glutamic acid-derived alkylzinc reagent **5b** was similar to that observed for **3b** (Table III).

Table III. Rate Constants for Elimination of Reagents 3a, 3b, 5a and 5b

Reagent	1st Order Rate Constant/ 10^{-6} s^{-1}	Reagent	2nd Order Rate Constant/10^{-6} $M^{-1}s^{-1}$
3a	9.0	**3b**	2.8
5a	24	**5b**	3.3

In the event, when preparative Negishi cross-coupling reactions of the two reagents **3a** and **3b** were carried out, the isolated yields of products **24** and **25** were broadly similar (Table IV) (*16*). Although reagent **3b** is more stable towards β-elimination than reagent **3a**, it is also appears to be less reactive in the cross-coupling reaction. Nonetheless, it does allow access to β^3-amino acids with a base-labile nitrogen protecting group.

The situation for the two glutamic acid derived reagents, **5a** and **5b**, is rather different. In this case, the reactivity of the reagent was increased by introduction of the trifluoroacetyl group, with the result that the alkylzinc reagent **5b** is extremely effective for the preparation of γ-amino acids **27** (Table V) (*17*). The increase in yield when using reagent **5b** compared to **5a** for the coupling with 2-fluoroiodobenzene, a normally inefficient substrate in such processes, is significant.

One possible explanation for the lower stability of reagent **5a**, compared to reagent **3a**, is that in the latter case one might expect enhanced coordination by the ester carbonyl group to zinc (forming a 6-membered ring **29**) compared to the situation with **5a** (forming a 7-membered ring **28**) (Figure 10). Evidence for this co-ordination has been provided through ^{13}C NMR studies. (*4*) Such coordination might be expected to compete with coordination by the Boc-group to zinc, thereby
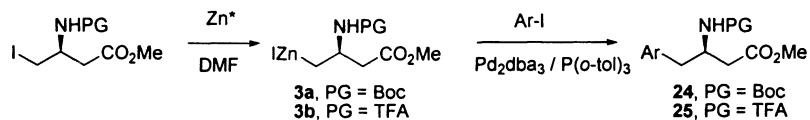
Figure 9a. Preparation of β³-amino acids 24 and 25 by Negishi Cross-coupling
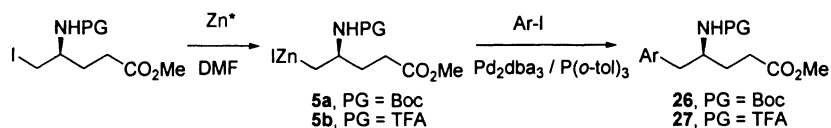
Figure 9b. Preparation of γ-amino acids 26 and 27 by Negishi Cross-coupling

Table IV. Comparison of Yields Obtained using Zinc Reagents 3a and 3b

Ar	Yield of 24 (%)[a] from Reagent 3a	Yield of 25 (%)[a] from Reagent 3b
4-MeC$_6$H$_4$	73	64
4-MeOC$_6$H$_4$	68	69
Ph	73	72
4-NCC$_6$H$_4$	-	77
1-Naphthyl	61	70
4-BrC$_6$H$_4$	58	53

[a] All yields are based on the starting alkyl iodide.

Table V. Comparison of Yields Obtained using Zinc Reagents 5a and 5b

Ar	Yield of 26 (%)[a] from Reagent 5a	Yield of 27 (%)[a] from Reagent 5b
4-MeOC$_6$H$_4$	68	79
2-H$_2$NC$_6$H$_4$	56	62
2-FC$_6$H$_4$	34	51
4-MeC$_6$H$_4$	68	79
Ph	68	88

[a] All yields are based on the starting alkyl iodide.

reducing the rate of elimination. The fact that the serine-derived reagent **1** (which could form a very stable 5-membered ring **30** through analogous internal coordination) is even less susceptible to elimination is entirely consistent with this hypothesis. Since the mechanism for elimination from the trifluoroacetamides **3b** and **5b** does not require such coordination, the position of the ester would not be expected to exert a substantial influence (as is indeed observed).

28, Less significant   **29**, Moderately significant   **30**, Most significant
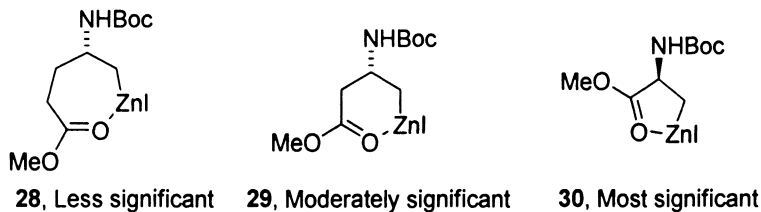
Figure 10. Internal Coordination by the Ester Carbonyl Group

Negishi Reactions of Alkylzinc Halides Tolerate Unprotected Phenols

As previously noted, the alkylzinc reagents **3b** and **5b** each contain an acidic trifluoroacetamide proton. The satisfactory results obtained in Negishi cross-coupling reactions with both reagents suggest that protonation of the carbon-zinc bond by the trifluoroacetamide proton is not a problematic side-reaction. Literature data for the pKa of carbamate protons (such as those found in **3a** and **5a**) suggests a value of around 25, when measured in DMSO (*18*), presumed to be a reasonable guide for the corresponding pKa values in DMF. The pKa of trifluoroacetamide in DMSO is 17, which suggests that electrophiles containing protons with pKa values higher than this might be tolerated in reactions with alkylzinc halides. The most intriguing candidate is phenol (pKa 18), so a study to determine the compatibility of unprotected phenols in Negishi cross-coupling reactions with alkylzinc halides was undertaken. Reactions of alkylzinc reagents **1a** and **2** with 4-iodophenol gave moderate yields of the coupled products (based on the alkyl iodide precursors to the corresponding alkylzinc iodides). Analogous reaction of alkylzinc reagent **3c** gave the product in excellent yield demonstrating that unprotected phenols are indeed tolerated (Figure 11) (*19*). It is possible that this tolerance has a kinetic origin, a topic for future research.

Conclusions

While the synthetic utility of alkylzinc reagents derived from proteinogenic amino acids has been clear for some time, recent work has started to shed light
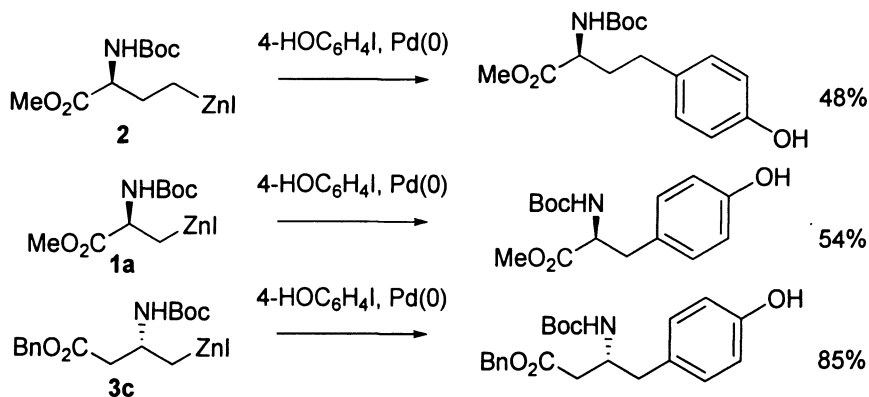
Figure 11. Negishi Cross-Coupling with Unprotected Iodophenols

on why such highly functionalized organometallic reagents can exist, and the structural characteristics that help to stabilize the reagents (specifically towards β-elimination of protected nitrogen functionality). Better understanding of these features promises to lead to the design of new, even more effective reagents.

References

1. Rilatt, I.; Caggiano, L.; Jackson, R. F. W. *Synlett* **2005**, 2701-2719.
2. Furstner, A.; Weidmann, H. *J. Org. Chem.* **1990**, *55*, 1363-1366.
3. Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. *J. Org. Chem.* **1992**, *57*, 3397-3404.
4. Jackson, R. F. W.; Moore, R. J.; Dexter, C. S.; Elliot, J.; Mowbray, C. E. *J. Org. Chem.* **1998**, *63*, 7875-7884.
5. Carrillo-Marquez, T.; Caggiano, L.; Jackson, R. F. W.; Grabowska, U.; Rae, A.; Tozer, M. J. *Org. Biomol. Chem.* **2005**, *3*, 4117-4123.
6. Dunn, M. J.; Jackson, R. F. W.; Pietruszka, J.; Turner, D. *J. Org. Chem.* **1995**, *60*, 2210-2215.
7. Smyth, M. S.; Burke, T. R., Jr. *Tetrahedron Lett.* **1994**, *35*, 551-554.
8. Malan, C.; Morin, C. *Synlett* **1996**, 167-168.
9. Ye, B.; Burke, T. R. *J. Org. Chem.* **1998**, *63*, 410-410.
10. Walker, M. A.; Kaplita, K. P.; Chen, T.; King, H. D. *Synlett* **1997**, 169-170.
11. Swahn, B.-M.; Claesson, A.; Pelcman, B.; Besidski, Y.; Molin, H.; Sandberg, M. P.; Berge, O.-G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1635-1640.
12. Perez-Gonzalez, M.; Jackson, R. F. W. *Chem. Commun.* **2000**, 2423-2424.

13. Dexter, C. S.; Hunter, C.; Jackson, R. F. W.; Elliott, J. *J. Org. Chem.* **2000**, *65*, 7417-7421.
14. Di Marco, V. B.; Bombi, G. G. *Mass Spectrom. Rev.* **2006**, *25*, 347-379.
15. Dexter, C. S.; Jackson, R. F. W.; Elliott, J. *J. Org. Chem.* **1999**, *64*, 7579-7585.
16. Jackson, R. F. W.; Rilatt, I.; Murray, P. J. *Chem. Commun.* **2003**, 1242-1243.
17. Rilatt, I. PhD, The University of Sheffield, 2006.
18. Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456-463.
19. Jackson, R. F. W.; Rilatt, I.; Murray, P. J. *Org. Biomol. Chem.* **2004**, *2*, 110-113.

Chapter 2

Synthesis of Heterocycle-Linked *C*-Glycosyl α-Amino Acids and *C*-Glycopeptides

Alessandro Dondoni and Alessandro Massi

Dipartimento di Chimica, Laboratorio di Chimica Organica, Università di Ferrara, Via L. Borsari 46, I–44100 Ferrara, Italy

Non-natural *C*-glycosyl α-amino acids are building-blocks for the co-translational modification of natural glycopeptides. These are key components of the complex machinery that operates in vital processes of all living organisms, ranging from eubacteria to eukaryotes. While a wide range of *C*-glycosyl amino acids have been synthesized, we have designed and prepared a small collection of a new class of these amino acids whose structure features a heterocycle ring holding the carbohydrate and the amino acid fragments. Isoxazole, triazole, tetrazole, and pyridine moieties were used as suitable linkers in this project owing to their easy construction via Huisgen cycloaddition and Hantzsch multicomponent reaction. Details on the synthesis of each family of these glycol-conjugates are given below, some emphasis being given to the efficiency and generality of each method employed. The potential of these glycosyl amino acids in non-natural glycopeptide synthesis is exemplified by the coupling of a pyridine-linked derivative with two natural amino acids to give a non-natural glycotripeptide.

Introduction

Protein glycosylation is a ubiquitous post-translational modification that has been evolutionary conserved from unicellular yeast to man in structure and biosynthetic pathway.(*1*) An impressive variety of carbohydrate-peptide linkages are found in glycoproteins distributed in essentially all living organisms, ranging from eubacteria to eukaryotes.(*2*) The structure of the oligosaccharide units present on the proteins are quite diverse, as they fall into two basic types defined by their linkage to the protein backbone. Specifically, *O*-linked oligosaccharides are bound to the hydroxyl group of amino acids threonine, serine, hydroxyproline, hydroxylysine, and tyrosine via an *N*-acetylgalactosamine residue, whereas *N*-linked oligosaccharides are bound to the amide group of asparagine via *N*-acetylglucosamine residue (Figure 1).

Oligosaccharide units of glycoproteins participate in folding of nascent proteins in endoplasmic reticulum, serve as protection from the action of proteases, and modulate the biological activity.(*3*) Moreover, the saccharide portion plays its own key role in a variety of biological events such as specific recognition sites for the intracellular targeting of proteins, the clearance of proteins from the plasma, and cell-cell interactions. Given the complexity and multiple functions of the oligosaccharide units in glycoproteins, it can reasonably be expected that genetic defects that impair the biosynthesis of these structures would be detrimental and result in clinical syndromes. This is in fact the case. For instance, following the Jaeken observations in 1980,(*4*) it is now clear that congenital underglycosylation of proteins causes severe problems in children and typically results in interference with normal development of the brain and functions of the nerve, liver, stomach, and intestinal systems. This disease which is called CDG-syndrome (congenital disorder of glycosylation) (*1*) is sufficient to demonstrate the importance of protein glycosylation and the need to obtaining more insights in this field. Indeed, the list of diseases due to abnormal protein glycosylation is growing steadily.(*5*) In this area of glycobiology and glycomedicine, synthetic organic chemistry is called to contribute with new and efficient synthetic tools that can furnish *O*- and *N*-glycopeptides with a well defined structure and composition. The isolation of homogeneous glycopeptides from natural glycoproteins is complicated by the presence of the latter as a mixture of glycoforms. Moreover, chemical synthesis can offer an entry to various non-natural glycopeptides in which the carbohydrate unit is bound to the polyamide backbone through an artificial tether resistant to chemical and enzymatic degradation. Synthetic glycopeptides can be used as probes in biological studies for a deeper understanding of the effect of glycosylation on protein structure and function in molecular detail.(*6*) These products can also result in new drug-discovery leads against carbohydrate-based metabolic disorders. Quite obviously, the key building blocks needed in a co-translational approach (*7*) to non-natural glycopeptides are non-natural glycosyl amino acids.
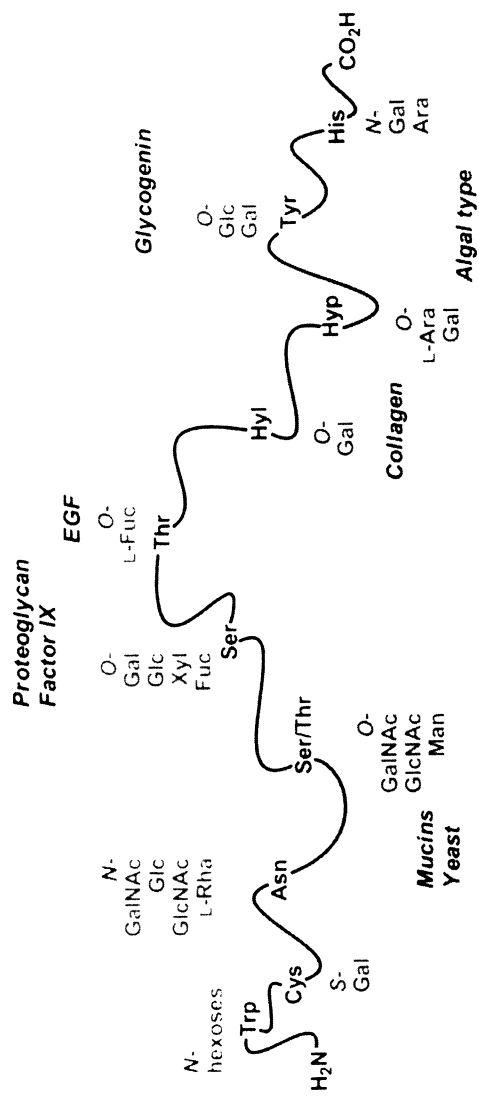
Figure 1. Forms of protein glycosylation. (See page 1 of color insert.)

Therefore several methods have been reported in the last two decades for the synthesis of *C*-glycosyl amino acids,(*8*) especially methylene isosteres of glycosyl serines (9) and ethylene isosteres of glycosyl asparagines.(*9b,10*) In the same vein we have recently designed a new and more elaborated class of artificial *C*-glycosyl amino acids which display a nitrogenated heterocyclic ring holding the carbohydrate and amino acid moieties (Figure 2). Isoxazole, triazole, tetrazole, and pyridine moieties were envisaged as suitable linkers in this project owing to their easy construction via Huisgen cycloaddition and Hantzsch multicomponent reaction. Hence, the general strategy that we planned to follow consisted of the one-step generation of the heterocyclic ring from suitable reaction partners which in addition to a reactive functionality incorporated a sugar or amino acid residue. We envisaged the heterocycle ring in the constructed glycopeptides to serve as rigid and robust linker as well as an active site for molecular recognition processes through H-bonding and dipolar interactions. In addition, the efficient construction of the heterocycle-tethered glycosyl amino acid architecture would allow the preparation of a collection of strictly related compounds in which structural and stereochemical diversity can be explored by varying the nature of the sugar residue as well as its position in the heterocyclic rings. Indeed, the search for structural changes and new biological properties of peptides induced by introduction of non-natural heterocycle amino acid fragments has been actively pursued since several years.(*11*)

A. Isoxazole-Linked *C*-Glycosyl α-Amino Acids

Among the variety of 1,3-dipolar cycloadditions (Huisgen reaction),(*12*) the thermally induced nitrile oxide-alkyne coupling to give substituted isoxazoles is one of the most powerful synthetic tool that has been exploited in a wide range of synthetic methodologies.(*13*) A recent improvement of this reaction relies on the use of Cu(I)-catalyst to gain a full control of the regioselectivity.(*14*) This promotes the cycloaddition to the status of a "click process". Hence, the isoxazole annulation from a sugar nitrile oxide and an ethynyl amino acid appeared a viable route to *C*-glycosyl isoxazole α-amino acids.(*15*) The feasibility of this program relied on the prompt access to various glycosyl nitrile *N*-oxides and their good reactivity with alkynes.(*16*) Accordingly, the *in situ* generation of *C*-galactosyl nitrile oxide **2a** (from aldoxime **1a** and *N*-bromosuccinimide and then Et₃N) in the presence of excess (10 equiv.) of alkyne **3** afforded the 3,5-disubstituted isoxazole **4a** (H-4, δ = 6.23 ppm, DMSO-*d₆*, 120 °C) as the sole regioisomer, while the side-product was the nitrile oxide cyclic dimer, i.e. the sugar furoxan **7a** (Scheme 1). Guided by earlier experience carried out in our laboratory on the transformation of the *N*-Boc oxazolidine ring into the α-amino acid (glycinyl) group via acid catalyzed ring opening and Jones
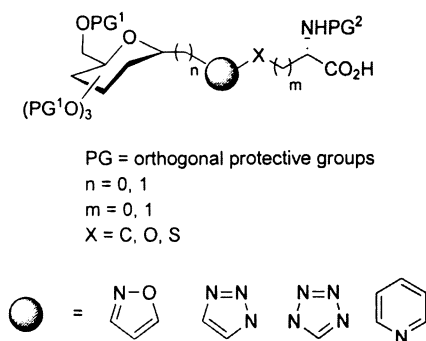
Figure 2. Heterocycle-tethered C-glycosyl amino acids.

oxidation of the alcohol intermediate, compound **4a** was readily transformed by this one-pot reaction sequence into the corresponding α-amino acid **5a**. This product was then isolated and characterized as the corresponding methyl ester **6a**. In order to demonstrate the potential of this glycosyl amino ester as an orthogonally protected substrate suitable for *N*-Boc peptide synthesis, debenzylation of the carbohydrate fragment was carried out in high yield by hydrogenation over Pd(OH)$_2$ without affecting the reducing agent sensitive isoxazole ring. The whole procedure described above was successfully repeated starting from the *C*-glucosyl nitrile oxide **2b** (Scheme 1).

A more straightforward approach to *C*-glycosyl amino esters **6a** and **6b** was then optimized by using the readily available ethynyl functionalized amino ester **8** as a partner of the 1,3-dipolar cycloadditions (1,3-DCRs) with sugar nitrile oxides. In fact, it was demonstrated that cyclocondensations of in situ generated nitrile oxides **2a** and **2b** with an excess of the alkyne **8** proceeded smoothly at room temperature furnishing the 3,5-disubstituted isoxazole **6a** and **6b** in good yield without any loss of stereochemical integrity at α-carbon of the glycinate moiety (Scheme 2).

B. Triazole-Linked *C*-Glycosyl α-Amino Acids

Another powerful reaction in the panoply of Huisgen 1,3-dipolar cycloadditions (*12*) is represented by the azide-alkyne coupling to give 1,2,3-triazoles. This reaction, however, met with less success in synthetic methodology than the nitrile oxide-alkyne coupling very likely for two main reasons. One reason is the so called azidophobia, that is the reluctance of many researchers to use organic azides because of the explosive character of some of them, especially those with low molecular weight. Another reason which, however, is quite substantial is the low selectivity of the reaction when terminal alkynes are
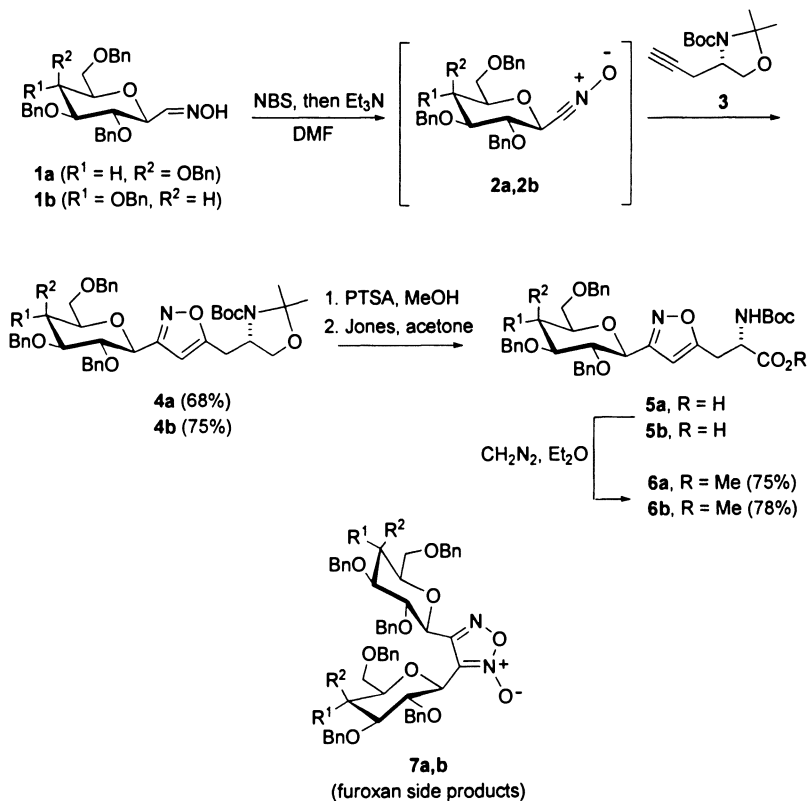
Scheme 1. Initial approach toward the synthesis of C-glycosyl isoxazole alanines.
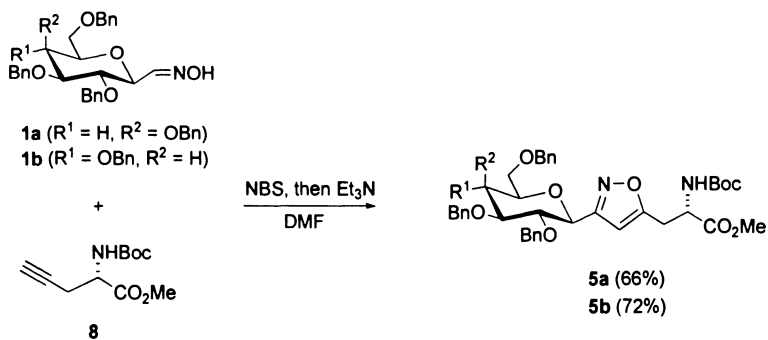
Scheme 2. Optimized synthesis of C-glycosyl isoxazole alanines.

employed. In the event mixtures of 1,4- and 1,5-disubstituted triazole regioisomers are in general obtained. Nevertheless, we considered quite important to introduce the triazole ring as a linker in the designed non-natural glycosyl amino acids of our project because of the numerous properties of this heterocycle. This, in addition of being a site for the formation of hydrogen bonding due to the presence of three basic nitrogen atoms, is quite stable under a variety of reaction conditions. Moreover, not present in natural products, the triazole ring is remarkably stable to metabolic transformations, thus making the triazole ligation an ideal tool for the assembly of compounds for biological testing. In a first attempt of execution of our program we experienced the above mentioned shortcoming, i.e. the lack of regioselectivity, of the thermal azide-alkyne coupling.(*15*) In the event the fusion process (120 °C) of a 1:1 mixture of the ethynyl *C*-galactoside **9a** and the azide **10** afforded the 1,5- and 1,4-disubstituted triazole regioisomers **11a** and **12a** respectively in 5:1 ratio and 80% overall yield (Scheme 3). The structure of these compounds was assigned on the basis of their ^{1}H and ^{13}C NMR spectra.(*17*)

After separation, the two regioisomers were submitted to the standard oxazolidine to glycinyl group unmasking procedure to give the corresponding *C*-galactosyl triazole α-amino acids which were isolated as the methyl esters **13a** and **14a**. One of these compounds was debenzylated by hydrogenolysis over Pd(OH)$_2$, thus demonstrating the orthogonality of the whole set of protecting groups and the stability of the triazole ring. Similar results were obtained by coupling the perbenzylated ethynyl *C*-glucoside **9b** with the azide **10** and subsequent elaborations as described above. While from the combinatorial synthesis viewpoint the above route can be regarded as an entry to pairs of regioisomers both showing interesting structures for biological testing, from the viewpoint of synthetic efficiency the disadvantage is apparent due to the need of a separation procedure. The control of the regiochemistry of the azide-alkyne cycloaddition in favor of the exclusive formation of the 1,4-disubstituted triazole regioisomer by Cu(I)-catalysis introduced independently by Sharpless (*18*) and Meldal (*19*) groups provides a simple solution to this longstanding problem. The use of this catalyst allows also performing the cycloaddition under mild conditions as well as with reduced reaction time while the yield becomes almost quantitative. Hence the azide-alkyne cycloaddition is considered the prototypical of the click reaction family as defined by Sharpless and his colleagues.(*20*) Accordingly, it was with our great delight that we observed that both the ethynyl *C*-glycosides **9a** and **9b** reacted readily with the azide **15** bearing the unmasked glycinate moiety in toluene at room temperature in the presence of CuI and the Hünig base diisopropylethyl amine (DIPEA) to give in one-step high yields of the corresponding *C*-glycosyl α-amino esters **14a** and **14b** tethered by the 1,4-disubstituted triazole ring (Scheme 4).

To broaden the scope of the azide-alkyne ligation for *C*-glycosyl-triazole amino acid synthesis, the need for selective access to 1,5-disubstituted triazole
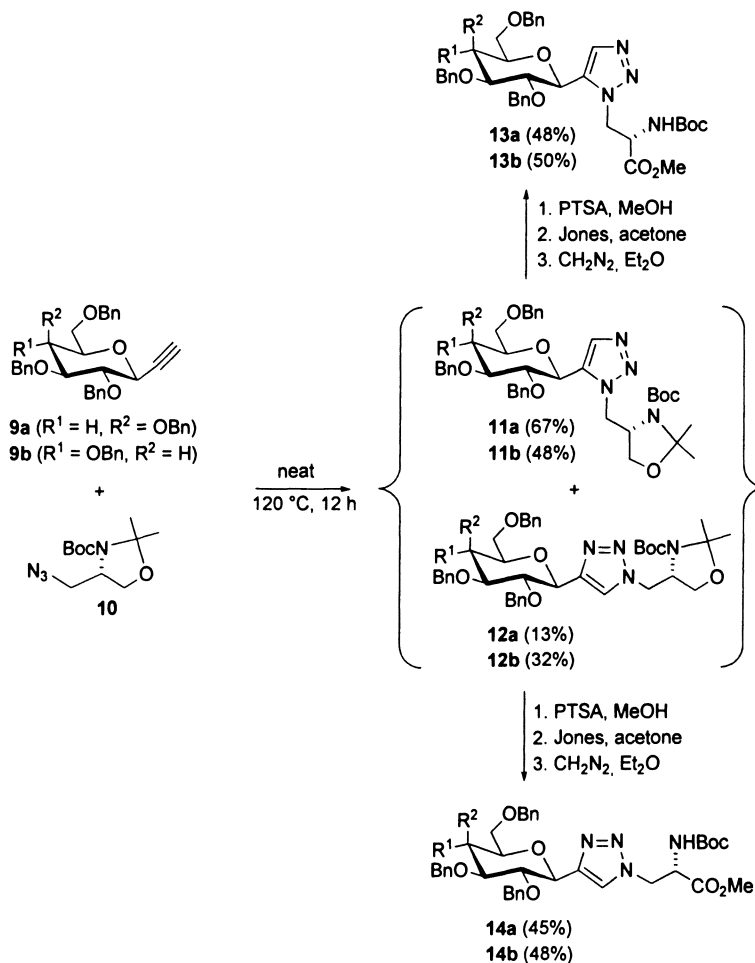
Scheme 3. Initial approach toward synthesis of C-glycosyl triazole alanines.

regioisomers became apparent. To this end we considered performing the cycloaddition in the presence of ruthenium catalysts that have been used by Fokin and Jia and their colleagues to control the 1,5-regioselectivity in triazole synthesis from azide-alkyne coupling.(*21*) Hence, optimal reaction conditions previously reported by these authors for the ruthenium-catalyzed cycloaddition of simple alkynes and azides were initially applied to the cycloaddition of more sophisticated reagents such as **9a** and **15**. Quite disappointingly, the use of Cp*RuCl(PPh$_3$)$_2$ catalyst (1 mol%), i.e. the pentamethyl analogue of less effective CpRuCl(PPh$_3$)$_2$ catalyst, in benzene at ambient temperature for 24 h or
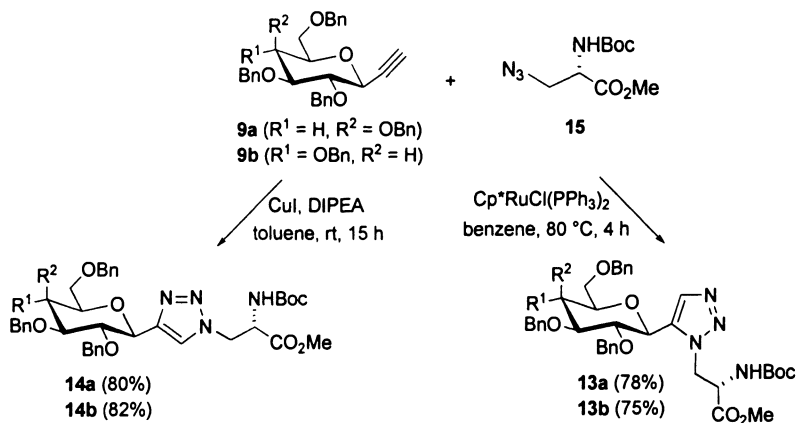
Scheme 4. Regioselective synthesis of C-glycosyl 1,5-and 1,4-triazole alanines.

at 80 °C for 2 h resulted in the isolation of unreacted components and a mixture of 1,4- and 1,5 regioisomers **13a** and **14a** (20% overall yield), respectively. Furthermore, utilization of a slightly higher loading of the catalyst (5 mol%) as well as application of longer reaction times produced similar unsatisfactory results. Fortunately enough, a suitable reaction window was found by performing the cycloaddition of **9a** and **15** in benzene at 80 °C for 4 h in the presence of 30 mol% of Cp*RuCl(PPh₃)₂ catalyst (Scheme 4). In fact, under these optimal conditions the 1,5-disubstitued triazole cycloadduct **13a** was isolated in good yield (78%) as the sole regioisomer. Similar results were then reproduced for the cycloaddition of *C*-glucosyl alkyne **9b** and azide **15** to give the amino ester **13b** (Scheme 4).(*22*)

C. Tetrazole-Linked *C*-Glycosyl α-Amino Acids

A difference in structure and activity of glycosyl amino acids with an azole linkage as well as glycopeptides derived therefrom may arise from the position/number of nitrogen atoms in the azole ring as well as the subtle changes in the electronic character of the heterocycle.(*23*) This suggested the introduction of a more densely nitrogenated heterocycle than the triazole ring. Therefore the use of tetrazole appeared to be quite appropriate. Moreover, the tetrazole moiety is quite stable under a variety of reaction conditions and can serve as metabolically stable surrogate for a carboxylic or amide group.(*24*) While the preparation of a small collection of *C*-glycosyl tetrazole amino acids is still underway, results have been obtained that indicate the feasibility of the

synthesis of these compounds.*(25)* The viable and simplest tetrazole synthesis is provided by the Huisgen-type azide-nitrile 1,3-dipolar cycloaddition.*(12)* The scope and efficiency of this reaction, however, are limited due to the need of using nitriles activated by strong electron-withdrawing groups and harsh reaction conditions. While the former point still remains a major drawback for this transformation, safety concerns have been recently addressed by Vilarrasa and Bosch, who reported on the first tentative metal-catalyzed "click" protocol for the organoazide-nitrile coupling.*(26)* These authors found that simple 1,5-disubstitued tetrazole derivatives can be prepared in excellent yields in dichloromethane at ambient temperature in the presence of 1-10 mol% of soluble $Cu_2(OTf)_2 C_6H_6$ catalyst, while 1,4-disubstituted tetrazoles are obtained as major regioisomers under heterogeneous conditions with 50-100 mol% of the same catalyst. Foregoing work in Sharpless *(27)* and our own laboratories *(28)* solved the problem of azide-nitrile ligation of more complex substrates. A two-step reaction sequence has been developed involving the cycloaddition of azides with an activated nitrile such as *p*-toluensulfonyl cyanide and then substitution of the tosyl group in the tetrazole thus formed by a suitable nucleophile. On applying this strategy to the synthesis of *C*-glycosyl tetrazole amino acids, we reacted the galactosylmethyl azide **16** with *p*-toluensulfonyl cyanide in the absence of solvent to give the 1,5-disubstituted tetrazole **17** (Scheme 5). This was reacted with the alcohol **18**, a masked serine form, under basic conditions to give the product **19** from which the amino ester **20** was unmasked by the aforementioned *N*-Boc oxazolidine ring cleavage and esterification. The quantitative removal of the benzyl groups $(Pd(OH)_2/H_2)$ from the carbohydrate moiety of **20** demonstrated the orthogonality of protective groups and the stability of the tetrazole ring. This reaction sequence appeared to be equally viable by using the thiol **21** as nucleophile (Scheme 6). In this way the *C*-galactosylmethyl tetrazole cystein **22**, i.e. the *S*-isostere of the amino ester **20** was prepared.*(25)*

D. Pyridine-linked *C*-Glycosyl α-Amino Acids

In earlier work from our laboratory we demonstrated the efficiency of the Hantzsch cyclocondensation for the synthesis of pyridine glycoconjugates and pyridylalanines.*(29)* To this end the dihydropyridine ring was constructed by the three-component thermal coupling comprising the aldehyde-ketoester-enamino ester system wherein one of the reagents was bearing a carbohydrate or amino acid fragment. The facile dehydrogenation of the dihydropyridine led to the target substituted pyridine. Consequently, we envisaged an entry to *C*-glycosyl-pyridine amino acids by the same Hantzsch-type pyridine synthesis using the above triad of reagents, one of which being decorated with the carbohydrate unit and the other with the amino acid group. While the design and synthesis of
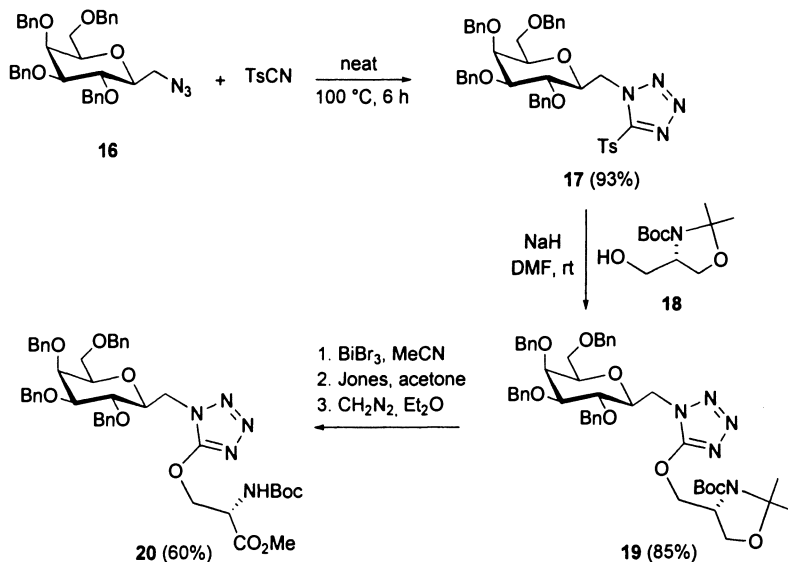
Scheme 5. Explorative studies toward the synthesis of C-glycosylmethyl tetrazole serines.
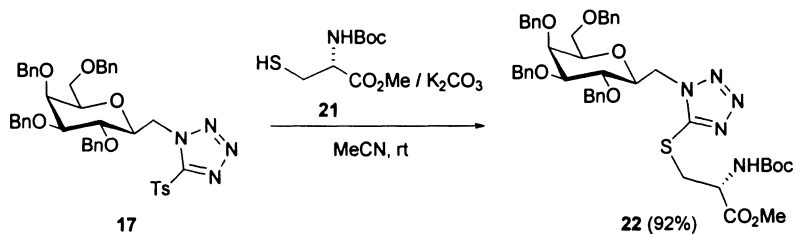
Scheme 6. Explorative studies toward the synthesis of C-glycosylmethyl tetrazole cysteines.

individual reagents have been reported in detail,(*30*) the essential of the results obtained thereafter are presented here.

A suitable substrate combination to perform the one-pot Hantzsch reaction was constituted of the *C*-galactosyl acetaldehyde **23**, the ketoester **24** functionalized with the protected glycinyl group, and *tert*-butyl aminocrotonate **25** (Scheme 7). The cyclocondensation of these reagents took place in *t*-BuOH at 70 °C to give after 24 h the carbohydrate and amino acid decorated dihydropyridine **26** as a mixture of diastereoisomers in fair yield (route A). This mixture was transformed into the target *C*-galactosyl-pyridine amino ester **27**, i.e. a pyridine-2-alaninate, by oxidation with pyridinium chlorochromate (PCC). The formation of a single final product demonstrated that the configuration of the anomeric center of the carbohydrate residue as well as of the asymmetric carbon of the glycinyl group remained unaltered throughout the whole reaction sequence.
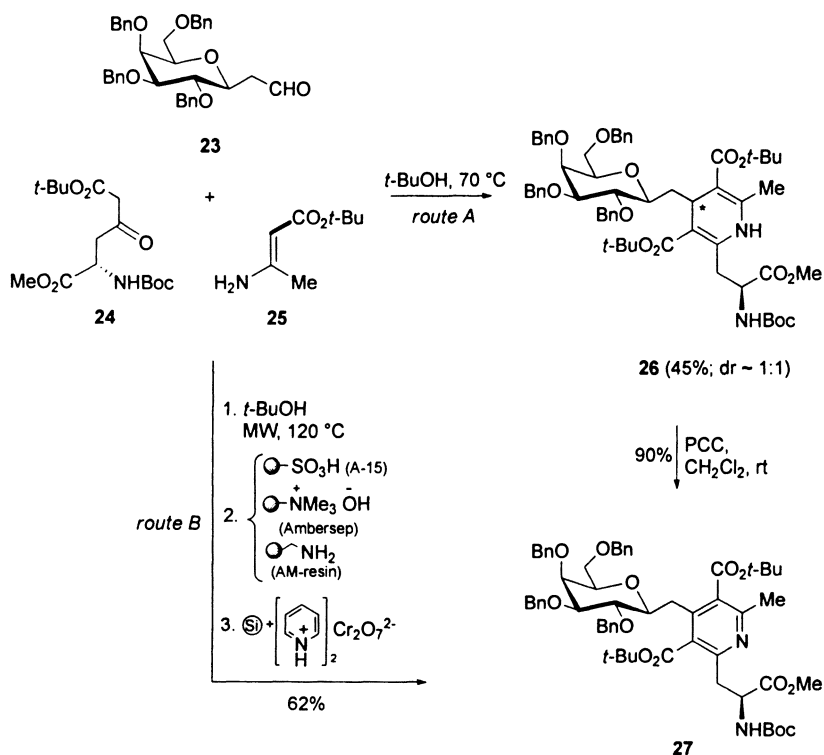
Scheme 7. Optimization of reaction conditions for the synthesis of 4-(C-glycosylmethyl)-2-(alaninyl)-pyridines.

Substantial improvement to the method efficiency was achieved by executing the cyclocondensation under MW irradiation and then performing product purification and oxidation using polymer supported sequestrants (Scheme 7, route B). MW irradiation at 120 °C reduced the cyclocondensation time from 24 to 1.5 h. Then, the reaction mixture was treated with a mixed-resin bed constituted of three polymer-bound reagents which were chosen as specific scavengers of unreacted material and side products. Specifically: (i) the supported sulfonic acid A-15 removed the residual enamine, (ii) the strong base Ambersep sequestered the ketoester, and (iii) the aminomethylated polystyrene (AM-resin) subtracted the aldehyde. This supported amine removed also the Knoevenagel adduct that was formed as initial condensation product between the aldehyde and the ketoester. After filtration of the resins, the diastereomeric dihydropyridines were oxidized to pyridine with PCC supported on silica gel. The overall yield of the isolated amino ester **27** was much higher than that registered in the two-step procedure (route A).

A different reagent combination was employed to prepare a regioisomer of **27**, i.e. the *C*-glycosyl pyridine-4-alaninate **30** (Scheme 8). The reagents employed were the aspartate semialdehyde **28**, the glycosylated ketoester **29**, and the already exploited valuable aminocrotonate **25**. This reaction was carried out under MW irradiation and processed using the same orchestrated sequence of polymer supported reagents illustrated in Scheme 7. The target amino ester **30** was isolated in 68% yield.

The efficiency of the routes illustrated in Schemes 7 and 8 was validated by preparation of other amino acids so that a collection of eight compounds shown in Figure 3 was obtained in which the elements of diversity were: (i) the *galacto* and *gluco* configurations of the pyranose ring, (ii) the α- and β-configurations at the anomeric center, and (iii) the positions of the carbohydrate and amino acid residues in the pyridine ring.

E. Pyridine-Linked *C*-Glycopeptides

In order to demonstrate the potential of the sugar amino esters presented in Figure 3 as orthogonally protected building blocks suitable for the co-translational synthesis of non-natural glycopeptides, the product **27** was selected as a prototype in this crucial validating test (Scheme 9). To this end, this compound was duly modified by selective hydrolysis of the ester functionality of the glycinyl group under very mild conditions (LiOH in THF at 0 °C). Then, the *N*-Boc protected pyridylalanine **37** obtained in this way was condensed with H-Phe-OEt under activation by (benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate (PyBOP) and the presence of DIPEA. This initial coupling step afforded the dipeptide **38**. The removal of the *N*-Boc group in this compound under mild acid conditions (diluted TFA) liberated the NH_2 group
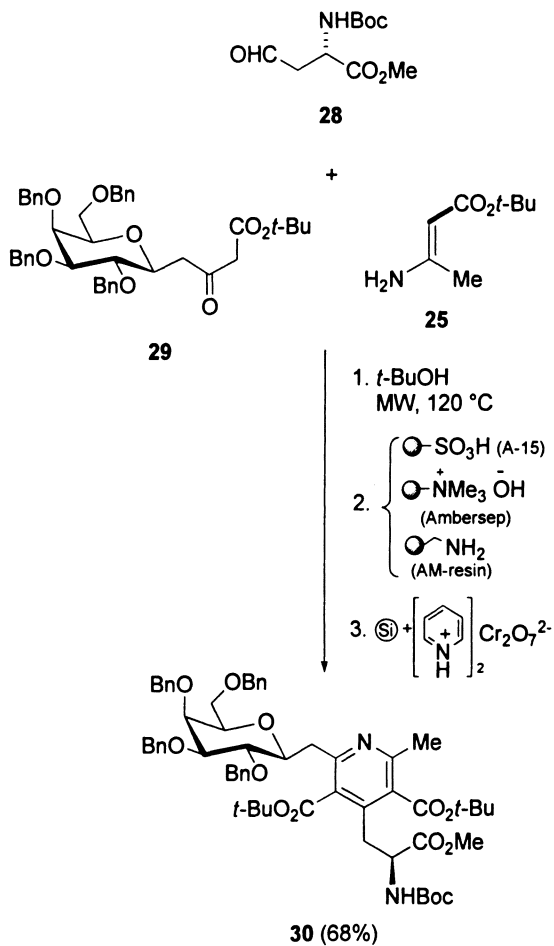
Scheme 8. Optimized conditions for the synthesis of
2-(C-glycosylmethyl)-4-(alaninyl)-pyridines.
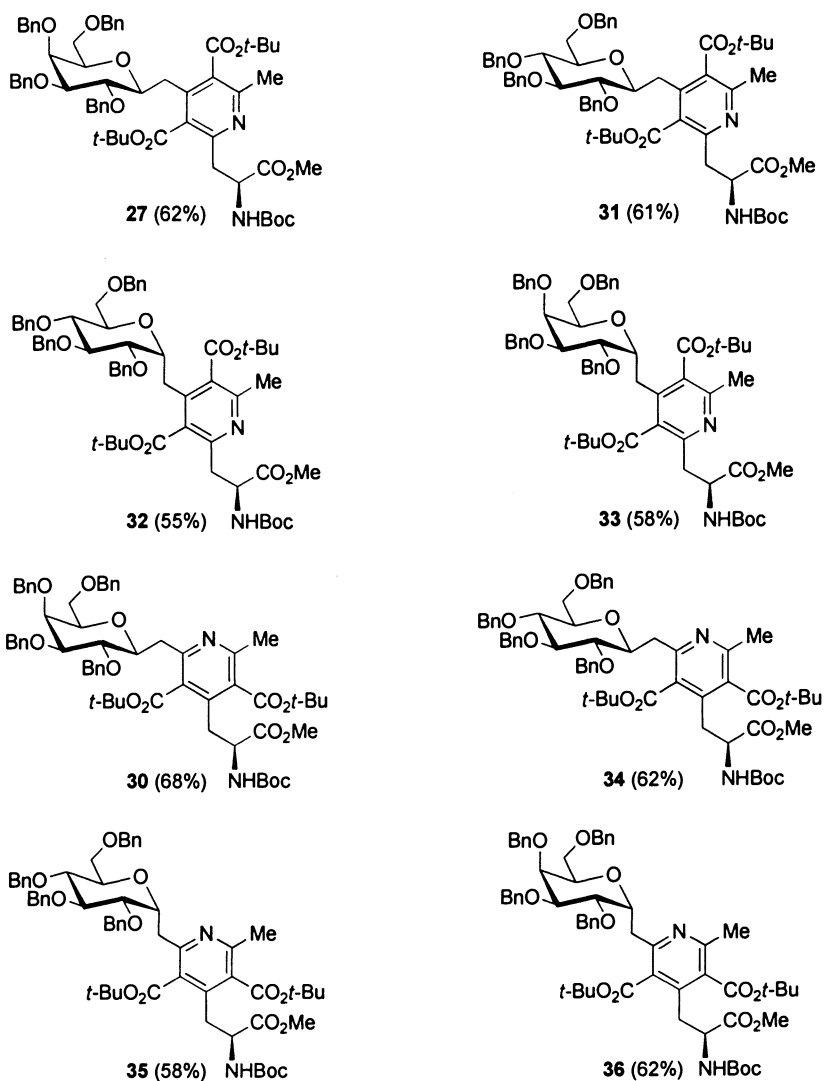
Figure 3. Pyridine-tethered C-glycosyl alanines prepared by Hantzsch three-component reactions.

that was used in the condensation with Boc-Ala-OH under the same coupling conditions illustrated above. The tripeptide **39** featuring a pendant galactosylmethyl residue linked through a rigid pyridine ring to the peptide backbone was isolated in 65% yield.
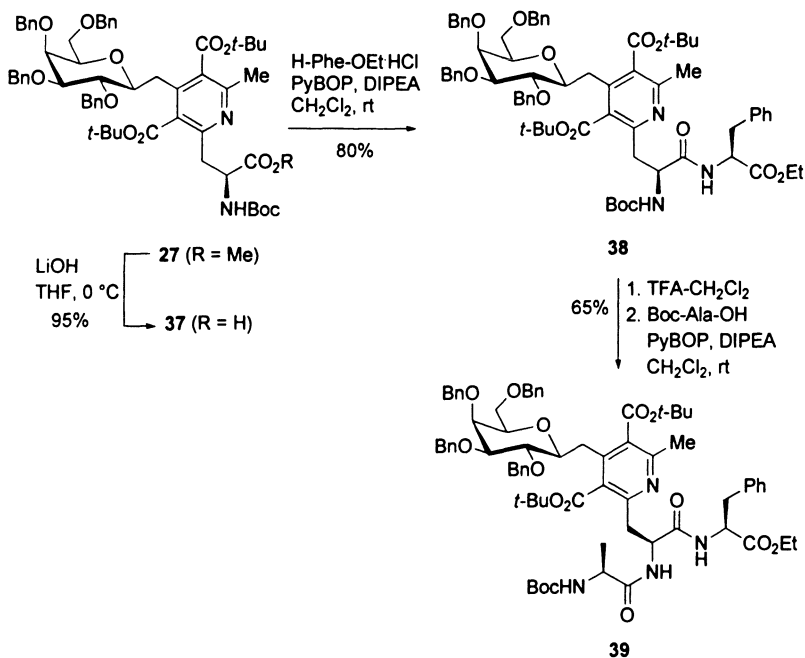
Scheme 9. Di- and tripeptide with embodied C-galactosylmethyl pyridylalanine.

Conclusions

We have reported in this short account some of our recent work that culminated with the synthesis of four types of carbohydrate-heterocycle-amino acid hybrids. The heterocycles that were considered as stable linkers of the two bioactive moieties were the heteroaromatic five- and six-membered ring isoxazole, triazole, tetrazole, and pyridine. In all cases the carbohydrate unit was anomerically bound to the heterocycle through a carbon-carbon bond. These compounds were prepared with the final goal to be introduced in glycopeptides. The main idea that spurred us to develop efficient entries to this new class of *C*-glycosyl amino acids is the involvement of the nitrogenated heterocycle ring in

structure modification of peptides as well as molecular recognition processes. The confirmation of this hypothesis indicates the direction for future research.

References

1. Lehle, L.; Strahl, S.; Tanner, W. *Angew. Chem. Int. Ed.* **2006**, *45*, 6802-6818.
2. Spiro, R. G. *Glycobiology* **2002**, *12*, 43R-56R.
3. Seitz, O. *ChemBioChem* **2000**, *1*, 214-216.
4. Jaeken, J.; Vanderschueren-Lodeweyckx, Casaer, P.; Snoeck, L.; Corbeel, L.; Egermont, E.; Eeckels, R. *Pediatr. Res.* **1980**, *14*, 179.
5. (a) Grünewald, S.; Matthijs, G.; Jaeken, J. *Pediatr. Res.* **2002**, *52*, 618-624. (b) Kornfeld, S. *J. Clin. Invest.* **1998**, *101*, 1293-1295.
6. Wong, C.-H. *J. Org. Chem.* **2005**, *70*, 4219-4225.
7. Zhang, Z. ; Gildersleeve, J.; Yang, Y.-Y; Xu, R.; Loo, J. A.; Uryu, S.; Wong, C.-H.; Schultz, P. G. *Science* **2004**, *303*, 301-373.
8. Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395-4421.
9. (a) Dondoni, A.; Marra, A.; Massi, A. *Chem.Commun.* **1998**, 1741-1742. (b) Dondoni, A.; Marra, A.; Massi, A. *J. Org. Chem.* **1999**, *64*, 933-944. (c) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380-2388. (d) Dondoni, A.; Mariotti, G.; Marra, A.; Massi, A. *Synthesis* **2001**, 2129-2137.
10. (a) Dondoni, A.; Massi, A.; Marra, A. *Tetrahedron Lett.* **1998**, *39*, 6601-6604. (b) Dondoni, A.; Mariotti, G.; Marra, A. *Tetrahedron Lett.* **2000**, *41*, 3483-3487. (c) Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, *67*, 4475-4486.
11. (a) Hsieh, K.; Jørgensen, E. C. *J. Med. Chem.* **1979**, *22*, 1199. (b) Van Batemburg, O. D.; Voskuyl-Holtcamp, I.; Schattenkerk, C.; Hoes, K.; Kerling, K. E. T.; Havinga, E. *Biochem. J.* **1977**, *163*, 385.
12. (a) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565-598. (b) Huisgen, R. *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 633-645.
13. *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*, Padwa, A.; Pearson, W. H.; Eds., Wiley : New York, 2003.
14. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216.
15. Dondoni, A.; Giovannini, P. P.; Massi, A. *Org. Lett.* **2004**, *6*, 2929-2932.
16. Dondoni, A.; Giovannini, P. P. *Synthesis* **2002**, 1701-1706.
17. The structure of 1,4- and 1,5-disubstituted triazole regioisomers was established by ^{13}C NMR analysis of cycloadduct on the basis of the observed $\Delta(\delta_{C4}-\delta_{C5})$ value, i.e. *ca.* 20 ppm for 1,4-regioisomers and *ca.* -5

ppm for 1,5-regioisomers, in agreement with spectral data of known compounds in the respective series. The validity of this empirical rule, i.e. $\Delta(\delta_{C4}-\delta_{C5})$ values large and positive for 1,4-regioisomers and small and negative for 1,5-regioisomer, was confirmed by further studies carried out in our laboratories. See: a) Dondoni, A.; Marra, A. *J. Org. Chem.* **2006**, *71*, 7546-7557. (b) Cheshev, P.; Dondoni, A.; Marra, A. *Org. Biomol. Chem.* **2006**, *4*, 3225-3227. (c) Nuzzi, A.; Massi, A.; Dondoni, A. *QSAR Comb. Sci.* **2007**, *26*, 1191-1199. (d) Marra, A.; Vecchi, A.; Chiappe, C.; Melai, B.; Dondoni, A. *J. Org. Chem.* **2008**, *73*, in press.

18. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.
19. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3062.
20. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021. (b) Kolb, H. C.; Sharpless, K. B. *Drug Disc. Today* **2003**, *8*, 1128-1137.
21. Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998-15999.
22. Dondoni, A.; Massi, A., unpublished results.
23. For a typical study of the structure-activity relationship of bioactive compounds containing various azoles including triazoles and tetrazole, see: Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953-970.
24. (a) Singh, H.; Chawla, A. S.; Kapoor, V. K.; Paul, D.; Malhotra, R. K. *Prog. Med. Chem.* **1980**, *17*, 151-183.(b) Yu, K.-L.; Johnson, R. L. *J. Org. Chem.* **1987**, *52*, 2051-2059. (c) Zabrocki, J.; Smith, G. D.; Dunbar, J. B.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 5875-5880.
25. Dondoni, A.; Massi, A.; Aldhoun, M., unpublished results.
26. Bosch, L.; Vilarrasa, J. *Angew. Chem. Int. Ed.* **2007**, *46*, 3926-3930.
27. Demko, Z. P.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 2110-2113.
28. Dondoni, A.; Marra, A. *Tetrahedron* **2007**, *63*, 6339-6345.
29. Dondoni, A.; Massi, A. *Acc. Chem. Res.* **2006**, *39*, 451-463 and references therein.
30. Dondoni, A.; Massi, A.; Aldhoun, M. *J. Org. Chem.* **2007**, *72*, 7677-7687.

Chapter 3

Asymmetric Synthesis of Amino Acids with a Tetrasubstituted Carbon Center via Memory of Chirality

Takeo Kawabata

Institute for Chemical Research, Kyoto University, Uji,
Kyoto 611–0011, Japan

Asymmetric synthesis of amino acid derivatives with a tetrasubstituted carbon center via memory of chirality is described. Intermolecular and intramolecular alkylation of α-amino acid derivatives proceeded highly enantioselectively in the absence of external chiral sources. The key intermediates for the asymmetric induction were chiral enolates with dynamic axial chirality.

1. Introduction: Memory of Chirality

The structure of enolates was long believed to be achiral because all four substituents are on the same plane as the enolate double bond. However, we had proposed the intrinsic chirality of enolate structures as shown in Figure 1 (*1,2*). Enolate **1** has axial chirality along the C(1)–C(2) axis and **2** has planar chirality comprising the enolate plane and a metal cation. Racemization of these chiral enolates readily takes place through rotation of the C(1)–C(2) or C(1)–O bond for **1** and **2**, respectively. Only for a limited time, these enolates can exist in chiral nonracemic forms. Because the chiral properties of these enolates are time- and temperature-dependent, we prefer to call this type of chirality "dynamic chirality" rather than conformational chirality. An asymmetric transformation based on dynamic chirality of the enolate structure is shown in Scheme 1. In order to realize an asymmetric transformation *via* the chiral enolate of type **1**, chiral ketone **3** was designed that would generate an axially chiral enolate **5** with relatively long half-life to racemization due to the restricted rotation of the C(1)–C(2) bond.[2] Treatment of **3** with potassium hydride in the presence of alkyl halide and 18-crown-6 gave optically active **4** in 48 ~ 67% ee.

Intervention of the chiral enolate intermediate with the chiral C(1)–C(2) axis (**5**) was suggested by an isolation of chiral nonracemic enol ether **6** of 43% ee. The enantiomeric purity of **6** decreased gradually at 21 °C ($t_{1/2}$ = 53 min, $\Delta G^{\ddagger}_{294}$ = 22.6 kcal/mol). Direct HPLC analysis of the reaction mixture showed that ee of **6** was at least 65% in the reaction medium below -20 °C. Thus, the chiral information of **3** appears to be *memorized* in the enolate intermediate **5** as dynamic axial chirality and then regenerated as central chirality in the product **4** (*3,4*).

axially chiral enolate

planar chiral enolate
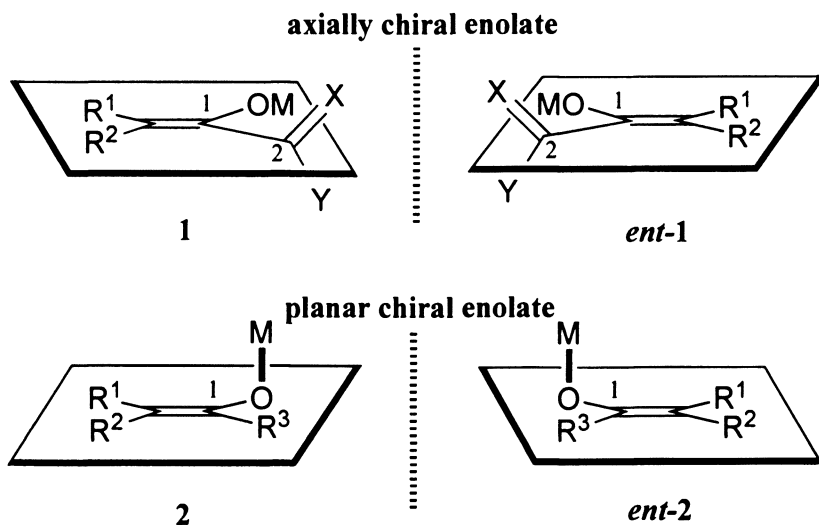
Figure 1. Dynamic Chirality of Enolate Structure

2. Strategy for Asymmetric α–Alkylation of α–Amino Acids via Memory of Chirality

Nonproteinogenic α,α–disubstituted–α–amino acids have attracted considerable attention because of their utility as conformational modifiers of biologically active peptides and as enzyme inhibitors (*5*). Typical methods for their asymmetric synthesis involve chiral auxiliary-based enolate chemistry (*6,7*). However, the most straightforward method for their synthesis would involve asymmetric α–alkylation of the parent α–amino acids without the aid of external chiral sources such as chiral auxiliaries or chiral catalysts. Since both L– and D–α–amino acids are readily commercially available, the synthetic route shown in Scheme 2 seems most attractive for the purpose. This process, however, usually gives racemic α–alkylated products from either L– or D–α–amino acids because the enolate formation eliminates the chiral information at C(2) and an *achiral enolate* common to both L– and D–series is
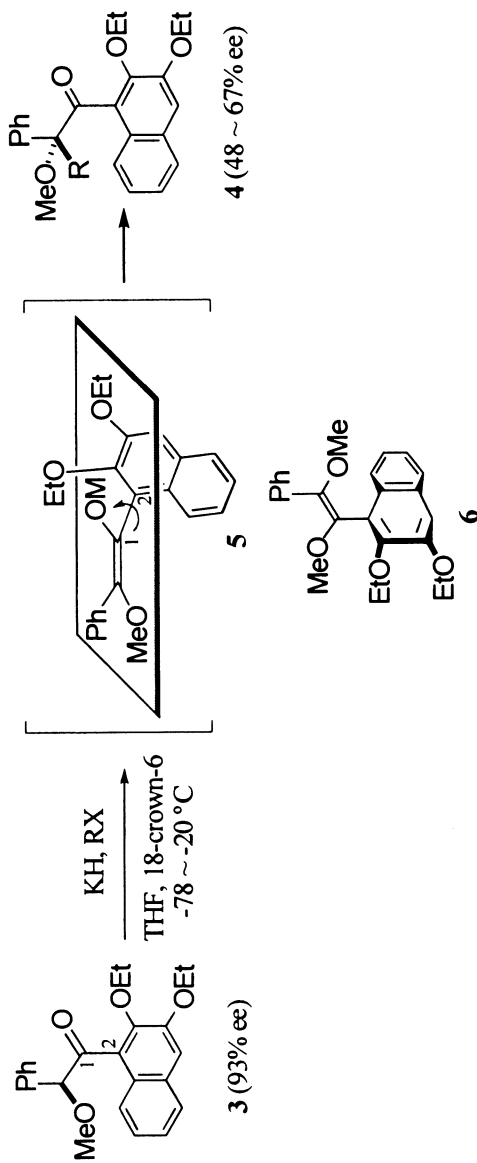
Scheme 1. Memory of Chirality in Alkylation of Ketone 3

formed (**A** = **B**). If the chirality of the starting materials is preserved in the enolate intermediate, L– and D–α–amino acids should give optically active L– and D– (or D– and L–) α,α–disubsituted α–amino acids. Enolates derived from protected α–amino acids are expected to have dynamic chirality (Figure 2). As shown in **C**, an enolate with axial chirality along the C(1)–N axis is expected when R^3 is different from R^4. An enolate with a chiral nitrogen atom is shown in **D**, where tight coordination of nitrogen to a metal cation creates a stereogenic nitrogen atom (8). An enolate with planar chirality comprising the enolate plane and a metal cation (**E**) is also possible. The choice of R^3 and R^4 in **C**, **D**, or **E** was expected to be the key for the generation of a chiral enolate, so the effects of the nitrogen substituents of phenylalanine on asymmetric alkylation were investigated in Chapter 3.

3. Asymmetric Intermolecular α–Alkylation of α–Amino Acids via Memory of Chirality

Based on the hypothetical enolate chirality shown in Figure 2, the effects of the nitrogen substituents of phenylalanine on asymmetric alkylation were thoroughly investigated, and selected examples were shown in Table I. Among several phenylalanine derivatives screened, compounds bearing an alkoxycarbonyl group on the nitrogen underwent α–methylation with significant asymmetric induction (entries 4–6). Existence of two substituents on the nitrogen seems promising for the asymmetric induction (entries 1 vs. 6). Since an alkoxycarbonyl group appears critical in the asymmetric induction, phenylalanine derivatives possessing *t*-butoxycarbonyl (Boc) group and the other substituent on the nitrogen were investigated (Table II). *N*–Me–*N*–Boc derivative **9a** was found to give the corresponding α–methylated product **10a** of 82% ee in 40% yield by treatment with lithium 2,2,6,6-tetramethylpiperidide (LTMP) followed by methyl iodide (entry 1). Although high asymmetric induction was achieved in the absence of external chiral sources (9), this procedure had drawbacks from the low chemical yield and the property of the *N*–Me group that is hardly removable. Other nitrogen substituents and conditions for asymmetric induction were further investigated (entries 2–8). The best result was obtained with *N*–methoxymethyl(MOM)–*N*–Boc derivative **9d**. Treatment of **9d** with potassium hexamethyldisilazide (KHMDS) in toluene-THF (4:1) at –78 °C for 30 min followed by the addition of methyl iodide afforded **10d** in 96% yield and in 81% ee (entry 8). Use of a toluene-THF (4:1) mixture as a solvent is crucial for both high yield and enantioselectivity (entries 6-8). α-Methylation of other α-amino acids with *N*–MOM–*N*–Boc substituents was carried out (Table III). α–Amino acid derivatives with aromatic side chains (**9d, 11, 13, 15,** and **17**) as well as aliphatic side chains (**19 and 21**) underwent α–methylation in a highly enantioselective manner (76 ~ 93% ee) and in good yields (78 ~ 96%). Removal of the
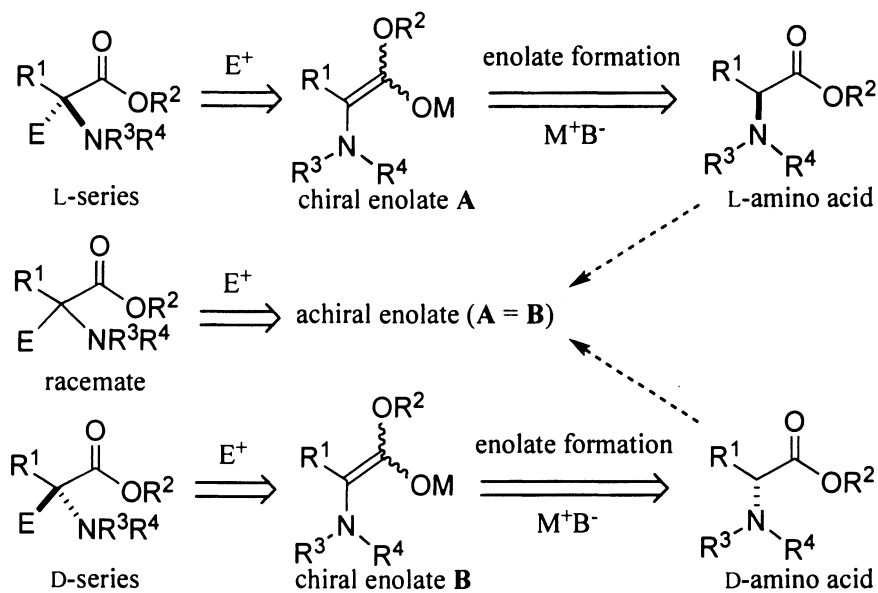
Scheme 2. Alkylation of α-Amino Acid Derivatives via Memory of Chirality
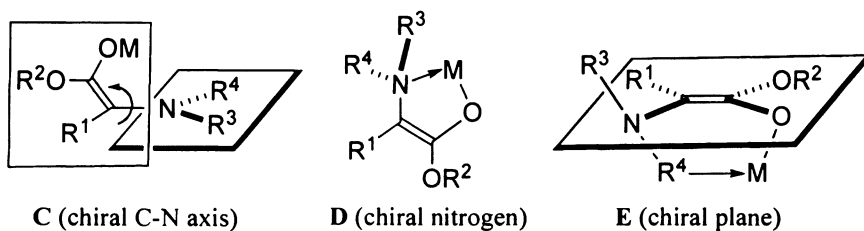
Figure 2. Dynamic Chirality of the Enolates Derived from α-Amino Acids

protective groups of **10d**, **14**, **20**, and **22** was readily accomplished in one step by treatment with 6 M aq HCl to give the corresponding α–methyl α–amino acids in 51 ~ 86% yields. Conversion of **16** into α–methyldopa was accomplished in the following three-step sequence, since the treatment of **16** with 6 M HCl gave the corresponding tetrahydroisoquinoline derivative: 1) TMSBr / Me$_2$S, 2) 1 M NaOH, 3) 47% aq HBr.

Table I. Effects of Nitrogen Substituents on Asymmetric α-Methylation of 7
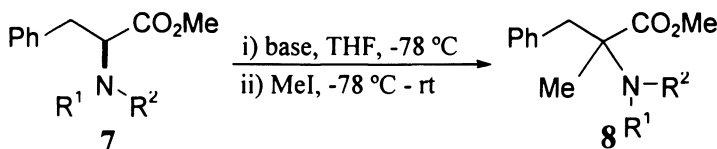
entry	R^1	R^2	base	yield (%)	ee (%)
1	H	CO$_2^t$Bu	LDA	57	~0
2	Me	CH$_2$Ph	LDA	45	~0
3	Me	COPh	LDA	50	12
4^b	Me	CO$_2$CH$_2$Ph	LHMDS	40	26
5	Me	CO$_2$Ad^c	LHMDS	38	35
6	Me	CO$_2$t-Bu	LHMDS	30	36

a) Lithium hexamethyldisilazide. b) Run in THF-DMF (10:1). c) 1-Adamantyl ester.

Table II. Asymmetric α-Methylation of N-Boc-Phenylalanine Derivatives 9 via Memory of Chitality
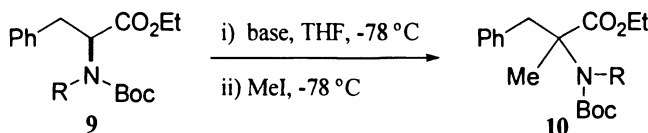
entry	R	substrate	base	product	yield (%)	ee (%)
1	Me	**9a**	LTMP^a	**10a**	40	82
2	CH$_2$OCH$_2$CH$_2$OMe	**9b**	LTMP^a	**10b**	51	10
3^c	CH$_2$OCH$_2$CH$_2$OMe	**9b**	KHMDS^b	**10b**	79	73
4	CH$_2$CH=CH	**9c**	LTMP^a	**10c**	24	54
5^c	CH$_2$CH=CH	**9c**	KHMDS^b	**10c**	66	31
6	CH$_2$OMe	**9d**	KHMDS^b	**10d**	93	36
7^d	CH$_2$OMe	**9d**	KHMDS^b	**10d**	47	75
8^c	CH$_2$OMe	**9d**	KHMDS^b	**10d**	96	81

a) Lithium 2,2,6,6-tetramethylpiperidide. b) Potassium hexamethyldisilazide. c) Run in toluene-THF (4:1). d) Run in toluene.

Table III. Asymmetric α-Methylation of N-Boc-N-MOM-α-Amino Acid Derivatives[a] via Meomory of Chirality

$$R\text{-}CH(CO_2Et)\text{-}N(MOM)(Boc) \quad \xrightarrow[\text{ii) MeI, -78 °C}]{\text{i) KHMDS / toluene-THF (4 : 1)}} \quad R\text{-}C(Me)(CO_2Et)\text{-}N(MOM)(Boc)$$
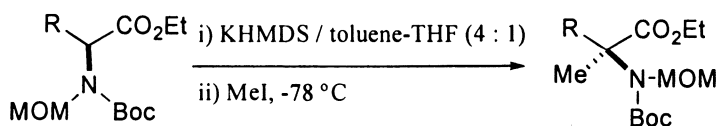
entry	R	substrate[b]	product	yield (%)	ee (%)[c]
1	PhCH$_2$ -	**9d**	**10d**	96	81(*S*)
2	*t*-BuOCO-imidazolyl-CH$_2$ -	**11**	**12**	83	93
3	MeOCH$_2$O-C$_6$H$_4$-CH$_2$ -	**13**	**14**	94	79 (*S*)
4	(MeO)$_2$C$_6$H$_3$-CH$_2$ -	**15**	**16**	95	80 (*S*)
5	(N-CH$_2$OMe-indol-3-yl)-CH$_2$ -	**17**	**18**	88	76
6	Me$_2$CH -	**19**	**20**	81	87 (*S*)
7	Me$_2$CHCH$_2$ -	**21**	**22**	78	78 (*S*)

a) A substrate was treated with 1.1 mol eq of KHMDS in toluene-THF (4:1) at −78 °C for 30 min (for **9d**, **11**, **13**, **15**, and **17**) or 60 min (for **19** and **21**) followed by 10 mol eq of methyl iodide for 16 - 17 h at −78 °C.   b) Ee of each substrate is > 99%.   c) Determined by HPLC analysis with chiral stationary phases. A letter in the parenthesis indicates the absolute configuration.

The stereochemical course of the α–methylation was retention in each case. The degree of asymmetric induction in the α–methylation was comparable among several different amino acids. This implies that the MOM and Boc groups at the nitrogen have a decisive effect on the stereochemical course of the reactions (*10*).

4. Mechanism for Asymmetric Intermolecular Alkylation via Memory of Chirality

A possible rationale for the present asymmetric induction involves participation of a mixed aggregate **F** in which the undeprotonated starting material acts as a chiral ligand of the potassium cation of the *achiral* enolate. To test the feasibility of **F**, a cross-over experiment between **9d** and **13** was done. A 1 : 1 mixture of *racemic* **9d** and (*S*)–**13** (>99% ee) was treated with KHMDS (1.1 equiv of the total amount of **9d** and **13**) followed by methyl iodide according to the protocol in Table III, affording *racemic* **10d** (79% yield) and (*S*)–**14** (74% ee, 79% yield). This clearly indicates that **F** is not responsible for the asymmetric induction.

F
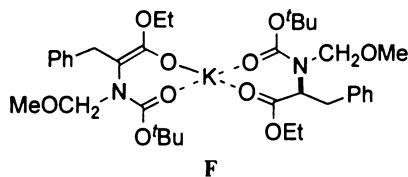
The structure and chiral properties of the enolate intermediate were then investigated. Treatment of **9d** with KHMDS (1.1 equiv) in toluene-THF (4:1) at −78 °C for 30 min followed by *t*-butyldimethylsilyl (TBS) triflate gave *Z*-enol silyl ether **23** and its *E*-isomer **24** in 56% and 27% yield, respectively (Scheme 3). Each of them exists as a mixture of *N*–Boc *E/Z* isomers; 4:1 for **23** and 5:1 for **24**. In the ^{1}H NMR spectra of both **23** and **24**, the methylene protons of the MOM groups appeared as AB quartets, which indicates the restricted rotation of the C(1)–N bonds. The rotational barrier of the C(1)–N bond of the major *Z*–isomer **23** was determined to be 16.8 kcal/mol at 92 °C by variable-temperature NMR measurements in d_8-toluene (400 MHz ^{1}H NMR, J_{AB} = 9.9 Hz, Δv_{AB} = 228.4 Hz, Tc = 365 K). *The restricted bond rotation brings about axial chirality in* **23** *(chiral C(1)–N axis)*. The half-life to epimerization of **23** was estimated to be 5 x 10^{-4} sec at 92 °C and ca. 7 days at −78 °C from the rotational barrier (*11*). This implies that the corresponding potassium enolate could also exist in an axially chiral form with a relatively long half-life to racemization at low temperatures. When **9d** was treated with KHMDS for 30 min at −78 °C, the reaction of the resulting enolate with methyl iodide gave **10d**

in 81% ee (Table III, entry 1). On the other hand, **10d** of 36% ee was obtained by 24-h base treatment. These results indicate that racemization of the enolate intermediate took place. The barrier to racemization was determined through the periodic quench of the enolate intermediate generated at −78 °C with methyl iodide. Figure 3 plots the logarithm of the relative ee's of **10d** as a function of time for base-treatment of **9d** and indicates a linear relationship between them (r^2 = 0.99), although the enolate is a 2 : 1 mixture of the *Z*– and *E*–forms. This suggests that the rates of racemization of the *Z*– and *E*–enolates are close to each other. The barrier was calculated from the slope ($2k$ = 5.34 x 10^{-4} min^{-1}) to be 16.0 kcal/mol at −78 °C, which matches well the rotational barrier (16.8 kcal/mol) of the C(1)–N bond of **23**. This suggests that the chirality of the potassium enolate also originates in the restricted rotation of the C(1)–N bond. Thus, *a chiral nonracemic enolate with dynamic axial chirality (G) is suggested to be the origin for the asymmetric induction*. The half-life to racemization of the chiral enolate **G** was 22 h at −78 °C, which is long enough for the chiral enolate to undergo asymmetric methylation (*10*). Support for the intervention of the axially chiral enolate intermediate was obtained from the reactions of **25** (Scheme 4). The *N*-di-Boc derivative **25** (>99% ee) gave racemic **26** in 95% yield by the treatment with KHMDS in toluene-THF at -78 °C. This result is consistent with the conclusions above, since the intermediary enolate generated from **25** can not be axially chiral along the C(1)-N axis.

The stereochemical course (retention) of the transformation of **9d** into **10d** may be explained by assuming (Scheme 5): 1) deprotonation occurs from the stable conformer **H** (**9d**)[12] where the C(α)-H bond is eclipsed with the N-C (MOM) bond to produce enantiomerically enriched chiral enolate **G**, and 2) an electrophile (methyl iodide) approaches from the sterically less demanding face (MOM) of the enolate double bond of **G**. When deprotonation takes place from the other stable conformation **I** (**9d**), where the C(α)-H bond is eclipsed with the N-C(Boc) bond, enolate *ent*-**G** would be produced. This leads to (*R*)-**10d** *via* electrophilic attack from the face of the MOM group. The difference in potential energies between **H** and **I** is estimated to be small (~1.0 kcal/mol) (*12*). Thus, the deprotonation step should critically affect the enantioselectivity (**G**/*ent*-**G** ratio) of formation of the chiral enolate. Table III shows that the enantio-selectivity of α-methylation is comparable among several α-amino acid derivatives irrespective of the side-chain structure. These results indicate that the difference in satiric bulk between Boc and MOM groups is essential for asymmetric induction.

5. Asymmetric Intramolecular α–Alkylation of α–Amino Acids via Memory of Chirality

Application of the principle of memory of chirality to intramolecular alkylation of α-amino acid derivatives would provide a concise access to cyclic
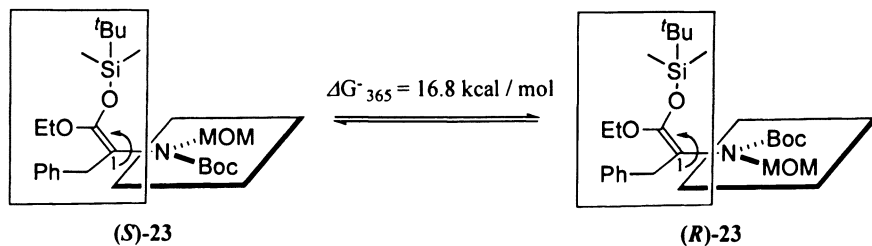
Scheme 3. Dynamic Axial Chirality of 23
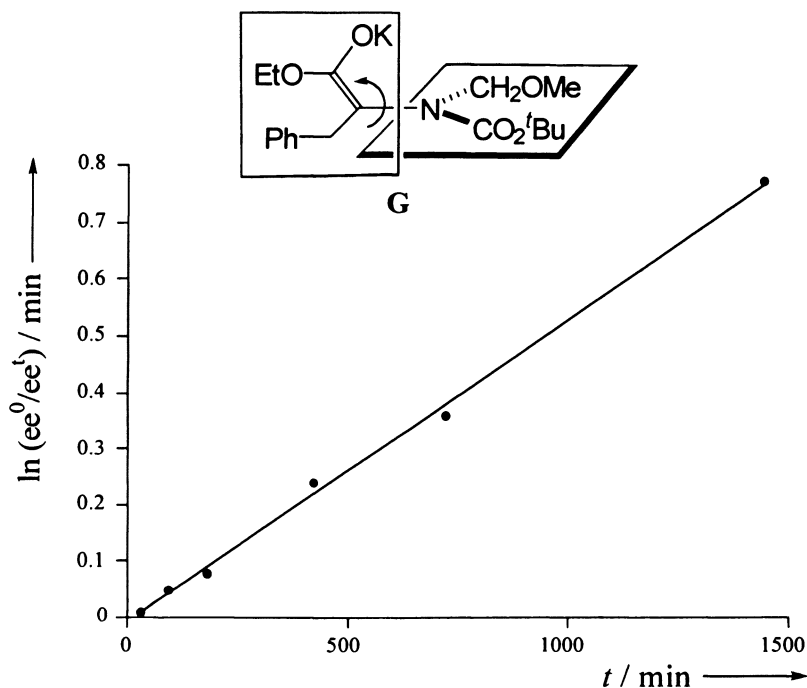
Figure 3. Measurement of the racemization barrier of chiral enolate **G**: Plot of logarithm of the relative ee value of **10d** (ln ee⁰ / ee') versus time (t) for KHMDS treatment of **9d**. ee⁰ : The ee value of **10d** obtained by the reaction of the enolate immediately after its generation from **9d** and KHMDS with methyl iodide. ee' : The ee value of **10d** obtained by the treatment of **9d** with KHMDS for the time indicated followed by addition of methyl iodide.
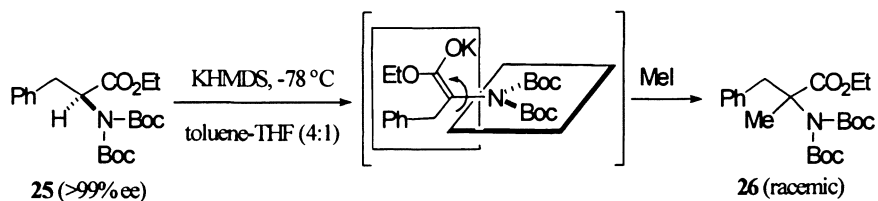
Scheme 4. Effects of the Nitrogen Substituents on Asymmetric Alkylation
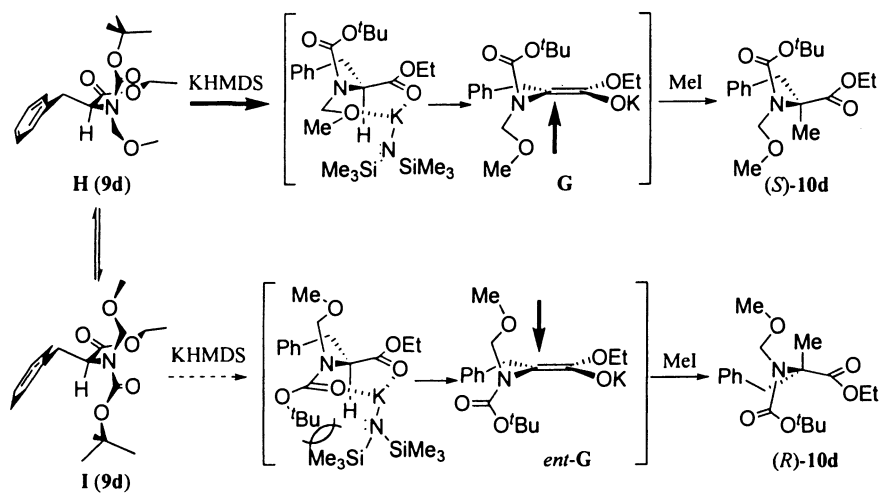
Scheme 5. Stereochemical Course of Asymmetric Alkylation via Memory of Chirality

amino acids with a tetrasubstituted stereo-center (Scheme 6). Cyclic amino acids are of increasing interest in the life-science industry (*13*). Incorporation of these compounds into peptides induces conformational constraint, and it provides an important tool for studying the relationships between peptide conformation and biological activity and for probing biological processes including protein folding (*14*). Cyclic amino acids with a tetrasubstituted stereo-center constitute a new class of non-natural amino acids with an even more constrained conformation (*15*). The simplest and ideal access to these molecules seems to involve asymmetric intramolecular alkylation of α-amino acid derivatives as shown in Scheme 6. However, such a route has rarely been examined, probably because of the anticipated production of racemic products due to a loss of chirality during the enolization step. Stoodley and co-workers reported an intramolecular reaction of an axially chiral enolate with an electrophilic diazo group to give 1,4,5-triazabicyclo-3-nonenes with retention of configuration.[4a,b] Enantioselective β-lactam synthesis has been reported based on memory effect of chirality of the parent amino acids (*16*). Here, asymmetric cyclization of α-amino acid derivatives according to the strategy in Scheme 6 is described. This provides a concise access to a variety of aza-cyclic amino acids with a tetrasubstituted stereo-center of high enantiomeric purity (*17*).
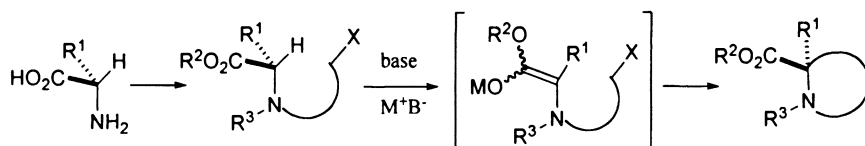
Scheme 6. Strategy for Asymmetric Cyclization via Memory of Chirality

The conditions for enantioselective cyclization were examined (Table IV). To preserve chirality during enolate formation and subsequent C-C bond formation, the choice of the protecting group on the nitrogen of α-amino acids is critical as already described in Table I. *N*-Boc-*N*-(3-bromopropyl)-phenyl-alanine derivative **27** was chosen as a precursor for the asymmetric cyclization because *N*-Boc group was essential for the generation of a chiral nonracemic enolate intermediate in the previously described *intermolecular* alkylation of α-amino acid derivatives (Chapter 3). Substrate **27** was prepared from (*S*)-phenylalanine ethyl ester through *N*-alkylation with 3-bromo-1-propanol, introduction of a Boc group to the nitrogen, and conversion of the hydroxy group into bromine without loss of enantiomeric purity (>99% ee). Treatment of **27** with KHMDS in THF at −78 °C gave α-benzylproline **28** in 47-98% ee (entries 1-3). Contrary to the asymmetric intermolecular alkylation of α−amino acid derivatives (Table II), polar solvent gave higher enantioselectivity. The reaction of **27** in DMF-THF (4:1) gave **28** in 98% ee and 94% yield with retention of configuration (entry 3).

Table IV. Asymmetric Intramolecular Alkylation of 27

Ph⌒CO₂Et (NH₃ Cl) →
i) Br-(CH₂)₃-OH, K₂CO₃
ii) (Boc)₂O, i-Pr₂NEt
iii) CBr₄, PPh₃
63% overall
→ Ph⌒CO₂Et, Boc–N⌒Br **27** → Table IV → Ph⌒CO₂Et, Boc–N (ring) **28**
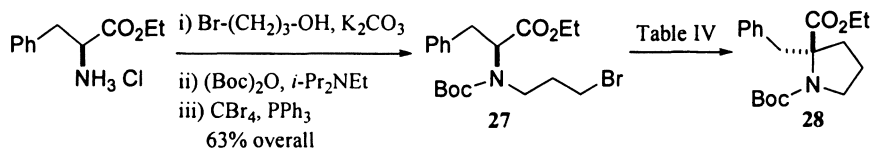
entry	base[a]	solvent	temp, time	yield (%)	ee[b,c] (%)
1	KHMDS	THF	-78 °C, 30 min	92	89
2	KHMDS	toluene	-78 °C, 2 hr	92	47
3	KHMDS	DMF-THF (4:1)	-60 °C, 30 min	94	98
4	LHMDS	DMF-THF (4:1)	-60 °C, 30 min	60	77
5	LTMP	DMF-THF (4:1)	-60 °C, 30 min	~0	-

a) 1.2 Equivs. of base were used. b) (S)-Isomer was obtained in every entry. c) Determined by HPLC analysis.

Table V. Asymmetric Cyclization of α-Amino Acid Derivatives[a]

R⌒CO₂Et, Boc–N–(CH₂)ₙ–X
→ KOH / DMSO / 20 °C →
R····(CO₂Et), Boc–N (CH₂)ₙ ring
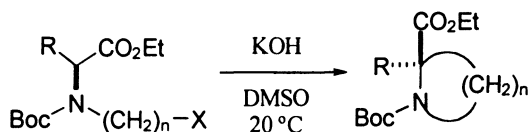
entry	substrate	R	n	X	time (h)[b]	product	yield (%)	ee (%)[c]
1	37	PhCH₂	2	Br	2	38	82	99 (R) [95]
2	45	MeSCH₂CH₂	2	Br	1	46	85	99 (S) [97]
3	50	Me₂CH	2	Br	3	51	79	99
4	27	PhCH₂	3	Br	2	28	91	99 (S) [98]
5	31	MeSCH₂CH₂	3	Br	2	32	91	98 (S) [97]
6	33	Me₂CH	3	Br	2	34	94	98 [94]
7	39	PhCH₂	4	Br	12	40	73	90 [97]
8	52	Me₂CH	4	Br	17	53	74	94
9	54	MeSCH₂CH₂	4	Br	4	55	86	88
10	56	PhCH₂	4	I	2	40	97	97
11	57	Me₂CH	4	I	8	53	90	98
12	58	MeSCH₂CH₂	4	I	3	55	89	97

a) A mixture of a substrate (0.25 mmol) and powdered KOH (prepared from 85% commercial KOH pellets from nacalai tesque, 0.75 mmol) in dry DMSO (anhydrous DMSO (H₂O < 0.005%) from Aldrich, 2.5 mL) was stirred vigorously at 20 °C for the time indicated in the Table. b) Time required for the consumption of the starting material checked by TLC. c) Ee of the corresponding N-benzoate determined by HPLC analysis. A letter in the parenthesis indicates the absolute configuration. Numbers in the brackets indicate ee of the product obtained by the reaction with KHMDS in DMF at -60 °C.

Examples of asymmetric cyclization of various amino acid derivatives via enantioselective intramolecular C-C bond are shown in Table V. Five-membered cyclization of phenylalanine, tyrosine, methionine, valine, and alanine derivatives took place by treatment with KHMDS in DMF-THF (4:1) at −60 °C for 30 min to give α-substituted prolines in 94-98% ee (entries 1-5). The four- and six-membered cyclization of phenylalanine derivatives 37 and 39 took place in high enantioselectivity to give azetidine 38 and piperidine derivatives (40) in 95% and 97% ee, respectively (entries 6 and 7). On the other hand, seven-membered cyclization took place in moderate enantioselectivity due to the slow rates of cyclization (entries 8-9). Treatment of 41 with KHMDS in DMF-THF (4:1) at −60 °C for 30 min gave azepane 42 in only 31% yield and in 83% ee, while prolonged reaction time (2h) gave 42 in 61% yield in the diminished enantioselectivity of 72% ee. The stereochemical course of the five-membered cyclization of 27 and 35 and the seven-membered cyclization of 41 was proven to be retention in each case.

6. Mechanism for Asymmetric Cyclization of α-Amino Acid Derivatives

A possible mechanisms for the asymmetric cyclization of 27 are shown in Scheme 7. Axially chiral enolate K may be responsible for the asymmetric induction according to the asymmetric intermolecular alkylation via memory of chirality (Chapter 4). Alternatively, concerted $S_{E}i$ process as shown in J might be possible. However, the latter (J) seems not feasible because N-bis-bromopropyl derivative 43 gave racemic 44 upon treatment with KHMDS (Scheme 8). This indicates critical importance of a chiral enolate intermediate for the asymmetric induction, since the enolate generated from 43 cannot be axially chiral along the C-N axis.

A conformational search of 27 gives two stable conformers 27A and 27B (Scheme 9) (18). Deprotonation of conformer 27A with KHMDS ($M^+R_2N^-$ =KHMDS), where the C(α)-H bond is eclipsed with the $N-C(CH_2CH_2CH_2Br)$ bond, via transition state X (M=K) would give an enantiomerically enriched enolate K with a chiral C-N axis, which undergoes intramolecular alkylation to give 28 with a total retention of configuration. Deprotonation of conformer 27B, where the C(α)-H bond is eclipsed with the N-C(Boc) bond, to give ent-K seems unfavorable due to the steric interaction between KHMDS and the Boc group. This rationale is consistent with the observed solvent effects (Table IV, entries 1-3), since deprotonation of 27B via chelation of the Boc-carbonyl group with a metal cation (transition state Y, M=K) becomes more significant in less coordinative solvents, resulting in decreased enantioselectivity. Enantio-selectivity in seven-membered ring formation depends on the reaction time (Table V, entries 8 and 9). This seems to be due to the partial racemization of a chiral enolate intermediate during relatively slow seven-membered-ring cyclization. The ee of the recovered 41 indicates time-dependent racemization of the intermediary enolate (Table V, footnotes e and f).
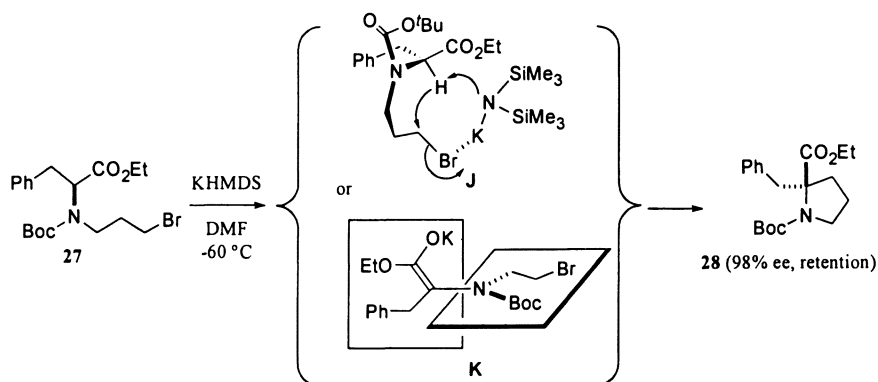
Scheme 7. Possible Mechanisms for Asymmetric Cyclization
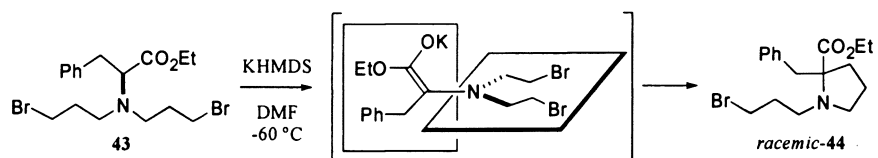
Scheme 8. Effects of Nitrogen Substituents on Asymmetric Cyclization

7. Stereochemical Diversity in Asymmetric Cyclization

Asymmetric cyclization of **27** proceeded in the diminished enantioselectivity in retention of configuration in less polar solvents (Table IV, entries 1-3). This could be explained by increasing extent of transition state **Y** (Scheme 9), which gives the product in inversion of configuration due to the favorable chelating of the Boc-carbonyl group with potassium cation (M=K) in less polar solvent. This rationale prompted us to investigate a hypothesis that enforcing chelation by the use of a lithium cation in less coordinative solvents should make transition state **Y** dominant and give the product with inversion of configuration. According to this hypothesis, the asymmetric cyclization of **27** with lithium amide bases was investigated (Table VI, entries 2-7). Treatment of **27** with LHMDS in DMF gave (S)-**28** with retention of configuration in decreased enantioselectivity (entries 1 vs. 2). The use of LHMDS in a less coordinative solvent (THF) gave (R)-**28** with inversion of configuration in 14% ee (entries 2 vs. 3). Upon treatment of **27** with LTMP in THF at −60 °C, (R)-**28** was obtained in 41% ee (entry 4). The corresponding reaction at 20 °C gave (R)-**28** in 91% ee and in 93% yield (entry 7). Against our expectation, the use of toluene instead of THF did not increase the enantioselectivity (entries 5 vs. 6) (19). Use of LDA also gave (R)-**28** in 82%ee (entry 7).

The conditions for enantiodivergent cyclization have been applied to various amino acid derivatives (Table VII). Five-membered cyclization of **27**, **31**, and **33** with KHMDS in DMF at −60 °C gave **28**, **32**, and **34** in 98, 97, and 95 % ee, respectively, with retention of configuration (entries 1, 3, and 5). On the other hand, use of LTMP in THF gave the cyclization products in 81~91% ee with inversion of configuration (entries 2, 4, and 6). Similar phenomena were observed in four-membered cyclization. Treatment of **37** with KHMDS in DMF at −60 °C gave **38** in 95 % ee with retention of configuration (entry 7), while that with LTMP at -20 °C gave **38** in 90 % ee with inversion of configuration (entry 8). Four-membered cyclization of methionine derivative **45** showed similar stereochemical results (entries 9 and 10). Five-membered spirocyclization showed somewhat different stereochemical behavior (Scheme 10). Treatment of **47** with KHMDS in DMF at −60 °C did not give **48**, due to the predominant β–elimination of HBr. Upon treatment of **47** with NaHMDS in THF at 20 °C, (R)-**48** (notation based on central chirality) was obtained with retention of configuration in 99% ee. On the other hand, treatment of **47** with LHMDS in toluene at 0 °C gave (S)-**48** in 94% ee with inversion of configuration (20).

8. Asymmetric Cyclization via Memory of Chirality at Ambient Temperature

Inter- and intramolecular alkylation of α-amino acid derivatives via memory of chirality proceeded in up to 98% ee via axially chiral enolate
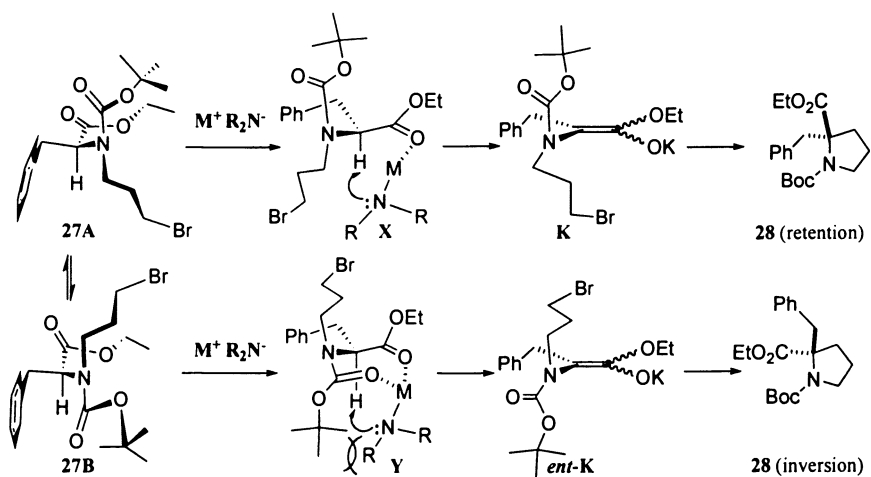
Scheme 9. Stereochemical Course of Asymmetric Cyclization via Memory of Chirality

Table VI. Effects of Base and Solvent on the Stereochemical Course of Asymmetric Cyclization of 27

$$27 \xrightarrow[\text{temp}]{\text{base, solvent}} 28$$

entry	base[a]	solvent	temp, time (h)	yield (%)	ee[b] (%)
1	KHMDS	DMF	-60 °C, 0.5	94	98 (S)
2	LHMDS	DMF	-60 °C, 0.5	60	77 (S)
3	LHMDS	THF	-60 °C, 1	10	14 (R)
4	LTMP	THF	-60 °C, 1	73	41 (R)
5	LTMP	THF	20 °C, 0.5	93	91 (R)
6	LTMP	toluene	20 °C, 0.5	90	77 (R)
7	LDA	THF	20 °C, 0.5	69	82 (R)

a) 1.2 Equivs. of base were used. b) Determined by HPLC analysis. The letter in parentheses indicates the absolute configuration.

Table VII. Enantiodivergent Asymmetric Cyclization of α-Amino Acid Derivatives

MeS $\diagdown\diagup$ CO_2Et \qquad MeS $\diagdown\diagup$ CO_2Et

Boc—N—Br \qquad Boc—N

45 \qquad **46**
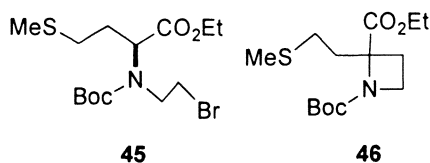
entry	substrate	base, solvent, temp	product	yield (%)	ee (%)[a]
1	27	KHMDS, DMF, –60 °C	28	94	98 (S)
2	27	LTMP, THF, 20 °C	28	93	91 (R)
3	31	KHMDS, DMF, –60 °C	32	92	97 (S)
4	31	LTMP, THF, -20 °C	32	92	81 (R)
5	33	KHMDS, DMF, –60 °C	34	91	95 (R)
6	33	LTMP, THF, 20 °C	34	91	87 (S)
7	37	KHMDS, DMF, –60 °C	38	61	95 (R)
8	37	LTMP, THF, -20 °C	38	69	90 (S)
9	45	KHMDS, DMF, –60	46	98	97 (S)
10	45	LTMP, THF, 0 °C	46	66	83 (R)

a) Ee was determined by HPLC analysis. The letter in parentheses indicates the absolute configuration.
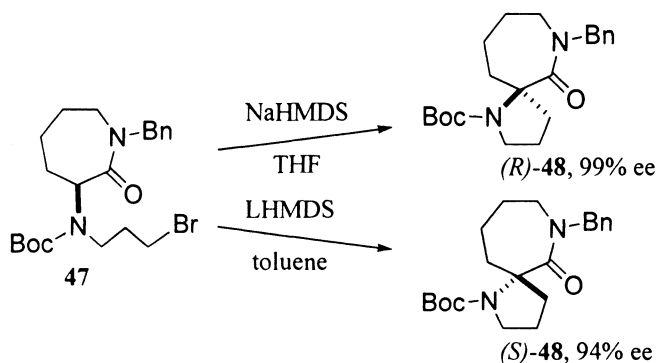
Scheme 10. Enantiodivergent Spirocyclization

intermediates, where enolate formation was usually performed at low temperatures such as -78 ~ -60 °C to maintain enantiomeric purity of the chiral enolates (chapters 3-5). Here, asymmetric cyclization that proceeds in up to 99% ee at 20 °C is described. The key to high asymmetric induction at ambient temperature was the use of powdered KOH in DMSO as a base. Surprisingly, the enantioselectivity of the asymmetric cyclization with powdered KOH in

DMSO at 20 °C was even higher than that of the corresponding reactions with KHMDS in DMF at -60 °C.

The transformation of **27** into **28** was chosen as a model reaction for the investigation of the temperature-dependence of asymmetric cyclization. Treatment of **27** with KHMDS at -60 °C in DMF gave **28** in 98% ee, while at 0 °C **28** was formed in only slightly diminished enantioselectivity of 93% ee (Table VIII, entries 1 *vs.* 2). Bases that contained a potassium cation were screened for the reactions in DMF (entries 3-5). To our surprise, treatment of **27** with powdered KOH (prepared by grinding commercial 85% KOH pellets with a mortar in a glove box) in DMF at 20 °C gave **28** in 98% ee (entry 5). Powdered KOH in DMSO was an excellent base for the transformation, and gave **28** in 99% ee and 91% yield (entry 6). No difference in the efficiency of asymmetric cyclization was observed between powdered KOH prepared from commercial 85% KOH pellets and that from commercial >99% KOH pellets. Powdered LiOH was not effective as a base, while powdered NaOH and powdered CsOH showed reactivity similar to powdered KOH (entries 7-9). The solvent effects in KOH-promoted asymmetric cyclization were investigated (entries 10-14). While the existence of 1% water in DMSO did not affect the efficiency of the asymmetric transformation (entries 6 *vs.* 10), an increase in the amount of water in DMSO decreased both yield and enantioselectivity of the cyclization (entries 11 and 12). Powdered KOH in THF was much less effective than that in DMSO (entries 12 and 13). Powdered KOH in EtOH did not promote cyclization, but rather promoted hydrolysis to give carboxylic acid **49** as a major product in 55% yield (entry 14). The difference in reactivity between powdered KOH in DMSO and powdered KOH in EtOH could be ascribed to the difference in the pKa's of H_2O in these solvents. The pKa's of H_2O in H_2O and in DMSO are known to be 16 and 31, respectively.[21] This means that KOH in DMSO is a strong base which can abstract the α–proton of esters (pKa 18~30) (*21,22*), while KOH in protic solvents is not.

Asymmetric four-, five-, and six-membered cyclization of various amino acid derivatives with powdered KOH in DMSO at 20 °C was examined (Table IX). Asymmetric four-membered cyclization of phenylalanine derivative **37** with powdered KOH in DMSO proceeded within 2 h at 20 °C to give azetidine **38** with a tetrasubstituted carbon center in 99% ee and 82 % yield with retention of configuration (entry 1). Similarly, four-membered cyclization of methionine and valine derivatives **45** and **50** gave azetidines **46** and **51**, respectively, in 99% ee (entries 2 and 3). Five-membered cyclization of **27**, **31**, and **33** with powdered KOH in DMSO proceeded to give pyrrolidines **28**, **32**, and **34**, respectively, in 98~99% ee and 91~94% yield (entries 4~6). Asymmetric four-membered cyclization of **37** and **45** and five-membered cyclization of **27**, **31**, and **33** took place in 94~98% ee upon treatment with KHMDS in DMF at -60 °C (Table IX, entries 1, 2, and 4-6). Surprisingly, asymmetric four- and five-membered cyclizations with powdered KOH in DMSO at 20 °C proceeded with higher enantioselectivity than those with KHMDS in DMF at -60 °C (*23*). This

**Table VIII. Effects of Bases, Temperature, and Solvents
on Asymmetric Cyclization of 27**

Ph⌒⌒CO$_2$H
Boc—N⌒⌒Br
49
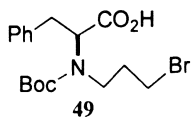
entry	base (mol equiv)	solvent	temp, time (h)	28, yield[a] (%)	28, ee[b] (%)
1	KHMDS (1.2)	DMF	-60°C, 0.5	94	98
2	KHMDS (1.2)	DMF	0 °C, 0.2	97	93
3	t-BuOK (1.5)	DMF	0~20 °C, 1	67	87
4	KH (2.0)	DMF	.0 °C, 0.2	76	89
5	KOH[c,d] (3.0)	DMF	20 °C, 2	89	98
6	KOH[c,d] (3.0)	DMSO	20 °C, 2	91	99
7	LiOH[c] (3.0)	DMSO	20 °C, 17	~0 (75)	-
8	NaOH[c] (3.0)	DMSO	20 °C, 2	81	97
9	CsOH[c] (3.0)	DMSO	20 °C, 2	64	99
10	KOH[c,d] (3.0)	DMSO:H$_2$O=100:1	20 °C, 2	98	99
11	KOH[c,d] (3.0)	DMSO:H$_2$O=10:1	20 °C, 1	76	97
12	KOH[c,d] (3.0)	DMSO:H$_2$O=4:1	20 °C, 1	12	93
13	KOH[c,d] (3.0)	THF	20 °C, 2	45 (45)	71
14	KOH[c,d] (3.0)	EtOH	20 °C, 23	~0[e] (8)	-

a) Numbers in the parentheses indicate the % recovery of **27**. b) Ee of the corresponding *N*-benzoate determined by HPLC analysis. (*S*)-isomer was obtained in each run. c) Powdered metal hydroxide was used. d) Prepared from commercial 85% KOH pellets from nakalai tesque. e) Carboxylic acid **49** was obtained in 55% yield.

could be ascribed to the high reactivity of the axially chiral enolate intermediate generated with powdered KOH in DMSO, which immediately undergoes cyclization before suffering from noticeable racemization (*see Chapter 9*). In contrast to the four- and five-membered cyclization, six-membered cyclization of **39** with powdered KOH in DMSO at 20 °C gave piperidine **40** with enantioselectivity lower than that observed in the reaction of **39** with KHMDS in DMF at -60 °C (entry 7). The enantioselectivity (88~94% ee) observed in the six-membered cyclization of bromides **39**, **52**, and **54** (entries 7-9) was improved by use of the corresponding iodides. Treatment of **56**, **57**, and **58** with powdered KOH in DMSO gave piperidines **40**, **53**, and **55**, respectively, in 97~98% ee and 89~97% yield (entries 10-12). Increase in enantioselectivity of cyclization of the iodides may be ascribed to their increased rates of cyclization. The similar effects of leaving groups on asymmetric alkylation via memory of chirality have reported by Carlier and coworkers (*24*).

9. Mechanistic and Kinetic Aspects of Asymmetric Cyclization of α-Amino Acid Derivatives at Ambient Temperature

The mechanistic aspects of asymmetric cyclization promoted by powdered KOH in MDSO was investigated. In chapter 6, axially chiral enolate **K** was proposed as the key intermediate for the asymmetric cyclization with KHMDS in DMF. While a mechanism via intermediate **K** is also expected for asymmetric cyclization with powdered KOH in DMSO, an alternative route may involve a concerted S_Ei process, as shown in **J** (KOH instead of KHMDS) (*25*) in Scheme 7. To investigate the validity of **J** and **K**, cyclization of **43** was again examined. Treatment of **43** with powdered KOH in DMSO at 20 °C for 5 h gave *racemic*-**44** in 27% yield together with 44% recovery of **43**. This again indicates that asymmetric cyclization with powdered KOH in DMSO also proceeds via axially chiral enolate intermediate **K**.

Table IX. Asymmetric Cyclization of α-Amino Acid Derivatives with Powdered KOH in DMSO at Ambient Temperature[a]
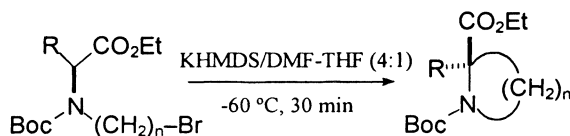
entry	substrate	R	n	product	yield (%)	ee (%)[b]
1	**27**[c]	$PhCH_2$	3	**28**	94	98 (*S*)
2	**29**	4-EtO-C_6H_4- CH_2	3	**30**	95	97
3	**31**	$MeSCH_2CH_2$	3	**32**	92	97
4	**33**	Me_2CH	3	**34**	78	94
5	**35**	CH_3	3	**36**	91	95 (*R*)
6	**37**	$PhCH_2$	2	**38**	61	95
7	**39**	$PhCH_2$	4	**40**	84	97
8	**41**[c]	$PhCH_2$	5	**42**	31[e]	83 (*S*)
9[d]	**41**[c]	$PhCH_2$	5	**42**	61[f]	72 (S)

a) A solution of substrate (0.25 mmol) in dry DMF (2.4 mL) was treated with 1.2 mole equiv. of KHMDS (0.50 M in THF) for 30 min at −60 °C. b) Ee was determined by HPLC analysis. A letter in the parenthesis indicates the absolute configuration. c) >99% ee. d) The reaction was run for 2 h. e) **41** (70% ee) was recovered in 52% yield. f) **41**(54% ee) was recovered in 17% yield.

The racemization behavior of **K** was investigated. In chapter 4, the barrier to racemization of the axially chiral enolate **G** was determined by periodic quenching of the enolate with methyl iodide (Figure 3). However, this protocol cannot be applied to enolate **K** because it undergoes cyclization immediately after it is generated. Compound **59**, an analogue of **27**, was used to estimate the racemization barrier of the axially chiral enolate **K** because the enolate **L** generated from **59** would not undergo cyclization (Figure 4). The barrier to racemization of a potassium enolate generated from **59** and KHMDS in DMF-THF (1:1) at -78 °C was determined through the periodic quenching of the enolate with methyl iodide (*26*). The barrier was calculated from the slope, $2k = 1.99$ x 10^{-3} min^{-1} (r^2=0.99), to be 15.5 kcal/mol at -78 °C. Based on the assumption that asymmetric cyclization of **27** with powdered KOH in DMSO proceeds via enolate **K** whose racemization barrier is comparable to **L**, enolate **K** is expected to undergo cyclization at 20 °C within ~10^{-3} sec after it is generated to give product **28** in 99% ee ($t_{99/100}$ at 20 °C = 3.0 x 10^{-4} sec, calculated from $\Delta G^{\ddagger}$=15.5 kcal/mol).[27] Since the asymmetric cyclization reactions in Table IX are also assumed to proceed via axially chiral enolates such as **K**, enolates generated with powdered KOH in DMSO in these reactions are expected to undergo rapid cyclization within ~10^{-2} sec at 20 °C to give products in ≥88% ee ($t_{88/100}$ at 20 °C = 3.8 x 10^{-3} sec, calculated from $\Delta G^{\ddagger}$=15.5 kcal/mol) (*27*). The extremely high reactivity of the enolates generated with KOH/DMSO could be ascribed to their amine-free structure (*28,29*).

The rate-determining step for the cyclization with powdered KOH in DMSO must be the enolate-formation step because the half-lives of racemization of the chiral enolate intermediates are supposed to be much shorter (< 0.1 sec) (*30*) than the time required for the reactions to be complete (2~12 h). Thus, axially chiral enolates such as **K** would form gradually, and once formed, would immediately undergo asymmetric cyclization (Scheme 11). Since axially chiral enolates suffer from time-dependent racemization, ee's of the products would correlate with the rate of cyclization of enolate intermediates; *i.e.* the faster the cyclization, the higher the enantioselectivity. Based on this assumption, the four-membered cyclization (99% ee, Table IX, entries 1-3) is expected to proceed faster than the corresponding six-membered cyclization (88~94% ee, Table 9, entries 7-9). Accordingly, enantioselectivities would be a measure of the rate of cyclization of the enolate intermediates (*31*), while the reaction time for asymmetric cyclization
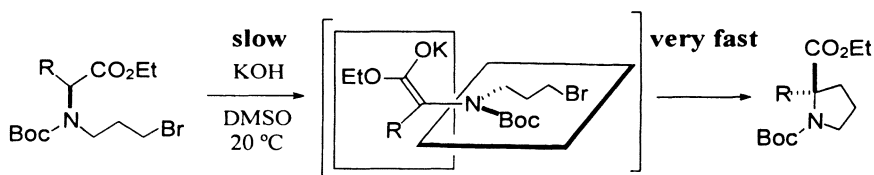
Scheme 11. Rate-Determining Enolate Formation in Asymmetric Cyclization with Powdered KOH in DMSO
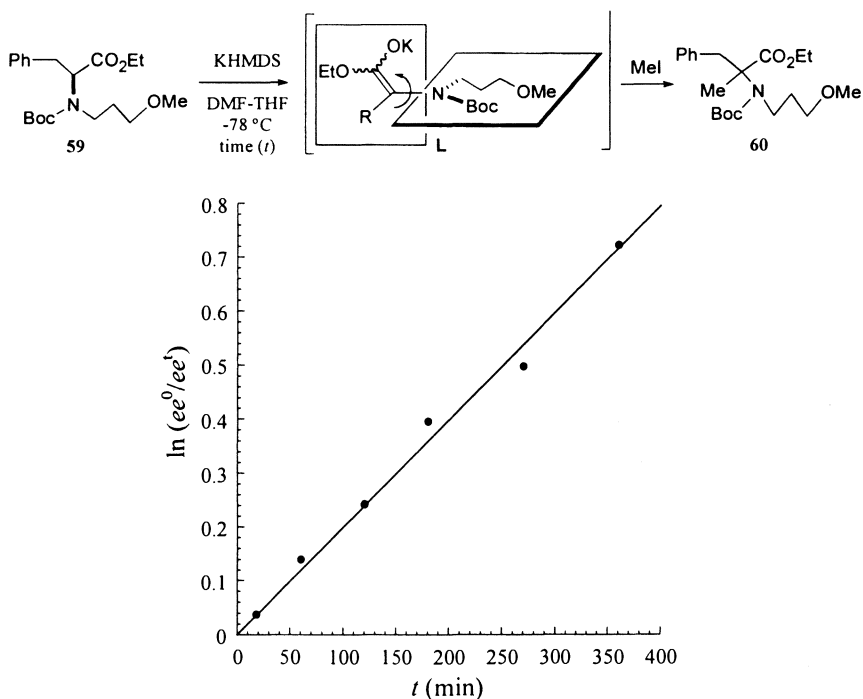
Figure 4. Measurement of the racemization barrier of chiral enolate L: Plot of ln (ee^0/ee^t) versus time (t) for base-treatment of **59**. ee^0 = the ee value of **60** obtained by the reaction of the enolate immediately after its generation from **59** and KHMDS with methyl iodide. ee^t = the ee value of **60** obtained by the treatment of **59** with KHMDS for the time indicated followed by addition of methyl iodide. Experiments were performed twice at the strictly controlled temperature. Ee^0 and each of ee^t are the average of two runs. The barrier to racemization was determined to be 15.5 kcal/mol at -78 °C from the slope, 2k = 1.99 x 10^{-3} min^{-1} (r^2 = 0.99).

with powdered KOH in DMSO depends on the rate of enolate formation. Higher enantioselectivity observed in the cyclization of the iodides than the corresponding bromide (Table IX, entries 7~12) are also consistent with expected higher rates of cyclization of the iodides.

Conclusion

Asymmetric intermelucular and intramolecular alkylation of α-amino acid derivatives via memory of chirality has been described. Chiral enaoltes with dynamic axial chirality are responsible for the asymmetric induction. The salient features of the present method are (1) chirality at the α–carbon (reaction

center) of the parent amino acids is the sole chiral source for the asymmetric alkylation, (2) all of the functionality of the parent amino acids are incorporated in the products of the reaction. Thus, the present method is advantageous in terms of atom economy as well as chirality economy.

Acknowledgment

References and Notes

1. a) Kawabata, T. *Yakugaku Zasshi*, **1995**, *115*, 700-715. b) Fuji, K.; Kawabata, T. *Chem. Eur. J.* **1998**, *4*, 373-376. c) Kawabata, T. *Reviews on Hetroatom Chemitry* **2000**, *22*, 33-58.
2. Kawabata, T.; Yahiro, K.; Fuji, K. *J. Am. Chem. Soc.* **1991**, *113*, 9694-9696.
3. For reviews on memory of chirality, see: (a) Kawabata, T.; Fuji, K.; *Topics in Stereochemistry* 2003, **23**, 175-205. (b) Zhao, H.; Hsu, D.; Carlier, P. R.; *Synthesis*, 2005, 1-16
4. For reactions related to memory of chirality, see: (a) Beagley, B.; Betts, M. J.; Pritchard, R. G.; Schofield, A.; Stoodley, R. J.; Vohra, S.; *J. Chem. Soc. Chem. Comm.* **1991**, 924-925. (b) Betts, M. J.; Pritchard, R. G.; Schofield, A.; Stoodley, R. J.; Vohra, S.; *J. C. S. Perkin 1* **1999**, 1067-1072. (c) Kawabata, T.; Chen, J.; Suzuki, H.; Nagae, Y.; Kinoshita, T.; Chancharunee, S.; Fuji, K.; *Org. Lett.* **2000**, *2*, 3883-3885. (d) Gerona-Navarro, G.; Bonache, M. A.; Herranz, R.; García-López, M. T.; González-Muñiz, R.; *J. Org. Chem.* **2001**, *66*, 3538-3547. (e) Bonache, M. A.; Gerona-Navarro, G.; Martín-Martínez, M.; García-López, M. T.; López, P.; Cativiela, C.; González-Muñiz, R.; *Synlett* **2003**, 1007-1011. (f) Carlier, P. R.; Zhao, H.; DeGuzman, J.; Lam, Polo, C.-H.; *J. Am. Chem. Soc.* **2003**, *125*, 11482-11483. (g) Kawabata, T.; Ozturk, O.; Suzuki, H.; Fuji, K.; *Synthesis* **2003**, 505-508. (h) Kawabata, T.; Kawakami, S.; Shimada, S.; Fuji, K.; *Tetrahedron* **2003**, *59*, 965-974. (i) Kawabata, T.; Chen, J.; Suzuki, H.; Fuji, K.; *Synthesis.* **2005**, *5*, 1368-1377
5. a) Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. *J. Med. Chem.* **1991**, *34*, 404-414. b) Altmann, K.-H.; Altmann, E.; Mutter, M. *Helv. Chim. Acta.* **1992**, *75*, 1198-1210. c) Mendel, D.; Ellman, J.; Schultz, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 4359-4360. d) Stilz, H. U.; Jablonka, B.; Just, M.; Knolle, J.; Paulus, E. F.; Zoller, G. *J.*

Med. Chem. **1996**, *39*, 2118-2122. e) Chinchilla, R.; Falvello, L. R.; Galindo, N.; Nájera, C. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 995-997.

6. For examples of chiral auxiliaries which utilize chirality of parent α-amino acids, see: a) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390-5398. b) Vedejs, E.; Fields, S. C.; Schrimpf, M. R. *J. Am. Chem. Soc.* **1993**, *115*, 11612-11613. c) Ferey, V.; Toupet, L.; Gall, T. L.; Mioskowski, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 430-431.

7. For examples of excellent catalytic methods for asymmetric synthesis of α,α–disubtituted α–amino acid derivatives have been developed; for examples, see: a) Kuwano, R. Ito, Y. *J. Am. Chem. Soc.* **1999**, *121*, 3236-3237. b) Ooi, T.; Takeuchi, M.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **2000**, *122*, 5228-5229.

8. Formation of a stereogenic nitrogen atom by coordination with a metal cation has been claimed: Sato, D.; Kawasaki, H.; Shimada, I.; Arata, Y.; Okamura, K.; Date, T.; Koga, K. *J. Am. Chem. Soc.* **1992**, *114*, 761-763.

9. Kawabata, T.; Wirth, T.; Yahiro, K.; Suzuki, H.; Fuji, K. *J. Am. Chem. Soc.* **1994**, *116*, 10809-10810.

10. Kawabata, T.; Suzuki, H.; Nagae, Y. Fuji, K. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2155-2157.

11. The half-life at -78 °C was roughly estimated on the assumption that $\Delta S^{\neq}$ of the restricted bond rotation is nearly zero.

12. The most stable conformation **H** of **9d** was generated by MCMM search with MM3* force field using MacroModel V6.0: a) Chang, G.; Guida, W. C.; Still, W. C. *J. Am Chem. Soc.* **1989**, *111*, 4379-4386. b) Kolossváry, I.; Guida, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 5011-5019.

13. Peterson, M.; Sauter, M.; Lonza, A.-G. *Chimia*, **1999**, *53*, 608-612.

14. (a) Degrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 51-124. (b) Williams, K. A.; Deber, C. M. *Biochemistry*, **1991**, *30*, 8919. (c) Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28*, 31. (d) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902-12908. (e) Goodman, M.; Shao, H. *Pure Appl. Chem.* **1996**, *68*, 1303-1308. (f) Hanessian, S.; McNaughton-Smith, G.; Lombert, H-G.; Lubell, W. D. *Tetrahedron*, **1997**, *53*, 12789-12854.

15. (a) Cativiela, C.; Díaz-de-Villegas, M. D. *Tetrahedron: Asymmetry*, **2000**, *11*, 645-732. (b) Park, K. -H.; Kurth, M. J. *Tetrahedron*, **2002**, *58*, 8629-8659. (c) Kotha, S. *Acc. Chem. Res.* **2003**, *36*, 342-351.

16. Gerona-Navarro, G.; Bonache, M. A.; Herranz, R.; García-López, M. T.; González-Muñiz, R. *J. Org. Chem.* **2001**, *66*, 3538-3547. (b) Bonache, M. A.; Gerona-Navarro, G.; Martín-Martínez, M.; García-López, M. T.; López, P.; Cativiela, C.; González-Muñiz, R. *Synlett* **2003**, 1007.

17. Kawabata, T.; Kawakami, S.; Majumdar, S.; *J. Am. Chem. Soc.* **2003**, *125*, 13012-13013.

18. The stable conformers **27A** and **27B** of **27** were generated by a molecular modeling search with AMBER* force field with the GB/SA solvation model for water using MacroModel V 6.0. The difference in potential energies between **27A** and **27B** is estimated to be 0.1 kcal/mol. A PM3 calculation with a polarized continuum model (water, ε=78.4) also gave stable structures similar to **27A** and **27B**.: (a) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127-6129. (b) *J. Comput. Chem.* **1991**, *12*, 620.

19. This could be ascribed to the difference in the aggregation state of LTMP in THF from toluene. For the discussion of the aggregate structure of LTMP, see: Hall, P. L.; Gilchrist, J. M.; Harrison, A. T.; Fuller, D. J.; Column, D. B.; *J. Am. Chem. Soc.* **1991**, *113*, 9575-9585.
20. Kawabata, T.; Matsuda, S.; Kawakami, S.; Monguchi, D.; Moriyama, K. *J. Am. Chem. Soc.*, **2006**, *128*, 15394-15395.
21. Bordwell, F. G. *Acc. Chem. Res*, **1988**, *21*, 456-463.
22. Mashchenko, N. V.; Matveeva, A. G.; Odinets, I. L.; Matrosov, E. I.: Petrov, E. S.; Terekhova, M. I.; Matveev, A. K.; Mastryukova, T. A.; Kabachnik, M. I. *Zhurnal Obshchei Khimii*, **1988**, *58*, 1973-1979.
23. Kawabata, T.; Moriyama, K.; Kawakami, S.; Tsubaki, K. *J. Am. Chem. Soc.*, **2008**, *130*, 4153-4157.
24. (a) MacQuarrie-Hunter, S.; Carlier, P. R. *Org. Lett.* **2005**, *7*, 5305-5308. (b) Carlier, P. R.; Lam, P. C.-H.; DeGuzman, J. C.; Zhao, H. *Tetrahedron: Asymmetry*, **2005**, *16*, 2998-3002.
25. It might be supposed that the active base in these reactions is potassium dimsylate generated in a small quantity from KOH and DMSO (pKa's of H_2O and DMSO in DMSO are 31 and 35, respectively; see reference 21). However, this seems not feasible because the dimsyl anion prepared from KH in DMSO showed a different reactivity in the reaction of **27**: Treatment of **27** with 3.0 mol equiv. of potassium dimsylate in DMSO at 20 °C for 2 h gave **28** in 99% ee and 68% yield together with the corresponding carboxylic acid of **28** in 25% yield.
26. Determination of the racemization barrier of the potassium enolate generated from **59** with KOH/DMSO is desirable, however, this was not possible. This is because (1) KOH/DMSO cannot be used at low temperatures suitable for the measurement of enolate racemization, and (2) KOH/DMSO cannot generate the enolate from **59** in a quantitative manner. Quantitative generation of the enolate in a much shorter period than the half-life of racemization of the enolate is indispensable for the measurement of the racemization barrier.
27. The racemization barrier at 20 °C was roughly estimated from that at -78°C based on the assumption that $\Delta S^{\neq}$ of the unimolecular racemization process (bond rotation along the chiral C-N axis) is nearly zero.
28. Amine-free lithium enolates have been known to be more reactive than the enolates in the presence of a secondary amine generated *in situ* by abstraction of α–proton of the carbonyl group with the lithium amide base, see: (a) Laube, T.; Dunitz, J. D.; Seebach, D. *Helv. Chim. Acta.* **1985**, *68*, 1373-1393. (b) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390-5398. (c) Aebi, J. D.; Seebach, D. *Helv. Chim. Acta.* **1985**, *68*, 1507-1518.
29. We suppose that a water molecule generated by deprotonation of the substrate with KOH may be excluded from the coordination sphere of the potassium enolate by strongly coordinating DMSO molecules.
30. The half-life of racemization was roughly estimated to be 0.02 sec at 20 °C based on the racemization barrier of chiral enolate **L** (15.5 kcal/mol).
31. For an example of show cyclization, seven-membered cyclization of a substrate derived from L-phenylalanine gave the product in 49% ee (25% yield) by treatment with powdered KOH in dry DMSO at 20 °C for 2 h.

Chapter 4

Asymmetric Synthesis of α-Substituted α-Amino Acids: Strecker and Claisen Approaches

Yasufumi Ohfune, Kazuhiko Sakaguchi, and Tetsuro Shinada

Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558–8585, Japan

Two efficient methods for the synthesis of a class of optically active α-substituted α-amino acids developed in our laboratories are disclosed. An asymmetric version of the Strecker synthesis of α-acyloxy ketones possessing an optically acitive amino acid as a chirality transferring acyl group produced acyclic and cyclic α-substituted serine analogs. Novel vinylsilane-containing α- or α,β-substituted α-amino acids were synthesized by the Claisen rearrangement of α-acyloxy-α-alkenylsilanes. These methods were used for the synthesis of biologically active amino acids and natural products.

There is an ever-growing interest in the synthesis, pharmacology, and conformational properties of non-proteinogenic amino acids (*1*). In particular, α-substituted α-amino acids have been the subject of numerous investigations over past decades (*2*). The structural feature common to these amino acids is the presence of an additional substituent at the α-position of the amino acid that sterically constrains the free rotation or conformational flexibility of its side chain or strictly fixes the conformation by a carbo- or a heterocyclic ring. Furthermore, α-substituted α-amino acids are often found in nature either in the free form or as constituents of biologically active compounds that are known as enzyme inhibitors, ion channel blockers, and antibiotics. Therefore, numerous attempts to synthesize the α-substituted α-amino acids have been performed (*3*).

Our interest in these amino acids is based on the synthesis of biologically active amino acids, such as α-substituted serine and glutamate, and natural products. The α-substituent of serine restricts its β-hydroxymethyl conformation which plays an important role in the biological activity when incorporated into peptides and proteins (*4*). L-Glutamic acid is a major excitatory neurotransmitter in mammalian CNS and plays an essential role for excitatory neurotransmission, the construction of memory and learning, and occasionally exhibits a potent neuroexcitotoxicity which causes serious brain diseases (*5*). We have been working on the design and synthesis of glutamate analogs (*6*) and natural products possessing an α-substituted α-amino acid sub-strucuture. Our recent achievement of these molecules is depicted in Figure 1 (*7-12*). In this chapter, two efficient methods, i.e., the asymmetric Strecker synthesis using α-acyloxy ketones and the ester-enolate Claisen rearrangement of α-acyloxy-α-alkenylsilanes, employed for the synthesis of these novel classes of amino acids and natural products, are described.

Asymmetric Strecker Synthesis of α-Substituted α-Amino Acid

The biosynthetic pathway of the α-substituted α-amino acid may involve the asymmetric transformation of an amino group of an α-amino acid to a ketone (*4b*). As a result, the chirality of the starting amino acid would be transferred to the prochiral ketone (eq 1).

Transfer of chirality & NH_2

(1)
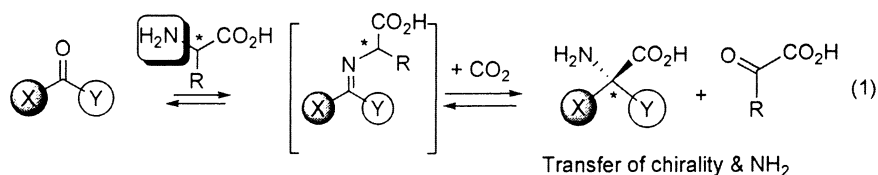
Influenced by the hypothetical biosynthetic route, our synthetic plan for these amino acids was an asymmetric version of the Strecker synthesis. The synthesis consists of the following sequence of transformations: (i) formation of a cyclic ketimine intermediate from an α-acyloxy ketone, having an L- or D-amino acid as the acyloxy group; (ii) stereoselective addition of a cyanide ion to 1,4-oxazine to give the α-amino nitrile; (iii) oxidative conversion to the α-imino nitrile; and (iv) removal of the chirality transferring amino acid as a pyruvate derivative and hydrolysis of the nitrile group under acidic conditions to give the β-hydroxy α-substituted α-amino acid (Scheme 1) (*7*).

The asymmetric synthesis of α-methylserine from acetol is depicted in Scheme 2. The treatment of the α-acyloxy ketone, possessing L-Val as the
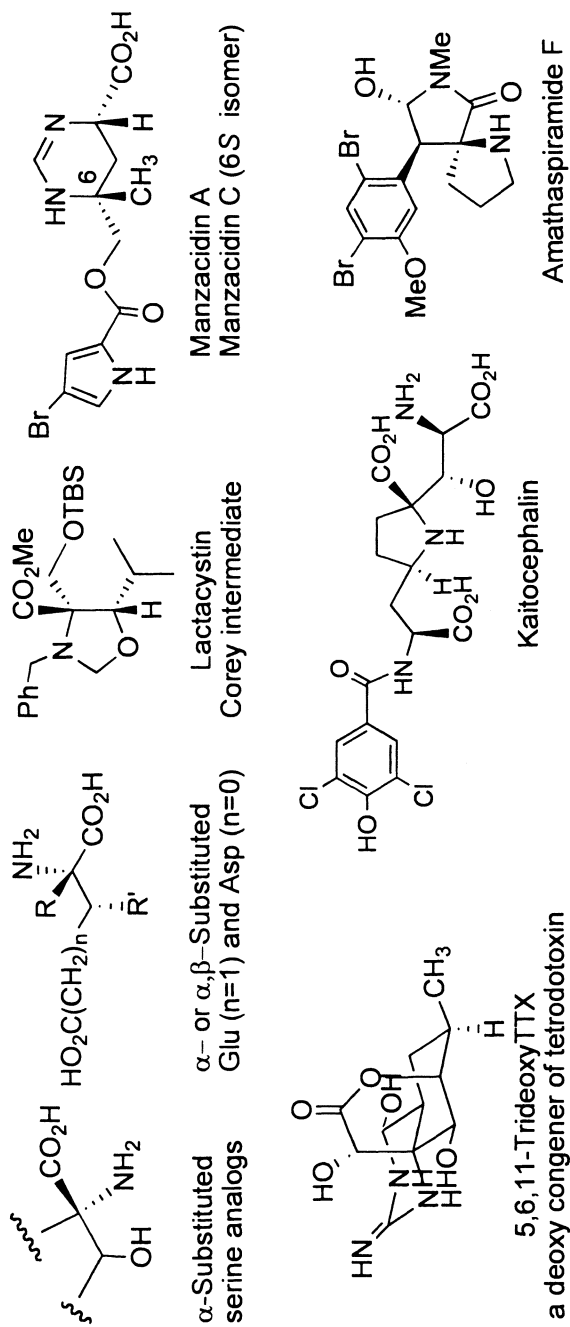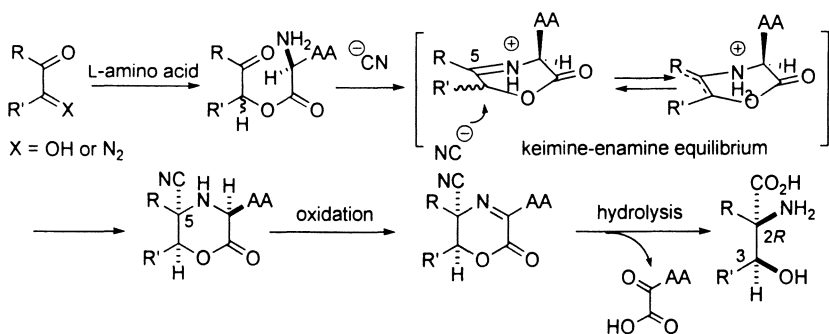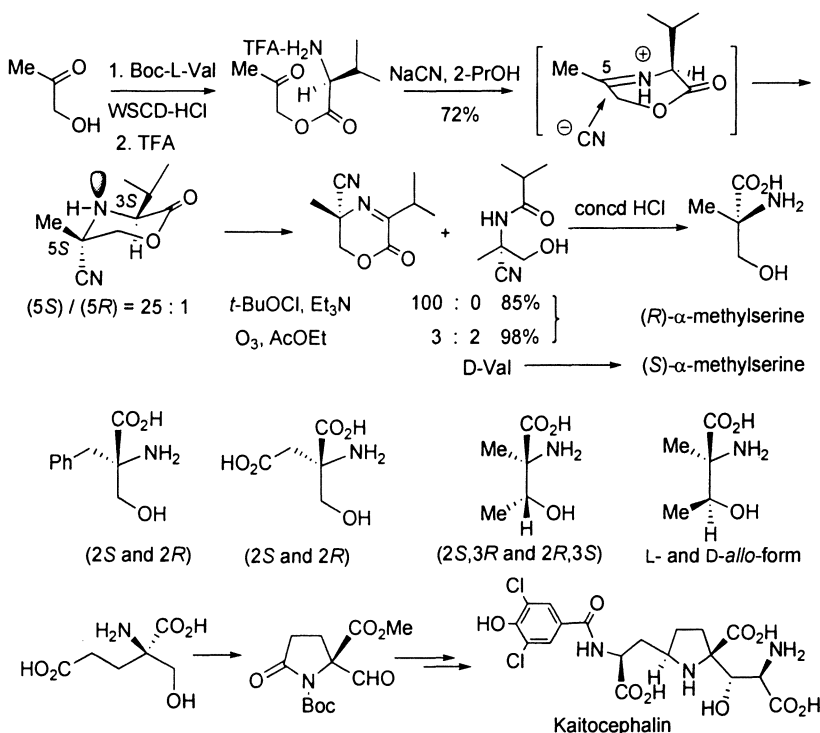
Figure 1. Structures of amino acids and natural products synthesized by the asymmetric Strecker synthesis and ester-enolate Claisen rearrangement.

Scheme 1. Asymmetric version of the Strecker synthesis: General scheme

acyloxy group, with NaCN in 2-propanol gave a mixture of cyclic α-amino nitriles in 72% yield ($5S/5R$ = 25:1). The conversion into (R)-α-methylserine required the initial oxidation of the α-amino nitrile and subsequent hydrolytic removal of the resulting α-imino nitrile and hydrolysis of the nitrile group. Upon oxidation of the α-amino nitrile, ozone was found to be a superior oxidant for the classical t-BuOCl/Et$_3$N method to give a mixture of the α-imino and α-amide nitriles in nearly quantitativet yield (*13*). Hydrolysis of the mixture gave (R)-α-methylserine (98%, 2 steps). The use of D-Val afforded (S)-α-methylserine. Thus, both enantiomers of α-methylserine were prepared from acetol in 5 steps (*13,14*). The reaction involves a ketimine intermediate, coexisting at equilibrium via an enamine, which was ascertained by deuterium and ^{13}CN incorporation experiments. The highly diastereoselective formation of the ($5S$)-isomer would be due to an attack of the cyanide ion on the sterically less hindered *si*-face of the ketimine with a boat-like conformation (*15*). In addition, stereoelectronic stabilization of the axial nitrile group, perpendicular to the lone pair electrons of the amino group, would also be attributed to the high stereoselectivity. This method was well applied to the synthesis of both enantiomers of α-benzylserine (*16*), α-hydroxymethylaspartic acid (*16*), α-hydroxymethylglutamic acid (*17*), and 4 enantiomers and diastereomers of α-methylthreonine (*14*). Among them, the α-hydroxymethylglutamic acid was employed for the total synthesis of the GluRs antagonist, kaitocephalin, via the α-substituted pyroglutamate (*11*).

Next, the syntheses of the cyclic serine analogs with a 5 or 6-membered ring were examined. The synthesis of 1-amino-2-hydroxycyclopentane-1-carboxylic acid (Ahp) was started with the Strecker synthesis of 2-acyloxycyclopentanone. The reaction underwent a stereoselective cyanide addition to the ketimine to exclusively give the *cis*-fused α-amino nitrile. In this case, the initial treatment of the acyloxy ketone with MgSO$_4$-NaOAc for the complete formation of the ketimine and the use of TMSCN and ZnCl$_2$ were crucial, otherwise, the reaction resulted in a decrease in yields (>50%). According to the routine transformation,

Me $\sim$ O 1. Boc-L-Val Me $\sim$ O NaCN, 2-PrOH
$\qquad$ OH WSCD-HCl $\qquad$ 72%
$\qquad$ 2. TFA

TFA-H$_2$N

(5S) / (5R) = 25 : 1 CN + HN $\sim$ O concd HCl CO$_2$H NH$_2$
$\qquad$ t-BuOCl, Et$_3$N 100 : 0 85% (R)-α-methylserine
$\qquad$ O$_3$, AcOEt 3 : 2 98%
$\qquad$ D-Val $\longrightarrow$ (S)-α-methylserine

Ph CO$_2$H NH$_2$ OH HO$_2$C CO$_2$H NH$_2$ OH Me CO$_2$H NH$_2$ Me OH H Me CO$_2$H NH$_2$ Me OH H

(2S and 2R) (2S and 2R) (2S,3R and 2R,3S) L- and D-allo-form

H$_2$N CO$_2$H HO$_2$C OH $\longrightarrow$ CO$_2$Me CHO O N Boc $\longrightarrow$ Kaitocephalin

Scheme 2. Asymmetric Strecker synthesis of α-methylserine and other α-substituted α-amino acids

(1R,2S)-Ahp was synthesized from the α-amino nitrile. The use of D-Phe afforded (1S,2R)-Ahp (*18*). (1R,2S)-Ahp was employed for the enantioselective synthesis of the mGluR2 agonist, LY-354740 (Scheme 3) (*19*).

The syntheses of the 6-membered ring analogs (Ahh) were started with α-acyloxycyclohexanone using L-Phe as the acyloxy group shown in Scheme 4. Contrary to the cyclopentane system, the reaction with NaCN in 2-propanol smoothly proceeded to give a mixture of the Strecker adducts which were composed of the (5S,6S)-isomer (*cis*) and *trans* isomer (*cis/trans* = 2~4:1, 85%) (*18*). Oxidation of the *cis* isomer with t-BuOCl using DABCO as the base followed by hydrolysis gave (1R,2S)-Ahh (*20*). Upon the cyanide addition process, we initially presumed that the composition of the ketimine intermediates (6R/6S = 2~4:1) would reflect the ratio of the resulting *cis* α-amino nitriles (form (6S)-ketimine) and its *trans* isomer (from (6R)-ketimine). However, isolation of the ketimines revealed that the ratio was nearly 1:1 by the ^{1}H NMR data. Further inspection of the ^{1}H NMR data and NOESY experiments suggested that the ketimines possess a boat-like conformation in which the phenyl group shielded
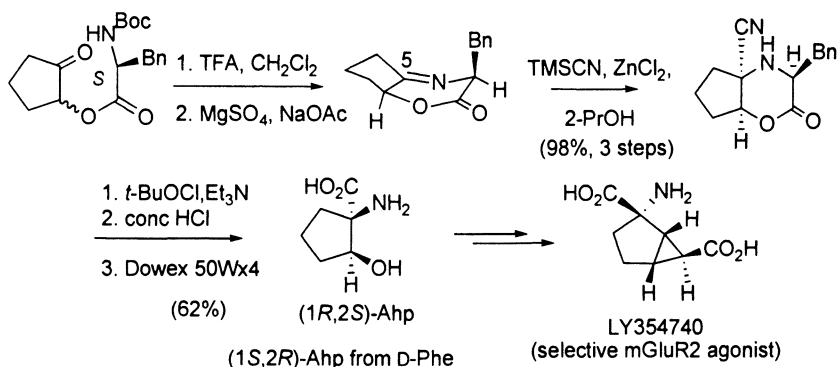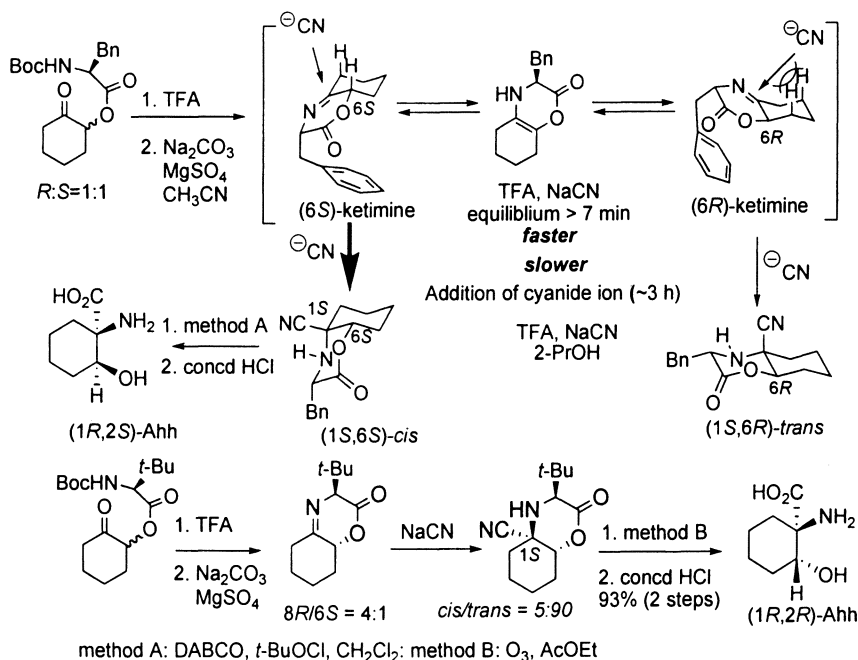
Scheme 3. Asymmetric synthesis of (1R,2S)-1-amino-2-hydroxycyclopentane carboxylic acid (Ahp)

the α-face (*15*). These results clearly indicated that the facial selectivity (1*R* versus 1*S*) is affected by the nature of the chirality transferring amino acid. Regarding the *cis/trans* selectivity, the cyanide addition to the ketimines would be the rate-determining step because the equilibrium between the (6*S*)- and (6*R*)-isomers is much faster (>7 min) than the cyanide addition (~3 h). On the other hand, (*S*)-*t*-Leu gave the corresponding (6*R*)-ketimine as the major isomer (6*S*/6*R* = 1:4). The bulky *t*-Bu group would restrict the formation of the (6*S*)-isomer due to steric reasons. In this case, the treatment with ZnCl$_2$ and TMSCN showed the highest *trans* selectivity for the α-amino nitrile formation (*cis/trans* = 5:90, 80%) and a small amount of the (1*S*)-isomer (5%) was produced as a byproduct presumably because the shielding of the α-face, such as that of L-Phe, was not available. Upon conversion of the (6*R*)-isomer to the *trans*-Ahh, oxidation with *t*-BuOCl was not successful but ozone produced the desired mixture of the α-imino and amide nitrile (*13*). Hydrolysis of the mixture gave *trans*-Ahh (*20*).

Cis-(1*R*,2*S*)-Ahh was incorporated into the Gly[2] position of Leu-enkephalin that exhibited a potent binding activity to cloned rat δ-opioid receptors (IC$_{50}$ = 0.01 nM), while its *trans* isomer was much less potent (IC$_{50}$ = 1.0 nM) (*21*). The stereochemical outcome investigated in the cyclohexane system enabled the construction of the multi-functional cyclohexane platform, key to the total synthesis of 5,6,11-trideoxyTTX (Scheme 5) (*10*).

Efficient Methods for the Synthesis of α-Acyloxy Ketones

Upon further application of the asymmetric Strecker synthesis into various classses of α-substituted α-amino acids, the conventional preparation of an α-

Scheme 4. Asymmetric synthesis of (1R,2S)-and (1R,2R)-1-amino-2-hydroxycyclohexane carboxylic acid (cis- and trans-Ahp)
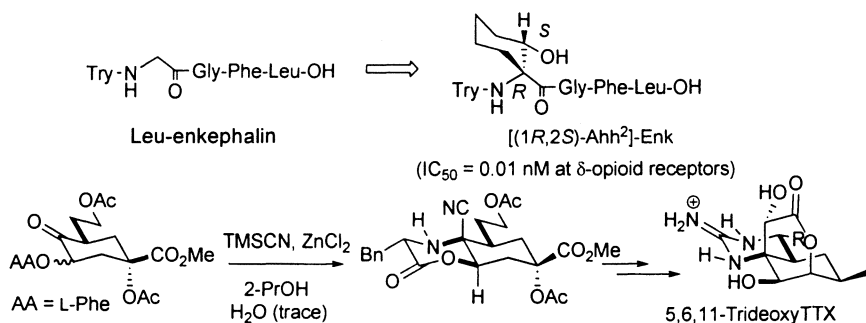
Scheme 5. Structure of cis-Ahh containing Leu-Enk and the Strecker synthesis of the cyclohexane platform during the total synthesis of 5,6,11-trideoxyTTX

acyloxy ketone is an important issue. The starting ketones can be prepared by esterification of an α-ketol with an *N*-protected amino acid or mono-esterification of a vicinal diol followed by oxidation. Occasionally, these methods incurred a problem, which is represented by the resistance of the hydroxy group to esterification by an α-amino acid.

