Supplementary Information

Content

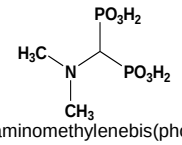
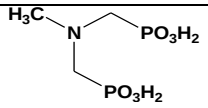
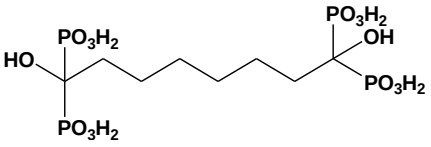
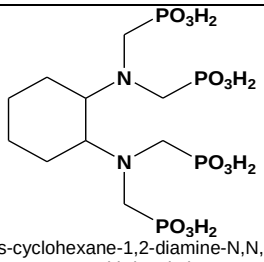
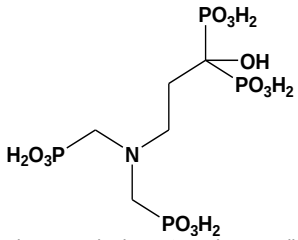
Single bisphosphonic acid	Diphosphonic acid
 N,N '-dimethylaminomethylenebis(phosphonic acid)[1]	 N-methyliminodimethylenephosphonic acid[2]
pK_{amine}= 13.4 $pK_{phosphonate}$= 9.18 $pK_{phosphonate}$= 5.01 $pK_{phosphonate}$= 1.30	pK_{amine}= 11.32 $pK_{phosphonate}$= 6.19 $pK_{phosphonate}$= 4.73
Double bisphosphonic acid	**Double diphosphonic acid**
 1,8-dihydroxyoctane-1,1,8,8-tetryltetraphosphinic acid[3]	 trans-cyclohexane-1,2-diamine-N,N,N,N -tetrakis(methyl- enephosphonic acid)[4]
$pK_{phosphonate}$= 12.14 $pK_{phosphonate}$= 10.98 $pK_{phosphonate}$= 7.28 $pK_{phosphonate}$= 6.42 $pK_{phosphonate}$= 3.06 $pK_{phosphonate}$= 2.21	pK_{amine}= 10.73 pK_{amine}= 9.89 $pK_{phosphonate}$= 6.86 $pK_{phosphonate}$= 5.93 $pK_{phosphonate}$= 4.95 $pK_{phosphonate}$= 2.92
Mixed	
 N,N-dimethylenephosphonate-1-hydroxy-3- aminopropylidenediphosphonate[5]	
pK_{amine}= nd $pK_{phosphonate}$= 9.79 $pK_{phosphonate}$= 7.04 $pK_{phosphonate}$= 5.91 $pK_{phosphonate}$= 4.94 $pK_{phosphonate}$= 2.36	

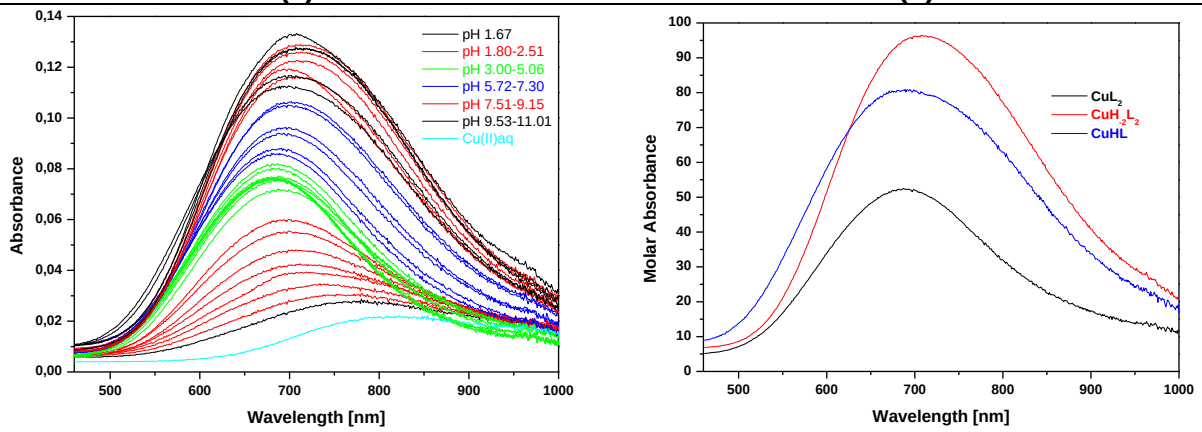
Figure S1. Approximate comparison of protonation constants of single and double bisphosphonic acids and diphosphonic acids. Chosen literature data.

[1] J. E. Bollinger, D. M. Roundhill, *Inorg. Chem.* **1994**, *33*, 6421–6424.
[2] B. Kurzak, A. Kamecka, K. Kurzak, J. Jezierska, P. Kafarski, *Polyhedron* **1998**, *17*, 4403–4413.
[3] M. Dyba, Jezowska-Bojczuk, E. Kiss, T. Kiss, H. Kozlowski, Y. Leroux, D. El Manouni, *J. Chem. Soc. Dalt. Trans.* **1996**, 1119–1123.
[4] J. Gałęzowska, R. Janicki, A. Mondry, R. Burgada, T. Bailly, M. Lecouvey, H. Kozłowski, *Dalton Trans.* **2006**, 4384–94.
[5] J. R. Zeevaart, D. R. Jansen, M. Filomena Botelho, A. Abrunhosa, C. Gomes, L. Metello, Z. I. Kolar, G. C. Krijger, W. K. A. Louw, I. C. Dormehl, *J. Inorg. Biochem.* **2004**, *98*, 1521–1530.

L^1/Cu^{2+}

Conditions; 0.1M KCl, [L]=2.7×10^{-3}M, [Cu^{2+}]=1.4×10^{-3}M, optical path: 1cm

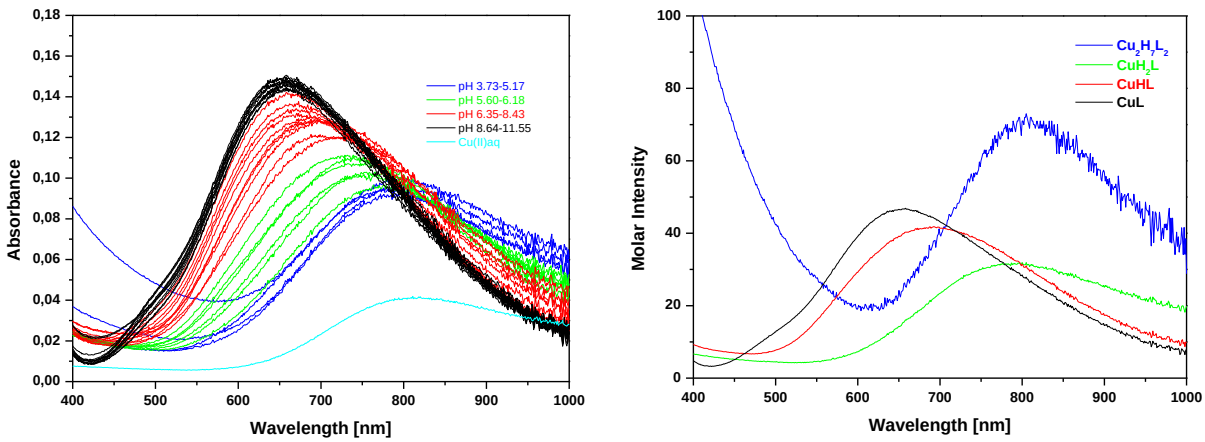
(a) **(b)**



L^2/Cu^{2+}

Conditions; 0.1M KCl, [L]=3.2×10^{-3}M, [Cu^{2+}]=3.2×10^{-3}M, optical path: 1cm, precipitation below pH 3.7

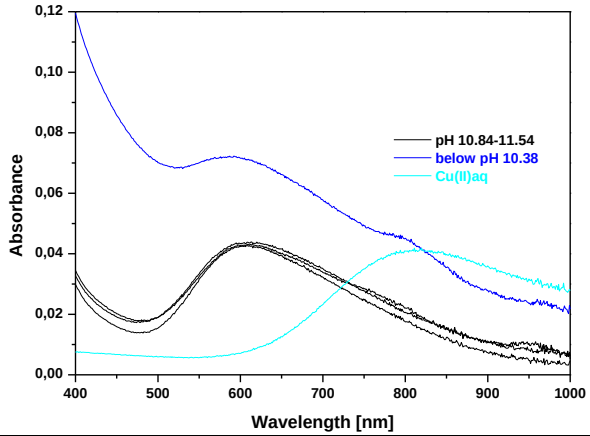
(a) **(b)**



L^3/Cu^{2+}

Conditions; 0.1M KCl, [L]=1.8×10^{-3}M, [Cu^{2+}]=8.4×10^{-4}M, optical path: 1cm, precipitation below pH 10.38

(a) **(b)**



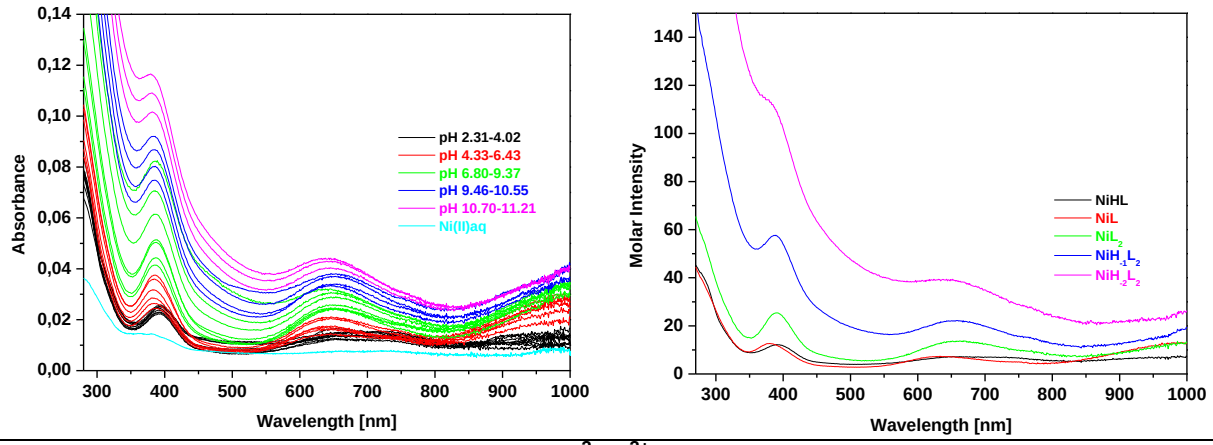
Not performed.

