



# Biocatalysis

## Preparation and functionalization of N-heterocycles

Michael Petersen and Andreas Kiener\*

Biotechnology Research, Lonza AG, CH-3930 Visp, Switzerland. E-mail: andreas.kiener@lonza.ch

Received 7th December 1998

### Summary

Biocatalysis has proven to be a useful supplementary technology for the chemical industry, allowing in some cases reactions which are not easily conducted by classical organic chemistry or in other cases allowing reactions which can replace several chemical steps. Highly chemo-, regio- and stereoselective bioconversions can simplify manufacturing processes to make them even more economically attractive and environmentally acceptable. The examples described are a selection of biocatalytic processes used for the preparation or modification of N-heterocycles.

### Introduction

Aromatic and aliphatic N-heterocycles are frequent structural motifs in nature's molecules and in man-made chemical active substances such as pharmaceuticals and agrochemicals. Nature's synthesis of amino acids, ribonucleosides, vitamins and the broad spectrum of biologically relevant compounds contrasts with the synthetic methods used by man. Efficient catalysis by enzymes results in highly selective reactions under mild conditions. However, the rules dictated by economics give the highest scores to technologies leading to cheap processes. Fortunately, processes generating less environmental damage are increasingly better financially rewarded due to the increasing costs of by-product and solvent disposal.

Organic chemistry and other manufacturing technologies are very innovative to further increase the environmental compatibility of chemical production technologies. During the past two decades biocatalysis has also proven to be a useful supplementary technology, allowing in some cases reactions which are not easily conducted by classical organic chemistry or in other cases allowing reactions which can replace several chemical steps. Highly chemo-, regio- and stereoselective bioconversions can simplify manufacturing processes to make them even more economically attractive and environmentally acceptable.

This article can not give a complete listing on all the different N-heterocyclic compounds produced by biocatalysis. It focuses on biocatalytic processes that are presently used as chemoenzymatic or entirely fermentative processes for the preparation of N-heterocycles to give examples of the broad applicability of biocatalysis today.

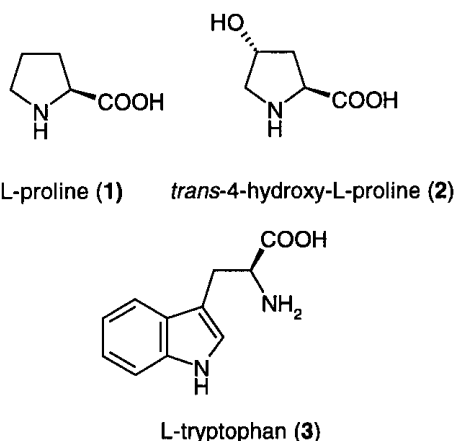
### 1 De novo synthesis of N-heterocyclic compounds

The isolation of substituted N-heterocycles from plants or other natural raw materials, as well as the production by micro-organisms grown on renewable resources such as glucose, are in principle ecologically very favorable manufacturing methods. The biosynthetic capabilities of living entities have found many commercial applications for the production of a wide variety of N-heterocycles. These processes have been reviewed by many authors in detail and therefore only a brief summary will be given in this article with references to recent publications.

N-Heterocycles are found in different classes of valuable natural compounds like amino acids, nucleosides, vitamins, and alkaloids. In many cases the desired substance is available from cheap natural raw materials by direct isolation. If the content is too low or no cheap raw material is available, complex biomolecules can be produced by fermentation. Several of the described compounds are produced by chemical, chemo-enzymatic or fermentative processes or isolated from natural sources simultaneously by different companies.

### L-Proline and hydroxy-L-proline

For L-proline (**1**) and *trans*-4-hydroxy-L-proline (**2**) (as well as cystine and tyrosine), isolation from collagen is still the most economical production method. The standard procedure for isolating an amino acid from an aqueous protein hydrolysate is chromatography on strong acidic ion-exchange resins. Even pharmaceutical grade amino acids are obtained by this procedure.<sup>1</sup> For the fermentative production of L-proline, micro-organisms of the genera *Brevibacterium*, *Corynebacterium* and *Serratia* have been used. Mutants of *Brevibacterium flavum* requiring isoleucine for



### Green Context

Many N-heterocycles are produced by nature, and are of interest due to their bio-activity. The use of biotechnology in their synthesis has been successfully applied to a range of these molecules. This review details syntheses of N-heterocycles from non-heterocyclic starting materials, and others involving modification of the ring structure. Many of these transformations represent useful extensions of the classical chemical routes, often with significantly improved environmental impacts. While many syntheses are impressive, effort is still required to push up product concentrations in the final fermentation solution. DJM

growth, and which are resistant to certain amino acid analogs, are capable of producing up to 40 g L<sup>-1</sup> of L-proline.<sup>2</sup>

### L-Tryptophan

Pathway engineering was performed in *Corynebacterium glutamicum* using molecular biological techniques to increase the production of L-tryptophan (**3**) up to 43 g L<sup>-1</sup>.<sup>3</sup> However, this amino acid is currently prepared with chemo-enzymatic methods discussed in a later section of the article.

### Ribonucleosides

Japanese companies have developed very efficient fermentation methods for the production of the ribonucleosides adenosine (**4**), guanosine (**5**), uridine (**6**), cytidine (**7**) and inosine (**8**). With the exception of inosine the ribonucleosides can also be prepared from polymeric ribonucleosides (RNA) isolated from yeast cells. Inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) are important purine nucleosides with flavor-enhancing activity. Both IMP and GMP are produced on a multiton per year scale.

Table 1 gives examples of biocatalysts developed for the preparation of ribonucleosides. With respect to ribonucleosides it is interesting to point out that presently no fermentation methods have been developed for the preparation of 2'-deoxyribonucleosides. The classical source for 2'-deoxyribonucleosides is hydrolyzed salmon sperm DNA.

