Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthesis of the pyridinyl analogues of dibenzylideneacetone (pyr-dba) via an improved Claisen-Schmidt condensation, displaying diverse biological activities as curcumin analogues

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yqlong@mail.shcnc.ac.cn

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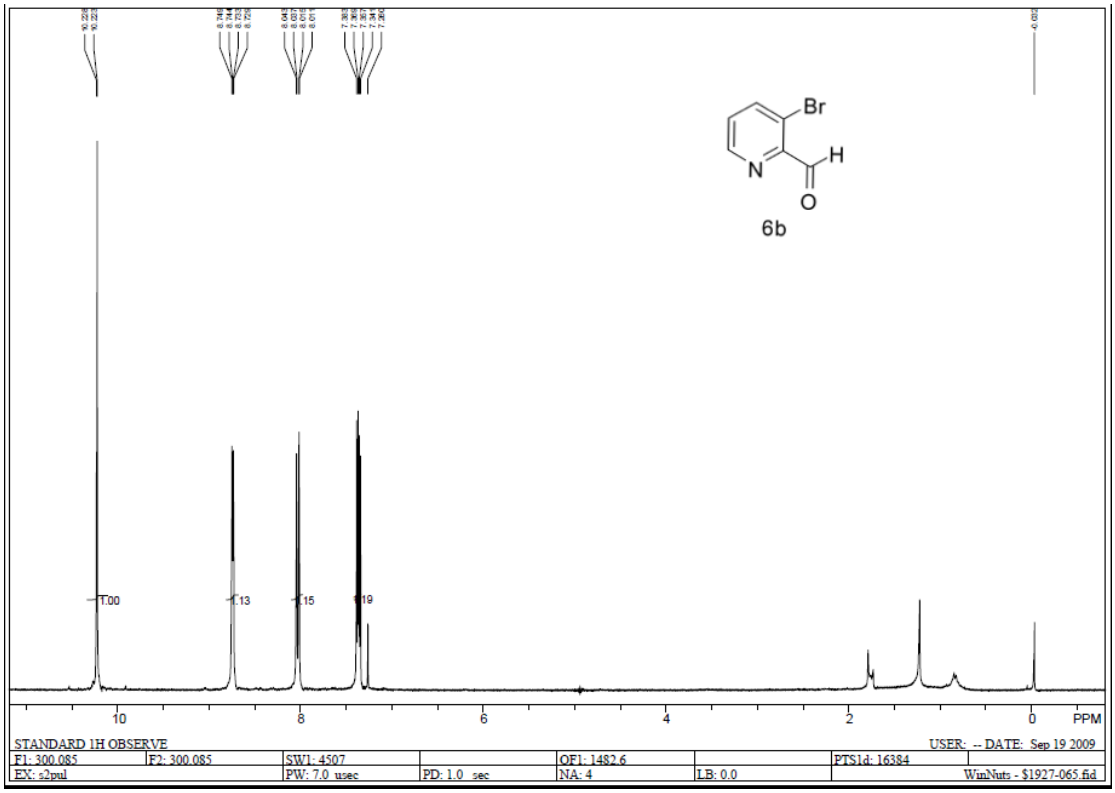
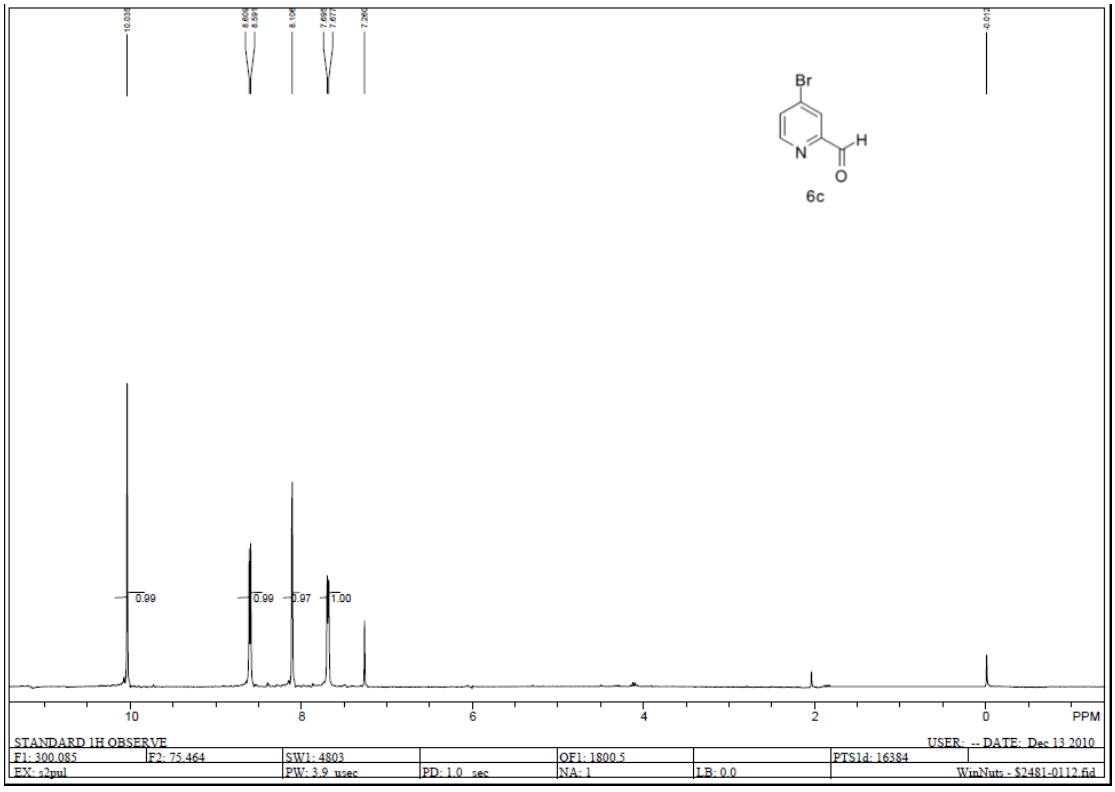
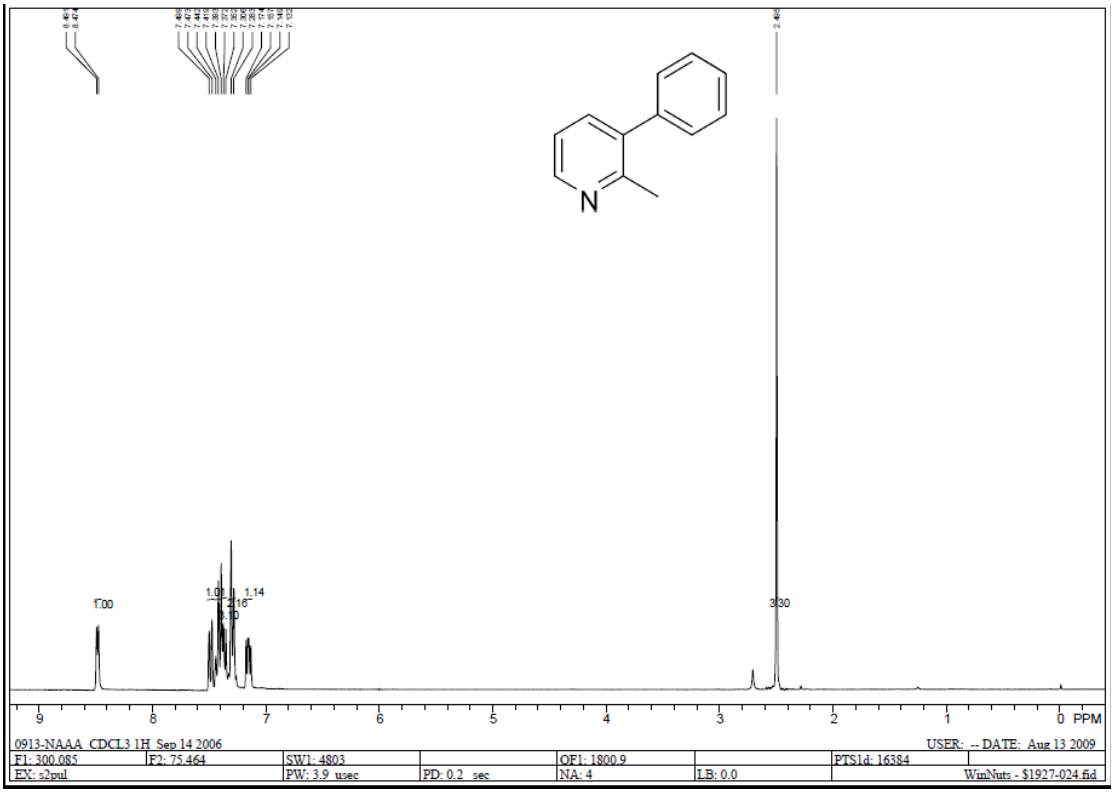
* To whom correspondence should be addressed. Tel: 86-21-50806876; Fax: 86-21-50807088.



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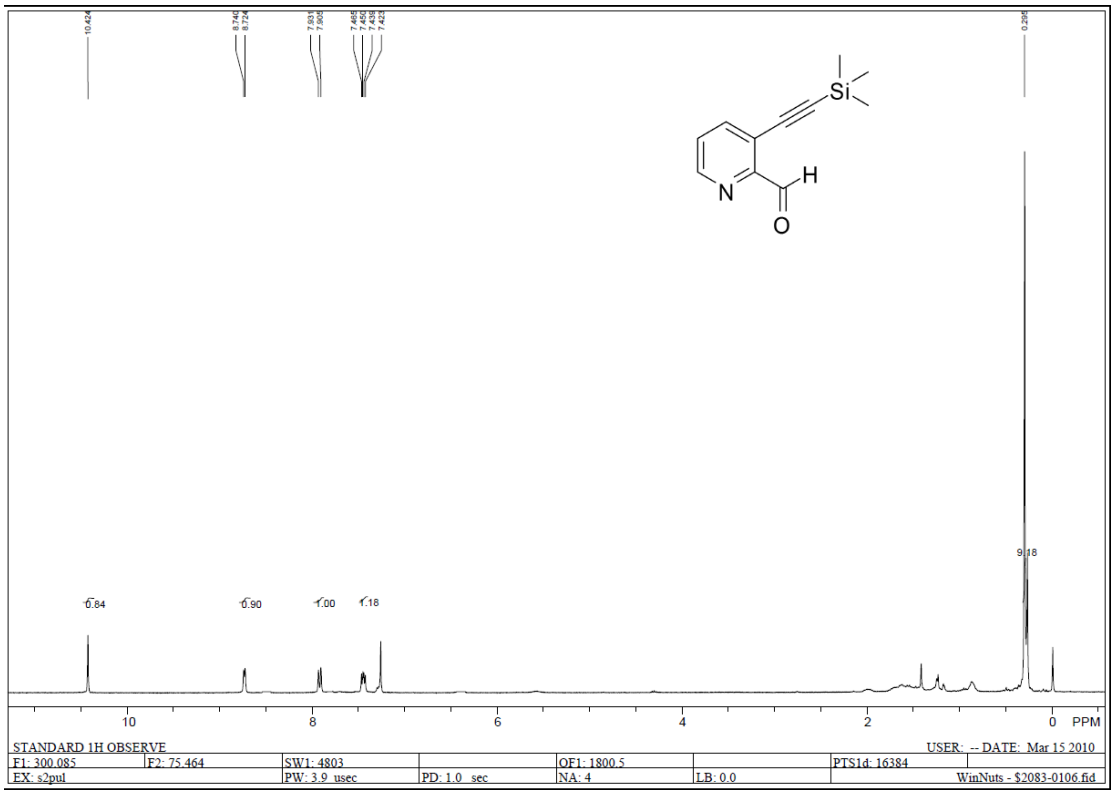
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H
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1.00
1.18
9.18
10
8
6
4
2
0 PPM
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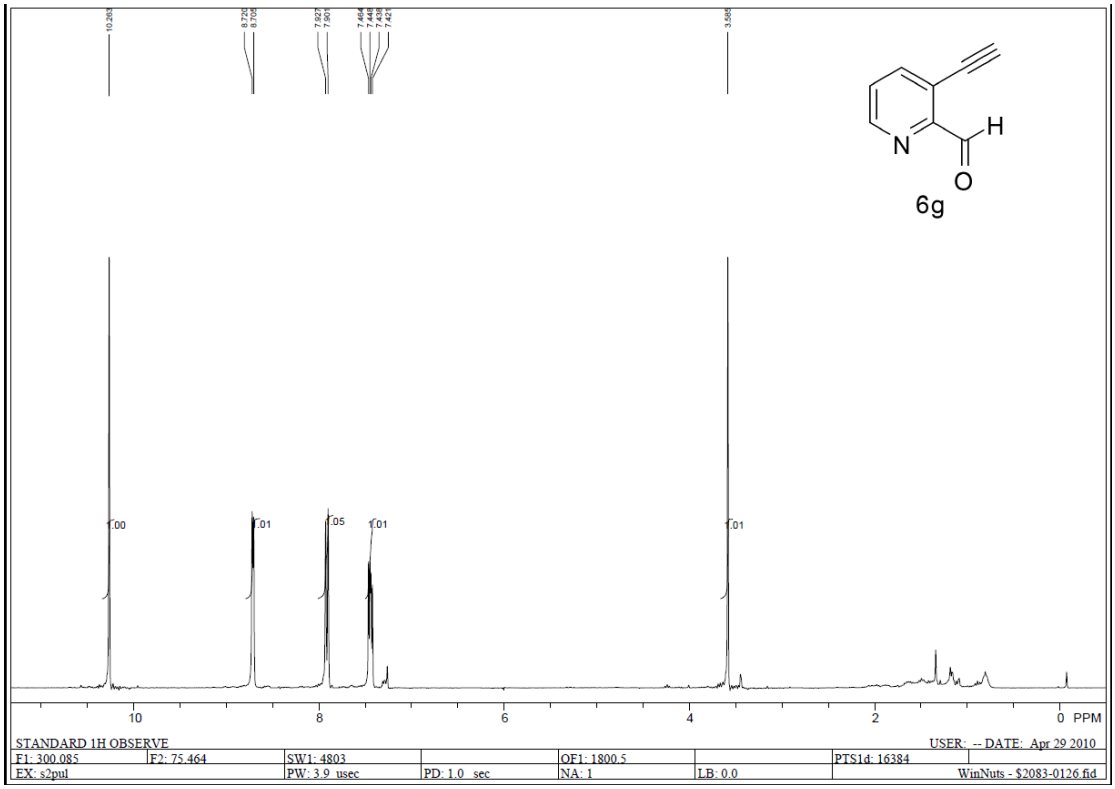

