

## Supporting Information

### Synthesis and *in vitro* evaluation of donepezil-based reactivators and analogues for nerve agent-inhibited human acetylcholinesterase

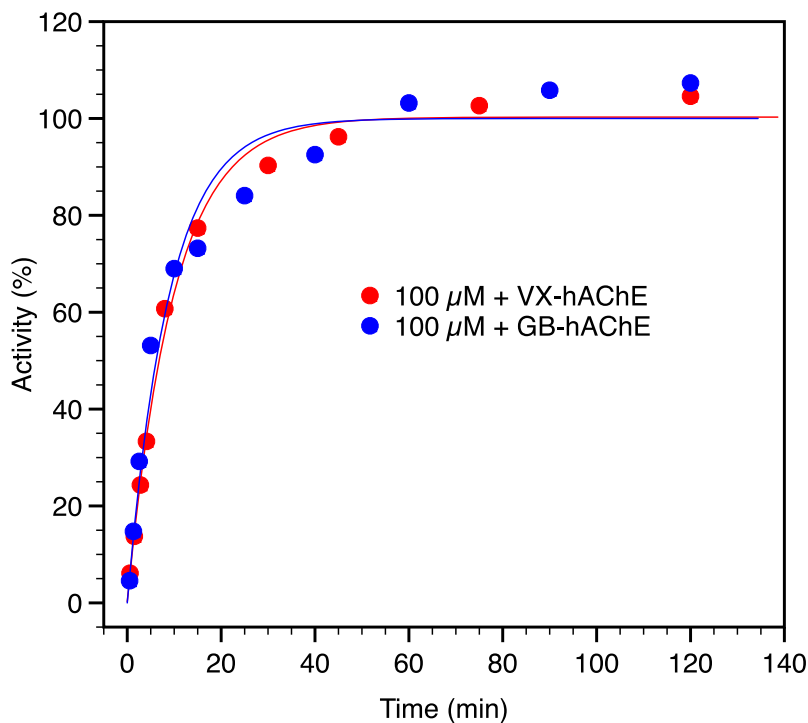
*Julien Renou, José Dias, Guillaume Mercey, Tristan Verdelet, Catherine Rousseau, Anne-Julie Gastellier, Mélanie Arboléas, Mélanie Touvrey-Loiodice, Rachid Baati, Ludovic Jean\*, Florian Nachon, Pierre-Yves Renard\**

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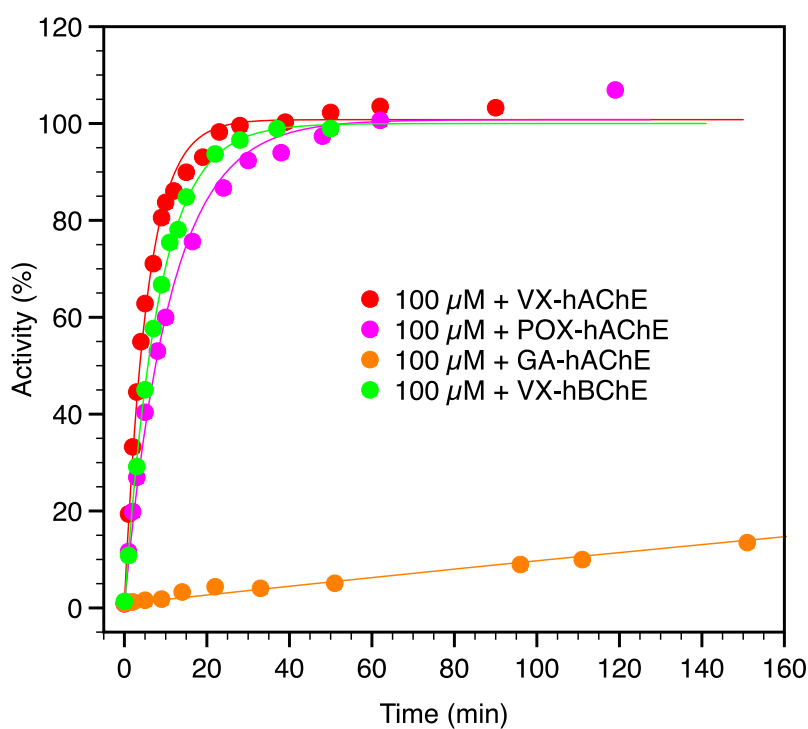
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## 1. Biological assays

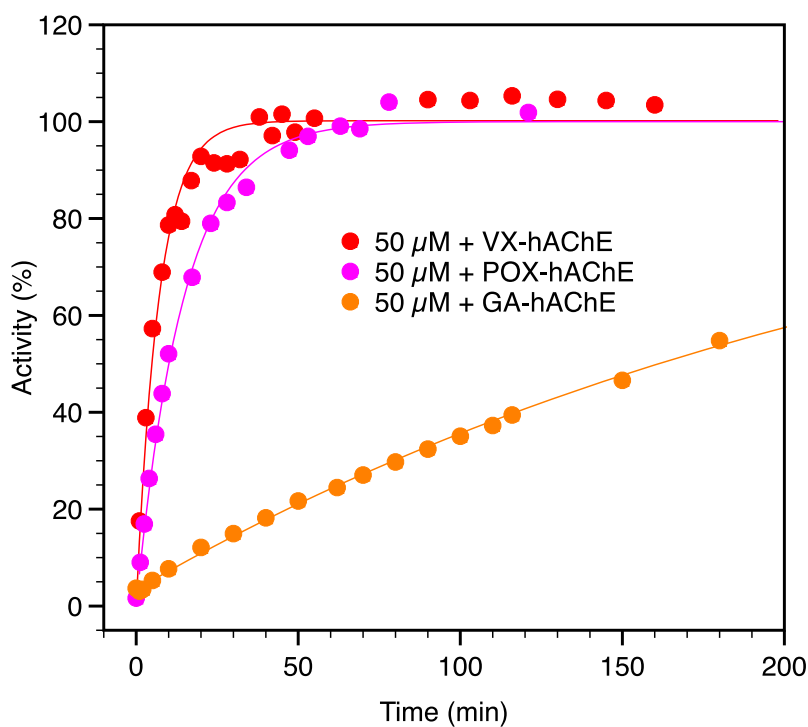
Time-dependent reactivation of VX-inhibited hAChE and GB-inhibited hAChE by 100  $\mu\text{M}$  of **1**, for the determination of  $k_{\text{obs}}$  (See Table 1 and experimental section).



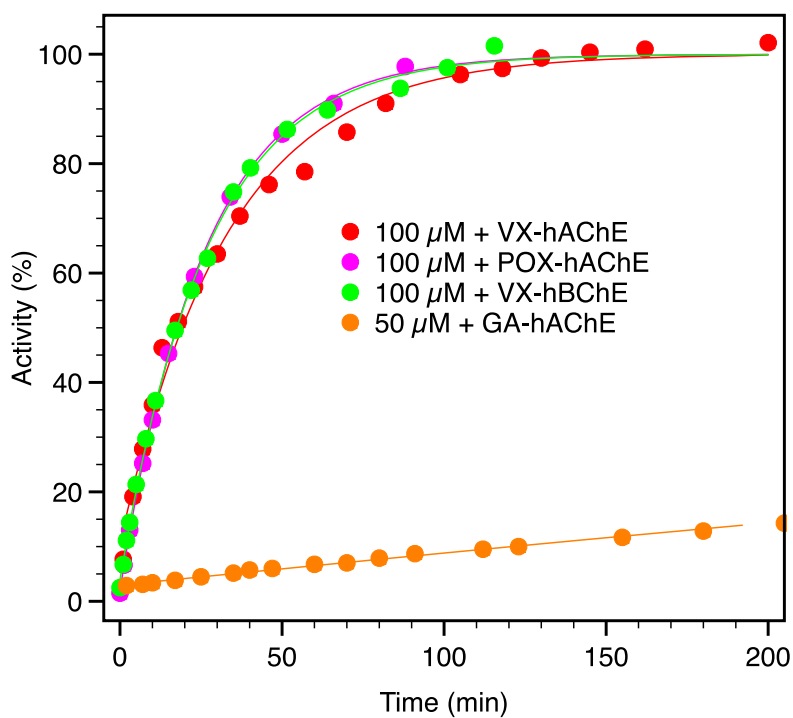
Time-dependent reactivation of VX, paraoxon (POX), tabun (GA)-inhibited hAChE and VX-inhibited hBChE by 100  $\mu\text{M}$  of **2**, for the determination of  $k_{\text{obs}}$  (See Table 1 and experimental section).



