

Supplementary Information

Synthesis, activity and metabolic stability of propan-2-one substituted tetrazolylalkanoic acids as dual inhibitors of cytosolic phospholipase A₂α and fatty acid amide hydrolase

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1. Metabolism of compounds 10 and 12 by rat liver S9 fraction

Incubation procedure: To a mixture of 125 μL S9 fraction (prepared as described recently,³² protein content 8 mg/mL) and 373 μL of a NADPH-solution (11.2 mg NADPH- Na_4 in a mixture of 9.5 mL of potassium phosphate buffer (0.1 M, pH 7.4), which contained 0.5 mM EDTA- Na_2 , and 0.5 mL of a 0.1 M magnesium chloride solution) was added 2 μL of a DMSO solution (5 mM) of the test compound (= parent compound). The final protein concentration in the sample was 2 mg/mL, the concentration of NADPH was 1 mM and the concentration of the parent compound was 20 μM . After incubation at 37 °C for 30 min, 1.0 mL acetonitrile was added. The mixture was vortexed and allowed to stand in an ice bath for 15 min. After vigorous vortexing, the mixture was centrifuged at 12000 g and 4 °C for 5 min. The supernatant of the metabolism sample was separated and subjected to HPLC-MS.

A control sample, in which the enzymatic activity of the S9 fraction was inactivated before the addition of the test substance, was prepared as follows: A mixture of 125 μL S9 fraction, 373 μL of a 19:1 (v/v) mixture of potassium phosphate buffer (0.1 M, pH 7.4), which contained 0.5 mM EDTA- Na_2 , and a 0.1 M magnesium chloride solution, and 1.0 mL of acetonitrile was treated with 2 μL of a DMSO solution (5 mM) of the test compound. This mixture was allowed to stand at room temperature for 30 min and in an ice bath for 15 min before centrifugation at 12000 g and 4 °C for 5 min. The supernatant was separated and subjected to HPLC-MS.

The extent of metabolism was determined by comparing the peak area of the test substance in the metabolism sample with that of the control sample.

HPLC-MS analysis: The HPLC/MS system used was from Shimadzu (Kyoto, Japan) and consisted of two LC-20ADXR HPLC-pumps, a SIL-30AC autosampler, and a LCMS-2020 single quad detector. Aliquots of 2 μL were injected onto a HICHROM ACE 3 C₁₈ column (2.1 mm inside diameter x 100 mm, particle size 3 μm) (HiChrom, Berkshire, UK) protected with a Phenomenex C18 guard column (3 mm inside diameter x 4 mm) (Phenomenex, Aschaffenburg, Germany). Autosampler temperature was 15 °C, column oven temperature was set to 30 °C. Gradient elution was used with solvent A (acetonitrile/water/formic acid, 10:90:0.1, v/v/v) and solvent B (acetonitrile/water/formic acid, 90:10:0.1, v/v/v): 0–3 min: isocratic run at 10% B, 3–15 min: linear gradient to 95% B, 15–18 min: isocratic run at 95% B, 18–20 min: linear gradient to 10% B, 20–28 min isocratic run at 10% B. Detection was performed in ESI negative mode. The effluents were directed to the mass spectrometer by a divert valve from minute 2.5 to minute 26.

HPLC-MS-ESI (negative) chromatograms:

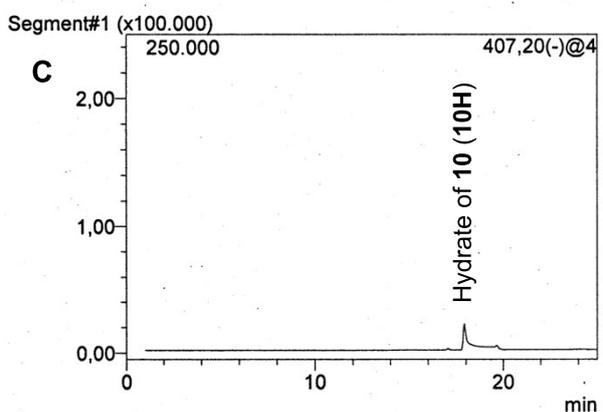
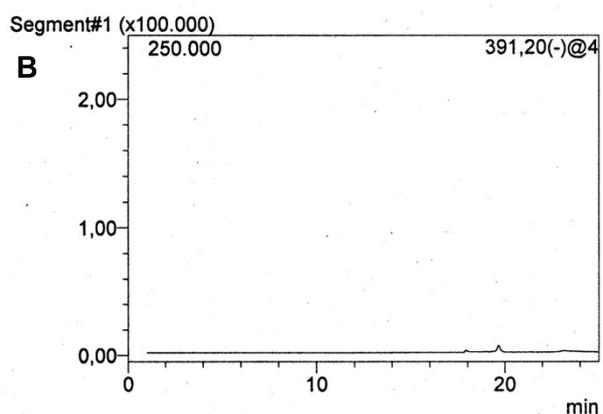
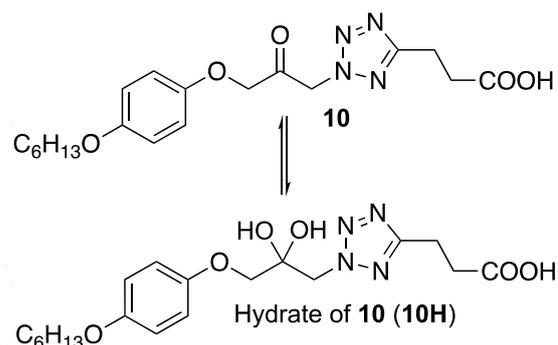
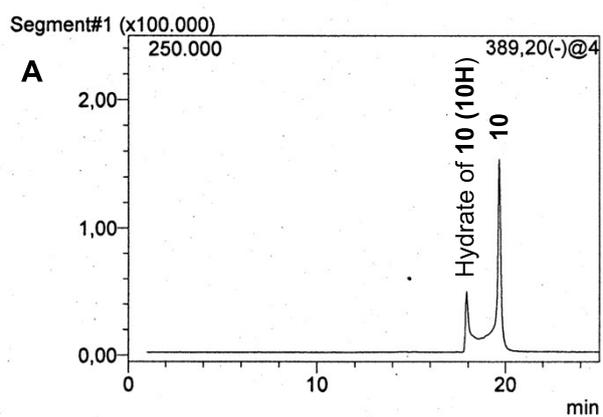


Figure 1 HPLC-MS ESI negative-chromatograms (SIM mode) obtained when enzymatic activity of the S9 fraction was inactivated by acetonitrile before addition of the test compound **10**; (A) m/z trace of the parent compound **10**; (B) m/z trace of the metabolite of **10** formed by reduction of the ketone group to an alcohol and of the (M+2) isotope of **10**; (C) m/z trace of the hydrate form of **10** (**10H**).

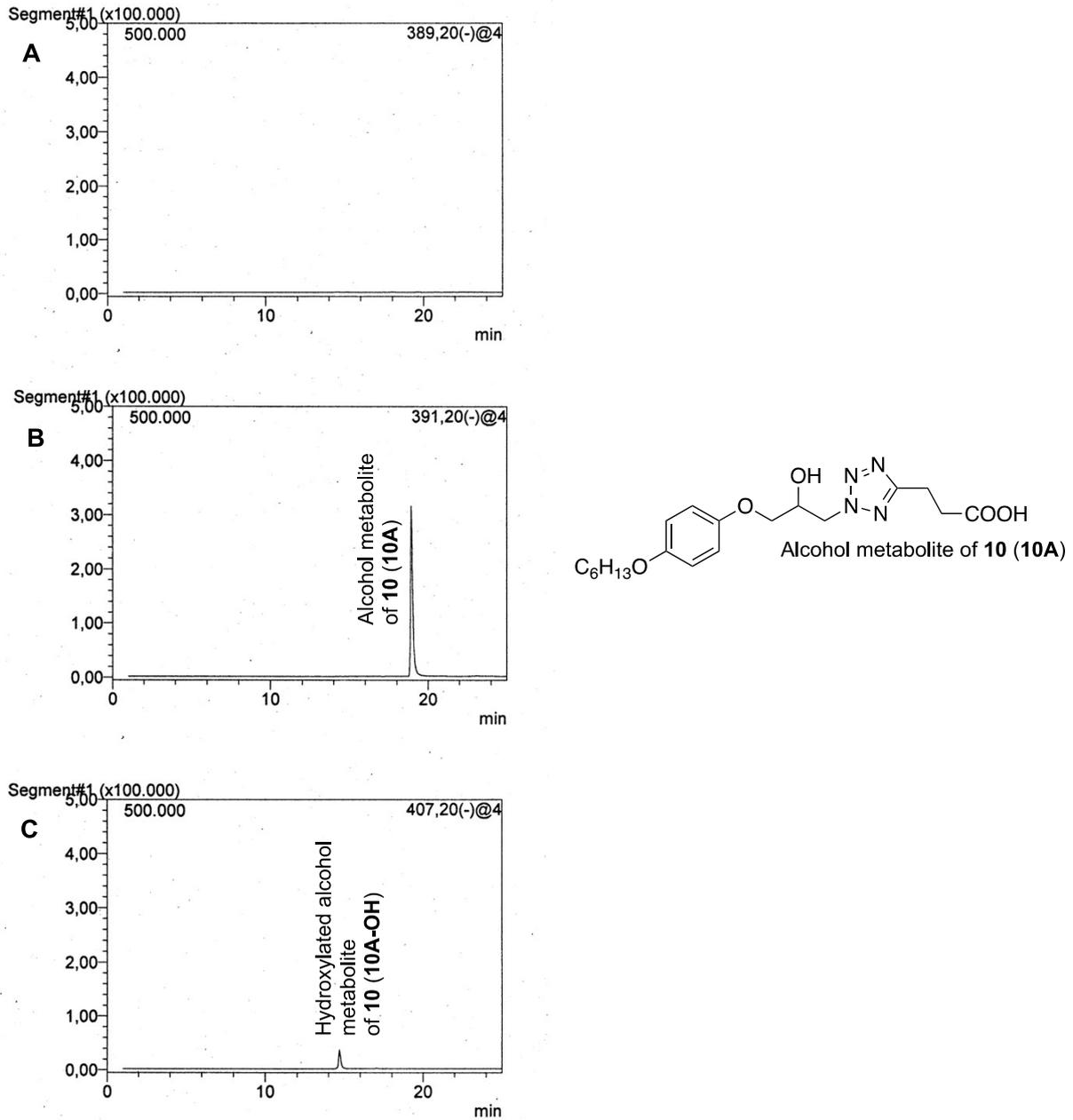


Figure 2 HPLC-MS ESI negative-chromatograms (SIM mode) obtained after incubation of **10** with rat liver S9 fraction in presence of NADPH; (A) m/z trace of the parent compound **10**; (B) m/z trace of the metabolite of **10** formed by reduction of the ketone group to an alcohol (**10A**); (C) m/z trace of a cytochrome P450 oxidation product of the alcohol metabolite of **10** (**10A-OH**).

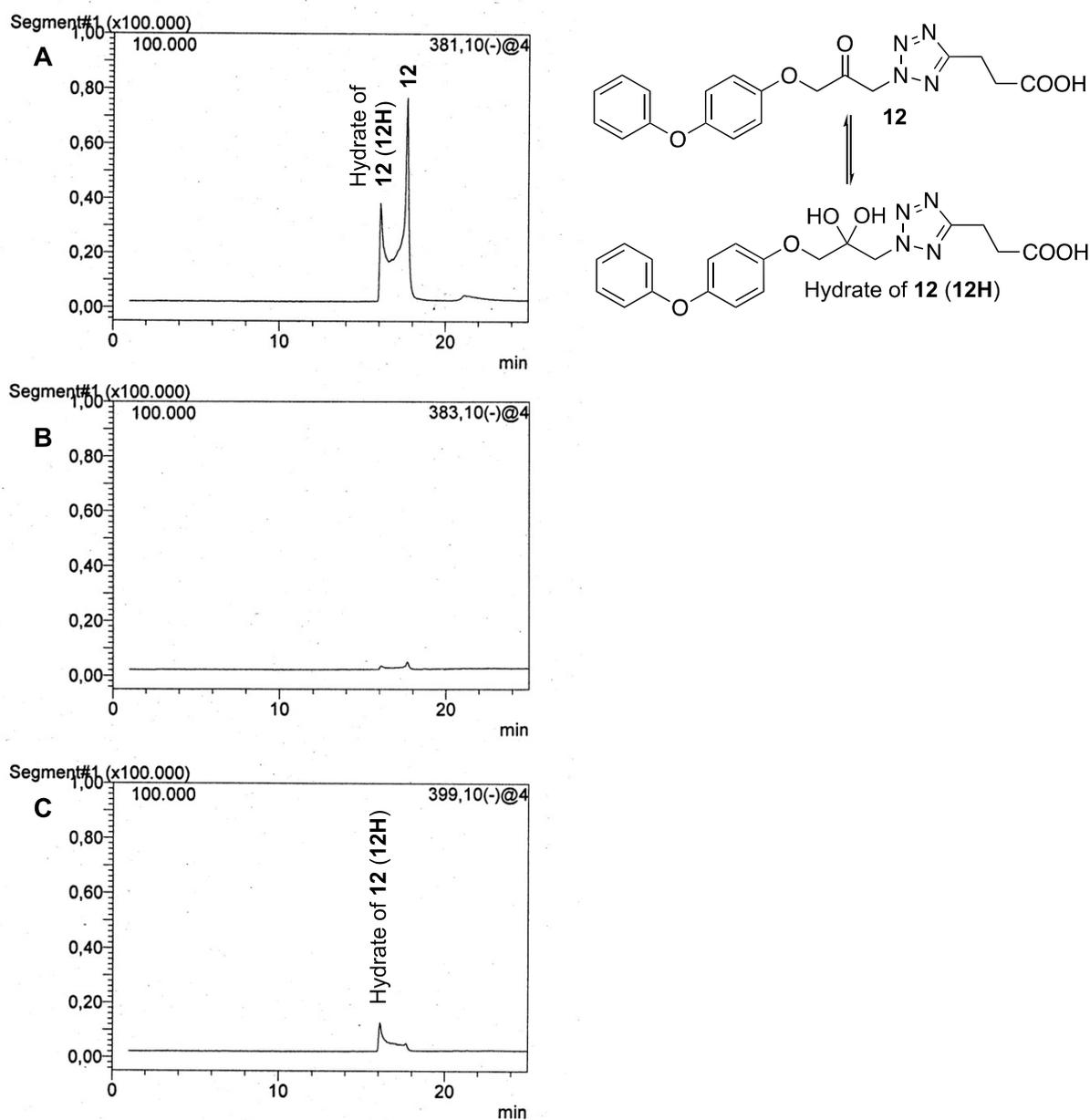


Figure 3 HPLC-MS ESI negative-chromatograms (SIM mode) obtained when enzymatic activity of the S9 fraction was inactivated by acetonitrile before addition of the test compound **12**; (A) m/z trace of the parent compound **12**; (B) m/z trace of the metabolite of **12** formed by reduction of the ketone group to an alcohol and of the (M+2) isotope of **12**; (C) m/z trace of the hydrate form of **12** (**12H**).

