

Supporting Information

Title: **Synthesis, Crystal Structure, Structure-Activity Relationships, and Antiviral Activity of a Potent SARS Coronavirus 3CL Protease Inhibitor**

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Content of SI: (1) HPLC and ¹HNMR spectral data for TG-0205221 and major compounds described in the article. (2) Summary of purity of these compounds analyzed by reverse phase HPLC.

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Summary of purity of key compounds:

The purity of the compound **1**, **2**, **3**, **4** was assessed by an HPLC using a Waters 2795 system, with a detector of Waters 2996 PDA, and a column of Agilent/ZORBAX Eclipse/XDB-C18/4.6X150mm. The mobile phase was employed with MeOH(65%-95%) and H₂O(35%-5%) at a flow rate of 1.0 ml/min.

The purity of the compound **9**, **11**, **13**, **14** and **15** was assessed by a preparative HPLC using a Gilson 215 system, with a detector of Gilson UV/VIS-156, and a column of LiChrospher[®] 100 RP-18e. The mobile phase was employed with MeOH/H₂O on various gradients at a flow rate of 10 ml/min and monitored at 210 nm.

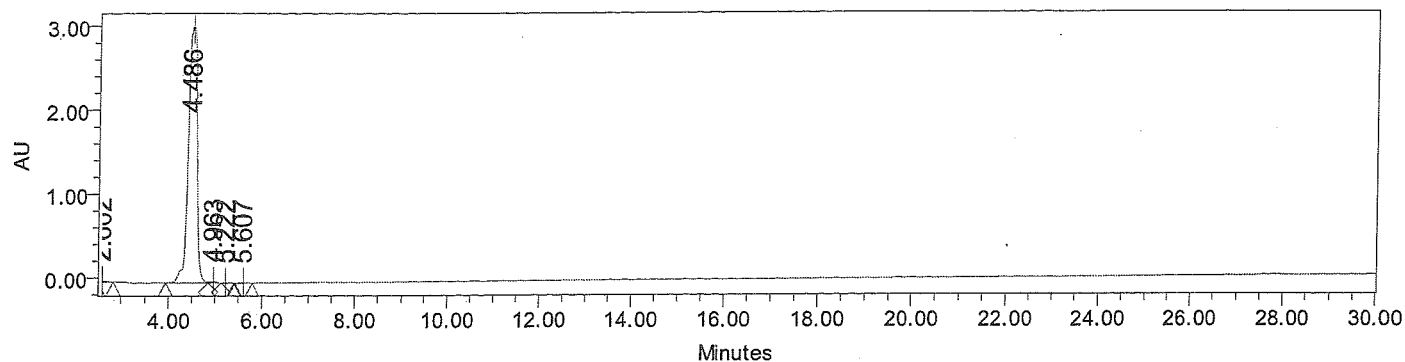
Compounds	Purity(HPLC)
1	>98%
2	>98%
3	>95%
4	>98%
9	>99%
11	>98%
13	>98%
14	>97%
15	>98%

SAMPLE INFORMATION

Sample Name: TG-3556
 Sample Type: Unknown
 Vial: 1:B,2
 Injection #: 1
 Injection Volume: 10.00 ul
 Run Time: 30.0 Minutes
 Sample Set Name: 123

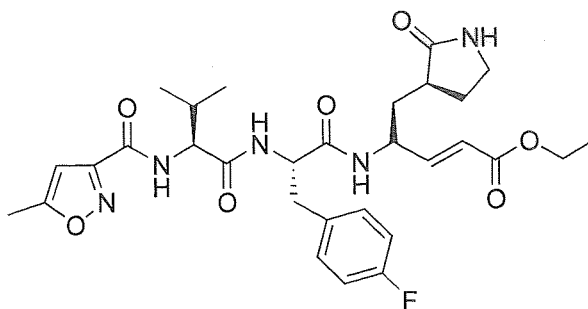
Acquired By: cfyeh
 Date Acquired: 2006/3/28 下午 04:04:52
 Acq. Method Set: TG0205628_PDA_1
 Date Processed: 2006/3/28 下午 04:41:35
 Processing Method: 5628 500
 Channel Name: W2996 210.0nm-1.2
 Proc. Chnl. Descr.: W2996 PDA 210.0 nm at 1.2

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1		2.602	121129	0.29
2		4.486	40859467	98.98
3		4.963	174558	0.42
4		5.222	61736	0.15
5		5.607	63315	0.15



