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Route Selection and Process Development for a 5-Piperazinylquinaldine Derivative for the Treatment of Depression and Anxiety

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ABSTRACT: 1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone, **1**, was identified as a potential drug for the treatment of depression and anxiety. Herein is described the work carried out to select the manufacturing route and the process research studies to optimize the key stages of route B. A particular focus is given to the genotoxic impurities, related to this route, as one of the intermediates of the manufacturing route was genotoxic and many genotoxic impurities can be formed in the process. Quality by Design principles were applied for the definition of the control strategy of these impurities.

1. INTRODUCTION

A new inhibitor of serotonin reuptake was recently identified as antidepressant.[1] 1-(3-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}phenyl)-2-imidazolidinone, **1**, is a presynaptic inhibitor 5-HT_1 receptor antagonist selected for the cure of depression and anxiety that reached early phase II trials.[2]

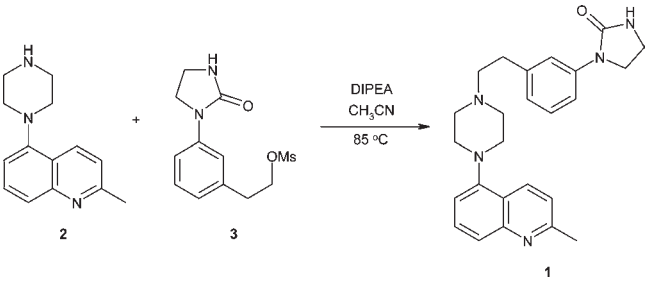
Herein is described the work carried out to select the manufacturing route and the process research studies to optimize stages 1 and 2 of this route (route B, see Scheme 7). A particular focus is given to the genotoxic impurities, related to this route, as one of the intermediates of the manufacturing route was genotoxic and many genotoxic impurities (see Table 1) can be formed in the process.

The principles of Quality by Design[3] have been applied to define the control strategy of these impurities. The concept of the control strategy was introduced in the ICH Q10 and consists of a set of controls, derived from current product and process understanding that assures process performance for obtaining drug substance that meets the Critical Quality Attributes (the measurable properties that are critical to ensuring patient safety and efficacy).

2. EARLY PROCESS

The early process to synthesise the 1-aryl-2-imidazolidinone **1** is depicted in Scheme 1[2] and allowed to produce the first batches of drug substance for toxicological use and to support the first time in human (FTIH) studies. In particular, the last stage of the Medicinal Chemistry synthesis involved the alkylation of 5-piperazinylquinaldine **2** with the mesylate **3** (Scheme 1). According to the GSK internal risk assessment process for genotoxins, the mesylate **3** is a structure of concern, proved to be mutagenic in the Ames test,[4] and therefore had to be controlled at a level below the threshold of toxicological concern (TTC, 1.5 μg/day) as at the time that these activities were undertaken the staged TTC had not yet been introduced, a default TTC approach was undertaken.[5]

Scheme 1. Early process for 1-aryl-2-imidazolidinone 1



The high level of the genotoxic mesylate **3** (>150 ppm) in the FTIH batch compelled us to purify it in order to meet the required specifications (in absence of a defined dose, 150 mg/day was considered, therefore an initial target specification limit of 10 ppm in the drug substance was adopted) as this mutagen had to be controlled down to an acceptable level. Two recrystallisations of the drug substance **1** from methyl ethyl ketone (MEK) were needed to lower the amount of the genotoxic impurity **3** down to an acceptable value (less than 10 ppm).

Although the synthesis depicted in Scheme 1 appealed to us for its convergent approach, we speculated that the extremely low tolerance of the genotoxic impurity **3** in the drug substance **1** might have represented a severe issue in view of manufacturing campaigns. Hence, we decided to explore alternative, but still convergent, routes to drug substance **1**. Looking at the nature of the starting materials shown in Scheme 1 (compounds **2** and **3**), and considering that the mesylate **3** was prepared from the ester **4**, two different approaches were envisaged (Schemes 2 and 3).

The first approach (Approach A, Scheme 2) consists of a reductive amination reaction. As a matter of fact, the required arylacetaldehyde **5** can be obtained either from the corresponding ester **4** or the alcohol **6**.

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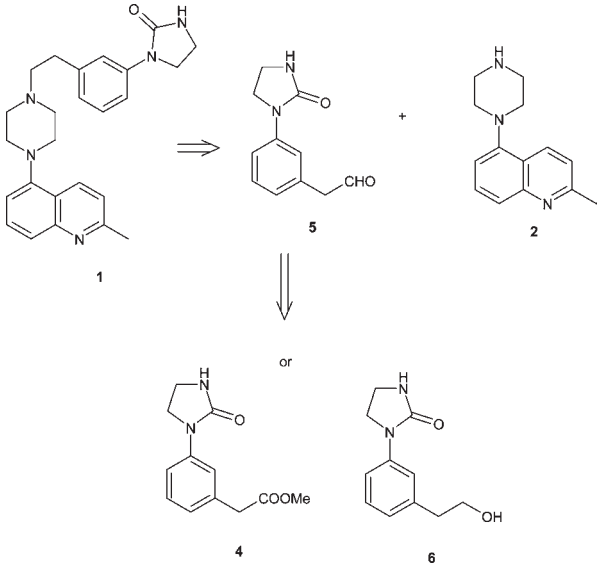
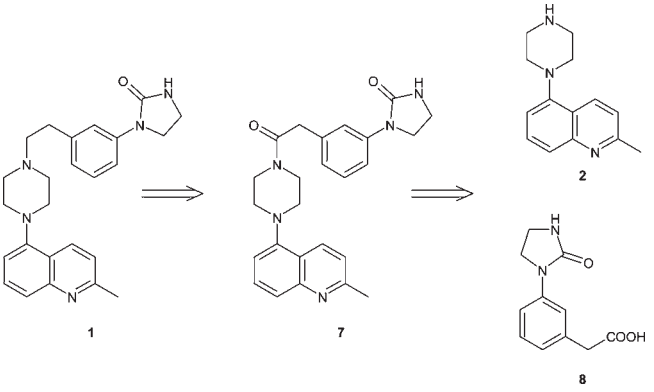
Scheme 2. Approach A



Scheme 3. Approach B



The success of Approach A depended, of course, on the ease of access to the arylacetaldehyde **5**. Unfortunately, both reduction of the ester **4** (e.g., with DIBAL-H) and oxidation of the alcohol **6** (e.g., with PDC, NaClO/TBAB, NaClO/TBAB/TEMPO, TBAB/TEMPO/$NaIO_4$, and under Swern conditions) resulted only in extremely complex reaction mixtures.

