Supporting Information

Microwave-Assisted Synthesis of Azacoumarin Fluorophores and the Fluorescence Characterization

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Table of contents

I. Determining Fluorescent Quantum Yield

The fluorescent quantum yield (QY) was measured relative to quinine sulfate (QY = 0.577) as a reference compound. The fluorescent spectra of samples were measured in PBS (2 μM) at 350 nm exciting-wavelength. Gain and slit bandwidths were applied for these samples, and these QY were calculated as equation,

$$QY = QY_{ref} \frac{\eta^2}{\eta^2_{\text{ref}}} \frac{I}{A} \frac{A_{ref}}{I_{ref}}$$

where QY_{ref} was the quantum yields of quinine sulfate, η was the refractive index of the solvent, I was the integrated fluorescence intensity and A was the absorbance at 350 nm excitation wavelength. The concentration of samples should be sufficiently diluted not to occur concentration quenching. Take into consideration inner filter effect the absorbance at 350 nm was kept in under 0.035.

II. Calculating the saturated concentration of compounds

Saturated solution in PBS was prepared by sonicating the solid compound for 30 min at 20 ℃. After we ensure that the solid was left in the solution, we filtrate the suspension to give the PBS saturated solution. The saturated concentration was determined from standard curves created by related peak area (detect at 340 nm) against known concentration of compounds in PBS.

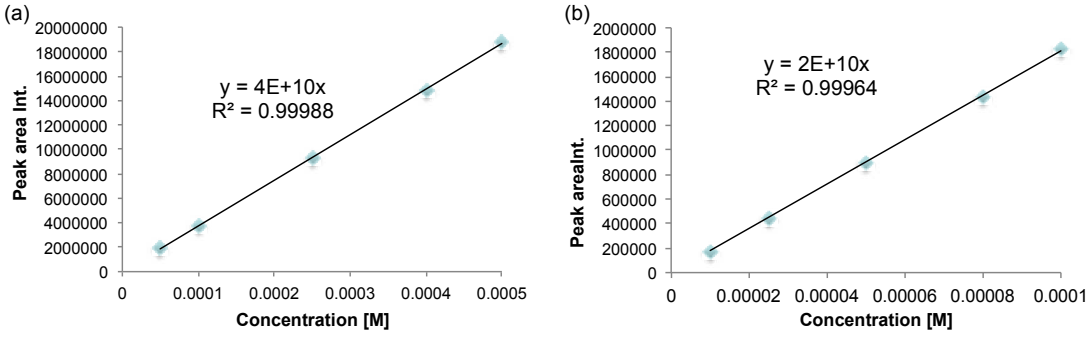


Figure S1. Standard curve of (a) 8-aza-hc and (b) hc.

Table S1. Saturated concentration (C_s) of 8-aza-hc and hc in PBS was calculated from each standard curve.

compd	Peak area	C_s [μM]
8-aza-hc	214615200	5365
hc	12537714	627

III. Determination of the pK_a value

The McIlvain buffer with a buffer capacity from pH 2.2 to 8.0 was used as solvent. The acidity of the sample solution was adjusted with addition of 10 μL of 2 M NaOH aq. and sequentially measured the pH values by a pH meter and the absorbance spectrum. The pK_a value was estimated from the titration curves of absorbance at 350 nm against pH value.

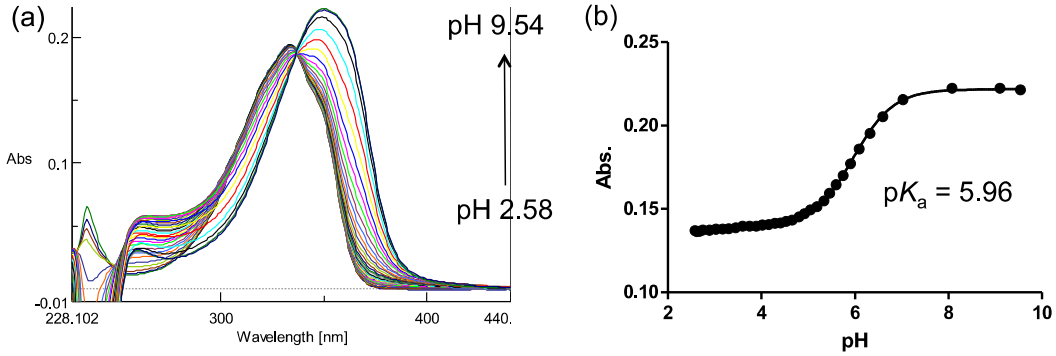
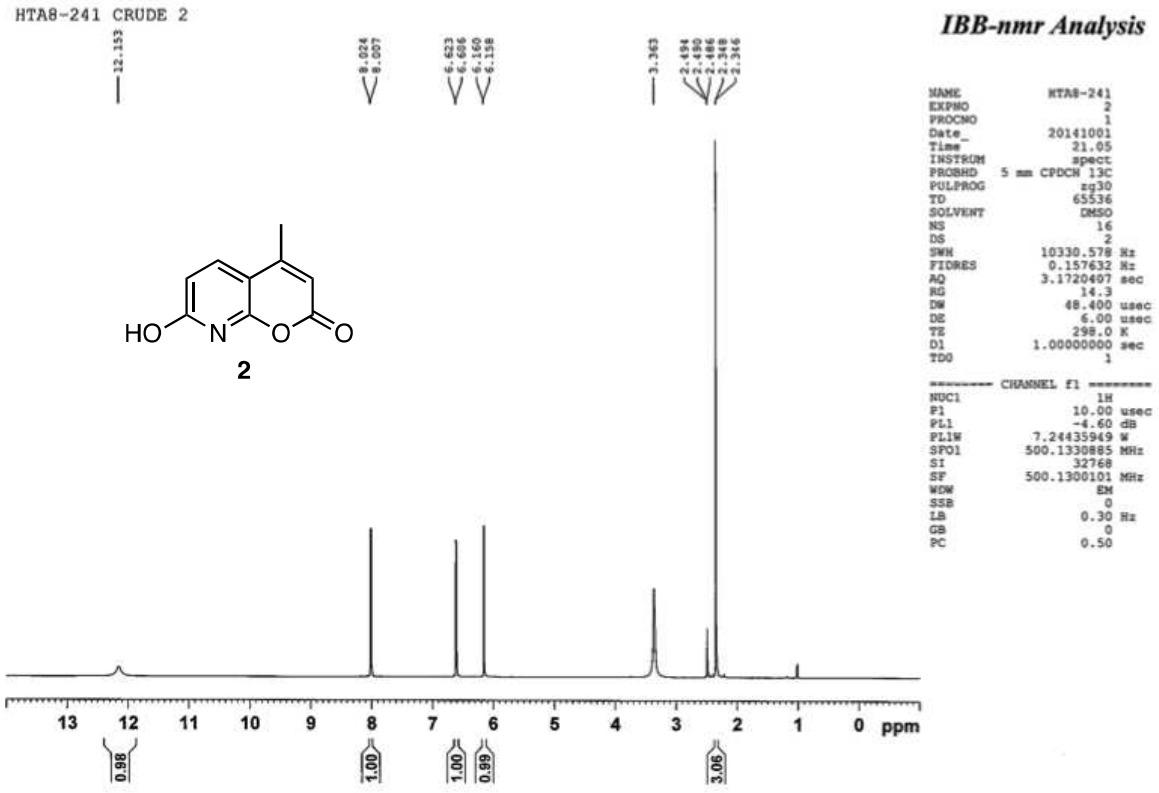


