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Preparation of Saxagliptin, a Novel DPP-IV Inhibitor

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Abstract:

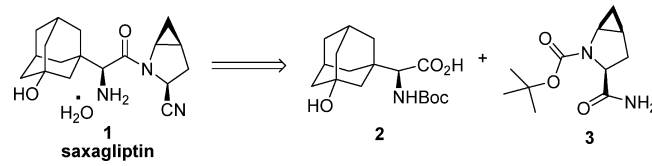
The commercial-scale synthesis of the DPP-IV inhibitor, saxagliptin (1), is described from the two unnatural amino acid derivatives 2 and 3. After the deprotection of 3, the core of 1 is formed by the amide coupling of amino acid 2 and methanoprolinamide 4. Subsequent dehydration of the primary amide and deprotection of the amine affords saxagliptin, 1. While acid salts of saxagliptin have proven to be stable in solution, synthesis of the desired free base monohydrate was challenging due to the thermodynamically favorable conversion of the free amine to the six-membered cyclic amidine 9. Significant process modifications were made late in development to enhance process robustness in preparation for the transition to commercial manufacturing. The impetus and rationale for those changes are explained herein.

Introduction

A recent epidemiological study suggests that the total number of people with diabetes worldwide is expected to double to 366 million by the year 2030.[1] This epidemic has spurred much research into alternative diabetes treatments exploiting the incretin effect, including the inhibition of dipeptidyl peptidase IV (DPP-IV).[2] DPP-IV is the primary enzyme responsible for degradation of incretins, such as glucagon-like peptide-1 (GLP-1), which is a hormone responsible for the glucose-dependent stimulation of insulin in the human body. DPP-IV inhibitors serve as effective glucose regulators primarily by increasing the endogenous concentration of GLP-1. The first of this new class of pharmaceuticals was approved by the FDA in 2006.[3] DPP-IV inhibitors have been clinically proven to lower blood glucose levels, increase glucose tolerance, and improve insulin response in patients with type 2 diabetes mellitus.[2,4] Saxagliptin (Onglyza, Bristol-Myers Squibb, AstraZeneca) is a highly potent, orally available reversible dipeptidyl peptidase IV (DPP-IV) inhibitor recently approved by the FDA for the treatment of type 2 diabetes mellitus.[5]

The initial route was a 15-step, convergent synthesis[5a] having the retrosynthetic plan shown in Scheme 1. Compounds **2** and **3** have been prepared on commercial scale by a number of contract manufacturers.[6] While the strategy of early drug deliveries was rapid syntheses to support preclinical activities and phase I clinical trials, as saxagliptin entered phase II, a greater emphasis was placed on defining and demonstrating a commercially viable synthetic process. Prior to process validation at the commercial manufacturing facility, further optimization was performed to increase process robustness and decrease EHS (environmental health and safety) impact. This report describes the final processes demonstrated during the validation campaign and highlights some of the key considerations during development.

Scheme 1. **Retrosynthetic analysis**



Results and Discussion

Scheme 1 shows the retrosynthetic plan for the preparation of saxagliptin, **1**, from the two proposed regulatory starting materials **2** and **3**. It is readily apparent that the cornerstone of this synthesis is the amide coupling. While many suitable amide- or peptide-coupling protocols are known,[7] our approach had to balance the typical hurdles found in commercial syntheses: API quality, cost of goods, yield, processing time, and process robustness. In addition, a change in the API final form from the benzoate salt to the free base monohydrate **1** required extensive changes to the final step of the synthesis. Due to the thermodynamically favorable cyclization of the free base monohydrate **1** form of the API to amidine **9**, detailed studies of the unit operations of the final step of the synthesis were required to define a robust process.

Deprotection of Methanoprolinamide. Hydrochloric acid was initially chosen for the removal of the Boc group to convert the methanoprolinamide **3** to **4**. The hygroscopic nature of the resulting HCl salt rendered it impractical to handle on larger scales. While many acids proved adequate for this conversion, methanesulfonic acid (MSA) provided a crystalline salt with

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(1) Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047–1053.

(2) (a) Idris, I; Donnelly, R. *Diabetes, Obes. Metab.* **2007**, *9*, 153–165. (b) von Geldern, T. W.; Trevillyan, J. M. *Drug Dev. Res.* **2006**, *67*, 627–642.

(3) FDA News Release P06−169, 2006.

(4) Langley, A. K.; Suffoletta, T. J.; Jennings, H. R. *Pharmacotherapy* **2007**, *27*, 1163–1180.

(5) (a) Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S.-P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hammann, L. G. *J. Med. Chem.* **2005**, *48*, 5025–5037. (b) Bristol-Myers Squibb and Astra Zeneca News Release, July 31st, 2009.

(6) (a) Vu, T. C.; Brzozowski, D. B.; Fox, R.; Godfrey, J. D.; Hanson, R. L.; Kolotuchin, S. V.; Mazzullo, J. A.; Patel, R. N.; Wang, J.; Wong, K.; Yu, J.; Zhu, J.; Magnin, D. R.; Augeri, D. J.; Hamann, L. G. Methods and Compounds Producing Dipeptidyl Petidase IV Inhibitors and Intermediates Thereof. Int. Pub. Num. WO2004052850, 2004. (b) Hanson, R. L.; Goldberg, S. L.; Brzozowski, D. B.; Tully, T. P.; Cazzulino, D.; Parker, W. L.; Lyngberg, O. K.; Vu, T. C.; Wong, M. K.; Patel, R. N. *Adv. Synth. Catal.* **2007**, *349*, 1369–1378.

(7) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827–10852.