Alternatively, the desired 1-aryl-2-imidazolidinone **1** could be obtained by reduction of the corresponding amide **7**, that in turn could be synthesized from the acid **8** and the 5-piperazinylquinaldine **2** (Approach B, Scheme 3).

Several amide coupling systems were tested to synthesise the amide **7**. For example, the latter was formed in 80−90% yield by using the DCC/HOBt coupling system. However, the resulting material was contaminated by 5−10% of dicyclohexylurea **9**, and by ∼5% of a byproduct which was tentatively identified as compound **10** (Figure 1).

The use of propane phosphonic acid anhydride (T3P) as the coupling agent gave complete conversion and a very clean reaction profile. A few solvents (DMF, EtOAc and dichloromethane) and a few bases (triethylamine and diisopropylethylamine) were screened at room temperature. The amide coupling was also investigated in absence of an added base, taking advantage of the three basic nitrogens in the piperazinylquinaldine **2**, but the reaction rate was

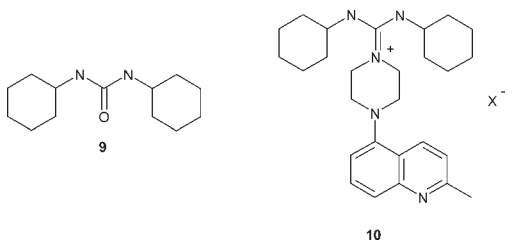
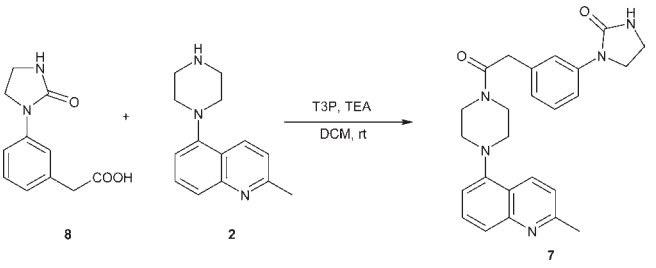
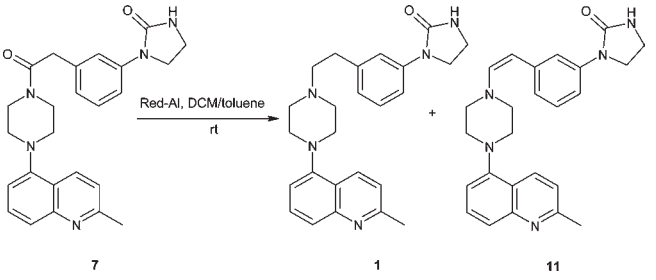


Figure 1. Impurities formed in the DCC/HOBt-mediated synthesis of **7**.

Scheme 4. T3P-mediated synthesis of amide 7



Scheme 5. Reduction of amide 7 by Red-Al

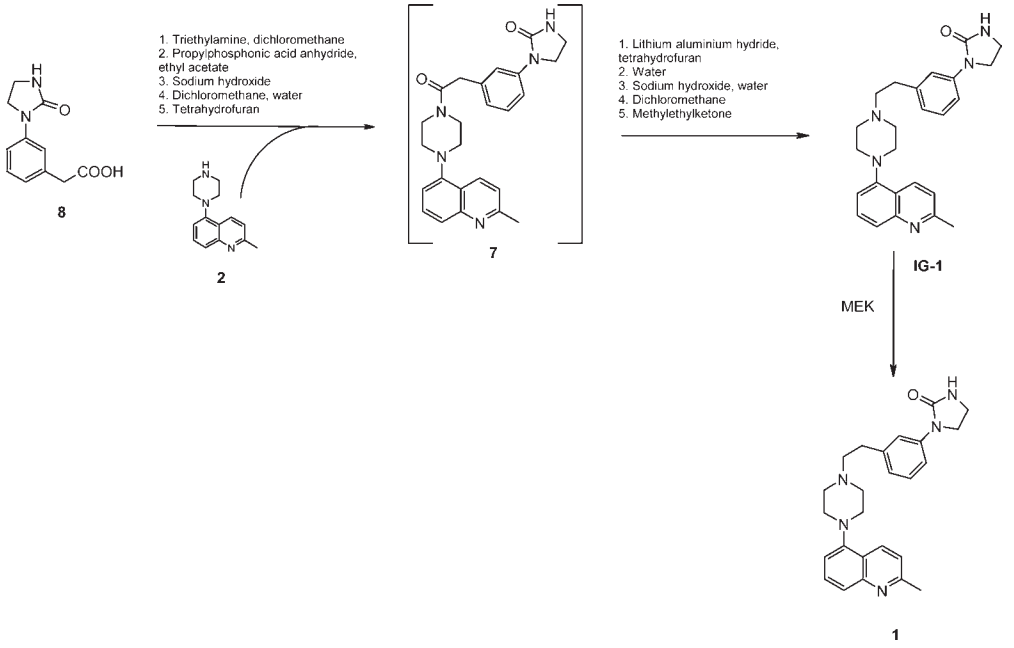


shown to be slower and did not go to completion. The reaction was best performed by adding the commercially available 50 wt % T3P in EtOAc to a stirred mixture of acid **8**, quinaldine **2** and triethylamine (TEA) in dichloromethane (DCM) at room temperature. After a careful setup of the whole process, the desired amide **7** was thus recovered in very high yields (>90%) and with high purity profile (>95% by HPLC and ^{1}H NMR), see Scheme 4.

With regard to the reduction of the amide **7** to prepare the drug substance **1**, both commercially available 1 M BH_3 in THF and 65 wt % sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) in toluene were tested. Since the latter furnished cleaner reaction profiles, this approach was explored in detail. The efficiency of the reduction reaction proved to be strongly affected by different parameters such as temperature, solvent, equivalents and addition mode of the reducing agent. Reverse addition of a solution of the amide **7** in CH_2Cl_2 to the Red-Al solution in toluene at room temperature gave eventually a clean and reproducible reaction. However, some residual starting material **7** (10−15%) and a major impurity that was identified as the compound **11** (5−10%) were still contaminating the drug substance **1** (Scheme 5). As a result, the crude compound **1** needed to be recrystallised at least twice from methyl ethyl ketone (MEK) in order to obtain pure material (>96% by HPLC and ^{1}H NMR).