**Table 1** Microbial strains developed for the production of ribonucleosides

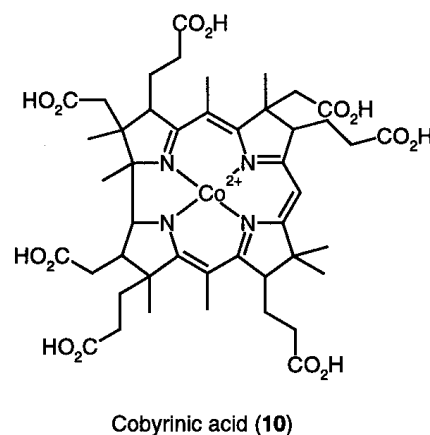
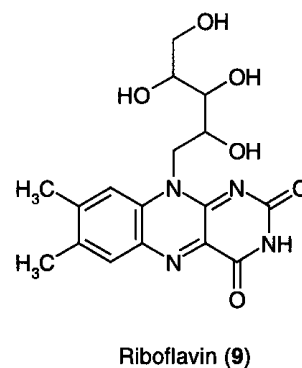
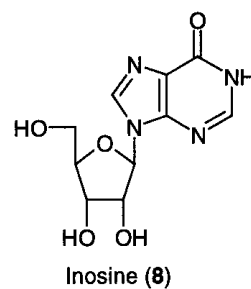
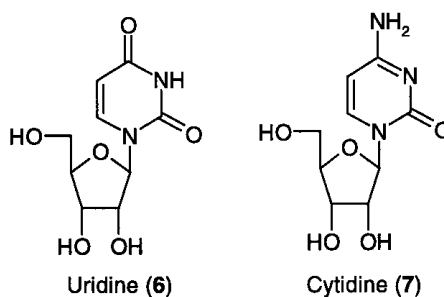
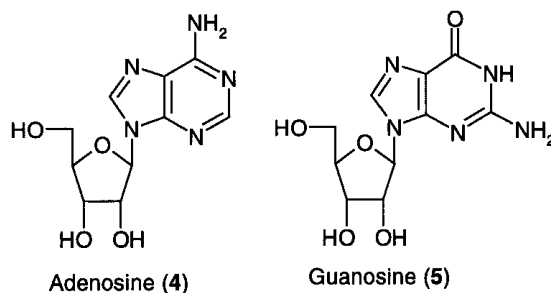
Ribonucleoside	Product conc. /g L <sup>-1</sup>	Microorganism	Ref.
Adenosine	16	<i>Bacillus subtilis</i>	4
Cytidine	19	<i>Bacillus subtilis</i>	5
Guanosine	20	<i>Bacillus subtilis</i>	6
Inosine	31	<i>Corynebacterium ammoniagenes</i>	7
Uridine	65	<i>Bacillus subtilis</i>	8

### Riboflavin

Vitamin B<sub>2</sub> (riboflavin) (**9**) is produced on a large scale by chemical synthesis or by a combined fermentative/chemical process. In recent years, novel microbiological production processes for the synthesis of riboflavin have been developed, some of which represent attractive alternatives to the current chemical production processes. BASF (Germany) uses the genetically modified fungus *Ashbya gossypii*<sup>9</sup> and Hoffmann-La Roche (Switzerland) a recombinant *Bacillus subtilis* strain. The product concentrations for both strains are over 10 g L<sup>-1</sup>. van Loon *et al.* have shown that the fermentation process offers significant environmental and economic advantages primarily because it uses predominantly natural renewable raw materials.<sup>10</sup>

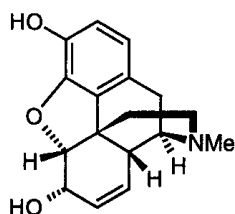
### Cobalamin (vitamin B<sub>12</sub>)

The chemical synthesis of cobyrinic acid (**10**), the core heterocycle of cobalamin, was the biggest project in organic synthesis until today. The groups of Woodward in Harvard and Eschenmoser in Zürich joined forces to prepare cobyrinic acid in 57 chemical steps from 1960 to 1972.<sup>11,12</sup> This precludes chemical production. In the past cobalamin was isolated from residues of antibiotic fermentations.<sup>13</sup> Today it is exclusively produced by fermentation. *Propionibacteria* and *Pseudomonada*<sup>14-16</sup> are frequently used and they produce >150 mg L<sup>-1</sup> of cobalamin inside the cells.



## Morphine

One of the most notable isoquinoline alkaloids is morphine (**11**) which is also the major alkaloid of opium produced from the plant *Papaver somniferum*. It is still the most effective painkiller available in medical practice. At the same time, it is addictive, and the mis-use of the diacetate, heroin, as a narcotic and euphoric drug can lead to the early death of the addict. The lethal dose in humans lies between 1 and 10 mg.

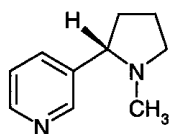


Morphine (**11**)

A review on the chemistry and biology of isoquinoline alkaloids has been published by Phillipson *et al.*<sup>17</sup>

## Nicotine

Dried leaves of the tobacco plants *Nicotiana rustica* and *N. tabacum* contain as much as 2–8% of enantiopure (*S*)-nicotine (**12**). When grown on fertile soils under irrigation for a period of several years, *N. rustica* can produce consistently 16.5 kg per 1000 m<sup>2</sup>. Surprisingly, this natural alkaloid has not been seriously considered as a potential renewable resource and text books on this topic generally lack references to (*S*)-nicotine. The only large scale application of nicotine is its use as an insecticide. Before the first synthetic insecticides came to the market approximately 2800 tons of (*S*)-nicotine per year were used as a crop protectant. The lack of any specific applications for nicotine as a renewable chemical may be because nicotine is very toxic, and the selective functionalization of nicotine at the pyridine or the pyrrolidine ring is difficult to control by chemical means. This restricts the chemical preparation of semi-synthetic pyridines using nicotine as a starting material.<sup>18</sup> Recently, selective biological oxidations were applied to prepare a series of nicotine analogues (see below).



