Supplementary Data

A novel strategy of chemical modification for rate enhancement of 10-23 DNAzyme: a combination of A9 position and 8-aza-7-deaza-2'-deoxyadenosine analogs

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Contents

Table S1. MALDI-TOF measurements of modified 10-23 deoxyribozymes modified with **1-5**.

No.	Modified 10-23 deoxyribozymes	MW (calculated)	MW (found)
DZ-00	5'-d(tgc tct cca GGC TAG CTA CAA CGA cct gca cct)-3'	9994.4	9990.5
DZ-1-5	5'-d(tgc tct cca GGC T**1**G CTA CAA CGA cct gca cct)-3'	9993.4	9994.8
DZ-1-9	5'-d(tgc tct cca GGC TAG CT**1** CAA CGA cct gca cct)-3'	9993.4	9991.1
DZ-1-11	5'-d(tgc tct cca GGC TAG CTA C**1**A CGA cct gca cct)-3'	9993.4	9993.3
DZ-1-12	5'-d(tgc tct cca GGC TAG CTA CA**1** CGA cct gca cct)-3'	9993.4	9991.7
DZ-1-15	5'-d(tgc tct cca GGC TAG CTA CAA CG**1** cct gca cct)-3'	9993.4	9989.4
DZ-2-5	5'-d(tgc tct cca GGC T**2**G CTA CAA CGA cct gca cct)-3'	9994.4	9995.5
DZ-2-9	5'-d(tgc tct cca GGC TAG CT**2** CAA CGA cct gca cct)-3'	9994.4	9995.9
DZ-2-11	5'-d(tgc tct cca GGC TAG CTA C**2**A CGA cct gca cct)-3'	9994.4	9990.1
DZ-2-12	5'-d(tgc tct cca GGC TAG CTA CA**2** CGA cct gca cct)-3'	9994.4	9991.2
DZ-2-15	5'-d(tgc tct cca GGC TAG CTA CAA CG**2** cct gca cct)-3'	9994.4	9995.5
DZ-3-5	5'-d(tgc tct cca GGC T**3**G CTA CAA CGA cct gca cct)-3'	10051.5	10051.8
DZ-3-9	5'-d(tgc tct cca GGC TAG CT**3** CAA CGA cct gca cct)-3'	10051.5	10051.1
DZ-3-11	5'-d(tgc tct cca GGC TAG CTA C**3**A CGA cct gca cct)-3'	10051.5	10052.2
DZ-3-12	5'-d(tgc tct cca GGC TAG CTA CA**3** CGA cct gca cct)-3'	10051.5	10053.2
DZ-3-15	5'-d(tgc tct cca GGC TAG CTA CAA CG**3** cct gca cct)-3'	10051.5	10053.4
DZ-4-5	5'-d(tgc tct cca GGC T**4**G CTA CAA CGA cct gca cct)-3'	10052.5	10053.5
DZ-4-9	5'-d(tgc tct cca GGC TAG CT**4** CAA CGA cct gca cct)-3'	10052.5	10053.4
DZ-4-11	5'-d(tgc tct cca GGC TAG CTA C**4**A CGA cct gca cct)-3'	10052.5	10053.7
DZ-4-12	5'-d(tgc tct cca GGC TAG CTA CA**4** CGA cct gca cct)-3'	10052.5	10050.1
DZ-4-15	5'-d(tgc tct cca GGC TAG CTA CAA CG**4** cct gca cct)-3'	10052.5	10053.1
DZ-5-9	5'-d(tgc tct cca GGC TAG CT**5** CAA CGA cct gca cct)-3'	10098.6	10099.1

Table S2. T_m measurements of the complexes formed between deoxyribozymes and the full-DNA substrate. [a]

Complex	T_m (°C)
10-23 DNAzyme +D19	51.7
DZ-2-9+D19	50.1
DZ-3-9+D19	50.7
DZ-4-9+D19	51.0
DZ-5-9+D19	51.0
DZ-3-5+D19	49.8

[a] D19: the full-DNA substrate, 5'-d (AGG TGC AGG ATG GAG AGC A)-3'. Tm measurement was conducted in the buffer consisting of 50 mM Tris-HCl (pH 7.5) and 2 mM $MgCl_2$. The solution was heated from 85 °C, after halting for 10 min, it was cooled to 10 °C at a rate of 0.5 °C/min. The UV absorbance was recorded at 260 nm during the cooling process. The T_m values were obtained from the melting curves, and each melting curve was fit to a non-self-complementary two-state model.

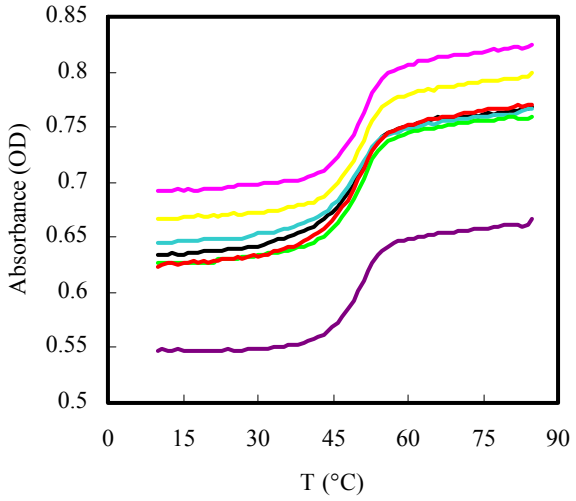


Fig. S1 The melting curves of the complex of the DNA substrate and 10-23 DNAzyme (black), **DZ-1-9** (purple), **DZ-2-9** (green), **DZ-3-5** (blue), **DZ-3-9** (yellow), **DZ-4-9** (red), and **DZ-5-9** (pink), respectively. The conditions for the measurement see the footnote under Table S2.

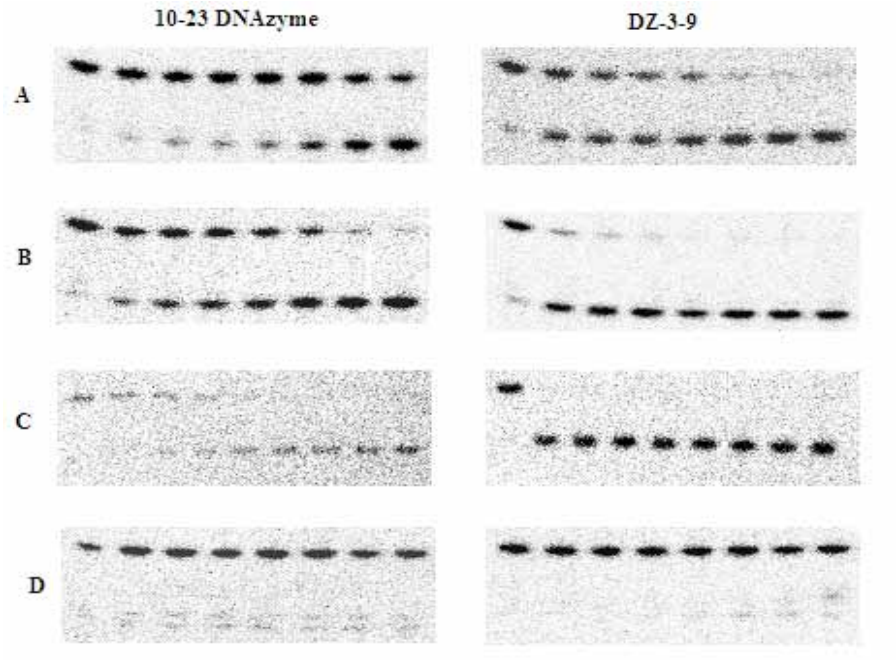


Fig. S2 The cleavage pattern of 10-23 DNAzyme and **DZ-3-9** in the presence of different Mg^{2+} concentrations. The reaction was conducted under single-turnover conditions in a buffer of 50 mM Tris-HCl, pH 7.5, containing 0.2 mM (A), 2 mM (B), or 50 mM (C) Mg^{2+}, as well as the control without Mg^{2+} (D). Time points for aliquots were 0, 15, 30, 45, 60, 120, 240, and 360 min from left to right. In each panel, the upper band was the substrate, and the lower band was the product.

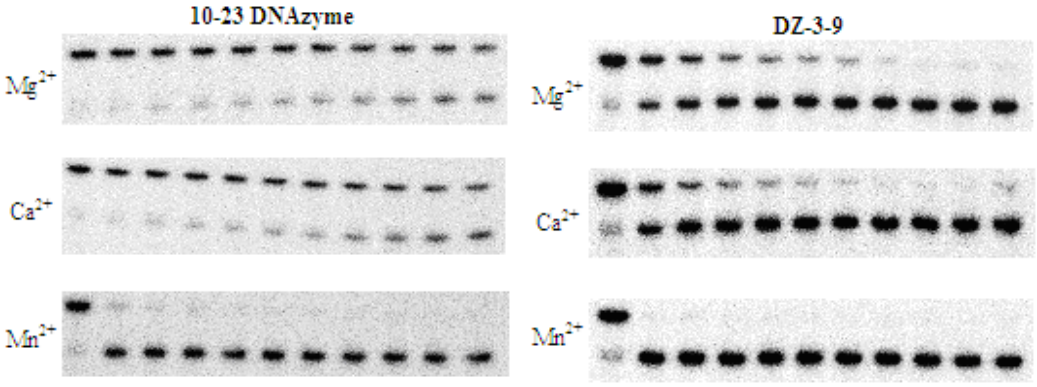
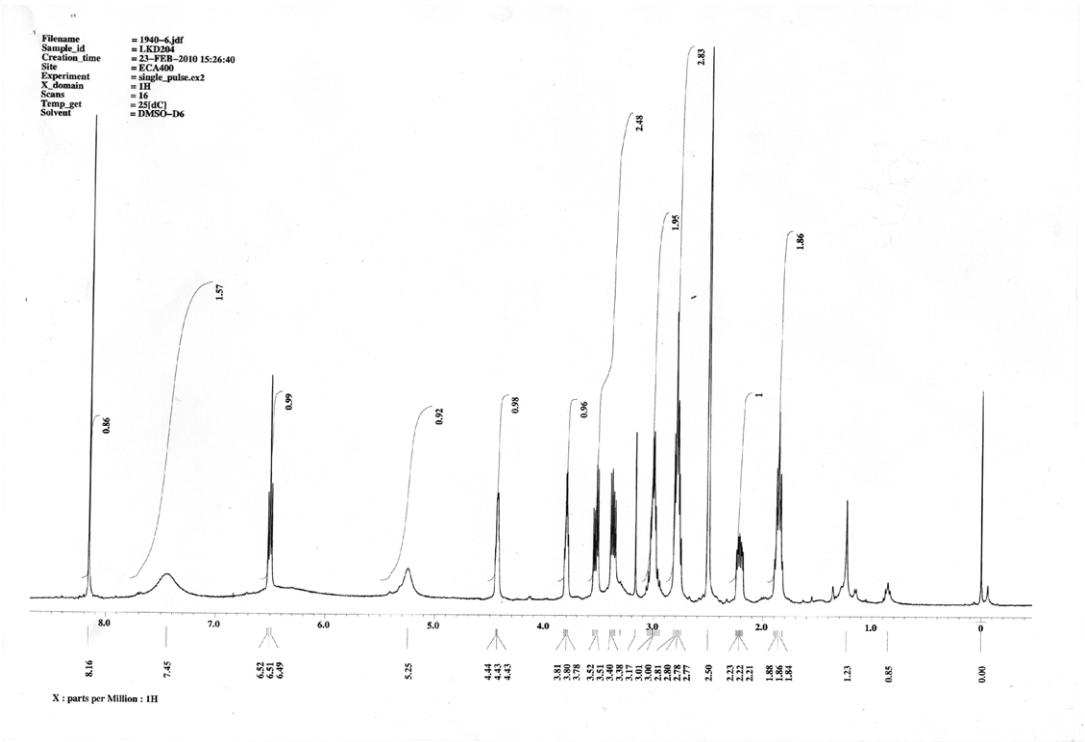
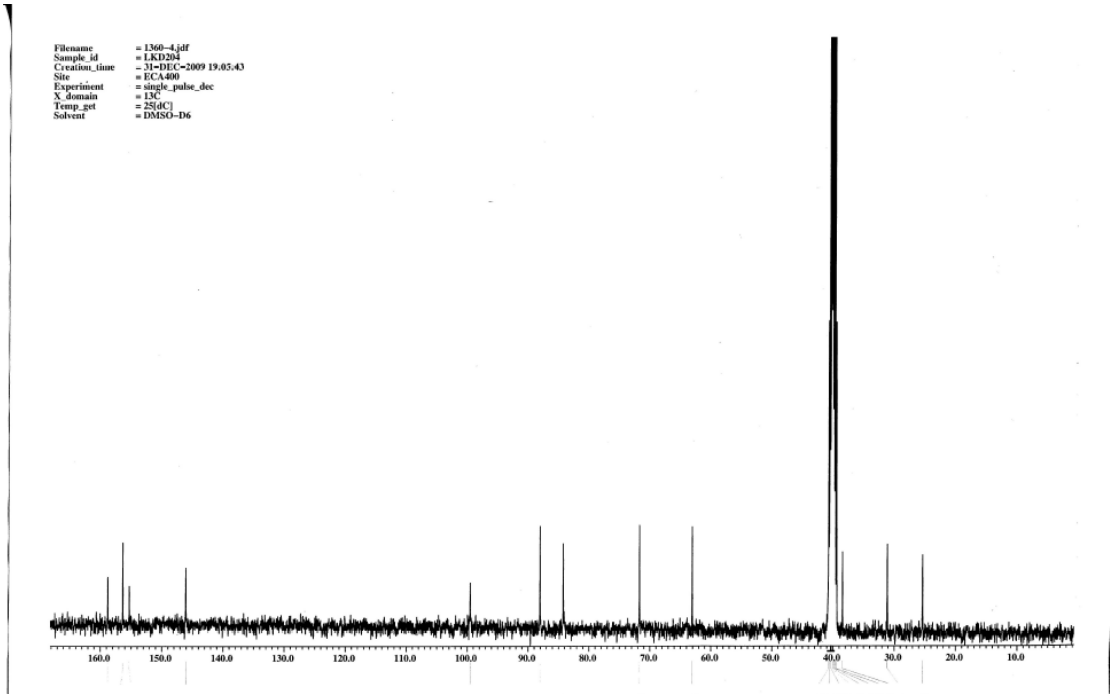


