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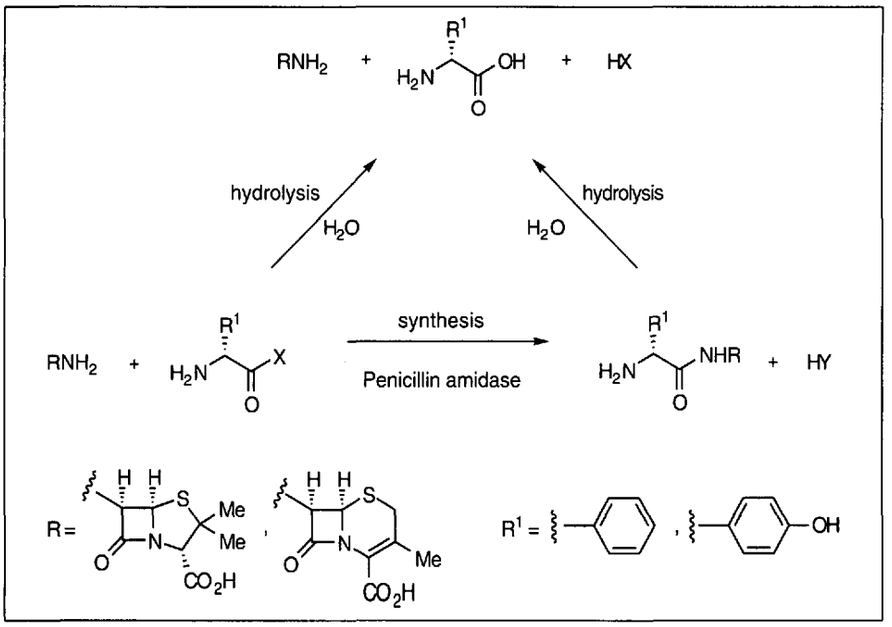
Scheme 5



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.

[2] F.P.J.T. Rutjes, H.E. Schoemaker, *Tetrahedron Lett.* **1997**, *38*, 677.

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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-

**Correspondence*: Prof. Dr. K. Drauz
Degussa AG
R + D Fine Chemicals
Specialty Chemicals Division
D–63403 Hanau

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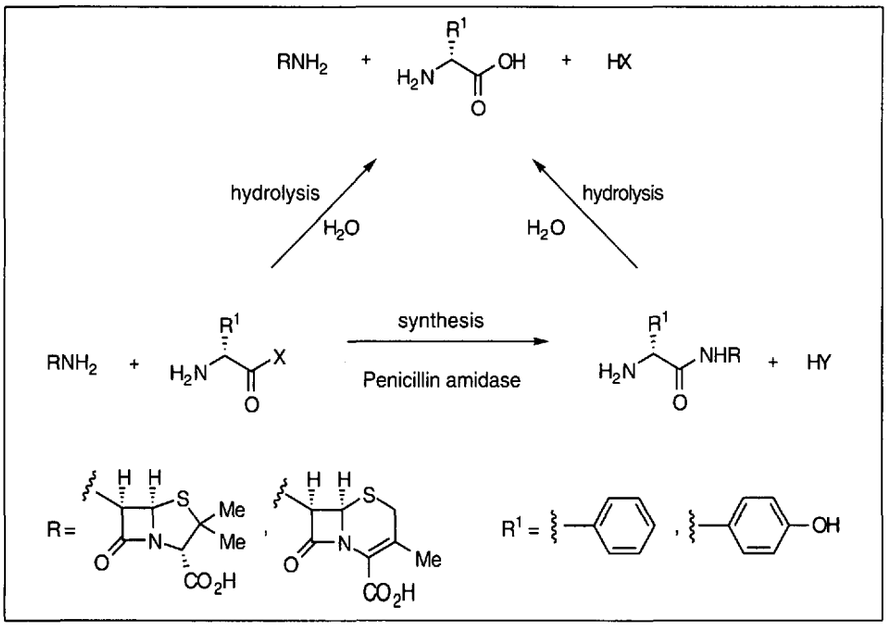
Scheme 5



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*.

