Supporting Information

Design and Synthesis of Imidazo[1,2-α][1,8]naphthyridine Derivatives as Anti-HCV Agents via Direct C-H Arylation

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Part 1: **General Methods and Materials**:

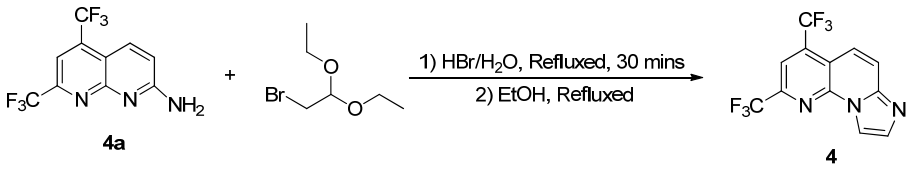
Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere under anhydrous conditions and all reagents were purchased from commercial suppliers without further purification. Solvent purification was conducted according to Purification of Laboratory Chemicals (Peerrin, D. D.; Armarego, W. L. and Perrins, D. R., Pergamon Press: Oxford, 1980).Yields refer to isolated yields.

Reactions were monitored by Thin Layer Chromatography on plates (GF254) supplied by Yantai Chemicals (China) using UV light as visualizing agent. If not specially mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China).

NMR spectra were recorded on Bruker AV400 instrument. TMS was used as internal standard for ^{1}H NMR (0 ppm), and solvent signal was used as reference for ^{13}C NMR (CHLOROFORM-D, 77.20ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet.

High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-Q/TOF MS.

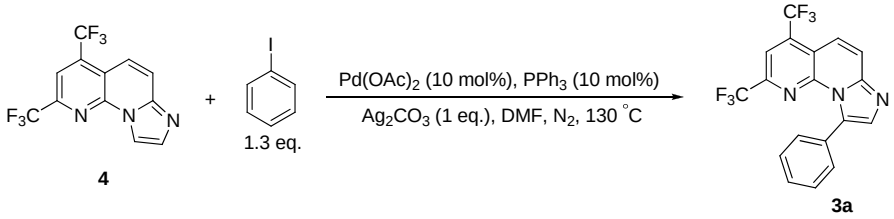
Part 2: **Experimental Details and Characteristic Data**:



Preparation of 2,4-bis(trifluoromethyl)imidazo[1,2-a][1,8]naphthyridin (4)[1]: Bromacetaldehyde diethyl acetal (11g, 55.8 mmol) was dissolved in water (40 mL) and hydrobromic acid (4 mL) was added. The mixture was refluxed for 30 minutes. After cooling the solution was extracted with ether. The organic layers were dried over anhydrous sodium sulfate and the ether was removed *in vacuo*. The bromoacetaldehyde was poured into a solution of 2.6g (18 mmol) of **4a** in dry ethanol (100 mL). The mixture was refluxed for 3 hours and evaporated under reduced pressure. The residue was dissolved in water, made basic with sodium carbonate and extracted with dichloromethane. After drying the organic layers were concentrated *in*

vacuo and submitted to chromatograph on silica gel (200-300 mesh). Elution with Petroleum ether (PE)/ethyl acetate (EA) = 5:1 gave 1.6 g of **4** (60%).

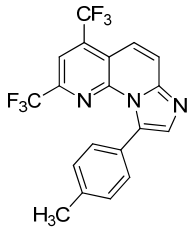
Characteristic data of 4: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.58 (s, 1H), 8.06 (s, 1H), 7.88 (d, 1H), 7.79 (t, 2H); ^{13}C-NMR (400MHz, $CDCl_3$): δ =146.2 (q, *J* = 37.0 Hz), 144.1, 144.0, 137.7 (q, *J* = 33.0 Hz), 134.1, 123.1, 122.4 (q, *J* = 274.0 Hz), 120.7 (q, *J* = 273.0 Hz), 119.0 (q, *J* = 3.0 Hz), 117.2, 113.9 (q, *J* = 2.0 Hz), 113.7 ppm; HRMS(ESI) m/z calcd for $C_{12}H_5F_6N_3$[M+H]$^+$: 306.0460; found: 306.0466.



Synthesis of single-arylation product 3a: A dry and nitrogen-flushed 10 mL Schlenk flask equipped with a mechanical stirrer, thermocouple and nitrogen/vacuum inlet was charged with **4** (50mg, 0.164 mmol), iodobenzene (43.5 mg, 0.213 mmol), palladium (II) acetate (3.7 mg, 0.0164 mmol), triphenylphosphine (4.3 mg, 0.0164 mmol), silver carbonate (45.5mg, 0.164 mmol), and anhydrous dimethylformamide (1.0 mL). The slurry was degassed with five vacuum/nitrogen back-fill cycles, heated to 130 °C, and aged for 3 h. The mixture was quenched with ice; then EtOAc (25 mL) were added to the crude reaction mixture, the organic phases were combined, dried over anhydrous sodium sulfate and then removed *in vacuo*. The residual was purified by column chromatography (PE/EA = 3:1) to give the desired compound **3a**.

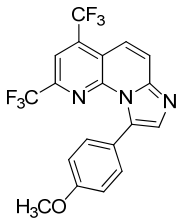
Notably, all of the following single-arylation products **3b-l** were synthesized using the similar procedure as compound **3a**.

Characteristic data of 3a: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.96 (s, 1H), 7.91 (d, 1H), 7.80 (d, 1H), 7.68 (s, 1H), 7.55 (m, 2H), 7.46 (m, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 145.4, 145.1, 144.8(q, *J* = 37.0 Hz), 137.2(q, *J* = 33.0 Hz), 135.0, 131.7, 130.7, 130.5, 128.6, 127.9, 123.6, 122.7(q,*J* = 274.0 Hz), 120.3(q, *J* = 274.0 Hz), 118.9(q, *J* = 3.0 Hz), 118.2, 113.6 (q, *J* = 3.0 Hz)ppm; MS (ESI) m/z calcd for $C_{18}H_{10}F_6N_3$ [M + H]$^+$: 382.07; found: 382.07.



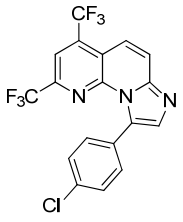
3b

Characteristic data of 3b: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.96 (s, 1H), 7.90 (d, 1H), 7.78 (d, 1H), 7.77 (s, 1H), 7.45 (d, 2H), 7.26 (d, 2H), 2.46 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 145.5, 145.0, 144.7 (q, *J* = 37.0 Hz), 138.6, 137.1 (q, *J* = 33.0 Hz), 134.9, 131.8, 130.3, 128.5, 127.7, 123.5, 122.5 (q, *J* = 274.0 Hz), 120.5 (q, *J* = 273.0 Hz), 118.7 (q, *J* = 2.0 Hz), 118.2, 113.6 (q, *J* = 3.0 Hz), 21.5 ppm; HRMS(ESI) m/z calcd for $C_{19}H_{11}F_6N_3$[M+H]$^+$: 396.0930; found: 396.0925.



3c

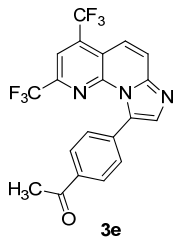
Characteristic data of 3c: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.96 (s, 1H), 7.90 (d, 1H), 7.78 (d, 1H), 7.65 (s, 1H), 7.49 (d, 2H), 6.98 (d, 2H), 3.90 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 160.1, 145.5, 144.9, 144.7 (q, *J* = 37.0 Hz), 137.1 (q, *J* = 33.0 Hz), 134.7, 131.9, 131.6, 123.5, 123.0, 122.5 (q, *J* = 274.0 Hz), 120.5 (q, *J* = 274.0 Hz), 118.6, 118.3, 113.6, 113.3, 55.8 ppm; HRMS(ESI) m/z calcd for $C_{19}H_{11}F_6N_3O$ [M+H]$^+$: 412.0879; found: 412.0875.



3d

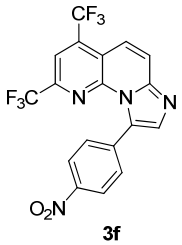
Characteristic data of 3d: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.98 (s, 1H), 7.90 (d, 1H), 7.78 (d, 1H), 7.67 (s, 1H), 7.49 (d, 2H), 7.42 (d, 2H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 145.3, 145.3, 144.9 (q, *J* = 37.0 Hz), 137.4 (q, *J* = 33.0 Hz), 135.1, 134.8, 131.8, 130.4, 129.2, 128.1, 123.5, 122.5 (q, *J* = 274.0 Hz), 120.4 (q, *J* = 273.0

Hz),119.2 (q, *J* = 2.0 Hz), 118.2, 113.8 ppm; HRMS(ESI) m/z calcd for $C_{18}H_8ClF_6N_3[M+H]^+$: 416.0384; found: 416.0389.



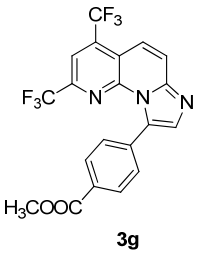
3e

Characteristic data of 3e: ^{1}H-NMR (400MHz, $CDCl_3$: δ = 8.03 (d, 1H), 7.99 (s, 1H), 7.92 (d, 1H), 7.84 (d, 1H), 7.72 (s, 1H), 7.67 (d, 2H), 2.67 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 197.9, 145.2, 145.1, 144.5 (q, *J* = 37.0 Hz), 137.6 (q, *J* = 33.0 Hz), 136.9, 135.5, 130.5, 127.8, 123.5, 122.4 (q, *J* = 274.0 Hz), 120.4 (q, *J* = 274.0 Hz), 119.5 (q, *J* = 3.0 Hz),118.2, 113.9 (q, *J* = 3.0 Hz), 26.9 ppm; HRMS(ESI) m/z calcd for $C_{20}H_{11}F_6N_3O[M+H]^+$: 424.0879; found: 424.0876.



3f

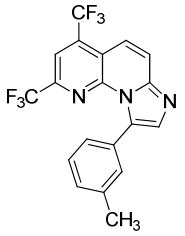
Characteristic data of 3f: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.32 (d, 2H), 8.02 (s, 1H), 7.96 (d, 1H), 7.88 (dd, 1H), 7.77 (d, 2H), 7.74 (s, 1H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 147.6, 145.7, 144.9, 144.9 (q, *J* = 38.0 Hz), 137.6 (q, *J* = 33.0 Hz), 137.2, 135.8, 131.0, 129.0, 123.4, 122.9, 122.1 (q, *J*= 274.0 Hz), 120.2 (q, *J* = 274.0 Hz), 119.7, 118.1, 113.9 ppm; HRMS(ESI) m/z calcd for $C_{18}H_8F_6N_4O_2$ $[M+H]^+$: 427.0624; found: 427.0621.



3g

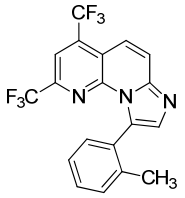
Characteristic data of 3g: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.12 (d, 2H), 7.99 (s, 1H), 7.93 (d, 1H), 7.84 (d, 1H), 7.73 (s, 1H), 7.65 (d, 2H), 3.98 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 167.0, 145.5, 145.2, 144.7 (q, *J* = 37.0 Hz), 137.4 (q, *J* = 33.0

Hz), 135.5, 135.3, 130.5, 130.3, 130.0, 129.0, 123.5, 122.4 (q, J = 274.0 Hz), 120.4 (q, J = 274.0 Hz), 119.3 (q, J = 2.0 Hz), 118.2, 113.8 (q, J = 2.0 Hz), 52.3 ppm; MS (ESI) m/z calcd for $C_{20}H_{12}F_6N_3O_2$ $[M + H]^+$: 440.07; found: 440.07.



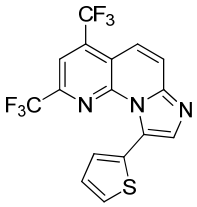
3h

Characteristic data of 3h: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.97 (s, 1H), 7.91 (d, 1H), 7.79 (d, 1H), 7.67 (s, 1H), 7.39 (s, 1H), 7.33 (t, 2H), 7.28 (m, 1H), 2.40 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 145.4, 145.0, 144.9 (q, J = 37.0 Hz), 137.3, 137.0 (q, J = 33.0 Hz), 134.9, 131.9, 131.2, 130.5, 129.4, 127.8, 127.3, 123.5, 122.5 (q, J = 273.0 Hz), 120.5 (q, J = 273.0 Hz), 118.8 (q, J = 2.0 Hz), 118.2, 113.6 (q, J = 2.0 Hz), 21.5 ppm; HRMS(ESI) m/z calcd for $C_{19}H_{11}F_6N_3[M+H]^+$: 396.0930; found: 396.0926.



3i

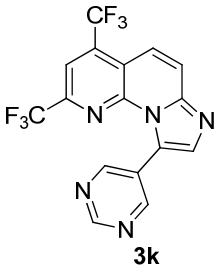
Characteristic data of 3i: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.92 (d, 2H), 7.80 (d, 1H), 7.64 (s, 1H), 7.40 (dd, 1H), 7.29 (d, 1H), 7.25 (m, 2H), 2.01 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 145.4, 145.0, 144.7 (q, J = 37.0 Hz), 138.6, 137.1 (q, J = 33.0 Hz), 134.8, 131.8, 130.3, 128.5, 127.7, 123.5, 122.5 (q, J = 273.0 Hz), 120.5 (q, J = 274.0 Hz), 118.7 (q, J = 3.0 Hz), 118.2, 113.6 (q, J = 2.0 Hz), 21.5 ppm; HRMS(ESI) m/z calcd for $C_{19}H_{11}F_6N_3[M+H]^+$: 396.0930; found: 396.0929.



3j

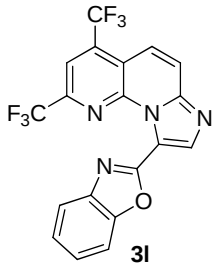
Characteristic data of 3j: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.01 (s, 1H), 7.90 (d, 1H), 7.83 (d, 1H), 7.79 (s, 1H), 7.52 (d, 1H), 7.39 (d, 1H), 7.16 (t, 1H); ^{13}C-NMR

(400MHz, $CDCl_3$): δ = 145.5, 145.3, 145.1 (q, *J* = 37.0 Hz), 137.3 (q, *J* = 33.0 Hz), 136.5, 130.8, 130.4, 127.7, 126.9, 124.4, 123.4, 122.5 (q, *J* = 274.0 Hz), 120.5 (q, *J* = 273.0 Hz), 119.4 (q, *J* = 2.0 Hz), 118.2, 113.8 ppm; HRMS(ESI) m/z calcd for $C_{16}H_7F_6N_3S[M+H]^+$: 388.0338; found: 388.0334.



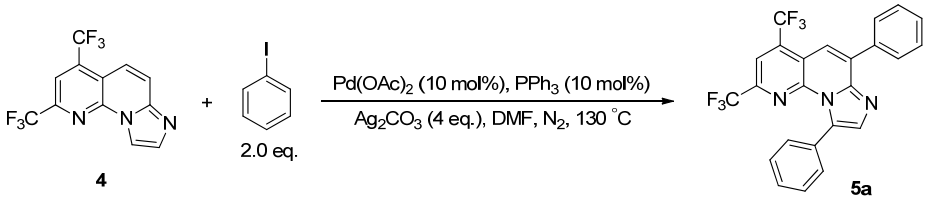
3k

Characteristic data of 3k: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.30 (s, 1H), 8.97 (s, 2H), 8.04 (s, 1H), 7.97 (d, 1H), 7.90 (d, 1H), 7.80 (s, 1H); HRMS(ESI) m/z calcd for $C_{19}H_8F_6N_4O[M+H]^+$: 384.06; found: 384.04.