Figure S2. UV-vis spectra for Cu^{2+} complexes in a broad pH range. (a) Absorption spectrophotometric titration *vs* pH (b) electronic spectra of species. *I*=0.1M (KCl), T=25(2)°C. Simulated plots were generated in *HypSpec2014* program. Singular values shown by the program were taken into account as an indication value of how many coloured species are in the equilibria.
Spectra of Cu^{2+}_{aq} under the same conditions ware additionally shown for comparison purposes.

L^1/Ni^{2+}

Conditions; 0.1M KCl, [L]=4.6×10^{-3}M, [Ni^{2+}]=2.2×10^{-3}M, optical path: 1cm

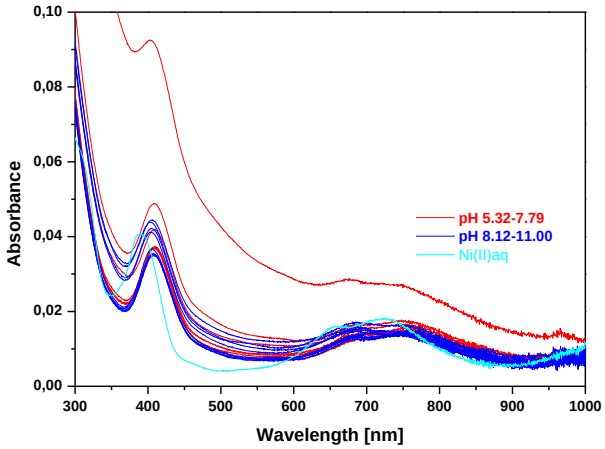
(a) **(b)**



L^2/Ni^{2+}

Conditions; 0.1M KCl, [L]=3.0×10^{-3}M, [Ni^{2+}]=3.2×10^{-3}M, optical path: 1cm, precipitation below pH 5.2

(a) **(b)**

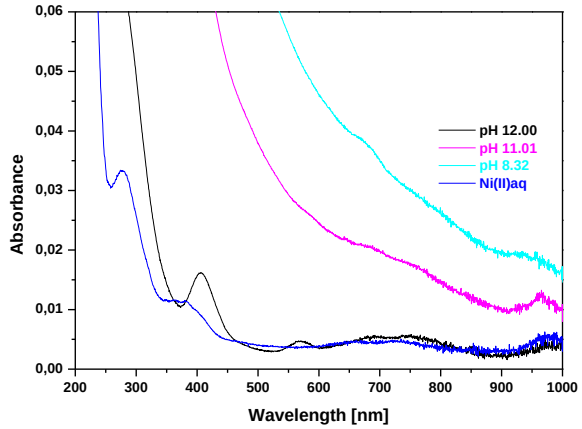


Not performed.

L^3/Ni^{2+}

Conditions; 0.1M KCl, [L]=2.0×10^{-3}M, [Ni^{2+}]=2.0×10^{-4}M, optical path: 1cm, precipitation below pH 12

(a) **(b)**



Not performed.

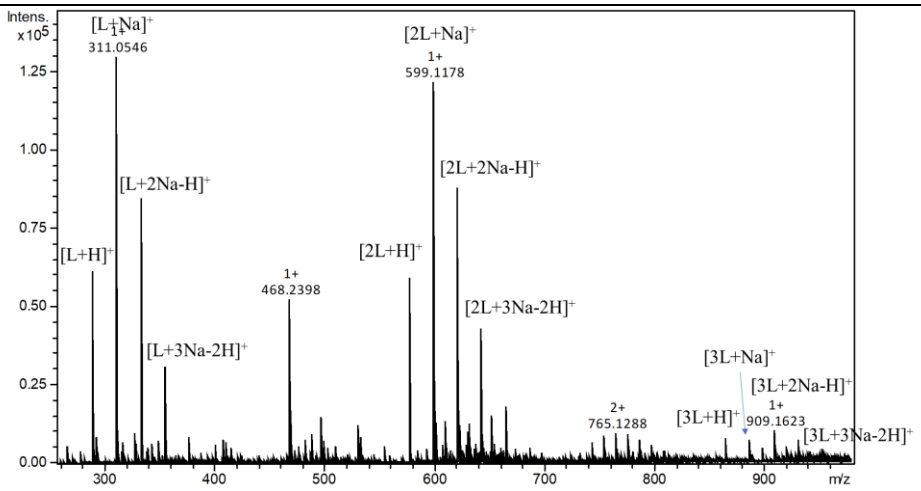
Figure S3. UV-vis spectra for Ni^{2+} complexes in a broad pH range. (a) Absorption spectrophotometric titration *vs* pH (b) electronic spectra of species. *I*=0.1M (KCl), T=25(2)°C. Simulated plots were generated in *HypSpec2014* program. Singular values shown by the program were taken into account as an indication value of how many coloured species are in the equilibria.
Spectra of Ni^{2+}_{aq} under the same conditions ware additionally shown for comparison purposes.

Table S1. Stability constants (logβ) of Cu^{2+} and Ni^{2+} complexes with L^1 and L^2 obtained by UV-vis titrations at 25°C and *I*=0.1M (KCl) based on absorbtion titrations shown on Figure S2 and S3.

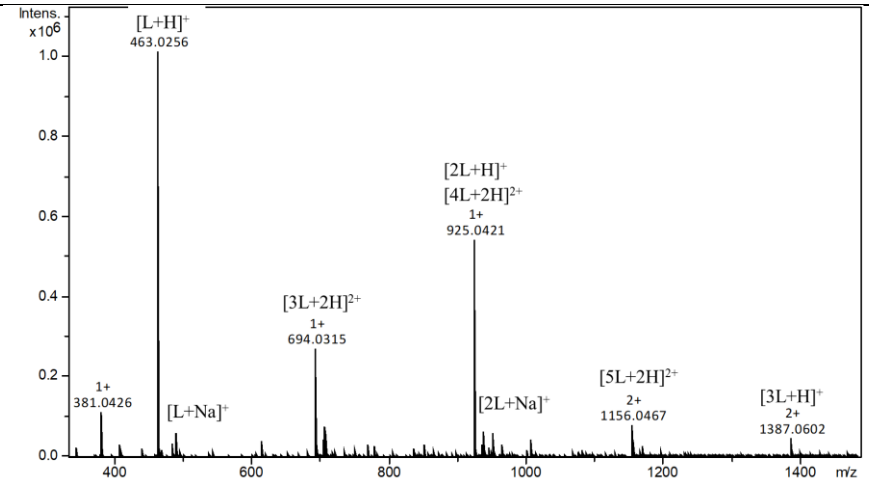
Species	L^1		L^2	
M:H:L	Cu^{2+}	Ni^{2+}	Cu^{2+}	Ni^{2+}
logβ[MH_2**L**]	-	-	26.05(2)	-
logβ[MH**L**]	19.09(4)	16.26(2)	19.80(2)	
logβ[M**L**]	nd	11.15(5)	10.79(5)	
logβ[MH_{-1}**L**]	-	-	-	
logβ[MH$\mathbf{L}_2$]	-	nd	-	
logβ[M$\mathbf{L}_2$]	18.64(2)	17.47(5)	15.2(5)	
logβ[$MH_{-1}\mathbf{L}_2$]	nd	8.75(5)	nd	
logβ[$MH_{-2}\mathbf{L}_2$]	-2.98(7)	-2.22(6)	-	
logβ[M_2**L**]	nd	-	-	
Polinuclear species	nd	-	nd	

Nd=not detected.

