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An Efficient Scale-up Synthesis of BMS-520, a Potent and Selective Isoxazole-containing S1P1 Receptor Agonist

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An Efficient Scale-up Synthesis of BMS-520, a Potent and Selective Isoxazole-containing $S1P_1$ Receptor Agonist

Xiaoping Hou, Juliang Zhu, Bang-Chi Chen, Scott H. Watterson, William J. Pitts, Alaric J. Dyckman, Percy H. Carter, Arvind Mathur and Huiping Zhang*

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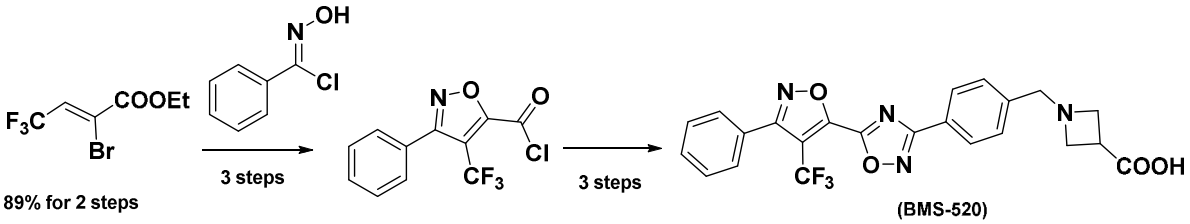


ABSTRACT: This paper reports an efficient scale-up synthesis of 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (BMS-520), a potent and selective isoxazole-containing $S1P_1$ receptor agonist. This process features a highly regioselective cycloaddition leading to a key intermediate, ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate, a chemo-selective hydrolysis of its regioisomers, as well as an improved method for 1,2,4-oxadiazole formation, relative to the original synthesis. The improved process was applied to the preparation of multiple batches of BMS-520 for preclinical toxicological studies.

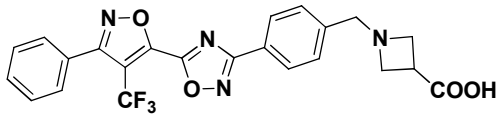
KEYWORDS: Regioselective dipolar cycloaddition, Chemo-selective hydrolysis, Substituted isoxazole synthesis, 1,2,4-oxadiazole, $S1P_1$ receptor agonist, Process optimization.

INTRODUCTION

Sphingosine 1-phosphate (S1P) is the endogenous ligand for the sphingosine 1-phophate receptors ($S1P_{1-5}$). S1P and its interaction with the S1P receptors plays an important role in a number of processes including vascular stabilization, heart development, lymphocyte homing, and tumor related angiogenesis.[1-2] Agonism of $S1P_1$, in particular, has been shown to play a significant role in lymphocyte trafficking from the thymus and secondary lymphoid organs, leading to immunosuppression, which has been demonstrated to provide a novel mechanism for the treatment of autoimmune diseases.[3-5]

During our drug discovery program, 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid **1** (BMS-520) was identified as a novel isoxazole-based $S1P_1$ receptor agonist,[6] providing highly potent $S1P_1$ receptor full agonism with an EC_{50} of 0.47 nM and selectivity against the $S1P_3$ receptor. Based on its in vitro potency and

selectivity, PD/PK data, and liability profile, BMS-520 (**1**) was selected for further biological evaluation. Herein, we outline a new and efficient scale up synthesis of **1** to support preclinical toxicology studies.



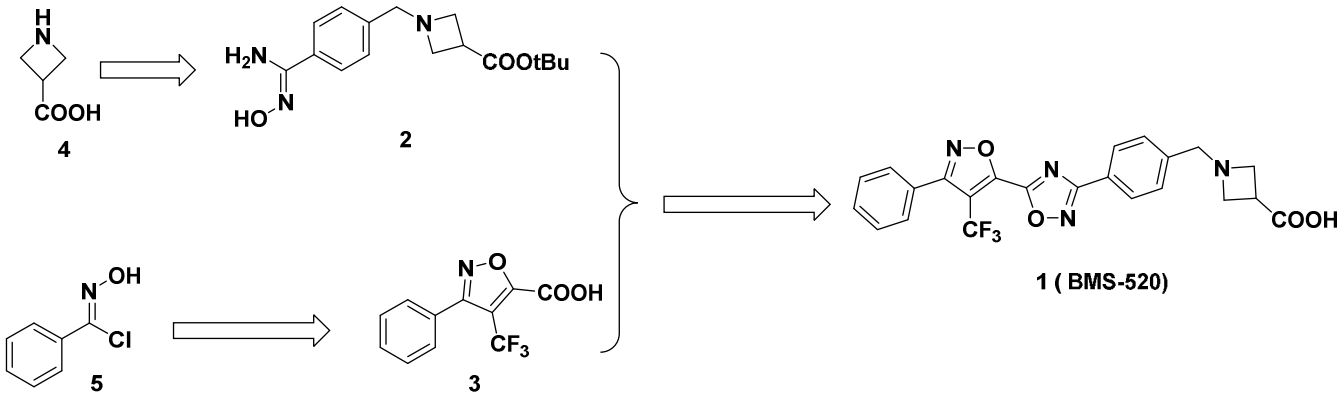
1 (BMS-520)

Figure 1. 1-(4-(5-(3-phenyl-4-(trifluoromethyl) -isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)-azetidine-3-carboxylic acid **1**.

RESULTS AND DISCUSSIONS

The preparation of **1** relied on a convergent synthesis (Scheme 1)[6] which required two advanced intermediates, tert-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate **2** and 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid **3**. Amidoxime **2**[8] was prepared from commercially available azetidine-3-carboxylic acid **4** in five linear steps utilizing our external collaborator. Our initial efforts were thus focused on a regiospecific and scalable synthesis of the requisite coupling partner **3**.