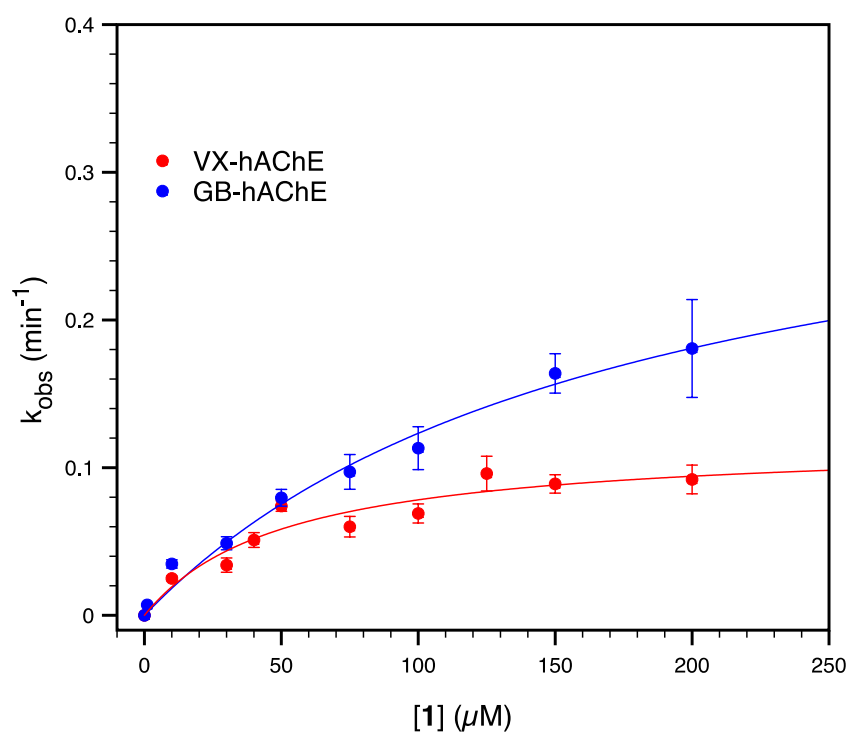
Time-dependent reactivation of VX-, paraoxon (POX-), tabun (GA)-inhibited hAChE by 50  $\mu\text{M}$  of **3**, for the determination of  $k_{\text{obs}}$  (See Table 1 and experimental section).



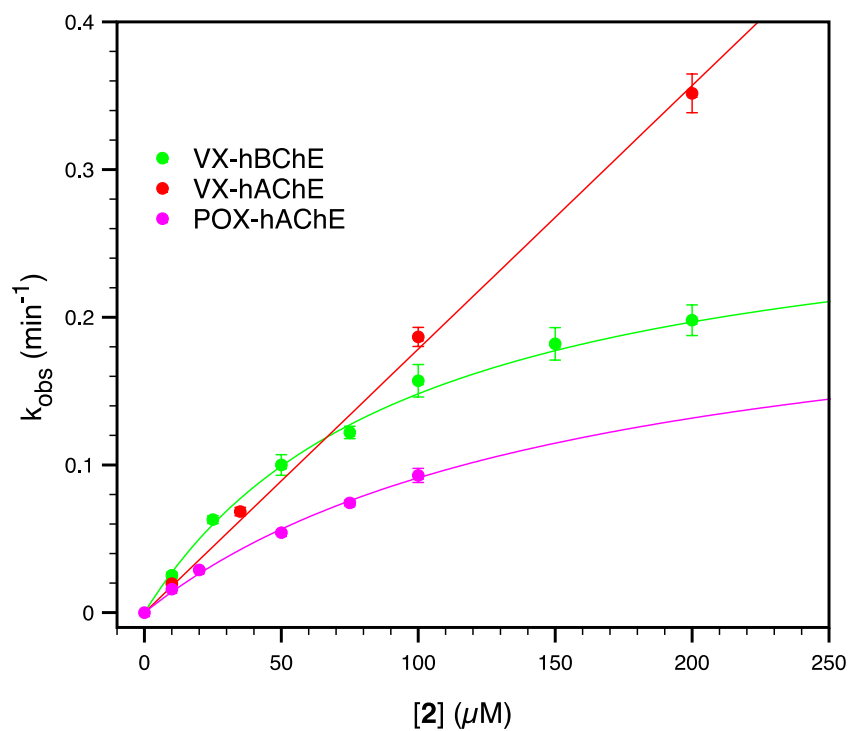
Time-dependent reactivation of VX-, paraoxon or POX-, tabun by 100  $\mu\text{M}$ , of GA-inhibited hAChE by 50  $\mu\text{M}$  of **4**, and VX-inhibited hBChE by 100  $\mu\text{M}$  of **4**, for the determination of  $k_{\text{obs}}$  (See Table 1 and experimental section).



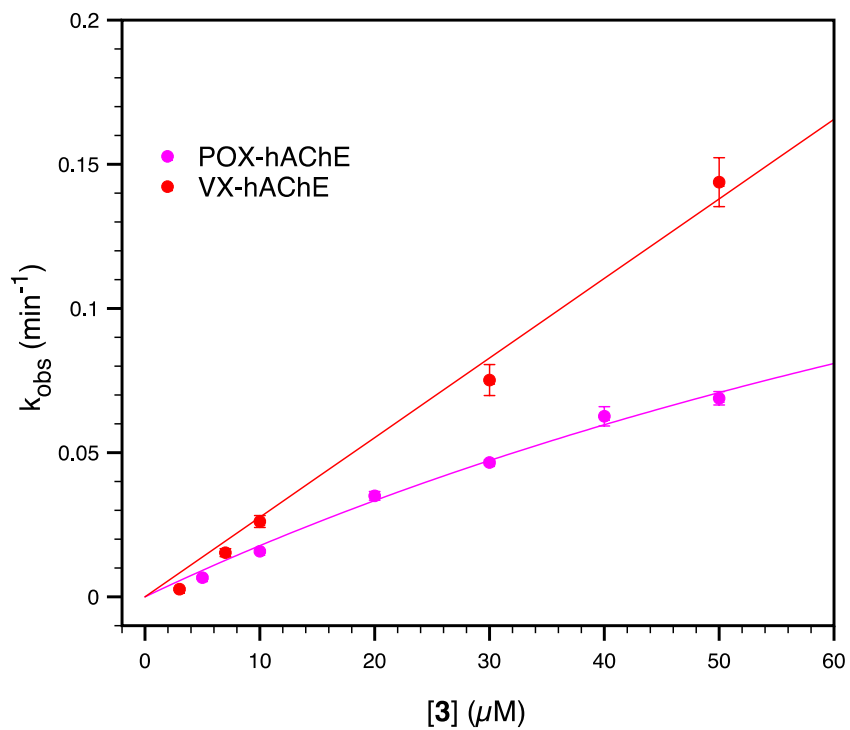
Concentration-dependent reactivation of VX- and GB-inhibited hAChE by compound **1** for the determination of  $k_r$ ,  $K_D$  and  $k_{r2}$  (See Table 1 and experimental section)