L[1]



L[2]



L[3]

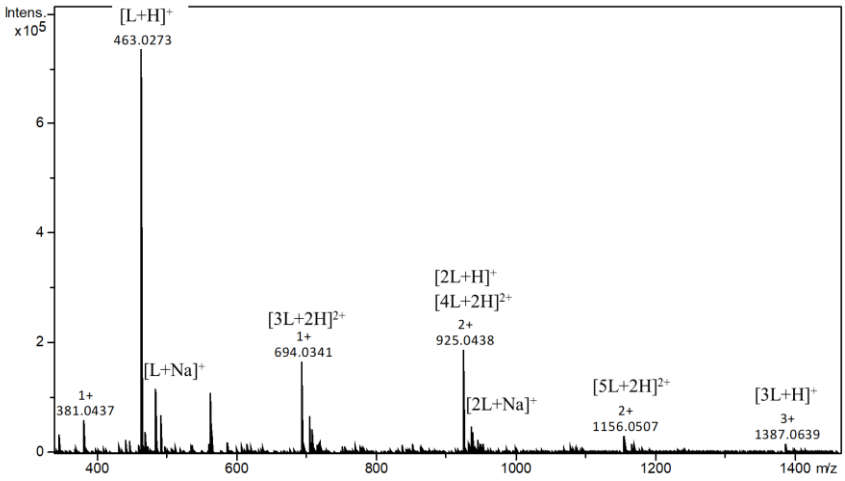


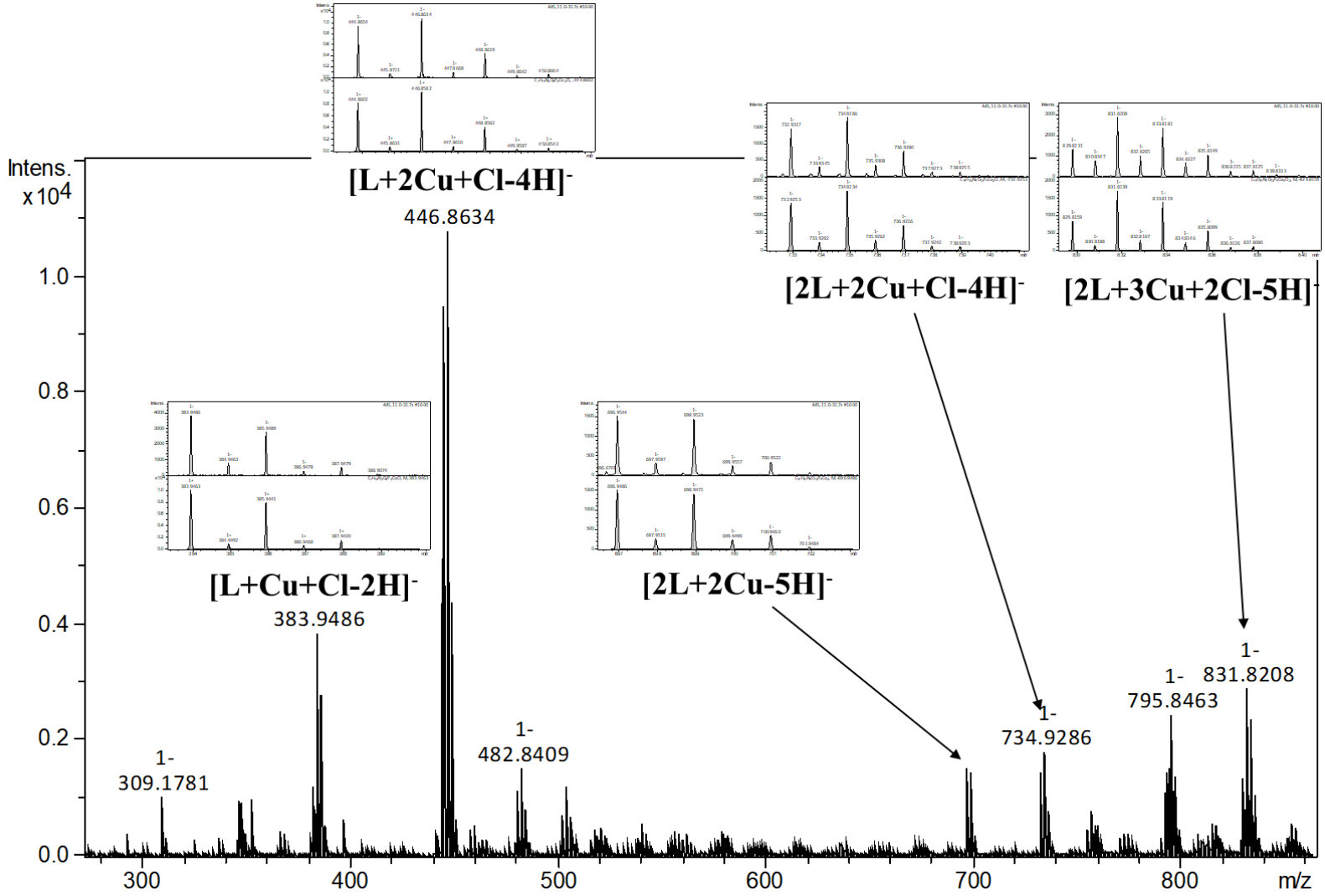
Figure S4. ESI-MS data for ligands alone.

Table S2. ESI-MS data for **L**^{1,2,3}/Cu^{2+}, Ni^{2+}, Ca^{2+} and Mg^{2+} complexes.

complex	calc.[a]	found [b]	err [ppm]	relative intensity [%]
		L^1 : Cu^{2+} (1-1)		
$[L+Cu+Cl-2H]^-$	383.9463	383.9486	5.99	36
$[L+2Cu+Cl-4H]^-$	444.8602	444.8654	11.69	100
$[2L+2Cu-5H]^-$	696.9486	696.9544	8.32	14
$[2L+2Cu+Cl-4H]^-$	732.9353	732.9317	-4.91	14
$[2L+3Cu+2Cl-5H]^-$	829.8159	829.8231	8.68	12
		L^1 : Cu^{2+} (2-1)		
$[L+Cu-3H]^-$	347.9707	347.9730	6.61	27
$[L+Cu+Cl-2H]^-$	383.9474	383.9482	2.08	62
$[L+2Cu+Cl-4H]^-$	444.8613	444.8655	9.44	6
$[2L+Cu-3H]^-$	636.0347	636.0382	5.50	46
$[2L+2Cu-5H]^-$	696.9486	696.9544	8.32	98
$[2L+2Cu+Cl-4H]^-$	732.9353	732.9282	-9.69	41
$[3L+3Cu-7H]^-$	1045.9266	1045.9322	5.35	21
		L^1 : Ni^{2+}		
$[L+Ni-3H]^-$	342.9764	342.9737	-7.87	66
$[2L+Ni-3H]^-$	631.0404	631.0376	-4.44	5
$[2L+Ni+Na-4H]^-$	653.0224	653.0193	-4.75	6
$[2L+Ni+K-4H]^-$	668.9963	668.9915	-7.17	12
		L^1 : Mg^{2+}		
$[L+Mg-3H]^-$	309.0261	309.0283	7.12	18
$[2L+Mg-3H]^-$	597.0901	597.0960	9.88	20
$[2L+Mg+K-4H]^-$	635.0460	635.0545	13.38	18
		L^1 : Ca^{2+}		
$[3L+Ca+Na-4H]^-$	635.0496	635.0506	1.57	11
		L^2 : Cu^{2+}		
$[2L+2Cu+2H]^{2+}$	523.9335	523.9382	8.97	2
$[3L+Cu]^{2+}$	724.4827	724.4888	8.42	5
$[3L+2Cu-2H]^{2+}$	754.9397	754.9467	9.27	2
$[2L+Cu-H]^+$	985.9459	985.9563	10.55	2
		L^2 : Ni^{2+}		
$[L+Ni-H]^+$ $[2L+2Ni-2H]^{2+}$	518.9393	518.9463	13.49	47
$[L+Ni-H+H_2O]^+$	536.9499	536.9567	12.66	25
		L^2 : Mg^{2+}		
$[L+Mg-H]^+$ $[2L+2Mg-2H]^{2+}$	484.9890	484.9971	16.70	30
$[L+Mg-H+H_2O]^+$	502.9996	503.0035	7.75	40
		L^2 : Ca^{2+}		
$[L+Ca-H]^+$	500.9665	500.9715	9.98	30
$[L+Mg-H+H_2O]^+$	518.9771	518.9827	10.79	15
		L^3 : Cu^{2+}		
$[L+Cu-H]^+$	523.9335	523.9428	17.75	14
$[L+Cu+Na-2H]^+$	545.9155	545.9241	15.75	8
		L^3 : Ni^{2+}		
$[L+Ni-H]^+$	518.9393	518.9446	10.21	3

[a] monoisotopic mass of the indicated ion formed by the ligand calculated by Compass DataAnalysis 4.2
[b] monoisotopic mass found experimentally on a compact™ mass spectrometer (Bruker Daltonics. Bremen. Germany.

