Evolution of Sitagliptin: Three Generations of Research

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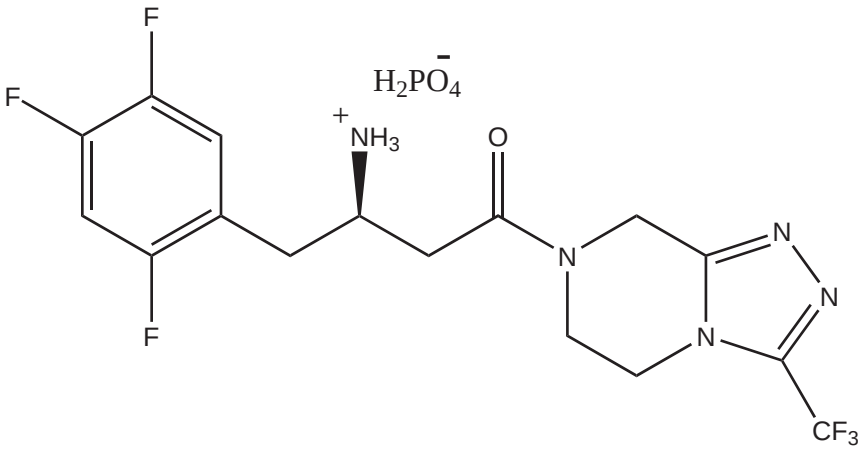

Figure 1: Chemical structure of Sitagliptin phosphate

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1 Introduction to Diabetes

Diabetes Mellitus is a common chronic metabolic disease characterized by defects in insulin secretion. Chronic hyperglycaemia [1] affects about 384 million people in the world [1] causing long-term damage and failure of organs, nerves, heart and blood vessels [2]. Major complications of diabetes include peripheral neuropathy [2] with risk of foot ulcers and amputation; retinopathy [3] and increased risk of cardiovascular diseases.

Majority of cases of diabetes fall into two main categories – type 1 diabetes and type 2 diabetes. Type 1 diabetes is characterized by the decreased secretion of insulin in β - cells of pancreas with consequent insulin deficiency leading to abnormalities in metabolism of carbohydrates, fat and proteins. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of insulin resistance [4] and an inadequate compensatory insulin secretory response [2].

1.1 Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM), previously known as non-insulin-dependent diabetes or adult-onset diabetes, accounts for 90-95% of the cases of diabetes. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. With an increasingly sedentary lifestyle and availability of high calorie diets, the number of incidents of T2DM is poised to increase to 439 million cases by 2030 [3]. The pathogenesis of T2DM involves three primary defects: insulin resistance, β cell dysfunction and hepatic glucose overproduction. These defects are principal targets of current and future therapy [4].

2 Biological rationale: Target identification

In the early 1990s, it was found that the incretin hormone Glucagon-like peptide-1 (GLP-1) was found to possess anti-hyperglycaemic properties. Nauck et. al showed that continuous intravenous infusion of GLP-1 could normalise blood glucose concentration in experimental subjects [5]. However, sub-cutaneous injections did not show the same efficacy. It was later shown that GLP-1 peptide was rapidly inactivated and degraded in presence of the enzyme Di-peptidyl peptidase IV (DPP IV). These results suggested that pharmacologically manipulating the in-vivo stability of GLP-1 would be a novel approach to treat T2DM.

Two strategies were devised to overcome the inherent drawbacks of instability of the native peptide –

1. Design more stable/DPP IV resistant analogues of GLP-1

2. Develop inhibitors of DPP IV

[1] Medical condition leading to presence of excess glucose in the bloodstream

[2] Medical condition resulting from the damage to peripheral nerves causing pain and numbness in hands and feet

[3] Medical condition leading to loss of vision

[4] Decreased responsiveness of body tissues to secreted insulin

Sub-cutaneous injections of stable GLP-1 analogues – exenatide (marketed as Byetta®)and liraglutide (marketed as Victoza®) have been shown to reduce HbA(1c)[5] and have been approved by US Food and Drug Administration (USFDA). Orally administered DPP IV inhibitor Sitagliptin (marketed as Januvia®) was identified as the lead compound after extensive research of *in-vitro* and *in-vivo* efficacies against DPP IV. Sitagliptin was approved by the USFDA for the treatment of T2DM in 2008.

This paper will delineate the three generations of process modifications undertaken for economic production of Sitagliptin.

3 Sitagliptin phosphate: 3 generations of research

Sitagliptin is the active ingredient in Januvia®, one of the foremost drugs used for the treatment of T2DM. Process chemistry experts Merck have collaborated with homogenous catalysis experts Solvias and biocatalysis experts Codexis to develop a new synthesis strategy for the large-scale manufacture of Sitagliptin phosphate. Figure 2 outlines the three generations of synthesis strategies developed by Merck and its partners.

