Supporting Information

Pharmacophore Guided Discovery of Small Molecule Human Apurinic/ Apyrimidinic Endonuclease 1 Inhibitors

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Supporting Information: **Optimization of Assay Conditions**

To determine optimal assay conditions for the evaluation of compounds against APE1 catalysis, we examined the activity of our purified APE1 protein in a variety of experimental conditions, including protein concentration, incubation time and temperature. Observing a variation in the concentration of APE1 used in inhibitor screening assays in prior studies reporting APE1 inhibitors, we sought to define a non-arbitrary concentration for our assays by performing dose response experiments at different protein concentrations. As observed in Figure S1, the IC_{50} value of compound **10** varies proportionally with enzyme concentration. Thus, we have reported all of our inhibitor IC_{50} values as assayed at an APE1 concentration of 0.5 nM, at which there is complete enzymatic cleavage of the 25-mer abasic substrate to cleaved product without subsequent APE1-mediated exonuclease activity on the cleaved product. Substrate concentration is the approximate K_m for the AP lesion[7]. Concentrations lower than 0.5 nM show low basal abasic site incision activity and result in submicromolar concentrations for all of our top APE1 inhibitors. Incision reactions were allowed to proceed for 10 minutes at 37ºC, considering the single-time point end measurement nature of our assay. Since reaction velocity will eventually decrease with substrate depletion, the effect of an inhibitor should be most prominent within the initial phase of the reaction. Longer incubation times of 20, 30, and 40 minutes at 37ºC, 30ºC, and at room temperature, respectively, results in a corresponding increase in IC_{50} value, whereas shorter incubation times produce lower values (data not shown).

Our report on APE1 assay optimization experiments delineates a need for standardized assay conditions when screening for inhibitors of APE1. In addition to our own inhibitors we have demonstrated a similar strong protein concentration-dependent effect on dose response to APE1 inhibition with 7-nitro-indole carboxylic acid, a compound formerly shown to inhibit APE1 activity *in vitro.*[29] The wild-type enzyme is a robust endonuclease that is highly efficient at incising the DNA-sugar backbone 5' to abasic sites, with an approximate maximal single-turnover rate of 850 s^{-1}.[35] Given the high enzyme velocity of APE1, it is perceived that the IC_{50} values of potential small molecule inhibitors to APE1 will be affected by the parameters of the screening assay.

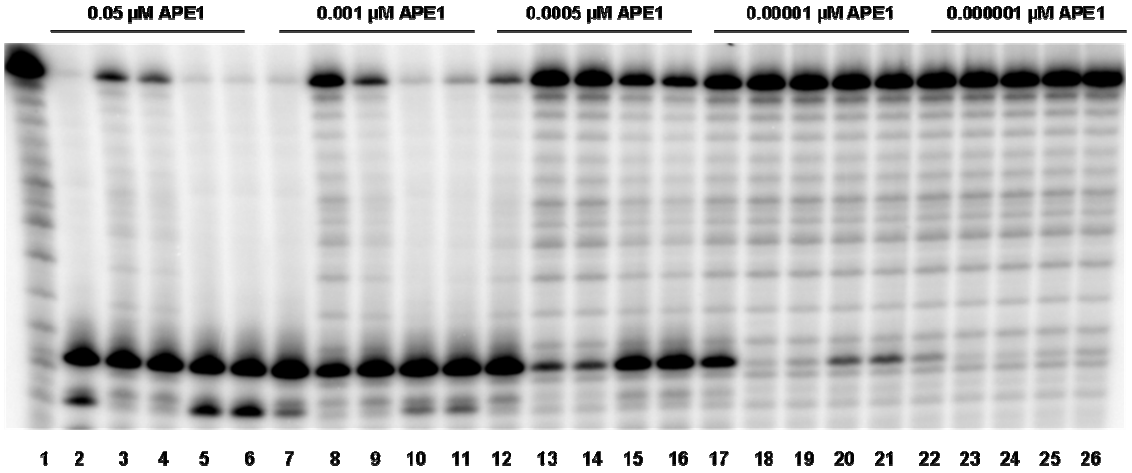
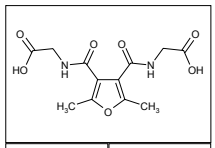


Figure S1. Compound **10** was tested at different concentrations of APE1. Lane 1: DNA only, lanes 2-6, 7-11, 12-16, 17-21 and 22-26 are as follows: DMSO control, compound **10** at 100, 33, 11, 3.7 µM, respectively. IC_{50} values for compound **10** at 0.0005 µM were obtained using further dilutions of the compound not shown in this figure. Percent inhibition at each dose was plotted on a log scale to obtain IC_{50} values.

Purity Information for Active Compounds. All compounds tested as part of this study were purchased from Asinex USA, North Carolina. These compounds are certified at a minimum purity of 90%. NMR data attesting to the purity of each of the active compounds is given below. All 1H-NMR have been recorded at 400MHz (Varian). The solvent used in all cases was DMSO-d6.

BAS 7201487



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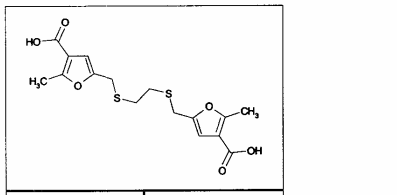
BAS 7201487	0502261
29.06.2003	Solv.DMSO

BAS 1172053

B



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[redacted]	
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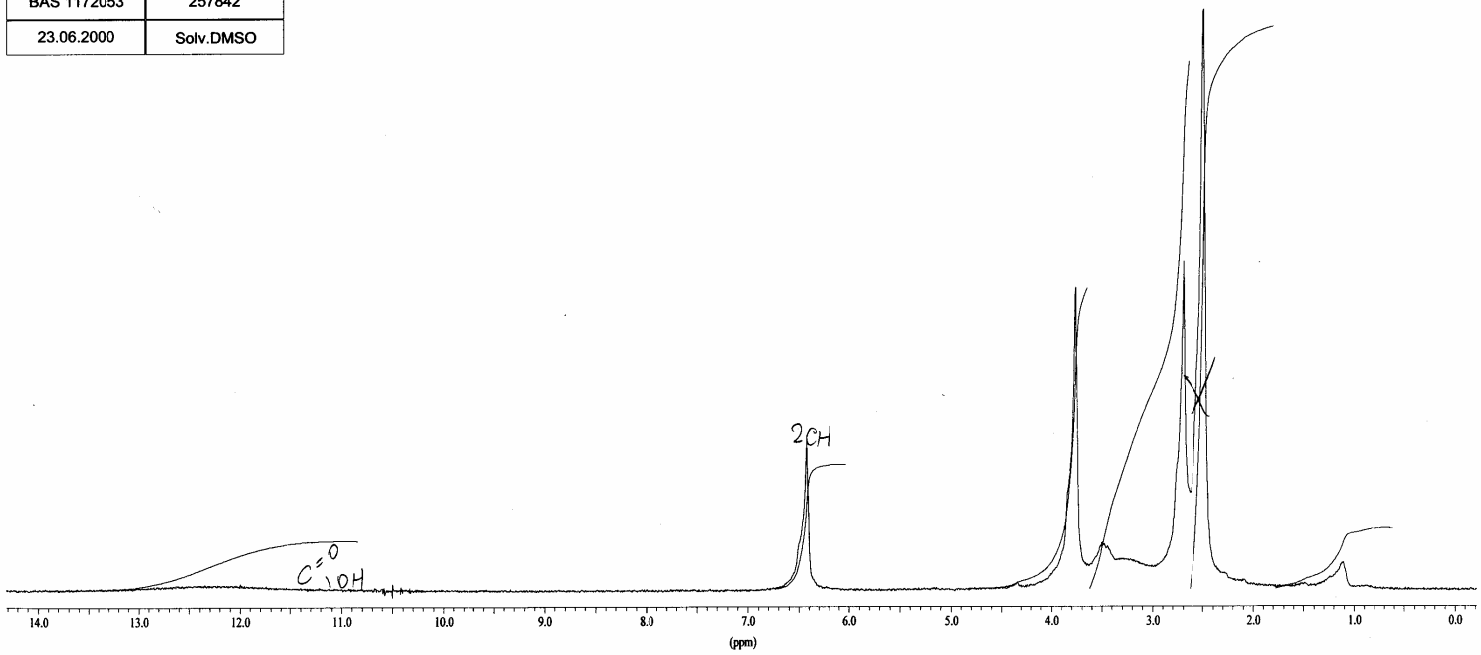
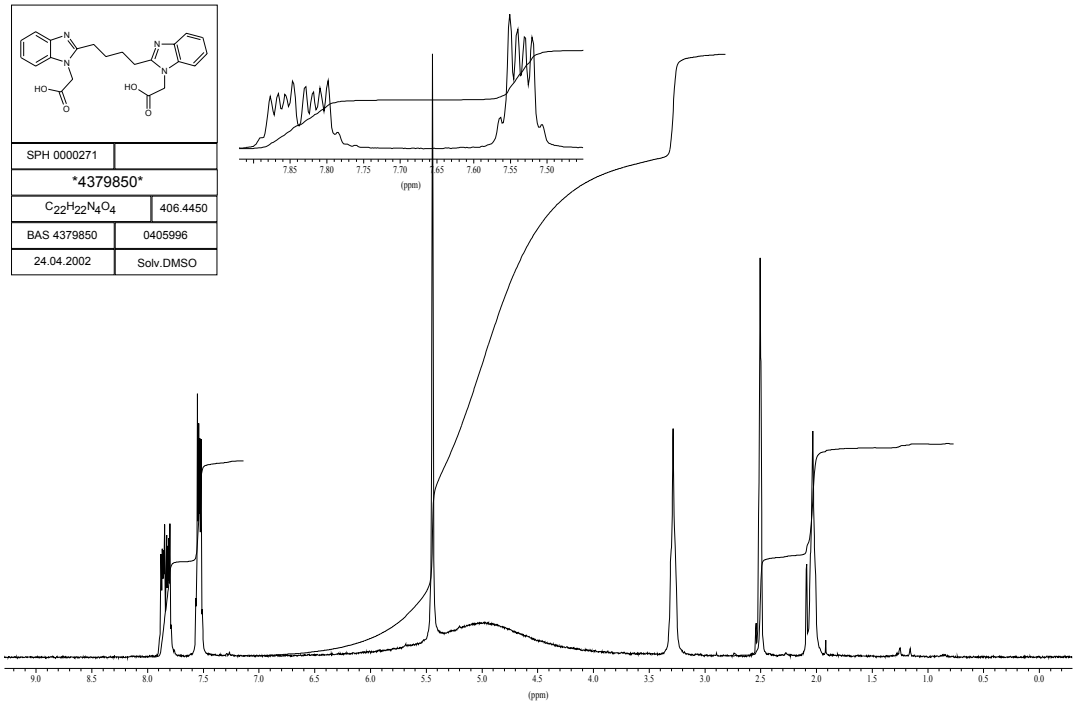
BAS 1172053	257842
23.06.2000	Solv.DMSO



B 4379850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BAS 4379850
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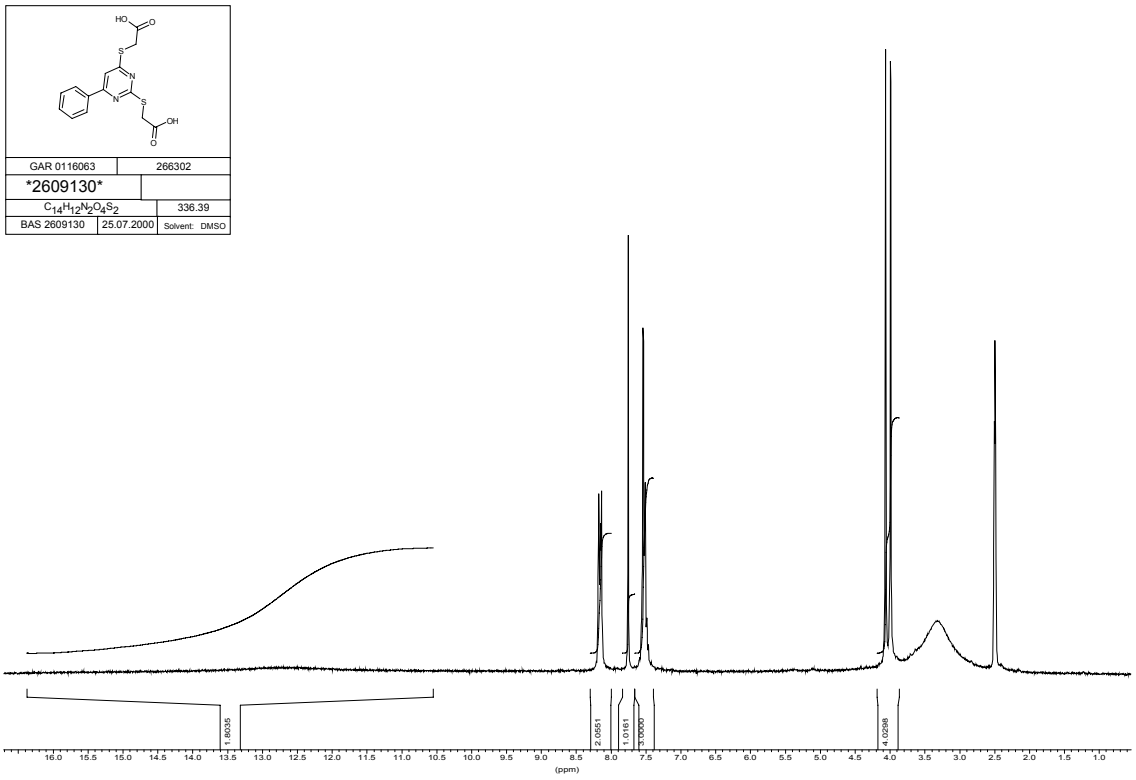
24.04.2002
Solv.DMSO
(ppm)
(ppm)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O
S
N
N
S
OH
O
GAR 0116063
266302
2609130
$C_{14}H_{12}N_2O_4S_2$
336.39
BAS 2609130
25.07.2000
Solvent: DMSO
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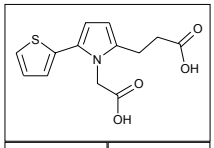
1.0161
3.0000
4.0298
(ppm)