Fig. S3 The effect of divalent metal ions on the cleavage reactions of 10-23 DNAzyme (A) and DZ-3-9 (B) under single turnover conditions, in a buffer of 50 mM Tris-HCl, pH 7.5, containing 0.2 mM divalent metal ion. Time points for aliquots were 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 min from left to right. In each panel, the upper band is the substrate, and the lower band corresponds to the product.

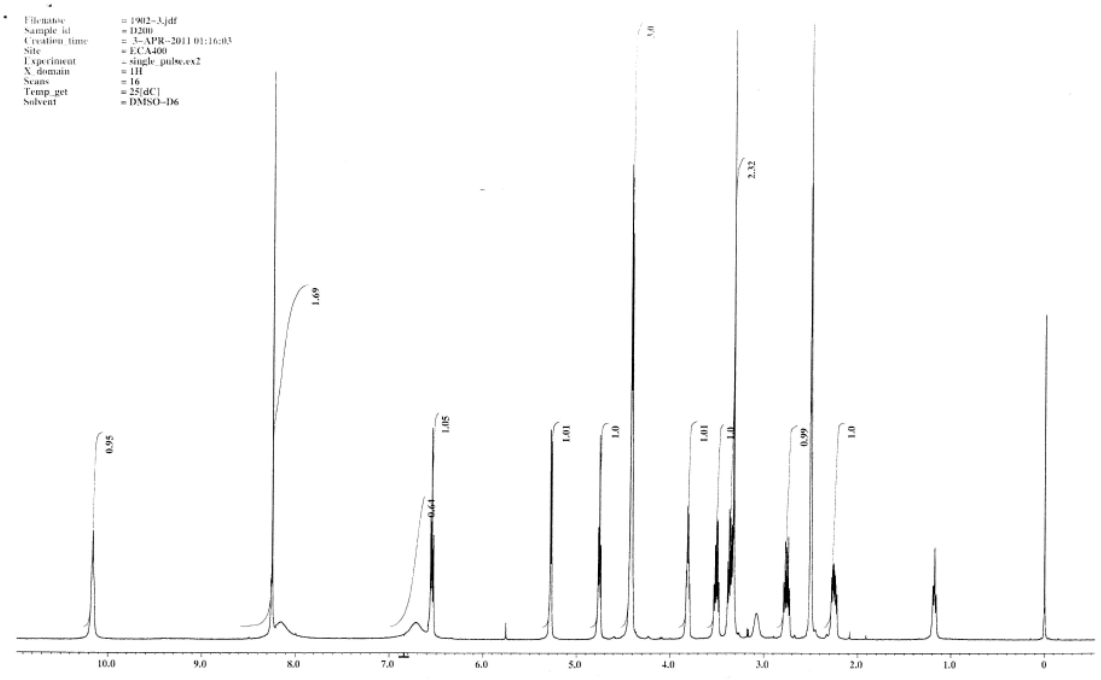
Compound 3 ^{1}H NMR



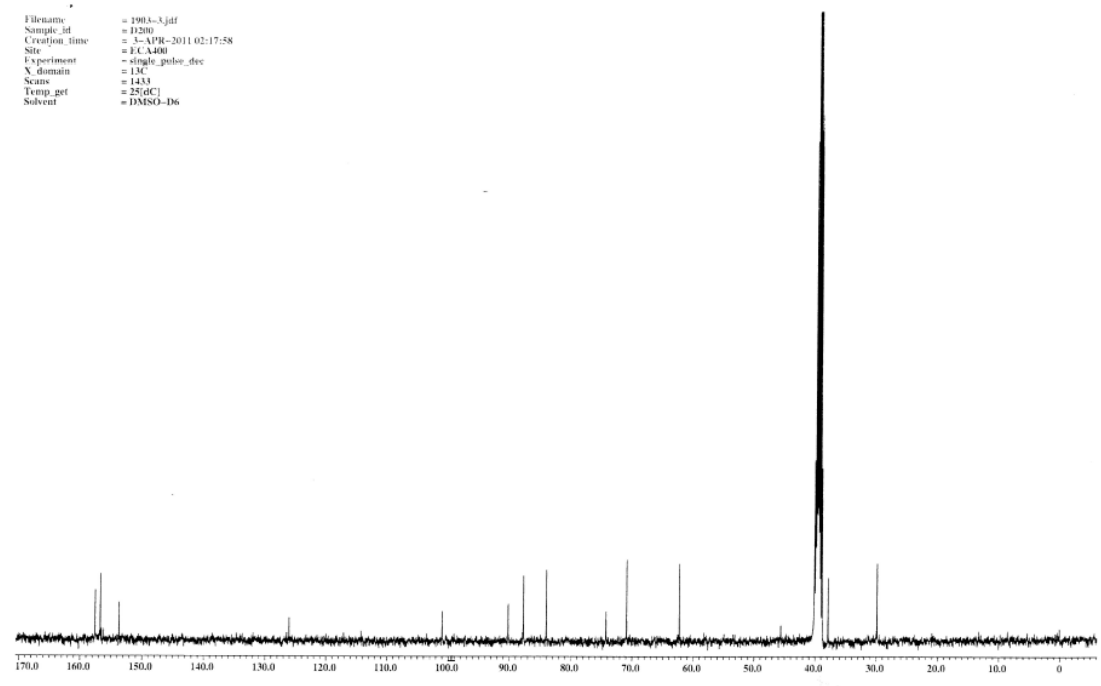
Compound 3 ^{13}C NMR



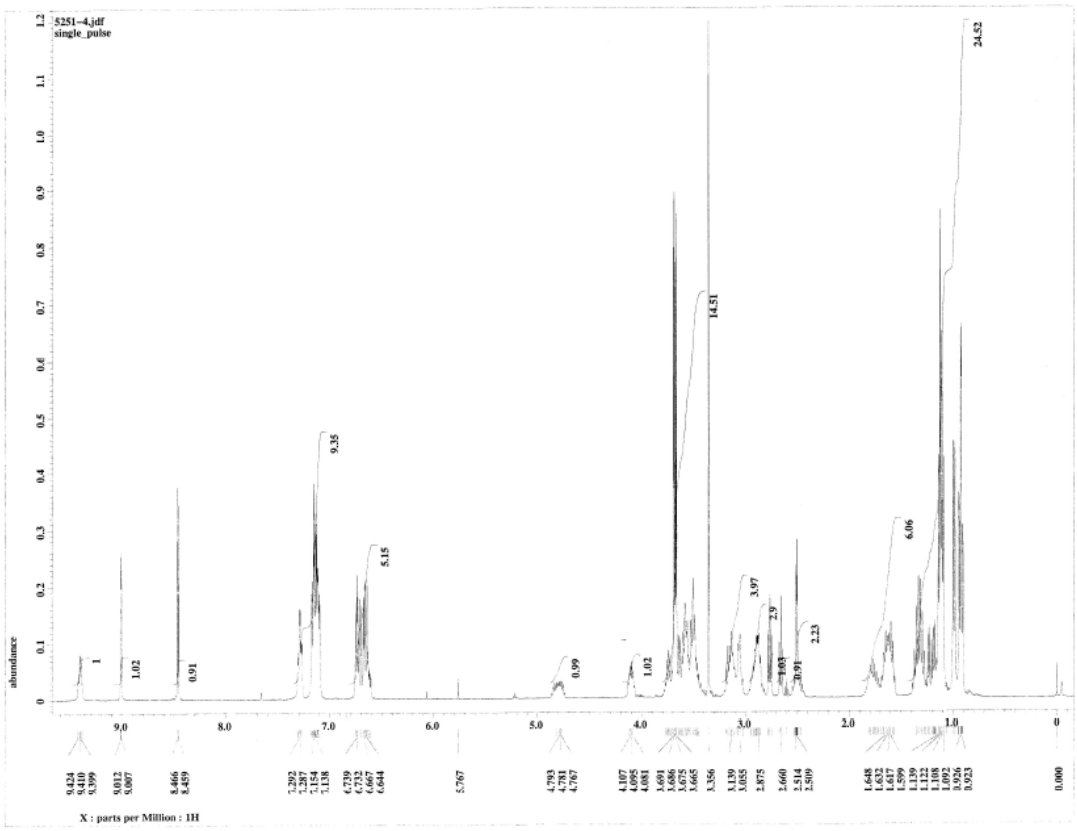
Compound 6 ^{1}H NMR



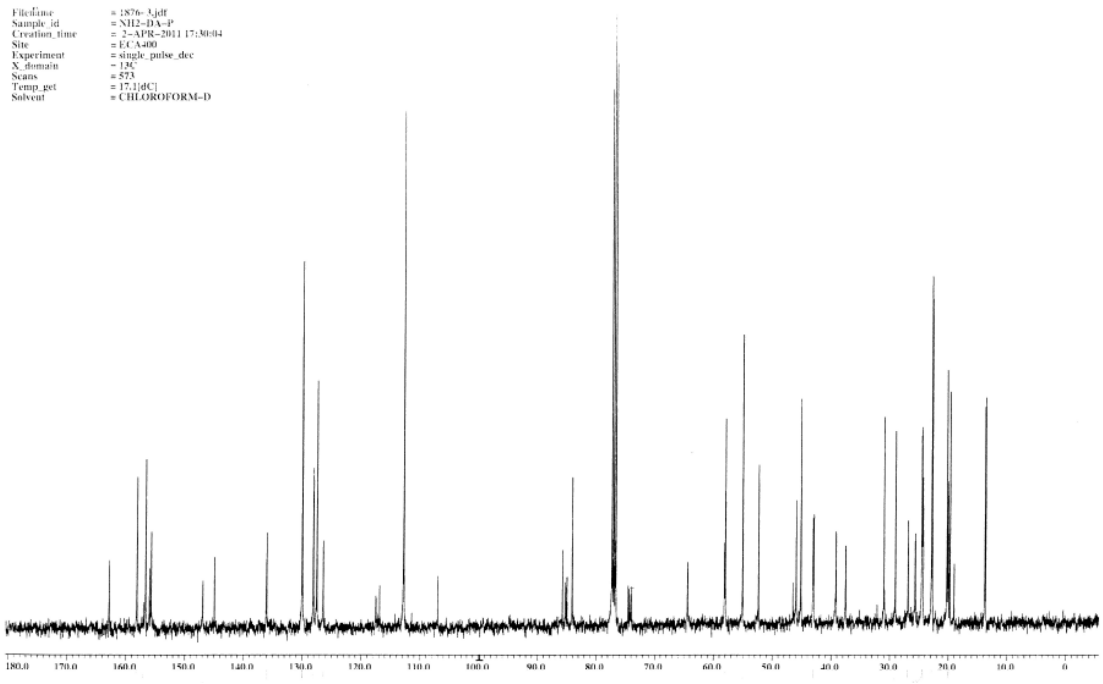
Compound 6 ^{13}C NMR



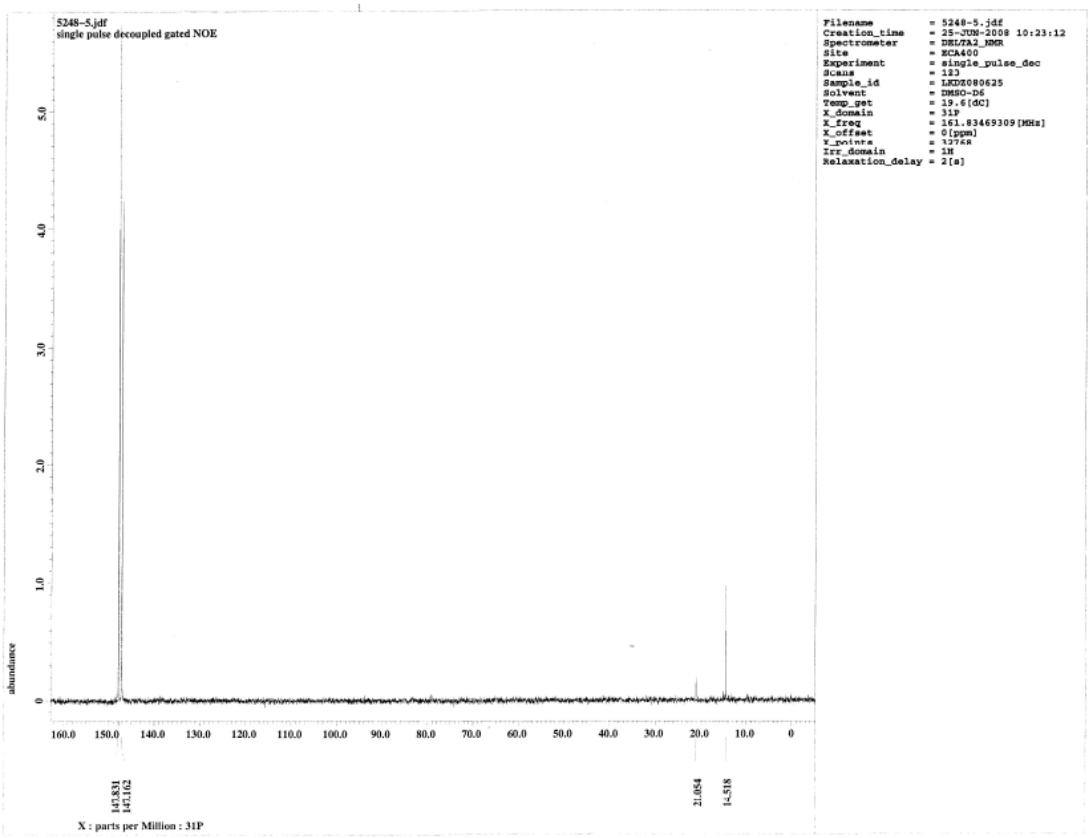
Compound **10** ^{1}H NMR



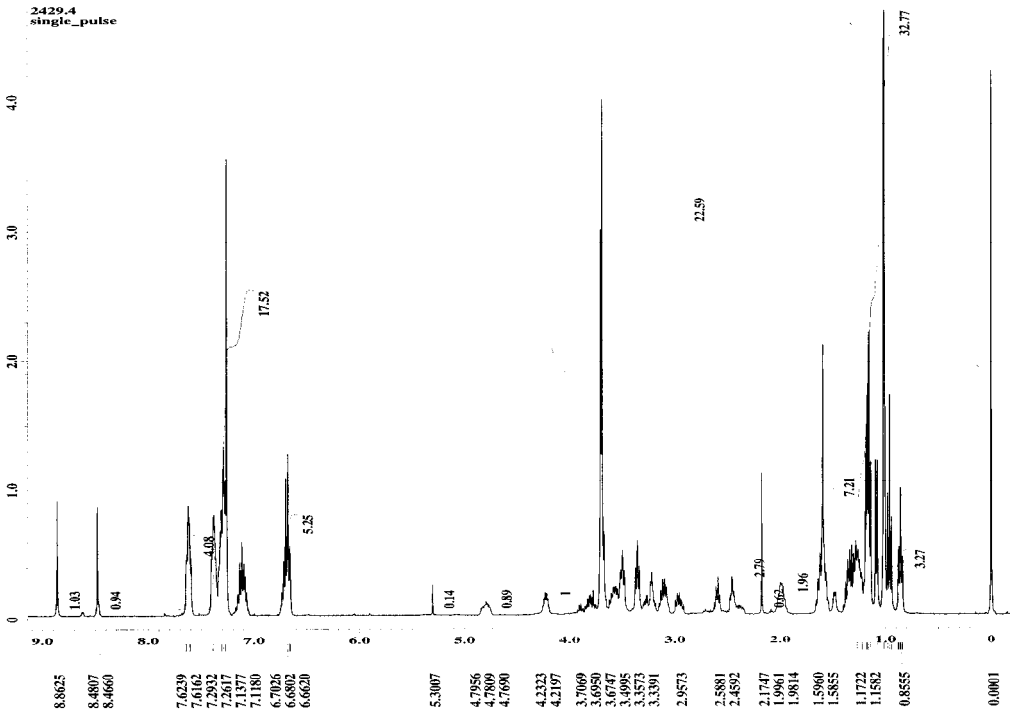
Compound **10** ^{13}C NMR



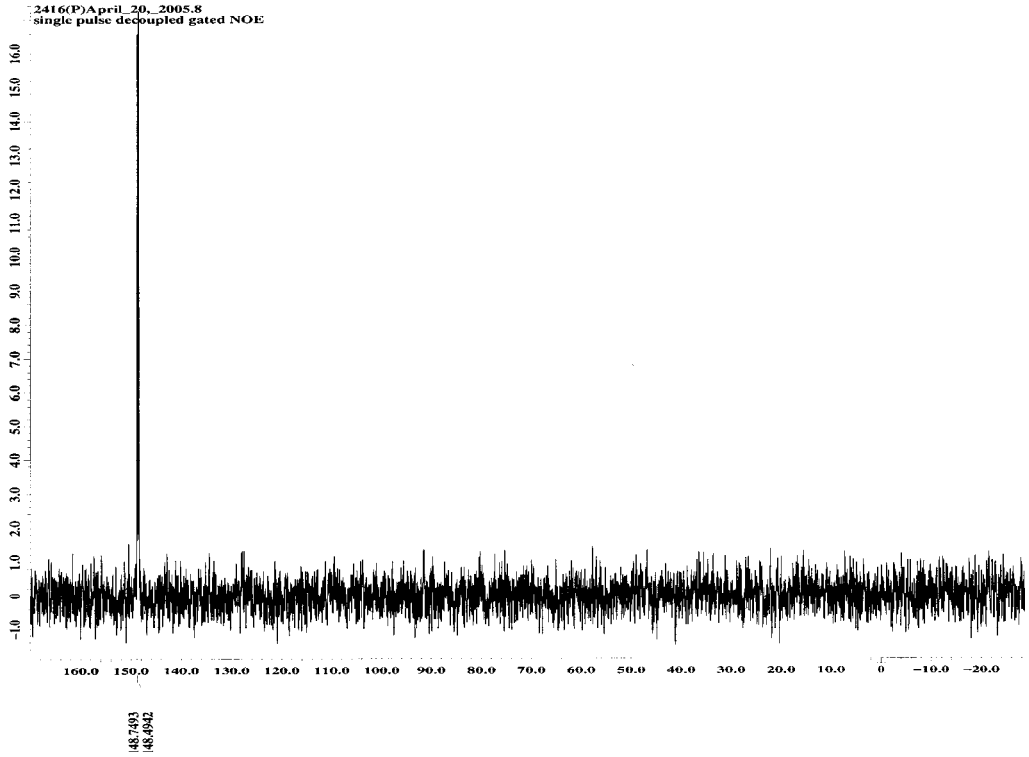
Compound **10** ^{31}P NMR



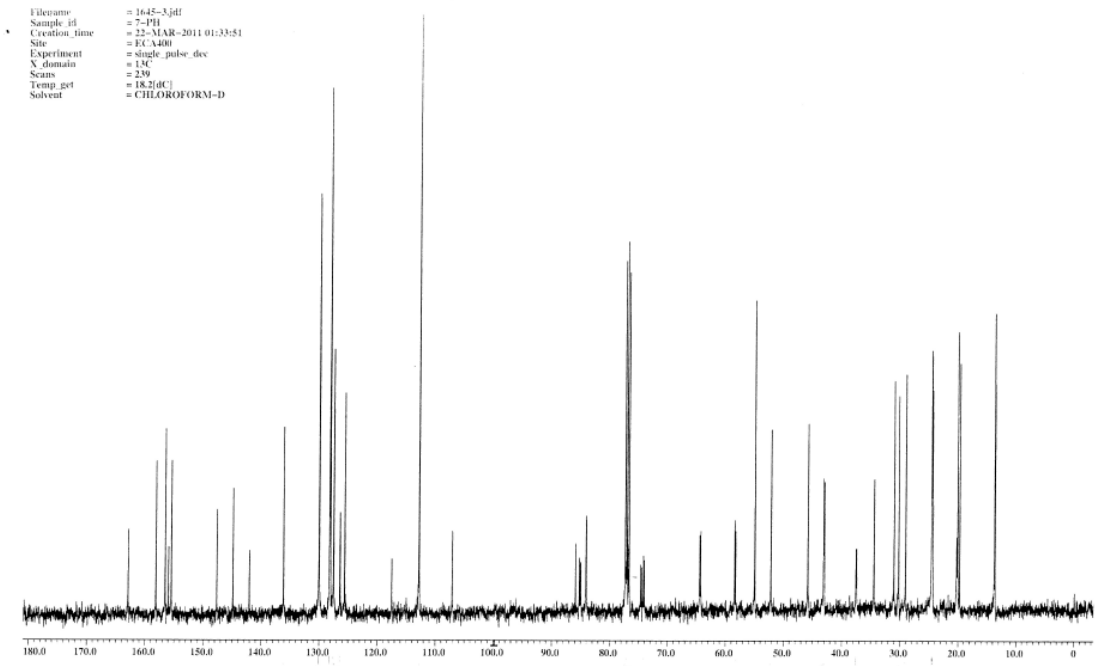