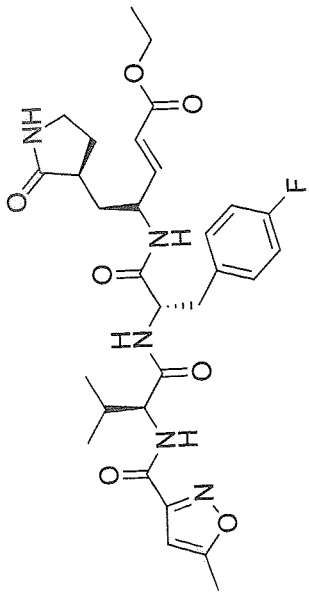
Compound 1

STANDARD 1H OBSERVE

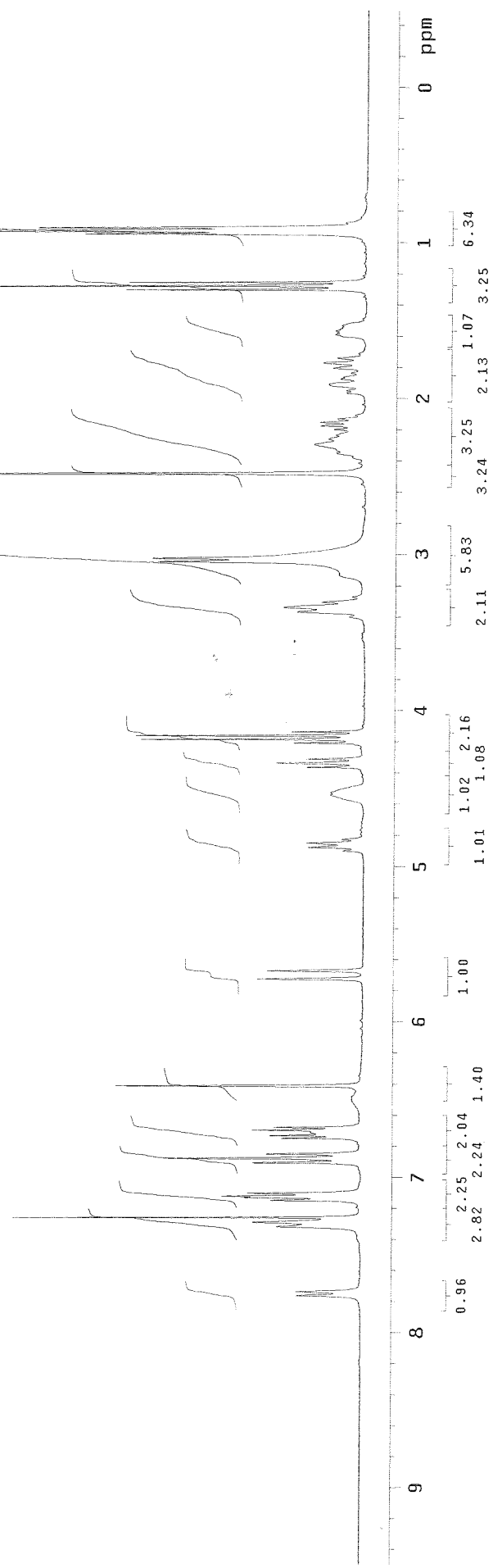
expl	stdlh	date	Mar 27 2006	DEC. & VT	300.063	0.872
solvent	CDCl3	file	exp	dn	H1	0.903
ACQUISITION	exp	dpwr	30	dof	0	0.923
sfrq	300.063	dm	nnn	dm	0	0.943
tn	2.668	dmf	200	dmf	200	1.254
np	25590	lb	0.10	lb	0.10	1.277
sw	4796.2	wtfile	not used	wtfile	not used	1.302
fb	not used	proc	4	proc	4	1.531
bs	not used	fn	57	fn	57	1.564
tpwr	7.5	werr	1.000	werr	1.000	1.576
pw	309.9	wexp	32	wexp	32	1.590
tof	32	wbs	32	wbs	32	1.740
nt	32	wnt	32	wnt	32	1.771
ct	32	gain	not used	gain	not used	1.805
alock	not used	flags	not used	flags	not used	1.862
il	n	sp	-150.1	sp	-150.1	1.875
in	n	wp	3000.5	wp	3000.5	1.907
dp	y	vs	117	vs	117	2.129
sc	0	sc	0	sc	0	2.152
wc	250	wc	250	wc	250	2.175
hzmm	12.00	hzmm	12.00	hzmm	12.00	2.198
is	1262.33	is	1262.33	is	1262.33	2.233
rfl	574.6	rfl	574.6	rfl	574.6	2.261
rff	0	rff	0	rff	0	2.293
th	3	th	3	th	3	2.350
ins	45.210	ins	45.210	ins	45.210	2.479
nm	ph	nm	ph	nm	ph	3.021

7.764 7.737 7.738 7.790 7.799 7.149 7.130 7.121 7.103 6.906 6.878 6.850 6.748 6.731 6.696 6.678 6.412

5.724 5.671 4.898 4.876 4.848 4.825 4.540 4.528 4.363 4.336 4.310 4.207 4.183 4.160 4.137 3.364 3.355 3.302 3.043 3.021 2.479 2.350 2.293 2.261 2.233 2.198 2.175 2.152 2.129 1.907 1.875 1.862 1.805 1.771 1.740 1.590 1.576 1.564 1.531 1.302 1.277 1.254 0.943 0.923 0.903 0.872



Compound 1

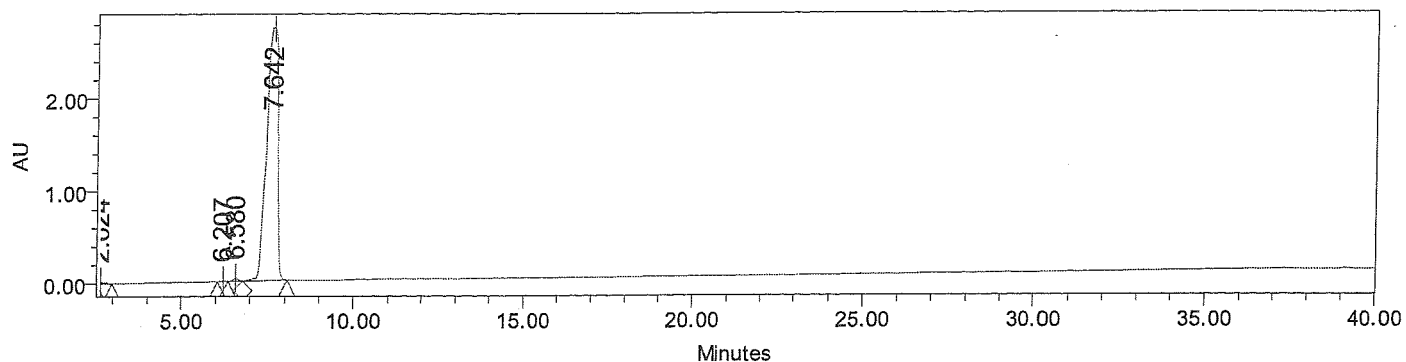


SAMPLE INFORMATION

Sample Name: TG-03299
 Sample Type: Unknown
 Vial: 1:A,6
 Injection #: 1
 Injection Volume: 10.00 ul
 Run Time: 40.0 Minutes
 Sample Set Name: 123

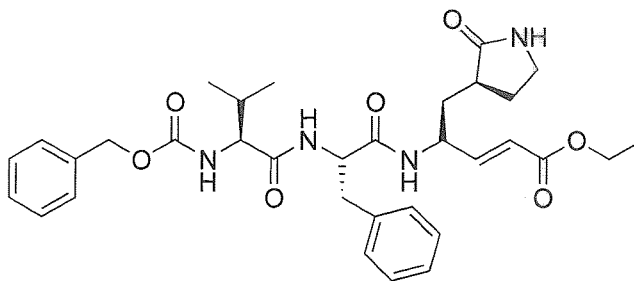
Acquired By: cfyeh
 Date Acquired: 2006/3/27 下午 06:13:34
 Acq. Method Set: TG0205628_PDA_1
 Date Processed: 2006/3/28 上午 09:57:38
 Processing Method: 5628 800
 Channel Name: W2996 210.0nm-1.2
 Proc. Chnl. Descr.: W2996 PDA 210.0 nm at 1.2

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1		2.624	287048	0.47
2		6.207	38734	0.06
3		6.580	305845	0.50
4		7.642	61049424	98.98



