Asymmetric Synthesis of (S)-2-Indolinecarboxylic Acid by Combining Biocatalysis and Homogeneous Catalysis

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Enantiopure heterocyclic amino acids are important building blocks for pharmaceuticals. For example, (S)-2-indolinecarboxylic acid (1; Figure 1) is a key intermediate for angiotensin 1-converting enzyme inhibitors (so called ACE inhibitors) that are used for the treatment of hypertension. Typical examples include Indolapril and Perindopril (Figure 1).

Figure 1. (S)-2-Indolinecarboxylic acid as intermediate for ACE inhibitors.

The synthesis of (S)-2-indolinecarboxylic acid (1) and derivatives is generally achieved by preparation of the racemic precursor using a Fischer indole synthesis followed by classical[1] or enzymatic[2] resolution. The yields in these methods never exceed 50%. An asymmetric synthesis approach would be much more appealing and indeed a number of potentially more economic routes could be considered. For example, direct asymmetric hydrogenation of a 2-indolecarboxylic acid derivative would be a very fast entry. Recently, mainly due to the work of Itoh, Kuwano, and co-workers, much progress has been made in the asymmetric hydrogenation of 2-substituted N-acyl indoles, allowing their conversion with high enantioselectivities.[3] However, these hydrogenations needed a chiral rhodium catalyst based on the bisphosphine TRAP ligand with a relatively low substrate/catalyst ratio of 100:1. In addition, the reaction needed the presence of expensive cesium carbonate. Use of PipPhos as a much cheaper ligand led to somewhat reduced enantioselectivities.[4]

A metal-catalyzed ring closure of an ortho-halophenylalanine derivative 2 could provide a very fast access to this class of compounds. Indeed, the palladium-catalyzed ring closure of ortho-bromophenylethylamines was reported by Buchwald and co-workers.[5] More recently, cheaper copper-catalyzed variants have also been reported.[6] However, the metal-catalyzed cyclization of enantiopure ortho-halophenylalanines has not been described. A possible problem in these cyclization reactions could be racemization. Palladium-catalyzed racemization of amines is well known,[7] although we could not find any examples of copper-catalyzed racemizations. Ma and co-workers reported the copper-catalyzed arylation of amino acids without racemization.[8] We decided to focus on the use of copper catalysts in view of the obvious cost advantage with respect to palladium. For reasons of cost and waste treatment the preference for the leaving group would be Cl > Br > I. We therefore focused on the use of chlorine and bromine as leaving groups.

Two routes can be envisaged to obtain the required phenylalanines in enantiopure form (Scheme 1). The enzyme phenylalanine ammonia lyase (PAL) catalyzes the enantioselective addition of ammonia to cinnamic acid.[9] The required cinnamates can be made either by a Perkin condensation (Scheme 1) or by a Heck reaction. Alternatively, an asymmetric hydrogenation approach could be envisaged. We have shown that the mono-

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dentate phosphoramidite ligands MonoPhos and, particularly, PipPhos are extremely suitable for the asymmetric hydrogenation of substituted 2-acetamidocinnamic acids,\textsuperscript{[10]} which, in turn, are made by the Erlenmeyer condensation of ortho-halo-benzaldehyde with N-acetylglycine, followed by hydrolysis of the intermediate benzylidene azlactone.

We started our research by screening catalysts and conditions for the copper-catalyzed ring closure of (S)-ortho-chlorophenylalanine (2a) and (S)-ortho-bromophenylalanine (2b). We were able to prepare 2a by asymmetric hydrogenation of 5a, using a rhodium–MonoPhos catalyst. The reaction proceeded in quantitative yield with enantioselectivities of 95–97\%.\textsuperscript{[10]} Hydrolysis of the amide using HCl/H\textsubscript{2}O gave 2a. Although 2b could be prepared in a similar way, we decided to use purchased material for the initial screening. Having developed the use of beta-diketones as ligands for the copper-catalyzed amination of heterocycles and primary and secondary amines,\textsuperscript{[11]} we soon found that, in this case, no ligand was necessary. Indeed, Ma et al. reported the copper-catalyzed amination of amino acids without ligands.\textsuperscript{[8]} The assumption is that the amino acid itself functions as a ligand. (S)-2-Bromophenylalanine 2b turned out to be a highly reactive substrate. In a first reaction with 6 mol\% of CuCl as catalyst and N,N-methylpyrrolidone (NMP) as solvent at 100 °C, all starting material was converted within 4 h and 1 was obtained in 93\% yield with 98.6\% ee (Table 1, entry 1). Leaving the reaction for a longer period not only led to a lower yield, we also detected considerable racemization of 1. In view of the fast reaction, we lowered the temperature to 80 °C and the amount of catalyst to 1 mol\%. Under these conditions, the reaction was practically finished within 2 h. The product was obtained in excellent yield and enantioselectivity (entry 2). If the substrate will be made using the enzymatic route, it would be highly advantageous to carry out the ring closure in water to avoid an unnecessary solvent switch.

