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Identification, Synthesis, and Strategy for Reduction of Potential Impurities Observed in Dabigatran Etexilate Mesylate Processes

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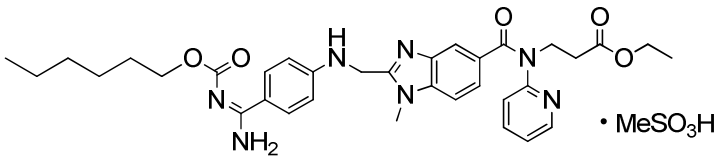
ABSTRACT: Synthetic impurities that are present in dabigatran etexilate mesylate were studied and possible pathways by which these impurities are formed during the manufacturing process were examined. The impurities were monitored by HPLC and their structures were determined by MS, ^{1}H and ^{13}C NMR. Potential causes for the formation of these impurities are discussed and strategies to minimize their formation are also described.

INTRODUCTION

Dabigatran etexilate mesylate (**1**) (Figure 1) is as an effective oral prodrug of the thrombin (Factor IIa) inhibitor dabigatran[1]. This drug has been approved by FDA and is widely used to reduce the risk of stroke in patients with non-valvular atrial fibrillation (AF). It is also approved by the EMEA for prevention of venous thromboembolism (VTE) events in adults who have undergone elective total hip or knee replacement surgery[2]. It is generally believed that the active pharmaceutical ingredient (API) can be contaminated by impurities, which may influence the quality and safety of the drug[3]. It has been found that the process employed in manufacturing **1** can result in the formation of certain impurities at a level of ≥ 0.10% (HPLC area)[4]. One major concern is to maintain the quality of the API while reducing the level of the impurities. It is therefore significant to isolate and characterize the corresponding impurities during the manufacturing process.

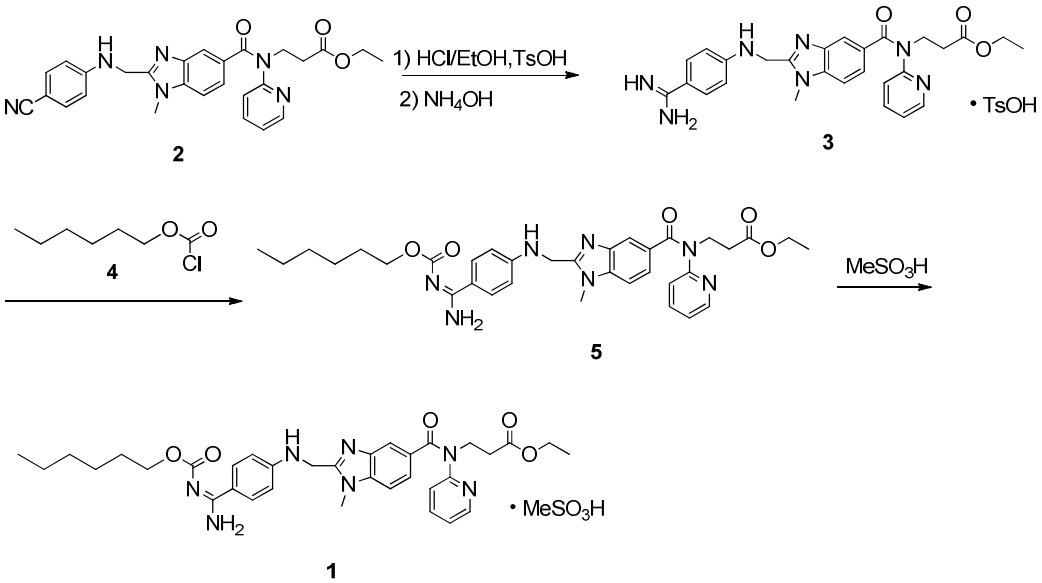


1

Figure 1. Structure of dabigatran etexilate mesylate (**1**).

Early experimental studies suggested that synthetic method outlined in Scheme 1 is the most advantageous and convenient route for the synthesis of **1**[5–8]. Cyano-compound **2** is first converted to the corresponding amidine intermediate **3** by Pinner reaction using HCl/EtOH. Then, Amidine **3** is reacted with n-hexyl chloroformate **4** to give dabigatran etexilate **5**. In the last step, the free base **5** reacts with methanesulfonic acid to produce its methanesulfonic acid salt **1**. A few impurities are identified. They can be produced by degradation, incomplete reactions, and side reactions[3, 9]. Contaminated starting ingredients, however are also major source of the impurities in **1**, such as n-hexyl chloroformate, which generally contains 5 impurities (see Scheme 3 for the impurities investigated in this work).