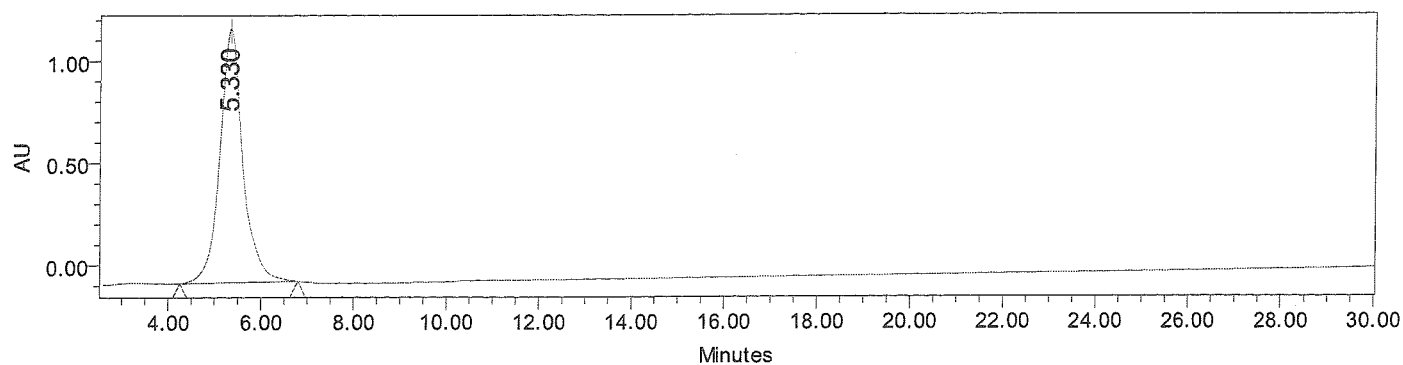
Compound 2

SAMPLE INFORMATION

Sample Name: TG-3250
 Sample Type: Unknown
 Vial: 1:B,3
 Injection #: 1
 Injection Volume: 10.00 ul
 Run Time: 30.0 Minutes
 Sample Set Name: 123

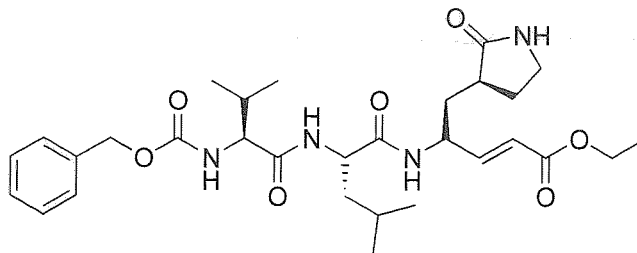
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 Date Acquired: 2006/3/28 下午 04:39:24
 Acq. Method Set: TG0205628_PDA_1
 Date Processed: 2006/3/28 下午 06:19:25
 Processing Method: 5628 500
 Channel Name: W2996 210.0nm-1.2
 Proc. Chnl. Descr.: W2996 PDA 210.0 nm at 1.2

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1		5.330	42856024	100.00

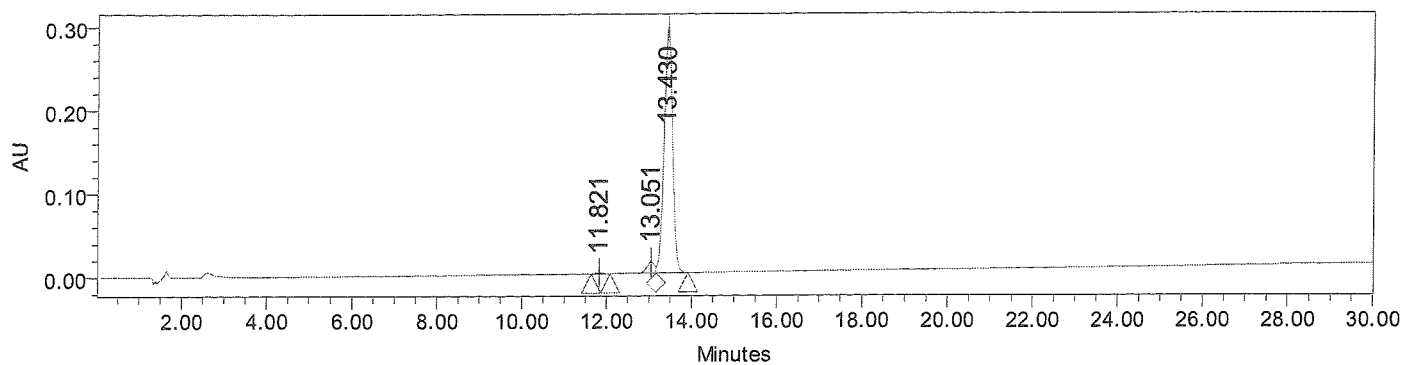


Compound 3

SAMPLE INFORMATION

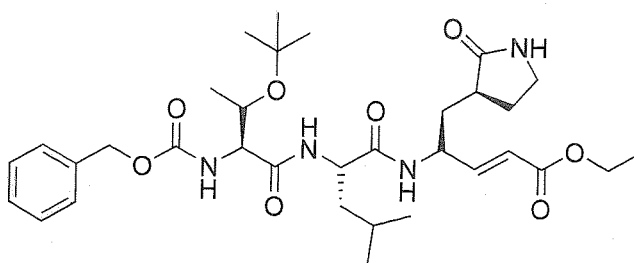
Sample Name: TG3770	Acquired By: cfyeh
Sample Type: Unknown	Date Acquired: 2006/3/30 下午 02:48:52
Vial: 1:A,1	Acq. Method Set: TG0205628_PDA_1
Injection #: 1	Date Processed: 2006/3/30 下午 03:34:24
Injection Volume: 10.00 ul	Processing Method: tg3770
Run Time: 30.0 Minutes	Channel Name: W2996 230.0nm-1.2
Sample Set Name: TG3770_032006	Proc. Chnl. Descr.: W2996 PDA 230.0 nm at 1.2

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1		11.821	10046	0.24
2		13.051	168616	4.03
3		13.430	4006570	95.73



Compound 4

0.922
 0.942
 0.949
 0.970
 0.998
 1.052
 1.071
 1.240
 1.254
 1.263
 1.287
 1.296
 1.524
 1.554
 1.583
 1.607
 1.619
 1.632
 1.647
 1.696
 1.734
 1.765
 1.806
 2.020
 2.060
 2.359
 2.462
 2.476
 3.220
 3.287
 3.309
 3.335
 4.134
 4.158
 4.181
 4.205
 4.453
 4.606
 5.053
 5.093
 5.116
 5.157
 5.899
 5.951
 5.956
 6.572
 6.793
 6.812
 6.846
 6.863
 7.259
 7.311
 7.332
 7.340
 7.353
 7.363
 7.395
 7.613
 7.624

