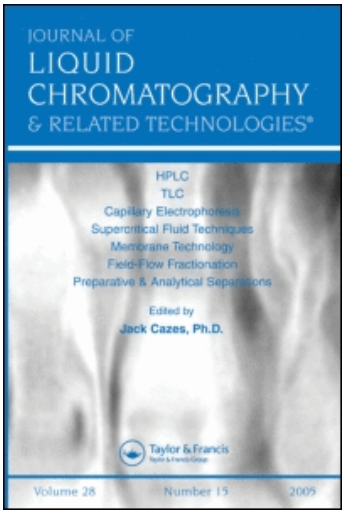

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Characterization and Control of Impurities in the Synthesis of an Amino Acid Drug Candidate

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Characterization and Control of Impurities in the Synthesis of an Amino Acid Drug Candidate

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Abstract: The identification of process related impurities and elucidation of the mechanism for their formation can provide invaluable input for the optimization of a pharmaceutical synthetic process. The final two steps for the synthesis of an amino acid drug candidate involve the formation of an aminonitrile followed by its hydrolysis to the amino acid. The degradation impurities were generated in both steps. The degradation products were characterized and the mechanisms for their formation were proposed. This information was then fed back to the process chemists to minimize impurity formation.

Keywords: Drug candidate, Identification of impurities, Degradation mechanism

INTRODUCTION

A critical aspect of drug development is the control and identification of impurities in the active pharmaceutical ingredient. Regulatory requirements dictate the control of impurities, and the identification, where possible, of impurities that exceed 0.1%. The identification of these impurities provides information towards their potential toxicity and the development of synthetic strategies to minimize their generation. Impurities in a drug substance may originate from

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raw materials, intermediates, by-products, solvents, or their degradation products. Common degradation pathways are hydrolysis, dehydration, oxidation, dimerization, or a combination thereof. Impurities associated with a drug substance are usually monitored through separation techniques, such as chromatography.

The identification of process related impurities can ultimately lead to elucidation of their mechanistic pathways. The first step in this process entails determination of structures for the impurities using tools such as NMR or LC-MS. With elucidated structures one can then attempt to propose a mechanism for their formation and to identify the synthetic steps that lead to their formation. This information can then be utilized to optimize the synthetic conditions so as to minimize the formation of impurities.

In this paper, we present a case study for the characterization of impurities generated during the final two synthetic steps of a polar amino acid drug candidate, (1R,2S,5S,6S)-2-amino-6-fluoro-4-oxobicyclo[3.1.0] hexane-2,6-dicarboxylic acid.[1] (**A**, Figure 1). This drug candidate **A** is one of a class of conformationally constrained, highly selective, and orally active group II metabotropic glutamate receptor (mGluR) agonists[2–4] targeted for the treatment of neurological diseases including schizophrenia and depression.[5,6] The synthetic steps involve the formation of an aminonitrile (**B**) followed by its hydrolysis to form the amino acid (**A**, Figures 1, 2).

Aminonitriles are commonly used as intermediates in the synthesis of amino acids.[7,8] The formation of optically active α-aminonitriles is usually performed through an asymmetric Strecker synthesis.[7,9] In this case (Figure 2), stereoselective aminocyanation was performed by reaction of the ketal ketone **(E)** with ammonia and trimethylsilyl cyanide (TMSCN) in the presence of titanium (IV) isopropoxide, as a catalyst. The titanium (IV) isopropoxide acts as both a dehydrating agent and Lewis acid to form an imine intermediate (**D**). The imine intermediate then reacts with TMSCN forming the aminonitrile (**B**). This aminonitrile is subsequently hydrolyzed to yield the amino acid using a solution of acetic acid and hydrochloric acid in water at 100°C. Impurities generated during both synthetic steps were identified and a mechanism for their formation proposed. This information was then fed back to the process chemists in order for them to optimize the process.