Scheme 1: Convergent Synthesis of 1.

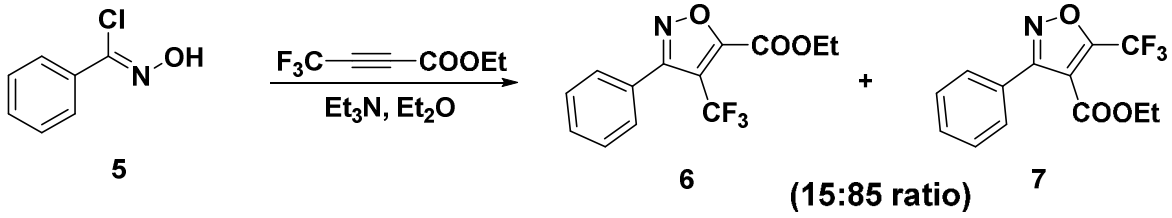


The original synthesis of 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid 1. An initial review of the literature at the time revealed that the only reported method for the preparation of ester **6** occurred through a 1,3-dipolar cycloaddition reaction between ethyl trifluorobutyno-2-ate and a nitrile N-oxide dipole generated in situ from N-hydroxybenzimidoyl chloride(**5**).[7] Unfortunately, this reaction favored the undesired regioisomer **7** in a ratio of 85:15, as depicted in scheme 2. In order to invert the regioselectivity of the [3+2]-cycloaddition reaction, the electronic pairing of the dipolarophile was inverted by replacing ethyl trifluorobut-2-ynate with 4,4,4-trifluorobut-2-yn-1-ol[8], as previously reported (Scheme 3).[6] This approach successfully resulted in a mixture of isomers **8** and **9** in a ratio of 9:1 favoring the desired isomer **8** with a typical yield of 60-65% after chromatographic separation. Isomer **8** was then oxidized to **3** in >90% yield with either Jones' Reagent or TEMPO/bleach.[6]

Although this route offered a very effective method for preparing acid **3** on moderate scale, there were a couple of aspects of the synthesis that were considered less desirable on larger scale. The first concern was that this approach relies on synthesizing non-commercially available 4,4,4-trifluorobut-2-yn-1-ol as a starting material. Upon preparation, the low boiling

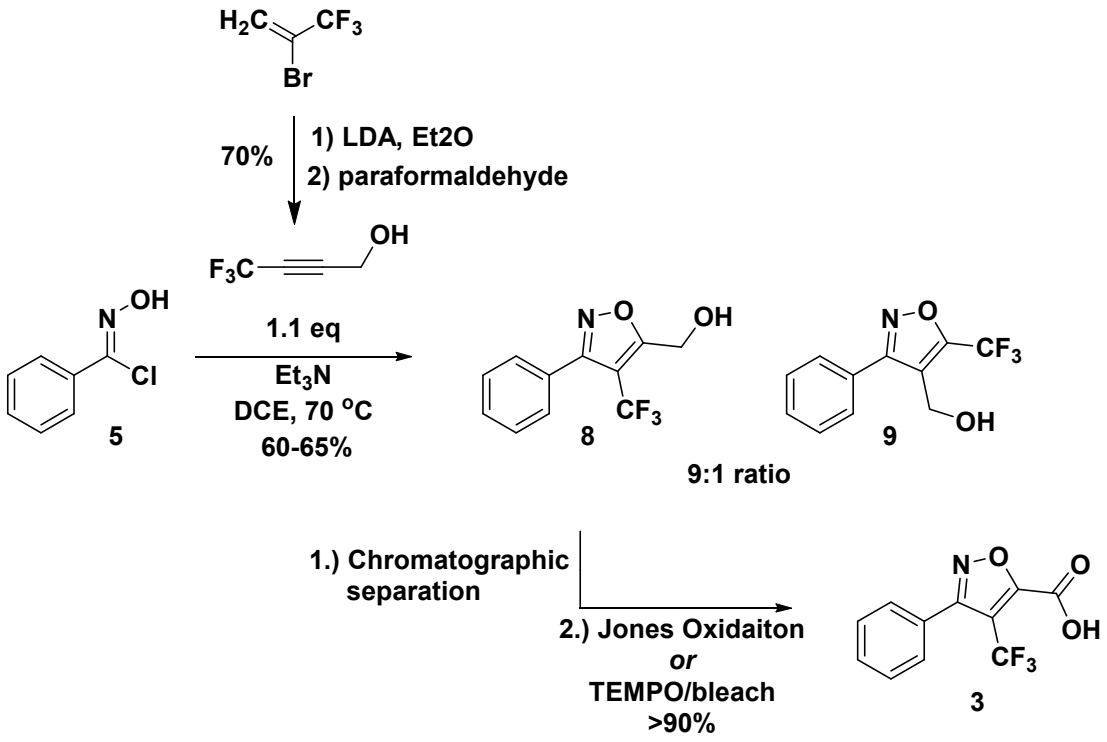
point crude product required a distillation for purification, which could result in variations in both yield and purity from batch to batch. A second concern was that a 9:1 ratio of isomers required chromatographic separation to isolate pure isomer **8**, which would be undesirable on scale. A final concern was that the alcohol requires oxidation to provide carboxylic acid **3**. As a result, a more efficient highly regio-selective alternative synthesis of 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid **3** was desired.

Scheme 2. Isoxazole regiosomeric distribution starting with ethyl trifluorobytyno-2-ate.