Changing the solvent from NMP to water also led to a very fast reaction and near perfect enantioretention (entry 3). In fact, under these circumstances, the reaction was so fast that even without any catalyst full conversion was obtained after 22 h (entry 4). We assume that, in this case, the reaction is catalyzed by trace amounts of metals in the tap water used as solvent. Using just 0.01 mol\% of CuCl turned out to be enough to obtain the product in excellent yield and ee after 5 h (entry 5).

We next turned our attention to the cyclization of (S)-ortho-chlorophenylalanine. This substrate turned out to be less reactive and with 1 mol\% of CuCl it took 40 h at 95 °C to achieve full conversion (Table 1, entry 7). In the presence of 4 mol\% of catalyst, the reaction was over in 4 h and the product was obtained in 95\% yield and 99\% ee after crystallization (entry 8). Not surprisingly, no conversion took place in the absence of catalyst (entry 6).

Having thus found scalable methods for the ring closure of both halogenated phenylalanines, we turned our attention to the enzymatic step. Various PAL enzymes were screened for activity in the conversion of 3a and 3b. It should be noted that the amination is an equilibrium reaction. Thus, to obtain high yields of the phenylalanine, it was necessary to work at high ammonia concentration; a condition that is not necessarily compatible with most enzymes. Nevertheless, screening of a range of PAL enzymes led to the discovery of suitable enzymes capable of converting both 3a and 3b in good yields (Figure 2).

Using the DSM plurigb technology,\textsuperscript{[12]} we were able to bring the enzymes to expression in E. coli, which allowed large-scale fermentation. The enzyme was not isolated in its pure form. Using whole E. coli cells as catalyst gave very good results. The enzymes converted 3a 2–3 times faster than 3b. For this reason, we decided to scale up the route based on the chloro compounds. Some PAL enzymes suffer from substrate and product inhibition. The former could be alleviated by feeding the substrate in small batches over time. After a total reaction time of 8.5 h, the desired 2a was obtained in a yield of 91\% with 99\% ee. The required 2-chlorocinnamic acid 3a was synthesized by Perkin condensation in 55\% yield (unoptimized).

This procedure is currently used for ton-scale production by DSM Pharma Chemicals.\textsuperscript{[13]} A comparison of the life-cycle analysis (LCA), which identifies the material, energy, and waste flows associated with the process to determine their environmental impact, of 1 made by the established (Fischer indole synthesis, classical resolution) and the current procedure showed that

\begin{table}
\caption{Copper-catalyzed ring closure of (S)-2-bromo and (S)-2-chlorophenylalanine.}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Entry & X & Solvent & CuCl [mol\%] & T [°C] & t [h] & Conv. [%]\textsuperscript{a} & Yield [%]\textsuperscript{b} & ee [%] \\
\hline
1 & Br & NMP & 6 & 100 & 4 & 100 & 93 & 98.6 \\
2 & Br & NMP & 1 & 80 & 2 & 99 & 96 & 99.5 \\
3 & Br & H\textsubscript{2}O & 2 & 95 & 2 & 100 & 81 & 99.6 \\
4 & Br & H\textsubscript{2}O & 0 & 95 & 5 & 37 & 39 & – \\
5 & Br & H\textsubscript{2}O & 0.01 & 95 & 5 & 100 & 95 & 99.5 \\
6 & Cl & H\textsubscript{2}O & 0 & 95 & 19 & 0 & – & – \\
7 & Cl & H\textsubscript{2}O & 1 & 95 & 22 & 40 & 88 & 98.3 \\
8 & Cl & H\textsubscript{2}O & 4 & 95 & 2 & 100 & 95 & 99.0 \\
\hline
\end{tabular}
\textsuperscript{[a]} Conversion of starting material as determined by HPLC; [b] HPLC yields; [c] yield of isolated product.
\end{table}
the carbon footprint of the new process is less than half that of the old process.[14] In particular, the use of organic solvents was greatly reduced because of the use of water as solvent. In addition, the new process is more economic.

In conclusion, the use of a combination of biocatalysis and homogeneous catalysis led to a very short synthesis route to enantiopure (S)-2-indolinecarboxylic acid. The environmental impact of this new process is greatly reduced in comparison to the previous process.

