Design, Synthesis and Biological Evaluation of Novel Pyrimidine Derivatives as Bone Anabolic Agents Promoting Osteogenesis via BMP2/SMAD1 Signaling Pathway

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Chemistry:

1. Material and Methods:

All materials and reagents were purchased from commercial suppliers from Sigma Aldrich or Alfa-Aesar or Spectrochem and used without further purification. All the glass apparatus were oven-dried prior to use. Silica gel (mesh size 100-200) was used for column chromatography and TLC was performed on Merck- pre-coated silica gel 60-F254 and aluminum oxide 60-F254 plates. The solvents of Thermo-Fisher were used for the column chromatography. The melting point was recorded with the COMPLAB melting point apparatus. All the synthesized compounds were fully characterized by ^{1}H, ^{13}C, ESI-MS, and ESI-HRMS analysis. ^{1}H spectrums were recorded at 300/400/500 MHz, and ^{13}C spectrums were recorded at 100/125 MHz. $CDCl_3$, and DMSO-d_6 were used as solvents for NMR recording, and tetramethyl silane as internal standards. Chemical shifts were reported in parts per million (ppm) downfield from solvent reference, and coupling constants (*J*) were measured in Hz. ESI-MS spectra were obtained as LCQ Advantage Ion Trap mass spectrometer (Finnigan Thermo Fischer Scientific) and high-resolution mass spectra (ESI-HRMS) were recorded on Agilent 6520 ESI-QTOP mass spectrometer.

2. Experimental procedure and characterization of chalcones (starting material):

A mixture of benzaldehyde (1.1 mmol), acetophenone (1 mmol), and 30% aqueous NaOH (2 mL) in 10 mL ethanol was stirred in a 50 mL round bottom flask at room temperature. The reaction progress was monitored by TLC (25% ethyl acetate in hexane). After the completion of the reaction, ethanol was evaporated by rotavapor. The obtained crude was washed with (3 x 15 mL) hot water and followed by MeOH (3 x 15 mL) on a Buckner funnel, dried under vacuum resulting in a pure product obtained with 91% to 94% yield.[S1]

3a. (*E*)-1-(4-bromophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3a** by the reaction of 2,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-bromoacetophenone (199 mg, 1 mmol) in the

presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3a** (354 mg, yield: 94%) was obtained as a yellow solid after crystallization in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, J = 15.6 Hz, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 15.6 Hz, 1H), 7.13 (s, 1H), 6.54 (s, 1H), 3.96 (s, 3H), 3.92(s, 6H). ^{13}C{1H} NMR (100 MHz, $CDCl_3$): δ 190.1, 154.8, 152.8, 143.3, 140.8, 137.6, 131.8, 130.0, 127.3, 119.8, 115.3, 111.6, 96.8, 56.6, 56.4, 56.1.

3b. (*E*)-1-(4-chlorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3b** by the reaction of 2,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-chloroacetophenone (154 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3b** (305 mg, yield: 92%) was obtained as a yellow solid after crystallization in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, J = 15.8 Hz, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 15.7 Hz, 1H), 7.12 (s, 1H), 6.52 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H). ^{13}C{1H} NMR (100 MHz, $CDCl_3$): δ 189.9, 154.8, 152.8, 143.3, 140.8, 138.6, 131.2, 129.9,128.8, 119.8, 115.3, 111.6, 96.8, 56.6, 56.4, 56.1.

3c. (*E*)-1-(4-fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3c** by the reaction of 2,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-fluoroacetophenone (138 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3c** (288 mg, yield: 91%) was obtained as a yellow solid after crystallization in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, J = 15.8 Hz, 1H), 8.06-8.01 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 15.7 Hz, 1), 7.16 (dd, J = 8.6 Hz, 1H), 7.12 (s, 1H), 6.53 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H). ^{13}C{1H} NMR (100 MHz, $CDCl_3$): δ 189.5, 165.4 (d, J^1 = 251.8 Hz), 154.8, 152.7, 143.4, 140.4, 135.2, 131.0 (d, J^3= 9.0 Hz), 119.9, 115.5 (d, J^2= 21.5 Hz), 111.6, 96.9, 56.6, 56.4, 56.1.

3d. (*E*)-1-(4-methoxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3d** by the reaction of 2,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-methoxyacetophenone (150 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3d** (298 mg, yield: 91%) was obtained as a yellow solid after crystallization in MeOH. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, J = 16.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 16.0 Hz, 1H), 7.13 (s, 1H), 6.98 (d, J = 8.0 Hz, 2H), 6.53 (s, 1H), 3.95 (s, 3H), 3.91

(s, 6H), 3.89 (s, 3H). $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$): 189.0, 163.1, 154.5, 152.3, 143.3, 139.3, 131.7, 131.1, 130.7, 124.3, 120.2, 115.7, 113.7, 111.5, 97.0, 56.6, 56.4, 56.0, 55.4.

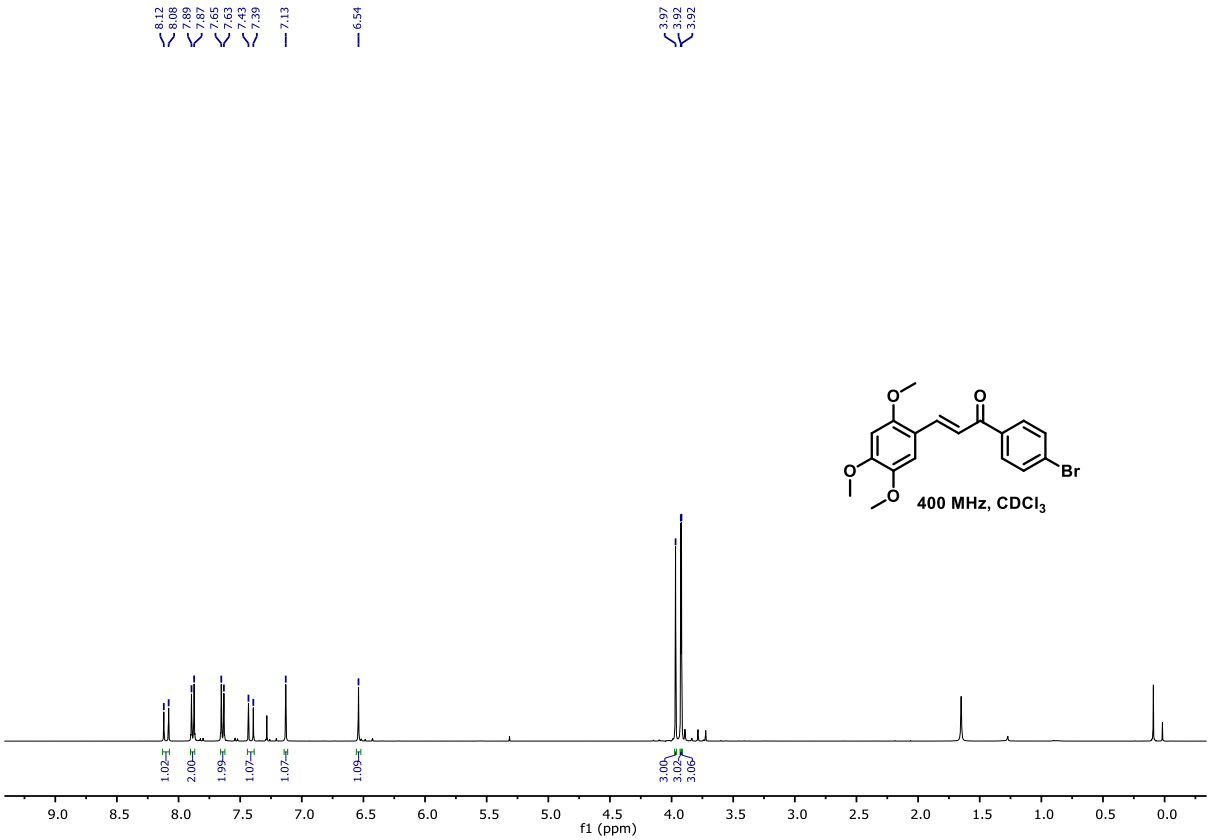
3e. (E)-1-(p-tolyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3e** by the reaction of 2,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-methylacetophenone (134 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3e** (296 mg, yield: 95%) was obtained as a yellow solid after crystallization in MeOH. M.P. 120°C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, J = 16.0 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 16.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.13 (s, 1H), 6.53 (s, 1H), 3.95 (s, 3H), 3.90 (s, 6H), 2.43 (s, 3H). $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$): δ 190.6, 154.6, 152.4, 143.3, 143.0, 139.7, 136.2, 129.1, 128.5, 128.3, 124.0, 120.4, 115.7, 111.5, 96.9, 56.6, 56.4, 56.0, 21.7.

3f. (*E*)-1-(4-bromophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3f** by the reaction of 3,4,5 tri methoxy benzaldehyde (215 mg, 1.1 mmol), and 4-bromoacetophenone (199 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3f** (3 mg, yield: 94%) was obtained as a yellow solid after crystallization in MeOH. ^1H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, J = 15.6 Hz, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 6.13 (s, 2H), 3.90 (s, 6H), 3.86 (s, 3H). $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$): δ 191.1, 163.4, 161.9, 138.1, 136.7, 131.6, 130.0, 126.9, 121.5, 106.5, 90.6, 55.9, 55.4.

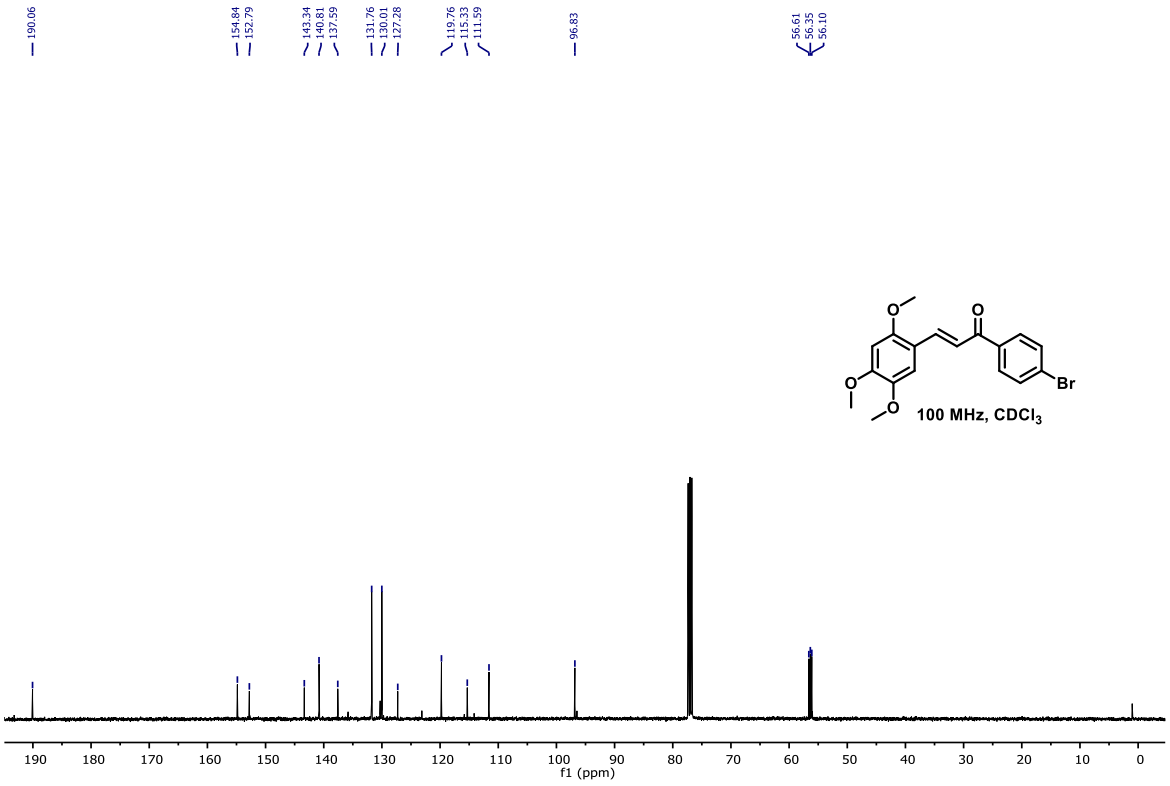
3g. (*E*)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one: Above General procedure was followed for the synthesis of **3g** by the reaction of 4-methoxy benzaldehyde (136 mg, 1.1 mmol), and 4-chloroacetophenone (154 mg, 1 mmol) in the presence of 30% aqueous NaOH (2 mL) in 10 mL ethanol for 12 hours at room temperature. Pure compound **3g** (252 mg, yield: 93%) was obtained as a white solid after crystallization in MeOH. ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, J = 8.6 Hz, 2H), 7.78 (d, J = 15.6 Hz, 1H), 7.59 (d, J = 8.7 Hz, 1H), 7.45 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 15.6 Hz, 1H), 6.93 (d, J = 8.8 Hz, 2H), 3.85 (s, 3H). $^{13}C\{1H\}$ NMR (100 MHz, $CDCl_3$): δ 189.2, 161.9, 145.2, 138.9, 136.8, 130.3, 129.8, 128.9, 127.5,119.2, 114.5, 55.4.

3. Spectral data for starting material

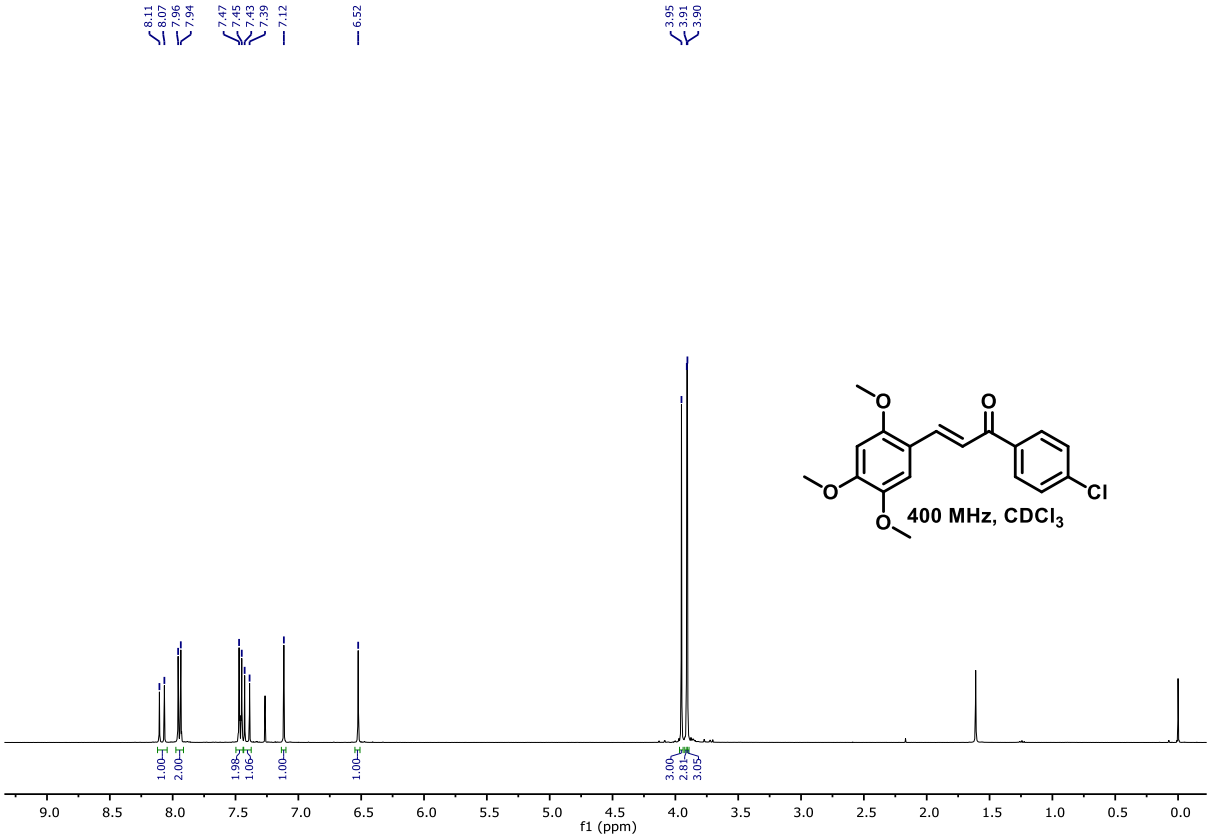
^{1}H NMR for 3a



^{13}C NMR for 3a



^{1}H NMR for 3b



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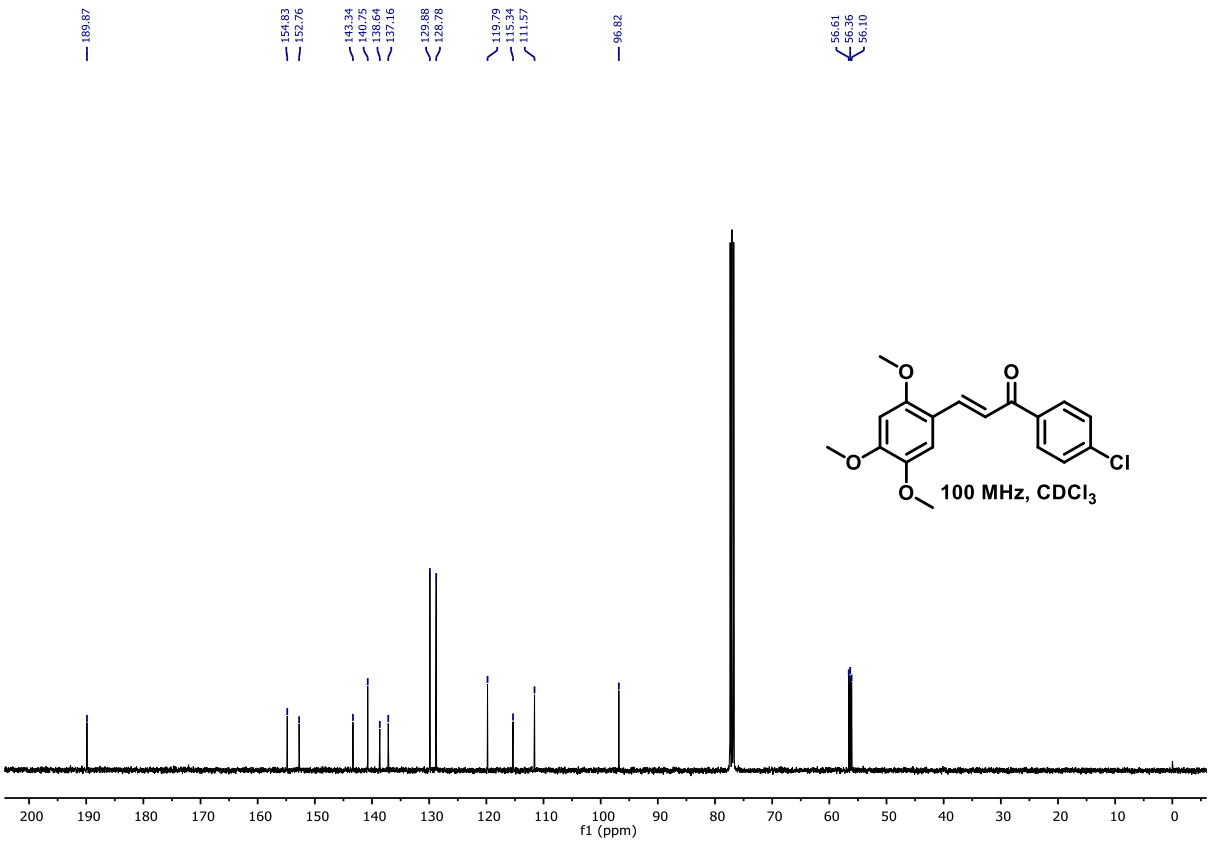
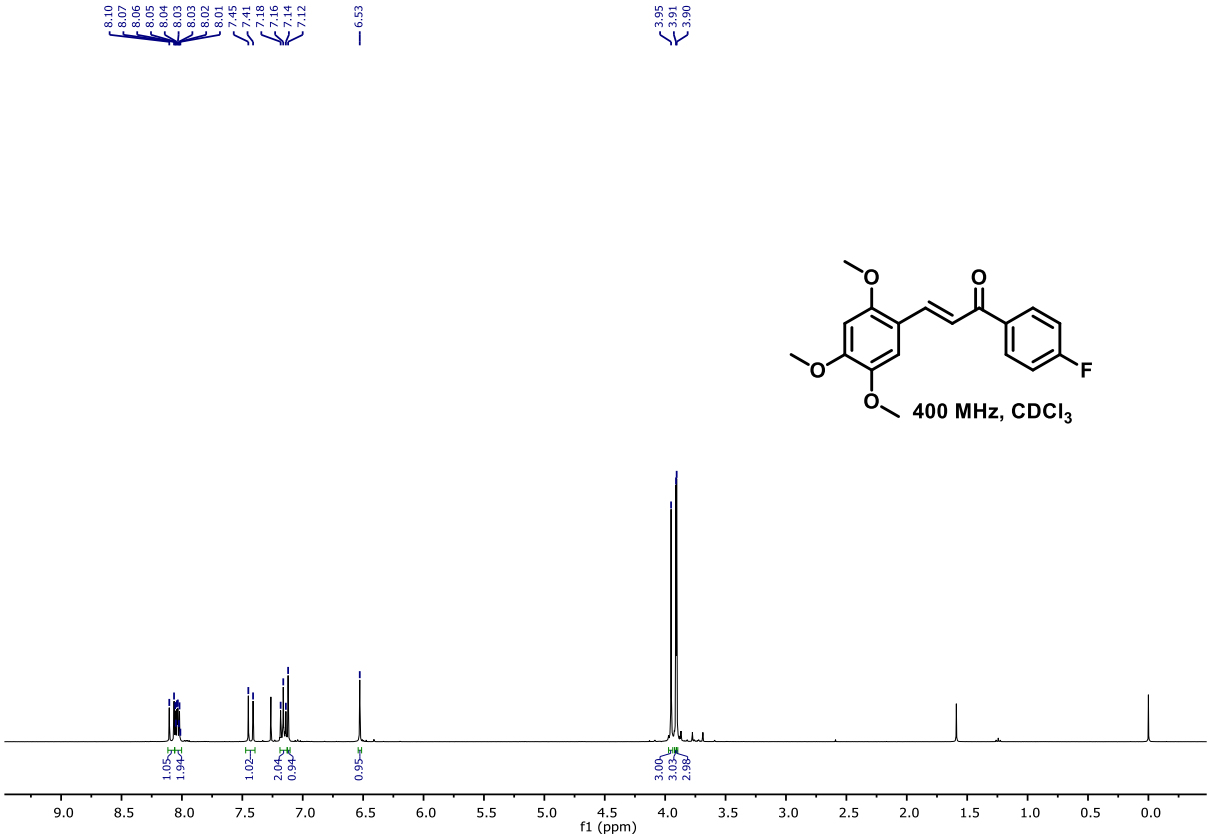


^{1}H NMR for 3c



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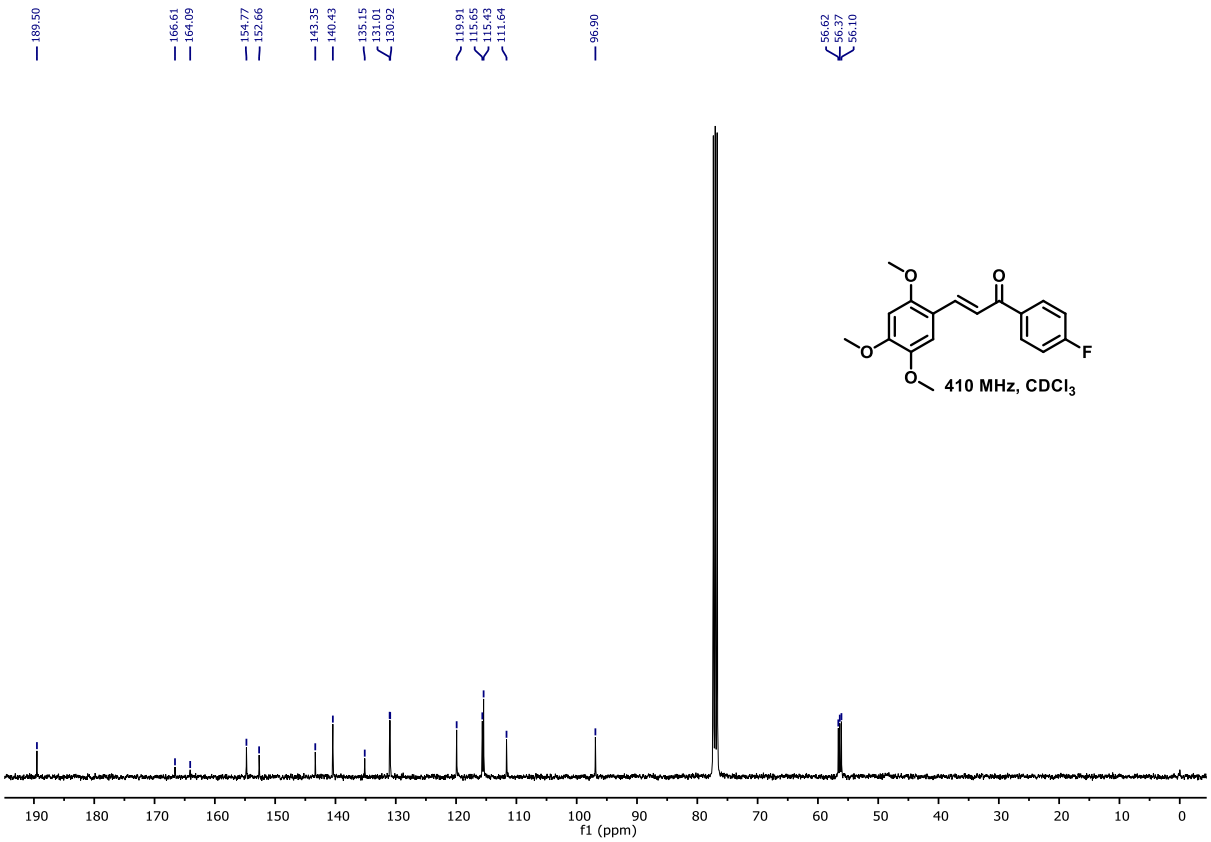
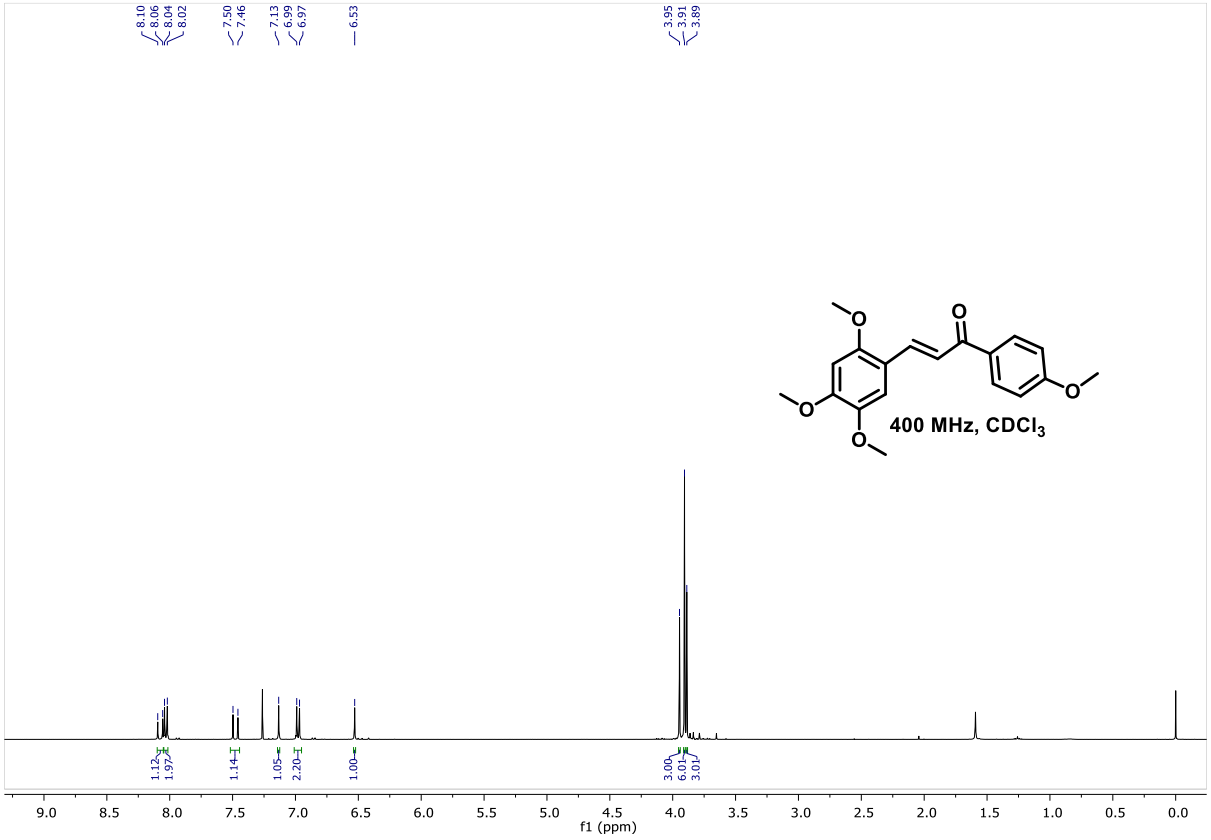
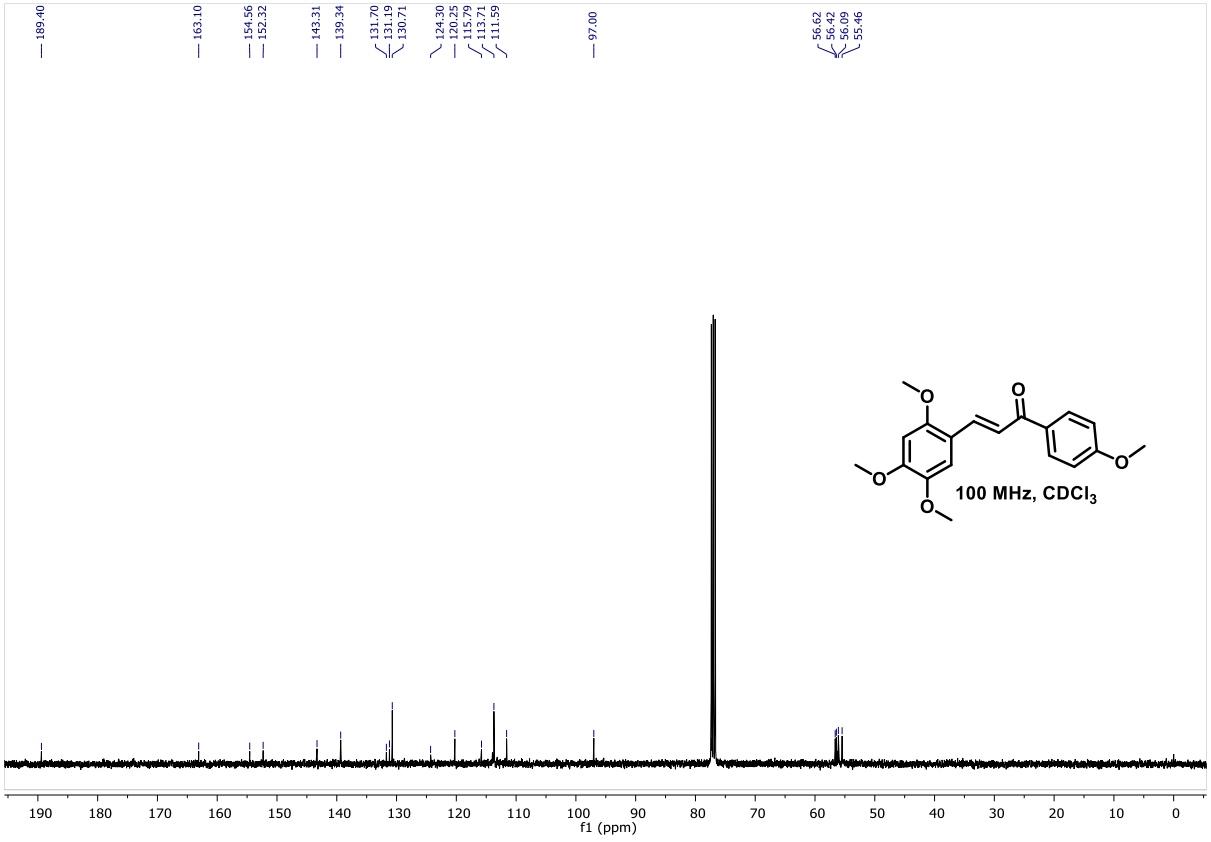