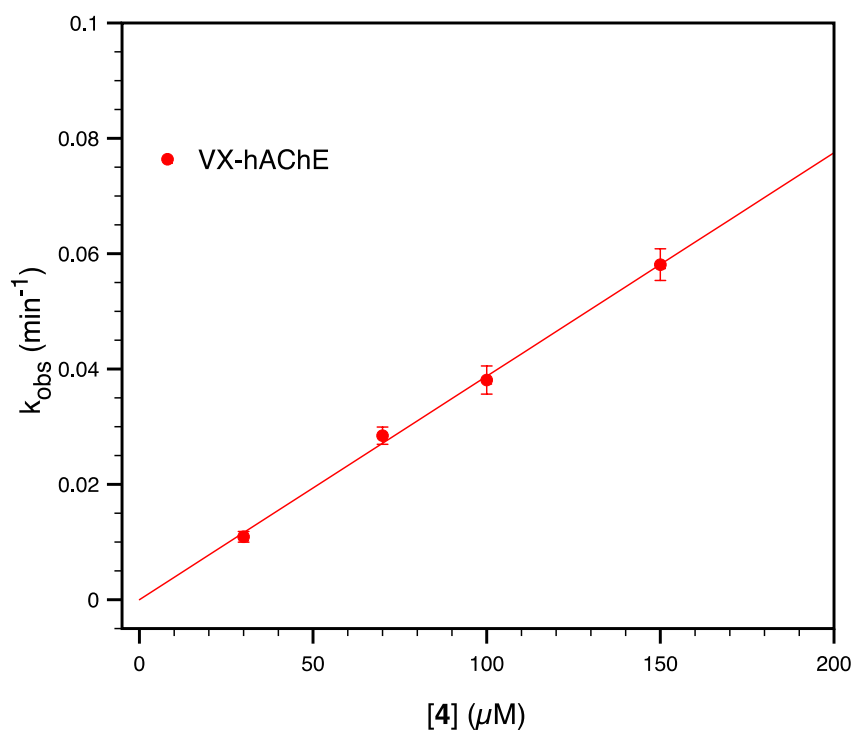
Concentration-dependent reactivation of VX-inhibited hBChE, VX-inhibited hAChE and paraoxon-inhibited hAChE by compound **2** for the determination of  $k_r$ ,  $K_D$  and  $k_{r2}$  (See Table 1 and experimental section)



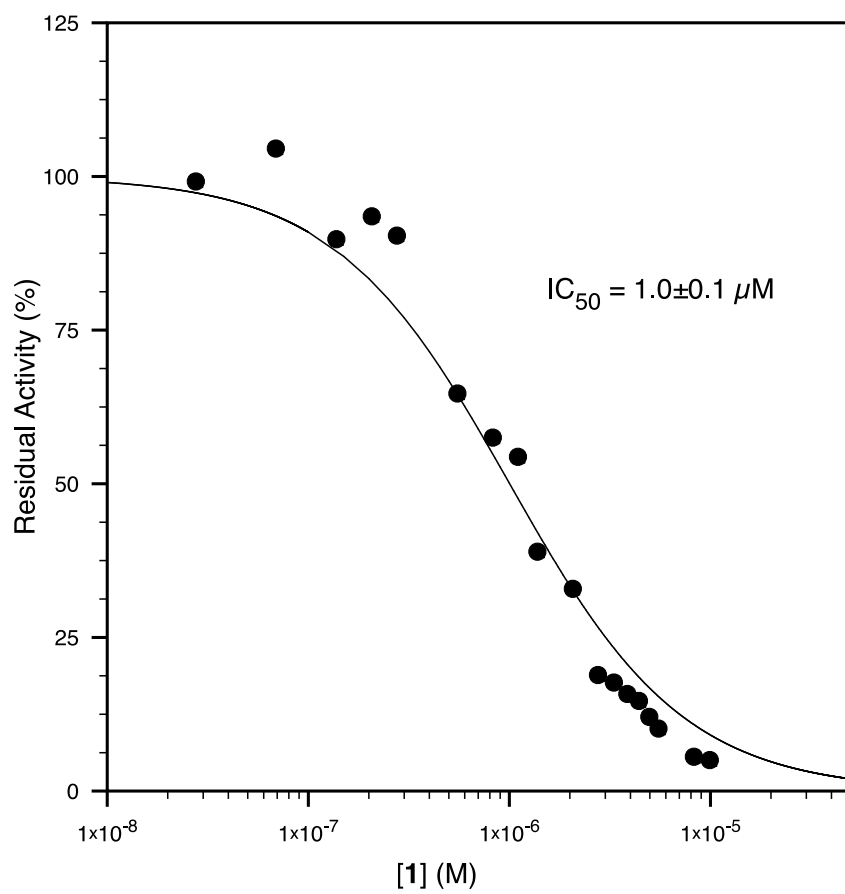
Concentration-dependent reactivation of VX- and paraoxon-inhibited hAChE by compound **3** for the determination of  $k_r$ ,  $K_D$  and  $k_{r2}$  (See Table 1 and experimental section)



Concentration-dependent reactivation of VX-inhibited hAChE by compound **4** for the determination of  $k_{r2}$  (See Table 1 and experimental section)



IC<sub>50</sub> of compound 1 for hAChE

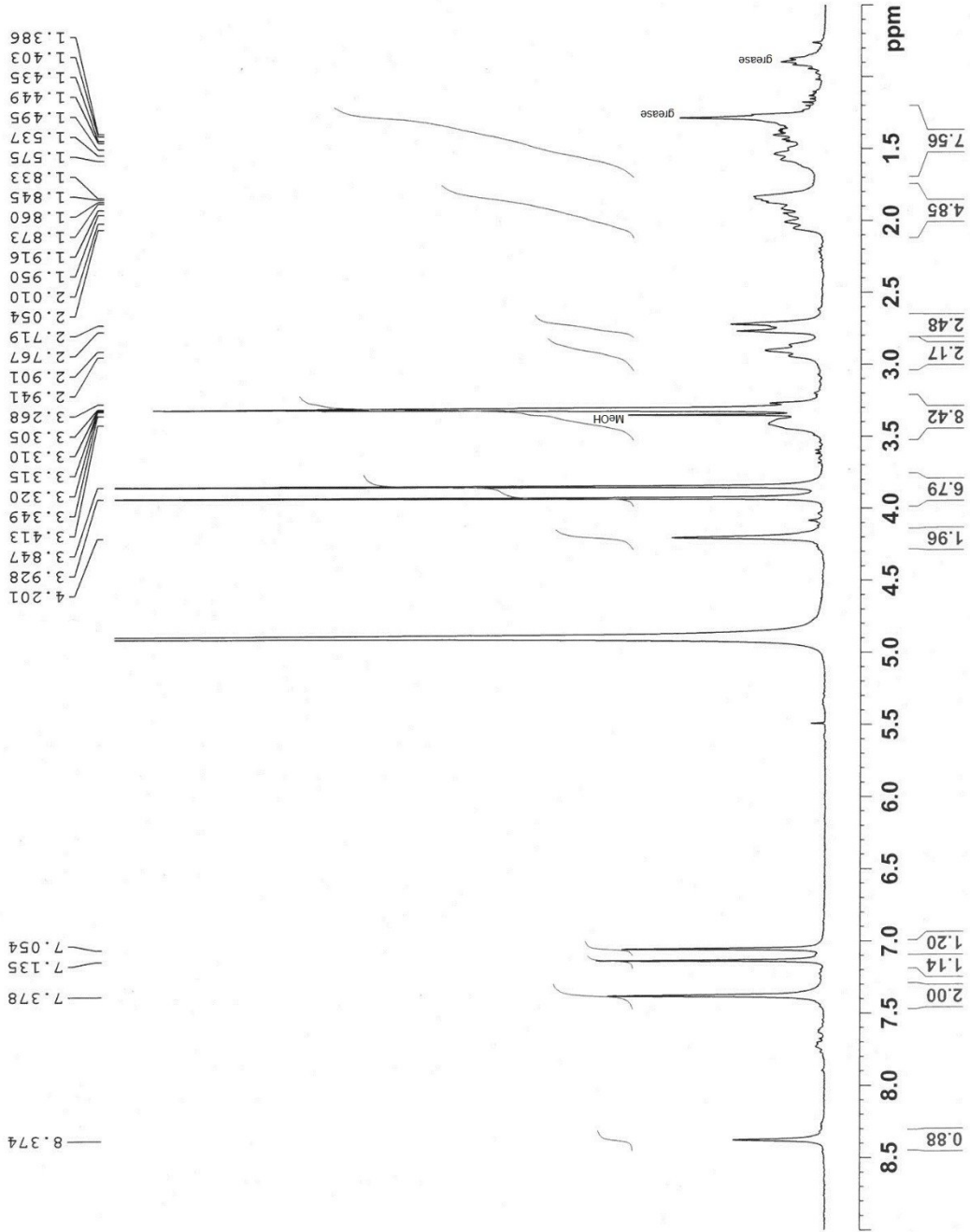
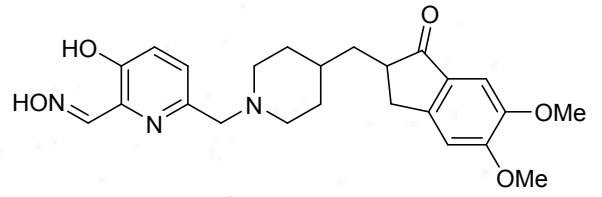