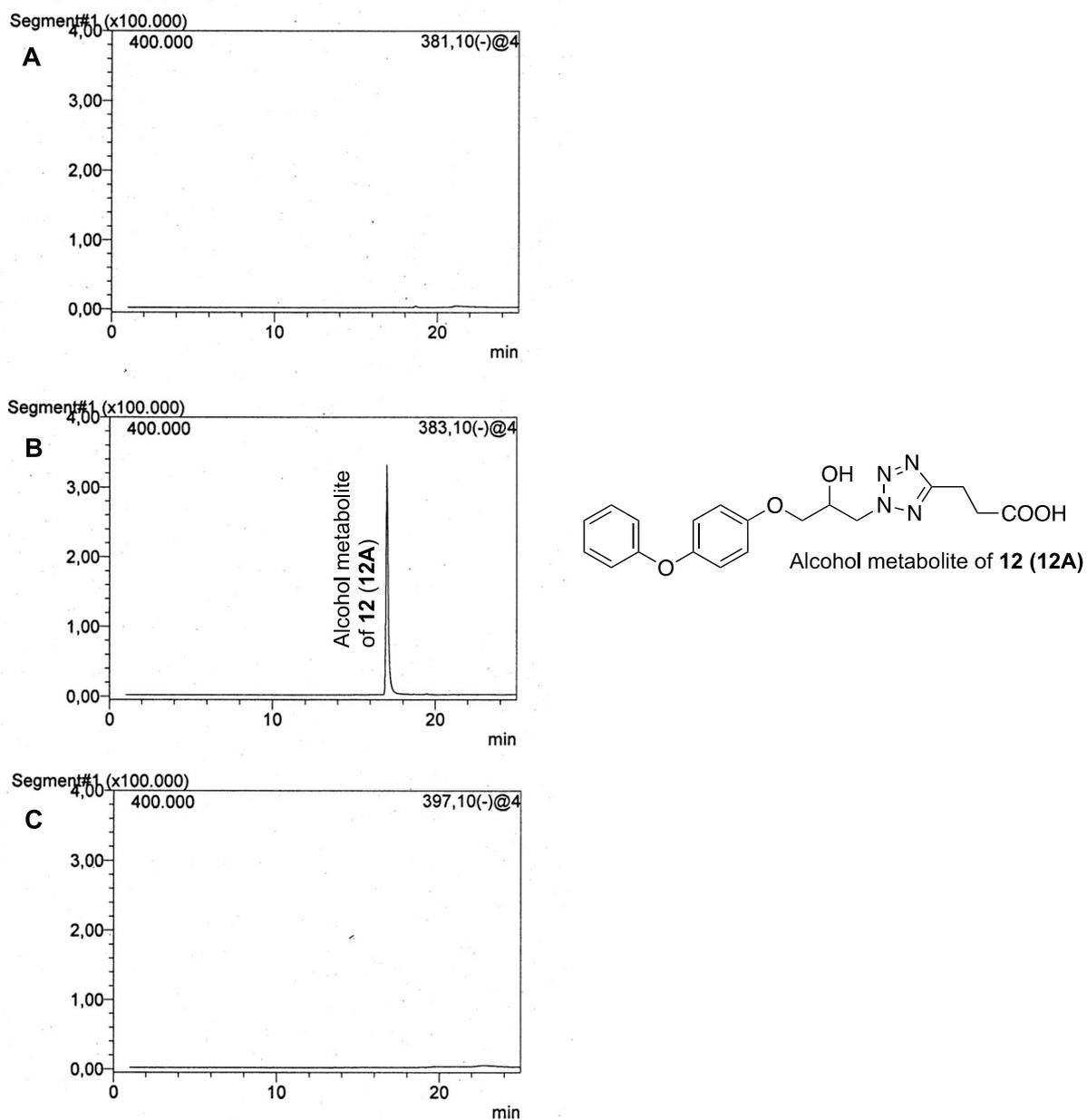
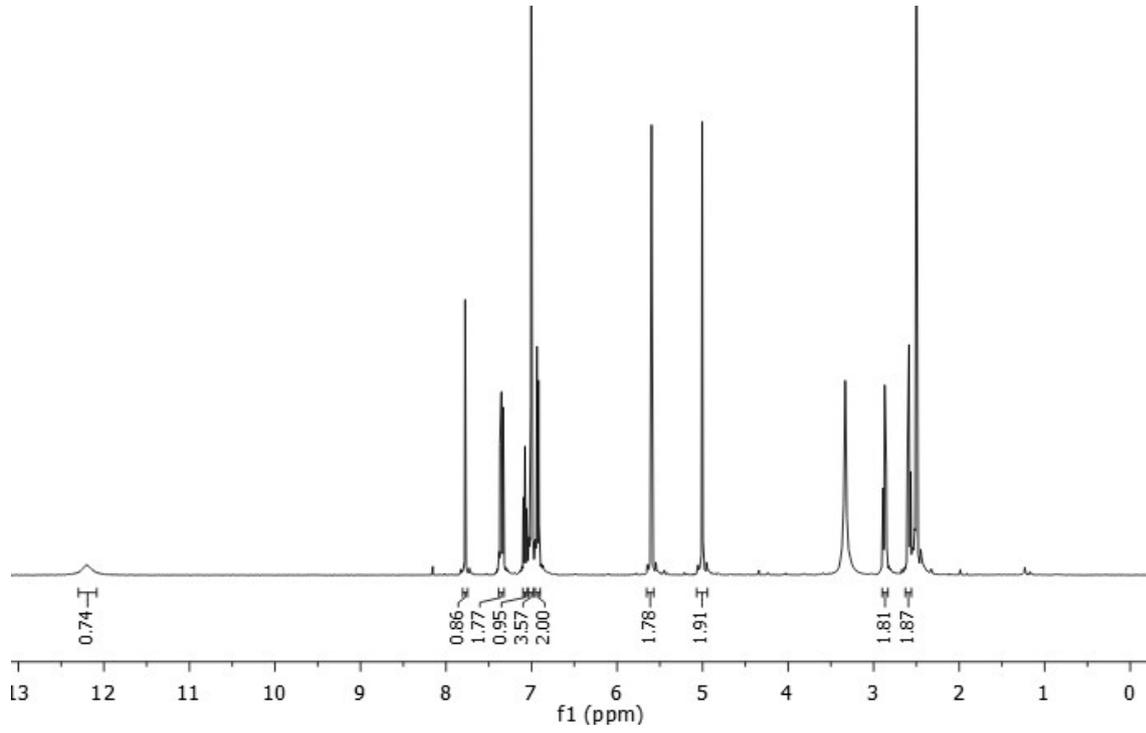
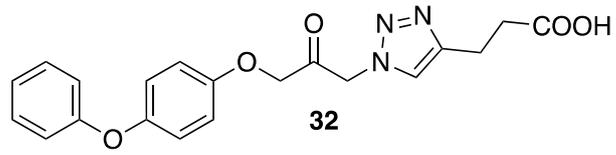


Figure 4 HPLC-MS ESI negative-chromatograms (SIM mode) obtained after incubation of **12** with rat liver S9 fraction in presence of NADPH; (A) *m/z* trace of the parent compound **12**; (B) *m/z* trace of the metabolite of **12** formed by reduction of the ketone group to an alcohol (**12A**); (C) *m/z* trace of a cytochrome P450 oxidation product of the alcohol metabolite of **12**.

Results: In the samples in which the microsomal enzymes were inactivated prior to addition of the test substance, only the parent compounds were detected (Figures 1 and 3). These appeared as double peaks, which is due to the fact that the activated ketones are in equilibrium with their hydrate forms. In the ESI source, the latter readily lose water, so that both forms are detected at the same mass-to-charge ratio.

In the samples in which the test compounds were incubated with the S9 fraction in the presence of NADPH, no parent compound could be detected after 30 min (Figures 2 and 4). Accordingly, large peaks of their alcohol metabolites formed by reduction of the ketone functions by carbonyl reductases appear. In the case of **10**, small amounts of a metabolite were detected (m/z 407.2) (Figure 2) that was probably formed by CYP P450-mediated oxidation of the alcohol metabolite of **10**.

2. Spectra of the new target compounds 32 and 44



eko972012, ME91, Ekodo
STANDARD 1H OBSERVE

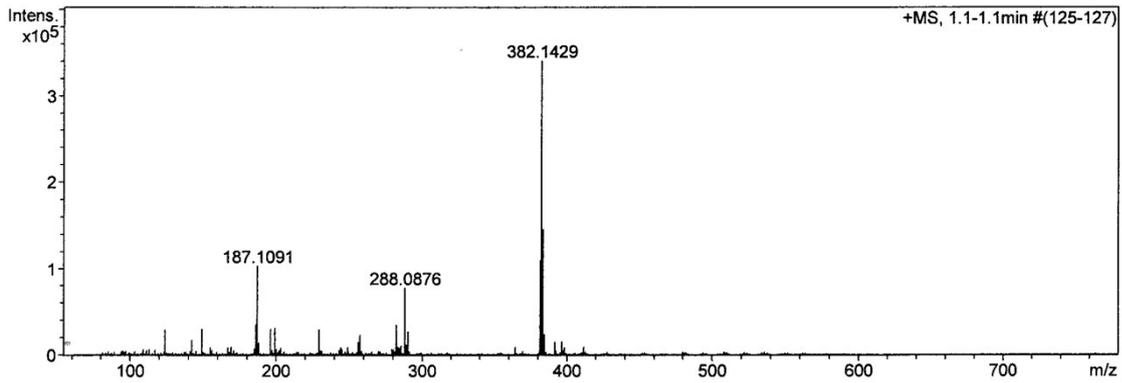
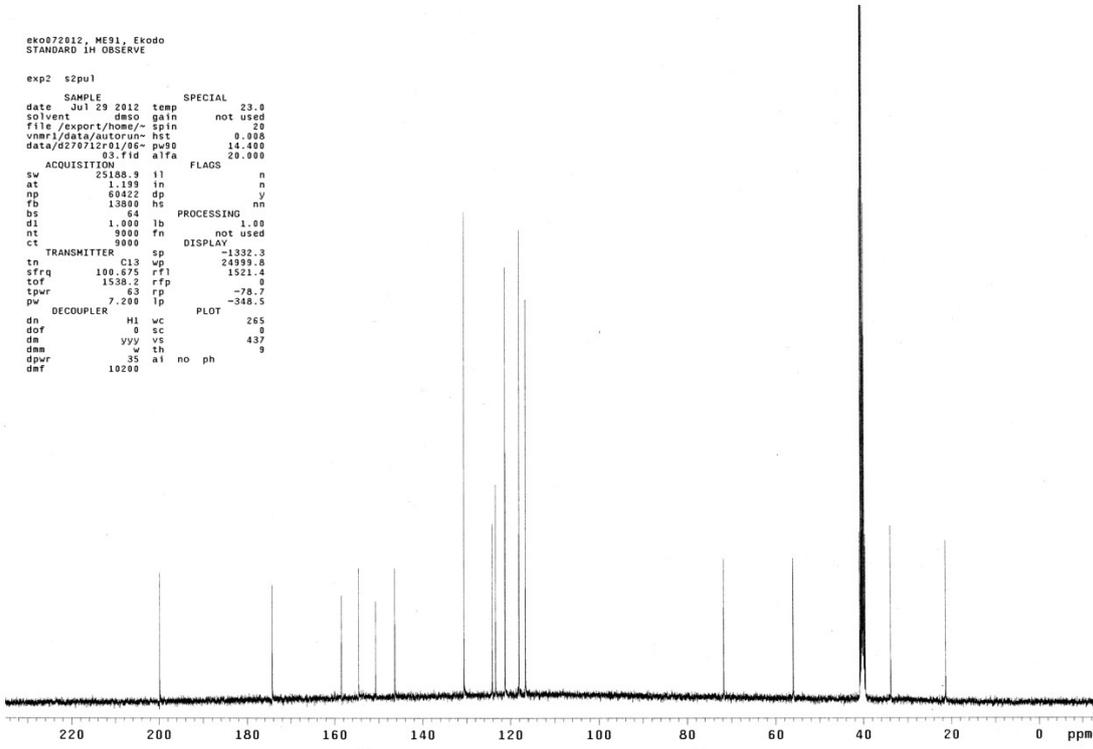
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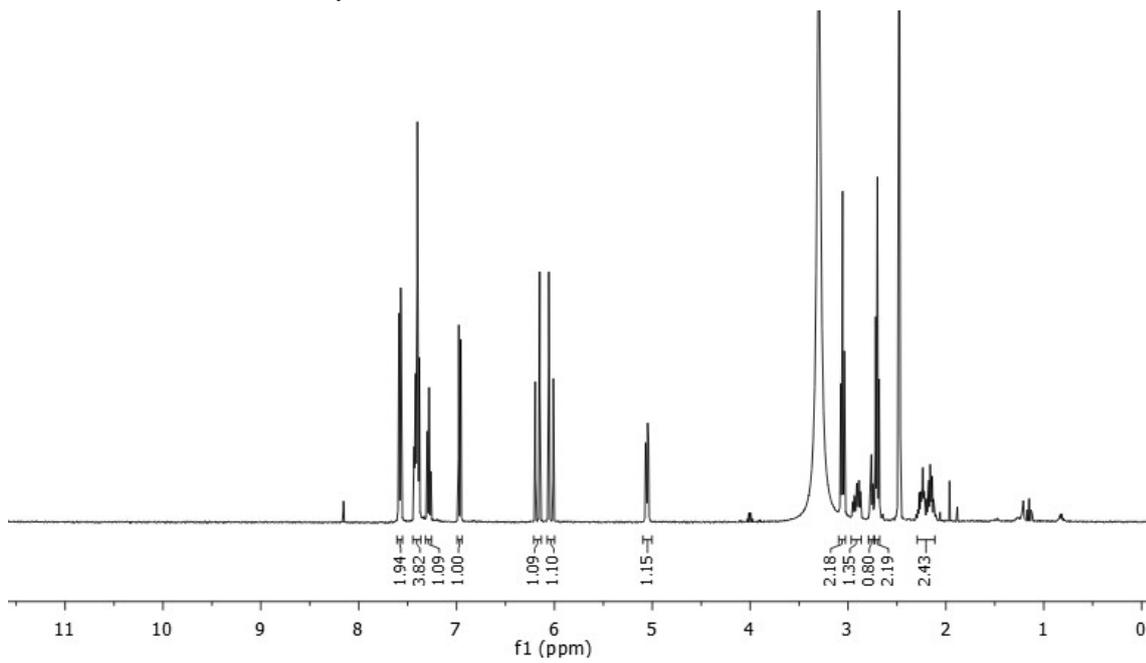
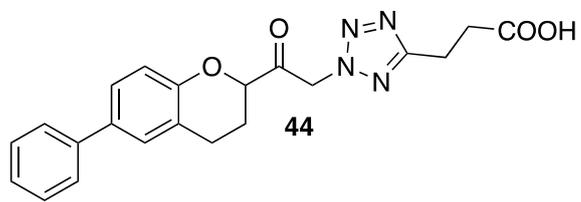
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          05.115 a1fa 20.000

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at 1.199 in n
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fb 13000 hs nm
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dl 1.000 lb 1.00
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ct 9000 DISPLAY -1332.3

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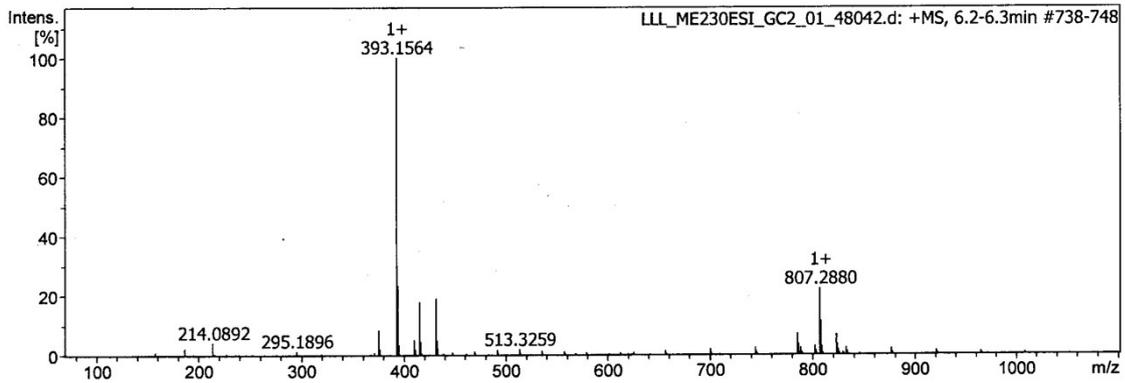
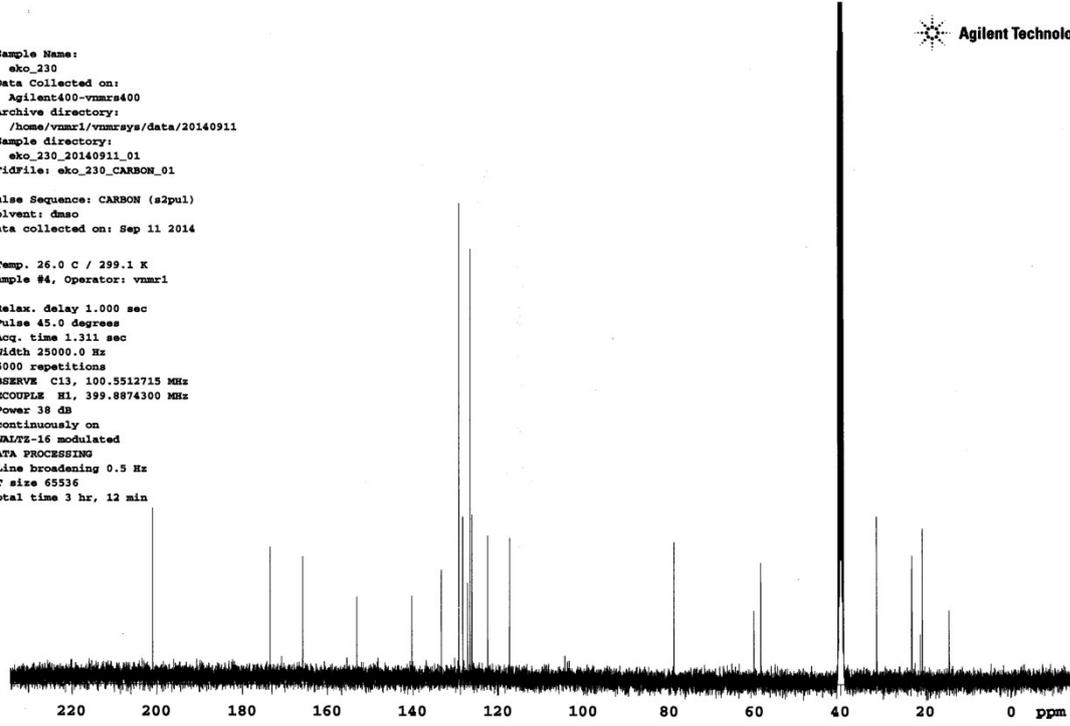


