Project Title: New Green Commercial Bio-Catalytic Route to Lipitor API

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Short Description/Milestones:
Pfizer has developed a new green bio-catalytic route to atorvastatin calcium, the active ingredient in the cholesterol lowering drug Lipitor. The new process is a shorter, greener, more efficient synthesis which avoids the use of hazardous reagents and reduces wastes associated with the previous process. This innovation will allow Pfizer to continue to produce this extraordinary medicine for patients around the world whilst providing significant environmental and worker safety benefits. This development, which occurred from 2006-2009 was submitted to the US FDA (and other worldwide regulatory authorities) in late 2009. US FDA approval for the new process was granted in April 2010. Commercial scale validation campaigns for API manufacture using the new process have been completed in the Pfizer manufacturing facilities in 2011. The transition to full scale commercial manufacture using the new process to supply Lipitor API will occur in 2012.

Category Eligibility:
This nomination is not eligible for the small business or academic award.

Focus Area:
The new process falls under the focus areas:
1) Greener Synthetic Pathways
2) Greener Reaction Conditions

US Component:
A team of Pfizer colleagues from various sites around the world were responsible for the ultimate success of the project. Pfizer's US-based colleagues contributed to this success by identifying an aldolase (DERA) enzyme that catalyzed the desired transformation and then evolved the DERA enzyme to make it commercially viable in terms of its catalytic efficiency and ability to operate under process conditions. They also developed the manufacturing process for the biocatalyst. Finally, the lactol material, which is the product of the DERA catalyzed reaction, is manufactured at a Pfizer US facility.

Abstract (Max 350 Word):
Since its launch in 1997 Lipitor, Pfizer's landmark cholesterol treatment has had a historic journey as the No. 1 prescribed branded cholesterol-lowering medication in the world. The success of Lipitor, together with Pfizer's commitment to continuous improvement, made Lipitor an obvious target for process improvement. Pfizer set a challenge to dramatically improve the efficiency and environmental performance of the manufacturing route to atorvastatin.

Pfizer recognized that optimizing the existing manufacturing process would not be enough to achieve the transformational changes that were sought. A completely new, more efficient and environmentally sustainable process was developed. This submission describes the result of that successful initiative whereby Pfizer developed a new commercial route to atorvastatin calcium API using a greener biocatalytic process.
The new green process incorporates a water based 2-deoxyribose-5-phosphate aldolase (DERA) enzyme in the very first part of the new route to make lactol. The presence of the nitrogen atom in the lactol avoids the necessity for cyanide or azide chemistry to introduce this later. The

original process required the use of a chiral starting material; in contrast the DERA enzyme sets both stereocenters with high selectivity in water at room temperature. This lactol is transformed into isopropyl acetonide atorvastatin (IAA) before a final conversion to atorvastatin calcium API product. The lactol to IAA conversion is extremely efficient in that it involves 4 high yielding chemical steps (oxidation, esterification, deprotection, Paal Knorr) with only the IAA being isolated as a solid. This highly efficient green process substantially reduces the environmental impact by eliminating hazardous steps and reducing/eliminating required chemicals. For example, there is no need for a high pressure hydrogenation step with its associated metal catalysts. The use of pyrophoric *n*-butyllithium is also avoided, as is its associated butane waste gas. The need for significant other reagents and solvents has either been eliminated or dramatically reduced. The new manufacturing process was approved by the FDA in April 2010. Commercial scale validation batches have been manufactured in 2011 and transition to full scale commercial manufacture has commenced.

1 Introduction:

The development of a new, more efficient manufacturing process to atorvastatin calcium API was targeted to help Pfizer meet its goals for Lipitor. The existing cyclopentyl process[1] is outlined in **Figure 1.1** below.

