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– Workbench Edition E 22c –

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(HOUBEN–WEYL)

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WORKBENCH EDITION VOLUME E 22 c

SYNTHESIS OF PEPTIDES AND PEPTIDOMIMETICS

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Preface

As we approach the 100th anniversary of Emil Fischer's first synthesis of peptides, we bring together in a new presentation the wide variety of topics which demonstrate how far our field has grown and indicate the challenges that lie ahead. These five volumes cover the core of peptide and peptidomimetic chemistry and bring to peptide scientists, comprehensive and critical presentations in the *Houben–Weyl* tradition. Thus, the chapters are not encyclopedic, but rather focused on the most effective routes for peptide and peptidomimetic synthesis.

In 1974, Professor Erich Wünsch edited two *Houben–Weyl* volumes on the Synthesis of Peptides. That was a monumental undertaking and provided a resource widely used by chemists to prepare peptides and their derivatives with maximum attention to yield and purity of products. The current volumes continue these principles but encompass a much larger effort in the area of the organic chemistry of peptides and peptidomimetics. We as editors followed the *Houben–Weyl* concept of emphasizing protocols and reactions. Procedures for the often-used protections, deprotections, activations, and peptide and peptidomimetic bond formations remain the critical theme in all of the chapters. In addition, we make certain that there is a continuity that joins ongoing productivity in research to the accomplishments of the pioneers of our field. This format sets the stage for future scientists to undertake important research.

The founders of the field of peptide research paid great attention to methodology and isolation of products. The development of analytical and spectroscopic tools has enabled peptide scientists to create molecules of incredible variety and purity. Current researchers are in many ways mainly concerned with the interface between organic and biological chemistry. Thus, there is a strong emphasis on biologically interesting peptides and peptidomimetics in our volumes. However, the protocols and methodology covered in these volumes can also be applied to design novel materials, nanostructures, liquid crystals, and molecular sensors. Peptides and peptidomimetics represent structures applicable to broad vistas of molecular designs.

Recent developments in nucleic acids and protein sciences have allowed researchers to unravel the mechanisms of the biological action of many peptide ligands. As a consequence, many analogues and mimetics of natural ligands have been designed and synthesized. In these volumes we have paid substantial attention to these advances. With the exciting developments in genomics, proteomics, and pharmaconomics, the field of peptide and peptidomimetic research is expanding into new areas of molecular diversity. As a result, peptides remain a key component of the molecules of life.

The five volumes simultaneously represent a tribute to the pioneers in our field led by Emil Fischer and to the key figures who guided us more recently including Vincent du Vigneaud and Bruce Merrifield. We also acknowledge the hard work and creativity of all the scientists who have made substantial contributions to the chemistry of peptides and peptidomimetics. They are too numerous to name here. Nearly one hundred authors wrote chapters to create these volumes. Finally, the volumes are a challenge for future scientists to continue to make exciting discoveries as the new century unfolds.

The volumes are divided into sections covering a broad spectrum of reactions involved in peptide chemistry, synthesis of building blocks and target structures. Volume E 22a covers the protection of main-chain and side-chain functional groups, methods of peptide bond formation, and aspects of peptide synthesis in solution and on solid supports. Volume E 22b includes synthesis of large peptides and proteins. Specific methods are illustrated for the preparation of cysteine peptides, peptidomimetics, and conjugates such as glycopeptides, lipopeptides, phosphopeptides, and sulfated peptides. Cyclic peptides are extensively treated in this section. Volume E 22b concludes with a section on purification, analysis, and spec-

trometry of peptidic structures. Quality standards and comparative syntheses are also presented. Volume E 22c deals with peptidomimetic structures including side-chain-modified and main-chain-altered structures. In this volume, secondary structure inducing mimetics and scaffolds are treated. The synthesis of peptide nucleic acid (PNA) structures is also contained in this volume. Volume E 22d deals with *de novo* designed peptide-like molecules including template-assisted formation of helices, sheets, and loops. In addition, coiled coils are covered. Reactive peptides are presented. These molecules are composed of peptidic structures containing a wide variety of reactive groups for biologically important targets. The series concludes with a chapter on peptide natural products which are illustrative of nature's formulation of biologically active peptides and peptidomimetics. These are points of departure for peptide chemists to design and synthesize analogues and mimetics. The final volume of the series, E22e is an index volume containing an authors' index, substance index and preparation index

