Waste Reduction at Source: A Look at Biocatalysts For Synthesis of *Lipitor*®

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Abstract: *In the development of "green" pharmaceuticals, the application of biocatalysis has revealed that fewer processing steps are involved, higher yields with less waste, and fewer costs associated with materials and energy. Biocatalysts have been favored because of their ability to catalyze almost any organic reaction in ambient conditions with minimal environmental costs associated with large-scale production. The chemical industry is currently applying biocatalysts in the design of pharmaceuticals, such as Lipitor®, to maximize efficacy while reducing toxicity.*

Introduction: The chemical industry is both diverse and complex, as it is the chief developer and producer of chemicals for commercial applications. The Chemical Manufacturers Association reports that the $419 billion industry produces over 70,000 products and with these valuable developments comes great concern about the repercussions involved with their synthesis, use, and disposal (Derry, 2006). Reducing pollution and resolving environmental issues has been a big concern for the industry, fortunately innovative chemists have embraced a new way of thinking, introducing a whole new concept known as "green chemistry." Green chemistry is environmentally friendly, minimizes waste by favoring renewable resources, and reduces energy consumption. Paul Anastas and John Warner were the first to coin a definition for green chemistry: "Applying fundamental knowledge of chemical processes and products to achieve elegant solutions with the ultimate goal of hazard-free, waste-free, energy efficient synthesis of non-toxic products without sacrificing efficacy of function" (Anastas & Warner, 1998). Likewise, in *Green Chemistry: Theory and Practice (1998)* Paul Anastas and John Warner instituted twelve principles to be used as guidelines in its application:

- It is better to prevent waste than to treat or clean up waste after it is formed.
- Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
- Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
- Chemical products should be designed to preserve efficacy of function while reducing toxicity.
- The use of auxiliary substances, e.g. solvents, separation agents, should be made unnecessary wherever possible and innocuous when used.
- Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperatures and pressure.
- A raw material feedstock should be renewable rather than depleting, whenever technically and economically practical.
- Unnecessary derivatization (blocking group, protection/deprotection, temporary modification of physical/chemical processes) should be avoided wherever possible.
- Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
- Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.

- Analytical methodologies need to be further developed to allow for real-time in-process monitoring and control prior to the formation of hazardous substances.
- Substances and the form of a substance used in a chemical process should be chosen so as to minimize the potential for chemical accidents, including releases, explosions and fires.

A major objective then, is to discover and develop products and processes that fulfill these very goals.

All organic substances have a carbon backbone that binds to other essential elements such as hydrogen and oxygen. Carbon atoms are capable of forming double bonds either naturally or through organic reactions. A great step forward for green chemistry was Yves Chauvin, Robert Grubbs and Richard Schrock's discovery of a chemical process called metathesis (Derry, 2006). In such reactions, catalyst molecules act to break and make double bonds between carbon atoms, creating many new molecules. Catalyzed reactions refine the nature and fate of synthetic reactions. Catalysts have incredible stability, can be used in small amounts, and are regenerated after each reaction, making them a remarkable asset for minimizing the amounts of chemicals involved in chemical processes. Metathesis applications are used daily in the chemical industry, especially in the production of complex molecules for pharmaceuticals because of their increased efficiency, are user friendly and more environmentally benign (Nobelprize.org, 2011).

When it comes to greening up chemistry, the pharmaceutical industry has the most to gain through improved chemical products and processes. Pharmaceutical production can be a dirty business, often requiring multiple and costly steps that involve the use of toxic chemicals and generation of waste. By reworking synthetic pathways, manufacturers can "improve the atom economy of reactions and reduce the number of reaction steps to achieve the same benefits" (ACS, 2011). As companies started to think about the triple bottom line of environmental sustainability, economic performance, and social responsibility, the pharmaceutical industry's interest in green chemistry activity grew (Thayer & Houston, 2009).

Enzyme-mediated processes involving biocatalysts offer many advantages over conventional, non-catalyzed methods and have become increasingly important as consumers become more enviro-conscious. Biocatalysts come with a myriad of amenities in the context of green chemistry: novelty, high yield, shorter process routes; increased selectivity; and lower temperatures and pressures. They are completely renewable, cause no harm to animals or humans, and generate drastically less waste per unit. With this in mind, there is no wonder why biocatalytic processes are starting to dominate the fine chemical and pharmaceutical industry.