(*S*)-Nicotine (**12**)

## Secondary metabolites

The fermentative production of secondary metabolites is a very important business. The scope of compounds manufactured by *de novo* biosynthesis is also extremely broad. As also mentioned previously, a detailed description of *N*-heterocycles produced in this manner is beyond the scope of this article.

$\beta$ -Lactam antibiotics are the most prominent class of compounds in the group of secondary metabolites. The total output from approximately 50 producers is estimated to be 10000–20000 tons per year. The bulk price for penicillin G is 25–30 \$/kg.<sup>19</sup>

For the preparation of a wide variety of commercial semi-synthetic antibiotics, the side-chain phenylacetic acid of penicillin G (**13**) and the *D*- $\alpha$ -aminoadipic acid of cephalosporin C (**14**) are first removed enzymatically to prepare the crucial intermediates 6-aminopenicillanic acid (**15**) and 7-aminocephalosporanic acid (**16**). Both enzymatic processes replace classical chemical routes which were applied for many years to hydrolyse the amide bonds attaching the side chains. The phenylacetic acid is cleaved off the penicillin G with the enzyme Penicillin G amidase.<sup>19,20</sup> 7-Aminocephalosporanic acid is prepared from cephalosporin C by a 3-step, 2-enzyme process. *D*-Amino acid oxidase deaminates cephalosporin C to give  $\alpha$ -keto-adipyl-7-aminocephalosporanic acid which then spontaneously forms glutaryl-7-aminocephalosporanic acid. Glutaryl-7-aminocephalosporanic acid acylase then converts this product to 7-aminocephalosporanic acid. H<sub>2</sub>O<sub>2</sub> produced by the oxidase reacts in the absence of catalase with the keto acid to glutaryl-7-aminocephalosporanic acid *via* oxidative decarboxylation. Glutaryl-7-aminocephalosporanic acid acylase then converts this product to 7-aminocephalosporanic acid. The yield of glutaryl-7-aminocephalosporanic acid from the first enzymic reaction is approximately 95%; the yield of 7-aminocephalosporanic acid from the second enzymic reaction is also approximately 95%.<sup>21</sup>

## 'Green' indigo production from glucose in *E. coli*

The production of indigo (**17**) by the fermentation of glucose is an interesting alternative to the production by chemical synthesis. The fermentation strategy of Genecor (USA) is to maximize the shikimic acid pathway followed by channeling this carbon through a mutated tryptophan synthase resulting in the production of indole. Cloned naphthalene dioxygenase is used to convert indole to indoxyl, which is spontaneously air oxidized to indigo.<sup>22</sup> This novel biosynthesis of indigo has, however, so far not been sufficiently economical to replace the present cheap chemical synthesis of indigo.

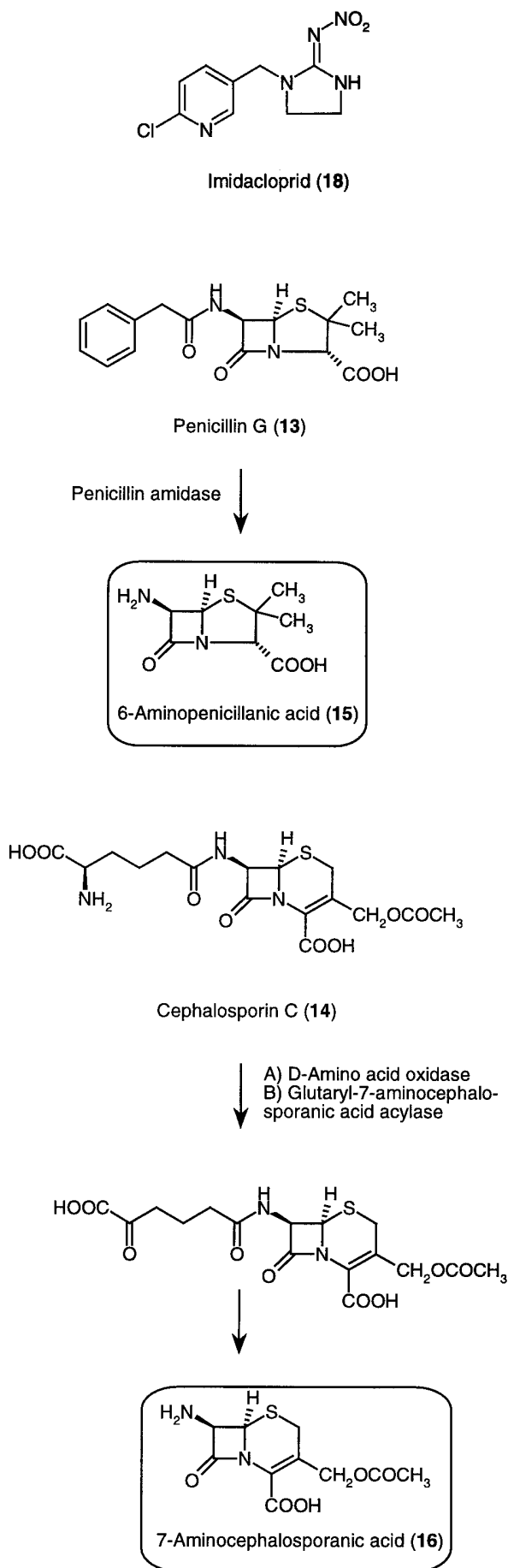
## 2 Bioconversions for the modification of substituted N-heterocycles

A wide variety of chemoenzymatic processes have been developed for the large scale preparation of functionalized heterocycles. Some of these biocatalytic processes were developed using enzymes naturally formed by micro-organisms degrading certain *N*-heterocycles. A review on the microbial metabolism of pyridines, quinoline and acridine was published recently by Kaiser *et al.*<sup>23</sup> Also, hydrolytic enzymes such as esterases, lipases, amidases and nitrile hydratases have been applied to prepare enantiomerically pure cyclic amino acids. The main advantage of most of the biocatalytic processes mentioned in this section is the high selectivity of the reaction which increases the yield compared to chemical routes and simplifies the purification of the desired products. As a consequence such biocatalytic routes are economically and ecologically favorable manufacturing methods.