This series on the synthetic aspects of peptides and peptidomimetics could not have come about without the dedication and commitment of my three coeditors, Arthur Felix, Luis Moroder, and Claudio Toniolo. Each brought a wealth of experience, insight, and knowledge to formulate the scope of the volumes and critically review each of the chapters. In addition, the coeditors and I wish to thank Dr. Guido F. Herrmann for his unflagging support throughout the project. The professional staff of Thieme, led by Dr. Fiona Shortt, have worked tirelessly to ensure that the volumes appear with great attention to detail consistent with the *Houben–Weyl* tradition.

As a final note, the success of this venture rests with you the scientists throughout the world. We will know if we have created a useful set of volumes by the attention paid to them. If we have succeeded, there can be no greater satisfaction.

Murray Goodman
Editor-in-Chief
San Diego, USA
May 2004

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Volume E 22c

8 Introduction to the Synthesis of Peptidomimetics

C. Toniolo and M. Goodman

The discovery of a multitude of naturally occurring, bioactive peptides has generated a rich source of pharmacophores from which medicinal chemists are developing new useful therapeutic drugs. After binding to an enzyme, or a membrane receptor, peptide-based inhibitors, neurotransmitters, immunomodulators, and hormones influence cell-to-cell communications and control a variety of vital functions such as metabolism, immune defense, digestion, respiration, sensitivity to pain, reproduction, and behavior.

As a result of intense research, a wealth of protection strategies and coupling methods is available for synthesis in solution and on solid supports. Peptides of up to almost fifty amino acid residues can now be prepared in sufficient quantities for pharmacological and clinical assays, and for clinical drugs and diagnostics. Thus, in the last few years new peptide-based treatments for a series of diseases have been established.[1,2]

Though naturally occurring peptides based on coded amino acids have been widely used as drugs, there are problems with the use of peptides as therapeutic agents. The problems mainly arise from their rapid metabolism by proteolysis, and their interactions at multiple receptors. As a result, peptide researchers have sought modifications of peptide structures to create more stable and bioavailable molecules.[3–27]

Given the extensive biology associated with peptides, it is not surprising that considerable effort has been devoted to the synthesis of *peptidomimetics* to overcome the unfavorable properties and therapeutic deficiencies of peptides. In particular, since the advent of solid-phase synthesis and, more recently, combinatorial chemistry, interest in peptidomimetic design and preparation has exploded. In theory, the peptide chemist is only limited by their imagination in the novel modifications that can be tailored for synthetic peptide analogues.

Peptidomimetics have found wide application as biostable, bioavailable, and often potent surrogates of naturally occurring peptides. They form the basis of important families of enzyme inhibitors and they act as receptor agonists and antagonists. Peptide chemists are also gaining a deeper understanding of the structural features of this class of compounds that influence their ability to permeate biological membranes and their pharmacokinetic properties.

Nanomolar affinity for a protein receptor can be achieved by a peptide having a few, appropriately oriented side chains. This implies that the peptide backbone is not an absolute requirement for this high affinity, thus justifying the search for peptidomimetics. These compounds imitate the topology of a peptide, but may partly, or completely, lack its amide backbone characteristics. It is the rationale for the use of peptidomimetics that the backbone scaffold places the amino acid side chains in a particular 3D-position to allow contact with the enzyme or receptor protein. The 3D-relationship of the side-chain groups in the peptide (topology) determines its binding and biological messages.

However, it was immediately recognized by peptide chemists that, even in the cases where a direct (backbone)peptide$\cdots$protein(backbone) interaction is not operative, the backbone conformation may dramatically influence the biological response. It is evident that the introduction of new, promising peptidomimetics is based primarily on the combined knowledge of the complementary conformational, topochemical, and electronic properties of the native peptide and of its address (in other words, of the receptor or the active site of the enzyme with which it interacts). Then, the design of peptidomimetics as potential bioactive compounds must take into particular account two structural factors: (i) a favorable fit (tertiary structure) with respect to the corresponding complementary spatial situation at the active site; (ii) the placement of structural elements (e.g., functional groups, polar and

for references see p 2

hydrophobic regions) in well-defined positions so that the required interactions (e.g., hydrogen bonds, electrostatic or hydrophobic interactions) can occur.