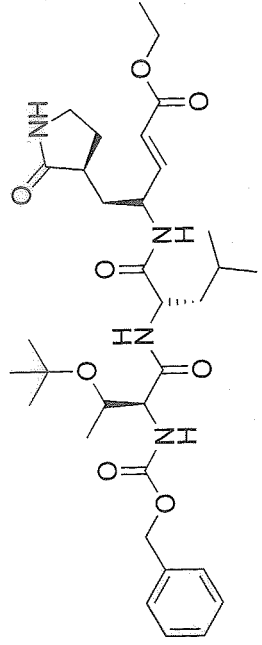
DEC. & VT

dfrq 300.063
 dn H1
 dpwr 30
 dof 0
 dm nnn
 dmm c
 dmf 200
 lb 0.10
 wfile
 proc ft
 fn not used

PROCESSING

SAMPLE

date Mar 30 2006
 solvent CDCl3
 file
 ACQUISITION exp
 sfrq 300.063
 th H1
 at 2.668
 np 25530
 sw 4796.2
 fb not used
 bs
 tpwr 57
 pw 7.5
 dl 1.000
 tof 309.9
 nt 32
 ct 32
 alock y
 gain not used
 fl n
 in n
 dp y

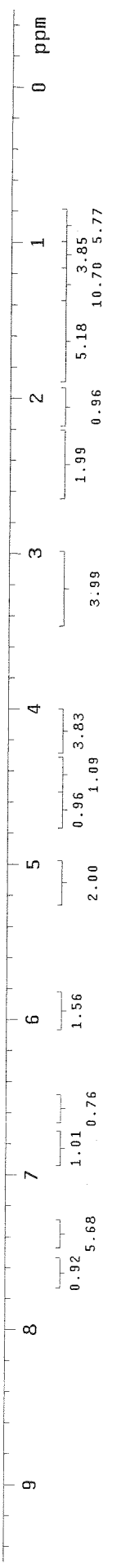


Compound 4

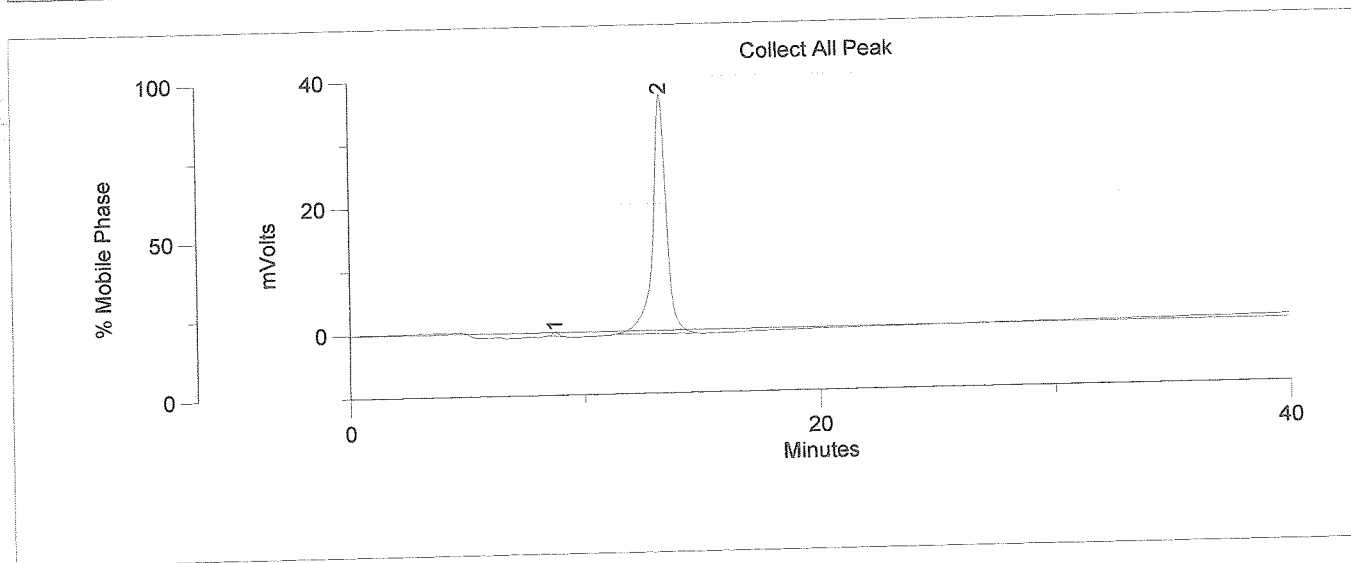
sp -150.1
 wp 3000.5
 vs 122
 sc 0
 wc 250
 hzmm 12.00
 ls 594.20
 rfl 574.6
 rfp 0
 th 2
 ins 50.250
 nm cdc ph

STANDARD 1H OBSERVE

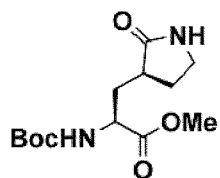
exp1 stdlh



	Inj. Number	Peak Name	R. Time	Area	Area %	Height	Height %	
1	2.00	1	8.73	22757.08	0.89	0.71	1.86	
2	2.00	2	13.25	2536807.25	99.11	37.74	98.14	



Custom Calculations:
 Injection Volume: 1000 ul
 Run Time: 40 Minutes
 Eluent System: 60 % - 100 % CH₃OH/ H₂O



Compound 9

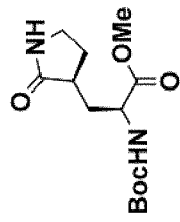
4.327 4.315 4.290 4.265 4.253 3.714 3.707 3.648 3.346 3.326 3.316 3.308 3.297 2.487 2.471 2.458 2.442 2.429 2.415 2.386 2.151 2.140 2.105 2.093 2.070 2.058 1.880 1.855 1.835 1.828 1.808 1.798 1.783 1.765 1.730 1.621 1.412 1.404