BAS 3633561



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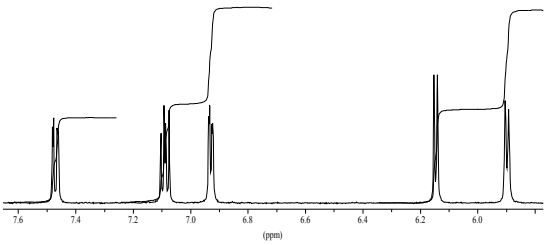
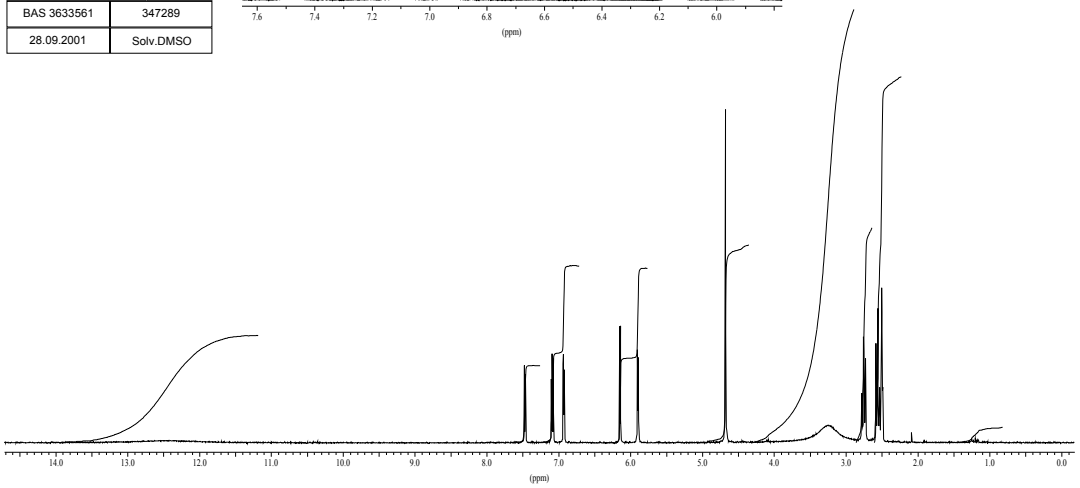
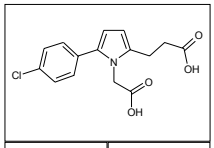
BAS 3633561	347289
28.09.2001	Solv.DMSO





BAS 3633558



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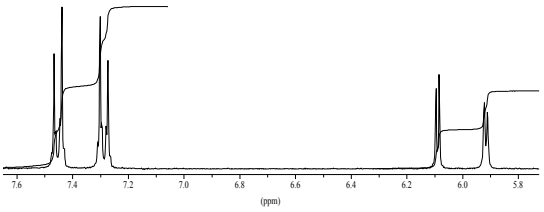
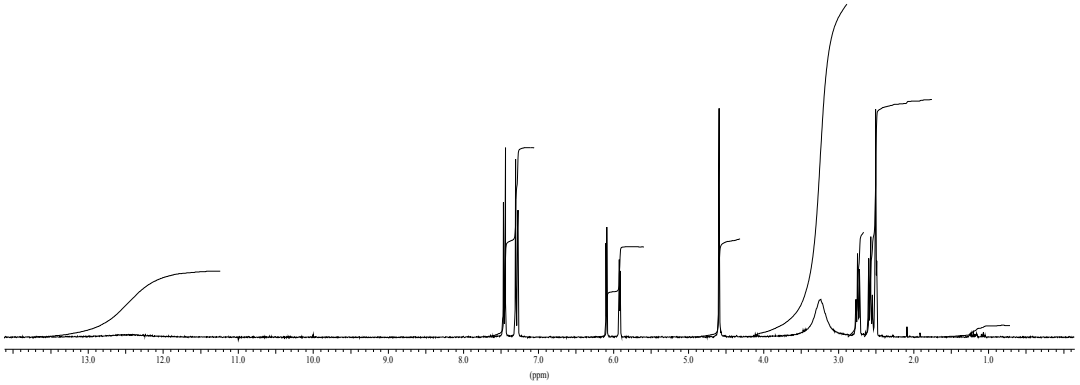
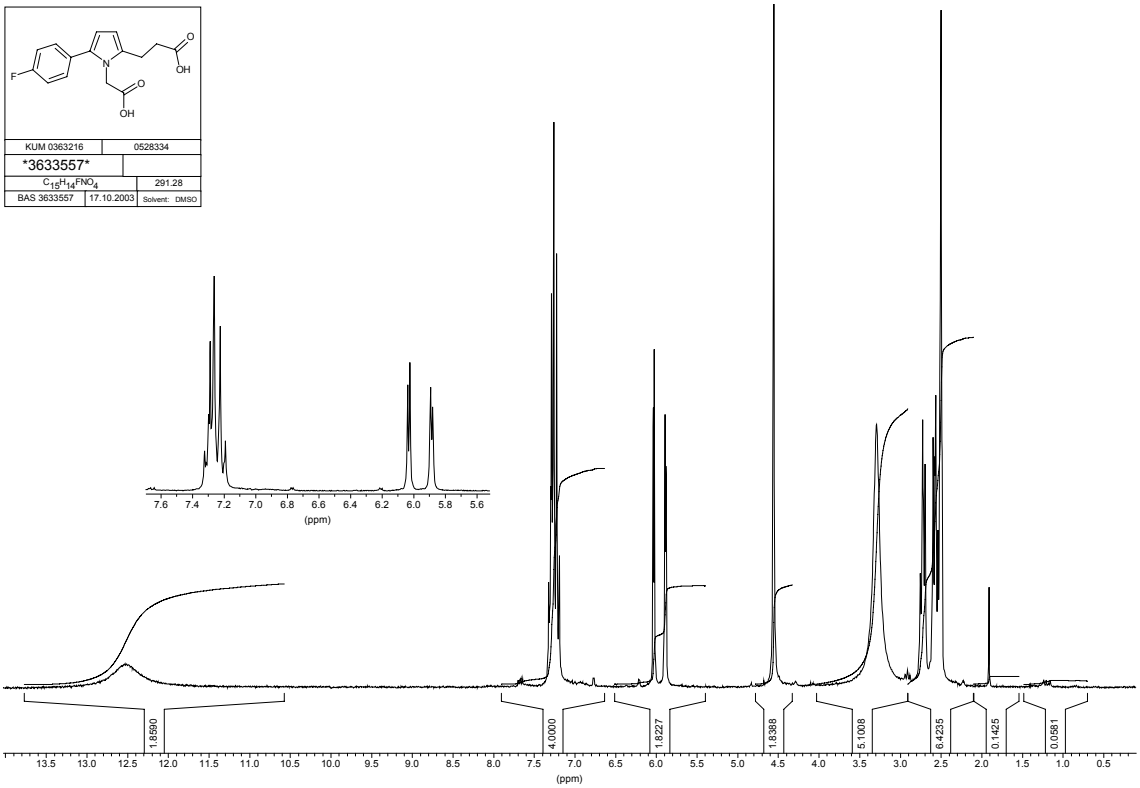
BAS 3633558	347287
28.09.2001	Solv.DMSO



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3633557
C15H14FNO4
291.28
BAS 3633557
17.10.2003
Solvent: DMSO
(ppm)
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4.0000
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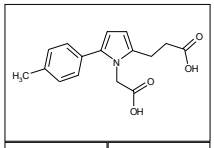
1.8388
5.1008
6.4235
0.1425
0.0581

BAS 3633556



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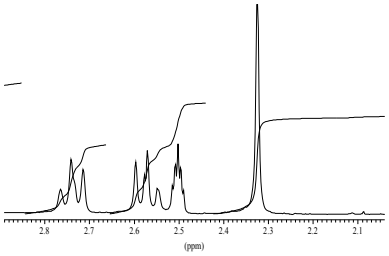
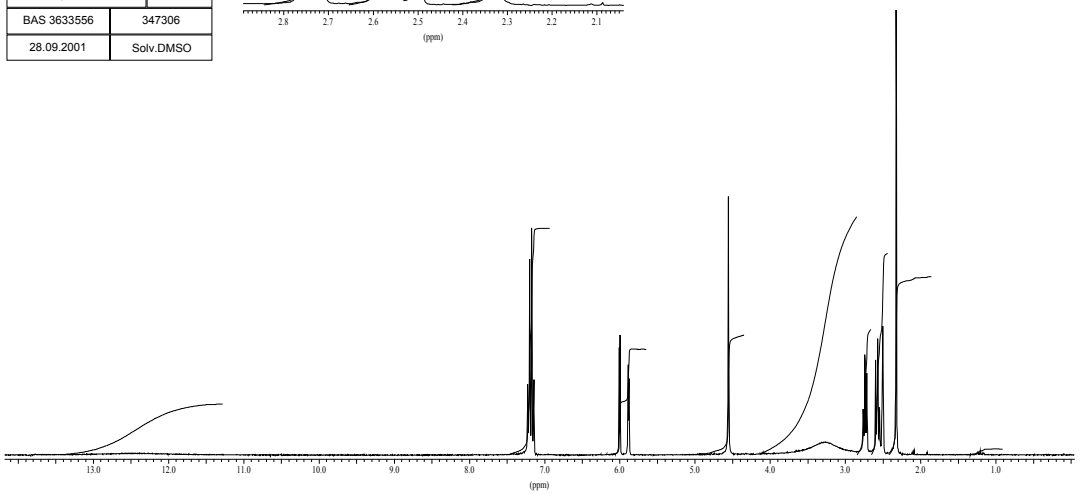
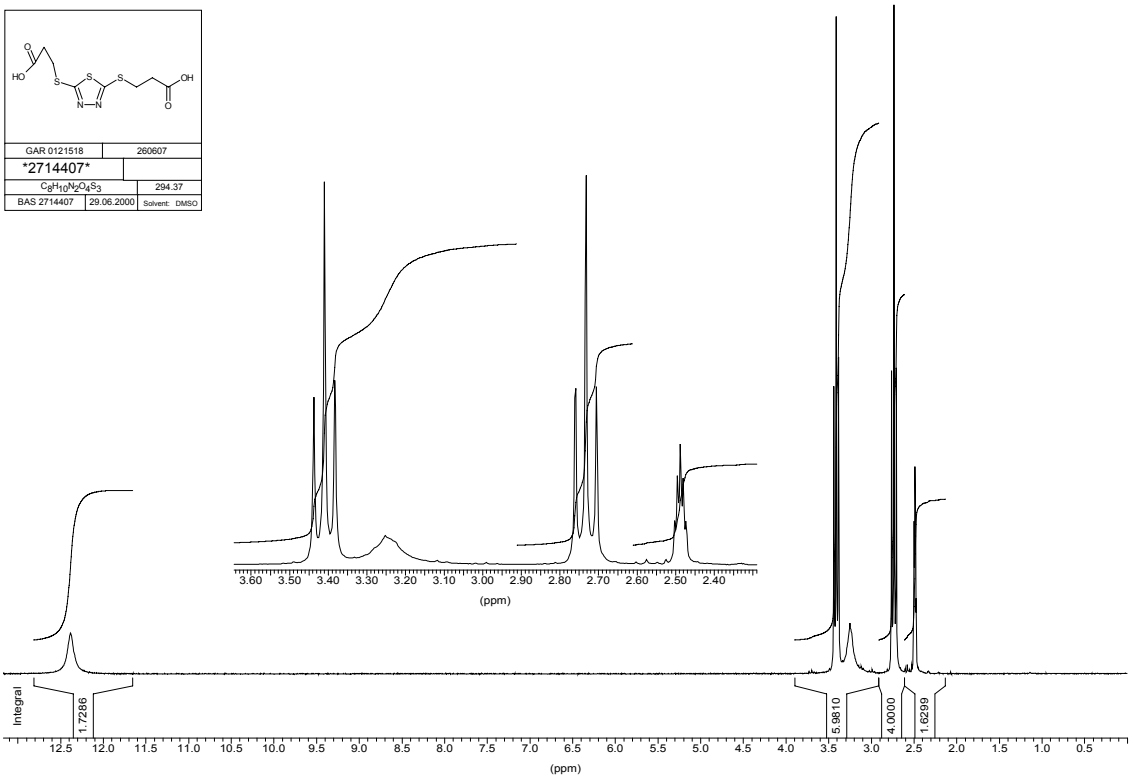
BAS 3633556	347306
28.09.2001	Solv.DMSO






GAR 0121518
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2714407
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294.37
BAS 2714407
29.06.2000
Solvent: DMSO
Integral
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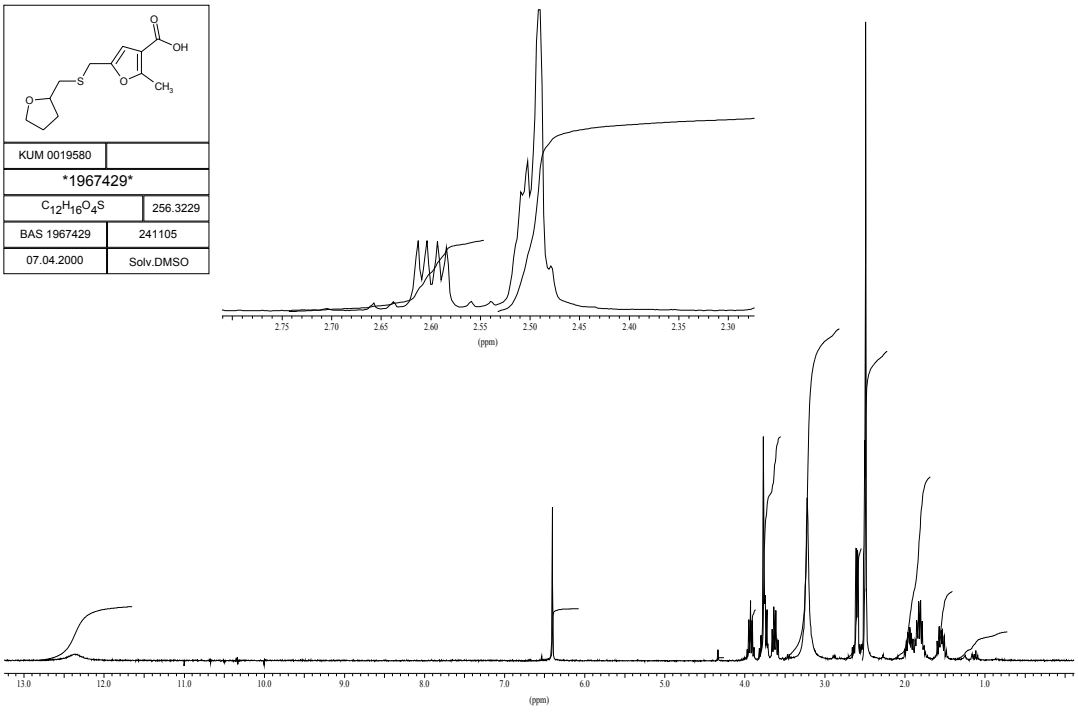
5.9810
4.0000
1.6299
(ppm)
(ppm)

