

SUPPORTING INFORMATION

Understanding the Cardioprotective Effects of Flavonols: Discovery of Relaxant Flavonols without Antioxidant Activity

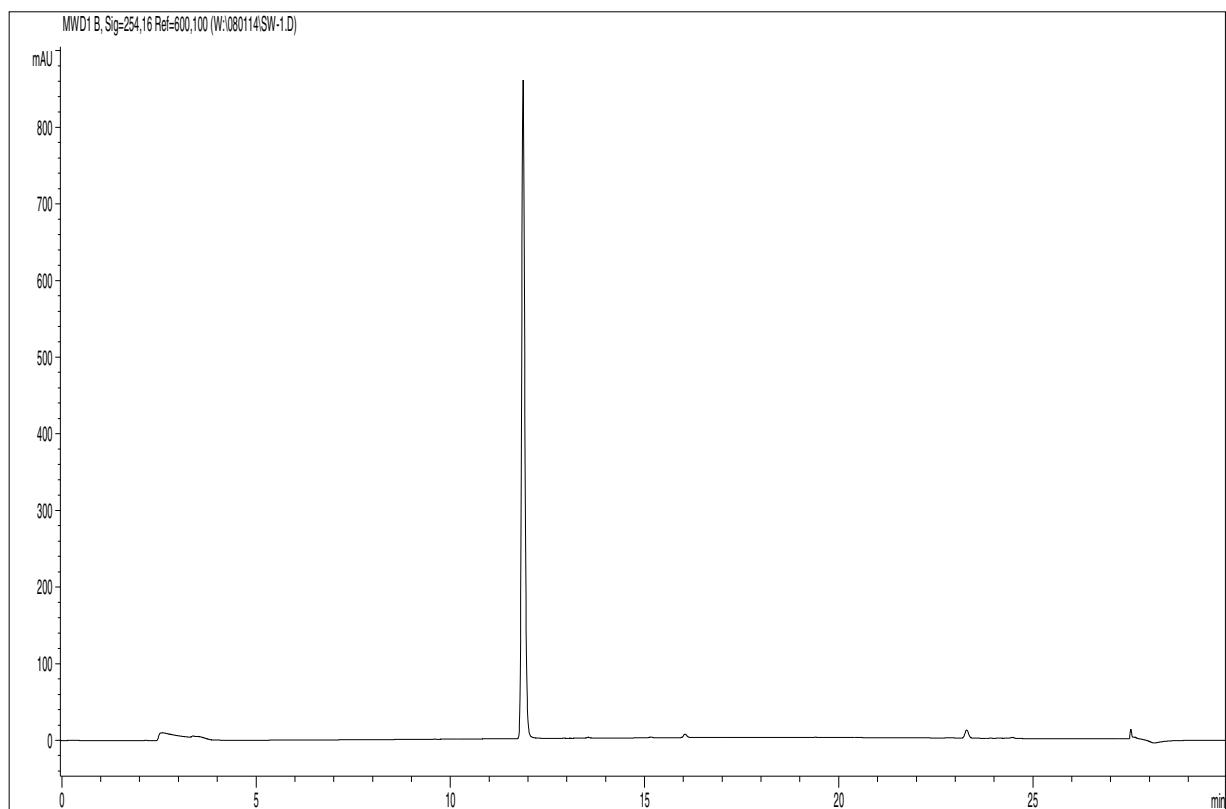
Chengxue Qin, Xingqiang Chen, Richard A. Hughes, Spencer J. Williams, Owen L. Woodman

Table. Combustion Analysis and/or HPLC Analysis Data for Key Compounds.

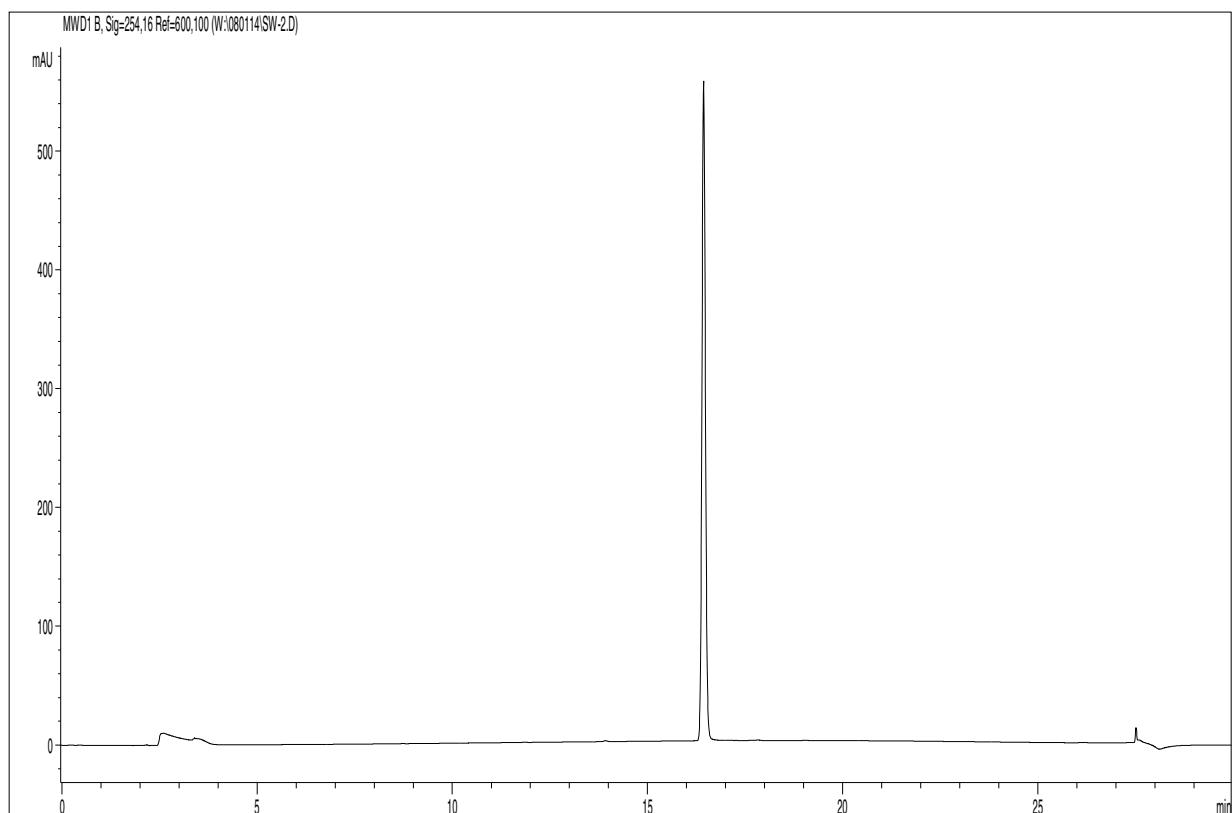
Compd #	Formula	Combustion analysis or HPLC analysis* data
1	C ₁₅ H ₁₀ O ₅	Anal. RP-HPLC: t _R = 11.9 min (97% purity)
2	C ₁₅ H ₁₀ O ₃	Anal. RP-HPLC: t _R = 16.4 min (99% purity)
3	C ₁₅ H ₁₀ O ₄	Anal. RP-HPLC: t _R = 13.4 min (98% purity)
4	C ₁₆ H ₁₂ O ₄	Anal. RP-HPLC: t _R = 16.6 min (96% purity)
5	C ₁₅ H ₉ ClO ₃	Anal. RP-HPLC: t _R = 18.4 min (99% purity)
6	C ₁₆ H ₉ F ₃ O ₃	Anal. RP-HPLC: t _R = 18.8 min (99% purity)
7	C ₁₆ H ₁₂ O ₃	Anal. RP-HPLC: t _R = 17.8 min (99% purity)
8	C ₁₉ H ₁₈ O ₃	Anal. RP-HPLC: t _R = 21.0 min (96% purity)
9	C ₁₅ H ₁₀ O ₃	Anal. RP-HPLC: t _R = 13.5 min (99% purity)
10	C ₁₆ H ₁₂ O ₄	Anal. RP-HPLC: t _R = 16.7 min (99% purity)
11	C ₁₅ H ₉ O ₃ Cl	Anal. RP-HPLC: t _R = 18.3 min (98% purity)
12	C ₁₆ H ₉ F ₃ O ₃	Anal. RP-HPLC: t _R = 18.5 min (99% purity)
13	C ₁₆ H ₁₂ O ₃	Anal. RP-HPLC: t _R = 17.7 min (99% purity)
14	C ₁₆ H ₁₂ O ₅	Anal. RP-HPLC: t _R = 13.5 min (96% purity) Calcd: C, 67.60; H, 4.25. Found: C, 67.62; H, 4.23.
15	C ₁₅ H ₉ BrO ₄	Anal. RP-HPLC: t _R = 15.2 min (97% purity) Calcd: C, 54.08; H, 2.72. Found: C, 54.04; H, 2.80.
16	C ₁₆ H ₉ F ₃ O ₄	Anal. RP-HPLC: t _R = 15.8 min (99% purity)
17	C ₁₆ H ₁₂ O ₄	Anal. RP-HPLC: t _R = 14.6 min (98% purity) Calcd: C, 71.64; H, 4.51. Found: C, 71.59; H, 4.58.
18	C ₁₉ H ₁₈ O ₄	Anal. RP-HPLC: t _R = 17.9 min (97% purity)

* HPLC analysis was performed by Mr John Karas (University of Melbourne) using an Agilent 1200 series instrument, with UV detector set at 254 nm, and a Zorbax Eclipse XDB-C18, 4.6×150mm, 5µm particle size column (eluting at 1 mL/min with a 25 min gradient of 0–100% B, where solvent A = 0.1% TFA in water and solvent B = 0.1% TFA in acetonitrile).

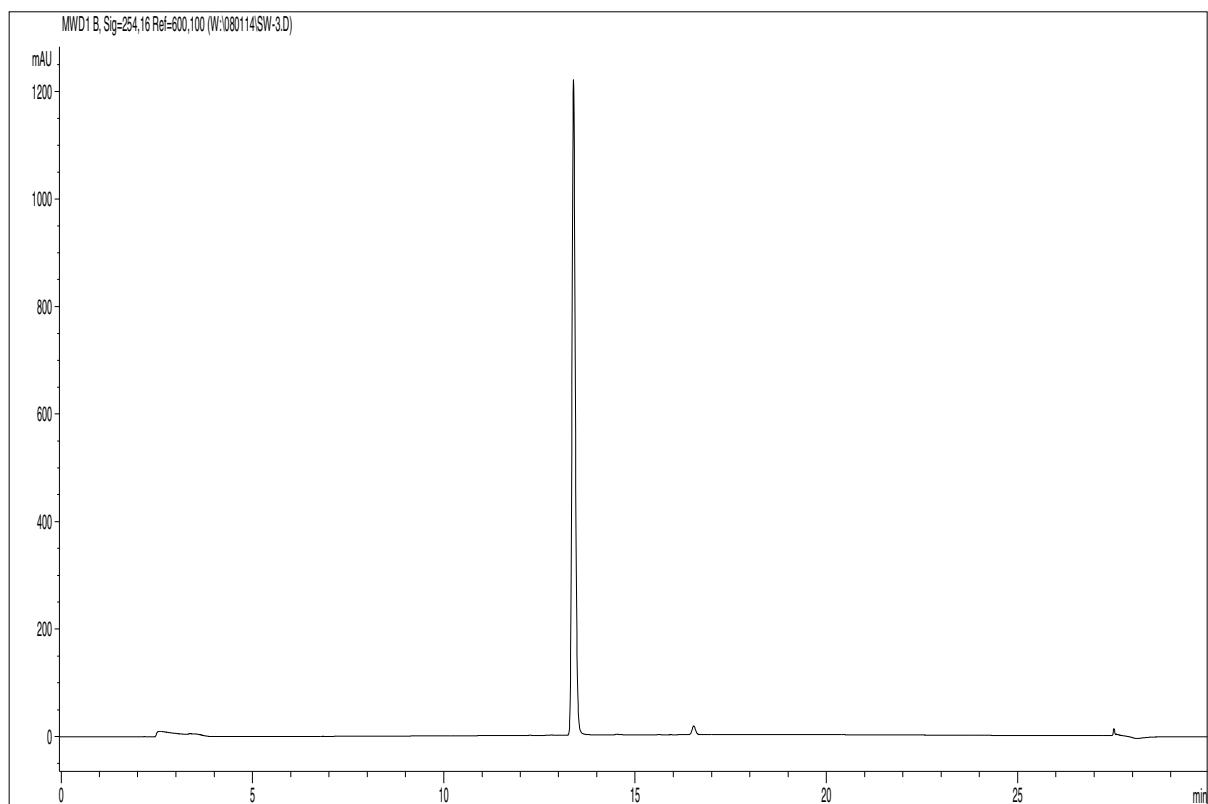
3',4'-Dihydroxyflavonol (1): purity > 97 %



