

Supporting Information

Nicotinic Acid Adenine Dinucleotide Phosphate Analogues Substituted on the Nicotinic Acid and Adenine Ribosides. Effects on ReceptorMediated Ca^{2+} Release

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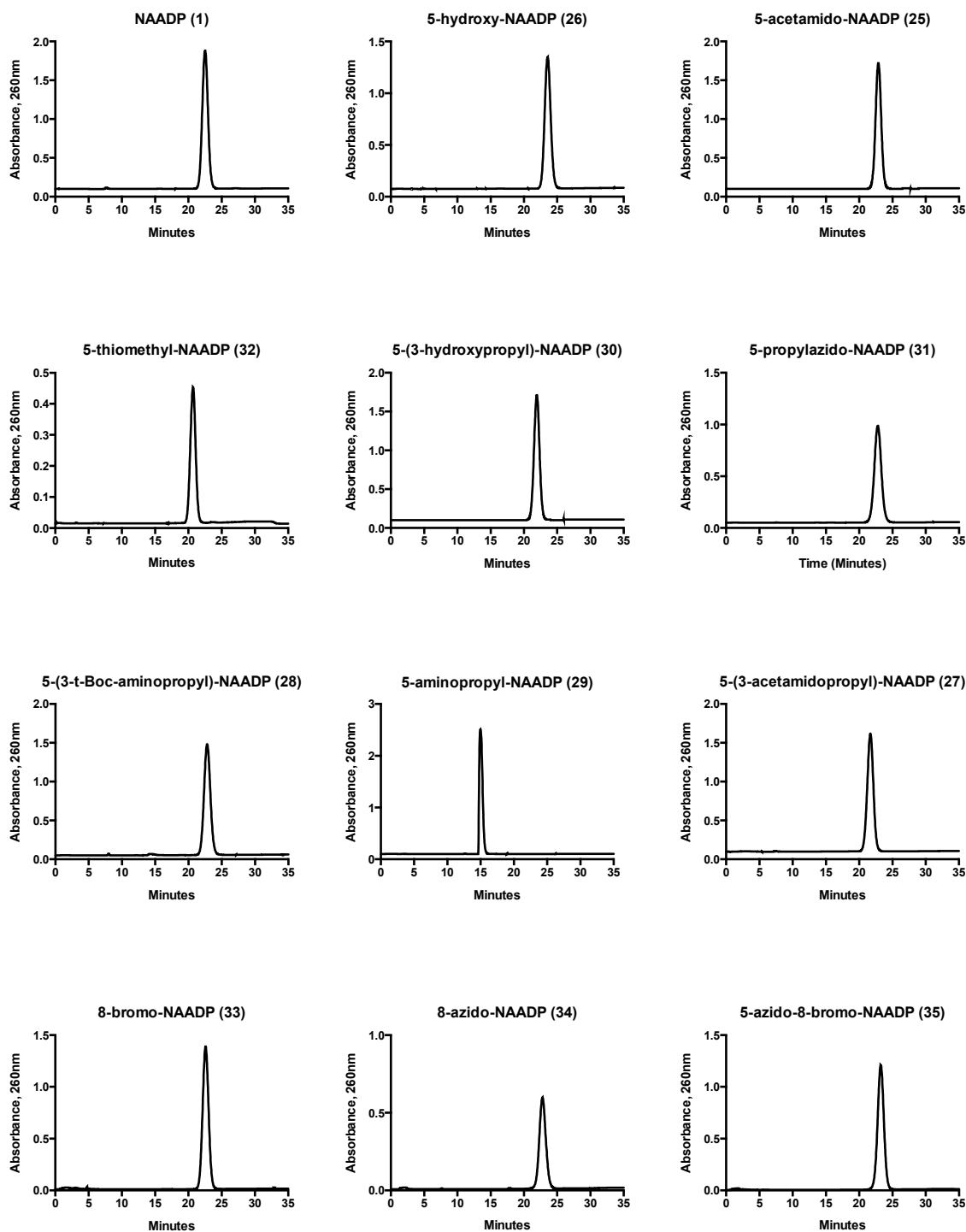


Figure S1. HPLC analysis of NAADP (**1**), and NAADP Derivatives **25-35** using an analytical column of AG MP-1 anion exchange resin (Bio-Rad, Hercules, CA) developed using gradients formed between water (solvent A) and 150 mM aqueous trifluoroacetic acid (solvent B) at a flow rate of 1 mL/min. The chromatography was described by Alexson et al., *Anal. Biochem.* **1981** 116, 357-360. The above chromatograms were generated immediately before biological evaluation.

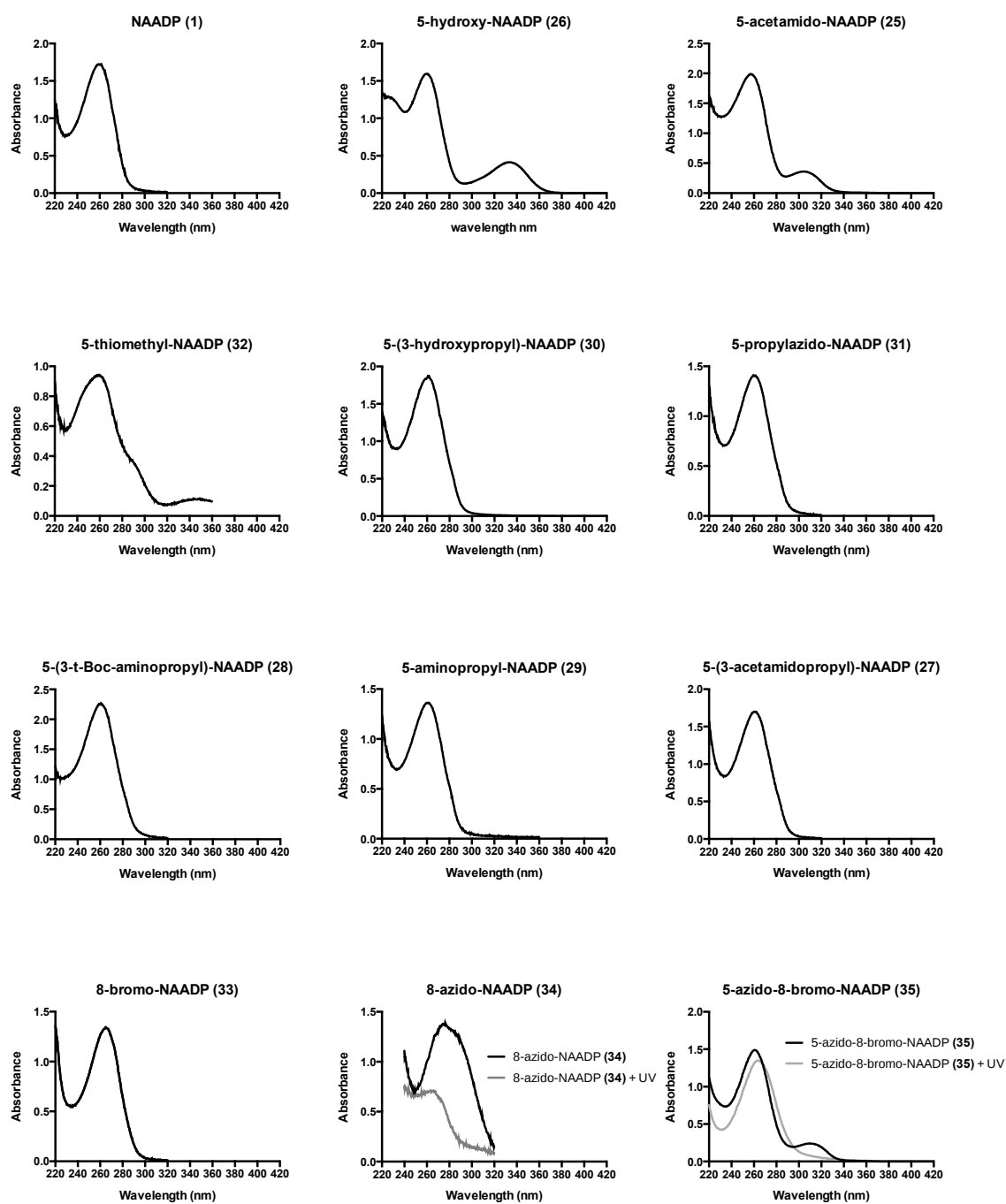
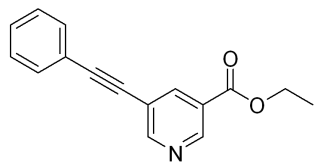
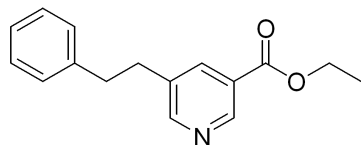
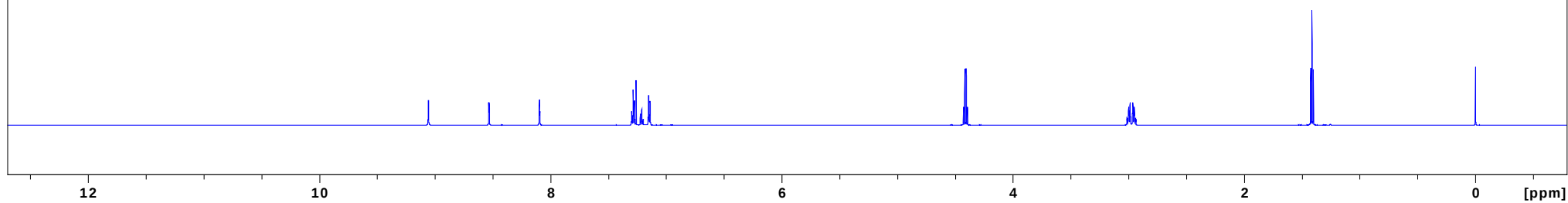


Figure S2. Ultra violet absorption spectra of NAADP **1** and NAADP analogs **25-35**, obtained during HPLC of purified dinucleotides using a diode array detector under the conditions described in Figure S1.

Compound	Formula	C (calcd)	H (calcd)	N (calcd)	C (found)	H (found)	N (found)
5-(2-Phenethynyl)nicotinic acid ethyl ester	C ₁₆ H ₁₃ NO ₂	76.48	5.21	5.57	76.21	5.41	5.54
5-(2-Phenethyl)nicotinic acid ethyl ester	C ₁₆ H ₁₇ NO ₂	75.27	6.71	5.49	75.48	6.80	5.40
2f	C ₁₄ H ₁₃ NO ₂	73.99	5.77	6.16	74.04	5.99	6.03
4	C ₁₃ H ₁₄ N ₂ O ₃	63.40	5.73	11.38	63.57	5.80	11.20
8	C ₁₆ H ₂₀ N ₂ O ₄ •0.1CH ₂ Cl ₂	61.81	6.51	8.95	61.83	6.42	8.60
11	C ₁₄ H ₂₀ N ₂ O ₄ •0.5CH ₂ Cl ₂	53.95	6.56	8.68	53.63	6.36	8.85
13	C ₁₁ H ₁₁ NO ₃	64.38	5.40	6.83	64.43	5.28	6.78

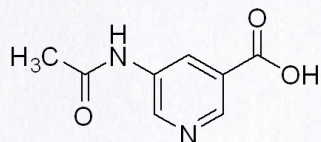
Figure S3. Elemental analysis results for synthetic derivatives described in the Experimental section.

**5-(2-phenylethynyl)-3-pyridinecarboxylic acid ethyl ester**¹H NMR (600 MHz, CDCl₃)

**5-(2-phenylethyl)-3-pyridinecarboxylic acid ethylester**¹H NMR (600 MHz, CDCl₃)

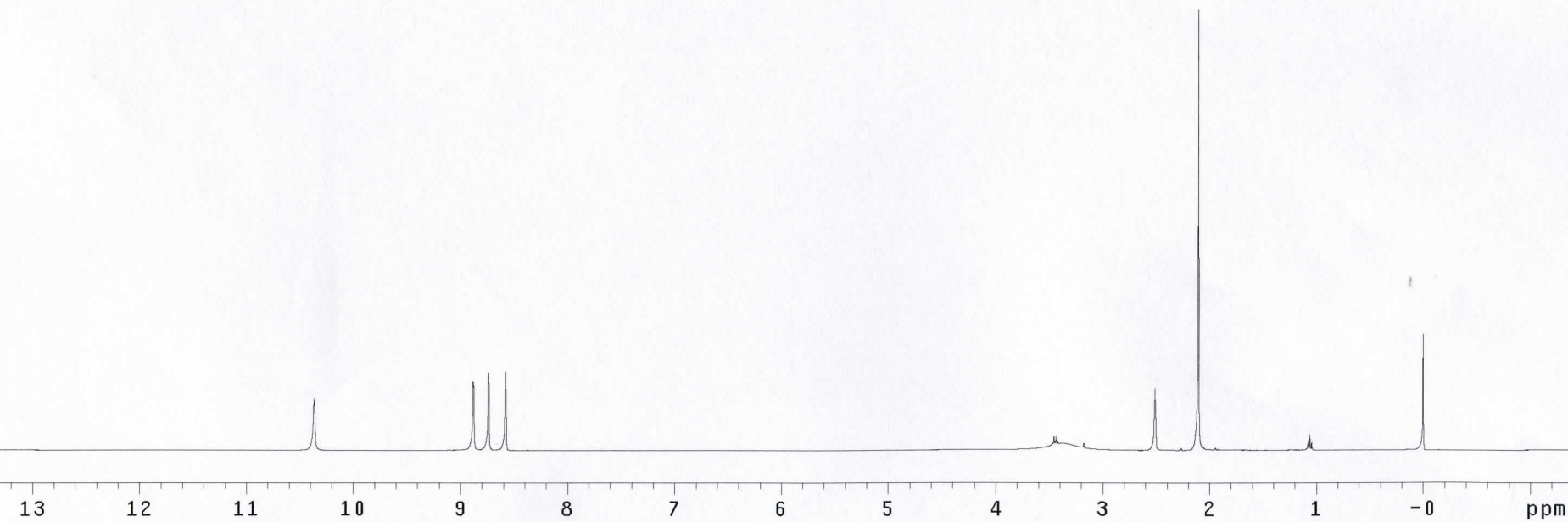
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Ambient temperature
File: n-acetyl-5aminonicotinicacid
NITY-400 "vxrs400"

Relax delay 2 000 sec
Pulse 51 1 degrees
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Width 5999 7 Hz
32 repetitions
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ATA PROCESSING
Line broadening 0 5 Hz
T size 65536
Total time 2 min 31 sec



2c

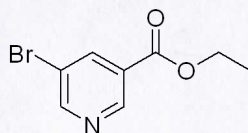
^1H NMR (400 MHz, d_6 -DMSO)



CS1=CC=C(C(=O)O)N=C1¹H NMR (400 MHz, CD₃OD)

