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INVESTIGATION OF THE 4-AMINO-α,α-DIHYDROXY-3,5-DICHLOROACETOPHENONE IMPURITY IN THE SYNTHESIS OF CLENBUTEROL HYDROCHLORIDE

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry at Virginia Commonwealth University.

by

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LIST OF ABBREVIATIONS

AcOH	-	Acetic Acid
ADAP	-	4-amino-3,5-dichloroacetophenone
ADBAP	-	4-amino-α-bromo-3,5-dichloroacetophenone
Addn	-	Addition
BICI	-	Boehringer Ingelheim Chemicals, Inc.
BIRG 0702	-	4-amino-α,α-dihydroxy-3,5-dichloroacetophenone
Br_2	-	Bromine
Cl_2	-	Chlorine
Clenbuterol	-	4-amino-α-[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol
dd	-	Doublet of doublets
Dibromo impurity	-	4-amino-α,α-dibromo-3,5-dichloroacetophenone
Dihydroxy Impurity	-	4-amino-α,α-dihydroxy-3,5-dichloroacetophenone
DoE	-	Design of Experiments
Eq	-	Equivalents
EtOAc	-	Ethyl Acetate
EtOH	-	Ethanol
g	-	Grams
hr(s)	-	Hour(s)
H_2O	-	Water
HCl	-	Hydrogen Chloride or Hydrochloride
HPLC	-	High Performance Liquid Chromatography
iPrOH	-	Isopropanol
J	-	Coupling constant
L	-	Liters
mHz	-	Mega Hertz
Min	-	Minutes
mL	-	Milliliters
mM	-	Millimolar
$NaBH_4$	-	Sodium Borohydride
NaOAc	-	Sodium Acetate
NMR	-	Nuclear Magnetic Resonance
PT	-	Proton Transfer
RT	-	Room Temperature
Rxn	-	Reaction
s	-	Singlet
TLC	-	Thin Layer Chromatography
vol	-	Volumes (i.e. 10 vol = 1 g solute per 10 mL solvent)

ABSTRACT

INVESTIGATION OF THE 4-AMINO-α,α-DIHYDROXY-3,5-DICHLOROACETOPHENONE IMPURITY IN THE SYNTHESIS OF CLENBUTEROL HYDROCHLORIDE

By Kerry Elizabeth Moore

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry at Virginia Commonwealth University.

Virginia Commonwealth University, 2014

Advisor: Dr. Scott Gronert, Department Chair and Professor, Chemistry

Clenbuterol Hydrochloride is a bronchodilator marketed as Spirospent® for Human Pharmaceuticals and as Ventipulmin® for Veterinary Pharmaceuticals by Boehringer Ingelheim.[1] This research investigates formation of the 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone impurity (Dihydroxy Impurity) in the synthesis of Clenbuterol Hydrochloride. The Dihydroxy Impurity has increased three-fold since the process was transferred to Boehringer Ingelheim Chemicals, Inc. in 1999.[1]

The Dihydroxy Impurity is proposed to be produced during an amination/reduction step from a reaction of water with the starting material.[9] Additionally, the Dihydroxy Impurity can be formed by the reaction of water with an impurity, 4-amino-α,α-didibromo-3,5-dichloroacetophenone (dibromo impurity), that is generated in the previous step.[9]

The formation of the dibromo impurity was investigated through a series of Design of Experiments (DoE) analyses. The results from these analyses, presented within, determined the

optimum bromination conditions to reduce the dibromo impurity. These conditions were able to reduce the dibromo impurity by 75%.

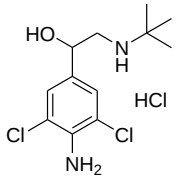
A series of water spiking experiments with both the starting material of the amination/reduction step and the dibromo impurity were performed to investigate the formation of the Dihydroxy Impurity. Based on the results, a mechanism for the formation of the Dihydroxy Impurity is presented. The three-fold increase of the Dihydroxy Impurity was concluded to be due to ≥ 15% water in the amination reaction mixture reacting with the starting material.

INTRODUCTION

Clenbuterol Hydrochloride

Clenbuterol Hydrochloride **1** (**Figure 1**), 4-amino-α-[(*tert*-butylamino)methyl]-3,5-dichlorobenzyl alcohol hydrochloride, is used as a decongestant and bronchodilator to treat breathing disorders, such as asthma.[1]

Figure 1: Clenbuterol Hydrochloride



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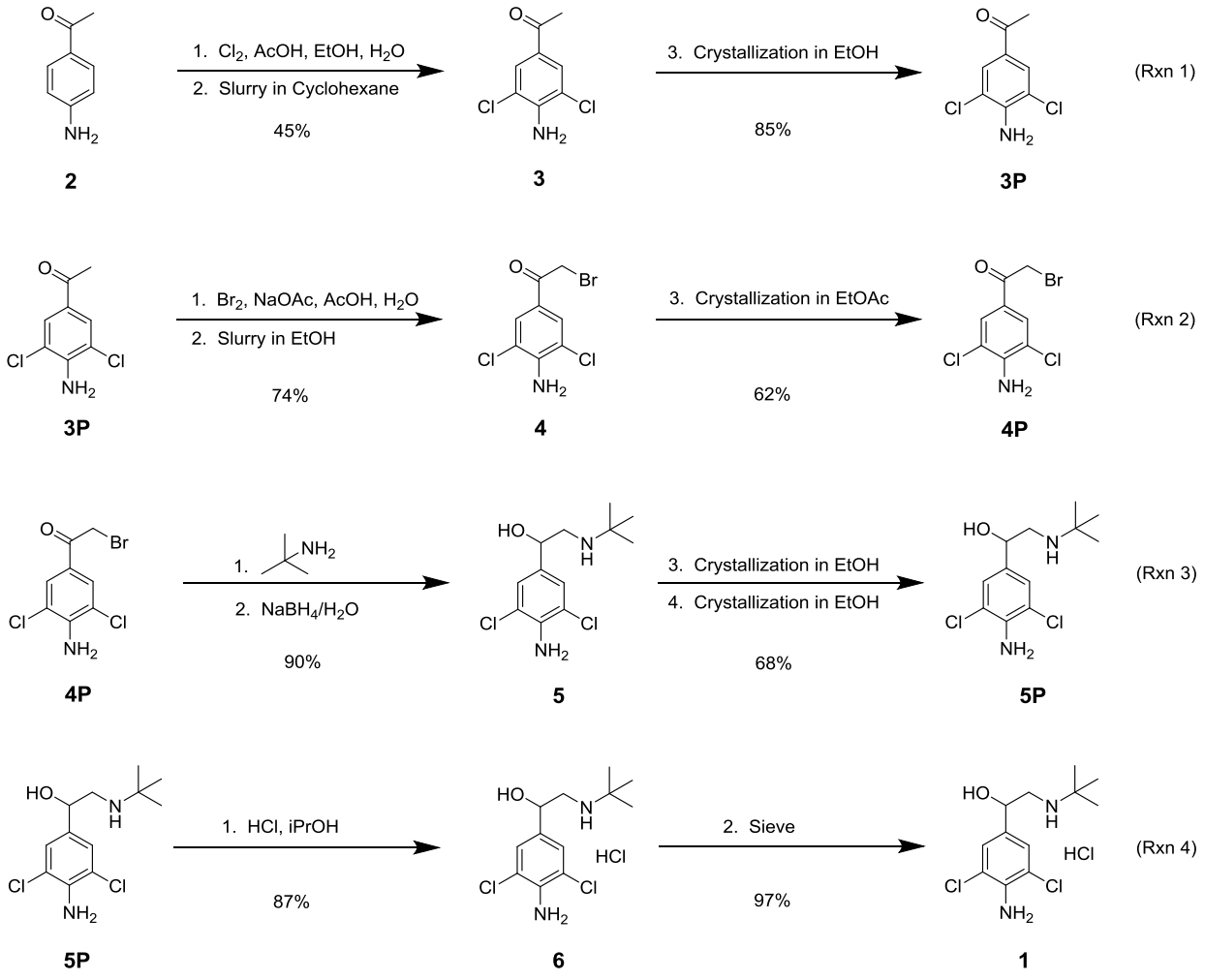
Clenbuterol Hydrochloride **1** was first synthesized at Thomae, a Boehringer Ingelheim research facility in Biberach, Germany, in 1967.[1] The synthesis of Clenbuterol Hydrochloride **1** was patented in the United States in 1970.[2] After comprehensive clinical trials, Clenbuterol Hydrochloride **1** was approved for the treatment of reversible airway obstruction in Germany in 1976 and later as a veterinary pharmaceutical for the treatment of bronchiolytic disorders in Germany in 1980.[1] Boehringer Ingelheim markets Clenbuterol Hydrochloride **1** as Spirospent® for Human Pharmaceuticals and as Ventipulmin® for Veterinary Pharmaceuticals.[1] Clenbuterol Hydrochloride **1** is not approved by the Federal Drug Administration for human use in the

United States, and the Clenbuterol Hydrochloride **1** currently made at Boehringer Ingelheim Chemicals, Inc. (BICI), in Petersburg, Virginia, primarily goes to Veterinary Pharmaceuticals.[1]

Overview of Clenbuterol Hydrochloride Process at BICI

The Clenbuterol Hydrochloride **1** process, shown in **Scheme 1**, was transferred from Thomae, in Biberach, Germany, to Boehringer Ingelheim Chemicals, Inc., in Petersburg, Virginia, in 1999. [1] Scheme 1 shows the 2013 average yields for each step.

Scheme 1: Clenbuterol Hydrochloride Process



The first step of the process (**Scheme 1, 2→3P**) involves the double chlorination of 4-aminoacetaphenone **2** through an electrophilic aromatic substitution reaction to yield 4-amino-3,5-dichloroacetophenone **3P** (ADAP).[1] The chlorination is followed by two purification steps: a slurry in cyclohexane to remove 2,4,6-trichloroaniline resulting from over chlorination[3] and a crystallization in ethyl acetate to remove the mono-chlorinated impurity, 4-amino-3-chloroacetophenone.[1] ADAP **3P** is analyzed by HPLC to ensure the removal these impurities.[4]

ADAP **3P** is brominated alpha to the ketone and purified twice (**Scheme 1, 3P→4P**) to yield 4-amino-α-bromo-3,5-dichloroacetophenone **4P**. The purpose of the purification steps was not described in the documentation associated with the transfer of the process. Additionally, neither of the intermediates, **4** nor **4P**, are analyzed for purity. Therefore, the purpose of the two purification steps and the purity of the product **4P** going into the next step are not fully clear.

In the third step of the process, the bromide of **4P** is displaced by *tert*-butylamine followed by reduction of the ketone moiety of **4P** with sodium borohydride ($NaBH_4$) to yield Clenbuterol Crude Free Base **5** (**Scheme 1, 4P→5P**), which is crystallized twice in ethanol in order to remove the 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone (dihydroxy impurity).[1] The formation of the dihydroxy impurity is discussed in more detail in the next section. The Clenbuterol Free Base **5P** intermediate is analyzed by HPLC to ensure the removal of the dihydroxy impurity before the material is processed further.[5]

The Clenbuterol Free Base **5P** is treated with hydrochloric acid in isopropanol to yield the hydrochloride salt, which is dried followed by the product being sieved to yield the final

product, Clenbuterol Hydrochloride **1** (**Scheme 1, 5P→1**). The final product is analyzed as per the specifications shown in **Table 1**.[6]

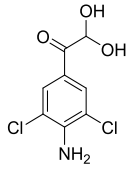
Table 1: Clenbuterol Hydrochloride Specifications[6]

Test		Acceptance Criterion
Description		White to yellowish powder or crystalline substance
Identification	IR	Compares to standard spectrum
	Test for Chlorides	Positive for Chlorides
Solubility		Soluble in water and ethanol, sparingly in acetone
Appearance of Solution	Clarity	Not more opalescent than reference II
	Color	Not more intensely colored than reference Y6
pH		5.0 – 6.5
Optical Rotation		-0.10° to +0.10°
Residue on Ignition		$\leq$ 0.1%
Heavy Metals		$\leq$ 10 ppm
Water Content		$\leq$ 0.5%
Titration Assay		99.0 – 101.0%
HPLC Assay		98.0 – 102.0%
HPLC Impurities	Individual (known)	$\leq$ 0.1%
	Individual (unknown)	$\leq$ 0.10%
	Total	$\leq$ 0.2%
Residual Solvents	Cyclohexane	$\leq$ 0.300%
	Ethanol	$\leq$ 0.010%
	Ethyl Acetate	$\leq$ 0.500%
	Isopropanol	$\leq$ 0.300%
	Toluene	$\leq$ 0.005%

History of the Dihydroxy Impurity

Dihydroxy impurity **7** (**Figure 2**) is observed during the testing of the Clenbuterol Free Base **5P** intermediate.

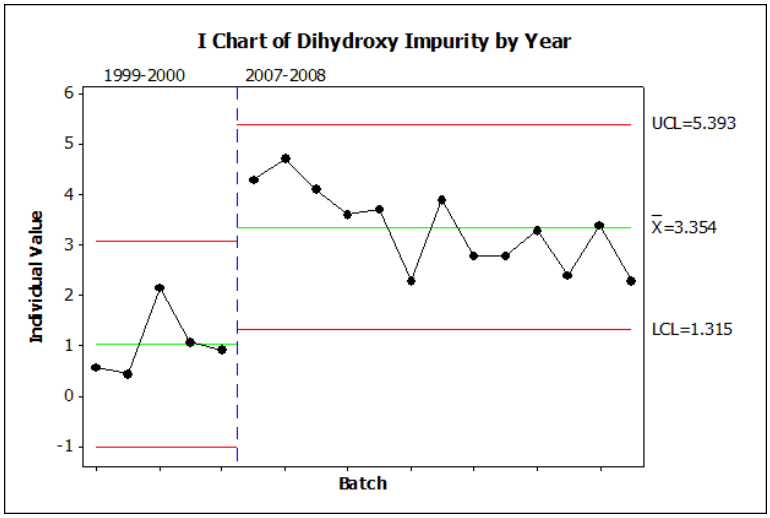
Figure 2: Dihydroxy Impurity



7

When the process was transferred to Petersburg, VA, the Clenbuterol Crude Free Base intermediate **5** contained an average of 1.0 % of dihydroxy impurity **7**; however, during campaigns from 2007 to 2008, the Clenbuterol Crude Free Base intermediate **5** contained over three times that amount of dihydroxy impurity **7**, an average of 3.4% (**Figure 3**).[7]