The option of telescoping the amide coupling reaction and the amide reduction was then implemented in view of a large scale production of the 1-aryl-2-imidazolidinone **1**. After fine-tuning of

Scheme 6. Route A



the reaction conditions, 1.1 kg of drug substance **1** was prepared in 30% yield after 3 recrystallisations of the crude **1**. Having successfully produced drug substance, this approach was defined as Route A for regulatory purposes and is detailed in Scheme 6.

3. DEVELOPMENT AND SCALE-UP OF ROUTE A

The low overall yields and the poor processability of the telescoped synthesis described above, prompted us to investigate alternative methods for the reduction of the amide **7**. Among the reducing agents screened, $LiAlH_4$ was superior in terms of conversion to the drug substance **1** and reaction profile. A telescoped coupling/amide reduction process was thus optimized on a laboratory scale (**Route A**). In particular, it involved a careful aqueous basic quench of the excess of hydride reagent to transform the aluminium-containing coproducts into inorganic salts that were easily removed by filtration over Celite (Scheme 6). By means of **Route A** several small batches (up to dozens of grams) of intermediate grade **IG-1**[6] and drug substance **1** were successfully prepared in 50−60% overall yield.

Unfortunately, the process depicted in Scheme 6 was not robust when scaled up on pilot plant scale. As a matter of fact, even though 4.65 kg of **IG-1** were prepared as shown in Scheme 6, the overall yield of the process (31.2%) was pretty low as compared to the laboratory scale experiments. The low recovery was mainly due to coprecipitation of **1** together with the aluminium-salts during the filtration of the quenched reaction mixture over Celite.

On the basis of these data, we were forced to seek a more robust, reproducible and efficient synthesis of the 1-aryl-2-imidazolidinone **1** in view of supplying a convenient and scalable process for future manufacturing.

4. DEVELOPMENT OF ROUTE B

As highlighted previously, Route A suffered from significant drawbacks. Some attempts were made to select and scale-up a new route, with the potential of being suitable for manufacturing production. After reviewing the literature, it was decided to work on the route with the same key step reported in Scheme 1 and detailed in Scheme 7, as it introduced a straightforward approach to compound **1**, this route was then defined as Route B for regulatory purposes. In addition, the known concerns about the genotoxic impurity **3** were somewhat discharged by a lowering of the projected effective dose in clinical studies. The therapeutic dose was lowered from 150 mg to 10 mg and to 3 mg after PhI clinical studies. As a result, the maximum level of mesylate **3** allowed in the API by the TTC approach increased from the initial 10 ppm to 500 ppm. This new limit was not considered a critical hurdle for this route and development studies started. The development studies for Stages 1 and 2 are detailed in this paper. The process research for Stage 3 is not discussed here, even though the experimental procedure is reported in the Experimental Section.

4.1. Genotoxic Risk Assessment. Once Route B was developed, a complete assessment of all the potential genotoxic compounds generated in the chemical steps summarized in Scheme 7, was carried out. A number of structures were identified as potential contaminants of the drug substance **1** (Table 1), including the previously mentioned mesylate **3**. The next step was to further assess the toxicity of these structures by means of the Ames test. In case of a positive result (the structure of concern is mutagenic) the molecule has to be controlled in the drug substance following the TTC approach, otherwise it has to be treated as a general impurity by following the ICH Q3A guideline. The results of the Ames test are also reported in Table 1.[7]

An additional structure of concern was the propyl mesylate, potentially formed from traces of methanesulfonic acid (from methanesulfonic anhydride used in Stage 1) and *n*-propanol (used as solvent in Stage 3). However, it was decided not to test this compound in the Ames test. As a matter of fact, the formation of this mesylate was considered negligible, as methanesulfonic acid, if present, would have been neutralised by the excess of the base and

Scheme 7. Route B

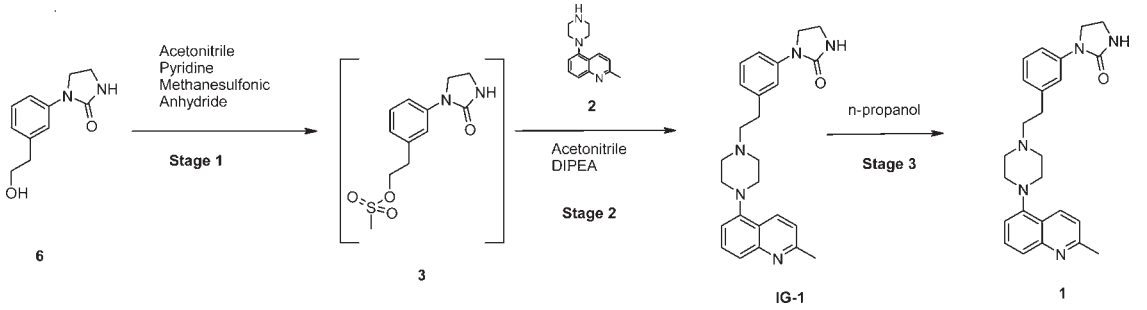
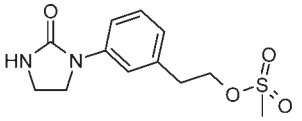
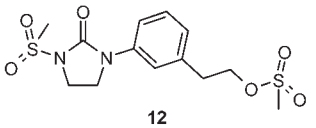
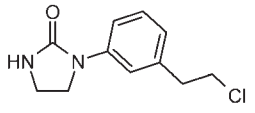
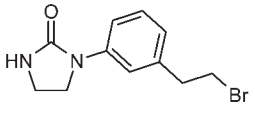
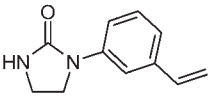


Table 1. Structures of concern

Compound	Origin	Ames test result
 3	Route intermediate	Mutagenic: control to TTC level
 12	From an excess of methanesulfonic anhydride	Mutagenic: control to TTC level
 13	From compound **3** when chloride ions are present in the reaction and in the starting material **6**	Mutagenic: control to TTC level
 14	From compound **3** and the hydrobromic acid present as contaminant in **2**	Mutagenic: control to TTC level
 15	From compound **3** in presence of a base	Not mutagenic: follow the ICH Q3A guidelines

in these conditions it was proved that esterification reactions with the involvement of methanesulfonic acid do not occur.[8]

As for the application of the Quality by Design principles, these impurities are drug substance-CQAs as, due to their toxicity, they have a direct impact on patient safety and thus, need to be controlled at the TTC level. Therefore, a control strategy[3] needs to be developed to ensure their control, and therefore the process development studies took into account the root causes for the formation of compounds **12**, **13**, and **14**.