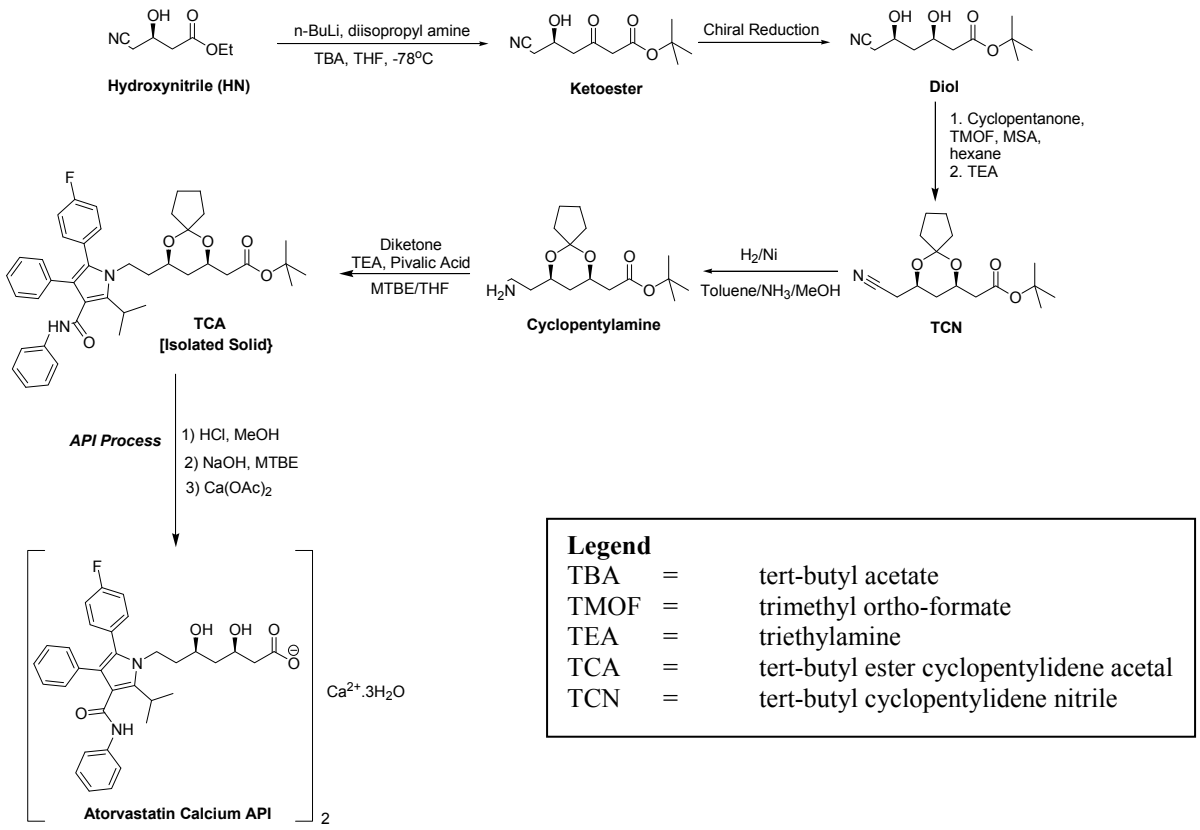

Fig 1.1 Existing Cyclopentyl Process

The most feasible way to achieve this goal was to completely redesign the atorvastatin manufacturing process by applying green chemistry principles and in particular by incorporating a water based, energy-efficient biocatalytic step. The process that ultimately delivered this goal utilizes a 2-deoxyribose-5-phosphate aldolase enzyme, referred to as DERA. Pfizer made the breakthrough discovery that protected amino aldehydes are excellent substrates for DERA. This was novel and unprecedented in the chemical literature. The presence of the nitrogen already in the lactol avoids the use of cyanide or azide chemistry to introduce this later. Thus as shown in **Figure 1.2**, an amino aldehyde (PPA) can sequentially react with two moles of Acetaldehyde to yield a masked form of the required Amine for the Paal Knorr reaction.

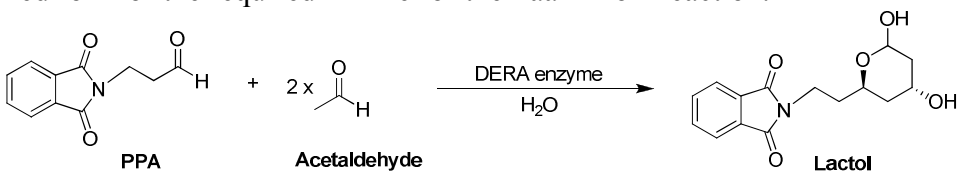

Fig 1.2 Bio-catalytic DERA Process

This stereochemically-pure lactol is then efficiently converted to the isopropyl acetonide atorvastatin (IAA) intermediate in 4 high yielding chemical steps (oxidation, esterification,

deprotection, Paal Knorr) with only the IAA being isolated. The isolated IAA is then converted to atorvastatin calcium API in the final step. Please see **Figure 1.3** below for the process overview from lactol to API.