Cu(II)/**L**[1] (1-1)


Intens.
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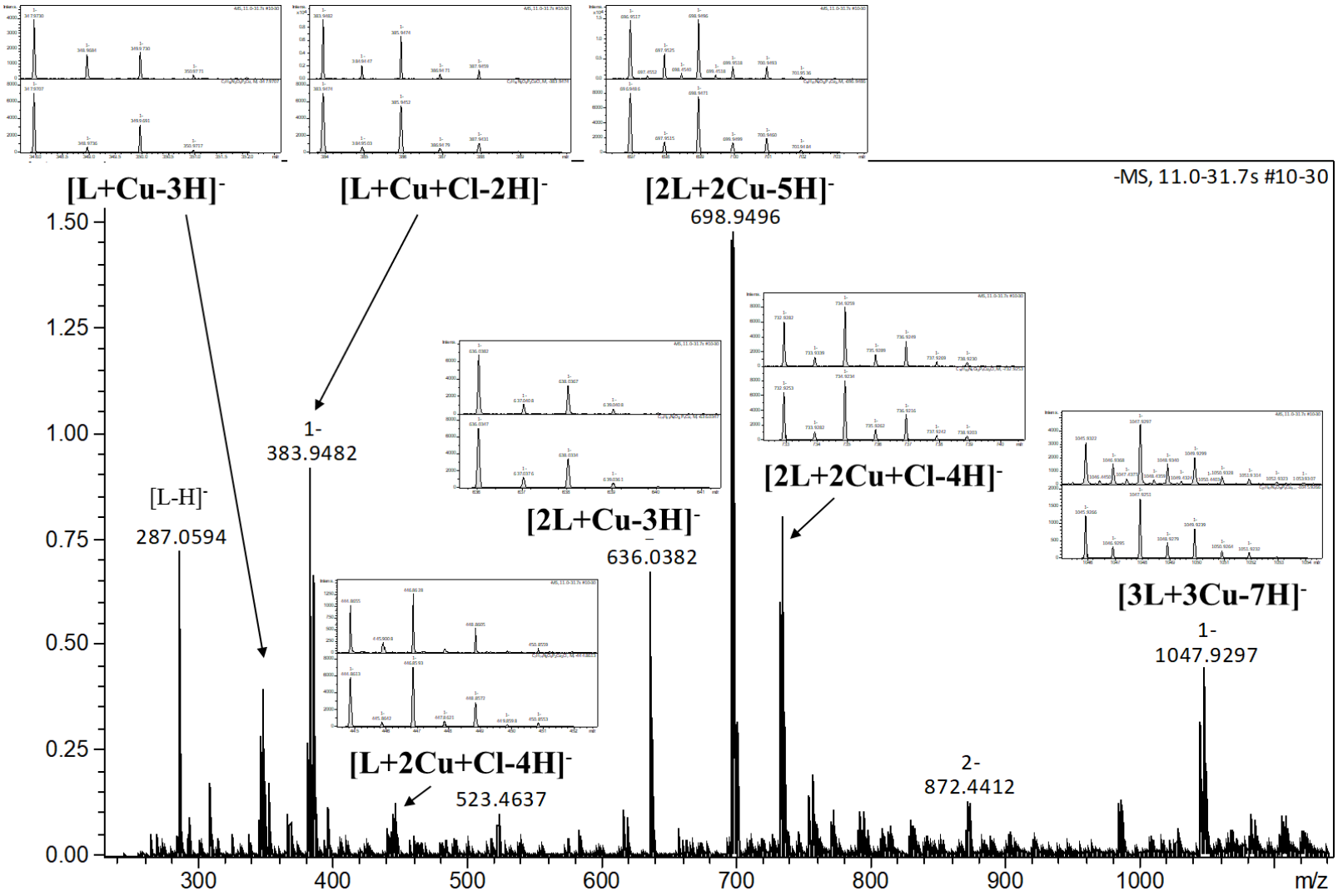
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800
m/z
[L+2Cu+Cl-4H]-
446.8634
[2L+2Cu+Cl-4H]-
[2L+3Cu+2Cl-5H]-
[L+Cu+Cl-2H]-
383.9486
[2L+2Cu-5H]-
1-
309.1781
1-
482.8409
1-
734.9286
1-
795.8463
1-
831.8208

Cu(II)/**L**[1] (2-1)


-MS, 11.0-31.7s #10-30
[L+Cu-3H]-
[L+Cu+Cl-2H]-
[2L+2Cu-5H]-
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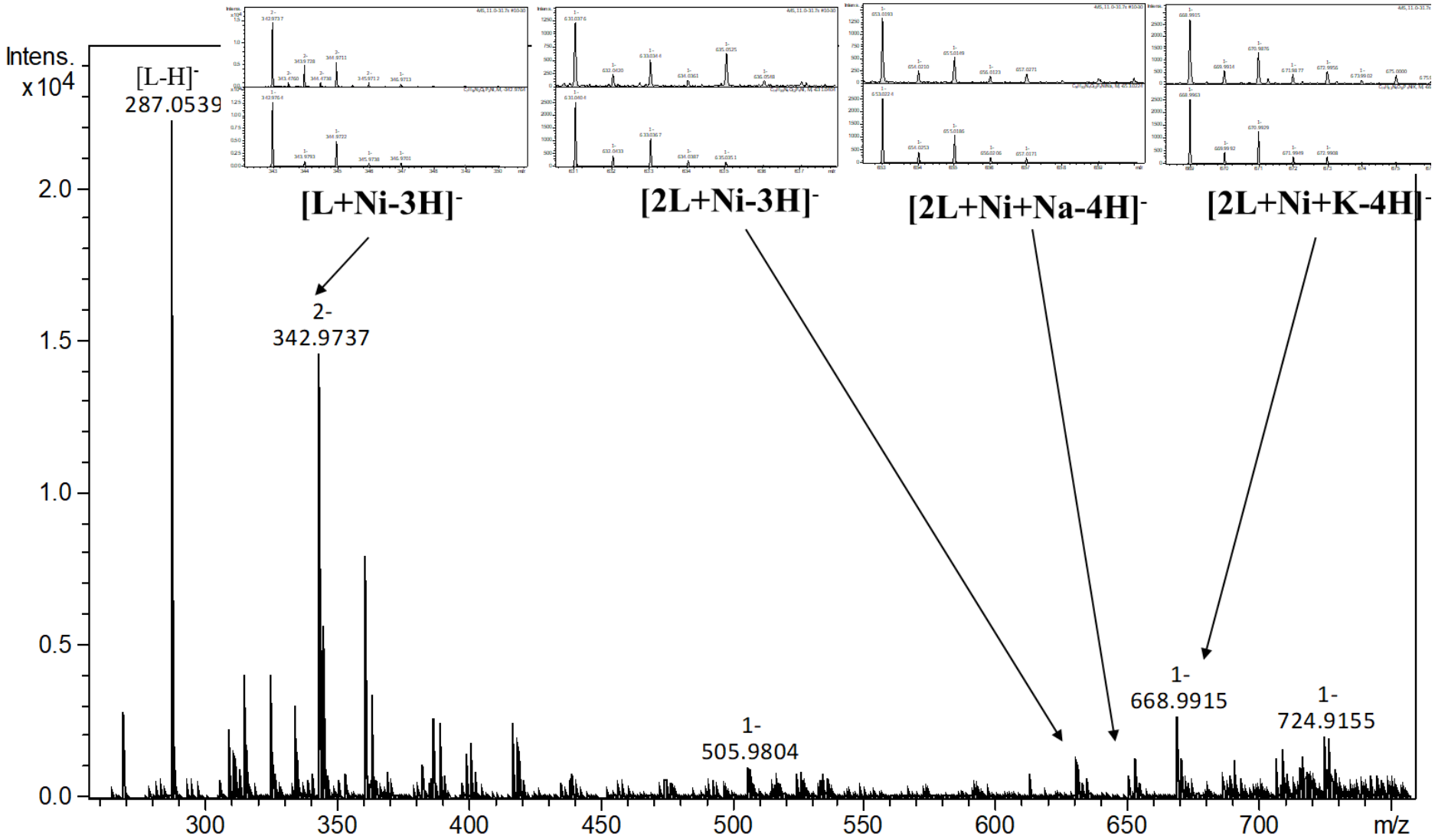
1-
383.9482
[L-H]-
287.0594
[2L+Cu-3H]-
636.0382
[2L+2Cu+Cl-4H]-
[3L+3Cu-7H]-
1-
1047.9297
[L+2Cu+Cl-4H]-
523.4637
2-
872.4412
1.50
1.25
1.00
0.75
0.50
0.25
0.00
300
400
500
600
700
800
900
1000
m/z

