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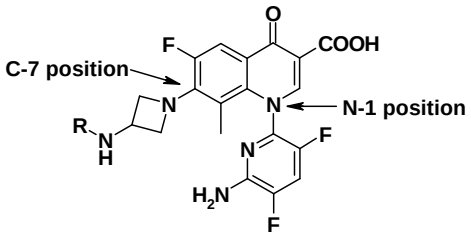
**Novel 7-(3-alkylaminoazetidin-1-yl)fluoroquinolones were designed, synthesized, and evaluated for their antibacterial activities and oral absorption rates. In this series of compounds, WQ-3810 was identified as an orally active fluoroquinolone with a potent *in vitro* activity.**




**10a**: R = H
**10b**: R = Me
**10c**: R = Et
**10d**: R = *n*-Pr
**10e**: R = *i*-Pr (WQ-3810)
**10f**: R = *t*-Bu

# Discovery of WQ-3810: Design, Synthesis, and Evaluation of 7-(3-alkylaminoazetidin-1-yl)fluoro-quinolones as Orally Active Antibacterial Agents

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**Kewords**

Antibacterial agent; Fluoroquinolone; Gram-negative bacteria; Oral absorption; Alkylaminoazetidine.

**Abstract**

Novel 7-(3-alkylaminoazetidin-1-yl)fluoroquinolones were designed, synthesized, and evaluated for their antibacterial activities and oral absorption rates. Against Gram-negative bacteria, **10a-e**, which have various alkyl groups containing different numbers of carbon atoms (C0-C3) at the C-7 alkylaminoazetidine position, showed potent and similar antibacterial activities, whereas the activity of **10f** (C4, *t*-Bu) was significantly lower than

those of **10a-e**. Conversely, the oral absorption rates of **10a-e** in rats increased depending on the number of carbon atoms in the alkyl groups; **10d** (C3, *n*-Pr) and **10e** (C3, *i*-Pr) had high oral absorption rates (> 90 % at 10 mg/kg). These results demonstrated that the introduction of alkyl groups onto C-7 aminoazetidine is useful for the improvement of the oral absorption rates of these drugs while maintaining their antibacterial activities. As a conclusion, from this series of fluoroquinolones, WQ-3810 (**10e**), having 3-isopropylaminoazetidine as the C-7 substituent, was identified as an orally active antibacterial agent with a potent *in vitro* activity.

## 1. Introduction

Since the discovery of norfloxacin as the first fluoroquinolone (FQ) [1], FQs have become a significant class of useful antibacterial agents. In an effort to improve the profiles of FQs as antibacterial drugs, a number of structure-activity relationships (SARs) have been established and reviewed [2-7]. In those SARs, aminopyrrolidines and piperazines were identified as the most effective C-7 substituents to enhance the antibacterial activities of FQs [2, 5]. The introduction of alkyl groups onto those C-7 substituents has also enhanced their antibacterial activities against Gram-positive bacteria [8], improved their pharmacokinetic profiles [5], and reduced their cytotoxicities against mammalian cells [7]. Furthermore, a recent study reported that the introduction of a methyl group at the geminal position of the amino group of C-7 aminopyrrolidine was effective for avoiding the mechanism-based inhibition of cytochrome P450 3A4 [9].

In our effort to create a useful FQ with a potent *in vitro* activity against refractory drug-resistant pathogens, we discovered the highly active compound (**10a**), whose structure is characterized by its unique N-1 and C-7 substituents, 2,4-difluoro-5-aminopyridine and 3-aminoazetidine, respectively. In the course of SAR studies of this series of FQs, we accumulated several important findings as follows: the introduction of an amino group in the N-1 substituent resulted in the reduction of the phototoxicity, even when groups (halogen atoms) that induce phototoxicity were introduced at the C-8 position [10]; a four-membered ring (aminoazetidine) substituent at the C-7 position gave a higher antibacterial activity than

a five-membered ring (aminopyrrolidine) substituent in combination with the N-1 substituent difluoro-aminopyridine; and the C-8 methyl group was effective for reducing the cytotoxicity without the loss of *in vitro* activity [11]. However, the proto-type compound **10a** has a defect in its pharmacokinetic properties, its poor oral absorption. Until now, there have been few studies evaluating the structure-pharmacokinetic relationships of FQs having a C-7 aminoazetidine. Jordi et al. [12-14] demonstrated that introduction of a methyl or ethyl group to the C-7 aminoazetidine improved the pharmacokinetic properties; the area under the plasma concentration-time curve (orally in mice) increased, but the antibacterial activity against Gram-negative bacteria was reduced. It is unclear how longer alkyl groups (> C3) effect the antibacterial activities and pharmacokinetics of 7-(3-alkylaminoazetidin-1-yl)FQs.

The aim of this study was to improve the oral absorption of **10a** by introducing an alkyl group and to clarify the effect of this introduction. To achieve this goal, we prepared various compounds with different alkyl groups (C0-C4) on the C-7 alkylaminoazetidine (**10a-f**) and evaluated their antibacterial activities, especially against Gram-negative bacteria, as well as their oral absorption rates in rats. In addition, we investigated the effects of the alkyl groups on the inhibitory activities of the drugs against DNA gyrase, which is one of the molecules targeted by FQs, and the physiochemical properties (solubility and membrane permeability) of the FQs.

## 2. Chemistry

WQ-3810 **10e** and its derivatives **10a-d** and **f** were synthesized according to previously reported methods [15, 16]. Scheme 1 shows the synthesis of 2-amino-6-*tert*-butylamino-3,5-difluoropyridine (**4**) as a N-1 substituent of **10a-f**. Stepwise aminations of 2,3,5,6-tetrafluoropyridine (**1**) with *tert*-butylamine and benzylamine gave di-protected diaminopyridine (**3**). The second amination with benzylamine required a high reaction temperature (160°C) due to the low activity for the amination at the C-6 position of **2**. The benzyl group of **3** was deprotected selectively by Pd/C-catalyzed hydrogenation to give the mono-protected diaminopyridine **4**. Scheme 2 shows the synthesis of 1-(3,5-difluoro-6-aminopyridin-2-yl)-8-methylquinolone carboxylic acid (**8**). The reaction of benzoylaceate (**5**) with triethyl orthoformate under reflux conditions and the removal of excess reagents gave benzoylacrylate (**6**). The reaction of **6** with **4** and subsequent cyclization under basic conditions gave quinolone ester **7**. To obtain 8-methylquinolone carboxylic acid (**8**), the deprotection of the *tert*-butyl group and the hydrolysis of the ethyl ester of **7** were achieved under acidic conditions.

As shown in scheme 3, compounds **10a-f** were obtained by aromatic nucleophilic substitution at the C-7 position of 8-methylquinolone carboxylic acid (**8**) with various 3-alkylaminoazetidines **9a-f**, which were synthesized according to a method similar to established procedures [17, 18]. The reactivity at the C-7 position of **8** is low due to the electron donating effect of the C-8 methyl group. We already reported that the C-7

89 substitution reaction of an 8-methylquinolone core with a 3-alkylaminoazetidine was

90 accelerated by the presence of $Li^+$ ions [19]. Accordingly, the syntheses of **10a-f** were conducted

91 in the presence of LiCl and/or LiOH

92

## 3. Results

### 3.1 *In vitro* activity

The half maximal inhibitory concentration ($IC_{50}$) and the minimum inhibitory concentration (MIC) values of **10a-f** and levofloxacin (LVFX) were determined for *Escherichia coli* DNA gyrase and Gram-negative bacteria (three strains of *E. coli* and 15 strains of 11 other Gram-negative bacteria), respectively (Table 1). **10a-f** have various alkyl groups of different lengths and sizes on the C-7 alkylaminoazetidine. These compounds similarly inhibited *E. coli* DNA gyrase potently with $IC_{50}$ values ranging from 0.078 to 0.33 mg/L. Conversely, the antibacterial activities of **10a-e** were higher than that of **10f**; the MIC values for *E coli* (3 strains) and other Gram-negative bacteria (11 species, 15 strains) were from 0.004 to 0.012 (**10a-e**) and 0.038 mg/L (**10f**) and from 0.061 to 0.21 (**10a-e**) and 0.57 mg/L (**10f**), respectively.

### 3.2 Oral absorption in rats

The urinary and biliary excretions (% of dose) of **10a-e** were measured following their oral (*p.o.*) or intravenous (*i.v.*) administration to rats (Table 2). The excretions of **10a-e** into bile were analyzed after the conversion of glucuronide metabolites of **10a-e** into their unmetabolized forms by alkaline hydrolysis. The total excretions of **10d** (C3, *n*-Pr) and **10e** (C3, *i*-Pr) in urine and bile after oral administration were 91 and 92 % of the dose, respectively, indicating their excellent oral absorption rates (> 90%). The high total recoveries (85-91%) of

113 **10a-c** following intravenous administration indicated that these compounds were primary
114 excreted in their unmetabolized forms or as glucuronide metabolites. Therefore, the oral
115 absorption rates (%) of **10a-c** were calculated by dividing their total recoveries (*p.o.*) by the
116 total recoveries (*i.v.*). The calculated oral absorption rates of **10a** (C0, non-alkylated), **10b** (C1,
117 Me), and **10c** (C2, Et) were 9.6, 31, and 76 %, respectively.
118

119 **3.3 Solubility and membrane permeability**

120 The solubilities and membrane permeabilities of **10a-e** were measured (Table 3). The
121 solubilities in Japanese Pharmacopoeia disintegration test solution 2 (JP2) were not very
122 high and ranged from 3.1 to 45 mg/L. The permeability coefficients ($P_{eff}$s) of **10d** and **10e**
123 in the parallel artificial membrane permeability assay (PAMPA) were $1.1 \times 10^{-6}$ and $1.2 \times$
124 $10^{-6}$ cm/s, respectively, which were four to 10 times higher than those of **10a-c** ($1.2 \times 10^{-7}$ to
125 $2.9 \times 10^{-7}$ cm/s).
126

## 4 Discussion

In this study, we attempted to improve the oral absorption of our proto-type FQ **10a** by performing several SAR studies. We determined the MICs against clinically important Gram-negative pathogens (18 strains of 12 species) and the oral absorption rates (in rats) of **10a-f**, which have various C-7 alkylaminoazetidine substituents (C0-4). Additionally, we evaluated their inhibitory activities against DNA gyrase, solubilities in JP2, and membrane permeabilities in a PAMPA study. DNA gyrase is the primary drug target of FQs [20], and the solubility and membrane permeability in the gastrointestinal tract are two major determining factors for the oral absorption of a drug.

In the MIC assay, the antibacterial activities of **10a-e**, containing a C0-C3 alkyl group, were found to be similar against Gram-negative bacteria; however, **10f** (C4, *t*-Bu) was significantly less potent (Table 1). To address the reason for the differences between the antibacterial activities of **10a-e** and **10f**, we measured the $IC_{50}$ values of **10a-f** against *E. coli* DNA gyrase and examined the correlation between their $IC_{50}$s and MICs for *E. coli* (three strains). Contrary to the higher MIC value of **10f** compared to those of **10a-e**, **10a-f** exhibited similar inhibition rates of *E. coli* DNA gyrase, although a good correlation between the $IC_{50}$ and MIC values of the FQs was reported [21]. This result suggested that the less potent antibacterial activity of **10f** was not due to its weaker inhibitory activity against the target enzyme.

