CHIMIA 51 (1997) Nr. 6 (Juni)

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Application of Enzymes in Industrial Organic Synthesis

Hans E. Schoemaker*, **Wilhelmus H.J. Boesten**, **Quirinus B. Broxterman**, **Eric C. Roos**, **Bernard Kaptein**, **Will J.J. van den Tweel**, **Johan Kamphuis**, **Emmo M. Meijer**, and **Floris P.J.T. Rutjes** (in part)[a])

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, cephalexin, 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 fluvalinate.

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*.

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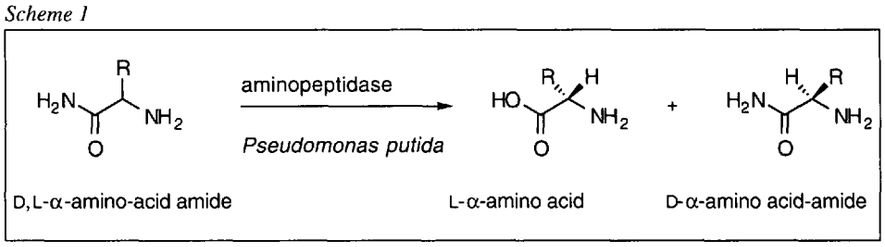
NL–1018 WS Amsterdam

Scheme 1



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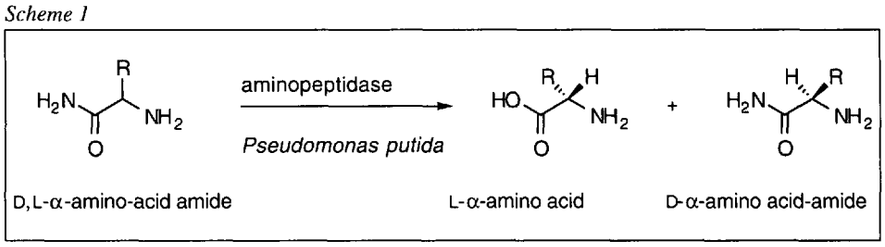
Scheme 1



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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*.

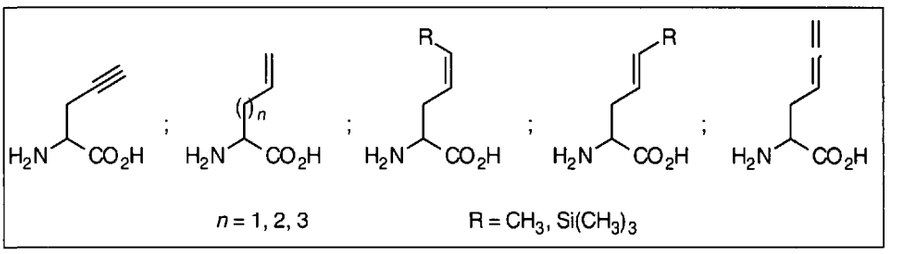
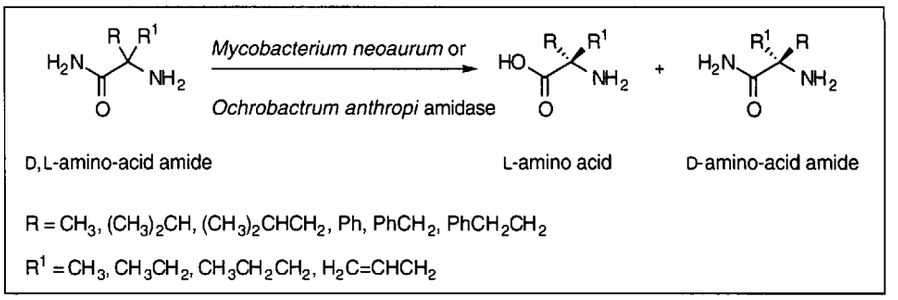


Figure. *Some recent examples of specifically functionalized amino acids*

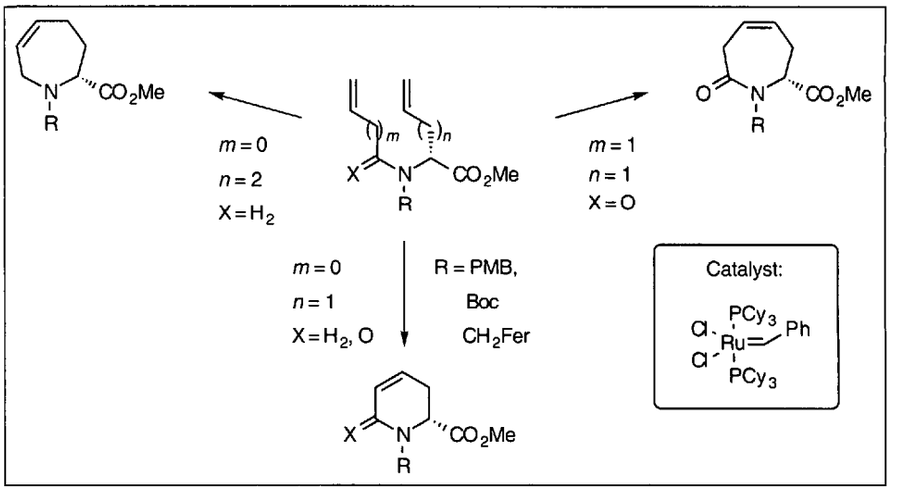
α,α-*Disubstituted Amino Acids*

Enantiomerically pure α,α-disubstituted α-amino acids, especially α-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 α-H-amino acids, we have developed procedures based on amidase preparations from *Mycobacterium neoaurum* (ATCC 25795) and *Ochrobactrum anthropi* (NCIMB 40321). A whole range of sterically highly crowded amino acids can now be prepared on a large scale according to *Scheme 2*.