Compound 14 ^{1}H NMR



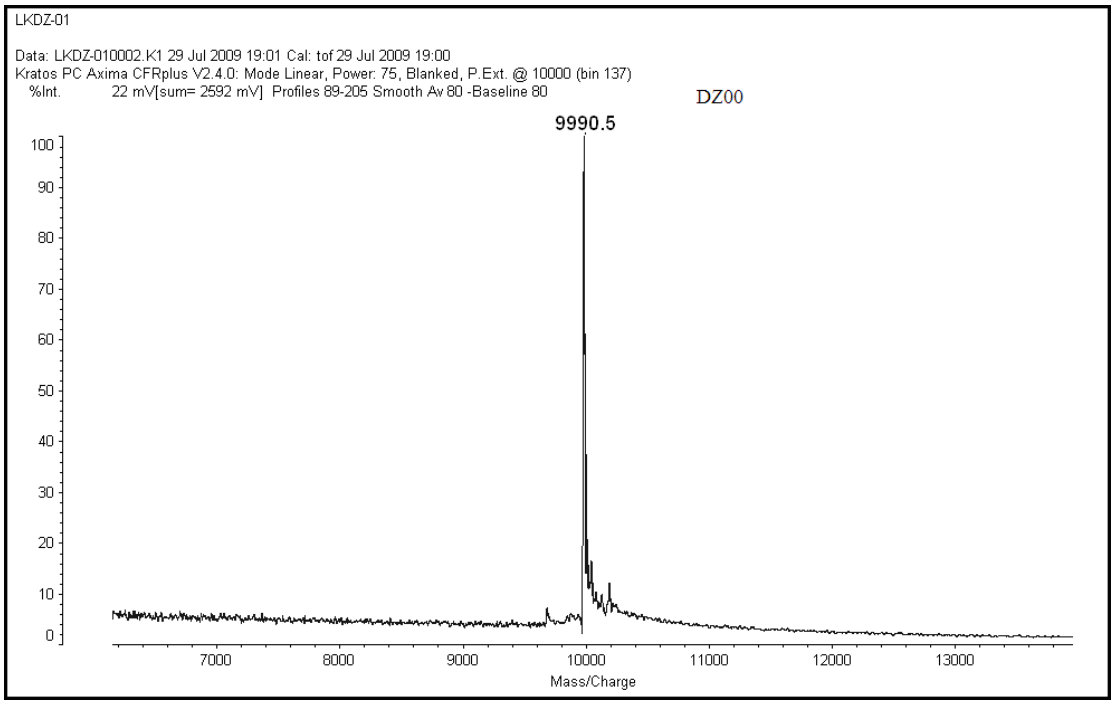
Compound **14**, ^{31}P NMR



Compound 14 ^{13}C NMR



MALDI-TOF MS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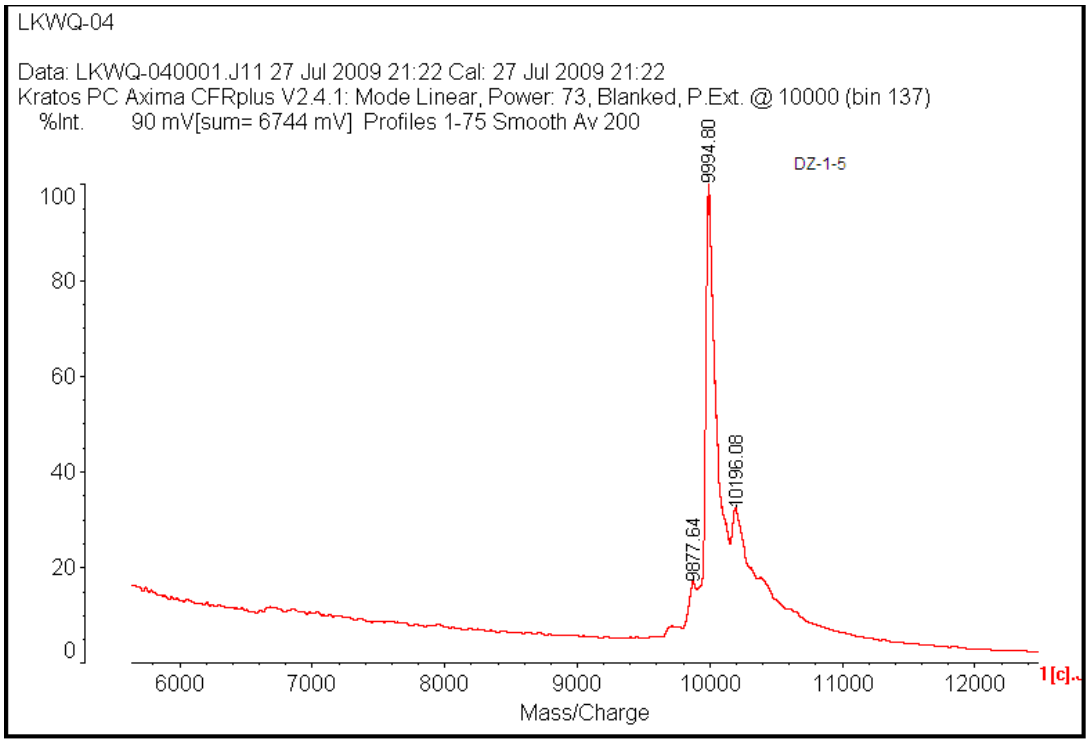
Data: LKDZ-010002.K1 29 Jul 2009 19:01 Cal: tof 29 Jul 2009 19:00
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 75, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 22 mV[sum= 2592 mV] Profiles 89-205 Smooth Av 80 -Baseline 80
DZ00
9990.5
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge


LKWQ-04
Data: LKWQ-040001.J11 27 Jul 2009 21:22 Cal: 27 Jul 2009 21:22
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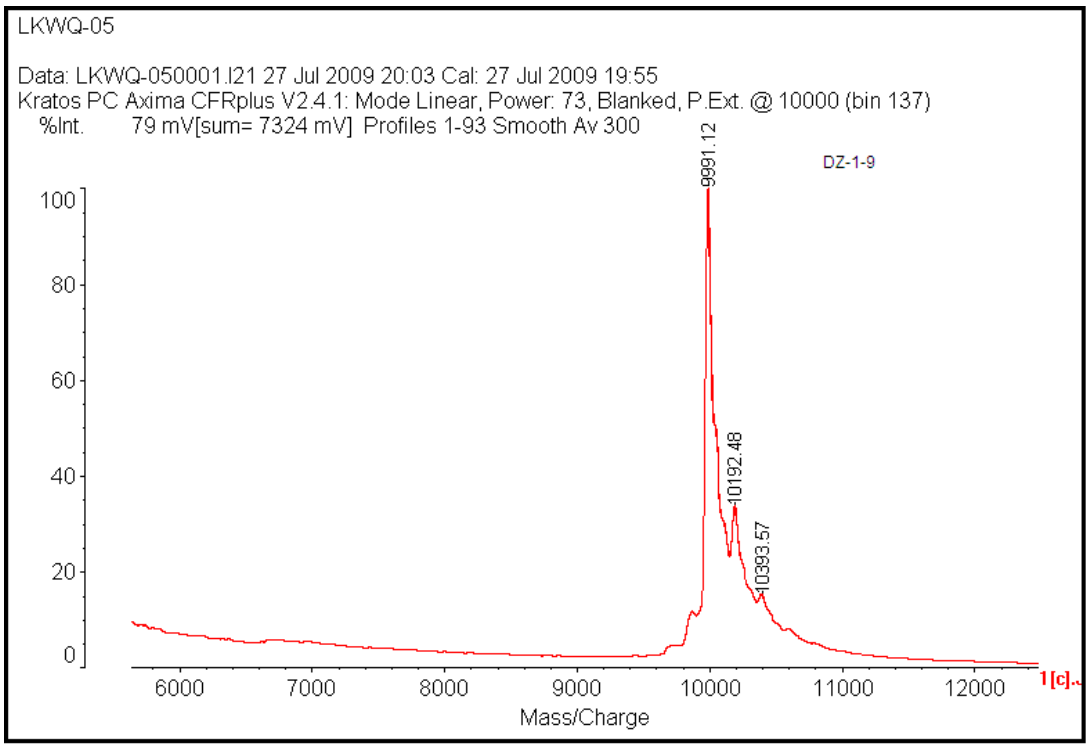
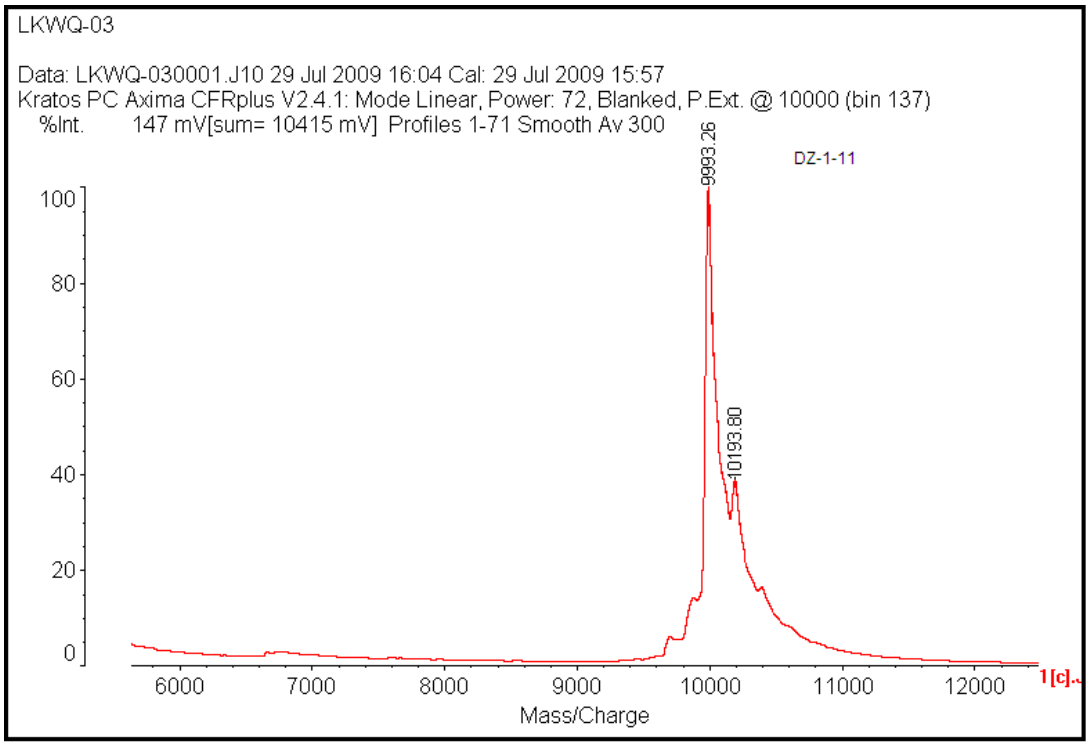
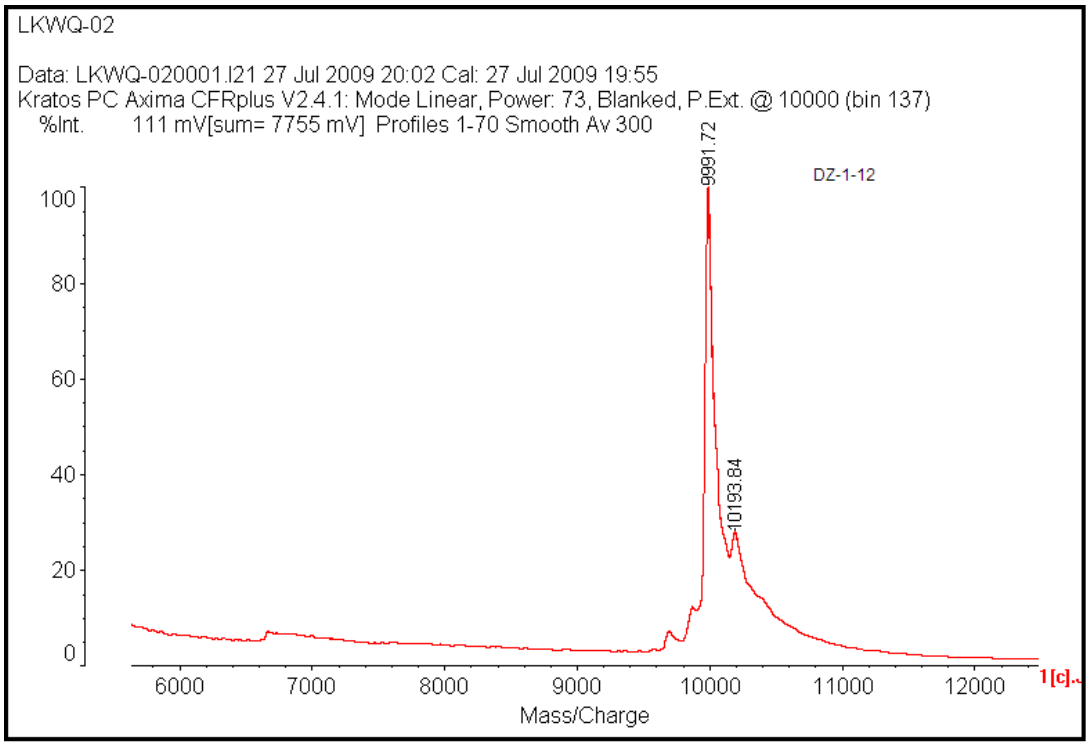
%Int. 90 mV[sum= 6744 mV] Profiles 1-75 Smooth Av 200
9994.80
DZ-1-5
10196.08
9877.64
100
80
60
40
20
0
6000
7000
8000
9000
10000
11000
12000
1[c]..
Mass/Charge