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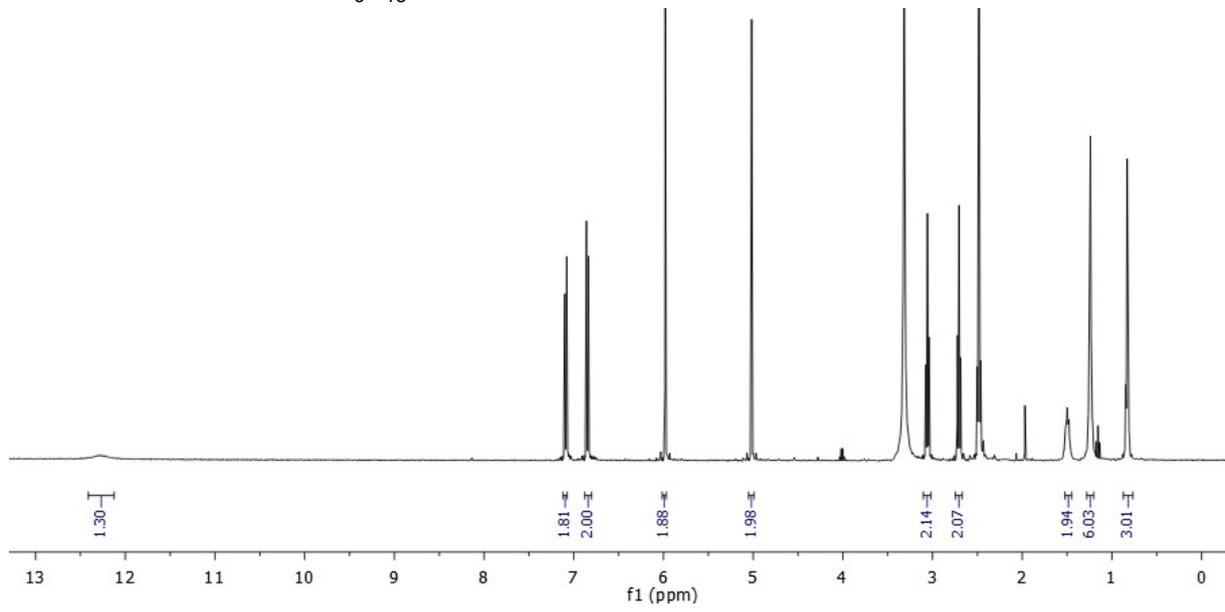
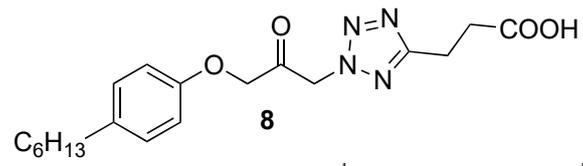
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Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
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Total time 3 hr, 12 min



3. ^1H and ^{13}C NMR spectra of the target compounds whose synthesis was already described in the patent applications DE102013016573 and WO2017093351

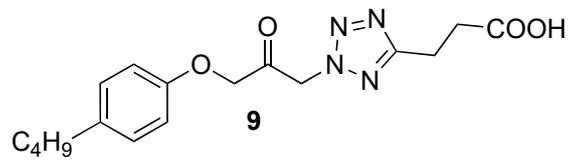
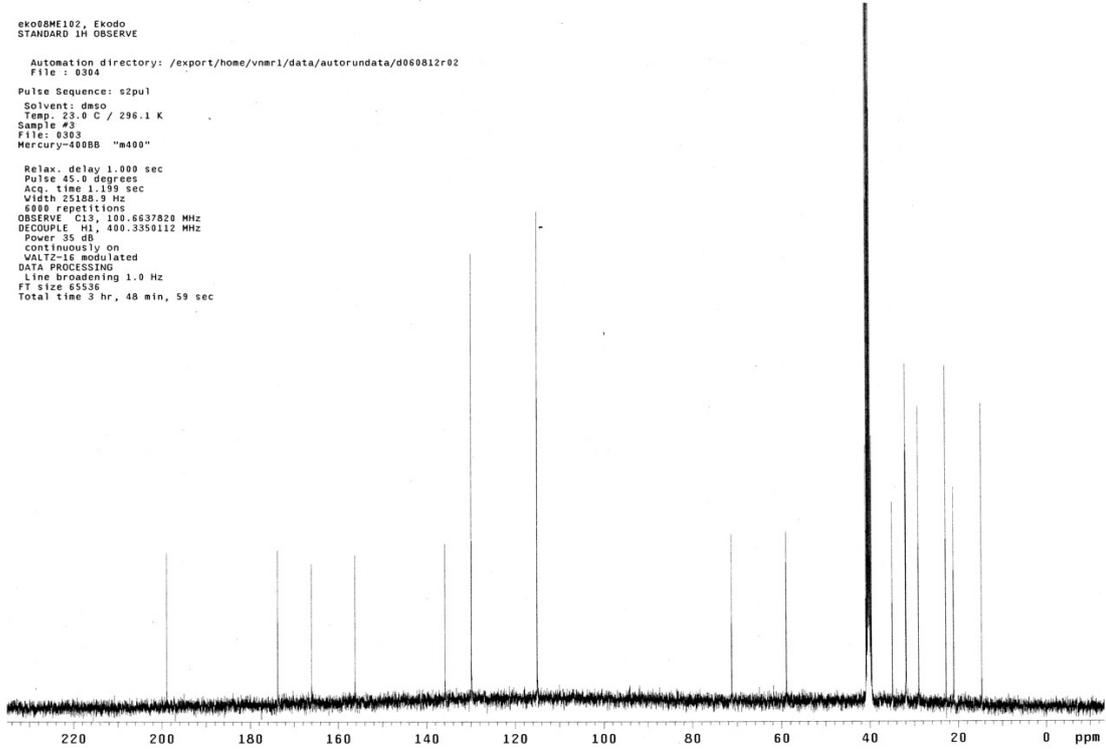


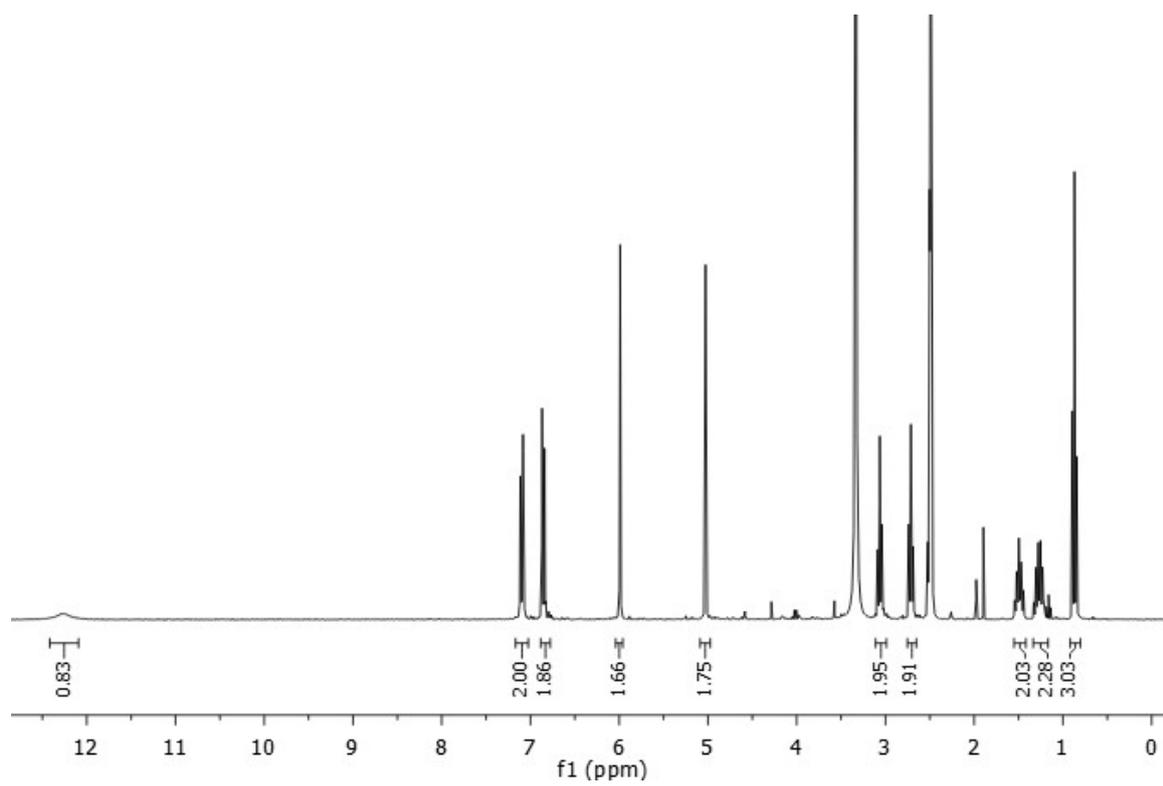
eko08ME102, Ekodo
STANDARD 1H OBSERVE

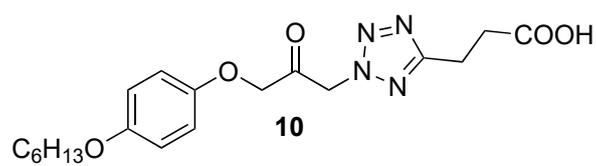
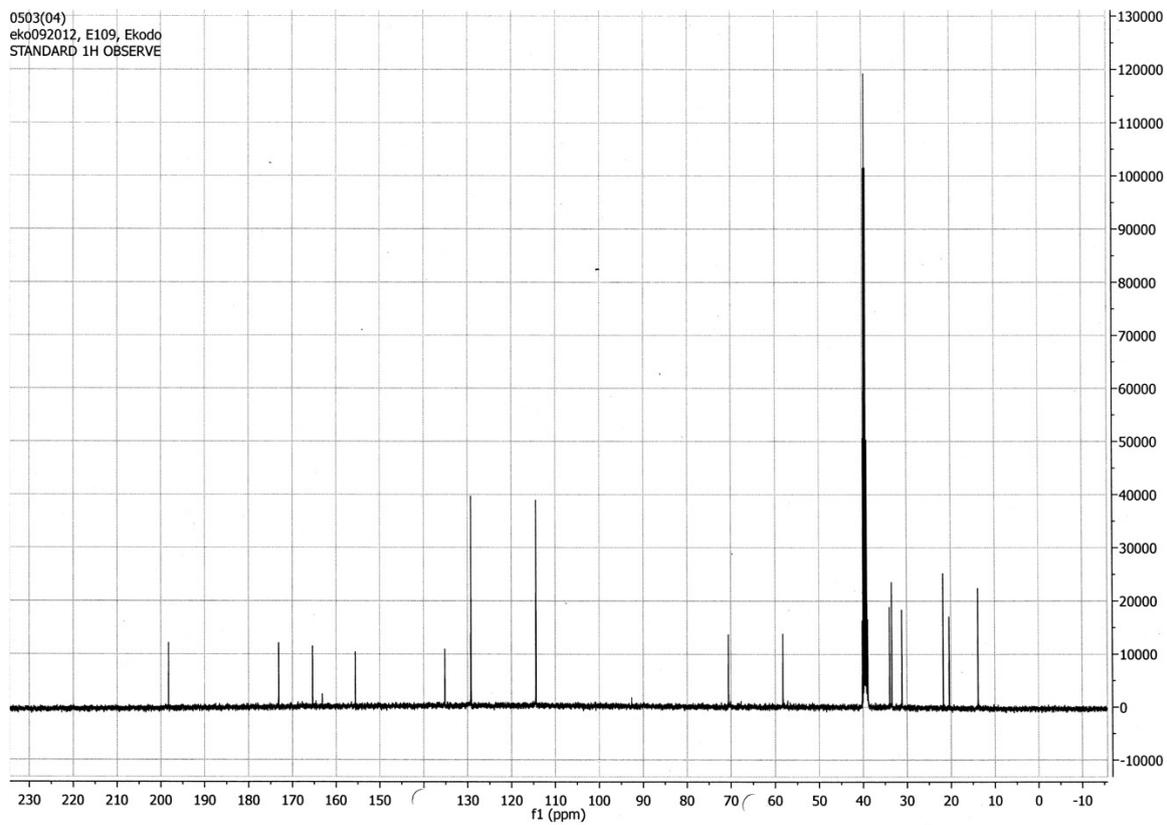
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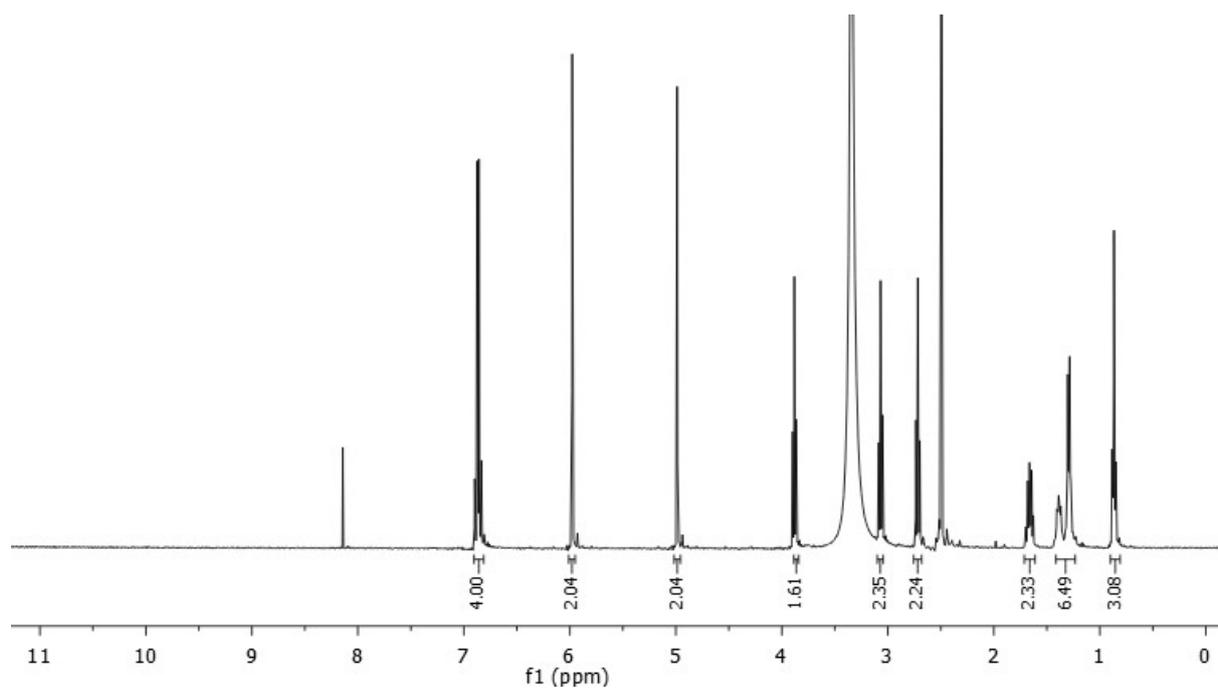
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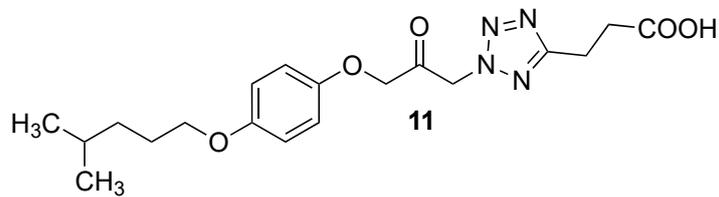
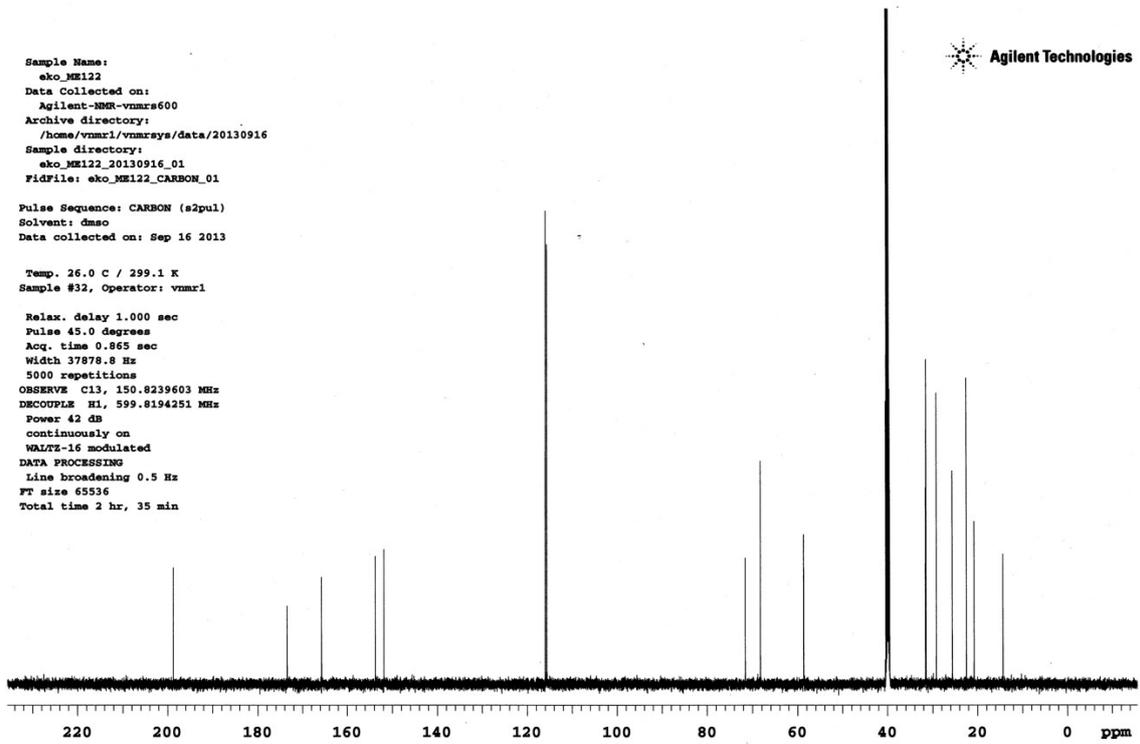
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DATA PROCESSING
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Total time 3 hr, 48 min, 59 sec