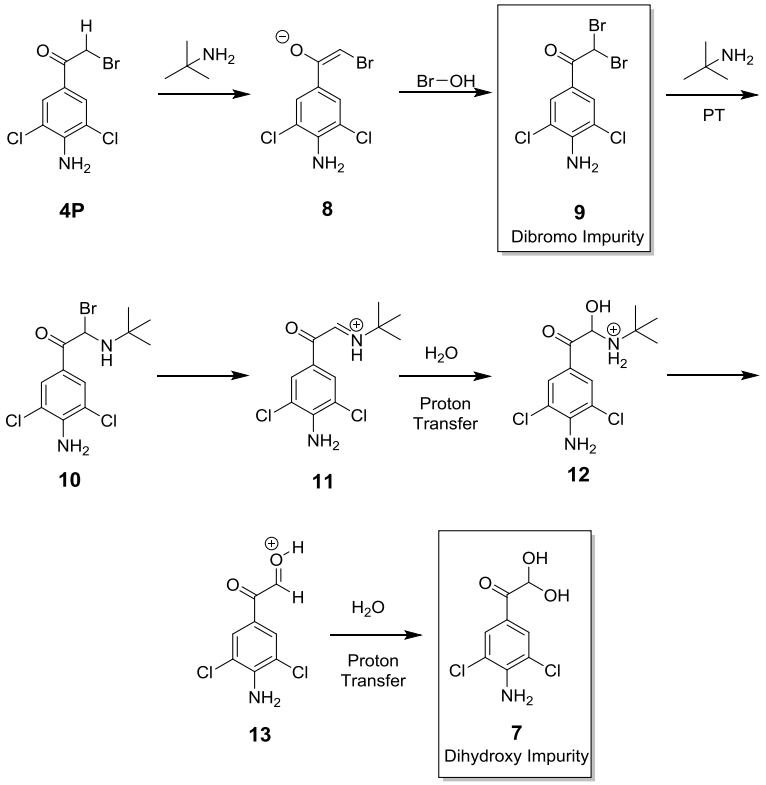
Figure 3: Increase in Dihydroxy Impurity

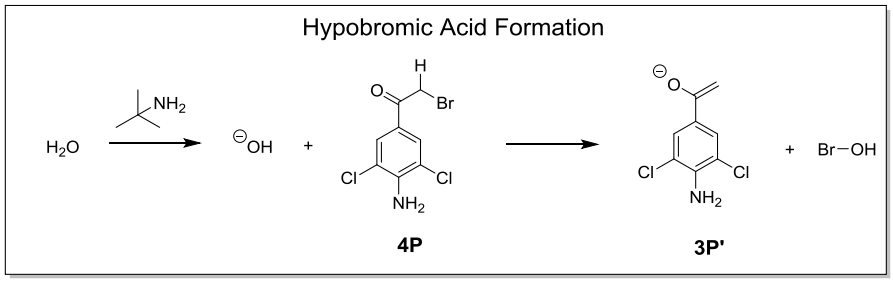


As a result of the three-fold increase of dihydroxy impurity **7**, a second crystallization was added in 2012 in order to get consistent quality and ensure that the Clenbuterol Free Base intermediate **5P** met specification.[8]

The proposed pathway for the formation of dihydroxy impurity **7** during the amination/reduction reaction (**Scheme 1, 4P→5P**) is shown in **Scheme 2** below: the reaction of the bromination product **4P** in a haloform-like reaction with hypobromic acid to form the 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**), which can then be hydrolyzed to the dihydroxy impurity **7**.[9]

Scheme 2: Potential Routes to the Dihydroxy Impurity





Hypobromic acid is proposed to be formed from an hydroxide anion attacking the bromide of ADBAP **4P**, as shown in the box at the bottom of **Scheme 2**. Additionally, a precursor to the dihydroxy impurity **7**, the dibromo impurity **9**, may also be coming into the amination/reduction reaction (**Scheme 1, 4P→5P**) as an impurity from the bromination reaction (**Scheme 1, Reaction 3P→4P**).[9] The carbonyl of dihydroxy impurity **7** is not reduced by sodium borohydride during the reduction reaction because it likely exists in the enol form under the reaction conditions.

The bromination product **4** or **4P** is not tested for purity and, therefore, it is not known how much of dibromo impurity **9** is generated in the bromination process and may be carried forward to make dihydroxy impurity **7**. Additionally, the water content going into the amination reaction (**Scheme 1, 4P→5, Step 1**) is not characterized.

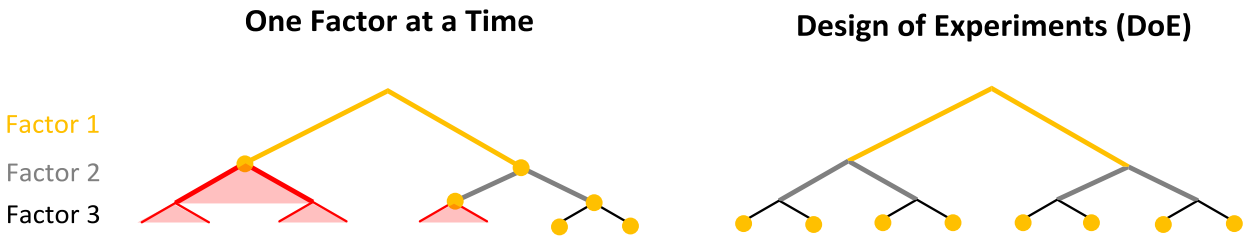
Research Objective

Since the Clenbuterol HCl **1** process was transferred to Boehringer Ingelheim Chemicals, Inc., dihydroxy impurity **7** has increased by over three-fold in the amination/reduction reaction (**Scheme 1, 4P→5P**).[7] It is proposed that dihydroxy impurity **7** is formed from a reaction of water with either bromination product **4P** or dibromo impurity **9** (**Scheme 2**). The purpose of this work is to understand the formation of dihydroxy impurity **7** by investigating dibromo impurity **9** formed in the bromination reaction (**Scheme 1, 3P→4P**) and the water content going into the amination/reduction step (**Scheme 1, 4P→5P**).

Design of Experiments

In the investigation of the dibromo impurity **9**, a statistical method of planning experiments and analyzing the data called Design of Experiments (DoE) was employed. DoE has benefits over the classic method of changing one factor at a time, such as looking at the entire range of all factors (also known as the design space) and investigating at the interactions between factors. A pictorial representation of how both the one factor at a time method and DoE work is shown below in **Figure 4**.

Figure 4: One Factor at Time vs. Design of Experiments



In the method of changing one factor at a time, Factor 1 (for example temperature) is tested with experiments (yellow dots) at high and low ranges until an optimum temperature is found based on the predetermined outputs. Factor 2 (for example concentration) is then tested at the optimum temperature and Factor 3 (for example equivalents of a reactant) is tested at the optimum temperature and concentration to determine the optimum conditions for the desired outputs; however, this method does not give any information about the interactions between the factors. The factors could interact in a way that the optimum conditions may not be explored by the one factor at a time method, as can be seen by the missing quadrants (red quadrants in **Figure 4**) in the one factor at time model.

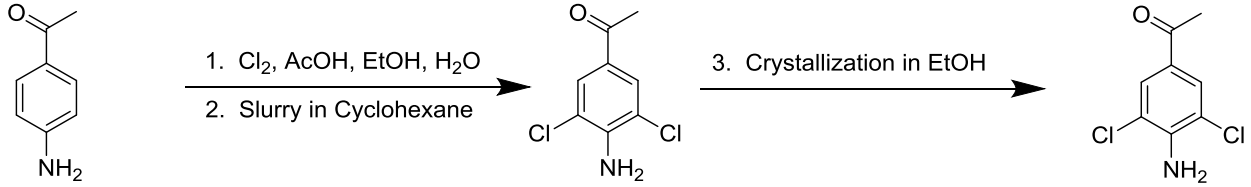
The DoE method inputs all of the factors initially and a series of experiments (yellow dots), testing the entire range of all factors, is generated in a random order typically using statistical software, such as Minitab©. Randomization of the experiments averages out the experimental error and the variation due to extraneous factors that are not controlled.[10] A full factorial DoE would run experiments with all variations of factors in their the defined range. A half factorial DoE would run half the experiments of a full factorial DoE, and a quarter factorial DoE would run a quarter of the experiments of a full factorial DoE. The lower factorial designs are useful to run a minimal number of experiments to determine what factors influence the output. Additionally, experiments may also be run in duplicate to estimate the experimental error or chance of variability.

RESULTS AND DISCUSSIONS

Chlorination

The first step of the Clenbuterol Hydrochloride **1** process (**Scheme 3**) is a double chlorination of 4-aminoacetaphenone **2** through an electrophilic aromatic substitution reaction and two purification steps to yield 4-amino-3,5-dichloroacetophenone **3P** (ADAP).[1]

Scheme 3: Chlorination



2 **3** **3P**

The double chlorination is followed by two purification steps: a slurry in cyclohexane to remove 2,4,6-trichloroaniline resulting from over chlorination[3] and a crystallization in ethyl acetate to remove the mono-chlorinated impurity, 4-amino-3-chloroacetophenone.[1]

Due to the ortho/para directing, electron donating, amino group and the meta directing, electron withdrawing, acetyl group, chlorination of 4-aminoacetophenone occurs primarily at the 3 and 5 positions over the 2 and 4 positions. Therefore, under chlorination would produce only the mono-chlorinated impurity, 4-amino-3-chloroacetophenone. Under these conditions, over chlorination does not result in the addition of chlorine to the 2 and 4 positions because the amino and acetyl groups do not direct that addition. Even though chlorides are ortho/para

directing and direct to the 2 and 4 position, chlorides are also deactivating. With another option available, over chlorination would result in the product, ADAP **3P**, undergoing a haloform-type reaction, eliminating chloroform with the addition of acetic acid to form an anhydride. With a good leaving group in place, the anhydride, the reaction would proceed through an S_NAr reaction to the 2,4,6-trichloroaniline impurity.[1,3]

The quantity, temperature, and rate of chlorine addition are critical to controlling both of these impurities.[1] It is apparent that an under charge of chlorine would produce the mono-chlorinated impurity, 4-amino-3-chloroacetophenone, while an over charge would produce the over-chlorinated impurity, 2,4,6-trichloroaniline.

The temperature during the chlorine addition is controlled between 0 - 8°C.[1] If the temperature goes below 0°C, then the rate of chlorination decreases causing an incomplete reaction, yielding the mono-chlorinated impurity, 4-amino-3-chloroacetophenone. In contrast, if the temperature goes above 8°C the rate of chlorination increases causing over chlorination, yielding the 2,4,6-trichloroaniline impurity.

The addition rate of the chlorine is between 3 – 8 hours, typically finishing around 7 hours.[1] The concentration of chlorine in the solution is determined by the addition rate; therefore, slower rates produce lower concentrations and faster rates produce higher concentrations. Lower concentrations of chlorine decrease the rate of chlorination and could result in an incomplete reaction; while higher concentrations of chlorine increase the rate of chlorination and could result in an over chlorination and the 2,4,6-trichloroaniline impurity.

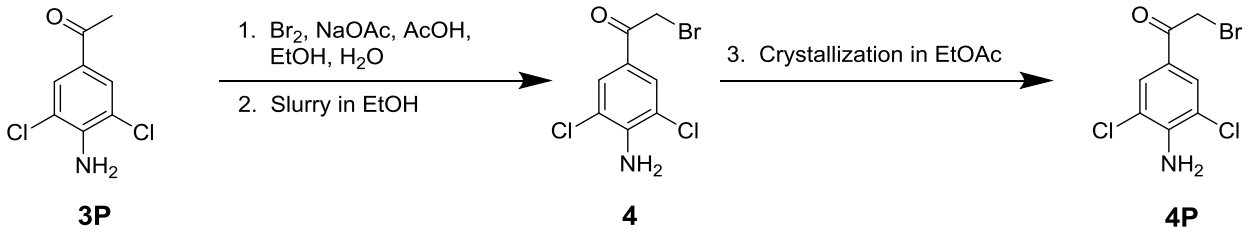
After the chlorination reaction and before the purification steps, higher amounts of the over chlorinated impurity, 2,4,6-trichloroaniline (30 – 50%), were observed in the lab scale reactions (40 g scale), versus < 0.5% in pilot plant scale reactions (12.3 kg scale, NOTE: two plant batches were tested from the September 2013 campaign during an investigation of failing in-process control samples for drying, the process is not routinely sampled and tested for purity prior to the slurry in cyclohexane). The likely reason for the difference in performance is the greater ability to control the amount of chlorine added and the addition rate of the chlorine gas on a larger scale. The slurry in cyclohexane was effectively able to remove all of the 2,4,6-trichloroaniline impurity formed in the lab runs.

It should also be noted that both the lab and pilot plant runs contained < 0.5% of the mono-chlorinated impurity, 4-amino-3-chloroacetophenone. Additionally, the samples tested from the plant after the chlorination reaction and before purification were ≥ 99.5% pure. These data suggest that the purification steps could potentially be removed from the pilot plant process pending additional data.

Bromination

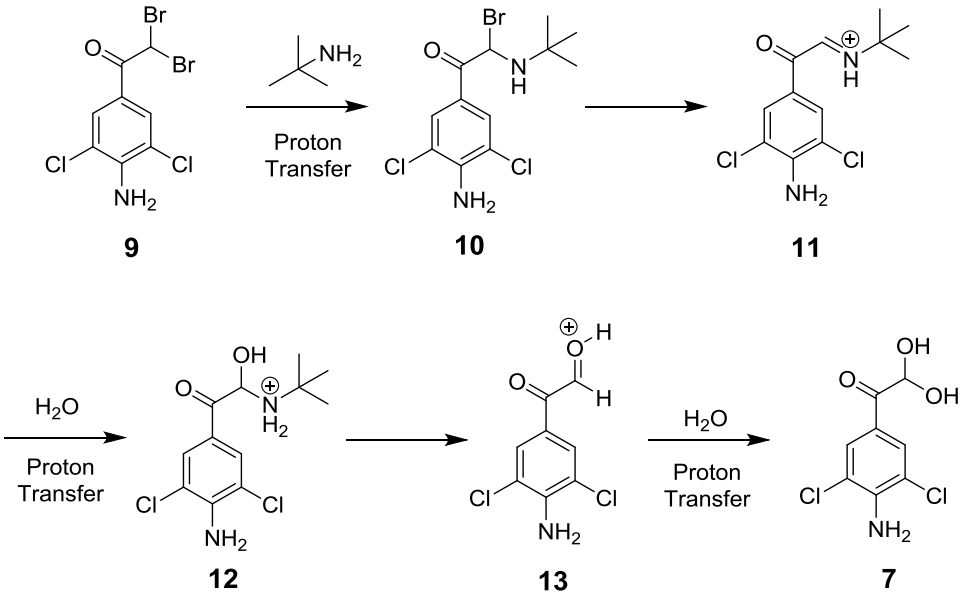
The second step of the Clenbuterol Hydrochloride **1** process (**Scheme 4**) is the alpha bromination of 4-amino-3,5-dichloroacetophenone **3P** (ADAP), followed by two purification steps to yield 4-amino-α-bromo-3,5-dichloroacetophenone **4P** (ADBAP).

Scheme 4: Bromination



Neither of the intermediates, **4** nor **4P**, are tested for purity in the current manufacturing process at Boehringer Ingelheim Chemicals, Inc.; therefore, the purpose of the two purification steps and the purity of the product **4P** going into the next step are not clear. As mentioned in the introduction (**History of the Dihydroxy Impurity**), one of the ways the impurity of interest, 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone (dihydroxy impurity **7**), is proposed to be formed is from an impurity generated during the bromination reaction, 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**, **Scheme 5**).