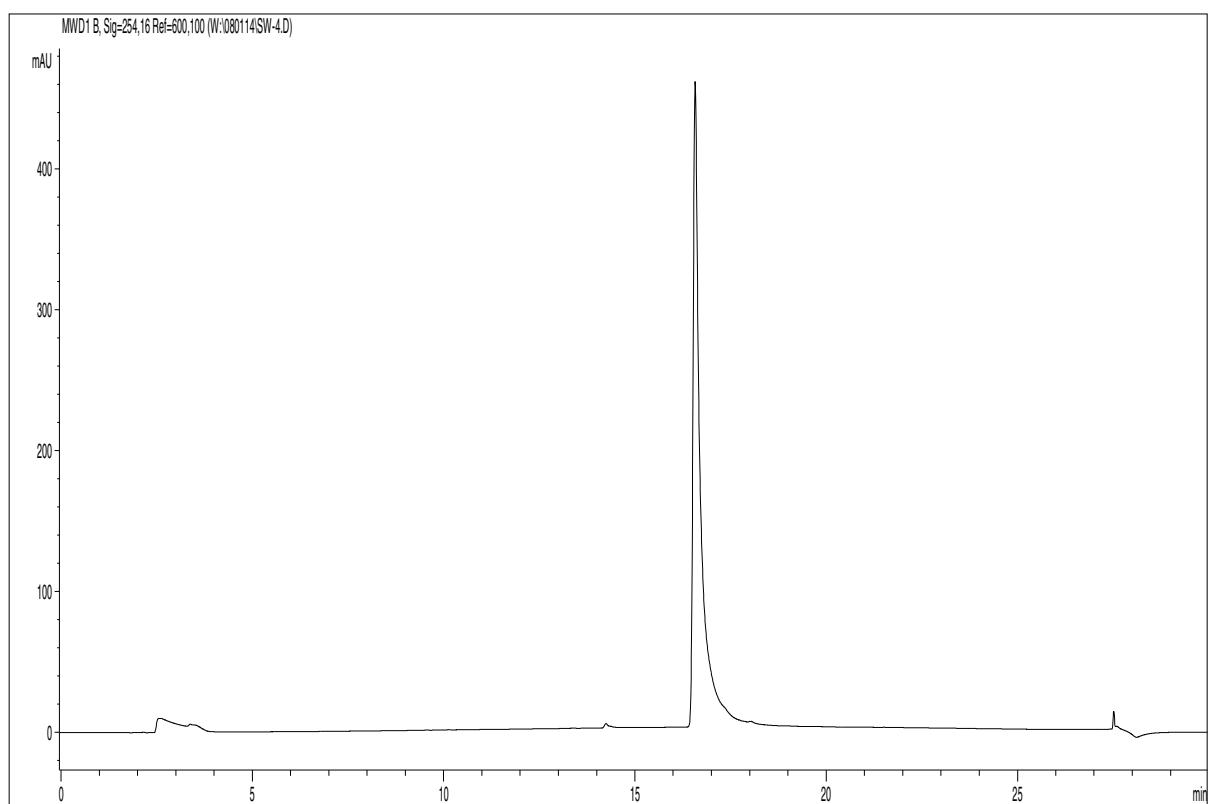
Flavonol (2): purity > 99 %



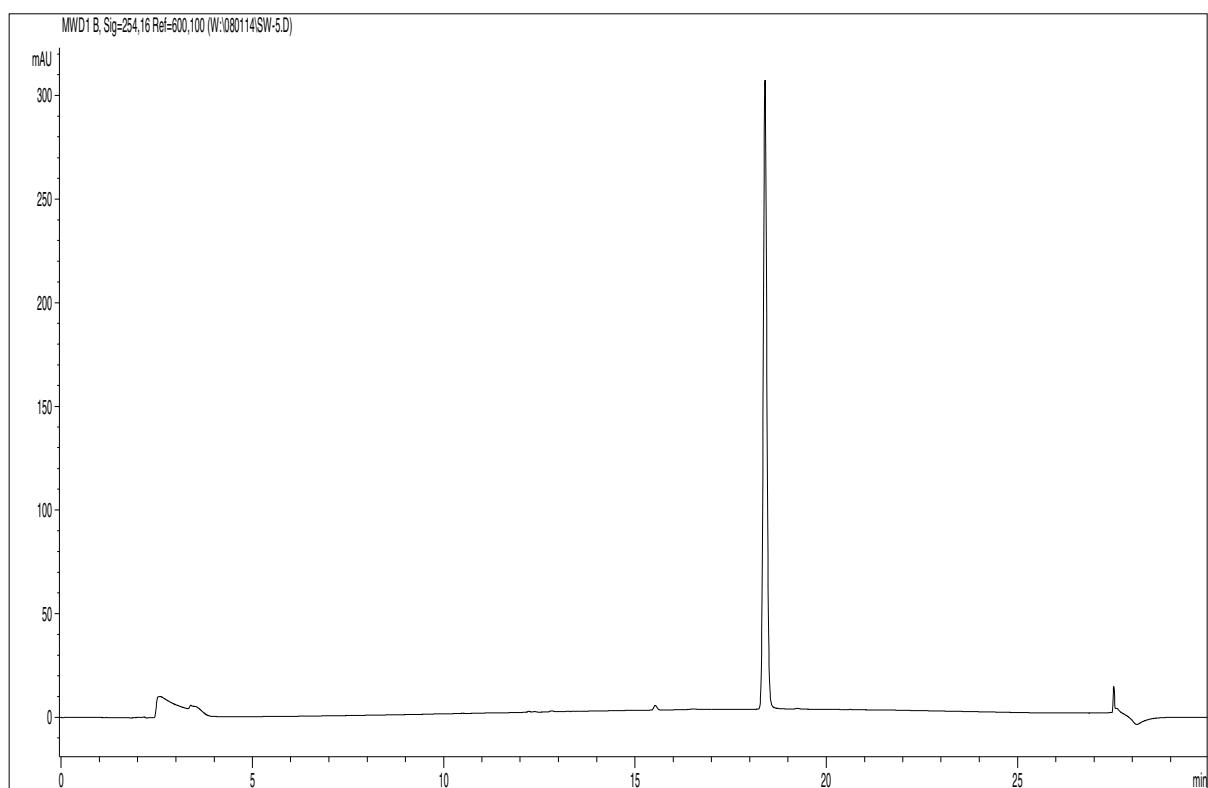
4'-Hydroxyflavonol (3): purity > 98%



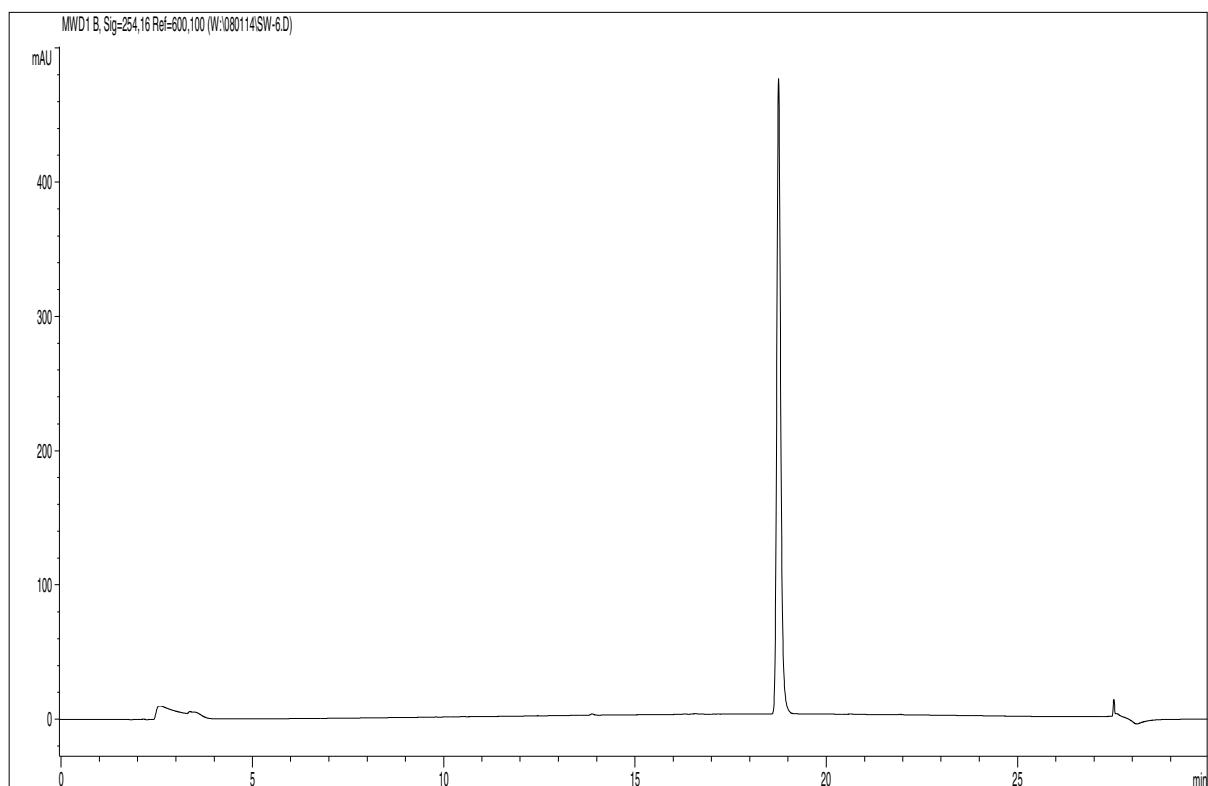
4'-Methoxyflavonol (4): purity > 96%



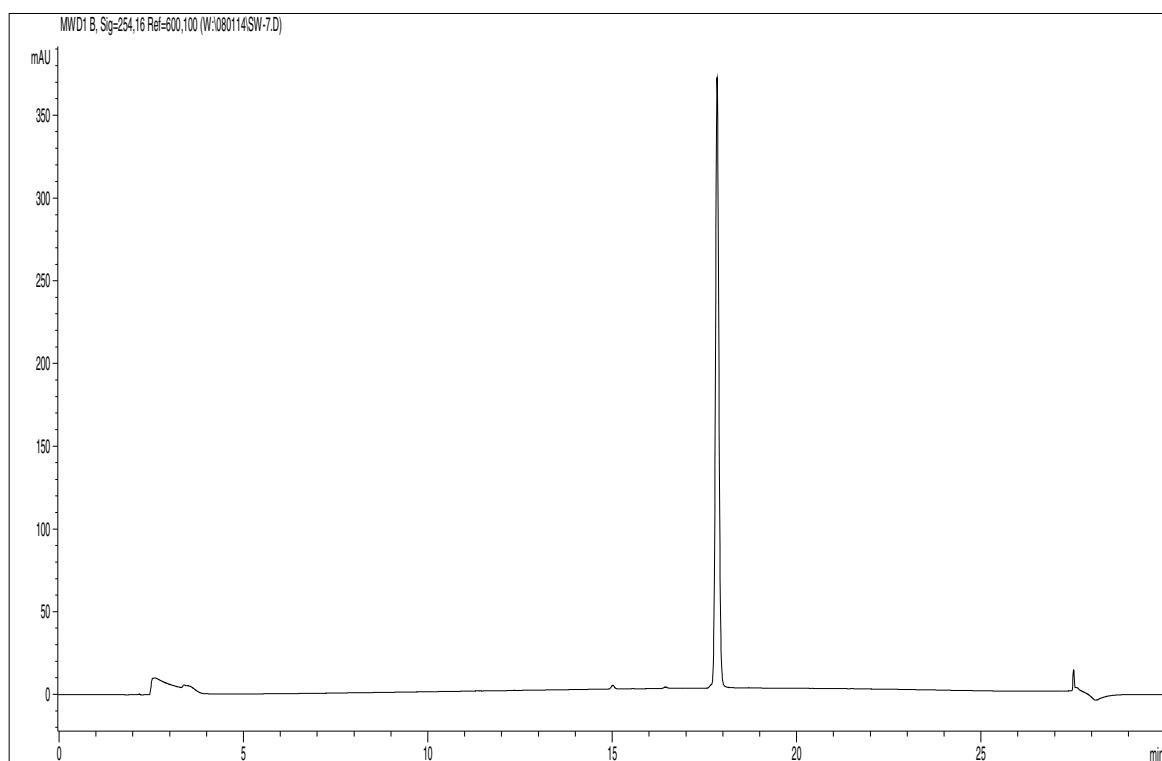
4'-Chloroflavonol (5): purity > 99%



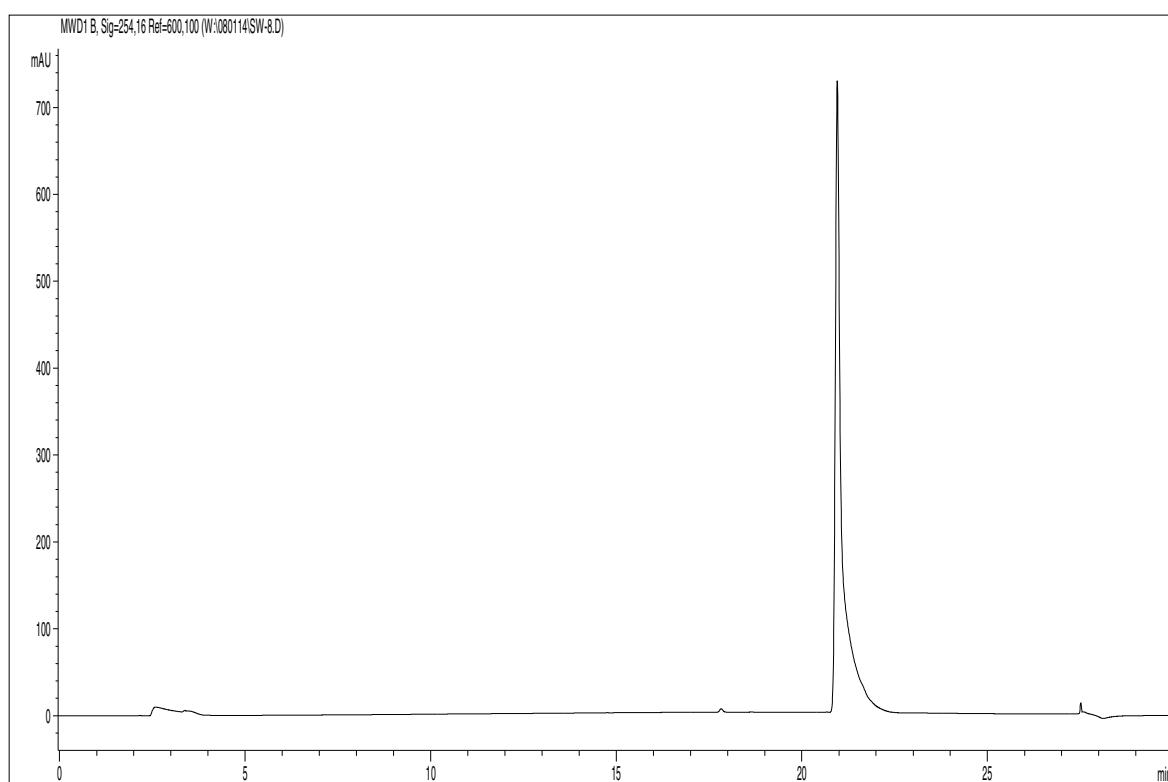
4'-Trifluoromethylflavonol (6): purity > 99%