To exert a potent antibacterial activity, a drug must have enough exposure to its target in addition to strong inhibition of the target molecules. The bacterial uptake of drugs is greatly

147 affected by components of the cell wall structure characteristic of bacteria. Especially,
148 Gram-negative bacteria have a hydrophilic outer membrane in the cell wall, which serves as
149 an effective permeability barrier against lipophilic antibiotics including FQs. The influence of
150 the lipophilicities of FQs on their uptake by Gram-negative bacteria has been studied [22-24],
151 and an inverse correlation between the lipophilicity of a FQ and its uptake by *E. coli* was
152 reported [24]. The cLogD values and the retention times of **10a-f** (data not shown) for
153 reverse-phase HPLC indicated that the lipophilicities of **10a-f** increased depending on the
154 number of carbon atoms in the alkyl groups, and **10f** has the highest lipophilicity. Therefore,
155 the impaired bacterial uptake of **10f** due to its higher lipophilicity might be a plausible reason
156 for its less potent antibacterial activity, although this speculation should be confirmed by the
157 future evaluation of the bacterial uptakes of **10a-f**.

158 The oral absorption rates of **10a-e** were evaluated in rats by measuring the total recoveries
159 (% of dose) of their unmetabolized forms and glucuronide metabolites. The total recoveries of
160 **10d** and **10e** (*p.o.*) were over 90%, indicating that both compounds were almost completely
161 absorbed from the gastrointestinal tract. The oral absorption rates (%) of **10a-e** increased with
162 an increase in the number of carbon atoms in the alkyl group. These results indicated that
163 introduction of alkyl groups at the C-7 aminoazetidine of **10a** enhanced the oral absorption by
164 increasing the lipophilicities of the compounds. The oral absorption of drugs depends on their
165 physicochemical properties. Considering the process of the intestinal absorption of drugs, the
166 solubility of a drug in gastrointestinal tract fluid and its intestinal membrane permeability

are two major determinants for its oral absorption. The dissolution of drugs in gastrointestinal fluid is the first requirement; only drug molecules dissolved in the fluid can be available for absorption across the intestinal membrane and thereafter be delivered to their site of action by systemic circulation. Due to the lipid nature of the intestinal membrane, a drug's lipophilicity is considered to be an important factor for its penetration. To address the reason for the improvement of the oral absorption of **10a** by the introduction of the alkyl groups, we evaluated the solubilities and permeabilities of **10a-e** (Table 3). The solubility study used JP2, which is one of the most commonly used test mediums as a simulated gastrointestinal tract fluid. The solubilities of **10a-e** in JP2 were not extremely high, and their solubilities were all at a similar level. The membrane permeabilities were evaluated using a PAMPA model, which has been reported to be useful for predicting the oral absorption rates of drugs [25]. In this report, a sigmoidal relationship has been observed between the $P_{eff}$ value in the PAMPA study and the oral absorption in humans; most of the drugs with $P_{eff}$ values greater than $1.0 \times 10^{-6}$ cm/s had good oral absorption rates. The $P_{eff}$ values of **10a-e** increased depending on the number of carbon atoms in the alkyl groups, and the values of **10d** ($1.1 \times 10^{-6}$ cm/s) and **10e** ($1.2 \times 10^{-6}$ cm/s) were greater than $1.0 \times 10^{-6}$ cm/s (Table 3). These results demonstrated that the oral absorption rates of **10a-e** in rats was in good correlation with the $P_{eff}$ values, suggesting that the introduction of alkyl groups to the C-7 aminoazetidine of **10a** improved the oral absorption through the enhancement of the membrane permeabilities of the drugs due to their increased lipophilicities.

## 5 Conclusion

This study evaluated the effects of various alkyl groups (C0-C4) of 7-(3-alkylaminoazetidin-1-yl)FQs **10a-f** on the anti-Gram-negative activities and oral absorption rates of the drugs. The alkyl group had little influence on the inhibitory activity against *E. coli* DNA gyrase, but a *t*-Bu group (**10f**, C4) decreased the antibacterial activity against Gram-negative bacteria, including *E. coli*, which might be due to its reduced bacterial uptake due to an excessive increase in its lipophilicity. Conversely, the increased lipophilicity by the introduction of alkyl groups enhanced oral absorption by enhancing the membrane permeability. Consequently, WQ-3810 (**10e**, *i*-Pr) was identified as an orally active FQ with a potent *in vitro* activity. Our subsequent evaluations have demonstrated that WQ-3810 is highly active against quinolone-resistant pathogens and has a good pharmacokinetic profile as well as a low potential for side effects related to this class of antibiotics [26, 27].

## 6 Experimental

### 6.1. Chemistry

#### 6.1.1. General Methods

All chemicals used were of reagent grade. Reactions were monitored by thin-layer chromatography on silica gel (Merck 60F 254) plates using UV light (254 nm) for detection. NMR spectra were recorded with a JEOL JNM-ECP 500 at 500 MHz ($^{1}$H NMR) and Varian VNMRS 500 at 125 MHz ($^{13}$C NMR). Chemical shifts are expressed in ppm ($\delta$) relative to internal tetramethylsilane, and the coupling constants ($J$ values) are given in hertz (Hz). Mass spectra (MS) were obtained with a Finnigan LTQ mass spectrometer with ESI.

#### 6.1.2. 2-(*tert*-Butylamino)-3,5,6-trifluoropyridine (2)

A mixture of 2,3,5,6-tetrafluoropyridine (**1**, 11.0 g, 72.8 mmol), *tert*-butylamine (18.5 g, 253 mmol) and MeCN (40 mL) was stirred at 60 °C for 3 d. The reaction mixture was evaporated, and the residue was partitioned between $CHCl_3$ (100 mL) and water (50 mL). The organic layer was separated, dried over $Na_2SO_4$ and evaporated to give **2** as pale yellow oil. (9.7 g, 66% yield); $^{1}$H NMR ($CDCl_3$): $\delta$ 1.45 (s, 9H), 4.40 (brs, 1H), 7.16 (ddd, $J$ = 7.0 Hz, 8.0 Hz, 9.0 Hz, 1H).

221 **6.1.3. 2-Benzylamino-6-(*tert*-butylamino)-3,5-difluoropyridine (3)**

222 A mixture of 2-(*tert*-butylamino)-3,5,6-trifluoropyridine (**2**, 9.7 g, 47.5 mmol), benzylamine
223 (15.5 g, 145 mmol) and *N*-methylpyrrolidone (20 mL) was stirred at 160 °C for 1 d. After
224 cooling to room temperature, the reaction mixture was diluted with $CHCl_3$. The solution was
225 washed with water (500 mL × 3), dried over $Na_2SO_4$ and evaporated to give crude **3** (16.5 g).
226 The obtained crude **3** was used for next reaction without further purification.

227

228 **6.1.4. 2-Amino-6-(*tert*-butylamino)-3,5-difluoropyridine (4)**

229 A mixture of crude 2-benzylamino-6-(*tert*-butylamino)-3,5-difluoropyridine (**3**, 10.7 g, 36.7
230 mmol), 10% Pd/C (1.1 g), conc. HCl (3.8g) and MeOH (60 mL) was stirred at room temperature
231 for 1 d under hydrogen atmosphere. After the catalyst was filtered off, the filtrate was
232 evaporated, and the residue was purified by silica gel column chromatography (2:1
233 $CHCl_3$/hexane → $CHCl_3$) to give **4** as pale brown oil. (3.3 g, 44% yield); $^1$H NMR ($CDCl_3$): $\delta$
234 1.43 (s, 9H), 4.11 (brs, 2H), 6.94 (t, $J$ = 10.0 Hz, 1H).

235

236 **6.1.5. Ethyl 1-[6-(*tert*-butylamino)-3,5-difluoropyridin-2-yl]-6,7-difluoro-8-methyl-4-oxo-**
237 **1,4-dihydroquinoline-3-carboxylate (7)**

238 A mixture of ethyl 2,4,5-trifluoro-3-methylbenzoyl acetate (**5**, 3.4 g, 13 mmol), acetic
239 anhydride (3.2 g, 31 mmol) and triethyl orthoformate (2.3 g, 16 mmol) was refluxed for 4 h.
240 The reaction mixture was evaporated to give a crude **6**, which was dissolved in EtOH (5 mL).

To the solution was added dropwise a solution of 2-amino-6-(*tert*-butylamino)-3,5-difluoropyridine (**4**, 2.7 g, 13 mmol) in EtOH (20 mL) at 0 °C, and the mixture was stirred at room temperature for 20 min. The reaction mixture was evaporated, and the residue was subjected to silica gel column chromatography (1:8 ethyl acetate/hexane). The fraction was evaporated, and the residue was dissolved in DMF (10 mL). To the solution was added $K_2CO_3$ (1.4 g, 9.8 mmol), and the mixture was stirred at 100 °C for 50 min. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was triturated with EtOH, and the precipitate was collected by filtration and washed with $Et_2O$ to give **7** as a pale yellow solid. (2.6 g, 44% yield); $^1$H NMR ($CDCl_3$): $\delta$ 1.34-1.48 (m, 12H), 1.82 (d, $J$ = 3.0 Hz, 3H), 4.40 (q, $J$ = 7.0 Hz, 2H), 4.75 (brs, 1H), 7.23 (t, $J$ = 9.0 Hz, 1H), 8.22 (t, $J$ = 10.0 Hz, 1H), 8.50 (s, 1H).

**6.1.6.**

**1-(6-Amino-3,5-difluoropyridin-2-yl)-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (8)**

A mixture of ethyl 1-[6-(*tert*-butylamino)-3,5-difluoropyridin-2-yl]-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**7**, 2.5 g, 5.5 mmol) and conc. HCl (10 mL) was refluxed overnight. After cooling to room temperature, the precipitate was collected by filtration and washed in turn

with EtOH and $Et_2O$ to give **8** as a pale yellow solid. (1.7 g, 84% yield); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.84 (s, 3H), 6.91 (brs, 2H), 8.03 (t, $J$ = 9.0 Hz, 1H), 8.25 (t, $J$ = 9.0 Hz, 1H), 8.93 (s, 1H).

### 6.1.7. 7-(3-Aminoazetidin-1-yl)-1-(6-amino-3,5-difluoropyridin-2-yl)-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (10a)

To a suspension of 3-aminoazetidine hydrochloride (**9a**, 283 mg, 2.0 mmol) in DMSO (2 mL) were added LiOH monohydrate (165 mg, 3.9 mmol), tetramethylguanidine (300 mg, 2.6 mmol), LiCl (165 mg, 3.9 mmol) and 1-(6-amino-3,5-difluoropyridin-2-yl)-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8**, 477 mg, 1.3 mmol), and the mixture was stirred at 30-35 °C for 40 h. The reaction mixture was washed with $Et_2O$ (5 mL × 5) by decantation to remove DMSO. To the residue was added water (3 mL), and the pH of the suspension was adjusted to pH 6 with aqueous 10% citric acid. The precipitate was collected by filtration, which was suspended in water (10 mL), and the pH of suspension was adjusted to pH 6 with AcOH. The suspension was stirred at 80 °C for 20 h. The precipitate was collected by filtration, which was suspended in EtOH (10 mL), and the suspension was refluxed for 2 h. The precipitate was collected by filtration, and dried under reduced pressure to give **10a** as a white solid. (220 mg, 40% yield); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.63 (s, 3H), 3.68-3.82 (m, 2H), 3.87-3.98 (m, 1H), 4.37-4.52 (m, 2H), 6.84 (brs, 2H), 7.76 (d, $J$ = 13.5 Hz, 1H), 7.96 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.71 (s, 1H); $^{13}$C

NMR (DMSO-$d_6$): $\delta$ 17.73 (s), 44.04 (d, $J$ = 5 Hz), 67.05 (s), 67.34 (s), 108.03 (s), 109.12 (d, $J$ = 22 Hz), 113.03 (d, $J$ = 4 Hz), 114.54 (dd, $J$ = 24, 21 Hz), 117.45 (d, $J$ = 7 Hz), 134.74 (dd, $J$ = 13, 4 Hz), 138.78 (s), 142.53 (dd, 250, 5 Hz), 145.40 (dd, 260, 5 Hz), 146.60 (d, $J$ = 14 Hz), 147.32 (d, $J$ = 10 Hz), 150.14 (s), 151.35 (d, $J$ = 247 Hz), 165.86 (s), 177.13 (d, $J$ = 3 Hz); MS-ESI (+): m/z 420 $[M+H]^+$.

