

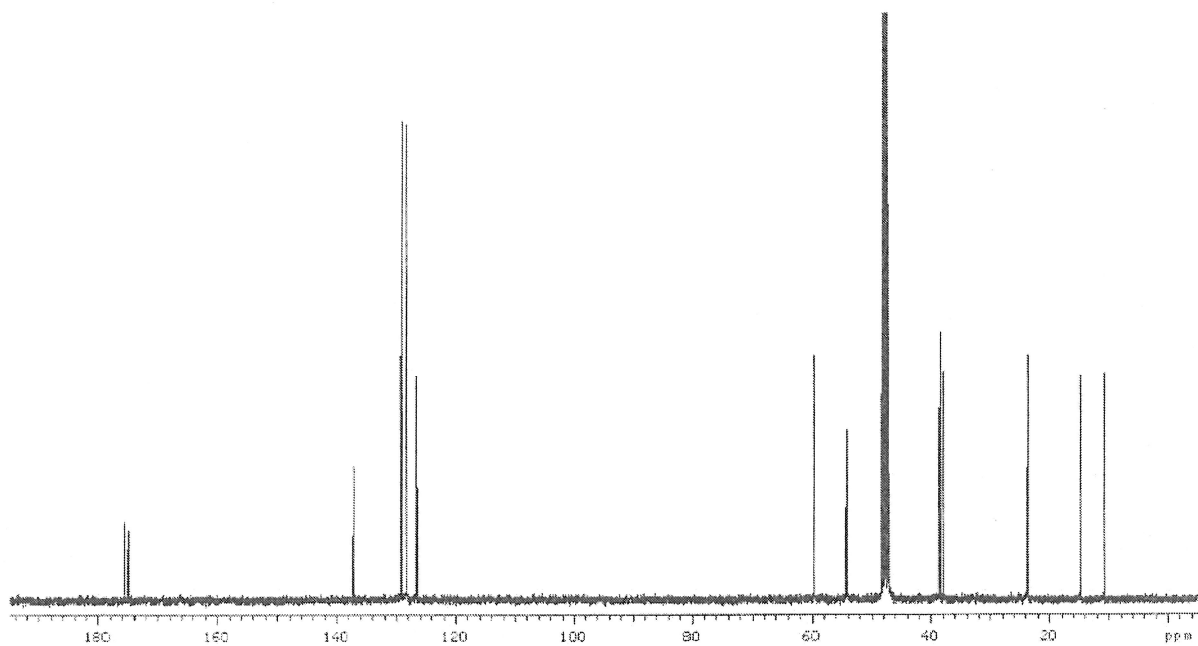
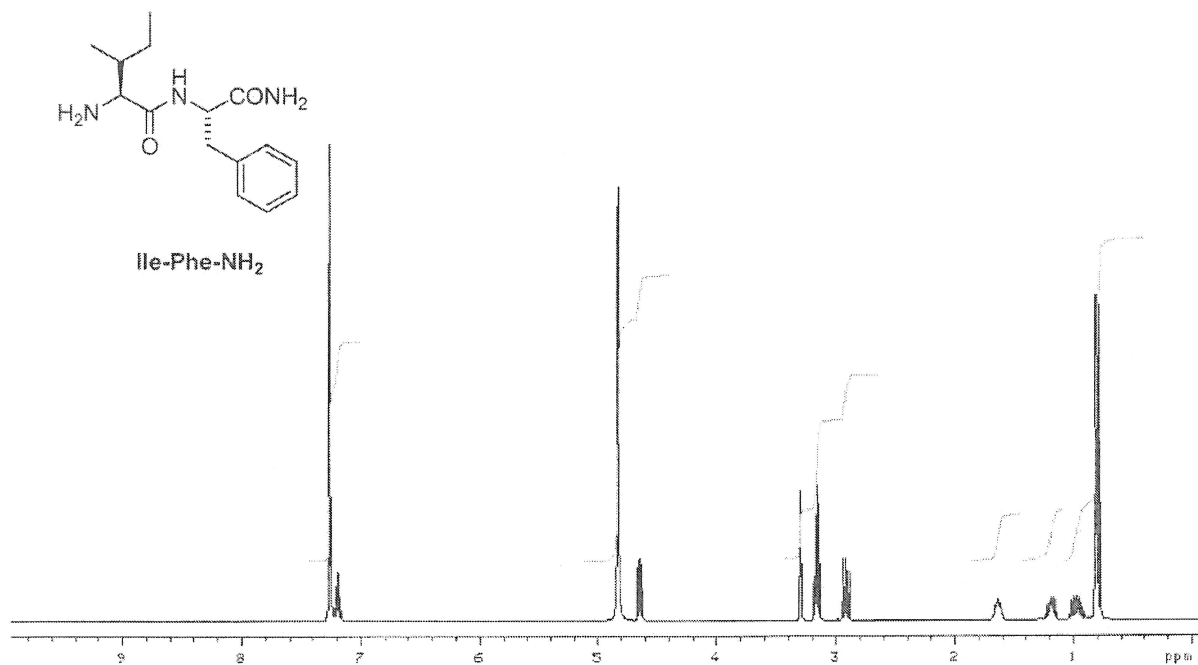
# Applications of 3-aminolactams: design, synthesis, and biological evaluation of a library of HIV1-protease inhibitors

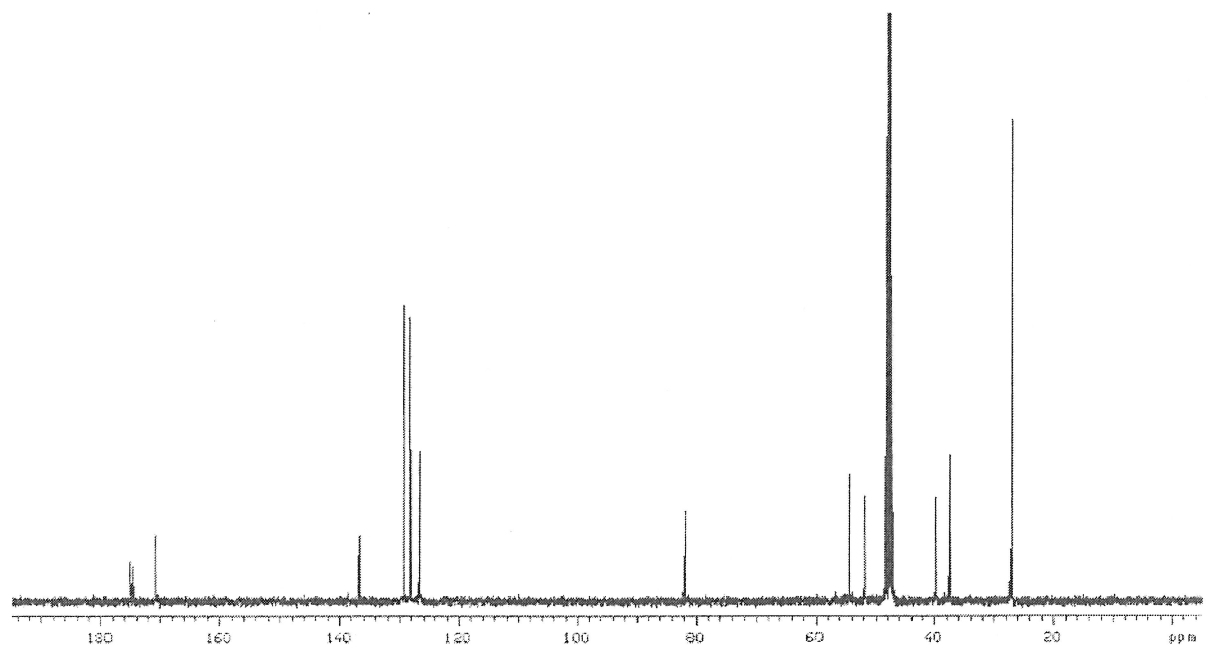
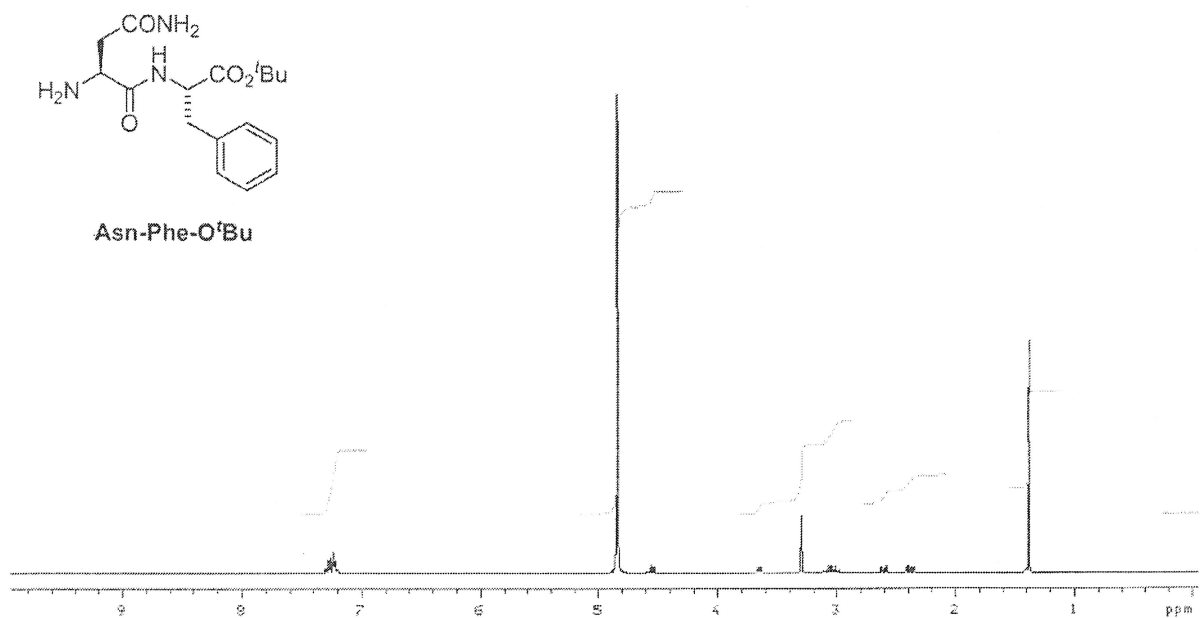
*Eulàlia Pinyol,<sup>a</sup> Silvia Frutos,<sup>a</sup> Dolors Grillo-Bosch,<sup>a</sup> Ernest Giralt,<sup>a,b</sup> Bonaventura Clotet,<sup>c</sup> Jose  
A. Esté,<sup>c</sup> Anna Diez<sup>a,d\*</sup>*

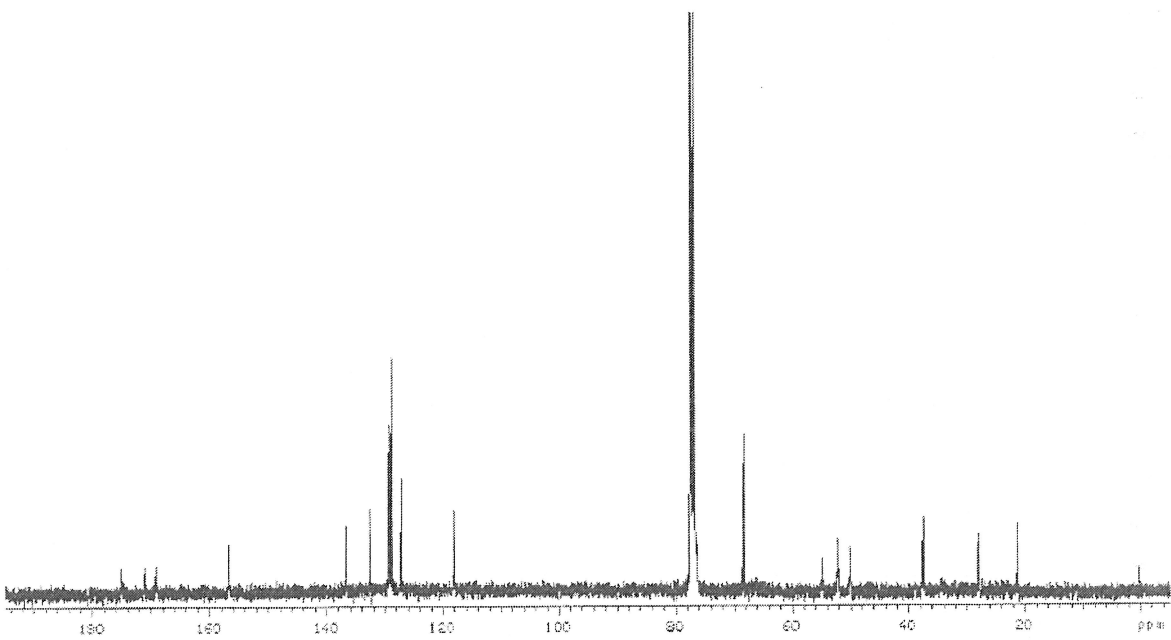
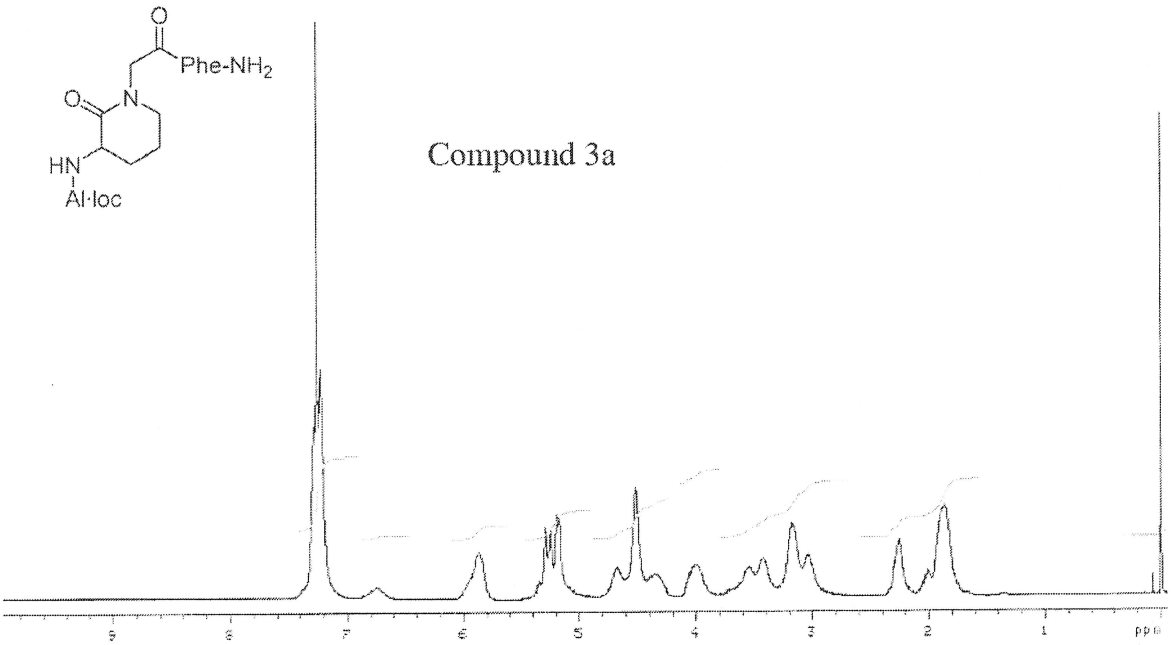
## Supplementary material

### Index

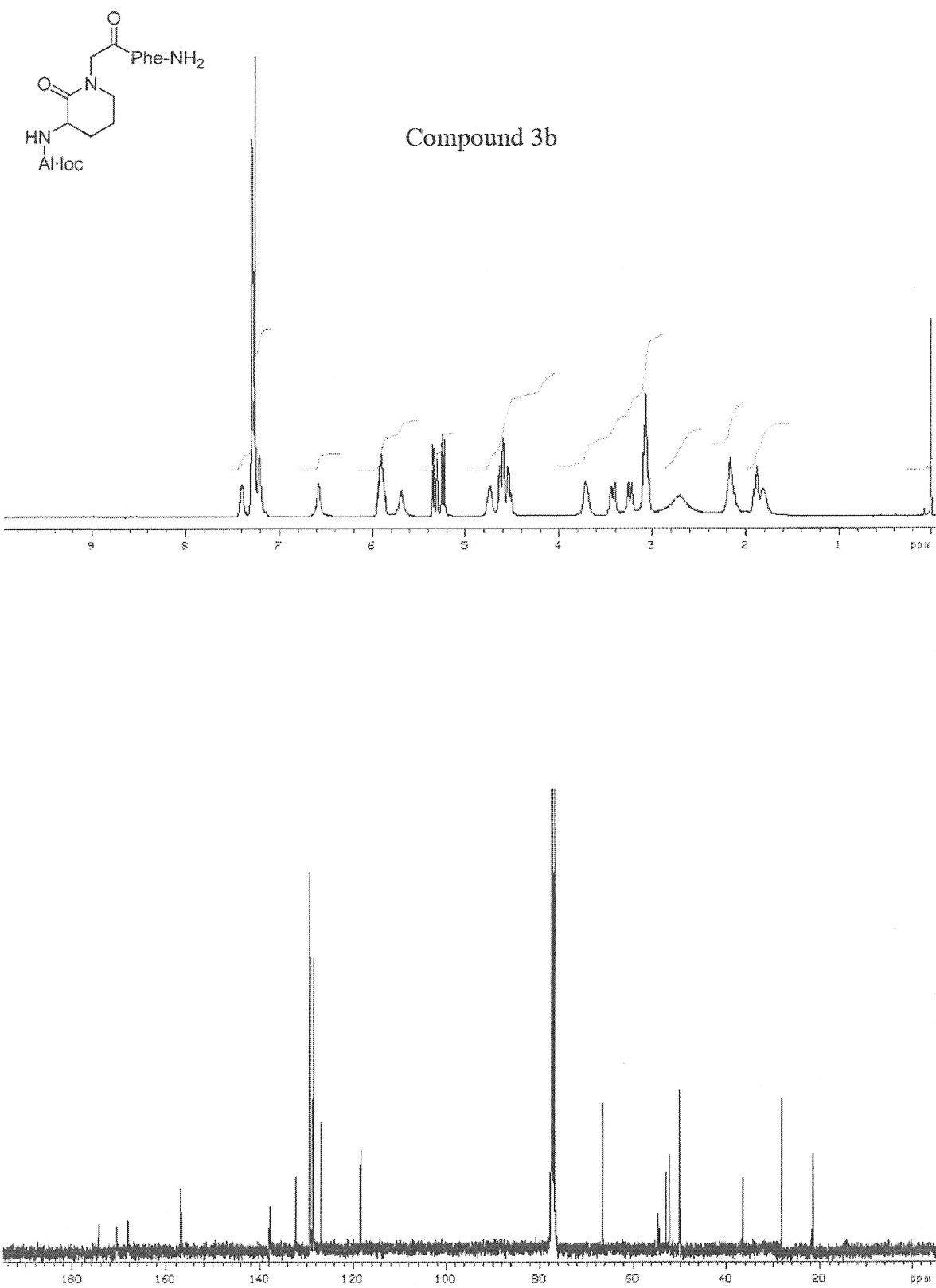
- <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of compounds **Ile-PheNH<sub>2</sub>** and **Asn-PheO<sup>t</sup>Bu**
- <sup>1</sup>H- and <sup>13</sup>C-NMR spectra, and HRMS of compounds **3a** and **3b**
- Analytical HPLC and MS of compounds **4-18cc**
- Cell culture activity against native HIV1 and resistant mutant IRL98DPRO