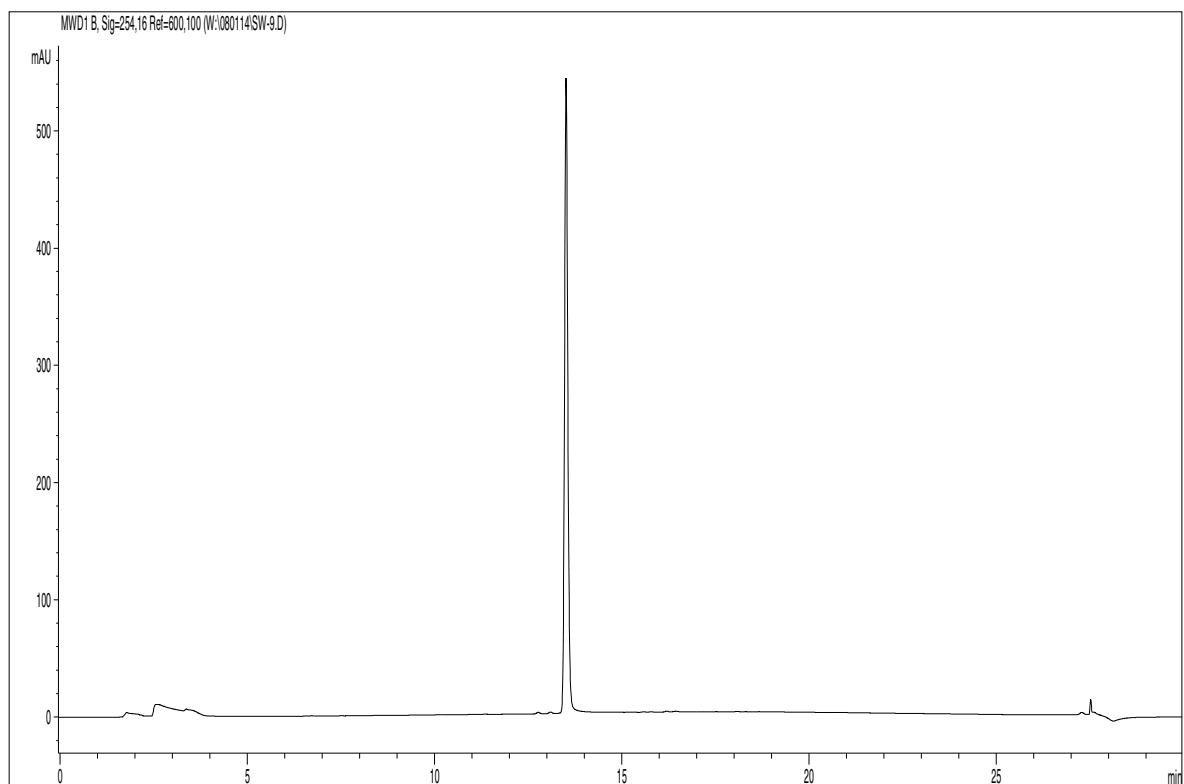
4'-Methylflavonol (7): purity > 99%



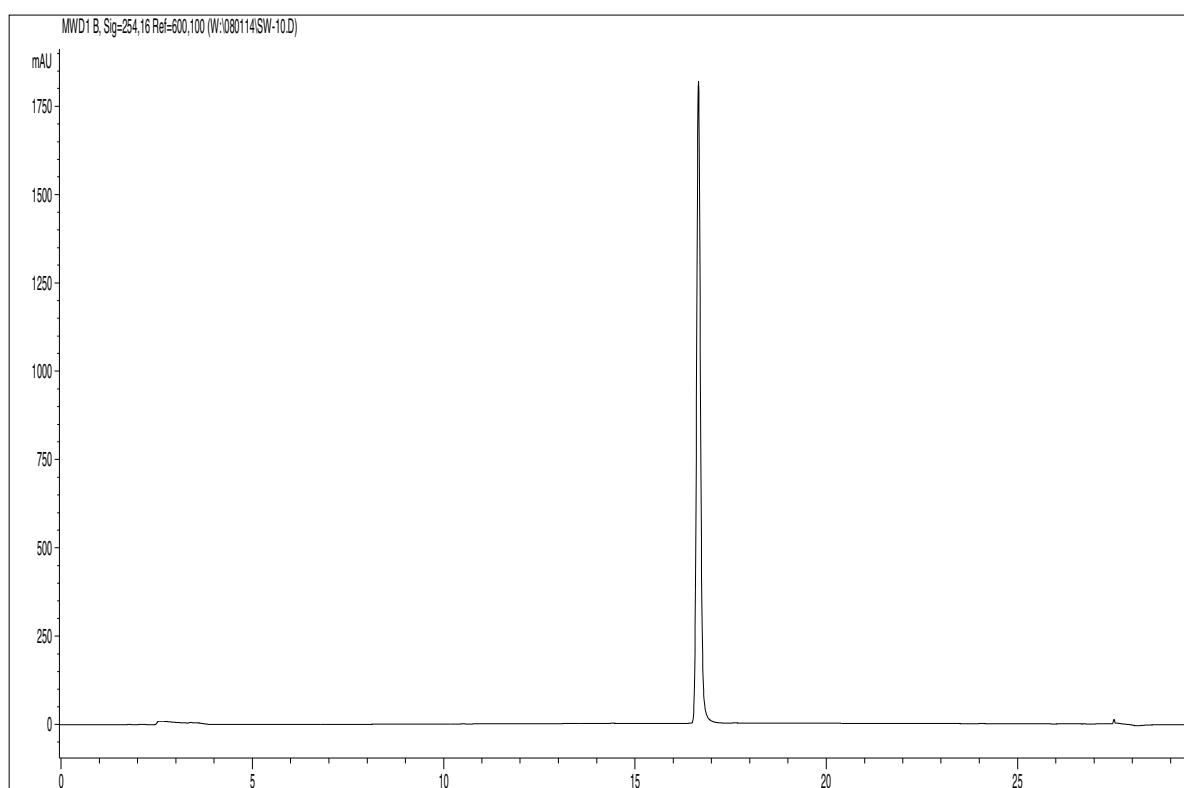
4'-tert-Butylflavonol (8): purity > 96%



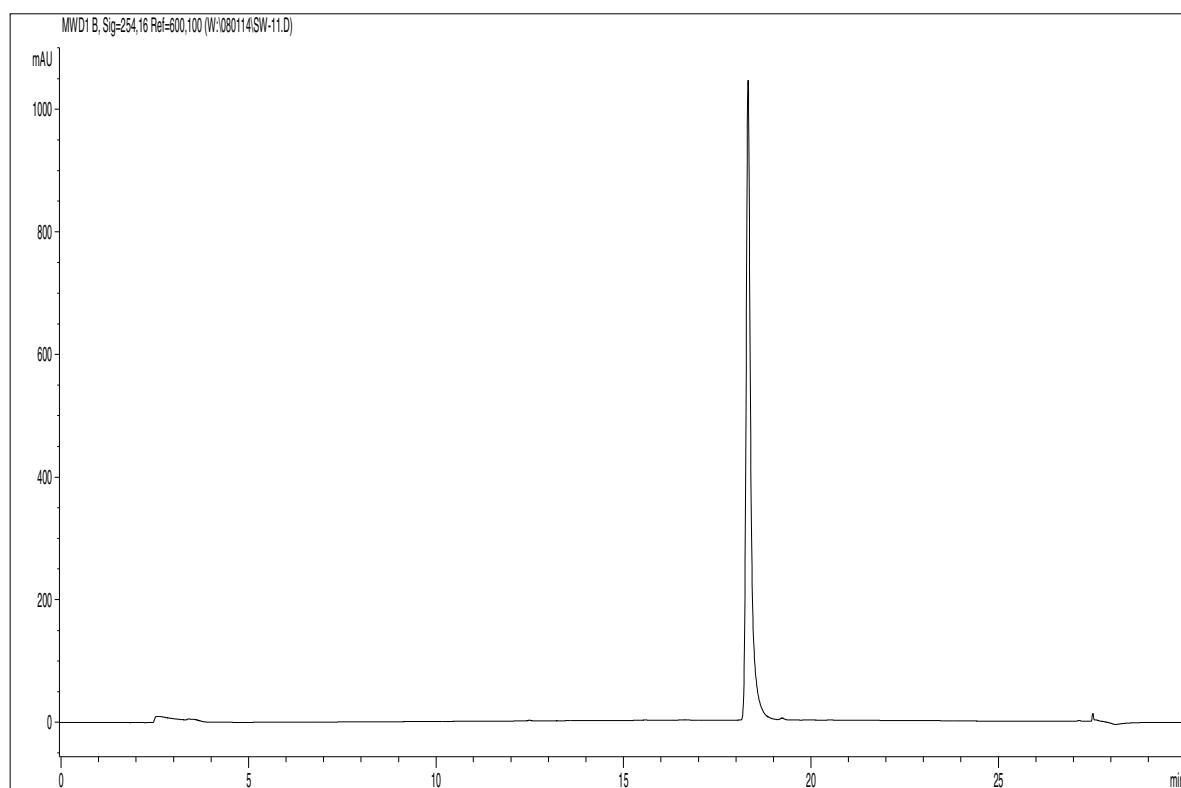
3'-Hydroxyflavonol (9): purity > 99 %



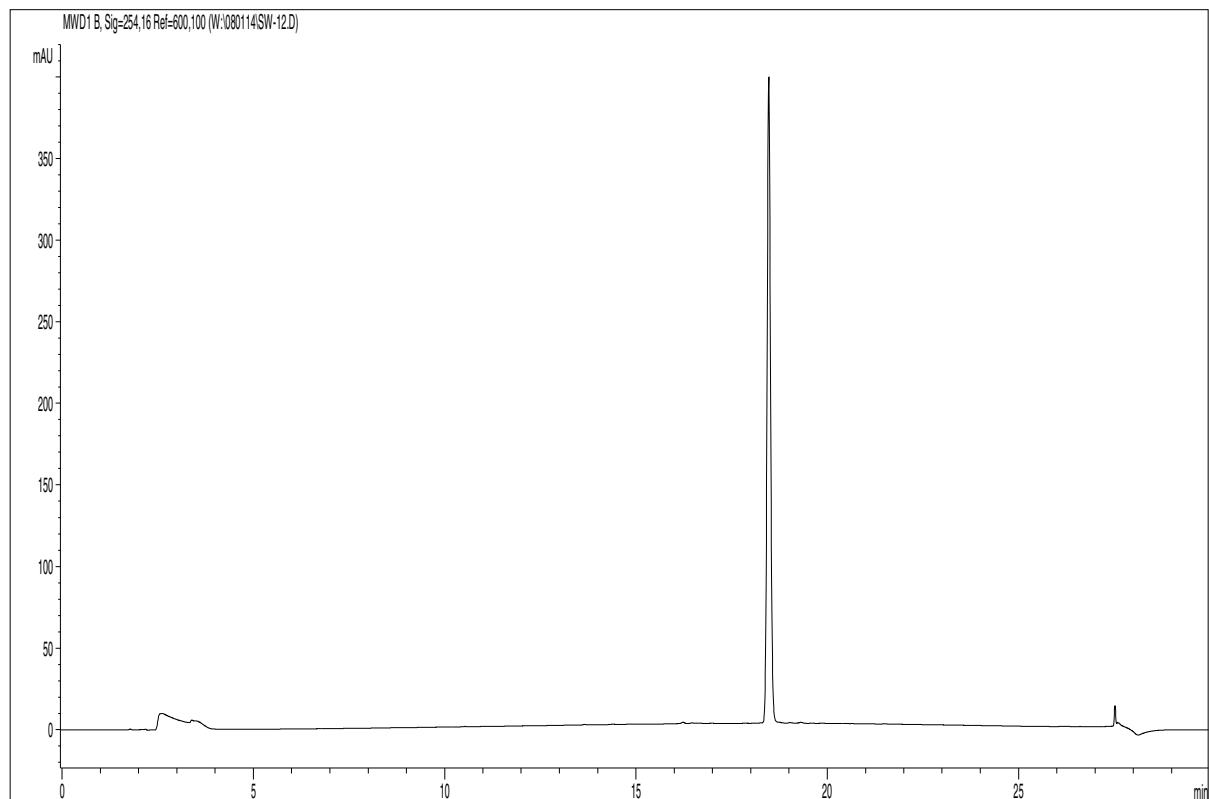
3'-Methoxyflavonol (10): purity > 99 %



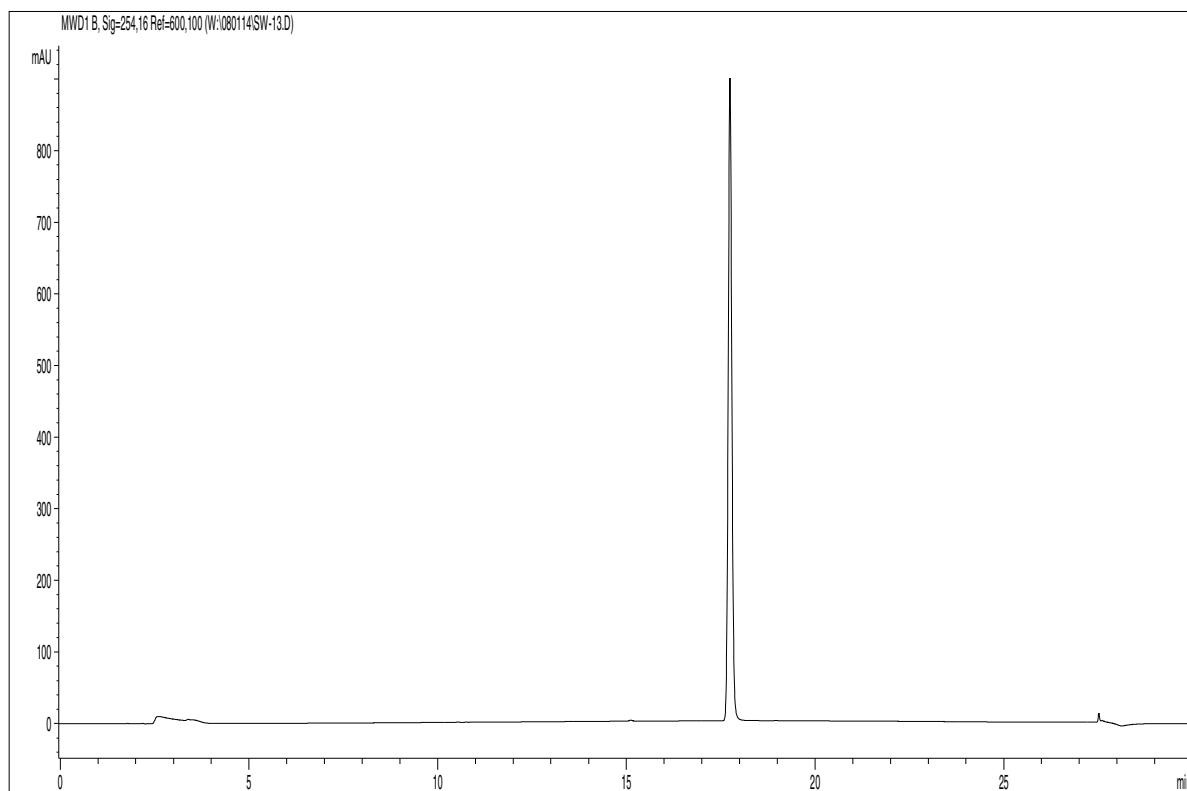
3'-Chloroflavonol (11): purity > 98%



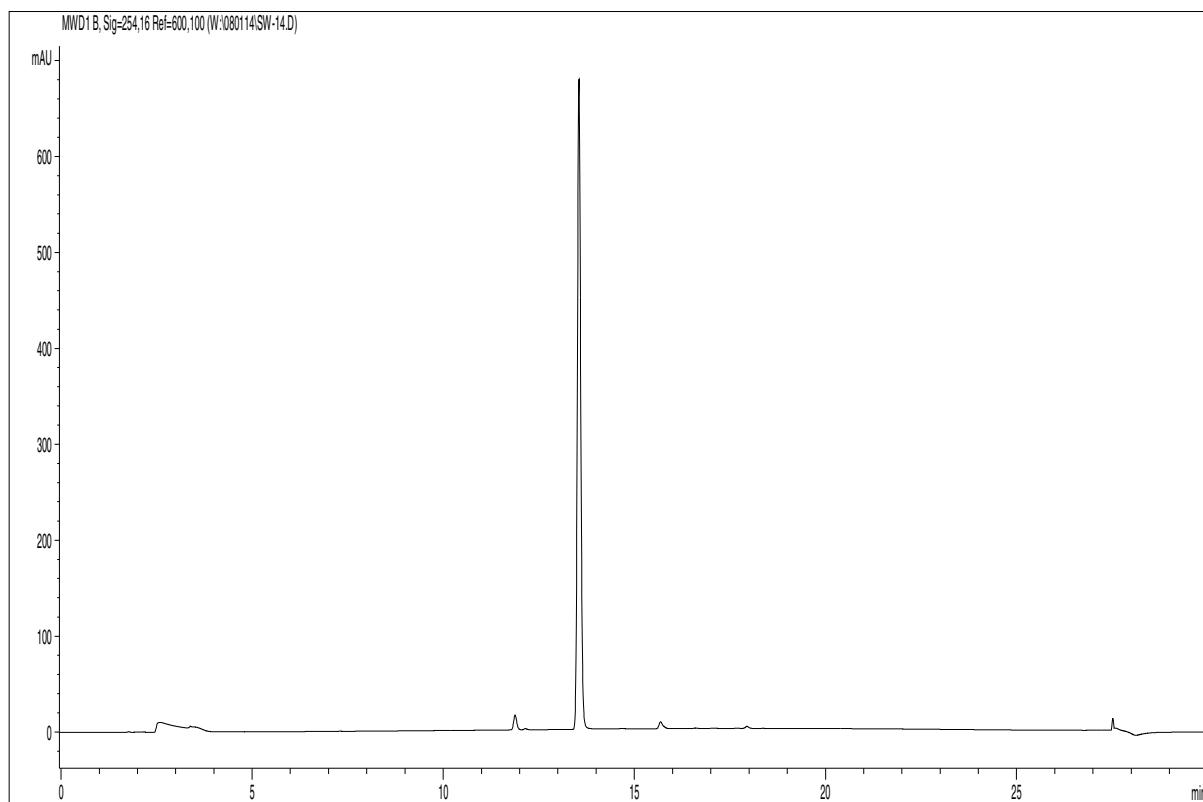
3'-Trifluoromethylflavonol (12): purity > 99%



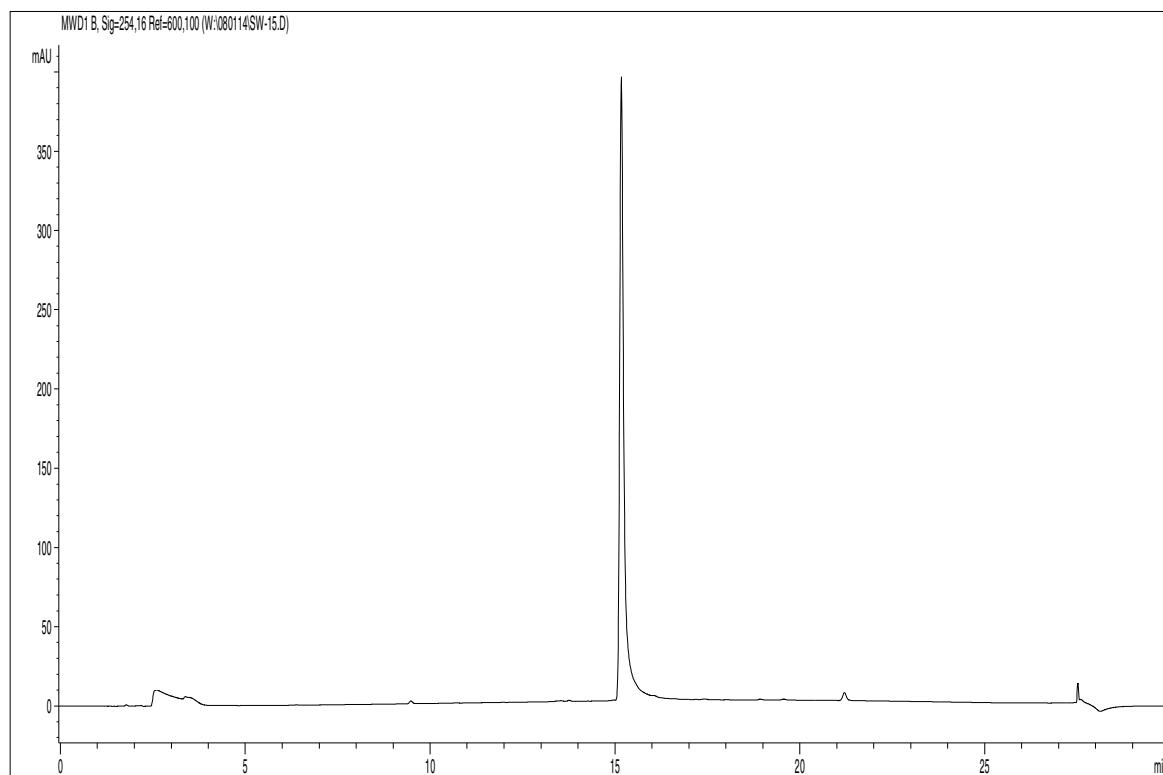
3'-Methylflavonol (13): purity > 99%



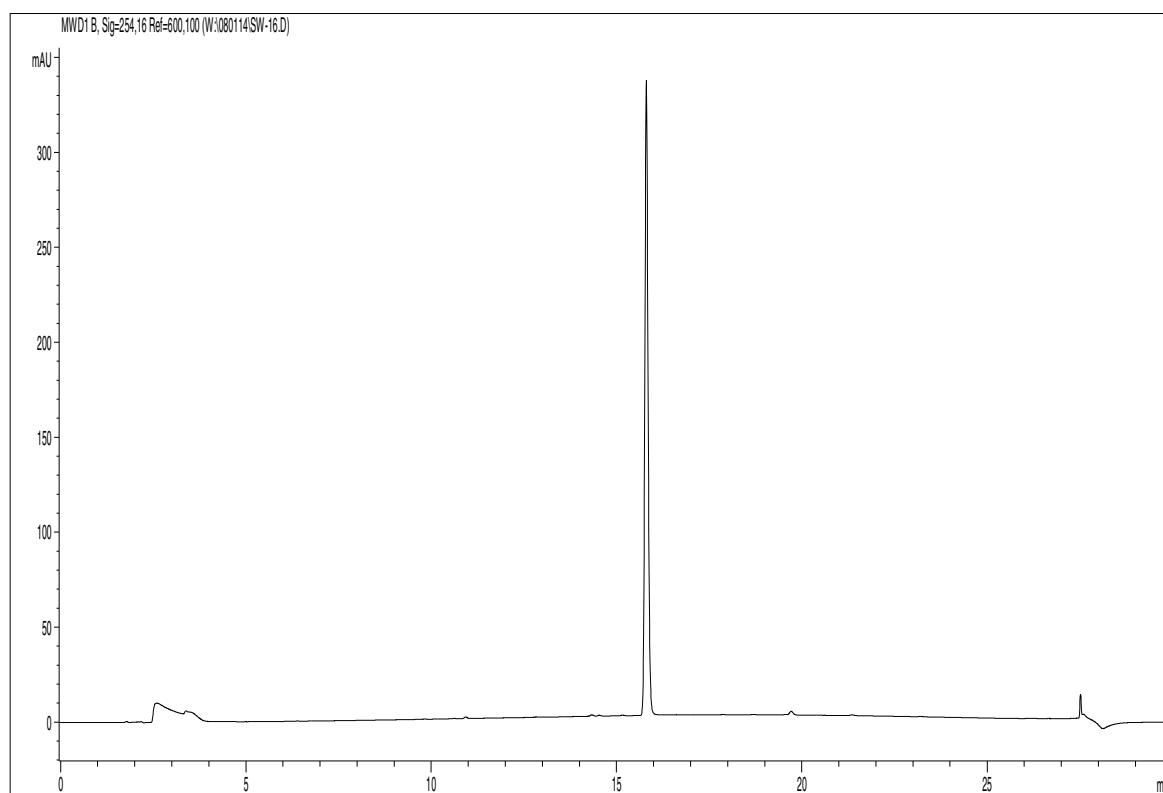
4'-Hydroxy-3'-methoxyflavonol (14): purity > 96%



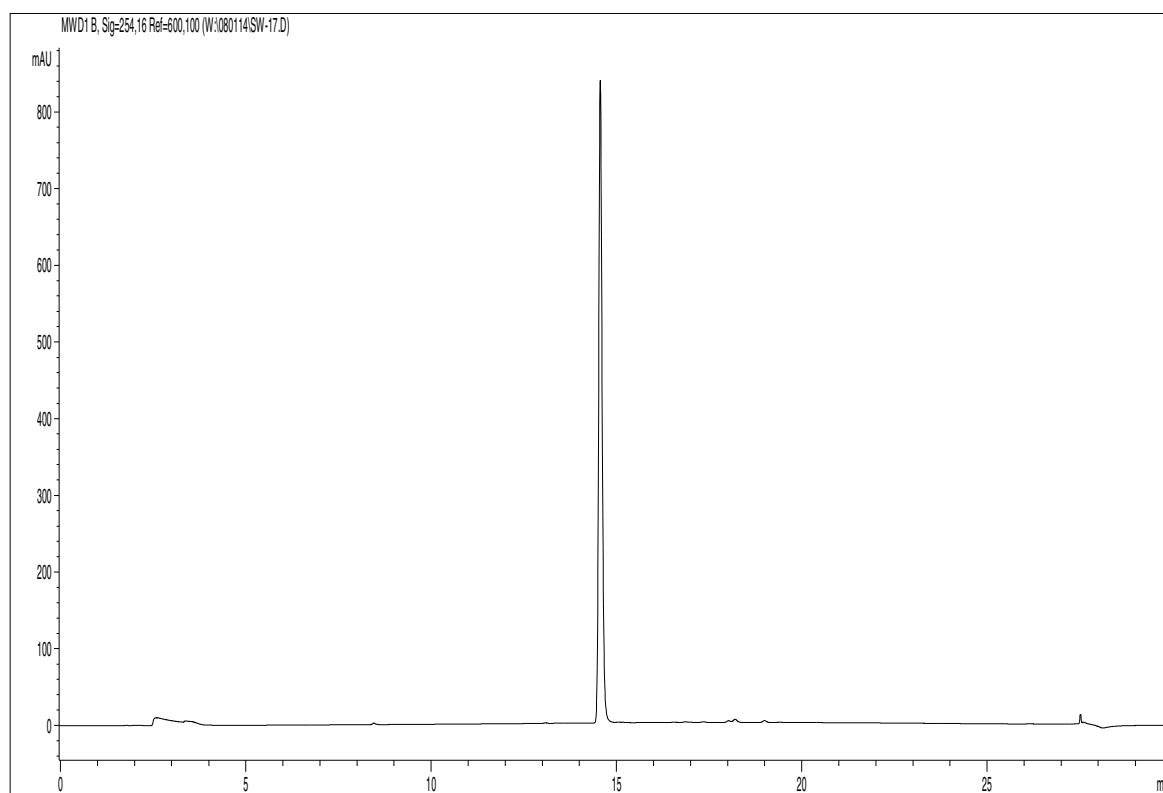
3'-Bromo-4'-hydroxyflavonol (15): purity > 97%



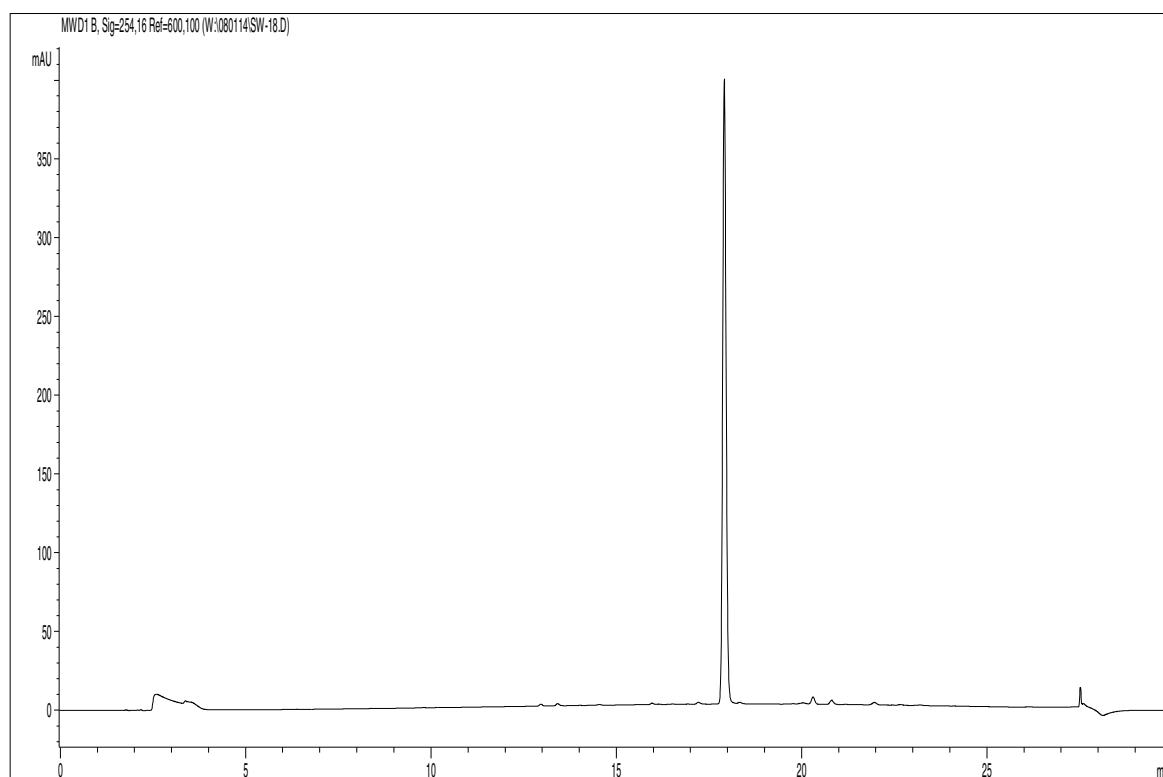
4'-Hydroxy-3'-trifluoromethylflavonol (16): purity > 99%



4'-Hydroxy-3'-methylflavonol (17): purity > 98%



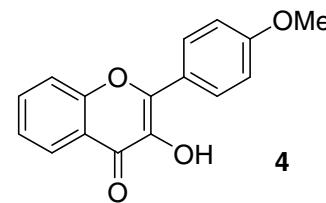
4'-Hydroxy-3'-tert-butylflavonol (18): purity > 97%



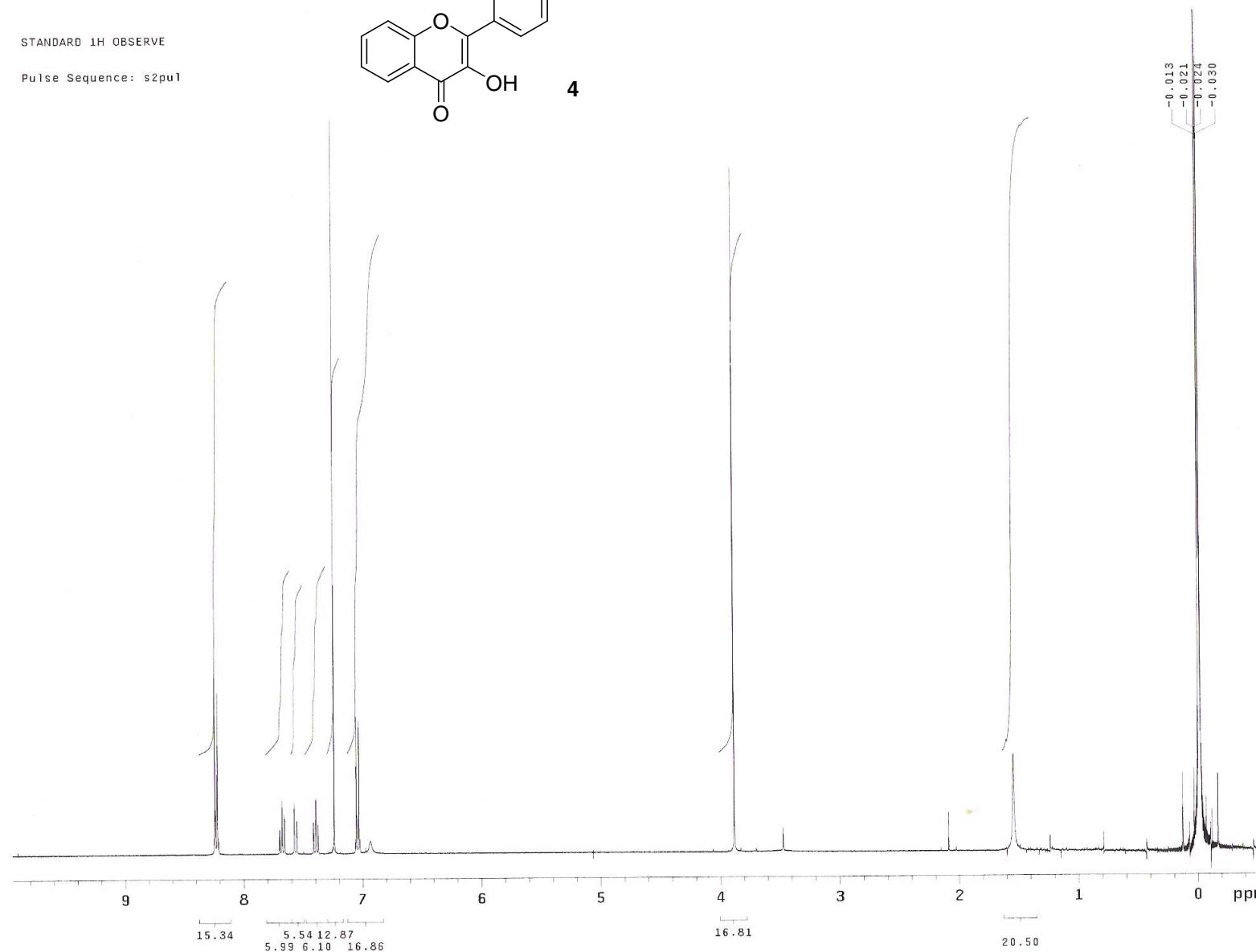
¹H NMR 400 MHz spectrum

STANDARD 1H OBSERVE

Pulse Sequence: s2pul



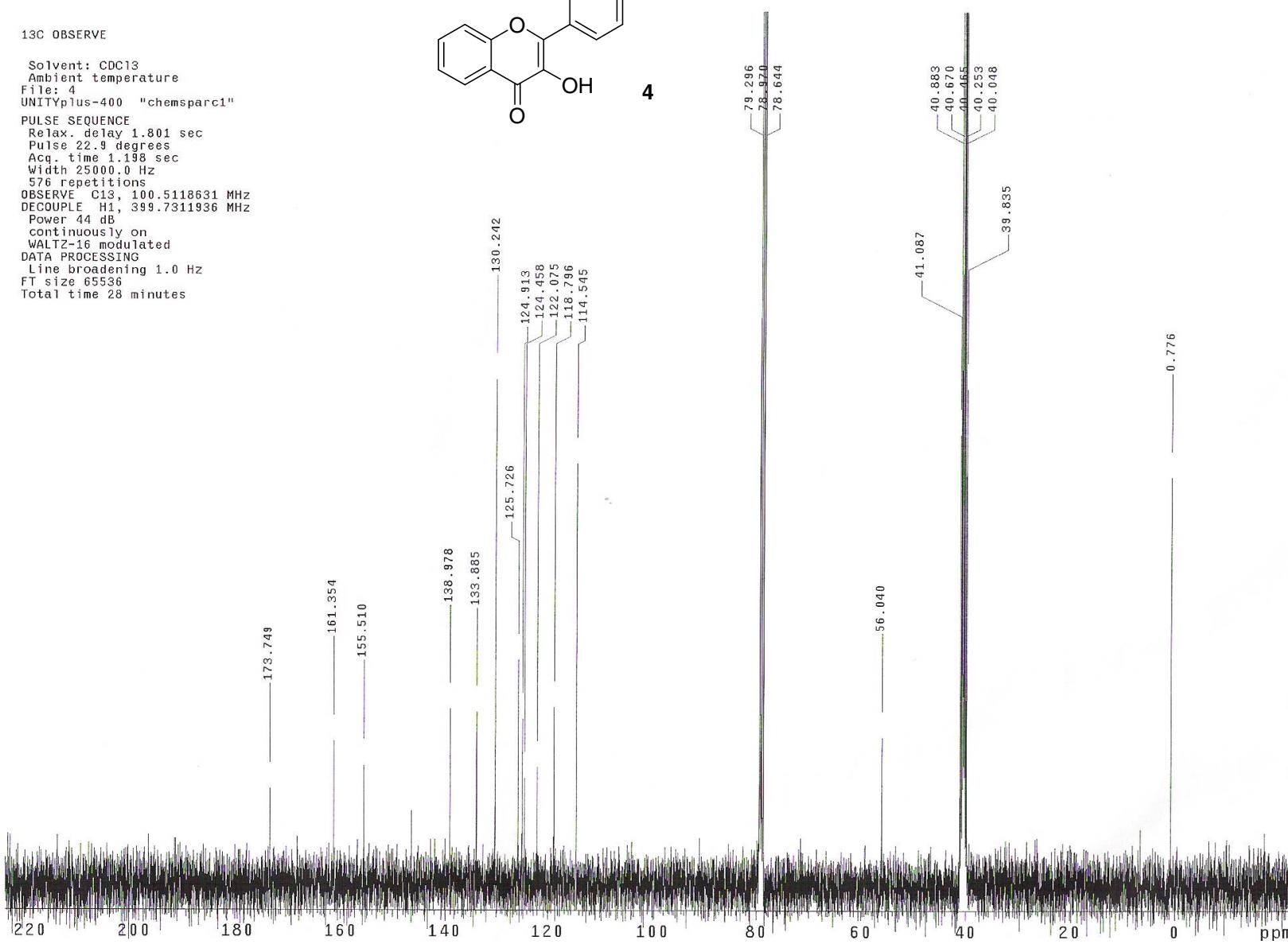
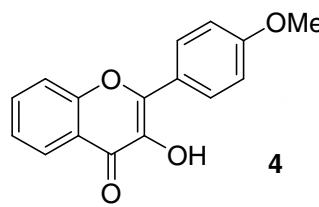
4