Scheme 1. Synthesis route of 1



2 **3** **4** **5** **1**

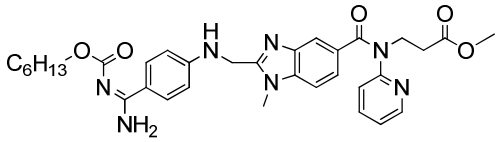
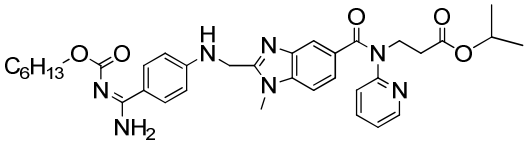
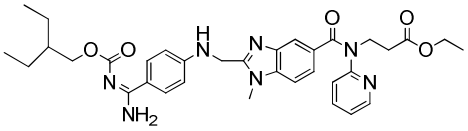
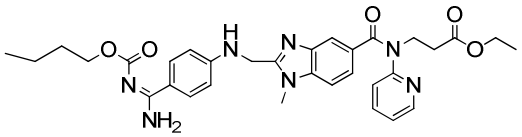
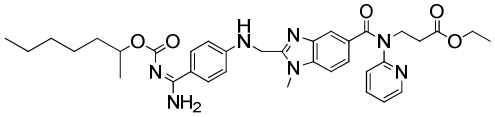
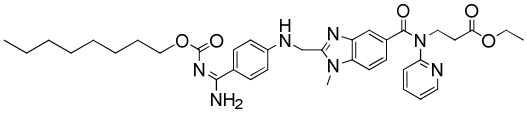
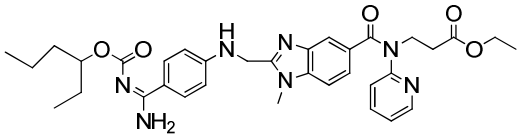
It is extremely challenging to identify impurities that formed in very small quantities in drug substances. The mechanism is also not straightforward due to the complexity of the conditions: multiple reactants and multiple impurities can coexist. This is strongly influenced by the reaction conditions. To solve the puzzle, the

potential impurities have been explored and synthesized. A mechanism of formation is here proposed and a practical strategy for lowering the concentration of impurities in the product is presented.

RESULTS AND DISCUSSION

HPLC was used to separate the impurities during the process of synthesizing **1**. Seven impurities were identified and are listed in Table 1. Their structures have been determined by MS, ^{1}H NMR, and ^{13}C NMR. It is essential to understand how these impurities are introduced in the drug, the mechanism by which the impurities form, and the strategies for detecting and assessing impurities. These points are elaborated upon as follows.

Table 1. Impurities detected during the synthetic process

Name	Structure	Relative Retention Time [a]
Impurity-A		8.07 min
Impurity-B		13.93 min
Impurity-C		9.39 min
Impurity-D		5.34 min
Impurity-E		13.42 min
Impurity-F		23.80 min
Impurity-G		8.67 min

[a] Ultimate 3000 HPLC (Dionex); column: C18 (5μm, 250mm×4.6mm); flow rate: 1.0 mL/min;

and detection at 225 nm; injection vol: 10 μL; mobile phase A (0.015mol/L KH_2PO_4, pH 6.0) and B (acetonitrile), 48:52 v/v; temp. 35 °C; dabigatran etexilate relative retention time: 10.47 min.

Impurities Identification and Plausible Pathways.

As shown in Table 1, impurities **A** and **B** are the homologous series of dabigatran etexilate methyl and isopropyl ester, which are similar to **1**. The two impurities were believed to arise from related impurities in the starting material. As shown in Figure 2, the methyl and isopropyl ester groups in impurities **A** and **B** can be traced back to compounds **6** and **7**, which may be the by-products of the Pinner reaction shown in Scheme 1[10]. It is generally believed that there are small amounts of methanol and isopropanol in HCl-ethanol solution, where the methyl and isopropyl ester moieties can be introduced as the by-products of intermediate **3** at the first step of synthesis (Scheme 1).

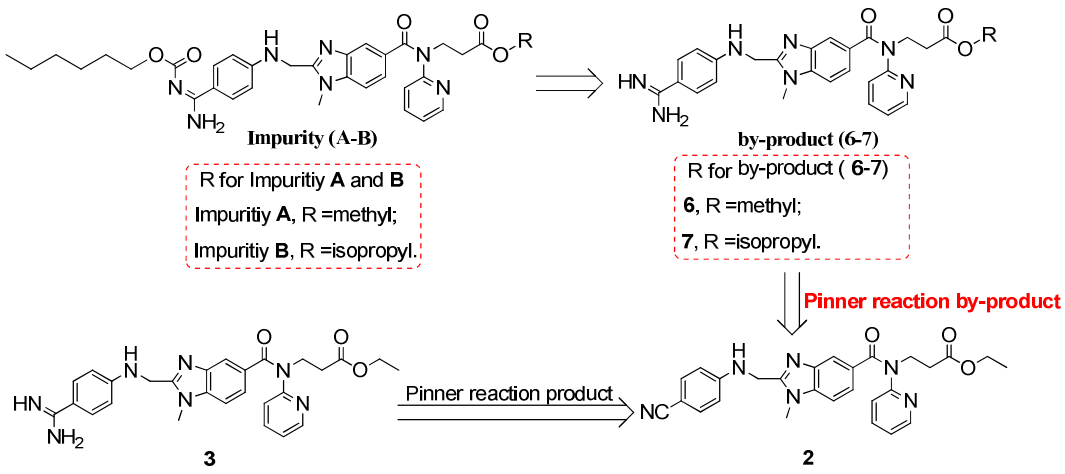


Figure 2. Possible pathway to impurity A and B

Impurities **C**, **D**, **E**, **F**, and **G** are structural analogues of **1**, where the n-hexyl group has been replaced by other alkyl groups. It is reasonable to believe that the 2-ethylbutyl moiety in impurity **C** is derived from the small amount of 2-ethylbutyl chloroformate, which is always present in the n-hexyl chloroformate **4**. After the amidation of amidine **3** with n-hexyl chloroformate **4**, the impurity **C** was found to be present at levels greater than 0.15% in the crude product **1**. Impurities **D**, **E**, **F**, and **G** were present at lower concentrations. They are also caused by the presence of other impurities (n-butyl chloroformate, heptan-2-yl chloroformate, n-octyl chloroformate, hexan-3-yl chloroformate) in the n-hexyl chloroformate **4**. Their concentrations were