A major problem in this area of research is represented by the conformational flexibility of most peptides, the high dependence of their conformation on environment, and the relationship between the conformation in solution and the receptor-bound conformation. To overcome these difficulties, a successful approach involves the preparation of conformationally constrained analogues. Synthetic, bioactive peptidomimetics specifically designed for this approach are characterized either by cyclization (main-chain-to-main-chain; side-chain-to-main-chain; or, side-chain-to-side-chain) or by the incorporation of amino acid or dipeptide building blocks that can explore only a very limited portion of conformational space. Corollary, useful properties of these systems often include prolonged biological activity as a result of an increased stability to proteolytic enzymes. By combining computer-assisted molecular modeling, modern spectroscopic and NMR techniques, and X-ray diffraction analysis it is now possible to design peptidomimetic drugs, the structural spectrum of which lies between that of slightly modified peptides and that of pure nonpeptide molecules. The preparation of such compounds requires the utilization of the complete arsenal of modern, synthetic organic chemistry in addition to the methodologies developed by peptide chemists.

This volume brings together most of these critical issues by highlighting recent advances in a number of core areas of peptidomimetic synthesis. Section 9 focuses on side-chain-modified peptides, Section 10 describes the preparation and use of a variety of peptide main-chain modifications. Combined side-chain- and main-chain-modified peptides are covered in Section 11. Finally, Section 12 provides chemistry leading to peptides incorporating secondary structure (β- and γ-turns, helices, β-sheets) mimetics and inducers.

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9 Side-Chain-Modified Peptides

A. M. Felix

The biological function of peptides and proteins depends on their native conformation. The side-chain functionalities of the α-amino acids that comprise peptides and proteins have profound effects on their properties. These functionalities reside in the 20 naturally occurring α-amino acids, which have different propensities for formation of the three major secondary structural conformations.[1] In addition to these naturally occurring α-amino acids whose primary structure enables the polypeptide to fold into a predictable secondary and tertiary structure, the incorporation of unnatural amino acids has opened important areas of research.

The large number of theoretically possible conformational states for a linear peptide is significantly reduced when the side chain of the peptide is modified with conformationally restricted α-amino acids. Conformationally restricted analogues of biologically active peptides may be more resistant to enzymatic degradation, or may be locked into a biologically active preferred conformation. Conformationally restricted peptides may result in other potential advantages including enhanced receptor selectivity, prolonged receptor binding, or increased hydrophobicity that may improve absorption and serve as important models for peptide design.[2]

The introduction of β-substituted analogues of natural amino acids into biologically important peptides enables their use as templates for peptidomimetics. Procedures for the synthesis of β-substituted analogues have been devised for several naturally occurring amino acids including β-substituted phenylalanines, 2′,6′-dimethyltyrosines, naphthylalanines, and cysteines, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids, and substituted phenylglycines, and glutamic acids (Section 9.1).

The pyrrolidine ring structure of proline is unique and places it in a special category among the natural α-amino acids since it leads to conformational restriction and a high probability of a *cis*-peptide bond preceding the proline residue in a polypeptide.[3] Proline and analogues of proline can play important roles in the de novo design of biologically important peptides and proteins. Procedures have been reported for the synthesis of nitrogen-containing three-membered rings (aziridine-2-carboxylic acids), four-membered rings (azetidine-2-carboxylic acids), five-membered rings (prolines), and six-membered rings [pipecolic acids (piperidine-2-carboxylic acids)], as well as those containing other heteroatoms (Section 9.2).

The incorporation of photoreactive amino acids into peptides (either during stepwise synthesis of the peptide or postsynthetically) enables the generation of a photoaffinity reagent that can bind to a receptor through carbene or nitrene intermediates. These peptides also contain tags (fluorophores, radionuclei, or biotin functions) which enable the identification of ligand-contact sites.[4] The synthesis of a variety of side-chain-modified phenylalanine- and benzophenone-containing photophores, and their use in the synthesis of the corresponding peptides (substance P, angiotensins, vasopressins, enkephalins), are reported (Section 9.3). The preparation of benzophenone-containing peptides using solid-phase methods either by direct insertion during synthesis or post-insertion after assemblage is described (Section 9.3.5.1).

