Biotransformations in chemical synthesis

Dr Tom Moody and Dr Stefan Mix of Almac introduce the SelectAZyme range*

The chemicals industry is under severe pressure to make its processes greener, reduce costs, minimise waste and shorten existing syntheses.1-3 To this end, Almac has devised the SelectAZyme** platform, which is so called because it provides a diverse, ‘A-Z’ range of enzymes including reductases, transaminases, hydrolases and nitrilases, from which Almac selects the optimum enzyme to provide an efficient and cost-effective process for scale-up.

Applications have been found for these enzymes in medicinal chemistry, metabolite synthesis and in the large-scale manufacture of specialty chemicals. Figure 1 shows some examples of SelectAZyme platform transformations.

The power of enzymes is their unequalled selectivity for the chemical reactions they catalyse.4 The phrase ‘paradigm shift’ has been used out of turn in many technological advances in recent years, but this is certainly not the case when related to biocatalysis. The paradigm shift for the acceptance of biocatalysis is persistent and at the forefront of research within the fine chemicals and pharmaceuticals industries.

This paradigm shift has resulted in biocatalysis becoming the work-horse of the chemists’ tool-box for chiral chemistry.5 Enzyme processes are now at the epicentre of key drivers in process design and economics, including:

- Determination of key cost contributors
- Route scouting
- ID and prioritisation of routes to be investigated
- Key reaction optimisation and Design of Experiments
- Investigation into waste stream management

The reason for the surge in the application of this green technology, in our view, is simply that success breeds success. Unlike ten years ago, we now have all the supporting technologies that can really make a difference in enzyme development, such as bioinformatics, enzyme evolution and high throughput screening.

Figure 1 - Examples of SelectAZyme platform transformations

Figure 2 - Timelines for using the SelectAZyme platform

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

Recent project

A typical project at Almac includes, for example, a Phase IIb compound, where nine steps of chemistry resulted in the formation of three chiral centres from a registered starting material with a global yield of 7.4%. Key to winning the project was the marriage of Almac’s synthetic, analytical and solid state chemistry and the introduction of SelectAZyme chemistry for induction of chirality. The project was initiated with clear objectives:

1. Increase productivity of the process by >50% kg/litre/day
2. A >20% reduction in waste
3. Removal of expensive and toxic solvents
4. Removal of heavy metals and subsequent contamination
5. Removal of the need for specialised equipment, in this case high pressure hydrogenation
6. Development of a process with consistent quality of product
7. Reduce the cost/kg of product

The original chemistry involved a myriad of steps, including a late stage classical resolution using an expensive amine-resolving agent and high pressure hydrogenation using metal catalysis. The late stage resolution resulted in huge volumes having to be processed until the seventh step.

Almac’s challenge was to make a scalable, lower volume route that had green and cost incentives for change. Almac completed route invention, proof of concept demonstration followed by hundreds of kilos scale-up prior to tonne manufacture. The revised route consisted of five steps, using three different SelectAZyme enzymes with a global yield of 23.4%.

From retrosynthetic analysis, it was demonstrated that the registered starting material could be made from feedstocks with no long-term supply issues that could be sourced readily from India and China. Having the proposed route on paper, the next step was to synthesise the key intermediates and begin enzyme screening.

The project involved an early stage bioresolution that resulted in a monoacid product with >96% ee. From this, a bioreduction step introduced another chiral centre.
Key to this enzyme screening was to find a carbonyl reductase (CRED) enzyme able to reduce the ketone of the desired enantiomer feedstock stereospecifically and not the undesired (2%) enantiomer from the bioreolution step. The CRED identified resulted in a stereospecific reduction and subsequent biopolish 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. Having developed the process, all stereoisomers (seven different products) were synthesised readily from other key SelectAZyme enzymes so that analytical development could be undertaken to determine the fate of these potential impurities.

Table 1 summarises the key advantages of the green enzyme process. This example shows clearly that biocatalysis offers an attractive approach for synthesis, resulting in greener processes with real benefits for both the environment and the costs of APIs.

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<th>Partnerships &amp; collaboration</th>
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| Almac has an ongoing agreement with DSM, granting each other access to their respective enzyme platform technologies, services and expertise for the manufacturing of APIs. This effectively unites Almac’s expertise in rapid enzyme identification, scale-up and implementation into early-phase projects with DSM’s in commercial, multi-tonne manufacturing bioprocesses. The completion of projects between them demonstrates, in our view, that there is a market for scalable green technologies to access difficult-to-make chiral chemicals.  

At the enzyme discovery stage, Almac has collaborated with University College London (UCL) in the area of metagenomics, a culture-independent technique used to extract the total DNA from environmental samples that can allow access to 99% of enzyme genes in these samples. Previous work at UCL has obtained a series of metagenomes from various unusual sources. The use of bioinformatic tools will allow the metagenomes concerned to be mined for enzymes usable in both synthetic chemistry and synthetic biology projects.  

Almac is also working with Celbius, an ultrasound company, on the development of ultrasound-assisted biotransformations to increase throughput and ultimately drive down the cost of manufacturing. This can ultimately help in bioremediation projects, through chemical synthesis to increased fermentation titres.

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| 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 enzymatic reaction systems could investigate and integrate the use of technologies that are known to speed up catalysis in other systems. One such approach Almac has taken, with Celbius, is the use of ultrasonication, which has been shown to increase the rate of some enzyme reactions greatly, sometimes by an order of magnitude.  

Ultrasonation can induce physical phenomena, such as cavitation and acoustic streaming. These lead to extreme conditions of liquid turbulence that can benefit mass transfer but also influence behaviour at the molecular level, such as protein conformation and secondary structure, and lower catalytic activation energies. If applied carefully, the beneficial effects, such as increased reaction rates, outweigh any negative damaging effects. After all, ultrasound is usually used as a destructive tool for protein recovery.  

Substrate engineering is also a candidate for further development, whereby the substrate is (reversibly) modified. The basis of this approach is to add extra structural functionality to the core molecule to be transformed, which can help to direct and bind the overall molecule in the enzyme active site. The core part of the molecule can then be positioned for productive catalysis. Once it is transformed, if required, the extra functionality can be removed.

Table 1 - Comparison of chemical & SelectAZyme routes to Phase IIb compound

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<tr>
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<th>Chemical route</th>
<th>SelectAZyme route</th>
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<tbody>
<tr>
<td>Number of steps</td>
<td>9</td>
<td>5</td>
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<tr>
<td>Catalyst type</td>
<td>Rhodium-based</td>
<td>1 x liquid enzyme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 x powdered enzymes</td>
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<tr>
<td>Volume efficiency (g/litre)</td>
<td>83</td>
<td>151</td>
</tr>
<tr>
<td>Solvent usage</td>
<td>DCM, MeTHF, EtOAc, DMF</td>
<td>EtOAc, toluene</td>
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<tr>
<td>Global yield (%)</td>
<td>7.4</td>
<td>23.4</td>
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Summary

The proven ability of biocatalytic technology to produce hard cost savings for pre-existing processes or to provide economic access to NCEs in the industrial sector ensures increased investment year on year in this area. The key difference between biocatalysis today and ten years ago is that we now have excellent supporting technologies that greatly simplify enzyme identification and development.  

It is for this reason that traditional chemical technologies will surely find it increasingly difficult to compete in the coming decade with biocatalysis, which, we must remember, is still in its infancy. Continued investment into evolution technologies and metagenomic programmes will continue to drive its growth.

References

2. T. Moody & S. Mix, Pharma, September 2011

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