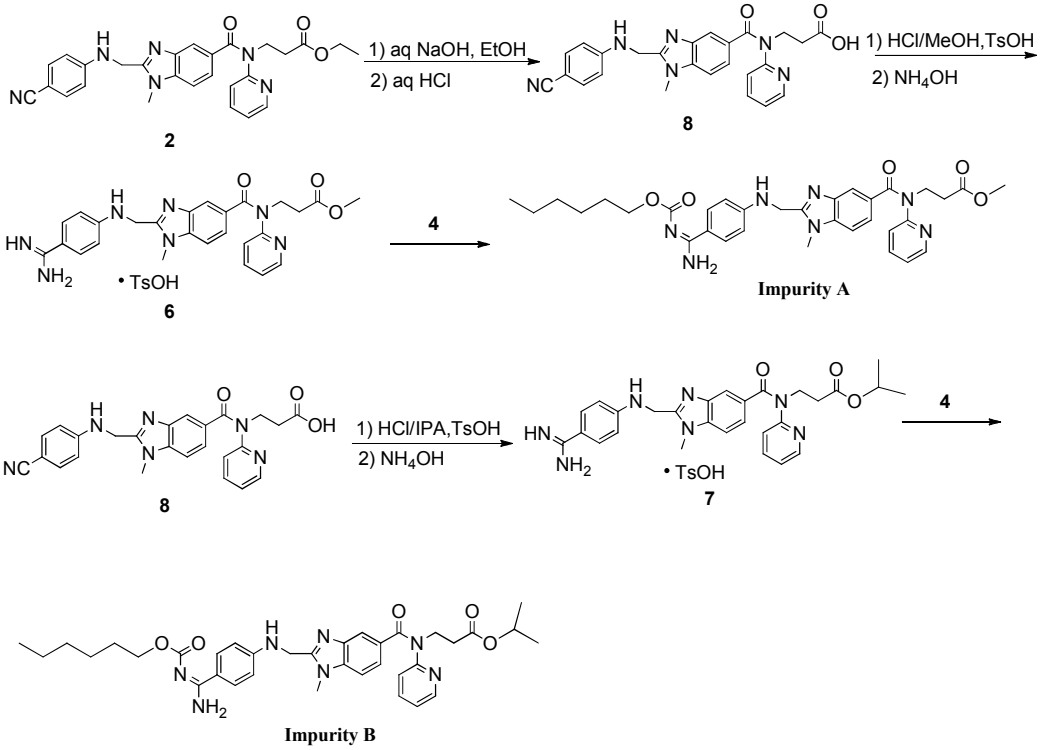
about 0.05–0.12% as indicated by HPLC. Unfortunately, it is virtually impossible to remove these specific impurities from the final product, and they can affect solubility.

Impurities Synthesis and Control.

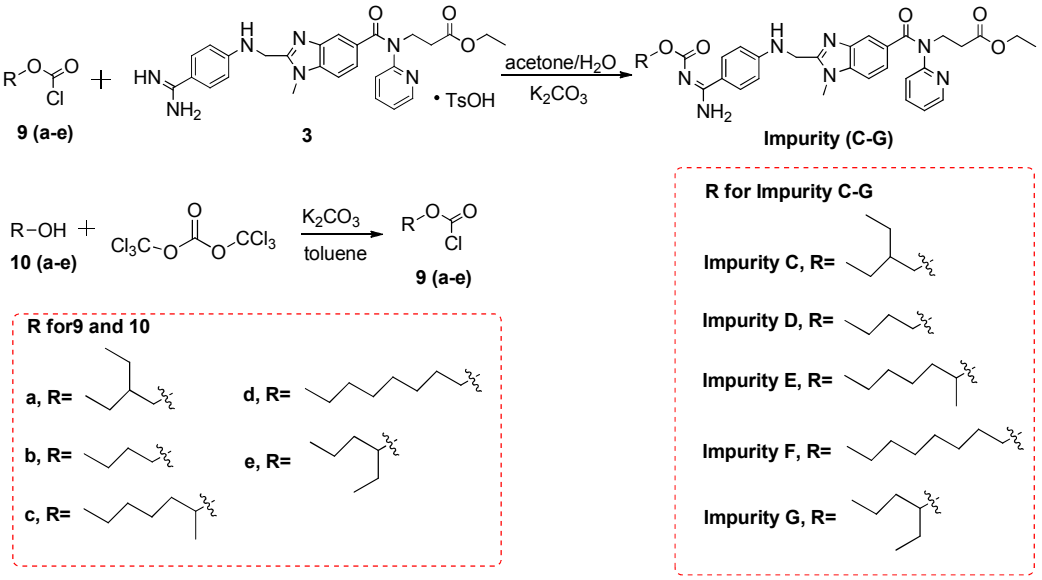
After identification of possible pathways for the formation of the impurities, the characteristics of the impurities which can influence the quality and safety of the drug were reevaluated. One path has been presented for the synthesis of all the investigated impurities. This model explains the quantities found and satisfies the analytical needs of regulatory requirements. However, the details of the formation of these impurities are not reported in previous studies, even though impurity **A** has been identified as related substance by Miralles and colleagues[3].