Ni(II)/**L**[1]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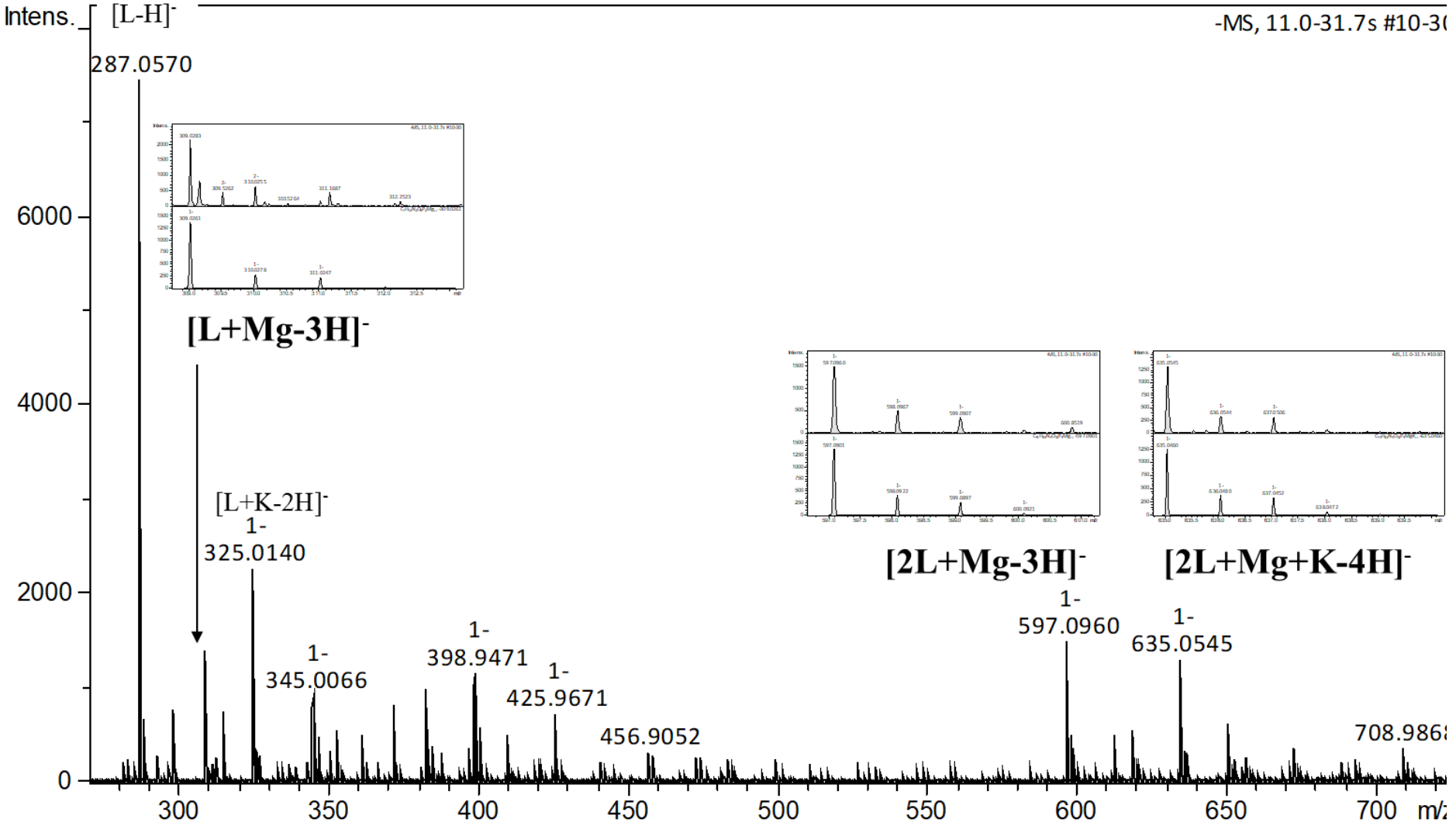
2.0
1.5
1.0
0.5
0.0
[L-H]-
287.0539
[L+Ni-3H]-
[2L+Ni-3H]-
[2L+Ni+Na-4H]-
[2L+Ni+K-4H]-
2-
342.9737
1-
505.9804
1-
668.9915
1-
724.9155
300
350
400
450
500
550
600
650
700
m/z

Mg(II)/**L**[1]


Intens.
[L-H]-
287.0570
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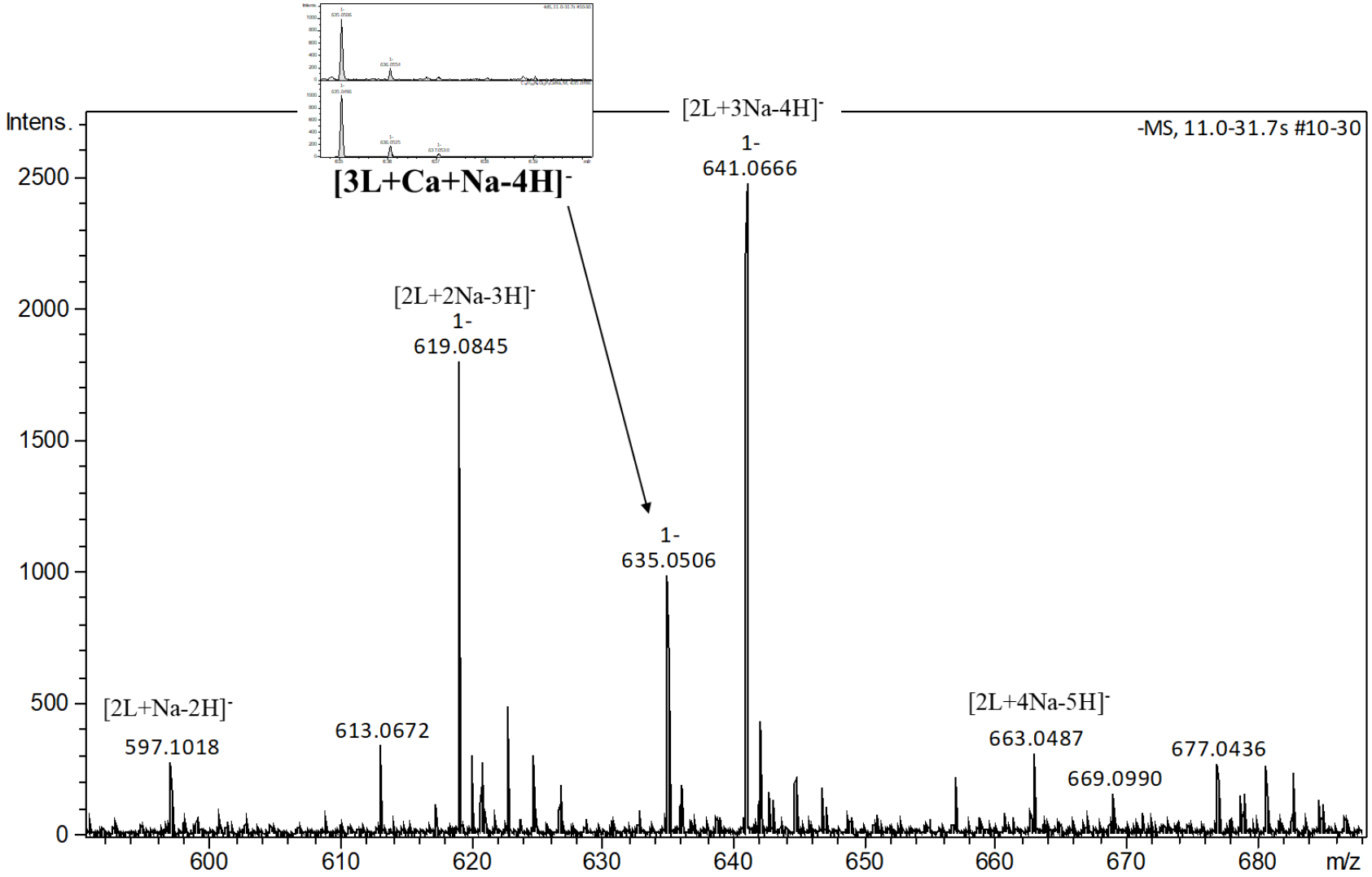
6000
4000
2000
0
[L+Mg-3H]-
[L+K-2H]-
1-
325.0140
1-
345.0066
1-
398.9471
1-
425.9671
456.9052
[2L+Mg-3H]-
[2L+Mg+K-4H]-
1-
597.0960
1-
635.0545
708.986
300
350
400
450
500
550
600
650
700
m/z

Ca(II)/**L**[1]


