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Efficient synthesis of the 2-amino-6-chloro-4-cyclopropyl-7-fluoro-5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione core ring system

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ABSTRACT

An optimized total synthesis of the 2-amino-6-chloro-4-cyclopropyl-7-fluoro-5-methoxy-pyrido[1,2-*c*]-pyrimidine-1,3-dione core structure of a new fluoroquinolone-like class of antibacterial agents is described. This synthesis is highlighted by a nearly quantitative ring-closing reaction to form the pyrido[1,2-*c*]pyrimidine core. This bicyclic ring system serves as a scaffold for a family of biologically active compounds.

Fluoroquinolones are a ubiquitous class of broad-spectrum antibacterial agents,[1] typically inhibiting DNA gyrase in Gram-negative bacteria and topoisomerase IV (TopoIV) in Gram-positive bacteria.[2] Newer 8-methoxy fluoroquinolones more effectively inhibit both DNA gyrase and TopoIV, thus retaining activity against many fluoroquinolone-resistant mutants.[3] Due to the need for improved spectrum antibacterial agents, and to combat antibiotic-resistance, several generations of quinolone-class antibacterial agents with varied core structures have been introduced (Fig. 1).[4]

Since the initial discovery of nalidixic acid, the quinolone class of antibacterial agents evolved to more potent fluoroquinolones.[5] Subsequent generations of the quinolone class of antibacterial agents have focused on various substitutions at the *N*-1, *C*-7, and *C*-8 positions (corresponding to R_1, R_3, and R_2, respectively, Fig. 1).[5,6]

Although a number of quinolone-like core ring structures have been pursued, because of the inherent requirement of a 3-carboxylate moiety for quinolone-class agents to retain activity,[6] there is limited precedent for modification of this portion of these structures. Recently, quinazoline-2,4-diones, fluoroquinolone-like agents lacking a cognate 3-carboxylate group (Fig. 1),[7] were reported to be potent broad-spectrum antibacterial agents.[8] The quinazoline-2,4-diones are also active against many fluoroquinolone-resistant gyrase mutants.[8d] A single patent has shown quinazoline 1,3-diones (pyrido[1,2-*c*]pyrimidine-1,3-diones) possess similar antibacterial activity.[9]

We have recently begun working to understand the structural requirements for select 8-methoxy fluoroquinolones to promote chromosome fragmentation and to rapidly kill cells.[10] To this end, we initiated a program to synthesize 8-methoxy quinazoline diones in order to evaluate these unique quinolone-like gyrase inhibitors for anti-mutant activity and rapid lethality.[8d] Described here is the first report of an optimized synthesis of the 2-amino-6-chloro-4-cyclopropyl-7-fluoro-5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione core structure, and elaboration at *C*-7 to give a 1,3-dione-based fluoroquinolone analog.

In the previously documented 1,3-dione synthesis,[9] a key ring-closing reaction to give 1,3-dione core proceeded in good yields (~85%) for 5-methyl-pyrido[1,2-*c*]pyrimidine-1,3-diones and poor yields (~15%) for 5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-diones from the corresponding 3-methyl and 3-methoxy cyanomethyl-substituted pyridine intermediates, which are also known intermediates in the synthesis of 2-pyridone analogs of fluoroquinolones.[11] Guided by these reports, we initially undertook the synthesis of 3-methoxy pyridine derivative **7** (Scheme 1).

Starting with commercially available 3-chloro-2,4,5,6-tetrafluoropyridine (**2**), reaction with lithium *t*-butoxide afforded desired *tert*-butoxy-substituted derivative **3** and the *C*-6 regioisomer in a 4:1 ratio, respectively. In the published report,[11b] conversion of chloride **3** to hydroxypyridine **4** was accomplished in two steps: hydrogenation to remove the chlorine followed by a hydroboration/oxidation sequence. We observed inconsistent results in the hydrogenation step, resulting in low conversion and incomplete reactions even at high catalyst loading. To avoid this problem, lithium/halogen exchange directly followed by hydroboration/oxidation was employed to consistently provide hydroxypyridine **4** from **3** in over 80% yield. Methylation of the resulting hydroxyl group was achieved via Mitsunobu reaction, as reported,[11] to give methoxypyridine **5** in excellent yield.