EXPERIMENTAL

Reagents and Samples

The amino acid and its intermediates were prepared by Process Research at Merck Research Labs. Methanol and acetonitrile (HPLC grade), acetic acid, phosphoric acid, and potassium phosphate monobasic were purchased from Aldrich (Milwaukee, WI). Potassium phosphate dibasic was acquired from

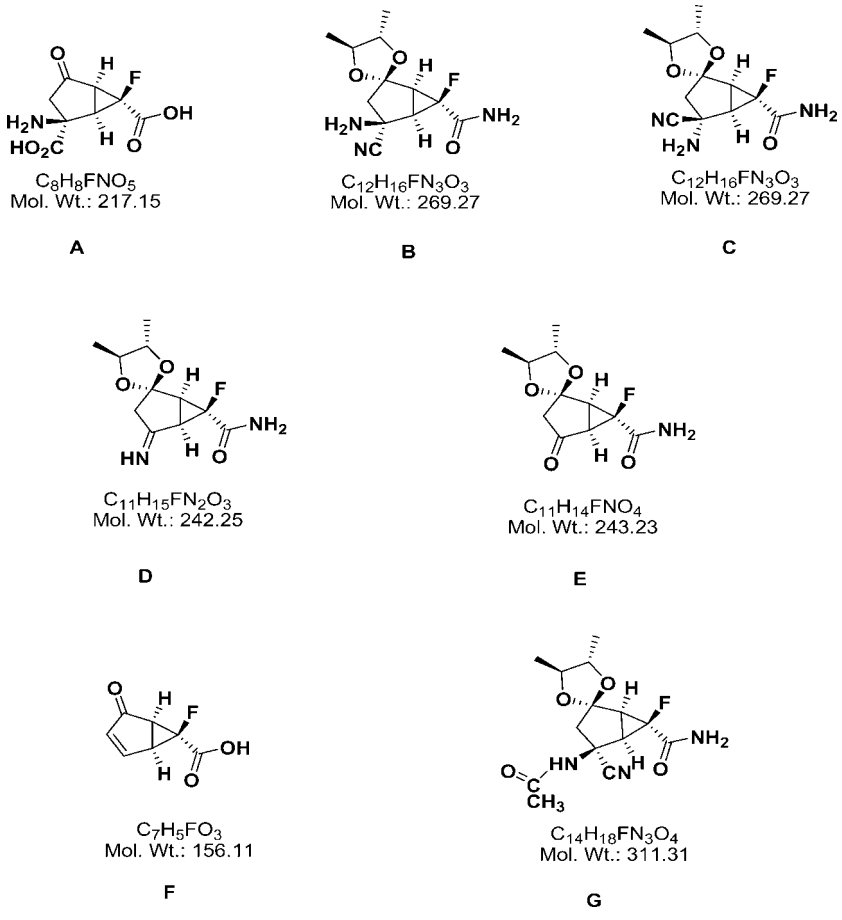


Figure 1. Structures of the drug substance (**A**), its intermediates (**B**), (**D**), (**E**), (**G**) and impurities (**C**), (**F**).

J. T. Baker (Phillipsburg, NJ). Highly purified water (HYDRO Service and Supplies, Garfield, NJ) was used throughout for the preparation of buffer and reagents.

Chromatographic Conditions

Analyses were performed with HP 1100 (Agilent, Palo Alto, CA) HPLC systems equipped with quaternary pump and photodiode array detection (DAD) systems. Data were collected using TurboChrom Navigator (Perkin Elmer, San Jose, CA).

HPLC separations were performed on YMC ODS-AQ or Atlantis HILIC Silica (Waters, Milford, MA) columns (150 × 4.6 mm, 3 μm particle size) at

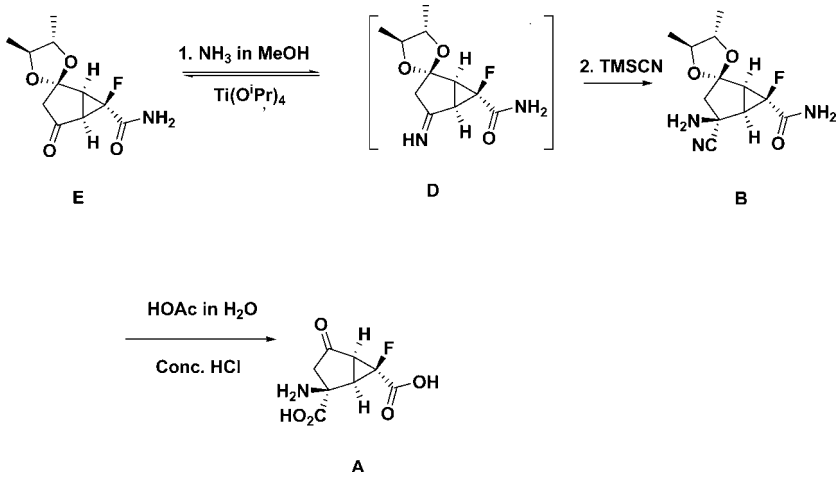


Figure 2. Synthetic scheme for the final two steps of the amino acid formation.

room temperature, with detection at 195 nm or 210 nm. Low wavelength detection had to be used because the aminonitrile has a low chromophore. The flow rate was kept at 1.00 mL/min. The sample concentration was 0.2 mg/mL for aminonitrile and 0.5 mg/mL for the amino acid compound. The injection volume was 10 μL. Acetonitrile was the organic modifier and the aqueous phase was water with 0.1% phosphate or acetate buffer.