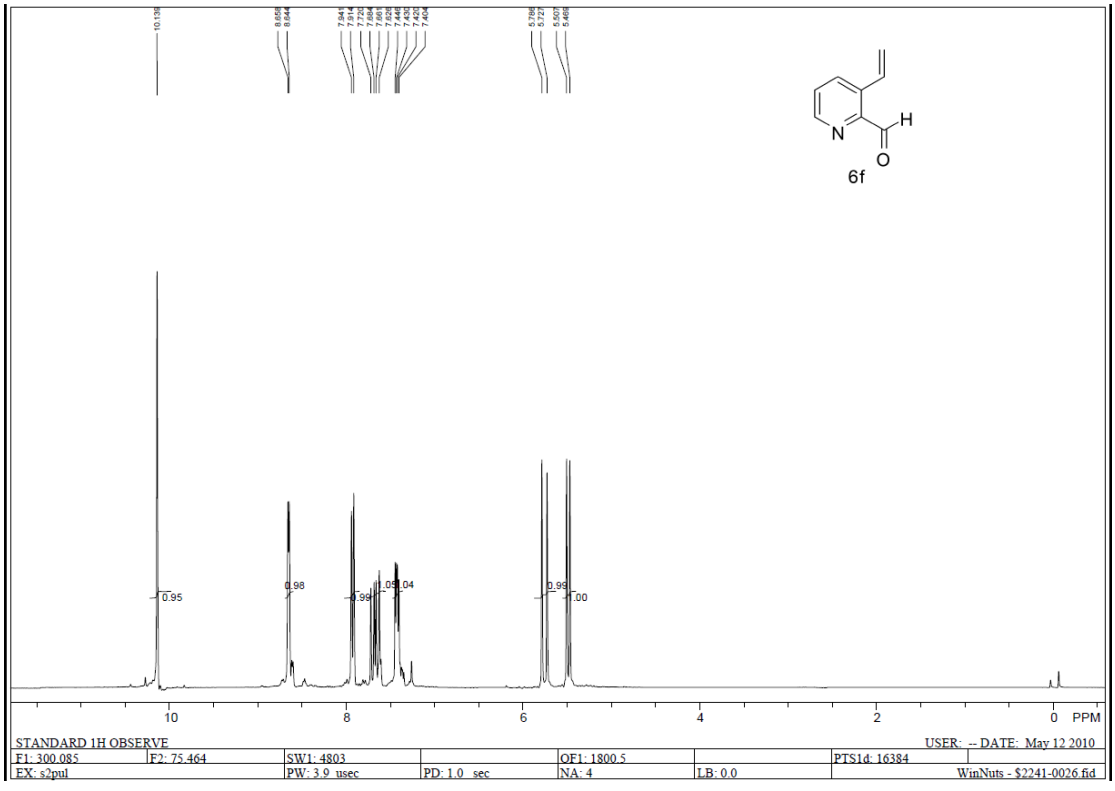

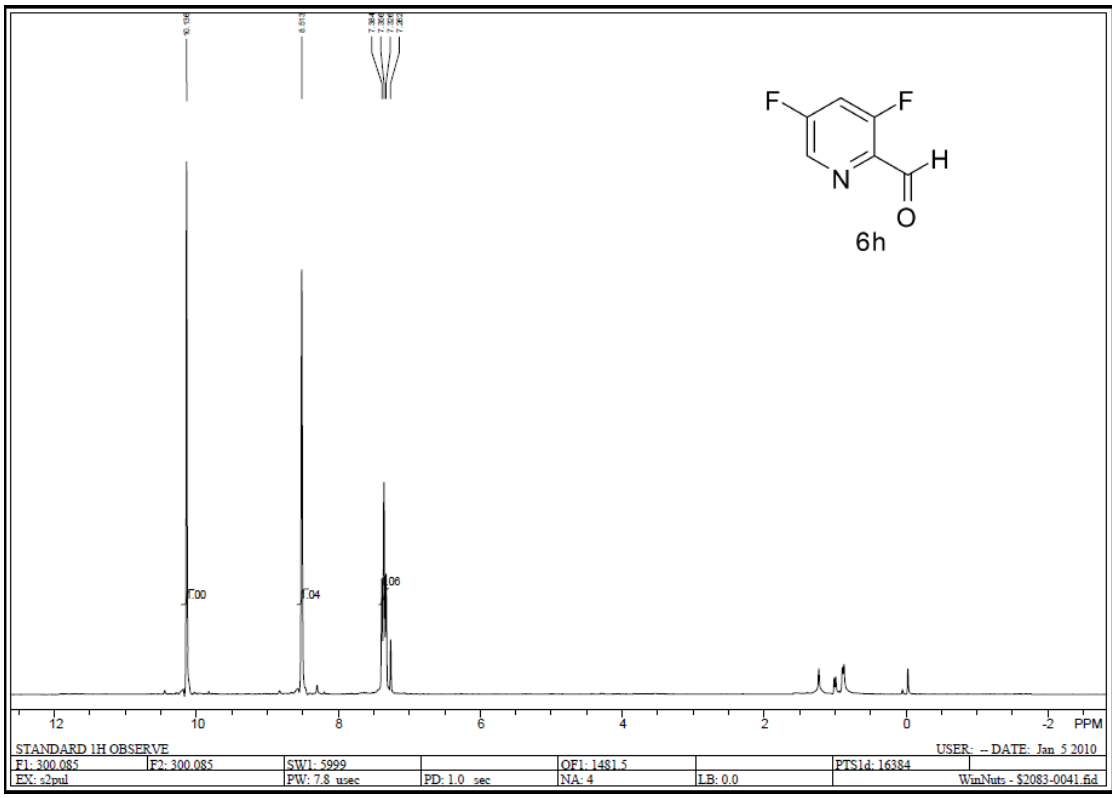

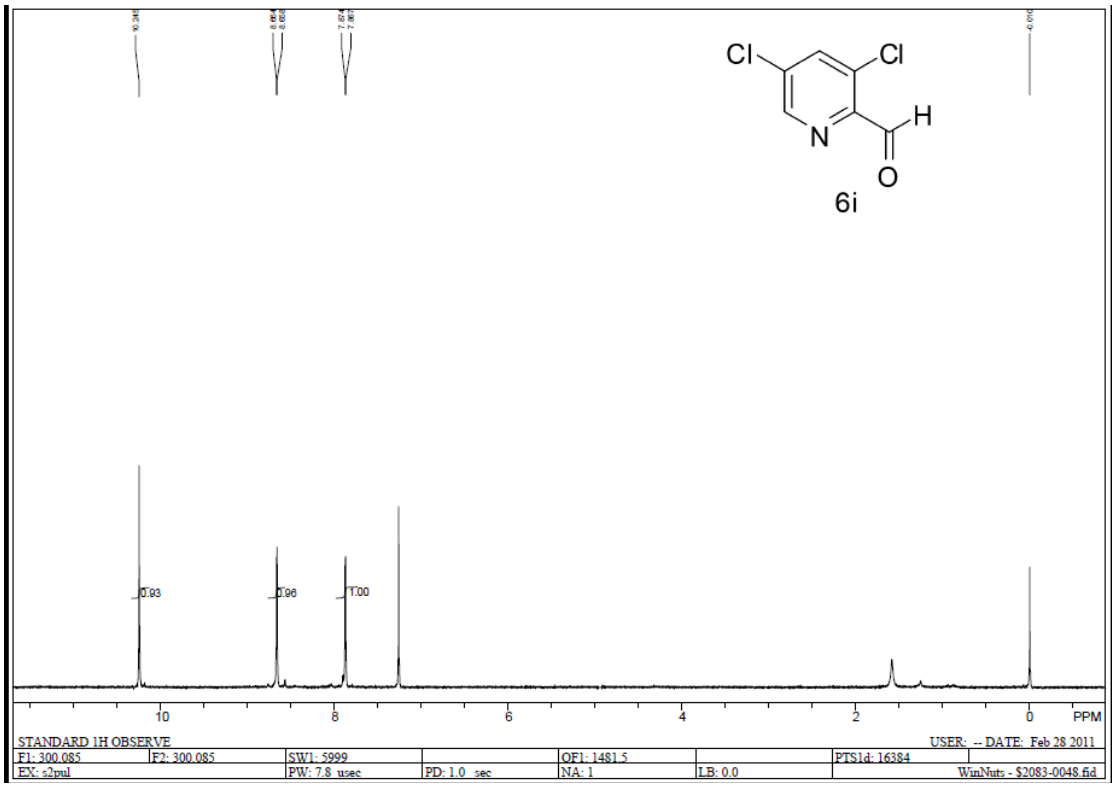

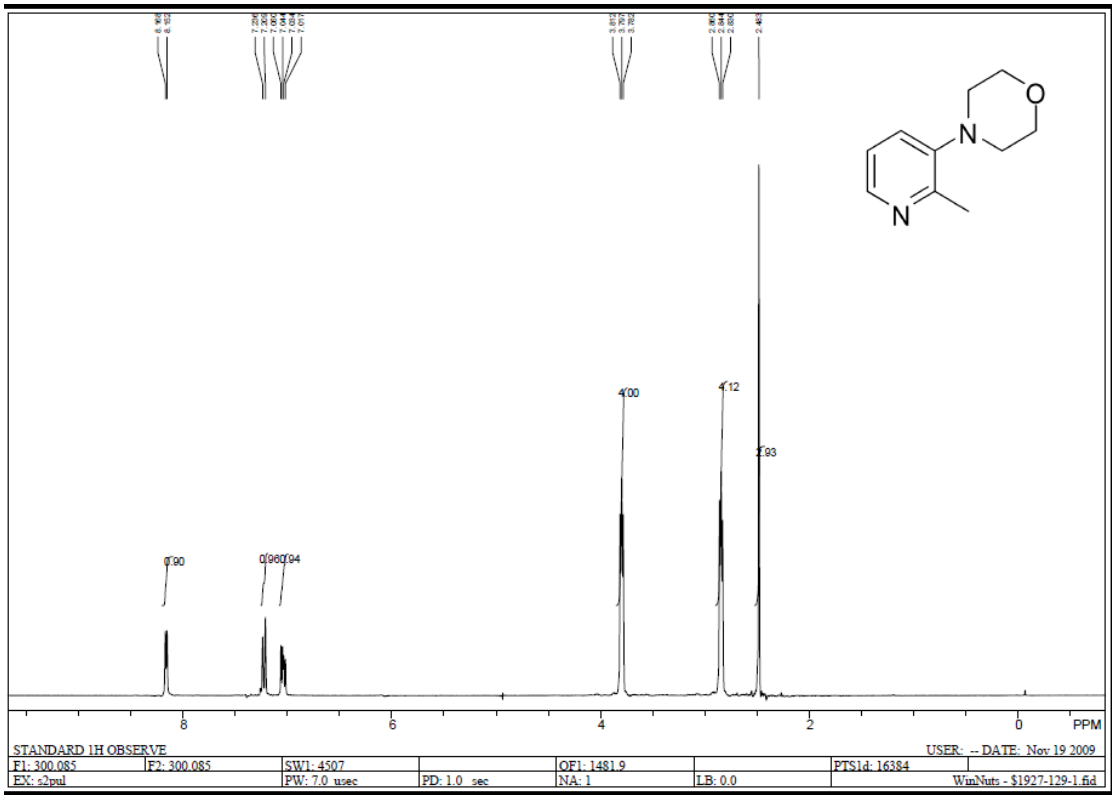

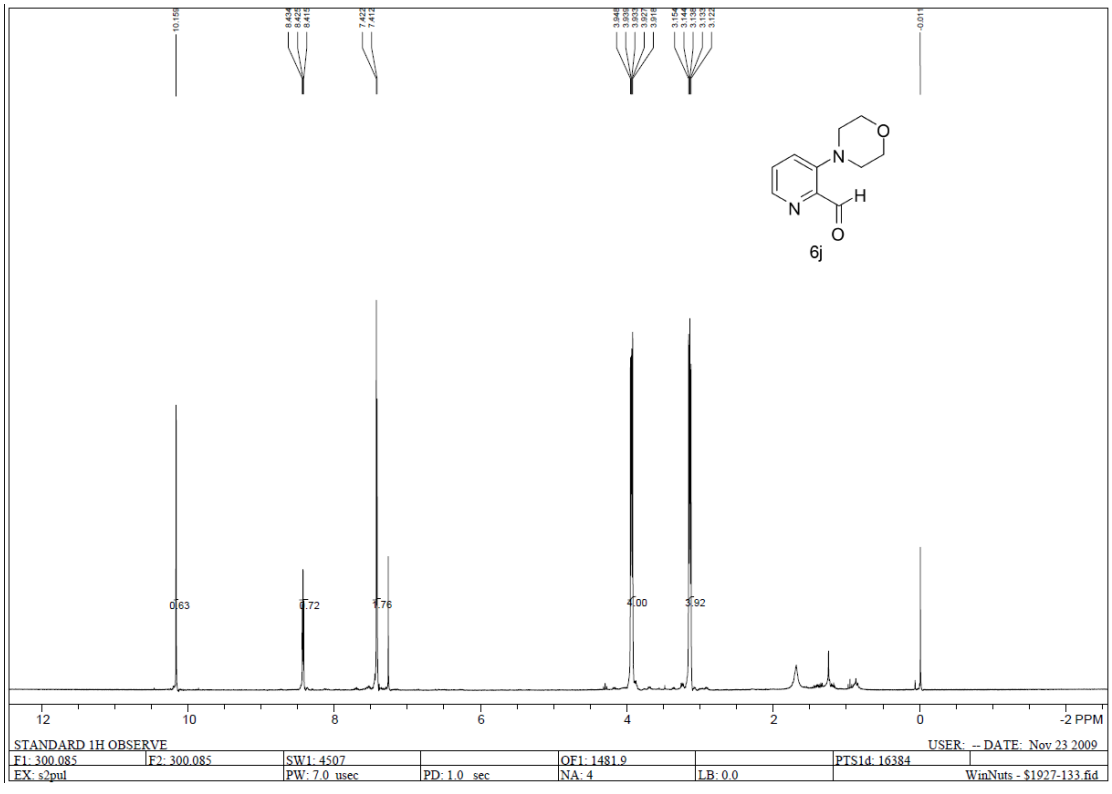

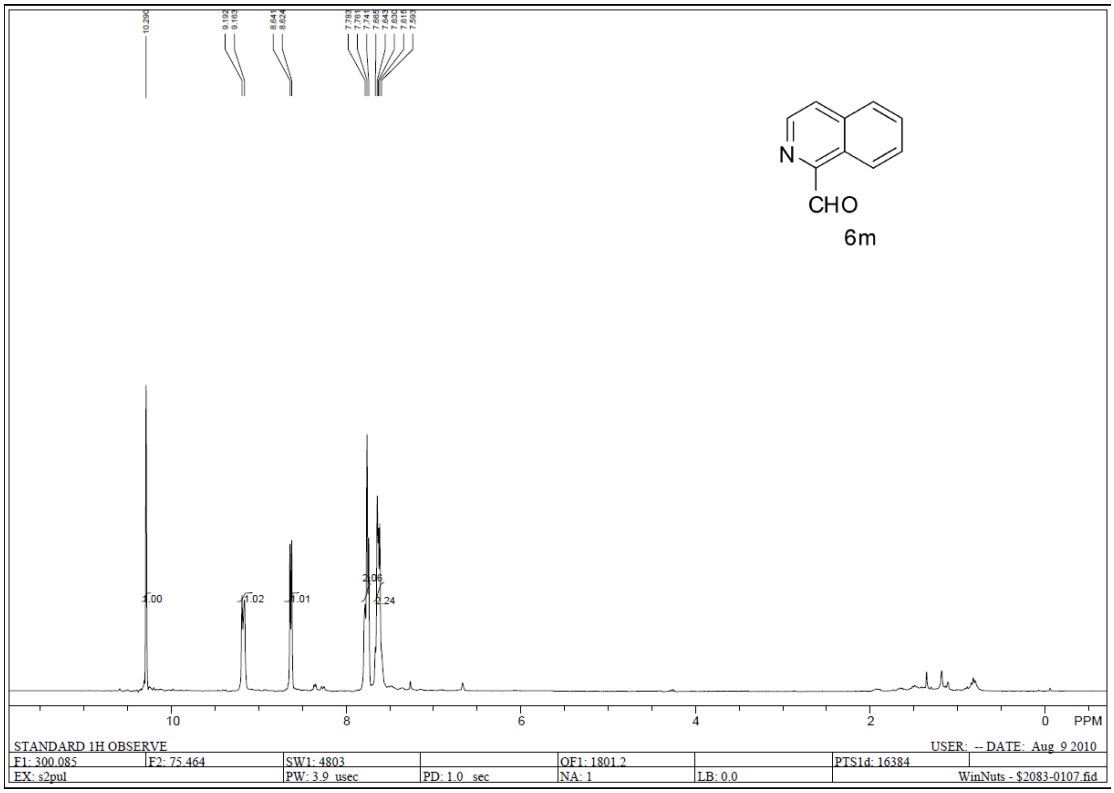

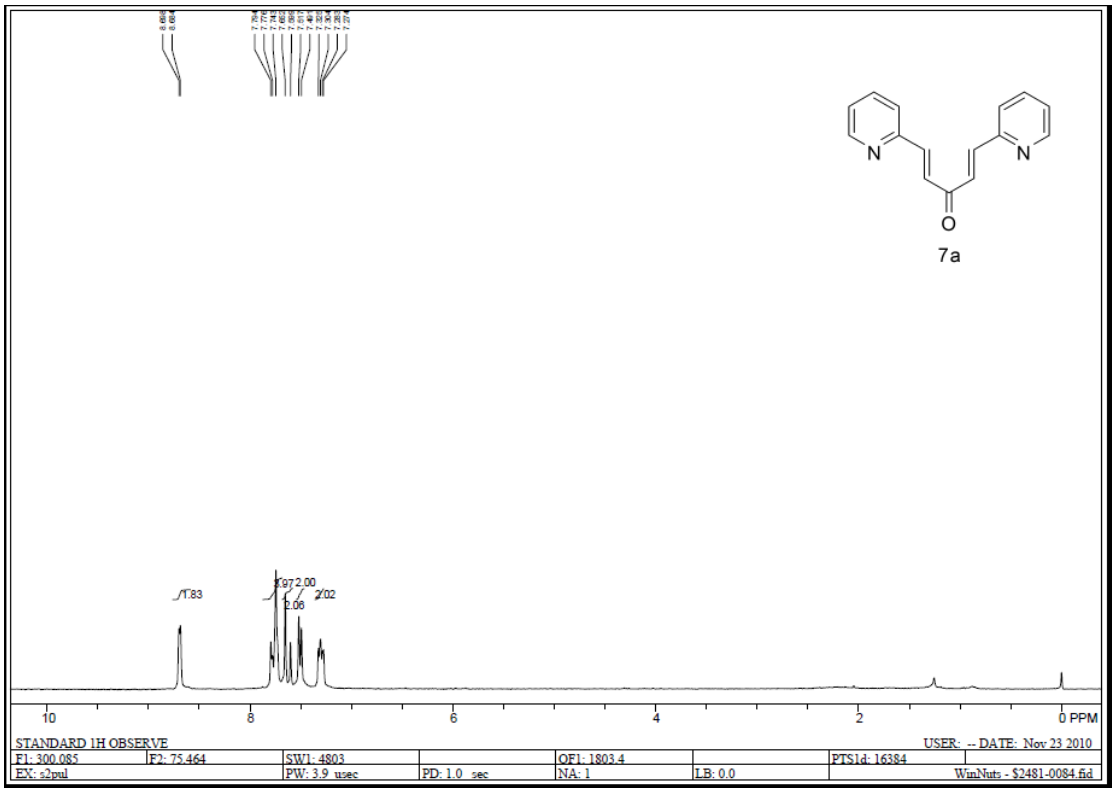