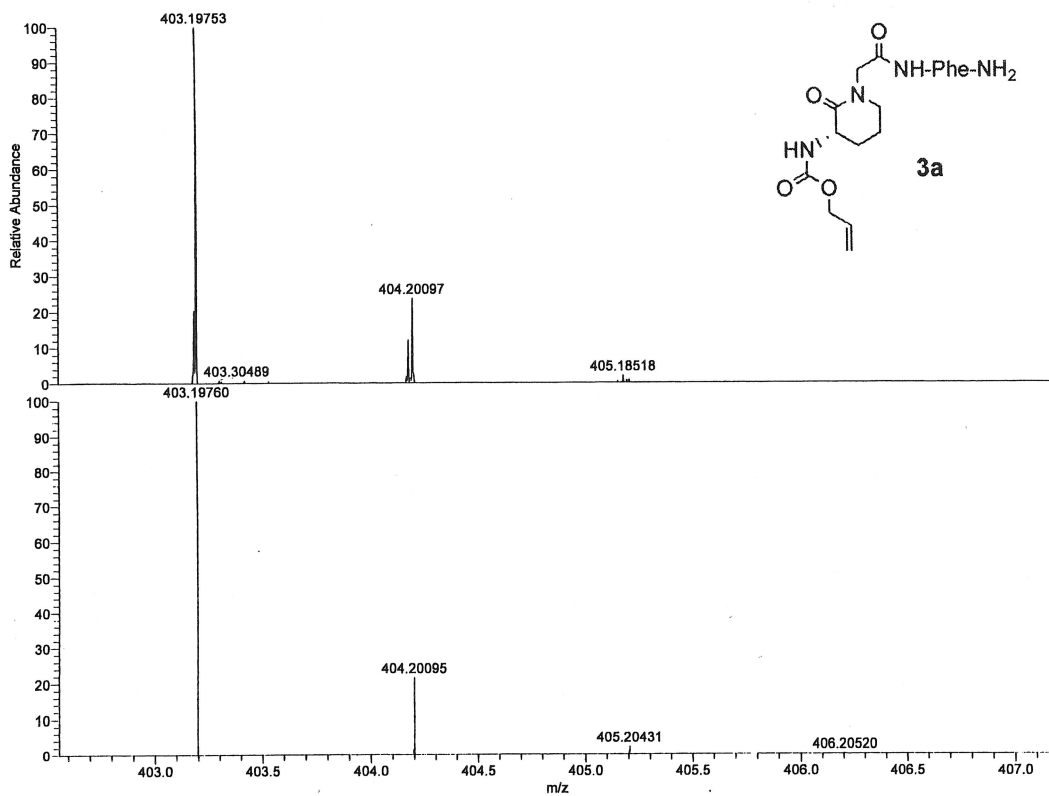






C:\Xcalibur\...1100525\_EP-223\_a

5/25/2010 7:07:29 PM



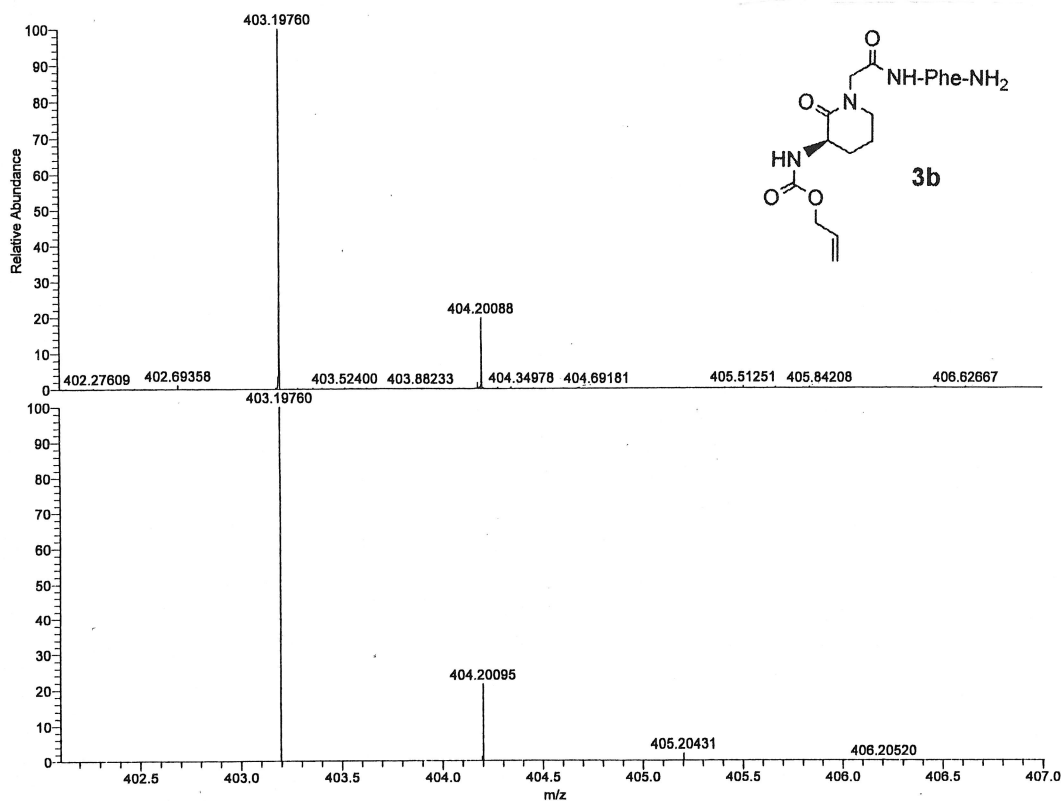
NL:  
1.50E8  
100525\_EP-  
223\_a#1 RT: 0.00  
AV: 1 T: FTMS + p  
NSI Full ms  
[100.00-1000.00]

NL:  
7.83E5  
C<sub>20</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub> +H:  
C<sub>20</sub>H<sub>27</sub>N<sub>4</sub>O<sub>5</sub>  
pa Chrg 1

C:\Xcalibur\...1100526\_EP-223\_b  
100526\_EP-223\_b

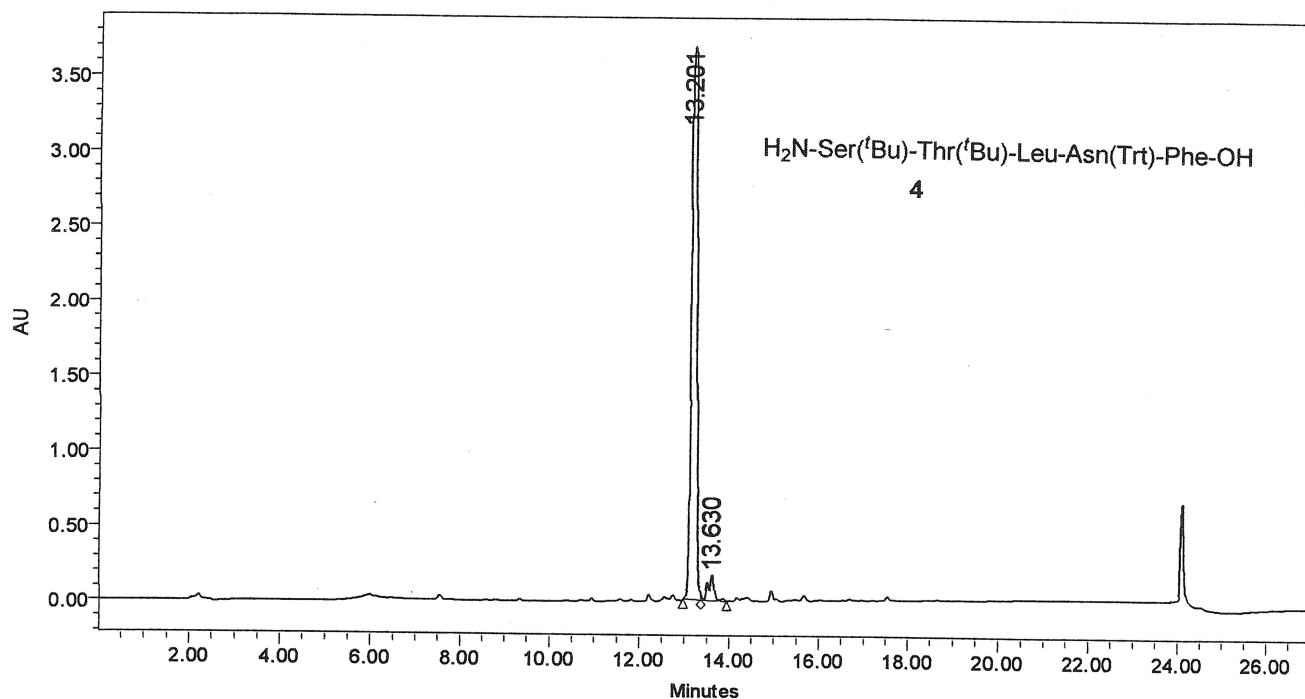
5/26/2010 5:27:38 PM

100526\_EP-223\_b



NL:  
1.03E8  
100526\_EP-223\_b#6-  
59 RT: 0.14-1.62  
AV: 54 T: FTMS + p  
NSI Full ms  
[200.00-2000.00]

NL:  
7.83E5  
C<sub>20</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub> +H:  
C<sub>20</sub>H<sub>27</sub>N<sub>4</sub>O<sub>5</sub>  
pa Chrg 1

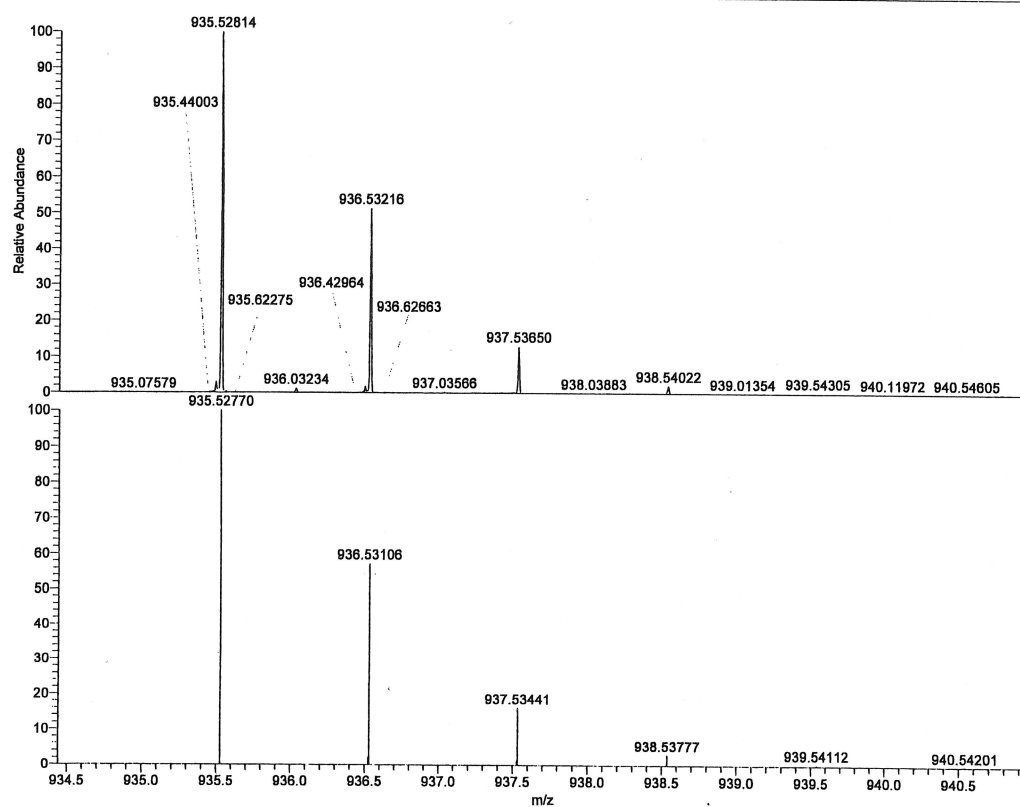


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	13.201	32746225	95.27	3689281	95.56
2	13.630	1624611	4.73	171277	4.44

C:\Xcalibur\100525\_EP-C1\_3bis  
100525\_EP-C1\_3bis

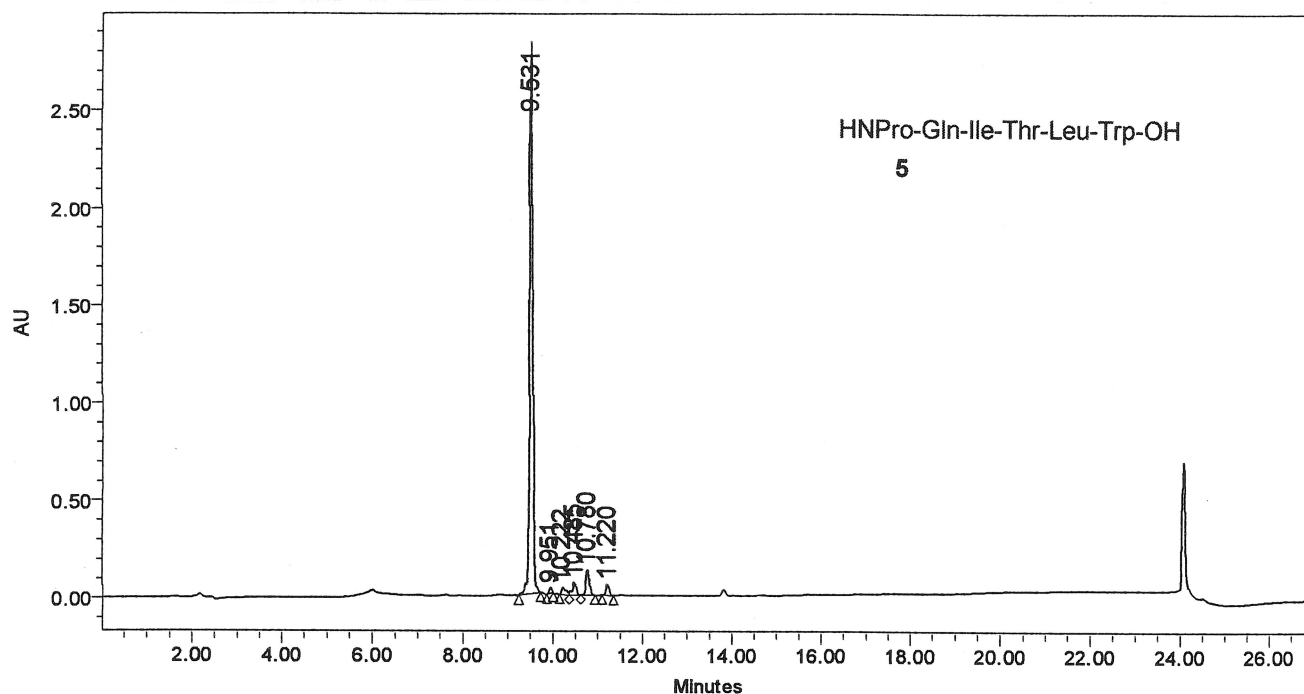
5/26/2010 2:43:12 PM

100525\_EP-C1\_3bis



NL:  
6.80E6  
100525\_EP-  
C1\_3bis#17-81 RT:  
0.45-2.25 AV: 65 T:  
FTMS + p NSI Full ms  
[200.00-2000.00]

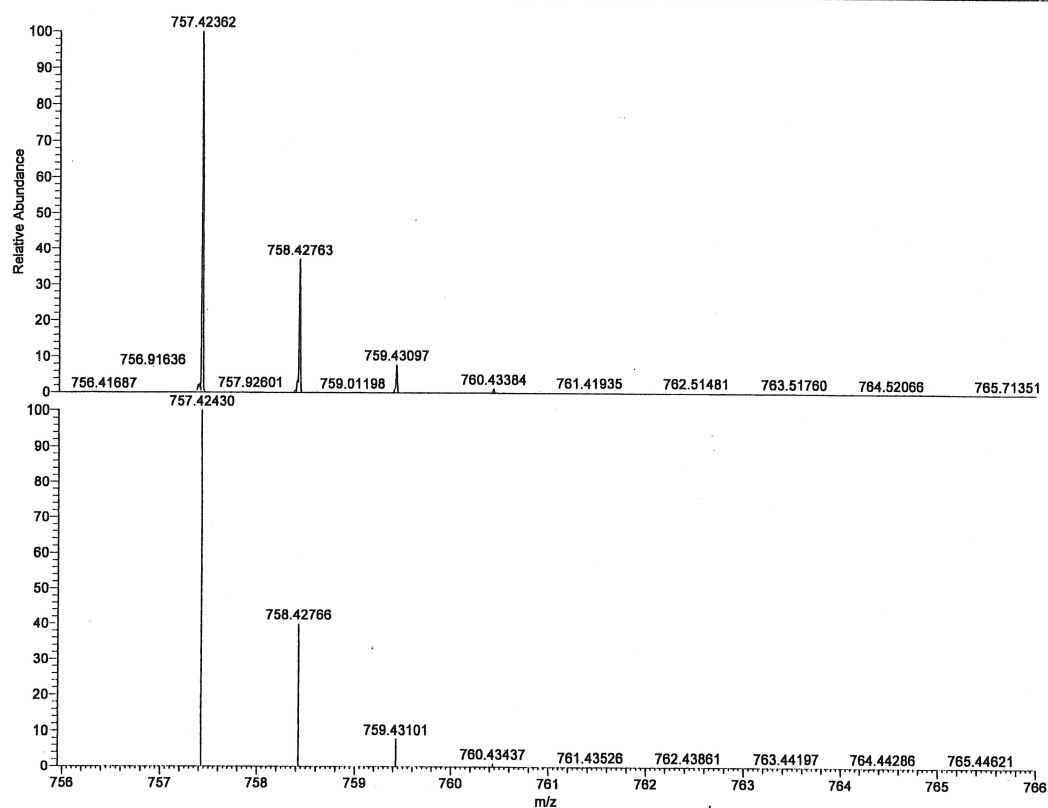
NL:  
5.37E5  
C<sub>53</sub>H<sub>70</sub>N<sub>8</sub>O<sub>8</sub>+H:  
C<sub>53</sub>H<sub>71</sub>N<sub>8</sub>O<sub>8</sub>  
pa Chrg 1