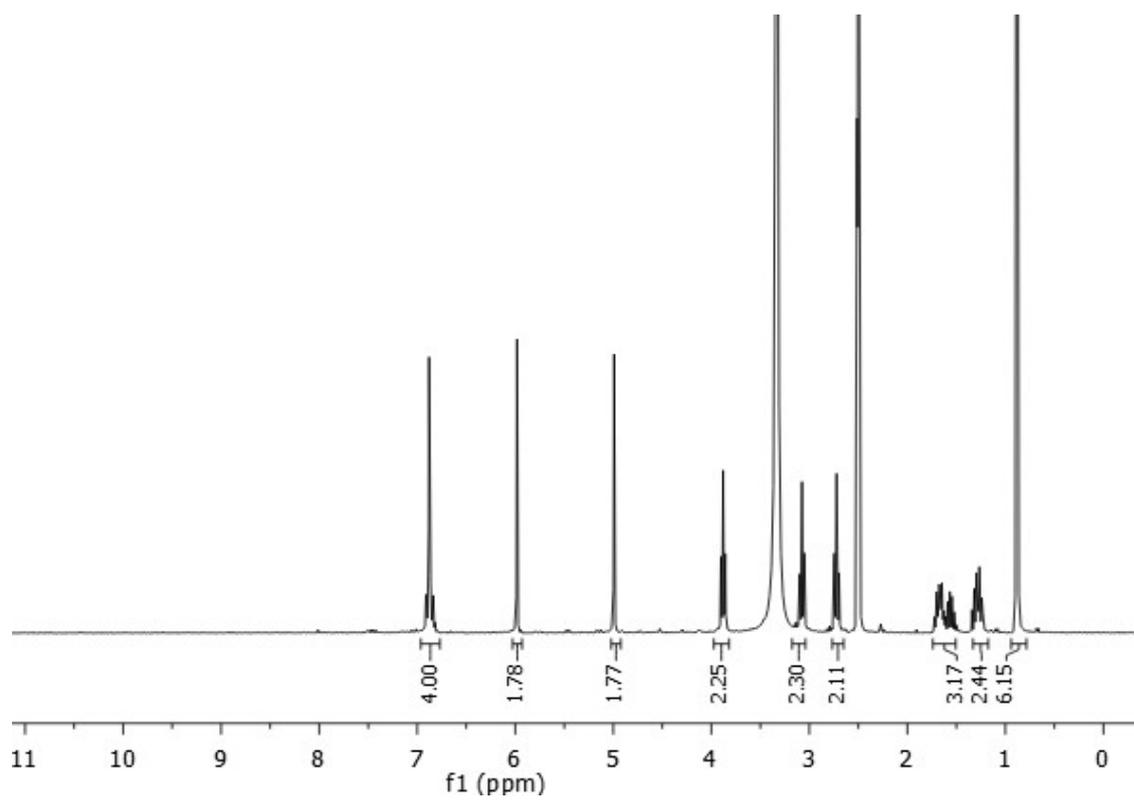










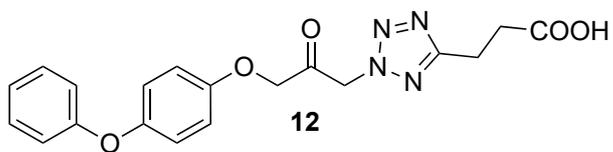
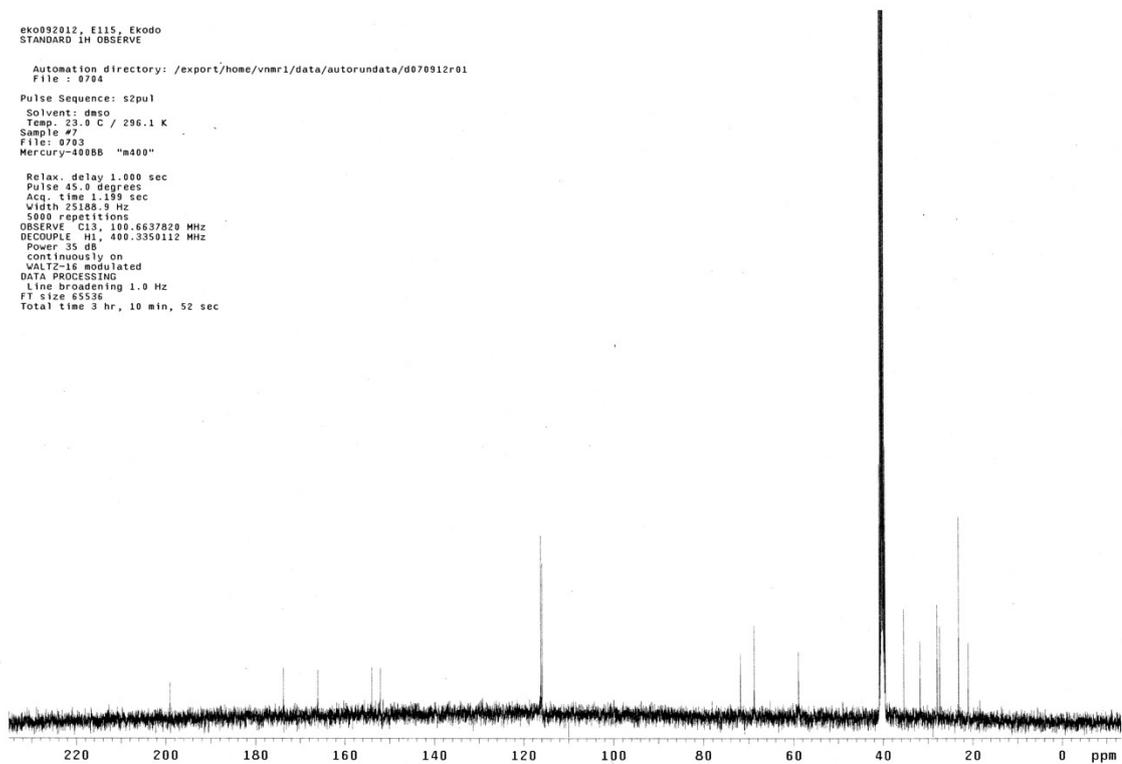


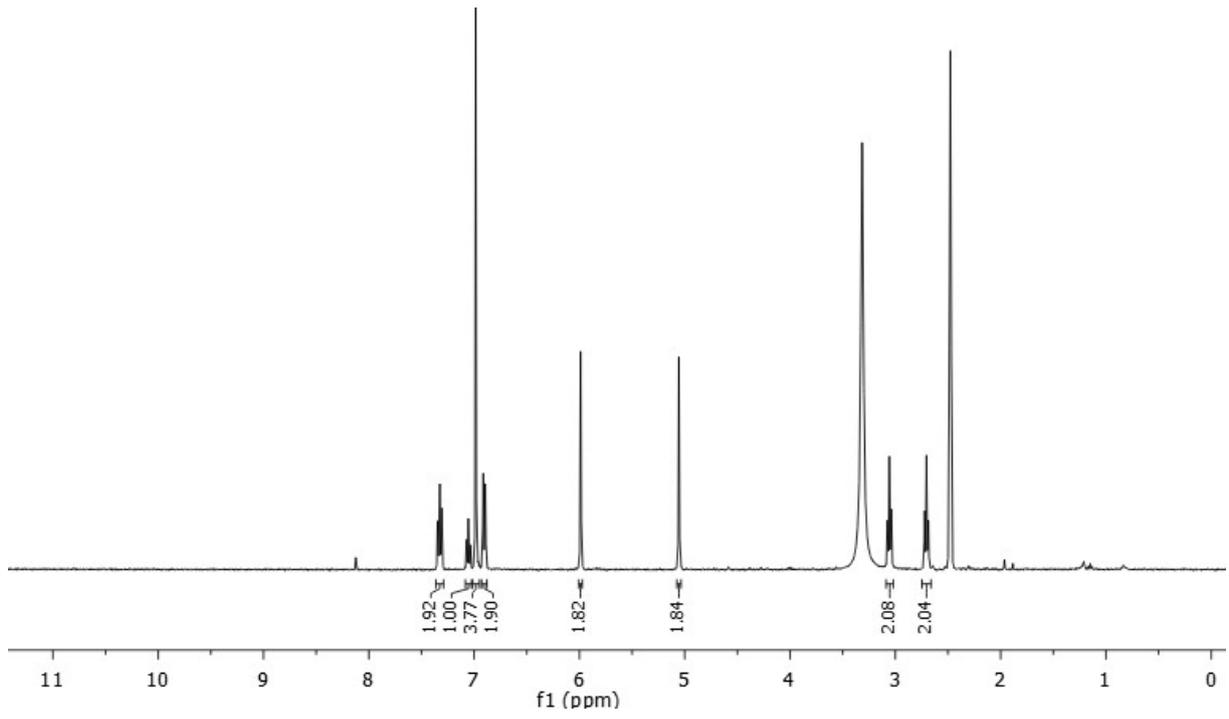
eko092012, F115, Ekodo
STANDARD 1H OBSERVE

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DECOUPLE H1, 400.3350112 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
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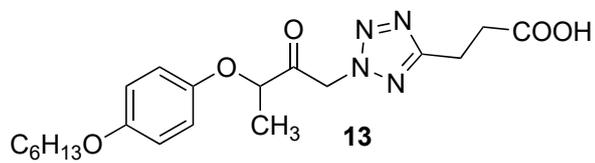
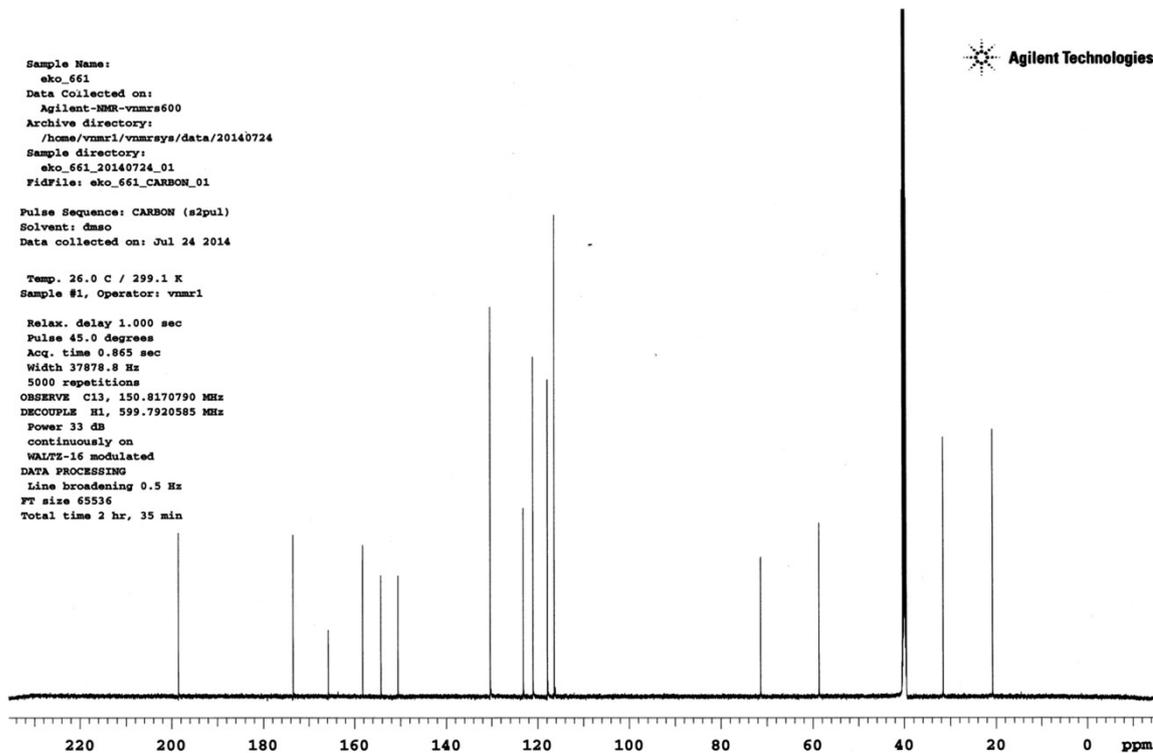