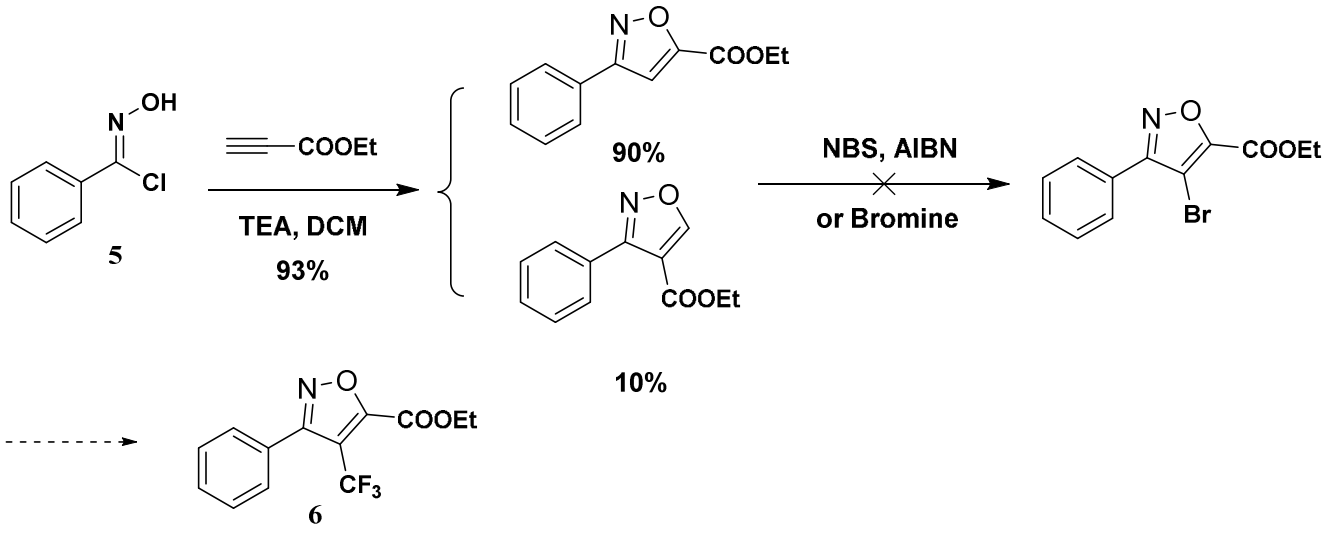
An alternative synthesis of 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid 3. It was anticipated that carboxylic acid **3** could be readily prepared from its corresponding ester **6** via hydrolysis. We envisioned two possible routes to improve access to trifluoromethylated isoxazoles like ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate **6**: 1) introduce the 4-CF_3 to a 3, 5-disubstituted isoxazole after the cycloaddition; or 2) improve access to the desired regioisomer **6** by exploring alternate cycloadditon approaches utilizing CF_3-containing starting materials.

Scheme 3. Original Synthesis of 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid **3**.



Our initial exploration focused on direct trifluoromethylation on the isoxaozle since this approach had been successful on a related isoxazole ester.[6,9] Additionally, we were encouraged to pursue this route as the dipolar cycloaddition provided the desired isoxazole regioisomer **10** as the predominant regioisomer in good yield. (Scheme 4).[10] However, all attempts to incorporate a bromine at C4 proved to be difficult, perhaps due to the electronic characteristics at C4. As a result, we turned our attention to exploring alternate CF_3-containing starting materials (Scheme 5).

Scheme 4. Late stage CF3 incorporation approach.

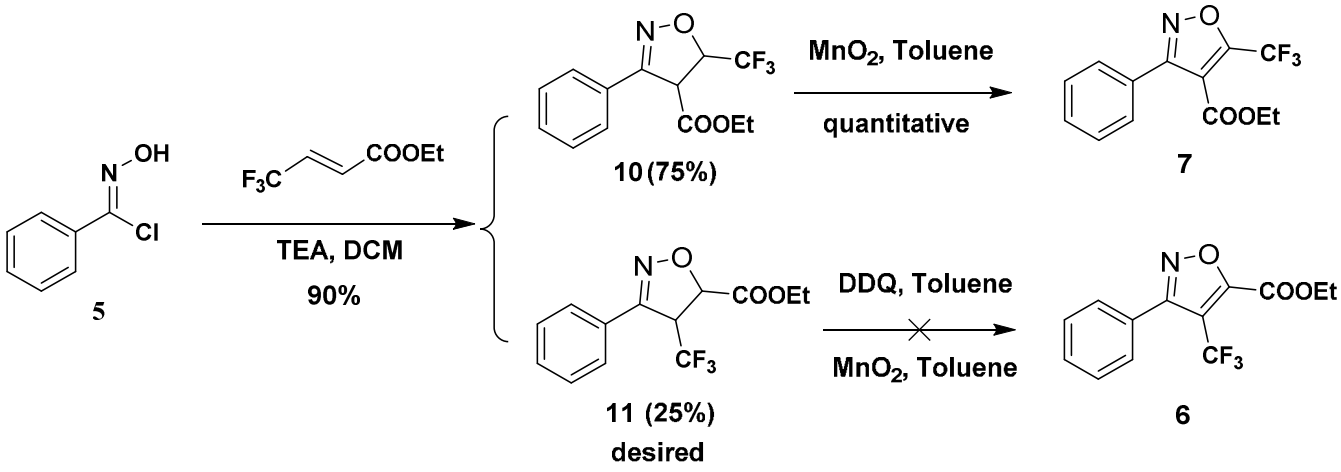


Since (E)-ethyl 4,4,4-trifluorobut-2-enoate is commercially available at a low cost, we planned to synthesize the corresponding dihydroisoxazole (**10** and **11**). The desired isomer could then be readily aromatized to ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate **6** (Scheme 5). Despite modest regioselectivity favoring the undesired dihydroisoxazole regioisomer **10** in a ratio of 1 : 4 (**10** : **11**), the dihydroisoxazoles were readily prepared in high yield. While the undesired dihydroisoxazole **10** could be readily aromatized with MnO_2 in quantitative yield, oxidation of the desired dihydroisoxazole **11** with either DDQ or MnO_2 failed to provide **6**. Due to the poor regioselectivity in the initial cycloaddition step, we decided not to pursue alternate oxidation conditions.