	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	9.531	14155639	88.94	2853021	89.97
2	9.951	122610	0.77	28280	0.89
3	10.222	269670	1.69	37675	1.19
4	10.485	371147	2.33	64155	2.02
5	10.780	715466	4.50	129831	4.09
6	11.220	281291	1.77	58158	1.83

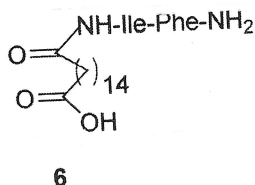
C:\Xcalibur\...100525\_EP-N1

5/25/2010 7:01:35 PM



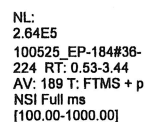
NL:  
2.41E6  
100525\_EP-N1#38-  
189 RT: 0.58-2.88  
AV: 152 T: FTMS + p  
NSI Full ms  
[100.00-1000.00]

NL:  
6.34E5  
PQITLW +H +H<sub>2</sub>O:  
C<sub>37</sub>H<sub>57</sub>N<sub>5</sub>O<sub>9</sub>  
pa Chrg 1

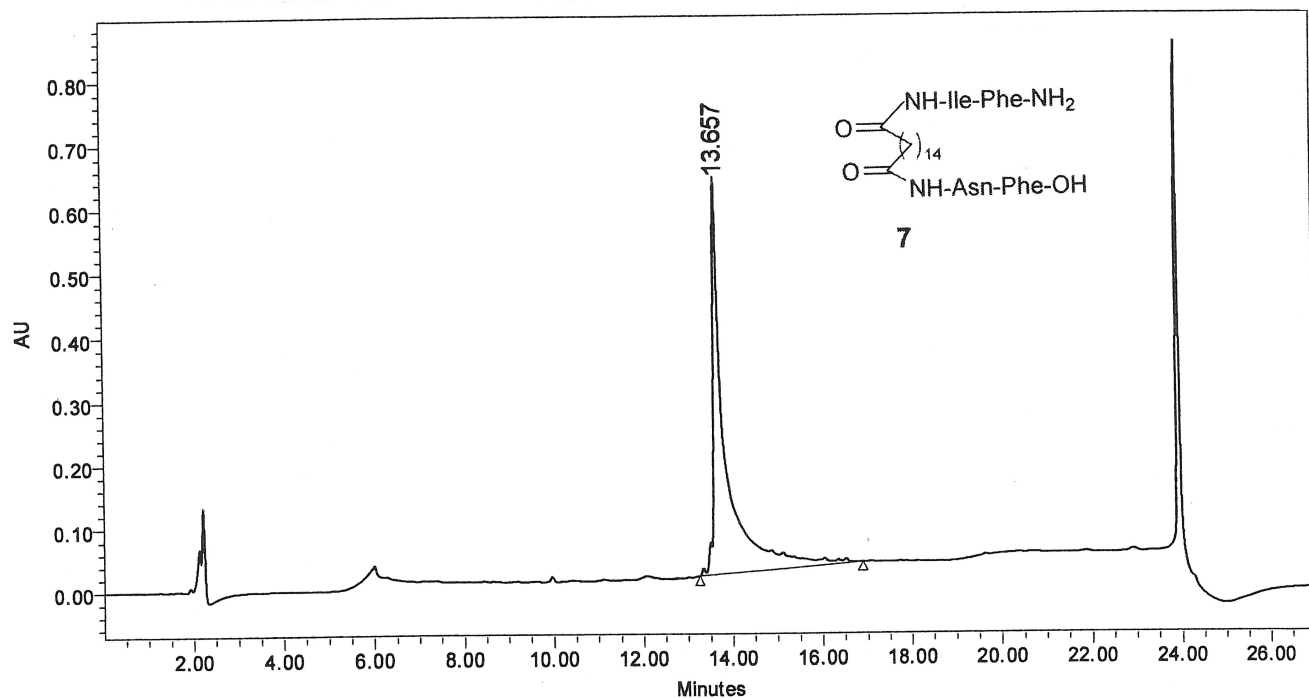


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	13.611	249224	8.72	45487
2	14.915	2162121	75.61	371125
3	15.184	448356	15.68	30124

5/25/2010 7:13:22 PM



NL:  
6.96E5  
C<sub>31</sub> h<sub>51</sub> n<sub>3</sub> o<sub>5</sub> +H:  
C<sub>31</sub> H<sub>52</sub> N<sub>3</sub> O<sub>5</sub>  
pa Chrg 1

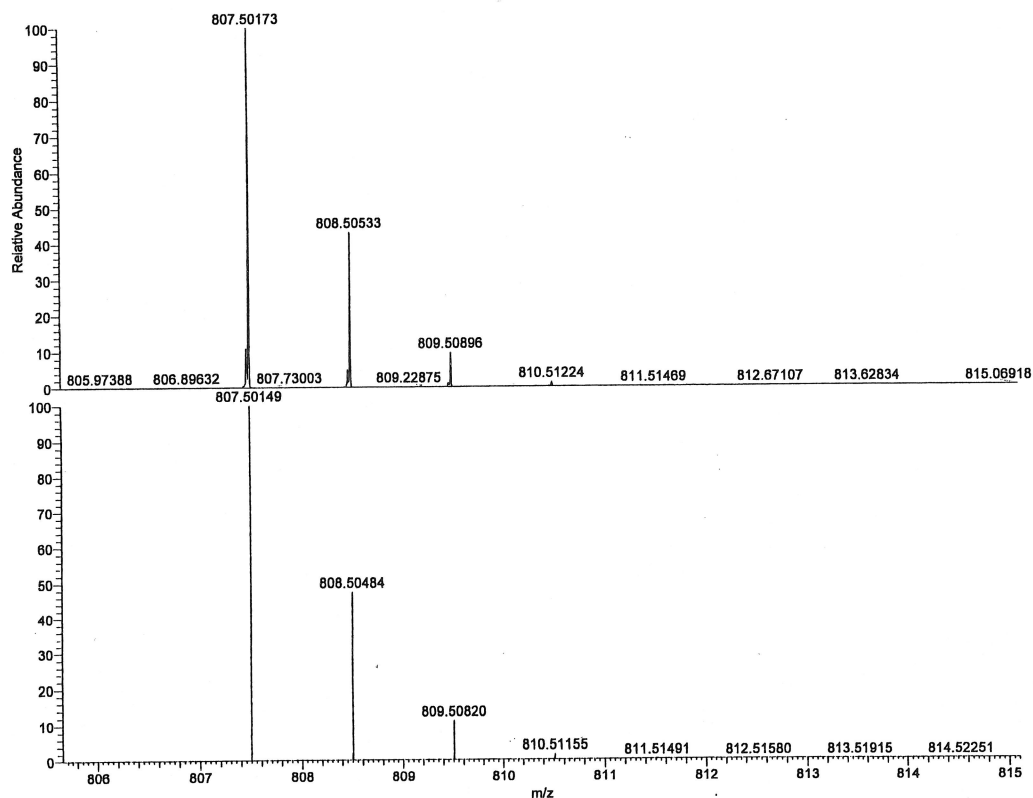


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	13.657	11706244	100.00	625670	100.00

100526\_EP-186\_p\_100526163726  
100526\_EP-186\_p

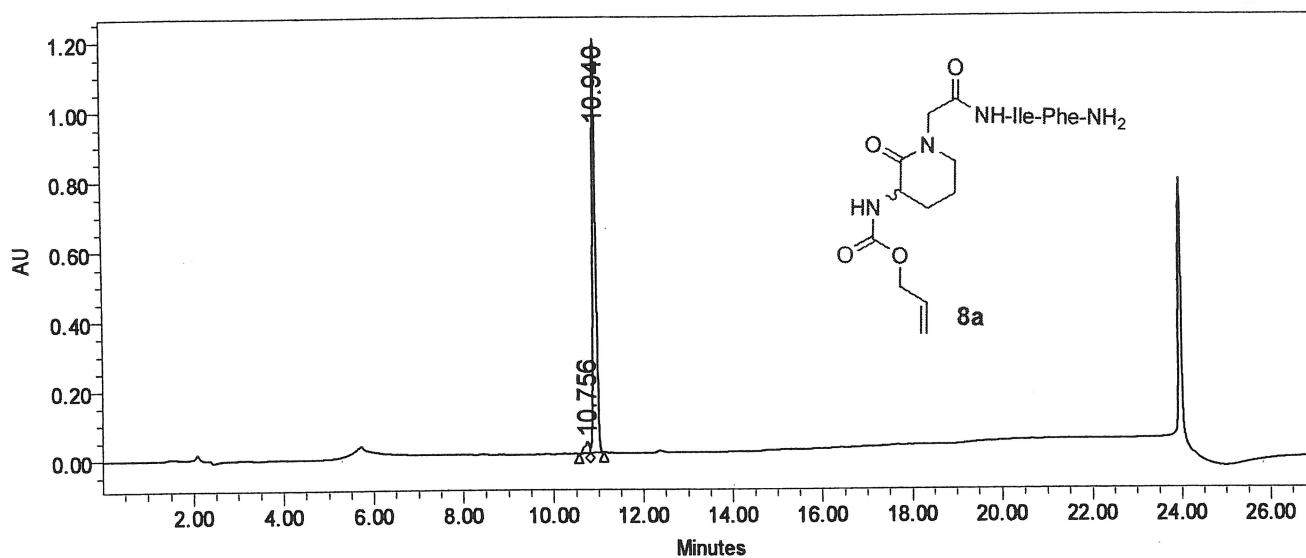
5/26/2010 4:37:26 PM

100526\_EP-186\_p



NL:  
8.13E5  
100526\_EP-  
186\_p\_100526163726#3-  
32 RT: 0.06-0.87 AV: 30  
T: FTMS + p NSI Full ms  
[200.00-2000.00]

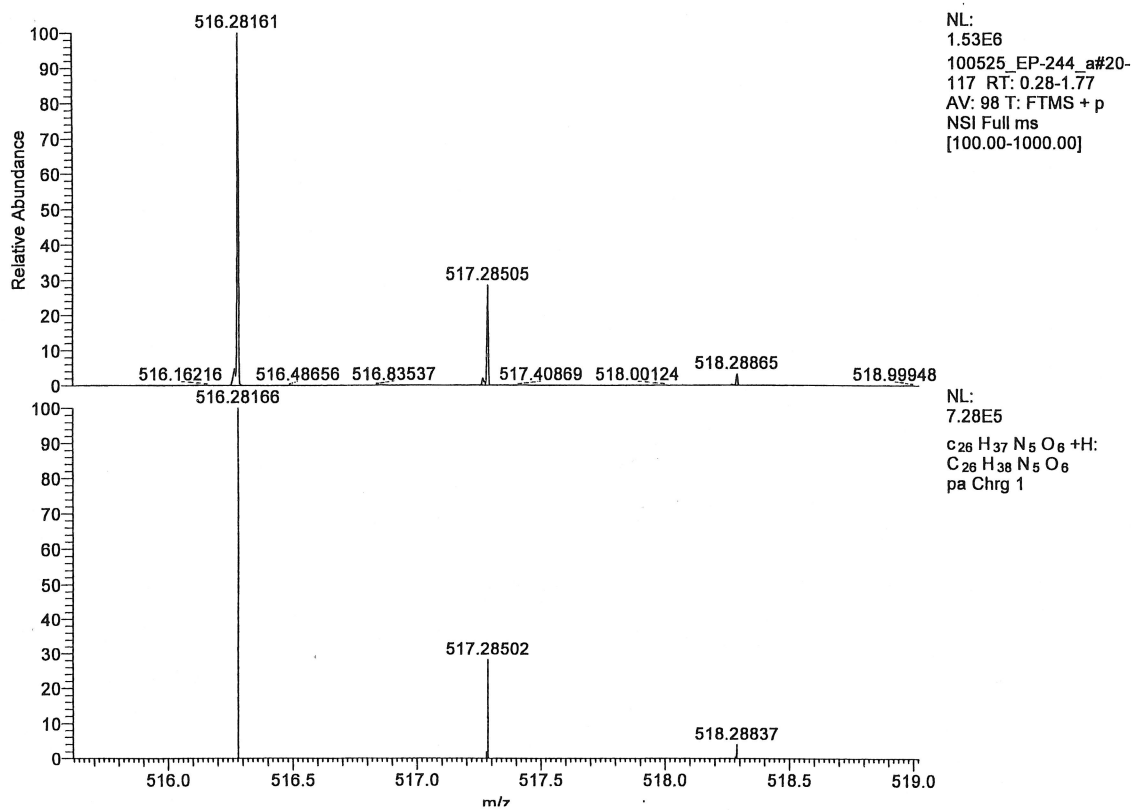
NL:  
5.93E5  
C<sub>44</sub>H<sub>66</sub>N<sub>6</sub>O<sub>8</sub>+H:  
C<sub>44</sub>H<sub>67</sub>N<sub>6</sub>O<sub>8</sub>  
pa Chrg 1

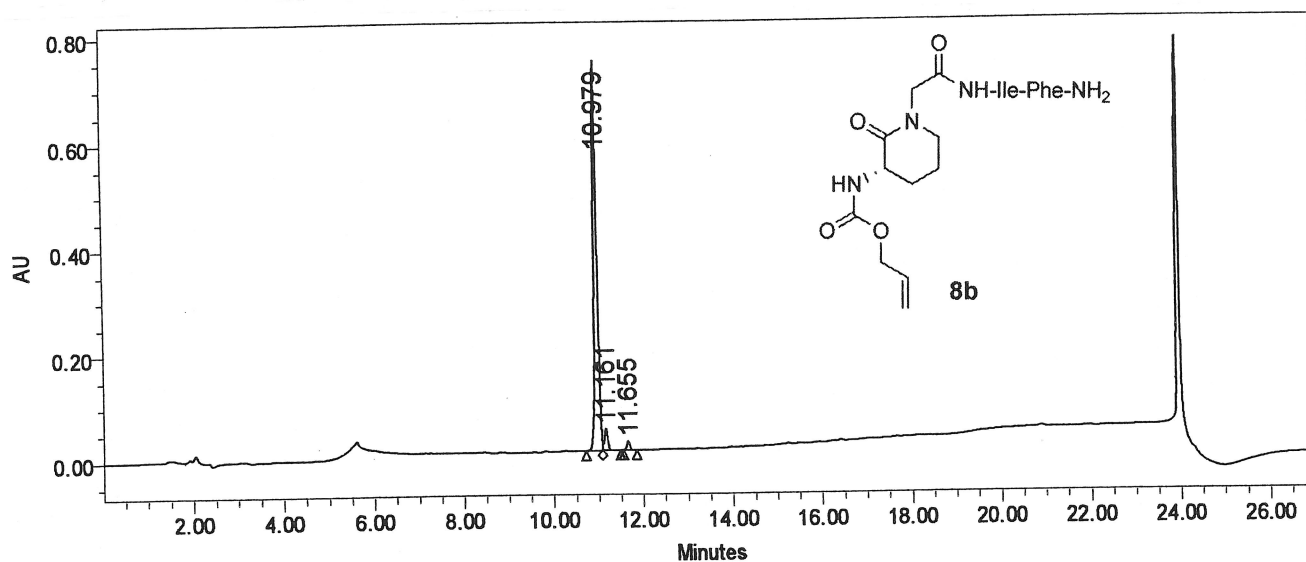


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	10.756	245752	3.97	32751
2	10.940	5939609	96.03	1192920