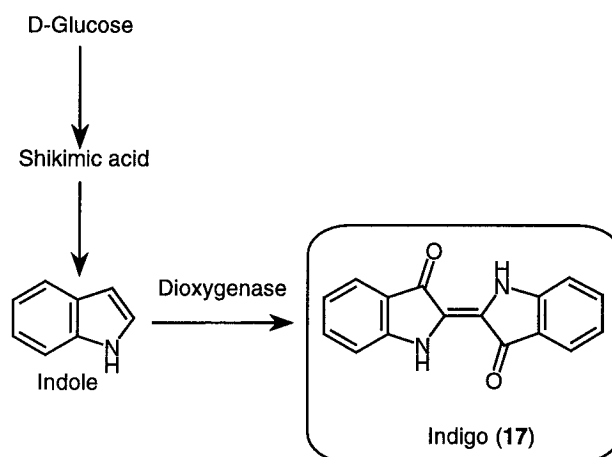
### Biocatalytic ring hydroxylation of aromatic N-heterocycles

The enzymatic hydroxylation of substituted pyridines and pyrazines occurs chemoselectively at the position  $\alpha$  to the ring nitrogen. The oxygen for the initial hydroxylation reaction is derived from H<sub>2</sub>O instead from O<sub>2</sub>. In the case of the dehydrogenase forming 6-hydroxynicotinic acid from nicotinic acid it was shown that this membrane bound enzyme system contains a molybdenum cofactor.<sup>24</sup> The volumetric productivity of these hydroxylations is generally very high and wild type cells can be used for these bioconversions (Table 2).

Several biotransformations with 3-substituted pyridines were developed to gain cheap access to building blocks used for the preparation of insecticides such as Imidacloprid<sup>TM</sup> (**18**) and analogues.<sup>32</sup>



Scheme 1



Scheme 2

6-Hydroxy-(*S*)-nicotine (19) was used as starting material for the preparation of 5,6-disubstituted nicotine analogues. Although the history of nicotine is well over 100 years old such disubstituted derivatives have not been described before, indicating that bioconversions can give access to novel building blocks. Pyrazinecarboxylic acid formed as an intermediate after the hydrolysis of 2-cyanopyrazine by a nitrilase is regiospecifically hydroxylated to 5-hydroxypyrazinecarboxylic acid (20). The chemical syntheses of 5-hydroxy- or 5-chloropyrazinecarboxylic acid are circumstantial.<sup>30</sup>

#### Enzymatic oxidation of alkyl groups on N-heterocycles

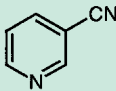
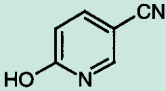
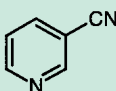
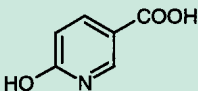
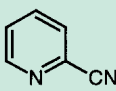
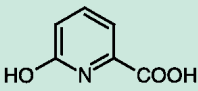
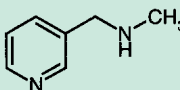
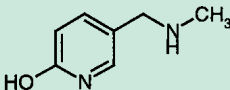
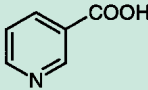
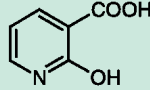
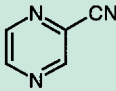
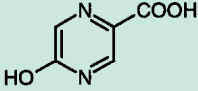

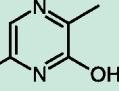
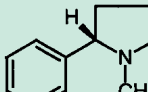
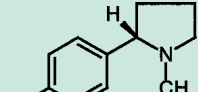
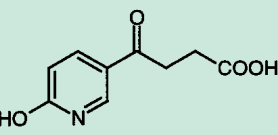
Chemical oxidation reactions used for the industrial-scale preparation of heteroaromatic monocarboxylic acids from heteroaromatic compounds bearing one or more alkyl groups are generally non-specific, leading to the formation of byproducts. For example, the oxidation of 5-ethyl-2-methylpyridine (24) with HNO<sub>3</sub> leads to the formation of pyridine-3-carboxylic acid (nicotinic acid). With this process it would not be possible to selectively oxidize the terminal methyl group of the ethyl function without oxidizing the methylene group or the methyl group in position 2. We discovered that micro-organisms grown on xylene as the sole carbon and energy source were capable of selectively oxidizing a single methyl group on heteroarenes. The oxidation of 2,5-dimethylpyrazine (21) to 5-methylpyrazine-2-carboxylic acid (22) is now performed commercially with wild type micro-organisms *Pseudomonas putida*. The oxidation of 2-chloro-3,6-dimethylpyrazine (23) is regiospecific because the substituent in *ortho* position to the methyl group prevents the hydroxylation by monooxygenase.<sup>33</sup>