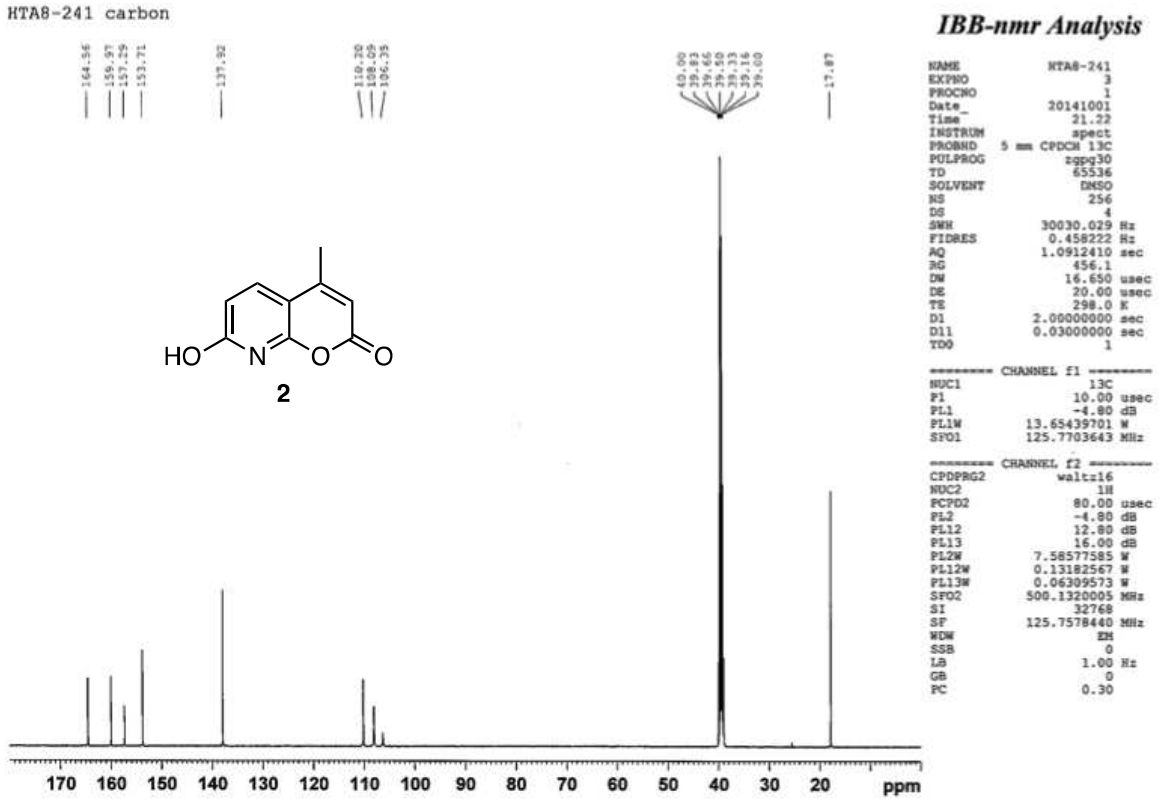
Figure S2. (a) the absorption spectra of 8-aza-hc aginst the pH values. (b) plot of pH to absorbance.

IV. ^{1}H-NMR and ^{13}C-NMR charts of compounds



IBB-nmr Analysis

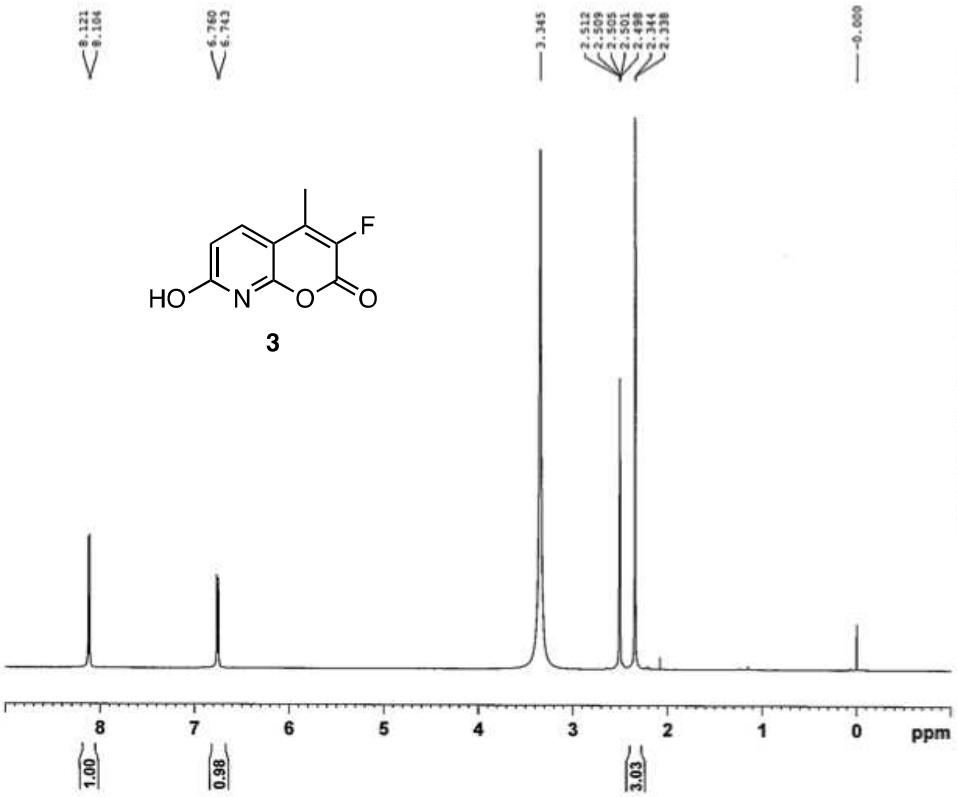
NAME	HTA8-241
EXPNO	2
PROCNO	1
Date_	20141001
Time	21.05
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300101 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50



IBB-nmr Analysis

NAME	HTA8-241
EXPNO	3
PROCNO	1
Date_	20141001
Time	21.22
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	256
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	456.1
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7578440 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30