Z:\data\...\100525\_EP-244\_a

5/25/2010 7:19:16 PM

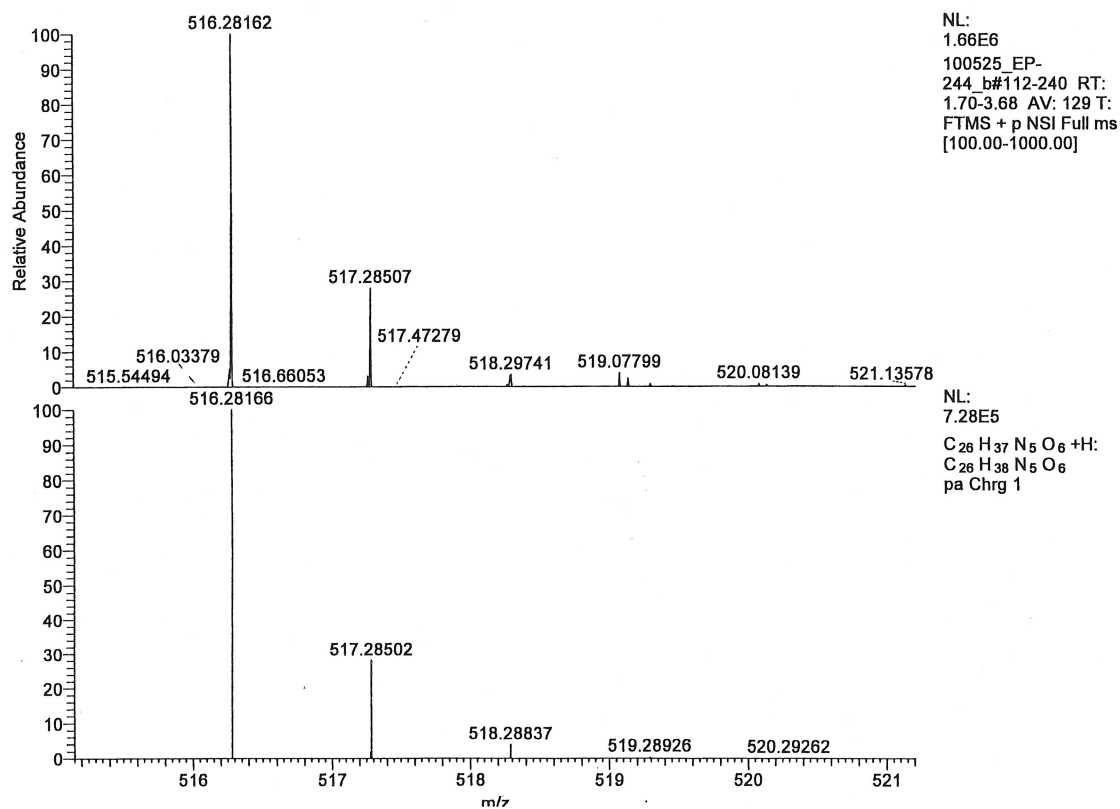




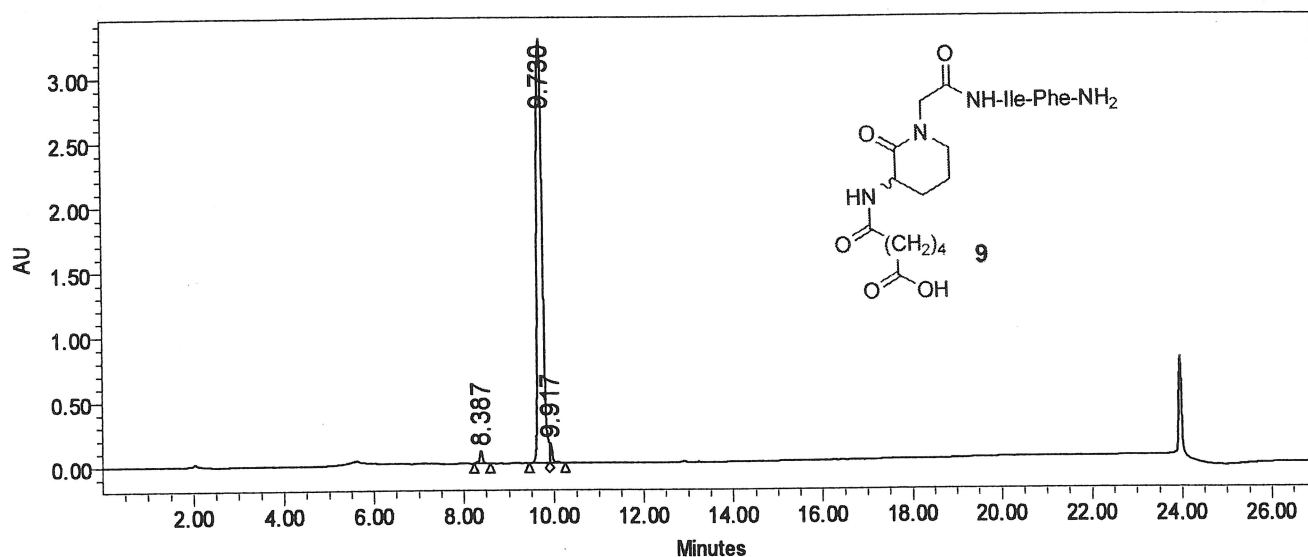
	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	10.979	3815145	92.81	730575
2	11.161	217374	5.29	41231
3	11.655	78286	1.90	16480

Z:\data\...100525\_EP-244\_b

5/25/2010 1:25:11 PM



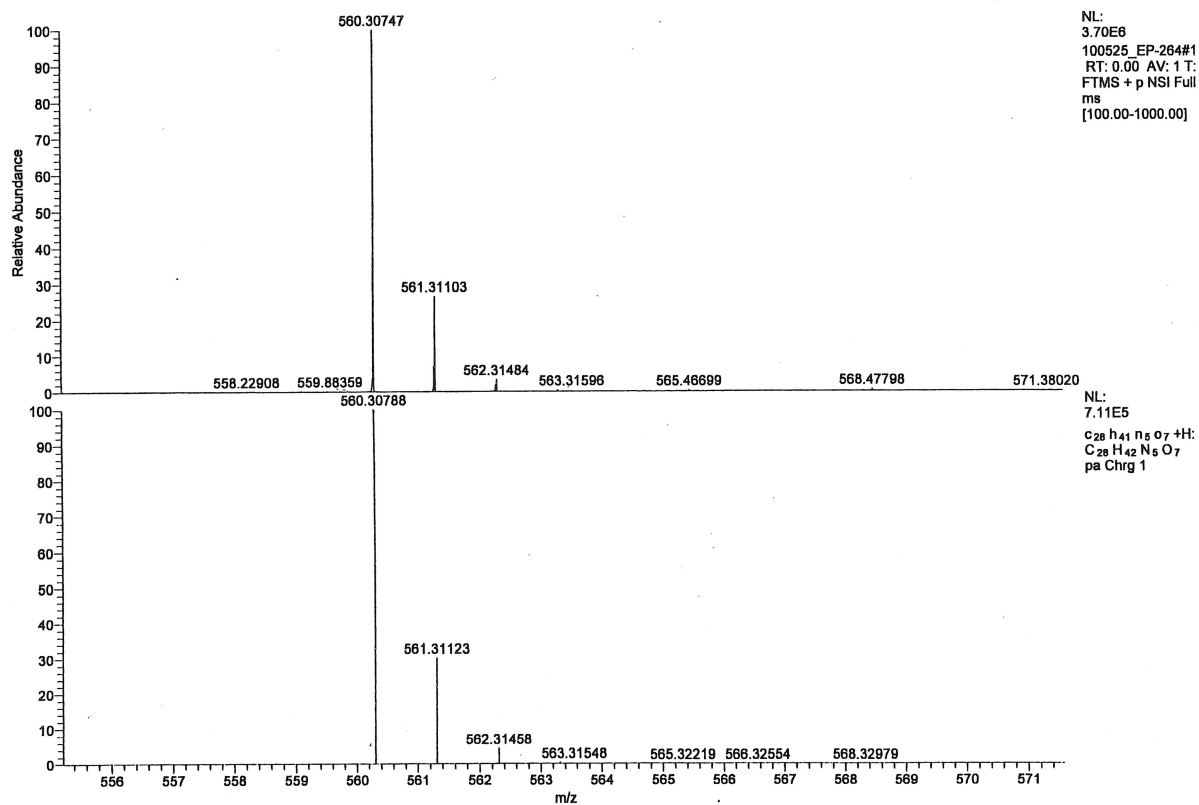


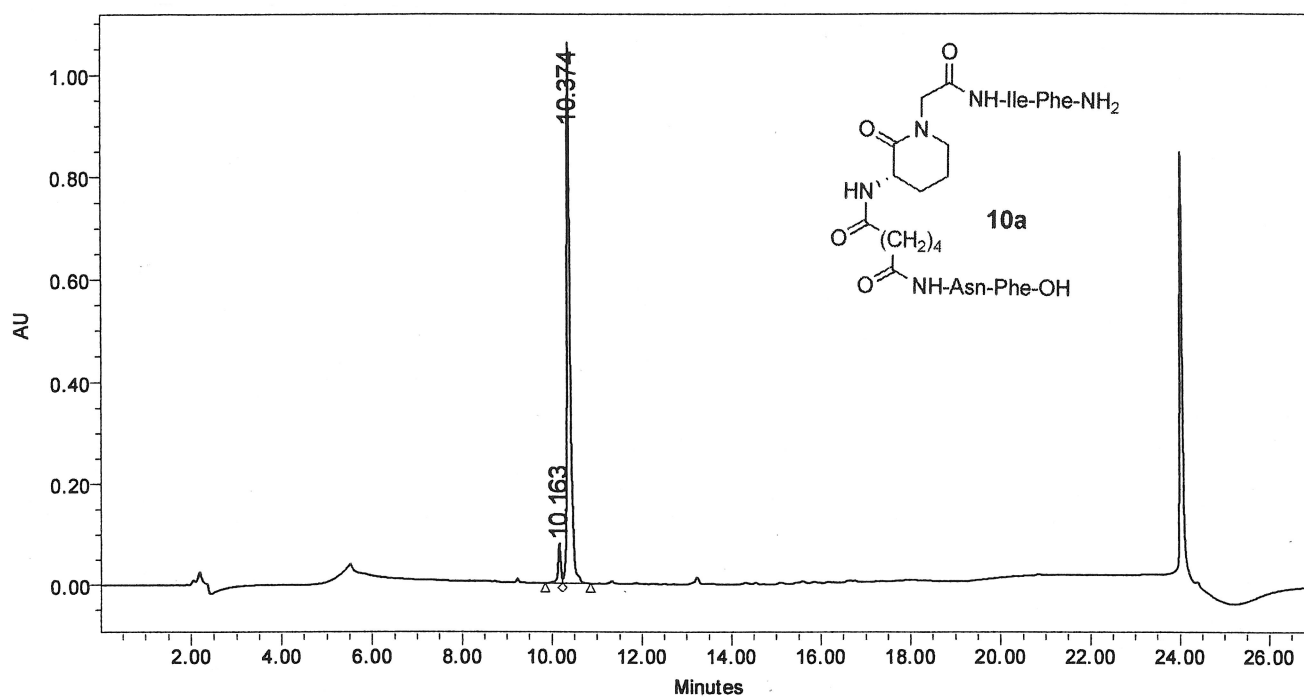


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	8.387	444367	1.54	94518
2	9.730	27827580	96.24	3284641
3	9.917	642270	2.22	154872

C:\Xcalibur\...100525\_EP-264

5/25/2010 7:31:08 PM



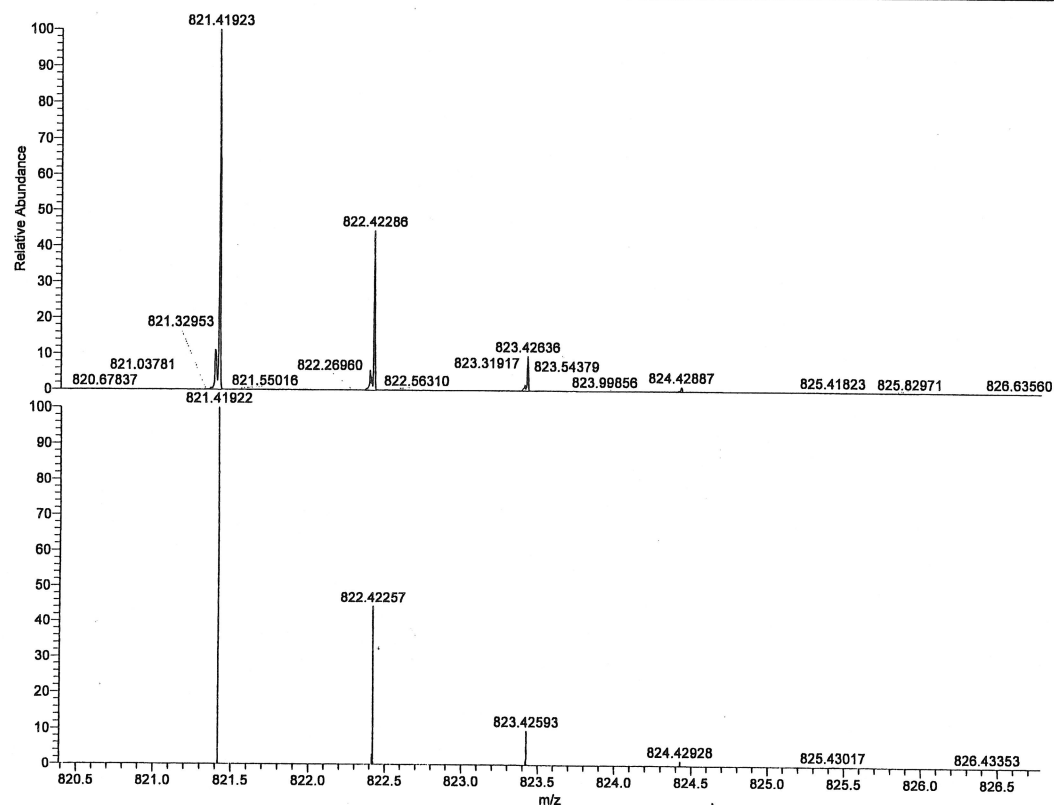


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	10.163	335501	5.79	77014	6.81
2	10.374	5455413	94.21	1054629	93.19

C:\Xcalibur\100526\_EP-245\_a  
100526\_EP-245\_a

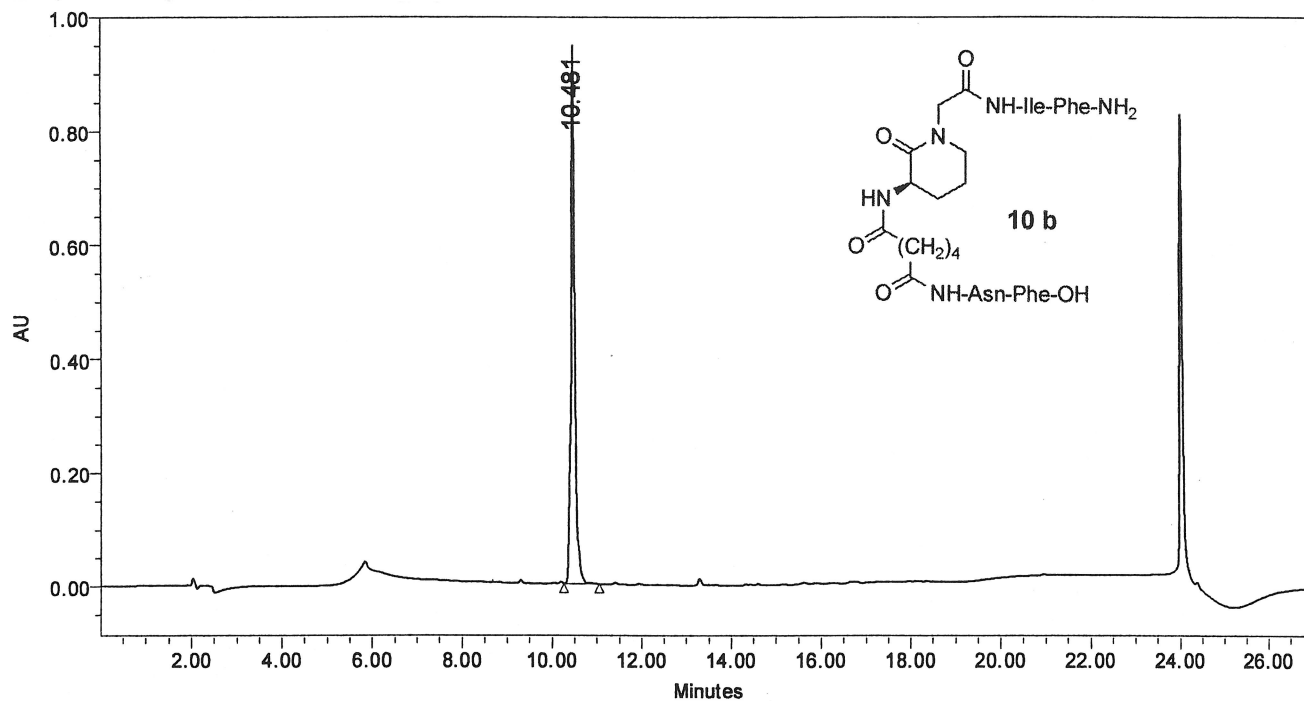
5/26/2010 4:44:20 PM

100526\_EP-245\_a



NL:  
2.19E8  
100526\_EP-245\_a#9-  
42 RT: 0.22-1.15  
AV: 34 T: FTMS + p  
NSI Full ms  
[200.00-2000.00]

NL:  
6.08E5  
 $\text{C}_{41}\text{H}_{56}\text{N}_8\text{O}_{10}^+ \text{H}^+$   
 $\text{C}_{41}\text{H}_{57}\text{N}_8\text{O}_{10}$   
pa Chrg 1

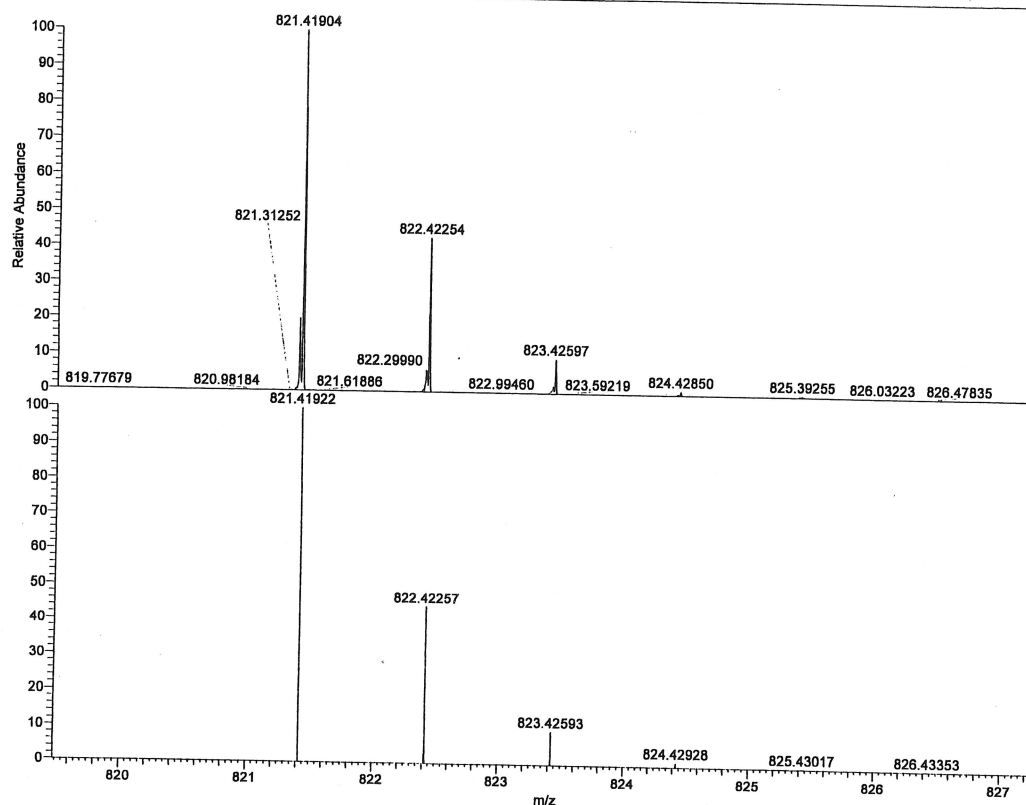


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	10.481	5557542	100.00	928108	100.00