**6.1.8.**

**1-(6-Amino-3,5-difluoropyridin-2-yl)-6-fluoro-8-methyl-7-[3-(methylamino)azetidin-1-yl]-4-oxo -1,4-dihydro-quinoline-3-carboxylic acid (10b)**

The title compound **10b** was prepared from 3-(methylamino)azetidine hydrochloride (**9b**, 310 mg, 2.0 mmol) according to the similar procedure for **10a**.

Pale yellow solid (380 mg, 67% yield); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.64 (s, 3H), 2.22 (s, 3H), 3.46-3.54 (m, 1H), 3.82-3.92 (m, 1H), 3.95-4.05 (m, 1H), 4.34-4.50 (m, 2H), 6.85 (brs, 2H), 7.75 (d, $J$ = 13.5 Hz, 1H), 7.96 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.71 (s, 1H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 17.71 (s), 33.41 (s), 50.80 (d, $J$ = 4 Hz), 64.22 (s), 64.42 (s), 108.03 (s), 109.13 (d, $J$ = 23 Hz), 112.81 (d, $J$ = 4 Hz), 114.57 (dd, $J$ = 24, 21 Hz), 117.41 (d, $J$ = 7 Hz), 134.77 (dd, $J$ = 13, 4 Hz), 138.83 (s), 142.53 (dd, $J$ = 250, 5 Hz), 145.35 (dd, $J$ = 259, 5 Hz), 146.60 (d, $J$ = 14 Hz), 147.16 (d, $J$ = 10 Hz), 150.15 (s), 151.26 (d, $J$ = 247 Hz), 165.86 (s), 177.12 (d, $J$ = 3 Hz); MS-ESI(+): m/z 434 $[M+H]^+$.

**6.1.9.**

**1-(6-Amino-3,5-difluoropyridin-2-yl)-7-[3(ethyl-amino)azetidin-1-yl]-6-fluoro-8-methyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (10c)**

The title compound **10c** was prepared from 3-(ethylamino)azetidine hydrochloride (**9c**, 1.6 g, 9.0 mmol) according to the similar procedure for **10a**.

Pale yellow solid (960 mg, 36% yield); $^{1}$H NMR (DMSO-$d_6$): $\delta$ 0.98 (t, $J$ = 7.5 Hz, 3H), 1.63 (s, 3H), 3.53-3.63 (m, 1H), 3.82-3.92 (m, 1H), 3.95-4.05 (m, 1H), 4.36-4.51 (m, 2H), 6.83 (brs, 2H), 7.75 (d, $J$ = 13.5 Hz, 1H), 7.94 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.70 (s, 1H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 15.77 (s), 17.69 (s), 45.66 (s), 49.24 (d, $J$ = 4 Hz), 64.80 (s), 65.05 (s), 109.18 (d, $J$ = 23 Hz), 112.85 (d, $J$ = 4 Hz), 114.54 (dd, $J$ = 24, 21 Hz), 117.83 (d, $J$ = 6 Hz), 134.94 (dd, $J$ = 13, 4 Hz), 138.75 (s), 142.51 (dd, $J$ = 249, 5 Hz), 145.32 (dd, $J$ = 260, 5 Hz), 146.56 (d, $J$ = 14 Hz), 146.93 (d, $J$ = 10 Hz), 149.96 (s), 151.20 (d, $J$ = 247 Hz), 165.87 (s), 176.98 (d, $J$ = 3 Hz); MS-ESI(+): m/z 448 $[M+H]^+$.

**6.1.10.**

**1-(6-Amino-3,5-difluoropyridin-2-yl)-6-fluoro-8-methyl-4-oxo-7-[3-(*n*-propylamino)azetidine-1-yl]-1,4-di-hydroquinoline-3-carboxylic acid (10d)**

To a suspension of 3-(*n*-propylamino)azetidine hydrochloride (**9d**, 2.7 g, 15 mmol) in DMSO (6.5 mL) were added LiOH monohydrate (1.2 g, 29 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (1.8 mL, 12 mmol) and

1-(6-amino-3,5-difluoropyridin-2-yl)-6,7-difluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carbo xylic acid (**8**, 1.1 g, 2.9 mmol), and the mixture was stirred at 60 °C for 3 h. The reaction mixture was washed with $Et_2O$ (5 mL × 5) by decantation to remove DMSO. To the residue was added water (2 mL), and the pH of the suspension was adjusted to pH 6 with aqueous 10% citric acid. The precipitate was collected by filtration, washed with EtOH and dried under reduced pressure to give **10d** as a pale yellow solid. (500 mg, 36% yield); $^1$H NMR (DMSO-$d_6$): $\delta$ 0.86 (t, $J$ = 7.5 Hz, 3H), 1.32-1.43 (m, 2H), 1.64 (s, 3H), 2.41 (t, $J$ = 7.5 Hz, 2H), 3.53-3.61 (m, 1H), 3.80-3.91 (m, 1H), 3.95-4.04 (m, 1H), 4.36-4.51 (m, 2H), 6.84 (brs, 2H), 7.76 (d, $J$ = 13.5 Hz, 1H), 7.95 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.71 (s, 1H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 12.20 (s), 17.70 (s), 23.14 (s), 48.95 (s), 49.31 (d, $J$ = 4 Hz), 64.51 (s), 64.76 (s), 108.02 (s), 109.14 (d, $J$ = 23 Hz), 112.90 (d, $J$ = 4 Hz), 114.55 (dd, $J$ = 24, 21 Hz), 117.46 (d, $J$ = 7 Hz), 134.75 (dd, $J$ = 13, 4 Hz), 138.81 (s), 142.51 (dd, $J$ = 250, 5 Hz), 145.44 (dd, $J$ = 259, 5 Hz), 146.60 (d, $J$ = 14 Hz), 147.16 (d, $J$ = 10 Hz), 150.17 (s), 151.26 (d, $J$ = 247 Hz), 165.79 (s), 177.13 (d, $J$ = 3 Hz); MS-ESI(+): m/z 462 $[M+H]^+$.

### 6.1.11. 1-(6-Amino-3,5-difluoropyridin-2-yl)-6-fluoro-7-[3-(isopropylamino)azetidin-1-yl]-8-methyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (10e)

The title compound **10e** was prepared from 3-(isopropylamino)azetidine hydrochloride (**9e**, 14.0 g, 75 mmol) according to the similar procedure for **10a**.

Pale yellow solid (12.0 g, 52% yield); $^1$H NMR (DMSO-$d_6$) $\delta$ 0.93 (d, $J$ = 6.0 Hz, 6H), 1.62 (s, 3H), 2.66-2.75 (m, 1H), 3.60-3.69 (m, 1H), 3.76-3.86 (m, 1H), 3.92-4.02 (m, 1H), 4.39-4.54 (m, 2H), 6.82 (s, 2H), 7.74 (d, $J$ = 14.0 Hz, 1H), 7.94 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.69 (s, 1H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 17.73 (s), 23.57 (s), 47.19 (s), 47.51 (d, $J$ = 4 Hz), 65.54 (s), 65.81 (s), 108.03 (s), 109.13 (d, $J$ = 23 Hz), 112.93 (d, $J$ = 4 Hz), 114.55 (dd, $J$ = 24, 21 Hz), 117.43 (d, $J$ = 7 Hz), 134.76 (dd, $J$ = 13, 4 Hz), 138.80 (s), 142.49 (dd, $J$ = 250, 5 Hz), 145.35 (dd, $J$ = 259, 5 Hz), 146.59 (d, $J$ = 14 Hz), 147.23 (d, $J$ = 10 Hz), 150.15 (s), 151.36 (d, $J$ = 247 Hz), 165.80 (s), 177.12 (d, $J$ = 3 Hz); MS-ESI(+): m/z 462 $[M+H]^+$.

**6.1.12.**

**1-(6-Amino-3,5-difluoropyridin-2-yl)-7-[3-(*tert*-butyl-amino)azetidin-1-yl]-6-fluoro-8-methyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (10f)**

The title compound **10f** was prepared from 3-(*tert*-butylamino)azetidine hydrochloride (**9f**, 145 mg, 0.7 mmol) according to the similar procedure for **10d**.

Pale yellow solid (45 mg, 58% yield); $^1$H NMR (DMSO-$d_6$): $\delta$ 1.09 (s, 9H), 1.65 (s, 3H), 3.86-4.07 (m, 2H), 4.07-4.18 (m, 1H), 4.47-4.65 (m, 2H), 6.85 (brs, 2H), 7.80 (d, $J$ = 13.5 Hz), 7.96 (dd, $J$ = 9.5 Hz, 9.5 Hz, 1H), 8.73 (s, 1H); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 17.75 (s), 29.86 (s), 44.54 (d, $J$ = 4 Hz), 50.59 (s), 66.84 (s), 67.11 (s), 109.19 (d, $J$ = 23 Hz), 113.08 (d, $J$ = 4 Hz), 114.50 (dd, $J$ = 24, 21 Hz), 118.10 (d, $J$ = 7 Hz), 135.00 (dd, $J$ = 13, 4 Hz), 135.59 (s), 138.66 (s), 142.49 (dd, $J$ = 250, 5

Hz), 145.26 (dd, $J$ = 259, 5 Hz), 146.56 (d, $J$ = 14 Hz), 146.92 (d, $J$ = 10 Hz), 149.91 (s), 151.20 (d, $J$ = 247 Hz), 165.96 (s), 176.91 (d, $J$ = 3 Hz); MS-ESI(+): m/z 476 $[M+H]^+$.

### 6.2 *in vitro* Activity

#### 6.2.1. Antibacterial activity

The *in vitro* antibacterial activities of **10a-f** and LVFX were evaluated against the following representative organisms: *E. coli* NIHJ, *E. coli* KC-14, *E. coli* 177, *Citrobacter freundii* IFO1268, *Klebsiella pneumoniae* KC-1, *K. pneumoniae* DT-S, *Salmonella typhimurium* IFO13245, *Enterobacter cloacae* IFO13535, *Proteus vulgaris* IFO3167, *Proteus mirabilis* IFO 3849, *Serratia marcescens* IFO3736, *S. marcescens* T-55, *Morganella morganii* W1026, *Providencia rettgeri* W1008, *Pseudomonas aeruginosa* IFO3445, *P. aeruginosa* E-2, *P. aeruginosa* 15846, and *Acinetobacter calcoaceticus* Ac-54. MICs were determined by an agar dilution method with Muller Hinton Agar (MHA, Becton Dickinson, NJ, USA) according to the standard method described by the Japanese Society of Chemotherapy [28]. Approximately $10^4$ cfu of organisms were inoculated onto the MHA plates containing the two-fold dilution series of **10a-f** and LVFX in the concentration range of 128 to 0.002 mg/L, and the plates were incubated at 37°C for 18 h. The MIC values were determined to be the lowest concentrations of **10a-f** and LVFX that yielded no visible growth of organisms on the MHA plates.

### 6.2.2. Inhibitory activity against DNA gyrase

The inhibitory activities of **10a-f** and LVFX against *E. coli* DNA gyrase were examined by the supercoiling assay [29]. After the preincubation of mixtures (20 μL) containing 35 mM Tris-HCl, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, 0.1 mg/mL albumin, 0.2 units of *E.coli* gyrase (New England Biolab, MA, USA), and a series of diluted solutions of the test compounds at 37°C for 5 min, 250 ng of relaxed pBR322 (Inspiralis, Norwich, UK) was added to initiate the supercoiling reaction. The reaction mixture was then further incubated at 37°C for 15 min. The reaction was terminated by the addition of loading buffer, which was followed by electrophoretic analysis in 1% agarose gels. The gels were viewed and photographed on an ImageQuant4000 instrument (GE Healthcare, Pollards Wood, UK). The intensities of the bands were calculated using ImageQuant TL (GE Healthcare). The $IC_{50}$ values were determined by nonlinear least-squares regression analysis using Kyplot (version 5.0, Keyence, Osaka, Japan).