We next turned our attention to exploring trifluoroalkene precursors substituted with a potential leaving group to facilitate formation of the desired isoxazole. We hypothesized that groups such as an OH or a Br could reverse the orientation of the dipolarophile during the cycloaddition through a combination of electronic and steric effects. Additionally, incorporation of a hydroxyl or Br was expected to significantly facilitate the aromatization step via elimination of H_2O or HBr as a driving force. Therefore, we proposed enoates **12**, **13**, and **14** for comparison

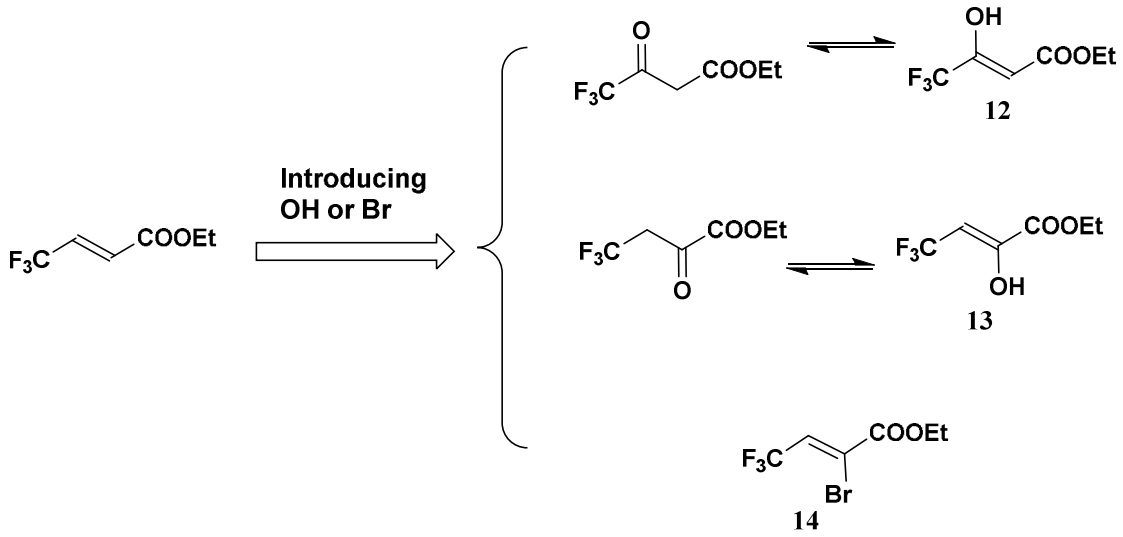
and further exploration (Scheme 6). Although enoate **12** was commercially available, it would likely favor the undesired regioisomer. In contrast, enoate **13** was expected to favor the desired regioisomer; nevertheless, it was not readily accessible, especially for a scale-up. Literature reports suggested that enoate **14** could be readily prepared through bromination followed by elimination.[11-12] It was anticipated that enoate **14** would favor the desired regioisomer. Thus, it was selected for further exploration.

Scheme 5. Building the heterocycle via aromatization approach.

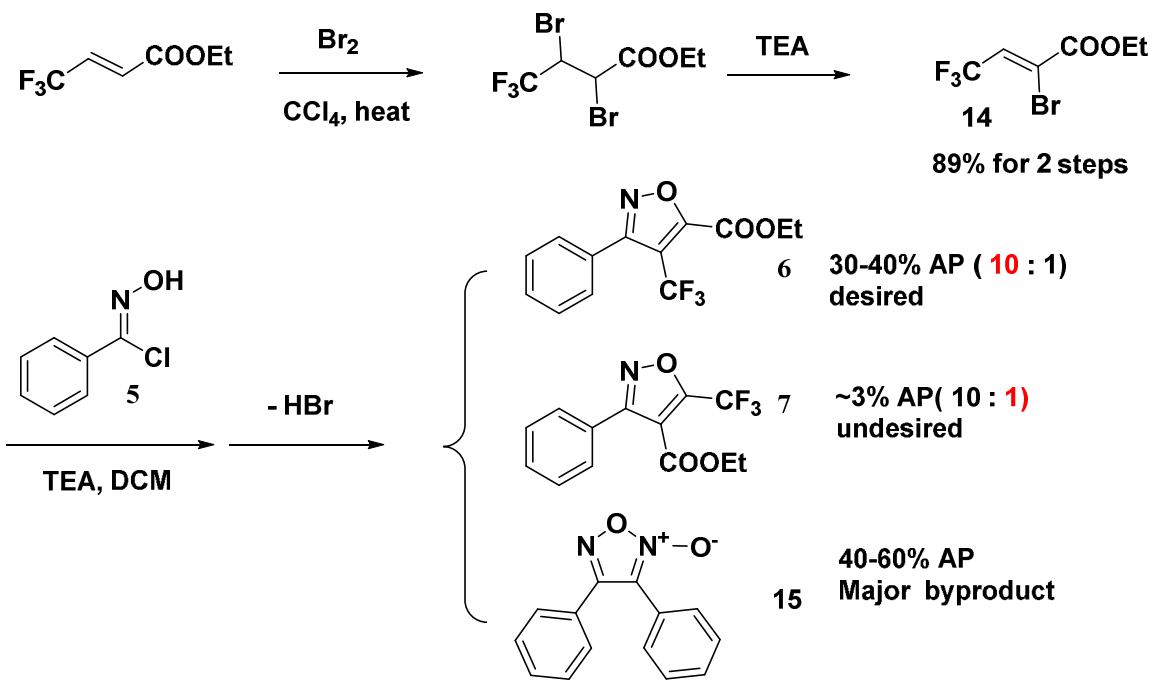


The initial pilot results were very encouraging, leading to the discovery of a novel, highly regioselective [3+2] cycloaddition (Scheme 7). As expected, the high regioisomer ratio (10:1) favored the desired isomer. Our efforts to optimize the regiochemical outcome of this reaction have been previously reported.[13] The cycloaddition, elimination, and aromatization sequence progressed very smoothly, and no intermediate dihydroisoxazole was observed. However, the yield was still low as a result of the formation of dimerization byproduct (**15**) during the reaction.