C:\Xcalibur\...100526\_EP-245\_b  
100526\_EP-245\_b

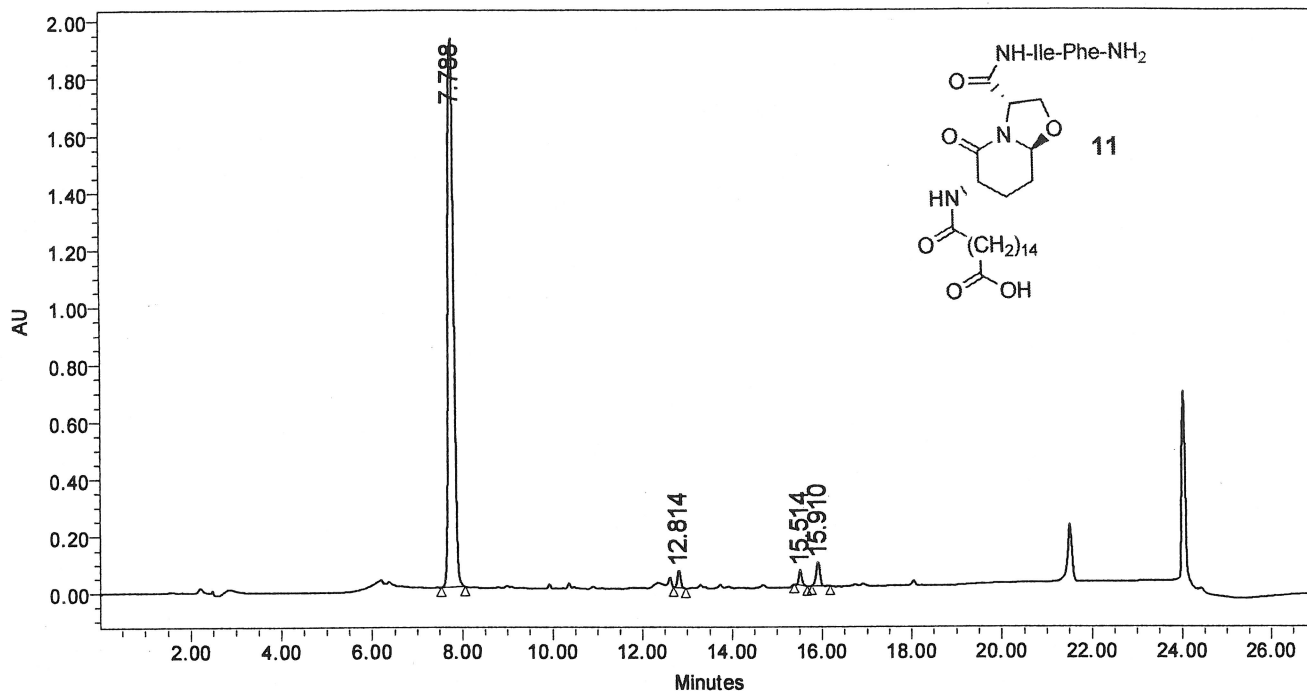
5/26/2010 4:50:10 PM

100526\_EP-245\_b



NL:  
4.29E6  
100526\_EP-245\_b#7-  
51 RT: 0.17-1.40  
AV: 45 T: FTMS + p  
NSI Full ms  
[200.00-2000.00]

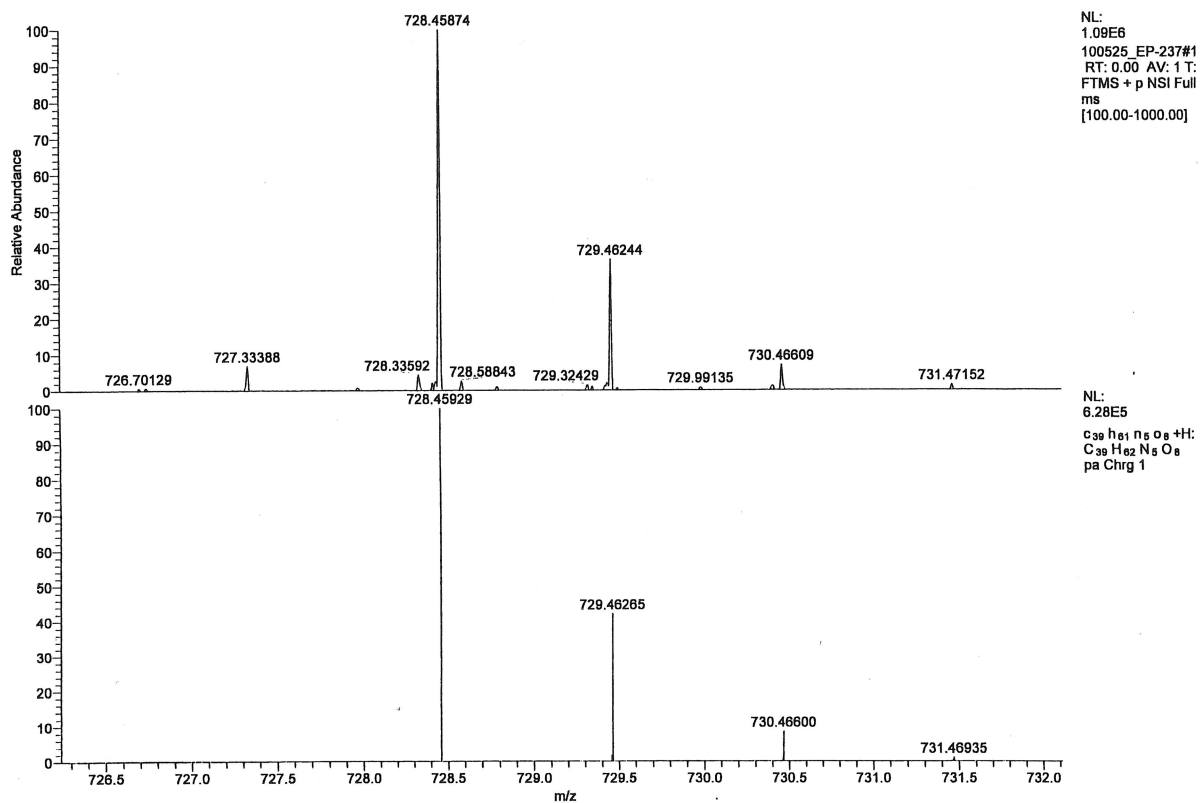
NL:  
6.06E5  
C<sub>41</sub>H<sub>58</sub>N<sub>8</sub>O<sub>10</sub> +H:  
C<sub>41</sub>H<sub>57</sub>N<sub>8</sub>O<sub>10</sub>  
pa Chrg 1

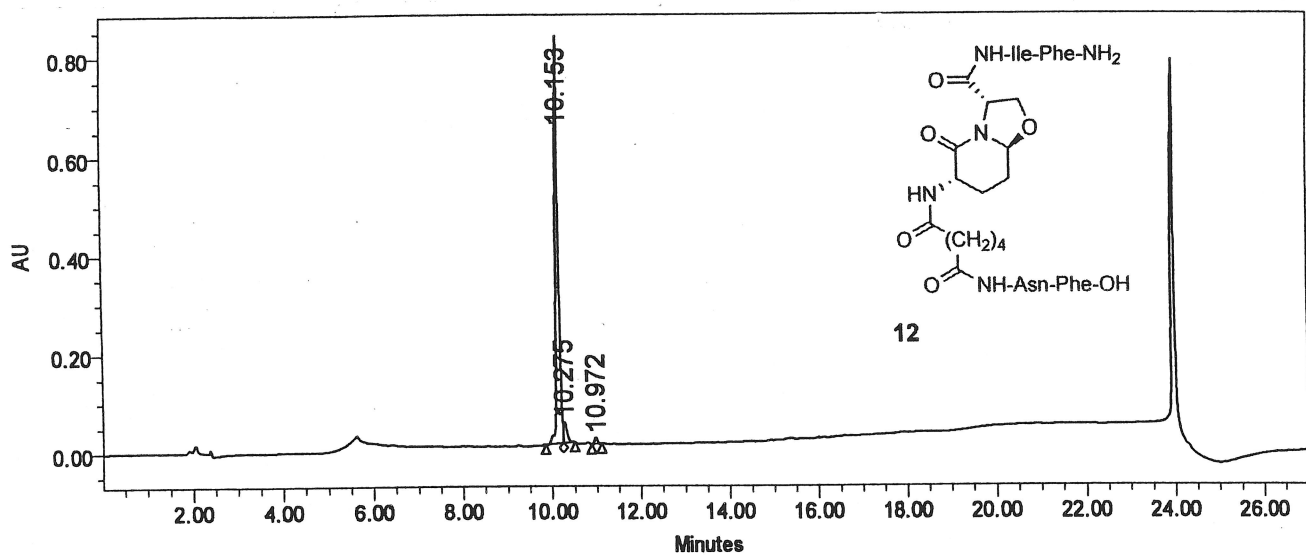


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )	% Height
1	7.788	16854006	94.17	1951498	90.88
2	12.814	299113	1.67	59479	2.77
3	15.514	246263	1.38	52268	2.43
4	15.910	497880	2.78	84106	3.92

C:\Xcalibur\...100525\_EP-237

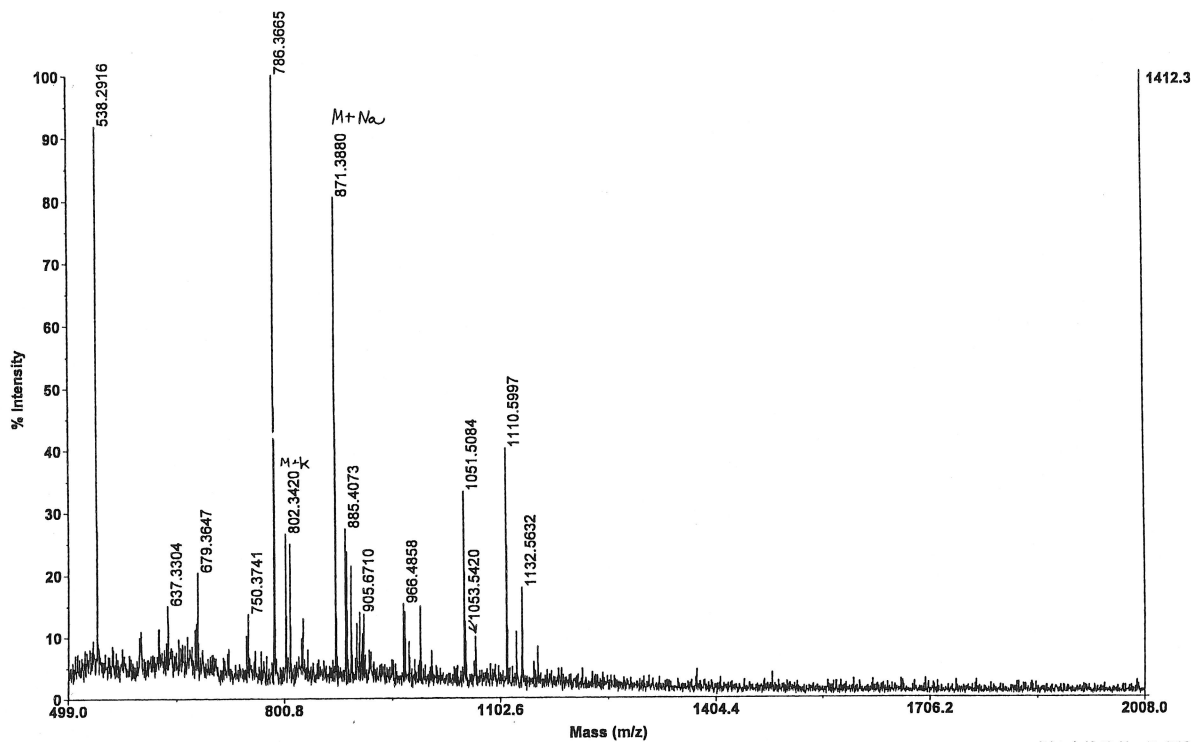
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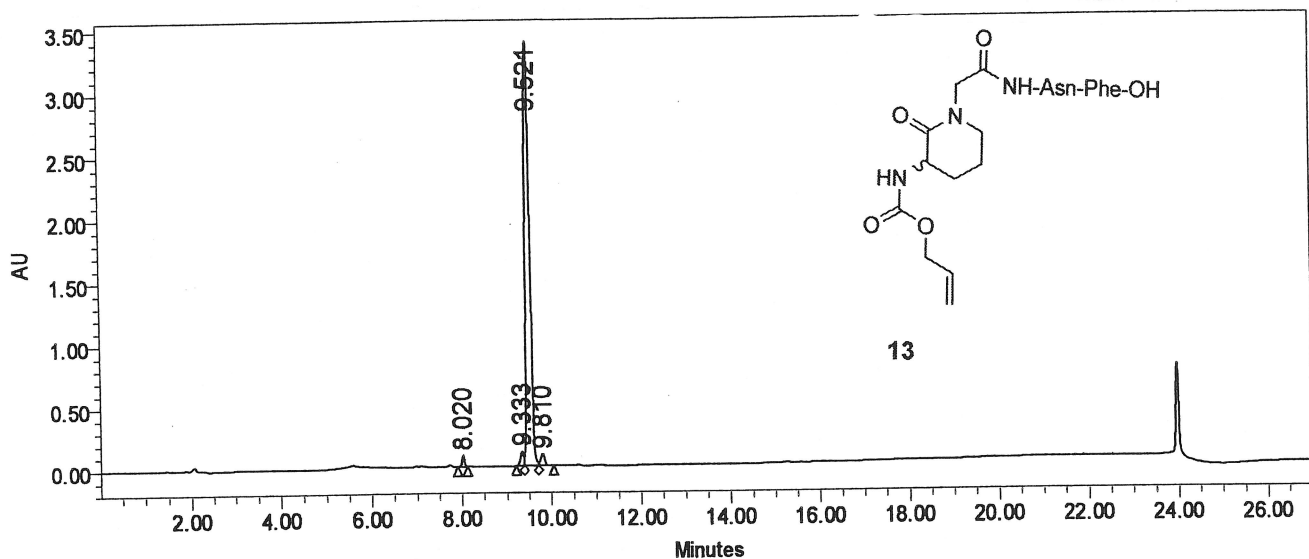




	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	10.153	3791289	93.14	810276
2	10.275	218889	5.38	41451
3	10.972	60264	1.48	12799

Applied Biosystems 4700 Proteomics Analyzer 347000059  
4700 Reflector Spec #1[BP = 786.4, 1412]