## 2. <sup>1</sup>H and <sup>13</sup>C NMR Spectra and HPLC

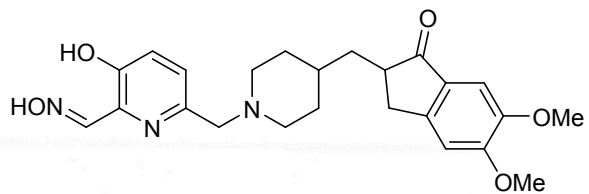
```

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PROCNO 1
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Time_ 10.46
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PROBHD 5 mm PABBO BB-
PULPROG ZG30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 138.22
DW 80.800 usec
DE 6.50 usec
TE 295.7 K
D1 1.00000000 sec
TD0 1

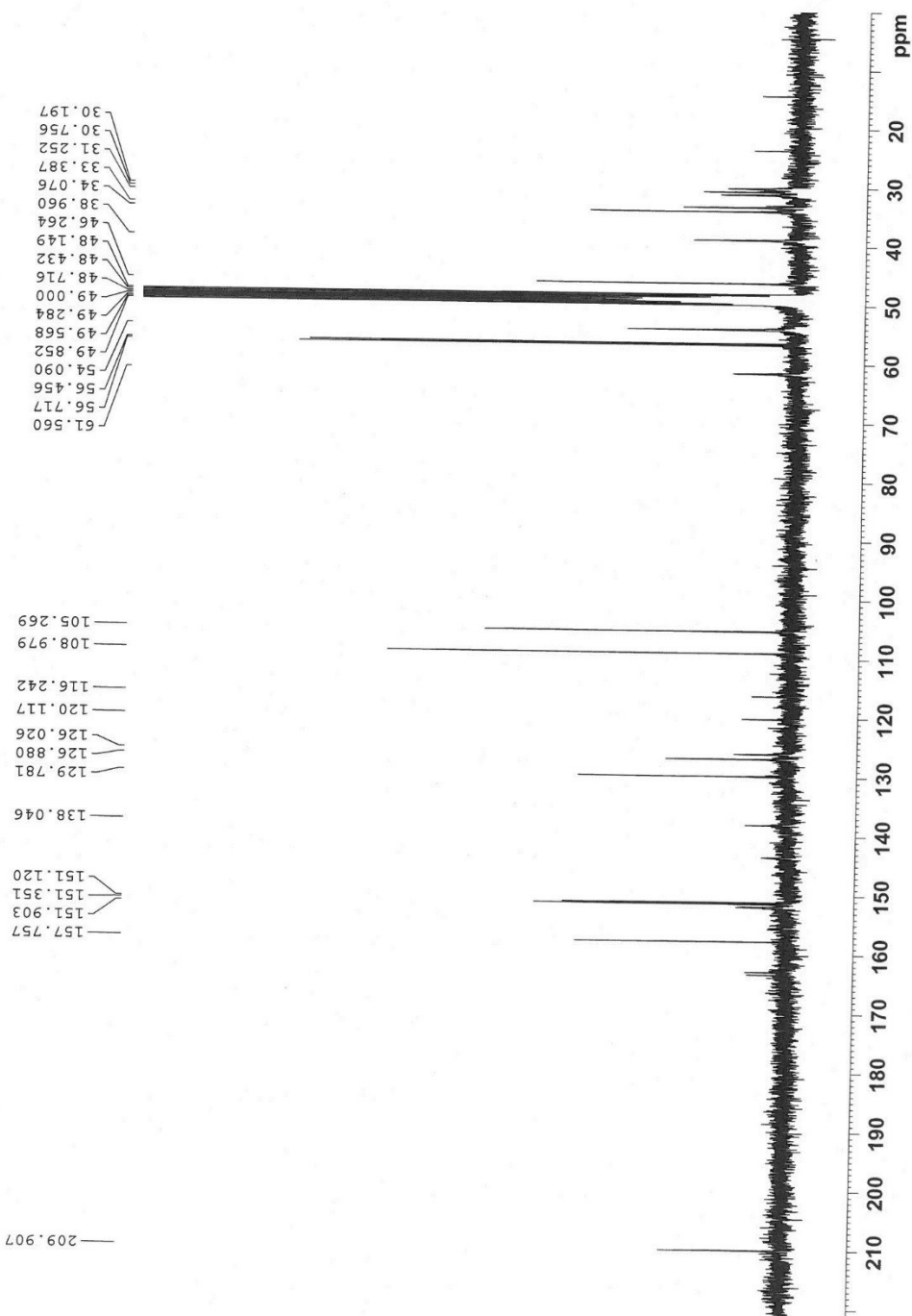
===== CHANNEL f1 =====
NUC1 1H
P1 15.50 usec
SI 65536
SF 300.1300047 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
  
```



FC 035 - 2

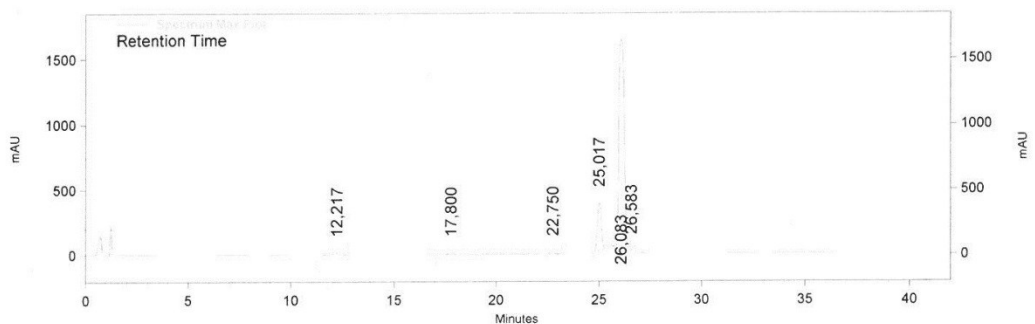


1



FC 035 - 2 C13

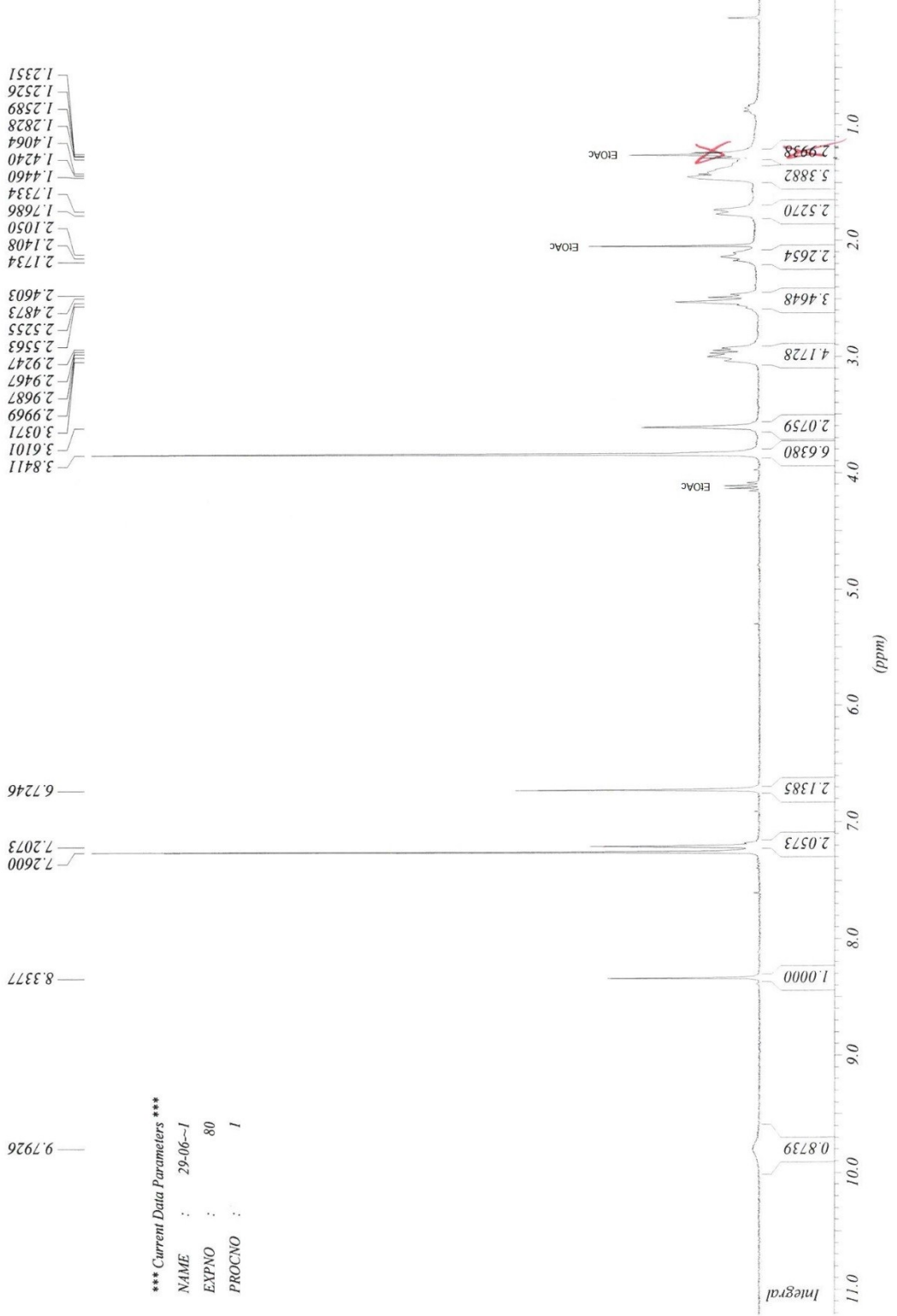
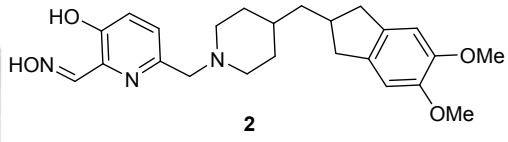