Scheme 6. Proposed CF_3 -containing enoates.



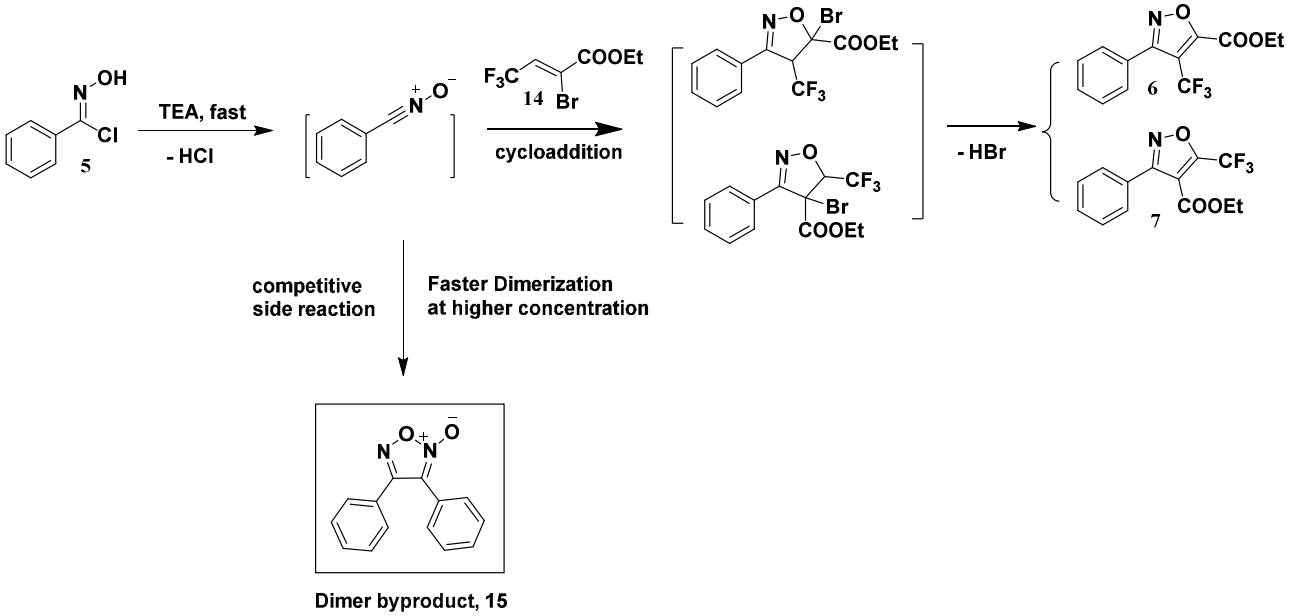
Scheme 7. Pilot regioselective [3+2] cycloaddition.



To optimize the [3+2] cycloaddition step, a mechanism was postulated based on both literature reports[14] and our own observations from the pilot experiments (Scheme 8). Kinetically, the initial elimination reaction of HCl from the starting chlorooxime occured quickly under the basic reaction conditions (TEA/DCM). This was evidenced by the disappearance of the

chlorooxime starting material **5** within minutes of base addition and a concomitant detectable molecular ion (LC/MS) corresponding to formation of the nitrile N-oxide. The [3+2] cycloaddition and elimination sequence and the dimerization were competitive processes, with the cycloaddition and elimination pathway occurring somewhat slower to that of the dimerization pathway, which was especially true when the concentration of nitrile N-oxide was higher. Therefore, our optimization rationale was to slow down the nitrile N-oxide formation, thus minimizing the dimerization process.

Scheme 8. Mechanism of [3+2] cycloaddition.[14-15]



To facilitate the slow release of the nitrile N-oxide, while maintaining the enoate **14** in large excess, we considered the micro-solubility of inorganic bases in different organic solvents under heterogeneous conditions at room temperature. Among the limited screening (Table 1), $KHCO_3$ and ethyl acetate provided an optimal combination for this [3+2] cycloaddition, which was further scaled up with isolated yields in the range of 72-82% and with high regioisomer selectivity (**6** : **7** = 20:1). However, the presence of ~4% of the undesired regioisomer **7** and

residual dimer **15** were difficult to separate by flash chromatography due to the non-polar nature of these compounds. To address this issue, we developed a robust, selective hydrolysis to remove all impurities, eliminating the need for column chromatography.