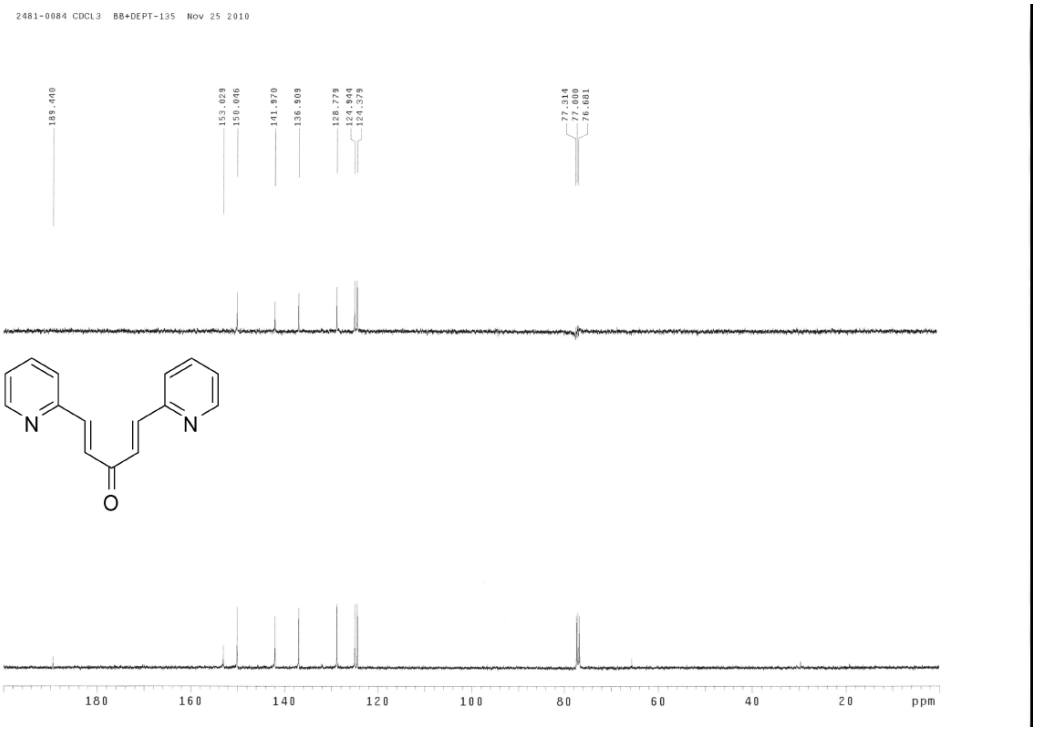

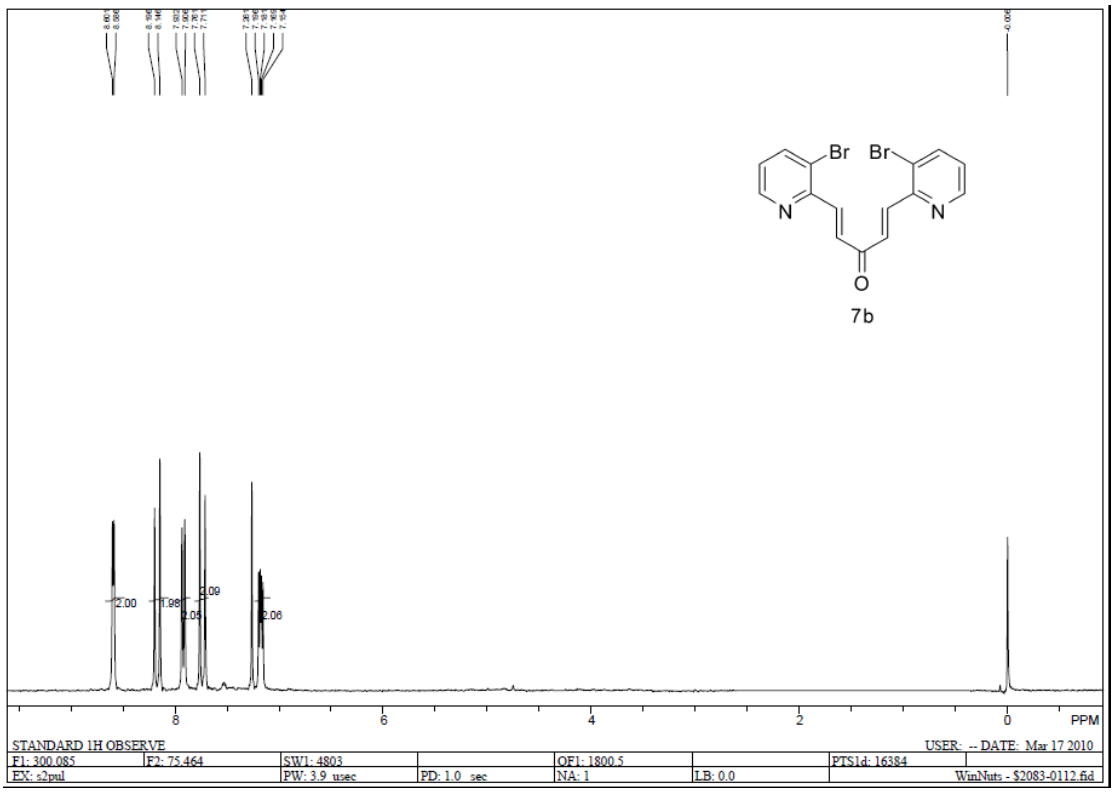

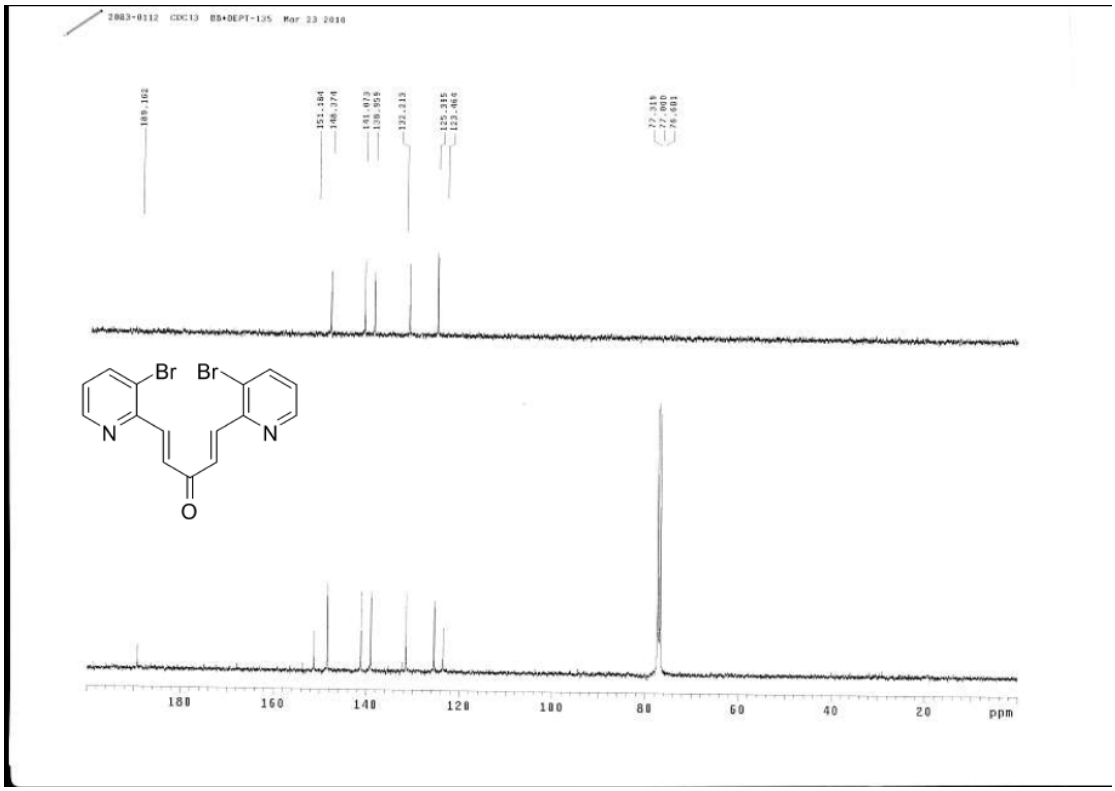

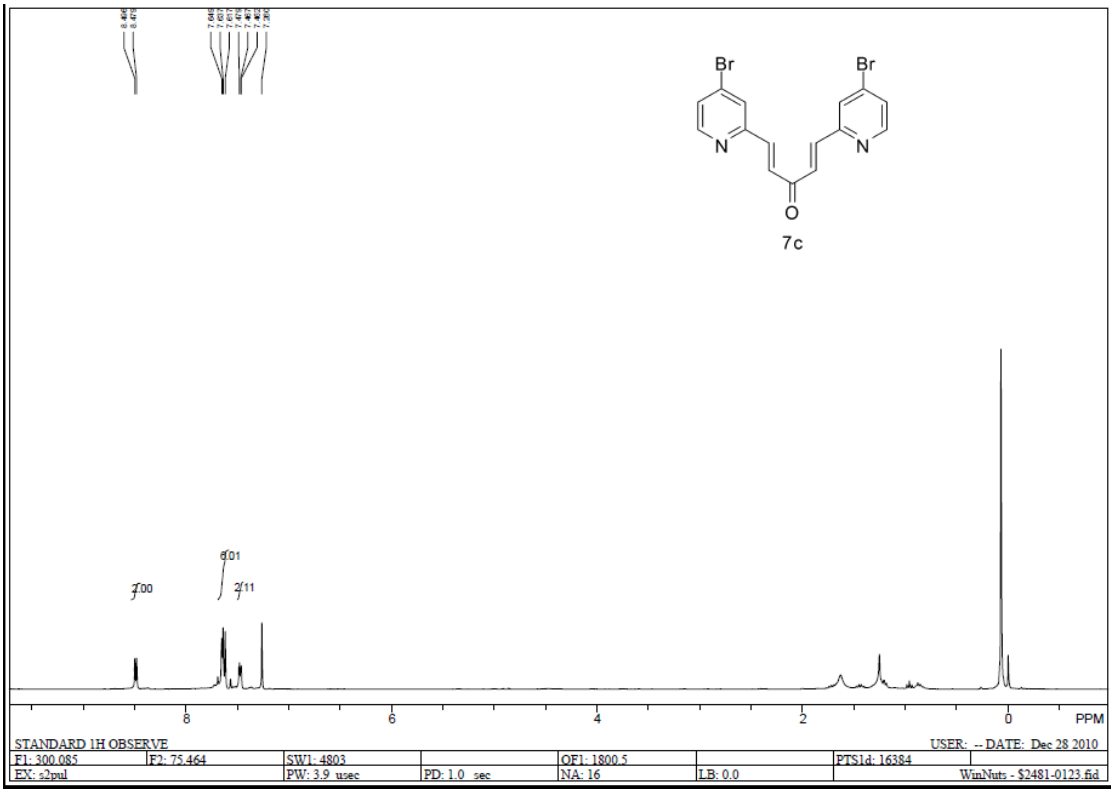
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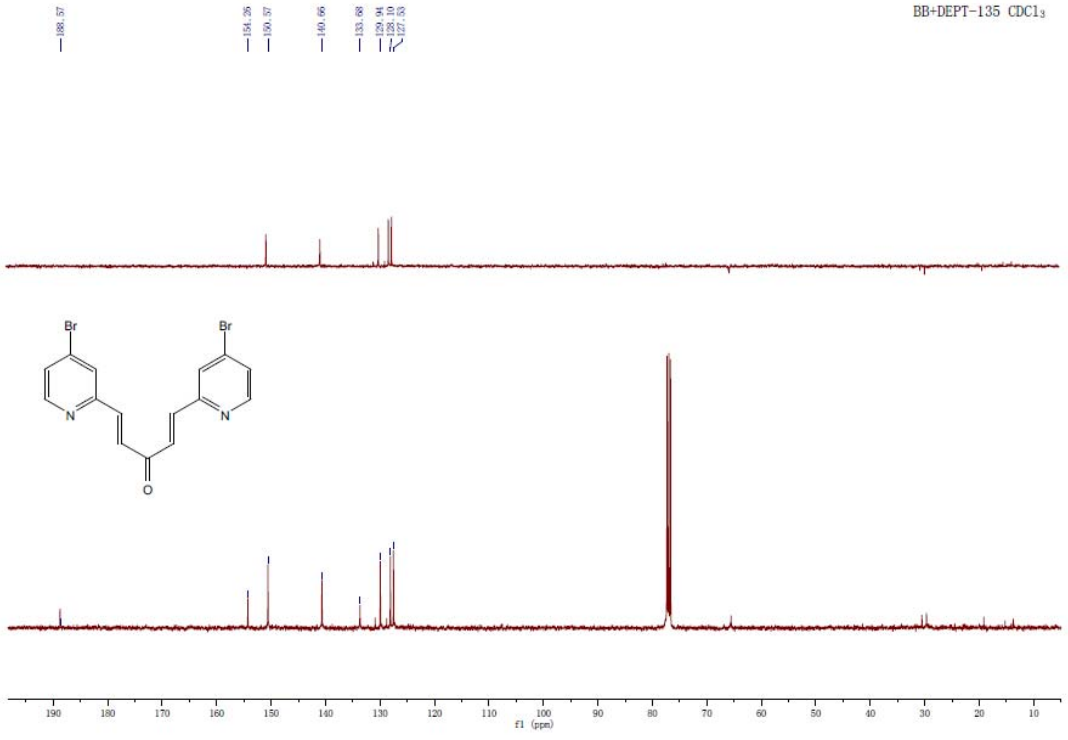
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N