Scheme 5: Potential Route to the Dihydroxy Impurity from the Dibromo Impurity



In order to better understand the formation of dibromo impurity **9**, samples were taken after each step during the production of the bromination process at Boehringer Ingelheim Chemicals, Inc. in the October 2013 pilot plant campaign, which consisted of two batches of ADBAP Crude **4** and two batches of ADBAP Pure **4P.** The samples were analyzed for purity (area % by HPLC). The results are summarized in **Table 2**.

Table 2: Chromatographic Purity of Bromination Steps

Step	Batch	ADAP (**3P**)	ADBAP (**4 or 4P**)	Dibromo Impurity (**9**)	All Other Impurities
Post Bromination	2034173	6.4%	75.5%	16.3%	1.8%
	2034176	4.9%	78.7%	13.8%	2.6%
Post Slurry	2034173	1.9%	93.9%	4.2%	0.0%
	2034176	1.5%	95.5%	3.0%	0.0%
Post Crystallization	2034313	1.1%	97.9%	1.0%	0.0%
	2034315	0.5%	99.1%	0.4%	0.0%

One of the goals of this research is to reduce the formation of dibromo impurity **9**, which according to the most recent campaign is formed in about 15% yield following the bromination reaction prior to purification. The first step in investigating the bromination reaction was to determine what factors influenced the formation of dibromo impurity **9**. The current bromination process[1] begins by heating a solution of ADAP **3P** in 20 volumes of acetic acid (20 volumes = 20 mL per 1 g of ADAP **3P**) to 62 ± 2°C. A bromine solution in acetic acid is prepared and split into three equal portions. The first portion of the bromine solution is added over about 15 minutes, followed by the addition of 0.2 equivalents of sodium acetate. The

second portion of the bromine solution is added over about 15 minutes, followed by the addition of another 0.2 equivalents of sodium acetate. The third portion of the bromine solution is added over about 30 minutes, and the reaction mixture is cooled to 30 ± 5°C. A portion of the acetic acid is distilled, the contents are cooled, water is added, and the slurry is held for about an hour prior to filtration. The product is washed with water and dried at 40 ± 10°C under vacuum.

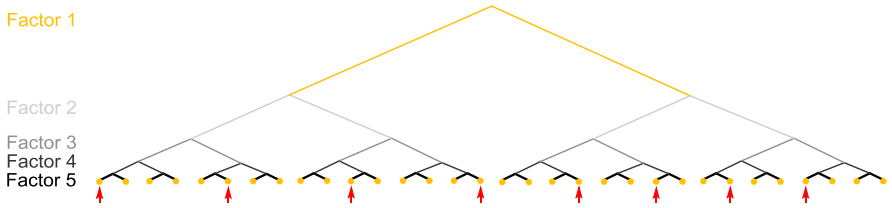
Sodium acetate is added as a buffer to avoid high concentrations of hydrogen bromide, which will produce overbromination.[1] A reaction was run in which the sodium acetate was added in one portion prior to the addition of the bromine solution. These conditions resulted in an incomplete reaction with over 60% starting material remaining. Based on the current process, the following factors were identified to potentially influence the formation of dibromo impurity **9**: temperature, equivalents of sodium acetate, portions of sodium acetate, rate of bromine addition, and concentration.

First Bromination Design of Experiments (DoE)

The purpose of the first bromination Design of Experiments (DoE) was to determine which of the factors: temperature, equivalents of sodium acetate, portions of sodium acetate, rate of bromine addition, and concentration, could affect the formation of 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**). Running experiments with these five factors in a DoE, a full factorial design would require 32 experiments (yellow dots in **Figure 5**);

however, a quarter-factorial design was chosen, which required only 8 experiments (red arrows in **Figure 5**).

Figure 5: Five Factor Full-Factorial vs Quarter-Factorial



The quarter-factorial design was chosen in order to scope out the design space and determine which factors influenced the formation of dibromo impurity **9** with the intent of later running a full-factor design with narrowed influencing factors. Two center points were also added to the quarter-factorial design, resulting in 10 experiments. The higher and lower limits for each of the five factors, which establish the design space, were based on the current process (**Table 3**).

Table 3: Factors for First Bromination DoE

Factor	Current Process	DoE Range
Temperature (°C)	62	30 - 70
Equivalents of NaOAc	0.4	0.2 – 0.6
Portions of NaOAc	2	1 – 3
Br_2 Addition Time (hrs)	1	0.5 – 3.0
Concentration (volumes*)	20	10 – 30

*20 volumes = 20 mL of acetic acid per 1 g of 4-amine-3,5-dichloroacetopheone

The predetermined output for this scoping DoE is primarily the percent of dibromo impurity **9** based on area percent by HPLC. However, the percent of starting material, product, and all other impurities were also analyzed.

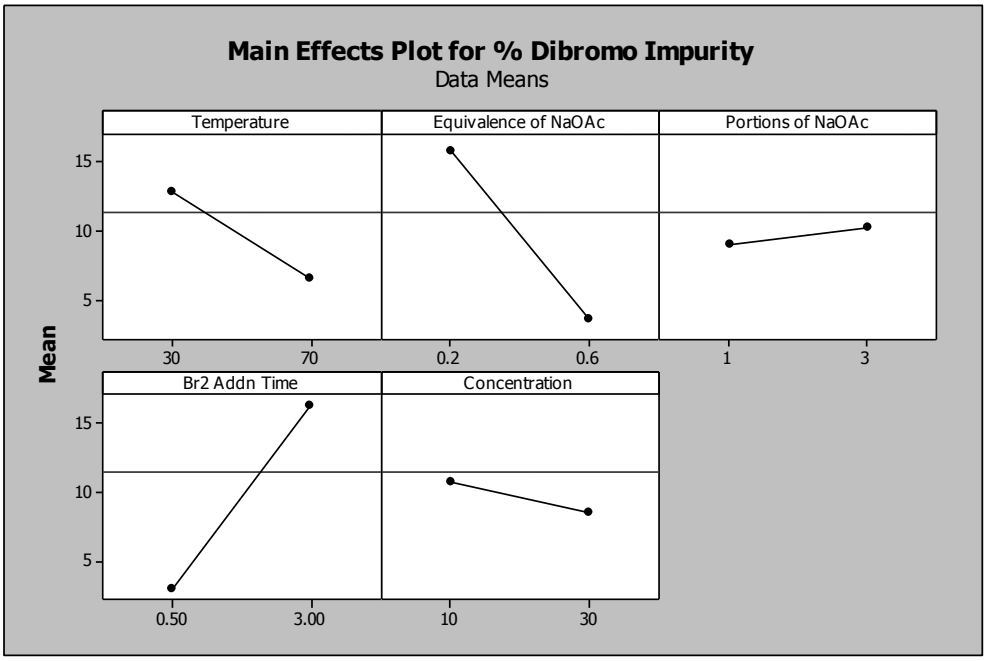
The DoE range was entered into the statistical program, Minitab©, and the list of experiments was generated with a random run order. The experiments were run on a 2 gram scale in the specified order. The raw data is summarized in **Table 4** and the Main Effects Plot for the percent of dibromo impurity **9** can be found in **Figure 6**.

Table 4: Raw Data for First Bromination DoE

Run Order	Temp (°C)	Equiv. NaOAc	Portions NaOAc	Br_2 Addn Time (hrs)	Conc. (Volumes*)	% Starting Material	% Product	% Dibromo Impurity	% Other
1	70	0.2	1	0.5	10	10	81	7	2
2	70	0.6	1	3.0	10	8	79	7	6
3	50	0.4	2	1.75	20	13	70	17	0
4	50	0.4	2	1.75	20	12	68	20	0
5	30	0.6	1	0.5	30	99	1	0	0
6	30	0.2	1	3.0	30	18	60	22	0
7	30	0.2	3	3.0	10	19	52	29	0
8	70	0.2	3	0.5	30	9	79	5	7
9	70	0.6	3	3.0	30	9	76	7	8
10	30	0.6	3	0.5	10	99	1	0	0

*10 volumes = 10 mL of acetic acid per 1 g of 4-amine-3,5-dichloroacetopheone

Figure 6: Main Effects Plot for Percent Dibromo Impurity



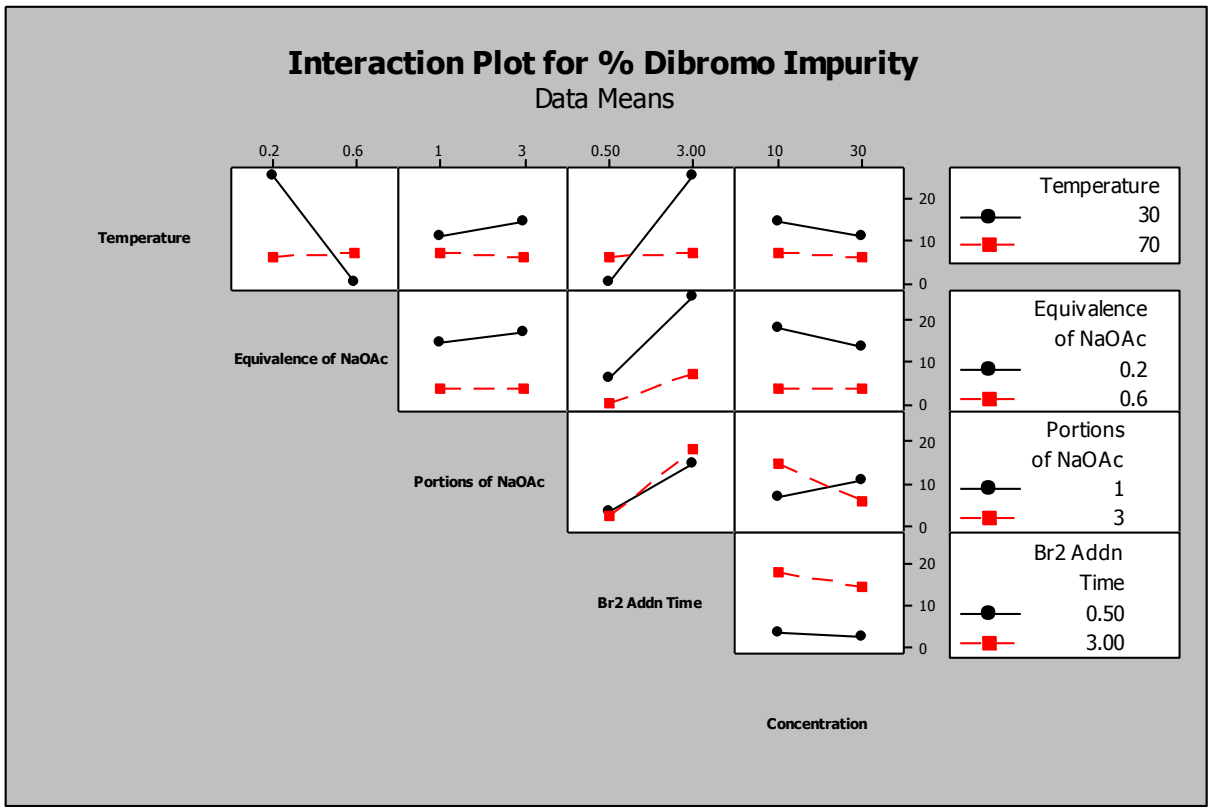
Reviewing the slope of the lines, it was determined that concentration and portions of sodium acetate had a minimal effect on the formation of dibromo impurity **9** due to the nearly horizontal slope. A horizontal slope indicates that experiments run at the higher and lower limits of that factor produced the same results for dibromo impurity **9**. On the other hand, temperature, equivalents of sodium acetate and bromine addition time produced steeper slopes indicating that these factors affect the results for dibromo impurity **9**.

Based on this data, higher temperatures, higher equivalents of sodium acetate and shorter bromine addition time conditions are predicted to produce lower amounts of dibromo impurity **9**. However, reviewing the raw data in **Table 4**, the experiments run with shorter bromine addition times at 30°C, resulted in incomplete reactions with no impurities. This could potentially skew the data of the first DoE, a consequence of running a quarter-factorial design. However, the quarter-factorial design was chosen to determine the influencing factors, not the

optimum conditions for reducing dibromo impurity **9**. The defining DoEs, run with a full-factorial design, will address the potential skewing of the data from the incomplete reactions.

One of the benefits of DoE is the ability to show the interactions between the factors. The interactions between the factors that influence the dibromo impurity **9** are shown in **Figure 7**.

Figure 7: Interaction Plot for Percent Dibromo Impurity



When analyzing the interaction plot, lines that cross determine if two factors have an interaction, with the greater interactions having a greater difference in slopes. For example, in the plot on the top left for the interaction between temperature/equivalents of sodium acetate, at higher temperatures (the red line), the equivalents of sodium acetate does not have

an effect; however at lower temperatures (the black line), the equivalents of sodium acetate does effect the amount of dibromo impurity **9**. Based on these data, the greatest interactions are between temperature/equivalents of sodium acetate and temperature/bromine addition time. These factors were also identified by the main effects plot to have an effect on the formation of dibromo impurity **9**, and were further investigated in the defining DoE.

It was noted that there were also interactions between portions of sodium acetate/bromine addition time and portions of sodium acetate/concentration; however, the interactions were smaller and the portions of sodium acetate and concentration did not have an effect on dibromo impurity **9** based on the main effects plot, so these factors were not included in future DoEs. For future experiments, these factors were held constant, concentration was set at the higher concentration, lower volumes, to reduce distillation time during the isolation and for simplicity the portions of sodium acetate was set at one portion.

Second Bromination Design of Experiments (DoE)

The purpose of the second bromination Design of Experiments (DoE) was to determine the optimum conditions for the bromination reaction by further examining the factors identified in the first scoping DoE to effect the formation of 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**): temperature, equivalents of sodium acetate and bromine addition time. This DoE was designed as a full-factorial with center points and duplicates, resulting in 18 experiments. The higher and lower limits for each of the three factors were based on both the current process and the first DoE (**Table 5**).

Table 5: Factors for Second Bromination DoE

Factor	Current Process	1st DoE Range	2nd DoE Range
Temperature (°C)	62	30 – 70	50 – 70
Equivalents of NaOAc	0.4	0.2 – 0.6	0.2 – 0.6
Br_2 Addition Time (hrs)	1	0.5 – 3.0	0.5 – 1.5

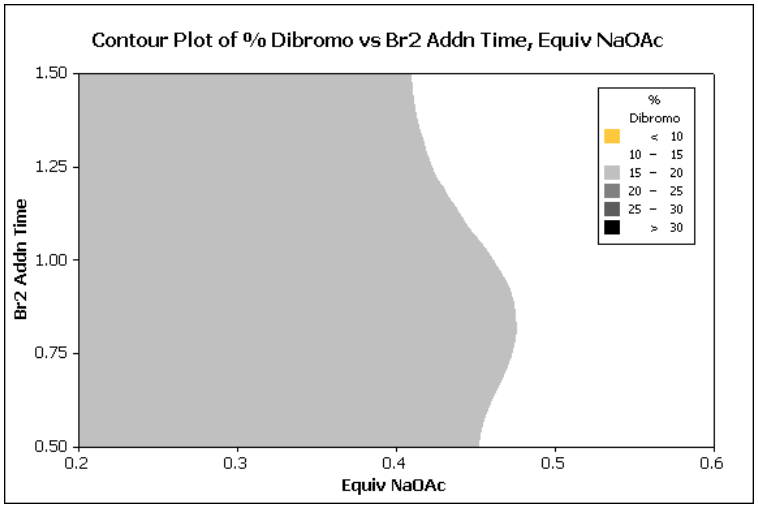
This DoE focused on higher temperatures to avoid the incomplete reactions that occurred in the first DoE. Additionally, the focus was moved to higher temperatures and shorter addition times based on the results of the first DoE. The predetermined outputs for this DoE are the percent of starting material, product, dibromo impurity **9**, and all other impurities based on area percent by HPLC.