3.1 First generation process

The initial process chemistry route towards Sitagliptin phosphate (**1**) is outlined in Scheme 1a, figure 2. Starting from the achiral β-keto ester **2**, asymmetry was introduced in the form of a hydroxy group in β-hydroxy acid **3** through a ruthenium catalyzed asymmetric hydrogenation using a modified (S)-Binap[6] catalyst. This was subsequently transformed into the requisite chiral amine center in **4** by using an EDC coupling / Mitsunobu sequence. This reaction has a total of eight steps and gave an overall yield of 52% [7]. This synthesis scheme was used to prepare 100 kgs of Sitagliptin phosphate which was used in early safety and clinical studies.

The first generation synthesis scheme lacked significantly in terms of efficiency. The primary concern being EDC coupling / Mitsunobu sequence which was not only a circuitous method of introducing the chiral amine center, but also generated copious amounts of waste resulting from the poor atom economy inherent to a Mitsunobu reaction.

3.2 Second generation process

Second generation process was developed in order to circumvent the tortuous nature of creating the β-amino acid moiety **5** (refer fig 2) via asymmetric hydrogenation of β-keto acid intermediate **3**. After evaluating the available literature for choosing a synthesis strategy to produce **1** with high enantioselectivity and productivity, Merck finalised on a synthesis strategy to use dehydrositagliptin (**12**) as the starting material. Further, Merck decided to collaborate with Solvias AG, Switzerland, to develop a novel homogenous catalysis synthesis route to produce large-scale quantities of **1** by addressing three main problems –

[5]Glycated haemoglobin

[6](2,2'-bis(diphenylphosphino)-1,1'-binaphthyl) is an organophosphorous catalyst widely used in asymmetric synthesis

1. Develop a short, concise synthesis of dehydrositaglpitin (**12**)

2. Discover a metal catalyst to render high enantioselectivity at low catalyst loadings

3. Develop a robust process to isolate the final product (**1**)

The key feature of the 2nd generation process is a three-step-one-pot synthesis of the enamine amide – dehydrositagliptin (**12**), which contains within its structure the entire skeleton of **1** with two hydrogen atoms in excess. However, at the time of this work, the asymmetric hydrogenation of an unprotected enamine was unprecedented. To explore the feasibility of a one-step conversion of crystallized **12** to **13** via asymmetric hydrogenation, a focused pilot screen of hydrogenation conditions on substrate **12** with a relatively small set of commercially available chiral bisphosphines in combination with Ir, Ru, and Rh salts was performed [8]. Using the screening results, rhodium catalyst was identified to give an excellent enantiomeric excess and high selectivity.

In collaboration with Solvias AG, Switzerland, an exhaustive screening of ligands was carried out and $[Rh(COD)Cl]_2$ gave the highest levels of enantioselectivity. However, an overall consideration of yield, enantioselectivity, reaction rate, and ligand cost led to a decision to pursue the $[Rh(COD)Cl]_2$-^{t}Bu-JOSIPHOS combination to deliver a viable hydrogenation process for the commercial manufacture of sitagliptin [8]. This reaction was performed at 250 psig pressure and 50°C to reduce the catalyst loading without sacrificing yield, enantioselectivity, or reaction rate. This step utilizes 0.15 mol% of the rhodium catalyst, and affords **13** in 98%yield and 95% enantiomeric excess [9].

Highlights of the second-generation synthesis process were –

1. Minimum number of operations (One-pot synthesis followed by asymmetric hydrogenation)

2. Greater overall yield (65%) compared to first generation process (52%)

3. Significant reduction in waste generated (50 kg waste / kg product)when compared to the first generation process (250 kg waste / kg product)

4. Complete elimination of aqueous waste stream

This synthesis was successfully used to launch the product but in spite of the major environmental savings, one significant environmental issue remained. Rhodium is an extremely rare metal that is only present in the earth crust at 4 ppb. This scarcity was also reflected in its price volatility and between early 2006 (when Januvia® was being brought to the market) and July 2008 (before the global recession) the price of rhodium increased three-fold. Hence, for both environmental and financial reasons, Merck started to look at other options [10].

