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# SUSTAINABLE MANUFACTURING ROUTES

***Sustainable manufacturing routes to life science compounds are becoming more important, as the costs of raw materials, energy and waste disposal continue to increase. Catalytic reactions can be used to advantage to address these factors. Design of the route takes a multi-disciplinary approach.***

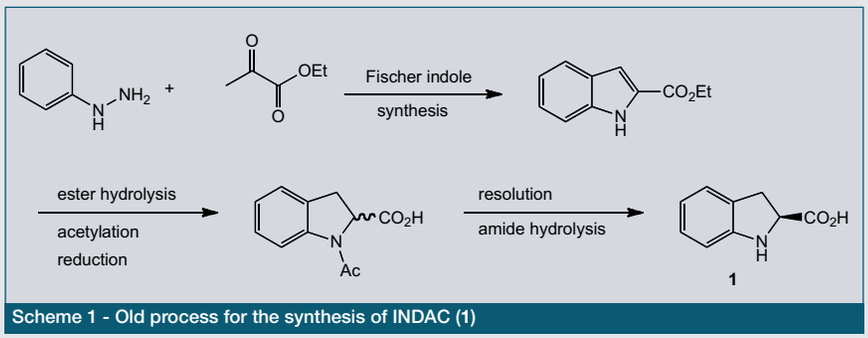
The production of pharmaceuticals and other life science molecules leads to a considerable amount of waste compared to commodity chemicals. As there is a cost associated with waste disposal, low yields and energy usage, minimising these factors can make the process cheaper and also greener [1]. In the pharmaceutical industry, drug candidates are becoming more complex and more sophisticated approaches are required to make the compounds [2]. The number of steps to an

active pharmaceutical ingredient (API) is 7-10 on average, with 15+ not uncommon for drugs in the development pipeline. The goal of a synthetic design is, therefore, to minimise the number of steps in a sequence.

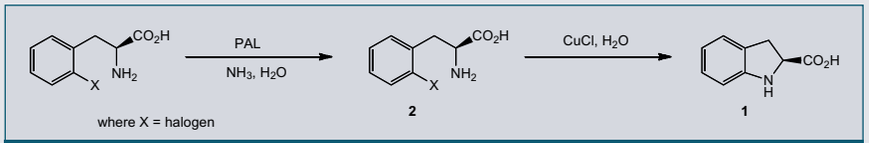
Today, many drugs contain a stereogenic centre, which can also be a significant synthetic challenge. Classical resolutions - often used in first generation manufacturing routes - are inefficient in that half of the material, the undesired isomer, has to be discarded or recycled. Methods that overcome this problem are asymmetric syntheses, deracemisations, or dynamic kinetic resolutions where the undesired isomer is avoided in some way to provide only the desired isomer; the 100% yield, 100% ee concept.

Route scouting requires a thorough knowledge of the literature with experts in many fields, including biocatalysis, chemical catalysis, organic chemistry, and reaction scale up. This broad background is required so that all possible approaches are considered without bias. Such bias is not uncommon, especially when it comes to enzymatic options in teams that lack experience or adequate capabilities in biocatalysis. In addition, intimate knowledge about running reactions at scale is necessary. It is easy to overlook a procedure that could be considered trivial, or a minor irritation in a laboratory environment, to find that it is a major problem or cost driver when run at scale. An example would be a cryogenic reaction. The use of catalytic, rather than stoichiometric agents, can help to reduce costs and make a process greener. For this purpose, biocatalysts are very useful, as they usually work under mild reaction conditions, can avoid the use (and disposal) of organic solvents, and do not require cryogenic or high temperature conditions.

Within DSM's InnoSyn® team, brainstorming is performed to generate a number of ideas. These ideas are then evaluated with respect to freedom to operate - i.e., do not infringe on other's patents - chances of success, and cost of performing the reaction(s) and putting it into practice. Typically, pharmaceutical products have been prepared by medicinal chemists in the early development phases by chemical routes, which are not always suitable to scale up. However, such a first generation synthesis route has been shown to work, even if only at small scale. This gives a basis for cost comparisons. Factors that need to be considered for cost improvements of first generation processes include solvent changes, the use of mixed solvents, chromatography steps, very dilute reactions, hazardous reactions or different starting materials for non-registered steps. Subsequent, lower cost routes can benefit from being convergent, rather than linear. In general, fewer steps will lead to cost reductions.



Scheme 1 - Old process for the synthesis of INDAC (1)



Scheme 2 - New process for the synthesis of INDAC (1)

The approach to achieve sustainable routes, as indicated above, is not limited to "new" products, but can also be used to improve existing processes. This is illustrated by the approaches to (*S*)-2,3-dihydro-1*H*-indole-2-carboxylic acid (**1**), abbreviated to INDAC. The original manufacturing process involved a two-step Fischer indole synthesis followed by a resolution. Not only did the resolution involve a low yield, but the indole nitrogen had to be protected to achieve separation, adding two steps to the overall sequence (Scheme 1).