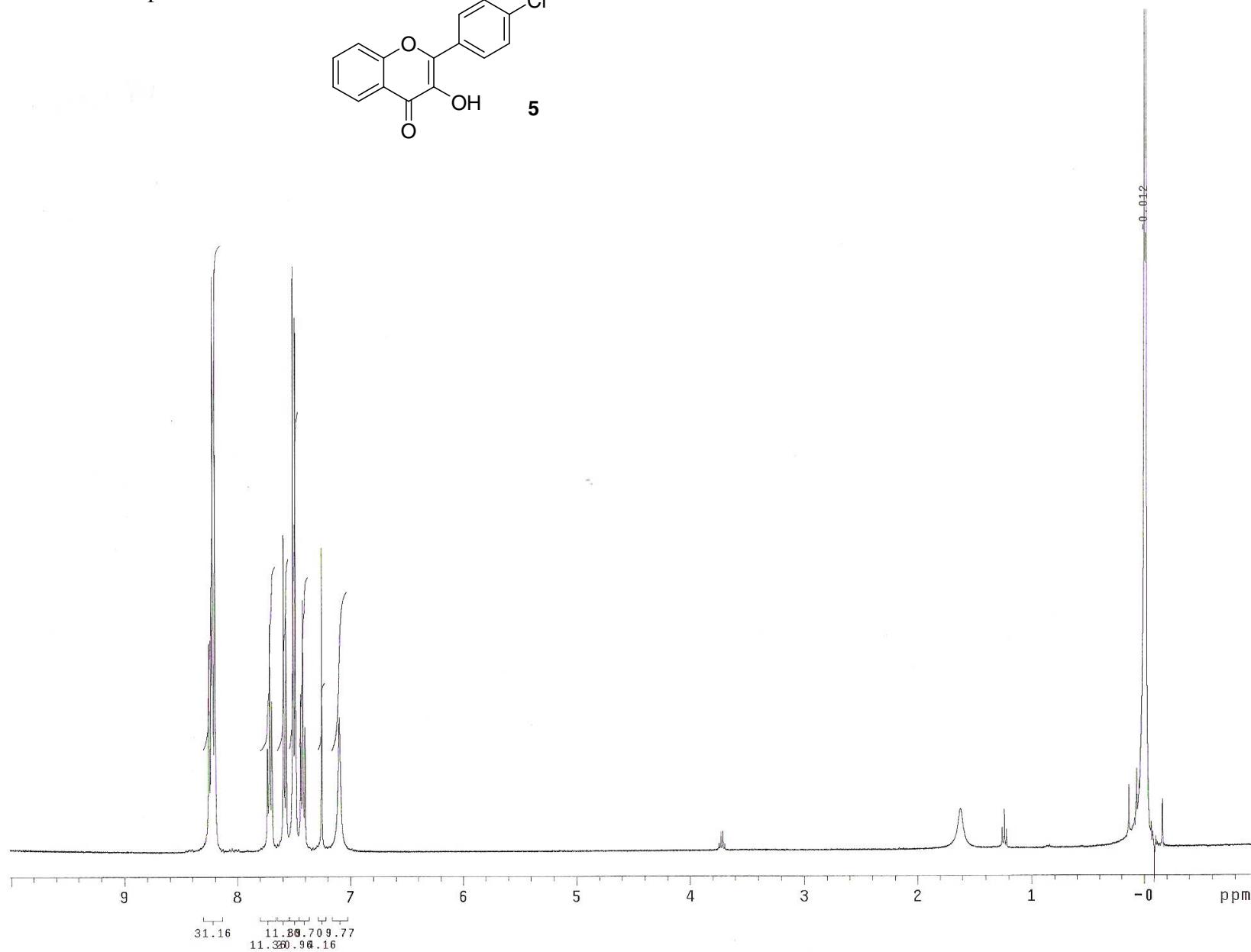
¹³C NMR 100 MHz spectrum

13C OBSERVE

Solvent: CDCl₃
Ambient temperature
File: 4
UNITYplus-400 "chemsparc1"
PULSE SEQUENCE
Relax. delay 1.801 sec
Pulse 22.9 degrees
Acq. time 1.198 sec
Width 25000.0 Hz
576 repetitions
OBSERVE C13, 100.5118631 MHz
DECOUPLE H1, 399.7311936 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 28 minutes



¹H NMR 400 MHz spectrum

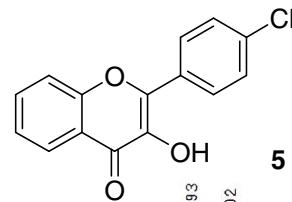


¹³C NMR 100 MHz spectrum

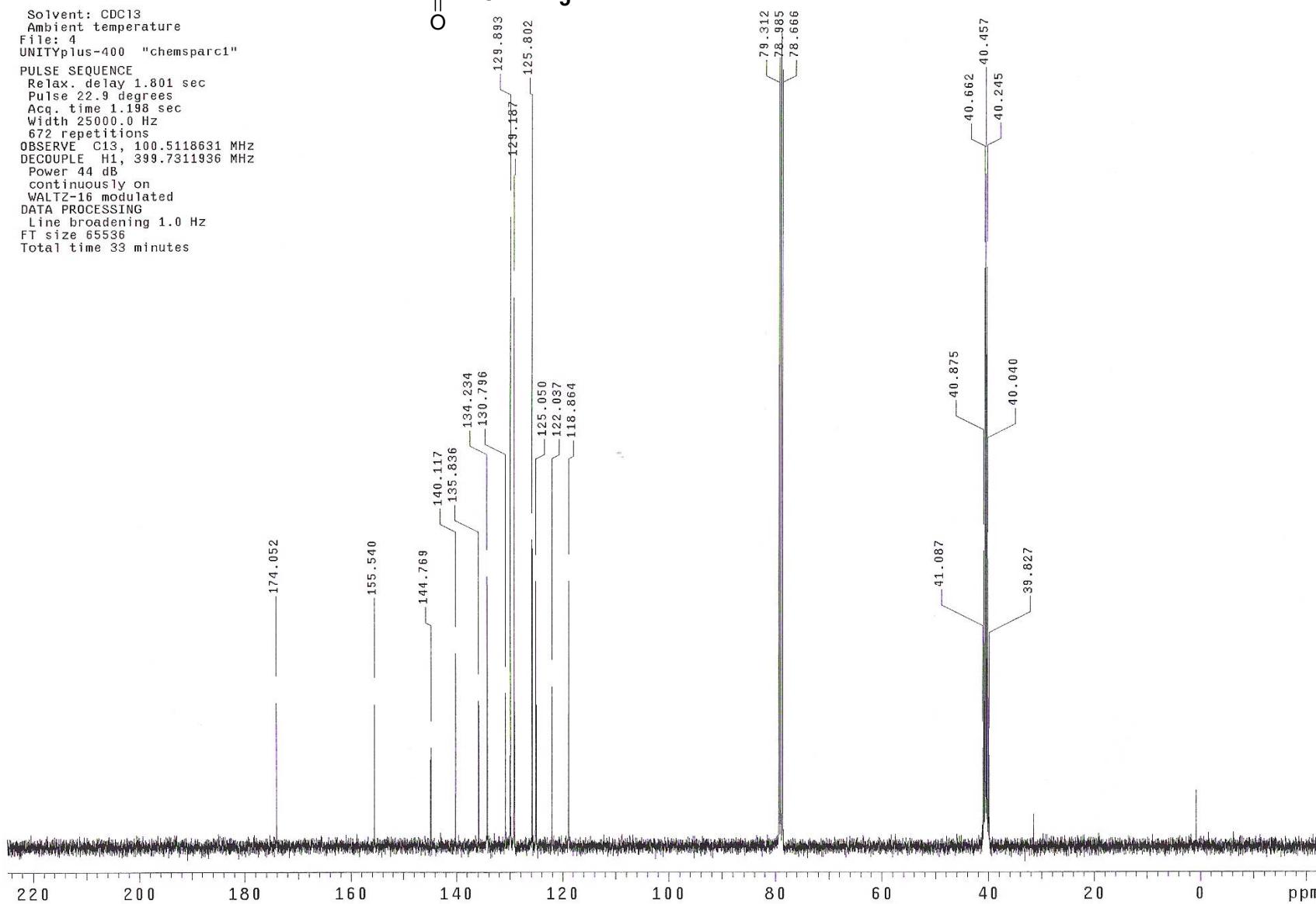
13C OBSERVE

Solvent: CDCl₃
Ambient temperature
File: 4
UNITYplus-400 "chemsparc1"

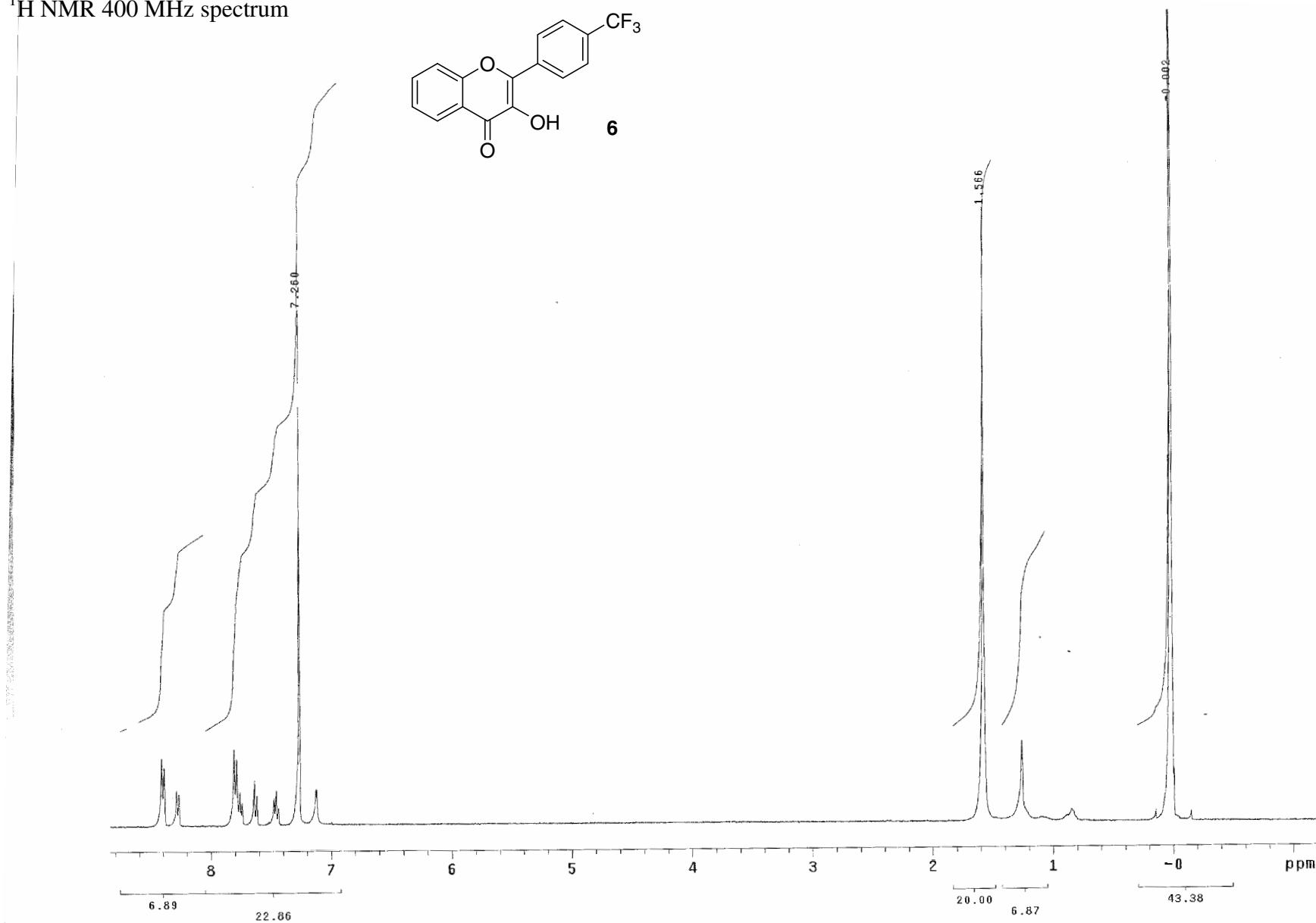
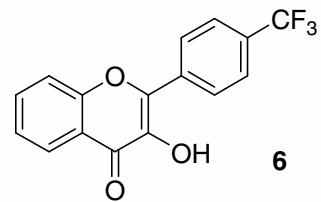
PULSE SEQUENCE
Relax. delay 1.801 sec
Pulse 22.9 degrees
Acq. time 1.198 sec
Width 25000.0 Hz
672 repetitions
OBSERVE C13, 100.5118631 MHz
DECOPLE H1, 399.7311936 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 33 minutes



5



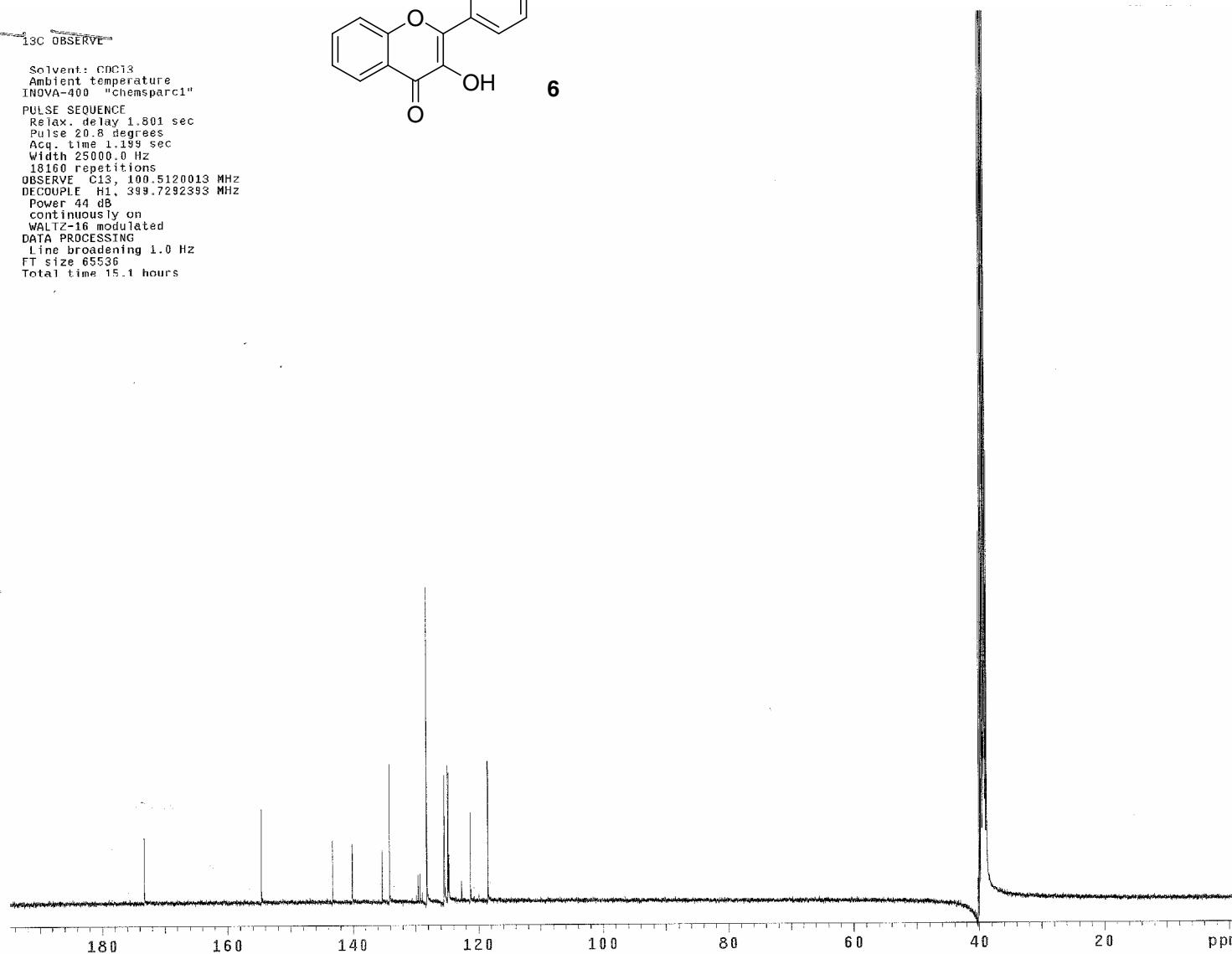
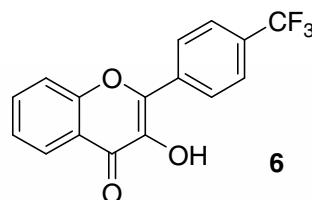
¹H NMR 400 MHz spectrum



¹³C NMR 100 MHz spectrum

13C OBSERVE

Solvent: CDCl₃
Ambient temperature
INOVA-400 "chemsparc1"
PULSE SEQUENCE
Relax., delay 1.801 sec
Pulse 20.8 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
18160 repetitions
OBSERVE C13, 100.5120013 MHz
DECOPPLE H1, 399.7292393 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 15.1 hours



¹H NMR 400 MHz spectrum

STANDARD 1H OBSERVE

Pulse Sequence: s2pul

Solvent: CDCl₃

Ambient temperature

Operator: sjw

INOVA-400 "inova400"