The initial approach was the insertion reaction of an α-diazo ketone into the carboxyl group of an α-amino acid which would provide simple access to the preparation of the α-acyloxy ketones required for the asymmetric Strecker synthesis. After a survey of several metal-catalysts, Cu(acac)$_2$ was found to effectively catalyze the insertion reaction under mild reaction conditions. Most of the diazo ketones reacted with a stoichiometric amount of not only the carboxylic acids, but also the *N*-protected α-amino acids to give the corresponding α-acyloxy ketones (*22*).

The next effort was the Au-catalyzed synthesis of the α-acyloxy ketones from α-acyloxy-α-alkynylsilane. It is well known that Au salts play a dual role for the [3,3] sigmatropic rearrangement of a propargyl ester and activation of the resulting allene to give a series of cascade reaction products via a cationic vinyl-Au species (an allyl cation) at the allenic terminus (*23*). Contrary to this, the Au-catalyzed reaction of an α-acyloxy-α-alkynylsilane gave an allene intermediate whose successive activation by the Au salt generated a vinyl cation at the allenic center. The unprecedented generation of the cation and its trapping with H$_2$O gave rise to an α-acyloxy-α'-silyl ketone. Treatment of a propargylsilane having Boc-L-Phe, Ala, or Val as the acyloxy group with Ph$_3$PAuOTf (3 mol%) in dioxane-H$_2$O (1 equiv) produced the α-acyloxy-α'-silyl ketones in good yields (*24*). The removal of the silyl group afforded the α-acyloxy ketone, a precursor for the asymmetric Strecker synthesis of α-methylthreonines. Thus, various types of α-acyloxy ketones as the precursor of the Strecker synthesis became available from the α-diazo ketones or α-acyloxy-α-alkynylsilanes (Scheme 6).

Claisen Approaches from α-Acyloxy-α-alkenysilanes

It is well-known that the carbocation β to silicon is stabilized by σ-π hyperconjugation, the so-called β-effect, which has provided various synthetic applications (*25*). Contrary to this, the carbocation α to silicon (α-silyl cation) has not been well investigated because of its presumed chemical instability which is indicated by the MP2 calculations: 18.3 kcal/mol less stable than the corresponding carbocation α to the methyl group (*26*). The reactions regarding the unprecedented α-silyl cation generated from the optically active α-hydroxy-α-alkenylsilanes have been extensively investigated over the past decade in our laboratories that include the acid-catalyzed rearrangement of the α-hydroxy-α-alkenylsilane (*27*), Pd-catalyzed alkylation (*28*) and ester-enolate Claisen rearrangement of an α-acyloxy-α-alkenylsilane (*29,30*). These reactions
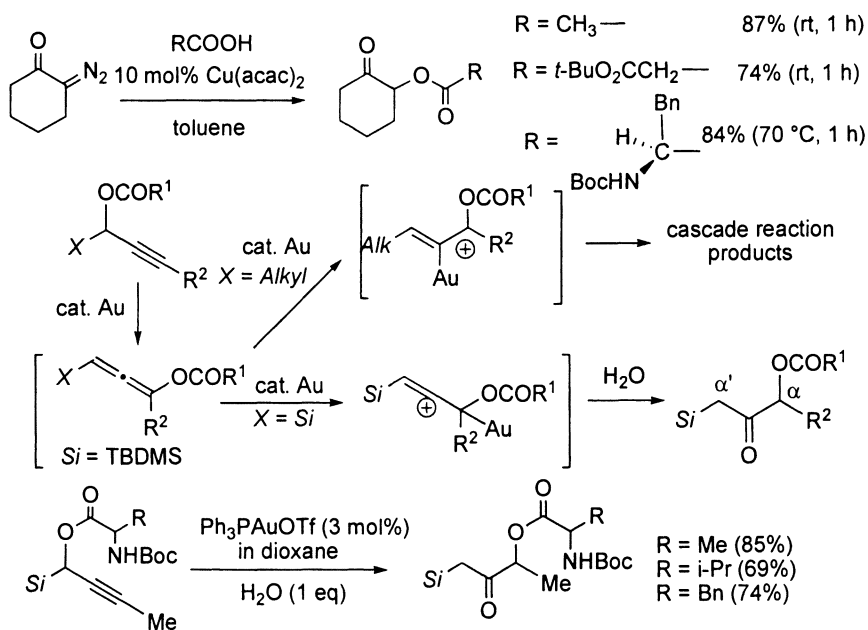
Scheme 6. Cu- and Au-catalyzed Synthesis of α-acyloxy ketones

proceeded through a putative α-silyl cation or π-allyl cation to afford the γ-substituted vinylsilanes with partial or complete transfer of the original chirality. The use of α-amino acid as the acyloxy group in the ester-enolate Claisen rearrangement was expected to produce the vinylsilane-containing α-amino acid, which can be viewed not only as a novel class of unusual amino acids but also as a useful synthetic block for biologically active compounds (Scheme 7).

The application of the ester-enolate Claisen rearrangement for the synthesis of the racemic α-amino acid had originally been reported by Kazmaier et al., (*31*) which is performed by the efficient control of the ester-enolate geometry by Zn^{2+}. According to Kazmaier's protocol, treatment of the ester-enolate from the *E*-isomer with LDA in the presence of $ZnCl_2$ in THF gave the rearrangement product in 92% yield as a single diastereomer with the 2*R*,3*S* configuration (*syn*). indicating that the chirality of the α-acyloxysilyl group was completely transferred to the product. The (*Z*)-isomer gave exclusively the (2*R*,3*R*)-isomer (*anti*). The fact that the reaction is highly diastereoselective, affording the *syn*- or *anti*-isomer suggests that the present reaction proceeded through a chair-like 6-membered ring transition state in which $ZnCl_2$ fixed the enolate configuration to the *Z* geometry and the sterically bulky TBDMS group located at an equatorial position to avoid severe steric repulsion with the enolate. Thus, the *syn*- and *anti*-diastereomers of the vinylsilane-containing amino acids were synthesized,
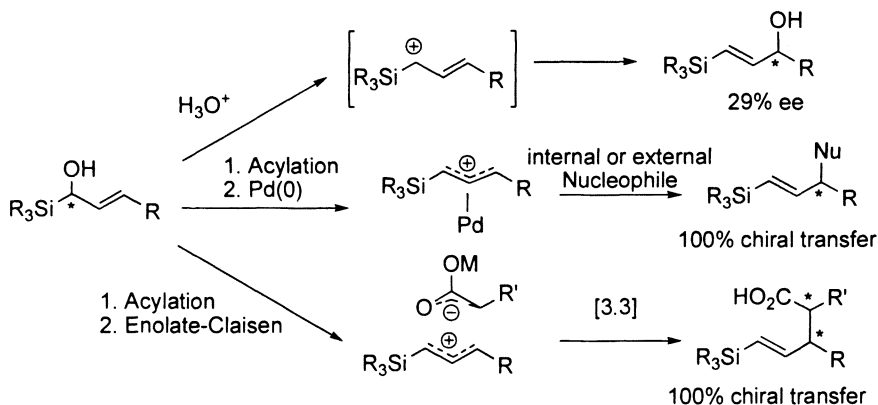
Scheme 7. Acid-catalyzed rearrangement of α-hydroxy-α-alkenyksilane, Pd-catalyzed alkylation and ester-enolate Claisen rearrangement of an α-acyloxysilane

respectively. Their TBDMS group can be removed with HBF$_4$ to give the (3*S*)- and (3*R*)-methyl allylglycines, respectively (Scheme 8).

The reaction tolerates various substituents at the vinylic terminus. As an example, we performed the synthesis of the optically active β-carbon-substituted analogs of *threo*-β-benzyloxy aspartate (TBOA) which is a potent blocker of excitatory amino acid transporter in mammalian CNS (*32*). Treatment of (*S,E*)-α-acyloxysilane with LDA and ZnCl$_2$ in THF yielded a rearrangement product (86%) as the sole diastereomer. Upon treatment with HBF$_4$, spontaneous desilylation and removal of the Boc group occurred to give the amino acid (68%). This was successfully converted to the TBOA-carbon analog (Scheme 9) (*30*).
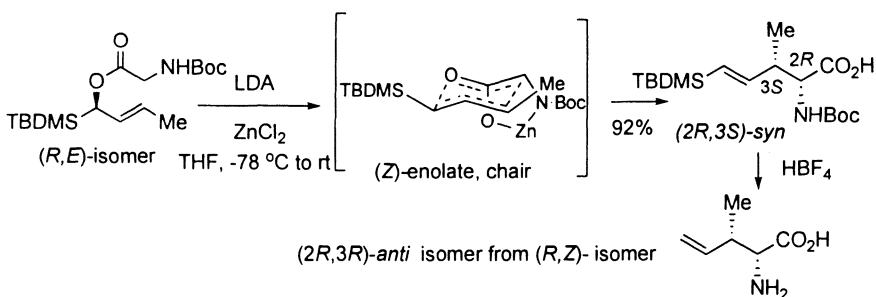
Scheme 8. Synthesis of vinylsilane-containing amino acids
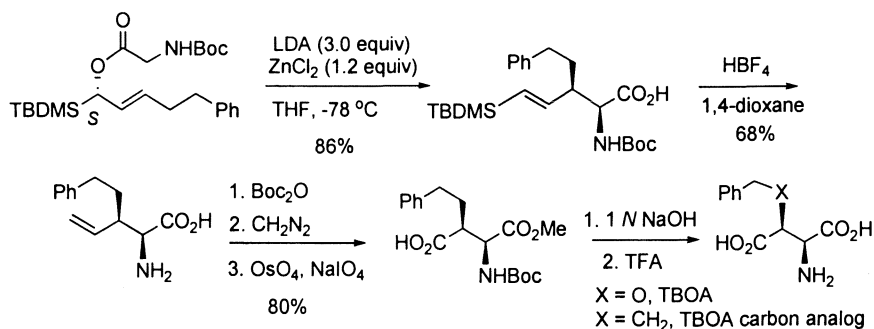
Scheme 9. Synthesis of TBOA carbon analog

Next, the feasibility of the reaction to prepare a vinylsilane-containing α-substituted α-amino acid was examined (Scheme 10). The enolate-Claisen rearrangement of the *N*-protected Phe as the acyloxy group would produce the corresponding vinylsilane-containing α,β-substituted α-amino acid. Thus, treatment of the (*E*)-isomer of Boc-L-phenylalanyloxylsilane under the same conditions allowed the desired rearrangement in a highly stereoselective manner to produce the *syn*-isomer. The reaction of the (*Z*)-isomer also gave the *anti*-isomer as a single diastereomer. The use of the (*S*,*E*)-isomer having the L-Ala moiety gave exclusively the (2*S*,3*S*)-isomer (*29*). This was converted to *threo*-α,β-dimethyl aspartate, an α-substituted analog of threo-β-hydroxyaspartate (THA) which is a transport blocker of L-Glu (*32*).

An extention of this method is the synthesis of the α-substituted-proline, which has attracted significant attention because this substructure was found in
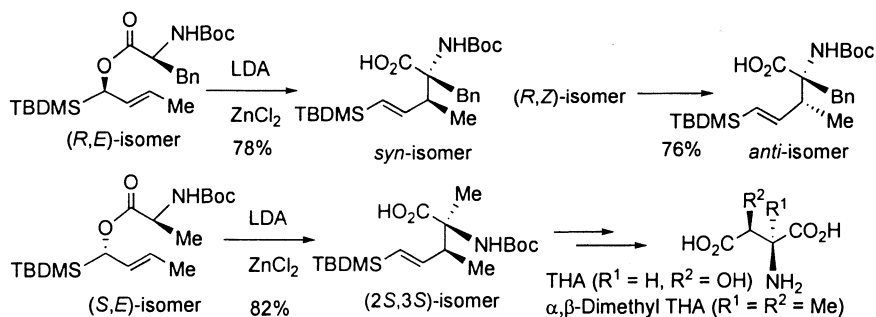
Scheme 10. Synthesis of vinylsilane-containing α-substituted α-amino acids

several natural products, such as kaitocephalin. Furthermore, this group can be viewed as a conformational modifier of Asp and Glu. However, the stereochemical outcome of the proline-containing Claisen rearrangement is not well-documented in which the enolate geometry cannot be controlled by Zn^{2+} since its base treatment produces a mono-anion enolate. The reaction using LHMDS gave the rearranged product (70%) whose optical purity was 71% ee with an S configuration (S/R = 85.5:14.5). The estimated activation energy of the model ester-enolate by the B3LPY/P6-31G* calculation suggested that the chair-like transition state with the (Z)-enolate is the most preferred pathway for the formation of the (S)-isomer, which is in good agreement with the experimental result. The (R)-enantiomer would be produced from the chair-E and/or boat-Z transition state. The addition of HMPA (23% in THF) was quite effective for the enantioselective transformation to afford the (S)-isomer with complete transfer of the starting chirality (>95% ee, 77%). The highly polar HMPA would contribute not only to the exclusive formation of the (Z)-enolate, but also to accelerate the rate of the rearrangement from the chair-Z transition state. The preferential formation of the (Z)-enolate in the presence of HMPA can be explained as kinetic deprotonation from the sterically less-hindered conformer (*33*). The product was converted to the cyclic analogs of L-Asp and L-Glu, respectively (Scheme 11) (*34*). Thus, the ester-enolate Claisen rearrangement of the optically active α- acyloxy-α-alkenylsilane is proven to be an efficient protocol for the synthesis of the vinylsilane containing α-substituted α-amino acids with complete transfer of the original chirality. The silane-containing amino acids were converted to biologically active amino acids and natural products, e,g., the total synthesis of amathaspyramide F (*12*).

Summary

Two methods for the synthesis of optically active α-substituted α-amino acids developed in our laboratories have been described. The Strecker approach starting from an achiral or a racemic α-acyloxy ketone afforded the optically pure α-substituted serine and threonine including their cyclic analogs and natural products. The Claisen approach using the optically active α-acyloxy-α-alkenylsilane furnished the novel vinylsilane-containing α- or α,β-substituted α-amino acids. The vinylsilane group was readily converted to other functional groups which led to the synthesis of the aspartate and glutamate analogs. The chiral transfer process common to these methods are highly diastereoselective for the construction of one or two consecutive chiral centers. Applications to the synthesis of biologically active natural products have been exemplified by the syntheses of the amino acids and natural products shown in Figure 1.
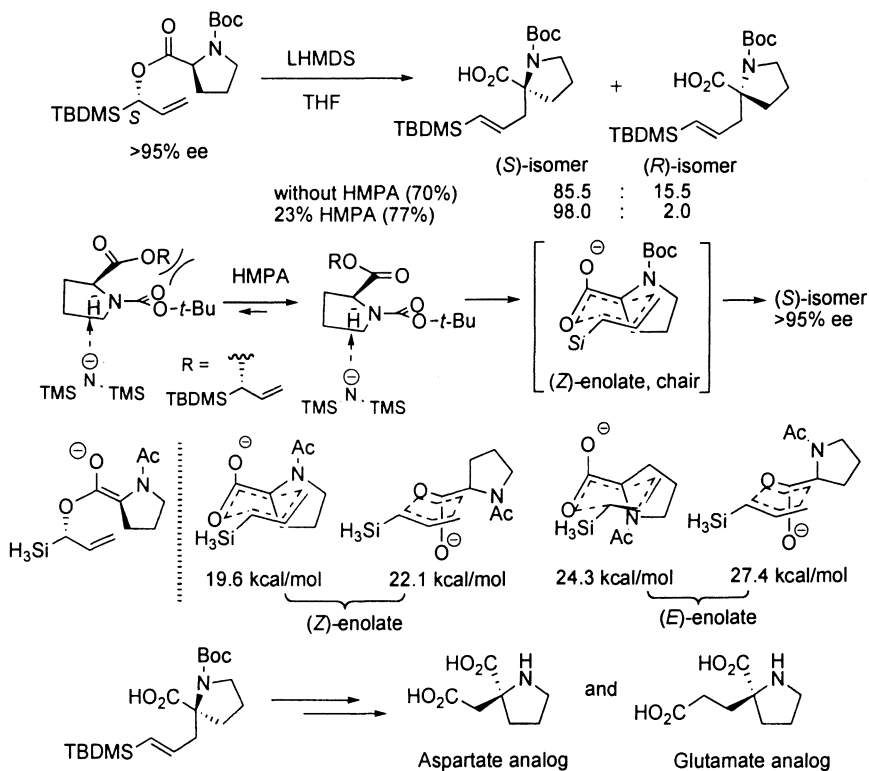
Scheme 11. Synthesis of α-substituted proline

Acknowledgement

References

1. (a) Hunt, S. *Chemistry and Biochemistry of the Amino Acids*; Barrett, G. C. Ed.; Chapman and Hall, London, 1985; p. 55. (b) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon, Oxford, 1989. (c) Ohfune, Y. *Acc. Chem. Res.* **1992**, *25*, 360.

2. *Asymmetric Synthesis of Sterically Constrained Amino Acids*; *Symposia-in-Print*, Hruby V. J.; Soloshonok, V. A. Eds. *Tetrahedron* **2001**, *57*, 6329.

3. For reviews: (a) Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2708. (b) Cativiela, C.; Diaz-de-Villegas, M. D. *Tetrahedon: Asymmetry* **1998**, *9*, 3517. (c) Kang, S. H.; Kang, S. Y.; Lee, H.-S.; Buglass, A. J. *Chem. Rev.* **2005**, *105*, 4537. (d) Ooi, T.; Maruoka, K. *Aldrichimica Acta*, **2007**, *40*, 77 and references cited therein.

4. (a) Cooper, G. M. *The Cell, A Molecular Approach*; Oxford University Press, Oxford, 1997. (b) Walsh, C. *Enzymatic Reaction Mechanisms,* W. H. Freeman and Company, San Francisco, 1979.

5. (a) Collingridge, G. L.; Lester, R. A. *Pharmacol. Rev.* **1989**, *40*, 143. (b) *Neurological disorders*; Parthasarathy, H. S. Ed. *Nature* **1999**, *399* (Suppl.), A1-A47.

6. Shimamoto, K.; Ohfune, Y. *J. Med. Chem.* **1996**, *39*, 407.

7. For reviews: (a) Ohfune, Y.; Shinada, T. *Eur. J. Org. Chem.* **2005**, 5127. (b) Ohfune, Y.; Shinada, T. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1115.

8. For the synthesis of lactacystin Corey intermediate: Iwama, S.; Gao, W.-G.; Shinada, T.; Ohfune, Y. *Synlett* **2000**, 1631.

9. For the synthesis of manzacidin A and C: Namba, K.; Shinada, T.; Teramoto, T.; Ohfune, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10708.

10. For the synthesis of 5,6,11-trideoxyTTX: Umezawa, T.; Hayashi, T.; Sakai, H.; Teramoto, H.; Yoshikawa, T.; Izumida, M.; Tamatani, Y.; Hirose, T.; Ohfune, Y.; Shinada, T. *Org. Lett.* **2006**, *8*, 4971.

11. For the synthesis of kaitocephalin: Kawasaki, M.; Shinada, T.; Hamada, M.; Ohfune, Y. *Org. Lett.* **2005**, *7*, 4165.

12. For the synthesis of amathaspyramide F: Sakaguchi, K.; Ayabe, M.; Watanabe, Y.; Kawamura, K.; Okada, T.; Shinada, T.; Ohfune, Y. *49th Symposium on the Chemistry of Natural Products*; Sapporo, 2007, Abstract p121.

13. This method well tolerates even sterically bulky α-amino nitriles: Namba, K.; Kawasaki, M.; Takada, I.; Iwama, S.; Izumida, M.; Shinada, T.; Ohfune, Y. *Tetrahedron Lett.* **2001**, *42*, 3733.

14. Moon, S.-H.; Ohfune, Y. *J. Am. Chem. Soc.* **1994**, *116*, 7405.

15. (a) Schulz, G.; Steglich, W. *Chem. Ber.* **1977**, *110*, 3615. (b) Sato, M.; Kitazawa, N.; Nagashima, S.; Kano, K.; Kaneko, C. *Chem. Lett.* **1992**, 485.

16. Horikawa, M.; Nakajima, T.; Ohfune, Y. *Synlett* **1998**, 609.

17. Kawasaki, M.; Namba, K.; Tsujishima, H.; Shinada, T.; Ohfune, Y. *Tetrahedron Lett.* **2003**, *44*, 1235.

18. Ohfune, Y.; Nanba, K.; Takada, I.; Kan, T.; Horikawa, M.; Nakajima, T. *Chirality* **1997**, *9*, 459.

19. Ohfune, Y.; Demura, T.; Iwama, S.; Matsuda, H.; Namba, K.; Shimamoto, K.; Shinada, T. *Tetrahedron Lett*, **2003**, *44*, 5431.

20. Shinada, T.; Kawakami, T.; Umezawa, T.; Matsuda, H.; Kawasaki, M.; Namba, K.; Ohfune. Y, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 768.
21. Horikawa, M.; Nakajima, T.; Shigeri, Y.; Ohfune,Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2027.
22. Shinada, T.; Kawakami, T.; Sakai, H.; Tekada, I.; Ohfune, Y. *Tetrahedron Lett.* **1998**, *39*, 3757.
23. For an example: Wang, S.; Zhang, L. *J. Am. Chem. Soc.* **2006**, *128*, 8414.
24. Sakaguchi, K.; Okada, T.; Shinada, T.; Ohfune, Y. *Tetrahedron Lett.* **2008**, *49*, 25.
25. (a) Siehl, H.-U.; Mueller, T. *The Chemistry of Organic Silicon Compounds*; Rappoport, Z.; Apeloig, Y. Eds.; Wiley: Chichester, UK, 1998; vol. 2, Chapter 12, p595. (b) Fleming, I.; Barbeto, A.; Walter, D. *Chem. Rev.* **1997**, *97*, 2063.
26. Apeloig, Y.; Stanger, A. *J. Am. Chem. Soc.* **1985**, *107*, 2806.
27. Sakaguchi, K.; Higashino, M.; Ohfune, Y. *Tetrahedron* **2003**, *59*, 6647.
28. (a) Sakaguchi, K.; Yamada, T.; Ohfune, Y. *Tetrahedron Lett.* **2005**, *46*, 5009. (b) Sakaguchi, K.; Okada, T.; Yamada, T.; Ohfune, Y. *Tetrahedron Lett.* **2007**, *48*, 3925.
29. Sakaguchi, K.; Suzuki, H.; Ohfune, Y. *Chirality*, **2001**, *13*, 357.
30. Sakaguchi, K.; Yamamoto, M.; Kawamoto, T.; Yamada, T.; Shinada, T.; Shimamoto, K.; Ohfune, Y. *Tetrahedron Lett*, **2004**, *45*, 5869.
31. (a) Kazmaier, U. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 998; b) Kazmaier, U. *J. Org. Chem.* **1996**, *61*, 3694.
32. (a) Shimamoto, K.; Shigeri, Y.; Yasuda-Kamatani, Y.; Lebrun, B.; Yumoto, N.; Nakajima, T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2407. (b) Shimamoto, K.; Shigeri, Y. *CNS Agents in Med. Chem.* **2006**, *6*, 59.
33. Ireland, R. E.; Wipf, P.; Armstrong, J. D., III. *J. Org. Chem.* **1991**, *56*, 650.
34. Sakaguchi, K.; Yamamoto, M.; Watanabe, Y.; Ohfune, Y. *Tetrahedron Lett.* **2007**, *48*, 4821.

Michael Addition Reactions between Nucleophilic Glycine Equivalents and Acrylic Acid Derivatives as a Practical and Generalized Approach to the Asymmetric Synthesis of β-Substituted-α-Amino Acids

Vadim A. Soloshonok, Hisanori Ueki, and Trevor K. Ellis

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019

Asymmetric Michael addition reactions between chiral/achiral nucleophilic glycine equivalents and chiral/achiral acrylic acid derivatives have been developed for the truly practical preparation of β-substituted pyroglutamic acids. The conceptually novel idea of the topographical control of the stereochemical outcome of asymmetric reactions, as a theoretical background of this chemistry is also described.

Interest in the β-substituted, or so called χ-constrained, tailor-made (*1*) amino acids started with the truly revolutionary studies by Prof. V. Hruby who introduced the concept of "the global and local constraint" for understanding peptide three dimensional (3D) structure-activity relationships (*2*). Thus, the peptide 3D structure depends on the following: peptide sequence (primary structure), conformation (secondary structure), and position of the amino acid side-chains. The importance of peptide amino acid sequence and its secondary conformation on the peptide bio-activity has been recognized for a long time. On the other hand the importance of the third factor, the position of the amino acid side-chains (so called χ–space) was virtually ignored (*2*). The paramount importance of the torsional angles ϕ (phi), ψ (psi) and ω (omega) (*3*) as well as the χ (chi) torsional angles of the amino acid side-chains (*2, 4*) in determining the 3D structure of the peptide backbone, (Figure 1) for understanding the peptide-structure-biological activity, has been demonstrated.
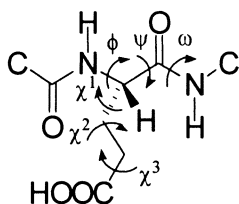
Due to only three χ dihedral angles, up to 27 (3×3×3) different bioactive conformations of the side-chain, are possible.

Figure 1. Depiction of ϕ, ψ, ω, and χ dihedral angles.

In sharp contrast to ϕ-, ψ- and ω-angles which can be controlled by the structure of amino acid residues and peptide secondary structure (e.g., global constraint via cyclization), the means to control the χ-angles is virtually undeveloped (*5*). As shown in Figure 1, a simple, 3-carbon-atom residue of glutamic acid in a peptide can have up to 27 possible biologically active side chain conformations resulting from three possible χ-angles. One may agree that limiting the number of possible conformations would add substantially to the elucidation of bio-activity in natural peptides and help enormously in the de novo design of peptides with pre-determined three-dimensional structure. However, as stated above, the control of the conformations in χ–space presents an enormous intellectual challenge. Currently, there is only one approach which looks promising in allowing control over the number of χ–conformers of the amino acid side-chains. Thus, it was demonstrated that the introduction of a methyl group in the β-position substantially enhances the population of one out of three [*gauche*-(+), *trans* and *gauche*-(-)] possible $\chi 1$-conformers (*2*). Furthermore, considering β-substituted prolines **1** (Figure 2), as proline-amino acid chimeras (*6*), it was demonstrated that regardless of *cis*- and *trans*-relative stereochemistry of **1** the corresponding (-)-*gauche* conformers are in principle, physically inaccessible (*7*). This promising data in controlling the number of conformation in χ–space clearly indicates the necessity for further investigations of various β-substituted amino acids. However as it turns out, the synthetic methodology for preparation of β-substituted amino acids in enantiomerically pure form is virtually undeveloped (*8-11*).

Here we describe our recent research on the development of highly stereoselective Michael addition reactions between nucleophilic glycine equivalents and acrylic acid derivatives as a practical and generalized approach to the asymmetric synthesis of β-substituted-α-amino acids.

As one may agree, the most straightforward and general approach to β-substituted α-mono acids might be the corresponding Michael addition reaction between properly protected nucleophilic glycine derivatives and α,β-unsaturated carboxylic acid derivatives (Scheme 1). Thus the resultant β-substituted glutamic acids **4** can be elaborated, via transformation of the ω-carboxylic group, to
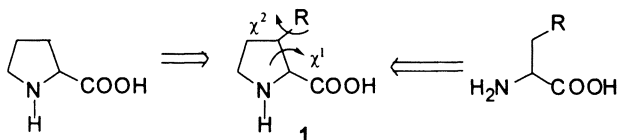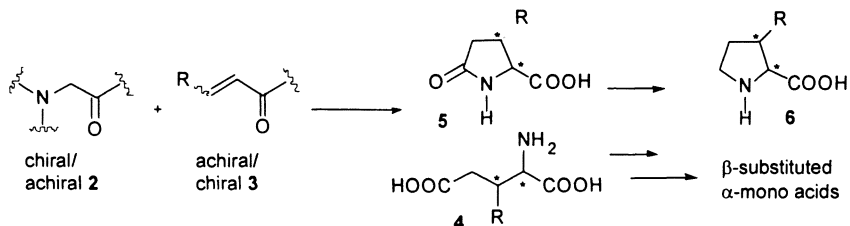
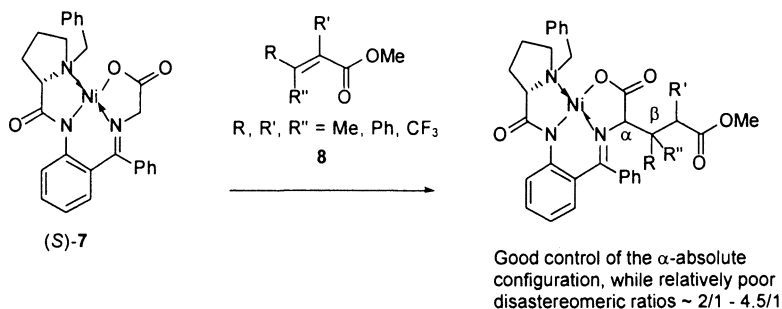*Figure 2. Proline-amino acid chimeras with constrained χ¹ angles.*

various desired α-amino acids. Moreover, easy cyclization of glutamic acids **4** to pyroglutamic acids **5** can provide a structural platform for very important β-substituted proline derivatives **6**. Indeed, numerous research groups have pursued this approach, with quite limited success (*9*).

Scheme 1. General approach to β-substituted α-mono acids via Michael addition reactions.

The major methodological problem of this approach is the necessity of simultaneous formation of two stereogenic centers in glutamic acids **4**. It was found that application of chiral glycine equivalents **2** (*12*) in the Michael addition reactions with achiral Michael acceptors **3** allowed for quite efficient (>98% ee) control of the α-stereogenic carbon in the corresponding products **4**, while the control of the stereochemistry of the β-stereogenic center was relatively poor, resulting in generally low stereochemical outcome. The Michael addition reactions between achiral glycine equivalents **2** and chiral Michael acceptors **3** were substantially less studied, however, it was shown that mode of the stereocontrol was opposite providing respected stereoselectivity the β-stereogenic center and low at the α-stereogenic carbon. Consequently, while β-substituted amino acids can be prepared by the literature methods, overall low diastereoselectivity and operationally inconvenient procedure rendered this type of amino acids prohibitively expensive (for instance, proline **5**, available from Acros 250 mg for $422 [R = Me] and 100 mg for $228 [R = Ph]) for systematic study of their promising ability to control peptide χ-space. This disappointing state of synthetic methodology prompted our group to address the challenge.

Among the literature examples (*9,12*), published on the diastereoselective Michael addition reactions between chiral glycine equivalents and achiral Michael acceptors, the application of chiral Ni(II)-complex **7** (Scheme 2), introduced by Professor Belokon (*13*), seemed, in our opinion, the most promising. Thus, despite the fact that the reactions of complex **7** with β-substituted alkyl acrylates **8** (*14*) showed rather poor (diastereomeric ratios ~ 2/1) stereoselectivity, its excellent structural features, reactivity and practicality rendered this compound as a potentially promising object. In fact, our prior experience with complex **7** in the development of asymmetric methods for preparation of various tailor-made amino acids (in particular fluorine- and phosphorus-containing derivatives) via alkylations (*15*) and aldol addition reactions (*16*) was quite satisfactory in terms of practicality and stereochemical outcome.

R, R', R" = Me, Ph, CF₃

8

Good control of the α-absolute configuration, while relatively poor disastereomeric ratios ~ 2/1 - 4.5/1

*Scheme 2. Reactions of Ni(II)-complex **7** with various alkyl acrylates.*

Our initial attempts (*17*) to improve the stereochemical outcome by optimizing reaction conditions (for instance, DBU as a base instead of NaOMe), though successful, lead to increased stereoselectivity (diastereomeric ratios ~ 4.5/1), however they remain unacceptable.

These results suggested that to be successful, we needed to develop a novel dimension in the chemistry of complex **7** and/or its derivatives. With this in mind, we decided to build on the advantageous features of complex **7** such as its virtually flat geometry, presence of Ni(II), and the (*Z*)-geometric homogeneity of the corresponding enolate. Thus, considering the Michael addition reactions between nucleophilic glycine equivalents and acrylic acid derivatives as a case of coupling of two unsymmetrically substituted trigonal centers, one can expect up to eighteen possible transition states to be involved in determining the stereochemical outcome. This large number of theoretically possible transition states resulting from the fact that starting nucleophilic glycine, in its enolate form, can react in two geometric (*Z/E*)-configurations as well as Michael acceptor in two conformations (*s-cis/s-trans*) (*18*). Simple mechanistic

rationalization suggested that complete control of the geometric/conformational homogeneity of glycine enolate and Michael acceptor might reduce the number of the possible transition states to only six. As one may agree, further limitation of the possible transition states to provide for formation of a single one was a much more formidable task. As mentioned above, the structure of complex **7** allows for the physical access to only (Z)-geometry of the corresponding enolate. Therefore, we focused on the structure of the Michael acceptors. Thus, taking advantage of the chemical structure of complex **7**, we assumed that the problem of a single transition state can be solved by the proper design of the corresponding Michael acceptor, which can co-ordinate to the Ni(II), via donor-acceptor type attractive interaction. Consequently, these mechanistic considerations highlighted two paramount requirements in the design of Michael acceptors: a) conformational homogeneity, and b) presence of partially negatively polarized group, capable of engaging in electrostatic attractive interactions with the Ni(II). Eventually, after careful consideration of numerous types of acrylic acid derivatives, we came up with pyrrolidin-2-one/oxazolidin-2-one containing structures **9** (Scheme 3).
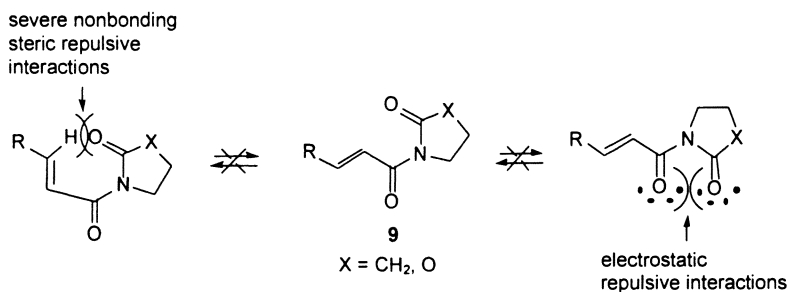
Scheme 3. Pyrrolidin-2-one/oxazolidin-2-one containing Michael acceptors

As demonstrated by single crystal X-ray analyses, compounds **9** exist exclusively in s-cis conformation (*18*). The conformational s-cis homogeneity of compounds **9** is a result of nonbonding steric repulsive interactions between the β-hydrogen of the C,C double bond and the carbonyl oxygen of the pyrrolidin-2-one or oxazolidin-2-one ring, and electrostatic repulsive interactions between the oxygen atoms of the corresponding carbonyl groups.

With these design considerations in hand, we prepared (*19*) various β-substituted derivatives **9** and studied their Michael addition reactions with complex **7** (Scheme 4).

We found that due to high electrophilicity of the Michael acceptors **9**, their reactions with complex **7** could be catalyzed by organic bases such as
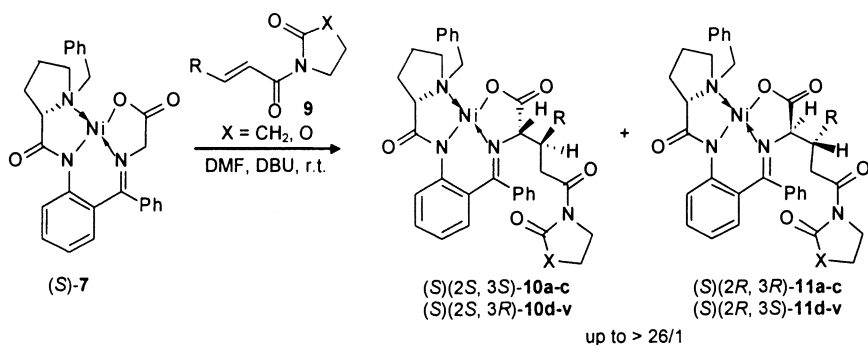
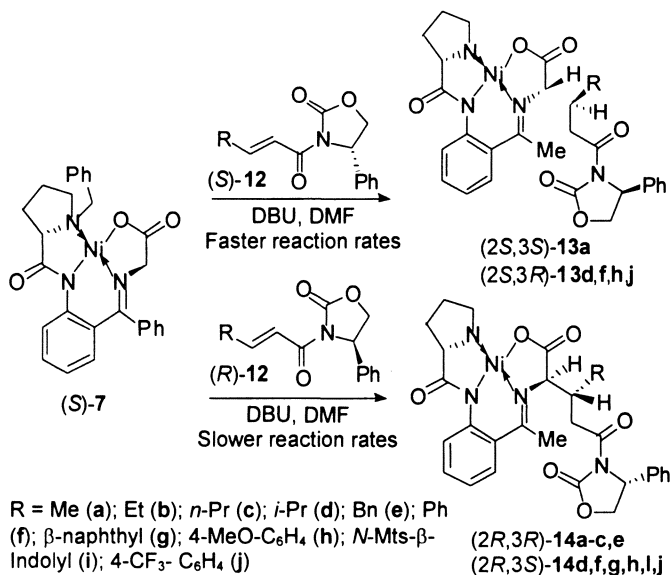R = Me (**a**), Et (**b**), *n*-Pr (**c**), *i*-Pr (**d**), Ph (**e**), α-naphthyl (**f**), β-naphthyl (**g**), 2-MeO-C$_6$H$_4$ (**h**), 3-MeO-C$_6$H$_4$ (**i**), 4-MeO-C$_6$H$_4$ (**j**), 2-CF$_3$-C$_6$H$_4$ (**k**), 3-CF$_3$-C$_6$H$_4$ (**l**), 4-CF$_3$-C$_6$H$_4$ (**m**), *N*-Mts-β-indolyl (**n**), C$_6$F$_5$ (**o**), 2,6-F$_2$-C$_6$H$_3$ (**p**), 2-F-C$_6$H$_4$ (**q**), 3,4-F$_2$-C$_6$H$_3$ (**r**), 4-MeO-C$_6$F$_4$ (**s**), 3,4-Cl$_2$-C$_6$H$_3$ (**t**), 4-NO$_2$-C$_6$H$_4$ (**u**)

*Scheme 4. Reactions of chiral Ni(II)-complex **7** with achiral homogeneous Michael acceptors **9**.*

triethylamine (slow) or DBU (fast). The stereochemical outcome of these reactions was found to depend on the nature of the β-substituent R ranging from reasonable (4/1) to excellent (>26/1) diastereoselectivity (*20*). It should be noted here that most of the reactions were extremely fast with the stereochemical outcome being kinetically controlled.

With these quite encouraging results in hand, we decided to reinforce the stereochemical outcome by using the chiral version of the Michael acceptors **9** and **12** (Scheme 5) which were easily prepared using our generalized and operationally convenient procedures (*19*). To our most complete satisfaction, the reactions of chiral Ni(II) complex **7** with chiral Michael acceptors **12** (Scheme 5) occurred at room temperature in the presence of catalytic amounts (15 mol %) of DBU with virtually complete (>25/1) chemical yields and stereoselectivity (*21*). It is important to note that the combination of (*S*)-configuration of complexes **7** with (*S*)-configuration of Michael acceptors **12**, provided for a case of "matched" stereochemistry, resulting in high reaction rates. The opposite combination of the (*S*)-absolute configuration of complexes **7** and (*R*)-configured Michael acceptors **12**, represented a "mismatched" case. However, in this case the diastereomeric purity of the products was not compromised giving rise to diastereomerically pure products with lower reaction rates.

A critical examination of the stereochemical outcome of the reactions between chiral complexes **7** and achiral Michael acceptors **9** vs. chiral complexes **7** and chiral **12**, one may assume that the reason for virtually
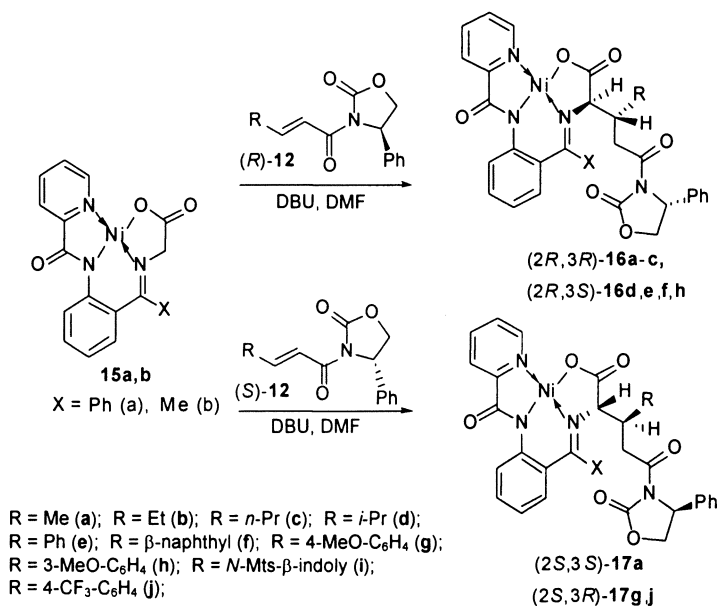
R = Me (a); Et (b); n-Pr (c); i-Pr (d); Bn (e); Ph (f); β-naphthyl (g); 4-MeO-C₆H₄ (h); N-Mts-β-Indolyl (i); 4-CF₃- C₆H₄ (j)

Scheme 5. Reactions of chiral Complex 7 with chiral Michael acceptors

complete stereoselectivity in the former case seemed to be connected with chirality of **12**. This assumption suggested that application of achiral analogs of complex **7** in combination with chiral Michael acceptors **12** might be a viable alternative option. To this end, we synthesized achiral Ni(II)-complexes **15a,b** (*22*) and studied their Michael addition reactions with **12** (Scheme 6).

We were pleased to find that according to our expectations the reactions of achiral complexes **15a,b** with chiral **12** occurred at generally high reaction rates furnishing diastereomerically pure addition products **16** and **17** in high chemical yield. It should be noted that the simultaneous formation of two stereogenic centers in products **16** and **17** was stereochemically complete regardless of the nature of the substituent R on the starting Michael acceptors **12** (*23*). These, as well other (*21-23*) results strongly suggested that we have found a unique mode of stereocontrol for addition reactions. As mentioned before, we actually have designed and anticipated this outcome based on the mechanistic considerations including the flat geometry of the complexes **7**, **15a,b** and Michael acceptors **9**, **12**. The detailed rational is described in our major publications (*21b*, *23c*). Here we present just a short version of our conclusions.

Considering three possible transition states **18**, **19** and **20** (Figure 3), one may agree that only transition state **20** can account for all stereochemical observations encountered in the study (*18-23*). In the transition state **20** the substituent R^2 at C-4 of the chiral oxazolidinone ring is pointing up and away

R = Me (**a**); R = Et (**b**); R = *n*-Pr (**c**); R = *i*-Pr (**d**);
R = Ph (**e**); R = β-naphthyl (**f**); R = 4-MeO-C₆H₄ (**g**);
R = 3-MeO-C₆H₄ (**h**); R = *N*-Mts-β-indolyl (**i**);
R = 4-CF₃-C₆H₄ (**j**);

*Scheme 6. Reactions of achiral Ni(II)-complexes **15a,b** and chiral Michael acceptors **12**.*

from any possible steric interactions with the rest of substituents on both the Ni(II)-complex and the Michael acceptor. In this position, the substituent R^2 does not directly control the facial diastereoselectivity of the Michael acceptor's C,C double bond via a stereodiscrimination process but works as a topographical feature, influencing the Ni(II)-complex ability to interact with each plane of the Michael acceptor. This mode of interaction, controlling the absolute configuration of the products, represents a topographical match or mismatch of two geometric figures, so we proposed to call it topographically controlled face diastereoselectivity (*21b*). The results obtained demonstrate that topographically controlled stereoselectivity is a much more powerful way to achieve stereocontrol in asymmetric reactions, as compared with the more common asymmetric synthetic approach in stereodiscrimination. An obvious advantage of the topographically controlled transition state is the virtually complete stereochemical outcome, and the extraordinary generality of the Michael additional reactions presented in the text.

With these results in hand and considering some minor issues of practically and large-scale applications, we decided that we still could improve on the physical and chemical properties of these Ni(II)-complexes. Practicality is defined by us as a *scalable* chemical process which can be conducted under *operationally convenient conditions* with *attractive cost structure*. Thus, we
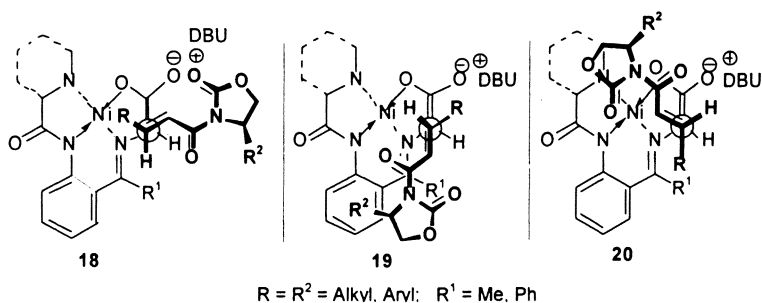
R = R² = Alkvl. Arvl: R¹ = Me. Ph

Figure 3. Possible (approach geometry) transition states in the addition reaction of Ni-(II)-complexes with chiral Michael acceptors.

arrived at our innovative idea of a modular approach (Scheme 7) for the design of nucleophilic equivalents of glycine and higher α-amino acids. Moreover, the methodological flexibility of this design would allow us to unify at least four major, currently orthogonal and conceptually different approaches under one synthetically powerful, flexible, and superior methodology for preparation of enantiomerically pure α-amino acids. In particular, we can use: (**A**) Asymmetric catalytic homologation of appropriately/carefully designed achiral derivatives **21** using a chiral base-catalyst. (**B**) Asymmetric stoichiometric homologation of chiral equivalents **21** using a chiral amine module in the design of ligands **21**. (**C**) Deracemization of racemic α-amino acids using specially designed chiral ligands **21** and racemic α-amino acids. (**D**) Asymmetric homologation of compounds **22** derived from achiral ligands **21** and optically pure α-amino acids using the memory of chirality/chiral relay principle (*24*).

The synthesis of starting ligands is extremely simple and can be conducted on very large-scale (Scheme 8). As illustrated in Scheme 8, we can control the desired physical properties of our glycine derivatives, simply by the appropriate choice of the "Amine Module".

As one may agree, the cost-structure of this design is also very attractive as most of these modular glycine derivatives can be prepared under $1 per 1g.

In connection with the synthesis of β-substituted glutamic acids and prolines (*25*), we studied the reactions of new generation of glycine equivalents with Michael acceptors **12** (Scheme 9). As one can see from the Scheme 9, the nature of the "Amine Module" had an unexpectedly strong effect of the reaction rate of these additions. However, the stereochemical outcome in all reactions studied was virtually perfect as only one stereoisomer of products **25** was observed in the reaction mixtures. These results allowed us to identify the corresponding glycine derivative **26** (Scheme 10) containing the piperidine moiety as the best starting compound.
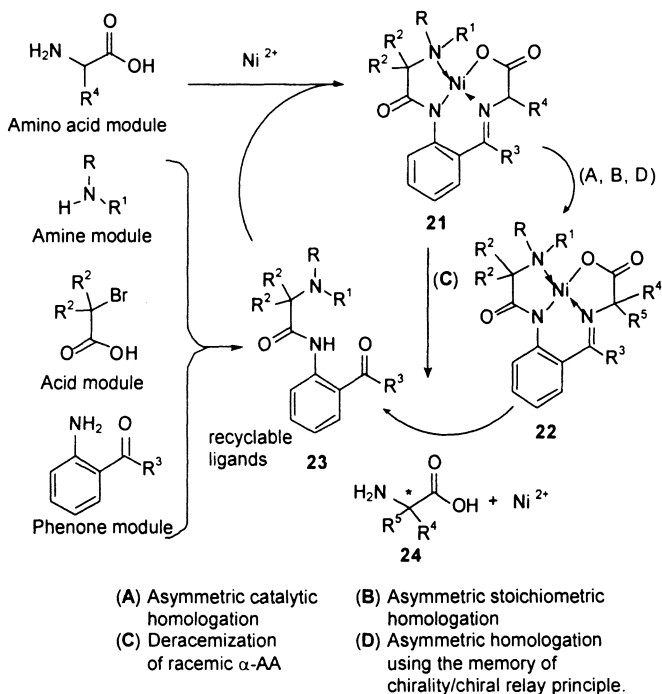
Scheme 7. Modular approach to the unified methodology for preparation of enantiomerically pure a-amino acids
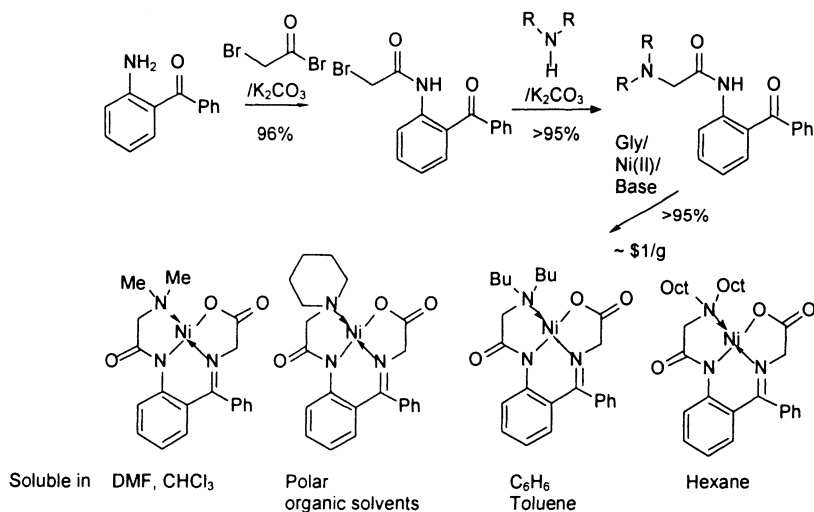

Scheme 8. Synthesis and physical properties of novel Modular glycine equivalents.

Scheme 9. The reaction of new generation of glycine equivalents
with Michael acceptors 12.
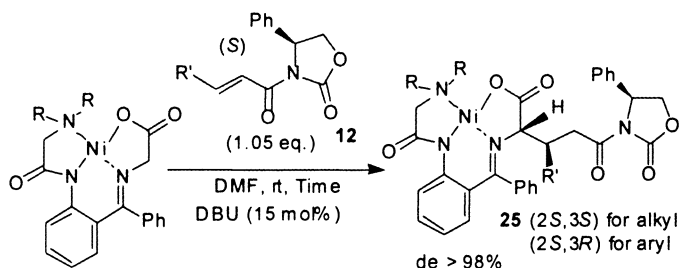
Entry	R	R'	Time	Conversion	Yield
1	O morpholine	4-MeOC₆H₄	2.25 h	>99	>95
2		i-Pr	18 h	>99	>79
3	piperidine	4-MeOC₆H₄	20 min	>99	>95
4		i-Pr	25 min	>99	>99
5	isoindoline	4-MeOC₆H₄	2.50 h	83	>78
6		i-Pr	26 h	60	>60
7	Me-N	4-MeOC₆H₄	2 h	>99	>99
8		i-Pr	26 h	62	>62
9	Ph, Ph-N	4-MeOC₆H₄	2.50 h	>99	>99
10		i-Pr	26 h	88	>88

Furthermore we have studied the generality of the reactions between glycine **26** and series of Michael acceptors **12**. Thus, the very high reactivity of the glycine equivalent **26** allowed us to use Michael acceptors of particularly high steric demand or electronic disadvantage. Just stating the extraordinary generality of these Michael additional reactions, as the best methodology developed to date for preparation of β-substituted glutamic acids, we must point out one exception. Thus, we were unable to produce the anticipated product in one example, in which a *tert*-butyl group was incorporated into the Michael acceptor. However, this limitation comes from the topographically controlled mechanism of these reactions. As one may assume, the *tert*-butyl group cannot minimize the repulsive steric interactions between the planes of Ni(II)-complex

Scheme 10. Michael addition reactions of piperidine containing glycine
equivalent with chiral acceptors 12.
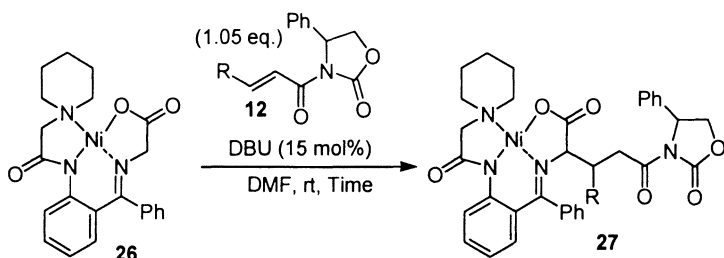
Entry	Chiral Aux.	R	Time	Conversion	Yield
1	S	4-MeO-C$_6$H$_4$	20 min	>99	>95
2	R	i-Pr	25 min	>99	>79
3	S	Me	3 min	>99	>95
4	S	Ph	3 min	>99	>99
5	S	2-MeO-C$_6$H$_4$	1.75 h	83	>78
6	S	2-CF$_3$-C$_6$H$_4$	1 h	60	>60
7	S	2,6-F$_2$-C$_6$H$_4$	4 min	>99	>99
8	S	N-Bn-Indolyl	20 h	62	>62
9	R	N-Ts-Indolyl	30 min	>99	>99
10	R	N-Tr-Imidazolyl	1 h	88	>88
11	S	tert-Bu	NR		

and Michael acceptor, making it impossible to form the corresponding transition state.

Finally, we would like to demonstrate the produce for disassembling the Ni(II) complex and recovery of the valuable products involved. As shown in Scheme 11, the disassembly of products **28** can be easily conducted under very mild conditions resulting in the formation of a product which can be readily separated. Usually resulting in the isolation of the target pyroglutamic acids no less than 93% chemical yield. The chiral auxiliary **30** can be almost completely recovered and reused for preparation of new portions of the Michael acceptors **32**. Finally, the modular ligands **31** also can be recovered and reused an
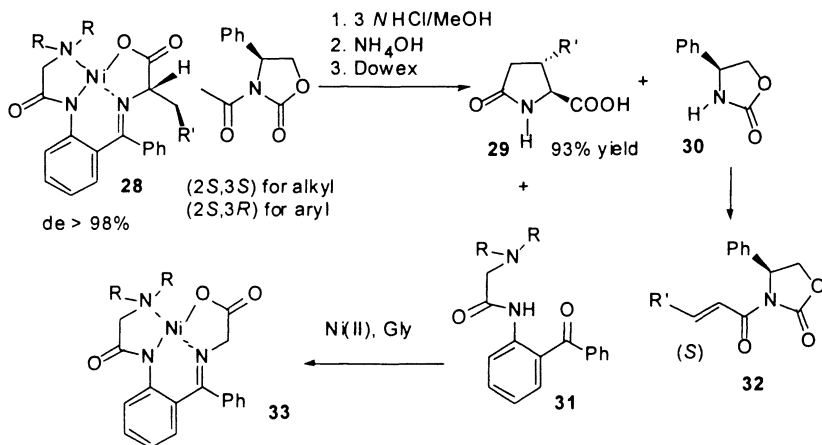
Scheme 11. Disassembling of the products 28

unlimited number of times for preparation of starting complexes **33** which are ready for production various amino acids.

In summary, the chemistry we have developed, is probably the first synthetic approach which can beat the enzymatic approach for industrial production of amino acids. The practically of this methodology has been tested on a kilogram-scale for several β-substituted pyroglutamic acids and prolines. Also, several modular glycine equivalents are now commercially available from TCI America and many other research groups are beginning to utilize these advantageous compounds for academic and industrial research.

Acknowledgements

References

1. The rapidly growing list of amino acids isolated from various natural sources makes the terms *unnatural*, or *non-proteinogenic* amino acids, which are most frequently used in the literature, dependent on the success of specific scientific achievements. For instance, amino acids containing the

most xenobiotic element fluorine have been shown to be synthesized by microorganisms. Therefore, the time independent term *tailor-made*, meaning rationally designed/synthesized amino acids, in the absence of a better definition, is most objective and suggested by us to be used in the literature. For original introduction of this term, see: ref. 17c.

2. For review on χ-(chi)-constrained amino acids, see: Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585-615.

3. (a) *Molecular Conformation and Biological Interactions*, Balaram, P.; Ramaseshan, S.; Eds.; Indian Academy of Science, Bangalore, 1991. (b) Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 283-438.

4. (a) Hruby, V. J. *Life Sci.* **1982**, *31*, 189-199. (b) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. M. *Biochemical J.* **1990**, *268*, 249-262. (c) Hruby, V. J. *Biopolymers* **1993**, *33*, 1073-1082. (d) Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. D. *Biopolymers* **1997**, *43*, 219-266.

5. (a) Scheraga, H. A. *Chem. Rev.* **1971**, *71*, 195-217. (b) Bloom, S. M.; Fasman, G. D.; DeLoze, C.; Blout, E. R. *J. Am. Chem. Soc.* **1962**, *84*, 458-463.

6. Damour, D.; Pulicani, J.-P.; Vuilhorgne, M.; Mignani, S. *Synlett* **1999**, 786-788.

7. Mosberg, H. I.; Lomize, A. L.; Wang, C.; Kroona, H.; Heyl, D. L.; Sobzyk-Kojiro, K.; Ma, W.; Mousigian, C.; Porreca, F. *J. Med. Chem.* **1994**, *37*, 4371-4383.

8. For excellent reviews on general asymmetric synthesis of α-amino acids, including β-substituted derivatives, see: For recent reviews on asymmetric synthesis of α-amino acids, see: (a) O'Donnell, M. J.; Esikova, I. A.; Mi, A.; Shullenberger, D. F.; Wu, S. *In Phase-Transfer Catalysis*, Halpern, M. E.; Ed.; ACS Symposium Series 659, American Chemistry Society: Washington, DC, 1997; Chapter 10; pp 124-135. (b) O'Donnell, M. J. Asymmetric Phase-Transfer Reactions. In *Catalytic Asymmetric Synthesis*, Ojima, I., Ed.; 2nd ed., Wiley-VCH: New York, 2000; Chapter 10; p 727-755. (c) Soloshonok, V. A. In *Biomedical Frontiers of Fluorine Chemistry*; Ojima, I.; McCarthy, J. R.; Welch, J. T., Eds.; ACS Books, American Chemical Society: Washington, D. C., 1996; Chapter 2. (d) O'Donnell, M. J. *Aldrichimica Acta* **2001**, *34*, 3. (e) Shioiri, T.; Hamada, Y. *Synlett* **2001**, 184-201. (f) Rutjes, F. P. J. T.; Wolf, L. B.; Schoemaker, H. E. *Perkin 1* **2000**, 4197-4212. (g) Abellan, T.; Chinchilla, R.; Galindo, N.; Guillena, G.; Najera, C.; Sansano, J. M. *Eur. J. Org. Chem.* **2000**, 2689-2697. (h) Yao, S. L.; Saaby, S.; Hazell, R. G.; Jorgensen, K. A. *Chem. Eur. J.* **2000**, *6*, 2435-2448. (i) Kazmaier, U.; Maier, S.; Zumpe, F. L. *Synlett* **2000**, 1523-1535. (j) Kawabata, T.; Fuji, K. *J. Synth. Org. Chem. Japan* **2000**, *58*, 1095. (k) Beller, M.; Eckert, M.; *Angew. Chem. Int. Ed.* **2000**, *39*, 1010-1027. (l) Sutherland, A.; Willis, C. L. *Nat. Prod. Rep.* **2000**, *17*, 621-631.

(m) Cativiela, C.; Diaz-de-Villegas, M. D. *Tetrahedron: Asymmetry* **2000**, *11*, 645-732. (n) Bouifraden, S.; Drouot, C.; El Hadrami, M.; Guenoun, F.; Lecointe, L.; Mai, N.; Paris, M.; Pothion, C.; Sadoune, M.; Sauvagnat, B.; Amblard, M.; Aubagnac, J. L.; Calmes, M.; Chevallet, P.; Daunis, J.; Enjalbal, C.; Fehrentz, J. A.; Lamaty, F.; Lavergne, J. P.; Lazaro, R.; Rolland, V.; Roumestant, M. L.; Viallefont, P.; Vidal, Y.; Martinez, J. *Amino Acids* **1999**, *16*, 345-379. (o) Calmes, M.; Daunis, J. *Amino Acids* **1999**, *16*, 215-250.

9. For general literature data on stereoselective Michael addition reactions between nucleophilic glycine equivalents and acrylic acid derivatives, see: Soloshonok, V. A. *Current Org. Chem.* **2002**, *6*, 341-364.

10. For most recent catalityc asymmetric approach to β-substituted amino acids, see: Kobayashi, S.; Tsubogo, T.; Saito, S.; Yamashita, Y. *Org. Lett.* **2008**, *10*, 807-809.

11. For other most recent and generalized approaches to some types β-substituted α-amino acids, see also the corresponding chapters in this book (by Professors K. J. Szabo, U. Kazmaier, and Y. Ohfune.)

12. For classical reviews on application of chiral nucleophilic glycine equivalents, see: (a) Duthaler, R. O. *Tetrahedron* **1994**, *50*, 1539-1650. (b) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: Oxford, 1989.

13. (a) Belokon, Yu. N. *Janssen Chimica Acta* **1992**, *10*, No 2, 4. (b) Belokon', Yu. N. *Pure Appl. Chem.* **1992**, *64*, 1917-1924.

14. Belokon, Yu. N.; Bulychev, A. G.; Ryzhov, M. G.; Vitt, S. V.; Batsanov, A. S.; Struchkov, Yu. T.; Bakhmutov, V. I.; Belikov, V. M. *J. Chem. Soc. Perkin Trans. 1* **1986**, 1865-1872.

15. (a) Soloshonok, V. A.; Belokon, Y. N.; Kukhar, V. P.; Chernoglazova, N. I.; Saporovskaya, M. B.; Bakhmutov, V. I.; Kolycheva, M. T.; Belikov, V. M. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1990**, 1630-1636; *Chem. Abstr.*, *114*: 7135d. (b) Soloshonok, V. A.; Svistunova, N. Y.; Kukhar, V. P.; Solodenko, V. A.; Kuzmina, N. A.; Rozhenko, A. B.; Galushko, S. V.; Shishkina, I. P.; Gudima, A. O.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1992**, 397-402; *Chem. Abstr.*, *118*: 255271m. (c) Soloshonok, V. A.; Belokon, Y. N.; Kuzmina, N. A.; Maleev, V. I.; Svistunova, N. Y.; Solodenko, V. A.; Kukhar, V. P. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1525-1529. (d) Kukhar, V. P.; Belokon, Y. N.; Svistunova, N. Y.; Soloshonok, V. A.; Rozhenko, A. B.; Kuzmina, N. A. *Synthesis* **1993**, 117-121. (e) Soloshonok, V. A.; Svistunova, N. Y.; Kukhar, V. P.; Kuzmina, N. A.; Popov, V. I.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1993**, 786-790. (f) Qiu, W.; Soloshonok, V. A.; Cai, C.; Tang, X.; Hruby, V. J. *Tetrahedron* **2000**, *56*, 2577-2582. (g) Tang, X.; Soloshonok, V. A.; Hruby, V. J. *Tetrahedron: Asymmetry* **2000**, *11*, 2917-2925. (h) Soloshonok, V. A.; Tang, X.; Hruby, V. J.; Meervelt, L. V. *Org. Lett.* **2001**, *3*, 341-343. (i) Soloshonok, V. A.;

Tang, X.; Hruby, V. J. *Tetrahedron* **2001**, *57*, 6375-6382. (j) Ellis, T. K.; Hochla, V. M.; Soloshonok, V. A. *J. Org. Chem.* **2003**, *68*, 4973-4976. (k) Ellis, T. K.; Martin, C. H.; Tsai, G. M.; Ueki, H.; Soloshonok, V. A. *J. Org. Chem.* **2003**, *68*, 6208-6214. (l) Taylor, S. M.; Yamada, T.; Ueki, H.; Soloshonok V. A. *Tetrahedron Lett.* **2004**, *45*, 9159-9162. (m) Soloshonok, V. A.; Yamada, T.; Ueki, H.; Moore, A. M.; Cook, T. K.; Arbogast, K. L.; Soloshonok, A. V.; Martin, C. H.; Ohfune, Y. *Tetrahedron* **2006**, *62*, 6412-6419. (n) Soloshonok, V. A; Boettiger, T. U.; Bolene, S. B. *Synthesis* **2008**, accepted for publication.

16. (a) Soloshonok, V. A.; Kukhar, V. P.; Galushko, S. V.; Kolycheva, M. T.; Rozhenko, A. B.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, 1166-1175; *Chem. Abstr.*, *115*: 136682z. (b) Soloshonok, V. A.; Kukhar, V. P.; Batsanov, A. S.; Galakhov, M. A.; Belokon, Y. N.; Struchkov, Y. T. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, 1548-1554; *Chem. Abstr.*, *115*: 256590q. (c) Soloshonok, V. A.; Kukhar, V. P.; Galushko, S. V.; Rozhenko, A. B.; Kuzmina, N. A.; Kolycheva, M. T.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, 1906-1913; *Chem. Abstr.*, *116*: 21426x. (d) Soloshonok, V. A.; Svistunova, N. Y.; Kukhar, V. P.; Gudima, A. O.; Kuzmina, N. A.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1992**, 1172-1175; *Chem. Abstr.*, *118*: 169180z. (e) Soloshonok, V. A.; Svistunova, N. Y.; Kukhar, V. P.; Kuzmina, N. A.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1992**, 687-693; *Chem. Abstr.*, *117*: 212905h. (f) Soloshonok, V. A.; Kukhar, V. P.; Galushko, S. V.; Svistunova, N. Y.; Avilov, D. V.; Kuzmina, N. A.; Raevski, N. I.; Struchkov, Y. T.; Pisarevsky, A. P.; Belokon, Y. N. *J. Chem. Soc. Perkin Trans 1* **1993**, 3143-3155. (g) Kukhar, V. P.; Luik, A. I.; Soloshonok, V. A.; Svistunova, N. Y.; Skryma, R. N.; Rybalchenko, V. V.; Belokon, Y. N.; Kuzmina, N. A. *Khim. Pharm. Zh.* **1994**, *27*(9), 35-37. (h) Soloshonok, V. A.; Avilov, D. V.; Kukhar, V. P.; Tararov, V. I.; Saveleva, T. F.; Churkina, T. D.; Ikonnikov, N. S.; Kochetkov, K. A.; Orlova, S. A.; Pysarevsky, A. P.; Struchkov, Y. T.; Raevsky, N. I.; Belokon, Y. N. *Tetrahedron: Asymmetry* **1995**, *6*, 1741-1756. (i) Soloshonok, V. A.; Avilov, D. V.; Kukhar', V. P. *Tetrahedron: Asymmetry* **1996**, *7*, 1547-1550. (j) Soloshonok, V. A.; Avilov, D. V.; Kukhar, V. P. *Tetrahedron* **1996**, *52*, 12433-12442. (k) Soloshonok, V. A.; Avilov, D. V.; Kukhar', V. P.; Meervelt, L. V.; Mischenko, N. *Tetrahedron Letters* **1997**, *38*, 4671-4674.

17. (a) Soloshonok, V. A.; Avilov, D. V.; Kukhar', V. P.; Meervelt, L. V.; Mischenko, N. *Tetrahedron Lett.* **1997**, *38*, 4903-4904. (b) Soloshonok, V. A.; Cai, C.; Hruby, V. J.; Meervelt, L. V.; Mischenko, N. *Tetrahedron* **1999**, *55*, 12031-12044. (c) Soloshonok, V. A.; Cai, C.; Hruby, V. J.; Meervelt, L. V. *Tetrahedron* **1999**, *55*, 12045-12058.

18. (a) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Tetrahedron Lett.* **2000**, *41*, 135-139. (b) Soloshonok, V. A.; Cai, C.; Hruby, V. J.; Meervelt, L. V.; Yamazaki, T. *J. Org. Chem.* **2000**, *65*, 6688-6696.

19. (a) Soloshonok, V. A.; Ueki, H.; Jiang, C.; Cai, C. Hruby, V. J. *Helv. Chim. Acta* **2002**, *85*, 3616-3623. (b) Cai, C.; Yamada, T.; Tiwari, R.; Hruby, V. J.; Soloshonok, V. A. *Tetrahedron Lett.* **2004**, *45*, 6855-6858.

20. (a) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Tetrahedron: Asymmetry*, **1999**, *10*, 4265-4269. (b) Cai, C.; Soloshonok, V. A.; Hruby, V. J. *J. Org. Chem.* **2001**, *66*, 1339-1350.

21. (a) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Angew. Chem. In. Ed. Engl.* **2000**, *39*, 2172-2175. (b) Soloshonok, V. A.; Cai, C.; Yamada, T.; Ueki, H.; Ohfune, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **2005**, *127*, 15296-15303.

22. (a) Ueki, H.; Ellis, T. K.; Martin, C. H.; Soloshonok, V. A. *Eur. J. Org. Chem.* **2003**, 1954-1957. (b) Ueki, H.; Ellis, T. K.; Martin, C. H.; Bolene, S. B.; Boettiger, T. U.; Soloshonok, V. A. *J. Org. Chem.* **2003**, *68*, 7104-7107.

23. (a) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Org. Letters* **2000**, *2*, 747-750. (b) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Tetrahedron Lett.* **2000**, *41*, 9645-9649. (c) Soloshonok, V. A.; Ueki, H.; Tiwari, R.; Cai, C.; Hruby, V. J. *J. Org. Chem.* **2004**, *69*, 4984-4990. (d) Yamada, T.; Okada, T.; Sakaguchi, K.; Ohfune, Y.; Ueki, H.; Soloshonok, V. A. *Org. Lett.* **2006**, *8*, 5625-5628.

24. (a) Soloshonok, V. A.; Ueki, H.; Ellis, T. K. *Tetrahedron Lett.* **2005**, *46*, 941-944. (b) Soloshonok, V. A.; Ueki, H.; Ellis, T. K.; Yamada, T.; Ohfune, Y. *Tetrahedron Lett.* **2005**, *46*, 1107-1110. (c) Soloshonok, V. A.; Ellis, T. K. *Synlett* **2006**, 533-538.(d) Ellis, T. K.; Ueki, H.; Yamada, T.; Ohfune, Y.; Soloshonok, V. A. *J. Org. Chem.* **2006**, *71*, 8572-8578.

25. Cai, M.; Cai, C. Mayorov, A. V.; Xiong, C.; Cabello, C. M.; Soloshonok, V. A.; Swift, J. R. Trivedi, D.; Hruby, V. J. *J. Peptide Research*, **2004**, *63*, 116-131.

Chapter 6

Novel Chiral Template for Preparation of α-Amino Acids: Practical Synthesis and Application

Kazumi Okuro, Yasuhiro Saka, Ikuhiro Suzuki, and Masaru Mitsuda[*]

Frontier Biochemical and Medical Research Laboratories, Corporate Research and Development Division, Kaneka Corporation, 1–8, Miyamae-machi, Takasago-cho, Takasago, Hyogo 676–8688, Japan

A novel chiral imidazolidinone-type template for synthesizing optically active α-amino acids has been developed. The template was prepared from α-phenylethylamine as a chiral source, 2-chloroacetamide, and 2,6-dichlorobenzaldehyde in 4 steps. The process includes crystallization-induced dynamic resolution (CIDR). The template reacted with electrophiles in a highly stereoselective manner under mild conditions to afford alkylated products that were further transformed in 2 steps into optically active α-amino acids.

Introduction

Biotechnology in the post-genomic era has been changing the ways that new drugs are discovered. Information regarding the human genome sequence offers new opportunities in medicinal research, including the field of resultant protein, peptide, and amino acid science. Approximately 20% of the new drugs approved in the last decade contain an α-amino acid moiety in their structure. In particular,

non-natural amino acids have attracted a great deal of attention as a key component of peptide mimetic agents, which improve stability and resistance toward metabolic degradation of the pharmaceuticals (*1*).

To date, a number of efficient methods, including biotransformations, for preparing optically active α-amino acids have been established (*2*). In particular, recent remarkable progress in asymmetric synthesis has provided a wide range of accesses to α-amino acids. Examples include enantioselective hydrogenation of dehydroamino acid derivatives catalyzed by chiral transition metals (*3*) and enantioselective alkylation of glycinate derivatives by a chiral-phase transfer catalyst (*4*). The process utilizing chiral glycine templates is also a candidate (*5*) because such a process is considered to be one of the most straightforward and reliable methodologies from the perspective of simple, quick, and routine procedures (*Figure 1*).
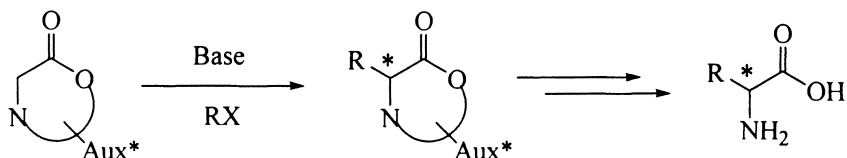

Figure 1. Synthetic outline of α-amino acids synthesis using a chiral glycine template

In this methodology, asymmetric induction is based on diastereoselective alkylation of the enolate generated from an optically active glycine derivative with electrophiles. In 1979, Schöllkopf et al. reported their pioneering studies on enantioselective synthesis of optically active α-amino acids using the symmetrical bis-lactim ether of L-alanine as a chiral glycine template (*5a*). Since then, a variety of glycine derivatives have been developed, some of which, including the bis-lactim ether, are now commercially available in reagent grade (*Figure 2*).
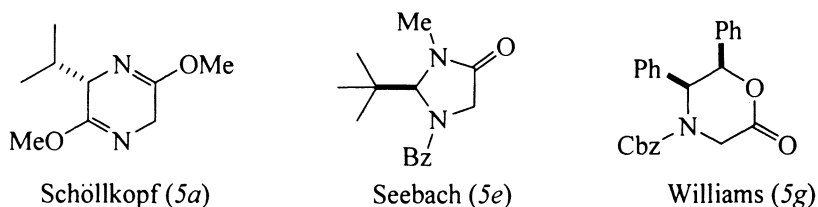

Schöllkopf (*5a*)   Seebach (*5e*)   Williams (*5g*)

Figure 2. Example of commercially available chiral glycine template

To our best knowledge (*6*), however, none of the known chiral glycine templates has been found in any industrial processes. There have been difficulties in their preparation or chemical conversion into a target α-amino acid on a large scale production. In general, extremely low temperature condition, below –60°C, is required in the asymmetric induction process (**Scheme 1**).
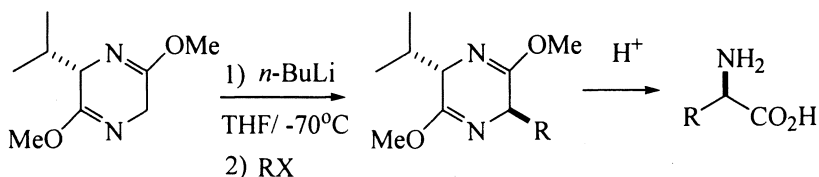
***Scheme 1.*** *Standard process to α-amino acid using Shöllkopf's template*

Development of a New Chiral Glycine Template

Under such circumstances, we envisioned the design of a novel chiral glycine template that can tolerate industrial-scale preparation. Consequently, we developed a chiral imidazolidinone **1**, a couple of enantiomers, easily converted to various chiral α-amino acids .

(2*R*,1′*R*)-**1**   (2*S*,1′*S*)-**1**

(*S*)-α-amino acid   (*R*)-α-amino acid
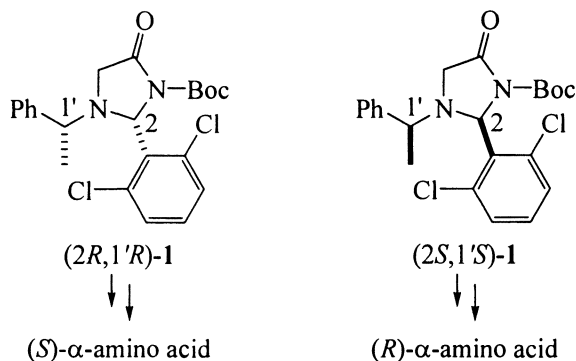
Preparation

When α-phenylethylamine (*R*)-**2** was heated with sodium iodide and 2-chloroacetamide **3** in acetonitrile, followed by condensation with 2,6-dichlorobenzaldehyde **5** in the presence of a catalytic amount of *p*-toluenesulfonic acid in toluene, a 1:1 diastereomeric mixture of imidazolidinone (2*RS*,1′*R*)-**6** was formed with regard to C-2 of the imidazolidinone ring (**Scheme 2**).
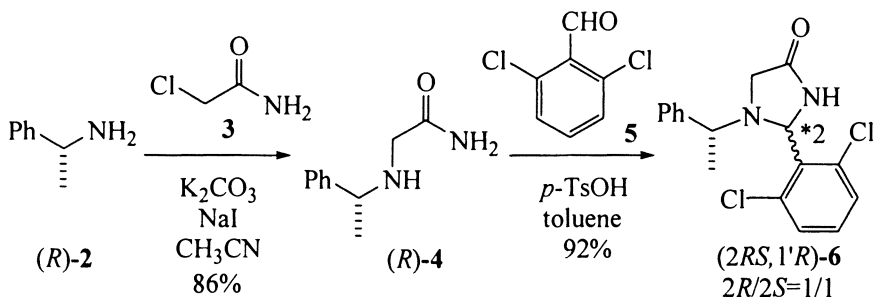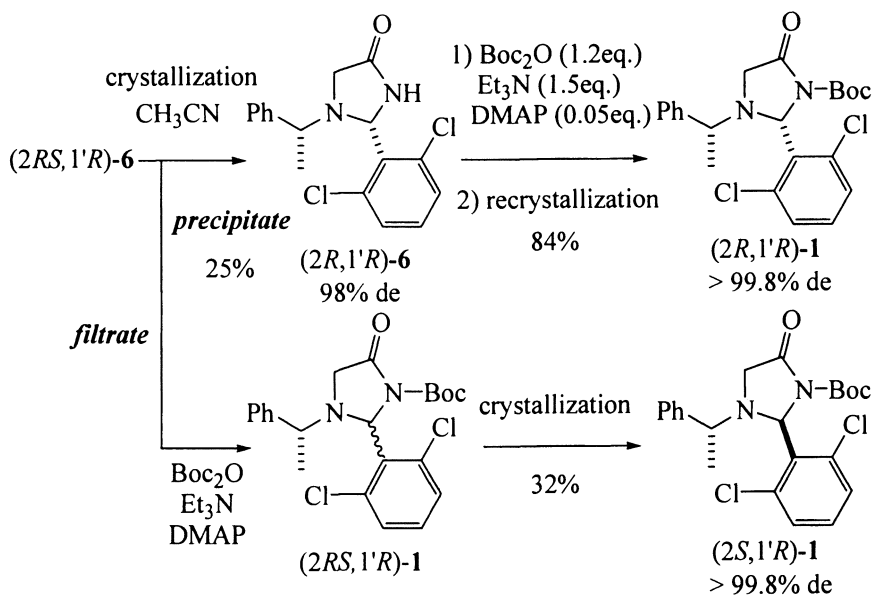
Scheme 2. Preparation of (2RS,1'R)-6

Simple crystallization of (2RS,1'R)-6 from acetonitrilè successfully increased the diastereomeric purity in up to 98% de, with (2R,1'R)-6 being isolated in 25%. Finally, it was treated with Boc₂O, followed by recrystallization to furnish our target chiral molecule (2R,1'R)-1 as a single diastereomer (>99.8% de). An isomer (2S,1'R)-1 could also be isolated by crystallization of the diastereomeric mixture of 1, which originated from the filtrate of crystallization of (2R,1'R)-6 (**Scheme 3**).

Reaction

The reaction of (2R,1'R)-1 with benzylbromide using LDA as a base in THF at –20 °C afforded alkylated product **7a**. Acid hydrolysis of *crude* **7a** and subsequent hydrogenolysis on Pd(OH)₂-C under the atmospheric pressure of H₂ delivered phenylalanine with 99.5% ee in 46% overall yield from (2R,1'R)-1, whose absolute configuration was found to be S (**Scheme 4**). It should be noted that the alkyation proceeded highly stereoselectively even at -20 °C, whereas alkylation of the chiral glycine templates previously developed[5] are typically carried out below -60 °C in order to achieve satisfactory stereoselectivity. When the preparation of phenylalanine was repeated employing (2S,1'R)-1 instead of (2R,1'R)-1, (R)-amino acid was produced in 99.8% ee.

The stereochemical outcomes of the reactions using (2R,1'R)-1 and (2S,1'R)-1, respectively, suggest that diastereoselectivity in the alkylation should be controlled by the chirality at C-2 on the imidazolidinone ring. In contrast, the diastereoselection of the alkylation could be explained by considering a chelated enolate intermediate, as shown in *Figure 3*. The metal cation originating from the base employed would form a chelation with two carbonyl oxygens. It is reasoned that on the basis of this model, the *re*-face might be shielded by a 2,6-dicholorophenyl group, resulting in exclusive attack to an electrophile from the *si*-face.

Scheme 3. *Preparation of (2R,1'R)- and (2S,1'R)-1 by simple crystallization*
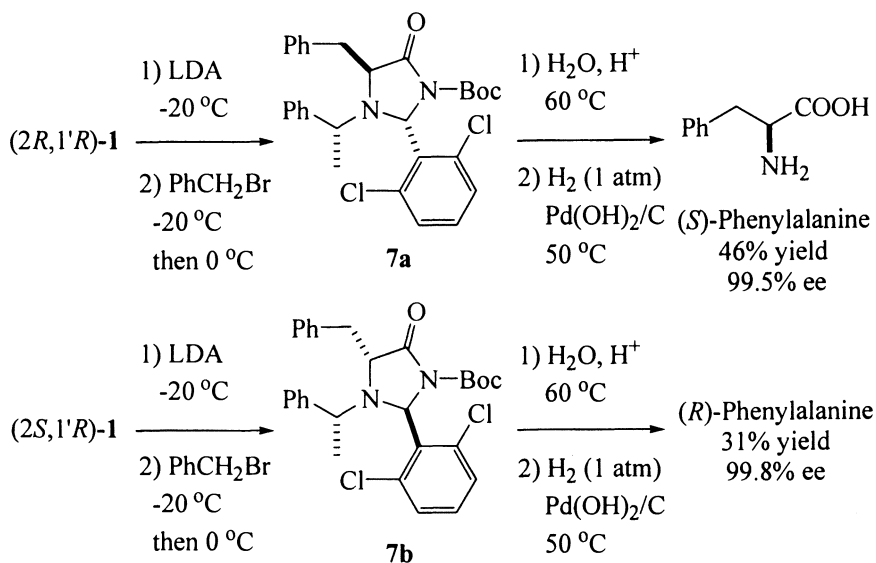
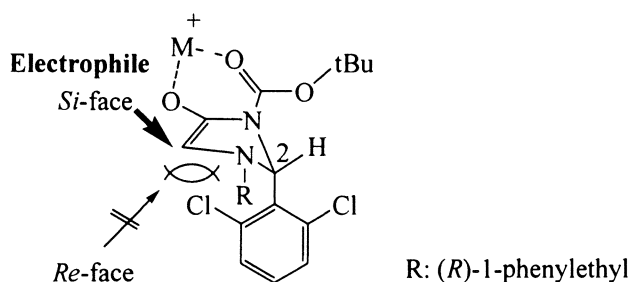**Scheme 4.** *Synthesis of phenylalanine using (2R,1'R) and (2S,1'R)-1*

Figure 3. Plausible mechanism of diastereoselective alkylation

Another interesting transformation to α-amino acids from the alkylated products could also be found that did not require hydrogenolysis to cleave the C-N bond at a α-phenylethylamino moiety. Upon exposure of **7a** to concentrated H_2SO_4 in chlorobenzene at room temperature for 1 hour could effect removal of a α-phenylethyl group on N-1. Successively, hydrolysis by the addition of water completed the conversion to (*S*)-phenylalanine in 83% yield with no substantial loss of optical purity. We succeeded in isolating an intermediate, 5-benzyl-3-(2,6-dichlorophenyl)imidazolidinone **8**, in 69% yield by work-up under basic conditions after treatment of **7a** with concentrated H_2SO_4 (**Scheme 5**).
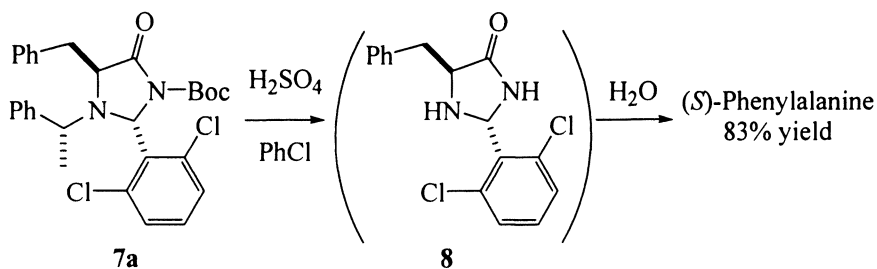
**Scheme 5.** *Transformation to α-amino acids by acid treatment*

Toluene could also be used as solvent in place of chlorobenzene. However, toluene underwent sulfonation by H_2SO_4, producing toluenesulfonic acid as a side product, which may cause difficult separation of the pure amino acid from the reaction mixture. No such sulfonation was observed for chlorobenzene.

Some examples have appeared upon acid-promoted removal of arylmethyl or α-arylethyl groups on nitrogen, but this is limited to the case in which the leaving group is activated by a methoxy group on the aromatic ring (*7*) unless the nitrogen is substituted by another electron-withdrawing group such as an acyl or

carbamoyl group (*8*). This unprecedented transformation under extremely mild conditions is undoubtedly useful for substrates having substituents sensitive to hydrogenolysis or heteroaromatic groups deactivating heterogeneous Pd catalyst.

Application of New Chiral Template
for α-Amino Acid Synthesis

Table I. α-amino acid synthesis using (2*R*,1'*S*)-1
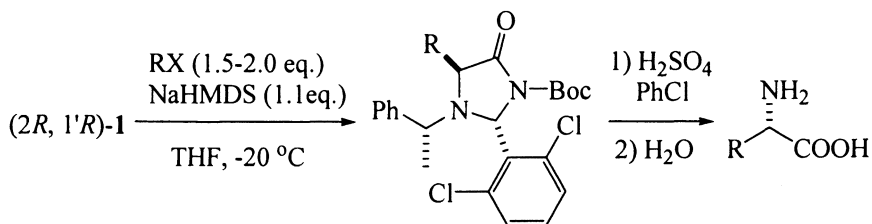
entry	RX	product (amino acid)	yield(%)^a	ee(%)^b
1	MeI	(*S*)-alanine	95	96
2	EtI	(*S*)-2-aminobutanoic acid	96	93
3	*i*-PrI	(*S*)-valine	90	98
4	*n*-BuI	(*S*)-norleucine	91	98
5	*n*-BuBr	(*S*)-norleucine	82	99
6	PhCH$_2$Br	(*S*)-phenylalanine	83	99
7	4-FC$_6$H$_4$CH$_2$Br	(*S*)-4-fluorophenylalanine	90	97
8	4-FC$_6$H$_4$CH$_2$Cl	(*S*)-4-fluorophenylalanine	86	96
9	4-ClC$_6$H$_4$CH$_2$Br	(*S*)-4-chlorophenylalanine	87	98
10	4-ClC$_6$H$_4$CH$_2$Cl	(*S*)-4-chlorophenylalanine	81	97
11	4-BrC$_6$H$_4$CH$_2$Br	(*S*)-4-bromophenylalanine	70	77

^{a} Based on (2*R*, 1' *R*)-1. ^{b} Determined by HPLC using a chiral column.

Under optimal conditions, a variety of α-amino acids were prepared that are recorded in **Table I**.

Both alkyl and benzylic-type α-amino acids were obtained with high enantiomeric excess in excellent overall yield except for (*S*)-4-bromophenylalanine (entry 11). A significant drop in enantiomeric excess was observed in the case of 4-bromophenylalanine synthesis. It appears that although the alkylation with 4-bromobenzylbromide proceeded highly stereoselectively as

with the reaction of other halides, a decrease in optical purity might occur in the conversion to the amino acid, and that this behavior is inherent in this substrate. Sterically hindered alkyl halide, *i*-propyl iodide, was also successfully employed, with L-valine being formed in 90% yield and 98% ee (entry 3).

Application of New Chiral Template for α,α-Disubstituted Amino Acid Synthesis

We next examined the construction of α,α-disubstituted α-amino acids, which have recently received a great deal of attention due to their importance in the field of peptide chemistry (*9*). When the first alkylation product **7a** was subjected to a second alkylation with methyl iodide, the precursor **9** of α-methylphenylalanine **10** was produced with high stereoselectivity (**Scheme 6**). The absolute configuration is obviously determined in the second alkylation step; thus, the methyl group will be introduced from the less hindered face opposite to the 2,6-dichlorophenyl group based on the discussion of stereocontrol, which is consistent with the fact that the final amino acid has an *R* configuration.
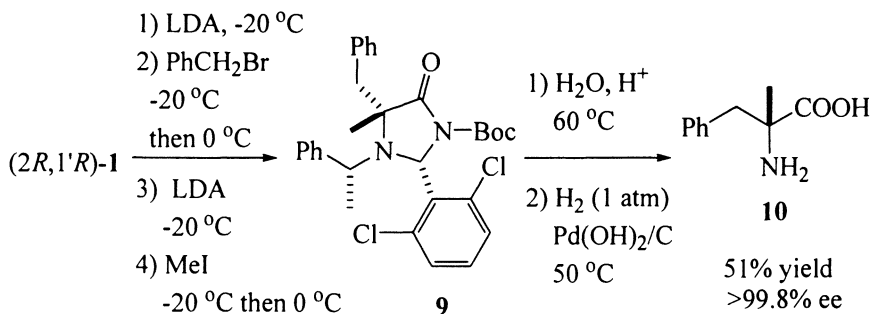
Scheme 6. Synthesis of α,α-disubstututed α-amino acid using (2R,1'R)-1

Practical Synthetic Method of Chiral Template 1

Encouraged by the potential (2*R*,1'*R*)-**1** (and (2*S*,1'*S*)-**1**) as a chiral glycine template, we then focused on the establishment of a more practical method to prepare (2*R*,1'*R*)-**1**, as our initial method relied on resolution of a 1:1 diastereomeric mixture by crystallization, with the chemical yield of the requisite diastereomer never exceeding 50%. We assumed that the intermediate **6** would be in equilibrium between diastereomers (2*S*,1'*R*) and (2*R*,1'*R*) in a presence of acid catalyst in solution phase. When diasteromers mixture of **6** was heated in *n*-

hexane/ethyl acetate containing a catalytic amount of trifluoroacetic acid (0.2 eq.) at 70 °C for 15 h, crystallization-induced dynamic resolution (CIDR) took place to afford (2R,1'R)-6 as a precipitate in >99% de and 84% yield based on total diastereomer amount of 6 charged (*Figure 4*). With this CIDR process, we were able to prepare 1kg of (2R,1'R)-1 and (2S,1'S)-1 in a laboratory flask without using column chromatography for purification in any steps.
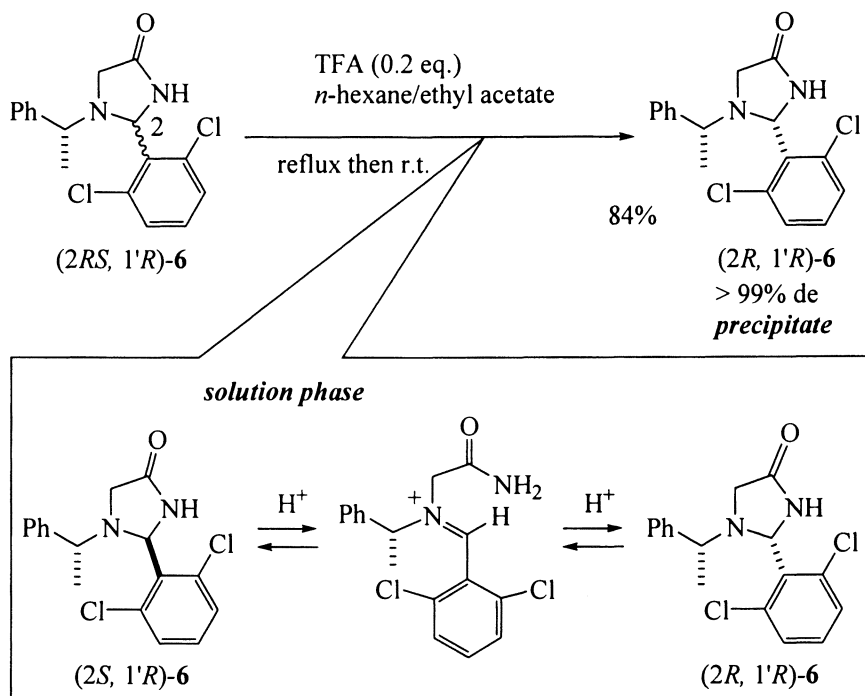

Figure 4. Practical preparation of (2R, 1'R)-6 by Crystalization- Induced Dynamic Resolution (CIDR)

Experimental Procedure for Preparation of 6

To a suspension of [(S)-(1-phenylethylamino)]acetamide (4) (618 g, 3.5 mol) in toluene (1400 mL) were added p-toluenesulfonic acid monohydrate (20 g, 0.1 mol) and 2,6-dichlorobenzaldehyde (5) (668 g, 3.8 mol) at 40 °C . The reaction mixture was refluxed for 24 h with azeotropic removal of the water formed. The reaction solution was concentrated under reduced pressure, and the resulting residue was allowed to recrystallize from n-hexane (466 mL)/ toluene

(180 mL) to yield a diastereomeric mixture (2*RS*,1'*S*)-**6** (1012 g, 87 % yield) as pale brown solid. A mixture of the above compound (1011 g, 3.0 mol) and trifluoroacetic acid (71 g, 0.6 mol) in ethyl acetate (900 mL)/ n-hexane (900 mL) was refluxed for 4 h, after which n-hexane (900 mL) was slowly added. The reaction mixture was further refluxed for 16 h, and then allowed to cool gradually to 29 °C over 50 h. The resultant precipitate was isolated by vacuum filtration, and dried to give 745 g of (2*S*,1'*S*)-**6** (74% yield, >99% de) as white crystals.

Summary

We have developed a novel chiral glycine template for synthesizing α-amino acid. The template undergoes highly stereoselective alkylation under mild conditions, leading to α-amino acid in an optically pure form. The synthetic process of the template, starting from commercially available inexpensive materials, involves crystalline-induced dynamic resolution (CIDR), which makes the methodology more practical and economical.

Reference

1. For reviews, see: (a) Moore, G. J. *Proceedings of the Western Phram. Soc.* **1997**, *40*, 115. (b) Ma, J. S. *Chimica Oggi* **2003**, *21*, 65. (c) Hodgson, D. R. W.; Sanderson, J. M. *Chem. Soc. Rev.* **2004**, *33*, 22.

2. For review, see: (a) Hruby, V. J.; Soloshonok, V. A. *Tetrahedron* **2001**, *57*, 6329. (b) Williams, R. M. *Synthesis of Optically Active α-Amino Acids* 1989, Pergmon Press. (c) Ogawa, J.; Ryono, A.; Xie, S.-X.; Vohra, R. M.; Indrati, R.; Miyakawa, H.; Ueno, T.; Ikenaka, Y.; Nanba, H.; Takahashi, S.; Shimizu, S. *Appl. Microbiol. Biotecnol.* **1999**, *52*, 797.

3. For reviews, see: (a) Ohkuma, T.; Noyori, R. *In Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; Wiley-VCH: Weinheim, 2000, pp 1. (b) Brown, J. M. *In Comprehnsive Asymmetric Catalysis Vol. 1*; Jacobsen, E. N., Pfaltz, A.; Yamamoto, H.,; Eds.Springer, Heidelberg, 2004, pp 121. (c) *The Handbook of Homogeneous Hydrogenation*; de Vries, J. G.; Elsevier, C. J., Eds.; Wiley-VCH: Weinheim, 2007. (d) Chi, Y.; Tang, W.; Zhang, X. *Modern Rhodium-Catalyzed Organic Reactions*; Evans, P. A.; Ed.; Wiley-VCH: Weinheim, 2005; pp 1. for recent examples, see: (e) Reetz, M. T.; Ma, J-A., Goddard, R. *Angew. Chem. Int. Ed.* **2005**, *44*, 412. (e) Giacmina, F.; Meetsma, A.; Panella, L.; Lefort, L.; de Vries, A. H. M. *Angew. Chem. Int. Ed.* **2007**, *46*, 1497. (f) Weis, M.; Waloch, C.; Seiche, W.; Breit, B. *J.*

Am. Chem. Soc. **2006**, *128*, 4188. (g) Chen, W.; Mbafor, W.; Roberts, S. M.; Whittall, J. *J. Am. Chem. Soc.* **2006**, *128*, 3922. (h) Zhang, W.; Zhang, X. *J. Org. Chem.* **2007**, *72*, 1010.

4. For reviews, see: (a) Maruoka, K.; Ooi, T. *Chem. Rev.* **2003**, *103*, 3013. (b) O'Donnell, M. J.; *Acc. Chem. Res.* **2004**, *37*, 506. (c) Lygo, B.; Andrews, B. I. *Acc. Chem. Res.* **2004**, *37*, 518. (d) Albanese, D. *Mini-Rev. Org. Chem.* **2006**, *3*, 195. (e) Vachon, J.; Lacour, J. *Chimica* **2006**, *60*, 266. for recent examples, see: (f) Ooi, T.; Kato, D.; Inamura, K.; Ohmatsu, K.; Maruoka, K. *Org. Lett.* **2007**, *9*, 3945. (e) Maruoka, K.; Ooi, T. *Chem. Commun.* **2007**, 1487. (f) Ooi, T.; Maruoka, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 4222. (g) Yong-Gang, W.; Maruoka, K. *Organic Process & Research Development* **2007**, *11*, 628. (h) Ooi, T.; Uematsu, Y.; Komeda, M.; Maruoka, K. *Tetrahedron* **2006**, *62*, 11425.

5. (a) Schöllkopf, U.; Hartwig, W.; Groth, U. *Angew. Chem. Int. Ed.* **1979**, *18*, 863. (b) Schöllkopf, U.; Hartwig, W.; Groth, U.; Westphalen, L. -O. *Liebigs. Ann. Chem.* **1981**, 696. (c) Leon-Romo, J. L.; Virues, C. I.; Avina, J.; Regla, I.; Juaristi, E. *Chirarity* **2002**, *14*, 144. (d) Schöllkopf, U. *Tetrahedron* **1983**, *39*, 2085. (e) Seebach, D.; Juaristi, E.; Miller, D. D.; Schickli, C.; Weber, T. *Helv. Chim. Acta* **1987**, *70*, 237. (f) Juaristi, E.; Leon-Romo, J. L.; Ramirez-Quiros, Y. *J. Org. Chem.* **1999**, *64*, 2914. (g) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547. (h) Dellaria, J. F.; Santarsiero, B. D. *J. Org. Chem.* **1989**, *54*, 3916. (i) Belokon, Y. N.; Sagyan, A. S. Diamgaryan, S. M.; Bakhmutov, V. I.; Belikov, V. M. *Tetrahedron* **1988**, *44*, 5507. (j) Orena, M.; Porzi, G.; Sandri, S. *J. Org. Chem.* **1992**, *57*, 6532. (k) Chinchilla, R.; Falvello, L. G.; Galindo, N.; Najera, C. *Angew. Chem. Int. Ed.* **1997**, *36*, 995. (l) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6767. (m) Ikegami, S.; Uchiyama, H.; Hayama, T.; Yamaguchi, M. *Tetrahedron* **1988**, *44*, 5333. (n) Myers, A. G.; Yang, B. H.; Chen, H, Gleason, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 9361. (o) Meyer, L.; Poirier, J. –M.; Duhamel, P.; Duhamel, L. *J. Org. Chem.* **1998**, *63*, 8094.

6. Soloshonok et al., have recently carried out extensive studies of the practical synthesis of α-amino acids using their unique modular nucleophilic glycine equivalents. (a) Ellis, T. K.; Ueki, H.; Yamada, T.; Ohfune, Y.; Soloshonok, V. A. *J. Org. Chem.* **2006**, *71*, 8572. (b) Soloshonok, V. A.; Yamada, T.; Ueki, H.; Moore, A. M.; Cook, T. K.; Arbogast, K. L.; Soloshonok, A. V.; Martin, C. H.; Ohfune, Y. *Tetrahedron* **2006**, *62*, 6412. (c) Soloshonok, V. A.; Cai, C.; Yamada, T.; Ueki, H.; Ohfune, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **2005**, *127*, 15296. (d) Taylor, S. M.; Yamada, T.; Ueki, H.; Soloshonok, V. A. *Tetrahedron Lett.* **2004**, *45*, 9159. (e) Soloshonok, V. A. *Current Organic Chemistry* **2002**, *6*, 341. and references are cited therein.

7. (a) Zhong, H. M.; Cohen, J. H.; Abdel-Magid, A.F.; Kenney, B. D.; Maryanoff, C. A.; Shah, R. D.; Villani Jr, F. J.; Zhang, F.; Zhang, X. *Tetrahedron Lett.* **1999**, *40*, 7721. (b) Buckle, D. R.; Rockell, C. J. M. *J. Chem. Soc. Perkin Trans. 1* **1982**, 627. (c) Kamijo, T.; Tamamoto, R.; Hirada, H.; Iizuka, K. *Chem Pharm. Bull.* **1983**, *31*, 1213. (d) Subramanyam, C. *Synth. Commun.* **1995**, *25*, 761.

8. Davies, S. G.; Haggitt, J. R.; Ichihara, O.; Kelly, R. J.; Leech, M. A.; Price Mortimer, A. J.; Robert, P. M.; Smith, A. D. *Org. Biomol. Chem.* **2004**, *2*, 2630. (b) Brehm, L.; Greenwood, J. R.; Hansen, K. B.; Nielsen, B.; Egebjerg, J.; Stensbol, T. B.; Brauner-Osborne, H.; Stok, F. A.; Kronborg, T. T. A.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2003**, *46*, 1350. (c) Brackenridge, I.; Davies, S. G.; Fenwick, D. R.; Ichihara, O.; Polywka, M. E. C. *Tetrahedron* **1999**, *55*, 533. (d) Brehm, L.; Greenwood, J. R.; Slok, F. A.; Holm, M. M.; Nielsen, B.; Geneser, U.; Stensbol, T. B.; Brauner-Osborne, H.; Begtrup, M.; Egebjerg, J.; Krogsgaard-Larsen, P. *Chirarity* **2004**, *16*, 452. (e) Sugiyama, S.; Morishita, K.; Chiba, M.; Ishii, K. *Heterocycles* **2002**, *57*, 637.

9. For examples: (a) Cronin, J.; Pizzareo, S. *Science* **1997**, *275*, 951. (b) Karle, I. L.; Balaram, P. *Biochemisrty* **1990**, *29*, 6747. (c) Karle, I. L.; Kaul, R.; Rao, R. B.; Raghothama, S.; Balaram, P. *J. Am. Chem. Soc.* **1997**, *119*, 12048. (d) Wolf, W. M.; Stasiak, M.; Leplawy, M. T.; Bianco, A.; Formaggio, F.; Cristina, M.; Toniolo, C. *J. Am. Chem. Soc.* **1998**, *120*, 11558. (e) Kochi, K. N.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2000**, *83*, 233. (f) Obrecht, D.; Abrecht, C.; Altorfer, M.; Bohdal,; U. Grieder, A.; Kleber, M.; Pfyffer, P.; Muller, K. *Hel. Chim. Acta* **1996**, *79*, 1315. (g) Mossel, E.; Formaggio, F.; Crisma, M.; Toniolo, C.; Broxterman, Q. B.; Boesten, W. H.; Kamphuis, J.; Quaedflieg, P. J. L. M.; Temussi, P. *Tetrahedron: Asymmetry* **1997**, *8*, 1305. (h) Horikawa, M.; Shigeri, Y.; Yumoto, S.; Yoshikawa, S.; Nakajima, T.; Ohfune, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2027. (i) Gibsen, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585.

Catalytic Approach

Chapter 7

Catalytic Enantioselective Synthesis of α,α-Disubstituted Amino Acids: The Strecker Reaction of Ketimines Using Chiral Poly-Gadolinium Complexes

Masakatsu Shibasaki and Motomu Kanai

Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan

Enantiomerically-enriched α,α-disubstituted amino acids are an important group of unnatural amino acids that are useful for pharmaceutical synthesis. The Strecker reaction of ketimines is a direct method for synthesizing α,α-disubstituted amino acids. This chapter summarizes the development, mechanistic insights, and application of the catalytic enantioselective Strecker reaction developed in our group.

Introduction

The catalytic enantioselective Strecker reaction is among the most straightforward methods for synthesizing enantiomerically enriched α-amino acids and their derivatives (*1*). More than a dozen examples have been reported using aldimines as substrates, which can produce α-monosubstituted amino acid derivatives (*1*). The catalytic enantioselective Strecker reaction of ketimines, however, is not as well established due to the lower reactivity and small difference (sterically and electronically) between the two substituents on the prochiral carbon of ketimines compared to aldimines. To achieve an efficient enantioselective Strecker reaction of ketimines, an asymmetric catalyst must be highly active and strictly enantioselective. The development of a chiral urea-catalyzed reaction by Jaconsen's laboratory in 2000 overcame this issue for the first time (*2,3*). In 2003 and 2004, we reported the most general and practical

catalytic enantioselective Strecker reaction of ketimines to date (*4*). In this chapter, we review the development and synthetic application of our reaction. Progress is actively ongoing in this field (*5*). The development of enantioselective catalysts that promote tetrasubstituted carbon-forming reactions is a new frontier in organic synthesis (*6*).

Development of Catalytic Enantioselective Strecker Reaction of Ketimines Using a Chiral Gadolinium Complex

In 2001, we developed a catalytic enantioselective cyanosilylation of ketones using a gadolinium (Gd) complex of D-glucose-derived ligand **1** (Scheme 1) (*7,8*). The optimized catalyst was produced from Gd(O^{i}Pr)$_3$ and **1** mixed in a 1:2 ratio. Based on the results of mechanistic studies, including labeling experiments and kinetic measurements, we proposed model **4** for the enantio-differentiating cyanation step: (1) the active catalyst is a TMS-containing 2:3 complex of Gd and **1**; (2) the active nucleophile is a gadolinium isocyanide generated through transmetalation from TMSCN; (3) the enantioselective cyanation proceeds through intramolecular transfer of cyanide to an activated ketone coordinating to the Lewis acidic Gd. The positions of both the activated nucleophile and the electrophile are determined by the asymmetric bimetallic complex, thereby affording high enantioselectivity.
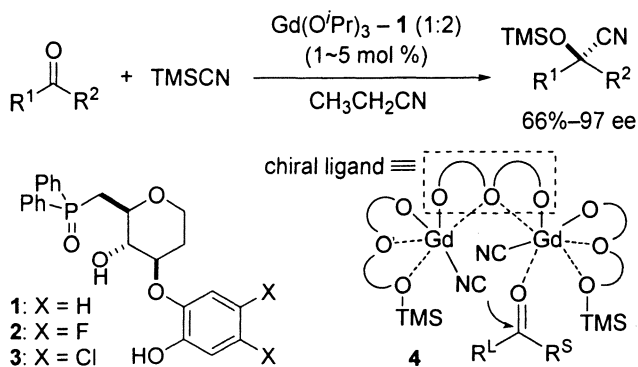

Scheme 1. Catalytic enantioselective cyanosilylation of ketones

We extended this catalysis to enantioselective Strecker reaction of ketimines. Initial optimization using acetophenone-derived ketimines allowed us to identify two important parameters for reaction efficiency: (1) *N*-phosphinoyl imines

produced high reactivity and enantioselectivity; (2) an electronically tuned catalyst generated from ligand **2** was more active and enantioselective than the catalyst derived from **1**. Thus, using 2.5 mol % catalyst, the product from acetophenone-derived ketimine was obtained in 94% yield with 95% ee (24 h) (*4a*). Heteroaromatic and aliphatic ketimines, however, afforded less satisfactory results.

Studies toward expanding substrate generality led us to identify beneficial effects of protic additives. Thus, using 1.5 equiv of TMSCN and 1 equiv of 2,6-dimethylphenol (conditions A), excellent enantioselectivity was produced from a wide range of ketimines, including aromatic, heteroaromatic, cyclic, and aliphatic ketimines (Table 1) (*4b*).

The protic additive significantly improved the catalyst activity as well as enantioselectivity. To gain insight into the additive effect, ESI-MS studies of the catalyst were performed in the presence of 2,6-dimethylphenol. A catalyst prepared from a 1:2 ratio of $Gd(O^iPr)_3$ and **2**, in the presence of TMSCN (15 equiv to the catalyst) in acetonitrile, mainly afforded a peak corresponding to the *O*-silylated 2:3 complex (Scheme 2, **5**). When 2,6-dimethylphenol (10 equiv) was added to the catalyst solution, the peak corresponding to **5** disappeared and a new peak corresponding to an *O*-protonated 2:3 complex **6** appeared (Scheme 2, eq. 1). Therefore, this protonated complex was a highly active enantioselective catalyst in this asymmetric Strecker reaction of ketimines.
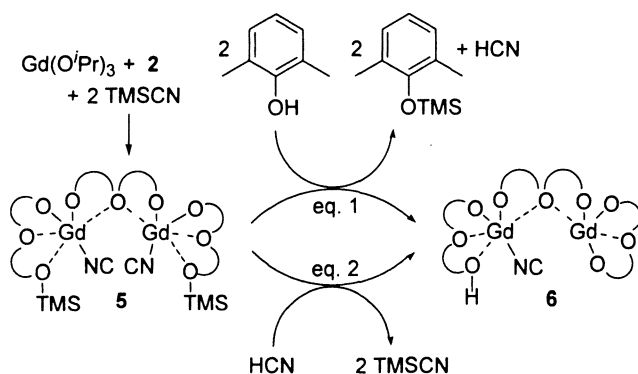
Scheme 2. Generation of proton-containing asymmetric catalyst

Based on this finding, we expected that the same active catalyst **6** would be generated through the reaction of HCN with **5** (Scheme 2, eq. 2). Protonolysis with HCN produces 2 equiv of TMSCN. Thus, only a catalytic amount of TMSCN is required if HCN is used as the proton source and stoichiometric

cyanide source. This would lead to a further advanced catalytic enantioselective Strecker reaction that might be applicable to industrial scale synthesis of α,α-disubstituted amino acids.
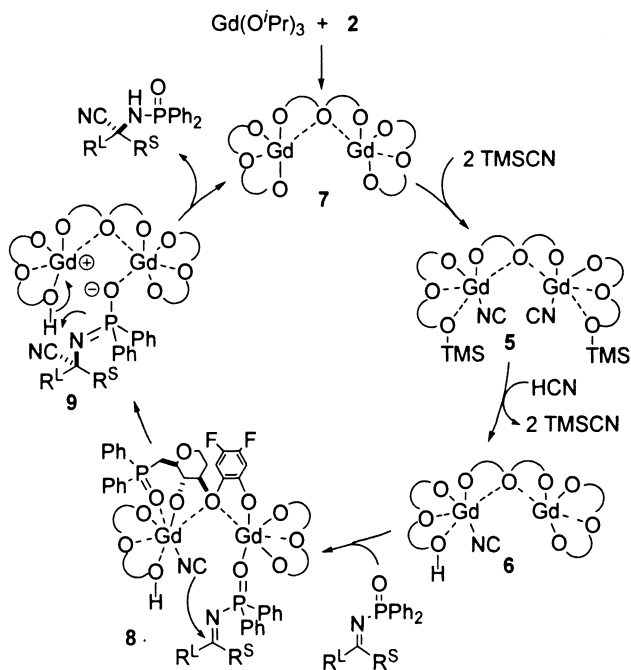
Table 1. Catalytic Enantioselective Strecker Reaction of Ketimines

entry	substrate	condition (cat. loading/mol%)	time (h)	yield (%)	ee (%)
1		A (1)	30	94	92
2		B (0.1)	19	97	90
3		A (1)	31	97	95
4		A (1)	21	93	93
5		B (1)	3	99	99
6		A (1)	22	92	92
7		A (1)	43	73	90
8		A (2.5)	2.5	91	80
9		A (1)	38	93	96

[a] Condition A: catalyst = Gd(O^{i}Pr)$_3$ (x mol %) + **2** (2x mol %); TMSCN (1.5 equiv), 2,6-dimethylphenol (1 equiv). Condition B: catalyst = Gd(O^{i}Pr)$_3$ (x mol %) + **2** (2x mol %); TMSCN (2–5 mol %), HCN (150 mol %).

As expected, the reaction proceeded smoothly in the presence of a catalytic amount of TMSCN and a stoichiometric amount of HCN (Table 1, condition B) (*4c*). The reaction time was significantly shortened compared to using 2,6-dimethylphenol as an additive (condition A). The higher reactivity allowed us to

reduce the catalyst amount to as low as 0.1 mol %, while maintaining excellent enantioselectivity. The difference in reactivity when using HCN or 2,6-dimethylphenol as the proton source might be partly due to the acidity of the additive. In the presence of HCN (pK_a = 9.2), the concentration of the active catalyst 6 should be higher than in the presence of 2,6-dimethylphenol (pK_a = 10).


Scheme 3. Catalytic cycle of enantioselective Strecker reaction of ketimines

We proposed the catalytic cycle under condition B as shown in Scheme 3. To the active catalyst 6, the substrate ketimine is incorporated and activated. An intramolecular cyanide transfer from the gadolinium isocyanide to the activated substrate defines the enantioselectivity (8), and the zwitter ionic intermediate 9 is generated. In this step, the characteristics of phosphinoyl ketimines—i.e., the cis/trans isomers equilibrate very rapidly at the reaction temperature (–40 °C) (*4a*)—should work advantageously in determining the reactive imine geometry. The intermediate 9 should collapse through intramolecular proton transfer, and the product was liberated with regeneration of gadolinium alkoxide complex 7.

From **7**, successive reactions with TMSCN and HCN reproduced the active catalyst **6**. The reaction did not proceed at all in the absence of a catalytic amount of TMSCN at –40 °C. This finding suggests that the active catalyst **6** is generated only through the silylated *pre*-catalyst **5**, and direct generation of **6** from the alkoxide complex **7** and HCN does not occur at –40 °C.

Synthetic Application

Due to the versatility of α,α-disubstituted amino acids in biologically active compounds, there are many potential synthetic targets for our reaction. We achieved catalytic asymmetric synthesis of sorbinil (**10**) (*4b*) and lactacystin (**11**) (*9*) using the catalytic enantioselective Strecker reaction of ketimines.
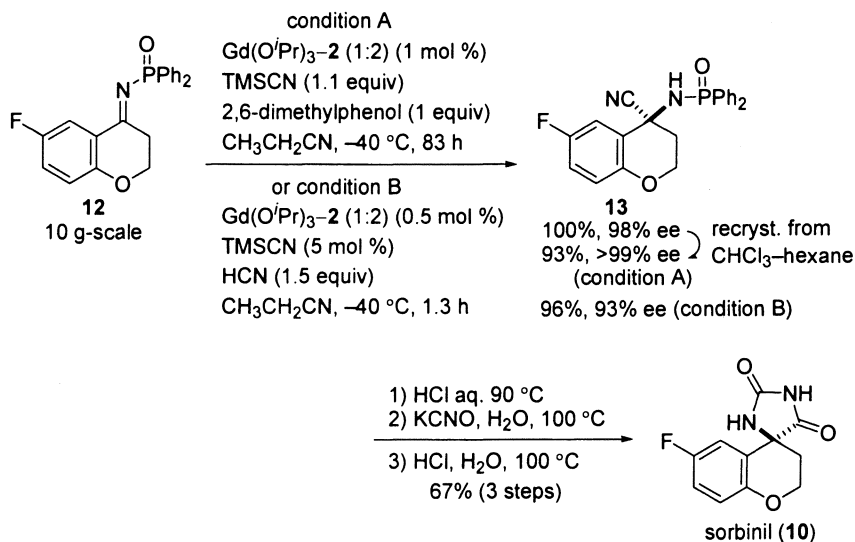
Scheme 4. Catalytic asymmetric synthesis of sorbinil

Sorbinil (**10**) is a potent inhibitor of aldose reductase developed by Pfizer, and could be a therapeutic agent for chronic complications of diabetes mellitus (such as neuropathy) (*10*). Sorbinil contains a chiral spirohydantoin structure, and its biologic activity resides in the (*S*)-enantiomer. A straightforward synthesis of sorbinil was achieved using the catalytic enantioselective Strecker reaction of ketimine **12** as a key step (Scheme 4). The reaction proceeded using 1 mol % of catalyst under condition A or 0.5 mol % of catalyst under condition

B, affording **13** with excellent enantioselectivity. Enantiomerically pure **13** was obtained by recrystallization of a crude mixture. The reaction was performed in 10 g-scale. Acid hydrolysis followed by hydantoin formation produced **10** in high overall yield. No silica gel chromatography was necessary from **12** to **10**.
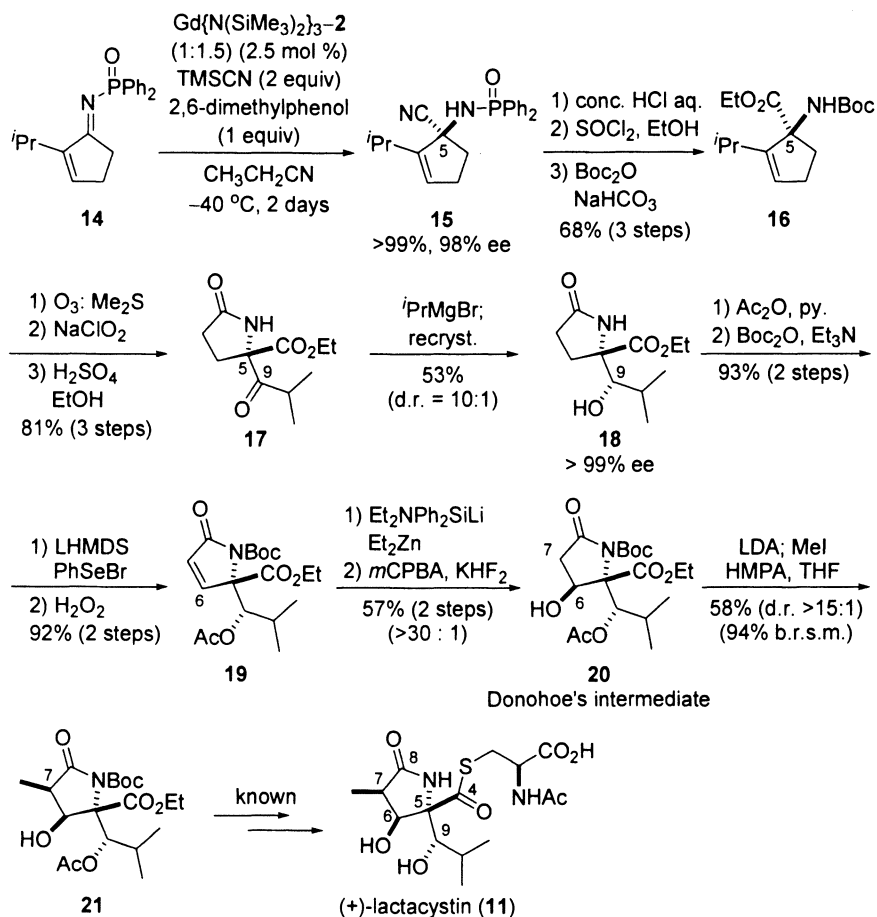
Scheme 5. Catalytic asymmetric total synthesis of lactacystin

(+)-Lactacystin (**11**) is a potent and selective proteasome inhibitor, isolated from the *Streptomyces* sp. by Omura et al (*11*). Due to its potent biological activity and complex chemical structure, many synthetic chemists study **11** as a synthetic target (*12*). We planned to utilize our catalytic enantioselective Strecker reaction for the construction of the tetrasubstituted carbon C-5 of

lactacystin. Based on this plan, α-hydroxy ketimines are an obvious starting point. This type of imine, however, is unstable and not isolable in a pure form. Thus, we utilized an enone-derived, stable **14** as a masked α-hydroxy ketimine (Scheme 5).

Imine **14**, containing a bulky isopropyl group at the α-position, was barely reactive under the Strecker reaction conditions, and optimization of the reaction conditions was necessary. It was found that the catalyst generated from Gd{N(SiMe$_3$)$_2$}$_3$ and **2** in a 2:3 ratio produced higher activity and enantioselectivity than the catalyst prepared from Gd(O^{i}Pr)$_3$. The modified catalyst preparation method afforded the active 2:3 complex in a pure form, which was detected by ESI-MS (*13*). The catalyst activity difference depending on the preparation methods was attributed to the purity of the active 2:3 complex. Thus, the Strecker reaction of **14** completed using 2.5 mol % catalyst in 2 days, and product **15** was obtained in quantitative yield with 98% ee.

Amidonitrile **15** was converted to the protected amino acid derivative **16**, which was further transformed to γ-lactam **17** through ozonolysis, oxidation, and cyclization. Stereoselective reduction of C-9 ketone using ^{i}PrMgBr (*14*) via a presumed cyclic transition state produced a diastereomixture of C-9 secondary alcohols with the desired α-alcohol **18** as the major isomer (d.r. = 10:1). Diastereomerically and enantiomerically pure **18** was obtained by recrystallization of the crude mixture from a toluene-hexane mixed solvent. After protection of the secondary alcohol and the lactam nitrogen atom with acetyl and Boc groups, respectively, selenenylation and oxidation produced the α,β-unsaturated lactam **19** in excellent yield. The β-hydroxyl group of C-6 was introduced via a stereoselective conjugate addition of the Et$_2$NPh$_2$Si group from the less hindered side of the enone, followed by Tamao-Fleming oxidation of the silicon with retension of configuration, producing enantiomerically pure known intermediate **20**. Methylation at C-7 of **20** under the conditions developed by Donohoe (*15*) produced the desired stereoisomer **21**, which was further converted to lactacystin following the procedures reported by Corey (*16*) and Donohoe.

Structural Studies of the Poly Gadolinium Asymmetric Catalysts

To elucidate the enantio-differentiation mechanism of the Gd catalyst, we studied crystallization of the catalytic species by varying the ligand structure and metal. Colorless, air-stable prisms were obtained from a propionitrile-hexane (2:1) solution of the complex prepared from a 2:3 ratio of Gd(O^{i}Pr)$_3$ and ligand **3** (crystal **A**: 80% yield). X-ray crystallographic analysis revealed that crystal **A**

was a 4:5 complex of Gd and **3** with a μ-oxo atom surrounded by four gadolinium atoms (Figure 1) (*17*). The tetranuclear structure was maintained in a solution state, and the sole peak observed by ESI-MS corresponded to crystal **A**.
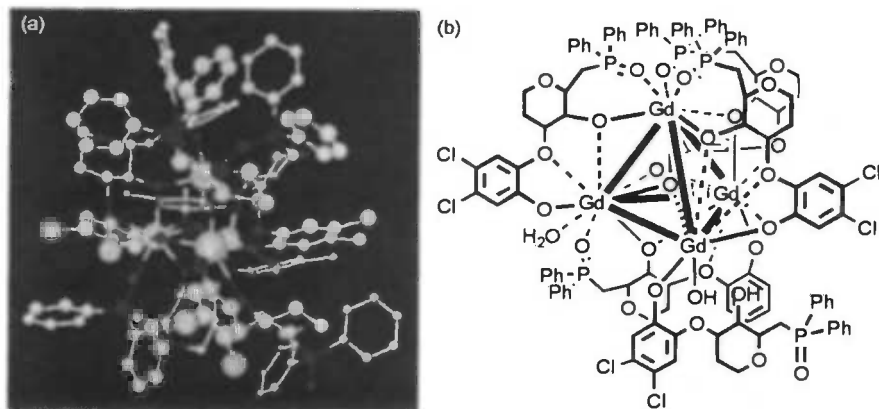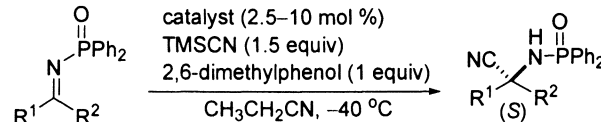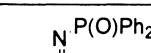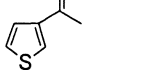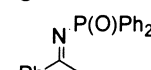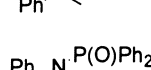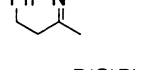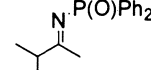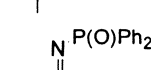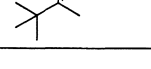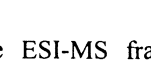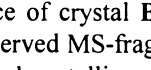

Figure 1. (a) Crystal structure of 4:5+oxo complex (crystal A) and (b) its chemical structure depiction.

Previously, the MS peak corresponding to crystal **A** was observed, concomitant with the 2:3 complex (active catalyst), in a solution prepared from a 1:2 ratio of Gd(O^{i}Pr)$_3$ and **1** or **2**. The high yield of crystal **A** suggested that there is a conversion process from the 2:3 complex to the 4:5+oxo complex (crystal **A**) during crystallization. Time-dependent conversion of the 2:3 complex to the 4:5+oxo complex in a solution state was observed by ESI-MS. This conversion was an auto-catalytic process. Thus, seeding the 4:5+oxo complex to a solution containing pure 2:3 complex (prepared from Gd{N(SiMe$_3$)$_2$}$_3$) markedly accelerated the formation of the 4:5+oxo complex. The 4:5+oxo complex is thermodynamically more stable than the active kinetically-formed catalyst, the 2:3 complex.

The function of crystal **A** as an enantioselective catalyst was evaluated by Strecker reaction of ketimines. To our surprise, enantioselectivity was completely reversed when crystal **A** was used as a catalyst, compared to the catalyst prepared *in situ* (Table 2) (*17*). This dramatic reversal in enantioselectivity was attributed to the change in the higher-order structure of the chiral polymetallic catalyst. The reaction rate was approximately 5~50 times slower than that using the catalyst prepared *in situ*. Due to the marked difference in catalyst activity, the major enantiomer obtained using the catalyst prepared from Gd(O^{i}Pr)$_3$ was (*S*), whereas the catalyst solution contained a mixture of the 2:3 complex and the 4:5+oxo complex.

Efforts to elucidate the structure of the active 2:3 complex continued. Colorless, air-stable prisms (crystal **B**) were obtained from a THF solution of La(O^{i}Pr)$_3$ and ligand **1** mixed in a 2:3 ratio (47% yield). The x-ray diffraction study revealed the structure to be a *pseudo* C$_2$-symmetric 6:8 complex of La and **1** [Figure 2(a)] (*17*). Six La atoms were arranged in line as a backbone, and eight chiral ligands were arranged around the backbone. This structure was maintained in solution, based on the fact that the parent peak corresponding to crystal **B** was observed by ESI-MS. In addition, two major fragment peaks corresponding to metal:ligand = 2:3 and 4:5+oxo complexes were observed.

Table 2. Dramatic Reversal of Enantioselectivity Depending on the Higher-Order Structure of the Polymetallic Catalyst

entry	substrate	catalyst (Gd mol %)	time/ h	yield/ %	ee/%	config.
1		Gd-3 (5)	0.5	99	98	(S)
2		crystal **A** (7)	2	99	91	(R)
3		Gd-3 (5)	0.5	99	88	(S)
4		crystal **A** (7)	1	93	96	(R)
5		Gd-3 (5)	2	99	87	(S)
6		crystal **A** (7)	2	95	87	(R)
7		Gd-2 (2.5)	2.5	91	80	(S)
8		crystal **A** (7)	12	99	82	(R)
9		Gd-3 (10)	2	92	74	(S)
10		crystal **A** (7)	14	99	98	(R)

The ESI-MS fragment patterns of crystal **B** provide insight into the relevance of crystal **B** to the optimized Gd catalyst (2:3 complex) in solution. The observed MS-fragments (2:3 and 4:5+oxo) can be viewed as subunits of the entire polymetallic assembly. The x-ray structure of crystal **B** supports this consideration: the distances between the second and the third La atoms from both ends of the 6:8 complex was significantly longer than those between the first and the second La atoms [Figure 2(b)]. In addition, the coordination number
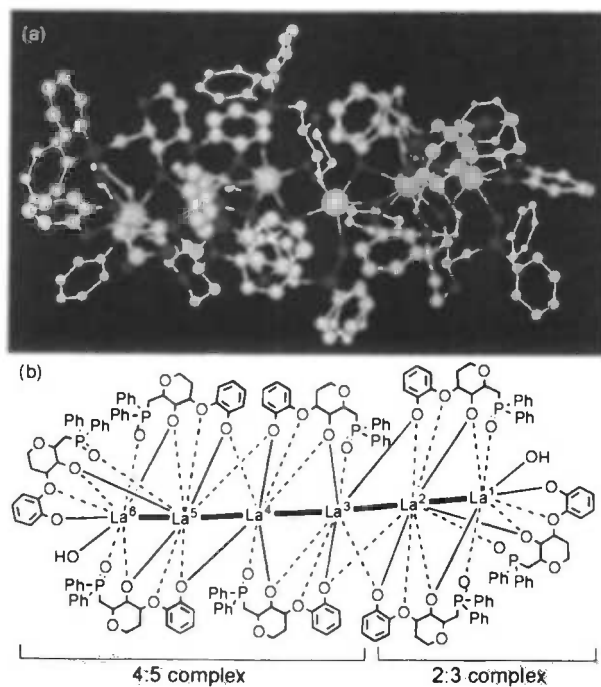
Figure 2. (a) Crystal structure of 6:8 complex (crystal B) and (b) its chemical depiction.

of the third La was smaller than those of the first and the second La, which might induce Lewis base coordination to the third La and dissociation of the terminal 2:3 subunits. Thus, the 6:8 complex (crystal **B**) is likely to be constructed via assembly of the 2:3 subunit and the 4:5+oxo subunit. The terminal 2:3 subunits of crystal **B** might correspond to the optimized active catalyst. This hypothesis is supported by the fact that crystal **B** (8 mol % La) promoted the catalytic enantioselective Strecker reaction of acetylthiophene-derived ketimine, giving the product with 26% ee. Configuration of the major product was the same (*S*) as when using a catalyst prepared *in situ*. Crystal **B** produced a similar level of enantioselectivity as when using a catalyst prepared *in situ* from La(O*i*Pr)₃ and **1** (16% ee). Therefore, crystal **B** represents one of the structures of the lanthanum catalyst in solution.

Although the three-dimensional structure of the actual catalyst (2:3 complex) has yet to be clarified, these results demonstrated that the higher-order structure of an artificial asymmetric polymetallic catalyst is a determining factor of the function (enantioselectivity and activity). This finding led to new chiral ligand design (**22**) (*18*). Whereas the Gd catalyst derived from **22** produced less

satisfactory results in the Strecker reaction of ketimines compared to **2**, it exhibited markedly higher catalyst activity and enantioselectivity in the asymmetric aziridine-opening reaction with TMSCN (Scheme 6). Despite the fact that ligands **2** and **22** contained the same chirality, enantiomer products were produced, which was totally unexpected. The enantio-switching was, however, attributed to the dramatic change in higher-order structure of the polymetallic complexes depending on the ligands. Studies are currently ongoing to identify novel functions of the catalyst derived from **22**.
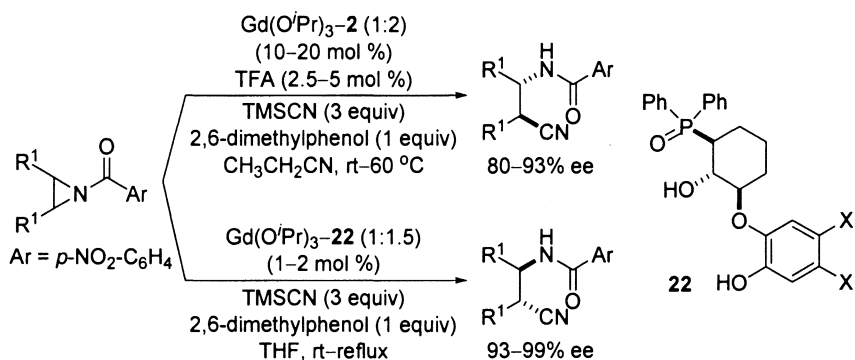
Scheme 6. Catalytic asymmetric aziridine-opening reaction with TMSCN: Ligand 22 designed from the higher-order catalyst structure consideration

Conclusion

In this review, we described the substrate scope, synthetic application, and mechanism of the catalytic enantioselective Strecker reaction of ketimines developed in our group. The catalyst contained higher-order structures constructed via modular self-assembly. The higher order structure was the determining factor of the asymmetric catalyst function. Our future research aims in this area include: (1) logical design of the assembly structure, and (2) identification of new biologic functions of chiral α,α-disubstituted amino acids in pharmaceutical design.

Acknowledgments

The research described in this review would not have been possible without the hard work of our collaborators, whose names are listed in the Reference section. We sincerely thank all of these people for their contribution.

References

1. For recent reviews, see: (a) Gröger, H. *Chem. Rev.* **2003**, *103*, 2795-2828. (b) Pino, C. *Angew. Chem. Int. Ed.* **2004**, *43*, 1764-1766. (c) Connon, S. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 1176-1178. (d) Shibasaki, M.; Kanai, M.; Mita, T. *Org. React.* In press.

2. Vachal, P.; Jacobsen, E. N. *Org. Lett.* **2000**, *2*, 867-870.

3. Vallée reproted the catalytic enantioselective Strecker reaction of ketimines in 2000. The reported example was, however, only acetophenone-derived ketimine. See: (a) Byrne, J. J.; Chavarot, M.; Chavant, P.; Vallée, Y. *Tetrahedron Lett.* **2000**, *41*, 873-876. (b) Chavarot, M.; Byrne, J. J.; Chavant, P. Y.; Y. Vallée *Tetrahedron Asymmetry* **2001**, *12*, 1147-1150.

4. (a) Masumoto, S.; Usuda, H.; Suzuki, M.; Kanai M.; Shibasaki, M. *J. Am. Chem. Soc.* **2003**, *125*, 5634-5635. (b) Kato, N.; Suzuki, M.; Kanai, M.; Shibasaki, M. *Tetrahedron Lett.* **2004**, *45*, 3147-3151. (c) Kato, N.; Suzuki, M.; Kanai, M.; Shibasaki, M. *Tetrahedron Lett.* **2004**, *45*, 3153-3155.

5. (a) Becker, C.; Hoben, C.; Kunz, H. *Adv. Synth. Catal.* **2007**, *349*, 417-424. (b) Blacker, J.; Clutterbuck, L. A.; Crampton, M. R.; Grosjean, C.; North, M. *Tetrahedron Asymmetry* **2006**, *17*, 1449-1456. (c) Rueping, M.; Sugiono, E.; Moreth, S. A. *Adv. Synth. Catal.* **2007**, *349*, 759-764. (d) Huang, J.; Liu, X.; Wen, Y.; Qin, B.; Feng, X. *J. Org. Chem.* **2007**, *72*, 204-208. (e) Huang, X.; Huang, J.; Wen, Y.; Feng, X. *Adv. Synth. Catal.* **2006**, *348*, 2579-2584. (f) Wang, J.; Hu, X.; Jiang, J.; Gou, S.; Huang, X.; Liu, X.; Feng, X. *Angew. Chem. Int. Ed.* **2007**, *46*, 8468-8470.

6. Riant, O.; Hannedouche, J. *Org. Biomol. Chem.* **2007**, *5*, 873-888.

7. Yabu, K.; Masumoto, S.; Yamasaki, S.; Hamashima, Y.; Kanai, M.; Du, W.; Curran, D. P.; Shibasaki, M. *J. Am. Chem. Soc.* **2001**, *123*, 9908-9909.

8. For reviews, see: (a) Shibasaki, M.; Kanai, M. *Org. Biomol. Chem.* **2007**, *5*, 2027-2039. (b) Shibasaki, M.; Kanai, M.; Matsunaga, S. *Aldrichimica Acta* **2006**, *39*, 31-39. (b) Kanai, M.; Kato, N.; Ichikawa, E.; Shibasaki, M. *Synlett* **2005**, 1491-1508.

9. Fukuda, N.; Sasaki, K.; Sastry, T. V. R. S.; Kanai, M.; Shibasaki, M. *J. Org. Chem.* **2006**, *71*, 1220-1225.

10. Sarges, R.; Howard, H. R.; Kelbaugh, P. R. *J. Org. Chem.* **1982**, *47*, 4081-4085.

11. Omura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113-116.

12. For a review, see: Shibasaki, M.; Kanai, M.; Fukuda, N. *Chem. Asian J.* **2007**, *2*, 20-38.

13. Suzuki, M.; Kato, N.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2005**, *7*, 2527-2530.

14. Kang S. H.; Jun, H.-S. *Chem. Commun.* **1998**, 1929-1930.

15. Donohoe, T. J.; Sintim, H. O.; Sisangia L.; Harling, D. J. *Angew. Chem. Int. Ed.* **2004**, *43*, 2293-2296.
16. Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677-10678.
17. Kato, N.; Mita, T.; Kanai, M.; Therrien, B.; Kawano, M.; Yamaguchi, K.; Danjo, H.; Sei, Y.; Sato, A.; Furusho, A.; Shibasaki, M. *J. Am. Chem. Soc.* **2006**, *128*, 6768-6769.
18. (a) Fujimori, I.; Mita, T.; Maki, K.; Shiro, M.; Sato, A.; Furusho, S.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2006**, *128*, 16438-16439. (b) Fujimori, I.; Mita, T.; Maki, K.; Shiro, M.; Sato, A.; Furusho, S.; Kanai, M.; Shibasaki, M. *Tetrahedron,* **2007**, *63*, 5820-5831.

Asymmetric Amino Acid Synthesis by the Asymmetric Alkylation of Glycine Derivatives with Chiral Spiro-Type and Simplified Phase-Transfer Catalysts

Keiji Maruoka

Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606–8502, Japan

This review illustrates our recent development on the design of various types of chiral phase transfer catalysts, which possess the high environmentally-benign property, and successfully applied them to practical asymmetric synthesis of useful amino acid derivatives.

1. Introduction

The chemical community has witnessed the exponential growth of phase transfer catalysis as a practical methodology for organic synthesis, featuring its simple experimental operations, mild reaction conditions, inexpensive and environmentally benign reagents and solvents, and possibility to conduct large-scale preparations (*1*). Nowadays, it appears to be a prime synthetic tool being appreciated in various fields of organic chemistry, and also find widespread industrial applications. On the other hand, the development of asymmetric phase transfer catalysis based on the use of structurally well-defined chiral, non-racemic catalysts has progressed rather slowly, despite its great importance to create a new domain in modern asymmetric catalysis by taking full advantage of structurally and stereochemically modifiable tetraalkylonium cations. However, recent enormous efforts toward this direction have resulted in notable achieve-

ments, making it feasible to perform various bond formation reactions under mild phase transfer-catalyzed conditions (2). Now, asymmetric phase transfer catalysis is certainly one of the hottest research area in asymmetric organocatalysis. This review illustrates our recent development on the design of various types of chiral phase transfer catalysts, which possess the high environmentally-benign property, and applied them to practical asymmetric synthesis of useful amino acid derivatives. For other synthetic applications of chiral phase transfer catalysts, the reader should consult the excellent published reviews of asymmetric phase-transfer catalysis (2).
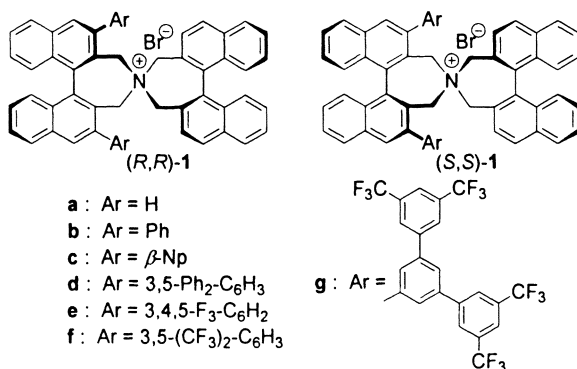
2. Design of Spiro-Type Chiral Phase Transfer Catalysts
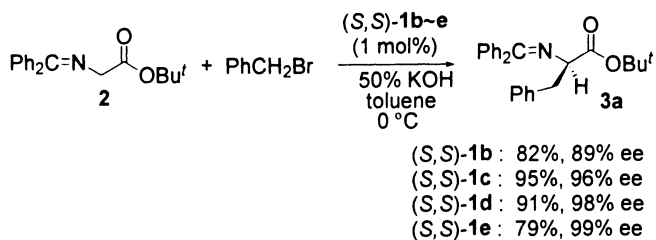
2-1. Asymmetric Synthesis of α-Alkyl-α-Amino Acids

Since the initial work of O'Donnell *et al.* in 1989 (3), asymmetric synthesis of α-alkyl-α-amino acids by asymmetric phase transfer alkylation of a prochiral protected glycine derivative using a chiral catalyst has become an attractive method for the preparation of both natural and unnatural amino acids (4). However, when we started asymmetric phase transfer chemistry in 1998, almost all the elaborated chiral phase transfer catalysts had been restricted to *cinchona* alkaloid derivatives, which unfortunately constituted a major difficulty in rationally designing and fine-tuning of catalysts to attain sufficient reactivity and selectivity. In this context, the structurally rigid, chiral spiro ammonium salts of type 1 derived from commercially available (*S*)- or (*R*)-1,1'-bi-2-naphthol have been designed as a new C_2-symmetric chiral phase transfer catalyst (Scheme 1) and successfully applied to the highly efficient, catalytic asymmetric synthesis of various α-alkyl-α-amino acids under mild phase transfer conditions (5).

Attempted benzylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester 2 with 1 mol% of symmetric (*S,S*)-1a in 50% aqueous NaOH–benzene (volume ratio = 1:3) at room temperature afforded the corresponding benzylation product 3a in 76% yield with 73% ee. Introduction of aromatic substituents (Ar) on the 3,3'-position of one binaphthyl subunit of the catalyst afforded a beneficial effect on the enantiofacial discrimination, and the similar reaction in toluene under the influence of (*S,S*)-1b gave the product 3a in 82% yield with 89% ee (Scheme 2). Switching the catalyst to (*S,S*)-1c and sterically more hindered (*S,S*)-1d further increased the enantioselectivity to 96% ee and 98% ee, respectively, and virtually complete stereochemical control was achieved using (*S,S*)-1e as catalyst (6,7). The lower chemical yield (79%) with (*S,S*)-1e was ascribed to the intervention of enolate oxidation by aerobic oxygen and was improved to 90% by simply performing the reaction under argon atmosphere. In the case of a reactive alkyl halide, the catalyst loading can be reduced to 0.2 mol% without loss of enantiomeric excess (7).
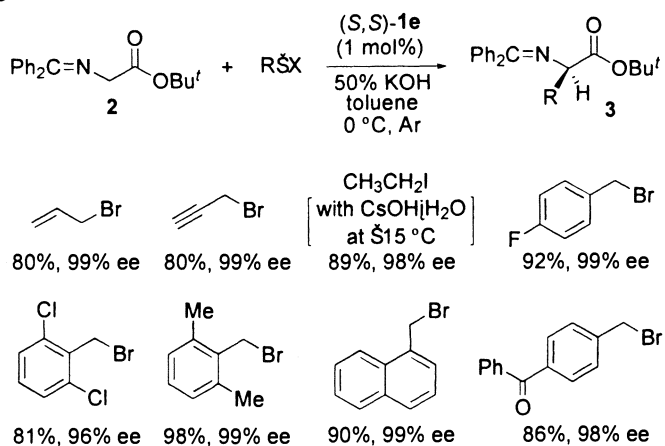
118

Scheme 1

(R,R)-1   (S,S)-1

a : Ar = H
b : Ar = Ph
c : Ar = β-Np
d : Ar = 3,5-Ph$_2$-C$_6$H$_3$
e : Ar = 3,4,5-F$_3$-C$_6$H$_2$
f : Ar = 3,5-(CF$_3$)$_2$-C$_6$H$_3$

g : Ar =

Scheme 2

Ph$_2$C=N—CH$_2$—C(=O)OBu^t + PhCH$_2$Br

(S,S)-1b~e
(1 mol%)
————————
50% KOH
toluene
0 °C

Ph$_2$C=N—C(=O)OBu^t ... Ph ... H   3a

(S,S)-1b : 82%, 89% ee
(S,S)-1c : 95%, 96% ee
(S,S)-1d : 91%, 98% ee
(S,S)-1e : 79%, 99% ee

Scheme 3

Ph$_2$C=N—CH$_2$—C(=O)OBu^t + RŠX

(S,S)-1e
(1 mol%)
————————
50% KOH
toluene
0 °C, Ar

Ph$_2$C=N—C(=O)OBu^t ... R ... H   3

80%, 99% ee   80%, 99% ee

CH$_3$CH$_2$I
[with CsOH¡H$_2$O]
at Š15 °C
89%, 98% ee

92%, 99% ee

81%, 96% ee   98%, 99% ee   90%, 99% ee   86%, 98% ee

(*S,S*)-**1e** is the catalyst of choice for the preparation of a variety of essentially enantiopure α-alkyl-α-amino acids by this transformation (Scheme 3). Facile asymmetric synthesis of α-alkyl-α-amino acids, which is usually inaccessible by enzymatic processes, becomes feasible by employing appropriate electrophiles such as *ortho*-disubstituted benzyl bromides. In the reaction with the simple alkyl halides such as ethyl iodide, use of aqueous cesium hydroxide (CsOH) as a basic phase at lower reaction temperature is generally recommended (*7*). Since both enantiomers of the catalyst of type **1** can be readily available from either (*R*)- or (*S*)-1,1'-bi-2-naphthol, a wide variety of natural and unnatural α-alkyl-α-amino acids can be synthesized in an enantiomerically pure form by the catalytic phase transfer alkylation of **2**.

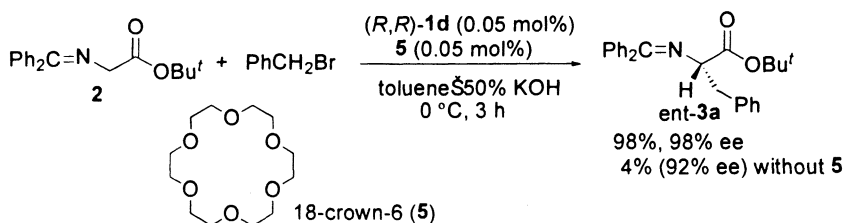The synthetic utility of chiral phase transfer catalysis of **1** was highlighted by the facile synthesis of L-Dopa ester **4** (R^2 = OH) and its analogue (Scheme 4), which have usually been prepared by either asymmetric hydrogenation of eneamides or enzymatic processes and tested as potential drugs for the treatment of Parkinson's disease. The successful asymmetric synthesis of natural tyrosine *tert*-butyl ester **4** (R^2 = H) in a similar manner strongly implies the feasibility of highly enantioselective synthesis of various L-Dopa analogues (*8*).