Mass Spectra were obtained using a Finnegan LCQ ion trap mass spectrometer (San Jose, CA) with an ESI ion source operating in the positive ion mode. Nitrogen was used as sheath gas. Helium was used as the dampening gas in the ion trap. MS parameters were optimized as follows: Spray voltage = 4.5 kV, Capillary voltage = 9V, tube lens offset = −5V, capillary temperature = 200°C. For MS/MS, the analyte in the ion trap was activated by increasing collision energy from 0–30% of 5V maximum tickle voltage. The scan rate was 5000 scans/min. Each spectrum was obtained with 70–140 scans.

RESULTS AND DISCUSSION

The first step investigated in the synthetic process for the amino acid involved the conversion of a ketal ketone (**E**) to form the aminonitrile (**B**) (Figure 2). The stability of the isolated aminonitrile (**B**) at room temperature was initially assessed in methanol, as this was the reaction/crystallization solvent. It was determined that the aminonitrile (**B**) degraded under these conditions over four days, to form two degradates (Figure 3). One degradate (**C**) (Figures 1, 3), eluted close to the aminonitrile peak and the other degradate (**E**)

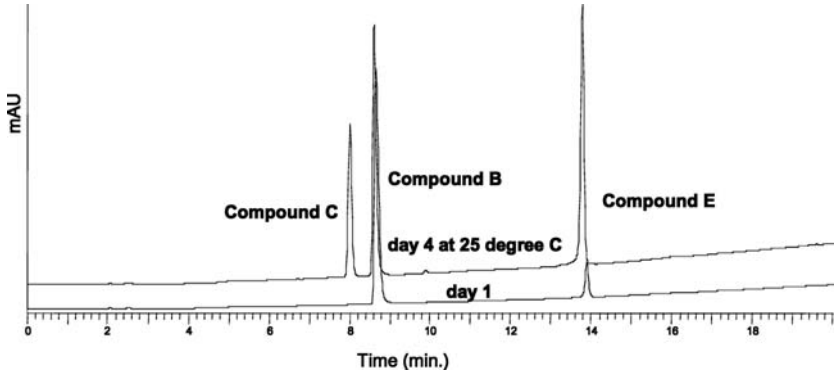


Figure 3. Stability of (**B**) in MeOH at 25°C. Chromatographic conditions: Waters YMC AQ HPLC column 150 x 4.6 mm, 3 μm particle size. Mobile phase (**A**)—0.1% Phosphoric acid in (**D**). I water; (**B**)—Acetonitrile; flow rate, 1 mL/min, 10 μL injection; 25° C; UV detection at 195 nm with gradient at 2% (**B**) to 20% (**B**) in 10 min., and then to 50% (**B**) in another 10 min.

eluted at the same retention time as that of the starting material ketal ketone (**E**). Mass spectrometry indicated that the first degradate (**C**) had a parent mass of 270 (M + 1), which is the same mass as the aminonitrile (**B**). The mass spectra of this degradate also closely matched that of the aminonitrile (**B**). The second degradate had a parent mass of 244 (M + 1), which is the same mass as the ketal ketone (**E**). The mass spectra of this degradate matched that of the ketal ketone (**E**).

The mass spectra of peak (**B**) and (**C**) showed the same parent mass peak. In addition, the authentic diastereomer (**C**) (synthesized by Process Chemist at Merck) was spiked in LC and its retention time is the same as the first degraded product. Based on the retention times and mass spectra, the first degradate was determined to be the diastereomer (**C**) (Figure 1) of the desired aminonitrile (**B**). Similarly, the second degradate was determined to be the starting ketal ketone (**E**). Aminonitriles are prone to retro-Strecker decomposition in water via its ketimine intermediate to reform the ketal ketone (Figure 4a, 4b).[7,10,11] The methanol used as the solvent possessed trace amounts of water. The presence of water led to epimerization of the aminonitrile (**B**) (Figure 4a).[7] The mechanism for the degradation of the aminonitrile (**B**) to form its diastereomer (**C**) and the ketal ketone (**E**) are proposed in Figure 4a and Figure 4b.