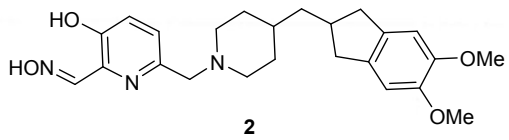
Spectrum Max  
 Plot Results  
 (System  
 (22/10/2012  
 16:29:17)  
 (Reprocessed))

Retention Time	Height	Height Percent	Area	Area Percent
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17,800	9789	0,490	185951	0,535
22,750	10654	0,533	160529	0,462
25,017	363916	18,218	5240816	15,082
26,083	1600511	80,122	28641544	82,423
26,583	0	0,000	0	0,000
Totals	1997600	100,000	34749617	100,000

Under these non-optimized HPLC conditions, the basic compound **1** is partially protonated and the purity (97.5%) was determined by the sum of the two peak areas of the protonated form ( $t_R = 26.08$  min) and of the unprotonated form ( $t_R = 22.48$  min). The UV absorption spectra were completely similar.



\*\*\* Current Data Parameters \*\*\*  
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 EXPNO : 80  
 PROCNO : 1

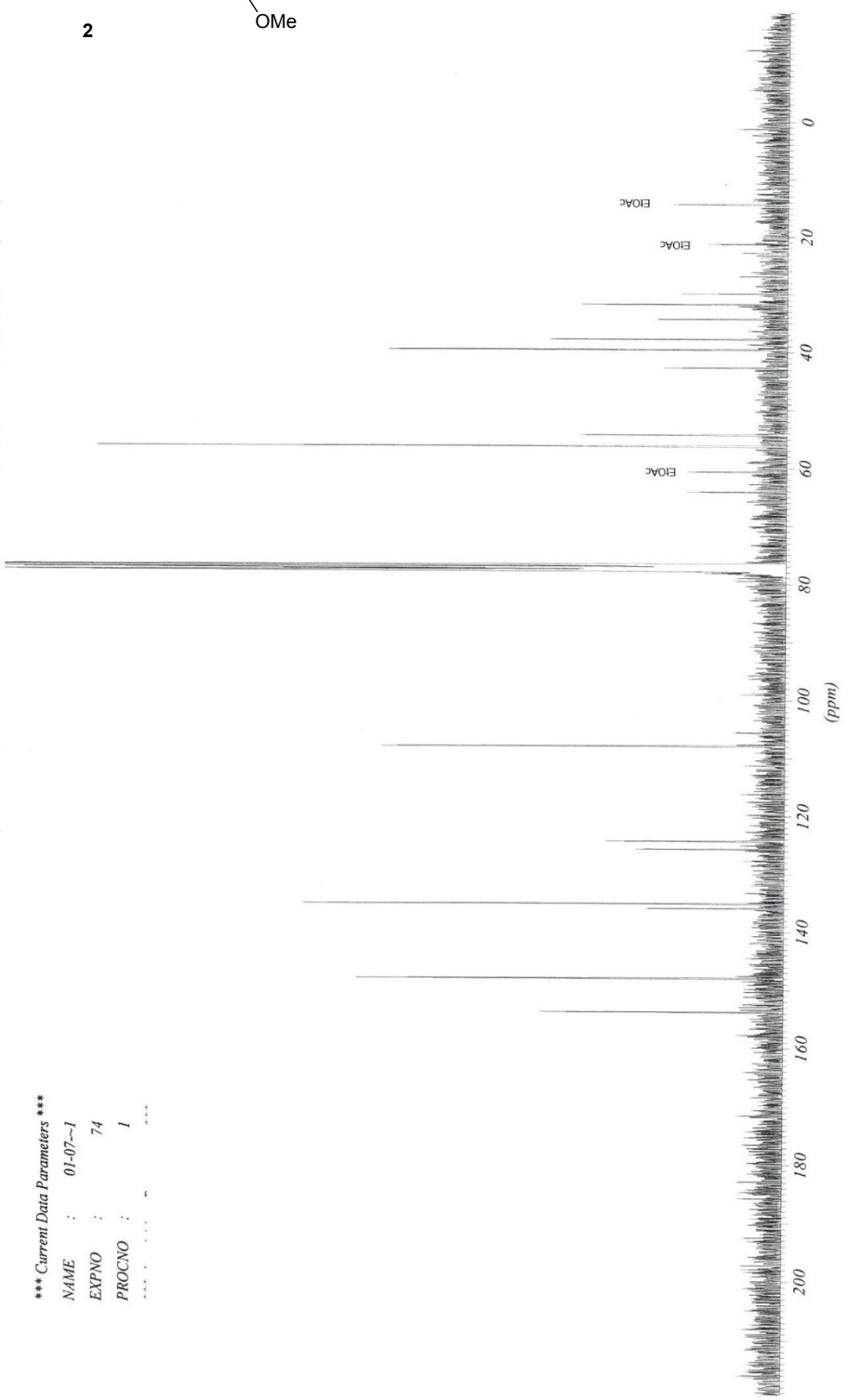


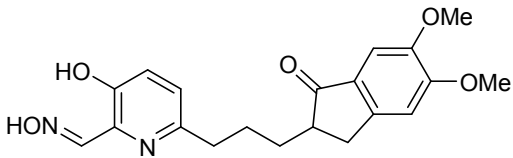
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 21.2160  
 29.8418  
 31.7328  
 34.2784  
 37.7840  
 39.5586  
 42.6496  
 54.2137  
 56.1847  
 60.5630  
 64.0904