Scheme 4
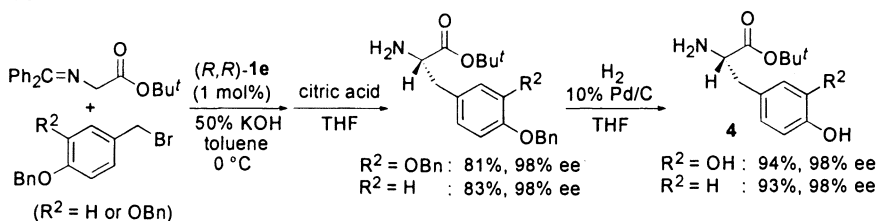
In order to fully induce the potential catalytic activity of *N*-spiro chiral ammonium salt such as **1d**, we have developed binary phase transfer catalysis using an appropriate achiral co-catalyst. For instance, the phase transfer-catalyzed alkylation of **2** with benzyl bromide under the influence of (*R,R*)-**1d** (0.05 mol%) turned out to be sluggish to give **3a** in only 4% yield (92% ee), while benzylation of **2** in the presence of 18-crown-6 (**5**) (0.05 mol%) under similar conditions proceeded smoothly to furnish **3a** in 98% yield with 98% ee (Scheme 5) (*9*). The origin of this dramatic rate enhancement would be the ability of the crown ether to extract KOH into toluene phase, thereby accelerating otherwise slow deprotonation process. Interestingly, achiral tetrabutyl- and tetraoctylammonium bromides are also employable for this purpose. Unfortunately, however, the origin of the acceleration effect by adding achiral tetrabutyl- and tetraoctylammonium bromides remains unclear.

Scheme 5

Ph$_2$C=N~~~C(=O)OBu^t + PhCH$_2$Br $\xrightarrow[\substack{\text{toluene–50\% KOH} \\ 0\,^\circ\text{C, 3 h}}]{\substack{(R,R)\text{-}\mathbf{1d}\ (0.05\ \text{mol\%}) \\ \mathbf{5}\ (0.05\ \text{mol\%})}}$ Ph$_2$C=N–CH(Ph)–C(=O)OBu^t

2

18-crown-6 (5)

ent-3a

98%, 98% ee
4% (92% ee) without **5**

In the series of this work, introduction of 3,3'-diaryl substituents to the parent symmetrical ammonium bromide **1a** is found to be crucially important for obtaining high enantioselectivity. In this reagrd, we have been interested in the possibility of examining the effect of adjacent 4,4'-substituents of the catalyst rather than 3,3'-substituents in the asymmetric phase transfer alkylations (*10*). Interestingly, even 4,4'-diaryl substituents of the catalysts of type **6** (Scheme 6) exhibited unexpectedly high asymmetric induction on such asymmetric phase transfer alkylations. For example, asymmetric alkylation of **2** with benzyl bromide in toluene-50% aqueous KOH under the influence of 1 mol% of catalyst (*S,S*)-**6** gave rise to benzylation product **3a** in 90% yield with 91% ee. The observed enantioselectivity is rather surprising compared to that (89% ee) using 3,3'-diphenyl-substituted **1b** under similar reaction conditions. Sterically more hindered 4,4',6,6'-tetrakis(3,5-diphenylphenyl)binaphthyl analogue (*S,S*)-**6b** was also prepared and applied to the asymmetric alkylation of **2** to furnish the alkylation product **3** with slightly higher enantioselectivity (96% ee) and shorter reaction time (*cf.*, 98% ee in the asymmetric benzylation of **2** with **1d** under similar phase transfer conditions).
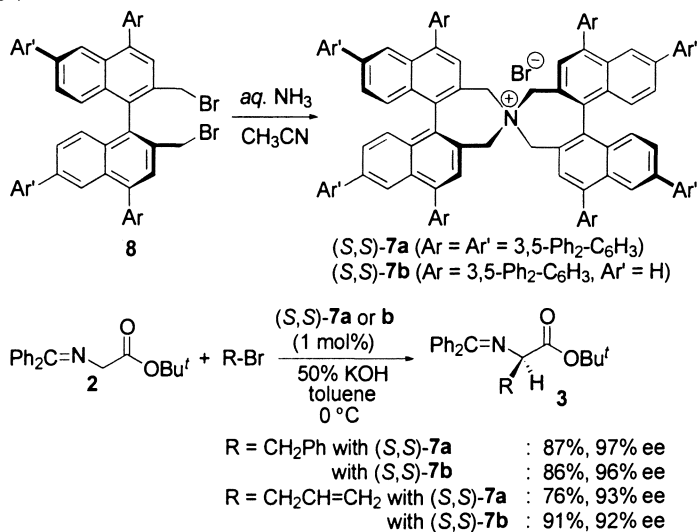
Scheme 6

(*S,S*)-**6a** (Ar = Ph)
(*S,S*)-**6b** (Ar = 3,5-Ph$_2$-C$_6$H$_3$)
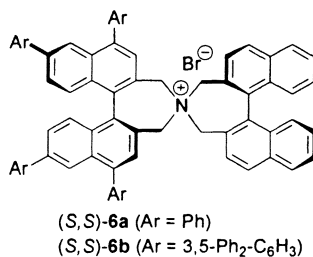
On the other hand, we were intrigued with the preparation of symmetrical *N*-spiro type catalyst to avoid the independent synthesis of two different binaphthyl-modified subunits required for **1**. Along this line, 4,4',6,6'-

tetraarylbinaphthyl-substituted ammonium bromide **7** was assembled through the reaction of aqueous ammonia with bis-bromide **8** on the basis of our study on the substituent effect of this type of salts. Evaluation of **7** as a chiral phase transfer catalyst in the alkylation of **2** uncovered its high catalytic and chiral efficiency (Scheme 7) (*11*).

Scheme 7

(*S,S*)-**7a** (Ar = Ar' = 3,5-Ph$_2$-C$_6$H$_3$)
(*S,S*)-**7b** (Ar = 3,5-Ph$_2$-C$_6$H$_3$, Ar' = H)

R = CH$_2$Ph with (*S,S*)-**7a** : 87%, 97% ee
          with (*S,S*)-**7b** : 86%, 96% ee
R = CH$_2$CH=CH$_2$ with (*S,S*)-**7a** : 76%, 93% ee
          with (*S,S*)-**7b** : 91%, 92% ee

2-2. Asymmetric Synthesis of α,α-Dialkyl-α-amino Acids

With this basic information at hand, our attention has been focused on the α,α-dialkyl-α-amino acid synthesis. We envisioned that two different side chains could be introduced directly to the aldimine Schiff base **9** derived from glycine in a highly enantioselective manner by appropriate chiral phase transfer catalysis (Scheme 8). This possibility of the one–pot asymmetric double alkylation has been realized by using C$_2$-symmetric chiral quaternary ammonium bromide (*S,S*)-**1** (*12*). Initial treatment of the toluene solution of **9** and (*S,S*)-**1c** (1 mol%) with allyl bromide and CsOH•H$_2$O and the subsequent reaction with benzyl bromide resulted in formation of the double alkylation product **10** in 61% yield with 87% ee after hydrolysis. It is of interest that the use of (*S,S*)-**1e** as catalyst under similar conditions enhanced both the chemical yield and the enantioselectivity to 80% and 98% ee, respectively (*12*). The distinct feature of this procedure is that it enables straightforward asymmetric synthesis of various α,α-dialkyl-α-amino acids including those otherwise inaccessible from the naturally occurring amino acids. Notably, in the double alkylation of **9** by the

addition of the halides in a reverse order, the absolute configuration of the product was confirmed to be opposite, indicating the intervention of the expected chiral ammonium enolate in the second alkylation stage (Scheme 8). This double alkylation procedure works well only for reactive alkyl halides.
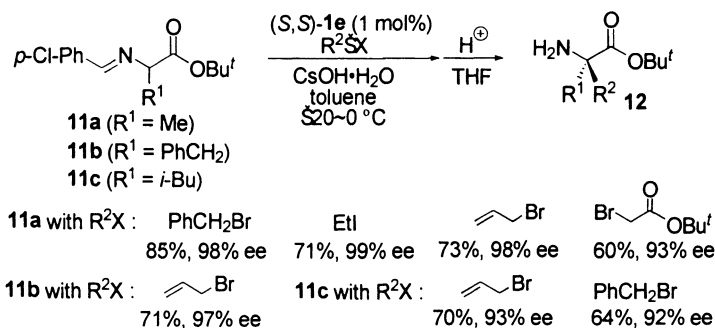
Scheme 8
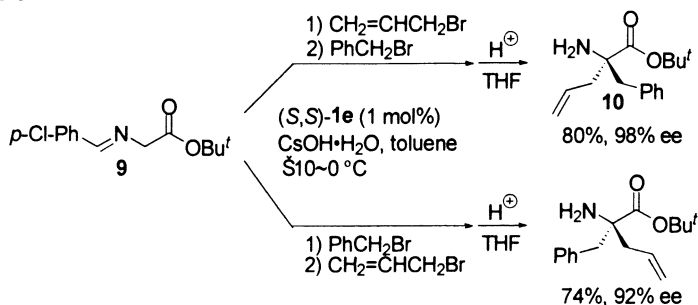
Since the stereochemistry of the newly created quaternary carbon center was apparently determined in the second alkylation process, the core of this method should be applicable to the asymmetric alkylation of aldimine Schiff base **11** derived from the corresponding α-amino acids. Indeed, rapid benzylation of *dl*-alanine–derived imine **11a** occurred in toluene with benzyl bromide and CsOH•H$_2$O using (*S*,*S*)-**1e** (1 mol%) as a catalyst, giving the alkylation product **12** (R^1 = Me, R^2 = CH$_2$Ph; 85%) in an almost enantiomerically pure form (98% ee). Other selected results illustrated in Scheme 9 demonstrate the remarkable efficiency and generality of this method (*12*). Use of *tert*-butyl α-bromoacetate as an alkylating agent allows facile enantioselective access to α-methyl aspartic acid and asymmetric synthesis of α-methyl tryptophan, an important amino acid for the design of dipeptoid with high affinity for the central cholecystokinin receptor, can also be realized. In addition, the phase transfer catalytic alkylation of aldimine Schiff base derived from other α-alkyl-α-amino acids such as *dl*-phenylalanine (**11b**) and *dl*-leucine (**11c**) also appeared to be feasible with high efficiency, providing the desired non-coded amino acid esters **12** with excellent asymmetric induction (Scheme 9).

2-3. Asymmetric Synthesis of β-Hydroxy-α-amino Acids

Although phase transfer catalytic enantioselective direct aldol reactions of glycine donor with aldehyde acceptors could provide an ideal method for the simultaneous construction of the primary structure and stereochemical integrity of β-hydroxy-α-amino acids, extremely important chiral units,

Scheme 9

11a with R²X :	PhCH₂Br	EtI	⟋⟋∼Br	Br∼C(O)OBuᵗ
	85%, 98% ee	71%, 99% ee	73%, 98% ee	60%, 93% ee

11b with R²X : ⟋⟋∼Br   **11c** with R²X : ⟋⟋∼Br   PhCH₂Br
                71%, 97% ee   70%, 93% ee   64%, 92% ee

especially from the pharmaceutical viewpoint, the examples reported to date are very limited. In this context, we were able successfully to realize an efficient, highly enantioselective direct aldol reaction of glycine Schiff base with aldehydes under phase transfer conditions using C_2-symmetric chiral quaternary ammonium salt **1**. Treatment of **2** with 3-phenylpropanal in toluene–1% NaOH aqueous solution in the presence of (*R,R*)-**1f** (2 mol%) and subsequent hydrolysis with 1 N HCl in THF resulted in the formation of the corresponding β-hydroxy-α-amino ester **13** in 76% isolated yield with the *anti/syn* ratio of 77:23, and the enantiomeric excess of the major *anti* isomer was determined to be 91% ee. Interestingly, use of (*R,R*)-**1g** possessing 3,5-bis[3,5-bis(trifluoromethyl)phenyl]phenyl substituent as a catalyst enhanced both diastereo- and enantioselectivities (*anti/syn* = 92:8, 96% ee for *anti* isomer) (Scheme 10) (*13*).

The initially developed reaction conditions using 2 equiv of aqueous base (1% NaOH *aq*) exhibited inexplicably limited general applicability in terms of aldehyde acceptors. For example, reaction of glycine derivative **2** with 4-

Scheme 10

(*R,R*)-**1f** : 76% (*anti/syn* = 77:23), 91% ee (*anti* isomer)
(*R,R*)-**1g** : 71% (*anti/syn* = 92:8), 96% ee (*anti* isomer)
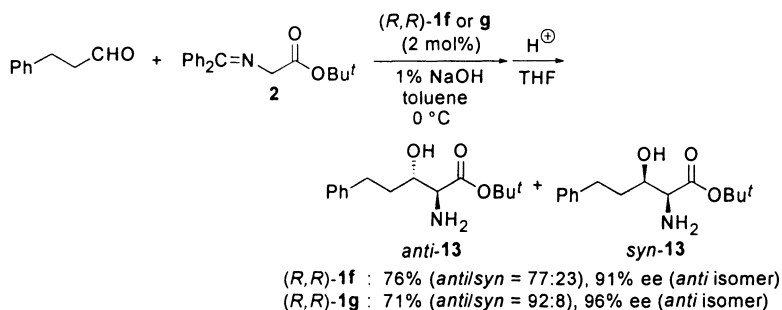
benzyloxybutanal gave the aldol product with low diastereoselectivity (*anti/syn* = 58:42; 82% ee for *anti* isomer). The mechanistic investigation revealed the intervention of an unfavorable yet inevitable retro aldol process involving chiral catalyst **1**. Based on this information, a reliable procedure has been established by use of the catalyst **1g** (2 mol%) with a catalytic amount of 1% NaOH (15 mol%) and ammonium chloride (10 mol%), which tolerates a wide range of aldehydes to afford the corresponding *anti*-β–hydroxy-α-amino esters almost exclusively in an essentially optically pure form (Scheme 11) (*14*).

Scheme 11

98% ee	97% ee	98% ee	99% ee
(82%, *anti/syn* = 96:4)	(80%, *anti/syn* = 94:6)	(73%, *anti/syn* = >96:4)	(54%, *anti/syn* = >94:6)
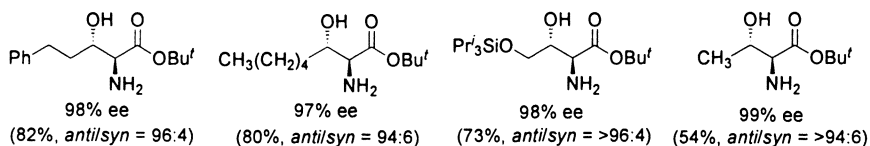
2-4. Asymmetric Conjugate Addition of Nitroalkanes

Asymmetric conjugate addition of α-anions of nitroalkanes to α,β-unsaturated esters is not an easy task. Accordingly, we developed the diastereo- and enantioselective conjugate addition of nitroalkanes to alkylidenemalonates **14** under mild phase transfer conditions by using chiral quaternary ammonium bromide **1g** as an efficient catalyst (Scheme 12) (*15*). This new protocol offers a practical entry to the facile synthesis of optically active γ-amino acid derivatives such as (*R*)-Baclofen and (*R*)-Rolipram.

3. Design of Simplified, Yet Very Efficient Chiral Phase Transfer Catalysts

Our further efforts toward the design of very efficient, chiral phase transfer catalyst have led to the discovery that chiral quaternary ammonium bromide **15** possessing flexible straight-chain alkyl groups instead of a rigid binaphthyl moiety functions as an efficient chiral phase-transfer catalyst. Most notably, the asymmetric alkylation of **2** with various alkyl halides proceeded smoothly under mild phase-transfer conditions in the presence of only 0.01–0.05 mol% of **15** to afford the corresponding alkylation products with excellent enantioselectivities (Scheme 13) (*16*).

Various alkyl halides are employable for the practical synthesis of α-alkyl- and α,α-dialkyl-α-amino acids (Scheme 14).
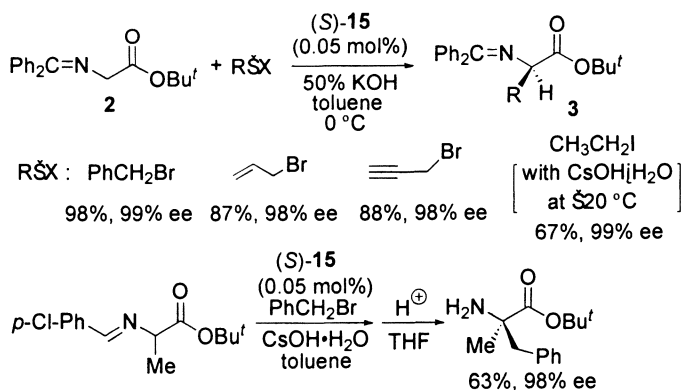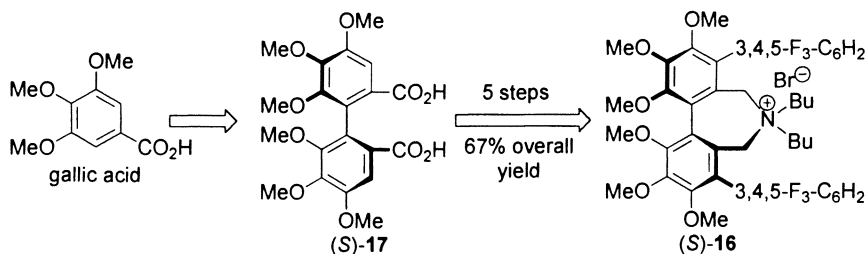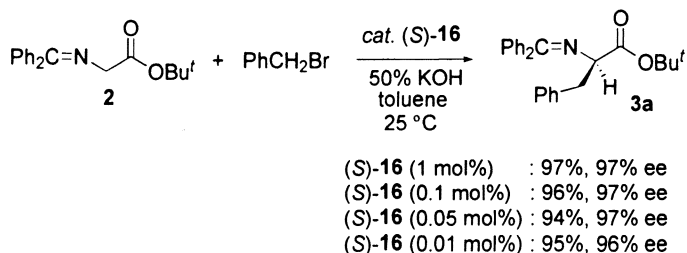
Scheme 12

$$\text{NO}_2 + \text{Ph} \overset{\text{CO}_2\text{Pr}^i}{\underset{\text{CO}_2\text{Pr}^i}{=}} \quad \xrightarrow[\substack{\text{Cs}_2\text{CO}_3 \\ \text{toluene} \\ 0\,°C}]{(S,S)\text{-}\mathbf{1g} \\ (1\,\text{mol\%})}$$

14

99% (*anti/syn* = 86:14)
97% ee (*anti* isomer)

γ-amino acids

(*R*)-Baclofen

MeO (*R*)-Rolipram

Scheme 13

$$\text{Ph}_2\text{C}=\text{N} \overset{O}{\underset{}{}}\text{OBu}^t + \text{PhCH}_2\text{Br} \xrightarrow[\substack{50\%\ \text{KOH} \\ \text{toluene} \\ 0\,°C}]{cat.\ (S)\text{-}\mathbf{15}}$$

2

3,4,5-F$_3$-C$_6$H$_2$

Br$^{\ominus}$
N$^{\oplus}$ Bu
Bu

3,4,5-F$_3$-C$_6$H$_2$

(S)-**15**

Ph$_2$C=N, OBu^t, Ph, H, **3a**

(S)-**15** (1 mol%)   : 99%, >99% ee
(S)-**15** (0.1 mol%) : 99%, 99% ee
(S)-**15** (0.05 mol%) : 98%, 99% ee
(S)-**15** (0.01 mol%) : 92%, 98% ee

Scheme 14

$$\text{Ph}_2\text{C}=\text{N} \overset{O}{\underset{}{}}\text{OBu}^t + \text{R}\check{\text{S}}\text{X} \xrightarrow[\substack{50\%\ \text{KOH} \\ \text{toluene} \\ 0\,°C}]{(S)\text{-}\mathbf{15} \\ (0.05\,\text{mol\%})}$$

2

Ph$_2$C=N, OBu^t, R, H, **3**

R$\check{\text{S}}$X : PhCH$_2$Br   Br   Br

98%, 99% ee   87%, 98% ee   88%, 98% ee

CH$_3$CH$_2$I
[with CsOH¡H$_2$O]
at Š20 °C
67%, 99% ee

$$p\text{-Cl-Ph} \overset{}{\underset{\text{Me}}{\diagup}}\text{N} \overset{O}{\underset{}{}}\text{OBu}^t \xrightarrow[\substack{\text{CsOH·H}_2\text{O} \\ \text{toluene}}]{(S)\text{-}\mathbf{15} \\ (0.05\,\text{mol\%}) \\ \text{PhCH}_2\text{Br}} \xrightarrow{\text{H}^{\oplus}} \text{THF}$$

H$_2$N, OBu^t, Me, Ph

63%, 98% ee

In designing practical phase-transfer catalysts, the ready availability of starting chiral sources is crucial. Accordingly, chiral phase-transfer catalyst **16** was conveniently prepared from the known, readily available (S)-4,5,6,4',5',6'-hexamethoxybiphenyldicarboxylic acid (**17**) derived from gallic acid. This catalyst (S)-**16** exhibited the high catalytic performance (0.01~1 mol%) in the asymmetric alkylation of 2 compared to the existing chiral phase-transfer

gallic acid → (S)-**17** → 5 steps, 67% overall yield → (S)-**16**

catalysts, thereby providing a general and useful procedure for highly practical enantioselective synthesis of structurally diverse natural and unnatural α-alkyl-α-amino acids (Scheme 15) (*17*).

Scheme 15

$$Ph_2C=N\text{—}CH_2\text{—}CO\text{—}OBu^t \quad \textbf{2} \quad + \quad PhCH_2Br \xrightarrow[\substack{50\% \text{ KOH} \\ \text{toluene} \\ 25\,^\circ\text{C}}]{cat.\ (S)\text{-}\textbf{16}} \quad Ph_2C=N\text{—}C(Ph)(H)\text{—}CO\text{—}OBu^t \quad \textbf{3a}$$

(S)-**16** (1 mol%)   : 97%, 97% ee
(S)-**16** (0.1 mol%) : 96%, 97% ee
(S)-**16** (0.05 mol%) : 94%, 97% ee
(S)-**16** (0.01 mol%) : 95%, 96% ee

Conclusions

This review overviews our recent development on the practical asymmetric synthesis of various useful organic molecules, particularly α-amino acids by designing several chiral phase transfer catalysts. Such achievements certainly provide valuable tools for the production of a wide variety of pharmaceutical intermediates. We believe that continuous efforts should be devoted for the rational design of various chiral organocatalysts including chiral phase transfer catalysts and their applications to synthetically useful transformations, which

would make great contributions to establish genuinely sustainable chemical processes within the context of forthcoming paradigm shift in worldwide production of highly valuable pharmaceutical substances in this century.

References

1. a) Dehmlow, E. V.;. Dehmlow, S. S. *Phase Transfer Catalysis*, 3rd ed.; VCH: Weinheim, 1993; b) Starks, C. M.; Liotta, C. L.; Halpern, M. *Phase–Transfer Catalysis*; Chapman & Hall: New York, 1994; c) Sasson, Y.; Neumann, R. Eds. *Handbook of Phase–Transfer Catalysis*; Blackie Academic & Professional: London, 1997; d) Halpern, M. E. Ed. *Phase–Transfer Catalysis*; ACS Symposium Series 659; American Chemical Society: Washington, DC, 1997.
2. a) O'Donnell, M. J. *Aldrichimica Acta* **2001**, *34*, 3; b) Maruoka, K.; Ooi, T. *Chem. Rev.* **2003**, *103*, 3013; c) O'Donnell, M. J. *Acc. Chem. Res.* **2004**, *37*, 506; d) Lygo, B.; Andrews, B. I. *Acc. Chem. Res.* **2004**, *37*, 518; e) Ooi, T.; Maruoka, K. *Angew. Chem. Int. Ed.*, **2007**, 46, 4222; f) Ooi, T.; Maruoka, K. *Aldrichimica Acta* **2007**, *40*, 77; g) Hashimoto, T.; Maruoka, K. *Chem. Rev.* **2007**, *107*, 5656.
3. O'Donnell, M. J.; Bennett D. W.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353.
4. Maruoka, K.; Ooi, T.; Kano, T. *Chem. Commun.* **2007**, 1487.
5. Maruoka, K. *J. Fluorine Chem.* **2001**, *112*, 95.
6. Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **1999**, *121*, 6519.
7. Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **2003**, *125*, 5139.
8. Ooi, T.; Kameda, M.;Tannai, H.; Maruoka, K. *Tetrahedron Lett.* **2000**, *41*, 8339.
9. Shirakawa, S.; Yamamoto, K.; Kitamura, M.; Ooi T.; Maruoka, K. *Angew. Chem. Int. Ed.* **2005**, *44*, 625.
10. Hashimoto, T.; Maruoka, K. *Tetrahedron Lett.* **2003**, *44*, 3313.
11. Hashimoto, T.; Tanaka Y.; Maruoka, K. *Tetrahedron: Asymmetry* **2003**, *14*, 1599.
12. Ooi, T.; Takeuchi, M.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **2000**, *122*, 5228.
13. Ooi, T.; Taniguchi, M.; Kameda, M.; Maruoka, K. *Angew. Chem. Int. Ed.*, **2002**, *41*, 4542.
14. Ooi, T.; Kameda, M.; Taniguchi, M.; Maruoka, K. *J. Am. Chem. Soc.* **2004**, *126*, 9685.
15. Ooi, T.; Fujioka, S.; Maruoka, K. *J. Am. Chem. Soc.* **2004**, *126*, 11790.
16. Kitamura, M.; Shirakawa, S.; Maruoka, K. *Angew. Chem. Int. Ed.* **2005**, *44*, 1549.
17. Han, Z.; Yamaguchi, Y.; Kitamura, M.; Maruoka, K. *Tetrahedron Lett.* **2005**, *46*, 8555.

Chapter 9

Catalytic Asymmetric Synthesis of α-Amino Acid Derivatives: Approaches toward Green Sustainable Methodology for Preparation of Optically Active Amino Acids

Yasuhiro Yamashita and Shū Kobayashi[*]

Department of Chemistry, School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 110–0033, Japan

Catalytic asymmetric synthesis of chiral α-amino acids using more environmental benign and green sustainable methods was described. Diastereo- and enantioselective Mannich-type reactions of α-hydrazono ester with silicon enolates for the synthesis of amino acid derivatives in aqueous media were achieved with a zinc fluoride–chiral diamine complex. The reaction successfully proceeded not only in an organic solvent/water-mixed system, but also in only pure water. Catalytic asymmetric 1,4-addition reactions and [3+2] cycloaddition reactions of glycine Schiff bases using chiral calcium complexes as catalysts were also developed. Calcium alkoxide-chiral bisoxazoline complexes worked well as chiral Brønsted bases to provide desired products in high enantioselectivities. Both concepts, asymmetric reactions using water as a solvent and using less harmful and ubiquitous metals as catalysts, are very important in design of new green chemical processes.

Introduction

Development of efficient methods to supply chiral amino acids, especially unnatural ones, is a valuable and much interested topic in synthetic organic chemistry, since chiral amino acids are not only basic components of proteins but also one of the most important tools or intermediates in biological and medicinal chemistry. Recently, some synthetically useful protocols based on highly enantioselective reactions have been disclosed using several chiral metal catalysts. On the other hand, development of environmentally benign chemical methods is urgently required in laboratories as well as industrial process at the conjuncture of pollution to earth environment (earth warming, ozone hole, etc.) mainly caused by human activity. A solution to those problems in synthetic chemistry is reducing or replacement of harmful organic materials to environmentally benign ones by technological revolution. For example, switch of organic solvents to water is quite attractive and promising because water is cheap, safe and no forming of CO_2 during its deactivating process. Alternatively, use of less harmful and ubiquitous metals instead of precious and harmful metals is a current important topic. In this chapter, we describe catalytic asymmetric synthesis of chiral α-amino acids using more environmental benign and green sustainable methods.

1. Catalytic asymmetric additions to α-iminoesters using chiral Zn catalysts in aqueous media.

Organic reactions in aqueous media have attracted a great deal of attention (1-4). Water is no doubt cheap, safe, and environmentally friendly when compared with organic solvents, and unique reactivity and selectivity, which cannot be obtained in organic solvents, are often observed in aqueous reactions. Moreover, from a synthetic viewpoint, these reactions have several advantages compared with reactions under anhydrous conditions, which are required in many conventional synthetic procedures. For examples, while it is necessary to dry solvents and substrates vigorously before use for many reactions in dry organic solvents, such drying is unnecessary for reactions in aqueous media.

Asymmetric Mannich-type reactions for the synthesis of amino acid derivatives have been studied in recent years, and high enantioselectivities have been achieved in organic solvents (5-9), however it has been difficult to realize catalytic asymmetric Mannich-type reactions in aqueous media. To examine the catalytic asymmetric Mannich-type reactions in aqueous media, N-acylhydrazone was chosen as an imine equivalent. N-acylhydrazones are readily prepared from aldehydes and N-acylhydrazines, and often isolated as much more stable crystals than the corresponding imines. It should be noted that hydrazines

such as the products of the Mannich reaction or allylation are interesting compounds, not only because hydrazines themselves can be used as unique building blocks, but also because N-N bond cleavage would lead to amine products (10) Furthermore, *N*-acylhydrazones have been successfully used in Sc(OTf)$_3$-catalyzed allylation in aqueous THF (11-13), indicating that they can be regarded as imine surrogates stable even in aqueous media. Here development of catalytic asymmetric Mannich-type reaction using chiral ZnF$_2$ catalysts in aqueous media is described (Scheme 1) (14-16).
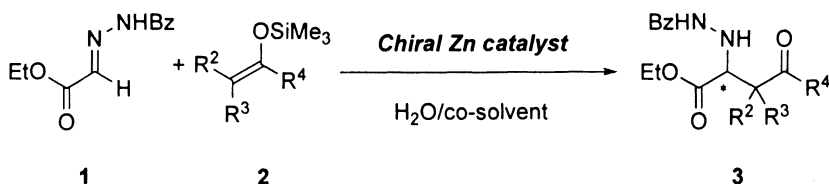
Scheme 1. Asymmetric Mannich Reaction of N-Acylhydrazone 1 in aqueous media

An important feature in design of a chiral ligand for Lewis acid-mediated asymmetric reactions in aqueous media is its binding affinity to metal cations. The strong binding ability of ethylenediamine to Zn^{2+} cation is noted (17), and various chiral analogues of ethylenediamine for catalytic asymmetric Mannich-type reactions were tested in aqueous media. After many trials, it was found that the combination of chiral diamine 4a (18) and Zn(OTf)$_2$ gave a low but significant ee (24%) in the reaction of α-hydrazono ester 1 with silyl enol ether 2a in H$_2$O/THF (1/9) (Table 1, entry 1). α-Hydrazono ester 1 and silicon enolate 2a were soluble in the aqueous THF media. The effect of the counteranions of Zn^{2+} was then examined, but typical Zn Lewis acids were not effective (Zn(ClO$_4$)$_2$, ZnBr$_2$ or ZnCl$_2$). However, it was remarkable to find that high enantioselectivity was obtained when ZnF$_2$ was used (entry 2) (19). Interestingly, a stoichiometric amount of ZnF$_2$ led to a low yield due to rapid hydrolysis of 2a, high enantioselectivity was maintained in spite of using a large excess of ZnF$_2$ compared with the chiral diamine (entry 3). The main reason of this low yield is hydrolysis of silicon enolate 2a in the current reaction system. It was exciting to find that 1 mol % of TfOH significantly suppressed the hydrolysis of 2a, increasing the yield dramatically (entry 3 vs 4). The same level of enantioselectivity was obtained when the amount of ZnF$_2$ was lowered to 50 mol % (entry 5), although the yield was decreased when the amount of ZnF$_2$ was reduced further (entry 6). Effective additives other than TfOH in the reaction of 1 with 2a were searched in aqueous THF. While several acids

showed no marked effect, it was interesting to find that the reactions using metal triflates as additives gave better yields than the reaction without any additives (entries 9-11 vs entry 3). Especially, alkali metal triflates gave higher yields than TfOH, and the best yield was obtained when NaOTf was employed (entry 9). These results indicate that TfOH acts not as a protic acid but as a triflate anion source. It is likely that òne fluoride anion of ZnF$_2$ is replaced with a triflate anion, and that ZnF(OTf) is generated in the reaction system. It is noted that ZnF(OTf) must be more Lewis acidic than ZnF$_2$.

Table 1. Optimization of Reaction Conditions in the Asymmetric Mannich-type Reaction
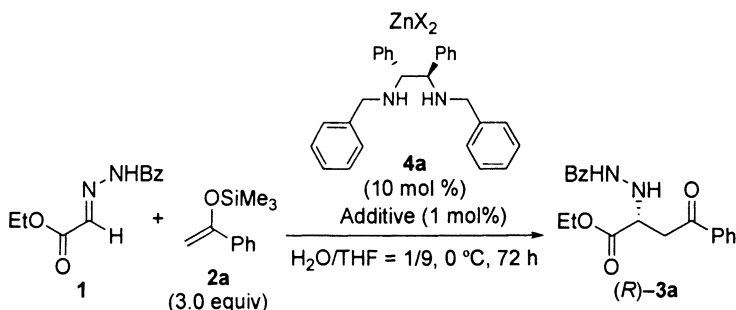
Entry	ZnX_2 (mol%)	Time (h)	Additive	Yield (%)	ee (%)
1[a]	Zn(OTf)$_2$ (10)	72	–	21	24
2[a]	ZnF$_2$ (10)	72	–	7	86
3	ZnF$_2$ (100)	72	–	19	90
4	ZnF$_2$ (100)	72	TfOH	93	92
5	ZnF$_2$ (50)	72	TfOH	89	92
6	ZnF$_2$ (30)	72	TfOH	34	89
7[b]	ZnF$_2$ (100)	72	TfOH	34	–
8	ZnF$_2$ (100)	12	TfOH	57	90
9	ZnF$_2$ (100)	12	LiOTf	69	90
10	ZnF$_2$ (100)	12	NaOTf	73	92
11	ZnF$_2$ (100)	12	KOTf	68	91

[a] 1.5 equiv of Si enolate and 12 mol% of chiral diamine were used. [b] The chiral diamine was not uesd.

In addition, chiral diamines also influenced on the rate of the Mannich-type reaction. Indeed, various chiral diamines derived from (1*R*,2*R*)-1,2-diphenylethylenediamine were tested in the reaction of **1** with **2a** (1.5 equiv) under the conditions shown in Table 2, it was found that diamine **4c** having

ortho MeO groups on its aromatic rings afforded much higher yield than **4a** (entry 3). Although the corresponding ortho tolyl ligand **4b** gave a comparable yield to **4a** (entry 2), **4c** gave higher enantioselectivity than **4a**. Moreover, it was found that the use of **4e** resulted in high yield and ee (entry 5), whereas **4f** gave comparable yield and ee to **4a** (entry 6). The above results demonstrate that the MeO group at the 2-position of the aromatic ring of chiral diamine ligands plays an essential role in attaining high yield and selectivity (20) When **4i** was employed, Mannich adduct (*R*)-**3a** was obtained in satisfactory yield with the highest enantioselectivity among the diamines examined in Table 2 (entry 9). Furthermore, it is noteworthy that the reaction using **4i** proceeded in high yield even in the absence of NaOTf (entry 10).

Table 2. Effect of Ligands

$$\text{1} + \underset{(1.5\ \text{equiv})}{\text{2a}} \xrightarrow[\text{H}_2\text{O/THF} = 1/9,\ 0\ ^\circ\text{C},\ 6\ \text{h}]{\substack{\text{4 (10 mol \%)}\\ \text{ZnF}_2\ (50\ \text{mol \%})\\ \text{NaOTf}\ (0.5\ \text{mol \%})}} (R)\text{-3a}$$
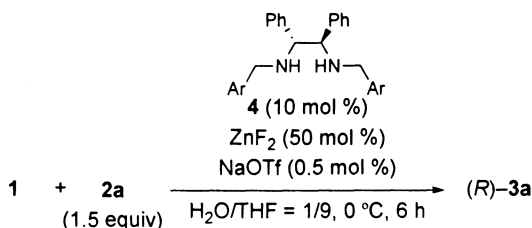
Entry	Ar (diamine)	Yield (%)	ee (%)
1	Ph (**4a**)	25	86
2	2-Me-C_6H_4 (**4b**)	23	57
3	2-MeO-C_6H_4 (**4c**)	83	93
4	2-EtO-C_6H_4 (**4d**)	35	94
5	2,5-$(MeO)_2$-C_6H_3 (**4e**)	84	93
6	3,5-$(MeO)_2$-C_6H_3 (**4f**)	19	86
7	3-MeO-2-naphthyl (**4g**)	43	91
8	2-MeO-4-^{t}Bu-C_6H_3 (**4h**)	53	92
9	2-MeO-5-^{t}Bu-C_6H_3 (**4i**)	72	96
10[a]	2-MeO-5-^{t}Bu-C_6H_3 (**4i**)	81	96

[a] Without NaOTf.

One of the interesting features of the present Mannich-type reactions is that high enantioselectivity can be obtained in spite of using a large excess of ZnF_2 with respect to the chiral diamine. Thus, reduced amounts of diamine **4i** in the reaction of **1** with **2a** (3.0 equiv) using ZnF_2 (100 mol %) were investigated. It was found that **4i** could be reduced to only 2 mol % without significant loss of the enantioselectivity (87% yield, 94% ee (20 h)).

The reaction systems were applied to diastereoselective reactions (Table 3). The silicon enolate **2b** derived from propiophenone reacted with **1** to afford **3b** in high yield with high diastereo- and enantioselectivities even in the absence of NaOTf (entries 1 and 2). On the other hand, the reaction of **1** with the (*Z*)-**2c** (**2c-Z**) using **4c** afforded the corresponding adduct with high *syn* selectivity and good ee (entry 3). In this reaction, the addition of NaOTf was essential for attaining high yield (entry 4). More over, it was surprise to find that the *anti* adduct was obtained with good diastereoselectivity by the reaction with the (*E*)-ketene silyl acetal (**2c-E**) under the same conditions as those of entry 3 (entry 5). The reactions using the (*E*)- and (*Z*)-silyl enol ethers derived from 3-pentanone (**2d-E** and **2d-Z**) were also examined. When **2d-E** was employed, the *anti* adduct was obtained selectively with high diastereo- and enantioselectivities (entry 6). In contrast, **2d-Z** was found to afford good *syn* selectivity (entry 7). Good stereospecificity was observed in the reactions using both **2c** and **2d**. It is noted that such stereospecificities are rare in catalytic asymmetric Mannich-type reactions (21), and that both *syn*- and *anti*-adducts can be easily prepared by simply changing the geometry of the silicon enolates. Furthermore, **2c** and **2d** proved to afford the opposite results with regard to the relationships between the geometry of the enolates and the relative configurations of the products. This interesting phenomenon is supposed to be due to the different steric influences of the Et and S^{t}Bu groups.

One of problems in the present reactions is that more than 50 mol % of ZnF_2 is required to obtain high yields. The catalytic use of fluoride anion seemed to be most challenging due to the great strength of the silicon-fluorine bond (592 kJ mol^{-1}) of Me_3SiF, which was generated by the activation of the silicon enolate with the fluoride anion of ZnF_2. However, the problem was overcome by using the highly active diamine **4i** and **4c**. It was exiting to find that, when 20 mol % of ZnF_2 was used in the reaction of **1** with **2a** using diamine **4i**, comparable yield and enantioselectivity with those using 100 mol % of ZnF_2 were obtained (Table 4, entry 2 vs 1). The use of **1a** instead of **4i** resulted in very low yield due to the rapid hydrolysis of **2a** (entry 3). Furthermore, when NaOTf (1 mol %) was added in this reaction, the yield was still low despite the fact that large amounts of silicon enolate **2a** remained after the reaction (entry 4). These results clearly indicate that the use of **4i** permitted the catalytic turnover of the fluoride anion. In addition, it was found that 20 mol % of ZnF_2 was sufficient to obtain high yield and stereoselectivity in the reaction with **2b** using diamine **4c** (entry 6). Therefore, diamines having ortho methoxy groups were effective not only for the acceleration of the reaction but also for the reduction of the ZnF_2 loading. It is noteworthy that the use of these diamines overcame the problems of the reaction using **4a**. On the other hand, it was revealed that NaOTf suppressed the hydrolysis of silicon enolates in the case using diamine **4i** as well as in the case using **4a**, and that the reaction

Table 3. Substrate scope of the reactions in aqueous media

Entry	Enolate	Time (h)	Product	Yield (%)	syn/anti	ee (%, syn/anti)
1	OSiMe₃ / Ph (2b, 1.5 equiv)	16	3b	87	95/5	92[a]
2[b]	2b, 1.5 equiv	20	3b	89	91/9	94[a]
3	OSiMe₃ / S^{t}Bu (2c-Z^c)	8	3c	80	97/3	87/27[d]
4[b]	2c-Z^c	8	3c	32	97/3	86/24[d]
5	OSiMe₃ / S^{t}Bu (2c-E^e)	20	3c	22	27/73	65/85[d]
6	OSiMe₃ / Et (2d-E^f)	72	3d	98	13/87	88/91
7	OSiMe₃ / Et (2d-Z^g)	72	3d	65	77/23	86/81

[a] Ee of syn adduct. [b] Without NaOTf. [c] $E/Z = 3/97$. [d] $(2R,3R)/(2R,3S)$. [e] $E/Z = 98/2$. [f] $E/Z = 76/24$. [g] $E/Z = 1/99$.

proceeded smoothly with a reduced amount of a silicon enolate without significant decrease in the yield. In fact, when 1.5 equiv of **2a** were employed, satisfactory results were obtained (entry 5).

A catalytic cycle of this Mannich-type reaction is proposed as depicted in Scheme 2. This reaction is likely to proceed with double activation where Zn^{2+} acts as a Lewis acid to activate **1** and at the same time, fluoride anion acts as a Lewis base to attack the silicon atom of the silicon enolate (working model **B**) (22-28). In other words, zinc amide **C** and Me_3SiF are formed first, and subsequent hydrolysis of **c** affords the Mannich adduct and ZnF(OH)–diamine

Table 4. The amount of ZnF$_2$

$$\text{EtO}\underset{O}{\overset{N^{\nearrow NHBz}}{\bigg|}}\text{H} \quad + \quad R\overset{OSiMe_3}{\underset{Ph}{=}} \xrightarrow[\substack{H_2O/THF = 1/9 \\ 0\,°C, 20\,h}]{\substack{ZnF_2 \\ \text{diamine 4 (10 mol \%)}}} \quad \text{EtO}\underset{O}{\overset{BzHN^{\searrow NH}}{\bigg|}}\underset{R}{\overset{O}{\bigg|}}Ph$$

	1		**2** (3.0 equiv)				(R)–**3a** or **3b**
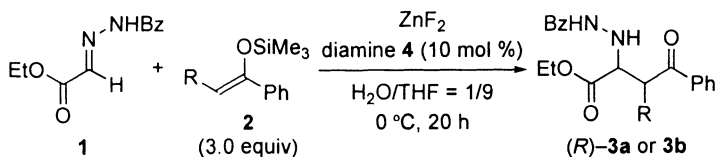
Entry	Enolate	ZnF$_2$ (mol %)	Diamine	Yield (%)	syn/anti	ee (%)
1	**2a**	100	**4i**	94	–	96
2	**2a**	20	**4i**	94	–	96
3	**2a**	20	**4a**	13	–	87
4[a]	**2a**	20	**4a**	25	–	79
5[b,c]	**2a**	20	**4i**	82	–	96
6	**2b**	20	**4c**	95	94/6	94[d]

[a] NaOTf (1 mol %) was added. Time = 72 h. [b] Enolate: 1.5 equiv. [c] NaOTf (4 mol %) was added. [d] Ee of *syn* adduct.

complex **D**. It is considered that a catalytic amount of the fluoride anion gives a high yield in this reaction probably because the catalytic turnover of the fluoride anion occurs. This apparently means that **D** reacts with Me$_3$SiF, regenerating ZnF$_2$–diamine complex a for the next catalytic cycle (fluoride-catalyzed mechanism) (29-31). Water may facilitate the dissociation of the fluoride anion from Zn^{2+}, thereby enhancing the catalytic activity of the chiral ZnF$_2$ complex.

The structure of the chiral catalyst is interesting point in this unique catalysis. Although the crystals of ZnF$_2$–**4c** complex suitable for XRD analysis were not prepared due to the low solubility of ZnF$_2$, a complex suitable for X-ray analysis was obtained from ZnCl$_2$ and **4c** (Figure 1). In this X-ray structure, the complex exhibits tetrahedral geometry, and the asymmetric information of the two asymmetric carbons on the ligand backbone is transferred to the two benzyl moieties on the nitrogen atoms upon coordination to the metal, which would play a key role for high stereoselectivity in catalysis. In addition, interactions between the MeO groups of **4c** and Zn^{2+} were not observed, a finding contrary to our initial expectation. Instead, the MeO groups proved to be located near the amino groups, suggesting hydrogen bonding between the MeO oxygen and the amino proton (N(1)...O(1) = 3.028 Å; N(2)...O(2) = 3.141 Å). On the basis of this X-ray crystal structure and the results that **4a** and **4c** gave similar enantioselectivities in Table 2, it is implied that the MeO groups of the diamine do not coordinate to Zn^{2+} even in the transition states in the asymmetric reactions.

The origin of this acceleration in the reactions using the diamine **4c** or **4i** was assumed as follows: The intramolecular hydrogen bonding effect (*vide*

Scheme 2. Proposed Catalytic Cycle
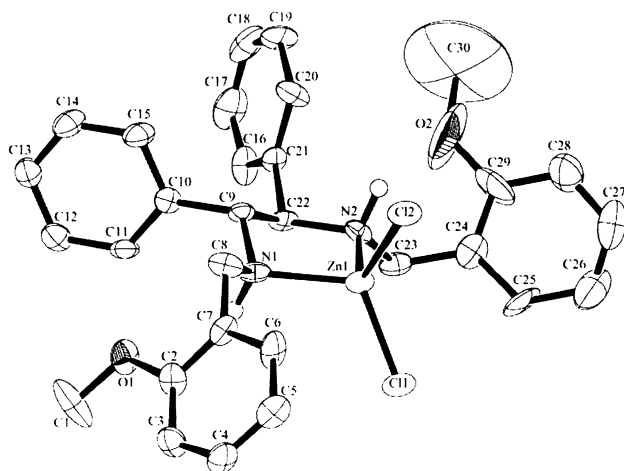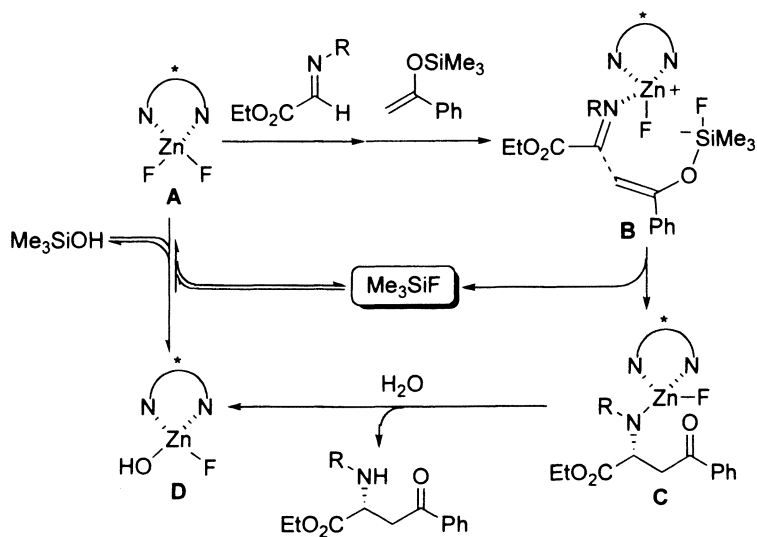
Figure 1. ORTEP drawing of [ZnCl₂–1c] moiety in the X-ray crystal structure of [ZnCl₂–4c•CH₂Cl₂] (Hydrogen atoms except NH and solvent of crystallization are omitted for clarity)

supra) or the electron-donating effect of the MeO groups seems to increase the basicity of the amino nitrogen atoms which coordinate to Zn^{2+} (32) Therefore, the use of these diamines could facilitate the dissociation of the fluoride anion from ZnF_2, leading to the improved catalytic activity of chiral ZnF_2 complexes.

While it was revealed that the Mannich-type reactions in aqueous THF gave high yields and high stereoselectivities in the presence of catalytic amounts of ZnF_2 and a chiral diamine, it is more desirable to use only water as a solvent. It was surprising to find that the reaction of 1 with 2a in only water, direct use of the catalytic system which was most effective in H_2O/THF (1/9), gave satisfactory results. Indeed, when ZnF_2 (100 mol %)–4i (10 mol %) was employed, Mannich adduct 3a was obtained in 91% yield with 95% ee (Table 5, entry 1). However, the reactions with 2b were found to proceed sluggishly in spite of the fact that a large amount of 2b remained after the reaction (entries 2 and 3). To accelerate the reaction, addition of surfactants was then investigated (33-37). While sodium dodecyl sulfate (SDS) or Triton X-405 was not effective (entries 4 and 5), it was remarkable that cetyltrimethylammonium bromide (CTAB) gave an excellent yield, and that it also improved the enantiomeric excess slightly (entry 6). It is noteworthy that a cationic surfactant is effective in this reaction (38,39), while an anionic surfactant has been reported to work well in Lewis acid-catalyzed aldol reactions (40-42). Moreover, it was found that 2 mol % of CTAB was sufficient for giving high yield and enantioselectivity (entry 7).

Table 5. Asymmetric Mannich-type reaction in pure water

Entry	Si enolate	Additive (mol %)	Time (h)	Yield (%)	syn/anti	ee (%, syn)
1	2a	–	20	91	–	95
2	2b	–	20	6	91/9	94
3	2b	NaOTf (1)	20	8	87/13	92
4	2b	SDS (5)	20	9	91/9	78
5	2b	Triton® X-405 (5)	20	10	91/9	93
6	2b	CTAB (5)	20	94	94/6	97
7	2b	CTAB (2)	20	93	94/6	96

Finally, the amount of ZnF_2 could be reduced to 10 mol % in the reactions with several silyl enol ethers derived from α-monosubstituted carbonyl compounds, although unsatisfactory results were obtained in the case of **2a**. The reaction with **2b** proceeded to give the desired adduct **3b** in high yield with high stereoselectivity even when the amount of **4c** was reduced to 5 mol % (Table 6, entry 1). However, further decrease in the ZnF_2 loading resulted in moderate yield (entry 2). When the silyl enol ethers derived from 4'-methoxypropiophenone and butyrophenone were employed, high yields and high diastereo- and enantioselectivities were obtained (entries 3 and 4). Furthermore, stereospecific, enantio- and diastereoselective reactions were achieved in only water by using only 10 mol % of ZnF_2 and **4c**. In fact, the reactions with **2d-E** and **2d-Z** proceeded to afford *anti*- and *syn*-adducts in good yields with high diastereo- and enantioselectivities, respectively, although longer reaction time was required (entries 5 and 6). It is noted that the products obtained in the present Mannich-type reactions were often highly crystalline, and one recrystallization afforded the diastereomerically and enantiomerically almost pure materials (entries 1, 3, 4 and 6).

As described above, diastereo- and enantioselective Mannich-type reactions of α-hydrazono ester **1** with silicon enolates for the synthesis of amino acid derivatives in aqueous media have been achieved with a ZnF_2–chiral diamine

Table 6. Substrate scope of the reactions using water as a solvent

Entry	Enolate	4c (mol %)	Time (h)	Product	Yield (%)	syn/anti	ee (%)[a]
1	**2b**	5	40	**3a**	87	93/7	96
2[b]	**2b**	5	40	**3a**	60	93/7	96
3[c]	**2e**[d]	10	40	**3e**	76	96/4	96
4[c]	**2f**	10	40	**3f**	74	96/4	96
5	**2d**	10	155	**3d**	75	13/87	92
6	**2d**	10	144	**3d**	63	90/10	98

[a] Ee of major diastereomer. [b] ZnF_2: 5 mol %. [c] E/Z = <2/>98. [d] PMP = *p*-methoxyphenyl.

complex. This reaction seems to proceed with double activation where Zn^{2+} acts as a Lewis acid to activate **1** and fluoride anion acts as a Lewis base to activate silicon enolates. The effect of the diamines having the MeO groups on their aromatic rings is noteworthy, and the advantageous points of using these diamines are as follows: (i) The reactions in aqueous THF were remarkably accelerated. (ii) The reaction with silicon enolate **2a** or **2b** proceeded in high yield even in the absence of TfOH or NaOTf, which was needed in the reactions using **4a**. (iii) The ZnF_2 loading could be reduced to 10–20 mol % without loss of yield and enantioselectivity, whereas more than 50 mol % of ZnF_2 was required in the reactions using **4a**. (iv) The reactions in water without any organic cosolvents proceeded smoothly to give high yields and high stereoselectivities. It is also noted that, in contrast to most asymmetric Mannich-type reactions, either *syn-* or *anti-*adducts were stereospecifically obtained from (*E*)- or (*Z*)-silicon enolates in the present reaction. These studies will provide a useful guide to the development of catalytic asymmetric carbon–carbon bond-forming reactions in water.

2. Chiral calcium-catalyzed asymmetric 1,4-addition and [3+2] cycloaddition reactions of α-aminoester Schiff bases.

Calcium, strontium, and barium belong to group 2 elements, alkaline earth metals, and are recognized as one of the most abundant elements in the natural world, for example in the sea or in the earth's crust (43). While they are familiar to our life, their application to organic synthesis has been limited. Characteristic points of the elements are, (i) lower electron negativity, (ii) stable oxidation state is 2, that means two bondings with anions are possible, (iii) many numbers of coordination sites. Among them, the first point, lower electron negativity, is an interesting nature from a view point of synthetic chemistry, because it usually leads to stronger Brønsted basicity of their counter anions. The elements are also less harmful compared to other heavy metals and much attractive in development of environmentally benign process. Based on these characteristic points, alkaline earth metals have quite recently been used for development of efficient asymmetric synthesis.

As a ligand design, the chiral bisoxazoline skeleton was selected as a model of a chiral ligand. Bisoxazoline derivatives are one of the most efficient and often employed chiral ligands in asymmetric catalysis (44). When the methylene-tethered bisoxazoline ligand (ex. **5a–5e**) is used, the alkaline earth metal base could deprotonate the methylene moiety of the ligand to form a rigid chiral complex in which two nitrogen atoms coordinate to the center metal in a bidentate fashion (Scheme 3).

Catalytic asymmetric carbon-carbon bond forming reactions using glycine ester derivatives are getting much attention to provide efficient synthetic routes to optically pure a-amino acid derivatives (45-47). From the first report on asymmetric alkylation using a chiral phase-transfer catalyst by O'Donnell *et al.* in 1978 (48), many highly strereoselective reactions have been developed. Among them, asymmetric Michael-type 1,4-addition of Schiff bases of glycine esters to α,β-unsaturated carbonyl compounds provides an efficient route to optically active glutamic acid derivatives. Although some successful examples have been reported in this reaction, excess amounts of bases or substrates are required to realize high conversions in most cases (49-59). However, conceptually a catalytic amount of a Brønsted base should work effectively in this reaction. It was envisioned that the alkaline earth metals were suitable for this reaction, and asymmetric 1,4-addition reactions of glycine derivatives to α,β-unsaturated carbonyl compounds using a chiral alkaline earth metal catalyst have been investigated. (60,61).
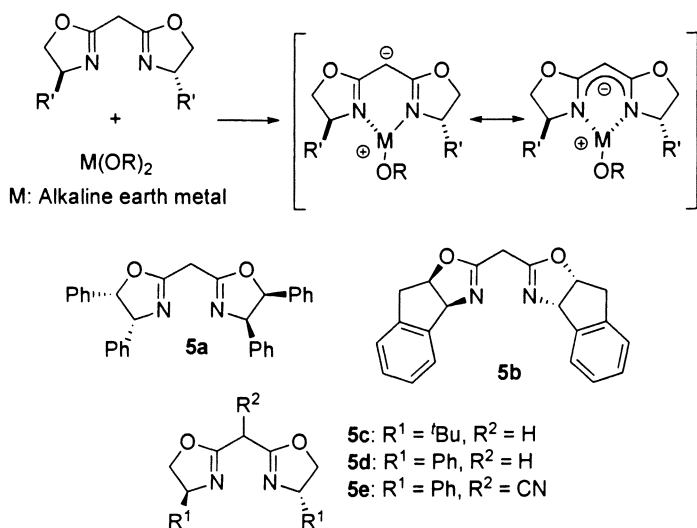
Scheme 3. Alkaline earth metal complexes prepared from bisoxazolidine ligands

First, the 1,4-addition of *N*-diphenylmethylideneglycine *tert*-butyl ester (7a) to methyl acrylate (6a) in THF using chiral alkaline earth metal alkoxides was examined. The reaction proceeded in moderate to good yields in the presence of the catalysts prepared from alkaline earth metal alkoxides and bisoxazoline

(Box) ligand **5a**, and a good enantioselectivity was observed in the reaction using calcium isopropoxide (Table 7, entry 2). While strontium and barium alkoxides showed higher reactivities, enantioselectivities were insufficient (entries 3 and 4). Those results indicated that the metal with smaller ionic radius, calcium, gave better chiral environments. Next, other Box ligands were investigated. The box ligand **5b** synthesized from 2-amino-1-indanol showed a little lower selectivity. Among the Box ligands tested, ligand **5d** gave the best result (82% ee). Furthermore, several typical solvents (Et$_2$O, TBME, Toluene, CH$_2$Cl$_2$, DME, CH$_3$CN) were tested, but THF gave the best results. Also effect of molecular sieves was examined, and it was found that the presence of molecular sieves was necessary for the reaction proceeding, and MS 4Å showed the best selectivity compared to other molecular sieves (3Å, 5Å, 13X). After tuning of some reaction parameters, finally high yield and high enantioselectivity (94% ee) were obtained (entry 9).

Table 7. Asymmetric 1,4-addition reaction of 7a with 6a using chiral alkaline earth metal complexes
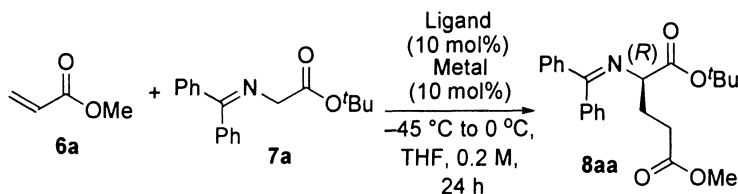
Entry	Metal	Ligand	Additive	Yield (%)	Ee (%)
1	Mg(OEt)$_2$	**5a**	MS 5Å	No Reaction	–
2	Ca(O^{i}Pr)$_2$	**5a**	MS 5Å	39	73[a]
3	Sr(O^{i}Pr)$_2$	**5a**	MS 5Å	76	29[a]
4	Ba(O^{t}Bu)$_2$	**5a**	MS 5Å	79	17[a]
5	Ca(O^{i}Pr)$_2$	**5b**	MS 5Å	24	71
6	Ca(O^{i}Pr)$_2$	**5c**	MS 5Å	19	6[a]
7	Ca(O^{i}Pr)$_2$	**5d**	MS 5Å	54	82
8	Ca(O^{i}Pr)$_2$	**5e**	MS 5Å	31	44
9[b]	Ca(O^{i}Pr)$_2$	**5d**	MS 4Å	88	94

[a] (*S*)-enantiomer was obtained. [b] The reaction was performed at –30 °C for 12 h using **7a** (1.2 equiv). The catalyst was prepared in optimized preparation method.

Substrate scope of this asymmetric 1,4-addition reaction is shown in Table 8. The effect of the ester parts of the Schiff bases was examined in the reaction with methyl acrylate. While the methyl ester showed higher reactivity,

enantioselectivity was lower than the reaction using the *tert*-butyl ester (entries 1 and 2). The ester part of acrylate was also examined. Ethyl acrylate and *tert*-butyl acrylate reacted with the Schiff base to afford the desired products in moderate to good yields with high selectivities (entries 3 and 4). The acrylamide with methoxy group on the N atom (Weinreb amide) also reacted with good enantioselectivity (entry 5). Next, the effect of substituents at the 2-position of the acrylates was investigated (62). The reaction of 2-methylacrylate with the glycine ester proceeded smoothly to afford two diastereomers in high yield with moderate diastereo- and excellent enantioselectivities (entry 6). 2-Ethylacrylate reacted smoothly, but lower enantioselectivity was observed (entry 7). It was found that the size of the substituents at the 2-position of acrylates affected the diastereoselectivity, and that the substrates with larger groups, 2-chloro- and 2-phenylacrylate, showed higher diastereoselectivitis (entries 8 and 9). 2-Methylacrylamide with *N*-methoxy group reacted with the glycine Schiff base **7a** to afford the 1,4-addition product in high yield with high diastero- and enantioselectivity (entry 10). In general, it was revealed that asymmetric 1,4-addition of glycine esters to acrylate derivatives proceeded well with high enantioselectivities.

Table 8. Substrate scope of the 1,4-addition reactions
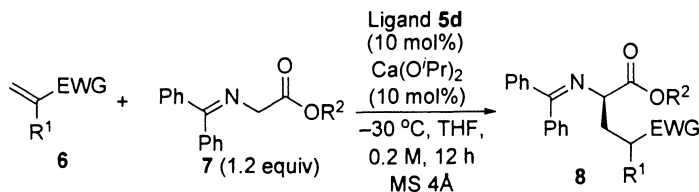
Entry	R^1	EWG	R^2	Product	Yield (%)	2,4- syn/anti	Ee (%)
1	H	CO_2Me	^tBu	**8aa**	88		94
2	H	CO_2Me	Me	**8ac**	quant		83
3[a,c]	H	CO_2Et	^tBu	**8ba**	56		95
4[a,c]	H	$CO_2{}^tBu$	^tBu	**8ca**	74		92
5[a]	H	CONMeOMe	^tBu	**8da**	46		87
6[b]	Me	CO_2Me	^tBu	**8ea**	93	61/39	99[d]/94[e]
7	Et	CO_2Me	^tBu	**8fa**	quant	63/37	86[d]/91[e]
8[a]	Cl	CO_2Me	^tBu	**8ga**	95	83/17	81[d]
9[a]	Ph	CO_2Me	^tBu	**8ha**	quant	91/9	84[d]
10[a,c]	Me	CONMeOMe	^tBu	**8ia**	83	91/9	85[d]

[a] 24 h. [b] **7a** (1.5 equiv) was used. [c] **6** (1.5 equiv) was used. [d] Value represents ee of the major product. [e] Value represents ee of the minor product.

Next, the asymmetric 1,4-addition reactions of crotonates using the chiral calcium catalyst were examined. Successful diastereo- and enantioselective 1,4-addition of glycine enolates to crotonates has not been reported yet (63-72). When the glycine *tert*-butyl ester **7a** was treated with methyl crotonate under the optimized reaction conditions, the reaction proceeded and the starting material was consumed rapidly; however, the obtained product was not the desired 1,4-addition product **8ka** but a substituted pyrrolidine derivative **9ka** as a single diastereomer unexpectedly. The structure was unambiguously determined by X-ray crystallographic analysis. Moreover, the enantioselectivity of the product was extremely high. The structure of the product indicated that [3+2] cycloaddition proceeded with high diastereo- and enantioselections (Scheme 4). This result was very remarkable for us, because only one small structural difference, hydrogen or methyl, on the terminal position of the acrylate determined the reaction course decisively. This interesting finding prompted to investigate catalytic asymmetric [3+2] cycloaddition of glycine esters using the chiral calcium catalyst further (73-91).
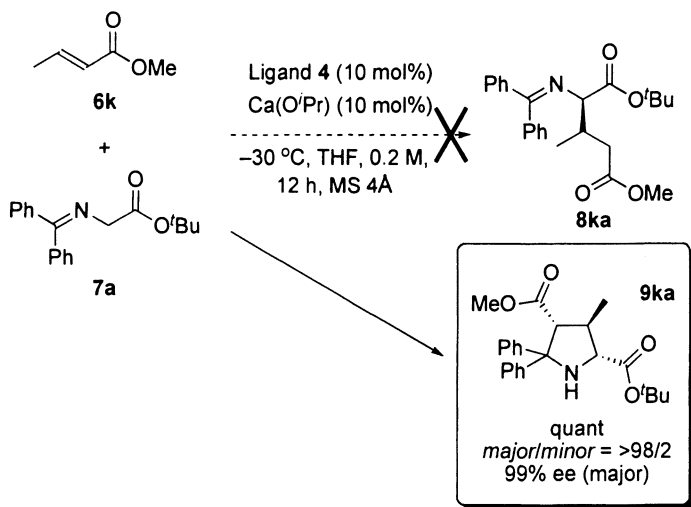
Scheme 4. Unexpected highly stereoselective [3+2] cycloaddition reaction

At the outset, the ester part of crotonates was examined. It was found that substrates with larger ester parts also reacted to afford the products, but that yields and selectivities decreased (Table 9, entries 1-3). Notably, the chiral calcium catalyst worked well in only 0.1 mol% catalyst loading (entry 1). Other β-substituted acrylates reacted in high enantioselectivities (entries 4-6). Not

only crotonates and their derivatives but also acrylamides afforded [3+2] cycloadducts **9** in good yields with very high enantioselectivities (entries 7–9). Substrates with bulkier amide-substituents showed slightly lower selectivities. It is noted that single diastereoisomers were obtained in all cases, and that this is a rare example of highly stereoselective [3+2] cycloaddition of *N*-diphenylmethylene-protected glycine derivatives (92).

Table 9. Asymmetric [3+2] cycloaddition of 7a to crotonates and acrylamides
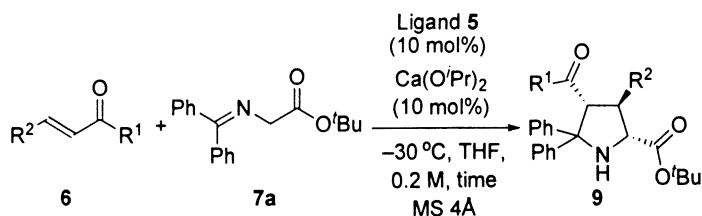
Entry	R^1	R^2	Ligand	Time (h)	Product	Yield (%)	Ee (%)
1	OMe	Me	**5d**	3	**9ma**	quant (quant)[a]	>99 (93)[a]
2	OEt	Me	**5d**	3	**9na**	98	98
3	O^{t}Bu	Me	**5d**	3	**9oa**	77	87
4	OMe	Et	**5b**	24	**9pa**	quant	95
5	OMe	^{t}Bu	**5b**	24	**9qa**	quant	99
6	OMe	Heptyl	**5b**	48	**9ra**	97	>99
7	NMe$_2$	H	**5d**	12	**9sa**	83	95
8	(morpholino)	H	**5d**	24	**9ta**	76	98
9	(piperidino)	H	**5d**	24	**9ua**	84	97
10	NCy$_2$	H	**5d**	24	**9va**	93	91

[a] 0.1 mol% catalyst. The reaction was carried out at 20 °C for 72 h. Ligand **5b** was used. 1,4-adduct was observed in the isolated product (3%).

Catalytic asymmetric [3+2] cycloaddition reactions of Schiff bases **7**, derived from aldehydes, were also examined (Table 10). In this reaction, ligand **5d** as well as ligands **5a** and **5b** worked well under the optimized reaction conditions. Halogen-substituted aromatic imines reacted smoothly to afford the

cycloadducts in good selectivites (entries 2, 3). In addition, the effect of substitution positions was examined, and among tolyl substrates, the *meta*-substituted one gave the highest enantioselectivities (entries 4–6). Bulkier imines showed higher selectivities, and the enantiomeric excess reached to 94% when the imine prepared from the 3,5-xylene derivative was used (entry 7). An electron rich imine also gave good enantioselectivity using ligand **5d** (entry 9), while 2-furaldehyde imine also gave high ee using ligand **5a** (entry 10). It should be noted that these reactions are the first successful examples of the asymmetric [3+2] cycloaddition of crotonates (93).

Table 10. Asymmetric [3+2] cycloaddition of various glycines
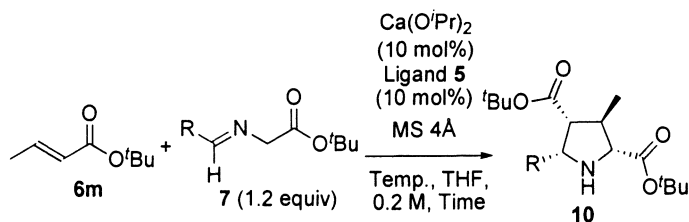
Entry	R	Ligand	Conditions	10	Yield (%)	ee (%)
1[a]	Ph	**5d**	10 °C, 3 h	**10me**	86	86
2	p-ClC$_6$H$_4$	**5b**	−20 °C, 8 h	**10mf**	92	82
3	p-BrC$_6$H$_4$	**5d**	10 °C, 3 h	**10mg**	95	86
4	p-MeC$_6$H$_4$	**5a**	−30 °C, 12 h	**10mh**	92	87[b]
5	m-MeC$_6$H$_4$	**5b**	−20 °C, 12 h	**10mi**	quant	91
6	o-MeC$_6$H$_4$	**5d**	10 °C, 3 h	**10mj**	86	78
7	3,5-(Me)$_2$C$_6$H$_3$	**5d**	10°C,12 h	**10mk**	quant	94
8	2-naphtyl	**5d**	10 °C, 3 h	**10ml**	97	92
9	p-MeOC$_6$H$_4$	**5d**	10 °C, 12 h	**10mm**	76	86
10	2-furyl	**5a**	−30 °C, 12 h	**10mn**	97	90[b]

[a] 1.1 equivalent of glycine ester were used. [b] The absolute configuration of the product was reversed.

The fact that the aldehyde-derived imines reacted with *tert*-butyl crotonate **(6m)** in [3+2] cycloaddition manner in high yields with perfect diastereoselectivities and high enantioselectivities encouraged to investigate the synthesis of pyrrolidine derivatives with chiral quaternary carbon centers. Based on a working model, a calcium enolate formed in the reaction of the

glycine ester with calcium isopropoxide, where the nitrogen atom of the enolate could coordinate to the calcium atom in a bidentate fashion. Therefore, it was assumed that the substituent at the α-position of the enolate did not affect the asymmetric environments significantly. In addition, since the bulkiness of the imine part improved the enantioselectivity in the previous investigation, it was envisioned that introduction of appropriate bulkiness at the α-position of the enolate might improve the enantioselectivity.

First, the reactions of alanine derivatives with α,β-unsaturated carbonyl compounds was investigated. The benzilidene protected alanine methyl ester reacted with methyl acrylate to afford the desired [3+2] adduct **12aa** in good enantioselectivity (Table 11, entry 1). In this reaction, bulkier ester parts also gave better effects, and the highest enantioselectivity was obtained using *tert*-butyl acrylate (entry 2). Contrastively, the selectivity did not depend on the structure of the alanine ester part (entries 2–4). The amino acid esters with simple alkyl substituents on their α-positions were also investigated (entries 5–9). 2-Ethylglycine, phenylalanine, norleucine, and leucine derivatives also gave good to high selectivities, although the valine derivative did not work well presumably due to a steric factor (entry 9). The reactions of the amino acid derivatives with α-substituents containing heteroatoms was also investigated. Methionine and O-*tert*-butyl serine derivatives also worked well, and good to high enantioselectivities were obtained (entries 10, 11). In all cases, single diastereomers were obtained exclusively. It is noted that this method is superior to other previous chiral pyrolidine synthesis via asymmetric [3+2] cycloaddition in terms of yields and diastereo- and enantioselectivities, and that chiral quaternary carbon centers have been constructed efficiently.

As a more challenging trial, asymmetric synthesis of pyrolidine derivatives containing contiguous chiral tertially and quaternary carbon centers was further investigated. This kind of complex molecule is usually difficult to be synthesized especially in an enantiomerically pure form, since steric bulkiness around the reaction site of substrates often prevents strict enantioselection. Firstly, the reaction of an alanine derivative with crotonates was examined. Remarkably, the desired reaction proceeded smoothly to afford the desired pyrrolidine derivatives as single diastereomers in good yields with excellent enantioselectivity (Table 12, entries 1, 2). Longer-chain α,β-unsaturated esters, methyl 2-pentenoate and methyl 2-heptenoate, also reacted with the alanine derivative, and excellent enantioselectivities were obtained (entries 3, 4). Next, the reactions of α-substituted α-amino acid esters were studied. In the reaction of 2-ethylglycine derivative **11d**, moderate yield with high enantioslectivity was observed (entry 5). On the other hand, the reactions of the amino ester with larger substituents proceeded slowly, and high enantioselectivities were also obtained (entries 6, 7). Interestingly, the amino ester derived from a bulky serine derivative reacted with α,β-unsaturated esters to afford the desired

Table 11. Asymmetric [3+2] cycloaddition of Schiff bases derived from α-amino acids
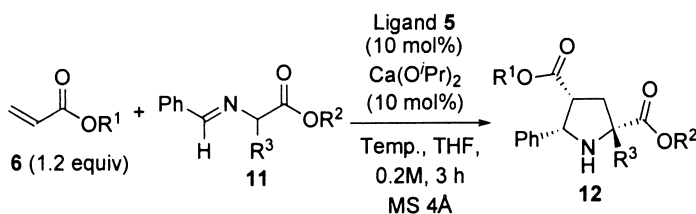
Entry	R^1	R^2	R^3	5	Temp.	12	Yield (%)	ee (%)
1	Me	Me	Me	5d	10 °C	12aa	95	70
2[a]	^{t}Bu	Me	Me	5d	10 °C	12ba	quant	90
3	^{t}Bu	Et	Me	5d	10 °C	12bb	quant	91
4	^{t}Bu	Bn	Me	5d	10 °C	12bc	93	90
5[a]	^{t}Bu	Me	Et	5b	–30 °C	12bd	82	96
6[a]	^{t}Bu	^{t}Bu	Bn	5b	–30 °C	12be	90	92
7[a]	^{t}Bu	Me	^{n}Bu	5b	–20 °C	12bf	94	94
8[a]	^{t}Bu	Me	^{i}Bu	5b	–30 °C	12bg	98	87
9	^{t}Bu	Me	^{i}Pr	5d	10 °C	12bh	32	59
10[a]	^{t}Bu	Me	$(CH_2)_2SMe$	5b	–20 °C	12bi	81	81
11[a,b]	^{t}Bu	^{t}Bu	CH_2O^tBu	5b	–20 °C	12bj	80	93

[a] 12 h, [b] L-Amino acid was used.

products in good yields with excellent enantioselectivities (entries 8, 9). These results clearly showed that the chiral calcium catalyst could construct highly sterically hindered and complicated carbon centers directly with high stereoselectivities. This is one of quite remarkable features of this chiral calcium catalyst compared to other chiral catalyst systems reported previously in asymmetric [3+2] cycloaddition.

While the reaction with crotonates afforded [3+2] cycloaddition adducts as a sole product in all cases as mentioned before, asymmetric 1,4-addition of glycine derivatives to crotonates is also an important method to synthesize branched α-amino acid derivatives, however successful examples are very limited. One key against this issue is modification of the imine part of the glycine Schiff base. The imine part of the glycine unit could affect the reaction course in the [3+2] cycloaddition reactions. To realize 1,4-addition, the reaction of a ketimine derived from glycine *t*-butyl ester with methyl crotonate was investigated. The reaction of a glycine derivative with more bulkier substituents at the imine part, which could prevent the cyclization step to afford the 1,4-addition product, was conducted. The glycine derivative 7s with *tert*-butyl

Table 12. Asymmetric synthesis of contiguous tertiary and quaternary carbon centers
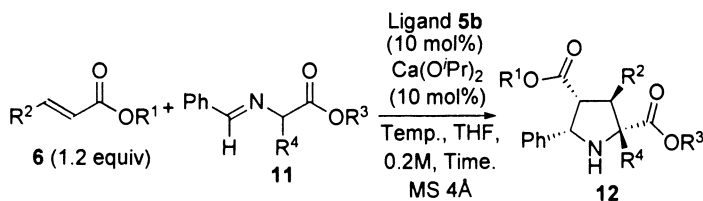
Entry	R^1	R^2	R^3	R^4	Conditions	12	Yield (%)	ee (%)
1	Me	Me	Me	Me	0 °C, 12 h	12ka	79	96
2	Et	Me	Me	Me	10 °C, 24 h	12la	55	93
3	Me	Et	Me	Me	0 °C, 12 h	12na	64	96
4[a]	Me	^{n}Bu	Me	Me	0 °C, 18 h	12wa	81	98
5	Me	Me	Me	Et	−20 °C, 24 h	12kd	41	89
6[a]	Me	Me	Me	^{n}Bu	−30 °C, 72 h	12kf	50	93
7[a]	Me	Me	^{t}Bu	Bn	−30 °C, 72 h	12ke	98	85
8[b]	Me	Me	^{t}Bu	CH_2O^tBu	10 °C, 24 h	12kj	80	97
9[a,b]	Me	^{i}Bu	^{t}Bu	CH_2O^tBu	0 °C, 24 h	12oj	87	95

[a] Catalyst (20 mol%) was used. [b] L-Amino acid derivative was used.

phenyl methylidene (94-98) as a protecting group was reacted with methyl crotonate. Expectedly, the 1,4-addition products were obtained exclusively as a single diastereomer with high enantioselectivity by using calcium-5b catalyst (Table 13, entry 1).

By using this glycine derivative 7s, the reactions with a couple of crotonate derivatives proceeded in a 1,4-addition manner. As shown in Table 13, not only crotonates, but also 2-pentenoate, 2-heptenoate and 5-methyl-2-hexenoate reacted with the glycine derivative in a 1,4-addition manner with good to high enantioselectivities (entries 3 - 6). A crotonate derivative substituted by benzyloxy group at the terminal methyl moiety also gave high selectivity (entry 7). In the case of a crotonamide with *N*-methoxy group, high yield and high selectivity were also obtained (entries 9 and 10). It should be noted that this is the first example of catalytic highly stereoselective 1,4-addition of a glycine enolate to crotonates.

A possible mechanism of the calcium-catalyzed asymmetric 1,4-addition is shown in Scheme 5. The chiral calcium complex deprotonated the α-position of the glycine derivative 7 to form chiral calcium enolate **F**, in which the imine nitrogen also coordinated to the calcium atom. The enolate then reacted with an α,β-unsaturated carbonyl compound **6** with high enantioselection to form the 1,4-addition product initially as a calcium enolate **G**. Further, the enolate **G** was

Table 13. Asymmetric 1,4-addition reactions with β-substituted α,β-unsaturated carbonyl compounds
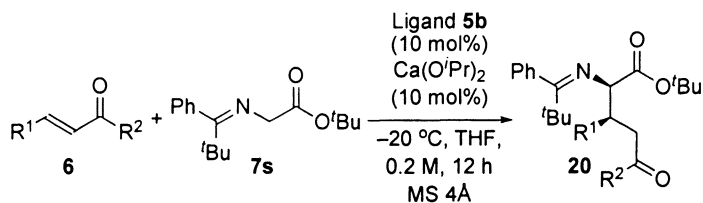
Entry	R^1	R^2	20	Dr	Yield (%)	Ee (%)
1	Me	OMe	20ms	>99/1	97 (93)[a]	99 (99)[a]
2	Me	OEt	20ns	>99/1	95	93
3	Et	OMe	20ps	>99/1	96	96
4	Et	OEt	20xs	>99/1	97	94
5[b]	^{n}Bu	OMe	20ys	>99/1	73	91
6[c]	^{i}Bu	OMe	20qs	>99/1	56	82
7	BnOCH$_2$	OEt	20zs	82/18	82	96
8	Me	NMeOMe	20as	>99/1	94	98
9[c]	Et	NMeOMe	20bs	>99/1	92	96
10[c]	^{i}Bu	NMeOMe	20gs	>99/1	89	95

[a] 2 mol%, [b] –20 °C, 48 h, [c] 24 h.
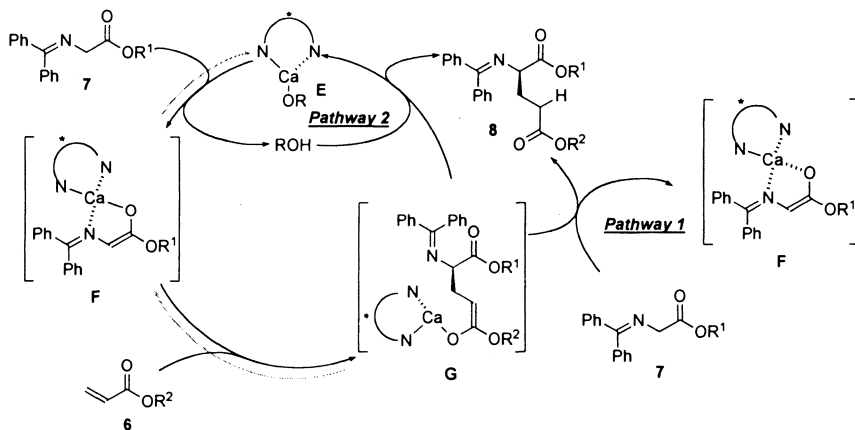
Scheme 5. Proposed catalytic cycle of the 1,4-addition reaction

protonated with glycine derivative **7** to afford the product **8** along with regeneration of the reactive calcium enolate **F** (Pathway 1). Alternatively, the free alcohol (*PrOH) quenched **G** to give **8** and the active calcium complex **E** (Pathway 2).

On the other hand, two reaction mechanisms are possible for the [3+2] cycloaddition reaction. One is a concerted pathway and the other is a stepwise pathway. Based on our success of the 1,4-addition reaction with crotonates using the Schiff base bearing the bulky substituent, we are now assuming the reaction proceeds in a stepwise pathway. The proposed catalytic cycle was shown in Scheme 6. Intramolecular cyclization of the calcium enolate **G** occurred to afford calcium amide intermediate **H**, which was protonated with the glycine derivative **7** or *PrOH to regenerate the active calcium catalyst **E**.

As shown above, catalytic asymmetric 1,4-addition reactions of glycine derivatives using chiral alkaline earth metal complexes as catalysts have been developed. It was found that chiral calcium-Box **5** complexes were effective for the reaction, and high yields and high enantioselectivities have been achieved. Compared to previously reported reaction systems, this catalyst system is simple because only a catalytic amount of a Brønsted base is employed. Furthermore, in the reactions of crotonates, [3+2] cycloaddition reactions proceeded by using
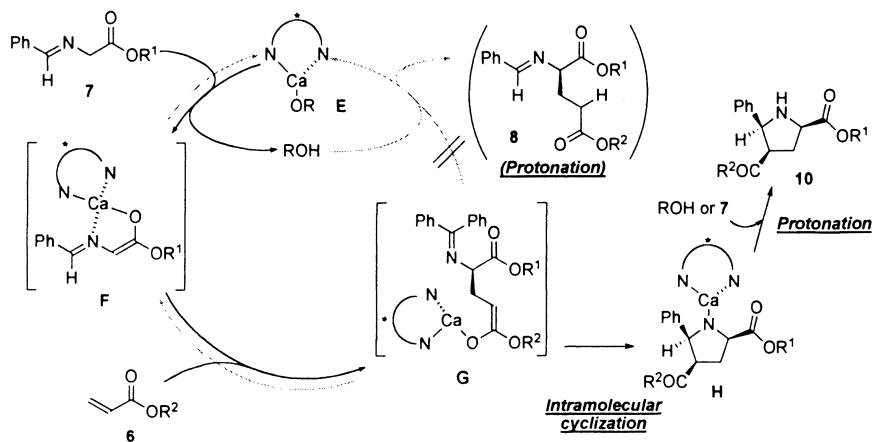
Scheme 6. Proposed catalytic cycle of the [3+2] cycloaddition reaction

the same reaction system to afford the chiral pyrrolidines in high yields with high diastereo- and enantioselectivities. It is remarkable that small difference of the substrate structure affected the reaction course and changed the product structure dramatically. Not only aldehyde-protected glycine derivatives, but

also ketoimine-protected glycine derivatives reacted with active olefins to afford the substituted pyrrolidines. Moreover, the first successful asymmetric [3+2] cycloaddition and 1,4-addition reactions of crotonates have been realized by modifying the protecting groups of the amine parts. Remarkably, the calcium catalyst could construct contiguous chiral tertially and quaternary carbon centers with high stereoselectivities in the [3+2] cycloaddition reactions of α-substituted α-amino acid derivatives and 3-substituted acrylates. It is noted that the current chiral calcium catalyst expanded possibilities of the asymmetric 1,4-addition and [3+2] cycloaddition reactions of α-amino acid derivatives. The reaction mechanisms were also investigated, and a stepwise mechanism, 1,4-addition and successive intramolecular cyclization, was proposed in the [3+2] cycloaddition.

Conclusion

We have described recent examples of environmentally benign processes in asymmetric synthesis of chiral α-amino acid derivatives. Both concepts, asymmetric reactions using water as a solvent and using less harmful and ubiquitous metals as catalysts, are very important in design of new green chemical processes. Chemical synthesis is now an indispensable methodology for human welfare to provide essentially fine chemical products such as drugs, materials, etc. However, recent problems in earth environment are also critical and unavoidable all over the world, therefore successive efforts for improvement of chemical process is necessary for further evolution of our technology to improve human life. We hope our research contribute to development of environmentally benign chemical processes.

References

1. Li, C.-J.; Chan, T.-H. *Organic Reactions on Aqueous Media*; John Wiley & Sons: New York, 1997.
2. *Organic Synthesis in Water*; Grieco, P. A. Ed.; Blackie Academic and Professional: London, 1998.
3. Lindström, U. M. *Chem. Rev.* **2002**, *102*, 2751.
4. Li, C.-J.; Chen, L. *Chem. Soc. Rev.* **2006**, *35*, 68.
5. Kobayashi, S.; Ishitani, H. *Chem. Rev.* **1999**, *99*, 1069.
6. Taggi, A. E.; Hafez, A. M.; Lectka, T. *Acc. Chem. Res.* **2003**, *36*, 10.
7. Kobayashi, S.; Ueno, M. in *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N.; Pfaltz, A.; Yamamoto H. Eds.; Supplement 1; Springer: Berlin, 2004; Chapter 29.5, pp. 143.
8. Friestad, G. K.; Mathies, A. K. *Tetrahedron* **2007**, *63*, 2541.

9. Verkade, J. M. M.; van Hemert, L. J. C.; Quaedflieg, P. J. L. M.; Rutjes, F. P. J. T. *Chem. Soc. Rev.* **2008**, *37*, 29.
10. Burk reported catalytic asymmetric hydrogenations of *N*-acylhydrazones: Burk, M. J.; Feaster, J. E. *J. Am. Chem. Soc.* **1992**, *114*, 6266.
11. Oyamada, H.; Kobayashi, S. *Synlett* **1998**, 249.
12. Kobayashi, S.; Sugita, K.; Oyamada, H. *Synlett* **1999**, 138.
13. Manabe, K.; Oyamada, H.; Sugita, K.; Kobayashi, S. *J. Org. Chem.* **1999**, *64*, 8054.
14. Kobayashi, S.; Hamada, T.; Manabe, K. *J. Am. Chem. Soc.* **2002**, *124*, 5640.
15. Hamada, T.; Manabe, K.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 7768.
16. Hamada, T.; Manabe, K.; Kobayashi, S. *Chem. Eur. J.* **2006**, *12*, 1205.
17. The binding constant of Zn^{2+} and ethylenediamine in water is 105.7 M−1: Martell, A. E.; Smith, R. M. *Critical Stability Constants*, Vol. 1–3, Plenum Press, 1974, 1975, 1977.
18. Alexaxis, A.; Mangeney, P. *Advanced Asymmetric Synthesis*, (Ed.: G. R. Stephenson), Chapman & Hall: London, **1996**, pp. 93-110 and references therein.
19. For review on transition metal fluoride complexes in asymmetric catalysis, see: Pagenkopf, B. L.; Carreira, E. M. *Chem. Eur. J.* **1999**, *5*, 3437.
20. Hamada, T.; Manabe, K.; Kobayashi, S. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 3927.
21. Although diastereoselectivities were not as high as the present reactions, our group also observed the stereospecificities in the asymmetric Mannich-type reactions in an organic solvent: Kobayashi, S.; Matsubara, R.; Nakamura, Y.; Kitagawa, H.; Sugiura, M. *J. Am. Chem. Soc.* **2003**, *125*, 2507.
22. For a similar type of double activation, see: Kobayashi, S.; Uchiro, H.; Shiina, I.; Mukaiyama, T. *J. Am. Chem. Soc.* **1991**, *113*, 4247. Also see ref. 23, 24.
23. Gauthier Jr. D. R.; Carreira, E. M. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2363.
24. Friestad, G. K.; Ding, H. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 4491.
25. For Lewis base activation of silicon enolates in Mannich-type reactions, see: Miura, K.; Tamaki, K.; Nakagawa, T.; Hosomi, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 1958. See also ref. 26.
26. Miura, K.; Nakagawa, T.; Hosomi, A. *J. Am. Chem. Soc.* **2002**, *124*, 536.
27. For recent reviews on double activation in asymmetric catalysis, see: Shibasaki, M.; Kanai, M.; Funabashi, K. *Chem. Commun.* **2002**, 1989. Also see ref. 28.
28. Ma, J.-A.; Cahard, D. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 4566.
29. Aoyama, N.; Hamada, T.; Manabe, K.; Kobayashi, S. *J. Org. Chem.* **2003**, *68*, 7329-7333. See also Ref. 21

30. A similar fluoride-catalyzed mechanism was partly proposed in aldol reactions catalyzed by TBAF or *tris*-(diethylamino)sulfonium difluorotrimethylsiliconate (TASF): Nakamura, E.; Shimizu, M.; Kuwajima, I.; Sakata, J.; Yokoyama, K.; Noyori, R. *J. Org. Chem.* **1983**, *48*, 932. See also ref. 31.

31. Noyori, R.; Nishida, I.; Sakata, J. *J. Am. Chem. Soc.* **1983**, *105*, 1598.

32. As for the *tert*-amines, the basicity of the nitrogen atom of 2'-methoxybenzylamine is known to be higher than that of benzylamine: Bartolini, M.; Bertucci, C.; Gotti, R.; Tumiatti, V.; Cavalli, A.; Recanatini, M.; Andrisano, V. *J. Chromatogr. A* **2002**, *958*, 59.

33. Fendler, J. H.; Fendler, E. J. *Catalysis in Micellar and Macromolecular Systems*; Academic Press: London, 1975.

34. *Mixed Surfactant Systems*; Holland, P. M.; Rubingh, D. N. Eds.; American Chemical Society: Washington, DC, 1992.

35. *Structure and Reactivity in Aqueous Solution* Cramer, C. J.; Truhlar D. G. Eds.; American Chemical Society: Washington, DC, 1994.

36. *Surfactant-Enhanced Subsurface Remediation*, Sabatini, D.A.; Knox, R. C.; Harwell, J. H. Eds.; American Chemical Society: Washington, DC, 1994.

37. *Reactions and Synthesis in Surfactant Systems*; Texter, J.; Ed.; Marcel Dekker: New York, 2001.

38. For selected examples of the reactions which were accelerated by the addition of a cationic surfactant, see: Behme, M. T. A.; Fullington, J. G.; Noel, R.; Cordes, E. H. *J. Am. Chem. Soc.* **1965**, *87*, 266. See also Ref. 39.

39. Nivalkar, K. R.; Mudaliar, C. D.; Mashraqui, S. H. *J. Chem. Res. (S)* **1992**, 98.

40. Kobayashi, S.; Wakabayashi, T.; Nagayama, S.; Oyamada, H. *Tetrahedron Lett.* **1997**, *38*, 4559.

41. Kobayashi, S.; Mori, Y.; Nagayama, S.; Manabe, K. *Green Chem.* **1999**, 175;

42. Manabe, K.; Mori, Y.; Wakabayashi, T.; Nagayama, S.; Kobayashi, S. *J. Am. Chem. Soc.* **2002**, *124*, 5640.

43. *Basic Inorganic Chemistry*, 3rd ed.; Cotton, F. A.; Wilkinson, G.; Gaus. P. L., Eds.; Wiley: New York, 1995.

44. Asymmetric reactions using Box-Ligand: Desimoni, G.; Faita, G.; Jørgensen, K. A. *Chem. Rev.* **2006**, *106*, 3561.

45. Maruoka, K.; Ooi, T. *Chem. Rev.* **2003**, *103*, 3013.

46. O'Donnel, M. J. *Acc. Chem. Res.* **2004**, *37*, 506.

47. Najera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584.

48. O'Donnell, M. J.; Boniece, J. M.; Earp, S. E. *Tetrahedron Lett.* **1978**, *30*, 2641.

49. Corey, E. J.; Noe, M. C.; Xu, F. *Tetrahedron Lett.* **1998**, *39*, 5347.

154

50. Zhang, F.-Y., Corey, E. J. *Org. Lett.* **2000**, *2*, 1097.
51. Ishikawa, T.; Araki, Y.; Kumamoto, T.; Seki, H.; Fukuda.; Isobe, T. *Chem. Commun.* **2001**, 245.
52. O'Donnell, M. J.; Delgado, F. *Tetrahedron.* **2001**, *57*, 6641.
53. Shibuguchi, T.; Fukuta, Y.; Akachi, Y.; Sekine, A.; Ohshima, T.; Shibasaki, M. *Tetrahedron Lett.* **2002**, *43*, 9539.
54. Arai, S.; Tsuji, R.; Nishida, A. *Tetrahedron Lett.* **2002**, *43*, 9535.
55. Akiyama, T.; Hara, M.; Fuchibe, Sakamoto, S.; Yamaguchi, K. *Chem. Commun.* **2003**, 1734.
56. Ohshima, T.; Shibuguchi, T.; Fukuta, Y.; Shibasaki, M. *Tetrahedron.* **2004**, *60* 7743.
57. Lygo, B.; Allbutt, B.; Kirton, E. H. M. *Tetrahedron Lett.* **2005**, *46*, 4461.
58. Arai, S.; Takahashi, F.; Tsuji, R.; Nishida, A. *Heterocycles.* **2006**, *67*, 495.
59. Ryoda, A.; Yajima, N.; Haga, T.; Kumamoto, T.; Nakanishi, W.; Kawahata, M.; Yamaguchi, K.; Ishikawa, T. *J. Org. Chem.* **2008**, *73*, 133.
60. Saito, S.; Tsubogo, T.; Kobayashi, S. *J. Am. Chem. Soc.* **2007**, *129*, 5364.
61. Kobayashi, S.; Tsubogo, T.; Saito, S.; Yamashita, Y. *Org. Lett.* **2008**, *10*, 807.
62. Stereoselective synthesis of 4-subsitituted glutamic acid derivatives: Belokon, Y. N.; Bespalova, N. B.; Churkina, T. D.; Císařová, I.; Ezernitskaya, M. G.; Harutyunyan, S.; Hrdina, R.; Kagan, H. B.; Kočovský, P.; Kochetkov, K. A.; Larionov, O. V.; Lyssenko, K. A.; North, M.; Polášek, M.; Peregudov, A. S.; Prisyazhnyuk, V. V.; Vyskočil, Š. *J. Am. Chem. Soc.* **2003**. *125*. 12860.
63. Stereoselective synthesis of 3-subsitituted glutamic acid derivatives: Soloshonok, V. A. *Curr. Org. Chem.* **2007**, *6*, 341. as a review. Other representative examples: Mauger, A. B. *J. Org. Chem.* **1981**, *46*, 1032. See Also Ref. 64-72.
64. Achqar, A. El; Boumzebra, M.; Roumestant, M.-L.; Viallefont, P. *Tetrahedron* **1988**, *44*, 5319.
65. Kanemasa, S.; Tatsukawa, A.; Wada, E.; Tsuge, O. *Chem. Lett.* **1989**, 1301.
66. Kanemasa, S.; Tatsukawa, A.; Wada, E. *J. Org. Chem.* **1991**, *56*, 2875.
67. Alvarez-Ibarra, C.; Csk, A. G.; Maroto, M.; Quiroga, M. L. *J. Org. Chem.* **1995**, *60*, 6700.
68. Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Tetrahedron* **1999**, *55*, 12031.
69. Ezquerra, J.; Pedregal, C. *J. Org. Chem.* **1999**, *64*, 6554.
70. Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Org. Lett.* **2000**, *2*, 747.
71. Herdeis, C.; Kelm, B. *Tetrahedron* **2003**, *59*, 217.
72. Soloshonok, V. A.; Cai, C.; Yamada, T.; Ueki, H.; Ohfune, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **2005**, *127*, 15296.
73. For some recent reviews and examples on catalytic asymmetric [3+2] cycloaddion reactions: Padwa, A.; Pearson, W. H. In *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; John Wiley & Sons: New York, **2002**. See also ref. 74-83.

74. Gothelf, K. V.; Jørgensen, K. A. *Chem. Rev.* **1998**, *98*, 863.
75. Coldham, I.; Hofton, R. *Chem. Rev.* **2005**, *105*, 2765.
76. Pandey, G.; Banerjee, P.; Gadre, S. R. *Chem. Rev.* **2006**, *106*, 4484.
77. Gotthelf, A. S.; Gothelf, K. V.; Hazell, R. G.; Jørgensen, K. A. *Angew. Chem. Int. Ed.* **2002**, *41*, 4236.
78. Oderaotoshi, Y.; Cheng, W.; Fujitomi, S.; Kasano, Y.; Minakata, S.; Komatsu, M. *Org. Lett.* **2003**, *5*, 5043.
79. Alemparte, C.; Blay, G.; Jørgensen, K. A. *Org. Lett.* **2005**, *7*, 4569.
80. Gao, W.; Zhang, X.; Raghunsth, M. *Org. Lett.* **2005**, 7, 4241.
81. Husinec, S.; Savic, V. *Tetrahedron: Asymmetry* **2005**, *16*, 2047.
82. Nájera, C.; Sansano, J. M. *Angew. Chem. Int. Ed.* **2005**, *44*, 6272.
83. Yan, X.-X.; Peng, Q.; Zhang, Y., Zhang, K.; Hong, W.; Hou, X.-L.; Wu, Y.-D. *Angew. Chem., Int. Ed.* **2005**, *45*, 1979.
84. Syntheses of quaternary carbon centers in asymmetric [3+2] cycloaddition reactions: Chen, C.; Li, X.; Schreiber, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 10174. See also Ref. 85-88.
85. Cabrera, S.; Arrayás, R. G.; Carretero, J. C. *J. Am. Chem. Soc.* **2005**, *127*, 16394.
86. Llamas, T.; Gómez-Arrayá, R.; Carretero, J. C. *Org. Lett.* **2006**, *8*, 1795.
87. Cabrera, S.; Arrayás, R. G.; Martín-Matute, Cossío, F. P.; Carretero, J. C. *Tetrahedron* **2007**, *63*. 6587.
88. Nájera, C.; Gracia, Retamosa, M. de .G.; Sansano, J. M. *Org. Lett.* **2007**, *9*, 4025;
89. Intramolecular symmetric [3+2] cycloadditon reaction: Stohler, R.; Wahl, F.; Pfaltz, A. *Synthesis* **2005**, 1431;
90. Organocatalytic asymmetric [3+2] cycloaddition reactions: Vicario, J. L.; Reboredo, S.; Badía, D.; Carrillo, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 5168. See also ref. 91.
91. Xue, M.-X.; Zang, X.-M.; Gong, L.-Z. *Synlett* **2008**, 691.
92. Example of [3+2] cycloadditon reaction using N-diphenylmethylene-protected glycine; see ref. 85 and 87.
93. Example of [3+2] cycloaddition reaction using crotonate; see ref. 84.
94. Duhamel, P.; Eddine, J. J.; Valonot, J.-Y. *Tetrahedron Lett.* **1984**, *25*, 2355.
95. Tsuge, O.; Kanemasa, S.; Yoshioka, M. *J. Org. Chem.* **1988**, *53*, 1384.
96. Duhamel, L.; Duhamel, P.; Fouquay, S.; Eddine, J. J.; Peschard, O.; Plaquevent, J.-C.; Ravard, A.; Solliard, R.; Valonot, J.-Y.; Vincens, H. *Tetrahedron* **1988**, *44*, 5495.
97. Kanemasa, S.; Uchida, O.; Wada, E. *J. Org. Chem.* **1990**, *55*, 4411.
98. Casas, J.; Grigg, R.; Nájera, Sansano, J. M. *Eur. J. Org. Chem.* **2001**, 1971.

Chapter 10

Transition Metal Catalyzed Reactions toward the Synthesis of Amino Acids and Peptides

Uli Kazmaier

Institut für Organische Chemie, Universität des Saarlandes, Saarbruecken, Germany

Chelated enolates are excellent nucleophiles for palladium-catalyzed allylic alkylations, which provide γ,δ-unsaturated amino acids in highly stereoselective fashion. The reaction proceeds easily at -78 °C which allows to suppress $\pi-\sigma-\pi$-isomerization of the corresponding π-allyl complexes. This opens up completely new reaction pathways. For example, either the olefin geometry from the allylic substrate can be transferred to the amino acid, or, based on the different reactivities of the two allylic positions, regioselective C–C-couplings become possible. In general, an excellent transfer of chirality is observed, even for terminal π-allyl complexes which react under substrate control. This approach can nicely be tranferred to peptides, what allows highly stereoselective peptide modification. By using stannylated allylic carbonates, the metallated amino acids and peptides can be further modified *via* Stille couplings, which allows the generation of chemical libraries.

Introduction

π-Allyl palladium complexes play an important role in modern organic synthesis (*1*). With respect to the different synthetic applications the allylic alkylation is probably the most popular one (*2*). Herein, a π-allyl complex is attacked by a nucleophile such as an amine or a stabilized carbanion (in general malonate). In contrast to the symmetrical malonates, reactions of β-keto esters, as well as all other unsymmetric nucleophiles, are more critical. Their reactions generate a stereogenic center which is configurationally labile (if α-CH is present), giving a mixture of stereoisomers. Even if the allylation proceeds in a highly stereoselective fashion, subsequent epimerization of the newly generated and configurationally unstable stereogenic center wastes all efforts. This problem can be circumvented by using alkylated nucleophiles, such as chiral pyrazinones (*3*) or substituted azlactones, such as 1. In the presence of chiral ligand **L-1** the substitution product **2** was obtained in excellent yield and selectivity. A wide range of substrates and azlactones were investigated by Trost et al. and the products were converted to several α-alkylated amino acids (*4*). Besides allylic acetates and carbonates also *gem* diacetates can be used for this purpose, as illustrated in a nice synthesis of sphingofungin F based on this approach (*5*).
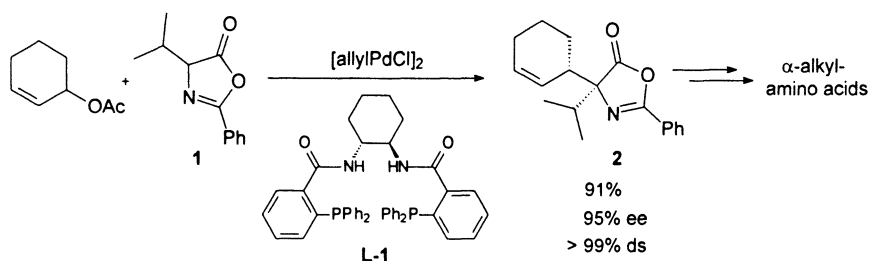
Scheme 1. Allylic alkylation of azlactones according to Trost et al.

How we Stumbled into Palladium-Chemistry

Our group is investigating chelated enolates of amino acid esters (such as **A**) as substrates for the synthesis of unnatural amino acids for some time (*6*). Chelation causes a marked enhancement of thermal stability without having any negative influence on the reactivity of these enolates. Due to the fixed enolate geometry, their conversions often proceed with a high degree of stereoselectivity. Excellent results are obtained in typical enolate reactions, such as aldol reactions (*7*) or Michael additions (*8*). But besides that, we are

especially interested in reactions, which cannot be carried out with non-chelated enolates. Thus, chelated enolates of allyl esters **3** (**A**) undergo Claisen rearrangement when being warmed up to room temperature providing *syn*-configured γ,δ-unsaturated amino acids **4** (Scheme 2) (*9*). If ester of chiral allyl alcohols are used, the chiral information can nicely be transferred to the new formed stereogenic centres (*10*). An alternative is the use of quinine (or quinidine) as a chiral ligand, coordinating to the chelating metal. Ee's up to 87 could be obtained, depending on the substitution pattern and the chelating metal salt used (*11*). Because the *N*-protected amino acids is formed via rearrange-ment, the next amino acid (or a peptide chain) can be coupled, allowing the direct incorporation of the new, unusual amino acid into a peptide.
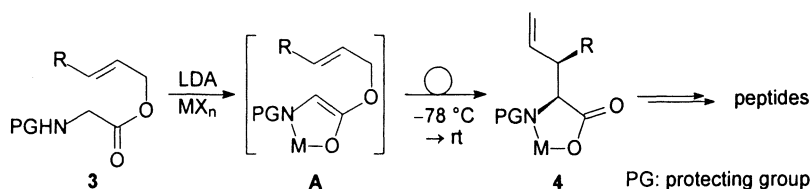
Scheme 2. Chelate Claisen-rearrangement of glycine allyl esters 3

With respect that the new amino acid later on should be incorporated into peptide, e.g. during a natural product or drug synthesis, it seems highly attractive to carry out the Claisen rearrangement directly with a peptide. In general, peptides contain other chiral amino acids, and if one is able to use this chiral information of the peptide chain to control the stereochemical outcome of the Claisen rearrangement, this would be an extremely straightforward approach towards stereoselective peptide modifications (*12*).

Therefore, we investigated the rearrangement of several different protected dipeptides. At the beginning we used Boc- and Cbz-protected methallylesters such as **5** as substrates and obtained only moderate yields of the rearrangement product (Scheme 3) (*13*). Nowadays we know that the methallylester was not the best choice, but at the early days we tried to find suitable catalysts to increase the yield. Based on the work of Overman *et al.* (*14*), we investigated the influence of Hg^{2+} and Pd^{2+} salts on the outcome of the reaction, although with no significant success. Because Pd^{2+} is known to form relatively stable peptide complexes (*15*), which might result in a deactivation of the catalyst, we also investigated the influence of Pd^0 on the reaction. And indeed, the "rearrangement product" **6** was obtained in high yield, albeit without some diastereoselectivity (50% *ds*).

The drop in selectivity, which was more dramatic with other chelating metal salts, was surprising and rose the question concerning the mechanism of the rearrangement. Does Pd^0 activate the substrate by coordination to the double

bond, as proposed for Pd^{2+}-catalysis, or are π-allyl-Pd-complexes involved in this reaction? To distinguish between these two possibilities we investigated the rearrangement of the corresponding crotyl ester **7**. Without Pd^0, the expected rearrangement product **8** was obtained exclusively, but in the presence of the catalyst, **8** was only the minor product. The unbranched peptide **9** was formed preferentially, which clearly indicates that the products are formed not *via* Claisen rearrangement, but an unselective intermolecular allylic alkylation of an unsymmetric π-allyl-Pd-complex.

Catalyst		
—	28%,	62% ds
PdCl$_2$(cod)	20%,	64% ds
Pd(PPh$_3$)$_4$	75%,	50% ds
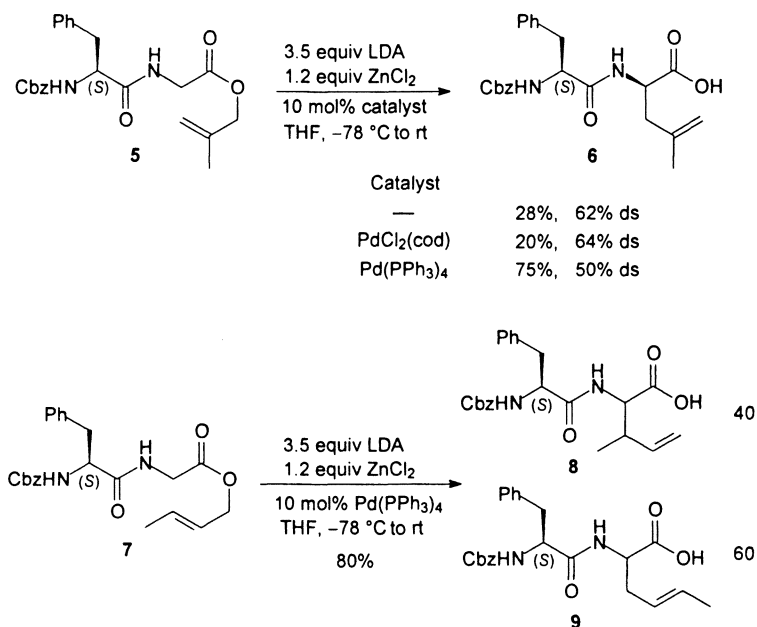
Scheme 3. Palladium-catalyzed peptide Claisen-"rearrangement"

Allylic Alkylations of Chelated Enolates

Based on theses interesting results, we focused our interest on the palladium catalyzed allylic alkylation. We hoped, that our chelated enolates can be used also for this important reaction. In contrast to the generally used malonates, with our enolates a stereogenic center is formed also at the α-position of the amino acid. Of course, the control of this stereogenic centre is not a trivial issue, but the products obtained are highly interesting structures, and therefore we decided to face this challenge.

The reaction of different *N*-protected glycine esters with allyl carbonates was examined. Besides carbonates, the corresponding acetates or benzoates can be used as well, but with the more reactive carbonates the yields and selectivities were usually better. To avoid transesterifications by the liberated alcoholate the glycine *tert*-butyl esters were chosen. Tfa- (trifluoroacetyl) was the best *N*-protecting group, with respect to yield and selectivity (*16*). Concerning the catalyst, the best results were generally obtained with allylpalladium chloride in the presence of triphenylphosphine. As a result of the high reactivity of the chelated enolates, the allylations already take place under very mild conditions at –78 °C, which has an extremely positive effect on the selectivity of the reaction.

In the reaction of **10** with the allyl carbonate **11** the yield was very good and the diastereoselectivity excellent (Scheme 4). The diastereomerically pure product **12** was accessible after a single crystallization step. As determined by X-ray structure analysis, the *anti*-product was preferentially obtained from the palladium-catalyzed allylic alkylation. Thus, two different procedures for the synthesis of substituted γ,δ-unsaturated amino acids are available, which complement each another. The *syn* diastereomers can be obtained by ester enolate Claisen rearrangement, and the *anti*-diastereomers *via* palladium-catalyzed allylic substitution.
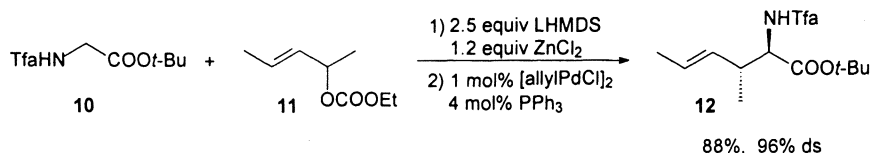
Scheme 4. Diastereoselective allylic alkylations

Synthesis of Stannylated Amino Acids *via* Allylic Alkylation

Although a wide range of substituents can be introduced diastereoselectively into a glycin ester *via* this protocoll, a even more flexible concept would be the introduction of a side chain which can be further modified later on. While heterofunctionalizations of the double bond are not a big deal, the more valuable C–C couplings, especially in a regioselective fashion are not a trivial issue. Crisp and Clink reported on Stille couplings using stannylated amino acids (*17*), a concept which is extremely suitable for this purpose, because Stille couplings proceed under neutral reaction conditions (*18*).

To use the allylic alkylation for the synthesis of such stannylated amino acids **14**, a straightforward approach towards the α-stannylated allyl carbonate **13** was required. In principle, stannylated allylic alcohols can easily be obtained

by hydrostannation of the corresponding propargylic alcohols (*19*). Unfortunately, with most methods isomeric mixtures are formed, containing the α-product as the minor isomer. But we were able to develope a new molybdenum catalyzed hydrostannation protocol which provided **13** in a highly regioselective fashion (*20*). The best results in the allylic alkylation were obtained, as usual, with the TFA-protected enolate **10'**, while the subsequent Stille couplings proceeded better with Cbz-protected derivatives **15** (Scheme 5). This approach allows the introduction of a wide range of different side chains (**16**, **17**) after only one single allylation step (*21*).
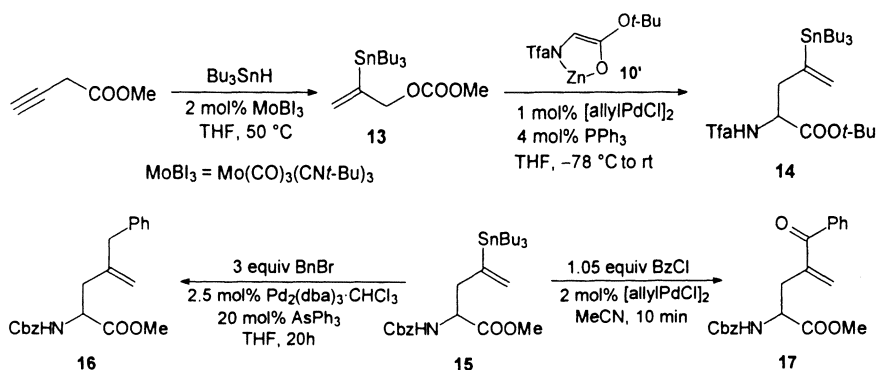
Scheme 5. Synthesis and reactions of stannylated amino acids

Asymmetric Allylic Alkylations in the Presence of Chiral Ligands

With respect that these unusual amino acids should be used as building blocks for drugs or natural products, an asymmetric version of this protocol is strongly desirable. One of the most popular approaches is the use of chiral ligands to introduce chirality. Substrates such as **18** or **20** with two identical substituents at both allyl termini form symmetrical, achiral π-allyl-palladium-complexes, and therefore the stereochemical outcome of the reaction can be controlled *via* chiral ligands on the palladium (*2*). This straightforward approach is elegant, but unfortunately more or less limited to such symmetrical substrates.

In order to assess the scope of this reaction, a representative set of substrates, including a large and a small acyclic and two cyclic allylic derivatives, was investigated (Scheme 6) (*22*). As chiral ligands phosphinooxazoline **L-2**, which is particularly suited for acyclic substrates, as well as phosphinomyrtanic acid **L-3**, which is better suited for cyclic compounds, were used (*23*).

High levels of selectivity were achieved with 1,3-diphenylallyl acetate (**18**) as substrate, especially with the chiral ligands **L-2**. Recrystallization of the crude product gave diastereo- and enantiomerically pure **19**. Allylic alkylations

of cyclic substrates such as cyclohexenyl acetate (**20**) led to cyclohexenyl glycine derivative **21**, respectively, which displays antibacterial activity and, therefore, is of particular interest (*24*). While **L-2** gave a nearly 1:1 diastereomeric mixture, with **L-3** the *syn*-product was formed preferentially. ee-Values of up to 93% could be obtained with these ligand, what is remarkable for such cyclic systems.
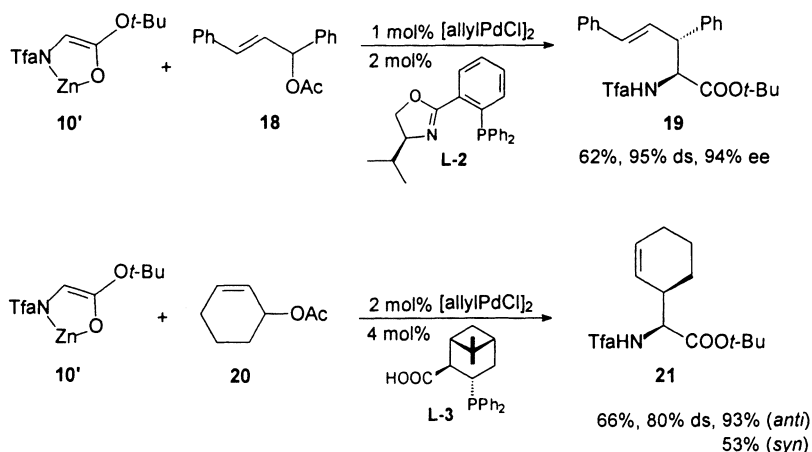
Scheme 6. Allylic alkylations in the presence of chiral ligands

Asymmetric Allylic Alkylations using Chiral Allylic Substrates

On the other hand, if allyl derivatives with different substituents are used, unsymmetrical π-allyl-palladium-complexes are formed. Attack of nucleophiles on these complexes in general provides mixtures of regioisomers, depending on the substitution pattern of the allyl moiety. Although this can be problematic, these substrates also have a big advantage: if optically active allyl substrates are used, the π-allyl-palladium-complexes formed are chiral, and nucleophilic attack on these complexes provides optically active substitution products.

Based on the reaction mechanism, the stereochemical control in the allyl fragment is not a problem (double inversion), in contrast to chiral centers formed in the 'nucleophile moiety'. The allylic substitutions with chiral allyl substrates (such as **21**) proceeded cleanly and with good yields (Scheme 7). The chirality could be transfered almost completely from the allyl derivative **21** to the product **22**. The only regioisomers obtained were those with the double bond in conjugation to the phenyl ring. The diastereoselectivities of the reaction were high, depending on the substitution pattern at the allyl moiety and the leaving
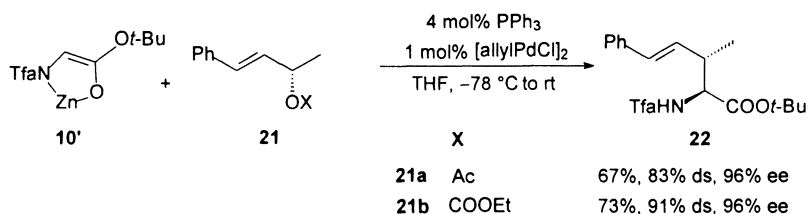
Scheme 7. Allylic alkylations using chiral allyl substrates

group. The methyl derivative was superior to other alkyl derivatives, probably because of steric reasons.

It was a striking feature that the diastereoselectivities obtained with the acetates were a little worse in comparison to those obtained with the carbonates. This is the result of the higher reactivity of the carbonates. While these react already at −78 °C (or even lower), the corresponding acetates in general start reacting around −60 °C.

Facing the π–σ–π-Isomerization Problem

Since the allylic substitution with chelated enolates already proceeds at these low temperatures, one might see a good chance to circumvent a nearly unsolved problem in palladium-catalyzed allylic alkylations: the π–σ–π-isomerzation. With respect to the reaction mechanism the configuration of the substitution product depends on the configuration of the π-allyl intermediates involved (25). Thus, the oxidative addition of palladium(0) to (E)-allyl-acetates and carbonates 21 leads to allyl complexes with syn/syn-configuration (21'), which reacts with nucleophiles to provide the corresponding (E)-substitution products such as 22 (Scheme 8). Quite different is the situation if (Z)-configured substrates such as 23 are used. In this case the anti/syn-complex 23' is formed. Reactions of 23' with nucleophiles would provide the (Z)-configured products 24 (attack a) or the (E)-configured product 24* (attack b) (26). But in general, these products are not obtained. Instead, the π–σ–π-isomerization causes a fast interconversion of the π-complexes via rotation of the σ-complexes A and B, normally preferring the syn-syn-complex 21', which gives rise to (E)-substitution products 22 and/or 22*.

A transfer of the (Z)-configuration from the allyl substrate 23 to the product 24 would only be possible if one could run the reaction at temperatures where isomerization reactions do not yet take place. Since the palladium-catalyzed allylic substitution with chelated enolates already proceeds at −78 °C, these
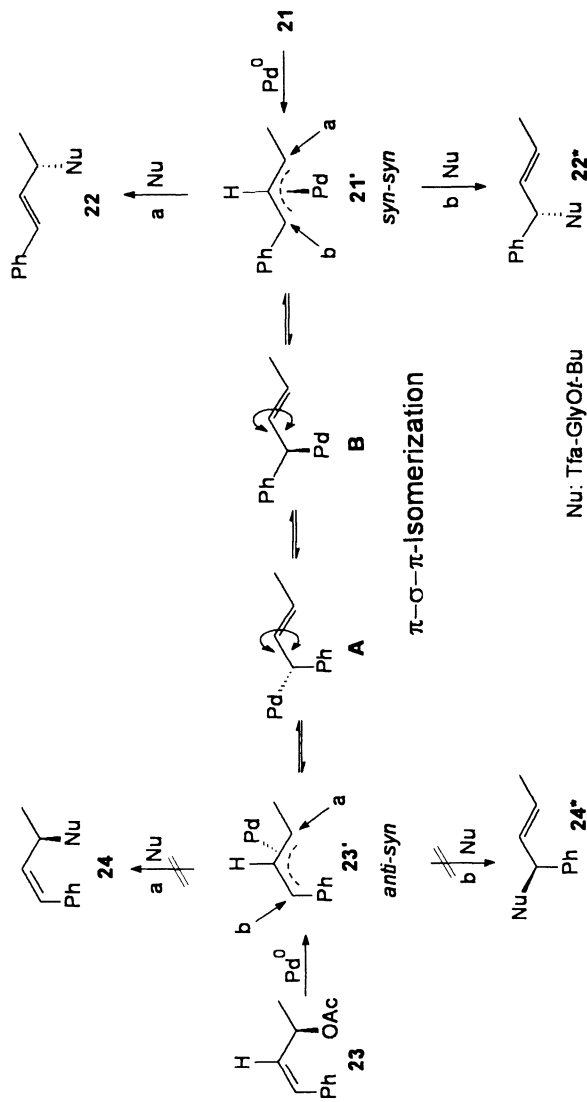
Scheme 8. π–σ–π-Isomerization of π-allyl-palladium complexes

nucleophiles have a good chance to fulfill these precondition. And indeed, reactions of (Z)-substrates with chelated enolate **10'** gave interesting results. The reaction with the (Z)-carbonate **25** almost exclusively yielded the desired (Z)-substitution product **24** (Z/E: > 99/1) (Scheme 9). The outstanding selectivities observed even surpassed the very good results of the reaction with the (E)-carbonate **21**. In contrast, the corresponding acetate **23** furnished a (E/Z)-mixture in a very low yield. The selectivities were markedly worse than those obtained with the carbonate **25**. This clearly indicated, that with the acetate both processes, direct allylic substitution and isomerization, compete with each other, while the carbonate reacts isomerization free.

Regioselective allylic alklyations

If the π-σ-π-isomerization can be suppressed like in this case, further interesting questions arise: What would happen to allylic substrates with (Z)-configuration and the same substituents at the allyl moiety (**26**)? In principle, there are two different reaction pathways, because the *anti/syn* π-allyl complex **26'** formed as an intermediate has two different allylic termini (Scheme 10). Nucleophilic attack at **a** (*anti*-position) would provide a product **27** with (E)-configuration of the double bond, whereas the attack at **b** (*syn*-position) would lead to a (Z)-double bond (**28**). Which position is the more reactive one, the *syn*-or the *anti*- position (*27*)?
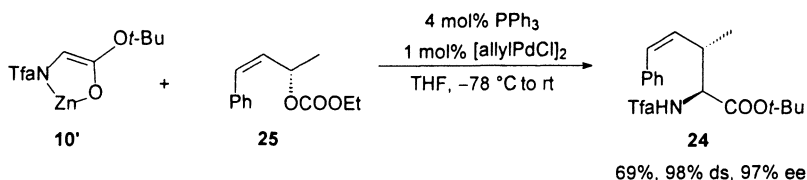
Scheme 9. Isomerization-free allylic alkylations

The clarification of this question is of major interest, because symmetrically substituted allyl derivatives are normally used in asymmetric catalyzed reactions. Even if enantiomerically pure allyl substrates are used, the chiral information gets lost during the reaction if, via π-σ-π-isomerization, an achiral *syn/syn* complex is formed. But if one can suppress the isomerization during the reaction of (Z)-configured substrates, and if one of the two allylic positions is pronouncedly more reactive than the other one, it should be possible to generate optically active compounds with these substrates as well.
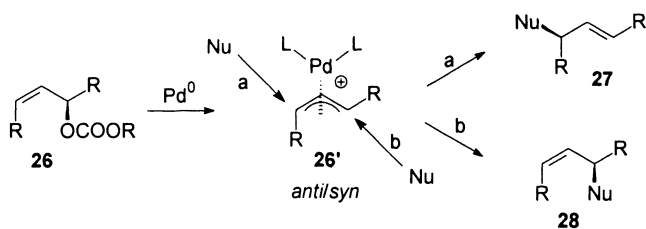
Scheme 10. Nucleophilic attack on anti/syn π-allyl complexes

The allylic alkylation with racemic **29** gave a very pleasing result (Scheme 11). The only product to be isolated was the (*E*)-configured product **30** with good yield and diastereoselectivity (*28*). The same product could also be obtained starting from the corresponding (*E*)-configured allylic substrate albeit, interestingly, with lower diastereoselectivity (65 % ds) (*16*). Keeping in mind, that the π-σ-π-isomerization should not play a significant role under these reaction conditions, one can conclude that the *anti* position is much more reactive than the *syn* position (*27*). For that reason, the investigation of a feasible transfer of chirality from optically active, symmetrically substituted (*Z*)-allyl substrates was obvious, and we scanned dimethyl derivatives such as (*S*)-**31**, which gave the chiral (*E*)-configured substitution product **32** exclusively in good yields. Also in these cases the selectivities, which the *anti*-products were formed with, were excellent. Moreover, the almost complete transfer of chirality shows that the reaction proceeds *via* the *anti/syn*-complex and not *via* the *syn/syn*-complex, which would inevitably lead to racemization.
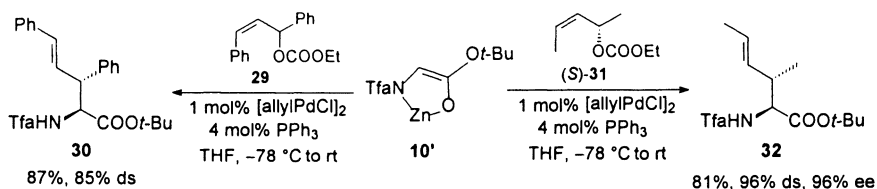
Scheme 11. Regioselective allylic alkylations using symmetrical allylic substrates

At this point, we became interested to see, whether the different reactivity of the two allylic positions can be utilized to control the regioselectivity (rs) of the nucleophilic attack on π-allyl complexes with similar substituents. First, we investigated the methyl propyl-substituted allylic carbonates **33** and **35** and found interesting results (Scheme 12). As we hoped in the reaction of substrate

33 the allylation product **34** was obtained nearly exclusively (98% rs) with a high preference for the *anti*-product (*29*). This is in good agreement with the observation made with carbonate **31**. Quite different was the situation if **35** was used as substrate. Nucleophilic attack at the more reactive *anti*-position of the π-allylpalladium complex should give rise to substitution product **36**, while attack at the *syn*-position should provide a (*Z*)-configured product (see Scheme 10). **36** was found to be the major product, albeit with a poor yield compared to the reaction of **33**, but instead of a byproduct bearing a (*Z*)-double bond, (*E*)-configured **34** was obtained in nearly equal amount. Moreover, the diastereoselectivities the *anti*-products are formed with were significantly lower. Obviously in this case, nucleophilic attack at the propyl-substituted *anti*-position of the metal complex is – probably for steric reasons – slower than the competing π-σ-π-isomerization, giving rise to the corresponding *syn/syn*-complex which shows no significant selectivity regarding the nucleophilic attack at the allylic positions.

If steric arguments are responsible for this different behavior, one might expect better results if the propyl group is replaced by a sterically less demanding one, such as a propynyl group. Therefore, substrate **37** was investigated and indeed, in the reaction with enolate **10'** only one regioisomer **38** could be observed, which was obtained with excellent stereoselectivity.
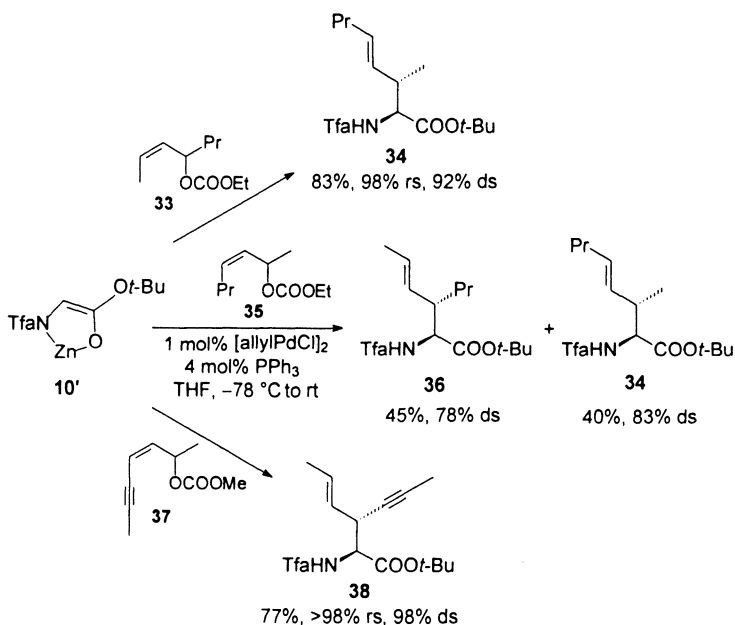
Scheme 12. Regioselective allylic alkylations using unsymmetrical allylic substrates

Reactions *via* terminal π-Allyl-Complexes

Terminal π-allyl-complexes show an even higher tendency towards isomerization than 1,3-disubstituted derivatives. Therefore, we were interested to see if with our nucleophiles, even with these substrates, isomerization can be suppressed, at least in part. And indeed, with THP-protected (*Z*)-butendiolcarbonate **39** the required amino acid **40** was obtained with a nearly perfect transfer of the olefin geometry (*30*). A similar result was obtained with the chiral substrate **41**, which also showed a significant chiral induction from the allyl substrate to the α-position of the new formed amino acid **42**.

This chiral induction could even be increased by switching to a sterically more demanding protecting group such as TBDPS (**43**). Interestingly, with this substrate the (*E*)-configured product (**44**) was formed exclusively. Obviously, the formation of the π-allyl-Pd-complex is sterically hindered and the reaction requires a higher temperature to set in, probably a temperature range were isomerization is a fast process.
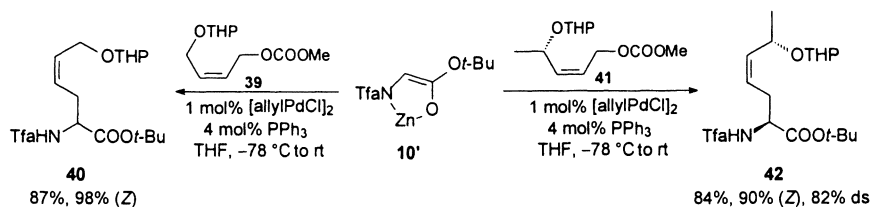
Scheme 13. Allylic alkylation with conservation of the (Z)-olefin geometry

But if isomerization takes place, it should be irrelevant if we use a (*Z*)- or an (*E*)-substrate **45**, or even the isomeric carbonate **46**. To prove this option we also investigated the reaction of these two isomers. Indeed, the result of the reaction was the same, independent of the configuration of the leaving group in **45**. The stereochemical outcome of the reaction is exclusively controlled by the stereogenic center adjacent to the π-allyl-complex (*31*).

This approach has been applied to the several (also α-methylated) amino acids such as **47–49**. It is worth to mention, that the diastereomer with the opposite configuration at the α-position can be obtained by using a coordinating protecting group as in compound **50** (*32*).

Stereoselective Peptide Modifications *via* Allylic alkylation

The high isomerization tendency of terminal π-allyl-complexes makes them good candidates for substrate controlled allylic alkylations. Because we came
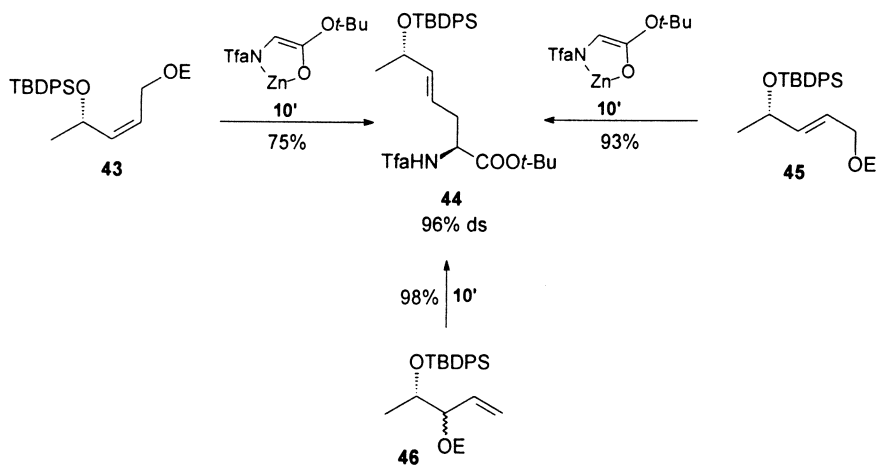
Scheme 14. Allylic alkylation with isomerization of the (Z)-olefin geometry
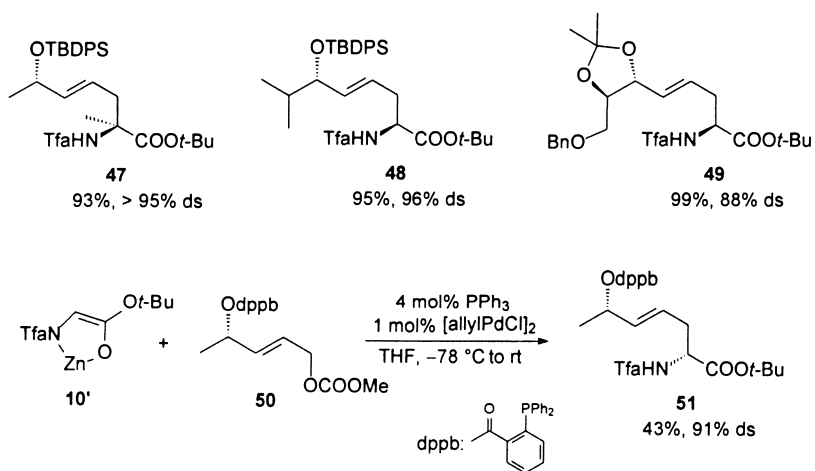
Scheme 15. Substrate-controlled allylic alkylations

into this business *via* our peptide modification program (*13*), we of course were highly interested to see, if we can use this approach also for the stereoselective modification of peptides. Based on the good results obtained with TFA-protected *t*-butyl glycinate **10** we used this protecting group combination also for our investigations of dipeptides **52**, and obtained excellent results. Most amino acids gave yields and selectivities in the 90's. The best results were obtained with the aromatic amino acids, such as phenylalanine (R = Bn), but even the smallest amino acid alanine (R = CH$_3$) gave up to 83 % ds (*33*).
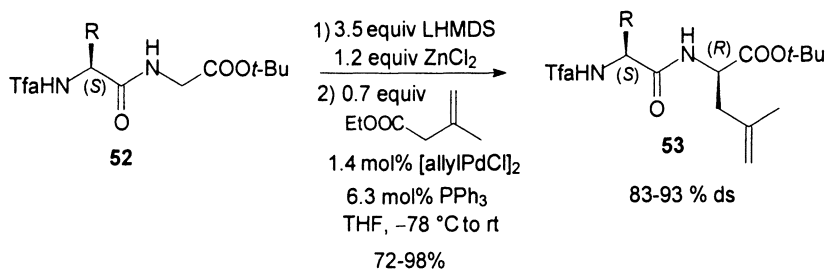
Scheme 16. Allylic Alkylations of Peptides

An (*S*)-amino acid in the peptide chain always induces an (*R*)-amino acid and the other way round. This can be explained *via* the formation of a metal peptide enolate complex, where the peptide coordinates three times towards the metal. In this enolate complex, one face of the enolate is shield by the side chain of the adjacent amino acid, generating the "opposite" configuration.

This approach is not only applicable to a wide range of different, also functionalized, peptides but also to rather complex allylic substrates, which allows the synthesis libraries of functionalized peptides, such as **54–59** (*34*).

To increase the flexibility of this protocol even further we took advantage of the Stille approach discussed above. With the stannylated allylcarbonates **60** the selectivites were even higher than with the "usual" substrates, probably because of the sterically more demanding stannylated π-allyl-complex (*35*).

The stannylated peptides **61** gave the different cross coupling products (**62**, **63**) in very high yield and without epimerization of the stereogenic centres. Last but not least the stannylated peptides **61** can undergo an umpolung *via* a tin-iodine-exchange. The halogenated peptides **64** obtained can now be coupled the other way round with several organometallics or in carbonylation reactions. In principle with only two to three steps large libraries of rather divergent peptides can be generated, what makes this approach interesting for the synthesis of drug like molecules and investigations of structure-activity relationships.
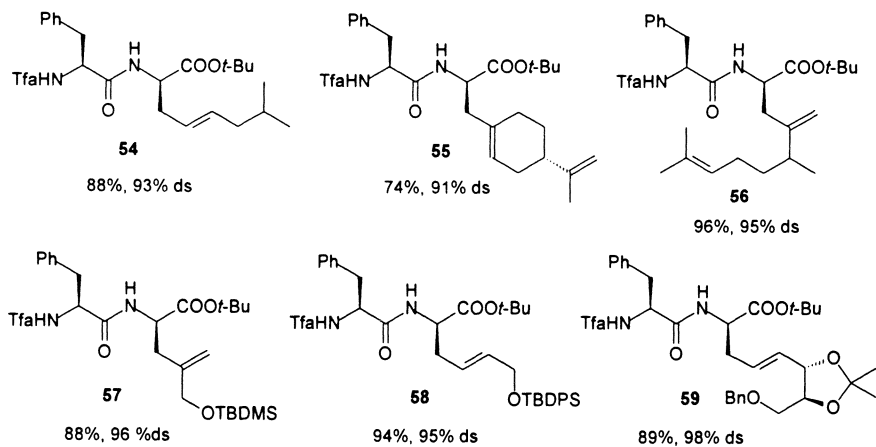
Scheme 17. Stereoselective modifications of dipeptides
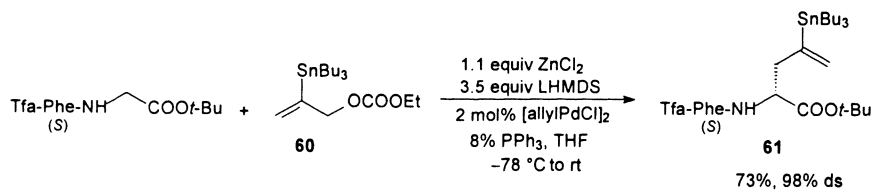
Scheme 18. Synthesis of stannylated peptides
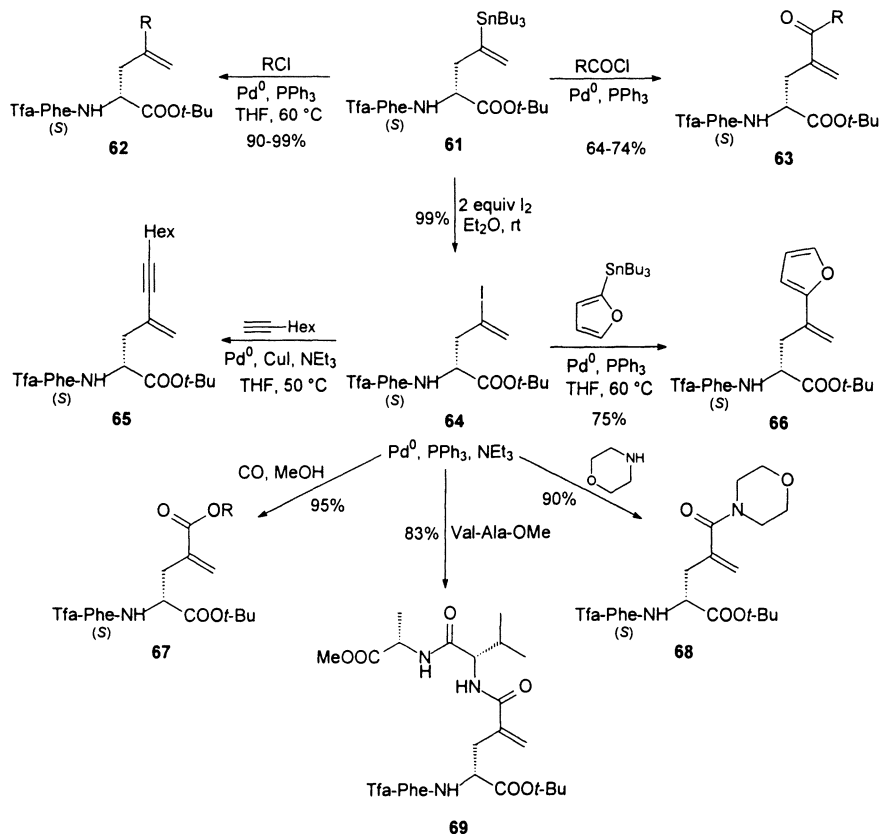
Scheme 19. Generation of Peptide Libraries from Stannylated Peptides.

Acknowledgements

References

1. (a) Hegedus, L. S. *Transition Metals in the Synthesis of Complex Organic Molecules*, University Science Books, Mill Valley, CA, 1994; *Organische*

Synthese mit Übergangsmetallen, VCH, Weinheim, 1995. (b) Kazmaier, U.; Pohlman, M. "Cross Coupling Reactions *via* π-Allyl-Intermediates" in *Metal Catalyzed C-C and C-N Coupling Reactions*; de Meijere, A.; Diederich F., Ed.; Wiley-VCH, Weinheim, 2004, pp 531-583, and references cited therein.

2. (a) Consiglio, G.; Waymouth, R. M. *Chem. Rev.* **1989**, *89*, 257-276. (b) Trost, B. M.; Van Vranken, D. L., *Chem. Rev.* **1996**, *96*, 395-422. (c) Helmchen, G. *J. Organomet. Chem.* **1999**, *576*, 203-214. (d) Pfaltz, A.; Lautens, M. In *Comprehensive Asymmetric Catalysis I–III*; Jacobsen, E. N.; Pfaltz, A.; Yamamoto, H., Eds., Springer, Berlin, 1999, pp. 833-884. (e) Acemoglu, L.; Williams, J. M. J. In *Handbook of Organopalladium Chemistry for Organic Synthesis*, Vol. 2; Negishi, E.-I.; de Meijere, A., Ed.; John Wiley, New York, 2002, pp. 1689-1705. (f) Tsuji, J. In *Handbook of Organopalladium Chemistry for Organic Synthesis*, Vol. 2; Negishi, E.-I.; de Meijere, A., Ed.; John Wiley, New York, 2002, pp. 1669–1688. (g) Trost, B. M.; Crawley, M. L. *Chem. Rev.* **2003**, *103*, 2921-2944. (h) Trost, B. M.; Jiang, C. *Org. Lett.* **2003**, *5*, 1563-1565. (i) Trost, B. M.; Xu, J. *J. Am. Chem. Soc.* **2005**, *127*, 2846-2847. (k) Trost, B. M.; Brennan, M. K. *Org. Lett.* **2006**, *8*, 2027-2030.

3. Abellán, T.; Nájera, C.; Sansano, J. M. *Tetrahedron Asym.* **1998**, *9*, 2211-2214.

4. Trost, B. M.; Ariza, X. *J. Am. Chem. Soc.* **1999**, *121*, 10727-10737.

5. Trost, B. M.; Lee, C. B. *J. Am. Chem. Soc.* **1998**, *120*, 6818-6819.

6. Reviews: (a) Kazmaier, U. *Amino Acids* **1996**, *11*, 283-299. (b) Kazmaier, U. *Recent Res. Dev. Org. Chem.* **1998**, *2*, 351-358.

7. (a) Grandel, R.; Kazmaier, U.; Nuber, B. *Liebigs Ann.* **1996**, 1143-1150. (b) Grandel, R.; Kazmaier, U. *Eur. J. Org. Chem.* **1998**, 409-417. (c) Grandel, R.; Kazmaier, U. *J. Org. Chem.* **1998**, *63*, 4524-4528.

8. (a) Mendler, B.; Kazmaier, U. *Org. Lett.* **2005**, *7*, 1715-1718. (b) Mendler, B.; Kazmaier, U.; Huch, V.; Veith, M. *Org. Lett.* **2005**, *7*, 2643-2646. (c) Mendler, B.; Kazmaier, U. *Synthesis* **2005**, 2239-2245. (d) Schmidt, C.; Kazmaier, U. *Eur. J. Org. Chem.* **2008**, 887-894.

9. (a) Kazmaier, U. *Angew. Chem.* **1994**, *106*, 1046-1047; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 998-999. (b) Kazmaier, U. *Liebigs Ann. Chem./Recueil* **1997**, 285-295.

10. (a) Kazmaier, U.; Schneider, C. *Synlett*, **1996**, 975-977. (b) Kazmaier, U.; Schneider, C. *Tetrahedron Lett.* **1998**, *39*, 817-818. (c) Kazmaier, U.; Schneider, C. *Synthesis* **1998**, 1321-1326.

11. (a) Kazmaier, U.; Krebs, A. *Angew. Chem.* **1995**, *107*, 2213-2214; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2012-2013. (b) Krebs, A.; Kazmaier, U. *Tetrahedron Lett.* **1996**, *37*, 7945-7946. (c) Kazmaier, U.; Mues, H.; Krebs, A. *Chem. Eur. J.* **2002**, *8*, 1850-1855.

12. For reviews on enolate-based peptide modifications see: (a) Seebach, D. *Angew. Chem.* **1988**, *100*, 1685-1715; *Angew. Chem. Int. Ed., Engl.* **1988**, *27*, 1624-1654. (b) Seebach, D. *Aldrichimica Acta* **1992**, *25*, 59-66. (c) Seebach, D.; Beck, A. K.; Studer, A., in *Modern Synthetic Methods, Vol. 7* (Eds.: B. Ernst, C. Leumann), Helvetica Chimica Acta/VCH, Basel/Weinheim, **1995**, pp. 1-179. (d) Severin, K.; Bergs, R. Beck, W. *Angew. Chem.* **1998**, *110*, 1722–1743; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1634-1654. (e) Seebach, D.; Beck, A. K.; Bossler, H. G.; Gerber, C.; Ko, S. Y.; Murtiashaw, C. W.; Naef, R.; Shoda, S.-I.; Thaler, A.; Krieger, M.; Wenger, R. *Helv. Chim. Acta* **1993**, *76*, 1564-1590. (f) Deska, J.; Kazmaier U. *Curr. Org. Chem.* **2008**, in press, and references cited therein.

13. (a) Kazmaier, U. *J. Org. Chem.* **1994**, *59*, 6667-6670. (b) Kazmaier, U.; Maier, S. *J. Chem. Soc., Chem. Commun.* **1998**, 2535-2536. (c) Kazmaier, U.; Maier, S. *J. Org. Chem.* **1999**, *64*, 4574-4575. (d) Kazmaier, U.; Maier, S. *Org. Lett.* **1999**, *1*, 1763-1766. (d) Kazmaier, U. Maier, S.; Zumpe, F. L. *Synlett* **2000**, 1523-1535.