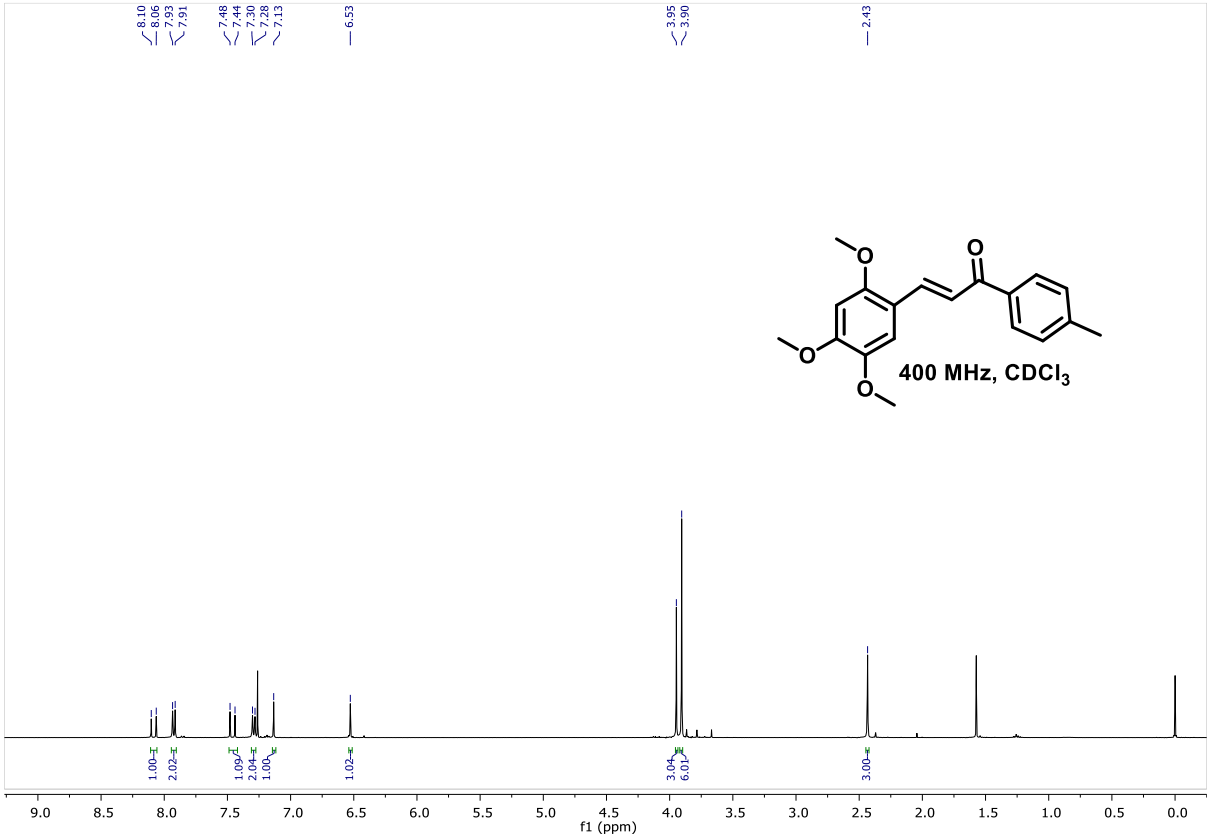
^{1}H NMR for 3d



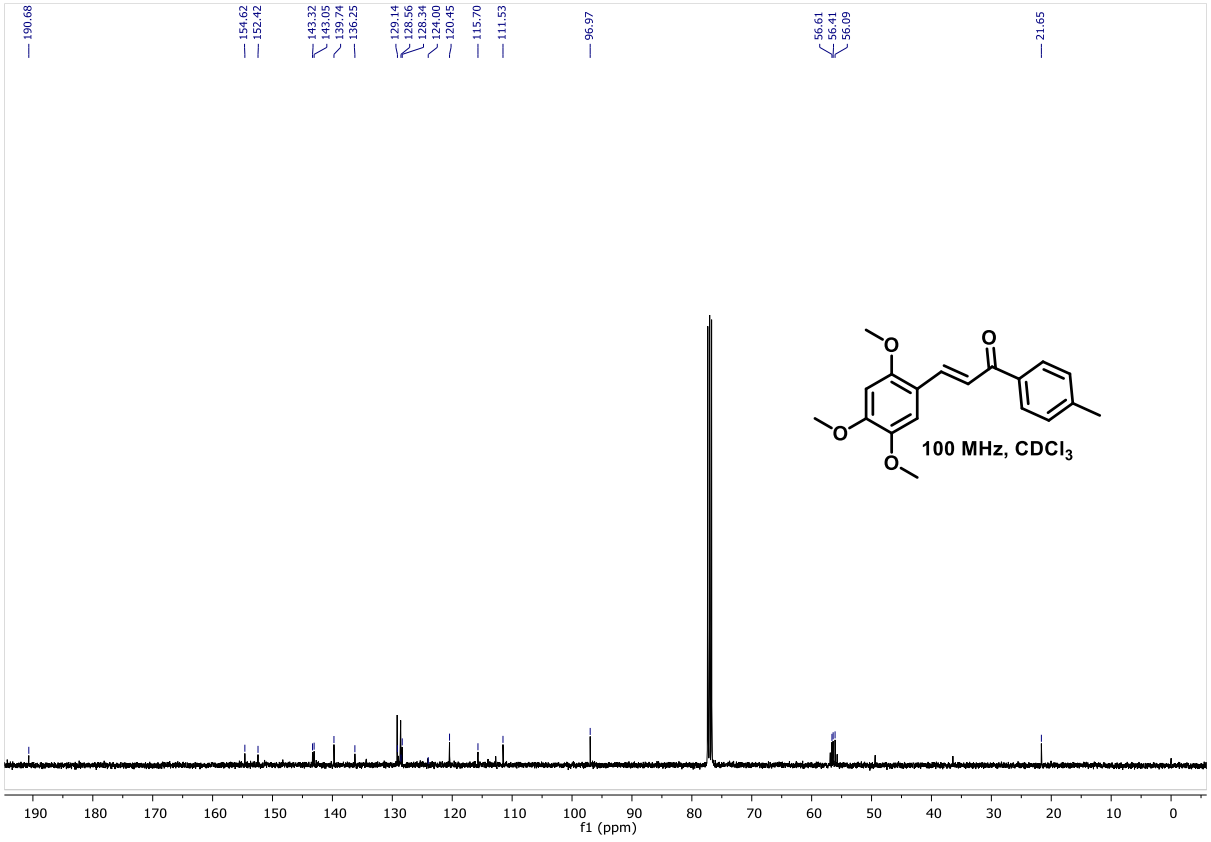
^{13}C NMR for 3d



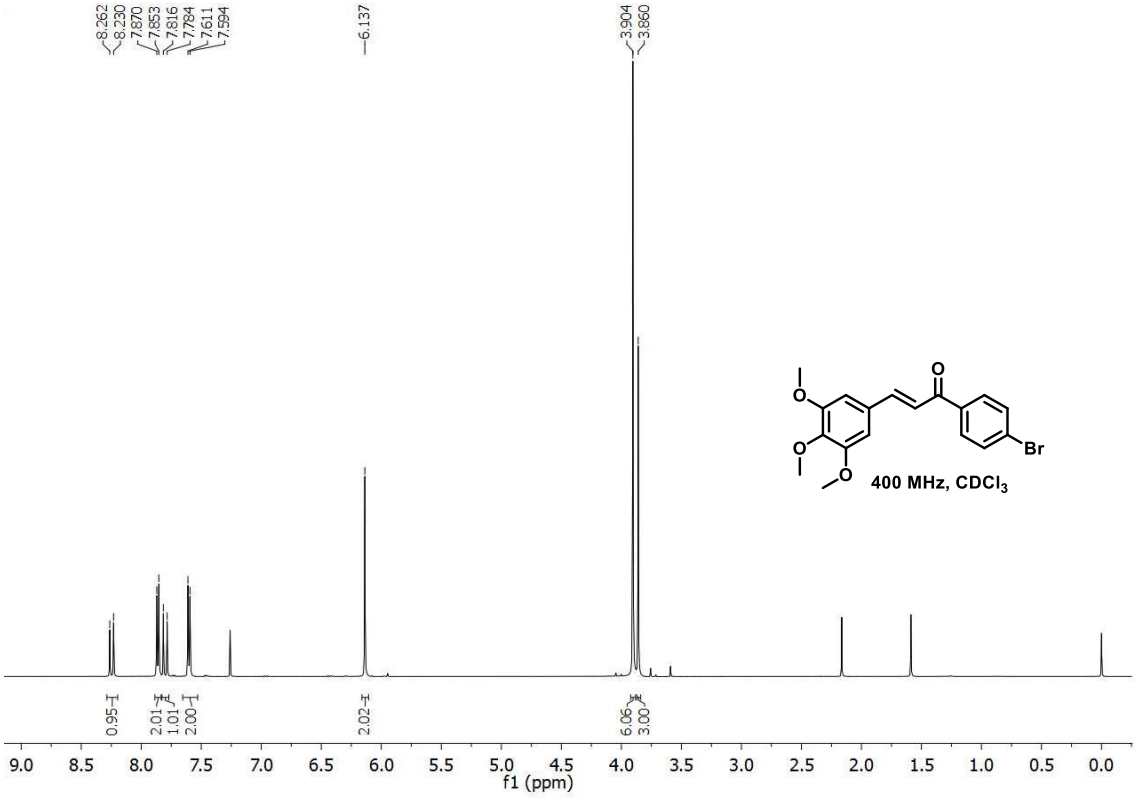
^{1}H NMR for 3e



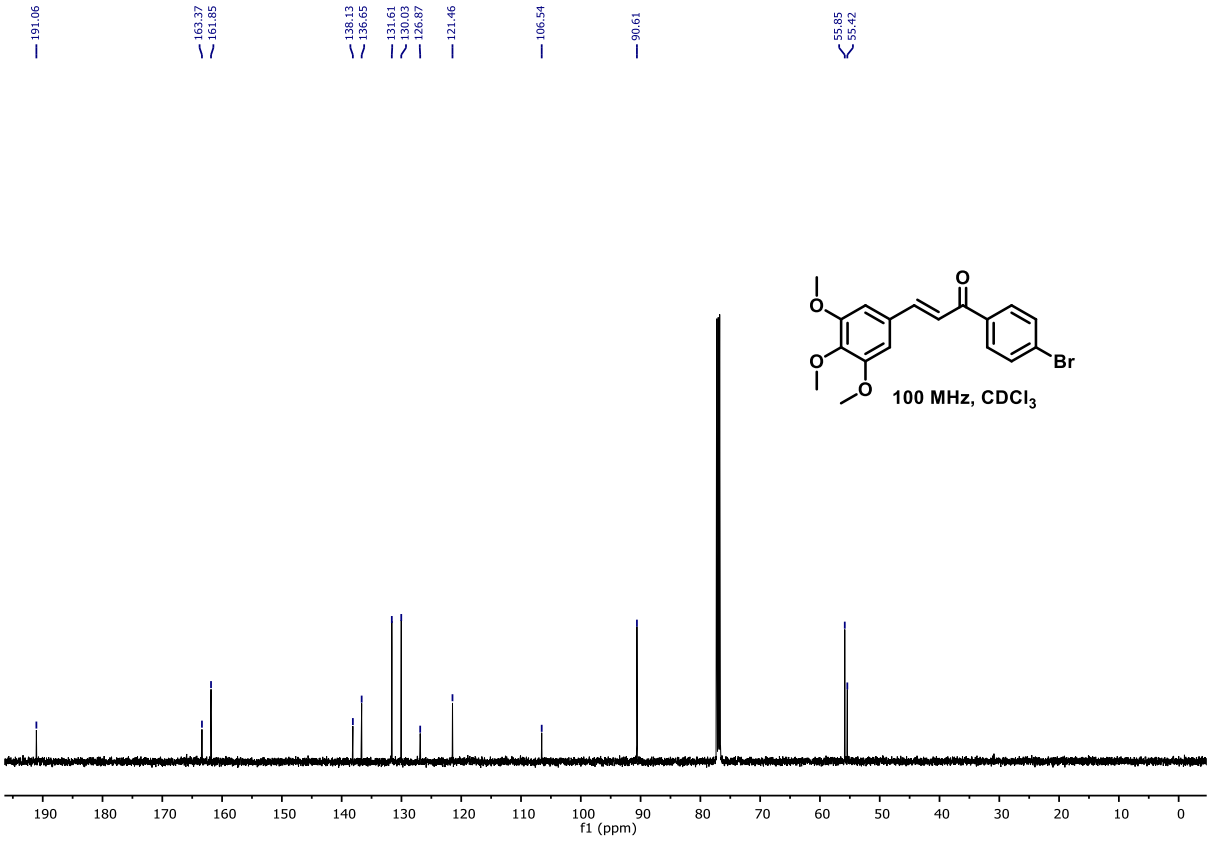
^{13}C NMR for 3e



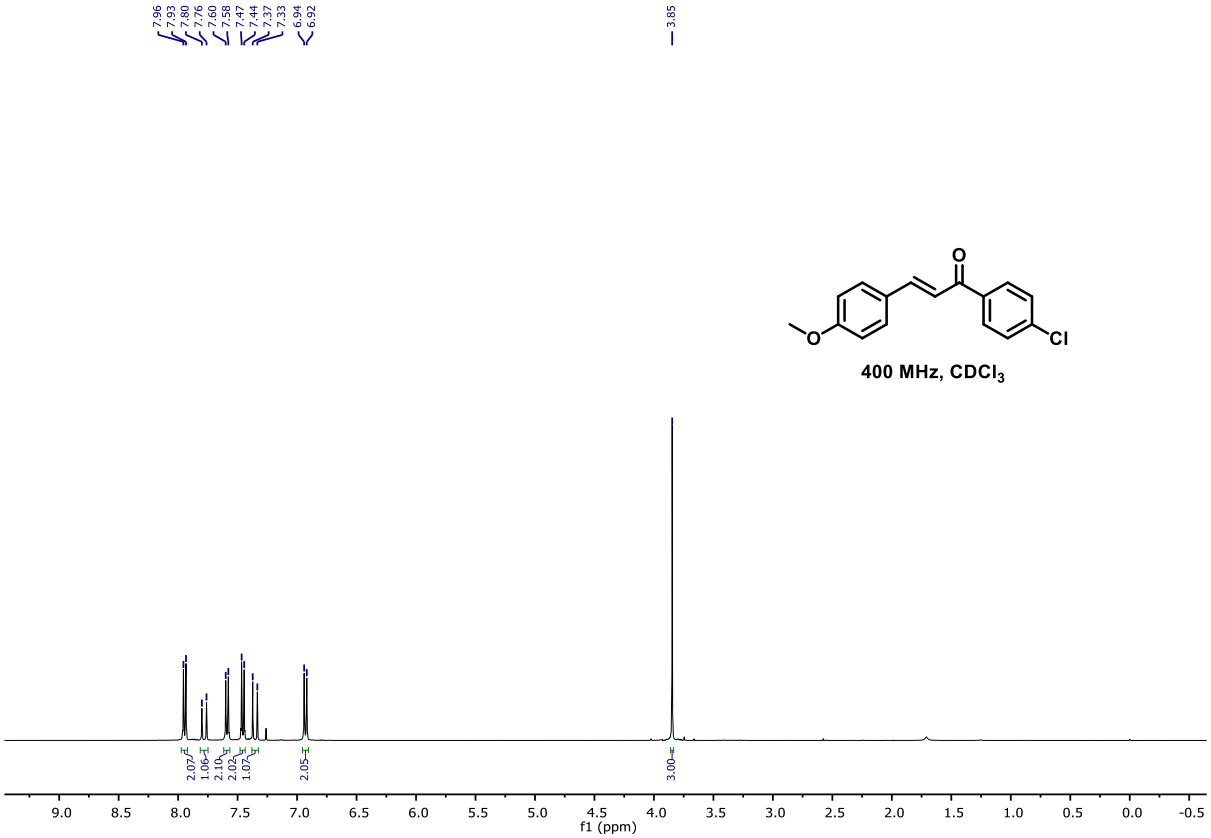
^{1}H NMR for 3f



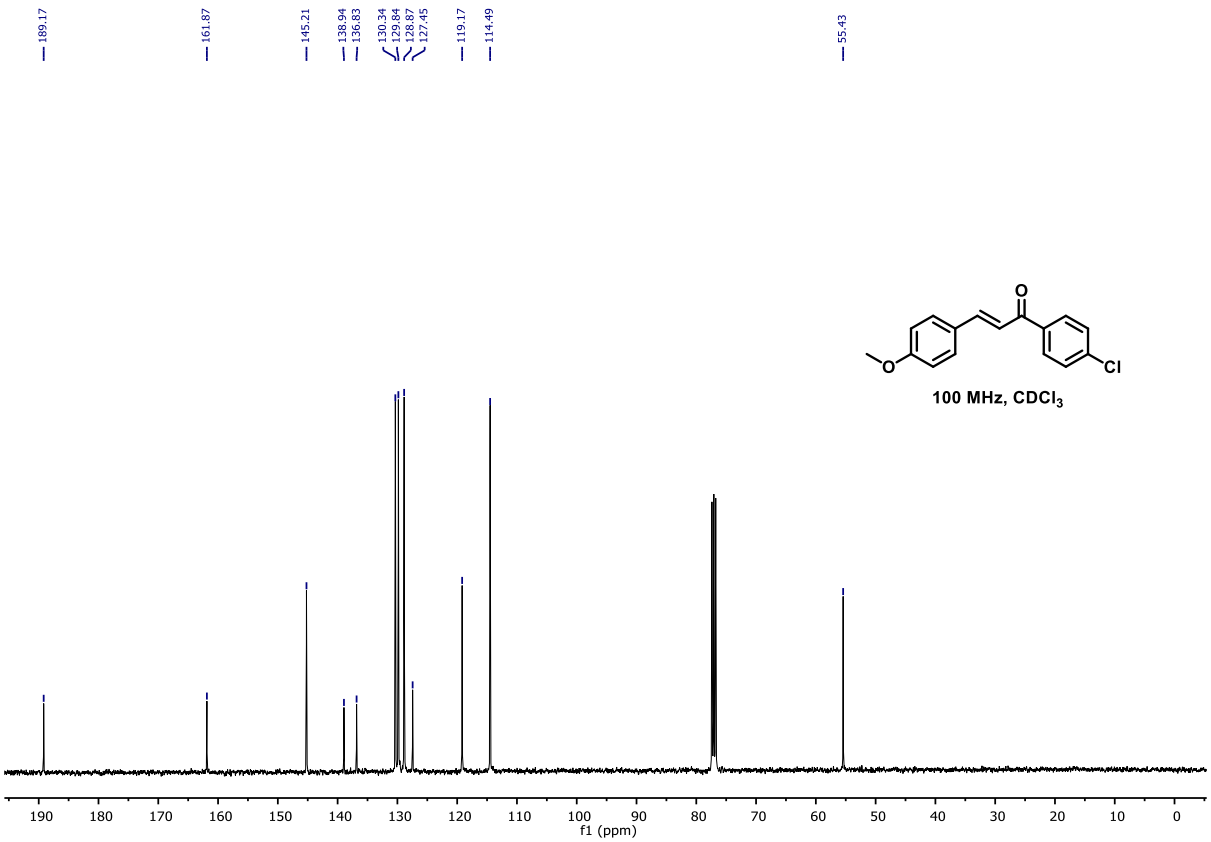
^{13}C NMR for 3f



^{1}H NMR for 3g

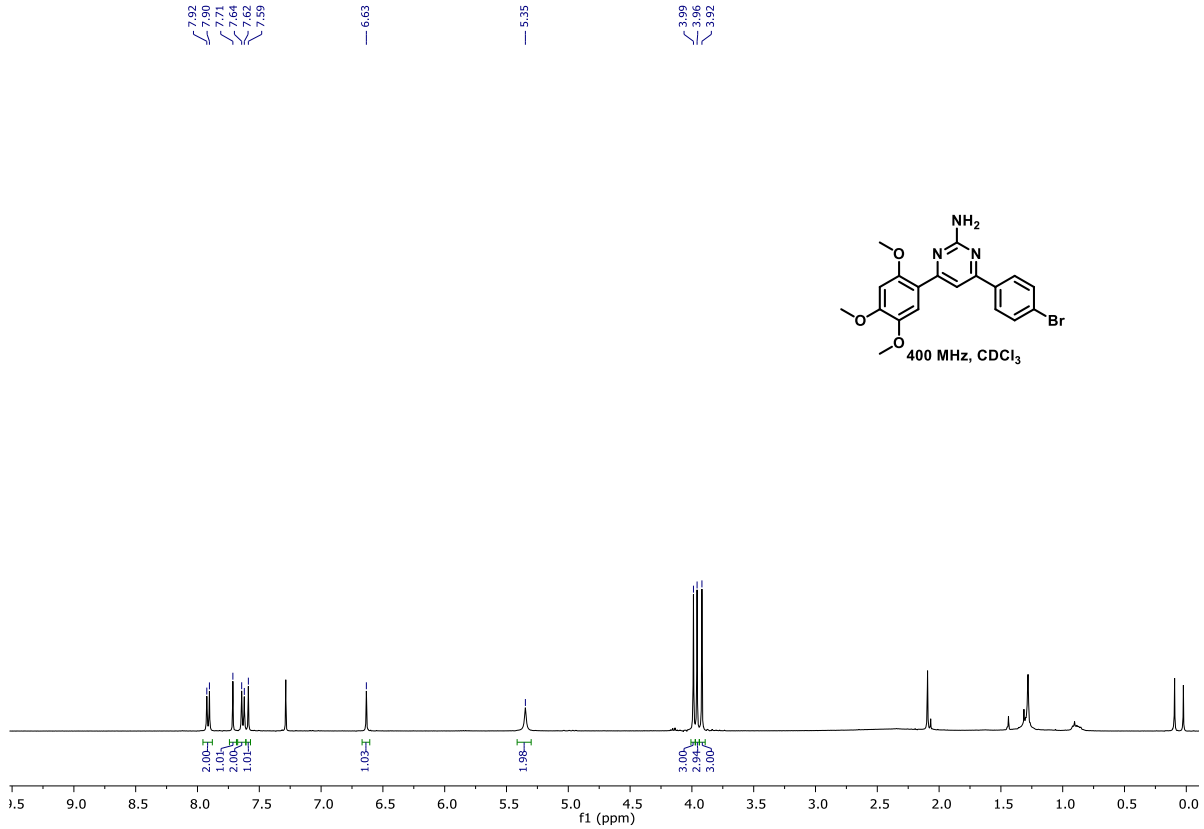


^{13}C NMR for 3g

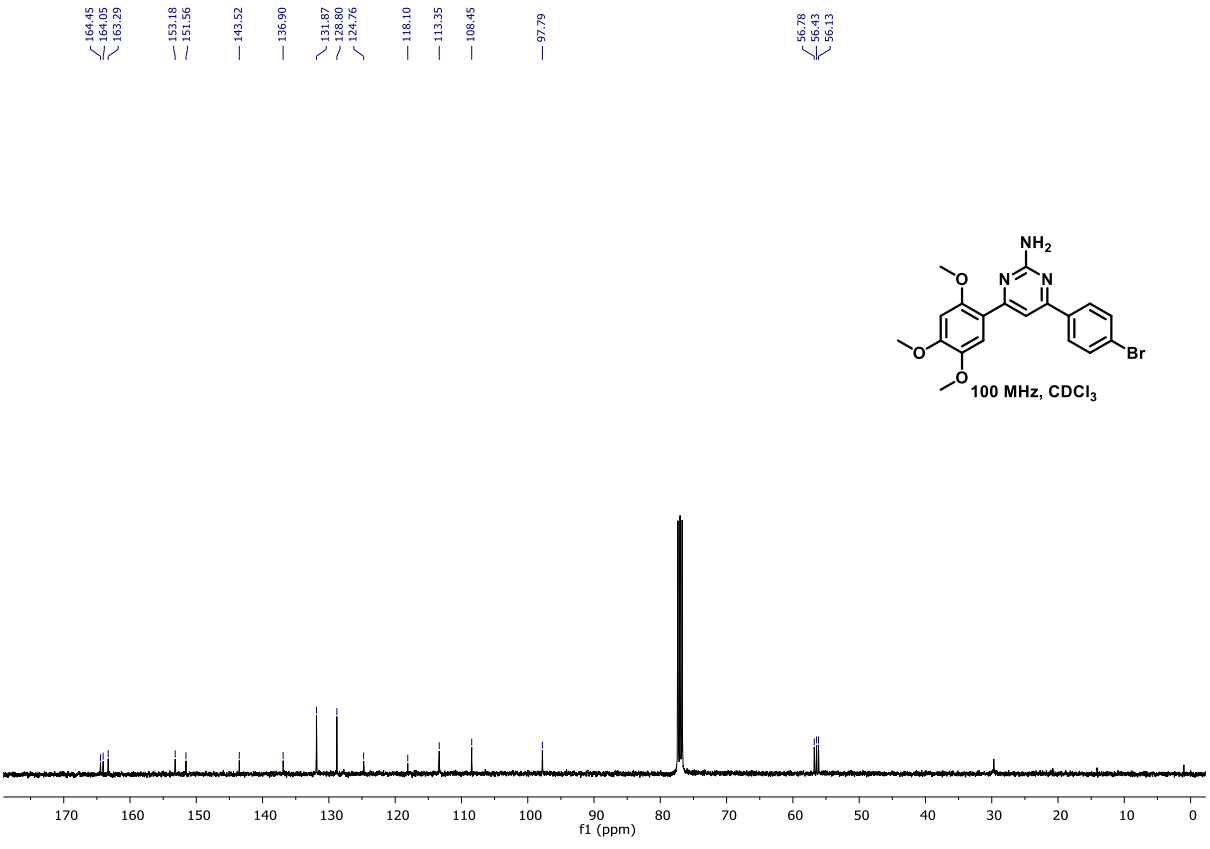


4. Spectral data for pyrimidine derivatives

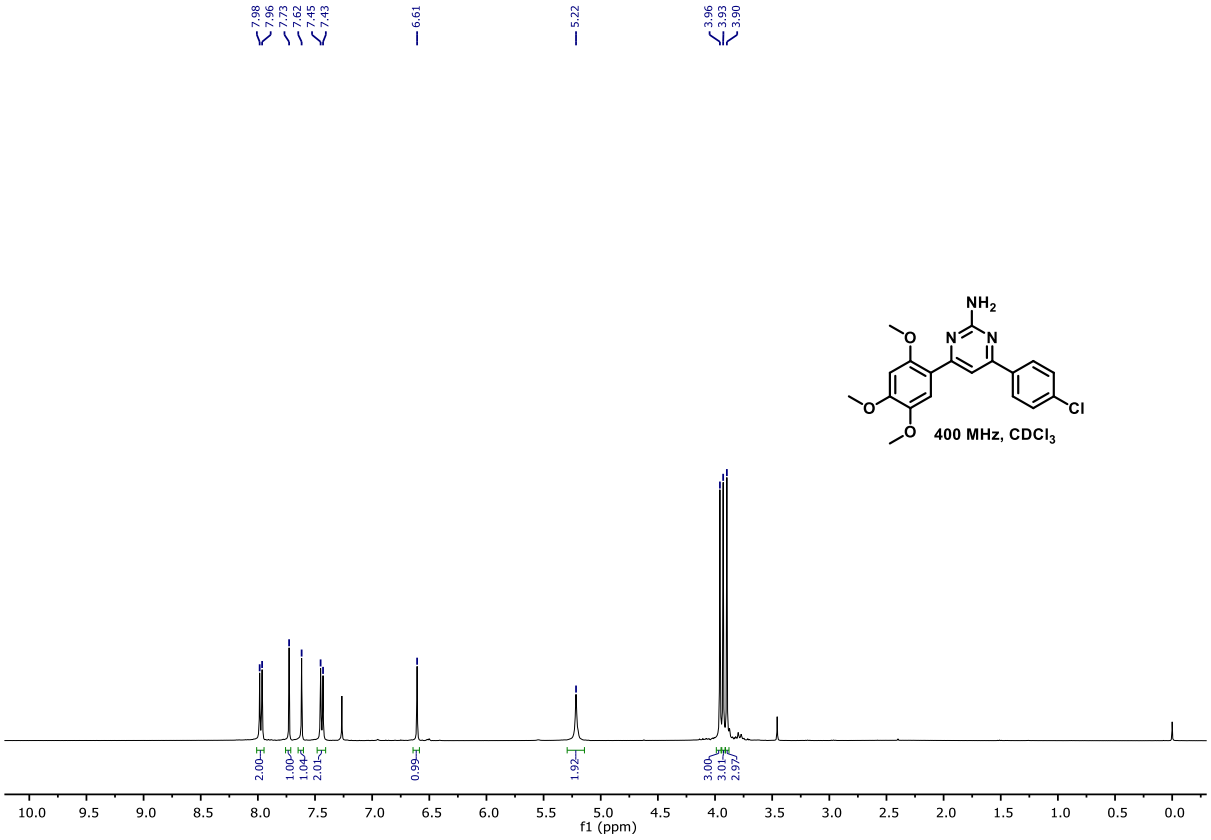
^{1}H NMR for 5a



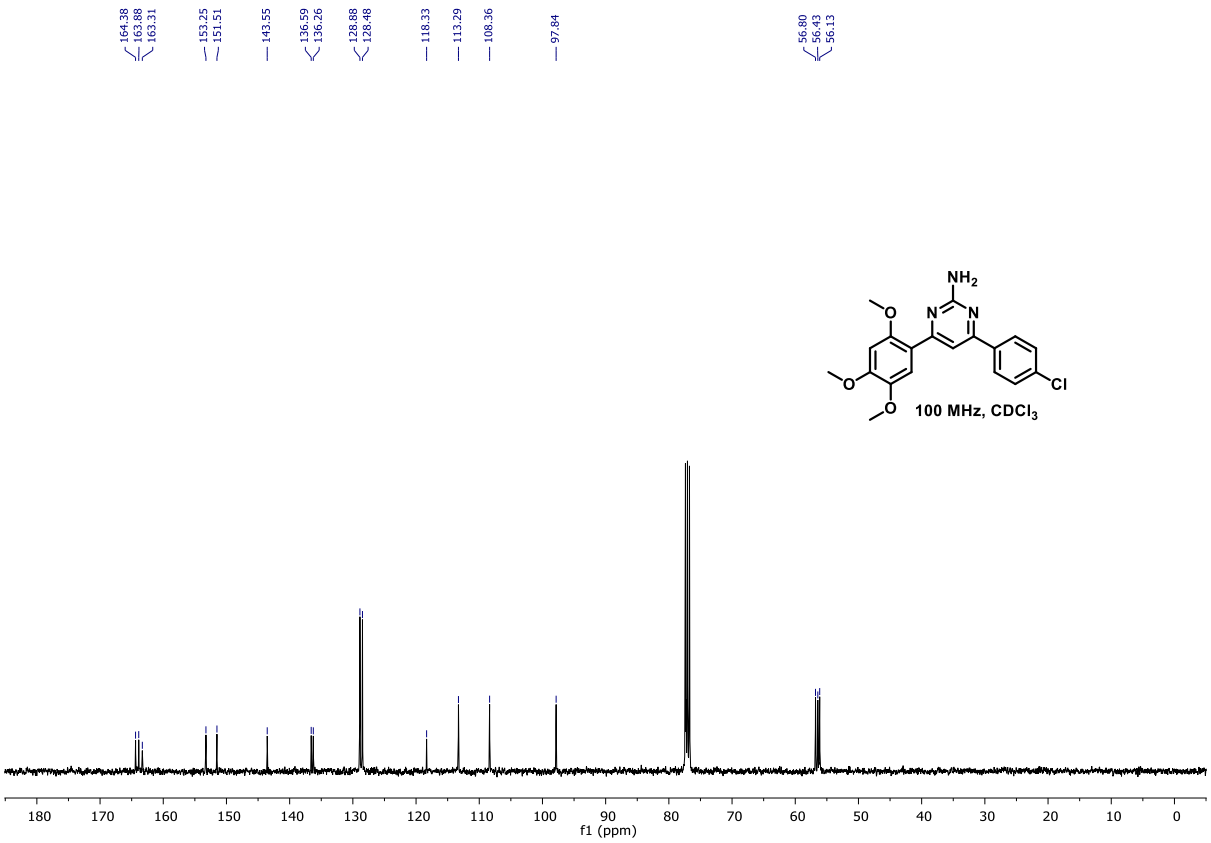
^{13}C NMR for 5a