NKTA B1967429



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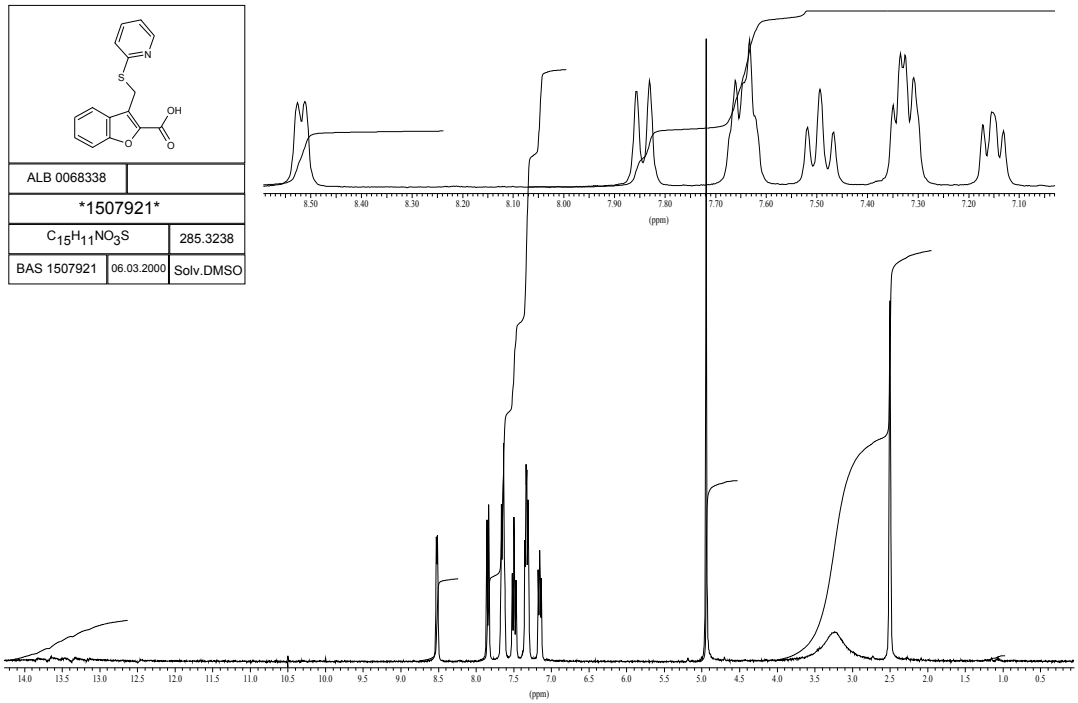

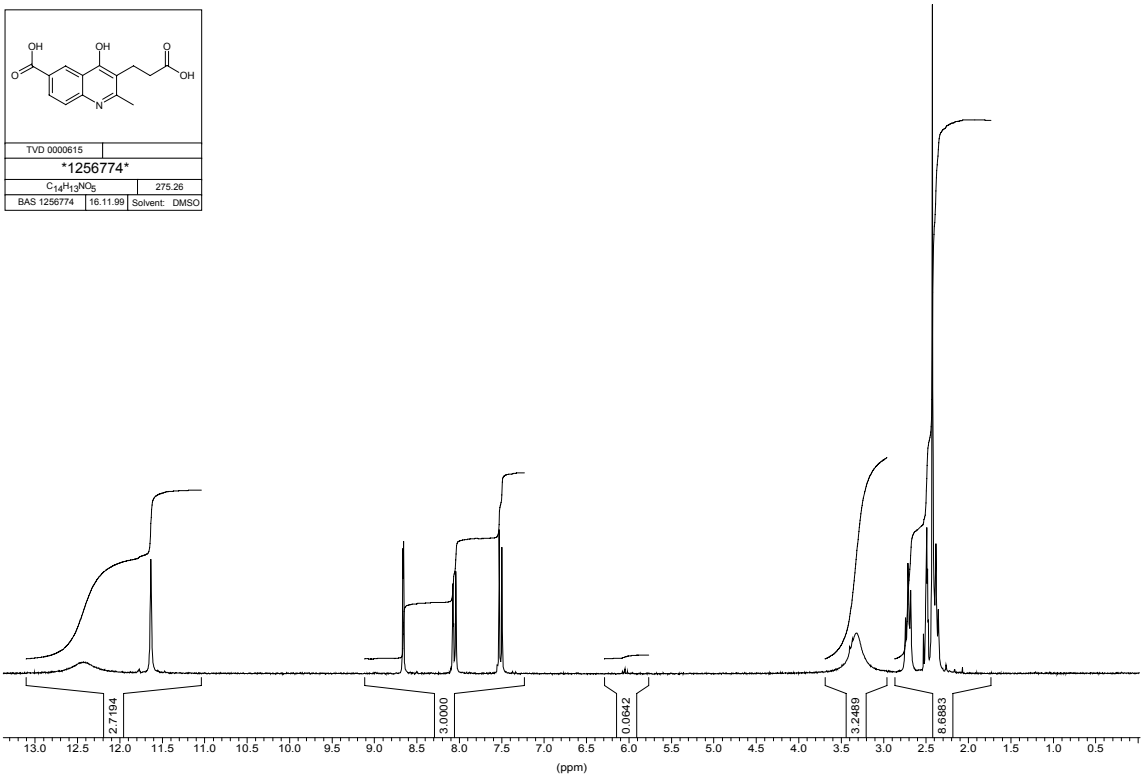
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BAS 1256774
16.11.99
Solvent: DMSO
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3.0000
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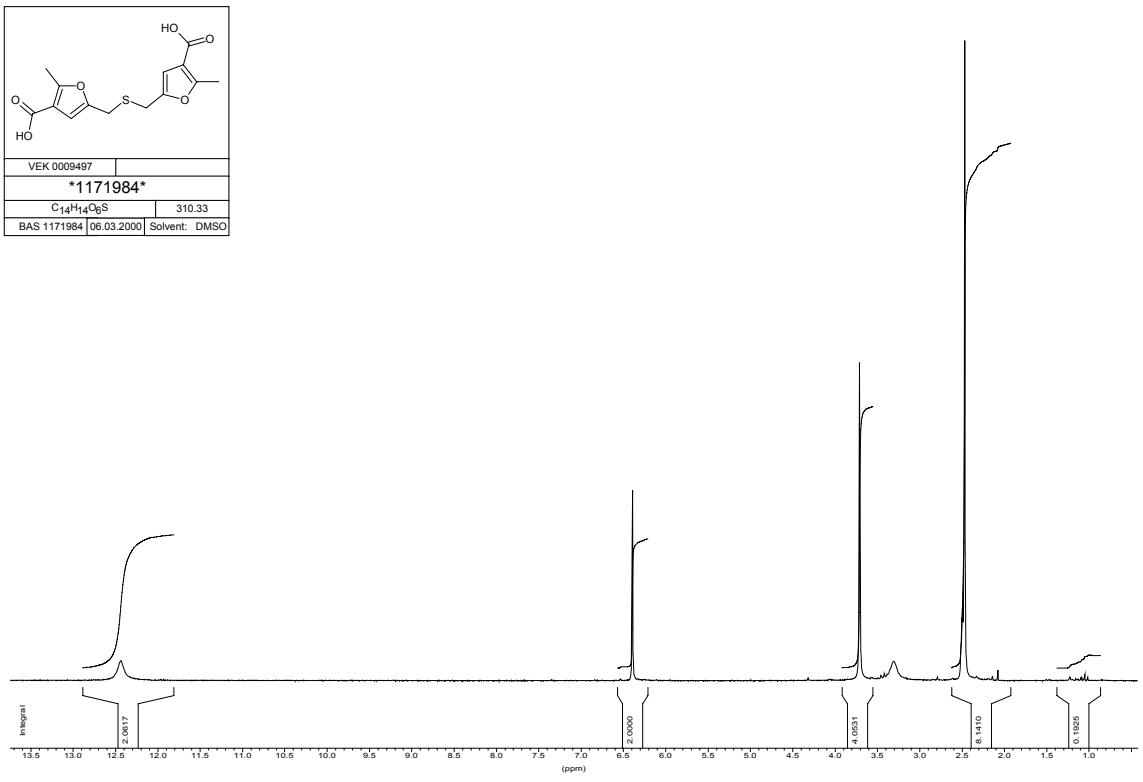
8.6883
(ppm)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O
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HO
S
O
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VEK 0009497
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Solvent: DMSO
Integral
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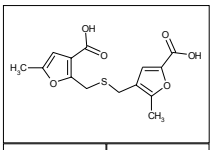
(ppm)

BAS 1171823



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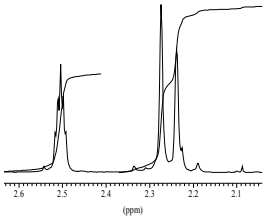
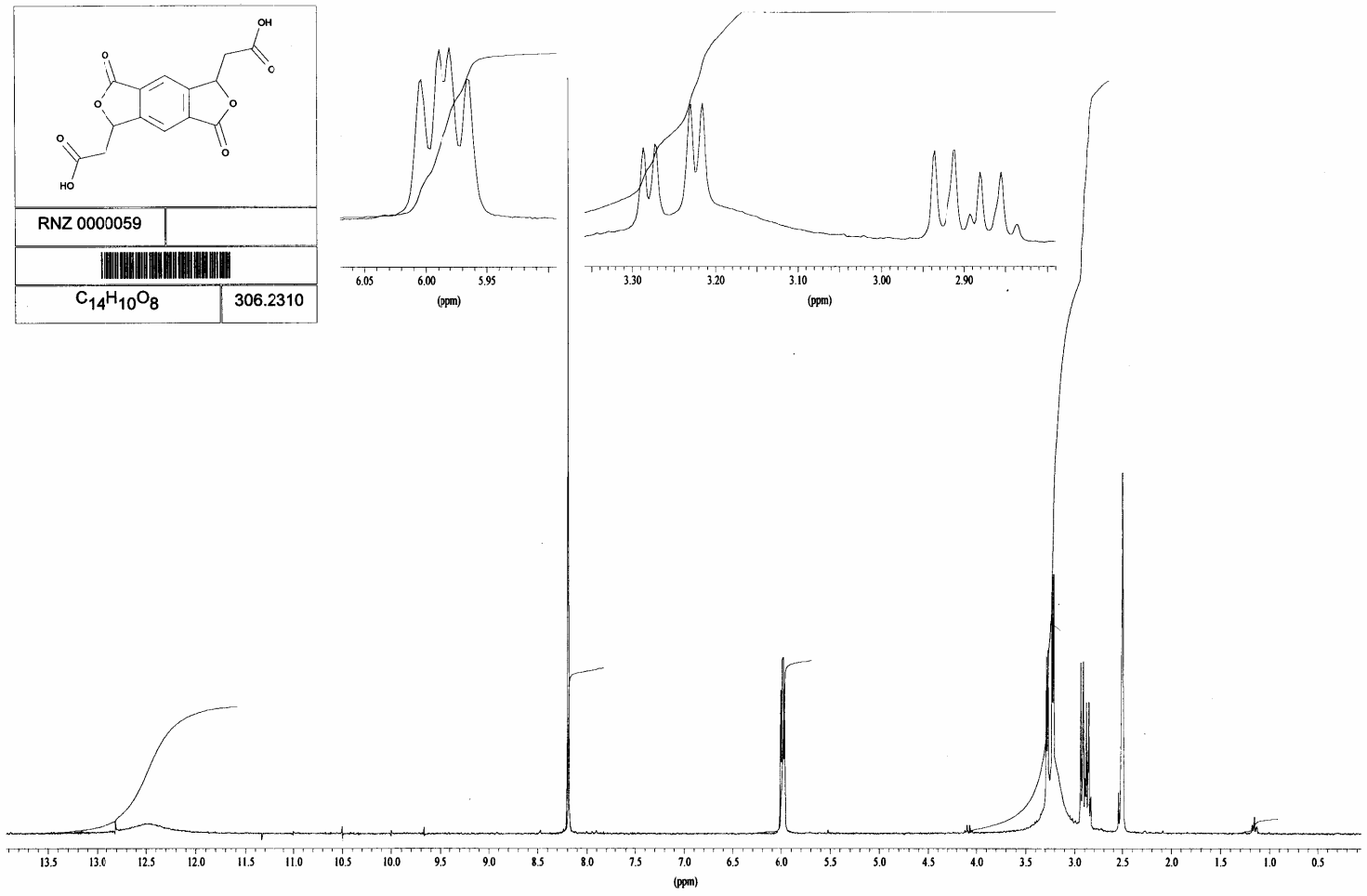
BAS 1171823	311750
07.05.2001	Solv.DMSO