Selective defluorination of **5** to give **6** was anticipated to be problematic due to similar reactivity at the *C*-6 and *C*-2 positions. Initial attempts at hydride displacement with Red-Al gave poor selectivity and consistently provided **6** in less than 25% yield. Previously, 3-methyl derivatives of **5** were reported to undergo selective displacement of fluorine by hydrazine followed by oxidation of the hydrazine intermediate to provide the 3-methyl version of **6** in good yields (47–91%).[11b] Here, reaction of 3-methoxy pyridine

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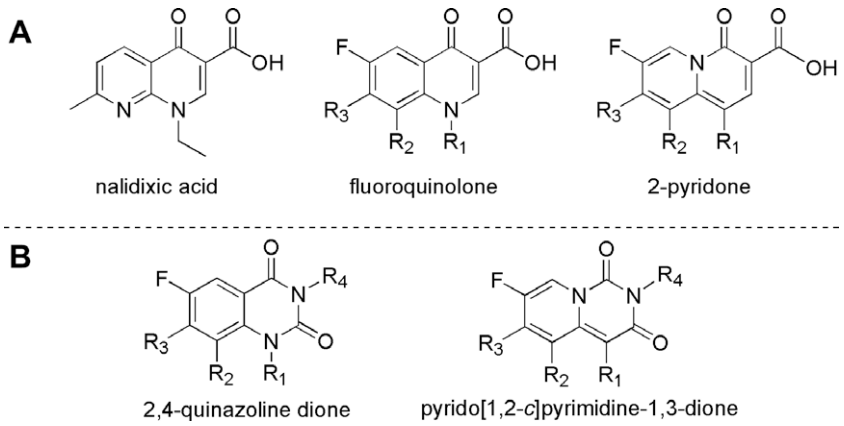


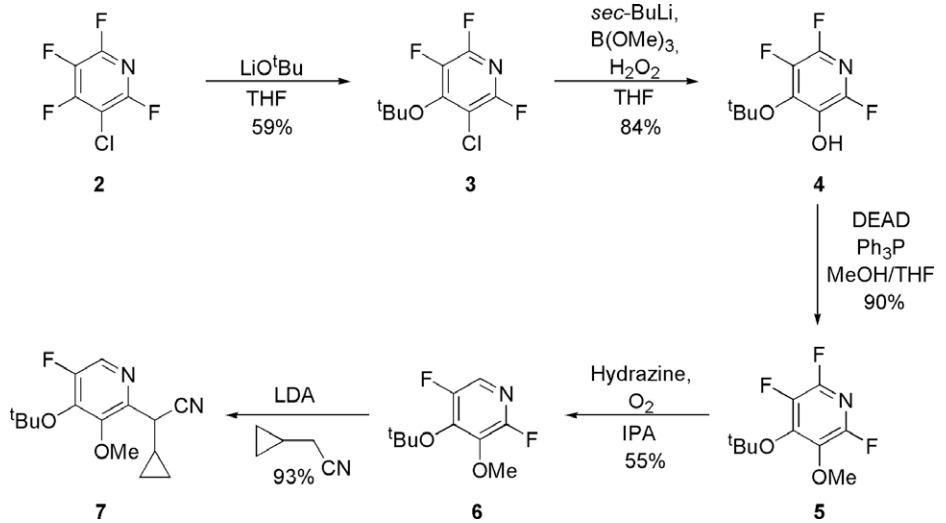
Figure 1. Quinolone-class antibacterial agents. (A) Representative structures of naphthyridone, fluoroquinolone, and 2-pyridone type cores. (B) Representative structures of newer quinazoline-dione type cores (R_4 = H or NH_2).

derivative **5** with hydrazine gave a 3:1 mixture of adducts at the desired and *C*-2 position, respectively. Reaction of the crude mixture with atmospheric oxygen and base over three days gave **6** and the corresponding 5,6-difluoro isomer, which were separated chromatographically. Installation of the cyclopropyl moiety was achieved as reported,[11] whereby reaction of **6** with the lithium anion of cyclopropylacetonitrile provided **7** in excellent yield.