107.9321  
 124.2236  
 125.5764  
 134.9950  
 135.8168  
 147.8973  
 153.7011

\*\*\* Current Data Parameters \*\*\*  
 NAME : 01-07-1  
 EXPNO : 74  
 PROCNO : 1

lv4-024





3

```

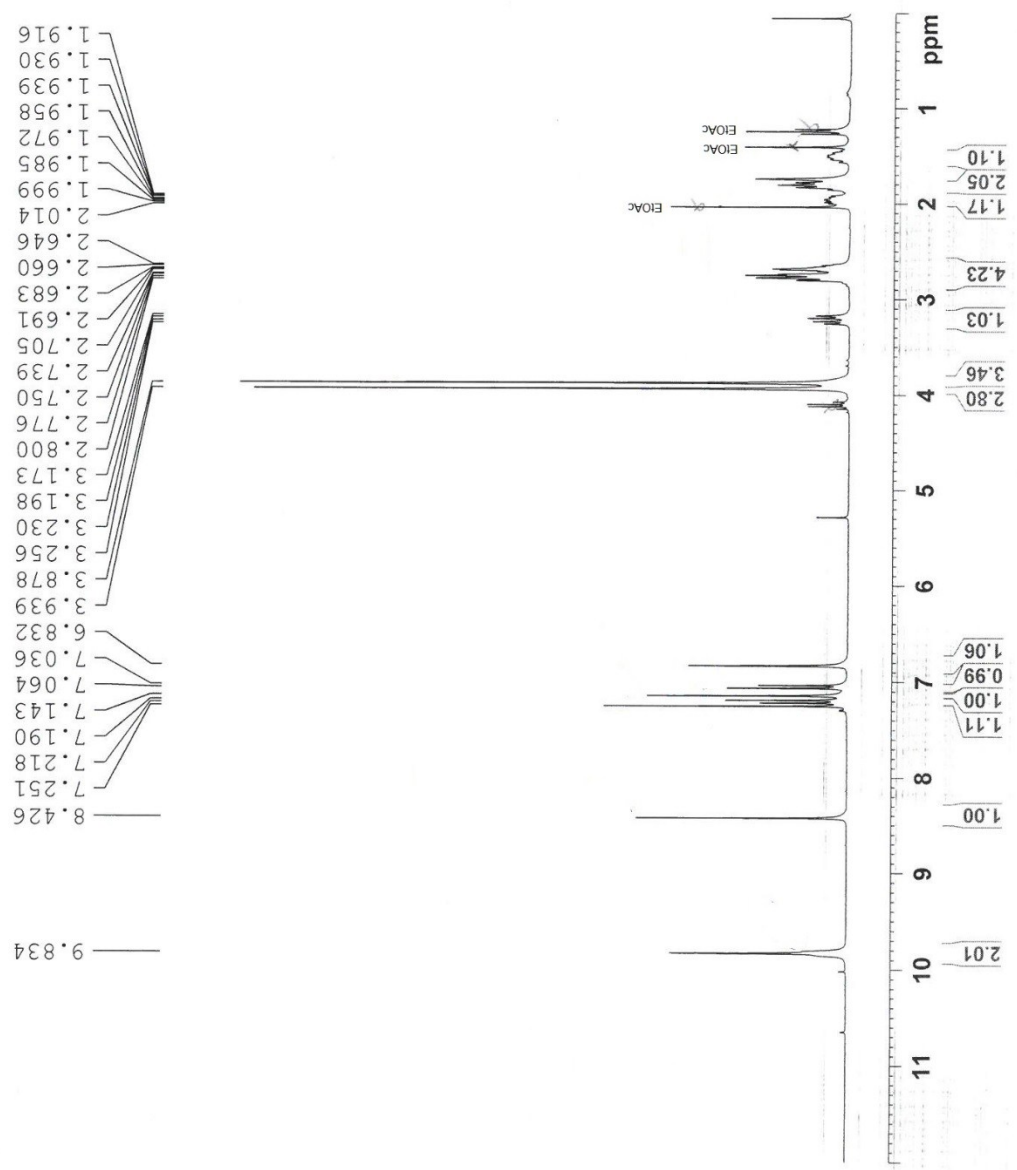
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EXPNO    88
PROCNO   1

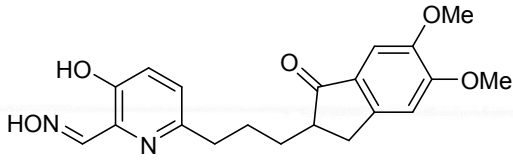
F2 - Acquisition Parameters
Date_    20100429
Time     19.45
INSTRUM spect
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PULPROG zg30
TD       65536
SOLVENT  CDCl3
NS       8
DS       2
SWH      6172.839 Hz
FIDRES   0.094190 Hz
AQ       5.3084660 sec
RG       287.4
DW       81.000 usec
DE       10.00 usec
TE       294.2 K
D1       1.00000000 sec
TD0      1

===== CHANNEL f1 =====
NUC1     1H
P1       10.62 usec
PL1      0.00 dB
SFO1     300.1318534 MHz

F2 - Processing parameters
SI       32768
SF       300.1300084 MHz
WDW      EM
SSB      0
LB       0.30 Hz
GB       0
PC       1.00
  
```

gml175f2





Current Data Parameters  
NAME gml175f2  
EXPNO 62  
PROCNO 1

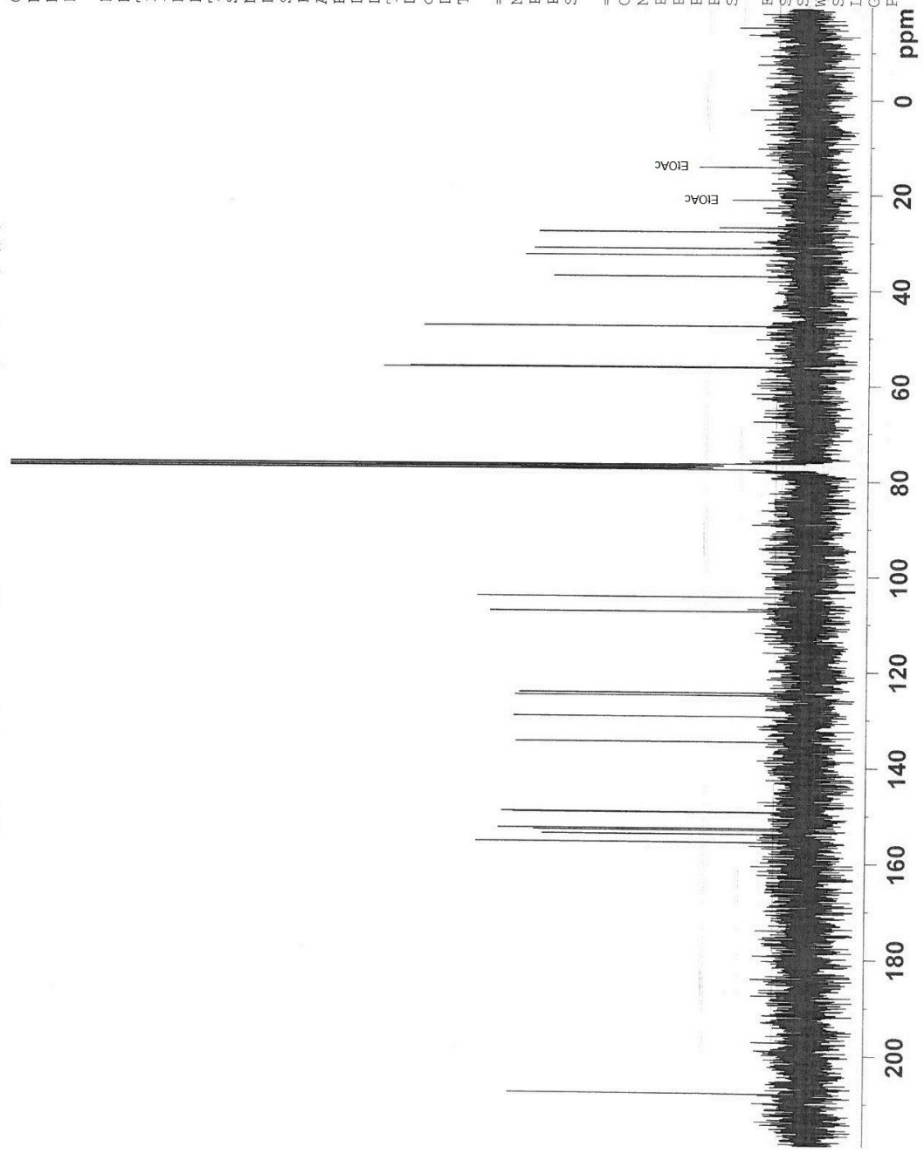
F2 - Acquisition Parameters  
Date\_ 20100430  
Time 14.35  
INSTRUM spect  
PROBHD 5 mm QNP 1H/1  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 1024  
DS 4  
SWH 17985.611 Hz  
FIDRES 0.274439 Hz  
AQ 1.8219508 sec  
RG 10321.3  
DW 27.800 usec  
DE 10.00 usec  
TE 295.2 K  
d1 2.00000000 sec  
d11 0.03000000 sec  
DELTA 1.89999998 sec  
TD0 1