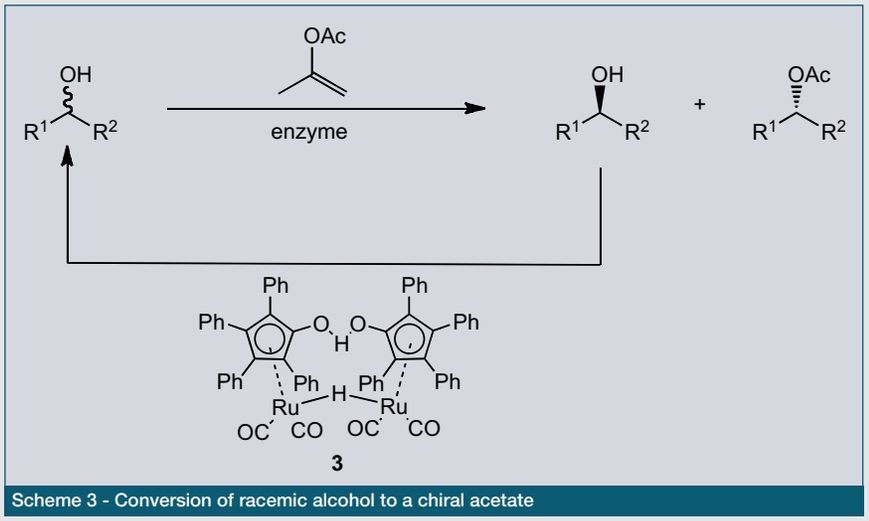
A route scouting exercise identified many possible alternatives ranging from the use of different biocatalysts, asymmetric hydrogenation to introduce the stereogenic centre, to the use of a chiral pool starting material. Some approaches were, at the time, considered to have a low probability of success, such as the asymmetric hydrogenation of indole-2-carboxylic acid. Biocatalytic approaches offered the most direct access to an acyclic α-amino acid (**2**). When access to the substrate and chances of success were evaluated together with the cost reduction potential and the projected costs of doing the necessary research and development, a method based on the use of phenylalanine ammonia lyase (PAL) was pursued. These enzymes add ammonia to a cinnamic acid derivative to form an α-amino acid with high stereocontrol (Scheme 2).

The second step in the process is a copper catalysed cyclisation. This approach was already being developed for other compounds [3]. At the beginning, the two reactions could be decoupled, as alternative routes to the amino acid **2** had been identified during the route scouting exercise.

The challenges of the route shown in Scheme 2 are to have access to an appropriate enzyme, which will produce the acid **2** with good quality and in a cost effective manner, and to telescope the enzymatic step to the metal-catalysed cyclisation. Looking at these challenges, it is obvious that both enzymatic and metal catalysis expertise is required to address these different aspects of the sequence.

The enzymatic step needs to be performed at >10% $NH_3$ and pH>10. These are quite extreme conditions that are required to drive the equilibrium of the reaction towards the product. Standard enzymes usually denature under such conditions. Indeed using standard phenylalanine ammonia lyases the enzymes not only rapidly loose activity but are also inhibited by the substrate.

One approach to address this limitation would be to improve the enzyme features by enzyme engineering, which is applied for several of our enzymatic processes




Scheme 3 - Conversion of racemic alcohol to a chiral acetate

which are, for example, based on designed pharmaPLEs®, hydroxynitrilases, PenG-acylases, dehydrogenases, aldolases and other enzymes. In this case, for the desired phenylalanine lyase, we turned to our network of enzyme suppliers and identified together with Verenium (former Diversa) a suitable enzyme. Cloning of this enzyme in one of our proprietary plu*Gb*ug® expression organisms and fermentation protocols as well as by use of a cheap and robust enzyme formulation, allowed our team to develop an efficient enzymatic step that provided the product at >90% yield in >95% ee.

*N*-Arylation reactions using stoichiometric amounts of copper have been known for a long time, and are called the Ullmann reaction or condensation. In the last decade this stoichiometric method has been replaced by catalytic ones based on the use of a palladium or copper catalysts. Copper is the cheaper metal. A range of experiments was performed to optimise the ring closure process. The most critical requirements to allow this step to be telescoped with the enzymatic transformation were: no solvent switch after the enzymatic reaction - the ring closure had to be performed in the presence of water; a high yield of the desired compound; and no racemisation of the stereogenic centre.