Synthetic peptides containing side-chain modification have also been used as molecular scaffolds for the preparation of multiple receptors and molecular devices.[5] These include the use of crown ethers, cyclodextrins, porphyrins, and peptides with metal-binding sites (including ferrocenyl and EDTA side chains) (Section 9.4). Cyclization procedures have been developed to prepare biologically active cycloisodityrosine peptides which contain 14- or 17-membered rings (Section 9.5). The use of tryptathionine, a cross-linking dipeptide consisting of side-chain-to-side-chain linked L-Trp-L-Cys that is present in phallotoxins,[6] a family of cyclic heptapeptides, is also described (Section 9.6).

for references see p 4

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9.1 Synthesis of Side-Chain Conformationally Restricted α-Amino Acids

V. J. Hruby, G. Han, and P. M. Gitu

The side-chain groups of the 20 naturally occurring α-amino acids, which are found in peptides and proteins that are the products of genes, have a wide range of chemical functionalities which make them readily adaptable as acids, bases, nucleophiles, electrophiles, chelators, charged groups, hydrophobes, hydrophiles, proton acceptors, proton donators, etc. These also tend to be quite conformationally flexible with energy barriers to rotation about their torsional angles χ^1 (α–β bond), χ^2 (β–γ bond), etc. generally less than 8 kcal·mol^{-1} and, therefore, they readily interconvert at physiological temperatures. The exception is proline which has a five-membered ring which restricts the conformation; even so, proline readily interconverts between envelope-like conformations rapidly on the NMR timescale. Why nature has chosen 19 amino acid side-chain groups (Gly has no side-chain group) that are so flexible is not known, but it has great significance.

In order to understand peptide–protein, peptide–nucleic acid, peptide–peptide, peptide–lipid, and peptide–sugar interactions, binding, and information transduction in biological systems, side-chain conformational restriction can provide a very powerful tool for design.[1–3] Of course, of the 20 naturally occurring amino acids found in proteins, three have β-disubstitutions; namely, valine which has β-dimethyl substitution and hence is prochiral at the β-carbon atom, threonine which can be viewed as β-methylserine and hence is chiral at the β-carbon atom, and isoleucine which has β-methyl and β-ethyl substitution, and hence has a β-chiral center, and can be viewed as a β-methyl derivative of α-aminopentanoic acid. The presence of a β-substitution for these amino acids does lead to additional torsional constraint about the χ^1 (C_α–C_β) torsional angle. Undoubtedly this has been exploited by nature in some way, but surprisingly little has been undertaken by peptide and protein chemists to examine this question, and to systematically evaluate the effect of β-substitution on protein folding or molecular recognition, until quite recently.

It has been known for many years, however, that the β-branched amino acids, especially valine and isoleucine, cause problems in synthesis,[4,5] and special care and additional reaction time are required when β-substituted amino acids are added to a growing peptide chain in synthesis. For example, in the synthesis of [2,4-diisoleucine]oxytocin efforts to couple the isoleucine to isoleucine by the azide method failed and only the rearranged product was obtained.[6] Also, it is much more difficult to hydrolyze peptide bonds formed between two or more contiguous β-substituted amino acids using standard 6 M HCl conditions. For example, in the hydrolysis of [2,4-diisoleucine]oxytocin (3 isoleucine residues adjacent to each other) complete hydrolysis takes 60 hours.

Synthetic Side-Chain Conformationally Constrained α-Amino Acids

Because β-substituted analogues of the naturally occurring amino acids and their analogues can lead to novel amino acids with conformationally restricted side-chain groups, they provide unique opportunities to explore (a) the topographical requirements [g(−), *trans*, or g(+)] for molecular recognition with a particular conformational scaffold (α-helix, β-turn, etc.), (b) the stereostructural requirements for information transduction for specific pharmacophore elements, (c) the dynamic requirements for binding and induction of conformational changes accompanying binding, (d) the effects of topographical constraint on peptide stability against proteolysis on bioavailability, and on peptide–membrane permeability, and (e) their use as templates for the preparation of peptidomimetics. Thus, their design, synthesis, and incorporation into bioactive peptides and/or peptides designed to

for references see p 49

explore topographical properties is an increasingly important area of research. In this section we examine a few of the various modes of side-chain topographical constraints which have been examined to date, with specific applications to the design of biologically active peptides.