Table 1. Optimization of [3+2] cycloaddition.[a]

Entry	Base	Solvent	Yield [b]
1	KOAc	ethyl acetate	41%
2	K_2CO_3	ethyl acetate	65%
3	$KHCO_3$	ethyl acetate	72%
4	KOAc	EtOH	27%
5	$KHCO_3$	DMF	13%
6	$KHCO_3$	50% aq. EtOH	trace

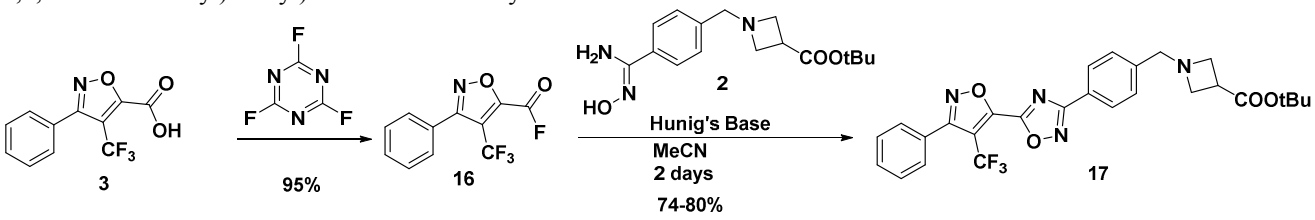
[a] Standard condition: 0.2 mmol Chloro-oxime, 3 eq. of base, and 0.2M concentration, RT, 16h.
[b] Yields determined by HPLC against a calibrated standard.

The initial hydrolysis was performed with LiOH in MeOH at RT overnight. This resulted in the hydrolysis of the undesired regioisomer **7**, the hydrolysis of the desired ester **6**, and a di-acid byproduct resulting from the hydrolysis of the CF_3 group under the basic conditions. Interestingly, we noticed that hydrolysis of the undesired regioisomer ester **7** was much slower. Consequently, we lowered the reaction temperature to 0^oC and used water compatible solvents like THF or dioxane instead of MeOH to avoid potential ester exchange. After 1.5-2 h, a complete chemo-selective hydrolysis was achieved. Undesired regioisomer **7** and any residual dimer were successfully removed with ether extraction.

The original synthesis of tert-butyl 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (17). As shown in Scheme 9, the

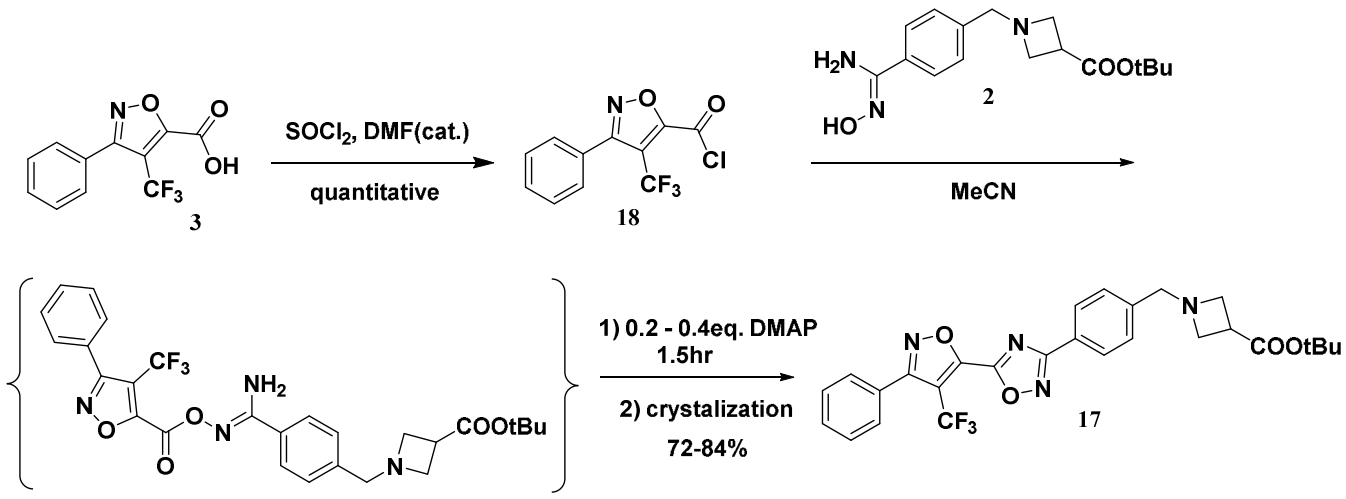
original synthesis utilized cyanuric fluoride to generate the acid fluoride intermediate **16**. Cyanuric fluoride can be quite corrosive and could potentially erode the glassware on a large scale. In addition, Hunig's base induced amidation/cyclization sequence to form the oxadiazole required a lengthy reaction time, although providing **17** in overall good yield..

Scheme 9. Original synthesis of the penultimate, tert-Butyl 1-(4-(5-(3-phenyl-4-(trifluoromethyl)-isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate.



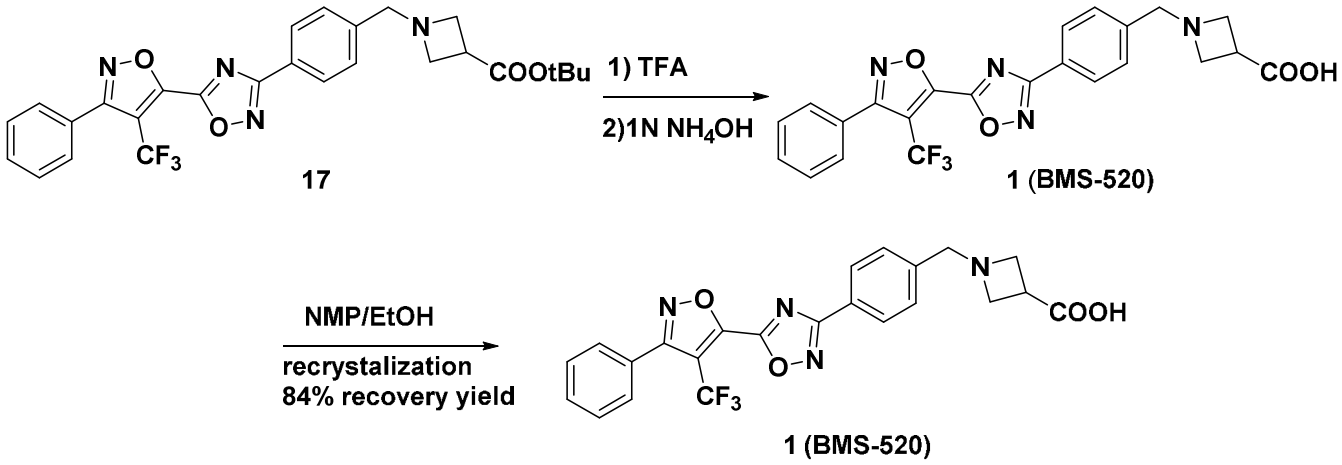
To address the aforementioned concerns, cyanuric fluoride was replaced with thionyl chloride to prepare the acyl chloride **18** in quantitative yield as an off-white solid which was easy to handle and relatively stable for a few months under anhydrous condition. Meanwhile, an efficient DMAP catalyzed 1,2,4-oxadiazole formation was developed which could directly crystallize the product **17** from the reaction mixture with high purity (>99%) and in good yield (72 - 84%).

