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Controlling the Genotoxins Ethyl Chloride and Methyl Chloride Formed During the Preparation of Amine Hydrochloride Salts from Solutions of Ethanol and Methanol

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Abstract:

The genotoxins ethyl chloride (EtCl) and methyl chloride (MeCl) were generated during the preparation of the hydrochloride salts of two tertiary amines in the presence of ethanol and methanol, respectively. In EtOH five batches of a tertiary amine hydrochloride were prepared on 0.3−18 kg scale using 37% aqueous HCl; residual EtCl was detected at less than 10 ppm in each batch. The preparation of the hydrochloride salt of another tertiary amine in MeOH on a 3 kg scale produced salt with 11−12 ppm of MeCl, and these higher levels precipitated investigations into controlling the levels of residual MeCl in batches of the HCl salt of the second amine. Four rework procedures were developed to reduce the level of MeCl in one batch of HCl salt. Generating the salts at a lower temperature (10 °C) was the key parameter to minimize the concentration of these impurities in this drug candidate when HCl was charged as 37% aqueous HCl. Control of the process was demonstrated by preparing a 30 kg batch containing 1 ppm of MeCl without rework processing; this level of MeCl is well within the guideline of ≤1.5 μg for the daily dosage of this drug candidate. The analytical methods to detect EtCl and MeCl, which were critical for the development of these processes, are also described.

Introduction

The need to control genotoxic impurities in drug substances and drug candidates is a growing concern of most pharmaceutical companies, and such regulatory concerns have been recently discussed in this journal.[1,2] Analytical and processing difficulties arise from specifications set at low levels; for example, a specification of 70 ppb was set by a pharmaceutical company for an especially potent genotoxin in a drug candidate.[3] In general a specification of 1.5 μg/day has been proposed for genotoxic impurities (daily exposure for clinical trials with duration of longer than 12 months), and this level poses analytical and process development challenges that could impede the development of new drugs.[4−7]

Genotoxins, or mutagens, are compounds that are potentially carcinogenic to humans by genetic mutations, rearrangement of chromosomal material, or other damage to DNA.[8] On a molecular level, genotoxins may alter genetic material by scission or covalent modification, and alkylating agents are readily flagged as genotoxins.[4,9] Toxicological testing of carcinogens can establish threshold dosages,[10] i.e., dosages below which there is considered to be no risk of inducing cancer. For example, data from rodent carcinogenicity studies have been used to calculate permissible daily exposure limits (PDEs) for residual solvents.[11] In the early stages of the development of a drug candidate, tight timelines and the unavailability of potentially genotoxic impurities (PGIs, sometimes termed GTIs) in sufficient quantities may preclude suitable toxicological testing to establish mechanisms and threshold dosage levels. PGIs without sufficient data to establish a threshold dose are treated as a category of carcinogens, and guidance in predicting

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carcinogenicity may be obtained from computer models.[12] Guidelines from the European Medicines Agency (EMEA) Committee for Medicinal Products for Human Use (CHMP) specify that if the formation of such impurities in a drug substance cannot be prevented, then these impurities should be removed by purification down to levels as low as reasonably practicable.[13] Impurities without sufficient mechanistic data to propose a threshold dosage may be subject to the "threshold of toxicological concern" (TTC) limit of not more than 1.5 μg/day (for clinical trials with duration more than 12 months). Quantifying and controlling impurities at concentrations of parts per million can pose daunting technological hurdles. Researchers from Lilly have reviewed investigations into detecting and controlling trace toxic impurities, including a detailed example on detection of formaldehyde in an intermediate and acceptable process tolerance that led to the removal of formaldehyde from the drug candidate.[14] To assess the efficacy of drug candidates, clinical trials using a slightly higher level of genotoxins have been proposed (a staged TTC) by members of the pharmaceutical community.[15] The concern of genotoxins was aired recently in the public press when nelfinavir mesylate was temporarily withdrawn from the European market due to contamination by ethyl mesylate.[16] The causes of this contamination have been discussed in detail,[17] and the EMEA recently requested that manufacturers consider the risk of sulfonate esters contaminating their marketed products.[18,19] There is considerable flux regarding the identification and control of PGIs and related compounds in drug candidates and APIs. For instance, although the FDA lists acetamide as a food additive,[20] control of acetamide in a drug candidate was filed as though acetamide were a genotoxin.[21] Jacobson-Kram and Jacobs have discussed the positions of the FDA on genotoxins,[22] and additional guidelines are anticipated.[23]