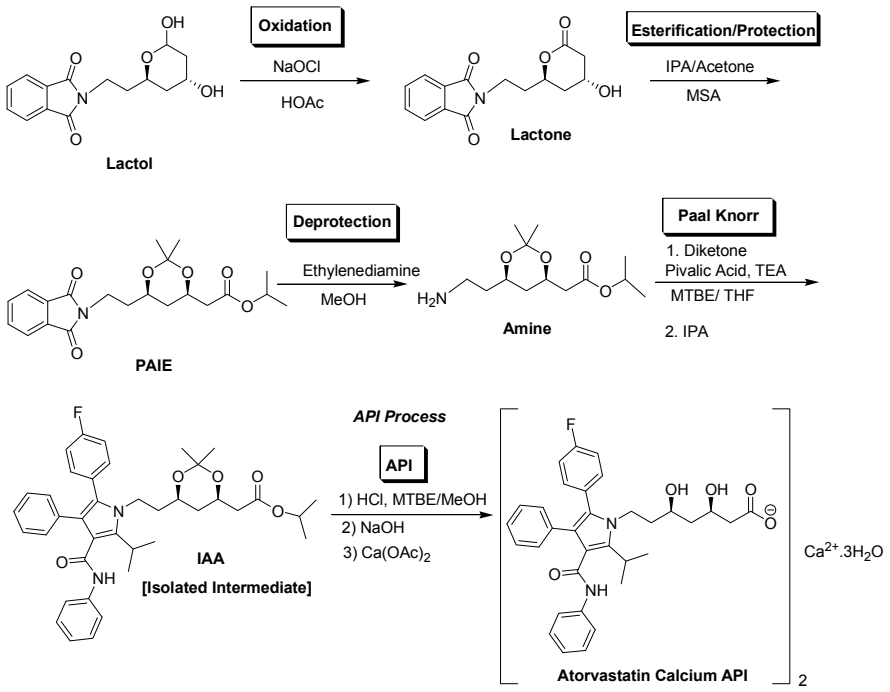

Fig 1.3 Lactol to IAA to API Process

The innovative biocatalytic technology described in this submission will allow Pfizer to continue to produce this extraordinary medicine for patients around the world whilst providing significant environmental and worker safety benefits.

2 Process Development

2.1 Development of the Biocatalytic Reaction

Development of a biocatalytic process typically begins with the identification of an enzyme suited to perform the reaction. Ideally an enzyme would be available "off the shelf", as is the case for many lipase enzymes. However, a DERA enzyme with the required activity was not commercially available. As such an enzyme with the desired activity level had to be found and developed. As a result, enzyme and process development were undertaken simultaneously by Pfizer. During the course of process development work, the starting substrate was changed due to problems with its enzymatic transformation and a new substrate (PPA) was developed - this necessitated a change in direction for engineering the enzyme. Also, the preferred enzyme form evolved from cell lysate form to concentrated whole cells form. As could be expected, the technical obstacles of the project presented significant challenges which the global project team, overcame each time.

A key to the success of DERA for atorvastatin was the development of an enzyme with sufficient specificity and activity to meet chemical efficiency targets as well as physical characteristics

compatible with manufacturing capabilities. Pfizer's Biocatalysis Center of Emphasis, initiated an enzyme engineering program to engineer a DERA enzyme with at least 10-fold greater activity than the initially identified enzyme. A rational, iterative process in which specific amino acids were changed to favorably increase enzyme activity was completed. The DERA project was Pfizer's first application of this new enzyme engineering technology and has opened up this development approach for other products.

Pfizer's API development groups brought their expertise to bear on the challenge in terms of microbial genetics, fermentation, downstream processing, piloting, and analysis. The optimum substrate (PPA) for the DERA enzyme needed to be identified for the lactol reaction. Once PPA had been identified, Pfizer then needed to develop a commercial synthesis to it from commodity chemicals. In addition to developing the lactol process, a new process from lactol to API needed to be developed. As stated above, Pfizer developed a process to IAA in 4 high yielding chemical steps (oxidation, esterification, deprotection, Paal Knorr) with only the final IAA intermediate being isolated. The IAA intermediate was converted in a single step to API.

2.2 Enzyme Process Development

The skills and experience of Pfizer's development groups combined well to design and deliver the DERA process. The development group's facilities include pilot plant facilities, with multiple fermentation vessels including two 5000 L fermenters and supporting downstream processing trains. These assets within Pfizer allowed rapid in-house biocatalyst development and material delivery at a large scale.

The initial process development work focused on providing enzyme preparations for development of the biocatalytic reaction. As the project progressed the DERA process was further refined including the following examples:

- genetic manipulations to express the enzyme in *E. coli*
- development and pilot scale manufacture of a suitable enzyme form
- development of a commercial fermentation/harvest process
- development of assays needed to support the enzyme and fermentation work

Vector/Culture Development

Early in the project, a DERA enzyme was identified that had sufficient activity to be a candidate for enzyme evolution. The particular enzyme was chosen for further development and was successfully engineered to improve activity >10 fold. In addition, a new *E. coli* expression system was designed and developed for large scale production. The expression system is particularly valuable to Pfizer in that it is designed to be "generic" for future Pfizer protein product candidates expressed in *E. coli.*

Large Scale Fermentation Development

Early in the project it was identified that DERA enzyme material would quickly be required for process development . A quick start was possible by relying on media and conditions previously developed for an *E. coli* expressed ketoreductase enzyme. A shake flask model was developed to rapidly screen new enzyme constructs and host organisms. Based on extensive in-house experience expressing recombinant proteins, fermentation conditions were further developed for each subsequent improved enzyme in vessels ranging from 2 L to 5000 L. The 5000 L fermenter

was used to produce the large amounts of enzyme required to support development activities and plant trials.