3l

Characteristic data of 3l: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.24 (s, 1H), 8.02 (d, 1H), 7.97 (d, 2H), 7.87 (t, 1H), 7.57 (t, 1H), 7.45 (m, 2H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 155.0, 151.3, 146.2, 146.1 (q, *J* = 38.0 Hz), 144.3, 141.5, 139.0, 138.0 (q, *J* = 34.0 Hz), 126.1, 124.9, 123.2, 122.3 (q, *J* = 274.0 Hz), 121.3, 120.7, 120.1 (q, *J* = 273.0 Hz), 117.8, 117.7, 114.5, 111.0 ppm; HRMS(ESI) m/z calcd for $C_{19}H_8F_6N_4O[M+H]^+$: 423.0675; found: 423.0671.

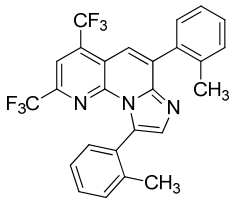


4 **5a**

Synthesis of double-arylation product 5a: A dry and nitrogen-flushed 10 mL Schlenk flask equipped with a mechanical stirrer, thermocouple and nitrogen/vacuum inlet was charged with **4** (50mg, 0.164 mmol), iodobenzene (67.0mg, 0.328mmol), palladium (II) acetate (3.7 mg, 0.0164 mmol), triphenylphosphine(4.3 mg, 0.0164

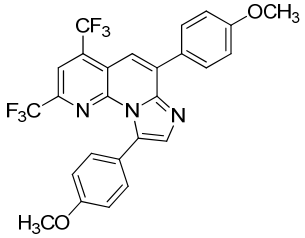
mmol), silver carbonate (182.2mg, 0.656 mmol) and anhydrous dimethylformamide(1.0 mL). The slurry was degassed with five vacuum/nitrogen back-fill cycles, heated to 130 °C, and aged for 3 h. The mixture was quenched with ice; thenEtOAc (25 mL) were added to the crude reactionmixture, theorganic phases were combined, dried over anhydrous sodium sulfate and then removed *in vacuo*. The residual was purified by column chromatography (PE/EA = 3:1) to give the desired compound **5a**. Notably, the following double-arylation products **5b-d**were synthesized using the similar procedure as compound **5a**.

Characteristic data of 5a: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.04 (d, 2H), 7.97 (s, 1H), 7.84 (s, 1H), 7.74 (s, 1H), 7.53-7.62 (m, 5H), 7.47 (d, 3H), 7.45 (m, 2H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 144.7, 144.3, 144.0 (q, *J* = 37.0 Hz), 136.7 (q, *J* = 33.0 Hz), 136.3, 135.5, 134.9, 132.0, 131.0, 130.5, 130.0, 129.6, 129.0, 128.6, 127.9, 122.7 (q, *J* = 274.0 Hz), 120.5 (q, *J* = 273.0 Hz), 118.5, 117.0, 113.8 ppm; HRMS(ESI) m/z calcd for $C_{24}H_{13}F_6N_3[M+H]^+$:458.1086; found: 458.1088.



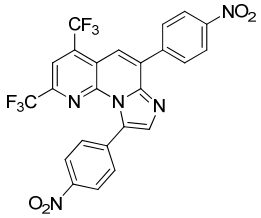
5b

Characteristic data of 5b: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.95 (s, 1H), 7.70 (s, 1H), 7.65 (s, 1H), 7.51 (d, 1H), 7.47-7.40 (m, 4H), 7.38 (d, 1H), 7.34 (d, 1H), 7.31 (d, 1H), 2.34 (s, 3H), 2.08 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ =144.9 (q, *J* = 37.0 Hz), 144.8, 144.3, 139.1, 137.1, 136.7, 136.5 (q, *J* = 33.0 Hz), 135.3, 134.5, 131.0, 130.7, 130.1, 129.7, 129.5, 129.2, 126.2, 125.4, 122.6 (q, *J* = 274.0 Hz), 120.4 (q, *J* = 273.0 Hz), 118.6, 117.9, 113.5, 20.5 ppm; HRMS(ESI) m/z calcd for $C_{26}H_{17}F_6N_3[M+H]^+$:486.1399; found: 486.1395.



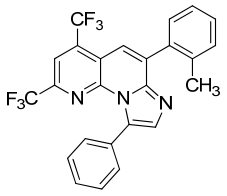
5c

Characteristic data of 5c: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.03 (d, 2H), 7.95 (s, 1H), 7.77 (s, 1H), 7.68 (s, 1H), 7.50 (d, 2H), 7.11 (d, 2H), 6.99 (d, 2H), 3.91 (s, 6H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 161.2, 160.1, 144.7, 144.3, 143.5(q, *J* = 37.0 Hz), 136.2 (q, *J* = 33.0 Hz), 135.8, 134.8, 134.6, 131.9, 131.1, 127.8, 123.3, 122.7 (q, *J* = 274.0 Hz), 120.6 (q, *J* = 273.0 Hz), 118.7, 115.7, 114.6, 114.5, 113.7, 113.5, 113.4, 55.7, 55.6 ppm; HRMS(ESI) m/z calcd for $C_{26}H_{17}F_6N_3O_2$[M+H]$^+$:518.1298; found: 518.1294.



5d

Characteristic data of 5d: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 8.46 (d, 2H), 8.34 (d, 2H), 8.23 (d, 2H), 8.06 (s, 1H), 7.96 (s, 1H), 7.82 (s, 1H), 7.76 (d, 2H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 148.8, 148.0, 145.4 (q, *J* = 37.0 Hz), 144.7, 144.4, 141.1, 138.8 (q, *J* = 33.0 Hz), 137.2, 135.9, 134.0, 131.3, 130.7, 129.9, 124.2, 123.2, 122.4 (q, *J* = 274.0 Hz), 120.3 (q, *J* = 273.0 Hz), 118.8, 118.0, 114.6 ppm; MS (ESI) m/z calcd for $C_{24}H_{12}F_6N_5O_4$ [M + H]$^+$:548.08; found: 548.08.

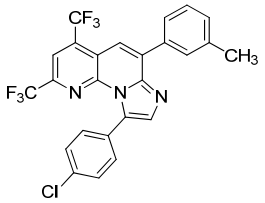


5e

Synthesis of sequential double-arylation product 5e: A dry and nitrogen-flushed 10 mL Schlenk flask equipped with a mechanical stirrer, thermocouple and nitrogen/vacuum inlet was charged with **4** (50 mg, 0.164 mmol), iodobenzene (43.5 mg, 0.213 mmol), palladium (II) acetate (3.7 mg, 0.0164 mmol), triphenylphosphine(4.3 mg, 0.0164mmol), silver carbonate (45.5mg, 0.164 mmol), and anhydrous dimethylformamide (1.0 mL). The slurry was degassed with five vacuum/nitrogen back-fill cycles, heated to 130 °C, and aged for 3 h. After the single arylation was completed (monitored by TLC), the second potion of 1-iodo-2-methylbenzene (71 mg, 0.328 mmol) and silver carbonate (91.0 mg, 0.328 mmol) were added to the reaction mixture. After stirring another 12 h, the mixture

was quenched with ice; thenEtOAc (25 mL) were added to the crude reaction mixture, the organic phases were combined, dried over anhydrous sodium sulfate and then removed *in vacuo*. The residual was purified by column chromatography (PE/EA = 3:1) to give the desired compound **5e**. Notably, **5f** was synthesized using the similar procedure as compound **5e**.

Characteristic data of 5e: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.99 (s, 1H), 7.69 (t, 2H), 7.58-7.61 (m, 2H), 7.37-7.7.50 (m, 7H), 2.33 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ =144.9, 144.7, 144.1 (q, *J* = 37.0 Hz), 137.2, 136.8 (q, *J* = 33.0 Hz), 136.7, 135.3, 135.2, 131.9, 131.0, 130.9, 130.5, 130.0, 129.5, 128.6, 127.9, 126.2, 122.6 (q, *J* = 274.0 Hz), 120.5 (q, *J* = 274.0 Hz), 118.6, 118.3, 113.7, 20.5 ppm; HRMS(ESI) m/z calcd for $C_{25}H_{15}F_6N_3[M+H]^+$: 472.1243; found: 472.1247.



5f

Characteristic data of 5f: ^{1}H-NMR (400MHz, $CDCl_3$): δ = 7.98 (s, 1H), 7.83 (s, 1H), 7.80 (s, 2H), 7.72 (s, 1H), 7.43-7.52 (m, 5H), 7.36 (d, 1H), 2.51 (s, 3H); ^{13}C-NMR (400MHz, $CDCl_3$): δ = 144.6, 144.0 (q, *J* = 37.0 Hz), 138.7, 136.6 (q, *J* = 33.0 Hz), 136.6, 135.3, 135.1, 134.7, 131.8, 130.9, 130.7, 130.1, 129.5, 129.0, 128.9, 128.1, 126.7, 122.6 (q, *J* = 273.0 Hz), 120. 5 (q, *J* = 274.0 Hz), 118.5, 117.2, 114.0, 21.8 ppm; HRMS(ESI) m/z calcd for $C_{25}H_{14}ClF_6N_3[M+H]^+$: 506.0853; found: 506.0852.

Reference:

[1] Gueiffier, H. Viols, Y. Blache, J. P. Chapat, T. Olivier, C. Jean, G. Florence, G. Grassy, G. Dauphin, *J. Heterocyclic. Chem.,* 1997, **34**, 765-771.

[2] (a) H. K. Cui, J. Qing, Y. Guo, Y. J. Wang, L. J. Cui, T. H. He, L. Q. Zhang and L. Liu, *Bioorg. Med. Chem.* 2013, **21**, 3547–3554; (b) Y. Wu, Q. J. Liao, R. G. Yang, X. W. Chen and X. L. Chen, *Virus Research,* 2011, **155**, 406–414.

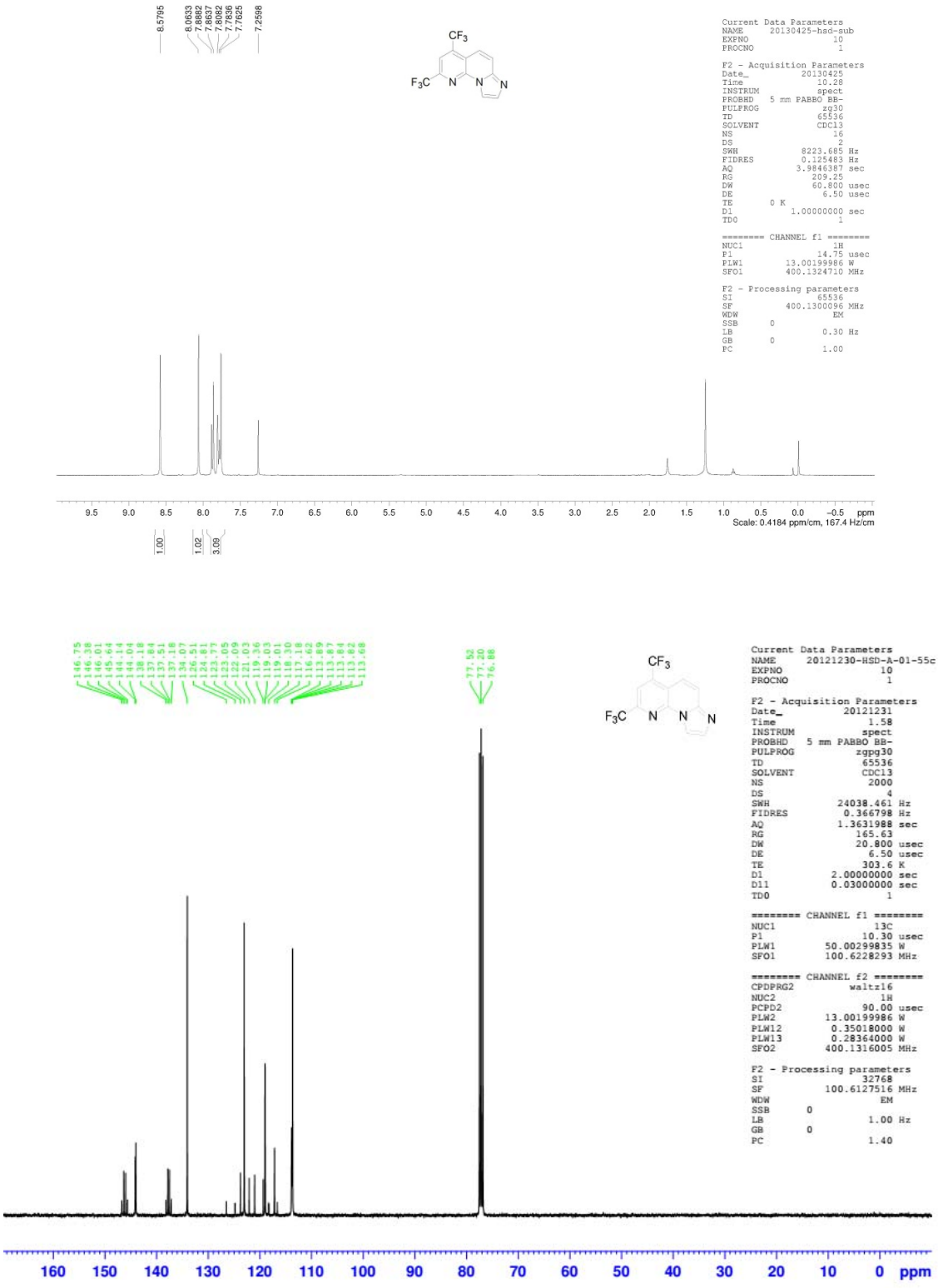
Part 3: Preparation of HCV Cell Culture (HCVcc-hRluc-JFH1) System

The HCVcc system was constructed by usingthe method developed aspreviously described.[2]In brief, The plasmid of pRluc-JFH-1was constructed as follows. Based on the plasmid of pJFH-1, a gift from Apath, L.L.C.， a humanized Renilla luciferase reporter gene was introduced between the 5'untranslated regions and HCV open reading frame, separated by a FMDV cleavage site. The plasmid of pRluc-JFH-1 was made through digestion with Xbal restrict ion enzyme, and used as a template for RNA transcription. The virus transcripts were prepared in vitro by using the Ambion MEGA script Kits, and then 10μg RNA was mixed with 400 mL of Huh7.5.1 at a concentration of 1×107 cells/mL. After electroporation, the Huh7.5.1 cells containing virus transcripts were seeded in a 10 cmdish. After cells were cultured for 4 days,the supernatant was collected and filteredto obtain the stock solution of virus hRluc-JFH-1. To obtain virus titer, the virus stocks were diluted at a gradient of 1:10, and incubated the Huh7.5.1 cells for 48 h at37 ℃. Then the cells were harvested and the luminescence was detected as manufacturer's protocol of Renilla-Glo™ Luciferase Assay System（Promega）.