8-aza-Fhc

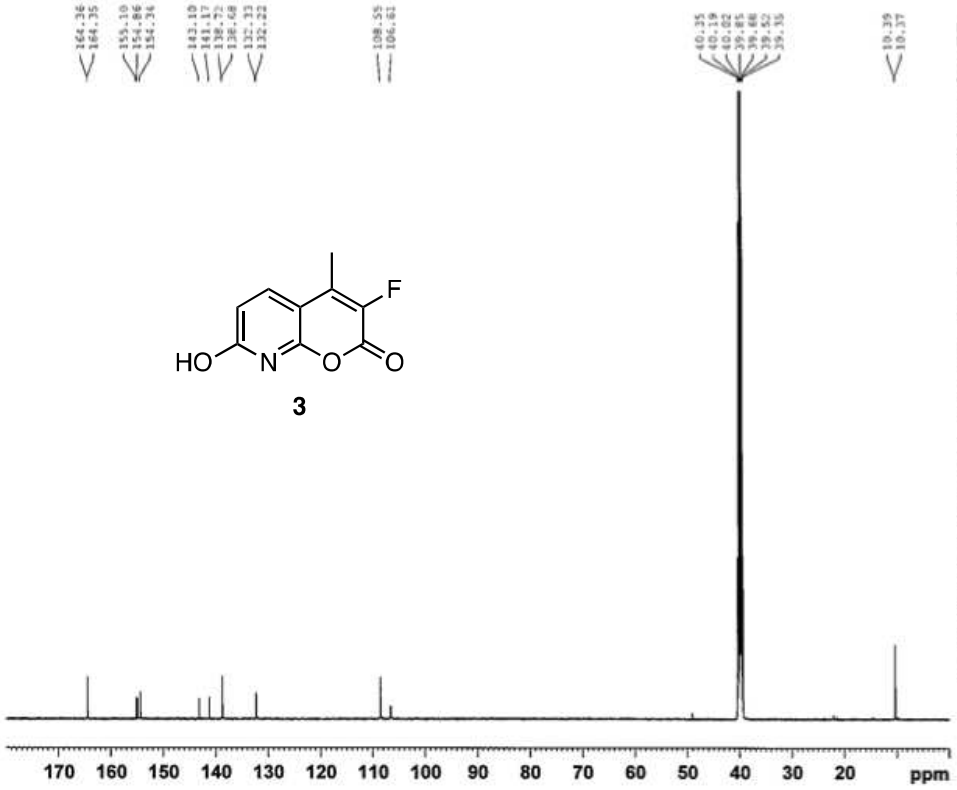


3

IBB-nmr Analysis

NAME	HTAsi
EXPNO	53
PROCNO	1
Date_	20160810
Time	12.14
INSTRUM	av500
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	22.6
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300037 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50

8-aza-Fhc carbon

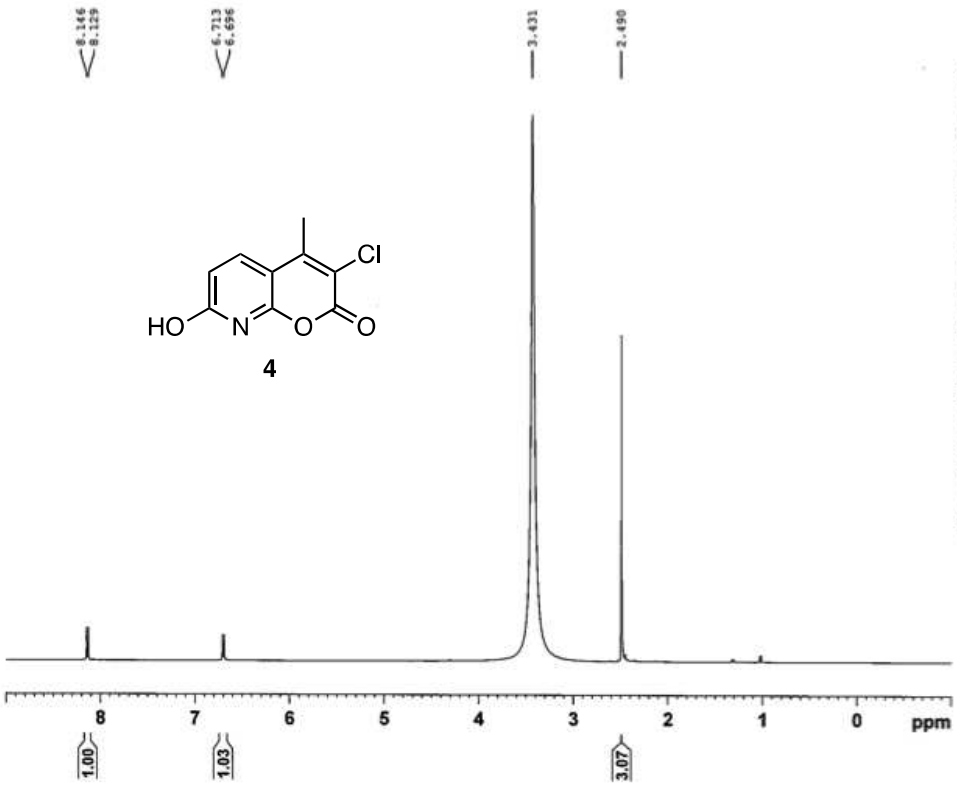


3

IBB-nmr Analysis

NAME	HTAsi
EXPNO	52
PROCNO	1
Date_	20160808
Time	10.58
INSTRUM	av500
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	64
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	161.3
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7577924 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30