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

**Correspondence*: Prof. Dr. K. Drauz
Degussa AG
R + D Fine Chemicals
Specialty Chemicals Division
D–63403 Hanau

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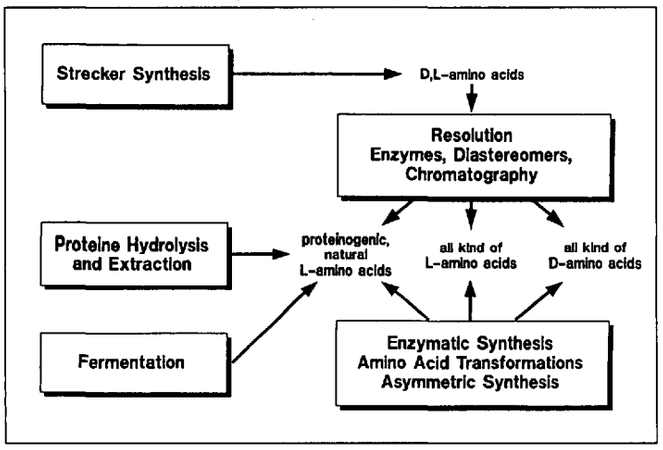
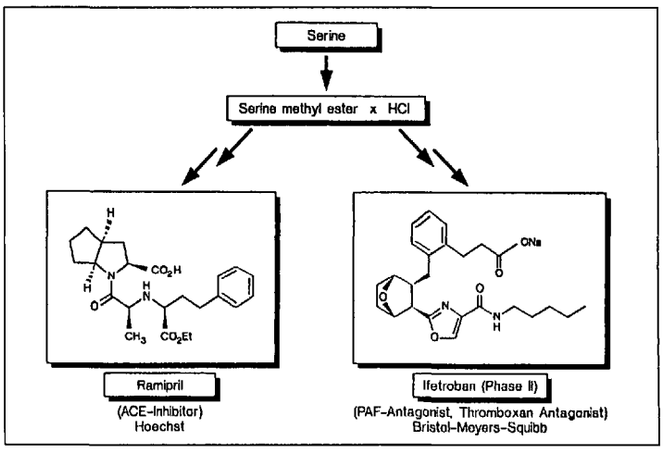
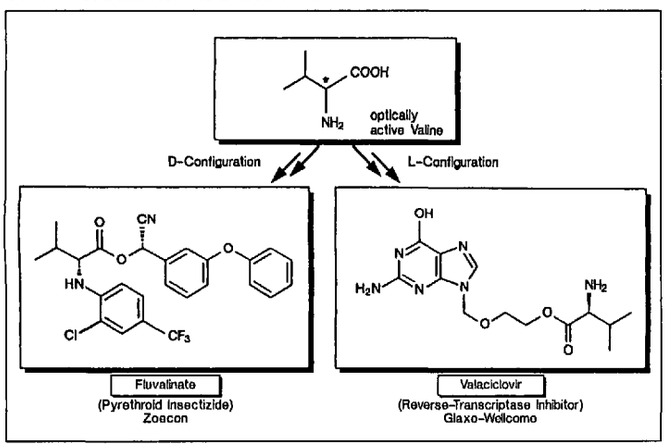
Scheme 1. *Basic Methods for Amino-Acid Synthesis*



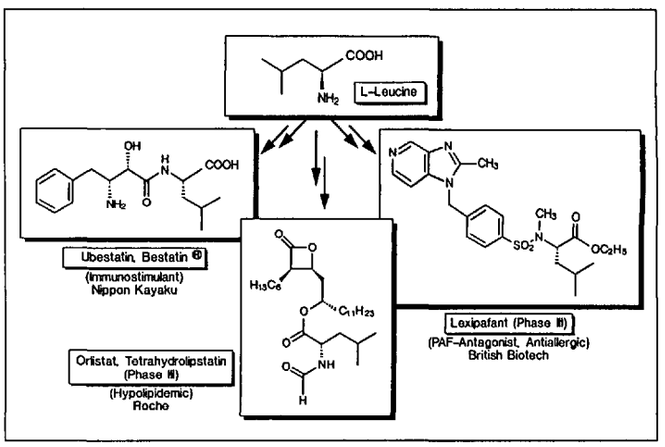
Scheme 2. *Bulk Actives Based on Ser*



Scheme 3. *Bulk Actives Based on* L- *and* D-*Val*



Scheme 4. L-*Leu-Based Pharmaceuticals*



merically pure compounds. The most efficient EPC synthesis is depending on the structure of a specific molecule.