However, biocatalysts are nothing new to the chemical industry. According to the American Chemical Society, the advantage of enzyme-catalyzed reactions has been well understood, but with limited technology, they have not been practical for use in industry (2011). Utilization of biocatalysts has been hampered by the "lack of consistent production and formulation, limited scope of substrates compared with classical chemical methods, limited stability and shelf life, and lack of easy separation and reuse" (ACS, 2011). Because biocatalysts are highly selective, use on the industrial scale can be limited because availability is highly restricted to those captured from nature (Thayer, & Houston, 2001). The speed of process development is often slower for biocatalytic processes than their chemical counterparts. Modern biotechnological tools now allow enzymes to be optimized for a desired reaction, but this optimization is often too costly and time consuming to meet industrial timelines; therefore, broad application remains elusive (Bio-Catalyst.com). Obstacles to overcome when using biocatalysts is to increase both productivity and stability while lowering the cost of production, thus

improving yield of economically viable chemical processes (Thayer & Houston, 2001)

Application of Biocatalysts: Atorvastatin, commercially known as *Lipitor*®, is a statin drug primarily indicated for use in patients at risk of heart attack, stroke, or heart disease by suppressing biosynthesis of cholesterol (Pub Med Health, 2010). Currently, *Lipitor*® is the largest selling pharmaceutical in the history of mankind, with annual sales of over $13 billion worldwide. Being that cardiovascular disease is one of the leading causes of death today, *Lipitor*® has succeeded in preventing countless cardiovascular events and saving the lives of millions. To maintain its status as a world-class leading drug, Pfizer researchers have reengineered the production process to develop a way to "reduce the use of chemicals by an order of magnitude" (Pfizer, 2009).

Traditionally, commercial processes designed for the synthesis of *Lipitor*® had a maximum yield of 95%. Production cost of product was as much as $85 per kilogram. An eleven step process, reaction conditions had to be set at 100°C, using methanol solvent and hydrogen gas, generating as much as 100 grams of waste per kilogram of product. Enantiomeric purity <95% after purification by fractional distillation resulted in incomplete conversion and by-product formation that significantly reduced product yield. Hydroxynitrile (HN), the key chiral building block for synthesis of the active ingredient, atorvastatin, found in *Lipitor*® required a resolution step with 50% maximum yield. They also need hydrogen bromide to generate a bromohydrin for cyanation. Substituting cyanide for halide under heated alkaline conditions, formed extensive byproducts.

Biocatalysts have emerged as a versatile tool that offers a variety of enantioselective routes to key chiral intermediates for the drug. Atorvastatin, the active ingredient in Lipitor, is a completely synthetic molecule with a chiral 3,5- dihydroxy acid side chain that accounts for 25% of the compounds molecular weight. This side chain also represents the greatest challenge for preparation of the drug *Lipitor*®. Taking into account that biocatalysis is inherently green, Presidential Green Chemistry Challenge Award winner, Codexis, developed a biocatalytic route to the *Lipitor*® intermediate, hydroxynitrile (HN), (IUPAC name: ethyl (*R*)-4-cyano-3-hydroxybutyrate). Biocatalysis of *Lipitor*® synthesis has shown to have excellent selectivity and yields, purification only at the last step, reduced requirements, reduced byproducts, mild reaction conditions, efficiency, and is green, safe, scalable and cost effective. The new HN synthesis doesn't require metal catalysts or chemical derivatization steps, and it is carried out at room temperature and pressure at neutral pH in water in much less time. Reaction time is 8 hours and because the product of the enzymatic process is enantiomerically and chemically pure, product yield is at least 99%. The new E factor, a measure of process sustainability, is 5.8, significantly lower than any of the previous routes to HN. The two-step, three-enzyme mechanism can recover high-quality product by directly extracting the reaction mixture because the enzymes are so active and stable. Codexis scientists used the company's MolecularBreeding accelerated directed-evolution technology to engineer three enzymes to accomplish the chemical reaction. The new HN synthesis starts with reduction of the keto group of ethyl 4-chloroacetoacetate feedstock to form a chiral chlorohydrin. The reduction is facilitated by two of the engineered enzymes, a ketoreductase and a glucose dehydrogenase. The enzymes work in tandem with nicotinamide adenine dinucleotide to convert the chloroketone to the chlorohydrin using glucose as a reductant, which avoids the need to use hydrogen. More pivotal is the ensuing step in which the third enzyme, a halohydrin dehalogenase, catalyzes substitution of chlorine with a cyano group to form hydroxynitrile. Both

enzymatic reaction steps take place under aqueous conditions at neutral pH and at atmospheric pressure and just above room temperature, Grate pointed out. Overall, the Codexis process provides more than 90% yield for each step and provides HN in high enantiomeric purity of >99%. In addition, the low enzyme loading avoids emulsion formation and allows product isolation by extraction rather than by more costly distillation (Ritter, 2006).