6.003

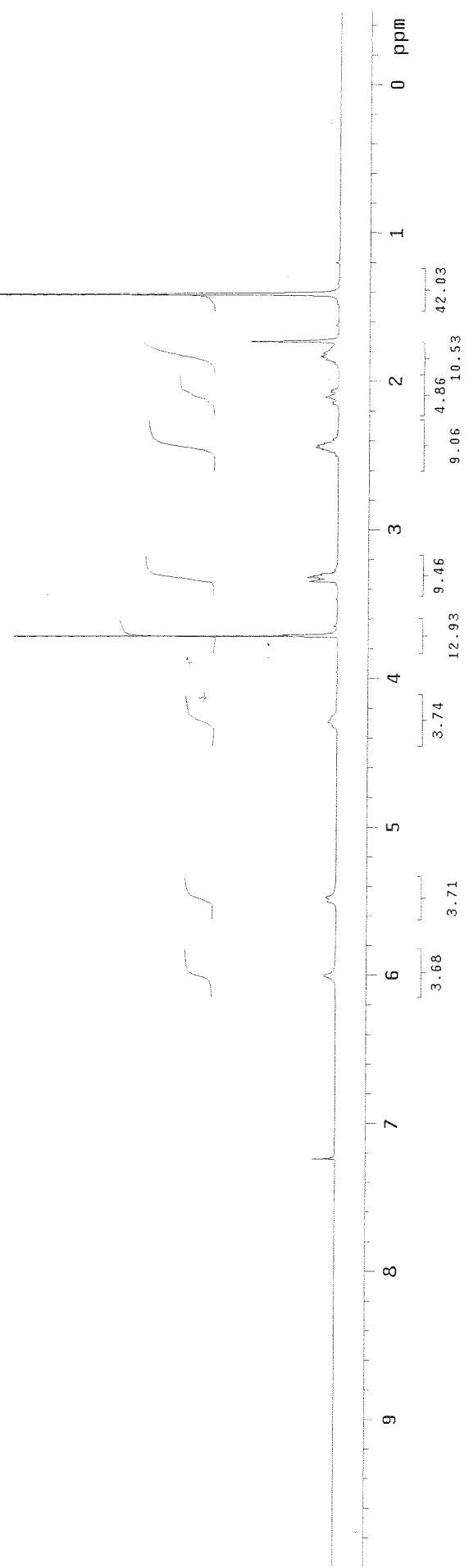
7.240 7.232

040819_BocMACDOOME
 expl stdin
 date Aug 19 2004
 solvent CDC13
 file /export/home/~
 syllao/040819_BocM~
 ACQ002.fid
 ACQUISITION
 sfrq 300.069
 tn H1
 at 1.708
 np 16384
 sw 4796.2
 fb not used
 bs 4
 tpwr 57
 pw 7.0
 dl 1.000
 tof 309.9
 nt 100
 ct 100
 alock V
 gain not used
 flags
 il n
 in n
 dp y
 DISPLAY
 sp -150.1
 wp 3150.4
 vs 151
 sc 0
 wc 250
 hzmm 12.60
 is 330.60
 rfl 2775.7
 rfp 2172.5
 th 1
 ins 100.000
 nm cdc ph

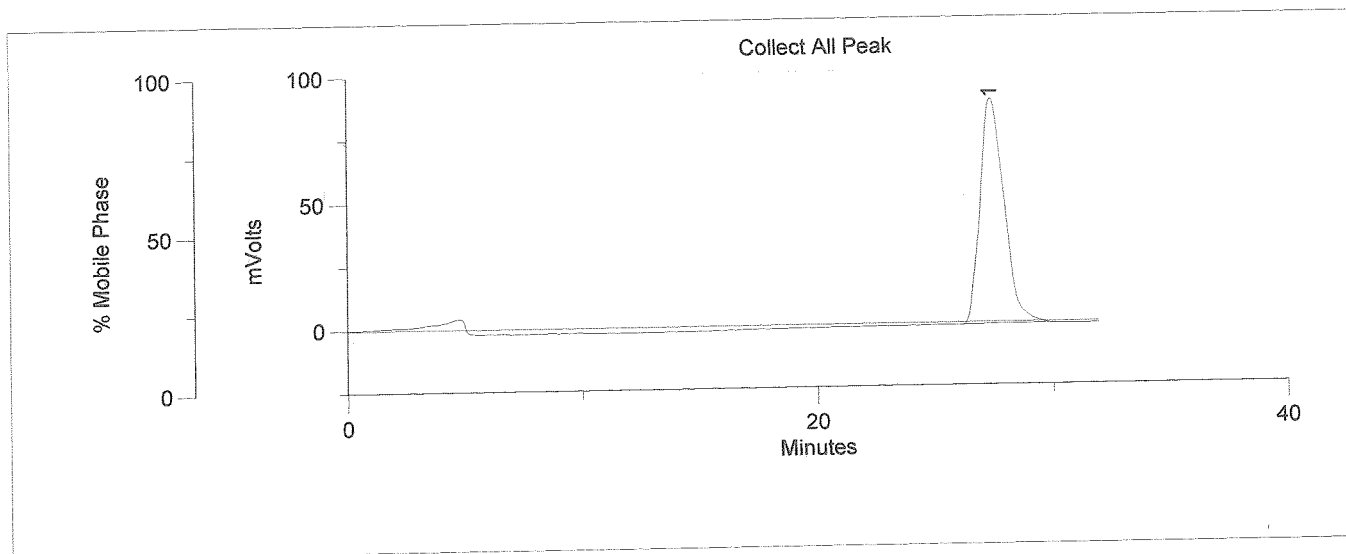
DEC. & VT
 dfrq 300.069
 dn H1
 dpwr 30
 dof 0
 dm nnn
 dmm C
 dmf 200
 PROCESSING
 lb 0.10
 wtfile
 proc ft
 fn not used



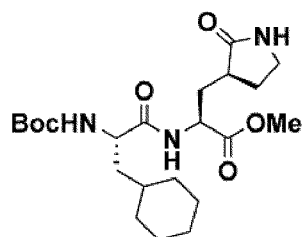
Compound 9



	Inj. Number	Peak Name	R. Time	Area	Area %	Height	Height %
1	2.00	1	27.33	10616901.00	100.00	89.00	100.00



Custom Calculations:
Injection Volume: 1000 ul
Run Time: 32 Minutes
Eluent System: 60 % - 92 % CH₃OH/ H₂O

**Compound11**

060330Boc_B_CH_A_MA_COOMe

expl stidh

SAMPLE date Mar 30 2006
solvent CDC13
file H1
ACQUISITION exp 30
dof 0
Sfrq 300.063
tn H1
at 2.668
np 25590
sw 4796.2
fb not used
bs 4
tpwr 57
pw 7.5
dl 1.000
tof 309.9
nt 128
ct 128
alock y
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -150.1
wp 3150.4
vs 150
sc 0
wc 250
hzmm 1.75
ts 725.30
rfi 2752.9
rfp 2172.4
th 5
ins 41.000
nm cdc ph