14. (a) Overman, L. E. *Angew. Chem.* **1984**, *96*, 565-573; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 579-587. (b) Overman, L. E.; Campbell, C. B. *J. Org. Chem.* **1976**, *41*, 3338-3340. (c) Overman, L. E.; Campbell, C. B.; Knoll, F. M. *J. Am. Chem. Soc.* **1978**, *100*, 4822-4834.

15. (a) Böhm, A.; Beck, W. *J. Organomet. Chem.* **1999**, *588*, 247-255. (b) Böhm, A.; Schreiner, B.; Steiner, N.; Urban, R.; Sünkel, K.; Polborn, K.; Beck, W. *Z. Naturforsch.* **1998**, *53b*, 191-205.

16. Kazmaier, U.; Zumpe, F. L. *Angew. Chem.* **1999**, *111*, 1572-1574; *Angew. Chem. Int. Ed.* **1999**, *38*, 1468-1470.

17. (a) Crisp, G. T.; Glink, P. T. *Tetrahedron Lett.* **1992**, 33, 4649-4652. (b) Crisp, G. T.; Glink, P. T. *Tetrahedron* **1994**, *50*, 3213-3234.

18. Stille, J. K. *Angew. Chem.* **1986**, *98*, 504-519; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508-523.

19. Review: Trost, B. M.; Ball, Z. T. *Synthesis* **2005**, 853-887.

20. (a) Kazmaier, U.; Schauß, D.; Pohlman, M. *Org. Lett.* **1999**, *1*, 1017-1019. (b) Kazmaier, U.; Pohlman, M.; Schauß, D. *Eur. J. Org. Chem.* **2000**, 2761-2766.

21. (a) Kazmaier, U.; Schauß, D.; Pohlman, M.; Raddatz, S. *Synthesis* **2000**, 914-917. (b) Kazmaier, U.; Schauß, D.; Raddatz, S.; Pohlman, M. *Chem. Eur. J.* **2001**, *7*, 456-464.

22. Weiss, T. D.; Helmchen, G.; Kazmaier, U. *Chem. Commun.* **2002**, 1270-1271.

23. Review: Helmchen, G. *J. Organomet. Chem.* **1999**, *576*, 203-214.

24. Cramer, U.; Rehfeldt, A. G.; Spener, F. *Biochemistry* **1980**, *19*, 3074-3080.

25. Consiglio, G.; Waymouth, R. M. *Chem. Rev.* **1989**, *89*, 257-276.

26. Corradini, P.; Maglio, G.; Musco, A.; Paiaro, G. *J. Chem. Soc., Chem. Commun.* **1966**, 618.

27. (a) Åkermark, B.; Hansson, S.; Vitagliano, A. *J. Am. Chem. Soc.* **1990**, *112*, 4587-4588. (b) Sjögren, M.; Hansson, S.; Norrby, P.-O.; Åkermark, B.; Cucciolito, M. E.; Vitagliano, A. *Organometallics* **1992**, *11*, 3954-3964.

28. Kazmaier, U.; Zumpe, F. L. *Angew. Chem.* **2000**, *112*, 805-807; *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 802-804. Kazmaier, U.; Zumpe, F. L. *Eur. J. Org. Chem.* **2001**, 4067-4076.

29. Kazmaier, U.; Pohlman, M. *Synlett* **2004**, 623-626.

30. Kazmaier, U.; Krämer, K. *J. Org. Chem.* **2006**, *71*, 8930-8953.

31. Kazmaier, U.; Lindner, T. *Angew. Chem.* **2005**, *117*, 3368-3371; *Angew. Chem. Int. Ed.* **2005**, *44*, 3303-3306.

32. Lindner, T.; Kazmaier, U. *Adv. Synth. Catal.* **2005**, 1687-1695.

33. Kazmaier, U.; Deska, J.; Watzke, A. *Angew. Chem.* **2006**, *118*, 4973-4976; *Angew. Chem. Int. Ed.* **2006**, *45*, 4855-4858.

34. Deska, J.; Kazmaier, U. *Chem. Eur. J.* **2007**, *13*, 6204-6211.

35. Deska, J.; Kazmaier U. *Angew. Chem.* **2007**, *119*, 4654-4657; *Angew. Chem. Int. Ed.* **2007**, *46*, 4570-4573.

Chapter 11

Development of Phase-Transfer-Catalyzed Asymmetric Strecker Reaction Based on the Molecular Design of Chiral Quaternary Ammonium Salts

Takashi Ooi

Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464–8603, Japan

Asymmetric Strecker reaction of aldimines using aqueous potassium cyanide (KCN) as a cyanide source has been devised through the molecular design of chiral phase-transfer catalyst **9** bearing a stereochemically defined tetranaphthyl backbone. Further, highly efficient, enantioselective synthesis of *N*-protected α-amino nitriles from the corresponding α-amido sulfones under similar biphasic conditions is also described, featuring its practical advantages.

Cyanation of imines, the second step in the Strecker reaction, represents one of the most direct and viable methods for the synthesis of α-amino acids and their derivatives. Primarily because of the importance of optically active α-amino acids (*1*), stereochemical control of this valuable carbon-carbon bond-forming reaction has been the focus of extensive study. Actually, numerous efforts have been devoted for the last decade to the development of chiral catalysts to effect the enantioselective Strecker reaction of aldimines and ketimines (*2*). However, most of the previously elaborated catalytic asymmetric Strecker methodologies rely on either alkylmetal cyanide such as trimethylsilyl cyanide or anhydrous hydrogen cyanide generally at low temperature, which poses an important problem to be addressed particularly when large-scale industrial applications are considered (*2,3*). In this regard, we have been

interested in the possibility of employing potassium cyanide (KCN) as a cyanide source within the context of our continuous research program toward the development of new and practical synthetic strategies in the realm of asymmetric phase-transfer catalysis *(4,5)*.   In this chapter, we describe the highly enantioselective Strecker reaction of *N*-arylsulfonyl aldimines under toluene-aqueous KCN biphasic conditions based on the molecular design of chiral quaternary ammonium salts **2** bearing tetranaphthyl backbone as an efficient phase-transfer catalyst *(6)*. Furthermore, we document that the in situ generation of the reactive imines from the corresponding α-amido sulfones leads to the improvement of both chemical yield and stereoselectivity *(7,8)*.

Reaction System and Use of *N*-Spiro-type Catalyst

We initiated our search for an appropriate reaction system with an imine of cyclohexanecarboxaldehyde (**1a**) as a representative substrate in order to realize its smooth cyanation under biphasic conditions consisting of organic solvent and KCN aqueous solution. For this purpose, a protecting group (PG) of the imine nitrogen was screened in the cyanation with 2 M KCN aqueous solution (1.2 equiv) in toluene-H₂O (volume ratio = 1:3) at 0 °C using tetrabutylammonium bromide (TBAB, 5 mol%) as catalyst. While none of the product formation was detected in the reactions with substrates having *p*-methoxyphenyl or benzhydryl group even after stirring at room temperature (entries 1 and 2 in Table 1), cyanation of *N-p*-toluenesulfonyl imine proceeded to give the desired protected α-amino nitrile **2a** (PG = SO₂Tol) in 76% isolated yield (entry 3). The marked decrease of the yield observed in the control experiment without TBAB suggested the essential role of the catalyst, extracting a cyanide anion from the aqueous phase as an ammonium cyanide with enhanced nucleophilicity (entry 4). These results led us to examine the possibility of asymmetric induction by the use of a series of *N*-spiro chiral quaternary ammonium bromide (**3a-3c**, 1 mol%, Figure 1) as catalyst, which were quite effective for the phase-transfer-catalyzed alkylation of glycine derivatives *(4e)*. Unfortunately, however, it turned out that the observed enantiomeric excesses were less than 10% ee, though **3a-3c** were reactive enough to catalyze the cyanation as also included in Table 1 (entries 5-7).

Design, Synthesis and Evaluation of New Catalysts

Based on these initial observations, we decided to design new chiral quaternary ammonium salts that would exert sufficient reactivity and stereoselectivity. Our prime concern at this stage was to endow the ammonium

Table 1. Effect of Nitrogen Protecting Group and Results of Asymmetric Cyanation Using (*R,R*)-3 as Catalyst
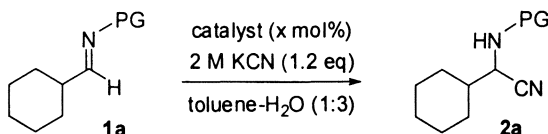
entry	PG	catalyst (x)	conditions	yield (%)	ee (%)^a
1	PMP	TBAB (5)	0 °C ~r.t., 24 h	n.r.	–
2	CHPh$_2$	TBAB (5)	0 °C ~r.t., 24 h	n.r.	–
3	SO$_2$Tol	TBAB (5)	0 °C, 30 min	76	–
4	SO$_2$Tol	–	0 °C, 30 min	15	–
5	SO$_2$Tol	3a (1)	0 °C, 10 h	77	rac.
6	SO$_2$Tol	3b (1)	0 °C, 8 h	91	10
7	SO$_2$Tol	3c (1)	0 °C, 7 h	82	3

^{a} Determined by chiral HPLC analysis (DAICEL Chiralpak AD-H). PMP = *p*-methoxyphenyl. TBAB = tetrabutylammonium bromide. n.r. = no reaction. Tol = *p*-tolyl.
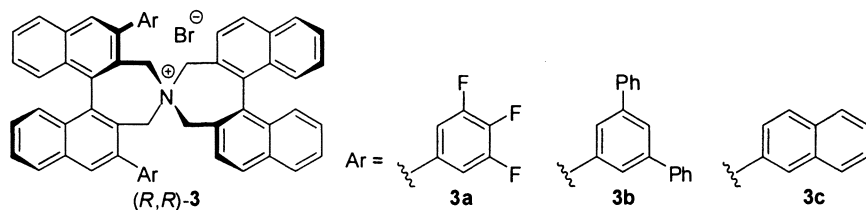
Figure 1. Representative N-Spiro-type Chiral Phase-Transfer Catalysts.

cation with an optimal structure for achieving both a facile anion extraction and a precise enantiofacial discrimination of the prochiral imine. Actual design plan toward this end was first to get rid of one of the two chiral binaphthyl subunits from the parent structure of *N*-sipro-type **3**, and replace it by simple alkyl substituents (R) to impart hydrophilicity to the salt (Figure 2). Second, introduction of *ortho*-substituted phenyl groups at 3,3'-positions of chiral binaphthyl unit was considered with the expectation that the *ortho*-substituents caused rotational restriction around the naphthyl-phenyl biaryl axes, which would provide a configurational bias to create a stereochemically defined molecular cavity over the nitrogen. According to this molecular design, three diastereomers are conceivable as exemplified in Figure 2 [R = Me, *ortho*-substituent = Ph (**4**)], and two symmetric isomers would offer a suitable binding pocket for the imine substrate, possibly exhibiting expected catalytic and chiral efficiencies.

(R,R)-3a (Ar = 3,4,5-F₃-C₆H₂)

asymmetric **4**   symmetric **4**
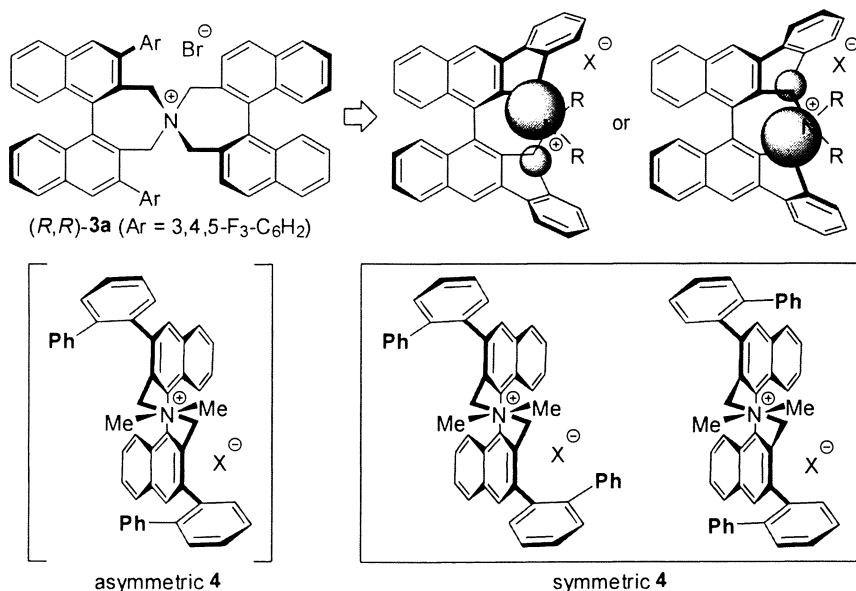
Figure 2. Design Plan of New Chiral Quatrenary Ammonium Salts.

The synthesis of **4** was then implemented in a six-step sequence starting from (*R*)-binaphthyl dicaboxylate **5** as illustrated in Scheme 1. The *ortho*-magnesiation-halogenation technique we developed (*9*) was effective for the neopentyl ester **5**, furnishing **6** in 71% yield. Subsequent reduction of the ester moieties, bromination and alkylation with methyl amine afforded the key intermediate **7**, which was subjected to the Suzuki-Miyaura cross-coupling conditions with *ortho*-phenylphenyl boronic acid. Although we assumed that three diastereomers might be formed after the installment of the 3,3'-*ortho*-phenylphenyl groups, the desired **8** was obtained as a mixture of two stereoisomers, symmetric and asymmetric ones, in a ratio of 4:1. We were fortunately able to obtain **4** in a pure form through the quaternization with methyl iodide and purification with silica gel column chromatography (*10*).

To unequivocally determine the three-dimensional molecular structure of **4**, the single crystal X-ray diffraction analysis was conducted after derivatization to the corresponding hexafluorophosphate (**4**-PF₆) and recrystallization from THF-hexane solvent system at 0 °C, revealing its (*R,R,R*) configuration (Figure 3). As expected, each phenyl substituent at 3,3'-positions of the binaphthyl unit is nearly perpendicular to the attached naphthalene ring, sticking over the central cationic nitrogen. Further, the pendant *ortho*-phenyl group is parallel to the other and contributes to extend the aromatic surface to offer an ample reaction cavity, which could hold the imine functionality within an ideal proximity to the
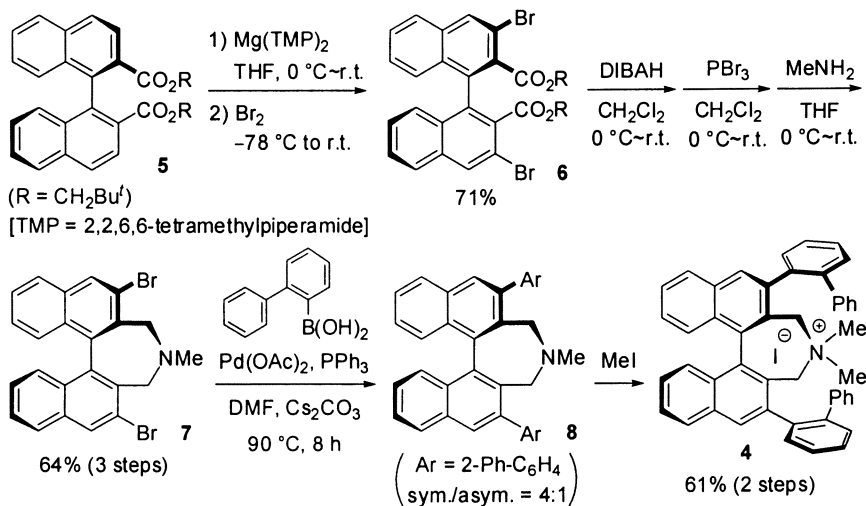
Scheme 1. Synthesis of Chiral Quaternary Ammonium Iodide 4.

ORTEP diagram of **4-PF₆**
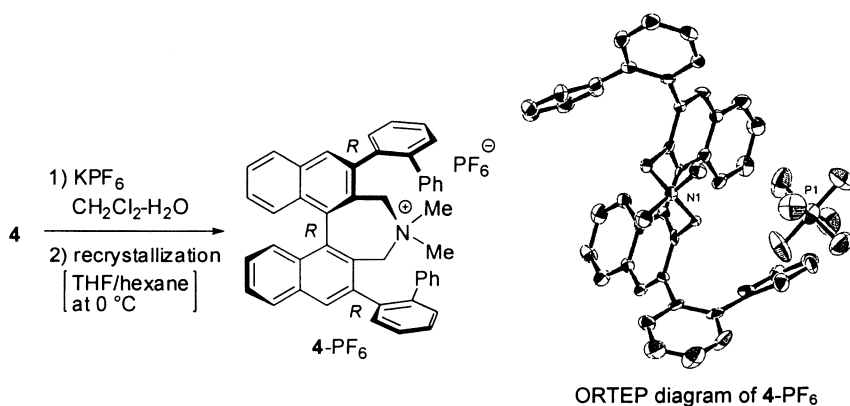
Figure 3. Preparation and Structural Determination of 4-PF₆.

cyanide anion located inside the chiral environment, enabling an efficient and stereoselective bond formation.

To examine this hypothesis, we evaluated the potential of **4** as a chiral phase-transfer catalyst by performing the asymmetric cyanation of **1a** (PG = SO_2Tol) under the biphasic conditions in the presence of **4**. Thus, a mixture of **1a** (PG = SO_2Tol) and 1 mol% of **4** in toluene-KCN aqueous solution (1.2 equiv) was vigorously stirred at 0 °C. The TLC monitoring indicated the complete consumption of the substrate after 2 h, and the desired protected amino nitrile **2a** (PG = SO_2Tol) was isolated in 84% yield with enantiomeric excess of 24% ee (entry 1 in Table 2). Here, employment of *N*-mesitylenesulfonyl imine **1a** (PG = SO_2Mes) enhanced the enantioselectivity to 57% ee, though longer reaction time was required (entry 2). These promising results apparently supported the direction of the molecular design of the catalyst, which led us to undertake further structural modification of **4** by replacing the 3,3'-substituents with 2-phenyl-1-naphthyl group for critical improvement of the stereoselectivity (**9a** in Figure 4). The chiral quaternary ammonium iodide **9a** possessing stereochemically homogeneous tetranaphthyl backbone was prepared in a manner similar to that of **4**. Subsequent crystallographic analysis uncovered its enlarged chiral molecular cavity as shown in Figure 4, implying the feasibility of more accurate enantiofacial recognition. Indeed, the catalyst **9a** exhibited high chiral efficiency in the reaction of **1a** (PG = SO_2Mes) with aqueous KCN under similar conditions to afford **2a** (PG = SO_2Mes) with 89% ee (entry 3). Moreover, the additional phenyl substituents at 4-positions of the outer naphthyl units (**9b** in Figure 4) were revealed to be associated with even more rigorous enantiocontrol (90% ee) (entry 4). Finally, we found that the introduction of electron-withdrawing trifluoromethyl group to all the appended phenyl groups (**9c**) delivered a beneficial effect on the enantioselectivity (entry 5), and the phase-transfer cyanation of **1a** (PG = SO_2Mes) with 1.5 equiv of KCN under the influence of **9c** was completed within 2 h to produce **2a** (PG = SO_2Mes) in 89% yield with 95% ee (entry 6).

Other selected examples listed in Table 3 clearly demonstrate the effectiveness of this asymmetric Strecker protocol for a variety of aliphatic aldimines. In general, 1 mol% of **9c** with 1.5 equiv of KCN was sufficient for the smooth reaction, and the corresponding protected amino nitrile **2** was obtained uniformly in high yield with excellent enantioselectivity. Emphasized is the fact that the present system nicely accommodates the substrates having α-*tert*-alkyl substituents such as pivalaldimine (entries 5-7), thereby enabling a facile synthesis of enantiomerically enriched *tert*-leucine and its various analogues, a series of considerably useful chiral building blocks not accessible by the asymmetric alkylation of glycine derivatives (*4*).

The mesitylenesulfonyl moiety of the optically active Strecker product **2** can be readily cleaved by the treatment with methanesulfonic acid in TFA-

Table 2. Optimization of the Asymmetric Strecker Reaction of 1a with Respect to Arylsulfonyl Group and Catalyst Structure
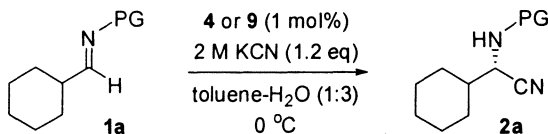
entry	PG	catalyst	react. time (h)	yield (%)	ee (%)^a (config)^b
1	SO$_2$Tol	4	2	84	24 (S)
2	SO$_2$Mes	4	8	81	57 (S)
3	SO$_2$Mes	9a	4	83	89 (S)
4	SO$_2$Mes	9b	4	90	90 (S)
5	SO$_2$Mes	9c	4	89	94 (S)
6^c	SO$_2$Mes	9c	4	89	95 (S)

^{a} Determined by chiral HPLC analysis (DAICEL Chiralpak AD-H). Mes = mesityl. ^{b} For assignment of the absolute configuration, see Scheme 2. ^{c} With 1.5 equiv of 2 M KCN aqueous solution.

ORTEP diagram of 9a

9b (Ar^1 = Ar^2 = Ph)
9c (Ar^1 = Ar^2 = p-CF$_3$-C$_6$H$_4$)
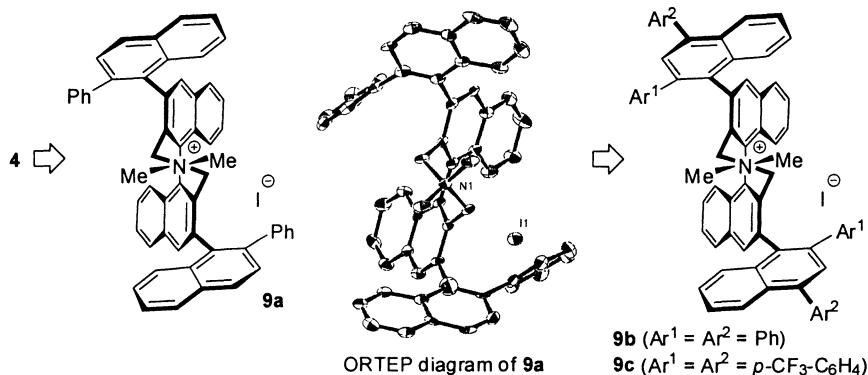
Figure 4. Modification of 4 to Tetranaphthyl-type 9 and Its Structural Determination by X-ray Analysis.

Table 3. Substrate Scope of the Asymmetric Strecker Reaction
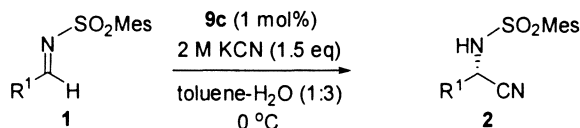
entry	1 (R^1)	react. time (h)	yield (%)	ee (%)^a
1	c-Oct	2	88	97
2	i-Pr	3	85	93
3	Ph(CH$_2$)$_2$	2	81	90
4	(CH$_3$)$_2$CHCH$_2$	3	82	88
5	t-Bu	3	94	94
6	Ph(CH$_3$)$_2$C	8	95	98
7	Ad	8	98	97

^{a} Determined by chiral HPLC analysis (DAICEL Chiralpak AD-H).
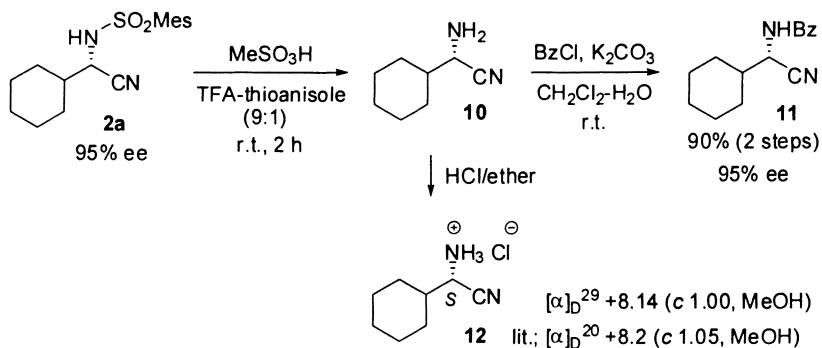
Scheme 2. Deprotection and Assignment of Absolute Configuration.

thioanisole at room temperature as exemplified in Scheme 2 (*11*), and complete preservation of the enantiomeric excess was confirmed by HPLC analysis after derivatization of amino nitrile **10** to its *N*-benzoate **11**. The absolute configuration of **2a** was assigned to be *S* by comparing the optical rotation of amino nitrile hydrochloride **12** with the reported value (*12*).

In Situ Generation of Sulfonyl Imines

As described above, the phase-transfer-catalyzed, highly enantioselective cyanation of *N*-mesitylenesulfonyl aldimines using aqueous KCN as a cyanide source has been devised through the molecular design of a chiral quaternary ammonium iodide **9c** as catalyst. Although this practical asymmetric Strecker protocol tolerates a wide range of aliphatic aldimines, the chemical yield and enantioselectivity were still insufficient in the reactions with secondary and, particularly, primary alkyl aldimines compared to tertiary alkyl ones (Table 3). We reasoned that it could be ascribed to the partial imine hydrolysis and uncatalyzed cyanation under biphasic conditions. To overcome these problems associated with the direct use of the reactive sulfonyl imines, we sought to employ *N*-mesitylenesulfonyl α-amido sulfones as a suitable starting substrate for the in situ generation of the imine under similar liquid-liquid phase-transfer conditions (*13,14*).

The requisite *N*-mesitylenesulfonyl α-amido sulfone, actually a synthetic precursor of the corresponding aldimine, can be readily prepared from aldehydes and mesitylenesulfonamide and easily purified by simple recrystallization (*6*). With cyclohexanecarboxaldehyde-derived α-amido sulfone **13a** as a representative substrate, initial attempt was made by exposure of **13a** to the biphasic conditions consisting of toluene and 2 M KCN aqueous solution (2 equiv) in the presence of **4** as catalyst. Complete consumption of **13a** was comfirmed after 2 h, and the desired *N*-mesitylenesulfonyl α-amino nitrile **2a** was produced in a quantitative yield with 60% ee (entry 1 in Table 4). Similar reactivity and selectivity were observed when the reaction was performed with 1.2 equivalents of KCN under otherwise identical condition (entry 2). The considerable improvement of the chemical yield and the enhanced enantioselectivity compared to the case with the imine as substrate clearly indicates that the intervention of imine hydrolysis and background cyanation is minimized, thereby releasing the full potential of the chiral phase-transfer catalysis of **4**. It should be noted that the amount of KCN can further be reduced to 1.05 equivalents without any detrimental effect on the reaction efficiency, thus solidifying the practical aspect of this system. Finally, switching the catalyst from **4** to **9c** enabled the quantitative isolation of highly enantioenriched **2a** as included in Table 4.

Table 4. Optimization of Reaction Conditions with Respect
to the Amount of KCN
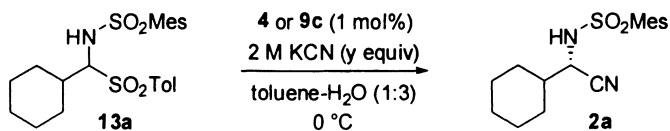
entry	Catalyst	KCN (y)	react. time (h)	yield (%)	ee (%)^a
1	4	2.0	2	99	60
2^b	4	1.2	1.5	99 (81)	61 (57)
3	4	1.05	1.5	99	63
4^b	9c	1.05	1.5	99 (89)	97 (95)

^{a} Determined by chiral HPLC analysis (DAICEL Chiralpak AD-H). ^{b} The results with the corresponding N-mesitylenesulfonyl aldimine as a starting substrate are shown in the parentheses.

    With the optimized conditions in hand, we conducted experiments to probe the scope of this asymmetric Strecker synthesis from α-amido sulfones, and representative results are summarized in Scheme 3. The reaction proceeded rapidly and cleanly with a variety of α-amido sulfones derived from α-branched and α-unbranched aldehydes including a highly enolizable one in the presence of 1 mol% of 9c and 1.05 equivalents of KCN aqueous solution, and the corresponding α-amino nitriles 2 were obtained in excellent chemical yields and constantly higher enantioselectivities. In particular, this method is quite effective for the asymmetric synthesis of an α-amino nitrile possessing a secondary alkyl α-substituent as represented by the cyanation starting from the substrate with N-benzyloxycarbonyl-4-piperidinyl group, in which the target product was isolated quantitatively in an essentially enantiopure form.

Conclusion

    We have achieved the phase-transfer-catalyzed, highly enantioselective cyanation of aldimines using aqueous KCN as an inexpensive and easy-to-handle cyanide source through the development of new chiral quaternary ammonium iodide 9c bearing a stereochemically defined tetranaphthyl backbone. Furthermore, remarkably efficient, high yielding asymmetric synthesis of N-mesitylenesulfonyl α-amino nitrile from the corresponding α-amido sulfones has also been accomplished under similar biphasic conditions in the presence of 9c, which obviates the preformation of the reactive aldimines having primary and

$$\underset{\substack{R^1 \quad SO_2Tol \\ \mathbf{13}}}{\overset{HN^{\diagup SO_2Mes}}{\bigwedge}} \quad \xrightarrow[\substack{toluene-H_2O \ (1:3) \\ 0\,°C, <2\,h}]{\substack{\mathbf{9c}\ (1\ mol\%) \\ 2\ M\ KCN\ (1.05\ eq)}} \quad \underset{\substack{R^1 \quad CN \\ \mathbf{2}}}{\overset{HN^{\diagup SO_2Mes}}{\bigwedge}}$$

primary

99%, 94% ee
(81%, 90% ee)

99%, 91% ee
(82%, 88% ee)

96%, 84% ee

93%, 85% ee

9c (Ar¹ = Ar² = 4-CF₃-C₆H₄)

secondary

99%, 98% ee
(88%, 97% ee)

99%, 97% ee
(85%, 93% ee)

99%, 97% ee
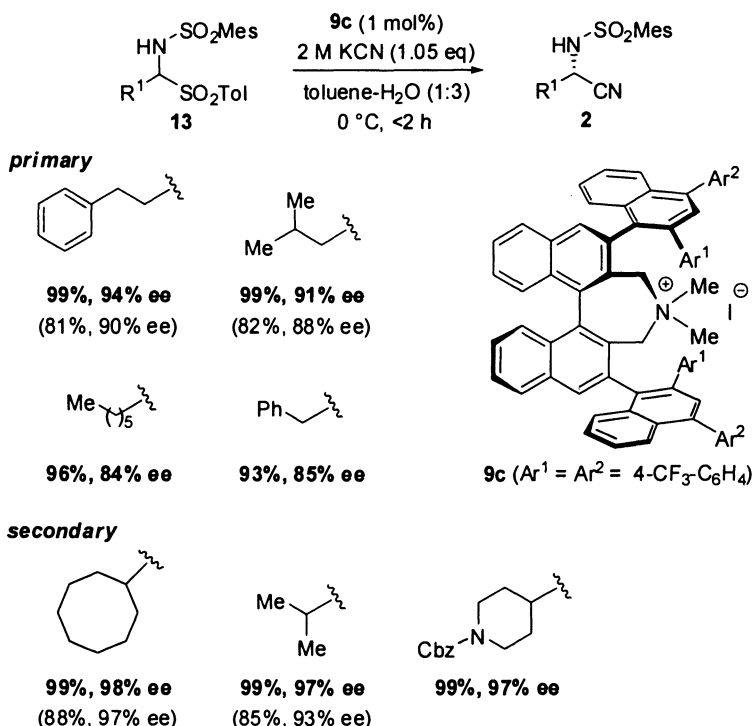
Scheme 3. Advantage and Generality of the Asymmetric Strecker Synthesis from α-Amido Sulfones. Results of the Reactions with Imines Are Shown in Parentheses.

secondary alkyl α-substituents and also allows for the reduction of KCN to nearly equimolar amount. This study represents an essentially new and powerful approach toward the asymmetric Strecker-type reactions, which holds distinctive practical advantages and should fulfill the recent continually increasing demand for the availability of a broad range of α-amino acids in diverse scientific disciplines.

Acknowlegements

References

1. For a recent review on the catalytic asymmetric synthesis of α-amino acids: Nájera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584-4671.
2. For reviews: (a) Enders, D.; Shilvock, J. P. *Chem. Soc. Rev.* **2000**, *29*, 359-373. (b) Yet, L. *Angew, Chem., Int. Ed.* **2001**, *40*, 875-877. (c) Gröger, H. *Chem. Rev.* **2003**, *103*, 2795-2828. (d) Spino, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 1764-1766. (e) Vachal, P.; Jacobsen, E. N. In *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N.; Pfaltz, A.; Yamamoto, H. Eds.; Springer: Berlin, 2004; Supplement 1, p 117-130.
3. For impressive studies on catalytic asymmetric acylcyanation of imines: (a) Pan, S. C.; Zhou, J.; List, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 612-614. (b) Pan, S. C.; List, B. *Org. Lett.* **2007**, *9*, 1149-1151 and recent examples of catalytic asymmetric Strecker reactions are cited therein.
4. For recent representative reviews on asymmetric phase-transfer catalysis: (a) O'Donnell, M. J. In *Catalytic Asymmetric Syntheses*, 2nd ed.; Ojima, I., Ed.; WILEY–VCH: New York, 2000; Chapter 10. (b) Shioiri T.; Arai, S. In *Stimulating Concepts in Chemistry*, Vogtle, F.; Stoddart, J. F.; Shibasaki, M., Eds.; WILEY-VCH: Weinheim, 2000; p. 123-143. (c) Albanese, D. *Mini-Rev. Org. Chem.* **2006**, *3*, 195-217. (d) Vachon, J.; Lacour, J. *Chimia* **2006**, *60*, 266-275. (e) Ooi, T.; Maruoka, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 4222-4266. (f) Hashimoto, T.; Maruoka, K. *Chem. Rev.* **2007**, *107*, 5656-5682. (g) *Asymmetric Phase Transfer Catalysis*, Maruoka, K. Ed.; WILEY-VCH: Weinheim, 2008.
5. For an example of using KCN in asymmetric synthesis: Belokon', Y. N.; Blacker, A. J.; Carta, P.; Clutterbuck, L. A.; North, M. *Tetrahedron* **2004**, *60*, 10433-10447 and references cited therein.
6. Ooi, T.; Uematsu, Y.; Maruoka, K. *J. Am. Chem. Soc.* **2006**, *128*, 2548-2549.
7. Ooi, T.; Uematsu, Y.; Fujimoto, J.; Fukumoto, K.; Maruoka, K. *Tetrahedron Lett.* **2007**, *48*, 1337-1340.
8. For a recent similar approach: Herrera, R. P.; Sgarzani, V.; Bernardi, L.; Fini, F.; Pettersen, D.; Ricci, A. *J. Org. Chem.* **2006**, *71*, 9869-9872.
9. Ooi, T.; Uematsu, Y.; Maruoka, K. *J. Org. Chem.* **2003**, *68*, 4576-4578.
10. For use of binaphthol derivatives with similar structural motif as a chiral metal ligand: Simonson, D. L.; Kingsbury, K.; Xu, M.-H.; Hu, Q.-S.; Sabat, M.; Pu, L. *Tetrahedron* **2002**, *58*, 8189-8193.
11. Costanzo, M. J.; Yabut, S. C.; Almond, H. R., Jr.; Andrade-Gordon, P.; Corcoran, T. W.; de Garavilla, L.; Kauffman, J. A.; Abraham, W. M.; Recacha, R.; Chattopadhyay, D.; Maryanoff, B. E. *J. Med. Chem.* **2003**, *46*, 3865-3876.
12. Effenberger, F.; Kremser, A.; Stelzer, U. *Tetrahedron: Asymmetry* **1996**, *7*, 607-618.

13. For recent excellent review on the utility of α-amido sulfones as precursors of *N*-acylimino derivatives: Petrini, M. *Chem. Rev.* **2005**, *105*, 3949-3977.

14. For pioneering study on the synthesis of *N*-protected α-amino nitriles from α-amido sulfones under phase-transfer conditions: Banphavichit, V.; Chaleawlertumpon, S.; Bhanthumnavin, W.; Vilaivan, T. *Synth. Commun.* **2004**, *34*, 3147-3160.

Chapter 12

Efficient Synthesis of α-Amino Acids via Organoboronate Reagents

Nicklas Selander and Kálmán J. Szabó[*]

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE–106 91, Stockholm, Sweden

This chapter describes the application of a three component coupling reaction of organoboronates, amines and appropriate aldehydes for synthesis of unnatural α-amino acids. This process can easily be combined with catalytic generation of organoboronates, and thus structurally diverse amino acids can be obtained from simple precursors, such as allylic alcohols, in a one pot process.

In the past decades there has been a large interest to design new general methods for synthesis of unnatural α-amino acids (1). One of the latest important developments in this field is based on application of organoboronates, first reported by Petasis and co-workers (Figure 1) (*1-3*).

$$\text{R-B(OH)}_2 \underset{\textbf{2}}{} + \text{R'}_2\text{NH} \underset{\textbf{3}}{} + \text{HOOC-CH=O} \underset{\textbf{4}}{} \longrightarrow \underset{R \quad \textbf{1} \quad COOH}{\overset{NR'_2}{\bigwedge}}$$
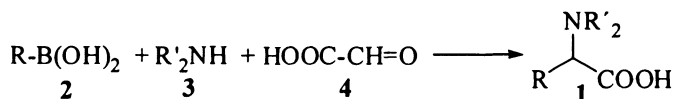
Figure 1. General scheme for the Petasis reaction obtaining α-amino acids

The key element of this process is a three component coupling reaction of organoboronates (**2**) with amines (**3**) and glyoxylic acid (**4**), affording α-amino

acids directly. This process is usually referred to as the borono-Mannich reaction or Petasis reaction (*4-6*). The structural diversity of the amino acids is created by the various substituents of the organoboronates. Practically all types of organoboronates, including aryl (*2,7,8*), vinyl (*1,7,9*) and allylboronates (*10-13*) undergo Petasis reaction, forming β, γ and δ-functionalized α-amino acids. Furthermore, as the coupling between allylboronates and *in situ* generated imines proceeds with a high regio- and stereoselectivity, the reaction is particularly suitable for synthesis of densely functionalized, stereodefined, unnatural α-amino acids. The reaction is environmentally benign, as the major waste product is non-toxic boric acid or its derivatives. Although aryl and vinylboronates are relatively easily accessible and air-stable organometallic reagents, functionalized allylboronates are more difficult to obtain, and their isolation is relatively cumbersome (*4*). Therefore, a one-pot procedure was designed (*10*) (*vide infra*) for synthesis of α-amino acids via in situ generated allylboronates.

α-Amino Acids Prepared from Isolated Organoboronates

Application of vinyl and arylboronic acids as substrates

Vinylboronic acids (such as **2a-b**) are highly reactive and versatile reaction partners for synthesis of structurally diverse amino acid derivatives (eq. 1-3) (*1,7,9*).
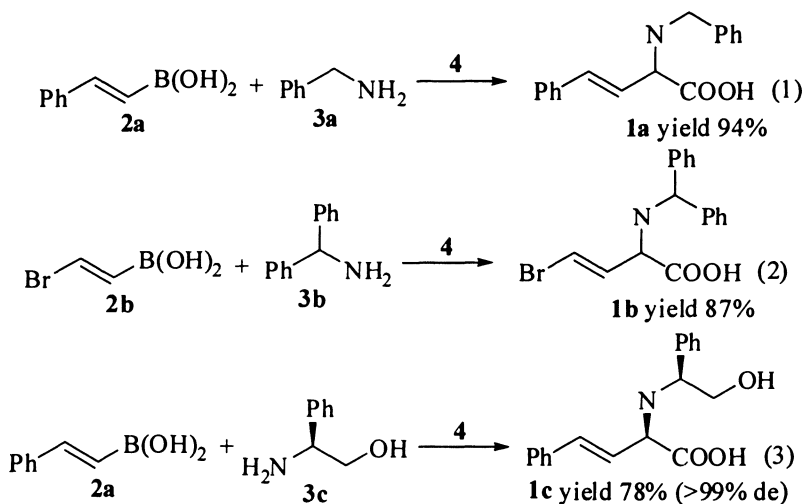
The reaction proceeds with high yields using primary (**3a-b**) and secondary amines, even bulky ones (eq. 2). By using cleavable amines (such as **3b**), the final products (such as **1b**) can easily be converted to free amino acids. Asymmetric versions of the reaction proceeds with fair to excellent stereoselectivities depending on the applied chiral amine component. For example, phenylglycinol **3c** was reacted with excellent diastereoselectivity and high yield. The product (**1c**) could easily be converted to enantiopure homophenylalanine.
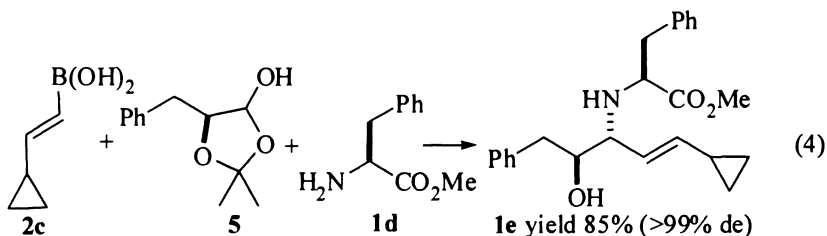
Schreiber and co-workers (*9*) employed a slightly modified version of the Petasis reaction to generate arrays of skeletally and stereochemically diverse small molecules. The three component coupling of cyclopropylvinylboronic acid **2c**, lactol **5** and phenylalanine derivative **1d** proceeded smoothly, affording exclusively the anti diastereomer of **1e**. The high stereoselectivity of the coupling reaction is probably due to the directing effects of the hydroxy group in the intermediate imine (formed from **5** and **1d**). The densely functionalized product **1e** was employed as a template in diversity oriented synthesis to generate libraries of skeletally diverse α-amino acid derivatives.
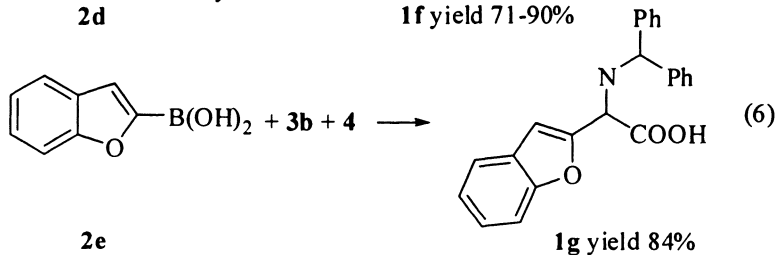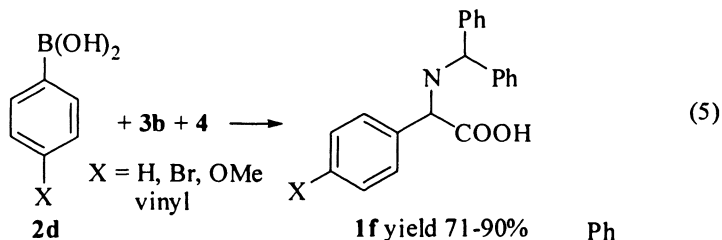
Phenylglycine derivatives (**1f**) can easily be prepared (eq. 5) from arylboronic acids (**2d**), the corresponding amine (**3**) and glyoxylic acid (**4**).(*2*) The analogous synthesis can be extended for preparation of heteroaromatic analogs, such as **1g**, using the corresponding heteroarylboronic acid derivative (e.g. **2e**) (*2,7,14-16*). Cleavage of the diarylmethyl group by hydrogenation or hydrolysis affords the free amino acids, which are valuable drug intermediates.

α-Amino acids from allylboronates

Kobayashi and co-workers (*11,12*) have shown that isolated allylboronates can also be employed as organoboronate components to prepare α-amino acids. Thus, the parent allylboronic acid **2f** reacted readily with aqueous ammonia and glyoxylic acid **4** to give amino acid **1h** in quantitative yield (eq. 7) (*12*). Despite of the high reactivity of allylboronates (*4*) toward aldehydes (such as **4**) the α-hydroxy acid analog did not form at all in this process.

$$\text{2f} \quad \diagup\!\!\diagdown\!\!\diagup\text{B(OH)}_2 + \text{NH}_3 \xrightarrow{\text{4}} \diagup\!\!\diagdown\!\!\diagup\overset{\overset{\displaystyle\text{NH}_2}{|}}{}\text{COOH} \qquad (7)$$

2f　　　　　　　　　　　　　　　**1h** quantitative yield

$$\text{2g} \quad \diagup\!\!\diagdown\!\!\diagup\text{B(OH)}_2 + \overset{\oplus}{\underset{\ominus}{\text{NH}_3}}\overset{}{\underset{\text{OOC}\quad\text{OH}}{}} \longrightarrow \diagup\!\!\diagup\overset{\text{NH}_2}{}\text{COOH} \qquad (8)$$

2g　　　　　　　　**6**　　　　　　　**1i** yield 95%

$$\text{2h} \quad \diagdown\!\!\diagup\!\!\diagdown\!\!\diagup\text{B(OH)}_2 + \overset{\oplus}{\text{NH}_3}\overset{}{\underset{\text{OOC}\quad\text{OH}}{}} \longrightarrow \diagup\!\!\diagup\overset{\text{NH}_2}{}\text{COOH} \qquad (9)$$

2h　　　　　　　　**6**　　　　　　　**1j** yield 93%
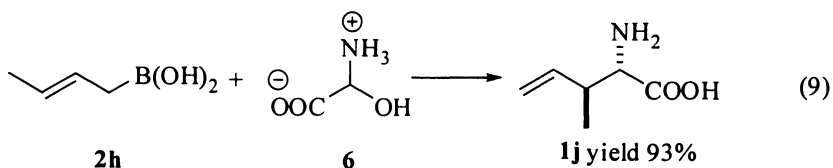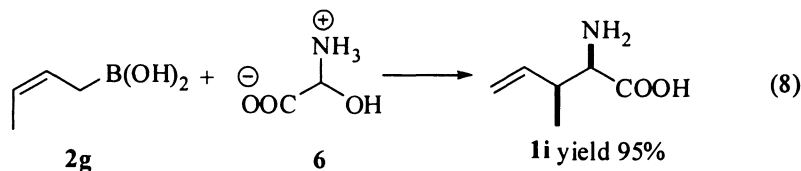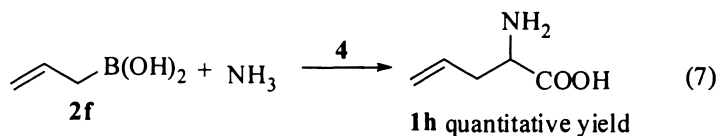
Application of crotylboronates **2g,h** (eqs. 8-9) with **6** led to formation of the branched allylic products with a high regioselectivity. As the coupling reaction creates new stereogenic carbons, two diastereomers are expected to form, however, the stereoselectivity of this reaction was found to be excellent. Starting from *cis*-crotylboronate **2g**, the *syn* stereoisomer **1i** was obtained, while the *trans* isomer **2h** gave the *anti* product **1j** selectively. This stereo- and regioselectivity is exactly the same as in the coupling reaction of allylboronates with aldehydes (*4,17*). The reaction provided the free α-amino acids directly, however this

caused a minor problem as well. It was found that the branched amino acid products (such as **1i-j**) tended to undergo aza-Cope rearrangement in the reaction mixture after reacting of **6**, resulting in the corresponding linear regioisomers. This side reaction could be avoided by quick purification. Thadani and co-workers (*13*) demonstrated that α-amino acid derivatives could be prepared in a similar way using a three component coupling of allylboronic acids (such as **2h**), glyoxylic acid (**4**) and ketones.

One-pot Synthesis of α-Amino Acids via in situ Generated Allylboronates

In the above described procedures, the α-amino acids have been prepared from isolated organoboronates. As the functional part of the amino acids is introduced by the organoboronate component, accessibility to the appropriate reagents is an important factor for a successful application of these synthetic routes. A large variety of arylboronic acids (such as **2d**) and some of the vinylboronates are commercially available (such as **2a**), and therefore the synthesis of the requested amino acids can relatively easily be performed starting from these reagents. However, allylboronates are much less easily accessible compounds. Currently, only the pinacolate ester of the parent allylboronic acid (**2f**) is commercially available. Besides, the selective synthesis and handling of many functionalized allylboronates are problematic (*18-21*). These problems can be solved by development of new efficient catalytic methods for generation of allylboronates followed by a Petasis reaction in a one-pot sequence. In this way, structurally diverse α-amino acids can be prepared from simple precursors without cumbersome isolation and handling of the allylboronate reagents.

Catalytic generation of allylboronates

In the past decades, allylboronates have been used in a wide array of synthetic applications (*4*). These reagents show a high selectivity in allylation reactions with aldehydes (*22-24*), ketones (*25,26*) and imines (*11,27*). Therefore, development of mild and selective methods for preparation of functionalized allylboronates is highly important (*18,28-31*). Recently, a versatile catalytic protocol to generate allylboronic acids, using allylic alcohol precursors has been reported by Szabó and co-workers (Figure 2) (*18*). Various substituted allylic alcohols (**7**) were converted to the corresponding allylboronic acids, using catalytic amounts of palladium pincer complex **9** and diboronic reagent **8**. Addition of catalytic amounts of *p*-toluenesulfonic acid (**10**) was shown to accelerate the borylation reaction. The regio- and stereoselectivity of these

transformations are excellent and the functional group tolerance is high. Both linear and branched alcohols gave the *trans* substituted linear allylboronic acid (**2i**).
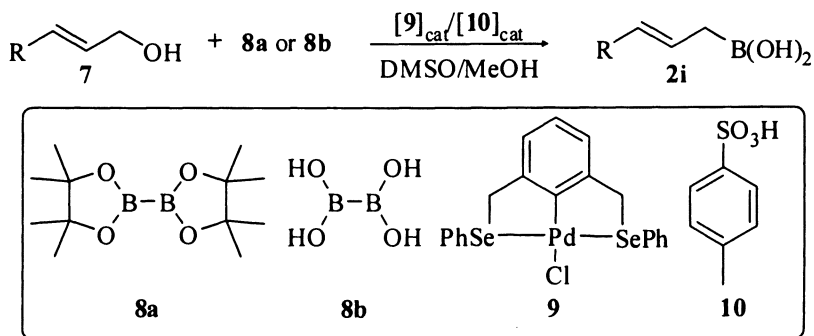
Figure 2. Palladium pincer catalyzed borylation of allylic alcohols.

Since the allylboronic acids are unstable under solvent-free conditions, they can be converted to the corresponding potassium trifluoro(allyl)borates and then isolated (*18*). Alternatively, one-pot reactions can be designed using the above method for *in situ* generation of allylboronates (**2i**). Accordingly, Szabó and co-workers (*20,32-34*) have published several applications using this concept for synthesis of homoallylic alcohols and α-amino acids.

α-Amino acids prepared from allylic alcohols

It was shown (*10*) that the mild conditions of the catalytic generation of organoboronates (Figure 2) are fully compatible with the reaction conditions of the Petasis reaction (*1-3*). Thus, the synthesis of α-amino acids from allylic alcohols could be performed as a sequential one-pot reaction, where the *in situ* generated allylboronic acids were coupled with *in situ* formed imines (*10*) (Figure 3).

These operationally simple reactions were conducted under mild conditions (typically at 50°C) by mixing allylic alcohol **7**, boronate source **8** and catalysts **9** and **10** in a mixture of DMSO and methanol, then (usually after 16 h) amine **3** and glyoxylic acid **4** were added and the reaction mixture was stirred 16 hours at ambient temperature. The reason for this sequential addition scheme was that amine **7** inhibited catalyst **9**, and therefore **7** was added to the reaction mixture after the catalytic boronation (Figure 2) had been completed (about 16 h). The transformations were performed using aryl and benzyl amines (such as **3b** and

3d), and thus the reaction afforded protected homoallylic amino acids **1k-q** (eq. 10-15) with excellent regio- and stereoselectivity. Because of the protection of the amino functionality, aza-Cope type rearrangements to the corresponding linear forms could be avoided (*vide supra*).
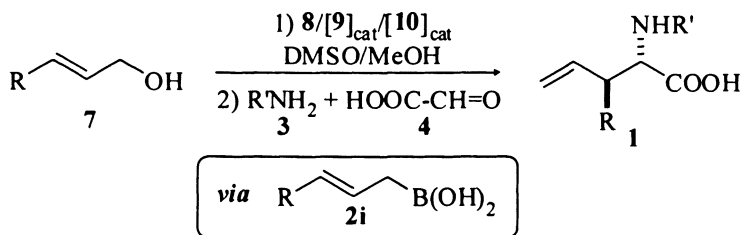
Figure 3. One-pot synthesis of α-amino acids via transient allylboronates.

Using the above concept, cinnamyl alcohol **7a** was converted to α-amino acid **1k** using pincer-complex catalyst **9** and *bis*-pinacolato diboron **8a** as boronate source, followed by addition of amine **3b** and **4**. The product (**1k**) was formed with an excellent anti stereochemistry, affording a phenylalanine derivative from **7a** in good yield. The reaction proceeded via *in situ* formation of cinnamylboronate (**2i**, R = Ph), which was not isolated (eq. 10).

1k yield 67 %
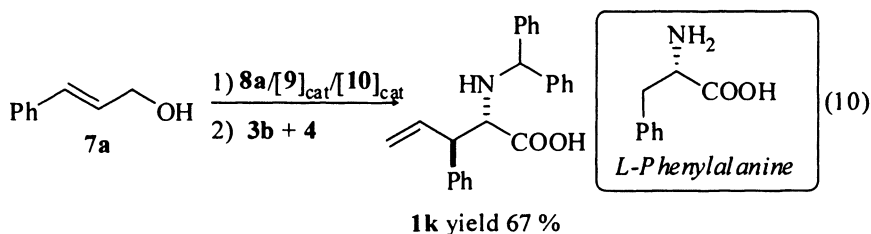
The reaction starting from **7a** readily proceeds with diboronic acid **8b** as well. Replacement of **3b** with aniline, provides the phenyl protected form **11** (eq. 11).

11 yield 78 %
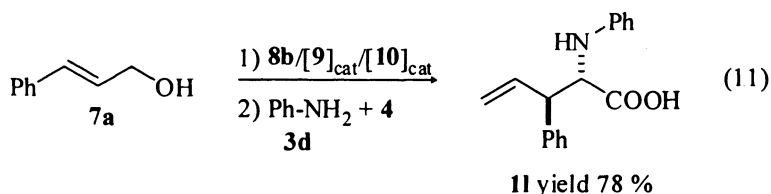
The alkyl substituted amino acid **1m** could also be easily prepared, starting from octenol **7b** (eq. 12) (*10*). In this one pot procedure, *p*-methoxyaniline (**3e**) was used as amine component. It was found (*10*), that the electron donating methoxy substituent has a significant accelerating effect on the Petasis reaction.

$$\text{(12)}$$
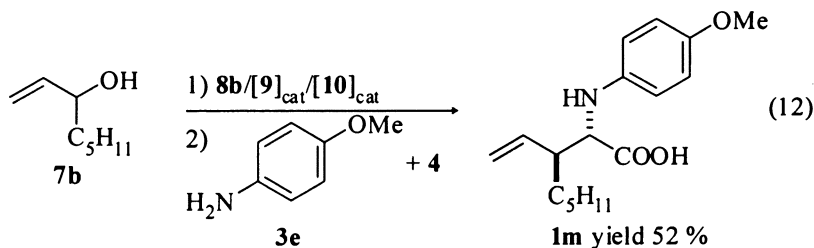
Tertiary alcohol **7c** could also be employed as precursor for the one-pot synthesis of valine analog **1n** (eq. 13). In this reaction, still a single regioisomer was formed, indicating that selective formation of a sterically congested branched product (such as **1n**) can readily be accomplished by this method.

$$\text{(13)}$$
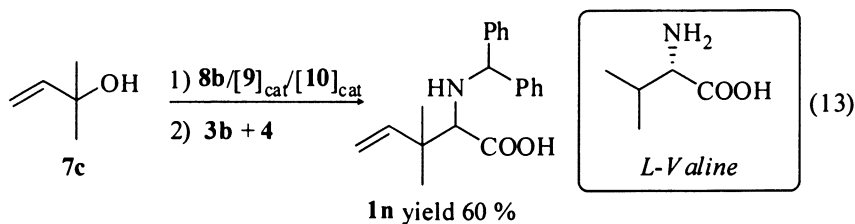
Protected hydroxy functionality was also introduced in the amino acid derivatives by applying allylic alcohol **7d** (*10*). The boronation (*18*) of di-allylic alcohol derivatives (such as **7d**) is particularly fast, and therefore the designated homo-serine analog **1o** can be prepared efficiently under mild conditions (eq. 14).

$$\text{(14)}$$
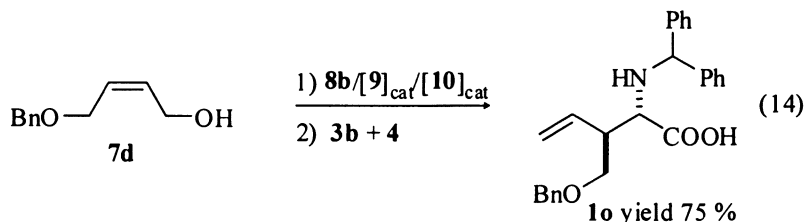
An interesting domino reaction can be triggered by using ester functionalized allylic alcohols **7e-f** (eq. 15) as precursors. When ethyl ester derivative **7e** was reacted under the standard reaction conditions, glutamic acid analogue **1p** was formed with high selectivity and in high yield. However, upon heating, product **1p** underwent lactam formation reaction, affording pyroglutamic acid derivative **1q**. Interestingly, starting from methyl ester derivative **7f**, the corresponding glutamic acid analogue (**1p**) could not be isolated, as it underwent spontaneous cyclization affording **1q** as the final product of the process. Accordingly, the reaction of **7f**, **8b**, **3b** and **4** triggers a spectacular four-step cascade, which can be performed as a one-pot reaction.

$$(15)$$
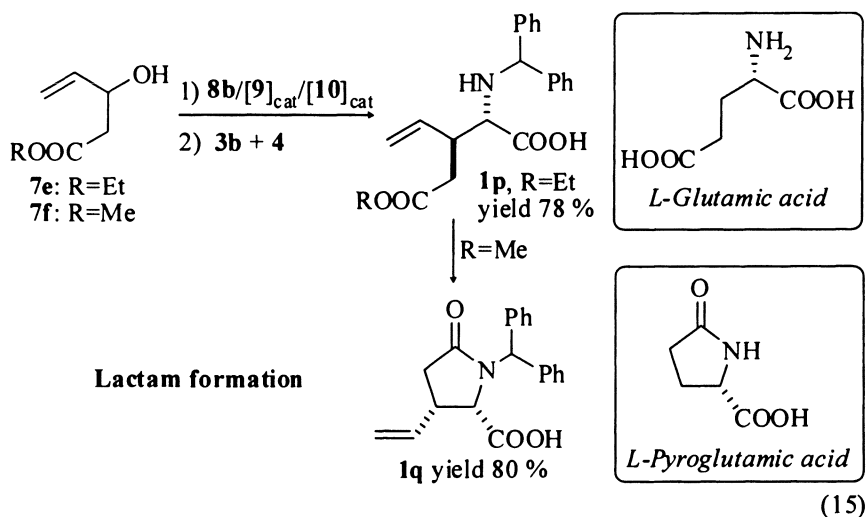
Mechanistic aspects

The one-pot synthesis of α-amino acids (**1**) from allylic alcohols (**7**) involves at least three cooperative processes (Figure 4): borylation of the allylic alcohol catalyzed by pincer complex **9** affording allylboronate **2i**; in situ formation of imine **13** from the corresponding amine (**3**) and glyoxylic acid **4**; and finally the coupling of allylboronate **2i** with imine **13** to give the final product **1**.

Hydroxy groups are very poor leaving groups, therefore **7** have to be activated for the catalytic boronation process. A possible way (*18*) can be the esterification of diboronic acid **8b** with **7** to give **11**. Using pinacolato ester **8a** as boronate source, the first step can be a partial transesterification of **8a** to the

corresponding derivative of **11**, which is probably catalyzed by the added toluenesulfonic acid **10**. The exact mechanism of the boronate transfer is not known, however considering the mechanism of the analog pincer complex catalyzed stannylation reaction (*35,36*), it is assumed that complex **9a** is transmetallated by a diboronate species (such as **8b** or **11**) to give boronate complex **9b**. Subsequently the boronate group is transferred from palladium to the allylic carbon atom of **12** to give **2i**. Thereafter, the Petasis sequence starts involving the coupling reaction of allylboronate **2i** with the *in situ* formed imine **13** to yield the α-amino acid product **1**.
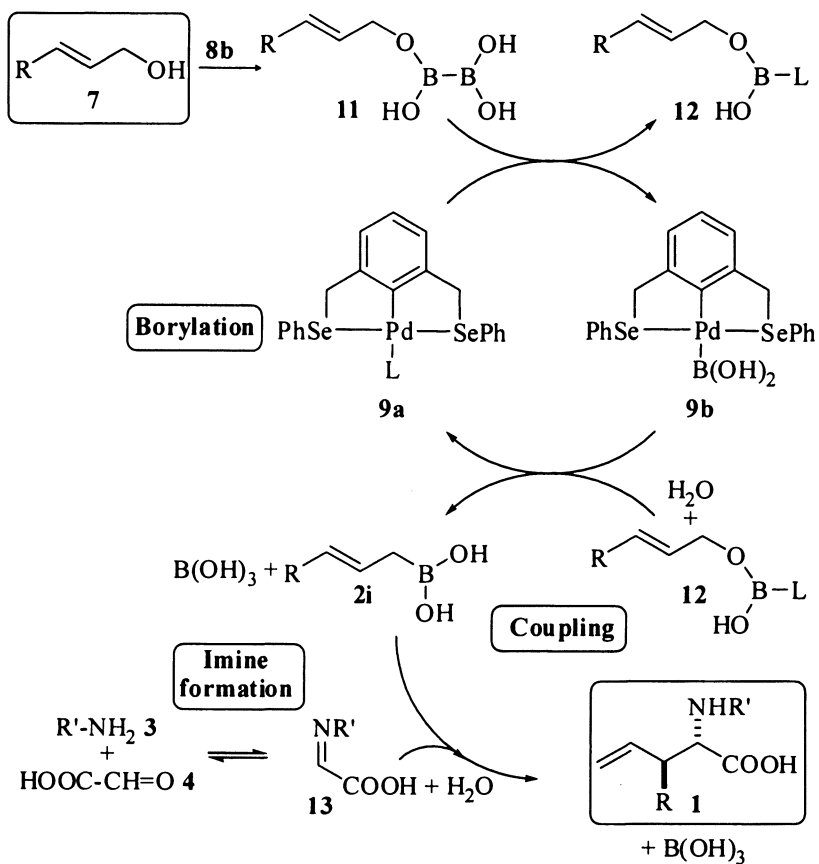
Figure 4. Schematic mechanism for the allylation of imines with allylic alcohols.

The mechanism of the imine formation and the addition of organoboronates was studied by Hutton and Hansen and their co-workers (*37,38*). It was proposed

that the coordination of the boronic acid to a heteroatom in the aldehyde (such as 4) is a prerequisite for an efficient coupling reaction. Based on ^{11}B-NMR studies, Hansen and co-workers (*38*) suggested formation of a tetra-coordinated boronate complex, in which the arylboronic acid coordinates to the hydroxy group of the glyoxylic acid. When functionalized allylboronates are used in the Petasis reaction, the same selectivity was observed for allylation of imines as in the corresponding coupling reaction of allylboronates and aldehydes (*4,17*). This selectivity involves formation of the branched product with *anti* stereoselectivity from allylboronic acids with *trans* double bond (eq. 9).

Conclusions and Outlook

As demonstrated in this chapter, organoboronates are very useful precursors for synthesis of functionalized unnatural α-amino acids. The functionalities can be introduced *via* the organoboronate component. As we have shown, certain organoboronates, such as allylboronates can be generated *in situ* in the reaction mixture of the Petasis reaction, affording α-amino acids directly from simple precursors, such as allylic alcohols (7). Thus, synthesis of α-amino acids *via* catalytic generation of allylboronates (**2f-i**) is a useful concept with a broad synthetic scope. A possible extension of this concept may involve catalytic generation of aryl (such as **2d-e**) and vinyl (such as **2a-c**) boronic acids. In particular, catalytic carbon-hydrogen bond based boronation reactions offer an attractive approach, as using these reactions, simple precursors (such as hydrocarbons) can be employed for synthesis of α-amino acids in a one-pot sequence. In fact, several one –pot procedures are described in the literature (*39-43*), in which the aryl and vinylboronates are generated *in situ* by carbon-hydrogen bond activation.

Application of the Petasis reaction for synthesis of enantio-pure α-amino acids is one of the most important area of interest. These reactions could be achieved by employment of phenylglycine (such as in eq. 3) or other chiral reaction components (eq. 4). However, the greatest challenge would be employment of chiral auxiliaries in catalytic amounts. In this respect, chiral diols (*44*) are probably promising reagents.

In summary, organoboronate based synthesis grants a simple and highly efficient route for preparation of functionalized unnatural α-amino acids. The reactions work well with *in situ* generated organoboronates, which allows application of very simple precursors, such as allylic alcohols. Further new development is expected in this area and also for design of the asymmetric versions of the introduced processes.

Acknowledgement

References

1. Petasis, N. A.; Zavialov, I. A. *J. Am. Chem. Soc.* **1997**, *119*, 445.
2. Petasis, N. A.; Goodman, A.; Zavialov, I. A. *Tetrahedron* **1997**, *53*, 16463.
3. Petasis, N. A.; Zavialov, I. A. *J. Am. Chem. Soc.* **1998**, *120*, 11798.
4. Hall, D. G. *Boronic Acids*; Wiley: Weinheim, 2005.
5. Petasis, N. A. *Aust. J. Chem.* **2007**, *60*, 795.
6. Kaiser, P. F.; Churches, Q. I.; Hutton, C. A. *Aust. J. Chem.* **2007**, *60*, 799.
7. Jourdan, H.; Gouhier, G.; Hijfte, L. V.; Angibaud, P.; Piettre, S. R. *Tetrahedron Lett.* **2005**, *46*, 8027.
8. Petasis, N. A.; Patel, Z. D. *Tetrahedron Lett.* **2000**, *41*, 9607.
9. Kumagai, N.; Muncipinto, G.; Schreiber, S. L. *Angew. Chem. Int Ed.* **2006**, *45*, 3635.
10. Selander, N.; Kipke, A.; Sebelius, S.; Szabó, K. J. *J. Am. Chem. Soc.* **2007**, *129*, 13723.
11. Sugiura, M.; Mori, C.; Hirano, K.; Kobayashi, S. *Can. J. Chem.* **2005**, *83*, 937.
12. Sugiura, M.; Hirano, K.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 7182.
13. Dhudshia, B.; Tiburcio, J.; Thadani, A. N. *Chem. Commun,* **2005**, 5551.
14. Naskar, D.; Roy, A.; Seibel, W. L.; Portlock, D. E. *Tetrahedron Lett.* **2003**, *44*, 8865.
15. Grigg, R.; Sridharan, V.; Thayaparan, A. *Tetrahedron Lett.* **2003**, *44*, 9017.
16. Follmann, M.; Graul, F.; Schäfer, T.; Kopec, S.; Hamley, P. *Synlett* **2005**, 1009.
17. Hoffmann, R. W. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 555.
18. Olsson, V. J.; Sebelius, S.; Selander, N.; Szabó, K. J. *J. Am. Chem. Soc.* **2006**, *128*, 4588.
19. Sebelius, S.; Olsson, V. J.; Szabó, K. J. *J. Am. Chem. Soc.* **2005**, *127*, 10478.
20. Selander, N.; Sebelius, S.; Estay, C.; Szabó, K. J. *Eur. J. Org. Chem.* **2006**, 4085.
21. Roush, W. R.; Walts, A. E.; Hoong, L. K. *J. Am. Chem. Soc.* **1985**, *107*, 8186.
22. Kennedy, J. W. J.; Hall, D. G. *Angew. Chem. Int. Ed.* **2003**, *42*, 4732.

23. Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207.

24. Denmark, S. E.; Fu, J. *Chem. Rev.* **2003**, *103*, 2763.

25. Schneider, U.; Kobayashi, S. *Angew. Chem. Int. Ed.* **2007**, *46*, 5909.

26. Lou, S.; Moquist, P. N.; Schaus, S. E. *J. Am. Chem. Soc.* **2006**, *128*, 12660.

27. Wada, R.; Shibuguchi, T.; Makino, S.; Oisaki, K.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2006**, *128*, 7687.

28. Sebelius, S.; Olsson, V. J.; Wallner, O. A.; Szabó, K. J. *J. Am. Chem. Soc.* **2006**, *128*, 8150.

29. Ishiyama, T.; Ahiko, T.-A.; Miyaura, N. *Tetrahedron Lett.* **1996**, *37*, 6889.

30. Ito, H.; Ito, S.; Sasaki, Y.; Matsuura, K.; Sawamura, M. *J. Am. Chem. Soc.* **2007**, *129*, 14856.

31. Burks, H. E.; Lu, S.; Morken, J. P. *J. Am. Chem. Soc.* **2007**, *129*, 8766.

32. Carosi, L.; Hall, D. G. *Angew. Chem. Int. Ed.* **2007**, *46*, 5913.

33. Sebelius, S.; Wallner, O. A.; Szabó, K. J. *Org. Lett.* **2003**, *5*, 3065.

34. Goldberg, S. D.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 807.

35. Kjellgren, J.; Sundén, H.; Szabó, K. J. *J. Am. Chem. Soc.* **2004**, *126*, 474.

36. Kjellgren, J.; Sundén, H.; Szabó, K. J. *J. Am. Chem. Soc.* **2005**, *127*, 1787.

37. Southwood, T. J.; Curry, M. C.; Hutton, C. A. *Tetrahedron* **2006**, *62*, 236.

38. Schlienger, N.; Bryce, M. R.; Hansen, T. K. *Tetrahedron* **2000**, *56*, 10023.

39. Cho, J.-Y.; Tse, M. K.; Holmes, D.; Maleczka, R. E.; Smith, M. R. *Science* **2002**, *295*, 305.

40. Mkhalid, I. A. I.; Coventry, D. N.; Albesa-Jove, D.; Batsanov, A. S.; Howard, J. A. K.; Perutz, R. N.; Marder, T. B. *Angew. Chem. Int. Ed.* **2006**, *45*, 489.

41. Tzschucke, C. C.; Murphy, J. M.; Hartwig, J. F. *Org. Lett.* **2007**, *9*, 761.

42. Olsson, V. J.; Szabó, K. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 6891.

43. Moran, W. J.; Morken, J. P. *Org. Lett.* **2006**, *8*, 2413.

44. Koolmeister, T.; Södergren, M.; Scobie, M. *Tetrahedron Lett.* **2002**, *43*, 5969.

Chapter 13

Synthesis of Amino Acid Derivatives via Asymmetric Hydrogenation

Hideo Shimizu, Izuru Nagasaki, Noboru Sayo, and Takao Saito

Corporate Research and Development Division, Takasago International Corporation, 1–4–11, Nishi-Yawata, Hiratsuka City, Kanagawa 254–0073, Japan

The development of asymmetric hydrogenation en route to α-amino acid derivatives is outlined with regard to the catalyst and substrate scope. The reaction that gives β-amino acid derivatives is also addressed as an extension of this technology.

Introduction

Asymmetric hydrogenation to give α-amino acid derivatives has been in the vanguard of asymmetric synthesis. In the development of a new ligand, a key parameter in asymmetric synthesis, the asymmetric hydrogenation of dehydroamino acid derivatives (typically 2-acetamidocinnamic acid and its ester) has frequently been used as a benchmark reaction to investigate the initial performance. Consequently, many promising systems that give α-amino acid derivatives in a highly enantioselective manner have been reported to date (*1*).

In general, asymmetric hydrogenation offers several advantages, including the use of a clean reducing agent and the low generation of waste, the feasibility of a high-density reaction to enable an efficient operation, and a relatively wide scope of reaction substrates. Although the number of industrial applications that use this reaction has increased (*2, 3*), further expansion seems likely, in light of the potential utility of this reaction. Obstacles to such expansion include the cost of adopting the catalysis and development time (*4*).

Considerable effort has been made to improve the value of asymmetric hydrogenation by eliminating these obstacles. This chapter deals with

representative examples of these efforts to reduce the cost in terms of the catalyst and substrate scope. The former helps to expand the pool of suitable catalysts and improve its performance, which directly impact the cost of the reaction. On the other hand, the latter should make it possible to apply asymmetric hydrogenation to a process in a suitable form, i.e., without additional adjustment such as protection/deprotection. The ability to skip redundant steps should help to reduce costs. In this chapter, the synthesis of β-amino acid derivatives is also addressed as an expansion of substrate scope in a new direction.

Excellent reviews have been published on other efforts to reduce the cost of the catalysis, i.e., on methodologies designed to easily separate and/or reuse catalysts, such as recoverable catalysts (i.e. immobilization) and unconventional reaction media (i.e. supercritical CO_2 and ionic liquid) (5, 6).

With regard to development time, high-throughput screening (HTS) has recently become popular for meeting the demand of chemists, especially those in the pharmaceutical industry (7). In this field, the time-to-market pressure has become severe, which forces process chemists to identify the best possible synthetic route in a shorter period of time. Under such circumstances, the time required to identify an optimal condition takes a high priority for asymmetric hydrogenation to be regarded as a potential key reaction. A conventional step-by-step approach with individual reactions can be too slow to meet such a time requirement, particularly in a case where there is no close empirical analogy. On the other hand, with the HTS system, where typically 96 reactions are carried out at a time, parameters such as ligands, catalyst precursors, solvents, and additives can be extensively screened quickly within a few days to identify an optimal system. In addition to identifying the ligand of choice in a short time, HTS has the potential to identify suitable catalyst systems for substrate types that have not yet been investigated. Some of the examples in this chapter were identified by HTS.

Progress in Catalyst Parameters

Chiral Ligands

A large part of the research on the asymmetric hydrogenation of dehydroamino acid derivatives involves the study of chiral ligands. In most cases, ligand development was carried out for the use of Rh catalysis. The development of chiral ligands was motivated by the need for higher performance (selectivity and productivity), easier accessibility and/or the circumvention of existing patents.

Here we provide an overview of some representative ligand types that are effective in the asymmetric hydrogenation of dehydroamino acid derivatives,

especially those used in Rh catalysis. Excellent reviews provide a detailed discussion for each ligand type (*8, 9, 10*).

Monodentate Ligands (11, 12)

The use of chiral monodentate ligands in asymmetric hydrogenation started at 1968 when PPh_3 of the Wilkinson catalyst ($RhCl(PPh_3)_3$) was replaced by optically active phosphines (*13, 14*). In the early stage of its history, a considerably high enantioselectivity (88% ee) was achieved in the asymmetric hydrogenation of a dehydroamino acid by using a monophosphine ligand, CAMP (*15*). However, monodentate ligands lost their prominent position in ligand development with the appearance of bidentate ligands. Although monodentate ligands continued to be studied (*16*), it was not until 2000 that ligands in this category were again highlighted. In that year, several groups reported the efficiency of BINOL-based phosphorus ligands (phosphonites (**1**) (*17, 18*), phosphites (**2**) (*19*) and phosphoramidites (*20*)) in the Rh-catalyzed asymmetric hydrogenation, to give excellent enantioselectivities comparable to bidentate counterparts. In a kinetic study, monodentate ligands showed rates comparable to DuPHOS, a representative bidentate ligand (*21*).

Many of these ligands can be prepared easily, which promoted their modification. In the case of MonoPHOS ligands, modification of the amine moiety is effective. For example, in many cases, ligands bearing piperidine (PipPHOS) give higher enantioselectivity than the parent MonoPHOS ligand (*22*). Meanwhile, various modifications of the diol moiety have been implemented to achieve higher performance. Representative examples are H_8-MonoPHOS (*23*) and SIPHOS (*24*). A later study showed that the diol moiety is not necessary for high enantioselectivity; ligands derived from catecol and optically active amine such as ligand **3** also give the products with high optical purity (*25*) (Figure 1).

Although the optimal ligand tends to vary with the substrate, this can be overcome by HTS, which identifies the ligand of choice in a short period of time (*26, 27*).

Bidentate Ligands

Since DIOP, the first ligand in this category, was reported (*28*), it has become generally recognized that C_2 symmetry and a bidentate property are the key to success in ligand development, and this has inspired researchers to design and synthesize various chiral bidentate ligands. Bidentate ligands can be classified into several categories.
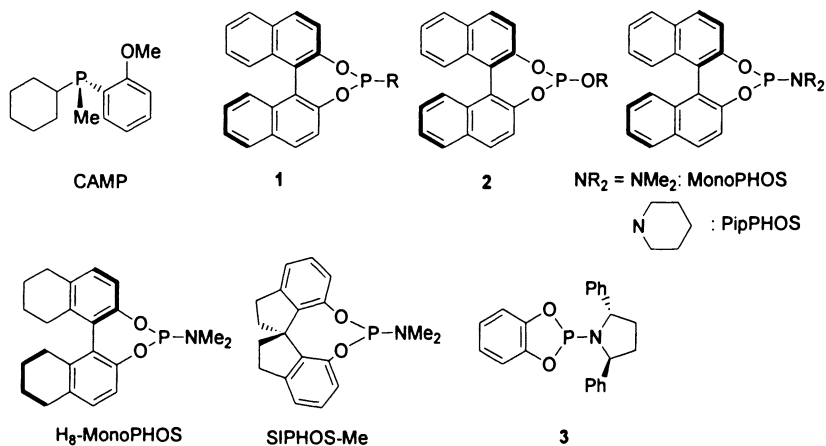
Figure 1. Monodentate ligands.

DIPAMP-Type Ligands (P-Chiral Ligands) (29)

Soon after the concept of C_2-symmetric bidentate ligands was reported, Knowles synthesized DIPAMP (30). It was revealed that the bidentate ligand gave higher enantioselectivity than the corresponding monodentate ligands. This success led to the L-Dopa process, the first industrial application to use asymmetric hydrogenation (31).

P-chiral ligands are attractive in the sense that we can expect high enantioselectivity by having chirality on the phosphorus atom, the closest position to the reaction center (metal atom) in the complex. However the development of this ligand type was slow, presumably due to the difficulty of its synthesis. A renaissance of P-chiral ligands was initiated by Imamoto, who reported BisP* (32). Since then, a variety of P-chiral ligands have been reported, such as MiniPHOS (33). Many ligands of this type are trialkylphosphine-type, which are sensitive to air. They are easier to handle when converted to the corresponding salts (34). Recently, it was discovered that the introduction of a quinoxaline backbone (Quinox P*) made the ligand stable in air while maintaining high chiral-recognition ability (35).

Other effective P-chiral ligands in the asymmetric hydrogenation of dehydro aminoacid derivatives include TangPhos (36), DuanPHOS (37), DisquareP* (38), and BIPNOR (39) (Figure 2).
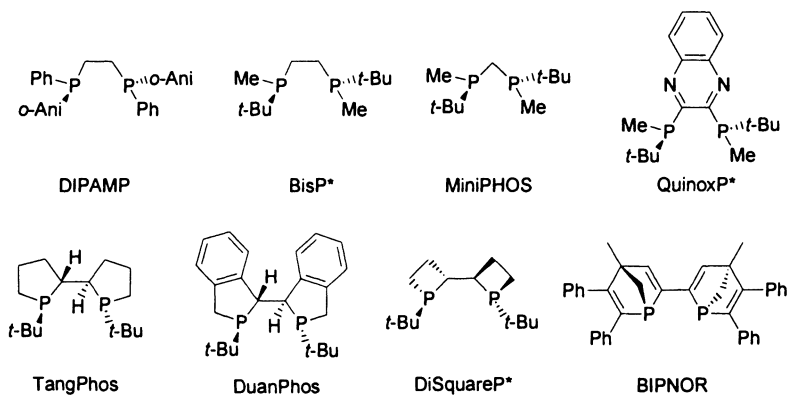
Figure 2. DIPAMP-type ligands.

BINAP-Type Ligands (*40*)

The first application of BINAP, the landmark ligand in a wide array of asymmetric synthesis, was the Rh-catalyzed asymmetric hydrogenation of dehydroamino acids and their esters (*41*). However, parent ligands with biaryl frameworks (with PPh$_2$ appendages) often fail to give high enantioselectivity in the reaction of acylated dehydroamino acid esters. Promising ligands with a biaryl backbone are those with dialkylphosphino appendages, such as BICHEP (*42*). Those which introduce substituents at the 3,3'-position are also attractive (*43*). For example, *o*-Ph-MeO-BIPHEP gave 98% ee in the asymmetric hydrogenation of methyl 2-acetamidocinnamate, while the parent MeO-BIPHEP gave 21% ee under identical conditions. Alternatively, the use of biheteroaromatic TMBTP reportedly gave high enantioselectivity (*44*) (Figure 3).

DuPHOS-Type Ligands (*45*)

DuPHOS and BPE (*46*) developed by Burk, are landmarks in this field. These ligands generally give a wide array of natural and unnatural amino acid derivatives with high optical purity (*47*). Catalytic activity is quite high and in some cases reaches a turnover number (TON) of 50,000.

The success of these hyperactive ligands ignited the extensive development of their descendants. In the early stage, the comparatively difficult availability of the corresponding 1,4-diol precursor led to the design of alternative ligands derived from easily available Mannitol. RoPHOS (*48*), KetalPHOS (*49*) and BASPHOS (*50*) are among these ligands. A 2,5-diaryl pholane version of BPE, Ph-BPE, was recently reported to give higher enantioselectivity than the parent alkyl-counterparts (*51*). Variation of the backbone has also been carried
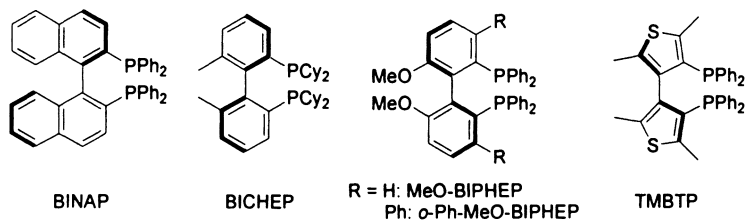
Figure 3. BINAP-type ligands.

out (Figure 4). UlluPHOS, with a thiophene backbone, has a geometry similar to that of DuPHOS, but has greater electronic availability, and provides a qualitatively faster reaction than DuPHOS (*52*). Cat*A*Sium M (MalPHOS) has a wider bite angle than Me-DuPHOS, and gives higher performance in some cases (*53*). Me-f-KetalPHOS, which shows high catalyst productivity, is also promising (*54*).
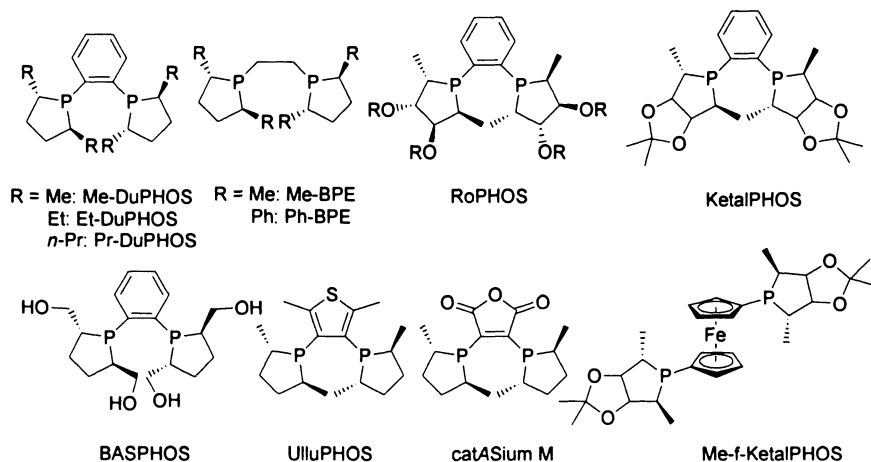
Figure 4. DuPHOS-type ligands.

Other phosphacycle ligands inspired by DuPHOS, such as ligand **4** (*55*), iPr-BeePHOS (*56*), and Me-FerroTANE (*57*), have also been reported (Figure 5).

Josiphos-Type Ligands (*58*)

The Josiphos family is one of the largest ligand libraries (*59*). Its modifications with various appendages can be easily prepared stereospecifically
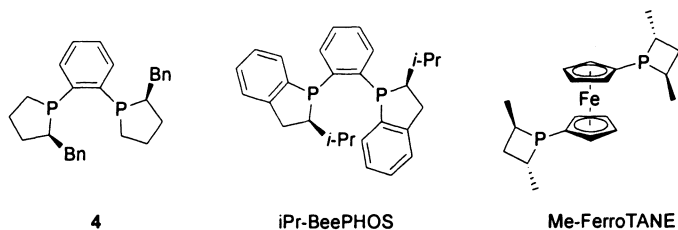
Figure 5. Other Phosphacycle ligands.

from an identical intermediate, which provides wide screening options. Success with the Josiphos family has led to other ferrocene-based diphosphine ligand families, including Taniaphos (*60*), Walphos (*61*) and Mandyphos (*62*). Many ligands in these families show promise in the asymmetric hydrogenation of dehydroamino acid derivatives. TRAP, a trans chelating ligand (*63*), is also an effective ligand in this reaction (Figure 6).
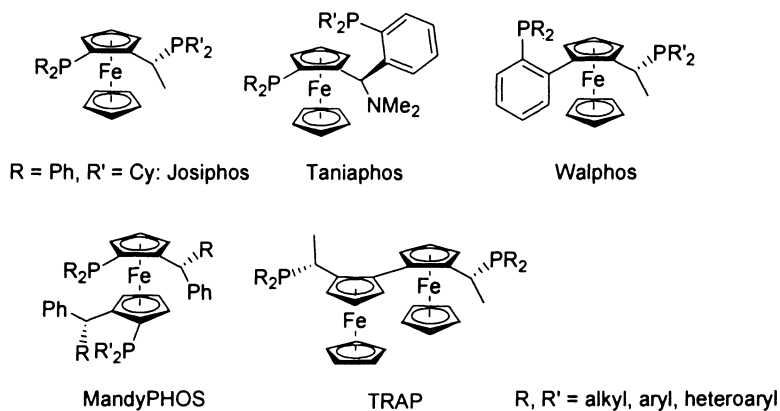
Figure 6. Josiphos-type ligands.

DIOP-Type Ligands (Including Bisphosphine Ligands with Other Chiral Backbones) (*64*)

As previously noted, DIOP offered several guidelines for subsequent ligand development (*28*). These included the introduction of a chiral backbone, which made synthesis of chiral ligands much easier.

Since DIOP, numerous other ligands with diversified backbones, which are effective in α-amino acid synthesis by asymmetric hydrogenation, have been
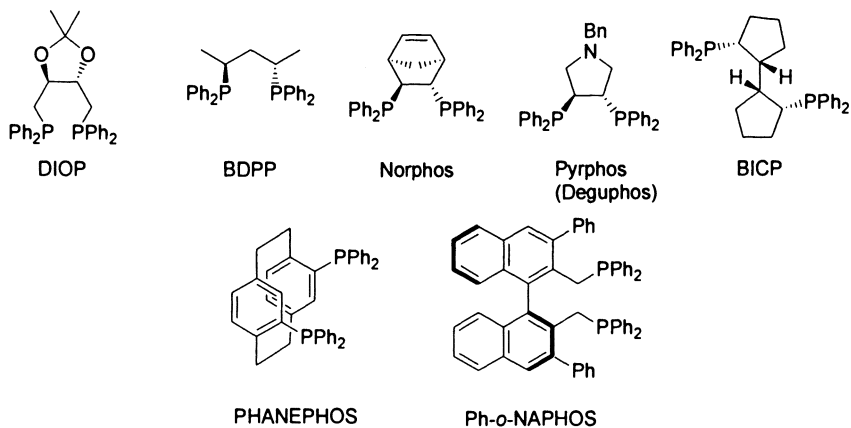
Figure 7. Ligands with various chiral backbones.

reported. Representative examples include BDPP (*65*), Norphos (*66*), Pyrphos (*67*), BICP (*68*), PHANEPHOS (*69*), and Ph-*o*-NaPHOS (*70*) (Figure 7).

Bisphosphine Ligands Based on Self-Assembly.

A new category of bidentate ligands based on self-assembly through hydrogen bonding or coordination bonding has recently been developed (*71*), and some have been applied to the asymmetric hydrogenation of dehydroamino acid derivatives. A representative example is depicted in Figure 8. An adenine-thymine base pair analogue was used for the scaffold of the bidentate ligand, which enabled the selective formation of heterodimeric ligands. With *m* aminopyridine ligands and *n* isoquinolone ligands, the screening of (*m* x *n*) combinations was feasible, and this could identify the ligand that gave ultimate enantioselectivity in the asymmetric hydrogenation of 2-acetamidoacrylate (*72*).

Ligands Other Than Bisphosphine (73)

Diphosphorus ligands other than diphosphine ligands also form a large category of bidentate ligands. Many ligands can be prepared from readily available diols, amino alcohols and diamines. These ligands have been applied to the asymmetric hydrogenation of dehydroamino acid derivatives, and give moderate to excellent enantioselectivities.

Backbones similar to those for diphosphine ligands are frequently adopted. Some representative examples are depicted in Figure 9 (BoPhoz (*74*), Ph-*O*-

m monomers

PivHN—N—PR^{1}R^2 *

+

O=N(H)—PR^{3}R^4 *

⟶ ⟵

PivN—N—PR^{1}R^2 *
H   H
O—N—PR^{3}R^4 *

m x n combinations

n monomers

R^1, R^2, R^3, R^4 = Aryl, Alkyl, Aryloxy
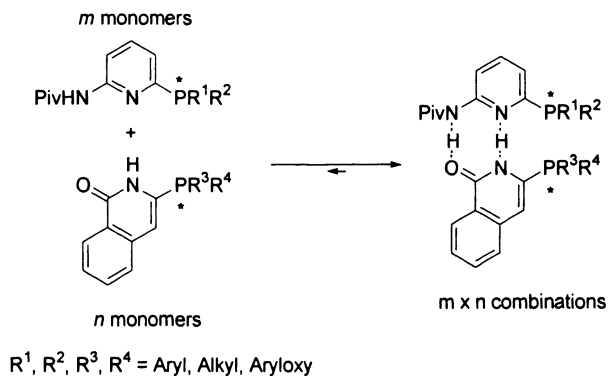
Figure 8. Bidentate ligands with hydrogen bonding

Fe—PPh$_2$ ... N—R, PR$_2$

BoPhoz

Ph, O—PPh$_2$, O—PPh$_2$, Ph

Ph-O-BINAPO

N(H)—PAr$_2$, N(H)—PAr$_2$

BDPAB

Ph$_2$P—N—''n-Bu, P, O O

nBu-QUINAPHOS

Ph, Ph, O, N—, Ph$_2$P, PPh$_2$

DPAMPP

Ph$_2$P—O, O—PPh$_2$
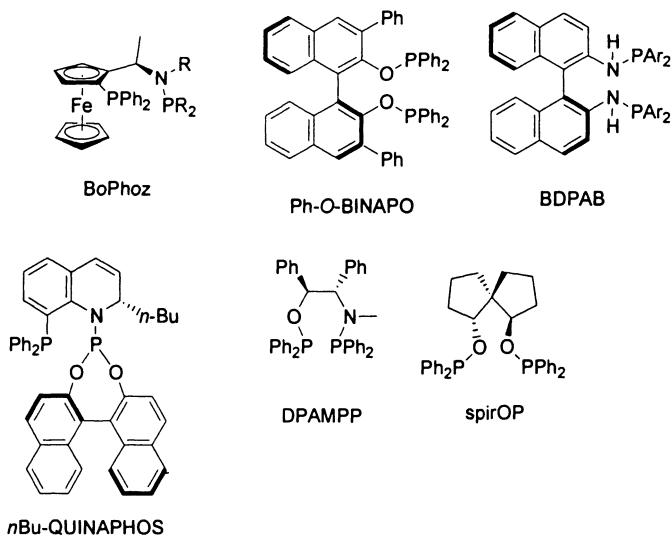
spirOP

Figure 9. Ligands other than bisphosphine.

BINAPO (*70*) BDPAB (*75*), *n*Bu-QUINAPHOS (*76*), DPAMPP (*77*), spirOP (*78*)).

Ligands derived from carbohydrates are advantageous in the sense that highly functionalized chirality can be introduced without optical resolution (*79*). A representative example is a series of ligands **5** derived from D-glucose (*80, 81, 82*). An extensive study on the phosphorus appendage showed that high electron density on the phosphorus atom was essential for high enantioselectivity in the

asymmetric hydrogenation of methyl 2-acetamido cinnamate. Enantioselectivities under identical conditions are shown in the Figure 10.

Although this ligand-type sometimes has a limitation that only one enantiomer is readily accessible, this can be overcome in part by designing its pseudo-enantiomers (*82*). For example, ligand **6** gives a product with an opposite configuration to **5** in high enantioselectivity.

As other ligands in this category, ligands derived from D-mannitol (DIMOP) (*83*) and trehalose (Ligand **7**) (*84*) have been reported to give high enantioselectivities.
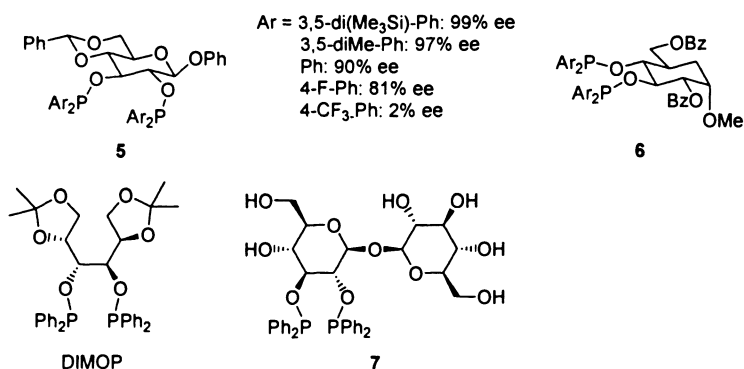
Figure 10. Ligands derived from carbohydrates.

Recent studies have shown that the chiral ligand need not necessarily be a diphosphorus ligand. Mixed phosphorus/sulfur ligands **8** and **9** (*85*) as well as a phosphorus/carbene ligand **10** (*86*) are effective ligands that give high enantioselectivities (Figure 11).

Metal Precursors

In contrast to the diversity in chiral ligands, the variation in metal precursors is rather limited. Most examples of Rh-catalyzed asymmetric hydrogenation
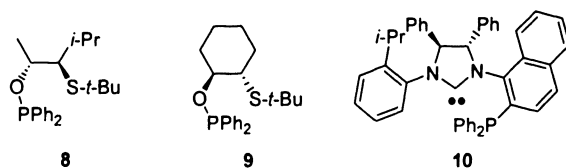
Figure 11. Mixed ligands.

have used cationic $[Rh(cod)_2]^+$ or $[Rh(nbd)_2]^+$ as a catalyst precursor, although a chiral ligand was prepared from the Wilkinson catalyst in the very early stage.

In some cases, $[Rh(nbd)_2]^+$ gave a faster reaction than $[Rh(cod)_2]^+$ due to a shorter induction time (*87*, *88*), while this difference reportedly became insignificant at lower catalyst loading (*89*).

Catalysts other than Rh–catalysis can also be used in asymmetric hydrogenation, but there are far fewer examples. Some Ru complexes associated with biaryl diphosphine ligands give high enantioselectivities in the asymmetric hydrogenation of acylated dehydroamino acid derivatives (*90, 91, 92*). A Ru complex generally gives the opposite enantiomer compare to its Rh counterpart when an identical chiral ligand is used (*93*). It has been suggested that Ru catalysis proceeds via a fundamentally different mechanism than Rh catalysis: Ru uses a monohydride mechanism (*94, 95*) whereas Rh uses a dihydride mechanism (*96, 97*). Ir catalysts ligated by a monodentate ligand (*98*), a mixed P/N (*99*), P/P (*100*) or P/carbene (*101*) ligand give high to excellent enantioselectivties. The reaction with Co catalysts has also been reported, albeit with low enantioselectivity (*102*).

Approaches Toward α-Amino Acid Derivatives

Dehydroamino Acids

Many examples of asymmetric hydrogenation of the acylated dehydroamino acid derivatives have been reported in the literature. Most of the Rh complexes bearing a chiral ligand discussed above give high enantioselectivity of more than 95% ee in the reaction of 2-acetamidocinnamic acid and its ester (Figure 12). With some ligands, such as DuPHOS (*47*), TangPhos (*36*), DuanPHOS (*37*), DiSquareP* (*38*), Me-f-KetalPHOS (*54*), Mandyphos (*62*) and BoPhoz (*74*), catalyst productivity (TON) exceeds 10,000. Detailed data have been provided in recent reviews (*1*). Here, we address the scope of the dehydroamino acid substrate.
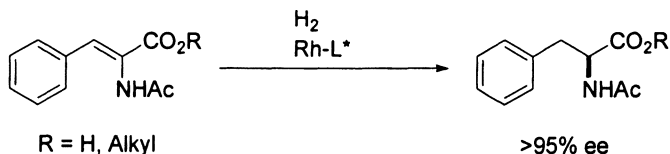
Figure 12. Asymmetric hydrogenation of 2-acetamidocinnamic acid and its ester.

Substituents at the β-Position

Generally, asymmetric hydrogenation tolerates a variety of substituents at the β-position. With DuPHOS-Rh complex, for example, substrates with aryl, heteroaryl (thienyl, furyl, pyrolyl, etc.) and alkyl substituents are hydrogenated with high enantioselectivity (usually >95% ee) (*45, 103*) (Figure 13).
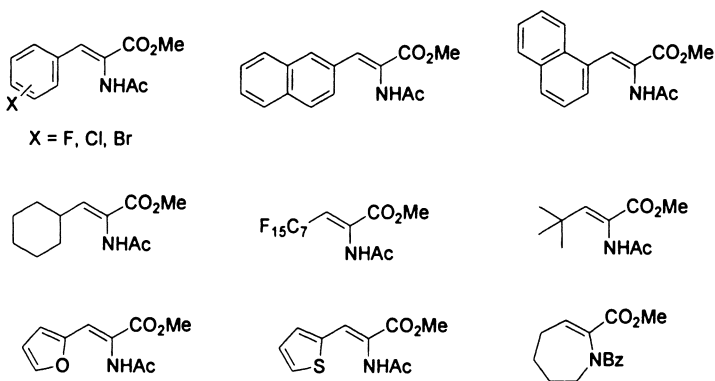
Figure 13. Substrates with substituents at the β-position.

The geometry of trisubstituted dehydroamino acid derivatives may strongly affect the reaction rate and selectivity. In extreme cases (i.e. Rh-BINAP complex), the reaction of (*E*)-substrate gives enantioselectivity opposite to that of its (*Z*)-counterpart (*93*). In such cases, it is necessary to prepare geometrically pure substrate by including a separation step. However, ligands such as DuPHOS overcome this situation: (*E*) and (*Z*) substrates give the identical configuration, both in high enantioselectivity (*47*). This phenomena makes it possible to use a mixture of (*E*)- and (*Z*)-isomers as a substrate for hydrogenation, thus avoiding tedious separation (Figure 14).
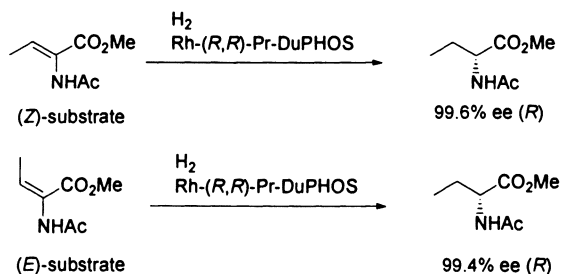
Figure 14. Asymmetric hydrogenation of (E)- and (Z)-substrates.

For some substrates, "tricks" are necessary to carry out the reaction. In Rh-DuPHOS-mediated asymmetric hydrogenation of a substrate with a 2-quinolyl group at the β-position, the addition of tetrafluoroboric acid was effective for overcoming the presumed catalyst inhibition by nitrogen atom (*104*). Although the substrate with a 2-pyridyl moiety was not hydrogenated in the presence of acid, the corresponding pyridine oxide was hydrogenated with high enantioselectivity (*105*) (Figure 15).

On the other hand, a substrate with a 6-methyl-2-pyridyl group was hydrogenated without any additives (*104*). For 3-pyridyl and 4-pyridyl substrates, the reaction in the presence of HBF₄ proceeded in good enantioselectivity with Rh-PROPRAPHOS complex (*106*).
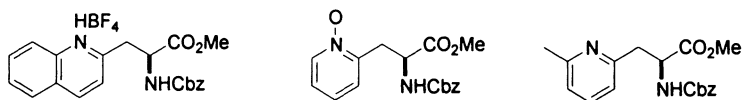
Figure 15. Hydrogenated compounds of quinoly- and pyridyl-type substrates.

For dehydroamino acid derivatives with another alkene site, Rh-DuPHOS predominantly hydrogenates the enamide moiety, although a longer reaction time results in the generation of overreduced product. With α,γ-dienamide ester, only the Rh-Et-Duphos system can give excellent chemoselectivity (>98%) among those screened (*107*) (Figure 16).

L* = Et-DuPHOS: 200 (99% ee)   : 1
Me-DuPHOS: 25 (97% ee)   : 1
DIPAMP: 2 (90% ee)   : 1
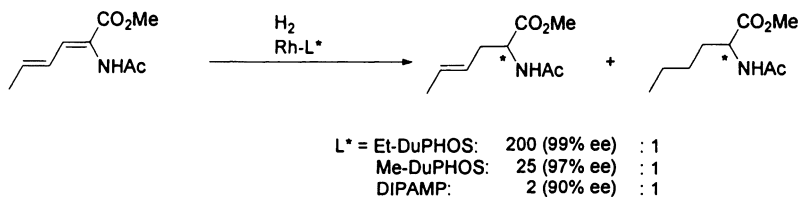
Figure 16. Asymmetric hydrogenation of an α,γ-dienamide ester.

Dehydroamino acid derivatives with two substituents, i.e., tetrasubstituted olefins, are challenging substrates.

Whereas Et-DuPHOS resulted in moderate enantioselection, Me-DuPHOS and Me-DPE generally give hydrogenated products with excellent optical purities (*108*) (Figure 17). TRAP (*63*), BisP* (*32*), PHANEPHOS (*109*) and mixed phosphorus/sulfur ligand (*85*) have also been reported to be effective with this type of substrate.
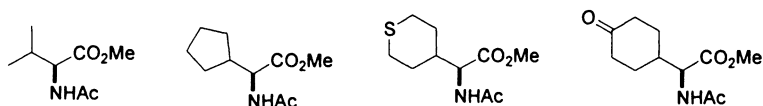
Figure 17. Hydrogenated compounds of tetrasubstituted olefins.

When the two β-substituents are different, diastereoselectivity is controlled by the geometry of the substrate, which provides a protocol for preparing four possible isomers through the proper choice of ligand and substrate (*108, 110*) (Figure 18).
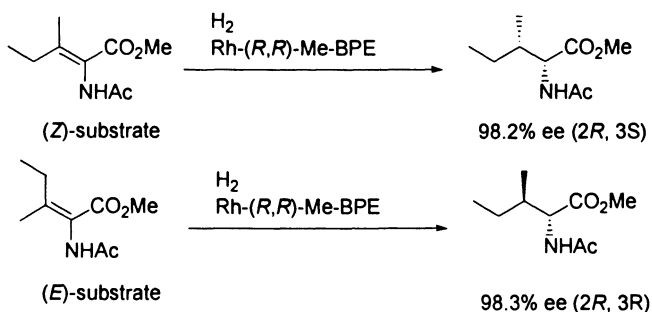
Figure 18. Asymmetric hydrogenation of substrates with two different β-substituents.

Amino Protective Groups

In most cases, acyl groups such as acetyl and benzoyl groups are used for the *N*-protecting group. Although the asymmetric hydrogenation of an acylated dehydroamino acid derivative shows promise, the desired targets often require another amino protecting group. In such cases, a protection/deprotection step is necessary. In some cases the hydrogenated target does not tolerate acidic conditions. For such cases, a milder condition for deprotection using a biocatalyst has been reported (*111*). However, multiple reaction steps remain an intrinsic disadvantage.

In response to this problem, efforts have been made to hydrogenate a variety of *N*-protected dehydroamino acid derivatives to best suit the process. It has been shown that Boc and Cbz groups give comparable performances, which enhances the synthetic value of the reaction (*103*). These protecting groups can be easily deprotected compared to acyl groups.

The scope of asymmetric hydrogenation has been expanded to *N*-sulfonylated dehydroamino acids. A high-throughput screening (HTS) system was a powerful tool for identifying the rather unusual combination of Ru and Josiphos, which gives the desired sulfonamide in 97% ee (*112, 113*) (Figure 19).
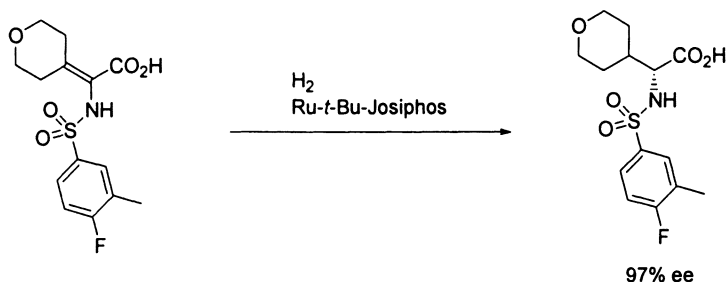
Figure 19. Asymmetric hydrogenation of an N-sulfonylated dehydroamino acid.

The asymmetric hydrogenation of unsaturated ureas was recently reported. In this case, the reaction with a Rh complex ligated by chiral phosphinoimidazoline ligand gave high enantioselectivity of >99% ee (*114*) (Figure 20).
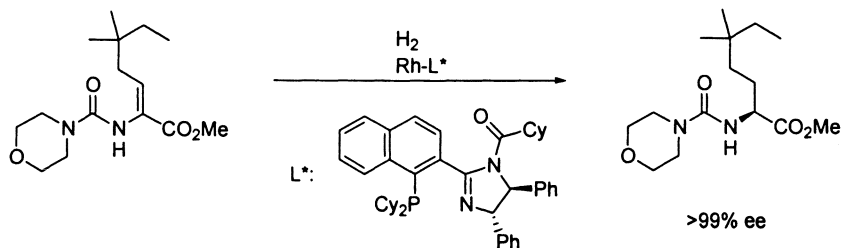
Figure 20. Asymmetric hydrogenation of an unsaturated ureas.

N-Aryl amino acids sometimes become target compounds. During the study of chiral switch of an herbicide, (*S*)-metolachlor, *N*-aryl enamide was subjected to asymmetric hydrogenation in the presence of Rh complex. Under the optimal conditions, the Rh-DuPHOS complex gave extremely high productivity (TON 50,000) as well as high enantioselectivity (96% ee) (*115*) (Figure 21).
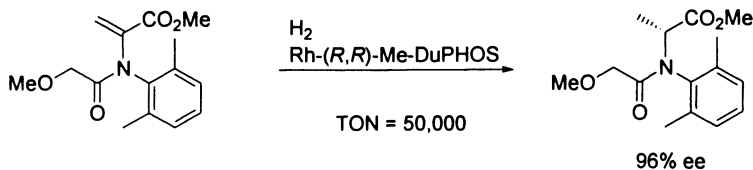
Figure 21. Asymmetric hydrogenation of an N-aryl enamide.

Imines / Including Reductive Amination

Fewer studies have been made on the asymmetric hydrogenation of imines to access α-amino acids.

A Rh-Et-DuPHOS complex can hydrogenate a variety of *N*-acyl hydrazone compounds with high enantioselectivity. The obtained α-hydrazino acid derivatives can be transformed to the corresponding α-amino acids (*116*) (Figure 22).
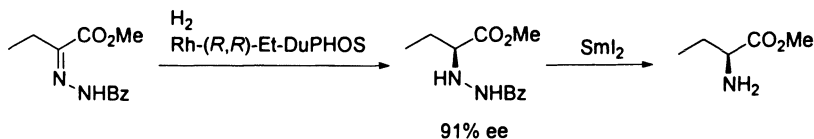
Figure 22. Asymmetric hydrogenation of an N-acyl hydrazone compound.

Recently, it was reported that a Pd-BINAP compound was an effective catalyst in the asymmetric hydrogenation of α-fluorinated iminoesters. With fluorinated alcohol as a solvent, the catalyst gave improved enantioselectivity up to 91% ee (*117*) (Figure 23).
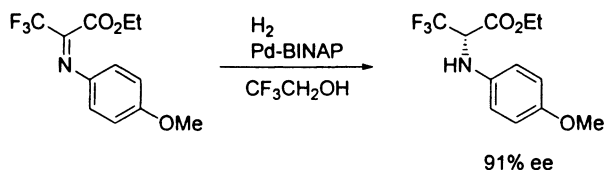
Figure 23. Asymmetric hydrogenation of an α-fluorinated iminoester.

The asymmetric hydrogenation of oximes has also been reported using Ir-DPAMPP complexes. Enantioselectivity exceeded 90% ee in some cases, but with low conversion (*118*).

Direct reductive amination is a reaction in which an α-amino acid can be obtained in one pot by asymmetric hydrogenation of the corresponding ketoester and an amine. The reaction remains challenging, although it has a potential advantage of reducing the reaction steps to the ultimate level in amino acid synthesis. A recent study with high-throughput screening shed some light on the reaction. Screening of 96 phosphorus ligands revealed that Norphos and Deguphos were ligands of choice that afforded high enantioselectivities when benzylamine and phenylpyruvic acid were used as substrates (*119*) (Figure 24).
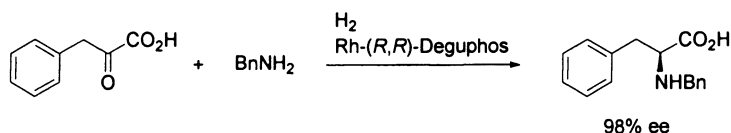
Figure 24. Direct reductive amination.

Dynamic Kinetic Resolution

Asymmetric hydrogenation of α-amino-β-ketoesters accompanied by dynamic kinetic resolution is a powerful tool for accessing α-amino acid derivatives bearing a hydroxyl group at the β-position. Ru (*120, 121, 122*), Ir (*123*) and Rh (*124*) catalysts associated with a biaryl ligand, such as BINAP, gave virtually one isomer out of four possibilities. The readers will find more details in other chapters of this book.

Expansion of the Scope to β-Amino Acid Derivatives

Here we discuss asymmetric hydrogenation en route to β-amino acid derivatives, as an extension of this technology. β-Amino acid derivatives are attracting attentions as chiral building blocks, especially in pharmaceutical industry (*125*). The asymmetric hydrogenation acylated dehydro-β-amino acid derivatives were reported after that of acylated dehydro-α-amino acid (*126*). In general, there is a gap between the (*E*)-isomer and its (*Z*)-counterpart in terms of enantioselectivity and reaction rate: the (*E*)-isomer reacts faster and shows higher enantioselectivity than the (*Z*)-isomer. This phenomenon is undesirable because the isomers will need to be separated to give high enantioselectivity. This problem was solved in part by the use of Rh-Tangphos complex, which gives high enantioselectivities for both enantiomers, and enables the use of an (*E/Z*) mixture as a substrate (*127*) (Figure 25).

$$H_2$$
Rh-(S,S,R,R)-TangPhos

NHAc
CO_2Me

E/ Z mixture

NHAc
CO_2Me

99.5% ee
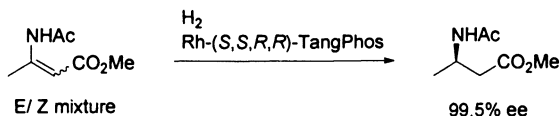
Figure 25. Asymmetric hydrogenation of an acylated dehydro-β-amino acid derivatives.

A recent breakthrough in this area is asymmetric hydrogenation of unprotected dehydro-β-amino acid derivatives. It was reported that a Rh-Josiphos (*128*) (Figure 26) and Ru-DM-SEGPHOS (*129*) could hydrogenate dehydro-β-amino esters in a highly enantioselective fashion. This methodology makes it possible to avoid both the separation of regioisomers and protection/deprotection of the acetyl group.

NH_2
CO_2Me

$$H_2$$
Rh-L*

NH_2
CO_2Me

96% ee

L*: (4-CF$_3$-Ph)$_2$P — Fe — P(t-Bu)$_2$
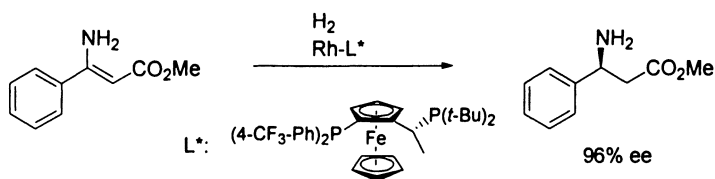
Figure 26. Asymmetric hydrogenation of unprotected dehydro-β-amino acid derivatives.

Direct reductive amination has been reported more recently. Exposition of a β-ketoester to asymmetric hydrogenation in the presence of ammonium acetate, acetic acid and a Ru-DM-SEGPHOS complex directly gave the corresponding β-amino ester in high enantioselectivity. With this protocol, a variety of β-aminoesters can be obtained in high optical purities (*129*) (Figure 27).

Concluding Remarks

Thousands of developments have been made to improve asymmetric hydrogenation. Most of this effort was directed toward making chiral ligands. As a result, a large library of chiral ligands, i.e., a powerful toolbox for tackling asymmetric hydrogenation, has been established. Thanks to this resource, the availability of catalysts and the performance of the reaction have been greatly improved. Additional efforts to expand the substrate scope, which was once rather limited to acylated dehydroamino acid derivatives, have provided new
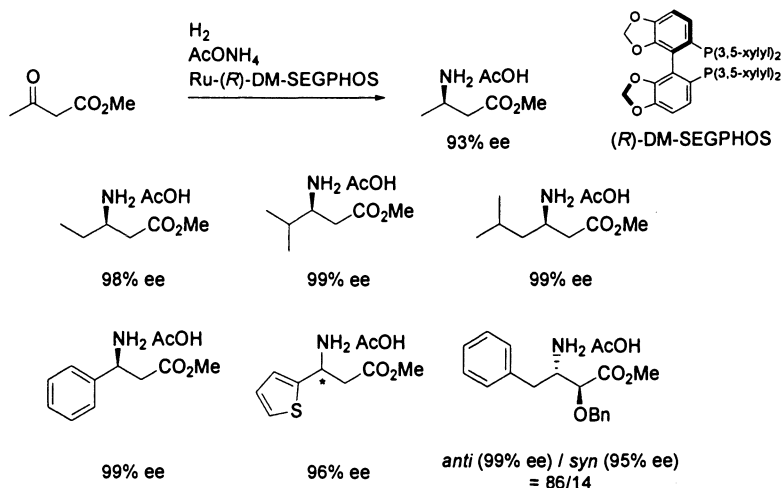
Figure 27. Direct reductive amination.

options in the application of asymmetric hydrogenation. With more options regarding the substrate type, it has become much easier for process chemists to fit asymmetric hydrogenation into their processes without redundancy.

These efforts, together with high-throughput screening, have helped to overcome obstacles in industrial application (i.e. the cost of adopting the catalysis and development time), which improved the value of asymmetric hydrogenation and inspires new development efforts. Thanks to this virtuous cycle, asymmetric hydrogenation will surely continue to evolve as a practical protocol. It will be interesting to see how this reaction changes in the future.

References

1. Nájera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584.
2. Thayer, A. M. *Chem. Eng. News* **2007**, *85*, *(32)*, 11.
3. Blaser, H.-U.; Spindler, F.; Thommen, M. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 3, 1279.
4. Blaser, H.-U. *Chem. Commun.* **2003**, 293.
5. Fan, Q.-H.; Li, Y.-M.; Chan, A. S. C. *Chem. Rev.* **2002**, *102*, 3385.
6. *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 3, Part V.
7. Jäkel, C.; Paciello, R. *Chem. Rev.* **2006**, *106*, 2912.
8. Tang, W.; Zhang, X. *Chem. Rev.* **2003**, *103*, 3029.

9. Ager, D. J. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 745.

10. *Phosphorus Ligands in Asymmetric Catalysis,* Börner, A. Ed., Wiley-VCH: Weinheim, Germany, 2008.

11. Jerphagnon, T.; Renaud, J.-L.; Bruneau, C. *Tetrahedron: Asymmetry* **2004,** *15,* 2101.

12. Van den Berg, M.; Feringa, B. L.; Minnaard, A. J. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 995.

13. Knowles, W. S.; Sabacky, M. J. *J. Chem. Soc. Chem. Commun.* **1968,** 1445.

14. Horner, L.; Siegel, H.; Büthe, H. *Angew. Chem. Int. Ed.* **1968,** *7,* 942.

15. Knowles, W. S.; Sabacky, M. J.; Vineyard, B. D. *J. Chem. Soc. Chem. Commun.* **1972,** 10.

16. Lagasse, F.; Kagan, H. B. *Chem. Pharm. Bull.* **2000,** *48,* 315.

17. Claver, C.; Fernandez, E.; Gillon, A.; Heslop, K.; Hyett, D. J.; Martorell, A.; Orpen, A. G.; Pringle, P. G. *Chem. Commun.* **2000,** 961.

18. Reetz, M. T.; Sell, T. *Tetrahedron Lett.* **2000,** *41,* 6333.

19. Reetz, M. T.; Mehler, G. *Angew. Chem. Int. Ed.* **2000,** *39,* 3889.

20. Van den Berg, M.; Minnaard, A. J.; Schudde, E. P.; Van Esch, J.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *J. Am. Chem. Soc.* **2000,** *122,* 11539.

21. Pena, D.; Minnaard, A. J.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *Org. Lett.* **2003,** *5,* 475.

22. Bernsmann, H.; van den Berg, M.; Hoen, R.; Minnaard, A. J.; Mehler, G.; Reetz, M. T.; de Vries, J. G.; Feringa, B. L. *J. Org. Chem.* **2005,** *70,* 943.

23. Van den Berg, M.; Minnaard, A. J.; Haak, R. M.; Leeman, M.; Schudde, E. P.; Meetsma, A.; Feringa, B. L.; de Vries, A. H. M.; Maljaars, E. P.; Willans, C. E.; Hyett, D.; Boogers, J. A. F.; Henderickx, H. J. W.; de Vries, J. G. *Adv. Synth. Catal.* **2003,** *345,* 308.

24. Fu, Y.; Guo, X.-X.; Zhu, S.-F.; Hu, A.-G.; Xie, J.-H.; Zhou, Q.-L. *J. Org. Chem.* **2004,** *69,* 4648.

25. Hoen, R.; van den Berg, M.; Bernsmann, H.; Minnaard, A. J.; de Vries, J. G.; Feringa, B. L. *Org. Lett.* **2004,** *6,* 1433.

26. Lefort, L.; Boogers, J. A. F.; de Vries, A. H. M.; de Vries, J. G. *Org. Lett.* **2004,** *6,* 1733.

27. Minnaard, A. J.; Feringa, B. L.; Lefort, L.; de Vries, J. G. *Acc. Chem. Res.* **2007,** *40,* 1267.

28. Dang, T. P.; Kagan, H. B. *Chem. Commun.* **1971,** 481.

29. Crépy, K. V. L.; Imamoto, T. *Adv. Synth. Catal.* **2003,** *345,* 79.

30. Knowles, W. S.; Sabacky, M. J.; Vineyard, B. D.; Weinkauff, D. J. *J. Am. Che. Soc.* **1975,** *97,* 2567.

31. Knowles, W. S. In *Asymmetric Catalysis on Industrial Scale,* Blaser, H. U., Schmidt, E. Eds.; Wiley-VCH: Weinheim, Germany, 2004, 23.

32. Imamoto, T.; Watanabe, J.; Wada, Y.; Masuda, H.; Yamada, H.; Tsuruta, H.; Matsukawa, S.; Yamaguchi, K. *J. Am. Chem. Soc.* **1998**, *120*, 1635.
33. Yamanoi, Y.; Imamoto, T. *J. Org. Chem.* **1999**, *64*, 2988.
34. Danjo, H.; Sasaki, W.; Miyazaki, T.; Imamoto, T. *Tetrahedron Lett.* **2003**, *44*, 3467.
35. Imamoto, T.; Sugita, K.; Yoshida, K. *J. Am. Chem. Soc.* **2005**, *127*, 11934.
36. Tang, W.; Zhang, X. *Angew. Chem. Int. Ed.* **2002**, *41*, 1612.
37. Liu, D. ;Zhang, X. *Eur. J. Org. Chem.* **2005**, 646.
38. Imamoto, T.; Oohara, N.; Takahashi, H. *Synthesis* **2004**, 1353.
39. Robin, F.; Mercier, F.; Ricard, L.; Mathey, F.; Spagnol, M. *Chem. Eur. J.* **1997**, *3*, 1365.
40. Shimizu, H.; Nagasaki, I., Saito, T. *Tetrahedron* **2005**, *61*, 5405.
41. Miyashita, A.; Yasuda, A.; Takaya, H.; Toriumi, K.; Ito, T.; Souchi, T.; Noyori, R. *J. Am. Chem. Soc.* **1980**, *102*, 7932.
42. Chiba, T.; Miyashita, A.; Nohira, H.; Takaya, H. *Tetrahedron Lett.* **1991**, *32*, 4745.
43. Wu, S.; He, M.; Zhang, X. *Tetrahedron: Asymmetry* **2004**, *15*, 2177.
44. Barbaro, P.; Bianchini, C.; Giambastiani, G.; Oberhauser, W.; Bonzi, L. M.; Rossi, F.; Dal Santo, V. *Dalton Trans.* **2004**, 1783.
45. Cobley, C. J.; Moran, P. H. In *Handbook of Homogeneous Hydrogenation*, de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 773.
46. Burk, M. J. *J. Am. Chem. Soc.* **1991**, 113, 8518.
47. Burk, M. J.; Feaster, J. E.; Nugent, W. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10125.
48. Holz, J.; Quirmbach, M.; Schmidt, U.; Heller, D.; Stürmer, R.; Börner, A. *J. Org. Chem.* **1998**, *63*, 8031.
49. Li, W.; Zhang, Z.; Xiao, D.; Zhang, X. *Tetrahedron Lett.* **1999**, *40*, 6701.
50. Holtz, J.; Heller, D.; Stürmer, R.; Börner, A. *Tetrahedron Lett.* **1999**, *40*, 7059.
51. Pilkington, C. J.; Zanotti-Gerosa, A. *Org. Lett.* **2003**, *5*, 1273.
52. Benincori, T.; Pilati, T.; Rizzo, S.; Sannicolò, F.; Burk, M. J.; de Ferra, L.; Ullucci, E.; Piccolo, O. *J. Org. Chem.* **2005**, *70*, 5436.
53. Holtz, J.; Monsees, A.; Jiao, H.; You, J.; Komarov, I. V.; Fischer, C.; Drauz, K.; Börner, A. *J. Org. Chem.* **2003**, *68*, 1701.
54. Liu, D.; Li, W.; Zhang, X. *Org. Lett.* **2002**, *4*, 4471.
55. Hoge, G. *J. Am. Chem. Soc.* **2003**, *125*, 10219.
56. Shimizu, H.; Saito, T.; Kumobayashi, H. *Adv. Synth. Catal.* **2003**, *345*, 185.
57. Marinetti, A.; Labrue, F.; Pons, B.; Jus, S.; Ricard, L.; Genêt, J.-P. *Eur. J. Inorg. Chem.* **2003**, 2583.
58. Blaser, H.-U.; Lotz, M.; Spindler, F. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 833.

59. Blaser, H.-U.; Brieden, W.; Pugin, B.; Spindler, F.; Studer, M.; Togni, A. *Top. Catal.* **2002**, *19*, 3.

60. Tappe, K.; Knochel, P. *Tetrahedron: Asymmetry* **2004**, *15*, 91.

61. Sturm, T.; Weissensteiner, W.; Spindler, F. *Adv. Synth. Catal.* **2003**, *345*, 160.

62. Spindler, F.; Malan, C.; Lotz, M.; Kesselgruber, M.; Pittelkow, U.; Rivas-Nass, A.; Briel, O.; Blaser, H.-U. *Tetrahedron: Asymmetry* **2004**, *15*, 2299.

63. Sawamura, M.; Kuwano, R.; Ito, Y. *J. Am. Chem. Soc.* **1995**, *117*, 9602.

64. Chi, Y.; Tang, W.; Zhang, X. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 853.

65. Herseczki, Z.; Gergely, I.; Hegedüs, C.; Szöllősy, Á.; Bakos, J. *Tetrahedron: Asymmetry* **2004**, *15*, 1673.

66. Brunner, H.; Pieronczyk, W.; Schönhammer, B.; Streng, K.; Bernal, I.; Korp, J. *Chem. Ber.* **1981**, *114*, 1137.

67. Nagel, U.; Kinzel, E.; Andrade, J.; Prescher, G. *Chem. Ber.* **1986**, *119*, 3326.

68. Zhu, G.; Cao, P.; Jiang, Q.; Zhang, X. *J. Am. Chem. Soc.* **1997**, *119*, 1799.

69. Pye, P. J.; Rossen, K.; Reamer, R. A.; Tsou, N. N.; Volante, R. P.; Reider, P. J. *J. Am. Chem. Soc.* **1997**, *119*, 6207.

70. Zhou, Y.-G.; Zhang, X. *Chem. Commun.* **2002**, 1124.

71. Breit, B. *Angew. Chem. Int. Ed.* **2005**, *44*, 6816.

72. Weis, M.; Waloch, C.; Seiche, W.; Breit, B. *J. Am. Chem. Soc.* **2006**, *128*, 4188.

73. Kok, S. H. L.; Au-Yeung, T. T.-L.; Cheung, H. Y.; Lam, W. S.; Chan, S. S.; Chan, A. S. C. In *Handbook of Homogeneous Hydrogenation,* de Vries, J. G., Elsevier, C. J. Eds. Wiley-VCH: Weinheim, Germany, 2007; Vol. 2, 883.

74. Boaz, N. W.; Mackenzie, E. B.; Debenham, S. D.; Large, S. E.; Ponasik, Jr., J. A. *J. Org. Chem.* **2005**, *70*, 1872.

75. Guo, R.; Li, X.; Wu, J.; Kwok, W. H.; Chen, J.; Choi, M. C. K.; Chan, A. S. C. *Tetrahedron Lett.* **2002**, *43*, 6803.

76. Franciò, G.; Faraone, F.; Leitner, W. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 1428.

77. Lou, R.; Mi, A.; Jiang, Y.; Qin, Y.; Li, Z.; Fu, F.; Chan, A. S. C. *Tetrahedron* **2000**, *56*, 5857.

78. Chan, A. S. C.; Hu, W.; Pai, C.-C.; Lau, C.-P. *J. Am. Chem. Soc.* **1997**, *119*, 9570.

79. Diéguez, M.; Pàmies, O.; Claver, C. *Chem. Rev.* **2004**, *104*, 3189.

80. Selke, R.; Pracejus, H. *J. Mol. Cat.* **1986**, *37*, 213.

81. RajanBabu, T. V.; Ayers, T. A.; Casalnuovo, A. L. *J. Am. Chem. Soc.* **1994**, *116*, 4101.

82. RajanBabu, T. V.; Ayers, T. A.; Halliday, G. A.; You, K. K.; Calabrese, J. C. *J. Org. Chem.* **1997**, *62*, 6012.

83. Chen, Y.; Li, X.; Tong, S.-K.; Choi, M. C. K.; Chan, A. S. C. *Tetrahedron Lett.* **1999**, *40*, 957.

84. Yonehara, K.; Hashizume, T.; Mori, K.; Ohe, K.; Uemura, S. *J. Org. Chem.* **1999**, *64*, 5593.

85. Evans, D. A.; Michael, F. E.; Tedrow, J. S.; Campos, K. R. *J. Am. Chem. Soc.* **2003**, *125*, 3534.

86. Bappert, E.; Helmchen, G. *Synlett* **2004**, 1789.

87. Börner, A.; Heller, D. *Tetrahedron Lett.* **2001**, *42*, 223.

88. Drexler, H.-J.; Baumann, W.; Spannenberg, A.; Fischer, C.; Heller, D. *J. Organomet. Chem.* **2001**, *621*, 89.

89. Cobley, C. J.; Lennon, I. C.; McCague, R.; Ramsden, J. A.; Zanotti-Gerosa, A. *Tetrahedron Lett.* **2001**, *42*, 7481.

90. Duprat de Paule, S.; Jeulin, S.; Ratovelomanana-Vidal, V.; Genêt, J.-P.; Champion, N.; Dellis, P. *Eur. J. Org. Chem.* **2003**, 1931.

91. Wu, J.; Pai, C. C.; Kwok, W. H.; Guo, R. W.;Au-Yeung, T. T. L.; Yeung, C. H.; Chan, A. S. C. *Tetrahedron: Asymmetry* **2003**, *14*, 987.

92. Wiles, J. A.; Daley, C. J. A.; Hamilton, R. J.; Leong, C. G.; Bergens, S. H. *Organometallics* **2004**, *23*, 4564.

93. Ikariya, T.; Ishii, Y.; Kawano, H.; Arai, T.; Saburi, M.; Yoshikawa, S.; Akutagawa, S. *J. Chem. Soc. Chem. Commun.* **1985**, 922.

94. Wiles, J. A.; Bergens, S. H. *Organometallics* **1998**, *17*, 2228.

95. Kitamura, M.; Tsukamoto, M.; Bessho, Y.; Yoshimura, M.; Kobs, U.; Widhalm, M.; Noyori, R. *J. Am. Chem. Soc.* **2002**, *124*, 6649.

96. Brown, J. M. *Chem. Soc. Rev.* **1993**, 25.

97. Halpern, J. *Precious Met.* **1995**, *19*, 411.

98. Jiang, X.; van den Berg, M.; Minnaard, A. J.; Feringa, B. L.; de Vries, J. G. *Tetrahedron: Asymmetry* **2004**, *15*, 2223.

99. Bunlaksananusorn, T.; Polborn, K.; Knochel, P. *Angew. Chem. Int. Ed.* **2003**, *42*, 3941.

100. Bunlaksananusorn, T.; Knochel, P. *J. Org. Chem.* **2004**, *69*, 4595.

101. Focken, T.; Raabe, G.; Bolm, C. *Tetrahedron: Asymmetry* **2004**, *15*, 1693.

102. Nindakova, L. O.; Shainyan, B. A.; Shmidt, F. K. *Russ. J. Org. Chem.* **2004**, *40*, 973.

103. Burk, M. J.; Gross, M. F.; Harper, T. G. P.; Kalberg, C. S.; Lee, J. R.; Martinez, J. P. *Pure Appl. Chem.* **1996**, *68*, 37.

104. Jones, S. W.; Palmer, C. F.; Paul, J. M.; Tiffin, P. D. *Tetrahedron Lett.* **1999**, *40*, 1211.

105. Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Org. Lett.* **2001**, *3*, 3157.

106. Döbler, C.; Kreuzfeld, H.-J.: Michalik, M.; Krause, H. W. *Tetrahedron: Asymmetry* **1996**, *7*, 117.

107. Burk, M. J.; Allen, J. G.; Kiesman, W. F. *J. Am. Chem. Soc.* **1998**, *120*, 657.

108. Burk, M. J.; Gross, M. F.; Martinez, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 9375.

109. Johnson, N. B.; Lennon, I. C.; Moran, P. H.; Ramsden, J. A. *Acc. Chem. Res.* **2007**, *40*, 1291.
110. Kuwano, R.; Okuda, S.; Ito, Y. *J. Org. Chem.* **1998**, *63*, 3499.
111. Cobley, C. J.; Johnson, N. B.; Lennon, I. C.; Mcgaue, R.; Ramsden, J. A.; Zanotti-Gerosa, A. In *Asymmetric Catalysis on Industrial Scale,* Blaser, H. U., Schmidt, E. Eds.; Wiley-VCH: Weinheim, Germany, 2004, 269.
112. Shultz, C. S.; Dreher, S. D.; Ikemoto, N.; Williams, J. M.; Grabowski, E. J. J.; Krska, S. W.; Sun, Y.; Dormer, P. G.; DiMichele, L. *Org. Lett.* **2005**, *7*, 3405.
113. Shultz, C. S.; Krska, S. W. *Acc. Chem. Res.* **2007**, *40*, 1320.
114. Busacca, C. A.; Lorenz, J. C.; Grinberg, N.; Haddad, N.; Lee, H.; Li, Z.; Liang, M.; Reeves, D.; Saha, A.; Varsolona, R.; Senanayake, C. H. *Org. Lett.* **2008**, *10*, 341.
115. Blaser, H.-U.; Spindler, F. *Top. Catalysis* **1997**, *4*, 275.
116. Burk, M. J.; Feaster, J. E. *J. Am. Chem. Soc.* **1992**, *114*, 6266.
117. Abe, H.; Amii, H.; Uneyama, K. *Org. Lett.* **2001**, *3*, 313.
118. Xie, Y.; Mi, A.; Jiang, Y.; Liu, H. *Synth. Commun.* **2001**, *31*, 2767.
119. Kadyrov, R.; Riermeier, T. H.; Dingerdissen, U.; Tararov, V., Börner, A. *J. Org. Chem.* **2003**, *68*, 4067.
120. Noyori, R.; Ikeda, T.; Ohkuma, T.; Widhalm, M.; Kitamura, M.; Takaya, H.; Akutagawa, S.; Sayo, N.; Saito, T. Taketomi, T.; Kumobayashi, H. *J. Am. Chem. Soc.* **1989**, *111*, 9134.
121. Mordant, C.; Dünkelmann, P.; Ratovelomanana-Vidal, V.; Genêt, J.-P. *Eur. J. Org. Chem.* **2004**, 3017.
122. Makino, K.; Goto, T.; Hiroki, Y.; Hamada, Y. *Angew. Chem. Int. Ed.* **2004**, *43*, 882.
123. Makino, K.; Hiroki, Y.; Hamada, Y. *J. Am. Chem. Soc.* **2005**, 127, 5784.
124. Makino, K.; Fujii, T.; Hamada, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 481.
125. Boge,T. C.; Georg, G. I.; In *Enantioselective Synthesis of β-Amino Acids*; Juaristi, E., Ed.; Wiley-VCH: New York, 1997, p. 1.
126. Juaristi, E.; Gutierrez-Garcia, V. M.; Lopez-Ruiz, H. In *Enantioselective Synthesis of β-Amino Acides,* 2nd Ed.; Juaristi, E.; Soloshonok, V. A., Eds.; Wiley & Sons: New York, 2004, p. 159.
127. Tang, W.; Zhang, X. *Org. Lett.* **2002**, *4*, 4159.
128. Hsiao, Y.; Rivera, N. R.; Rosner, T.; Krska, S. W.; Njolito, E.; Wang, F.; Sun, Y.; Armstrong, III, J. D.; Grabowski, E. J. J.; Tillyer, R. D.; Spindler, F.; Malan, C. *J. Am. Chem. Soc.* **2004**, *126*, 9918.
129. Shimizu, H.; Nagasaki, I.; Matsumura, K.; Sayo, N.; Saito, T. *Acc. Chem. Res.* **2007**, *40*, 1385.

Chapter 14

Stereoselective Synthesis of *anti*-β-Hydroxy-α-Amino Acids Using *anti*-Selective Asymmetric Hydrogenation

Yasumasa Hamada and Kazuishi Makino

Graduate School of Pharmaceutical Sciences, Chiba University, Yayoi-cho, Inage-ku, Chiba 263–8522, Japan

anti-β-Hydroxy-α-amino acids are efficiently synthesized from chirally labile α-amino-β-keto esters using ruthenium- and iridium-catalyzed asymmetric hydrogenation through dynamic kinetic resolution, which is capable of the stereocontrolled construction of two consecutive stereocenters at one reaction.

Introduction

β-Hydroxy-α-amino acids with *syn* or *anti* stereochemistry are common structural units widely found as a component of biologically active natural products, especially cyclodepsipeptides (*1*). Their importance and usefulness as building blocks for synthesis of natural products and medicines have prompted a search for better methods in the stereocontrolled construction of two consecutive stereocenters (*2*). We have been working on total synthesis of naturally occurring cyclodepsipeptides with biologically interesting activities. In this research we needed an efficient synthesis of *anti*-β-hydroxy-α-amino acids. Although to date various methods for synthesis of *anti*-β-hydroxy-α-amino acids have been reported and are roughly classified into asymmetric aldol reactions in a diastereoselective or enantioselective manner (*3*), diastereoselective synthesis from the Garner aldehyde (*4*), enantioselective synthesis using Sharpless asymmetric dihydroxylation as a key step (*5*), and etc (*6*) as shown in Figure 1, these methods need long reaction steps and/or contain tedious operation. Simple and straightforward synthesis of *anti*-β-hydroxy-α-amino acids still remains to

be developed. In our efforts on synthesis of *anti*-β-hydroxy-α-amino acids, we have found new and efficient asymmetric hydrogenation. In this article we focus on our developed diastereo- and enantioselective synthesis of *anti*-β-hydroxy-α-amino acids using asymmetric hydrogenation through dynamic kinetic resolution.
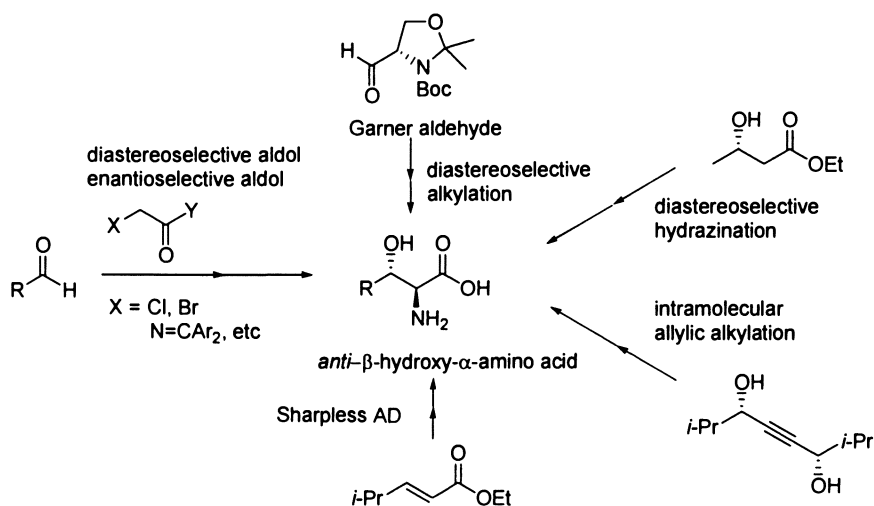
Figure 1. Literature procedures for anti-β-hydroxy-α-amino acids

Synthesis of *anti*-β-Hydroxy-α-Amino Acids using Noyori Asymmetric Hydrogenation

The dynamic kinetic resolution (DKR) (*7*) in the asymmetric hydrogenation of racemic ketones is an efficient method for obtaining theoretically optically pure alcohols from racemic ketones in 100% yield, which was reported by Noyori and coworkers for the first time (*8*). Especially, asymmetric hydrogenation of α-substituted-β-ketoesters via DKR is an excellent procedure with the ability to construct two adjacent stereocenters in a stereocontrolled fashion at one reaction. Noyori and coworkers reported the highly stereoselective synthesis of *syn*-β-hydroxy-α-amino acids from chirally labile α-acylamino-β-ketoesters using this method. At the beginning of our study, we synthesized all four stereoisomers of 3-hydroxyleucine using the Noyori method (*9*). This method is efficient for obtaining *syn*-β-hydroxy-α-amino acids, but for *anti*-β-hydroxy-α-amino acids it has a substantial disadvantage that one more step for

the inversion of the C3 stereocenter is needed. Therefore, we focussed on a direct and straightforward construction of *anti*-β-hydroxy-α-amino acids by asymmetric hydrogenation via DKR.
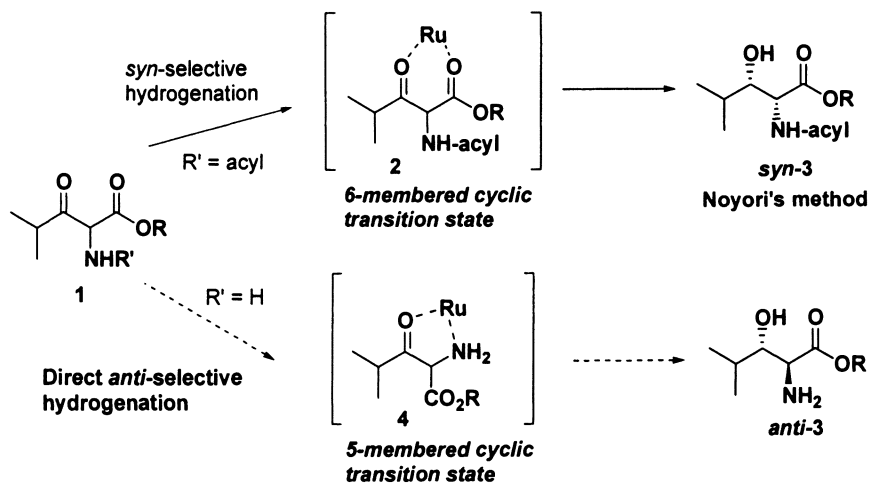
Figure 2. Direct anti-Selective Hydrogenation

As shown in Figure 2, we envisioned hydrogenation via the 5-membered cyclic transition state **4** using the 2-amino substituent of α-amino-β-keto ester **1** as a directing group, which would directly produce *anti*-β-hydroxy-α-amino acid ester in place of the *syn* product generated via the 6-membered cyclic transition state **2** (*10*). In fact, asymmetric hydrogenation of α-amino-β-ketoester hydrochloride **5** with Ru-(*S*)-BINAP in methanol at 50°C for 48 h gave *anti*-β-hydroxy-α-amino acid ester **6** in almost perfect diastereoselectivity and moderate enantioselectivity (Table 1, entry 1). With this encouraging result in hand, we extensively surveyed the optimized conditions for the *anti*-selective hydrogenation with high enantioselectivity. Methylene chloride is the solvent of choice for the enantioselectivity and isopropanol and *n*-propanol also give satisfactory results. The ester function affected the chemical yield due to low solubility of the starting hydrochloride salt. Finally, benzyl ester was most effective in terms of chemical yield and enantioselectivity (entry 2).

This method was then applied to various substrates to clarify the generality of the hydrogenation. The starting α-amino-β-keto ester hydrochlorides were easily prepared by the following four methods: (1) acid hydrolysis of 4-alkoxycarbonyloxazoles derived from carboxylic anhydrides and isocyanoacetic

Table I. *anti*-Selective Hydrogenation through DKR

$$R^1 \underset{\underset{NH_2 \cdot HCl}{5}}{\overset{O \quad O}{\bigvee}} OR^2 \xrightarrow[\text{solvent, 50 °C, 48 h}]{\begin{array}{c}H_2 \text{ (100 atm)}\\ [RuCl_2(S)\text{-binap}](dmf)_n\end{array}} R^1 \underset{\underset{NH_2 \cdot HCl}{6}}{\overset{OH \quad O}{\bigvee}} OR^2 \xrightarrow[\text{THF, rt, 1 h}]{BzCl, TEA} R^1 \underset{NHBz}{\overset{OH \quad O}{\bigvee}} OR^2$$

entry	R^1	R^2	solvent	yield [%]	anti:syn	ee [%][a]
1	*i*Pr	Me	MeOH	71	99:1	56
2	*i*Pr	Bn	CH_2Cl_2	87	>99:1	96
3	cyclobutyl	Bn	*n*PrOH	92	83:17	81
4	cyclopentyl	Bn	*n*PrOH	85	97.5:2.5	95
5	cyclohexyl	Bn	CH_2Cl_2	85	>99:1	97
6	cycloheptyl	Bn	*n*PrOH	86	97:3	97
7[b]	*n*Pr	Bn	*n*PrOH	76	97:3	91
8	*t*Bu	Bn	*n*PrOH	89	96:4	79
9[c]	cyclohexyl	Me	CH_2Cl_2	92	98:2	95

[a] Value of the *anti*-amino acid ester.
[b] (*R*)-MeO-BIPHEP was used at room temperature.
[c] The reaction was carried out by using 0.4 mol catalyst under 30 atm hydrogen pressure.

acid esters, (2) base-mediated N-C acyl migration of *N-t*-butoxycarbonyl-*N*-acylglycine esters and then acid deprotection (*11*), and (3) acylation of the benzophenone ketimine derived from a glycine ester in the presence of a strong base followed by acid hydrolysis (*12*), (4) acylation of acylaminomalonic acid half ester and then deprotection (*13*). Under our reaction conditions, the substrates with a secondary alkyl group, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl substituent, at the α-position of the ketone carbonyl group were the most suitable ones and afforded the *anti* products with high diastereo- and enantioselectivities in excellent chemical yields (entry 3-6). Furthermore, it was found that the hydrogenation of the cyclohexyl substrate was completed in 6 h even with a substrate/catalyst ratio of 250 under 30 atm of hydrogen (entry 9). (2*R*,3*R*)-Cyclohexyl β-hydroxy-α-amino acid obtained by the asymmetric hydrogenation is an important intermediate of the anti-HIV substance (GW873140/ONO-4128) with CCR5 antagonist activity. In the case of the *n*-alkyl substrate at the C 4 position, MeO-BIPHEP in place of BINAP was used due to improving the stereoselectivity (entry 7). The hydrogenation of the *t*-Bu substrate was carried out in *n*-propanol to give the product in 89% yield and 79% ee with 96:4 diastereoselectivity (entry 8).

A few month later, Genêt and coworkers have reported *anti*-selective asymmetric hydrogenation using the Ru catalyst (*14*). This method is essentially same to our *anti*-selective asymmetric hydrogenation. Then, they have applied

this hydrogenation as a key step to a stereoselective synthesis of sulfobacin A (Figure 3) (*15*). Zhang and coworkers have reported an example of *anti*-selective hydrogenation of the α-phthalimino-β-keto ester (*16*).
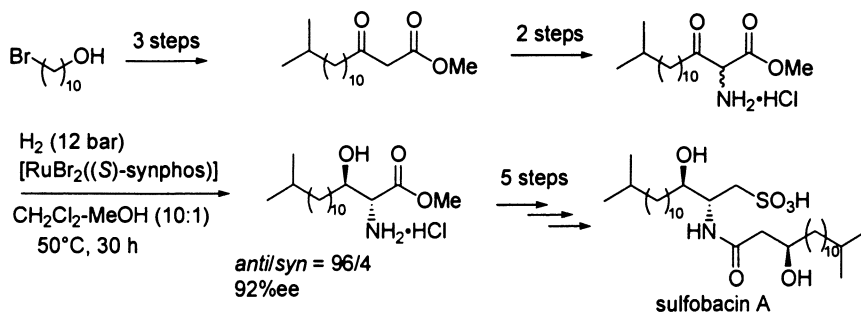
Figure 3. Synthesis of Sulfobacin A by Genêt et al.