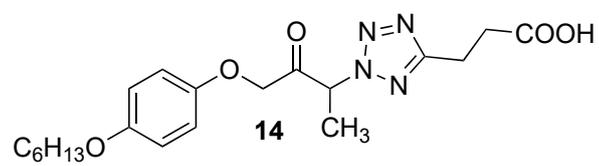
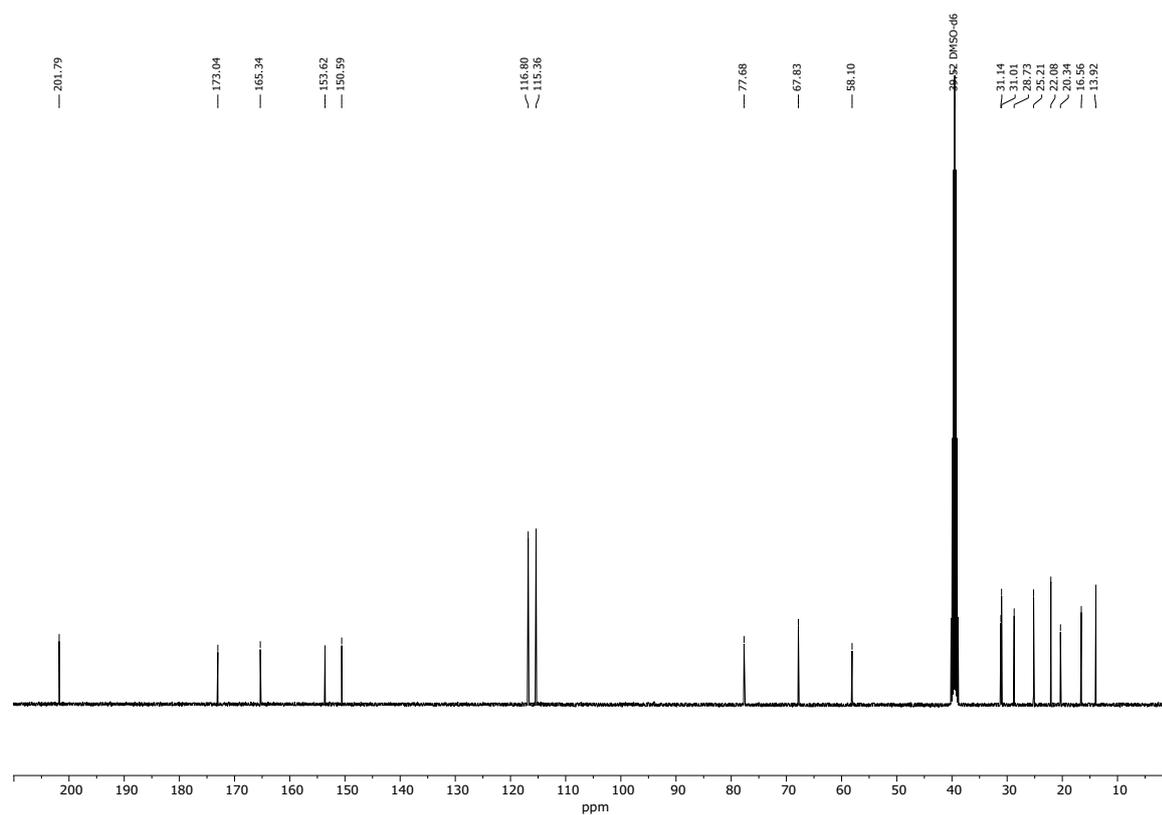
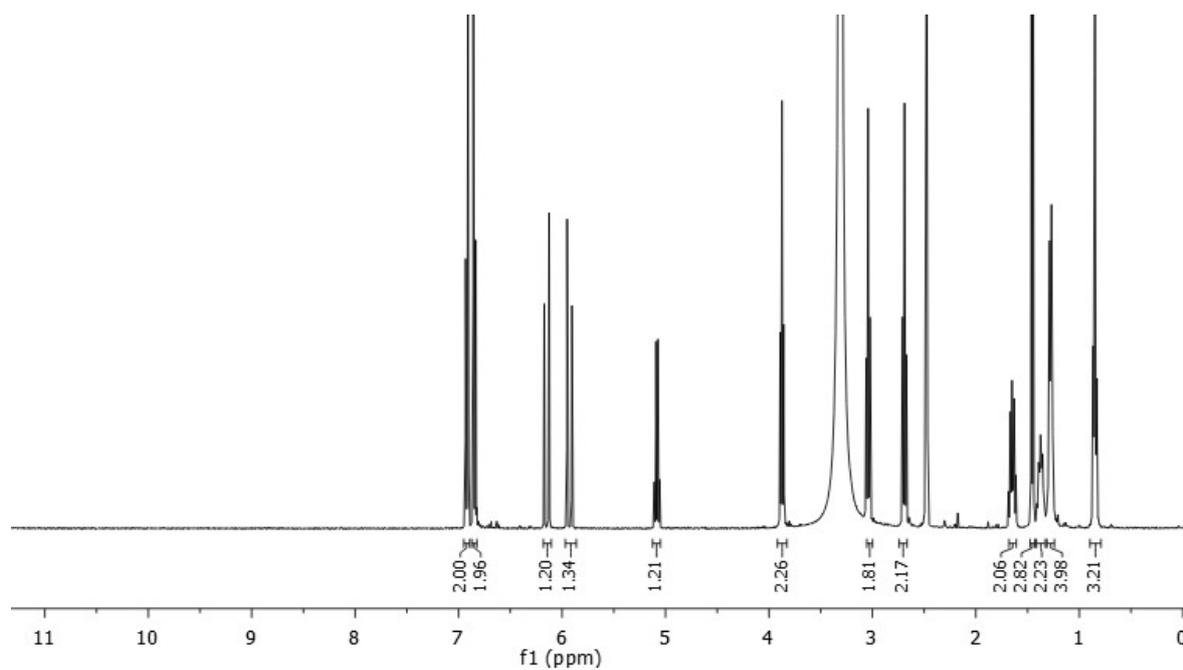
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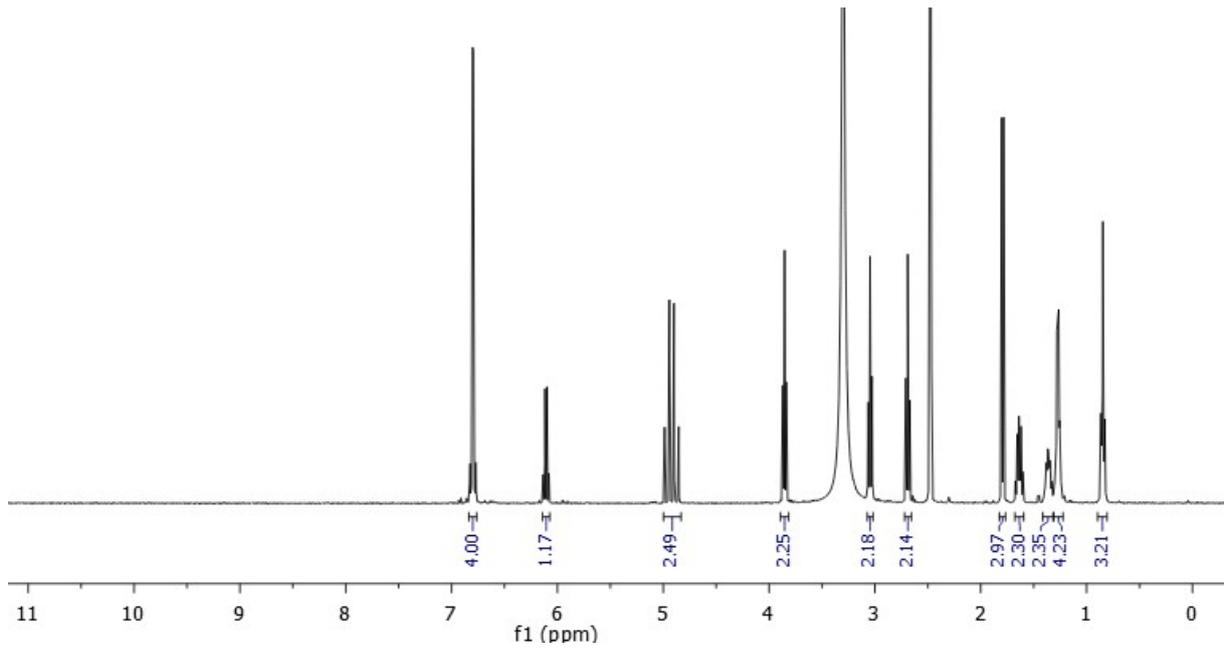
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DATA PROCESSING
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FT size 65536
Total time 2 hr, 35 min





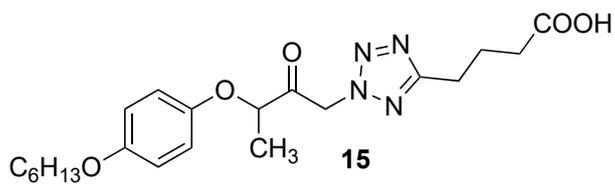
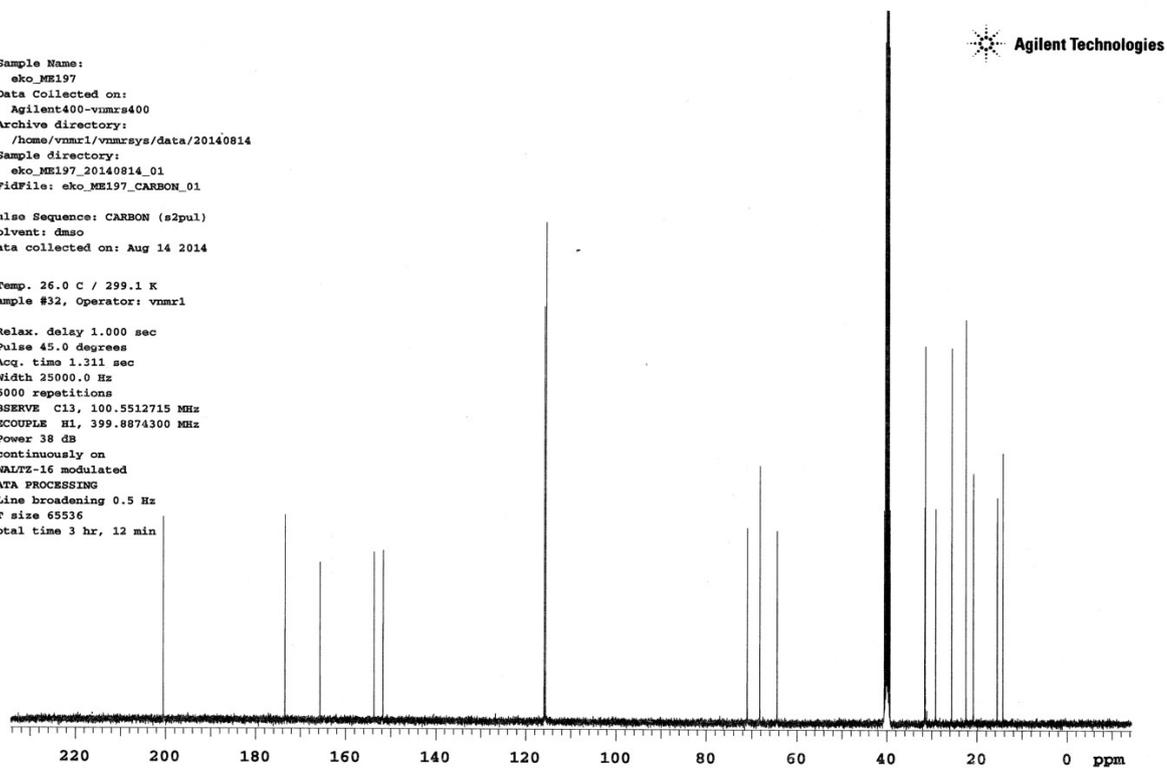


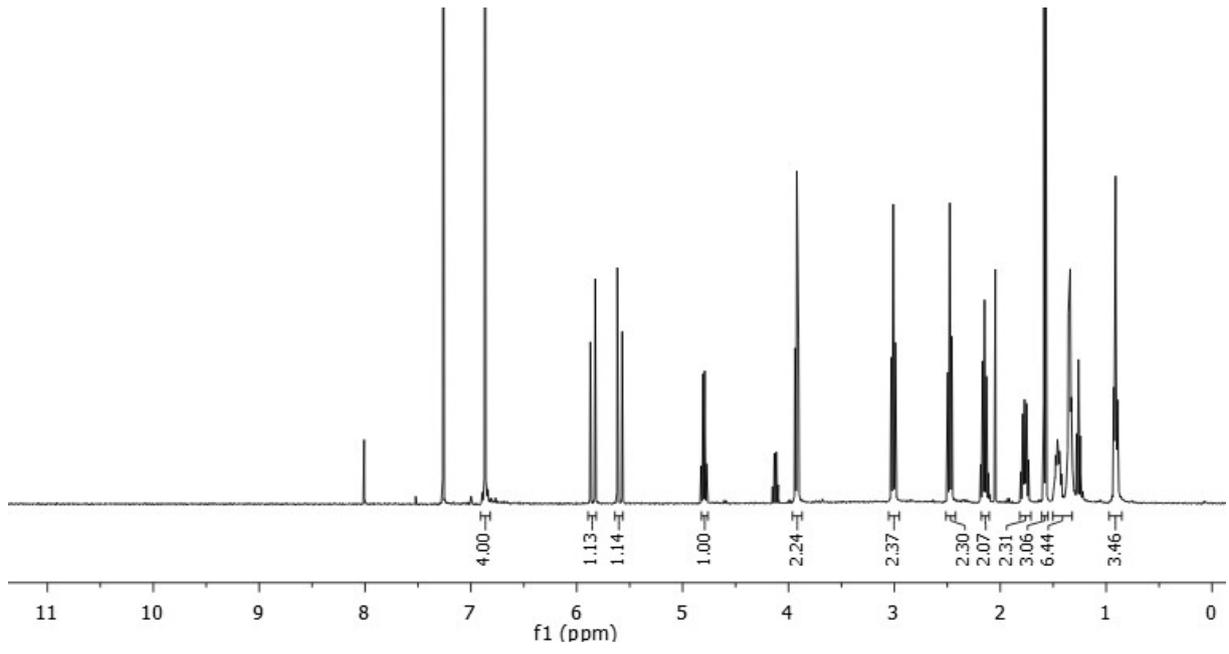
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Temp. 26.0 C / 299.1 K
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Line broadening 0.5 Hz
Ft size 65536
Total time 3 hr, 12 min

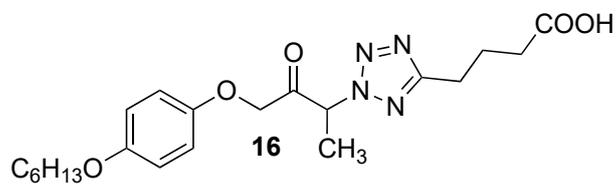
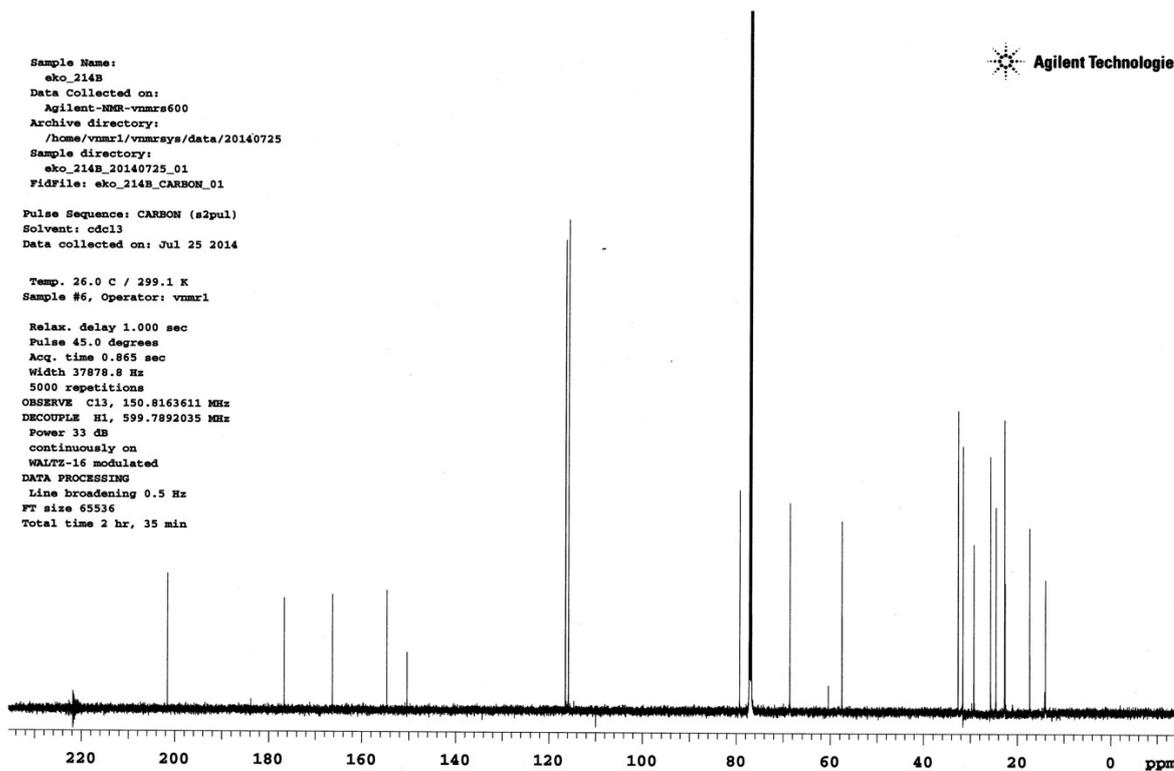


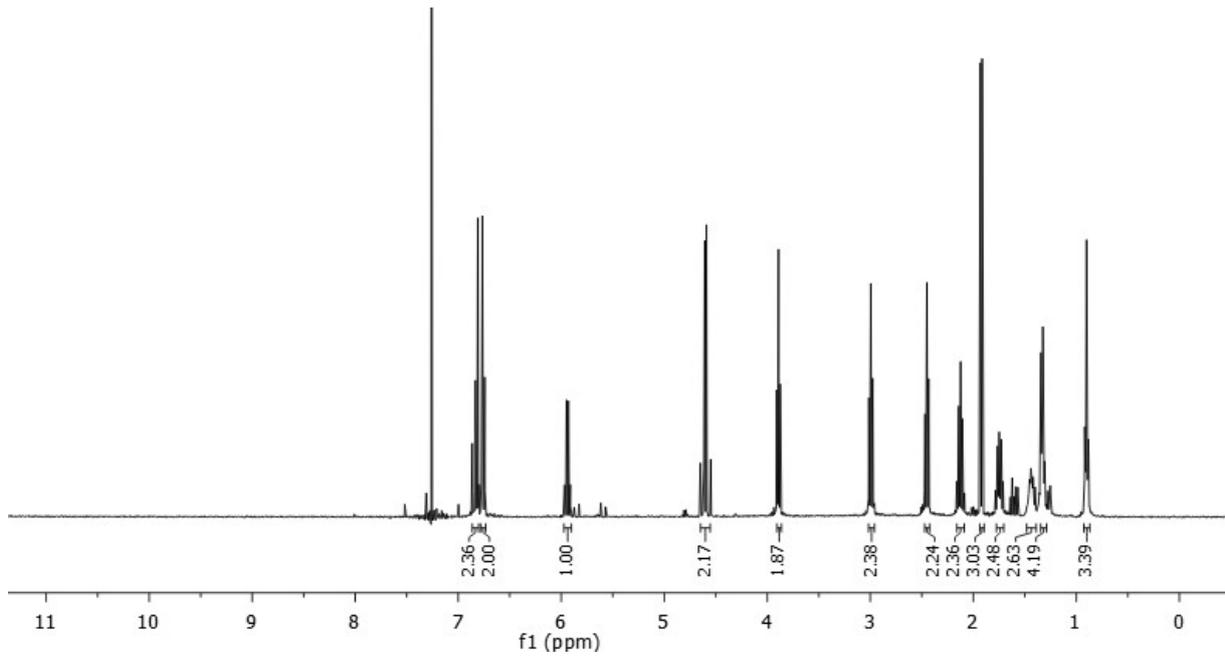


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Solvent: cdcl3
Data collected on: Jul 25 2014