3.3 Improved second generation process

In order to reduce one more step from the previous process, Merck chemists devised an improvement to the second generation process by isolating the crystallized the β-keto amide **11**

and enantio-selectively transforming it into **13** via ruthenium catalyzed asymmetric reductive amination [11]. Although this process eliminated one unit operation from the previous process, the following inherent dis-advantages of the second generation process were still retained –

1. The improved second generation process still relied on a transition-metal mediated catalysis
2. This process necessitated the use of specialized high-pressure equipment
3. Complete removal of transition metal from the product stream proved to be very cost-intensive
4. Low stereocontrol in the process necessitated incorporation of an additional crystallization step

To circumvent the drawbacks of the second generation process, Merck collaborated with the Biocatalysis experts in Codexis Inc., USA to design a highly evolved bio-catalyst to convert **11** to **13** (3rd generation process).

3.4 Third generation process

Merck envisioned that the preparation of Sitagliptin could be further streamlined and made more environment-friendly using the cutting-edge technology of custom-engineering an enzyme to enantio-selectively convert the β-keto amide (**11**) into the product (**13**). Oftentimes, biocatalysis is constrained by shortcomings such as low turnover numbers[7], instability towards the conditions of chemical processes and post-reaction purification problems [12]. In one of the most successful collaborative research in pharmaceutical industry, Merck and Codexis co-developed a protein-engineering approach to develop a transaminase enzyme for enantioselective transamination of β-keto amide.

Not surprisingly, initial screening with a variety of commercially available transaminases failed to identify an enzyme that could reduce **11** to **13** due to steric interference around the ketone. In order to induce transaminase activity with ketoamide (**11**) as the substrate, a "substrate-walking" approach was employed [8]. Briefly,"substrate-walking" approach is used to gradually open up the binding-site of the enzyme through forced enzyme-evolution[8] . A substrate with less steric-hindrance around the ketone group was used as the start-up substrate. Start-up substrate had a methyl group that replaced the tri-fluorophenyl group of **11**. An (R)-selective transaminase ATA-117 effected a modest 4% conversion of the start-up substrate to the corresponding amine [12]. Although the activity of the enzyme was low, this laid the foundation for further evolution and optimization of the enzyme through subsequent mutations and screening [8].

[7]Maximum number of chemical conversions of substrate per second

[8]A protein-engineering tool employing 'directed evolution' to produce mutant enzymes with increased efficiency

In addition to the forced-evolution techniques, molecular modeling was employed to identify residues with active sites that could interact with the tri-fluorophenyl group [12]. Biocatalyst libraries were generated by using iterative directed-evolution[9]. These libraries were screened using stringent high-throughput screening conditions with a goal to identify an enzyme with good productivity of **13**. After eleven rounds of evolution and additional enzyme-optimization, a mutant with 75-fold increase in activity was identified [8]. At this point, optimal co-solvents required for the reaction were identified as DMSO and methanol [12].

Further optimization of the enzyme had to meet three specific targets –

1. Meet process targets (tolerance to solvents DMSO, acetone, iso-propylamine)
2. Stable at elevated temperatures
3. Amenable to expression in *Escherichia coli* (manufacturing host)

Consequently, a chemo-enzymatic strategy was developed as a preferred manufacturing route to Sitagliptin. The new process involves the treatment of β-keto amide (**11**) with iso-propylamine in 50% DMSO in presence of 4.5 wt% of the mutant enzyme at 50°C. This reaction affords 92% assay yield with >99.95% enantiomeric excess [12]. The final product **1** is isolated as a monohydrate phosphate salt with an overall yield of 88%. The process of using a new enzymatic catalysis step for the manufacture of Sitagliptin was approved by the US FDA five years from the start of the work, in April 2012.

4 Conclusion

Sitagliptin phosphate (JANUVIA^{TM}) was approved as the first in-class selective DPP IV inhibitor for the treatment of type-2 diabetes mellitus in October 2006 (approval for the latest synthesis process was granted in April 2012). Sitagliptin phosphate is an important addition to the arsenal of anti-hyperglycaemic drugs available to practitioners [8] and is prescribed in combination with Metformin[10] (JANUMET^{TM}) as the second-line or third-line therapy in patients with mildly elevated A1C[11] levels who do not reach their A1C goal with first-line therapy. The 2014 sales of Januvia & Janumet stood at $4.35 billion.

The evolution of manufacturing process of Sitagliptin is an impressive show-case of Green chemistry, process intensification and industrial asymmetric catalysis and deserves the wide acclaim garnered. The synthetic route to sitagliptin has been re-evaluated and optimized multiple times since the compound entered development. Each successive route has achieved greater efficiency with a reduced environmental impact. The team was not limited by precedented chemistry in their search for an optimal synthesis and, as described above, made several groundbreaking discoveries in the fields of catalytic asymmetric reduction and enzymatic biotransformation.