N
O
190
180
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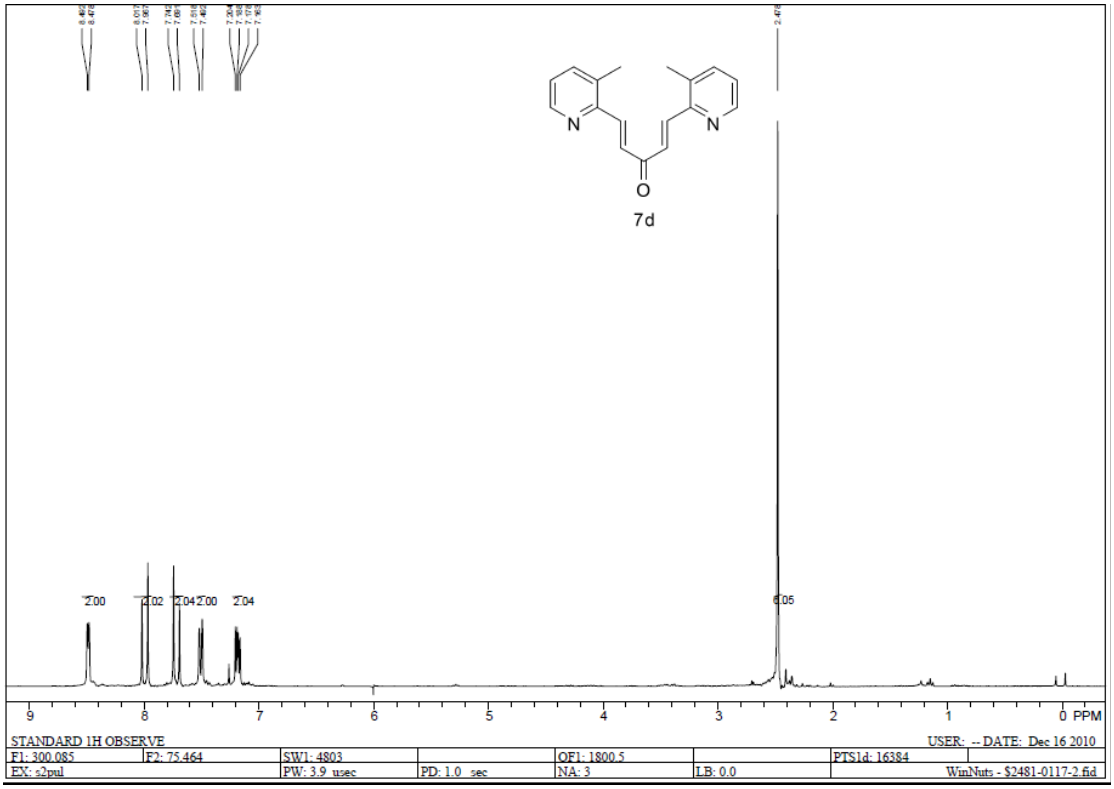
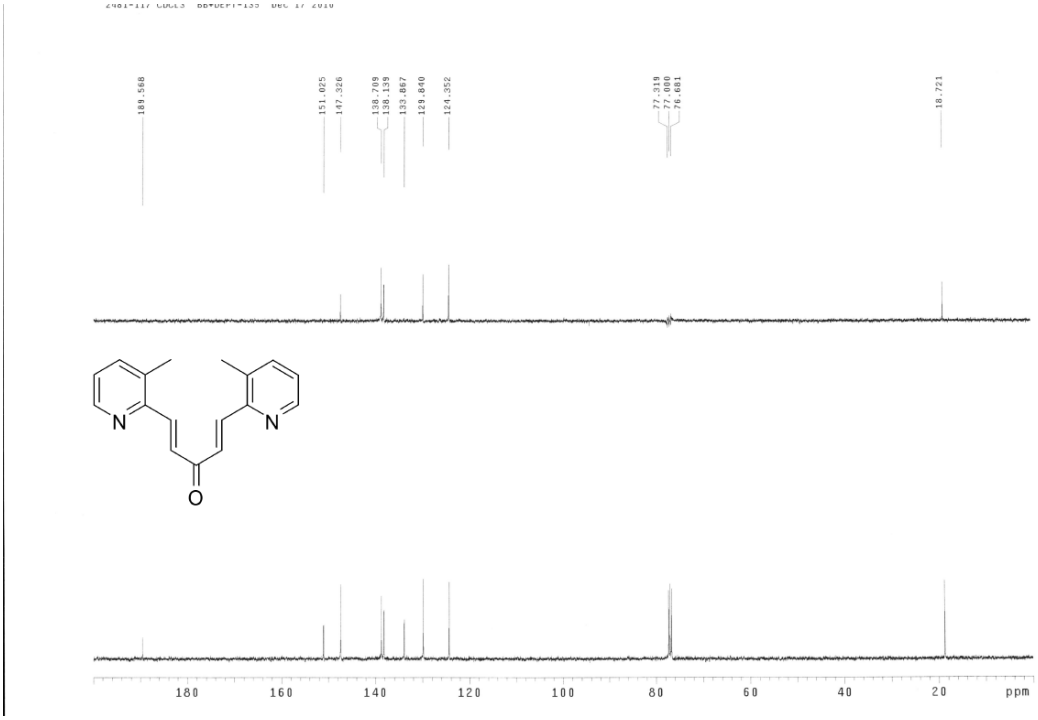
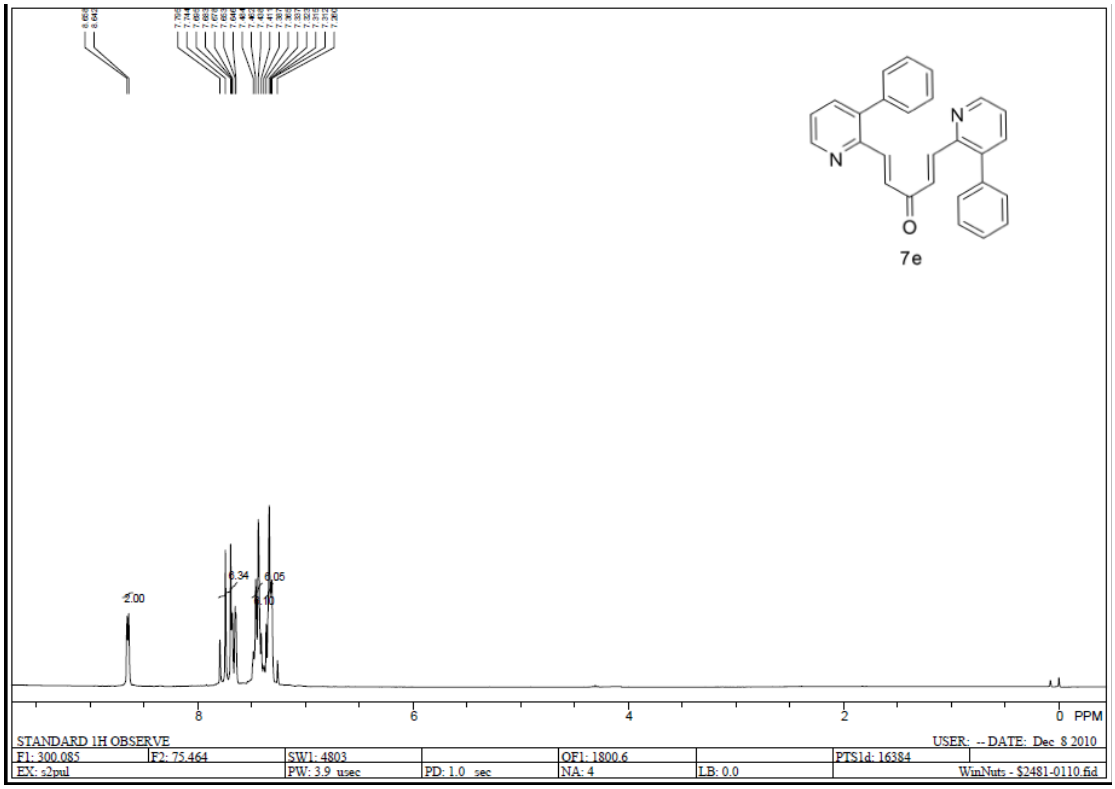
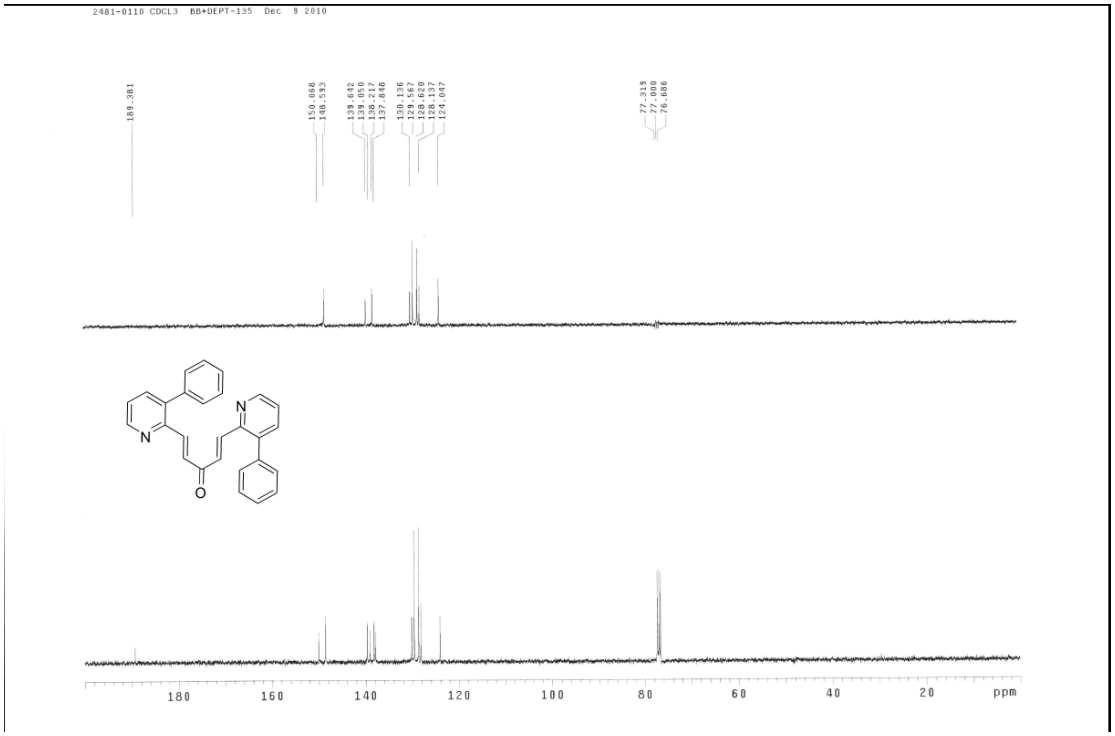
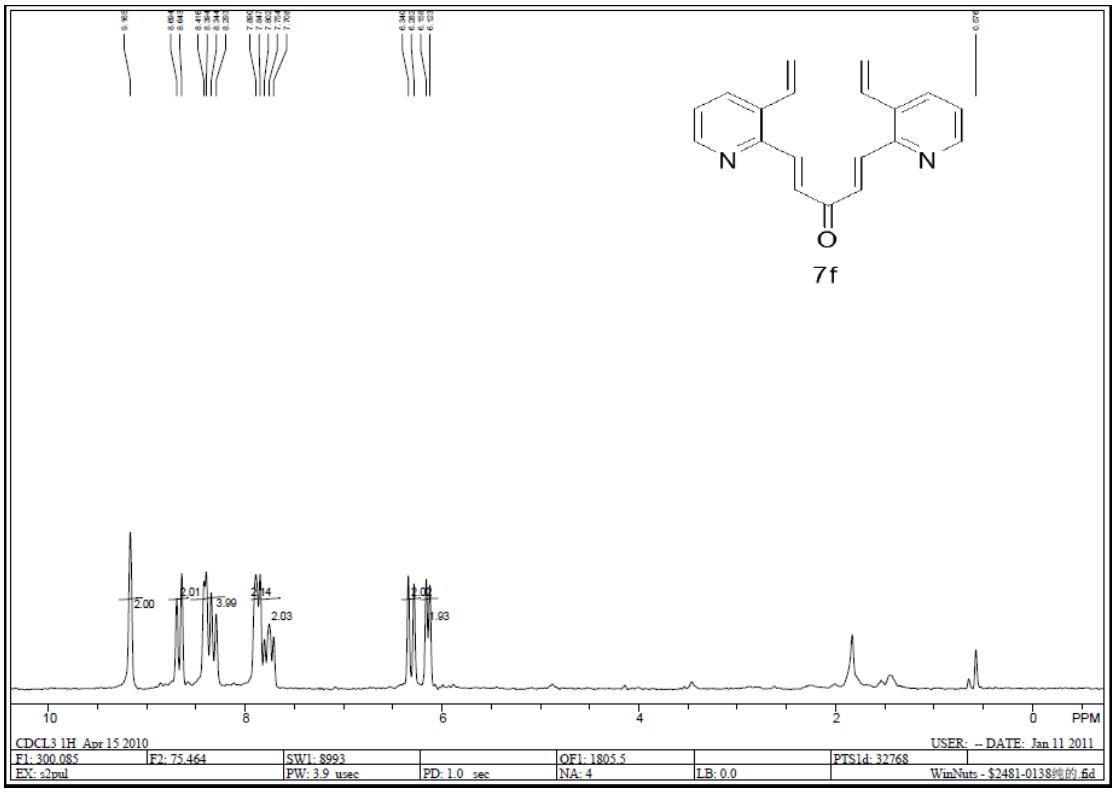
10
f1 (ppm)







