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Greener, sustainable processes using efficient biocatalyst purification tools at Almac Sciences

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This article describes how Almac Sciences have used biocatalysts as easy-to-use tools for rapid purification from mg to tonne scale, free from IP constraints for their customers.

The need for green, economic, robust, scaleable and reliable processes for the synthesis of chiral APIs and intermediates prompted Almac to build and fully integrate a biocatalysis group into its service portfolio. The dedicated group based at the Belfast site has expertise in gene identification, expression, fermentation and biocatalyst production. This is followed by the efficient use of biocatalysts to produce clean and sustainable routes to complex chiral APIs and intermediates. The biocatalysis group is closely linked to process and GMP pilot plant chemistry groups where biocatalyst discovery and development is integrated with screening, route definition and subsequent scale-up. The group applies expertise to complex chemical processes, rapidly implementing biocatalytic steps to significantly improve the yield and reduce waste for multi-stage syntheses.

Almac has launched its biocatalysis offering consisting of biocatalyst screening, bio-process development and optimisation, synthesis of chiral APIs and intermediates right through to supply of easy-to-use biocatalyst kits and bulk biocatalyst supply (1,2).

"The launch of biocatalyst screening kits last year provided our customers with a deeper understanding of biocatalysis benefits. The biocatalyst option is now Almac's first choice for scale-up of chemistry involving chirality and provides efficient, clean and robust processes", notes Biocatalysis Team Leader Dr. Tom Moody.

As anticipated, the business has advanced from the sale of biocatalyst screening kits, to screening and optimisation of custom transformations, and finally to the supply of 10s and 100s of kilograms of chiral intermediates. For example, preliminary screening was carried out to demonstrate that a carbonyl reductase (CRED) (3) process could replace a resolution for the preparation of a chiral alcohol. Having identified a CRED at small scale, Almac prepared 120 g of the chiral alcohol for integration into its API process development programme followed by manufacture of 30 kg for incorporation into the GMP API manufacture for Phase I clinical trials. Continuing on from this success, the group is now diversifying its application of biocatalysts in product purification to remove bottlenecks from API synthesis.

PRODUCT ISOLATION CAN STILL BE THE BOTTLENECK IN CHEMICAL SYNTHESIS

Often when carrying out chemical synthesis of a multi-step process, impurities arise. Purification at any scale can be the bottleneck for a process, particularly when preferred methods such as crystallisation, distillation and precipitation techniques fail to deliver the desired weight/weight

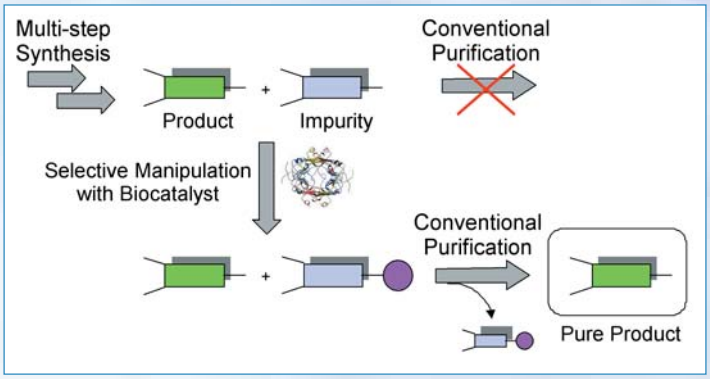


Figure 1 – Selective impurity modification (biopolishing) and subsequent removal

Figure 2 – Enzyme kits including pre-formulated enzymes and user manuals. Scale-up quantities are also available

(wt/wt) purity required for the product. When simple crystallisation, partitions and distillations are not good enough to yield the desired purity, Almac has again turned to the biocatalyst for help. Using the unique ability of a biocatalyst to selectively recognise differences in structure and shape, it is possible to selectively modify impurities in the presence of desired products.

APPLICATION OF BIOCATALYST TECHNOLOGY FOR SELECTIVE MODIFICATION

This application of the biocatalyst's ability to selectively modify functional groups has been exploited at Almac and used in the purification of compounds ranging from medicinal chemistry scaffold synthesis right through to kg GMP manufacture in Almac's pilot plant to the tonne manufacture of chiral building blocks.

"Biocatalysis can provide mild, regio- and chemoselective conditions that can modify the impurities, thereby altering their physical properties and facilitating in the purification processes, which can often not be matched by other chemical techniques" notes biocatalysis team leader Dr. Tom Moody.