Pulse 36.0 degrees

Acq. time 3.744 sec

Width 6000.6 Hz

64 repetitions

OBSERVE H1, 399.7636710 MHz

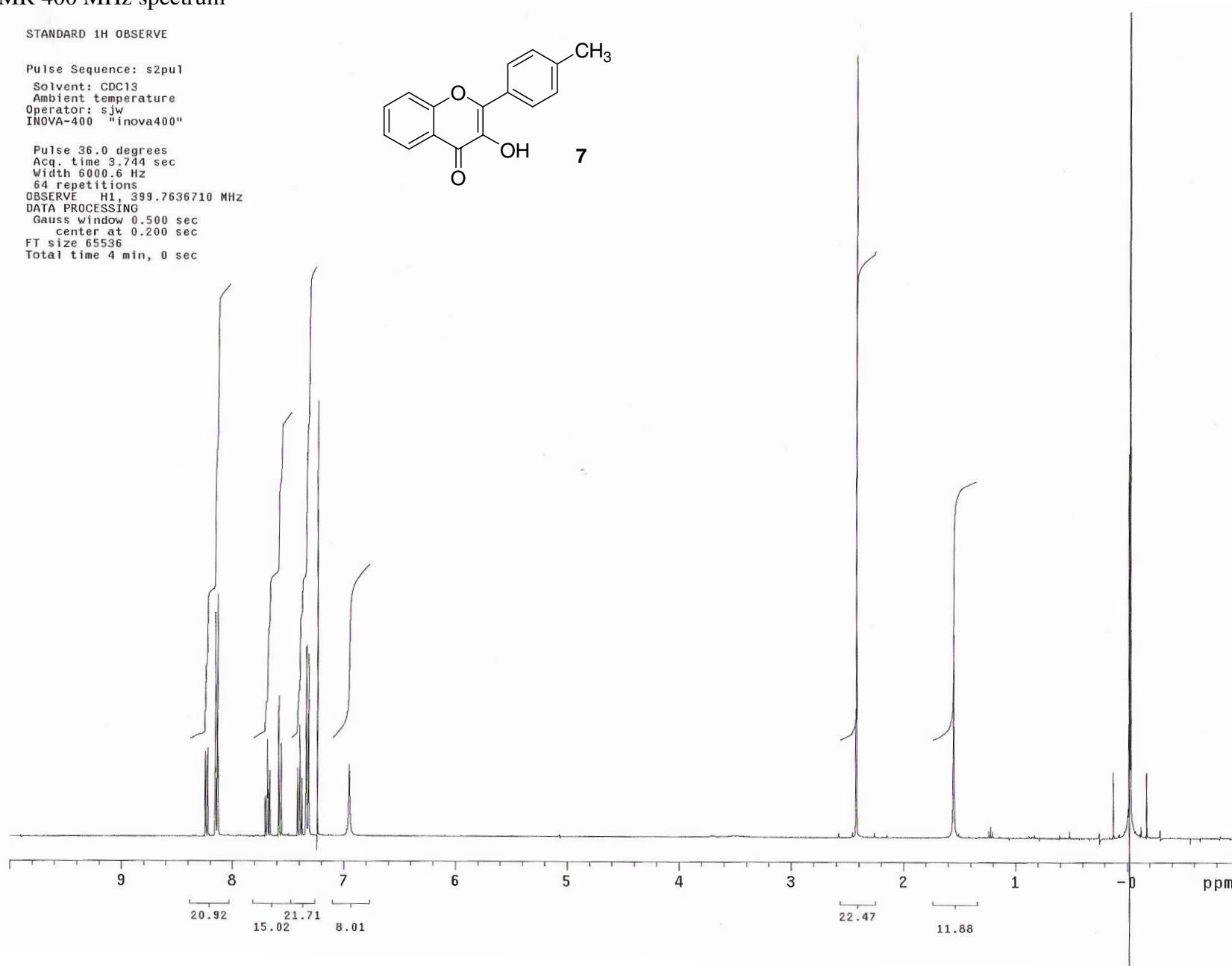
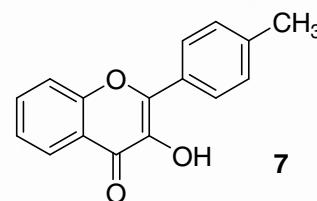
DATA PROCESSING

Gauss window 0.500 sec

center at 0.200 sec

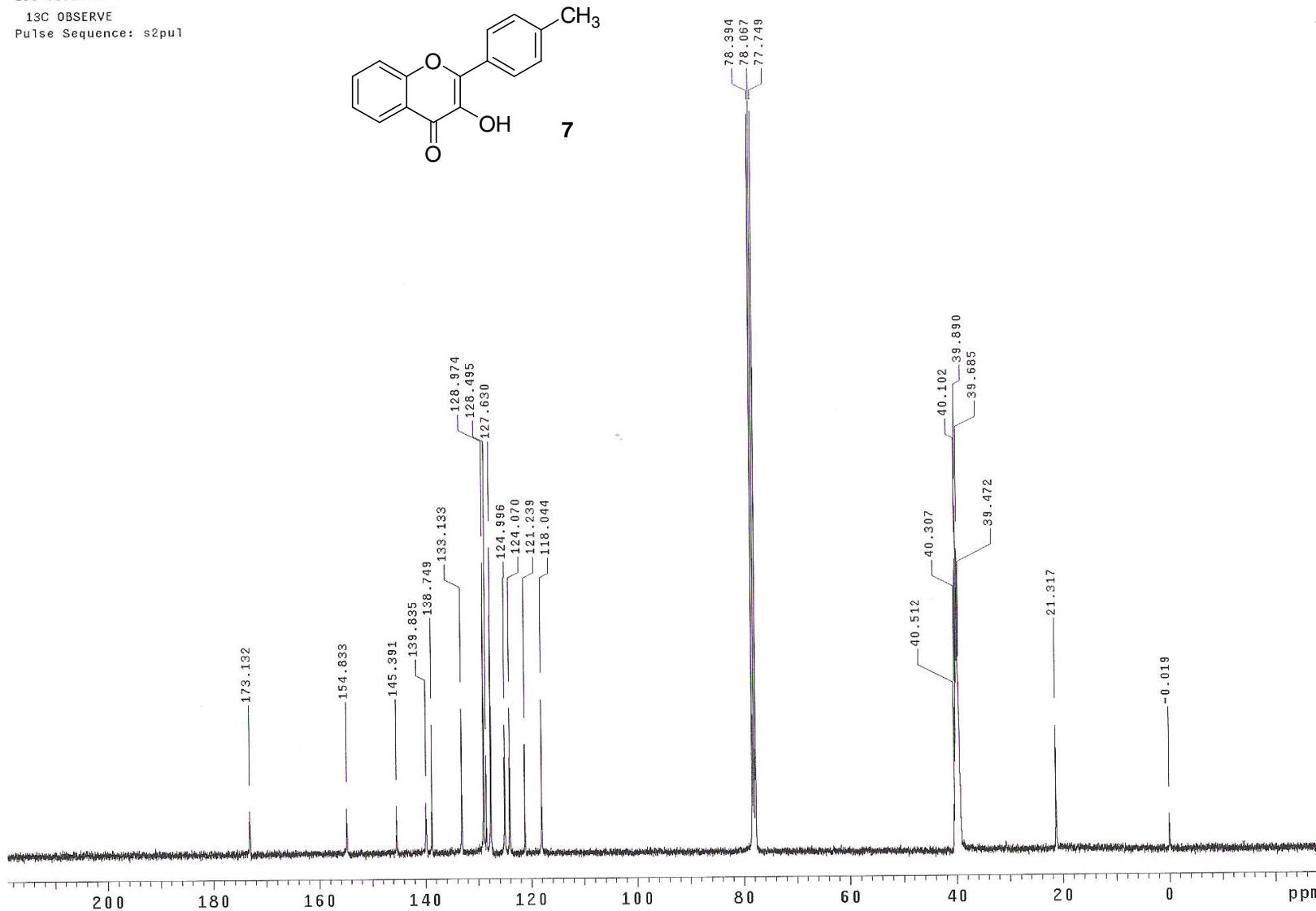
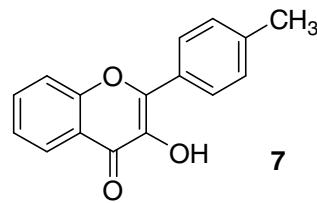
FT size 65536

Total time 4 min, 0 sec

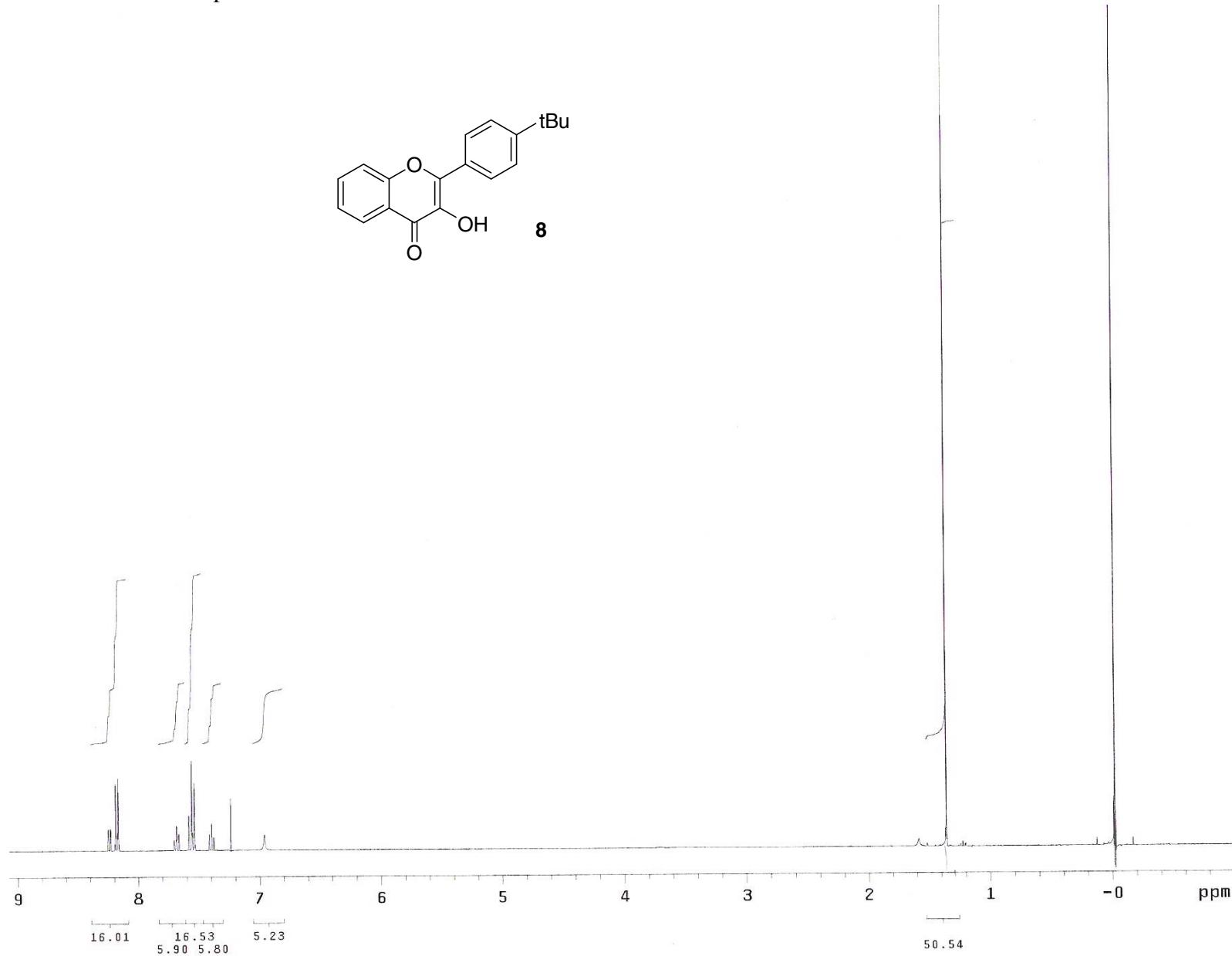


¹³C NMR 100 MHz spectrum

13C OBSERVE
13C OBSERVE
Pulse Sequence: s2pul



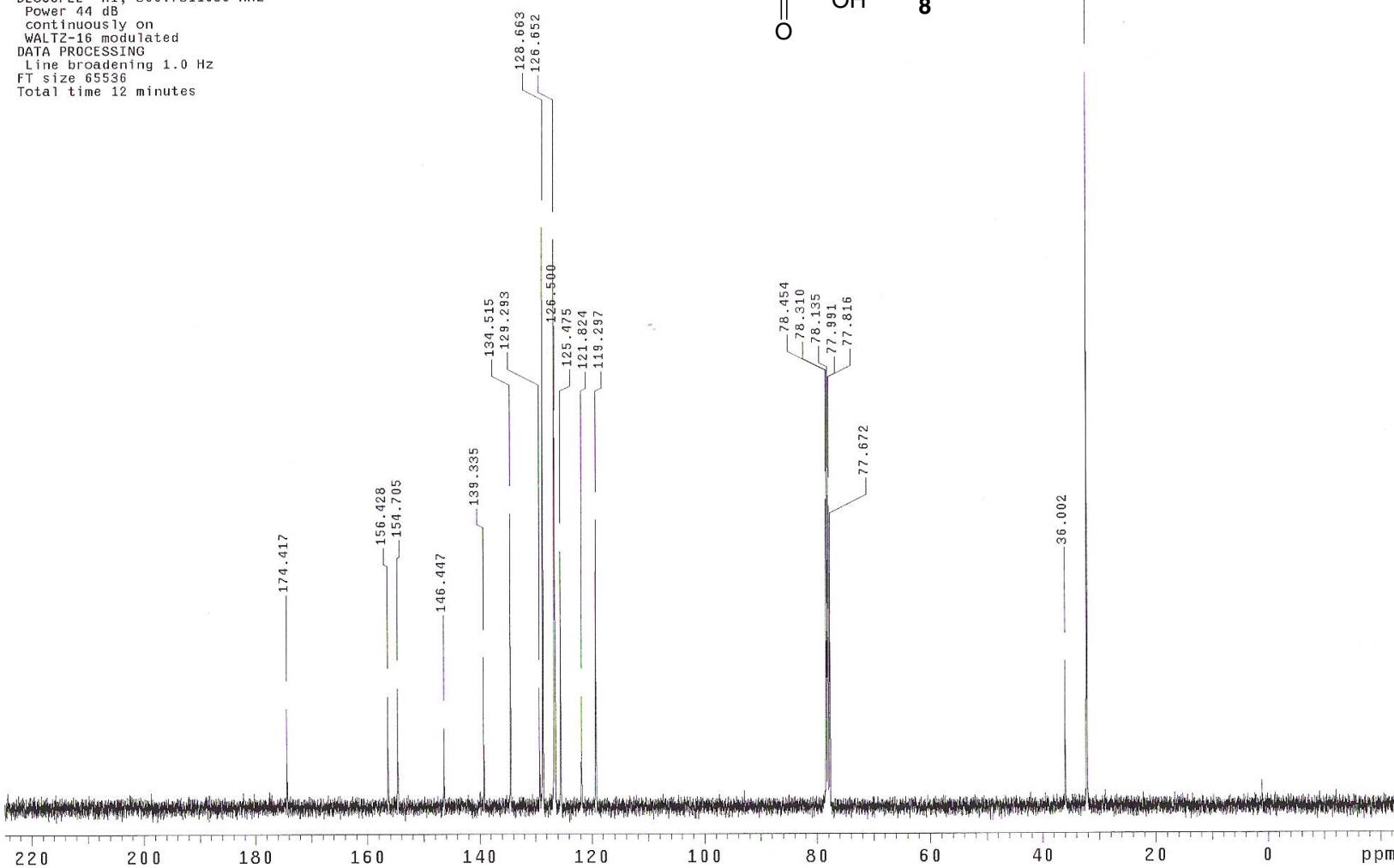
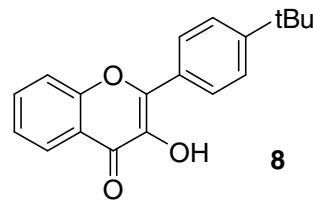
¹H NMR 400 MHz spectrum



¹³C NMR 100 MHz spectrum

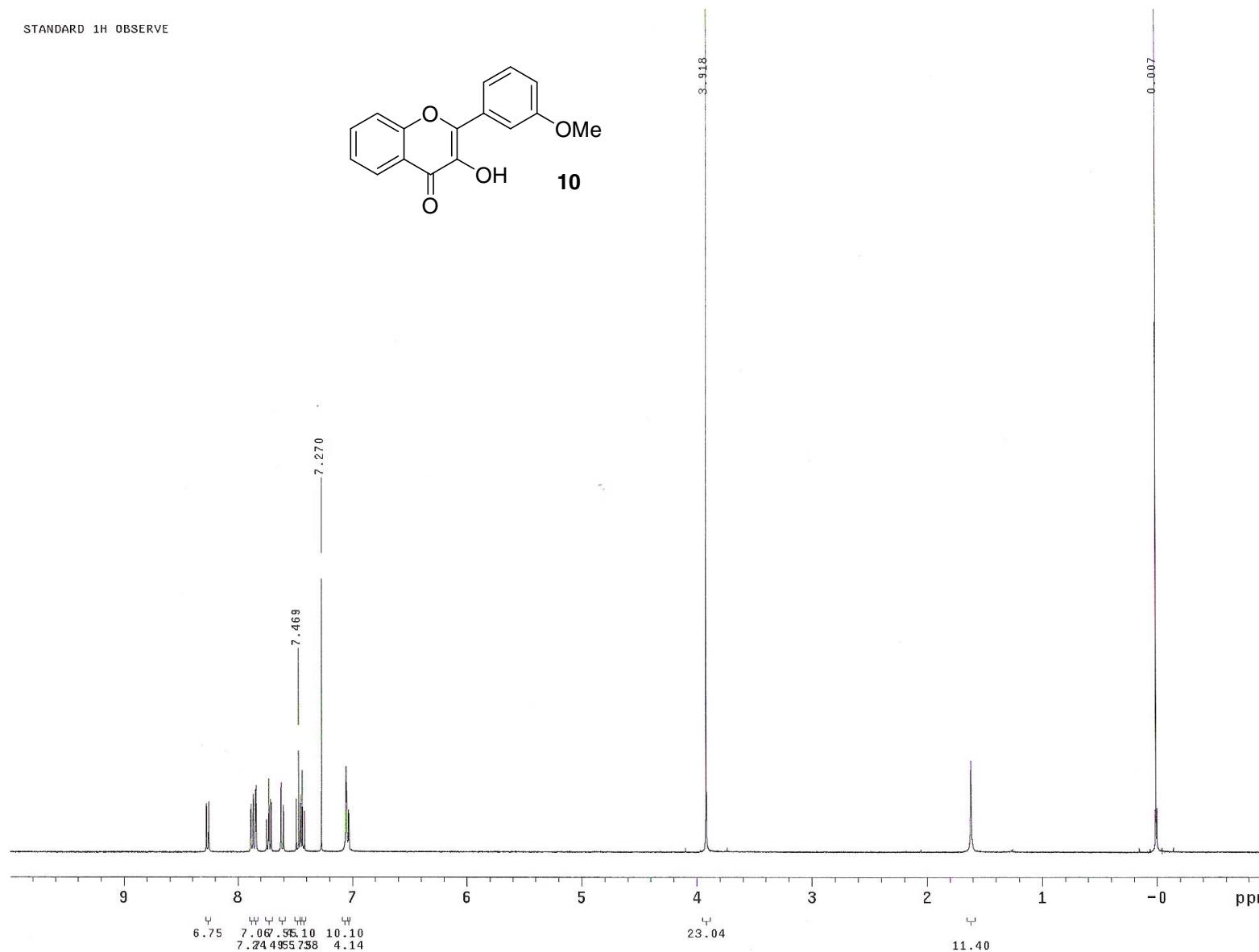
13C OBSERVE

Solvent: CDCl₃
Ambient temperature
UNITYplus-400 "chemsparc1"
PULSE SEQUENCE
Relax. delay 1.801 sec
Pulse 22.9 degrees
Acq. time 1.198 sec
Width 25000.0 Hz
240 repetitions
OBSERVE C13, 100.5118631 MHz
DECOPPLE H1, 399.7311936 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 12 minutes



¹H NMR 400 MHz spectrum

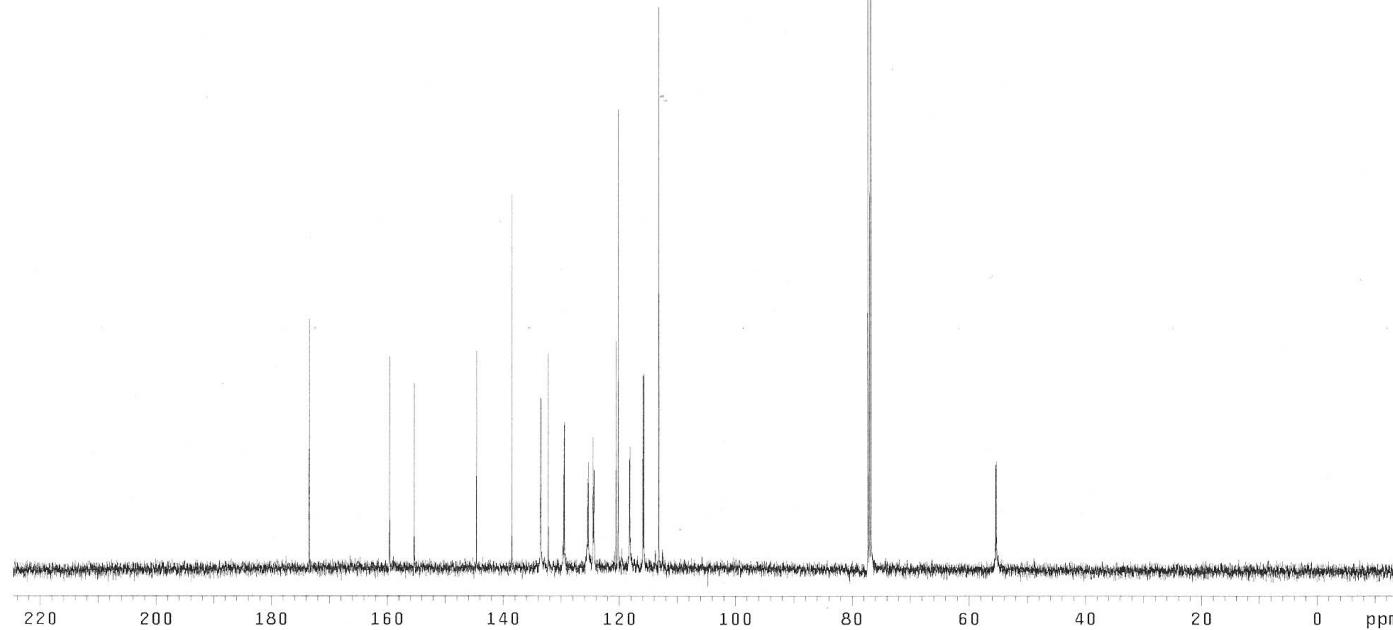
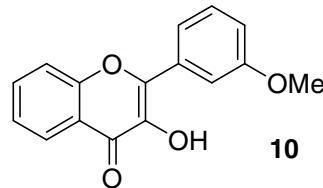
STANDARD 1H OBSERVE