Synthesis of *anti* β-Hydroxy-α-Amino Acids using Chiral Iridium Catalysts

First-generation Ir Catalyst

In the case of R = alkyl, the asymmetric hydrogenation using the Ru-axially chiral phosphine smoothly proceeds to afford an *anti*-β-hydroxy-α-amino acid ester with excellent diastereomeric and enantiomeric purity. However, when R is phenyl group, slow reaction and no enantioselectivity were observed. In order to overcome the difficulty, we briefly surveyed transition metals for the *anti*-selective asymmetric hydrogenation of the α-amino-β-keto ester 7, as shown in Table II. Interestingly, in addition to the known ruthenium catalyst, rhodium (Rh) and iridium (Ir) proved to be excellent catalysts for highly *anti*-selective hydrogenation through DKR (entries 1-3) (*17,18,19*). Especially, the Ir catalyst was the promising candidate for the asymmetric hydrogenation of the α-amino-β-keto ester (entry 3). Therefore, we carried out optimization of the Ir-catalyzed *anti*-selective asymmetric hydrogenation.

The procedure for preparation of the catalyst was critical for its catalytic activities. The most active catalyst was made prior to the hydrogenation by mixing [Ir(cod)Cl]₂ with BINAP in methylene chloride at 23°C for 10 min. Acetic acid was the solvent of choice for the diastereoselectivity (Table III, entry 1). The presence of sodium acetate affected the enantioselectivity dramatically (entry 2). Among several chiral phosphines, MeO-BIPHEP was most efficient for the enantioselectivity (entry 3). Moreover, the addition of an iodide anion

Table II. Screening of Transition-metals
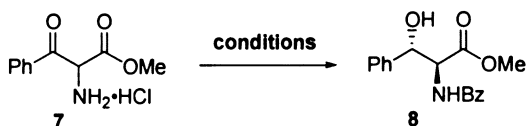
entry	conditions	yield (%)	dr anti : syn	ee (%)
1	RuCl$_2$[(S)-binap](dmf)$_n$ (4 mol%) H$_2$ (100 atm) MeOH, 50 °C, 48 h	31	93 : 7	0
2	[Rh(cod)Cl]$_2$ (3 mol%) (S)-BINAP (4 mol%) H$_2$ (50 atm) MeOH-Benzene, rt, 48 h	44	98 : 2	8
3	[Ir(cod)Cl]$_2$ (3 mol%) (S)-BINAP (4 mol%) H$_2$ (50 atm) MeOH-Benzene, rt, 48 h	87	96 : 4	45

source, especially sodium iodide, in the preparation of the Ir catalyst led to the maximized enantioselectivity (entry 4). This method was then applied to various aromatic substrates. The *anti*-selective hydrogenation via DKR using 3 mol % of the Ir-(S)-MeO-BIPHEP-I catalyst proceeded with almost complete diastereoselectivities under 100 atm of hydrogen in the presence of sodium acetate (1 equiv) in acetic acid at 27-30°C to afford the aromatic *anti*-β-hydroxy-α-amino esters with high enantioselectivities in excellent yields. However, high hydrogen pressure (100 atm) and tedious degassing operation by freeze-thaw cycles in the preparation of the catalyst are essential for smooth reaction and make it difficult to run this hydrogenation in a practical sense.

Second-generation Ir Catalyst

In our effort to expand the utility of the first-generation Ir-catalyst, we examined again various additives except sodium iodide. Among them, the addition of NaBARF to the iridium catalyst prepared from [IrCl(cod)]$_2$ and (S)-MeO-BIPHEP effected increase of the isolated yield to 100%, but the diastereo- and enantioselectivity remained at a similar level with the case of the reaction under high hydrogen pressure (100 atm) (entry 5). Finally, we found an unusual relationship between hydrogen pressure and enantioselectivity that lowering

Table III. Asymmetric *anti*-Selective Hydrogenation using the Ir Catalysts

Reaction scheme: substrate **7** (phenyl group with O, O, OMe, NH₂·HCl) → 1. Ir-ligand-additive complex (3 mol%), H₂, AcOH, rt; 2. Bz₂O, TEA, THF → product **8** (OH, O, OMe, NHBz), *anti:syn* = >99:1

entry	ligand	additives (equiv)	H_2 (atm)	time (h)	yield (%)	ee (%)
First-generation Ir catalyst						
1	(S)-BINAP	-	100	48	81	27
2	(S)-BINAP	NaOAc (1)	100	3	90	69
3	(S)-MeO-BIPHEP	NaOAc (1)	100	3	79	77
4	(S)-MeO-BIPHEP	NaOAc (1), NaI (0.06)	100	24	82	90
Second-generation Ir catalyst						
5	(S)-MeO-BIPHEP	NaOAc (1), NaBARF (0.03)	100	3	quant	74
6	(S)-MeO-BIPHEP	NaOAc (1), NaBARF (0.03)	60	12	quant	80
7	(S)-MeO-BIPHEP	NaOAc (1), NaBARF (0.03)	30	24	quant	84
8	(S)-MeO-BIPHEP	NaOAc (1), NaBARF (0.03)	4.5	24	quant	93
9	(S)-MeO-BIPHEP	NaOAc (1), NaBARF (0.03)	1	96	91	92

hydrogen pressure enhanced enantioselectivity (entries 6-9). Under 4.5 atm of hydrogen the enantioselectivity was improved to 93% ee. Furthermore, the reaction proceeded even under 1 atm of hydrogen with similar stereoselectivity in excellent chemical yield. This second-generation catalyst, Ir-(*S*)-MeO-BIPHEP-BARF complex, can be readily prepared by mixing [IrCl(cod)]₂, (*S*)-MeO-BIPHEP, and NaBARF in methylene chloride at 23°C for 1 h and can be easily handled without a strictly degassed anhydrous condition. The catalyst loading can be lowered from 3 mol % to 0.5 mol % without loss of the yield and diastereo- and enantioselectivities. A survey of several chiral phosphines revealed that (*S*)-MeO-BIPHEP was most efficient in terms of chemical yield and enantioselectivity. Interestingly, when the Ir-PHOX catalyst developed by the Pfaltz group (*20*) was applied to the asymmetric hydrogenation of the α-amino-β-keto ester hydrochloride salt **7**, no reaction was observed.

Under the optimized reaction conditions, the present catalytic direct *anti*-selective asymmetric hydrogenation under low hydrogen pressure was applied to various aromatic substrates as shown in Table IV. The hydrogenation was carried out by using the second-generation Ir-(*S*)-MeO-BIPHEP-BARF catalyst

Table IV. *anti*-Selective Asymmetric Hydrogenation using the Second-generation Ir Catalyst

Reaction scheme:

1. Ir-(S)-MeO-BIPHEP-BARF (1 mol%)
H₂ (4.5 atm), NaOAc (1 eq), AcOH
96 h, rt
→
2. Bz₂O, TEA, THF

Substrate: R–CO–CH(NH₂HCl)–CO–OMe
Product: R–CH(OH)–CH(NHBz)–CO–OMe

quant.
dr = >99:1
92% ee (90% ee)ᵃ

97%
dr = >99:1
90% ee (88% ee)ᵃ

R = OBn, quant., dr = >99:1, 93% ee (94% ee)ᵃ
R = Me, 92%, dr = >99:1, 92% ee (94% ee)ᵃ
R = t-Bu, 97%, dr = 99:1, 91% ee
R = Br, 96%, dr = >99:1, 82% ee (75% ee)ᵃ

94%.
dr = >99:1
90% ee (86% ee)ᵃ

94%
dr = >99:1
96% ee (92% ee)ᵃ

R = Me, quant., dr = >99:1, 90% ee (87% ee)ᵃ
R = Cl, 76%, dr = >99:1, 74% ee
R = F, 67%, dr = >99:1, 67% ee

61
dr = >99:1
84% ee (88% ee)ᵃ

quant.
dr = >99:1
91% ee

33%
dr = >99:1
85% ee

ᵃ The values in the parenthese are the results of the first-generation Ir catalyst.

in the presence of sodium acetate (1 equiv) in acetic acid under 4.5 atm of hydrogen at 23°C for 96 h. The yields and enantioselectivities were improved in comparison with our previous data by using the first-generation Ir catalyst. The introduction of an electron-withdrawing group at the para or meta position on the phenyl ring resulted in a slight decrease of the enantionselectivity, but the *anti*-selectivity was excellent. The cationic Ir complex was also applicable to heteroaromatic substrates containing a sulfur or oxygen atom. In the case of aliphatic substrates, such as R = *n*-Pr and cyclohexyl substrates, at the C4 position, no or low desired reaction was observed. Surprisingly, hydrogenation of the hindered substrate with a *tert*-butyl group efficiently proceeded to provide *anti*-β-hydroxy-α-amino acid ester with >99:1 diastereoselectivity in quantitative yield and 91% ee. It is noted that this result is the highest value for the *tert*-butyl substrate and is superior to that of the Ru-BINAP catalyzed *anti*-selective hydrogenation developed by us.

Mechanism of the *anti*-Selective Asymmetric Hydrogenation

In order to elucidate origin of the extremely high *anti*-selectivity, we briefly carried out the hydrogenation of deuterio substrate as shown in Figure 4. In the case of the hydrogenation via the enol form **13**, the deuterium at the α-position would be removed by tautomerization and the hydrogen for hydrogenation would be provided from gaseous hydrogen to afford the deuterium-free product **12b** instead of the ketone reduction product **12a** after workup.
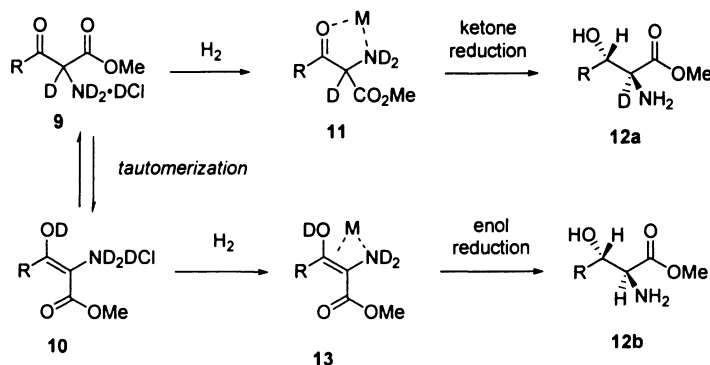
Figure 4. Isotope labeling experiment

Initially, we investigated Ru-catalyzed asymmeric hydrogenation using the cyclohexyl substrates as shown in Figure 5. When the deuterio substrate **14** was hydrogenated with Ru-(*S*)-BINAP at 50°C for 1 h in methylene chloride, the D/H ratio at the α-position of the product was 18:82, supporting that the Ru-catalyzed *anti*-selective asymmetric hydrogenation takes place through the reduction of the enol form. In contrast *syn*-selective asymmetric hydrogenation of **16** as a control experiment gave the D/H ratio of 66:34, the paralell result to the Noyori experiment, which supports that the hydrogenation proceeds via the ketone reduction. These experiments clearly indicate that the *syn*- and *anti*-selective asymmetric hydrogenations proceed via substantially different mechanism.

Next, we carried out the Ir-catalyzed asymmetric hydrogenation using the deuterio aromatic substrate **18**. The hydrogenation using the Ir-(*S*)-MeO-BIPHEP-BARF catalyst in the presence of sodium acetate in acetic acid under one hydrogen pressure at 23°C afforded an equal mixture of two corresponding β-hydroxy-α-amino acid esters **19a** and **19c** in 17% yield. Surprisingly, **19c** has a deuterium at the β-position, which seems to be derived from the D/H exchange on the catalyst (*21*). However, the D/H ratio at the α-position was 100:0. This

236

anti-Selective Hydrogenation
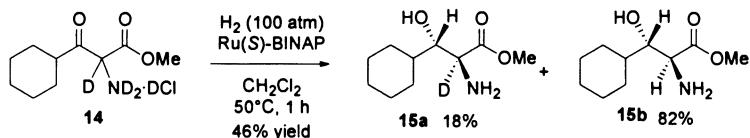
syn-Selective Hydrogenation
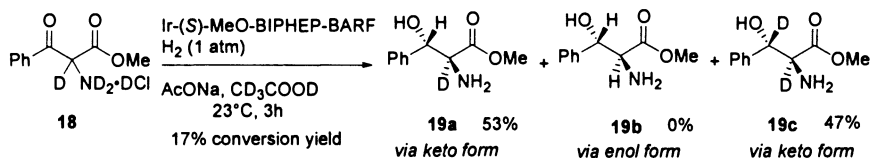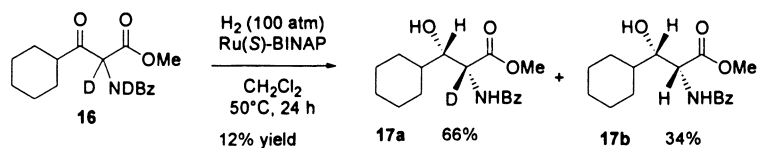
Figure 5. Isotope labeling experiments using the Ru and Ir Catalysts

result clearly supports that the Ir-catalyzed asymmetric hydrogenation of the α-amino-β-keto esters takes place through reduction of the ketone double bond to produce the β-hydroxy-α-amino acid esters with *anti*-stereochemistry.

Conclusion

The above *anti*-selective asymmetric hydrogenation of chirally labile α-amino-β-ketoesters using the Ru- and Ir-axially chiral phosphine catalysts provides simple and straightforward access to important *anti*-β-hydroxy-α-amino acids. Both processes are complementary each other on their scope and limitation. The Ru-catalyzed asymmetric hydrogenation of α-amino-β-ketoesters via DKR is the first example of giving *anti*-β-hydroxy-α-amino acids and the Ir-catalyzed asymmetric hydrogenation is the first example of hydrogenation via dynamic kinetic resolution by the Ir catalyst. Especially, the second-generation Ir catalyst is robust and can be easily used without care, which undergoes mild hydrogenation under low hydrogen pressure. It is noted that this method does not require special instruments and techniques and can be carried out even by use of the hydrogen-balloon technique. The product *anti*-β-hydroxy-

α-amino acids are useful as building blocks for the synthesis of various pharmaceuticals and natural products.

References

1. Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441-4482.
2. a) Williams, R. M.; *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: 1989; pp 1-133. b) Makino, K.; Hamada. Y. *J. Synth. Org. Chem.* **2005**, *63*, 1198-1208. c) Najera, C.; Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584-4671.
3. a) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E.; *Tetrahedron Lett.* **1987**, *28*, 39-42. b) Soloshonok, V. A.; Kukhar, V. P.; Batsanov, A. S.; Galakhov, M. A.; Belokon, Y. N.; Struchkov, Y. T. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, *7*, 1548-1554. c) Soloshonok, V. A.; Kukhar, V. P.; Galushko, S. V.; Rozhenko, A. B.; Kuz'mina, N. A.; Kolycheva, M. T.; Belokon, Y. N. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1991**, *8*, 1906-1913. d) Corey, E. J.; Lee, D. –H.; Choi, S. *Tetrahedron Lett.* **1992**, *33*, 6735-6738. e) Kanemasa, S.; Mori, T.; Tatsukawa, A. *Tetrahedron Lett.* **1993**, *34*, 8293-8296. f) Iwanowicz, E. J.; Blomgren, P.; Cheng, P. T. W.; Smith, K.; Lau, W. F.; Pan, Y. Y.; Gu, H. H.; Malley, M. F.; Gougoutas, J. Z. *Synlett*, **1998**, 664-666. g) Panek, J. S.; Masse, C. E. *J. Org. Chem.* **1998**, *63*, 2382-2384. h) Felice, P. D.; Porzi, G.; Sandri, S. *Tetrahedron: Asymmetry*, **1999**, *10*, 2191-2201. i) Jackson, B.G.; Pedersen, S. W.; Fisher, J. W.; Misner, J. W.; Gardner, J. P.; Staszak, M. A.; Doecke, C.; Rizzo, J.; Aikins, J.; Farkas, E.; Trinkle, K. L.; Vicenzi, J.; Reinhard, M.; Kreoeff, E. P.; Higginbotham, C. A.; Gazak, R. J.; Zhang, T. Y. *Tetrahedron*, **2000**, *56*, 5667-5677. j) Ooi, T.; Taniguchi, M.; Kameda, M.; Maruoka, K. *Angew. Chem. Int. Ed.* **2002**, *41*, 4542-4544. k) Yoshikawa, N.; Shibasaki, M. *Tetrahedron* **2002**, *58*, 8289-8298. l) Kobayashi, J.; Nakamura, M.; Mori, Y.; Yamashita, Y.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 9192-9193. m) Ooi, T.; Kameda, M.; Taniguchi, M.; Maruoka, K. *J. Am. Chem. Soc.* **2004**, *126*, 9685-9694. n) Thayumanavan, R.; Tanaka, F.; Barbas III, C. F. *Org. Lett.* **2004**, *6*, 3541-3544.
4. a) Herold, P. *Helvetica Chimica Acta* **1988**, *71*, 354-362. b) Shimizu, M.; Wakioka, I.; Fujisawa, T. *Tetrahedron Lett.* **1997**, *38*, 6027-6030.
5. Kolb, H. C.; VanNieuwenhze,, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *92*, 2483-2547.
6. a) Guanti, G.; Banfi, L. Narisano, E. *Tetrahedron* **1988**, *44*, 5553-5562. b) Kuwano, R.; Okuda, S.; Ito, Y. *J. Org. Chem.* **1998**, *63*, 3499-3504. c) Amador, M.; Ariza, X.; Garcia, J.; Ortiz, J. *Tetrahedron Lett.* **2003**, *43*, 2691-2694.

7. For reviews on dynamic kinetic resolution, see a) Noyori, R.; Tokunaga, M.; Kitamura, M. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 36-56; b) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475-1490; c) Pellissier, H. *Tetrahedron* **2003**, *59*, 8291-8327.

8. Noyori, R.; Ikeda, T.; Ohkuma, T.; Widhalm, M.; Kitamura, M.; Takaya, H.; Akutagawa, S.; Sayo, N.; Saito, T.; Taketomi, T.; Kumobayashi, H. *J. Am. Chem. Soc.* **1989**, *111*, 9134-9135.

9. Makino, K.; Okamoto, N.; Hara, O.; Hamada, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1757-1762.

10. Makino, K.; Goto,T.; Hiroki, Y.; Hamada, Y. *Angew. Chem. Int. Ed.* **2004**, *43*, 882-884.

11. Hara, O.; Ito, M.; Hamada, Y. *Tetrahedron Lett.* **1998**, *39*, 5537-5540.

12. Singh, J.; Gordon, T. D.; Earley, W. G.; Morgan, B. A. *Tetrahedron Lett.* **1993**, *34*, 211-214.

13. Krysan, D. J. *Tetrahedron Lett.* **1996**, *37*, 3303-3306.

14. a) Mordant, C.; Dunkelmann, P.; Ratovelomanana-Vidal, V.; Genêt, J. P. *Chem.Commun.* **2004**, 1296-1297. b) Mordant,C.; Dunkelmann, P.; Ratovelomanana-Vidal, V.; Genêt, J. -P. *Eur. J. Org. Chem.* **2004**, 3017-3026.

15. Labeeuw, O.; Phansavath, P.; Genêt, J. -P. *Tetrahedron: Asymmetry* **2004**, *15*, 1899-1908.

16. Lei, A.; Wu, S.; He, M.; Zhang, X. *J. Am. Chem. Soc.* **2004**, *126*, 1626-1627.

17. Makino, K.; Hiroki, Y.; Hamada, Y. *J. Am. Chem. Soc.*, **2005**, *127*, 5784-5785.

18. Makino, K.; Fujii, T.; Hamada, Y. *Tetrahedron: Asymmetry*, **2006**, *17*, 481-485.

19. Makino, K.; Iwasaki, M.; Hamada, Y. *Org. Lett.* **2006**, *8*, 4573-4576.

20. Lightfoot, A.; Schnider, R.; Pfaltz, A. *Angew. Chem. Int. Ed.* **1998**, *37*, 2897-2899.

21. Dalenburg, L.; Götz, R. *Eur. J. Inorg. Chem.* **2004**, 888-905.

Chapter 15

Catalyst Screening for the Synthesis of Amino Acids

David J. Ager[1], Laurent Lefort[2], and Johannes G. de Vries[2]

[1]DSM Pharmaceutical Products, PMB 150, 9650 Strickland Road,
Suite 103, Raleigh, NC 27615
[2]DSM Pharmaceutical Products, Innovative Synthesis and Catalysis,
P.O. Box 18, 6160 MD, Geleen, The Netherlands

Asymmetric hydrogenation is a powerful method for the synthesis of α-amino acids and was the first transition metal catalyzed reaction to provide high enantioselectivity. Unfortunately, there is no catalyst that can reduce every possible substrate with high enantioselectivity and screening has to be performed to find a useful candidate. The use of monodentate BINOL-based ligands such as MonoPhos™, which can be prepared by a very short synthetic sequence, allows for large libraries of potential ligands to be accessible within a few hours. Further diversity can be created by screening catalysts based on mixtures of these monodentate ligands. These catalysts can then be used to prepare a wide variety of amino acid derivatives.

Introduction

Amino acids, peptides and peptidomimetics occur in a wide range of biologically active compounds and present a challenge for asymmetric synthesis. Knowles and his coworkers at Monsanto developed the first chemical asymmetric synthesis of amino acids based on rhodium-catalyzed olefin hydrogenation, which rivals enzymatic methods in terms of enantioselectivity and culminated in the production of the drug, L-Dopa (1), for the treatment of Parkinson's disease (Scheme 1).[1] The enamide substrate is prepared by the classical Erlenmeyer reaction and the desired Z-isomer 2 crystallizes from the reaction mixture.[2] The asymmetric catalyst is based on the chiral bisphosphine, R,R-DIPAMP (3), that has chirality at the phosphorus atoms, and can form a 5-membered chelate ring with rhodium. The asymmetric reduction of the Z-enamide, proceeds in 96% ee.[3] The pure isomer of the protected amino acid intermediate 4 can be obtained upon crystallization from the reaction mixture as it is a conglomerate.[4]
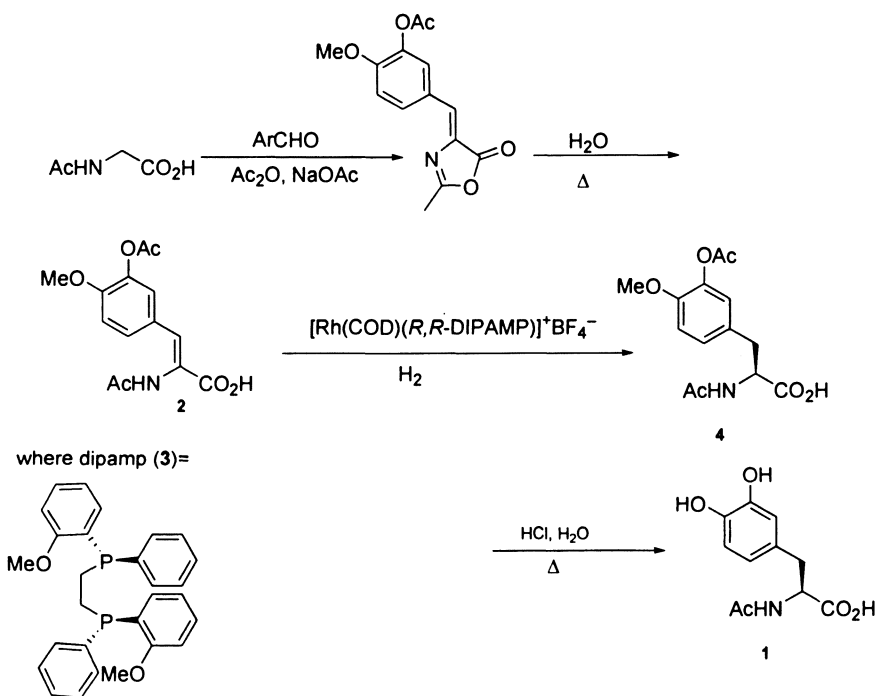
Scheme 1.

Although the catalyst system is amenable to the preparation of a wide variety of amino acids, especially substituted phenylalanine derivatives,[5,6] a major shortcoming of the approach is the need to have just the *Z*-enamide isomer **5a** as the substrate. Knowles' catalyst gives slow reactions with low asymmetric induction if an *E*-enamide **5b** is used as the substrate.[4] Fortunately, the general method for the preparation of the enamides is stereoselective to the *Z*-isomer when an aryl aldehyde is used. Other approaches to the alkene are also available such as the Heck or Suzuki reactions.

The need to prepare the substrate in a stereoselective manner was overcome by the use of the DuPhos (**6**) family of ligands. For substrates that possess a single β-substituent (e.g., either R^2 or R^3 = H), the Me-DuPhos-Rh and Et-DuPhos-Rh catalysts were found to give enantioselectivities of 95-99% at the alpha-center for a wide range of amino acid derivatives.[7,8] Hydrogenation of just the *E*- or *Z*-enamide isomers allows the generation of the β-stereogenic center with high selectivity. The more electron-rich Me-BPE (**7**, R = Me) catalysts can be used to access β-branched amino acids (Scheme 2, R^2, $R^3 \neq$ H).[9,10]

5a R^3 = H
5b R^2 = H
5c R = Me, R^1 = Me, R^2 = Ph, R^3 = H
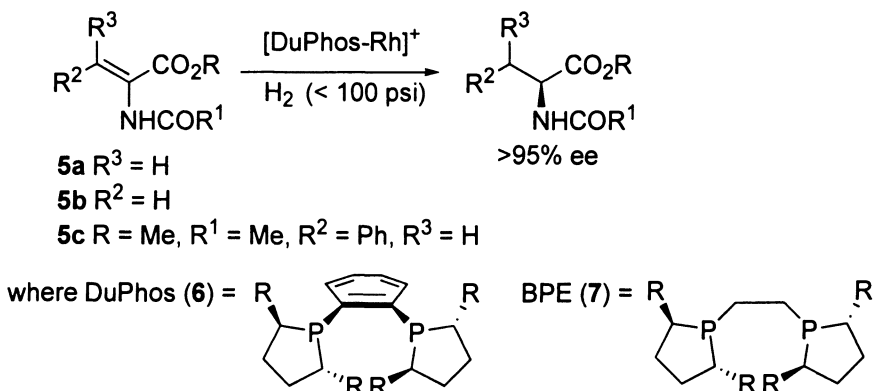
Scheme 2.

The DuPhos reduction has been used to prepare a wide range of amino acids with substituted aromatic, heteroaromatic, alkyl, fluoroalkyl, and other functionalized organic groups.[9-16] Polyamino acids can also be accessed.[17-20]

There is no comprehensive catalyst system to perform an asymmetric hydrogenation under optimal conditions. Although the systems mentioned above, as well as others,[21] provide adequate selectivity and rates across a wide range of enamides, some optimization may be required to obtain an economical, commercial synthesis. For these large-scale reactions, not only does

enantioselectivity have to be considered but turnover number (TON), the amount of catalyst that is required, and turnover frequency (TOF), the amount of time it takes to go round the catalytic cycle, are also important factors. Both of these considerations relate to cost; how much catalyst has to be bought is related by the TON while equipment costs and time are connected to TOF. A different ligand, therefore, may be required for each separate substrate. In addition, the optimal reaction conditions may also vary. As with most process chemistry procedures, the solution comes back to looking at many different conditions; in other words, the variables need to be screened.

For the catalysts themselves, there are a number of metals known to perform asymmetric hydrogenations to provide amino acid derivatives although rhodium and iridium are the most common. To achieve high enantioselectivity under optimal conditions, a wide variety of ligands needs to be screened.

Many asymmetric hydrogenation catalysts are bisphosphines and prepared by long synthetic sequences. Thus, to obtain a sample of such a ligand requires investment in time and money. Some of these ligands can be bought either as the free ligand or as a metal complex; others require synthesis, as they are not commercially available. Samples of these ligands and catalysts can be kept for future screening use. However, many phosphines are oxygen sensitive and can degrade over time. Although this can be addressed, especially by the use of an inert atmosphere and temperature control, this approach requires careful monitoring and space. In addition, for screening only small amounts of the ligand will be required and dispensing these small amounts can be problematic. An alternative method is to make the ligand and then use it straightaway. This alleviates storage problems. The monodentate MonoPhos family of ligands **8** is set up to fulfill the requirements due to their ready accessibility.

Rhodium-Catalyzed Hydrogenations

Rhodium-based catalysts with the monodentate phosphoramidite ligands have been found to provide high enantioselectivities for the asymmetric hydrogenation of dehydroamino acid substrates. Unlike bidentate ligands, there can be a strong solvent effect.[22] The stereogenic backbone of the majority of phosphoramidite ligands is derived from 2,2'-binaphthol (BINOL) (**9**), which is readlily available as either isomer (Scheme 3).[23,24]

The intermediate phosphoryl chloride **10** is stable enough to be able to use in stock solutions. This convenient phenomenon extends to derivatives of BINOL. The biphenol **9** is derived from dimerization of 2-naphthol and the antipodes are then separated.[25] 2-Naphthol is readily substituted at the 3- and 6-positions due to the directing nature of the hydroxy group. Although a little more difficult, substitution can also be introduced at the 4-position.[26] Dimerization and resolution then gives access to the general BINOL-based ligands **11**.

Scheme 3.

11
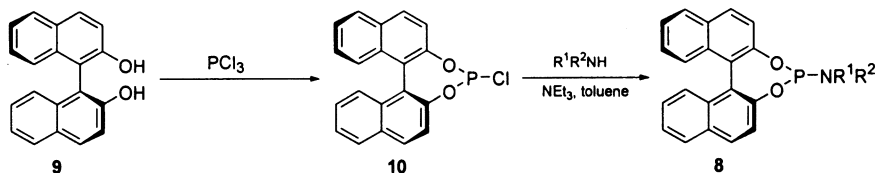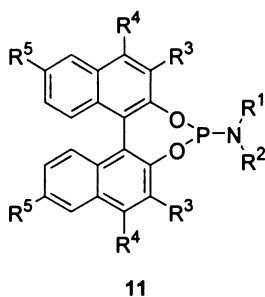
To alleviate the problems associated with ligand storage and dispensing, a method was sought where the ligand synthesis could be automated. The structure of the ligands themselves allows for coupling to a solid support either through the biphenol (e.g., R^5 attached to a polymer) or the amine (R^1). None of these approaches were successful. A polymeric base did provide ligands that gave comparable results in the reduction to when purified ligands were used. However, as the purpose of the base is to drive the synthesis of the phosphoramidite to completion, triethylamine is competent in a solvent, such as toluene, where the resultant salt is insoluble.[27] For asymmetric hydrogenations, the presence of chloride ion can be detrimental,[28] but the triethylamine hydrochloride precipitates out and can be removed by filtration.

The addition of the reagents for ligand synthesis can be performed by a robot and the reactions performed in 96-well plates. The use of an oleophobic filter then allows subsequent removal of the salt by-product by application of a vacuum. Comparison studies showed that parallel synthesis of the ligands, with only filtration to remove the salt by-product and no further purification, gave results in the reductions that were close to the analogous reactions with purified ligands.[27] Thus, for the reduction of the enamide (**5c**) to prepare phenylalanine methyl ester, use of the purified ligand **8** ($R^1 = R^2 = Et$) gave 99% ee with quantitative conversion in dichloromethane under 5 bar of hydrogen; whereas use of the ligand prepared in the robot from which only the salts were removed by filtration led to 96% ee and 100% conversion under identical conditions. On average ee's are ~5% lower using the robot-prepared ligands. However, the

relative order of selectivity and reactivity remains the same, making this methodology an excellent screening tool.

As phosphoramidite ligands are monodentate and simple to prepare, there is a second aspect of the approach that can be exploited. The results from the first round of screening can be used to design and investigate more ligands in a second round of screening. The time required for this series of experiments is the same as the first screen; no long ligand syntheses are required. Thus, multiple rounds of experiments can be performed to not only optimize enantioselectivity but also reaction conditions. This is illustrated in Figure 1.
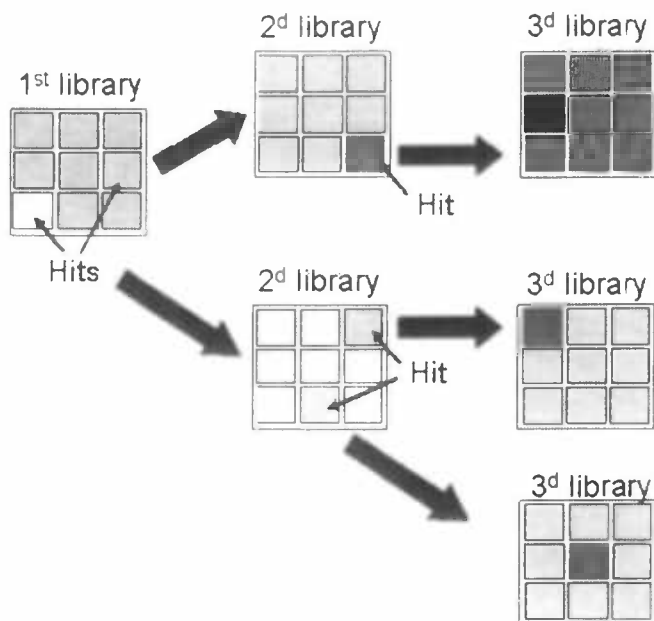
Figure 1. Iterative approach to ligand screening.

Monodentate ligands such as phosphoramidites and phosphites have a third property, which can be useful and also greatly increases the diversity space. Unlike bidentate bisphosphine ligands, which bind tightly to the metal, monodentate ligands provide a more dynamic system. In these, the most reactive system can be made up from a mixed ligand system. This is illustrated by the reduction of the α,β-unsaturated carboxylic acid 12 in the synthesis of the renin inhibitor, Aliskirin™ (13) (Scheme 4).[29]
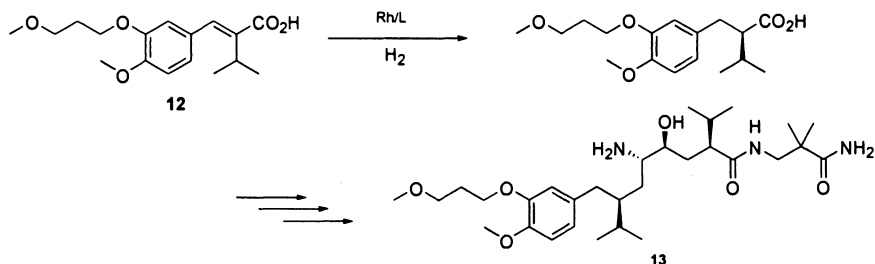
Scheme 4.

The initial screen was performed with eight different phosphoramidite ligands. Although the enantioselectivity was lower than required, it was the reaction rate that gave cause for concern. However, it could be seen that the use of 3,3'-disubstituted BINOL gave the best stereoselection. A second screen was performed but with 96 different achiral electron-donating ligands being added to the system; MonoPhos™ itself (**8**, $R^1 = R^2 = Me$) was used as the phosphoramidite. The results showed that the addition of an arylphosphine not only increased the reaction rate but also provided better enantioselectivity. Further rounds of screening lead to the use of the piperidine ligand **11** ($R^1, R^2 = -(CH_2)_5-$, $R^3 = Me$, $R^4 = R^5 = H$) with tri(*m*-tolyl)phosphine in a ratio of 2:1.[29]

As stated above, the monodentate ligands lead to complex equilibria being established. The presence of an achiral ligand can give rise to a rhodium complex, which is viable to perform the reduction but would lead to racemic product. From the initial screening results the complex arising from two moles of **11** interacting with the metal leads to a system that is slow in performing the desired asymmetric hydrogenation. The use of the 2:1 ratio gives enough of the "mixed" ligand complex, which is the active catalyst to result in good reaction rate while not compromising the enantioselectivity.

The use of mixed ligand systems provides an extremely large number of possibilities. Just 10 different ligands provide 55 different combinations. In independent studies, Reetz and coworkers independently found that mixtures of monodentate ligands can provide improved enantioselectivity in selected cases.[30]

This phenomenon has been applied to the reduction of β-amino acid derivatives where the use of a mixed ligand system gave much better rates and enantioselectivity compared to the use of two moles of a single ligand (Scheme 5).[31]

Phosphoramidites based on a BINOL backbone have proven to be extremely useful ligands for a wide variety of asymmetric hydrogenations, as summarized in Table I.[32,33] Unlike most bidentate ligands, there can be a marked solvent effect for these reductions. The enantioselectivity for the reduction of the dehydroamino ester **5c** with MonoPhos (**8**, $R^1 = R^2 = Me$) was 70% in methanol but a simple switch to dichloromethane resulted in an ee of >95%.[22] Thus, a

NHAc

R ⤳CO₂Me

$$\xrightarrow[\text{H}_2]{\text{Rh(COD)}_2\text{BF}_4,\ \text{L}^1\text{L}^2}$$

NHAc

R ⤳CO₂Me
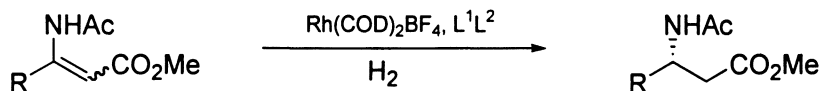
where R = Me or Ph

Scheme 5.

change in solvent can be used to advantage. An increase in hydrogen pressure increases the reaction rate for hydrogenations with **11**, but does not adversely affect the stereoselectivity of the reduction.

Iridium Catalysts

For the asymmetric reduction of enamides **5c** to α-amino acid derivatives, the much cheaper metal iridium can be used rather than rhodium (Scheme 6). Based on the work of Crabtree, [Ir(cod)Cl]₂ was treated with two equivalents of a MonoPhos ligand to provide [Ir(cod)LCl], which, in turn, was treated with a second ligand. No viable hydrogenation catalysts were found until a bulky ligand was used and the chloride was not displaced by a second ligand. Further screening studies showed that the BINOL backbone was not required and a simple bisphenol could be used as long as there was a large group in the 3- and 3'-positions.[42]

Subsequent studies suggest that there is only one phosphoramidite ligand bound to the metal in the active species; the precursor only has one present, the addition of more ligand does not give a rate acceleration, non-linear effects are absent, and there is no mixed ligand effect.

Summary

In summary, the monodentate phosphoramidite ligands have been established as a versatile family of ligands to perform asymmetric hydrogenations.[43-46] The ease of synthesis and ready access to a very large number of family members provides for the screening of many ligands and mixtures without taking a large amount of time. The second advantage is the simple synthesis keeps costs for the ligand to a minimum. These systems have been, and continue to be, implemented at industrial scale. However, work still needs to be performed to understand the mechanism of the reductions and provide more insight into the controlling factors.

Table I. Asymmetric hydrogenations with phosphoramidite ligands based on the BINOL backbone.

Substrate	Product	Reference(s)
Ar–CH=C(CO$_2$H)(NHAc)	Ar–CH$_2$–CH(CO$_2$H)(NHAc)	22,34,35
Ar–CH=C(CO$_2$R)(NHAc)	Ar–CH$_2$–CH(CO$_2$R)(NHAc)	22,24,27,34-36
R–CH=C(CO$_2$H)(NHAc)	R–CH$_2$–CH(CO$_2$H)(NHAc)	22,34,35
R–CH=C(CO$_2$R^1)(NHAc)	R–CH$_2$–CH(CO$_2$R^1)(NHAc)	22,34-36
R–C(NHAc)=CH–CO$_2$R^1	R–CH(NHAc)–CH$_2$–CO$_2$R^1	27,37
AcHN–CH=C(CO$_2$R^1)(R)	AcHN–CH$_2$–C(CO$_2$R^1)(R)	38
R–CH=C(CO$_2$H)(R^1)	R–CH$_2$–CH(CO$_2$H)(R^1)	39
HO$_2$C–CH$_2$–C(=CH$_2$)–CO$_2$H	HO$_2$C–CH$_2$–CH(CH$_3$)–CO$_2$H	22,24,35
R^{1}O$_2$C–CH$_2$–C(=CH$_2$)–CO$_2$R	R^{1}O$_2$C–CH$_2$–CH(CH$_3$)–CO$_2$R	22
Ar–C(NHAc)=CH–R	Ar–CH(NHAc)–CH$_2$–R	24,35,40
Ar–C(OAc)=CH–R	Ar–CH(OAc)–CH$_2$–R	41
Ar–C(OCONMe$_2$)=CH–R	Ar–CH(OCONMe$_2$)–CH$_2$–R	41

$$Ph \overset{\displaystyle CO_2Me}{\underset{\displaystyle NHAc}{=}} \quad \xrightarrow[\text{CH}_2\text{Cl}_2,\ H_2\ (5\ bar)]{\text{[Ir(COD)(L)Cl]}} \quad Ph \overset{\displaystyle CO_2Me}{\underset{\displaystyle NHAc}{\bigwedge}}$$

5c

ee 98%

where L =

Scheme 6.

References

1. Ager, D. J. In *Handbook of Homogeneous Hydrogenation*; de Vries, J. G., Elsevier, C. J., Eds.; Wiley-VCH: Weinheim, 2007; Vol. 2; p 745.
2. Herbst, R. M.; Shemin, D. *Org. Synth.* **1943**, *Col. Vol. II*, 1.
3. Knowles, W. S. *Angew. Chem., Int. Ed.* **2002**, *41*, 1998.
4. Knowles, W. S. *Acc. Chem. Res.* **1983**, *16*, 106.
5. Ager, D. J.; Laneman, S. A. In *Asymmetric Catalysis on Industrial Scale*; Blaser, H.-U., Schmidt, E., Eds.; Wiley-VCH: Weinheim, 2004; p 259.
6. Laneman, S. A.; Froen, D. E.; Ager, D. J. In *Catalysis of Organic Reactions*; Herkes, F. E., Ed.; Marcel Dekker: New York, 1998; Vol. 75; p 525.
7. Burk, M. J.; Bienewald, F. In *Transition Metals for Organic Synthesis and Fine Chemicals*; Bolm, C., Beller, M., Eds.; VCH: Weinheim, 1998; p 13.
8. Burk, M. J.; Ramsden, J. A. In *Handbook of Chiral Chemicals*; 2nd ed.; Ager, D. J., Ed.; CRC: Taylor Francis: Boca Raton, 2005; p 249.
9. Burk, M. J.; Gross, M. F.; Martinez, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 9375.
10. Hoerrner, R. S.; Askin, D.; Volante, R. P.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 3455.
11. Stammers, T. A.; Burk, M. J. *Tetrahedron Lett.* **1999**, *40*, 3325.
12. Masquelin, T.; Broger, E.; Mueller, K.; Schmid, R.; Obrecht, D. *Helv. Chim. Acta* **1994**, *77*, 1395.

13. Jones, S. W.; Palmer, C. F.; Paul, J. M.; Tiffin, P. D. *Tetrahedron Lett.* **1999**, *40*, 1211.

14. Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Tetrahedron: Asymmetry* **2001**, *12*, 2385.

15. Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. *Org. Lett.* **2001**, *3*, 3157.

16. Roff, G.; Lloyd, R. C.; Turner, N. J. *J. Am. Chem. Soc.* **2004**, *126*, 4098.

17. Hiebl, J.; Kollmann, H.; Rovenszky, F.; Winkler, K. *J. Org. Chem.* **1999**, *64*, 1947.

18. Maricic, S.; Ritzén, A.; Berg, U.; Frejd, T. *Tetrahedron* **2001**, *57*, 6523.

19. Rizen, A.; Basu, B.; Chattopadhyay, S. K.; Dossa, F.; Frejd, T. *Tetrahedron: Asymmetry* **1998**, *9*, 503.

20. Shieh, W.-C.; Xue, S.; Reel, N.; Wu, R.; Fitt, J.; Repic, O. *Tetrahedron: Asymmetry* **2001**, *12*, 2421.

21. Tang, W.; Zhang, X. *Chem. Rev.* **2003**, *103*, 3029.

22. van den Berg, M.; Minnaard, A. J.; Schudde, E. P.; van Esch, J.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 11539.

23. Arnold, L. A.; Imbos, R.; Mandoli, A.; de Vries, A. H. M.; Naasz, R.; Feringa, B. L. *Tetrahedron* **2000**, *56*, 2865.

24. Bernsmann, H.; van den Berg, M.; Hoen, R.; Minnaard, A. J.; Mehler, G.; Reetz, M. T.; de Vries, J. G.; Feringa, B. L. *J. Org. Chem.* **2005**, *70*, 943.

25. Brunel, J. M. *Chem. Rev.* **2005**, *105*, 857.

26. Hovorka, M.; Ščigel, R.; Gunterova, J.; Tichý, M.; Závada, J. *Tetrahedron* **1992**, *48*, 9503.

27. Lefort, L.; Boogers, J. A. M.; de Vries, A. H. M.; de Vries, J. G. *Org. Lett.* **2004**, *6*, 1733.

28. Cobley, C.; Lennon, I. C.; Praquin, C.; Zanotti-Gerosa, A. *Org. Proc. Res. Develop.* **2003**, *7*, 407.

29. Boogers, J. A. M.; Felfer, U.; Kotthaus, M.; Lefort, L.; Steinbauer, G.; de Vries, A. H. M.; de Vries, J. G. *Org. Proc. Res. Develop.* **2007**, *11*, 585.

30. Reetz, M. T.; Sell, T.; Meiswinkel, A.; Mehler, G. *Angew. Chem., Int. Ed.* **2003**, *42*, 790.

31. Peña, D.; Minnaard, A. J.; Boogers, J. A. M.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *Org. Biomol. Chem.* **2003**, *1*, 1087.

32. Minnaard, A. J.; Feringa, B. L.; Lefort, L.; de Vries, J. G. *Acc. Chem. Res.* **2007**, *40*, 1267.

33. Ager, D. J.; de Vries, A. H. M.; de Vries, J. G. *Plat. Metal Rev.* **2006**, *50*, 54.

34. Ager, D.; van den Berg, M.; Minnaard, A. J.; Feringa, B. L.; de Vries, A. H. M.; Willans, C. E.; Boogers, J. A. M.; de Vries, J. G. In *Methodologies in Asymmetric Catalysis*; Malhotra, S. V., Ed.; American Chemical Society: Washington DC, 2004; Vol. ACS Symposium Series 880; p 115.

35. van den Berg, M.; Minnaard, A. J.; Haak, R. M.; Leeman, M.; Schudde, E. P.; Meetsma, A.; Feringa, B. L.; de Vries, A. H. M.; Maljaars, C. E. P.; Willans, C. E.; Hyett, D.; Boogers, J. A. M.; Henderickx, H. J. W.; de Vries, J. G. *Adv. Synth. Catal.* **2003** *345*, 308.

36. Panella, L.; Aleixandre, A. M.; Kruidhof, G. J.; Robertus, J.; Feringa, B. L.; de Vries, J. G.; Minnaard, A. J. *J. Org. Chem.* **2006** *71*, 2026.

37. Peña, D.; Minnaard, A. J.; de Vries, J. G.; Feringa, B. L. *J. Am. Chem. Soc.* **2002** *124*, 14552.

38. Hoen, R.; Tiemersma-Wegman, T.; Procuranti, B.; Lefort, L.; de Vries, J. G.; Minnaard, A. J.; Feringa, B. L. *Org. Biomol. Chem.* **2007** *5*, 267.

39. Hoen, R.; Boogers, J. A. M.; Bernsmann, H.; Minnaard, A. J.; Meetsma, A.; Tiemersma-Wegman, T. D.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2005** *44*, 4209.

40. van den Berg, M.; Haak, R. M.; Minnaard, A. J.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *Adv. Synth. Catal.* **2002** *344*, 1003.

41. Panella, L.; Feringa, B. L.; de Vries, J. G.; Minnaard, A. J. *Org. Lett.* **2005** *7*, 4177.

42. Giacomina, F.; Meetsma, A.; Panella, L.; Lefort, L.; de Vries, A. H. M.; de Vries, J. G. *Angew. Chem., Int. Ed.* **2007** *46*, 1497.

43. Eberhardt, L.; Armspach, D.; Harrowfield, J.; Matt, D. *Chem. Soc. Rev.* **2008** *37*, 839.

44. van den Berg, M.; Feringa, B. L.; Minnaard, A. J. In *Handbook of Homogeneous Hydrogenation*; de Vries, J. G., Elsevier, C. J., Eds.; Wiley-VCH: Weinheim, 2007; Vol. 2; p 995.

45. de Vries, J. G. In *Handbook of Chiral Chemicals;* 2nd ed.; Ager, D. J., Ed.; CRC: Taylor Francis: Boca Raton, 2005; p 269.

46. Jerphagnon, T.; Renaud, J.-L.; Bruneau, C. *Tetrahedron: Asymmetry* **2004** *15*, 2101.

Chapter 16

Asymmetric Synthesis of α-Amino Acids Using Polymer-Supported Cinchona Ammonium Salts as Phase-Transfer Catalysts

Rafael Chinchílla[*] and Carmen Nájera[*]

Departamento de Química Orgánica, Facultad de Ciencias, and Instituto de Sintesis Orgánica (ISO), Universidad de Alicante, P.O. Box 99, 03080 Alicante, Spain

Cinchona ammonium salts from cinchonine, cinchonidine, quinine and quinidine alkaloids have been anchored to polymers and used as phase-transfer catalysts in the enantioselective alkylation of N-diphenylmethylene glycine esters to afford enantioenriched α-amino acid derivatives. These supported catalysts can be separated from the reaction medium and reused, being promising from an industrial viewpoint.

Introduction

Quaternary ammonium salts derived from Cinchona alkaloids have evolved as economic and readily available chiral catalysts in several enantioselective processes (*1*) performed using the mild, simple and easily scalable phase-transfer catalysis (PTC) methodology (*2*). Especially, intensive studies have been performed on their use in the enantioselective alkylation of N-diphenylmethylene glycine esters 1, which afford α-alkylated derivatives 2 thus driving to a variety of natural and of non-natural α-amino acids after hydrolysis (Figure 1) (*3*). Both enantiomers of 2 can be obtained by using as catalysts cinchonine/quinidine-derived salts [driving generally to (*R*)-2] or cinchonidine/quinine-derived salts

[driving generally to (*S*)-2], these alkaloids being considered as pseudoenantiomers.

Although small amounts of these not too expensive solution phase catalyst are consumed working under PTC conditions, reusable polymer supported catalysts might have more advantages in a large scale production from both practical and economical viewpoints (*4*). This article presents a revision of the use of polymer-anchored ammonium salts from Cinchona alkaloids as recyclable catalysts for the enantioselective alkylation of glycinates **1** under PTC conditions.
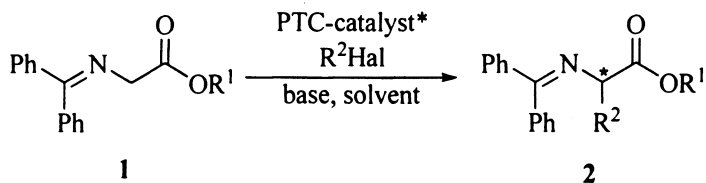
Figure 1. Enantioselective α-alkylation of glycine imines under PTC conditions.

Polymer-Supported Cinchona-derived salts

Based on the connection site to the polymer, the supported Cinchona-derived ammonium salts can be classified into four categories (Figure 2). These anchored systems are employed as PTC catalysts in the enantioselective benzylation reaction of glycinates **1** to give enantioenriched phenylalanine derivatives **3** (Figure 3), this reaction being always carried out as a model for testing the performance of the PTC catalyst.

N-Anchored Ammonium Salts

The attachment of a Cinchona alkaloid to a polymer through the nitrogen atom of the quinuclidine system is the most direct way for creating a chiral supported ammonium salt. Thus, the first enantioselective PTC-promoted alkylation of glycinate **1a** using a polymer-bound Cinchona ammonium salt was performed back in 1998 using cinchonine and quinine-derived ammonium salts **4a** and **5c** (Figure 4), obtained by treating the alkaloid with the Merrifield resin (*5*). However, the achieved enantioselections were very low under the employed reaction conditions (10 mol% of catalyst, CH₂Cl₂, 50% aq NaOH, 20 °C). Thus, when using the cinchonine-derived salt **4a** as PTC catalyst, only a 9% *ee* of the corresponding alkylated product (*R*)-**3** was obtained in the benzylation reaction
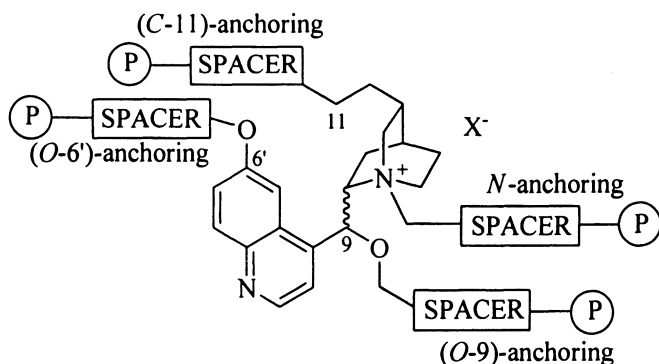
Figure 2. Options for the attachment of ammonium derivatives of Cinchona alkaloids to polymers.

1a: R = t-Bu
1b. R = iPr

3
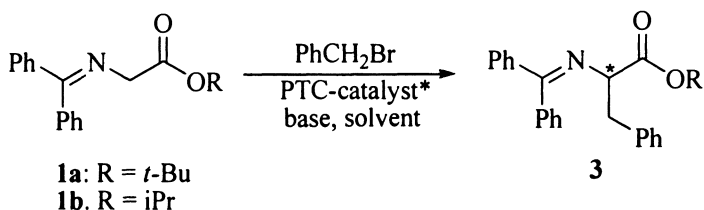
Figure 3. Model enantioselective benzylation of glycinates 1.

of 1a (Figure 3), whereas a 6% ee of its enantiomer (S)-3 was obtained using the quinine derivative 5c (5). These enantioselections could be improved slightly under the same reaction conditions using the cinchonine derivative 4a as well as its pseudoenantiomeric cinchonidine derivative 5a (Figure 4) up to 12 and 27% ee, respectively, by increasing the amount of catalysts up to 100 mol% (Table 1, entries 1 and 4) (6). These supported catalysts were reused up to three times.

The poor enantioselections obtained using these Merrifield-derived PTC catalysts were dramatically improved by changing the reaction conditions and the starting glycinate 1 (7). Thus, when the benzylation reaction (10 mol% of catalyst, toluene, 25% aq NaOH, 0 °C) was carried out on the isopropyl glycinate 1b using the cinchonine salt 4a as PTC catalyst, a 40% ee of (R)-3 could be obtained, whereas a 90% ee of (S)-3 was obtained using the supported cinchonidine salt 5a (Table 1, entries 2 and 6). Other substituted benzyl bromides, as well as allyl and propargyl halides were used as electrophiles achieving ee's in the range 24-66%. In addition, yields were much higher and reaction times considerably shorter than when using the previous procedure, something probably related to the less strict sterical requirements imposed to the

4a: R = H, X = Cl
4b: R = Allyl, X = Br

PS = crosslinked polystyrene

5a: $R^1 = R^2 = H$, X = Cl
5b: $R^1 = H$, R^2 = allyl, X = Br
5c: $R^1 = OMe$, $R^2 = H$, X = Cl
5d: $R^1 = H$, $R^2 = 4\text{-}O_2NC_6H_4CO$, X = Cl
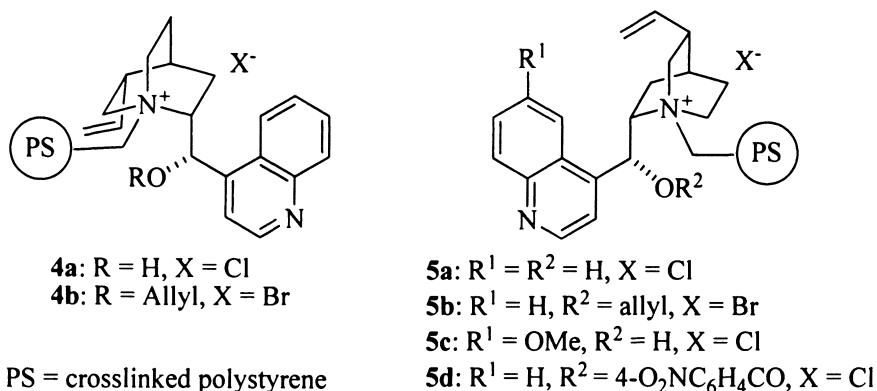
Figure 4. Merrifield N-anchored Cinchona ammonium salts.

polymeric catalyst by the isopropyl group in **1** when compared to the *tert*-butyl group. In fact, only a 58% *ee* for (*S*)-**3** was obtained starting from the *tert*-butyl derivative **1a** in 36 h reaction time. When the *O*-allylated ammonium salts **4b** and **5b** were used as catalysts under this reaction conditions, lower enantioselections were achieved (Table 1, entries 3 and 7), as well as when using the quinine-derived salt **5c** (Table 1, entry 8) (*8*).

In addition, a Merrifield-supported *p*-nitrobenzoate (*O*-9)-esterified cinchonidine salt **5d** has afforded also a very low enantioselection in the

Table 1. Enantioselective benzylation of glycinates 1 using catalysts 4 and 5

Ent.	1	Cat.	Solvent	Base	T (°C)	t (h)	Yield (%)	ee (%)
1	1a	4a	CH$_2$Cl$_2$	50% aq KOH	20	48	55	12 (*R*)
2	1b	4a	PhMe	25% aq NaOH	0	24	85	40 (*R*)
3	1b	4b	PhMe	25% aq NaOH	0	24	45	32 (*R*)
4	1a	5a	CH$_2$Cl$_2$	50% aq NaOH	20	48	48	27 (*S*)
5	1a	5a	PhMe	25% aq NaOH	25	36	80	58 (*S*)
6	1b	5a	PhMe	25% aq NaOH	0	17	90	90 (*S*)
7	1b	5b	PhMe	25% aq NaOH	0	140	23	50 (*S*)
8	1a	5c	CH$_2$Cl$_2$	50% aq NaOH	20	20	48	9 (*S*)
9	1b	5c	PhMe	25% aq NaOH	0	96	81	20 (*S*)
10	1a	5d	CH$_2$Cl$_2$	50% aq KOH	0	24	27	3 (*R*)

benzylation of *tert*-butyl glycinate **1a** (Table 1, entry 10), this result being raised up to 18% *ee* for (*R*)-**3** when working in the absence of an organic solvent (*9*). Moreover, it is interesting than when an *epi*-cinchonidine derivative similar to **4a** but with opposed C-9 stereochemistry was employed as PTC catalyst, a very low enantioselection (8% *ee*) was obtained but also in favour of (*S*)-**3** (*8*).

Other supported *N*-benzylated ammonium salts **6-9** were prepared by *N*-alkylation of cinchonidine using different commercial halogenated polymers (*10*) (Figure 5), such as crosslinked polystyrene-bound triphenylchloromethane (affording **6**), chloromethylated 1-[4-(4-vinylphenoxy)butoxy]-4-vinylbenzene-crosslinked polystyrene (JandaJel™-Cl) (affording **7**), Wang-Br resin (affording **8**) and chloromethylated polyethylene glycol-polystyrene copolymer (ArgoGel™-Cl) (affording **9**). In addition, the 9-anthracenylmethylated polymeric cinchonidine derivative **10** was prepared. All these polymer-bound cinchonidine ammonium salts were used in the benzylation of isopropyl glycinate **1b** giving rise to enantioselections in the range 2-70% *ee* for the (*S*)-**3** enantiomer (Figure 5), and could be separated by filtration and reused.

Cinchonidine and cinchonine were also *N*-quaternized using chloromethylated polystyrene-grafted polypropylene (chloromethylated Synphase™ lanterns) (*8*). These derivatized Lanterns **11** and **12** were added to the reaction flask, being employed as PTC catalysts in the benzylation of **1b**. Thus, when the cinchonidine-supported lantern **11** was used under the above reaction conditions (10 mol% catalyst, toluene, 25% aq NaOH, 0 °C) but working at room temperature, the final (*S*)-**3** was obtained with identical enantioselectivity than when using its Merrifield resin-attached counterpart **5a** (66% *ee*, 83% yield in 24 h), although lowering the reaction temperature also lowered the *ee* and increased considerably the reaction time (*8*). These lantern-attached ammonium salts have been tweezers-separated after the reaction and reused up to three times, observing just a 3% lowering in yield and *ee* between first and third run.

The role of the spacer between the *N*-quaternization moiety and the Cinchona alkaloid in the enantioselectivity of the PTC alkylation of glycinate **1a** (10 mol% catalyst, toluene, 50% aq KOH, 0 °C) has been investigated preparing a series of *N*-attached cinchonine (**13a**) (*11-13*), quinidine (**13b**) (*11,13*), cinchonidine (**14a**) (*11,13*) and quinine-derived (**14b**) (*11,13*) polymeric salts (n = 4, 6, 8) (Figure 7). The best result (81% *ee*, 60% yield in 27 h) was achieved using the cinchoninium iodide bound to polystyrene with a four-carbon spacer **13a** (n = 4) (*11-13*). Surprisingly, the same enantiomer (*R*)-**3** was always obtained irrespective of the catalyst used (*11*), thus revealing the strong influence of the 'superstructure' of the polymer in the sense of the enantioselectivity. The use of other activated or inactivated halides as electrophiles gave lower enantioselectivities, and recycling of the catalyst gave rise to longer reaction times and lower enantioselections (*13*).

6a: R = H (10 h, 59%, 70% *ee*)
6b: R = Allyl (120 h, 7, 2% *ee*)

7 (10 h, 71%, 56% *ee*)

8 (120 h, 63, 54% *ee*)

9a, R = H (20 h, 91%, 64% *ee*)
9b, R = Allyl (60 h, 28%, 8% *ee*)

10 (32 h, 94%, 70% *ee*)
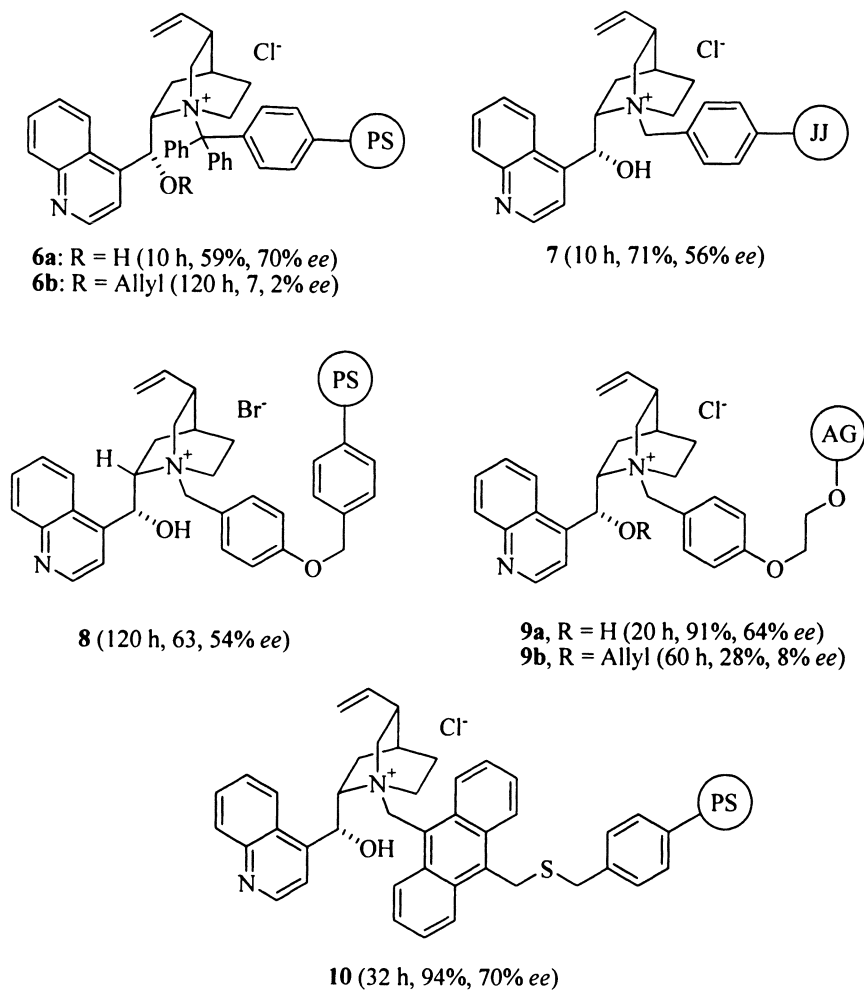
JJ = JandaJel™
AG = ArgoGel™

Figure 5. Supported cinchonidine-derived salts. In parenthesis, reaction times, yields and enantioselectivities for (S)-3 achieved when used as PTC catalysts in the benzylation of 1b (10 mol% catalyst, toluene, 25% aq NaOH, 0 °C).
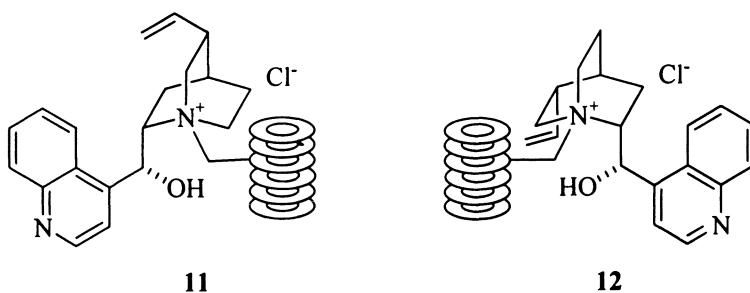
Figure 6. Synphase™ lantern N-bonded cinchonidine and cinchonine-derived ammonium salts.

13a: R = H
13: R = OMe
n = 4, 6, 8
14a: R = H
14b: R = OMe
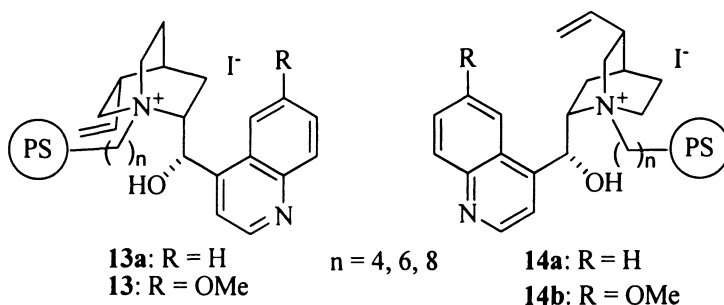
Figure 7. Cinchona-derived ammonium salts attached to differently spaced crosslinked polystyrene beads.

The nature of the support in these anchored supported catalysts has also been changed using a poly(ethylene glycol) (PEG) non-crosslinked matrix type, thus attempting an increase in the solubilising properties in different solvents. Thus, MeO-PEG-OH (M_w 5000) has been used as polymeric phase for the synthesis of the ammonium salts from cinchonidine **15a** (*13-15*) and quinine **15b** (*13,14*) (Figure 8), including the O-substituted surrogates **15c** and **15d** (*14*). The pseudoenantiomeric cinchonine and quinidine counterparts were also obtained (*13,14*).

These PEG-anchored ammonium salts have been used as PTC catalysts in the model benzylation reaction of **1a**, the cinchonidine-derived system being more efficient. Thus, when the PEG-supported cinchonidine salt **15a** was employed as catalyst at 20 °C, a moderate enantiosectivity for (*S*)-**4** was observed (56% ee) (*15*), being considerably improved when working at 0 °C (81% *ee*) (*13,14*) (Figure 8). These polymers could be separated after the reaction by precipitation with ether, although reusing gave slightly lower yields and *ee*'s (*15*).

15a: $R^1 = R^2 = H$ [(S)-3: 84%, 81% ee, 0 °C]
15b: $R^1 = OMe$, $R^2 = H$ [(S)-3: 78%, 33% ee, 0 °C]
15c: $R^1 = H$, $R^2 = Allyl$ [(S)-3: 83%, 73% ee, 20 °C]
15d: $R^1 = H$, $R^2 = Bn$ [(S)-3: 78%, 57% ee, 20 °C]
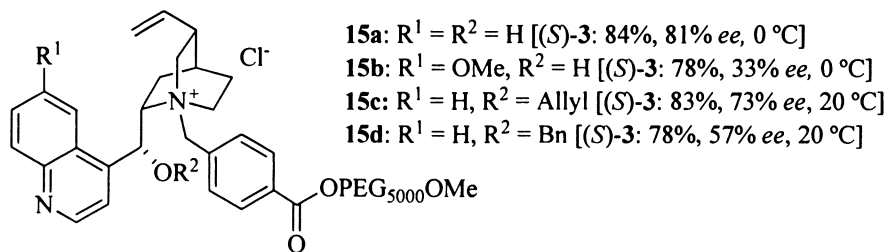
Figure 8. N-PEG-supported Cinchona quaternized salts. In brackets, yields and enantioselectivities achieved when used as PTC catalysts in the benzylation of 1a (10 mol% catalyst, toluene, 50% aq KOH).

Dimeric PEG-supported Cinchona ammonium salts from cinchonidine and quinine **16a** and **16b**, respectively, have been prepared by reaction of the alkaloid with diacetamido-PEG2000 chloride (Figure 9). The linking PEG group acts as a surfactant which can mix the organic components in water, thus allowing the enantioselective alkylation of **1a** in 1M KOH at room temperature and in the absence of any organic solvent in only 6 h (*16*). The quinine derivative **16b** has performed better than its cinchonidine counterpart in the model benzylation reaction of **1a** and has been used as catalysts in the alkylation reaction of **1a** using different benzyl, allyl and alkyl halides in high yields and with very good enantioselectivities of the corresponding (S)-2 in the range 82-97% *ee*. The related dimeric cinchonine derivative has also been prepared and gave rise to a slightly lower enantioselection for the *R*-enantiomer. The catalysts could be recovered by precipitation with ether and reused without loss of activity.

(*O*-9)-Anchored Ammonium Salts

As Cinchona ammonium salts have allowed to obtain very high enantioselectivities in the PTC-promoted enantioselective alkylation of glycinate **1a** when the *N*-quaternization moiety is a 9-anthracenylmethyl group (*3*), keeping this structural area intact by attaching the polymer to the alkaloid in other position was a logical reasoning. Thus, the free C-9 hydroxyl group in the alkaloid can be easily deprotonated with a base such as sodium hydride and can react with the Merrifield resin to afford the corresponding (*O*-9)-anchored ammonium salts (*12,13*). Only the supported cinchonidine-derived salt **17a** (Figure 10) afforded good results in the benzylation reaction of **1a** working at low temperature (10 mol% catalyst, toluene, CsOH, -50 °C), giving rise to (S)-3 in 64% yield and 93% *ee*. The corresponding cinchonine, quinine and quinidine

16a: R = H [(S)-**3**: 95%, 75% *ee*]
16b: R = OMe [(S)-**3**: 98%, 83% *ee*]
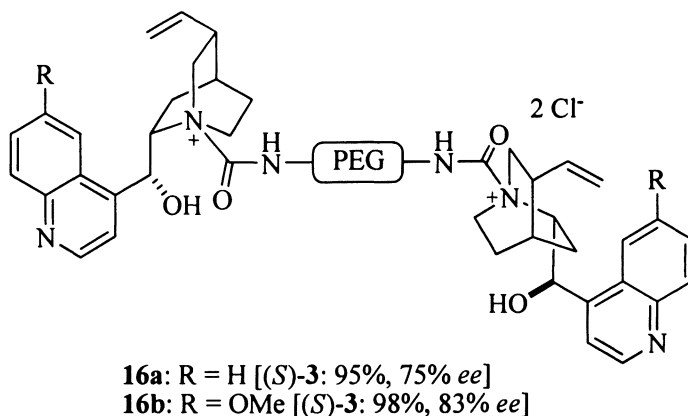
Figure 9. Dimeric PEG-supported cinchonidine and quinine-derived ammonium salts. In brackets, yields and enantioselectivities achieved when used as PTC catalysts in the benzylation of 1a (10 mol% catalyst, 1M KOH, rt, 6 h).

derivatives gave much lower enantioselections (23-36%), the pseudoenantiomeric effect being observed. Recycling of the catalyst resulted in a certain loss of activity after the third run (*13*). This polymer deterioration was avoided by *O*-linking the ammonium salt to Synphase™ lanterns, which allowed a higher stability, although with lower enantioselectivities (up to 78% *ee*) (*13*). In addition, *O*-linking the cinchonidinium moiety to polystyrene through a 4-carbon spacer to give the polymeric salt **17b** did not improve the results, and a 81% *ee* of (*S*)-**3** was obtained under the same reaction conditions than when using **17a** (*13*).

Very good enantioselectivities have been achieved using the (*O*-9)-Merrifield supported dihydrocinchonidine-derived ammonium salts **18** which have been quaternized with benzyl systems containing hydrogen bond-inducing groups (Figure 10) (*17*). The *N*-oxy derivative **18c** showed the highest enantioselectivity for (*S*)-**3** (**18a**, 91% *ee*; **18b**, 93% *ee*; **18c**, 95% *ee*) with yields in the range 81-88% and working under solid-liquid PTC conditions (20 mol% catalyst, toluene, 50% aq KOH, 0 °C). The alkylation of **1a** has also been performed using **18c** as PTC catalyst with other benzyl, allyl and alkyl halides, affording the corresponding (*S*)-amino acid derivatives with 76-96% *ee*. The supported catalyst **18c** could be recovered by filtration and reused five times with no decrease in the enantioselectivity.

The ether linkage of the Cinchona ammonium salt to the polymer has been changed by an ester by grafting the cinchonidinium salt to a cross-linked carboxypolystyrene resin to afford the (*O*-9)-anchored species **19a** (Figure 11) (*13*). In addition, soluble non-crosslinked homopolymeric polystyrene-supported

17a: X = Cl, n = 1
17b: X = I, n = 4

18a: X-Y = C-F
18b: X-Y = C-CN
18c: X-Y = N$^+$-O$^-$
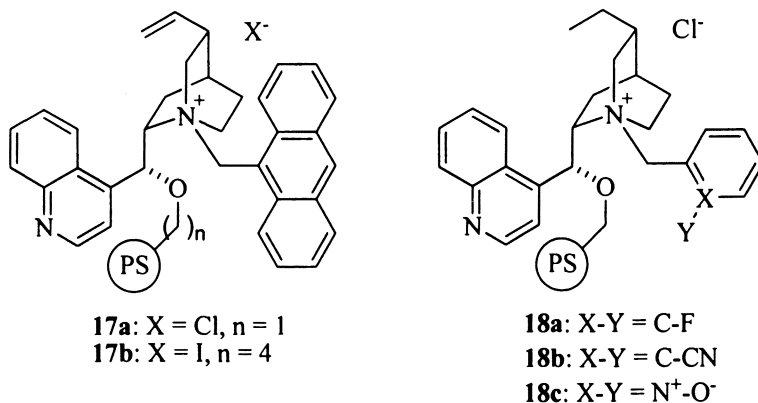
Figure 10. (O-9)-Crosslinked polystyrene-bonded cinchonidine and dihydrocinchonidine-derived ammonium salts.

ammonium salts from dihydrogenated cinchonidine (**19b**) and quinine (**19c**) have been prepared by polymerization of the dihydro alkaloid O-9-(4-vinylbenzoate) and further quaternization (*13*). When all these ammonium salts were used as PTC catalysts in the benzylation of **1a** (10 mol% catalyst, toluene, 50% aq KOH, 0 °C), long reaction times were observed (120 h) for the insoluble catalyst **19a** and only 30% *ee* for (*S*)-**3**. The soluble analogues were more efficient, with reaction completed in 24 h, and more enantioselective (**19b**, 85% *ee*; **19c**, 45% *ee*). The pseudoenantiomeric counterparts from cinchonine, dihydrocinchonine and dihydroquinidine have also been prepared, the highest enantioselectivity for (*R*)-**3** being achieved with the dihydroquinidine-derived salt (67% *ee*). However, attempts to reuse these catalysts failed to give good conversions, indicating strong degradation of the catalyst structure. Moreover, the use of a PEG support to get soluble catalysts, as the case of the (O-9)-anchored cinchonidinium-derivatives **20a** and **20b** (Figure 11) did not improve the best result obtained with the former PTC catalysts in the preparation of (*S*)-**3** under the same reaction conditions (**20a**, 54% *ee*; **20b**, 62% *ee*) (*13*).

(O-6')-Anchored Ammonium Salts

Demethylation of the methoxy group at C-6' of quinine or quinidine (or their derivatives) provides an aromatic hydroxyl group, suitable for connection to polymers. Thus, a quaternary ammonium salt from 6'-*nor*-dihydroquinine has been attached to the Merrifield resin after sodium hydride treatment, and the resulting (O-6')-anchored ammonium salt **21** (Figure 12) has been attempted in

19a: R^1 = H, R^2 = CH=CH$_2$,
    R^3 = crosslinked polystyrene
19b: R^1 = H, R^2 = CH$_2$CH$_3$,
    R^3 = polystyrene homopolymer
19c: R^1 = OMe, R^2 = CH$_2$CH$_3$,
    R^3 = polystyrene homopolymer

20a: X=H$_2$
20b : X = O
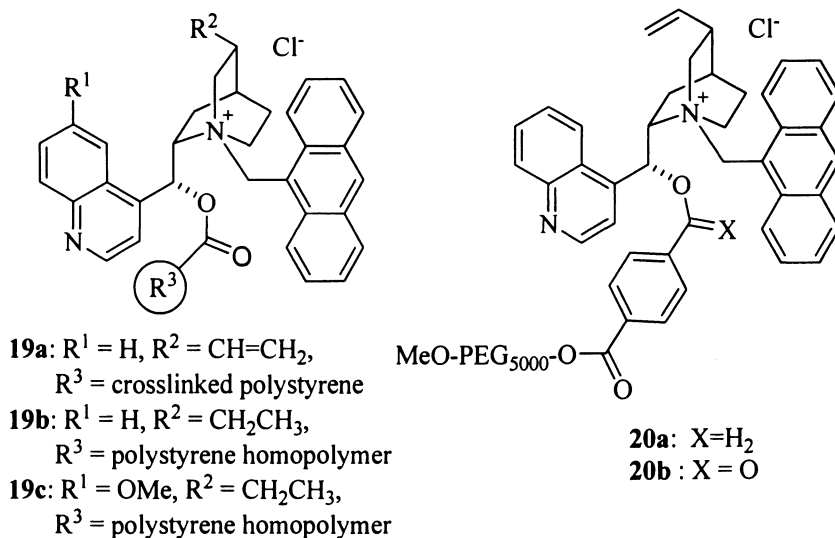
Figure 11. Cinchonidine-derived (O-9)-anchored ammonium salts.

the enantioselective benzylation of **1a**, but failed to promote enantioselectivity higher than 2% (*13*). Similarly, 6'-*nor*-quinine has been attached to differently modified PEG supports, affording ammonium salts **22** (Figure 12) (*18*). When these polymeric compounds were used in the benzylation reaction of **1a**, a poor enantioselectivity for (*S*)-**3** (12% *ee*) was achieved using **22b** as catalyst and working under solid-liquid PTC conditions at very low temperature (10 mol% catalyst, toluene, CsOH, -78 °C), whereas a moderate enantioselection was observed using **22a** as catalyst (64% *ee*). However, attempts to recycle the supported catalyst **22a** were unsuccessful due to strong decomposition.

(*C*-11)-Anchored Ammonium Salts

Cinchonine and cinchonidine have been anchored to a resin through the vinyl group after its transformation into a terminal alkyne and coupling to the Merrifield resin (*13*). When the obtained polymeric ammonium salts, such as the cinchonine-derived **23** (Figure 13) was employed in the PTC benzylation of **1a**, a 73% *ee* of (*R*)-**3** was isolated after 26 h (10 mol% catalyst, toluene, 50% aq KOH, 0 °C). However, the corresponding cinchonidine pseudoenantiomer gave rise to poor enantioselectivity [11% *ee* for (*S*)-**3**], their recyclability being not mentioned (*13*). In addition, the vinyl group of cinchonidine has been used in performing a copolymerization reaction with acrylonitrile to give the co-
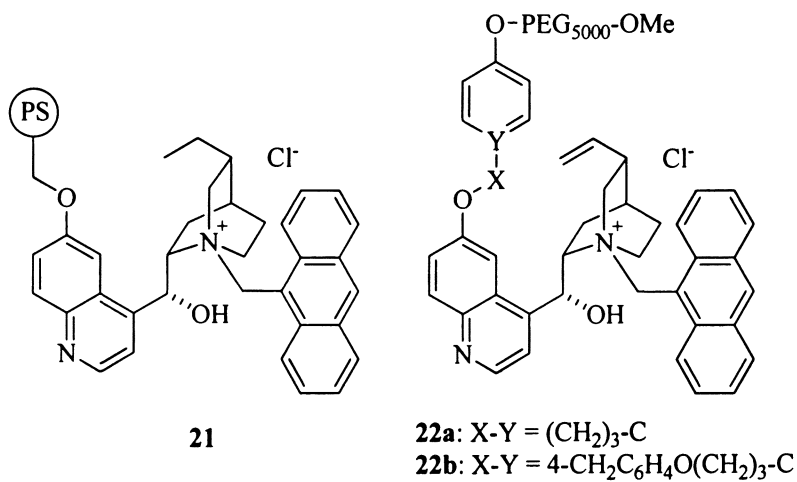
Figure 12. Cinchonidine-derived (O-6')-anchored ammonium salts.

21

22a: X-Y = $(CH_2)_3$-C
22b: X-Y = 4-$CH_2C_6H_4O(CH_2)_3$-C

23   **24**
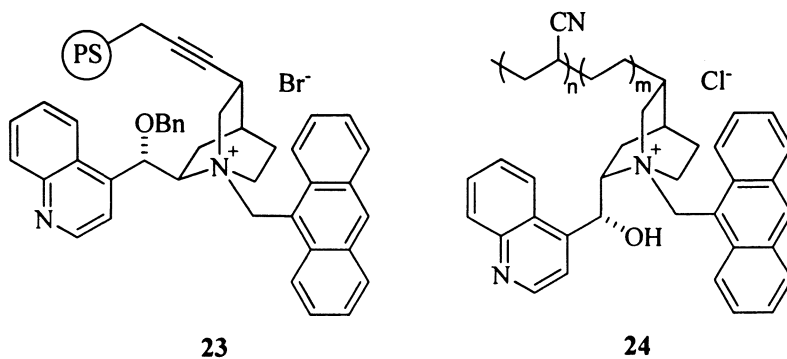
Figure 13. Cinchona-alkaloid-derived (C-11)-bonded ammonium salts.

polymeric ammonium salt **24** after *N*-quaternization (Figure 13). This insoluble catalyst yielded up to 71% *ee* of (*S*)-**3** after the benzylation of the isopropyl derivative **1b** (10 mol% catalyst, toluene/chloroform, 50% aq KOH, -20 °C), being separated and reused (*10*).

Conclusions

Supporting Cinchona ammonium salts to polymers represents a convenient procedure for achieving an easy separation from the reaction mixture and recyclability when employed as PTC catalyst in important processes such as the enantioselective preparation of amino acids by alkylation of glycinate imines. Although a series of different approaches to anchored Cinchona ammonioum salts have been prepared in the last years, few of them have shown really efficient in this process. Still a supported catalyst able to perform this alkylation reaction in short reaction times, achieving high yields and very high enantioselectivities with all kind of electrophiles and retaining its properties after continuous recycling is a non-reached target. No doubt that more research will be devoted to this topic in near future.

References

1. Kacprzap, K.; Gawronski, J. *Synthesis* **2001**, 961.
2. (a) Dehmlow, E. V.; Dehmlow, S. S. *Phase Transfer Catalysis*, third ed.; VCH: Weinheim, 1993. (b) Starks, C. M.; Liotta, C. L.; Halpern, M *Phase Transfer Catalysis—Fundamentals, Applications and Industrial Perspectives*; Chapman and Hall: New York, 1994. (c) *Phase Transfer Catalysis Symposium Series,* vol. 659; Halpern, M., Ed.; American Chemical Society: Washington, DC, 1995.
3. (a) O'Donnell, M. J. *Aldrichimica Acta* **2001**, *34*, 3. (b) *Chem. Rev.* **2003**, *103*, 3013. (c) O'Donnell, M. J. *Acc. Chem. Res.* **2004**, *37*, 506. (d) Lygo, B.; Andrews, B. I. *Acc. Chem. Res.* **2004**, *37*, 526. (e) Nájera, C. Sansano, J. M. *Chem. Rev.* **2007**, *107*, 4584. (f) Ooi, T.; Maruoka, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 4222.
4. Benaglia, M.; Puglisi, A.; Cozzi, F. *Chem. Rev.* **2003**, *103*, 3401.
5. Wang, Y.; Zhang, Z.; Wang, Z. Meng, J.; Hodge, P. *Chin. J. Polym. Sci.* **1998**, *16*, 356.
6. Zhang,, Z.; Wang,, Y.; Wang, Z.; Hodge, P. *React. Funct. Polym.* **1999**, *41*, 37.
7. Chinchilla R.; Mazón, P.; Nájera, C. *Tetrahedron: Asymmetry* **2000**, *11*, 3277.
8. Chinchilla R.; Mazón, P.; Nájera, C. *Adv. Synth. Catal.* **2004**, *346*, 1186.

9. Li, L.; Zhang, Z.; Zhu, X.; Popa, A.; Wang, S. *Synlett* **2005**, 1873.
10. Chinchilla R.; Mazón, P.; Nájera, C. *Molecules* **2004**, *9*, 349.
11. Thierry, B.; Plaquevent, J.-C.; Cahard, D. *Tetrahedron: Asymmetry* **2001**, *12*, 983.
12. Thierry, B.; Perrard, T.; Audouard, C.; Plaquevent, J.-C.; Cahard, D. *Synthesis* **2001**, 1742.
13. Thierry, B.; Plaquevent, J.-C.; Cahard, D. *Mol. Diversity* **2005**, *9*, 277.
14. Thierry, B.; Plaquevent, J.-C.; Cahard, D. *Tetrahedron: Asymmetry* **2003**, *14*, 1671.
15. Kim, D. Y.; Huh, S. C. *Bull. Korean Chem. Soc.* **2004**, *25*, 347.
16. Wang, X.; Yin, L.; Yang, T.; Wang, Y. *Tetrahedron: Asymmetry* **2007**, *18*, 108.
17. Shi, Q.; Lee, Y.-J.; Kim, M.-J.; Park, M.-K.; Lee, K.; Song, H.; Cheng, M.; Jeong, B.-S.; Park, H.; Jew, S. *Tetrahedron Lett.* **2008**, *49*, 1380.
18. Danelli, T.; Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F.; Tocco, G. *Tetrahedron: Asymmetry* **2003**, *14*, 461.

Synthesis and Application of Chiral α-Amino Acids by Kinetic Resolution of Urethane-Protected α-Amino Acid *N*-Carboxyanhydrides with Modified Cinchona Alkaloid Catalysts

Jianfeng Hang[1], Yutaka Ishii[2], Yoshiro Furukawa[2,*], and Li Deng[1,*]

[1]Department of Chemistry, Brandeis University,
Waltham, MA 02454–9110
[2]Research Laboratories, Daiso Company, Ltd., 9-Otakasu-cho,
Amagasaki-shi 660–0842, Japan

A general and efficient kinetic resolution of urethane-protected α-amino acid N-carboxyanhydrides (UNCA) that generates highly enantiomerically enriched α-amino acid derivatives in excellent yields is developed. Numerous UNCAs with different N-carbamoyl protecting groups and with a wide range of α-alkyl, α-aryl as well as α-alkenyl side chains are resolved with enzyme-like efficiency via this strategy. This modified cinchona alkaloid-catalyzed kinetic resolution of UNCA via alcoholysis provides a versatile and reliable route to optically active amino acid derivatives that are suitably protected for further synthetic elaborations. The reaction utilizes readily accessible substrates, cheap reagents, commercially available and fully recyclable catalysts and simple experimental protocols. These characteristics of the reaction render it a highly efficient, flexible and practical method for the asymmetric synthesis of α-amino acids. The further successful improvement of this cinchona alkaloid-catalyzed alcoholysis into a practical tool for large scale synthesis is made possible through the development of new and easily accessible cinchona alkaloid-based catalysts. The practical utility of this

new resolution method for large scale synthesis is highlighted in a multi kilogram scale asymmetric synthesis of propargylglycine, an unnatural amino acid that is a useful pharmaceutical intermediate but difficult to prepare using metal-catalyzed asymmetric hydrogenation of dehydro-amino acids.

Introduction

Acyl-transfer reactions use cheap reagents to transform readily available starting materials into useful and easily purified products. These characteristics, in combination with high enantioselectivity, enable acyl-transfer reactions to become highly valuable methods for asymmetric synthesis.[1] The development of synthetic catalysts to mimic lipases/esterases with the goal of further expanding the scope of asymmetric acyl transfer catalysis is of both conceptual and practical significance for asymmetric catalysis.[2] Although effective synthetic catalysts for the kinetic resolution of racemic alcohols using acyl-transfer reaction have emerged,[3] efforts to develop small molecular-catalyzed kinetic resolutions of racemic amino acids derivatives have met with limited success despite their great potential in asymmetric synthesis of amino acids.

Fu and co-worker reported that the dynamic kinetic resolution of azlactones can also be effected by alcoholysis in the presence of a planar-chiral ferrocene-based DMAP derivatives.[4] Recently, urea-based bifunctional organocatalysts also were used successfully by Berkessel and co-worker for this reaction.[5] Pirke and Synder showed that kinetic resolution by esterification of N-acylated α-amino acids proceed rapidly at hydrocarbon/water interfaces in the presence of a proline-derived chiral selector.[6] Notte and Sammakia described that the kinetic resolution of α-trifluoroacetamido N-acyl oxazolidinethiones by a chiral O-nucleophilic acyl transfer catalyst.[7]

In this account, we describe the development of a highly efficient kinetic resolution of urethane-protected α-amino acid N-carboxy anhydrides (UNCAs) for the asymmetric synthesis of a broad range of α-amino acids with modified cinchona alkaloid catalysts[8] and its practical scale applications.[9]

The design of a kinetic resolution of UNCA via cinchona alkaloid-catalyzed alcoholysis

In 2000, some of us discovered that modified cinchona alkaloids[10] (1.1-1.7, Figure 1) catalyze a highly enantioselective and general desymmetrization of cyclic anhydrides via asymmetric alcoholysis.[11] The sense of asymmetric induction with respect to the chirality of the cinchona alkaloid is consistently

predictable as summarized in Scheme 1. Whereas (DHQD)$_2$AQN-catalyzed reactions give hemiesters **1.9** in 74-99% yields and 92-98% ee, *ent*-**1.9** can be obtained in similar yields (71-95%) and equal or slightly lower ee (86-98%) with (DHQ)$_2$AQN.

Building on these results, we developed an efficient parallel kinetic resolution of monosubstituted succinic anhydrides **1.10** (Scheme 2).[12] A wide array of 2-alkyl and 2-aryl succinic anhydrides were effectively resolved, generating two optically active hemiesters **1.11** and **1.12** by two simultaneous enantioselective and regioselective alcoholysis of the two enantiomers of the anhydrides as shown in Scheme 2.

The analysis of the absolute configuration of the alcoholysis products derived from the succinic anhydrides **1.8** and **1.9** demonstrated that the removal of one of the two substituents on the *meso* succinic anhydrides **1.8** does not change how the (DHQD)$_2$AQN-catalyzed alcoholysis recognizes the cyclic anhydrides **1.9** (Figure 2). We reasoned that this efficient and general cinchona alkaloid-catalyzed asymmetric alcoholysis might be used to differentiate the two enantiomers of other cyclic anhydrides such as the *O*- and *N*-carboxyanhydrides (**1.13** and **1.14**, Figure 2). We became particularly interested in the kinetic resolution of *N*-carboxyanhydrides **1.14** *via* cinchona alkaloid-catalyzed alcoholysis to generate optically active *N*-protected α-amino acid derivatives (Scheme 3). The alcoholysis of **1.14** generates the *N*-protected amino ester **1.15** and CO$_2$. With the amino group suitably protected, ideally with some protecting group which is commonly used in amino acid and peptide chemistry, amino ester **1.15** will not interfere with the cinchona alkaloid-catalyzed alcoholysis by either initiating a polymerization of **1.14** or promoting a competitive amine-catalyzed alcoholysis of low enantioselectivity. Moreover, after the completion of the kinetic resolution, the remaining enantiomerically enriched **1.14** can be converted to the *N*-protected amino acid **1.16** by hydrolysis (Scheme 3). The resulting reaction mixture, consisting of the Brønsted-basic amine catalyst, the acidic amino acid **1.16** and the neutral amino ester **1.15**, can be separated using simple extractive procedures to give **1.15**, **1.16** and the recovered cinchona alkaloid catalyst in desired chemical and optical purity. In light of the well established procedures for the conversion of racemic amino acids to **1.14**, the development of an efficient kinetic resolution of **1.14** will establish a novel and practically attractive strategy for the asymmetric synthesis of α-amino acids.

Identification of Optimal Catalyst and Solvent for the kinetic resolution of urethane-protected α-amino acid *N*-carboxyanhydride (UNCA).

When the *N*-protecting group in **1.14** is a carbamoyl group (P = Z, Boc, Fmoc, Alloc), the compound is called a urethane-protected α-amino acid *N*-
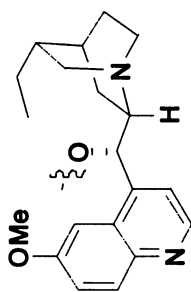
DHQD(dihydroquinidyl)
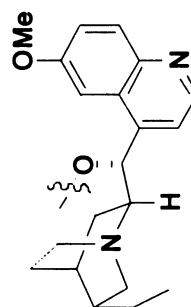
DHQ(dihydroquinyl)
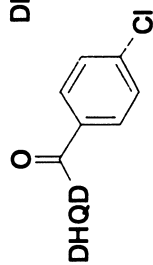
DHQD-CLB
1.1
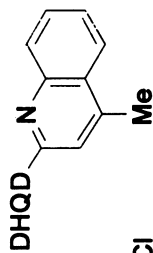
DHQD-MEQ
1.2
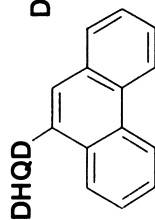
DHQD-PHN
1.3
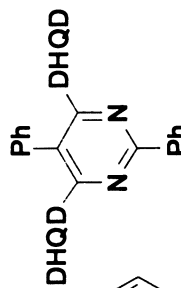
(DHQD)₂PYR
1.4
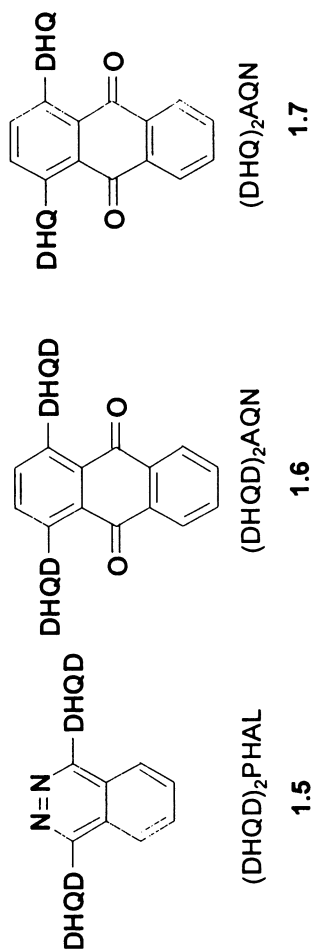
Figure 1. Structures of some commercially available modified cinchona alkaloids
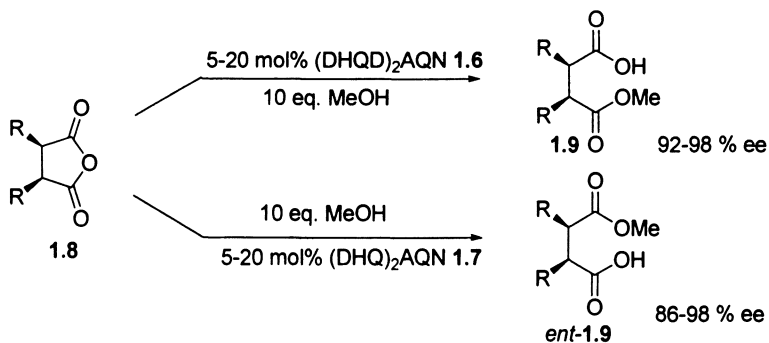
Scheme 1. Enantioselective desymmetrization of meso succinic anhydrides

carboxyanhydride (UNCA). Optically pure UNCAs derived from naturally occurring proteinogenic α-amino acids were first synthesized by Fuller and collaborators[13] in 1990 and many are commercially available now.[14] They are used as chiral building blocks in peptide synthesis,[13b,15] the total synthesis of natural products[16] and the synthesis of other compounds.[17]

As shown in Scheme 4, the synthetic routes toward UNCAs **1.14** are straightforward and flexible. The NCA **1.21** is the key intermediate. Treatment of racemic α-amino acids **1.17** with phosgene[13] or triphosgene[18] (method A) provides NCAs in quantitative yields. When N-protected Z-, Boc- and Alloc-amino acids (**1.18, 1.19, 1.20**) are available, NCAs **1.21** can be synthesized directly from these acids by simply treating them with thionyl chloride or oxalyl chloride (method B). Converting NCAs **1.21** into N-Z-, N-Fmoc- and N-Alloc-UNCAs is realized by reaction with the corresponding chloroformates in the presence of N-methylmorpholine (NMM) (method C). The combination of pyridine and Boc$_2$O is employed (method D) for the synthesis of N-Boc-UNCAs. Following the aforementioned routes, we prepared more than 30 racemic UNCAs conveniently from racemic α-amino acids **1.17** or N-Z-protected α-amino acids **1.18** in 60-88% yields in most cases.

We selected N-Z-phenylalanine NCA **1.14a** as a model substrate in the initial evaluation of key reaction parameters to establish optimal conditions for the kinetic resolution. Reaction of **1.14a** with methanol (0.55 equiv) at room temperature in ether in the presence of (DHQD)$_2$AQN **1.6** (10 mol %) and 4Å molecular sieves[19] provided the desired methyl ester **1.15a** in 82% ee at 40% reaction conversion, indicating that the kinetic resolution proceeded with a selectivity factor (s) of 16 (entry 1, Table 1.1). Following this promising lead, we subsequently found that the enantioselectivity of the kinetic resolution can be dramatically improved by carrying out the (DHQD)$_2$AQN-catalyzed alcoholysis at low temperature. At - 60 °C the enantioselectivity of the kinetic resolution was found to reach a level (s = 79, entry 2, Table 1.1) comparable to
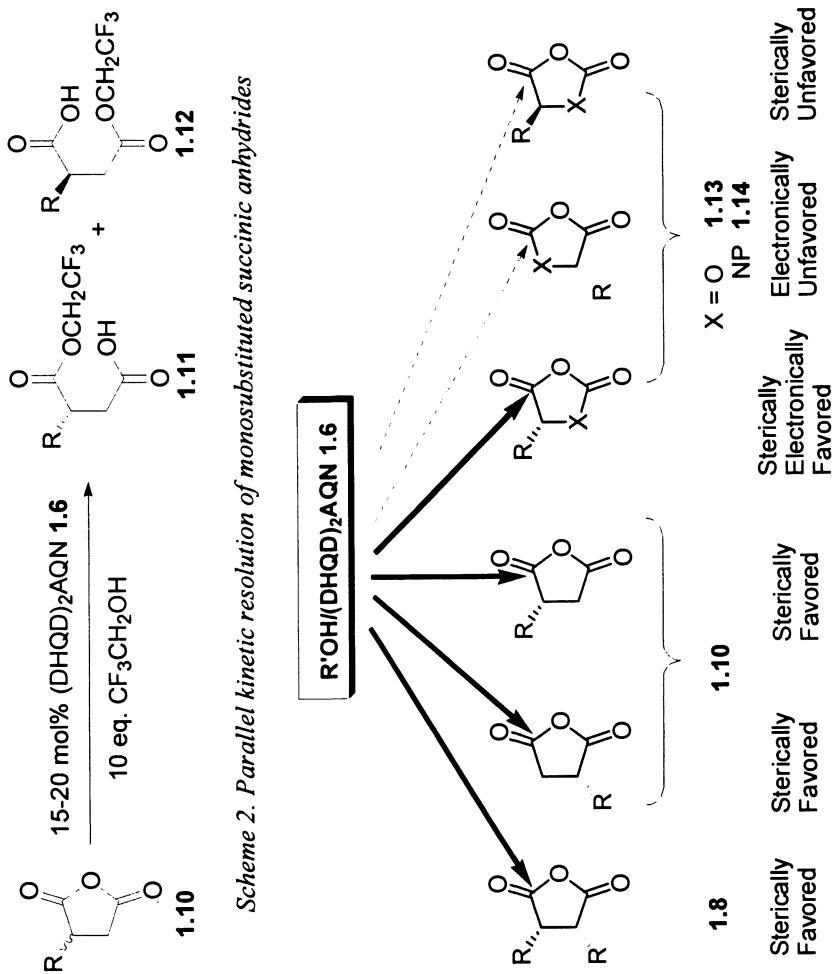
Scheme 2. Parallel kinetic resolution of monosubstituted succinic anhydrides

Figure 2.
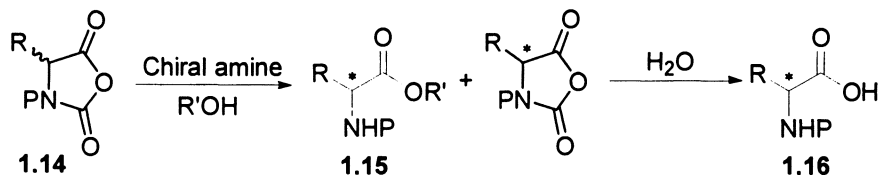
Scheme 3. Kinetic resolution of N-carboxyanhydride 1.14

that of an efficient enzyme-catalyzed kinetic resolution. In the previous cinchona alkaloid-catalyzed alcoholysis of cyclic anhydrides, 4Å molecular sieves were not employed. However, in our preliminary studies of kinetic resolution of **1.14a** with (DHQD)$_2$AQN, when the reaction was repeated without 4Å molecular sieves, it was sometimes found to proceed with lower enantioslectivity. We reasoned that the moisture in the substrate or from air during monitoring the reaction conversion and ee could hydrolyze UNCA to a relatively strong acid (compared to hemiesters); this acid could then form a salt with the basic catalyst, thus lowering the real catalyst loading in the reaction. A possible solution to this problem is to use a desiccant such as 4Å molecular sieves to remove trace amounts of water in the reaction mixture. As we expected, with the introduction of 4Å molecular sieves, the kinetic resolution provided reproducible results.

A variety of natural and modified cinchona alkaloids were screened for their abilities to mediate the kinetic resolution of **1.14a** *via* alcoholysis. The results are summarized in Table 1. While (DHQD)$_2$AQN **1.6**, a modified biscinchona alkaloid, stands as the most effective in our catalyst screening, a modified monocinchona alkaloid, DHQD-PHN **1.3**, is also found to be a highly effective catalyst (entry 3, Table 1). However, (DHQ)$_2$AQN **1.7** (entry 5, Table 1) is less effective for the kinetic resolution compared to its pseudoenantiomer **1.6**. Particularly notable is the finding that excellent enantioselectivity could be achieved with quinidine **1.22**, a natural cinchona alkaloid (entry 4, Table 1). Interestingly, under the same conditions, reactions with other closely related chiral amines including DHQD-CLB **1.1**, DHQD-MEQ **1.2**, (DHQD)$_2$PYR **1.4**, (DHQD)$_2$PHAL **1.5** and an achiral amine quinuclidine **1.23**, gave only minuscule conversions (1-6 %, Table 1).

After the most effective catalyst was identified, the influence of solvents on the kinetic resolution was studied (Table 2). Not surprisingly, *tert*-butyl methyl ether (TBME) gives excellent results for the kinetic resolution similar to those obtained with diethyl ether. However, when THF was used, the selectivity and reactivity of the reaction decreased dramatically. Another finding of this study is that in terms of the solubility of the UNCA substrate at low temperature, toluene is the best choice of solvent. In general, nonpolar solvents are significantly better than polar solvents.

Table 1. Catalyst screening for kinetic resolution of 1.14a [a]

1.14a   Catalyst
MeOH (0.55 eq.)
Et$_2$O, 4Å MS   1.15a   (S)-1.14a

Quinidine 1.22   Quinuclidine 1.23
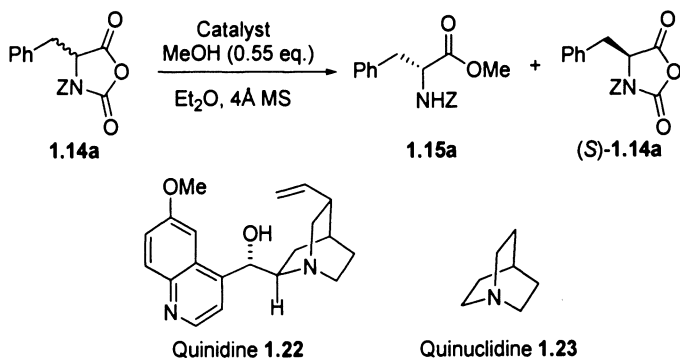
Entry	Catalyst & Loading/mol%		T/°C	Time/h	Conv/%[b]	ee of 1.15a/%[c,d]	s[e]
1	(DHQD)$_2$AQN	1.6	10	25	1	42	80	16
2	(DHQD)$_2$AQN	1.6	10	-60	22	50	92	79
3	DHQD-PHN	1.3	20	-60	22	45	91	47
4	Quinidine	1.22	20	-60	18	38	86	22
5	(DHQ)$_2$AQN	1.7	10	-60	18	38	84[f]	19
6	DHQD-CLB	1.1	20	-60	22	1	-	-
7	DHQD-MEQ	1.2	20	-60	22	4	-	-
8	(DHQD)$_2$PYR	1.4	10	-60	18	6	-	-
9	(DHQD)$_2$PHAL	1.5	10	-60	22	1	-	-
10	Quinuclidine	1.23	20	-60	18	4	-	-

[a] The reaction was performed with 1.14a (0.1 mmol) in ether (7.0 mL). [b] Determined by GC analysis. [c] Determined by HPLC analysis. [d] The absolute configuration of 1.15a was determined by comparison of its sign of optical rotation with the literature value. [e] The selectivity factor s was calculated using the equation $s = k_f/k_s = \ln[1 - C(1 + ee)]/\ln[1 - C(1 - ee)]$, where ee is the percent enantiomeric excess of the product (1.15a) and C is the conversion. [f] ee of (S)-1.15a.

P =

(Z),

(Boc),

(Alloc),

(Fmoc)
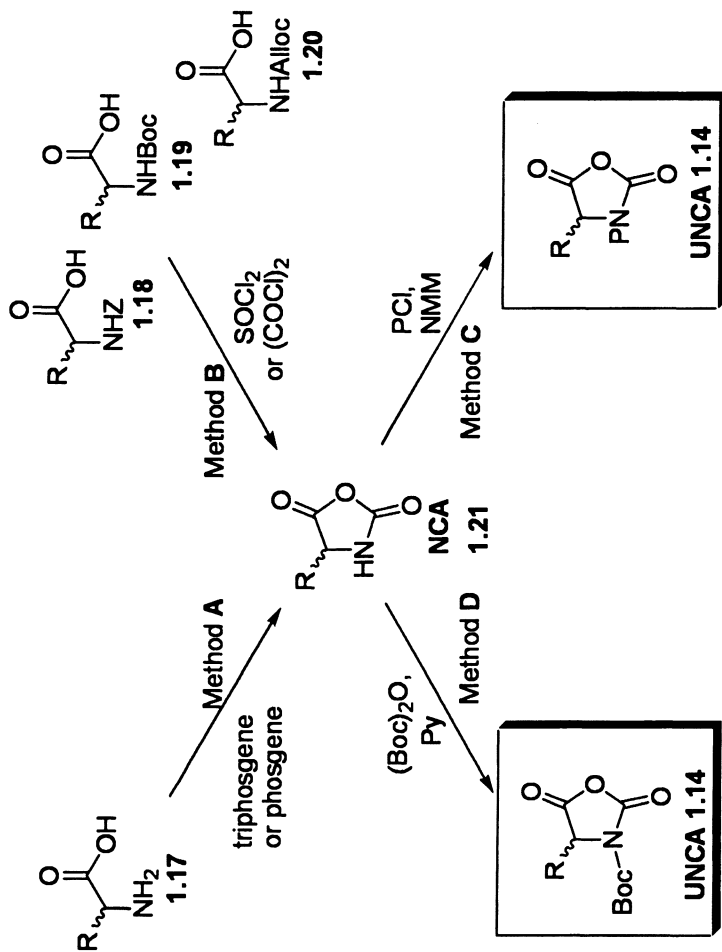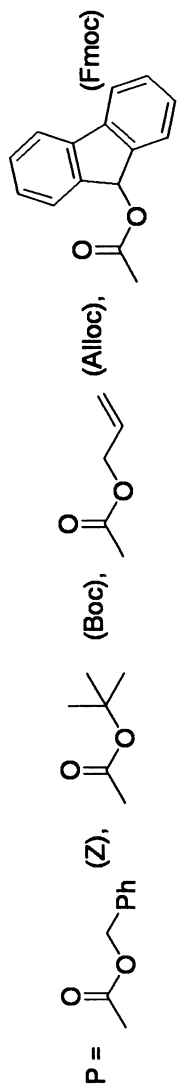
Scheme 4. Synthesis of UNCA

Table 2. Solvent screening for kinetic resolution of 1.14a [a]
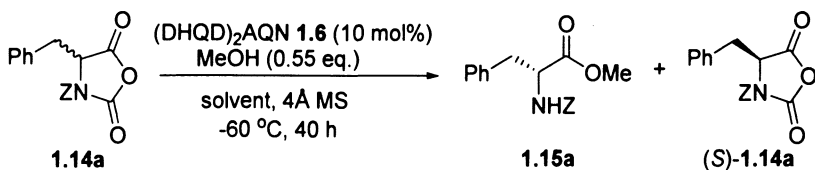
Solvent	Conv./%	ee of **1.15a**/%	*s*
Et$_2$O	48	94	79
TBME	47	91	60
toluene	48	88	41
THF	13	89	19
CH$_2$Cl$_2$	53	74	16
DMF	47	3	1.1

[a] For details, see footnotes in Table 1.

Preparative Reaction and Catalyst Recycle

The practical features of the kinetic resolution were demonstrated in a preparative scale resolution of **1.14a** (4.0 mmol). Under the optimized conditions, the modified cinchona alkaloid-catalyzed alcoholysis of **1.14a** proceeded cleanly with an s of 114 (s = 79 for a 0.1 mmol scale kinetic resolution, see entry 2, Table 1). When half of **1.14a** was consumed, aqueous HCl (2N) was added to the reaction mixture and the resultant mixture was allowed to warm to room temperature. The basic catalyst (DHQD)$_2$AQN **1.6** was transformed to its hydrochloric salt and stayed in the aqueous phase, while the neutral product **1.15a** and enantioenriched **1.14a** were the only two detectable species remaining in the organic phase. The mixture of **1.15a** and **1.14a** concentrated from the organic phase was subjected to a solution of water in THF. Under this mild hydrolysis condition, **1.14a** was converted to the corresponding amino acid **1.24a** selectively. Acid **1.24a** was then conveniently separated from the neutral amino ester **1.15a** by an extraction with basic sodium carbonate solution followed by neutralization with concentrated HCl. After this facile extractive procedure, **1.15a** and **1.24a** were obtained quantitatively as pure compounds. Catalyst (DHQD)$_2$AQN was recovered from its hydrochloric salt also in a quantitative fashion by a basification and a subsequent extraction. The recovered catalyst was used directly for another preparative resolution on the same scale, showing no detectable deterioration in catalytic activity and selectivity (cycle 2, Table 3).

Table 3. Preparative scale kinetic resolution of 1.14a with recycled (DHQD)₂AQN
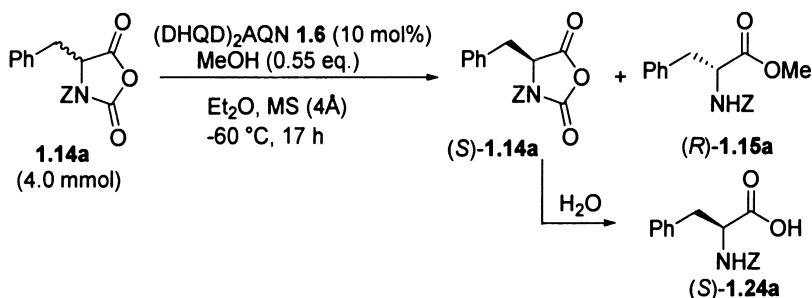
| Cycle | Conv[a] | ee (yield [b]) /% | | s |
		1.15a	**1.24a**	
1	51	93 (48)	98 (48)	114
2 [c]	52	91 (49)	98 (47)	104

[a] The conversion, calculated using the equation: $C = 100 \times ee_{1.14a}/(e_{1.14a} + e_{1.15a})$, is consistent with that determined experimentally. [b] Isolated yield. [c] Reaction is performed with recycled (DHQD)₂AQN
SOURCE: Reproduced from reference 8a. Copyright 2001 American Chemical Society.

Kinetic Resolution of α-Alkyl and α-Aryl UNCAs

The scope of the reaction was found to be extremely general. Clean kinetic resolutions of extraordinarily high enantioselectivities were attainable with an extensive range of UNCAs (Table 4). Following the same extractive procedure used for the isolation of **1.15a** and **1.24a**, the optically active α-amino esters **1.15** and amino acids **1.24** derived from kinetic resolutions of racemic **1.14** were routinely obtained with a combined yield of greater than 90%. Both α-alkyl- and aryl- substituted UNCAs were resolved with exceptional enantio-selectivities. The presence of heteroatoms (entry 7, Table 4) and heterocycles (entry 5, Table 4) in the substrates has no negative effect on the efficiency of the kinetic resolution. Even with a substrate bearing an α-branched alkyl side chain, the resolution can be accomplished with a synthetically useful enantioselectivity at 0 °C (entry 8, Table 4). Furthermore, the reaction is remarkably tolerant of structural variations of the protecting group, thus permitting the efficient syntheses of Z-, Alloc-, Boc-, and even the base-sensitive Fmoc-protected amino acids and esters in high optical purity and excellent yields. Among all the cases examined, (*R*)-**1.15** and (*S*)-**1.24** were obtained consistently from the (DHQD)₂AQN-catalyzed kinetic resolution of racemic **1.14 (a-c, e, g-n)**.

Table 4. KR of 1.14 via modified cinchona alkaloid-catalyzed alcoholysis [a]

X = H, 1.14a
F, 1.14b
Cl, 1.14c
Br, 1.14d

1.14e
1.14f
1.14g
1.14h

1.14i
1.14j
1.14k
1.14o

P = Fmoc, 1.14l
Boc, 1.14m
Alloc, 1.14n
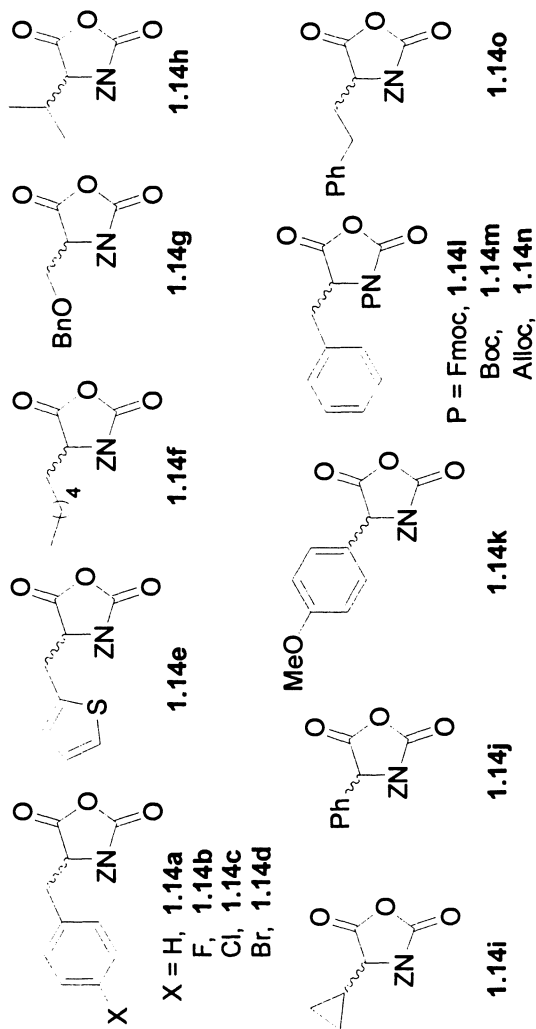
entry	1.14	temp (°C)	Time (h)	conv[b] (%)	ee (yield)/%[c]		s[d]
					(S)-1.24	(R)-1.15	
1	a[e]	-60	17	51	98 (48)	93 (48)	114
2	b	-78	31	50	93 (42)	92 (48)	79

3	c	-60	18	52	97ᵍ(43)	88 (52)	59
4	d	-78	45	51	92ᵍ(39)	87 (51)	45
5	e	-78	25	50	95 (47)	94 (49)	115
6	f	-60	37	51	94ᵍ(42)	91 (49)	78ⁱ
7	g	-78	72	52	96 (44)	89 (49)	69
8	hᶠ	0	22	59	96 (40)	67 (58)	19
9	i	-52	16	53	98 (41)	87 (51)	66
10	jᵍ	-78	16	46	84 (46)	97 (45)	170
11	kᵍ	-78	85	56	95 (43)	74 (56)	23
12	l	-78	46	51	96 (47)	92 (50)	93
13	m	-40	15	59	98 (41)	67 (56)	19
14	n	-60	15	50	91 (45)	91 (45)	67
15	o	-60	36	54	96ʰ(41)	81 (53)	35

[a] Unless noted, the reaction was performed by treatment of **1.14** (0.1 mmol) with (DHQD)$_2$AQN (10 mol%) and methanol (0.52-1.0 eq.) in ether (7.0 mL). [b] The calculated conversion, C = 100 x ee$_{1.14}$/(ee$_{1.15}$ + ee$_{1.14}$). is consistent with experimental value. The ee's of **1.24** and unreacted **1.14** are assumed to be equal. [c] Isolated yield. [d] See footnote "e"of Table 1. [e] The reaction employed 4.0 mmol of **1.14a**. [f] The reaction employed DHQD-PHN **1.3** (20 mol %). [g] Ethanol was used as the nuclephile. [h] Absolute configurations are assigned by analogy. [i] s was determined to be 33 for kinetic resolution of **1.14f** with (DHQ)$_2$AQN **1.7**.

Improvement of Cinchona Alkaloids Catalyst

The first problem for industrial application was the availability of the modified cinchona alkaloid catalysts (DHQD)$_2$AQN and (DHQ)$_2$AQN. These were originally developed as chiral ligands for Sharpless' Os-catalyzed asymmetric dihydroxylation of simple olefins.[20] Although these ligands are available on a laboratory scale,[21] it is difficult to obtain them in the quantities typically required for industrial applications due to the difficulty of their synthesis.

Initially we explored the use of the inexpensive quinidine itself as a catalyst in the kinetic resolution of UNCAs, even though this resulted in a little lower enantioselectivities. However, upon careful analysis of reactions in which recovered quinidine was reused as a catalyst, we discovered that the purity of the catalyst gradually decreased. We determined that the decreasing purity was caused by a reaction of the UNCA with the secondary alcohol group in quinidine. When kinetic resolutions of UNCAs were performed using these adducts as the catalyst, we found that the enantioselectivity of the alcoholysis was almost completely lost, confirming that the reuse of quinidine catalyst is not practical.

Thus, we decided to return to the original method which uses quinidine derivatives in which the secondary alcohol is protected. We synthesized various simple O-protected quinidine derivatives and screened them in the kinetic resolution of N-Z-phenylalanine UNCA **2.1** with ethanol. Consequently, we determined that O-alkylated quinidine derivatives displayed high enantioselectivity in this kinetic resolution (Table 5). Above all, O-propargylquinidine (OPQD) and O-t-butoxycarbonylmethyl quinidine proved to be more enantioselective catalysts than (DHQD)$_2$AQN. The fact that these simple O-alkylated quinidines can be much more easily prepared than O-arylated ones such as (DHQD)$_2$AQN is particularly attractive.[22] Cinchona alkaloids are typically reacted with alkylation agents such as benzyl and allyl halides to yield N-alkylated and N-, O-bisalkylated derivatives. These compounds have found significant utility as phase-transfer organocatalysts in the asymmetric alkylation of glycine benzophenone imines derivatives for the synthesis of chiral α-amino acids.[23] When the alkylation is carried out in NaH-DMF, the resulting alkoxide on the 9-position in quinidine is more nucleophilic than the quinuclidine nitrogen, and O-alkylation takes place rather than N-alkylation.[24] The catalytic activity of the corresponding quinine derivatives, O-propargylquinine (OPQ), was also superior to that of (DHQ)$_2$AQN. Thus, the O-propargyl derivatives of quinine and quinidine (OPQ and OPQD, respectively) could be readily prepared on large scale without using column chromatography for purification (Scheme 5). These catalysts are stable and can be readily recycled *via* pH controlled extractions and

Table 5. Screening of O-alkylated cinchona alkaloid catalysts in kinetic resolution of UNCA 2.1[a]
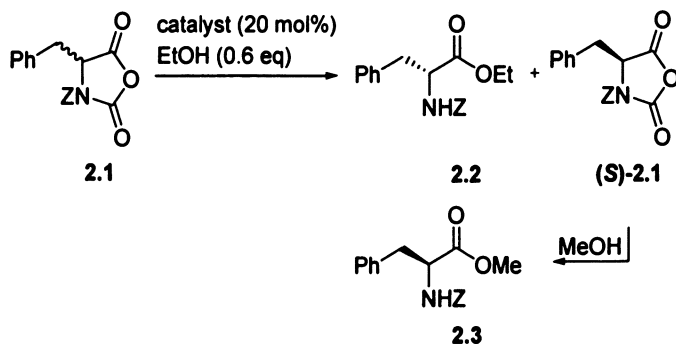
cinchona alkaloid catalyst	conv/%	ee (yield)/%		s[c]
		ester 2.2	(S)-2.1[b]	
(DHQD)$_2$AQN[d]	54	83 (48)	98 (40)	46
O-Me-QD	57	74 (51)	99 (35)	30
O-Bn-QD	58	70 (50)	98 (34)	22
O-allyl-QD	55	80 (49)	99 (37)	40
O-propargyl-QD (OPQD)	52	90 (45)	99 (45)	83
O-^{t}BuO$_2$CCH$_2$-QD	51	93 (44)	98 (45)	114
(DHQ)$_2$AQN[d,e]	57	74 (48)	98 (38)	30
O-propargyl-Q (OPQ)[e]	53	86 (47)	98 (42)	55

[a] The reaction was performed with **2.1** (1.0 eq), catalyst (0.2 eq), ethanol (0.6 eq) in ether at -60 °C. [b] Yield and enantiomeric excess of (S)-**2.1** were measured as the corresponding methyl ester **2.3**, see experimental section for detail. [c] Selectivity factors, $s = kf/ks = \ln[1-C(1+ee)]/\ln[1-C(1-ee)]$, where ee is the percent enantiomeric excess of ester and C is the conversion. [d] 0.1eq Catalyst was used. [e] An opposite enantiomeric pair was obtained with (DHQ)$_2$AQN and OPQ as the catalysts. QD = quinidine, Q = quinine, DHQD = dihydroquinidine, DHQ = dihydroquinine
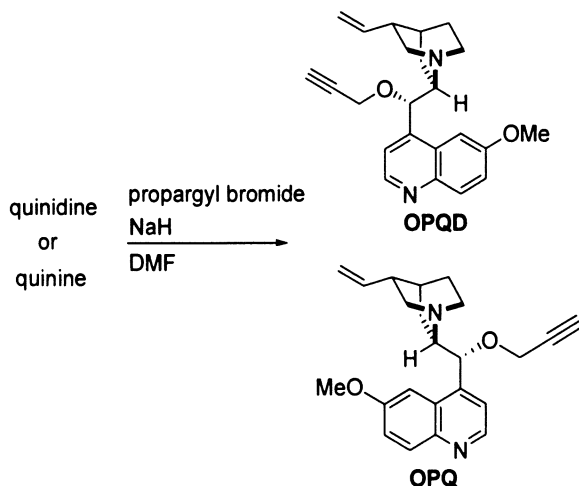
Scheme 5. Preparation of improved cinchona alkaloids catalyst OPQD
and OPQ

are thus suitable for industrial application. We carried out the synthesis of OPQ
on pilot plant scale and successfully prepared 100 kg of catalyst.[25]

Large-Scale preparation of (S)-N-Boc-propargylglycine

We selected propargylglycine, an unnatural amino acid, for large-scale
production by kinetic resolution of UNCAs with the newly developed modified
catalyst, because this amino acid is a useful pharmaceutical intermediate and
difficult to prepare using Rh-BINAP catalyzed asymmetric hydrogenation of
dehydroamino acids.[26]

In our original procedure,[8a] the UNCAs are prepared from racemic amino
acids via a reaction with phosgene, followed by N-carbamate-protection. Since
their hydroscopic nature makes many amino acids difficult to handle and
phosgene is highly toxic, an alternative route for efficient, large-scale synthesis
was developed. Our optimized procedure for the preparation of (S)-N-Boc-
propargylglycine 2.10 is shown in Scheme 6. Racemic N-Z-propargylglycine 2.6,
prepared in 52% overall yield from racemic propargyl glycine 2.5 following a
literature procedure,[27] was protected in situ as a benzyl carbamate. A one-pot
process consisting of cyclization induced by thionyl chloride[28] and subsequent
N-Boc protection provided the desired racemic N-Boc-propargylglycine UNCA
2.8 in an overall yield of 62%.
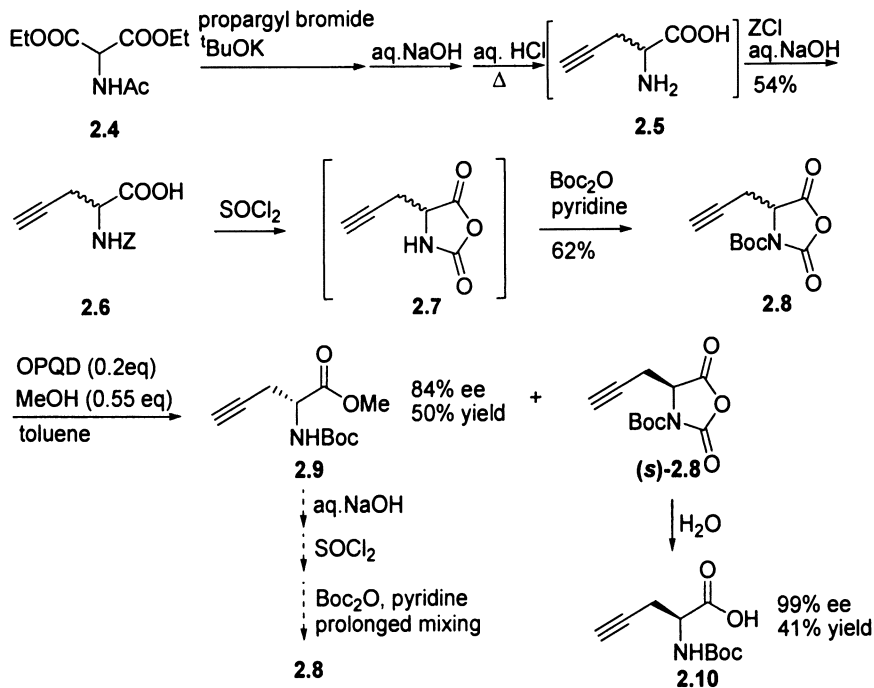
Scheme 6. Optimized procedure for the preparation of (S)-N-Boc-
propargylglycine **2.10**

The alcoholysis of racemic **2.8** (1.0 equiv) with OPQD (0.20 equiv) and methanol (0.55 equiv) at -60 °C on a 50 L scale, followed by hydrolysis of unreacted (S)-**2.8**, afforded (S)-N-Boc-propargylglycine **2.10** in 41% yield and with 99% ee *via* a simple extraction procedure. The catalyst was easily recovered quantitatively by treatment of the aqueous solution with NaOH followed by extraction with toluene. The recovered catalyst was pure according to ¹H NMR, and could be used for repeating the kinetic resolutions to give the desired product with consistently high ee and yield. Although ee of the byproduct (R)-N-Boc-propargylglycine ester **2.9** is lower, it can be easily recycled as the racemic starting material **2.8** by regenerating the UNCA via hydrolysis of **2.9** racemized and stirring this under basic conditions at room temperature for a few hours.

If the desired product is the enantiopure amino ester, esterification of the enantiopure amino acid obtained from the kinetic resolution would be necessary. We have developed a convenient 'continuous alcoholysis' procedure to directly generate the desired enantiopure amino ester from the resolution process.
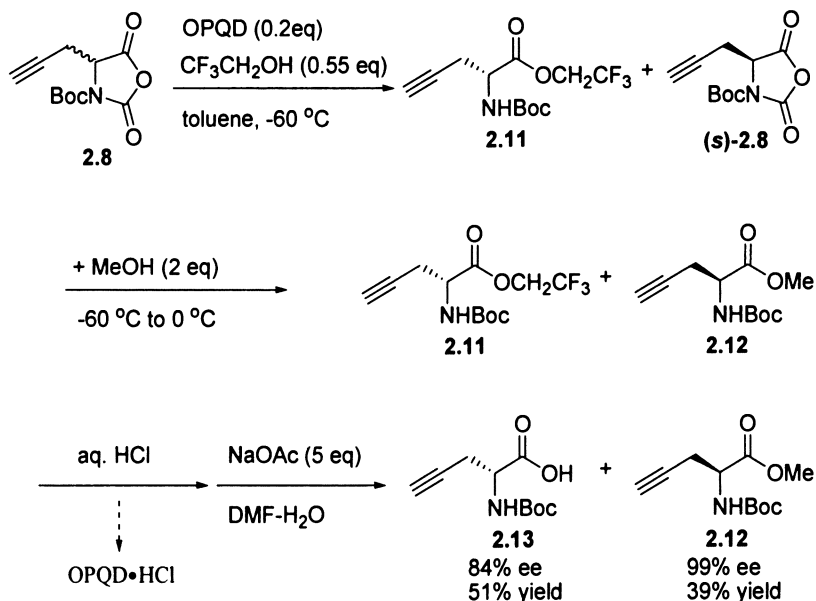
*Scheme 7. Synthesis of Enantiopure Amino Ester **2.12** via Continuous Alcoholysis of UNCA **2.8***

Specifically, enantiopure **2.12** (>99% ee) was easily obtained by the following sequence: 1) kinetic resolution of racemic **2.8** with OPQD and CF_3CH_2OH, as described above, to produce the (*S*)-**2.8** in >99% ee; 2) methanolysis of unreacted (*S*)-**2.8** in situ at -60 °C to 0 °C to form enantiopure amino ester **2.12**; and 3) selective hydrolysis of **2.11** in the mixture of **2.11** and **2.12** in DMF-aq. NaOAc (Scheme 7). This sequence provides an attractive route to (*S*)-*N*-Boc-propargylglycine **2.12** in 39% overall isolated yield from racemic **2.8**.

References

1. (a) Hæffner, F.; Norin, T. *Chem. Pharm. Bull.* **1999**, *47*, 591. (b) Faber, K.; *Biotransformations in Organic Chemistry*, 3rd ed.; Springer-Verlag: New York, 1997. (c) Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd: Oxford, 1994.
2. (a) Keith, J. M.; Larrow, J. F.; Jacobsen, E. N. *Adv. Synth. Catal.* **2001**, *343*, 5. (b) Spivey, A. C.; Maddaford, A.; Redgrave, A. J. *Org. Prep. Proced. Int.* **2000**, *32*, 331.
3. Vedejs, E.; Jure, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 3974.

4.   Liang, J.; Ruble, J. C.; Fu, G. C. *J. Org. Chem.* **1998**, *63*, 3154.

5.   (a) Berkessel, A.; Cleemann, F.; Mukherjee, S.; Müller, T. N.; Lex, J. *Angew. Chem. Int. Ed.* **2005**, *44*, 807. (b) Berkessel, A.; Mukherjee, S.; Cleemann, F.; Müller, T. N.; Lex, J *Chem. Commun.* **2005**, 1898.

6.   (a) Pirkle, W. H.; Synder, S. E. *Org. Lett.* **2001**, *3*, 1821. (b) Synder, S. E.; Pirkle, W. H. *Org. Lett.* **2002**, *4*, 3283.

7.   Notte, G. T.; Sammakia, T. *J. Am. Chem. Soc.* **2006**, *128*, 4230.

8.   (a) Hang, J.; Tian, S-K.; Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2001**, *123*, 12696. (b) Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2002**, *124*, 2870. (c) Hang, J.; Deng, L. *Org. Lett.* **2002**, *4*, 3321. (d) Hang, J.; Deng, L. *Synlett,* **2003**, *12*, 1927.

9.   Ishii, Y.; Fujimoto, R.; Mikami, M.; Murakami, S.; Miki, Y.; Furukawa, Y. *Org. Process Res. Dev.* **2007**, *11*, 609.

10. These commercially available catalysts were originally developed by Sharpless group and applied as ligands in the Os-catalyzed dihydroxylation of simple olefins. See: (a) Johnson, R. A.; Sharpless, K. B. Catalytic Asymmetric Synthesis, 2nd ed.; Ojima, I., Ed.; VCH: New York, 2000; p 357. (b) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. Chem. Rev. 1994, 94, 2483. (c) Kolb, H. C.; Sharpless, K. B. Transition Metals for Organic Synthesis; Beller, M., Bolm, C., Eds.; Wiley-VCH: Weinheim, Germany, 1998; Vol. 2, p 219. (d) Marko, I.; Svendsen, J. S. Comprehensive Asymmetric Catalysis; Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Springer-Verlag: Berlin, Germany, 1999; Chapter 20.

11. (a) Chen, Y.; Tian, S.-K.; Deng, L. *J. Am. Chem. Soc.* **2000**, *122*, 9542. (b) Choi, C.; Tian, S.-K.; Deng, L. *Synthesis* **2001**, 1737.

12. Chen, Y.; Deng, L. *J. Am. Chem. Soc.* **2001**, *123*, 11302.

13. (a) Fuller, W. D.; Cohen, M. P.; Shabankareh, M.; Blair, R. K.; Goodman, M.; Naider, F. R. *J. Am. Chem. Soc.* **1990**, *112*, 7414. (b) Fuller, W. D.; Goodman, M.; Naider, F. R.; Zhu, Y.-F. *Biopolymers (Pept. Sci.)* **1996**, *40*, 183.

14. ISOCHEM. website: http://www.isochem.fr

15. (a) Mellor, S. L.; Wellings, D. A.; Fehrentz, J.-A.; Paris, M.; Martinez, J.; Ede, N. J.; Bray, A. M.; Evans, D. J.; Bloomberg, G. B. In *Fmoc Solid Phase Peptide Synthesis* Chan, W. C., White, P. D., Eds.; Oxford University Press: Oxford, UK, 2000; 37. (b) Auvin-Guette, C.; Frerot, E.; Coste, J.; Rebuffat, S.; Jouin, P.; Bodo, B. *Tetrahedron Lett.* **1993**, *34*, 2481. (c) Zhu, Y.-F.; Fuller, W. D. *Tetrahedron Lett.* **1995**, *36*, 807. (d) Fehrentz, J.-A.; Genu-Dellac, C.; Amblard, M.; Winternitz, F.; Loffet, A.; Martinez, J. *J. Pept Sci.* **1995**, *1*, 124. (b) Auvin-Guette, C.; Frerot, E.; Coste, J.; Rebuffat, S.; Jouin, P.; Bodo, B. *Tetrahedron Lett.* **1993**, *34*, 2481.

16. (a) Davies, S. C.; Dixon, D. J. *J. Chem. Soc., Perkin Trans. 1.* **1998**, 2635. (b) Zhu, J.; Ma, D. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 5348.

17. (a) Fehrentz, J.-A.; Califano, J.-C.; Amblard, M.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1994**, *35*, 569. (b) Fehrentz, J.-A.; Bourdel, E.; Califano, J.-C.; Chaloin, O.; Devin, C.; Garrouste, P.; Lima-Leite, A.-C.; Llinares, M. Rieunier, F.; Vizavonna, J.; Winternitz, F.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1994**, *35*, 1557. (c) Fehrentz, J.-A.; Pothion, C.; Califano, J.-C.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1994**, *35*, 9031. (d) Chevallet, P.; Fehrentz, J.-A.; Kiec-Kononowicz, K.; Devin, C.; Castel, J.; Loffet, A.; Martinez, J. *Lett. Pept Sci.* **1996**, *2*, 297. (e) Pothion, C.; Fehrentz, J.-A.; Aumelas, A.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1996**, *37*, 1027. (f) Paris, M.; Fehrentz, J.-A.; Chevallet, P.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1996**, *37*, 8489. (g) Pothion, C.; Fehrentz, J.-A.; Chevallet, P.; Loffet, A.; Martinez, J. *Lett. Pept Sci.* **1997**, *4*, 241. (h) Paris, M.; Fehrentz, J.-A.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1998**, *39*, 1569. (i) Paris, M.; Pothion, C.; Michalak, C.; Martinez, J.; Fehrentz, J.-A. *Tetrahedron Lett.* **1998**, *39*, 6889. (j) Audin, P.; Pothion, C.; Fehrentz, J.-A.; Loffet, A.; Martinez, J.; Paris, J. *J. Chem. Research (S)*, **1999**, 282.
18. Daly, W. H.; Poché, D. *Tetrahedron Lett.* **1988**, *29*, 5859.
19. The role of 4Å molecular sieves in the reaction is only for the removal of trace amount of water in the reaction mixture. The reaction of freshly prepared dry UNCA under strict moisture-free condition without molecular sieves provides the same high enantioselectivity.
20. Recent review: Marko, I. E.; Svendsen, J. S. In *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N., Pfaltz, A., Yamamoto, H. Eds.; Springer: Heidelberg, 1999; Chapter 20.
21. Although Sharpless's ligands can be purchased from Aldrich, they are too expensive: $(DHQD)_2AQN$: price $74.8 / 500 mg (2007 catalogue).
22. Becker, H.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **1996**, *35*, 448.
23. (a) O'Donnell, M. J. *Acc. Chem. Res.* **2004**, *37*, 506. (b) Lygo, B.; Andrews, B. J. *Acc. Chem. Res.* **2004**, *37*, 518.
24. An example of O-alkylated cinchona alkaloid: Blaser, H. U.; Jalett, H. P.; Wiehl, J. *J. Mol. Catal.* **1991**, *68*, 215.
25. CAUTION: Although our reaction conditions in NaH-DMF mixtures are controlled under safety ranges, examples of explosive accidents in NaH-DMF mixtures, and their evaluation of the reaction hazard and the safety treatment have been reported, see: (a) Buckley, J.; Webb, R. L.; Laird, T.; Ward, R. J. *Chem. Eng. News* **1982**, *60 (28)*, 5. (b) DeWall, G. *Chem. Eng. News* **1982**, *60 (37)*, 5 and 43. (c) Laird, T. *Chem. Ind.* **1986**, *17*, 134. (d) Kinoshita, N.; Takeuchi, H.; Kawai, N. *Proc. Annu. Meet. Jpn. Soc. Saf. Eng.* **2000**, Abstr. 15.

26. Ohkuma, T.; Kitamura, M.; Noyori, R. In *Catalytic Asymmetric Synthesis,* 2nd ed.; Ojima, I. Ed.; WILEY-VCH: New York, 2000.
27. Leukart, O.; Caviezel, M.; Eberle, A.; Escher, E.; Tun-kyi, A.; Schwyzer, R. *Helv. Chim. Acta* **1976**, *59*, 2181.
28. Greenstein, J. P.; Winitz, M. In *Chemistry of the Amino Acids*; John Wiley and Sons: New York, 1961; Vol. 2. pp 861.

Chapter 18

Stereoselective Synthesis of Quaternary, α-Vinyl Amino Acids and Their α-(2'Z-Fluoro)vinyl Congeners: Promising Candidates for PLP Enzyme Inactivation

David B. Berkowitz, Kannan R. Karukurichi,
Roberto de la Salud-Bea, Gourhari Maiti, Jill M. McFadden,
and Michelle L. Morris

Department of Chemistry, University of Nebraska,
Lincoln, NE 68588–0304

This chapter provides an overview of a program directed at the synthesis of amino acids (AA's) bearing a vinyl substituent in place of the usual α-proton. These quaternary amino acids are of interest, in and of themselves, as mechanism-based inhibitors of pyridoxal phosphate dependent enzymes, and also as building blocks for natural product or peptide synthesis. Importantly, these unnatural amino acids also serve as synthetic precursors for α-halovinyl AA's and α-oxiranyl AA's. Stereoselective synthetic methodology development in this area is highlighted and the initial performance of the α-(2'Z-fluoro)vinyl trigger in an amino acid decarboxylase (AADC) active site is described. Interestingly, at least for *H. alvei* lysine decarboxylase (LDC), inactivation with this trigger, along the α-branch, displays a marked enantio-dependence, with the *L*-antipode giving efficient inactivation, and its mirror image behaving as a simple substrate.

The structural root of the family (*1*) of α-vinyl AA's is α-vinylglycine, a natural product isolated from mushrooms (*2*) that inactivates a number of pyridoxal phosphate (PLP)-dependent transaminase enzymes (*3,4*) and 1-aminocyclcopropane-1-carboxylate (ACC) synthase (*5*). The ability of this β,γ-unsaturated amino acid to inactivate PLP enzymes that normally labilize the C-α-H bond probably inspired the design and development of γ-vinyl-GABA (gamma-aminobutyric acid), or Vigabatrin, as a suicide substrate for GABA transaminase (*6*), an enzyme that normally labilizes the Cγ-H bond (Figure 1). Elegant early mechanistic studies by Silverman (*7*) and John (*8*), suggested that this trigger functioned by a transamination/conjugate addition mechanism. Indeed, this mechanism has now been confirmed by x-ray crystallographic studies of the adducted enzymes, for both α-vinylglycine (*9*) and γ-vinyl-GABA (*10*), with these structures firmly establishing the active site lysine as the nucleophile captured, in both cases.

As is illustrated in Figure 1, if one now replaces the α-hydrogen in vinylglycine with a normal amino acid side chain, one arrives at a quaternary, a-vinyl AA design for potential inhibitors of PLP-dependent decarboxylases or retroaldolases, i.e. enzymes that cleave the Cα-CO_2- or Cα-R bonds, respectively. At the same time, such inactivator candidates should be completely innocuous toward PLP-enzymes that are mechanistically constrained to α-deprotonate, including transaminases, racemases, β- and γ-eliminases, and β- and γ-replacement enzymes. This inherent specificity makes such an inactivator design all the more attractive and this inspired the groups at Merck (*11*) and Marion-Merrell Dow (*12,13*), and our own group (*14*) to pursue this design for AADC inactivation. The same substructure appears in the 1-amino-2-methylenecyclcopropane-1-carboxylate cleverly designed by Liu and coworkers as an ACC deaminase inactivator (*15,16*).

This design is also attractive because the vinylic α-branch is readily functionalizable, making quaternary α-vinyl AA's the centerpieces of a program directed at the development of new mechanism-based inactivators (MBI's) for AADC's. As is illustrated in Figure 1, the vinyl trigger can be exchanged for various halovinyl triggers, and for diastereomerically homogeneous oxiranyl triggers. Hence, dihydroxylation of *N*-Cbz-proected α-vinyl AA's (17), followed by selective mesylation of the primary alcohol and ring closure allows one to obtain cleanly the *erythro*- and *threo*-oxiranyl AA's (*18*), with potential as either MBI's for PLP enzymes or affinity reagents directed at other active sites, within carefully tailored peptidyl contexts. On the other hand, treatment of *N*-trifluoroacetyl-protected α-vinyl AA's with PhSeX, followed by selenide oxidation and selenoxide elimination, leads preferentially either to α-[2'*E*-chloro(bromo)]vinyl AA's or α-[1'-chloro(bromo)]vinyl AA's (*19*), under thermodynamic, or kinetic conditions (*20,21*), respectively. Most recently, we have demonstrated that one can selectively exchange out the terminal vinyl
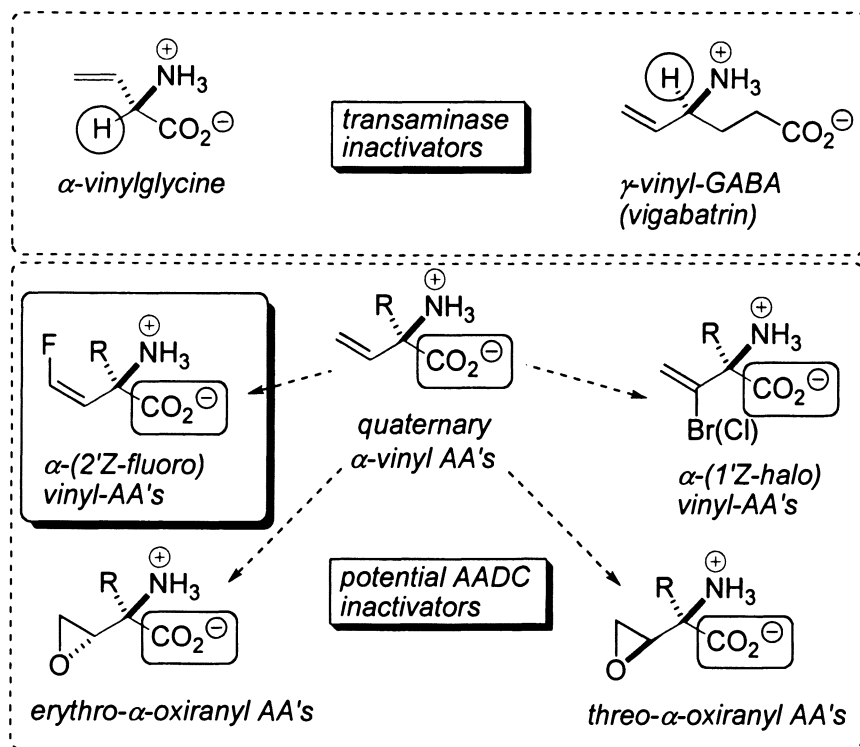
Figure 1. From α-vinylglycine to quaternary α-vinyl AA's

carbon atom by an ozonolysis/McCarthy fluoromethylenation (*22,23*) sequence that results in the corresponding α-(2'Z-fluoro)vinyl AA's (*24*).

So, while the divergent nature of this chemistry is attractive, this places the real synthetic burden here on the development of efficient and stereoselective routes into the the parent, α-vinyl AA's. Pioneering work in this area was performed by the groups of Schöllkopf (*25*), Seebach (*26-28*), Hegedus (*29*) and Williams (*30*). Additionally, there were early indications from the work of Metcalf (*31*) and Steglich (*32*) that, using α,β-unsaturated sulfones as vinyl cation surrogates, one could achieve a formal α-vinylation of suitably protected AA-derived ester enolate equivalents. It should also be noted that a number of groups have demonstrated the synthetic utility of α-vinylic amino acids as building blocks for synthesis (*33-41*), providing additional applications beyond their role as key precursors to new enzyme inactivators.