In amino-acid chemistry, *Strecker* synthesis is one of the classical methods making racemates. Hydrolysis of cheap proteins like keratin or gelatine, followed by extraction with ion-exchange chromatography is yielding proteinogenic amino acids. Genetically coded amino acids could be produced by fermentation. Asymmetric synthesis and amino-acid transformations like the conversion of L-Arg into L-Orn are completing the set of methods (*Scheme 1*).

Chiral amino acids can be used as starting materials for peptides and peptide derivatives, β- and γ-amino acids, chiral amines, chiral heterocycles, amino alcohols, and other chiral auxiliaries and all types of C- or N- modified derivatives. A few examples are given in the *Schemes 2–5*.

In case of producing L-Met, protein hydrolysis or fermentation cannot be used. The method of choice is the enzymatic resolution of *N*-acetyl-D,L-Met, which is produced from acrolein in a five-step reaction. The resolution process is carried out in an enzyme membrane reactor using a native amino acylase [1].

Peptides and peptide derivatives are highly active drugs having, *e.g.*, antidiabetic, cardiovascular, antibacterial, antiviral, anti-inflammatory, and antitumor activity.

LH-RH	1 Glp	2 His	3 Trp	4 Ser	5 Tyr	6 Gly	7 Leu	8 Arg	9 Pro	10 Gly	NH_2
Agonists											
Buserelin						D-Ser-(tBu)					
Naferelin						D-2-Nal					
Lutrelin						D-Trp	N-MeLeu			Pro-NHC_2H_5(1-9)	
Leuprolide						D-Leu					
Tryptorelin						D-Trp				Pro-NHC_2H_5(1-9)	
Goserelin						D-Ser(tBu)					
Histrelin						D-His(Bzl)				Pro-NHC_2H_5(1-9)	
Antagonists											
Cetrorelix	Ac-D-Nal-D-Cpa-D-Pal					D-Cit				D-Ala – NH_2	
Antarelix	Ac-D-Nal-D-Cpa-D-Pal					D-Hci		Lys(iPr)		D-Ala – NH_2	
Antide	Ac-D-Nal-D-Cpa-D-Pal				Lys(Nic)	D-Lys(Nic)		Lys(iPr)		D-Ala – NH_2	
Ramorelix	Ac-D-Nal-D-Cpa-D-Trp					D-Ser(Rha)				Azagly–NH_2	

Glp = Pyroglutamic acid **Pal** = 3'-Pyridylalanine **Rha** = Rhamnosyl
Nal = 2'-Naphthylalanine **Nic** = Nicotinoyl **Cpa** = (4-Chlorophenyl)alanine
Hci = Homocitrulline

Fig. 1. *LH-RH agonists and antagonists*

LH-RH agonists and antagonists are effective compounds in especially treating sex-hormone-dependent cancers like prostate and mamma carcinoma (*Fig. 1*).

Cetrorelix, a phase-two candidate of *ASTA Medica*, is inhibiting tumor growth by blocking LH and FSH release in the