The stability of both pure diastereomers (**B**, **C**) in methanol was investigated. A freshly prepared sample of pure (**B**) in methanol at 0.2 mg/mL showed no (**C**) to be present by HPLC. When this sample was aged for 4 days at room temperature, (**C**) was formed (resulting in a (**B**) to (**C**) ratio of 2:1), along with the ketal ketone (**E**) (Figure 3). A freshly prepared sample of pure (**C**) in methanol at 0.2 mg/mL showed no (**B**) to be present

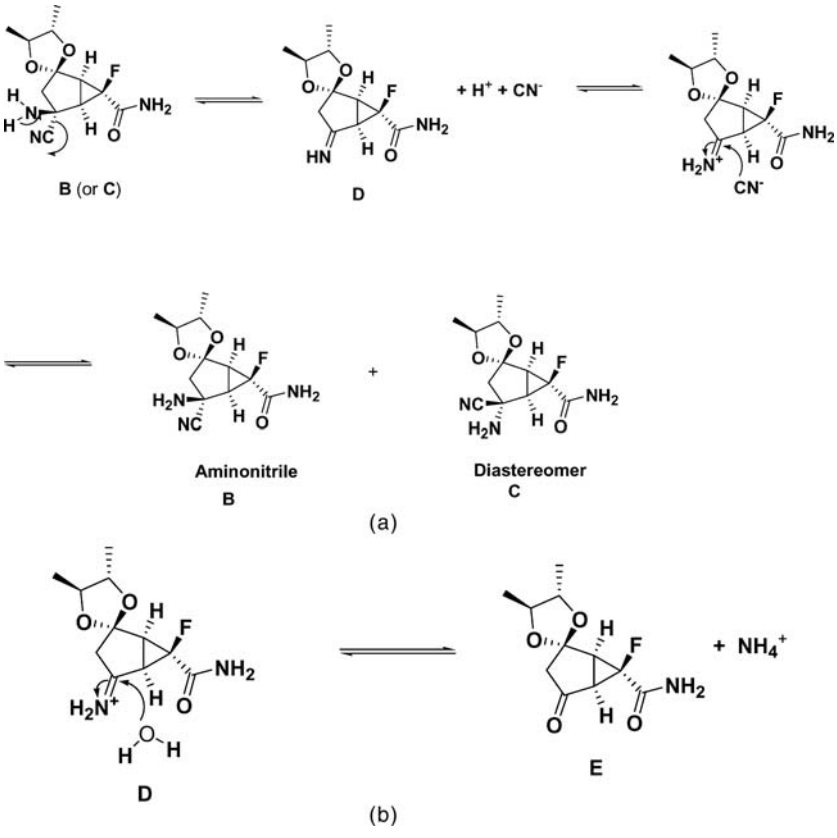


Figure 4. (a) Mechanism of aminonitrile rearrangement to its disastereomer. (b) Mechanism of aminonitrile degradation to ketal ketone.

by HPLC. But when the sample was aged for 4 days at room temperature, (**B**) was formed (resulting in a (**B**) to (**C**) ratio of 1:16), along with ketal ketone (**E**) (Figure 5). A typical 80% yield of (**B**), for the reaction of ketal ketone (**E**) to aminonitrile (**C**), suggests that though (**B**) is the kinetically controlled isomer, (**C**) is the thermodynamically favored isomer. This observation is consistent with gas phase ab initio calculations for (**B**) and (**C**), as shown in Figure 6. The results indicate that (**C**) is more stable than (**B**) by 1.34 kcal/mol, which is consistent with our experimental observations.

To support this mechanism for degradation, (**B**) was converted to an acetamidonitrile (**G**), shown in Figure 7 and the stability of (**G**) in methanol was determined after aging four days. The expectation was that the presence of the acetamide moiety will prohibit the formation of the ketimine intermediate and, thus, no degradation to the ketal ketone or the diastereomer (**C**) would occur. The acetamidonitrile (**G**) was indeed found to be