We developed perhaps the most general approach to the formal α-vinylation, in which N-benzoyl-protected α-amino ester-derived dianions are condensed with ethylene oxide, as convenient vinyl cation equivalent (*42*). The

intermediate β-alkoxy ester cyclizes, *in situ*, to the corresponding, quaternary, α-substituted homoserine lactone, bearing the side chain of the starting of the amino acid (Figure 2). Note that one can chemoselectively cleave these lactones, nucleophilically, at the β-carbon using a new, borane-free, phenylselenolate reagent, developed for this purpose (*43*). Selenoxide pyrolysis then installs the vinyl group. Interestingly, quite an array of functionalized amino acid side chains, including those of lysine, homoserine, histidine and DOPA can be carried as the α-branch on the dianionic intermediates. This route gives racemic, quaternary, α-vinylic amino esters. Reduction to the corresponding alcohols, allows for a partial resolution through a lipase-mediated "reverse tranesterification" procedure with vinyl acetate as acyl donor (*44*).
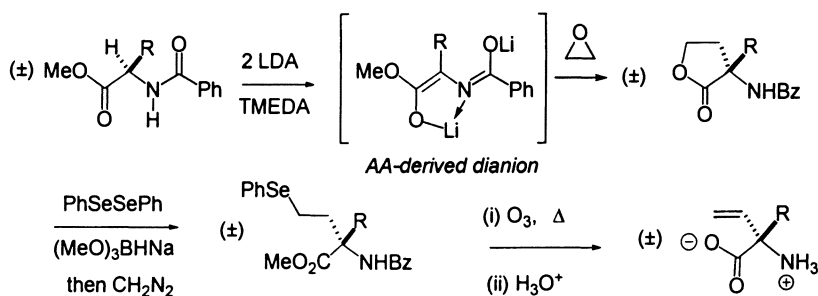
Figure 2. Formal α-vinylation of AA-derived dianions – Ethylene oxide as a vinyl cation equivalent

Encouraged by the success of this AA-derived dianion alkylations, we next moved to engineer a stereoselective version of this chemistry. Initially, it was found the corresponding alanine-derived dianion, bearing a phenmenthyl ester auxiliary could be used to introduce a wide variety of side chains with high levels of diastereoselection (*45*). This provided a useful route into α-methyl AA's (*46-59*), themselves of interest for engineering helical secondary structure in peptides (*60-62*), and for unnatural amino acid mutagenesis (*63*). Attempts to alkylate the analogous chiral dianion from N-Bz-phenylalanine showed little diastereoselectivity, thereby motivating us to turn the tables. Thus, rather than α-vinylate a chirally-biased AA-derived dianion, we chose to examine the α-alkylation of a chiral vinylglycine-derived dianionic dienolate (Figure 3). After some experimentation, it was found that the β-naphthylmenthyl d'Angelo ester auxiliary [(*64,65*) derived from pulegone] confers a significant facial bias upon its derivative N-Bz-vinylglycinate enolate. Imidate chelation is believed to control enolate geometry, such that *si*-face alkylation is favored and quaternary *L*-α-vinyl amino acids are produced (*66,67*). More recently, we have turned to

the analogous Comins auxiliary (derived from cyclohexene oxide) which is simply the nor-methyl analogue of the d'Angelo auxiliary, and which, via lipase-mediated kinetic resolution, is available in both antipodal forms (68). With this modification, this vinylglycine-derived dienolate alkylation methodology provides access to both L- and D-quaternary α-vinyl AA's (69).
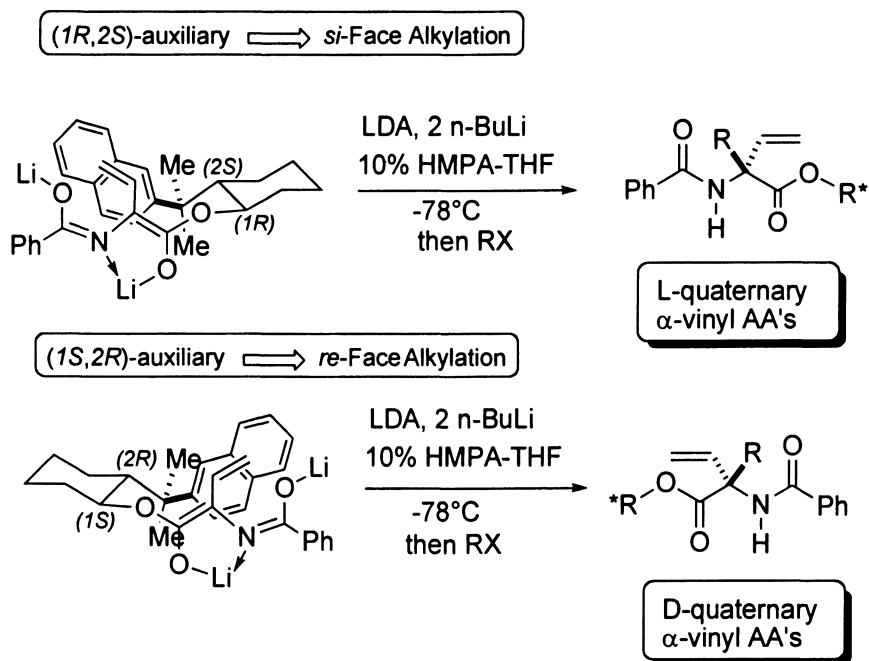
Figure 3. Selective synthesis of L- and D-quaterary, α-vinyl AA's via alkylation of chiral vinylglyine-derived dianionic dienolates – acyclic stereocontrol

While the aforementioned methodology serves as an excellent example of acyclic stereocontrol, and demonstrates significant generality with respect to side chain electrophile, it requires auxiliary synthesis, installation and removal. Thus, a complementary "self reproduction of chirality" [SRC (70); alternatively dubbed "self generation of stereocenters" (SRS) (26). Note: A referee has suggested that the terms "memory of chirality" or "chiral relay" might also be used to describe this strategy] approach was developed, in parallel. Beginning from L-vinylglycine, itself accessible from L-homoserine (71), for example (39,72-77), a low-temperature episelenonium-mediated cyclization masks the double bond, but preserves the α-stereocenter [Figure 4 (78)]. The resultant

enantiomerically enriched *cis-* and *trans*-oxazolines are separable chromato-graphically, but give rise to enantiomeric enolates upon α-deprotonation. Enolate alkylation occurs with virtually complete 1,2-stereoinduction via the new β-stereocenter, allowing for introduction of the side chain with either *D-* or *L-*stereochemistry, depending upon the choice of oxazoline precursor. The subsequent alkene-unmasking sequence leads consecutively to the corresponding protected the α-(2'*E*-phenylseleno)vinyl- and the α-(2'*E*-tributylstannyl)vinyl-AA's. The latter are useful building blocks for chain-extended β,γ-unsaturated amino acids, via transition metal-mediated cross coupling chemistry (*79*). Interestingly, the direct transformation of vinylselenides to vinyl stannanes uncovered here was unprecedented, and constitutes a new method for the installation of vinyl stannanes, in general.

While the methods illustrated in Figures 3 and 4 allow for the highly stereoselective construction of quaternary, α-vinyl AA's, both use stoichiometric chirality, in the form of either a chiral auxiliary, or a chiral educt, respectively. Hence, recent efforts in the group have focused on the development of transition metal catalyzed processes that lead into β,γ-unsaturated amino acids. As is depicted in Figure 5, one such approach involves a formal aza-Claisen rearrangement of *N-p*-methoxyphenyl (PMP)-trifluoroacetimidates. Early work, as part of the M.S. studies of Huijie Li in this group in 2001, demonstrated the particular utility of this new imidate functionality for fashioning quaternary allylic amines, via Pd(II)-catalyzed rearrangement (*80,81*). Subsequently, Overman disclosed an asymmetric rearrangement of such imidates, though not toward quaternary centers (*82*). This result, when combined with a number of reports from the Overman group (*83-85*), as well as the Hayashi (*86*) and Kang (*87*) groups, on asymmetric allylic imidate rearrangements suggests that asymmetric variants of this new Pd(II)-mediated route to quaternary, α-vinyl AA's may be achievable.

Another recent foray into catalytic, transition metal-mediated bond constructions toward β,γ-unsaturated AA's is illustrated in Figure 6. Here an intramolecular allylic amination of an internal carbamate nucleophile, presumably upon an intermediate π-allyl-metal species serves to for the Cα-N bond. This reaction served as the very first targeted transformation for a project on our laboratory on *In Situ Enzymatic Screening* (ISES) (*88-90*). In ISES approaches to combinatorial catalysis, enzymes serve as catalytic reporters to provide an immediate spectrophotometric readout upon the performanance of the transition metal catalyst being screened. This screen was performed in multiple cuvette format with automation, to parallel process a number of candidate transition metals, ligands and *N*-protecting groups for the targeted trans-formation.

Ni(0) emerged as the best non-Pd catalyst screened, with the combination of a Ni(cod)$_2$ pre-catalyst complex, bis-1,4-(diphenylphosphino)butane (dppb), and *p*-methoxyphenyl *N*-protecting group emerging as optimal from the original
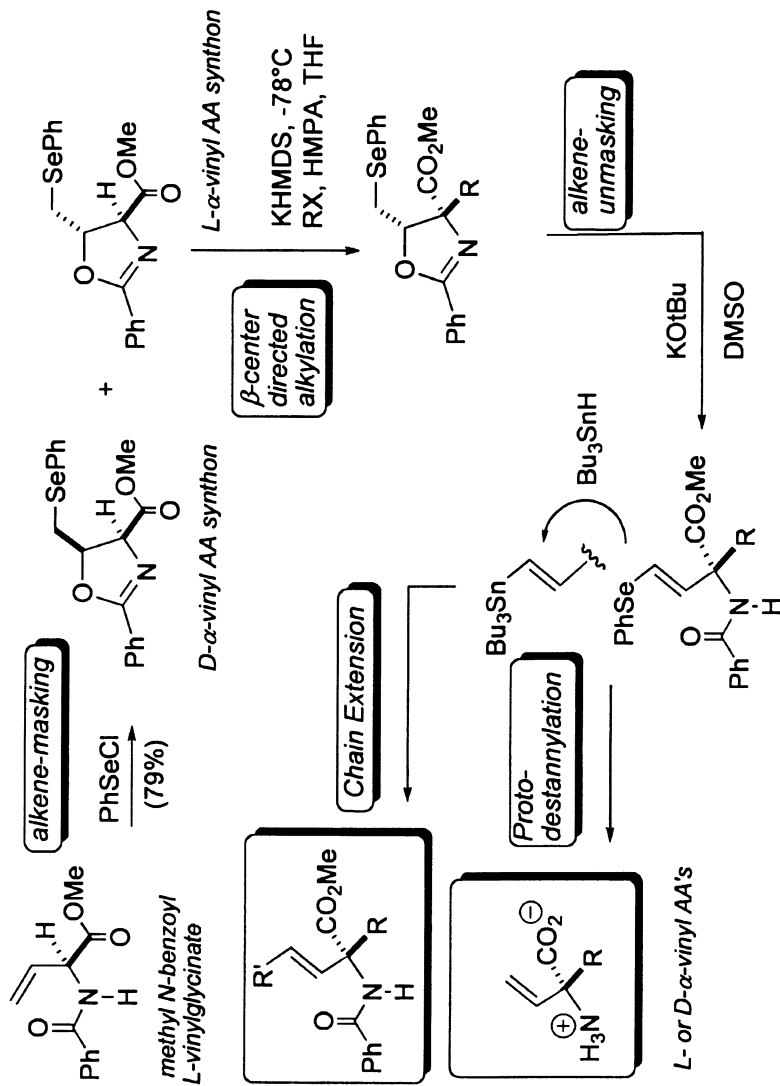
Figure 4. Self reproduction of chirality approach to L- and D-quaternary, α-vinyl AA's – side chain introduction with absolute 1,2-stereoinduction
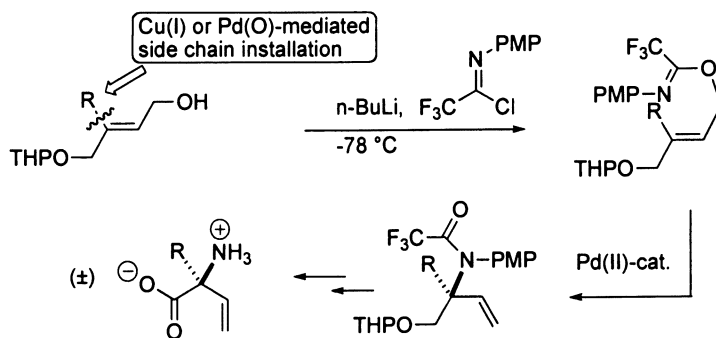
Figure 5. A Pd(II)-mediated allylic imidate rearrangement route to quaternary
α-vinylic AA's.
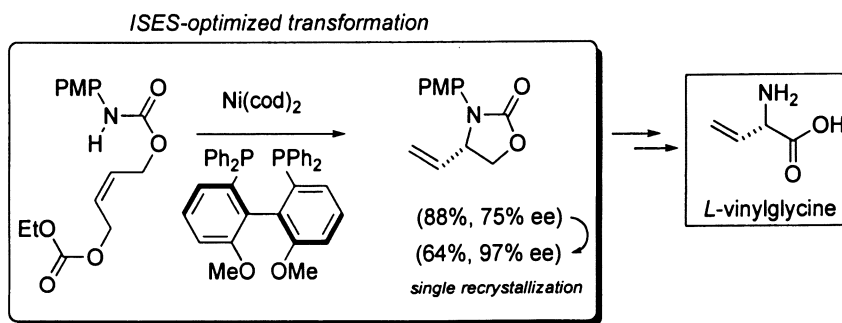
Figure 6. ISES identifies the first asymmetric Ni(0)-mediated allylic amination
– catalytic asymmetric entry into L-vinylglycine.

ISES experiments. This suggested that one might be able to develop the first known asymmetric allylic amination reaction with Ni(0). Indeed, follow-up work, involving the screening of bidentate *P,N*- (*91*) and *P,P*-ligands with central, planar and axial chirality turned up a number of enantioselective systems, with the methoxy-BIPHEP ligands supporting especially rapid catalysis, while maintaining enantioselection (Figure 6). With a single recrystallization, this led to the first, asymmetric Ni(0)-mediated synthesis of *L*-vinylglycine (*92*), complementing reports in this direction, by Trost (*93,94*), Alper (*95*) and Hayashi (*96*), with Pd(0).

With a number of methods in hand to construct the key, quaternary, α-vinylic amino acids, it became important to establish an efficient mechanism for preserving the absolute stereochemistry and parlaying the α-vinyl trigger into a terminally fluorinated halovinyl trigger. As is delineated in Figure 7, it was readily established that the combination of ozonolysis to the protected, quaternary, α-formyl AA, and McCarthy fluoromethylenation (*22*) served this purpose well (*24*). Moreover, the olefination reaction was found to be highly stereoselective in this quaternary AA context, giving the *E*-fluorovinyl phenyl sulfones, which ultimately translate into α-(2'*Z*-fluoro)vinyl AA final products.
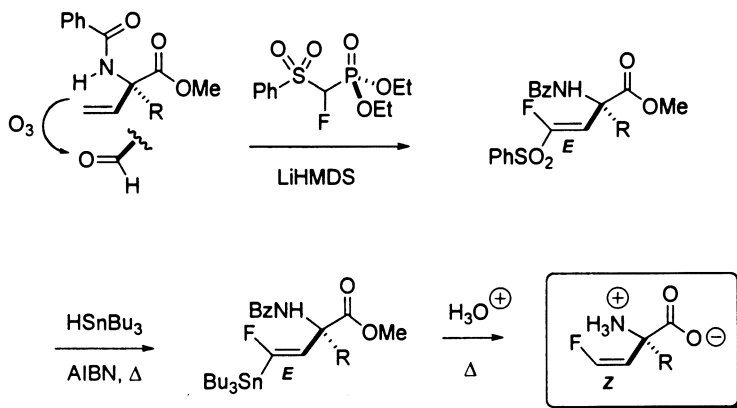
Figure 7. From enantiomerically enriched α-vinyl amino acids to the corresponding α-(2'Z-fluoro)vinyl amino acids

These quaternary, 2'-fluorovinyl AA's (*97*) are designed to be delivered to their cognate AADC's through side chain recognition and transimination to external aldimine **I**. Mechanism-based inactivation is then postulated to follow, provided that α-decarboxylation and then errant protonation occur (Figure 8). If protonation of the putative quinonoid intermediate (**II**) occurs at the α-position,

then simple turnover to α-(2Z'-fluoro)vinylcadaverine (FVC) occurs. Indeed, this is precisely what is seen with the *D*-antipode of α-(2'Z-fluoro)vinyllysine (FVL) in the active site of lysine decarboxylase (LDC) from the bacterium *H. alvei*, chosen as model AADC (*69*). On the other hand, errant protonation at C_4' of the PLP cofactor opens up an addition/elimination pathway for enzyme alkylation. Alternatively, Cγ-protonation could lead to inactivation via electrophilic (α,β-unsaturated iminium ion (**VIII**) formation) and/or nucleophilic pathways [Metzler enamine mechanism (*98-100*)].
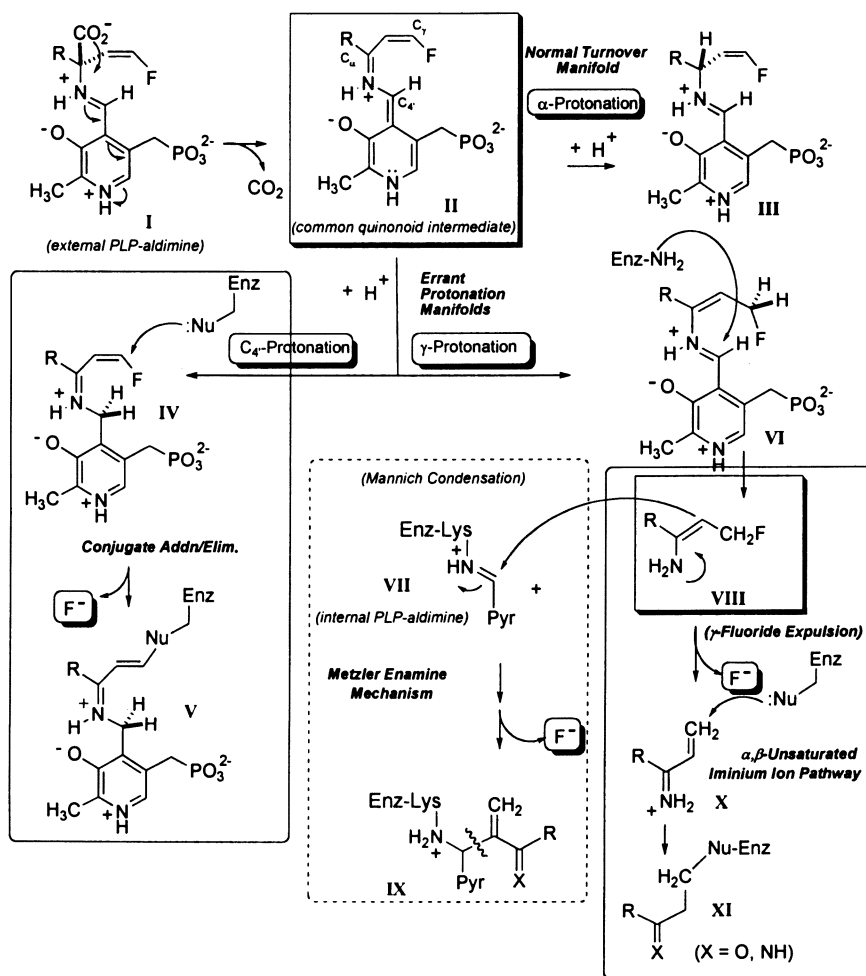
Figure 8. Proposed AADC inactivation pathways for α-(2'Z-fluoro)vinyl-AA's

Note that fluorine is postulated to serve an important mechanistic role in this trigger, particularly in the two electrophilic mechanisms, but fluorine has an ancillary analytical role here. Namely, each errant protonation is projected to lead to fluoride release, detectable by fluoride electrode or by ^{19}F NMR. As can be seen in Figure 9, one can thus discern valuable partition ratio information directly from a ^{19}F NMR spectrum of LDC inactivation with L-FVL. The FVC turnover product displays a clearly resolved signal indicative of α-protonation. All told, the combination of 1 in 3.2 errant protonations, followed by 1 in 5 of these leading to inactivation of LDC gives an overall partition ratio of 16 ± 2. The high errant protonation rate seen in this model AADC active site is promising, as, by design, altered protonation is required for trigger actuation. Given this, the high enantioselectivity of inactivation and the favorable inactivation kinetics seen ($t_{1/2} \sim 3$ min), it will be of interest to examine this trigger in other AADC active sites.
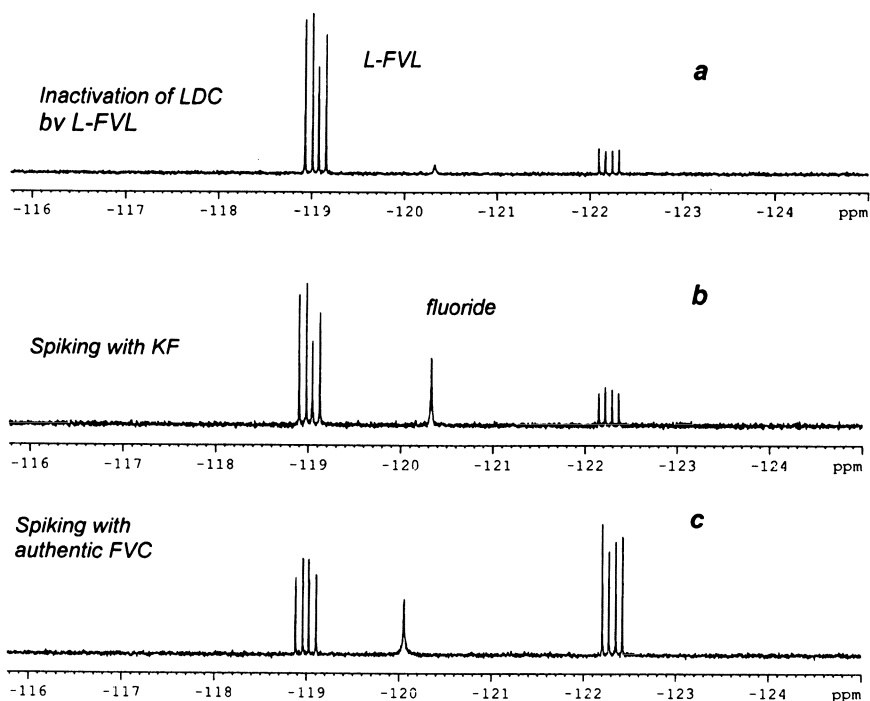
Figure 9. Inactivation of LDC with L-α-(2'Z-fluoro)vinyllysine as observed by ^{19}*F NMR (564 MHz; a), and spiking with KF (b) and FVC (c) standards*

Acknowledgement

References

1. Berkowitz, D. B.; Charette, B. D.; Karukurichi, K. R.; McFadden, J. M. *Tetrahedron: Asymmetry* **2006**, *17*, 869-882.
2. Dardenne, G.; Casimir, J.; Marlier, M.; Larsen, P. O. *Phytochemistry* **1974**, *13*, 1897-1900.
3. Gehring, H.; Rando, R. R.; Christen, P. *Biochemistry* **1977**, *16*, 4832-6.
4. Soper, T. S.; Manning, J. M.; Marcotte, P. A.; Walsh, C. T. *J. Biol. Chem.* **1977**, *252*, 1571-1575.
5. Feng, L.; Kirsch, J. F. *Biochemistry* **2000**, *39*, 2436-2444.
6. Jung, M. J.; Palfreyman, M. G. *Vigabatrin*; Raven: New York, 1995.
7. Nanavati, S. M.; Silverman, R. B. *J. Am. Chem. Soc.* **1991**, *113*, 9341-9349.
8. De Biase, D.; Barra, D.; Bossa, F.; Pucci, P.; John, R. A. *J. Biol. Chem.* **1991**, *266*, 20056-20061.
9. Capitani, G.; Tschopp, M.; Eliot, A. C.; Kirsch, J. F.; Gruetter, M. G. *FEBS Lett.* **2005**, *579*, 2458-2462.
10. Storici, P.; De Biase, D.; Bossa, F.; Bruno, S.; Mozzarelli, A.; Peneff, C.; Silverman, R. B.; Schirmer, T. *J. Biol. Chem.* **2004**, *279*, 363-373.
11. Greenlee, W. J.; Taub, D.; Patchett, A. A. *Tetrahedron Lett.* **1978**, 3999-4002.
12. Danzin, C.; Casara, P.; Claverie, N.; Metcalf, B. W. *J. Med. Chem.* **1981**, *24*, 16-20.
13. Ribereau-Gayon, G.; Danzin, C.; Palfreyman, M. G.; Aubry, M.; Wagner, J.; Metcalf, B. W.; Jung, M. J. *Biochem. Pharmacol. FIELD Full Journal Title:Biochemical Pharmacology* **1979**, *28*, 1331-5133.
14. Berkowitz, D. B.; Jahng, W.-J.; Pedersen, M. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2151-2156.
15. Zhao, Z.; Chen, H.; Li, K.; Du, W.; He, S.; Liu, H.-w. *Biochemistry* **2003**, *42*, 2089-2103.
16. Karthikeyan, S.; Zhou, Q.; Zhao, Z.; Kao, C.-L.; Tao, Z.; Robinson, H.; Liu, H.-w.; Zhang, H. *Biochemistry* **2004**, *43*, 13328-13339.
17. Berkowitz, D. B.; Pedersen, M. L. *J. Org. Chem.* **1994**, *59*, 5476-5478.
18. Berkowitz, D. B.; Pedersen, M. L. *J. Org. Chem.* **1995**, *60*, 5368-5369.

19. Berkowitz, D. B.; Pedersen, M. L.; Jahng, W.-J. *Tetrahedron Lett.* **1996,** *37,* 4309-4312.
20. Raucher, S. *Tetrahedron Lett.* **1977,** 3909-3912.
21. Raucher, S. *J. Org. Chem.* **1977,** *42,* 2950-2951.
22. McCarthy, J. R.; Huber, E. W.; Le, T.-B.; Laskovics, F. M.; Matthews, D. P. *Tetrahedron* **1996,** *52,* 45-58.
23. McCarthy, J. R.; Matthews, D. P.; Stemerick, D. M.; Huber, E. W.; Bey, P.; Lippert, B. J.; Snyder, R. D.; Sunkara, P. S. *J. Am. Chem. Soc.* **1991,** *113,* 7439-7440.
24. Berkowitz, D. B.; De la Salud-Bea, R.; Jahng, W.-J. *Org. Lett.* **2004,** *6,* 1821-1824.
25. Schoellkopf, U.; Groth, U. *Angew. Chem.* **1981,** *93,* 1022-1023.
26. Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem., Int. Ed.* **1997,** *35,* 2708-2748.
27. Seebach, D.; Buerger, H. M.; Schickli, C. P. *Liebigs Annalen der Chemie* **1991,** 669-684.
28. Weber, T.; Aeschimann, R.; Maetzke, T.; Seebach, D. *Helv. Chim. Acta* **1986,** *69,* 1365-1377.
29. Colson, P. J.; Hegedus, L. S. *J. Org. Chem.* **1993,** *58,* 5918-5924.
30. Williams, R. M. *Aldrichimica Acta* **1992,** *25,* 11-13, 15-25.
31. Metcalf, B. W.; Bonilavri, E. *J. Chem. Soc., Chem. Commun.* **1978,** 914-915.
32. Steglich, W.; Wegmann, H. *Synthesis* **1980,** 481-483.
33. Paleo, M. R.; Aurrecoechea, N.; Jung, K.-Y.; Rapoport, H. *J. Org. Chem.* **2003,** *68,* 130-138.
34. Itaya, T.; Hozumi, Y. *Chem. Pharm. Bull.* **1998,** *46,* 1094-1101.
35. Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* **1998,** *63,* 2133-2143.
36. Denniel, V.; Bauchat, P.; Danion, D.; Danion-Bougot, R. *Tetrahedron Lett.* **1996,** *37,* 5111-5114.
37. Itaya, T.; Shimizu, S. *Chem. Pharm. Bull.* **1995,** *43,* 398-402.
38. Crisp, G. T.; Glink, P. T. *Tetrahedron* **1992,** *48,* 3541-3556.
39. Krol, W. J.; Mao, S. S.; Steele, D. L.; Townsend, C. A. *J. Org. Chem.* **1991,** *56,* 728-731.
40. Meffre, P.; Vo Quang, L.; Vo Quang, Y.; Le Goffic, F. *Tetrahedron Lett.* **1990,** *31,* 2291-2294.
41. Jones, R. J.; Rapoport, H. *J. Org. Chem.* **1990,** *55,* 1144-1146.
42. Pedersen, M. L.; Berkowitz, D. B. *J. Org. Chem.* **1993,** *58,* 6966-6975.
43. Pedersen, M. L.; Berkowitz, D. B. *Tetrahedron Lett.* **1992,** *33,* 7315-7318.
44. Berkowitz, D. B.; Pumphrey, J. A.; Shen, Q. *Tetrahedron Lett.* **1994,** *35,* 8743-8746.
45. Berkowitz, D. B.; Smith, M. K. *J. Org. Chem.* **1995,** *60,* 1233-1238.
46. Scott, W. L.; Zhou, C.; Fang, Z.; O'Donnell, M. J. *Tetrahedron Lett.* **1997,** *38,* 3695-3698.

47. Ellis, T. K.; Ueki, H.; Yamada, T.; Ohfune, Y.; Soloshonok, V. A. *J. Org. Chem.* **2006**, *71*, 8572-8578.
48. Smith, N. D.; Wohlrab, A. M.; Goodman, M. *Org. Lett.* **2005**, *7*, 255-258.
49. Ellis, T. K.; Martin, C. H.; Tsai, G. M.; Ueki, H.; Soloshonok, V. A. *J. Org. Chem.* **2003**, *68*, 6208-6214.
50. Ojima, I.; Komata, T.; Qiu, X. *J. Am. Chem. Soc.* **1990**, *112*, 770-774.
51. Storcken, R. P. M.; Panella, L.; van Delft, F. L.; Kaptein, B.; Broxterman, Q. B.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Adv. Synth. Catal.* **2007**, *349*, 161-164.
52. Avenoza, A.; Busto, J. H.; Corzana, F.; Peregrina, J. M.; Sucunza, D.; Zurbano, M. M. *Tetrahedron: Asymmetry* **2004**, *15*, 719-724.
53. Belokon, Y. N.; Bespalova, N. B.; Churkina, T. D.; Cisarova, I.; Ezernitskaya, M. G.; Harutyunyan, S. R.; Hrdina, R.; Kagan, H. B.; Kocovsky, P.; Kochetkov, K. A.; Larionov, O. V.; Lyssenko, K. A.; North, M.; Polasek, M.; Peregudov, A. S.; Prisyazhnyuk, V. V.; Vyskocil, S. *J. Am. Chem. Soc.* **2003**, *125*, 12860-12871.
54. Najera, C.; Abellan, T.; Sansano, J. M. *Eur. J. Org. Chem.* **2000**, 2809-2820.
55. Ma, D.; Ding, K. *Org. Lett.* **2000**, *2*, 2515-2517.
56. Alonso, F.; Davies, S. G.; Elend, A. S.; Haggitt, J. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 257-264.
57. Studer, A.; Seebach, D. *Liebigs Ann. Chem.* **1995**, 217-222.
58. Ojima, I.; Chen, H. J. C.; Qiu, X. *Tetrahedron* **1988**, *44*, 5307-5318.
59. Georg, G. I.; Guan, X.; Kant, J. *Tetrahedron Lett.* **1988**, *29*, 403-406.
60. Peggion, C.; Formaggio, F.; Crisma, M.; Toniolo, C.; Kaptein, B.; Broxterman, Q. B.; Kamphuis, J. *J. Pept. Sci.* **1999**, *5*, 547-554.
61. Altmann, E.; Altmann, K. H.; Nebel, K.; Mutter, M. *Int. J. Pept. Protein Res.* **1988**, *32*, 344-351.
62. Yokum, T. S.; Gauthier, T. J.; Hammer, R. P.; McLaughlin, M. L. *J. Am. Chem. Soc.* **1997**, *119*, 1167-1168.
63. Mendel, D.; Ellman, J.; Schultz, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 4359-4360.
64. Dumas, F.; Mezrhab, B.; d'Angelo, J.; Riche, C.; Chiaroni, A. *J. Org. Chem.* **1996**, *61*, 2293-2304.
65. Mezrhab, B.; Dumas, F.; d'Angelo, J.; Riche, C. *J. Org. Chem.* **1994**, *59*, 500-503.
66. Berkowitz, D. B.; McFadden, J. M.; Sloss, M. K. *J. Org. Chem.* **2000**, *65*, 2907-2918.
67. Jones, M. C.; Marsden, S. P.; Subtil, D. M. M. *Org. Lett.* **2006**, *8*, 5509-5512.
68. Comins, D. L.; Salvador, J. M. *J. Org. Chem.* **1993**, *58*, 4656-4661.
69. Karukurichi, K. R.; De la Salud-Bea, R.; Jahng, W. J.; Berkowitz, D. B. *J. Am. Chem. Soc.* **2007**, *129*, 258-259.

302

70. Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243-1250.
71. Berkowitz, D. B.; Smith, M. K. *Synthesis* **1996**, 39-41.
72. Badorrey, R.; Cativiela, C.; Diaz-de-Villegas, M. D.; Galvez, J. A. *Synthesis* **1997**, 747-749.
73. Griesbeck, A. G.; Hirt, J. *Liebigs Ann. Chem.* **1995**, 1957-1961.
74. Hallinan, K. O.; Crout, D. H. G.; Errington, W. *J. Chem. Soc. Perkin Trans. 1* **1994**, 3537-3543.
75. Pellicciari, R.; Natalini, B.; Marinozzi, M. *Synth. Commun.* **1988**, *18*, 1715-1721.
76. Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425-1428.
77. Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817-4820.
78. Berkowitz, D. B.; McFadden, J. M.; Chisowa, E.; Semerad, C. L. *J. Am. Chem. Soc.* **2000**, *122*, 11031-11032.
79. Berkowitz, D. B.; Chisowa, E.; McFadden, J. M. *Tetrahedron* **2001**, *57*, 6329-6343.
80. Li, H. M.S., University of Nebraska, 2001.
81. Berkowitz, D. B.; Wu, B.; Li, H. *Org. Lett.* **2006**, *8*, 971-974.
82. Overman, L. E.; Owen, C. E.; Pavan, M. M.; Richards, C. J. *Org. Lett.* **2003**, *5*, 1809-1812.
83. Calter, M.; Hollis; Keith, T.; Overman, L. E.; Ziller, J.; Zipp, G. G. *J. Org. Chem.* **1997**, *62*, 1449-1456.
84. Anderson, C. E.; Overman, L. E.; Richards, C. J.; Watson, M. P.; White, N. *Org. Synth.* **2007**, *84*, 139-147.
85. Watson, M. P.; Overman, L. E.; Bergman, R. G. *J. Am. Chem. Soc.* **2007**, *129*, 5031-5044.
86. Uozumi, Y.; Kato, K.; Hayashi, T. *Tetrahedron: Asymmetry* **1998**, *9*, 1065-1072.
87. Kang, J.; Yew, K. H.; Kim, T. H.; Choi, D. H. *Tetrahedron Lett.* **2002**, *43*, 9509-9512.
88. Berkowitz, D. B.; Bose, M.; Choi, S. *Angew. Chem. Int. Ed.* **2002**, *41*, 1603-1607.
89. Dey, S.; Powell, D. R.; Hu, C.; Berkowitz, D. B. *Angew. Chem. Int. Ed.* **2007**, *46*, 7010-7014.
90. Dey, S.; Karukurichi, K. R.; Shen, W.; Berkowitz, D. B. *J. Am. Chem. Soc.* **2005**, *127*, 8610-8611.
91. Berkowitz, D. B.; Shen, W.; Maiti, G. *Tetrahedron: Asymmetry* **2004**, *15*, 2845-2851.
92. Berkowitz, D. B.; Maiti, G. *Org. Lett.* **2004**, *6*, 2661-2664.
93. Trost, B. M.; Fandrick, D. R.; Brodmann, T.; Stiles, D. T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6123-6125.
94. Trost, B. M.; Bunt, R. C. *J. Am. Chem. Soc.* **2000**, *122*, 5968-5976.
95. Larksarp, C.; Alper, H. *J. Am. Chem. Soc.* **1997**, *119*, 3709-3715.

96. Hayashi, T.; Yamamoto, A.; Ito, Y. *Tetrahedron Letters* **1988**, *29*, 99-102.
97. Silverman, R. B.; Bichler, K. A.; Leon, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 1253-1261.
98. Bhattacharjee, M. K.; Snell, E. E. *J. Biol. Chem.* **1990**, *265*, 6664-6668.
99. Ueno, H.; Likos, J. J.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4387-4393.
100. Likos, J. J.; Ueno, H.; Feldhaus, R. W.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4377-4386.

Enzymatic Approach

Chapter 19

Preparation of Chiral Amino Acid Intermediates for Synthesis of Pharmaceutical Compounds Using Amino Acid Dehydrogenases

Ronald L. Hanson

Process Research and Development, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903

Amino acid dehydrogenases are useful for preparation of L- or D-α-amino acids from the corresponding α-keto acids. The reactions require NH_3, NAD(P)H and a second enzyme, usually formate or glucose dehydrogenase, for regeneration of the NAD(P)H. The enzymes are effective at high substrate concentrations and have very high enantioselectivity. Examples of the use of amino acid dehydrogenases for the preparation of some chiral intermediates used for the synthesis of drug candidate compounds are described.

Introduction

α-Amino acids are useful intermediates for preparation of many pharmacologically active compounds. Several enzymatic approaches have been applied for the preparation of both D and L-amino acids including hydantoinases combined with carbamoylases or HNO_2, (1-4) N-acylases, (1,5) amidases, (6,7) hydrolysis of esters with esterase or proteases, (8-10) transaminases, (11,12) and amino acid dehydrogenases. Of these approaches, we have found the reductive amination of α-keto acids using amino acid dehydrogenases to be one of the most useful methods because the enzymes have good stability, broad substrate specificity and very high enantioselectivity, are effective at high substrate concentrations, and the reactions have favorable equilibria which are further enhanced when the reductive aminations are coupled to a suitable enzymatic cofactor regeneration system. Recent excellent reviews on the amino acid dehydrogenases cover the sources, substrate specificities and other

properties of available enzymes, enzyme structures and reaction mechanisms, and some examples of their synthetic utilities (13,14).

An example of a reductive amination reaction is shown in figure 4. Performing reductive amination reactions requires first screening a collection of amino acid dehydrogenases against the intended keto acid substrate to find the most effective enzyme. Commercially available enzymes may be screened or a suitable enzyme may be found by screening cell extracts from likely source strains in a culture collection. In cases where the gene sequence for the amino acid dehydrogenase has been reported, the gene can be cloned from the source organism using PCR, and the enzyme expressed in E. coli or other host.

A second enzyme is needed for regeneration of the pyridine nucleotide cofactor. For most enzymes, the required cofactor is NADH but NADPH is required in some cases. Yeast formate dehydrogenase is commonly used for NADH regeneration, (15-17) and glucose dehydrogenase usually from Bacillus species may be used for either NADH or NADPH regeneration (18,19). Mutated formate dehydrogenases from Pseudomonas (20) and Saccharomyces cerevisiae (21) capable of NADPH regeneration have also been described. Formate dehydrogenase has the advantage that the product CO_2 is easy to remove from the reaction. Glucose dehydrogenase has the advantages of working with either NAD or NADP and having a much higher specific activity than formate dehydrogenase. Amino acid dehydrogenase reactions have $E^{o'}$ of -0.13 to -0.14 V (22). Using the glucose dehydrogenase ($E^{o'}$ of -0.45) (22) or formate dehydrogenase reactions ($E^{o'}$ of -0.42V) (22) to regenerate NADH provides a large $\triangle E^{o'}$ to drive the reaction to completion. Phosphite dehydrogenase has also been recently introduced for regeneration of NADH (23) and a genetically evolved version for regeneration of NADPH has also been reported (24). Glucose, formate and phosphite dehydrogenases are all commercially available. When the enzymes are prepared by fermentation rather than obtained from a commercial supplier, it may be advantageous to clone and express both the amino acid dehydrogenase and cofactor regeneration enzymes in the same host.

Reaction conditions are chosen that are compatible with both enzymes and also with the stability of the keto acid substrate. Because the reactions go nearly to completion, the product is readily isolated either by direct crystallization or by adsorption at low pH on a Dowex-50 H^+ resin, washing with water, then elution with NH_4OH solution. Some examples of the use of amino acid dehydrogenases to prepare chiral intermediates for synthesis of drug candidate compounds are given below.

L-β-Hydroxyvaline

L-β-hydroxyvaline (1), an intermediate used for synthesis of the monobactam antibiotic drug candidate tigemonam (Figure 1), was initially prepared from racemic β-hydroxyvaline by a classical chemical resolution

method. A commercially available (although expensive) leucine dehydrogenase from *Bacillus* species was then found to carry out the reductive amination of α-keto-β-hydroxyisovalerate to give L-β-hydroxyvaline (*25*). *Bacillus* strains from our culture collection were screened to find an alternative source of a suitable enzyme. Reductive amination activity for α-keto-β-hydroxyisovalerate was found in most of the *Bacillus* strains screened (including *B. megaterium, B. subtiis, B. cereus, B. pumilus, B. licheniformis,B. thuringiensis, B. brevis*) with the highest specific activity found in *B. sphaericus* ATCC 4525. Either formate dehydrogenase from *Candida boidinii* or glucose dehydrogenase from *Bacillus megaterium* was used for regeneration of NADH. pH 8.5 was optimal for both glucose dehydrogenase from *Bacillus megaterium* and leucine dehydrogenase from *Bacillus sphaericus*. For the enzyme from *B. sphaericus* ATCC 4525, the apparent Km for α-keto-β-hydroxyisovalerate was 11.5 mM (sufficiently low considering that the reaction was run at 0.25 to 0.5 M of the keto acid) and the apparent V_{max} for α-keto-β-hydroxyisovalerate was 41% of the value for α-ketoisovalerate (reported to be the best substrate for reductive amination by leucine dehydrogenase), making leucine dehydrogenase from *B. sphaericus* ATCC 4525 very suitable for synthesis of this intermediate.

L-6-Hydroxynorleucine

The initial synthesis of Vanlev (Figure 2), a vasopeptidase inhibitor intended for the treatment of hypertension required L-6-hydroxynorleucine (2) as an intermediate (*26*). In the initial route, racemic N-acetyl-6-hydroxynorleucine was treated with L-acylase from hog kidney to prepare the required amino acid. Because the resolution with acylase gave a theoretical maximum yield of only 50% and required separation of the desired product from the unreacted enantiomer at the end of the reaction, we subsequently tried to prepare the amino acid by reductive amination of the corresponding ketoacid (*27*) 2-Keto-6-hydroxyhexanoic acid (in equilibrium with its cyclic hemiketal form) was prepared by chemical synthesis starting from 4-chloro-1-butanol, which was O-protected, then converted to a Grignard reagent which was added to diethyl oxalate, followed by hydrolysis of the ester and deprotection of the hydroxyl group. Initial screening, with formate dehydrogenase for regeneration of NADH, showed that phenylalanine dehydrogenase from *Sporosarcina* sp. and beef liver glutamate dehydrogenase converted 2-keto-6-hydroxyhexanoic acid completely to L-6-hydroxynorleucine. Leucine dehydrogenase partially purified from *Bacillus sphaericus* ATCC 4525 and alanine dehydrogenase from *Bacillus subtilis* were not active. Additional screening with spectrophotometric enzyme assays (i.e., monitoring the rate of NADH oxidation in the reaction) of commercially available amino acid dehydrogenases and extracts of 132 cultures
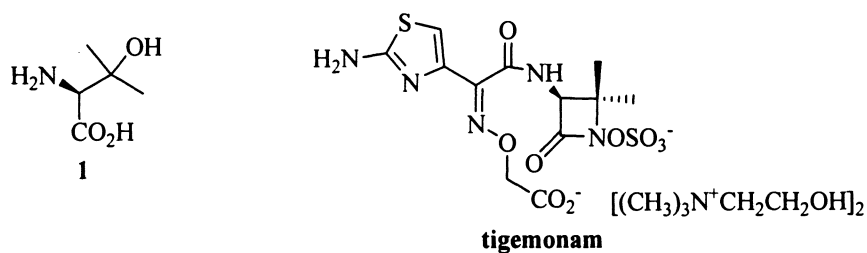
Figure 1. Structures of L-β-hydroxyvaline and tigemonam
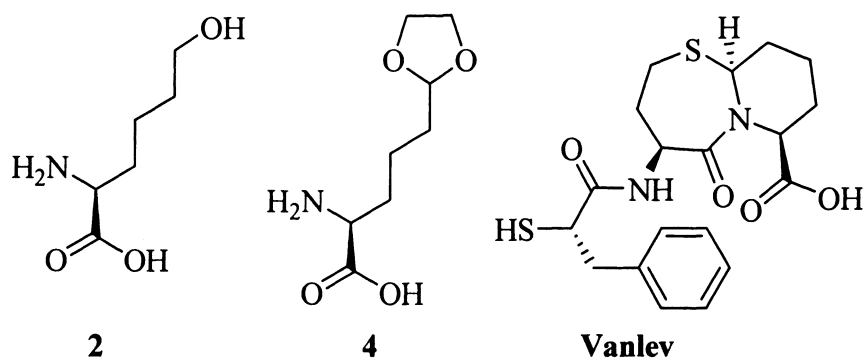
Figure 2. Structures of L-6-hydroxynorleucine 2, L-allysine ethylene acetal 4, and Vanlev (omapatrilat, BMS 186716)

from our collection identified several other active enzymes, with extract from *Thermoactinomyces intermedius* ATCC 33205 found to contain the most active enzyme. This strain has been reported to be a source of thermostable phenylalanine dehydrogenase (*28*) as well as leucine dehydrogenase (*29*).

Beef liver glutamate dehydrogenase was used for preparative reactions at 10% total substrate concentration. NADH was regenerated using glucose dehydrogenase from *Bacillus megaterium*. The optimum pH for glutamate dehydrogenase with this substrate was determined to be about 8.8, and glucose dehydrogenase as stated above has a broad pH optimum centered at about 8.5. The reaction, carried out at pH 8.75, was complete in about 3 h with a reaction yield of 89% and ee >99%. The product of the glucose dehydrogenase reaction is gluconolactone which is hydrolyzed to gluconic acid. NH_4OH was added from a pH stat to maintain pH 8.75 during the reaction and was used to follow the time course of the reaction.

Chemical synthesis and isolation of 2-keto-6-hydroxyhexanoic acid required several steps. In a second more convenient process (Figure 3), the ketoacid was prepared by treatment of racemic 6-hydroxynorleucine (produced by hydrolysis of commercially available 5-(4-hydroxybutyl) hydantoin) with D-amino acid oxidase and catalase. After the ee of the remaining L-6-hydroxynorleucine had risen to >99%, the reductive amination procedure was used to convert the mixture containing 2-keto-6-hydroxyhexanoic acid and L-6-hydroxynorleucine entirely to L-6-hydroxynorleucine with yields of 91 to 97% and ee >99%. Sigma porcine kidney D-amino acid oxidase and beef liver catalase or *Trigonopsis variabilis* whole cells (source of oxidase and catalase) (*30*) were used successfully for this transformation.

L-Allysine Ethylene Acetal

In the original route to Vanlev, L-6-hydroxynorleucine was coupled with S-acetyl-N-Cbz-L-homocysteine, then oxidized to the aldehyde (*26*). In the final route, a process for the production of L-allysine ethylene acetal (4) by enzymatic reductive amination of the corresponding keto acid 3 was developed and scaled up to avoid this oxidation step (Figure 4) (*31*). Screening of commercially available amino acid dehydrogenases as well as some strains from our culture collection showed that glutamate, alanine, leucine and phenylalanine dehydrogenases (listed in order of increasing effectiveness) gave some of the desired product. An extract of *Thermoactinomyces intermedius* ATCC 33205 was an effective source of phenylalanine dehydrogenase (PDH) for the reaction. For this process formate dehydrogenase (FDH) was used for regeneration of NADH. *Candida boidinii* grown on methanol was the initial source for the FDH. Although both the glucose and formate dehydrogenase reactions are equally effective for regeneration of NADH, the easy removal of CO_2 compared to
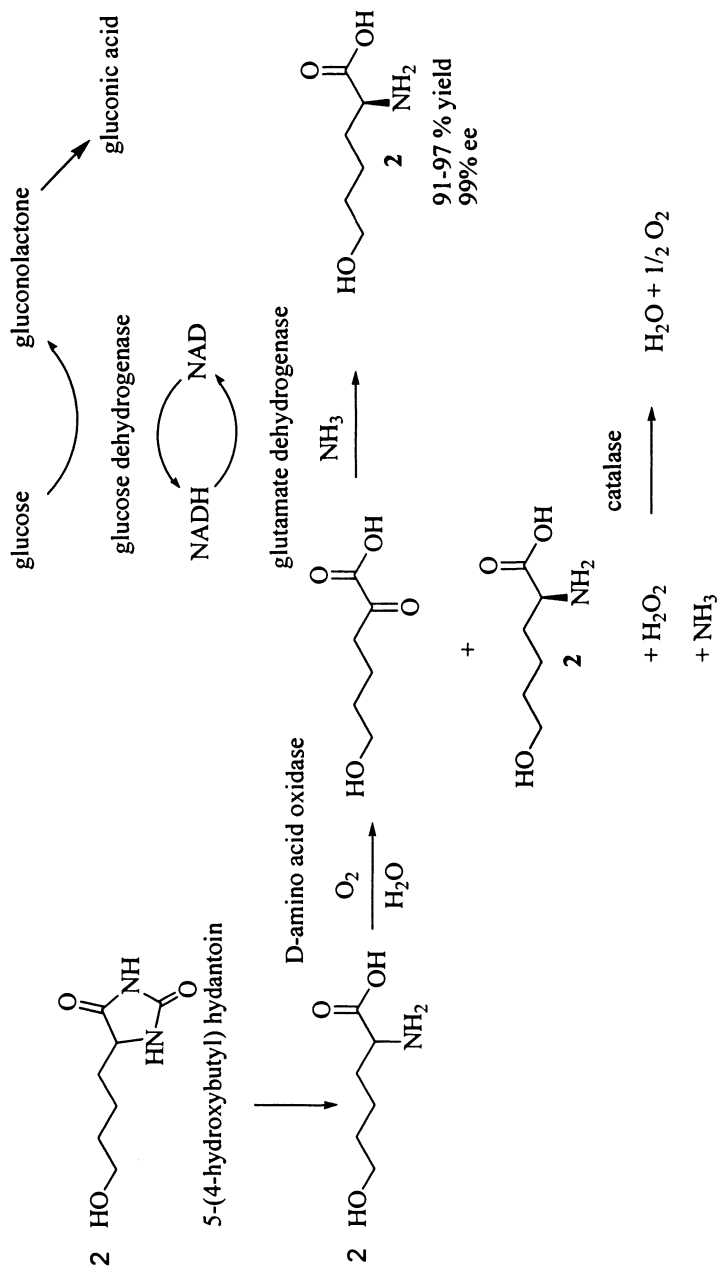
Figure 3. Preparation of L-6-hydroxynorleucine from 5-(4-hydroxybutyl) hydantoin
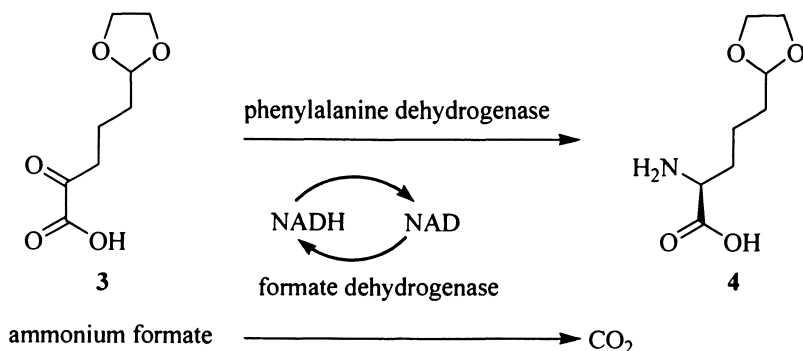
Figure 4. Conversion of keto acid acetal 3 to amino acid acetal 4.
Phenylalanine dehydrogenase catalyzes reductive amination of the keto acid.
Formate dehydrogenase is used for regeneration of NADH.

gluconic acid made this a preferable method for isolation of the amino acid product by direct crystallization from water/methanol.

T. intermedius IFO14230 (ATCC 33205) was first identified as a source of PDH by Ohshima *et al.(28)*. The enzyme was purified and characterized *(28)* then cloned and expressed in *E. coli* by the same workers *(32)*. The enzyme was reported to be moderately specific for deamination of phenylalanine and to carry out the amination of some keto acids at a much lower rate than amination of phenylpyruvate *(28)* In our screening, the enzyme was the most effective amino acid dehydrogenase identified for the reductive amination of the keto acid acetal.

Wet cells, heat-dried cells, extracts and immobilized enzymes were all useful for the reaction, but heat-dried cell preparations were the simplest and most convenient enzyme source to use. Heat-dried cells were produced by drying the cells under vacuum at 54 °C, then milling to <10 mesh. Activities were stable after storage for extended periods at 5 °C. Our initial procedure for the conversion of 3 to 4 used dried *T. intermedius* as a source of PDH and heat-dried *C. boidinii* as a source of FDH. Problems with scale-up of the *T. intermedius* fermentation and a need for more productivity, provided the impetus for cloning the *T. intermedus* enzyme using PCR to amplify the published sequence. Heat-dried recombinant *E. coli* containing cloned *T. intermedius* PDH inducible with isopropylthiogalactoside and again using heat-dried *C. boidinii* as a source of FDH provided a large increase in productivity for the fermentation and the reductive amination. Although the kinetics of the reaction were about the same, when using the same number of units of PDH, the higher specific activity of the recombinant *E. coli* compared to the *Thermoactinomyces intermedius* decreased the amount of dried cells required and made isolation of the product easier.

Like *Candida boidinii*, *Pichia pastoris* also produces a formate dehydrogenase when grown on methanol. Expression of the cloned *T. intermedius* phenylalanine dehydrogenase in *Pichia pastoris* under control of the alcohol oxidase promoter allowed both phenylalanine dehydrogenase and formate dehydrogenase to be produced together in the same fermentation, and they were conveniently produced in a suitable ratio for the reaction. To carry out this fermentation, the culture was initially grown on 1% glycerol for about 16h until glycerol was exhausted from the medium, then a methanol feed was begun to maintain the methanol concentration at about 0.1% for 48 h to induce production of both enzymes. After the induction period the cells were concentrated by cross-flow microfiltration, washed with buffer and spray-dried. The dried cells were added to the reaction mixture as a source of the two enzymes.

The optimum pH for the reductive amination of **3** by PDH from *T. intermedius* ATCC 33205 was about 8.7. Because **3** was much more stable under the reaction conditions at pH 8.0 than at 8.7, the reaction was carried out at pH 8.0. This pH was also well suited to the use of FDH from *C. boidinii*, which was reported to have a broad pH optimum of 7.5 to 8.5 (*33*). FDH from *P. pastoris* was reported to have a pH optimum of 6.5-7.5 (*34*), or 7.5 (*35*), with more than 80% of maximal activity at pH 8 (*34,35*).

The procedure using heat-dried cells of *Escherichia coli* containing cloned PDH and heat-dried *C. boidinii* was scaled up without any problems. A total of 197 kg of **4** was produced in three 1600-L batches using a 5% concentration of **3** with an average yield of 91.1 M % and ee >98%. The procedure with *P. pastoris* was also scaled up to produce 15.5 kg of **4** in 97 M % yield and ee >98% in a 180-L batch using 10% keto acid concentration. Subsequent to this work the *Pichia pastoris* FDH was cloned and expressed in *E. coli* together with the *T. intermedius* PDH to allow both enzymes to be induced with β-isopropyl-thio-D-galactoside (IPTG) (*36*).

The route using the amino acid acetal **4** for preparation of the bicyclic intermediate for Vanlev is summarized in Figure 5. The amino acid acetal was converted to the dimethyl acetal methyl ester, then coupled with N-protected homocystine to give a dipeptide dimer. The dimer was converted to the monomer with dithiothreitol or tributylphosphine. Acidification of the monomer gave the aldehyde which cyclized to the bicyclic intermediate with concomitant hydrolysis of the ester.

L-3-Hydroxyadamantylglycine

L-3-Hydroxyadamantylglycine (**6**) is an intermediate required for the dipeptidyl peptidase-IV inhibitor, Saxagliptin, a drug candidate for treatment of Type-2 diabetes (Figure 6). This amino acid was originally prepared using an asymmetric Strecker amino acid synthesis (*37*). For an alternative route,

314
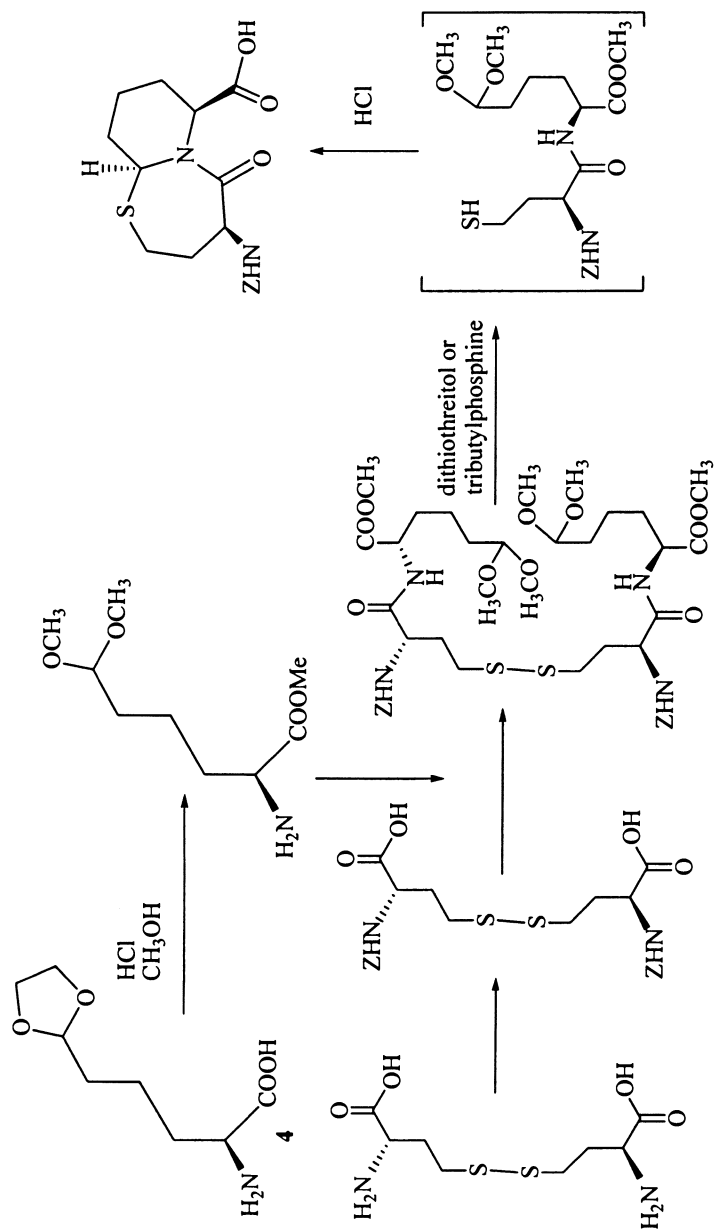
Figure 5. Conversion of amino acid acetal to the bicyclic intermediate
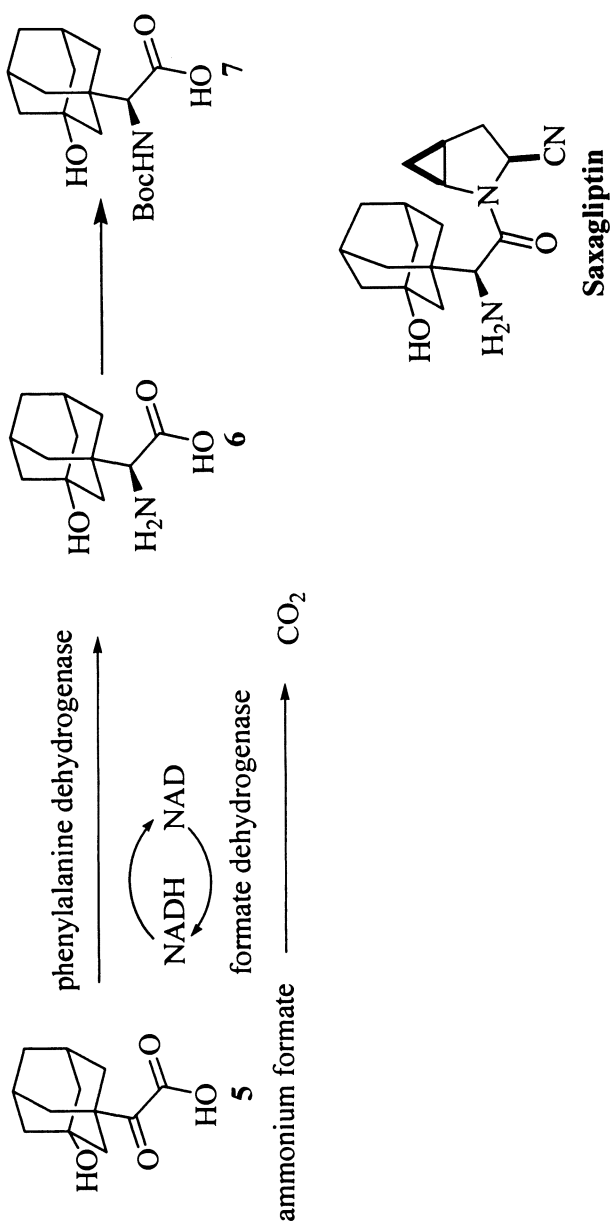
Figure 6. Preparation of S-3-hydroxyadamantylglycine, an intermediate for Saxagliptin (BMS-477118)

3' end of native PDH gene and corresponding amino acids

AAC	AGC	GCA	AGG	AGG	TAA
Asn	Ser	Ala	Arg	Arg	Stop

3' end of PDHmod gene and corresponding amino acids (changed or new amino acids in bold):

AAC	AGC	GCG	**GAG**	**GGG**	**TAC**	**CTC**	**GAG**	**CCG**	**CGG**
Asn	Ser	Ala	**Glu**	**Gly**	**Tyr**	**Leu**	**Glu**	**Pro**	**Arg**

CGG	**CCG**	**CGA**	**ATT**	**AAT**	**TCG**	**CCT**	**TAG**
Arg	**Pro**	**Arg**	**Ile**	**Asn**	**Ser**	**Pro**	Stop

Figure 7. Comparison of 3' ends of native pdh gene with modified gene reisolated from Pichia pastoris SC16176 (Reproduced with permission from reference 36. Copyright 2007 Wiley-VCH Verlag GmbH and Company.)

screening of amino acid dehydrogenases indicated that the recombinant phenylalanine dehydrogenase from *Thermoactinomyces intermedius* expressed in *Pichia pastoris* SC16176 previously used to prepare the amino acid acetal **4** was also the most effective enzyme for reductive amination of keto acid **5** (*36*). In this case cell extract was much more effective than the heat-dried cells, in contrast to the results for the reductive amination of **3**. The more bulky substrate **5** may have difficulty in entering cells. Surprisingly the enzyme expressed in *Pichia pastoris* was much more effective than the enzyme expressed in *E. coli* even when the activity of the enzyme from *E. coli* (assayed with phenylpyruvate as substrate) was much higher than that expressed in *Pichia*. After reisolation of the *pdh* gene from *Pichia pastoris* SC16176 and comparison with the original published sequence, this anomaly was found to be due to a modification of the original enzyme that had inadvertently been introduced during the cloning procedure. This modification consisted of 2 amino acid changes at the C-terminus and a 12-amino acid extension of the C-terminus (Figure 7). The modified enzyme was more effective with keto acid **5** than the original enzyme but had a lower specific activity than the original enzyme with phenylpyruvate. In addition the heat stability of the modified enzyme was decreased, with all activity lost after 1h at 60 °C compared to complete activity loss after 1 h at 70 °C for the original enzyme. Since the reaction was run at 40 °C to accomodate the lower stability of formate dehydrogenase, this change was of little consequence.

Production of multi-kg batches of L-3-hydroxyadamantylglycine was originally carried out with extracts of *Pichia pastoris* SC16176 containing the modified phenylalanine dehydrogenase (PDHmod) inducible with methanol together with the endogenous FDH produced during growth of the cells on methanol. The isolated yield (using Dowex 50 (H$^+$)) of the amino acid was 96% with ee >99%. Later, for further scaleup of the production of **6**, PDHmod from *Pichia pastoris* SC16176 was expressed in *E. coli* together with FDH from *Pichia pastoris* with both enzymes under the control of the *tac* promoter inducible by IPTG. With sufficiently pure keto acid substrate, the process was carried out at 10% keto acid concentration. The Boc-protected amino acid **7** is the required intermediate for the synthetic route, and amino acid **6** was converted directly to the Boc-derivative before isolation, then isolated by an extraction procedure. To support the development of Saxagliptin, more than 1000 kg of intermediate **7** has been prepared using this enzymatic process.

Modification of Amino Acid Dehydrogenases to Change Substrate Specificity

Although the modification of PDH to produce an enzyme more effective for preparation of L-3-hydroxyadamantylglycine was introduced inadvertently, enzyme engineering and directed evolution have been applied to amino acid dehydrogenases to change the substrate specificities. A hexapeptide sequence believed to be important for substrate recognition in PDH from *Thermoactinomyces intermedius* was replaced with the corresponding sequence from the leucine dehydrogenase of *Bacillus stearothermophilus* by Soda and coworkers (*38*) Activities were decreased for both aromatic and aliphatic amino acids and keto acids compared to the wild-type enzyme although the decreases were larger for the aromatic substrates. In addition the altered enzyme was found to be a monomer/dimer mixture in contrast to the hexamer structure of the original enzyme. This group also created a chimeric enzyme by combining the amino-terminal domain of PDH from *Thermoactinomyces intermedius* with the carboxy-terminal domain of leucine dehydrogenase from *Bacillus stearothermophilus* (*39*) The chimeric enzyme had a broader substrate specificity that was a mixture of the specificities from the two parent enzymes, although lower activity compared to the parent enzyme with the preferred substrates of each of the parent enzymes.

Engel and coworkers used site directed mutagenesis based on structural analysis to construct single and double mutants with G124A and L307V changes in PDH from *Bacillus sphaericus* with the amino acid replacements being the corresponding amino acids from leucine dehydrogenase. K_{cat}/K_m for phenylpyruvate was decreased for all three mutant enzymes but increased for

aliphatic keto acids, with almost a 100-fold increase in k_{cat}/K_m for α-ketovalerate in the double mutant compared to the wild type (40) Three other mutants of *B. sphaericus* PDH with substitutions at N145 had improved activity compared to the wild type with some keto acids but less activity with others (41) and were used for preparation of three *p*-substituted L-phenylalanine derivatives (42). The L307V mutant was also used for the synthesis of a *p*-substituted L-phenylalanine (43).

All of the naturally occurring amino acid dehydrogenases with wide substrate specificity described to this time convert keto acids exclusively to the the L-enantiomers. However D-amino acid dehydrogenases have recently been developed by Novick and coworkers (44). Their approach was to start with *meso*-diaminopimelic D-dehydrogenase from *Corynebacterium glutamicum*, an enzyme with strict D-specificity but limited substrate range, and use directed evolution to broaden the substrate range of the enzyme while preserving the D-selectivity. After five mutations from three rounds of mutagenesis and screening they achieved the desired result of an enzyme able to produce a variety of D-amino acids with high ee from α-keto acid precursors. In this case the enzyme requires NADPH as cofactor and glucose dehydrogenase was used for cofactor regeneration.

Conclusion

α-Amino acids are useful synthons for preparing many compounds with pharmacological activity. Reductive amination of α-keto acids using amino acid dehydrogenases is a technology that has been widely applicable at Bristol-Myers Squibb in various synthetic routes. In addition to the examples above, similar procedures were used to prepare six other non-proteinogenic amino acid intermediates, including a D-amino acid (45) The preparation of the keto acid substrates are described in the references for the examples cited. Addition of a Grignard reagent containing the amino acid R- group to diethyloxalate followed by saponification is a useful procedure (27,31). Oxidation of a methyl ketone (10) and hydrolysis of an α,α-dichloro ester are other effective methods (46). In cases where synthesis of the keto acid is difficult, another approach is to prepare the racemic amino acid by chemical methods, treat with a D- or L-amino acid oxidase (27,47) to convert the unwanted enantiomer to the keto acid and then treat the mixture with an L- or D-amino acid dehydrogenase to convert the mixture entirely to the desired enantiomer.

References

1   May, O.; Verseck, S.; Bommarius, A.; Drauz K. *Org. Proc. Res. & Dev.* **2002**, *6*, 452-457.

2. Altenbuchner, J.; Siemann-Herzberg, M.; Syldak, C. *Curr. Opin. Biotechnol.* **2001**, *12*, 559-563.
3. Drauz, K. *Chimia* **1997**, *51*, 310-314.
4. Pietzsch, M.; Syldatk, C. in *Enzyme Catalysis in Organic Synthesis (2ⁿᵈ Edition)*; Drauz, K.; Waldmann, H., Eds.; Wiley-VCH: Weinheim, Germany 2002; Vol. 2, pp 761-799.
5. Moriguchi, M.; Ideta, K. *Applied Environ. Microbiol.* **1988**, *54*, 2767-2770
6. Komeda, H.; Asano, Y. *Eur. J. Biochem.* **2000**, *267*, 2028-2035.
7. Yamaguchi, S.; Komeda, H.; Asano, Y. *Appl. Environ. Microbiol.* **2007**, *73*, 5370-5373.
8. Bennett, D. J.; Buchanan, K. I.; Cooke, A.; Epemolu, O.; Hamilton, N. M.; Hutchinson, E. J.; Mitchell, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 362-365.
9. Miyazawa, T.; Iwanaga, H.; Yamada, T.; Kuwata, S. *Biotechnol. Lett.* **1994**, *16*, 373-378.
10. Larionov, O. V.; de Meijere, A. *Adv. Synth. Catal.* **2006**, *348*, 1071-1078.
11. Taylor, P. P.; Pantaleone, D. P.; Senkpeil, R. F.; Fotheringham, I. G. *Trends Biotechnol.* **1998**, *16*, 412-418.
12. Rozzell, J. D.; Bommarius, A. S. in *Enzyme Catalysis in Organic Synthesis (2ⁿᵈ Edition)*; Drauz, K.; Waldmann, H., Eds.; Wiley-VCH: Weinheim, Germany 2002; Vol. 2, pp 873-893.
13. Bommarius, A. S in *Enzyme Catalysis in Organic Synthesis (2ⁿᵈ Edition)*; Drauz, K.; Waldmann, H., Eds.; Wiley-VCH: Weinheim, Germany 2002; Vol 3, pp 1047-1063.
14. Ohshima, T.; Soda, K. in *Stereoselective Biocatalysis*, Patel, R. N., Ed.; Marcel Dekker, New York, 2000; pp 877-902.
15. Shaked, Z.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7104-7105
16. Kula, M. R.; Wandrey, C. *Methods Enzymol.* **1987**, *136*, 9-21.
17. Bommarius, A. S.; Drauz, K.; Hummel, W.; Kula, M. R.; Wandrey, C. *Biocatalysis* **1994**, *10*, 37-47.
18. Wong, C-H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028-4031.
19. Weckbecker, A.; Hummel, W. in *Methods in Biotechnology, Microbial Enzymes and Biotransformations*; Barredo, J. L., Ed.; Humana Press, Totawa, NJ, 2005; Vol. 17, pp 225-237.
20. Tishkov, V. I.; Galkin, A. G.; Fedorchuk, V. V.; Savitsky, P. A.; Rojkova, A. M.; Gieren, H.; Kula, M-R. *Biotechnol. Bioeng.* **1999**, *64*, 187-193.
21. Serov, E.; Popova, A. S.; Fedorchuk, V. V.; Tishkov, V. I. *Biochem J.* **2002**, *367*, 841-847.
22. Walsh, C. *Enzymatic Reaction Mechanisms*, W. H. Freeman, New York, **1979**; p 313.
23. van der Donk, W. A.; Zhao, H. *Curr. Opin. Biotechnol.* **2003**, *14*, 421-426.
24. Johannes, T. W.; Woodyer, R. D.; Zhao, H. *Biotechnol. Bioeng.* **2007**, *96*, 18-26.

320

25. Hanson, R. L.; Singh, J.; Kissick, T. P.; Patel, R. N.; Szarka, L. J.; Mueller, R. H. *Bioorg. Chemistry* **1990**, *18*, 116-130.
26. Robl, J. A.; Sun, C.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Asaad, M.M.; Cheung, H. S.; Aboa-Offei, B. E.; Smith, P.L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A. ; Trippodo, N. C. *J. Med. Chem.* **1997**, *40*, 1570-1577.
27. Hanson, R. L.; Schwinden, M. D.; Banerjee, A.; Brzozowski, D. B.; Chen, B.-C; Patel, B. P.; McNamee, C. G.; Kodersha, G. A.; Kronenthal, D. R.; Patel, R. N.; Szarka, L. J. *Bioorg. Med. Chem.* **1999**, *7*, 2247-2252.
28. Oshima, T.; Takada, H.; Yoshimura, T.; Esaki, N.; Soda, K. *J. Bacteriol.* **1991**, 173, 3943-3948.
29. Oshima, T.; Nishida, N.; Bakthavatsalam, S.; Kataoka, K.; Takada, H.; Yoshimura, T.; Esaki, N.; Soda, K. *Eur. J. Biochem.* **1994**, 222, 305-312.
30. Wei, C.; Huang, J.; Tsai, Y. *Biotechnol. Bioeng.* **1989**, 34, 570-574.
31. Hanson, R. L.; Howell, J. M.; LaPorte, T. L; Donovan, M. J.; Cazzulino, D. L.; Zannella, V.; Montana, M. A.; Nanduri, V. B.; Schwarz, S. R.; Eiring, R. F.; Durand, S. C.; Wasylyk, J. M.; Parker, W. L.; Liu, M. S.; Okuniewicz, F. J.; Chen, B.-C.; Harris, J. C.; Natalie, K. J.; Ramig, K.; Swaminathan, S.; Rosso, V. W.; Pack, S. K.; Lotz, B. T.; Bernot, P. J.; Rusowicz, A.; Lust, D. A.; Tse, K. S.; Venit, J. J.; Szarka, L. J.; Patel, R. N. *Enzyme Microb. Technol.* **2000**, *26*, 348-358.
32. Takada, H.; Yoshimura, T.; Ohshima, T.; Esaki, N.; Soda, K. *J. Biochem.* **1991**, *109*, 371-376.
33. Schütte, H.; Flossdorf, J.; Sahm, H.; Kula, M-R. *Eur. J. Biochem.* **1976**, *62*, 151-160.
34. Hou, C. T.; Patel, R. N.; Laskin, A. I.; Barnabe, N. *Arch. Biochem. Biophys.* **1982**, *216*, 296-305.
35. Allais, J.J.; Louktibi, A.; Baratti, J. *Agric. Biol. Chem.* **1983**, *47*, 2547-2554.
36. Hanson, R. L.; Goldberg, S. L.; Brzozowski, D. B.; Tully, T. P; Cazzulino, D.; Parker, W. L.; Lyngberg, O. K.; Vu, T. C.; Wong, M. K.; Patel, R.N. *Adv. Syn. Catal.* **2007**, *349*, 1369-1378.
37. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2005**, *48*, 5025-5037.
38. Kataoka, K.; Takada, H.; Yoshimura, T.; Furuyoshi, S.; Esaki, N.; Ohshima, T.; Soda, K. *J. Biochem* **1993**, *114*, 69-75.
39. Kataoka, K.; Takada, H.; Tanizawa, K.; Yoshimura, T.; Esaki, N.; Ohshima, T.; Soda, K. *J. Biochem* **1994**, *116*, 931-936.
40. Seah, S. Y. K.; Britton, K. L.; Rice, D. W.; Asano, Y.; Engel, P. C. *Eur. J. Biochem.* **2003**, *270*, 4628-4634.
41. Busca, P.; Paradisi, F.; Moynihan, E.; Maguire, A. R.; Engel, P. C. *Org. Biomol. Chem.* **2004**, *2*, 2684-2691.

42. Paradisi, F.; Collins, S.; Maguire, A. R.; Engel, P. C. *J. Biotechnol.* **2007**, *128*, 408-411.
43. Chen, S.; Engel, P. C. *Enz. Microb. Technol.* **2007**, *40*, 1407-1411.
44. Vedha-Peters, K.; Gunawardana, M.; Rozzell, J. D.; Novick, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 10923-10929.
45. Hanson, R. L. Bristol-Myers Squibb, unpublished results.
46. Godfrey, J.D.; Fox , R. T.; Buono, F. G.; Gougoutas, J. Z.; Malley, M. F. *J. Org. Chem.,* **2006**, *71*, 8647-8650.
47. Massad, G.; Zhao, H.; Mobley, H. L. T. *J. Bacteriol.* **1995**, *177*, 5878-5883.

Chapter 20

Chemo-Enzymatic Synthesis of Unnatural Amino Acids

Ian V. Archer, S. Alison Arnold, Reuben Carr, Ian G. Fotheringham[*], Robert E. Speight, and Paul P. Taylor

Ingenza Ltd., Wallace Building, Roslin BioCentre, Roslin, Midlothian, EH25 9PP, Scotland, United Kingdom

A general chemo-enzymatic process has been developed to prepare enantiomerically pure L- and D-amino acids in high yield by deracemisation of racemic starting materials. The method has been developed from initial academic studies to be a robust, scalable industrial process. Unnatural amino acids, in high optical purity, are a rapidly growing class of intermediates required for pharmaceuticals, agrochemicals and other fine chemical applications. However, no single method has proven sufficiently adaptable to prepare these compounds generally at large scale. Our approach uses an enantioselective oxidase biocatalyst and a non-selective chemical reducing agent to effect the stereoinversion of one enantiomer and can result in an enantiomeric excess of > 99 % from a starting racemate, and product yields over 90 %. The current approach compares very favourably to resolution methods which have a maximum single pass yield of 50 %. Efficient methods have been developed to adapt the biocatalyst used in this process towards new target compounds and to optimise key factors which improve the process efficiency and offer competitive economics at scale.

Introduction

The market for chiral intermediates is undergoing double-digit annual growth and is expected to reach at least $30 billion by the year 2010. This rapidly increasing demand for these compounds is mainly to support the development of new, single enantiomer pharmaceuticals and agrochemicals. In parallel, the emergence of biocatalysis as a viable approach to manufacture chiral compounds at large scale is also increasing significantly.[i] The high enantio- and regioselectivity of biocatalysts is ideally suited to chiral organic synthesis and the continuing advances of methods in gene isolation and expression, microbial strain engineering, enzyme evolution and bioinformatics are broadening the availability of new biocatalysts for industry.[ii] However, the complexities of biocatalyst production and bioprocess optimisation almost always pose significant hurdles for process implementation, particularly in the rapid time frames required by industry. It is therefore essential to co-develop, along with the appropriate synthetic activities, the enabling technologies required for biocatalyst production, improvement and formulation. It is also highly advantageous if a biocatalytic transformation or process can be readily adapted towards a family of target molecules. Such a "platform" approach reduces the development time for each subsequent application and mitigates against the loss of individual opportunities through the failure of specific products. Ingenza, an Edinburgh, UK based biocatalyst and bioprocess development SME, has addressed each of these key criteria in developing a deracemisation bioprocess platform, which is adaptable to new amino acid targets required at large scale by the pharmaceutical and agrochemical industries. Unnatural amino acids are playing an increasingly significant role in pharmaceutical development.[iii] Ingenza's biocatalytic routes to these compounds now include the water-based deracemisation of racemic mixtures of amino acids by the stereo-inversion of the undesired enantiomer, achieved through the concerted use of an oxidase biocatalyst and a chemical reducing catalyst. This process yields a single enantiomer of the amino acid, in very high optical purity and near quantitative yield.

Background to the Amino Acid Deracemisation Process

The deracemisation process, which is now being commercialised by Ingenza, derives from earlier academic studies in the United States, Japan, and notably in the biological chemistry laboratory of Professor Nicholas Turner at the School of Chemistry at Edinburgh University. The method employs the concerted use of a highly enantioselective amino acid oxidase biocatalyst and a non-enantioselective chemical reducing agent or catalyst (**Figure 1**). The imine,

or in some cases the keto acid, which is generated exclusively from one enantiomer of the target compound by the oxidase, can be converted in equal proportions to both enantiomers by the reductant. The progression of this reaction ultimately results in the near complete depletion of the enantiomer which is a substrate for the oxidase, with a concomitant increase of the opposite enantiomer. The process involves no substrate recycling and results in the stereo-inversion of one enantiomer to yield the desired enantiomer, typically in > 99% e.e. and conversions which can approach 100%. The major advantages of the technology lie in the co-ordinated action of proven industrial oxidase biocatalysts and supported metal catalysts and high-throughput screening methods to identify and adapt suitable oxidase biocatalyst for each new target.
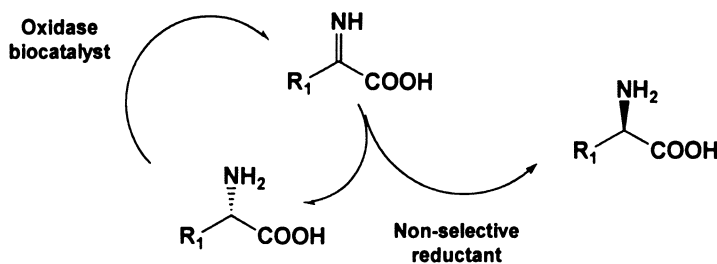
Figure 1. Principle of chemo-enzymatic amino acid deracemisation showing enantioselective oxidation and non-selective reduction, resulting in the stereoinversion of one enantiomer.

Chemo-enzymatic deracemisation of amino acids was first described in 1971 when Hafner and Wellner[iv] reported the production of L-alanine and L-leucine from their corresponding D-enantiomers, through the combined use of D-amino acid oxidase (D-AAO) from porcine kidney and $NaBH_4$. Subsequently Soda et al[v] carried out a more efficient demonstration of the principle, producing L-proline and L-pipecolic acid in > 98% ee by deracemisation of racemic mixtures, again using D-AAO and $NaBH_4$. However, the first attempts to develop a practical deracemisation process were carried out by the Turner group, which carried out a programme of process improvement, particularly by introducing more appropriate reducing agents, achieving a 99% yield and 99% ee of L-proline from DL-proline[vi] using pig kidney DAAO and only three molar equivalents of $NaCNBH_4$. Similar results were observed in the deracemisation of DL-piperazine-2-carboxylic acid, which could be converted to the L-enantiomer in 86% yield and 99% ee using DAAO and $NaCNBH_4$.[vi] There then followed in 1999, a collaboration between the Turner research group and NSC Technologies,

a US fine chemical manufacturer, which further significantly enhanced the versatility, efficiency and economic potential of the deracemisation process. In this collaboration, amine-boranes and catalytic transfer hydrogenation using Pd/C[vii] were introduced to the process, and proved much more effective reducing agents for the deracemisation of cyclic and acyclic amino acids. Amine-boranes, while extremely effective in this chemistry – demonstrating superb activity and selectivity –are unfortunately very expensive. Additionally, the optimal amine-borane (NH_3-BH_3) is not available at the scale required for commercial manufacture. Accordingly, the use of supported metal catalysts in transfer hydrogenation, which was introduced by Dr Scott Laneman of NSC Technologies, has proved to be a particularly versatile and economical approach and provided one basis for the development of the current process. Since 2003, when Ingenza began to develop amino acid deracemisation towards commercialisation, the process development has focused entirely upon the use of catalytic transfer hydrogenation, because of the extensive knowledge base available regarding the industrial use, recovery and recycling of the metal catalysts and the highly favourable large-scale process economics offered by this approach.

A second critical improvement to the performance and versatility of this technology derived from the introduction of engineered microbes to produce the biocatalyst required for the process. Engineered strains of *Escherichia coli* which express enzymes enantioselective for either L- or D-amino acids facilitate the deracemisation of DL-amino acids to yield either D- or L-amino acids as products of the deracemisation process. The initial recombinant bacterial strains, which were constructed by NSC Technologies for this application, did not express an amino acid oxidase enzyme but rather a cloned gene encoding L-amino deaminase (L-AAD).[viii] The L-AAD enzyme originates from *Proteus myxofaciens* and carries out amino acid oxidation by a different catalytic mechanism to that of oxidases, and does not produce detectable levels of hydrogen peroxide. Not producing the hydrogen peroxide by-product is a limitation of this L-AAD with respect to the directed evolution studies described below. However the broad range of L-amino acids which can be converted to their corresponding keto acids by the native L-AAD enzyme nevertheless renders it very appropriate for this industrial application.[viii] The deracemisation of DL-leucine to D-leucine in a 98% yield and 99% ee.[ix] was then demonstrated using recombinant cells which expressed L-AAO and 20 equivalents of ammonia borane as the reductant. The time course of this deracemisation is shown in Figure 2.

Recently a number of D- and L- amino acid oxidase encoding genes, including the D-AAO of *Trigonopsis variabilis* and the L-AAO of *Synechococcus sp.*, have been cloned by Ingenza and expressed in recombinant microbes for application in this general process.
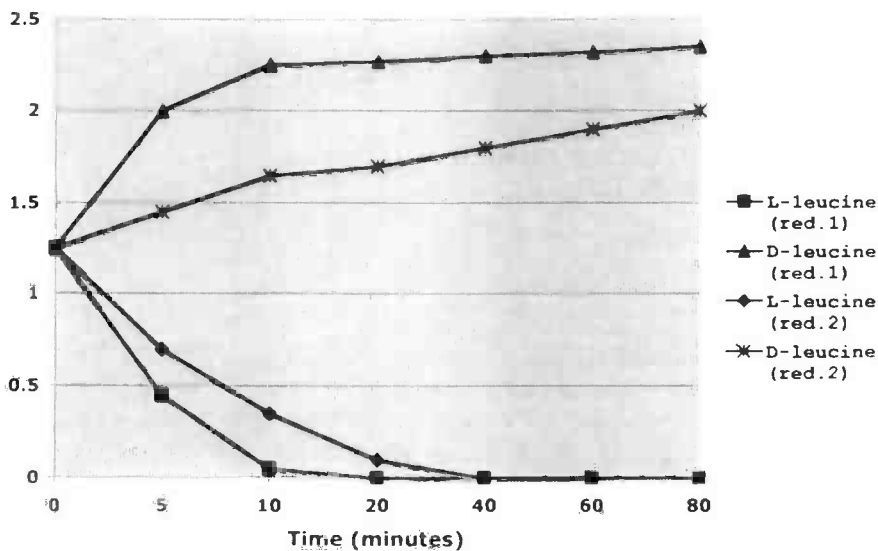
Figure 2. Deracemisation of DL-leucine using L-AAO and 20 equiv. of NH₃-BH₃
(red.1) or t-BuNH₂-BH₃ (red.2)

Production and Laboratory Evolution of Oxidase Biocatalysts

Importantly, the use of cloned genes which encode various oxidases, permits methods of enzyme evolution to be used efficiently to identify mutated variants of the biocatalysts which display improved or adapted properties. Directed evolution methods can be used to generate biocatalyst variants which show activity towards natural and unnatural amino acids and amines that are not good substrates for the native enzymes, thereby enabling the adaptation of the process towards industrial targets. Useful variants may also show improvements in properties suitable for industrial biocatalysts, such as greater stability or resistance to product inhibition under process conditions. The methods used to improve and adapt the oxidase biocatalysts are described below. By coupling random gene mutation with a powerful and very high-throughput *in vitro* and *in situ* selection, highly process suitable amino acid or amine oxidases have been evolved from the wild type enzymes. The high enantioselectivity of the native enzymes is typically retained following laboratory evolution. The screening procedure takes advantage of the fact that members of the oxidase family produce hydrogen peroxide as a reaction by-product. The presence of hydrogen peroxide (and therefore oxidase activity) can be detected colorimetrically by the addition of peroxidase and a substrate that yields a coloured product[x] as shown schematically in Figure 3.
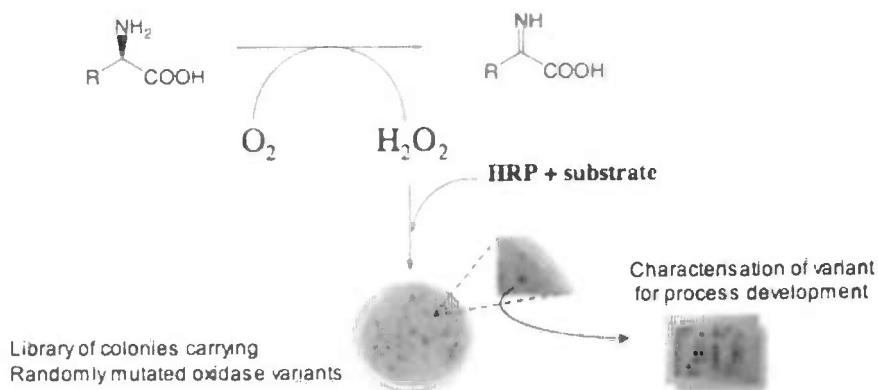
Figure 3. Solid phase colony and liquid phase micro-titre plate colorimetric screening for improved oxidase activity.

This screen can be carried out in a solid phase format using plated bacterial colonies which carry randomly mutated oxidase isolates. Ingenza has now optimised this approach to enable 10^5-10^6 isolates to be rapidly and economically screened for activity directly towards the substrate of interest, in a single experiment. The appearance of darker coloured colonies in the solid phase screen indicates improved activity of an oxidase variant towards a specific target substrate. The activity of the variant towards a range of target substrates can then be characterised kinetically in a micro-titre plate based assay. Increases in activity, substrate range and biocatalyst stability can be achieved by tailoring the assay conditions to the desired trait over multiple cycles of this process.

The laboratory evolution approach was used to alter the substrate range of the amine oxidase from *Aspergillus niger* (MAO-N).[x] In this case, the wild-type enzyme displayed a narrow substrate specificity with activity observable only towards simple achiral amines, such as 1-pentylamine and benzylamine. Libraries of randomly mutated variants of MAO-N were screened for activity towards α-methylbenzylamine. A single residue change in the enzyme (Ser336Asn) was found to significantly expand the substrate specificity of MAO-N so that α-methylbenzylamine and other larger, chiral substrates were accepted. Although α-methylbenzylamine was the substrate used in the screen, the variant displayed higher activity towards other amines, such as α-methylcyclohexylamine. In each case, the variant was found to display high (*S*)-enantioselectivity, enabling its use in deracemisation reactions.[x] Subsequent work has further enhanced and improved the activity of MAO-N variants towards different amines, such as (*S*)-1,2,3,4-tetrahydro-1-methylisoquinoline by incorporating additional mutations, through successive rounds of laboratory evolution.[xi]

In a recent example, Ingenza has improved the activity of *Trigonopsis variabilis* D-AAO (TvDAAO) over one hundred-fold towards a commercially important unnatural amino acid, using ten rounds of laboratory evolution. The evolved variant contains eleven distinct mutations (3.1% of the protein) that were individually shown to be beneficial at each round of evolution. The mutations were selected from randomly generated error-prone PCR libraries, as well as targeted libraries, in which individual amino acids or small groups were selected for saturation mutagenesis. Regions for saturation mutagenesis were rationally selected using sequence alignment and structural homology modelling of closely related oxidases, for which actual resolved structures were available. Additionally, mutated positions in variants selected from error-prone PCR libraries were then targeted for saturation mutagenesis to further optimise the amino acid substitution at these important positions in the protein. The high throughput colony-based colorimetric screen was then used to both improve the activity of the TvDAAO enzyme towards the commercially important unnatural amino acid and also, by adapting the assay conditions, to screen for variants that displayed increased thermal stability. The wild-type TvDAAO enzyme displays a high K_M (> 300 mM) towards the target substrate but in the first round of mutation and selection, two amino acid substitutions were identified that reduced the K_M to < 2 mM. Subsequent rounds of laboratory evolution were then used to improve the turnover rate of the enzyme towards the substrate, whilst retaining the low K_M, by increasing the stringency of the screening step and by using both random and targeted mutation strategies. Despite the activity of the evolved variants towards the substrate being improved to industrially acceptable levels, the operational stability of the enzyme was then found to be low. By exposing the colonies containing the libraries of randomly mutated enzymes to increased temperature (60°C for one hour) prior to colorimetric screening with the substrate, more stable variants could be selected. The increased stability was confirmed by a time course assay of the residual activity of TvDAAO variants following exposure of the protein to various temperatures. A specific variant, named WT1 TvDAAO, was produced that contained only stabilising mutations and therefore retained the substrate specificity of the wild-type enzyme. The stability of this variant was compared to the wild-type enzyme at various temperatures. Figures 4a and 4b compare the residual activity (initial reaction rate at 10 mM substrate concentration, expressed in milli optical density units (mOD) per minute) for the wild-type and variant WT1 TvDAAO enzymes.

Figure 4 demonstrates that whereas the wild-type enzyme is almost completely inactivated after ten minutes at 55.8°C, the WT1 variant retains activity, even at 65.6°C and is significantly more stable at lower temperatures. It was found that the WT1 variant was also more stable to oxidation by either hydrogen peroxide or oxygen gas, indicating a possible common mechanism of denaturation under different sources of stress.

Improvements in the level of oxidase biocatalysts produced by recombinant strains can also be readily detected using this colorimetric screen. Such
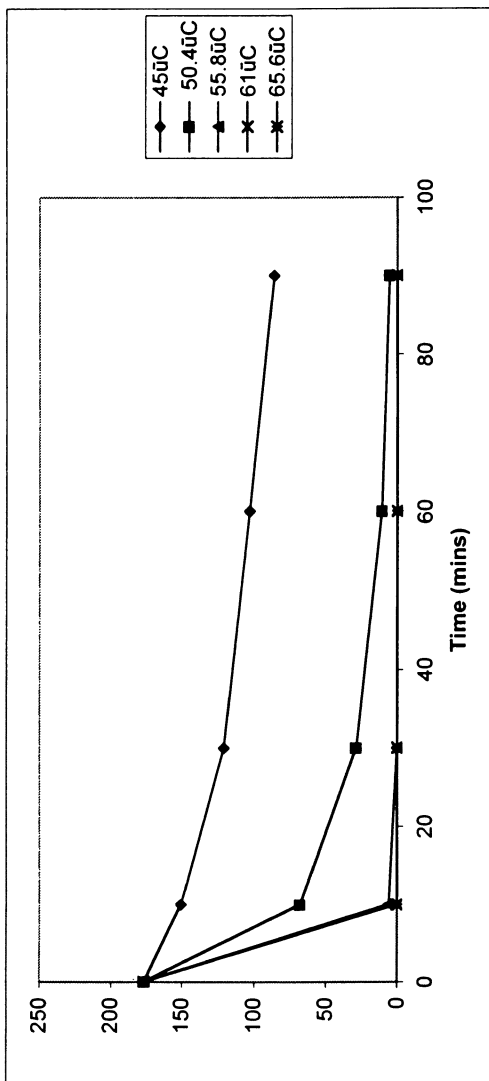
Figure 4a. Wild type Tv. D-AAO heat treatment assay.
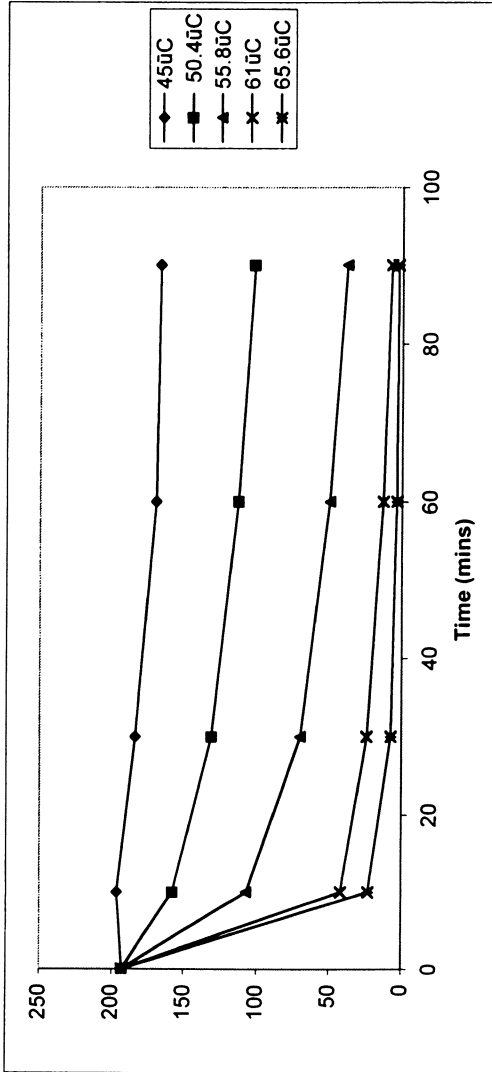
Figure 4b. Variant WT1 Tv. D-AAO heat treatment assay.

improvements are typically due to alterations to regulatory regions which control gene expression or codon changes within the oxidase genes which improve the efficiency of transcription or translational of the gene. Further rounds of directed evolution and the combination of individual beneficial mutations, generates the industrially appropriate biocatalysts required for the deracemisation process.

Enabling Technologies

To fully exploit the potential of any bioprocess, many critical enabling technologies are required to bring the laboratory scale discoveries, such as those outlined above, to full manufacturing scale, in areas of significant market opportunity. This aspect is often understated in the development of new biocatalytic methods. Key enabling technologies include the means to isolate and improve relatively fragile natural enzymes to generate robust industrial biocatalysts; an understanding of the major cost factors in biocatalyst production and formulation; rapid bioprocess optimisation using statistical experimental analysis, and the ability to adapt successful bioprocesses into platform technologies to manufacture multiple targets. Ingenza therefore, has acquired considerable expertise in molecular biology, high-throughput screening, fermentation, bioprocess development and synthetic chemistry for its overall bioprocess development. Each of these areas has been addressed and optimised, to establish an efficient and cost-effective operating philosophy for the deracemisation process.

Fermentation Optimisation

Initial lab scale fermentation of oxidase producing strains was carried out using complex growth medium, chemical inducers and antibiotics to maintain the plasmid borne oxidase genes within the host bacterium. The resulting cost contribution of the biocatalyst to the overall bioprocess is in excess of $100 per kg of amino acid product. This was clearly an unacceptable cost basis, for all but a few very high-value products and so each of these aspects required significant enhancement. A high cell density fed-batch fermentation was developed to culture the recombinant $E.coli$ strains required to produce the native and evolved oxidases. This fermentation resulted in a microbial biomass in excess of 90 gL^{-1} compared to the earlier 5 gL^{-1} . Plasmid vectors were also modified to become completely segregationally stable, thereby eliminating the need for antibiotics to be included in the culture medium. Finally, the induction of enzyme production was regulated by a simple temperature shift during the fermentation, eliminating the need for more costly chemical induction. Therefore when an oxidase biocatalyst is adapted towards a specific substrate of interest, it can then be produced routinely and efficiently under a standard fermentation protocol.

Biomass produced in fermentation is harvested, broken by mechanical lysis and clarified by centrifugation with the assistance of flocculants. The resulting lysate can be used directly in the deracemisation reaction.

Deracemisation Process Optimisation

Successful industrial bioprocesses must meet aggressive cost targets and, for broad acceptance, must be sufficiently robust to enable routine manufacture. Ideally, processes must be compatible with existing equipment and manufacturing practices, while opportunities for process intensification may be sought. In order to establish deracemisation as a competitive manufacturing route for unnatural amino acids, many process conditions required optimisation in addition to the successful biocatalyst production and directed evolution described above. Ingenza has concentrated upon the optimisation of the operating parameters for the process. Principally these parameters included substrate and catalyst loading, reaction conditions such as temperature, pH, aeration and agitation, catalyst formulation (free or immobilised enzyme and reducing catalyst), catalyst recycling, process scale-up (mass-transfer issues) and product recovery and purification. One early example, the deracemisation of L-pipecolic acid, exemplifies this approach.

A detailed screen of catalysts under various process conditions resulted in a pleasing deracemisation reaction yielding L-pipecolic acid in > 99% ee and > 80|% yield. Figure 5a shows a timecourse for this reaction run in triplicate. It is apparent from the timecourse that early in the reaction D-pipecolic acid (D-pip) is consumed at the same rate as the L-enantiomer (L-pip) is formed. However in the latter stages D-pipecolic acid is consumed but not converted to its enantiomer. A detailed examination of the reaction mixture allowed the identification of an impurity (imp) which began to accumulate at around the same time and appeared to account for this loss of yield. An adjustment to the addition of the reductant, ammonium formate, (Figure 5b) allowed us to eliminate both the yield loss and impurity to produce L-pipecolic acid in >99% enantiomeric excess and >95% yield.

In a further example, deracemisation was used to prepare L-2-aminobutyric acid from DL-2-aminobutyric acid as the starting material. Statistical design of the experimental procedures enabled a rapid screen of over 40 supported metal catalysts to be conducted with varying levels of ammonium formate and biocatalyst. These experiments established the ideal metal catalyst for the reaction as well as the appropriate balance of chemical and biocatalysts. At substrate loading of 2M, the L-2-aminobutyric acid can be recovered at > 99 % ee in 95 % isolated yield. Through catalyst re-use and efficient reprocessing, the metal catalyst contributes $|< \$10 \text{ kg}^{-1}$ to the product cost. It is worthwhile comparing the cost contribution of metal catalysis ($< \$10 \text{ kg}^{-1}$) with that of the amine-boranes used in the earlier deracemisation process, which would contribute $>> \$1,000 \text{ kg}^{-1}$ of product.
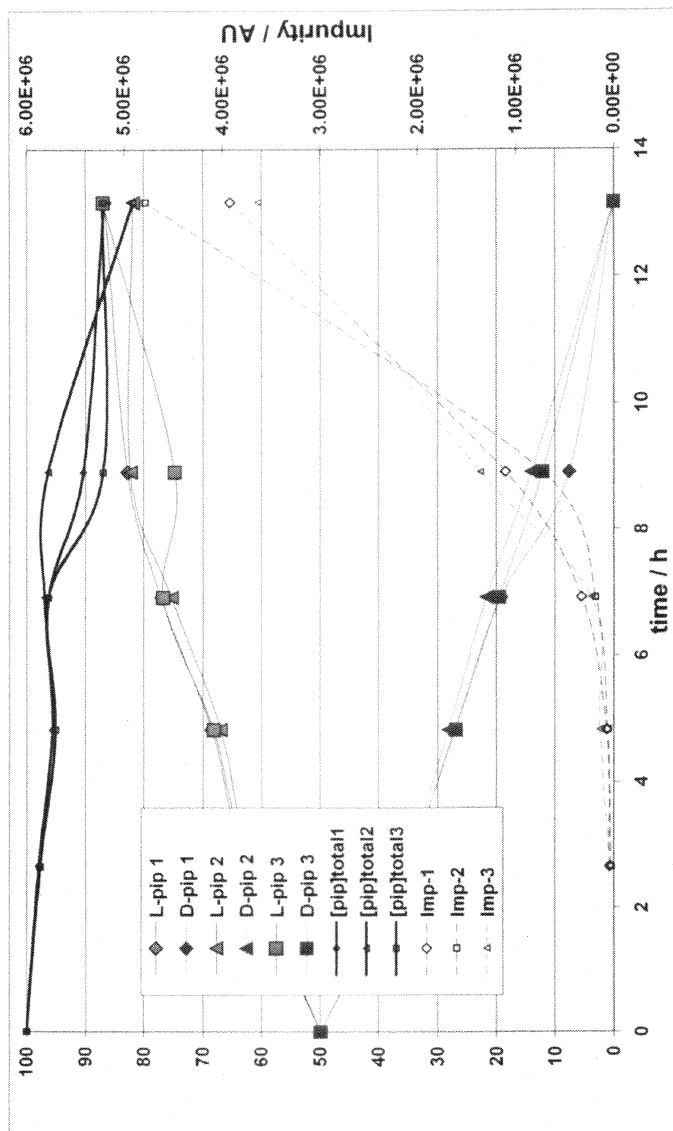
Figure 5a. Deracemisation of DL-pipecolic acid using immobilised wild-type D-AAO and Pd-catalysed transfer hydrogenation
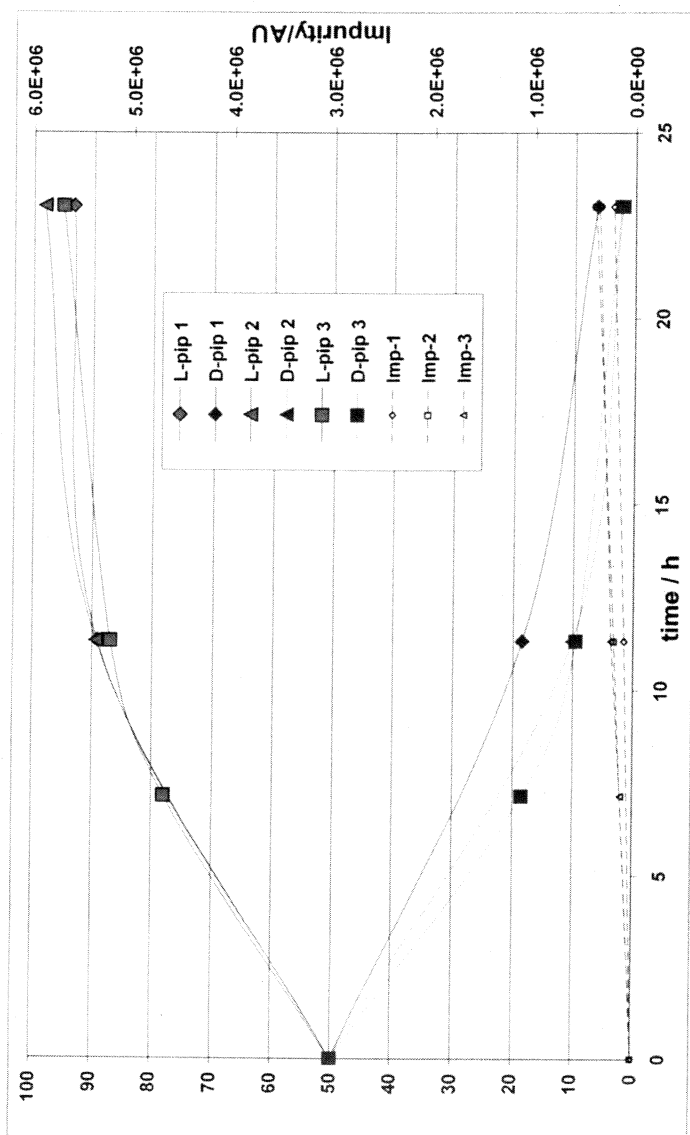
Figure 5b Deracemisation of DL-pipecolic acid using immobilised wild-type D-AAO and Pd-catalysed transfer hydrogenation, with adjusted reductant feed.

Scale-up and Future Prospects for Oxidase Based Chemo-enzymatic Deracemisation

Ingenza currently operates amino acid deracemisation routinely at 1-3 L litre scale in its Roslin facility. At this scale, the process parameters can be fully optimised for subsequent operation at multi-thousand litre scale, using a combination of the enabling technologies and process optimisation strategies described above. Ingenza along with its parent organisation, Chicago based, Richmond Chemical Corporation, is conducting a multi-hundred kilogram pilot scale manufacturing trial of the first commercial product of the deracemisation process, in preparation for full scale operation of the process in 2009. The process conditions being used for this compound are expected to be suitable, for a number of additional unnatural amino acids in development.

The generality of the metal catalysts used for the reduction step of the deracemisation process means that the breadth of process application depends on the availability of suitably active and enantioselective oxidase biocatalysts. Accordingly, screening programmes are in progress to identify novel amino acid oxidases from soil and marine microbes. Novel isolates, detected on the basis of colorimetric or specific cell growth based screens are characterised for activity towards a panel of commercial targets.

It has been shown by the laboratory evolution of the MAO-N towards new substrates and the demonstration that the chemo-enzymatic deracemisation process is equally applicable to chiral amines. Chiral amines are also of major industrial value in the fine chemical arena, in view of their significance as pharmaceutical intermediates, resolving agents,[xii] chiral auxiliaries/bases[xiii] and catalysts for asymmetric synthesis.[xiv] Ingenza is currently evolving amine oxidases such as MAO-N towards commercially important chiral amines and is using the process development know-how resulting from amino acid deracemisation to develop amine deracemisation as a parallel platform bioprocess.

Conclusions

Biocatalysis now offers an increasingly realistic option for the manufacture of new chiral molecules at large scale. Regulatory directives from the FDA have increased the number of chiral molecules required by the pharmaceutical industry, for single-isomer pharmaceuticals which offer lower dosage and reduced side effects. Demands for single-isomer agrochemicals are also increasing, due to environmental pressures. In addition to these drivers, the timing for new biocatalytic applications for industry is highly appropriate from a technology standpoint. Advances in molecular biology, bioinformatics and

microbial genomics have enabled access to large amounts of uncharacterised genetic material, which encodes an enormous array of novel enzymes, providing the raw material for new biocatalysts. Methods of enzyme evolution including increasingly diverse screening strategies and efficient gene mutation and recombination, have shortened the timeframe for the improvement of native enzymes. However, a rapid development timeframe is also critical for biocatalysis to compete with more established synthetic chemical methods. Biocatalysis continues to be regarded as a "last resort" when synthetic chemical approaches have failed or are too costly. Accordingly, the development of adaptable platform bioprocesses such as oxidase based chemo-enzymatic deracemisation are vitally important to enhance the acceptance and growth of biocatalysis in industrial applications.

References

i   R.N. Patel, Current Opinion in Biotechnology **2001**, 12, 587-604

ii   S. Panke, M. Held & M. Wubbolts, *Current Opinion in Biotechnology* **2004**, *15*, 272-279

iii S.C. Stinson, *Chem. Eng. News* **2001**, *79*, 79

iv E.W. Hafner & D. Wellner, *Proc. Nat. Acad. Sci. USA* **1971**, *68*, 987-991

v   J.W. Huh, K. Yokoigawa, N. Esaki & K. Soda, *J. Ferment. Bioeng.* **1992**, *74*, 189

vi T. Beard & N.J. Turner, *Chem. Commun.* **2002**, *246*

vii S.A. Laneman, P.P. Taylor, W. Liu & D.J. Ager, US Patent 6,365,380, **2000**

viii D. P. Pantaleone, A.M. Geller, Taylor, P. P. *J. Mol. Cat. B: Enzym.* **2001**, *11*, 795-803;

ix F-R. Alexandre, D.P. Pantaleone, P.P. Taylor, I.G. Fotheringham, D.J. Ager, N.J. Turner. *Tetrahedron Lett* **2002**, 43:707-711.

x   M. Alexeeva, A. Enright, M.J. Dawson, M. Mahmoudian, N.J. Turner. *Angew Chem Int Ed Engl* **2002**, *41*:3309-3312.

xi R. Carr, M. Alexeeva, A. Enright, T. S. C. Eve, M. J. Dawson and N. J. Turner. *Angew. Chem. Int. Ed.,* **2003**, *42*, 4807-4810.

xii J.W. Nieuwenhuijzen, R.F.P. Grimbergen, C. Koopman, R.M. Kellogg, T.R. Vries, K. Pouwer, E. van Echten, B. Kaptein, L.A. Hulshof & Q. B. Broxterman, *Angew. Chem.* **2002**, *114*, 4457; *Angew. Chem. Int. Ed.* **2002**, *41*, 4281

xiii K.W. Henderson, W. J. Kerr & J.H. Moir, *Chem. Commun.* **2000**, *479*

xiv M.F.A. Adamo, V.K. Aggarwal & M.A. Sage, *J. Am. Chem. Soc.* **2000**, *122*, 8317

Chapter 21

Integrated Solutions of Unnatural α-Amino Acids

Takahiro Ohishi[1], Masahiko Yamada[2], and Masanobu Sugawara[2]

[1]Frontier Materials Development Laboratories, Corporate R&D Division,
Kaneka Corporation, 5–1–1, Torikai-Nishi, Settsu-shi,
Osaka 566–0072, Japan
[2]Fine Chemicals Division, Healthcare Business Unit, Kaneka Corporation,
3–2–4, Nakanoshima, Kita-ku, Osaka 530-8288, Japan

Our approaches to the synthesis of non-natural chiral aliphatic
α-amino acid derivatives are demonstrated. The present
efficient methods involve asymmetric induction, chiral pool,
and dynamic resolution. The integration of our noted expertise
in biotechnology with synthetic technology is a key factor for
the production of useful pharmaceuticals.

Introduction

Recent progress in medicinal chemistry has enhanced the frequent
application of non-natural chiral amino acids for use as drug intermediates (1, 2).
Typically, they have been derived from natural amino acids, and thus have
structures that are similar to, but different from, those of natural L-amino acids.
Traditional synthetic approaches involve the transformation of natural L-amino
acids. While amino acids that contain aromatic groups can be produced
effectively and relatively easily by asymmetric catalysis (3), new aliphatic amino
acids often require a specific methodology.

This report describes our method for producing several non-natural aliphatic
chiral α-amino acids through the use of optimum methods including both bio-
and synthetic technologies, such as asymmetric induction, chiral pool, and
dynamic resolution. The synergistic combination of biotechnology and synthetic
technology will also be demonstrated with regard to α-methylcysteine.
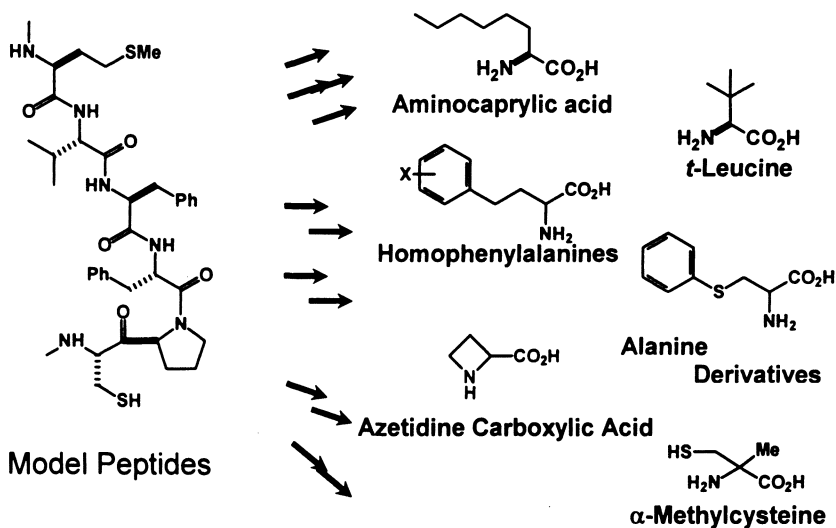
Figure 1. Examples of non-natural chiral amino acids described in this chapter

Specific Solutions

1. Biotechnology: General Methods for the Preparation of L-α-Amino Acids

A generic solution for the synthesis of non-natural L-α-amino acids has been sought for many years. In this section, we introduce a "one pot enzymatic deracemization" system for amino acid production. A previously reported method showed that racemic mixtures of amino acids are converted to the corresponding L-amino acids (4). The mechanism is an oxidation-reduction sequence, where the oxidation of racemic amino acids to α-keto acids or their imino derivatives, as well as subsequent NADH-dependent reductive amination, produced the desired chiral amino acids through the use of multi-enzyme system. Despite its apparent cost-effectiveness, no industrial application of this method has been reported. The crucial issues include not only the difficulty of constructing the enzyme system but also the need for robust coenzyme recycling. Irreversible formate-driven NADH-regeneration shifted the reversible enzymatic reaction to lead to the desired amino acids in the present case (Scheme 1).

A successful NAD recycling system was obtained by using durable formate dehydrogenases (FDH) isolated from specific soils, wherein FDH could survive under high substrate concentrations even in the presence of substrates, especially electrophiles (halogenated hydrocarbons, for example) (5). Thus, FDH allowed the system to produce non-natural L-α-amino acids in high concentrations.
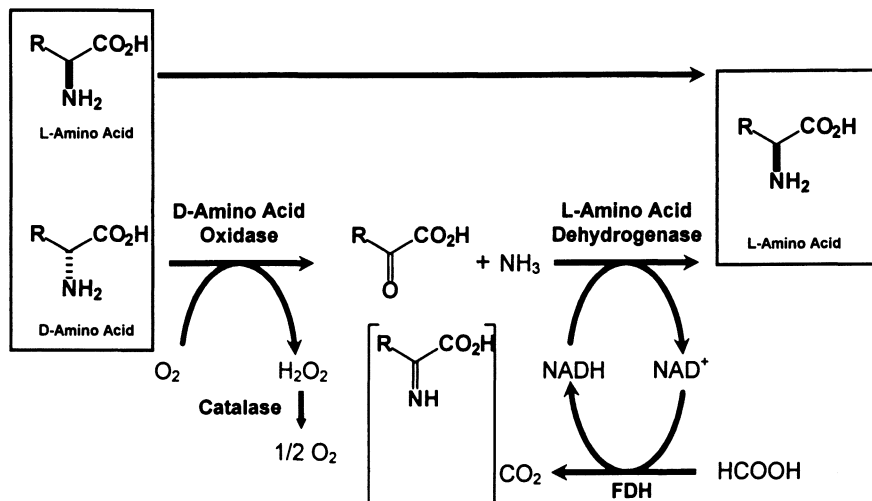
Scheme 1. One-pot enzymatic deracemization

Both aliphatic and aromatic L-α-amino acids can be produced by selecting appropriate dehydrogenases from our libraries, since the stereoselectivities of dehydrogenase marketedly depend on their nature: phenylalanine dehydrogenases gives a chiral aromatic amino acid series, whereas leucine dehydrogenase gives a chiral aliphatic amino acid series. The combination of a reductase library and coenzyme regeneration has already been discussed in a previous report (6).

With the present system taken into account, we are now producing natural L-α-amino acids commercially. α-Keto acids can also be used as the key substrates to this end (7).

Other important remaining issues are the recent increasing demands for the productions of chiral cyclic imino acids and chiral quaternary amino acids, which could not be obtained by the enzymatic method mentioned above. The following sections discuss the scope and limitations of these methods.

Synthetic Technology

1. Asymmetric induction: synthesis of chiral homophenylalanines

Optically active homophenylalanines, a chemical class of one-carbon-longer amino acids, are key constituents for a variety of pharmaceuticals such as classical angiotensin converting enzyme (ACE) inhibitors and neuronal transmitters (8-12).

Despite of several previous reports, synthetic methods for both (R)- and (S)-homophenylalanines are quite limited (13-20). We achieved a highly efficient and stereoselective synthesis of (R)- and (S)-homophenylalanines through a stereoinduced aza-Michael addition using chiral phenethylamine as the key step. This simple protocol was successfully applied to the synthesis of enalapril-type ACE inhibitors, but lacked consistent stereoselectivity (12).

Table 1. Aza-Michel-type addition of phenethylamine (PEA) to p-methoxy-benzoylacrylic acid

Entry	Reaction conditions PEA (equiv.)	temp (°C)	Precipitate yield of SS (%)	de of adducts (%)	Filtrate yield of SR (%)
1	(1.0)	30	38	96	30
2	(1.0)	40	61	90	n.d.
3	(1.1)	40	71	97	15
4	(1.1)	50	78	98	n.d.
5	(1.1)	60	90	97	n.d.

The present successful synthesis consists of three steps: 1) Preparation of substituted benzoylacrylic acids using Friedel-Crafts acylation with maleic anhydride (21); 2) Stereoinduced aza-Michael-type addition using both commercially available (R)- and (S)-phenethylamines; and 3) One-pot reductions of both keto and phenethyl groups using H_2/Pd-C. This overall reaction sequence is considered to be of general use for providing substituted homophenylalanines due to the availability of (R)- and (S)-phenethylamines.

Our initial trial of the key aza-Michael addition using 4-aryl-4-oxo-2-butenoates (benzoylacrylic esters) resulted in poor stereoselectivity, consistent with previous reports (22, 23). We anticipated that the zwitterionic character of the resultant adducts would exhibit the different solubilities between the two diastereomers.

Table 1 lists the successful results for the present aza-Michael addition between p-methoxybenzoylacrylic acid and (S)-phenethylamine in ethanol solvent. Most of the (S,S)-diastereomer precipitated out from the ethanol solution at 30 °C for 16 h after the addition of (S)-phenethylamine, as we

expected (Entry 1). The yields of the (S,S)- and (S,R)- adducts, the latter of which dissolved in ethanol, were 38% and 30%, respectively, which indicates that there was no significant stereoselectivity in this case.

Supposed that Michael addition is a reversible reaction, the yield of the desired (S,S)-adduct would gradually increase through disproportionation due to this addition-elimination sequence. Eventually, optimization of the temperature and the molar ratio gave remarkable results; 90% yield and 97% de (Entry 5). A slight excess (0.1 eq.) of (S)-phenethylamine should act as a basic catalyst to promote the present reversal reaction.

The de of the (S,S)- diastereomer in the whole system gradually increased to >80% in accordance with the duration of the reaction, as illustrated in Fig. 1. The phenomenon of increasing de is based on dynamic resolution: preferential precipitation of the (S,S)-isomer and epimerization of the α-position proceeded smoothly through the reversal aza-Michael addition (24).
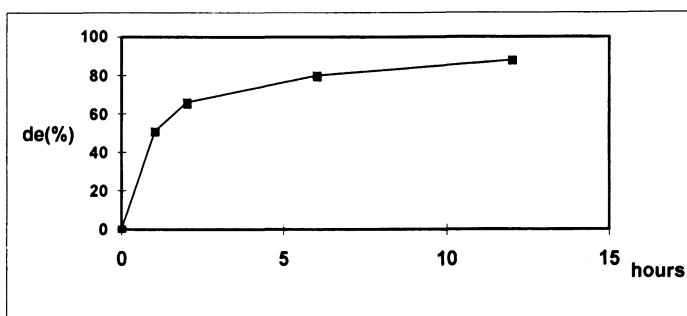
Figure 2. Time profile of aza-Michael addition (PEA: 1.2eq; 60 °C)

To the best of our knowledge, this is the first example of the aza-Michael addition for the preparation of chiral amino acids (25). The reaction of other substituted benzoylacrylic acids as well as non-substituted benzoylacrylic acid, also gave products with high optical purity (90-99%de), as shown in Table 2, depending on the appropriate solvents (ethanol and methanol).

The final stage for the synthesis of chiral homoarylalanines invloves double hydrogenations. Scheme 2 exemplifies the conversion of p-methoxyben-zoylacrylic acid-derived (S,S)-adduct to S-homo-p-methoxy-phenylalanine: deoxygenation of the ketone group and removal of the 1-methyl-p-methoxy-benzyl group proceeded smoothly in a one-pot manner while maintaining the newly created stereochemistry.

In conclusion, we established a practical three-step synthesis for substituted optically active homophenylalanines from simple aromatic compounds (26).

Table 2. Aza-Micheal addition using substituted benzoylacrylic acids

Entry (%)	X=	Solvent	de (%)	Yield	Entry	X=	Solvent	de (%)	yield (%)
					5	p-Cl	EtOH	99	85
1	p-OMe	EtOH	98	90	6	p-NO$_2$	EtOH	95	85
2	p,m-(OMe)$_2$	MeOH	95	80	7	p-Me	EtOH	97	95
3	p-H	MeOH	94	80	8	p-Ph	MeOH	99	87
4	p-F	MeOH	90	70					

2. Chiral pool: Chloroalanine and azetidine carboxylic acid

a) β-Chloroalanine

β-Substituted alanines are now widely used in many receptor modulators and protease inhibitors because of the potential accessibility to a variety of amino acid derivatives (27-29). Several methods for the conversion of L-serine to such precursors have been attempted (Scheme 3). However, the most straightforward route starting from protected L-serine through its sulfonate was not successful due to substantial racemization (30). Another important intermediate, L-serine lactone was a candidate for further functionalization of the β-position with a retention of chirality: this preparation requires expensive and/or toxic DMAD (dimethyl acetylenedicarboxylate) reagent and low-temperature conditions (31).

Our alternative method uses another atom-economical activated serine, L-β-chloroalanine. Since the only conventional method reported to date requires four long, tedious steps (32), route minimization was essential. Surprisingly, without any protection procedure, L-β-chloroalanine was directly and quantatively produced from L-serine quantitatively using halogenation chlorination reagents (33, Scheme 4).

The L-β-chloroalanine thus obtained has been shown to be an efficient chiral synthon for producing a variety of non-natural amino acids. Nucleophilic substitution of thiolate anions to L-β-chloroalanine proceeded smoothly to give a series of S-alkyl or S-aryl-L-cysteine derivatives without any undesirable racemization (33). In addition, protected aziridine derivatives, derived from L-β-chloroalanines, can accommodate carbanion nucleophiles such as acetylides in carbon-elongation reactions.

These substituted compounds were smoothly converted to the corresponding aliphatic amino acids. Since the generality of catalytic asymmetric synthesis is still considerably limited, the present method provides a new and useful avenue for the production of various less-accessible chiral aliphatic amino acids (34, Scheme 5).
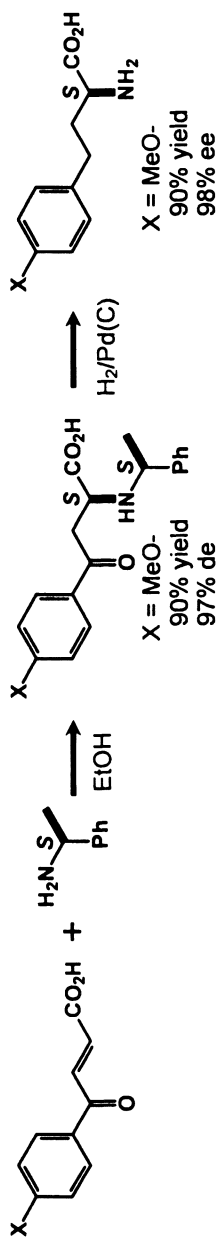
Scheme 2. Synthesis of (S)-homo-p-methoxyphenylalanine through asymmetric aza-Michael addition.

344
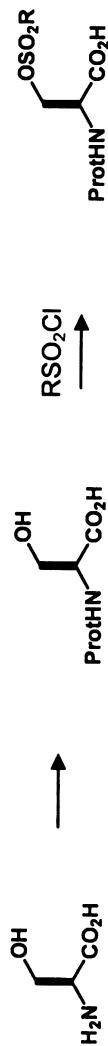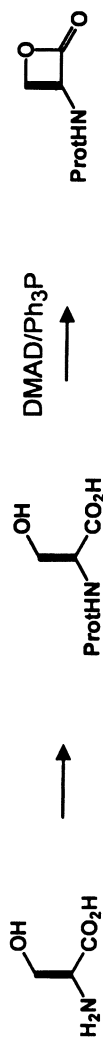
Scheme 3. *Reported methods for the preparation of activated L-serines*

"Direct Chlorination" in Kaneka

Reference: Conventional Chlorination
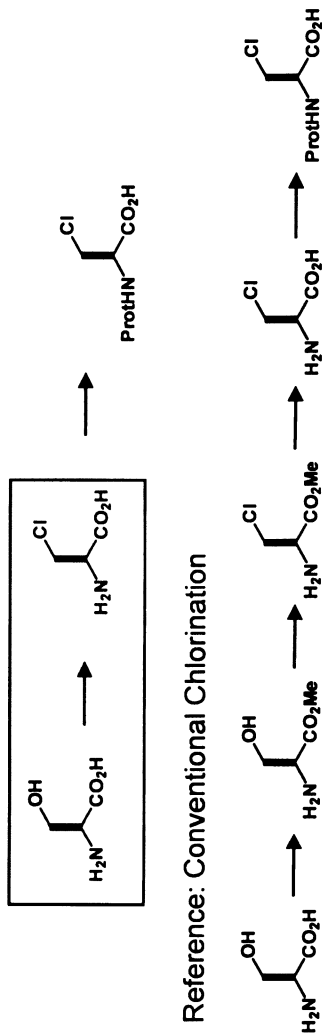
Scheme 4. Comparison of direct chlorination with reported methods

346

Sulfur Nucleophiles

Carbon Nucleophiles
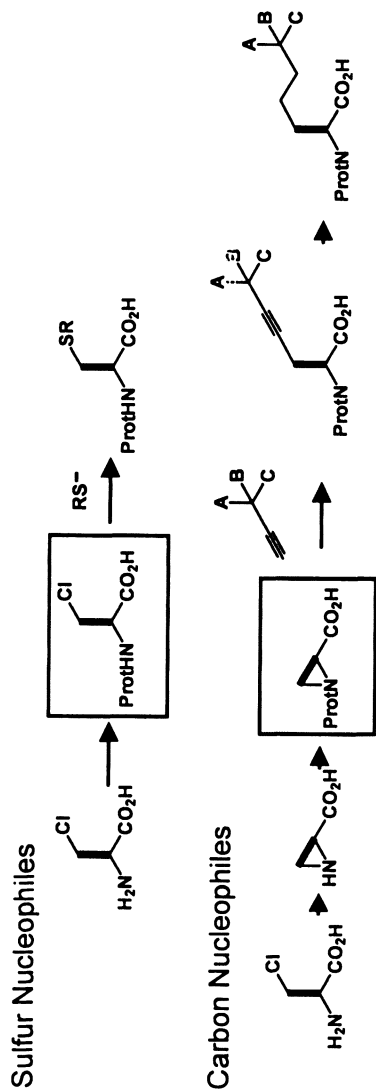
Scheme 5. Application of chloroalanine to nucleophilic substitution reactions
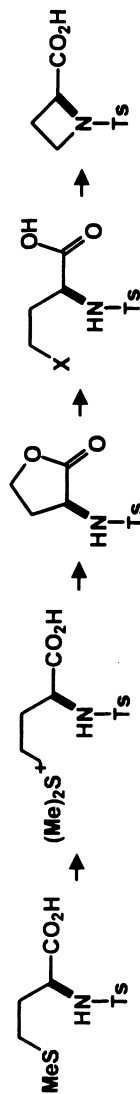
Scheme 6. A typical procedure for preparing azetidine carboxylic acid

b) Azetidine Carboxylic Acid

Azetidine carboxylic acid is widely used as a precursor for protease inhibitors and CNS (central nervous system) agents (35-39). Interestingly, it is a natural amino acid that is obtained in S-adenosyl-L-methionine biosynthesis (40).

While there are two reported methods, there are still some limitations from the standpoint of process chemistry: one method involves tedious and unsafe liquid ammonia-sodium deprotection of toluenesulfonamide (41) and the other requires inefficient optical resolution (42) (Scheme 6).

The pharmaceutical demands and a lack of practical method prompted us to investigate new methodologies. The first example involves a route starting from L-aspartic acid, wherein the whole synthesis requires inexpensive materials including L-aspartic acid (42, 43). The need for a large amount of reducing agents led us to develop an even better process. In the second valid method, (R)-2-chlorobutyrolactam was used as the key intermediate, and is readily obtained from commercially available raw material, such as (S)-2-hydroxybutyrolactam or methyl (R)-aminochlorobutyrate (44-46).

We are now improving the total costs, including route screening, based on the methods described above (Scheme 7).

3. Combination: α-Methylcysteine

α-Methylcysteine is a valuable building block for the synthesis of several natural products such as mirabazoles A-C, tantazoles A-F, thiangazole, etc. (47-55). Several members of these unique thiazoline/thiazole-containing alkaloid families have been shown to exhibit unusually high inhibitory activities against HIV-1 protease *in vitro* (56).

Since it is difficult to construct tetra-substituted carbon in an optically pure form, there have been few examples of α-methylcysteine. The first reported synthesis of an optically pure α-methylcysteine derivative used L-valine as a chiral auxiliary, and alanine was converted at the α-carbon through asymmetric thiomethylation (57). More recently, Singh *et al.* reported a method that involved asymmetric methylation to 2-phenylthiazoline using Oppolzer's camphorsultam as a chiral auxiliary (58).

Two other methods have used Seebach's "self-reproduction of chirality" approach to stereoselectively thiomethylate oxazolidinone 5 derived from alanine (49) or to methylate a thiazoline derivative of cysteine 6 (59, 60). These strategies give the products with good optical purities while asymmetric alkylation requires expensive reagents such as butyl lithium and must be carried out under extremely low-temperature conditions (−78 °C). In sum, the application of these methods to industrial processes for the mass production of optically pure α-methylcysteine might not yet be economically practical.

"Org Process R&D" Method - 1
An Aspartic Acid Route

L-Asp →
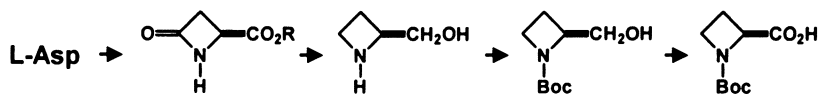
"Org Process R&D" Method - 2
An Aminochlorobutanoic Acid Route
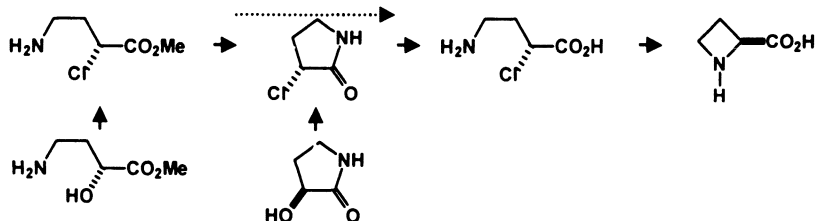
Scheme 7. Two methods for the synthesis of azetidine carboxylic acids

Completely different approaches have also been reported. One method uses the regioselective ring-opening of chiral aziridine **7** (61) or β-lactone **8** (62, 63) with a thiolate nucleophile. Kedrowski synthesized α-methylcysteine from dimethyl 2-methylmalonate *via* enzymatic resolution of the diester **9** followed by Curtius rearrangement (64). These methods involve some difficult-to-secure and/or hazardous reagents for industrial-scale production, such as azide compounds, Sharpless catalyst, PLE, etc.

Asano *et al.* reported the L-stereoselective hydrolysis of racemic α-methylcysteinamide by amidase from *Xanthobacter flavus* NR303 strain (65). After hydrolysis, L-α-methylcysteine was treated with acetone and isolated as (*R*)-2,2,4-trimethylthiazoline-4-carboxylic acid. However, as far as we know, no applications have been reported.

In this section, we describe our method for the synthesis of optically pure α-methylcysteine.

Hydantoinase process

The enzymatic production of optically pure D-amino acids from 5-substituted hydantoins, called the hydantoinase process, is one of the most practical, scalable, and environmentally benign methods. We have pioneered the industrial production of D-amino acids using the hydantoinase process. This process was first introduced for the production of D-*p*-hydroxyphenylglycine (D-
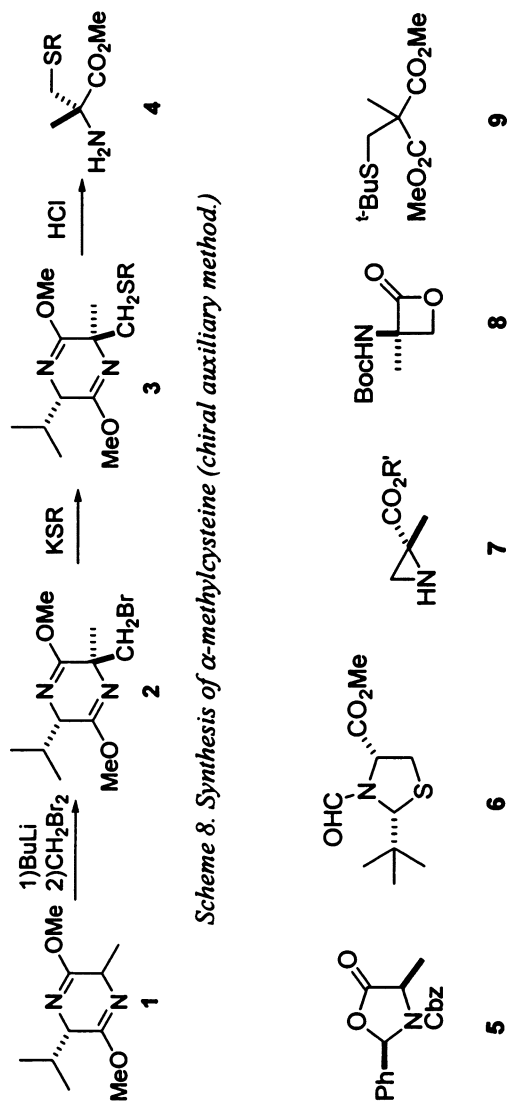
Scheme 8. Synthesis of α-methylcysteine (chiral auxiliary method.)

Figure 3. Precursors for α-methylcysteine

HPG) (6, 66-68), a non-natural amino acid that is used as a side chain of semisynthetic β-lactam antibiotics. We tried to extend our hydantoinase technology to the synthesis of optically pure α-methylcysteine and established two practical processes.

D-Selective hydrolysis of Hydantoin

Hydantoinase generally catalyzes enantioselective hydrolysis of hydantoin derivatives (the normal reaction). The hydrolysis of thio-substituted hydantoine (Scheme 9, Y=SH, SBn, St-Bu) was hamperd because the low solubility of the substrates in an aqueous media made the reaction slow.

The D-selective hydrolysis of chloro-substituted hydantoin (Scheme 9, Y=Cl) has been reported to proceed smoothly (69). The higher solubility of the substrate compared to thio-substituted hydantoins in an aqueous solution probably plays an important role in the stereoselective reaction. Therefore, we thus treated chloro-substituted hydantoin with an immobilized hydantoinase prepared from *Bacillus sp.* KNK245 strain according to the immobilization-culture in a patent application (70) to obtain L-5-chloromethyl-5-methyl hydantoin in 40.5% yield (97%ee). A thio group was then introduced by using various kinds of thio-nucleophiles, such as NaSH, BnSH, and t-BuSH, in moderate yields. Finally, L-α-methylcysteine was produced by the appropriate deprotection reaction of the thio-substituted hydantoins in good yields.
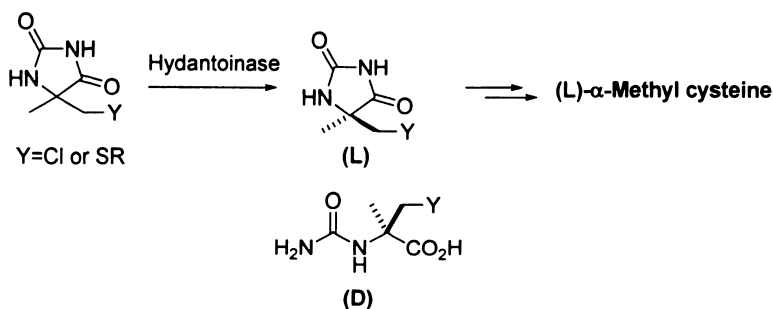
Scheme 9. D-Selective hydroxysis of hydantoin

D-Selective cyclization of *N*-carbamoyl amino acids

Inspired by a former application of the enantioselective reverse reaction (71), we examined the hydantoinase reverse reaction (72): the fact that *N*-carbamoyl-D, L-α-methylcysteine derivatives show good solubility in aqueous NaOH was also encouraging.

Hydantoin **11** was easily prepared from chloroacetone **10** by a previously described procedure (73). This compound was then hydrolyzed by aqueous NaOH and *N*-carbamoylated with KCNO to give *N*-carbamoyl-D, L -α-methylcysteine derivative **12**, where the sequence was carried out in one pot.

The next step is the enzymatic resolution of **12** with hydantoinase. To our surprise, the reverse reaction of **12** catalyzed by hydantoinase gave L-**12** and D-**11** with excellent selectivities (D-**11** : 97.8%ee, L-**12**: 99.5%ee) and yields (D-**11**: 43.9%; L-**12**: 49.6%). Compound **12** was treated with the immobilized hydantoinase in an aqueous solution, stirred at 40 °C, and kept at pH 6.5. The difference in solubility between **12** and **11** gave our process an advantage for scale-up. As the reaction proceeded, D-**12** appeared as a white precipitate and could be easily removed from L-**15** by filtration. The filtrate was acidified to give L-**12** as white crystals. We prepared multigram quantities of L-**12** and D-**11** to demonstrate the potential of our method for large-scale production (72).

Both of the t-butyl protected amino acid isomers were obtained. L-**12** was dissolved in aqueous LiOH solution and refluxed for 41hr. After being cooled to room temperature, the insoluble substance was filtered off and the filtrate was neutralized to give L-**13** as white crystals (75.5%, 99.8%ee). D-**13** was obtained by a similar procedure to afford D-**13** as white crystals (85.7%, 99.9%ee). The optical purities of D-**11** and L-**12** were increased by crystallization of D-**13** and L-**13**.
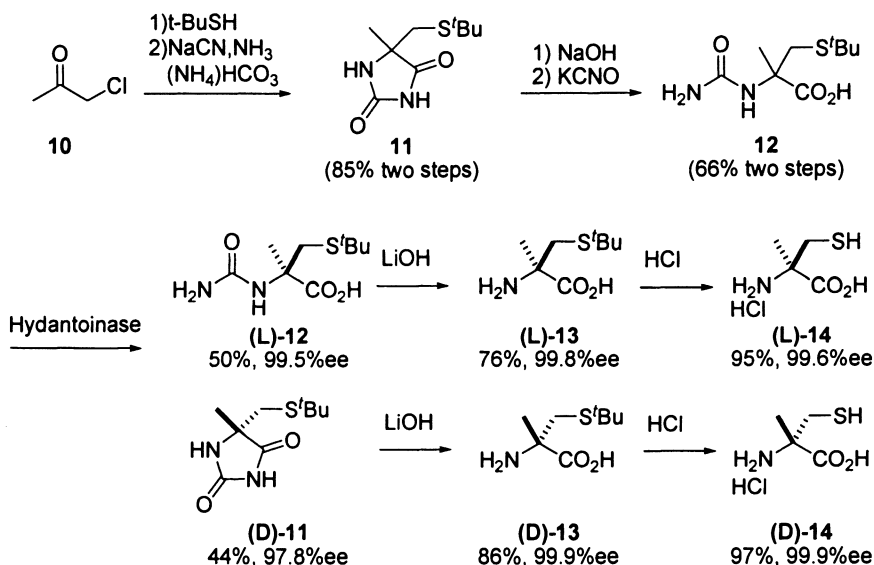
Scheme 10. D-Selective cyclization and synthesis of α-methylcysteine

352

Finally, the t-butyl group was cleaved from **13** to give **14**. Generally, alkyl thioethers show difficult deprotection and only a few methods are known for *S*-t-butyl-cysteine derivatives. Yajima *et al.* reported that the combination of a hard acid (TMSOTf (74, 75) or TMSBr (76)) and a soft nucleophile such as thioanisole can cleave the t-butyl group of cysteine derivatives. However, none of the methods described above are practical for production on an industrial level because they used expensive and/or hazardous reagents. We investigated many reagents and conditions for deprotection of the t-butyl group of **13** and found that the t-butyl group could be efficiently cleaved simply by heating with conc. HCl (77).

Thus, **13** was dissolved in conc. HCl, and the resultant solution was refluxed for 45 hr. After the reaction mixture was cooled to room temperature and concentrated, the remaining water was removed by azeotropic distillation with toluene to give **14** as white crystals. The optical purity of L-**14** was 99.6%ee, and that of D-**14** was 99.9%ee (78).

In summary, two efficient methods for the asymmetric synthesis of α-methylcysteine from chloroacetone, a commercially available and inexpensive compound, were successfully achieved with excellent optical purities and in overall good yields. In these new methods which use a hydantoinase process, no expensive and/or difficult-to-secure reagents were used, and all steps proceeded under mild conditions. In addition, no chromatographic purification was required. Hence, we believe that both of our methods are suitable for large-scale production.

Conclusion

Since there has recently been a greater diversity in aliphatic non-natural amino acids in the pharmaceutical field, access to a variety of technologies is even more necessary to fulfill the demands. Two indispensable aspects, continuous fundamental research and the availability of tool-box technologies to investigate specific amino acids, should be crucial for the production of non-natural chiral amino acids. Given the technology that some organizations have at their disposal, such as biotechnology or the synthetic technology typically described here, almost all of the issues regarding these exotic amino acids may eventually be addressed by combining these technologies or selecting the best technologies. We believe that Kaneka will maintain its prominence as a company that can provide such integrated solutions.

Acknowledgement

described here is strongly due to our superb management personnel, including Dr. Takehisa Ohashi, Dr. Junzo Hasegawa, Mr. Kazunori Kan, Dr. Yasuyoshi Ueda, Dr. Kenji Inoue, Mr. Noboru Ueyama, and Dr. Satomi Takahashi. We are also indebted to many dedicated biochemists and synthetic chemists, essentially those cited in the Reference sections.

References

1. Izawa, K.; Onishi, T. *Chem. Rev.* **2006**, *206*, 2811.
2. Leung, N.; Abbenante, G; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
3. Burk, M. J. *J. Am. Chem. Soc.* **1991**, *113*, 8518.
4. Nakajima, N.; Esaki, N; Soda, K. *J. Chem. Soc. Chem. Commun.* **1990**, *13*, 947.
5. Nanba, H.; Takaoka, Y.; Hasegawa, J. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2145.
6. Nanba, H.; Yosohara, Y.; Hasegawa, J.; Takahashi, S. *Org. Process Res. Dev.* **2007**, *11*, 503.
7. Thayer, A. *Chem. Eng. News* Aug 14, **2006**, 29.
8. Kim, T. -S.; Hague, A. B.; Lee, T. I.; Lian, B.; Tegley, C. M.; Wang, X.; Burgess, T. L.; Qian, Y-X.; Ross, S.; Tagari, P.; Lin, C-H.; Mayeda, C.; Dao, J.; Jordan, S.; Mohr, C.; Cheetham, J.; Viswanadhan, V.; Tasker, A. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 87.
9. McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Bromme, D. *Nat. Struc. Biol.* **1997**, *4*, 105.
10. Sahoo, S. P.; Caldwell, C. G.; Chapman, K. T.; Surette, P. L.; Esser, C. L.; Kopka, I. E.; Polo, S. A.; Sperow, K. M.; Niedwiechki, L. M.; Izquierdo-Martin, M.; Chang, B. C.; Harrison, R. K.; Stein, R. L.; MacCoss, M.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2441.
11. Urbach, H.; Henning, R. *Tetrahedron Lett.* **1984**, *25*, 1143.
12. Teetz, V.; Rolf, G. *European Patent EP* 79022.
13. Draper, R. W.; Donald Hou, D.; Iyer, R.; Lee, G. M.; Liang, J. T.; Mas, J. L; Eugene J.; Vater, E. J. *Org. Process Res. Dev.* **1998**, *2*, 186.
14. Ehrlich, P. P.; Jeffrey, W. R.; Michaelides, R. M. *J. Org. Chem.* **1997**, *62*, 2782.
15. Petatis, N. A.; Zavialov, I. A. *J. Am. Chem. Soc.* **1997**, *119*, 445.
16. Yato, M.; Homma, K; Ishida, A. *Heterocycles* **1995**, *41*, 17.
17. Cecchi, R.; Croci, T.; Bogegrain, R.; Boveri, S.; Baroni, M.; Boccardi, G.; Guimbard, J. L.; Guzzi, U. *Eur. J. Med. Chem.* **1994**, *29*, 259.
18. Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiarz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511.
19. Norlander, J. E.; Payne, M. J.; Njoroge, F. G.; Vishwanath, G. R. H.; Laikos, G. D.; Balk, M. A. *J. Org. Chem.* **1985**, *50*, 3619.

20. Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. *J. Org. Chem.* **1992**, *57*, 3397.
21. Berliner, E. *Organic Reactions;* John Wiley & Sons, Inc.: New York, NY, 1949; Vol 5, pp229-289.
22. Shustov, G. V.; Rauk, A. *Tetrahedron Asymmetry* **1996**, *7*, 699.
23. Davies, S. G.; Ichihara, O. *Tetrahedron Asymmetry* **1991**, *2*, 183.
24. Yamada, M.; Nagashima, N.; Hasegawa, J.; Takahashi, S. *Tetrahedron Lett.* **1998**, *39*, 9019.
25. Anderson, N. G. *Org. Process Res. Dev.* **2005**, *9*, 800.
26. Stinson, S. *Chem. Eng. News* Jan 18, **1999**, 69.
27. Seko, T.; Kato, M.; Kohno, H.; Ono, S.; Hashimura, K.; Takimizu, H.; Nakai, K.; Maegawa, H.; Katsube, N.; Masaaki Toda, M. *Bioorg. Med. Chem.* **2003**, *11*, 1901.
28. Li, J.; Thurairatnam, S.; Aldous, D. J. *World Patent WO* 2003/42197.
29. Samunen, J.; Ali, F.; Romoff, T.; Calvo, R.; Sorenson, E.; Vasko, J.; Storer, B.; Berry, D.; Bennett, D.; Strohsacker, M.; Powers, D.; Stadel, J.; Nichols, A. *J. Med. Chem.* **1991**, *34*, 3114.
30. Sasaki, N. A.; Hashimoto, C.; Potier, P. *Tetrahedron Lett.* **1987**, *28*, 6069.
31. *Org. Synthesis* **1992**, *70*, 1.
32. Plattner, A.; Boller, A.; Frick, H.; Fürst, A.; Hegedüs, B.; Kirchensteiner, H.; Majnoni, St.; Schläpfer, R.; Spiegelberg, H. *Helv. Chim. Acta* **1957**, *40*, 1531.
33. Yamashita, K.; Inoue, K.; Kinoshita, K.; Ueda, Y.; Murao, H. *World Patent WO* 1999/33785.
34. Sugawara, M.; Fujii, A.; Okuro K.; Saka, Y.; Nagashima, N.; Inoue, K.; Takeda, T.; Kinoshita K.; Moroshima, T.; Fuse, Y.; Ueda, Y. *World Patent WO* 2001/60795.
35. Tang, W.; Zhang, X. *Chem. Rev.* **2003**, *103*, 3029.
36. Morrissette, M. M. M.; Stauffer, K.; Williams, P. D.; Lyle, T. A.; Vacca, J. P.; Krueger, J. A.; S. Dale Lewis, D. S.; Lucas, B. J.; Wong, B. K.; White, R. B.; Miller-Stein, C.; Lyle, E. A.; Wallace, A. A.; Leonard, Y. M.; Welsh, D. C.; Lynch, J. J.; Daniel R.; McMasters, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4161.
37. Patel, D. V.; Trias, J ; Odowd, H.; Raju, B. G.; Gao, H. *World Patent WO* 2004/07444.
38. Zhihua, S.; Mark, M.; Lanter, J. C. *World Patent WO* 2003/29247.
39. Lin, N. -H. Yun, H.; Mark, H.; W.; Ryther, K.; Yihong, L.; Hao, B. *World Patent WO* 1997/46554.
40. Shimizu, S.; Yamada, H. *Trends Biotechnol.* **1984**, *2*, 137.
41. Miyoshi, M.; Sugano, H.; Fujii, T.; Ishihara, T; Yoneda, N. *Chem. Lett.* **1973**, 5.
42. Turner, K. *Org. Process Res. Dev.* **2001**, *5*, 100.
43. Awaji, H.; Matsumoto, S.; Inoue, K.; Matsuo, K. *World Patent WO* 1998/47867.

44. Turner, K. *Org. Process Res. Dev.* **2005**, *9*, 244.
45. Honda, T.; Nagashima, N. *World Patent WO* 2000/69817.
46. Kondo, T.; Ueyama, N. *World Patent WO* 2003/14081.
47. Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron Lett.* **1991**, *32*, 2593.
48. Akaji, K.; Kuriyama, N.; Kiso, Y. Aka *J. Org. Chem.* **1996**, *61*, 3350.
49. Walker, M. A.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 5566.
50. Parsons, R. L.; Heathcock, C. H. *Tetrahedron Lett.* **1994**, *35*, 1383.
51. Parsons, R. L.; Heathcock, C. H. *Tetrahedron Lett.* **1994**, *35*, 1379.
52. Carmeli, S.; Paik, S.; Moore, R. E.; Patterson, G. M. L.; Yoshida, W. Y. *Tetrahedron Lett.* **1993**, *34*, 6681.
53. Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Corbett, T. H.; Valoriote, F. A. *J. Am. Chem. Soc.* **1990**, *112*, 8195.
54. Jansen, R.; Kunze, B.; Reichenbach, H.; Jurkiewicz, E.; Hunsmann, G.; Höfle, G. *Liebigs Ann. Chem.* **1992**, 357.
55. Jansen, R.; Schomburg, D.; Höfle, G. *Liebigs Ann. Chem.* **1993**, 701.
56. Jurkiewicz, E.; Jansen, R.; Kunze, B.; Trowitzsch-Kienast, W.; Forche, E.; Reichenbach, H.; Holfe, G.; Hunsmann, G. *Antiviral Chem. Chemother.* **1992**, *3*, 189.
57. Groth, U.; Schöllkopf, U. *Synthesis* **1983**, *1*, 37.
58. Singh, S.; Rao, S. J.; Pennington, M. W. *J. Org. Chem.* **2004**, *69*, 4551.
59. Pattenden, G.; Thom, S. M.; Jones, M. F. *Tetrahedron* **1993**, *49*, 2131.
60. Mulqueen, G. C.; Pattenden, G.; Whiting, D. A. *Tetrahedron* **1993**, *49*, 5359.
61. Shao, H.; Zhu, Q.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 790.
62. Fukuyama, T.; Xu, L. *J. Am. Chem. Soc.* **1993**, *115*, 8449.
63. Smith, N. D.; Goodman, M. *Org. Lett.* **2003**, *5*, 1035.
64. Kedrowski, B. L. *J. Org. Chem.* **2003**, *68*, 5403.
65. Inoue, A.; Komeda, H.; Asano, Y. *Adv. Synth. Catal.* **2005**, *347*, 1132.
66. Yamada, H.; Takahashi, S.; Kii, Y.; Kumagai, H. *J. Ferment. Technol.* **1978**, *56*, 484
67. Takahashi, S.; Kii, Y.; Kumagai, H.; Yamada, H. *J. Ferment. Technol.* **1978**, *56*, 492
68. Takahashi, S.; Ohashi, T.; Kii, Y.; Kumagai, H.; Yamada, H. *J. Ferment. Technol.* **1979**, 57, 328
69. Ohishi, T.; Nanba H.; Sugawara, M.; Izumida, M.; Honda, T.; Mori, K; Yanagisawa, S; Inoue, K. *World Patent WO* 2004/22766.
70. Nanba, H. Yajima, K.; Takano, M.; Yamada, Y.; Ikenaka, Y.; Takahashi, S. *World Patent WO* 1996/20751.
71. Takahashi, S.; Yamada, Y.; Ueda, Y.; Katayama, Y.; Shimada, Y.; Watanabe, K. *US Patent US* 4812406.
72. Ohishi, T.; Nanba, H.; Sugawara, M.; Izumida, M.; Honda, T.; Mori, K.; Yanagisawa, S.; Ueda, M.; Nagashima, N.; Inoue, K. *Tetrahedron Lett.* **2007**, *48*, 3437.

73. Oh, C-H.; Kang, Y-K.; Park, S-W.; Cho, J-H. *Bull. Korean Chem. Soc.* **1988**, *9*, 231.

74. Yajima, H.; Fujii, N.; Funakoshi, S.; Watanabe, T.; Murayama, E.; Otaka, A. *Tetrahedron* **1988**, *44*, 805.

75. Fujii, N.; Otaka, A.; Ikemura, O.; Hatano, M.; Okamachi, A.; Funakoshi, S.; Sakurai, M.; Shioiri, T.; Yajima, H. *Chem. Pharm. Bull.* **1987**, *35*, 3447.

76. Fujii, N.; Otaka, A.; Sugiyama, N.; Hatano, M.; Yajima, H. *Chem. Pharm. Bull.* **1987**, *35*, 3880.

77. Almstead reported that t-butyl group of thio-bisphosphonate was cleaved simply by heating with conc. HCl. Almstead, N. G. *Phosphorus Sulfur Silicon Relat. Elem.* **1999**, 144-146, 325.

78. The optical purities were determined by the procedure in Ref. 72.

Chapter 22

Enzymatic Synthesis of Unnatural Amino Acids

Mari Hara Yasuda[1,*], Makoto Ueda[1], Kazuya Okano[2], Hisaaki Mihara[3], and Nobuyoshi Esaki[3]

[1]Mitsubishi Chemical Group Science and Technology Research Center, Inc., 1000 Kamoshida-cho, Aoba-ku, Yokohama 227–8502, Japan
[2]API Corporation, 2–3–4 Nihonbashi, Chuo-ku, Tokyo 103–0027, Japan
[3]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611–0011, Japan

In this paper, novel enzymatic syntheses of unnatural amino acids are described. The first to be described is a new enzymatic system for the synthesis of optically pure N-methyl-L-amino acids from α-keto acids. The second is the production of cyclic amino acids from the corresponding diamino acids. In both these systems, the novel enzyme, N-methyl-L-amino acid dehydrogenase from *Pseudomonas putida* ATCC12633 was crucial. The final synthesis described is a biocatalytic asymmetric reduction via dynamic kinetic resolution for the production of β-hydroxy-D-amino acids. This reaction system is applicable to a wide range of substrates and all of the products exhibit high enantio- and diastereomeric purities. The enzyme in this system is a short-chain dehydrogenase that is used for chiral alcohol production. In this study, substrate design was an important step.

Amino acids and unnatural (nonproteinogenic) amino acids have been used in many pharmaceutical products (*1–3*). Recently, compounds with a peptidic structure have been developed by many pharmaceutical companies. In order to increase activity, proteolytic stability, or membrane permeability, unnatural amino acids are used (*2*).

The chemical and biochemical processes involved in the synthesis of unnatural amino acids have been studied by many groups (*4–6*). In general, biological processes have advantages over chemical processes such as high enantiomeric purity, mild conditions, and non-toxic reagents.

We have developed many enzymatic processes for the synthesis of pharmaceutical intermediates. In this paper, novel enzymatic syntheses of unnatural amino acids are described.

1. *N*-methyl-L-amino acids (NMA)

Optically active *N*-methyl-L-amino acids (NMA) occur as functionally critical components of several biologically active natural peptides such as dolastatins (*7*) and didemnins (*8*). These peptides have been studied because they inhibit certain aspects of viral proliferation, tumor cell growth, and proliferative immune responses (*7, 8*). Therefore, NMA are useful building blocks in the chiral-specific synthesis of medicines and pesticides.

Many methods for the preparation of optically active NMA have been reported and a review article on synthetic preparation of the NMA has been published (*9*). First type is nucleophilic displacement of α-halocarboxylic acid by methylamine. Fischer *et al.* prepared *N*-methylalanine, *N*-methylleucine, and *N*-methylphenylalanine by the nucleophilic displacement of bromide by methylamine from optically active (*R*)-α-bromo acids [Figure 1 (1)] (*10*). Second type is *N*-methylation using reductive amination. Quitt *et al.* reported the production of *N*-methyl amino acids via *N*-benzyl derivatives [Figure 1 (2)] (*11*). The sequence involved conversion of optically active amino acids into their benzyl derivatives followed by methylation and hydrogenolysis. Recently, Silva *et al.* reported a reductive methylation method using aqueous formaldehyde and zinc [Figure 1 (3)] (*12*). Dorow *et al.* reported reductive alkylation of scalemic azide [Figure 1 (4)] (*13*). Reddy *et al.* reported a new synthetic method that involves the reductive cleavage of *N*-protected oxazolidinones using hydrogen in the presence of Pd/C [Figure 1 (5)] (*14*). However, each procedure has its drawbacks, which include the excessive number of steps required to prepare the substrate, problems of racemization, harsh reaction conditions, and the instability of acid-sensitive substrates. Thus, currently, there is no satisfactory method available for the industrial production of optically pure NMA under mild conditions.

Although enzymes that synthesize *N*-methyl-L-alanine (*15*) and *N*-methyl-L-glutamic acid (*16*) have been reported, none have been utilized for the modification of amino acids with other *N*-alkyl groups. The ability to synthesize a variety of optically pure *N*-alkyl-L-amino acids from a common intermediate would be useful in the development of pharmaceutical agents.

Recently, we identified a gene encoding a novel *N*-methyl-L-amino acid dehydrogenase (NMAADH, EC 1.5.1.21, GeneBank accession no. AB190215) in *Pseudomonas putida* ATCC12633 and succeeded in the functional expression of the enzyme in host *Escherichia coli* BL21 (DE3) cells (*17*). The enzyme converts a α-keto acid and methylamine into the corresponding NMA in an NADPH-dependent manner. We also established an NADPH regeneration system in an enzyme-coupled system employing glucose dehydrogenase (GDH) from *Bacillus subtilis*, and reported the synthesis of optically active *N*-methyl-L-phenylalanine from phenylpyruvic acid and methylamine (*18*). Using this system, *N*-methyl-L-alanine is also produced and the productivity is sufficiently good to enable commercial production. Table 1 shows the substrate specificity of NMAADH. This result indicates NMAADH has a wide range of substrate specificity, so that various N-methylated unnatural amino acids can be produced by this system. In situations where α-keto acids are not available, we established a process starting from amino acids (Scheme 1). We combined D-amino acids oxidase and NMAADH and produced *N*-methyl-L-methionine from D-methionine.

2. Cyclic amino acids (Cyclic imino acids) (CAA)

Cyclic amino acids (CAA) are also components of many drugs. Thioproline is the starting material for synthesis of the antagonists of α4β1 integrin (*19*). L-Pipecolic acid is a component of several immunosuppressants, such as Tacrolimus (*20*).

Several methods for the chemical preparation of optically active CAA have been reported. Ring closure by the alkylation of amino group of chiral amino acid is a feasible method to synthesize CAA. Ohtani *et al.* reported photocatalytic synthesis of pipecolic acid from *N*, ω-substituted α,ω-diamino carboxylic acids by intramolecular reductive amination (*21*) [Figure 2 (1)]. Shiraiwa *et al.* reported 1, 4-thiazane-3-carboxylic acid synthesis from cysteine by intramolecular nucleophilic substitution (*22*) (Figure2 (2)). Ring expansion of chiral aziridine is also useful method. Kogami *et al.* reported 3-morpholinecarboxylic acid was synthesized by nucleophilic ring opening of aziridine and following ring closing reaction (*23*) (Figure2 (3)). The combination of asymmetric alkylation of chiral auxiliary derived from glycine and following ring closing reaction is also useful as the general method for the preparation of
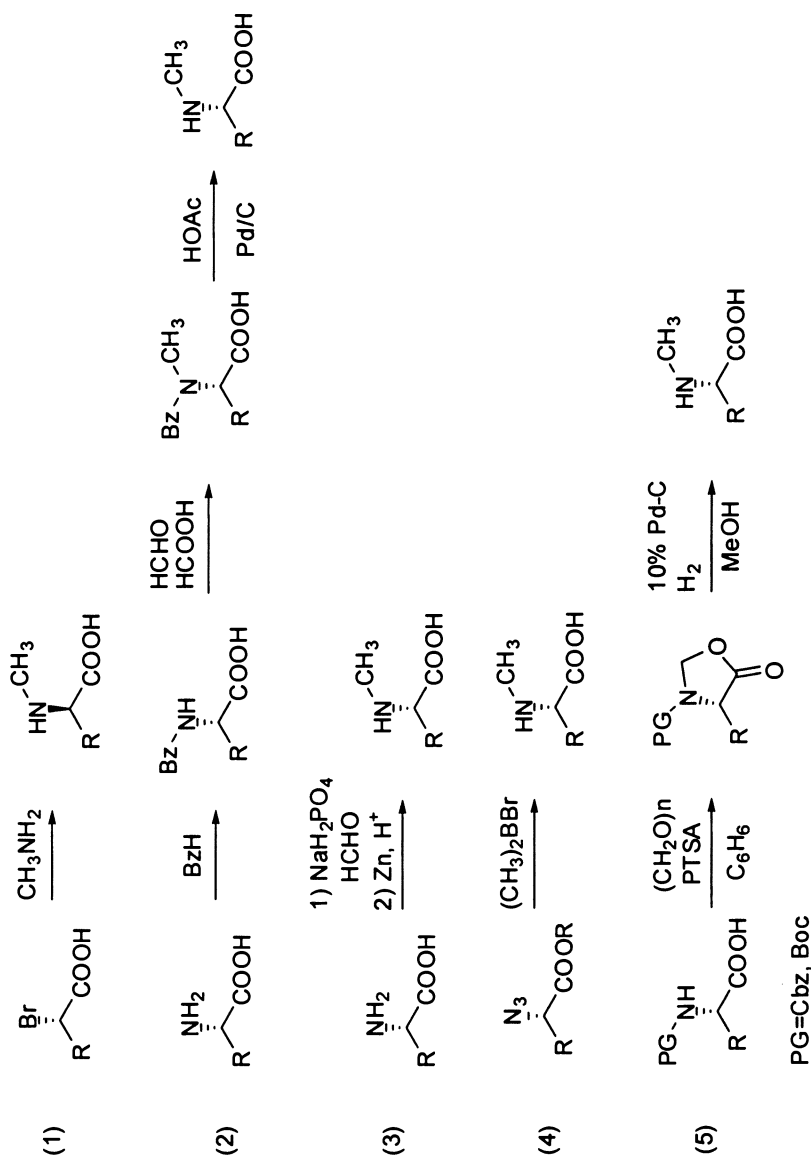
Figure 1. *Various preparation methods for N-methyl amino acids*

Table 1. Substrate specificity of NMAADH

R	Relative activity (%)
CH_3	100
$C_6H_5CH_2$	30
$CH_3CH(CH_3)CH_2$	9
$(CH_3)_2CH$	0
$CH_3CH_2CH(CH_3)$	0
CH_3CH_2	29
$CH_3(CH_2)_2$	16
$CH_3(CH_2)_3$	52
$CH_3(CH_2)_4$	7
$HOOCCH_2$	94
$HOCH_2$	6
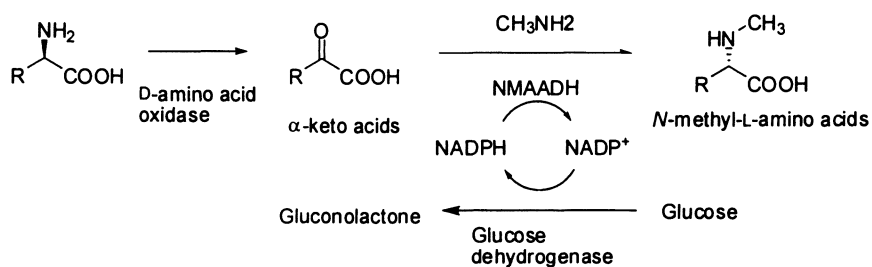
Scheme 1. Enzymatic preparation of NMA from D-amino acids
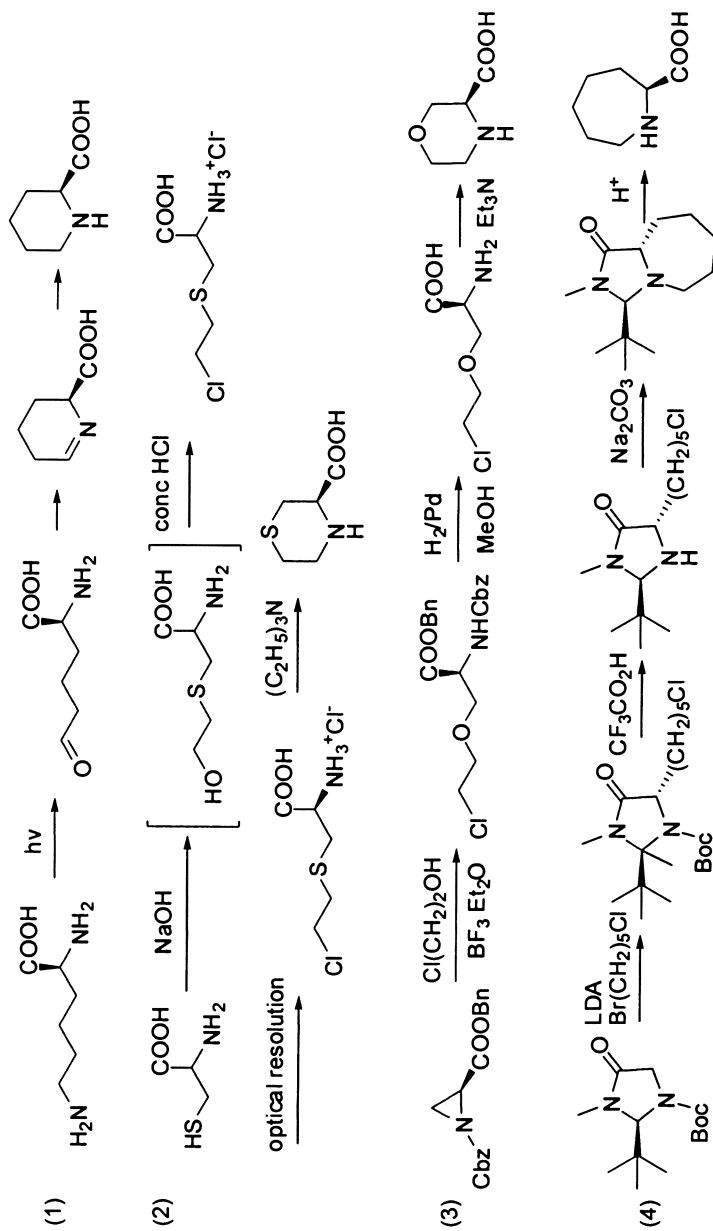
Figure 2. Reported preparation methods of cyclic amino acids

CAA. Seebach *et al.* reported the synthesis of azepane-2-carboxylic acid from *tert*-butyl 2-(*tert*-butyl)-3-methyl-4-oxo-1-imidazoline-carboxylate *(24)* (Figure2 (4)).

Enzymatic preparation has also been reported. The conversion of ornithine into L-proline by ornithine cyclase in *Clostridium* PA 3679 *(25)*, the preparation of L-pipecolic acid from L-lysine by recombinant *Escherichia coli (26)*, and the production of 1,4-thiazane-3-carboxylic acid, piperazine-2-carboxylic acid, and 5-hydroxypiperidine-2-carboxylic acid by recombinant *Escherichia coli*-expressed ornithine cyclase of *Streptomyces* species *(27)* have been reported. However, these chemical and enzymatic preparations have the disadvantage of requiring chiral compounds as reaction substrates. This is because the enantiomeric purity of the product depends on the purity of the substrate, that is, these enzymes react only with L-form of amino acids.

We established a CAA production method using NMAADH, GDH, and amino acid oxidase *(28)*. In this system (Scheme 2) many types of CAA [(S)-**4a**–**g**] are produced from both enantiomers of α,ω-diamino acids (**1**) or CAA (**4**). Most starting compounds used in this method are commercially available. For the production of 7-membered CAA, L-homolysine (**1d**) and L-aminopropyl-cysteine (**1f**) were prepared from L-lysine and L-cysteine, respectively, according to known methods *(29, 30)*.

α,ω-Diamino acids (**1**) were oxidized to α-keto acids (**2**) by L-amino acid oxidase (LAO) or lysine oxidase (LO), and these were spontaneously converted to a cyclic imine structure (**3**). The cyclic amine was subsequently reduced to CAA by NMAADH.

β-hydroxy-α-amino acids (HAA)

β-Hydroxy-α-amino acids (HAA) are found in natural amino acids such as serine or threonine, and, in recent times, unnatural types of HAA have been used as pharmaceutical intermediates. (2R, 3R)-2-amino-3-hydroxy-3-cyclohexyl-propanoic acid (Figure 3, **5**) is a key component of the CCR5 antagonist ONO-4128 *(31)*. ONO-4128 has unique spirodiketopiperazine structure and shows high binding affinity to CCR5 and exhibit very low toxicity. Since ONO-4128 had received considerable attention as a promising candidate for the development of a new HIV drug, much effort was paid to establish the industrial synthetic process. Especially many types of synthetic routes of **5** had been reported by various groups in the worldwide.

HAA have two adjacent chiral centres; thus, two types of synthetic control are required, diastereoselectivity *(anti)* and enantioselectivity *(R,R)*. Consequently, optical resolution is initially performed (Figure 4). Various technologies, such as simulated moving bed (SMB) chromatographic separation, enzymatic enantioselective ester hydrolysis, and diastereomer salt formation
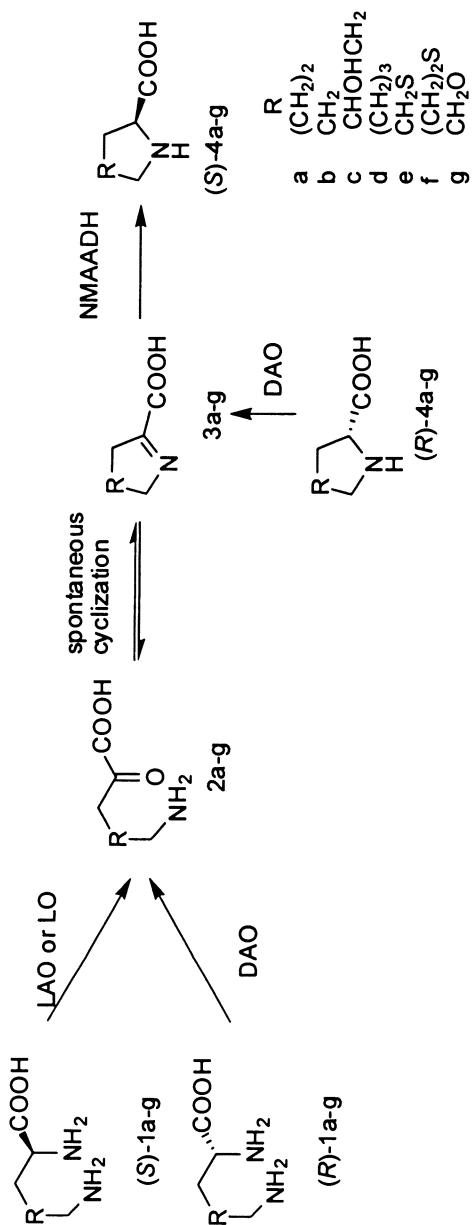
Scheme 2
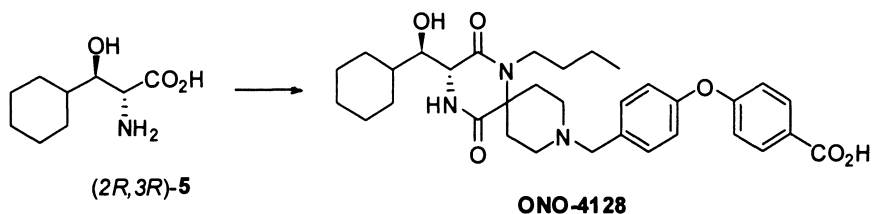
Figure 3. HIV drug, ONO-4128

were studied by Tanaka *et al.* (*32*). In addition, they also looked at diastereomer formation by a ring-opening reaction with a chiral amine via nucleophilic attack and obtained an adduct with (*R*)-phenetylamine (**8**). Both chiral epoxide [(2*S*, 3*R*)-**7**] and **8** can be transformed into (2*R*,3*R*)-**5** by the usual method. Yamamoto prepared (2*R*,3*R*)-**5** (99% ee) by cleaving the unnecessary L-form in the racemic substrate (**5**), which was easily prepared from epoxide (**7**) via short steps using *Pseudomonas putida*-derived L-phenylserine aldolase (*33*).

The asymmetric aldol reaction of an enolate-bearing chiral auxiliary is a well-employed method for the preparation of chiral HAA via short steps. Matsunaga reported the enantioselective aldol reaction of cyclohexylaldehyde using a titanium enolate derived from iminoglycinate (**9**), and succeeded in obtaining the chiral aldol product (**10**), which on subsequent hydrolysis with acid produced ethyl ester (**11**) with a high ee (97.3% ee as the N-Boc form) [Figure 5 (1)] (*34*). Ooi reported the direct asymmetric aldol reaction of a glycinate Schiff base (**12**) with an aldehyde catalyzed by a chiral quaternary ammonium salt [Figure 5 (2)]. *Tert*-Butyl ester (**14**) was obtained in high selectivity (98% ee); however, an excess amount (5 eq) of aldehyde was required to achieve the high conversion (*35*). These chemical methods require an N-protected glycine derivative. In contrast, biocatalytic asymmetric aldol reactions enable the direct aldol reaction between an aldehyde and an unprotected glycine. Recently, Steinreiber reported an improved enzymatic procedure using D-threonine aldolase derived from *Alcaligenes xylosoxidans* [Figure 5 (3)] (*36*). The product was the D-*syn*-form with high enantiopurity but relatively low diastereoselectivity. No example regarding the high enatio- and diastereoselective preparation of **5** using a biocatalytic aldol reaction has been reported.

Nishiyama prepared chiral oxirane (**17**) by the Sharpless oxidation of allyl alcohol (**16**), derived from the olefin **15**, and obtained (2*R*,3*R*)-**5** by subsequent alcohol oxidation and ring-opening reactions (*37*). Riela synthesized chiral diol (**19**) by the Sharpless asymmetric dihydroxylation of an unsaturated ester (**22**) and prepared (2*R*,3*R*)-**5** via the aminolysis of cyclic sulfite derived from **20** (*38*). These "oxidation" strategies have demonstrated their utility in organic synthesis; however, the overall process requires several steps (Figure 6).
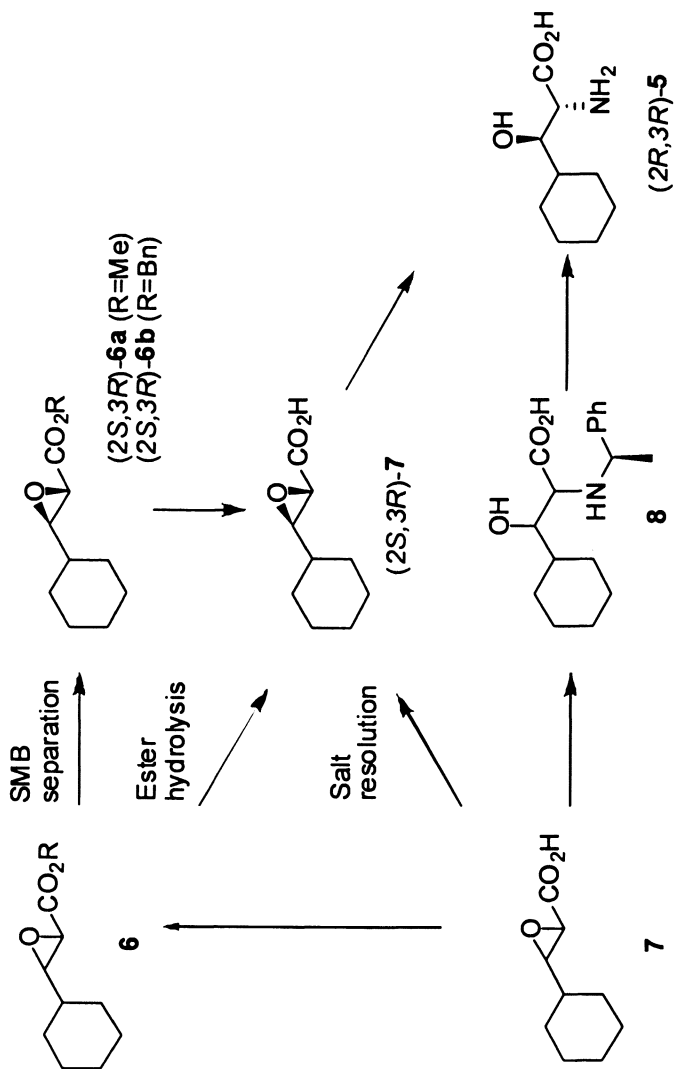
Figure 4. Optical resolution methods to prepare (2R, 3R)-5
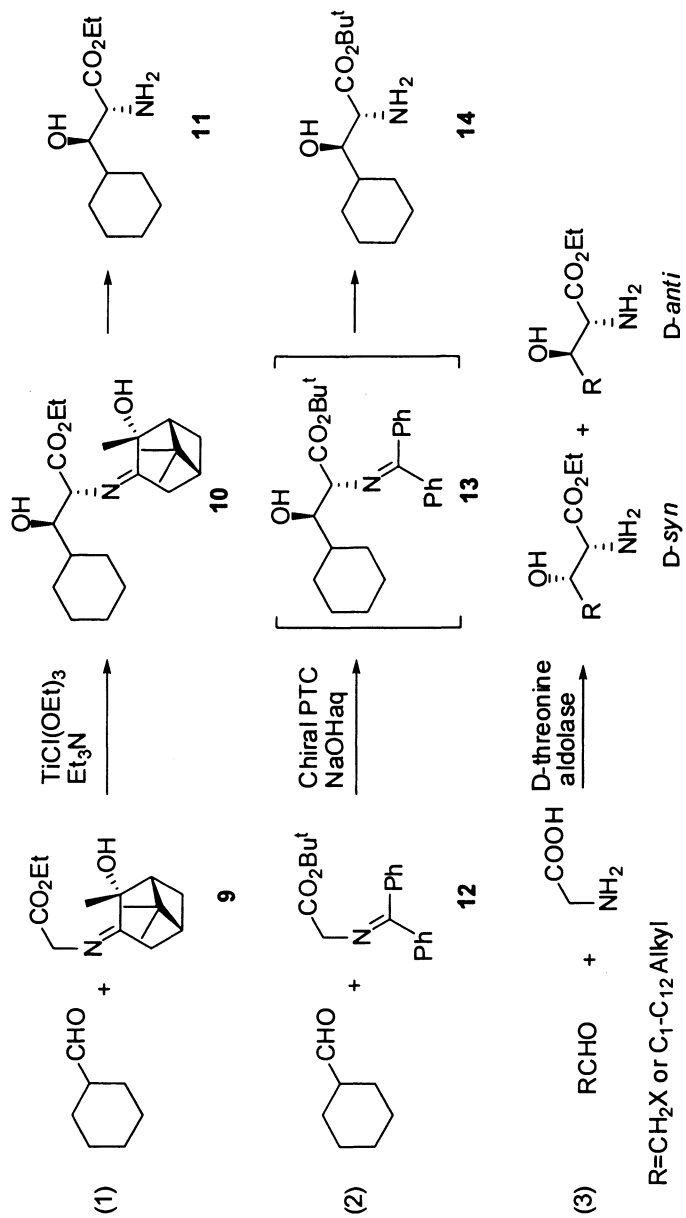
Figure 5. Asymmetric aldol reaction methods to prepare (2R,3R)-5
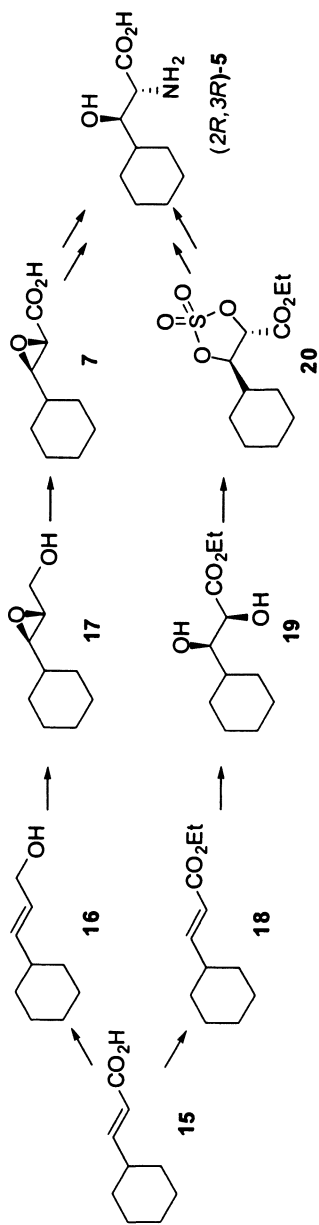
Figure 6. Asymmetric oxidation methods to prepare (2R,3R)-5

Asymmetric reduction via dynamic kinetic resolution (DKR)—the asymmetric reaction accompanying the racemization of the neighboring atom to prochiral carbon—is often used to control two adjacent chiral centers. Noyori studied the asymmetric hydrogenation of N-acyl-α-amino-β-keto (**21a**) esters using a Ru-BINAP catalyst and selectively obtained the *syn* isomer (**22a**) (*39*). In 2004, Makino and Mordant independently reported the highly *anti*-selective DKR of β-keto-α-amino esters (Table 2). They found that the *anti* isomer was obtained selectively by using the hydrochloric salt of β-keto-α-amino esters as the substrate instead of N-acyl-α-amino-β-keto esters. Makino reported that **21b** was hydrogenated and selectively yielded the *anti* form (95% de) using a Ru-BINAP catalyst (*40*). Mordant found that the same selectivity was achieved in the hydrogenation of β-keto-α-amino esters by using the SynPhos ligand (*41*). Furthermore, Katsuura reported that the C3-TunePhos ligand exhibited higher selectivity in the reduction of **21c** (*42*).

Table 2. Asymmetric reduction by chemical catalysts

Compound	R^1	R^2	R^3	Catalyst	yield %	syn : anti	ee %	ref
21a	Me	Ac	Me	(R)-BINAP	100	99 : 1	98	38
21b	cyclohexyl	H	Me	(R)-BINAP	84	2.5 : 97.5	96	39
21c	cyclohexyl	H	Et	(R)-C3-TunePhos	100	1 : 99	99.5	41

Similar to the asymmetric reduction by chemical catalysts, biocatalytic asymmetric reduction accompanying DKR has been reported by several groups. Fadnavis *et al.* reported that Baker's yeast could also reduce N-acyl-α-amino-β-keto esters, but they found that most of the starting material decomposed through the hydrolysis of ester to acid and subsequent decarboxylation to form β-ketoacetoamide (*43*). Therefore, only a small amount (10%) of the corresponding aminoalcohol was obtained. Fadnavis *et al.* also studied the reduction of α-azido-β-keto esters using Baker's yeast and isolated alcohol dehydrogenase (*44*). The major product of the reduction reaction was the D-form of anti-hydroxyamino acids; however, the highest diastereoselectivity was only 92% de (pH 4). When the reaction was carried out at pH 7, no diastereoselectivity was observed.

As a first step in the preparation of (2R,3R)-**5**, we screened biocatalysts and substrates. As biocatalysts, we had previously prepared a "reductase library" that

contains *Escherichia coli* transformants harboring the carbonyl reductase and glucose dehydrogenase genes in a co-expression system.

We synthesized various aminoketone derivatives (Table 3) and subjected these to enzymatic reaction. When the *N*-benzoyl compound (**21f**) was used, the biocatalytic reduction catalyzed by carbonyl reductase from *Exiguobacterium* sp. (*45*) yielded the *anti* form of **22** with a good selectivity; however, a by-product (**23**) was still formed. We estimated that decarboxylation occurred via hydrolysis of the ester by esterases produced by *Escherichia coli*. Therefore, we searched for a substrate that did not cause hydrolysis. Consequently, we found that when *N*-benzoyl isopropyl ester (**21g**) was used, an excellent yield and selectivity were achieved (99% yield, >98% de, >99% ee) (*46*).

Table 3. Asymmetric reduction by biocatalysts

Entry.	R^1	R^2	R^3	Yield %	de %	ee %
21b	cyclohexyl	H	Me	0		
21d	cyclohexyl	Boc	Et	23	90	N.D.
21e	cyclohexyl	Ac	Et	2	26	
21f	cyclohexyl	Bz	Et	53	86	99
21g	cyclohexyl	Bz	iPr	99	>98	>99

After the primary screening of substrates and enzymes, we started the scale-up research for pilot production. The main issues in the bioprocess were the continuous control of decarboxylation, the improvement of the reaction speed, and reducing the cost of NADPH—the cofactor of reductase. We found that ammonium sulfate or sodium sulfate was a good additive to inhibit the esterase reaction. In order to reduce the quantity of NADPH used and also to improve the reaction speed, we have applied the method of directed evolution to the enzyme. The gene that encodes the wild-type enzyme was subjected to random mutagenesis using error prone polymerase chain reaction (*47*). We screened the mutants library and selected best one. After sequencing DNA, the best mutants turned out to have amino acid substitutions at position 88. Then we applied saturation mutagenesis to the point (*48*). We found that the variant F88I, namely phenylalanine at position 88 was exchanged into isoleucine, showed more than 10 times higher activity. Repeating m,utagenesis, finally, we established a novel robust enzyme system that hardly requires NADPH and used it for the pilot production of (*2R,3R*)-**5**.

Our reaction system is applicable to a wide range of substrates. Table 4 shows the purity of the products from various substrates using our system. Surprisingly, our enzyme exhibited high enantio- and diastereoselectivity not only toward aliphatic and aromatic substituents but also toward small and bulky substituents. Thus, we can conclude that biocatalytic asymmetric reduction via DKR is the most versatile method for the production of optically pure HAA.

Table 4. Scope of our system for HAA

R	de %	ee %
methyl	100	98.3
ethyl	100	99.8
isopropyl	100	100
n-propyl	100	99.6
tert-butyl	100	100
decyl	100	100
Phenyl	100	100
pCl-phenyl	100	100
2-thienyl	100	99.7
2-naphtyl	100	100

Summary

In this article, we described the synthetic route of unnatural amino acids. Numerous synthetic methods have been developed according to the demands of pharmaceutical use. We demonstrated the use of a new enzyme that enabled us to establish novel economical synthetic routes. Even with known enzymes, the design of specific substrates for particular enzymes would be a breakthrough in the development of new production routes. The efficient combination of chemical and biological syntheses will provide a wide range of amino acid derivatives in a cost-effective and environmentally friendly manner.

References

1. Wagner, I.; Musso, H. *Angew. Chem. In. Ed. Engl.* **1983**, *22*, 816-828.
2. Rabasseda, X.; Martel, A. M.; Castañer, J. *Drugs Fut.* **1997**, *22*, 371-377.

3. Fairlie, D. P.; Abbenante, G.; Marych, D. R. *Curr. Med. Chem.* **1995**, *2*, 654-686.

4. *Chirality in Industry;* Collins, A. N.; Sheldrake, G. N.; Crosby, J. Eds.; John Wiley & Sons: New York, **1992**.

5. *Chirality in Industry II;* Collins, A. N.; Sheldrake, G. N.; Crosby, J. Eds.; John Wiley & Sons: New York, **1997**.

6. Pantaleone, D. P.; Dukes T. D.; Vogler K. R.; Ton J. L.; Beacom D. R.; McCarthy S. L.; Meincke B. E.; Behrendt L. K.; Senkpeil R. F.; Fotheringham I. G. In *Applied Biocatalysis in Specialty Chemicals and Pharmaceuticals*; Saha, B. C.; Demirjian, D. C. Eds.; ACS Symposium Series; American Chemical Society: Washington, D. C, 2000; Vol. 776, pp 165-175.

7. Rinehart, K. L. Jr.; Gloer, J. B.; Cook, J. C. Jr. *J. Am. Chem. Soc.* **1981**, *103*, 1857-1859.

8. Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, R. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151-9170.

9. Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Chem. Rev.* **2004**, *104*, 5823-5846.

10. Fischer, E.; Mechel, L. V. *Chem. Ber.* **1916**, *49*, 1355-1366

11. Quitt, P.; Hellerbach, J.; Vogler, K. *Helv. Chim. Acta* **1963**, *46*, 327-333

12 da Silva, R. A.; Estevam, I. H. S.; Bieber, L. W. *Tetrahedron Let.* 2007, *48*, 7680-7682.

13. Dorow, R. L.; Gingrich, D. E. *J. Org. Chem.* **1995**, *60*, 4986-4987.

14. Reddy, G. V.; Rao, G. V.; Iyengar, D. S. *Tetrahedron Lett.* **1998**, *39*, 1985-1986.

15. Lin, M. C. M.; Wagner, C. *J. Biol. Chem.* **1975**, *250*, 3746-3751.

16. Shaw, W. V.; Tsai, L.; Stadtman, E. R. *J. Biol. Chem.* **1966**, *241*, 935-945.

17. Mihara, H.; Kakutani, R.; Muramatsu, H.; Yasuda, M.; Ueda, M.; Kurihara, T.; Esaki, N. *FEBS Journal* **2005**, *272*, 1117-1123.

18. Muramatsu, H.; Mihara, H.; Kakutani, R.; Yasuda, M.; Ueda, M.; Kurihara, T.; Esaki, N. *Tetrahedron Asymm.* **2004**, *15*, 2841-2843.

19. Lin, L.S.; Lanza, T. Jr.; McCauley, E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 133-136.

20. Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583-5601.

21. Ohtani, B.; Tsuru, S.; Nishimoto, S.; Kagiya, T.; Izawa, K. *J. Org. Chem.* **1990**, *55*, 5551-5553.

22. Shiraiwa, T.; Tadokoro, K.; Tanaka, H.; Nanba, K.; Yokono, N.; Shibazaki, K.; Kubo, M.; Kurokawa, H. *Biosci. Biotech. Biochem.* **1998**, *62*, 2382-2387.

23. Kogami, Y.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2963-2965.

24. Seebach, D.; Dziadulewicz, E.; Behrendt, L.; Cantoreggi, S.; Fitzi, R. *Liebigs Ann. Chem.* **1989**, 1215-1232.

25. Costilow, R. N.; Laycock, L. *J. Bio. Chem.* **1971**, *246*, 6655-6660.

26. Fujii, T.; Aritoku, Y.; Agematu, H.; Tsunekawa, H. *Biosci. Biotech. Biochem.* **2002**, *66*, 1981-1984.

27. Thibaut, D.; Doring, V.; Marliere, P. **2002**, World Patent no. WO 02/101003.

28. Yasuda, M.; Ueda, M.; Muramatsu, H.; Mihara, H.; Esaki, N. *Tetrahedron Asymm.* **2006**, *17*, 1775-1779.

29. Piper, J. R.; Rose, L. M.; Johnston, T. P.; Grenan, M. M. *J. Med. Chem.* **1979**, *22*, 631-639.

30. Ezekiel, A. D. **1972**, German Patent Appl. no. DE2217895.

31. Takaoka, Y.; Nishizawa, R.; Shibayama, S.; Sagawa, K.; Matsuo, M. **2004**, World Patent no. WO200426873.

32. Tanaka, H.; Nishikawa, K.; Hamasaki, R.; Oda, H.; Okano, T.; Nakajima, T. **2005**, World Patent no. 2005040099.

33. Yamamoto, H.; Nakajima T.; Tanaka, H. **2006**, Japanese Patent Appl. no. 2006006216.

34. Matsunaga, N.; Hisaichi, K.;Takaoka, Y. **2005**, Japanese Patent Appl. no. 2005298337.

35. Ooi, T.; Kameda, M.; Taniguchi, M.; Maruoka, K. *J. Am. Chem. Soc.*, **2004**, *126*, 9685-9694.

36. Steinreiber, J.; Fesko, K.; Mayer, C.; Reisinger, C.; Schurmann, M.; Griengl, F. *Tetrahedron* **2007**, *63*, 8088-8093.

37. Nishiyama, T.; Kobayashi, K.; Takaoka, Y. **2003**, Japanese Patent Appl. no. JP0355358.

38. Alonso, M.; Santacana, F.; Rafecas, L.; Riera, A. *Org. Process Res. Dev.* **2005**, *9*, 690-693.

39. Noyori, R.; Ikeda, T.; Ohkuma, T.; Widhalm, M.; Kitamura, M.; Takaya, H.; Akutagawa, S.; Sayo, N.; Saito, T.; Taketomi, T.; Kumobayashi, H. *J. Am. Chem. Soc.* **1989**, *111*, 9134-9135.

40. Makino, K.; Goto, T.; Hiroki, Y.; Hamada, Y. *Angew. Chem. Int. Ed.* **2004**, *43*, 882-884.

41. Mordant, C.; Dünkelmann, P.; Ratovelomanana-Vidal, V.; Genet, J. P. *Chem. Commun.*, **2004**, 1296-1297.

42. Washio, N.; Hirao, S.; Katsuura, A. **2006**, Japanese Patent Appl. no. JP06206569.

43. Fadnavis, N. W.; Vadivel, S. K.; Bhalerao, U. T. *Tetrahedron Asymm.* **1997**, *8*, 2355-2359.

44 Fadnavis, N. W.; Vadivel, S. K.; Sharfuddin, M.; Bhalerao, U. T. *Tetrahedron Asymm.* **1997**, *8*, 4003-4006.

45. Wada, M.; Yoshizumi, A.; Furukawa, Y.; Kawabata, H.; Ueda, M.; Takagi, H.; Nakamori, S. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1481-1488.

46. Hara, M.; Hiraoka, H.; Ueda, M.; Sasaki, T. **2005**, Japanese Patent Appl. no. 2005-185819
47. (a) Leung, D. W.; Chen, E.; Goeddel, D. V. *Technique (Philadelphia)* **1989**, *1*, 11-15. (b) Eckert, K. A.; Kunkel, T. A. *PCR Meth. Appl.* **1991**, *1*, 17-24. (c) Cadwell, R. C.; Joyce, G. F. *PCR Meth. Appl.* **1994**, *3*, S136-S140.
48. (a) Kammann, M.; Laufs, J.; Schell, J.; Gronenborn, B. Nucleic Acids Res. 1989, 17, 5404. (b) Reetz, M. T.; Jaeger, K.-E. *Top. Curr. Chem.* **1999**, *200*, 31-57.

Chapter 23

Production of Amino Acids Using Wild Type and Recombinant Whole Cell Catalysts: Using Platform Technologies for Enhancing Production Efficiency

S. Verseck, U. Becker, K. Doderer, S. Oßwald, and W. Wienand

Department of Process Research and Development, Health and Nutrition, Evonik Degussa GmbH, Hanau, Germany

The use of enzymes and whole cell biocatalysts has proven valuable in production of enantiomercally pure proteinogenic and non-proteinogenic L- and D- amino acids, and their derivatives. These chemicals are of great interest as building blocks for pharmaceuticals, cosmetics, and agriculture products. In addition to the increasing importance of single enantiomers of chiral active ingredients this development is a result of recent advances in molecular biological methods like recombinant enzyme expression, high throughput DNA sequencing and enzyme evolution technologies. This chapter gives an introduction of important technologies for Evonik Degussa for the production of amino acids by biocatalysis.

Evonik Degussa GmbH, and especially the business unit Health and Nutrition has a long tradition in producing amino acids and their derivatives by chemical and biotechnological means. Biotechnological production of amino acids today serves a market with strong prospects of growth. Well known are fermentation processes, which are now widely established in industrial scale for the production of proteinogenic amino acids (*1*). In this chapter we will focus on the production of amino acids via biocatalysis and biotransformation.

Most of the customers of the business line Exclusive Synthesis & Catalysts are pharma-companies which use the amino acids as precursors for various API's. This fact leads to following challenges for the production of fine chemicals: As a supplier Evonik Degussa has only limited insights into the need of different customers and their in-house developments. When customers request a certain compound, they want to know whether an enzymatic reaction of interest is feasible or not, they want to have price indications and, a sample. And all that in a quite short time line of less than 10 weeks. That means no time consuming work like screening for suitable enzymes or tailoring of existing enzymes via evolutionary techniques are possible in the first step of testing the feasibility. Therefore, to be able to respond on customer requests we need enzymes off-the-shelf, scaleable expression systems and predictable cost structures, means we need enzyme platform technologies. In the following article we will introduce different enzyme platforms for the production of amino acids via whole cell biocatalysts.

The application of biotechnological methods for the production of fine chemicals was pushed by the rapid progress in the fields of micro- and molecular biology, e.g. the genetical screening for new enzymes by gene probes, the activity screening, or the screening in metagenome libraries (2, 3, 4, 5). In recent years new technologies have been developed to accelerate the screening for desired biocatalysts by High-Throughput-Screening (HTS) methods (6, 7). Also new developments on the field of enzyme optimization, e.g. better understanding of the mechanism how enzymes work (rational design) and/or applying random methods like error prone PCR or gene shuffling (directed evolution) lead to more efficient biocatalysts (8, 9, 10).

The advantages of biotransformation driven by biocatalysts are proceeding at mild reaction conditions, for instance at neutral pH and room temperature. Most of the processes proceed in aqueous media, which helps to avoid organic solvents. Enzymatic reactions are highly substrate specific and highly effective so that by-product formation is low or not detectable, and leads to an easier product isolation. Moreover, many reactions are chemo-, regio- or enantioselective so that biocatalysts are preferably used for the production of pure enantiomeres (11, 12).

There has been a growing number of biocatalytic processes for the production of enantiomerically pure intermediates for the pharmaceutical industry during the last decade. In addition to the increasing importance of single enantiomers of chiral active ingredients this development is a result of recent advances in technologies stated above. A particularly efficient approach is the application of recombinant microbial whole-cell biocatalysts so-called "designer cells" providing each enzyme at the optimum amount. Even two and three-step biotransformations can be accomplished combining and adapting enzymes from different sources. In this manner all of the required enzymes can be produced in only one fermentation, and cell disruption as well as clearification/concentration of the enzyme solution is dispensable. Furthermore

the separation of the biocatalyst after biotransformation can easily be done by flocculation and filtration of the biomass. Substantial substrate and product transfer limitations through the cell membranes have never been observed using frozen or spray dried biocatalysts.

All above stated features together leads to savings in material, in energy, and less formation of waste and pollution. That makes biotechnological processes favorable for the sustainable production of fine chemicals and especially enantiomerically pure amino acids (*11, 12, 13*).

The Acylase Platform

The resolution of N-acetyl DL-amino acids by use of immobilized acylases is one of the first industrial large scale processes for the production of L-amino acids (*14*) The numerous investigations on L-acylase (acylase I) have been summarized in several review-articles (*15, 16, 17*). Acylases are commercially available from porcine kidneys and, more important, from the fungi *Aspergillus oryzae* and *Aspergillus melleus*. The *Aspergillus* enzymes are provided by Amano in Japan. The stability tests in a repeated batch-fashion (*15, 18, 19*) showed that the enzyme from *Aspergillus oryzae* is more resistant towards deactivation and oxidation than the enzyme from porcine kidney. At Degussa a large scale acylase process have been established since the 1980's for the production of L-methionine. Enantiomerical pure amino acids are interesting compounds in infusion solutions, as feed and food additives, as intermediates for pharmaceuticals, cosmetics, and pesticides, and as chiral synthons in organic synthesis (*20*). Several tons per year of L-methionine are produced by this enzymatic conversion with an enzyme membrane reactor (EMR) (*21*). Beside this important amino acid other proteinogenic and non-proteinogenic L- and D-amino acids can produced by this method, e.g. L-α-aminobutyric acid, L-valine, L-phenylalanine, D-serine or D-valine.

The starting materials in the acylase process are N-acetyl-D,L-amino acids which are chemical synthesized by acetylation of D,L-amino acids with acetyl chloride or acetic anhydride in alkali in a Schotten-Baumann reaction. The enantiomerically pure L-amino acids are formed by a kinetic resolution reaction of this racemic mixture wherein only the N-acetyl-L-amino acid is deacetylated. This reaction is catalysed by a stereospecific L-acylase from *Aspergillus oryzae* and produces the L-amino acid, acetic acid and N-acetyl-D-amino acid. After separation of the L-amino acid by crystallization, the remaining N-acetyl-D-amino acid has to be racemized by physical or chemical means under severe conditions (high temperature, low pH) to form the N-acetyl-D,L-amino acids and will be used for a next cycle of the process (figure 1). The use of a D-specific acylase also makes D-amino acids accessible.

It has to be pointed out that the enzyme catalyzed kinetic resolution produces a yield of about 50% referring to the staring material, and the recycling

procedure is a fairly energy and chemical consuming process so that there was still need to enhance the performance of this process.

The Acylase/N-Acylamino Acid Racemase Process

Application of a cheap N-acylamino acid racemase would have to be considered as a breakthrough for the acylase technology. If N-acetylamino acids could be selectively racemized by an enzyme in presence of an optically active amino acid, then N-acetyl-D,L-methionine could be converted completely into L-methionine by the combined action of a racemase with L-aminoacylase without any intermittent separation step (Figure 1). Such a N-acetylamino acid racemase (AAR) activity was found by Tokuyama et al. (*22*) in various actinomycetes strains. The gene for the N-acetylamino acid racemase from *Amycolatopsis sp*. TS-1-60 was cloned, overexpressed in Escherichia coli, and the gene product was characterized (*23, 24*). The requirement for a high concentration of divalent metal ions for enzyme activity, substrate inhibition at concentrations exceeding 50 mM and inhibition by L-methionine at less than 100 mM severely restrict the use of this enzyme in a commercial process (*25*).

In order to obtain N-acetylamino acid racemases with different properties various actinomycetes strains were examined in a genetic screening. As a result of that screening we obtained an AAR from *Amycolatopsis orientalis* subsp. *lurida* which was successfully overexpressed in *E. coli* (*26*). This N-acylamino acid racemase catalyzed the racemization of various industrial important N-acylamino acids, which are listed in Table 1.

Besides N-acetyl-D- and L-methionine, the N-acyl derivatives of aromatic amino acids such as N-acetyl-D- and L-phenylalanine, N-acetyl-L-tyrosine and N-chloroacetyl-L-phenylalanine were effective substrates. The derivatives N-acetyl-D-naphthylalanine, N-acetyl-L-tert-leucine and N-benzyloxycarbonyl-L-phenylalanine were not racemized. In contrast, L-methionine was no substrate for N-acylamino acid racemase (*26*).

An important characteristic of the enzyme is, that the AAR from A. orientalis subsp. lurida exhibited substrate inhibition at concentrations of N-acetyl-D-methionine exceeding 200 mM in contrast to 50 mM for the racemase from *Amycolatopsis sp*. TS-1-60 27. This fact is important for the use of AAR in an industrial racemization process, because the reactor loading with the substrate could be much higher with the new enzyme.

The Hydantoinase Platform

The hydantoinase process was first introduced in the 70-ies for the production of D-amino acids such as D-phenylglycine and *p*-OH-phenylglycine
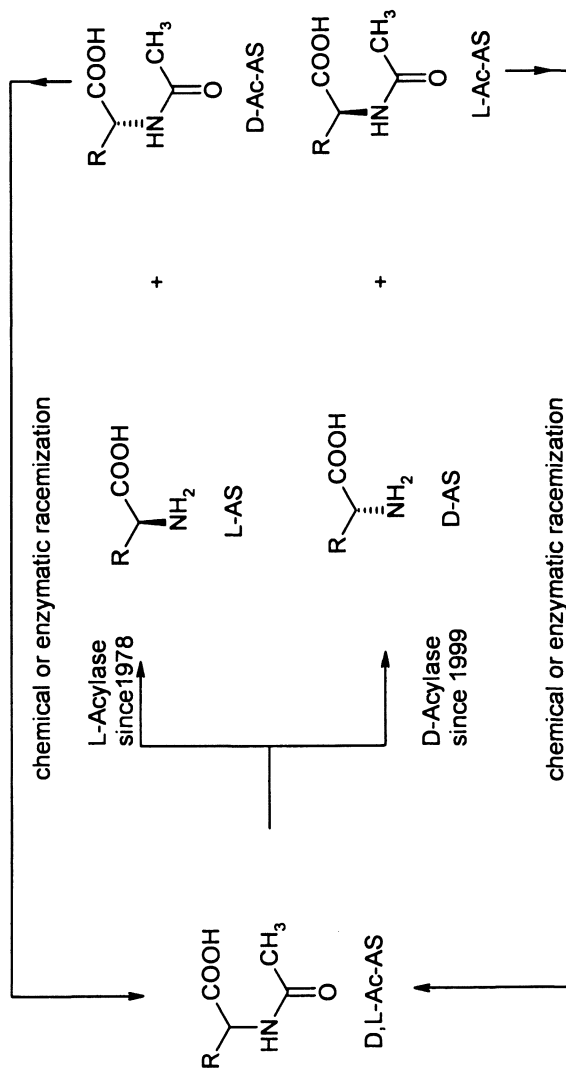
Figure 1. L-Amino acid/D-amino acid production with acylases.

**Table 1. Substrate specificity of N-acylamino acid racemase from
A. orientalis subsp. lurida. The specific activity with
N-acetyl-D-methionine was taken as 100%.**

Substrate	Relative activity (%)
N-acetyl-D-alanine	3
N-acetyl-D-aminobutyric acid	11
N-acetyl-D-methionine	100
N-acetyl-D-naphthylalanine	0
N-acetyl-D-phenylalanine	76
N-acetyl-D-valine	83
N-acetyl-L-tert-leucine	0
N-acetyl-L-methionine	130
N-acetyl-L-phenylalanine	30
N-acetyl-L-tyrosine	30
N-acetyl-L-valine	22
N-benzyloxycarbonyl-L-phenylalanine	0
N-chloracetyl-L-phenylalanine	7

(27, 28) and L-amino acids like L-Tryptophan (29) . Today, a production volume
of >1000 tons per year is reached for each of the above mentioned amino acids
which are used as side chains for the β-lactame antibiotics ampicillin and
amoxicilin.

The D-hydantoinase process, of which the reaction scheme is shown in
figure 2, is an excellent example of a dynamic kinetic resolution process.
As 5'-monosubstituted hydantoins racemize spontaneously or enzyme-catalyzed
(30, 31) under conditions used for biotransformation a 100% yield of optically
pure D- or L-amino acid can be reached. Another advantage of the hydantoinase
route is that racemic 5'-monosubstituted hydantoins can be easily synthesized
from cheap starting materials. In addition, if the decarbamoylation step is done
enzymatically with carbamoylases waste and by-product formation is extremely
low (CO_2 and NH_4 are the only by-products) which is also advantageous in the
product isolation step (32). All these features make the hydantoinase route very
attractive for the industrial production of optically pure artificial amino acids,
e.g. aromatic D-amino acid or D-serine.

So far, low space-time-yields and high biocatalyst costs prevented the
production of L-amino acids based on the hydantoinase process (33, 34, 35).
Therefore, we have expanded our hydantoinase platform for the production of L-
amino acids focusing on strain development and process optimization by
biochemical engineering (36). Despite significant progress in reducing the
biocatalyst production cost, increasing the activity of the biocatalyst and
improving the space-time-yield process economics were still prohibitive for
commercialization of this process.
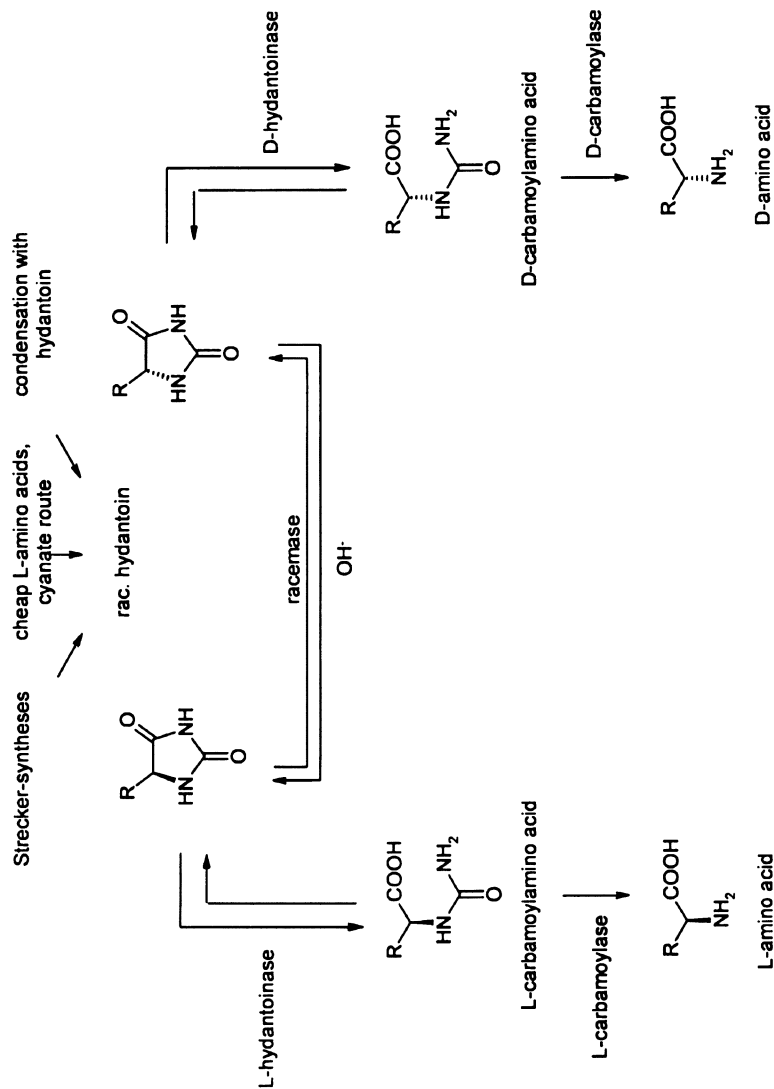
Figure 2. D-Amino acid/L-amino acid production using hydantoinases, carbamoylases and hydantoin racemase.

We have developed a new generation of an L-hydantoinase process based on a tailor-made recombinant whole-cell biocatalyst. The biocatalyst costs have been reduced by designing recombinant *E. coli* cells overexpressing a hydantoinase, carbamoylase and hydantoin racemase from *Arthrobacter sp.* DSM 9771. Despite this progress the D-selectivity of the hydantoinase for DL-methylthioethylhydantoin (DL-MTEH) was significantly limiting the space-time-yield of the L-hydantoinase process (*37, 38*). As screening did not provide us with better hydantoinases we intended to invert the enantioselectivity of the hydantoinase by directed evolution (*39*). The productivity of the process could be dramatically improved using the recombinant *E. coli* coexpressing the newly designed L-selective hydantoinase mutant with an L-carbamoylase and a hydantoin racemase. These improvements have been confirmed at industrial scale and resulted in a process for the production of various natural and non-natural L-amino acids, e.g. L-methionine or L-phosphotricine.

The Amino acid dehydrogenase technology platform

Evonik Degussa started its L-*tert*-leucine production more than 20 years ago. This 1st generation process is based on purified leucine dehydrogenase (LeuDH) from *Bacillus cereus* and formate dehydrogenase (FDH) from *Candida boidinii* for cofactor regeneration along with the enzyme membrane reactor concept (*40*). Using this enzyme combination a broad range of amino acids are accessible in excellent yields and enantioselectivities (figure 3). Especially for extremely bulky amino acids like L-*tert*-leucine, other 2,2,2-trialkylamino acids and L-neopentylglycine this can be a superior approach (*41*).

To meet future market growth for this amino acids a 2nd generation whole-cell biocatalytic process has been developed at Degussa. LeuDH whole-cell catalysts with FDH or glucose dehydrogenase (GDH) for cofactor regeneration have been constructed and tested. Due to the approx. 50-fold specific activity of GDH the latter has turned out to be much more efficient. A pilot production of L-neopentylglycine has been run on multi- kg scale at 42 g/L substrate concentration with an whole-cell LeuDH/GDH biocatalyst reaching > 95% conversion within one day and an optical purity of more than 99.8% ee. The starting material α-keto acid is easily accessible through condensation of pivalaldehyde with hydantoin and successive hydrolysis .

Another interesting enzyme which catalyze the cofactor-dependent reductive amination of α-keto acids to the corresponding L-amino acids is the Phenylalanine dehydrogenase (PheDH). Due to restricted substrate specificity of PheDH from *Thermoactinomyces intermedius* (main substrate is L-Phe/Phenylpyruvat), only the PheDH from *Rodococcus. rhodocrous M4* is interesting from a synthetic point of view. PheDH differs significantly from all the LeuDHs, as it converts aromatic substrates as well as aliphatic keto acids

$$CO_2 \qquad NADH \; + \; H^+ \qquad \text{(trimethyl pyruvic acid)}$$

trimethyl pyruvic acid

FDH
formate dehydrogenase

LeuDH
leucine dehydrogenase

$$HCO_2NH_4 \qquad NAD^+$$
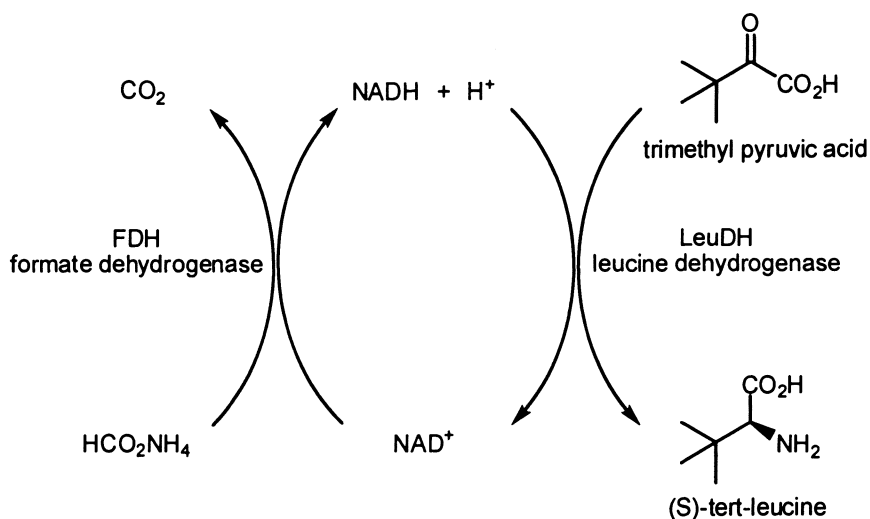
(S)-tert-leucine

Firure 3. Production of L-tert-leucine from trimethyl pyruvate, 1ˢᵗ generation process. The Leucine dehydrogenase catalyses reductive ammination of ammonium trimethyl pyruvate and the formate dehydrogenase performs cofactor regenerates of NAD⁺ to NADH by oxidation of of formate to carbon dioxide.

which are also accepted by LeuDH. Remarkably, the specific activity of PheDH from many bulky aliphatic α-keto acids is even higher than that of LeuDH (*42*).

Other Platform Technologies

Two other amino acids are preferably produced by applying enzymatic conversion steps (figure 4). L-Aspartic acid is formed by the aspartase catalysed addition of ammonia to fumaric acid. This reaction leads directly to L-aspartate, which is required for synthesis of the sweetener Aspartame in large quantities. The resulting aspartate serves also as staring material for enzymatic production of L-alanine by using immobilized aspartate β-decarboxylase from *Pseudomonas dacunhae* (*43, 44*).

3,4-Dihydroxyphenyl-L-alanine (L-DOPA) is another amino acid produced via a lyase catalysed reaction. Whole cells of *Erwinia herbicola* containing tyrosine phenol lyase catalyse the stoichiometric conversion of phenols and alanine or a mixture of pyruvate and ammonia to tyrosine derivatives (figure 5). More than 250 tons L-DOPA are produced per year, approximately half of it by the enzymatic process. DOPA is mainly used in the treatment of Parkinson's disease (*45, 46*).

384
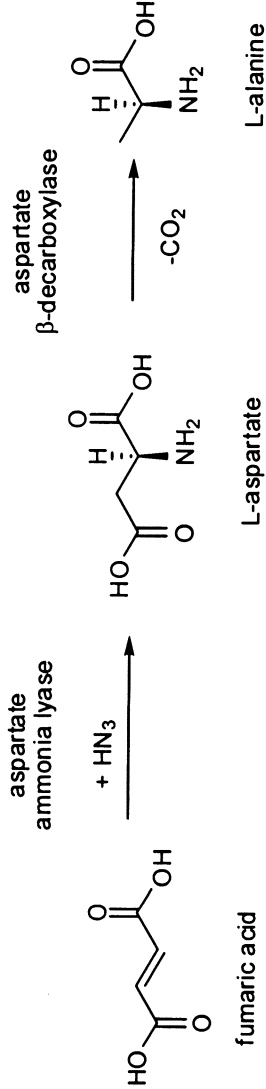
Figure 4. Enzymatic production of L-aspartate and L-alanine based on fumaric acid as starting material.
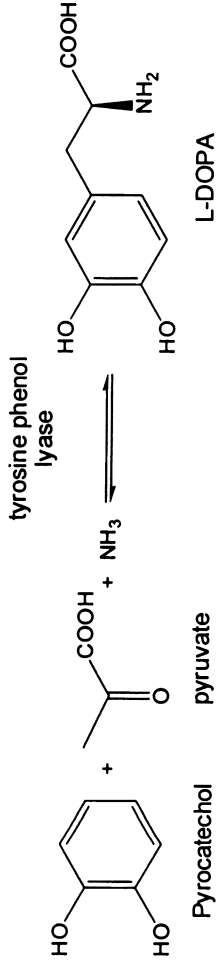
Figure 5. Enzymatic production of L-DOPA with pyrocatechol, pyruvate and ammonia as the starting materials

L-cysteine can be produced from racemic 2-amino-Δ^2-thiazoline-4-carboxylic acid (DL-ATC) via an whole cell process with *Pseudomonas* sp. cells containing an ATC-hydrolase and also an ATC-racemase. This enzyme combination enables a dynamic kinetic resolution with > 90% yield (*47*).

Another process for the preparation of enatiomercally pure amino acids by use of whole cell biocatalysts was proposed by Weckbecker and Hummel (*48, 49*). In that recombinant system an bacterial D-amino acid oxidase is coexpressed with LeuDH from *Bacillus cereus* in an *E. coli* strain. The desired L-amino acid, here L-methionine for example, is produced through enzymatic resolution of DL-amino acid, using both coordinately formed enzymes. The process will lead to nearly 100% conversion of the racemic mixture of DL-amino acids which is the big advantage applying such a whole cell catalysts for the production of L-amino acids (figure 5). For further details of other industrial applications of D-amino acids refer (*50, 51*).

A third example is the synthesis of chiral α-amino acid starting from α-keto acids via transamination performed by transaminases (aminotransferases). Such an reaction has already been reported by NSC Technologies (*52, 53, 54*) and can be used for the preparation of L- and D-amino acids whereas the transferred amino group comes from an inexpensive amino donor, e.g. L-glutamic acid. Transaminases requires an enzyme bound cofactor, most commonly pyridoxal phosphate, for proper function. The substrate specificity is broad, allowing the conversion of large variety of keto acid substrates to desired amino acid products with high enantioselectivity (*55, 56*).

An example for an efficient asymmetric transamination process is the production of L-alanine (figure 6), which is carried out in a continuous manner starting from prostereogenic pyruvat, and L-glutamate as amino donor, with a high space-time-yield of 4.8 kg/l · d (*55, 56*). Several non-proteinogenic α-amino acids, e.g. L-phosphinotricine, L-homophenyalanine, and L-tert-leucine have been also produced by the transamination technology.

The problem of asymmetric synthesis of amino acids is the equilibrium of the reaction, which doesn't lie completely on the side of the desired amino acid. Hence, only small amounts of enantiomerically pure amino acids are formed (*55, 56*, 57, 58, 59). The problem of incomplete reactions have been solved by coupling the transaminase reaction with another irreversible conversion step that consumes the α-keto acid formed (*60*). An example for such a coupled reaction is the decarboxylation of oxalacetate, which is the α-keto acid byproduct when using L-aspartate as amino donor (57, 58). Another keto acid consuming helper reaction was recently published by the group of Bornscheuer (*60*) which combines a transaminase with an pyruvate decarboxylase, here alanine serves as amino donor (figure 7). This system has the advantage that no cofactor recycling is required compared to other known procedures using lactate dehydrogenase for the equilibrium shift (*61*).
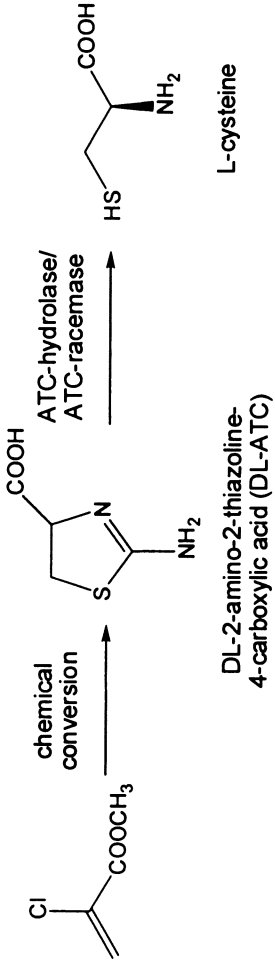
Figure 6. Enzymatic production of L-cysteine via dynamic kinetic resolution
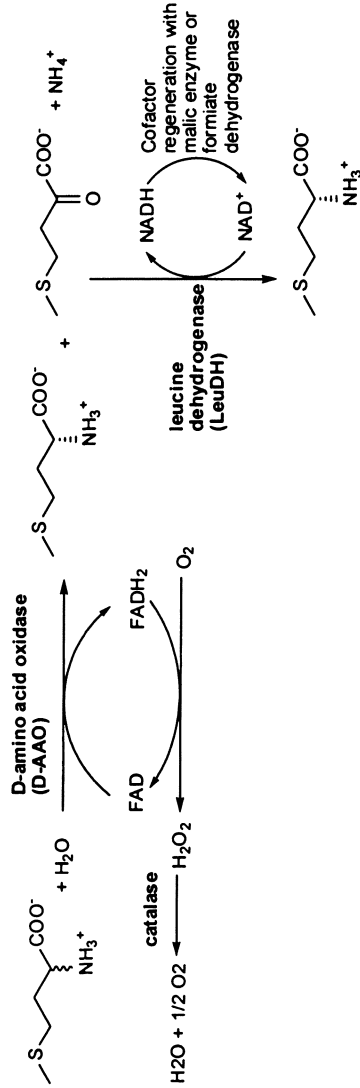
Figure 7. Enzymatic production of L-methionine by enzymatic resolution of DL-methionine via D-amino acid oxidyase and leucine dehydrogenase.

Synthesis of β-Amino Acids

This chapter deals not with a process performed by whole cell technology but with a enzymatic synthesis of the more and more important class of β-amino acids, which are attractive building blocks for a new generation of API's.

Staring point of β-amino acid synthsesis are racemic β-phenylalanines, which can be easily obtained by condensation of substituted benzaldehyde with malonic acid and ammonium acetate, for instant (figure 8). An subsequent esterification proceeds via *n*-propanol and the resulting esters are cleaved with lipase in a biphasic solvent system to give the corresponding substituted enantiomerically pure β-amino acids (*62*). This Process has been shown to be very stable and supplies the desired products in a very high enantiomeric purity with an ee greater of 99% and high substance concentrations of about 250 g/l (*63*). These positive parameters have made a substantial contribution to improve space-time-yield of the synthesis of this completely new class of industrial products that can be now manufactured in large quantities and high quality in an economically attractive way.
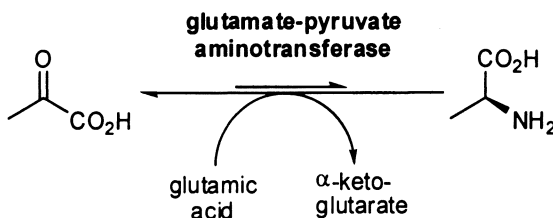
Figure 8. Aminotransferase catalyzed asymmetric synthesis of L-alanine.

Summary and Outlook

In the chapters above we provide examples for the development of platform technologies for the production of optically pure amino acids. Two different dynamic kinetic resolution processes for the production of natural and non-natural L-amino acids have been established at Evonik Degussa. The first process is based on the resolution of 5'-monosubstituted hydantoins and the second based on the resolution of N-acetyl amino acids using an acylase in combination with a racemase. These platform technologies have been recently expanded by a process using oxido-reductases and a co-factor regeneration systems for production of amino acids and chiral molecules starting from pro-chiral ketones. Whether the oxidoreductase platform, the L-hydantoinase process or the combination of an L-acylase with and N-acetyl-amino acid racemase (or other new technologies) is superior depends strongly on the requested specific

product and is influenced by the biocatalyst properties as well as the cheapest access to desired substrates. All approaches together provide us with a high degree of flexibility for the production of a large number of different L- or D-amino acids, which is especially important for fast changing product demands typical for the fine chemical industry.

Enzyme catalysts will be the preferred production method for non-proteinigenic and non-natural amino acids and their derivatives. Modern methods such as high throuput screening (HTS) of new enzymes and directed evolution will allow development of customized, highly selective, and stable enzymes and whole cell biocatalysts, as well as efficient and sustainable production of industrial relevant amino acids.

A. LDH reaction
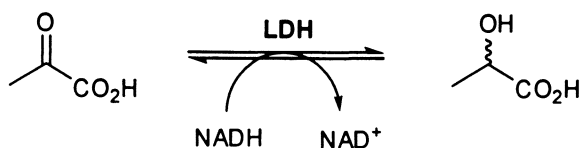
B. PDC reaction
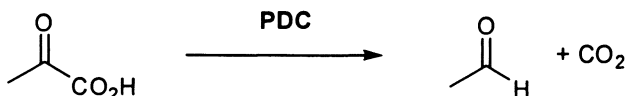
Figure 9. Pyruvate consuming helper reactions shifting the equilibrium in transaminase catalyzed asymmetric synthesis: A. Lactate dehydrogenase (LDH) and B. Pyruvate decarboxylase (PDC).

References

1. Leuchtenberger, W.; Huthmacher, K.; Drauz, K. Biotechnological production of amino acids and derivatives: current status and prospects. Applied Microbiology and Biotechnology **2005**, *69*, 1-8.
2. Cowan, D.A. Microbial genomes - the untapped resource. Trends in Biotechnology 2000, *18*, 14-16.
3. Gabor, E.; Liebeton, K.; Niehaus, F.; Eck, J.; Lorenz, P. Updating the metagenomics toolbox. Biotechnology Journal **2007**, *2*, 201-206.
4. Pace, N.R. A molecular view of microbial diversity and the biosphere. Science **1997**, *276*, 734-740
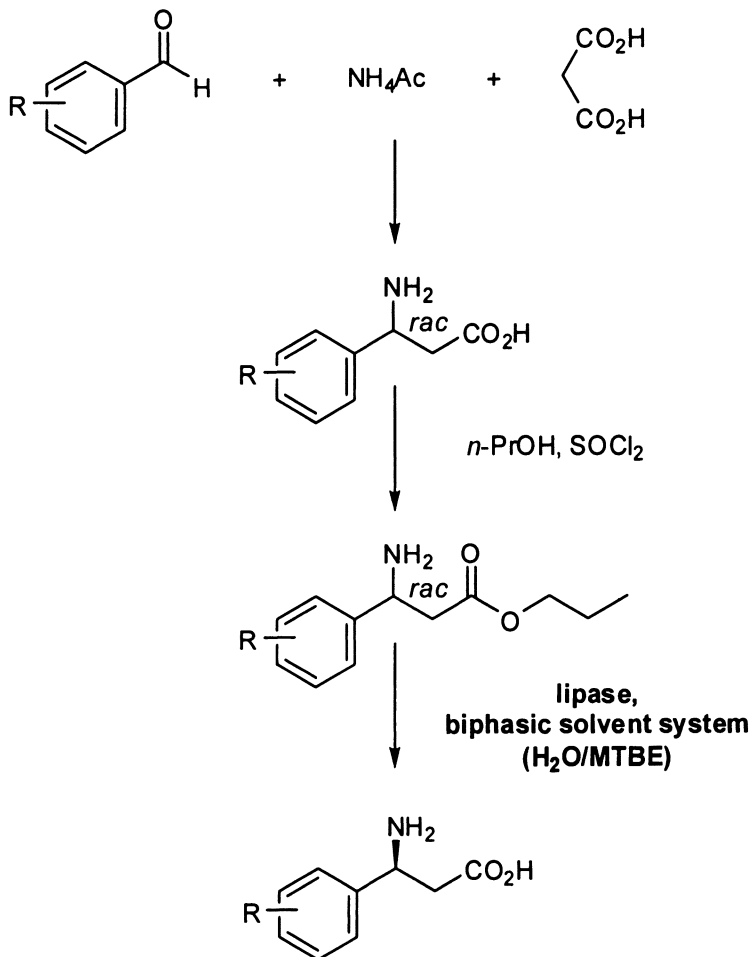
Figure 10. Enzymatic resolution of β-amino acid esters with lipase yielding enantimerically pure β-amino acids.

5. Sharma, R.; Ranjan, R.; Kapardar, R.K.; Grover, A. 'Unculturable' bacterial diversity: an untapped resource. Current Science **2005**, *89*, 72-77.

6. Olsen, M.; Iverson, B.; Georgiou, G. High-throughput screening of enzyme libraries. Current Opinion in Biotechnology **2000**, *11*, 331-337.

7. Wahler, D.;. Reymond J-L High-throughput screening for biocatalysts. Current Opinion in Biotechnology **2001**, *12*, 535-544.

8. Chirumamilla, R.R.; Muralidhar, R.; Marchant, R.; Nigam, P. Improving the quality of industrially important enzymes by directed evolution. Molecular and Cellular Biochemistry **2001**, *224*, 159-168.

9. Farinas, E.T.; Bulter, T.; Arnold, F.H. Directed enzyme evolution. Current Opinion in Biotechnology **2001**, *12*, 545-551.

10. Miyazaki, K.; Arnold, F.H. Exploring nonnatural evolutionary pathways by saturation mutagenesis: rapid improvement of protein function. Journal of Molecular Evolution **1999**, *49*, 716-720.

11. Bull, A.T.; Bunch, A.W.; Robinson, G.K. Biocatalysts for clean industrial products and processes. Current Opinion in Microbiology **1999**, *2*, 246-251.

12. *The Application of Biotechnology to industrial sustainability*; OECD report **2001**, OECD publications.

13. Koller, G.; Fischer, U.; Hungerbuehler, K. Assessing Safety, Health, and Environmental Impact Early during Process Development. Industrial & Engineering Chemistry Research **2000**, *39*, 960-972.

14. Chibata, I.; Tosa, T.; Sato T.; Mori T. Production of L-Amino Acids by Aminoacylase Adsorbed on DEAE-Sephadex. Methods in Enzymology **1976**, *44*, 746-759.

15. Chenault, K.; Dahmer, J.; Whitesides, G.M. Kinetic Resolution of Unnatural and Rarely Occurring Amino Acids: Enantioselective Hydrolysis of N-Acyl Amino Acids Catalyzed by Acylase I. Journal of the American Chemical Society **1989**, *111*, 6354 -6364.

16. Groeger, U.; Bommarius, A. S. Hydrolysis of N-Acyl Amino Acids. *Handbook of Enzyme Catalysis in Organic Synthesis*, Drauz K.; H. Waldmann H. Eds., VCH, Weinheim, Basel, Deerfield Beach, **1995**, 393-408.

17. Bommarius, A.S. Hydrolysis of N-acylamino acids. *Enzyme Catalysis in Organic Synthesis (2nd Edition)*, Drauz, K.; Waldmann, H. Eds.; VCH, Weinheim, Basel, Deerfield Beach, **2002**, 741-761.

18. C. Wandrey, C.; Flaschel, E. Acylase L-Methionine System. *Process development and Economic Aspects in Enzyme Engineering*. Adv. Biochem. Eng., *12*, Ghose, T.K.; Fiechter, A.; Blakebrough, N. Eds.; Springer, Berlin, **1979**, 147-218.

19. Gentzen, I.; Löffler H.G.; Schneider, F. Purification and partial characterization of Aminoacylase from Aspergillus oryzae; *Metalloproteins* U. Eser U. Ed.; Thieme, Stuttgart, **1979**, 270-274.

20. Bommarius, A.S.; Drauz, K.; Klenk, H.; Wandrey, C. Operational Stability of Enzymes - Acylase-Catalyzed Resolution of N-Acetyl Amino Acids to Enantiomerically Pure L-Amino Acids. Annuals of the New York Acadademy of Science (Enzyme Eng. XI), **1992**, *672*, 126-36.

21. Bommarius, A.S.; Schwarm, M.; Drauz, K. The membrane reactor for the acylase process – from laboratory results to commercial scale. Chimica Oggi **1996**, *14*, 61-64.

22. Tokuyama, S.; Hatano, K.; Takahashi, T. Discovery of a novel enzyme, N-acylamino acid racemase in an actinomycete: screening, isolation, and identification. Bioscience, Biotechnology, and Biochemistry **1994**, *58*, 24-27.

23. Tokuyama, S.; K. Hatano, K. Purification and Properties of Thermostable N-Acylamino Acid Racemase from Amycolatopsis sp. TS-1-60. Applied Microbiology and Biotechnology **1995**, *42*, 853-859.

24. Tokuyama, S.; K. Hatano, K. Cloning, DNA Sequencing and Heterologous Expression of the Gene for Thermostable N-Acylamino Acid Racemase from Amycolatopsis sp. TS-1-60 in Escherichia coli. Applied Microbiology and Biotechnology **1995**, *42*, 884-889.

25. Palmer, D.R.J.; Garrett, J.B.; Sharma, V.; Meganathan, R.; Babbitt, P.C.; Gerlt, J.A. Unexpected Divergence of Enzyme Function and Sequence: "N-Acylamino Acid Racemase" Is o-Succinylbenzoate Synthase. Biochemistry **1999**, *38*, 4252-4258.

26. Verseck, S.; Bommarius, A.; Kula, M.-R. Screening, overexpression and characterization of an N-acylamino acid racemase from Amycolatopsis orientalis subsp. lurida. Applied Microbiology and Biotechnology **2001**, *55*, 354-361.

27. Cecere, F.; Galli, G.; Della Penna, G.; Rappuoli, B. **1976**, DE 2422737.

28. Takahashi, S.; Kii, Y.; Kumagai, H.; Yamada, H. Microbial transformation of hydantoins to amino acids. II. Purification, crystallization and properties of hydantoinase from Pseudomonas striata. Journal of Fermentation Technology **1978**, *56(5)*, 492-8.

29. Sano, K.; Yokozeki, K.; Eguchi, C.; Kagawa, T.; Noda, I.; Mitsugi, K. Enzymic production of L-tryptophan from DL-indolylmethylhydantoin. Part I. Enzymic production of L-tryptophan from L- and DL-5-indolylmethyl-hydantoin by newly isolated bacterium. Agricultural and Biological Chemistry **1977**, *41(5)*, 819-2.

30. Kato, M.; Omine, H.; Yamamoto, H. Miyoshi, T. Microbial production of L-amino acids. Jpn. Kokai Tokkyo Koho **1986**, 5 pp.

31. Pietzsch, M.; Syldatk, C. Hydrolysis and formation of hydantoins. Enzyme Catalysis in Organic Synthesis (2nd Edition) **2002**, 761-799.

32. Syldatk, C.; May, O.; Altnbuchner, J.; Mattes, R.; Siemann, M. Microbial hydantoinases - industrial enzymes from the origin of life? Applied Microbiology and Biotechnology **1999**, *51*, 293-309.

33. Gross, C.; Syldatk, C.; Wagner, F. Screening method for microorganisms producing L-amino acids from DL-5-monosubstituted hydantoins. Biotechnology Techniques **1987**, *1*, 85-90.

34. Nishida, Y.; Nakamichi, K.; Nabe, K.; Tosa, T. Enzymic production of L-tryptophan from DL-5-indolylmethylhydantoin by Flavobacterium sp. Enzyme and Microbial Technology **1987**, *9*, 721-725.

35. Cotoras, D.; Wagner, F. Stereospecific hydrolysis of 5-monosubstituted hydantoins. Eur. Congr. Biotechnol., 3rd **1984**, 1 351-356.

36. Wagner, T.; Hantke, B.; Wagner, F. Production of L-methionine from D,L-5-(2-methylthioethyl)hydantoin by resting cells of a new mutant strain of Arthrobacter species DSM 7330. Journal of Biotechnology **1996**, *46*, 63-68.

37. May, O.; Siemann, M.; Pietzsch, M.; Kiess, M.; Mttes, R.; Syldatk, C. Substrate-dependent enantioselectivity of a novel hydantoinase from Arthrobacter aurescens DSM 3745: Purification and characterization as new member of cyclic amidases. Journal of Biotechnology **1998**, *61*, 1-13.

38. Volkel D; Wagner F Reaction mechanism for the conversion of 5-monosubstituted hydantoins to enantiomerically pure L-amino acids. Annals of the New York Academy of Sciences **1995**, *750*, 1-9.

39. May, O.; Nguyen, P.T.; Arnold, F.H. Inverting enantioselectivity by directed evolution of hydantoinase for improved production of L-methionine. Nature Biotechnology **2000**, *18*, 317-320.

40. Kula, M.-R.; Wandrey, C. Continuous enzymic transformation in an enzyme-membrane reactor with simultaneous NADH regeneration. Methods in Enzymology **1987**, *136* (Immobilized Enzymes Cells, Pt. C), 9-21.

41. Bommarius, A.S.; Schwarm, M.; Drauz, K. Biocatalysis to amino acid-based chiral pharmaceuticals - examples and perspectives. Journal of Molecular Catalysis B: Enzymatic **1998**, *5*, 1-11.

42. Krix, G.; Bommarius, A. S.; Drauz, K.; Kottenhahn, M.; Schwarm, M.; Kula, M.-R. Enzymic reduction of .alpha.-keto acids leading to L-amino acids, D- or L-hydroxy acids. Journal of Biotechnology **1997**, *53*, 29-39.

43. Calton, Gary J. The enzymic preparation of L-alanine. Biocatal. Prod. Amino Acids Deriv. **1992**, 59-74.

44. Calton, Gary J. The enzymic production of L-aspartic acid. Biocatal. Prod. Amino Acids Deriv. **1992**, 3-21.

45. Kumagai, H.; Yamada, H.; Matsui, H.; Ohkishi, H.; Ogata, K. Tyrosine phenol lyase. I. Purification, crystallization, and properties. The Journal of biological chemistry **1970**, *245(7)*, 1767-7172.

46. Koyanagi, T.; Katayama, T.; Suzuki, H.; Nakazawa, H.; Yokozeki, K.; Kumagai, H. Effective production of 3,4-dihydroxyphenyl-L-alanine (L-DOPA) with Erwinia herbicola cells carrying a mutant transcriptional regulator TyrR. Journal of biotechnology **2005**, 115(3), 303-306.

47. Sano K.; Yokozeki K.; Tamura F.; Yasuda N.; Noda I.; Mitsugi K. Microbial conversion of DL-2-amino-delta2-thiazoline-4-carboxylic acid to L-cysteine and L-cystine: screening of microorganisms and identification of products. Applied and environmental microbiology **1977**, 34(6), 806-810.

48. Weckbecker, C.; Hummel, W. Making L from D- in a single cell. Elements *06* **2004**, 34-37 (http://www.degussa.de/NR/rdonlyres/2C97116E-1E21-4A0F-922D-A19F697ED887/0/elements_06_en.pdf).

49. Geueke, B.; Weckbecker, A.; Hummel, W. Overproduction and characterization of a recombinant D-amino acid oxidase from Arthrobacter protophormiae. Applied Microbiology and Biotechnology **2007**, *74*, 1240-1247.

50. Pilone, M.S.; Pollegioni, L. D-amino acid oxidase as an industrial biocatalyst. Biocatalysis and Biotransformation **2002**, *20*, 145-159.

51. Fotheringham, I.; Archer, I.; Carr, R.; Speight, R.; Turner, N. J. Preparative deracemization of unnatural amino acids. Biochemical Society Transactions **2006**, *34*, 287-290.

52. Ager, D.J.; Fotheringham, I.G.; Laneman, S.A.; Pantaleone, D.P.; Taylor, P.P. The large-scale synthesis of unnatural amino acids. Chimica Oggi **1997**, *15*, 11-14.

53. Taylor, P.P.; Pantaleone, D.P.; Senkpeil, R.F.; Fotheringham I.G. Novel biosynthetic approaches to the production of unnatural amino acids using transaminases. Trends in biotechnology **1998**, *16*, 412-418.

54. Ager, D.J.; Fotheringham, I.G.; Li, T.; Pantaleone, D.P.; Senkpeil, R.F. The large scale synthesis of "unnatural" amino acids. Enantiomer **2000**, *5*, 235-243.

55. Rozzell, J.D.; Bommarius, A.S.. Transaminations. *Enzyme Catalysis in Organic Synthesis (2nd Edition)* **2002**, Rozzell, D.J.; Wagner, F.; Eds. *2* 873-893.

56. Crump, S.P.; Rozzell, J.D. Biocatalytic production of amino acids by transamination. Biocatal. Prod. Amino Acids Deriv. **1992**, 43-58.

57. Rozzell, J.D. Immobilized aminotransferases for amino acid production. Methods in Enzymology **1987**, *136* (Immobilized Enzymes Cells, Pt. C), 479-497.

58. Crump, Steven P.; Heier, Jeffery S.; Rozzell, J. David. The production of amino acids by transamination. Biocatalysis **1990**, Abramowicz, D.A. Ed.; 115-33.

59. Li, T.; Kootstra, A.B.; Fotheringham, I.G. Nonproteinogenic alpha-Amino Acid Preparation Using Equilibrium Shifted Transamination. Organic Process Research & Development **2002**, *6*, 533-538.

60. Hoehne, M.; Kuehl, S.; Robins, K.; Bornscheuer, U.T. Efficient Asymmetric Synthesis of Chiral Amines by Combining Transaminase and Pyruvat Decarboxylase. ChemBioChem **2008**, *9*, 1-3.

61. Shin, J.S.; Kim, B.G. Asymmetric synthesis of chiral amines with omega-transaminase. Biotechnology and bioengineering **1999**, *65*, 206-211.

62. Groeger, H.; Drauz, K. Methods fort he Enantioselective Biocatalytic Production of L-Amino Acids on an Industrial Scale. Asymmetric Catalysis on Industrial Scale **2003**. Blaser, H.-U.; Schmidt, E. Eds.; Wiles-VHC, Weinheim, 131-147.

63. Groeger, H. Beta Effect Stimulates Pharmaceutical Research. Elements *06* **2004**, 8-11 (http://www.degussa.de/NR/rdonlyres/2C97116E-1E21-4A0F-922D-A19F697ED887/0/elements_06_en.pdf).

Chapter 24

Synthesis of Optically Active α-Methyl Amino Acids Using Biotransformation as a Key Step

Masanobu Yatagai, Takayuki Hamada, Hiroyuki Nozaki, Shinji Kuroda, Kenzo Yokozeki, and Kunisuke Izawa

AminoScience Laboratories, Ajinomoto Company, Inc., 1–1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210–8681, Japan

A new method for the synthesis α-methyl-L-cysteine was realized by the enzymatic resolution of racemic 2,4-dimethyl-4-methoxycarbonylthiazoline, which can be derived from chloroacetone in 4 steps. α-Methyl-L-cysteine hydrochloride with high optical purity was successfully obtained by hydrolyzing (4R)-2,4-dimethyl-4-methoxycarbonylthiazoline. In an alternative approach, a new enzymatic synthesis of α-methyl-L-serine was discovered in the stereoselective hydroxymethylation of L-alanine. The chemical conversion of α-methyl-L-serine to α-methyl-L-cysteine is also demonstrated.

α-Methyl-α-amino acids are a class of nonproteinogenic α,α-disubstituted α-amino acids, and some compounds, such as (*S*)-α-methyl tyrosine, (*S*)-isovaline, and (*S*)-α-methyl aspartic acid, are found in nature (1). These amino acids are well known as important building blocks, especially in bioorganic chemistry.
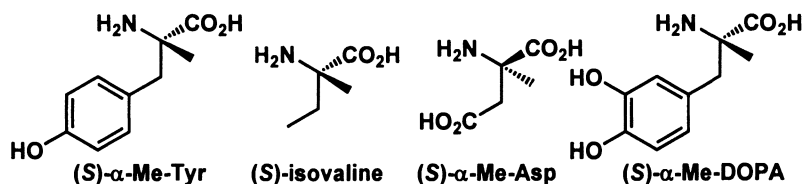
Figure 1. Examples of α-methyl α-amino acids

The peptides in α-methyl-α-amino acids show limited rotation around N-Cα (φ) and Cα-carbonyl (ψ) bonds, and this stabilizes the conformation of the peptide backbone (2). These effects make them remarkably resistant to enzymatic degradation.
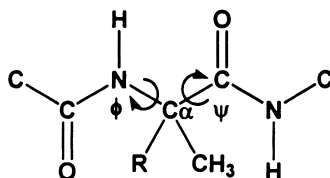
Figure 2. Peptide bond with α-methyl-α-amino acid

The antihypertensive drug (*S*)-α-methyl DOPA, which inhibits an aromatic amino acid decarboxylase, is a typical drug application (3).

α-Methyl Cysteine

α-Methyl cysteine plays an important role in peptide chemistry by forming constrained cyclic structure with disulfide bridging. Natural products with thiazoline rings exhibit antitumor and anti-HIV activities. Thiazoline com-

pounds derived from α-methyl cysteine have also been reported as drug candidates. (4)

deferitrin   desferrithiocin
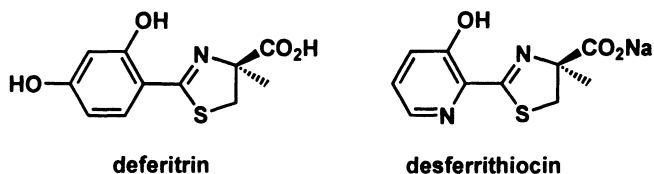
Figure 3. Drug candidates derived from α-methyl cysteine

Synthetic Methods for α-Methyl Cysteine

Many methods for the synthesis of α-methyl cysteine have been reported so far. Schöllkopf's alkylation of chiral bislactim ether (5) and Pattenden's self-reproduction of L-cysteine's chirality (4a) are well-known techniques for the asymmetric synthesis of α-methyl cysteine. These methods offer a convenient approach to the chiral form, but require relatively expensive reagents such as lithium base. The enzymatic hydrolysis of α,α-disubstituted malonate and subsequent Curtius rearrangement has been demonstrated for the effective synthesis of protected chiral α-methyl cysteines (6). Optical resolution of racemic intermediates has also been extensively studied (7). In these approaches, sulfur atom is introduced by reaction with sodium hydrogensulfide, *tertiary-*butylthiol, benzylthiol, or thioacetic acid. However, these sulfur reagents often cause odor issues and require special care to contain them (8).

New Synthetic Method for α-Methyl-L-Cysteine

The goal of our study was to satisfy the following concepts and develop a new method for the synthesis of α-methyl-L-cysteine **1** with a potential industrial application. This method does not require expensive alkylating reagents. The sulfur atom is imported from an odorless material. Chirality is realized by enzymatic resolution or synthesis. Our preliminary routes to α-methyl-L-cysteine **1** are outlined in *Scheme 1*.

Odorless thiourea **3** was selected as a sulfurizing reagent and converted to 2-amino-4-methyl-4-methoxycarbonylthiazoline **4**. Optical resolution of this racemic intermediate **4** was studied. An alternative synthetic route was also studied, in which chiral α-methyl-L-cysteine **1** could be derived from α-methyl-L-serine **6**. Soil samples were screened to find a new enzyme to catalyze the enantioselective hydroxymethylation of alanine **5**.
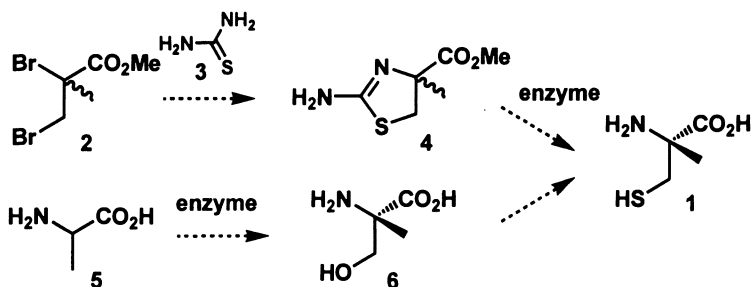
Scheme 1. Presumed synthetic routes for α-methyl-L-cysteine

Synthesis of α-Methyl Cysteine via 2-Amino-4-Methylthiazolines

While dibromides **2** and **9** were prepared in good yields by the bromination of **7** or **8**, thiazolines **4** and **10** were not obtained by the reaction with thiourea, and the starting material was recovered in all cases (*Scheme 2*).
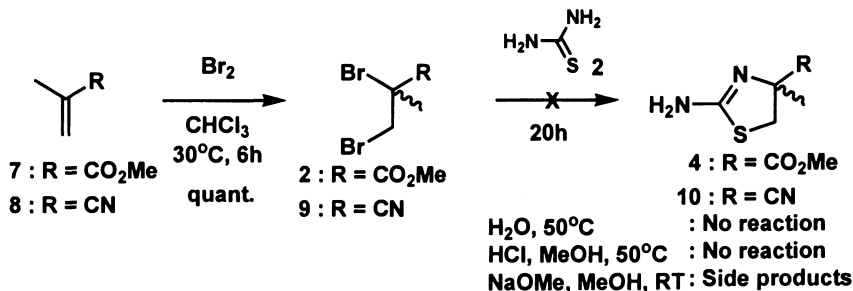
Scheme 2. Synthesis of 2-amino-4-methylthiazolines

From chloroacetone **11**, 2-amino-4-methylthiazoline-4-carboxylic acid **15** was obtained in 4 steps (*Scheme 3*). Hydrocyanation of **11** was followed by amination, and the amino nitrile **13** was hydrolyzed to α-methyl-β-chloroalanine **14** under acidic conditions. The reaction with thiourea gave **15** in 90% yield.

Conversion of **15** to α-methyl-DL-cysteine was tried, but the desired compound was not obtained. Electrolysis (9), reaction with nitrous acid and subsequent hydrolysis (10), and acidic hydrolysis (11) were all ineffective for this compound, even with enzymatic hydrolysis which has been applied for the conversion of 2-amino-thiazoline-4-carboxylic acid to L-cysteine on an industrial scale (12). Finally, the first presumed route for α-methyl-L-cysteine **1** had to be
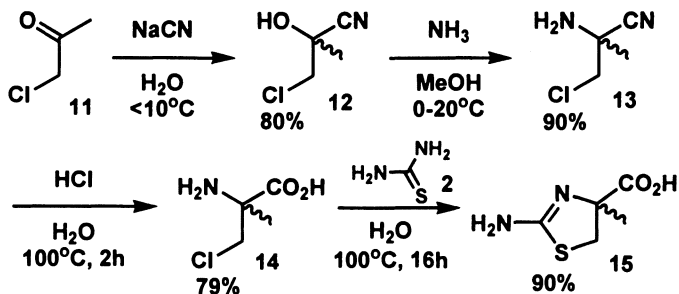
Scheme 3. Synthesis of 2-amino-4-methyl-4-hydroxymethylthiazoline

changed. In the revised route (*Scheme 4*), thiobenzamide **17** or thioacetamide **18** was used instead of thiourea **2**. No problem was expected in hydrolysis of the thiazoline compounds **21** and **22** to **1** (*Scheme 4*) based on the reported results (13).
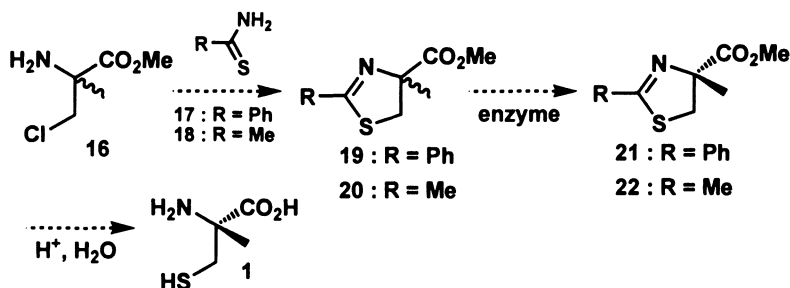
Scheme 4. Revised route to α-methyl-L-cysteine

Synthesis of α-Methyl Cysteine via 2,4-Dimethyl-4-Methoxycarbonyl-thiazoline

The reaction of thiobenzamide **17** and α-methyl-β-chloroalanine **14** was conducted in methanol with heating, and the desired product **23** was obtained in 32% yield (*Scheme 5*). The choice of methanol solvent was important in this reaction, since the reaction did not proceed in solvents other than methanol. The same conditions were applied for the reaction of thioacetamide **18**, but a complex mixture was obtained. On the other hand, in the reaction with the methyl ester **16**, the desired compound **20** was obtained in 82% yield (*Scheme 5*).
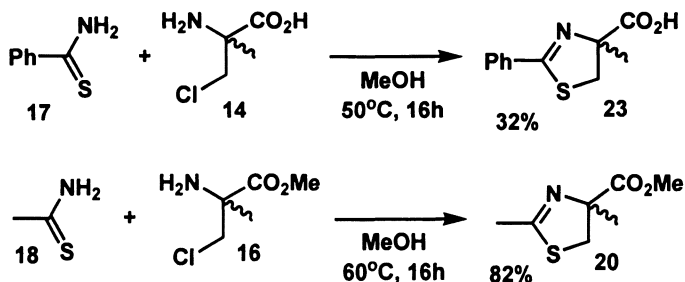
Scheme 5. Synthesis of thiazoline compounds

Reaction of **16** with an equivalent amount of thioacetamide **18** in methanol with heating at 60°C for 24 hours gave the thiazoline **20** in 87% yield. The hydrochloride of racemic α-methyl cysteine **24** was confirmed to be generated from acidic hydrolysis of the thiazoline **20**.
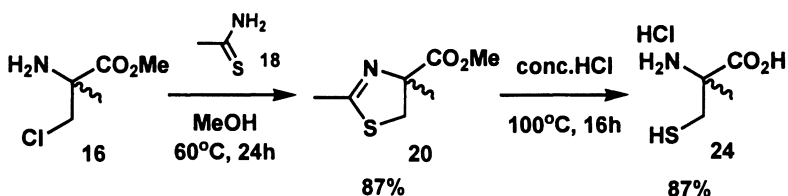
Scheme 6. Acidic hydrolysis of 2,4-dimethyl-4-methoxycarbonylthiazoline

Enantioselective Hydrolysis of 2,4-Dimethyl-4-Methoxycarbonylthiazoline

Commercially available enzymes were screened for the enantioselective hydrolysis of 2,4-dimethyl-4-methoxycarbonyllthiazoline **20** (*Scheme 6*). The substrate **20** was added to 10% wt/wt enzyme and reacted in phosphate buffer at 30°C for 24 hours. The optical purity of the products was then monitored. In the screening, three proteases stereoselectively hydrolyzed an ester and afforded **22** in higher than 90% ee (*Table 1*). The following issues were noted for scale-up. Ester hydrolysis proceeded stereoselectively in a diluted condition (1% w/v), but predominant hydrolysis of the thiazoline ring was observed under a higher concentration (15% w/v). After the hydrolysis of the enantiomer was completed, **22** also tended to be hydrolyzed.
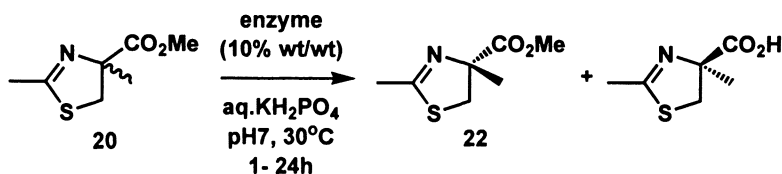
Scheme 7. Enantioselective hydrolysis of 2,4-dimethyl-4-methoxycarbonylthiazoline

Table 1. Optical Yields of 22 in Screening Enzymes

Enzyme	Reaction Time		
	1h	*4h*	*24h*
Protease P	39% ee (40%)	80% ee (51%)	91% ee (68%)
Prolether FG-F	23% ee (27%)	61% ee (42%)	99% ee (58%)
Orientase 22BF	-	96% ee (52%)	99% ee (75%)

Conversion of 2,4-dimethyl-4-methoxycarbonylthiazoline is given in the parentheses.

Synthesis of α-Methyl-L-Cysteine

Racemic α-methyl-β-chloroalanine methyl ester **16** was condensed with thioacetamide **18** in 87% yield, and (4R)-2,4-dimethyl-4-methoxycarbonylthiazoline **22** was obtained in 97% ee via enzymatic resolution. Although the purification of the hydrochloride of α-methyl-L-cysteine **1** was difficult in the final step, crystallization of **25** was identified as an effective purification process. The isolated carboxylic acid **25** was hydrolyzed under acidic conditions, and the hydrochloride of α-methyl-L-cysteine **1** was obtained in good yield and higher than 99% ee (14).

α-Methyl-L-Serine

A new enzymatic synthesis of α-methyl-L-serine **6** and chemical conversion to α-methyl-L-cysteine **1** were also studied (*Scheme 9*).

Wilson and Snell reported the hydroxymethylation of alanine as a reversible reaction (15). An enzyme, α-methyl-L-serine hydroxytransferase, which was isolated from soil bacterium, catalyzed the reversible cleavage of α-methyl-L-serine to formaldehyde and D-alanine (*Scheme 10*). In this reaction, tetrahydrofolate is added as a cofactor, which takes in the formaldehyde to form
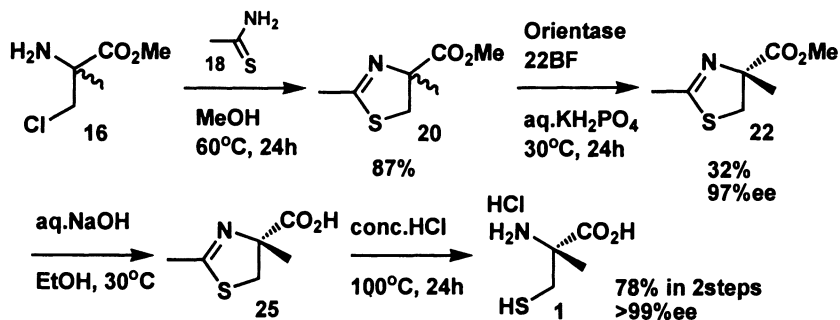
Scheme 8. Synthesis of a-methyl-L-cysteine
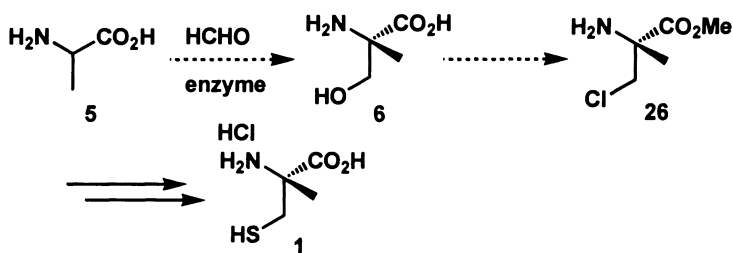
Scheme 9. Synthesis of α-methyl-L-cysteine via α-methyl-L-serine
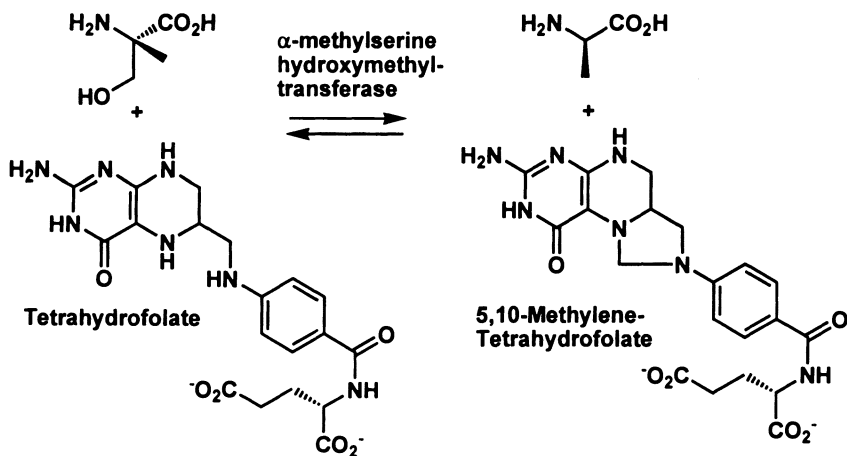
Scheme 10. Reversible hydroxymethylation of D-alanine (12)

aminal, 5.10-methylenetetrahydrofolate. This aminal is also reversibly hydro-lyzed to formaldehyde and tetrahydrofolate. We expected to find a new enzyme to catalyze the enantioselective hydroxymethylation of L-alanine from the screening of soil samples.

Finding New Enzymes

One hundred stocked soil samples were screened for new enzymes with the ability to catalyze hydroxymethylation of alanine. In this screening, micro-organisms were obtained from soil enrichment cultures, and the cell-free extracts were assayed for the ability of enzymes to release formaldehyde from α-methyl-DL-serine 27 (*Scheme 11*). Six kinds of enzymes were shown to release formaldehyde. Three enzymes were characterized to be independent with regard to tetrahydro-folate co-factor, and thus three quite new enzymes were obtained. Three were identified to be *Bosea sp.*, *Ralstonia sp.*, and *Variovorax paradoxus*, respectively.
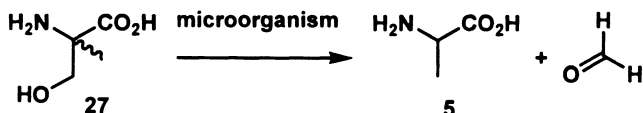
Scheme 11. Screening soil samples for the metabolism of a-methyl-DL-serine

The enzymes were isolated from the cell-free extracts, using three chromatographies, such as ion-exchange, hydrophobic interaction, and hydroxyapatite chromatography, and were finally confirmed to be electrophoretically pure. The enzymes were then analyzed to determine their amino acid sequences, and the encoding genes were cloned. Eventually, the cloned genes were respectively transferred to *E.coli.* and overexpressed to make recombinant cells. The three kinds of recombinant cells were used for the synthesis of α-methyl-L-serine **6**.

Enzymatic Synthesis of α-Methyl-L-Serine

The recombinant cells were respectively mixed with L-alanine **28** and formaldehyde, and reacted in phosphate buffer at 30°C (*Scheme 12*). The results are summarized in *Table 2*.

As described in *Table 2*, optically pure α-methyl-L-serine **6** was obtained in all cases with excellent reaction yields (16). A small amount of D-alanine was

H_2N—CO_2H (28) + (HCHO) $\xrightarrow[\substack{100mM\ KPB\ (pH7.4) \\ 0.1mM\ PLP,\ 30°C}]{\text{Recombinant cell}}$ H_2N—CO_2H / HO (6)

Scheme 12. Synthesis of α-methyl-L-serine using recombinant cells

observed in the reaction mixture, but these enzymes did not catalyze the hydroxymethylation of D-alanine in separate experiments. An enzyme derived from *Bosea sp.* was used for kg-scale synthesis and α-methyl-L-serine **6** was successfully obtained.

Table 2. Synthesis of α-Methyl-L-Serine Using Recombinant Cells

Origin	L-Ala (mmol)	HCHO (mmol)	α-Me-L-Ser yield (%)
Varivorax	15	14	98
Bosea	15	15	95
Ralstonia	60	60	89

Synthesis of α-Methyl-L-Cysteine from α-Methyl-L-Serine

The chlorination conditions for α-methyl serine **6** were screened. In the first trials using thionyl chloride or phosphorous pentachloride, the desired β-chlorinated product was not obtained. These results suggest that the bulkiness around the quaternary carbon could interfere with the substitution on the β-carbon.

As depicted in *Scheme 13*, a methansulfonate **29** was prepared from α-methyl-L-serine **6** in several steps, and reacted with thioacetamide **18**. Under the same conditions as in the reaction of α-methyl-β-chloroalanine methylester **16**, no desired product was observed in the reaction mixture.
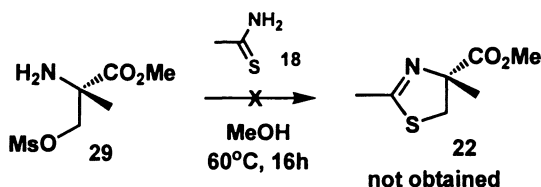
Scheme 13. Reaction of the mesylate with thioacetamide

Finally, the chlorination of N-Cbz-α-methyl-L-serine methylester **31** proceeded with triphenylphosphine in carbontetrachloride upon heating at 80°C for 16 hours (*Figure 14*). The desired product **32** was isolated in 37% yield. After acidic hydrolysis of the Cbz-group, α-methyl-β-chloroalanine methylester **26** was converted to the hydrochloride of α-methyl-L-cysteine **1** through the reaction with thioacetamide and subsequent acidic hydrolysis. Although the conditions have not been optimized and the yield is low, a new method was realized for synthesizing α-methyl-L-cysteine **1**.
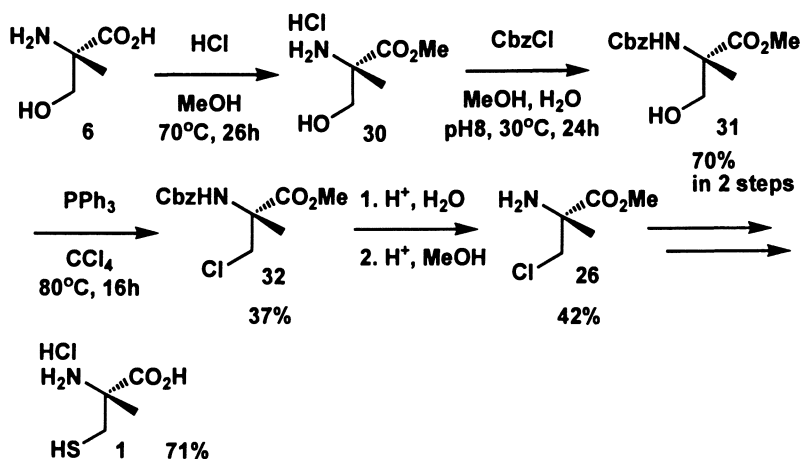
Scheme 14. Synthesis of a-methyl-L-cysteine from a-methyl-L-serine

α-Methyl-L-Serine as Chiral Building Block

There have been many studies on the chemical transformation of chiral α-methyl serine into advanced compounds. In those studies, activated compounds such as β-lactone **33**, sulfamidate **34** and aziridine **35** were synthesized as key intermediates (17). The aldehydes **36** and **37** derived from α-methyl-L-serine **6** are also interesting intermediates for the alkylation by Grignard reagents or Wittig reagents, etc. (18).

Conclusions

New methods for the synthesis of α-methyl-L-cysteine **1** and α-methyl-L-serine **6** with biotransformation in key steps were developed. With the use of
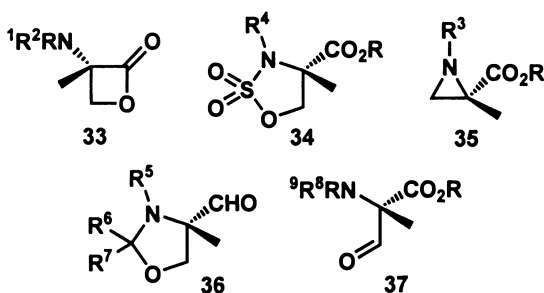
Figure 3. Chiral building blocks derived from α-methyl-L-serine

odorless thioacetamide **18**, racemic 2,4-dimethyl-4-methoxycarbonylthiazoline **20** was prepared, and D-selectively hydrolyzed to (4*R*)-2,4-dimethyl-4-methoxycarbonylthiazoline **22** with some commercially available enzymes. After the stepwise hydrolysis of **22**, the hydrochloride of α-methyl-L-cysteine **1** was obtained in high optical purity.

By screening soil samples, we identified new enzymes that could catalyze the hydroxymethylation of L-alanine **28** independent of the unstable cofactor tetrahydrofolate, and the one-pot synthesis of α-methyl-L-serine **6** was realized using recombinant cells. Finally, the synthesis of α-methyl-L-cysteine **1** was demonstrated starting from α-methyl-L-serine **6**.

References

1. (a) Khosla, M. C.; Stachowiak, K.; Smeby, R. R.; Bumpus, F. M.; Piriou, F.; Lintner, K. Fermandjian, S. *Proc. Nat. Acad. Sci. USA*, **1981**, *78*, 757-760. (b) Valle, G.; Crisma, M.; Toniolo, C.; Beisswenger, R.; Rieker, A.; Jung, G. *J . Am. Chem. Soc.* **1989**, *111*, 6828-6833. (c) Altmann, K-H.; Altmann, E.; Mutter, M. *Helv. Chim. Acta* **1992**, *75*, 1198-1210.
2. Shao, H.; Zhu, Q.; Goodman, M. J.; *J. Org. Chem.* **1995**, *60*, 790.
3. Levine, R. J.; Sjoerdsma, A. J. *J. Pharmacol. Exp. Ther.* **1964**, *146*, 42-47.
4. (a) Pattenden, G.; Thom, S. M.; Jones, M. F. *Tetrahedron* **1993**, *49*, 2131-2138. (b) Bergeron, R. J.; Wiegand, J.; McManis, J. S.; Bharti, N. *J. Med. Chem.* **2006**, *49*, 7032-7043.
5. Groth, U.; Schöllkopf, U. *Synthesis* **1983**, 37-38.
6. (a) Fukuyama, T.; Xu, L. *J. Am. Chem. Soc.* **1993**, *115*, 8449-8450. (b) Kedrowski, B. L. *J. Org. Chem.* **2003**, *68*, 5403-5406
7. (a) Ohishi, T.; Nanba, H.; Sugawara, M.; Izumida, M.; Honda, T.; Mori, K.; Yanagisawa, S.; Ueda, M.; Nagashima, N.; Inoue, K. *Tetrahedron Lett.*

2007, *48*, 3437-3440. (b) Preparation of 2-aryl-4-methylthiazol-(4*S*)-carboxylic acids via the condensation of 2-methyl-D-cysteine and arylnitriles: Krich, S.; Rieder, A.; Heu, F.; Steinbauer, G. Eur. Pat. Appl. EP 1302467 A2, April 16, **2003**. (c) Manufacture of optically active 2-alkyl-D-cysteine esters by enzymatic resolution and chemical synthesis: Jpn. Kokai Tokkyo Koho, JP 2006000006 A, January 5, **2006**.

8. Dietsche, T.J.; Gorman, D.B.; Orvik, J.A.; Roth, G.A.; Shiang, W.R. *Org. Process Res. Dev.* **2000**, *4*, 275-285.

9. Behringer, Hans; Zillikens, Paul. *Justus Liebigs Ann. Chem.* **1951**, *574*, 140-156.

10. DL-Cysteine: Irie, Y.; Kakizaki, F.; Eguchi, C. Jpn. Kokai Tokkyo Koho, JP 58010557 A January 21, **1983**.

11. 2-Mercaptoethylamine salts: Noguchi, Y.; Noda, E.; Nakayama, S.; Yamamoto, I.; Kawasaki, N. Jpn. Kokai Tokkyo Koho, JP1982009758 A January 19, **1982**.

12. Sano, K.; Eguchi, C.; Yasuda, N.; Mitsugi, K. *Agric. Biol. Chem.* **1979**, *43*, 2373-2374.

13. Bergeron, R. J.; Wiegand, J.; McManis, J. S.; McCosar, B. H.; Weimar, W. R.; Brittenham, G. M.; Smith, R. E. *J. Med. Chem.* **1999**, *42*, 2432-2440.

14. (a) Production methods of thiazoline compounds and optically active α-alkylcysteine: Hamada, T.; Yatagai, M. Eur. Pat. Appl. EP1721902 A1, November 15, **2006**. (b) Hamada, T.; Yatagai, M.; Izawa, K. To be submitted.

15. Wilson, E. M.; Snell, E. S. *J. Biol. Chem.* **1962**, *237*, 3171-3179.

16. (a) Enzymatic production of L-serine derivative by using microbial 2-methylserine hydroxymethyltransferases: Kuroda, S.; Nozaki, H.; Watanabe, K.; Yokozeki, K.; Imabayashi, Y.: PCT Int. Appl. WO 2006123745 A1 November 23, **2006**. (b) Bacterial 2-methylserine hydroxymethyl-transferases and their use for producing β-hydroxy amino acids: Nozaki, H.; Kuroda, S.; Watanabe, K.; Yokozeki, K. Eur. Pat. Appl. EP 1724356 A1, November 22, **2006**.

17. (a) Smith, N. D.; Goodman, M. *Org. Lett.* **2003**, *5*, 1035-1037. (b) Burgaud, B. G. M.; Horwell, D. C.; Padova, A.; Pritchard, M. C. *Tetrahedron* **1996**, *52*, 13035-13050. (c) Boulton, L. T.; Stock, H. T.; Raphy, J.; Horwell, D. C. *J. Chem. Soc. Perkin Trans. 1*, **1999**, 1421-1430.

18. (a) Avenoza, A.; Cativiela, C.; Peregrina, J.; Sucunza, D.; Zurbano, M. M. *Tetrahedron: Asymmetry* **1999**, *10*, 4653-4661. (b) Avenoza, A.; Cativiela, C.; Corzana, F.; Peregrina, J. M.; Zurbano, M. M. *Tetrahedron: Asymmetry* **2000**, *11*, 2195-2204.

Chapter 25

A Novel Method of Amino Acids in a Cell Factory

Motoko Hayashi and Hiroaki Yamamoto

Himeji Research Center, Daicel Chemical Industries, Ltd., 1239 Shinzaike, Aboshi-ku, Himeji, Hyogo 671–1283, Japan

A novel method for the synthesis of amino acids is described: deracemization from racemate to a single enantiomer with only one recombinant *E. coli*. The plasmid harbored 4 genes required for the reaction, which code D-amino acid oxidase, L-amino acid dehydrogenase, formate dehydrogenase, and catalase on a single operon. With this recombinant *E. coli*, the entire deracemization process was carried out in a single cell, *i.e.*, "cell factory."

Introduction

Generally, many steps are needed to synthesize a desired compound. A "one-pot reaction" is a strategy that seeks to achieve sequential reactions in a single reactor. There is no need for purification at each step. Instead, the reaction must be checked, the next compounds are added, and the reaction conditions are adjusted.

Enzymatic reactions are attractive methods in organic synthesis because they exhibit high chemo-, regio-, and enantioselectivity. Such high selectivity reduces the need for purification. Another attraction is that such reactions can be carried out under the ambient conditions, *i.e.*, under similar conditions. Thus, the reactions with some enzymes are suitable for use as one-pot reactions.

Amino acids are popular starting materials for asymmetric synthesis, (the so-called chiral pool method) because they are natural compounds with chiral centers (1). Since amino acids have common structures, we can plan a process from amino acids regardless of whether they are natural or unnatural. When we

need an unnatural aromatic amino acid, we can easily synthesize it, *e.g.* by amination of the corresponding α-keto acid. On the other hand, we cannot use this synthetic route for industrial production due to the cost of the corresponding α-keto acid when we need an unnatural amino acid with an alkyl chain (Figure 1). As a result, we select deracemization from a racemic amino acid, where the undesired enantiomer is converted into the desired one. Turner reported one-pot amino acid synthesis by deracemization (2). This process consisted of the
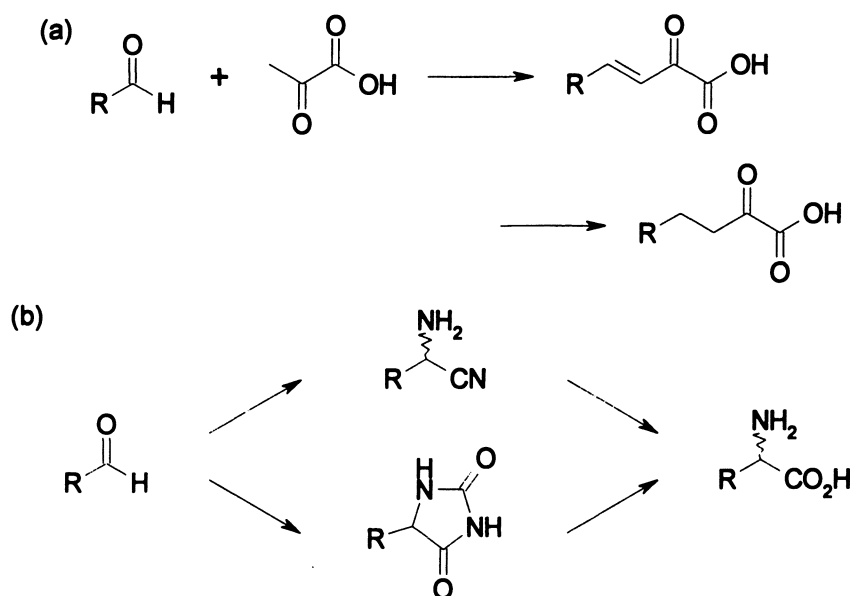
Figure 1. Synthesis of (a) α-keto acid and (b) racemic amino acid.
(a) R = Aromatic group: α-Keto acid should be obtained without side reactions.
R = Aliphatic group: Aldol condensation should occur since aliphatic aldehyde
has α-hydrogen(s). (b) Racemic amino acid is obtained by Strecker reaction.

enantioselective oxidation of D-amino acid to imine by D-amino acid oxidase and the reduction of imine to racemic amino acid by a hydride reducing agent or metal catalyst (Scheme 1).

We used an unnatural amino acid, L-norvaline (L-2-aminopentanoic acid, L-Nva), as a target material. We designed a reaction so that all of the steps of deracemization for the production of L-Nva could be performed using only enzymes (3, 4). We used whole recombinant cells as a biocatalyst, since living cells, which contain enzymes, can multiply by cultivation. In addition, since the
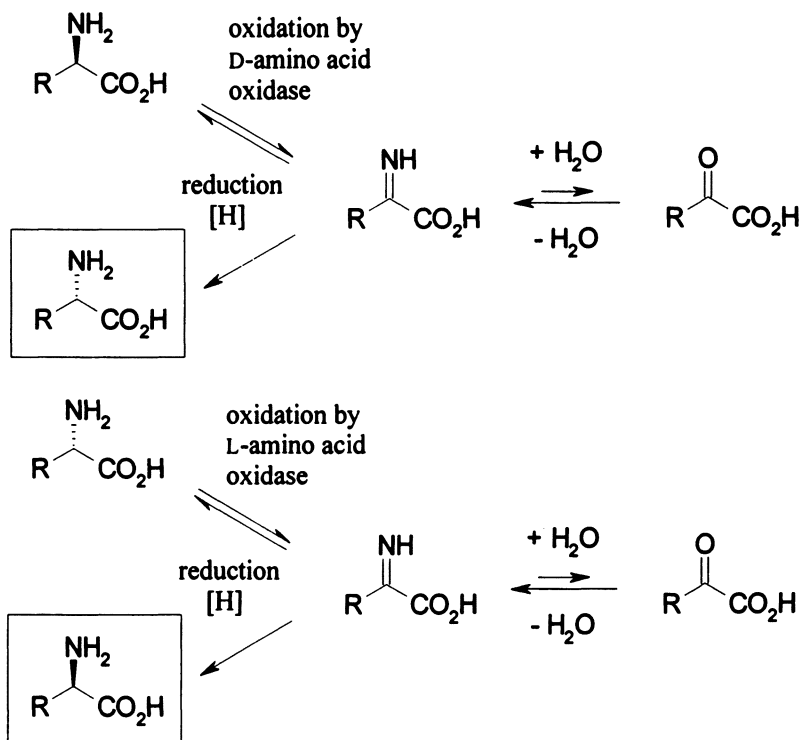
Scheme 1. Reproduced from reference 2. By permission of The Royal Society of Chemistry.

genes coding the necessary enzymes were expressed on a single operon, all of the necessary catalysts could be obtained by a single cultivation. Since all of the steps of deracemization proceeded in a single cell rather than in one pot, we call this approach "a cell factory."

Results and Discussion

Screening of Enzymes

Several routes are possible for deracemization (Scheme 2). Our enzyme library had one D-amino acid dehydrogenase, two D-amino acid oxidases, six amino acid dehydrogenases, and two amino acid transaminases. Enzyme screening was performed with these enzymes. *Escherichia coli* was used as a host microorganism for the expression of these genes.

Route 1
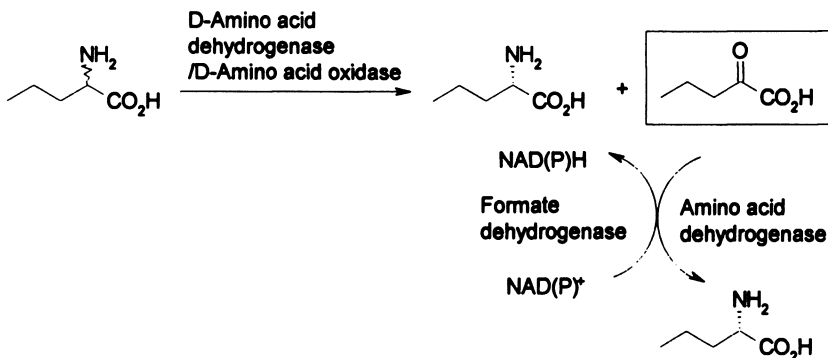
Route 2
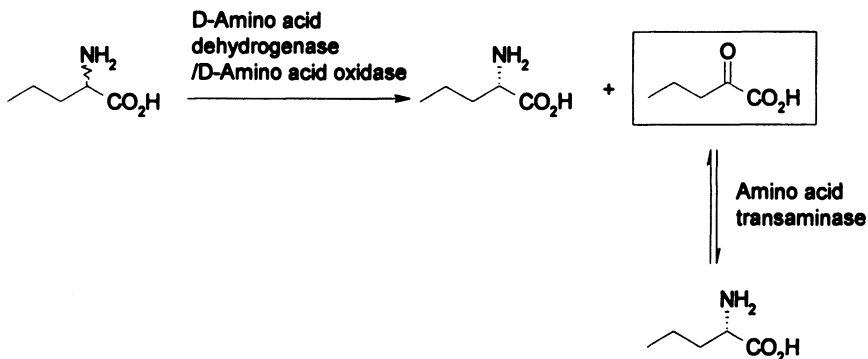
Scheme 2

The enzyme for the first step [conversion of D-Nva to 2-oxopentanoic acid (2-ketovaleric acid, KVA)] might be a D-amino acid dehydrogenase or a D-amino acid oxidase. Since the high expression of these enzymes affects the metabolism of D-amino acid and inhibits the synthesis of cytomembrane, they might be toxic for the host. In fact, since transformants that expressed D-amino acid dehydrogenase with high efficiency did not exhibit constant enzymatic activities, D-amino acid oxidase from *Candida boidinii* (CbDAO) (5) was selected. Fortunately, CbDAO was not inhibited by L-Nva.

The second step (conversion of KVA, the product of the first step, to L-Nva) was thought to be transamination with amino acid transaminase and reductive amination with L-amino acid dehydrogenase. Transamination is an equilibrium reaction and L-glutamate is usually used as an amino-donor. If L-aspartate is used as an amino-donor, the resulting oxaloacetate may be decomposed into

pyruvate, and thus the equilibrium is probably shifted to the right (Scheme 3). In fact, the reaction with transformants that highly expressed amino acid transaminase and L-aspartate as an amino-donor did not show the expected equilibrium shift. In the best result, 17 equiv. of L-glutamate was needed to reach >99% conversion with a branched-chain transaminase from *Escherichia coli*, which is unfavorable for an industrial process.

We also screened amino acid dehydrogenase, including alanine dehydrogenases (AlaDH), leucine dehydrogenases (LeuDH), and phenylalanine dehydrogenase (PheDH). AlaDHs exhibited low activities for KVA. Although LeuDHs had adequate enzymatic activity, a reaction with over 4% substrate did not run to completion due to substrate inhibition or inactivation of the enzyme by a high concentration of substrate. PheDH from *Thermoactinomyces intermedius* (TiPheDH) (6) had sufficient activity and inhibition or inactivation was not observed. It also showed perfect enantioselectivity, in that the D-form
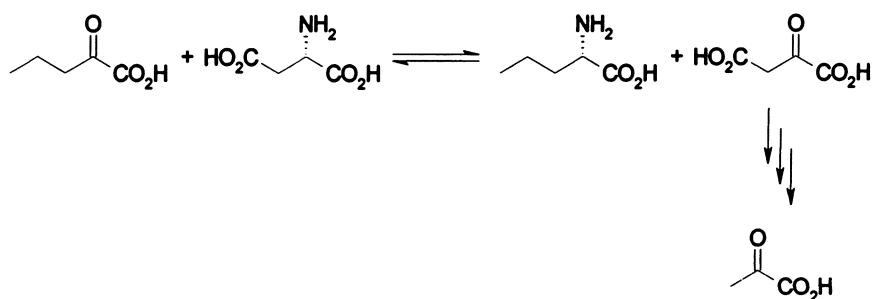
Scheme 3

was not detected in the reaction. A mutant of formate dehydrogenase from *Mycobacterium vaccae* (McFDH) which was modified to resist organic solvents was used to regenerate coenzyme NADH (7), since this reaction was essential for reductive amination with L-amino acid dehydrogenase.

In summary, three enzymes (CbDAO to oxidize D-Nva, TiPheDH to produce L-Nva, and McFDH to regenerate coenzyme) were selected.

Coexpression of Three Genes Comprising a Single Operon

We constructed plasmids that expressed the genes of the above three enzymes. Figure 2 shows maps of these plasmids. pSE420U is a versatile vector that enables the expression of these three genes like an operon (8). The first plasmid contained a CbDAO-gene, an McFDH-gene, and a TiPheDH-gene, in
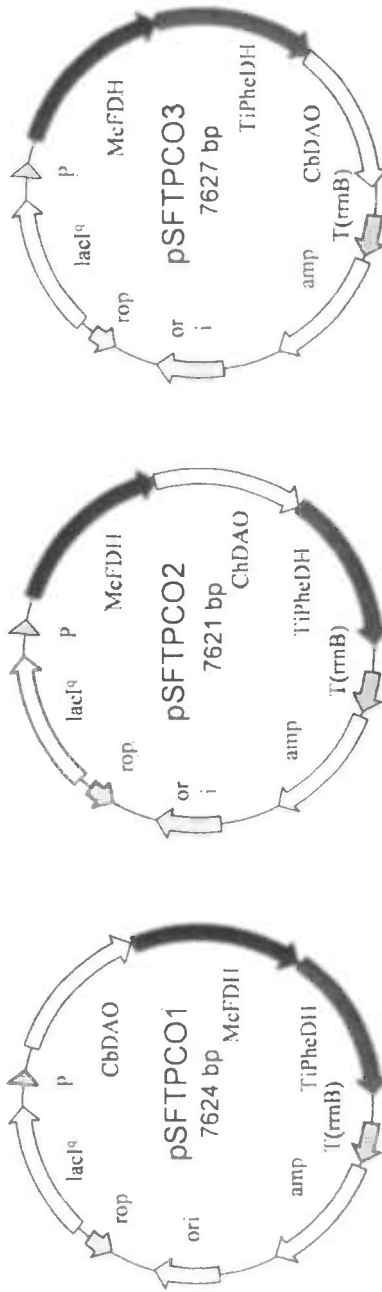
Figure 2. Maps of the plasmids pSFTPCO1, pSFTPCO2, and pSFTPCO3 harbored 3 genes, CbDAO, TiPheDH, and McFDH. CbDAO, D-amino acid oxidase from Candida boidinii; McFDH, formate dehydrogenase from Mycobacterium vaccae; TiPheDH, phenylalanine dehydrogenase from Thermoactinomyces intermedius; P, trc promoter; T(rrnB), rrnB terminator; amp, ampicilli- resistance gene; ori, origin of replication; rop, rop protein gene; lacI^q, lactose repressor.

that order, and was named pSFTPCO1. The second contained an McFDH-gene, a CbDAO-gene, and a TiPheDH-gene and was named pSFTPCO2. The third contained an McFDH-gene, a TiPheDH-gene, and a CbDAO-gene and was named pSFTPCO3. Escherichia coli HB101 was transformed with each plasmid. The McFDH gene was put at the first or second cistron, since it had less molecular activity than the others. The obtained transformants were cultured and the activities of enzymes were determined. Cells reached full-broth under the general conditions, and the activities of the enzymes were expressed with higher efficiency (Table I).

Deracemization with the Three Enzymes

We attempted to deracemize DL-Nva with the above recombinant E. coli that coexpressed the three genes. Since the oxidation with D-amino acid oxidase required oxygen, the speed of stirring was an important factor in the reaction: the harder a reaction mixture was stirred, the faster D-Nva was decreased. DO was 0% in the oxidation of D-Nva, and an increase in DO indicated the disappearance of D-Nva. However, the oxidation product, KVA, did not disappear with further prolongation of the reaction. An analysis of the reaction mixture showed that L-NVA was produced in 60% yield and KVA was produced in 3% yield. These results suggested that the steric inversion of D-Nva into the L-form occurred only partly, and by-product(s) was formed. When D-Nva was used as a substrate, the total yield of D-, L-Nva, and KVA was only 40%. When Nva and the other materials were stirred with E. coli harboring an empty vector, pSE420U, almost all Nva remained in the mixture. These results may be accounted for by the decomposition of Nva and/or an intermediate by hydrogen peroxide. Hydrogen peroxide is generated along with oxidation by oxidase, and it must be decomposed to increase the yield. The best pH for the reaction was 8, since the reaction was slow below pH 7 and the product yield became low above pH 9. This supports the notion that hydrogen peroxide decomposed Nva and/or an intermediate since it is stable under a basic conditions. In addition, butyrate, which is the oxidation product of KVA with hydrogen peroxide, was detected in the reaction mixture.

Improvement of Transformant Coexpressing Four Genes of Enzymes

To increase the yield of L-Nva, two methods were considered. First, the activities of TiPheDH and McFDH could be increased, and the reaction from KVA to L-Nva could be accelerated. Plasmids were constructed with different orders of the genes. Although these plasmids gave different balances of enzymatic activities, the reaction yields of L-Nva were reduced. Thus, the

Table I. Enzymatic activities of transformants

Plasmid	McFDH	TiPheDH	CbDAO	EcKatE
pSFTPCO1	0.46	5.56	3.15	-
pSFTPCO2	0.53	3.27	0.66	-
pSFTPCO3	0.63	4.07	0.75	-
pSCCBDO1	-	-	2.01	218
pSFCPCO1	0.63	5.30	2.40	399

NOTE: Units are Units per mg-protein.

decomposition of KVA and/or Nva with hydrogen peroxide was faster than the conversion to L-Nva with TiPheDH and McFDH. Otherwise, pSFTPCO1 gave the best balance of enzymatic activities.

The other method was the removal of hydrogen peroxide from the reaction mixture. Hydrogen peroxide is an oxidant, and a scavenger may be added to the reaction mixture to remove it. On the other hand, catalase can convert hydrogen peroxide to oxygen. Therefore, E. coli was transformed by the plasmid pSCCBDO1 comprised of the genes for D-amino acid oxidase and catalase from E. coli (EcKatE) (9). The enzymatic activities could be measured without disturbing each other (Table I).