Scheme 10. Improved 1, 2, 4-oxadiazole formation.



Generally, in a scale-up synthesis for a toxicological batch, the final step is the most important step in terms of purification. In this case, although deprotection of a t-butyl group is well established in the literature, purification could be difficult due to the zwitterionic properties of **1**. Original deprotection was accomplished with neat TFA followed by neutralization with 1N NaOH, producing very fine particles that resulted in very slow filtration that could trap impurities. Additionally, the original purification required two trituration/slurrying steps in MeOH to achieve the desired purity and crystalline form. To improve this process, 1N aqueous NH_4OH was used rather than NaOH to neutralize the TFA salt to generate a larger particle size to allow for a smooth filtration (Scheme 11). Additionally, the TFA-ammonium salt is more soluble in organic solvents than the TFA sodium salt, thus making it easier to remove. Furthermore, a new efficient recrystallization (NMP/EtOH) was developed to afford the product with high purity.

Scheme 11. Improved deprotection and purification.



In summary, we discovered a novel, practical, scalable, and highly selective [3+2] cycloaddition to prepare ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate **6**. In addition, its regioisomers (**6**, **7**) could be chemo-selectively hydrolyzed under a mild condition. Furthermore, an improved 1,2,4-oxadiazole formation gave the penultimate **17** in high yield with a simplified purification. The improved deprotection and the new recrystallization condition offered a smooth filtration providing the highly pure crystalline product. This process was utilized to prepare multiple batches of **1** for toxicological studies.

EXPERIMENTAL SECTION

All reagents were obtained from Aldrich Chemical Company and used without further purification unless otherwise stated. All reactions were performed under a nitrogen atmosphere. All glassware was dried and purged with nitrogen or argon before use. All reactions were monitored by Shimadzu LCMS system using the following method: Phenomenex C18 column 10 μm 4.6 X 50 mm. Solvent: A = 10% methanol/90% water with 0.1% TFA; B = 90% methanol/10% water with 0.1% TFA. Gradient: 0-100% B over 4 min. Flow: 4 mL/min,

Wavelength: 220 nm. HPLC analyses were performed using a Shimadzu system (model SPD 10AV). All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 MHz or 400 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as the solvents.

Ethyl 2,3-dibromo-4,4,4-trifluorobutanoate. Bromine (18.39 ml, 357mmol) was added drop-wise to a solution of (E)-ethyl 4,4,4-trifluorobut-2-enoate (50 g, 297mmol) and carbon tetrachloride (50 ml). The resulting dark red solution was heated to gentle reflux for 4 hours. Another 2ml bromine was added and continued to reflux until HPLC analysis showed no starting material remaining. The reaction mixture was concentrated. The crude product was directly used in the next step. HPLC (XBridge 5µ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 2.96 and 3.19 minutes.

(Z/E)-ethyl 2-bromo-4,4,4-trifluorobut-2-enoate 14. The above-prepared ethyl 2,3-dibromo-4,4,4-trifluorobutanoate (297mmol) was diluted with 200mL hexane, cooled to 0^{o}C. TEA (49.7 ml, 357mmol) was added drop-wise. A voluminous white precipitates formed during addition. Stirring was continued for 2 hours (LC showed a complete conversion), then, the solid was filtered off, washed with hexane (3X50mL), and discarded. The filtrate was carefully concentrated to yield the crude product as yellowish oil. The crude product was passed through a short silica gel pad with 10% ethyl acetate/hexane. (Z/E)-ethyl 2-bromo-4,4,4-trifluorobut-2-enoate (65.5 g, 265mmol, 89 % yield for two steps) was obtained as colorless oil. Alternatively, the crude product could be purified through distillation (85^{o}C / ~60 mmHg). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 (q, 1H, J = 7.28 Hz), 4.35 (q, 2H, J = 7.11 Hz)), 1.38 (t, 3H, J = 7.15 Hz)); HPLC (XBridge 5µ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 %

H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.09 minutes.

Ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate 6. (Z/E)-Ethyl 2-bromo-4,4,4-trifluorobut-2-enoate (39.7 g, 161mmol) and (Z)-N-hydroxybenzimidoyl chloride (30 g, 193mmol) were dissolved in ethyl acetate (150mL). Subsequently, potassium hydrogen carbonate (32.2 g, 321mmol) was added in portions and stirred overnight at room temperature. Ethyl acetate was removed in vacuum. The residue was re-suspended in 300mL hexane and stirred for 10miutes. After filtration, the filtering cake was washed with hexane (3X30mL). The filtrate was concentrated in vacuum to give crude product, which was further purified with flash chromatography to generate 33g product (72%) as yellowish oil. Bp. 111^{o}C / ~0.7 torr; ^{1}HNMR was consistent with the reference spectrum. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (m, 5H), 4.53 (q, 2H, J = 7.28 Hz), 1.46 (t, 3H, J = 7.15 Hz); MS *m/e* 286.1($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.57 minutes.