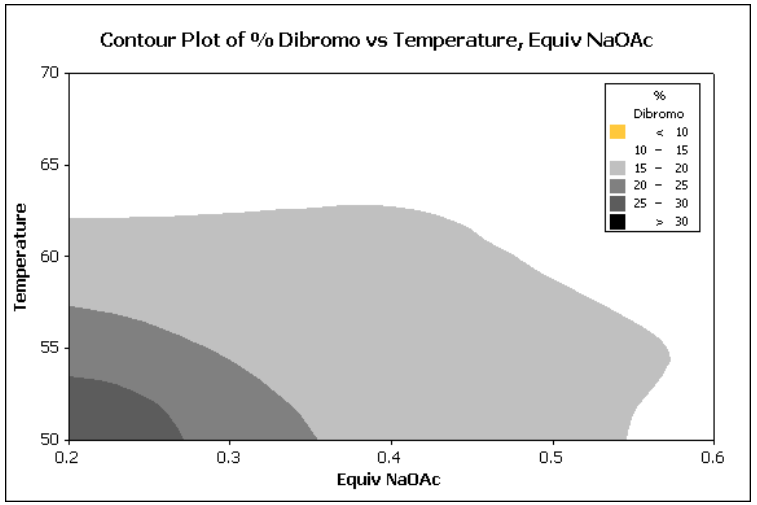
The DoE range was entered into the statistical program, Minitab©, and the list of experiments was generated with a random run order. The experiments were ran on a 2 gram scale in the specified order; the raw data is summarized in **Table 6** and the contour plots for percent of dibromo impurity **9** can be found in **Figure 8**.

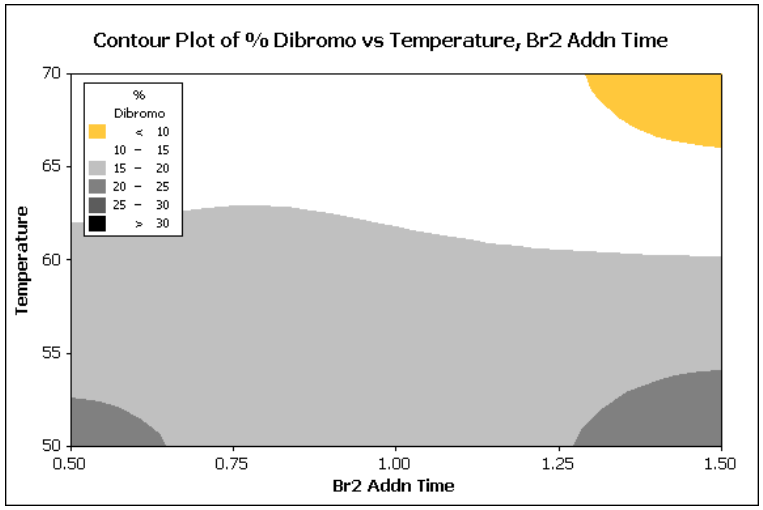
Table 6: Raw Data for Second Bromination DoE

Run Order	Temp (°C)	Equiv. NaOAc	Br_2 Addn Time (hrs)	% Starting Material	% Product	% Dibromo Impurity	% Other
1	50	0.2	1.5	13.5	60.4	26.1	0.0
2	50	0.2	1.5	13.3	60.0	26.7	0.0
3	70	0.6	0.5	34.1	53.6	11.7	0.6
4	70	0.6	1.5	10.2	77.7	11.7	0.4
5	50	0.6	0.5	36.7	47.8	15.3	0.2
6	50	0.6	1.5	38.4	45.2	16.3	0.1
7	60	0.4	0.75	9.6	74.0	16.3	0.1
8	50	0.6	1.5	36.8	47.2	15.8	0.2
9	70	0.2	1.5	8.8	79.7	6.7	4.8
10	70	0.2	0.5	8.3	78.4	12.4	0.9
11	70	0.6	1.5	6.5	84.0	5.1	4.4
12	60	0.4	0.75	9.8	76.0	13.9	0.3
13	70	0.6	0.5	32.5	53.4	14.0	0.1
14	50	0.2	0.5	13.8	61.0	25.2	0.0
15	50	0.2	0.5	13.3	56.5	30.2	0.0
16	70	0.2	0.5	8.2	78.4	11.6	1.8
17	50	0.6	0.5	38.8	48.6	11.9	0.7
18	70	0.2	1.5	8.4	75.7	11.0	4.9

Figure 8: Contour Plots for Percent Dibromo Impurity





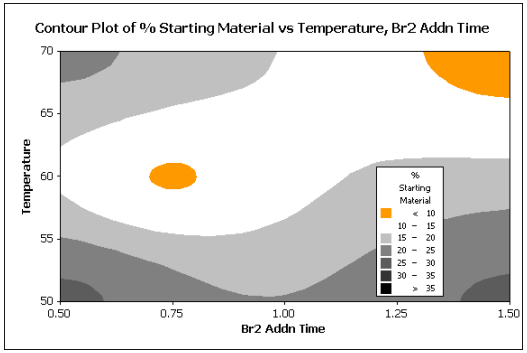


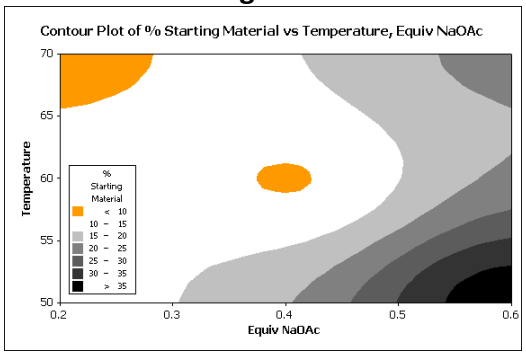
From “Contour Plot of % Dibromo vs Br_2 Addn Time, Equiv NaOAc,” it was determined that the formation of dibromo impurity **9** is reduced with higher equivalents of sodium acetate at all bromine addition times, as with from “Contour Plot of % Dibromo vs Temperature, Equiv NaOAc”. Therefore, it was concluded that higher equivalents of sodium acetate will minimize dibromo impurity **9** throughout the entire temperature and bromine addition design space. It was determined from “Contour Plot of % Dibromo vs Temperature, Br_2 Addn Time” that the formation of dibromo impurity **9** is reduced with higher temperatures and longer bromine addition times. The result of longer bromine addition time conflicts directly with the result from the first DoE, which leads us to conclude the data from the first DoE was somewhat skewed due to incomplete reactions at 30°C temperatures.

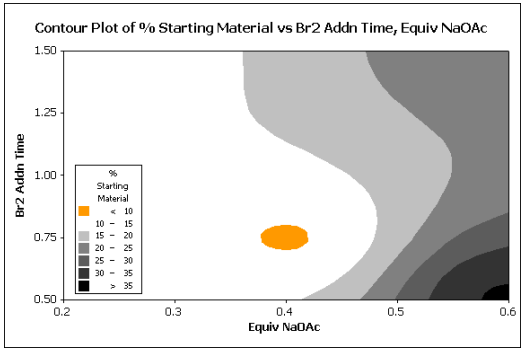
In order to find the optimum conditions, the other outputs (percent of starting material, product and all other impurities) were also analyzed (**Figure 9**).

Figure 9: Remaining Contour Plots for Starting Material, Product and All Other Impurities

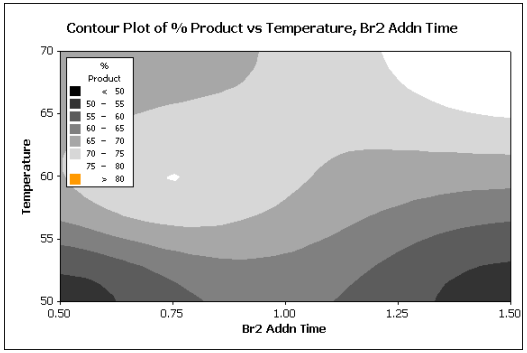
Starting Material

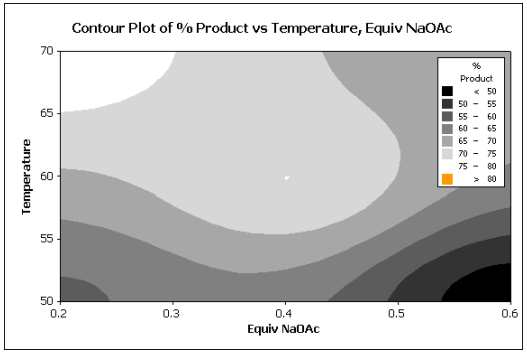


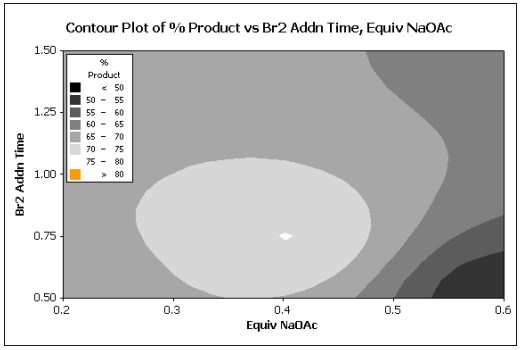




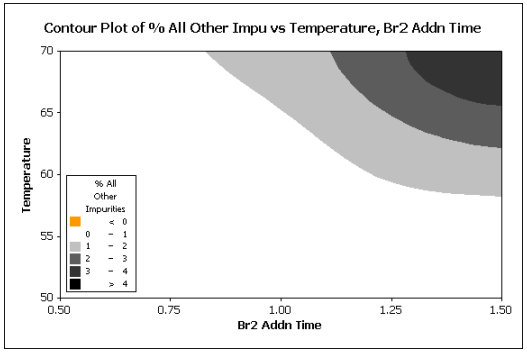
Product

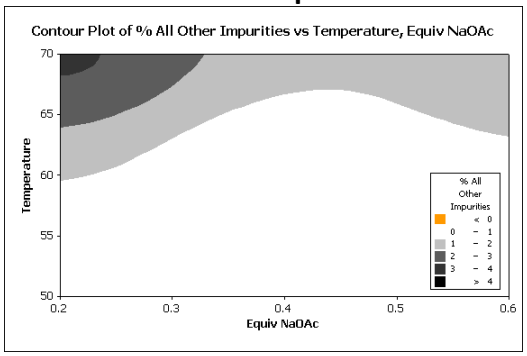


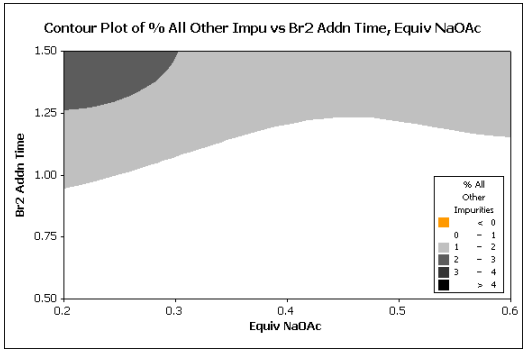




All Other Impurities







In alignment with the reduction of dibromo impurity **9**; reaction completion, in terms of reducing the percent of starting material and increasing the percent of product, also favored higher temperatures and longer bromine addition times. In contrast, reaction completion favored lower equivalents of sodium acetate; however, with the goal of reducing dibromo impurity **9**, using higher equivalents of sodium acetate would be a concession to sacrifice reaction progress for impurity reduction. Higher equivalents of sodium acetate was also favored in terms of percent of all other impurities as well as dibromo impurity **9**. It is important to note that while dibromo impurity **9** decreases with higher temperatures and longer bromine additions times, the percent of all other impurities increases.

Based on the results of the first two DoEs, the optimum conditions for the bromination reaction to reduce dibromo impurity **9** are higher temperatures (70°C), higher equivalents of sodium acetate (0.6 equivalents), and longer bromine addition times (1.5 hours). Since the optimum conditions exist at the corner of the design space, a third DoE was run keeping the equivalents of sodium acetate constant at 0.6 equivalents and examining higher temperatures and longer bromine addition times to better understand the limits of the optimum conditions.

Third Bromination Design of Experiments (DoE)

The purpose of the third bromination Design of Experiments (DoE) was to determine the limits of the optimum conditions to reduce 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**) with respect to temperature and bromine addition time. This DoE was designed as a full-factorial with center points and duplicates, resulting in 10 experiments. The

higher and lower limits for each of the three factors were based on both the current process and the previous DoEs (**Table 7**).

Table 7: Factors for Third Bromination DoE

Factor	Current Process	1st DoE Range	2nd DoE Range	3rd DoE Range
Temperature (°C)	62	30 – 70	50 – 70	70 – 90
Br_2 Addition Time (hrs)	1	0.5 – 3.0	0.5 – 1.5	1.5 – 3.0

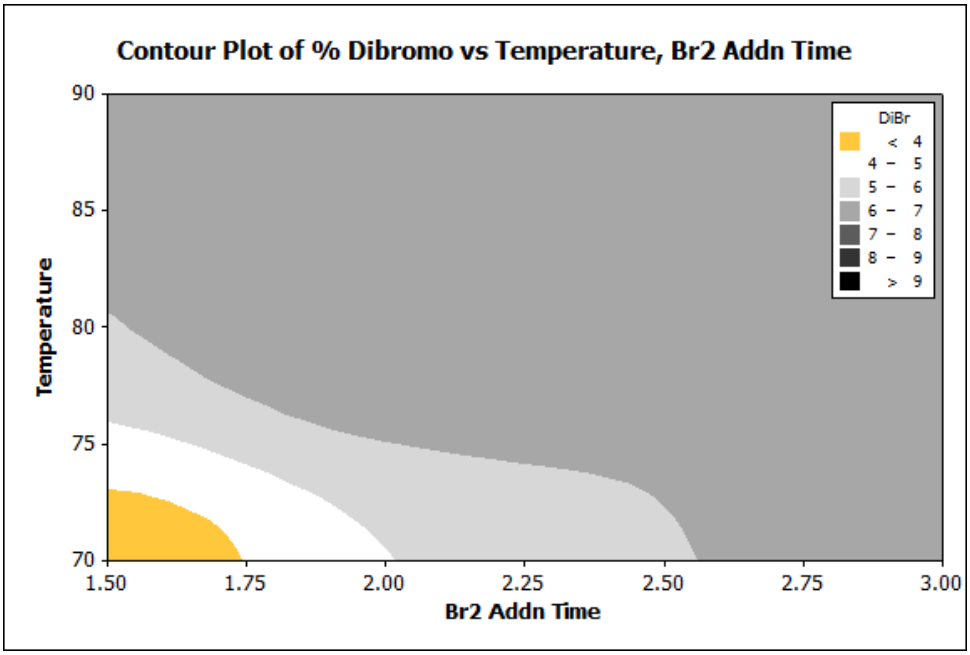
This DoE focused on higher temperatures and longer bromine addition times since the optimum conditions determined by the second bromination DoE were on the corner of the design space. The predetermined outputs for this DoE are the same as with the second bromination DoE: the percent of starting material, product, dibromo impurity **9**, and all other impurities based on area percent of the analytes by HPLC.