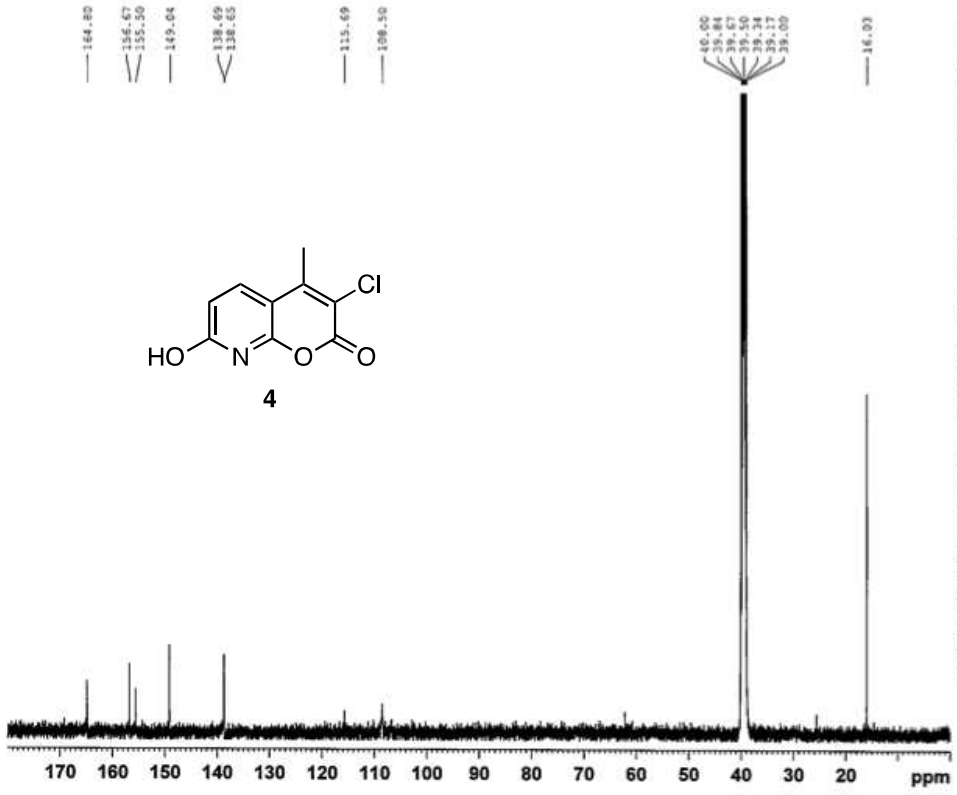
HTA11-349



IBB-nmr Analysis

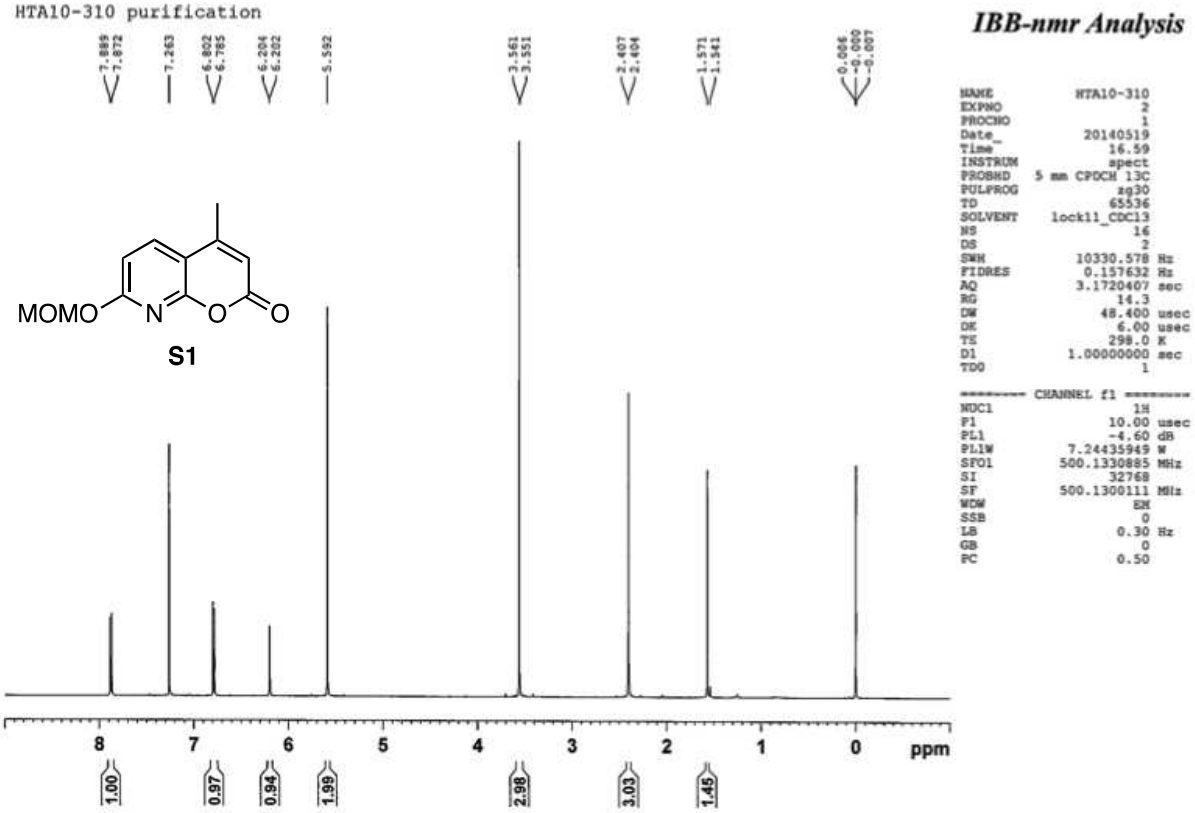
NAME	HTA11-349
EXPNO	1
PROCNO	1
Date_	20140722
Time	10.08
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	lock11_CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
========	CHANNEL f1 ========
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1323859 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50

HTA11-349 carbon



IBB-nmr Analysis

NAME	HTA11-349
EXPNO	2
PROCNO	1
Date_	20140722
Time	10.30
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	lock11_CDCl3
NS	256
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	362
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
========	CHANNEL f1 ========
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
========	CHANNEL f2 ========
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7584323 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30


HTA10-310 purification
IBB-nmr Analysis
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7.872
7.263
6.802
6.785
6.204
6.202
5.592
3.561
3.551
2.407
2.404
1.371
1.341
0.006
0.000
-0.007
MOMO
N
O
O
S1
NAME HTA10-310
EXPNO 2
PROCNO 1
Date_ 20140519
Time 16.59
INSTRUM spect
PROBHD 5 mm CPDCH 13C
PULPROG zg30
TD 65536
SOLVENT lock11_CDCl3
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 14.3
DW 48.400 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 10.00 usec
PL1 -4.60 dB
PL1W 7.24435945 W
SFO1 500.1330885 MHz
SI 32768
SF 500.1300111 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 0.50
8
7
6
5
4
3
2
1
0
ppm
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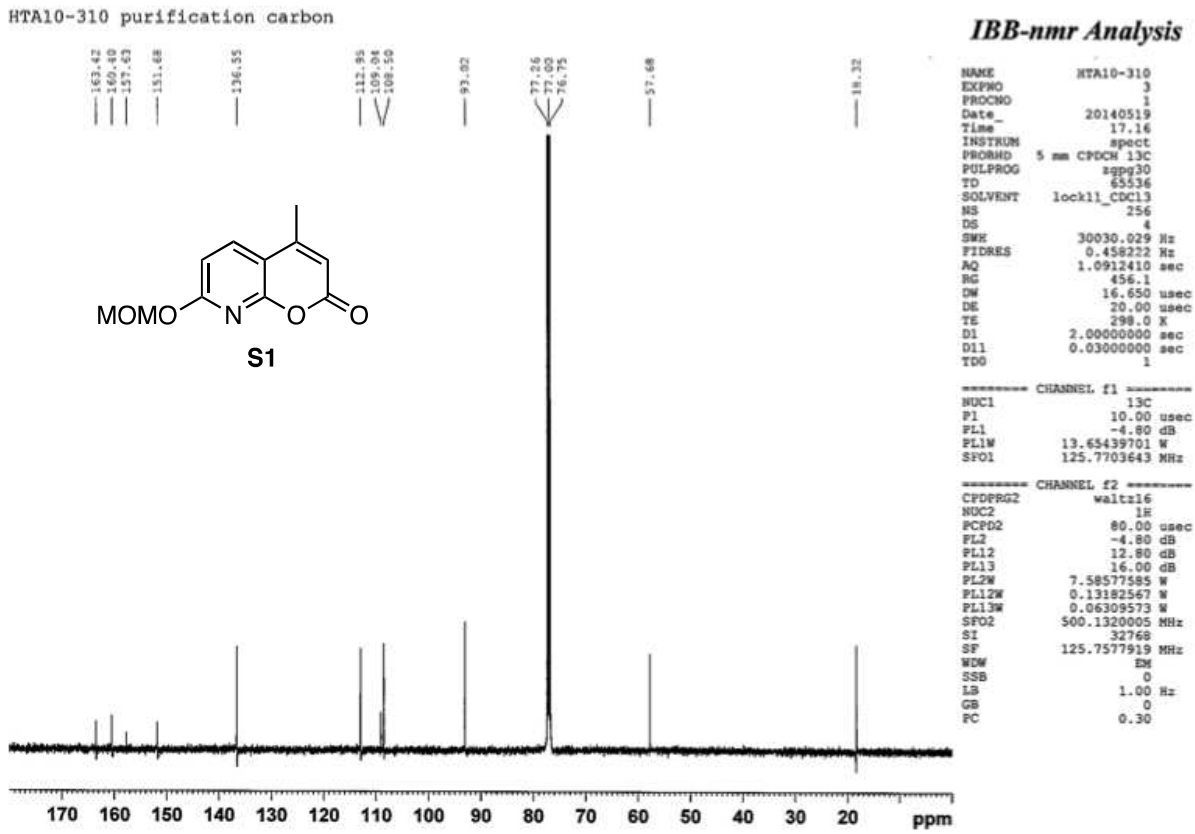
0.97
0.94
1.99
2.98
3.03
1.45