Genotoxins have affected route design,[24] reagent selection,[25] and the optimal form of the drug substance. Salts of basic drug substances are often preferred because of their increased solubility in aqueous systems and increased bioavailability. (For example, the solubilities of cocaine as the free base and the phosphate salt are 8.3 mg/mL and 435 mg/mL, respectively).[26] Unfortunately salt formation in the presence of alcohols such as the solvents MeOH, EtOH, and *i*PrOH can generate genotoxic alkylating agents, e.g., alkyl chlorides and alkyl mesylates from salt formation in the presence of HCl and methanesulfonic acid.[9] When alcoholic solvents are unavoidable during salt formation in order to control polymorphism, solubility, and stability for formulation, salts of phosphoric and sulfuric acid may be similarly rejected: alkyl phosphates and dialkyl sulfates are well-known alkylating reagents, and monomethyl sulfate has also shown alkylating ability.[27] To avoid generating genotoxic alkylating agents, some have proposed developing free bases of amines as the drug substance; the history of citrates, succinates, or even oxalates (e.g., escitalopram oxalate)

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(17) http://www.emea.europa.eu/humandocs/PDFs/EPAR/Viracept/Viracept-H-164-Z-109-AR.pdf (accessed March 29, 2009).

(18) http://www.emea.europa.eu/Inspections/docs/4471408en.pdf (accessed March 29, 2009).

(19) (a) Prior to the discovery of batches of nelfinavir mesylate that were contaminated with ethyl mesylate, toxicological data were not available to establish a threshold dose response for ethyl mesylate. Roche subsequently conducted extensive toxicological studies on ethyl mesylate, and on the basis of these data calculated that the permitted threshold dosage in humans was 370 times higher than the highest level of ethyl mesylate found in those batches of nelfinavir mesylate: http://www.roche-hiv.com/portal/eipf/pb/hiv/Roche-HIV/2008?paf_pageId=re7283195&paf_gear_id=20000003&paf_dm=full&faq_mode=detailed&doc_id=re7300002/re71700003/re73300004/re731001/faq/FAQ_00983.headline (accessed March 29, 2009); http://www.aids2008.org/Pag/Abstracts.aspx?AID=16184 (accessed March 29, 2009). (b) After reviewing the toxicological data, the EMEA concluded that monitoring patients that had been inadvertently exposed to high levels of ethyl mesylate in these batches of nelfinavir mesylate was not necessary. http://www.emea.europa.eu/humandocs/PDFs/EPAR/Viracept/38225608en.pdf (accessed March 29, 2009).

(20) (a) Acetamide is non-mutagenic (negative in Ames assay) and thus is not a genotoxin. The International Agency for Research on Cancer (IARC) has classified acetamide as possibly carcinogenic to humans (Group 2B) based on rodent toxicity data and thus is controlled to a threshold level of <5 μg/day. http://www.inchem.org/documents/iarc/vol71/053-acetamide.html (accessed May 5, 2008). (b) According to the FDA's EAFUS (Everything Added to Food in the U.S.) database (http://vm.cfsan.fda.gov/~dms/eafus.html), "The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS (Generally Recognized as Safe)." In this table acetamide is listed in the EAF category, meaning "There is reported use of the substance, but it has not yet been assigned for toxicology literature search." (Accessed May 19, 2008). (c) The Flavor and Extracts Manufacturers Association Expert Panel, an independent organization, has included acetamide in the GRAS (Generally Recognized as Safe) list, with the average maximum use level of 5 ppm for baked goods. http://members.ift.org/NR/rdonlyres/B9066605-9760-4E67-82BA-B2AA8D343020/0/0805gras22_complete.pdf (accessed 5/20/08).

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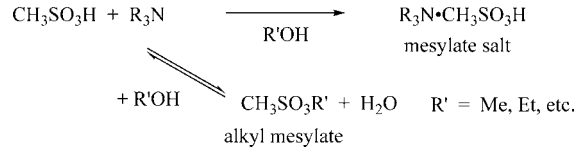
Scheme 1. **Byproducts and equilibrium for mesylate salt formation in alcoholic solvents**



as drug substances may encourage the development of other acid salts of amines.[28,29]

Salt formation in an alcoholic solvent may be unavoidable if that solvent is essential for formation of the desired morphology. Thus, controlling the amount of byproduct alkylating agents in the drug candidate becomes necessary.