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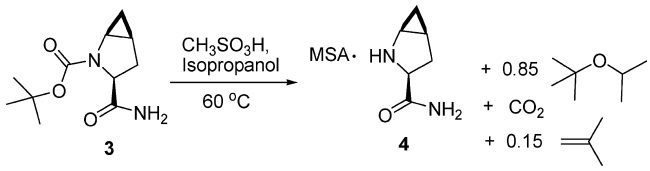
Scheme 2. **Deprotection of methanoprolinamide 3**



3 **4**

excellent physical properties for both isolation and storage (Scheme 2). A broad solvent screen was conducted and revealed that alcoholic solvents afforded the highest-quality MSA salt **4**. Isopropanol was ultimately selected, as it provided high solubility of the starting material **3**, excellent product quality, and an essentially quantitative yield of **4**. Our preference was to utilize nonalcoholic solvents in combination with MSA, to avoid the formation of genotoxic methanesulfonate esters.[8] However, the purification and yield provided by isopropanol outweighed the liabilities of using this combination, as extremely low levels of isopropyl methanesulfonate (IPMS) were observed in the product **4**.[9] In fact, levels of IPMS in **4** prepared on multikilogram (multikg) scales were typically <5 ppm (limit of detection), despite the presence of ~1000 ppm in the filtrate solution. In addition, tolerance studies demonstrated that the coupling process could withstand >1000 ppm of IPMS in MSA salt **4** and still produce **7** with no detectable IMPS found by gas chromatography.

In manufacturing, a mixture of isopropanol and **3** was heated to 60 °C prior to a controlled addition of MSA. This provided a nearly addition-controlled reaction during the MSA charge,[10] resulting in facile control of off-gassing on scale and preventing the isobutylene byproduct from overwhelming the plant's thermal oxidizer. Analysis of the off-gas indicated that the expected 1 equivalent of CO_2 was accompanied by 0.15 equivalent of isobutylene. As a result of the low water content of the isopropanol, no *tert*-butanol was formed, and the majority (0.85 equiv) of the *tert*-butyl cation reacted with isopropanol to form isopropyl *tert*-butyl ether. Due to the extremely low solubility of product **4** in isopropanol, the crystallization consistently initiated prior to the completion of the MSA charge. The poor chromophore of the deprotected product and the heterogeneity of the slurry formed during the reaction made it difficult to use traditional in-process monitoring by HPLC area percent conversion. Thus, the conversion of **3** to **4** was monitored by quantitation of the residual starting material remaining in the supernatant. Upon reaction completion, typically 3 hours at 60 °C, the slurry was cooled to 20 °C and the product isolated on a filter dryer or centrifuge, with isopropanol used as the wash solvent. Using this process, over 1.7 MT (15 batches) has been prepared to date with batch scales up to 205 kg and an average isolated yield of 94%.

Amide Coupling. Commercial-scale amide couplings are widespread in the pharmaceutical and biotechnology industries.[11] The original synthesis of saxagliptin utilized 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBt) for the coupling to form amide **5**. In an effort to identify a lower-cost alternative to EDC, several alternative conditions were evaluated.[12] While carbodiimides and methanesulfonyl chloride proved effective, many other typical coupling reagents failed to yield clean conversions to amide **5**. The first pilot-plant campaign utilized methanesulfonyl chloride (MsCl), where the intermediate mixed anhydride was initially formed as a solution in ethyl acetate in the presence of diisopropylethylamine (DIPEA). Once formed, methanoprolinamide **4** and catalytic HOBt were added to complete the coupling.[6a] Optimization of both solvent selection and workup conditions for this process provided solution yields of **5** in 95−99%.

As saxagliptin proceeded through development, it became apparent that the economics of scale would drive down the cost of EDC into the competitive realm.[13] Therefore, we fully developed both the MsCl and EDC routes for the coupling reaction to allow a complete, comparative evaluation. Ultimately, the exceptional yield and robustness of the EDC/HOBt process outweighed the small impact on the cost of API production and was selected for the commercial route. It is worth noting that the poor chromophores of **2** and **4**, along with the incompatibility of the activated ester to HPLC conditions, required the coupling reaction to be monitored by the increase in the concentration of the product, amide **5**, as opposed to the more typical percent conversion of starting materials to product. The EDC-activated ester of **2** proved to be somewhat unstable when monitored by react-IR and required an equivalent of HOBt hydrate to provide optimal yield. The solution yield of amide **5** was found to be directly proportional to the equivalents of HOBt charged. For example, 0.5 equiv of HOBt generated an approximately 50% solution yield. This instability also prevented the replacement of HOBt with less reactive alternative reagents, such as 5-nitro-2-hydroxypyridine,[14] as the EDC-activated ester decomposed at a faster rate than it reacted with either the HOBt alternative or amine **4**.

Due to the instability of the activated ester of **2**, a general trend during development of the process was that coupling conditions that afforded a faster rate of reaction generally gave higher yields of **5**. All orders of addition were evaluated, and interestingly, it was found that the best process was to charge all four solids (**2**, **4**, EDC, and HOBt) to the reactor prior to the sequential addition of solvent and base. Acetonitrile was found to be the preferred solvent for this reaction and required only 1.1 L/kg of total solids. This small amount of solvent was feasible because the use of 2.2 equivalents of DIPEA reacts to

(8) Glowienke, S.; Frieauff, W.; Allmendinger, T.; Martus, H.; Suter, W.; Mueller, L. *Mutat. Res.* **2005**, *581*, 23–24.

(9) The combined mother liquor and cake washes had <1000 ppm of IPMS on scale.

(10) Lab-scale gas evolution studies showed that no significant gas was evolved until after ~15% of the MSA was added and that, if the MSA addition was spread over 1 h or longer, ~90% of the reaction would occur during the acid charge. This allowed a high level of control over the evolution of carbon dioxide and isobutylene.