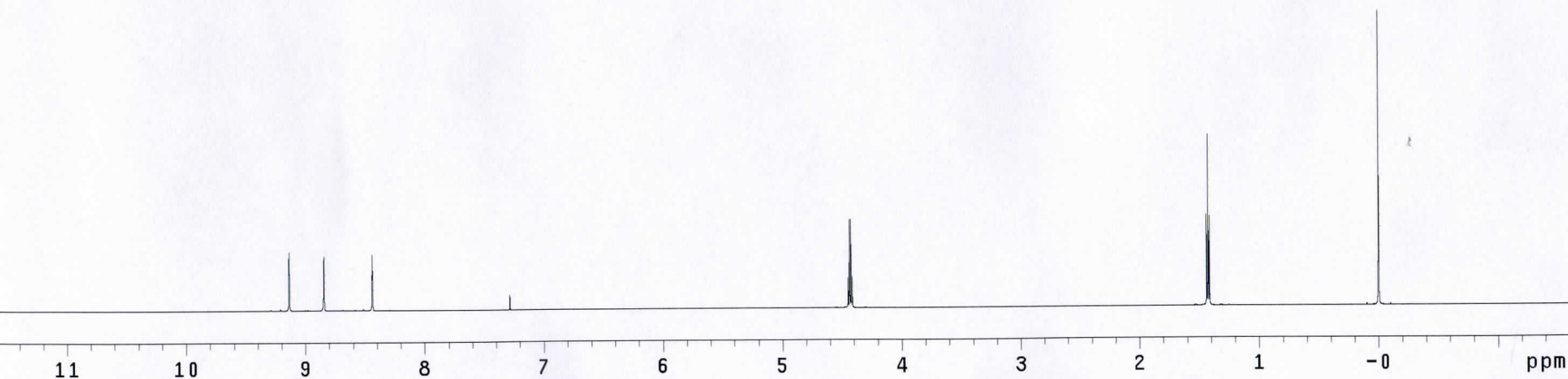
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NOVA-600 "inova-wkst"

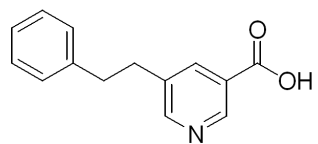
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Pulse 31 3 degrees
Acq time 3 000 sec
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16 repetit ons
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DATA PROCESSING
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FT size 131072
Total time 1 min, 4 sec



2e

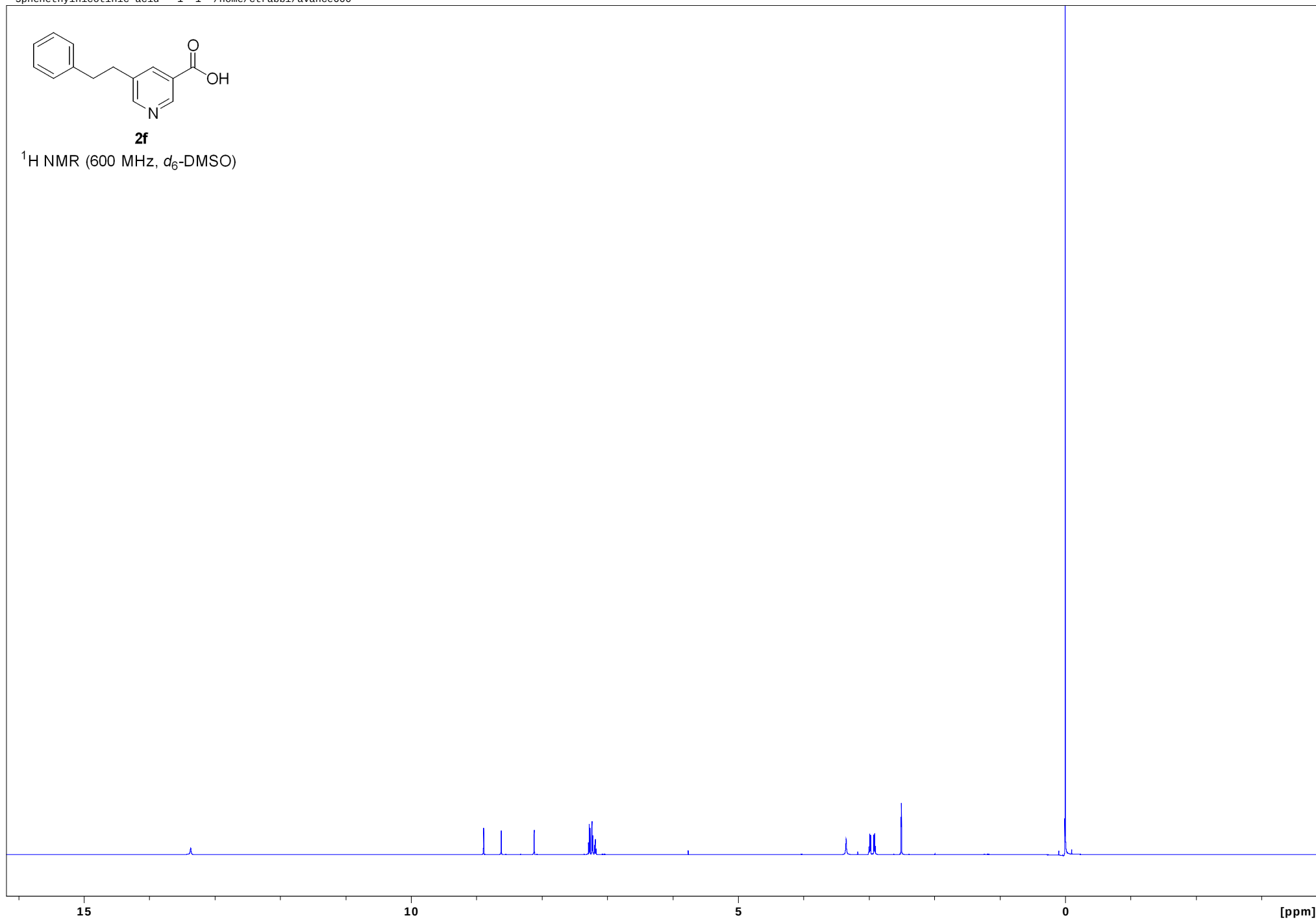
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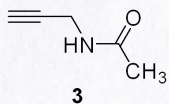


2f

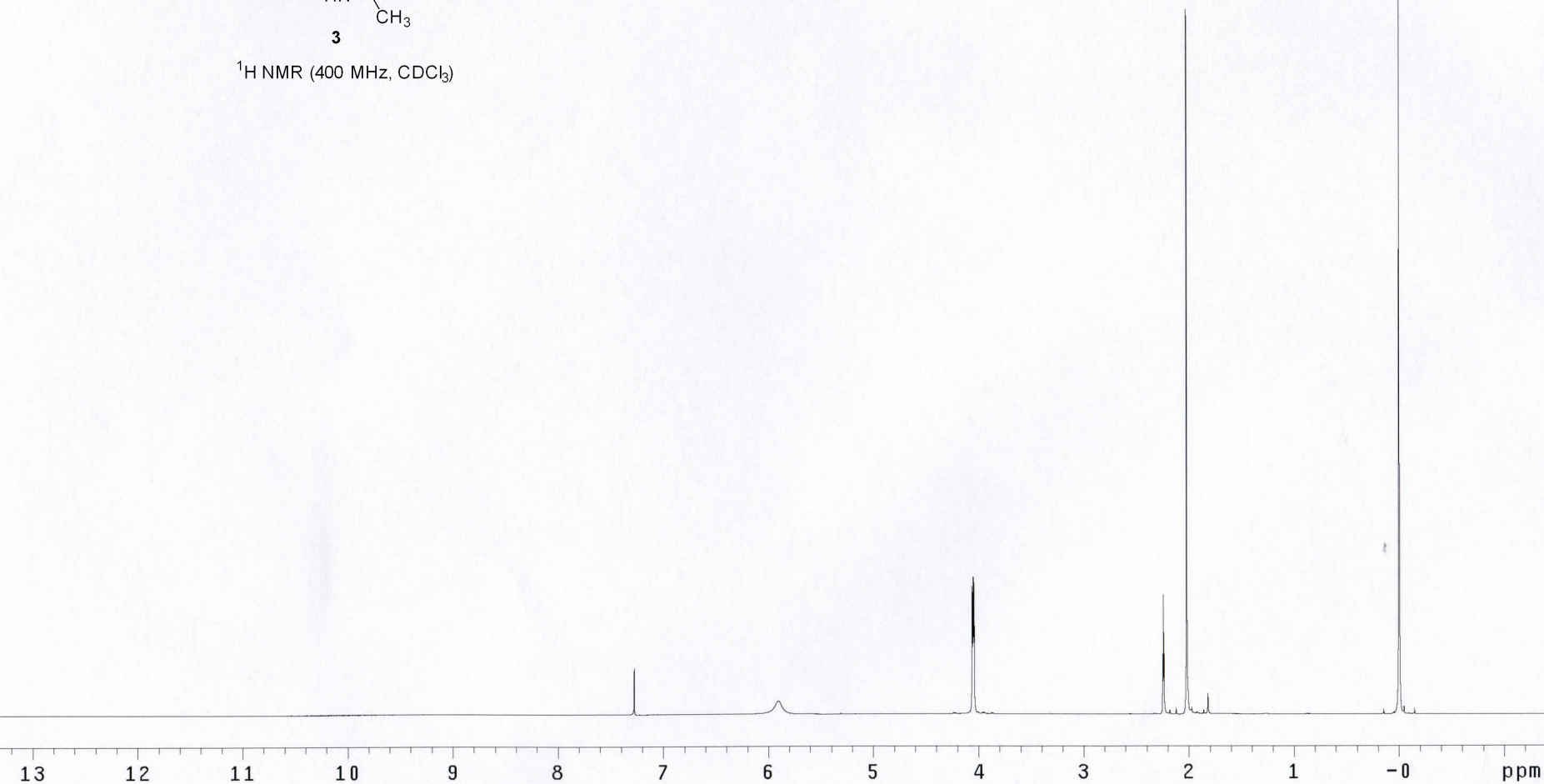
^1H NMR (600 MHz, d_6 -DMSO)



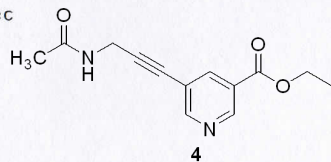
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DATA PROCESSING
Line broadening 0.5 Hz
T size 65536
Total time 2 min 31 sec



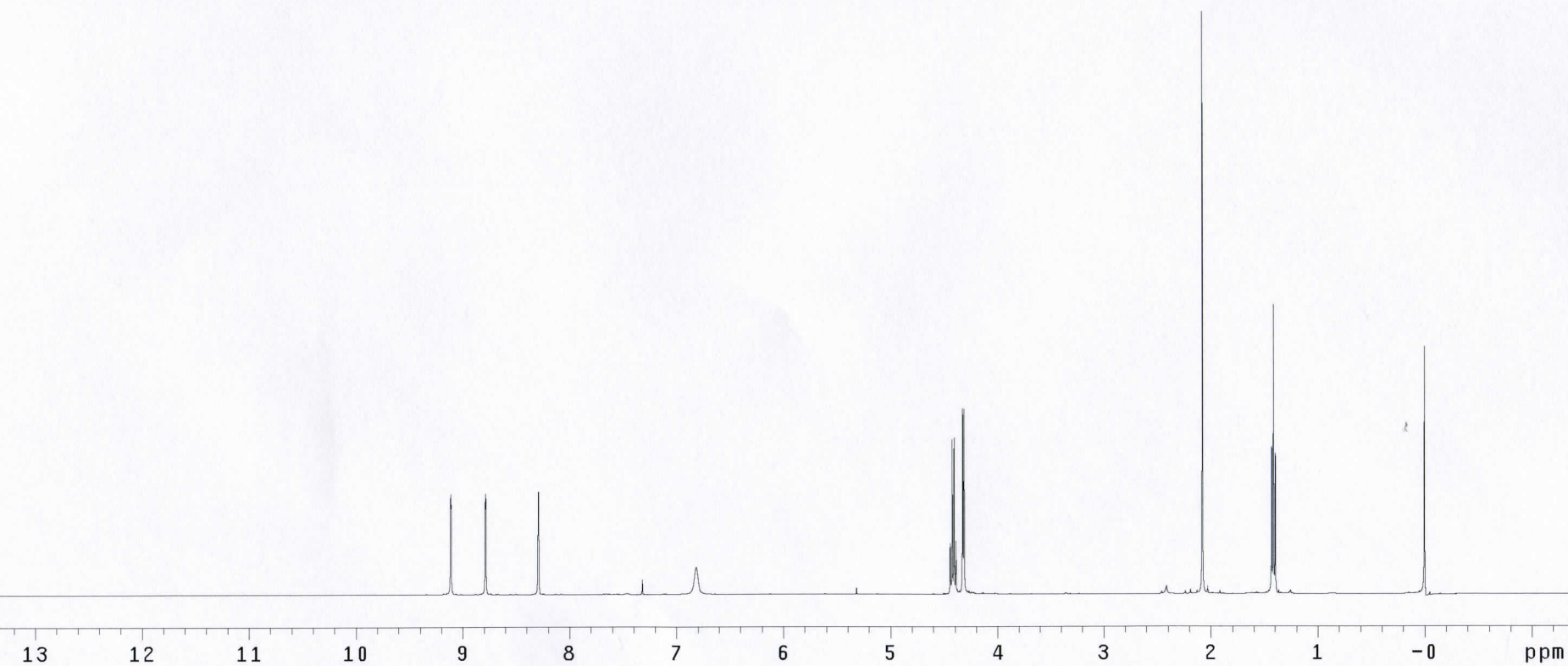
¹H NMR (400 MHz, CDCl₃)



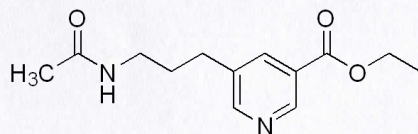
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Size 65536
Total time 2 min 31 sec