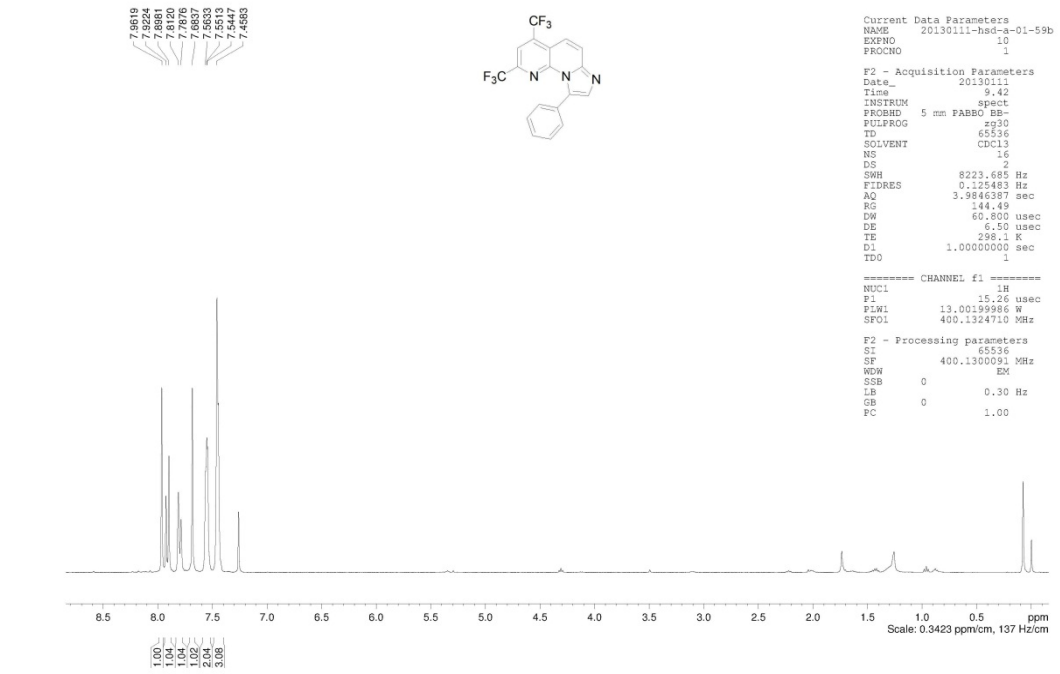
Viral inhibition assay

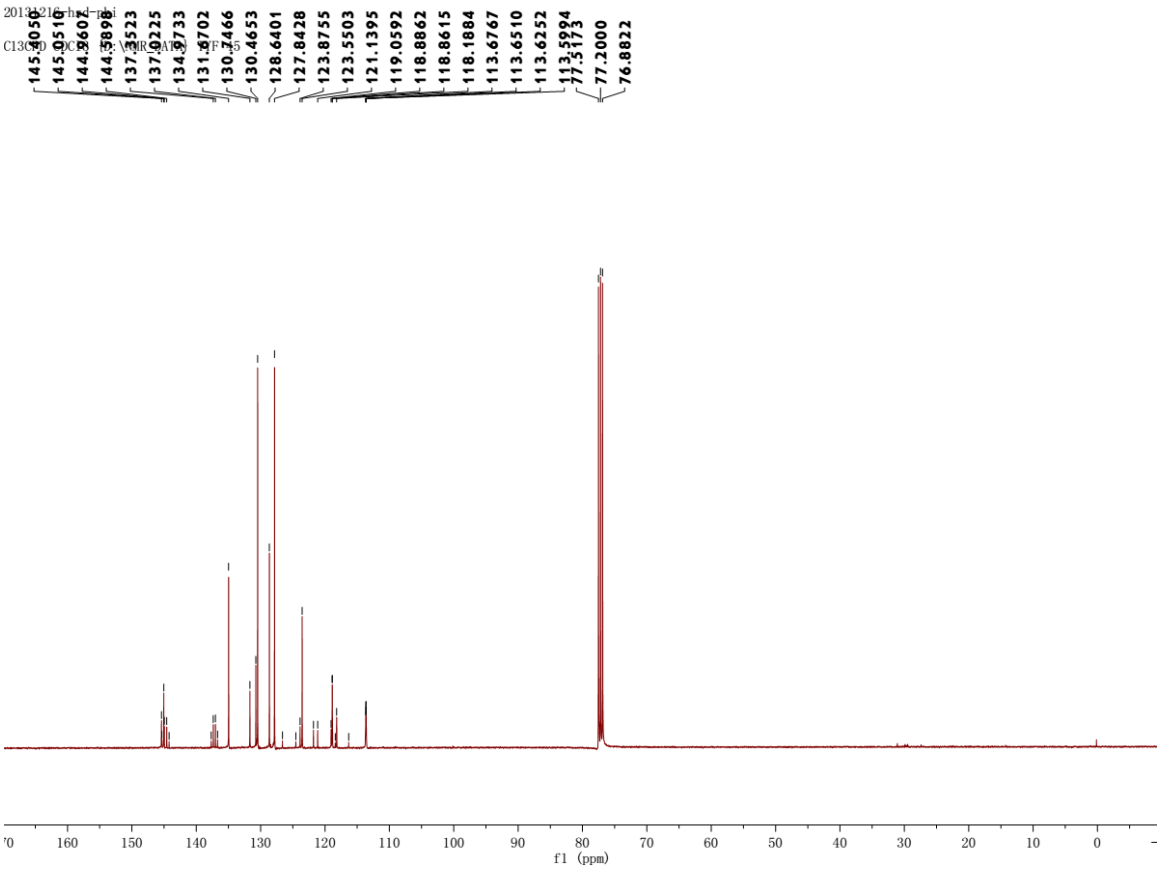
Huh 7.5.1 cells were seeded in 96-well plates at a density of2 ×104 cells per well at 37℃ overnight. All of the synthetic compounds were diluted with DMSO to 10 mM of stock solution. For the HCVcc system, the initial concentration of compounds was20μM and then diluted at a gradient of 1:8 to the concentrationsranging from 20μM to 0.6nM. Serial diluted compoundswere mixed with a certain titer of HCVcc-hRluc-JFH1virus.For the viral inhibition assay, the final concentration ofHCVcc-hRluc-JFH1 virus was diluted to the numbers of relativeluminescence units (RLU) ranging from 20,000 to 50,000 RLU.The mixture was added to Huh7.5.1 cells and the cells werecultured for 2 days at 37℃. Then the cells were harvested and the luminescence was detected as manufacturer's protocol of Renilla-Glo™Luciferase Assay System. EC_{50} is the concentration of compound at which the HCV luminescence level in the Huh7.5.1 cells is reduced by 50%. Each data point represents the average of three replicates in cell culture. The values of EC_{50} was plotted by the GraphPad Prism 5 software.

Part 4: NMR Spectra

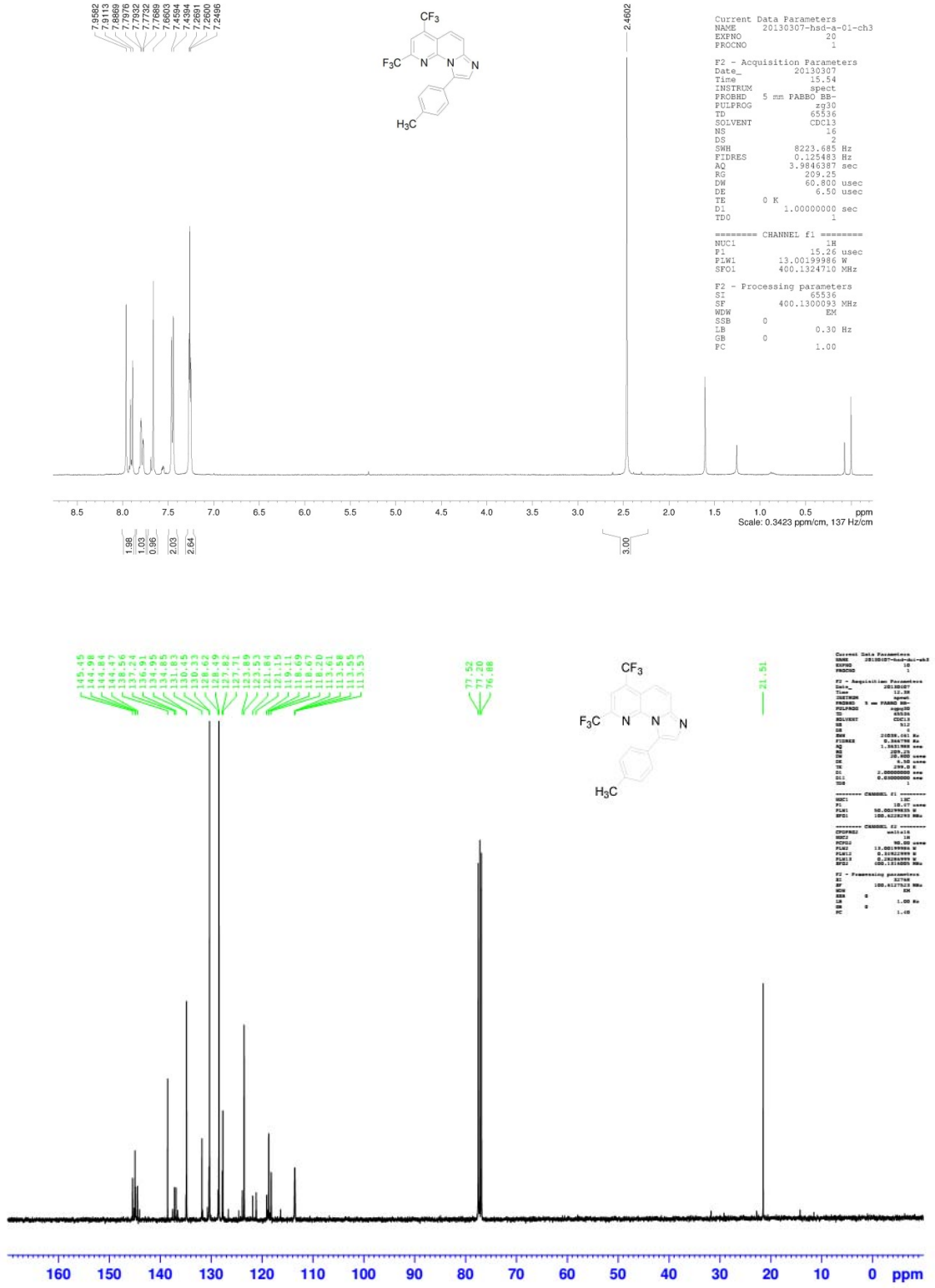


^{1}H-NMR and ^{13}C-NMR Spectra for Compound 4 (400Hz, CD_3Cl)

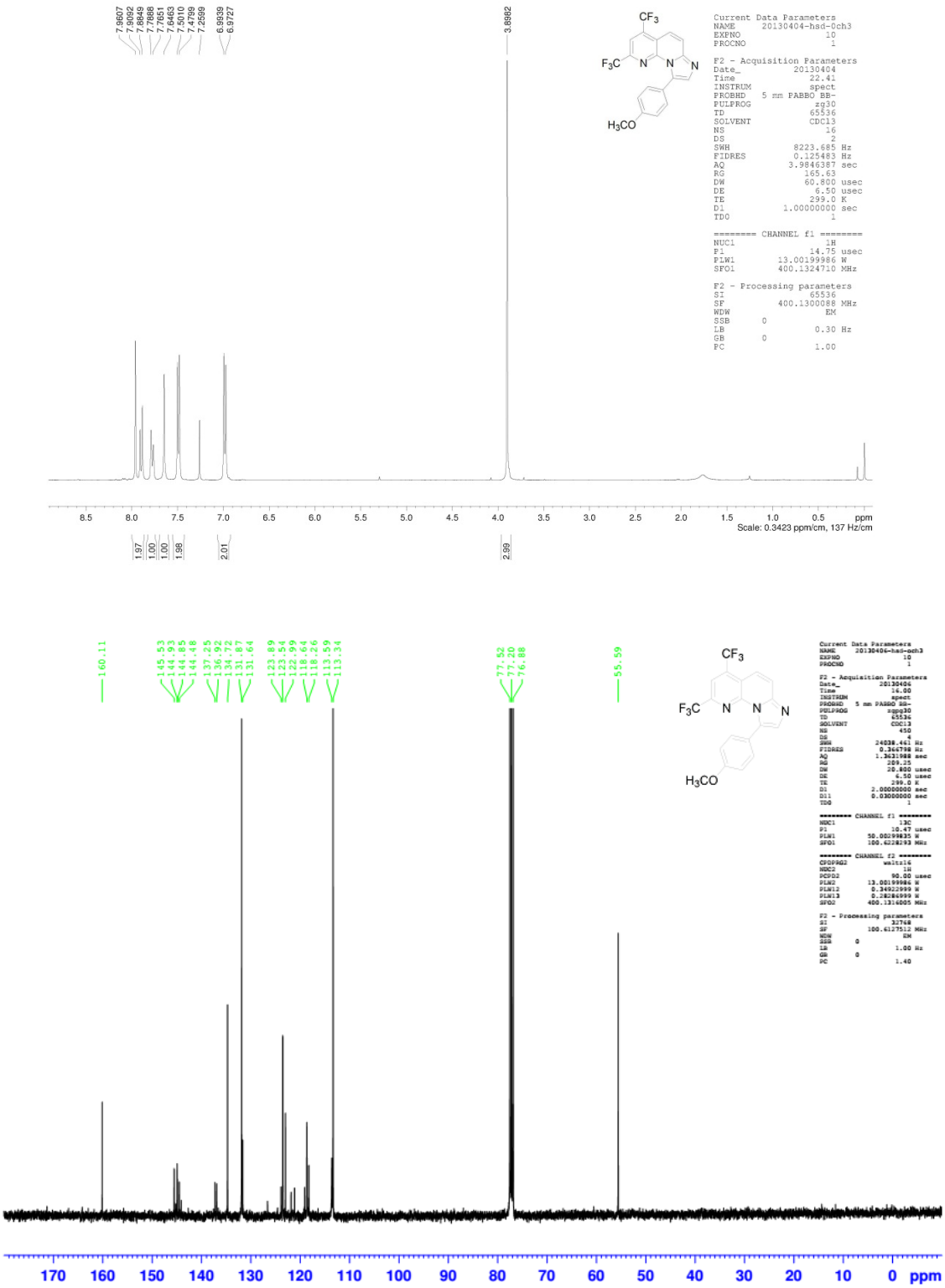




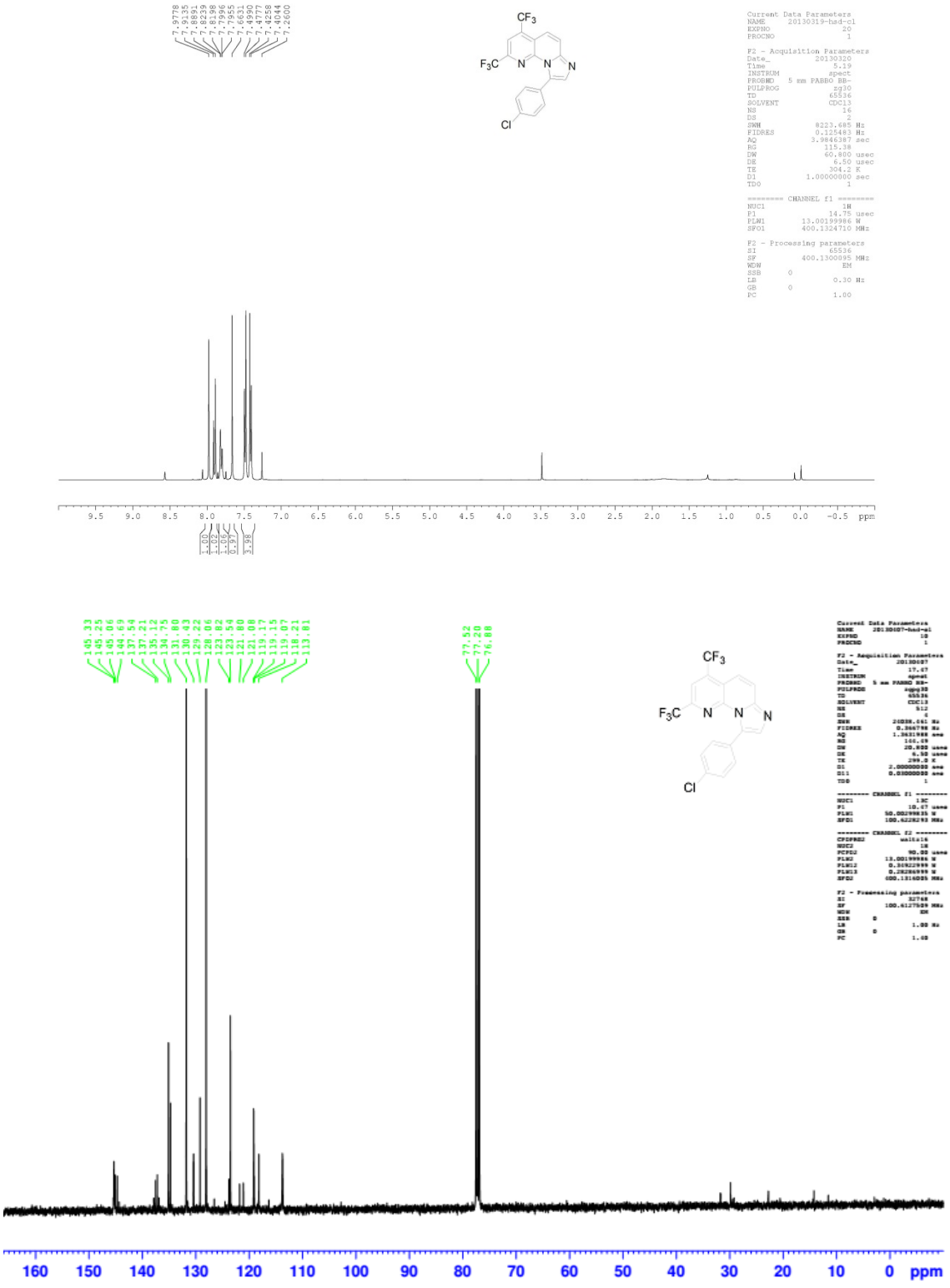
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3a (400Hz, CD_3Cl)