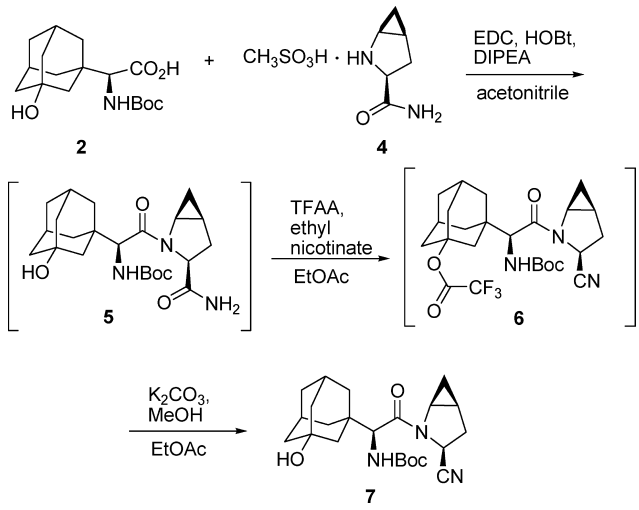
(11) Carey, J. S.; Laffan, D.; Thomson, C.; Williams, M. T. *Org. Biol. Chem.* **2006**, *4*, 2337–2347.

(12) Carbodiimides and sulfonyl chlorides (mesyl and tosyl) were the only two groups of coupling agents found suitable for this conversion. Examples of failed reagents are: chloroformates, piv chloride, $POCl_3$−DMF, 2-chloro-4,6-dimethoxy-1,3,5-triazine, *N*,*N*′-carbonyldiimidazole. Specifically, mixed anhydrides suffered from nonselective reactivity with amine **4**.

(13) While kilogram quantities typically cost ~$2000/kg, manufacturing-scale quantities typically run an order of magnitude less at $100 to $200 per kg.

(14) Dunn, P. J.; Hoffmann, W.; Kang, Y.; Mitchell, J. C.; Snowden, M. J. *Org. Process Res. Dev.* **2005**, *9*, 956–961.

Scheme 3



generate the free amines of **4** and EDC, which are both oils under these conditions. Even at scales >100 kg, the solids rapidly dissolve during the DIPEA addition and are completely in solution within minutes of the addition. While the sequential addition of acetonitrile and DIPEA was demonstrated multiple times on approximately 100 kg scale, the following, more robust process was ultimately developed. A mixture of DIPEA, acetonitrile (3 L/kg of **2**), and a small amount of EtOAc (1 L/kg), which provides a homogeneous solution, was added to the four solids. Since **2** and EDC react immediately upon addition of solvent, premixing the acetonitrile, ethyl acetate, and DIPEA allowed for immediate dissolution of HOBt and the MSA salt **4**, thereby precluding degradation of the EDC activated ester prior to the DIPEA charge. After addition of the DIPEA solution, the coupling reaction was >90% complete after approximately 30 min. Aging the reaction for a total of 2−3 h gave consistent solution yields of >95% of amide **5**. Typical yield losses during the subsequent aqueous work up were <5%.

Nitrile Formation. Amide **5** is an amorphous glass which required telescoping the coupling process leading to **5** into the subsequent dehydration step to form the corresponding nitrile **7** (Scheme 3). After the coupling reaction, ethyl acetate was added to allow an acidic aqueous workup to remove the basic byproducts of the process. Not surprisingly, the residual HOBt remaining in the organics reacts with the trifluoroacetic anhydride (TFAA) used for the amide dehydration, forming the corresponding trifluoroacetate triazole. While this species is still an effective dehydrating agent, it proved to have a higher affinity toward acylation of the adamantane alcohol. This expected side reaction made complete conversion of the amide to nitrile **6** difficult in the presence of large amounts of residual HOBt. Taking advantage of the acidic nature of HOBt, two aqueous potassium bicarbonate washes were utilized to remove 90% of the HOBt used for the coupling reaction.[15] This process modification eliminated the issue of incomplete amide dehydration.

(15) Potassium bicarbonate allowed for higher concentrations of salts than sodium bicarbonate and therefore reduced product loss to the aqueous layer. Though potassium carbonate washes were also effective, they were not chosen to avoid the possibility of epimerization during the subsequent distillation.

Initially, trifluoroacetic anhydride and pyridine were the preferred reagents for this reaction. Pyridine played a dual role as both an initial activating agent for the trifluoroacetic anhydride and, subsequently, as a base for the trifluoroacetic acid byproduct. The reaction was quite robust using these reagents and easily converted >99.5% of amide **5** to the corresponding nitrile **6** at pilot-plant scales of >100 kg. However, due to concerns over the odor and toxicity of pyridine at manufacturing scale, we investigated alternative bases. The identification of a direct substitute base was not as straightforward as initially expected, especially as pyridine participates in the mechanism as an activating agent.[16] Table 1 summarizes a number of bases, or classes of base, that were evaluated and rejected on the basis of deficiencies in reactivity or general environmental, health, and safety concerns. Typical tertiary alkyl amines (DIPEA, triethylamine, *N*-methylmorpholine, etc.) are oxidized by trifluoroacetic anhydride via a hydrogen transfer mechanism, with generation of the toxic byproduct fluoral.[17] In addition, 2- and 4-alkyl pyridines are reactive with trifluoroacetic anhydride.[18] One of the more interesting replacements considered was potassium trifluoroacetate, as this salt has been shown to form a dimer with trifluoracetic acid.[19] An initial hypothesis was that this phenomenon might allow potassium trifluoroacetate to replace pyridine by acting as a buffer for the trifluoroacetic acid generated during the dehydration. Unfortunately, this system had no tolerance for the slight excess of TFAA, 10 mol %, required for complete conversion of the amide and resulted in acylation of the carbamate nitrogen and loss of the Boc protecting group. While numerous organic and inorganic bases were considered and screened, 3- and 5-substituted pyridines were the only class of bases that satisfied reactivity requirements. Both 3-methoxypyridine and ethyl nicotinate were found to be effective replacements for pyridine, but ethyl nicotinate was chosen for its superior toxicity profile[20] (very limited toxicity data were available for 3-methoxypyridine) and nonoffending odor.