Compared to the product **1**, replacement of the terminal ethyl propionate moiety with methyl or isopropyl propionate can produce impurities **A** and **B**. It is desirable that impurities **A** and **B** be synthesized from the same starting material (Cyano-compound **2**) of product **1**. As shown in Scheme 2, cyano-compound **2** was hydrolyzed by aqueous sodium hydroxide in ethanol, and the carboxylic acid compound **8** can be furnished by acidification with aqueous hydrochloric acid. Impurity precursor **6** was synthesized by treating compound **8** with 10 N hydrochloride methanol. It should be mentioned that precursor **6** has been obtained by one-pot synthesis, including amidine formation by Pinner reaction and acid-catalyzed esterification reaction during this synthesis. Finally, the coupling of compound **6** with n-hexyl chloroformate **4** gave the desired impurity **A**. A similar protocol was utilized for impurity **B**, where the difference was that the 10 N hydrochloride methanol was replaced with 10 N hydrochloride isopropanol for impurity **B** (Scheme 2). Results showed that the entire process of the formation of impurities **A** and **B** started from **2** with overall yields of about 60% and 51%, respectively without resorting to separation by column chromatography in any step.

Scheme 2. Synthesis of impurity A and B



Scheme 3. Synthesis of impurities C–G



Impurities **C–G** contain different side chains from product **1.** It is favorable to utilize the available compound **3** and alkyl chloroformate **9a–e** as starting material. As shown in Scheme 3, these impurities can be obtained in good yields and high purity (yields: 72–80%, purities: 97–99%) through the dabigatran etexilate synthetic route. Regarding alkyl chloroformate **9a–e,** they can be easily synthesized from alkyl

alcohols **10a**–**e** with triphosgene in toluene[11]. The structures of all these impurities were determined by mass spectroscopy, ^{1}H NMR, and ^{13}C NMR, and relative retention times were established using HPLC.

To improve the safety and quality of the drug, the concentrations of these impurities must be reduced to levels accepted by the International Conference on Harmonization (ICH)[12]. Impurities **A** and **B** are introduced during the first Pinner reaction step. They can be removed in the following reaction process. It is conceivable to use the solvent crystallization as a form of purification. Unfortunately, owing because of the low solubility of impurities **A** and **B** in many organic solvents, isolation of these impurities from the product **1** proved difficult. Similarly, impurity precursors **6** and **7** could not be reduced from the amidine intermediate **3**. However, these two impurities can be removed from dabigatran etexilate **5** through recrystallization.

In order to identify solvents suitable for purification, several solvents were selected and the results are shown in Table 2. Entries 1–3 showed that acetone, acetonitrile, and methyl ethyl ketone suffered a unfavorable effects on recrystallization (impurities **A**, **B** $>$ 0.10%). The ester solvent system (entries 4–8) gave better results and removed more of impurities **A** and **B**. It was here observed that ethyl acetate (12 mL/g) (enter 6) was the best solvent system for this recrystallization process. It is noticed that levels of impurities **A** and **B** could be reduced to below 0.1%, when the product was obtained by the conditions reported in entry 6 of Table 2.

Table 2. Solvent screening for purification of dabigatran etexilate (5)

enter	solvent	ratio (mL/g)	recrystallization results of **5** by HPLC[a] (%)		
			5	**Imp A**	**Imp B**
1	acetone	8	99.43	0.12	0.13
2	acetonitrile	12	99.38	0.14	0.16
3	methyl ethyl ketone	10	99.45	0.12	0.14
4	ethyl acetate	8	99.65	0.09	0.10
5	ethyl acetate	10	99.68	0.09	0.09

6	ethyl acetate	12	99.73	0.04	0.05
7	n-butyl acetate	12	99.56	0.08	0.12
8	isopropyl acetate	12	99.59	0.09	0.11

[a] Input quality: purity is 99.27%, Imp **A** and Imp **B** content 0.15% and 0.19% respectively

Impurity control by specification of reagents is common in pharmaceutical manufacturing. It is essential to lower the concentration of reagent impurities to a very low level and ensure that the derivative impurities that form during API are under control. It has been found that impurities **C–G** are derived from the material n-hexyl chloroformate **4** in the amidation reaction process, where other alkyl chloroformate **9a–e** may be present. Therefore, the key factor is the supply and purity of the material **4,** which can be restricted and monitored by GC.

To investigate the effect of the degree of purity of **4**, different amounts of impurities in **4** were used to synthesize the final product **1**. Results are summarized in Table 3. It was here noted that product **1** was of high quality (entry 3–5) when the alkyl chloroformate **9a–e** in **4** was below 0.10% by GC. Otherwise, the high concentration of alkyl chloroformate **9a–e** in **4** would cause substantial amounts of impurities **C–G** to form in **1** (enter 1–2). These results indicated that it is essential to obtain highly pure **4**.