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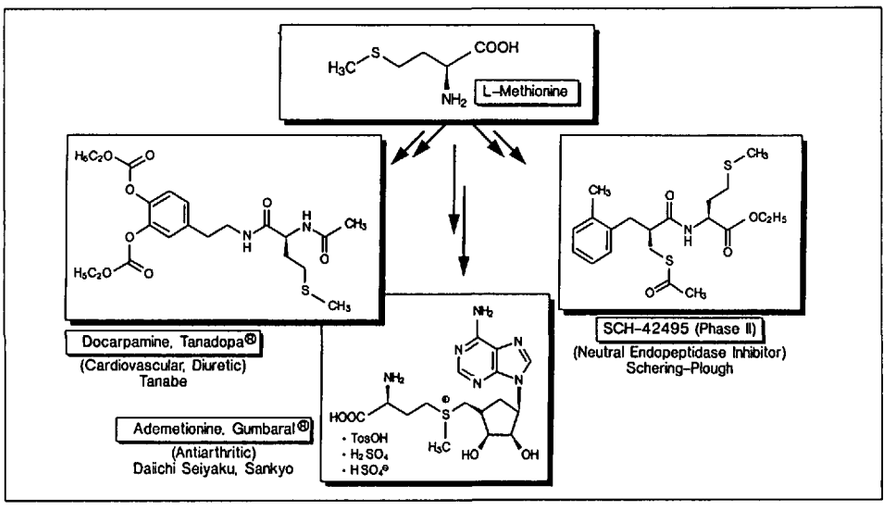
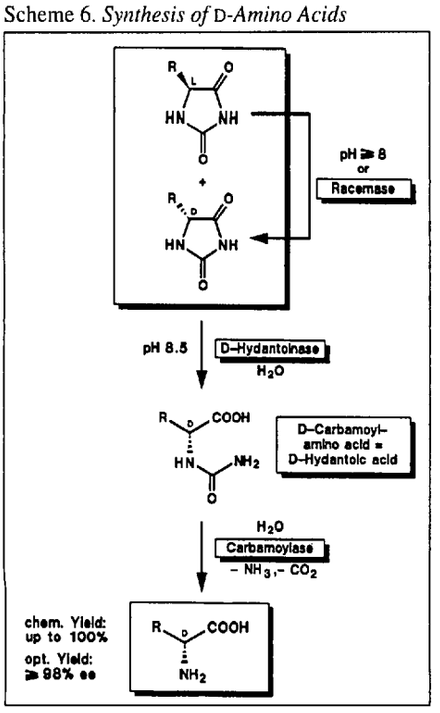
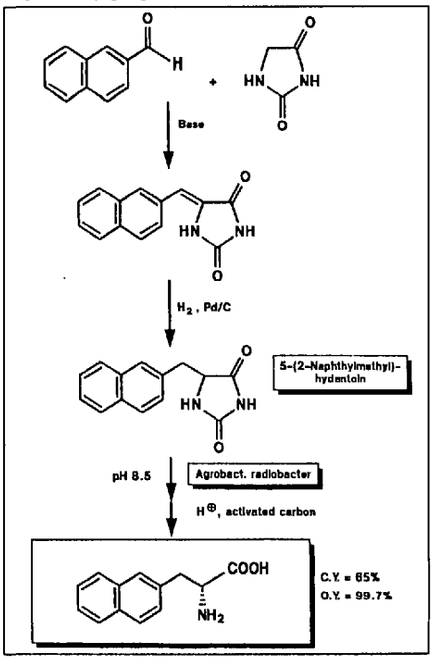
Scheme 5. L-*Met-Based Bulk Actives*



Scheme 6. *Synthesis of* D-*Amino Acids*



Scheme 7. *Synthesis of* D-*Nal* (= D-2-amino-3-naphth-2'-ylpropanoic acid)