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Data: LKWQ-020001.I21 27 Jul 2009 20:02 Cal: 27 Jul 2009 19:55
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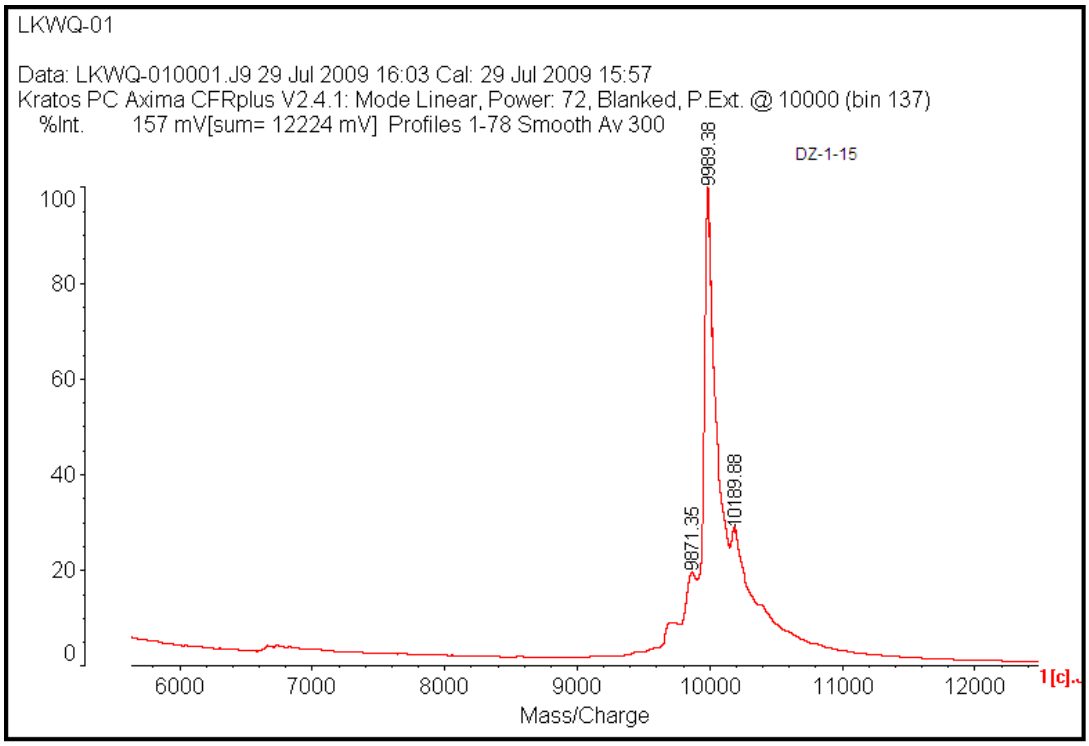
%Int. 111 mV[sum= 7755 mV] Profiles 1-70 Smooth Av 300
9991.72
DZ-1-12
10193.84
100
80
60
40
20
0
6000
7000
8000
9000
10000
11000
12000
Mass/Charge
1[c]..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Data: LKWQ-010001.J9 29 Jul 2009 16:03 Cal: 29 Jul 2009 15:57
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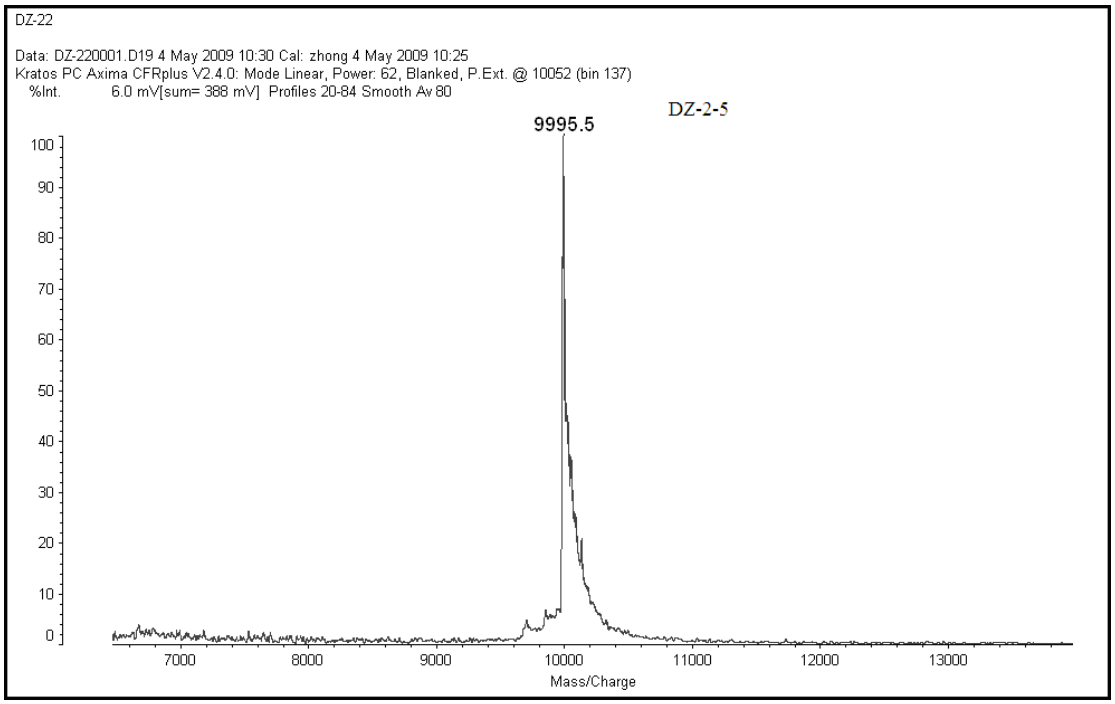
%Int. 157 mV[sum= 12224 mV] Profiles 1-78 Smooth Av 300
9989.38
DZ-1-15
10189.88
9871.35
100
80
60
40
20
0
6000
7000
8000
9000
10000
11000
12000
Mass/Charge
1[c]..


DZ-22
Data: DZ-220001.D19 4 May 2009 10:30 Cal: zhong 4 May 2009 10:25
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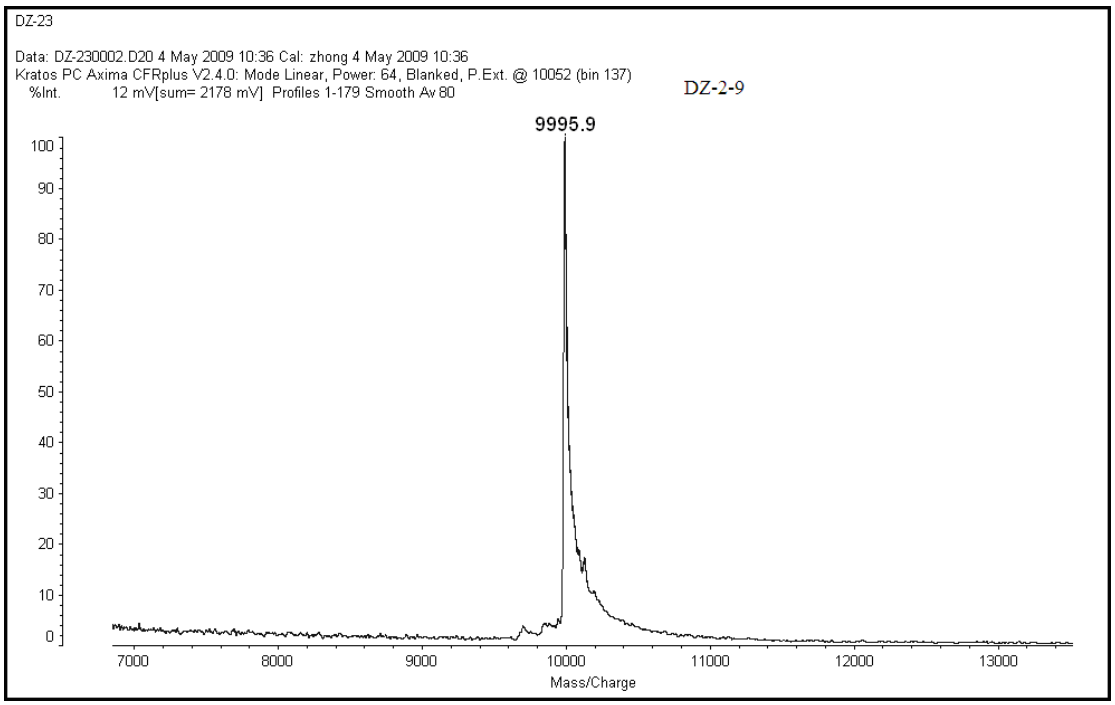
%Int. 6.0 mV[sum= 388 mV] Profiles 20-84 Smooth Av 80
DZ-2-5
9995.5
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge


DZ-23
Data: DZ-230002.D20 4 May 2009 10:36 Cal: zhong 4 May 2009 10:36
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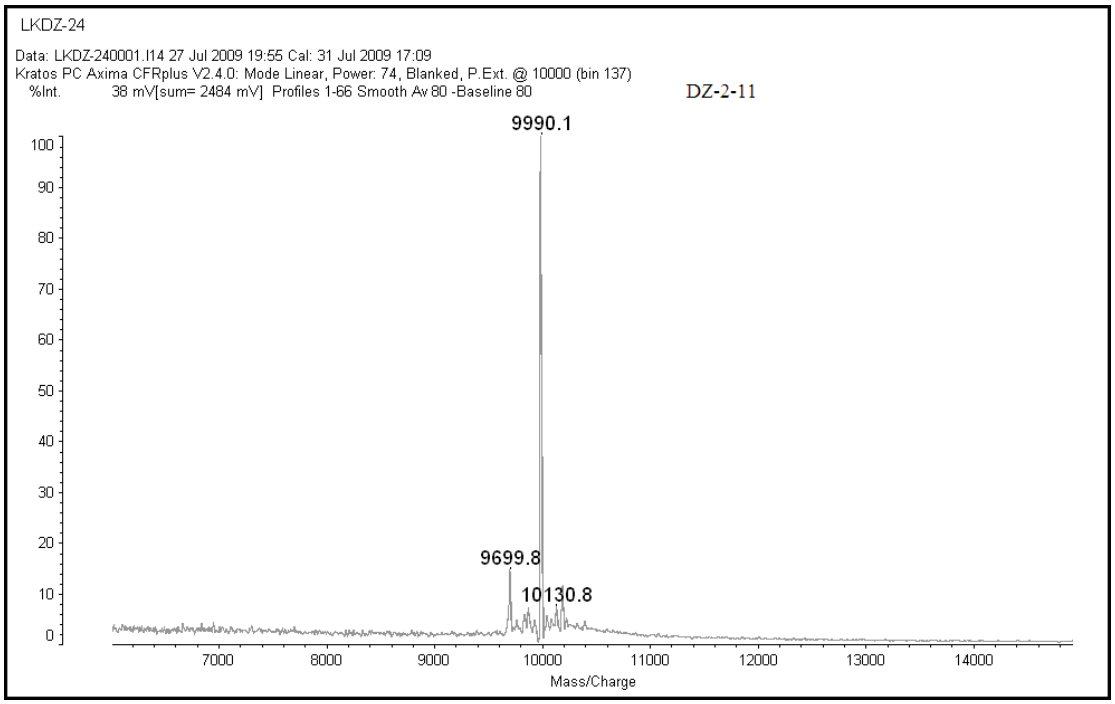
%Int. 12 mV[sum= 2178 mV] Profiles 1-179 Smooth Av 80
DZ-2-9
9995.9
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge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Data: LKDZ-240001.I14 27 Jul 2009 19:55 Cal: 31 Jul 2009 17:09
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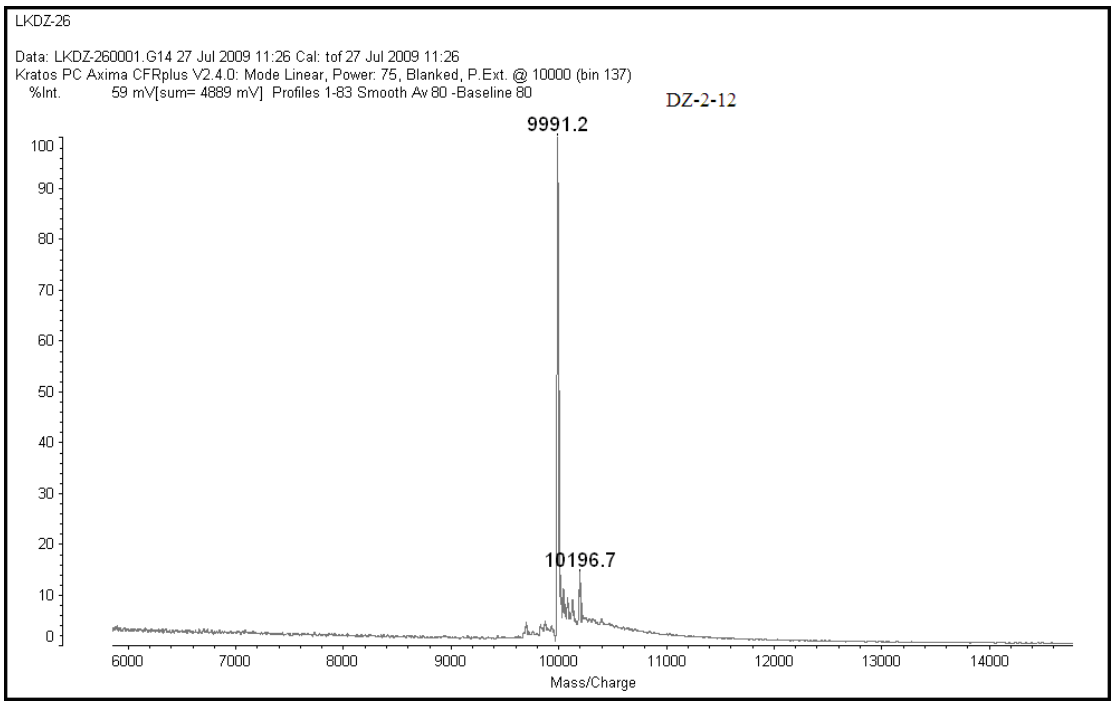
%Int. 38 mV[sum= 2484 mV] Profiles 1-66 Smooth Av 80 -Baseline 80
DZ-2-11
9990.1
9699.8
10130.8
Mass/Charge