Experimental Section

3-(2-Chlorophenyl)acrylic acid (3a): A mixture of KOAc (18.4 g, 0.19 mol) in 2-chlorobenzaldehyde (70.3 g, 0.50 mol) was heated to 145 °C. Next, acetic anhydride (76.5 g, 0.75 mol) was added over a period of 1 h. After 0.50 mol of acetic anhydride had been added, the mixture became clear. The mixture was then stirred at 145 °C during 8 h. The hot reaction mixture was poured into a mixture of 6.4 wt% aqueous NaOH (670 g, 1.1 mol, 2.1 equivalents based on 2-chlorobenzaldehyde) and toluene (200 mL) at 80 °C. The final pH was 7.4. After separation of the organic phase, the water layer was again extracted with toluene (100 mL) at 80 °C. The combined water phases were acidified to pH 4.6 with 25 wt% aqueous H2SO4 (380 g). Crystallization started at pH 6.4. The mixture was cooled to 25 °C and the product was isolated by filtration, washed with water (100 mL) and dried under reduced pressure at 50 °C. 3a was obtained as an off-white solid (100.2 g, 0.55 mol, 55%).

(S)-2-amino-3-(2-chlorophenyl)propionic acid (2a): 3a (1.8 g, 10 mmol) was dissolved in 13 vol% aqueous NH3 (0.5 L) and the pH adjusted to 11 with 25 wt% aqueous H2SO4 (0.2 L, pH adjusted to 11 with 25 wt% aqueous H2SO4, substrate solution). E. coli cells (130 g) containing the phenylalanine ammonia lyase gene of R. glutinis were resuspended in 13 vol% aqueous ammonia (0.2 L, pH adjusted to 11 with 25 wt% aqueous H2SO4). The cell suspension was added to the substrate solution and the volume adjusted to 1 L with 13 vol% aqueous ammonia. The reaction was stirred at 200 rpm and 30 °C in a closed vessel. During the first 1 h, every 5 min a further portion of 3a (0.91 g, 5.0 mmol) was added to the reaction medium. Then, the feeding was continued for another 7 h with the addition of 3a (0.91 g, 5.0 mmol) every 25 min. After a total reaction time of 8.5 h, the cells were removed by centrifugation. The yield of the reaction (determined using HPLC) was approximately 91%. The reaction mixture contained approximately 18.1 g (91 mmol) of 2-chlorophenylalanine. The supernatant (850 mL, pH 10.8) was concentrated under reduced pressure (150 to 1 mPa; bath temperature = 60 °C). After removal of about 65% of the water, a large quantity of precipitate was formed (pH 7.5). The precipitate was removed by filtration and stirred in water to remove inorganic salts. The resulting solid material was dried to give the crude product (14.9 g). This product contained 57 wt% 2a (8.5 g, 47% yield), 99% ee, 24 wt% water (as crystal water), and 1.7 wt% 3a. The mother liquor contained the remaining 9.5 g of 2a.[15] 3-(2-Chlorophenyl)allyl alcohol (1a): To a mixture of K2CO3 (18.4 g, 133 mmol) and CuCl (15 mg, 0.015 mmol, 1 mol %) in 2-chlorobenzaldehyde (70.3 g, 0.50 mol) was added acetic anhydride (76.5 g, 0.75 mol) over a period of 1 h. The mixture was then stirred at 145 °C during 18 h. The hot reaction mixture was poured into a mixture of 6.4 wt% aqueous NaOH (670 g, 1.1 mol, 2.1 equivalents based on 2-chlorobenzaldehyde) and toluene (200 mL) at 80 °C. The final pH was 7.4. After separation of the organic phase, the water layer was again extracted with toluene (100 mL) at 80 °C. The combined water phases were acidified to pH 4.6 with 25 wt% aqueous H2SO4 (380 g). Crystallization started at pH 6.4. The mixture was cooled to 25 °C and the product was isolated by filtration, washed with water (100 mL) and dried under reduced pressure at 50 °C. 3a was obtained as an off-white solid (100.2 g, 0.55 mol, 55%).

(S)-2,3-dihydro-1H-indole-2-carboxylic acid (1; Table 1, entry 7): A flask was charged successively with 2a (3.00 g, 15.0 mmol), K2CO3 (2.17 g, 15.7 mmol), CuCl (15 mg, 0.015 mmol, 1 mol %) and H2O (15 g). The reaction was flushed with argon and then kept under a slow stream of argon. The reaction mixture was stirred and heated to 95 °C and kept at this temperature for 22 h. Samples were taken regularly and analyzed by HPLC. The conversion after 22 h was approximately 40%. Water (15 mL) was then added and the mixture stirred and heated for an additional 18 h. HPLC indicated full conversion of (S)-2-chlorophenylalanine. The reaction mixture was cooled to about 25 °C. The pH of the solution was then decreased from 7.6 to about 3.5 by addition of 5 M aqueous HCl. The resultant precipitate was isolated by filtration and washed with 0.01 M aqueous HCl (2 × 10 mL), and dried to afford 1 (1.88 g, 11.5 mmol, 76.6% yield, > 99% ee).

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[15] The remainder of the solid material is inorganic salts. Improvements of the isolation procedure gave higher yields. The best overall yields for the PAL reaction and the ring closure (60%) were obtained by telescoping the enzymatic reaction (after removal of the enzyme by filtration) into the follow-up step.

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