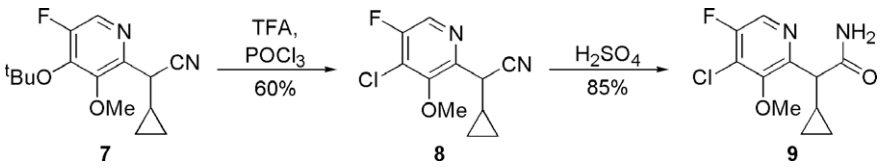
Having improved yields in preparing methoxypyridine intermediate **7**, conversion to the requisite precursor for cyclization was undertaken (amide **9,** Scheme 2). Trifluoroacetic acid-mediated deprotection of the *tert*-butyl group followed by treatment of the resulting phenol with $POCl_3$ afforded chloropyridine **8** in reasonable yield (Scheme 2). Sulfuric acid catalyzed hydrolysis of the nitrile provided amide **9**, however it was observed that extended reaction times at 100 °C led to over-hydrolysis, resulting in formation of the corresponding carboxylic acid. Lowering the reaction temperature to 70 °C combined with careful monitoring of the reaction negated this problem.

Treatment of amide **9** with phosgene and potassium *tert*-butoxide was previously reported to effect cyclization and give the 5-methoxy-pyrido[1,2-*c*]pyrimidine ring system (**10**) in 15% yield.[9] The 3-methyl version of **9** was reported to give 85% yield of the corresponding 5-methyl-pyrido[1,2-*c*]pyrimidine ring system under the same conditions. Following this precedent, amide **9** was treated with phosgene and potassium *tert*-butoxide as reported. At cryogenic temperatures, no reaction was observed. Somewhat surprisingly, upon warming to 0 °C clean formation of nitrile **8** was observed (Scheme 3). While there is precedent for this type of dehydration using $COCl_2$,[12] the observation that no desired product was formed was unexpected. Varying combinations of temperature, phosgene stoichiometry, and the base used gave either no reaction or provided nitrile **8**.

Fearing that phosgene was too reactive as the carbonyl source for efficient formation of the bicyclic ring system, attempts were made to use the less reactive carbonyl equivalent, carbonyldiimidazole (CDI). Using CDI in place of phosgene under the aforementioned conditions afforded no cyclization product at −78 °C, and dehydration to nitrile **8** proceeded slowly at room temperature. However, replacing potassium *tert*-butoxide with DBU while employing CDI led to conversion of **9** to cyclized product **10** in ~20% yield, as determined by analytical HPLC. All attempts to drive the reaction to further increase cyclization resulted in dehydration back to the nitrile (using forcing conditions) or no improvement in percent conversion (HPLC-based yields following modification of stoichiometry, solvent, reaction times, etc.). Recognizing that there was a reported difference in yield of 70% between the formation of the 5-methoxy **10** and the corresponding 5-methyl derivative,[9] it seemed likely that steric interactions between the methoxy and cyclopropyl groups here were interfering with ring closure. The reason for the reac-



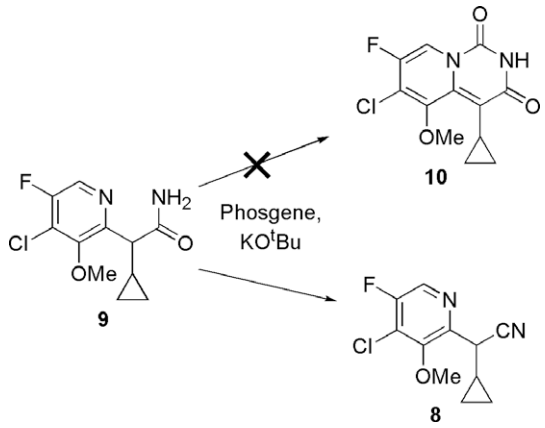
Scheme 1.



Scheme 2.