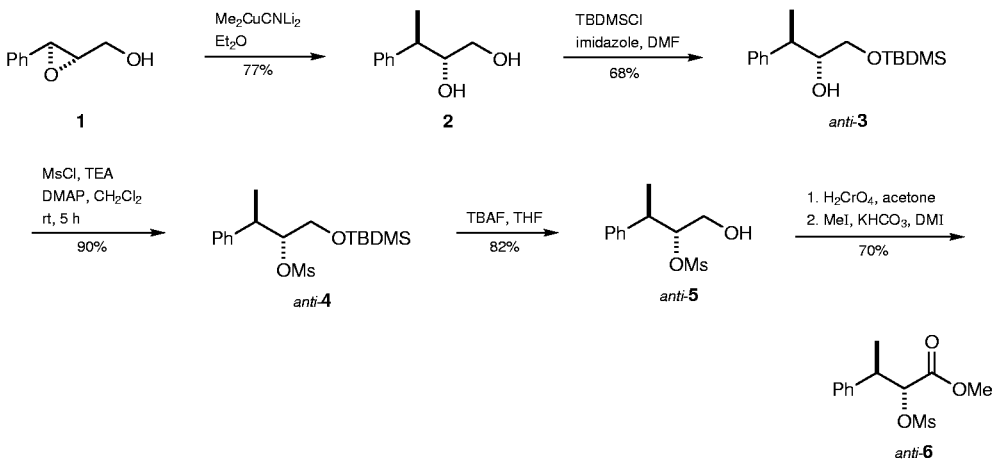
9.1.1 β-Methylphenylalanine

There is increasing evidence[3,7–19] demonstrating the important impact on bioactivity that β-methylphenylalanines can have when incorporated into bioactive peptides. Asymmetric synthesis of these compounds with well-established chemistry and economic starting materials has become well developed. Among the reported syntheses, the chiral auxiliary assisted asymmetric pathway[20–22] remains the most well-developed approach, while other pathways have only been briefly explored, because either too many steps were involved[23] or poor stereoselectivity was obtained.[24]

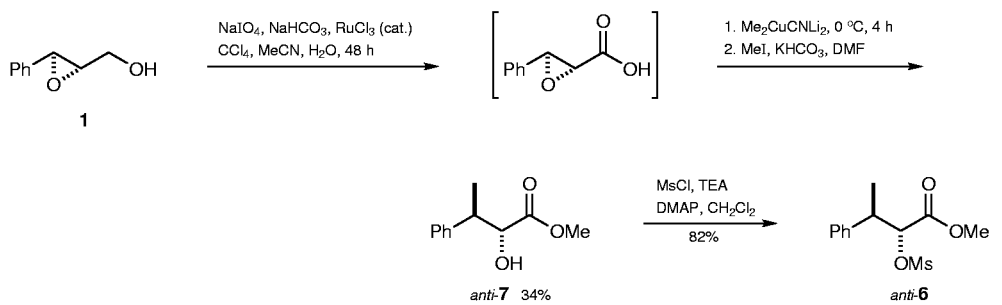
9.1.1.1 Asymmetric Epoxide Ring Opening

N^{α}-Boc-protected β-methylphenylalanines can be prepared from diastereoselective ring opening of epoxide derivatives, followed by selective protection, oxidation, and functional group substitutions (Schemes 1–5). Low yields in some steps limit the applications of this method.[23]

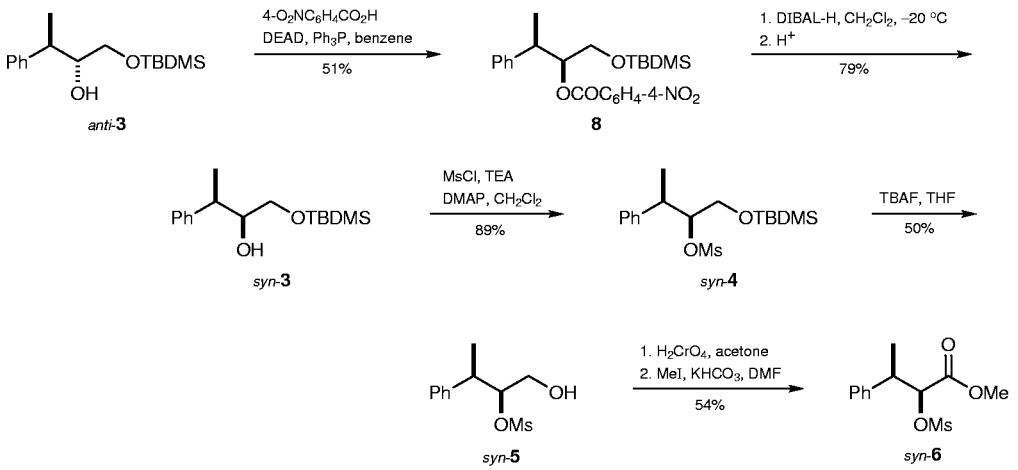
Scheme 1 Synthesis of the *anti*-Intermediate[23]