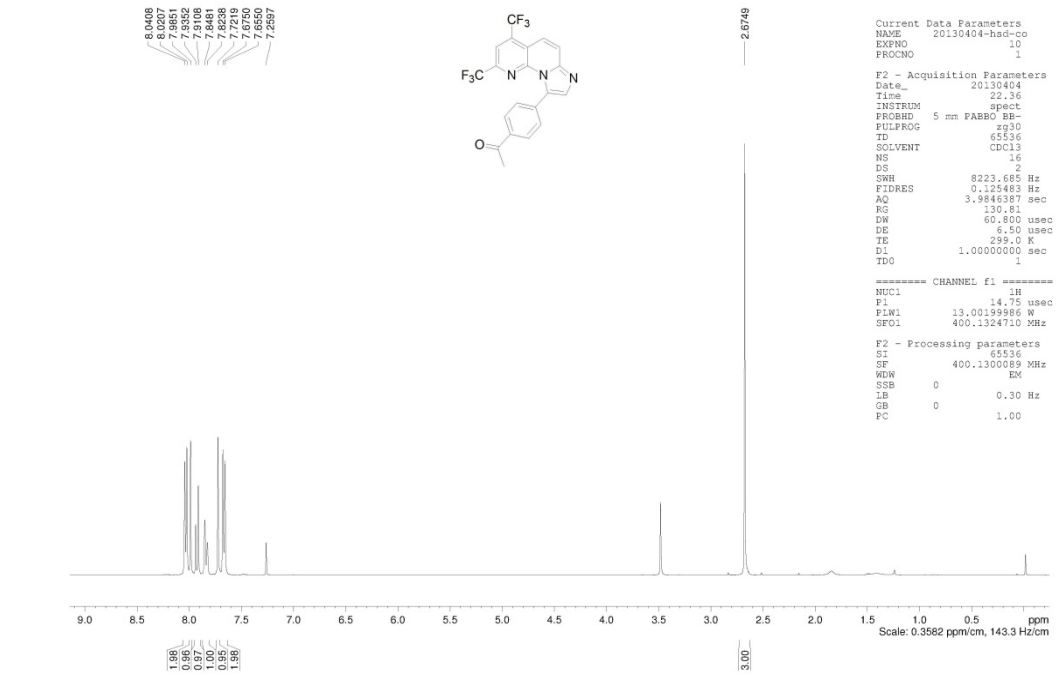
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3b (400Hz, CD_3Cl)

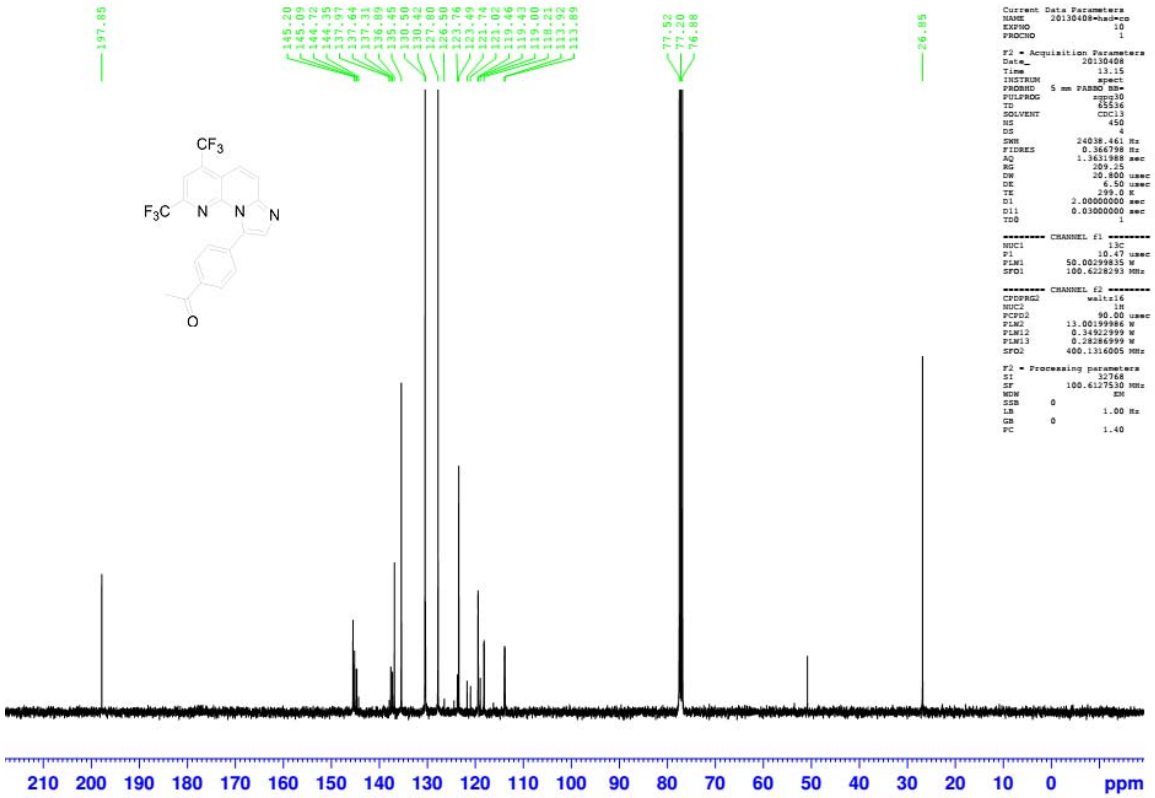


^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3c (400Hz, CD_3Cl)

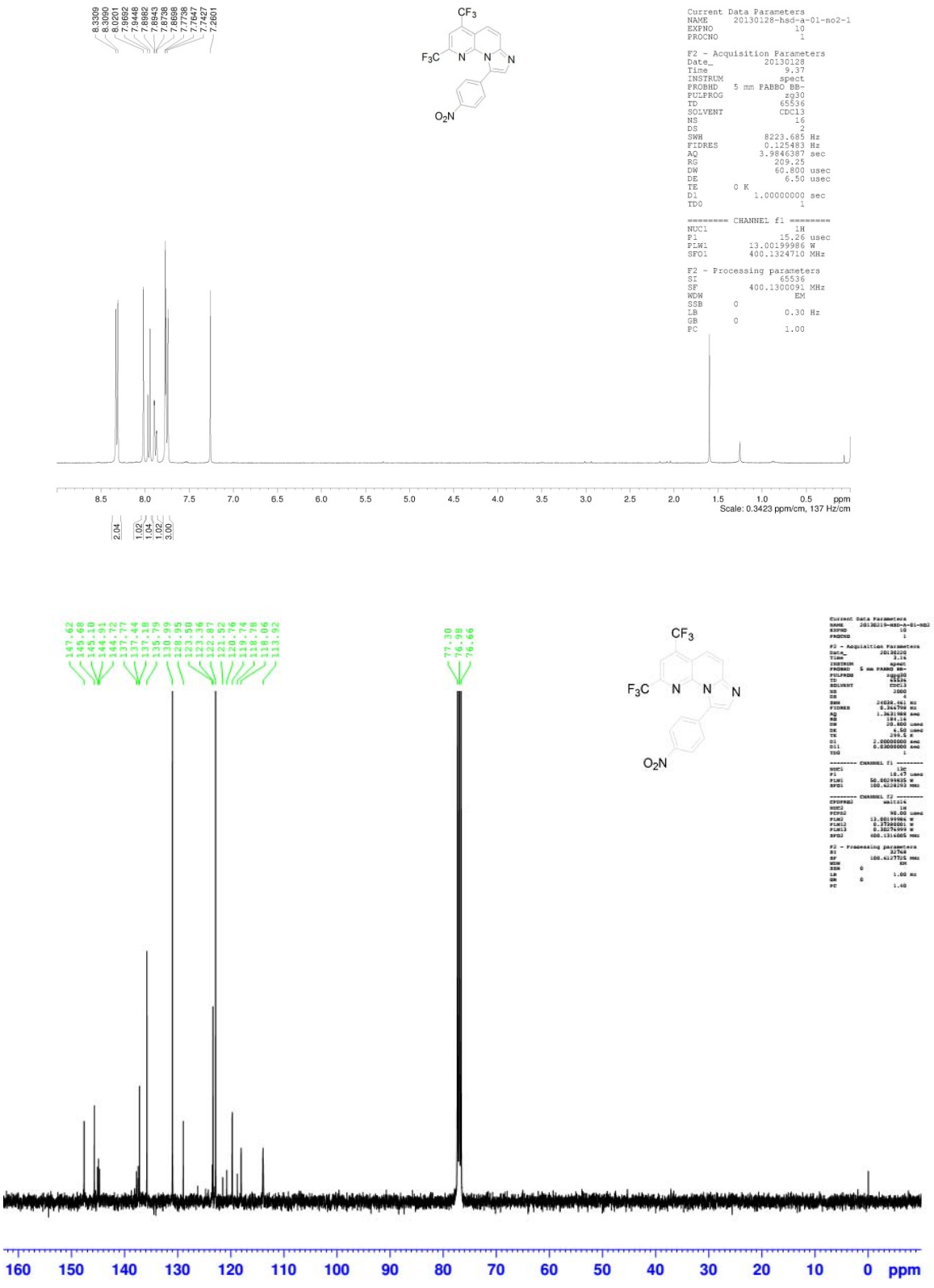


^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3d (400Hz, CD_3Cl)