Intens.
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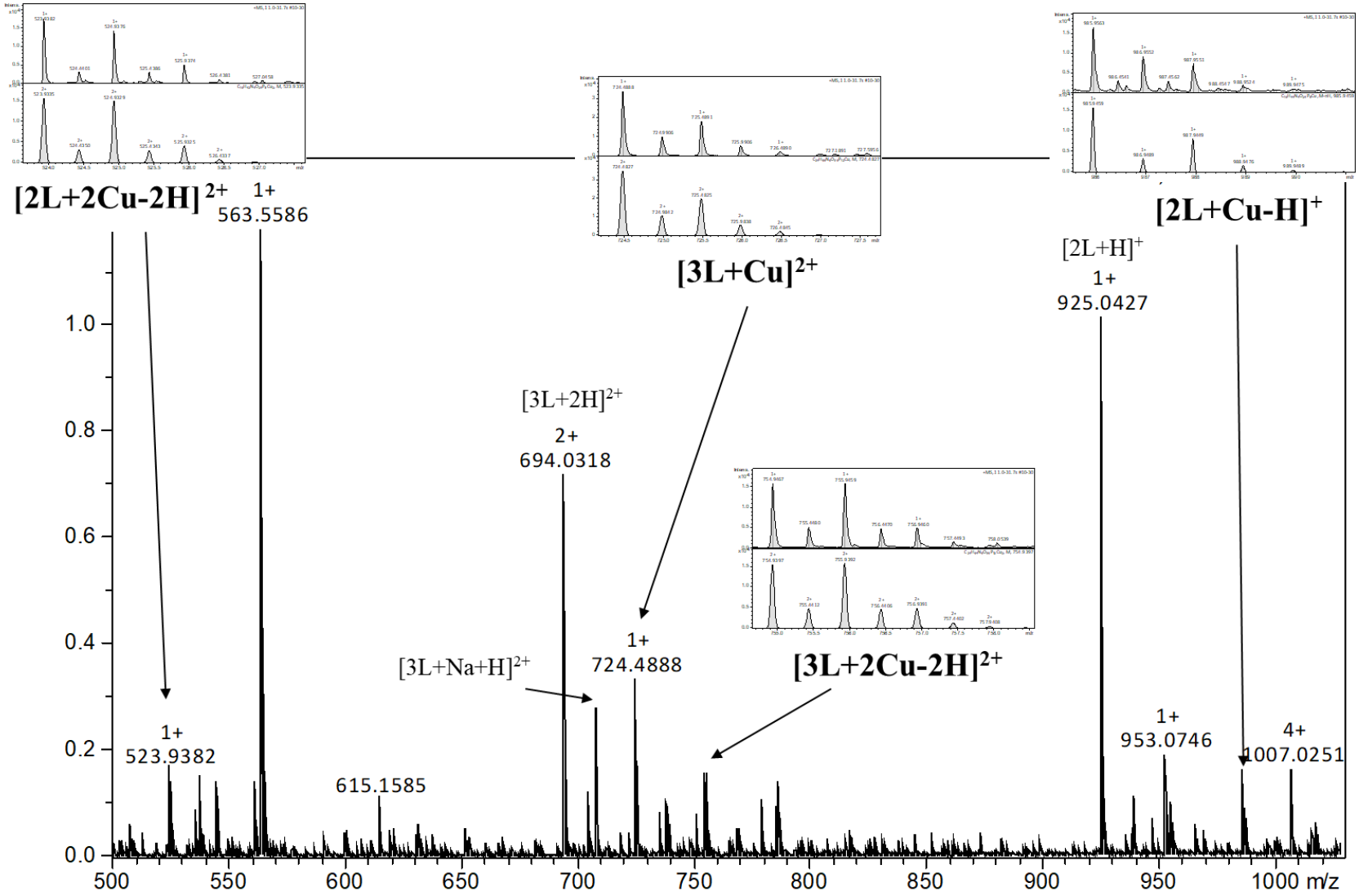
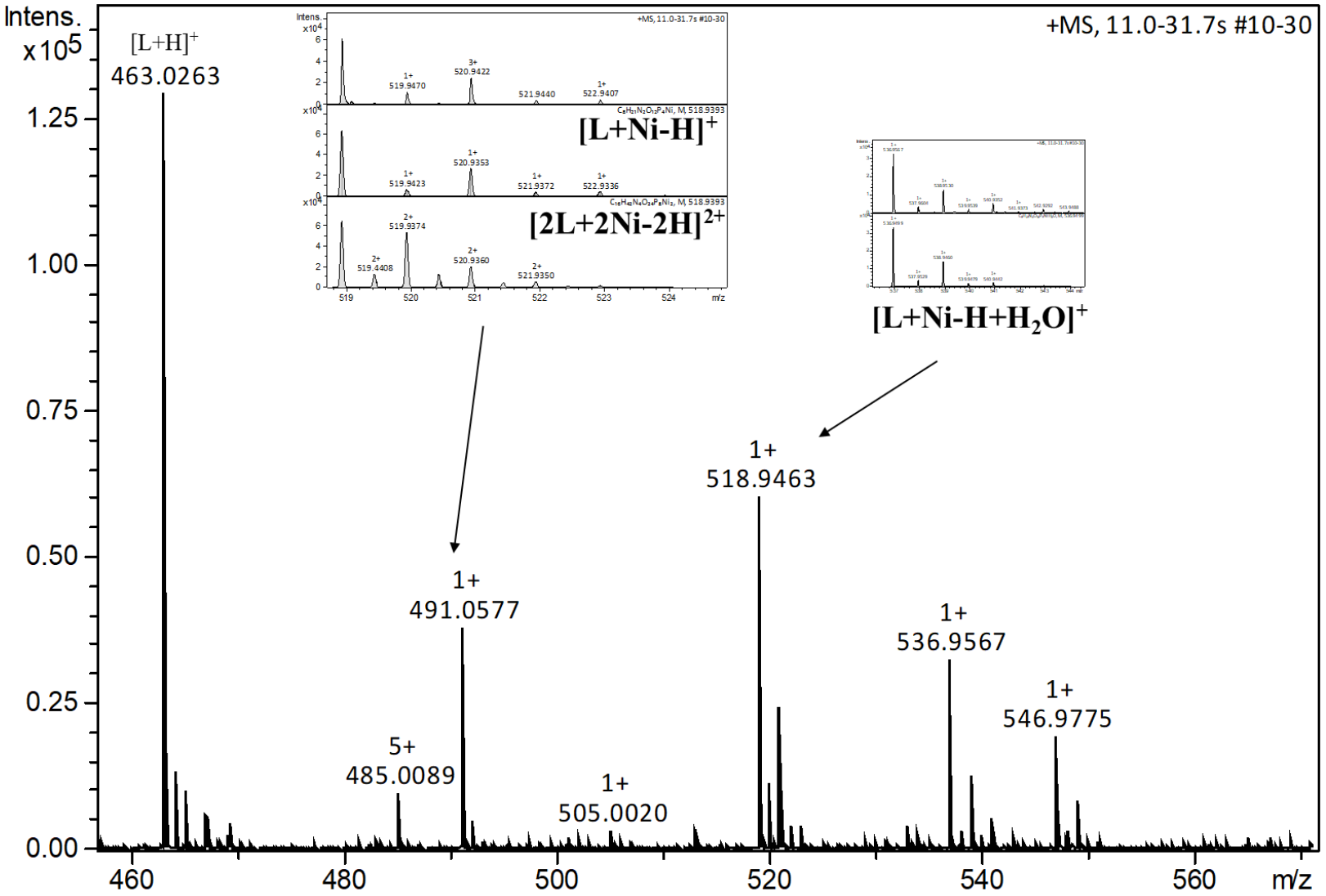
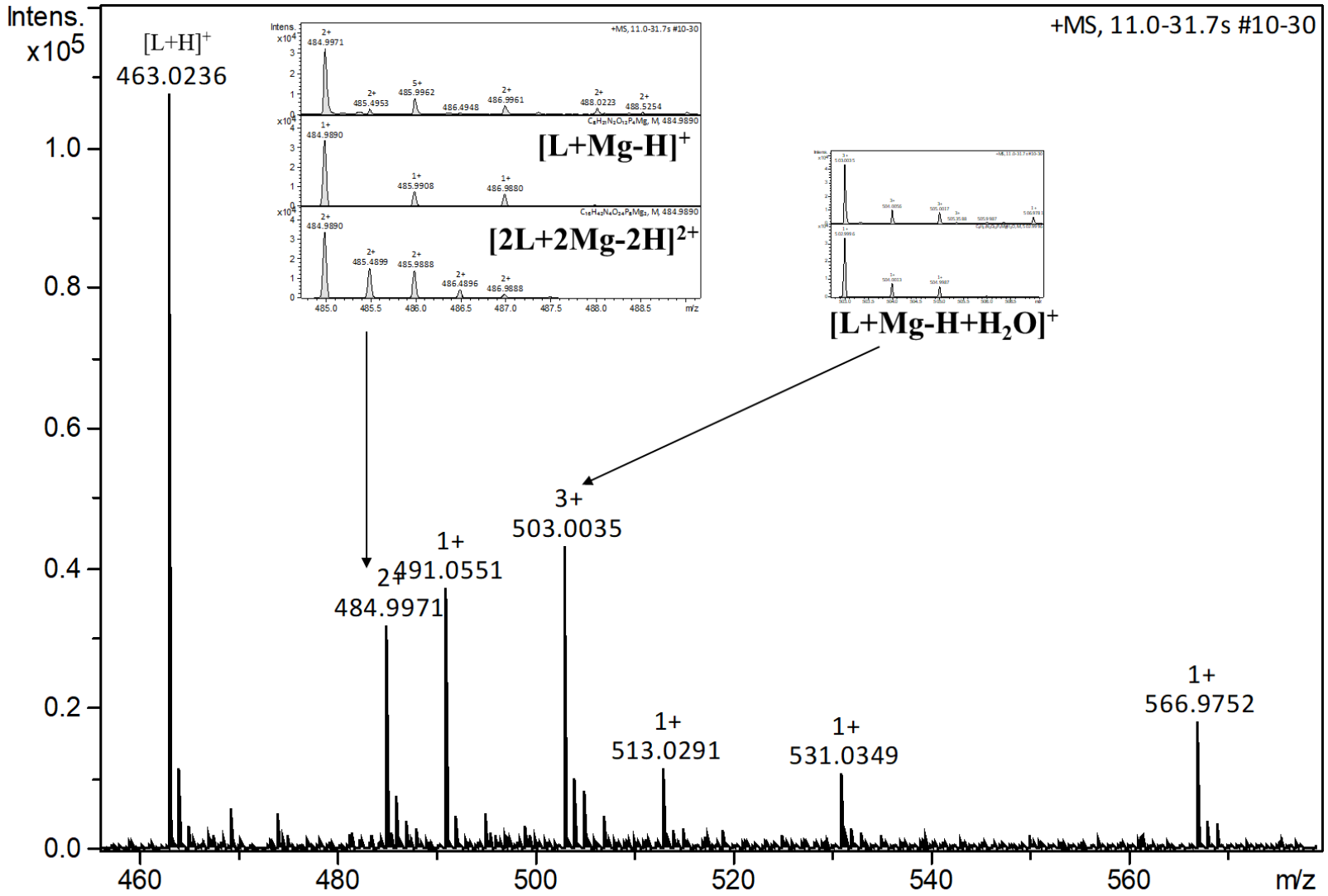
2000
1500
1000
500
0
600
610
620
630
640
650
660
670
680
m/z
-MS, 11.0-31.7s #10-30
[3L+Ca+Na-4H]-
[2L+3Na-4H]-
1-
641.0666
[2L+2Na-3H]-
1-
619.0845
1-
635.0506
[2L+Na-2H]-
597.1018
613.0672
[2L+4Na-5H]-
663.0487
669.0990
677.0436