The DoE range was entered into the statistical program, Minitab©, and the list of experiments was generated with a random run order. The experiments were performed on a larger scale than previous DoEs, a 6 gram scale, in the specified order; the raw data is summarized in **Table 8** and the contour plot for percent of dibromo impurity **9** can be found in **Figure 10**.

Table 8: Raw Data for Third Bromination DoE

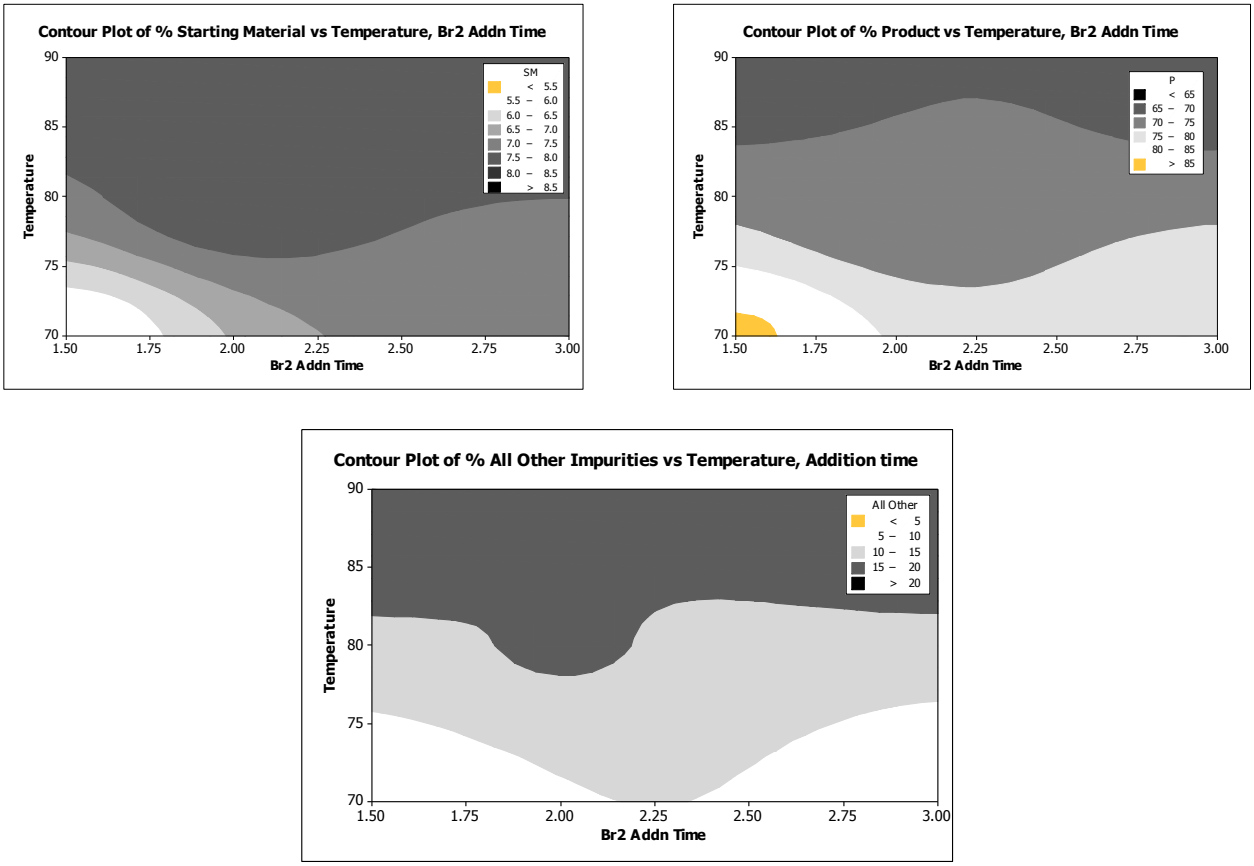
Run Order	Temp (°C)	Br_2 Addn Time (hrs)	% Starting Material	% Product	% Dibromo Impurity	% Other
1	70	3.0	7.6	78.9	7.4	6.1
2	70	1.5	5.9	81.9	5.2	7.0
3	80	2.0	7.2	72.7	6.3	13.8
4	90	3.0	8.8	60.7	6.3	24.2
5	90	1.5	7.5	67.7	6.4	18.4
6	80	2.0	8.6	67.4	7.2	16.8
7	70	3.0	6.8	81.1	5.0	7.1
8	90	1.5	8.3	64.8	6.2	20.7
9	90	3.0	7.0	72.4	6.7	13.9
10	70	1.5	5.2	89.3	1.9	3.6

Figure 10: Contour Plots for Percent Dibromo Impurity



From the contour plots of percent dibromo impurity **9** versus temperature and addition time in both the second and third bromination DoEs (**Figure 8** and **Figure 10**), it was concluded that there is a tight range for the optimum conditions to reduce dibromo impurity **9** with a temperature range of 67 - 78°C and a bromine addition time range of 1.25 – 1.75 hrs. The contour plots for starting material, product, and all other impurities, can be found below in **Figure 11**.

Figure 11: Contour Plots for Starting Material, Product and All Other Impurities



In alignment with the reduction of dibromo impurity **9**; reaction completion, in terms of reducing the percent of starting material and increasing the percent product, also favored lower temperatures (70°C) and shorter bromine addition times (1.5 hrs). Lower temperatures

and shorter bromine addition times were also favored in terms of percent of all other impurities as well as dibromo impurity **9**.

As a result of the three bromination DoEs, the optimum conditions for the bromination reaction to reduce the formation of dibromo impurity **9**, compared to the current process, is summarized in **Table 9.**

Table 9: Optimum Bromination Conditions

Factor	Current Process	Optimum Conditions
Temperature (°C)	62	70
Equivalents of NaOAc	0.4	0.6
Portions of NaOAc	2	1
Br_2 Addition Time (hrs)	1	1.5
Concentration (volumes)	20	10

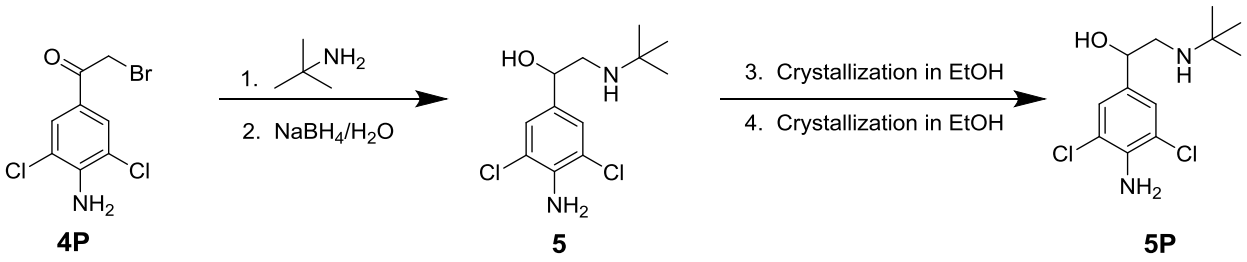
The optimum conditions reduced dibromo impurity **9** by 75% with comparable yields. Additionally, these conditions would allow for the removal of one of the purification steps, benefitting both time and yield. One of the purification steps would need to remain in place to ensure no water is carried over into the amination/reduction reaction.

Amination/Reduction

In the third step of the Clenbuterol Hydrochloride **1** process (**Scheme 6**), the bromide of 4-amino-α-bromo-3,5-dichloroacetophenone **4P** (ADBAP) is displaced by *tert*-butylamine

followed by reduction of the ketone moiety of **4P** with sodium borohydride ($NaBH_4$) to yield Clenbuterol Crude Free Base **5**.

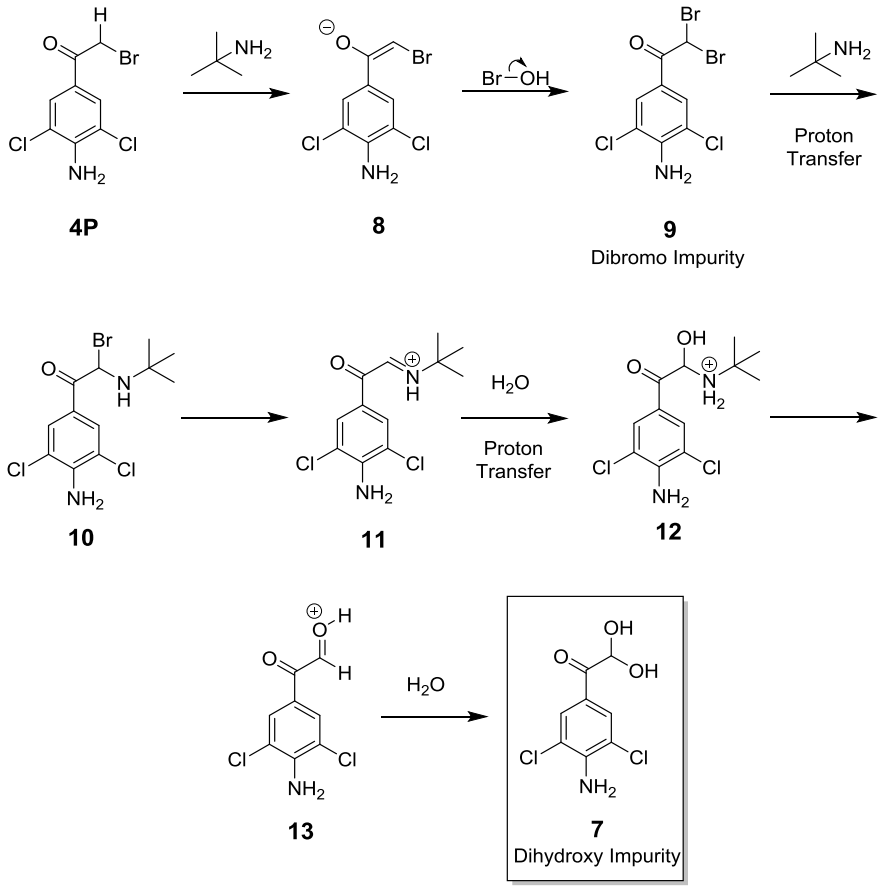
Scheme 6: Amination/Reduction



The amination/reduction reaction (**4P→5**), is run by slowly adding ADBAP **4P** to *tert*-butylamine (12.5 equivalents) while keeping the temperature between 20 - 28°C. The reaction mixture is held at this temperature for at least 30 minutes prior to the addition of a solution of sodium borohydride (3.1 equivalents assuming 4 hydrides) in water (2 volumes) while keeping the temperature between 20 - 28°C. The reaction mixture is held at this temperature for at least 30 minutes prior to the addition of water (8 volumes). The slurry is then held for at least an hour prior to filtration. The product is washed with water and dried at 40°C under vacuum.

The crude product **5** is recrystallized twice in ethanol in order to remove the 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone (dihydroxy impurity **7**). Dihydroxy Impurity **7**, formed in the amination/reduction reaction (**4P→5**), was proposed to result from a reaction of water with either the starting material (**4P**) or dibromo impurity **9** as illustrated in **Scheme 7**.

Scheme 7: Potential Routes to the Dihydroxy Impurity



The addition of water during the amination reaction was proposed to enhance the formation of dihydroxy impurity **7**. A series of water spiking studies during the amination reaction were designed to test this hypothesis starting with both dibromo impurity **9** and ADBAP **4P**. Under this hypothesis, it was expected that the addition of more water would produce more of dihydroxy impurity **7**. The dibromo impurity **9** used in the amination water spiking experiments was produced with the optimum bromination conditions (See **Third Bromination DoE** Section) using 2 equivalents of bromine; the product was 95% pure by HPLC and was also tested for water content by Karl Fischer and found to have ≤ 0.1% water. The results of the water spiking experiments with dibromo impurity **9** are summarized in **Table 10**.

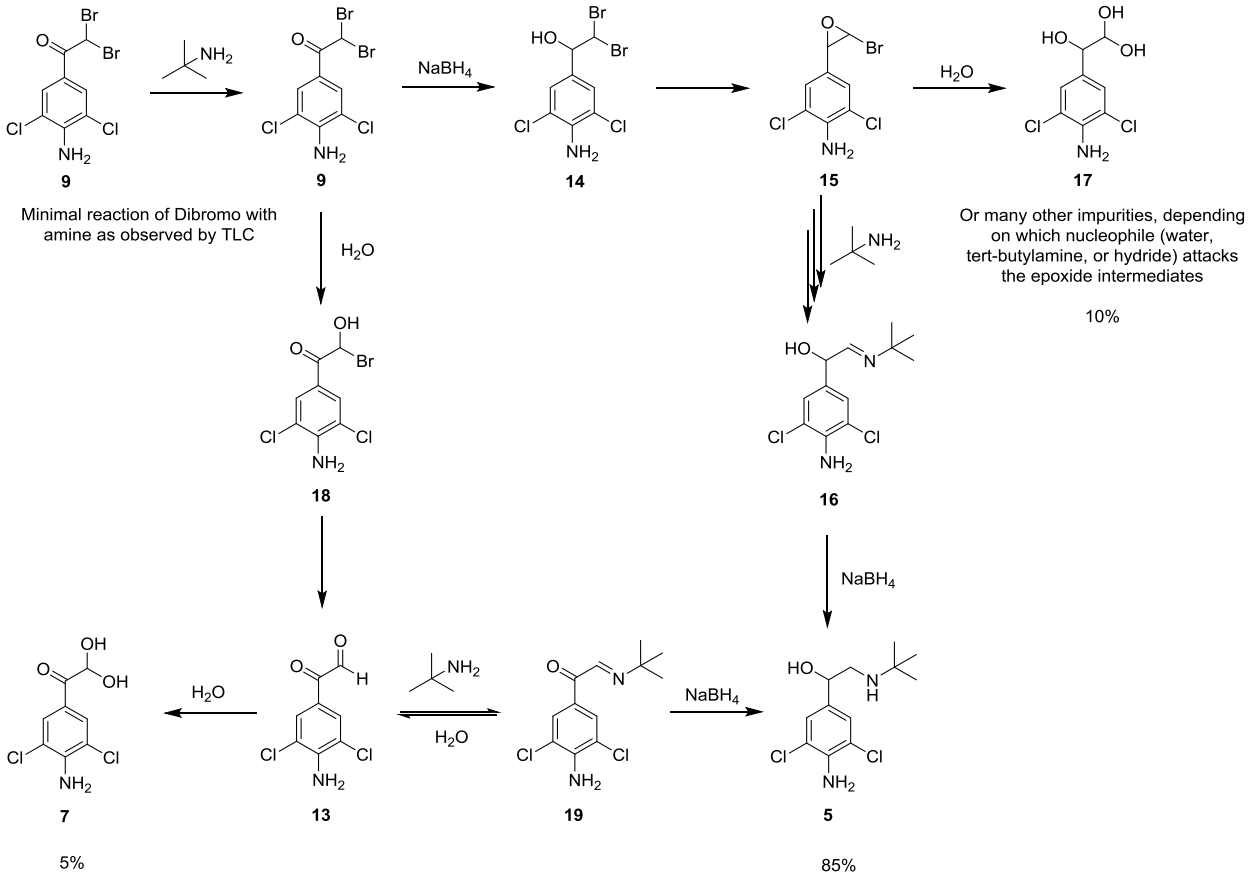
Table 10: Amination Water Spiking Experiments with Dibromo Impurity

% Water	% Dihydroxy 7	% Clenbuterol 5	% All Other Impurities
0%	4.8%	85.9%	9.3%
5%	1.8%	95.6%	2.6%
10%	2.0%	94.1%	3.9%
15%	1.4%	97.3%	1.3%

The results of the water spiking experiments in the amination reaction with dibromo impurity **9** were not as expected. Dibromo impurity **9** was not expected to form the product, Clenbuterol **5.** Monitoring the reaction by thin-layer chromatography (TLC) showed no reaction occurred when dibromo impurity **9** was added to *tert*-butylamine; however, once the water sodium borohydride solution was added, the primary product, Clenbuterol **5**, was formed. There are two pathways in which dibromo impurity **9** could lead to Clenbuterol **5**.