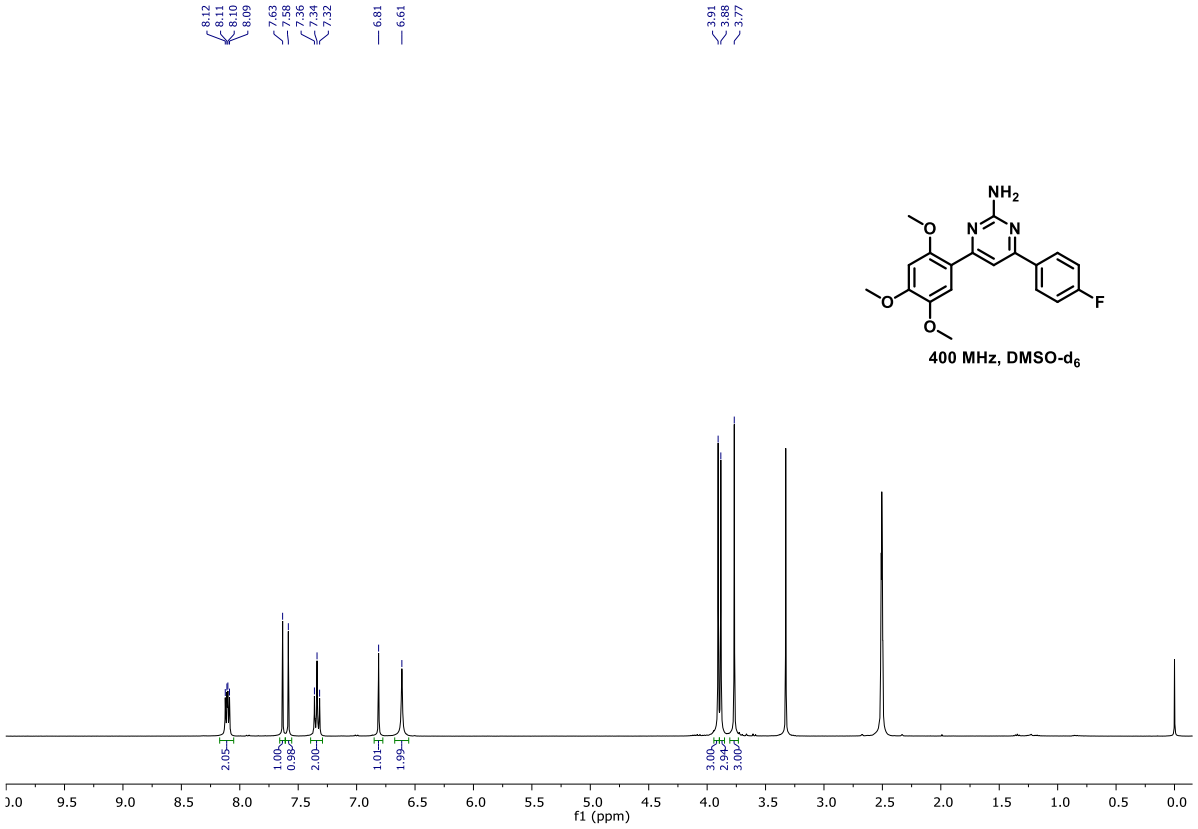
^{1}H NMR for 5b



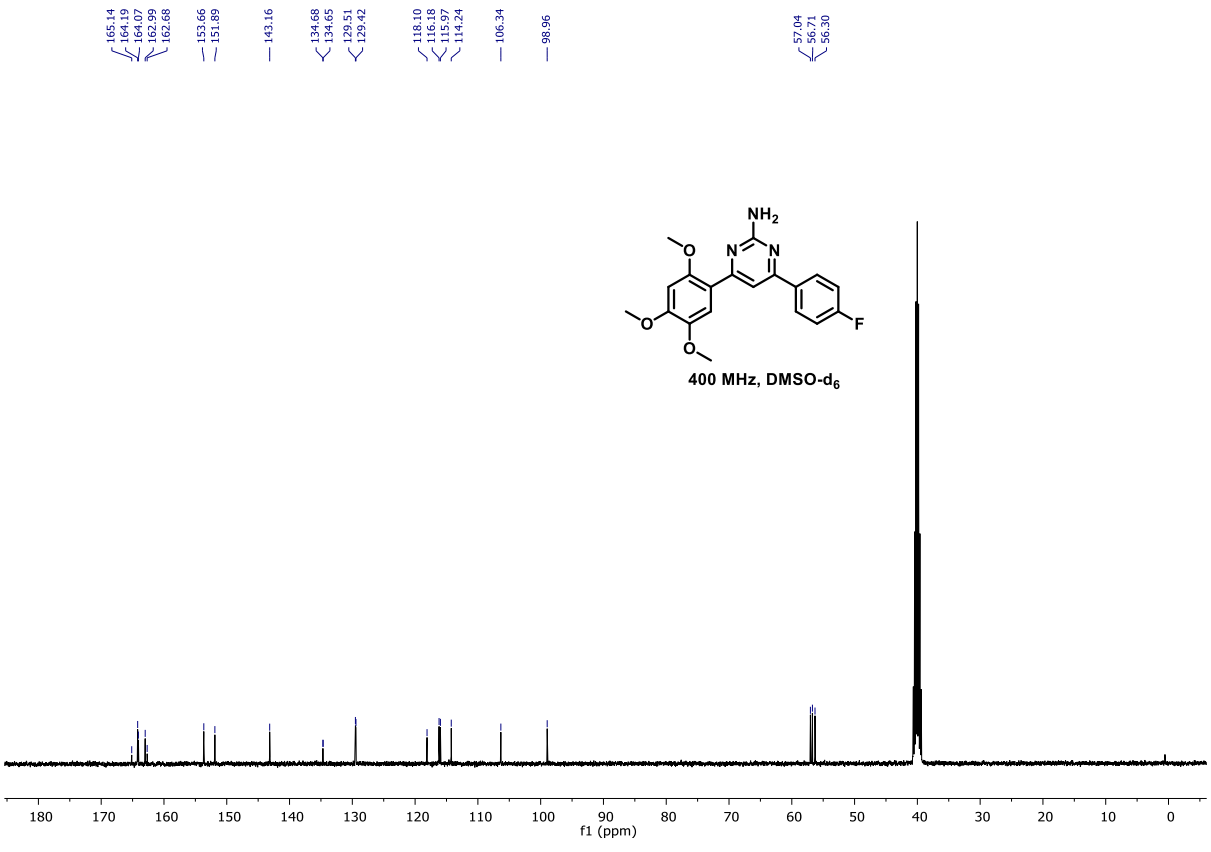
^{13}C NMR for 5b



^{1}H NMR for 5c

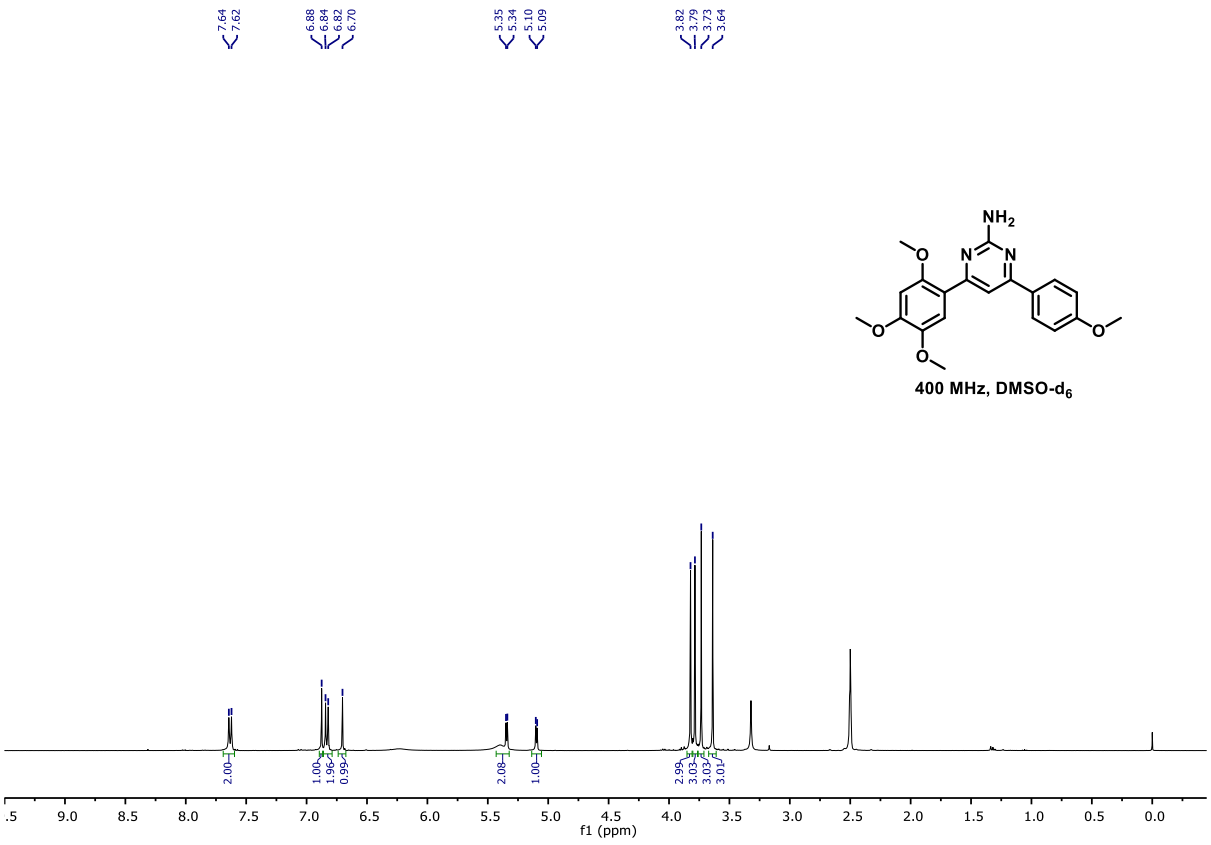


^{13}C NMR for 5c

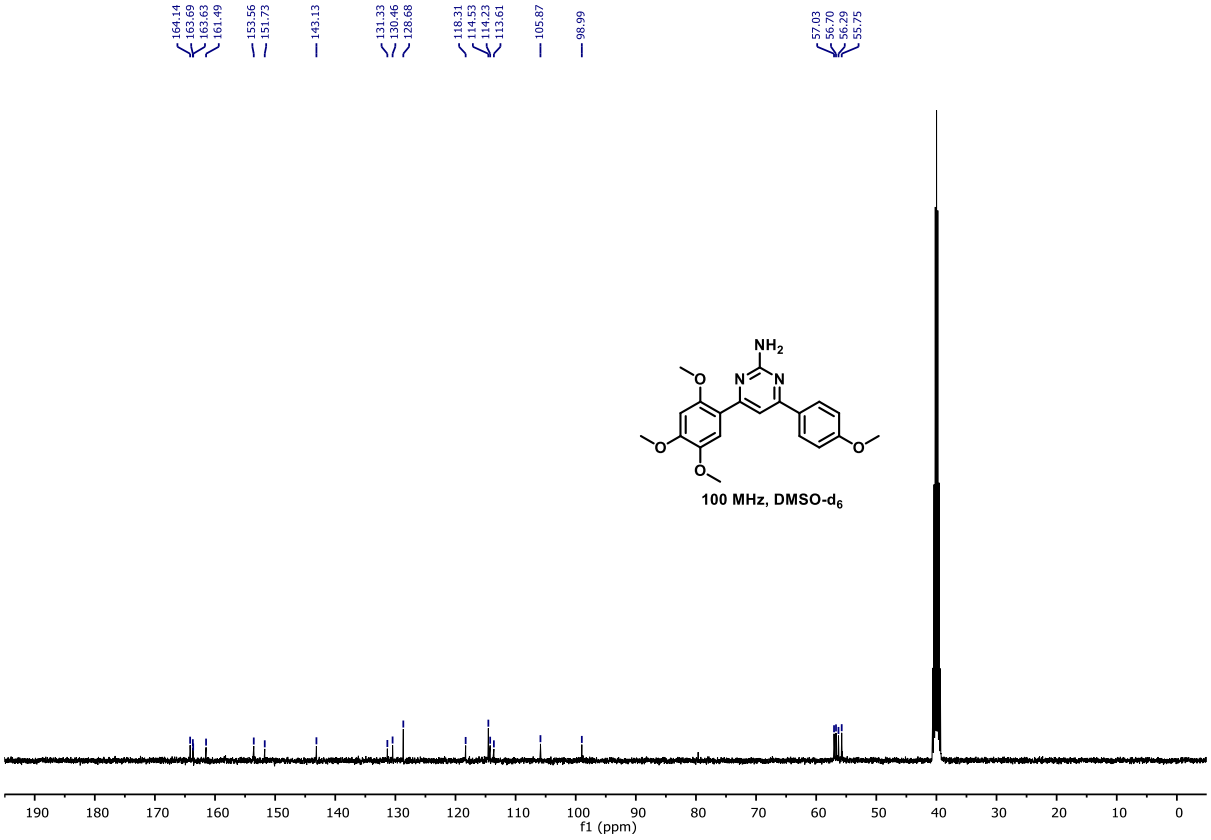


^{19}F NMR for 5c

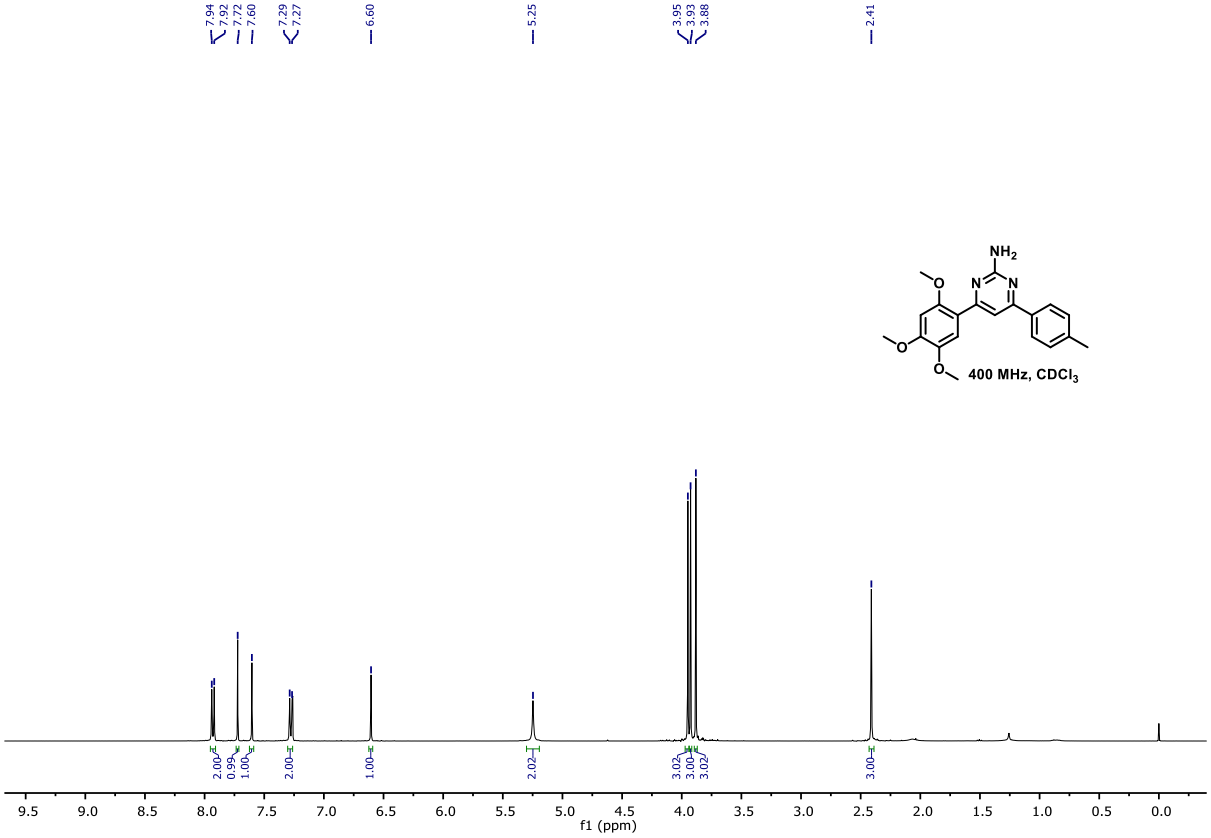
^{1}H NMR for 5d



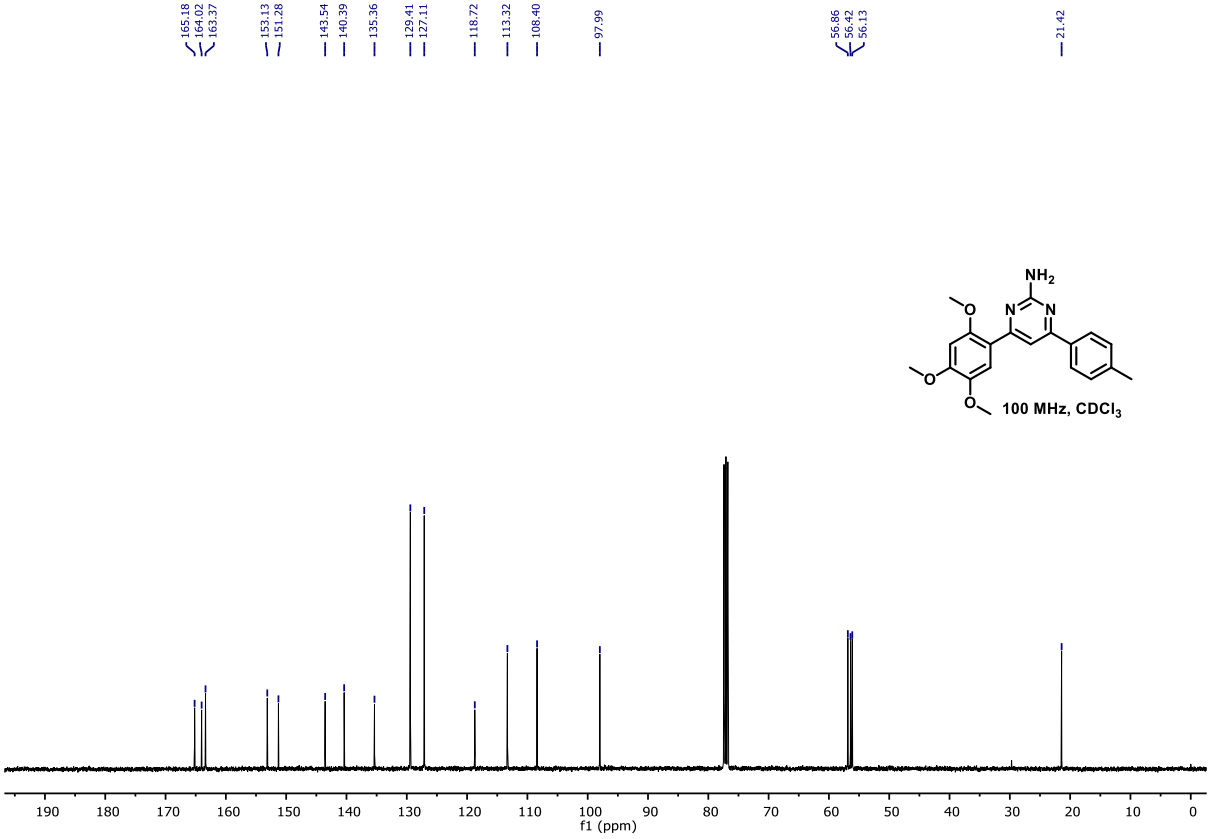
^{13}C NMR for 5d



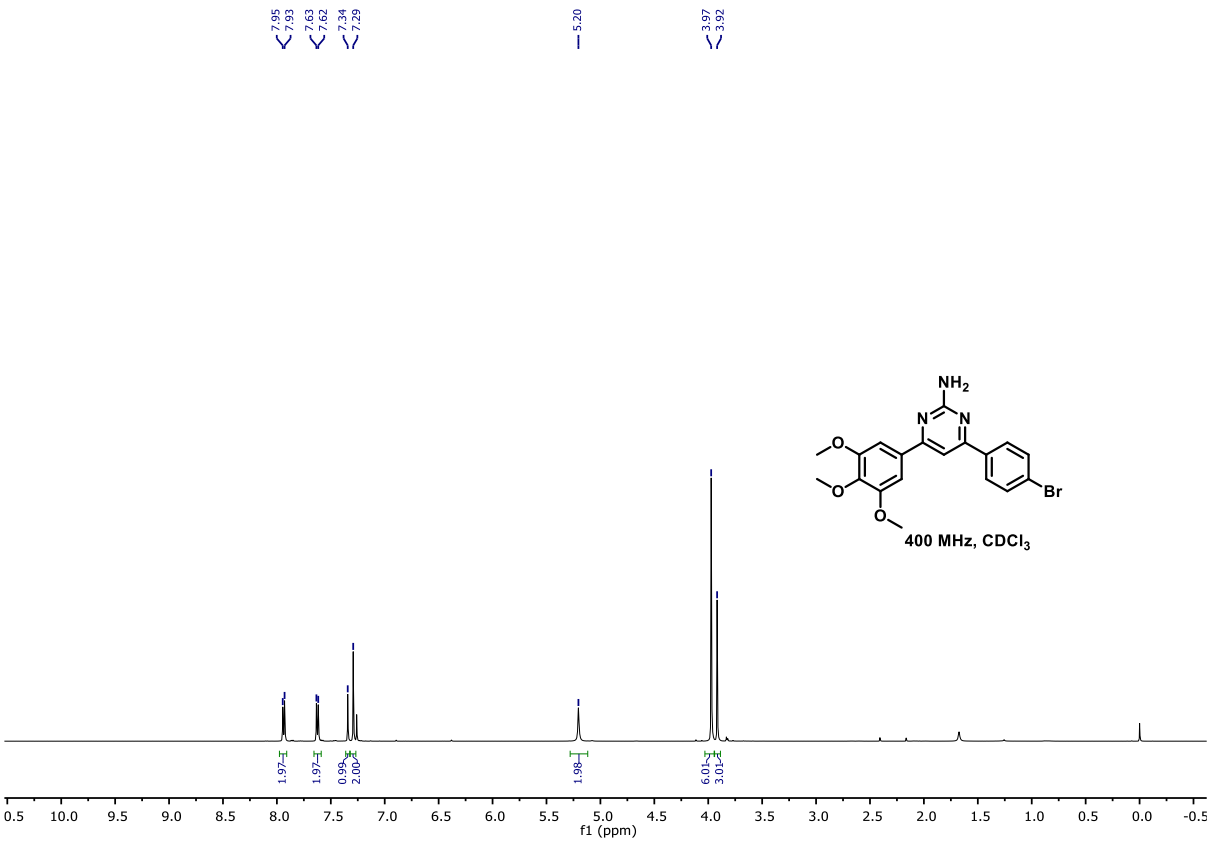
^{1}H NMR for 5e



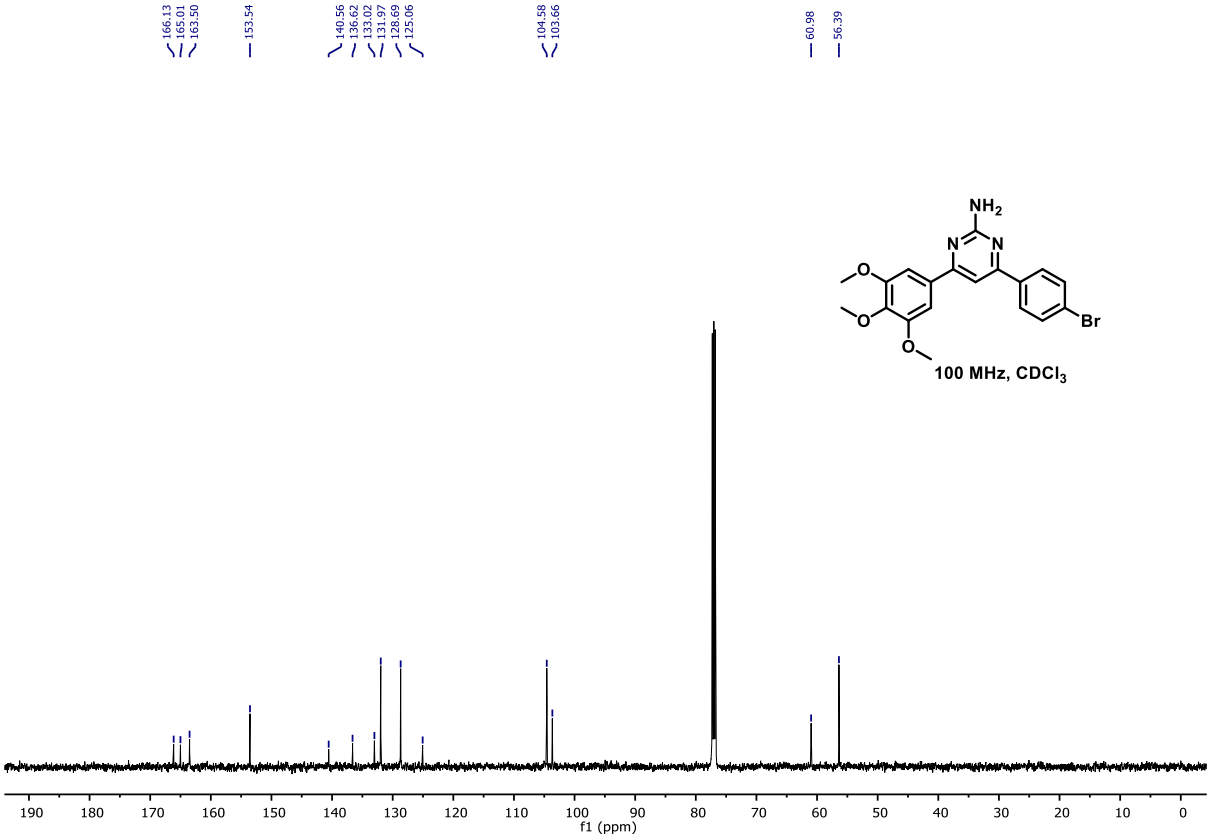
^{13}C NMR for 5e



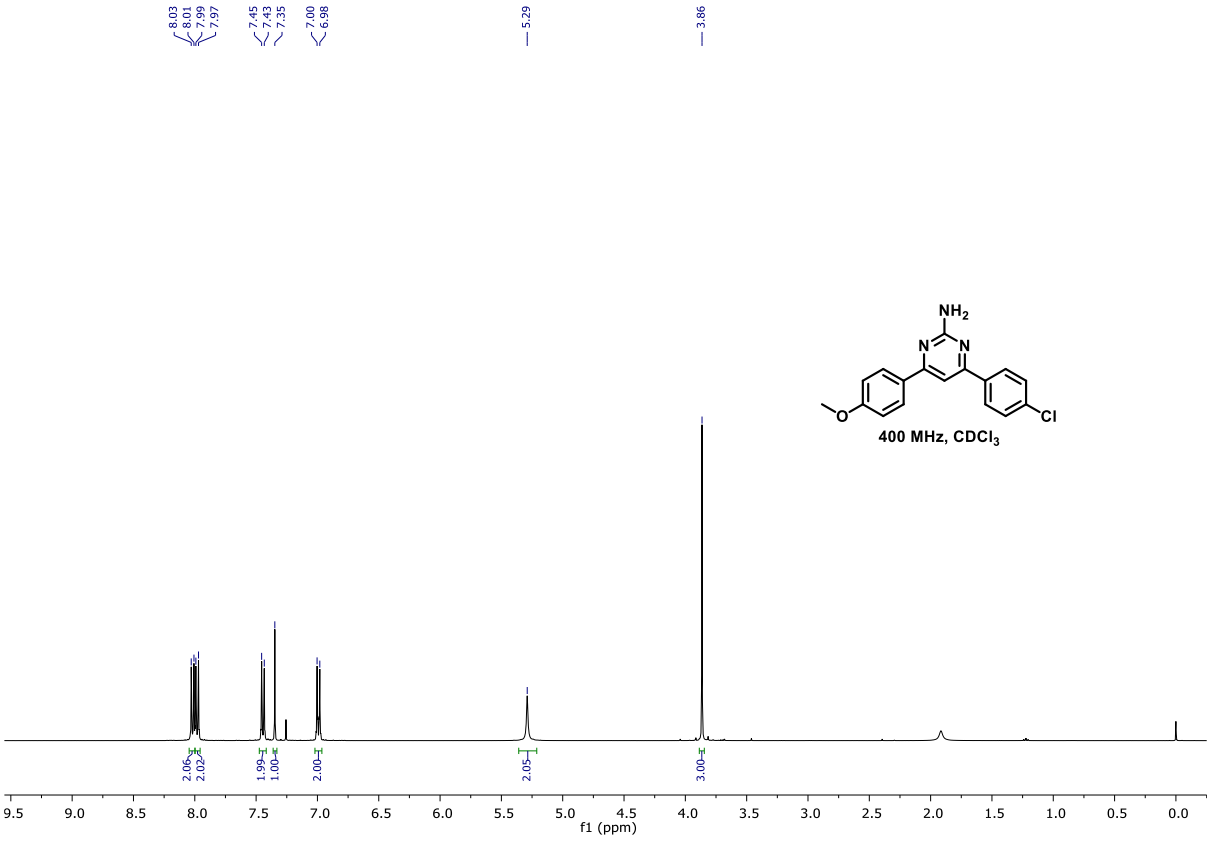
^{1}H NMR for 5f



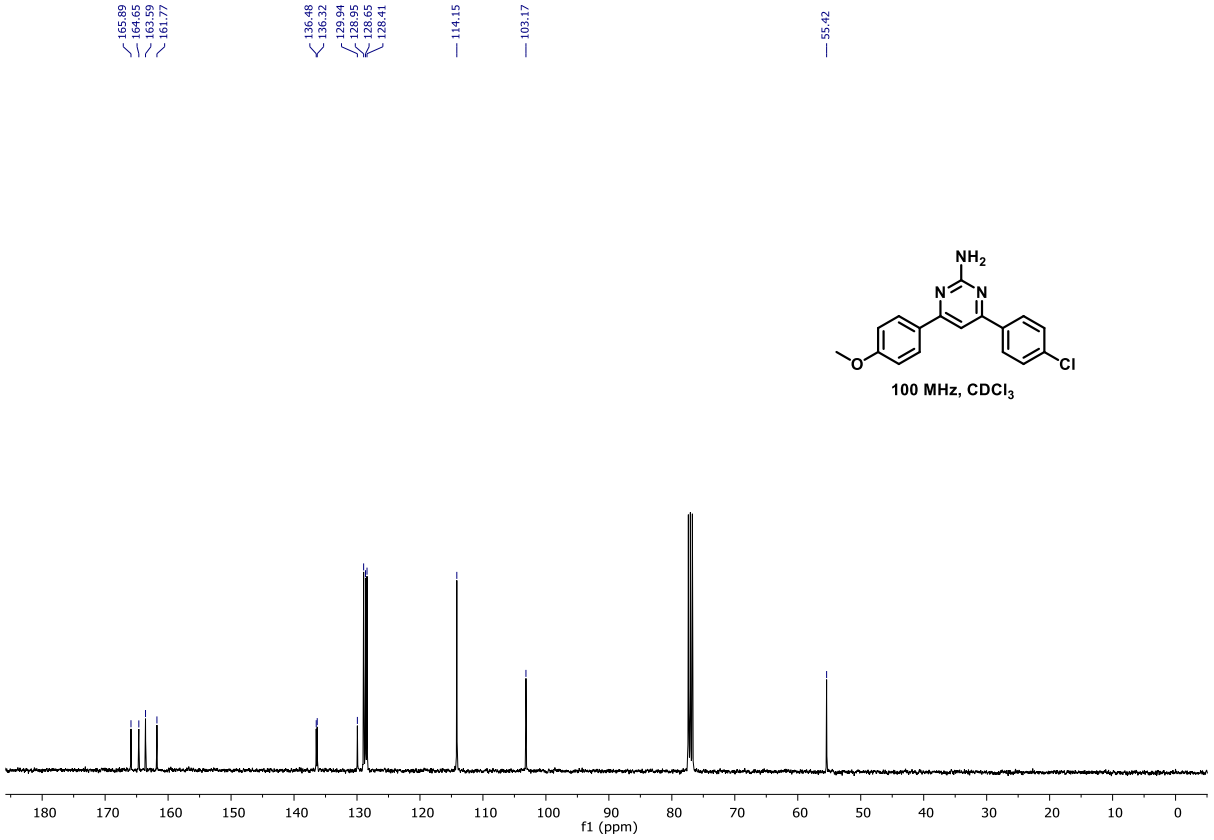
^{13}C NMR for 5f



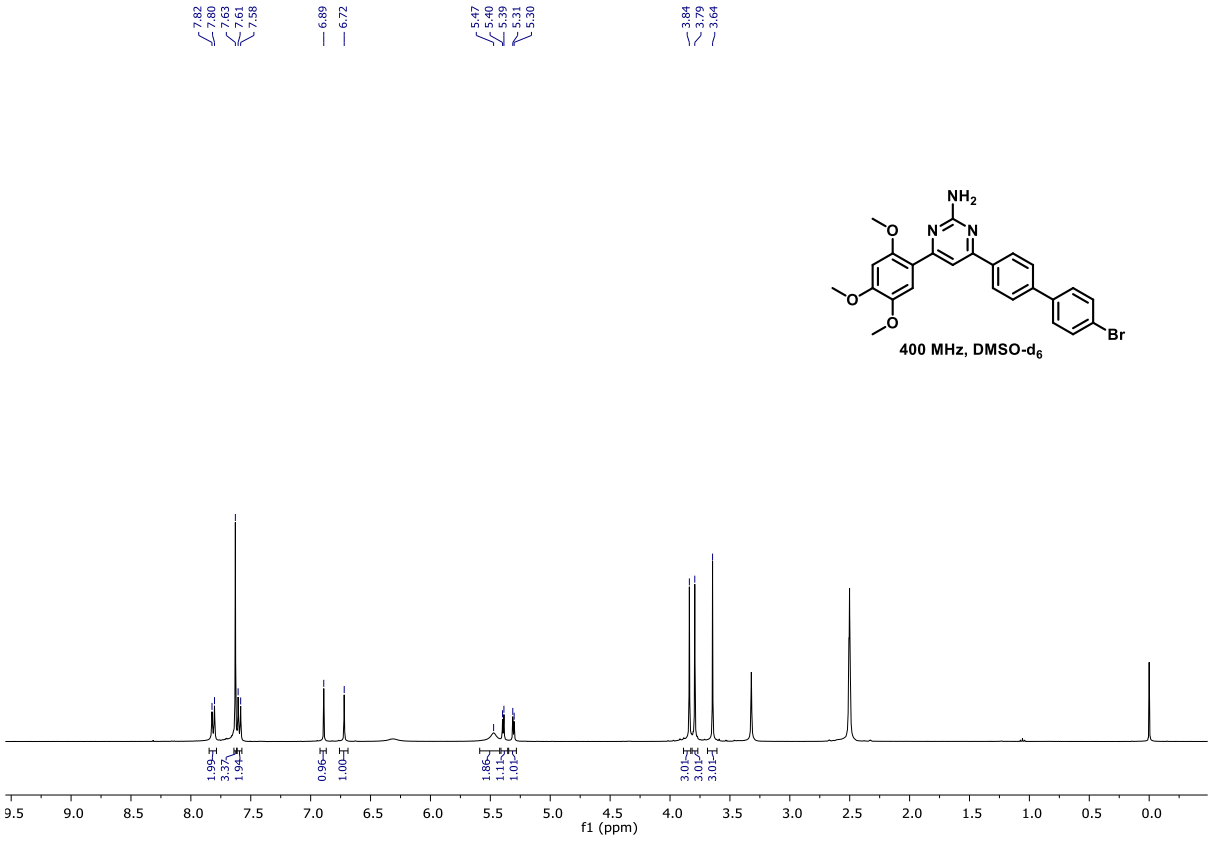