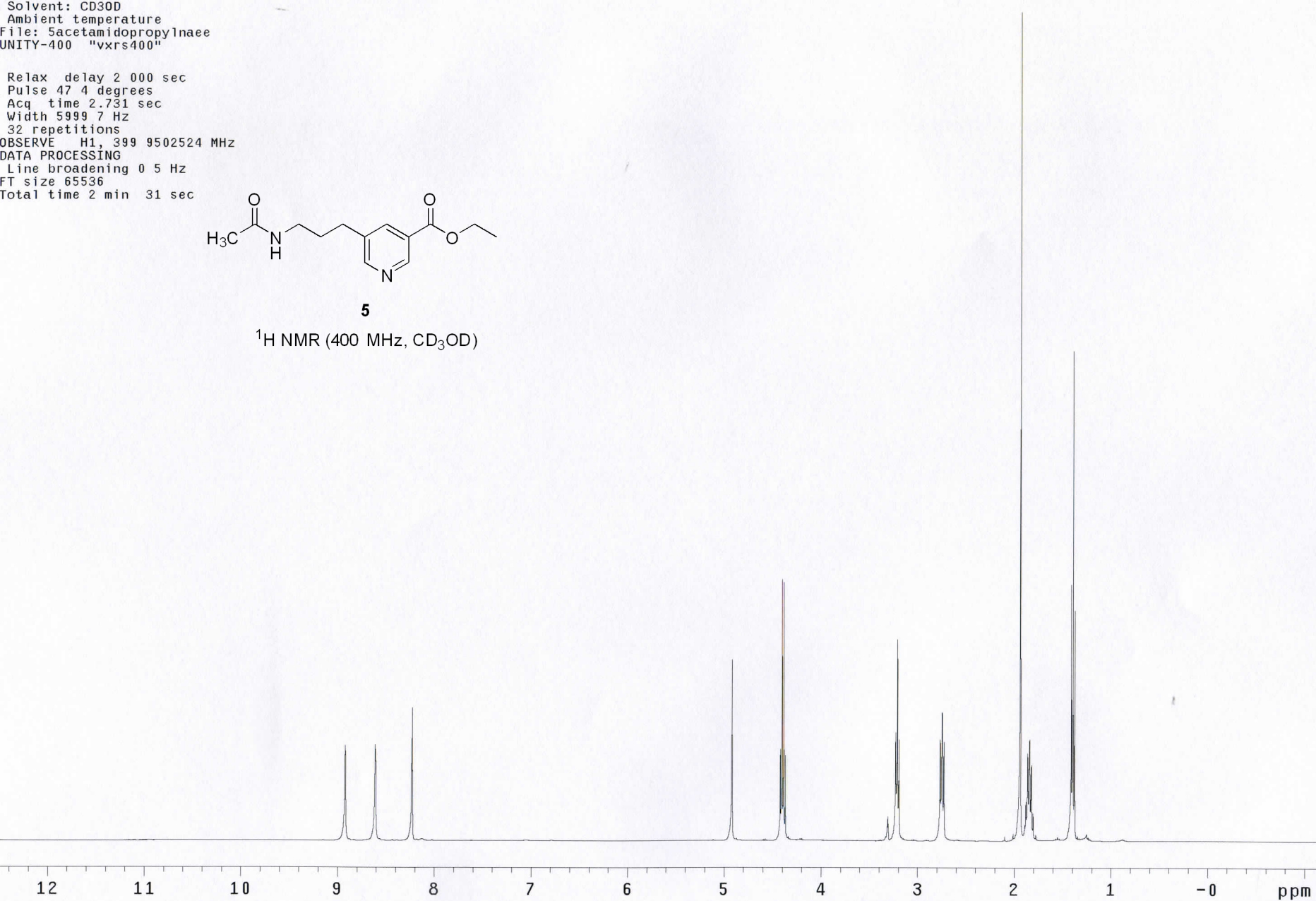
¹H NMR (400 MHz, CDCl₃)



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Total time 2 min 31 sec
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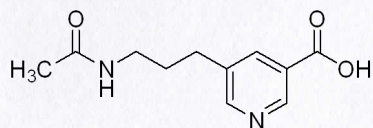


5

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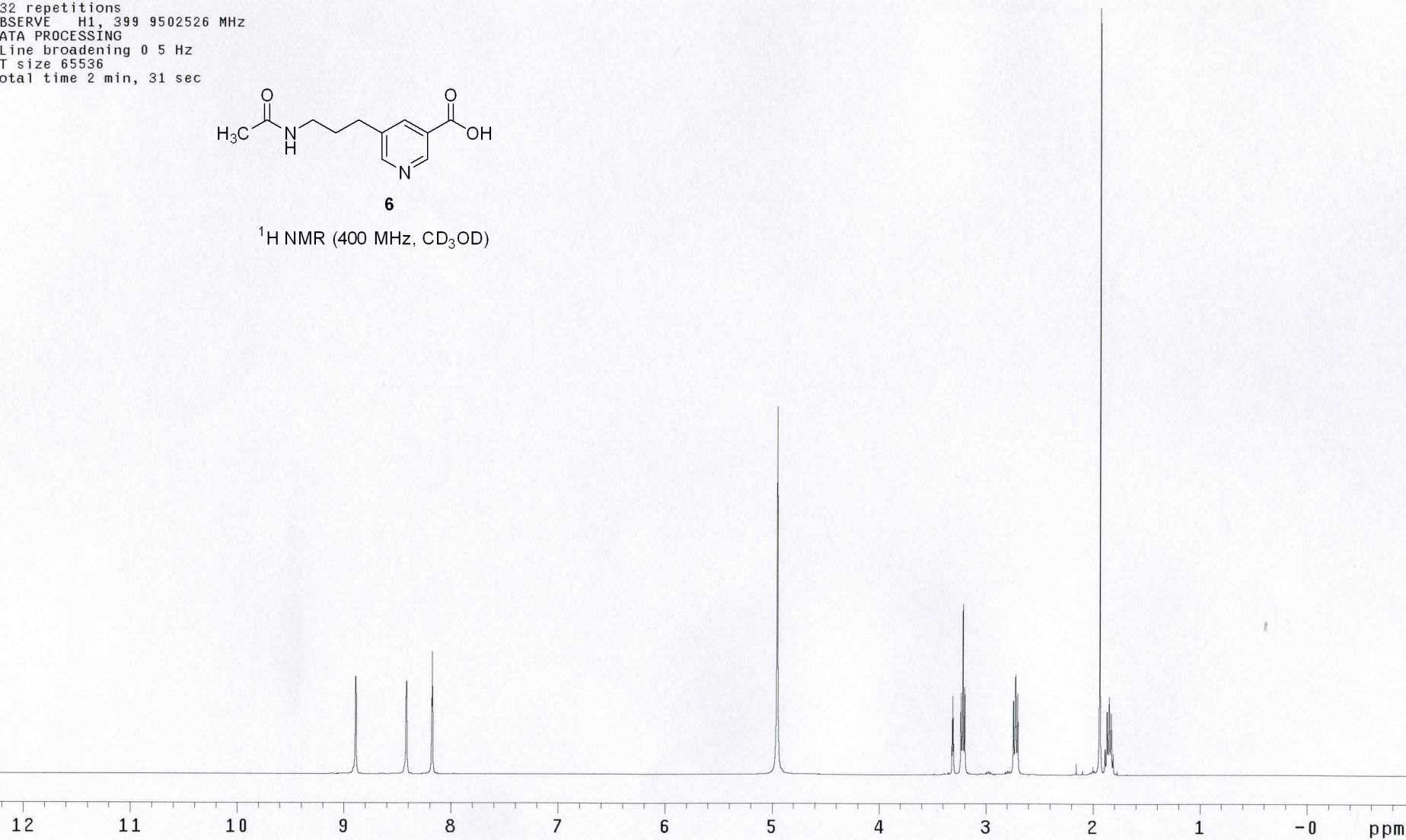
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File: 5acetamidopropylna
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Total time 2 min, 31 sec

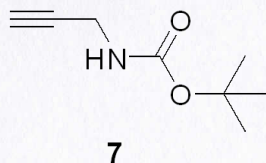


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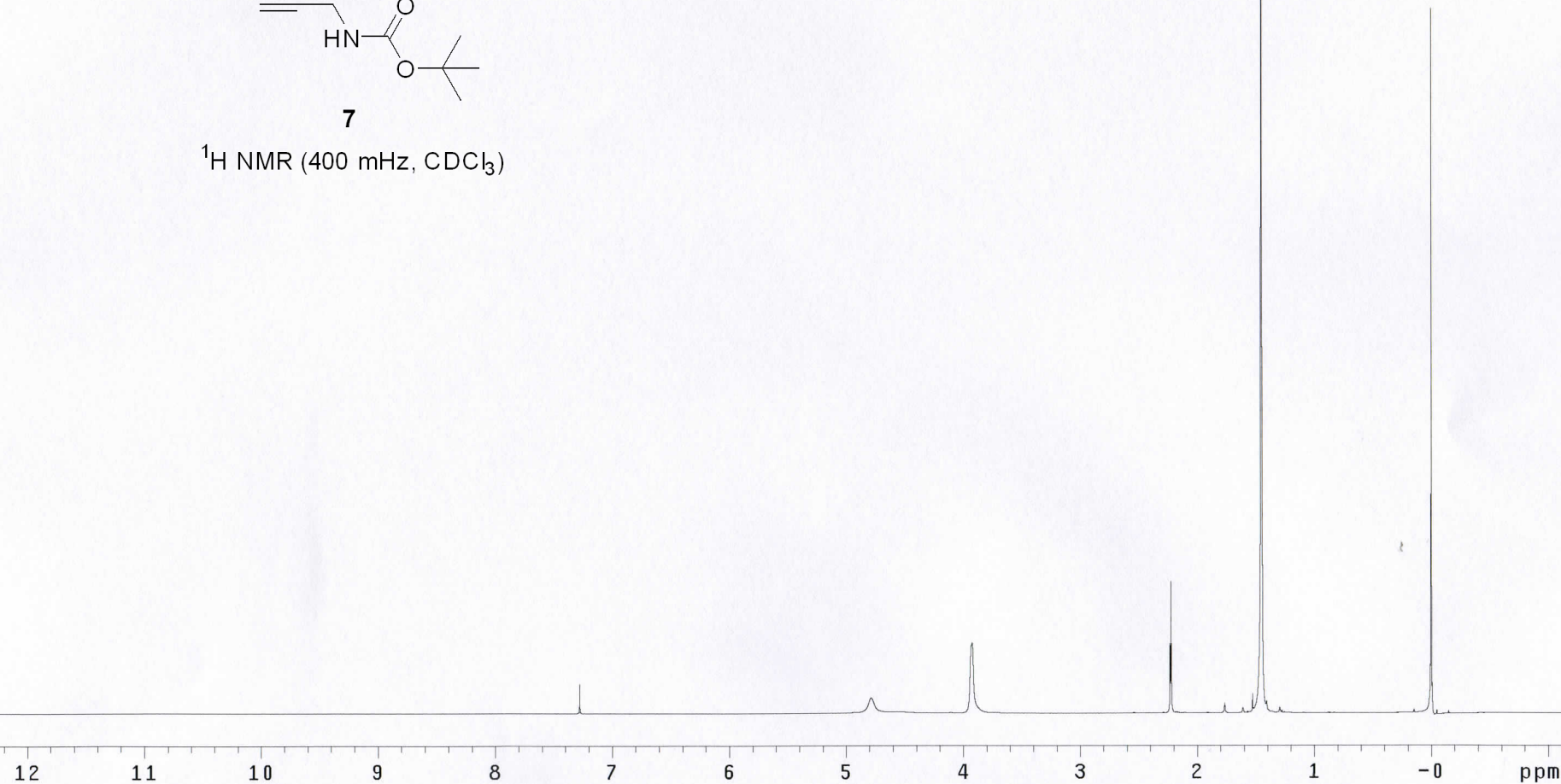
¹H NMR (400 MHz, CD₃OD)



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Ambient temperature
File: tbocacetylene
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Pulse 47 4 degrees
Acq time 2 731 sec
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32 repetitions
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Total time 2 min 31 sec

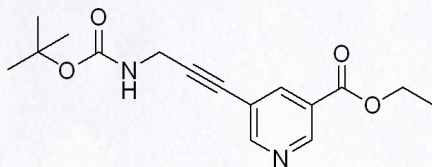


¹H NMR (400 MHz, CDCl₃)



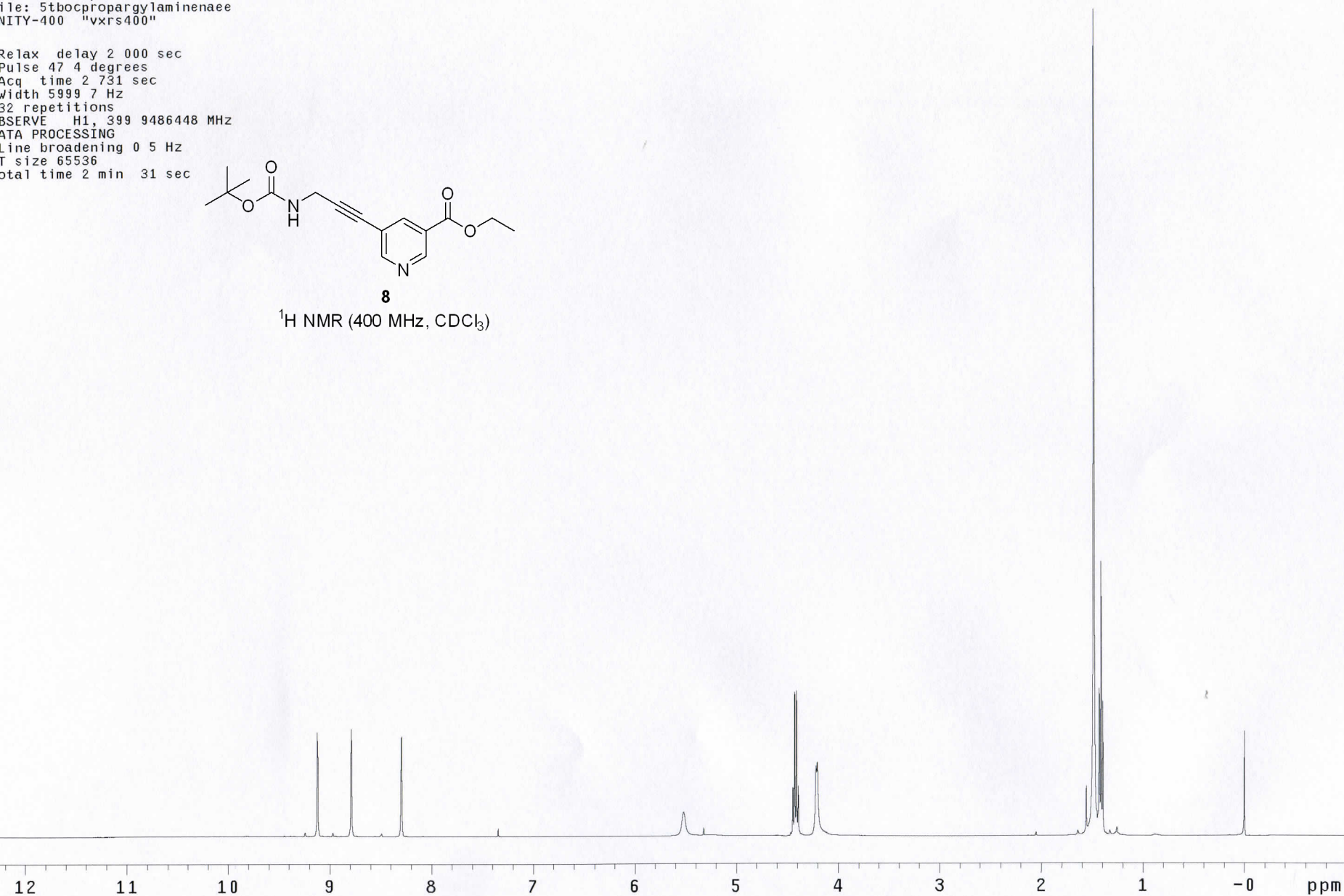
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Ambient temperature
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T size 65536
Total time 2 min 31 sec