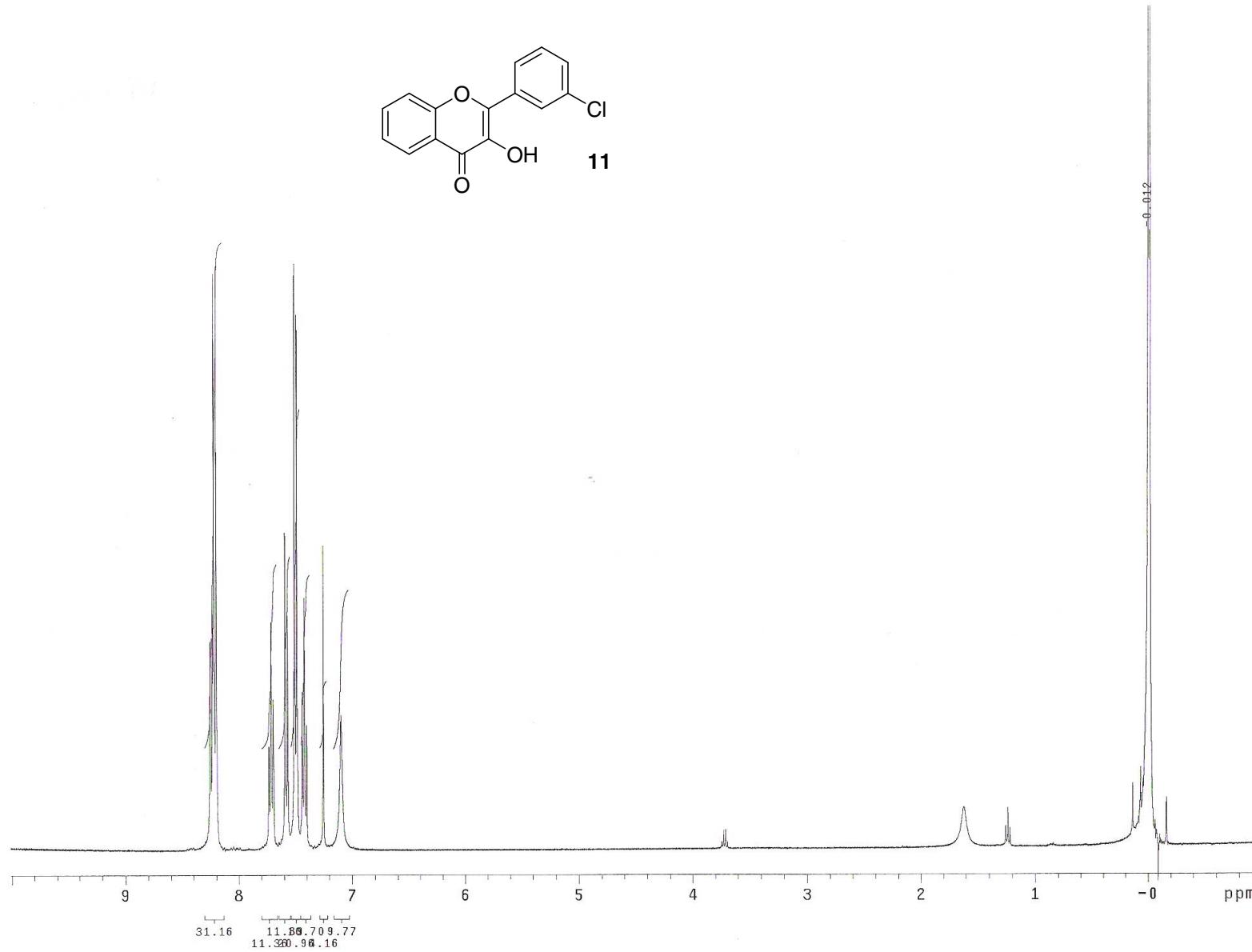
¹³C NMR 100 MHz spectrum

Proton Standard Parameters
File: Carbon
Pulse Sequence: s2pul
Solvent: cdcl₃
Temp. 25.0 C / 298.1 K
Operator: sjw
INOVA-500 "bio500"

Pulse 45.0 degrees
Acq. time 1.500 sec
Width 30188.7 Hz
832 repetitions
OBSERVE C13, 125.7781812 MHz
DECOUPLE H1, 500.2140810 MHz
Power 44 dB
continuously on
WALTZ=16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 2 hr, 5 min, 44 sec



¹H NMR 400 MHz spectrum

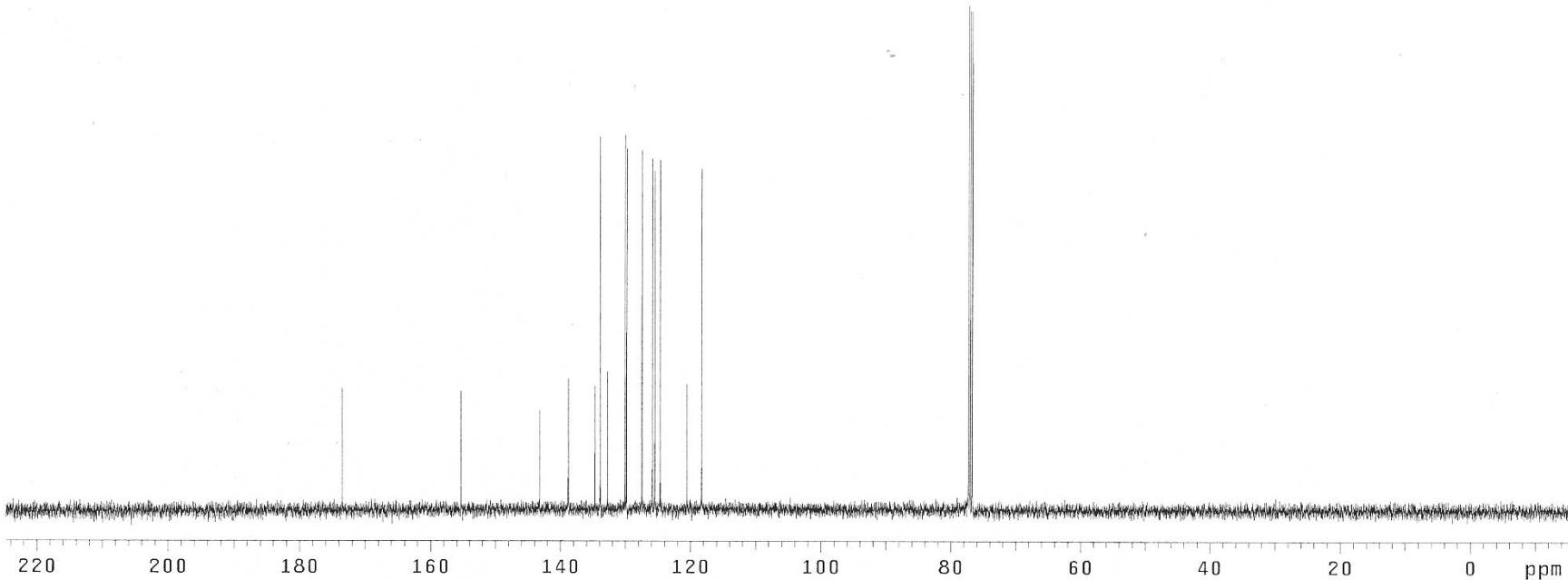
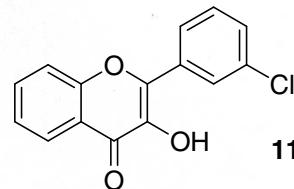


¹³C NMR 100 MHz spectrum

pad=10 run with findz0 before acquisition
pad=10 run with gradshim before acquisition

Solvent: cdc13
Temp. 25.0 C / 298.1 K
Sample #43, Operator: sjw-xc
File: 3pri-Cl-Flav-C_Carbon-Std_01
INOVA-500 "chem500"

Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 1.500 sec
Width 30154.5 Hz
256 repetitions
OBSERVE C13, 125.6459915 MHz
DECOPLE H1, 499.6875700 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 12 min, 50 sec

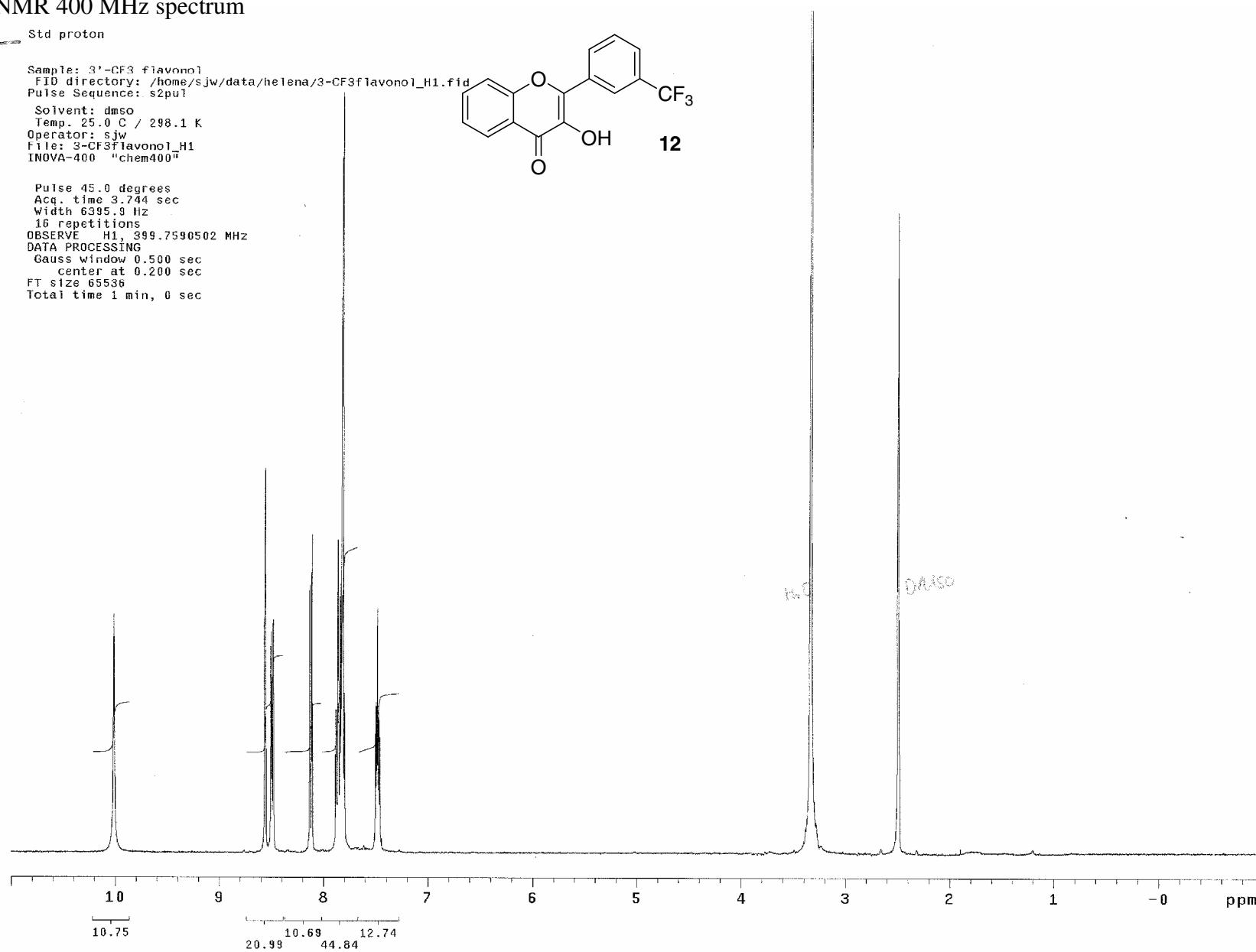
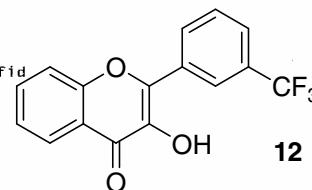


¹H NMR 400 MHz spectrum

Std proton

Sample: 3'-CF₃ flavonol
FID directory: /home/sjw/data/helena/3-CF₃flavonol_H1.fid
Pulse Sequence: s2pul
Solvent: dmso
Temp. 25.0 C / 298.1 K
Operator: sjw
File: 3-CF₃flavonol_H1
INOVA-400 "chem400"

Pulse 45.0 degrees
Acq. time 3.744 sec
Width 6395.9 Hz
16 repetitions
OBSERVE H1, 399.7590502 MHz
DATA PROCESSING
Gauss window 0.500 sec
center at 0.200 sec
FT size 65536
Total time 1 min, 0 sec



¹³C NMR 100 MHz spectrum
Std proton

Sample: 3'-CF₃ flavonol
FID directory: /home/sjw/data/helena/3-CF₃flavonol_C13.fid

Pulse Sequence: s2pul

Solvent: dmso

Temp. 25.0 C / 298.1 K

Operator: siw

File: 3-CF₃flavonol_C13

INOVA-400 "chem400"

Relax. delay 1.700 sec

Pulse 45.0 degrees

Acq. time 1.300 sec

Width 24125.5 Hz

15936 repetitions

OBSERVE C13, 100.5194705 MHz

DECOPPLE H1, 399.7610547 MHz

Power 36 dB

continuously on

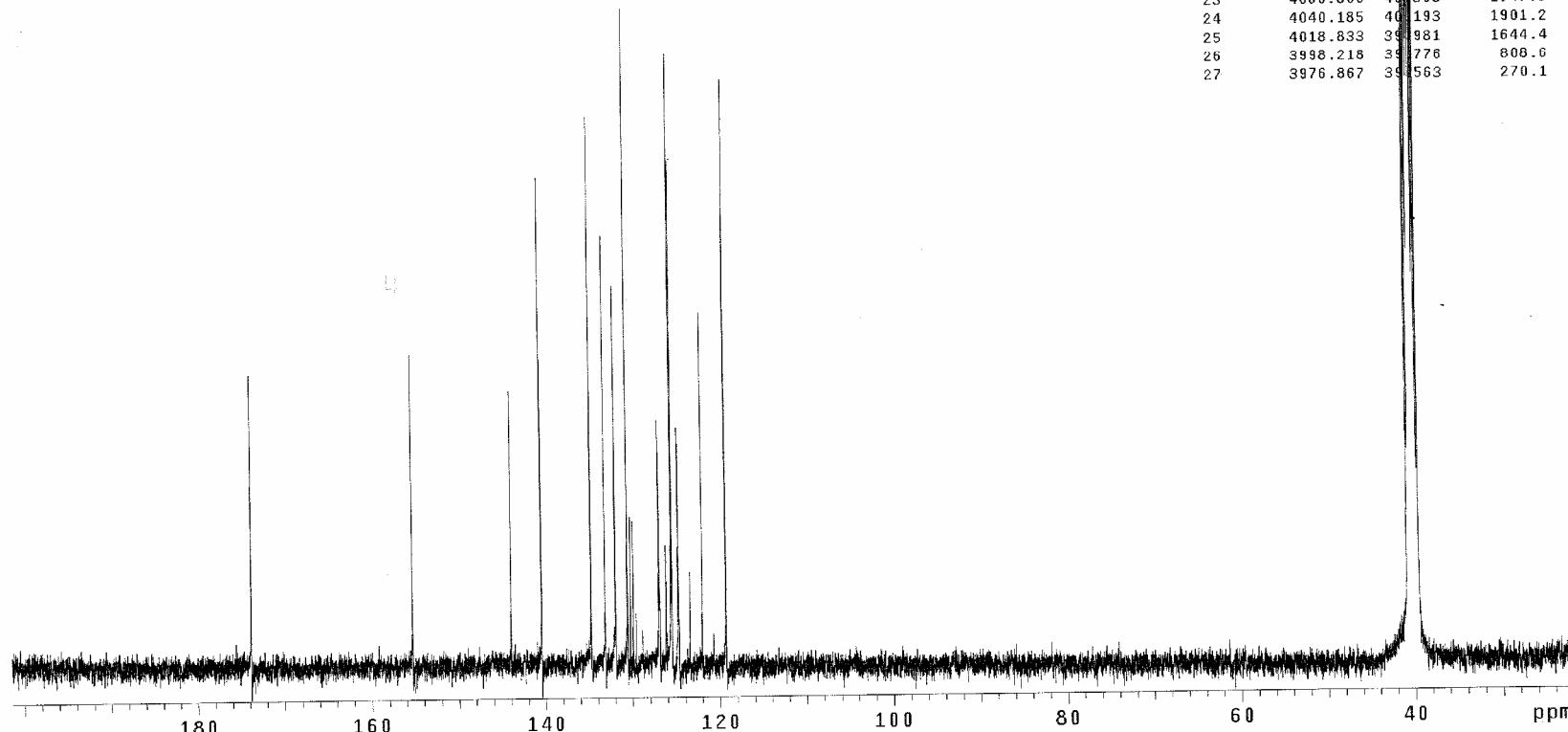
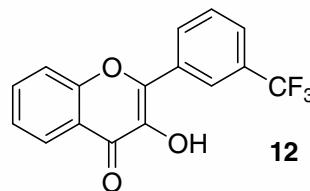
WALTZ-16 modulated

DATA PROCESSING

Line broadening 1.0 Hz

FT size 65536

Total time 16 hr, 42 min, 58 sec

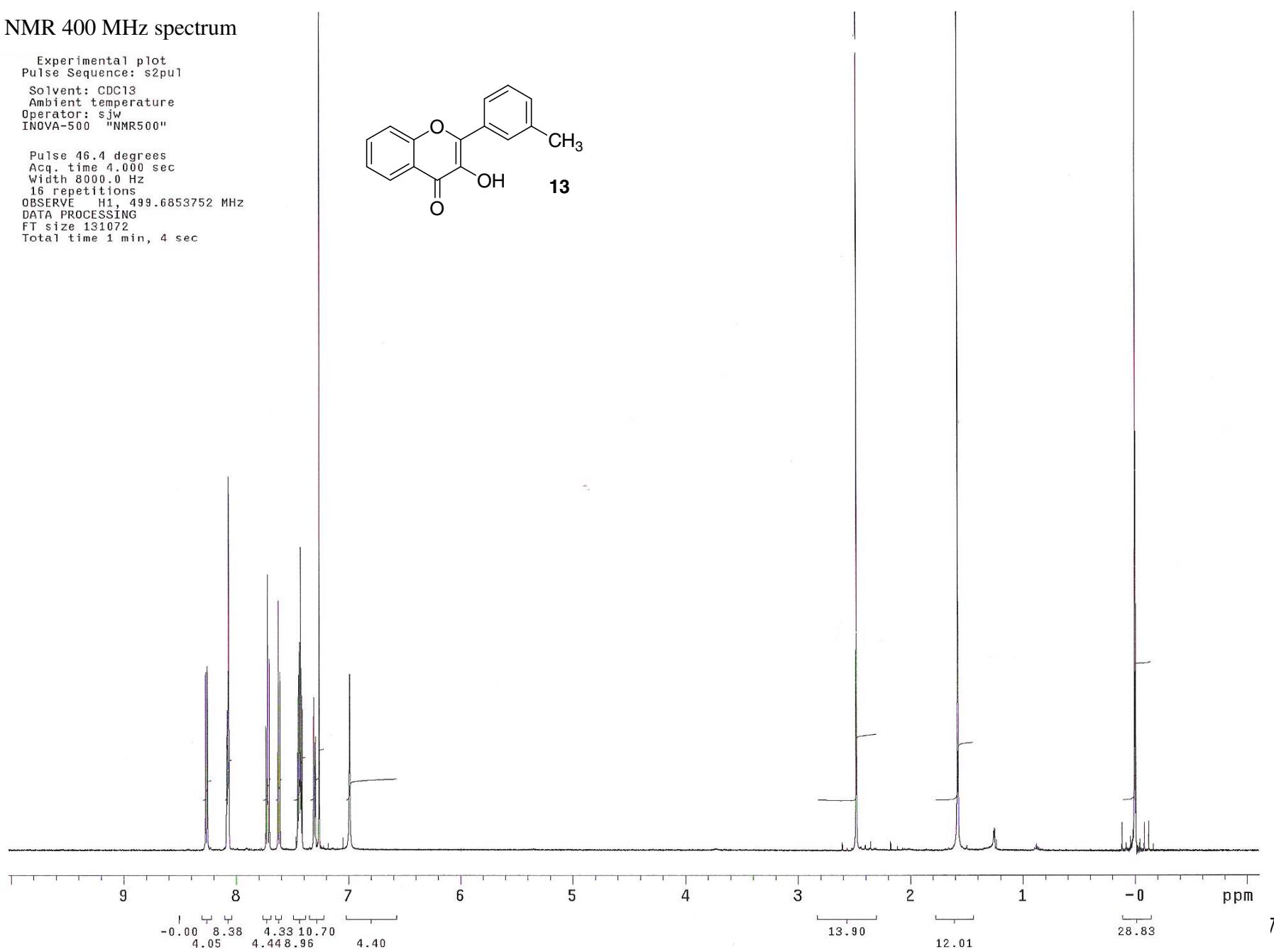
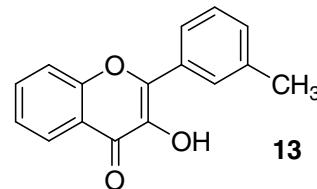