[9]Method used in protein-engineering that mimics the process of natural selection to evolve proteins or nucleic acids towards a user-defined goal

[10]First-line therapy prescribed for T2DM

[11]Blood test that provides information of blood glucose over past 3 months

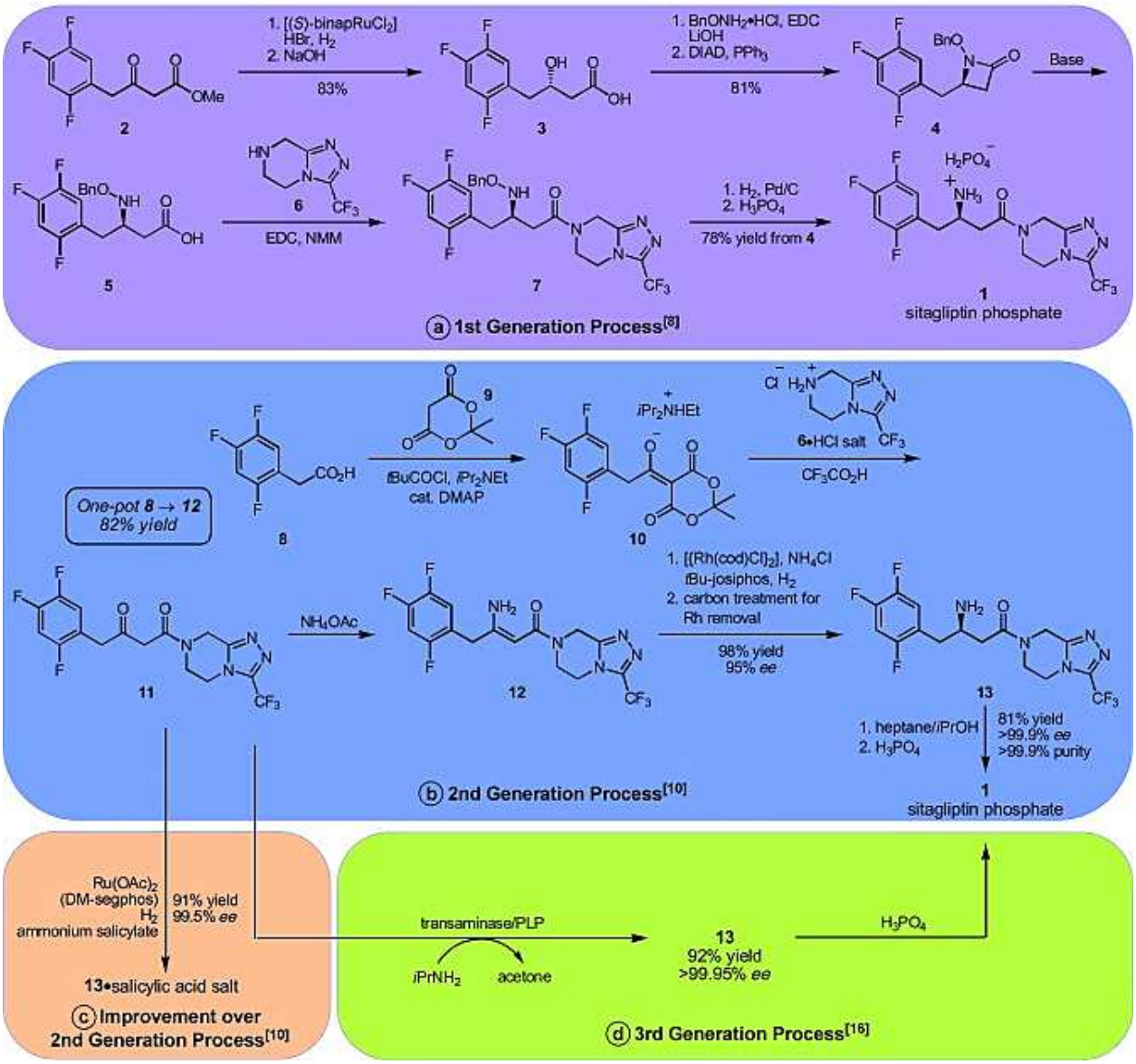

Figure 2: Synthesis scheme of Sitagliptin phosphate

Three generations of process research and development towards the manufacture of sitagliptin phosphate **1**. a) 1st generation process. b) 2nd generation process. c) Improvement upon the 2nd generation process. d) 3rd generation process. Bn=benzyl, binap=2,2-bis(diphenylphosphino)-1,1-binaphthyl, cod=1,5-cyclo-octadiene, DIAD=di-isopropyl azodicarboxylate, DMAP=4-dimethylaminopyridine, NMM=N-methylmorpholine, segphos=(4,4-bi-1,3-benzodioxole)-5,5-diylbis(diphenylphosphine). Figure adapted from [6]

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