Because of its lack of nucleophilicity, ethyl nicotinate appears to have no reactivity with trifluoroacetic anhydride by ^{1}H NMR and does not play a role as an activating agent in the dehydration of the amide. With TFAA as the dehydrating reagent (as opposed to the TFAA−pyridine complex formed in the original process) the typical conversion of amide **5** to nitrile **6** was approximately 98%. Even up to 2.3 equiv of TFAA did not drive the reaction to complete conversion, and larger amounts were not compatible with the Boc protected amine. The switch from pyridine to ethyl nicotinate resulted in an approximately 2% yield loss due to lower conversion, but ethyl nicotinate is far more friendly from an EHS perspective.

The apparently simple change from pyridine to ethyl nicotinate required an extensive change in the workup. Pilot-plant batches utilizing pyridine only required a single water

(16) Anthoni, U.; Christensen, D.; Christophersen, C.; Nielsen, P. H. *Acta Chem. Scand.* **1995**, *49*, 203–6.
(17) Schreiber, S. L. *Tetrahedron Lett.* **1980**, *21*, 1027.
(18) Kiwase, M.; Teshima, M.; Saito, S.; Tani, S. *Heterocycles* **1998**, *48*, 2103.
(19) Clark, J. H.; Emsley, J. *J. Chem. Soc., Dalton Trans.* **1974**, *11*, 1125–9.
(20) LD 50: >2005 mg/kg (rat), cutaneous irritation: nonirritant (rabbit), ocular irritation: nonirritant (rabbit). See the MSDS at Seppic (Seppic, Inc., Fairfield, NJ).

Table 1. **Pyridine alternatives for dehydration reaction**

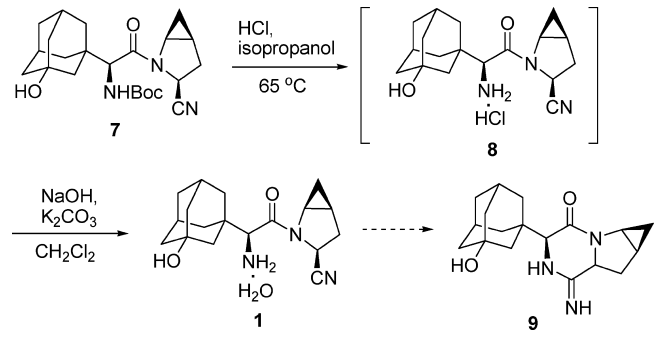
base	successful dehydration	TFAA compatible	comments
tertiary amines	yes	no	redox reaction with TFAA[17]
potassium trifloroacetate	yes	yes	no tolerance of excess TFAA
phosphates	no	yes	heterogeneous conditions
2- or 4-alkyl pyridines	na	no	acylation of alkyl pyridine
3- or 3,5-alkyl pyridines	yes	yes	odor concerns
2,2′-bipyridine	NA	yes	mutagen
quinoline	NA	yes	mutagen
dimethylaminopyridine	no	yes	low reaction conversion
3-acetoxypyridine	no	yes	poor reaction conversion
3-cyanopyridine	no	yes	poor reaction conversion
3-methoxypyridine	yes	yes	some lots had strong odor
ethyl nicotinate	yes	yes	excellent toxicity data

wash with a small hydrochloric acid charge to protonate the excess pyridine. Since the trifluoroacetic acid salt of pyridine has a favorable partition coefficient between water and ethyl acetate, >90% of the pyridine was removed by this single aqueous wash. The trifluoroacetic acid salt of ethyl nicotinate proved to be more difficult to remove, as it is freely soluble in ethyl acetate. We decided that the best approach would be to first remove the TFA byproduct with another base. A salt screen of inorganic bases and tertiary amines with higher pK_a's than that of ethyl nicotinate revealed that tetramethylethylenediamine (TMEDA) forms a water-soluble bis-TFA salt that is completely insoluble in ethyl acetate. As a result, the final procedure for the ethyl nicotinate process incorporated a water and TMEDA charge after the dehydration reaction and a subsequent phase split. Hydrochloric acid (2 N) was then charged to extract the corresponding ethyl nicotinate hydrochloride salt, which possesses a far more favorable partition coefficient between water and ethyl acetate than its TFA analogue. This low pH wash (pH 2−2.5) effectively removed approximately 90% of the ethyl nicotinate while avoiding loss of the Boc protecting group.

The pyridine and ethyl nicotinate processes both resulted in trifluoroacetylation of the adamantane alcohol, requiring a subsequent hydrolysis. To avoid any possible racemization of the nitrile-bearing stereogenic center,[21] aqueous potassium carbonate was utilized for the hydrolysis of **6** to alcohol **7**. A small amount of methanol was added to improve the phase transfer of the 25 wt % potassium carbonate into the ethyl acetate layer. After approximately 1 h at 40 °C, the hydrolysis was complete, and there was no detectable epimerization. The reaction was then cooled to 15 °C in preparation for the subsequent phase split. While an ethyl acetate/heptane crystallization was feasible, an isopropanol/water crystallization proved to be far superior at removing a number of polar byproducts formed in this three-step telescope as well as trace impurities present in the two starting materials. After an efficient solvent exchange from ethyl acetate to isopropanol, water was added to initiate crystallization and to adjust to a final solvent composition of approximately 75% water and 25% isopropanol. The average overall yield for the conversion of **2** to **7** using the ethyl nicotinate-based dehydration on scale-up to 280 kg was 78%, and the purity was >99.7%, with the only significant impurity being ∼0.15% of the intermediate amide **5**. To date,

(21) Epimerization of the nitrile stereogenic center was observed at pH >12 under warm, alcoholic conditions.

Scheme 4



10 batches and over 1.2 MT have been prepared with the described process.