^{1}H NMR for 5g



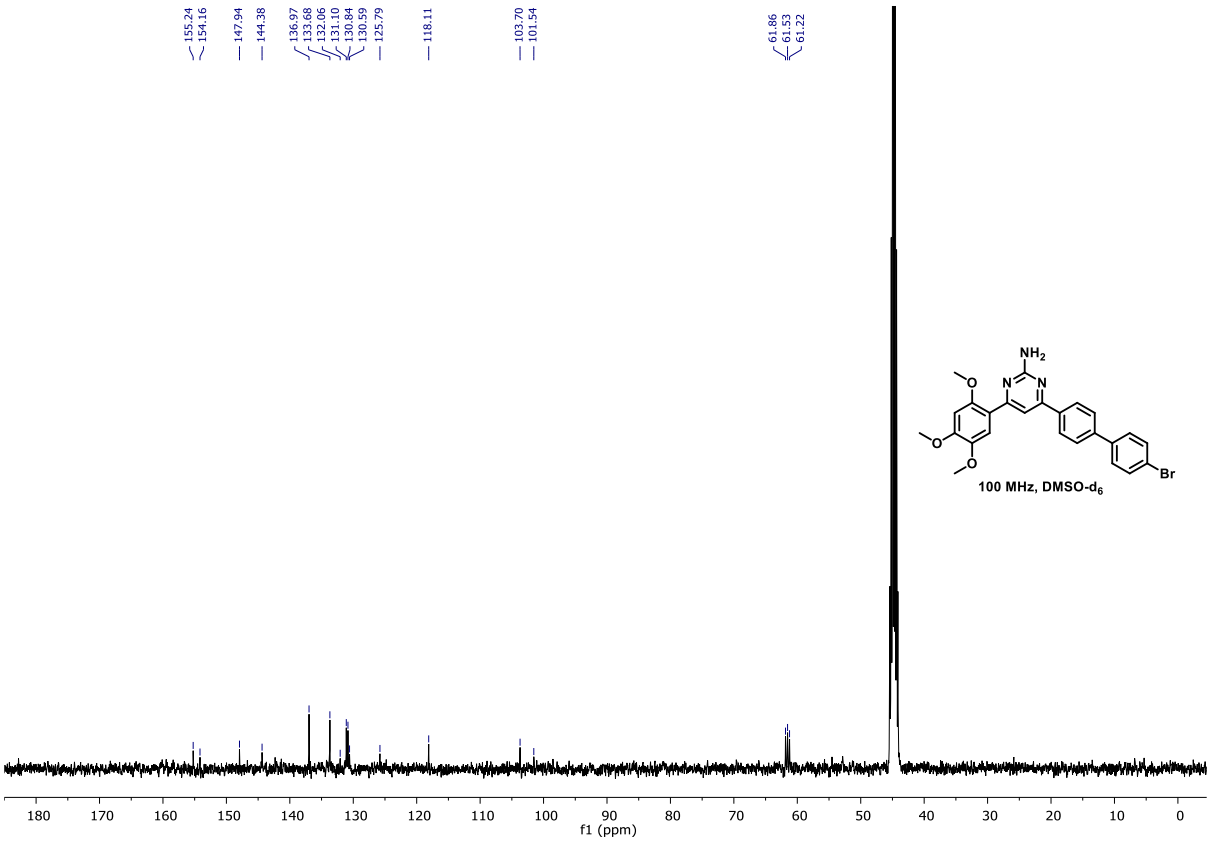
^{13}C NMR for 5g



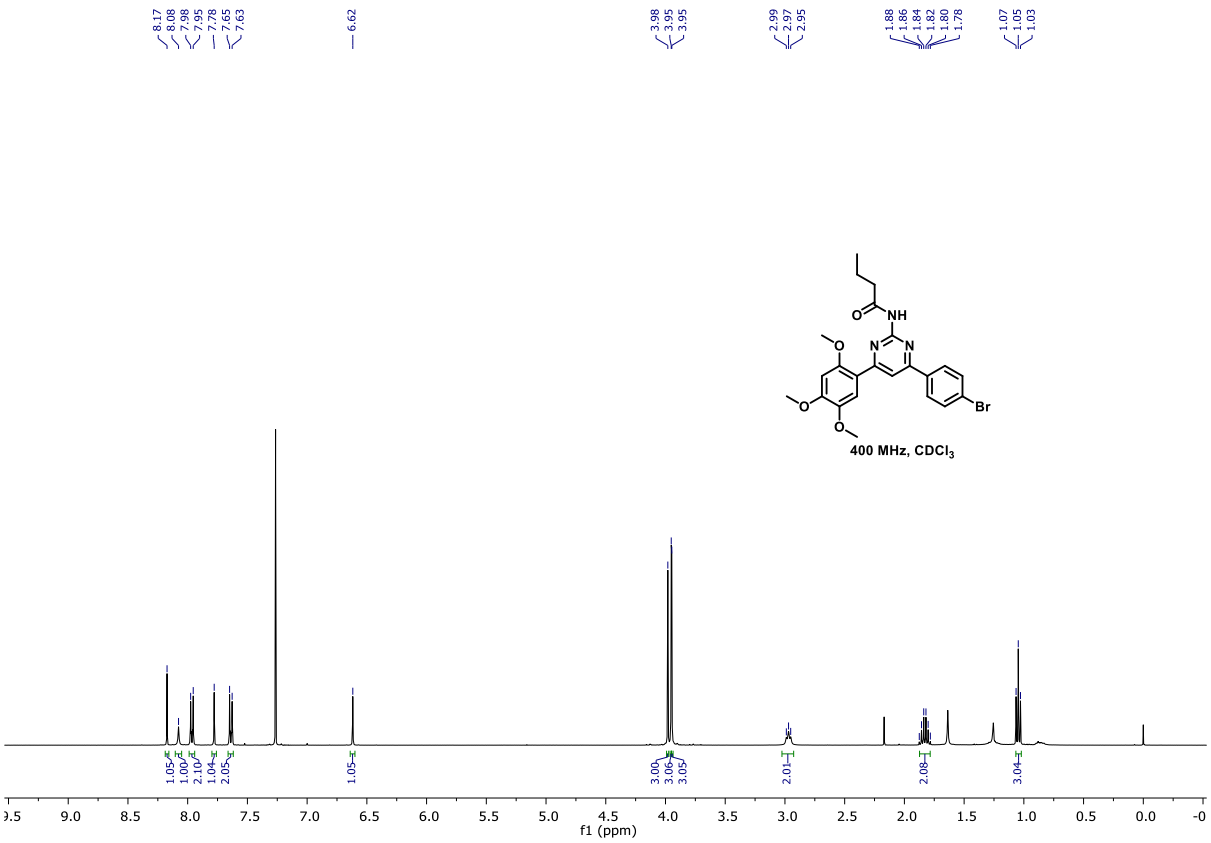
^{1}H NMR for 5h



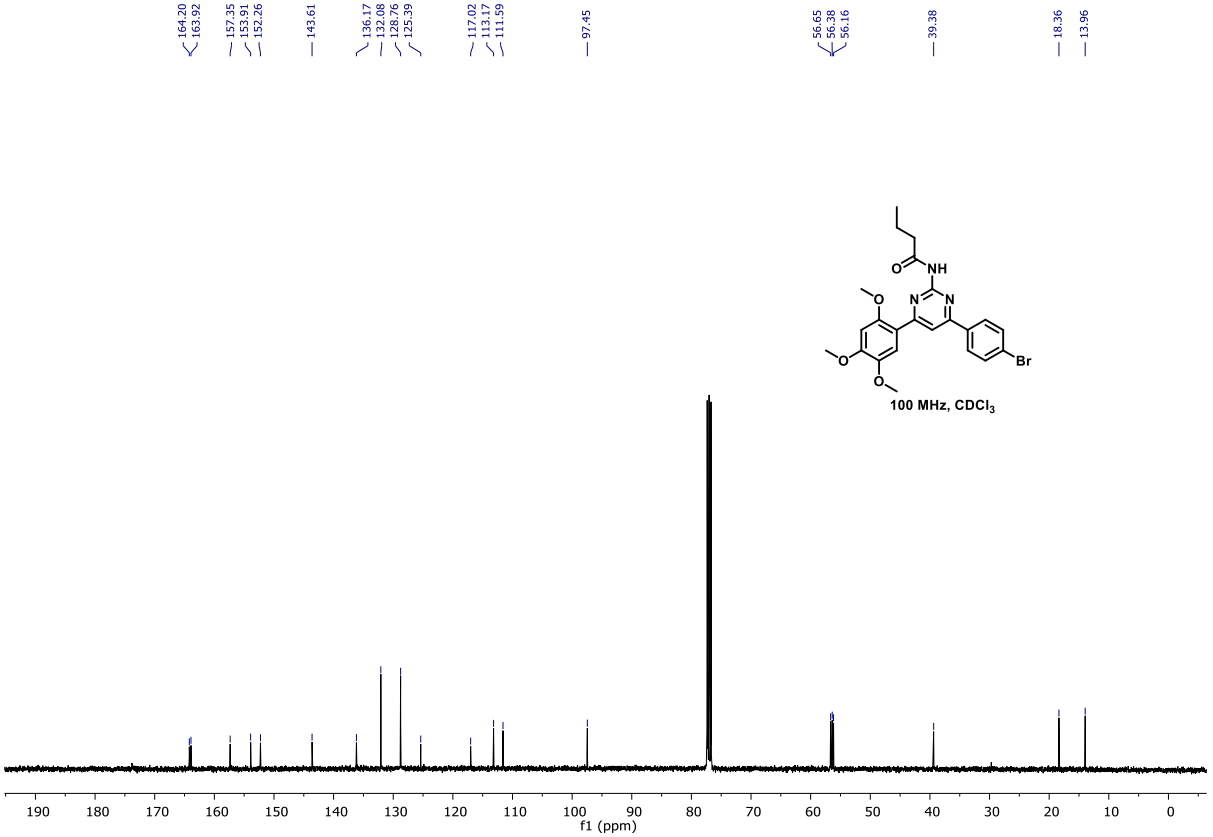
^{13}C NMR for 5h



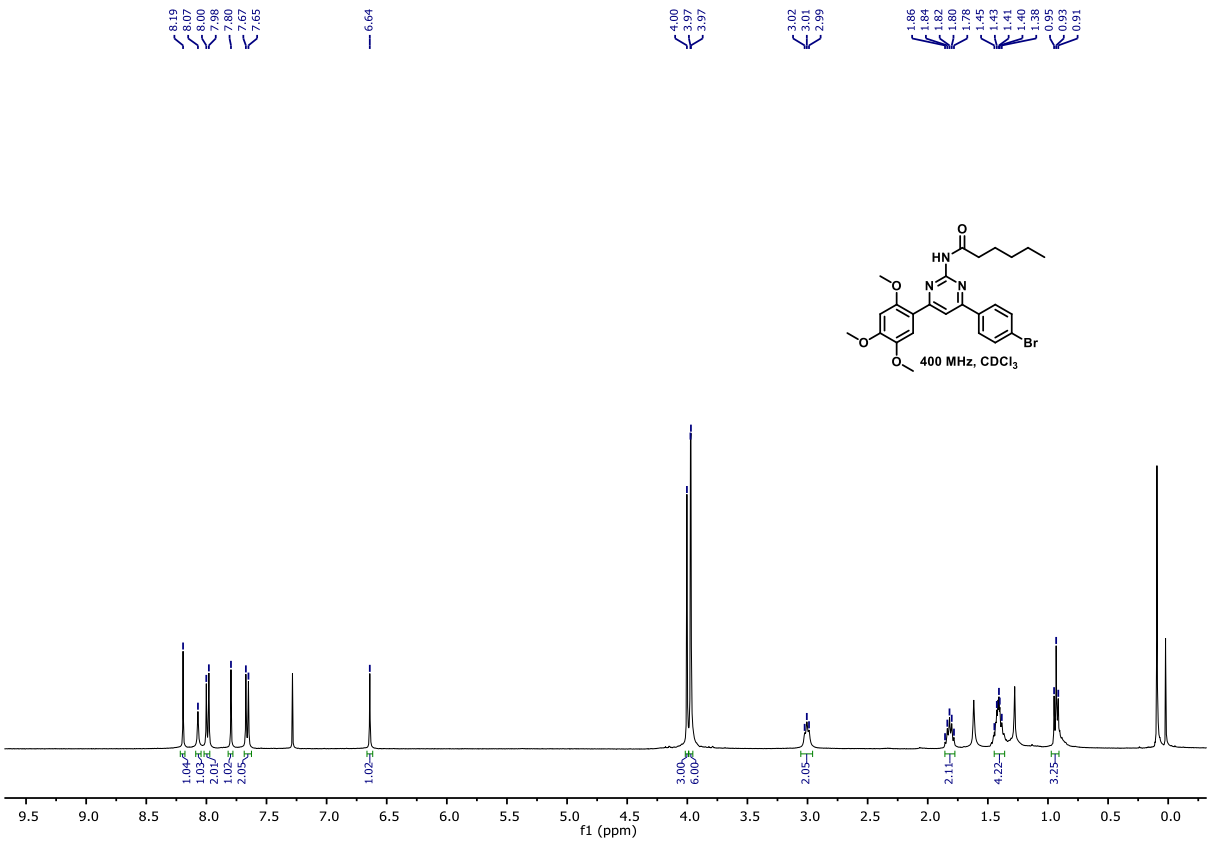
^{1}H NMR for 7a



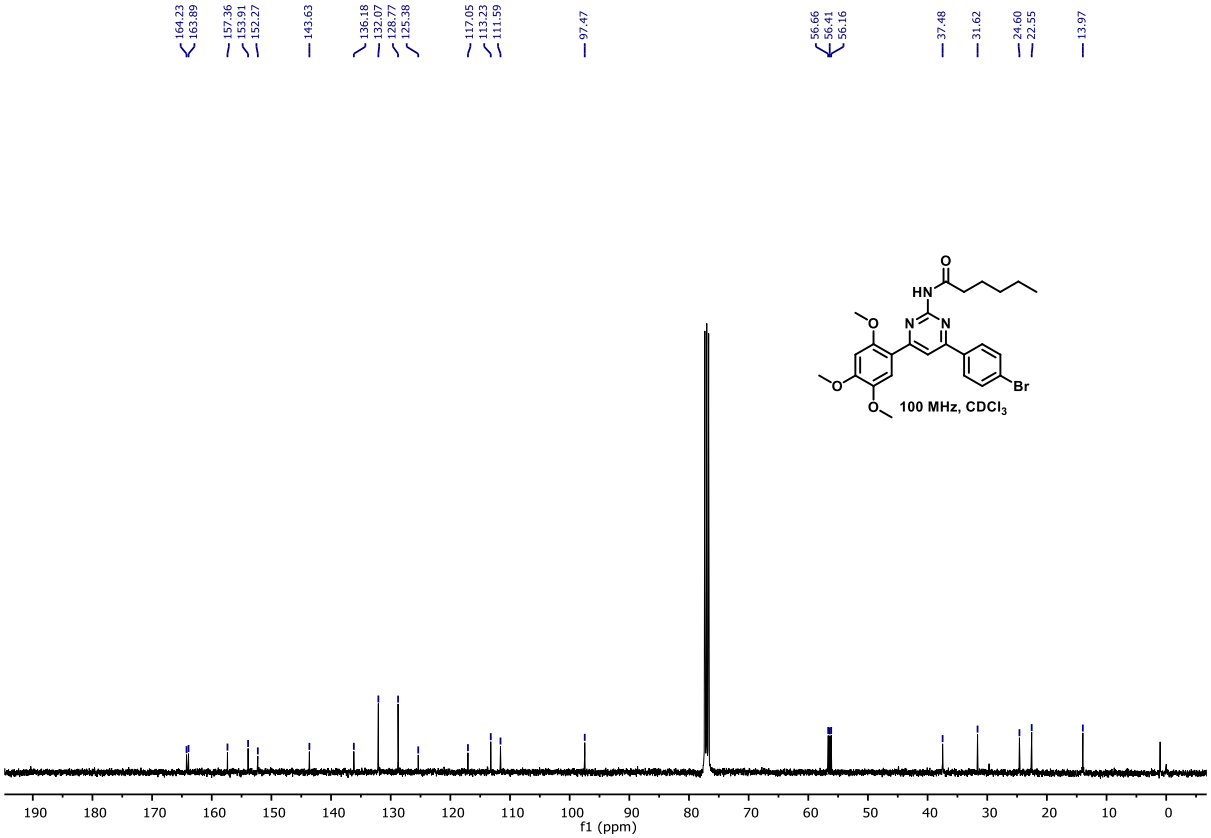
^{13}C NMR for 7a



^{1}H NMR for 7b



^{13}C NMR for 7b



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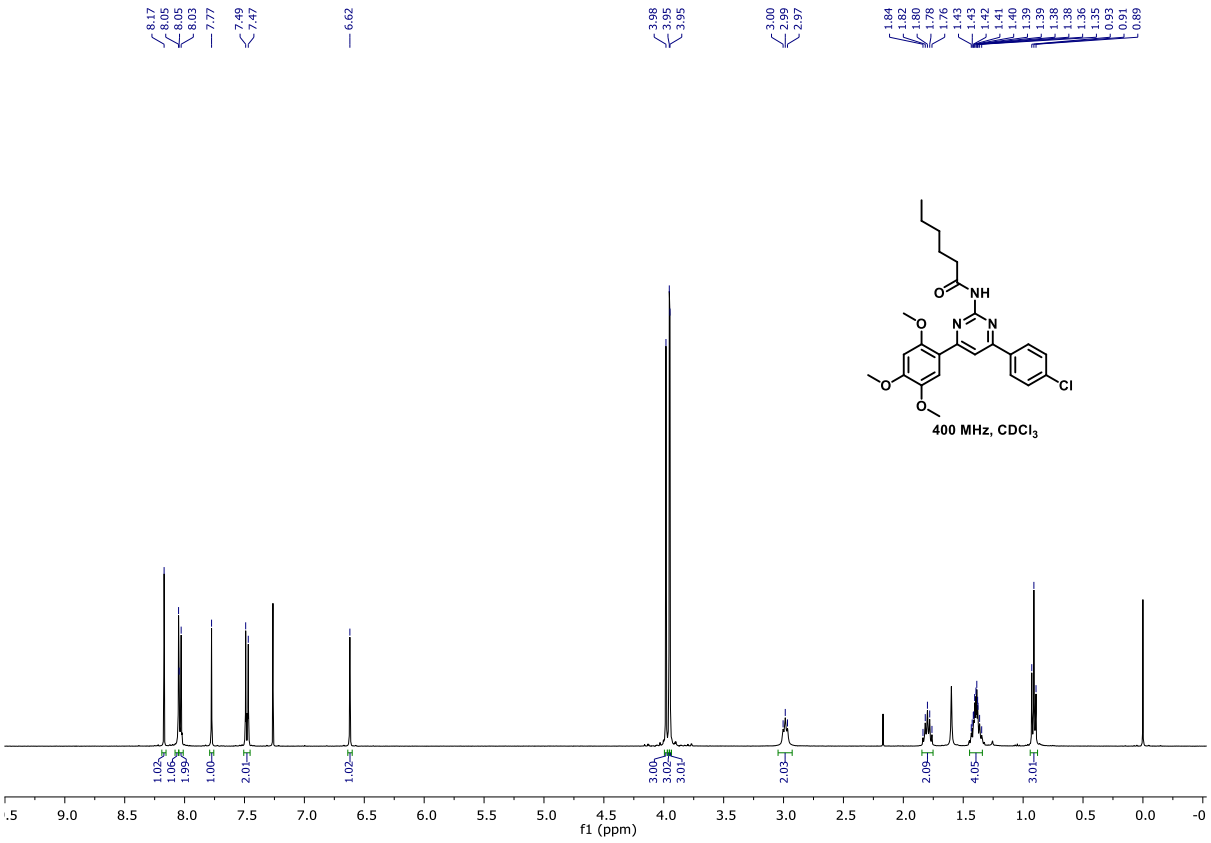

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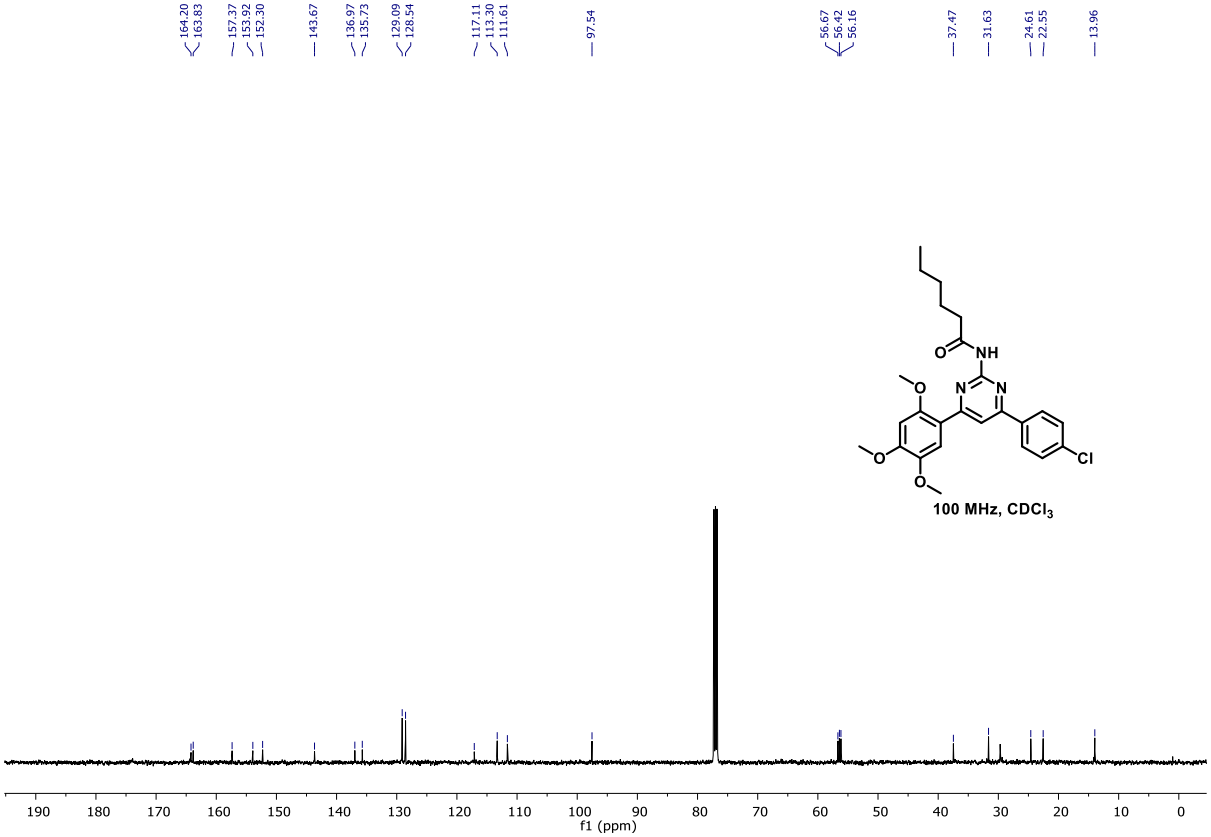
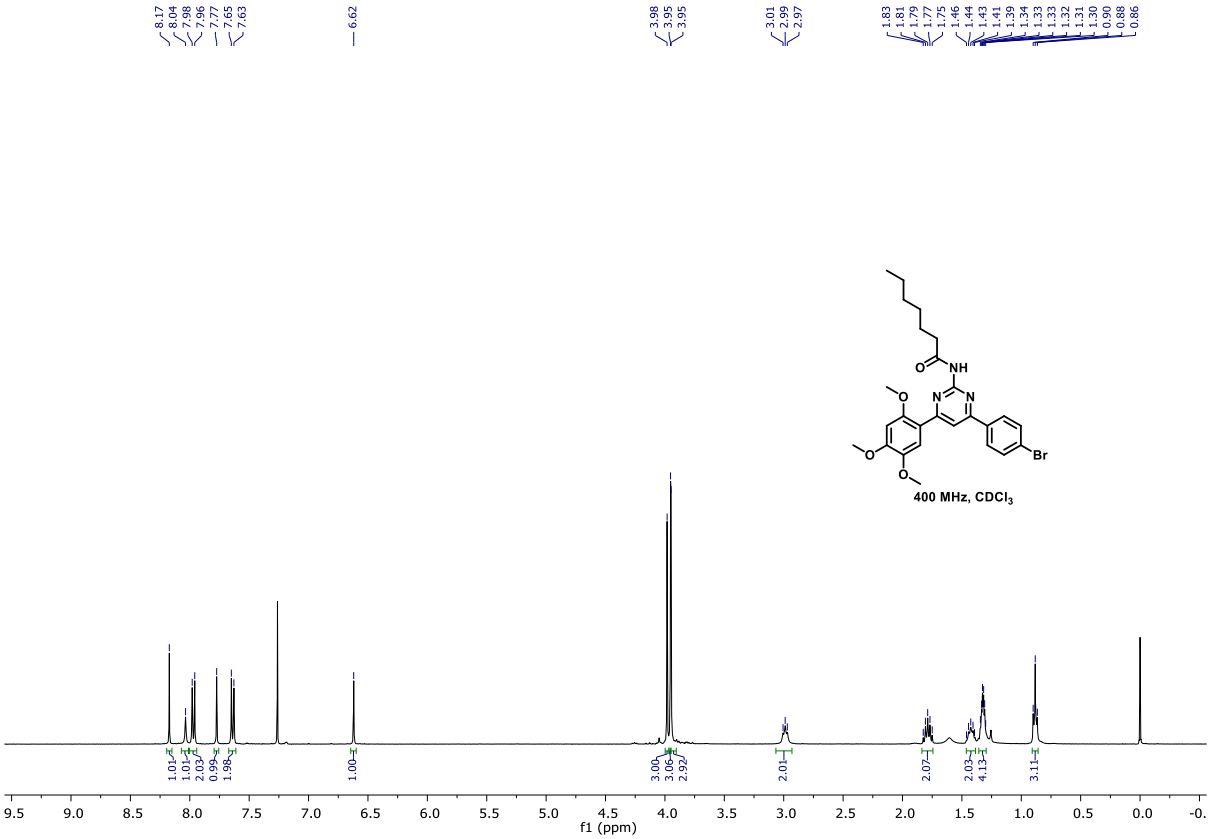
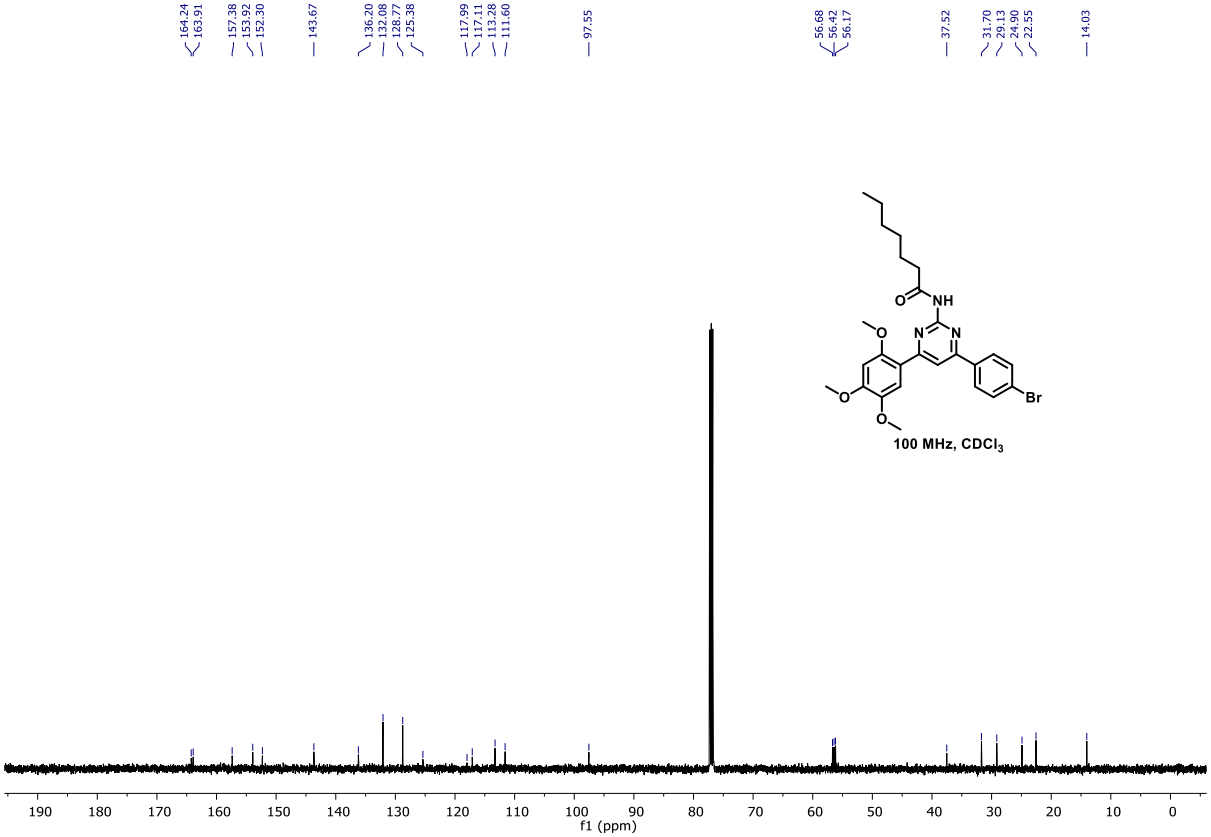