Table 3. Analytical data for dabigatran etexilate mesylate using different qualities of 4

enter	input						output					
	Content of impurities in **4** by GC (%)						Quality of **1** by HPLC (%)					
	4	**9a**	**9b**	**9c**	**9d**	**9e**	**1**	**Imp C**	**Imp D**	**Imp E**	**Imp F**	**Imp G**
1	98.83	0.19	0.06	0.15	0.11	0.12	99.45	0.15	0.07	0.10	0.08	0.10
2	98.83	0.19	0.06	0.15	0.11	0.12	99.38	0.16	0.05	0.12	0.09	0.11
3	99.25	0.05	ND	0.06	0.03	0.04	99.70	0.05	ND	0.01	ND	0.02
4	99.25	0.05	ND	0.06	0.03	0.04	99.74	0.04	ND	ND	ND	0.02
5	99.25	0.05	ND	0.06	0.03	0.04	99.73	0.04	ND	ND	0.01	0.01

CONCLUSION

In summary, seven observed and potential impurities of dabigatran etexilate mesylate (**1**) were here synthesized and characterized. The origins of formation impurities **A–G** during the preparation of **1** were also mapped out. In addition, a strategy to lower the concentrations of these impurities to levels accepted by ICH is here proposed. This information would be immensely useful for process chemists working in this area.

EXPERIMENTAL SECTION

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) spectrometer with TMS as an internal standard. Chemical shift (d values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an Agilent 6210 TOF spectrometer. Uncorrected melting points were determined on an electrothermal melting point apparatus. Solvents and reagents were used without any pretreatment. Reaction progress and chemical purity were evaluated by HPLC analysis using a Ultimate 3000 (Dionex) C18（5μm, 250mm×4.6mm）with a mobile phase A (0.015mol/L KH_2PO_4, pH 6.0) and B (acetonitrile), 48:52 v/v; and detection at 225 nm; flow: 1.0 mL/min; temp. 35 ℃.

Synthesis of Impurity A. A solution of sodium hydroxide (2.6g, 65.0mmol) in water (40mL) was added dropwise to compound **2** (20.0g, 41.5mmol) in ethanol (160mL). The mixture was stirred at room temperature for 2h. The mixture was concentrated in vacuo to remove ethanol (about 100mL), the residue was then diluted with water (120mL) and adjusted to pH 5-6 by 10% hydrochloric acid solution. The precipitate was isolated and washed with water (30mL) to afford compound **8** (18.1g, 96.1%) as white crystals. Mp 210-212 ℃; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.38-8.37 (dd, J_1 = 1.2Hz, J_2 = 4.8Hz, 1H), 7.58-7.54 (m, 1H), 7.48-7.47 (m, 2H), 7.45 (s, 1H), 7.40-7.38 (d, J = 8.4Hz, 1H), 7.21-7.19 (m, 1H), 7.17-7.16 (m, 1H), 7.13-7.10 (dd, J_1 = 4.8Hz, J_2 = 7.2Hz, 1H), 6.97-6.95 (d, J = 8.0Hz, 1H), 6.84-6.81 (d, J = 8.4Hz, 2H), 4.60-4.59 (d, J = 5.6Hz, 2H), 4.20-4.16 (t, J = 7.6Hz, 2H), 3.76 (s, 3H), 2.64-2.60 (t, J

= 7.6Hz, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 172.8, 170.5, 156.1, 153.3, 151.7, 148.7, 140.9, 137.8, 137.2, 133.3, 129.7, 122.9, 122.0, 121.2, 120.4, 119.6, 112.5, 109.4, 96.9, 43.5, 39.7, 33.1, 29.9; MS *m/z* 455 $[M + H]^+$, 477 $[M + Na]^+$.

Compound **8** (9.0g, 19.8mmol) and *p*-toluenesulfonic acid (4.5g, 23.8mmol) were dissolved in 10 mol/L HCl-methanol solution (20mL), the mixture was stirred for 8h at room temperature. The mixture cooled to 0℃, and ammonium hydroxide (30mL) was added dropwise. The reaction mixture was stirred for 5h at room temperature. Water (30mL) was added to this mixture and stirred for another 5h, the precipitate was isolated and washed with water (50mL) to afford compound **6** (8.1g, 84.2%) as white crystals. Mp 188-190 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.65 (br, 4H), 8.39-8.38 (dd, J_1 = 1.2Hz, J_2 = 4.8Hz, 1H), 7.64-7.62 (d, J = 8.8Hz, 2H), 7.57-7.53 (m, 1H), 7.51-7.49 (m, 3H), 7.41-7.39 (m, 1H), 7.31-7.29 (t, J = 5.6Hz, 1H), 7.18-7.15 (dd, J_1 = 1.2Hz, J_2 = 8.4Hz, 1H), 7.12-7.10 (m, 3H), 6.92-6.89 (d, J = 8.4Hz, 1H), 6.88-6.86 (d, J = 8.8Hz, 2H), 4.65-4.64 (d, J = 5.6Hz, 2H), 4.24-4.21 (t, J = 7.2Hz, 2H), 3.77 (s, 3H), 3.53 (s, 3H), 2.72-2.69 (t, J = 7.2Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 171.4, 170.3, 164.4, 155.9, 153.3, 153.0, 148.6, 144.9, 140.8, 138.1, 137.8, 137.2, 129.6, 129.4, 128.2, 125.4, 122.8, 121.9, 121.2, 119.5, 113.4, 111.8, 109.5, 56.0, 51.3, 44.4, 32.8, 29.9, 20.7; MS *m/z* 486 $[M + H]^+$.