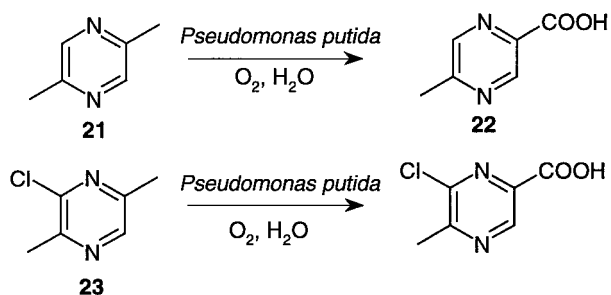
We demonstrated that *Pseudomonas oleovorans* previously grown on *n*-octane catalyzes the oxidation of ethyl groups on heteroaromatic compounds to the corresponding acetic acid derivatives. The oxidation of 5-ethyl-2-methylpyridine (24) to 5-methylpyridine-3-acetic acid (25) illustrates the selectivity of this reaction very well because no byproducts were detected after the reaction was complete.<sup>34</sup>

#### Chemoenzymatic synthesis of L-tryptophan

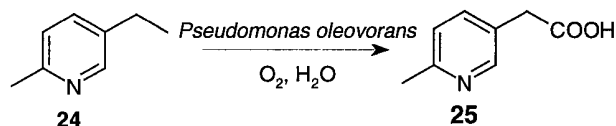
An alternative process to the *de novo* biosynthesis of L-tryptophan (3) is the use of L-tryptophan indole synthase ('tryptophanase') from *Proteus rettgeri*. This micro-organism accumulates this enzymes up to 6% of the total soluble protein. L-Tryptophan is synthesized from indole, pyruvate and ammonia. For the production of tryptophan inosine was added to the reaction mixture. Tryptophan formed an insoluble complex with ino-

**Table 2** Microbial conversions of substituted pyridines

Starting material	Product	Product concentration (enzymes) Biocatalyst
		45 g L <sup>-1</sup> (Hydroxylase) <i>Comamonas testosteroni</i> <sup>25</sup>
		40 g L <sup>-1</sup> (Nitrilase & Hydroxylase) <i>Agrobacterium</i> sp. <sup>26</sup>
		55 g L <sup>-1</sup> (Nitrilase & Hydroxylase) <i>Alcaligenes faecalis</i> <sup>27</sup>
		25 g L <sup>-1</sup> (Hydroxylase) <i>Arthrobacter ureafaciens</i> <sup>28</sup>
		6.5 g L <sup>-1</sup> (Hydroxylase) <i>Proteobacteria</i> <sup>29</sup>
		40 g L <sup>-1</sup> (Nitrilase & Hydroxylase) <i>Agrobacterium</i> sp. <sup>30</sup>
		8 g L <sup>-1</sup> (Hydroxylase) <i>Rhodococcus erythropolis</i> <sup>31</sup>
		30 g L <sup>-1</sup> (Hydroxylase) <i>Arthrobacter oxydans</i> <sup>18</sup>
		15 g L <sup>-1</sup> (Several enzymatic steps) <i>Pseudomonas</i> sp. <sup>18</sup>



**Scheme 3**



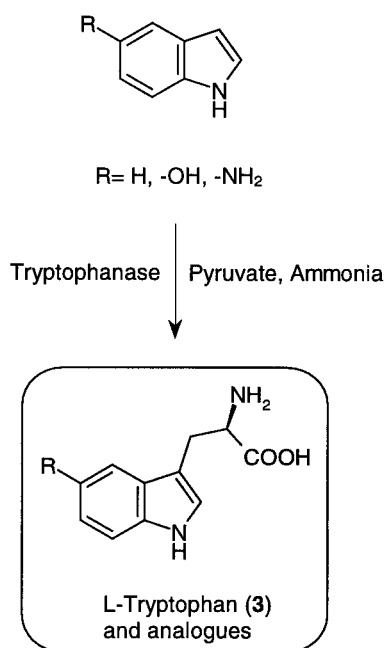
**Scheme 4**

was formed.<sup>35</sup> Tryptophanase also accepts 5-aminoindole and 5-hydroxyindole as substrates.

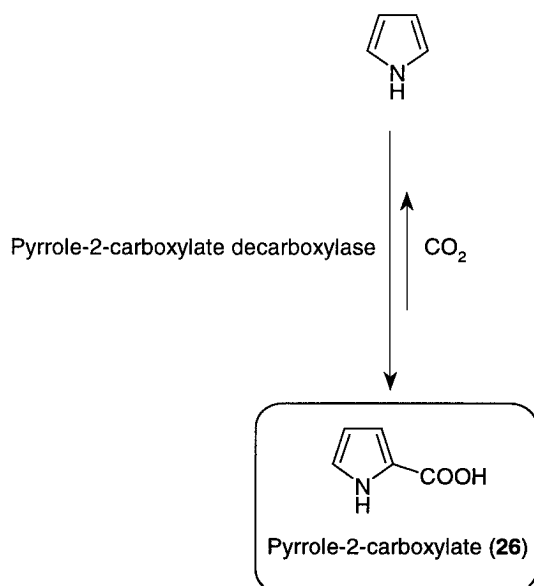
#### Microbial synthesis of pyrrole-2-carboxylate

Pyrrole-2-carboxylate (**26**) was synthesized from pyrrole using the carboxylation reaction of the reversible pyrrole-2-carboxylate decarboxylase from *Bacillus megaterium*. By addition of high amounts of bicarbonate, the reaction equilibrium was shifted

sine and therefore the equilibrium of the reaction was shifted to the product side and in this case up to 100 g L<sup>-1</sup> of tryptophan



Scheme 5

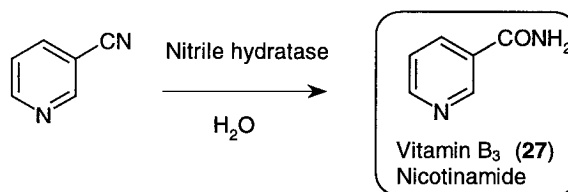


Scheme 6

towards pyrrole-2-carboxylate. A unique feature of this enzyme is its requirement for an organic acid, such as acetate, propionate, butyrate or pimelate.<sup>36</sup>