A significant effort was expended in trying to identify and optimize analytical methods capable of detecting the mutagenic compounds **3**, **12**, **13**, and **14** at the TTC level (500 ppm), as detection in the drug substance **1** was necessary.

4.2. Development of Stage 1 of Route B. The studies carried out to develop a suitable process for Stage 1 and the approach followed to control the related genotoxins are reported in this paragraph.

*4.2.1. Control of Genotoxin **13** in Compound **6**.* Compound **6** is the input material for the generation of the mesylate **3** and one of the starting materials of the route. As for the risk assessment described in Table 1, the genotoxic compound **13** might also be formed if residual chloride ions are contained in the starting material **6**, therefore, to avoid any contamination of the starting material:

- A control of chloride ions in compound **6** was introduced as part of the control strategy by means of a specification.
- A change control procedure for the manufacturing process of the starting material **6** was put in place in agreement with the starting material supplier.

4.2.2. Selection of the Solvent and the Base. The mesylation reaction of Scheme 7 involved the combination acetonitrile and pyridine but propionitrile and 2-picoline were finally selected. The rationale for the change is described below. Pyridine was the

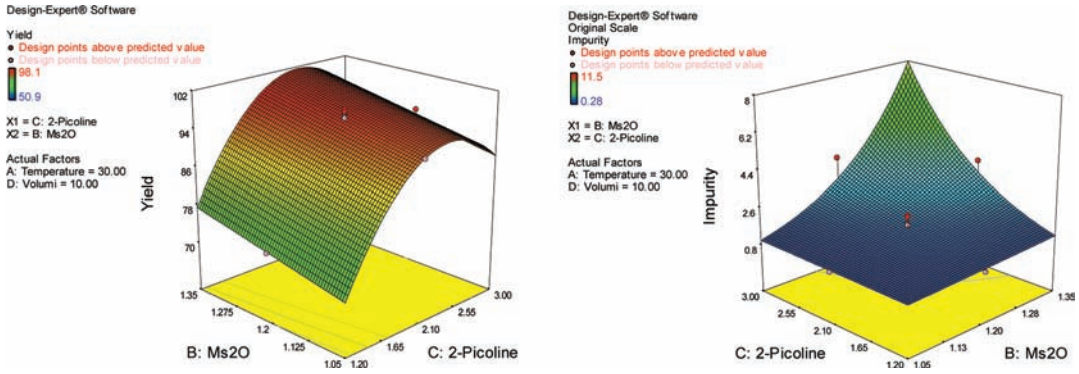


Figure 2. Result of the multivariate study.

Table 2. Ranges for the Stage 1 multivariate study

process parameter	range	centre point
temperature	20−40 °C	30 °C
methanesulfonic anhydride	1.05−1.35 equiv	1.2 equiv
2-picoline	1.2−3 equiv	2.1 equiv
concentration	7−13 vol	10 vol

selected reagent according to a base screening for stage 1 but 2-picoline represents a safer, less toxic alternative with similar physical properties and comparable reaction profile. Proprionitrile is similar to acetonitrile and gave complete conversion to the mesylate **3**. Moreover, propionitrile is immiscible with water, allowing a simple workup without the use of any additional cosolvent. Despite the fact that propionitrile is not classified by ICH and recommended limits are not available, this solvent was not a problem for the process as the drying conditions (vacuum, 50−55 deg) were selected to allow its complete removal prior to the last step (the Stage 3 recrystallization).[9] Finally, methanesulfonic anhydride was preferred over methanesulfonyl chloride as it does not produce chloride ions in the reaction that can be responsible for the formation of genotoxin **13** (see Table 1).

Initially the process consisted in the portionwise addition of the solid methanesulfonic anhydride to the slurry of 2-picoline and alcohol **6**, this was decided to avoid an excess of methanesulfonic anhydride in presence of the starting material **6** and 2-picoline as it was considered critical for the formation of the bis-mesylate **12**.

4.2.3. Optimisation of the Reaction Parameters. A DoE study (Fractional Factorial Design) was carried out to identify the main factors that affected the yield and the formation of the impurity **12** in the synthesis of compound **3**. Since strong curvature was observed (Figure 2), an upgrade to a Central Composite Design was performed as second step of the investigation. Previous process knowledge and a risk assessment allowed the selection of a series of process parameters and ranges for the DoE study (see Table 2).

The main impurity observed in this step was the bis-mesylate impurity **12** (Table 1). This impurity was also a drug substance-CQA, as it was carried through the entire process contaminating the drug substance, and therefore it needs to be controlled following the TTC approach.[5]

Analysis of the data showed a strong curvature of the yield (ranging from 78 to 97%), that was mainly due to the quantity of 2-picoline. Other factors affecting the yield were the quantity of methanesulfonic anhydride and the interaction between the quantity of 2-picoline and the reaction volume. The main factors affecting the formation of the impurity **12** were the temperature, the quantity of 2-picoline and methanesulfonic anhydride, along with the interaction between these two parameters. Hence, as target value for the process parameters, a compromise between the higher yield and the lower amount of bis-mesylate **12** was sought and the centre points of the ranges shown in Table 2 were selected. Only in the case of the equivalents of methanesulfonic anhydride, the amount was reduced to 1.15 to minimize the formation of the impurity **12**.

Some work was then carried out on the workup conditions. As introduced in Table 1, the presence of compound **3** and chloride ions resulted in the formation of the genotoxin **13**. It was speculated that the hydrochloric acid previously used in the hot workup (40 °C) of stage 1 was a source of chloride ions. Because of that, aqueous sulphuric acid was used in place of hydrochloric acid to avoid the formation of the chloride impurity **13**. The use of sulphuric acid might in turn generate the corresponding sulphate impurity. This was not formed or was undetected in our analytical method.

4.3. Development of Stage 2 of Route B. The studies carried out to develop a suitable process for Stage 2 and the approach followed to control the related genotoxins are reported in this paragraph.