Cu(II)/**L**^{2}

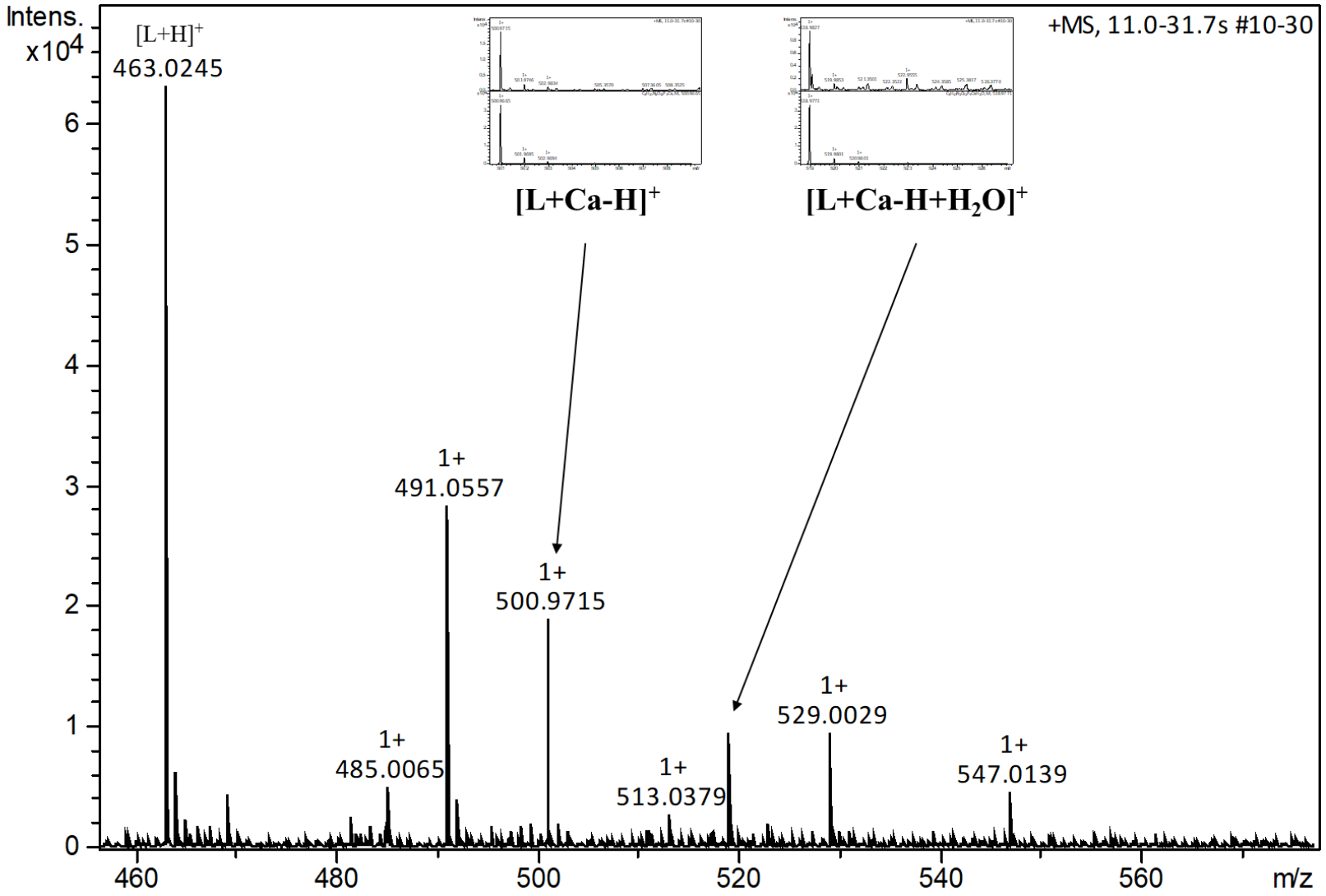

[2L+2Cu-2H]2+
1+
563.5586
[3L+Cu]2+
[2L+Cu-H]+
[2L+H]+
1+
925.0427
[3L+2H]2+
2+
694.0318
[3L+2Cu-2H]2+
[3L+Na+H]2+
1+
724.4888
1+
523.9382
615.1585
1+
953.0746
4+
1007.0251
1.0
0.8
0.6
0.4
0.2
0.0
500
550
600
650
700
750
800
850
900
950
1000 m/z


Intens.
x10^5
1.25
1.00
0.75
0.50
0.25
0.00
460
480
500
520
540
560
m/z
[L+H]+
463.0263
+MS, 11.0-31.7s #10-30
[L+Ni-H]+
[2L+2Ni-2H]2+
[L+Ni-H+H2O]+
1+
518.9463
1+
491.0577
5+
485.0089
1+
505.0020
1+
536.9567
1+
546.9775

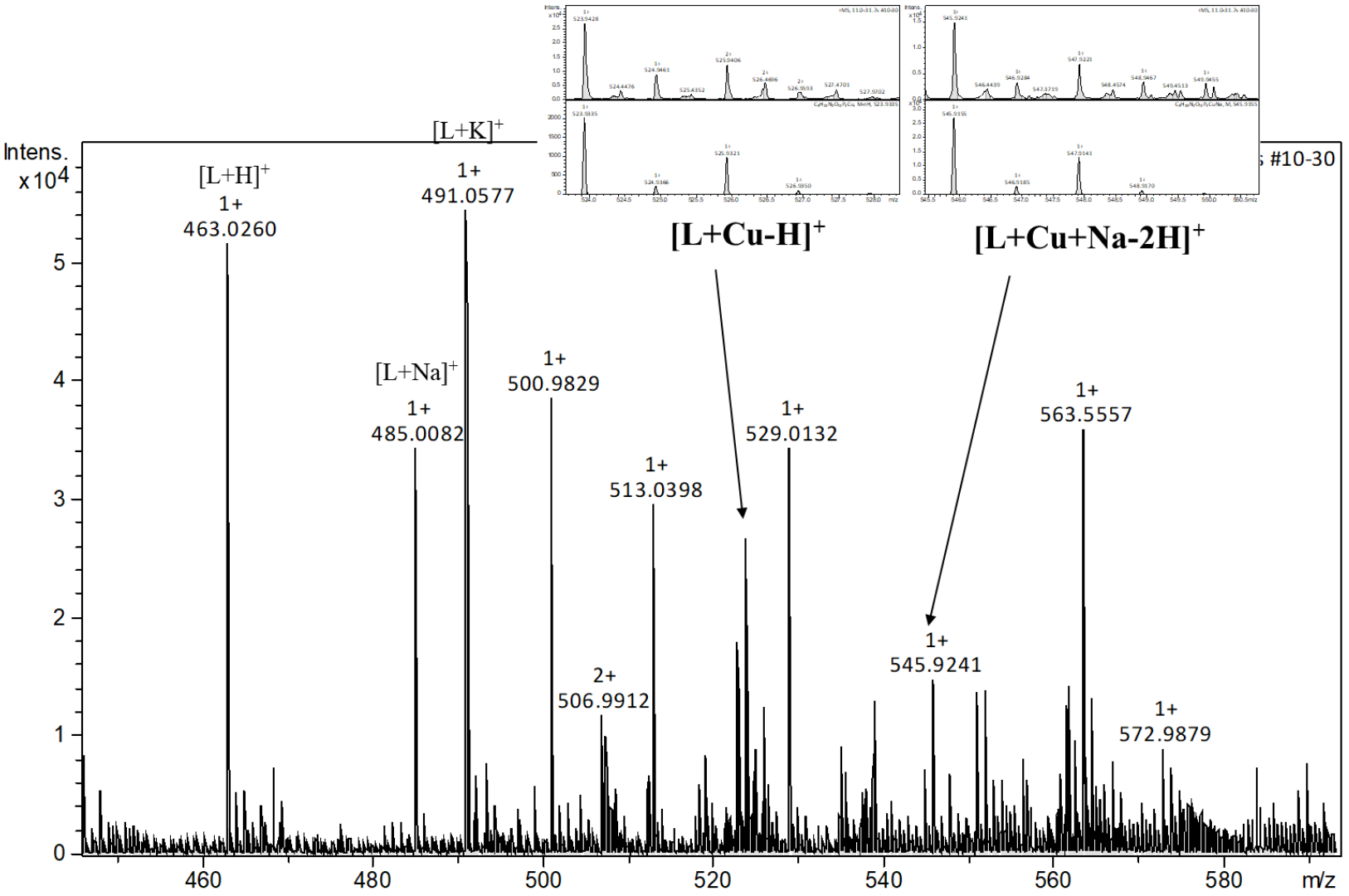
Mg(II)/**L**^{2}


Intens.
x10^5
1.0
0.8
0.6
0.4
0.2
0.0
460
480
500
520
540
560
m/z
[L+H]+
463.0236
+MS, 11.0-31.7s #10-30
[L+Mg-H]+
[2L+2Mg-2H]2+
[L+Mg-H+H2O]+
3+
503.0035
1+
491.0551
2+
484.9971
1+
513.0291
1+
531.0349
1+
566.9752