Deprotection of Primary Amine. The final step in the preparation of the drug substance was the deprotection of **7** (Scheme 4). As expected, many acids facilitated this Boc deprotection. In addition to providing a clean, fast reaction, hydrochloric acid was ultimately chosen due to the preferential characteristics of the chloride salts formed upon neutralization. Both potassium and sodium chloride were easily purged, and just as importantly, these helped to lower the concentration of **1** in the aqueous waste by increasing the ionic strength of the aqueous phase. A solvent screen showed that both acetonitrile and isopropanol gave exceptionally clean reaction profiles, but the ICH Q3C class 2 status of acetonitrile made the selection of isopropanol obvious. Somewhat surprisingly, the use of methanol gave small amounts of the corresponding carboxylic acid, presumably due to imidate formation and subsequent hydrolysis. Initial pilot-plant campaigns used 2 L of isopropanol per kilogram of **7** and 1.4 equiv of 2 N HCl. A final process improvement was to switch to a mixture of 1 L of isopropanol and 1 L of water per kg of **7**, in conjunction with using concentrated HCl. The reduction in isopropanol was important, as it reduced the amount of water in the organic phase after extraction of the free amine **1**. This was critical in reducing the cycle time of the subsequent drying distillation, and thus limited cyclic amidine formation. HCl salt **8** was quite stable under the acidic deprotection conditions of 60 to 70 °C, as the product is much less prone to cyclization under acidic conditions. Similar to the synthesis of methanoproline **4**, concentrated hydrochloric acid (1.4 equiv) was added to a 65 °C solution of **7** to allow nearly addition control of gas evolution. In addition to the standard one equivalent of CO_2, this process formed 0.4 equiv of isobutylene and 0.6 equiv of *tert*-butanol. Unlike the

deprotection of **3**, the presence of water in this reaction prevented the formation of any detectable isopropyl *tert*-butyl ether. At the end of the reaction, additional water was added at 65 °C to prevent formation of an unstirrable slurry of the HCl salt **8** upon cooling.

Monohydrate Formation and Crystallization. Early clinical deliveries of the active drug were of the benzoate salt. A change in the final form was required due to formulation requirements. The free base monohydrate **1** was selected as the preferred form for commercial development. While **1** is stable as a solid, handling it in solution was initially challenging due to the more facile conversion of the free base to the cyclic amidine **9**. Detailed process-enabling kinetic studies to understand and control this undesired conversion were performed and will be the topic of a future publication.

Concentrated sodium hydroxide (50 wt %) was utilized to neutralize the first equivalent of hydrochloric acid. The final pH adjustment was carried out using 25 wt % potassium carbonate to allow a buffered titration of the remaining 0.4 equiv of HCl. Both of these base additions were carried out in the presence of methylene chloride. Despite the general undesirability of using an ICH Q3C class 2 solvent in an API step, methylene chloride was chosen as it was the only process-friendly solvent identified to efficiently extract **1** from an aqueous solution. In addition, the product is quite stable in this solvent and tolerates distillation under atmospheric conditions. As has been made apparent by the use of concentrated acids and bases, minimal water is used for this process to minimize product loss to the aqueous layer.[22] In addition, 1.25 kg of sodium chloride per kg of starting material was added to further reduce losses in the extraction and to reduce the amount of water present in the organic extract.

The methylene chloride solution of **1** was distilled under atmospheric conditions to remove water down to <0.4 equiv. This prevented crystallization of monohydrate **1** upon subsequent addition of ethyl acetate. This operation carries the greatest risk of forming the undesired cyclic amidine **9**. The small change in reaction solvent mentioned earlier, using 1 L of IPA per kilogram (kg) of **7** rather than 2 L per kg, had a profound effect on this distillation. The reduction in isopropanol lowered the initial water content of the organic phase enough to cut the methylene chloride consumption by approximately 50% and significantly reduced the cycle time and thus the formation of cyclic amidine **9**. After a polish filtration to remove residual solid salts, a controlled water addition was performed to induce crystallization of the monohydrate **1**. A constant volume distillation, charging fresh ethyl acetate at approximately the distillation rate, was utilized to complete the solvent switch from methylene chloride to ethyl acetate. While this type of distillation is known to reduce solvent usage,[23] it was chosen here primarily to minimize the adherence of solids to the walls of the reactor during the distillation and continued crystallization. Although this is an atypical crystallization protocol for an API, the nature of the formulation allowed for a broad range of acceptable particle sizes. A small amount of water was added postdistillation to ensure enough water was present to achieve

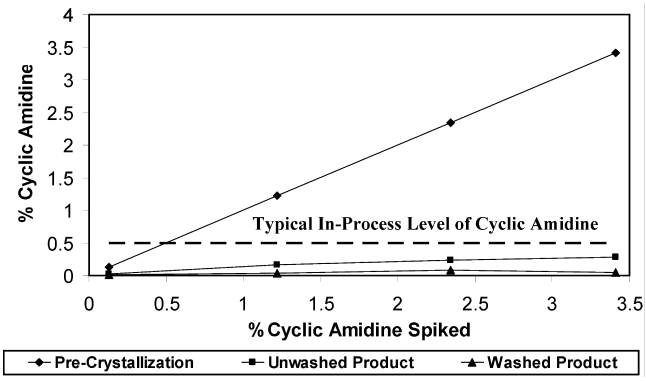


Figure 1. **Tolerance study of cyclic amidine impurity in the crystallization and cake wash.**

the target 1.5−2.0% water. In addition to assuring proper hydration of the product, the addition of water enhanced removal of the ~0.4% cyclic amidine **9** formed during the process, and minimized yield losses. For these same reasons, the cake wash was composed of 2% water in ethyl acetate. Figure 1 displays results of a tolerance study in which various amounts of the cyclic amidine impurity **9** was spiked into precrystallization process streams in order to test the ability of the crystallization and cake wash to reject this impurity. As demonstrated in the graph, the crystallization readily purges that impurity at levels above 3%, as compared to a typical in-process level of 0.5%. The typical yield for this process on scale was 86−90%, with >99.9% purity and <0.1% cyclic amidine. This final procedure was significantly greener due to waste reduction (70% less in total waste, 60% less methylene chloride) and yield improvement compared with the original synthesis of the monohydrate.