HTA10-310 purification carbon
IBB-nmr Analysis
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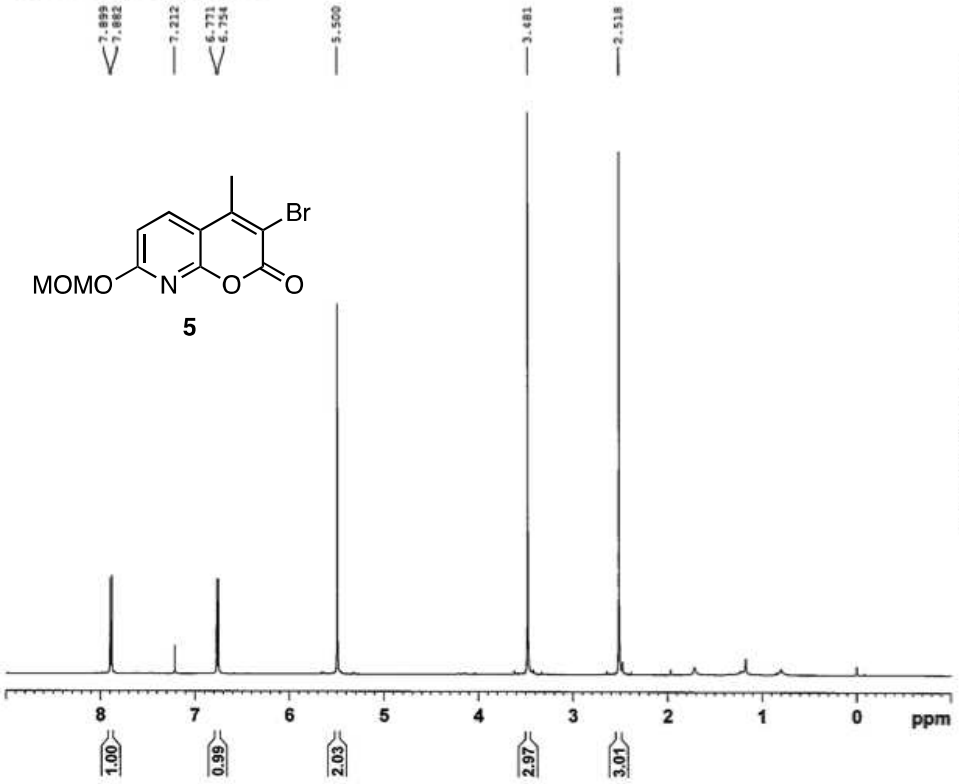
157.63
151.68
136.55
112.95
109.04
108.50
93.02
77.26
77.00
76.75
57.68
18.32
MOMO
N
O
O
S1
NAME HTA10-310
EXPNO 3
PROCNO 1
Date_ 20140519
Time 17.16
INSTRUM spect
PROBHD 5 mm CPDCH 13C
PULPROG zgpg30
TD 65536
SOLVENT lock11_CDCl3
NS 256
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0912410 sec
RG 456.1
DW 16.650 usec
DE 20.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 10.00 usec
PL1 -4.80 dB
PL1W 13.65439701 W
SFO1 125.7703643 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.80 dB
PL12 12.80 dB
PL13 16.00 dB
PL2W 7.58577585 W
PL12W 0.13182567 W
PL13W 0.06309573 W
SFO2 500.1320005 MHz
SI 32768
SF 125.7577919 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 0.30
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

HTA11-350 purification

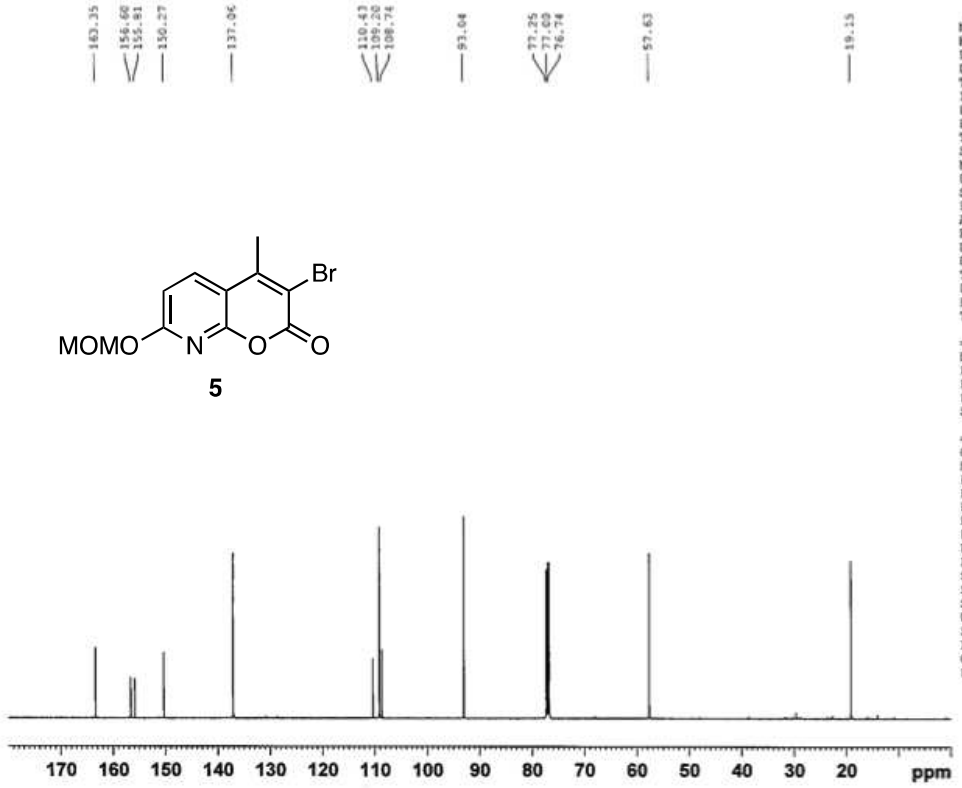


5

IBB-nmr Analysis

NAME	HTA11-350
EXPNO	6
PROCNO	1
Date_	20141001
Time	21.28
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	lock11_CDC13
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300374 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50