Control strategies have recently been described to limit the amounts of mesylate esters[7,9,30] and trifluoroacetate esters[25] found in APIs. In general, water is added to hydrolyze the ester formed or to shift the equilibrium away from the ester (see Scheme 1 for this approach with methanesulfonic acid). Formation of methyl methanesulfonate from MeOH and methanesulfonic acid is reduced at lower temperatures in the presence of small amounts of water.[31] For APIs controlling the amount of byproduct alkyl sulfonates and other esters with alkylating potential remains a concern.

Strategies to control the amounts of alkyl halides and benzyl halides in APIs have been reviewed.[32] We are not aware of any quantitative data on controlling the genotoxins EtCl and MeCl in amine hydrochlorides formed in EtOH or MeOH, and we present such data herein.

Results and Discussion

A series of compounds targeted for CNS effects were prepared. From this series the hydrochloride salts of two tertiary amines (structures are currently proprietary) were prepared in either EtOH or MeOH, with MTBE added as an antisolvent after the addition of HCl. The HCl salts were selected over other salts due to the high solubilities of those salts in water and the anticipated higher bioavailabilities. Nonalcoholic solvents were screened for polymorph formation, but salt formations from EtOH and MeOH were developed due to the superior stabilities of polymorphs from these solvents. Crystallizing the HCl salts from EtOH or MeOH necessitated monitoring and controlling the concentrations of EtCl and MeCl in these two tertiary amine salts. Note: byproduct alkyl chlorides and ethers often have extremely low flash points, and care must be taken for safe operations (Scheme 2). MeCl and Me_2O can be prepared in a practical manner from reaction of MeOH and HCl.[33]

Scheme 2. **Byproducts and equilibrium for hydrochloride salt formation in alcoholic solvents**

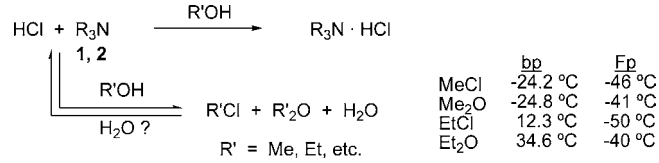


Table 1. **[MeCl] in initial scale-up batches of 2·HCl**

batch	scale (kg)	temperature for salt formation (°C)	[MeCl] in isolated **2**·HCl[a] (ppm)
1	0.3	10	<LOD
2	0.3	10	3.2
3	3.0	25	11.1
4	3.0	25	11.9

[a] For this method the LOQ was 2.5 ppm, and the LOD was 0.75 ppm.

Developing Headspace GC Analysis for EtCl and MeCl. As an essential part of the process development program, reliable headspace GC (HS-GC) methods were developed for the detection and quantification of the byproducts EtCl and MeCl in the isolated products. Each of these methods utilized a capillary column with a flame ionization detector (FID). Every effort was made to obtain suitable sensitivity with a FID which would then allow the quantification of the process solvents in the same chromatographic method (rather than requiring a separate method on an electron capture detector (ECD)). Both HS-GC methods were validated where suitable recovery was obtained at the proposed 10 ppm specification limit. For EtCl the method was validated as a limit test, with a limit of quantitation (LOQ) at 10 ppm being appropriate for that stage of development of **1**·HCl. For MeCl in **2**·HCl the LOQ and limit of detection (LOD) were 2.5 and 0.75 ppm, respectively.

Controlling the Amount of EtCl in the Hydrochloride Salt of Tertiary Amine 1. Salts of a tertiary amine with $pK_a = 9.67$ were screened for high solubility in aqueous systems, and the hydrochloride salt was selected. (In water the solubilities of **1**·HCl and the free base **1** were >600 mg/mL and 0.3 mg/mL, respectively.) Solvent screening for polymorphs indicated that the hydrochloride salt from EtOH/MTBE was the most stable, and these solvents were selected to crystallize **1**·HCl. To avoid the inconvenience of charging gaseous HCl on scale, HCl added was charged as 37% aq HCl. The amount of EtCl in drug candidate **1**·HCl was consistently less than 10 ppm in four batches run at 0.3−4 kg scale, and in a fifth batch run at 18 kg scale.