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10
8
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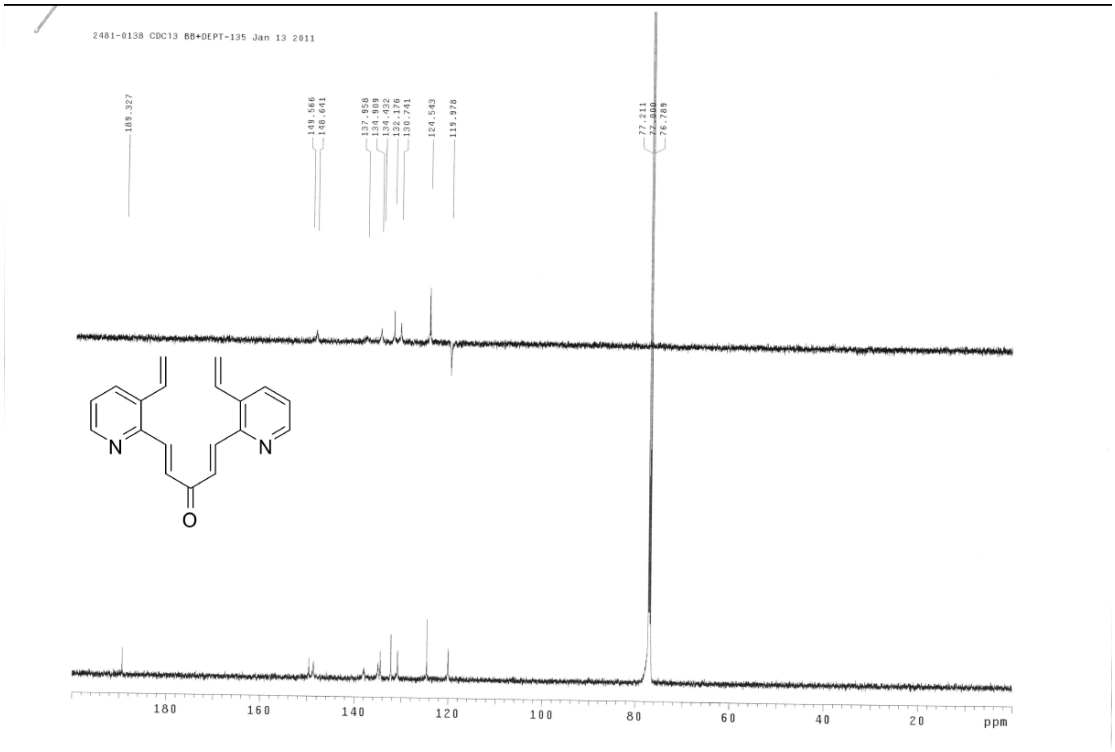
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60
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20
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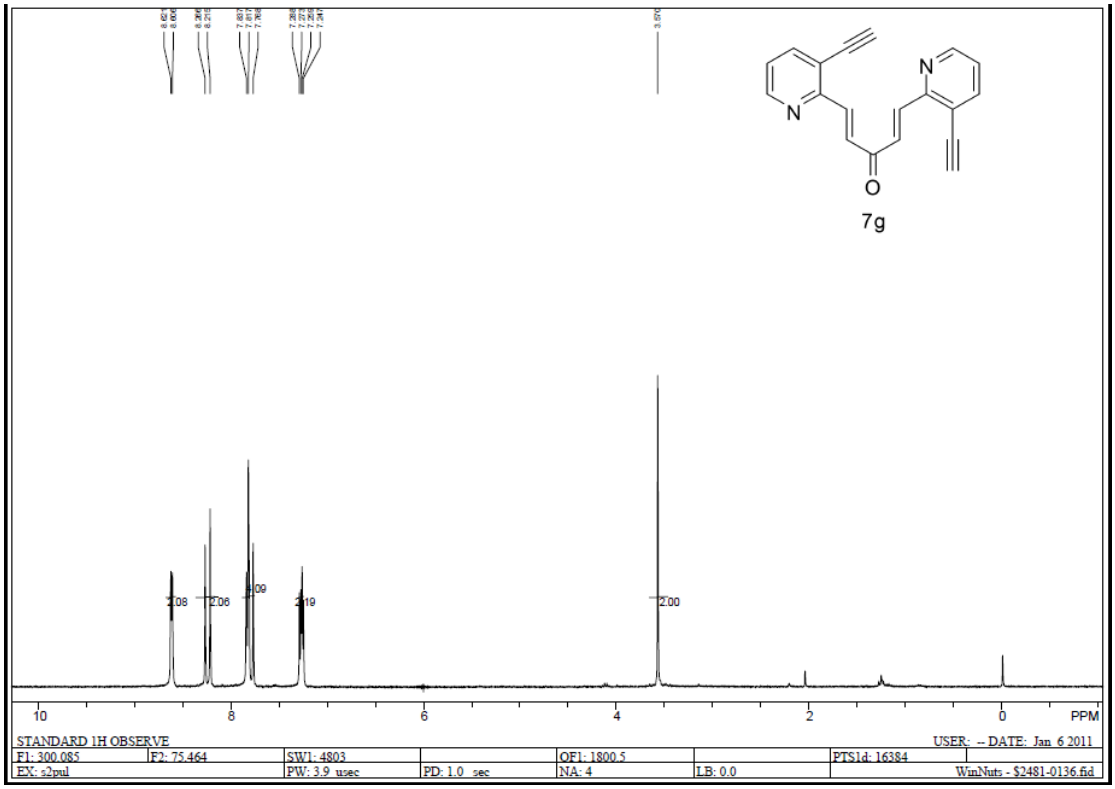
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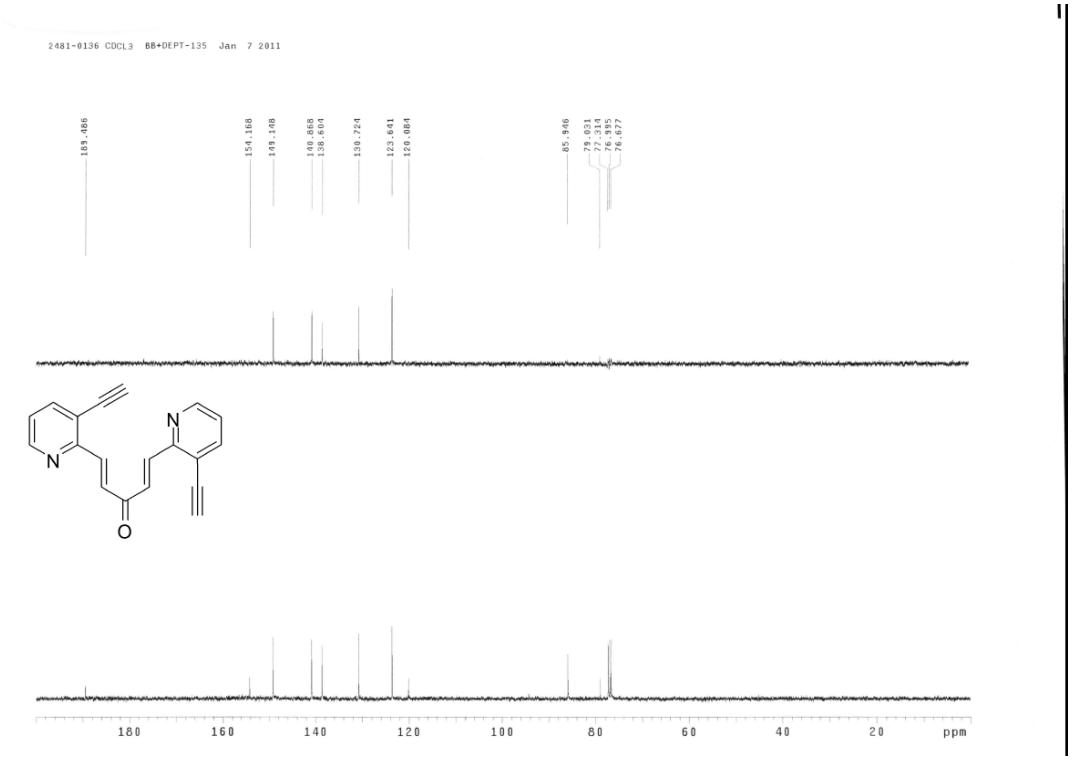
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138.684
130.724
123.641
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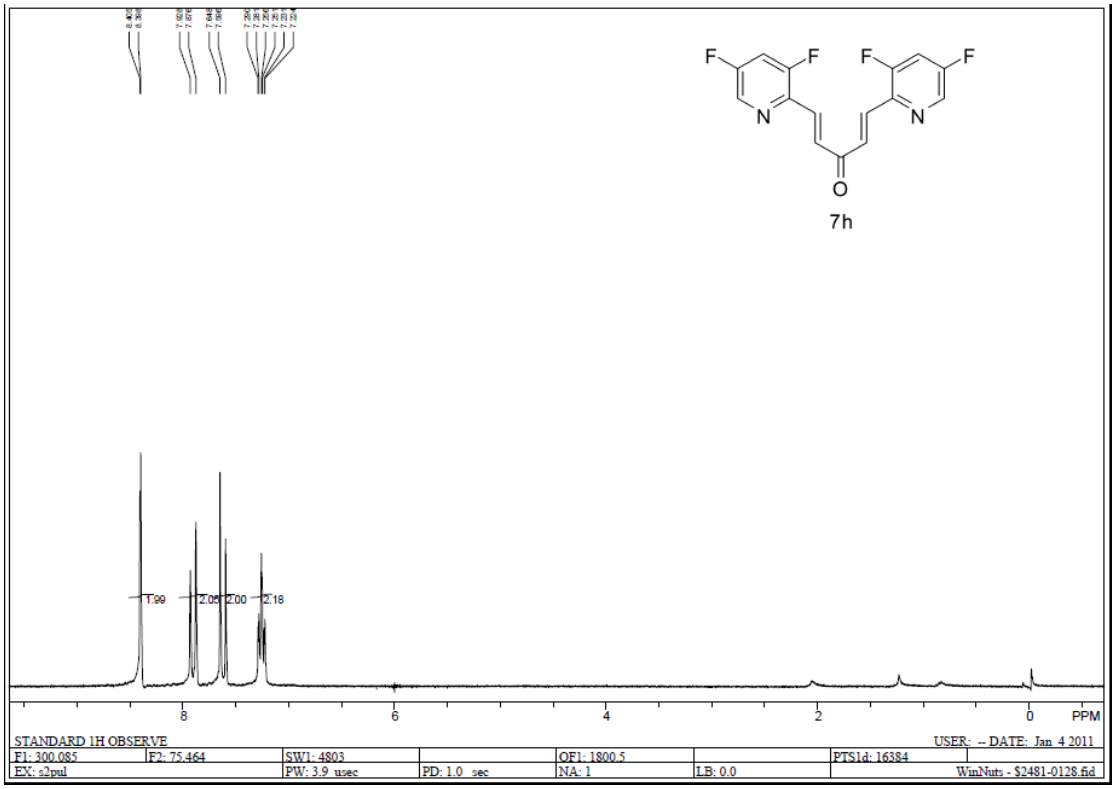
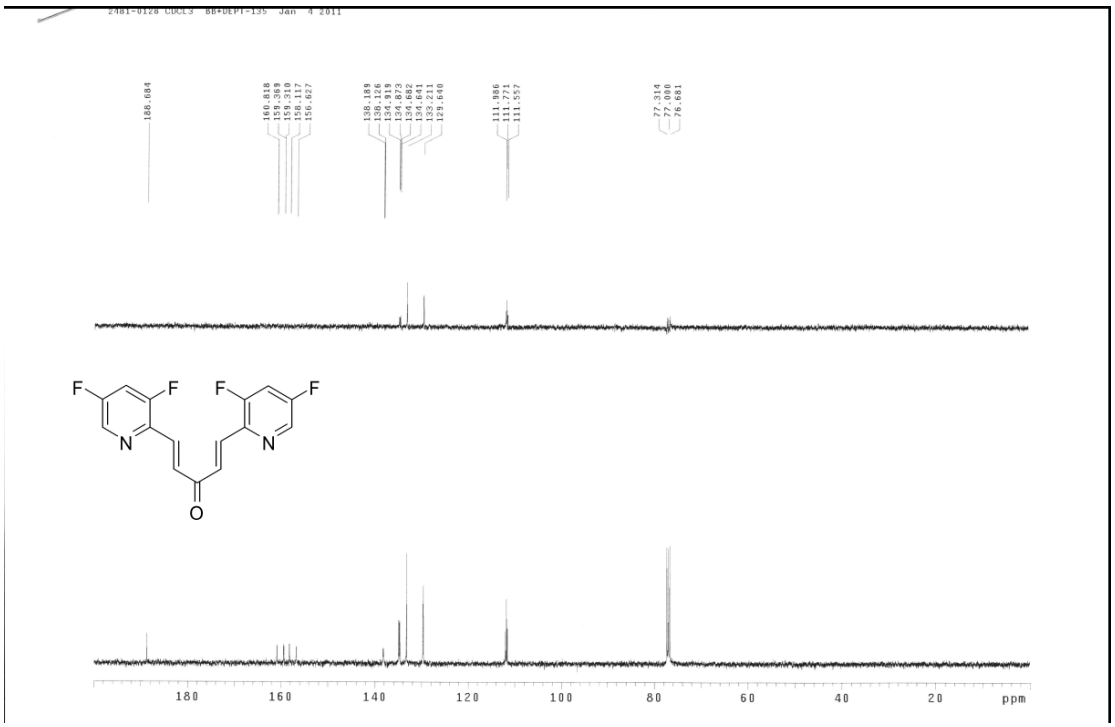
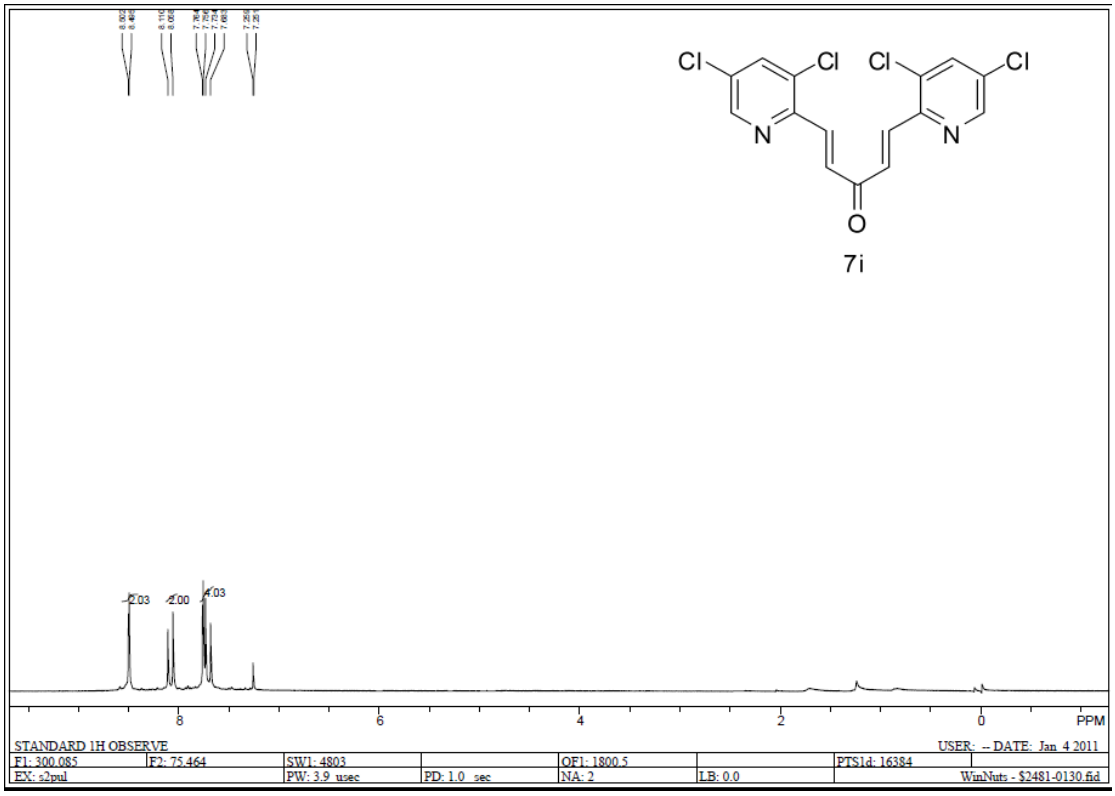
20
ppm