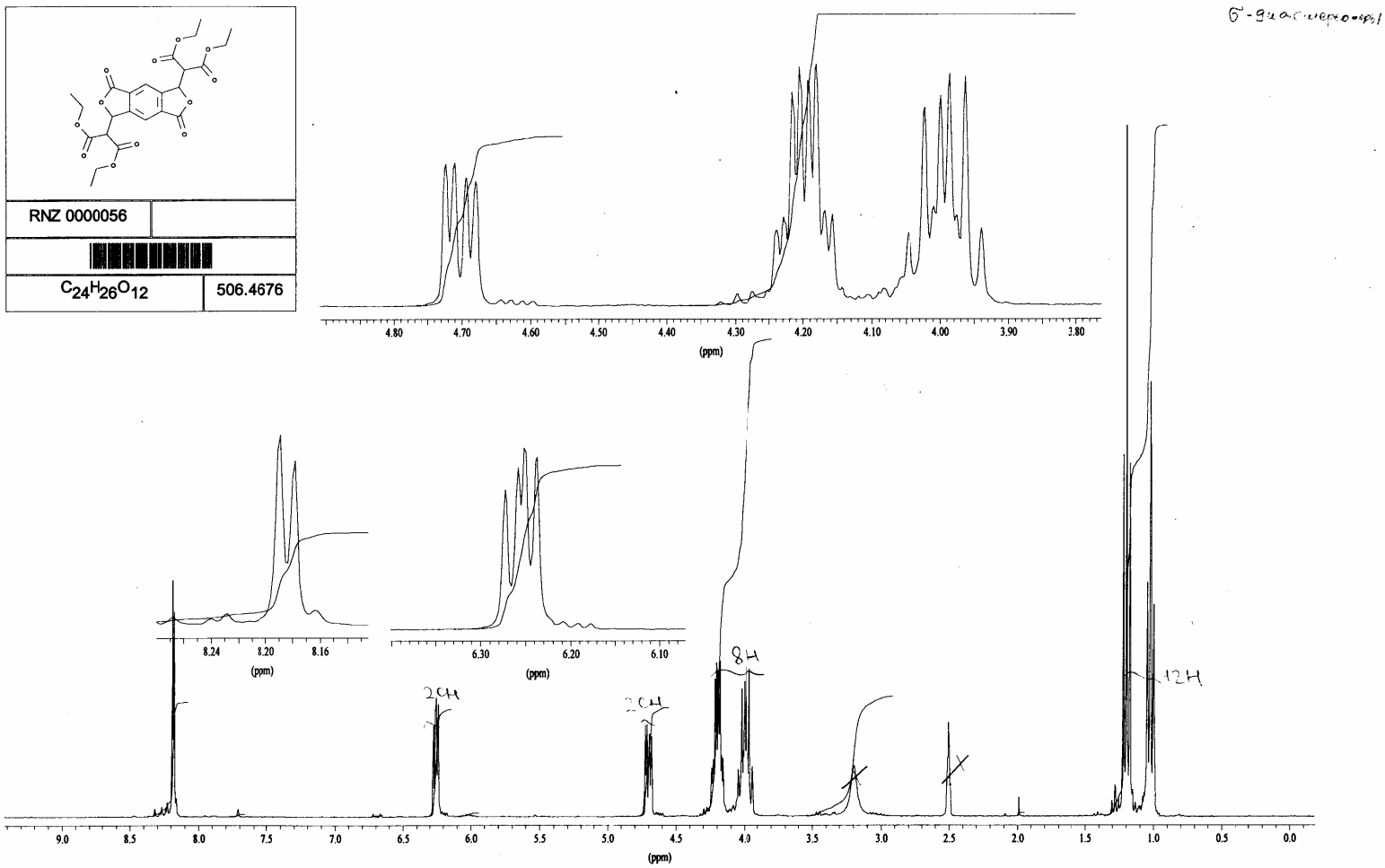
B

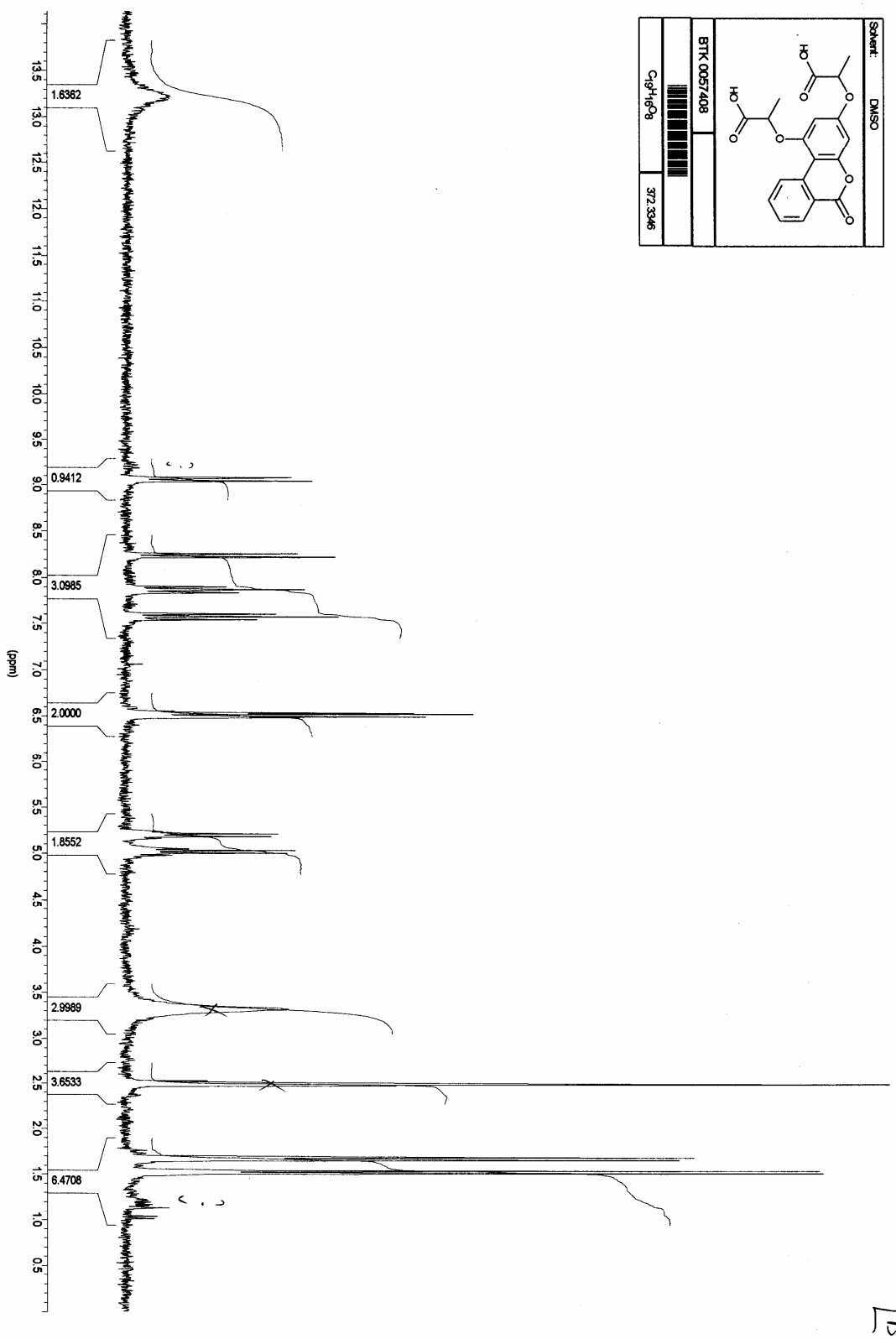
NKTA RNZ 00059



RNZ 56

B

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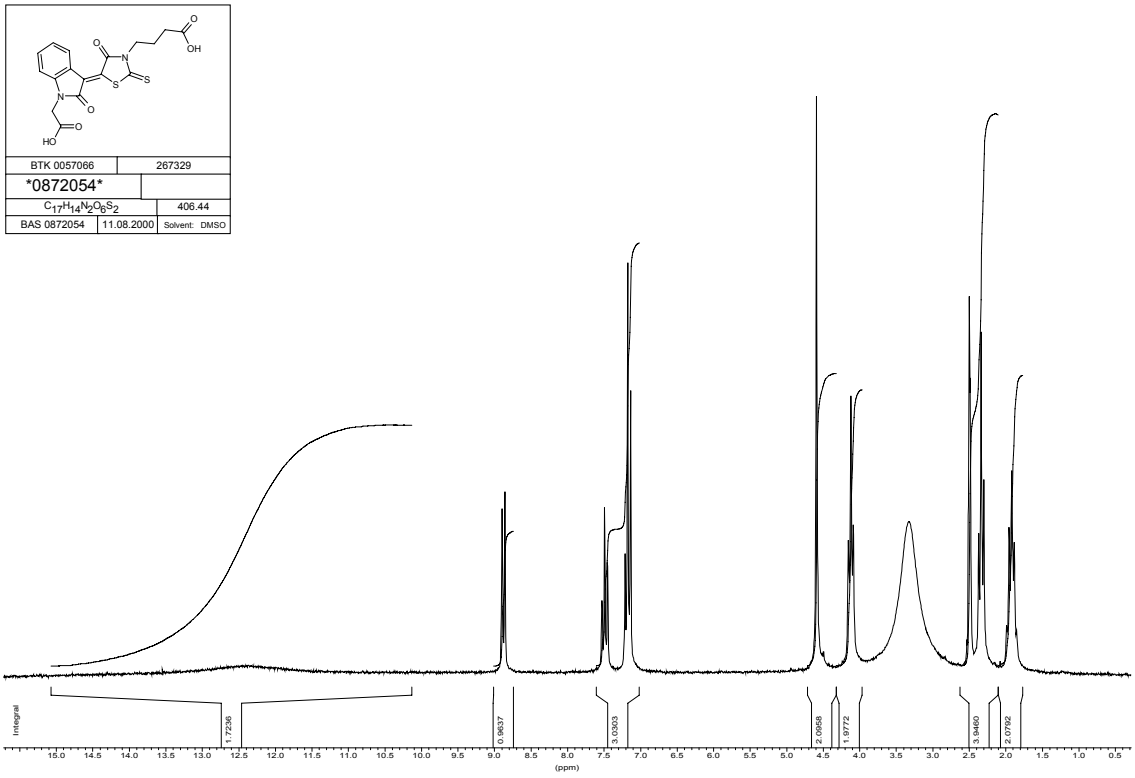
2.0000
1.8552
2.9989
3.6533
6.4708
(ppm)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267329
0872054
$C_{17}H_{14}N_2O_6S_2$
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BAS 0872054
11.08.2000
Solvent: DMSO
Integral
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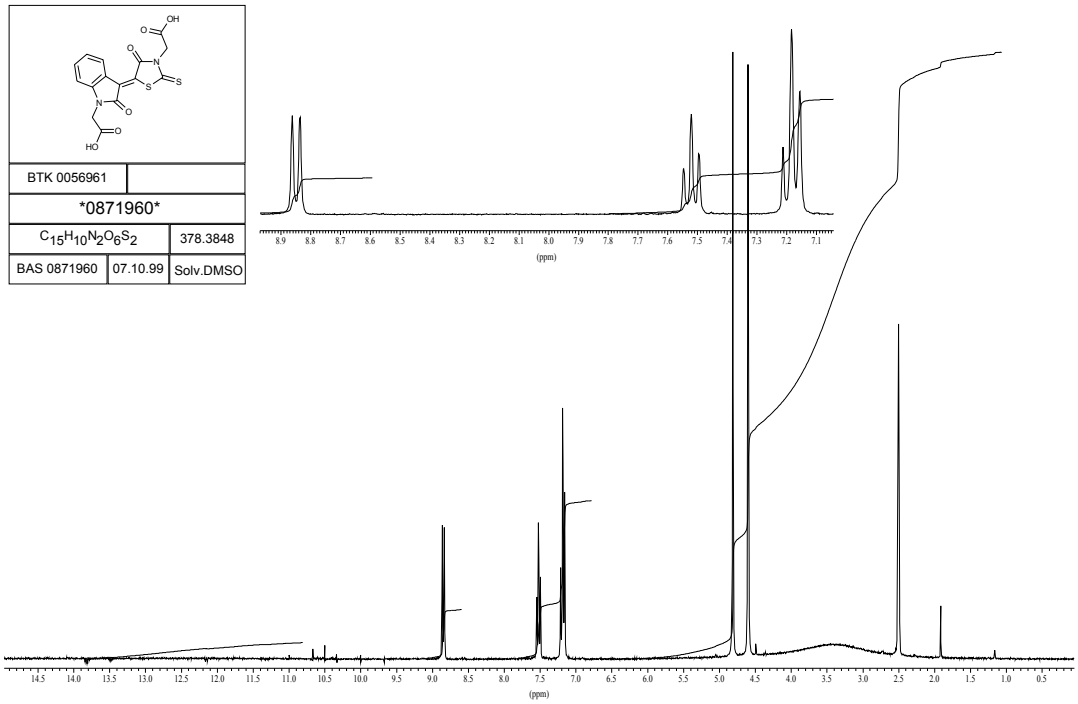
(ppm)

NKTA B0871960 IN DMSO



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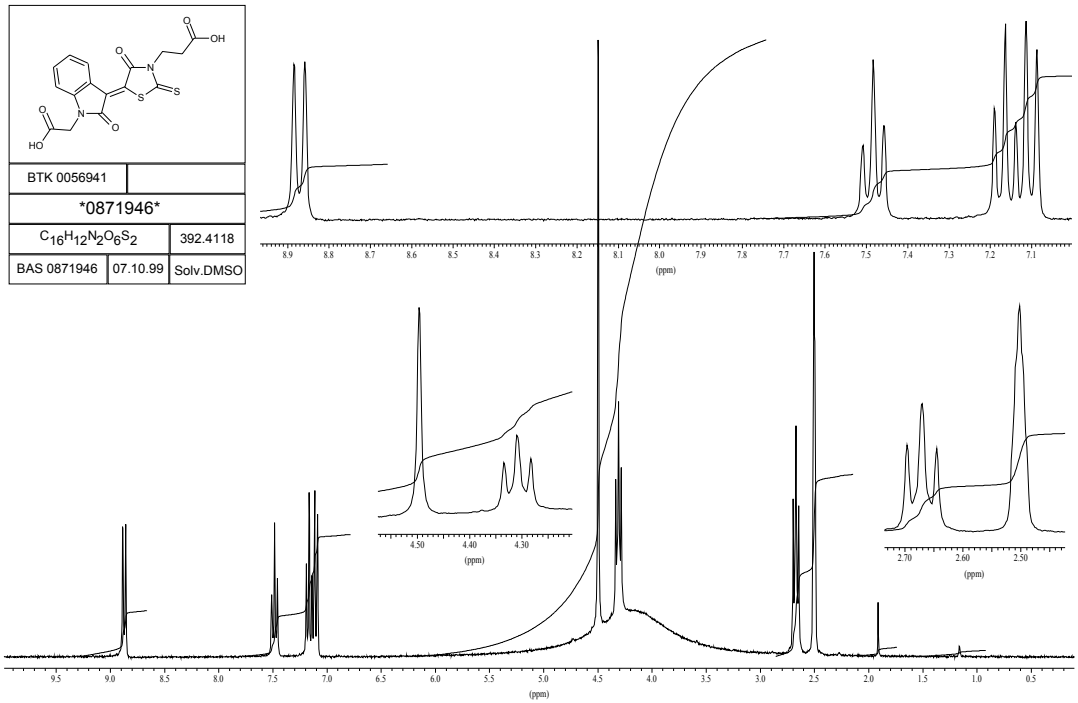