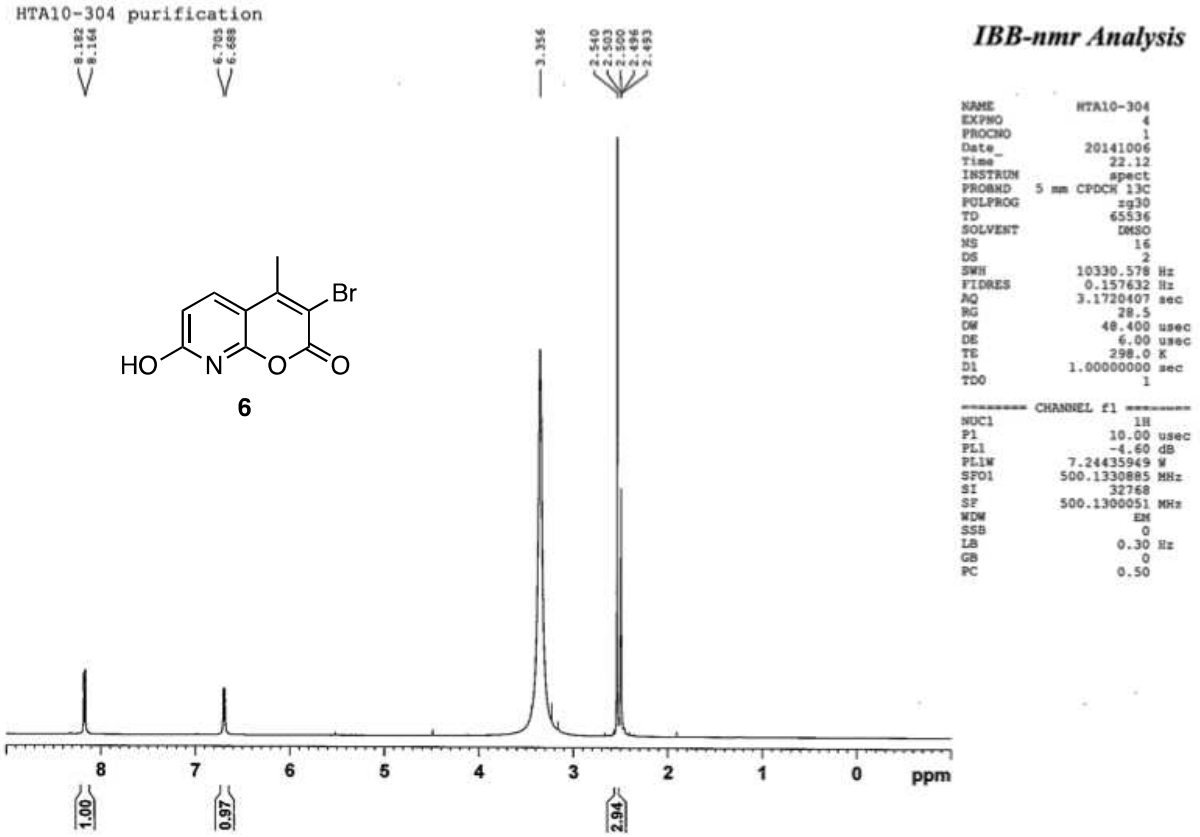
HTA11-350 purification carbon



5

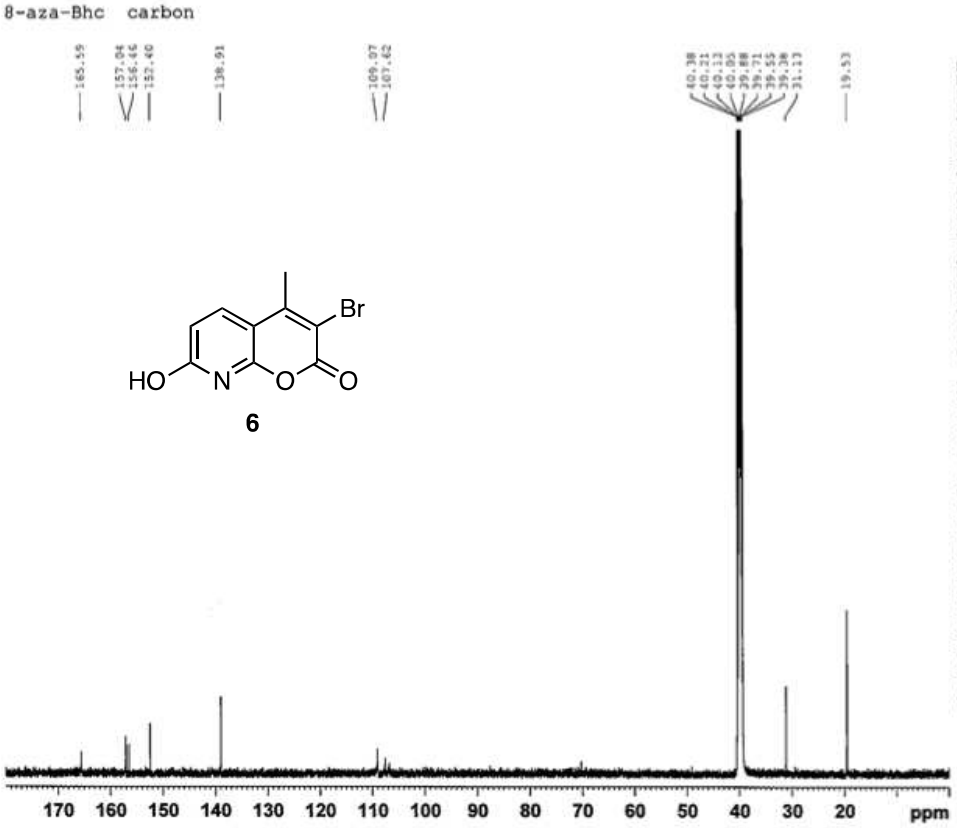
IBB-nmr Analysis

NAME	HTA11-350
EXPNO	7
PROCNO	1
Date_	20141001
Time	21.44
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	lock11_CDC13
NS	256
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	256
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7578010 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30



IBB-nmr Analysis

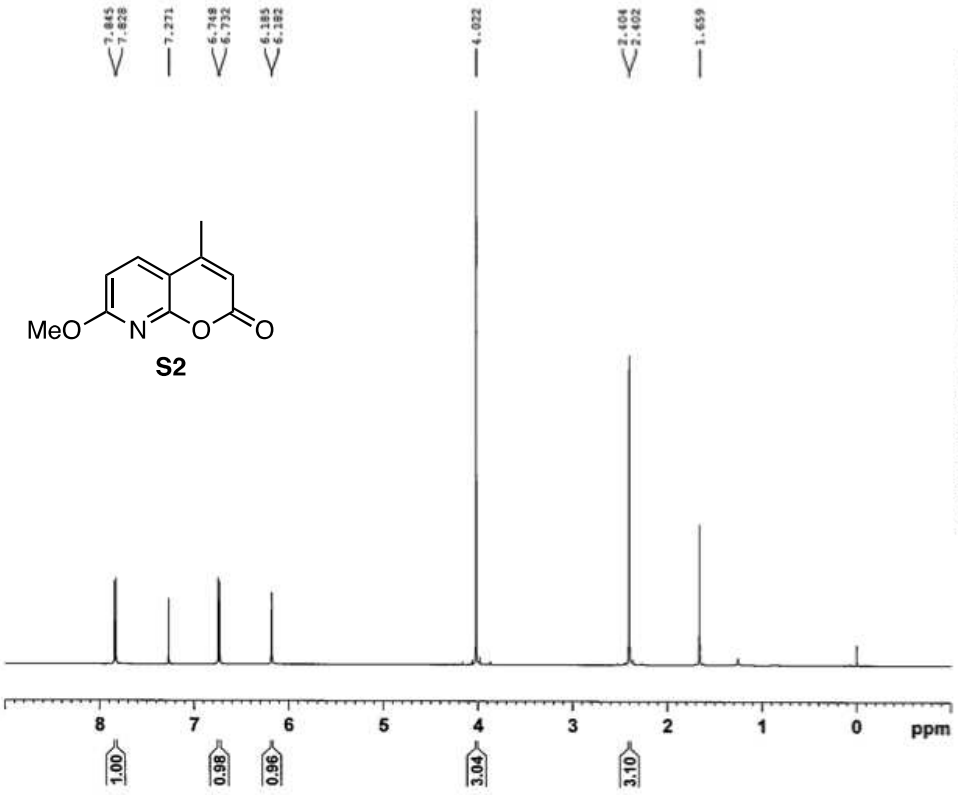
NAME	HTA10-304
EXPNO	4
PROCNO	1
Date_	20141006
Time	22.12
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	28.5
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300051 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50



IBB-nmr Analysis

NAME	HTA si
EXPNO	53
PROCNO	1
Date_	20160805
Time	15.26
INSTRUM	av500
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	64
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	181
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7577924 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30