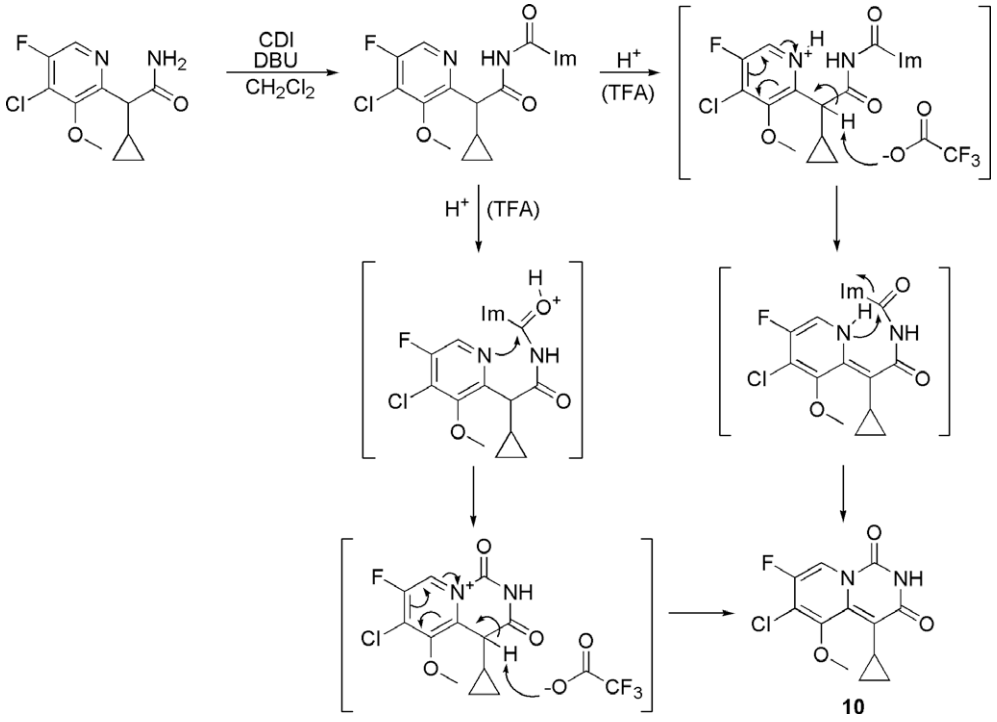
tion stalling at 15–20% conversion in both the previous report and in our hands was unclear.

Resigned to a low yield in the cyclization step, the reaction to form **10** from **9** using DBU and CDI was performed on large scale. Upon quenching this reaction with water, as previously reported, no cyclized product (**10**) was observed despite the reaction displaying a typical HPLC profile of 20% conversion prior to quench. Realizing that reaction aliquots analyzed by HPLC were diluted/quenched with mobile phase containing 0.1% TFA, we explored the effect of quenching this 'base-catalyzed' cyclization reaction with aqueous acidic rather than water. Brief optimization of reaction conditions, in particular the acid used and the acid quench procedure, led to reproducible preparation of **10** in over 90% isolated yield.[15] This near quantitative conversion of **9** to **10**, as indicated by a HPLC analysis, and high isolated yield of **10** (92–95%) are the significant improvements over the previously reported 15% yield[9] of **10** at this late stage of the synthesis.

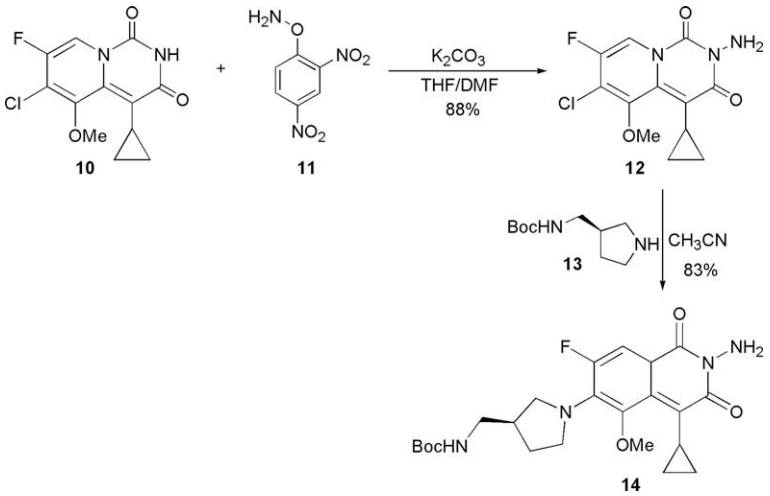


Scheme 3.