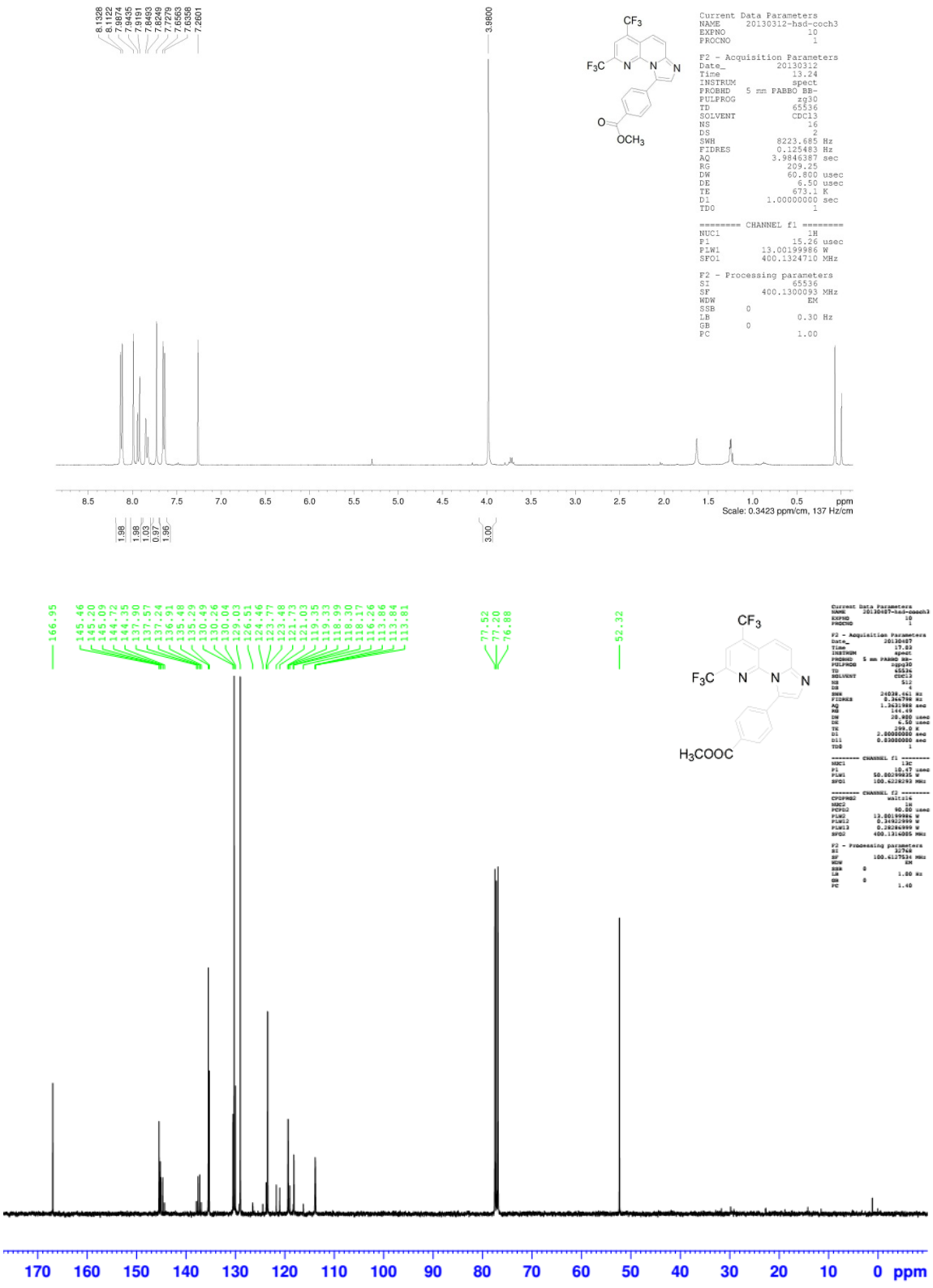




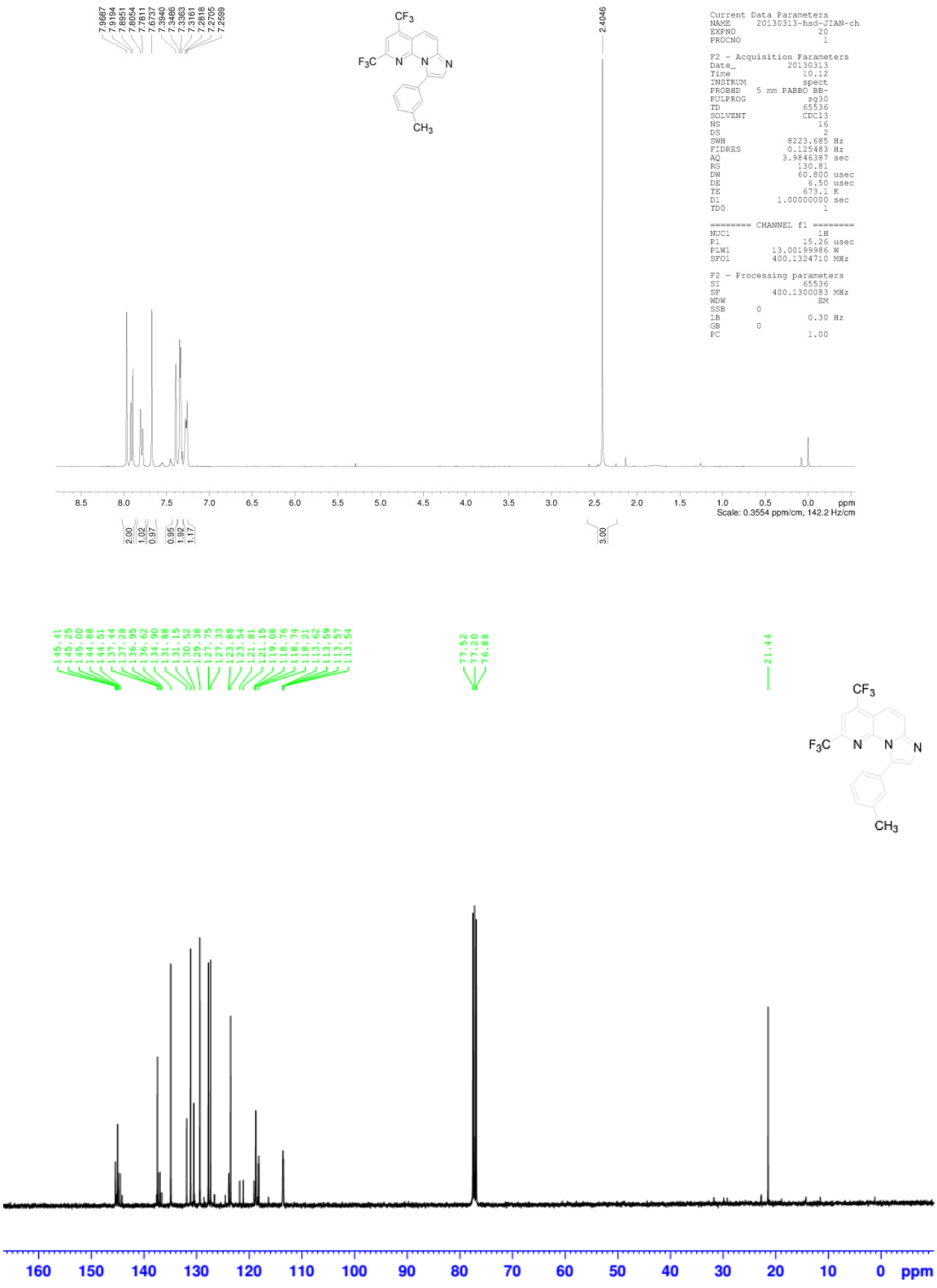
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3e (400Hz, CD_3Cl)



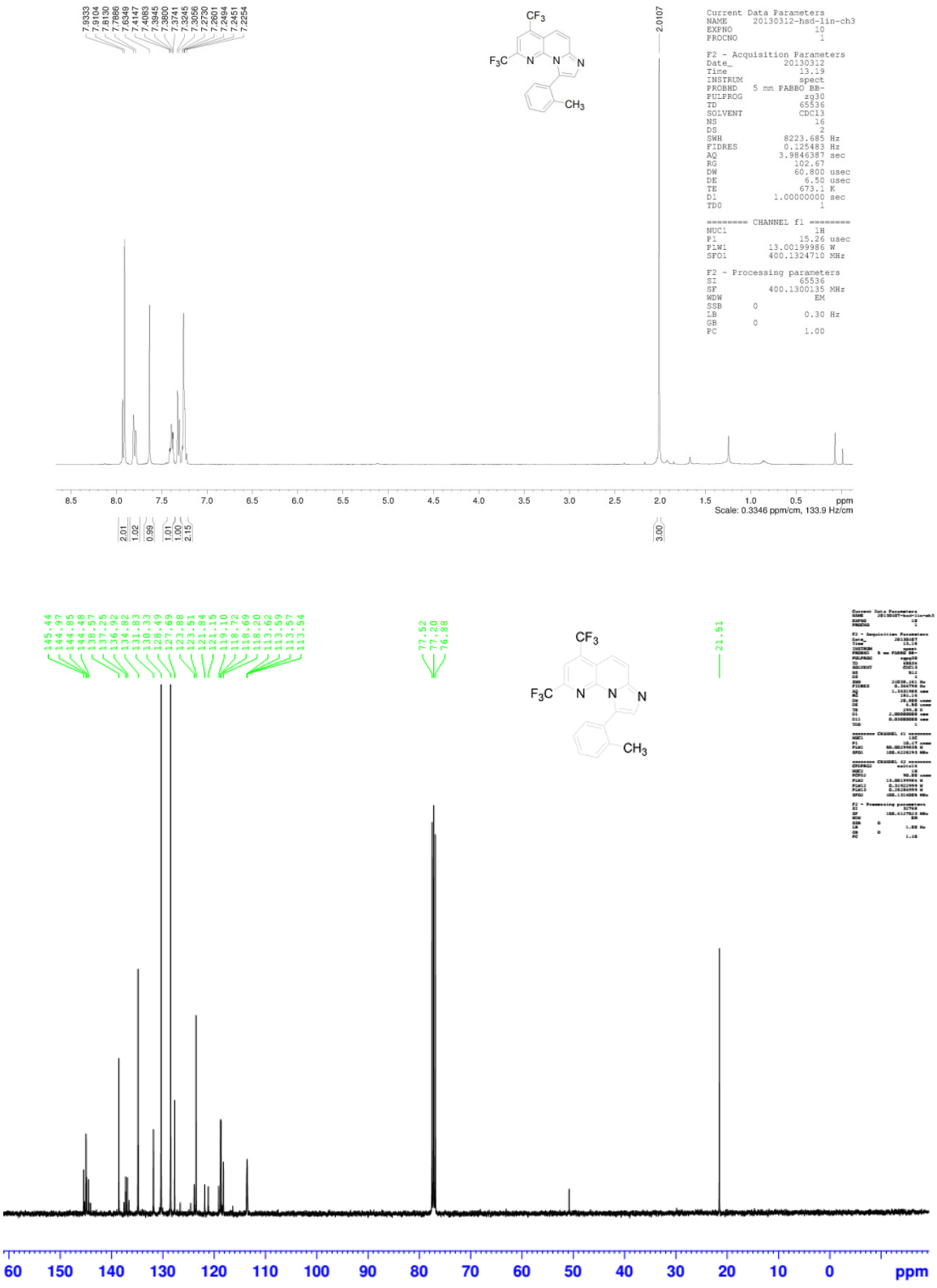
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3f (400Hz, CD_3Cl)



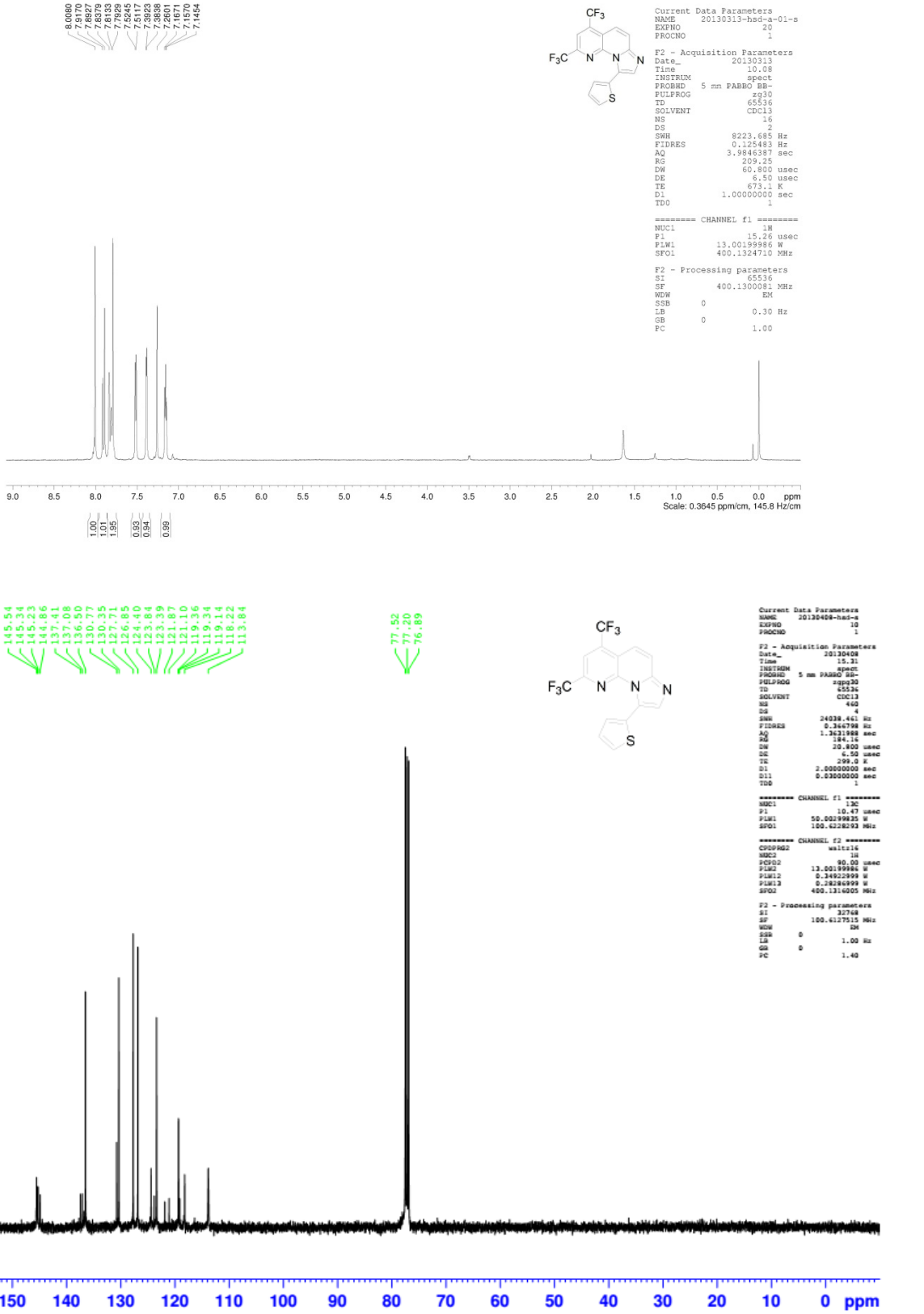
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3g (400Hz, CD_3Cl)



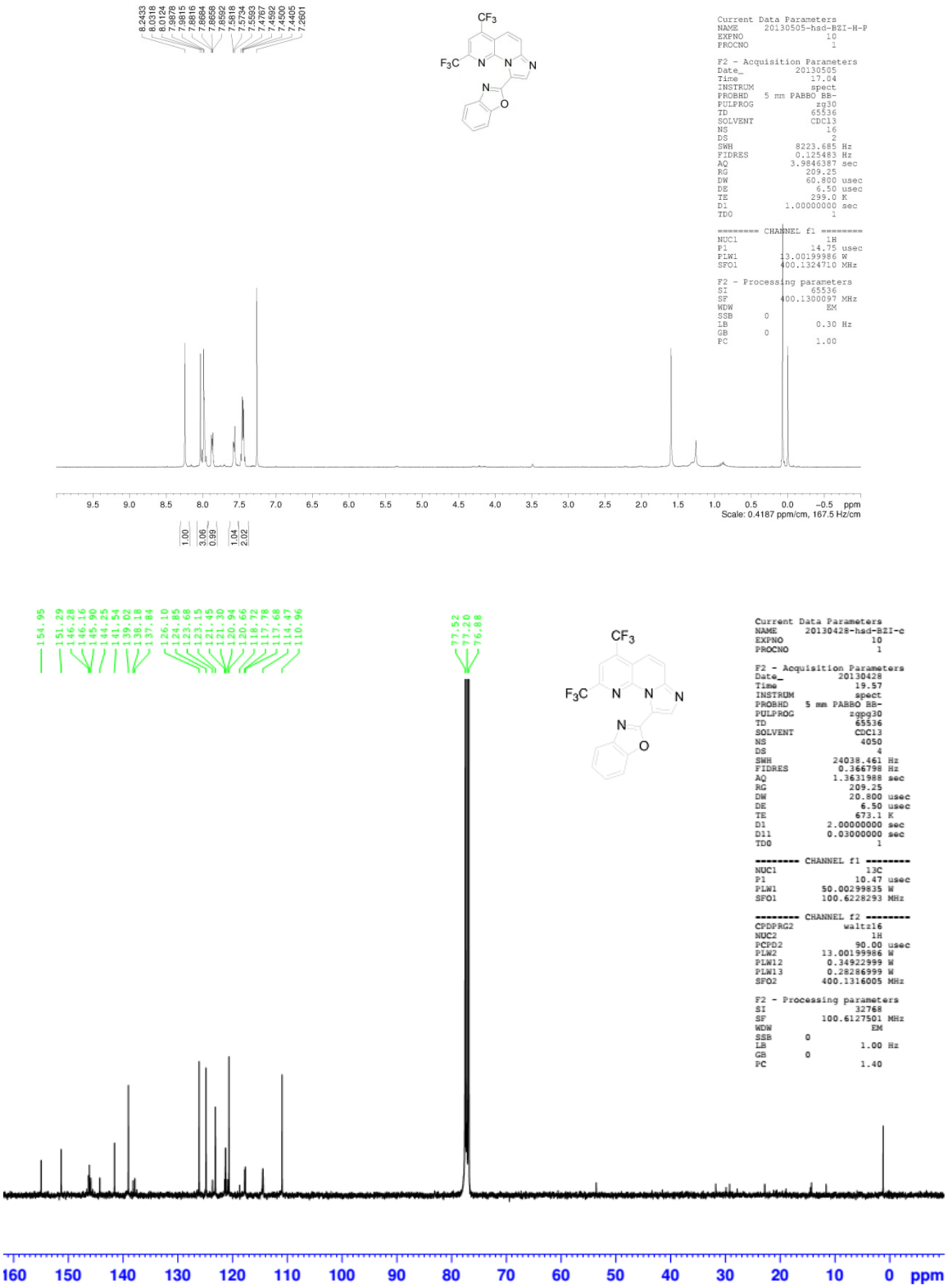
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3h (400Hz, CD_3Cl)



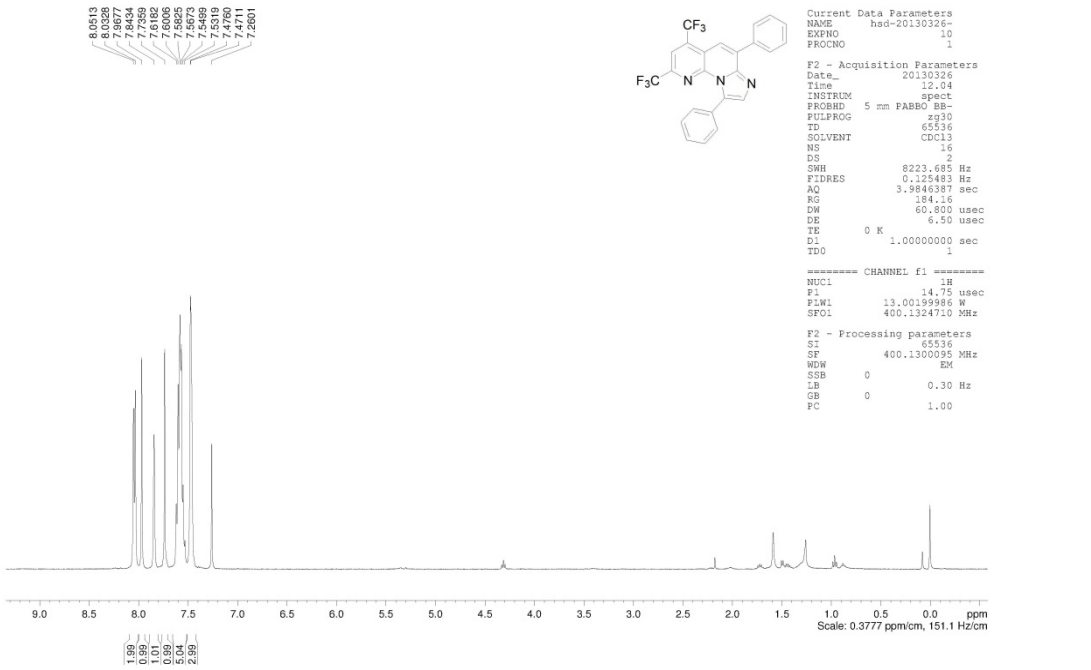
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3i (400Hz, CD_3Cl)