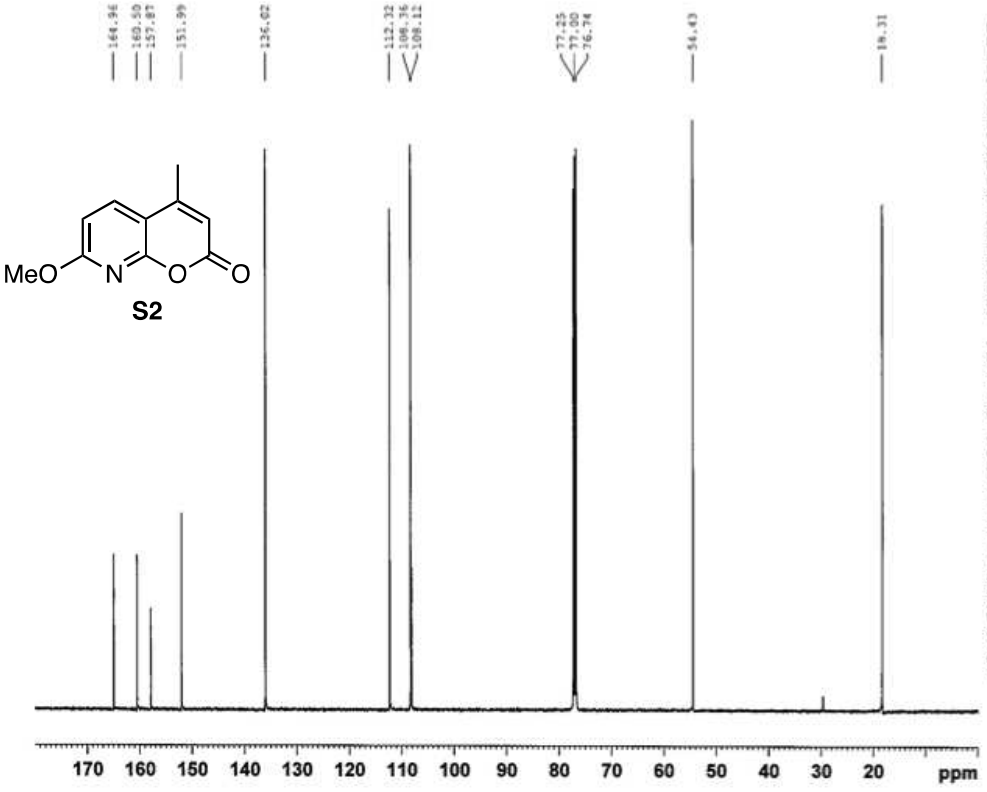
HTA11-394 purification



IBB-nmr Analysis

NAME	HTA11-394
EXPNO	2
PROCNO	1
Date_	20140902
Time_	11.17
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zg30
TD	65536
SOLVENT	lock11_CDC13
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300081 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50

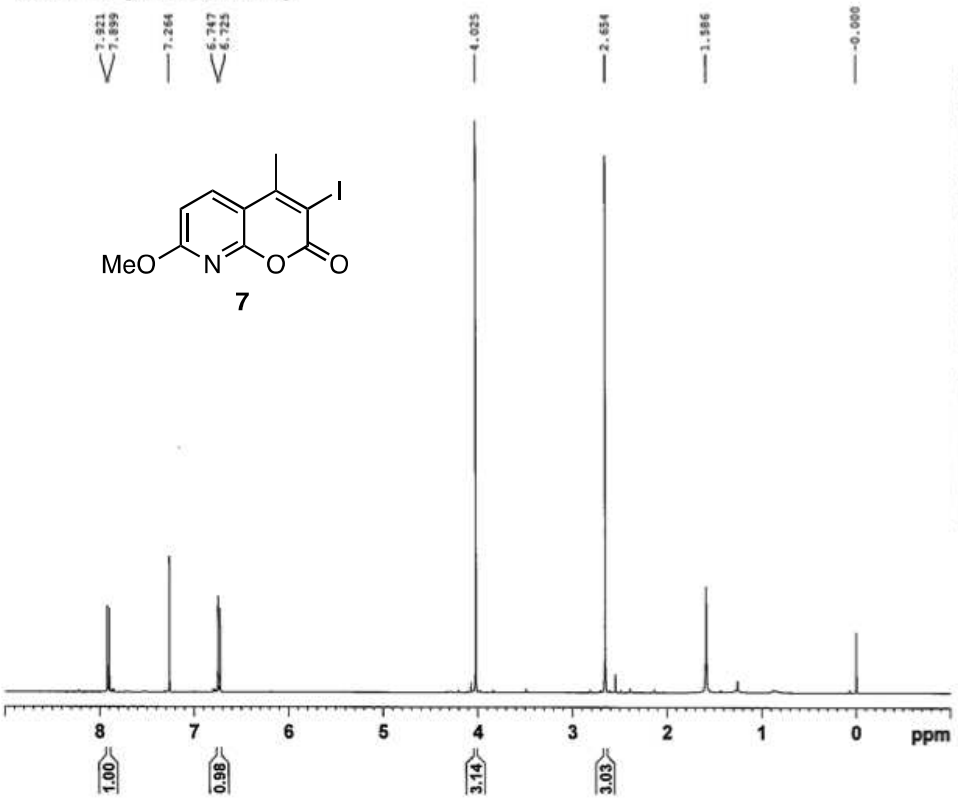
HTA11-391 purification carbon



IBB-nmr Analysis

NAME	HTA11-391
EXPNO	4
PROCNO	1
Date_	20141001
Time_	22.31
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	lock11_CDC13
NS	256
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	228.1
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7577983 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30