In the first pathway (**Scheme 8, 9→15→5**), the ketone moiety of dibromo impurity **9** is reduced by sodium borohydride to form the hydroxy intermediate **14**, which undergoes an intramolecular S_N2 reaction to form epoxide **15**. *Tert*-butylamine opens the epoxide and form hydroxyl imine **16** by eliminating bromide. The imine moiety of intermediate **16** is then reduced by sodium borohydride to produce Clenbuterol Free Base **5**. It should also be noted that there are other nucleophiles in the solution (hydride and water) that could open the epoxide intermediate **15** to form other impurities such as trihydroxy **17** (**Scheme 8, 15→17**). This may explain the larger percentage of other impurities seen in these reactions.

Scheme 8: Dibromo Impurity under Amination/Reduction Conditions



The second pathway (**Scheme 8, 9→ 13→5**) to form Clenbuterol Free Base **5** from dibromo impurity **9** is by reacting first with water to produce hydroxyl bromide intermediate **18** This would be the case with the amination spiking experiments with 5 – 15% water. It is also a possibility in the amination reaction with 0% water that hydroxyl bromide intermediate **18** forms after the addition of the water sodium borohydride solution, in which dibromo impurity **9** may react with water prior to sodium borohydride. Hydroxyl bromo intermediate **18** collapses to form aldehyde **13**, which then reacts with water or *tert*-butylamine leading to Dihydroxy Impurity **7** or keto-imine intermediate **19** respectively.

In the amination spiking experiments with 5 – 15% water, where aldehyde intermediate **13** would be formed prior to the addition of the water sodium borohydride solution, *tert*-butylamine is in larger excess, thus favoring the equilibrium toward keto-imine **19** and towards Clenbuterol Free Base **5** by reduction with sodium borohydride. This explains why water spiking experiments with 5 – 15% water produce less dihydroxy impurity **7** than the experiment with 0% water. Additionally, during the amination reaction, water acts as a catalyst in the formation of keto-imine **19**, which explains why the 5 – 15% water spiking experiments produced similar amounts of dihydroxy impurity **7**.

In the amination reaction with 0% water, aldehyde intermediate **13** may be reached after the addition of the water sodium borohydride solution. In this case, water would be in larger excess and would drive toward the formation of dihydroxy impurity **7** (**Scheme 8, 13→7**). dihydroxy impurity **7** is not significantly greater in the amination reaction of dibromo impurity **9** with 0% water, because the majority would follow the first pathway (**Scheme 8, 9→15→5**) due to the higher reactivity of sodium borohydride.

Based on these data, dibromo impurity **9** primarily goes to form product under the amination/reduction conditions. During the amination reaction, water catalyzes the formation of keto-imine **19**, which leads to Clenbuterol Free Base **5** post reduction. Therefore, it was concluded that the source of three-fold increase of dihydroxy impurity **7** was not due to the carry over of dibromo impurity **9** with ADBAP **4P**. In addition, the pathway to dihydroxy impurity **7** likely does not go through dibromo impurity **9** as originally proposed.

The same amination water spiking experiments were also run with ADBAP **4P** to investigate the formation of dihydroxy impurity **7** from the reaction of water with ADBAP **4P**. The ADBAP **4P** used in these experiments was produced with the optimum bromination conditions as determined by the series of Design of Experiments (DoEs), and was purified as per the current process to ≤ 0.2% dibromo impurity **9**. The ADBAP **4P** was also tested for water content by Karl Fischer and found to have ≤ 0.1% water. The results of the water spiking experiments with ADBAP **4P** are summarized in **Table 11**.

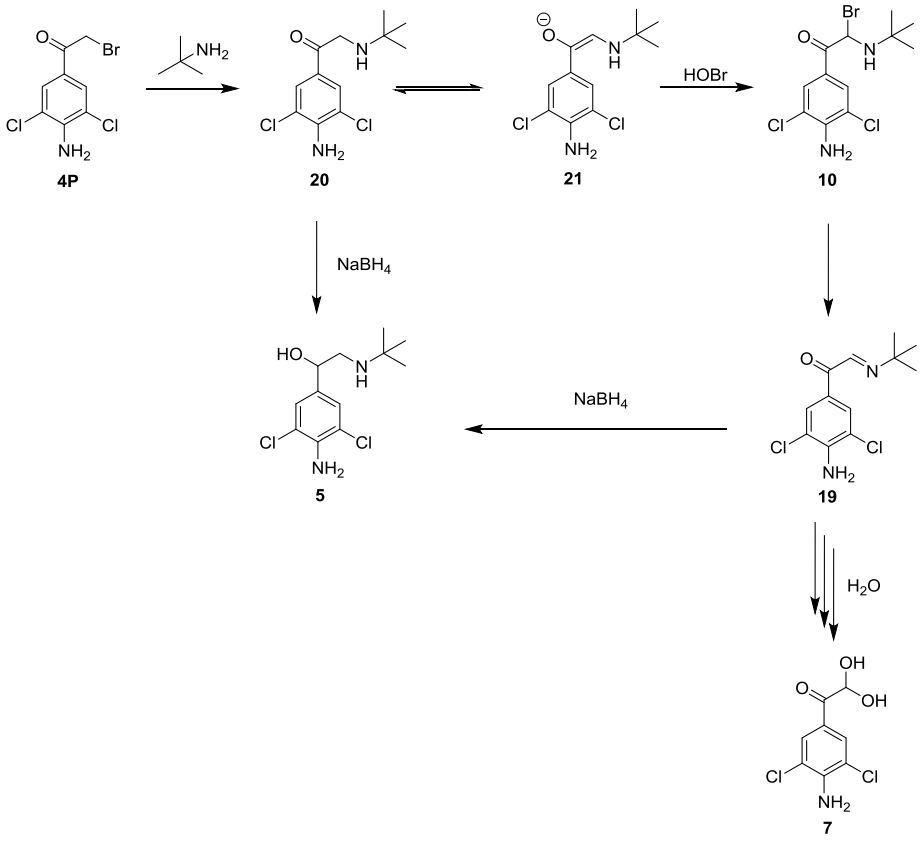
Table 11: Amination Water Spiking Experiments with ADBAP

% Water	% Dihydroxy 7	% Clenbuterol 5	% All Other Impurities
0%	0.9%	98.6%	0.5%
5%	0.8%	98.6%	0.6%
10%	0.8%	98.4%	0.8%
15%	1.8%	97.4%	0.8%

The results of the amination water spiking experiments with ADBAP **4P** were also not as expected; however, further insight into the mechanism of the formation of dihydroxy impurity **7** was gained. It was expected that without water present in the amination reaction, no dihydroxy impurity **7** would be formed. Similar amounts of dihydroxy impurity **7** (about 1%) were formed in the experiments with 0 – 10% water, which indicates that some dihydroxy impurity **7** is inherent in the current process. Dihydroxy impurity **7** was seen by monitoring with Thin Layer Chromatography (TLC), which additionally indicates that dihydroxy impurity **7** is inherent to the process and not formed on analysis by HPLC. Based on these data, the

amination reaction can tolerate up to 10% water without forming any additional dihydroxy impurity **7**. This amount of dihydroxy impurity **7** (about 1%) aligns with the amount see in the original transfer of the process in 1999 as well. The proposed pathway from ADBAP **4P** to both dihydroxy impurity **7** and Clenbuterol Free Base **5** is shown below in **Scheme 9**.

Scheme 9: ADBAP under Amination/Reduction Conditions



The pathway from ADBAP **4P** to keto-amine **20** through amination followed by reduction to Clenbuterol Free Base **5** is the desired pathway for this reaction. However, with *tert*-butyl amine in excess, keto-amine **20** is in equilibrium with enol **21**. Enol **21** then reacts with hypobromic acid, an in situ generated electrophile from a reaction of *tert*-butylamine hydrobromide salt (a byproduct of the amination reaction **4P→20**) and water, to form the

bromo amine intermediate **10**, which collapses to form keto-imine **19**. Keto-imine **19** may be reduced to form Clenbuterol Free Base **5** or may react with water to form dihydroxy impurity **7**.

The formation of hypobromic acid requires a certain amount of water to be present, likely to disassociate the hydrobromide from the *tert*-butylamine hydrobromide salt. Based on the data in **Table 11**, the water needed to disassociate the hydrobromide salt is either at least 15% water in the amination reaction or the water added with the aqueous sodium borohydride solution. Again, this means that some dihydroxy impurity **7** is inherent to the process because there will always be water present at some point in the reaction mixture.

Inverse addition experiments were run by adding the solution of *tert*-butylamine and keto-amine **20** to the aqueous sodium borohydride solution. The purpose of these experiments was to determine if dihydroxy impurity **7** is inherent to the process or if by increasing the amount of hydride relative to keto-amine **20** with the inverse addition could drive the reaction towards Clenbuterol Free Base **5**. The inverse addition experiments showed formation of dihydroxy impurity **7** throughout the addition as determined by HPLC analysis of aliquots from the reaction mixture. The isolated product from the inverse addition experiments contained about 10% of dihydroxy impurity **7**, confirming that dihydroxy impurity **7** is inherent to the process. The inverse addition experiment has a greater ratio of water to *tert*-butylamine hydrobromide salt, which would facilitate the disassociation of the hydrobromide salt and thus explains the higher percentage of dihydroxy impurity **7** for the inverse addition.

The three-fold increase from about 1% to about 3% of dihydroxy impurity **7** is due to ≥ 15% water in the amination reaction reacting with the starting material, ADBAP **4P**. The 1% of

dihydroxy impurity **7** present during the initial transfer of the process in 1999 to Boehringer Ingelheim Chemicals, Inc. is inherent to the process and formed from water added during the reduction step. Reducing the presence of water in the amination reaction to ≤ 10% would generate only the amount of dihydroxy impurity **7** inherent to the process (about 1%), which could then be reduced to meet the specification for dihydroxy impurity **7** with only a single crystallization in ethanol.

Formation of Dihydroxy Impurity in Plant Batches

The previous section concluded that the 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone impurity (dihydroxy impurity **7**) is due to the presence of ≥ 15% water in the amination reaction mixture reacting with 4-amino-α-bromo-3,5-dichloro-acetophenone (ADBAP **4P**). The amination/reduction process is currently run on a scale of about 5 kg in a 100 L vessel. At this scale, the 15% of water necessary to increase the formation of dihydroxy impurity **7** is about 60 mL. The ADBAP **4P** produced in the plant was tested for water content by Karl Fischer and found to have ≤ 0.1% water. Additionally, the tert-butylamine used in the plant is also tested for water content by Karl Fischer to a specification of ≤ 0.5% water.[11] Therefore, the incoming materials are not the source of the ≥ 15% water in the amination reaction.

The current process at Boehringer Ingelheim Chemicals, Inc. uses one vessel; therefore, the amination reaction of any batch other than the first batch is performed in a vessel that previously contained water. Steps have been put in place to dry the reactor under heat and

vacuum between batches; however, based on recent batches having around 2% dihydroxy impurity **7**, this may not be sufficient. The configuration of the vessel could allow for hold up of about 60 mL without being seen. Two efficient solutions for ensuring no water carry over from one batch to another are: (1) rinse with water miscible solvent such as methanol or acetone and dry the vessel under heat and vacuum between batches or (2) use a two vessel system in which no water would enter the vessel used for the amination reaction. Additionally, the same pump is used to charge *tert*-butylamine and water in the current process. The pump and associated lines could also carry over some water to the next batch. A separate pump for *tert*-butylamine should be used to eliminate the introduction of water to the amination reaction. With the preventative actions in place to ensure there is no carryover of water from the vessel or the pump used to charge *tert*-butylamine, the amount of dihydroxy impurity **7** should decrease to the amount that is inherent to the process and the amount that was seen in the original transfer in 1999, 1% dihydroxy impurity **7**.

CONCLUSIONS

The chlorination process was primarily run to build starting material for an investigation into 4-amino-α,α-dibromo-3,5-dichloroacetophenone (dibromo impurity **9**) formed in the bromination process. During these experiments it was determined that the control of the chlorine addition in the plant greatly exceeds the control in laboratory scale additions. With better control of the chlorine addition, the plant is able to control both of the main impurities to minimal levels that both of the chlorination purification steps could potentially be eliminated from the process. This change would need additional data from plant batches tested by the quality control group and a quality assessment of the impact to the bromination process with the product of the chlorination coming out of a different solvent.

The bromination process was optimized through a series of Design of Experiments (DoEs) to reduce the formation of dibromo impurity **9**. The optimum conditions reduced the formation of dibromo impurity **9** by 75% from the current process while maintaining similar yields. This could potentially allow for one of the bromination purification steps to be eliminated. One of the purification steps would need to remain in place to ensure water is not carried through to the amination/reduction process. This change would also need additional experiments to ensure no quality impact to the amination/reduction process.

Through a series of water spiking experiments of the amination reaction with both 4-amino-α-bromo-3,5-dichloro-acetophenone (ADBAP **4P**) and dibromo impurity **9,** it was determined that the three-fold increase in 4-amino-α,α-dihydroxy-3,5-dichloroacetophenone (dihydroxy impurity **7**) is due to the presence of ≥ 15% water in the amination reaction reacting with ADBAP **4P**. The process can tolerate ≤ 10% of water in the amination reaction without forming additional dihydroxy impurity **7**. There is about 1% of dihydroxy impurity **7** that is inherent to the process and is formed from the water added in the reduction step. The majority of dibromo impurity **9** carried into the process as an impurity in ADBAP **4P** goes on to form product, Clenbuterol Free Base **5**. The current manufacturing process in the plant was evaluated and preventative actions to ensure there is no carryover of water from the vessel or the pump used to charge *tert*-butylamine were suggested to reduce dihydroxy impurity **7** to the amount (about 1%) that is inherent to the process.