DEC. & VT

dfrq 300.063

dn H1

dpwr 30

dof 0

dm nnn

dmm c

dmf 200

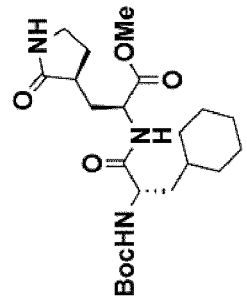
PROCESSING

lb 0.10

wfile

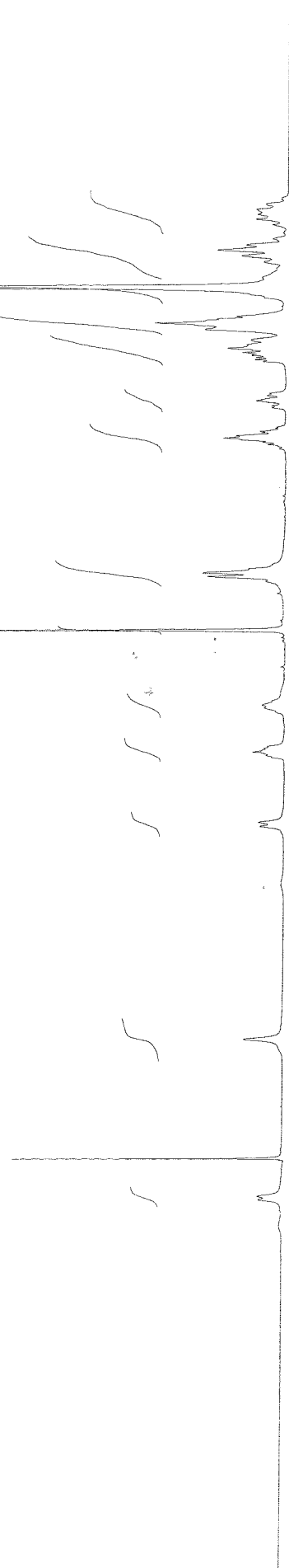
proc ft

fn not used



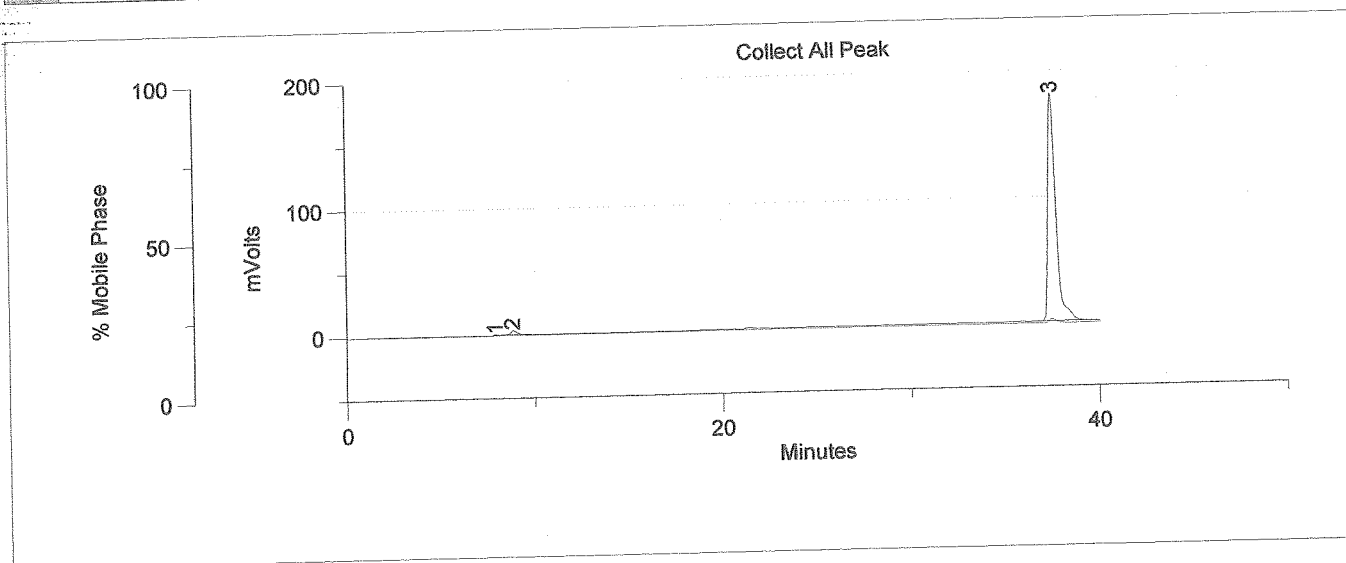
Compound11

3.702
3.340
3.316
3.288
2.414
2.399
1.863
1.858
1.844
1.828
1.814
1.790
1.759
1.690
1.646
1.619
1.600
1.405
1.196
1.156
1.124

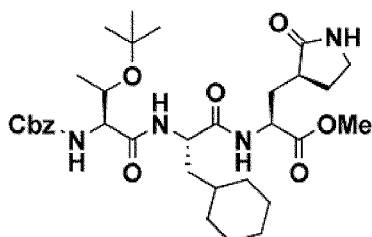


9 8 7 6 5 4 3 2 1 0 ppm

	Inj. Number	Peak Name	R. Time	Area	Area %	Height	Height %	
1	2.00	1	7.87	8520.01	0.10	0.63	0.34	
2	2.00	2	8.82	122462.50	1.38	3.35	1.82	
3	2.00	3	37.50	8732244.00	98.52	179.56	97.83	



Custom Calculations:
Injection Volume: 2000 ul
Run Time: 40 Minutes
Eluent System: 60 % - 100 % CH₃OH/ H₂O



Compound 13

```

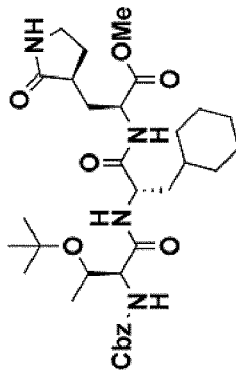
0504082ThrBuCHAMACDOME
expl stdih
date Apr 8 2005 dfrq 300.069
solvent CDC13 dn H1
file /export/home/~ dpwr 30
syllao/0504082Thr~ dof 0
BuCHAMACDOME.fid dm nnn
ACQUISITION dmm 200
sfrq 300.069 H1
tn at 1.708 lb wtfile 0.10
np 16384 wfile
sw 4796.2 proc ft
fb not used fn not used
bs 4
tpwr 57 werr
pw 7.0 wexp
dl 1.000 wbs
tof 309.9 wnt
nt 100
ct 0
alock not used
gain V
flags
il n
in n
dp y
DISPLAY
sp -150.1
wp 3150.4
vs 150
sc 0
wc 250
hzmm 2.61
is 1037.76
rfl 2775.7
rfp 2172.5
th 2
ins 100.000
nm cdc ph

```

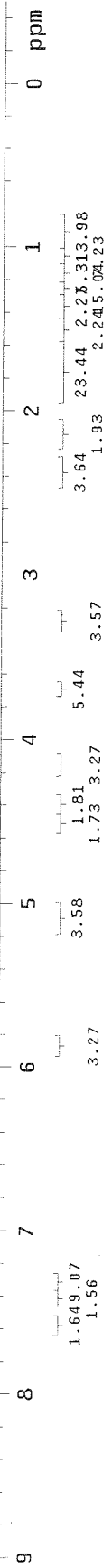
DEC. & VT

7.592
7.572
7.420
7.394
7.351
7.345
7.322
7.312
7.293
7.275
7.240
5.872
5.076
5.035
4.485
4.473
4.438
4.411
4.393
4.366
4.151
4.151
3.697
3.271