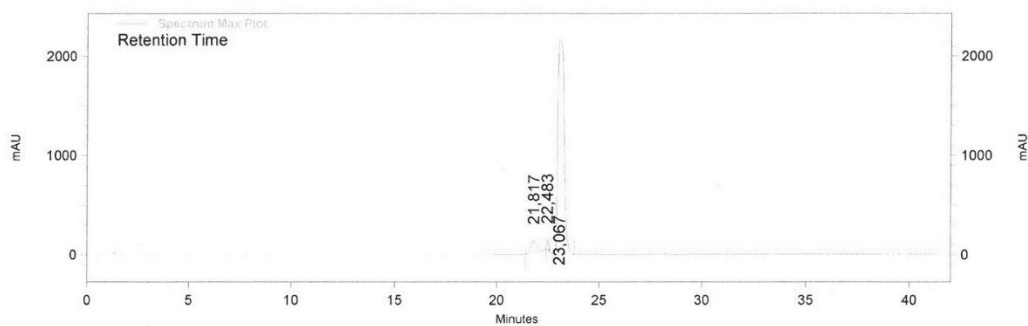
==== CHANNEL f1 =====  
NUC1 13C  
P1 6.75 usec  
PL1 0.00 dB  
SFO1 75.4752953 MHz

==== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 100.00 usec  
PL2 0.00 dB  
PL12 19.48 dB  
PL13 18.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
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SF 75.4677490 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

gml175f2

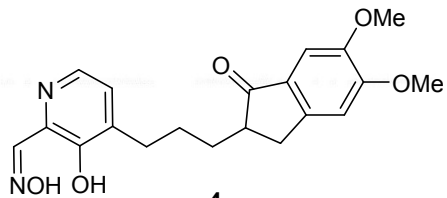




Spectrum Max  
 Plot Results  
 (System  
 (25/08/2011  
 10:25:25)  
 (Reprocessed))

Retention Time	Height	Height Percent	Area	Area Percent
21,817	110534	4,704	2383820	4,891
22,483	85799	3,652	545911	1,120
23,067	2153255	91,644	45807685	93,989
Totals	2349588	100,000	48737416	100,000

Under these non-optimized HPLC conditions, the basic compound **3** is partially protonated and the purity (95.1%) was determined by the sum of the two peak areas of the protonated form ( $t_R = 23.07$  min) and of the unprotonated form ( $t_R = 22.48$  min). The UV absorption spectra were completely similar



