Biocatalysis is a Key Technology for Successful Chiral Synthesis at Almac

Dr Tom Moody, Head of Biocatalysis and Dr Stefan Mix, Biocatalysis Team Leader at Almac, describe the latest biocatalytic technologies developed by the company and how they are assisting in API development. The technology is used to support its broader chemical offerings for the synthesis of advanced chiral intermediates and APIs for pharmaceutical and biotech customers.

It is now well-recognised that the need for green, economic, robust and scaleable processes is at the forefront of customer research plans for the synthesis of chiral APIs and intermediates. The chemical industry is under severe pressure to make their chemical processes greener, lower costs, minimise waste and shorten existing syntheses. At Almac, the selectAZyme™ platform provides a diverse, A-Z range of enzymes including redox transaminases, hydrolases, nitrilases and many others.

In collaboration with their customers, Almac selects the optimum enzyme to provide an efficient and cost-effective process for scale-up. These enzymes are finding uses in applications from A-Z in medicinal chemistry, metabolite synthesis and in the large-scale manufacture of specialty chemicals. The selection of enzymes is their unequalled selectivity for the chemical reactions they catalyze.

Biocatalysis is becoming the workhorse of chiral synthesis at Almac and is now at the epicentre of key drivers in the company’s synthetic, screening, enzyme production, process optimisation and manufacture to GMP standard. For production of biocatalysts beyond pilot plant capabilities, Almac uses its UK and European specialist fermentation partners for multi-thousand-litre-scale production. The company’s synthetic platforms, whether further optimisation is required.

In the timely delivery of batches of API to support clinical trials, options to simplify or shorten the synthetic route are always welcome. For example, (S)-2-bromocyclohex-2-enol (1) is assigned to a decahydroquinoline alkaloid assigned to a decahydroquinoline alkaloid of the selectAZyme™ technology is a first choice for Almac when looking at chiral synthesis, and enzymes now offer myriad chemical transformations, as shown in Figure 1.

Another key advantage of running these processes is the timeline required for implementation. From selection of a selectAZyme™ catalyst to actual manufacture of product, timelines are similar to those of conventional chemistry optimisation and scale-up. Typical timelines are shown in Figure 2.

Figure 1. Examples of selectAZyme™ platform transformations.

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Figure 2. Timelines for using the selectAZyme™ platform.

Biocatalysis and API Clinical Trials Supply

In the timely delivery of batches of API to support clinical trials, options to simplify or shorten the synthetic route are always welcome. For example, (S)-2-bromocyclohex-2-enol (1) is frequently encountered as the starting point in a number of natural product syntheses, including (S)-trans-3-triket (2), the name assigned to a decahydroquinoline alkaloid isolated from the skin of dendrobatid frogs. Blechert and colleagues prepared a 0.5 g scale in 95% yield and 99.5% e.e. using a CBS reduction followed by chromatographic purification. Almac had API requirement to synthesise 100 g quantities of a novel therapeutic agent currently under development, and wanted to evaluate the use of a CRED enzyme for this screening of the selectAZyme™ CRED kit identified an enzyme that exhibited high conversion and high enantioselectivity, albeit using a glucose / glucose dehydrogenase coupled system. There are a number of reaction parameters to consider when developing a CRED reduction, including temperature, pH, cofactor regeneration and % substrate loading. Systematic evaluation of these parameters identified good reaction progress at 30°C (lower temperatures gave slower reaction progress; higher temperatures also gave slower reaction progress, presumably due to denaturation of the enzyme), pH 6.6-6.9 (pH 8 gave significantly slower progress) and 20 volumes of solvent.

Figure 3. Examples of selectAZyme™ platform transformations.

Delivery of this project required access to a 100 kg scale of a high enantiomeric purity. On lab scale, this had been readily achieved by a traditional diastereomeric resolution. However, as this chemistry was developed for scale-up it quickly became apparent that this resolution wasn’t working as required, with low yields and challenging filtration issues being observed. To ensure that the committed delivery date for the API was met, work started on an enzymatic resolution approach, while continuing to work on improving the crystallisation. Following a selectAZyme™ hydrolyse enzyme screen, a lipase was identified that converted the undesired enantiomer to an acetate ester, simply by running the reaction in ethyl acetate (both as acyl donor and solvent). The two product compounds (desired enantiomer and acylated undesired ester) were readily separated and, following some focussed work, the lipase catalyst was successfully applied on scale, leading to on-time delivery of API of the required purity.