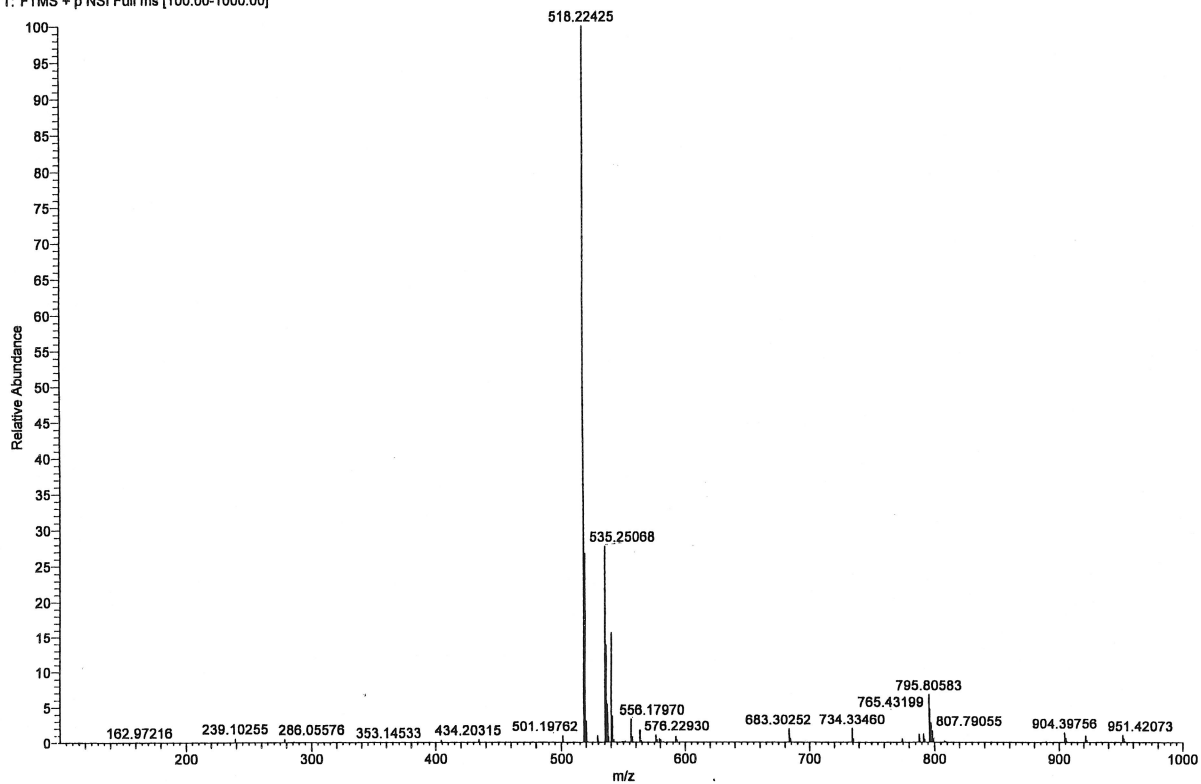


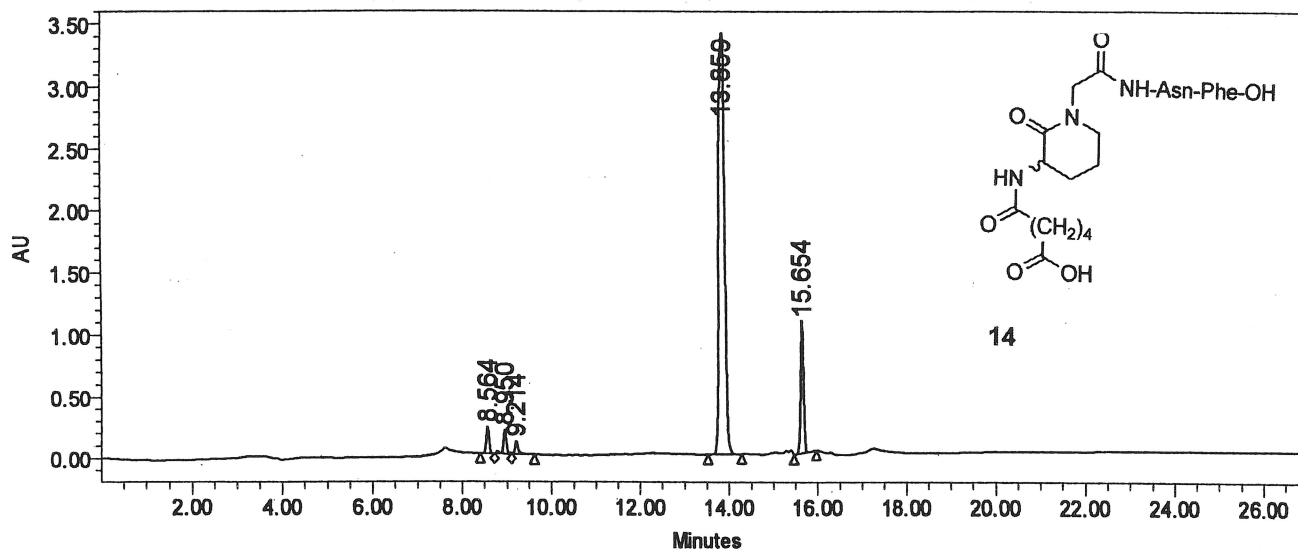
	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	8.020	344923	1.28	86578
2	9.333	580780	2.16	112660
3	9.521	25350726	94.38	3396601
4	9.810	583583	2.17	95411

C:\Xcalibur\...1100525\_EP-259

5/25/2010 7:42:56 PM

100525\_EP-259 #160-309 RT: 2.53-4.80 AV: 150 NL: 1.14E7  
T: FTMS + p NSI Full ms [100.00-1000.00]

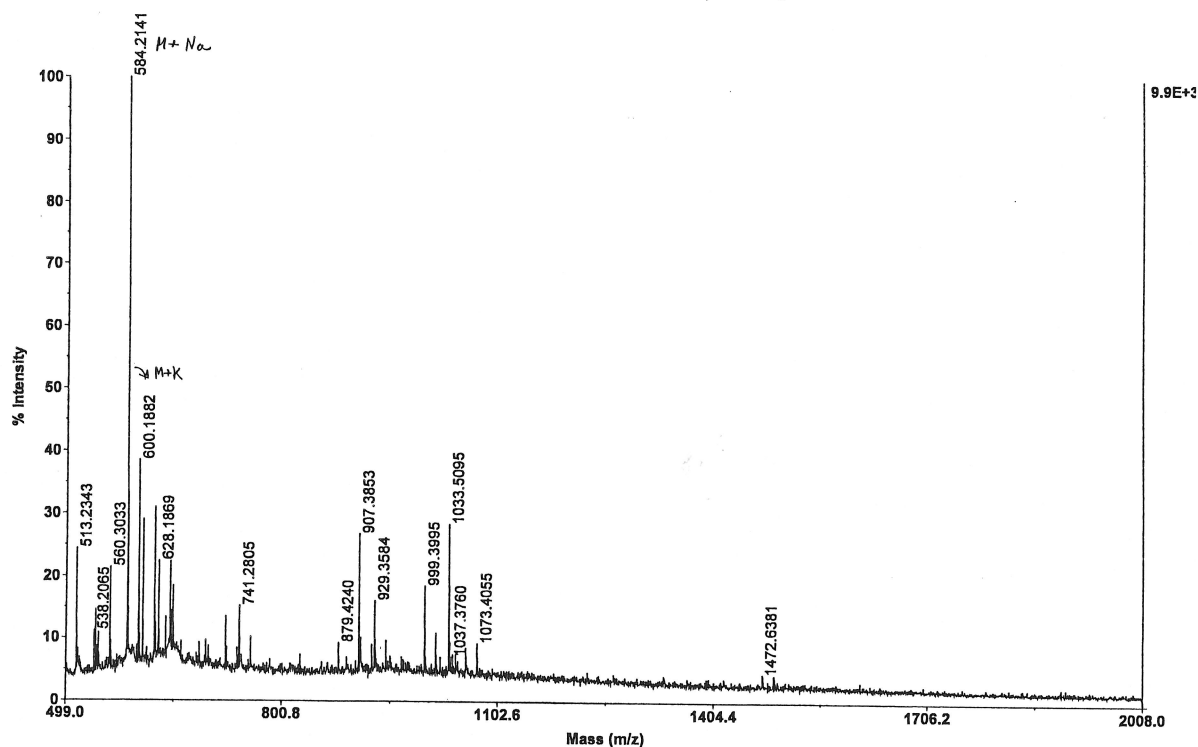


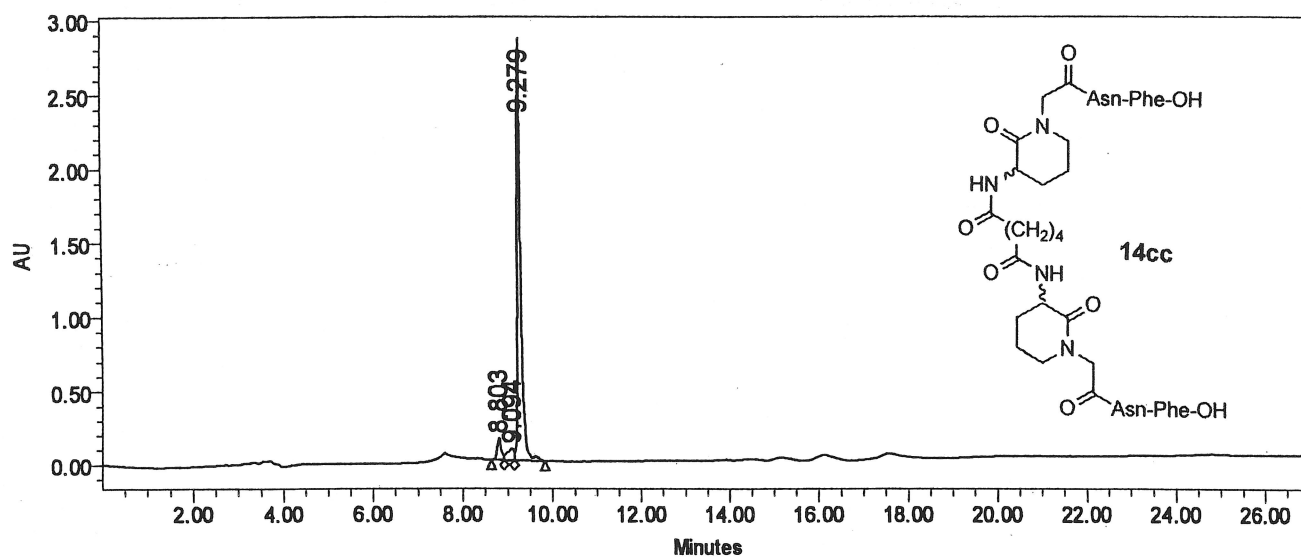


	RT (min)	Area (μV*sec)	% Area	Height (μV)
1	8.564	940600	2.42	213587
2	8.950	938482	2.42	186957
3	9.214	533721	1.37	101471
4	13.859	31460676	81.00	3410503
5	15.654	4967638	12.79	1063396

Applied Biosystems 4700 Proteomics Analyzer 347000059

4700 Reflector Spec #1[BP = 584.2, 9872]

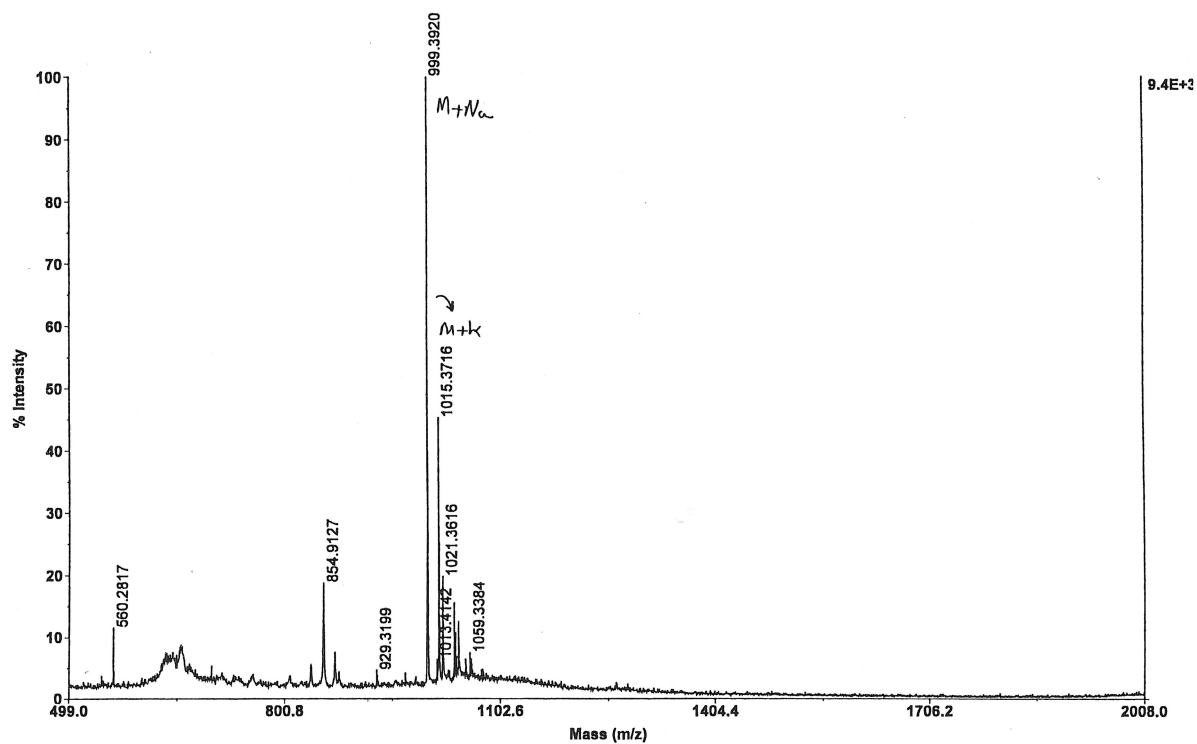




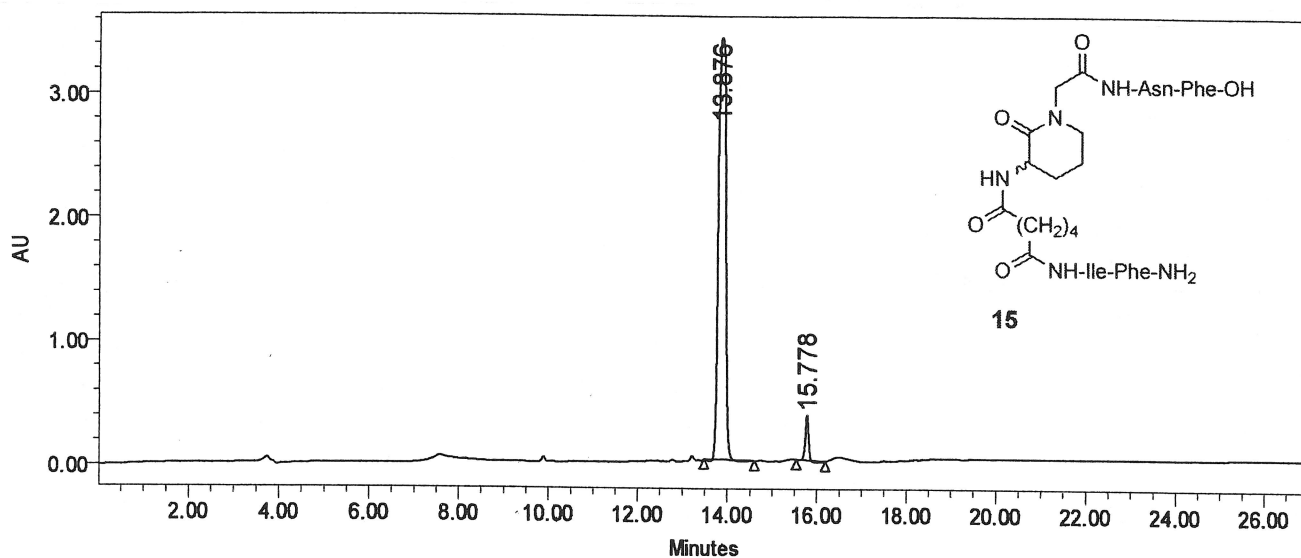
	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	8.803	928054	5.29	146927
2	9.094	813905	4.64	81326
3	9.279	15806622	90.07	2850049

Applied Biosystems 4700 Proteomics Analyzer 347000059

4700 Reflector Spec #1[BP = 999.4, 9354]





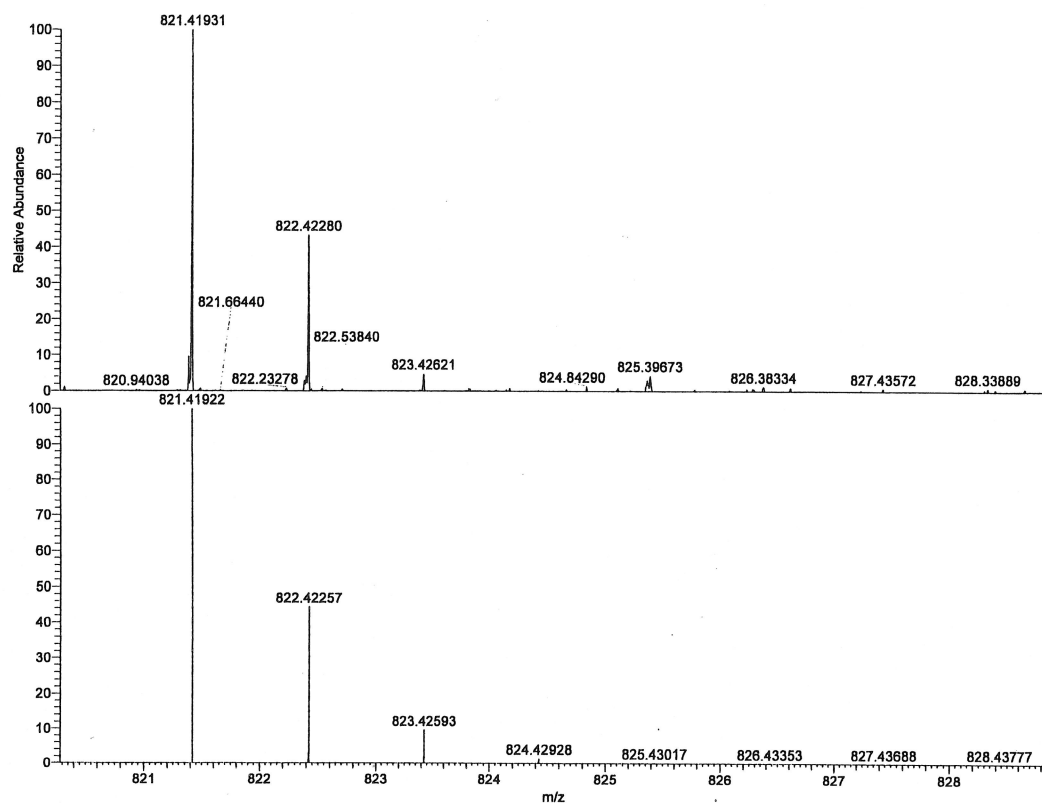


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	13.876	39063716	95.09	3410341
2	15.778	2015687	4.91	361998

C:\Xcalibur\...100526\_EP-262  
100526\_EP-262

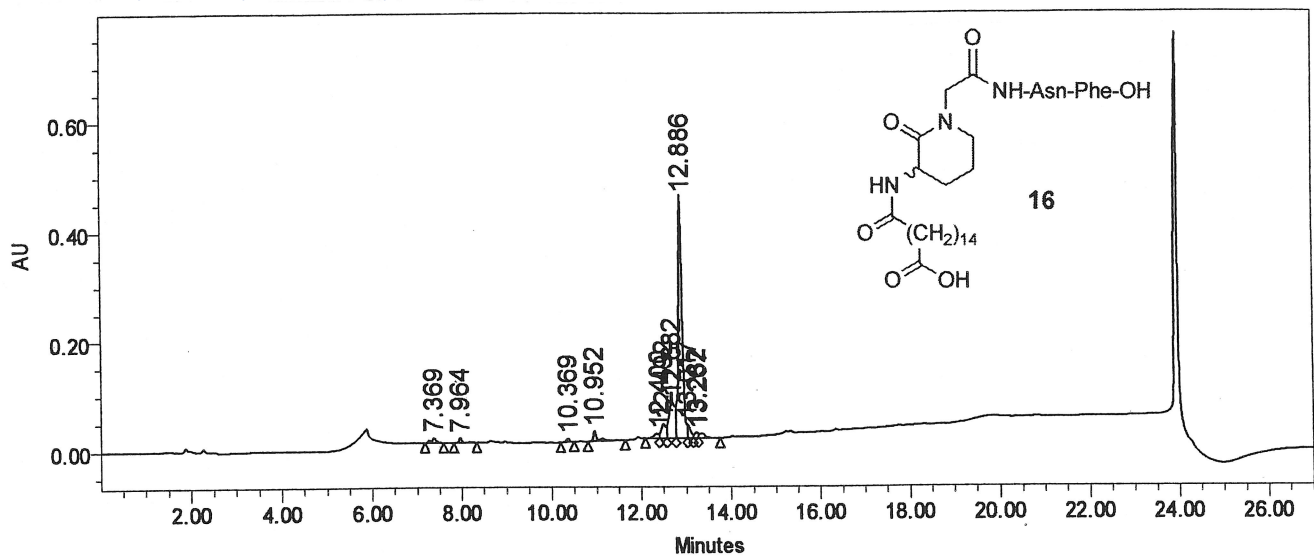
5/26/2010 5:22:26 PM

100526\_EP-262



NL:  
4.84E5  
100526\_EP-262#12-  
36 RT: 0.31-0.98  
AV: 25 T: FTMS + p  
NSI Full ms  
[200.00-2000.00]