LKDZ-26
Data: LKDZ-260001.G14 27 Jul 2009 11:26 Cal: tof 27 Jul 2009 11:26
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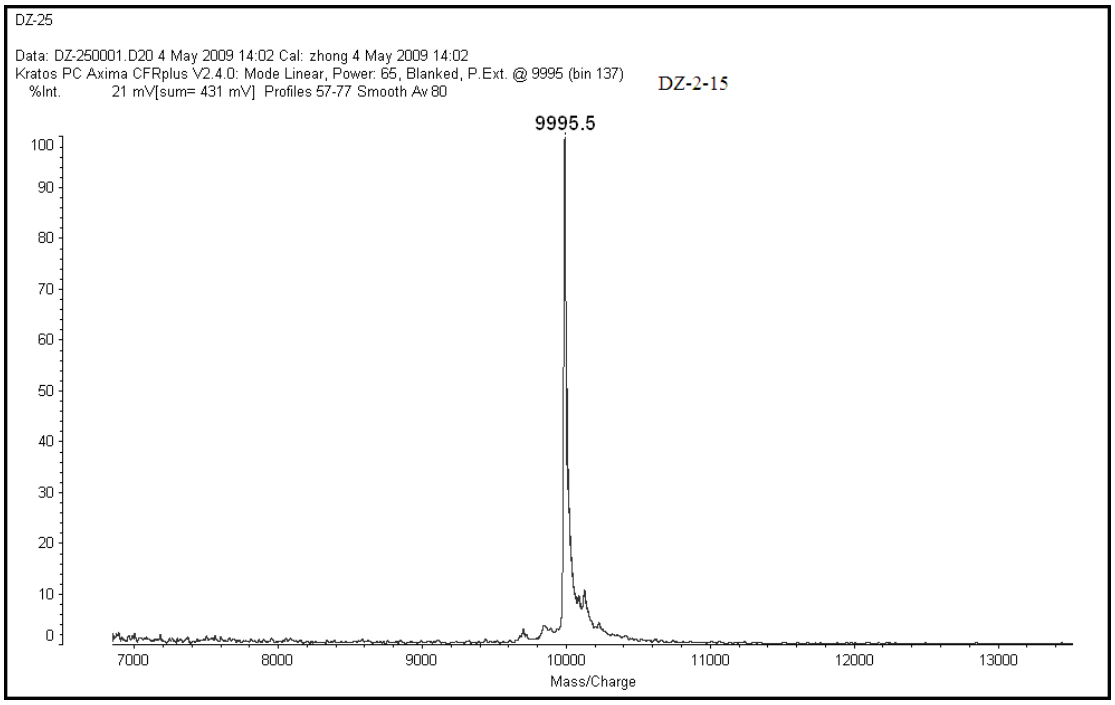
%Int. 59 mV[sum= 4889 mV] Profiles 1-83 Smooth Av 80 -Baseline 80
DZ-2-12
9991.2
10196.7
Mass/Charge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Data: DZ-250001.D20 4 May 2009 14:02 Cal: zhong 4 May 2009 14:02
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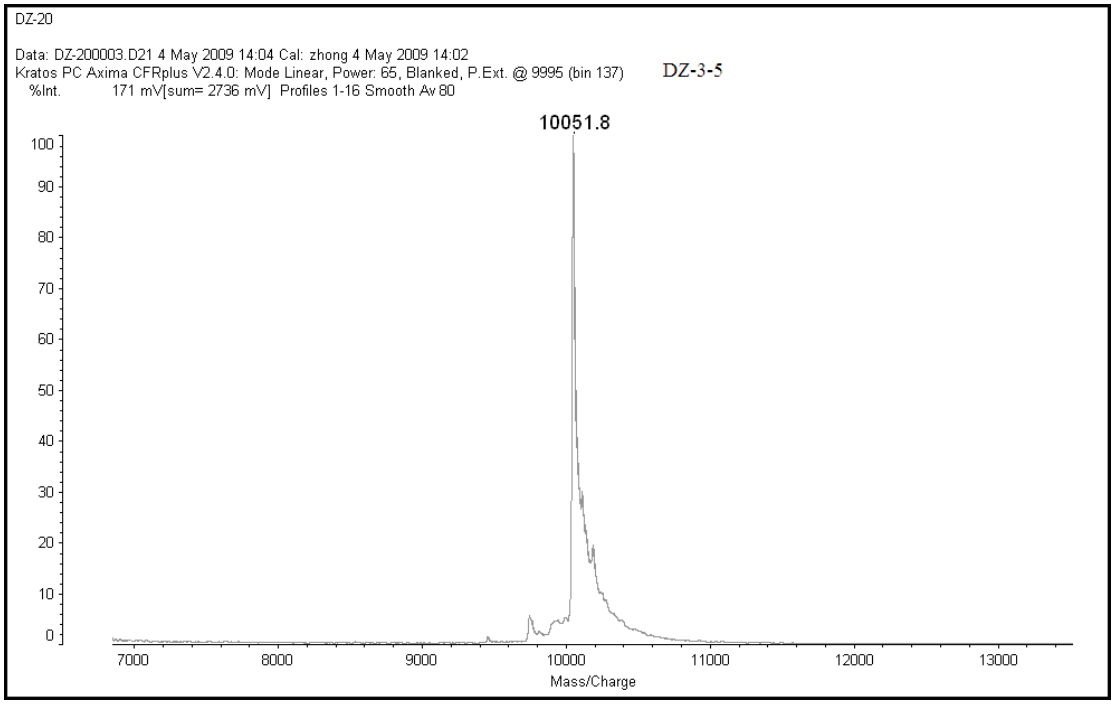
%Int. 21 mV[sum= 431 mV] Profiles 57-77 Smooth Av 80
DZ-2-15
9995.5
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge


DZ-20
Data: DZ-200003.D21 4 May 2009 14:04 Cal: zhong 4 May 2009 14:02
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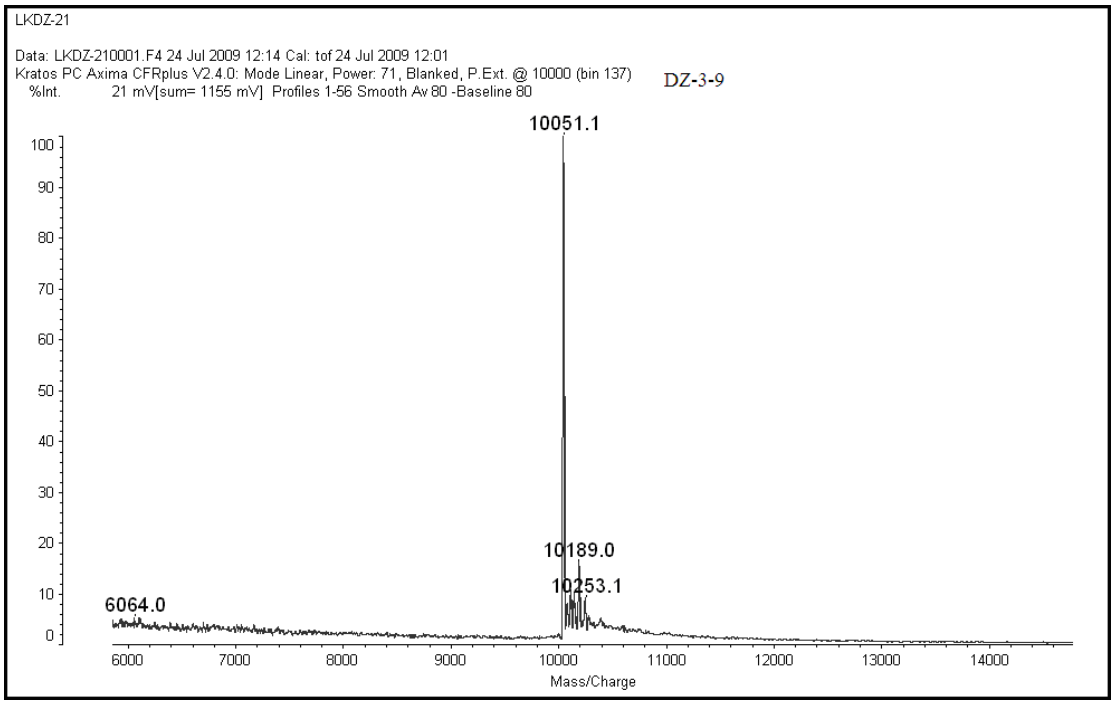
%Int. 171 mV[sum= 2736 mV] Profiles 1-16 Smooth Av 80
DZ-3-5
10051.8
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge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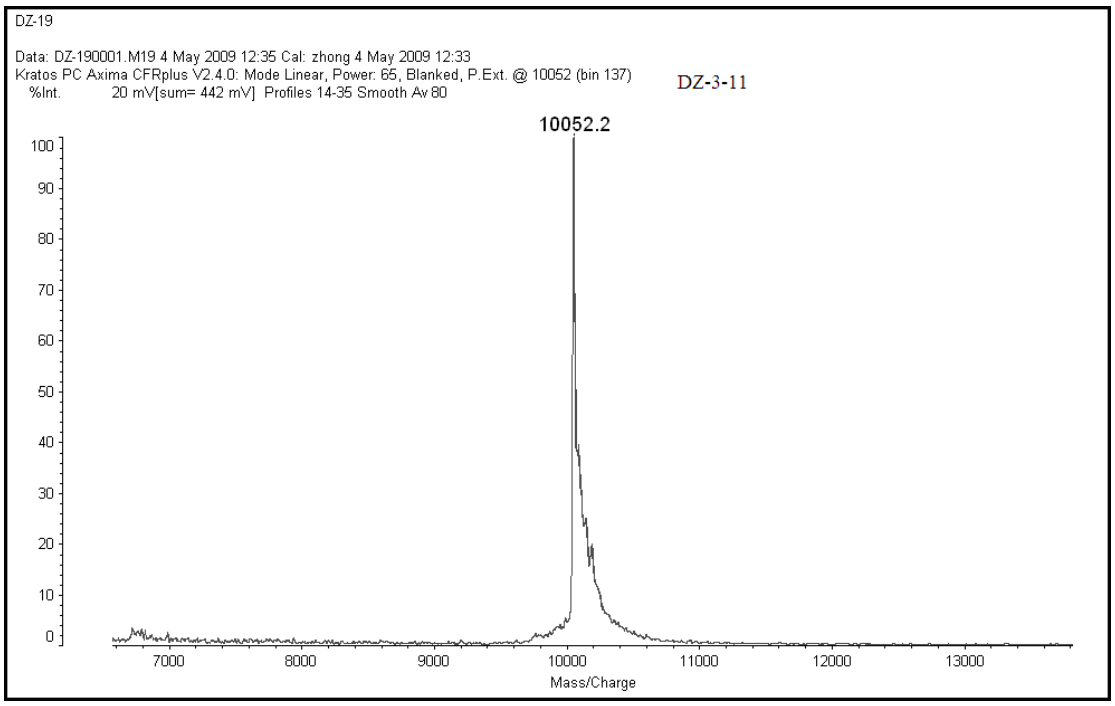
Data: LKDZ-210001.F4 24 Jul 2009 12:14 Cal: tof 24 Jul 2009 12:01
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 71, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 21 mV[sum= 1155 mV] Profiles 1-56 Smooth Av 80 -Baseline 80
DZ-3-9
10051.1
10189.0
10253.1
6064.0
Mass/Charge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Data: DZ-190001.M19 4 May 2009 12:35 Cal: zhong 4 May 2009 12:33
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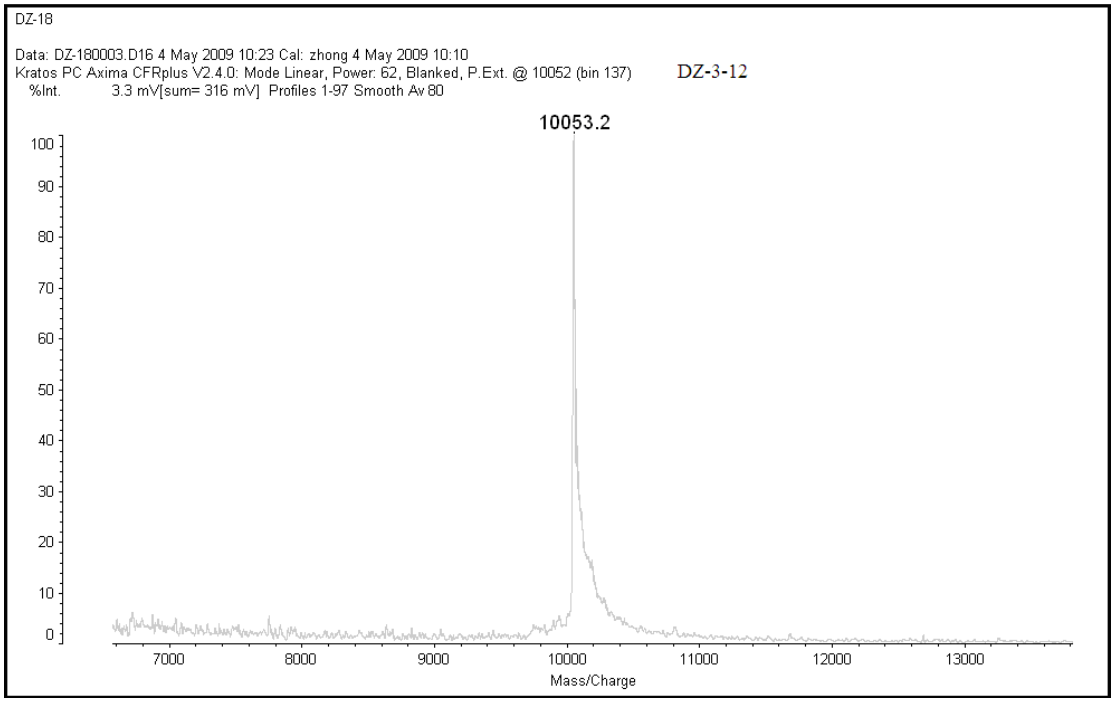