Controlling the Amount of MeCl in the Hydrochloride Salt of Tertiary Amine 2. The hydrochloride salt of a second tertiary amine **2** ($pK_a = 9.22$, with significant absorption at 254 nm) was similarly selected for development. (In water the solubilities of **2**·HCl and the free base **2** were >600 mg/mL and 0.2 mg/mL, respectively.) Solvent screening showed that crystallization from MeOH gave the most stable polymorph, and MTBE was added as antisolvent to further crystallize the product. We were concerned that using anhydrous HCl in

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Table 2. **Removal of MeCl from a batch of 2·HCl containing 11.9 ppm MeCl**

		results		
entry	conditions[a]	recovery (%)	purity (% AUC)	MeCl[b] (ppm)
1	stirred suspension at reflux for 2 h	92	>99	1.6
2	added water (45 mL) to dissolve solids	95	>99	ND
3	added MeOH (8 vol.) to dissolve the solids and distill off MeOH (8 vol.) at atmospheric pressure	99	>99	<1.0
4	added MeOH (8 vol.) to dissolve the solid and distill off MeOH (8 vol.) under reduced pressure	97	>99	<1.0

[a] Procedure: **2**·HCl (100 g, containing 11.9 ppm of MeCl) was suspended in MeOH (4 vol.) and heated to reflux. After the operations indicated in the table above, MTBE (8 vol.) was added over 15 min; the mixture was cooled to 10 °C over 2 h, filtered, and washed with 2 cake volumes of MTBE. The products were dried in a vacuum oven at 45 °C for ~24 h. [b] The values for residual MeCl in the salt are estimates, below the LOQ (2.5 ppm). The LOD was 0.75 ppm.

Table 3. **Effect of reaction conditions on MeCl content in the preparation of 2·HCl**

	conditions[a]					results		
entry	HCl (equiv)	temp (°C)	agitation rate (rpm)	HCl addition (min)	agitation after MTBE (h)	purity (% AUC)	yield (%)	[MeCl] (ppm)
1	1.5	35	60	80	2	>99	87	23.8
2	1.5	10	60	80	2	>99	90	3.3
3	1.5	10	~250	80	2	>99	84	3.0
4	1.5	10	60	300	2	>99	92	3.7
5	1.5	10	60	80	60	>99	90	ND
6	1.5	3	60	80	2	>99	90	ND
7	1.2	10	60	80	2	>99	84	ND
8	1.5	10	60	20	2	>99	94	1.2

[a] Procedure: The free base **2** was dissolved in MeOH (4 vol.), and 37% aq HCl was added. MTBE (8 vol.) was added over 15 min; the slurry was cooled to 10 °C over 2 h, filtered, and washed with 2 cake volumes of MTBE. The products were dried in a vacuum oven at 45 °C for ~24 h.

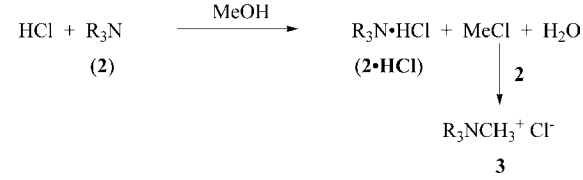
MeOH would produce unacceptably high levels of the byproduct MeCl in the isolated salt. As above, we substituted 37% aq HCl for anhydrous HCl to minimize potential MeCl formation. Controlling the concentration of MeCl in the isolated salt was a concern: higher amounts of MeCl found in the subsequent, larger batches could have arisen due to increased scale, higher temperature, or other factors not understood (Table 1).

Attempts to reduce the MeCl level in **2**·HCl by extended drying under vacuum were largely unsuccessful. Drying another batch at 85 °C and 30 inHg of vacuum reduced the level of MeCl from 80 ppm to 70 ppm, but required 12 days. Extended drying was expected to be of little value for the scale-up of the process (this batch of **2**·HCl was prepared by an unoptimized process not discussed here). It is possible MeCl was trapped inside the API particles but no attempt was made to reduce the particle size by grinding or other processing, in order to avoid both yield losses and increased exposure of personnel to the API candidates.