Future of Biocatalysts: Codexis' evolved enzymes have "improved the volumetric productivity of the reduction reaction by approximately 100-fold and that of the cyanation reaction by approximately 4,000-fold" (Ritter, 2006). This reengineered process involves many environmental and health benefits by reducing waste, solvent usage, energy consumption, and need for specialized manufacturing equipment, and increasing worker safety (EPA, 2011). Goals for industrial processing such as "sustainable development", "green chemistry," or "environmentally benign manufacturing," would be difficult to attain without the availability of biocatalysts that perform optimally in ambient conditions (Bommarius & Riebel, 2004). Increasing emphasis on becoming "green" through the use of environmentally benign products and processes means that pharmaceutical companies will embrace the evolution of biocatalysts, particularly in the production of atorvastatin. In a report by Dr. Nicholas Derry (2007), he discusses the viability of many green chemistry processes that were once frowned upon, but records indicate that industrial-scale biocatalytic processes more than doubled between 1992 and 2002. (Straathof, 2002) and have proved to be profitable as consumers yearn to be more environmental friendly. Moreover, there are significant economic benefits from utilizing the revised process of HN production for the synthesis of atorvastatin, as the pharmaceutical industry is based on maximum efficacy with minimum toxicity.

The future looks bright for *Lipitor*®, as it continues to grow steadily and dominate the market in cardiovascular medications. The latest generation of *Lipitor*® that involves green by design processes enables access to global markets that have expectations for cleaner and efficient products.

References

American Chemical Society (ACS). (2011).

- Chemical society to honor "Heroes of Chemistry" during national meeting. http://portal.acs.org:80/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=222&content_id=WPCP_010675&use_sec=true&sec_url_var=region1&__uuid=3b11aeb5-b955-4d8c-aa63-cf11f04d71e8 (accessed 29 June 2011).
- Greening up process chemistry. http://portal.acs.org:80/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=445&content_id=CNBP_026112&use_sec=true&sec_url_var=region1&__uuid=d35f770f-f99c-4429-9866-f2f4fcf07299 (accessed 29 June 2011).

Affholter, J., Apel, W. A., Arnold, F., et. Al. (1999). New Biocatalysts: Essential tools for a sustainable 21st century chemical industry. Council for Chemical Research.

Bio-catalyst.com Time for biocatalysis and bio-based processes? *Biocatalysis:How enzymes, biofuels, and sustainable chemistry are changing the world*. http://www.bio-catalyst.com/hot-and-new-in-biocatalysis/biocatalysis/time-for-biocatalysis/ (accessed 30 June 2011).

Bommarius, A. S. & Riebel, B. R. (2004). Inroduction to Biocatalysts.

http://media.wiley.com/product_data/excerpt/48/35273034/3527303448.pdf (accessed 22 June 2011).

Derry, N. (2007). Green chemistry. *Victorian Curriculum and Assessment Authority, Units 1-2.*

Environmental Protection Agency. (2011). Green Chemistry. http://www.epa.gov/gcc/pubs/pgcc/winners/grca06.html (accessed 16 June 2011).

Greenberg, W. A., Varvak, A., Hanson, S. R., Wong, K., Huang, H., Chen, P., and Burk, M. A. (2004). Development of an efficient, scalable, aldolase-catalyzed process for enantioselective synthesis of statin intermediates. http://www.pnas.org/content/101/16/5788.full.pdf (accessed 30 June 2011).

Nobelprize.org. (2011). Press Release: The Nobel Prize in chemistry 2005. http://nobelprize.org/nobel_prizes/chemistry/laureates/2005/press.html (accessed 30 June 2011).

Pfizer. (2009). Pfizer 2008 Annual Report. http://media.pfizer.com/files/annualreport/2008/annual/review2008.pdf (accessed 16 June 2011).

PubMedHealth. (2010). Atorvastatin. http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0000009/ (accessed 16 June 2011).

Ritter, S. K. (2006). Going green keeps getting easier. *Science & Technology, 84,* 24-27.

Roumeliotis, G. (2006). Green Lipitor building block synthesis wins kudos for Codexis. Pharma Technologist.com. http://www.in- pharmatechnologist.com/Processing-QC/Green-Lipitor-building-block- synthesis-wins-kudos-for-Codexis (accessed 1 July 2011).

Straathof, A. J., Panke, S., & Schmid, A. (2002). The production of fine chemicals by biotransformations. *Current Opinion of Biotechnology, 13,* 548–556.

Thayer, A. M. & Houston, E.

- (2001). Biocatalysis. *Chemical and Engineering News, 79*, 27-34. http://pubs.acs.org/cen/coverstory/7921/7921biocatalysis.html (accessed 30 June 2011).
- (2009). Sustainable syntheses. Chemical and Engineering News. http://portal.acs.org/portal/fileFetch/C/CNBP_024675/pdf/CNBP_024675.pdf (accessed 30 June 2011).