The biocatalyst can be used to selectively modify the impurity leaving the desired product untouched. The modification of the impurity by the biocatalyst or **biopolishing** as Almac has coined it, results in physical property changes and the now modified impurity can be removed using conventional purification techniques as shown in Figure 1. More importantly, a simple solvent or aqueous wash is all that is required to remove the impurity and to yield the desired wt/wt purity of product.

Biopolishing can be used to increase both chemical and optical purities. The initial identification of a biopolish route involves biocatalyst screening and subsequent identification of a selective and active biocatalyst using conventional analytical techniques such as LC and/or GC. Development of the screening result into a process for kg and tonne manufacture can now be completed under timelines that are comparable to other chemistry scale-up timelines.

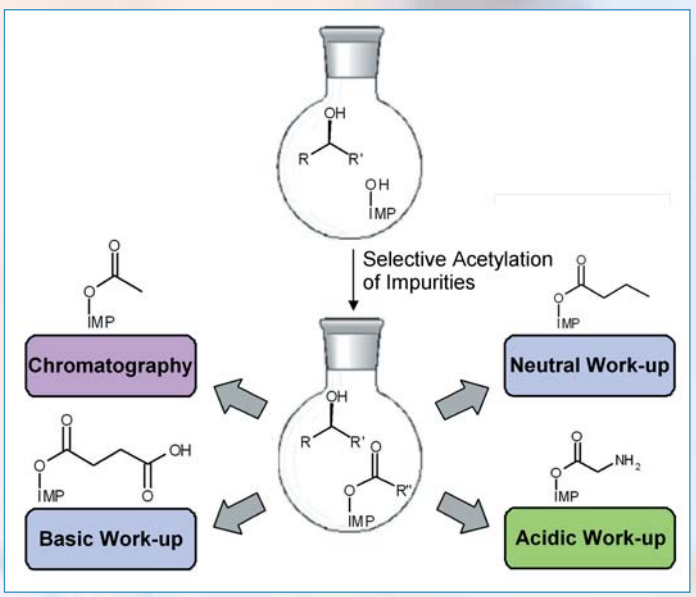


Figure 3 – Work-up techniques used for the separation of mixtures of enantiomers or diastereoisomers

RAPID BIOCATALYST IDENTIFICATION USING BIOCATALYST-SCREENING KITS

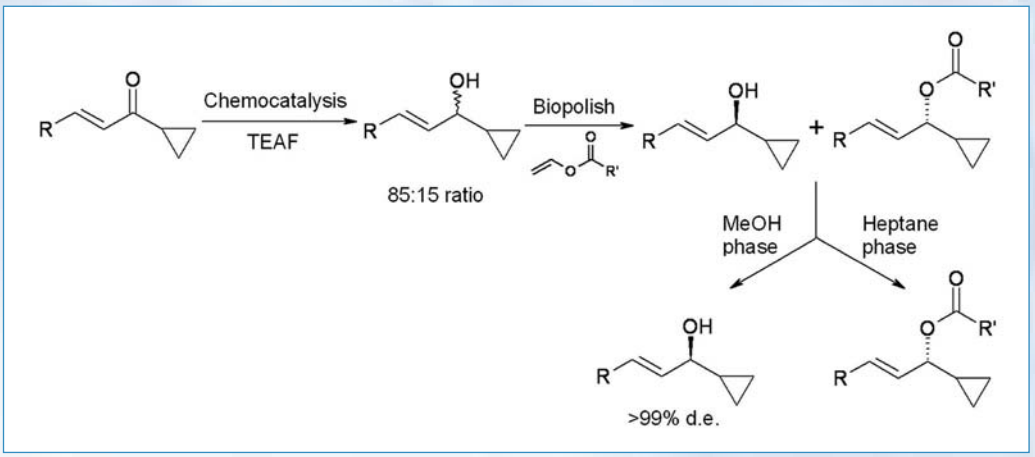
Almac offers a range of biocatalyst kits that have diverse substrate tolerance, are stable in organic solvent and at elevated temperatures and are commercially available at large scale. The screening kits have been built for ease of use and contain all the necessary reagents to carry out a screen to demonstrate selectivity and activity of the biocatalyst for a certain biotransformation and require no specialised equipment. There is also a detailed user guide included. The kits are available ready for shipment as shown in Figure 2.