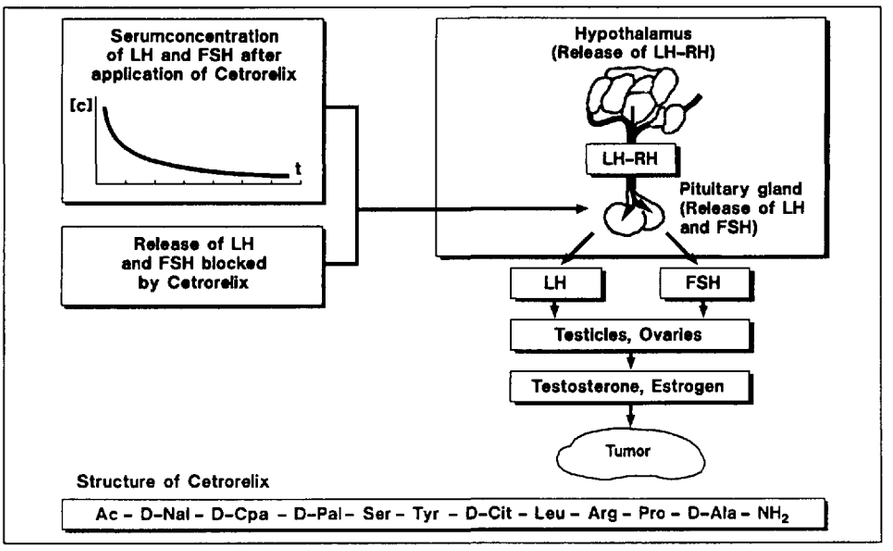
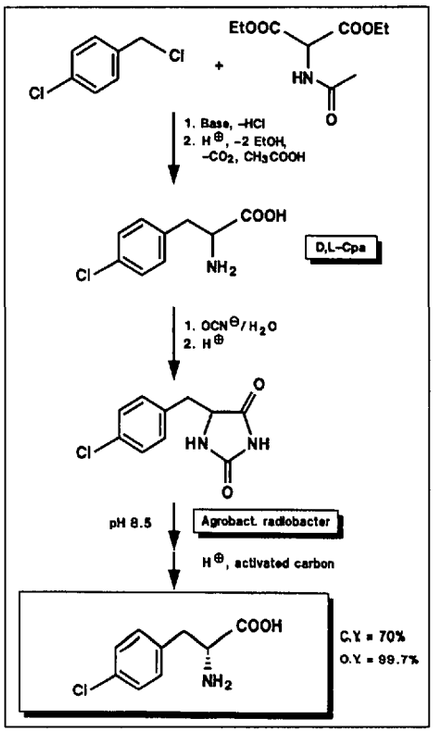
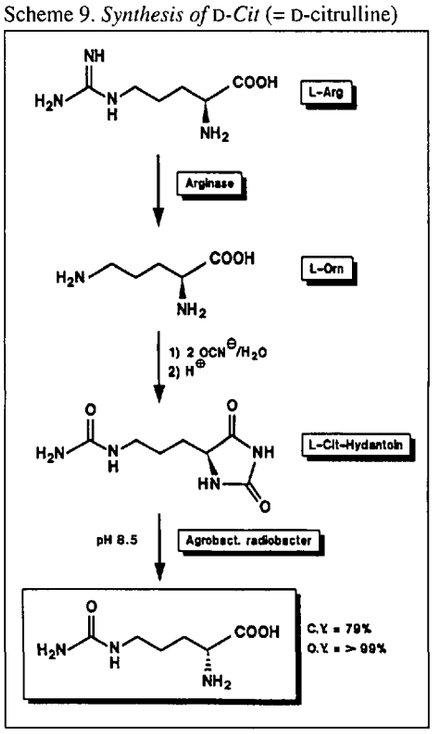


Fig. 2. *Antitumor activity of Cetrorelix*

Scheme 8. *Synthesis of* D-*Cpa* (= D-2-amino-3-(4-chlorophenyl)propanoic acid)



Scheme 9. *Synthesis of* D-*Cit* (= D-citrulline)



pituitary gland directly after application. This compound is a decapeptide consisting out of five L- and D-amino acids [2] (*Fig. 2*).

When we started our first attempts of synthesizing Cetrorelix, a solid-phase synthesis was used to get quick access to this compound. As the need increased during clinical development, we switched to a

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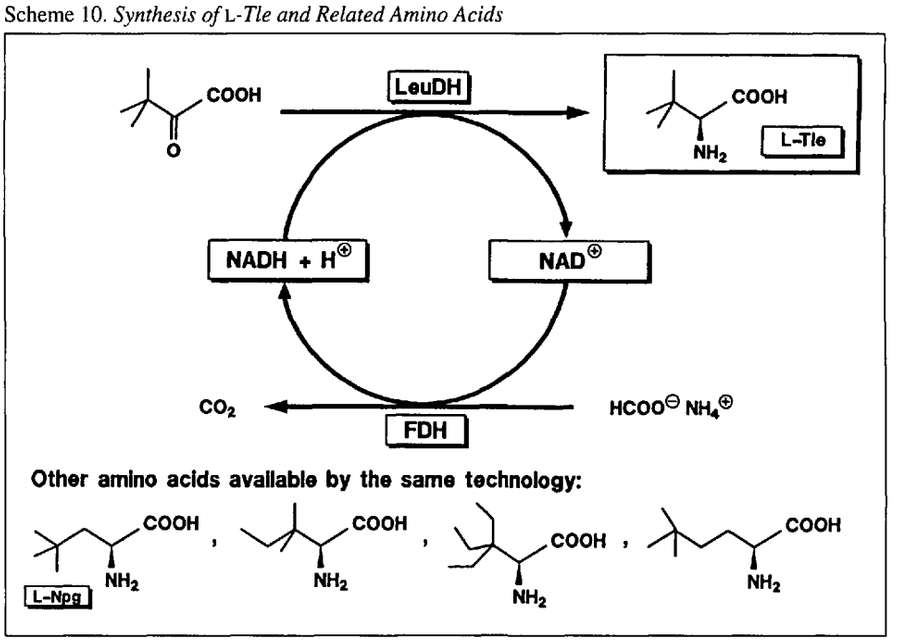
classical convergent liquid-phase synthesis. We also developed new methods producing the special D-amino acids of Cetrorelix (*Scheme 6*).