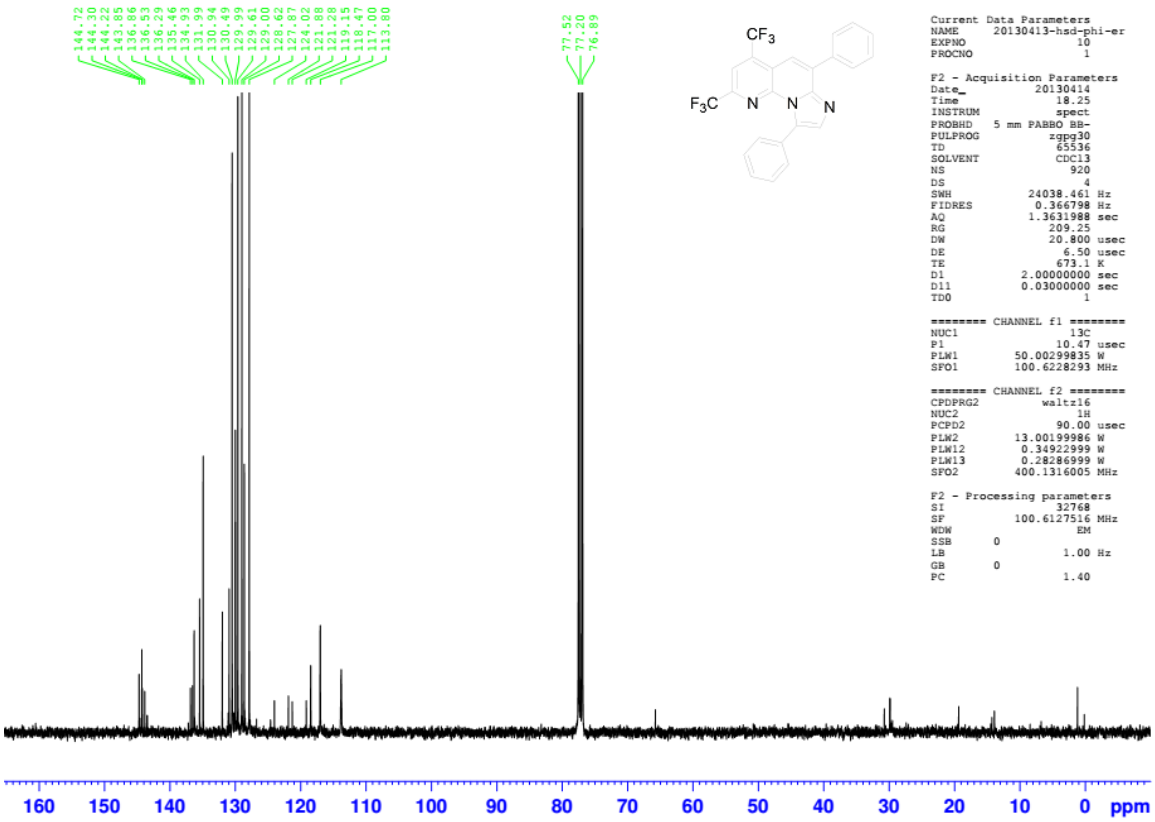


^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3j (400Hz, CD_3Cl)

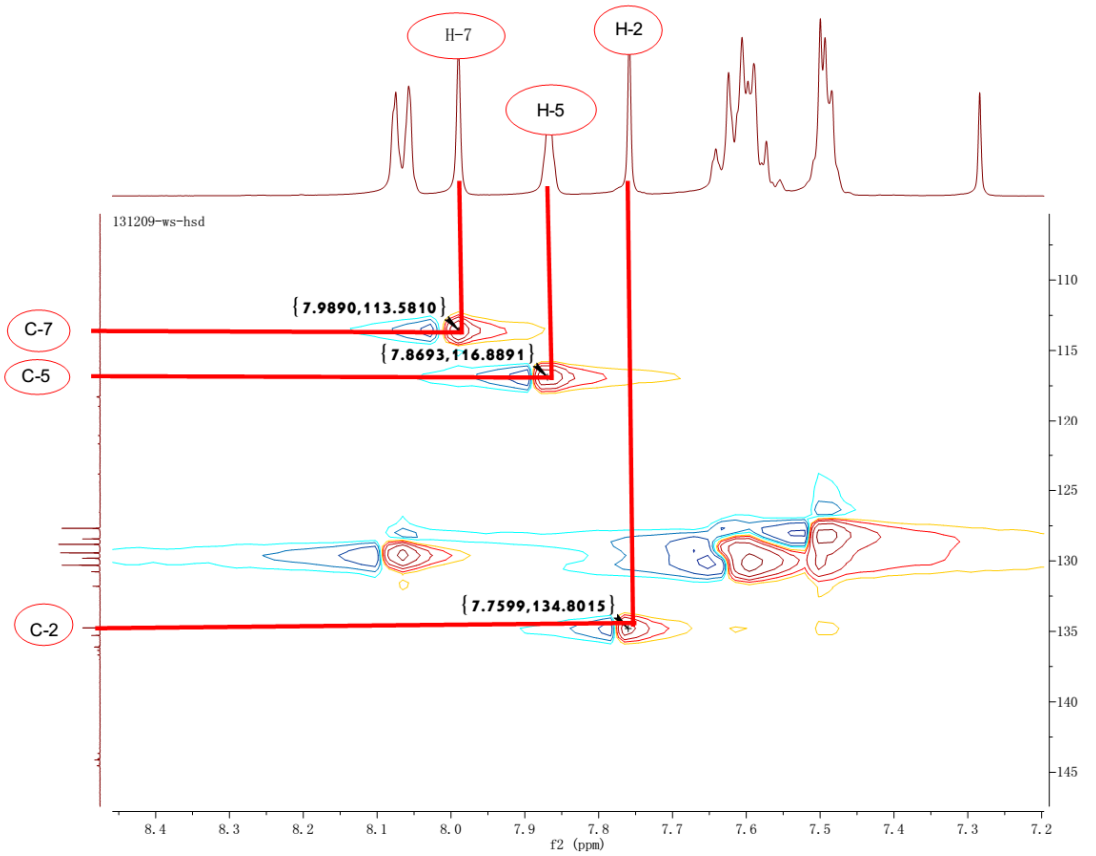


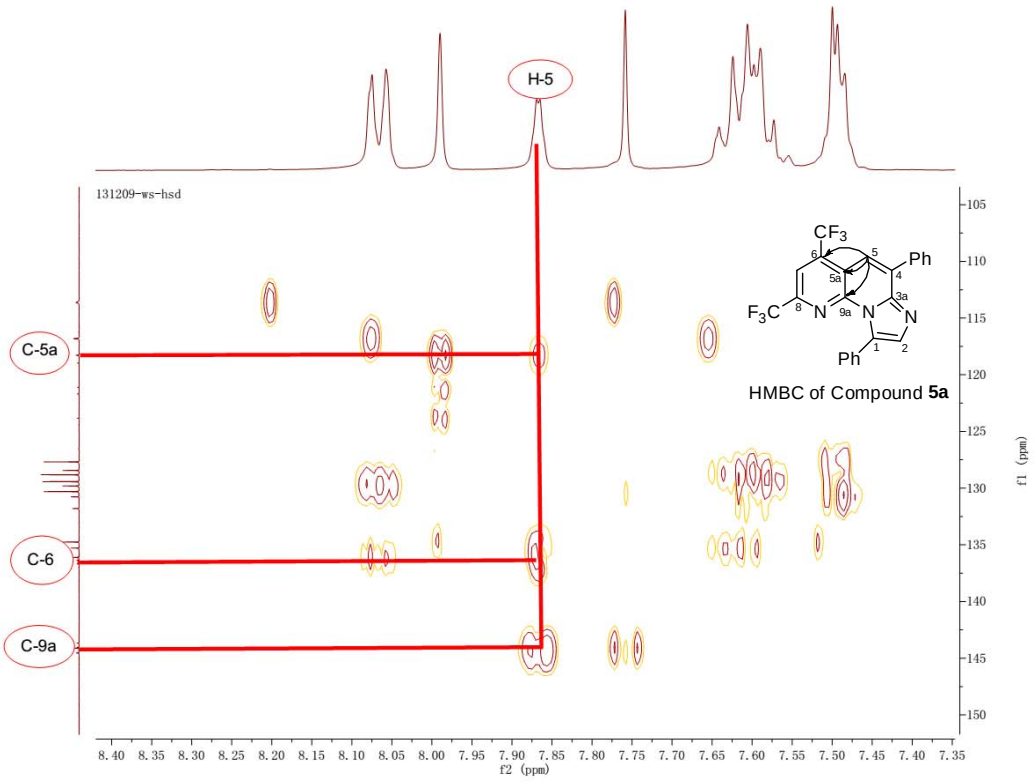
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 3l (400Hz, CD_3Cl)



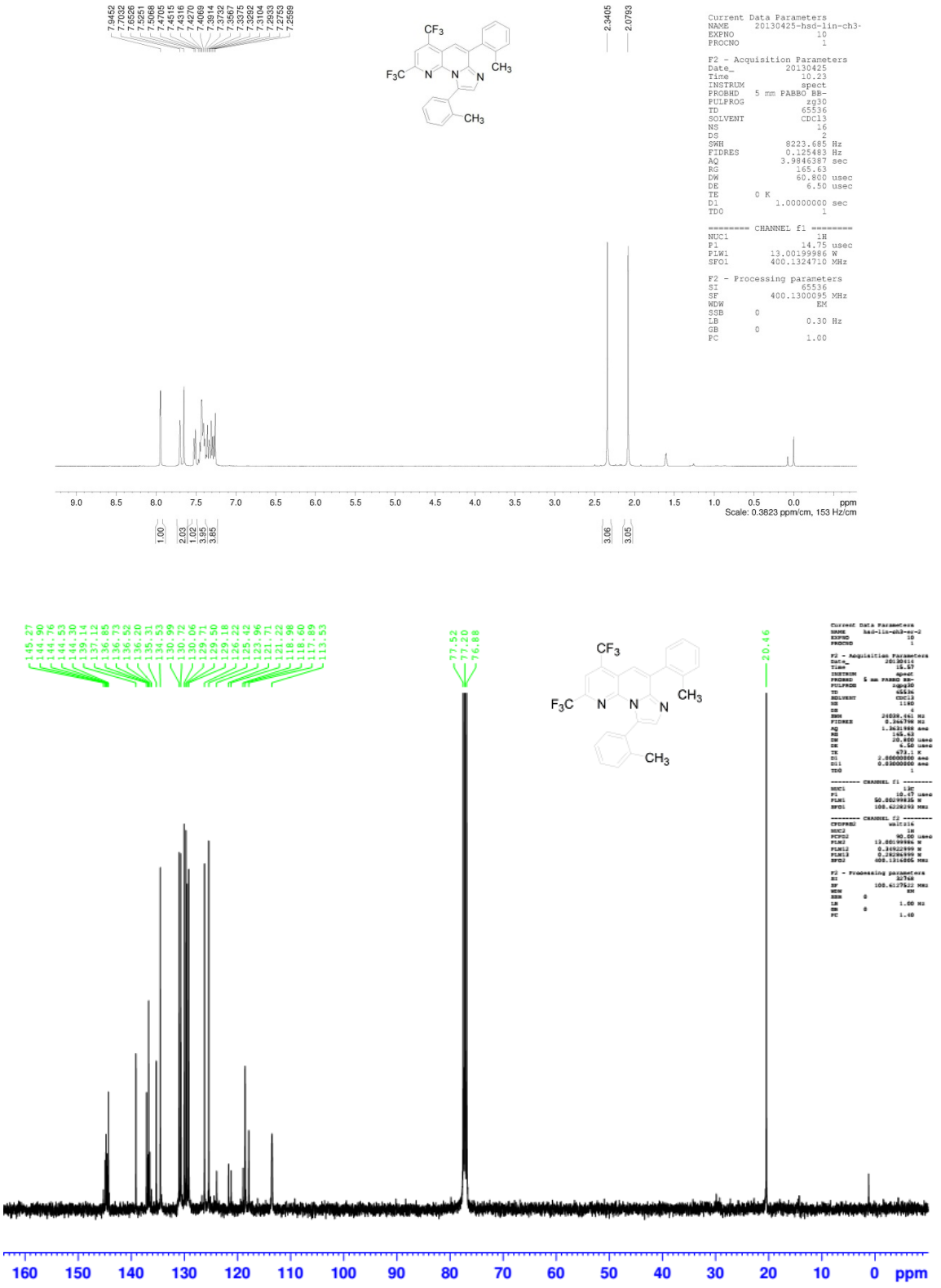


^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5a (400Hz, CD_3Cl)