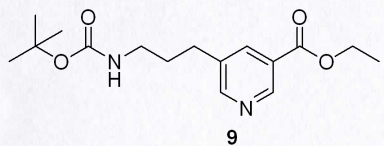
8

¹H NMR (400 MHz, CDCl₃)

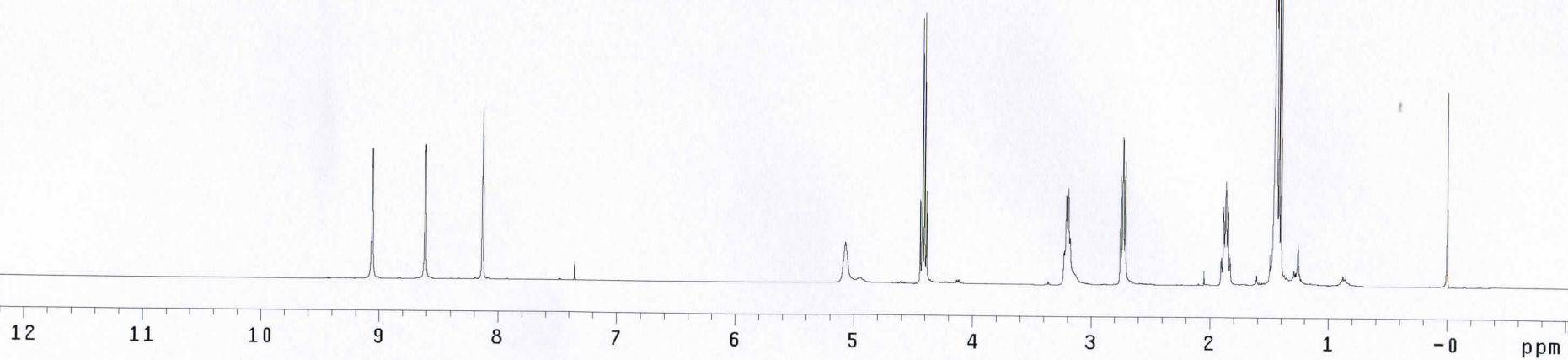


Solvent: CDC13
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File: 5tbocpropylaminoNAEE
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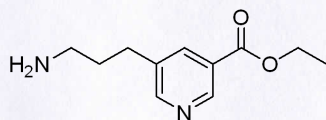


^1H NMR (400 MHz, CDCl_3)



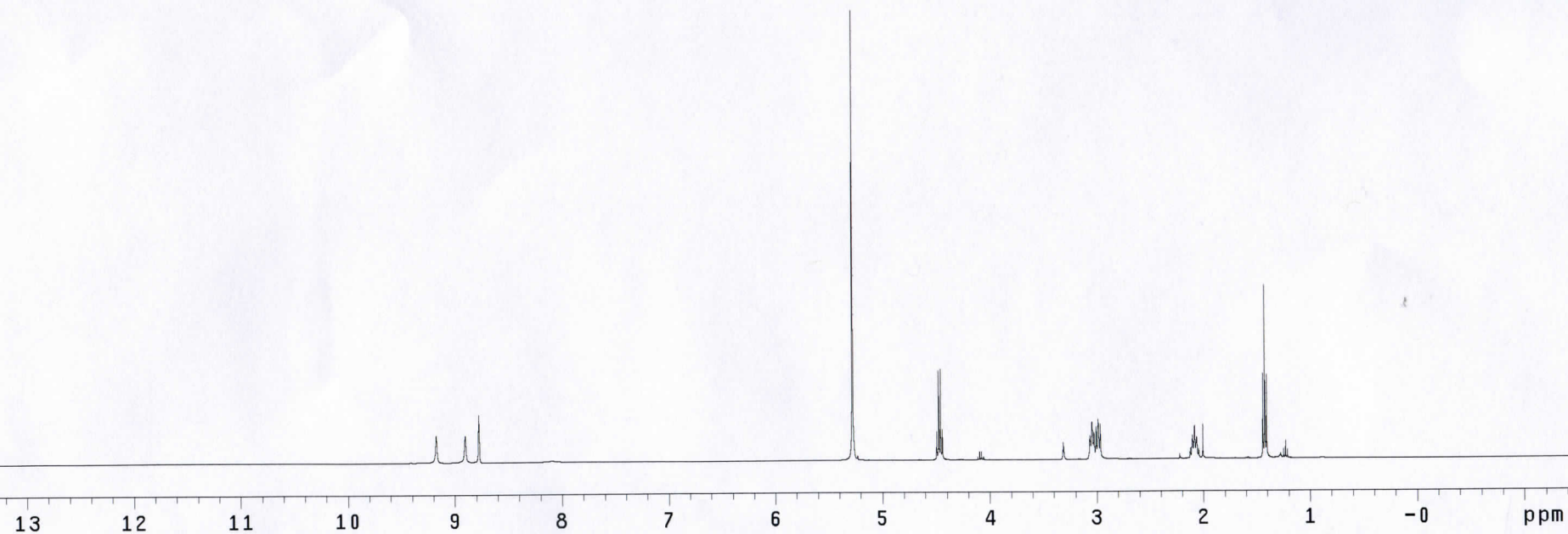
ambient temperature
le: 5propylaminonae
OVA-600 "inova-wkst"

relax delay 2 000 sec
pulse 47 4 degrees
cq time 2 731 sec
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2 repetitions
SERVE H1, 399 9502524 MHz
TA PROCESSING
line broadening 0 5 Hz
size 65536
total time 2 min, 31 sec



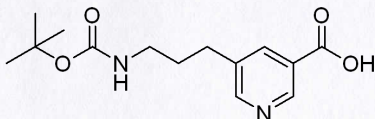
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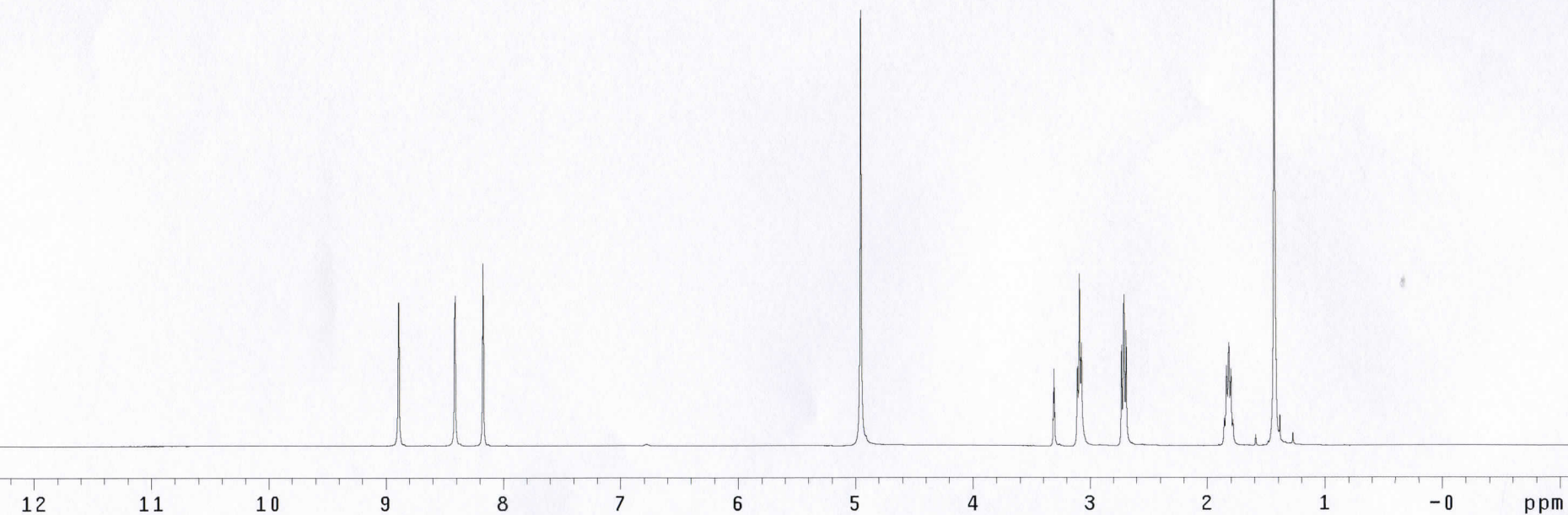
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T size 65536
Total time 2 min 31 sec



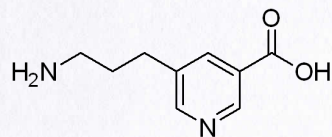
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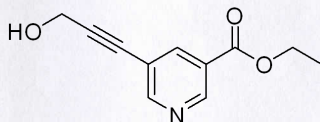


12

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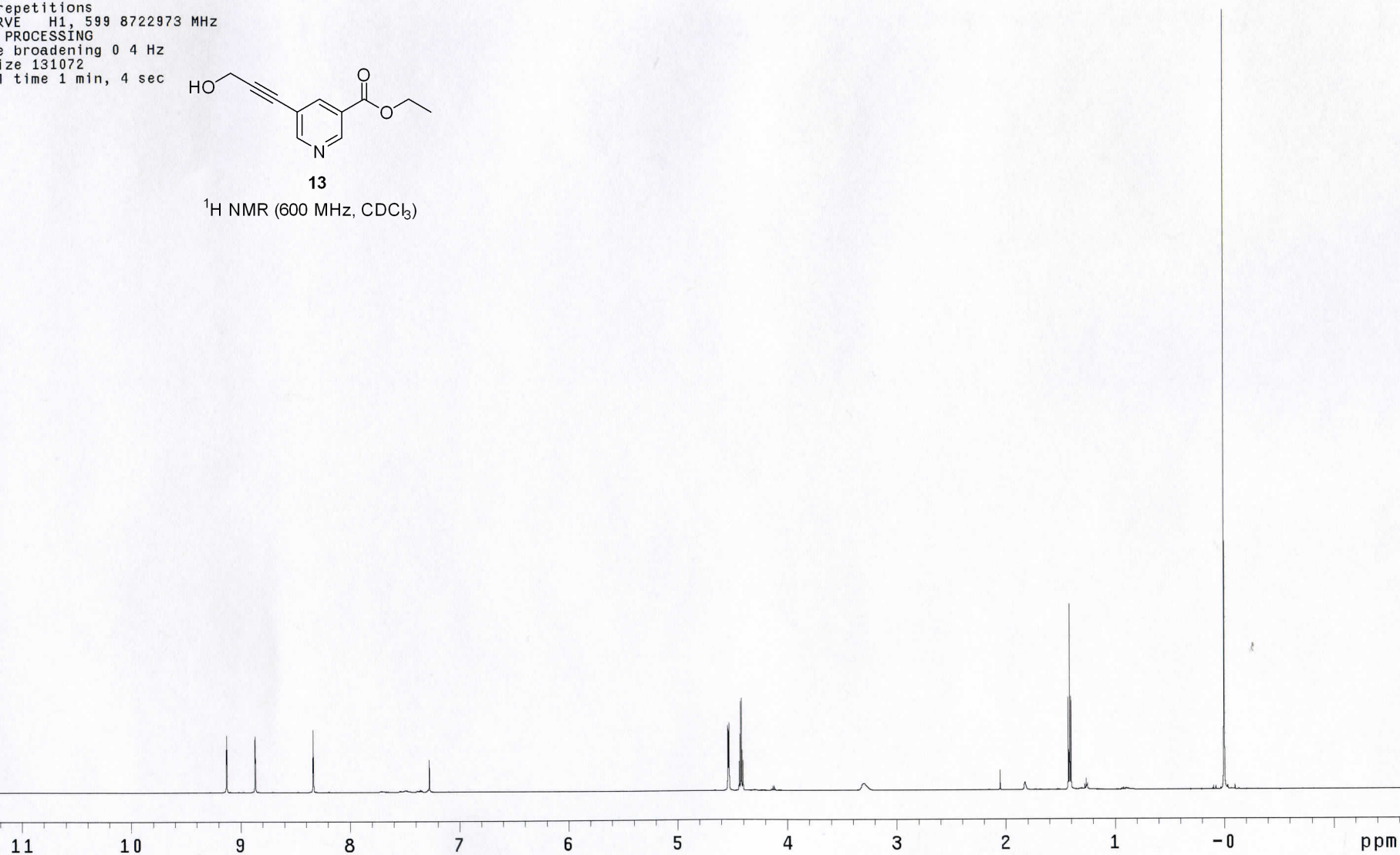
11 10 9 8 7 6 5 4 3 2 1 -0 ppm

Ambient temperature
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Pulse 30 9 degrees
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ATA PROCESSING
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r size 131072
otal time 1 min, 4 sec

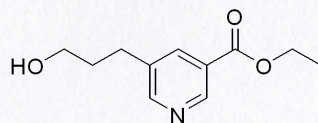


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¹H NMR (600 MHz, CDCl₃)

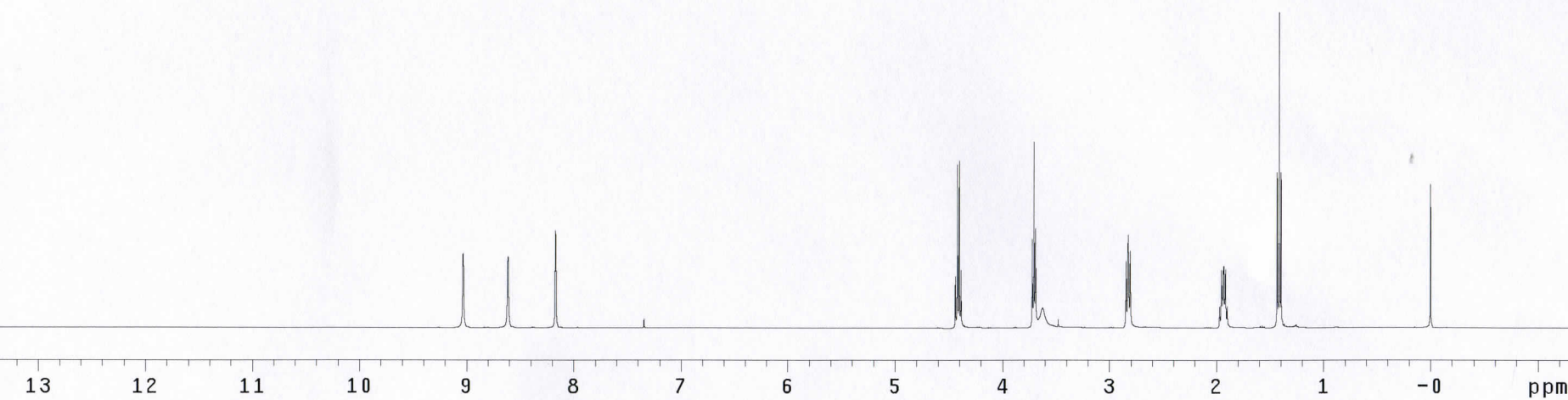


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Acq time 2 731 sec
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DATA PROCESSING
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T size 65536
Total time 2 min 31 sec