Compound **6** (8g, 16.5mmol) and potassium carbonate (6.8g, 49.5mmol) were dissolved in acetone (100mL) and water (50mL), compound **4** (3.0g, 18.1mmol) was added at room temperature and stirring was continued for 2h. The mixture was heated to 50℃ and the organic phase was separated, and concentrated in vacuo. The residue was finally crystallized in EtOAc (60mL) to give impurity **A** (7.6g, 75.2%) as a white solid. Purity by HPLC 97.42%, Mp 88-90 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.86 (br, 2H), 8.39-8.38 (d, J = 4.0 Hz, 1H), 7.81-7.79 (d, J = 8.4Hz, 2H), 7.56-7.52 (t, J = 7.2Hz, 1H), 7.48 (s, 1H), 7.40-7.38 (d, J = 8.4Hz, 1H), 7.18-7.16 (d, J = 8.0Hz, 1H), 7.13-7.10 (m, 1H), 6.91-7.89 (d, J = 8.0Hz, 2H), 6.78-6.76 (d, J = 8.4Hz, 2H), 4.60-4.59 (d, J = 5.2Hz, 2H), 4.24-4.21 (t, J = 6.8Hz, 2H), 4.00-3.97 (t, J = 6.4Hz, 2H), 3.77 (s, 3H), 3.53 (s, 3H), 2.72-2.69 (t, J = 6.8Hz, 2H), 1.60-1.57 (m, 2H), 1.29 (m, 6H), 0.88-0.85 (t, J = 6.4Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 171.4,

170.4, 166.4, 164.2, 155.9, 153.7, 151.5, 148.6, 140.8, 137.8, 137.2, 129.4, 129.1, 122.8, 122.0, 121.2, 121.1, 119.5, 111.4, 109.4, 64.1, 51.3, 44.4, 32.8, 30.9, 29.9, 28.5, 25.2, 21.9, 13.8; MS *m/z* 614 $[M + H]^+$.

Synthesis of Impurity B. Compound **8** (9.0g, 19.8mmol) and *p*-toluenesulfonic acid (4.5g, 23.8mmol) were dissolved in 10 mol/L HCl-isopropanol solution (20mL), the mixture was stirred for 8h at room temperature. The mixture cooled to 0℃, and ammonium hydroxide (30mL) was added dropwise. The reaction mixture was stirred for 5h at room temperature. Water (30mL) was added to this mixture and stirred for another 5h, the precipitate was isolated and washed with water (50mL) to afford compound **7** (7.5g, 73.7%) as white crystals. Mp 201-203 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.67 (br, 4H), 8.40-8.39 (d, J = 4.8Hz, 1H), 7.68-7.66 (d, J = 8.8Hz, 2H), 7.57-7.54 (m, 2H), 7.51-7.49 (m, 2H), 7.41-7.39 (d, J = 8.4Hz, 1H), 7.30-7.28 (t, J = 5.6Hz, 1H), 7.19-7.17 (d, J = 8.4Hz, 1H), 7.12-7.10 (m, 3H), 6.93-6.90 (d, J = 8.4Hz, 1H), 6.88-6.86 (d, J = 8.8Hz, 2H), 4.85-4.81 (m, 1H), 4.64-4.63 (d, J = 5.6Hz, 2H), 4.25-4.22 (t, J = 7.2Hz, 2H), 3.78 (s, 3H), 2.71-2.68 (t, J = 7.2Hz, 2H), 2.29 (s, 3H), 1.15-1.13 (d, J = 6.4Hz, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 171.2, 170.5, 166.8, 164.4, 156.1, 154.1, 153.0, 148.9, 144.6, 140.8, 138.2, 137.6, 137.0, 129.6, 129.1, 128.4, 126.0, 123.0, 122.8, 121.9, 121.1, 119.7, 114.0, 111.8, 109.5, 68.0, 56.0, 51.3, 44.6, 32.8, 29.9, 20.7, 19.6; MS *m/z* 514 $[M + H]^+$.

Compound **7** (7.5g, 14.6mmol) and potassium carbonate (6.1g, 43.8mmol) were dissolved in acetone (100mL) and water (50mL), compound **4** (2.6g, 16.1mmol) was added at room temperature and stirring was continued for 2h. The mixture was heated to 50℃ and the organic phase was separated, and concentrated in vacuo. The residue was finally crystallized in EtOAc (60mL) to give impurity **B** (6.7g, 71.6%) as a white solid. Purity by HPLC 97.56%, Mp 103-105 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.85 (br, 2H), 8.41-8.40 (d, J = 3.6Hz, 1H), 7.85-7.83 (d, J = 8.4Hz, 2H), 7.56-7.52 (m, 2H), 7.40-7.38 (d, J = 8.4Hz, 1H), 7.20-7.18 (d, J = 8.4Hz, 1H), 7.13-7.10 (dd, J_1 = 5.2Hz, J_2 = 6.8Hz, 1H), 6.94-6.92 (m, 1H), 6.90 (s, 1H), 6.81-6.79 (d, J = 8.8Hz, 2H), 4.86-4.81 (m, 1H), 4.62-4.60 (d, J = 5.2Hz, 2H), 4.27-4.24 (t, J = 6.8Hz, 2H), 4.02-3.99 (t, J = 6.8Hz, 2H), 3.78 (s, 3H), 2.70-2.66 (t, J = 7.2Hz, 2H), 1.61-1.55 (m,

2H), 1.35-1.29 (m, 6H), 1.15-1.13 (d, J = 6.4Hz, 6H), 0.90-0.85 (m, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 170.9, 170.8, 166.9, 164.7, 156.5, 154.2, 152.0, 149.1, 141.4, 138.3, 137.7, 129.9, 129.6, 123.3, 122.6, 121.7, 120.0, 111.9, 109.9, 67.8, 64.6, 44.8, 33.8, 31.5, 30.4, 29.0, 25.7, 22.5, 21.9, 14.3; MS *m/z* 642 $[M + H]^+$.

