The western piperazine fragment is derived from a readily available pyrazine by reduction of the aromatic ring. While we have had success with both asymmetric catalytic reduction and enzymatic routes to the required (S)-piperazinecarboxamide, the method of choice is a classical resolution/racemization. Resolution is achieved by crystallization of the bis(l-pyroglutamic acid) salt. The undesired enantiomer is then racemized with base and recycled. Selective acylation (Boc₂O) at the distal nitrogen yields the piperazine ready for coupling (Scheme 7).

Simply heating the two key fragments (piperazine + epoxide) followed by removal of the Boc group from the distal nitrogen produces the penultimate intermediate in 94% yield. Alkylation with picolyl chloride and sulfate salt formation give Indinavir Sulfate, the active ingredient of CRIXIVAN® with greater than 99% enantiomeric and chemical purity (Scheme 8).

The chemistry described here is the work of an heroic team of scientists whom I have the honor to represent. I would especially like to acknowledge the team leaders from Merck’s Process Research Department: Drs. David Askin, Thomas R. Verhoeven, and R.P. (Skip) Volante; they and their colleagues have much to be proud of.

Received: May 5, 1997


Abstract. Aminopeptidase- and amidase-based methods for the production of enantioselectively pure amino acids, intermediates for pharmaceuticals and agrochemicals, are discussed. Furthermore, enzymatic syntheses of the dipeptide sweetener aspartame and semisynthetic antibiotics (such as ampicillin, amoxicillin, cephalaxin, and cefadroxil) are highlighted.

Introduction

There is an ever increasing demand for enantioselectively pure compounds used as building blocks but also as active ingredients in the pharmaceutical and agrochemical industry. In addition to relying on the existing chiral pool, production methods include resolution processes via diastereomeric salt formation, asymmetric homogeneous catalysis, asymmetric synthesis, and kinetic resolution processes with enzymes. In this paper, we will concentrate on the use of amidases and aminopeptidases in (industrial) synthesis. In addition, recent results with further transformations of unsaturated amino acids will be discussed.
The western piperazine fragment is derived from a readily available pyrazine by reduction of the aromatic ring. While we have had success with both asymmetric catalytic reduction and enzymatic routes to the required (S)-piperazinecarboxamide, the method of choice is a classical resolution/racemization. Resolution is achieved by crystallization of the bis(l-pyroglutamic-acid) salt. The undesired enantiomer is then racemized with base and recycled. Selective acylation (BocO) at the distal nitrogen yields the piperazine ready for coupling (Scheme 7).

Simply heating the two key fragments (piperazine + epoxide) followed by removal of the Boc group from the distal nitrogen produces the penultimate intermediate in 94% yield. Alkylation with picolyl chloride and sulfate salt formation give Indinavir Sulfate, the active ingredient of CRIVITIVAN® with greater than 99% enantiomeric and chemical purity (Scheme 8).

The chemistry described here is the work of an heroic team of scientists whom I have the honor to represent. I would especially like to acknowledge the team leaders from Merck’s Process Research Department: Drs. David Askin, Thomas R. Verhoeven, and R.P. (Skip) Volante; and their colleagues have much to be proud of.

Received: May 5, 1997


Application of Enzymes in Industrial Organic Synthesis


Abstract. Aminopeptidase- and amidase-based methods for the production of enantiomerically pure amino acids, intermediates for pharmaceuticals and agrochemicals, are discussed. Furthermore, enzymatic syntheses of the dipeptide sweetener aspartame and semisynthetic antibiotics (such as ampicillin, amoxicillin, cephalaxin, and cefadroxil) are highlighted.

Introduction

There is an ever increasing demand for enantiomerically pure compounds used as building blocks but also as active ingredients in the pharmaceutical and agrochemical industry. In addition to relying on the existing chiral pool, production methods include resolution processes via diastereomeric salt formation, asymmetric homogeneous catalysis, asymmetric synthesis, and kinetic resolution processes with enzymes. In this paper, we will concentrate on the use of amidases and aminopeptidases in (industrial) synthesis. In addition, recent results with further transformations of unsaturated amino acids will be discussed.

Kinetic Resolutions with Aminopeptidases and Amidases

α-H-Amino Acids

Amino acids have proven to be a versatile class of intermediates for a wide variety of enantiomerically pure pharmaceuticals and agrochemicals. Classical examples include D-phenylglycine and D-(p-hydroxyphenyl)glycine, used as building blocks for semisynthetic antibiotics, and L-phenylalanine, one of the constituents of the dipeptide sweetener aspartame. Production capacities for these types of applications typically are in the order of thousands of tons per year. Other amino acids, produced on a somewhat smaller scale, are utilized in various pharmaceutical and agrochemical applications. For example, D-valine is used in the synthesis of the pyrethroid insecticide fluvatinate.

Whereas the naturally occurring amino acids can often be most conveniently produced using microbial production methods (fermentation), synthetic amino acids or amino acids with the nonnatural D-configuration have to be prepared using chemical or chemo-enzymatic techniques. One particularly useful approach, discovered by DSM in 1975, is the use of an aminopeptidase present in Pseudomonas putida ATCC 12633 according to Scheme 1.