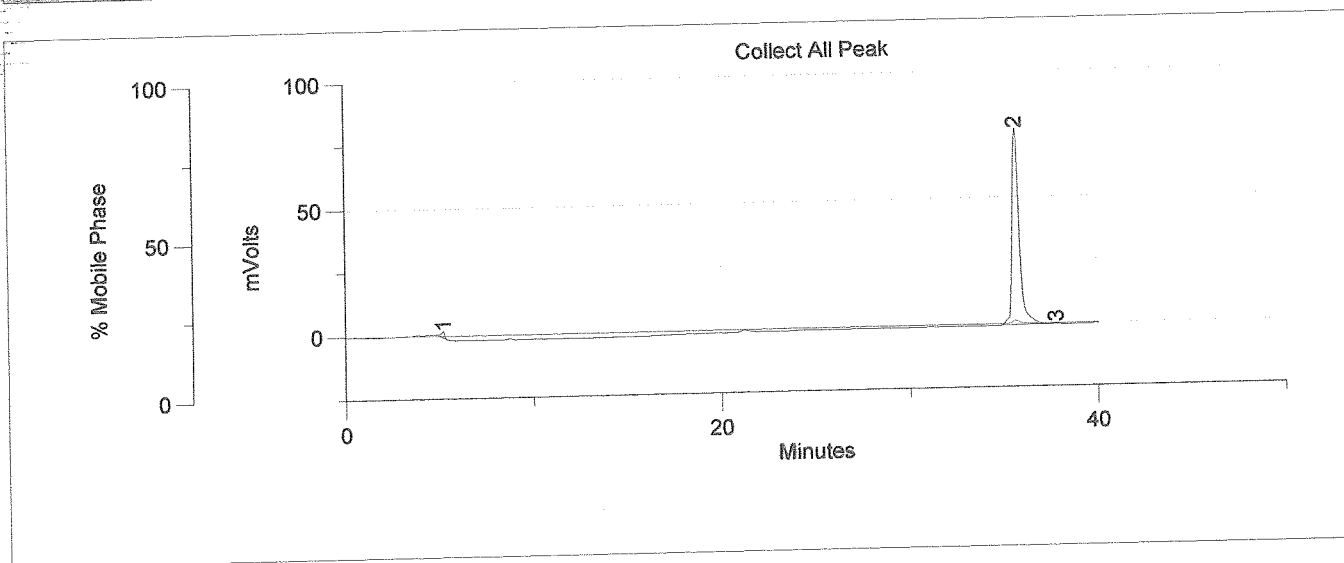
1.818
1.777
1.707
1.662
1.564
1.545
1.535
1.515
1.500
1.488
1.469
1.408
1.336
1.311
1.246
1.197
1.158
1.127
1.065
1.045
1.004
0.963
0.932
0.899
0.860



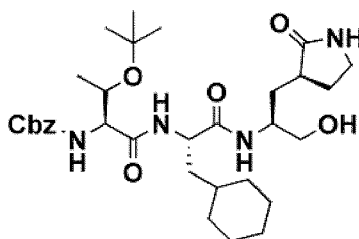
Compound 13



	Inj. Number	Peak Name	R. Time	Area	Area %	Height	Height %	
1	2.00	1	5.18	57515.81	1.59	2.45	3.04	
2	2.00	2	35.64	3543661.75	97.94	77.62	96.29	
3	2.00	3	37.76	17180.00	0.47	0.54	0.67	



Custom Calculations:
 Injection Volume: 2000 ul
 Run Time: 40 Minutes
 Eluent System: 60 % - 100 % CH₃OH/ H₂O