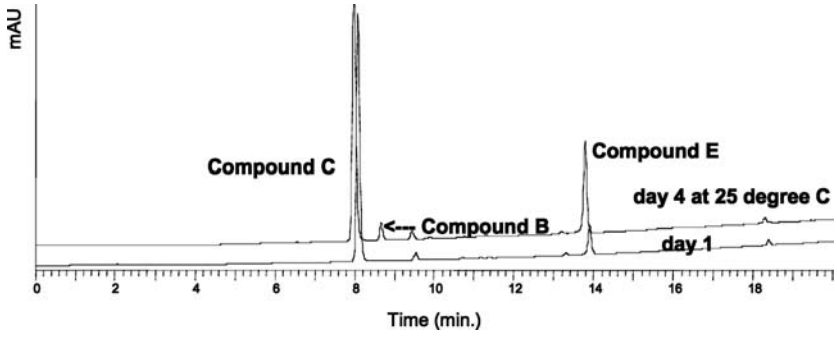


Figure 5. Stability of (**C**) in MeOH at 25°C. Chromatographic conditions are the same as in Figure 3.

stable in methanol with no formation of either the diastereomer (**C**) or the ketal ketone (**E**).

The mechanism was further confirmed through additional mass spectrometry data. A sample of (**B**) in methanol was introduced to a mass spectrometer by direct infusion in ESI positive mode. Among the peaks observed was a peak of m/z 270 (M + H) representing (**B**), as well as a peak of m/z of 243 (M + H). MS/MS of the peak of m/z 270 (M + H), (E% = 0–30%) did not produce a fragment of m/z 243 (M + H). This indicates that the peak at m/z 243 (M + H) is from a component in the solution, rather than a fragmentation product. The mass of 243 corresponds to that of the ketimine intermediate ($\mathbf{DH}^+$). This peak was not observed during LC-MS, as the intermediate (**D**) is very labile in aqueous environment of LC mobile phase buffer, leading to its conversion back to the ketone ketal (**E**).

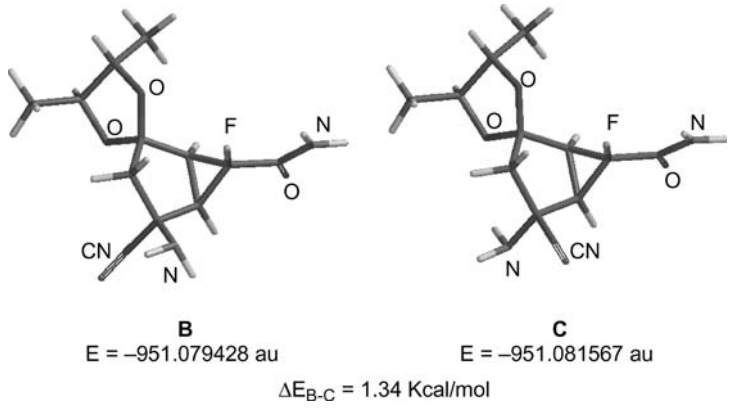
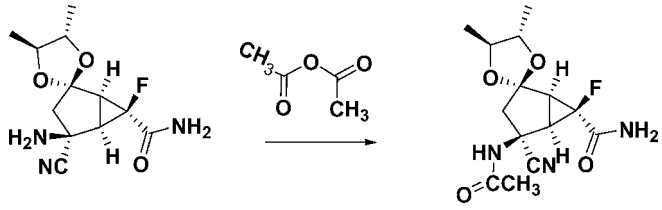


Figure 6. The calculated structures of (**B**) and (**C**). The calculation of molecular energies was performed on a PC Spartan 04 using an HF/6-31G* basis set.



B G

Figure 7. Synthetic scheme for the formation of (**G**) from (**B**).

Elucidation of the degradation pathways of the aminonitrile (**B**) to form its diastereomer (**C**) and the ketal ketone (**E**), allowed the process chemists to further optimize the reaction conditions to avoid formation of ketone (**E**) and the diastereomer **C**, therefore achieving a higher yield and quality of product. This elucidation was also helpful from an analytical perspective. Knowing the instability of aminonitrile in methanol led to studies to determine its stability under the aqueous conditions utilized for the chromatographic analysis (0.1% phosphoric acid). This study showed extensive degradation of the aminonitrile to the ketal ketone after two days. There was no evidence of diastereomer formation, which was expected, given that the high levels of water would favor the pathway to the ketal ketone. The run time and sample tray temperature (5°C) of HPLC conditions were consequently optimized, and samples were prepared fresh prior to injection to minimize degradation and avoid over estimating the level of ketal ketone (**E**) present in the product mixture.