*4.3.1. Control of Genotoxin **14** in Compound **2**.* The risk assessment identified the presence of hydrobromic acid in the starting material **2** as a root cause for the formation of the genotoxin **14**, therefore a basic wash was introduced in the key step of the formation of the starting material **2**.

4.3.2. Selection of the Solvent and the Base. A multivariate analysis started to identify some combinations of solvents and bases. A Principal Components Analysis (PCA)[10] was employed to ensure diversity in bases and solvents for the screening. Fourteen solvents and eleven bases were selected, knowing that below 70 °C the conversion was low and the reaction rate slow. In particular the choice of the solvents was restricted to those with a boiling point higher than 70 °C. As for the bases, primary and secondary amines were not selected, because they might react with the mesylate **3**. In Table 3 the solvents selected are shown.

4.3.2.1. Experimental Studies. It is worth noting that this approach would have required 154 experiments (14 solvents × 11 bases) to test all the possible combinations. In order to reduce the number of experiments the Partial Least Square (PLS) approach was used[11] and only 20 experiments were run. In these experiments a mixture of equimolar compounds **2** and **3** and the base (2 equiv) in the solvent (12.5 vol) was stirred at ∼80 °C. Each reaction was

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Table 3. Solvent and base screening

run	solvent	base	conversion (24 h)	note
1	isopropyl acetate	DBU	54.2	gum
2	toluene	TEA	85.8	suspension/gum
3	butanone	*N*-methylpiperidine	69.9	solution
4	1-butanol	tributylamine	93.2	solution
5	2-methyl-THF	2,3-lutidine	62.9	suspension
6	MIBK	4-methylmorpholine	68.6	suspension
7	*n*-butyl acetate	pyridine	65.0	suspension/gum
8	isooctane	4-methylmorpholine	58.6	solid/gum
9	ethylene glycol	DABCO	21.4	solution
10	NMP	DIPEA	95.0	solution
11	isooctane	DMAP	45.5	gum
12	DMSO	DBU	51.6	solution
13	ethylene glycol	*N*-methylpiperidine	76.1	solution
14	*n*-butyl acetate	4-phenylmorpholine	48.0	suspension
15	MIBK	2,3-lutidine	68.3	suspension/gum
16	2-methyl-THF	pyridine	66.6	suspension/gum
17	anisole	tributylamine	88.8	solution
18	propionitrile	TEA	95.2	solution
19	ethyl acetate	*N*-methylpiperidine	74.7	solution
20	1-butanol	DIPEA	95.6	solution

sampled and analysed by HPLC after 3 and 6 h; after ~24 h the reactions were cooled to room temperature and each reaction mixture treated with DMF until complete dissolution, sampled and analysed. The obtained data are reported in Table 3, only the conversion after 24 h is reported as in the previous check points (3 and 6 h) the reaction was not complete. The conversion of each reaction is calculated by HPLC comparing the % area of the peak of compound **1** versus compound **2**.

4.3.2.2. Analysis of the Data. Analysis of the data using the PLS approach showed that the yield was mainly affected by the factors correlated to the bases; in particular trialkylamine, such as TEA, DIPEA, tripropylamine (not in the original design) and tributylamine, gave the most promising results and, among these, DIPEA and tributylamine were chosen. Moreover, looking at the solvents, a correlation between the polarity and the yield was observed. According to this, among the analysed solvents, 1-butanol, anisole and propionitrile were chosen, because they permitted precipitation of the product after cooling to room temperature, allowing an easy isolation of the desired product (DMSO, NMP and ethylene glycol gave a solution after cooling).

4.3.2.3. Selection of Propionitrile and DIPEA. Further experiments, where all the combinations of the three solvents and the two bases were tested, gave very similar results (almost complete conversion of the mesylate **3** was observed in all the cases). However propionitrile gave the highest yield after precipitation (88% with respect to the mesylate **3**). In addition it was selected as it allowed the direct precipitation of the compound **IG-1** at room temperature. DIPEA was selected as more commercially available with respect to tributylamine. This combination was considered for the optimisation of the reaction conditions.

It has to be highlighted once again that the application of PCS/PLS approach is extremely useful for the investigation of a complex experimental space, in this case it is described how a complex experimental space generated by the selection of 14 solvents and 11 bases, could be successfully explored with a minimal number of experiments (20 versus 154 potentially required to test all the combinations) and identifying a successful combination of solvent and base (propionitrile/DIPEA).

Table 4. Factor Ranges for the definition of the stoichiometry of stage 2

	factor	unit	range low	range high
A	compound **2**	equiv	1	1.4
B	DIPEA	equiv	1.2	1.8
C	concentration	vol	5	10
D	temperature	°C	kept constant (at reflux)	

Table 5. Hydrolysis of the mesylate 3 in aqueous Na_2CO_3 (5% wt)

time (h)	alcohol **6** (%)	mesylate **3** (%)
0	0	100
0.5	4	96
1.5	9	91
4.5	25	75
24	70	30

4.3.3. Optimisation of the Reaction Parameters. A DoE was carried out to identify the main factors that could affect the final yield of Stage 2 (see Table 4). The initial process knowledge and some risk assessment activities allowed to identify three factors for the multivariate study: the amount of starting material **2**, the amount of DIPEA and the concentration of the mesylate **3** in propionitrile. The mesylate **3** was used in substoichometric quantity in the study to increase the chance to complete the reaction and minimize the risk of contamination of the residual mesylate **3** in the compound **IG-1**. The temperature was kept at reflux to increase the solubility of the reactants and the reaction rate.

A 2 Level Factorial design (Full design) with one block, two center points and ten reactions, was used to study the reaction.

Analysis of the obtained data, carried out by using the software Design Expert 7.0, did not give a significant model, mainly because the yield range was narrow (between 67.6% and 73.0% calculated with respect to the alcohol **6** by using phenanthrene as internal standard) and the variability of the two centre points quite high, if compared to the yield range. Thus, the reaction was considered robust enough in the measured range.