Initial experiments using the 2-bromophenylalanine revealed already a very high yield for the ring closure in water, with only 0.01 mol% of CuCl and no racemisation; control experiments in NMP revealed a decrease in ee. The more cost effective 2-chlorophenylalanine gave a 78% yield of ring-closed product at full conversion using 4 mol% of CuCl. Careful examination revealed the formation of a dimeric compound - an unprecedented intermolecular *N*-arylation of the product with the 2-chlorophenylalanine; this could be suppressed by the addition of organic bases. Again, no racemisation took place using these conditions. Removal of traces of copper and other impurities by pH switches, filtration and crystallisation techniques delivered the (*S*)-2,3-dihydro-1*H*-indole-2-carboxylic acid (**1**) as a white solid.

The second generation route has only two steps from the commercial cinnamic acid derivative, while the first generation method had seven steps. In addition to significant cost reductions, there is over a 15% increase in capacity throughput. Environmental factors can also be calculated to highlight the comparisons. The second generation process generates less waste with a reduction in the Process Mass Intensity (PMI) of 32%. The carbon footprint of the new process shows a reduction of 55%. Life Cycle Analysis (LCA), as determined by the Eco-Indicator 99, shows an 80% reduction.

The second example of biocatalysis and chemocatalysis working together is a more general approach, popularised by Bäckvall [4]. The power of this approach comes from the wide range of lipases and esterases available for the enantioselective hydrolysis of esters. This simple transformation, in its various forms, can allow access to chiral alcohols, carboxylic acids or esters. However, the process is a resolution and the enantiomer that is not a substrate is unaffected by the enzyme. To move towards the 100% yield, 100% ee concept, the unreacted or undesired isomer has to be recycled. This can be done by a racemisation reaction. For the secondary alcohol, this can be achieved by a redox sequence. A transition metal catalyst, such as the Shvo catalyst (**3**), will oxidise the alcohol to the corresponding ketone by hydrogen abstraction. The reduced catalyst can then add the hydrogen back to the ketone to reform the secondary alcohol (Scheme 3). As the transition metal catalyst is achiral, there is no face selectivity in the reduction and racemic product is produced. In other words, the unreactive alcohol isomer is converted to racemic alcohol. This allows the enzyme then to remove its preferred enantiomer from the reaction cycle. Fortunately, the transition metal catalyst does not oxidise the product

esters. The transition metal step is usually the slow one. The driving force for the conversion is still the enzymatic step, and one way to push the reaction to the product side is to use an acylating agent such as isopropenyl acetate where the by-product, acetone, can simply be removed by distillation. This is easily achieved by running the reaction under reduced pressure [5].

Other ligands and transition metal catalysts can also be used to speed up the racemisation process [6]. One useful aspect of the approach is that higher concentrations still give good conversions while the throughput is increased.

The design of manufacturing routes for pharmaceutical and life science compounds requires many facets. DSM provides all of the necessary skill sets. Innovation is required from scientists who are experts in specific areas. Substrates still need to be made and reactions telescoped (coupled in sequence) together. This requires a broad knowledge of organic chemistry and in some cases, biochemistry and catalysis. A major factor for the development of green and sustainable processes is a foundation in process chemistry performed at scale. This is not available at many smaller companies or in some academic groups, yet it can play a very significant role in selecting the most promising manufacturing route. The examples above do not highlight some newer engineering approaches, such as the use of microreactors or flow reactors to allow hazardous reactions to be performed in a safer manner. As the reaction parameters can be controlled better than in batch reactors, advantages are now being seen from the green aspects of this approach, as large excess of reagents are no longer required.

Although we are making progress towards sustainable manufacturing routes to life science compounds, there is still a long way to go to reach similar carbon footprint numbers to those seen for bulk chemicals, which is our ultimate goal.

With the increase in cost pressures seen by life science products, including pharmaceuticals, the use of green and sustainable routes can have a major impact. Technology advances now allow these approaches to have significant cost reductions over more traditional methods when the whole process is taken into consideration. The key technologies for sustainable manufacture, biocatalysis, chemocatalysis - including homogeneous catalysis - and microreactors continue to develop at a rapid rate. For many companies, the challenge remains in the maintenance and integration of all these technologies. The DSM InnoSyn team continues to embrace new developments in these key technology areas, which, in turn, allows them to be integrated into route scouting. This results in shorter, greener, sustainable and more cost-effective synthetic routes. The remaining question of when to invest in route scouting is simple to answer from the DSM perspective; the earlier the better. The sooner a more efficient route is implemented, the earlier pay-off in terms of cost, as well as sustainability, safety, and environmental impact can be realised.

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RIASSUNTO

***Processi di produzione sostenibili***

*I processi di produzione sostenibili stanno diventando molto importanti a causa dell'aumento dei costi delle materie prime, dell'energia e dello smaltimento dei rifiuti. La catalisi può risultare un'applicazione molto utile in questo settore, anche attraverso un approccio multidisciplinare.*