Analytical Method Development

Evaluating expression and production of enzymes requires suitable assay methods. For naturally-occurring enzymes, substrates and reaction chemistries are often known. However when enzyme modifications are done, the typical enzyme assays need to be redeveloped. That was also the case for DERA. A rapid microtiter plate-based assay was developed for tracking enzyme levels in fermentation time courses and throughout the isolation. These data were then used to optimize enzyme expression and recovery.

2.3 Safety & Environmental Benefits

The project's use of improvements in recombinant technology and fermentation improvements yielded a nine fold increase in specific enzyme activity. These improvements lowered the total amount of the DERA enzyme material needed for the bioconversion and dramatically increased the volumetric efficiency of the process.

By its design the DERA process yields significant reductions in hazardous materials in comparison to the existing cyclopentyl process. For example;

- Pyrophoric raw materials such as *n*-butyllithium, Raney nickel and highly flammable hydrogen gas are not used in the DERA Atorvastatin process and thereby reduce the safety hazards that our production staff need to manage.
- In addition the new process will avoid the hazardous transportation of 18,000 kg of Raney nickel and 530,000 kg of *n*-butyllithium solution by road and sea per year - again reducing the safety and environmental concerns both inside and outside Pfizer.
- The existing process involves the generation of hazardous butane gas which needs special venting and end of line incineration. The new process avoids the formation of butane completely.
- In comparison to the previous atorvastatin process the new DERA process has eliminated the need for certain solvents/reagents and minimised the use of others quite dramatically. The following are an example of eliminations and reductions that are projected on an annual basis;
    - 1,038,000 kg of hexane – completely eliminated
    - 772,000 kg ethyl acetate – >70% reduction
    - 476,000 kg of hydrochloric acid – completely eliminated
    - 400,000 kg of tetrahydrofuran – >60% reduction
    - 315,000 kg of t-butyl acetate – completely eliminated
    - 280,000 kg of di-isopropylamine – completely eliminated
    - 205,000 kg of trimethylothoformate – completely eliminated
    - 162,000 kg cyclopentanone – completely eliminated
- By avoiding the need for cryogenic reaction conditions the new process has also removed the need for 334,000 kg of liquid nitrogen per year.

3 Scientific Impact

- The discovery that amino aldehydes are good substrates for DERA is very significant, not just for Atorvastatin but for other valuable chemical entities.
- The project team kept a continuous focus on the bond-forming and bond-breaking chemistries to increase atom economy and greatly reduce processing time by telescoping four chemical transformations from lactol to IAA (the final isolated intermediate). Enzyme engineering to improve the properties of the DERA enzyme, and substrate engineering, to select the optimum substrate (PPA), were both utilised to develop the key biocatalytic transformation that set both stereocenters in atorvastatin in one reaction step.
- The project's use of improvements in recombinant DNA technology and fermentation improvements increased enzyme specific activity nine fold. These improvements lowered the total amount of the DERA enzyme material needed for the bioconversion and dramatically increased the volumetric efficiency of the process.
- In late 2009 the new process was submitted to the US FDA for regulatory approval for its commercial use (and other worldwide regulatory authorities). US FDA approval for the DERA process was granted in April 2010. EMEA approval in Europe was granted in September 2010. Hence the nominated technology has undergone detailed scrutiny by major regulatory agencies and has been approved. The process has been optimized since 2010 and commercial scale batches were manufactured in 2011. The transition to full scale commercial manufacture using the new process to supply Lipitor API will occur in 2012.
- The experience gained in the development of this biocatalytic route to Atorvastatin has further advanced Pfizer's knowledge of developing and commercializing enzymatic reactions. Pfizer recognizes the value and broad applicability of biocatalysis and Green Chemistry to achieve environmentally responsible and sustainable manufacture of its compounds.

4 Summary

The DERA project exemplifies the innovation and dedication Pfizer is committing to develop and incorporate biocatalysis and green chemistry into its production processes. DERA is an excellent example of co-operation among many groups across Pfizer who are working to develop multiple green chemistry approaches simultaneously and bringing them together to create an environmentally sustainable manufacturing process for one of the world's most successful medicines. The DERA process demonstrates that through careful design and conscious consideration of green chemistry principles, highly efficient pharmaceutical production processes can be realised with excellent environmental stewardship.