Based on the necessity of acid for ring-closure in this system, the exact mechanism of the cyclization reaction remains unclear. However, it is postulated that after initial base-catalyzed *N*-acylation of the amide by CDI, protonation of the pyridine ring upon addition of acid is the driving force behind dearomatization, affording formation of secondary amine, and thus the second, ring-closing, acylation (Scheme 4). Similarly, it is possible that acid is serving to simply activate the carbonylimidazole leaving group, driving cyclization. These hypotheses are supported both by the fact that no product formation is observed until after addition of acid to the reaction, which can be confirmed visually due to color change, and that there is an intermediate species that can be hydrolyzed back to starting material. Aqueous acid quenches gave varying yields based on acid concentration, suggesting a competing reaction between hydrolysis and ring-closure. In addition, quenching the reaction with anhydrous acidic resulted in varying yields based on time of reaction, indicating a slow buildup of intermediate species.



Scheme 4.



Scheme 5.

Having discovered that cyclization of amide **9** to give the pyrido[1,2-*c*]pyrimidine-1,3-dione core structure requires acid, it is now unclear if the previous report for forming 5-methoxy- and 5-methyl-pyrido[1,2-*c*]pyrimidine-1,3-dione ring systems using phosgene and potassium *tert*-butoxide to effect cyclization was fully base-mediated. It is possible that acid-mediated cyclization similarly occurred, as a result of HCl generated during quenching of the reaction (containing excess phosgene) with water. Further exploration and optimization of this reaction using variably substituted pyridine derivatives is planned.

With the penultimate 5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione derivative (**10**) in hand, the remainder of the synthesis to prepare 1,3-dione derivatives for future microbiological studies was straightforward (Scheme 5). To this end, *N*-amination of **10** was achieved using *O*-(2,4-ditrophenyl)hydroxylamine (**11**), which was prepared via literature procedure,[13] to supply 1,3-dione core structure **12** in high yield.[15] Having completed an optimized synthesis of the title core ring system, installation of a *C*-6 side chain was accomplished by simply heating a solution of (*R*)-3-*N*-Boc-aminomethyl pyrrolidine (**13**) and **12** to produce **14**,[15] demonstrating the ability of the 1,3-dione core to serve as a scaffold for a future library of quinolone-like compounds.

In summary, the studies here provide the first optimized synthetic route to the 5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione core ring system.[14] Further elaboration to give the *C*-6 substituted pyrimidine-1,3-dione as a representative analog of quinolone-like antimicrobial 1,3-diones is demonstrated. Synthesis of the 5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione core here, while originally guided by patent and literature precedent in similar syntheses, ultimately affords a significant improvement over previous reports. By simplifying early steps in the synthesis of intermediates that allows for more facile processing, and highlighted by a major improvement to the key ring-closing step, we have improved the overall yield of this synthesis from 1.2% to 8.0%.

Acknowledgment

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15. Experimental details for final quinazoline dione products **10**, **12**, and **14.** For 6-Chloro-4-cyclopropyl-7-fluoro-5-methoxy-pyrido[1,2-*c*]pyrimidine-1,3-dione (**10**): To a solution of **9** (100 mg, 0.38 mmol) in dry dichloromethane (4 mL) was added 1,1′-carbonyldiimidazole (184 mg, 1.14 mmol) followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (117 μL, 0.76 mmol). The resulting solution was stirred under argon for 2 h at room temperature, cooled in a water/ice bath, and quenched by drop-wise addition of a solution of TFA in dichloromethane (3 mL of 10% w/w) causing a brilliant yellow color to persist. The reaction was transferred to a separatory funnel, diluted with