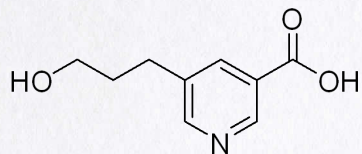


14

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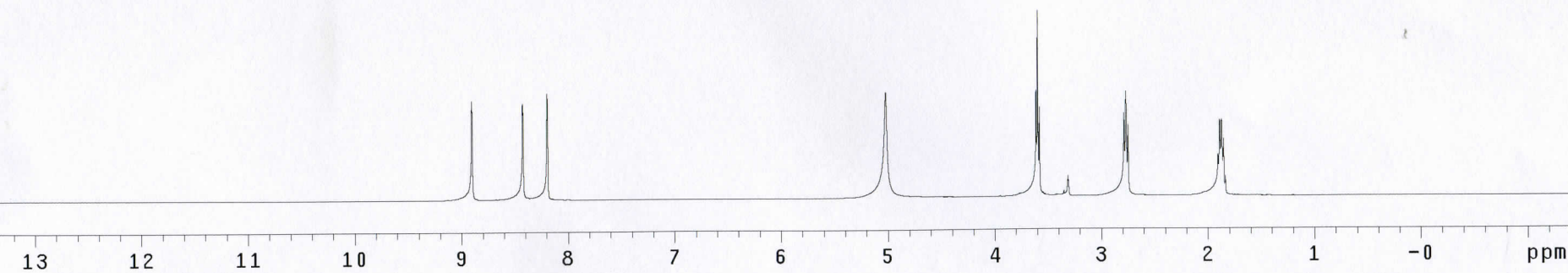


Solvent: CD3OD
Ambient temperature
File: 5propyloxyNA
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Line broadening 0 5 Hz
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Total time 2 min 31 sec

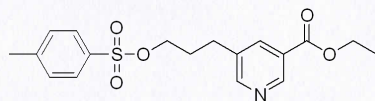


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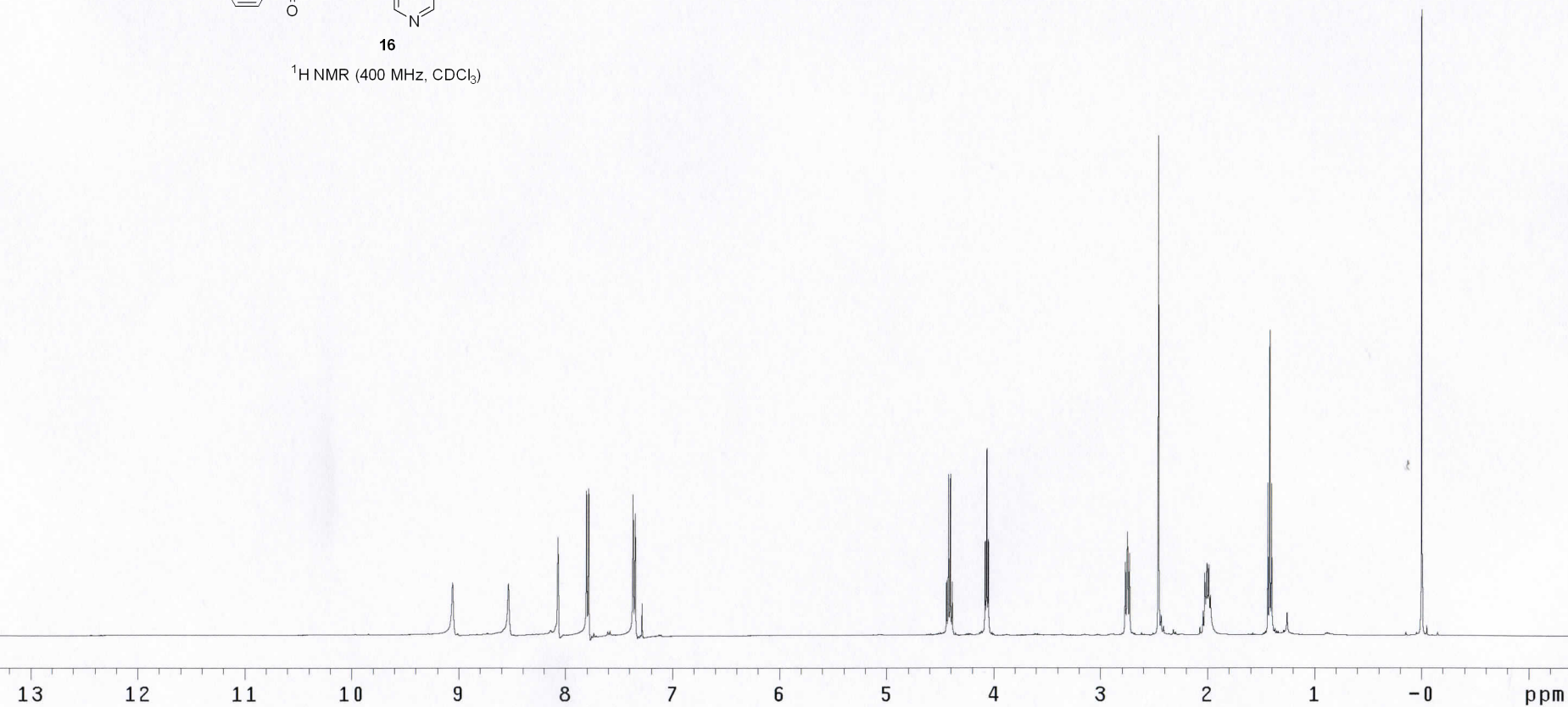


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Ambient temperature
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Pulse 47 4 degrees
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32 repetitions
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ATA PROCESSING
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r size 65536
otal time 2 min 31 sec

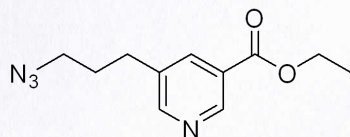


16

¹H NMR (400 MHz, CDCl₃)

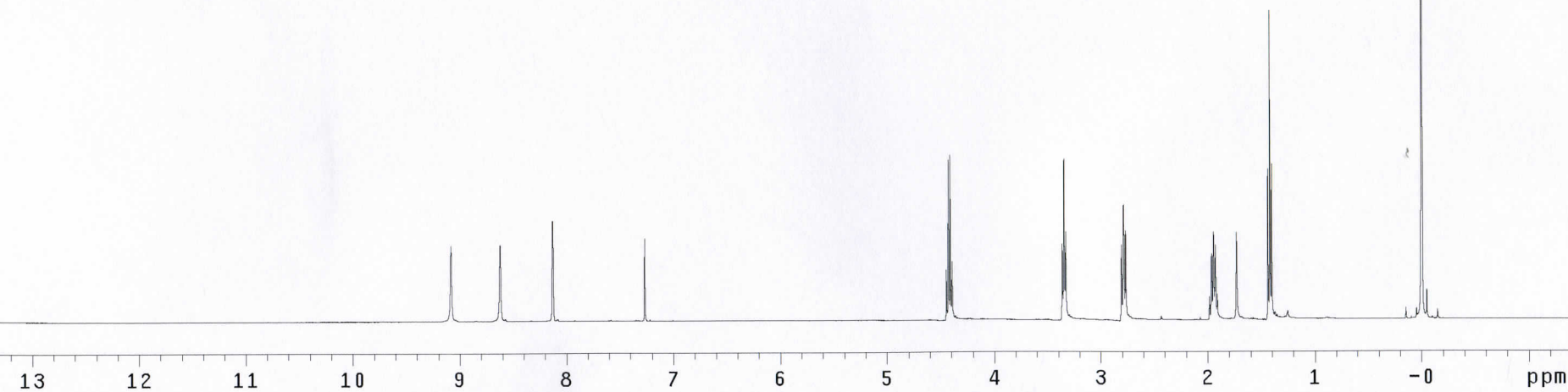


Solvent: CDCl₃
Ambient temperature
le: nmr2-84
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ATA PROCESSING
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T size 65536
otal time 2 min, 31 sec



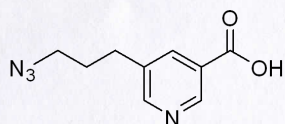
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¹H NMR (400 MHz, CDCl₃)



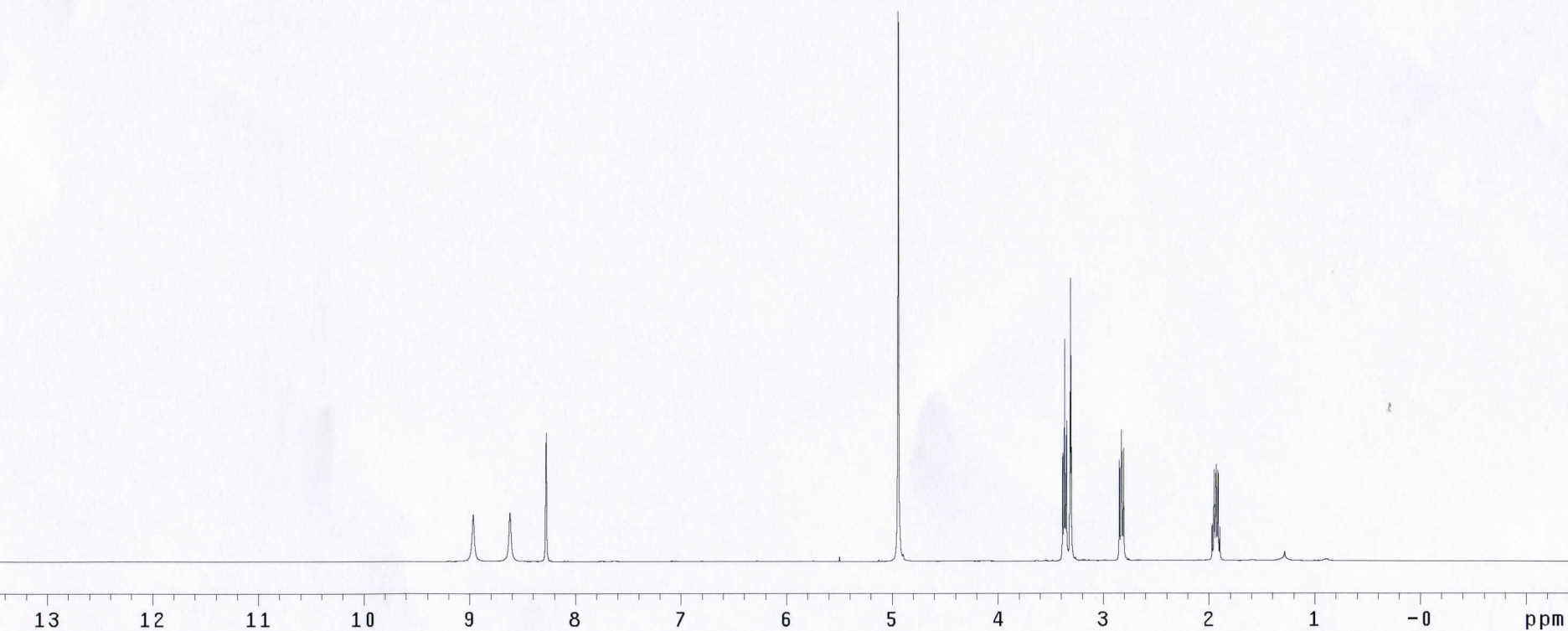
Ambient temperature
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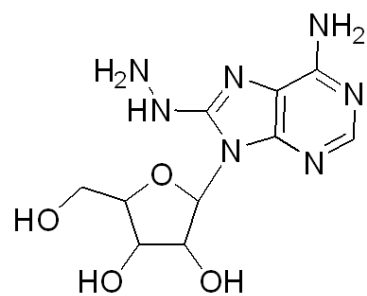
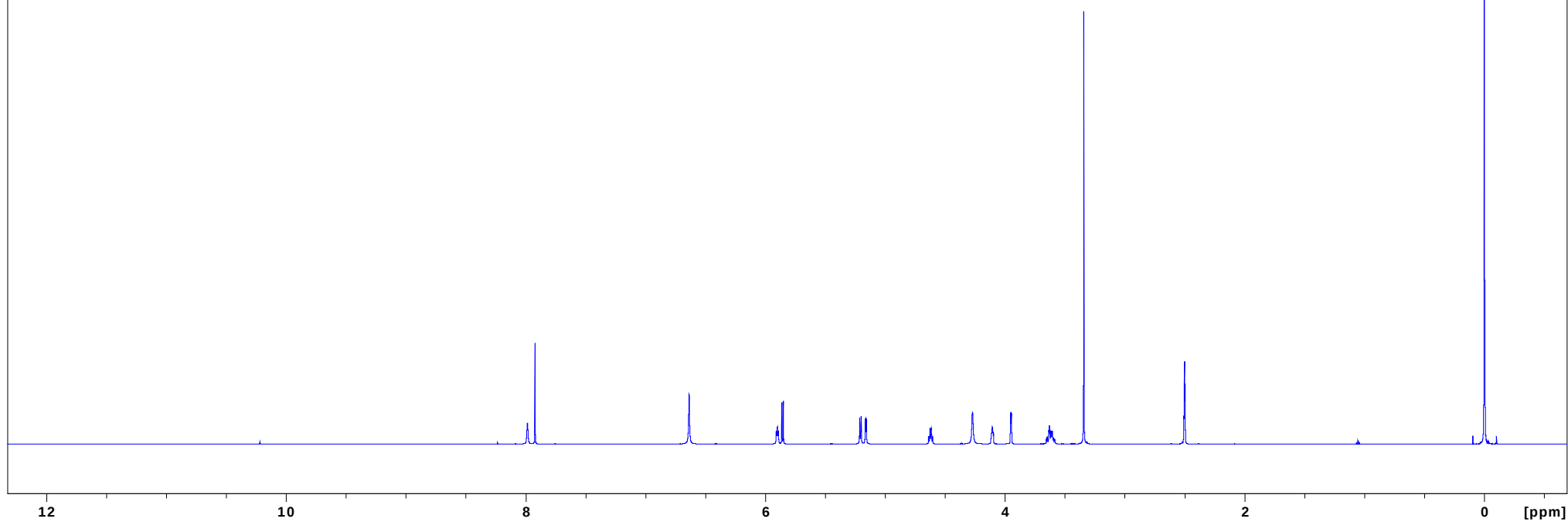
Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999 7 Hz
32 repetitions
OBSERVE H1, 399 9502526 MHz
DATA PROCESSING
Line broadening 0 5 Hz
FT size 65536
Total time 2 min 31 sec