4

```

Current Data Parameters
NAME      gm293f1
EXPNO    65
PROCNO   1

F2 - Acquisition Parameters
Date_    20100908
Time     17.40
INSTRUM spect
PROBHD   5 mm QNP 1H/1
PULPROG zg30
TD       65536
SOLVENT  CDCl3
NS       15
DS       0
SWH      6172.839 Hz
FIDRES   0.094190 Hz
AQ       5.3084660 sec
RG       512
DW       81.000 usec
DE       10.00 usec
TE       614.2 K
D1       2.0000000 sec
TD0      1

===== CHANNEL f1 =====
NUC1     1H
P1       10.62 usec
PL1      0.00 dB
SFO1     300.1318534 MHz

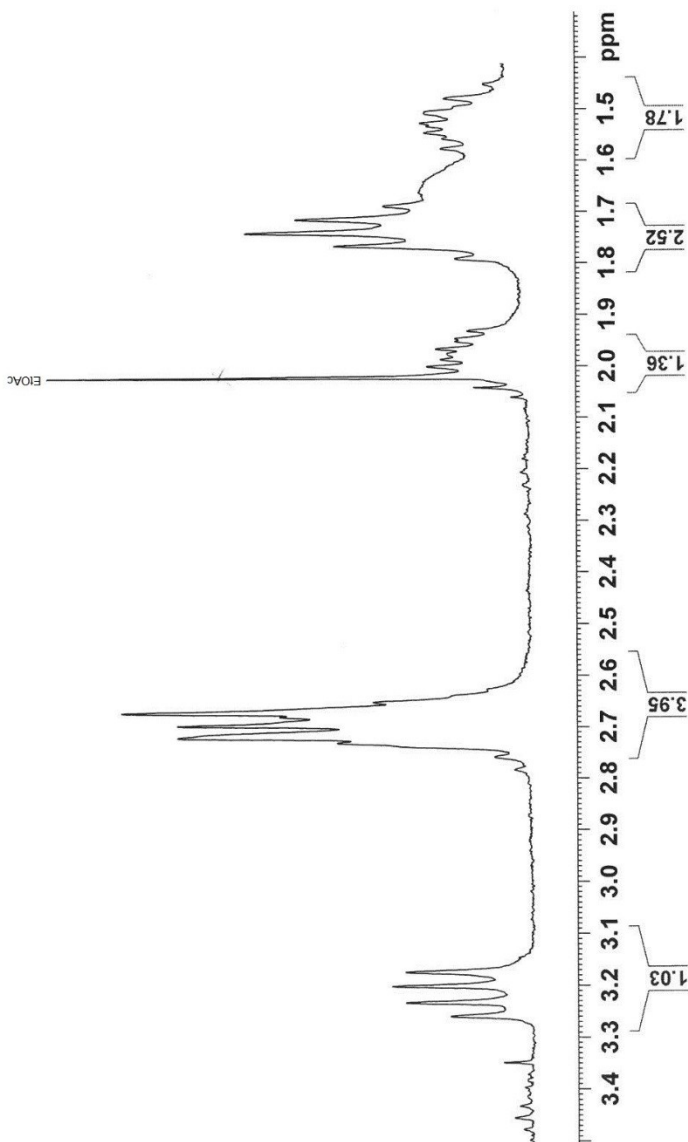
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SSB      0
LB       0.30 Hz
GB       0
PC       1.00
  
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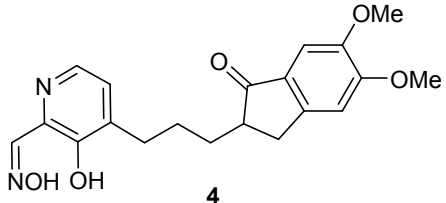
gm293f1

2.041  
2.000  
1.987  
1.975  
1.966  
1.951  
1.946  
1.931  
1.791  
1.766  
1.740  
1.714  
1.688  
1.662  
1.577  
1.557  
1.545  
1.532  
1.526

2.730  
2.719  
2.695  
2.679  
2.670  
2.651

3.258  
3.232  
3.200  
3.173





4

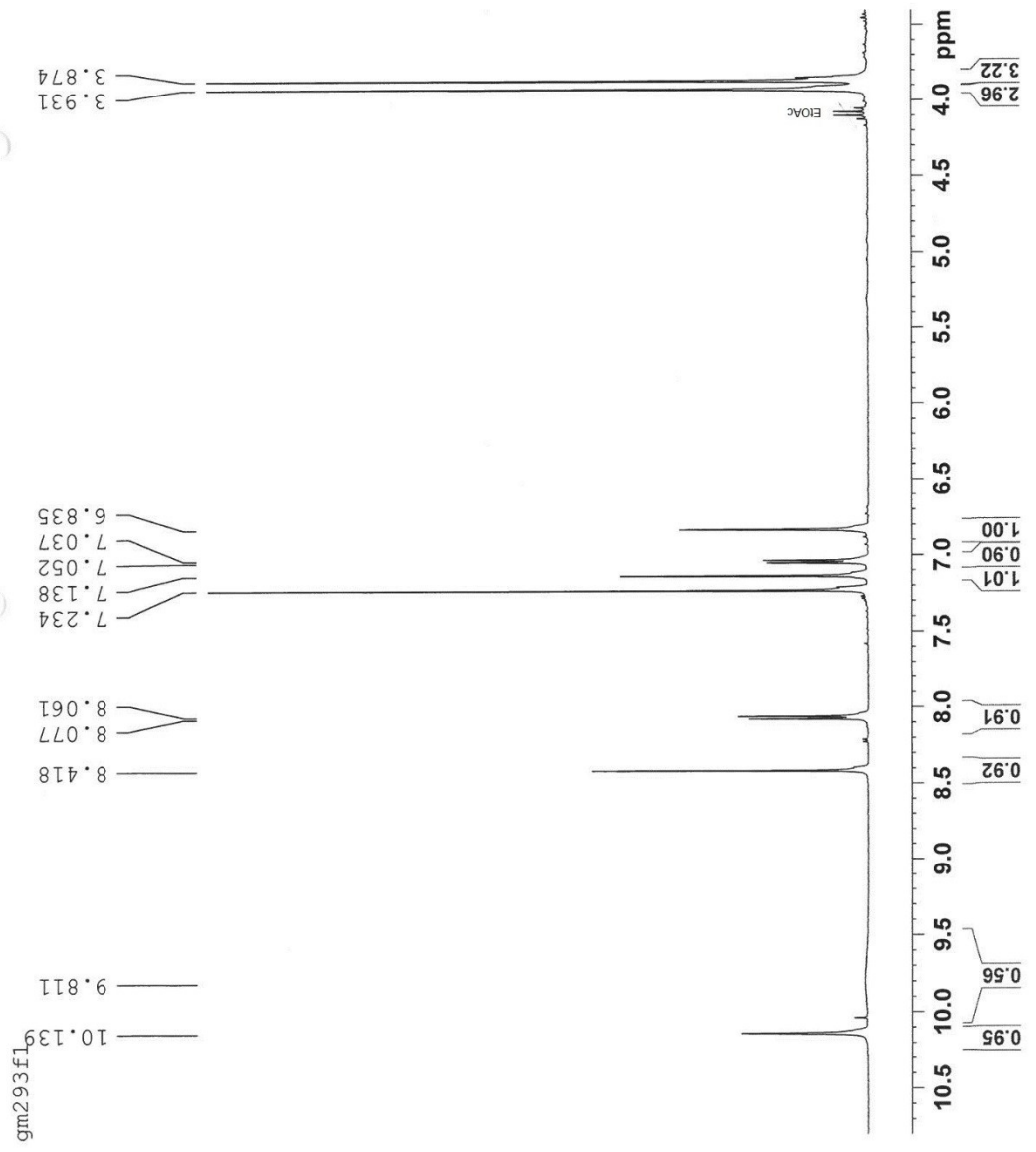
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Current Data Parameters
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EXPNO     65
PROCNO    1

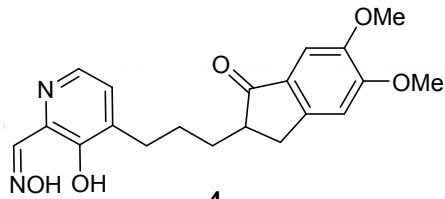
F2 - Acquisition Parameters
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Time      17.40
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PULPROG   zg30
TD         65536
SOLVENT   CDCl3
NS         15
DS         0
SWH        6172.839 Hz
FIDRES     0.094190 Hz
AQ          5.3084660 sec
RG          512
DW          81.000 usec
DE          10.00 usec
TE          614.2 K
D1          2.0000000 sec
TD0         1

===== CHANNEL f1 =====
NUC1       1H
P1         10.62 usec
PL1        0.00 dB
SFO1       300.1318534 MHz

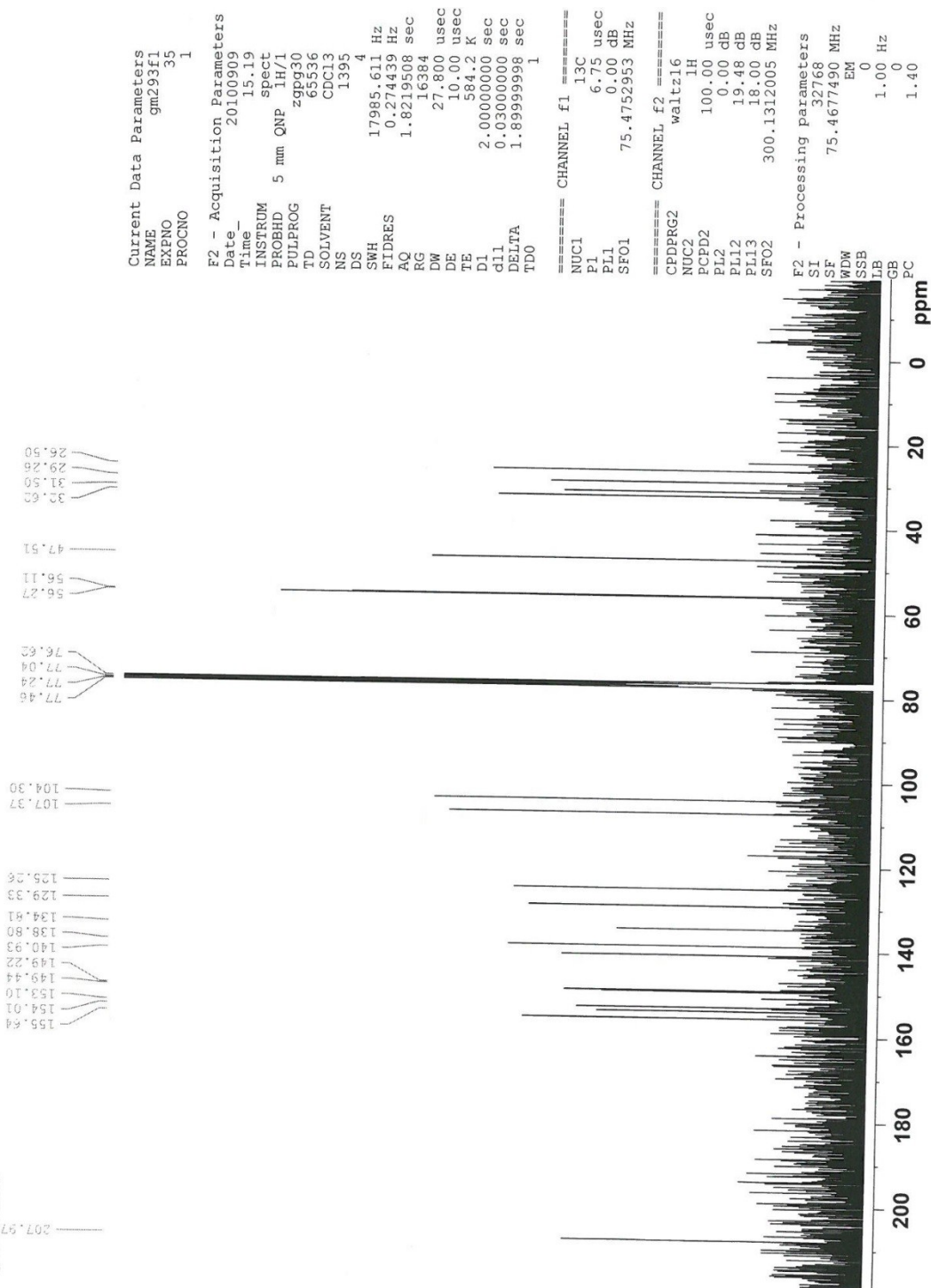
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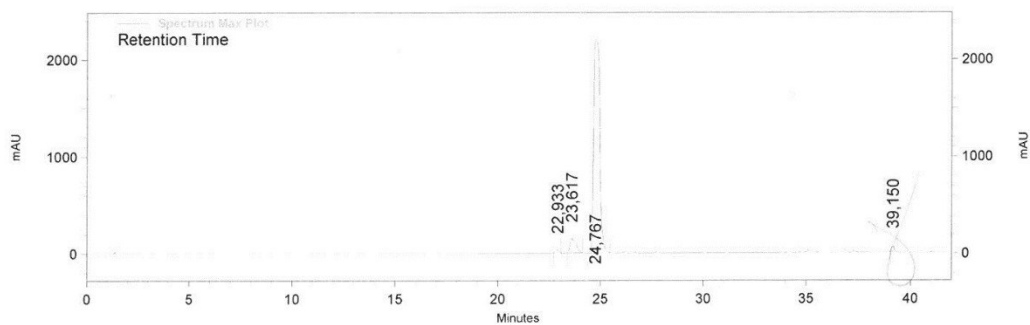






gm293f1c





Spectrum Max  
 Plot Results  
 (System  
 (25/08/2011  
 10:52:10)  
 (Reprocessed))

Retention Time	Height	Height Percent	Area	Area Percent
22,933	30150	1,234	321232	0,655
23,617	145957	5,972	2916355	5,945
24,767	2201644	90,079	45255968	92,247
39,150	66369	2,715	565928	1,154
Totals	2444120	100,000	49059483	100,000

Under these non-optimized HPLC conditions, the basic compound **4** is partially protonated and the purity (98.2 %) was determined by the sum of the two peak areas of the protonated form ( $t_R = 24.76$  min) and of the unprotonated form ( $t_R = 23.62$  min). The UV absorption spectra were completely similar.