### 6.3. Excretion and oral absorption in rats

Six-week old male Sprague-Dawley rats (Japan SLC and Charles River Japan, Japan) were used. Before the study, the animals had been acclimated to the laboratory conditions for at least 1 week. **10a-e** were orally or intravenously administered at doses of 10 mg/kg and 5 mg/kg, respectively, to rats fasted overnight. **10a-e** were suspended in 0.5% methylcellulose for oral administration or dissolved in 0.04 N NaOH for intravenous injection. Urine samples

were collected from intact rats individually housed in a metabolic cage for up to 24 h post-dose. Bile samples were collected from bile duct-cannulated rats individually kept in a Bollmann cage for 24 h post-dose. The volumes of the urine and bile samples were recorded, and the samples were stored at -30°C until analysis. The urine and bile samples were analyzed as follows: bile samples (100 μL) were alkaline-treated by the addition of 10 μL of 1 N NaOH with incubation at 37°C for 1 h. Intact and alkaline-treated samples were diluted with HPLC mobile phase and spiked with an internal standard. After centrifugation, portions of the supernatants were analyzed by HPLC [LC-10A System, Shimadzu; Kyoto, Japan; analytical column, ODS-80TM, 5 μm, 4.6 × 150 mm, Tosoh, Tokyo, Japan; flow rate, 1 mL/min; column temperature, 40°C; mobile phase, A (MeCN), B (distilled water containing 20 mM sodium 1-decansulfonate, 40 mM phosphoric acid, and 0.2% (v/v) triethylamine), isocratic A/B (40/60); detection wavelength, 290 nm]. The extent of excretion (% of dose) was calculated by dividing the amount of **10a-e** excreted in the urine and bile by the administered dose. The oral absorption rate (%) was calculated by dividing the total urinary and biliary excretions of **10a-e** (p.o.) by the total excretion (i.v.).

### 6.4 Solubility in JP2

Suspensions of **10a-e** were prepared by adding excess amounts of **10a-e** into 0.6 mL of JP2, which is a 50 mM potassium dihydrogenphosphate solution at a pH of 6.8. Each suspension was incubated at 25°C for 24 h with shaking to prepare a thermodynamically equilibrated

420 solution, which was followed by filtration through a 0.45 μm Millex HV membrane filter
421 (Millipore, MA, USA). The concentrations of **10a-e** in the filtrate were measured with HPLC
422 and defined as the solubilities. HPLC separation was achieved on a UPLC BEH C18 column
423 (1.7 μm 2.1 × 100 mm, Waters) using gradient elution at a flow rate of 0.3 mL/min. The mobile
424 phases consisted of 10% acetonitrile/90% water containing 0.1% formic acid (A) and
425 acetonitrile containing 0.1% formic acid (B). The following HPLC gradient program was used:
426 starting at 15% mobile phase B, a linear gradient was run beginning at 0.5 min to reach 40%
427 B at 3 min, with the solvent composition being returned to 15% in 0.2 min and then
428 re-equilibration for 1.8 min. The column temperature was set at 40°C, and UV detection was
429 applied at 290 nm.
430
431 **6.5 Membrane permeability in PAMPA**
432 The passive membrane permeabilities of **10a-e** were evaluated using a PAMPA plate system
433 (BD Biosciences, CA, USA). Ten micromolar solutions of **10a-e** in 300 μL of phosphate buffered
434 saline (PBS) containing 5% MeOH were added into the donor plate. The acceptor plate filled
435 with 200 μL of PBS was placed on the donor plate, which was followed by incubation at 25°C
436 for 5 h. The solutions of both plates were analyzed by HPLC as described above (section 6.4).
437 The permeability coefficients ($P_{eff}$) of **10a-e** were calculated by following formula [30]:
438 $P_{eff} = -\ln[1-C_a(t)/C_{equ}]/[A\times(1/V_d+1/V_a)\times t]$
439 $C_{equ} = [C_d(t)\times V_d+C_a(t)+ V_a]/[V_d+V_a]$

where $C_d(t)$ and $C_a(t)$ are the concentrations of **10a-e** in the solutions of the donor and acceptor plates at time t, respectively. $V_d$ and $V_a$ are the volumes of the solutions in the donor and acceptor plates, respectively, $A$ is the filter surface area (0.3 $cm^2$), and $t$ is the incubation time (18,000 s).

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**Table 1**

$IC_{50}$ and GM-MIC values of **10a-f** for *E. coli* DNA gyrase and Gram-negative bacteria

| Comp. | $IC_{50}$ (mg/L) | GM-MICs (mg/L)[a] | |
|---|---|---|---|
| | DNA gyrase[b] | *E.coli*[c] | Other bacteria[d] |
| **10a** | 0.12 | 0.004 | 0.061 |
| **10b** | 0.24 | 0.006 | 0.077 |
| **10c** | 0.078 | 0.006 | 0.12 |
| **10d** | 0.19 | 0.012 | 0.21 |
| **10e (WQ-3810)** | 0.20 | 0.010 | 0.17 |
| **10f** | 0.33 | 0.038 | 0.57 |
| **LVFX** | 0.17 | 0.019 | 0.12 |

[a]geometric mean MICs

[b]one wild type strain

[c] three strains

[d]15 strains of 11 Gram-negative bacteria, which names are described in Experimental session

**Table 2**

Oral absorption rates (%) of **10a-e** in rats

| Comp. | Excretion of **10a-e** in urine and bile (% of dose) | | | | | | Oral absorption rate[d] (%) |
|---|---|---|---|---|---|---|---|
| | p.o. (10 mg/kg) | | | i.v. (5 mg/kg) | | | |
| | Urine[a] | Bile[a, b] | Total[c] | Urine[a] | Bile[a, b] | Total[c] | |
| **10a** | 4.0 ± 0.42 | 4.2 ± 1.3 | 8.2 | 44 ± 8.0 | 41 ± 7.1 | 85 | 9.6 |
| **10b** | 13 ± 2.1 | 16 ± 2.8 | 28 | 49 ± 7.2 | 41 ± 7.1 | 90 | 31 |
| **10c** | 20 ± 2.4 | 49 ± 5.9 | 69 | 41 ± 5.0 | 51 ± 5.4 | 91 | 76 |
| **10d** | 17 ± 5.4 | 74 ± 14 | 91 | n.t. | n.t. | n.t. | >90 |
| **10e (WQ-3810)** | 24 ± 1.6 | 68 ± 1.5 | 92 | n.t. | n.t. | n.t. | >90 |

[a]Each value represents mean ± S.D. for three rats.

[b]Excreted amount of **10a-e** in bile was measured after the complete conversion of the glucuronide metabolites of **10a-e** to their unmetabolized forms by alkaline hydrolysis.

[c]Total value was calculated by adding the mean values of **10a-e** in urine and bile.

[d]Oral absorption rate was calculated by dividing the total excretions (% of dose) of **10a-e** (p.o.) by the total excretions (i.v.).

n.t., not tested.

**Table 3**

Solubility in JP2[a] and Peff[b] in PAMPA[c] of **10a-e**

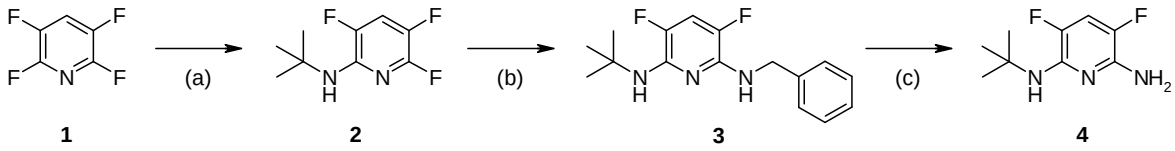
| Comp. | Solubility in JP2 (mg/L) | Peff ($10^{-6}$cm/s) |
|---|---|---|
| **10a** | 27 ± 1.4 | 0.12 ± 0.028 |
| **10b** | 3.1 ± 0.089 | 0.19 ± 0.048 |
| **10c** | 45 ± 0.72 | 0.29 ± 0.086 |
| **10d** | 41 ± 3.6 | 1.1 ± 0.23 |
| **10e (WQ-3810)** | 41 ± 2.3 | 1.2 ± 0.10 |

Each value represents mean ± S.D (n=3)

[a]Japanese Pharmacopoeia disintegration test solution 2 (pH 6.8)

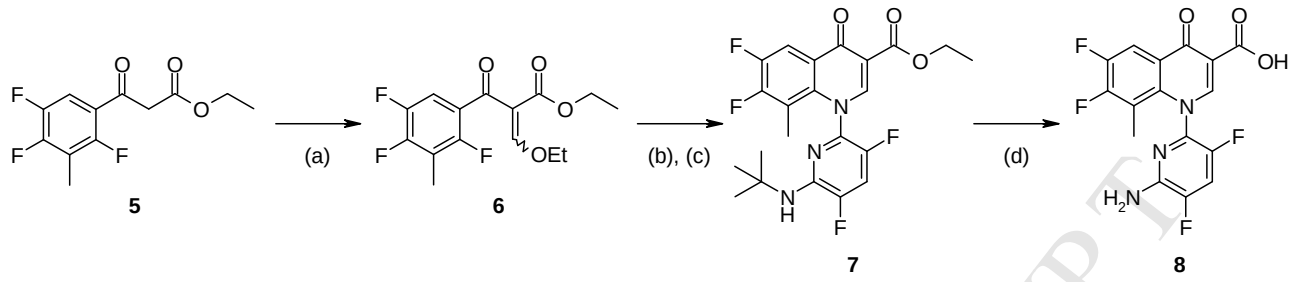
[b]Permeability coefficient

[c]Parallel artificial membrane permeability assay

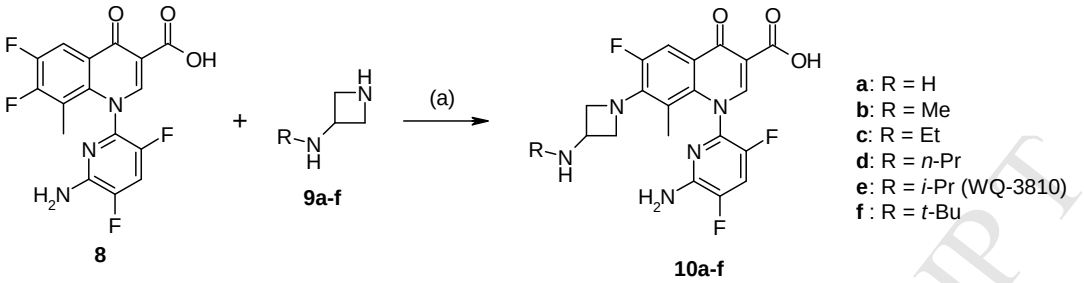



**1** **2** **3** **4**

**Scheme 1.** Synthesis of compound **4**. Reagents and conditions: (a) *t*-$BuNH_2$ in MeCN; (b) $BnNH_2$ in NMP; (c) 10% Pd/C, conc. HCl in MeOH.




**Scheme 2.** Synthesis of compound **8**. Reagents and conditions: (a) $CH(OEt)_3$, $Ac_2O$; (b) **4** in EtOH; (c) $K_2CO_3$ in DMF; (d) conc. HCl.




**Scheme 3.** Synthesis of compounds **10a-f**. Reagents and conditions: (a) TMG, LiOH, LiCl in DMSO or DBU, LiOH in DMSO for **10d** and **10f**.