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Cl
Cl
Cl
N
N
O
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2.00
4.03
8
6
4
2
0
PPM
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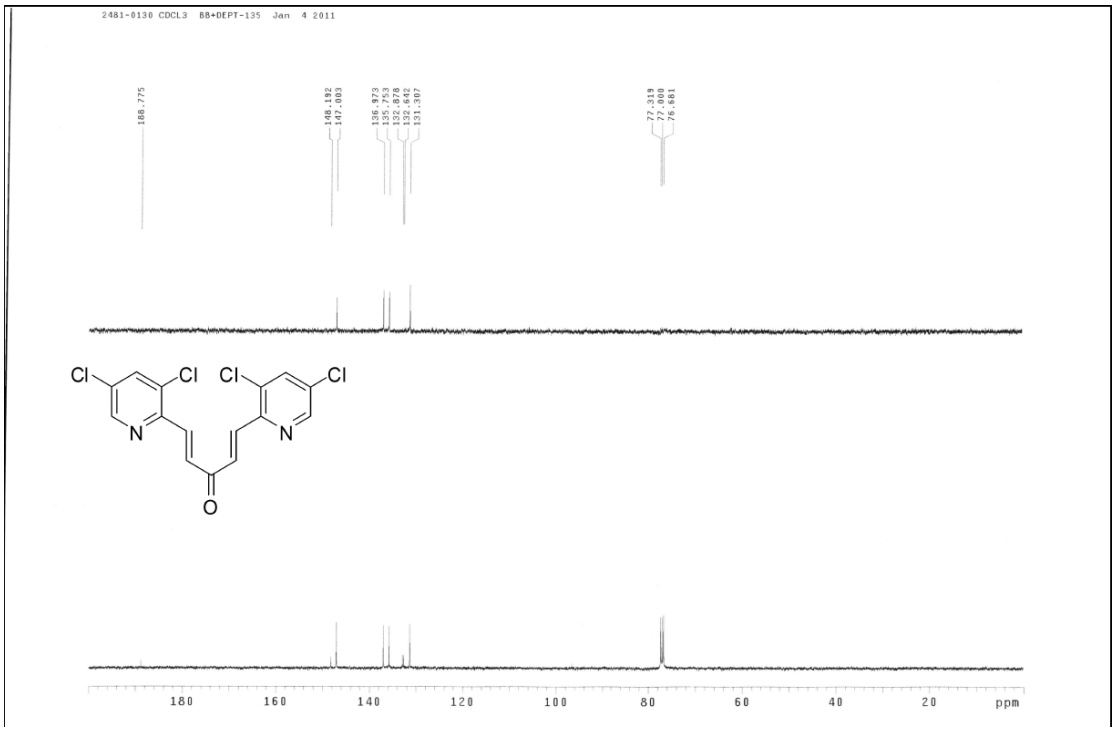
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Cl
Cl
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N
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140
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60
40
20
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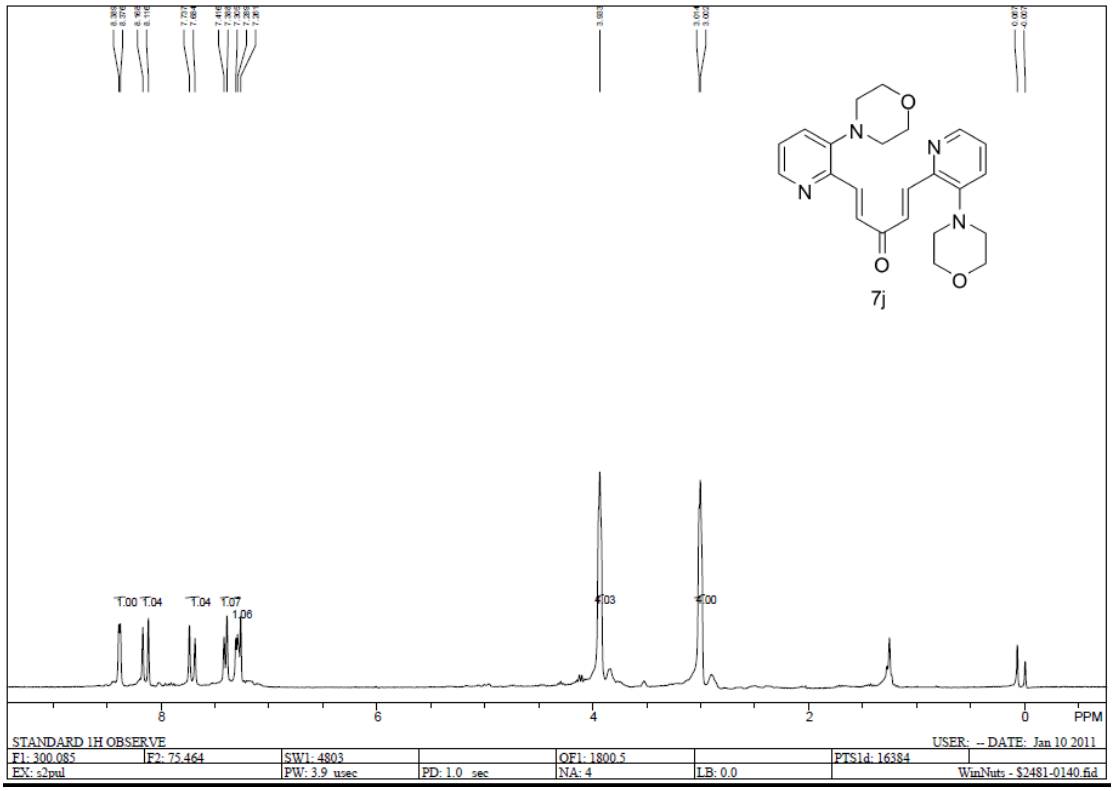
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4.03
4.00
8
6
4
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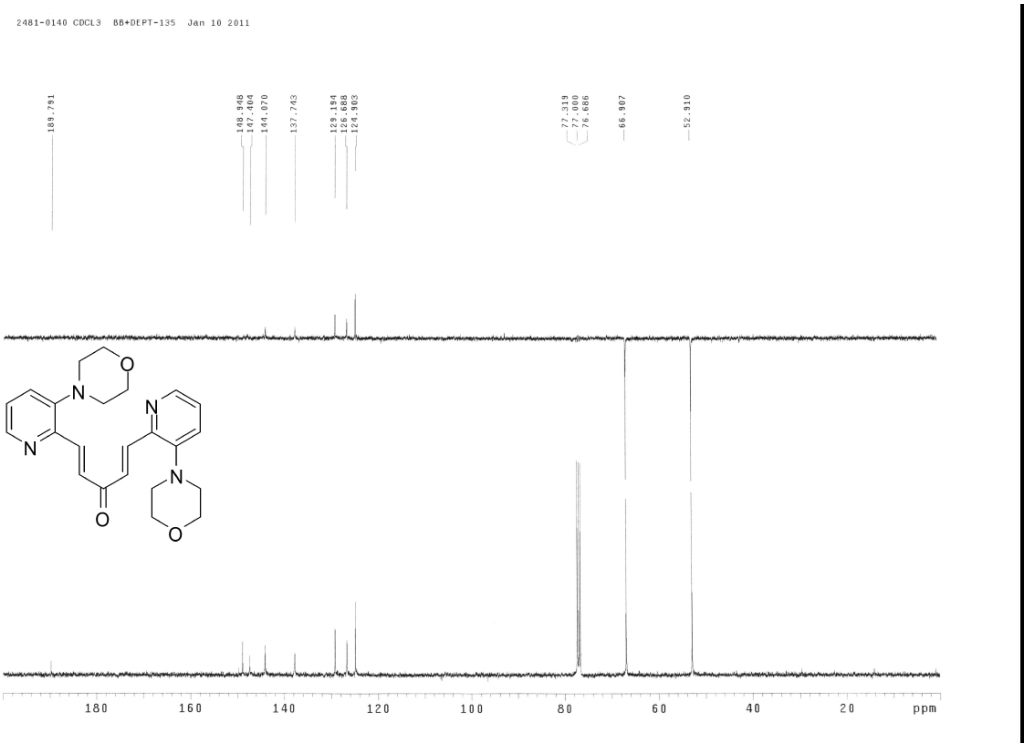
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140
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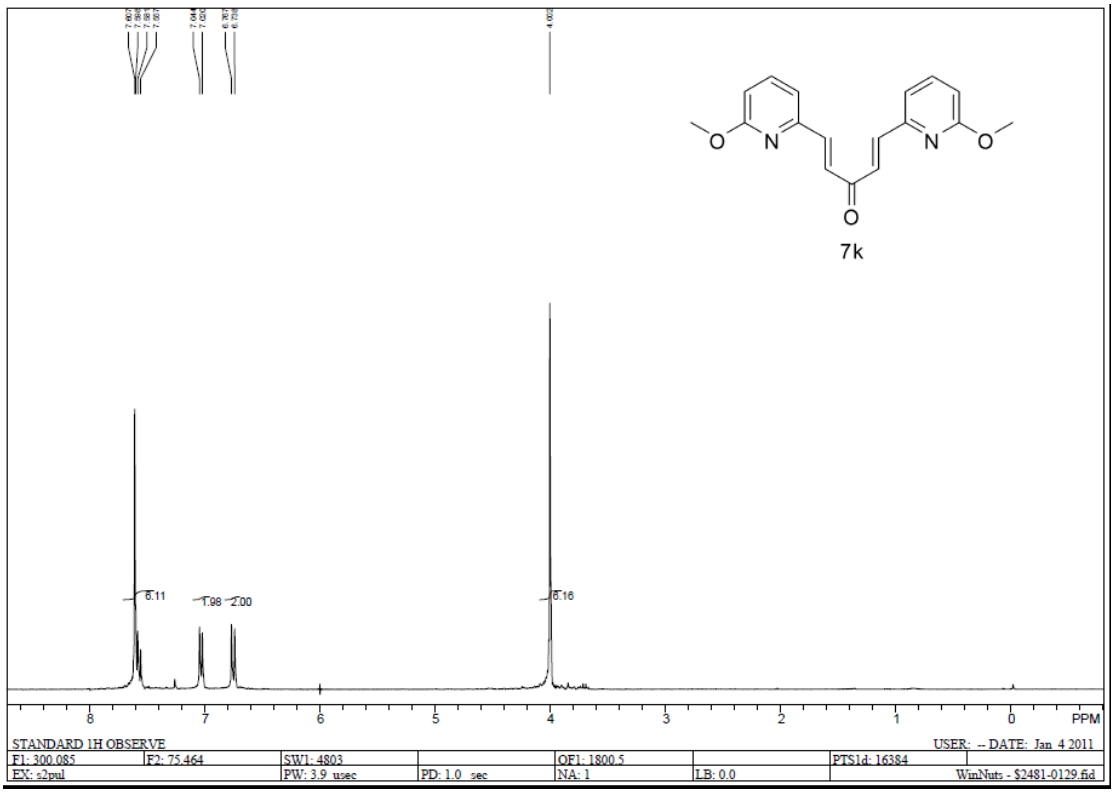
20
ppm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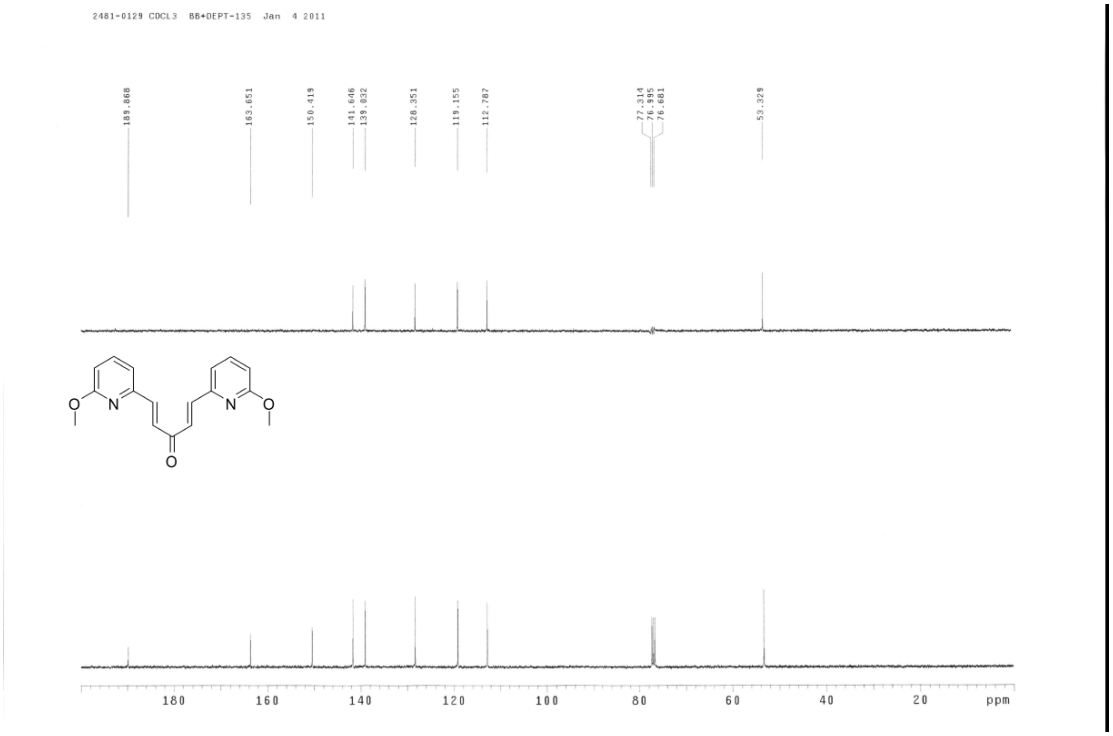