After testing and comparing different methods, we have chosen a biocatalytical route using a whole cell system which can convert a racemic hydantoin to the D-amino acid in up to 100% optical and chemical yield. This biocatalyst contains a D-hydantoinase and a D-carbamoylase as well as a racemase which together contribute to this reaction [3].

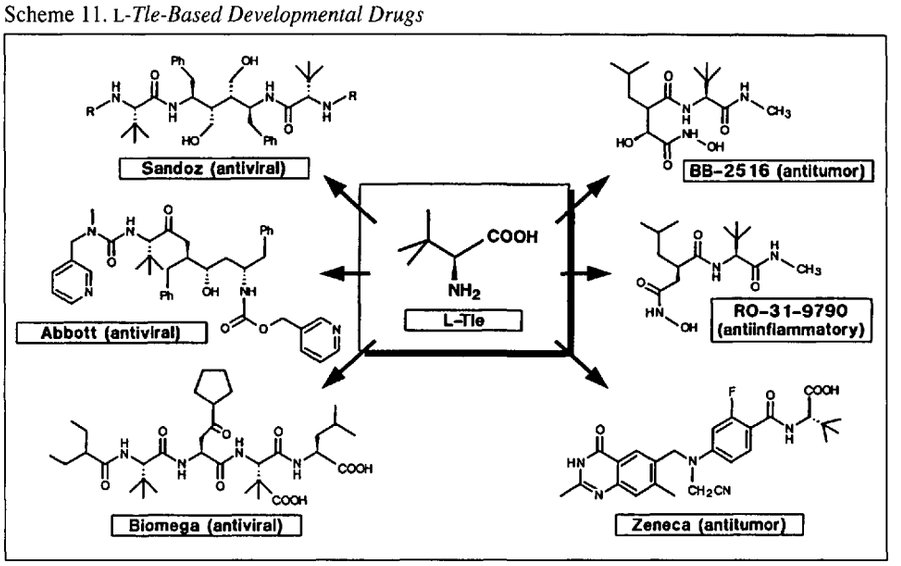
Schemes 7–9 are showing the different routes towards the hydantoins and the broad substrate specifity of our hydantoinase system. The most elegant synthesis is the three-step conversion of L-Arg to D-Cit, using two highly selective biocatalytical transformations.

Other highly specific bioconversions are based on NADH-depending oxidoreductases. Ketones could be reduced to chiral alcohols, α-keto acids to chiral α-hydroxy acids, and in the presence of ammonia, α-keto acids are converted by reductive amination to L-amino acids. The cofactor is regenerated by a second enzyme formate dehydrogenase (FDH), which is oxidizing formic acid to CO_2. Leucine dehydrogenase also accepts bulky-side-chain α-keto acids and therefore can be used for the synthesis of bulky-side-

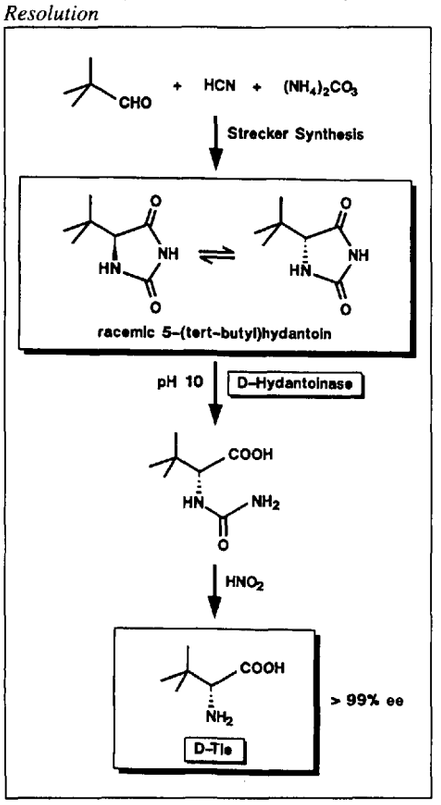
Scheme 10. *Synthesis of* L-*Tle and Related Amino Acids*