Drying the Monohydrate. Drying of organic hydrates has been discussed in the literature,[24] and in this case drying of the monohydrate free base **1** required an in-depth understanding of the thermodynamics in order to avoid formation of a known, undesired anhydrous crystalline form. To avoid concerns over the kinetics of form conversions, relative humidity conditions were established under which the desired monohydrate was the thermodynamically preferred crystal form. The relative humidity required to maintain the monohydrate was primarily established by monitoring the form conversion of a physical mixture of the monohydrate and anhydrous forms in slurries with varying water content or activity. Those experiments were supplemented by dynamic vapor sorption (DVS), drying experiments, and solubility measurement of both forms as a function of water content. All of these investigations suggested that the relative humidity should be maintained above approximately 4.5% in order to preserve the monohydrate indefinitely. For operational reasons, a decision was made to monitor the dew point rather than the relative humidity during drying. At the 40 °C dryer jacket temperature, 4.5% relative humidity corresponds to a dew point of approximately −8 °C. Several options for providing the required humidity were considered. Because the azeotrope between water and ethyl acetate (~3.6 wt % water at 25 mmHg,

(22) The water solubility of the monohydrate is 19 mg/mL.
(23) Gentilcore, M. J. *Chem. Eng. Proc.* **2002**, 56–59.
(24) (a) Cypes, S. H.; Wenslow, R. M.; Thomas, S. M.; Chen, A. M.; Dorwart, J. G.; Corte, J. R.; Kaba, M. *Org. Process Res. Dev.* **2004**, *8*, 576–582. (b) Variankaval, N.; Lee, C.; Xu, J; Calabria, R.; Tsou, N.; Ball, R. *Org. Process Res. Dev.* **2007**, *11*, 229–236.

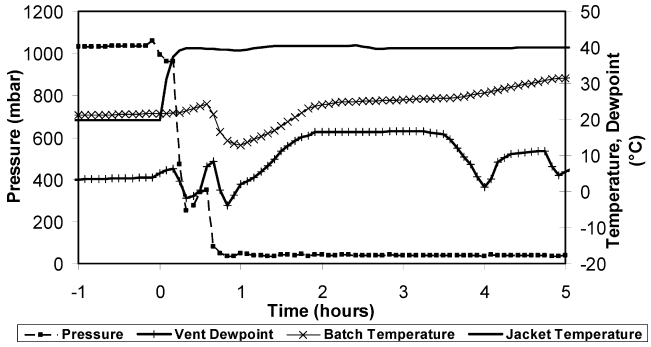


Figure 2. **Pilot-plant drying trends.**

6.3 wt % water at 250 mmHg)[25] is greater than the solubility of water in ethyl acetate (3.3 wt %) a water-wet ethyl acetate cake wash alone does not provide sufficient excess water to allow for a robust drying process across multiple pressures. Although a humid nitrogen sweep did provide the requisite humidity, this approach required the use of a portable reactor and was ultimately replaced with the following simpler approach. After the cake was washed with 2 wt % water in ethyl acetate and deliquored, a small addition of water (0.04 kg/kg **1**) was charged to the agitated wetcake just prior to drying. A key to the success of this procedure was that the addition of water raised the concentration beyond the azeotropic composition during vacuum drying. This promoted the preferential removal of ethyl acetate during drying and allowed for a robust drying process on pilot- and manufacturing scale that consistently delivered the desired monohydrate. The low solubility of **1** in water also made this operation feasible, without concern of changing the physical properties of the wet cake.

The dew point in the dryer vent line and the cake temperature provided insight into the drying process on scale. Figure 1 shows the trends of a pilot-plant batch that was dried in a filter dryer with 40 °C on the jacket and 40 mbar pressure. Evaporative cooling initially lowered the cake temperature as ethyl acetate was rapidly removed from the powder. As the ethyl acetate was depleted, the cake warmed until it essentially reached water's boiling point under these conditions. The dew point followed a similar trend since water was driven from the cake as it warmed. The plateaus in batch temperature and dew point represent the portion of drying where the excess water was being removed from the cake. Once the surface water was dried from the cake, the batch temperature then rose and the dew point fell, giving independent confirmation that the dryer was ready to be sampled for analytical verification of drying completeness. In this example, the dew point fell precipitously once the excess water was removed from the cake. This occurred because there was a slight nitrogen sweep into the headspace of the dryer, corresponding to about 1 dryer volume turnover every 30 min. In the absence of such a sweep, or similar leak from the atmosphere, the dew point above the dry cake will remain at roughly the same value as during the water drying period. As demonstrated in Figure 2, this approach ensured adequate humidity, well beyond the thermodynamic limit for form conversion, and ensured the preservation of the desired monohydrate while maintaining an acceptable overall drying cycle time.

(25) Horsley, L. H. *Azeotropic Data III*; Advances in Chemistry Series, 116, American Chemical Society: Washington D.C., 1973; p 21.

Conclusions

From the purchased unnatural amino acid derivatives **2** and **3**, the synthesis of the DPP-IV inhibitor saxagliptin involves five chemical transformations and three isolations. Significant hurdles, such as analytical challenges due to poor UV chromophores and a thermodynamically favorable cyclization, were overcome to provide a robust and efficient synthesis of saxagliptin. In addition, vital changes were made for environmental and safety concerns, including replacement of pyridine with the more benign base, ethyl nicotinate. The key step in this convergent synthesis is the amide coupling, which links the adamantane and proline-based cores. In the optimized commercial process, the overall yield from the proposed starting materials is approximately 65% and represents a significant improvement over earlier process versions. The safe implementation and robustness of the processes have been validated by multiple process demonstrations in both pilot and manufacturing facilities. Specifically, nine batches (>600 kg) of saxagliptin, **1**, have been prepared by the described processes, all having >99.9% purity, and the correct monohydrate form.