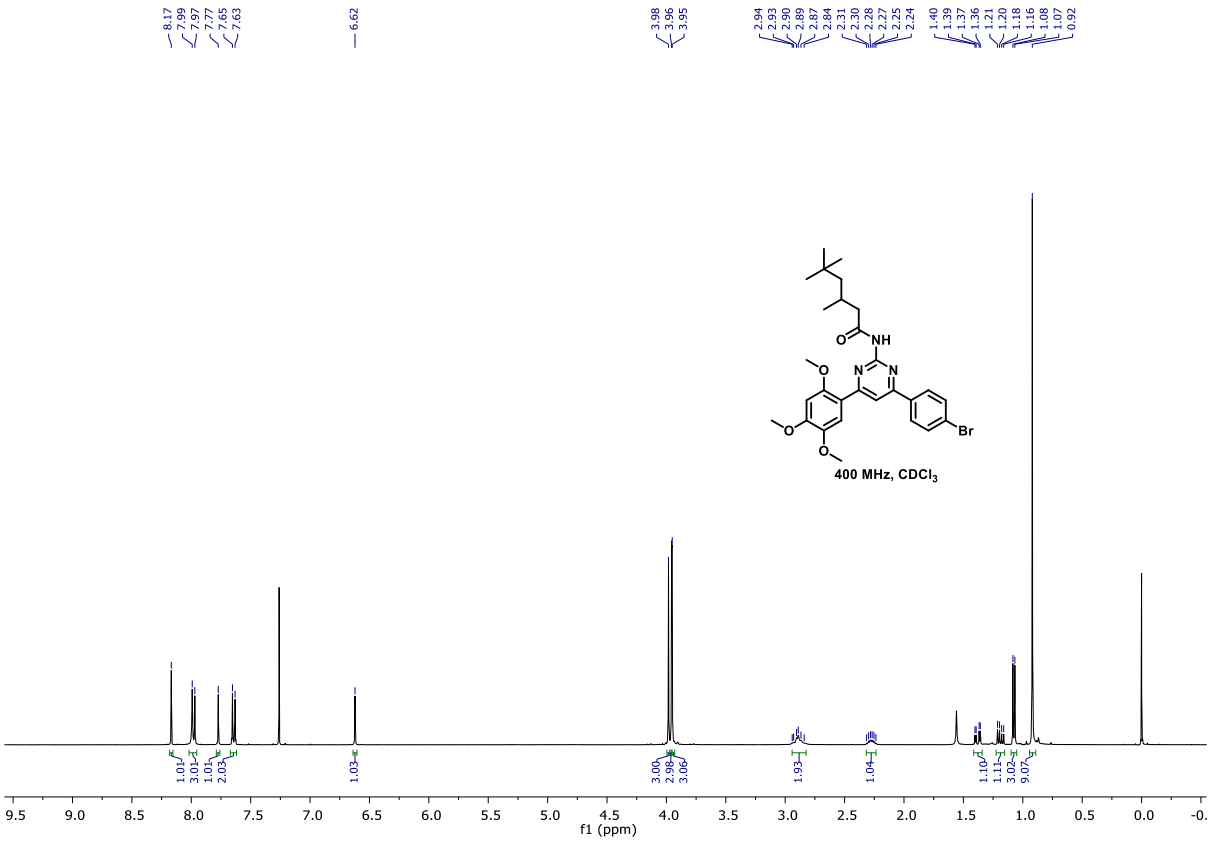
^{1}H NMR for 7d



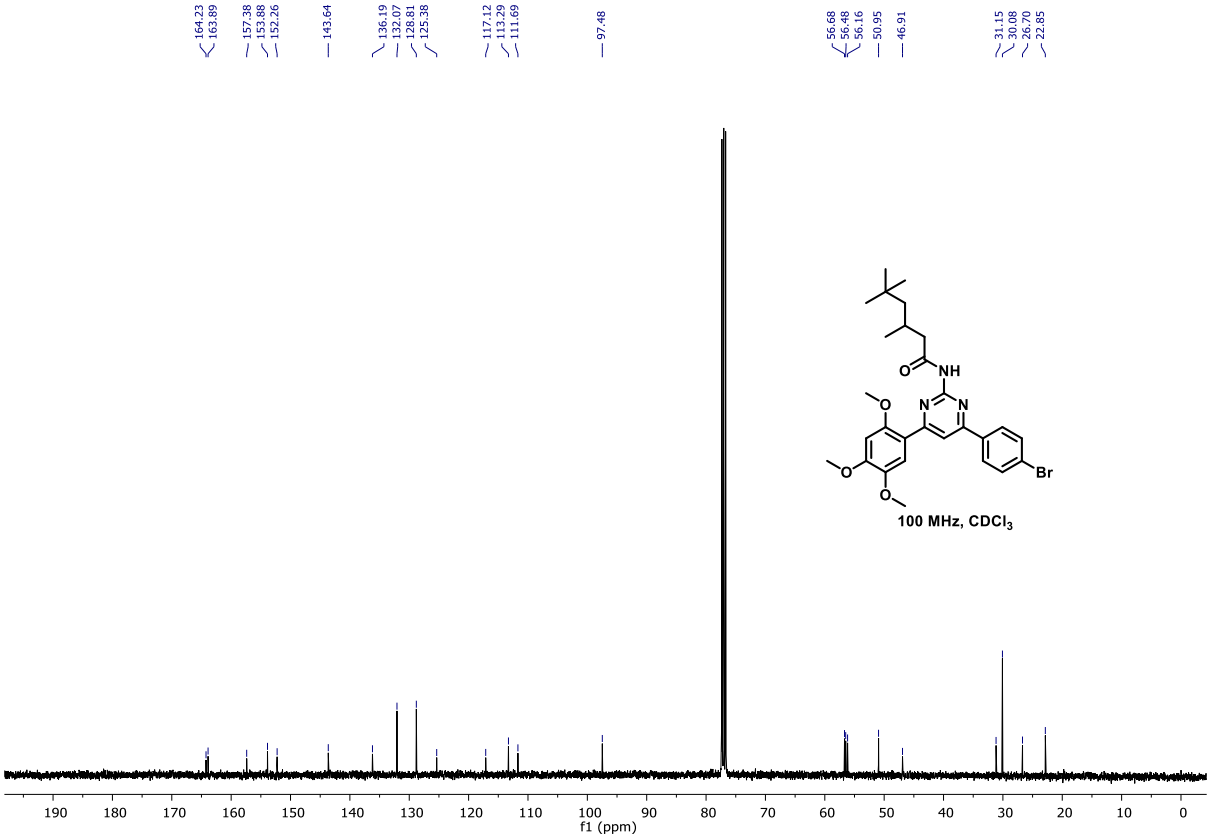
^{13}C NMR for 7d



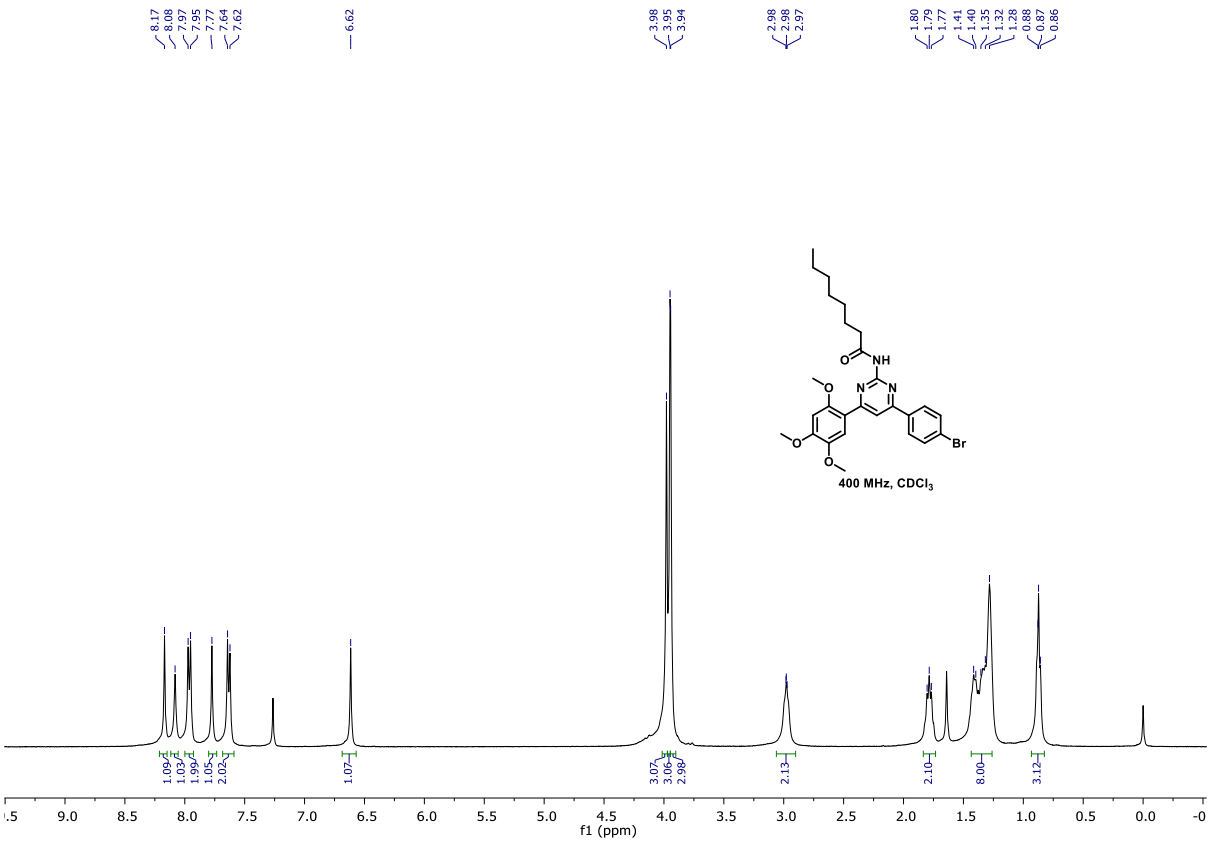
^{1}H NMR for 7e



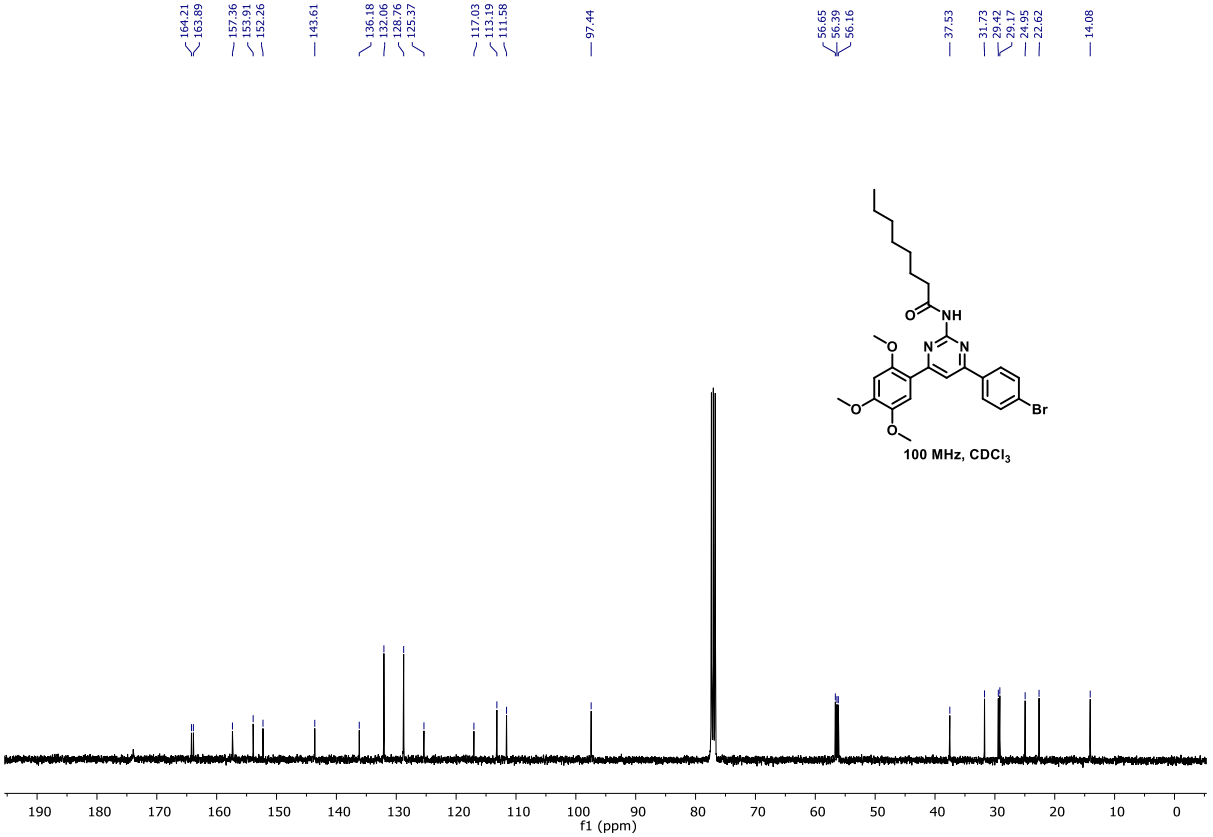
^{13}C NMR for 7e



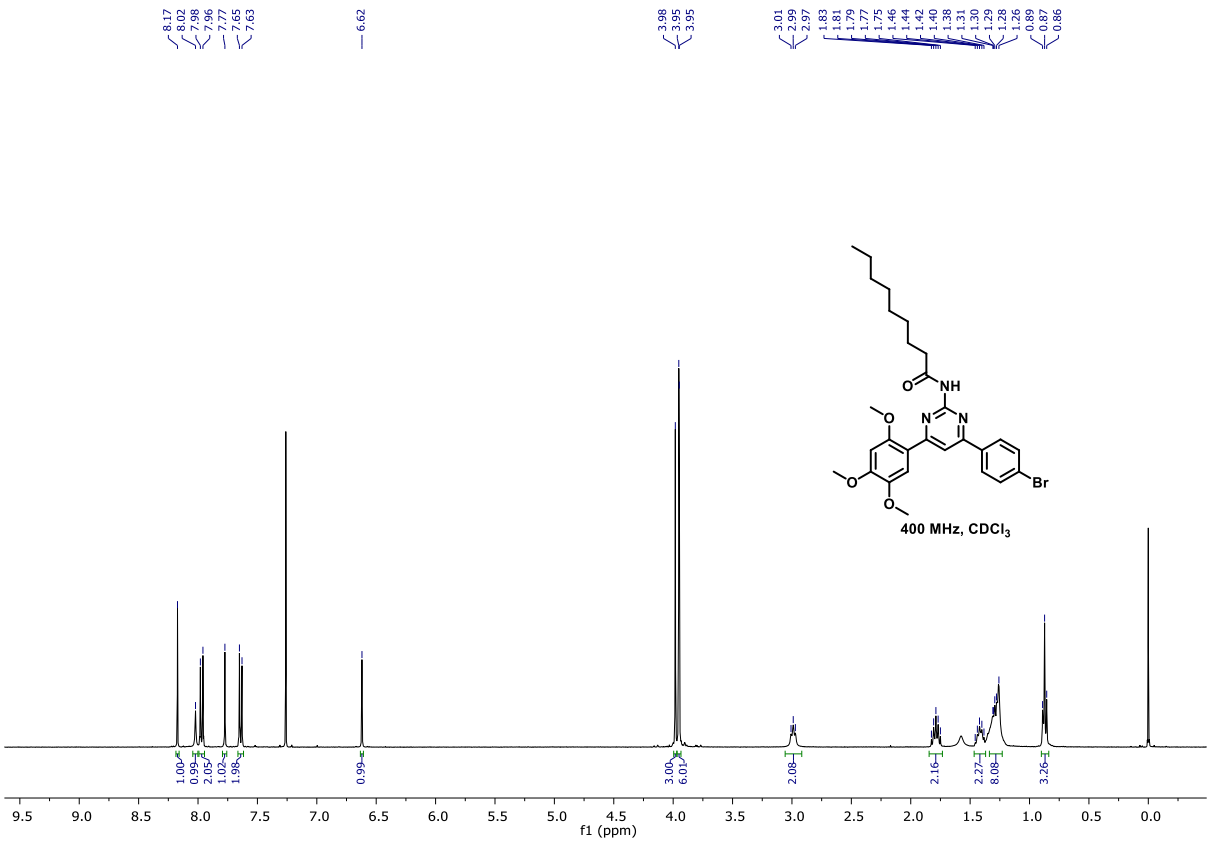
^{1}H NMR for 7f



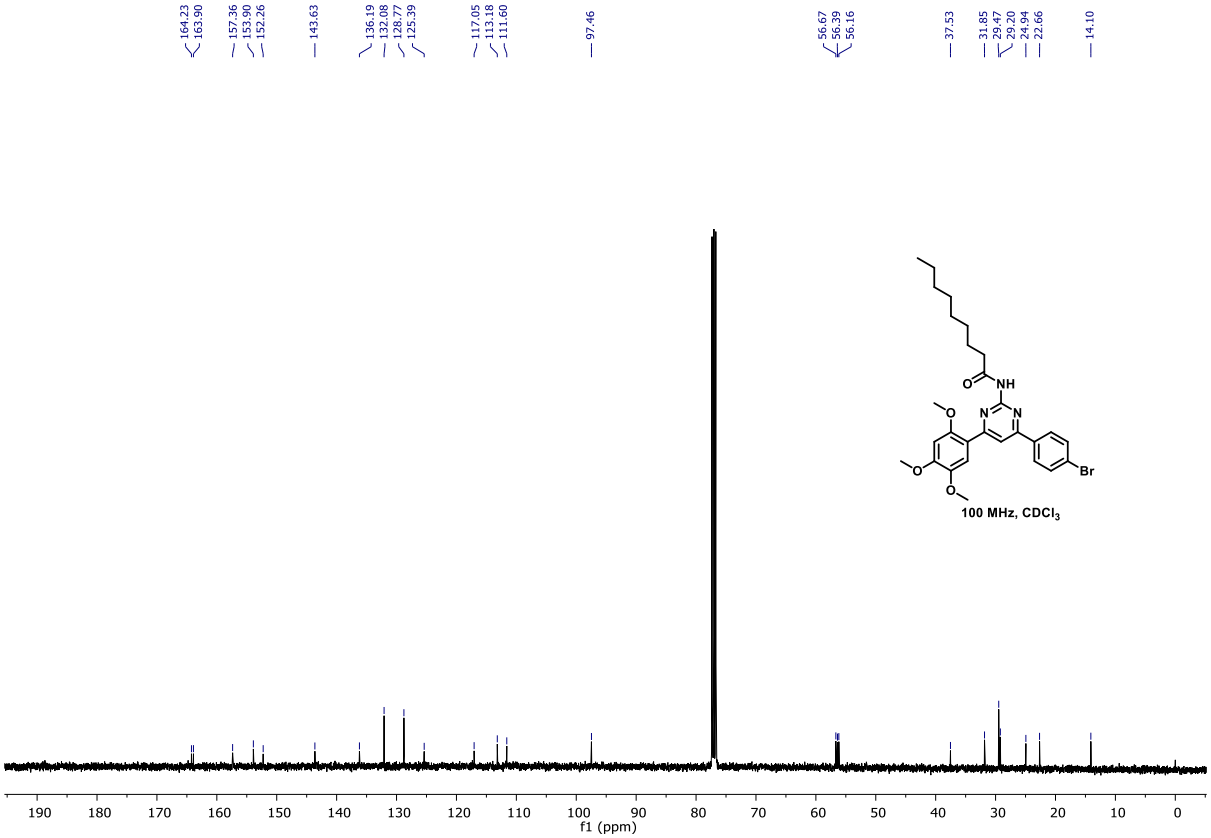
^{13}C NMR for 7f



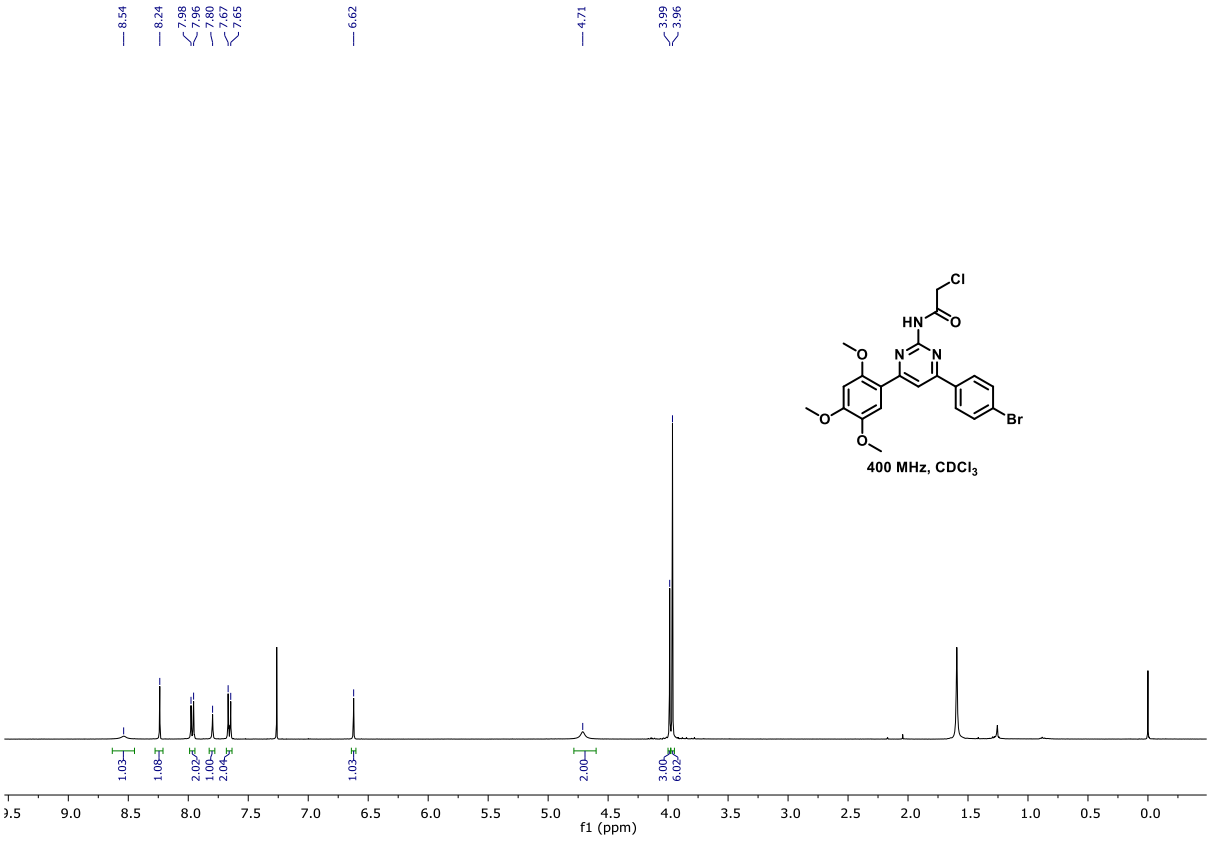
^{1}H MNR for 7g



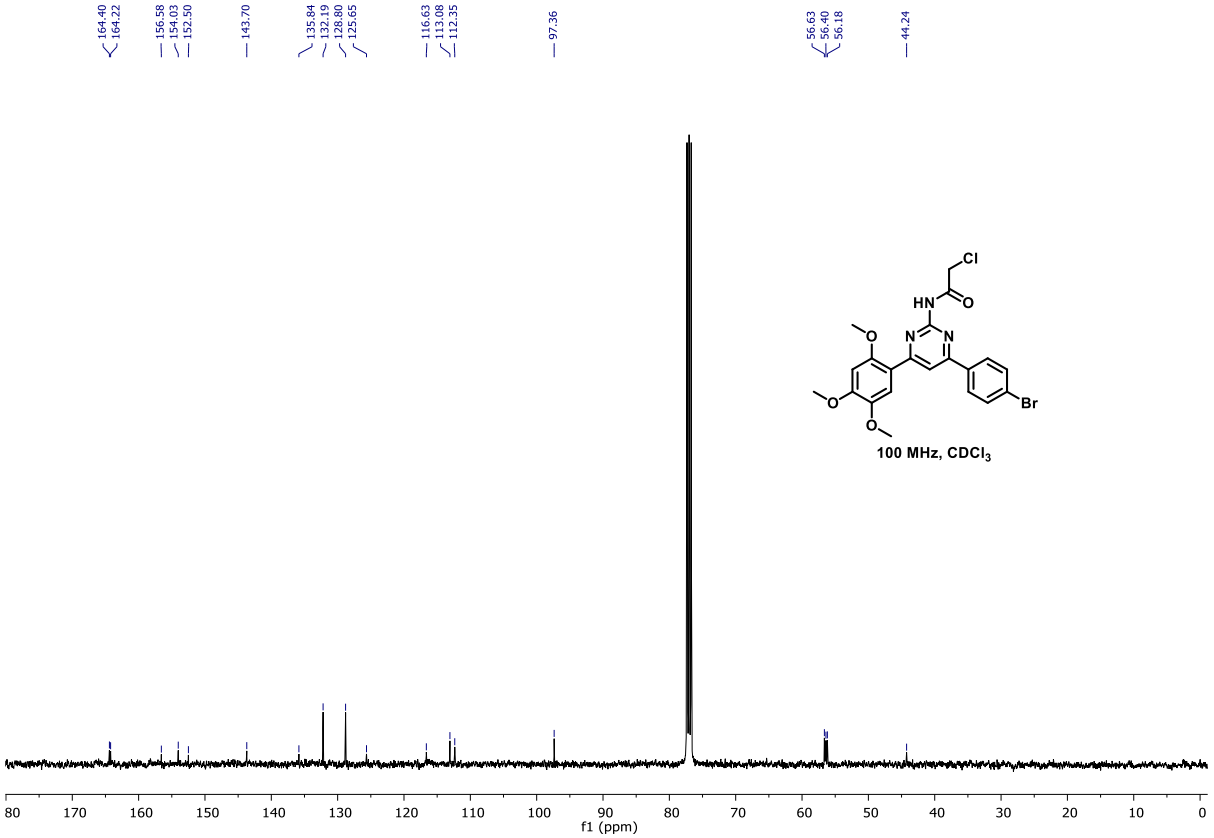
^{13}C NMR for 7g



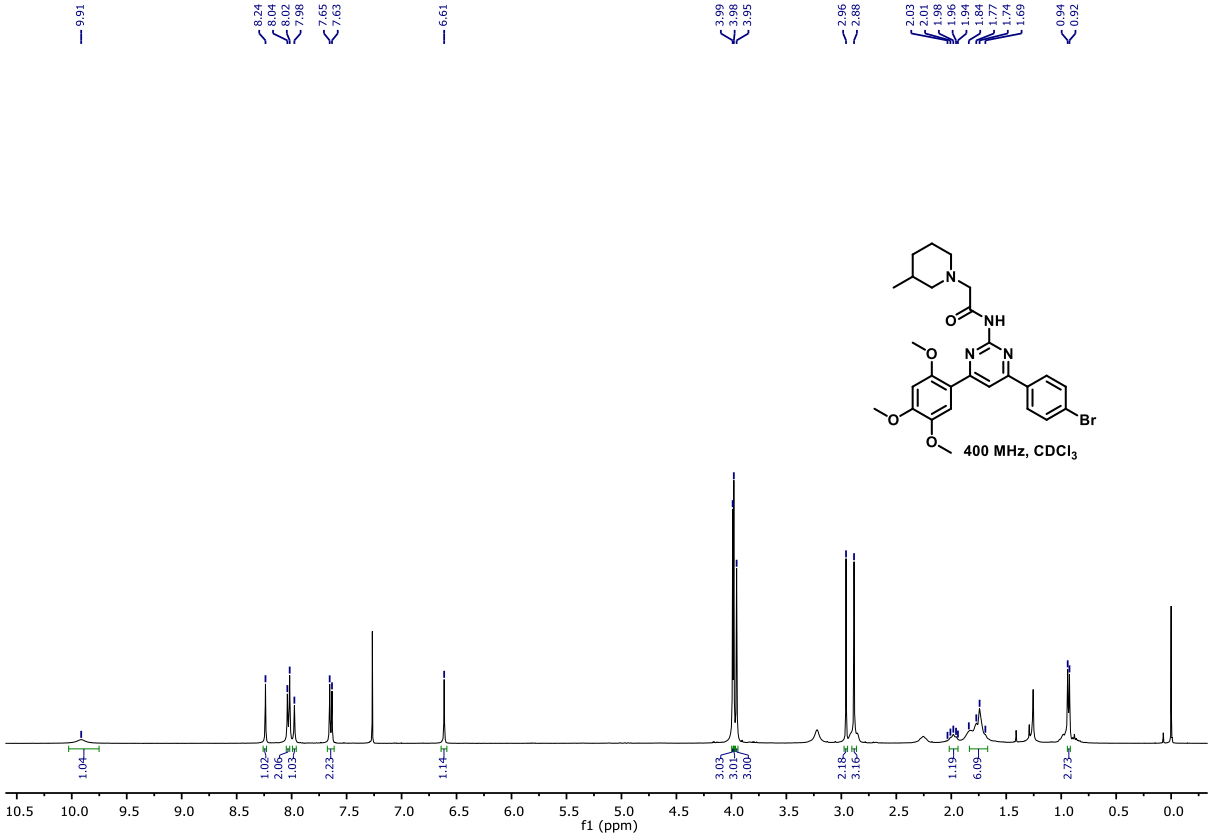
^{1}H NMR for 5’



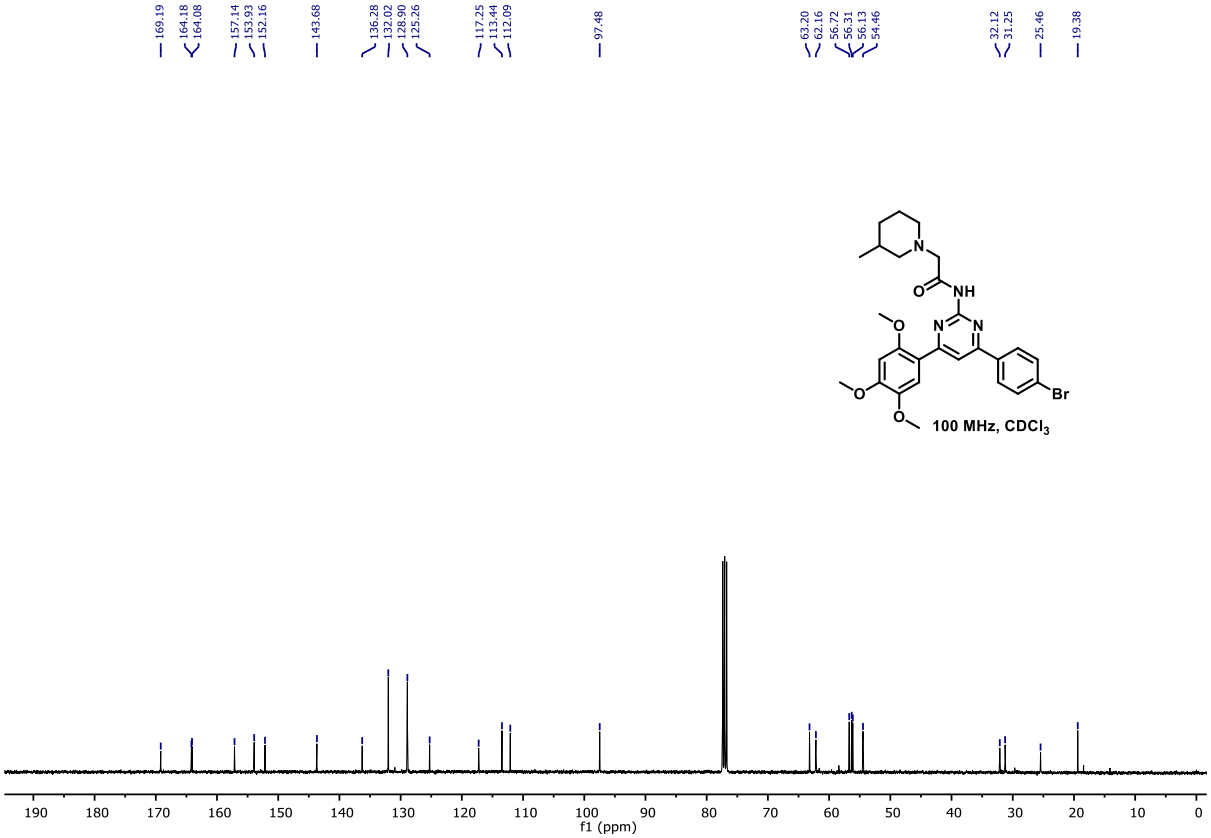
^{13}C NMR for 5’