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76.995
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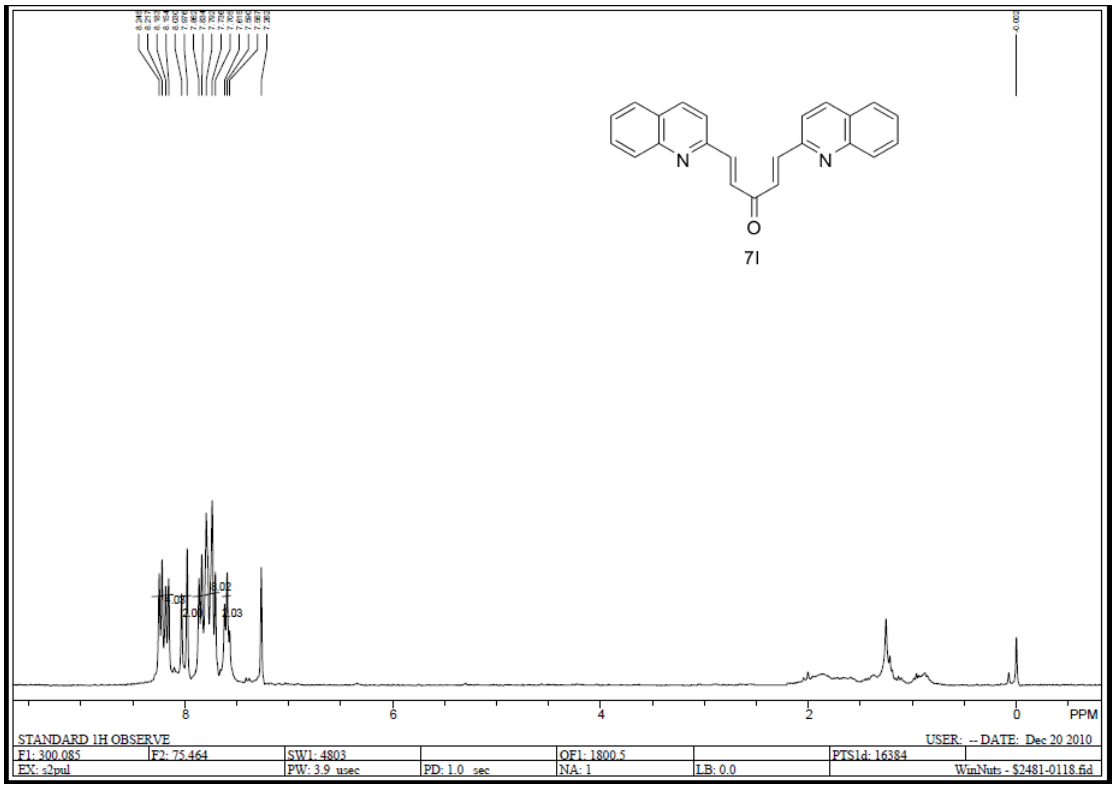
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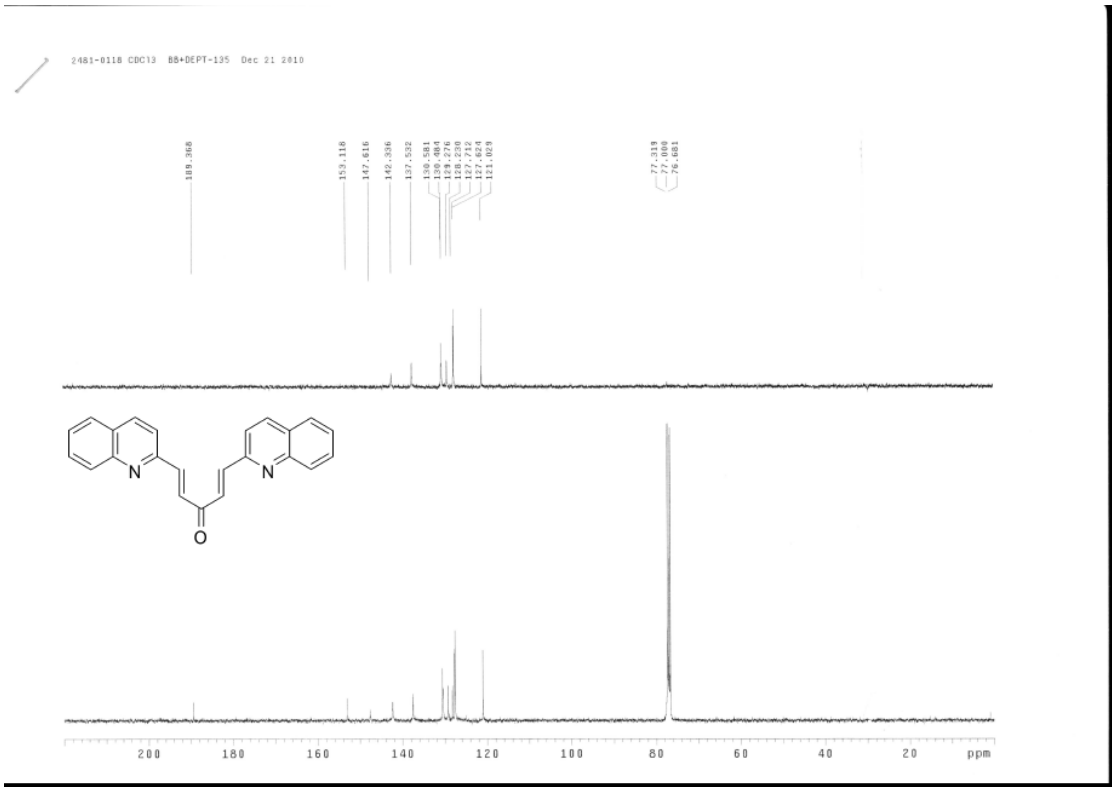
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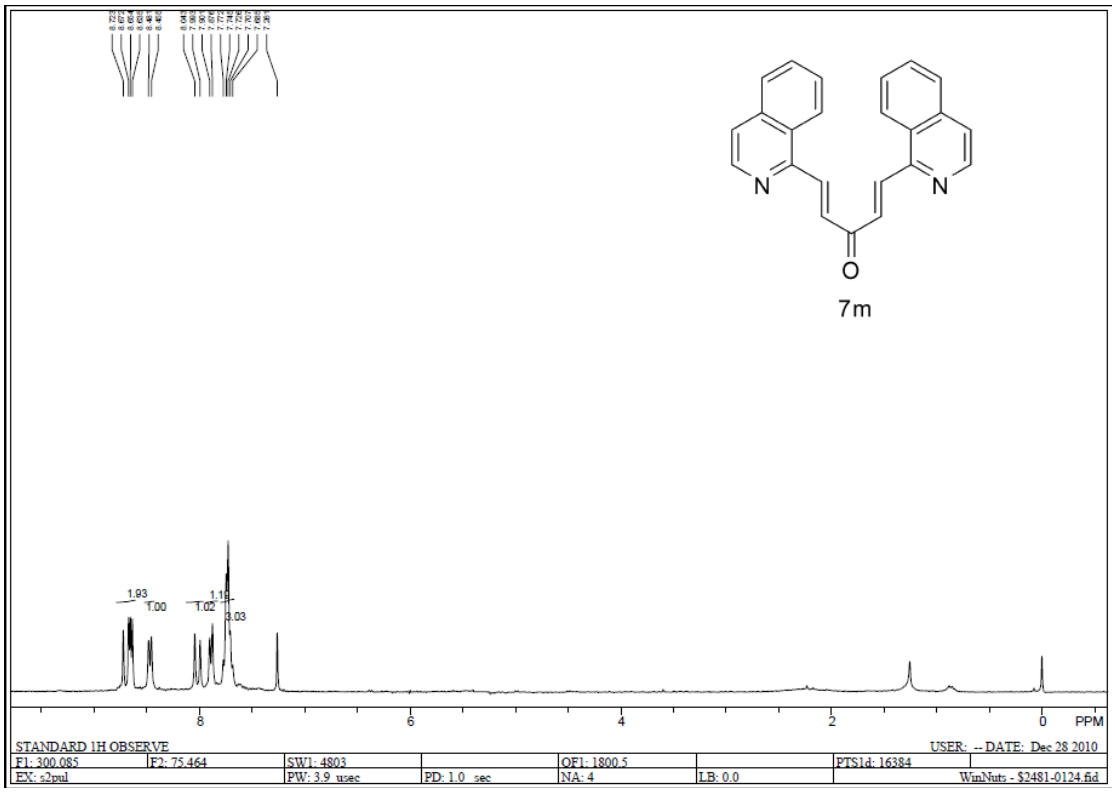
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1.02
3.03
8
6
4
2
0
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STANDARD 1H OBSERVE
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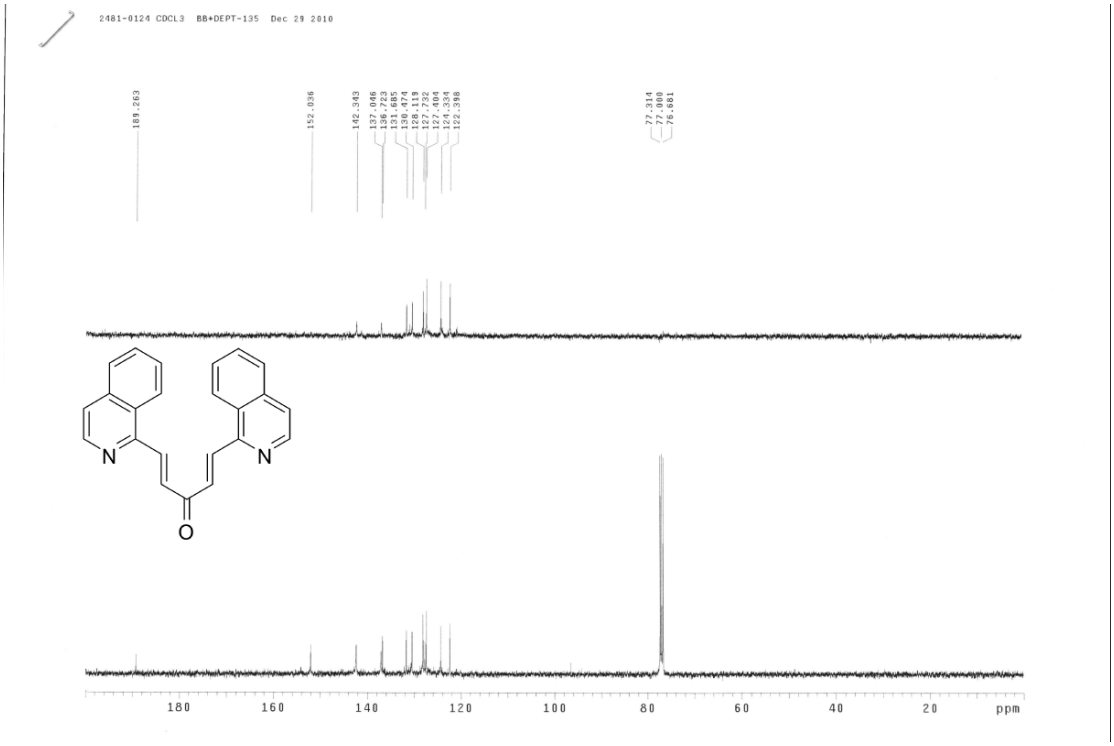
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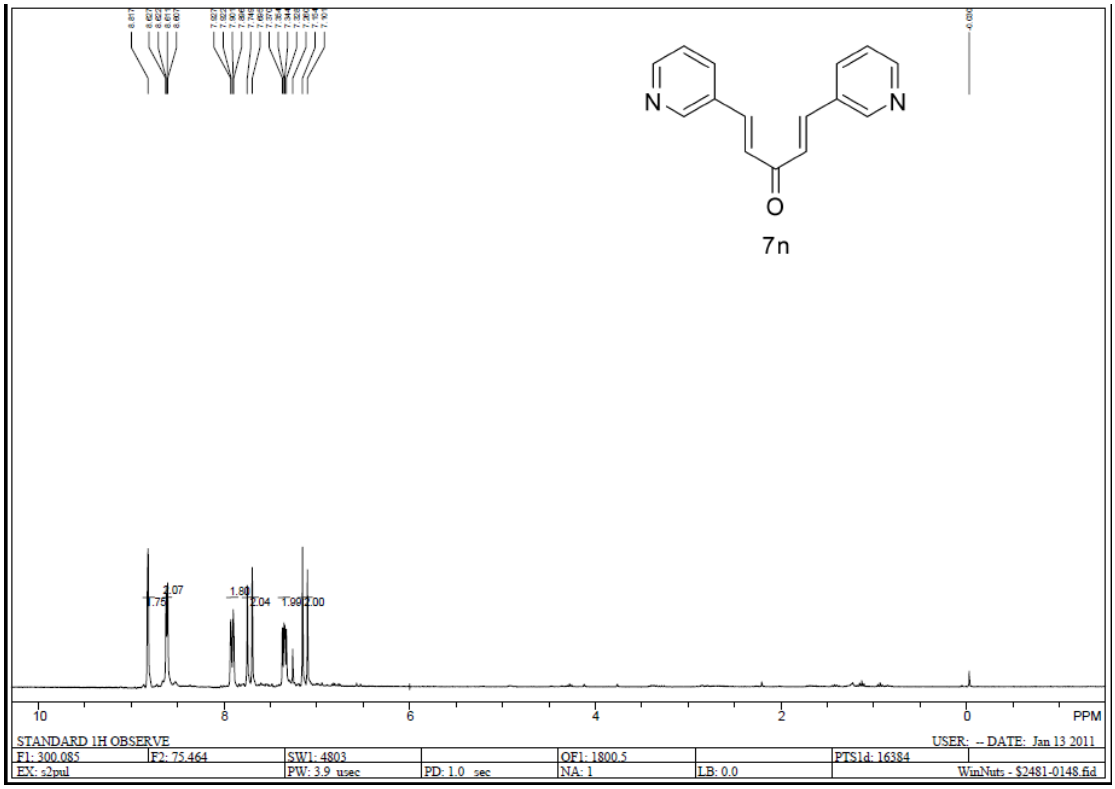
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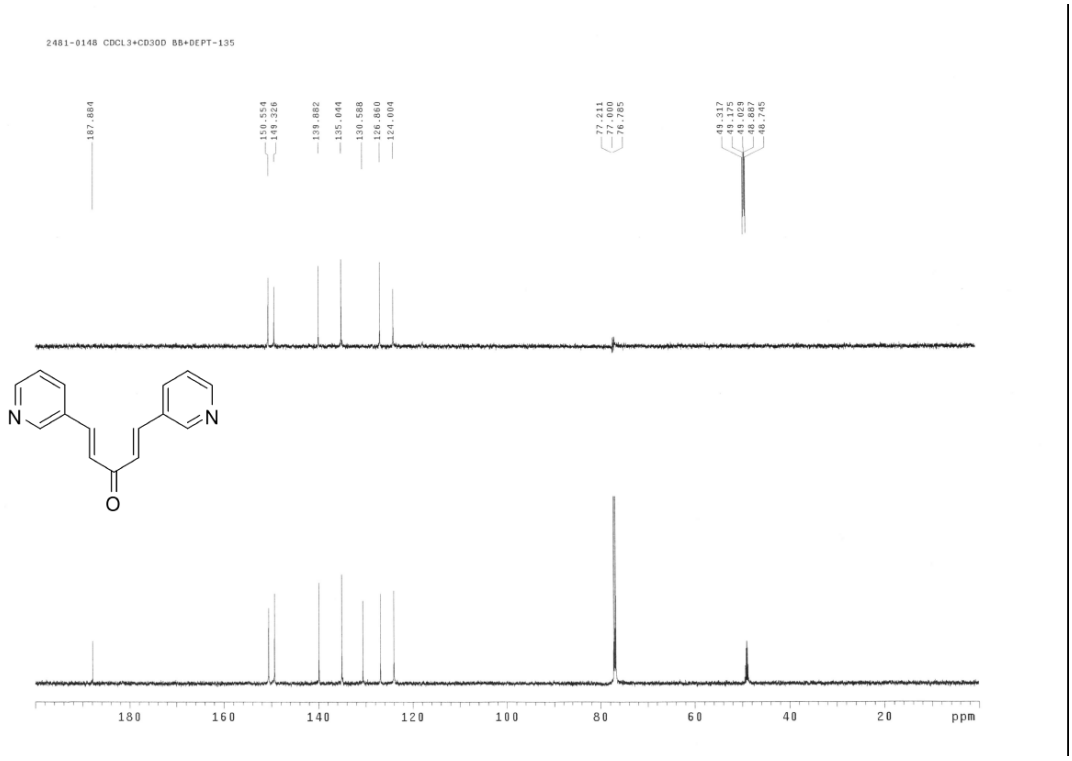
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76.785
49.317
49.125
48.933
48.887
48.745
N
N
O
180
160
140
120
100
80
60
40
20
ppm