#### Vitamin B<sub>3</sub> from 3-cyanopyridine

Nicotinamide, or vitamin B<sub>3</sub> (27), is an essential component of enzymes catalyzing hydrogenation and dehydrogenation reactions. Nicotinamide is also used as a vitamin supplement for food and animal feed. One commercial process for the manufacture of nicotinamide is alkaline hydrolysis of 3-cyanopyridine. The hydrolysis is normally carried out with a catalytic amount of sodium hydroxide. A typical yield of this process is 96%, but nicotinic acid can be up to 4% in the product. An alternative biological method for the hydrolysis of 3-cyanopyridine to nicotinamide involves micro-organisms that produce nitrile hydratase. The use of the bacterial nitrile hydratase formed by *Rhodococcus rhodochrous* J1 for the industrial production of ca. 20000 tons of



Scheme 7

acrylamide per year was developed in Japan.<sup>37</sup> The same biocatalyst can be used for the hydrolysis of 3-cyanopyridine in the form of immobilized cells. The absolute selectivity of the bioconversion is the main advantage over the chemical process. No nicotinic acid is formed as a byproduct. Lonza (Switzerland) has built a plant in China producing ca. 3000 tons of nicotinamide per year with this biocatalyst.

#### Enantiomerically pure cyclic amino acids

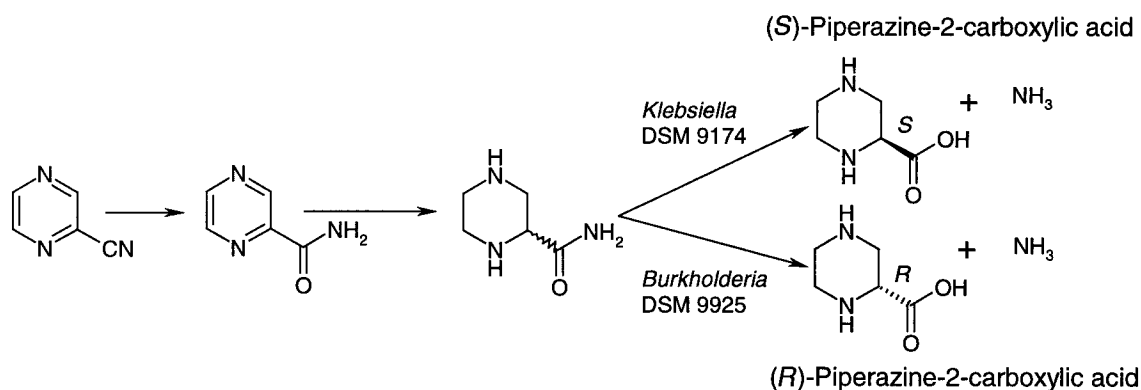
Non-proteinogenic amino acids such as piperidine-2-carboxylic acid (pipercolic acid) (28), piperazine-2-carboxylic acid (29) and D-proline are precursors of numerous bio-active compounds.

The best chemical route to the pure enantiomers of pipercolic acid and piperazine-2-carboxylic acid is classical resolution of the racemate by fractional crystallization of diastereomeric salts. Asymmetric syntheses for (*S*)-pipercolic acid have been developed as well as a kinetic resolution of (*R,S*)-*n*-octylpipercolate using a partially purified *Aspergillus niger* lipase. (*S*)-Piperazine-2-carboxylic acid has also been prepared by kinetic resolution of racemic 4-(*tert*-butoxycarbonyl)piperazine-2-carboxamide with leucine aminopeptidase. Both of these biocatalytic processes have practical disadvantages in that the preparation of the racemic starting materials is complicated and the availability of the biocatalysts for large scale production is limited. Whole cells of wild-type micro-organisms that contain stereospecific amidases have been used for the kinetic resolution of racemic piperazine-2-carboxamide and racemic piperidine-2-carboxamide to the corresponding enantiomerically pure carboxylic acids. These kinetic resolutions are attractive because the starting materials can be easily prepared from 2-cyanopyrazine and 2-cyanopyridine via the corresponding aromatic carboxamides. Furthermore, the micro-organisms can be grown in fermenters on the racemic carboxamides at the same time as the biotransformations are taking place.

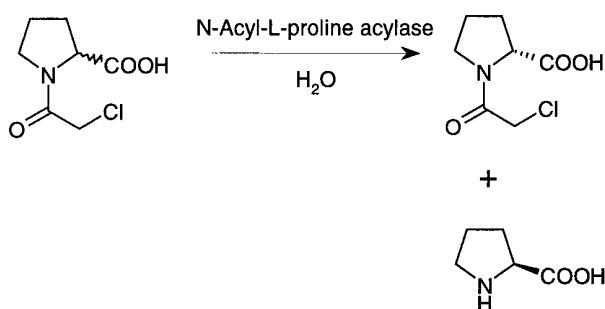
*N*-Acyl-L-proline acylases have been used for the production of *N*-acyl-D-proline and L-proline from racemic *N*-acylproline. The *N*-acyl-L-proline acylase from *Comamonas testosteroni* has a broad substrate specificity and was used for the hydrolysis of *N*-chloroacetyl-derivatives of azetidine-2-carboxylic acid, proline and pipercolic acid.<sup>38</sup>

An analogous biocatalytic process was developed for the production of CBZ-D-proline which can be used directly for subsequent coupling reactions. A further advantage of this process is the high overall yield of the biotransformation since the solubility of CBZ-D-proline is very low in the aqueous broth.<sup>39</sup>