The hydrolysis of the aminonitrile (**B**) to the amino acid (**A**) was also studied. Hydrolysis was performed in aqueous solutions of acetic acid and hydrochloric acid at 100°C, resulting in the formation of the hydrochloride salt of the drug substance. This step also included a rinse of the amino acid with hot water. As a precursor to scaling this reaction, the stability of the amino acid in these solutions was evaluated. A significant impurity was observed to form within 30 minutes. At 4 hours, chromatographic analysis indicated the formation of greater than 12% by area total of this impurity (Figure 8). Mass spectrometric analysis indicated that the mass of this impurity was 156. This mass corresponded to a loss of ammonia and carbon dioxide and was determined to be (**F**), which originates from the degradation of (**A**), via an elimination of ammonia[12–14] followed by decarboxylation.[15] The formation pathway is depicted in Figure 9. Because a significant amount of impurity formation was observed, the reaction temperature for the hydrolysis of aminonitrile to drug substance was decreased from 100°C to 75°C in order to minimize the impurities and maximize the yield. The formation of impurity (**F**) was decreased significantly (from 12% to about 1% in four hours), and the yield of amino acid (**A**) was greatly improved.

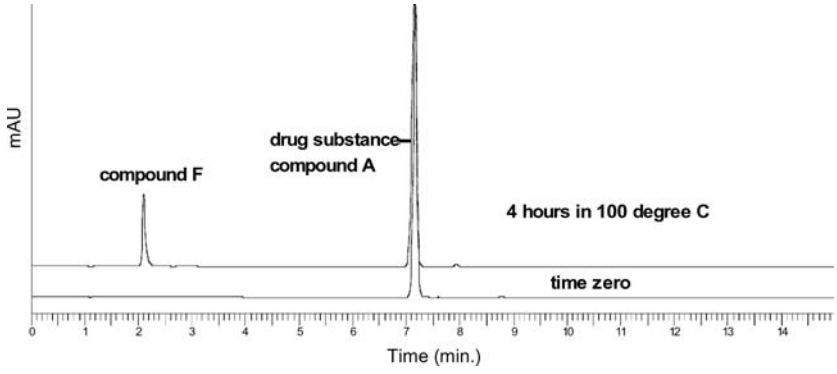


Figure 8. The formation of hydrolysis degradate of the drug substance in 100°C water. Chromatographic conditions: Atlantic Hilic Silica, 3 μm, 150 × 4.6 mm column. Mobile phase (**A**)—0.1% Phosphoric acid in (**D**). I water; (**B**)—Acetonitrile; flow rate, 1 mL/min, 10 μL injection; 25° C; UV detection at 210 nm with gradient at 95% (**B**) to 60% (**B**) in 7 min, and hold for another 8 min.

CONCLUSION

A diastereomeric impurity (**C**) and the starting ketal ketone (**A**) were observed to form from solutions of an aminonitrile (**B**) in methanol. The pathway for the generation of the diastereomer of aminonitrile and the ketal ketone was proposed through an initial formation of a ketimine intermediate. An investigation of the subsequent hydrolysis step led to the elucidation of the major impurity formation. The proposed mechanistic pathways provided insight, and the information was used to optimize the reaction conditions for both penultimate and final drug substance formation. The yields of the desired

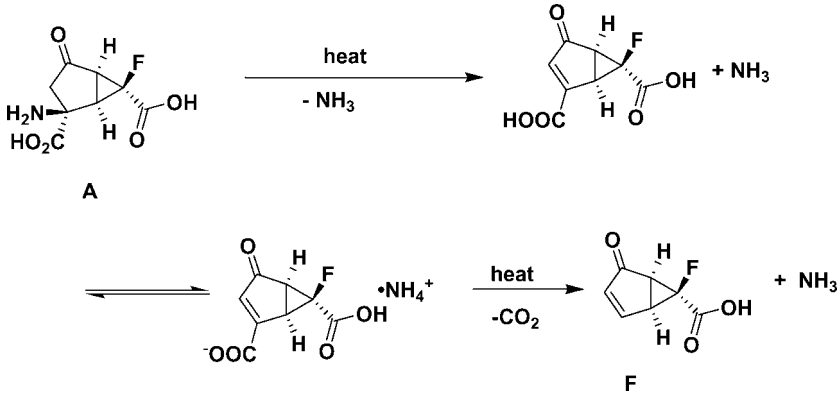


Figure 9. The proposal pathway for the formation of (**F**).

compounds were improved, and impurity generation was significantly minimized through optimizing holding time of aminonitrile in methanol and lowering the hydrolysis temperature of aminonitrile to amino acid drug substance.

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