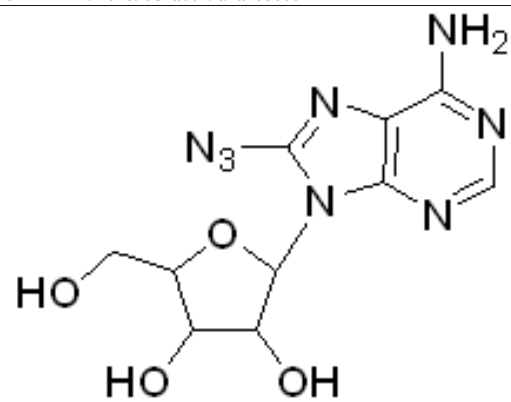
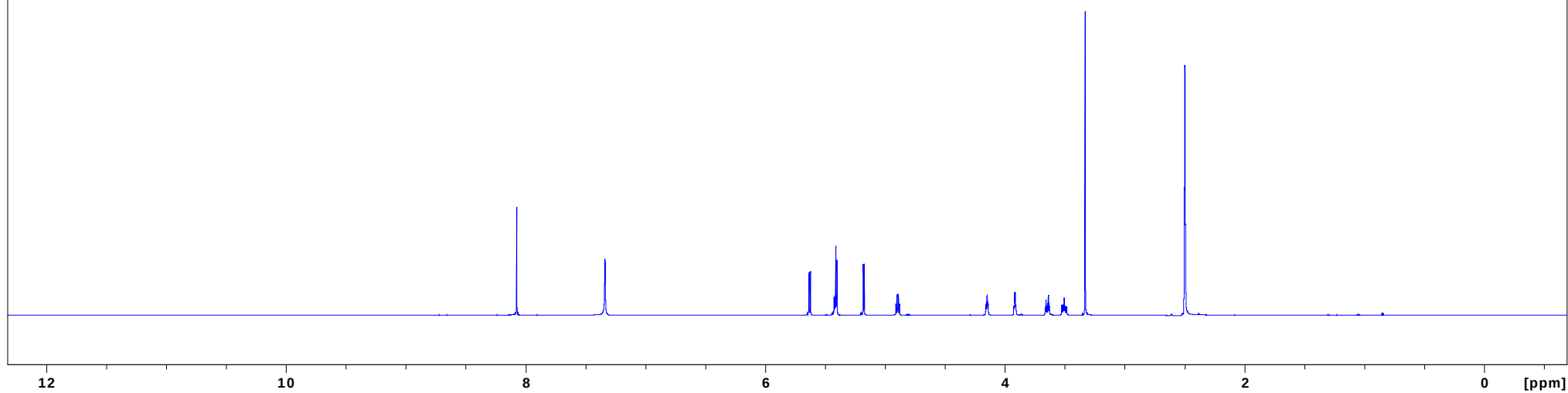


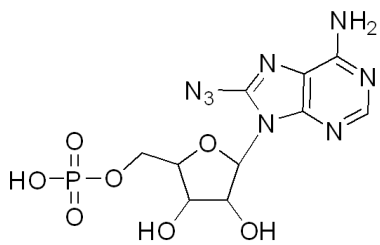
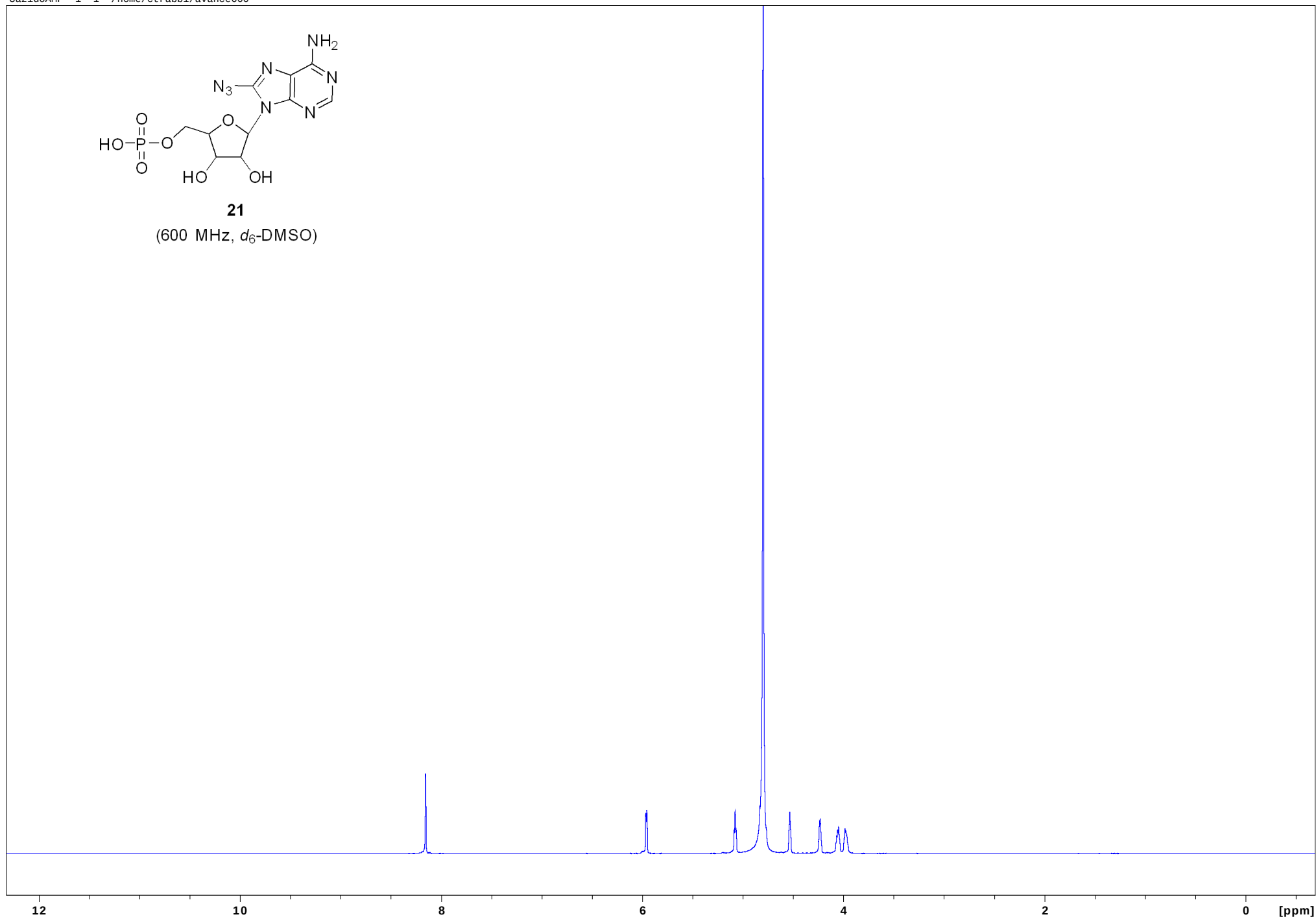
18

^1H NMR (400 MHz, CD_3OD)



**19** ^1H NMR (600 MHz, d_6 -DMSO)

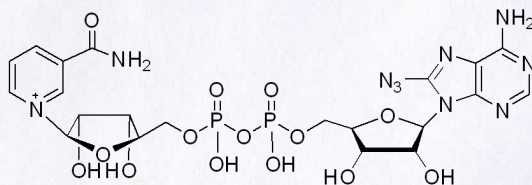
**20**(600 MHz, d_6 -DMSO)

**21**(600 MHz, d_6 -DMSO)

S31

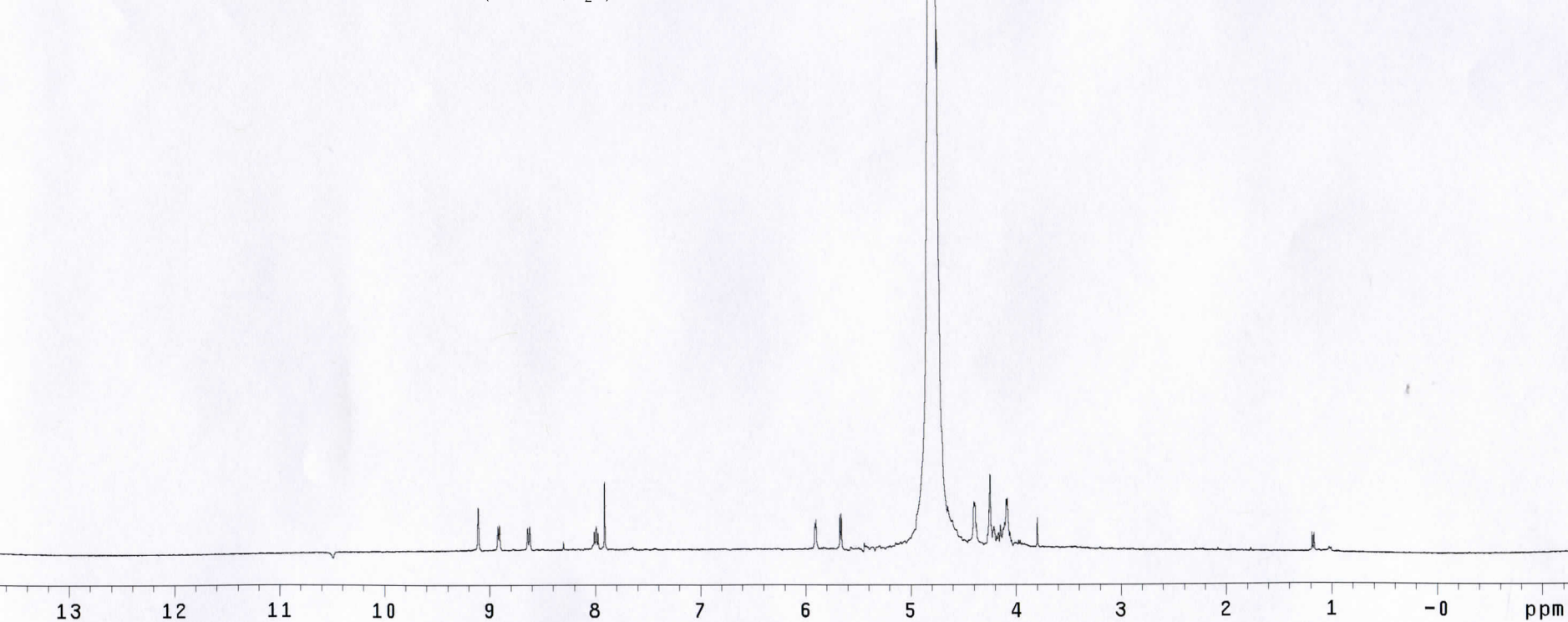
Ambient temperature
File: 8AzidoNAD
INOVA-600 "inova-wkst"

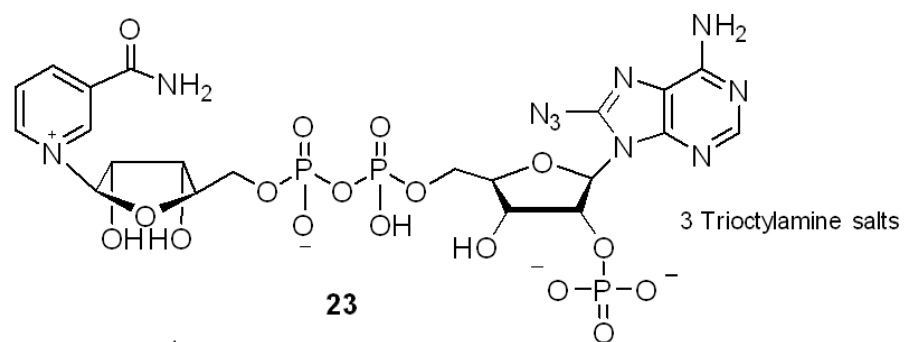
Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999.7 Hz
32 repetitions
OBSERVE H1, 399 9496436 MHz
DATA PROCESSING
Line broadening 0 5 Hz
FT size 65536
Total time 2 min, 31 sec



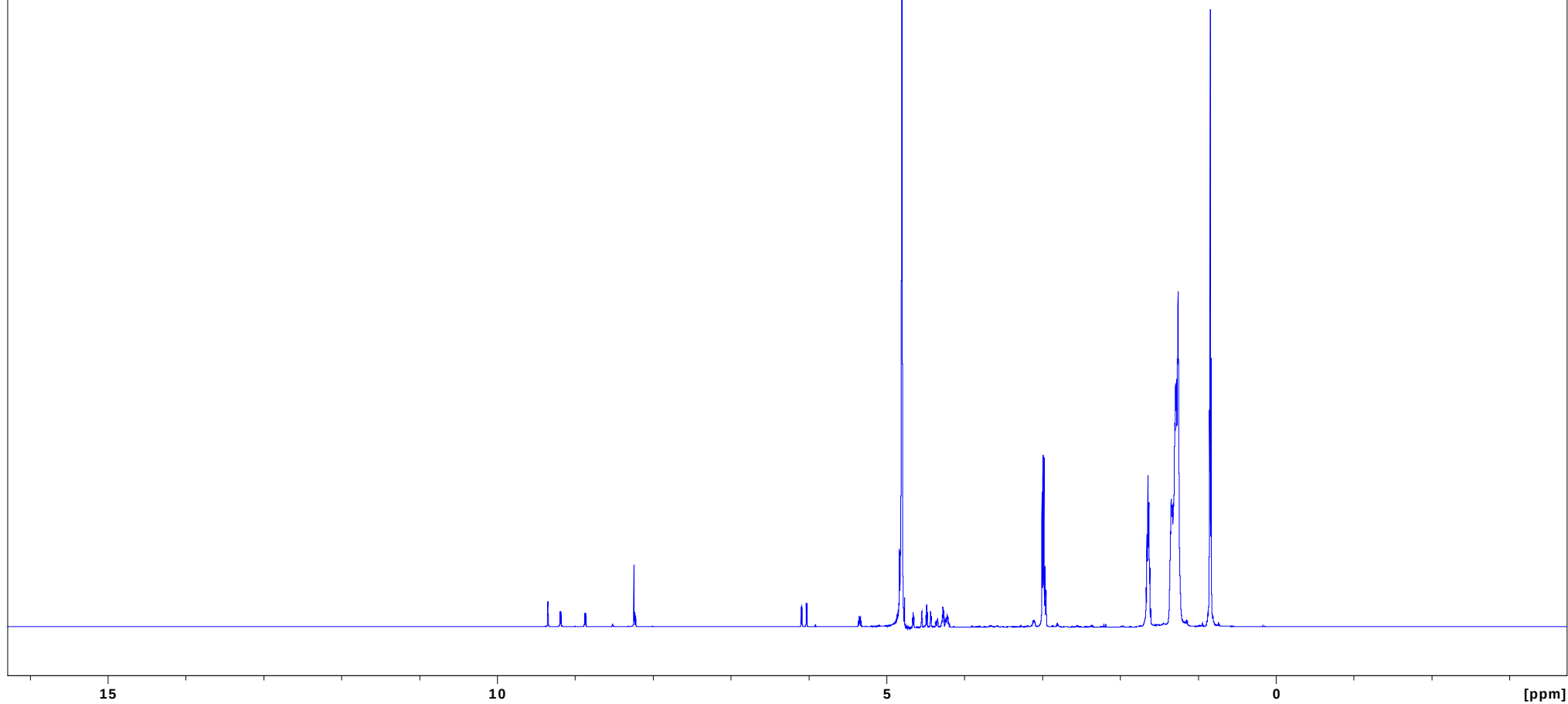
22

¹H NMR (600 MHz, D₂O)