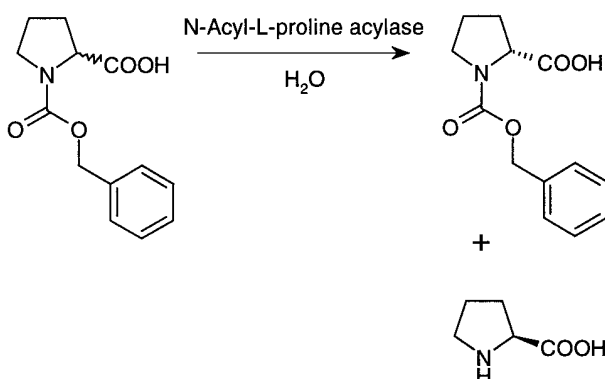
The obvious disadvantage of these kinetic resolutions of racemic D,L-proline derivatives is the low maximal theoretical yield of 50%. A new asymmetric route for the production of D-proline was developed from L-arginine as starting material. In a first chemical step L-arginine was converted to (*S*)-5-[amino-



Scheme 8



Scheme 9

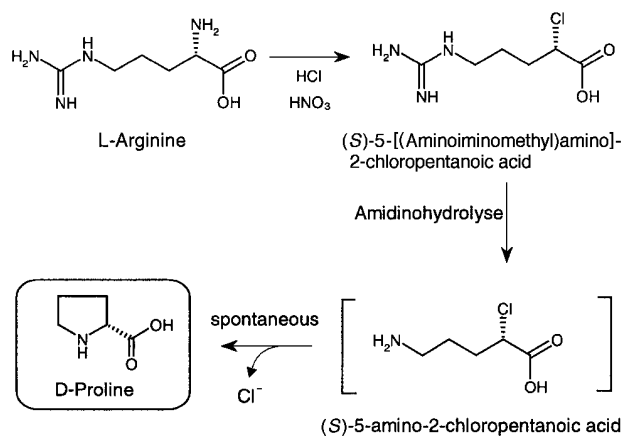


Scheme 10

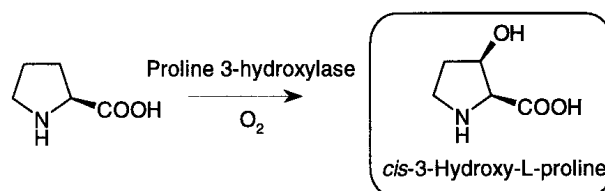
iminomethyl)amino]-2-chloropentanoic acid. This compound was then used to screen for micro-organisms capable of accepting this molecule as a nitrogen source for growth. *Pseudomonas aeruginosa* could convert (S)-5-[(aminoiminomethyl)amino]-2-chloropentanoic acid to (S)-5-amino-2-chloropentanoic acid, which spontaneously cyclized with inversion of configuration to D-proline.<sup>40</sup>

#### Production of non-proteinogenic hydroxyprolines

Among eight isomers of hydroxyproline, only *trans*-4-hydroxy-L-proline is abundant in nature as a component of collagens produced by higher organisms. It is known that procollagen-proline dioxygenase hydroxylates L-proline residues of procollagen post-translationally to *trans*-4-hydroxy-L-proline. Recently a novel enzyme was found capable of hydroxylating free L-proline to *cis*-3-hydroxy-L-proline. The purified enzyme showed properties of a 2-oxoglutarate-dependent dioxygenase.<sup>41</sup>



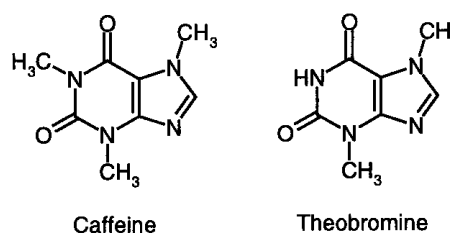
Scheme 11



Scheme 12

#### Demethylation of caffeine to theobromine

The caffeine content of the bean of *Coffea canephora* (dry basis) is ca. 2.0% and is currently a by-product of the manufacture of caffeine-free coffee. Theobromine is the principle alkaloid of cacao beans which contain 1.5–3% of this base.<sup>42</sup> The selective biological demethylation of caffeine to theobromine was achieved by caffeine-degrading bacteria. *Pseudomonas putida* strains can produce nearly 20 g L<sup>-1</sup> theobromine from caffeine.<sup>43</sup> The presence of zinc ions is necessary to inhibit the further degradation of the accumulating theobromine.



## Conclusions

There are several other N-heterocyclic compounds which can be manufactured by microbial catalysts. The examples described are only a small selection of biocatalytic processes which are presently used for the preparation of N-heterocycles. Due to the advances of molecular biology it may be possible in future to modify the natural producers like plants, animals, microorganisms to produce related but chemically distinct compounds as a renewable raw material basis for the chemical industry.

Biocatalysis has proven to be a useful supplementary technology for the chemical industry, allowing reactions which are not easily conducted by classical organic chemistry. The range of applications is not limited to natural compounds like amino acids, nucleosides, or vitamins. Bioconversions can also be used to perform chemical reactions which are in the domain of classic organic chemistry. The examples of nitrile hydratizing microbial strains used for the production of acrylamide or nicotinamide on an industrial scale show that biocatalysts can compete even with cheap traditional catalysts such as mineral acids and bases.