Temp. 26.0 C / 299.1 K
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DATA PROCESSING
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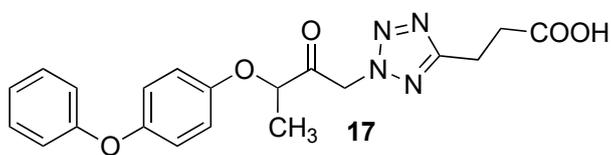
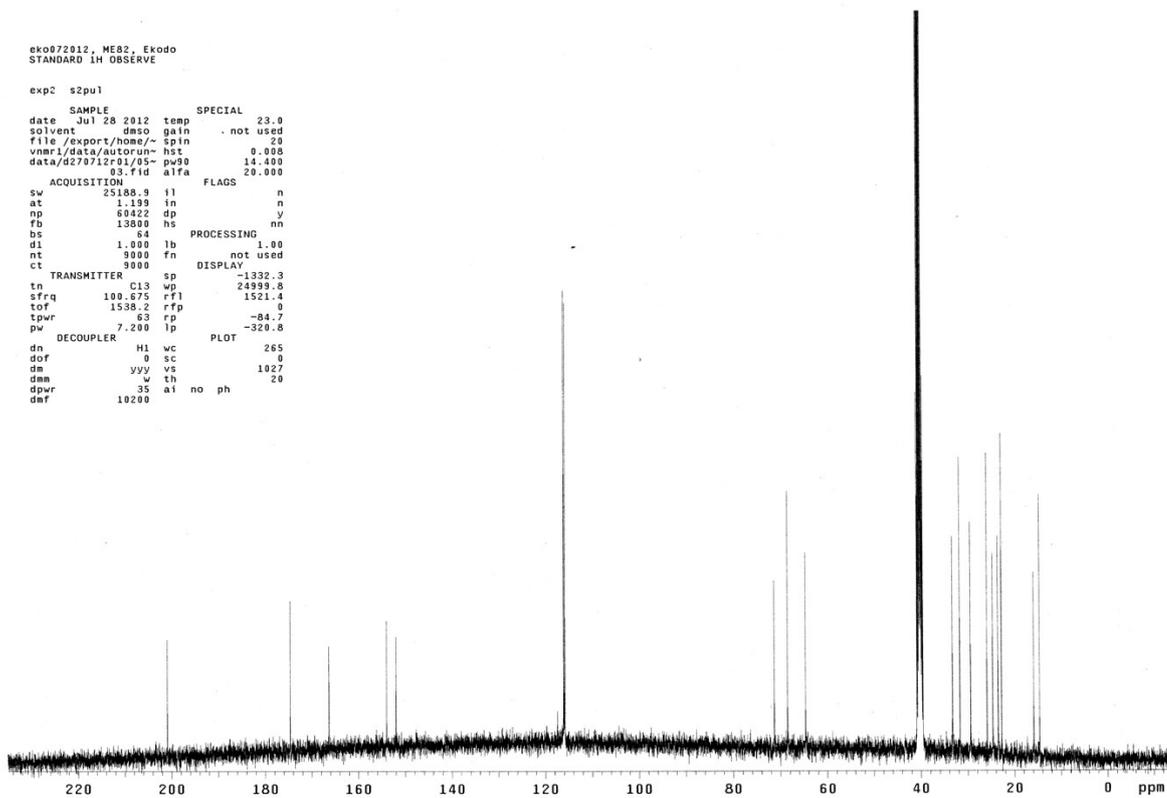


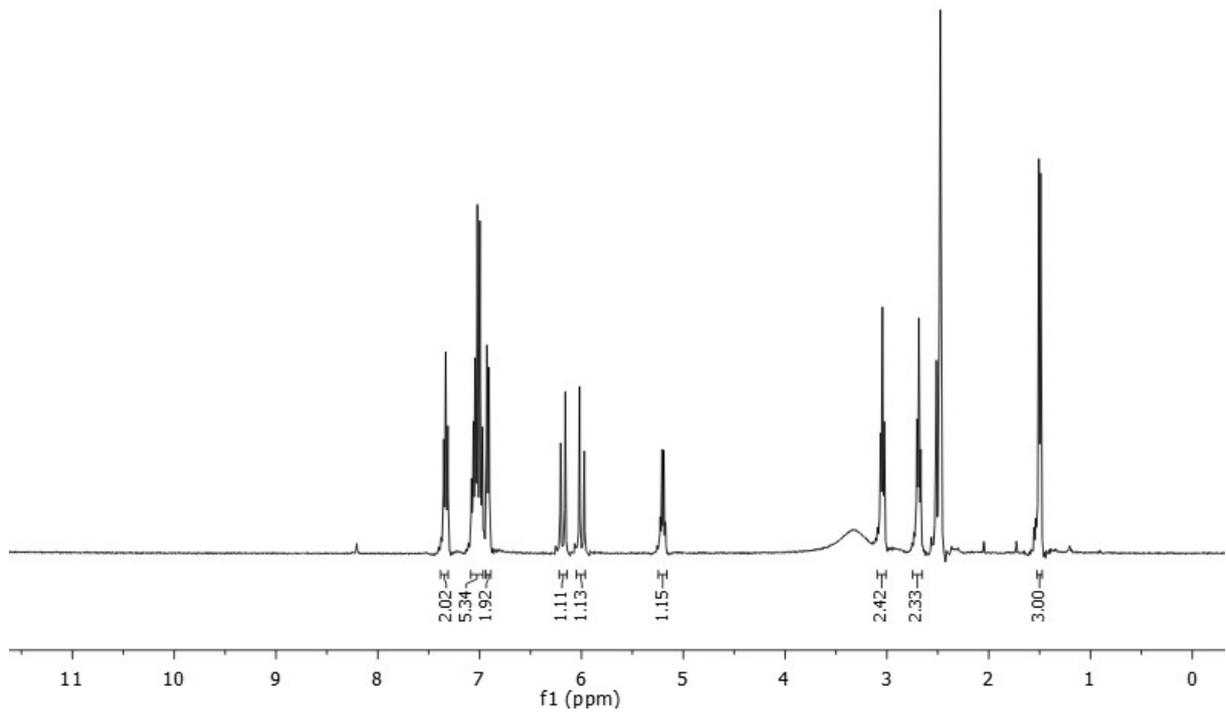


eko072012, ME82, Ekodo
STANDARD 1H OBSERVE

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at	1.199	in	n
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bs	64		
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tof	1538.2	rfp	1521.4
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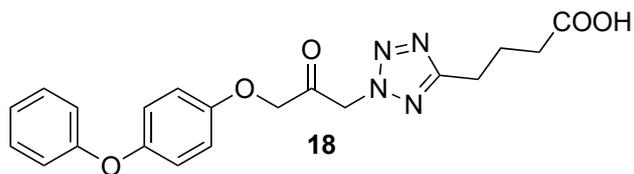
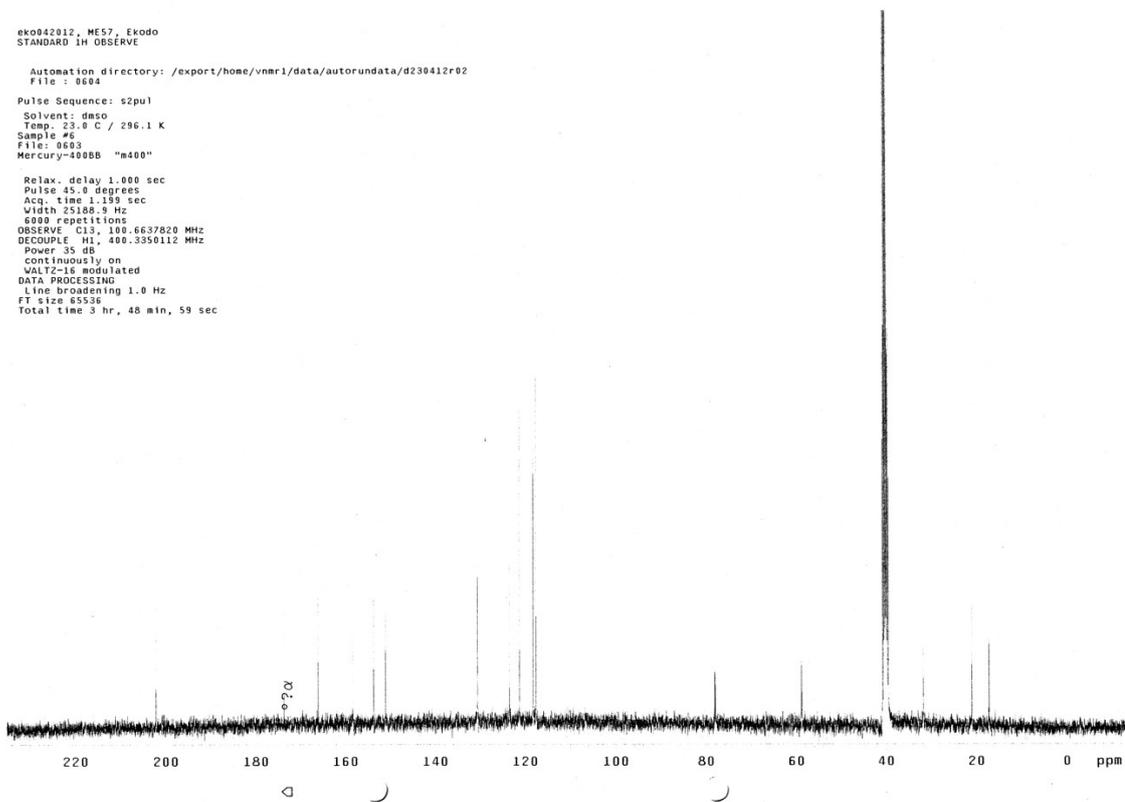


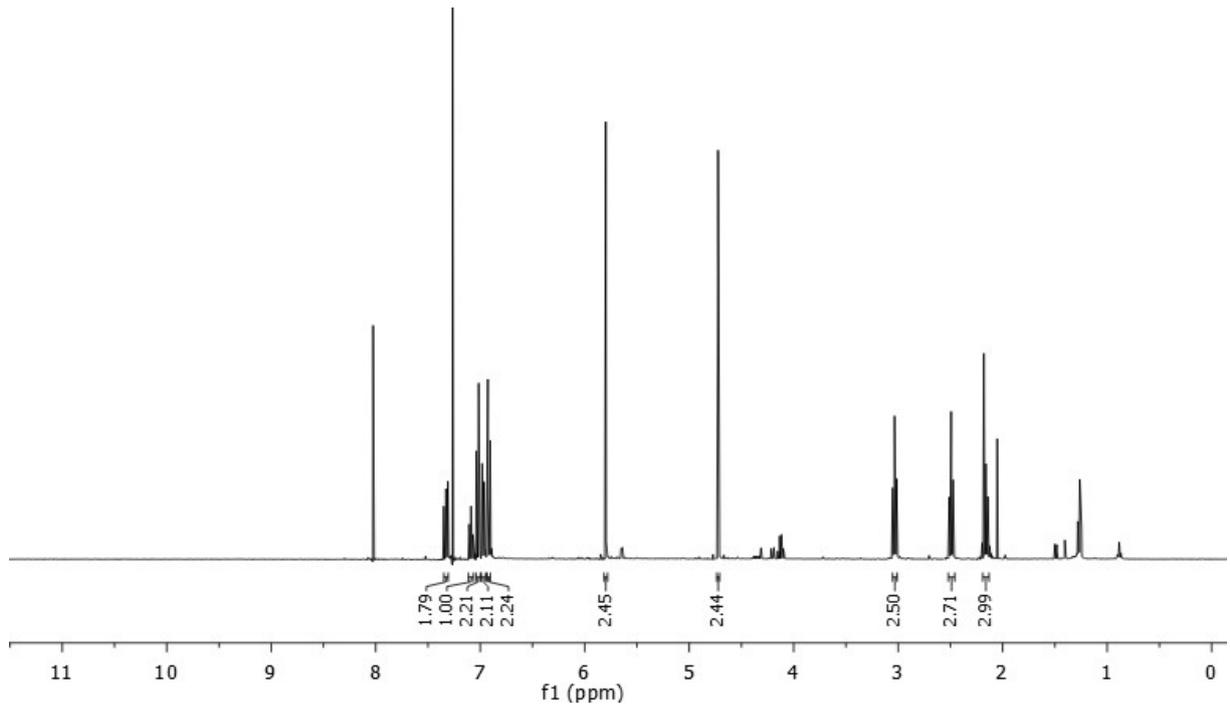
eko042012_ME57_Ekodo
STANDARD 1H OBSERVE

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Pulse Sequence: s2pul
Solvent: dms
Temp: 23.0 C / 296.1 K
Sample #6
File: 0603
Mercury-400BB "m400"

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DECOUPLE H1 400.3350112 MHz
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continuously on
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DATA PROCESSING
Line broadening 1.0 Hz
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Total time 3 hr, 48 min, 59 sec





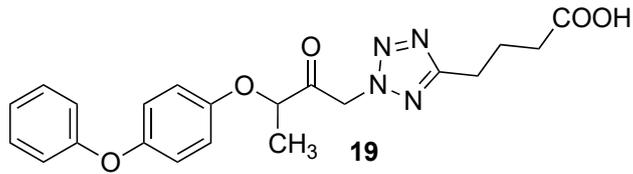
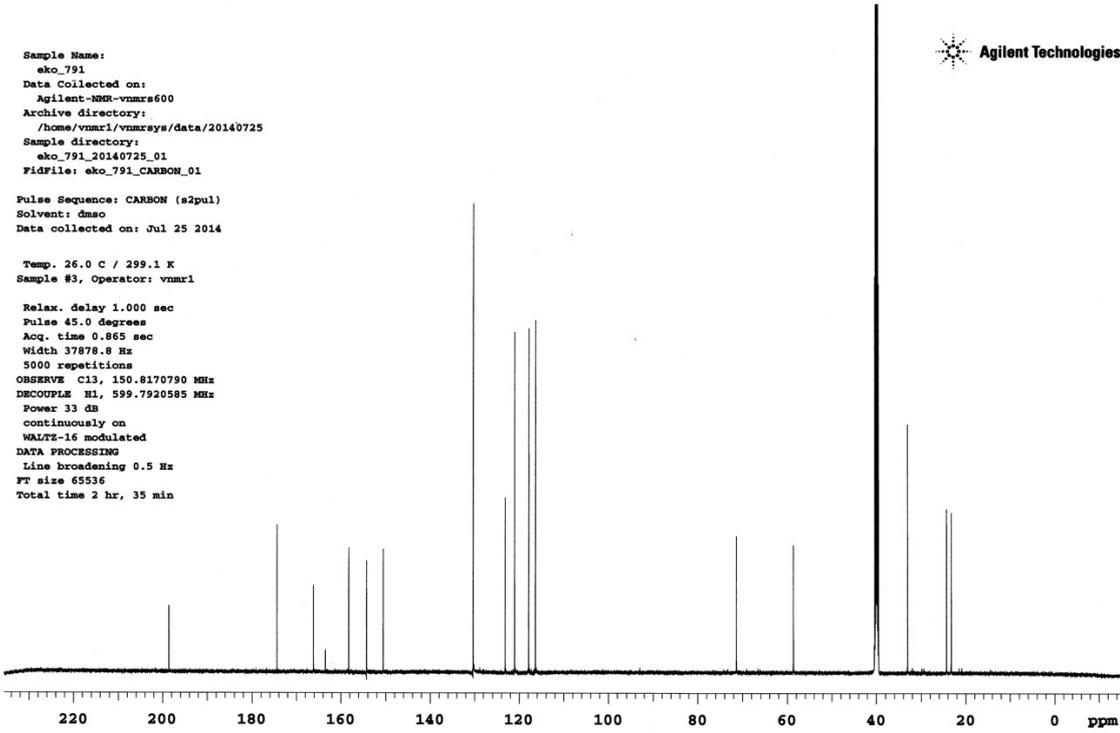
Sample Name:
eko_791
Data Collected on:
Agilent-MMR-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/data/20140725
Sample directory:
eko_791_20140725_01
FidFile: eko_791_CARBON_01