Screening inhibitors of NF-κB based TLR4 signal pathway

1. Cell lines

   293/ NF-κB-Luc: incubated with DMEM medium,10% Gibco FBS

   Hek293 stably transfected with NF-κB binding DNA fragment recombinant luciferase reporter system.

2. Materials

   Lipopolysaccharides from Escherichia coli 055:B5 (LPS, Sigma L6529-1MG)

   Stock solution:1 mg/ml diluted in sterile PBS, stored at -20℃

   Work solution: dilute the stock solution into cell culture medium to 10μg/ml

   Compound library

3. Methods

   1) Seed 293/ NF-κB-Luc cells in 96 well plate,3×10^5/ml, 100μl/well, incubate at 37℃, 5% CO_2 overnight
   2) Add screening compound solution 100μl to each well, incubate for 15 minutes, set **BLANK** and **CONTROL** well in the meanwhile
   3) Add LPS solution 20μl to get end concentration 1μg/ml, incubate for 6 hrs
   4) Lyse the cells using 1×CCLR buffer **20**μl/well according to the luciferase assay system kit, pipette then transfer to 96 well lumitrac plate
   5) Add **40**μl assay substrate per well, read RLU immediately among 10 minutes
   6) Calculate the inhibition rate of the compound

$$Inhibition\ Rate = \frac{RLU_{LPS} - RLU_{COMPOUND}}{RLU_{LPS} - RLU_{BLANK}} \times 100\%$$

4. Results：

Fig1-6 shows the inhibition rate of compounds **7a-n** (LCX-94, LCX-95, LCX-99, LCX-100, LCX-101, LCX-102, LCX-103, LCX-104, LCX-105, LCX-109, LCX-110, LCX-111, LCX-112, LCX-113) to 293/ NF-κB-Luc cells at 100uM, 10uM, 1uM, 0.1uM, 0.01uM working concentration.

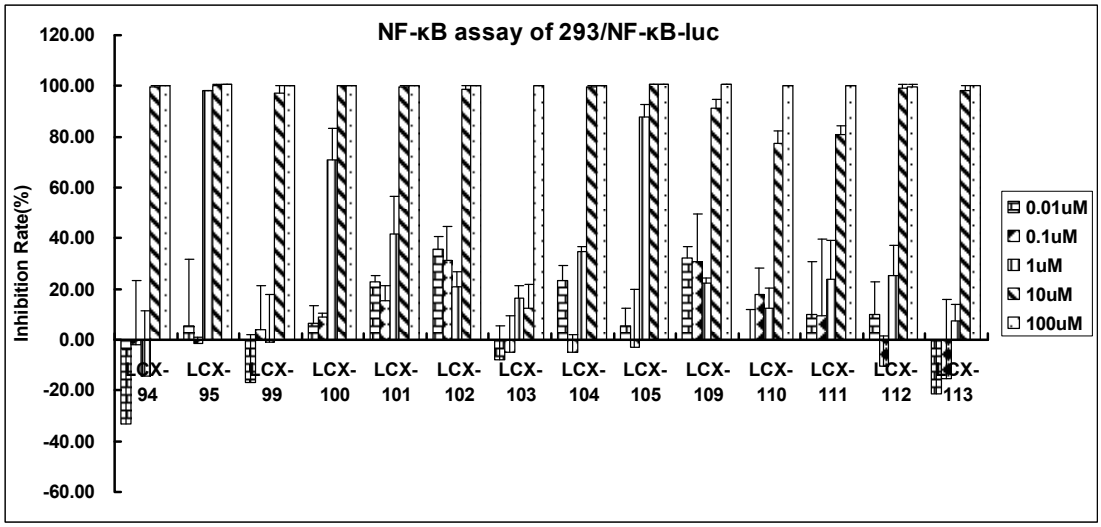

Fig 1

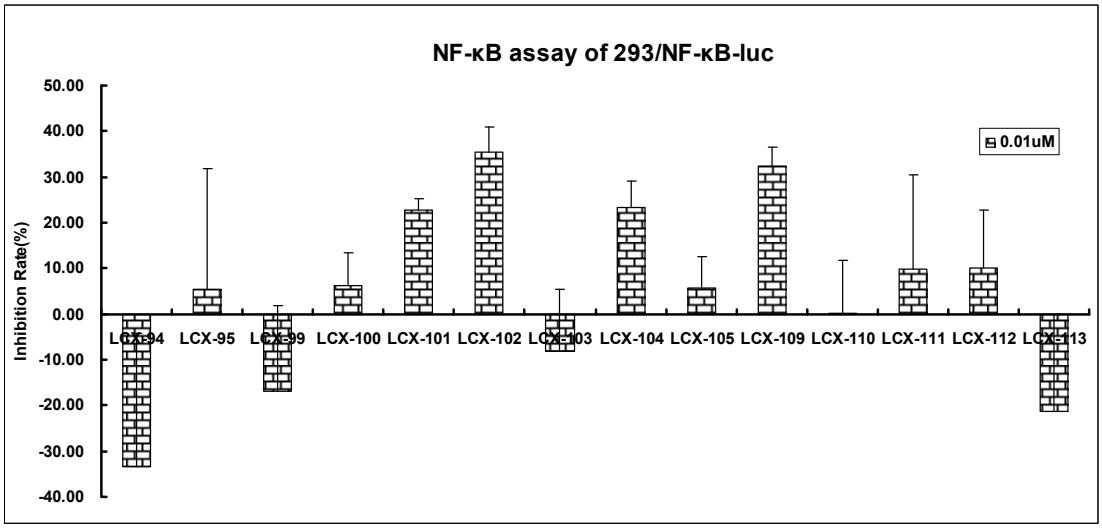

Fig 2

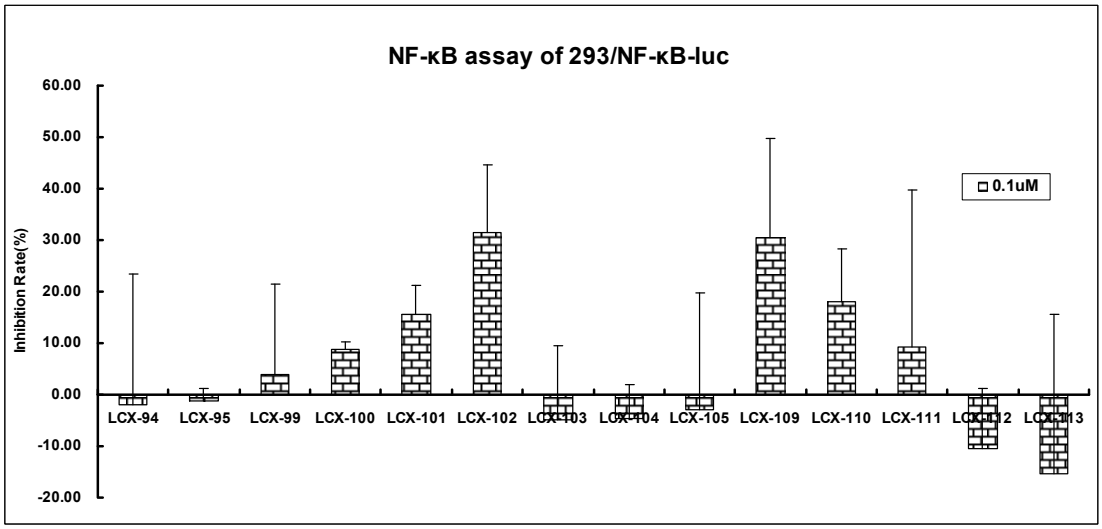

Fig 3

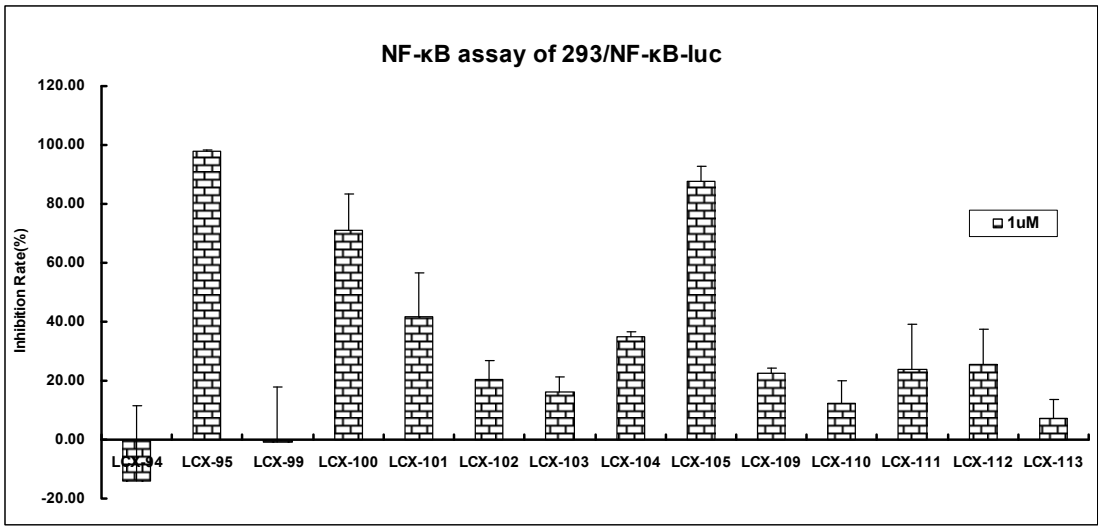

Fig 4

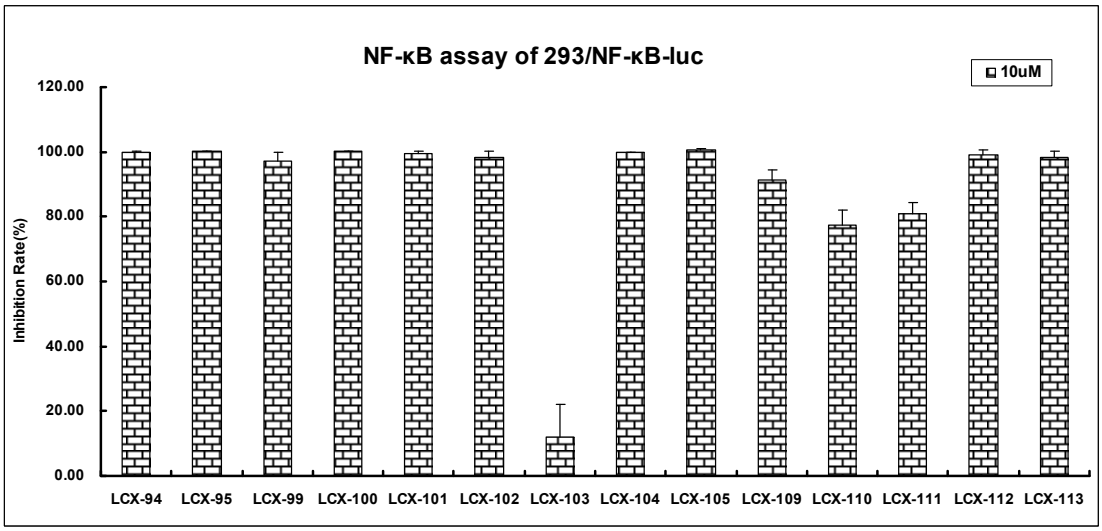

Fig 5

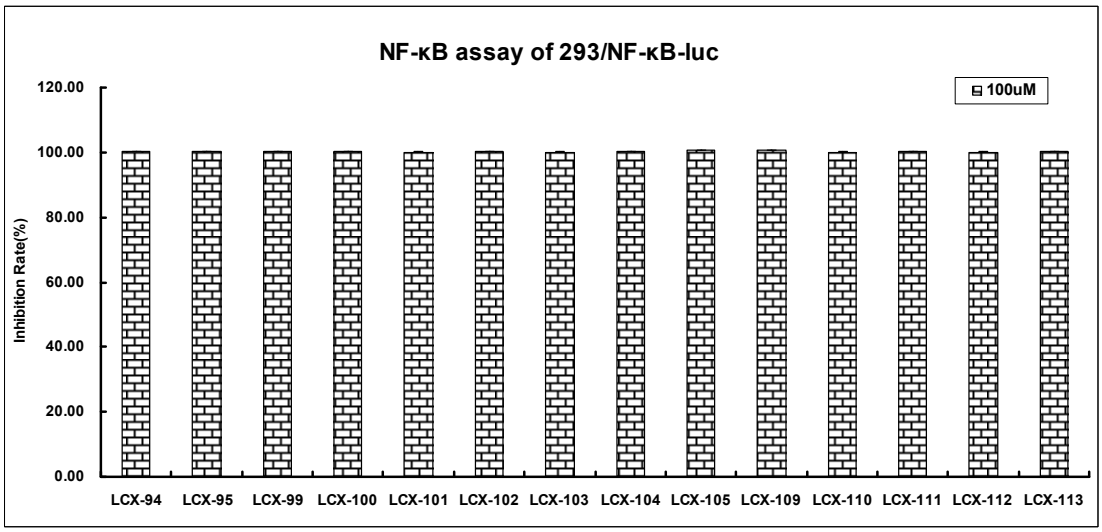

Fig 6

Cytotoxicity assay. (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), was used to assess cytotoxicity. HCT116 colon cancer cells were seeded in 96-well microtiter plates and allowed to attach overnight. Cells were subsequently treated with continuous exposure to corresponding drugs for 72 h. An MTT solution (final concentration of 0.5 mg/mL) was added to each well and cells were incubated for 4 h at 37°C. After removal of the supernatant, DMSO was added and the absorbance was read at 570 nm. The IC_{50} was then determined for each drug from a plot of log (drug concentration) versus percentage of cells killed.

LEDGF/p75 – IN AlphaScreen assay. The AlphaScreen assay was performed according to the manufacturer's protocol (Perkin – Elmer, Benelux). Reactions were performed in 25 µl final volume in 384-well Optiwell microtiter plates (Perkin-Elmer). The reaction buffer contained 25 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, 0.01% (v/v) Tween-20 and 0.1% (w/v) bovine serum albumin. His_6-tagged IN (300 nM final concentration) was incubated with the inhibitory compound for 30 min at 4°C. The compounds were added in varying concentrations spanning a wide range from 0.1 µM up to 100 µM. Afterwards, 100 nM Flag-LEDGF/p75 was added and incubation was prolonged for an additional hour at 4°C. Subsequently, 5 µl of Ni-chelate-coated acceptor beads and 5 µl anti-Flag donor beads were added to a final concentration of 20 µg/ml of both beads. Proteins and beads were incubated for 1 h at 30°C in order to allow association to occur. Exposure of the reaction to direct light was omitted as much as possible and the emission of light from the acceptor beads was measured in the EnVision plate reader (Perkin-Elmer) and analyzed using the EnVision manager software.