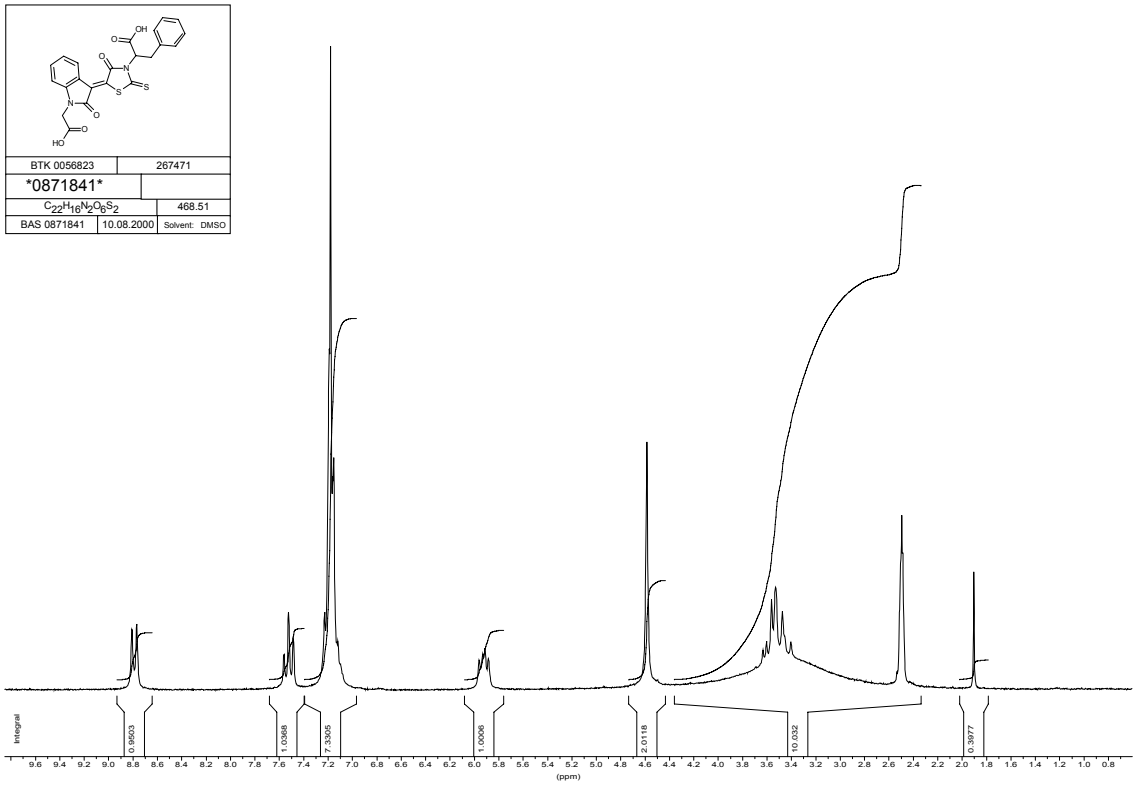
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267471
0871841
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BAS 0871841
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Solvent: DMSO
Integral
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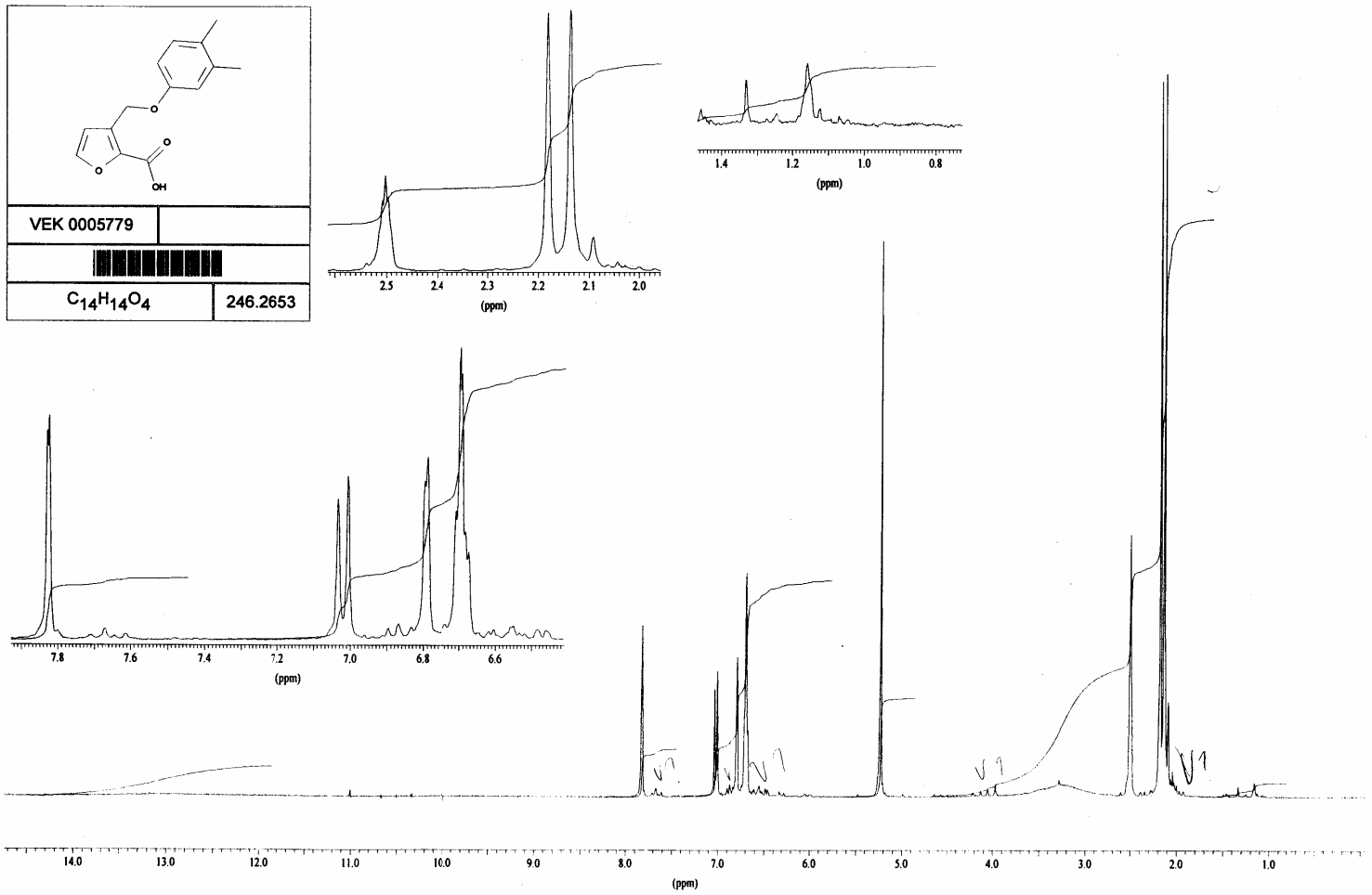
(ppm)

VEK 5779

B



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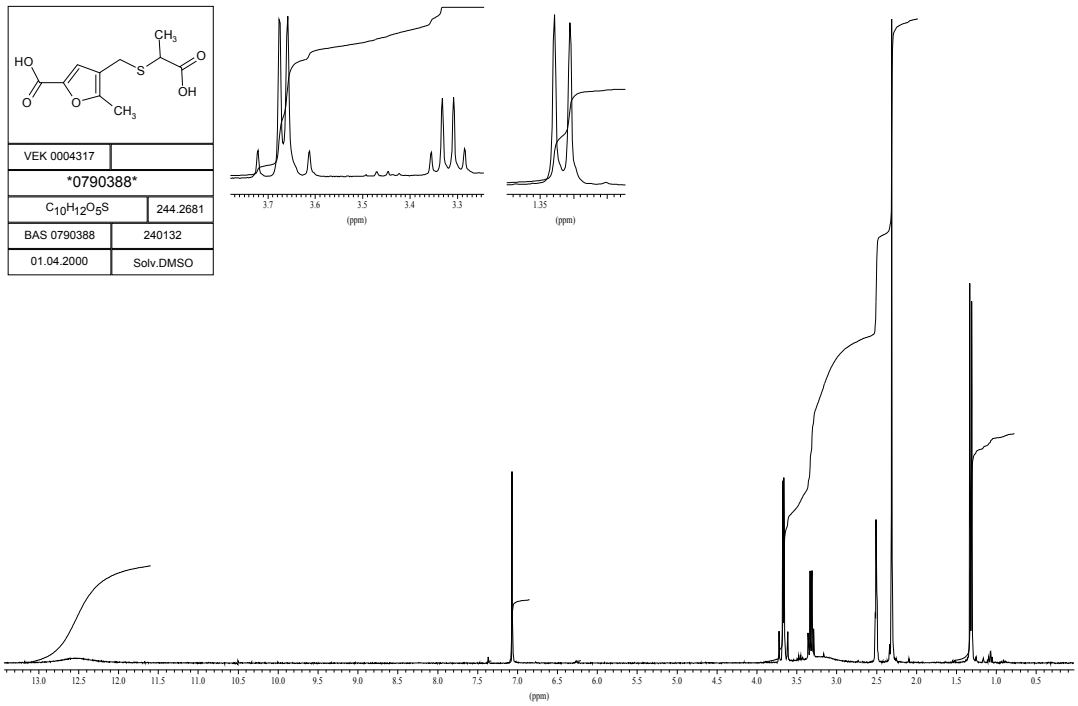
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O
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O
CH3
S
CH3
O
OH
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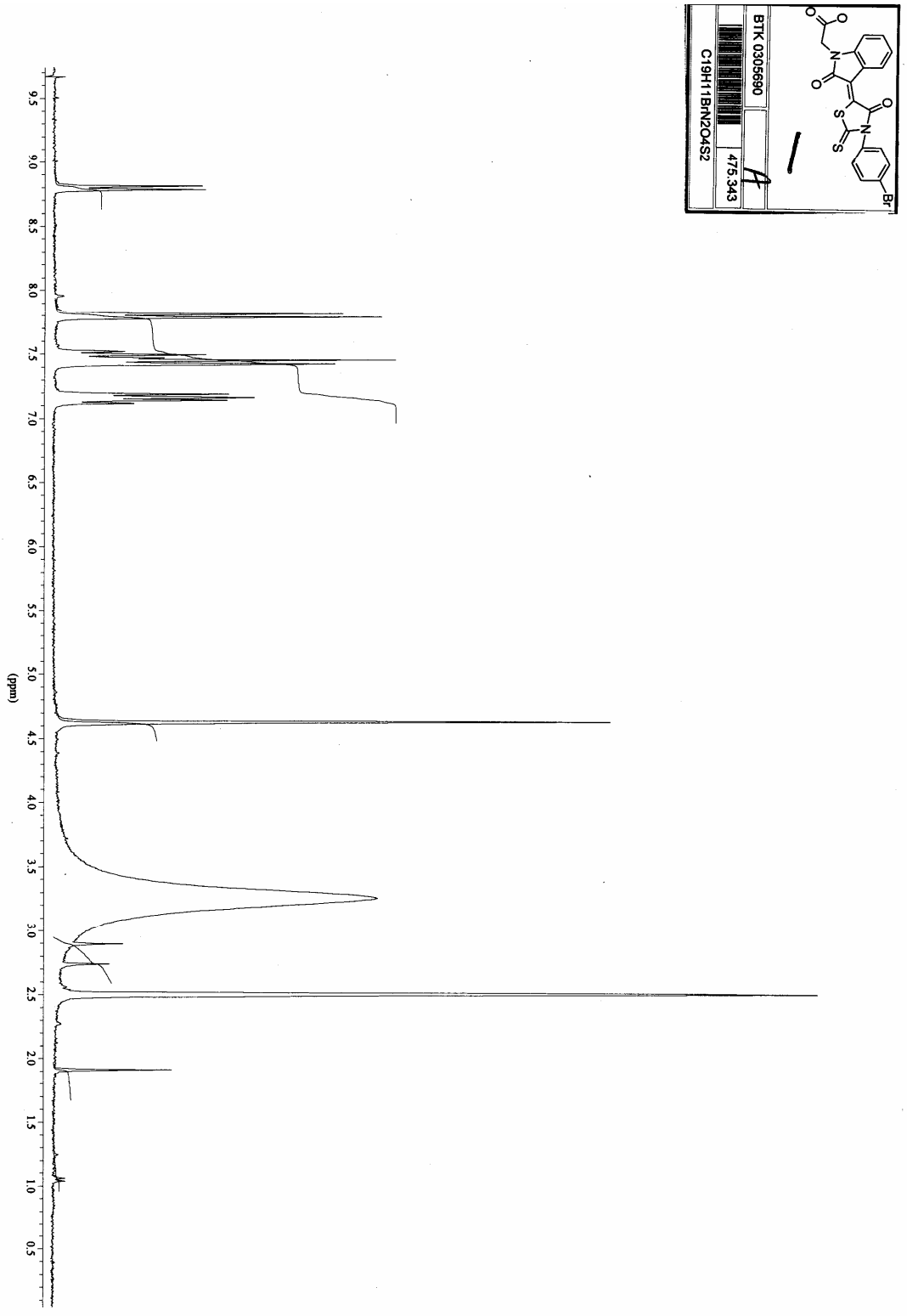
244.2681
BAS 0790388
240132
01.04.2000
Solv.DMSO
3.7
3.6
3.5
3.4
3.3
(ppm)
1.35
(ppm)
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)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Br
(ppm)
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9.0
8.5
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7.5
7.0
6.5
6.0
5.5
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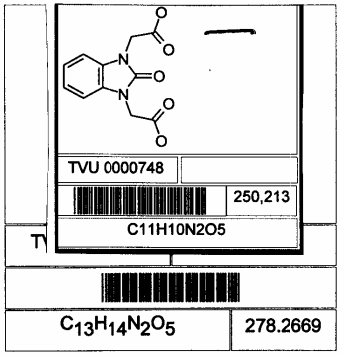
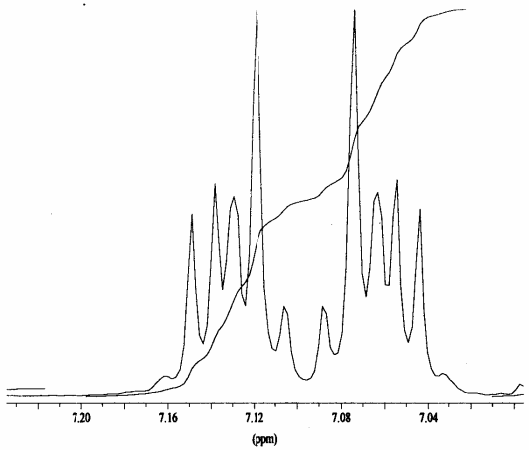
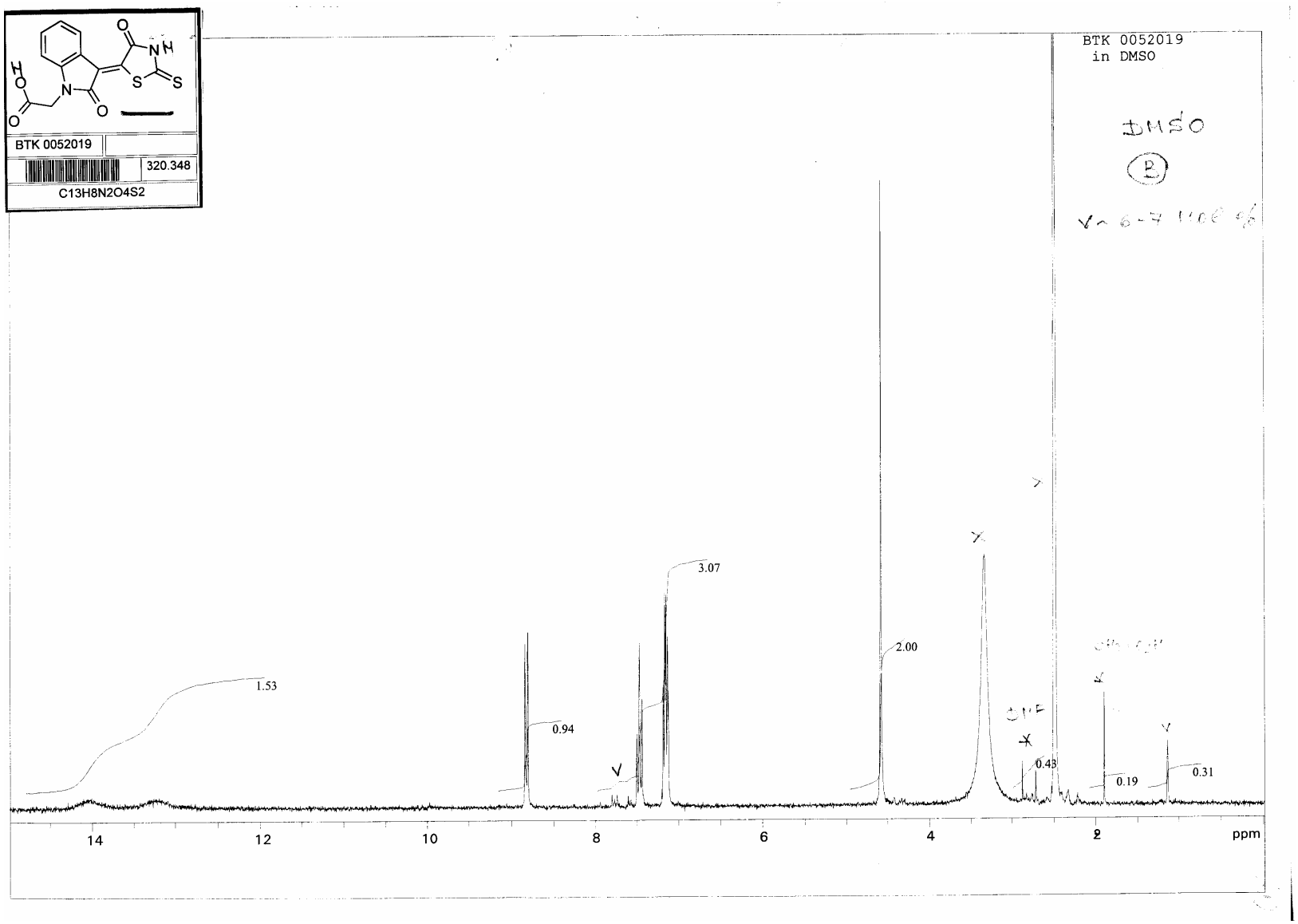
1.5
1.0
0.5