¹H NMR 400 MHz spectrum

Experimental plot
Pulse Sequence: s2pul

Solvent: CDCl₃
Ambient temperature
Operator: sjw
INOVA-500 "NMR500"

Pulse 46.4 degrees
Acq. time 4.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.6853752 MHz
DATA PROCESSING
FT size 131072
Total time 1 min, 4 sec



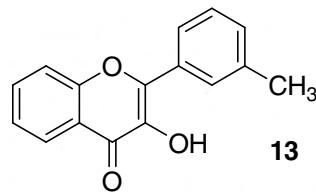
Plot 1

¹³C NMR 100 MHz spectrum

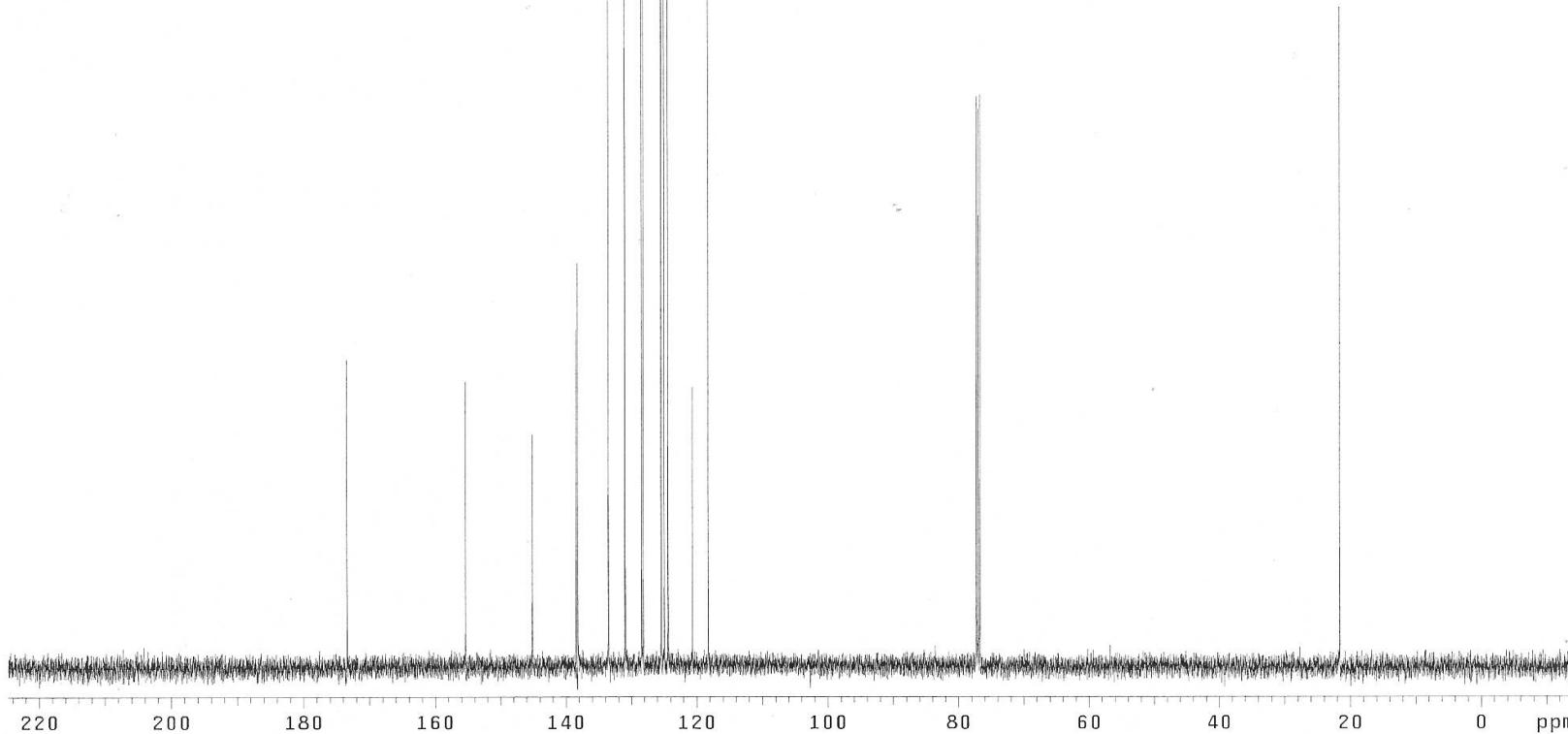
pad=10 run with findz0 before acquisition
pad=10 run with gradshim before acquisition

Solvent: cdcl₃
Temp. 25.0 C / 298.1 K
Sample #42, Operator: sjw-xc
File: 3pri-Me-Flav-C_Carbon-Std_01
INOVA-500 "chem500"

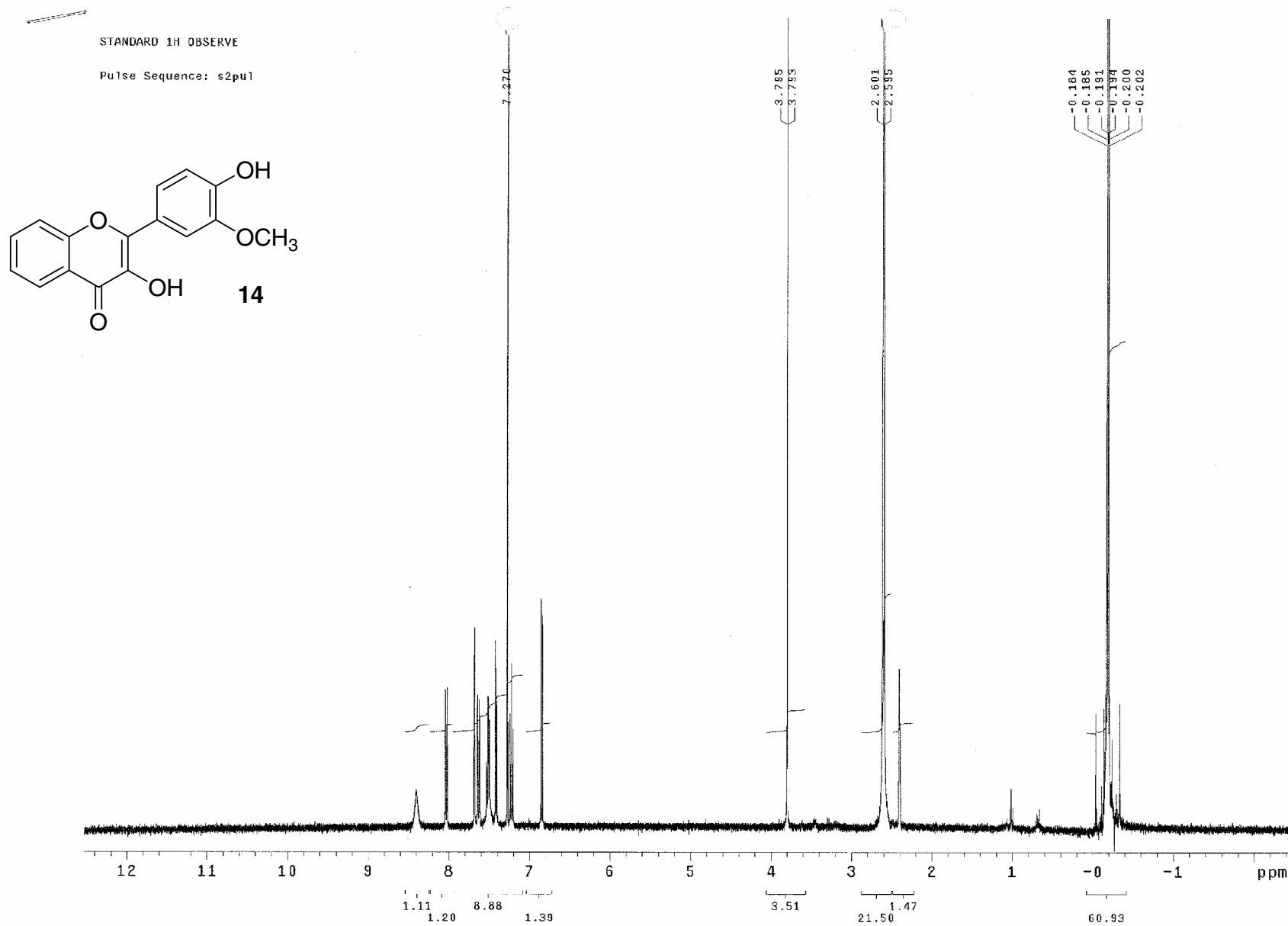
Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 1.500 sec
Width 30154.5 Hz
256 repetitions
OBSERVE C13, 125.6459948 MHz
DECOPPLE H1, 499.6875700 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 12 min, 50 sec



13



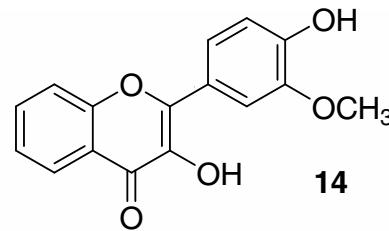
¹H NMR 400 MHz spectrum



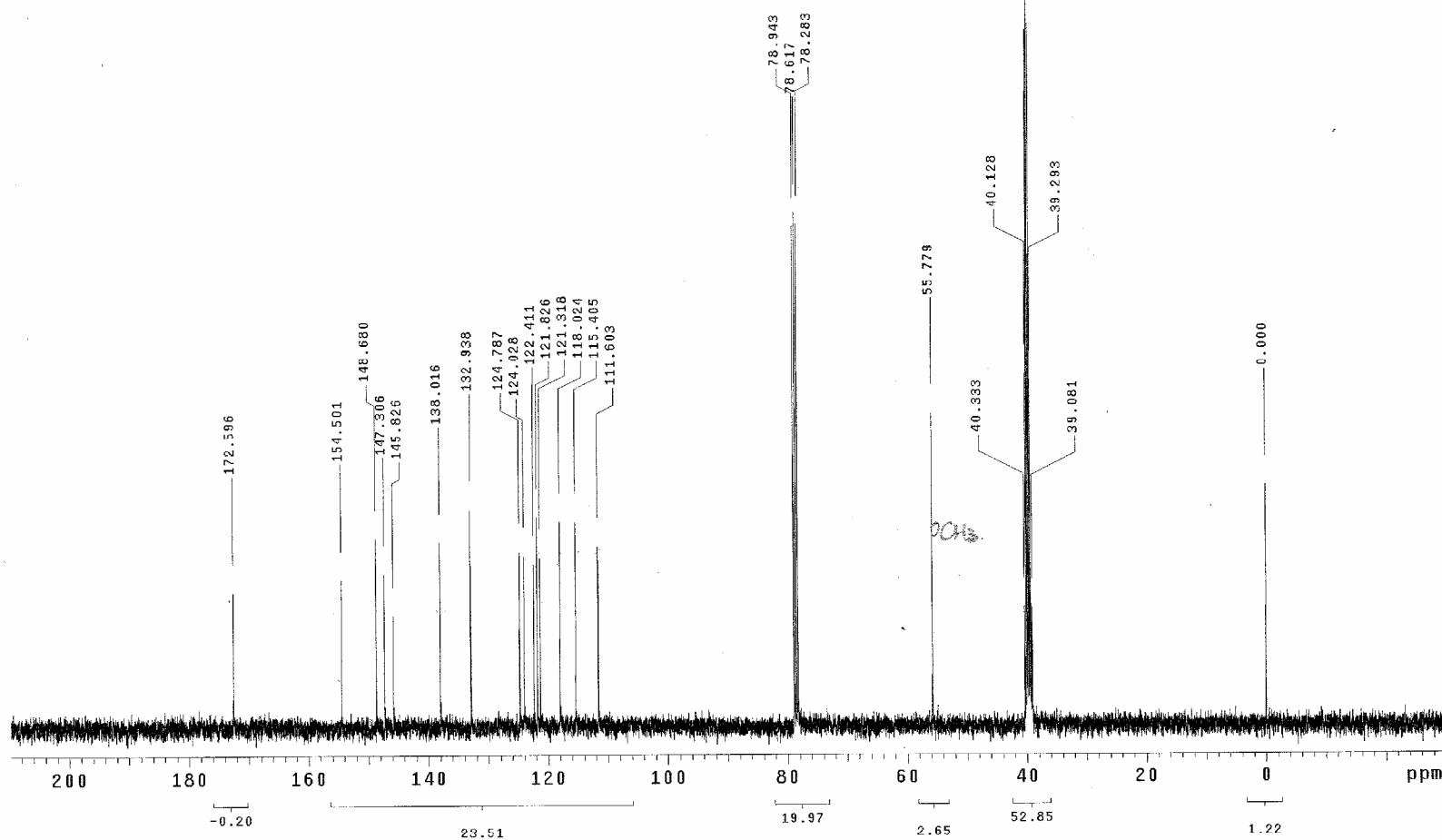
¹³C NMR 100 MHz spectrum

13C OBSERVE

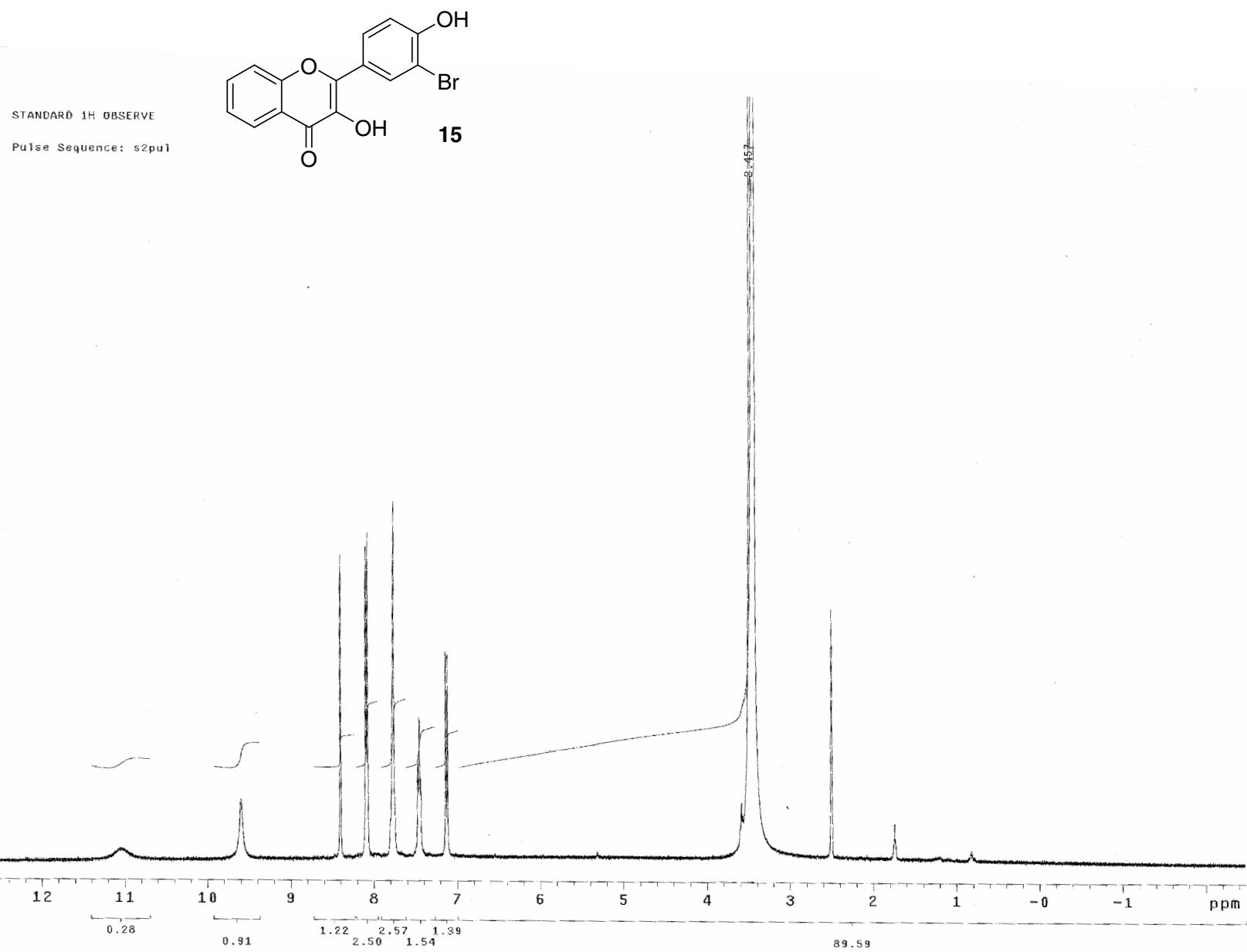
Pulse Sequence: s2pul



14



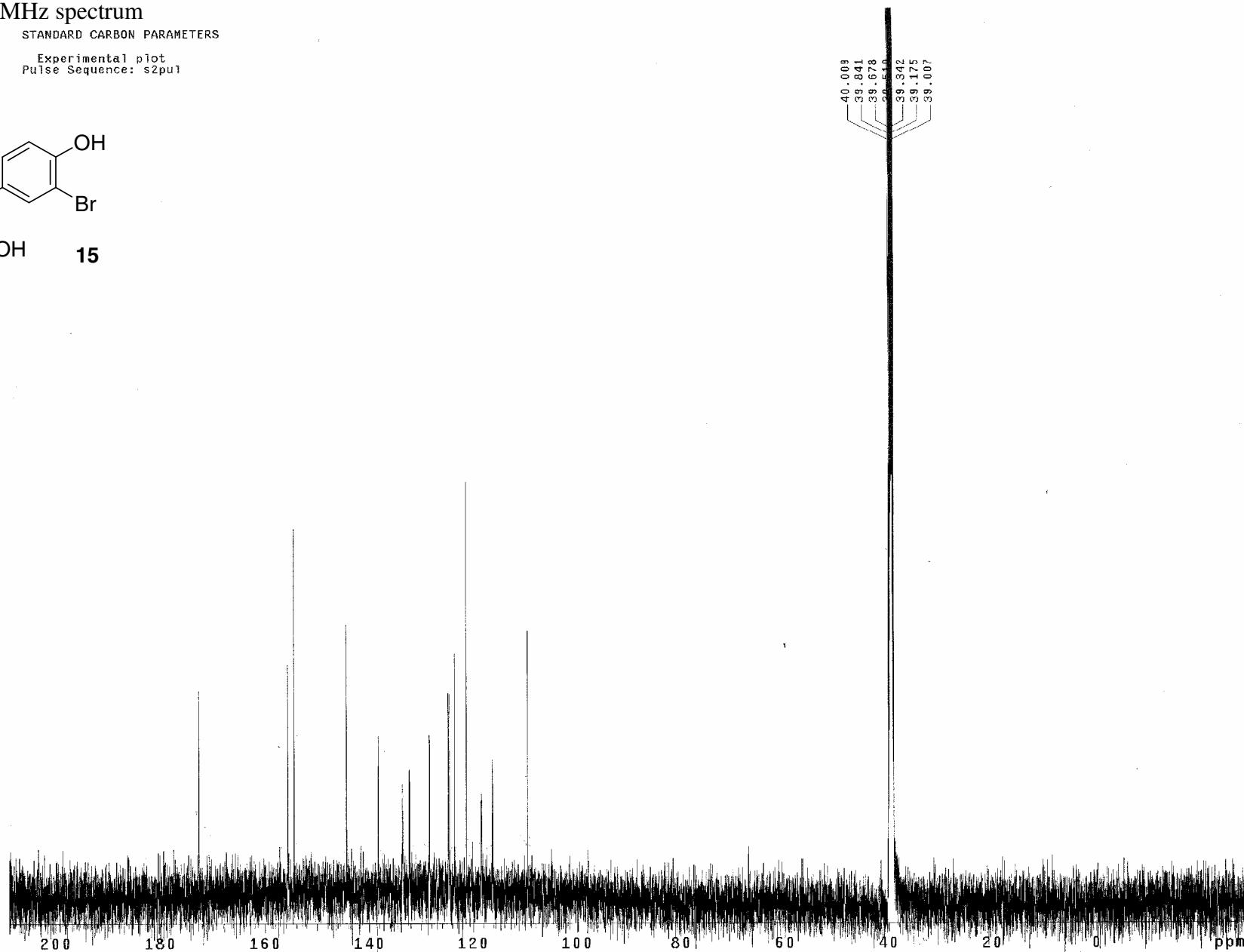
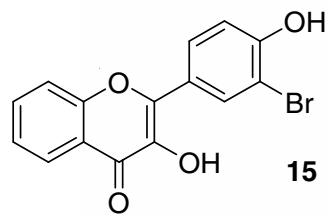
¹H NMR 400 MHz spectrum