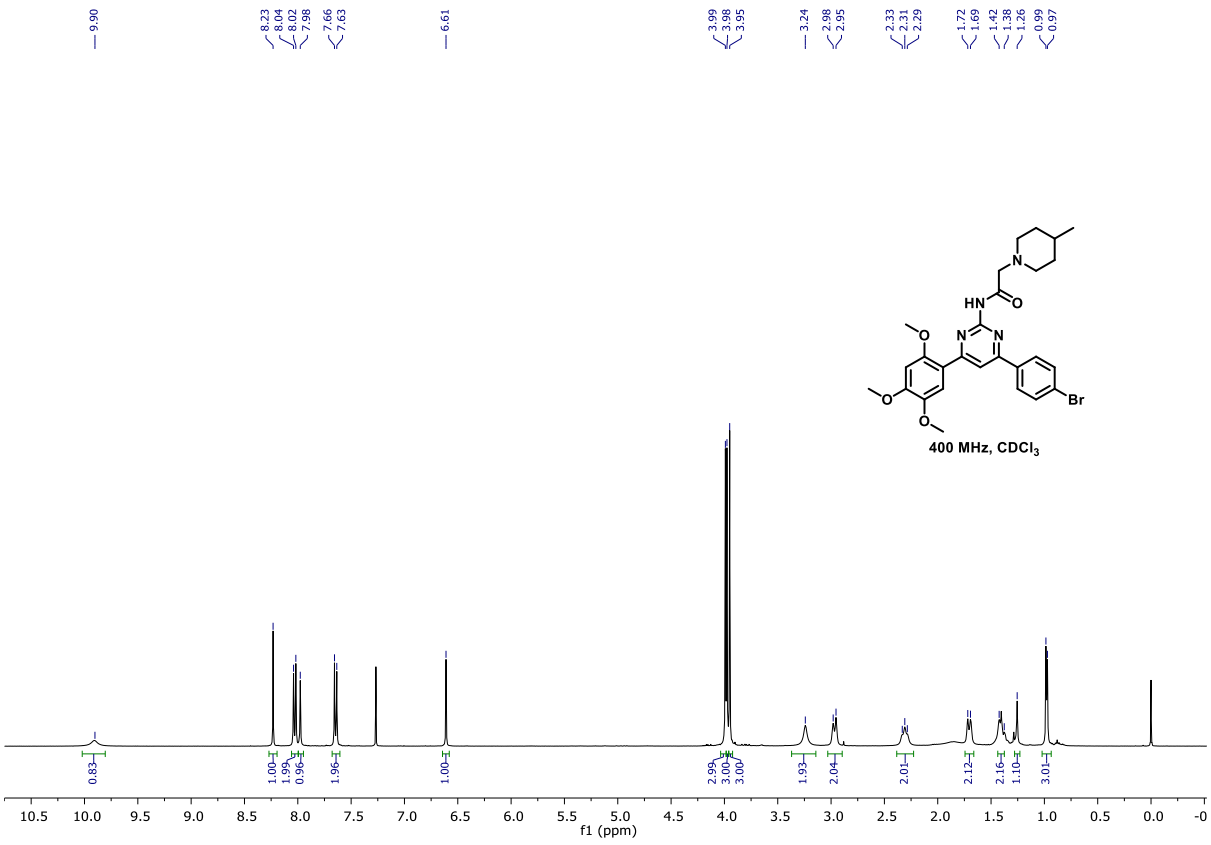
^{1}H NMR for 8



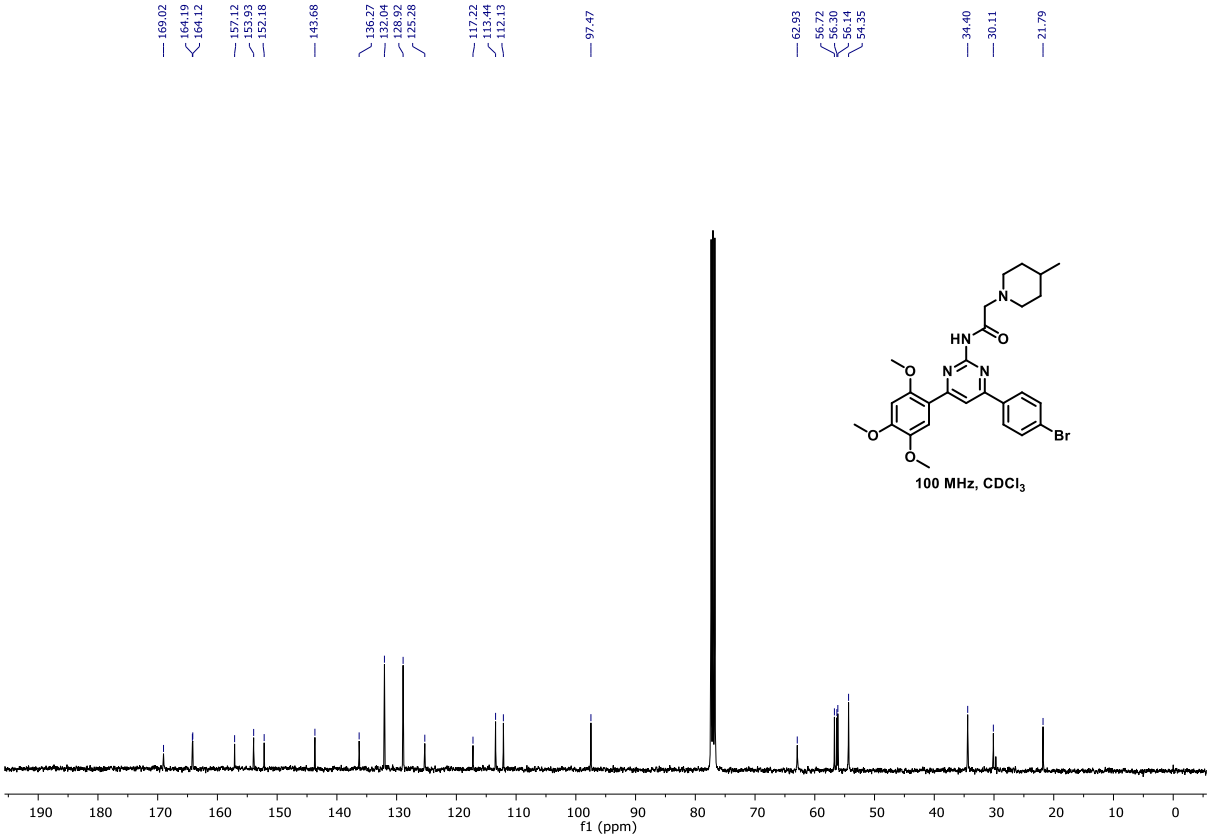
^{13}C NMR for 8



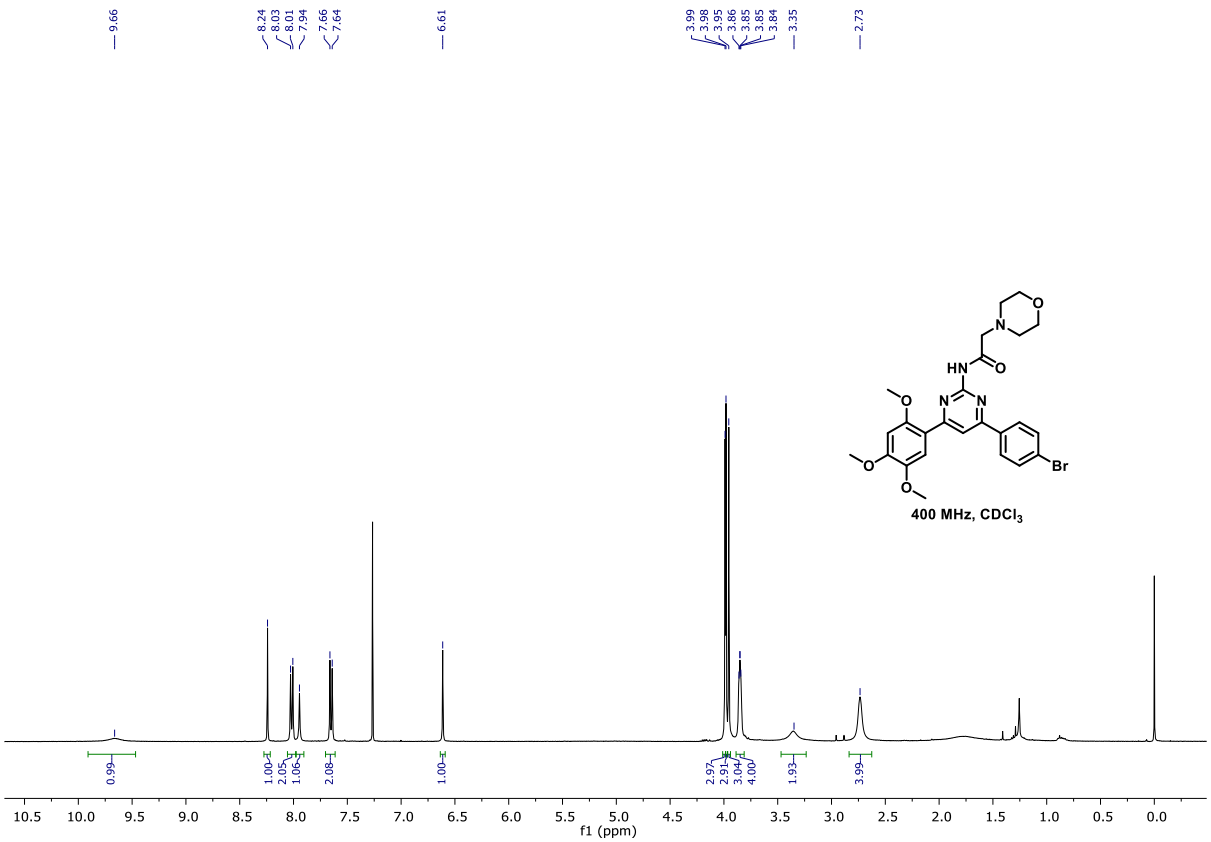
^{1}H NMR for 9



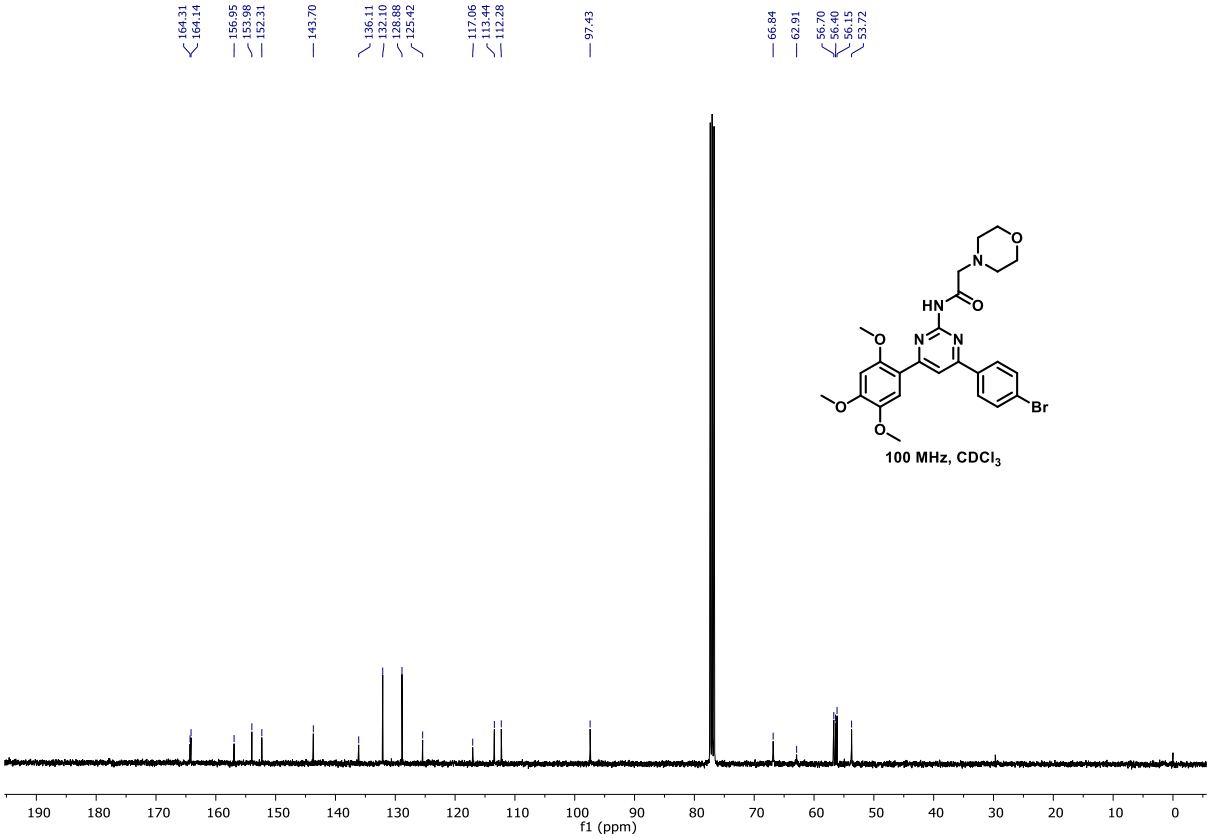
^{13}C NMR for 9



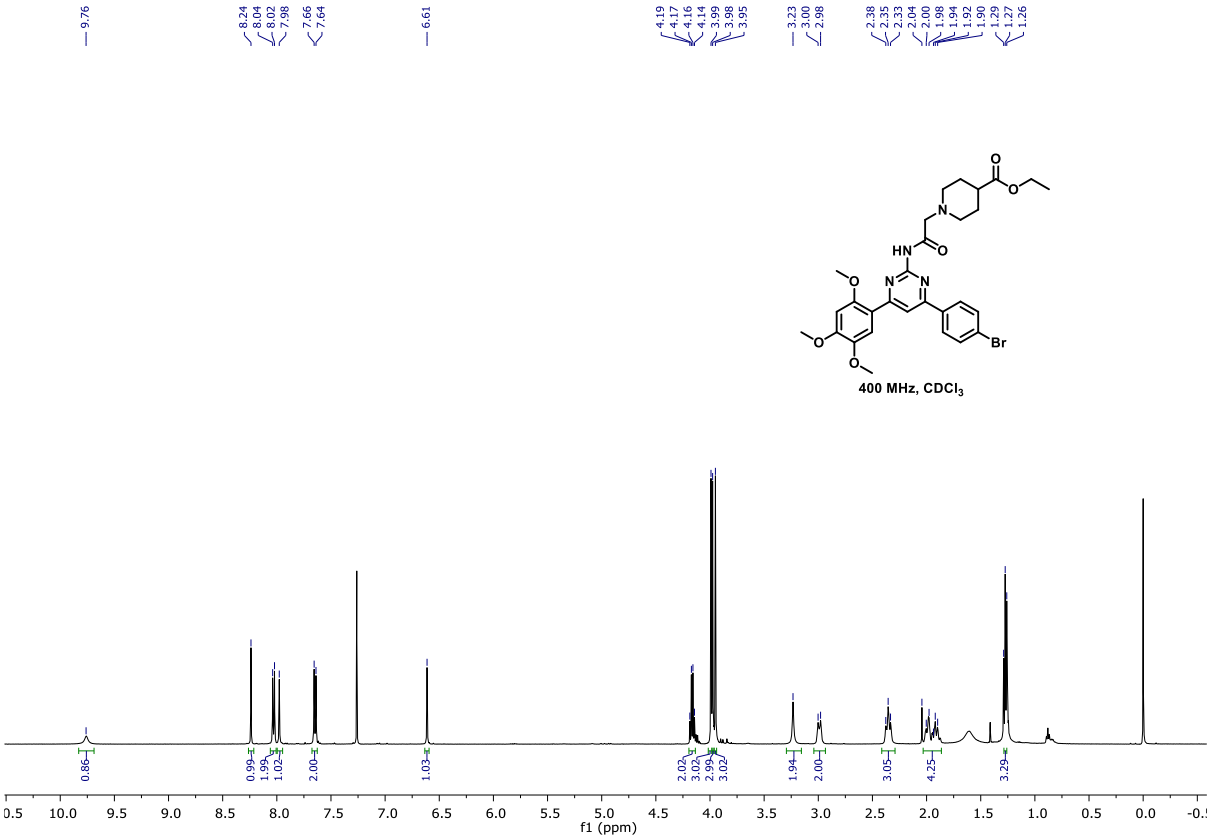
^{1}H NMR for 10



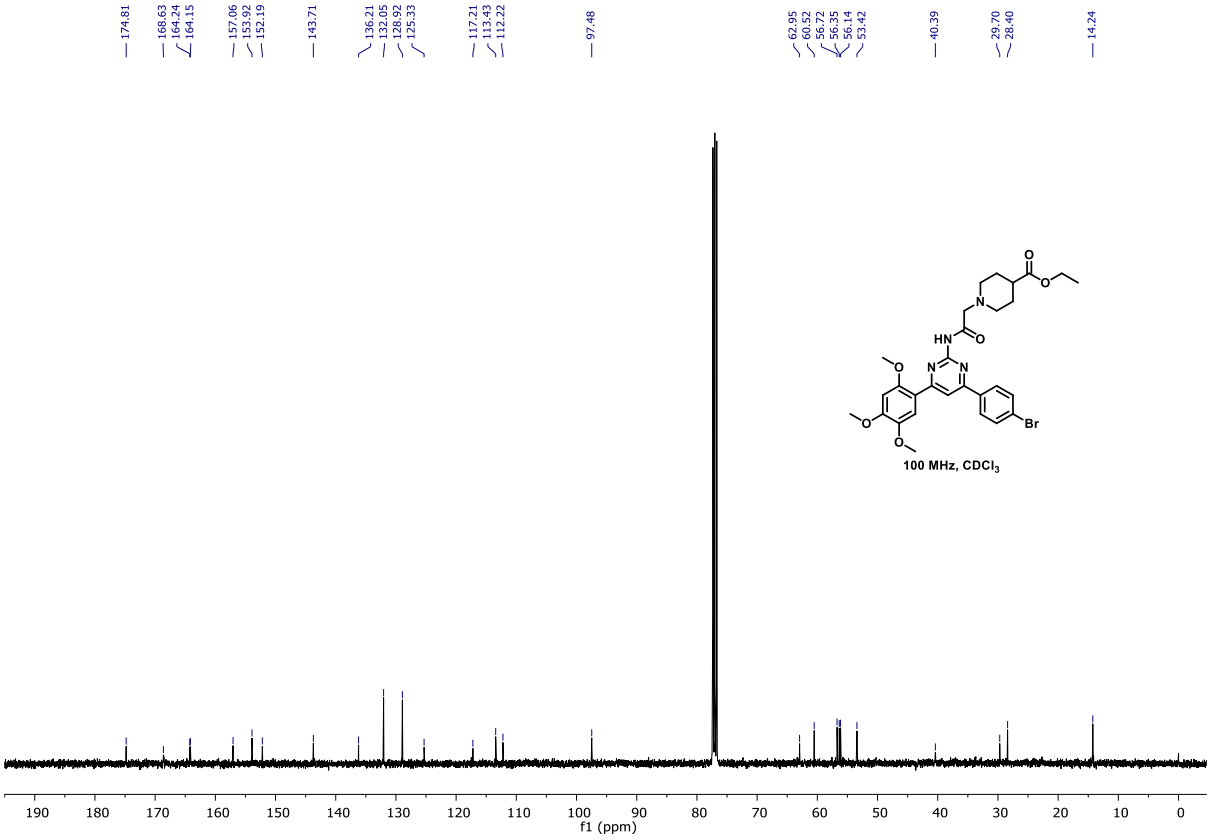
^{13}C NMR for 10



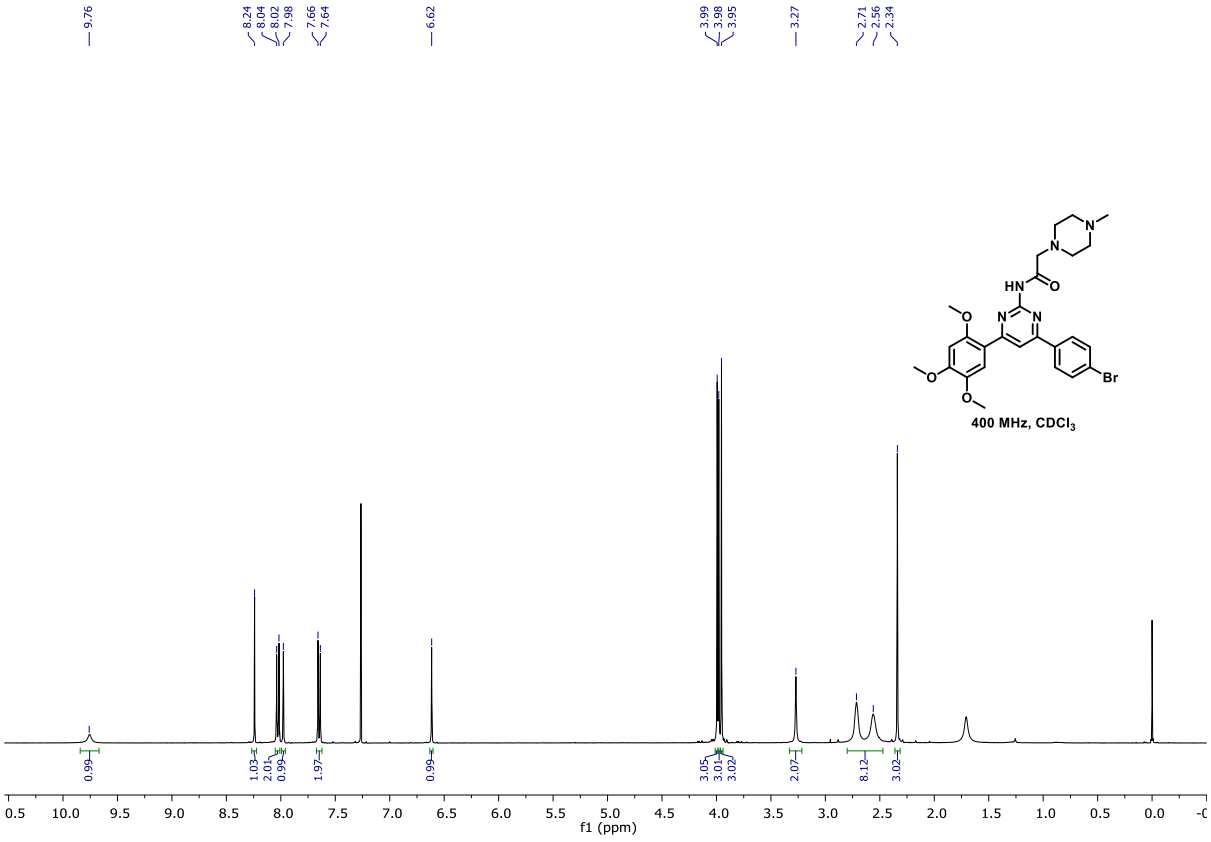
^{1}H NMR for 11



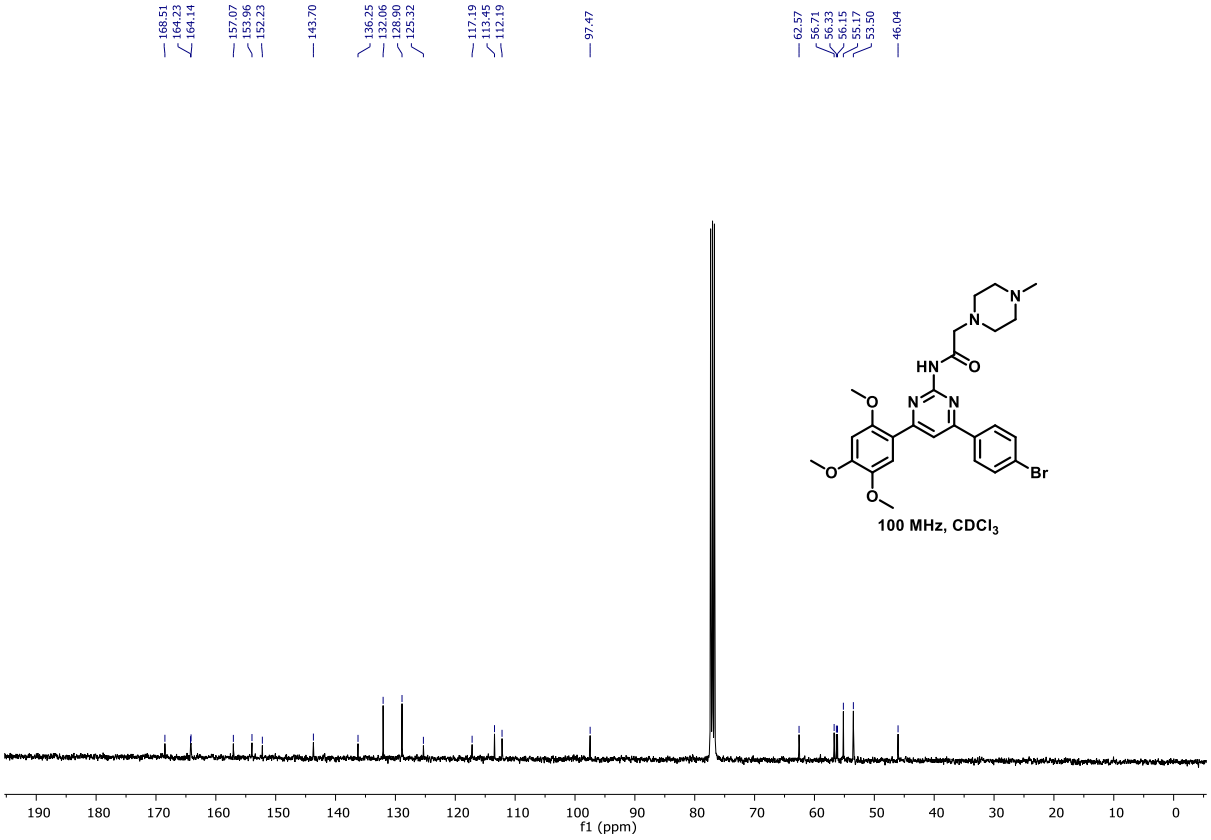
^{13}C NMR for 11



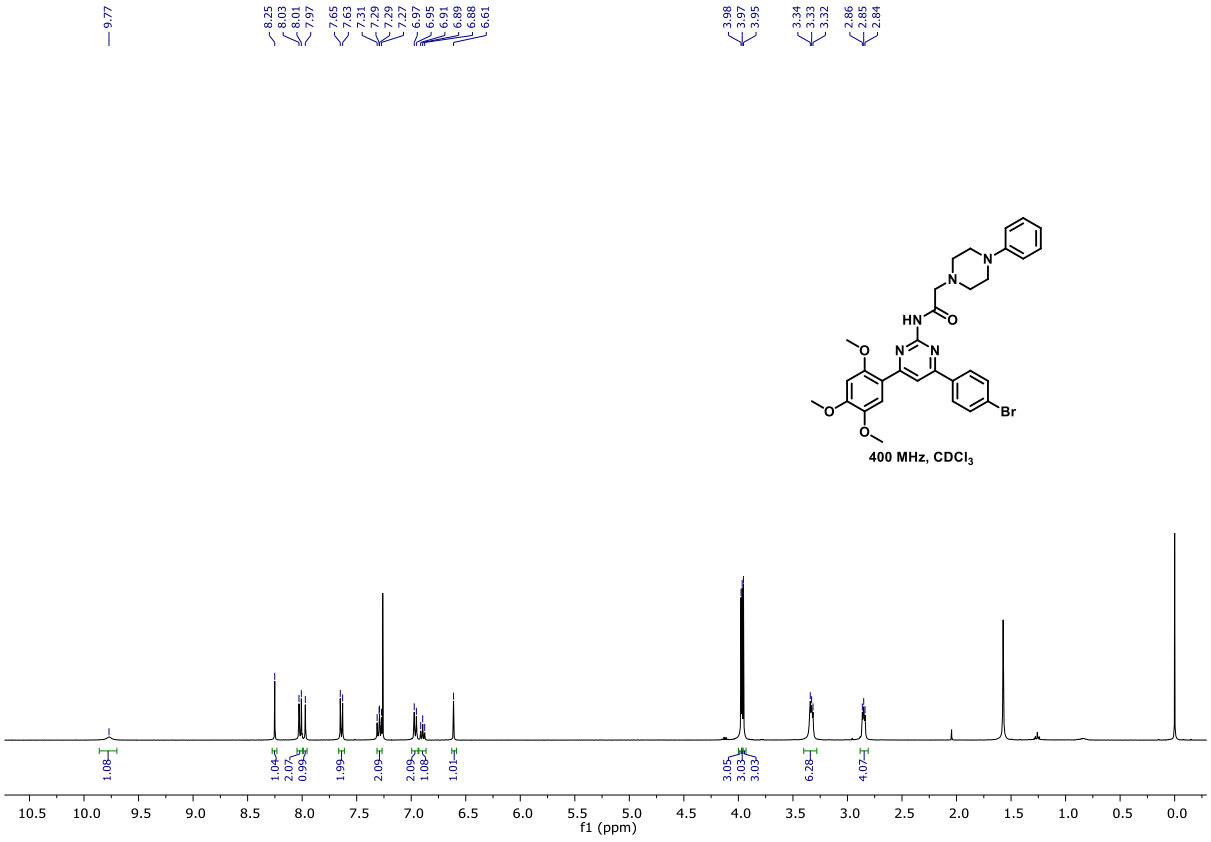
^{1}H NMR for 12



^{13}C NMR for 12



^{1}H NMR for 13



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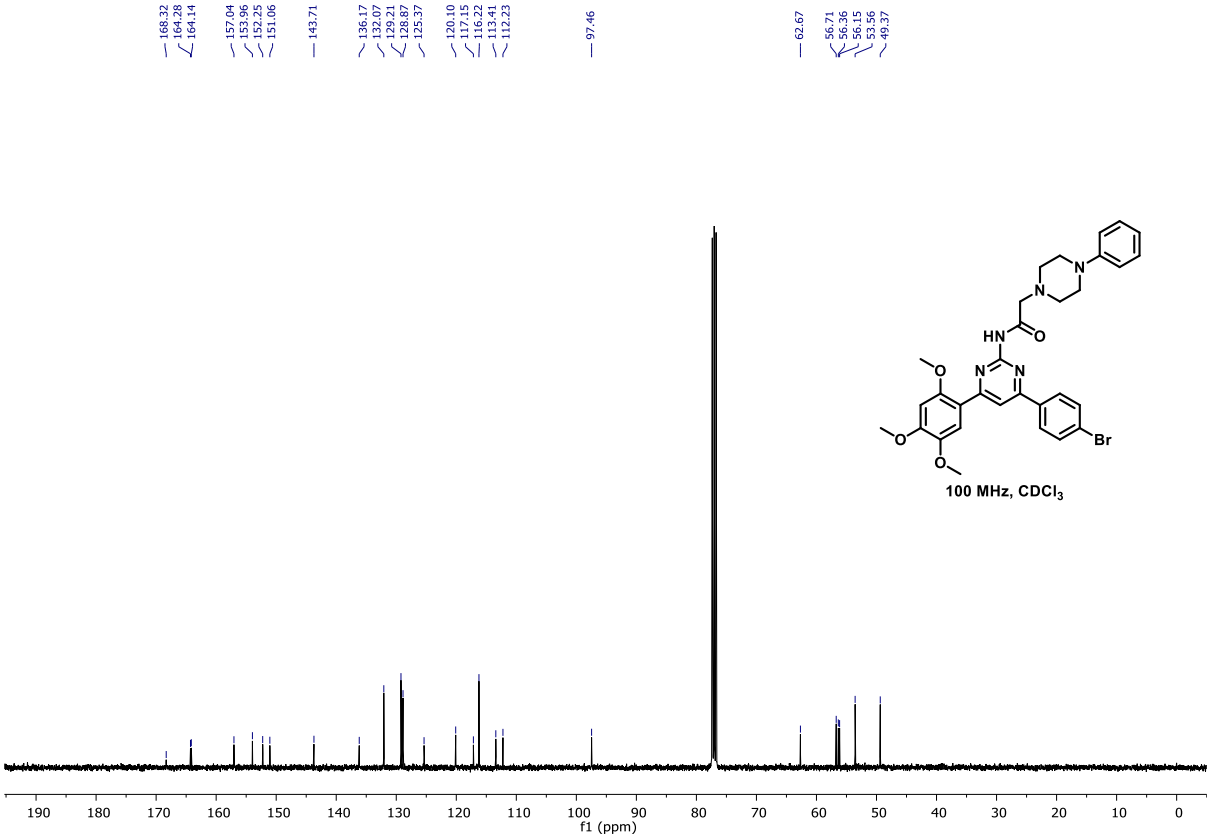

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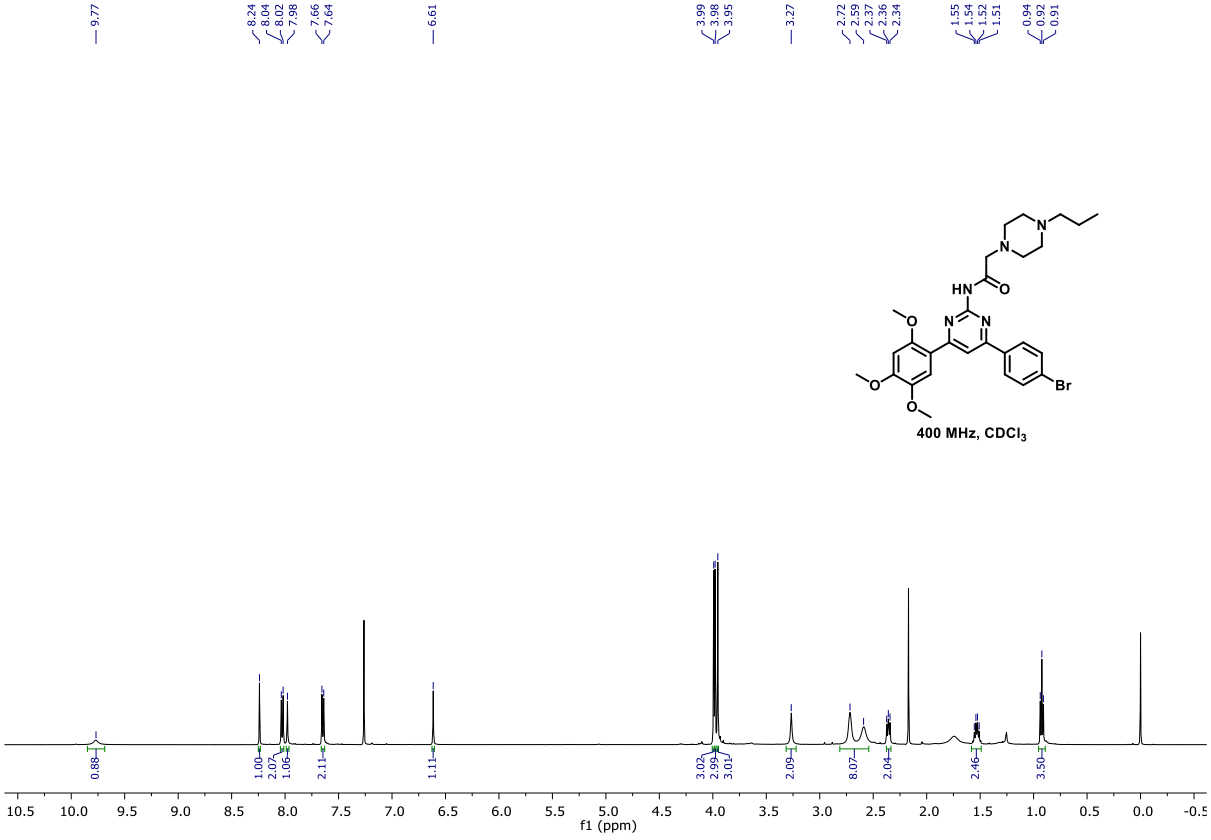

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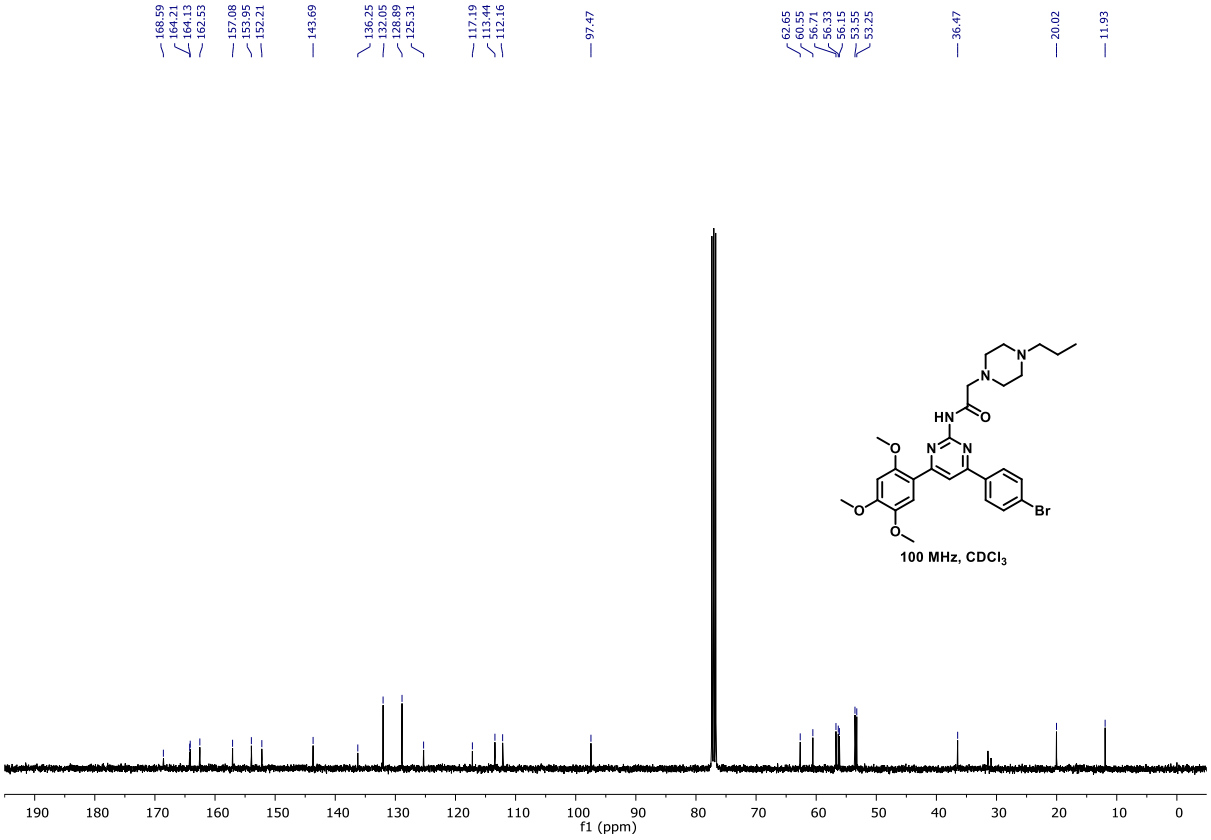
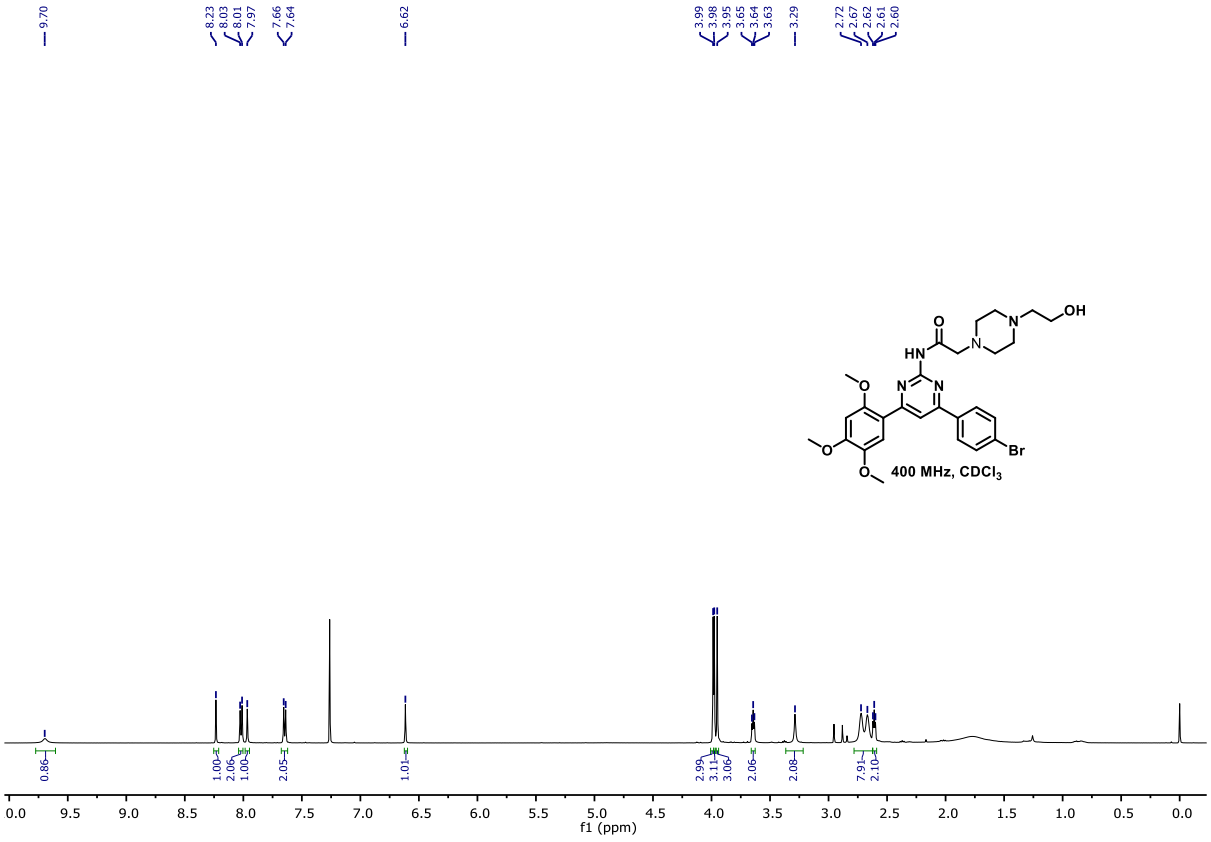
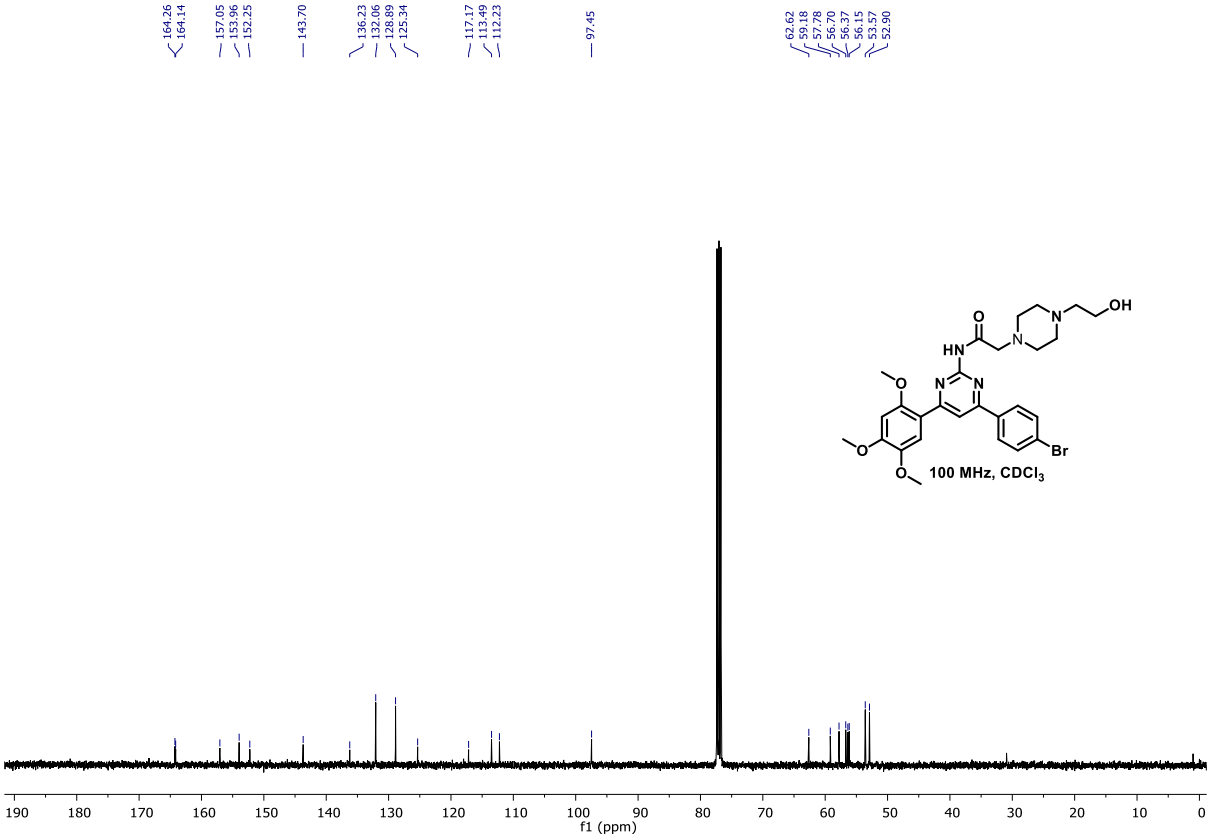