General procedure for the preparation of Impurities C-G A mixture of triphosgene (1.7g, 5.7mmol), potassium carbonate (1.5g, 11mmol) and dimethyl formamide (0.3g, 4.1mmol) as a catalyst in toluene (10mL) was cooled to 0℃, and stirred for 1h. A solution of alkyl alcohols **10a-e** (1.1g, 11mmol) in toluene (10mL) was added dropwise to this mixture. Then the reaction mixture was stirred for 5h. This mixture was added to a solution of **3** (5.0g, 10mmol) and potassium carbonate (4.1g, 30mmol) in acetone (60mL) and water (30mL). The reaction was stirred for 2h at room temperature. The mixture was heated to 50℃ and the organic phase was separated, and concentrated in vacuo. The residue was finally crystallized in EtOAc (50mL) to give impurities **C-G**.

Impurity C. Yield 75.4% as a white solid. Purity by HPLC 99.00%, Mp 95-97 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.85 (br, 2H), 8.40-8.38 (dd, J_1 = 1.6Hz, J_2 = 4.8Hz, 1H), 7.81-7.79 (d, J = 8.8Hz, 2H), 7.57-7.52 (m, 1H), 7.50-7.48 (m, 1H), 7.41-7.39 (d, J = 8.4Hz, 1H), 7.18-7.16 (dd, J_1 = 1.2Hz, J_2 = 8.4Hz, 1H), 7.14-7.11 (dd, J_1 = 4.8Hz, J_2 = 7.2Hz, 1H), 6.91-6.89 (d, J = 8.0Hz, 1H), 6.88 (s, 1H), 6.79-6.77 (d, J = 8.8Hz, 2H), 4.61-4.59 (d, J = 5.6Hz, 2H), 4.25-4.22 (t, J = 6.8Hz, 2H), 4.02-3.96 (q, J = 7.2Hz, 2H), 3.94-3.93 (d, J = 6.0Hz, 2H), 3.77 (s, 3H), 2.71-2.67 (t, J = 7.2Hz, 2H), 1.56-1.46 (m, 1H), 1.40-1.28 (m, 4H), 1.15-1.12 (t, J = 6.8Hz, 3H), 0.90-0.86 (t, J = 7.2Hz, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 170.9, 170.3, 166.4, 164.3, 156.0, 153.6, 151.5, 148.6, 140.8, 137.8, 137.2, 129.3, 129.1, 127.9, 125.5, 122.7, 122.0, 121.2, 121.1, 119.4, 111.4, 109.4, 66.0, 59.9, 44.3, 40.0, 33.0, 29.8, 22.8, 13.9,10.8; MS *m/z* 628 $[M + H]^+$.

Impurity D. Yield 77.3% as a white solid. Purity by HPLC 98.53%, Mp 104-106 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.94-8.81 (br, 2H), 8.40 (m, 1H), 7.85-7.83 (d, J = 8.4Hz, 2H), 7.57-7.52 (m, 2H), 7.40-7.38 (d, J = 8.4Hz, 1H), 7.19-7.17 (d, J = 8.4Hz, 1H), 7.13-7.10 (t, J = 5.6Hz, 1H), 6.99 (s, 1H), 6.92-6.90 (d,

J = 7.6Hz, 1H), 6.81-6.79 (d, *J* = 8.4Hz, 2H), 4.61-4.60 (d, *J* = 4.8Hz, 2H), 4.27-4.24 (t, *J* = 6.8Hz, 2H), 4.02-3.97 (m, 4H), 3.78 (s, 3H), 2.73-2.69 (t, *J* = 5.6Hz, 2H), 1.62-1.55 (m, 2H), 1.40-1.31 (m, 2H), 1.15-1.12 (t, *J* = 7.2Hz, 3H), 0.93-0.89 (t, *J* = 7.2Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 171.5, 170.8, 166.9, 164.7, 156.5, 154.2, 152.1, 149.1, 141.4, 138.3, 137.7, 129.8, 129.7, 123.2, 122.5, 121.7, 121.6, 120.9, 120.0, 111.9, 109.9, 64.3, 60.9, 60.5, 44.8, 33.6, 31.1, 30.4, 19.2, 14.4, 14.1; MS *m/z* 600 $[M + H]^+$.