DZ-3-11
10052.2
Mass/Charge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Data: DZ-180003.D16 4 May 2009 10:23 Cal: zhong 4 May 2009 10:10
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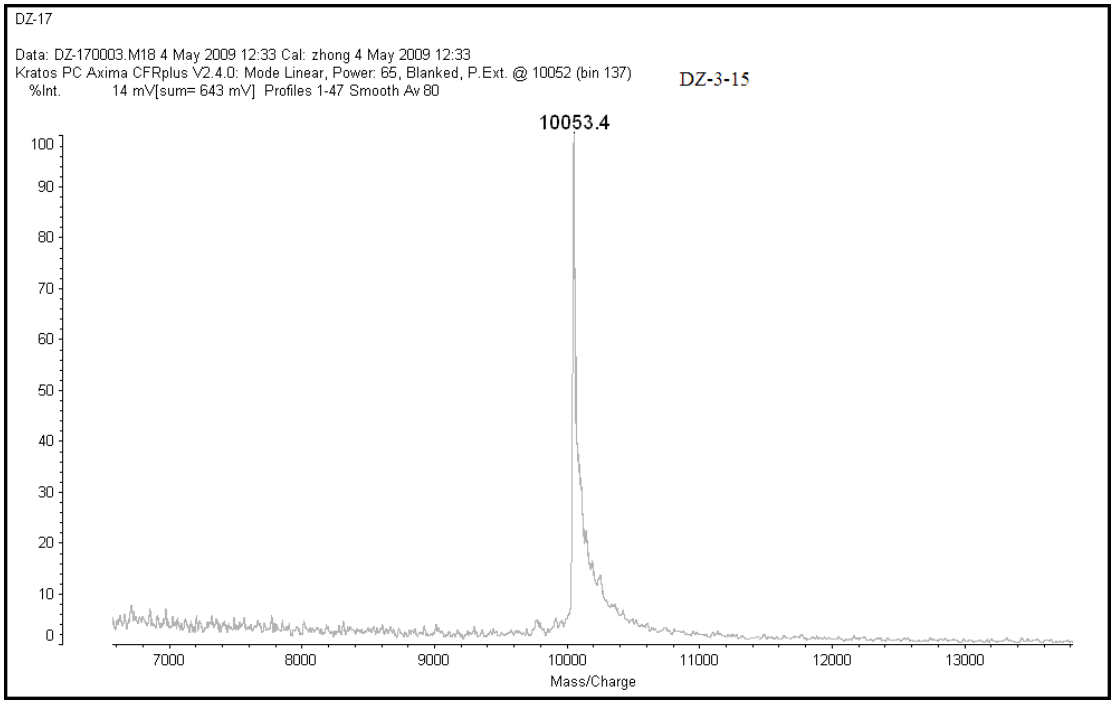
%Int. 3.3 mV[sum= 316 mV] Profiles 1-97 Smooth Av 80
DZ-3-12
10053.2
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge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Data: DZ-170003.M18 4 May 2009 12:33 Cal: zhong 4 May 2009 12:33
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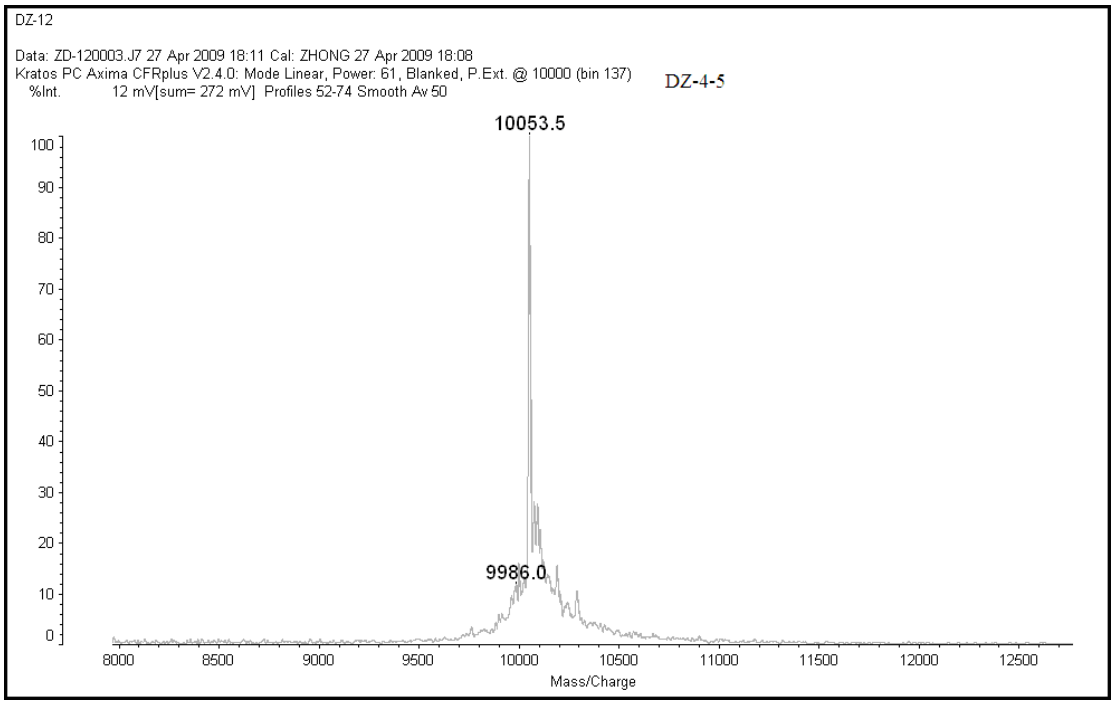
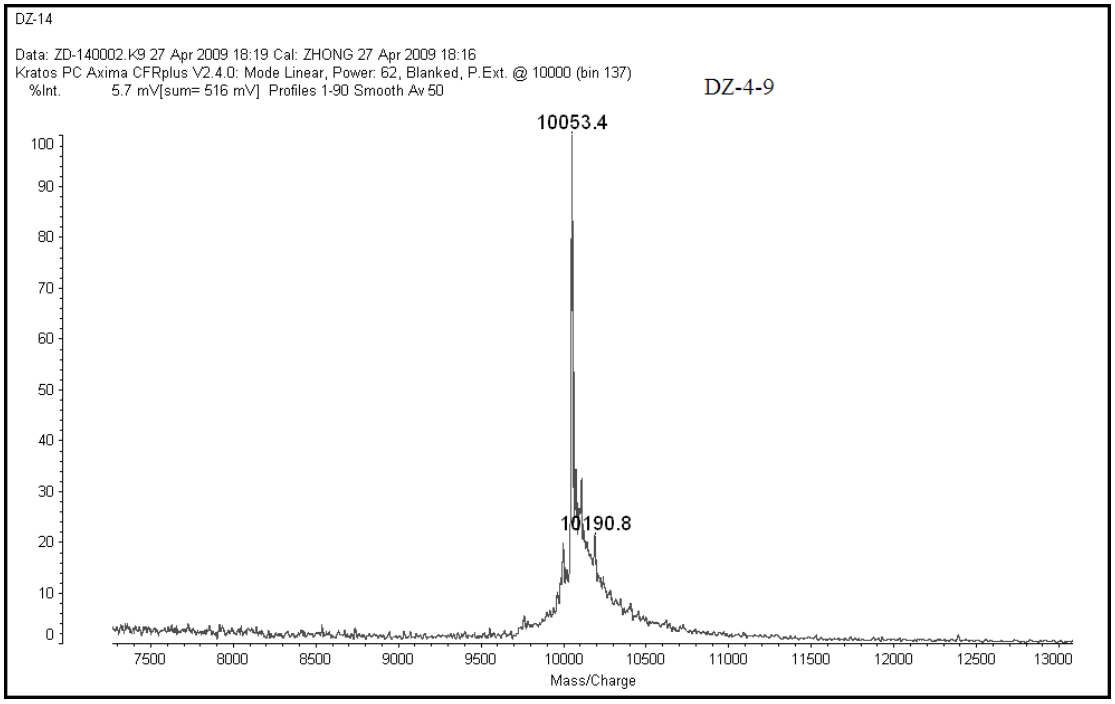
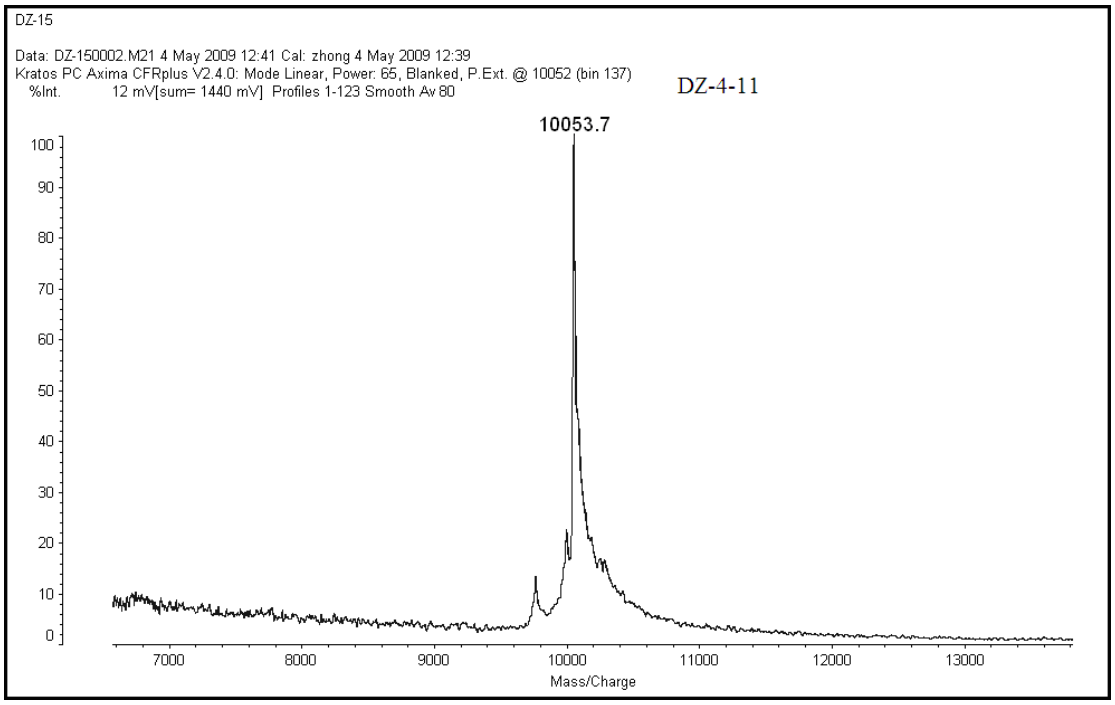
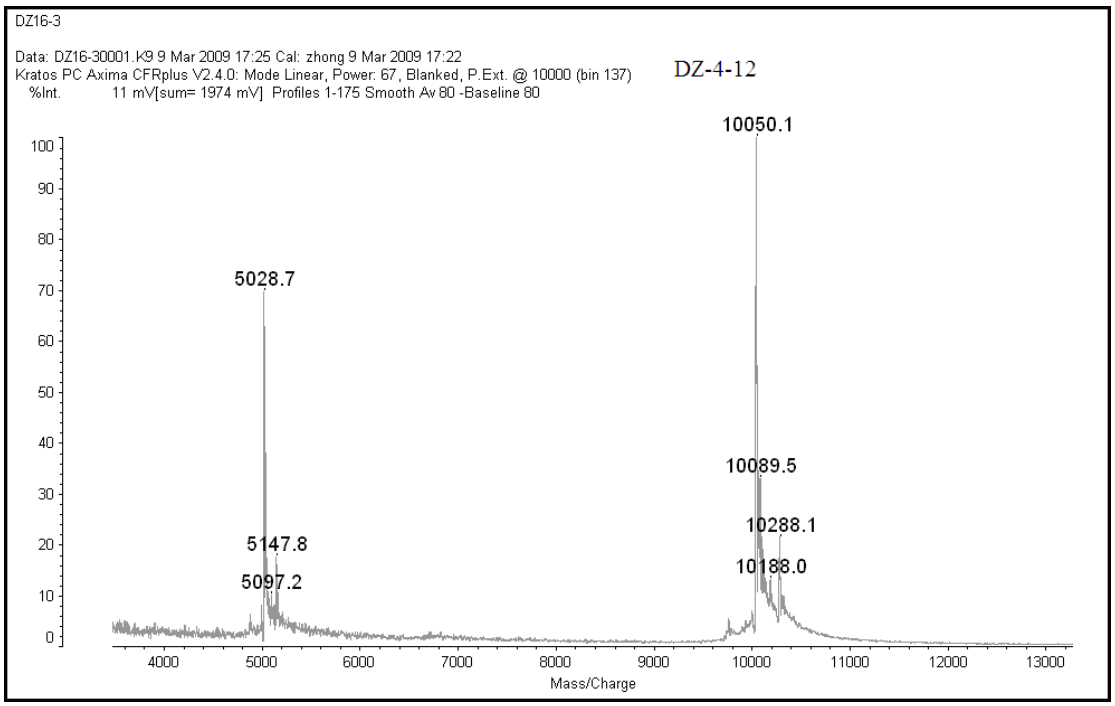
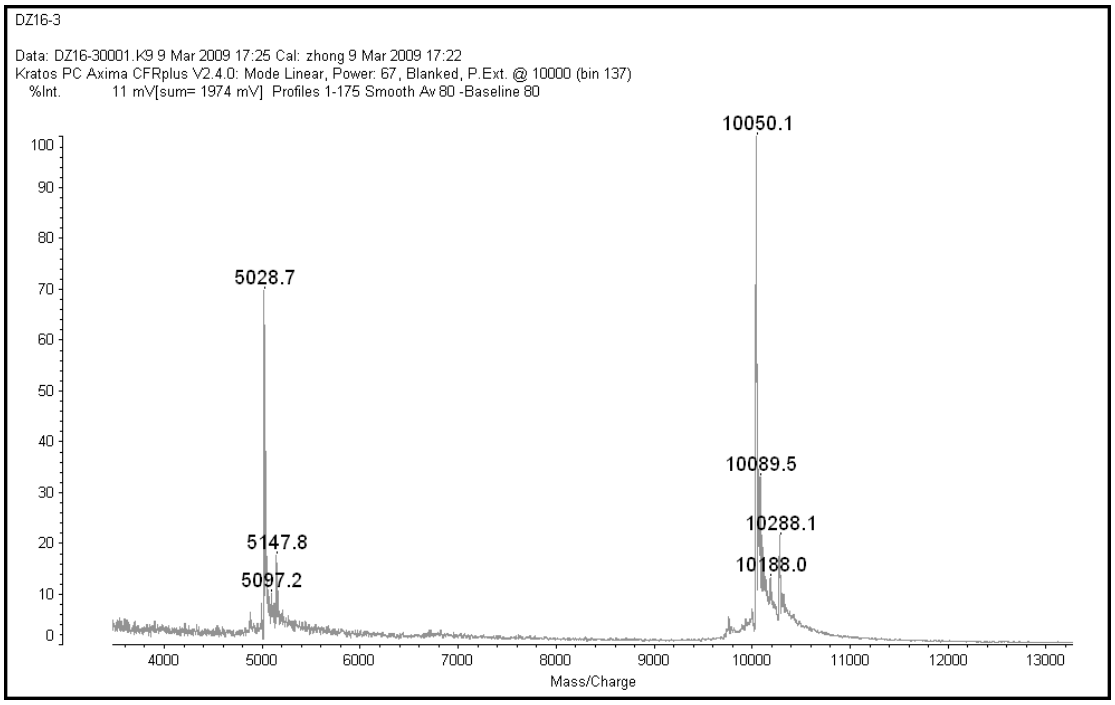
%Int. 14 mV[sum= 643 mV] Profiles 1-47 Smooth Av 80
DZ-3-15
10053.4
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge