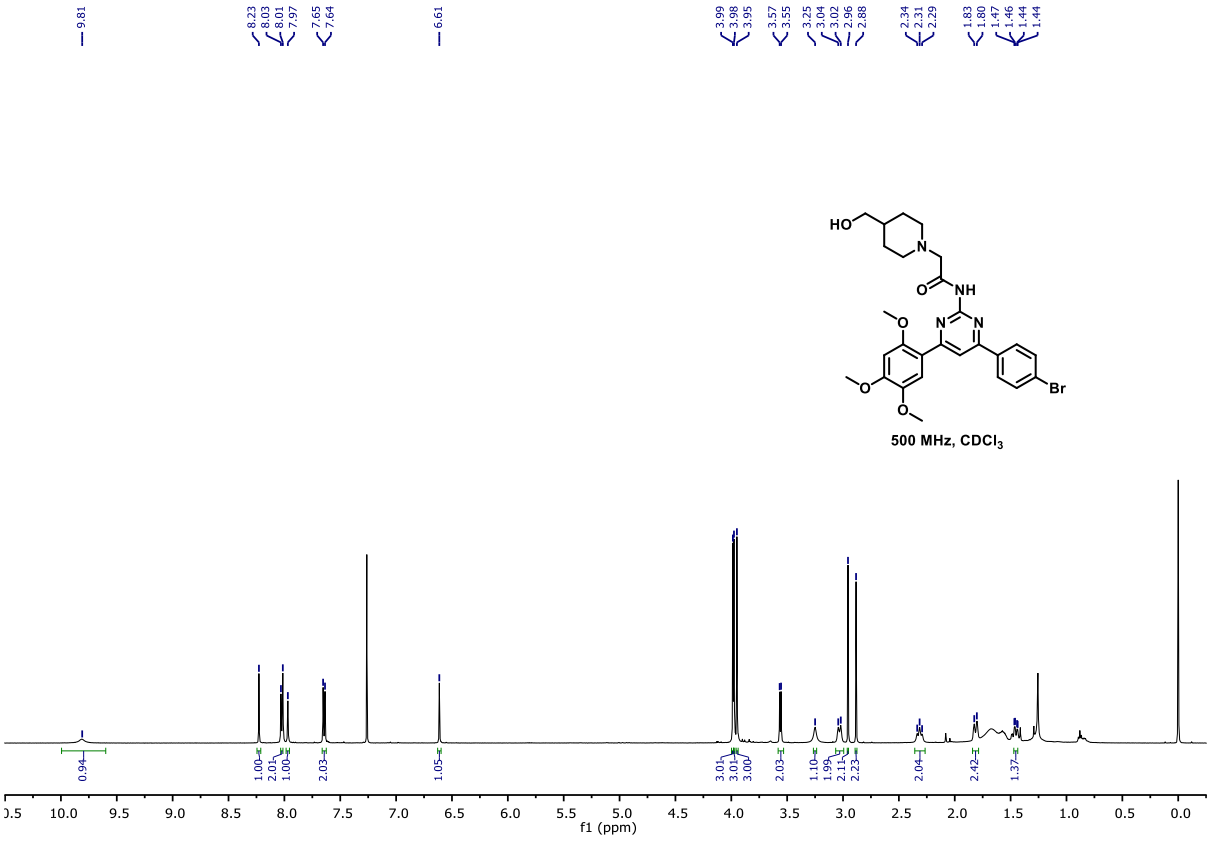
^{1}H NMR for 15



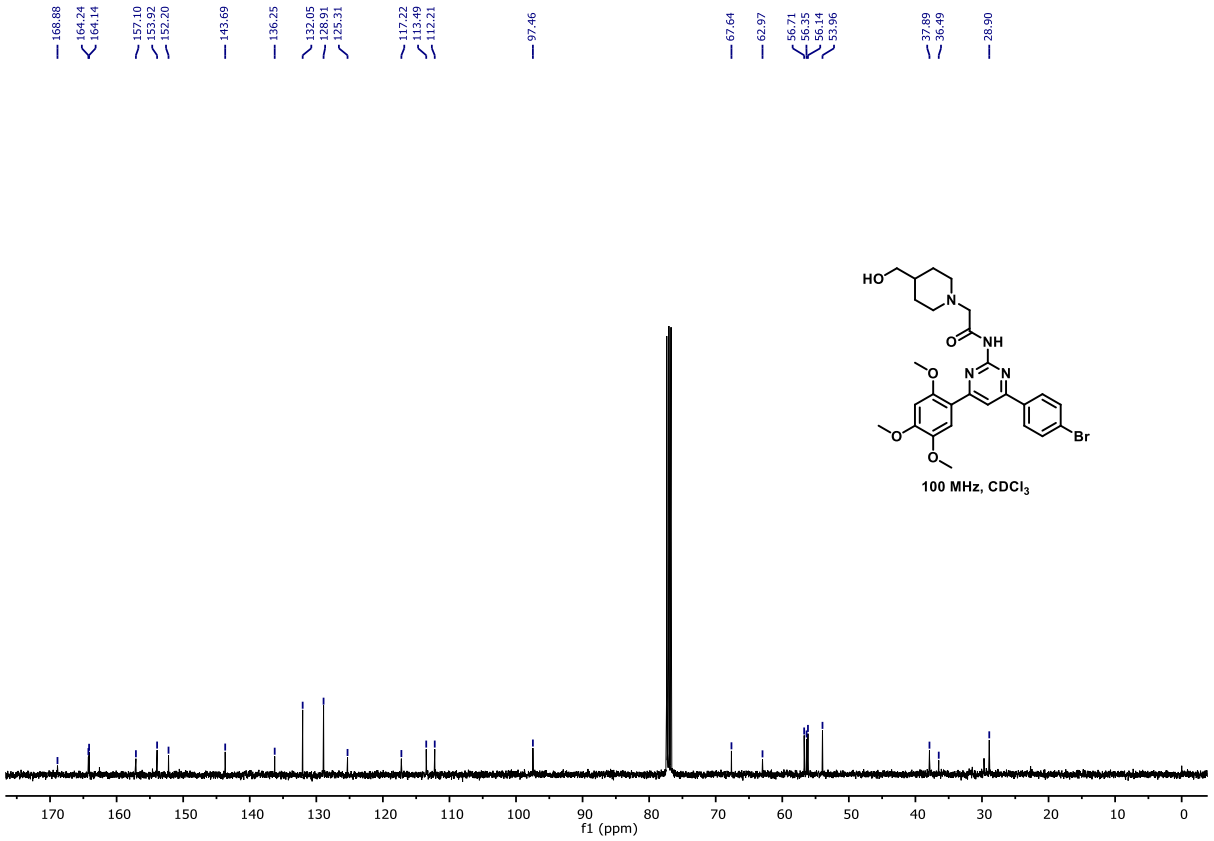
^{13}C NMR for 15



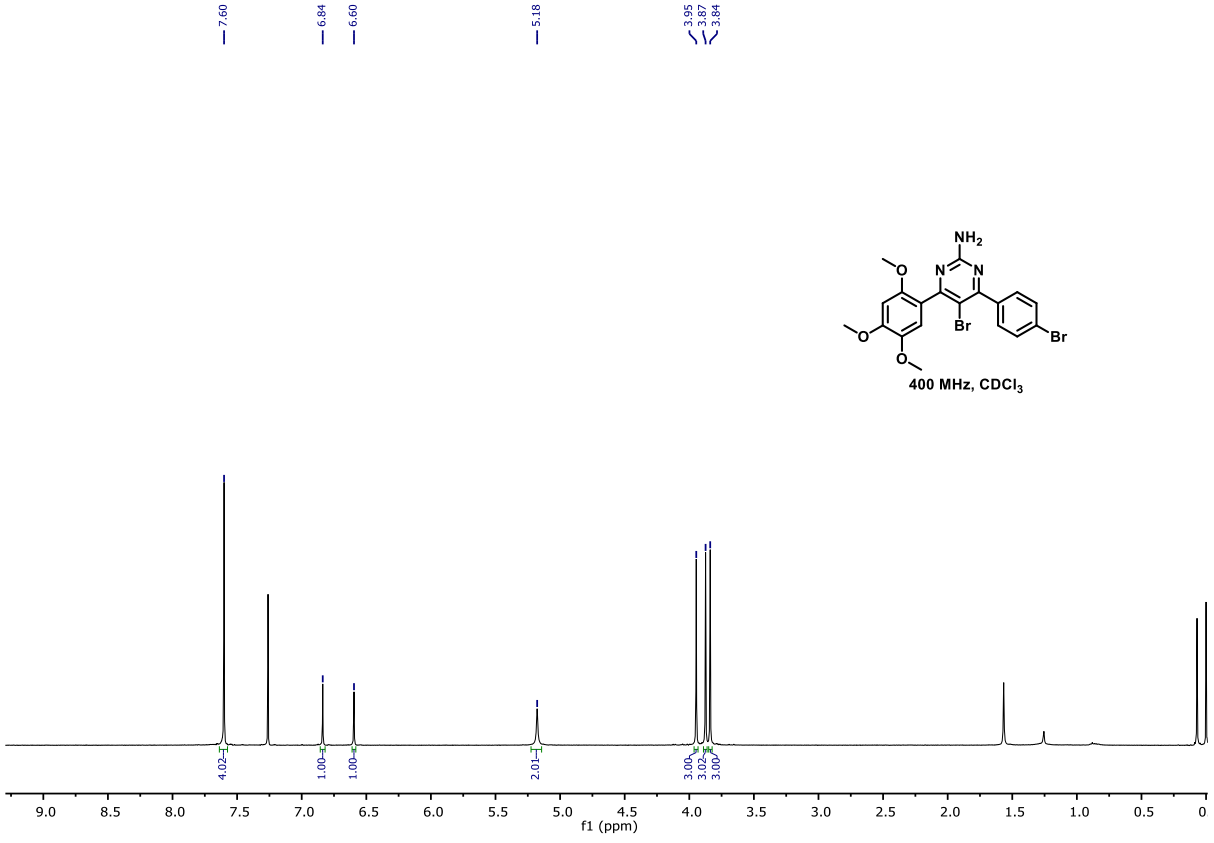
^{1}H NMR for 16



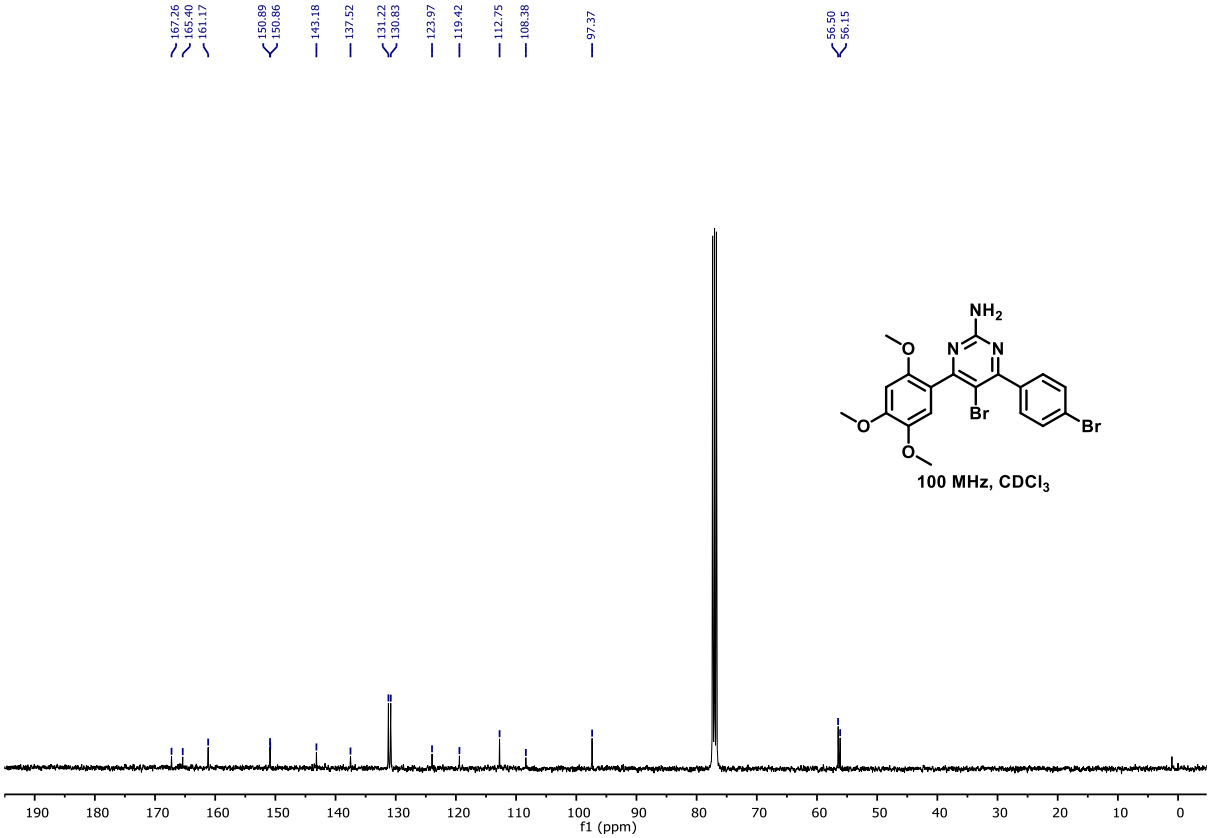
^{13}C NMR for 16



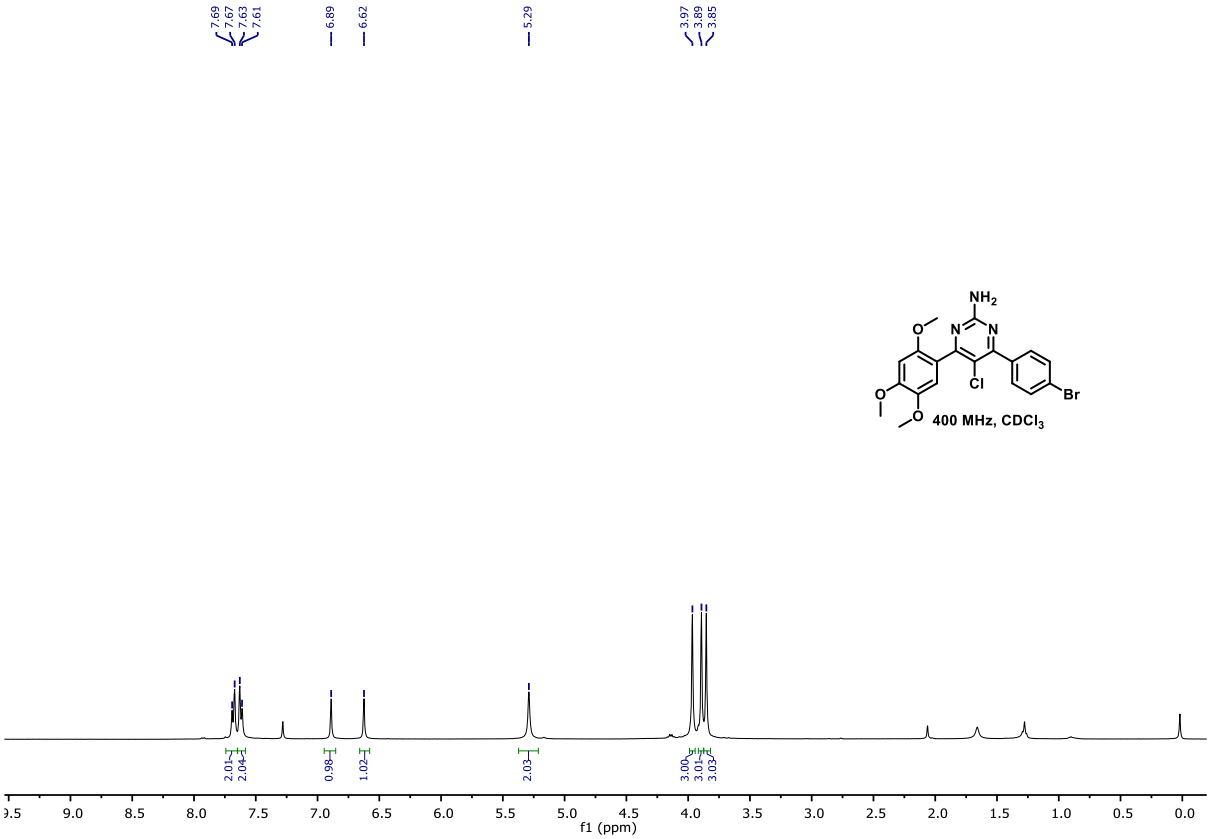
^{1}H NMR for 17a



^{13}C NMR for 17a



^{1}H NMR for 17b



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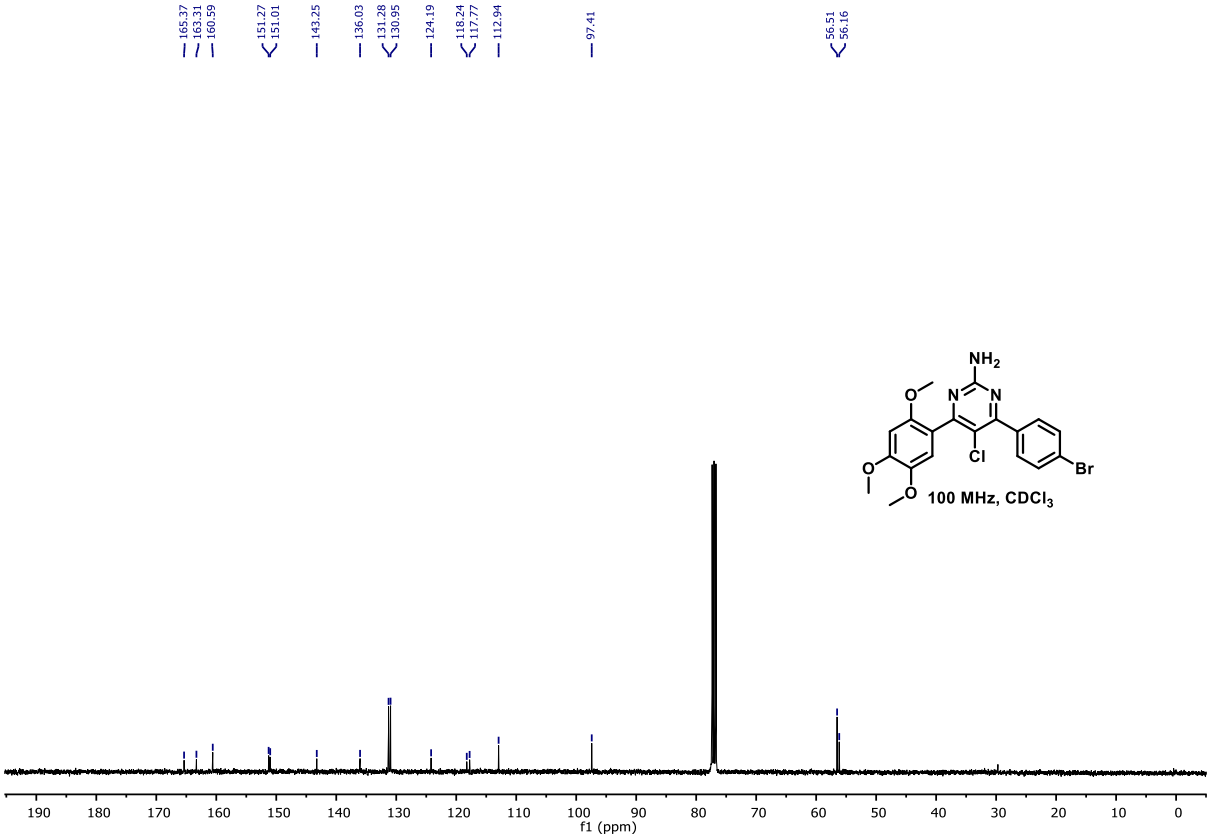
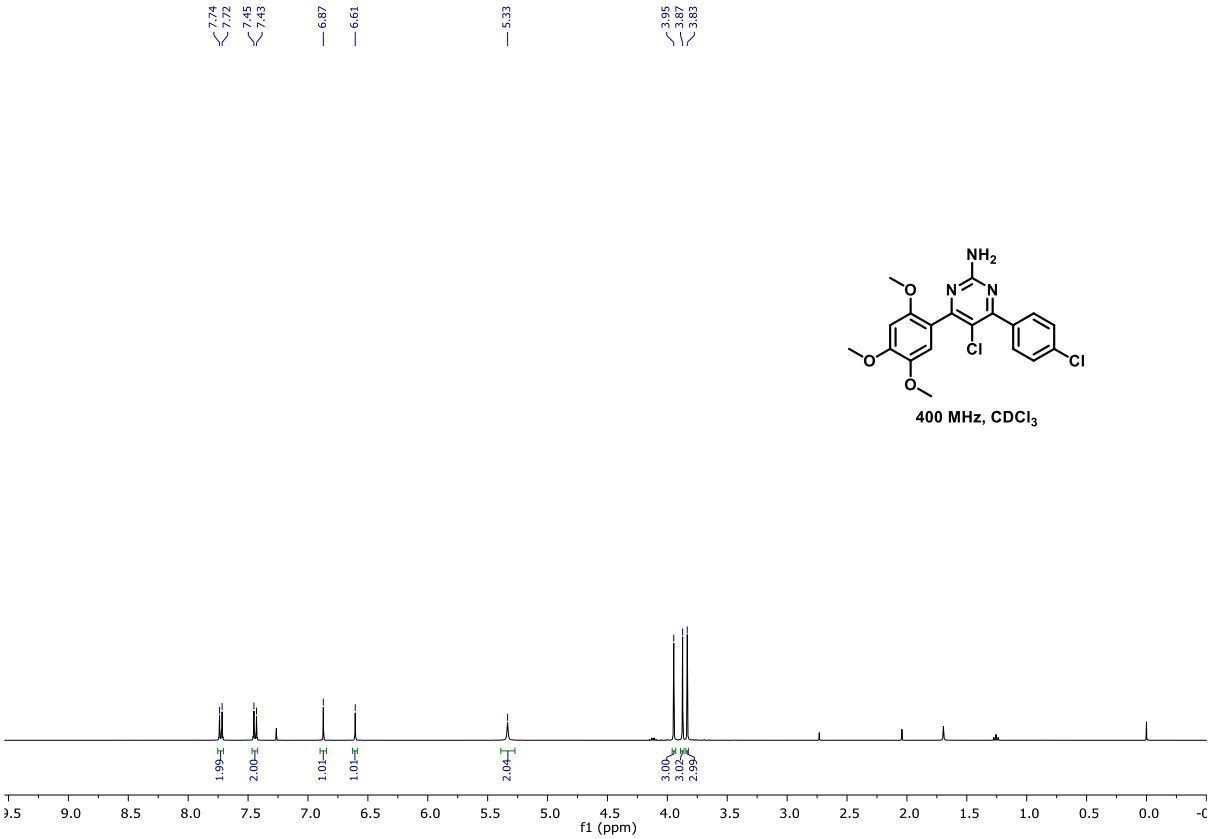


^{1}H NMR for 17c



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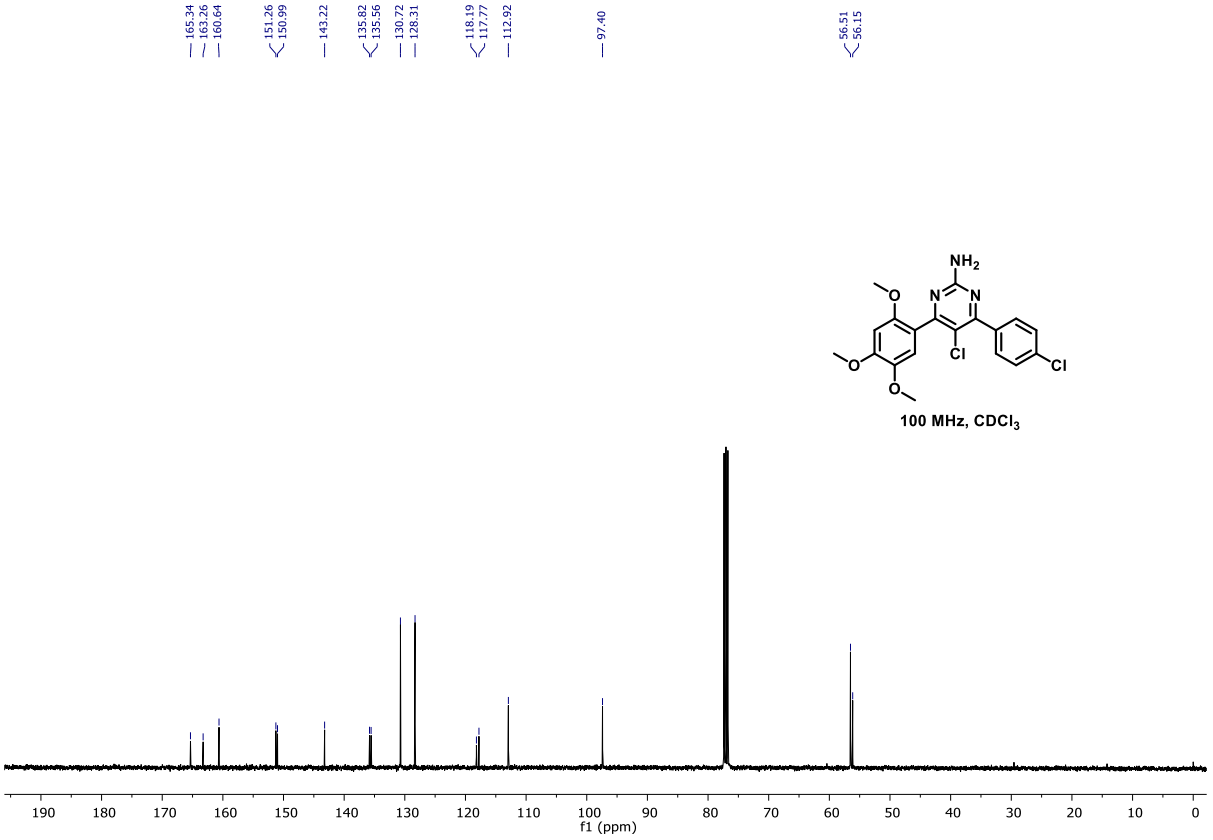
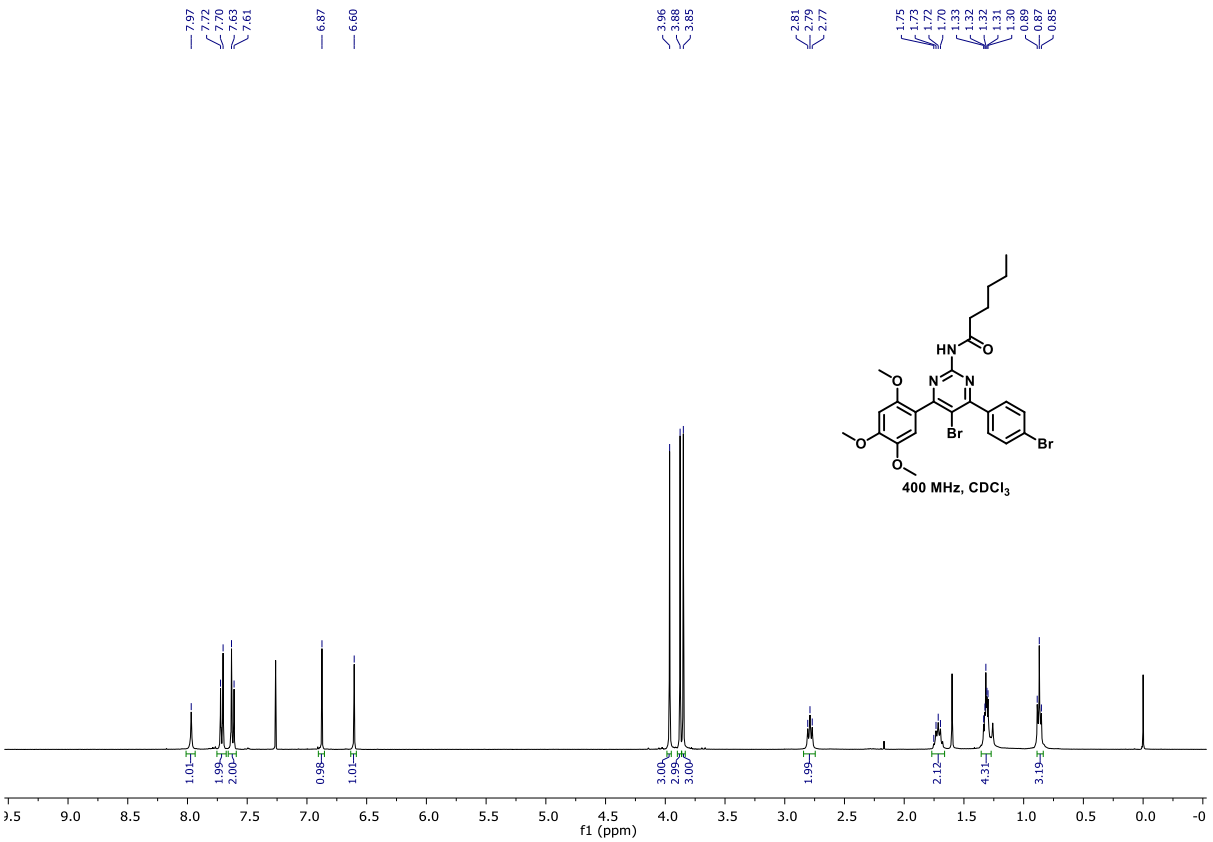


^{1}H NMR for 18a



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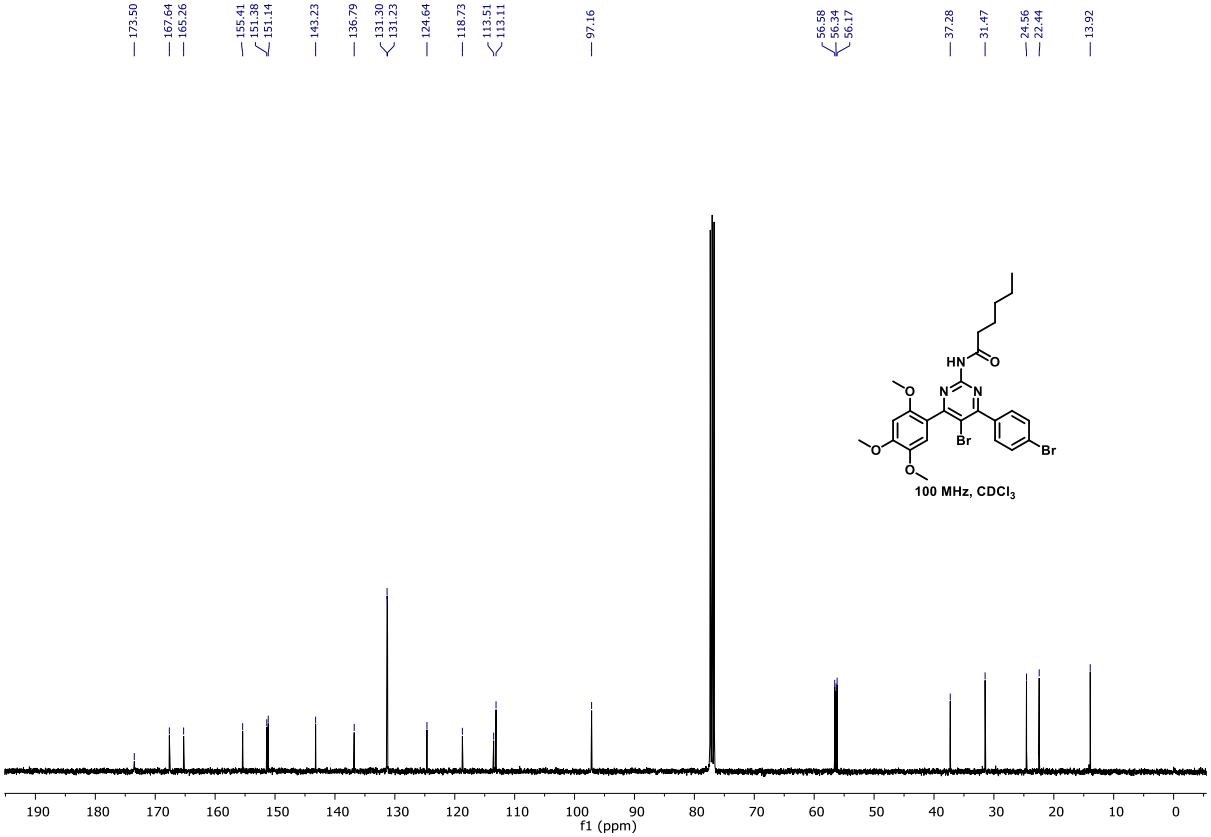
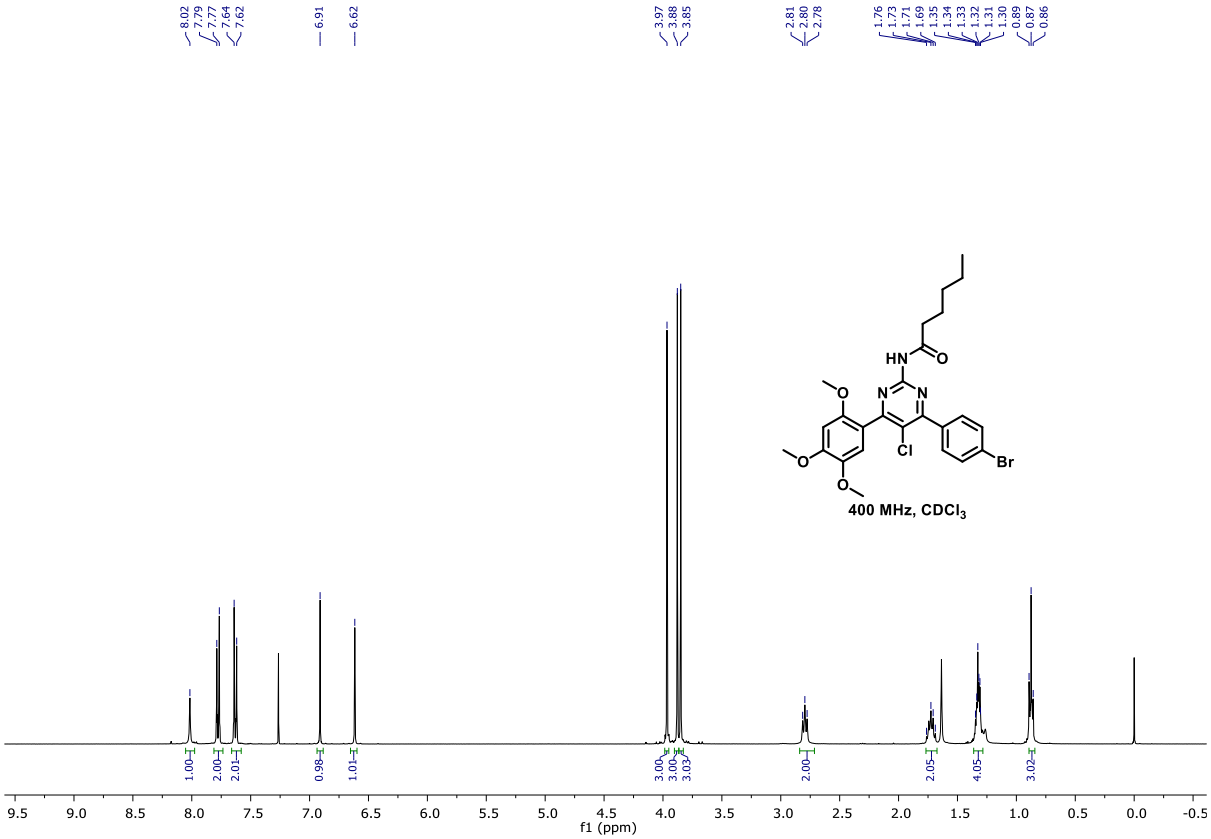