¹H NMR 400 MHz spectrum

STANDARD CARBON PARAMETERS

Experimental plot
Pulse Sequence: s2pul

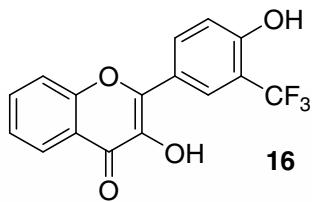


¹H NMR 400 MHz spectrum

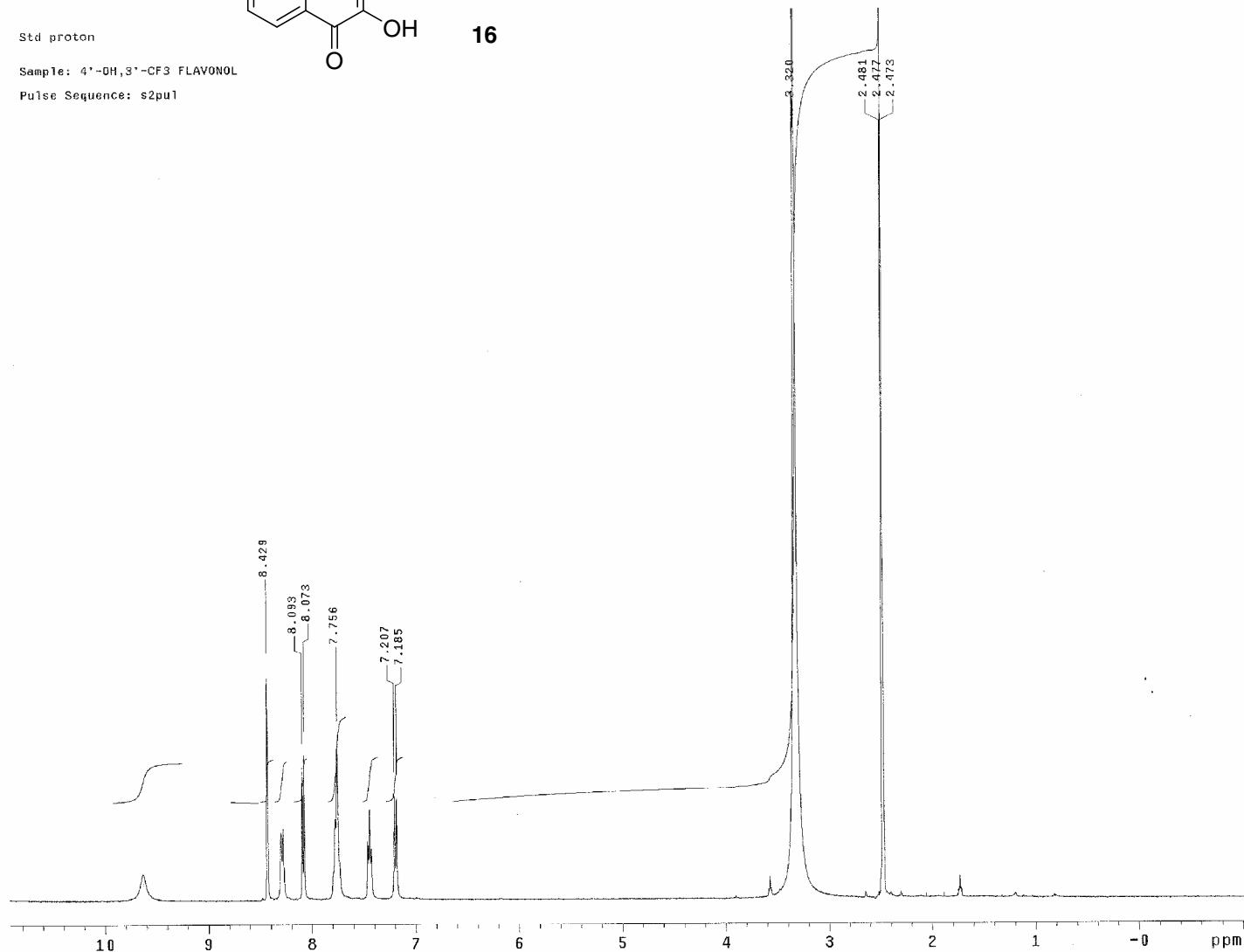
Std proton

Sample: 4'-OH,3'-CF₃ FLAVONOL

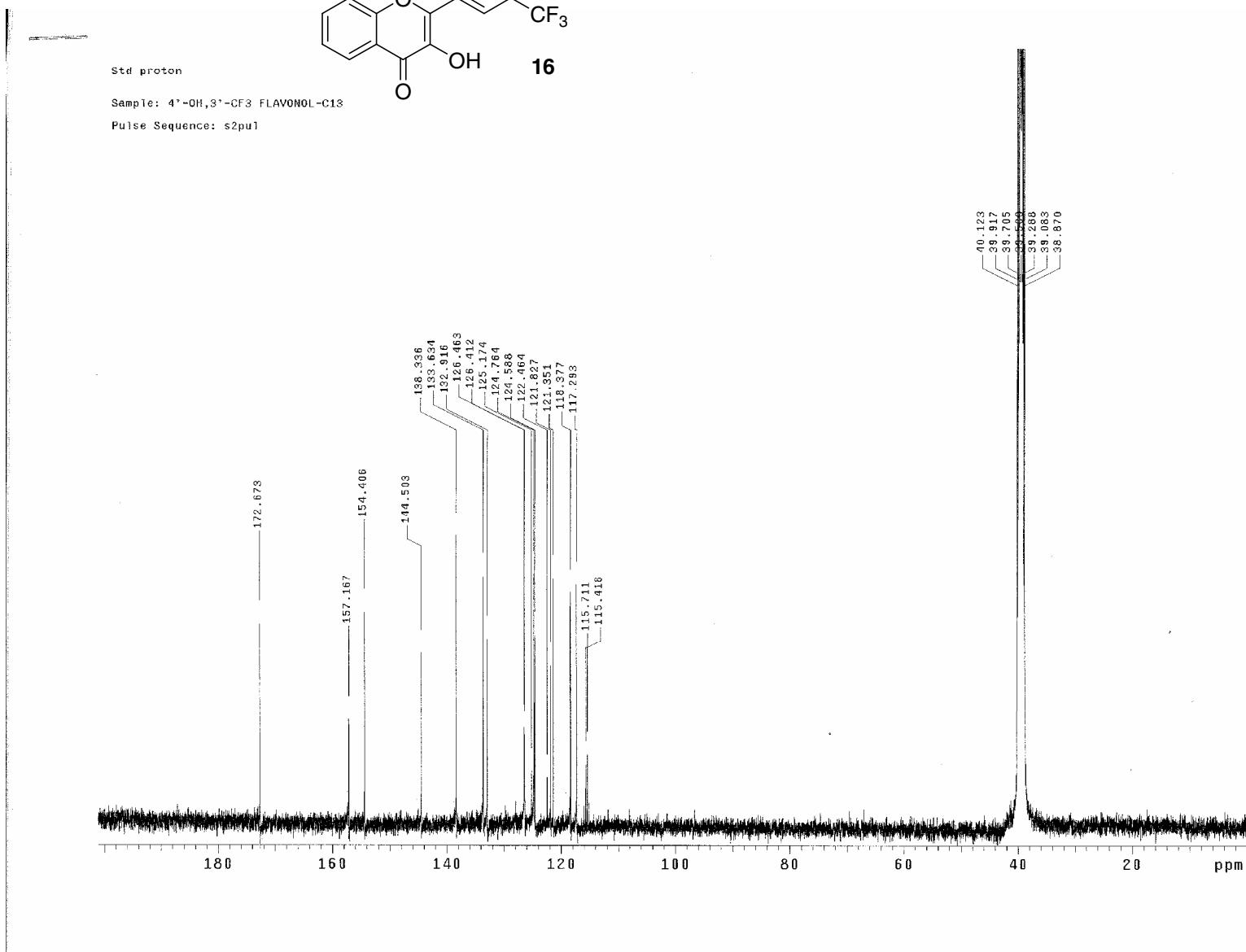
Pulse Sequence: s2pul



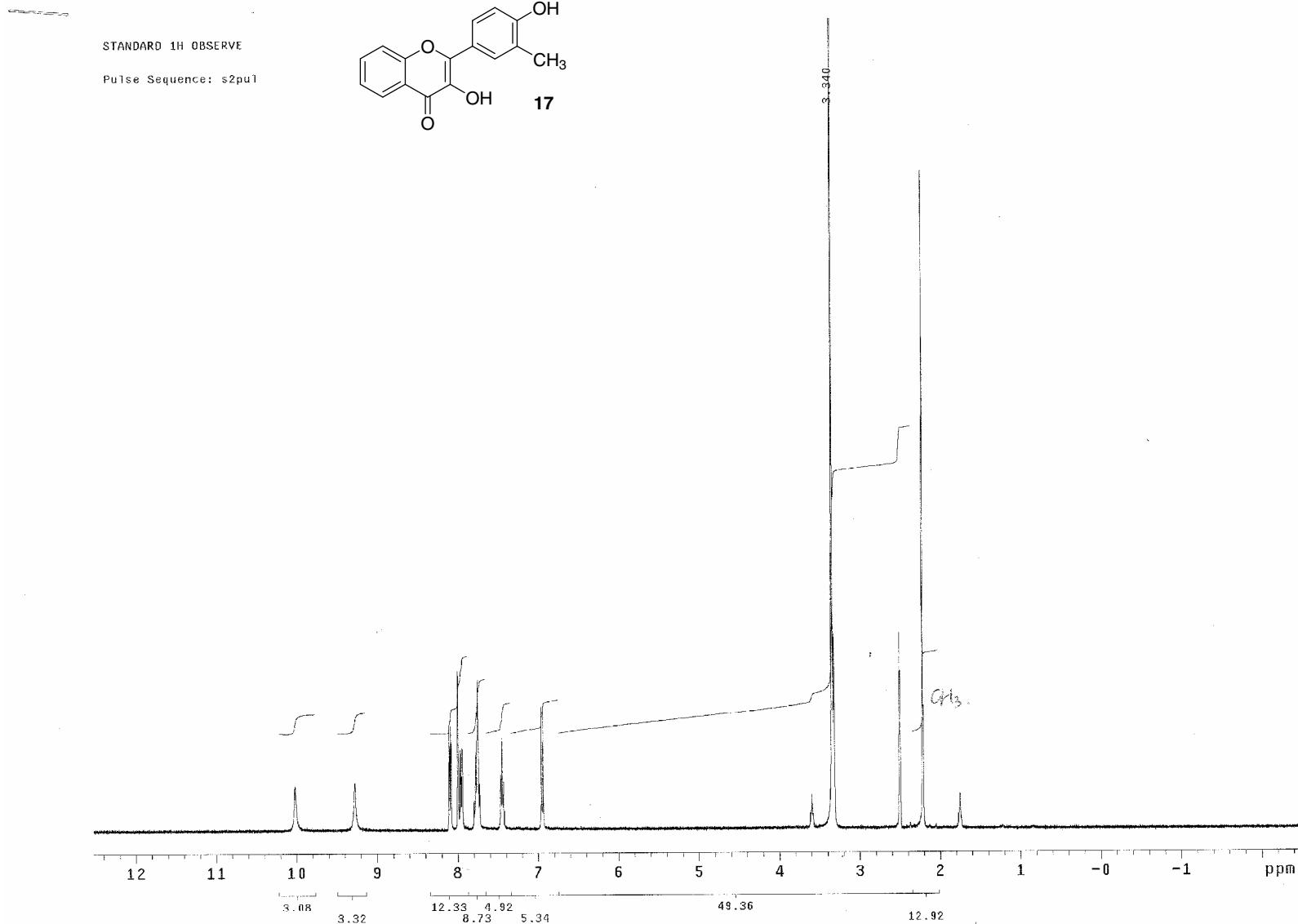
16



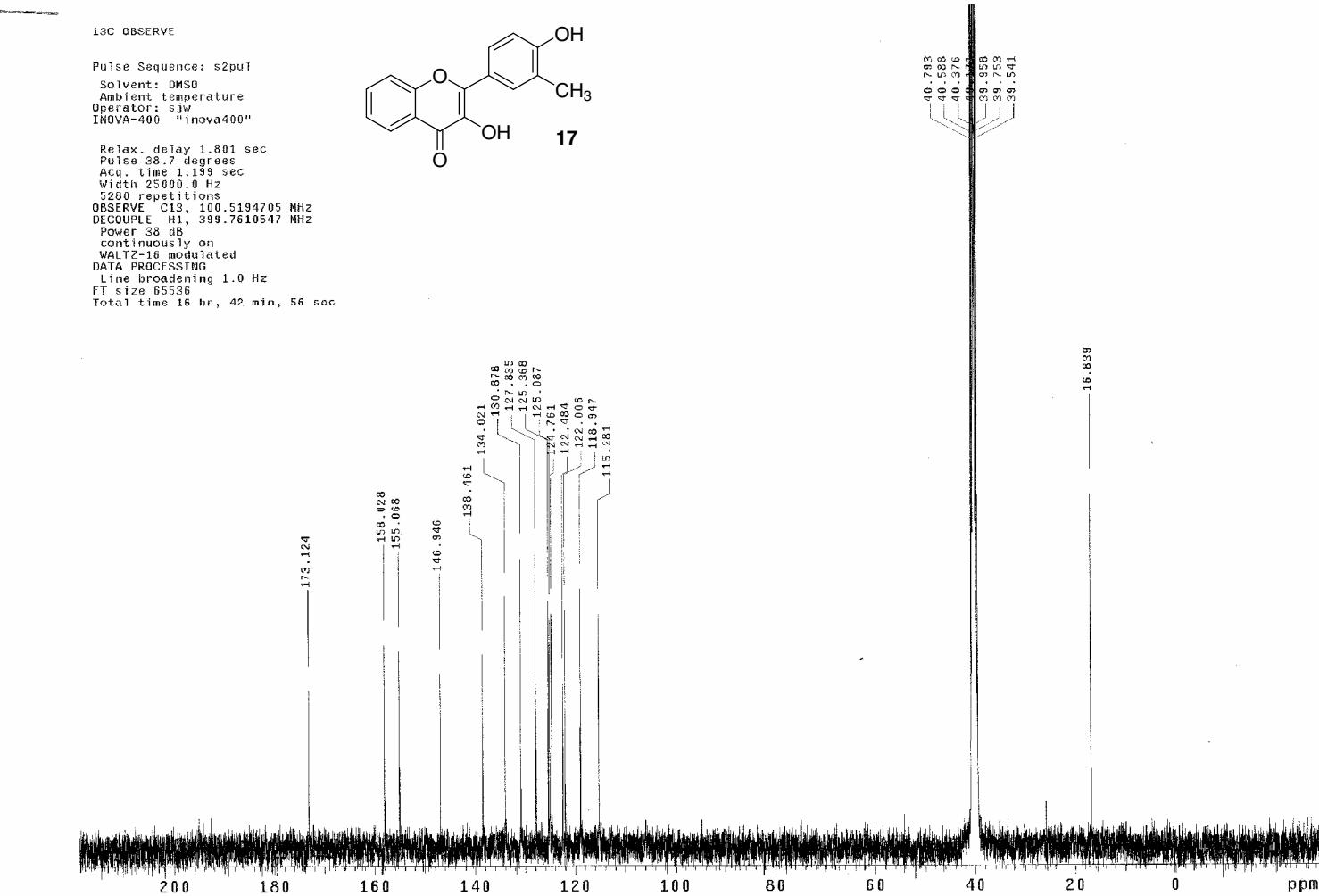
¹³C NMR 100 MHz spectrum



¹H NMR 400 MHz spectrum



¹³C NMR 100 MHz spectrum

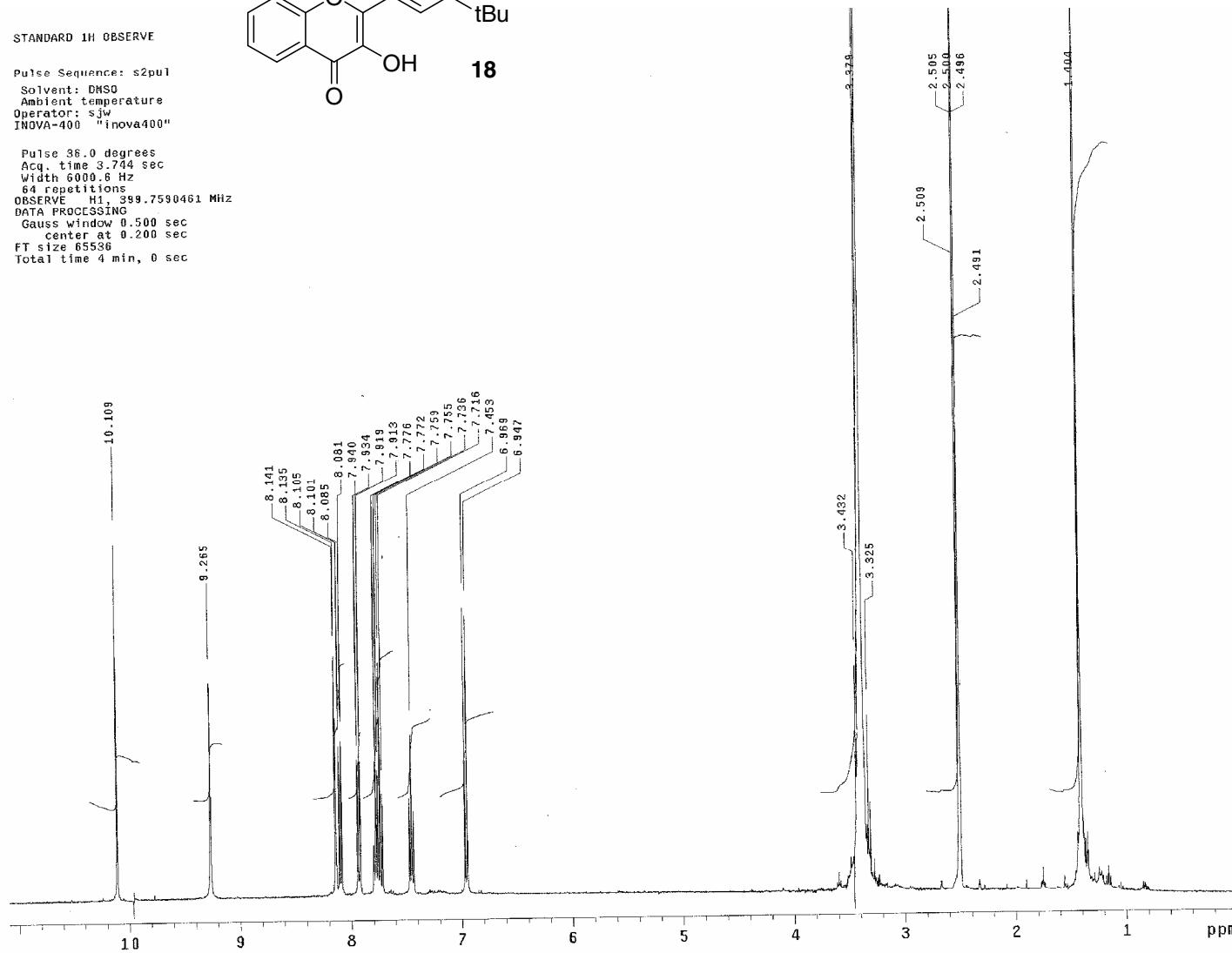
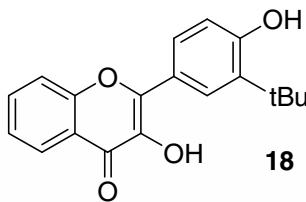


¹H NMR 400 MHz spectrum

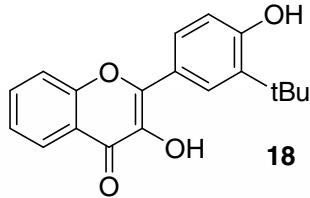
STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Operator: sjw
INNOVA-400 "inova400"

Pulse 90.0 degrees
Acq. time 3.744 sec
Width 6000.6 Hz
64 repetitions
OBSERVE H1, 399.7590461 MHz
DATA PROCESSING
Gauss window 0.500 sec
center at 0.200 sec
FT size 65536
Total time 4 min, 0 sec



¹³C NMR 100 MHz spectrum



13C OBSERVE

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Operator: SJW
INOVA-400 "innova400"

Relax. delay 1.801 sec
Pulse 38.7 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
18960 repetitions
OBSERVE C13, 100.5195331 MHz
DECOPLE H1, 399.7610547 MHz
Power 38 dB
continuously on
WALTZ8 simulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 16 hr, 42 min, 56 sec