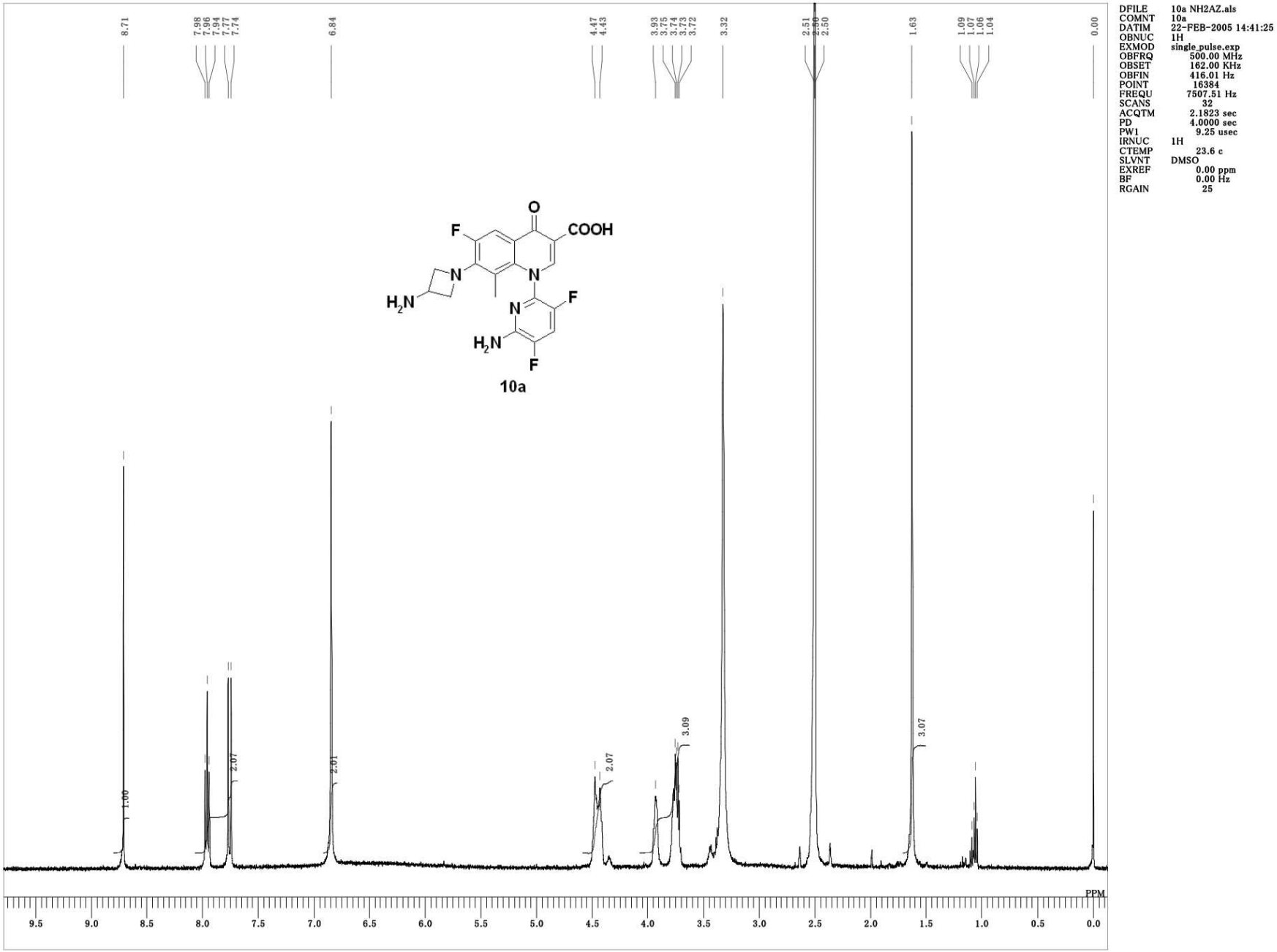
We synthesized and evaluated 7-(3-alkylaminoazetidin-1-yl)fluoroquinolones.

Introduction of alkyl groups at the C-7 aminoazetidine enhanced the oral absorption.

WQ-3810 was identified as an orally active fluoroquinolone.

WQ-3810 showed a potent antibacterial activity against Gram-negative bacteria.

10a







10a

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COMNT 10a
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OBNUC 1H
EXMOD single_pulse.exp
OBFRQ 500.00 MHz
OBSET 162.00 KHz
OBFIN 416.01 Hz
POINT 16384
FREQU 7507.51 Hz
SCANS 32
ACQTM 2.1823 sec
PD 4.0000 sec
PW1 9.25 usec
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BF 0.00 Hz
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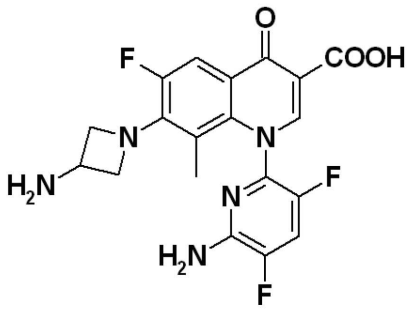


10a




| | | | | |
|---|---|---|---|---|
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5/14/2012 11:43:18 AM

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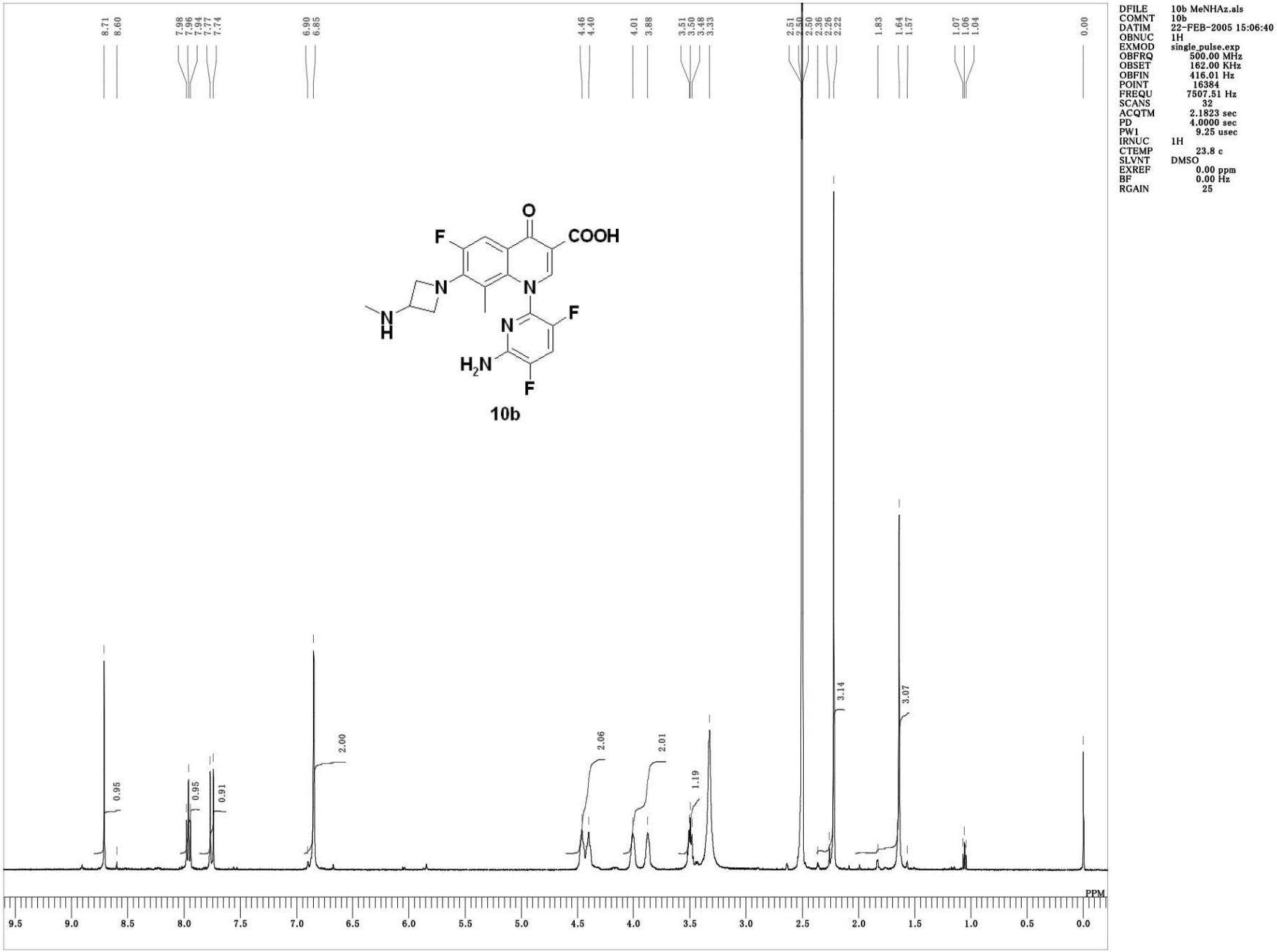
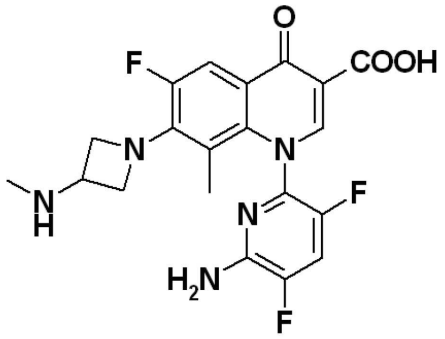
10a