EXPERIMENTAL DATA

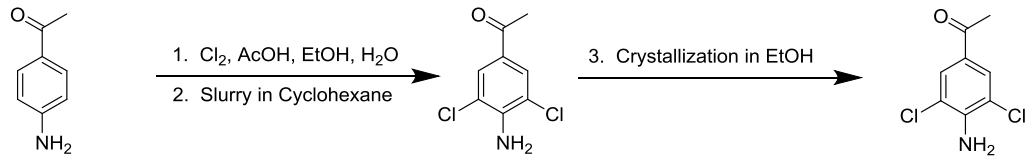
General Experimental Information

Commercial and reagent grade chemicals were used as obtained. All reactions were conducted under nitrogen. The 4-amino-3,5-dichloroacetophenone **3P** used in the first and second bromination Design of Experiments was produced in the lab using the chlorination general procedures described below. The 4-amino-3,5-dichloroacetophenone **3P** used in the third bromination Design of Experiments was produced in the Bay 31 pilot plant at Boehringer Ingelheim Chemicals, Inc. (BICI), IA129 batch 2033835. The chlorination procedures were carried out in a 1 L Lab-Max Jacketed Reactor with content temperature control. The bromination and reduction/amination procedures were carried out in round-bottomed flasks utilizing a J-KEM connected to an oil bath for temperature control. Temperatures of 0°C were obtained with ice/water bath. Amination/Reduction reactions were monitored by thin layer chromatography (TLC) using Whatman MKC18F precoated silica gel plates with a 30% ethyl acetate in hexanes solvent system.

Process monitoring during the chlorination and solid intermediates from the chlorination and bromination reactions were analyzed for purity versus analytical standards by high performance liquid chromatograph (HPLC) on an Agilent 1100 Series system using a Zorbax SD-C18 column (4.6 x 150 mm, 5 µm particle size) and gradient from 2 - 100% acetonitrile in

water over 30 min with a 1 mL/min flow rate. Products from the bromination reaction were analyzed for water content by a Mettler Toledo Coulometric Karl-Fischer Autotitrator with Aquastar® Coulomat A and Aquastar® Coulomat C. Products from the amination/reduction were analyzed by HPLC on an Agilent 1100 Series system using an Inertsil ODS-2 column (4.6 x 150 mm, 5 µm particle size) and a mobile phase 5mM 1-Decane Sulfonic Acid Sodium Salt in 25 mM Potassium Phosphate Buffer (pH 3), Methanol, Acetonitrile (69:16:15) over 70 minutes with a 1.5 mL/min flow rate as per intermediate specification.[5] H^1 NMR spectra were recorded on Varian Mercury-300 (300 mHz) at ambient temperature. Chemical shifts are referenced to residual chloroform (δ = 7.26 ppm).

Chlorination



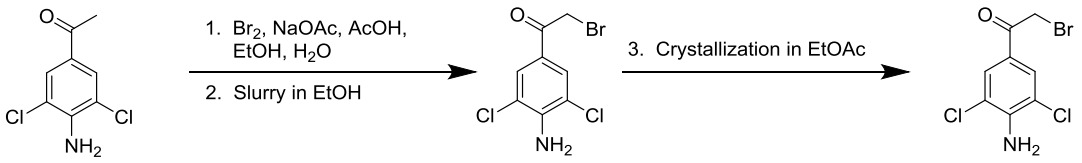
2 **3** **3P**

General Procedure for 4-Amino-3,5-dichloroacetophenone (3): Synthesized according to procedure described in BICI master production record for ADAP Pure.[12] A mixture of 4-aminoacetophenone **2** (1 eq), acetic acid (9.8 vol), ethanol (1.95 vol), and water (0.5 vol) was cooled to 0°C. Chlorine gas (1.8 eq) was added slowly keeping temperature between 4-10°C. The reaction progress was monitored by HPLC. Water (15.25 vol) was added and the resulting slurry was stirred at 5°C for at least 1 hr prior to vacuum filtration and washing with water (18.75 vol). The solids were dried at 40°C under vacuum overnight. The crude product was then reslurried in cyclohexane (6.25 vol) by heating to 55°C for at least 30 min prior to vacuum

filtration and washing with cyclohexane (1.25 vol). The solids were dried at 40°C under vacuum overnight to afford the titled compound.

General Procedure for 4-Amino-3,5-dichloroacetophenone (3P). Synthesized according to procedure described in BICI master production record for ADAP Recrystallized.[13] A mixture of 4-amino-3,5-dichloroacetophenone **3** (1 eq) and ethanol (12.3 vol) was heated to reflux and slowly cooled to 0°C prior to vacuum filtration and washing with ethanol (2.9 vol). The solids were dried at 40°C under vacuum overnight to afford the titled compound: H^1 NMR ($CDCl_3$, 300 MHz) δ 7.80 (s, 2 H), 4.91 (s, 2 H), 2.48 (s, 3 H).

Bromination



General Procedure for the Synthesis by the Current Conditions of 4-Amino-α-bromo-3,5-dichloroacetophenone (4). Synthesized according to procedure described in BICI master production record for ADBAP Crude.[14] A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (20 vol) was heated to 62°C. A solution of bromine (1 eq) in acetic acid (0.6 vol compared to bromine) was added over a specified amount of time (1 hrs) with sodium acetate (0.4 eq) added in 2 portions in the middle of the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with a temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was

cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C. The crude product was reslurried in ethanol (4 vol) by heating to 55°C and stirring for at least 30 min. The mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with ethanol (3 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound.

First Bromination Design of Experiments General Procedure for 4-Amino-α-bromo-3,5-dichloroacetophenone: A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (10 – 30 vol) was heated to 30 - 70°C. A solution of bromine (1 eq) in acetic acid (0.6 vol compared to bromine) was added over a specified amount of time (0.5 – 3 hrs) with a specified number of portions (1 – 3) of sodium acetate (0.2 – 0.6 eq) added equally spaced throughout the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with a temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound.

Second Bromination Design of Experiments General Procedure for 4-Amino-α-bromo-3,5-dichloroacetophenone: A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (10 vol) was heated to 50 - 70°C. A solution of bromine (1 eq) in acetic acid (0.6 vol

compared to bromine) was added over a specified amount of time (0.5 – 1.5 hrs) with sodium acetate (0.2 – 0.6 eq) added in the middle of the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with a temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound.

Third Bromination Design of Experiments General Procedure for 4-Amino-α-bromo-3,5-dichloroacetophenone: A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (10 vol) was heated to 70 - 90°C. A solution of bromine (1 eq) in acetic acid (0.6 vol compared to bromine) was added over a specified amount of time (1.5 – 3 hrs) with sodium acetate (0.6 eq) added in the middle of the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with a temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound.

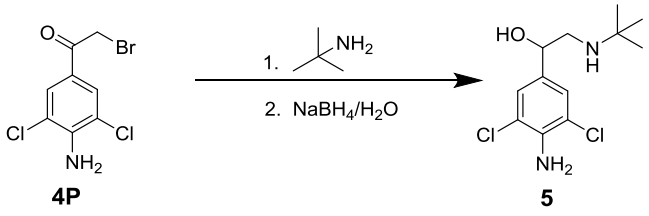
General Procedure for the Optimized Synthesis of 4-Amino-α-bromo-3,5-dichloroacetophenone (4): A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (10 vol) was heated to 70°C. A solution of bromine (1 eq) in acetic acid (0.6 vol compared

to bromine) was added over a specified amount of time (1.5 hrs) with sodium acetate (0.6 eq) added in the middle of the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with a temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C. The crude product was reslurried in ethanol (4 vol) by heating to 55°C and stirring for at least 30 min. The mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with ethanol (3 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound.

General Procedure for the Synthesis of 4-Amino-α-bromo-3,5-dichloroacetophenone 4P. Synthesized according to procedure described in BICI master production record ADBAP Pure.[15] A mixture of 4-amino-α-bromo-3,5-dichloroacetophenone **4** (1 eq), ethyl acetate (5.3 vol), celite (0.04 wt%), and carbon (0.08 wt%) was heated to reflux. The mixture was vacuum filtered to remove celite and carbon and washed with ethyl acetate (2.6 vol). The resulting solution was heated to 45°C and vacuum distilled (50 mbar) until about 3 vols remained. The mixture was cooled to 0°C and stirred for at least 2 hrs prior to vacuum filtration and washing with ethyl acetate (2 vol). The solids were then dried under vacuum at 40°C overnight to afford titled compound: H^1 NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 2 H), 5.05 (s, 2 H), 4.29 (s, 2 H).

General Procedure for the Optimized Synthesis of 4-Amino-α,α-dibromo-3,5-dichloroacetophenone (Dibromo Impurity 9): A mixture of 4-amino-3,5-dichloroacetophenone **3P** (1 eq) in acetic acid (10 vol) was heated to 70°C. A solution of bromine (2 eq) in acetic acid (0.6 vol compared to bromine) was added over a specified amount of time (1.5 hrs) with sodium acetate (0.4 eq) added in the middle of the addition of the bromine solution. The mixture was cooled to 30°C, and distilled under vacuum (50 mbar) with temperature of 55°C to a minimum volume. After cooling to 40°C, water (12.5 vol) was added and the mixture was cooled to room temperature and stirred for at least 1 hr prior to vacuum filtration and washing with water (at least 12.5 vol). The solids were dried under vacuum at 40°C overnight to afford titled compound: H^1 NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 2 H), 6.51 (s, 1 H), 5.11 (s, 2 H).

Amination/Reduction

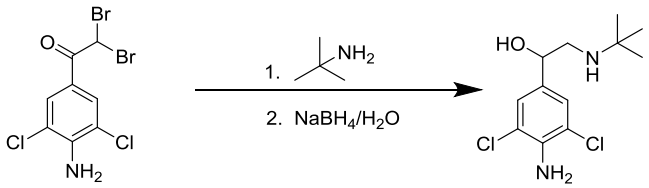


4P **5**

General Procedure for the Water Spiking studies of 4-Amino-α-bromo-3,5-dichloroacetophenone 4P in the Synthesis of Clenbuterol Free Base (5). Synthesized according to procedure described in BICI master production record for Clenbuterol Crude Free Base (0 eq water).[16] To a solution of *tert*-butylamine (12.5 eq) spiked with water (0 – 0.15 eq) at room temperature, 4-amino-α-bromo-3,5-dichloroacetophenone **4P** (1 eq) was added slowly keeping the temperature between 20 - 28°C. The reaction was monitored by TLC. The mixture was

stirred for about 30 min. A solution of sodium borohydride (3.1 eq assuming 4 hydrides) in water (2 vol) was added dropwise keeping temperature between 20 - 28°C. The reaction was monitored by TLC. The mixture was stirred for about 30 min and water (8.3 vol) was added. The mixture was cooled to 5°C and held for at least 2 hrs prior to vacuum filtration and washing with water (23 vol). The solids were then dried under vacuum at 40°C overnight to afford titled compound. NMR spectrum completed on material that was twice recrystallized in ethanol: H^1 NMR ($CDCl_3$, 300 MHz) δ 7.18 (s, 2 H), 4.43 (dd, *J* = 3.3, 9.0 Hz, 1 H), 4.38 (s, 2 H), 2.83 (dd, *J* = 3.6, 8.4 Hz, 1 H), 2.49 (dd, *J* = 3.6, 8.7 Hz, 1 H), 2.38 (s, 2 H), 1.09 (s, 9 H).

General Procedure for Inverse Addition experiments of 4-Amino-α-bromo-3,5-dichloroacetophenone 4P in the Synthesis of Clenbuterol Free Base (5). To a solution of *tert*-butylamine (12.5 - 25 eq) at room temperature, 4-amino-α-bromo-3,5-dichloroacetophenone **4P** (1 eq) was added slowly keeping the temperature between 20 - 28°C. The reaction was monitored by TLC. The mixture was stirred for about 30 min. The *tert*-butylamine solution was added dropwise to a solution of sodium borohydride (3.1 eq assuming 4 hydrides) in water (2 vol) keeping temperature between 20 - 28°C. The mixture was stirred for about 30 min and water (8.3 vol) was added. The mixture was cooled to 5°C and held for at least 2 hrs prior to vacuum filtration and washing with water (23 vol). The solids were then dried under vacuum at 40°C overnight to afford titled compound.



9 **5**

General Procedure for the Water Spiking studies of 4-Amino-α,α-dibromo-3,5-dichloroacetophenone 9 in the Synthesis of Clenbuterol Free Base 5. To a solution of *tert*-butylamine (12.5 eq) spiked with water (0 – 0.15 eq) at RT, 4-amino-α,α-bromo-3,5-dichloroacetophenone **9** (1 eq) was added slowly keeping the temperature between 20 - 28°C. The reaction was monitored by TLC. The mixture was stirred for about 30 min. A solution of sodium borohydride (3.1 eq assuming 4 hydrides) in water (2 vol) was added dropwise keeping temperature between 20 - 28°C. The reaction was monitored by TLC. The mixture was stirred for about 30 min and water (8.3 vol) was added. The mixture was cooled to 0°C and held for at least 2 hrs prior to vacuum filtration and washing with water (23 vol). The solids were then dried under vacuum at 40°C overnight to afford titled compound.