## References

- 1 A. Kleemann, W. Leuchtenberger, B. Hoppe and H. Tanner, *Amino Acids*, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. W. Gerhartz, VCH Verlagsgesellschaft, Weinheim, 1985.
- 2 F. Yoshinaga, *Prog. Ind. Microbiol.*, 1986, **24**, 117.
- 3 R. Katsumata and M. Ikeda, *Bio/Technology*, 1993, **11**, 921.
- 4 K. Haneda, A. Hirano, R. Kodaira and S. Ohuchi, *Agric. Biol. Chem.*, 1971, **35**, 1906.
- 5 S. Asahi, Y. Tsunemi and M. Doi, *Biosci. Biotechnol. Biochem.*, 1995, **59**, 2123.
- 6 K. Miyagawa, H. Kimura, K. Nakahama, M. Kikuchi, M. Doi, S. Akiyama and Y. Nakao, *Bio/Technology*, 1986, **4**, 225.
- 7 H. Mori, A. Iida, T. Fujio and S. Teshiba, *Appl. Microbiol. Biotechnol.*, 1997, **48**, 693.
- 8 M. Doi, Y. Tsunemi and S. Asahi, *Biosci. Biotechnol. Biochem.*, 1994, **58**, 1608.
- 9 P. Philippsen, M. Pompejus and H. Seulberger, *WO 98/29539*, 1998.
- 10 A. P. G. M. van Loon, H.-P. Hohmann, W. Bretzel, M. Hübeline and M. Pfister, *Chimia*, 1996, **50**, 410.
- 11 W. Friedrich, *Handbuch der Vitamine*, Urban & Schwarzenberg, Munich, 1987.
- 12 S. Fukui and S. Shimizu, *Antibiotics, Vitamins and Hormones*, ed. F. Korte and M. Goto, Georg Thieme Verlag, Stuttgart, 1977.
- 13 C. Spalla, in *Biotechnology of Vitamins, Pigments and Growth Factors*, ed. E. J. Vandame, Elsevier, Amsterdam, 1989.
- 14 T. Karuhara and I. Nakamura, *Adv. Biochem. Eng. Biotechnol.*, 1984, **29**, 35.
- 15 H. C. Friedmann and L. M. Cagen, *Annu. Rev. Microbiol.*, 1970, **24**, 159.
- 16 J. Florent and L. Ninet, in *Microbial Technology*, ed. H. J. Pepler and D. Perlman, Academic Press, New York, 1979.
- 17 J. D. Phillipson, M. F. Roberts and M. H. Zenk, *The Chemistry and Biology of the Isoquinoline Alkaloids*, Springer-Verlag, Berlin, 1985.
- 18 J.-P. Roduit, A. Wellig and A. Kiener, *Heterocycles*, 1997, **45**, 1687.
- 19 M. Ohno, M. Otsuka, M. Yagisawa, S. Kondo, H. Öppinger, H. Hoffmann, D. Sukatsch, L. Hepner and C. Male, *Antibiotics*, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. W. Gerhartz, VCH Verlagsgesellschaft, Weinheim, 1985.
- 20 J. Danzig, W. Tischer and C. Wandrey, *Chem. Eng. Technol.*, 1995, **18**, 256.
- 21 U. Giesecke, F. Wedekind and W. Tischer, *Dechema Biotechnol. Conf.*, 1992, Pt. B, 609.
- 22 A. Berry, S. Battist, G. Chotani, T. Dodge, S. Peck, S. Power and W. Weyler, *Proc. Biomass Conf. Am.: Energy, Environ., Agric. Ind.*, 1995, p. 1121.
- 23 J.-P. Kaiser, Y. Feng and J.-M. Bollag, *Microbiol. Rev.*, 1996, **60**, 483.
- 24 M. Nagel and J. R. Andreesen, *Arch. Microbiol.*, 1990, **154**, 605.
- 25 M. Yasuda, T. Sakamoto, R. Sashida, M. Ueda and Y. Morimoto, *Biosci. Biotechnol. Biochem.*, 1995, **59**, 572.
- 26 A. Kiener, *EP 0,504,819 A2*, 1992.
- 27 A. Kiener, R. Glöckler and K. Heinzmann, *J. Chem. Soc., Perkin Trans. I*, 1993, 1201.
- 28 T. Ishikawa, K. Maeda, K. Hayakawa and T. Kojima, *J. Mol. Catal. B*, 1996, **1**, 173.
- 29 M. Ueda and R. Sashida, *J. Mol. Catal. B*, 1998, **4**, 199.
- 30 M. Wieser, K. Heinzmann and A. Kiener, *Appl. Microbiol. Biotechnol.*, 1997, **48**, 174.
- 31 A. Kiener, Y. van Gameren and M. Bokel, *USP 5,284,767*, 1994.
- 32 S. Kagabu, K. Moriya, K. Shibuya, Y. Hattori, S. Tsuboi and K. Shiokawa, *Biosci. Biotechnol. Biochem.*, 1992, **56**, 362.
- 33 A. Kiener, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 774.
- 34 A. Kiener, *USP 5,242,816*, 1993.
- 35 H. Yamada and H. Kumagai, *Adv. Appl. Microbiol.*, 1975, **19**, 249.
- 36 M. Wieser, T. Yoshida and T. Nagasawa, *Tetrahedron Lett.*, 1998, **39**, 4309.
- 37 T. Nagasawa and H. Yamada, *Trends Biotechnol.*, 1989, **7**, 153.
- 38 U. Groeger, K. Drauz and H. Klenk, *Angew. Chem.*, 1992, **104**, 222.
- 39 M. Sauter, D. Venetz, F. Henzen, D. Schmidhalter, G. Pfaffen and O. Werbitzky, *PCT/WO 97.33987*, 1997.
- 40 C. Bernegger-Egli, K.-S. Etter, F. Studer, F. Brux and O. M. Birch, *J. Mol. Catal. B, Enzymatic*, 1999, **6**, 359.
- 41 H. Mori, T. Shibasaki, K. Yano and A. Ozaki, *J. Bacteriol.*, 1997, **179**, 5677.
- 42 R. Viani, *Coffee*, in *Ullmann's Encyclopedia of Industrial Chemistry*, ed. W. Gerhartz, VCH Verlagsgesellschaft, Weinheim, 1986.
- 43 Y. Asano, T. Komeda and H. Yamada, *Biosci. Biotechnol. Biochem.*, 1993, **57**, 1286.

Paper 8/09538H