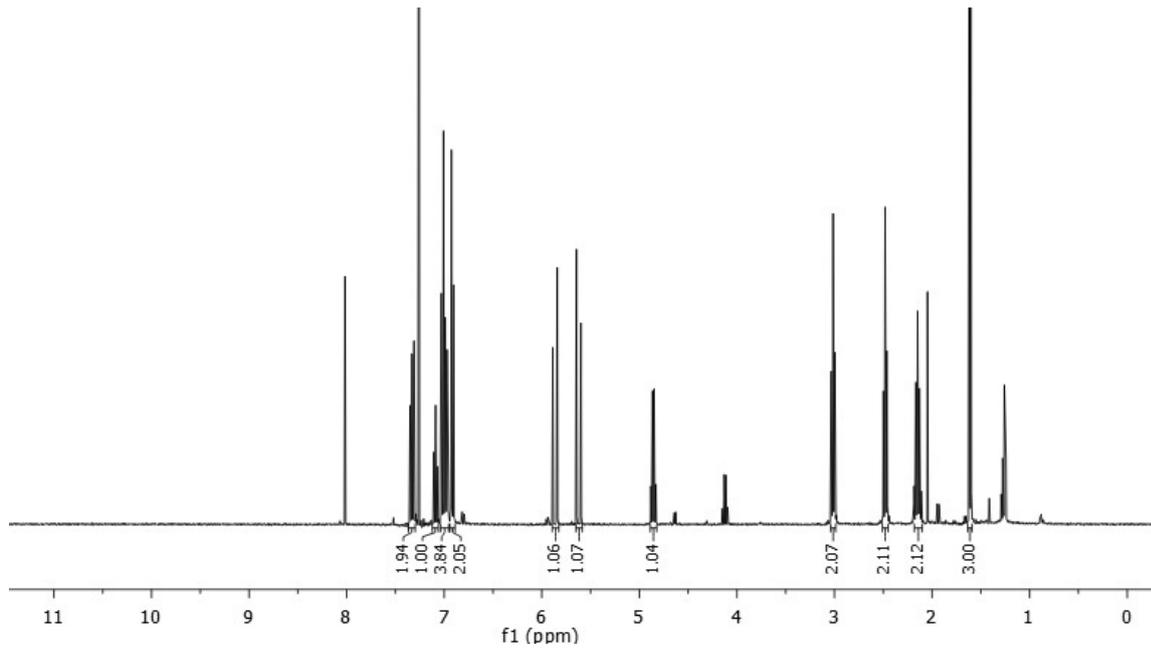
Pulse Sequence: CARRON (s2pul)
Solvent: dmsc
Data collected on: Jul 25 2014

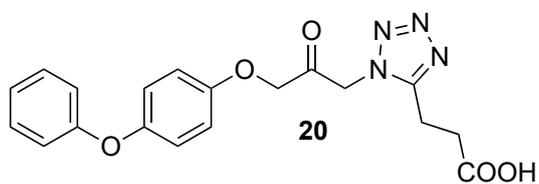
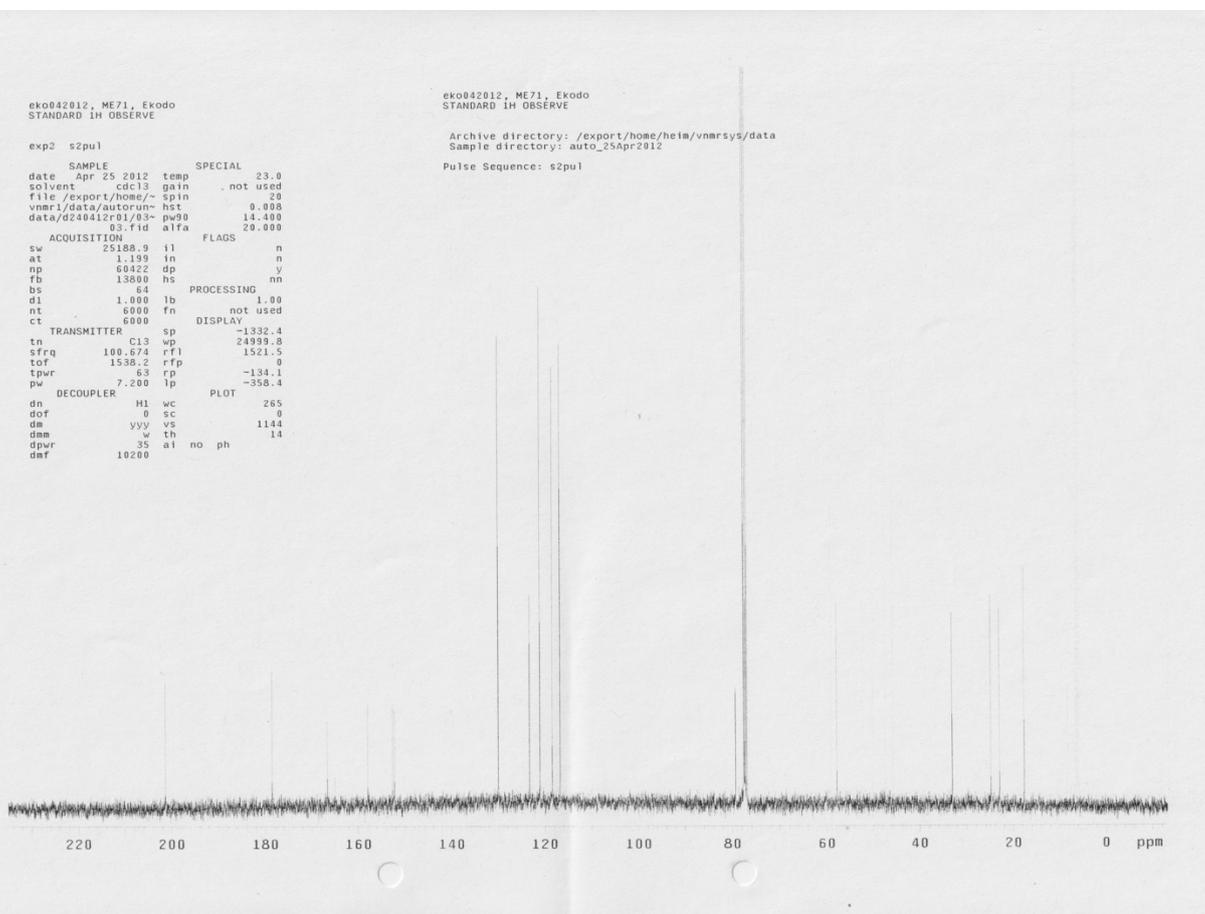
Temp. 26.0 C / 299.1 K
Sample #3, Operator: vnmr1

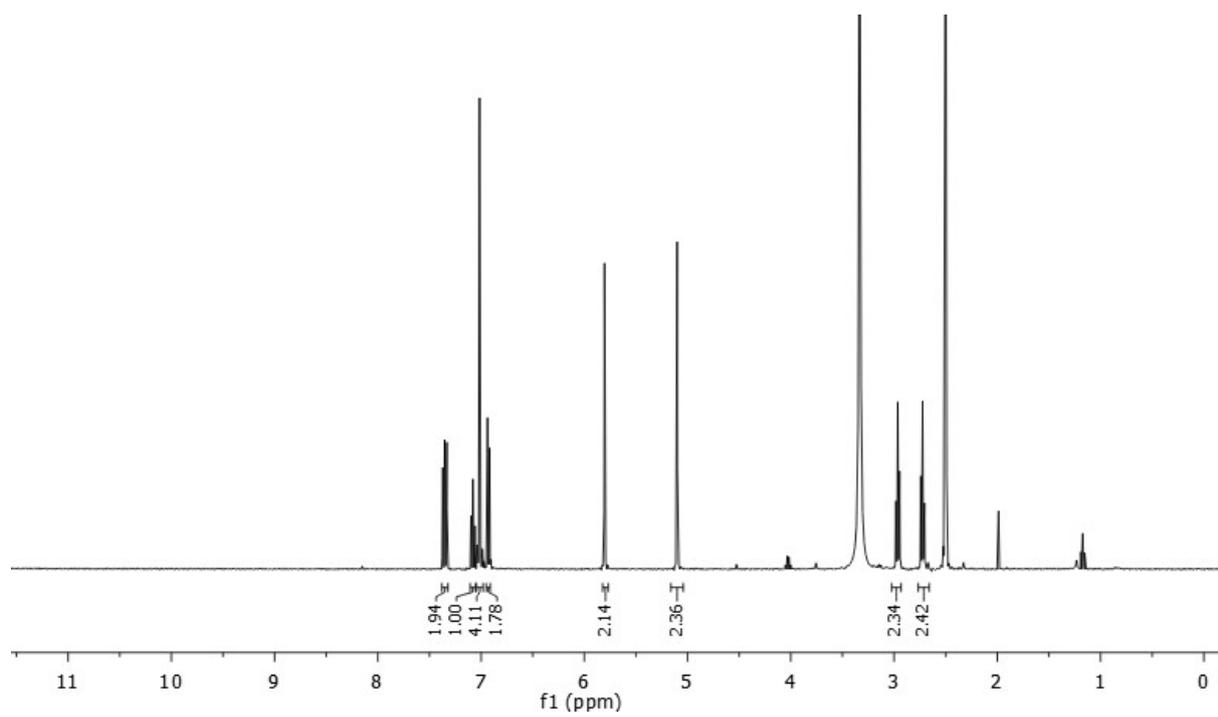
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.865 sec
Width 37876.8 Hz
5000 repetitions
OBSERVE C13, 150.8170790 MHz
DECOUPLE H1, 599.7920585 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 35 min

 Agilent Technologies







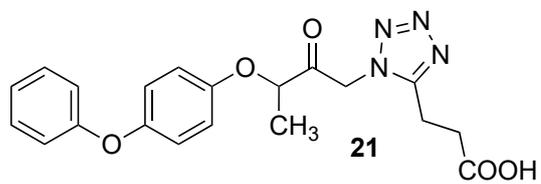
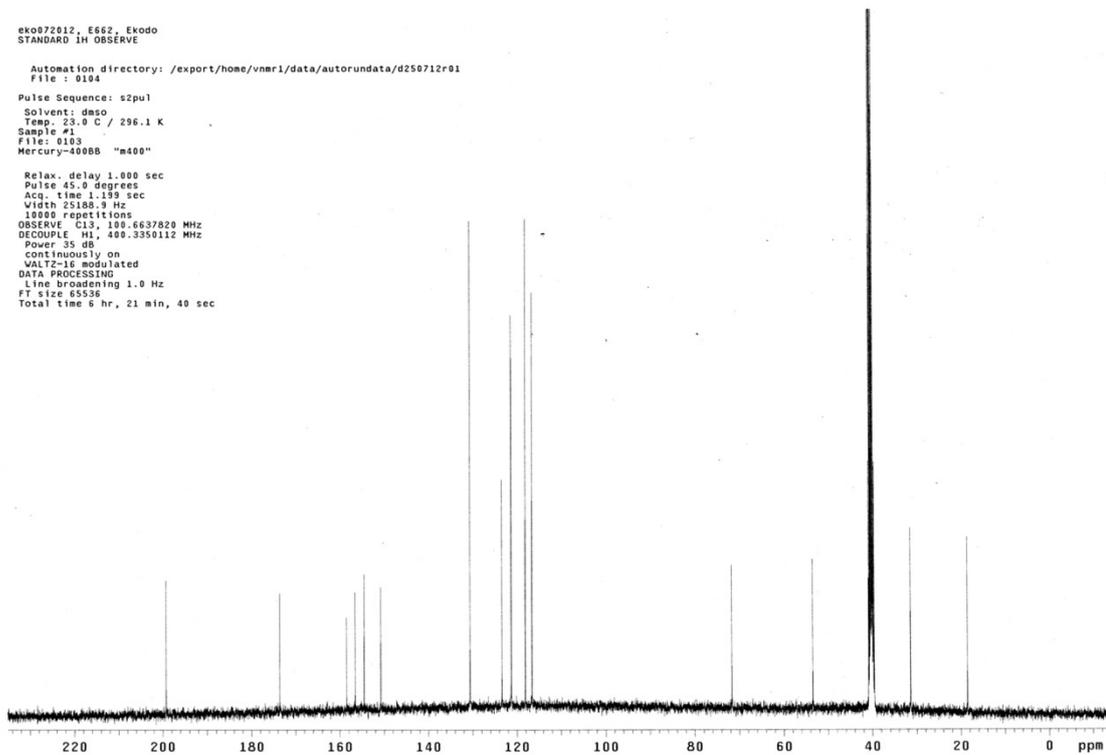


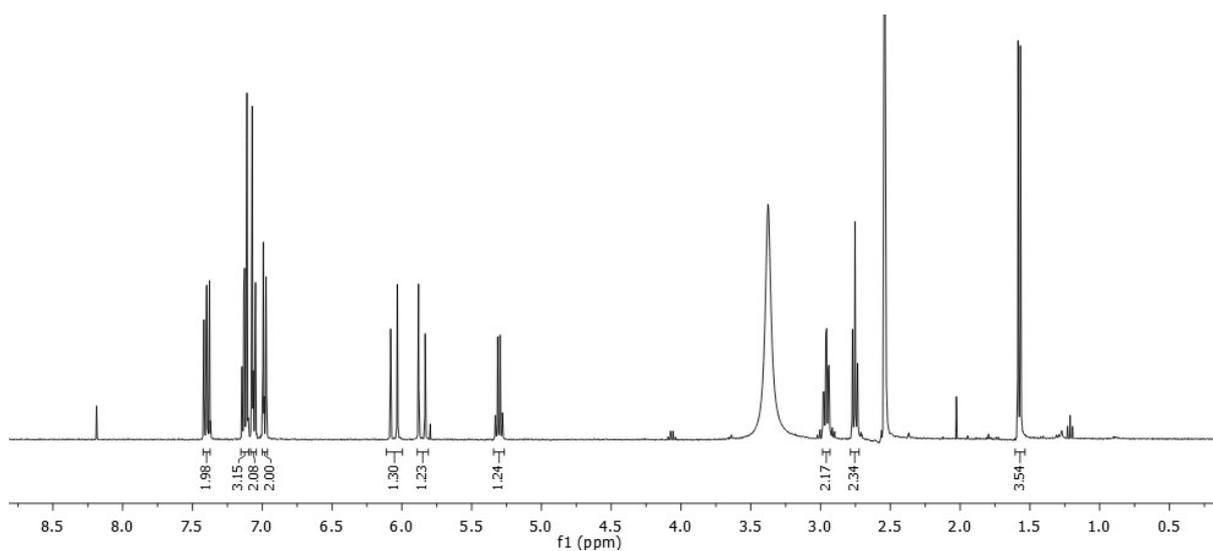
eko072012, E662, Ekodo
STANDARD 1H OBSERVE

Automation directory: /export/home/vnmr1/data/autorundata/d250712r01
File: 0194

Pulse Sequence: s2pu1
Solvent: dms0
Temp: 23.0 C / 296.1 K
Sample #1
File: 0103
Mercury-4000B "m400"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 25188.3 Hz
10000 repetitions
OBSERVE C13, 100.627820 MHz
DECOUPLE H1, 400.3350112 MHz
Power 35 db
continuously on
VOLTAGE modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 6 hr, 21 min, 40 sec