Experimental Section

L-*cis*-4,5-Methanoprolineamide Methanesulfonic Acid Salt (4). Isopropyl alcohol (808 kg) and **3** (135 kg, 600 mol) were charged to a glass-lined reactor. An additional isopropyl alcohol charge (39 kg) was added via "spray balls" to remove solids from the walls. The slurry was then heated to 60 °C, where most if not all of the solids dissolved. Methanesulfonic acid (74.54 kg, 776 mol) was charged while maintaining the temperature between 55 and 65 °C. This continuous feed was spread over 30 min to control gas evolution. Isopropanol (10 kg) was used to rinse the methanesulfonic acid charge lines. The product began to crystallize out of solution at this point. The reaction was held at 60 °C for 3 h. The reaction was sampled to ensure there was <3 mg/mL of **3** remaining. The slurry was cooled to 20 °C over one hour and held for an additional hour. The product was then filtered using four loads on a centrifuge. Each cake load was washed with isopropanol (50 kg). The solids were dried at 50 °C in a conical dryer to give **4** as an off-white product (124 kg, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.67 (s, 1H), 4.46 (dd, J_1 = 7.8 Hz, J_2 = 3.0 Hz, 1H), 3.31 (ddd, J_1 = 7.0 Hz, J_2 = 6.0 Hz, J_3 = 2.5 Hz, 1H), 2.62−2.52 (m, 1H), 2.38 (s, 3H), 2.12 (dd, J_1 = 10.9 Hz, J_2 = 2.8 Hz, 1H), 1.75 (ddd, J_1 = 10.9 Hz, J_2 = 9.1 Hz, J_3 = 5.3 Hz, 1H), 0.84 (m, 1H), 0.61 (ddd, J_1 = 7.3 Hz, J_2 = 5.0 Hz, J_3 = 2.5 Hz, 1H) ^{13}C NMR (100 MHz, MeOD) δ 171.43, 60.33, 38.17, 36.61, 31.58, 17.05, 8.84. Analysis Calculated for $C_7H_{14}N_2O_5S$: C, 35.29; H, 5.92; N, 11.76; S, 13.46. Found: C, 35.12; H, 5.86; N, 11.68; S, 13.55.

(*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinamide (5). Acetonitrile (318 kg), *N*,*N*-diisopropylethylamine (118 kg, 913 mol), and ethyl acetate (122 kg) were charged to a glass-lined reactor to prepare a homogeneous solution. To a separate glass-lined reactor were charged **2** (135

kg, 415 mol), **4** (97 kg, 436 mol), HOBt (62 kg, 405 mol), and EDC (87 kg, 454 mol). The DIPEA solution was then added to the solids, followed by an ethyl acetate rinse (45 kg). The jacket of the reactor was maintained at 25 °C during and post addition, allowing the exotherm of the base addition to take the reaction to ~40 °C. The resulting solution was held for 3 h. Ethyl acetate (1094 kg), 2 N hydrochloric acid (217 kg, 415 mol), water (208 kg), and brine (483 kg) were charged and allowed to agitate for 20 min. After settling for 30 min, the lower aqueous layer was removed (1162 kg). Potassium bicarbonate solution (20 wt %, 770 kg) was charged and stirred for 20 min. After settling for 30 min, the lower aqueous layer was removed. The potassium bicarbonate wash was repeated, and the aqueous waste was combined (1750 kg). A sample was taken for HPLC analysis to determine the solution yield of **5** (373 mol, 90%). The jacket of the reactor was set to 90 °C, and vacuum was pulled to ~350 mbar. The ethyl acetate solution was distilled down to ~540 L. Once the target volume was reached, ethyl acetate (1500 kg) was charged at a rate such that the volume in the reactor remained constant. At the end of the distillation, the solution was diluted with an additional ethyl acetate (973 kg) charge. A Karl Fisher test was performed to ensure the water content of the organics was <0.05% and was suitable for proceeding to the subsequent dehydration reaction. Analytical data were consistent with previously reported data.[5]