10b

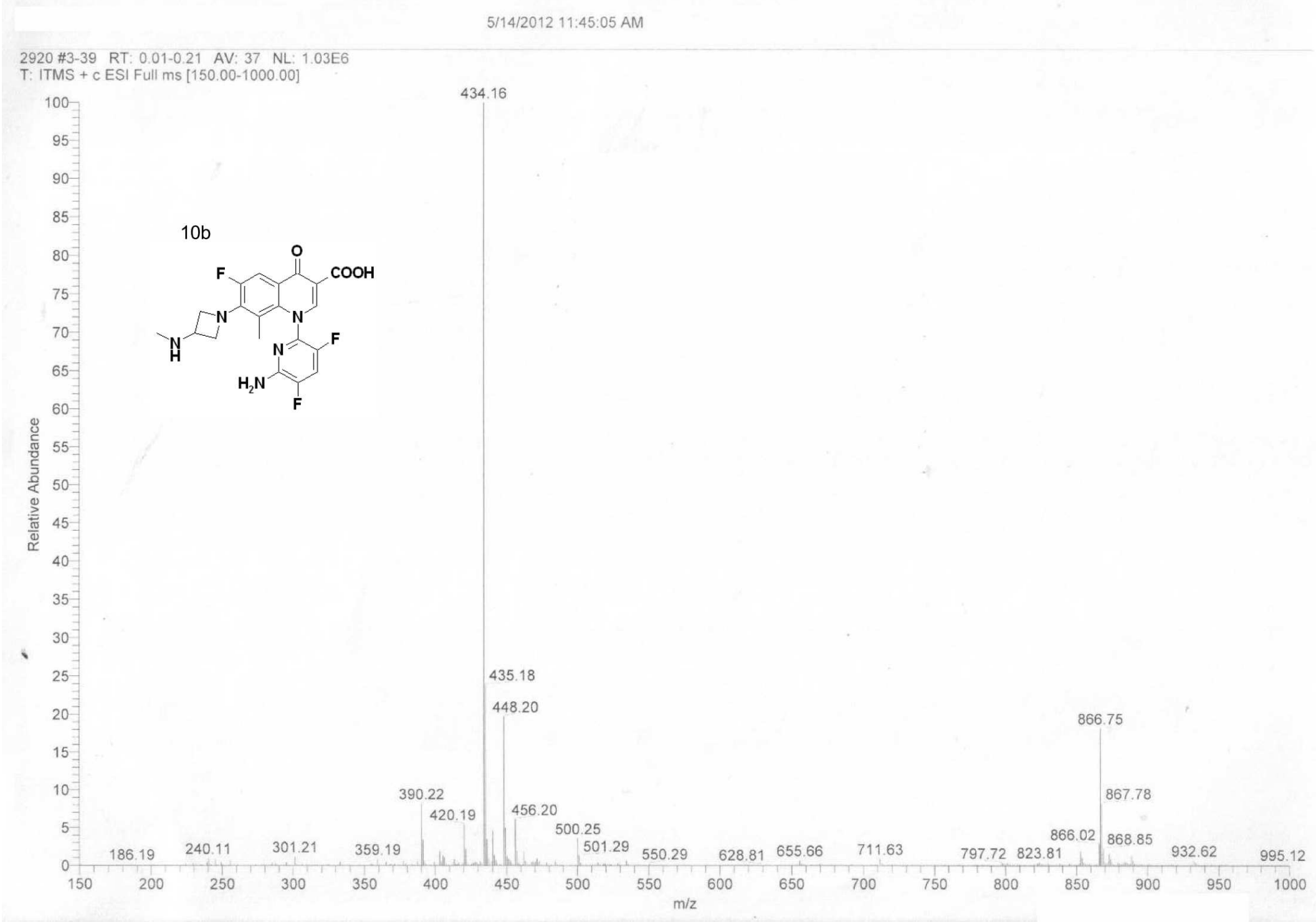




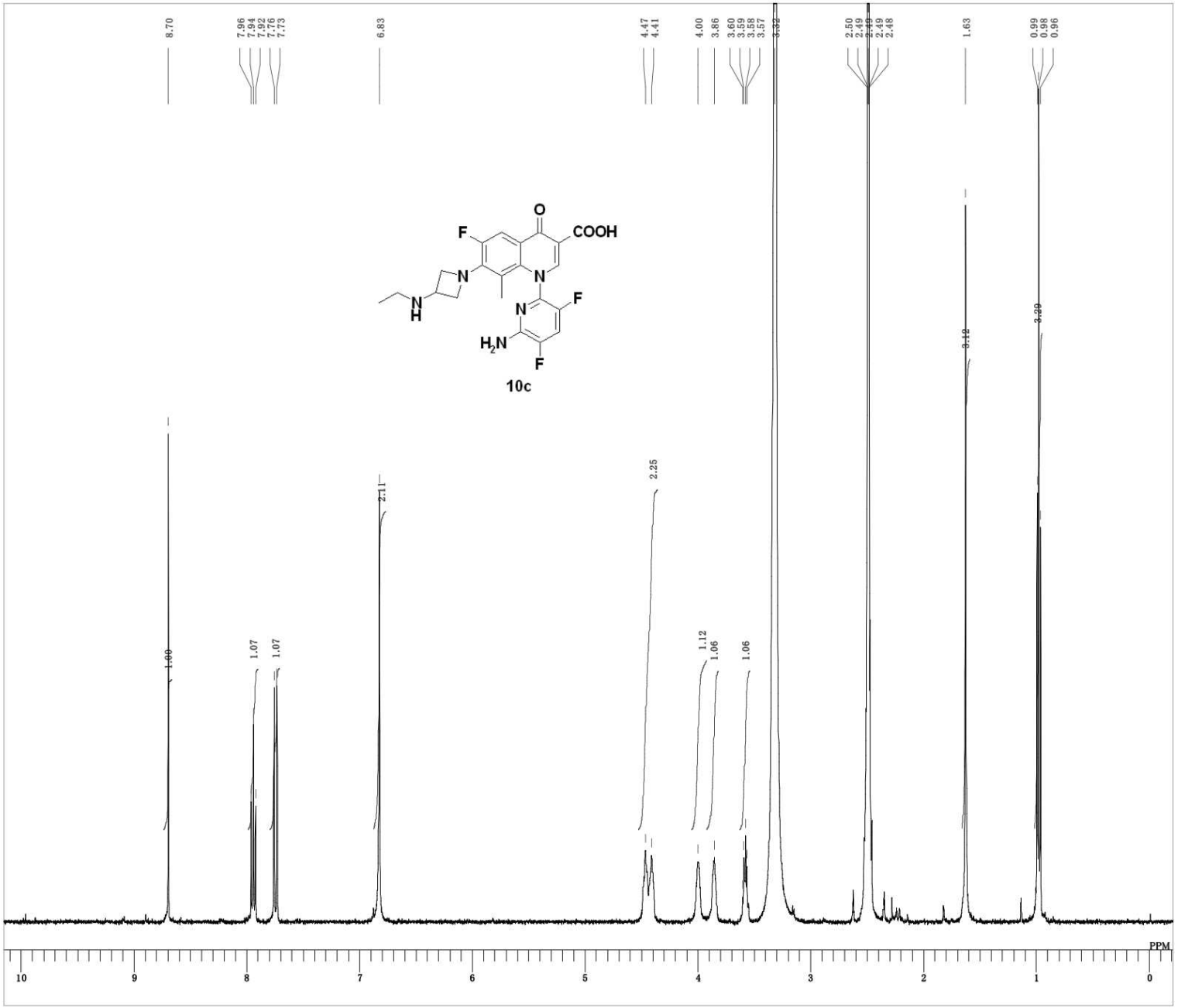

10b




| | | | | |
|---|---|---|---|---|
| PULSE SEQUENCE<br>Relax. delay 1.000 sec<br>Pulse 45.0 degrees<br>Acq. time 1.049 sec<br>Width 31250.0 Hz<br>20000 repetitions | OBSERVE C13, 125.6686970<br>DECOUPLE H1, 499.7781880<br>Power 39 dB<br>continuously on<br>WALTZ-16 modulated | DATA PROCESSING<br>Line broadening 0.5 Hz<br>FT size 65536<br>Total time 11.4 hours | | Solvent: dmso<br>Ambient temperature<br>Sample #2, Operator: vnmr1<br>File: 150415-10b-13C_CARBON_01<br>VNMRS-500 "Varian-NMR" |


5/14/2012 11:45:05 AM
2920 #3-39 RT: 0.01-0.21 AV: 37 NL: 1.03E6
T: ITMS + c ESI Full ms [150.00-1000.00]
10b
O
F
COOH
N
N
N
F
N
H
$H_2N$
F
Relative Abundance
m/z
434.16
435.18
448.20
866.75
867.78
390.22
420.19
456.20
500.25
501.29
186.19
240.11
301.21
359.19
550.29
628.81
655.66
711.63
797.72
823.81
866.02
868.85
932.62
995.12

Single Pulse Experiment

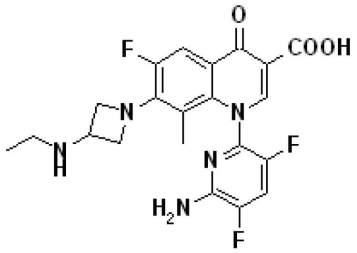






10c

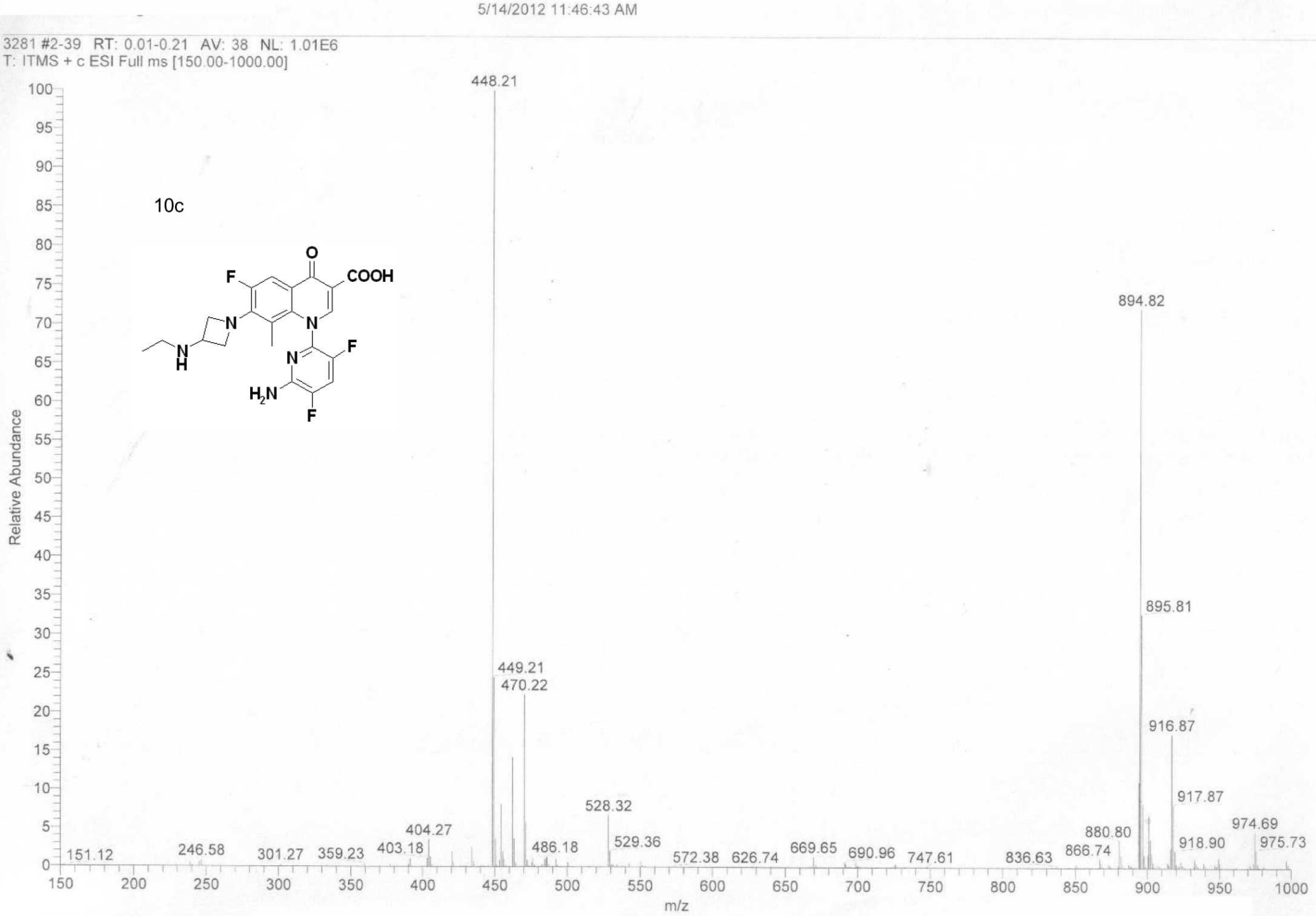
DFILE 10c Et.als
COMNT Single Pulse Experiment
DATIM 9-JUN-2005 13:41:59
OBNUC 1H
EXMOD single_pulse.exp
OBFRQ 500.00 MHz
OBSET 162.00 KHz
OBFIN 416.01 Hz
POINT 16384
FREQU 7507.51 Hz
SCANS 32
ACQTM 2.1823 sec
PD 4.0000 sec
PW1 9.25 usec
IRNUC 1H
CTEMP 25.2 c
SLVNT DMSO
EXREF 2.49 ppm
BF 0.00 Hz
RGAIN 22