In order to salvage batches of **2**·HCl that contained high levels of MeCl, several approaches were examined and found to readily remove MeCl (Table 2). These results suggest that MeCl entrapped in the solids was either being removed or transferred to the mother liquids, or the MeCl was being hydrolyzed or otherwise decomposed under the rework conditions. Conditions in entry 2, adding a small amount of H_2O to dissolve the salt before adding MTBE, gave **2**·HCl with the lowest amount of MeCl and provided simple, high-throughput operations. A one kilogram demonstration run afforded a 92% recovery of **2**·HCl with <1.0 ppm of MeCl.

Experiments were undertaken to determine how to minimize the [MeCl] in isolated **2**·HCl without rework. As shown in Table 3, five parameters were examined: temperature of salt formation, agitation rate, duration of HCl addition, equivalents of HCl, and length of agitation time after MTBE addition. Salt formation at a higher temperature (35 °C) produced **2**·HCl contaminated with the highest level of MeCl; this may be due to accelerated formation of MeCl at higher temperatures. Experiments at lower temperatures produced **2**·HCl salt with less than 4 ppm of MeCl. Increasing the stirring time after adding MTBE slightly lowered the amount of residual MeCl (entries 2 and 5). Decreasing the HCl charge from 1.5 to 1.2 equiv reduced the level of MeCl, but with a lower yield (entries 2 and 7). Agitation rate and the duration of the HCl addition time had no significant impact. No attempt was made to correlate the [MeCl] in isolated **2**·HCl with particle size. These data suggest that the lower temperature of salt formation was the primary key in producing **2**·HCl of lower MeCl content.

Scheme 3. **Formation of quaternary ammonium salt 3**



The optimized process, with salt formation at 10 °C, was scaled up and produced 30 kg of **2**·HCl under cGMP conditions. Only 1 ppm of MeCl was detected, indicating that the level of MeCl was successfully controlled on scale. No recrystallization or further treatment was necessary. No problems are anticipated with further scale-up of this process.

Additional Studies. Several studies were conducted after successful preparation of **1**·HCl and **2**·HCl on scale. A possible impurity from generation of MeCl is the quaternary ammonium salt **3** formed by methylation of **2**

Table 4. **Effects of temperature and [HCl] on EtCl and MeCl content in salts[a]**

entry	substrate	solvent	[HCl]	[RCl] from salt formation at 10 °C	[RCl] from salt formation at 35 °C
1	**1**	EtOH	gaseous	<10 ppm EtCl	<10 ppm EtCl
2	**1**	EtOH	37% aq	<10 ppm EtCl	<10 ppm EtCl
3	**1**	EtOH	6 N aq	<10 ppm EtCl	<10 ppm EtCl
4	**2**	MeOH	anhyd	88 ppm MeCl	–
5	**2**	MeOH	37% aq (3.5 equiv of H_2O)	1 ppm MeCl	23.8 ppm MeCl
6	**2**	MeOH	6 *N* aq (8.1 equiv of H_2O)	17 ppm MeCl	44 ppm MeCl

[a] 1.5 equiv of HCl was charged in each experiment.

(Scheme 3). The iodide analogue of this impurity was prepared by reaction of the free base **2** with MeI, and an HPLC method was developed which could detect **3** at 0.015% (AUC) at 254 nm. In the above five development batches and the 30 kg scale-up batch of **2**·HCl generated under routine conditions, no **3** was detected.

Varying the concentration of HCl for the salt formation produced some unexpected results (Table 4) when compared with earlier data. In EtOH, higher temperatures during salt formation and the use of more concentrated HCl did not produce higher levels of EtCl in isolated **1**·HCl. However, we confirmed that using anhydrous HCl in MeOH led to high levels of MeCl in isolated **2**·HCl (entry 4). Unexpectedly, use of lower molarity HCl (6 N HCl in place of 12 N HCl (37% aq HCl)) in MeOH led to higher (17−44 ppm) levels, in spite of the additional water present in the aq HCl. The higher levels using more dilute HCl may have been due to a delayed crystallization, as the salt did not crystallize until MTBE was added and crystallization proceeded at a higher rate than before, possibly occluding MeCl.[34] In contrast, solids of **2**·HCl began to crystallize during the HCl addition when 37% aq HCl was charged to the solution of **2** in MeOH.

