IN-VITRO MULTI-ENZYMATIC CASCADE REACTIONS: DESIGN, ADVANCES, AND APPLICATIONS

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INTRODUCTION:

Since the early 1900s, biochemists have recognized the synthetic utility of enzymatic transformations in synthesis, applied towards the development of small chiral substrates, and common biological building blocks such as amino acids (Figure 1).[1-2] Originating from the early study of glycolytic processes,[3] multi-enzymatic cascades (enzymatic processes incorporating two or more enzymatic steps for the production of valuable chemical compounds) offer potentially unparalleled efficiency, selectivity, and safety of operation for the synthetic chemist. However, practical application of this field has historically lagged behind traditional chemical transformations, due first to the poor definition and characterization of enzymes, and later to limited substrate scope, low loading capacities, and incompatibilities with conventional organic solvents. It was not until the "third wave" of biocatalysis that protein engineering enabled substantial progress towards addressing these limitations, enabling enzymatic transformations under practical conditions compatible with applications such as process-scale synthesis.

This seminar will address the principles of practical cascade design,[4-6] advancement of enzymatic cascade technology throughout its young history,[3, 7] and showcase its applications through the discussion of landmark cascade processes for the synthesis of Atvorastatin (Lipitor®), and Islatravir (an HIV reverse transcriptase translocation inhibitor), in comparison to their conventional synthetic routes.[8-9]

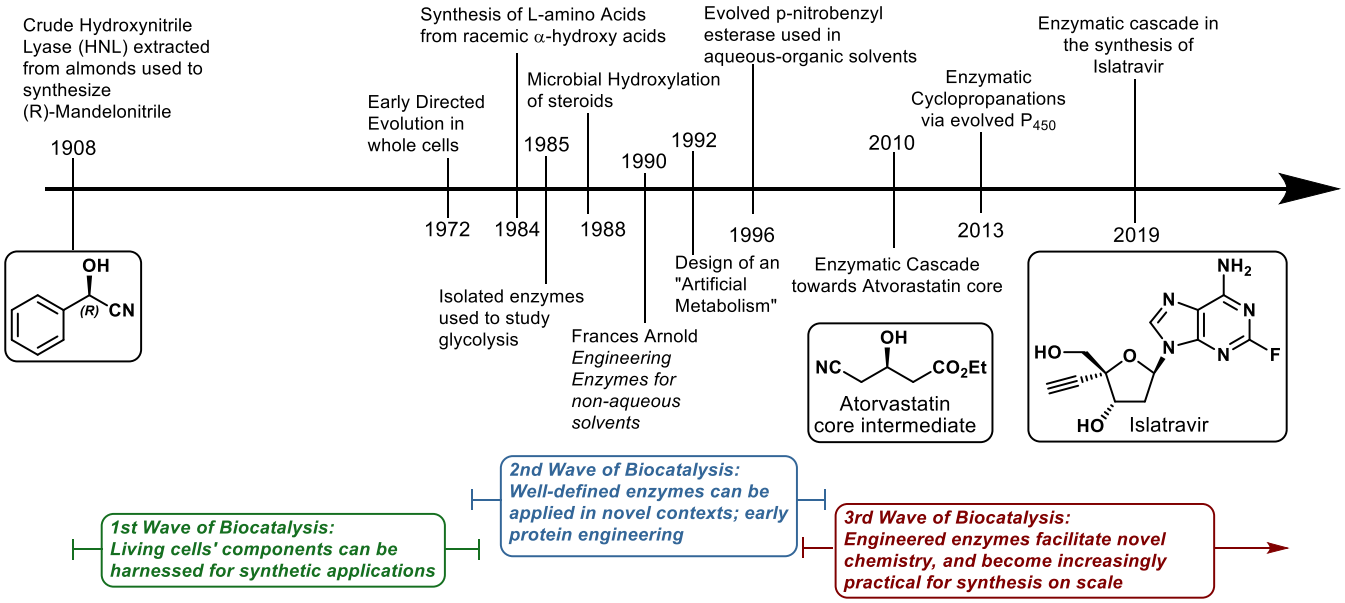

Fig. 1: Timeline of biocatalytic advances enabling enzymatic cascades.