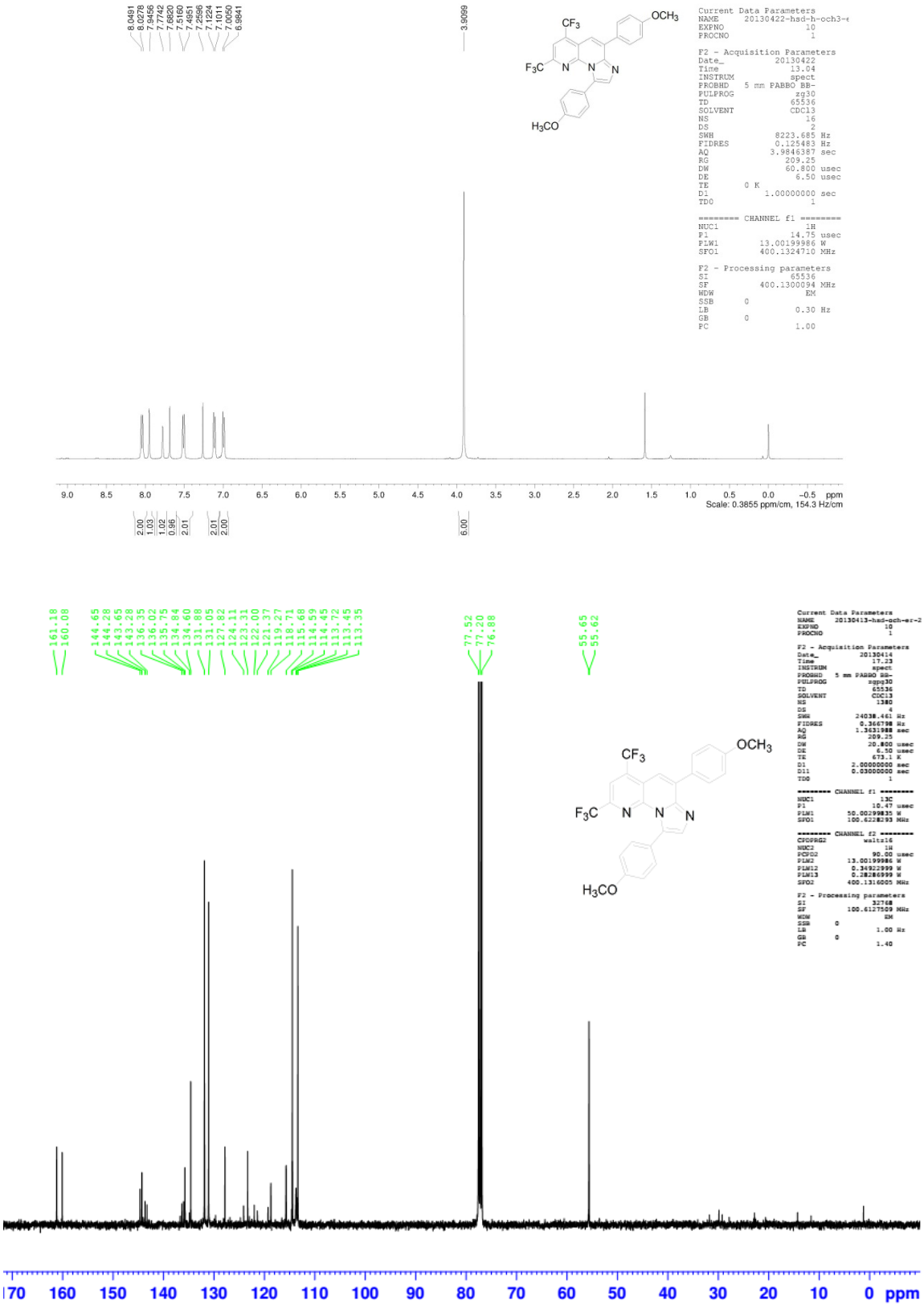




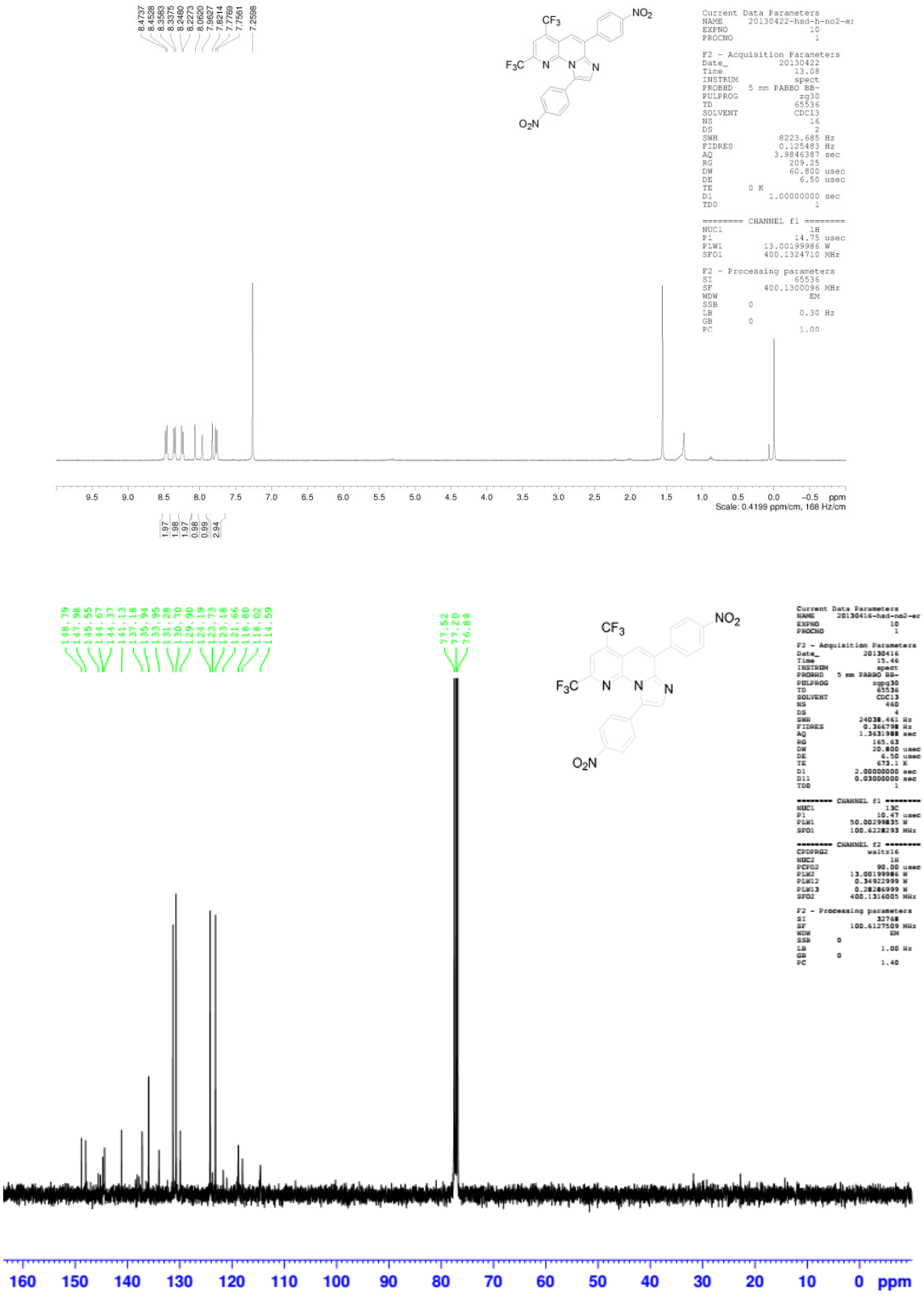
HSQC and HMBC Spectra for Compound 5a (400Hz, CD_3Cl)



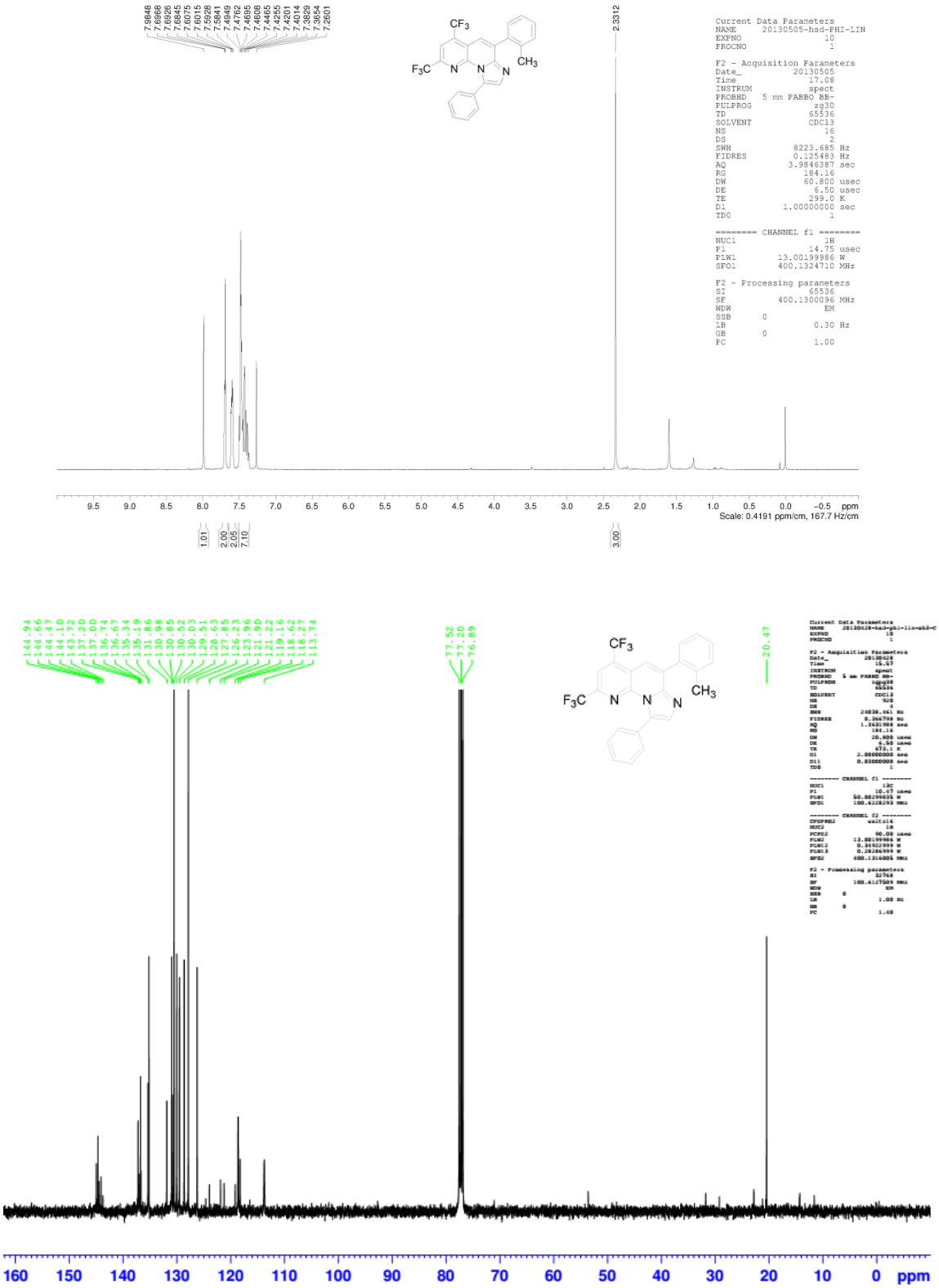
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5b (400Hz, CD_3Cl)



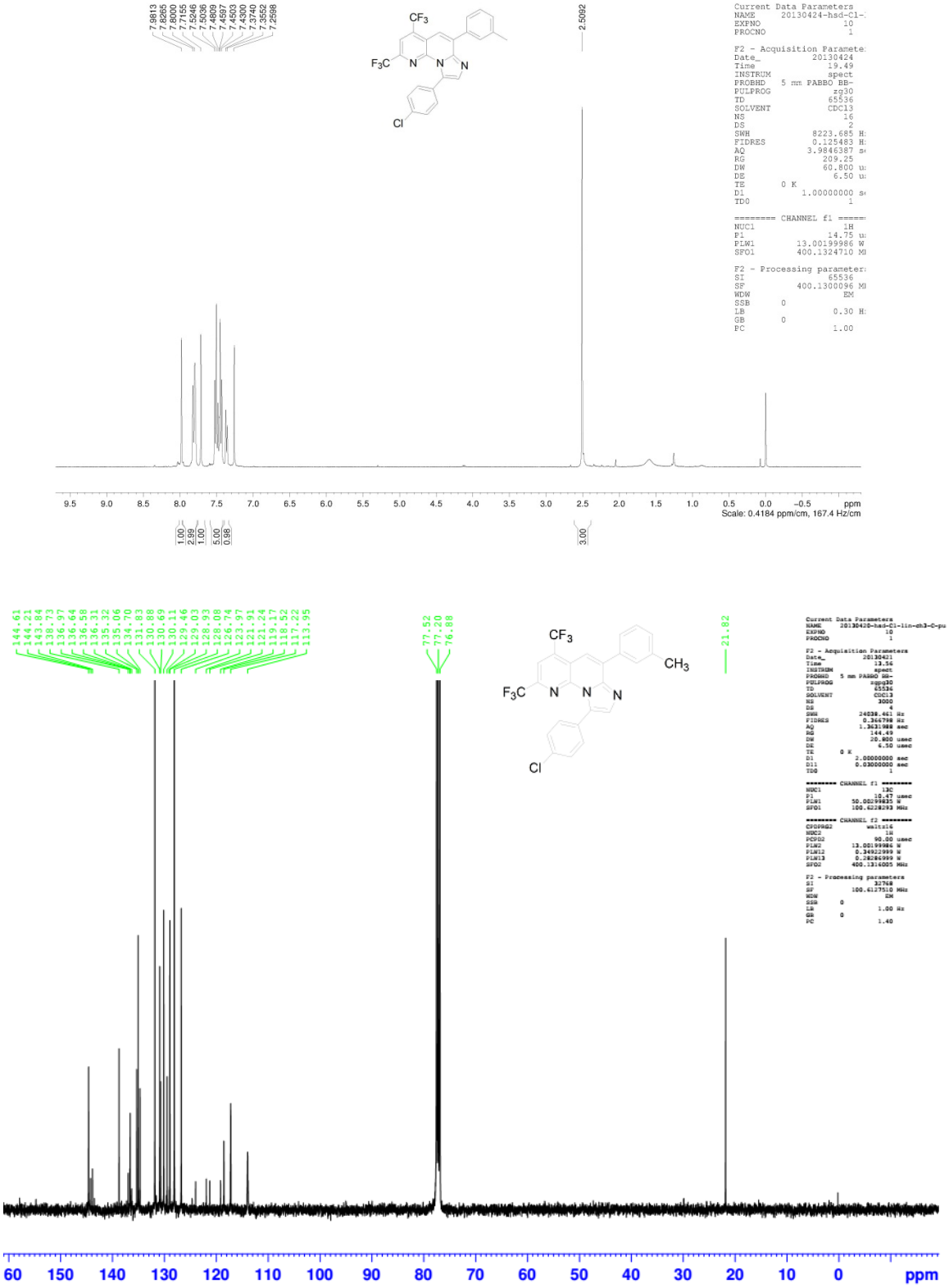
^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5c (400Hz, CD_3Cl)



^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5d (400Hz, CD_3Cl)



^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5e (400Hz, CD_3Cl)



^{1}H-NMR and ^{13}C-NMR Spectra for Compound 5f (400Hz, CD_3Cl)

Part 5:X-ray Crystallographic Studies for Compound 3a

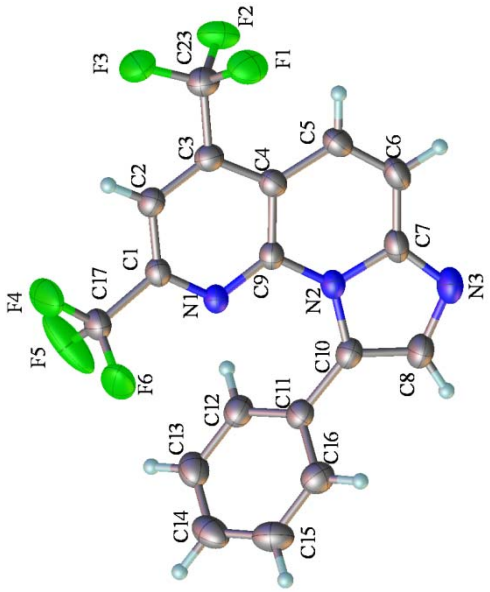


Table 1: Crystal data and structure refinementforCompound **3a**

Identification code	Compound **3a**
Empirical formula	C_{18} H_9 F_6 N_3
Formula weight	381.28
Temperature 173(2) K	
Wavelength 0.71073 A	
Crystal system, space group	Monoclinic, P2 (1)/c
Unit cell dimensionsa = 14.868(4) Aalpha = 90 deg.	
b = 9.506(3) Abeta = 111.598(3) deg.	
c = 11.977(4) A gamma = 90 deg.	
Volume	1574.0(8) A^3
Z, Calculated density	4, 1.609 Mg/m^3
Absorption coefficient	0.148 mm^-1
F (000) 768	
Crystal size	0.43 x 0.35 x 0.19 mm
Theta range for data collection	2.60 to 27.49 deg.
Limiting indices	-18<=h<=19, -12<=k<=11, -15<=l<=9

Reflections collected / unique	10544 / 3570 [R (int) = 0.0365]
Completeness to theta = 27.49	98.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.0000 and 0.6469
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3570 / 36 / 272
Goodness-of-fit on F^2	1.140
Final R indices [I>2sigma (I)]	R1 = 0.0554, wR2 = 0.1412
R indices (all data)	R1 = 0.0627, wR2 = 0.1472
Largest diff. peak and hole	0.298 and -0.254 e.A^-3

Table 2: Atomic coordinates (x 10^4) and equivalent isotropicdisplacementparameters (A^2 x 10^3) for Compound **3**.U(eq) is defined as one third of the trace of the orthogonalizedUij tensor.

	x	y	z	U (eq)
F(1)	7915(1)	7784(1)	6802(1)	53(1)
F(2)	8757(1)	8696(1)	5873(1)	53(1)
F(3)	7251(1)	9173(1)	5310(1)	59(1)
F(4)	5042(2)	6726(3)	2079(3)	77(1)
F(4A)	4924(4)	5736(14)	2298(5)	100(5)
F(5)	5760(2)	5812(7)	1050(2)	97(2)
F(5A)	5575(5)	6800(7)	1315(7)	83(3)
F(6)	5161(2)	4553(3)	2044(3)	78(2)
F(6A)	5592(4)	4619(6)	1326(6)	78(3)
N(1)	7161(1)	4667(1)	3260(1)	30(1)
N(2)	8568(1)	3470(2)	4379(1)	30(1)
N(3)	9772(1)	2027(2)	5463(2)	46(1)
C(1)	6587(1)	5775(2)	3117(2)	31(1)
C(2)	6798(1)	6927(2)	3881(2)	33(1)
C(3)	7654(1)	6904(2)	4863(2)	33(1)

C(4)	8282(1)	5743(2)	5088(2)	31(1)
C(5)	9156(1)	5574(2)	6135(2)	38(1)
C(6)	9669(1)	4376(2)	6288(2)	41(1)
C(7)	9382(1)	3281(2)	5429(2)	36(1)
C(8)	9199(1)	1388(2)	4414(2)	43(1)
C(9)	7983(1)	4657(2)	4219(1)	28(1)
C(10)	8454(1)	2221(2)	3724(2)	34(1)
C(11)	7698(1)	1892(2)	2551(2)	33(1)
C(12)	7535(1)	2721(2)	1536(2)	38(1)
C(13)	6818(2)	2375(2)	453(2)	45(1)
C(14)	6267(2)	1183(2)	360(2)	48(1)
C(15)	6444(2)	321(2)	1347(2)	51(1)
C(16)	7156(2)	675(2)	2441(2)	43(1)
C(17)	5649(1)	5708(2)	2042(2)	38(1)
C (23)	7893 (1)	8137 (2)	5708 (2)	40 (1)

Table 3: Bond lengths [A] and angles [deg] for Compound **3a**.