(*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (7). Ethyl nicotinate (198 kg, 1.3 kmol) was charged to the dry solution of **6** above and was cooled to −10 °C. Trifluoroacetic anhydride (165 kg, 788 mol) was added over 30 min to keep the temperature of the reaction <10 °C. After holding for 30 min, a sample was taken to confirm reaction completion by HPLC. Water (945 kg) and tetramethylethylenediamine (69 kg, 590 mol) were charged. The heterogeneous solution was then warmed to 15 to 20 °C and allowed to separate. The lower aqueous layer (1110 kg) was removed and the organics were recooled to 5 °C. Hydrochloric acid (2 N, 683 kg, 1.3 kmol) was added slowly to keep the batch temperature <10 °C. The mixture was agitated for 20 min prior to allowing the layers to separate for 1 h. The bottom aqueous layer (1067 kg) was removed. Aqueous potassium carbonate (25 wt %, 688 kg, 1.2 kmol) was then charged, followed by methanol (213 kg). The mixture was warmed to 40 °C and held for 1 h. Once the reaction was determined complete by HPLC, the reactor was cooled to 15 °C. After settling for 30 min, the bottom aqueous layer (606 kg) was removed. Hydrochloric acid (2 N, 6 kg) was added to adjust the pH to 6−8 and brine (483 kg) was added. After settling for 30 min, the bottom aqueous layer (600 kg) was removed. The reactor jacket was set to 80 °C and pressure to 300 mbar. The organics were distilled down to 540 L. Once the target volume was reached, isopropanol (810 kg) was added at a rate equal to the distillation rate. A sample for GC analysis was taken to ensure that the ratio of isopropanol to ethyl acetate was >95:5 wt %. The solution was then further concentrated to 450 L, keeping the temperature 35−50 °C. Once the target volume was reached, the vacuum distillation was stopped and water (300 kg) was added at 40−50 °C. The batch was slowly cooled to 40 °C, and **7** (1.4 kg) was added to initiate nucleation. Water (600 kg) was added continuously over 2 h, maintaining a batch temperature of 40 °C. The resulting slurry was cooled to 15 °C and held for 1 h. The batch was filtered on a filter dryer and washed with a 75% water, 25% isopropanol mixture (2 × 250 kg). The solids were dried at 50 °C to give **7** as a white solid (134 kg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$-d_6) δ 5.30 (d, J = 9.9 Hz, 1H), 5.02 (dd, J_1 = 10.6 Hz, J_2 = 2.3 Hz, 1H), 4.44 (d, J = 9.9 Hz, 1H), 3.86−3.79 (m, 1H), 2.55 (ddd, J_1 = 16.4 Hz, J_2 = 10.6 Hz, J_3 = 5.8 Hz, 1H), 2.35 (dd, J_1 = 13.6 Hz, J_2 = 2.3 Hz, 1H), 2.26−2.20 (m, 2H), 1.87 (ddd, J_1 = 13.2 Hz, J_2 = 6.5 Hz, J_3 = 6.5 Hz, 1H), 1.77 (dt, J_1 = 11.6 Hz, J_2 = 2.3 Hz, 1H) 1.75−1.42 (m, 12H), 1.41 (s, 9H), 1.05 (dd, J_1 = 6.8 Hz, J_2 = 4.3 Hz, 2H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.99, 155.84, 119.28, 80.00, 68.61, 58.66, 46.32, 45.12, 44.42, 44.31, 41.22, 38.04, 37.56, 37.06, 35.22, 30.51, 30.25, 30.23, 28.39, 17.83, 13.57. MS (FAB) *m*/*z* 416 $[M + H]^+$.

(*S*)-3-Hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (1). To a glass-lined reactor was charged **7** (82.5 kg, 199 mol), isopropanol (64.8 kg), water (82.5 kg), and 37% hydrochloric acid (3.9 kg, 39.7 mol). The batch was heated to 65 °C, and hydrochloric acid (23.5 kg, 238 mol) was added over 30 min, followed by a water flush (16.5 kg). After 90 min at 65 °C, water (165 kg) was charged, and an HPLC sample was taken to determine reaction completion. The batch was cooled to 25 °C, and methylene chloride (660 kg) was added. Sodium hydroxide (10 N, 24.8 kg, 199 mol) was charged with a water (16.5 kg) line flush. Potassium carbonate (24 wt %, 43.9 kg, 79 mol) was added to adjust to pH 9, followed by a water flush (16.5 kg). Sodium chloride (103 kg, 1.8 kmol) was charged and allowed to agitate for 30 min. The layers were allowed to separate, and the lower organic layer was transferred to a clean, glass-lined reactor. An atmospheric distillation was performed to reduce the total volume to 250 L. Ethyl acetate (74 kg) was added, and the solution was filtered through a 1 μm line filter. Ethyl acetate (74 kg) was used to chase the reactor and transfer lines. Water (2.2 kg) was then added, and the solution was aged for 30 min to induce crystallization. Another water charge (2.2 kg) was performed, followed by an additional 20 min age. Water (17.5 kg) was charged, and the pressure was set to 300 mbar. The jacket of the reactor was set to 60 °C, and distillation was initiated at 20 °C. Ethyl acetate (520 kg) was charged at a rate to keep the volume in the reactor constant during the distillation. The pressure was reduced periodically during the distillation to maintain the target 15−30 °C batch temperature. The final pressure set point was 100 mbar. After completion, water (6.2 kg) was charged, and the batch was cooled to 5 °C. The slurry was filtered on a filter dryer and washed with wet ethyl acetate (6.2 kg water, 148 kg EtOAc). The monohydrate **1** was dried at 40 °C. The dew point in the dryer was monitored by a dew point hygrometer, Vaisala HMP360 series probe. A small water charge (2.8 kg) was added during drying to ensure that the dew point in the dryer remained >−8 °C. After the cake temperature had risen above 20 °C, a dry nitrogen purge equivalent to two dryer volume turnovers per hour was initiated. The nitrogen purge primarily served to clear the dryer headspace of water vapor following removal of surface water from the drug substance. By this mechanism the dew point measurement dropped precipitously once the cake

was dry, and served as an independent signal to the cake temperature that the cake was dry. Monohydrate **1** was isolated as a white solid (58.2 kg, 88%). ^{1}H NMR (400 MHz, CD_2Cl_2-d_6) δ 5.25 (dd, $J_1 = J_2 = 1.0$ Hz, 1H), 4.93 (dd, $J_1 = 10.6$ Hz, $J_2 = 2.3$ Hz, 1H), 3.55−3.50 (m, 1H), 3,35 (s, 1H), 2.45 (ddd, $J_1 = 16.1$ Hz, $J_2 = 10.9$ Hz, $J_3 = 5.6$ Hz, 1H), 2.25 (dd, $J_1 = 13.6$ Hz, $J_2 = 2.5$ Hz, 1H), 2.18−2.10 (m, 2H), 1.83−1.42 (m, 15H), 1.40−1.27 (m, 3H) 1.0−0.87 (m, 2H) ^{13}C NMR (100 MHz, CD_2Cl_2) δ 173.43, 120.15, 68.83, 60.90, 46.57, 45.51, 45.08, 45.01, 41.62, 38.15, 37.92, 37.35, 35.88, 30.98, 30.93, 30.80, 18.00, 13.69. MS (FAB) *m*/*z* 316 $[M + H]^+$.

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