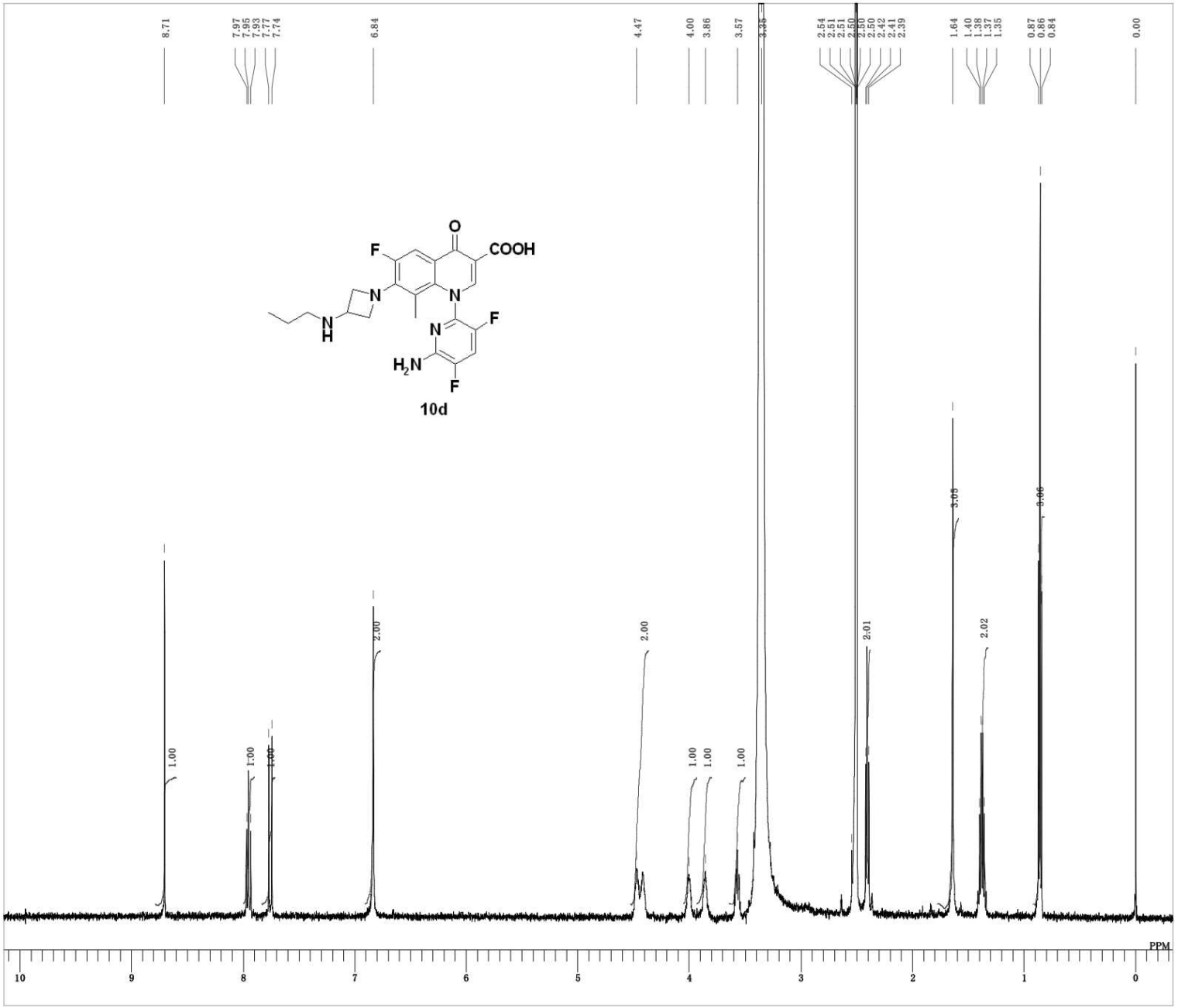

10c



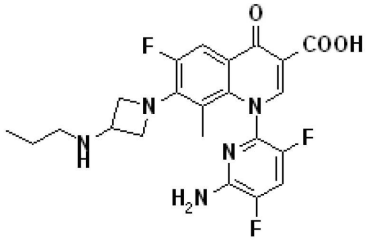

| | | | | |
|---|---|---|---|---|
| PULSE SEQUENCE<br>Relax. delay 1.000 sec<br>Pulse 45.0 degrees<br>Acq. time 1.049 sec<br>Width 31250.0 Hz<br>20000 repetitions | OBSERVE C13, 125.6686970<br>DECOUPLE H1, 499.7781880<br>Power 39 dB<br>continuously on<br>WALTZ-16 modulated | DATA PROCESSING<br>Line broadening 0.5 Hz<br>FT size 65536<br>Total time 11.4 hours | | Solvent: dmso<br>Ambient temperature<br>Sample #4, Operator: vnmr1<br>File: 150526-10c-13C_CARBON_01<br>VNMRS-500 "Varian-NMR" |



Single Pulse Experiment



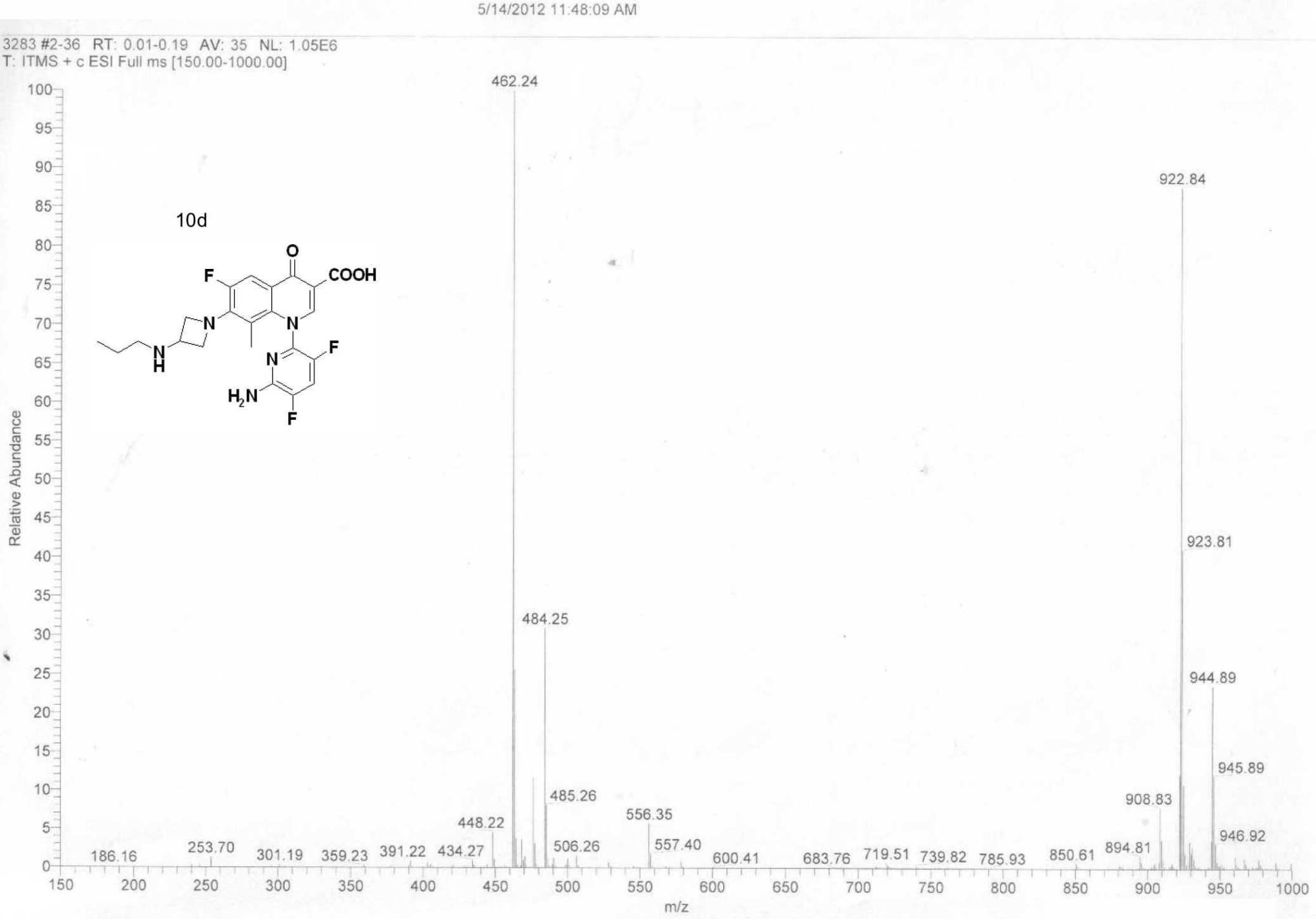

DFILE 10d nPr.als
COMNT Single Pulse Experiment
DATIM 30-SEP-2004 08:19:10
OBNUC 1H
EXMOD single_pulse.exp
OBFRQ 500.00 MHz
OBSET 162.00 KHz
OBFIN 416.01 Hz
POINT 16384
FREQU 7507.51 Hz
SCANS 16
ACQTM 2.1823 sec
PD 4.0000 sec
PW1 9.25 usec
IRNUC 1H
CTEMP 25.2 c
SLVNT DMSO
EXREF 0.00 ppm
BF 0.00 Hz
RGAIN 20



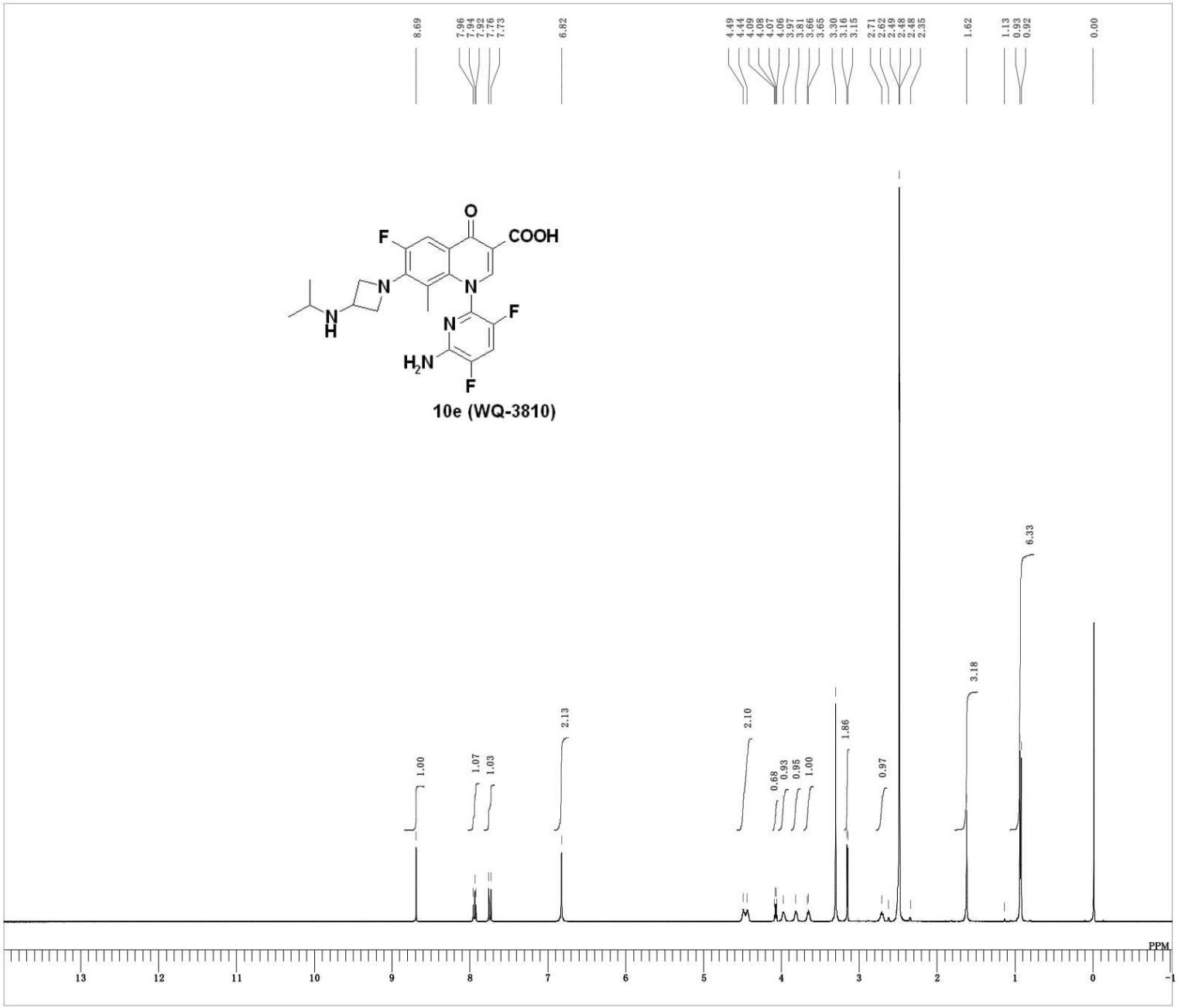

10d

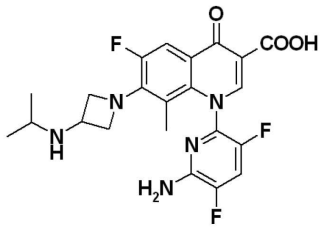



| | | | | |
|---|---|---|---|---|
| PULSE SEQUENCE<br>Relax. delay 1.000 sec<br>Pulse 45.0 degrees<br>Acq. time 1.049 sec<br>Width 31250.0 Hz<br>20000 repetitions | OBSERVE C13, 125.6686970<br>DECOUPLE H1, 499.7781880<br>Power 39 dB<br>continuously on<br>WALTZ-16 modulated | DATA PROCESSING<br>Line broadening 0.5 Hz<br>FT size 65536<br>Total time 11.4 hours | | Solvent: dmso<br>Ambient temperature<br>Sample #4, Operator: vnmr1<br>File: 150603-10d-13C_CARBON_01<br>VNMRS-500 "Varian-NMR" |