Verification experiments confirmed these data and hypotheses. It was demonstrated that the concentration was mainly affecting the reaction rate, with more concentrated reaction conditions giving a faster conversion of the mesylate **3**. Moreover, the reaction was stable with time. The following target value was then selected, based on the latter results:

compound **2**: 1.1 mol equiv versus the mesylate **3**
DIPEA: 1.8 mol equiv versus the mesylate **3**
temperature: reflux of propionitrile
concentration of the mesylate **3**: 10 volumes (this parameter was selected to minimise the likelihood of precipitation of the **IG-1** during the workup procedure)

4.3.4. Studies on the Workup Procedure. As the reaction was optimised in propionitrile, a series of aqueous washings were used to remove all the reaction components that could have affected the

Scheme 8. Route B, final process

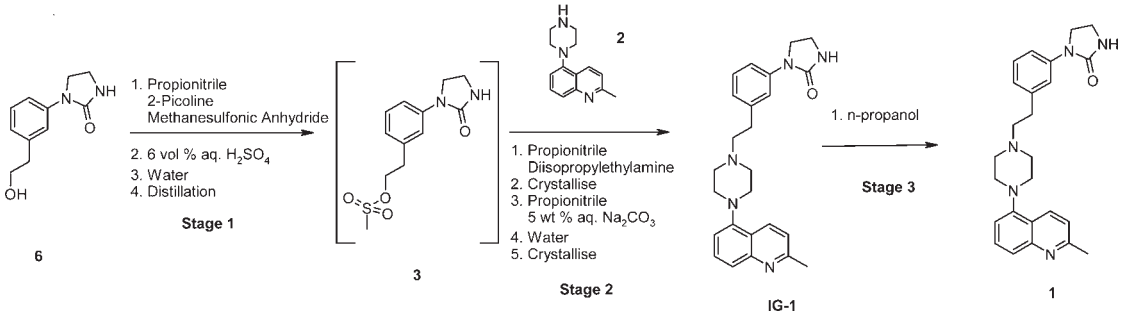

Table 6. Level of genotoxins in the kilo-lab and pilot-plant batches

			level of genotoxins (ppm)[b]					
run	batch[a]	scale	**3**	**12**	**13**	**14**	total impurities (% a/a)	yield[c] (%)
1	IG-1	kilo lab	<5	<5	<50	<5	1.3	76
2	DS-1	kilo lab	<5	<5	<5	<5	0.6	86
3	IG-2	kilo lab	<50	<5	<500	<5	1.7	78
4	DS-2	kilo lab	<5	<5	<250	<5	1.1	88.5
5	IG-3	pilot plant	<50	<5	<50	<5	0.9	70
6	DS-3	pilot plant	<50	NA	<50	<5	1.0	80

[a] IG is Intermediate grade and DS is Drug Substance. DS-1 was prepared from IG-1, DS-2 from IG-2, and DS-3 from IG-3. [b] The method for the detection of these genotoxins was developed as a limit test by LC/MS (ESI positive, SIM mode). [c] Yield from compound **2** for entries 1, 3, and 5. Yield from IG-1 for entries 2, 4 and 6.

crystallization of **1**. Since the mesylate **3** was the most critical to be eliminated due to its toxicity, a washing with sodium carbonate was introduced with the objective of hydrolyzing **3** (see Table 5). The reaction was studied over 24 h. Aqueous sodium carbonate (5% wt) was added to a representative sample of mesylate **3** (1 equiv) reaction mixture in propionitrile (10 vol) at 83 °C. The biphasic mixture was kept for 24 h at 83 °C and evaluated against an internal standard (anthracene). It was found that 70% of the mesylate was hydrolyzed in the workup conditions in 24 h. Thus, the workup can be used as a step to significantly reduce the mesylate **3**.

Considering the rate of reaction and the reaction conditions, 15 min stirring at 83 °C were selected for the optimised process.

5. ROUTE B, FINAL PROCESS AND SCALE-UP RESULTS

The final process after the development work is reported in Scheme 8

This process was scaled up to kilo lab (50−200 g input of starting material **2**) and pilot plant (up to 5 kg input of starting material **2**), the results obtained are reported in the following paragraphs

5.1. Final Process Details. The process depicted in Scheme 8 was implemented with some minor changes. Stage 1 was operated with the amount of solvent/reagents suggested by the DoE studies. The alcohol **6** was dissolved in propionitrile (10 vol) and submitted to the mesylation reaction in the presence of methanesulfonic anhydride (1.15 equiv) and 2-picoline (2.1 equiv). At first, the process included the portionwise addition of the solid methanesulfonic anhydride to the slurry of 2-picoline and alcohol **6**. However, methanesulfonic anhydride is a very hygroscopic material that is better manipulated only once it is charged into a reactor. Then, the reverse addition of the 2-picoline to a slurry of the alcohol **6** and methanesulfonic anhydride at 30 °C was introduced in order to have an easier process for the pilot scale-up. In addition to a lower quantity of methanesulfonic anhydride and the reverse addition previously introduced, temperature control kept the bismesylate impurity **12** below the level of concern. This required the slow and controlled addition of 2-picoline in order to keep the mesylation reaction at a temperature below 40 °C. Under these conditions complete conversion (>99%, HPLC) of **6** to the mesylate **3** was achieved in 30 min, and the bismesylate impurity was 1.4% or lower (HPLC). Workup was performed by diluting with propionitrile (10 vol) and extracting with aqueous sulphuric acid solution and water. Removal of water via azeotropic distillation (<1 wt %) gave a red solution ready for the subsequent nucleophilic substitution (Stage 2). It was found that water content higher than 1 wt % in propionitrile resulted in slow conversion rates for the formation of **IG-1**.

For Stage 2, piperazinylquinaldine **2** (1.1 equiv) and DIPEA (1.8 equiv) were added to the solution of the mesylate **3**, the reaction mixture was heated at reflux (about 94 °C) for at least 10 h to achieve complete conversion. A quench with a 5% wt Na_2CO_3 aqueous solution at 83 °C was introduced to remove any residual mesylate **3** and methanesulfonic acid, produced by the mesylation reaction (Stage 1). After separation, the dark red residue was finally washed with water at 83 °C. The implementation of a warm workup was needed to keep the compound **IG-1** in solution. When **IG-1** crashed out accidentally in the presence of water, for instance during the washes at temperatures lower than 60 °C, it crystallized in the undesired monohydrate form.[12] Instead, the correct anhydrous form was obtained after the warm aqueous washes and azeotropic removal of water *via* propionitrile distillation.