Summary and Conclusions

The presence of the genotoxic byproducts EtCl and MeCl in API candidates were concerns for the preparation of two hydrochloride salts generated in EtOH and MeOH. No problem occurred for one amine HCl prepared in EtOH; however, when the hydrochloride salt of another tertiary amine was prepared in MeOH using HCl(g), residual MeCl in the isolated **2**·HCl was >80 ppm. When HCl was charged as a 37% aq HCl solution, the levels of residual MeCl in initial batches were <4 ppm, but initial scale-up led to 11−12 ppm levels of MeCl. In order to salvage a batch of **2**·HCl produced on scale four rework procedures were developed, with recrystallization producing **2**·HCl with slightly lower amounts of residual MeCl. No MeCl was detected in the isolated **2**·HCl reworked by recrystallization (1 kg batch).

To preclude the need to rework future batches of **2**·HCl, we discovered that adding 37% aq HCl to a methanolic solution of the tertiary amine **2** at a lower temperature was key to minimizing the amount of MeCl in isolated **2**·HCl. Other factors investigated, including agitation time, agitation rate, and addition rate of HCl, were found to have little to no effect. These conditions were used to produce 30 kg of **2**·HCl under cGMP conditions, resulting in isolated **2**·HCl containing only 1 ppm MeCl.

In conclusion, we successfully prepared 18 and 30 kg batches of hydrochloride salts that were contaminated with the genotoxins, EtCl and MeCl, at <10 ppm and 1 ppm, respectively. The levels of these residual PGIs were comfortably below the staged TTC requirements based on anticipated human dosages.[15] For salt formation from methanolic solutions the critical parameters were using 37% aq HCl and maintaining the slurry at 10 °C during the HCl addition. Charging 1.5 equiv of HCl produced **2**·HCl in slightly higher yield than did charging 1.2 equiv of HCl; charging more than 1.5 equiv of HCl would be expected to produce higher levels of MeCl. While the low levels of alkyl chlorides in the isolated salts are partially consistent with the presence of water suppressing formation of alkyl chlorides, other factors such as product particle size and kinetics of crystallization cannot be ruled out.[35] Kinetic studies into the rate of alkyl chloride formation at various temperatures in the presence of H_2O would clarify how readily H_2O can reduce the burden of alkyl chloride formation, and whether the presence of the amine salts encourages the formation of the alkyl chlorides in the alcoholic solvents. These examples of our successful scale-up of two amine hydrochloride salts from EtOH and MeOH with control of residual EtCl and MeCl may be generally useful for the production of other amine hydrochlorides from alcoholic solvents.

Experimental Section

Preparation of 2·HCl on 30 kg Scale. A mixture of amine **2** (30.9 kg) and methanol (85.4 kg) was agitated for 1 h to afford a slightly cloudy solution, which was transferred by vacuum into a second kettle through a 1.2 μm in-line filter, followed by a rinse with methanol (12.6 kg). The contents were cooled to 0−10 °C, and HCl (37 wt %, 17 kg) was added over 2 h at 0−10 °C. The mixture was stirred at 0−10 °C for 1.5 h, and MTBE (182 kg) was added over 1 h at 0−10 °C. The resulting suspension was agitated at 0−10 °C for 2.5 h and filtered. The filter cake was washed with MTBE (2 × 46.2 kg), conditioned for 1 h, and dried under vacuum at 35−45 °C for 14 h to afford

(34) No extended drying studies or residual solvent assays were carried out for these batches. All large-scale batches had residual solvent levels well below the ICH guidelines.

(35) Temperature cycling of the suspension, as suggested by a reviewer, was not examined as treatment to reduce MeCl in isolated **2**·HCl. We thank the reviewer for this suggestion.

the desired product **2**·HCl salt as a white crystalline solid [30.8 kg, 88%, 99.8% (AUC) purity].[36]

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(36) The crystallization was carried out under a nitrogen blanket, and off-gases were scrubbed through a caustic solution in an effort to trap and neutralize any HCl and MeCl given off. Operations were performed under standard explosion-proof scale-up conditions. Diluting the off-gases with nitrogen mitigated the risk from generating the volatile and flammable byproducts Me_2O and MeCl. No effort was made to follow the generation of Me_2O and MeCl.

Supporting Information Available

Equipment and conditions for the headspace GC assays of MeCl, EtCl, and solvents involved in preparing hydrochloride salts. This material is available free of charge via the Internet at http://pubs.acs.org.

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