5/14/2012 11:48:09 AM
3283 #2-36 RT: 0.01-0.19 AV: 35 NL: 1.05E6
T: ITMS + c ESI Full ms [150.00-1000.00]
10d
O
F
COOH
N
N
N
F
N
H
H2N
F
Relative Abundance
m/z
462.24
922.84
923.81
484.25
944.89
945.89
485.26
908.83
556.35
448.22
946.92
506.26
557.40
186.16
253.70
301.19
359.23
391.22
434.27
600.41
683.76
719.51
739.82
785.93
850.61
894.81

WQ-3810

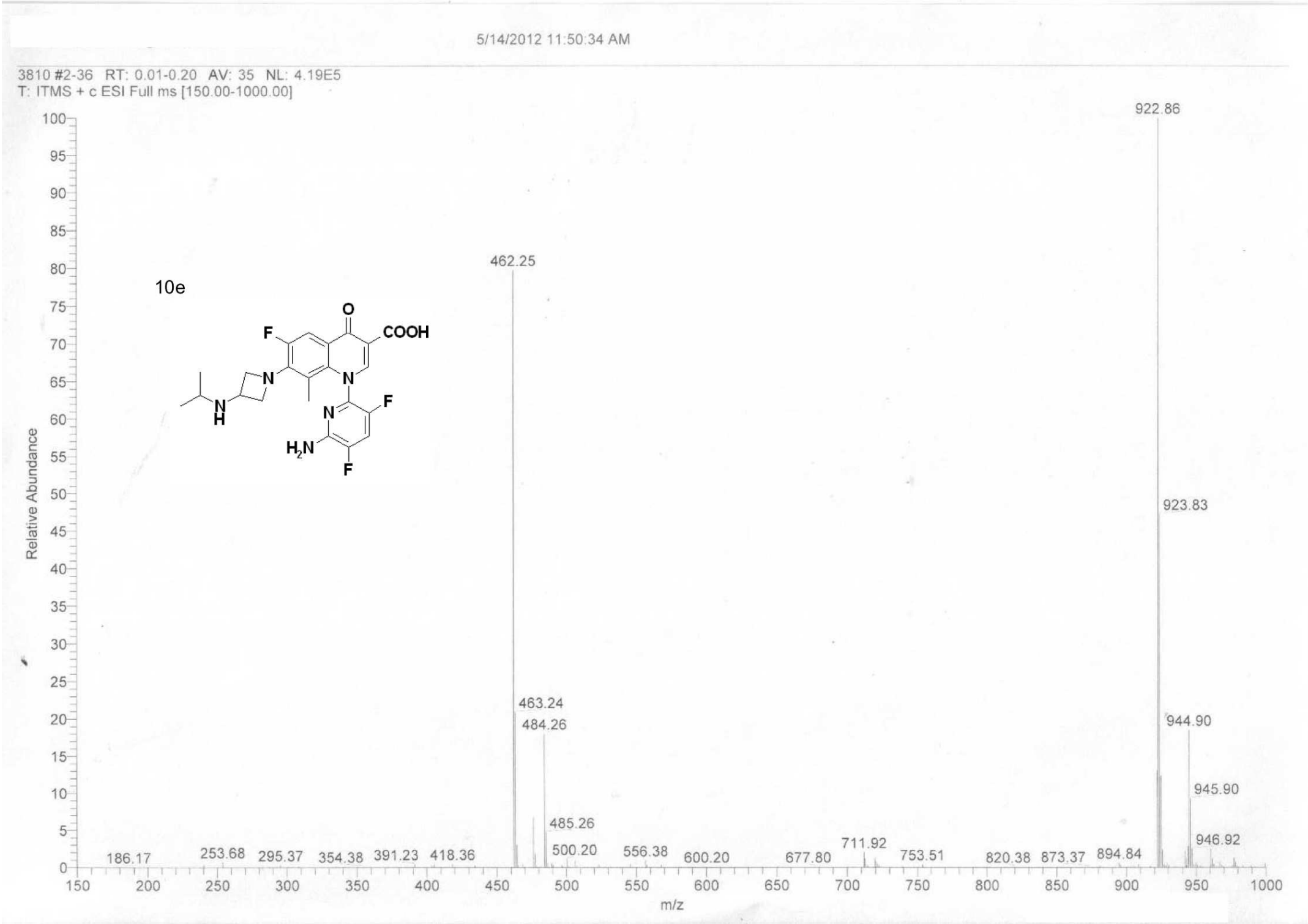




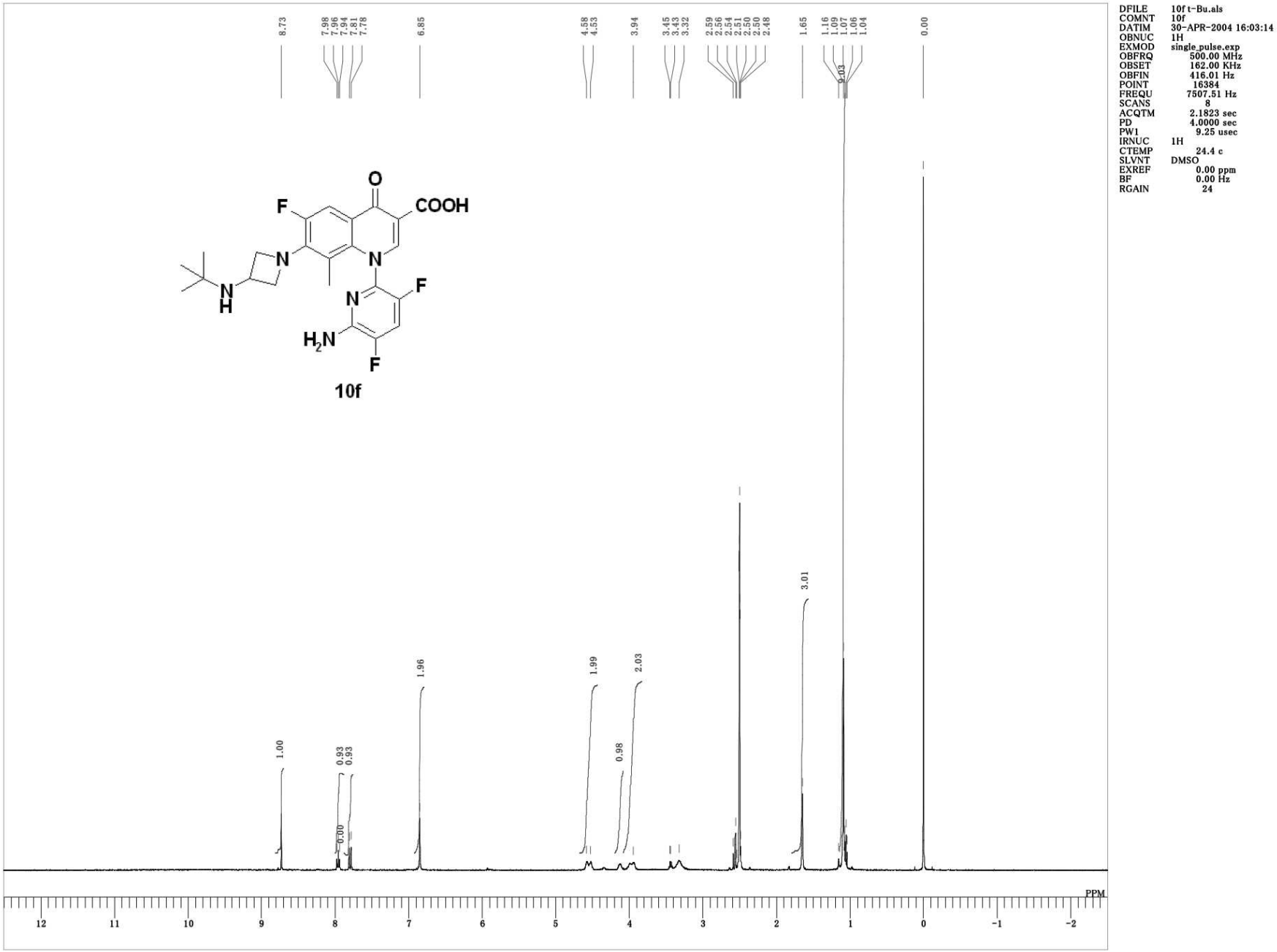

10e

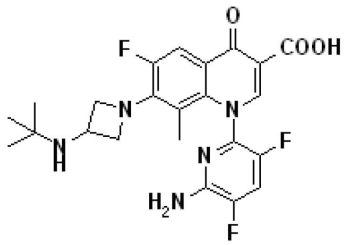



| | | | | |
|---|---|---|---|---|
| PULSE SEQUENCE<br>Relax. delay 1.000 sec<br>Pulse 45.0 degrees<br>Acq. time 1.049 sec<br>Width 31250.0 Hz<br>20000 repetitions | OBSERVE C13, 125.6686970<br>DECOUPLE H1, 499.7781880<br>Power 39 dB<br>continuously on<br>WALTZ-16 modulated | DATA PROCESSING<br>Line broadening 0.5 Hz<br>FT size 65536<br>Total time 11.4 hours | | Solvent: dmso<br>Ambient temperature<br>Sample #8, Operator: vnmr1<br>File: 150605-10e-13C_CARBON_01<br>VNMRS-500 "Varian-NMR" |


5/14/2012 11:50:34 AM
3810 #2-36 RT: 0.01-0.20 AV: 35 NL: 4.19E5
T: ITMS + c ESI Full ms [150.00-1000.00]
10e
Relative Abundance
m/z
922.86
462.25
923.83
463.24
484.26
944.90
945.90
946.92
485.26
500.20
186.17
253.68
295.37
354.38
391.23
418.36
556.38
600.20
677.80
711.92
753.51
820.38
873.37
894.84

10f






10f




| | | | | |
|---|---|---|---|---|
| PULSE SEQUENCE<br>Relax. delay 1.000 sec<br>Pulse 45.0 degrees<br>Acq. time 1.049 sec<br>Width 31250.0 Hz<br>20800 repetitions | OBSERVE C13, 125.6686970<br>DECOUPLE H1, 499.7781880<br>Power 39 dB<br>continuously on<br>WALTZ-16 modulated | DATA PROCESSING<br>Line broadening 0.5 Hz<br>FT size 65536<br>Total time 11.8 hours | | Solvent: dmso<br>Ambient temperature<br>Sample #4, Operator: vnmr1<br>File: 150518-10f-13C_CARBON_01<br>VNMRS-500 "Varian-NMR" |