BIOPOLISHING APPLICATION: EE AND DE POLISHING

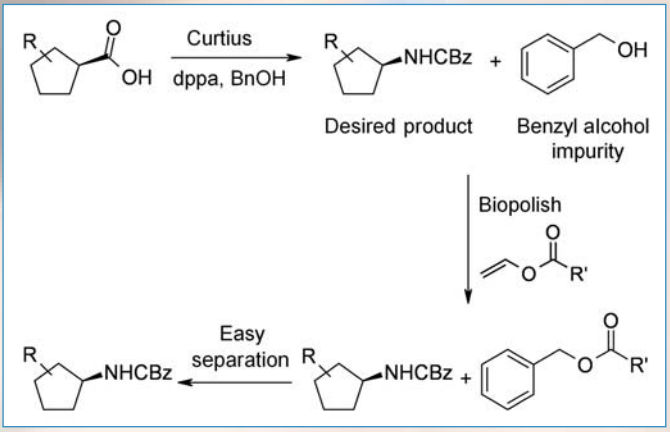
If the asymmetric route to a compound does not deliver the desired optical purity, then the ee or de can be biopolished to optical purity. For example, in the case of alcohols, hydrolase biocatalysts can selectively acetylate the hydroxyl group to the corresponding ester. Using different acyl agents such as vinyl esters, esters and anhydrides, the hydroxyl group can be modified in such a way for easy removal as shown in Figure 3.

Hydrolase enzymes can tolerate a wide range of substrates as well as acetylating reagents. This tolerance has been exploited by the introduction of different acyl donors to ease in the separation of mixtures of enantiomers and diastereoisomers under neutral, basic and acidic conditions. This acyl donor selection is key to the facile removal of the impurity and can be tuned to other R-groups already present on the product. As shown in Figure 3, the use of long alkyl chain acyl donors can result in a "tag" that can be partitioned between solvents. Alternatively, the use of succinic anhydride results in the preparation of the hemi-succinate, which can be removed by a basic work-up. An alternative donor is the application of cheap protected amino acids. The introduction of an amine tag means the impurity can be removed using an acidic work-up (4).

An example of this technology was used at 10 kg scale after an asymmetric reduction of an enone. The enone was reduced using a chemocatalyst in a triethylamine / formic acid mix to yield a ratio of 85:15 in favour



Scheme 1 – Biopolishing optical purity



Scheme 2 – Selective acetylation of benzyl alcohol impurity

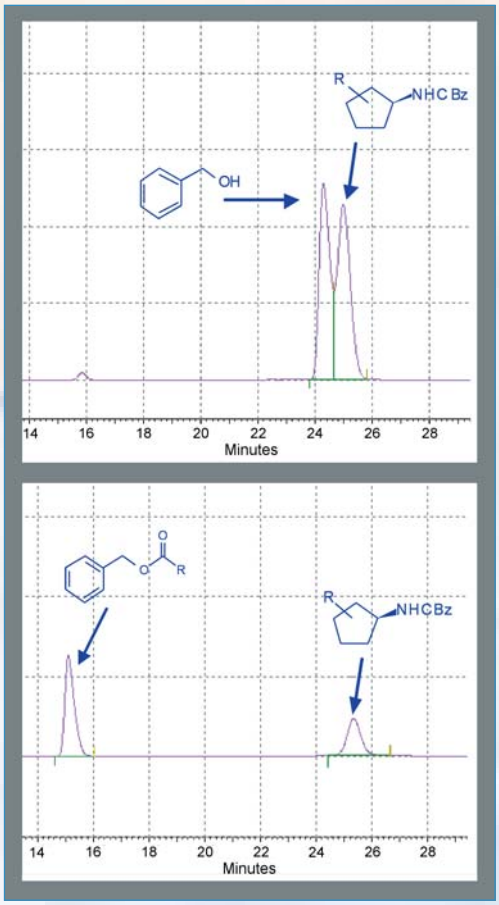
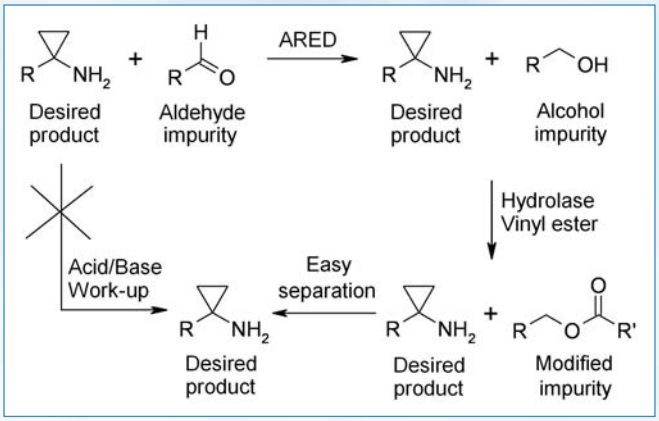