Scheme 11. L-*Tle-Based Developmental Drugs*



Scheme 12. *Synthesis of* D-*Tle* via *Enzymatic Resolution*



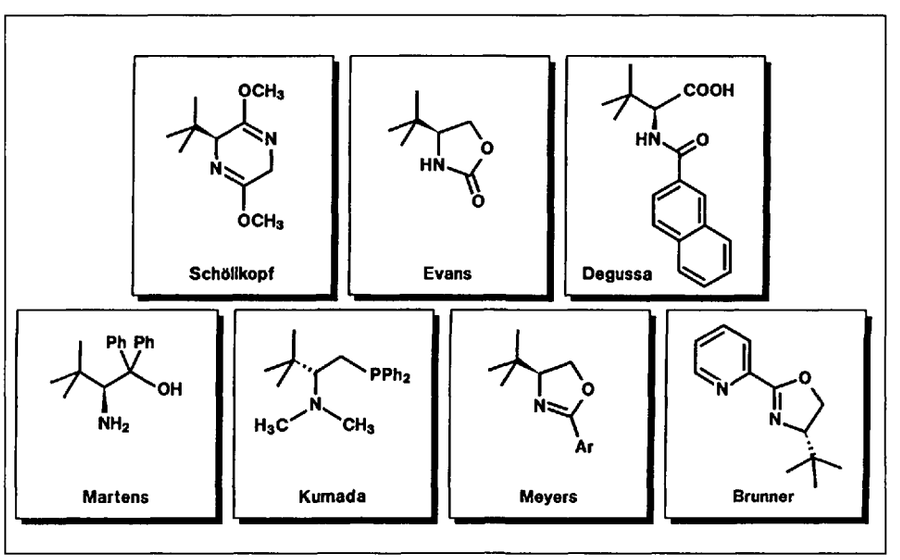


Fig. 3. *Tle-based chiral auxiliaries 1*

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chain amino acids like L-Tle (= L-2-amino-3,3-dimethylbutanoic acid) and others [4] (*Scheme 10*).

L-Tle is inducing special propertities in biologically active compounds like enhanced lipophilicity and increased half-life time by decreasing enzymatic degradation. *Scheme 11* is showing a selection of interesting developmental candidates based on L-Tle.