dichloromethane (8 mL), and washed with water (5 mL). The organic was collected and the aqueous back-extracted with water (5 mL). The combined organic was washed with water (5 mL) and dried in vacuo to a bright yellow solid. The solid was dissolved in EtOAc and separated by flash chromatography (SiO_2, 3:1 EtOAc/hexanes) to give 102 mg (93%) of **10** as a crystalline yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ) 10.40 (s, 1H), 8.19 (d, J = 5.1 Hz, 1H), 3.83 (s, 3H), 1.83–1.74 (m, 1H), 1.02–0.96 (m, 2H), 0.64–0.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 162.2, 149.2 (d, J = 1.6 Hz), 147.1 (d, J = 2.2 Hz), 146.4 (d, J = 242.1 Hz), 142.6, 124.5 (d, J = 22.5 Hz), 110.5 (d, J = 42.8 Hz), 106.6, 61.0, 8.8, 7.6; HRMS (ESI+) calcd for $C_{12}H_{10}ClFN_2O_3$, 284.0364; *m/z* found, 284.0360.

For 2-Amino-6-chloro-4-cyclopropyl-7-fluoro-5-methoxy-pyrido[1,2-*c*]-pyrimidine-1,3-dione (**12**): To a solution of **10** (35 mg, 0.12 mmol) in dry THF (1 mL) were added **11** (35 mg, 0.18 mmol), potassium carbonate (33 mg, 0.24 mmol), and dry DMF (250 μL). The suspension was heated to 80 °C for 2 h and judged complete by TLC. The reaction was cooled to room temperature and the slurry was filtered through a glass wool plug. The residual solids were washed with THF (2 mL), the filtrates combined, and concentrated in vacuo to a brown oil. Separation by flash chromatography (SiO_2, 100% EtOAc) gave 32 mg (88%) of **12** as a bright yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ) 8.28 (d, J = 5.1 Hz, 1H), 5.56 (s, 2H), 3.84 (d, J = 0.3 Hz, 3H), 1.90–1.81 (m, 1H), 1.05–0.99 (m, 2H), 0.63–0.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 158.2, 149.1 (d, J = 1.6 Hz), 146.7 (d, J = 242.3 Hz), 144.7 (d, J = 2.2 Hz), 139.4, 123.7 (d, J = 22.2 Hz), 110.5 (d, J = 43.6 Hz), 105.0, 61.1, 8.8, 8.1; HRMS (ESI+) calcd for $C_{12}H_{11}ClFN_3O_3$, 299.0473; *m/z* found, 299.0461.

For [1-(2-Amino-4-cyclopropyl-7-fluoro-5-methoxy-1,3-dioxo-2,3-dihydro-1*H*-pyrido[1,2-*c*]pyrimidin-6-yl)-pyrrolidin-3-ylmethyl]-carbamic acid *tert*-butyl ester (**14**): To a solution of **12** (30 mg, 0.10 mmol) in dry acetonitrile (1 mL) was added **13** (60 mg, 0.30 mmol). This solution was heated to 85 °C and stirred for 24 h. The solution was concentrated in vacuo to an oil and purified by flash chromatography (SiO_2, 9:1 EtOAc/MeOH) to give 39 mg (83%) of **14** as a bright yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ) 8.19 (d, J = 10.0 Hz, 1H), 5.43 (s, 2H), 4.82–4.73 (m, 1H), 3.80–3.72 (m, 3H), 3.53–3.45 (m, 1H), 3.43 (s, 3H), 3.29–3.23 (m, 2H), 2.51–2.39 (m, 1H), 2.15–2.05 (m, 1H), 1.78–1.67 (m, 2H), 1.46 (s, 9H), 1.00–0.91 (m, 1H), 0.88–0.80 (m, 1H), 0.47–0.33 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 158.2, 156.1, 146.5 (d, J = 245.2 Hz), 145.0 (d, J = 1.6 Hz), 142.1, 136.8 (d, J = 12.6 Hz), 132.6 (d, J = 6.6 Hz), 111.8 (d, J = 46.3 Hz), 96.3, 79.6, 60.5, 55.4 (d, J = 7.1 Hz), 51.6 (d, J = 7.1 Hz), 42.6, 39.6, 29.2 (d, J = 1.5 Hz), 28.4, 8.94 (d, J = 5.9 Hz), 8.33; HRMS (ESI+) calcd for $C_{22}H_{30}FN_5O_5$, 463.2231; *m/z* found, 463.2227.