Figure 4 – Top: HPLC trace of product and benzyl alcohol impurity,
Bottom: HPLC trace of product and acetylated benzyl alcohol after biopolishing



Scheme 3 – Application of ARED and hydrolase biopolishing to remove aldehyde impurities

of the desired diastereoisomer. After a hydrolase screen, a biocatalyst was selected which selectively acetylated the undesired diastereoisomer using a long alky chain acyl donor as shown in Scheme 1. Simple partition in a methanol / heptane extraction system yielded the desired diastereoisomer with >99% de. The unwanted acetylated diastereoisomer was washed away in the heptane layer after 2 x10 vol extractions.

BIOPOLISHING APPLICATION: MEDICINAL CHEMISTRY SMALL SCALE APPLICATIONS

Almac has used hydrolases to speed up scaffold synthesis to deliver material for further library synthesis. For example, Almac has used biopolish technology for the rapid purification of a chiral amine scaffold synthesised from the corresponding acid. Typically when performing Curtius rearrangements to yield the desired CBz protected amine, a slight excess of benzyl alcohol is required to push the reaction to completion. Benzyl alcohol can be difficult to remove from the product using even chromatographic techniques. The benzyl alcohol can be biopolished using hydrolase biocatalysts, which can selectively acetylate the benzyl alcohol in the presence of other functionalities present on the product as shown in Scheme 2.

The modification alters the physical properties of the impurity making the chromatography very easy and separable on short timelines as shown from the HPLC traces in Figure 4.

BIOPOLISHING APPLICATION: SCALEABLE AND ROBUST PROCESSES FOR PILOT PLANT

Biopolishing has also been applied to the purification of cyclopropyl amines at scale as shown in Scheme 3. The amine product was contaminated with an aldehyde by-product and simple acid/base extraction failed to separate them. The amine was very sensitive to acid and prone to ring expansion. The mixture was biopolished using a two-biocatalyst system. The first biocatalyst used was an aldehyde reductase, A111 from Almac's aldehyde reductase (ARED) kit, which was used to reduce the aldehyde to the desired primary alcohol followed by solvent extraction into *tert*-butyl methyl ether (TBME). The TBME layer was azetroped dry and a hydrolase biocatalyst was then added in the presence of vinyl decanoate resulting in the selective acetylation of the primary alcohol. The acetylated impurity was easily removed by solvent swapping to heptane, were the product precipitated out of solution and the impurity remained in the TBME solvent. Simple filtration yielded the product with excellent wt/wt purity.

SUMMARY

The development and availability at scale of easy-to-use and robust biocatalysts for organic synthesis has resulted in an explosion of the number of uses and applications in synthetic labs in the pharmaceutical and fine chemical industries. Almac's biocatalysis group has demonstrated the use of biocatalysts as powerful separation tools for the purification of APIs and intermediates at mg to tonne scale. The power of a biocatalyst to selectively discriminate between products and impurities through selective hydrolysis, acetylation, reduction or a combination of them has led to the rapid delivery of pure compound to the customer. This technique can be applied to mixtures of compounds where a "handle" for biopolishing is available for the biocatalyst to modify, such as ketone, ester, acid, nitrile, amide or hydroxyl moieties.

Almac is applying this technology routinely in its labs and is now investigating its application in the selective modification of peptides to aid in downstream purification, which can be a bottleneck in peptide synthesis. Typically screens take 2-3 weeks for biocatalyst selection followed by 2-4 weeks process optimisation for the delivery of a process that would yield 10s of kilograms in a pilot plant.

REFERENCES

1) Moody T.S., Taylor S.J.C. ***sp2 magazine* 2009**, December, 30-2
2) Moody T.S., Taylor S.J.C. ***Speciality Chemicals* 2009**, January, 51-3
3) Alanvert E., Doherty C., Moody T.S., Nesbit N., Rowan A.S., Taylor S.J.C., Vaughan F., Vaughan T., Wiffen J.,Wilson I. ***Tetrahedron Asymm.* 2009**, *20*, 2462-6
4) Brossat M., Moody T.S., de Nanteuil F., Taylor S.J.C., Vaughan F. ***Org. Process Res. Dev.* 2009**, *13* (4), 706-9