Another typical project at Almac includes, for example, a Ph2b compound where nine steps of chemistry resulted in the isolation of three chiral centres from a registered starting material with a global yield of 74%. Key to winning the project was the marriage of Almac’s synthetic, analytical and solid-phase expertise in the design and synthesis of a chiral intermediate leading to stereospecifically reduce the ketone of the desired enantiomer feedstock and not the undesired (2R) enantiomer from the biocatalytic step. The CRED identified resulted in a stereospecific reduction and subsequent biopisolation of the diastereomeric mixture. The remaining undesired ketone was easily removed using conventional work-up at the next step. The process ran from start to finish using two solvent combinations. The project involved six pairs of stereoisomers (seven different products) were synthesised readily from one key intermediate. It is expected that the synthetic and development could be undertaken to determine the fate of these potential impurities. The summarised advantages of the green enzyme process are shown in the table below –

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Traditional</th>
<th>Green Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>85%</td>
<td>95%</td>
</tr>
<tr>
<td>Purity (%)</td>
<td>98%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Enzyme cost</td>
<td>$100/kg</td>
<td>$50/kg</td>
</tr>
<tr>
<td>Solvent cost</td>
<td>$20/kg</td>
<td>$10/kg</td>
</tr>
<tr>
<td>Overall cost</td>
<td>$120/kg</td>
<td>$60/kg</td>
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It is clear from the example described herein, that biocatalysis offers an attractive approach for synthesis resulting in greener processes with real benefits for both the environment and costs of your APIs. The increased demand for oxidant-free metabolism synthesis has resulted in more and more research and published literature in the area of FAD enzymes.

In practical terms, the Phase I metabolites has led to a greater diversity of off-the-shelf catalysts that...
can be used by the synthetic chemist. The ease of access of P₄₅₀ enzymes even from published gene sequences meant Almac was able to complete a carbon-13 project where the customer required the corresponding carbon-13 metabolite (Figure 4). Almac simply identified the P₄₅₀ gene sequence from the literature and obtained the synthetic gene. The gene was cloned and expressed and the isolated enzyme obtained from fermentation was used in the isotope lab to easily access the customer metabolite incorporating the desired carbon-13 labelled sites.

Figure 4: P₄₅₀ mediated hydroxylation of a carbon-13 run on a multi-ton scale, providing green commercial manufacturing bioprocesses.

DSM’s experience of more than 30 early-phase projects complements and expertise for the manufacturing of enzyme platform technologies, services each company access to their respective Partnerships and Collaboration. The Almac/DSM agreement grants Partnerships and Collaboration.

The Almac/DSM agreement grants

Partnerships and Collaboration. The Almac/DSM agreement grants

the biocatalysis business is to ensure to the customer security of supply of the catalysts for repeat manufacture. Today, we are now in a position to implement a biocatalysis-based synthesis for a new compound right from medicinal chemistry through to GMP manufacture – biocatalysis is our ‘first point of call’ for any project involving chiral moieties because it’s as quick or as quicker than other processes to develop, and is predictable upon scale-up.”

Moody adds: “In the past, due to customer demands for rapid supply, we often had to employ an inferior technology to produce the compound of interest, resulting in a sub-optimum process that ‘we were stuck with’. This resulted in a process that was not easily changed and lacked the potential for further cost reductions using the biocatalytic option. Nowadays, biocatalytic methods can be developed and employed just as quickly as chemocatalytic methods. Therefore development timelines are now the same as in a chemocatalytic approach, and biocatalysis is the first method explored when developing a synthetic route to a new compound at Almac.”

Summary

The existing pool of recombinant enzymes, both in the literature and available commercially, provides an ample resource from which to develop this technology further. In our opinion, the future focus for enzyme research needs to be on applying new technologies at the molecular level. However, this should also be integrated with methodologies aimed at improving biotransformations at the reaction level, including both physical and chemical approaches.  Future development of enzymic reaction systems could investigate and integrate the use of technologies that are known to speed up catalysis in other systems. Almac’s continued investment into evolution technologies and metagenomic programmes further confirm the company’s commitment to green technologies that offer solutions for their customers. This commitment is being driven by market needs and stresses, and the key to future success is flexibility and rapid response to change. Biocatalysis is a maturing technology and is certainly aiding future success stories for the rapid supply and delivery of chiral intermediates, fine chemicals and APIs by Almac.

References

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Tom Moody (Head of Biotransformation & Isotope Chemistry) received his 1st Class BSc (Hons) (1998) in Chemistry and a PhD in Physical Organic Chemistry (2001) from The Queen’s University of Belfast (QUB). He has completed a Masters Degree with Distinction in Business specializing in business strategy. He has earned numerous awards, including a Foundation Award, The AGB Scientific Award for Best Oral Presentation at the 53rd Irish University Chemistry Research Colloquium, and the Best PhD thesis at QUB. He also holds the position of honorary lecturer at Queen’s University, Belfast and the recipient of the 2012 BMI technology award.

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