F(1)-C(23)	1.341(2)
F(2)-C(23)	1.337(2)
F(3)-C(23)	1.331(2)
F(4)-C(17)	1.335(3)
F(4A)-C(17)	1.225(5)
F(5)-C(17)	1.261(3)
F(5A)-C(17)	1.333(5)
F(6)-C(17)	1.317(3)
F(6A)-C(17)	1.327(5)
N(1)-C(1)	1.326(2)
N(1)-C(9)	1.333(2)
N(2)-C(9)	1.394(2)
N(2)-C(10)	1.399(2)

N(2)-C(7)	1.400(2)
N(3)-C(7)	1.320(2)
N(3)-C(8)	1.373(3)
C(1)-C(2)	1.387(2)
C(1)-C(17)	1.512(2)
C(2)-C(3)	1.379(2)
C(2)-H(2)	0.9500
C(3)-C(4)	1.407(2)
C(3)-C(23)	1.503(2)
C(4)-C(9)	1.416(2)
C(4)-C(5)	1.444(2)
C(5)-C(6)	1.345(3)
C(5)-H(5)	0.9500
C(6)-C(7)	1.414(3)
C(6)-H(6)	0.9500
C(8)-C(10)	1.365(2)
C(8)-H(8)	0.9500
C(10)-C(11)	1.474(2)
C(11)-C(16)	1.388(3)
C(11)-C(12)	1.393(2)
C(12)-C(13)	1.382(3)
C(12)-H(12)	0.9500
C(13)-C(14)	1.378(3)
C(13)-H(13)	0.9500
C(14)-C(15)	1.382(3)
C(14)-H(14)	0.9500
C(15)-C(16)	1.389(3)
C(15)-H(15)	0.9500
C(16)-H(16)	0.9500

C(1)-N(1)-C(9)	117.32(14)
C(9)-N(2)-C(10)	132.01(14)
C(9)-N(2)-C(7)	121.14(14)
C(10)-N(2)-C(7)	106.34(14)
C(7)-N(3)-C(8)	104.72(15)
N(1)-C(1)-C(2)	124.27(15)
N(1)-C(1)-C(17)	115.16(14)
C(2)-C(1)-C(17)	120.57(15)
C(3)-C(2)-C(1)	117.65(15)
C(3)-C(2)-H(2)	121.2
C(1)-C(2)-H(2)	121.2
C(2)-C(3)-C(4)	120.99(15)
C(2)-C(3)-C(23)	118.51(16)
C(4)-C(3)-C(23)	120.49(16)
C(3)-C(4)-C(9)	115.10(15)
C(3)-C(4)-C(5)	125.09(16)
C(9)-C(4)-C(5)	119.76(16)
C(6)-C(5)-C(4)	119.72(17)
C(6)-C(5)-H(5)	120.1
C(4)-C(5)-H(5)	120.1
C(5)-C(6)-C(7)	121.34(17)
C(5)-C(6)-H(6)	119.3
C(7)-C(6)-H(6)	119.3
N(3)-C(7)-N(2)	111.60(16)
N(3)-C(7)-C(6)	129.15(17)
N(2)-C(7)-C(6)	119.24(16)
C(10)-C(8)-N(3)	112.93(17)
C(10)-C(8)-H(8)	123.5

N(3)-C(8)-H(8)	123.5
N(1)-C(9)-N(2)	116.87(14)
N(1)-C(9)-C(4)	124.63(15)
N(2)-C(9)-C(4)	118.49(15)
C(8)-C(10)-N(2)	104.41(16)
C(8)-C(10)-C(11)	128.24(17)
N(2)-C(10)-C(11)	127.35(14)
C(16)-C(11)-C(12)	118.70(17)
C(16)-C(11)-C(10)	118.68(16)
C(12)-C(11)-C(10)	122.56(16)
C(13)-C(12)-C(11)	120.73(18)
C(13)-C(12)-H(12)	119.6
C(11)-C(12)-H(12)	119.6
C(14)-C(13)-C(12)	120.10(19)
C(14)-C(13)-H(13)	119.9
C(12)-C(13)-H(13)	119.9
C(13)-C(14)-C(15)	119.88(19)
C(13)-C(14)-H(14)	120.1
C(15)-C(14)-H(14)	120.1
C(14)-C(15)-C(16)	120.1(2)
C(14)-C(15)-H(15)	119.9
C(16)-C(15)-H(15)	119.9
C(11)-C(16)-C(15)	120.39(19)
C(11)-C(16)-H(16)	119.8
C(15)-C(16)-H(16)	119.8
F(4A)-C(17)-F(5)	131.7(3)
F(4A)-C(17)-F(6)	58.7(6)
F(5)-C(17)-F(6)	109.3(3)
F(4A)-C(17)-F(6A)	109.0(6)

F(5)-C(17)-F(6A)	56.8(4)
F(6)-C(17)-F(6A)	56.4(4)
F(4A)-C(17)-F(5A)	106.1(6)
F(5)-C(17)-F(5A)	48.1(4)
F(6)-C(17)-F(5A)	137.5(3)
F(6A)-C(17)-F(5A)	102.4(5)
F(4A)-C(17)-F(4)	46.2(5)
F(5)-C(17)-F(4)	107.4(3)
F(6)-C(17)-F(4)	103.0(3)
F(6A)-C(17)-F(4)	134.7(3)
F(5A)-C(17)-F(4)	64.1(4)
F(4A)-C(17)-C(1)	114.0(3)
F(5)-C(17)-C(1)	113.55(19)
F(6)-C(17)-C(1)	111.69(17)
F(6A)-C(17)-C(1)	113.7(2)
F(5A)-C(17)-C(1)	110.6(3)
F(4)-C(17)-C(1)	111.41(18)
F(3)-C(23)-F(2)	106.34(16)
F(3)-C(23)-F(1)	107.02(16)
F(2)-C(23)-F(1)	106.16(15)
F(3)-C(23)-C(3)	112.02(15)
F(2)-C(23)-C(3)	112.61(16)
F(1)-C(23)-C(3)	112.26(16)

Symmetry transformations used to generate equivalent atoms:

Table 4: Anisotropic displacement parameters (A^2 x 10^3) for Compound **3a**.The anisotropic displacement factor exponent takes the form:-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12].

	U11	U22	U33	U23	U13	U12
F(1)	71(1)	53(1)	39(1)	-10(1)	24(1)	-4(1)
F(2)	56(1)	47(1)	54(1)	-14(1)	17(1)	-19(1)
F(3)	67(1)	38(1)	61(1)	-13(1)	11(1)	10(1)

F(4)	50(2)	67(2)	81(2)	-22(2)	-17(1)	31(1)
F(4A)	30(2)	220(14)	52(3)	2(5)	18(2)	4(4)
F(5)	48(1)	211(7)	26(1)	4(2)	7(1)	-6(2)
F(5A)	77(4)	64(4)	68(4)	32(3)	-23(3)	-6(3)
F(6)	51(2)	54(2)	89(2)	14(2)	-23(2)	-19(1)
F(6A)	61(4)	64(4)	66(4)	-31(3)	-27(3)	22(3)
N(1)	29(1)	29(1)	28(1)	3(1)	7(1)	3(1)
N(2)	28(1)	30(1)	30(1)	3(1)	6(1)	3(1)
N(3)	39(1)	43(1)	46(1)	9(1)	5(1)	11(1)
C(1)	28(1)	31(1)	30(1)	3(1)	8(1)	2(1)
C(2)	32(1)	29(1)	37(1)	1(1)	11(1)	3(1)
C(3)	36(1)	30(1)	33(1)	-2(1)	12(1)	-2(1)
C(4)	30(1)	32(1)	30(1)	0(1)	9(1)	-2(1)
C(5)	37(1)	40(1)	31(1)	-1(1)	5(1)	-4(1)
C(6)	34(1)	46(1)	33(1)	5(1)	1(1)	0(1)
C(7)	30(1)	40(1)	35(1)	9(1)	6(1)	5(1)
C(8)	43(1)	36(1)	45(1)	5(1)	10(1)	11(1)
C(9)	27(1)	28(1)	28(1)	4(1)	9(1)	3(1)
C(10)	35(1)	29(1)	37(1)	3(1)	12(1)	5(1)
C(11)	34(1)	30(1)	36(1)	0(1)	12(1)	5(1)
C(12)	44(1)	33(1)	38(1)	2(1)	15(1)	5(1)
C(13)	50(1)	47(1)	35(1)	-1(1)	12(1)	12(1)
C(14)	40(1)	60(1)	41(1)	-17(1)	10(1)	5(1)
C(15)	49(1)	47(1)	57(1)	-14(1)	22(1)	-12(1)
C(16)	47(1)	37(1)	45(1)	0(1)	18(1)	-2(1)
C(17)	34(1)	37(1)	37(1)	1(1)	5(1)	6(1)
C (23)	44(1)	36(1)	39(1)	-4(1)	13(1)	-2(1)

Table 5: Hydrogen coordinates (x 10^4) and isotropicdisplacement parameters (A^2 x 10^3) for Compound **3a**.

	x	y	z	U (eq)
H (2)	6369	7704	3732	40
H(5)	9367	6307	6713	46
H(6)	10235	4264	6987	49
H(8)	9310	464	4192	52
H(12)	7922	3532	1589	46
H(13)	6705	2959	-229	54
H(14)	5767	954	-382	58
H(15)	6077	-515	1277	61
H (16)	7273	81	3117	51

Table 6: Torsionangles [deg] for Compound **3a**.

C(9)-N(1)-C(1)-C(2)	1.6(2)
C(9)-N(1)-C(1)-C(17)	-177.80(15)
N(1)-C(1)-C(2)-C(3)	-1.1(3)
C(17)-C(1)-C(2)-C(3)	178.27(16)
C(1)-C(2)-C(3)-C(4)	-0.9(3)
C(1)-C(2)-C(3)-C(23)	-179.42(16)
C(2)-C(3)-C(4)-C(9)	2.1(2)
C(23)-C(3)-C(4)-C(9)	-179.37(15)
C(2)-C(3)-C(4)-C(5)	-175.14(17)
C(23)-C(3)-C(4)-C(5)	3.4(3)
C(3)-C(4)-C(5)-C(6)	175.94(18)
C(9)-C(4)-C(5)-C(6)	-1.2(3)
C(4)-C(5)-C(6)-C(7)	1.4(3)
C(8)-N(3)-C(7)-N(2)	0.0(2)
C(8)-N(3)-C(7)-C(6)	179.0(2)
C(9)-N(2)-C(7)-N(3)	172.84(16)
C(10)-N(2)-C(7)-N(3)	0.2(2)
C(9)-N(2)-C(7)-C(6)	-6.3(3)
C(10)-N(2)-C(7)-C(6)	-178.96(17)
C(5)-C(6)-C(7)-N(3)	-176.7(2)
C(5)-C(6)-C(7)-N(2)	2.3(3)
C(7)-N(3)-C(8)-C(10)	-0.2(2)
C(1)-N(1)-C(9)-N(2)	178.26(14)
C(1)-N(1)-C(9)-C(4)	-0.1(2)
C(10)-N(2)-C(9)-N(1)	-1.6(3)
C(7)-N(2)-C(9)-N(1)	-172.12(15)
C(10)-N(2)-C(9)-C(4)	176.89(17)
C(7)-N(2)-C(9)-C(4)	6.4(2)
C(3)-C(4)-C(9)-N(1)	-1.7(2)
C(5)-C(4)-C(9)-N(1)	175.76(16)
C(3)-C(4)-C(9)-N(2)	179.96(14)
C(5)-C(4)-C(9)-N(2)	-2.6(2)
N(3)-C(8)-C(10)-N(2)	0.3(2)
N(3)-C(8)-C(10)-C(11)	-179.80(18)
C(9)-N(2)-C(10)-C(8)	-171.82(17)
C(7)-N(2)-C(10)-C(8)	-0.28(19)
C(9)-N(2)-C(10)-C(11)	8.3(3)
C(7)-N(2)-C(10)-C(11)	179.82(17)
C(8)-C(10)-C(11)-C(16)	53.5(3)
N(2)-C(10)-C(11)-C(16)	-126.6(2)
C(8)-C(10)-C(11)-C(12)	-123.4(2)
N(2)-C(10)-C(11)-C(12)	56.5(3)
C(16)-C(11)-C(12)-C(13)	3.0(3)

C(10)-C(11)-C(12)-C(13)	179.95(17)
C(11)-C(12)-C(13)-C(14)	-1.4(3)
C(12)-C(13)-C(14)-C(15)	-1.1(3)
C(13)-C(14)-C(15)-C(16)	1.8(3)
C(12)-C(11)-C(16)-C(15)	-2.2(3)
C(10)-C(11)-C(16)-C(15)	-179.27(18)
C(14)-C(15)-C(16)-C(11)	-0.2(3)
N(1)-C(1)-C(17)-F(4A)	118.6(7)
C(2)-C(1)-C(17)-F(4A)	-60.9(7)
N(1)-C(1)-C(17)-F(5)	-69.8(4)
C(2)-C(1)-C(17)-F(5)	110.8(4)
N(1)-C(1)-C(17)-F(6)	54.3(3)
C(2)-C(1)-C(17)-F(6)	-125.1(3)
N(1)-C(1)-C(17)-F(6A)	-7.3(5)
C(2)-C(1)-C(17)-F(6A)	173.3(5)
N(1)-C(1)-C(17)-F(5A)	-121.9(5)
C(2)-C(1)-C(17)-F(5A)	58.7(6)
N(1)-C(1)-C(17)-F(4)	168.8(3)
C(2)-C(1)-C(17)-F(4)	-10.6(3)
C(2)-C(3)-C(23)-F(3)	-5.7(2)
C(4)-C(3)-C(23)-F(3)	175.73(16)
C(2)-C(3)-C(23)-F(2)	-125.54(18)
C(4)-C(3)-C(23)-F(2)	55.9(2)
C(2)-C(3)-C(23)-F(1)	114.72(19)
C(4)-C(3)-C(23)-F(1)	-63.8(2)

Symmetry transformations used to generate equivalent atoms:

Table 7: Hydrogen bonds for Compound **3a** [A and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)