3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid 3. Ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate (40g, 140 mmol) was dissolved in THF (240 mL) and Water (24.01 mL), then cooled to 0°C (internal temperature). Subsequently, LiOH monohydrate (5.04 g, 210 mmol) was added in portions and monitored with HPLC. The reaction mixture was stirred overnight at 0^{o}C. LC showed 17% AP of starting material. Then, 0.1eq (336mg) of LiOH was added at 0^{o}C and gradually warmed up to rt for 3 hours. The reaction mixture was re-cooled to 0^{o}C, diluted with 200mL ether, then, 200mL water. The aqueous phase was separated and extracted with ether (2X50mL). The organics were combined and washed with water (3X30mL), then discarded. The aqueous phase were combined, cooled to 0^{o}C, and then neutralized with 1N

HCl, extracted with ether (3x200mL). Ether layers were combined and dried with Na_2SO_4. After concentration, 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid (29.5 g, 114 mmol, 81 % yield) were obtained as pale solid (purity 99%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44 - 7.68 (m, 5H), 6.2-7.0 (br, 1H); MS *m/e* 257.8($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 2.45 minutes.

3-Phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl chloride 18. 3-Phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid (29.2g, 114 mmol) was suspended in thionyl chloride (100 ml, 1370 mmol) at RT. The reaction mixture was heated up to 90^{o}C for 24 hours. The reaction was monitored with HPLC (Note: all HPLC sample was prepared by quenching an aliquot of reaction mixture with anhydrous methanol). HPLC at 24 hour indicated that the reaction was completed (peak to peak conversion). The reaction mixture was cooled down to RT, diluted with 100mL anhydrous toluene, then concentrated in vacuum. The yellow oil residue was further pumped overnight under high vacuum to afford a gray solid (31.3g, 114mmol, 100% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.50 - 7.63 (m, 5H). HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.29 minutes (methyl ester derivatized from MeOH quench).

tert-Butyl 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate 17 (the penultimate). A 1 liter 3-necked round bottom flask was charged with a suspension of tert-butyl 1-(4-(N'-hydroxycarbamimidoyl)benzyl)azetidine-3-carboxylate (38.1 g, 125 mmol) in dry acetonitrile (222 mL) at room temperature under N_2. 3-phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl

chloride (31.3 g, 114 mmol) was added in portions. The residual acyl chloride was rinsed with acetonitrile (2x30ml). The reaction was slightly exothermic at this stage, which warmed up the reaction mixture from 25°c to 36°c. A light brown solution was observed. After 30 minutes stirring, 4-Dimethylaminopyridine (4.86 g, 39.7 mmol) was added. The reaction mixture was heated to 65°c and stirred for 1.5 hour. HPLC indicated the reaction was complete. Subsequently, the reaction mixture was cooled to 0°c using ice-bath. The slurry was filtered and rinsed with cold acetonitrile (2x30ml) and dried in vacuum for 2 hours, then at 50°c for additional 4 hours. tert-butyl 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (40g, 1st crop, 76 mmol, 66.9 % yield) was obtained as a white solid with >99% purity. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1. 47 (s, 9H) 3. 28 -3. 37 (m, 3H), 3. 60 (br. s. , 2H), 3. 74 (br. s. , 2H), 7. 49 (d, J=7. 70 Hz, 2H), 7. 53 - 7. 62 (m, 3H), 7. 69 (d, J=7. 15 Hz, 2H), and 8. 16 (d, J=7. 70 Hz, 2H); MS *m/e* 527.1($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.47 minutes.

1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid 1 (BMS-520). tert-Butyl 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylate (83g, 158 mmol) was added to a stirring trifluoroacetic acid (200mL, 2596 mmol) in small portions. The reaction mixture was stirred at room temperature for 45 min. HPLC showed the deprotection was complete. The reaction mixture was concentrated under vacuum to remove TFA. The residue was cooled to 0°C, neutralized slowly with 1N ammonium hydroxide to control the temperature below 8°C and adjust pH = 7. Fine precipitates were observed. After filtration, the solid was rinsed with 100mL water. The sample was dried in vacuum overnight. 1-(4-(5-(3-

phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (57.8 g for 1st crop, 121 mmol, 77 % yield, 98.2% purity) was obtained as a white solid.

Recrystallization: 1-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)benzyl)azetidine-3-carboxylic acid (55.8g, 119 mmol) was suspended in NMP (140 mL) at room temperature, then heated up to 90^{o}C. A homogeneous solution was observed. Then, ethanol (308mL) was added at 90^{o}C slowly by using an addition funnel. After addition, the solution was naturally cooled down to RT overnight. The suspension was further cooled to 0^{o}C in ice bath for 1 hour, and then filtered. The solid was rinsed with cold EtOH (3X100mL). The sample was dried in vacuum oven at 50^{o}C overnight to a constant weight (50.4g for 1st crop, 90% yield, 99.6% purity). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3. 20 - 3. 46 (m, 5H), 3. 66 (s, 2H), 7. 53 (d, J=8. 25 Hz, 2H), 7. 60 - 7. 70 (m, 5H), and 8. 06 (d, J=7. 70 Hz, 2H); MS *m/e* 471(M+H^{+}); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.14 minutes; Anal. Calcd for $C_{23}H_{17}N_4O_4F_3$ • 0.01 EtOH: C, 58.72; H, 3.65; N, 11.90. Found: C, 58.63; H, 3.41; N, 11.84.

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