Impurity E. Yield 71.7% as a white solid. Purity by HPLC 99.02%, Mp 86-88 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.89 (br, 2H), 8.40-8.39 (m, 1H), 7.82-7.80 (d, *J* = 8.8Hz, 2H), 7.57-7.52 (m, 1H), 7.49 (s, 1H), 7.41-7.39 (d, *J* = 8.4Hz, 1H), 7.18-7.16 (dd, J_1 = 1.2Hz, J_2 = 8.8Hz, 1H), 7.14-7.11 (m, 1H), 6.94-6.91 (m, 1H), 6.89 (s, 1H), 6.79-6.77 (d, *J* = 8.4Hz, 2H), 4.77-4.69 (m, 1H), 4.61-4.59 (d, *J* = 5.2Hz, 2H), 4.26-4.22 (t, *J* = 6.8Hz, 2H), 4.02-3.96 (q, *J* = 7.2Hz, 2H), 3.78 (s, 3H), 2.71-2.68 (t, *J* = 7.2Hz, 2H), 1.56-1.51 (m, 1H), 1.49-1.42 (m, 1H), 1.36-1.33 (m, 6H), 1.19-1.17 (d, *J* = 6.4Hz, 3H), 1.15-1.12 (t, *J* = 7.2Hz, 3H), 0.91-0.84 (m, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 170.9, 170.3, 166.4, 163.9, 156.0, 153.7, 151.5, 148.6, 140.8, 137.8, 137.2, 129.3, 129.1, 122.7, 122.0, 121.1, 119.5, 111.3, 109.3, 69.9, 59.9, 44.3, 35.6, 33.0, 31.1, 29.9, 24.6, 21.9, 20.0, 13.9, 13.8; MS *m/z* 642 $[M + H]^+$.

Impurity F. Yield 73.4% as a white solid. Purity by HPLC 98.57%, Mp 85-87 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.84 (br, 2H), 8.40-8.39 (d, *J* = 4.0Hz, 1H), 7.81-7.79 (d, *J* = 8.4Hz, 2H), 7.56-7.53 (t, *J* = 7.2Hz, 1H), 7.48 (s, 1H), 7.41-7.39 (d, *J* = 8.8Hz, 1H), 7.18-7.16 (d, *J* = 8.4Hz, 1H), 7.14-7.11 (dd, J_1 = 5.2Hz, J_2 = 6.8Hz, 1H), 6.91-6.89 (d, *J* = 7.6Hz, 2H), 6.79-6.76 (d, *J* = 8.8Hz, 2H), 4.61-4.59 (d, *J* = 5.2Hz, 2H), 4.25-4.22 (t, *J* = 7.2Hz, 2H), 4.02-3.96 (m, 4H), 3.77 (s, 3H), 2.71-2.67 (t, *J* = 7.2Hz, 2H), 1.62-1.56 (m, 2H), 1.27 (m, 10H), 1.15-1.12 (t, *J* = 7.2Hz, 3H), 0.88-0.85 (m, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 170.9, 170.3, 166.4, 164.2, 155.9, 153.6, 151.5, 148.6, 140.8, 137.7, 137.2, 129.3, 129.1, 122.7, 122.0, 121.2, 119.5, 111.4, 109.3, 64.1, 59.9, 44.3, 33.0, 31.2, 29.8, 28.7, 28.6, 25.5, 22.0, 13.9, 13.8; MS *m/z* 656 $[M + H]^+$.

Impurity G. Yield 79.6% as a white solid. Purity by HPLC 97.28%, Mp

110-112 °C; ^{1}H NMR (400Hz, DMSO-d_6) δ 8.84 (br, 2H), 8.39-8.38 (d, J = 3.6Hz, 1H), 7.81-7.79 (d, J = 8.4Hz, 2H), 7.56-7.52 (m, 1H), 7.48 (s, 1H), 7.40-7.38 (d, J = 8.4Hz, 1H), 7.18-7.16 (m, 1H), 7.13-7.10 (dd, J_1 = 5.2Hz, J_2 = 6.8Hz, 1H), 6.91-6.86 (m, 2H), 6.78-6.76 (d, J = 8.8Hz, 2H), 4.69-4.63 (m, 1H), 4.60-4.59 (d, J = 5.2Hz, 2H), 4.25-4.21 (t, J = 6.8Hz, 2H), 4.01-3.96 (q, J = 7.2Hz, 2H), 3.77 (s, 3H), 2.70-2.67 (t, J = 7.2Hz, 2H), 1.59-1.46 (m, 4H), 1.38-1.23 (m, 2H), 1.15-1.11 (t, J = 7.2Hz, 3H), 0.90-0.84 (m, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 171.0, 170.4, 166.5, 164.2, 156.0, 153.7, 151.5, 148.6, 140.9, 137.8, 137.2, 129.4, 129.1, 122.8, 122.1, 121.3, 121.2, 119.5, 111.4, 109.4, 74.2, 60.0, 44.3, 35.6, 33.1, 29.9, 26.8, 18.2, 13.9, 13.8, 9.6; MS *m/z* 628 $[M + H]^+$.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, MS spectra of impurities **A-G**, and the HPLC results of the mixture impurities **A-G** and product **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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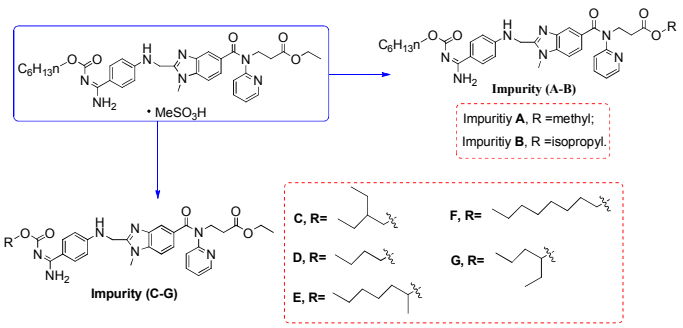

Table of Contents graphic