A

NKTA TVU00748






BTK 0052019
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C13H8N2O4S2
BTK 0052019
in DMSO
DMSO
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2.00
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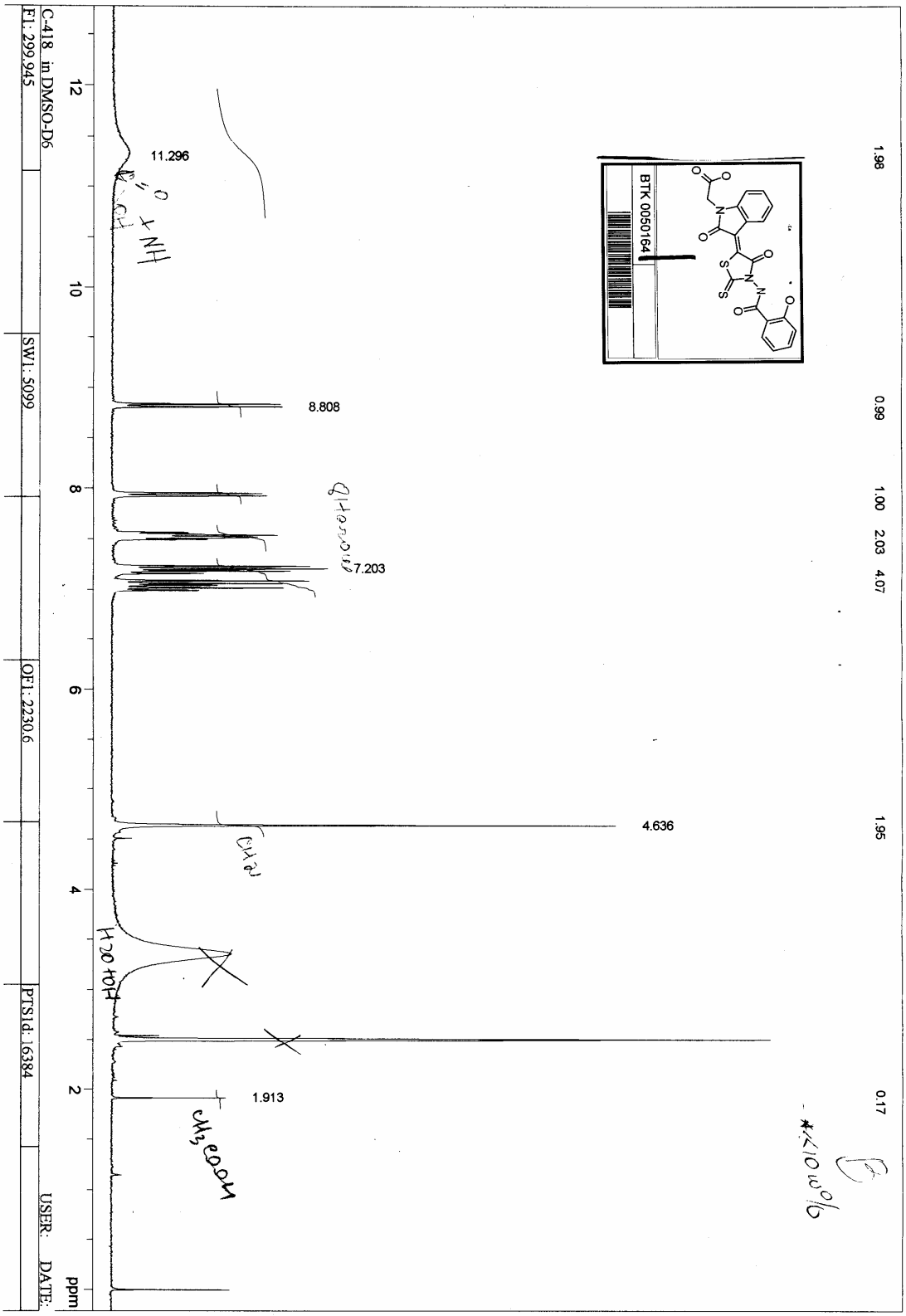
0.31
14
12
10
8
6
4
2
ppm


C-418 in DMSO-D6
F1: 299.945
SW1: 5099
OF1: 2230.6
PTS1d: 16384
USER:
DATE:
BTK 0050164
11.296
8.808
7.203
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1.913
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0.99
1.00
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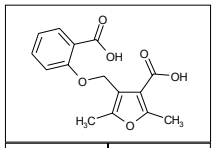
4.07
1.95
0.17
ppm