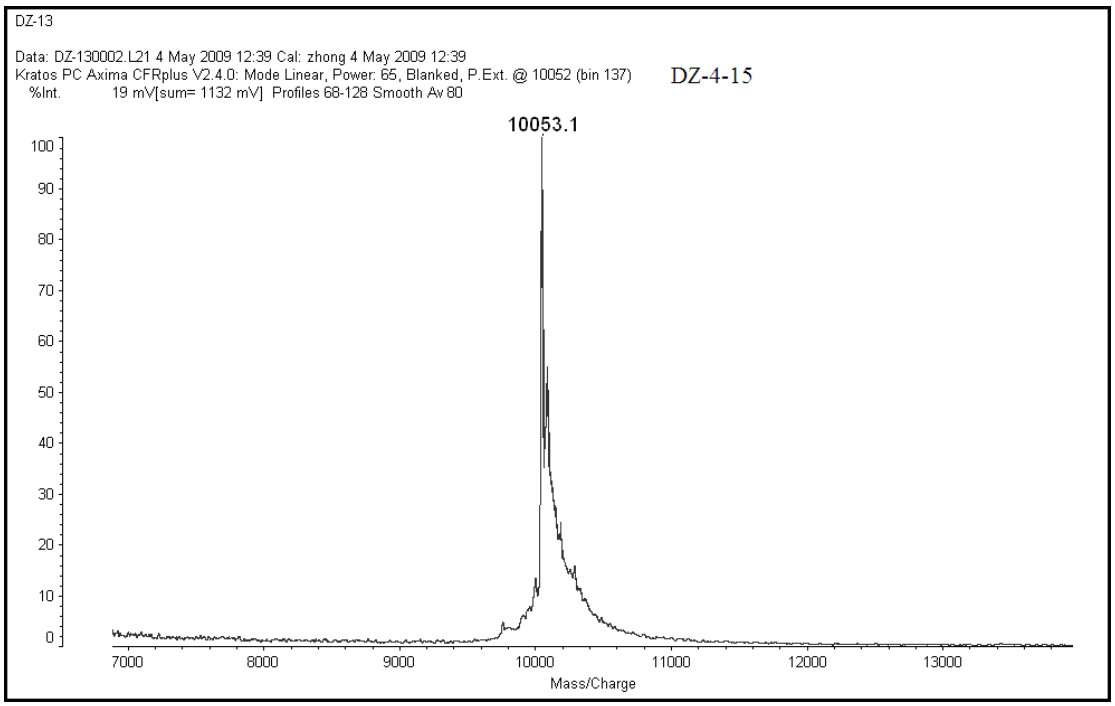

DZ-12
Data: ZD-120003.J7 27 Apr 2009 18:11 Cal: ZHONG 27 Apr 2009 18:08
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 61, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 12 mV[sum= 272 mV] Profiles 52-74 Smooth Av 50
DZ-4-5
10053.5
9986.0
100
90
80
70
60
50
40
30
20
10
0
8000
8500
9000
9500
10000
10500
11000
11500
12000
12500
Mass/Charge

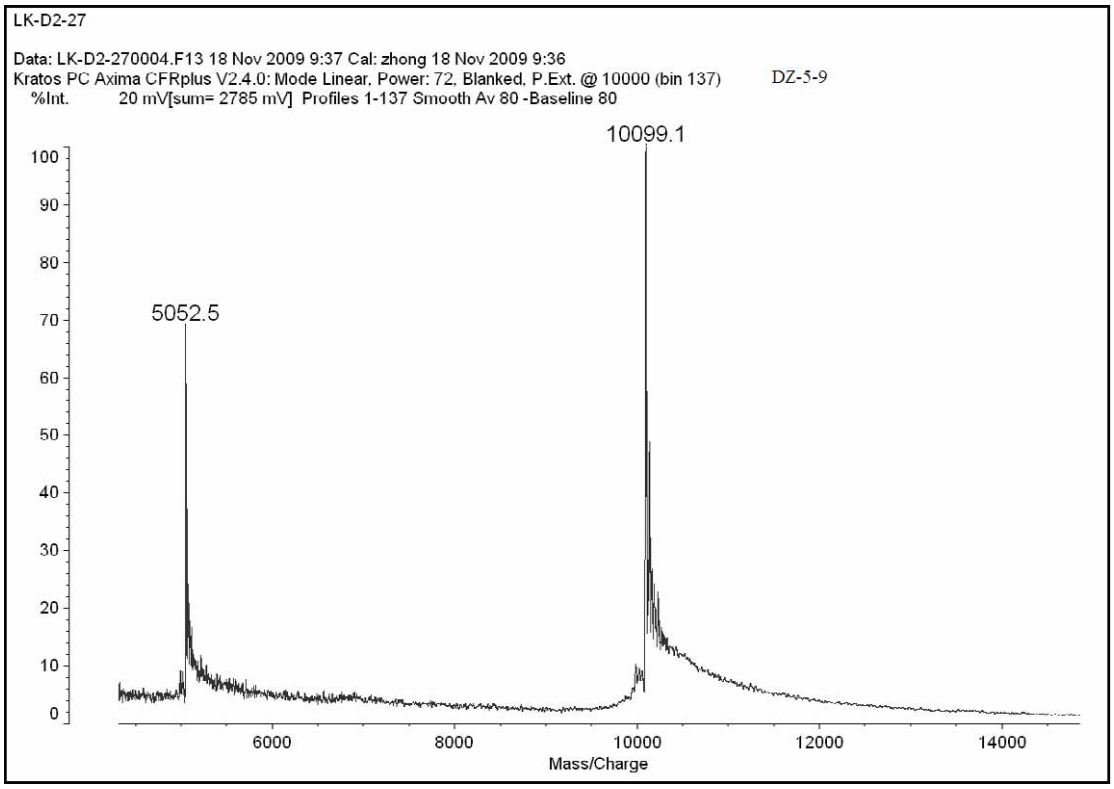

DZ-14
Data: ZD-140002.K9 27 Apr 2009 18:19 Cal: ZHONG 27 Apr 2009 18:16
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 62, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 5.7 mV[sum= 516 mV] Profiles 1-90 Smooth Av 50
DZ-4-9
10053.4
10190.8
100
90
80
70
60
50
40
30
20
10
0
7500
8000
8500
9000
9500
10000
10500
11000
11500
12000
12500
13000
Mass/Charge


DZ-15
Data: DZ-150002.M21 4 May 2009 12:41 Cal: zhong 4 May 2009 12:39
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 65, Blanked, P.Ext. @ 10052 (bin 137)
%Int. 12 mV[sum= 1440 mV] Profiles 1-123 Smooth Av 80
DZ-4-11
10053.7
100
90
80
70
60
50
40
30
20
10
0
7000
8000
9000
10000
11000
12000
13000
Mass/Charge


DZ16-3
Data: DZ16-30001.K9 9 Mar 2009 17:25 Cal: zhong 9 Mar 2009 17:22
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 67, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 11 mV[sum= 1974 mV] Profiles 1-175 Smooth Av 80 -Baseline 80
DZ-4-12
10050.1
5028.7
10089.5
10288.1
5147.8
10188.0
5097.2
100
90
80
70
60
50
40
30
20
10
0
4000
5000
6000
7000
8000
9000
10000
11000
12000
13000
Mass/Charge


DZ16-3
Data: DZ16-30001.K9 9 Mar 2009 17:25 Cal: zhong 9 Mar 2009 17:22
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 67, Blanked, P.Ext. @ 10000 (bin 137)
%Int. 11 mV[sum= 1974 mV] Profiles 1-175 Smooth Av 80 -Baseline 80
10050.1
5028.7
10089.5
10288.1
5147.8
10188.0
5097.2
Mass/Charge


DZ-13
Data: DZ-130002.L21 4 May 2009 12:39 Cal: zhong 4 May 2009 12:39
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 65, Blanked, P.Ext. @ 10052 (bin 137)
%Int. 19 mV[sum= 1132 mV] Profiles 68-128 Smooth Av 80
DZ-4-15
10053.1
Mass/Charge


LK-D2-27
Data: LK-D2-270004.F13 18 Nov 2009 9:37 Cal: zhong 18 Nov 2009 9:36
Kratos PC Axima CFRplus V2.4.0: Mode Linear, Power: 72, Blanked, P.Ext. @ 10000 (bin 137)
DZ-5-9
%Int. 20 mV[sum= 2785 mV] Profiles 1-137 Smooth Av 80 -Baseline 80
10099.1
5052.5
100
90
80
70
60
50
40
30
20
10
0
6000
8000
10000
12000
14000
Mass/Charge