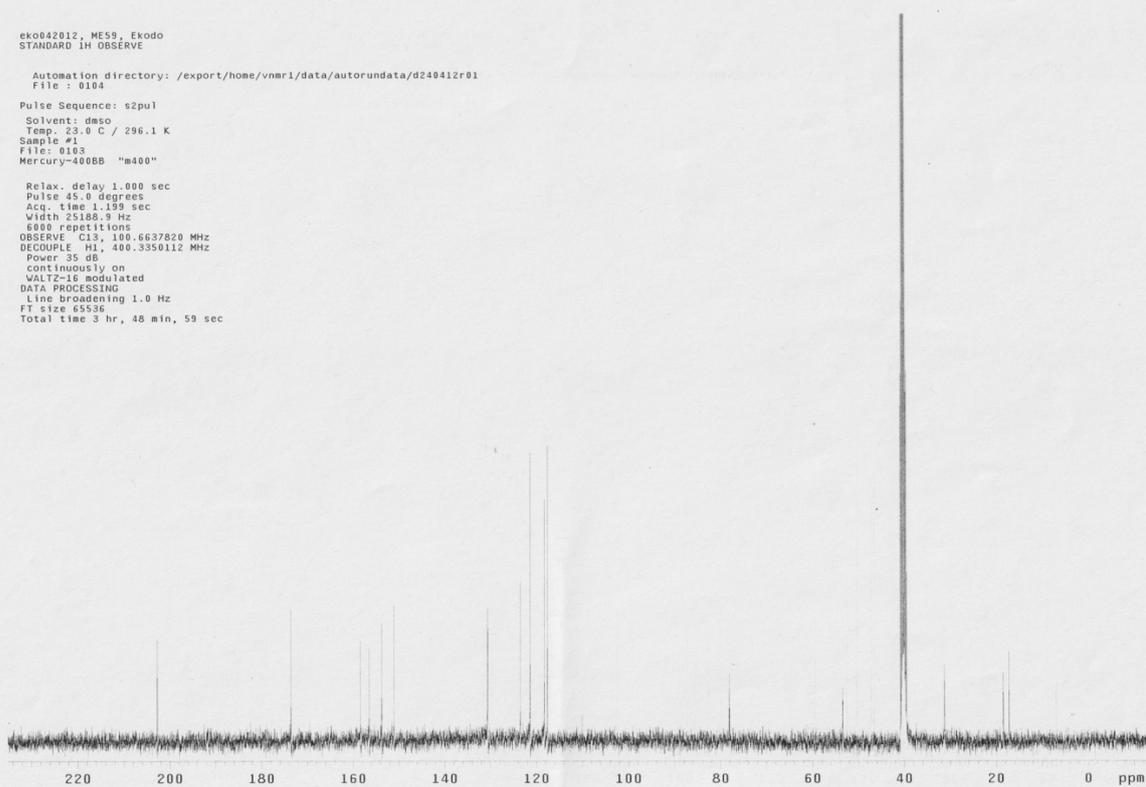


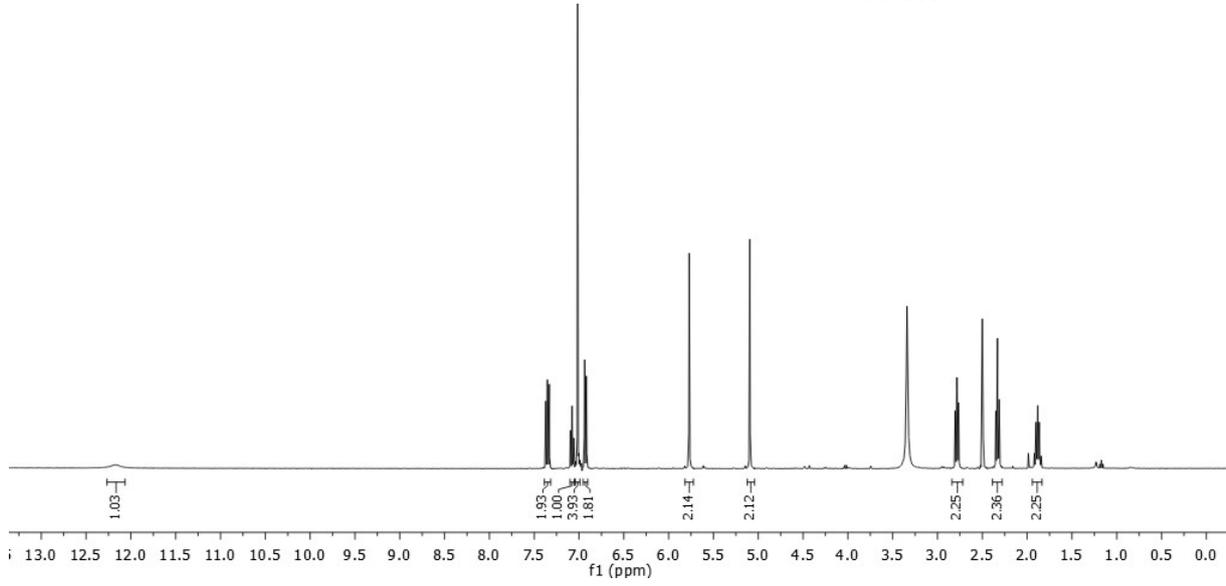
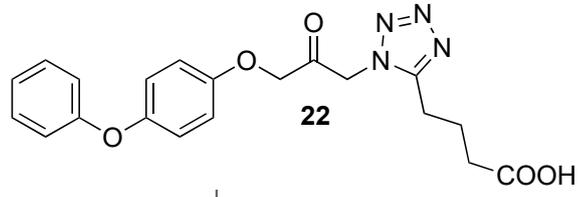
eko042012, ME59, Ekodo
STANDARD 1H OBSERVE

Automation directory: /export/home/vnmr1/data/autorundata/d240412r01
File: 0104

Pulse Sequence: s2pul
Solvent: dms0
Temp: 23.0 C / 296.1 K
Sample #1
File: 0103
Mercury-400BB "m400"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 23168.8 Hz
6000 repetitions
OBSERVE C13, 100.6637820 MHz
DECUPLE H1, 400.3350112 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 3 hr, 48 min, 59 sec



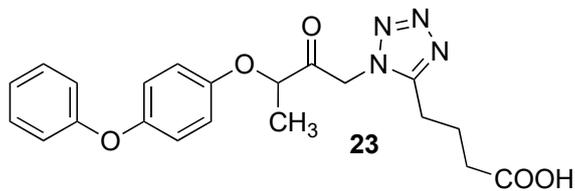
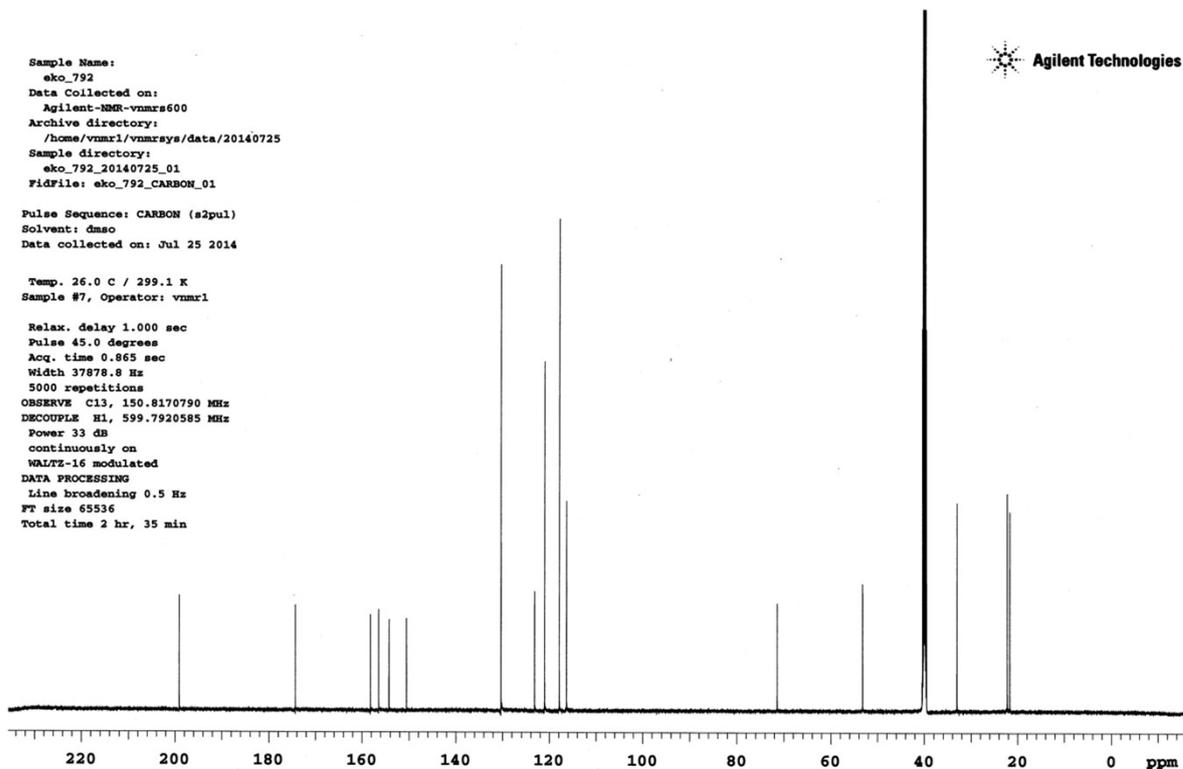


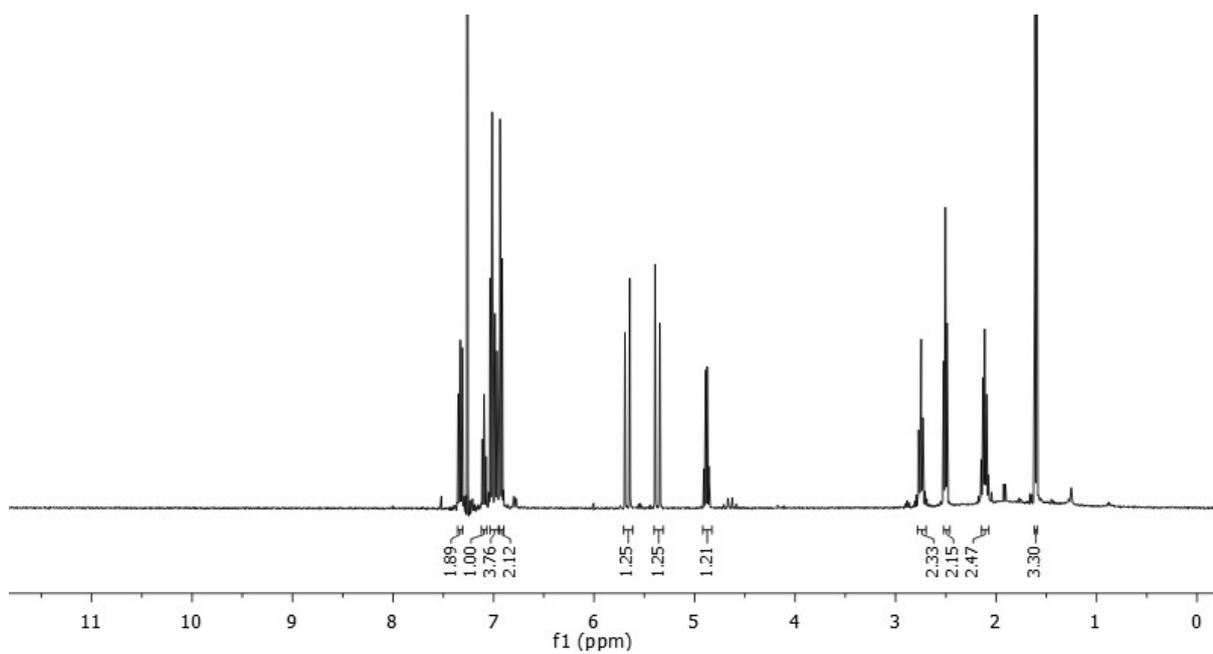
Sample Name:
eko_792
Data Collected on:
Agilent-MMR-vnmrs600
Archive directory:
/home/vnmr1/vnmrsys/data/20140725
Sample directory:
eko_792_20140725_01
Fidfile: eko_792_CARBON_01

Pulse Sequence: CARBON (s2pul)
Solvent: dmsc
Data collected on: Jul 25 2014

Temp. 26.0 C / 299.1 K
Sample #7, Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.865 sec
Width 37878.8 Hz
5000 repetitions
OBSERVE C13, 150.8170790 MHz
DECOUPLE H1, 599.7920585 MHz
Power 33 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 35 min



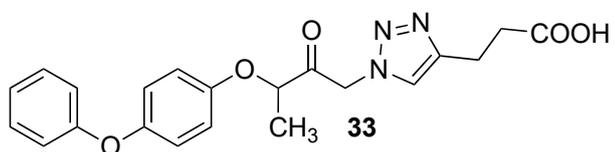
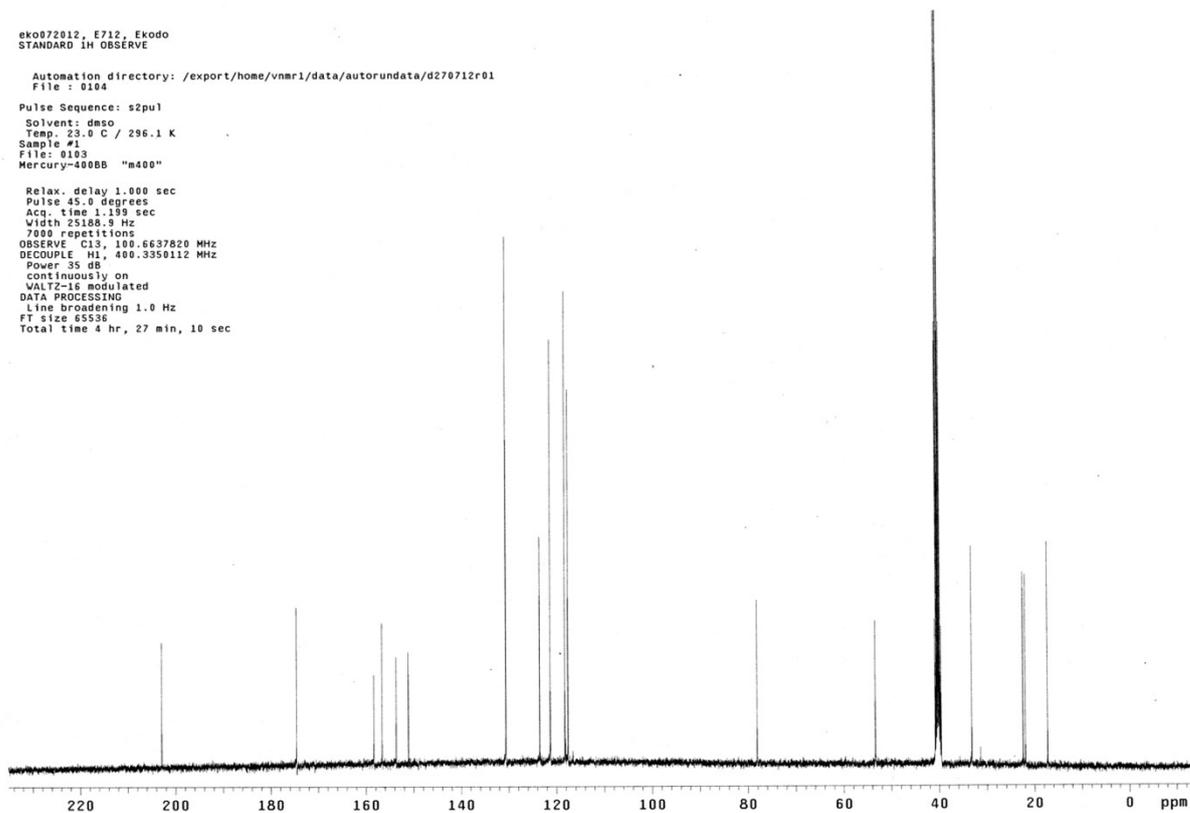


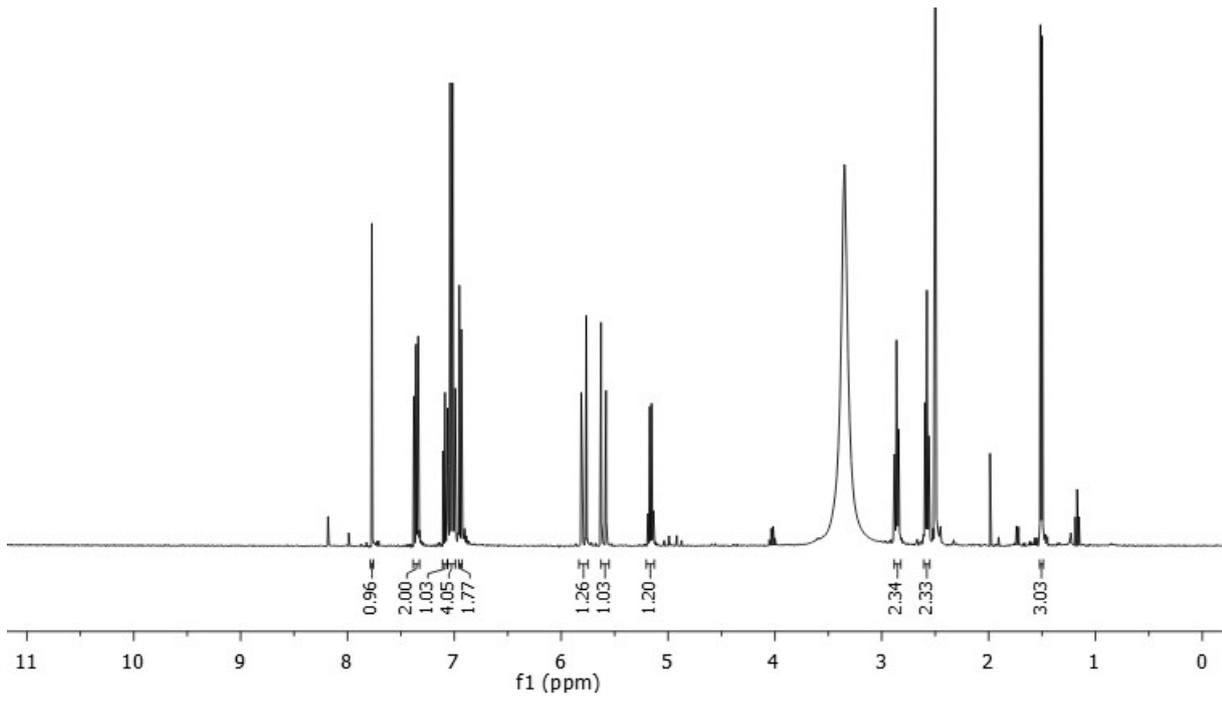
eko072012_E712_Ekodo
STANDARD IH OBSERVE

Automation directory: /export/home/vnmr1/data/autorundata/d270712r01
File: 0104

Pulse Sequence: s2pu1
Solvent: dmsd
Temp: 23.0 C / 296.1 K
Sample #1
File: 0103
Mercury-400BB "m400"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.199 sec
Width 23185.8 Hz
7000 repetitions
OBSERVE C13, 100.627820 MHz
DECOUPLE H1, 400.3350112 MHz
Power 35 dB
continuously on
VATZ-is modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 4 hr, 27 min, 10 sec





sk08ME93_Ekodo
STANDARD 1H OBSERVE

Automation directory: /export/home/vnmr1/data/autorundata/d060812r02
File : 0204

Pulse Sequence: s2pul
Solvent: dms0
Temp: 23.0 C / 296.1 K
Sample #2
File: 0203
Mercury-400BB "m400"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.139 sec
Width 25188.9 Hz
6000 repetitions
OBSERVE C13, 100.6237820 MHz
DECUPLE H1, 400.3350112 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 3 hr, 48 min, 59 sec