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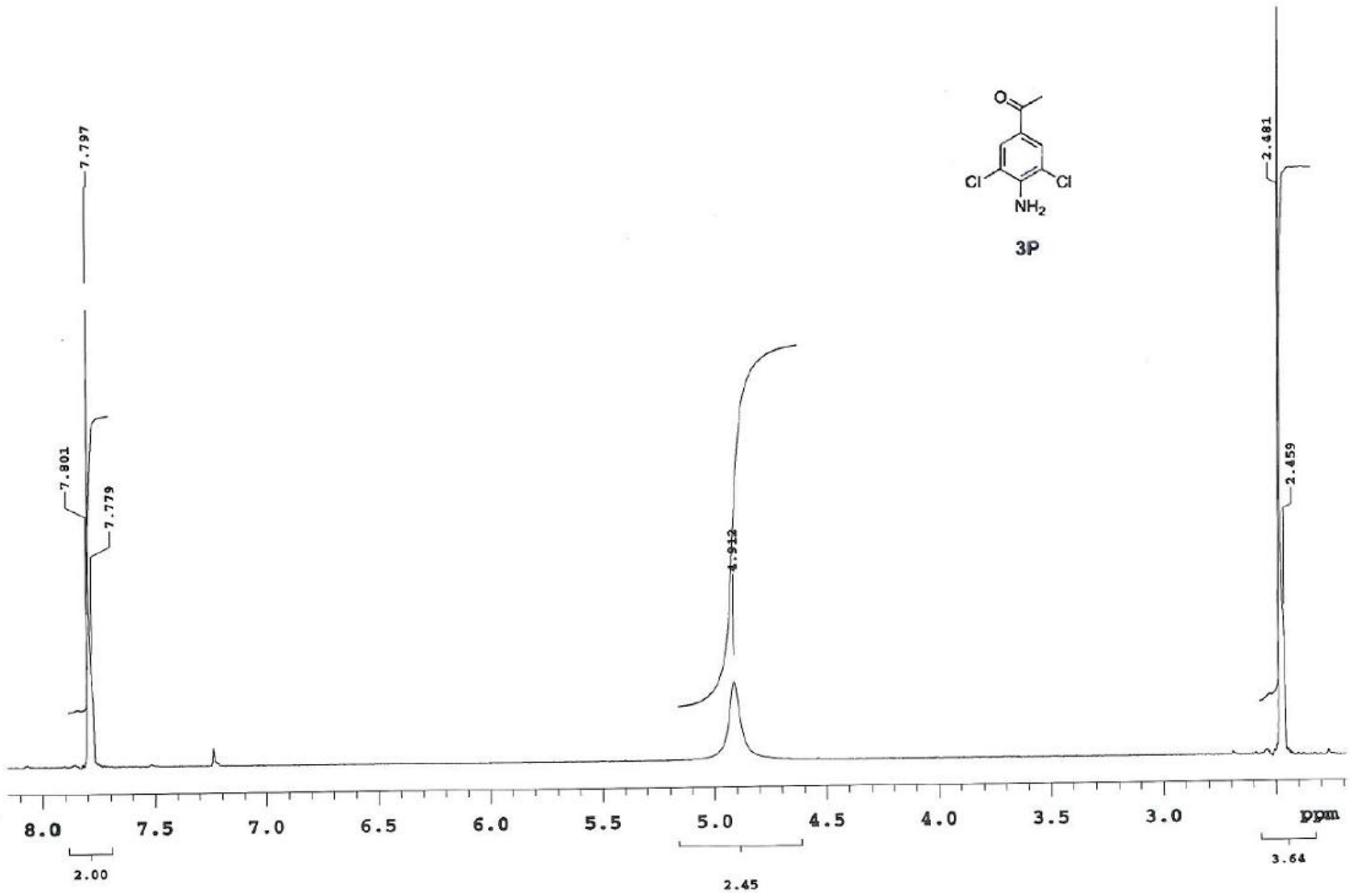
APPENDIX A: NMR SPECTRA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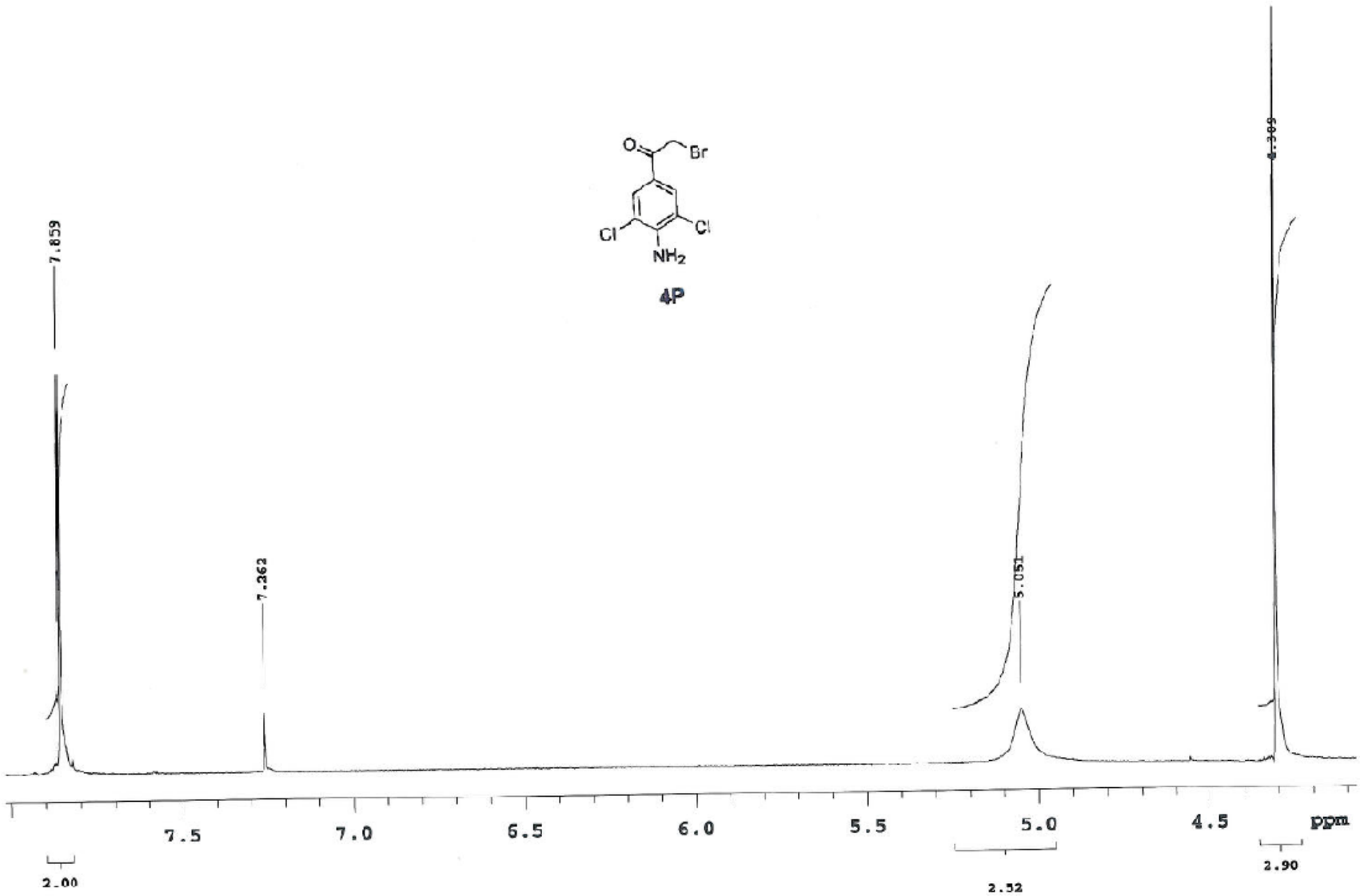
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ppm
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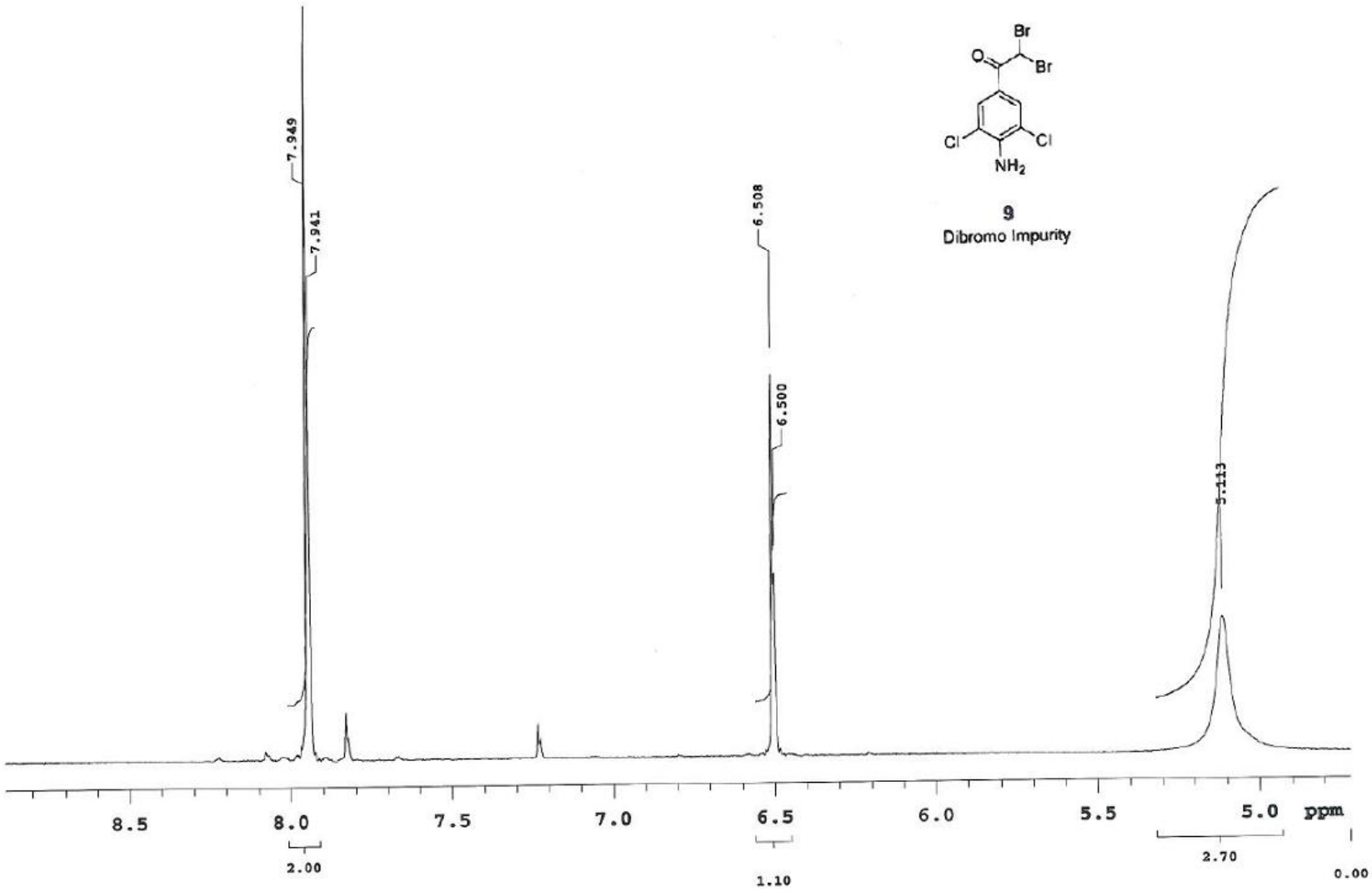
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Br
Cl
Cl
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7.941
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6.500
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7.5
7.0
6.5
6.0
5.5
5.0
ppm
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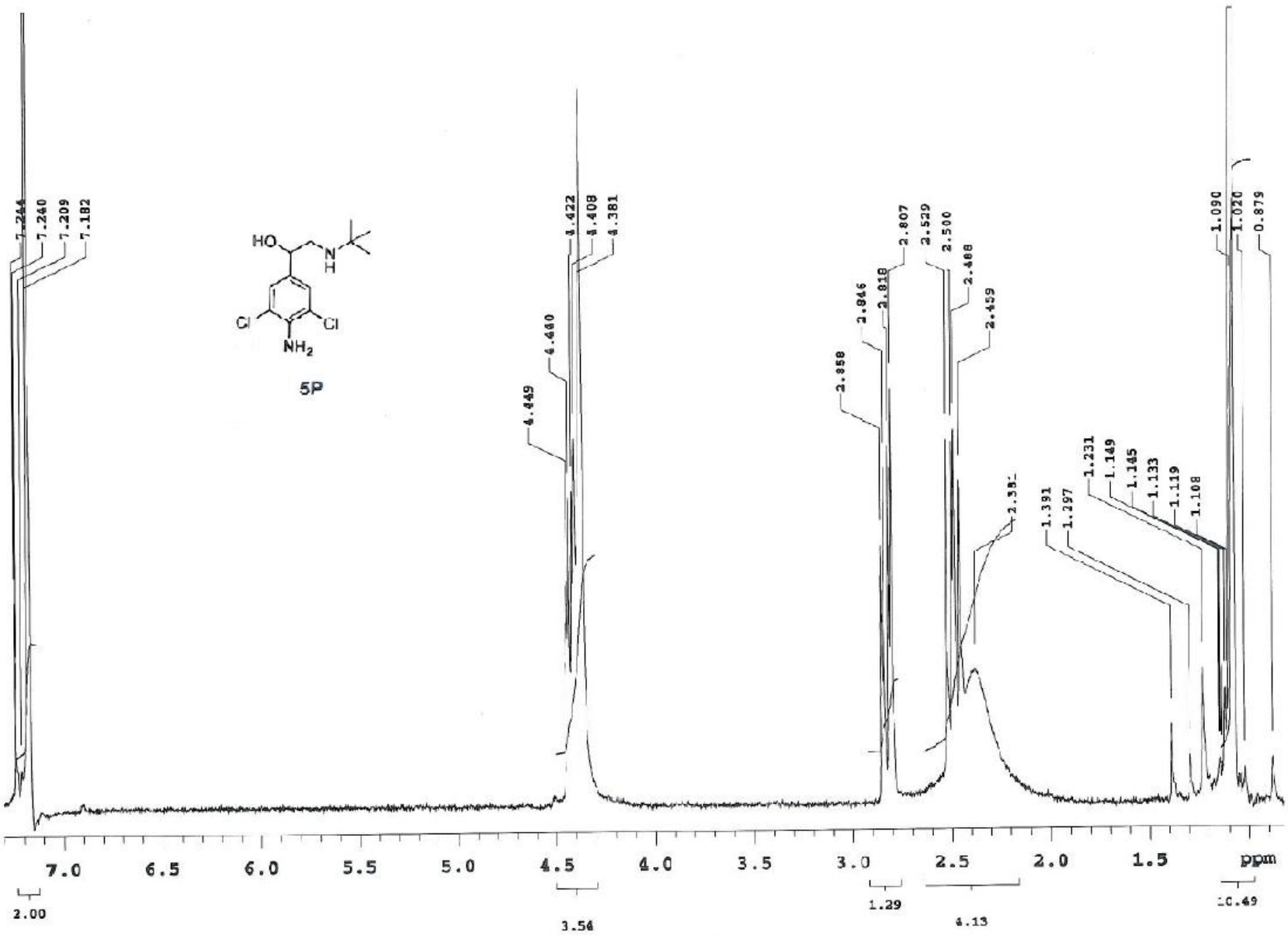
1.10
2.70
0.00


HO
N
H
Cl
Cl
NH2
5P
7.244
7.240
7.209
7.182
4.449
4.440
4.422
4.408
4.381
2.858
2.846
2.818
2.807
2.529
2.500
2.468
2.459
2.358
1.391
1.297
1.231
1.149
1.145
1.133
1.119
1.108
1.090
1.020
0.879
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
2.00
3.54
1.29
4.13
10.49

VITA

Kerry Elizabeth Moore was born on March 21, 1983, in Richmond, Virginia. She graduated from Midlothian High School in Midlothian, Virginia in 2001. She received her Bachelor of Science degree in chemistry from James Madison University in Harrisonburg, Virginia in 2005. She subsequently attended Pennsylvania State University for a doctoral program in chemistry, but left in 2007 to join the chemical development group at GlaxoSmithKline in King of Prussia, Pennsylvania. Moving back to Virginia, she worked for Pharmaceutical Product Development, Inc. in Richmond, Virginia starting in 2008. In 2009, she began work at Boehringer Ingelheim Chemicals, Inc. in Petersburg, Virginia, where she currently holds a position in the process chemistry group. She entered the Virginia Commonwealth University Master of Science program in chemistry in January of 2013.