NL:  
6.06E5  
C<sub>41</sub>H<sub>58</sub>N<sub>8</sub>O<sub>10</sub> +H:  
C<sub>41</sub>H<sub>57</sub>N<sub>8</sub>O<sub>10</sub>  
pa Chrg 1

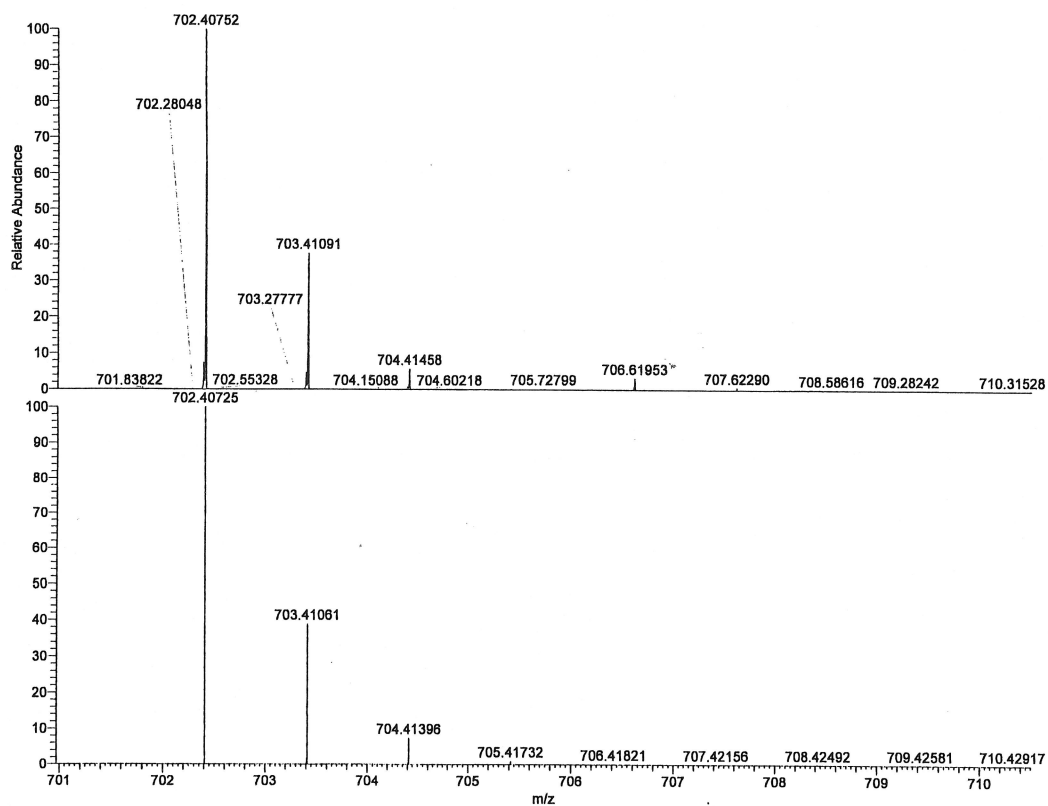


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	7.369	68498	1.71	9446
2	7.964	46975	1.17	9402
3	10.369	31016	0.77	6386
4	10.952	117645	2.93	18285
5	12.400	60922	1.52	7479
6	12.492	186115	4.64	25165
7	12.682	612315	15.26	66415
8	12.886	2645553	65.94	440895
9	13.017	116966	2.92	24354
10	13.232	55672	1.39	10255
11	13.267	70288	1.75	7911

C:\Xcalibur\...100526\_EP-260\_p  
100526\_EP-260\_p

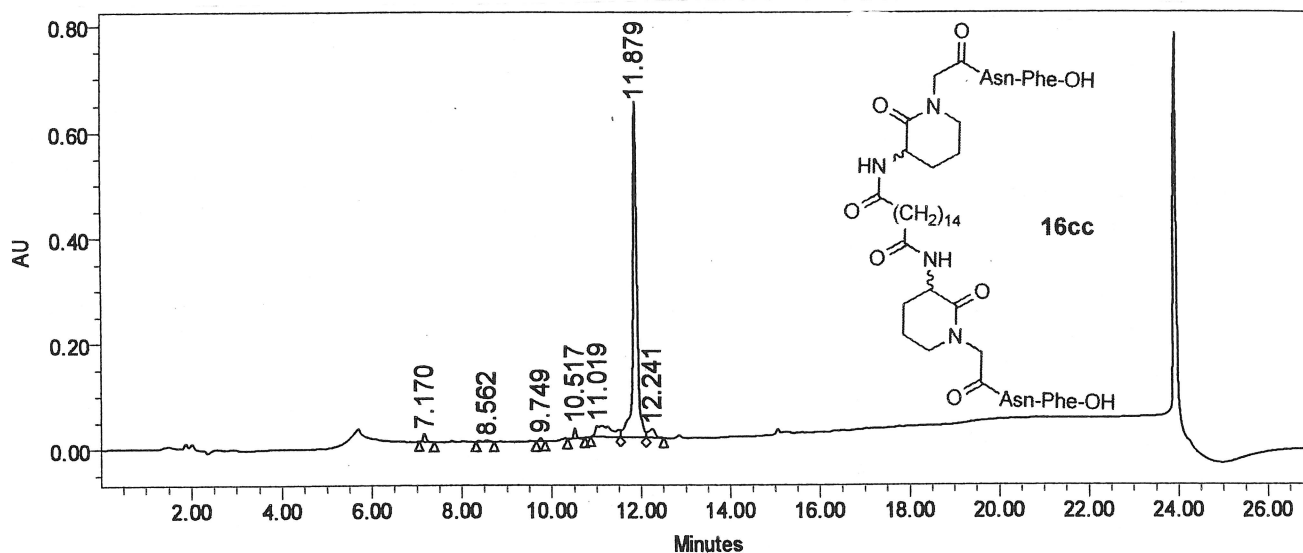
5/26/2010 3:13:13 PM

100526\_EP-260\_p



NL:  
3.47E5  
100526\_EP-260\_p#73-  
277 RT: 2.03-7.77  
AV: 205 T: FTMS + p  
NSI Full ms  
[200.00-2000.00]

NL:  
6.48E5  
C<sub>38</sub>H<sub>55</sub>N<sub>5</sub>O<sub>9</sub> + H:  
C<sub>38</sub>H<sub>55</sub>N<sub>5</sub>O<sub>9</sub>  
pa Chrg 1



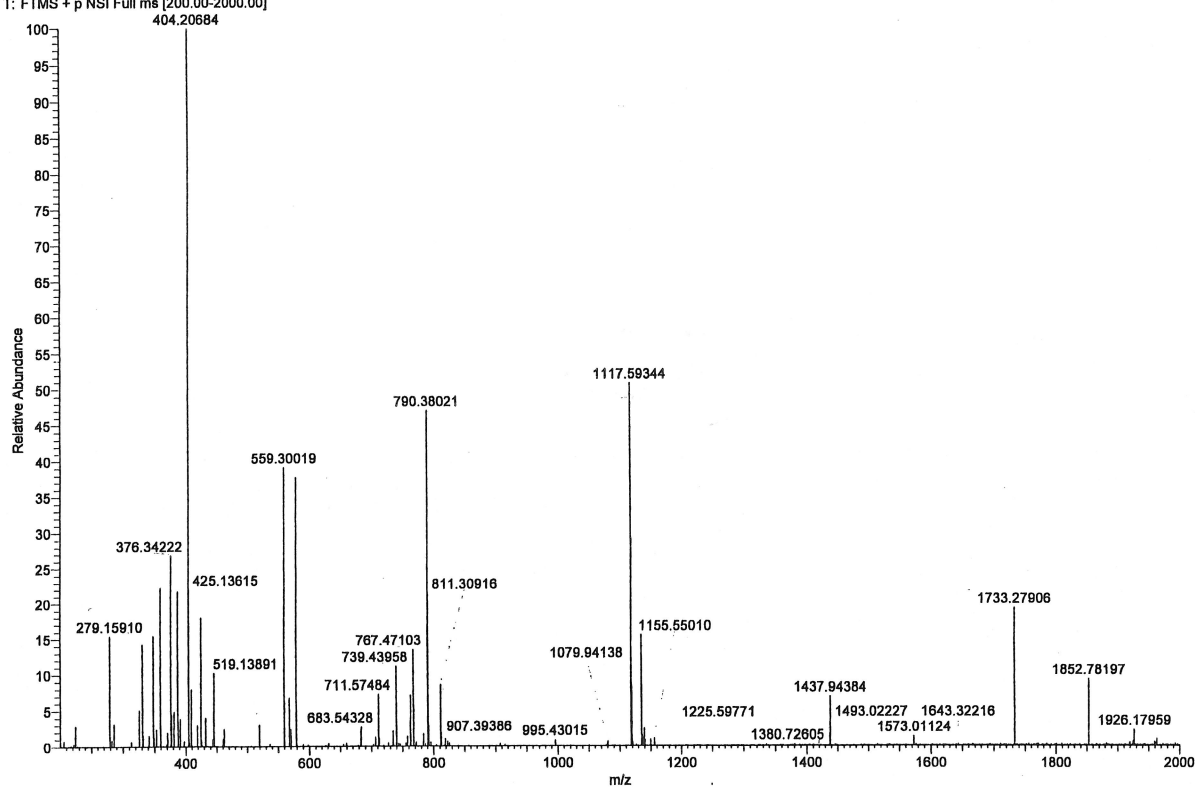
	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	7.170	73452	1.44	15522
2	8.562	34060	0.67	3191
3	9.749	24905	0.49	6372
4	10.517	86506	1.70	19420
5	11.019	580013	11.37	21407
6	11.879	4119101	80.73	632868
7	12.241	184316	3.61	16971

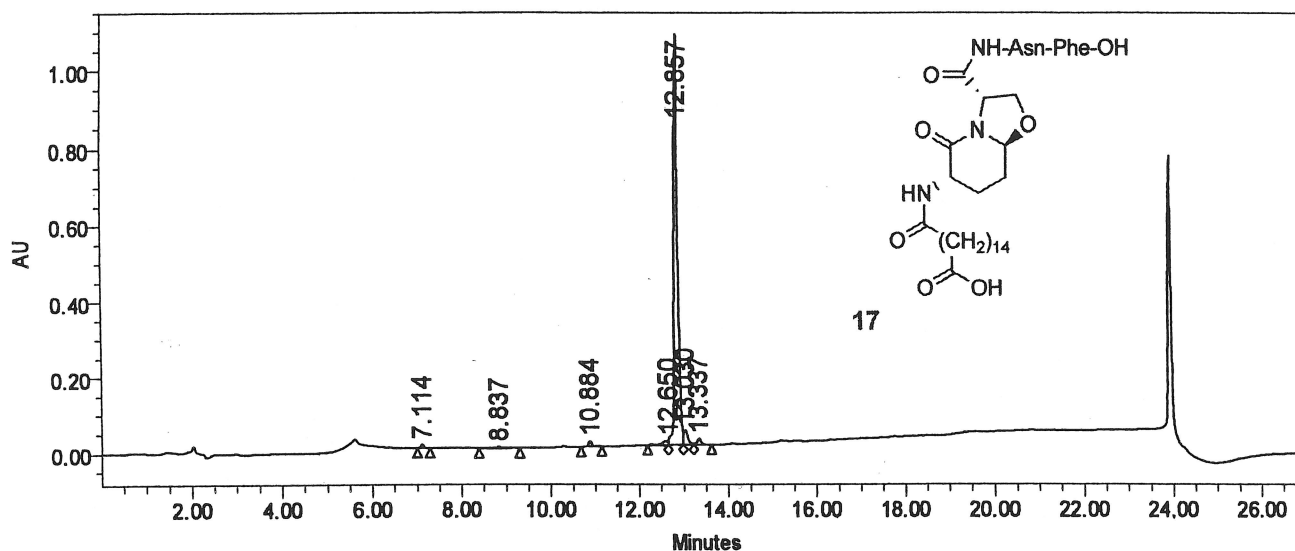
C:\Xcalibur\...100526\_EP-260\_cc  
100526\_EP-260\_cc

5/26/2010 3:32:50 PM

100526\_EP-260\_cc

100526\_EP-260\_cc #15-93 RT: 0.39-2.60 AV: 79 NL: 1.04E5  
T: FTMS + p NSI Full ms [200.00-2000.00]



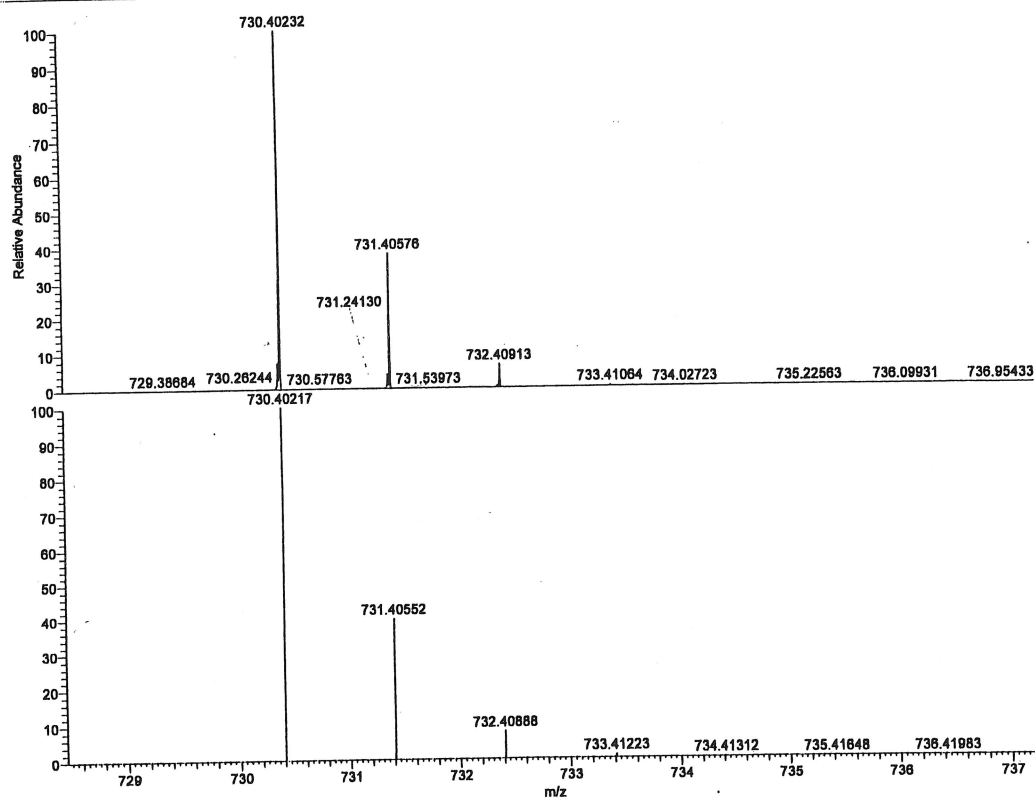


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	7.114	49238	0.72	10024
2	8.837	35795	0.52	4135
3	10.884	81793	1.19	14433
4	12.650	111827	1.63	12277
5	12.857	6217855	90.54	1081714
6	13.030	263182	3.83	39124
7	13.337	107980	1.57	16410

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100526\_EP-253\_cc

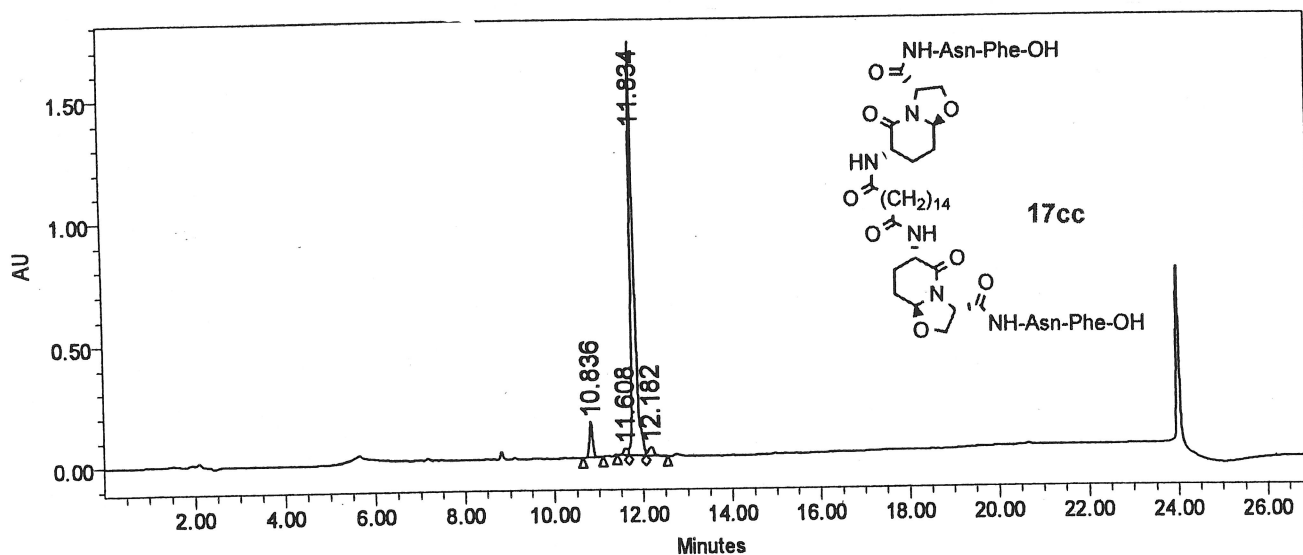
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NL:  
6.69E5  
100526\_EP-  
253\_cc#15-47 RT:  
0.39-1.29 AV: 33 T:  
FTMS + p NSI Full ms  
[200.00-2000.00]