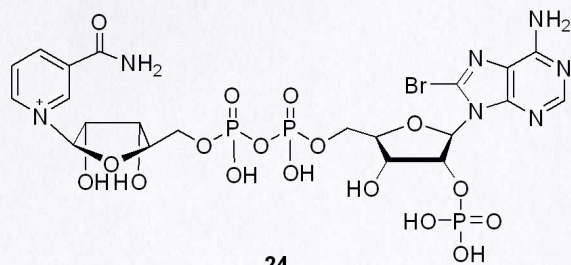


¹H NMR (600 MHz, D₂O)



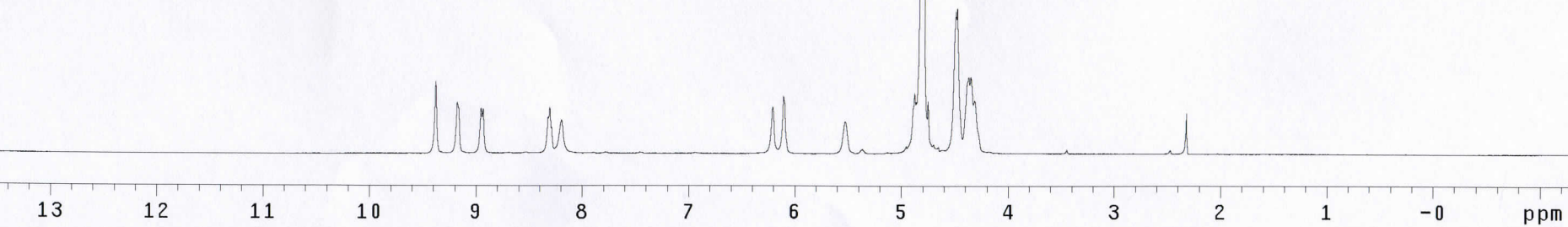
Solvent: D2O
Ambient temperature
File: 8-BrNADP
UNITY-400 "vxrs400"

Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999 7 Hz
32 repetitions
OBSERVE H1, 399.9496465 MHz
DATA PROCESSING
Line broadening 0.5 Hz
F2 size 65536
Total time 2 min 31 sec

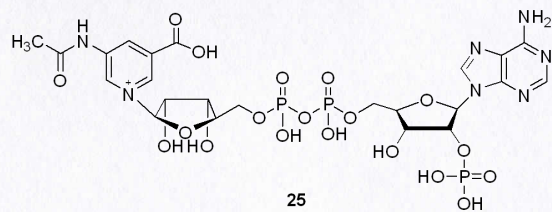


24

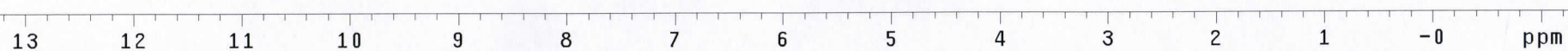
^1H NMR (400 MHz, D_2O)



Solvent: D2O
 Ambient temperature
 File: nacetylnaadp
 NITY-400 "vxrs400"
 Relax delay 2.000 sec
 Pulse 51.1 degrees
 Acq time 2.731 sec
 Width 5999.7 Hz
 32 repetitions
 OBSERVE H1, 399.9496409 MHz
 DATA PROCESSING
 Line broadening 0.5 Hz
 F size 65536
 Total time 2 min 31 sec

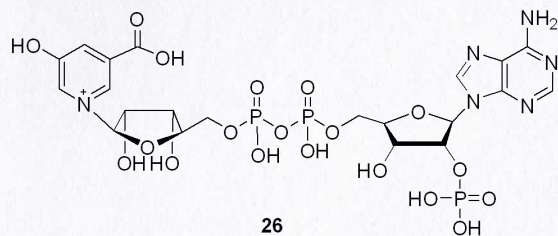


25
¹H NMR (400 MHz, D₂O)



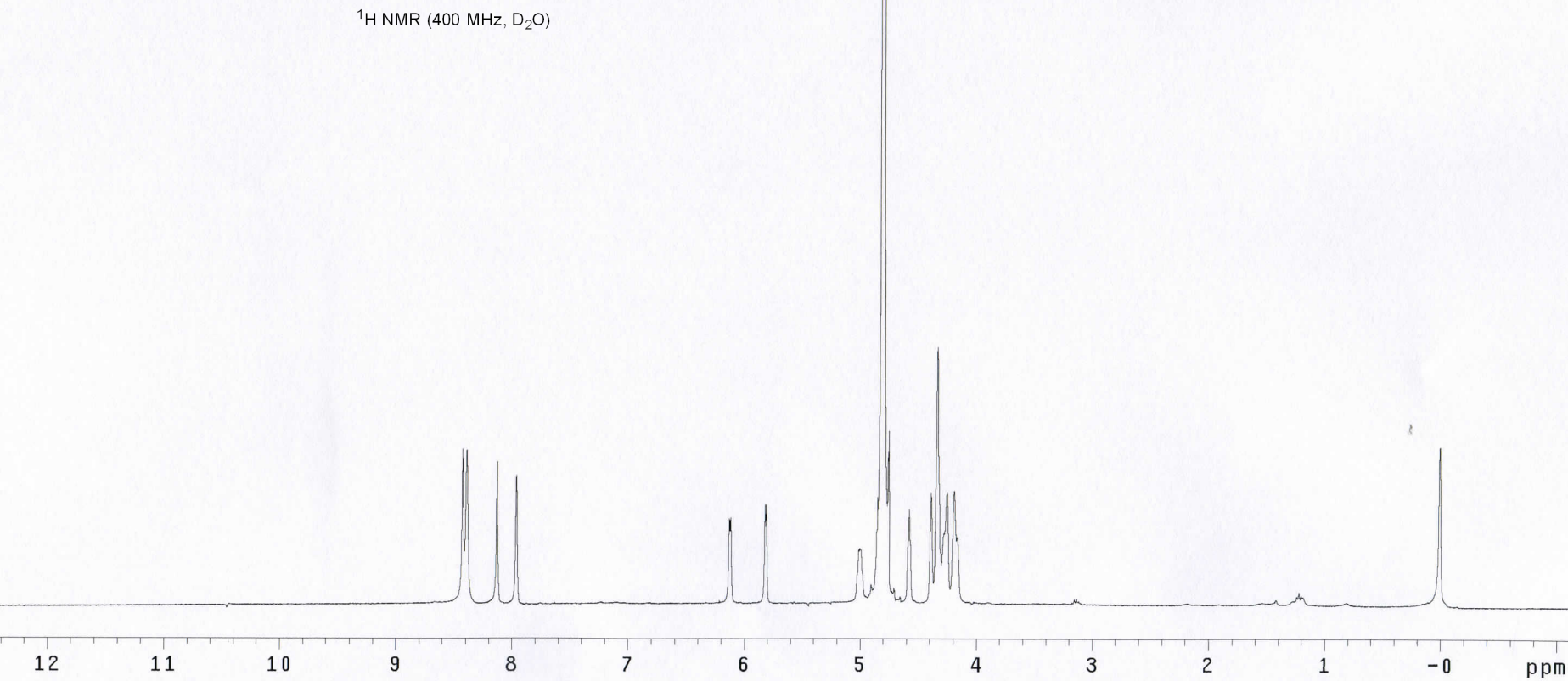
Solvent: D2O
Ambient temperature
File: 5-oh-naadpresent
NITY-400 "vxrs400"

Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999 7 Hz
64 repetitions
BSERVE H1, 399 9496465 MHz
ATA PROCESSING
Line broadening 0 5 Hz
T size 65536
Total time 5 min 3 sec



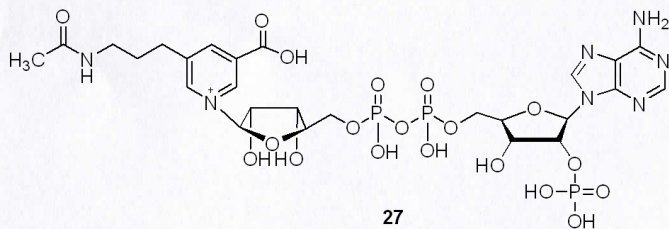
26

¹H NMR (400 MHz, D₂O)

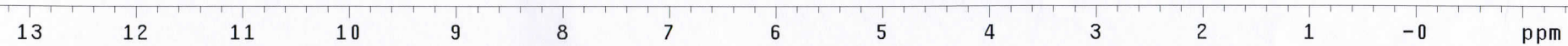


Solvent: D2O
Ambient temperature
File: 5acetamidopropylnaadp
NITY-400 "vxrs400"

Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999 7 Hz
8 repetitions
B1, 399 9497065 MHz
ATA PROCESSING
Line broadening 0 5 Hz
T size 65536
Total time 5 min 3 sec

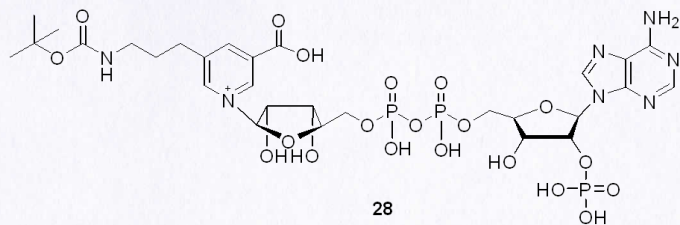


27
¹H NMR (400 MHz, D₂O)

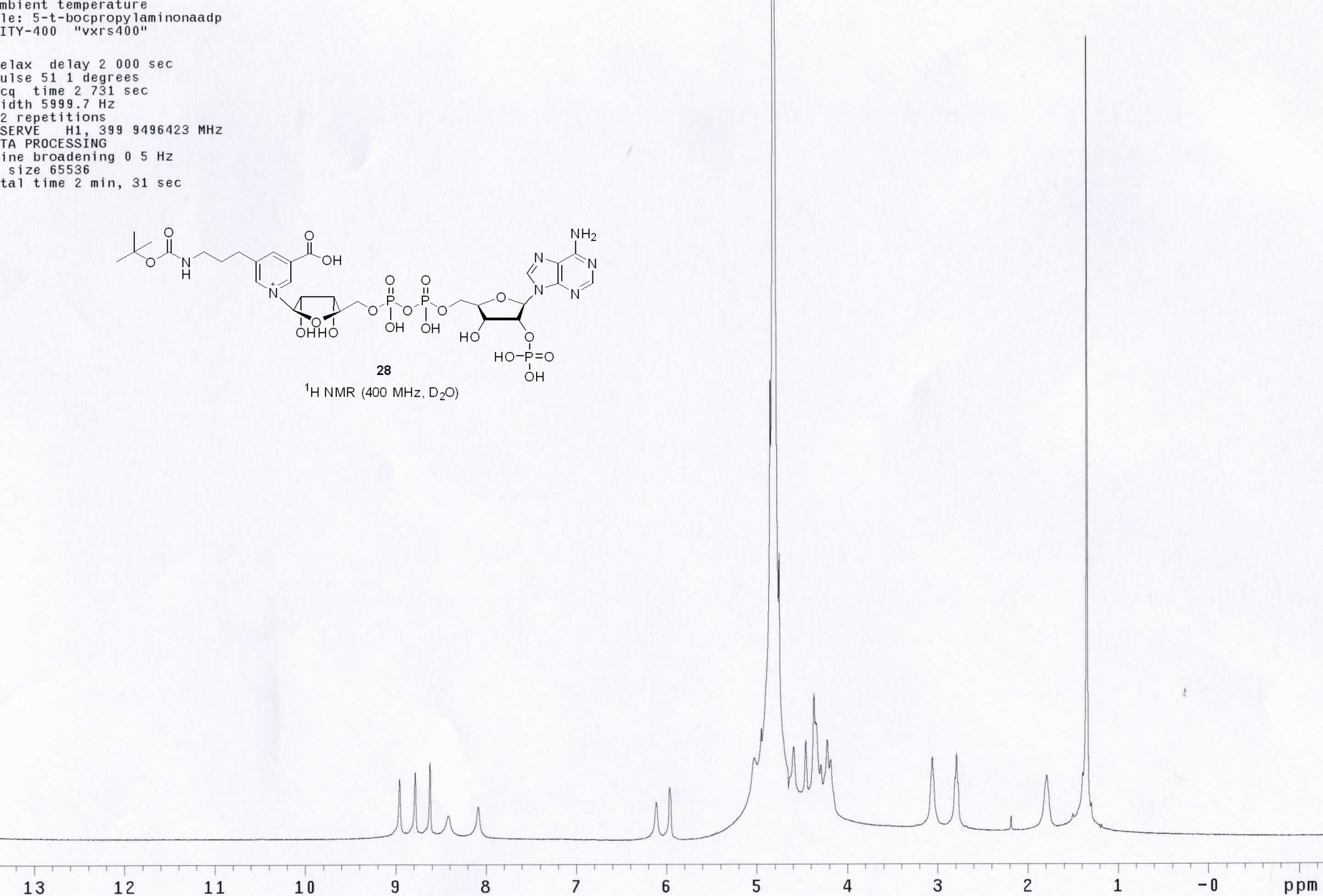


ambient temperature
le: 5-t-bocpropylaminonaadp
ITY-400 "vxrs400"

relax delay 2 000 sec
pulse 51.1 degrees
cq time 2.731 sec
width 5999.7 Hz
2 repetitions
SERVE H1, 399.9496423 MHz
TA PROCESSING
line broadening 0.5 Hz
size 65536
total time 2 min, 31 sec

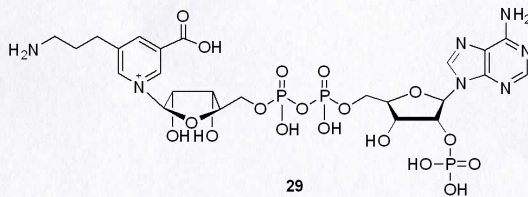


28
¹H NMR (400 MHz, D₂O)