The fourth gene, catalase, was inserted into the above plasmid pSFTPCO1, and the resulting plasmid was named pSFCPCO1. Its gene was placed in the fourth frame which is furthest from the promoter since it showed high activity (Figure 3). In general, the farther a gene is from the promoter, the lower its expression. Its transformant showed an ash green appearance because of heme in catalase. The activities of the enzymes are shown in Table I. Those of all the enzymes were expressed with high efficiency even in the transformant with pSFCPCO1.

Deracemization in a Cell Factory

The deracemization process consists of two steps: oxidation of D-Nva to KVA and reductive amination of KVA to L-Nva. We attempted to carry out these reactions under the same conditions. Under aerobic conditions, L-Nva was obtained in 77% yield with 100% e.e. However, the yield of KVA remained at 21% and prolongation of the reaction did not convert it to L-Nva. These facts indicate that while catalase was effective, L-amino acid dehydrogenase and formate dehydrogenase were not.
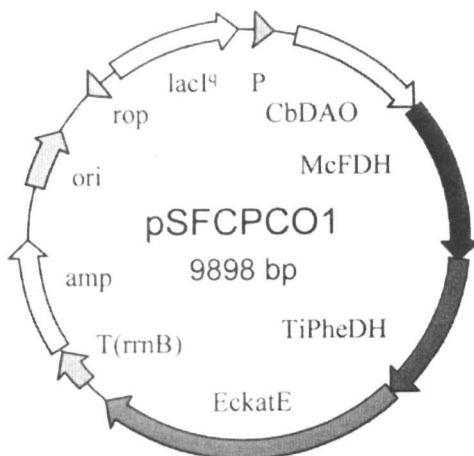
*Figure 3. Map of the plasmid pSFCPCO1, which harbors 4 genes,
CbDAO, TiPheDH, McFDH, and EcKatE.
EcKat E, catalase E from Escherichia coli.*

In general, enzymatic reduction prefers anaerobic conditions, since NADH reduced with FDH might be reoxidized by a respiratory chain. A two-step reaction was then carried out. We sought to achieve the oxidation of D-Nva to KVA in the first step under aerobic conditions. Next, aeration was stopped and reductive amination of KVA to L-Nva occurred in the second step. In the first step, L-Nva was obtained in 60% yield, KVA was obtained in 32% yield, and D-Nva was not detected. The conversion of KVA to L-Nva was achieved in the additional reaction step, and L-Nva was obtained in a final yield of 93% with >99.9% e.e. (8). The time course of deracemization process was shown in Figure 4. The deracemization of Nva was carried out in a single cell that expressed four enzymes. Racemic Nva was incorporated into a cell and L-Nva was released, and this cell acted as an amino acid-producing factory.

Conclusions

Deracemization from racemate to a single enantiomer is a simple process because an additional step, such as racemization in kinetic resolution, is not necessary. Deracemization with a single cell is a versatile process because it can be applied to other target compounds with suitable sets of enzymes.
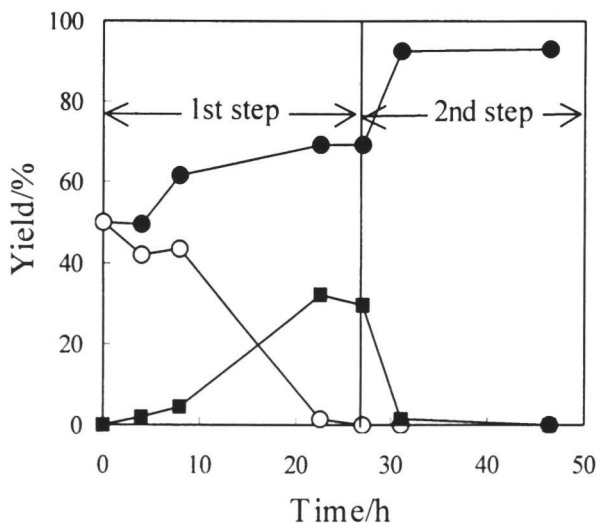
Figure 4. Time course of deracemization process.
Closed circle, L-Nva; open circle, D-Nva; closed square, KVA. 1st step is
indicated the reaction with aeration, and 2nd step is indicated without aeration.

References

1. (a) K. Nakanishi, A. Takeuchi, and R. Matsuno, *Appl. Microbiol. Biotechnol.*, **1990**, *32*, 633-636. (b) U. Nagel, *Angew. Chem. Int. Ed.*, **1984**, *23*, 435-436.
2. (a) F.-R. Alexandre, D. P. Pantaleone, P. P. Taylor, I. G. Fotheringham, D. J. Ager, and N. J. Turner, *Tetrahedron*, **2002**, *43*, 707-711. (b) T. Beard and N. J. Turner, *Chem. Commun.*, **2002**, 246-247. (c) A. Enright, F.-R. Alexandre, G. Roff, A. G. Fotheringham, M. J. Dawson, and N. J. Turner, *Chem. Commun.*, **2003**, 2636-2637. (d) G. J. Roff, R. C. Lloyd, and N. J. Turner, *J. Am. Chem. Soc.*, **2004**, *126*, 4098-4099.
3. N. Nakajima, N. Esaki, and K. Soda, *J. Chem. Soc. Chem. Commun*, **1990**, 947-948.
4. For a review, see: R. N. Patel, *Biomolecular Engineering*, **2001**, *17*, 167-182 and references cited therein.
5. H. Yurimoto, T. Hasegawa, Y. Sakai, and N. Kato, *Yeast*, **2000**, *16*, 1217-1227.
6. H. Takeda, T. Yoshimura, T. Ohshima, N. Esaki, and K. Soda, *J. Biochem.*, **1991**, *109*, 371-376.

7. H. Yamamoto, K. Mitsuhashi, N. Kimoto, Y. Kobayashi, and N. Esaki, *Appl. Microbiol. Biotechnol.*, **2005**, *67*, 33-39.
8. M. Hayashi, H. Yamamoto, and N. Kimoto, WO patent, 132145, 2006.
9. I. v. Ossowski, M. R. Mulvey, P. A. Leco, A. Borys, and P. C. Loewen, *J. Bacteriol.*, **1991**, *173*, 514-520.

Application of Enantiomerically Pure α-Amino Acids in the Total Synthesis of Complex Natural Products and Peptides

Chapter 26

New Tricks in Amino Acid Synthesis: Applications to Complex Natural Products

Robert M. Williams and Cameron M. Burnett

Department of Chemistry, Colorado State University,
Fort Collins, CO 80523

We report the application of diphenyloxazinone glycinate chiral templates to asymmetric syntheses of cylindro-spermospin, 7-*epi*-cylindrospermopsin, 7-deoxycylindro-spermopsin, and spirotryprostatins A and B. Synthetic studies toward quinine, nakadomarin A, and palau'amine using these templates are also described.

We have previously described (*1*) the use of commercially available diphenyloxazinones **1** and **2** as versatile templates for the asymmetric synthesis of amino acids and natural products containing amino acids. Herein we will detail the use of these templates for the synthesis of complex natural products, many with no apparent amino acid functionality.

(+)-1, R = Cbz
(+)-2, R = Boc

(-)-1, R = Cbz
(-)-2, R = Boc
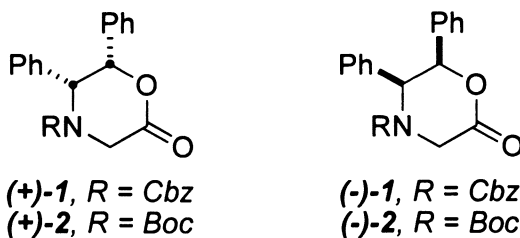
Figure 1. The diphenyloxazinone glycine templates.

Cylindrospermopsin

Cylindrospermopsin (3) was isolated from the marine cyanobacterium *Cylindrospermopsis raciborskii* in 1992 (2). Other members of the family include 7-*epi*-cylindrospermopsin (4), isolated from *A. ovalisporum* (3), and 7-deoxycylindrospermopsin (5), isolated from *C. raciborskii* (4). While 3 and 4 were shown to be equipotent hepatotoxic glutathione biosynthesis inhibitors, the deoxy analogue 5 showed no activity. During the course of our efforts, Snider (5) and Weinreb (6) had reported racemic syntheses of 3, while White (7) had synthesized 4 in optically active form.

cylindrospermopsin (3): R_1 = OH, R_2 = H
7-epi-cylindrospermopsin (4): R_1 = H, R_2 = OH
7-deoxycylindrospermopsin (5): R_1 = R_2 = H
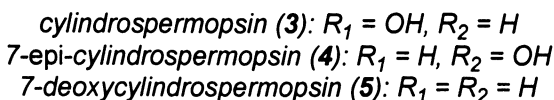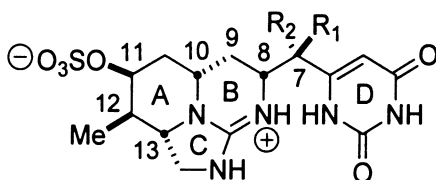
Figure 2. Members of the cylindrospermopsin family.

We began by alkylation of the enolate of (+)-2 with homoallyl iodide to yield alkene 6 (Scheme 1), planning for the new chiral center to set by relay the remaining stereocenters of the molecule. Removal of the chiral template via Birch reduction furnished protected amino acid 7; treatment with acetyl chloride cleaved the Boc group and converted the acid to its mixed anhydride, whose LAH reduction furnished the free amino alcohol 8. Treatment with phenyl bromoacetate under basic conditions gave oxazinone 9, which was unstable to dimerization; immediate oxidation with *m*-CPBA yielded the stable nitrone 10.

At this point we attempted our key intramolecular nitrone 1,3-dipolar cycloaddition and were gratified to obtain the desired adduct 11 in 78% yield as a single diastereomer, albeit as a 10:1 mixture with the regioisomer resulting from *endo* cyclization. The stereochemical outcome, confirmed by X-ray analysis, results from suprafacial addition to the alkene by the nitrone in an *exo* fashion, as shown in the transition state A. This cycloadduct contained the necessary stereochemistry for the A ring of the cylindrospermopsin family. (8)

The lactone of 11 was reduced to the corresponding lactol, and reductive amination under hydrogen gave the free amine while also reducing the labile N-

O bond; treatment with *p*-nitrophenylcarbonate yielded the urea **12** (Scheme 2). After much experimentation, we found that subjection of the diol to TEMPO and PhI(OAc)$_2$, along with a catalytic amount of MsOH to aid disproportionation of the nitroxyl radical, gave the desired aldehyde **13** resulting from selective oxidation of the primary alcohol. Treatment of **13** with lithiated nitromethane provided the corresponding β-hydroxynitro compound as a mixture of diastereomers; treatment with acetic anhydride resulted in elimination to the nitroalkene as well as protection of the secondary alcohol, and *in situ* conjugate reduction with sodium borohydride gave the nitroalkane **14**. Refluxing in neat TFA removed the PMB protection, and conversion to the *O*-ethyl isourea **15** was effected with Meerwein's salt.

TBAF-mediated nitroaldol reaction of **15** with the dimethoxypyrimidine aldehyde **16** gave the adduct **17**; immediate quenching was required to prevent retro-nitroaldol reaction and equilibration to an equimolar mixture of all four possible diastereomers. The quenched adduct **17** was immediately hydrogenated to effect reductive guanidinylation of the nitro group onto the isourea, yielding **18** as an inseparable 1:0.8 mixture of diastereomers. Acidic hydrolysis of the acetyl group and pyrimidines produced a separable mixture. Finally, treatment of **18** with sulfur trioxide-pyridine and molecular sieves in DMF gave a moderate yield of 7-*epi*-cylindrospermopsin (**4**) as a 2:1 mixture with the bis-sulfate (*9*). Cylindrospermopsin (**3**) was synthesized in a similar fashion (*10*).

Nitroaldol reaction between **15** and benzylpyrimidine **19** with cesium fluoride and acetic anhydride produced directly the *E*-nitroalkene **20** (Scheme 3). Treatment with sodium borohydride effected conjugate reduction, and hydrogenation gave the pyrimidine **21** as an equimolar mixture of diastereomers at C8. Cleavage of the acetate and sulfonation yielded 7-deoxy-cylindrospermopsin (**5**) and its diastereomer **22**. With this material in hand, we showed that the proposed structure for **5** was incorrect, and that contrary to previous reports, **5** showed potent protein synthesis inhibitory activity (*11*).

Quinine

Quinine (**23**, Figure 3) is among the most famous alkaloids due to its antimalarial properties and has long been the target of synthetic research. Stork closed the quinuclidine ring at the N1-C6 bond in his stereoselective synthesis (*12*), while Jacobsen (*13*) and Kobayashi (*14*) used the classical Rabe N1-C8 disconnection. Our plans called for a novel intramolecular S$_N$2′ reaction to install the C3-C4 bond.

Our synthesis began with formation of the silyl enol ether **24** of lactone (+)-**2** in near-quantitative yield (Scheme 4). Treatment of **24** with TBAF in the presence of aldehyde **25** led to a diastereoselective aldol reaction to give the thermodynamic adduct, containing the correct C8 and C9 stereochemistry as confirmed by X-ray crystallographic analysis of the TES-protected alcohol **26**.
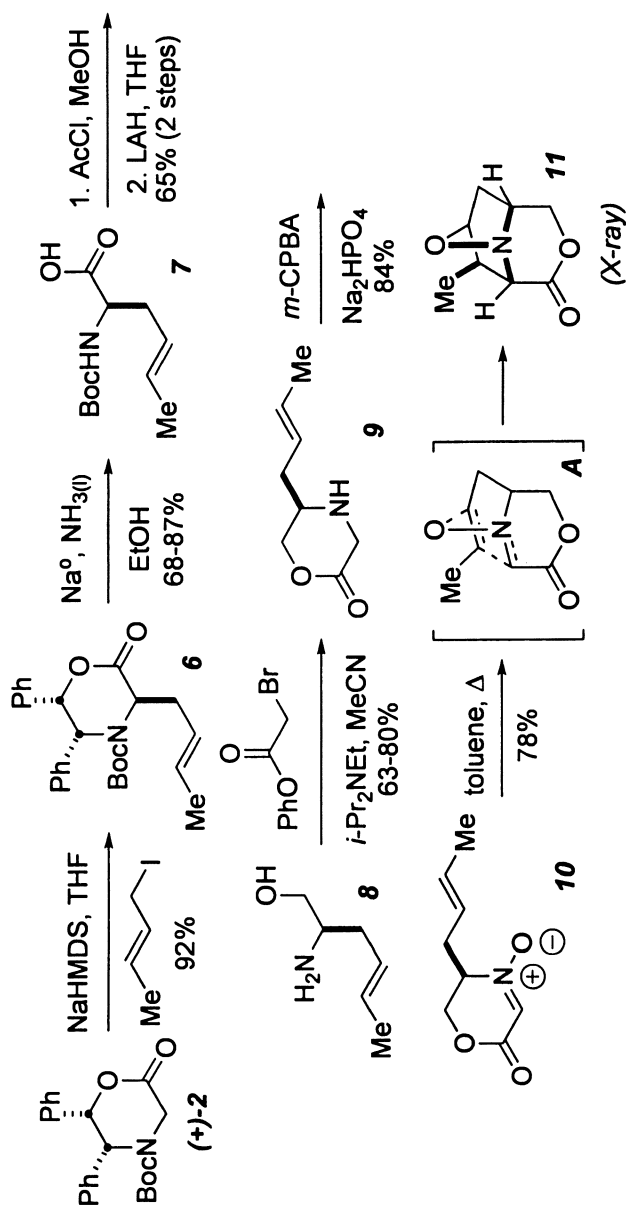
Scheme 1. Synthesis of the A-ring of cylindrospermopsin.

Scheme 2. Synthesis of 7-epi-cylindrospermopsin (4).
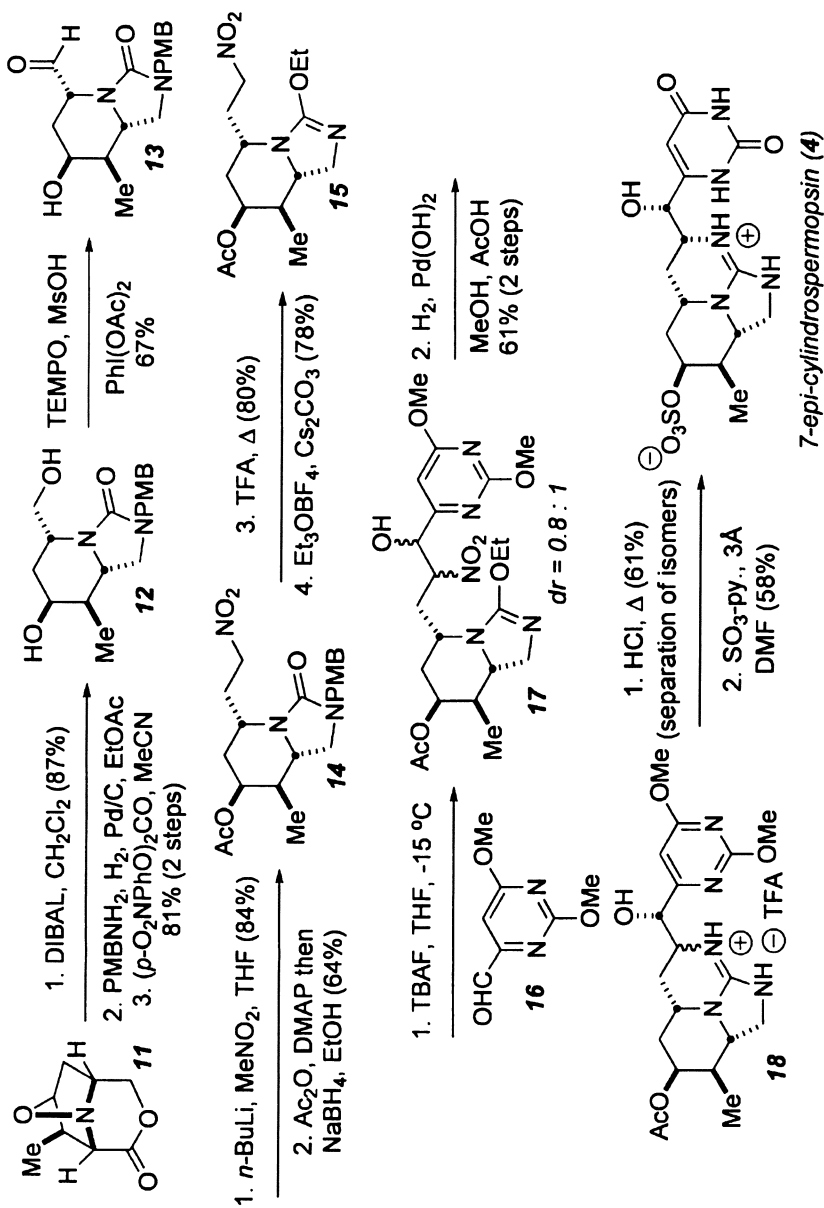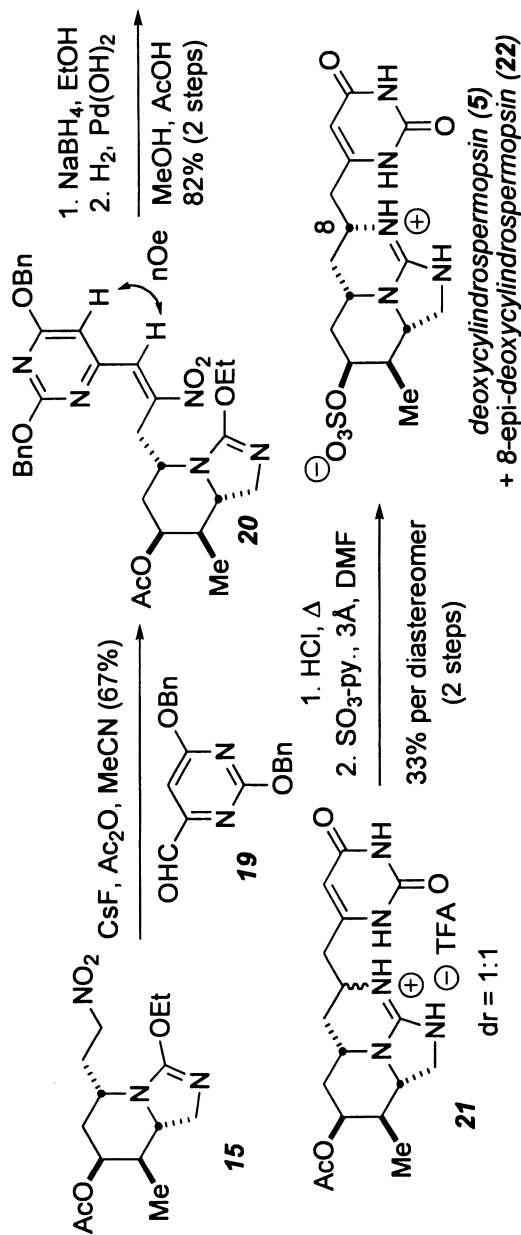
Scheme 3. Synthesis of 7-deoxycylindrospermopsin (5).
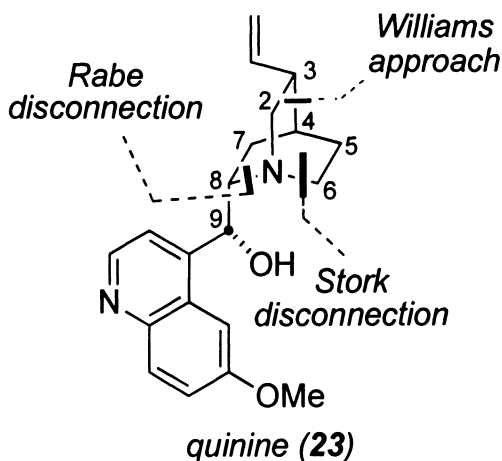
Figure 3. Retrosynthetic disconnections of quinine.

Removal of the *N*-Cbz group followed by a three-step protocol to remove the chiral auxiliary yielded the fully protected amino acid **27**. Direct reduction of the ester to the aldehyde **28**, followed by a Grignard reaction, produced a single diastereomeric alcohol. It was subsequently found that the stereochemistry of the secondary alcohol was crucial for a successful piperidine-forming reaction, and the product directly obtained from this sequence did not lead to the desired piperidine system. Thus, the resultant alcohol was oxizided and diastereoselectively reduced to secure **29** with the opposite carbinol stereochemistry, which led to a productive piperidine-forming reaction. Acetate protection, conversion of the benzyl ether to the corresponding mesylate **30** in two steps, and treatment with sodium hydride effected cyclization to the piperidine ring of **31** in excellent yield.

At this point we converted the acetate of **31** to the corresponding *O*-TMS ether substrate **32** (Scheme 5). Thus, treatment of **31** with lithium aluminum hydride, followed by treatment with trimethylsilyl triflate and N-alkylation, provided **32** in high yield for the three steps. Acidic removal of the *O*-TMS residue and oxidation to the ketone **33** followed by formation of the silyl enol ether furnished the key substrate **34**. Treatment of **34** with tributyltin fluoride in the presence of Pd(II) and a phosphine ligand effected the desired C3-C4 closure to the quinuclidine and installed the vinyl group with the correct relative stereochemistry. The incipient keto-quinuclidine proved rather labile to purification and handling and was immediately reduced to furnish 7-hydroxyquinine **35** (*15*). We are currently examining methods for deoxygenation of the quinuclidine **35** to yield quinine **24**.
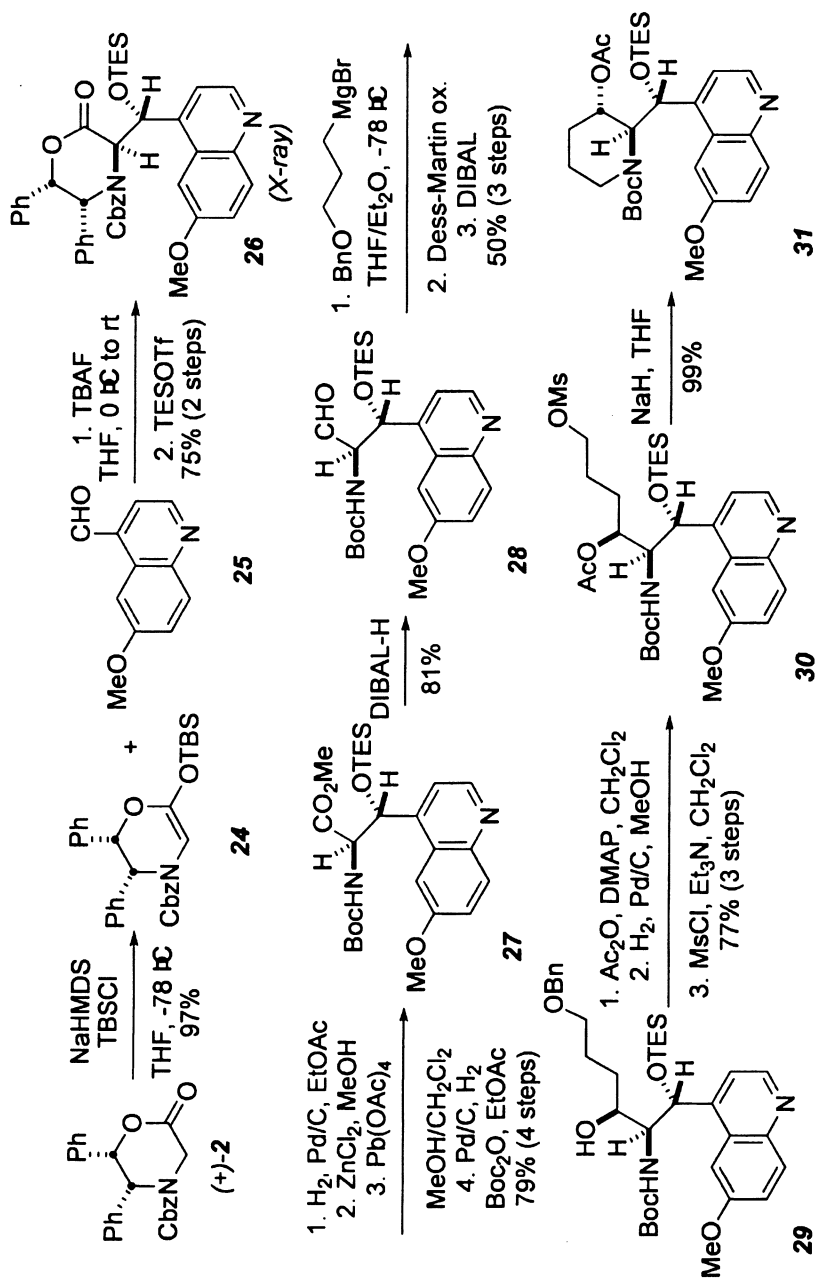
Scheme 4. Synthesis of the piperidine 31.

428
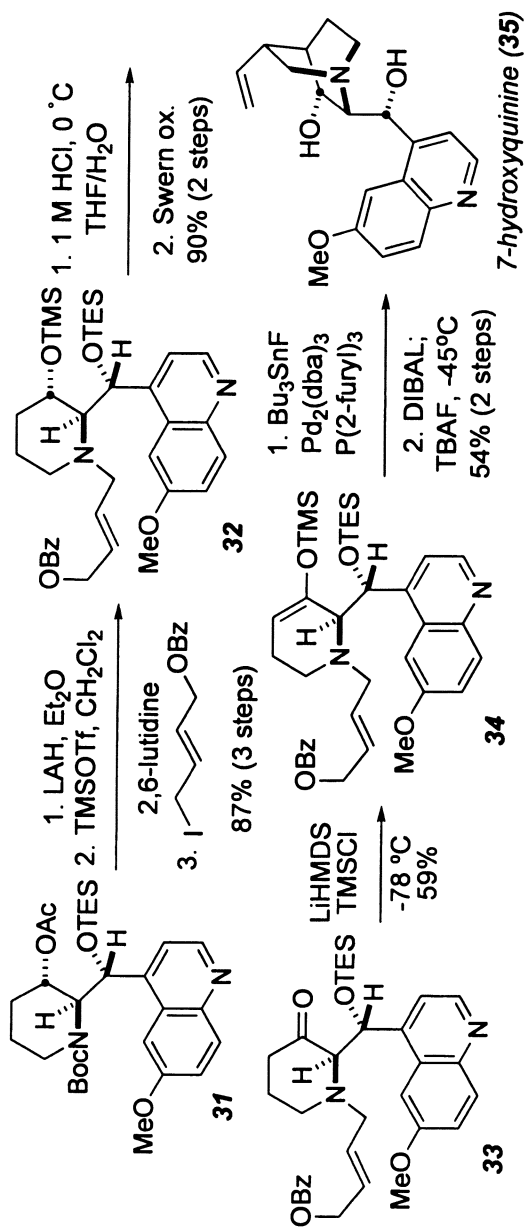
Scheme 5. Synthesis of 7-hydroxyquinine (35).

Spirotryprostatins A and B

We have developed concise asymmetric syntheses of spirotryprostatins A and B, structurally interesting antimitotic arrest agents isolated by Osada (*16*) that have attracted considerable attention from the synthetic community (*17*). Our approach deploys a novel three-component azomethine ylide cycloaddition strategy to assemble the prenylated *spiro*-oxindole pyrrolidine in a single stereocontrolled step. Our synthesis of spirotryprostatin B commenced with condensation of oxazinone **36** with aldehyde **37** to generate the incipient iminium species, whose deprotonation yields the azomethine ylide **39** (Scheme 6). Dipolar cycloaddition reaction with the readily available dipolarophile **38** provided the tetracyclic cycloadduct **40** in excellent yield. This reaction sets four consecutive stereogenic centers, including the quaternary *spiro*-ring junction, whose stereochemistry was confirmed by X-ray crystallographic analysis.

Hydrogenation of **40** in the presence of palladium chloride quantitatively cleaved the chiral auxiliary from **40** (Scheme 7) to furnish the free amino acid **41**. Fortuitously, we found that **41** could be selectively coupled with D-proline benzyl ester without the need to protect the proline amine of **41**. This is presumably a manifestation of the severe steric hindrance of the proline amine on both faces, which obviates self-condensation. Removal of the benzyl ester and cyclization gave dioxopiperazine **42** in excellent yield. Treatment with two equivalents of tosic acid in refluxing toluene effected regioselective elimination of methanol to furnish alkene **43**. The final oxidative decarboxylation of **43** to spirotryprostatin B proved extremely difficult and required the extensive evaluation of methods to effect this seemingly trivial transformation. Standard Kochi-type oxidative decarboxylation methods led to significant decomposition and over-oxidation side products. Eventually, we found that Barton-modified Hunsdiecker conditions effected the oxidative decarboxylation and installed the desired alkene in the central pyrrolidine ring. In the event, lithium iodide cleaved the ethyl ester of **43** to yield the corresponding acid. Subjection of the resultant acid **44** to a Barton-modified Hunsdiecker protocol installed the desired alkene in modest, but reproducible, yield. Finally, the proline residue was epimerized under thermodynamic conditions (2:1 favoring the natural stereochemistry) to complete the synthesis of spirotryptostatin B (**44**) (*18*).

We next pursued a similar strategy toward spirotryprostatin A, but unfortunately observed that the Hunsdiecker reaction on a substrate corresponding to **43** completely failed in this case. In light of this, we decided to do away with the troublesome carboalkoxy residue entirely.

Thus, dipolar cycloaddition of the labile exocyclic alkene **45**, prepared *in situ* from the corresponding β-TMS alcohol, with the ylide derived from condensation of **36** and **37** gave the desired adduct **46** along with the methanol elimination product (Scheme 8). Hydrogenation removed the bibenzyl auxiliary, and thermodynamic epimerization at C9 yielded an inseparable mixture of diastereomers **47** that was carried forward to dioxopiperazine **48**, which could
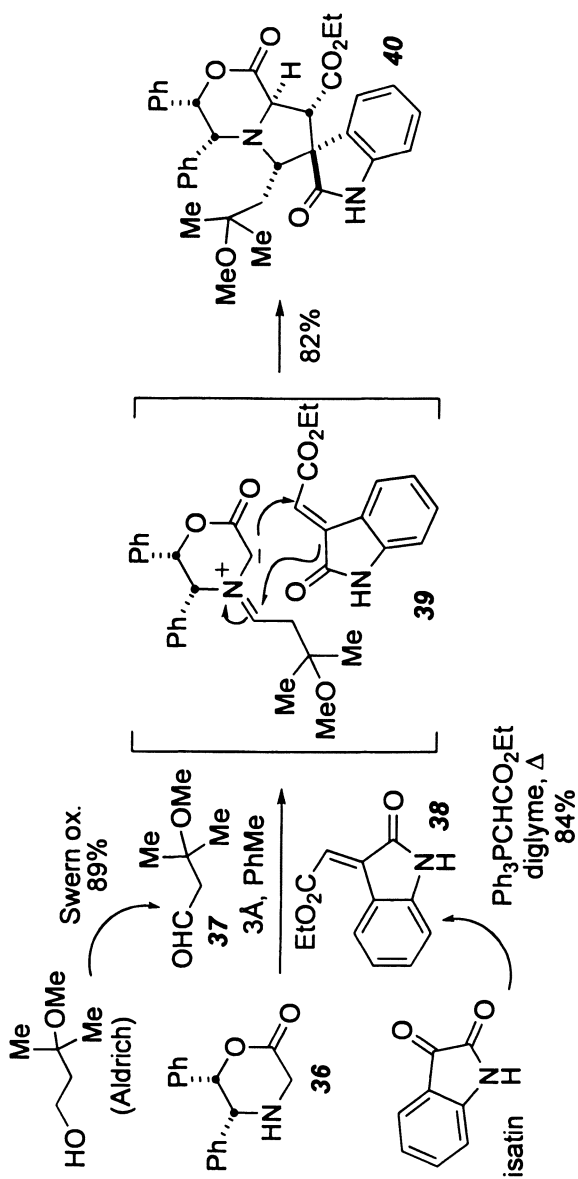
Scheme 6. Synthesis of the spiro ring junction.
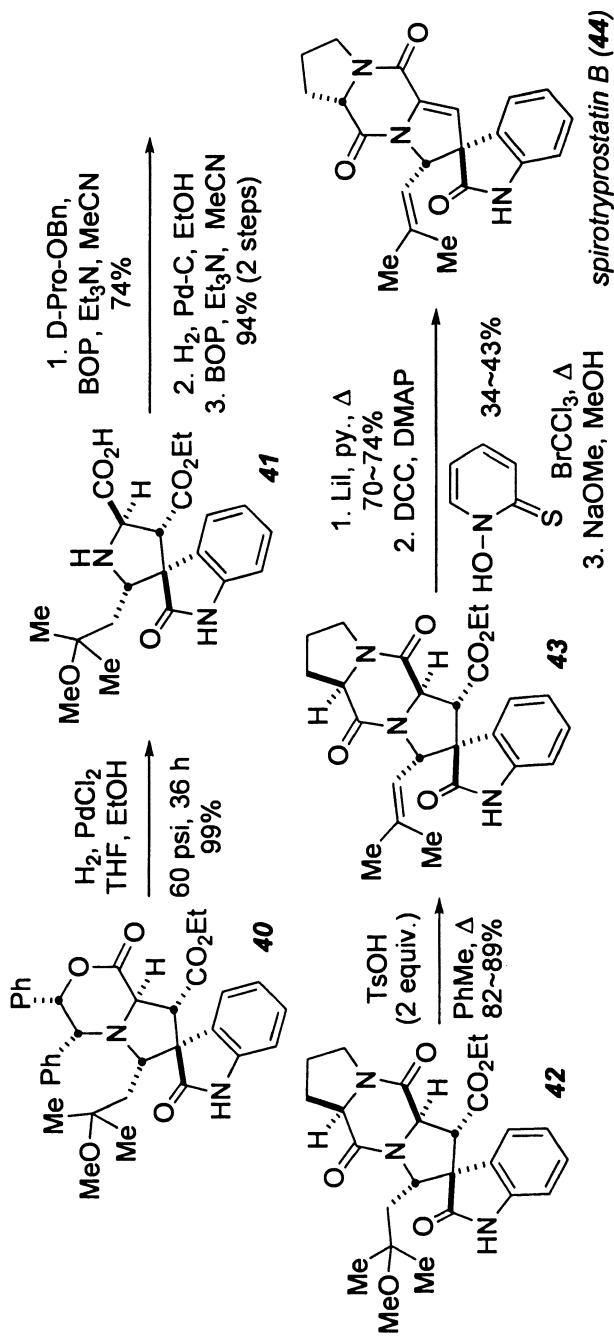
Scheme 7. Completion of spirotryprostatin B (44).
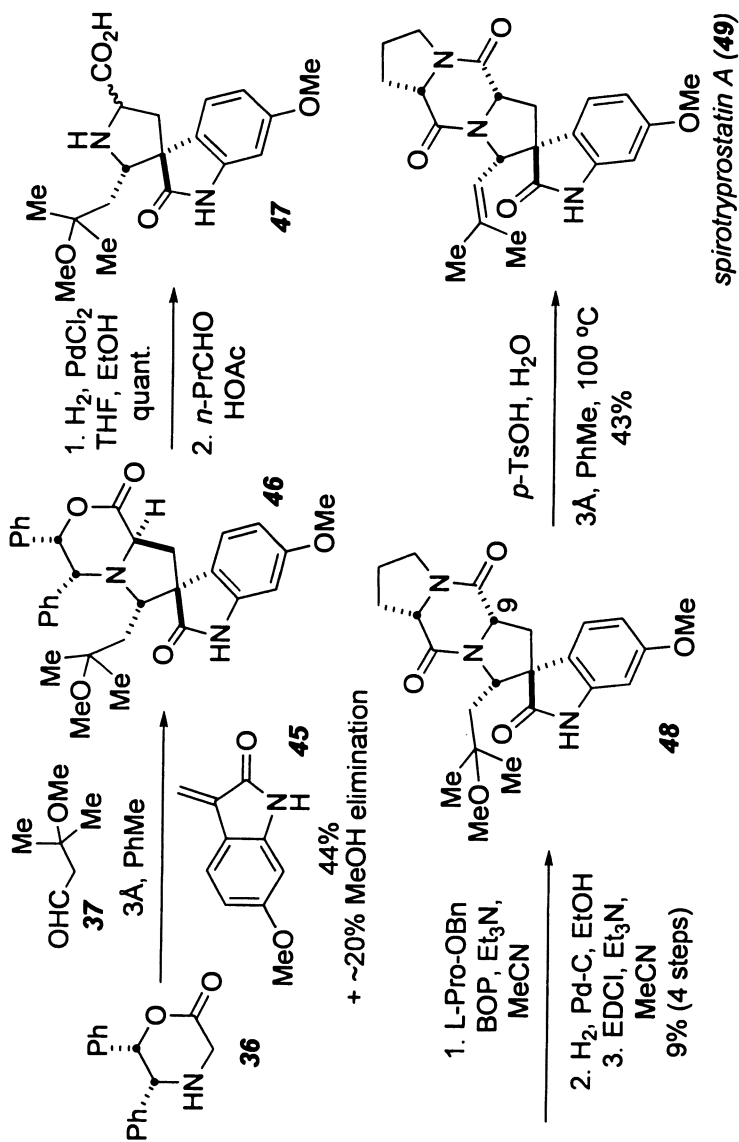
Scheme 8. Synthesis of spirotryprostatin A (49).

be separated from its C9 diastereomer. Treatment of **48** with tosic acid effected methanol elimination to complete the synthesis of spirotryprostatin A (**49**) (*19*).

Nakadomarin

We recently applied the dipolar cycloaddition methodology to synthesis of the ADE ring system of the manzamine alkaloid nakadomarin A (*20*). The requisite aldehyde **50** could be generated easily from protected mannitol, but the exocyclic enone dipolarophile **51** proved highly reactive and unstable to polymerization. We then deployed a two-step procedure to generate **51** *in situ*, which permitted the dipolar cycloaddition with diphenyloxazinone **36** to yield spirocycle **52** containing the AD ring system (Scheme 9).

The chiral auxiliary was easily cleaved with Pearlman's catalyst to give amino acid **53** (Scheme 10). Acylation of the amine yielded alkene **54**, and installation of the methyl ester and acetonide deprotection gave diol **55**, which was converted to diene **56**. Ring-closing metathesis with Grubbs' second-generation catalyst completed the ADE ring system, giving alkene **57**.

The ketone of **57** was converted in a two-step process to alkene **58** (Scheme 11); reduction of the ester to the alcohol and reoxidation to the aldehyde **59** allowed installation of the desired alkyne **60**. Epoxide alkylation of the alkyne gave β-hydroxyalkyne **61** *(21)*, but the yields of this reaction have proven variable and the reaction capricious to scale-up.

Currently, alternatives to this homologation are being evaluated such that the planned palladium-mediated conversion to the furan system can be interrogated. If successful, this will allow subsequent closure of the B ring, positioning the molecule for the acylation-metathesis sequence planned to complete the synthesis of nakadomarin A (**62**).

Palau'amine

Palau'amine (**63**, Figure 4) is a highly complex, densely functionalized alkaloid, with a carbon-to-nitrogen ratio approaching 2:1. The presence of six fused rings, including a spiro junction, a guanidine hemiaminal, and a guanidine hemi-amino aminal, and potent biological activity (*22*) makes the molecule a highly challenging yet inviting synthetic target, currently being pursued by many groups (*23*). We have recently undertaken studies utilitzing an intramolecular dipolar cycloaddtion strategy to assemble the difficult cyclopentane core.

Our approach begins with α-acylation of oxazinone (-)-**2** to yield alkene **64** (Scheme 12). *N*-Boc removal followed by addition of paraformaldehyde

434
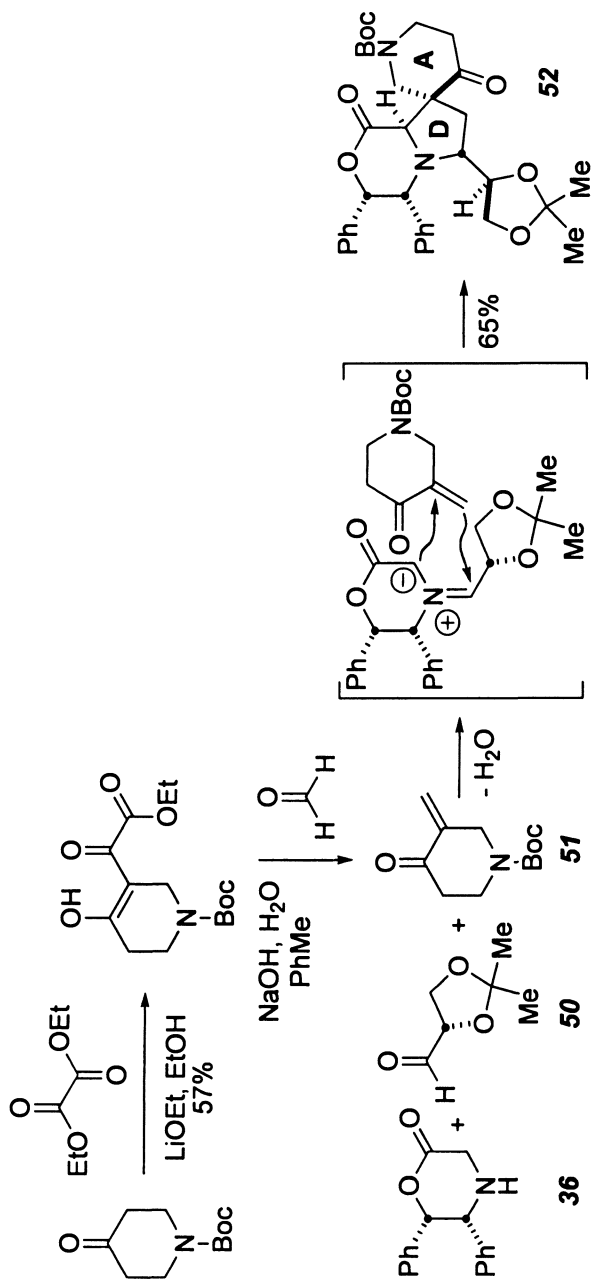
Scheme 9. Dipolar cycloaddition toward the nakadomarin ring system.
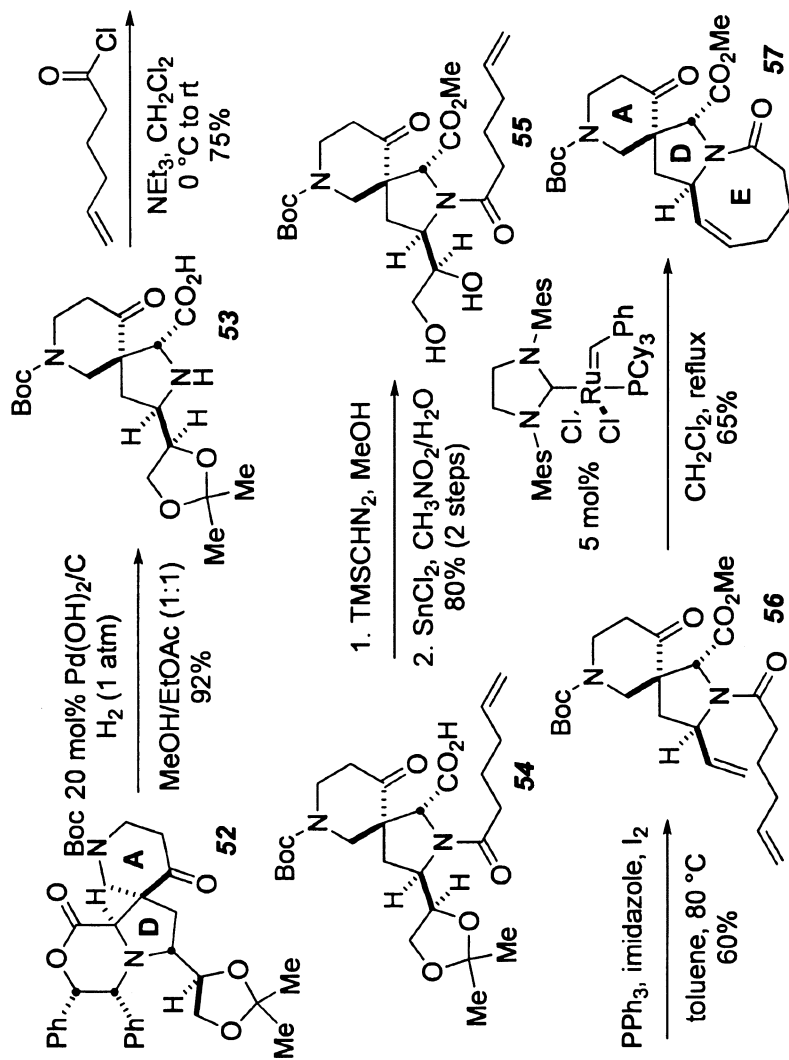
Scheme 10. Completion of the ADE ring system of nakadomarin A.
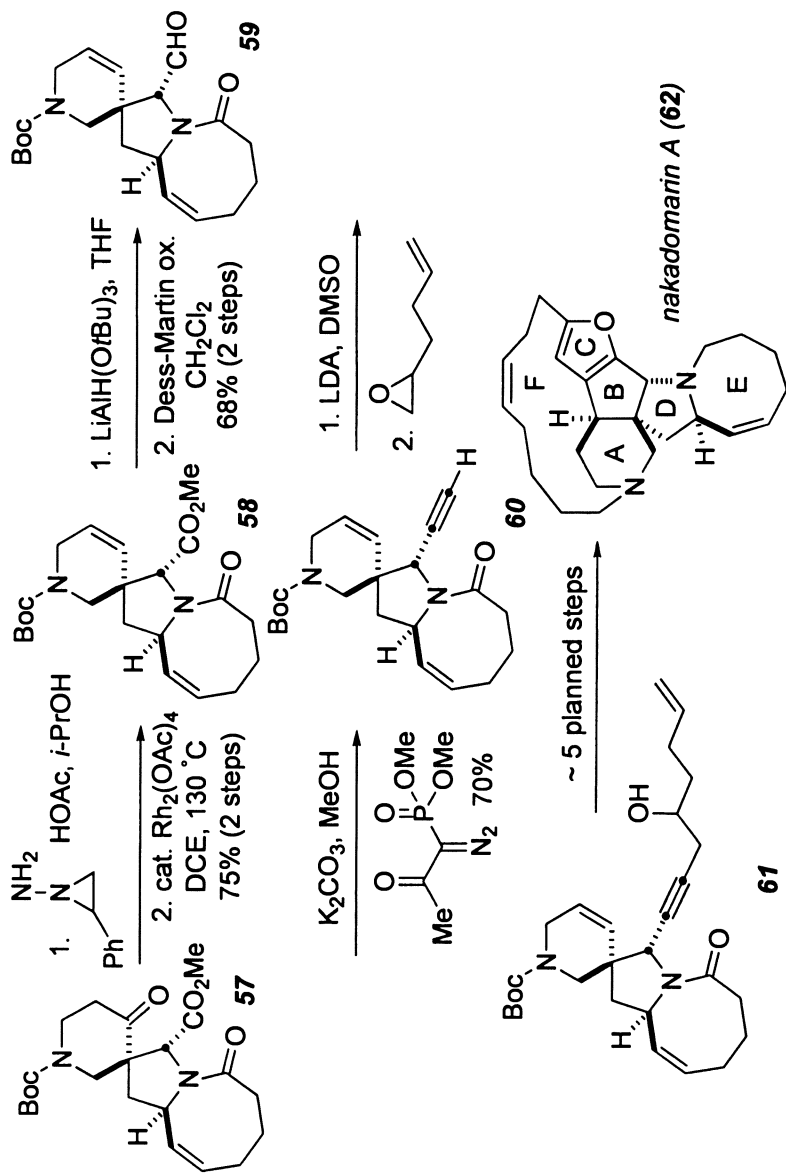
Scheme 11. Progress toward nakadomarin A (62).
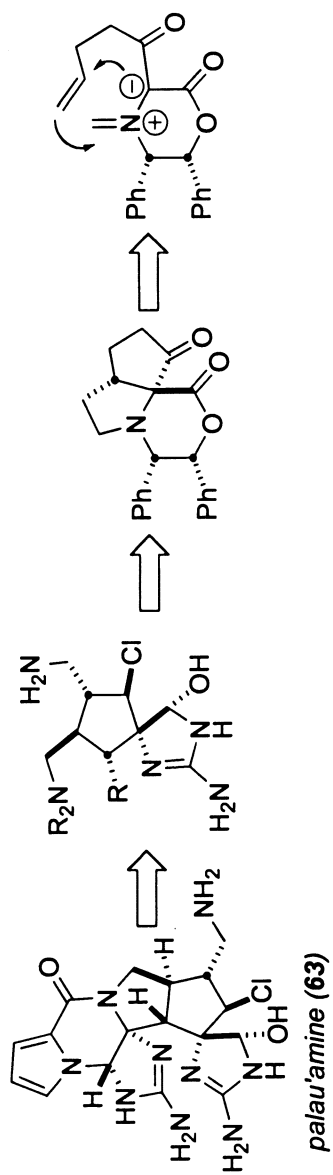
Figure 4. Retrosynthetic analysis for palau'amine (63).

438
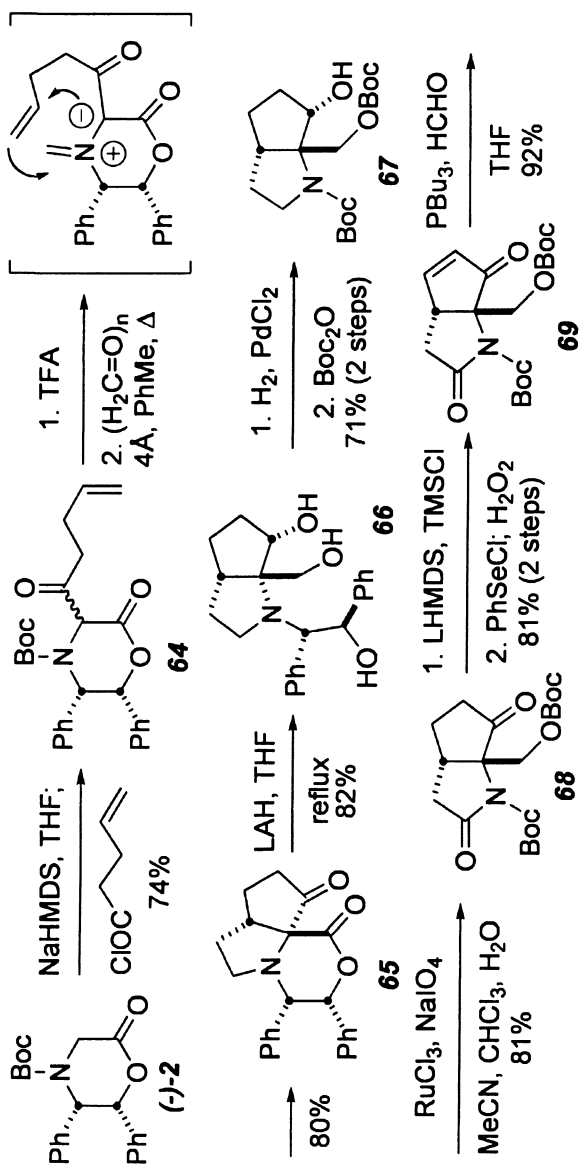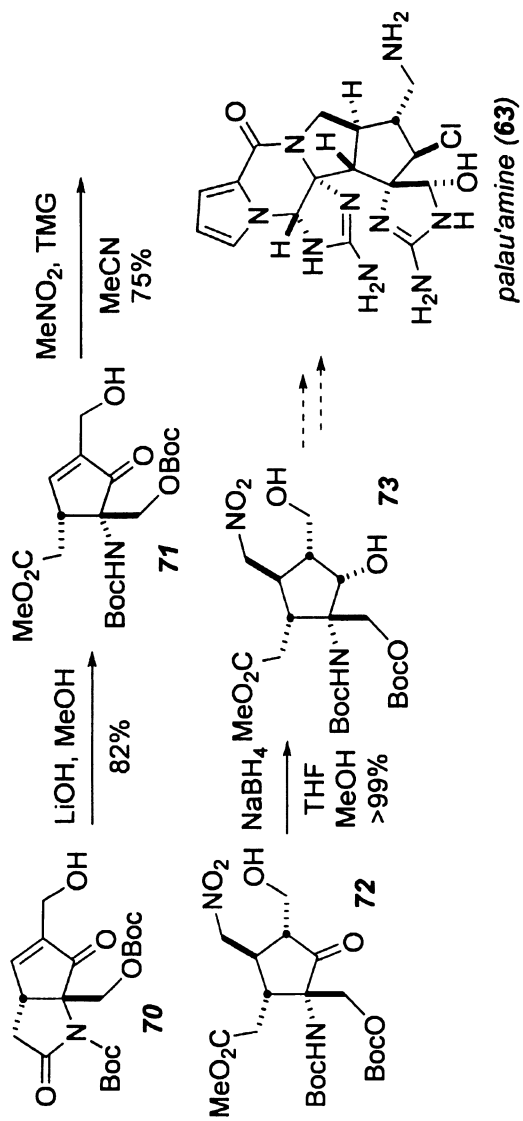
Scheme 12. Initial approach to palau'amine.

furnishes the incipient iminium species, whose deprotonation generates the azomethine ylide; spontaneous intramolecular dipolar cycloaddition afforded the desired tricyclic species **65** as a single diastereomer. Treatment of **65** with lithium aluminum hydride furnished diol **66**, which was subsequently subjected to hydrogenation to remove the bibenzyl residue. Reaction of the resultant amino alcohol with Boc anhydride effected acylation of both the nitrogen and the primary alcohol to give **67**. Treatment of **67** with ruthenium trichloride not only oxidized the secondary alcohol, but also effects the oxidation α to the ring nitrogen to provide the ketoamide **68** *(24)*. Conversion of the ketone to enone **69** under standard conditions followed by a Bayliss-Hillman reaction furnished alcohol **70**, which was subjected to saponification to provide the ester **71**. Conjugate addition of nitromethane to enone **71** in the presence of tetramethyl guanidine proceeded in a diastereoselective manner to give **72**, which was followed by reduction of the ketone to alcohol **73**. Compound **73** embodies all of the requisite carbon atoms and suitably poised functionality to serve as a reasonable substrate from which palau'amine might be constructed. Studies are currently underway to effect the end-game conversion of **73** to palau'amine and congeners.

In parallel with this approach, several alternative routes were being concomitantly evaluated for installation of the two aminomethyl arms. As shown in Scheme 13, enone **69** was subjected to dipolar cycloaddition to give isoxazolidine **74**; treatment with DBU restored the enone while liberating the *N*-hydroxyl moiety, which was converted to its acetate **75**. Conjugate addition of nitromethane allowed closure of the resultant α-anion onto the *N*-acetoxy species to give protected *spiro*-aziridine **76**. Studies are similarly underway to futher elaborate this tricyclic system to palau'amine.

In conclusion, the diphenyl oxazinone templates (**1**, **2**) have proven to be versatile and useful systems from which numerous complex natural products and their analogs can be constructed in a stereocontrolled, asymmetric manner. We continue to explore and exploit the chemistry of this versatile glycine template to the asymmetric total synthesis of many different families of biomedically significant nitrogenous substances.

Acknowledgements
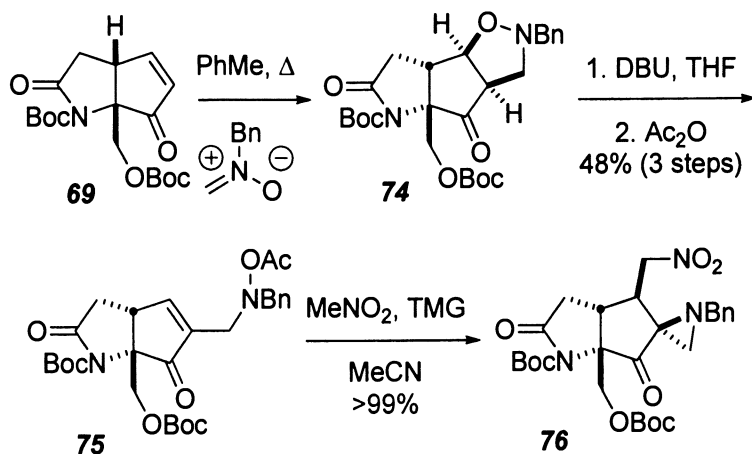
Scheme 13. Revised approach to the D ring of palau'amine.

References

1. a) Williams, R. M. *Aldrichimica Acta* **1992**, *25*, 11; b) Williams, R. M. *Advances in Asymmetric Synthesis*, JAI Press Volume 1 (pp 45-94), A. Hassner, Ed., **1995**.
2. Ohtani, I.; Moore, R. E.; Runnegar, M. T. C. *J. Am. Chem. Soc.* **1992**, *114*, 7941.
3. Banker, R.; Teltsch, B.; Sukenik, A.; Carmeli, S. *J. Nat. Prod.* **2000**, *63*, 387.
4. Norris, R. L.; Eaglesham, G. K.; Pierens, G.; Shaw, G. R.; Smith, M. J.; Chiswell, R. K.; Seawright, A.; Moore, M. R. *Environ. Toxicol.* **1999**, *14*, 163.
5. Xie, C. Y.; Runnegar, M. T. C.; Snider, B. B. *J. Am. Chem. Soc.* **2000**, *122*, 5017.
6. a) Heintzelman, G. R.; Fang, W. K.; Keen, S. P.; Wallace, G. A.; Weinreb, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 8851. b) Heintzelman, G. R.; Fang, W. K.; Keen, S. P.; Wallace, G. A.; Weinreb, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 3939.
7. a) White, J. D.; Hansen, J. D. *J. Am. Chem. Soc.* **2002**, *124*, 4950. b) White, J. D.; Hansen, J. D. *J. Org. Chem.* **2005**, *7*, 1963.
8. Looper, R. E.; Williams, R. M. *Tetrahedron Lett.* **2001**, *42*, 769.
9. Looper, R. E.; Williams, R. M. *Angew. Chem.* **2004**, *116*, 2990.
10. Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Tetrahedron* **2006**, *62*, 4549.

11. Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Angew. Chem.* **2005,** *117,* 3947.

12. Stork, G.; Niu, D.; Fujimoto, R. A.; Koft, E. R.; Balkovec, J. M.; Tata, J. R.; Dake, G. R. *J. Am. Chem. Soc.* **2001,** *123,* 3239.

13. Raheem, I. T.; Goodman, S. N.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2004,** *126,* 706.

14. Igarashi, J.; Katsuka, M.; Wang, Y.-G.; Acharya, H. P.; Kobayashi, Y. *Tetrahedron Lett.* **2004,** *45,* 3783.

15. Johns, D. M.; Mori, M.; Williams, R. M. *Org. Lett.* **2006,** *8,* 4051.

16. a) Cui, C. B.; Kayeka, H.; Osada, H. *J. Antibiot.* **1996,** *49,* 832. b) Cui, C. B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996,** *51,* 12651.

17. For other syntheses of the spirotryprostatins, see: (a) von Nussbaum, F.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2000,** *39,* 2175; (b) Overman, L. E.; Rosen, M. D. *Angew. Chem. Int. Ed.* **2000,** *39,* 4596; (c) Wang, H.; Ganesan, A. *J. Org. Chem.* **2000,** *65,* 4685; (d) Bagul, T. D.; Lakshmaiah, G.; Kawabata, T.; Fuji, K. *Org. Lett.* **2002,** *4,* 249; (e) Meyers, C.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2003,** *42,* 694; (f) Miyake, F. Y.; Yakushijin, K.; Horne, D. A. *Org. Lett.* **2004,** *6,* 4249; (g) Miyake, F. Y.; Yakushijin, K.; Horne, D. A. *Angew. Chem. Int. Ed.* **2004,** *43,* 5357; (h) Trost, B. M.; Stiles, D. T. *Org. Lett.* **2007,** *9,* 2763.

18. a) Sebahar, P. R.; Williams, R. M. *J. Am. Chem. Soc.* **2000,** *122,* 5666. b) Sebahar, P. R.; Osada, H.; Usui, T.; Williams, R. M. *Tetrahedron* **2002,** *58,* 6311.

19. (a) Onishi, T.; Sebahar, P. R.; Williams, R. M. *Org. Lett.* **2003,** *5,* 3135; (b) Onishi, T.; Sebahar, P. R.; Williams, R. M. *Tetrahedron* **2004,** *60,* 9503.

20. Kobayashi, J.; Watanabe, D.; Kawasaki, N.; Tsuda, M. *J. Org. Chem.* **1997,** *62,* 9236.

21. Ahrendt, K. A.; Williams, R. M. *Org. Lett.* **2004,** *6,* 4539.

22. Kinnel, R. B.; Gehrken, H. P.; Swali, R.; Skoropowski, G.; Scheuer, P. J. *J. Org. Chem.* **1998,** *63,* 3281.

23. Köck, M.; Grube, A.; Seiple, I. B.; Baran, P. S. *Angew. Chem. Int. Ed.* **2007,** *46,* 6586.

24. Carlsen, P. H. G.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981,** *46,* 3936.

Chapter 27

β-Substituted D-Leucines and Their Relevance in the Total Synthesis of Natural and Unnatural Aeruginosins

Juan R. Del Valle[1], Xiaotian Wang[2], Karolina Ersmark[3], and Stephen Hanessian[2,*]

[1]Department of Chemistry and Biochemistry, New Mexico State University, 1175 North Horseshoe Drive, MSC 3C, Las Cruces, NM 88003
[2]Department of Chemistry, Université de Montréal, P.O. Box 6128, Station Centre-ville, Montréal, Québec H3C 3J7, Canada
[3]Medivir AB, P.O. Box 1086, SE–141 22, Huddinge, Sweden

Methods for the synthesis of diastereomerically pure β-chloro and β-methyl-D-leucines and their derivatives are described. The incorporation of these residues in natural and non-natural analogues is shown with relevance to their *in vitro* thrombin inhibitory activities.

A large number of natural products contain unusual amino acid moieties as part of their oftentimes intricate structures. Among these are a select group of biologically active peptides that contain a chlorine atom in the side–chain of an amino acid, or as a substituent in the carbon framework.[1] In rare instances, Nature has produced peptide structures that harbor a β-chloroamino acid residue. The paucity of natural products bearing these residues may be testament to the susceptibility of β-chloro, and related amino acids to undergo elimination across the α and β carbons. Indeed, dehydroamino acids are abundant in Nature and play an important role in the biosynthesis of various non-proteinogenic amino acids, and D-amino acids.[2] To date, β-chloroamino acids have only been found
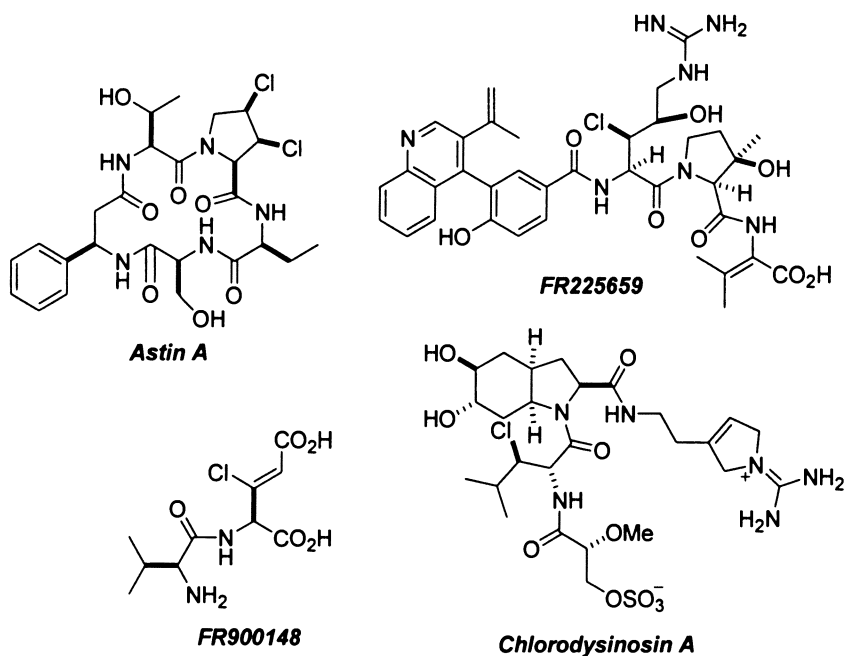
Figure 1. Structures of astin A, FR900148, FR225659, and chlorodysinosin A.

in a select number of natural product families which include the astins,[3] FR900148,[4] FR225659,[5] and chlorodysinosin A,[6] a new member of the aeruginosins[7] (Figure 1).

The aeruginosins, derived from cyanobacterial waterblooms and marine sponges, belong to a family of so-called linear peptides incorporating novel non-proteinogenic α-amino acids. Several efforts have been made to synthesize members of this class of natural products and details of seven total syntheses of various aeruginosins have been summarized in a recent review.[7] The first aeruginosin to be discovered was aeruginosin 298A (Table 1), which was isolated from *Microcystis aeruginosa* by Murakami and coworkers[8] in 1994. This finding was followed by the isolation and characterization of so far 20 more natural compounds belonging to the same structural family gathered from geographically different aquatic sources.[7] Most of the aeruginosins are comprised of four distinct subunits: a *C*-terminal arginine mimetic, a 6-mono or 5,6-dihydroxy-2-octahydroindole carboxamide (Choi or OHChoi), a bulky hydrophobic amino acid, and an *N*-terminal hydroxyl or acidic group. This pharmacophoric combination of subunits is in part responsible for the *in vitro* inhibitory activities against serine proteases, especially those with trypsin-like substrate specificity. Particular attention has been directed to the inhibition of
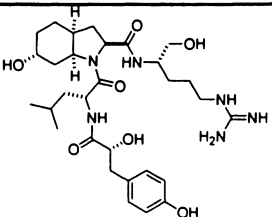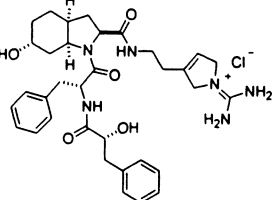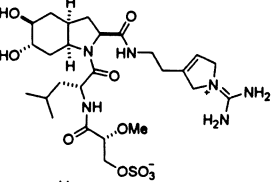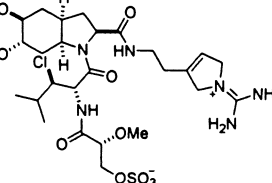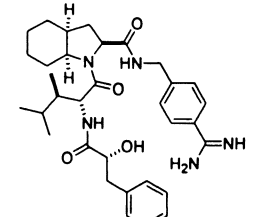
thrombin and other blood coagulation factors (Table 1).[7] Nearly all aeruginosins incorporate a D-configured α-amino acid with a bulky hydrophobic side-chain in the P3 position. Interestingly, it was found that the presence of a 3-chloro substituent in this position, as in the (3R)-chloro-D-leucyl residue in chlorodysinosin A, gave a several-fold increase in inhibitory activity against some coagulation factors compared to the hydrogen-substituted dysinosin A (Table 1). In fact, chlorodysinosin A is the most potent inhibitor of thrombin *in vitro*, among all other members of the aeruginosin family.

So far, four X-ray crystal structures of aeruginosins in complex with thrombin have been solved. A hirugen-thrombin complex with aeruginosin 298A was reported in 1998,[9] followed by co-crystal structures of thrombin with dysinosin A,[10] oscillarin,[11] and chlorodysinosin A[12] (Table 1). They all show similar binding modes to the thrombin active site (Figure 2). The basic P1 side-chain in oscillarin, dysinosin A, and chlorodysinosin A, is positioned in the S1 pocket forming a strong interaction from the terminal guanidino group to an aspartic acid residue at the bottom of the S1 pocket. The P2 position in the octahydroindole subunit is well accommodated in the S2 pocket, but without any hydrogen bonds formed between the enzyme and the 6- (Choi) or 5,6- (OHChoi) hydroxyl groups. The P3 D-α-amino acid occupies the so-called D-S3 subsite, which is a relatively large hydrophobic pocket. Moreover, the P3-P4 amide of the aeruginosins binds to the backbone of a glycine residue in the thrombin active site in an antiparallel β-strand fashion, and the *N*-terminal acidic sulfate group in dysinosin A and chlorodysinosin A form hydrogen bonds to arginine residues on the surface of thrombin. The overall co-crystal structures and binding modes of dysinosin A and chlorodysinosin A, differing only in the presence of the P3 β-chlorine atom in chlorodysinosin A, are very similar. A subtle difference is a shift in orientation of the side-chains of the two active site residues Glu192 and Arg173 in thrombin. The conformation of the D-S3 pocket, as well as the binding mode of the P3 leucine and 3-chloroleucine residues is identical in the dysinosin A and chlorodysinosin A complexes respectively (Figure 3A). The enhanced binding of chlorodysinosin A in the D-S3 subsite has been suggested to arise from a favorable position of the chlorine atom compared to the same D-Leu residue in dysinosin A (Figure 3A). Molecular dynamics simulation suggested that the chlorine substituent stabilizes the χ^1 dihedral angle in the β-chloro-D-leucyl side-chain in the bioactive conformation (Figure 3B). Additionally, the accommodation of the hydrophobic D-S3 site could also be accompanied by a release of water resulting in a gain in entropy of binding.

Synthetic Approaches to β-Chloroleucine

The identification of a new amino acid, β-chloro-D-leucine (Cleu), as a component of chlorodysinosin A,[6] prompted us to explore efforts toward the

Table 1. In Vitro Enzyme Inhibitory Activities of Natural and Synthetic Aeruginosins

Compound	Structure	Enzyme IC$_{50}$ (μM)[a]		
		Thrombin (FIIa)	FVIIa	FXa
Aeruginosin 298A		0.5	nd	nd
Oscillarin		0.028	3.9	nd
Dysinosin A		0.046	0.326	5
Chlorodysinosin A		0.0057	0.039	1.54
31		0.022	nd	nd

[a] nd = not determined

A.
Dysinosin A-thrombin complex

B.
Oscillarin-thrombin complex

C.
Chlorodysinosin A-thrombin complex
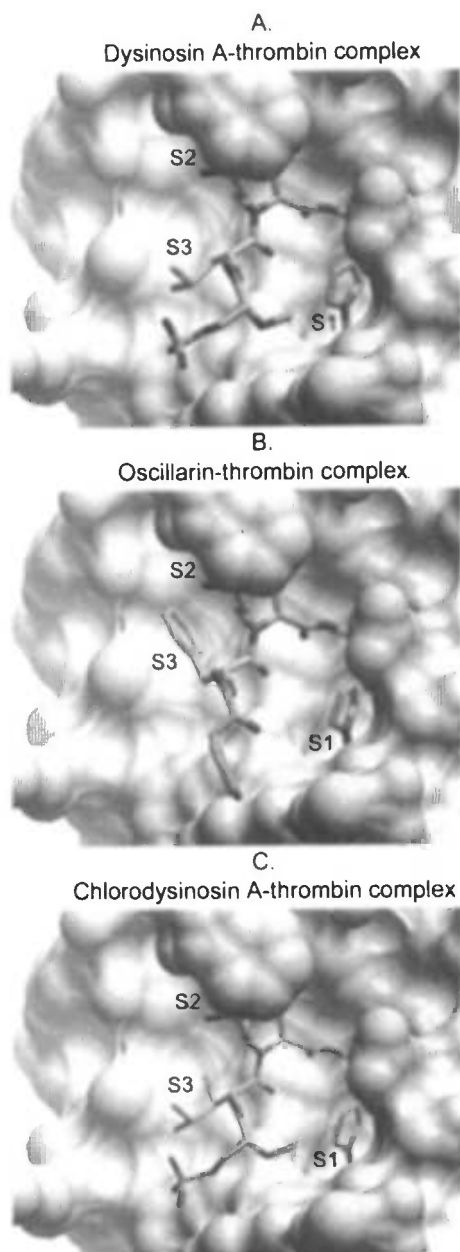
Figure 2. Connolly surface representations from X-ray crystal structures of thrombin in complex with dysinosin A,[10] oscillarin,[11] and chlorodysinosin A,[12] panels A-C, respectively. Molecular structures are outlined in Table 1. (See page 2 of color insert.)
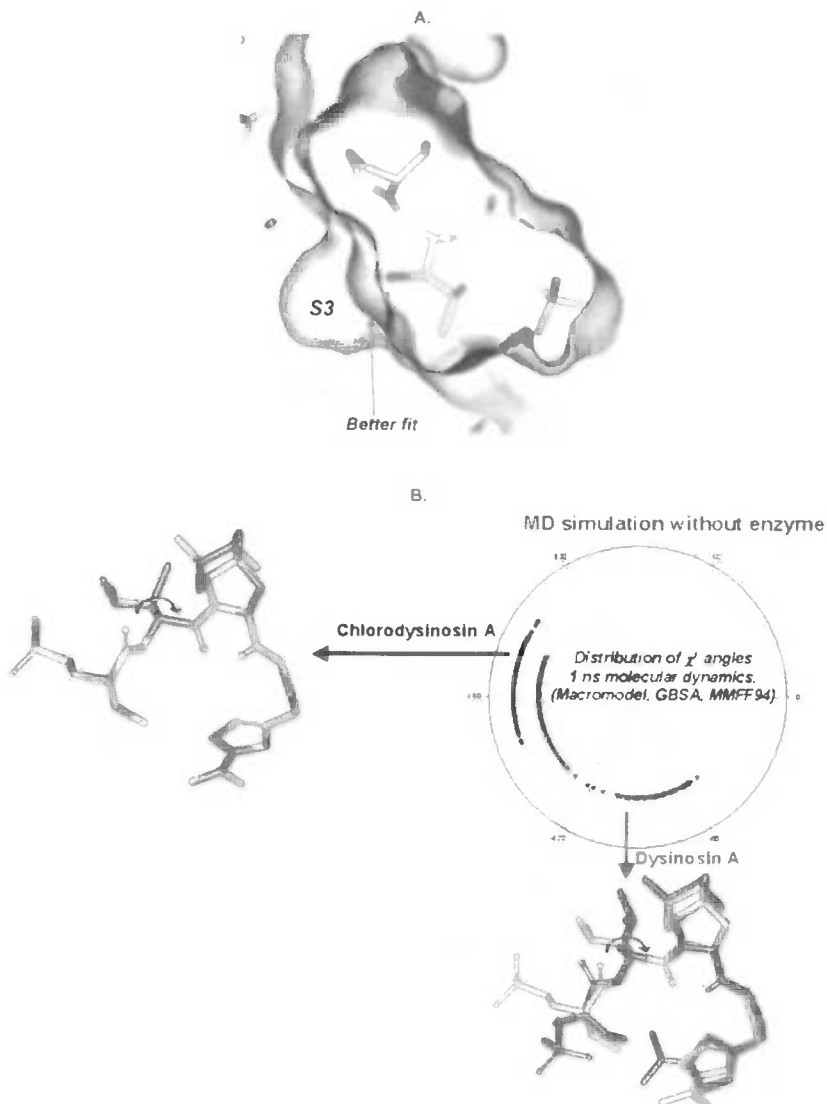
Figure 3. Panel A) Space filling model of the P3 side chains in the S3 pocket from an overlay of the X-ray crystal structures of dysinosin A (green) and chlorodysinosin A (pink) in complex with thrombin. Panel B) Molecular dynamics simulations of chlorodysinosin A (yellow and pink) and dysinosin A (yellow and green) without enzyme. Distribution of χ^{1} dihedral angles in the P3 amino acid. (See page 3 of color insert.)

synthesis of Cleu-containing peptides. We anticipated that the incorporation of Cleu in chemically modified natural and non-natural aeruginosin congeners, would also enhance their inhibitory activities toward the coagulation cascade factors. Furthermore, it appears that aeruginosins 205A and 205B also contain a Cleu residue based on preliminary spectral data.[13] In fact, the synthesis and incorporation of Cleu into aeruginosin analogs has resulted in new, highly potent inhibitors of various coagulation cascade factors.[14]

Prior to the isolation of chlorodysinosin A in 2003,[6] there existed only one report on the synthesis of β-chloroleucine (Cleu). In 1960 Shive et al.[15] described a synthesis of racemic Cleu en route to an isopropyl analog of the *Streptomycete* antibiotic cycloserine. As depicted in Scheme 1, an isomeric mixture of 4-methyl-2-pentenoic acids was obtained via Knoevenagel condensation followed by decarboxylation. A two-step aminohydroxylation then provided racemic hydroxyleucine, which underwent chlorination with PCl_5 to obtain Cleu as the hydrochloride salt.
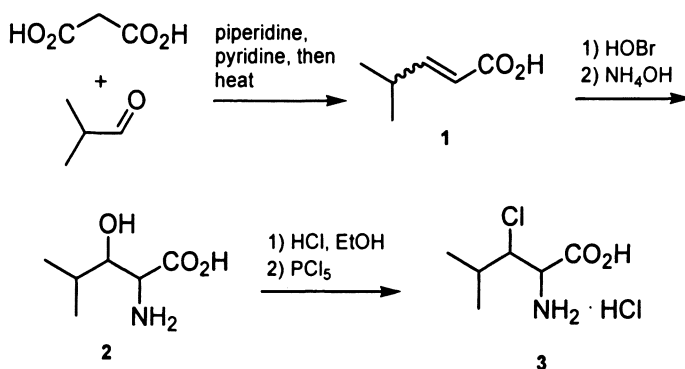
Scheme 1. Shive's synthesis of racemic 3-chloroleucine.

An alternative and stereoselective strategy for the introduction of halogens onto the β-carbon of α-amino acids relies on the regioselective cleavage of β-lactones. Vederas and coworkers[16] have shown that magnesium halides are effective in the ring-opening of an *N*-tosyl lactone such as **5** which can be prepared from L-threonine (Scheme 2).

Based on this strategy, Bonjoch and coworkers[17] recently completed the synthesis of both Cleu diastereomers starting from (2*R*,3*S*)-3-hydroxyleucine (Hleu), which was obtained in five synthetic steps via a kinetic resolution protocol according to Hamada et al.[18] As shown in Scheme 3, Hleu was protected as the benzyloxycarbamate and subjected to lactonization with HBTU to provide compound **7**. Regioselective opening of **7** with chloride ion gave
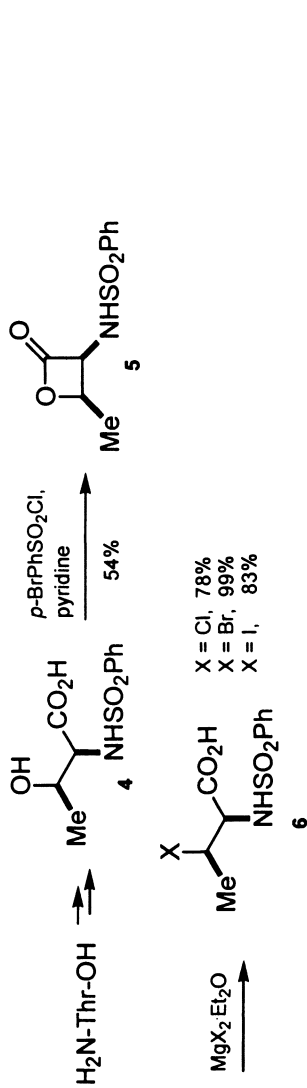
Scheme 2. Vederas' lactone strategy toward branched 3-halo-amino acids.

X = Cl, 78%
X = Br, 99%
X = I, 83%
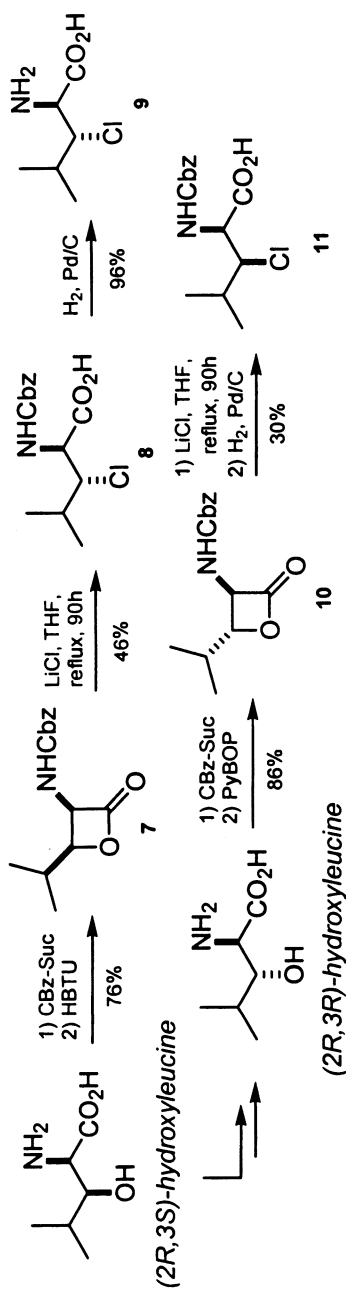
Scheme 3. Bonjoch's synthesis of 3-chloroleucines.

(2R,3S)-hydroxyleucine

(2R,3R)-hydroxyleucine

(2R,3R)-Cleu in 44% yield after Cbz deprotection. The (2R,3S)-Cleu isomer **11** was obtained in a similar fashion from (2R,3R)-Hleu.

Concurrent to Bonjoch's stereoselective synthesis of the diastereomers isomers of Cleu, Hanessian and coworkers utilized a regioselective aziridine opening strategy toward the Cleu-containing dipeptide fragment of chlorodysinosin A[12] (Scheme 4). Epoxy alcohol **12**, obtained on large scale via Sharpless asymmetric epoxidation[19] of the corresponding allylic alcohol, was converted in an efficient three-step procedure to the aziridine **14** in 68% overall yield. To study the regio- and stereoselectivity of the key chlorinolysis of **14**, various protecting groups were introduced to activate the carbon-nitrogen bond and facilitate ring-opening.

Previously, Bonini and coworkers[20] had reported the regioselective opening of N-Boc aziridines with MgBr$_2$ to give Boc-protected brominated β-amino alcohols. Efforts to open N-Boc-aziridine **15a** with MgCl$_2$ and CeCl$_3$ met with no success (Table 2). However, the combination of an N-toluenesulfonyl (Ts) group and CeCl$_3$ as a chloride source[21] provided a 2:1 mixture of regioisomeric chlorosulfonamides in 69% overall yield. Although chromatographic separation allowed for the isolation of the desired 3-chloro isomer in 40% yield, anticipated compatibility issues between the chloro and toluenesulfonamide groups during a subsequent N-Ts deprotection step led to the evaluation of other N-protecting groups (Table 2). Ultimately, the best isomeric ratios were obtained in the case of the N-t-butylsulfonamide derivative (N-Bus).[22a] By employing excess CeCl$_3$ and extending the reaction time, regioselective aziridine cleavage occurred concomitant with TBS removal. Thus, (2S,3R)-N-Bus-3-chloroleucinol was obtained as the major isomer in 80% directly from compound **15d** (Table 2, entry 7). Under the same conditions the alcohol analogue also gave the same regioisomer (Table 2, entry 8). It is possible that the preferred attack at the β-position of the aziridine is due to a more favorable trajectory of approach of the chloride ion. A steric effect of the OTBS group can be ruled out due to the results observed with the hydroxyl analog (compare entries 7,8, Table 1). The regio- and stereochemistry of the product was confirmed by single-crystal x-ray diffraction. Since it was first reported as a new protective group for primary amines by Weinreb and coworkers,[22a] the N-Bus group has only been exploited in a few cases.[22b-f]

Chlorodysinosin A and Non-Natural Congeners

With (2S,3R)-N-Bus-3-chloroleucinol in hand, elaboration of the chlorodysinosin A N-terminal fragment proceeded by deprotection of the N-Bus group under acidic conditions followed by amide coupling to (R)-3-tert-butyldiphenylsilyloxy-2-methoxypropionic acid (Scheme 5). Many attempts were made to oxidize the primary hydroxymethyl group of **18** to the intended carboxylic acid. Typical conditions such as TEMPO/chlorite/hypochlorite,
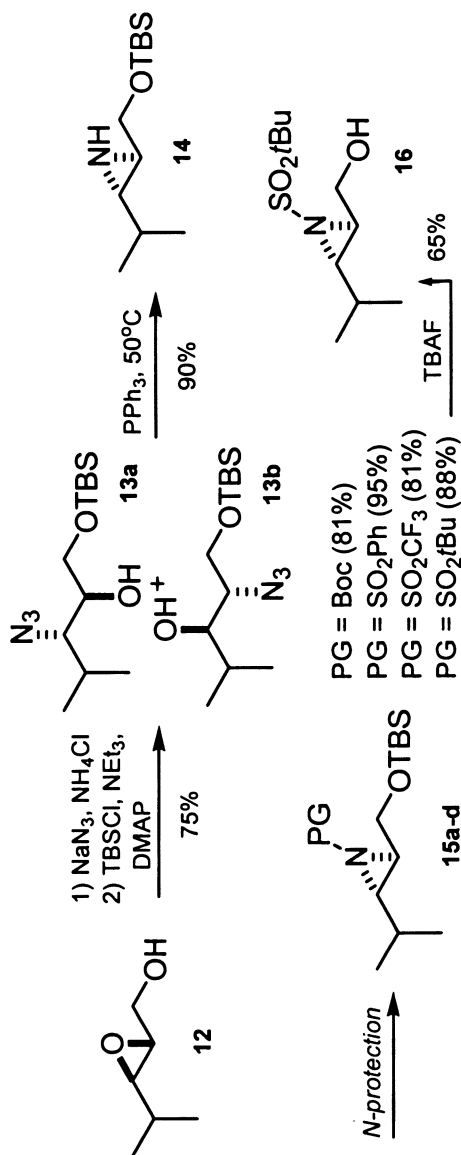
Scheme 4. Synthesis of N-Bus aziridines.

Table 2. Regioselective Aziridine Opening Reactions

entry	PG	conditions	yield	3-Cl : 2-Cl	R'
1	Boc	10 eq. MgCl$_2$, Et$_2$O, 72h, rt	no rxn	-	TBS
2	Boc	1.1 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 24h	no rxn	-	TBS
3	SO$_2$Ph	10 eq. MgCl$_2$, Et$_2$O, 72h, rt	no rxn	-	TBS
4	SO$_2$Ph	1.1 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 24h	69%	2:1	TBS
5	SO$_2$CF$_3$	1.1 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 24h	57%	1:1	TBS
6	SO$_2$tBu	2.0 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 48h	56%	> 10:1	5:1 H:TBS
7	SO$_2$tBu	4.0 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 72h	80%	> 10:1	H
8	SO$_2$tBu *(no TBS)*	4.0 eq. CeCl$_3$·7H$_2$O, MeCN, 90°C, 24h	74%	10:1	H
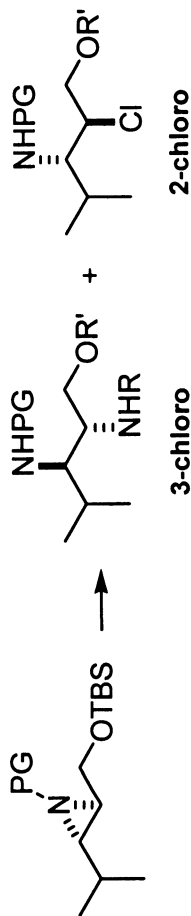
Swern or Dess-Martin oxidation followed by hypochlorite, and $RuCl_3/NaIO_4$ led to low yields and a preponderance of unsaturated products resulting from β-elimination. Finally, we adopted a method developed by Grabowski, Reider and coworkers[23] (H_5IO_6 and catalytic CrO_3) which worked admirably well to provide the β-chlorodipeptide carboxylic acid subunit **19**.

The next step was the assembly of **19** with the octahydroindole core structure **20**, previously prepared from L-pyroglutamic acid.[24] Under several conditions of amide formation, only low yields of the desired compound **21** were obtained, the main products being produced by elimination. Presumably, the active ester intermediates were particularly susceptible to elimination of HCl across the α and β carbons. Fortunately, when DEPBT[25] was used as the coupling reagent in conjunction with 2,6-lutidine, amide bond formation proceeded smoothly in 55-60% yield to give **21** without appreciable HCl elimination.[26]

Completion of the synthesis of chlorodysinosin A is depicted in Scheme 6. Unmasking the carboxylic acid functionality in **20** required the strict avoidance of alkaline conditions. In fact, many milder conditions to obtain the free carboxylic acid were fraught with problems. We also explored sodium hydroperoxide and enzymatic methods, and the $MeTeAlMe_2$ complex recently introduced by Corey and coworkers.[27] Under the latter conditions, methyl ester cleavage was attended by rapid deprotection of the MOM ether. Finally, ester **21a** could be converted into acid **22** under neutral conditions by the action of excess trimethyltin hydroxide in dichloroethane.[28] The reaction proceeds through a four-center intermolecular transfer of the methoxy group onto the tin with intermediate formation of the tributyltin ester that undergoes hydrolysis to the acid. Amide coupling to the *N*-Boc amidino pyrroline (Adc) subunit proceeded in high yield in the presence of PyBOP to give **23a**. Finally, silyl ether cleavage, tin-ether mediated sulfation,[29] and global deprotection provided the crude product, which was purified by RP-HPLC to give chlorodysinosin A in 38% overall yield from **23b**.

The discovery of the "chlorine effect" in chlorodysinosin A led to the investigation of other β-branched residues in place of Cleu. We were particularly interested in replacing the chlorine atom with a *C*-methyl group, to validate the proposed benefits of a relatively small hydrophobic β-substituent. To achieve this objective we explored the cleavage of aziridines with *O*- and *N*-protecting groups with excess Me_2CuLi. In all instances mixtures of 3-methyl and 2-methyl leucinols were obtained which could be chromatographically separated (Table 3). By employing the *N*-Ts-*O*-TIPS aziridine, the desired β-methyl product could be obtained in 38% yield after chromatographic purification.

The synthesis of a new aeruginosin analog featuring octahydroindole carboxylic acid (Oic), (3*R*,2*R*) 3-methylleucine (β-MeLeu), and a P1 benzamidine subunits is shown in Scheme 7. Thus, removal of the *N*-Ts protection with Na in liquid ammonia and coupling to protected phenyl lactic
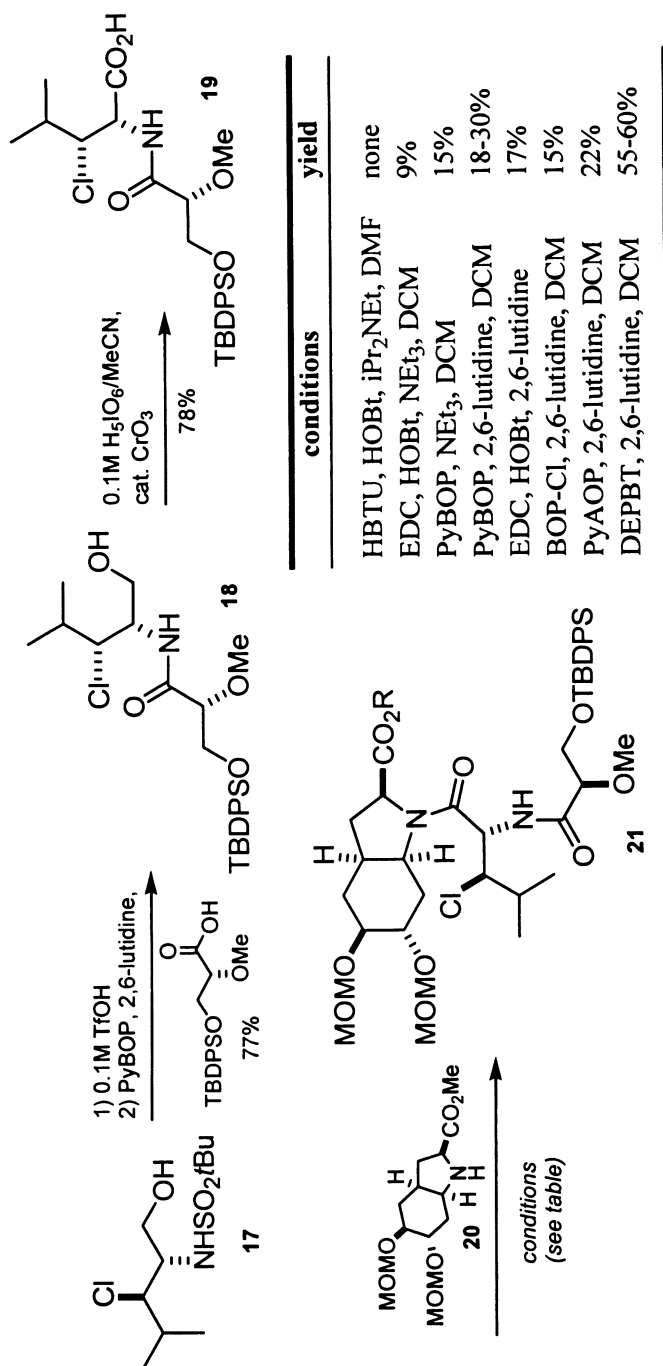
conditions	yield
HBTU, HOBt, iPr$_2$NEt, DMF	none
EDC, HOBt, NEt$_3$, DCM	9%
PyBOP, NEt$_3$, DCM	15%
PyBOP, 2,6-lutidine, DCM	18-30%
EDC, HOBt, 2,6-lutidine	17%
BOP-Cl, 2,6-lutidine, DCM	15%
PyAOP, 2,6-lutidine, DCM	22%
DEPBT, 2,6-lutidine, DCM	55-60%

Scheme 5. Synthesis and coupling of the chloroleucine fragment 19.

1) 0.1M TfOH
2) PyBOP, 2,6-lutidine,
77%

0.1M H$_5$IO$_6$/MeCN,
cat. CrO$_3$
78%

conditions
(see table)

17 **18** **19** **20** **21**

22a R = Me
22b R = H

Me₃SnOH, 75°C
78%

23a R = TBDPS
23b R = H

TBAF
85%

PyBOP, 2,6-lutidine
93%

1) SO₃·py, Bu₂SnO
2) TFA/DCM
3) RP-HPLC
38%
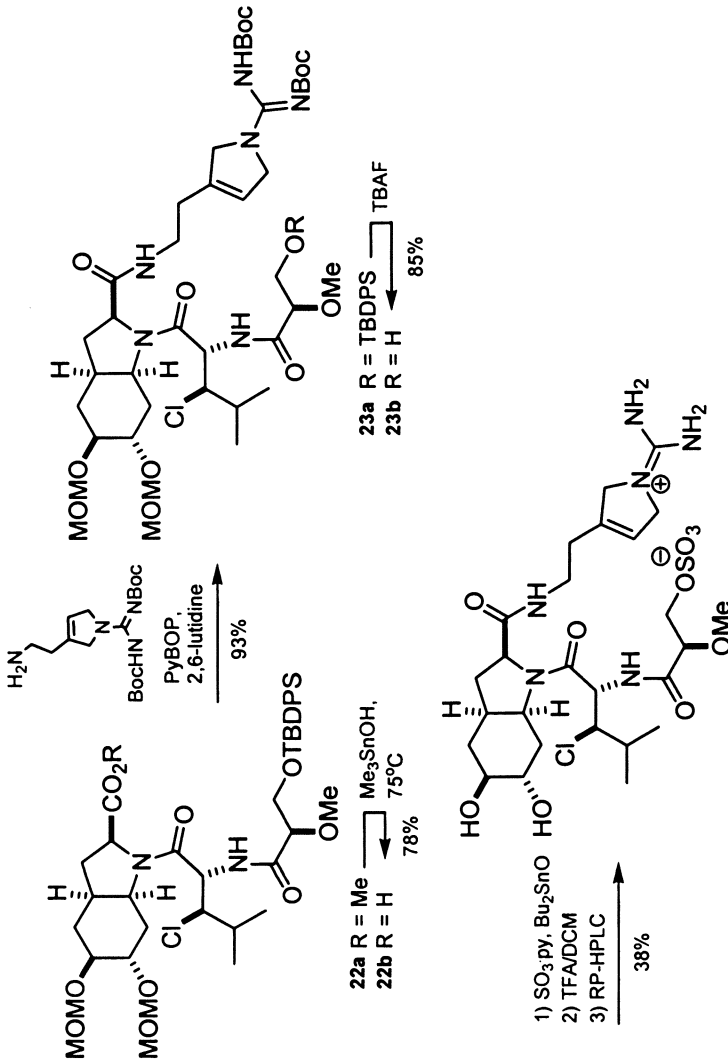
chlorodysinodin A

Scheme 6. Synthesis of chlorodysinosin A.

acid gave the amide **26** in 71% yield (2 steps). Cleavage of the silyl ether was followed by oxidation to the acid, and amide coupling to **29** to give protected analog **30**. Finally, hydrogenolysis of the Cbz group and cleavage of the MOM ether under acidic conditions gave **31** in 47% yield. Following a similar route, analogue **32** was prepared by employing the regioisomeric 2-*C*-methyl β-amino acid isomer obtained in the cuprate opening reactions (Table 3).

Table 3. Reaction of *N*-Sulfonyl Aziridines with Lithium Dimethylcopper(II)
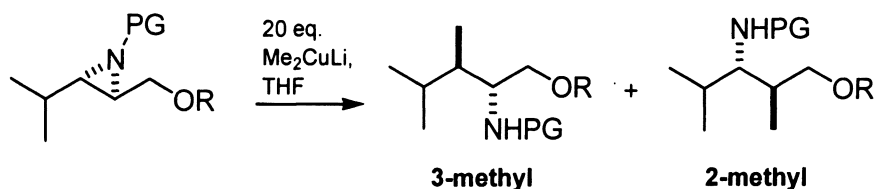
entry	PG	R	Time (h)	Temp (°C)	yield (%)	3-Me : 2-Me
1	Tos	H	16	-10 to 0	96	0 : 1
2	Tos	TBS	16	-10 to 0	89	1 : 1.8
3	Tos	TBS	16	-10 to RT	nd	1 : 2.3
4	Tos	TBDPS	24	-10 to RT	nd	1 : 3
5	Tos	TES	24	-10 to RT	nd	1 : 1.6
6	Tos	TIPS	24	-10 to RT	84	1 : 1.2
7	Bus	TIPS	24	-10 to RT	53	1 : 2

Compound **31** displayed an IC_{50} of 22 nM toward thrombin, compared to **32** which was significantly less active (IC_{50} = 17µM). These results confirm that a small alkyl branch at P3 in simpler aeruginosin analogs can replace the chlorine atom in chlorodysinosin A, and lead to excellent inhibition of thrombin *in vitro*. In addition, the removal of the hydroxyl groups from the Choi subunit, and the replacement of the more synthetically challenging Adc arginine mimetic for a benzamidine unit as in **31** are well tolerated.[14]

In conclusion, we have described methods for the regio- and stereocontrolled syntheses of β-substituted D-leucines, including chloro and *C*-methyl analogs in diastereomerically pure forms. The incorporation of these residues at the P3 subunit in natural and synthetic aeruginosins results in potent *in vitro* inhibitory activity against the blood coagulation factors such as thrombin.
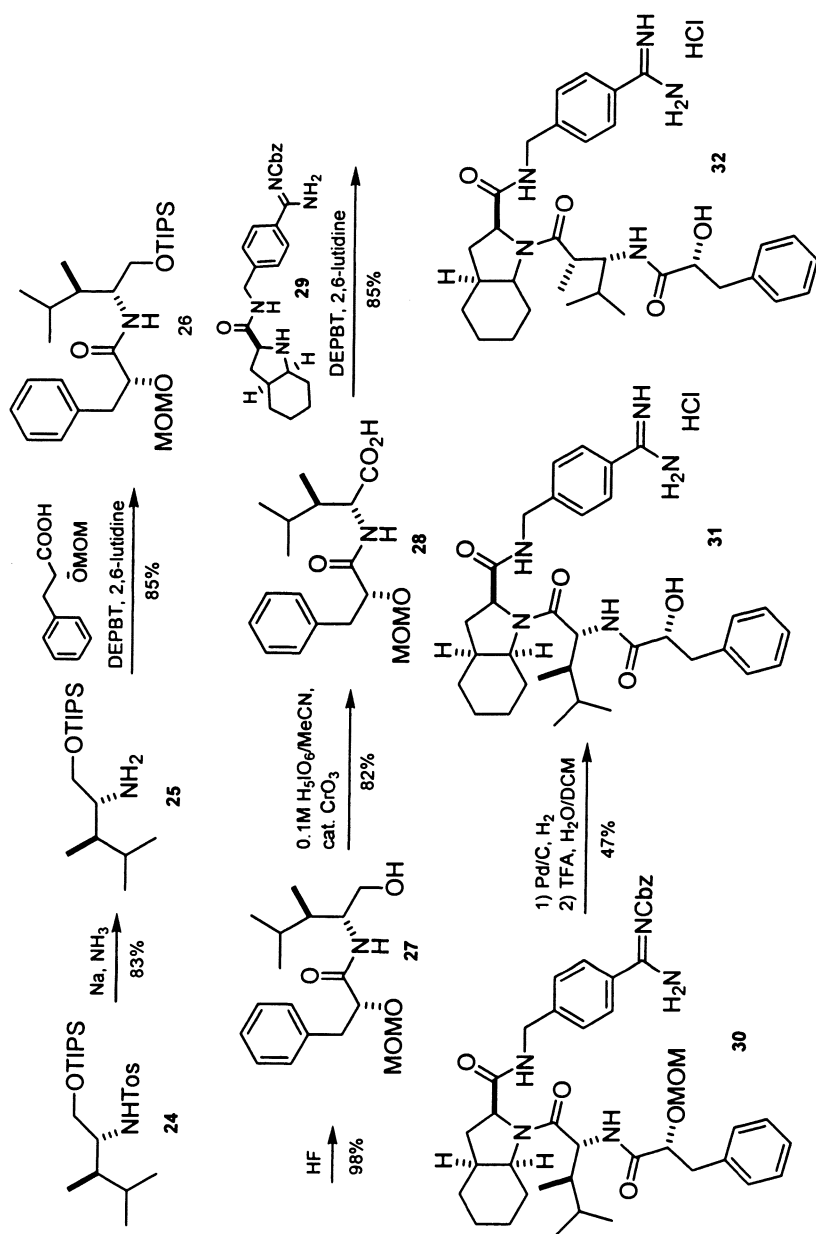
Scheme 7. Synthesis of methylleucine aeruginosin analogues 31 and 32.

Acknowledgments

References

1. (a) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793. (b) Gribble, G. W. *J Nat. Prod.* **1992**, *55*, 1353. (c) Gribble, G. W. *Environ. Sci. Technol.* **1994**, *28*, 310. (d) Vaillancourt, F. H.; Yeh, E.; Vosburg, D. A.; Garneau-Tsodikova, S.; Walsh, C. T. *Chem. Rev.* **2006**, *106*, 3364. (e) van Pée, K. H. *Ann. Rev. Microbiol.* **1996**, *50*, 375.

2. (a) Dunn, M. J.; Gomez, S.; Jackson, R. F. W. *J. Chem. Soc. Perkin Trans. 1* **1995**, 1639. (b) Gelb, M. H.; Lin, Y. K.; Pickard, M. A.; Song, Y. H.; Vederas, J. C. *J. Am. Chem Soc.* **1990**, *112*, 4932. (c) Murkin, A. S.; Tanner, M. E. *J. .Org. Chem.* **2002**, *67*, 8389. (d) Srinivasan, A.; Stephenson, R. W.; Olsen, R. K. *J. Org. Chem.* **1977**, *42*, 2253. (e) Srinivasan, A.; Stephenson, R. W.; Olsen, R. K. *J. Org. Chem.* **1977**, *42*, 2256. (f) Murphy, C. D. *Nat. Prod. Rep.* **2006**, *23*, 147. (g) Neumann, C. S.; Fujimori, D. G.; Walsh, C. T. *Chem. Biol.* **2008**, *15*, 99.

3. (a) Morita, H.; Nagashima, S.; Takeya, K.; Itokawa, H. *Chem. Phar. Bull.* **1993**, *41*, 992. (b) For synthetic studies on the β-chloroproline residue found in astin natural products see: Schumacher, K. K.; Jiang, J. J.; Joullie, M. M. *Tetrahedron: Asymmetry* **1998**, *9*, 47.

4. (a) Kuroda, Y.; Okuhara, M.; Goto, T.; Okamoto, M.; Yamashita, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1980**, *33*, 267. (b)Kuroda, Y.; Okuhara, M.; Goto, T.; Yamashita, M.; Iguchi, E.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J Antibiot.* **1980**, *33*, 259.

5. Ohtsu, Y.; Sasamura, H.; Tsurumi, Y.; Yoshimura, S.; Takase, S.; Hashimoto, M.; Shibata, T.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 682.

6. Goetz, G. H.; Harrigan, G. G.; Likos, J. J.; Kasten, T. P. PCT WO03/051831; *Chem Abst.* **2003**, *139*, 47155.

7. Ersmark, K.; Del Valle, Juan R.; Hanessian, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 1202.

8. Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994**, *35*, 3129.

9. Steiner, J. L. R.; Murakami, M.; Tulinsky, A. *J. Am. Chem. Soc.* **1998**, *120*, 597.

10. Carroll, A. R.; Pierens, G. K.; Fechner, G.; de Almeida Leone, P.; Ngo, A.; Simpson, M.; Hyde, E.; Hooper, J. N. A.; Boström, S.-L.; Musil, D.; Quinn, R. J. *J. Am. Chem. Soc.* **2002**, *124*, 13340.

11. Hanessian, S.; Tremblay, M.; Petersen, J. F. W. *J. Am. Chem. Soc.* **2004**, *126*, 6064-6071.

12. Hanessian, S.; Del Valle, J. R.; Xue, Y.; Blomberg, N. *J. Am. Chem. Soc.* **2006**, *128*, 10491.

13. (a) Valls, N.; Vallribera, M.; Font-Bardia, M.; Solans, X.; Bonjoch, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1241. (b)Toyooka, N.; Nakazawa, A.; Himiyama, T.; Nemoto, H. *Heterocycles* **2003**, *59*, 75.

14. Hanessian, S.; Ersmark, K.; Wang, X.; Del Valle, J. R.; Blomberg, N.; Xue, Y.; Fjellström, O. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3480.

15. Hayashi, K.; Skinner, C. G.; Shive, W. *J. Org. Chem.* **1961**, *26*, 1167.

16. Pansare, S. V.; Vederas, J. C. *J. Org. Chem.* **1989**, *54*, 2311.

17. Valls, N.; Borregan, M.; Bonjoch, J. *Tetrahedron Lett.* **2006**, *47*, 3701.

18. Makino, K.; Okamoto, N.; Hara, O.; Hamada, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1757.

19. (a) Caldwell, C. G.; Bondy, S. S. *Synthesis-Stuttgart* **1990**, 34. (b) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.

20. Righi, G.; Franchini, T.; Bonini, C. *Tetrahedron Lett.* **1998**, *39*, 2385.

21. Sabitha, G.; Babu, R. S.; Rajkumar, M.; Reddy, C. S.; Yadav, J. S. *Tetrahedron Lett.* **2001**, *42*, 3955.

22. (a) Sun, P.; Weinreb, S. M.; Shang, M. Y. *J. Org. Chem.* **1997**, *62*, 8604. (b)Furkert, D. P.; Husbands, S. M. *Org. Lett.* **2007**, *9*, 3769. (c) Gontcharov, A. V.; Liu, H.; Sharpless, K. B. *Org. Lett.* **1999**, *1*, 783. (d) Hanessian, S.; Ritson, D. J. *J. Org. Chem.* **2006**, *71*, 9807. (e) Hodgson, D. M.; Humphreys, P. G.; Miles, S. M.; Brierley, C. A. J.; Ward, J. G. *J. Org. Chem.* **2007**, *72*, 10009. (f) Hodgson, D. M.; Humphreys, P. G.; Ward, J. G. *Org. Lett.* **2006**, *8*, 995.

23. Zhao, M. Z.; Li, J.; Song, Z. G.; Desmond, R.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 5323.

24. Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L. *J. Am. Chem. Soc.* **2002**, *124*, 13342.

25. (a) Li, H. T.; Jiang, X. H.; Ye, Y. H.; Fan, C. X.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91. (b) Fan, C. X.; Hao, X. L.; Ye, Y. H. *Syn. Comm.* **1996**, *26*, 1455.

26. For examples of the utility of DEPBT for difficult amide bond formations see: (a) Jiang, W. L.; Wanner, J.; Lee, R. J.; Bounaud, P. Y.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 1877. (b) Ye, Y. H.; Li, H. T.; Jiang, X. H. *Biopolymers* **2005**, *80*, 172.

27. (a) Reddy, L. R.; Fournier, J. F.; Reddy, B. V. S.; Corey, E. J. *J. Am. Chem. Soc.* **2005**, *127*, 8974. (b) Reddy, B. V. S.; Reddy, L. R.; Corey, E. J. *Tetrahedron Lett.* **2005**, *46*, 4589.

28. (a) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem. Int. Ed.* **2005**, *44*, 1378. (b) see also: David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

29. (a) Lubineau, A.; Lemoine, R. *Tetrahedron Lett.* **1994**, *35*, 8795. (b) Sanders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 16391.

Chapter 28

Pure Peptides via Fluorocarbon Capping Technology: A Hands-On Discussion

Vittorio Montanari

Current address: PPG Industries, Inc., Coatings Innovation Center, Allison Park, PA 15101

Pure peptides are obtained on the solid phase by a capping technique that consists in treating the resin with a solution of a reactive iodonium salt, followed by base, at difficult coupling steps. Wide applicability is anticipated. This paper focuses on how to practice and extend this method with best speed, economy, and probability of success. The origins and chemical background of the method are also outlined.

Solid phase methodology permits the routine synthesis of long peptide sequences both in the laboratory and on a commercial scale (1,2). The crude peptide is purified by HPLC to remove various reaction impurities and the shorter sequences, or deletions, which result from incomplete coupling steps. Deletions, that differ from the desired sequence by only one unit, can be very difficult to separate. Repeated HPLC purification is frequently necessary at the expense of much material, solvent, and labor.

Recently, an unprecedented purification protocol was reported, consisting of treating the resin, at critical coupling steps, with an iodonium salt that caps unreacted chain ends with inert fluorocarbon residues.

At the end of the synthesis, when the peptide is separated from the resin and dissolved in aqueous medium, the capped deletions precipitate out of solution and are just filtered off. Proof of concept experiments and synthetic protocols have been published (3-5). Herein, focus is on the practical aspects of using the iodonium salt. The experimenter who wishes to use this new method will be confident of the results and aware of the peculiar reactivity of the iodonium compounds such as **1a-c**, from the discussion below.

Capping in solid phase peptide synthesis

The final purification of a polymer synthesized on the solid phase can be approached by two routes. One is to attach a "handle" after the final condensation-deprotection step (6). After detaching the crude peptide from the resin, only the desired compound is separated via this handle, which then has to be detached. The advantage is high final purity, the disadvantage, that detachment of the handle and extra separation work are necessary.

The other route is to cap the resin, after a condensation step but prior to deprotection, with a large excess of a reactive compound: typically, acetic anhydride.

Let us consider in detail what are the advantages and disadvantages of a typical acetic anhydride capping.

The reaction time is comparable to a coupling step: a common protocol is to cap twice for 10 minutes each. The capping is highly efficient; essentially all the unreacted chain ends become acetylated. The acetyl groups are inert under peptide coupling and final cleavage conditions. However, it is not practical to cap after every step in a long synthesis. The reason is empirical: the resin's performance is always degraded by repeated treatment with acetic anhydride. The loss in quality and yield of the final peptide is unacceptable if capping is done too many times. Thus, it may be preferred to perform the capping only in those couplings, which are presumed or known to be the most difficult. This may not solve the principal problem that capping is meant to address, that is, separation of (n-1) deletions: even if acetylated, these can be so similar to the full-length peptide that separation remains difficult and wasteful.

Other capping reagents, such as active esters of benzoic or myristic acid, produce derivatives whose retention times on HPLC are sufficiently different; they are much less reactive, however.

Summarizing, a capping technology for the purification of polymers synthesized in the solid phase has considerable practical value, if certain requirements are met:

- Resin integrity: the ability of the resin to swell in solvents must not be diminished by the capping steps.
- Reactivity: the unreacted chain ends must be capped totally. If the time to achieve this is very long, repeated coupling could be a better choice.
- Robust caps: the caps must be unchanged through all the subsequent steps.
- Separation: the purpose of capping is to achieve purification, normally this means baseline separation in HPLC.

None of the common capping protocols consistently meets these requirements. Several groups attempted to improve the capping technology for

the synthesis of peptides and other polymers, making use of the "fluorous" separation principle (7-10).

It is well known from the work of Curran and associates (11,12) that attaching a sufficiently long fluorocarbon tag to an organic molecule makes it easily separable by means of fluorocarbon-derivatized, or "fluorous" silica gel. Unfortunately, the vast amount of work carried out on small molecules has only rarely been extended to peptides, which tend to be entrained by the fluorous silica gel.

$$R_f CH_2-I \quad \oplus \quad \ominus N \begin{array}{c} SO_2CF_3 \\ SO_2CF_3 \end{array} \quad \begin{array}{ll} Rf = CF_3 & \textbf{1a} \\ Rf = C_7F_{15} & \textbf{1b} \\ Rf = C_8F_{17} & \textbf{1c} \end{array}$$
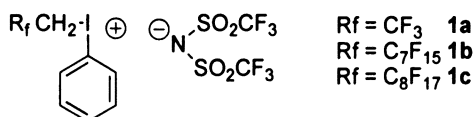
Figure 1. Structures of reactive fluoroalkyl iodonium compounds 1a-c

In the Kumar laboratory, efforts to achieve a "fluorous" separation by means of the reactive fluoroalkylating reagent **1b**, were initially frustrated by this fact. Success soon came with the realization that every time peptides were derivatized with **1b**, they became insoluble in water or largely aqueous media. Thus, a capping based on simple centrifugation was developed, with no need for any chromatography. Herein, it will be called a fluorous capping, following the usage in the original papers (3-5), although use of fluorous (11, 12) stationary phases is *not* involved.

Examples of routine fluorous capping

In this section, two proof of concept experiments are recalled (Figures 2 and 3).

Routine means that no changes are made to a typical peptide synthesis, except for the short capping step itself. Figure 2 shows the HPLC trace of the 22-unit peptide Ac-NH-RAVKVYADAAEDESAEAFALEF-CONH₂, prepared by the Boc method in two parallel versions at the same time. Both versions had the same three deliberately introduced deletions. The deletions were capped in one case with acetic anhydride, in the other with the reagent **1b**. The difference in purity was extreme: the acetyl-capped peptide showed the large deletions in its HPLC trace, as expected, but the fluorous-capped one, after filtration, was essentially pure. The substantial solid residue in the latter case (Figure 4) was found to consist of the fluorous-capped impurities by means of ESI mass spectroscopy (3-5).

Figure 3 describes the equally successful capping of a 10-unit peptide, where the last coupling is difficult and was deliberately reduced to less than 80% completion (Figure 5). Figure 3 highlights the fact that the fluorous-capped
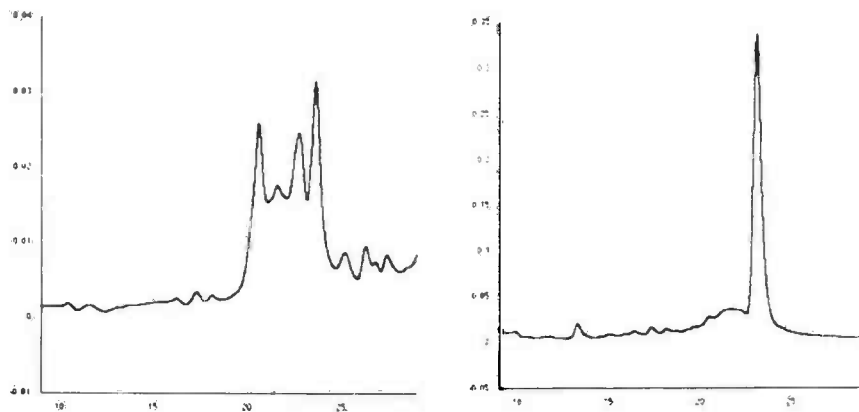
Figure 2. A 22-unit peptide with three intentional deletions (see text for the sequence) acetyl-capped (left) or capped with 1b then centrifuged (right)
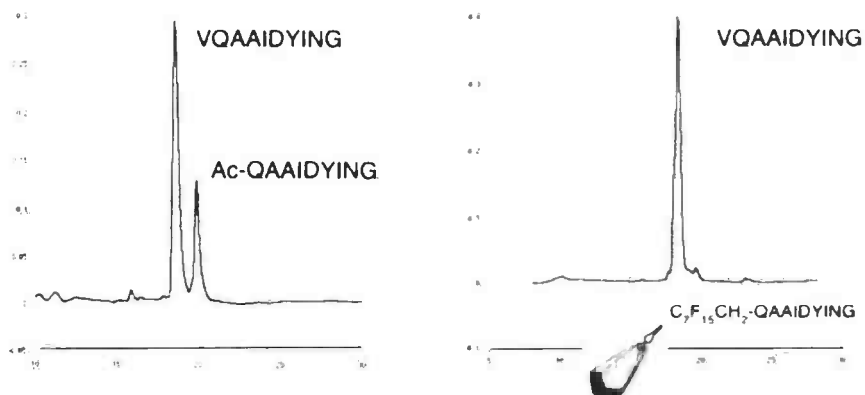
Figure 3. Successful capping in Fmoc chemistry using 1b: the fluorous-capped deletions "sink" out of solution
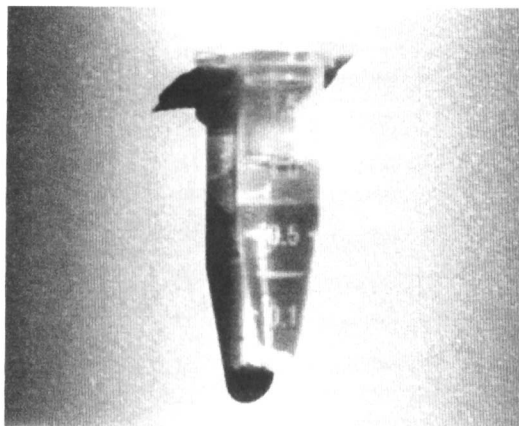
Figure 4. Centrifuged vial, where the deletions capped with 1b or 1c precipitated as a white, insoluble pellet.

deletion is present in the crude product, just as much as the acetyl-capped one is in the parallel version. The fluorous-capped deletion is easily centrifuged out (Figure 4), leaving a single HPLC peak (4,5).

Fmoc chemistry is the more widely used, but also much more challenging to a fluorous capping method because the fluorous tags may be sensitive to the conditions of Fmoc group deprotection.

Typically, Fmoc deprotection is carried out by piperidine in DMF, for a total time of up to 40 minutes at each step. These conditions are harsh relative to possible dehydrofluorination reaction.

Let us consider then, which fluorocarbon structures may be adept at end-capping in solid phase peptide synthesis.

Stability of fluorous residues under peptide synthesis conditions

The simplest fluorous capping reagents we can think of using in solid phase peptide synthesis are trifluoroacetic anhydride or one of its higher analogs. In practice, this is not possible because perfluoroalkyl amides are readily hydrolyzed. The capping would thus be reversible and give no result overall.

One can interpose hydrocarbon spacers between the perfluorocarbon residue and the carbonyl. The use of such reactants for peptide capping has not been reported. Dehydrofluorination under basic conditions is possible by cumulative long exposure to piperidine/DMF. If dehydrofluorination occurs, an electrophilic alkene results, which is liable to further decomposition. In the best of cases, the overall result would be no capping reaction. Dehydrofluorination of the
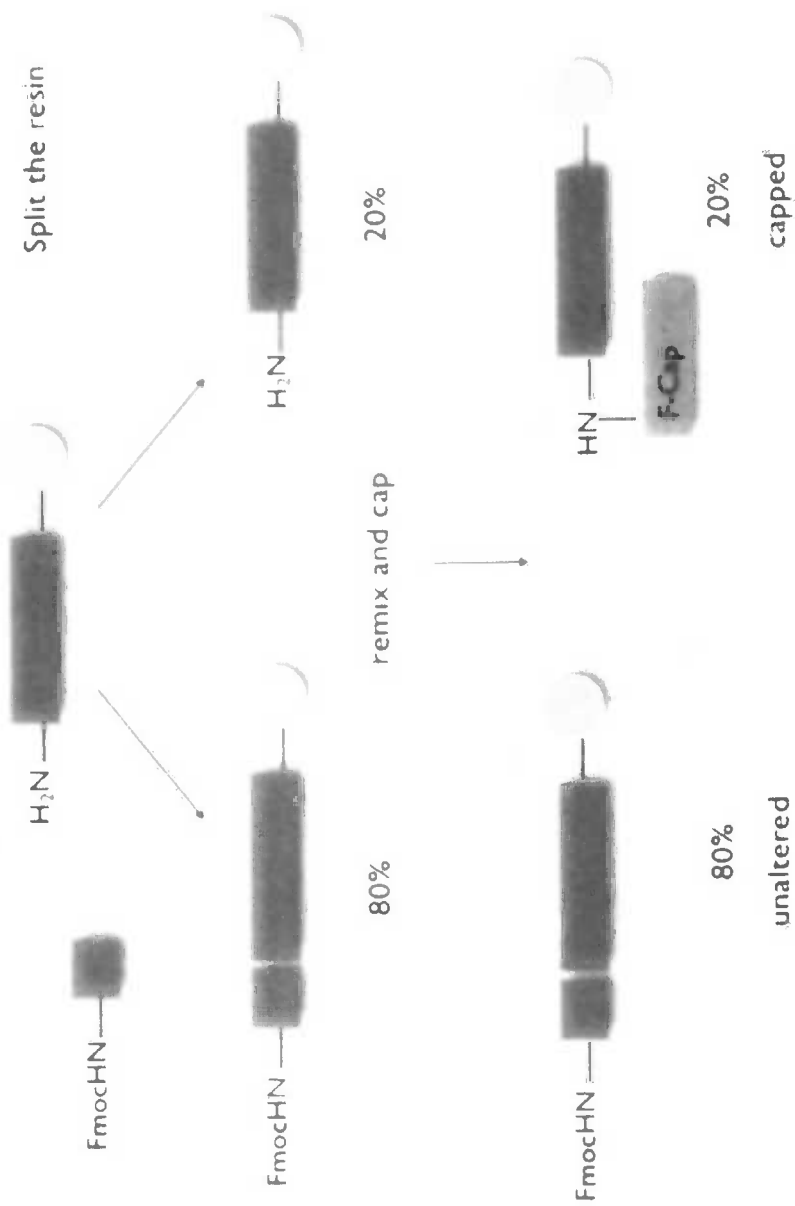
Figure 5. Schematic description of capping in the Fmoc case.

perfluoroalkylmethylene residues did not occur. In control experiments, model compounds were intact after hours in piperidine-DMF solution. In peptide synthesis, there was no evidence of degradation of either peptide or resins.

Figure 7 shows the capping scheme involving the iodonium salt: the resin is first soaked for about 3 minutes with a solution of the iodonium compound, then a base is added to trigger the alkylation. The coproducts are washed off and the peptide synthesis resumes in the usual manner.

In the course of many experiments, degradation of the resin's performance was not observed. In fact, in all comparative experiments the quality of the fluorous-capped peptide was better than that of the acetyl-capped one.

Figure 7 implies a mechanistic hypothesis, whereby the iodonium salt complexes the free amino groups without reaction, and then the heterocyclic base causes the alkylation to proceed. Note that the iodonium and the base *are not added together.*

The reasons for this are best seen in the earlier use of compound **1c** in synthesis, briefly discussed below.
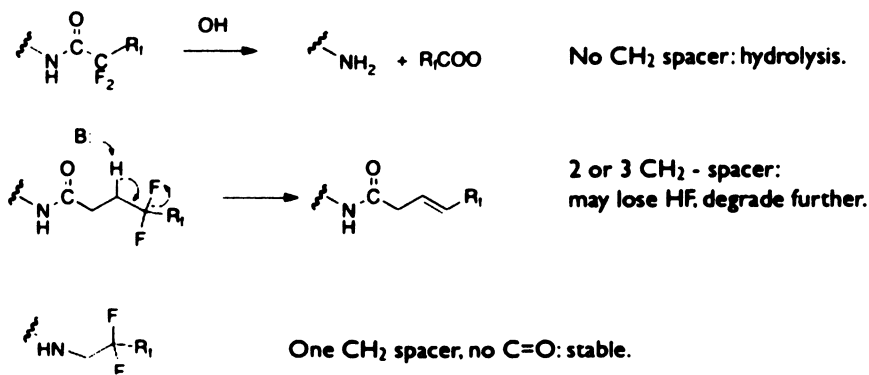
Figure 6. Possible fluorous capping residues

Alkylation of simple amino acids by iodonium salt 1a in water

The first examples of extremely reactive iodonium compounds, the perfluoroalkylmethylene iodonium triflates, were reported by Umemoto and Gotoh (13), who demonstrated several rapid alkylation reactions. For example, *N*-trifluoroethyl aniline was prepared from aniline directly in the presence of hindered bases.
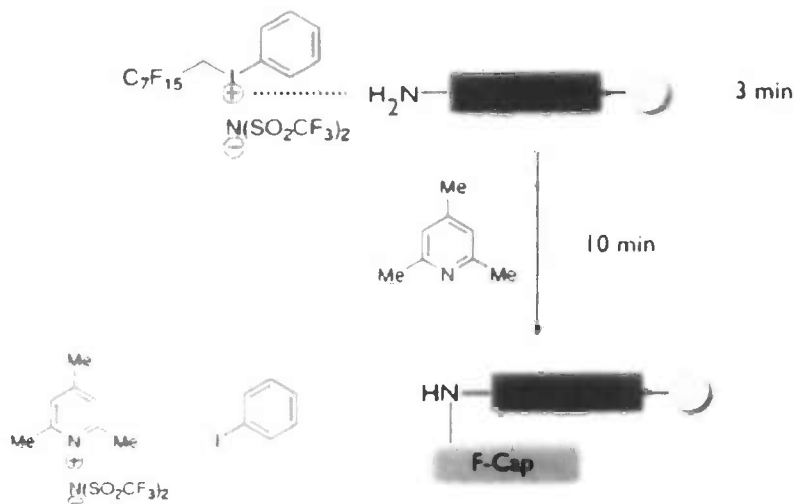
Figure 7. The fluorous capping step in solid phase peptide synthesis

DesMarteau and Montanari, while studying the trifluoromethane sulfonimide superacid and its salts, prepared the iodonium triflimide salt **1a** (14,15). This was found to be surprisingly water-stable. Not only: it could quickly alkylate nucleophilic substrates in water. The clearest evidence of this capability was the first synthesis of *N*-trifluoroethyl amino acids.

Figure 8 shows how these amino acids were synthesized. The *N*-alkylation is promoted by bicarbonate, in a biphasic dichloromethane-water medium. The amino acid is presumably able to complex the salt **1c**, but not sufficiently basic to promote the alkylation step. At the interface of organic solvent and water, bicarbonate decomposes the complex to the final products. When a hindered base such as collidine was used in a homogeneous organic phase, the yield of *N*-trifluoroethyl amino acids was low to zero, due to reaction of the hindered base itself with **1a**.

Thus, if **1b** or **1c** and collidine were introduced *at the same time* in a peptide capping step, collidine would prevail in the same way and become *N*-fluoroalkyl collidinium triflimide salt. This reaction is undesired in this context, but has been exploited elsewhere to prepare ionic liquids (16).

Properties of the *N*-trifluoroethyl amino acids

N-perfluoroalkylmethylene groups are inert. The attempt to condense *N*-trifluoroethyl amino acid esters with electrophiles failed under a variety of
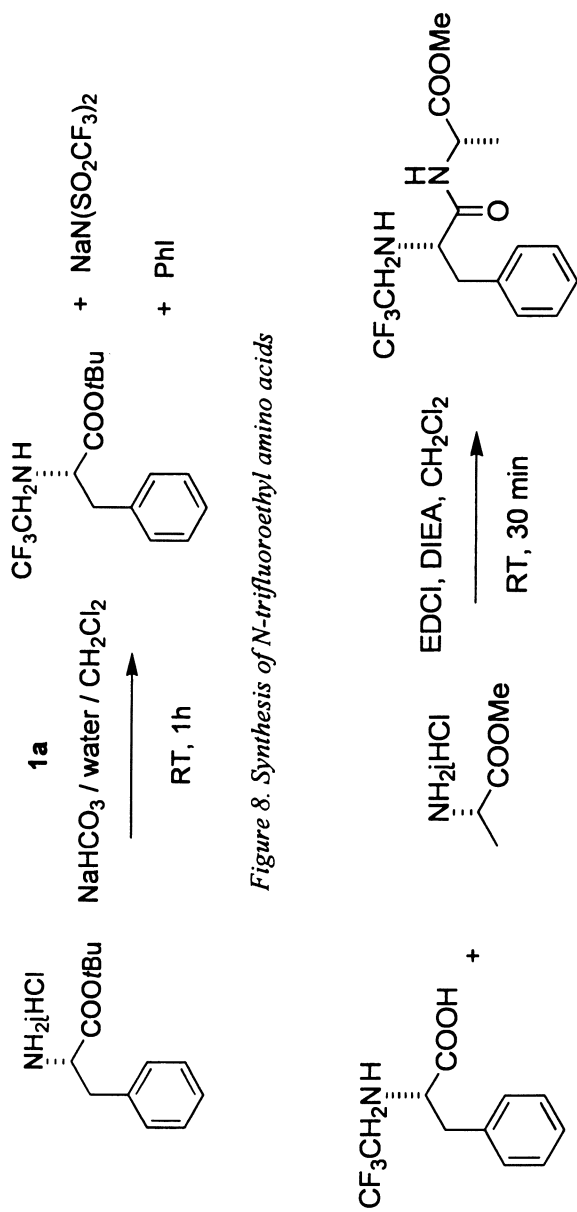
Figure 8. Synthesis of N-trifluoroethyl amino acids

Figure 9. Condensation of N-trifluoroethyl amino acids with nucleophiles

conditions (15). The *N*-trifluoroethyl group is so unreactive that condensation of the free carboxyl group with nucleophiles, including other amino acids, proceeded in high yields (Figure 9). The condensation reactions were so selective that usually the products required no purification besides conventional removal of coproducts (14, 15).

The method is versatile, as shown in the synthesis of the compound NSC 718800 (Figure 10), which had *in vitro* activity against leukemia cells (15, 17).

Synthesis of the iodonium salts

The synthesis consists of very easy steps, none of which requires special precautions. Detailed procedures were published (4,15).

In the published syntheses, the triflimide acid was sublimed from its lithium salt, but it is now commercially available. In the scheme of Figure 12, only the $R_fCH_2I(OCOCF_3)_2$ intermediates are not commercial compounds, but their synthesis presents no problem. They do not decompose rapidly even well above 100 °C (15) and are stable for at least one year at 4°C. On this basis, it is hoped that **1a-c** will become commercially available soon (18).

A further improvement in peptide capping technique.

Considering the mechanistic scheme and the reactivity of **1a-c** as discussed above, Kumar and Montanari (4) succeeded in using only a minimum amount of **1c** in preparing pure H_2N-GNQWAVGHLC-CONH$_2$, an analog of the peptide sequence Bombesin 5-14 (Figure 12).

As usual, the Bombesin analog was prepared in parallel with acetyl and fluorous capping. Three deletions were introduced. In this case, the solution of **1c** used to soak the resin was drained, collected and reused in subsequent capping steps. The concept was to use only as much **1c** as the unreacted amino groups on the resin would complex. The solution of collidine base was then added on top of the drained resin. As seen by the HPLC traces, the results remained good with much less consumption of reagent (4). We may call this the "flow" method of using reagents **1** in solid-phase synthesis.

Conclusions and recommendation for future work

The fluorous capping protocol that was demonstrated for peptides should be useful for other polymers that are synthesized stepwise on the solid phase by Boc or Fmoc chemistries. Compounds like **1a-c** are extremely reactive, yet entirely compatible with the existing methodology, resulting in clean syntheses for a
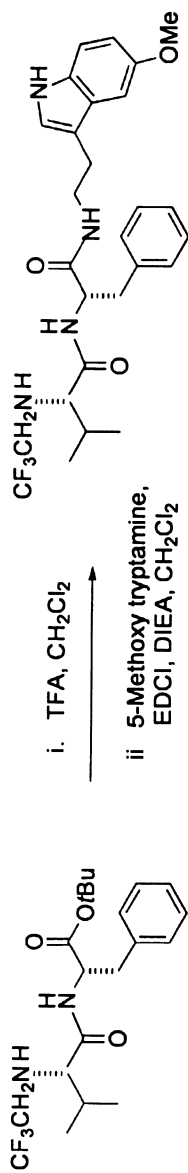
Figure 10. Synthesis of the bioactive compound NSC 718800
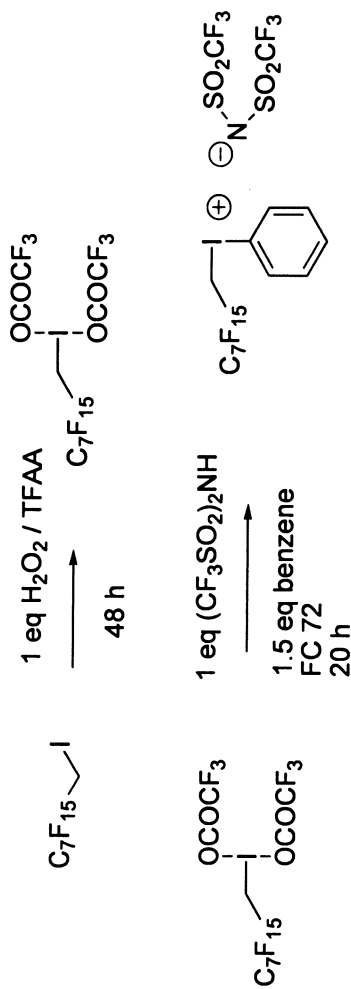
Figure 11. Synthetic scheme for 1a-c.
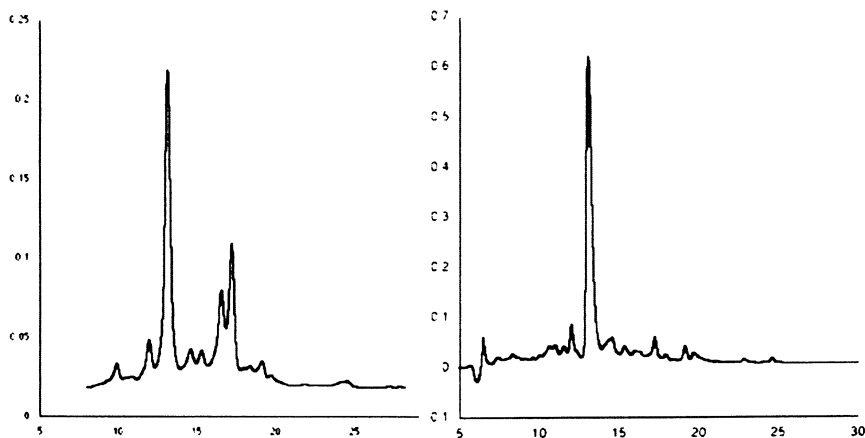
Figure 12. Bombesin analog, acetyl (left) and fluorous (right) capped

negligible increase in overall time. This may be exploited in solution work as well, analog to the synthesis of *N*-trifluoroethyl amino acids and their derivatives shown above.

There are many opportunities yet to be explored between the two proven extremes of small molecules and resin beads.

Summarizing the principal findings:

- One terminal fluorocarbon chain can drastically change the solubility of a large, ionic macromolecule enabling complete separation.
- Reactive iodonium salts install perfluoroalkylmethylene residues on nucleophilic termini in short reaction times. These residues are inert.
- The easy, two-step reaction guideline: a) allow the iodonium salt to find and complex its target, thereafter b) add a base to trigger reaction, has been consistently effective.

On this basis, frequent use of these reagents in chemical research, to purify macromolecules *during their synthesis* and to prepare novel fluorinated entities, is anticipated.

Acknowledgements

References

1. *The Peptides: Analysis, Synthesis, Biology*; Gross, E.; Meienhofer, J., Eds; Academic Press: London, 1979.
2. Andersson, L.; Blomberg, L.; Flegel, M.; Lepsa, L.; Nilsson, B.; Verlander, M. *Biopolymers (Peptide Science)* **2000**, *55*, 227-250.
3. Montanari, V.; Kumar, K. *J. Am. Chem. Soc.* **2004**, *126*, 9528-9529.
4. Montanari, V.; Kumar, K. *Eur. J. Org. Chem.* **2006**, 874-877.
5. Montanari, V.; Kumar, K. *J. Fluorine Chem.* **2006**, *127*, 565-570.
6. Bang, D.; Kent, S. B. *Proc. Nat. Acad. Sci. USA* **2005**, *102*, 5014.
7. Filippov, D. V.; vanZoelen, D. J.; Oldfield, S. P.; van der Marel, G. A.; Drijfhout, J. W.; van Boom, J. H.; Noort, D.; Overkleeft, G. S.; *Tetrahedron Lett.* **2002**, *43*, 7809.
8. de Visser, P. C.; van Helden, D. V.; Filippov, D. V.; van der Marel, G. A.; Overkleeft, G. S.; Drijfhout, J. W.; van Boom, J. H. *Tetrahedron Lett.* **2003**, *44*, 9013.
9. Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K. Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114.
10. Miura, T.; Goto, K. T.; Hosaka, D.; Inazu, T. *Angew. Chem. Int. Ed.* **2003**, *42*, 2047.
11. Luo, Z. Y.; Zhang, Q. S.; Oderatoshi, Y.; Curran, D. Science **2001**, *291*, 1766-1769.
12. Zhang, W. *Chem. Rev.* **2004**, *104*, 2531.
13. Umemoto, T. *Chem. Rev.* **1996**, *96*, 1757 and refs. therein.
14. DesMarteau, D. D.; Montanari, V. *J. Fluorine Chem.* **2001**, *109*, 19-23 and refs. therein.
15. Montanari, V. Ph.D. thesis, Clemson University, Clemson, SC, 2002.
16. Zhang, J.; Martin G. R.; DesMarteau, D. D. *Chem. Commun.* **2003**, *18*, 2334-2335.
17. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=403672.
18. Kumar, K. K.; Montanari, V. Patent WO 2005 118527 to Tufts University.
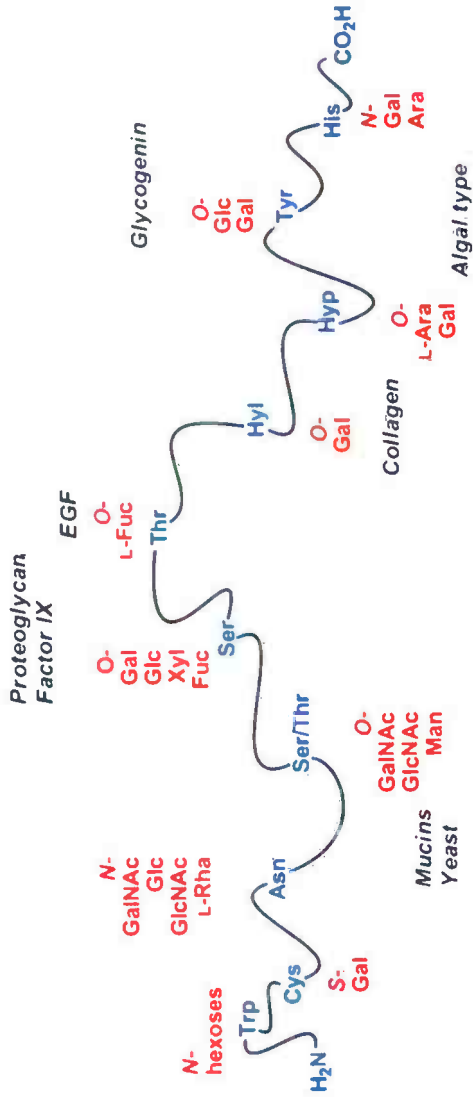
Indexes

Author Index

Subject index

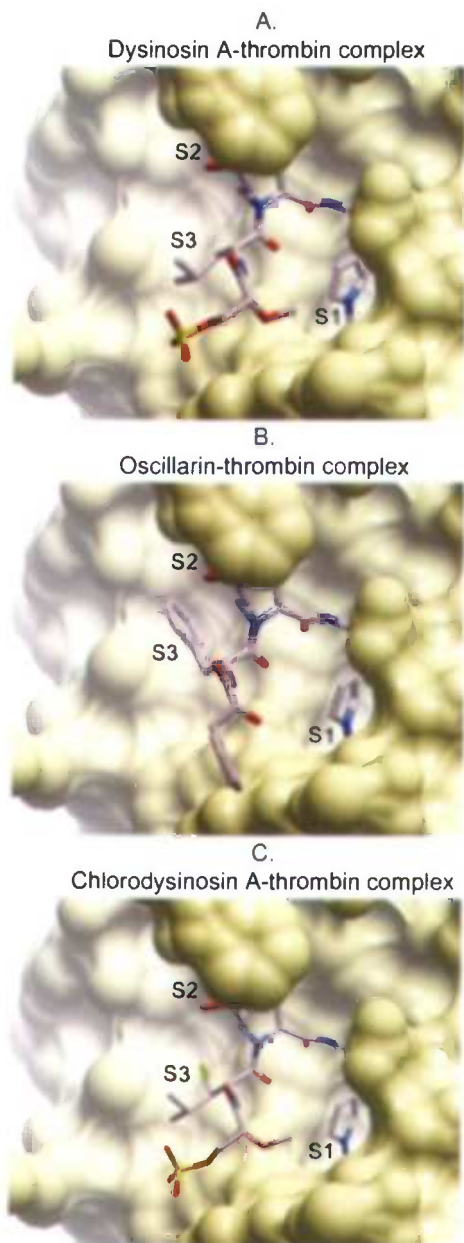*Figure 2.1. Forms of protein glycosylation.*

Figure 27.2. Connolly surface representations from X-ray crystal structures of thrombin in complex with dysinosin A,[10] oscillarin,[11] and chlorodysinosin A,[12] panels A-C, respectively. Molecular structures are outlined in Table 1.
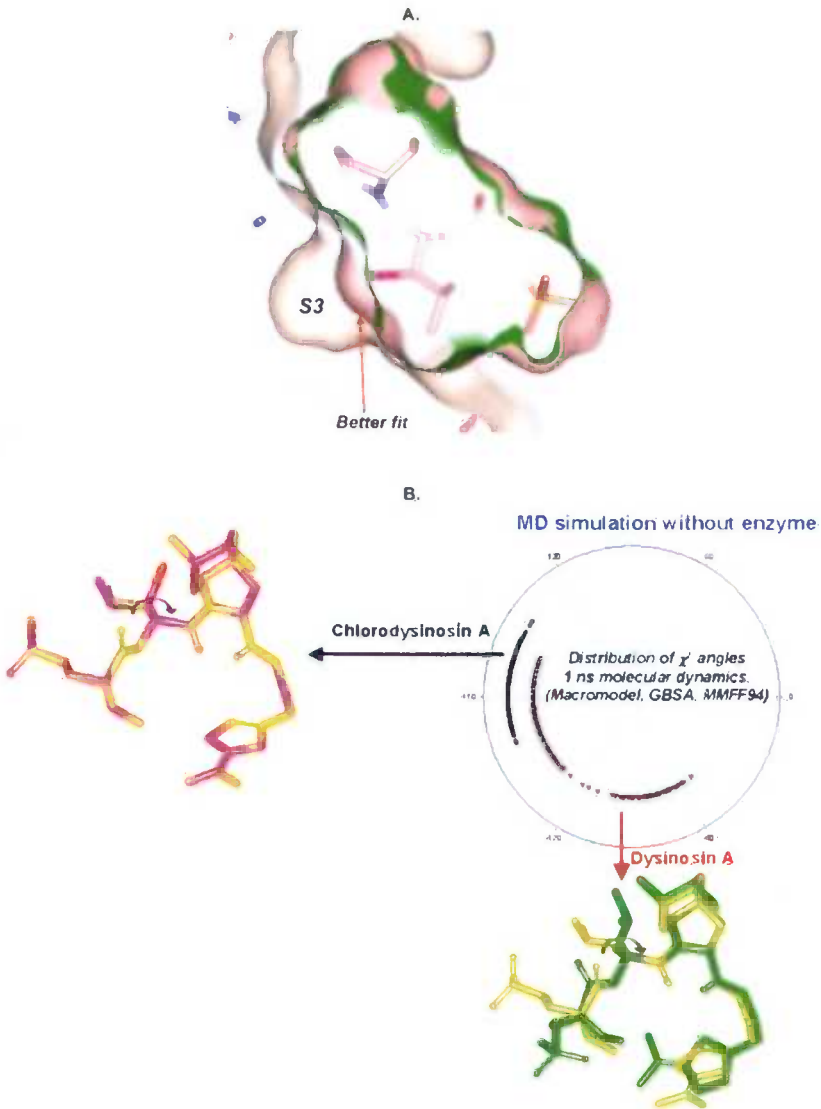
Figure 27.3. Panel A) Space filling model of the P3 side chains in the S3 pocket from an overlay of the X-ray crystal structures of dysinosin A (green) and chlorodysinosin A (pink) in complex with thrombin. Panel B) Molecular dynamics simulations of chlorodysinosin A (yellow and pink) and dysinosin A (yellow and green) without enzyme. Distribution of χ^1 dihedral angles in the P3 amino acid.