NL:  
6.39E5  
C<sub>37</sub>H<sub>58</sub>N<sub>5</sub>O<sub>10</sub><sup>+</sup>  
C<sub>37</sub>H<sub>58</sub>N<sub>5</sub>O<sub>10</sub>  
pa Chrg 1



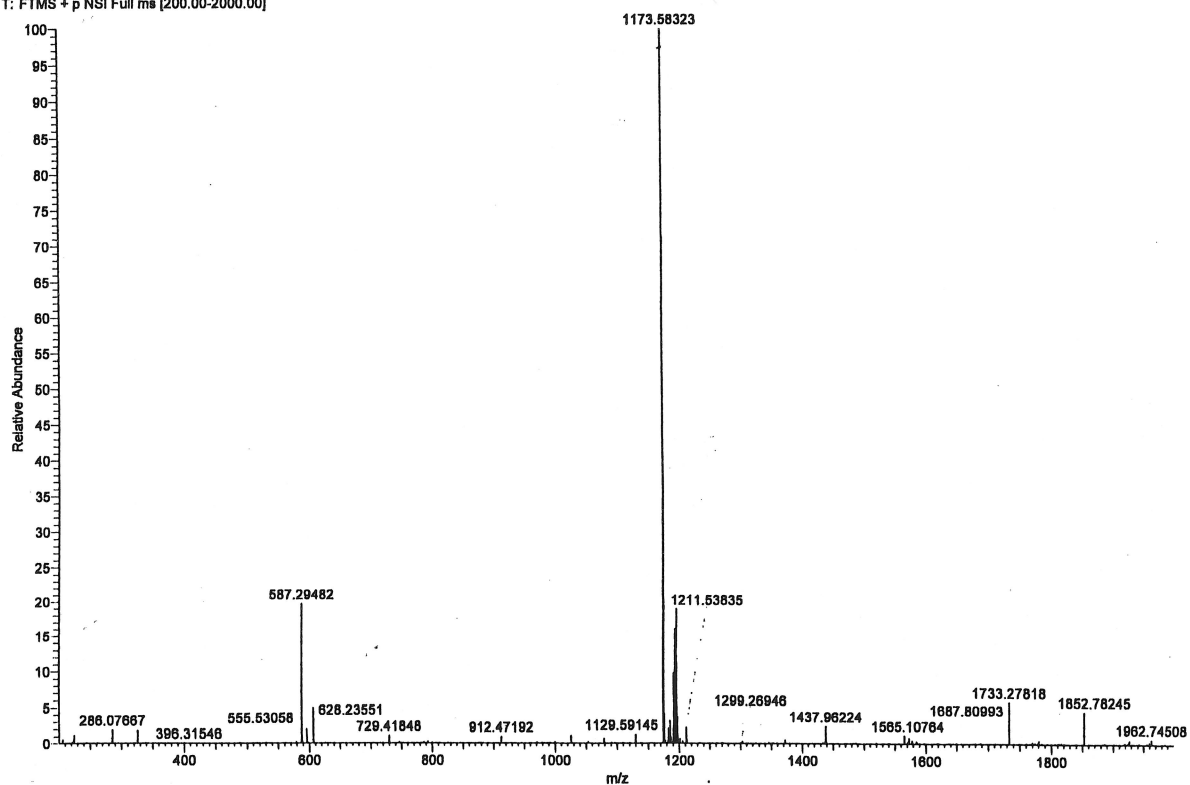
	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	10.836	746301	6.73	145566
2	11.608	204316	1.84	27445
3	11.834	9830308	88.63	1679777
4	12.182	309911	2.79	35082

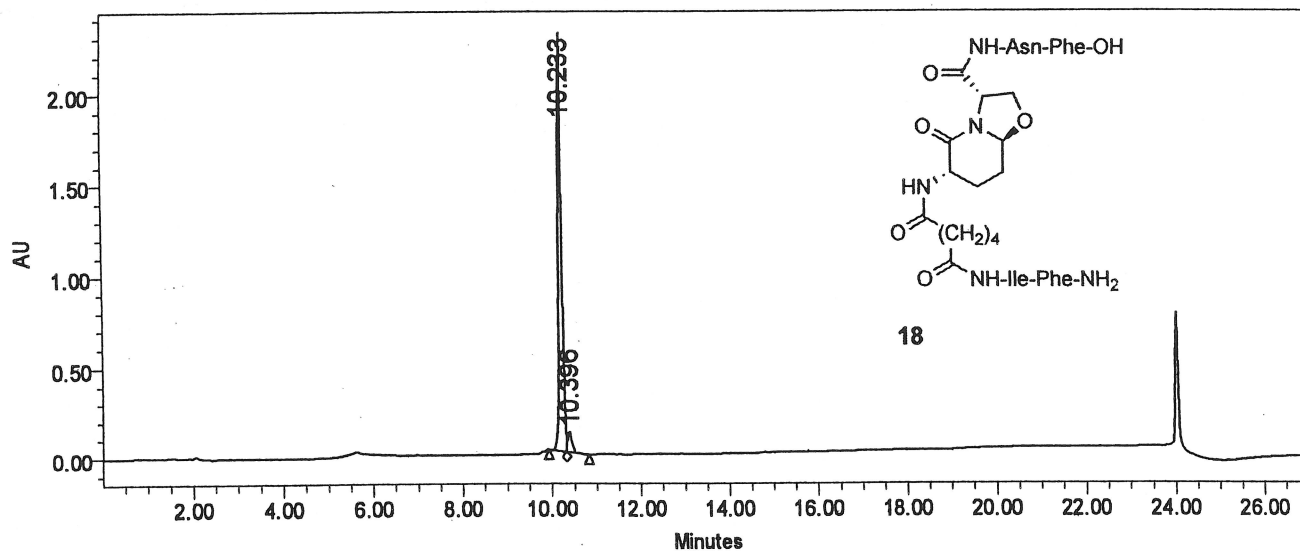
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100526\_EP-253\_p #4-31 RT: 0.08-0.84 AV: 28 NL: 2.01E6  
T: FTMS + p NSI Full ms [200.00-2000.00]

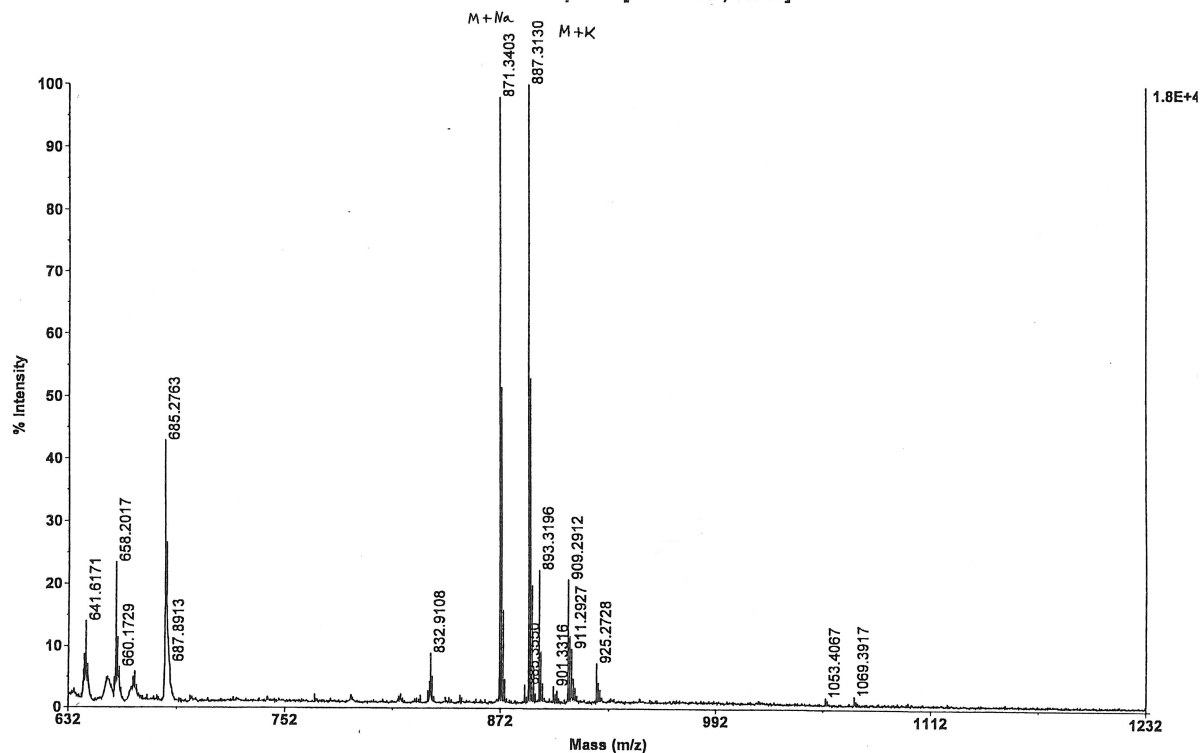


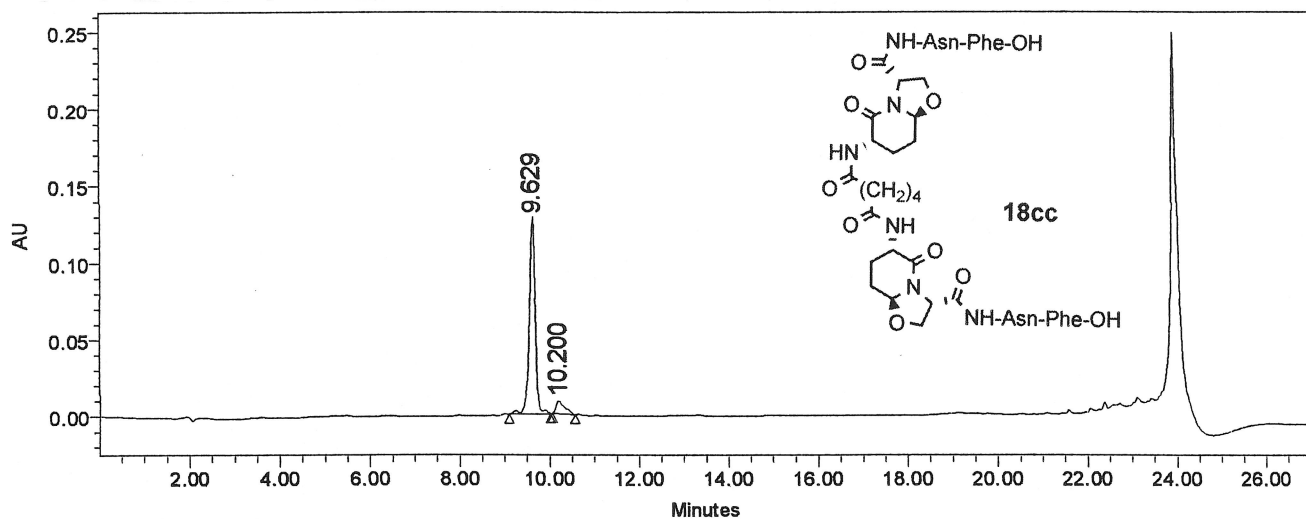


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	10.233	12315684	94.33	2281723
2	10.396	740102	5.67	113089

Applied Biosystems 4700 Proteomics Analyzer 347000059

4700 Reflector Spec #1[BP = 887.3, 18256]



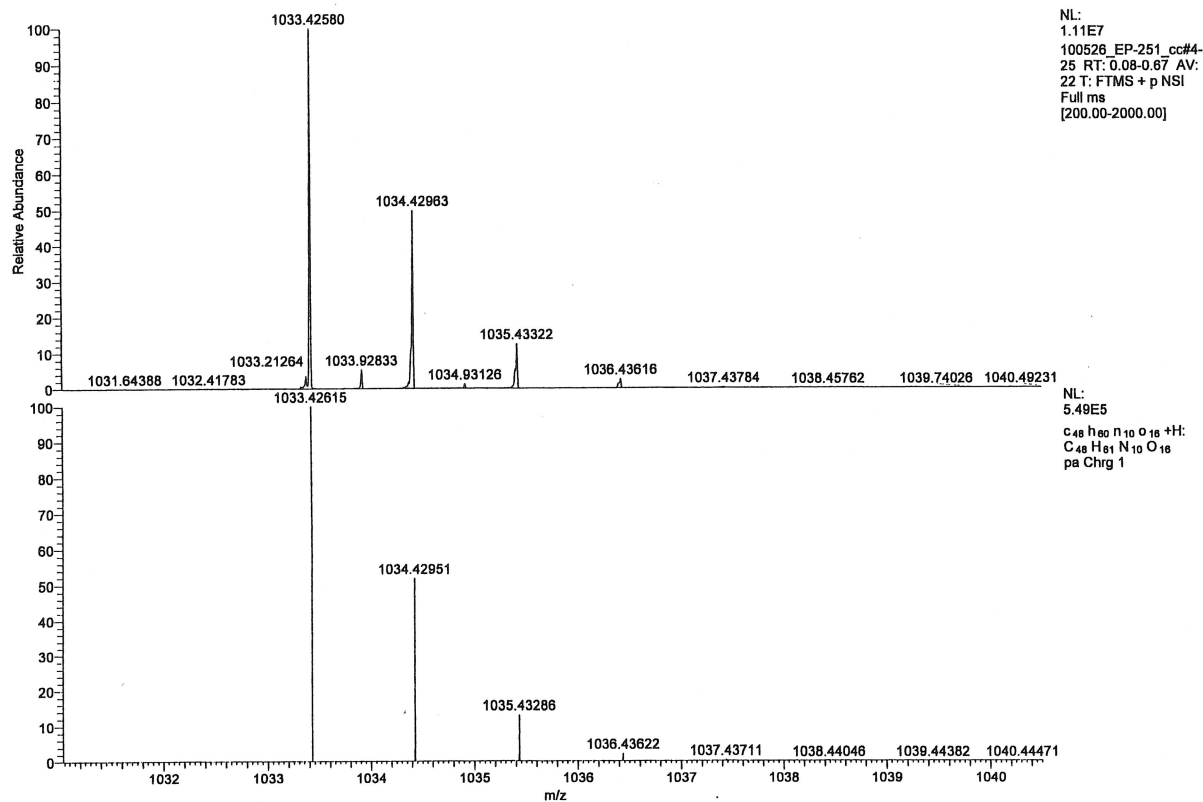


	RT (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	9.629	1156513	91.41	128055
2	10.200	108729	8.59	8175

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### Anti-HIV activity in cell culture<sup>1</sup> (EC<sub>50</sub>)

All compounds were tested in cultures cells infected with wild type HIV1 virus. The four most potent compounds were then tested on cells infected with the multidrug resistant HIV1 strain IRL98DPRO.<sup>2</sup> The test was based on the MTT colorimetric method that measures cellular proliferation,<sup>3</sup> specifically applied to HIV1-PR inhibitors.<sup>4</sup> The screening allowed determination of the 50% effective concentration (EC<sub>50</sub>) of the compounds as well as their 50% cytotoxic concentration (CC<sub>50</sub>). The results (Table 1) showed that four of our compounds (**6**, **14**, **15** and **16cc**) had antiviral activity on the wild type virus at the micromolar scale.

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(1) These tests were carried out at the IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona (Spain).

(2) Moncunill, G.; Armand-Ugon, M.; Clotet-Codina, I.; Pauls, E.; Ballana, E.; Llano, A.; Romagnoli, B.; Vrijbloed, J. W.; Gombert, F. O.; Clotet, B.; De Marco, S.; Esté, J. A.. Anti-HIV activity and resistance profile of the CXCR4 chemokine receptor 4 antagonist POL3026. *Mol. Pharmacol.* **2008**, 73, 1264-73.

(3) a. Gonzalez-Ortega, E., Ballana, E., Badia, R., Clotet, B., and Este, J. A. (2011) *Antiviral Res* **92**, 479-483. b. Mosmann, T.; Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, 65, 55-63. c. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; DeClerq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, 20, 309-321.



(4) Davis, D.A.; Brown, C.A.; Singer, K.E.; Wang, V.; Kaufman, J.; Stahl, S.J.; Wingfield, P. Maeda, K. Harada, S.; Yoshimura, K.; Kosalaraksa, P.; Mitsuya, H.; Yarchoan, R. Inhibition of HIV-1 replication by a peptide dimerization inhibitor of HIV-1 protease. *Antiviral Res.* **2006**, 72, 89-99.

Name	Activity	MW (g/mol)	CC50 (µg/mL)	EC50 (wild type)	EC50 (mutant22)	Fold Resistance "R"
AZT	Reverse transcriptase inhibitor	276.24	>1	1.09 nM	2.90 nM	2.6 <sup>a</sup>
AMD 3100	Fusion inhibitor	502.78	>5	2.00 nM	8.00 nM	4 <sup>a</sup>
Ritonavir	Protease inhibitor	720.94	>1	47.16 nM	>1 µg/ml	19
Indinavir	Protease inhibitor	613.79	>1.2	8.15 nM	0.85 µM	104
<b>6</b>	Protease inhibitor	573.76	>125	97.17 µM	132 µM	1,36
<b>14</b>	Protease inhibitor	561.58	>125	2.96 µM	>125 µg/ml	-
<b>15</b>	Protease inhibitor	820.93	>125	2.23 µM	>125 µg/ml	-
<b>16cc</b>	Protease inhibitor	1117.29	>125	87.04 µM	>125 µg/ml	-

a. AZT and AMD3100 are scarcely affected by the mutation of the protease, since they have different targets.

**Table 1.** Cell culture activity against native HIV1 and resistant mutant IRL98DPRO.