^{1}H NMR for 18b



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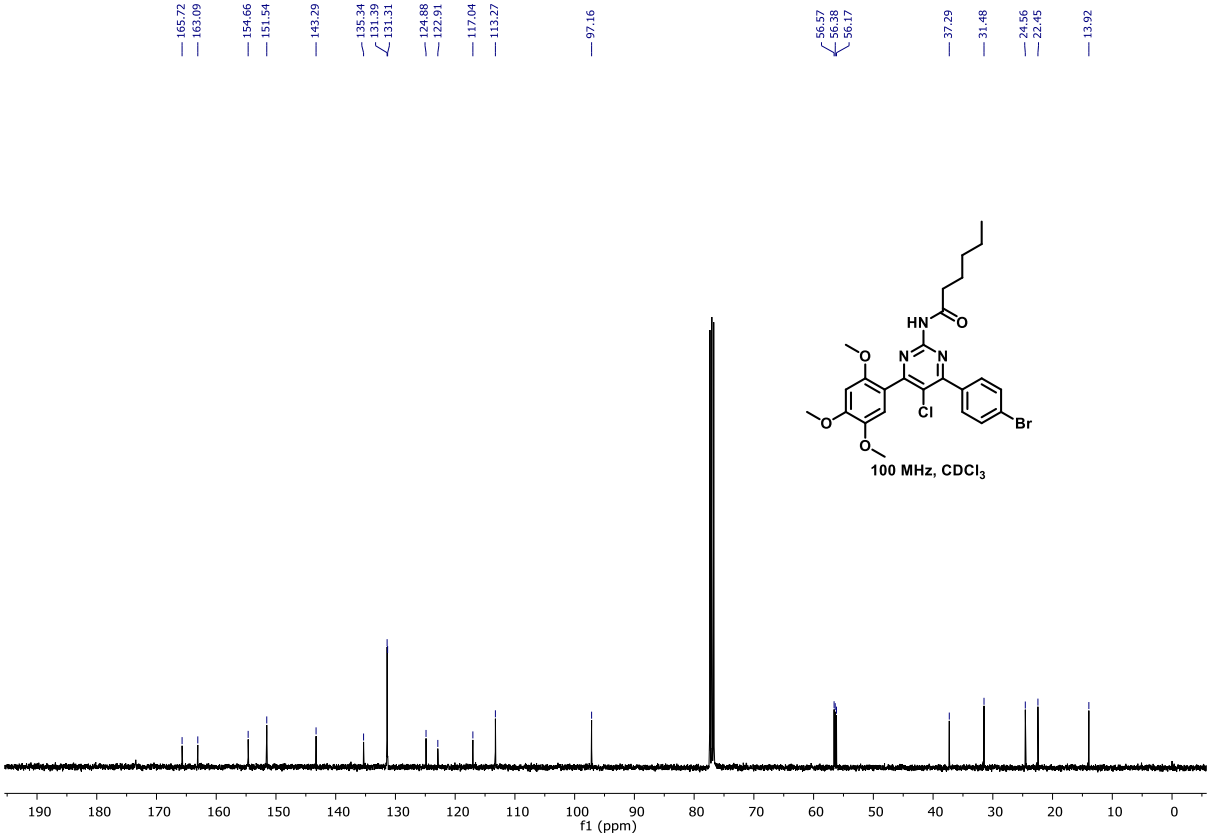
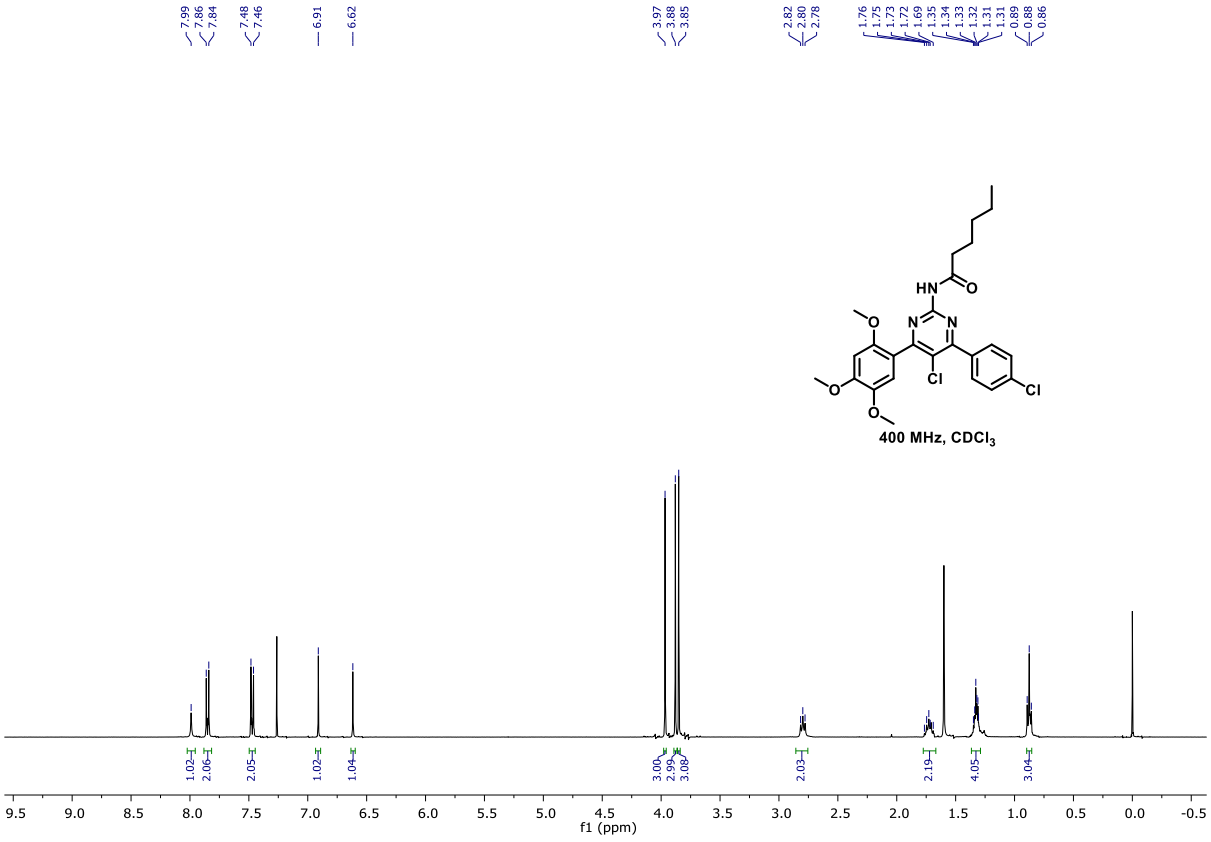
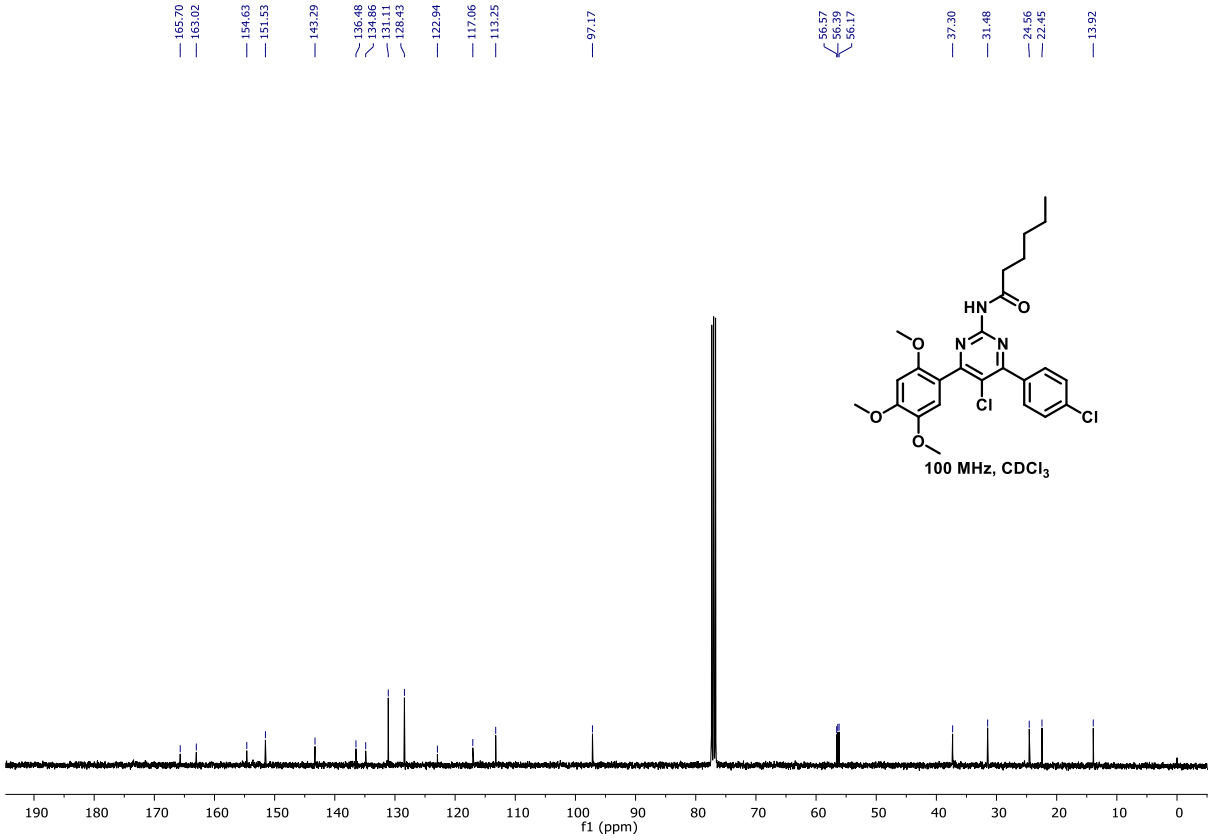


^{1}H NMR for 18c



^{13}C NMR for 18c



5. Representative HPLC profile for active compounds:

HPLC profile for 5a

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Acq. Operator   : Dr. Anil Kumar K.S.          Seq. Line :   4
Acq. Instrument : Instrument 1                  Location : Vial 13
Injection Date  : 1/15/2018 4:39:44 PM               Inj :   1
                                              Inj Volume : 3.000 µl
Acq. Method     : C:\CHEM32\1\DATA\MANISHA-15-01-2018-3 2018-01-15 15-42-16\ACN-WATER-90-10.M
Last changed    : 1/15/2018 4:06:19 PM by Dr. Anil Kumar K.S.
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\ACN-WATER-90-10.M
Last changed    : 1/15/2018 3:42:15 PM by Dr. Anil Kumar K.S.
Method Info     : OSDD
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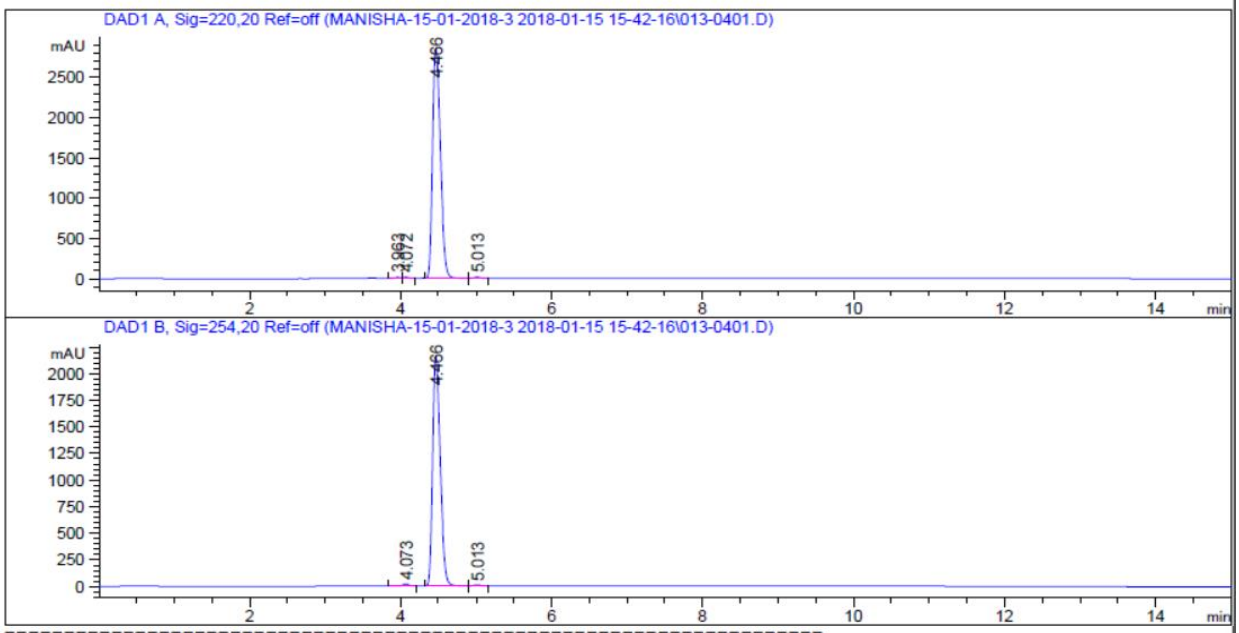


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=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.963 BV    0.0735   73.05510   15.36156   0.3529
   2   4.072 VB    0.0747   88.53242   18.22295   0.4276
   3   4.466 VV    0.1138 2.04355e4  2844.93433  98.7021
   4   5.013 VB    0.0958  107.14032   17.40480   0.5175

Totals :                  2.07043e4  2895.92363
Signal 2: DAD1 B, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.073 BB    0.0838  119.31906   21.17112   0.7881
   2   4.466 VV    0.1067 1.49449e4  2160.34351  98.7057
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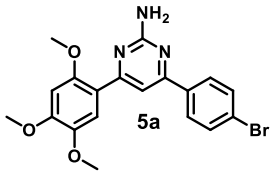

HPLC profile for 5b

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Acq. Instrument : Instrument 1                  Location : Vial 62
Injection Date  : 4/23/2019 3:55:25 PM               Inj :   1
                                              Inj Volume : 4.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FARHEEN-23-04-2019-2 2019-04-23 15-41-32\ACN-WATER-90-10.M
Last changed    : 4/22/2019 3:53:32 PM by Dr. Anil Kumar K.S.
Analysis Method : C:\CHEM32\1\METHODS\ACN-WATER-90-10.M
Last changed    : 4/22/2019 3:53:32 PM by Dr. Anil Kumar K.S.
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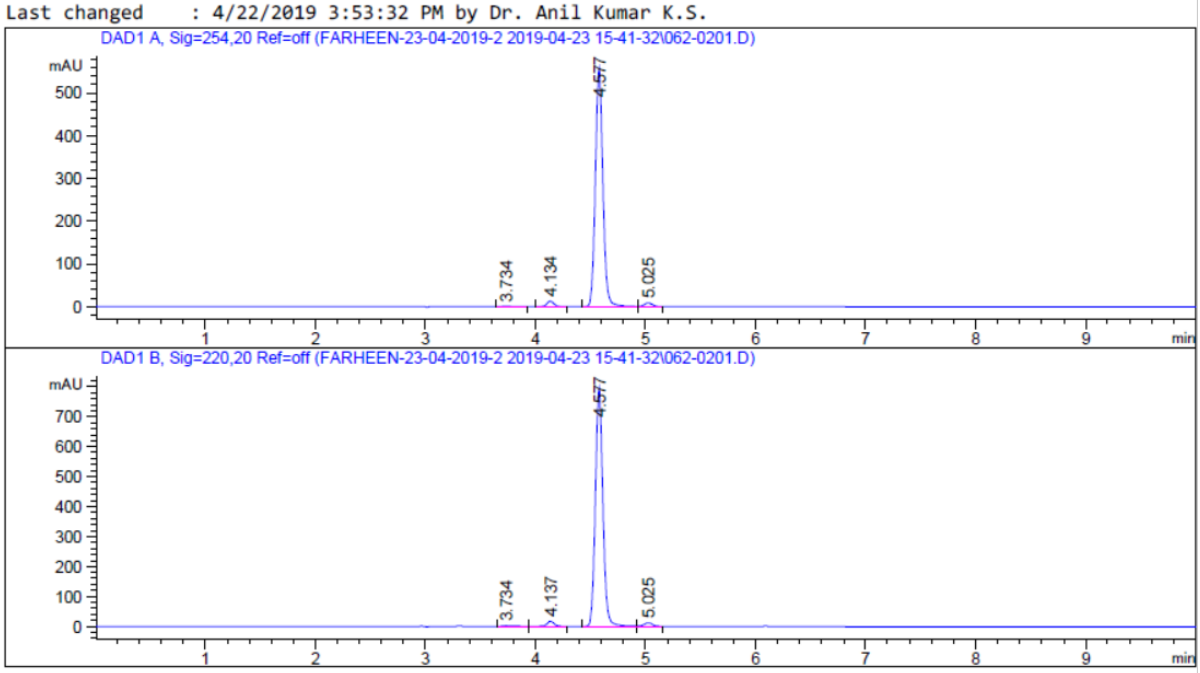


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======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.734 BB    0.0799    7.16487    1.30755   0.2596
   2   4.134 BV    0.0671   54.69370   12.49866   1.9817
   3   4.577 BV    0.0735 2654.92212  558.16913  96.1966
   4   5.025 VB    0.0775   43.11236    8.44967   1.5621

Totals :                  2759.89305  580.42501
Signal 2: DAD1 B, Sig=220,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.734 BB    0.0823   12.74366    2.17628   0.3235
   2   4.137 BV    0.0712   78.36575   17.19351   1.9891
   3   4.577 BV    0.0735 3787.06909  795.76733  96.1266
   4   5.025 VB    0.0773   61.48838   12.09904   1.5608

Totals :                  3939.66688  827.23616
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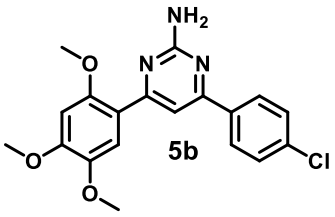

HPLC profile for 7b

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Acq. Instrument : Instrument 1                  Location : Vial 7
Injection Date  : 4/13/2018 5:06:35 PM              Inj :   1
                                               Inj Volume : 12.000 µl
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                  .M
Last changed    : 4/13/2018 4:48:42 PM by Dr. Anil Kumar K.S.
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METH-WATER-90-10-NEW.M
Last changed    : 4/13/2018 3:23:59 PM by Dr. Anil Kumar K.S.
Method Info     : OSDD
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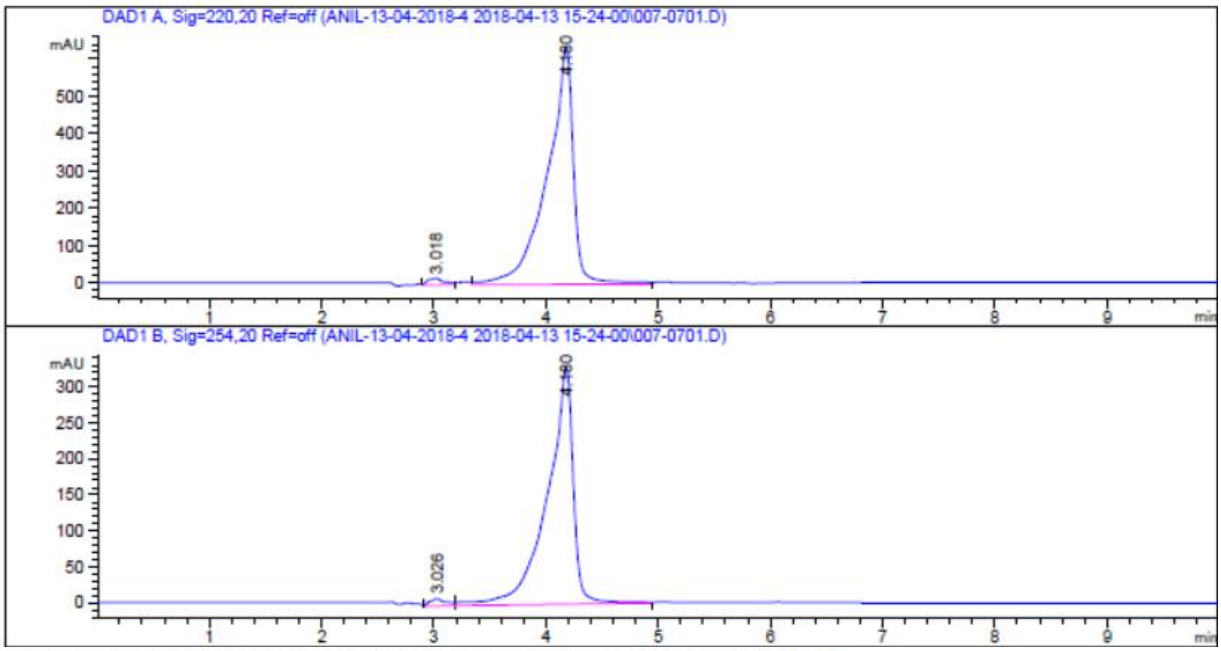


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==================================================================
                    Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,20 Ref=off

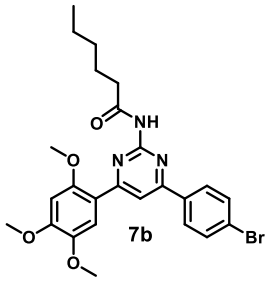
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.018 VV    0.1272  178.84203   18.43100   1.7011
   2   4.180 VV    0.2173 1.03346e4   636.95422  98.2989

Totals :                  1.05134e4   655.38523

Signal 2: DAD1 B, Sig=254,20 Ref=off

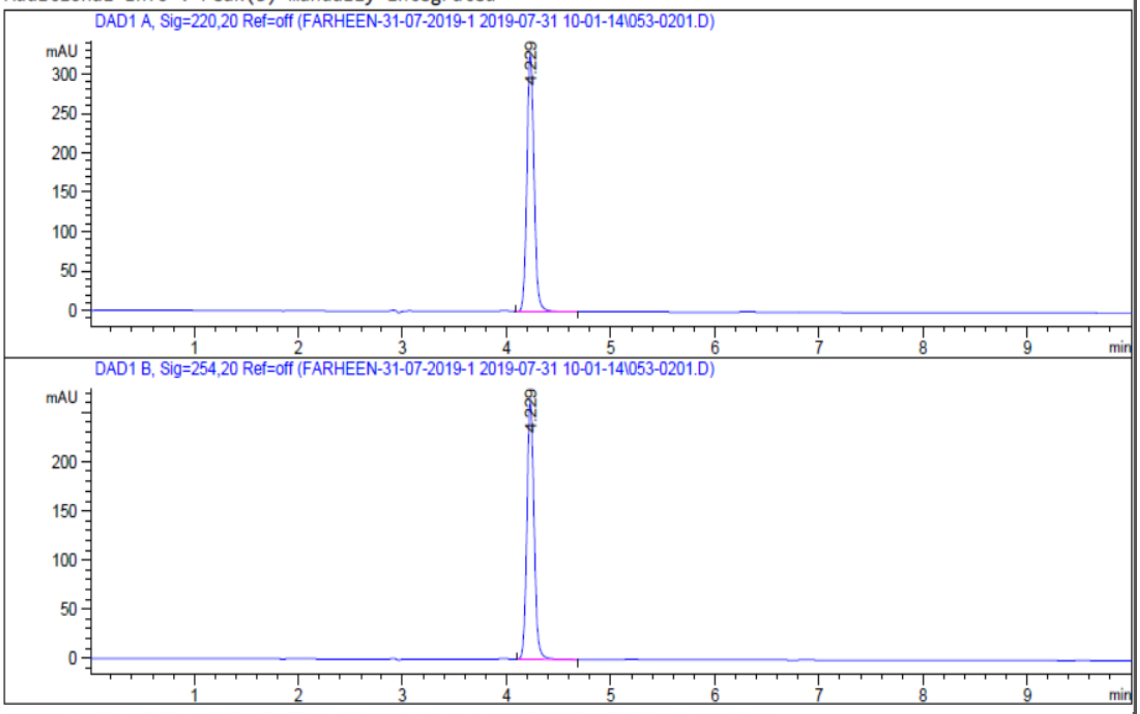
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.026 BV    0.1238   94.90692   10.27026   1.7543
   2   4.180 VV    0.2161 5315.11816  329.63553  98.2457

Totals :                  5410.02509  339.90578
```



HPLC profile for 17c

```
====================================================================
Acq. Operator   : Dr. Anil Kumar K.S.          Seq. Line :   2
Acq. Instrument : Instrument 1                  Location : Vial 53
Injection Date  : 7/31/2019 10:14:59 AM              Inj :   1
                                               Inj Volume : 4.000 µl
Method          : C:\CHEM32\1\DATA\FARHEEN-31-07-2019-1 2019-07-31 10-01-14\ACN-WATER-90-10.M
                  (Sequence Method)
Last changed    : 7/8/2019 3:51:28 PM by Dr. Anil Kumar K.S.
Additional Info : Peak(s) manually integrated
```




```
====================================================================
                        Area Percent Report
====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=220,20 Ref=off

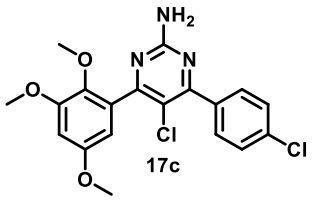
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.229 VB    0.0737 1561.06116  326.74161 100.0000

Totals :                  1561.06116  326.74161

Signal 2: DAD1 B, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.229 BB    0.0736 1252.55359  262.63843 100.0000

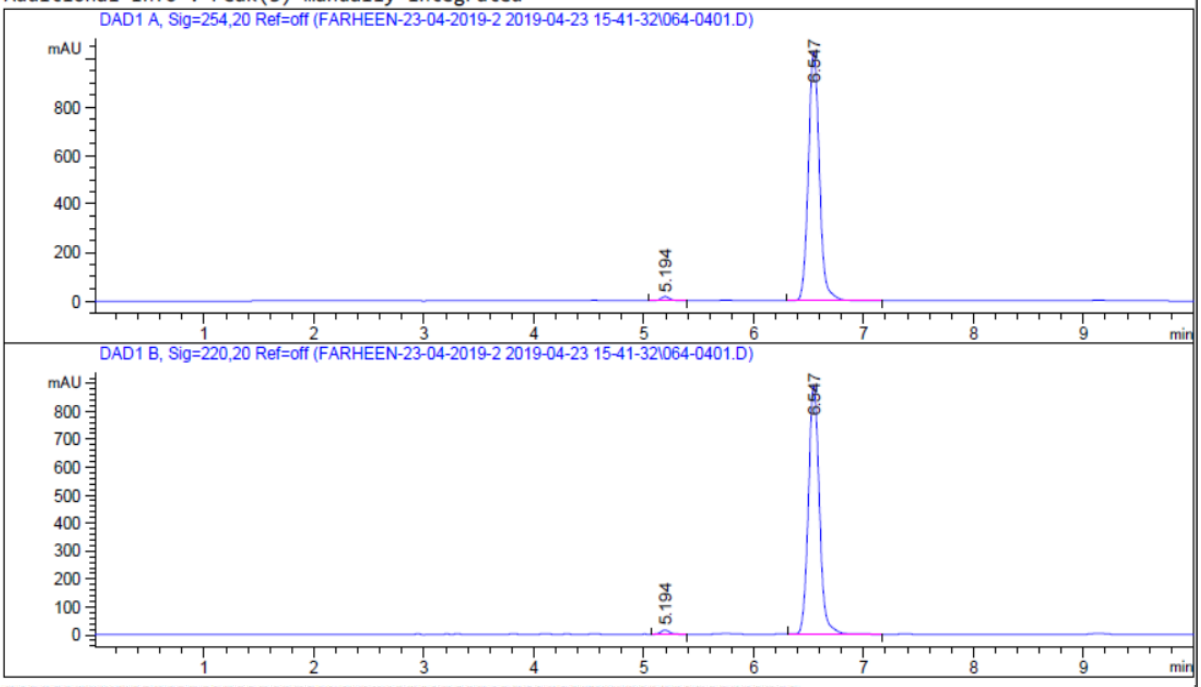
Totals :                  1252.55359  262.63843
```



17c

HPLC profile for 18a

```
====================================================================
Acq. Operator   : Dr. Anil Kumar K.S.          Seq. Line :   4
Acq. Instrument : Instrument 1                  Location : Vial 64
Injection Date  : 4/23/2019 4:19:32 PM               Inj :   1
                                              Inj Volume : 4.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FARHEEN-23-04-2019-2 2019-04-23 15-41-32\ACN-WATER-90-10.M
Last changed    : 4/22/2019 3:53:32 PM by Dr. Anil Kumar K.S.
Analysis Method : C:\CHEM32\1\METHODS\ACN-WATER-90-10.M
Last changed    : 4/22/2019 3:53:32 PM by Dr. Anil Kumar K.S.
Additional Info : Peak(s) manually integrated
```




```
====================================================================
                        Area Percent Report
====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

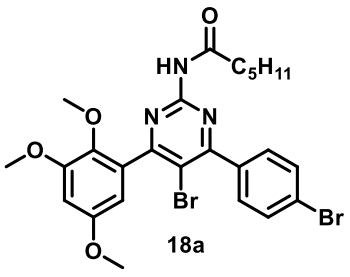

Signal 1: DAD1 A, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.194 BB    0.0850   95.30827   17.11275   1.3162
   2   6.547 BB    0.1072 7145.82861 1026.98547  98.6838

Totals :                   7241.13689 1044.09822
Signal 2: DAD1 B, Sig=220,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.194 VB    0.0851   84.18088   15.09448   1.3375
   2   6.547 VB    0.1073 6209.93066  890.89746  98.6625

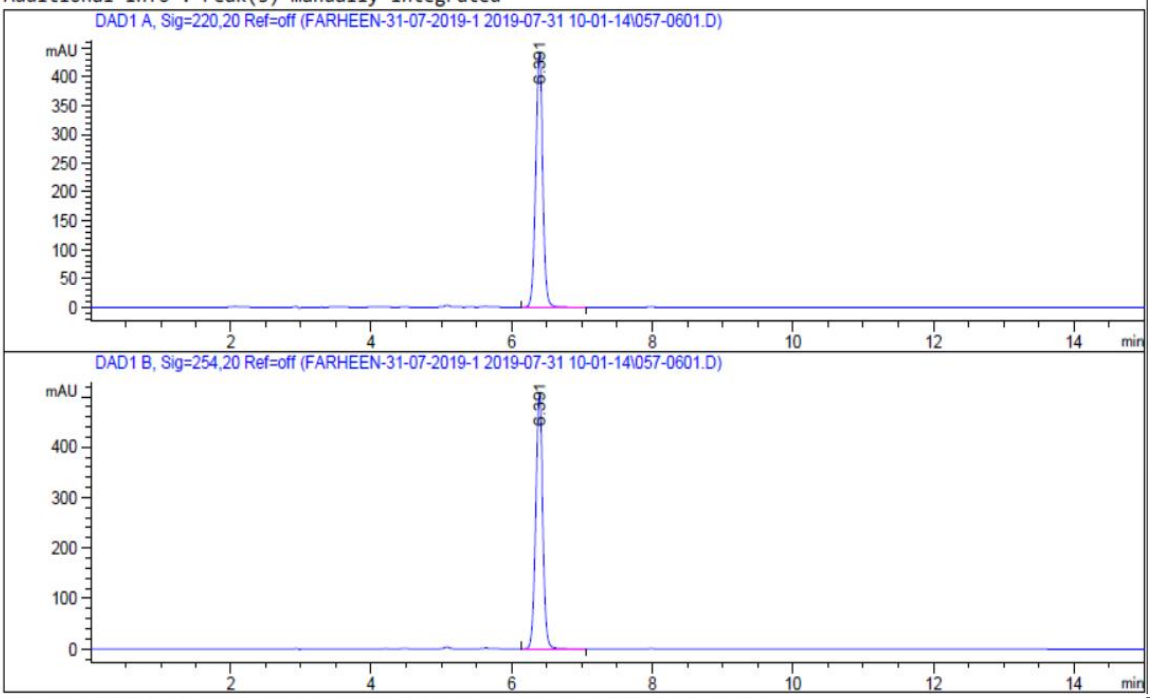
Totals :                   6294.11154  905.99194
```



18a

HPLC profile for 10c

```
Acq. Operator   : Dr. Anil Kumar K.S.          Seq. Line :   6
Acq. Instrument : Instrument 1                  Location : Vial 57
Injection Date  : 7/31/2019 11:03:15 AM              Inj :   1
                                              Inj Volume : 4.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FARHEEN-31-07-2019-1 2019-07-31 10-01-14\ACN-WATER-90-10.M
Last changed    : 7/31/2019 11:01:59 AM by Dr. Anil Kumar K.S.
                  (modified after loading)
Analysis Method : C:\CHEM32\1\DATA\FARHEEN-31-07-2019-1 2019-07-31 10-01-14\ACN-WATER-90-10.M
                  (Sequence Method)
Last changed    : 7/8/2019 3:51:28 PM by Dr. Anil Kumar K.S.
Additional Info : Peak(s) manually integrated
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

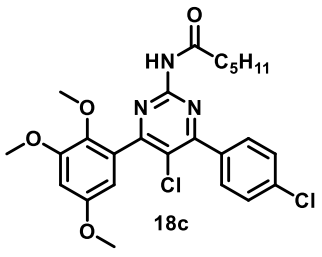

Signal 1: DAD1 A, Sig=220,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.391 BB    0.1101 3107.97852  441.68982 100.0000

Totals :                  3107.97852  441.68982
Signal 2: DAD1 B, Sig=254,20 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.391 BB    0.1100 3549.26367  505.02533 100.0000

Totals :                  3549.26367  505.02533
```



18c

6. ALP activity for rest compounds

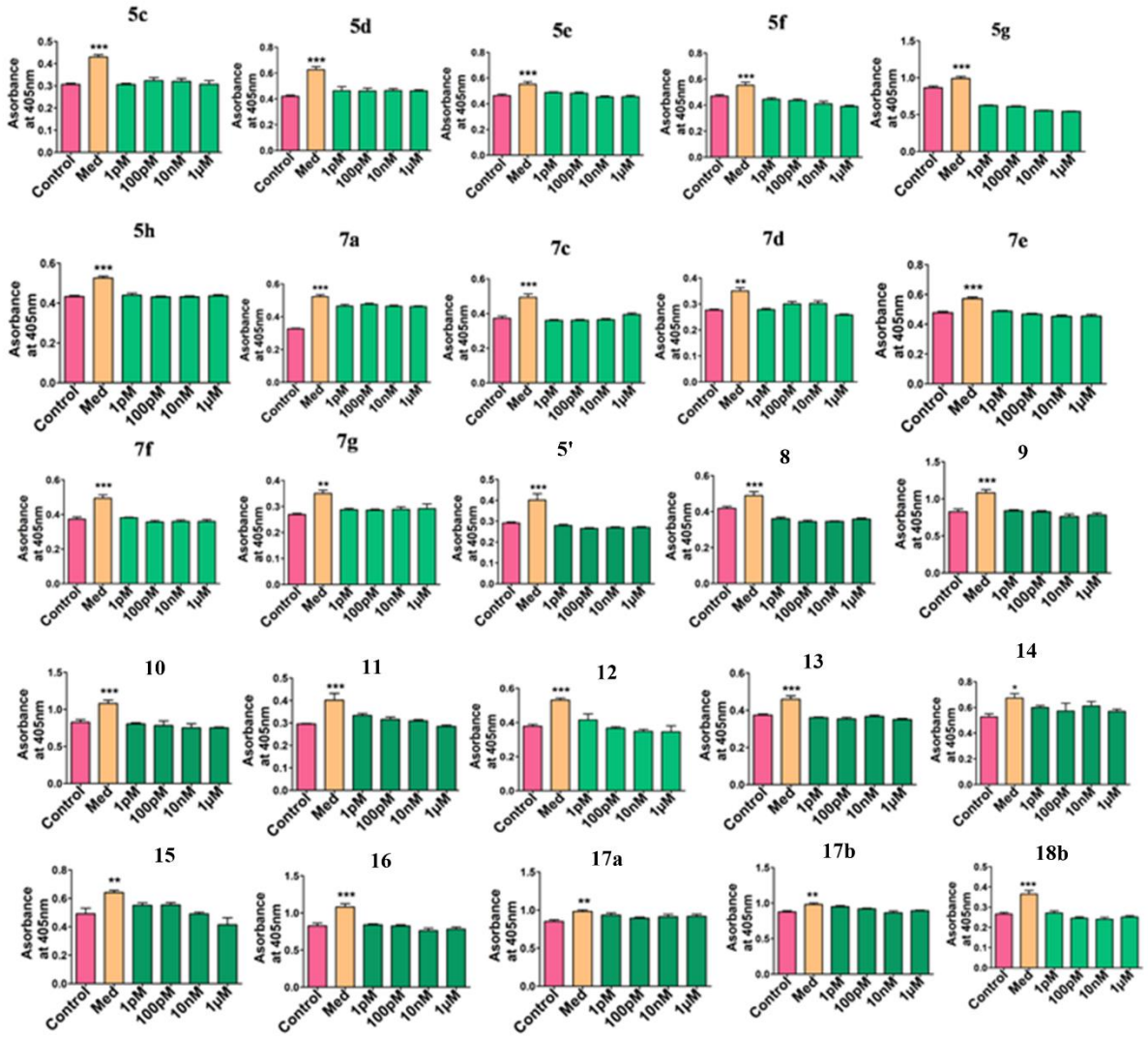


Figure: S1. ALP activities of the inactive compounds.

7. Table S1: Sequence of primer for real time-PCR

S. No.	Gene name	Sequence
1.	Glyceraldehyde-3-phosphate dehydrogenate (GAPDH)	F-5′-AGCTTGTCATCAACGGGAAG-3′ R-5′-TTTGATGTTAGTGGGGTCTCG-3′
2.	Bone Morphogenetic protein 2 (BMP-2)	F-5′-CGGACTGCGGTCTCCTAA-3′ R-5′-GGGGAAGCAGCAACACTAGA-3′
3.	Type 1 collagenase (Col-1)	F-5′-CATGTTCAGCTTTGTGGACCT-3′ R-5′-GCAGCTGACTTCAGGGATGT-3′
4.	Runt related transcription factor 2 (RUNX-2)	F-5′-CCCGGGAACCAAGAAATC-3′ R-5′-AGATAGGAGGGGTAAGACTGG-3′

8. Reference:

1. Kumar, R.; Mohanakrishnan, D.; Sharma, A.; Kaushik, N. K.; Kalia, K.; Sinha, A. K.; Sahal, D. Reinvestigation of structure--activity relationship of methoxylated chalcones as antimalarials: Synthesis and evaluation of 2,4,5-trimethoxy substituted patterns as lead candidates derived from abundantly available natural β-asarone. *Eur. J. Med. Chem*. **2010**, *45*, 5292-5310.