Compound 14

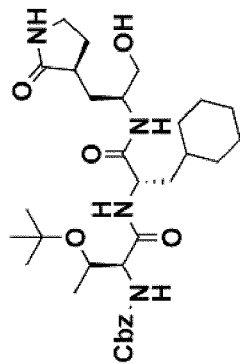
```

050317_HPLC1st_01
expl stdih
SAMPLE
date Mar 17 2005
solvent CDC13
file /export/home/~
syllao/050317_HPLC~
1st_01.fid
nnn
dm
dmm
dmf
sfrq 300.069
tn H1
at 1.366
np 16384
sw 5995.2
fb not used
bs 4
tpwr 57
pw 7.0
dl 1.000
tof 309.9
nt 1000
ct 272
alock y
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -150.4
wp 3149.8
vs 150
sc 0
wc 250
hzmm 1.81
ls 769.62
rfl 3375.2
rff 2172.5
th 5
ins 100.000
nm cdc ph

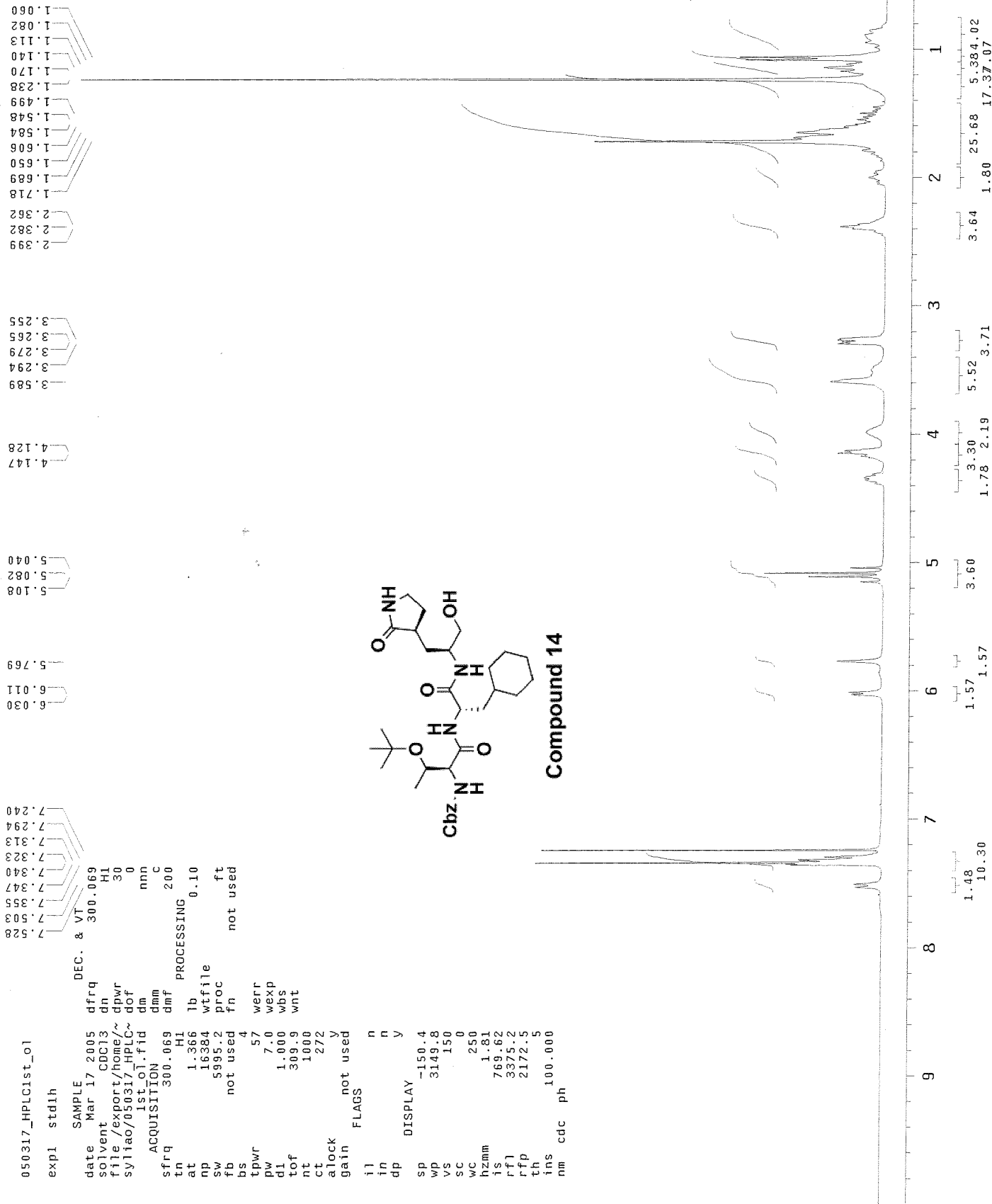
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DEC. & VT

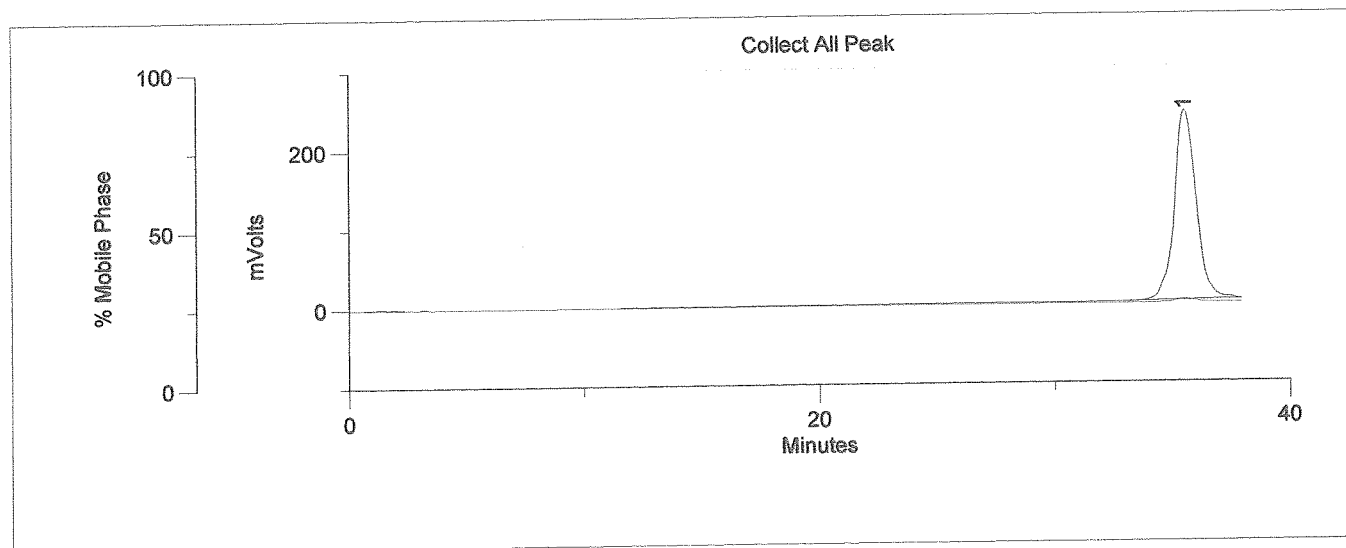
dfrq 300.069
dn H1
dpwr 30
dof 0
dm nnn
dmm C
dmf 200
PROCESSING 0.10
lb wtfile
ft
proc
fn not used



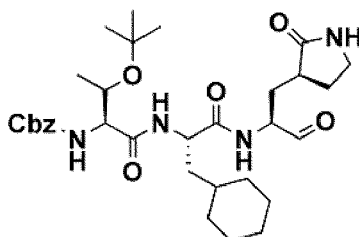
Compound 14



	Inj. Number	Peak Name	R. Time	Area	Area %	Height	Height %	
1	1.00	1	35.53	25452462.00	100.00	239.55	100.00	



Custom Calculations:
Injection Volume: 1000 ul
Run Time: 38 Minutes
Eluent System: 60 % - 98 % CH₃OH/ H₂O



Compound 15

NO STANDARD 1H OBSERVE

7.407
7.388
7.340
7.318
7.298
7.240

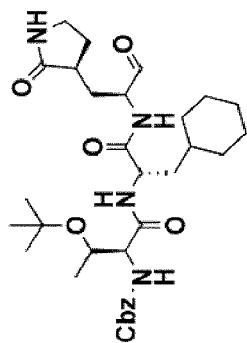
5.885

5.146
5.106
5.077
5.036

4.438
4.423
4.395
4.361
4.162

3.325
3.299
2.443
2.411
2.357
2.024
1.932
1.868
1.842
1.771
1.707
1.670
1.608
1.589
1.560
1.309
1.244
1.160
1.130
1.079
1.060
0.975
0.944
0.911
0.875
0.856

SAMPLE date Dec 28 2005 dfrq 300.063
solvent CDC13 dn H1
file exp 30
ACQUISITION exp 30
dof 0
dm nnn
tn 300.063
at 2.868
np 25590
sw 4796.2
fb not used
bs 0.10
tpwr 4
pw 57
di 7.5
tof 1.000
nt 309.9
ct 128
alock 128
gain not used
flags
il n
in n
cp y
DISPLAY
sp -150.1
wp 3300.6
vs 179
sc 0
wc 250
hzmm 13.20
is 609.62
rfi 580.4
rff 0
th 4
ins 100.000
nm cdc ph



Compound 15