HTA11-362 purification up

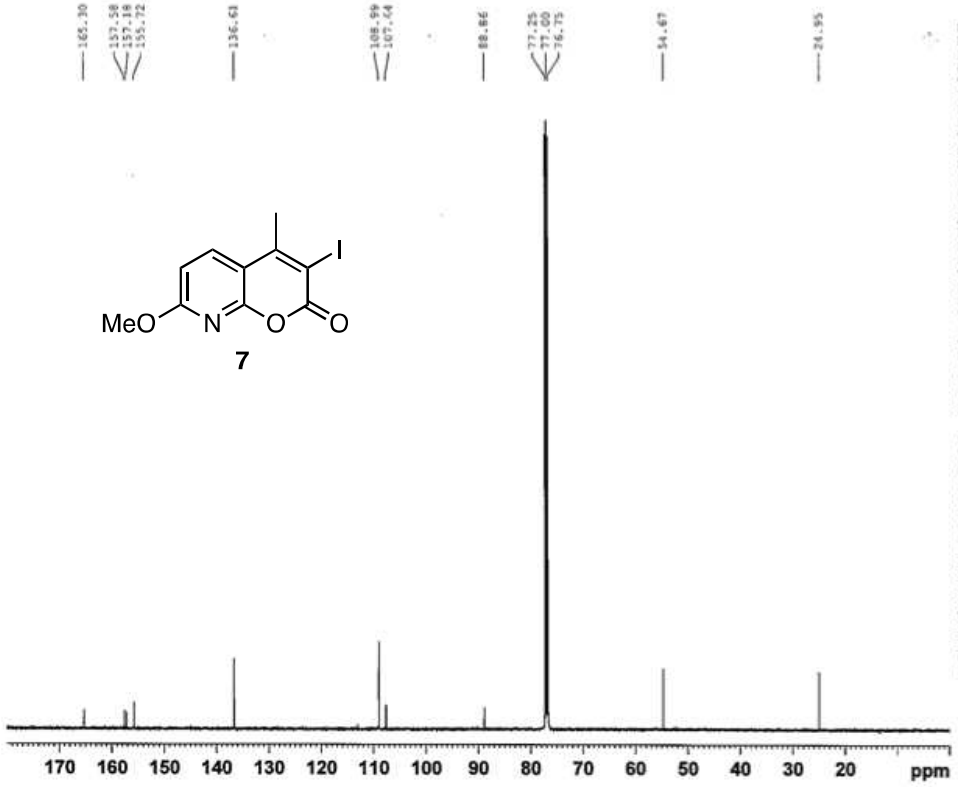


7

IBB-nmr Analysis

NAME	HTA11-362
EXPNO	2
PROCNO	1
Date_	20140804
Time_	20.10
INSTRUM	av400
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	203
DW	60.800 usec
DE	6.50 usec
TE	300.6 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.00 usec
PL1	-1.80 dB
PL1W	14.82738590 W
SFO1	400.1324710 MHz
SI	32768
SF	400.1300077 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

HTA12-416 purification carbon

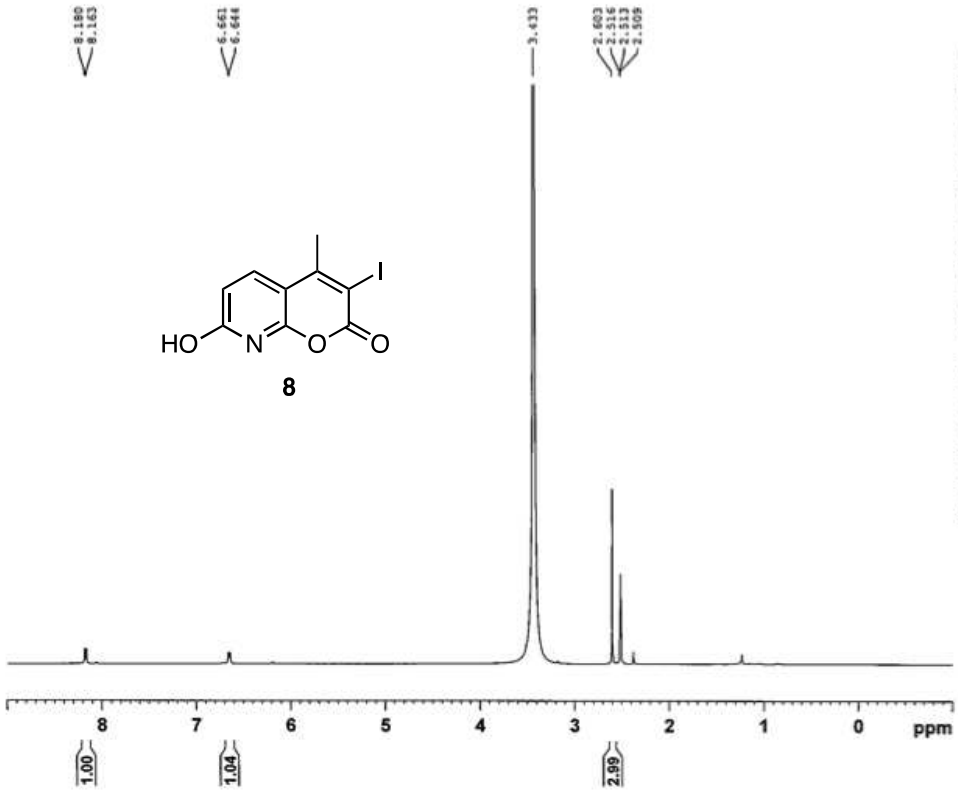


7

IBB-nmr Analysis

NAME	HTA12-416
EXPNO	4
PROCNO	1
Date_	20141006
Time_	22.08
INSTRUM	spect
PROBHD	5 mm CPDCH 13C
PULPROG	zgpg30
TD	65536
SOLVENT	lock11_CDCl3
NS	256
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	256
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7577928 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30

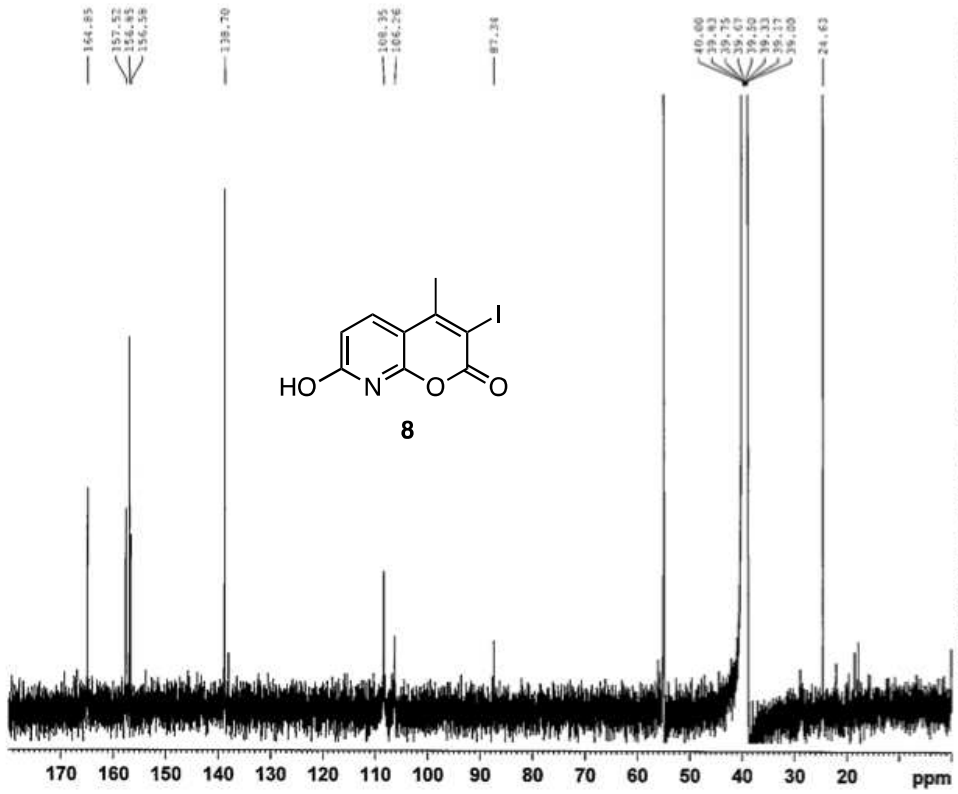
HTA17-802 purification



IBB-nmr Analysis

NAME	HTA17-802
EXPNO	1
PROCNO	1
Date_	20160808
Time	10.38
INSTRUM	av500
PROBHD	5 mm CPDCN 13C
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.50

HTA17-802 purification carbon



IBB-nmr Analysis

NAME	HTA17-802
EXPNO	4
PROCNO	1
Date_	20160808
Time	15.11
INSTRUM	av500
PROBHD	5 mm CPDCN 13C
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1024
DS	4
SWH	30030.029 Hz
FIDRES	0.458222 Hz
AQ	1.0912410 sec
RG	161.3
DW	16.650 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.00 usec
PL1	-4.80 dB
PL1W	13.65439701 W
SFO1	125.7703643 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	7.58577585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7578495 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	0.30