BAS 469505



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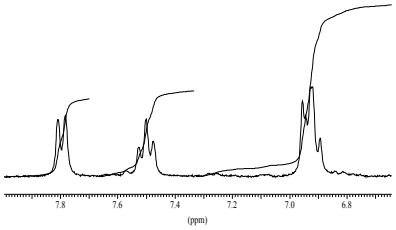
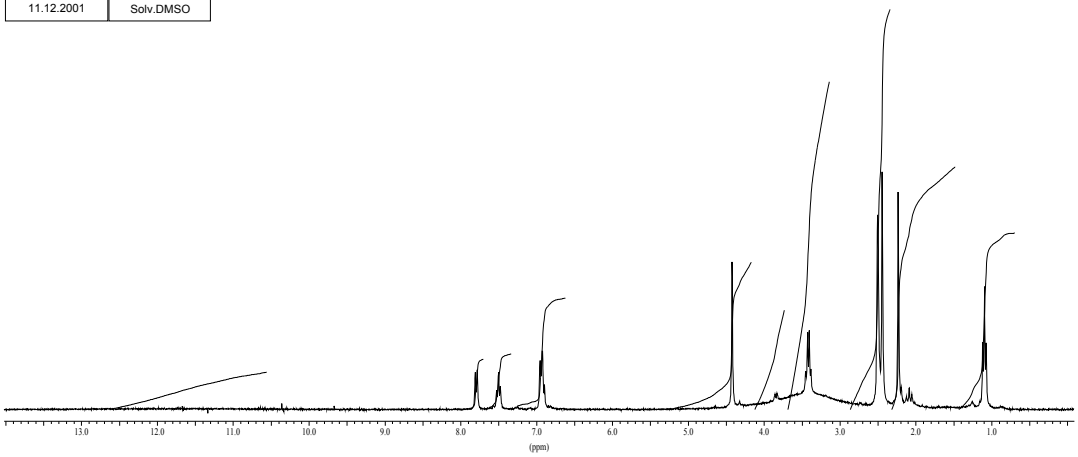
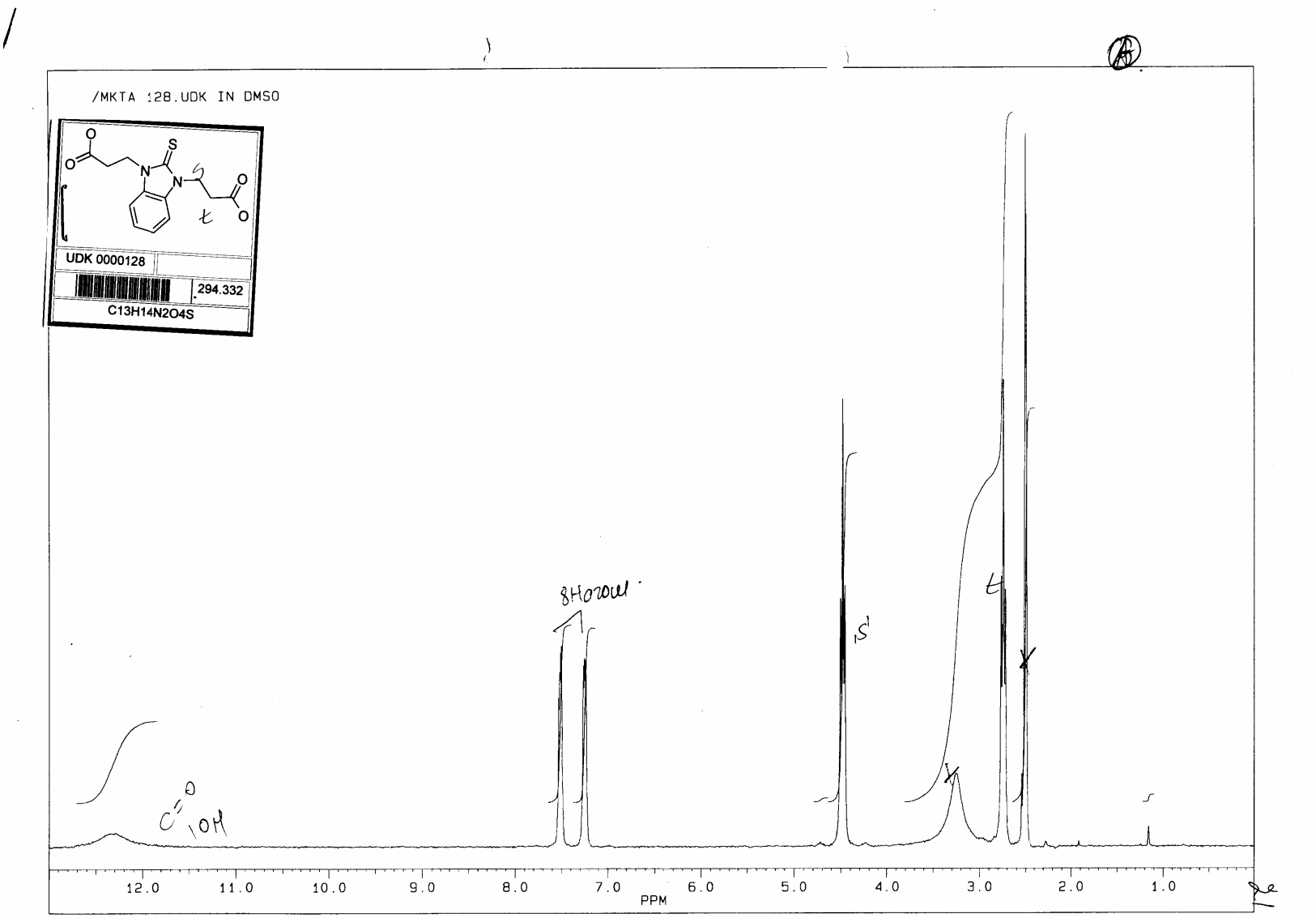
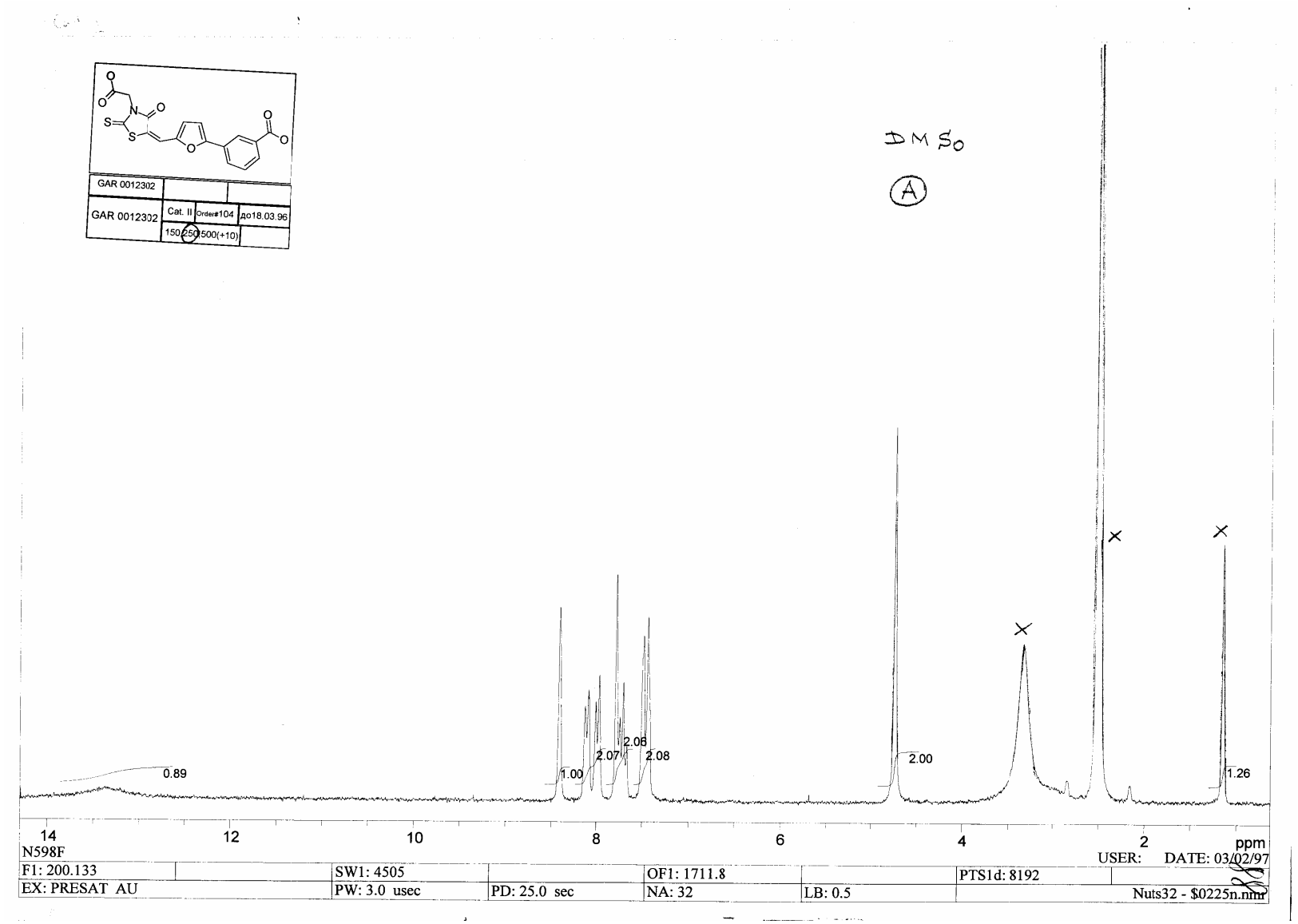
BAS 0469505	0365532
11.12.2001	Solv.DMSO

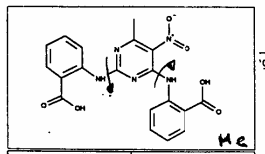





/MKTA 128.UDK IN DMSO
UDK 0000128
294.332
C13H14N2O4S
8H ortho
1S
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
PPM

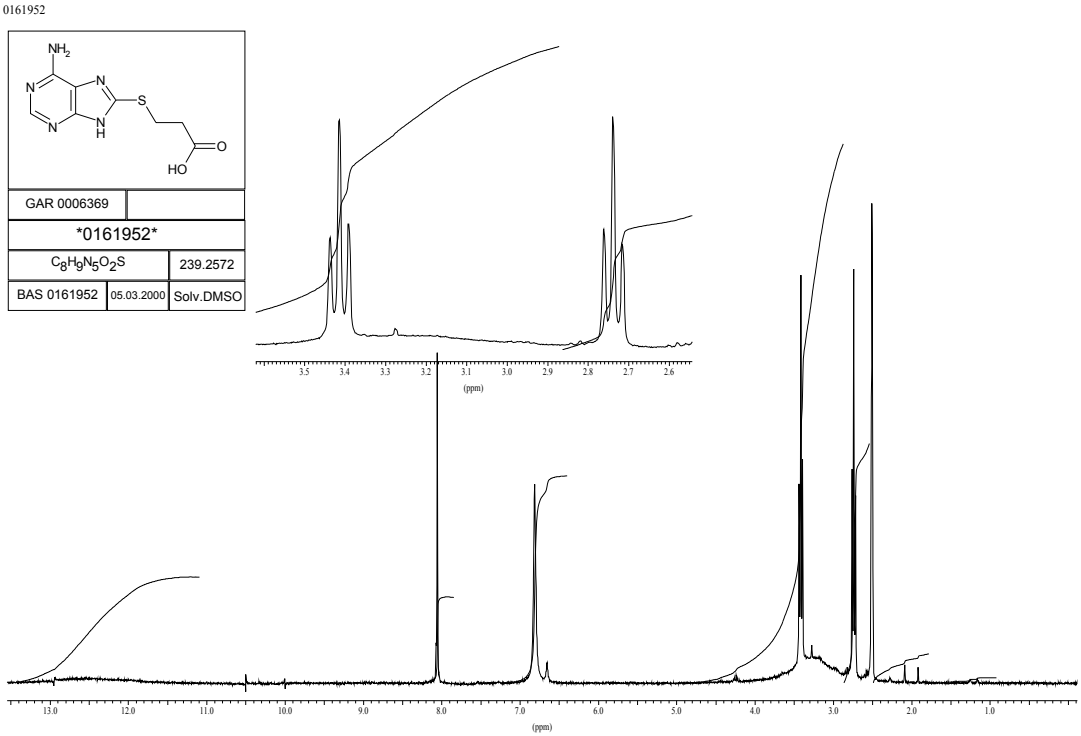

GAR 0012302
GAR 0012302 Cat. II Order#104 до18.03.96
150 250 500(+10)
DMSO
A
X
X
X
0.89
1.00
2.07
2.08
2.08
2.00
1.26
14
12
10
8
6
4
2
ppm
N598F
USER:
DATE: 03/02/97
F1: 200.133
SW1: 4505
OF1: 1711.8
PTS1d: 8192
EX: PRESAT AU
PW: 3.0 usec
PD: 25.0 sec
NA: 32
LB: 0.5
Nuts32 - $0225n.nmr