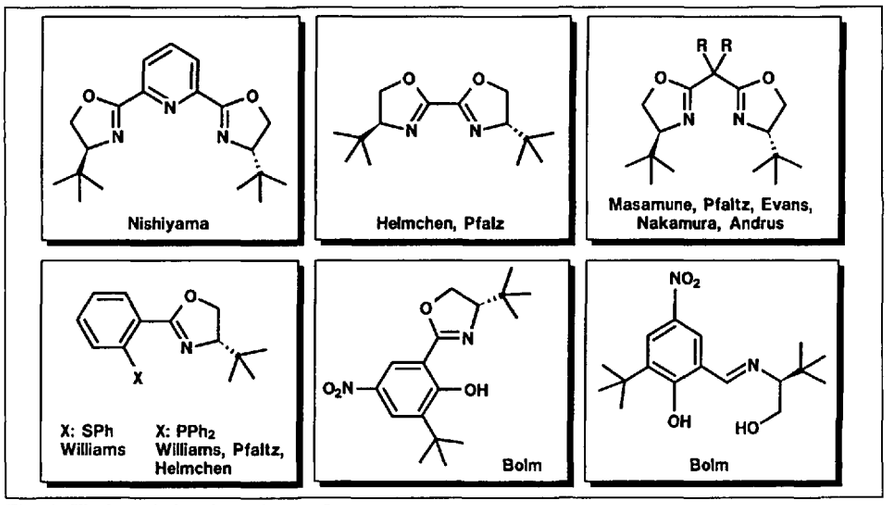

Fig. 4. *Tle-based chiral auxiliaries 2*

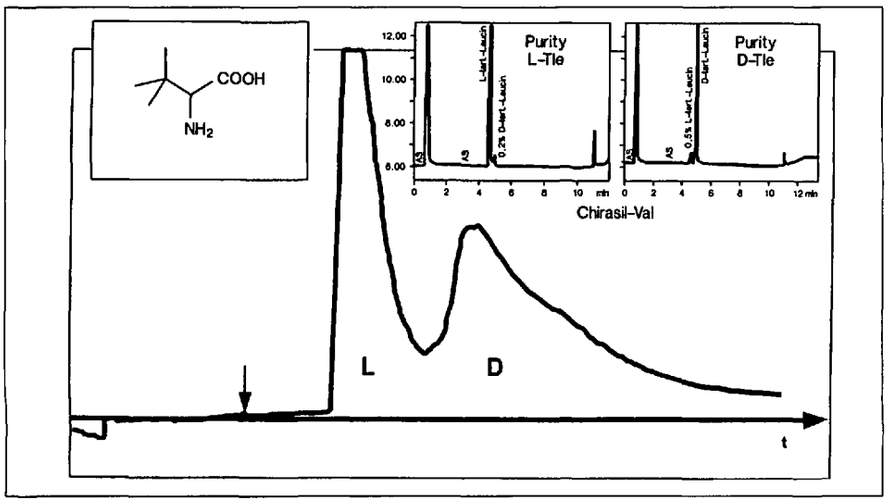

Fig. 5. *Chromatographic separation of* D,L-*Tle*

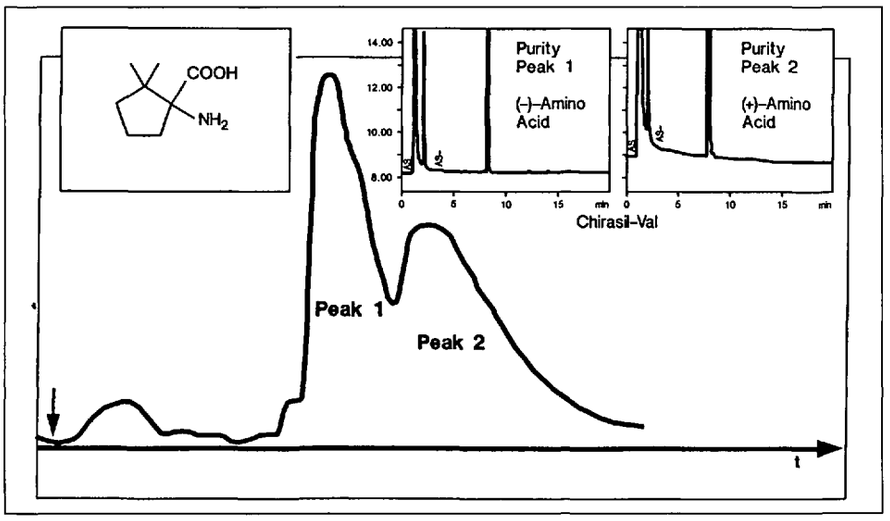

Fig. 6. *Chromatographic separation of* D,L-*1-amino-2,2-dimethylcyclopentane-1-carboxylic acid*

Because of its *tert*-butyl group, L-Tle and derivatives are also effective chiral auxiliaries inducing asymmetry or resolving agents [5] (*Figs. 3* and *4*).

The synthesis of highly pure D-Tle is difficult, because normal enzymatic or chemical resolution processes are giving low yields and moderate optical purities [4] (*Scheme 12*).

After an intensive screening, we found a single D-hydantoinase suitable for converting *rac*-5-(*tert*-butyl)hydantoin to carbamoyl-D-Tle which can be deprotected with HNO_2 to the free amino acid yielding a very satisfying ee value.

Very recently, we developed a chiral chromatographic system for separating racemic bulky-side-chain amino acids directly into both pure L- and D-enantiomers. By this way, D,L-Tle and analogous compounds could be resolved in a preparative scale giving both enantiomers in > 99% ee [6] (*Figs. 5* and *6*).

The author would like to thank Prof. *Carsten Bolm*, *Angelika Magnus*, and *Arno Classen*, RWTH Aachen for the very fruitful collaboration in asymmetric chemistry synthesizing and using amino acids, Dr. *Kurt Günther* and *Stefan Merget* for their skillful analytical and preparative chromatographic separations, and my coworkers Dr. *Michael Bernd*, Dr. *Kyriakos Makryaleas*, Dr. *Thomas Müller*, Dr. *Matthias Kottenhahn*, Dr. *Michael Schwarm*, and Dr. *Andreas Bommarius* for their excellent synthetic work.

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