Scheme 2



Scheme 3

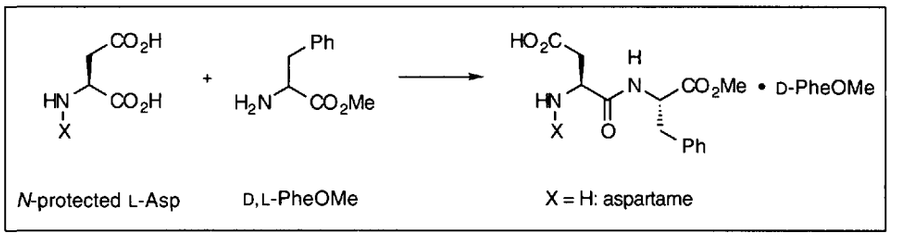


Application of Unusual Amino Acids in Organic Synthesis

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].

Scheme 4



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 α-carboxy group is involved in peptide bond formation, the β-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-(*p*-hydroxyphenyl)glycine, respectively, using chemical procedures. Also, semisynthetic cephalosporines like cephalexin and ce-

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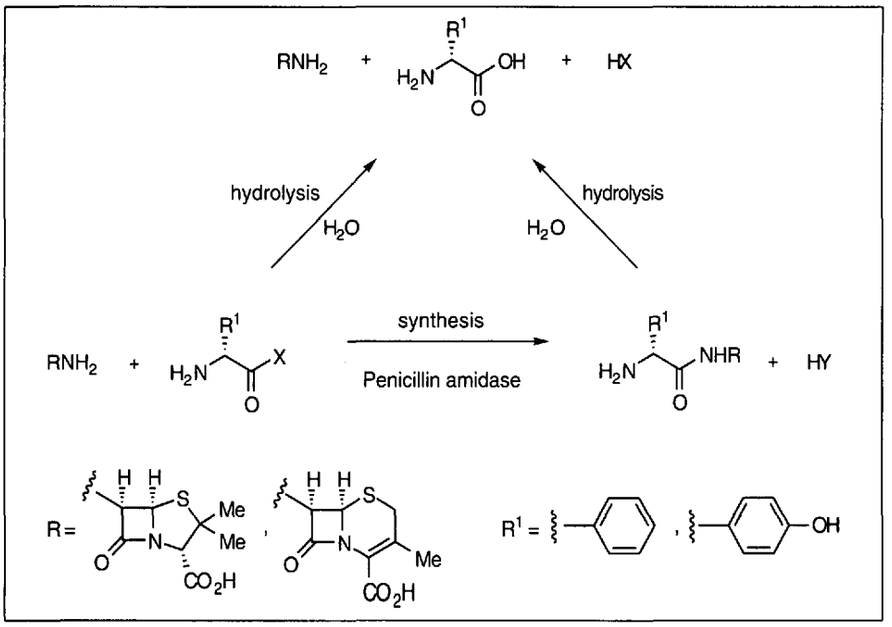
Scheme 5



fadroxil are derived from D-amino-acid derivatives and 7-amino-desacetyloxy-cephalosporanic acid (= 7-ADCA). From an environmental point of view, it is highly desirable to replace the chemical processes by an enzymatic method in which a D-phenylglycine or D-(*p*-hydroxyphenyl)-glycine derivative is coupled in water to the β-lactam unit under kinetic control. This approach is depicted in *Scheme 5*.

Due to the resemblance in physical properties of both starting materials, products, and side products – all contain both amino and carboxylic-acid functionalities –, in combination with the well-known instability of the β-lactam nucleus under aqueous conditions, careful downstream processing is of utmost importance for success. Moreover, an optimal ratio between synthesis and hydrolysis, an intrinsic property of the enzyme used, is one of the key success factors for industrial application of this enzymatic approach, which has been developed by *Chemferm*, the joint venture between *DSM* and *Gist-Brocades*.

Conclusions

It has been shown that both enzymatic hydrolysis and synthesis of amide bonds offers attractive commercial opportunities. Advantages of the enzymatic approach include both stereoselectivity and regioselectivity of the enzyme preparations used, in combination with the fact that enzymes may catalyze the formation of peptide bonds in aqueous solution. Environmentally benign processes for the production of semisynthetic antibiotics and related compounds will be part of the realm of industrial synthesis soon.

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[1] For a recent review article, see *e.g.*: H.E. Schoemaker, W.H.J. Boesten, B. Kaptein, E.C. Roos, Q.B. Broxterman, W.J.J. van den Tweel, J. Kamphuis, *Acta Chem. Scand.* **1996**, *50*, 225, and ref. cit. therein.

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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. Fermention 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-

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