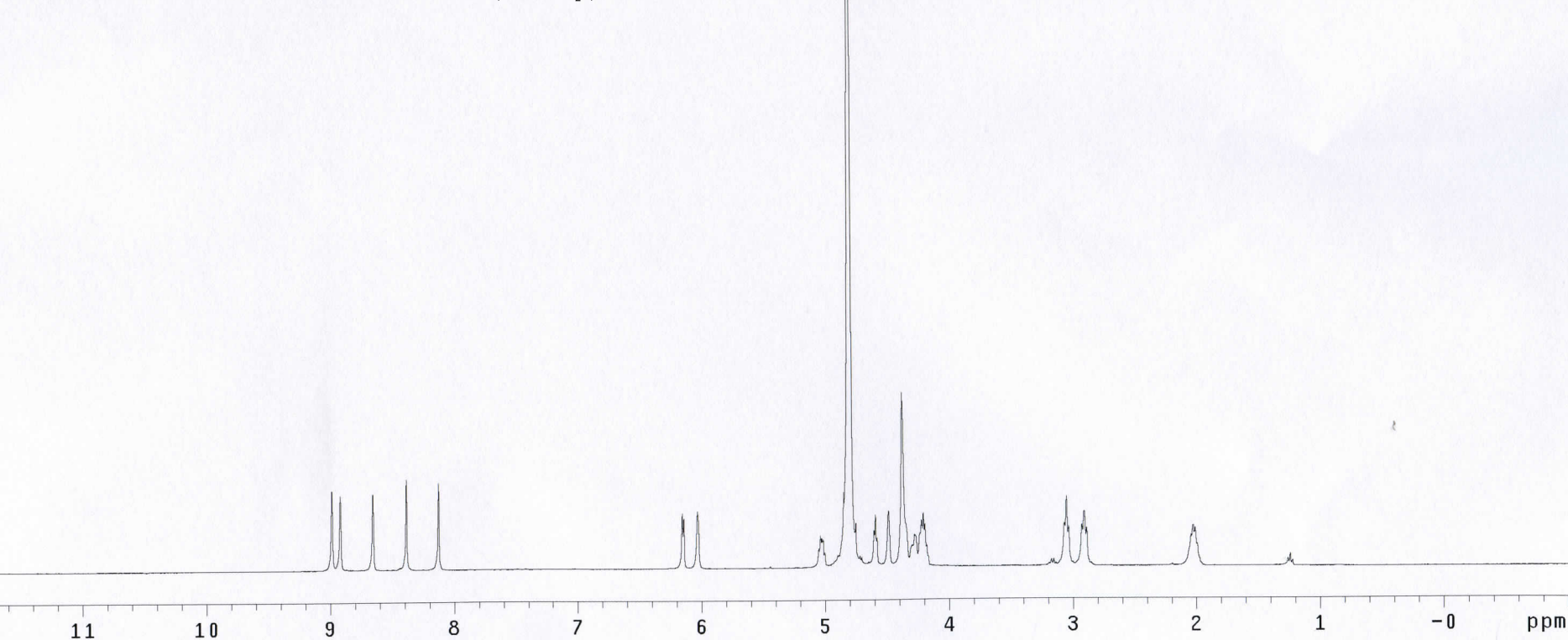
Ambient temperature
File: 5-propylaminonaadp
UNITY-400 "vxrs400"

Relax delay 2 000 sec
Pulse 51 1 degrees
Acq time 2 731 sec
Width 5999 7 Hz
32 repetitions
OBSERVE H1, 399 9496442 MHz
DATA PROCESSING
Line broadening 0 5 Hz
FT size 65536
Total time 2 min, 31 sec

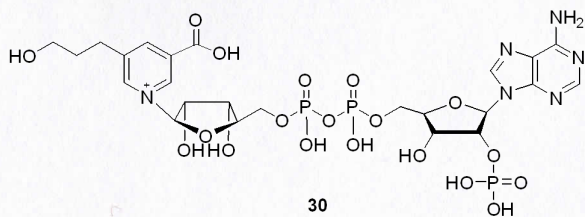


29

^1H NMR (400 MHz, D_2O)



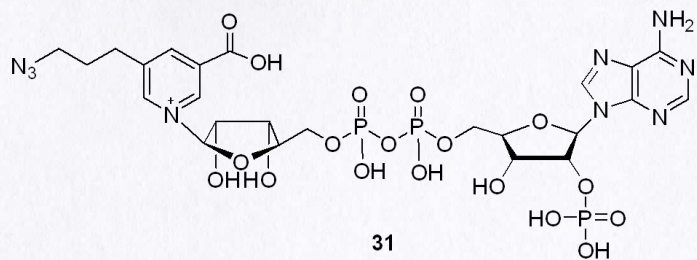
Solvent: D2O
 Ambient temperature
 File: 5propyloxynaadp
 NITY-400 "vxrs400"
 Relax delay 2.000 sec
 Pulse 51 1 degrees
 Acq time 2 731 sec
 Width 5999 7 Hz
 32 repetitions
 SSERVE H1, 399 9496416 MHz
 DATA PROCESSING
 Line broadening 0 5 Hz
 F size 65536
 Total time 2 min 31 sec



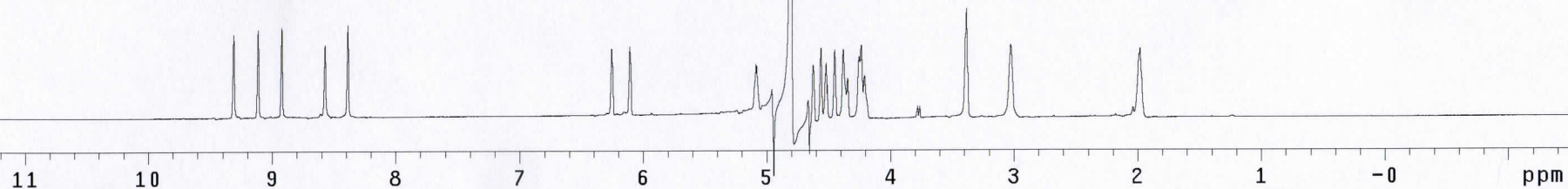
30
¹H NMR (400 MHz, D₂O)

10 9 8 7 6 5 4 3 2 1 -0 ppm

Ambient temperature
File: 5propylazidonaadp
NOVA-600 "inova600"
Relax delay 1 000 sec
Pulse 31 8 degrees
Acq time 3 000 sec
Width 8000 0 Hz
64 repetitions
BSSERVE H1, 599 8737615 MHz
DATA PROCESSING
Line broadening 0 4 Hz
T size 131072
Total time 1 hr 6 min 48 sec

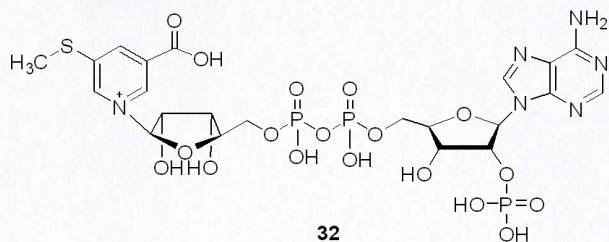


31
 ^1H NMR (400 MHz, D_2O)

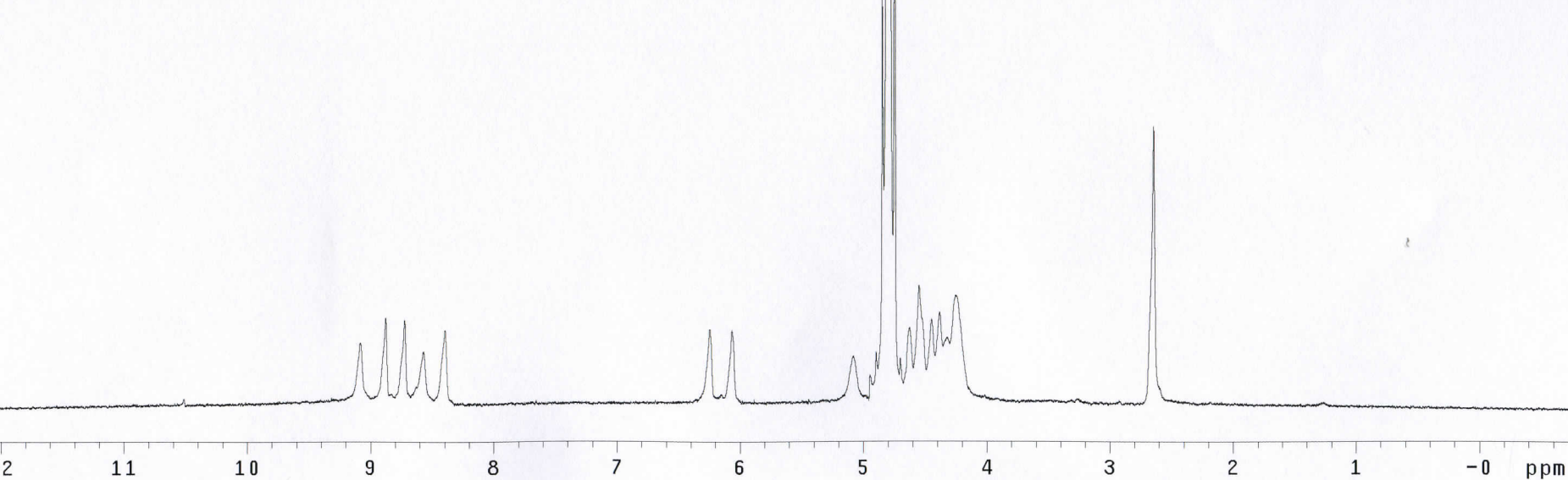


Solvent: D2O
Ambient temperature
File: 5-thiomethoxyNAADP
NITY-400 "vxrs400"

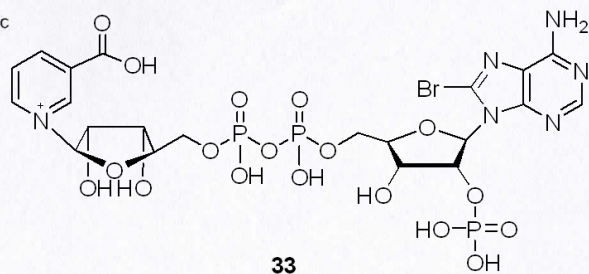
Relax delay 2 000 sec
Pulse 47 4 degrees
Acq time 2 731 sec
Width 5999 7 Hz
32 repetitions
Bserve H1, 399 9496409 MHz
ATA PROCESSING
Line broadening 0 5 Hz
T size 65536
Total time 2 min 31 sec



^1H NMR (400 MHz, D_2O)



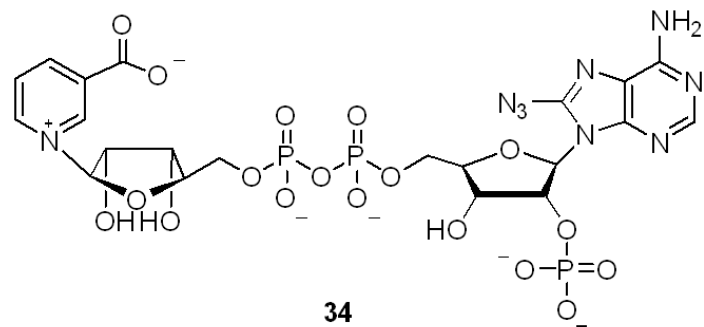
Solvent: D2O
 Ambient temperature
 le: 8brnaadp
 NITY-400 "vxrs400"
 Relax delay 2 000 sec
 Pulse 47 4 degrees
 Acq time 2 731 sec
 Width 5999 7 Hz
 32 repetitions
 SSERVE H1, 399 9496436 MHz
 ATA PROCESSING
 ine broadening 0 5 Hz
 r size 65536
 otal time 2 min 31 sec



33

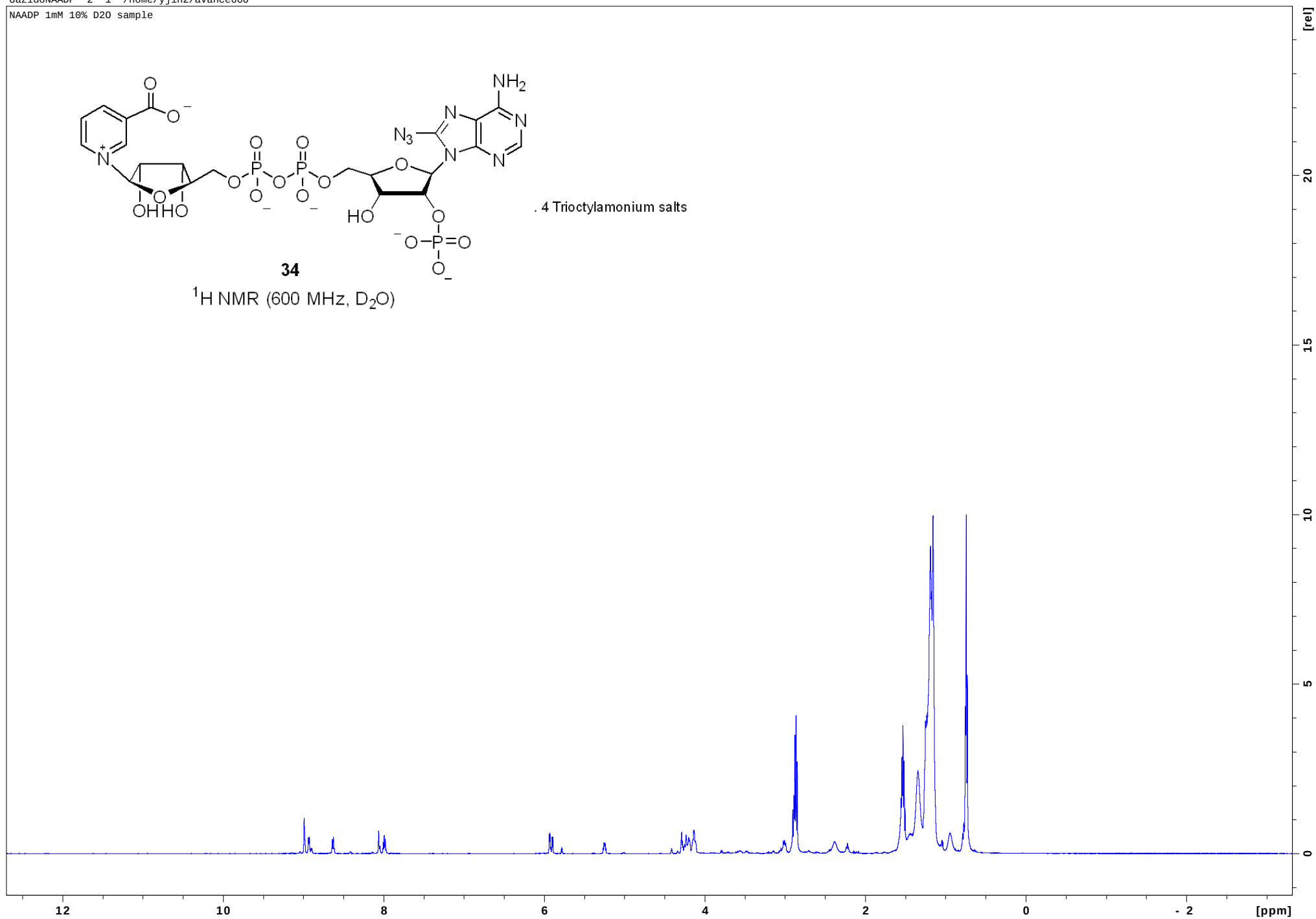
^1H NMR (400 MHz, D_2O)

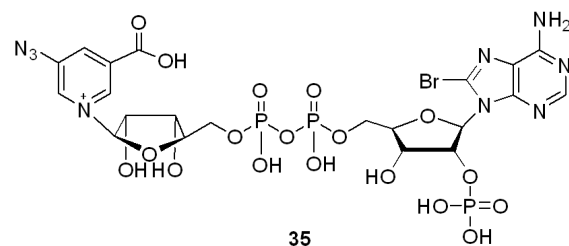
13 12 11 10 9 8 7 6 5 4 3 2 1 -0 ppm



. 4 Trioctylammonium salts

34
¹H NMR (600 MHz, D₂O)





35
 1H NMR (600 MHz, D_2O)