ENZYMATIC CASCADES: ROUTES TO PHARMACEUTICAL COMPOUNDS[8-9]:

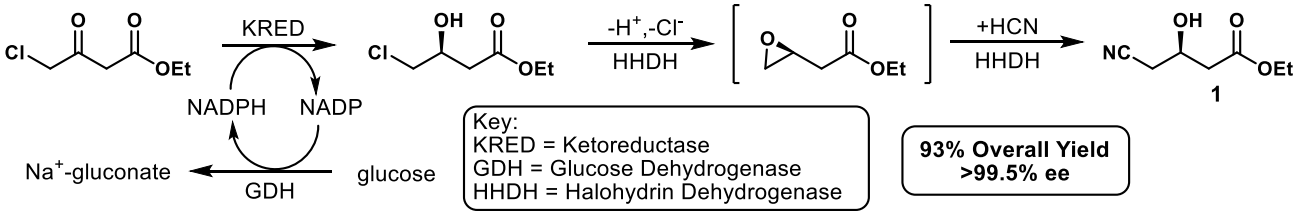

Figure 2: Enzymatic cascade for the production of Atvorastatin core scaffold.

Atvorastatin Calcium (Lipitor®) is a cholesterol lowering medication exceeding $10 billion in annual sales. Conventional synthetic routes and preliminary enzymatic routes have been investigated towards improved synthetic methods, although all practical routes require the use of core scaffold **1**. Sheldon *et. al.* proposed the use of an enzymatic reduction, dehydrogenation, and cyanation cascade (Figure 2). Enabled by several iterations of DNA shuffling, optimized variants of ketoreductase, glucose dehydrogenase, and halohydrin dehydrogenase enzymes delivered core scaffold **1** in 93% overall yield, while avoiding the need for kinetic resolution, chiral pool starting materials, or asymmetric chemical hydrogenation native to previous processes.

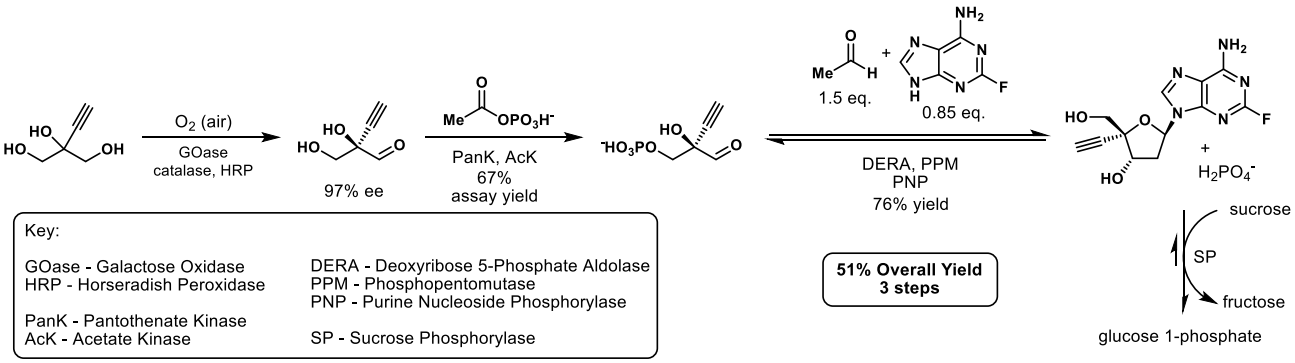

Figure 3: Enzymatic cascade for the production of Islatravir.

The HIV reverse transcriptase translocation inhibitor Islatravir exhibits exceptional potency and long duration of action, prompting substantial interest into its process-scale production. Conventional organometallic routes have previously required 12 to 18 steps, struggling with inefficient protecting group manipulations, and facing difficulties controlling anomeric stereochemistry. The novel enzymatic cascade reported by Merck employs five engineered enzymes and four auxiliary enzymes in order to synthesize Islatravir in three steps and 51% overall yield (Figure 3).

References:

1. *Protein Sci* **2002,** *11* (2), 292-300.
2. *Ann. NY Acad. Sci.* **1984,** *434* (1), 091-094.
3. *ACS Catalysis* **2018,** *8* (3), 2385-2396.
4. *Chemical Reviews* **2018,** *118* (1), 270-348.
6. *Angew. Chem. Int. Ed. Engl.* **1992,** *31* (5), 614-616.
7. *Journal of Biotechnology* **1985,** *2*, 257-273.
8. *Green Chemistry* **2010,** *12* (1), 81-86.
9. *Science* **2019,** *366* (6470), 1255.