*Correspondence: Prof. Dr. H.E. Schoemaker
DSM Research
P.O. Box 18
NL-6160 MD Geleen
NL-6160 MD Geleen
AIMS, University of Amsterdam
Nieuwe Achtergracht 129
NL-1018 WS Amsterdam

© Neue Schweizerische Chemische Gesellschaft
ISSN 0009–4293

308 NSCS SPRING MEETING 97: INDUSTRIAL ASYMMETRIC SYNTHESIS

Chemia 51 (1997) 308–310

This enzymatic resolution process – including synthesis of the starting materials and recycling of the undesired isomer – has been reviewed on several occasions [1]. In addition to simple alkyl- and aryl-substituted amino acids, the method can be applied for a wide variety of specifically functionalized amino acids, of which some recent examples are depicted in the Figure.

\( \alpha, \alpha \)-Disubstituted Amino Acids

Enantiomerically pure \( \alpha, \alpha \)-disubstituted \( \alpha \)-amino acids, especially \( \alpha \)-methyl-substituted amino acids, are of increasing interest for the agrochemical and pharmaceutical industry. These compounds and their derivatives may act as enzyme inhibitors and several are antagonists of receptors. Analogous to the enzymatic resolution of \( \alpha \)-H-amino acids, we have developed procedures based on amidase preparations from \textit{Mycobacterium neoaurum} (ATCC 25795) and \textit{Ochrobactrum anthropi} (NCMB 40321). A whole range of sterically highly crowded amino acids can now be prepared on a large scale according to Scheme 2.

Application of Unusual Amino Acids in Organic Synthesis

In the past decades, we have illustrated the potential of the enzyme-mediated synthesis of enantiomerically pure amino-acid derivatives in different applications in the field of pharmaceuticals, agrochemicals, novel sweeteners, chiral ligands for homogeneous catalysis, etc.

Recently, we have shown that enantiomerically pure linear olefinic amino-acid derivatives can also be selectively converted to highly functionalized 6- and 7-membered ring amino acids using the ring-closing olefin metathesis catalyst developed by Grubbs. The principle of the approach is depicted in Scheme 3 [2].

Thermolysin-Catalyzed Synthesis of the Sweetener Aspartame: Enzymatic Peptide Synthesis Under Thermodynamic Control

The dipeptide sweetener aspartame is produced by Holland Sweetener Company (HSC), the joint venture between DSM and TOSOH, on a more than 2000 tons per year scale using a thermolysin-catalyzed peptide bond-forming reaction.

The system is under thermodynamic control due to the formation of an addition compound (salt) of N-protected aspartame and a second equivalent of phenylalanine methyl ester, as depicted in Scheme 4. The enzyme is both regioselective – only the \( \alpha \)-carboxy group is involved in peptide bond formation, the \( \beta \)-carboxy group remains unaffected – and stereoselective. Even if D,L-phenylalanine methyl ester is used as a substrate, only the N-protected L,L-dipeptide is formed. This is one of the rare examples of industrial peptide synthesis under thermodynamic control.

Enzymatic Synthesis of Semisynthetic Antibiotics: Enzymatic Peptide Bond Formation under Kinetic Control

Traditionally, semisynthetic penicillins like ampicillin and amoxicillin are prepared from 6-aminopenicillic acid (= 6-APA) and D-phenylglycine or D-(\( \alpha \)-hydroxyphenyl)glycine, respectively, using chemical procedures. Also, semisynthetic cephalosporines like cephalixin and ce-
Chiral Amino Acids: A Versatile Tool in the Synthesis of Pharmaceuticals and Fine Chemicals

Karlheinz Drauz*

Abstract. Methods of preparing enantiomerically pure amino acids especially focusing on amino-acylase-based resolution of D,L-acetylamino-acid precursors, synthesis of D-amino acids using a hydantoinase system, and the cofactor-dependent enzymatic reductive amination of α-keto acids to L-amino acids are described. Examples are given for bulk actives, based on L- and D-amino acids and peptides. L- and D-Tle (= L-/D-2-amino-3,3-dimethylbutanoic acid) are important molecules for synthesizing drugs and a great variety of chiral auxiliaries. A new chromatographic separation of bulky-side-chain amino acids in a preparative scale is described, giving both enantiomers in > 99% ee.

Enantiomerically pure compounds (EPC) can be made by different methods. The resolution of racemates using enzymes, optically active acids or bases forming diastereomeric salt pairs or chromatographic systems are common methods. The chiral pool offers a great variety of natural chiral substances which could be transformed to advanced derivatives; amino acids and sugars are the most prominent examples. Fermentation is using the metabolism of living cells, producing highly effective dedicated compounds based on sugar or advanced precursors.

Induction of asymmetry using enzymes or chiral auxiliaries in ideally catalytic amounts can provide all types of enantio-

Received: April 11, 1997