40

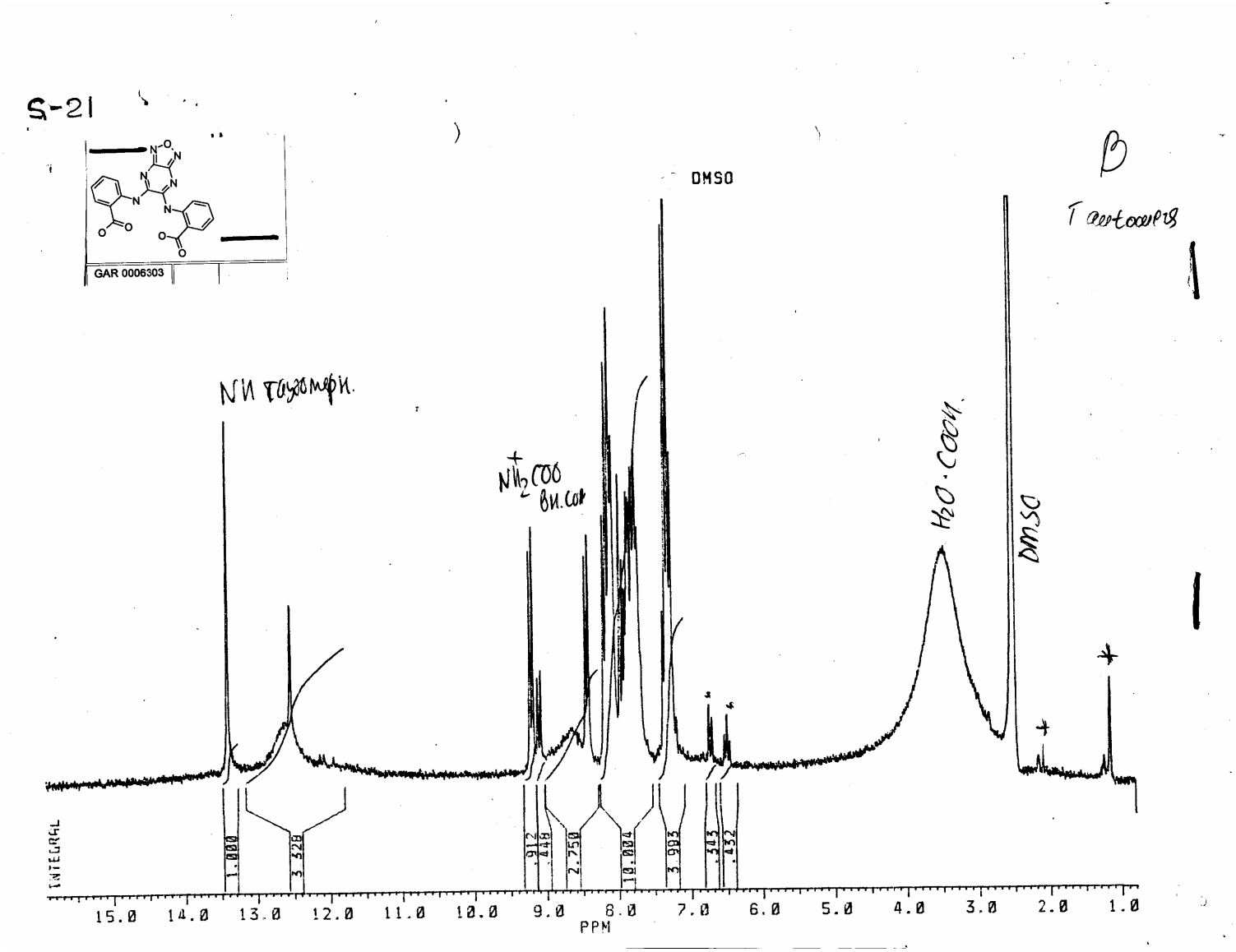


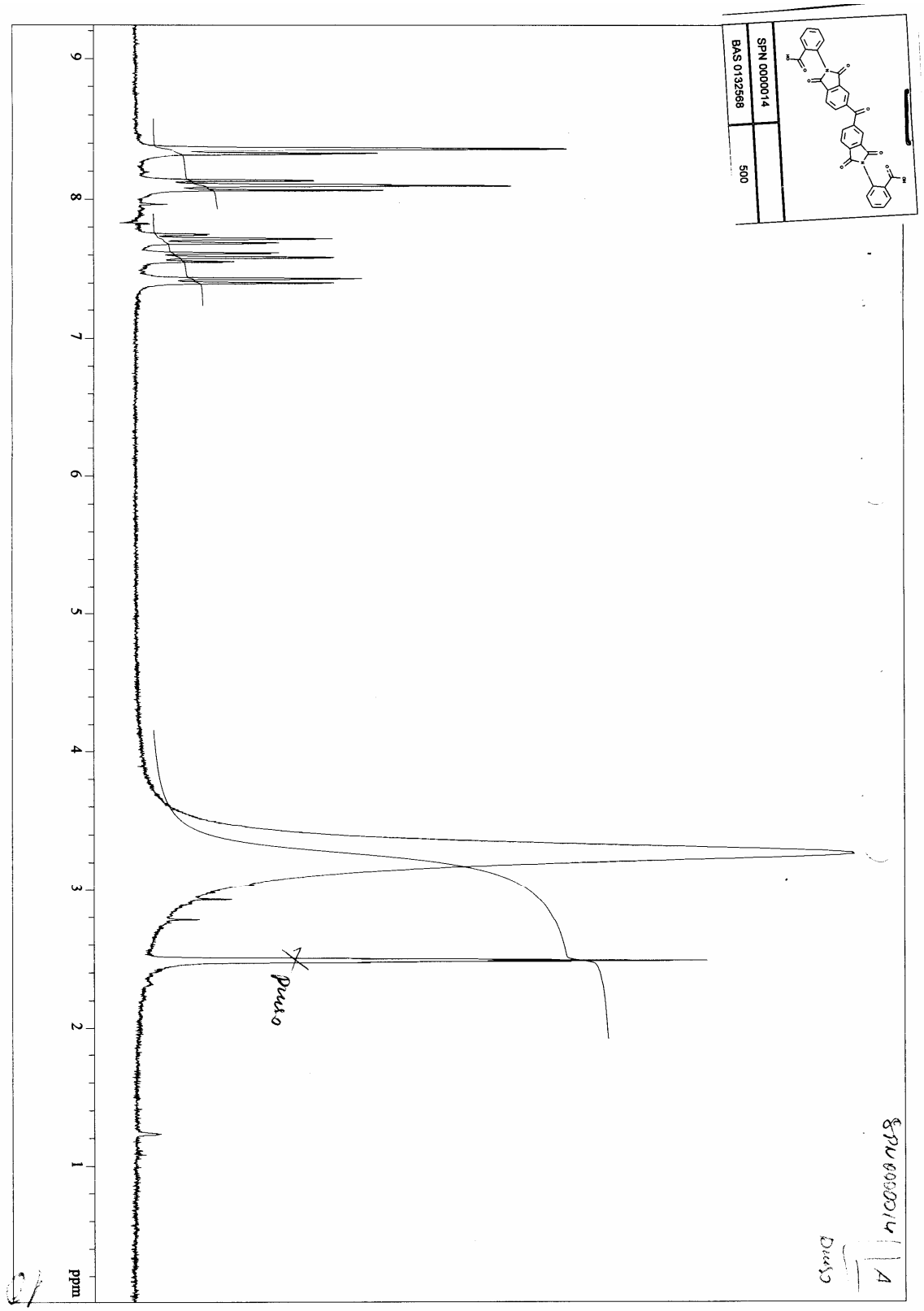
$C_{19}H_{15}N_5O_6$	
AG-690/4446003	BMW0006585
SBSS5 Ord# 12	500 mg

42

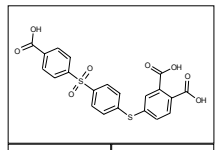

0161952
NH2
N
N
S
N
H
N
O
HO
GAR 0006369
0161952
C8H9N5O2S
239.2572
BAS 0161952
05.03.2000
Solv.DMSO
(ppm)
(ppm)

S-21

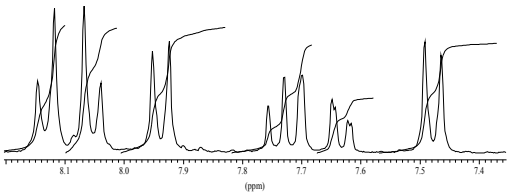

GAR 0006303
DMSO
NH tautomer.
NH_2^+COO Bu.cat
$H_2O \cdot CoCl_2$
DMSO
Tautomers
INTEGRAL
1.000
3.328
.912
.448
2.750
10.004
3.903
.343
.432
15.0 14.0 13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
PPM

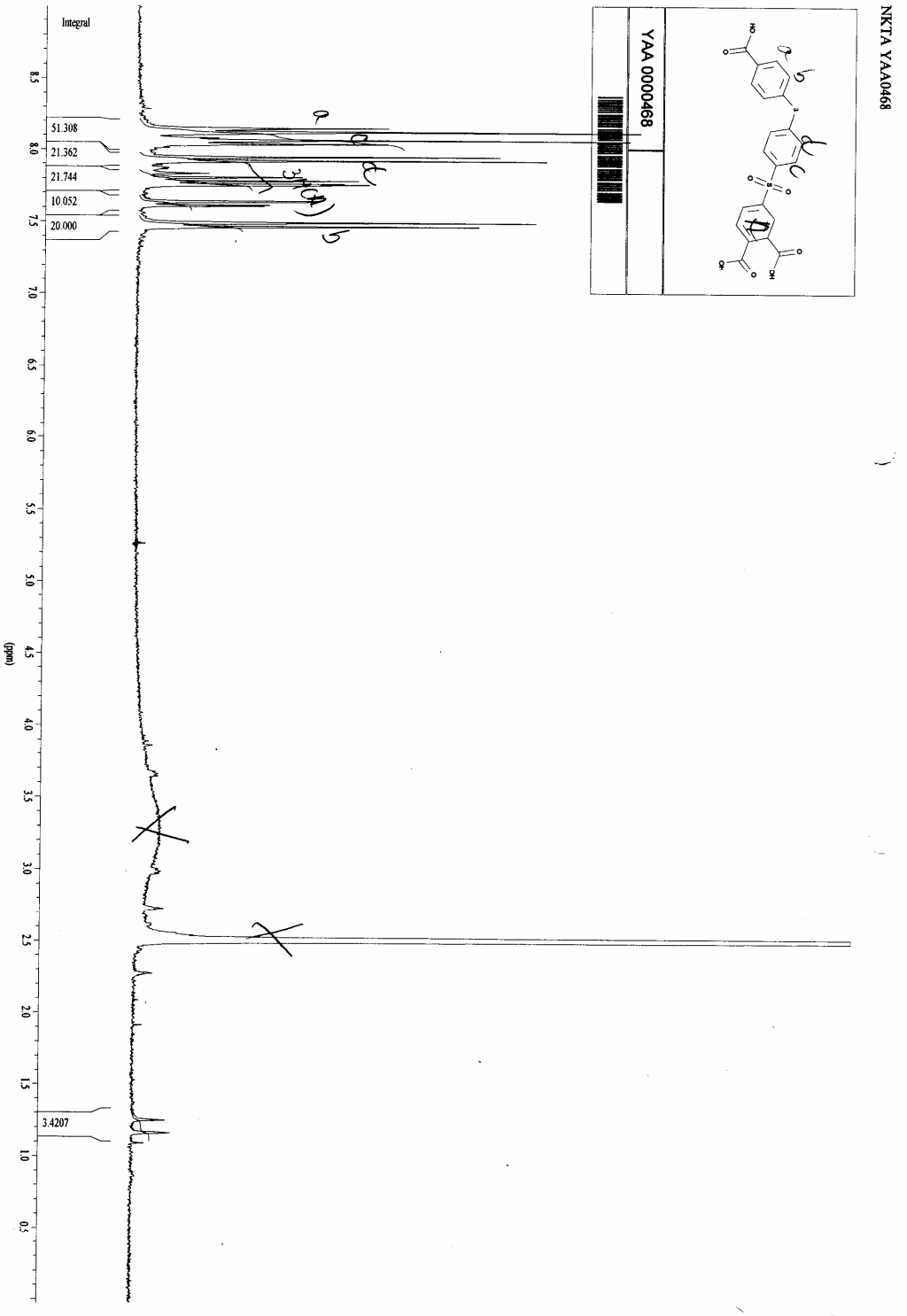

SPN 0000014
BAS 0132568
500
ppm
puro

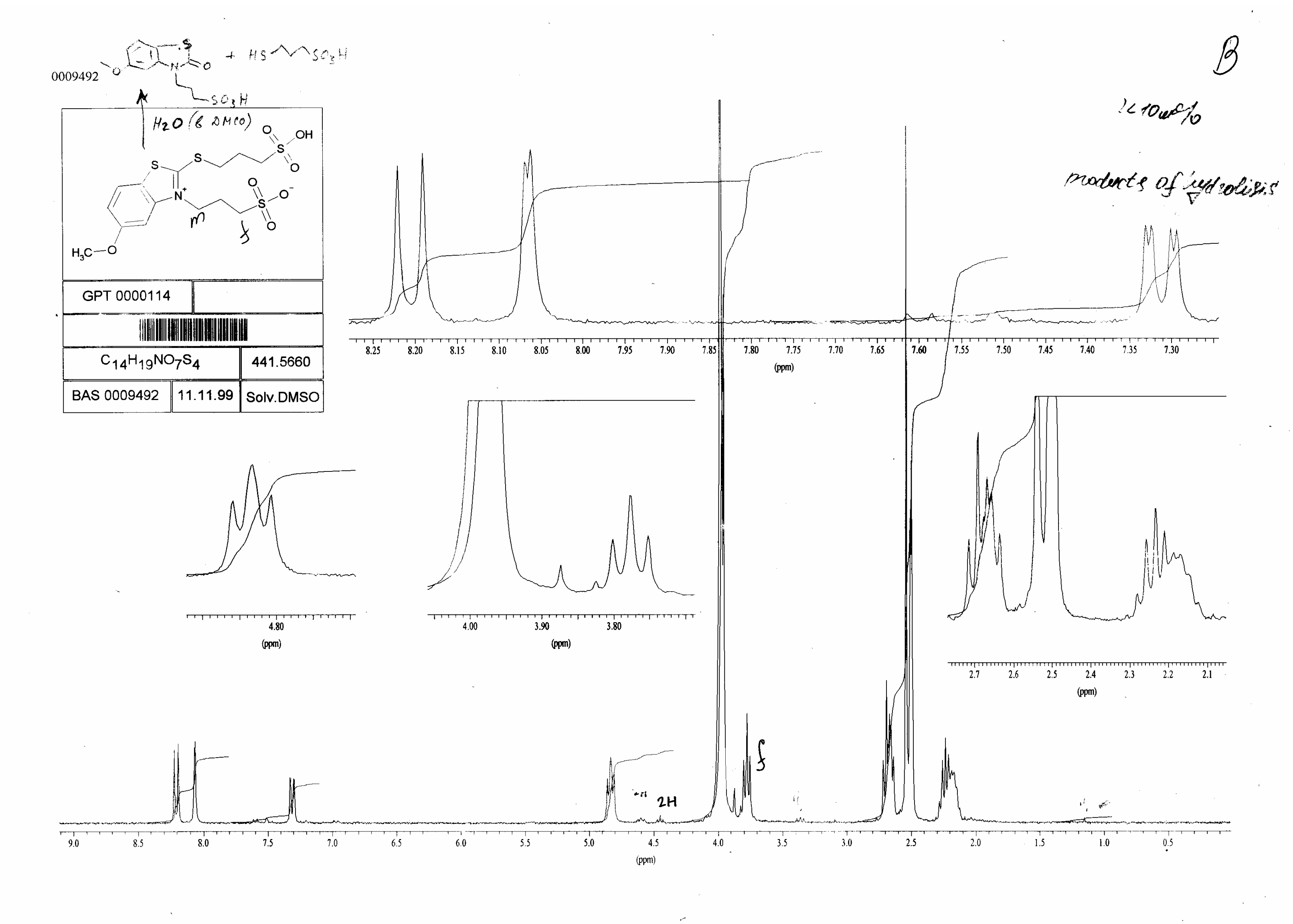
NKTA B0100837



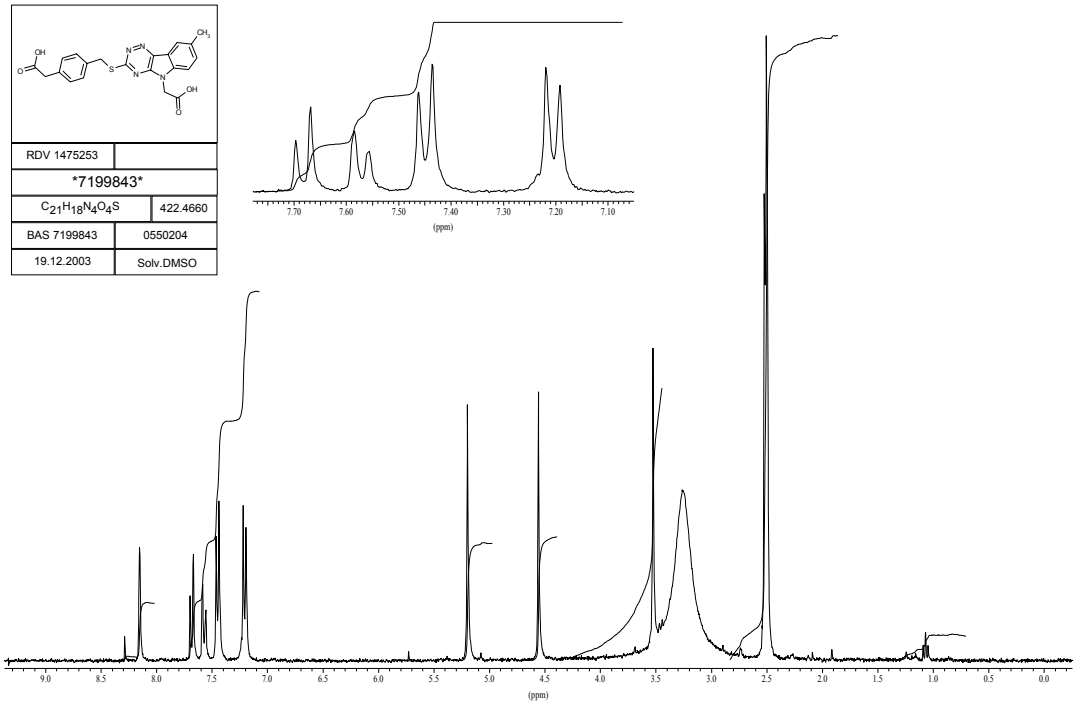
YAA 0001424	
0100837	
$C_{21}H_{14}O_8S_2$	458.4689
BAS 0100837	305114
06.04.2001	Solv.DMSO




NKTA YAA0468
YAA 0000468
Integral
51.308
21.362
21.744
10.052
20.000
3.4207
(ppm)


0009492
+ HS SO3H
SO3H
H2O (6 DMSO)
OH
H3C—O
B
<10wt%
products of hydrolysis
GPT 0000114
C14H19NO7S4
441.5660
BAS 0009492
11.11.99
Solv.DMSO
2H
(ppm)

BAS 7199843



RDV 1475253	
7199843	
$C_{21}H_{18}N_4O_4S$	422.4660
BAS 7199843	0550204
19.12.2003	Solv.DMSO