5.2. Process Scale-Up Results. The results of the scale-up of the optimised process in kilo lab and pilot plant are summarized in Table 6. Regarding the yield a small decrease of the yield was obtained at a plant scale, but this was not considered critical for this project phase. The impurity profile gave a slight improvement from kilo lab batches versus pilot plant (runs 1 and 3 versus 5) while the difficulty of the crystallization process in Stage 3 to remove general impurities was confirmed (run 1 compared with 2, run 3 compared with 4, run 5 compared with 6).

As can be seen from Table 6, the level of the genotoxins (**3**, **12**, **13**, **14**) were controlled properly by the optimised Stage 1 and 2 process (entries 1, 3 and 5) as the data referred to the analyses of intermediate grade material (**IG-1** in Scheme 8). In addition, the final crystallization in 1-propanol carried out as the last step (Stage 3 in Scheme 8) seemed to further reduce the level of mutagens **3** and **13** (run 1 compared with 2, run 3 compared with 4). Regarding the mutagens **12** and **14** their level was not a concern.

For genotoxin **14**, the process selected to prepare the starting material **2** was considered to be appropriate for its control, so any process change introduced would have been risk assessed and tested on the appropriate scale before approval.

The same approach was used for the starting material **6** where the amount of chloride ions was introduced as a test in the release specification. In addition, any change introduced in the process to **6** would have been risk assessed and tested on the appropriate scale before approval.

In conclusion, the optimised process allowed us to prepare the target compound in acceptable yield, confirming full control of the impurities and of the genotoxins present in the process.

5.3. Control Strategy for Genotoxins. In summary, the following control points have been defined, these are elements of the control strategy for genotoxins in the drug substance **1**

For the intermediate mesylate **3**:

- Stage 2 stoichiometry: the mesylate **3** is substoichiometric with respect to compound **2** in Stage 2 (see section 4.3.3).
- Stage 2 workup: the sodium carbonate washing temperature is 83 °C (see section 4.3.4).

For the bismesylate impurity **12**:

- Stage 1 reaction parameters: the excess of methanesulfonic anhydride is minimised (1.15 equiv), the amount of 2-picoline is 2.1 equiv, and the temperature is 30 °C (see 4.2.2).
- Stage 1 addition order: 2-picoline is added to a slurry of the alcohol **6** and methanesulfonic anhydride at 30 °C (see section 4.2.2).

For the chlorinated impurity **13**:

- Specification: the control of chloride ions in starting material **6** is introduced (see section 4.2.1).
- Change control procedure: a change control procedure for the process of the starting material **6** was put in place (see section 4.2.1).
- Stage 1 reactant: methanesulfonic anhydride is used instead of methanesulfonyl chloride as reagent in this step (see section 4.2.2).
- Stage 1 work up conditions: a sequence of washing, avoiding chloride ions, was optimised (see section 4.2.3).

For the brominated impurity **14**:

- Starting material process: a basic wash was introduced in the key step of the formation of the starting material **2** (see section 4.3.1).

It is recommended that analytical data on the level of these mutagens in the drug substance **1** are collected for the development and scale up batches so that, for the commercial product, the removal of the testing of these mutagens in the drug substance can be considered after the appropriate risk assessment in agreement with the current regulatory requirements.

6. CONCLUSION

The activities for the identification of the manufacturing route and the optimisation of the process for stages 1 and 2 to the drug substance **1** are discussed in this paper. The process allowed the preparation of the target compound in good yield, in addition no scale up problems were encountered confirming full control on the impurities present in the process. Once again, the importance of the use of a DOE approach coupled with the PLC/PLS tools to build the knowledge in support of a robust and scalable manufacturing process was confirmed.

Finally, a particular focus is given to the genotoxic impurities as they needed to be controlled below the TTC (lower than 1.5 μg/day). The studies carried out to build a robust control strategy for genotoxic impurities are reported. As for the general impurities, the optimised process allows complete control of their level in the drug substance. In spite of these promising initial data, it is recommended that data on the performance of the process in controlling these genotoxic impurities are collected throughout the development phase. If the robustness of the control strategy is confirmed and the risk of contamination is fully under control, there is the opportunity, according to the current regulatory requirements, to remove these drug substance-CQAs (or some of them) from the drug substance specification through the full application of the QbD principles to manufacturing processes.

7. EXPERIMENTAL SECTION

NMR spectra were recorded on a 600 MHz Varian Inova 600 spectrometer. HPLC analysis of the intermediates and reaction monitoring were carried out on Agilent Series 1200 (Agilent). Generic acidic HPLC method used: column type Luna C18; mobile phase A: 0.05% TFA/water and B: 0.05%. TFA/acetonitrile; gradient: 0 min 100% A to 8 min 95% B; flow 1 mL/min; column temperature 40 °C; detector UV DAD @ 220 nm. Mass spectra analyses were performed on Agilent 1100 LC/MS equipped with a Waters ZQ single quadrupole, operating in ESI positive mode, SIM acquisition. Commercially available reagents and solvents were purchased from ordinary chemical suppliers and used without purification. The starting materials, i.e. 5-piperazinylquinaldine **2**, methyl arylacetate **4**, arylethanol **6**, and arylacetic acid **8**, were supplied by external contractors on multikilogram scale.

Preparation of 5-Piperazinylquinaldine 1 (IG-1). *Stage 1.* The alcohol **6** (1 equiv) and the methanesulfonic anhydride (1.15 equiv, 0.96 wt) were suspended in propionitrile at 20 °C (10 vol). The slurry was warmed up to 30 °C and kept at 30 °C with vigorous stirring and under a flow of nitrogen for at least 15 min. 2-Picoline (2.1 equiv, 1.01 vol) was added dropwise, maintaining the internal temperature below 40 °C. At the end of the 2-picoline addition, a solution was obtained, and within a few minutes a suspension appeared. The mixture was heated to 40 °C. After 30 min the reaction mixture was quenched at 40 °C with 6% vol/vol aqueous sulphuric acid (3 vol) and the temperature increased to 50 °C; after separation the organics

were washed with water (3 vol) (during the wash the internal temperature must be kept at 48 ± 2 °C). To the washed organic phase propionitrile (10 vol) was added, and the resulting solution was concentrated to 10 vol by distillation at atmospheric pressure. The propionitrile solution was cooled to 60 ± 2 °C.