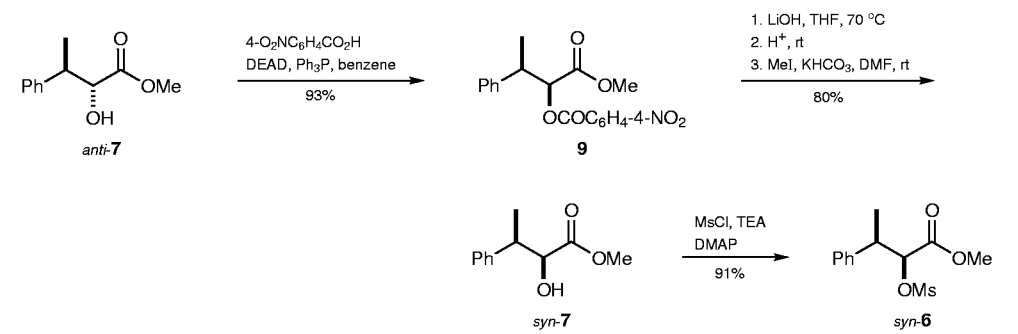
Scheme 2 Improved Synthesis of the *anti*-Intermediate[23]



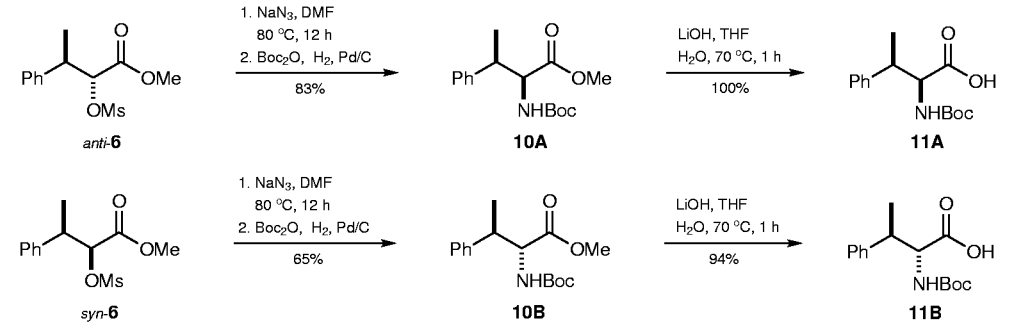
Scheme 3 Synthesis of the *syn*-Intermediate[23]



Scheme 4 Improved Synthesis of the *syn*-Intermediate[23]



Scheme 5 Synthesis of N^{α}-Boc-β-methylphenylalanines[23]



(2*R*,3*R*)-3-Phenylbutane-1,2-diol (2):[23]

CAUTION: *Cyanide salts can be absorbed through the skin and are extremely toxic. Appropriate safety precautions and first aid procedures should be adopted when handling cyanide salts.*

To a stirred slurry of CuCN (2.69 g, 0.03 mol) in anhyd Et_2O at −68 °C was added 1.6 M MeLi in Et_2O (37.5 mL, 0.06 mol), and the mixture was stirred at this temperature for 1 h. A soln of (2*S*,3*R*)-2,3-epoxy-3-phenylpropan-1-ol (**1**)[25,26] (≥99% ee; 1.5 g, 10 mmol) in Et_2O (36 mL) was added via cannula at −68 °C, and the mixture was allowed to warm to 0 °C over 3 h under N_2. When no starting material could be detected by TLC, sat. aq NH_4Cl (80 mL) and NH_4OH (10 mL) were added to the mixture, and the

for references see p 49