Stage 2. The piperazine quinaldine **2** (1.1equiv, 1.2 wt) and *N*, *N*-diisopropylethylamine (1.8 equiv, 1.5 vol) were added to the solution of the mesylate **3** at 60 °C. The resulting mixture was heated at reflux (approximately 94 °C) for at least 10 h. The solution was sampled for analysis by HPLC (IPM expected: >99% conversion to drug substance **1** with respect to the mesylate). The solution was cooled to 83 ± 2 °C and diluted with proprionitrile (10 vol). The solution was washed at 83 ± 2 °C with 5 wt % aqueous sodium carbonate (5 vol) and stirred for 15 min, and the lower aqueous phase was separated. To the warm organic phase was added slowly water (5 vol), keeping the internal temperature at 83 ± 2 °C, and was stirred at 83 ± 2 °C for at least 5 min. The lower aqueous phase was separated. The warm organic phase (about 20 vol) was concentrated by distillation at atmospheric pressure to 10 vol with a Dean−Stark (about 4 h) (KF < 0.5 wt/wt), while the internal temperature reached 95 ± 2 °C. The slurry was cooled to 83 ± 2 °C and diluted with propionitrile (5 vol) and the temperature was increased to 95 ± 2 °C to give a solution. Then the temperature was adjusted to 83 ± 2 °C and seeded with drug substance **1** (0.005 wt). The suspension was cooled to 20 ± 2 °C over approximately 1 h. The slurry was stirred for 1 to 2 h at 20 ± 2 °C then the solid was collected by filtration. The cake was washed with propionitrile (2 × 3 vol). All the washes were carried out at 20 ± 2 °C. The damp cake was dried in a vacuum oven at 50−55 °C overnight (yield 70% mol).

Preparation of 5-Piperazinylquinaldine 1. *Stage 3.* **IG-1** (1 wt) was dissolved in 1-propanol (15 vol) by heating to 85 °C; then the solution was passed through a clarification line-filter (5 μm). The line-filter was washed with hot 1-propanol (2 vol). The collected filtrates were concentrated to 9 vol by distillation at atmospheric pressure. The resulting solution was then cooled to 70 °C (internal temperature) and seeded with drug substance **1** (0.0025 wt). The suspension was cooled down to 20 °C (internal temperature) over approximately 1 h. The slurry was stirred for 2 h at 20 °C (internal temperature), then the solid was collected by filtration. The cake was washed successively with 1-propanol (2 vol), 1-propanol/isooctane 1:1 (2 vol), and isooctane (2 vol). All the washes were carried out at 20 °C. The damp cake was dried in a vacuum oven at 50−55 °C overnight. (yield 80% mol).

^{1}H NMR (600 MHz, DMSO-d_6) δ 2.64 (m, 2 H), 2.63 (s, 3 H), 2.74 (br s, 4 H), 2.78 (m, 2 H), 3.04 (br s, 4 H), 3.39 (m, 2 H), 3.84 (dd, J = 8.94, 7.01 Hz, 2 H), 6.89 (d, J = 7.70 Hz, 1 H), 6.91 (m, 1 H), 7.11 (dd, J = 6.60, 1.92 Hz, 1 H), 7.21 (t, J = 7.97 Hz, 1 H), 7.39 (m, 2 H), 7.46 (t, J = 1.65 Hz, 1 H), 7.59 (m, 2 H), 8.34 (d, J = 8.80 Hz, 1 H); m/z 416.

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(4) Mesylate **3** was tested in a bacterial mutation screening assay (Ames test) with *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2*uvrA*(pKM101) in the presence and absence of S9-mix. Mesylate **3** was mutagenic in the Ames test in strains TA100, TA1535 and WP2*uvrA*(pKM101) when tested in the presence and absence of S9-mix. The maximum concentration tested was 5000 μg/plate, in accordance with current guidelines.

(5) (a) Draft Guidance for Industry. *Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches*. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, December 2008. (b) *Guideline on the Limits of Genotoxic Impurities*, CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, 28 June 2006. (c) *Question and Answers on the CHMP Guideline on Genotoxic Impurities*, CHMP/SWP/431994/2007; Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency (EMEA): London, January 2008, . (d) Elder, D. P.; Harvey, J. S. *Org. Process Res. Dev.* **2010**, *14*, 1037–1045.

(6) IG-1 is the Intermediate grade drug substance, the acronym indicates the compound before the final crystallization step.

(7) It is worth noting that the Ames test defines if a substance is mutagenic (it changes the genetic material), and this concept is slightly different from the concept of genotoxicity (a substance that has negative effect on the cell's genetic material). For the purposes of the discussion in this contribution a positive result in the AMES test identifies a mutagenic compound, and this compound is considered a genotoxin.

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(9) As mentioned above the wet cake drying conditions in Stage 2 (vacuum at 50−55 °C) were selected to allow full removal of propionitrile. This is confirmed by the NMR where, after drying, is not seen any residual left in IG-1 (if the ^{13}C satellite peaks of the ^{1}H NMR resonances of the IG-1are considered, up to 0.5% mol of propionitrile can be detected, this limit corresponds to 0.1% w/w of propionitrile in IG-1, that, on a dose of 3 mg/day is equal to a daily intake of 3 μg/day).

In addition, Stage 3, the recrystallization step in *n*-propanol, introduces an additional step where the amount of residual propionitrile is further reduced.

(10) Each class of compounds (for example solvents and reagents) can be described by relevant chemical descriptors (e.g., dielectric constant, boiling point, log *P*, etc.) that generate vectors in a multi-dimensional space. The PCA is a statistical approach that allows reducing the descriptors to three principal components; in this way, solvents that are expected to behave similarly are close in space. For a more statistical discussion: Eriksson, L.; Johansson, E.; Kettaneh-Wold, N., Wold, S. *Multi- and Megavariate Data Analysis, Principles and Applications*; Umetrics Academy, 2001; pp 43−48.

(11) Partial Least Squares (PLS) regression is a multivariate data analysis technique which can be used to relate several response variables to several explanatory variables. In this context this is used to find relations among chemical properties of solvent and base. The PLS model can be used to predict the outcome of the experiment for other combinations of solvent, reagent and/or catalyst not evaluated in the initial array. See also Eriksson, L.; Johansson, E.; Kettaneh-Wold, N., Wold, S., *Multi- and Megavariate Data Analysis, Principles and Applications*; Umetrics Academy, 2001; pp 43−48.

(12) The IG-1 compound as monohydrate form was not a problem for this process; even in case of its precipitation, it could be fully removed in the following Step 3 where it was demonstrated that its formation was due to the amount of water left in the *n*-propanol solution before starting the precipitation.