Ca(II)/**L**^{2}


Intens.
x10^4
[L+H]+
463.0245
+MS, 11.0-31.7s #10-30
[L+Ca-H]+
[L+Ca-H+H2O]+
1+
491.0557
1+
500.9715
1+
485.0065
1+
513.0379
1+
529.0029
1+
547.0139
0
1
2
3
4
5
6
460
480
500
520
540
560
m/z

Cu(II)/**L**^{3}


Intens.
x10^4
[L+H]+
1+
463.0260
[L+K]+
1+
491.0577
[L+Na]+
1+
485.0082
1+
500.9829
1+
513.0398
1+
529.0132
2+
506.9912
1+
545.9241
1+
563.5557
1+
572.9879
[L+Cu-H]+
[L+Cu+Na-2H]+
#10-30
0
1
2
3
4
5
460
480
500
520
540
560
580
m/z

Ni(II)/**L**^{3}

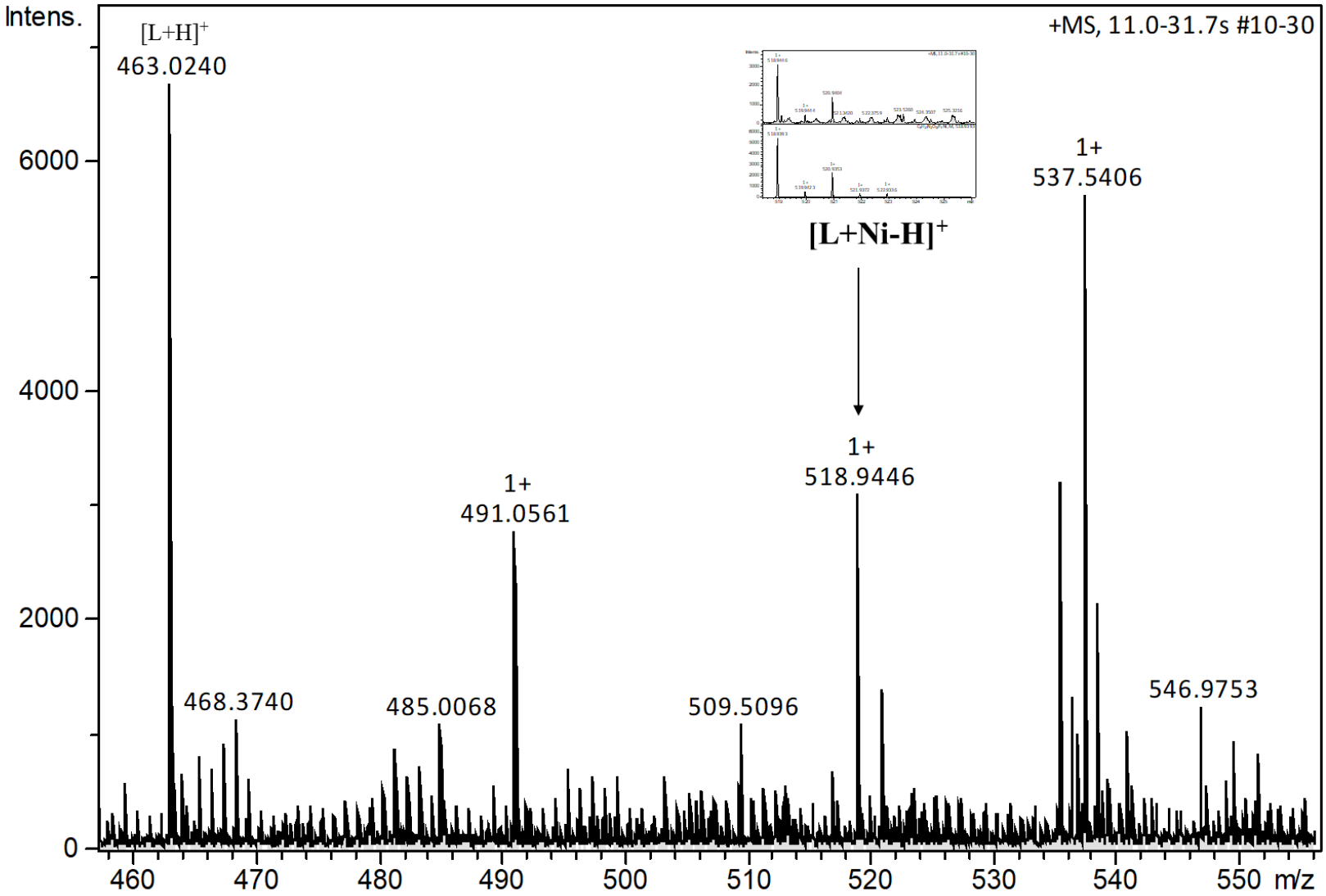
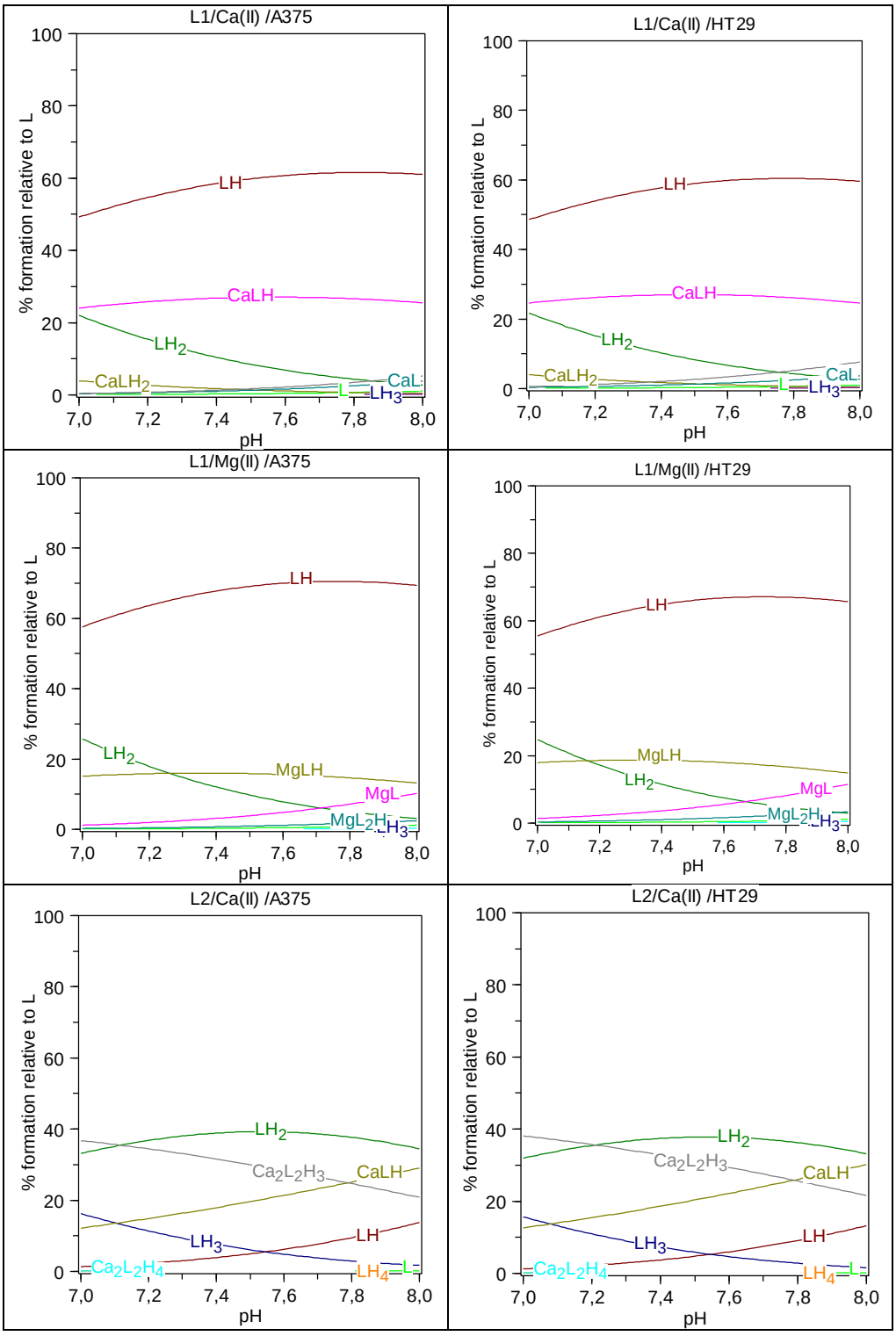


Figure S5. ESI-MS data for $M^{2+}/\mathbf{L}^{1-2}$ complexes. Enlarged pictures: experimental (higher panel) *vs* simulated (lower panel) isotopic pattern of the complexes.

Table S3. Experimentally measured molar extinction coefficients (ε) and absorbance maxima (λmax) for ligands which concentration was determined by UV-vis. Background solution: HEPES buffer (50 mM. I = 0.16 M). path length = 1 cm. T = 25°C.

Ligand	λmax (nm)	ε (mM^{-1} cm^{-1})
L^1	229	61
L^2	230	390
L^3	232	708


L1/Ca(II) /A375
L1/Ca(II) /HT29
L1/Mg(II) /A375
L1/Mg(II) /HT29
L2/Ca(II) /A375
L2/Ca(II) /HT29
% formation relative to L
pH
LH
CaLH
LH2
CaLH2
MgLH
MgL
LH3
LH4
Ca2L2H3
Ca2L2H4

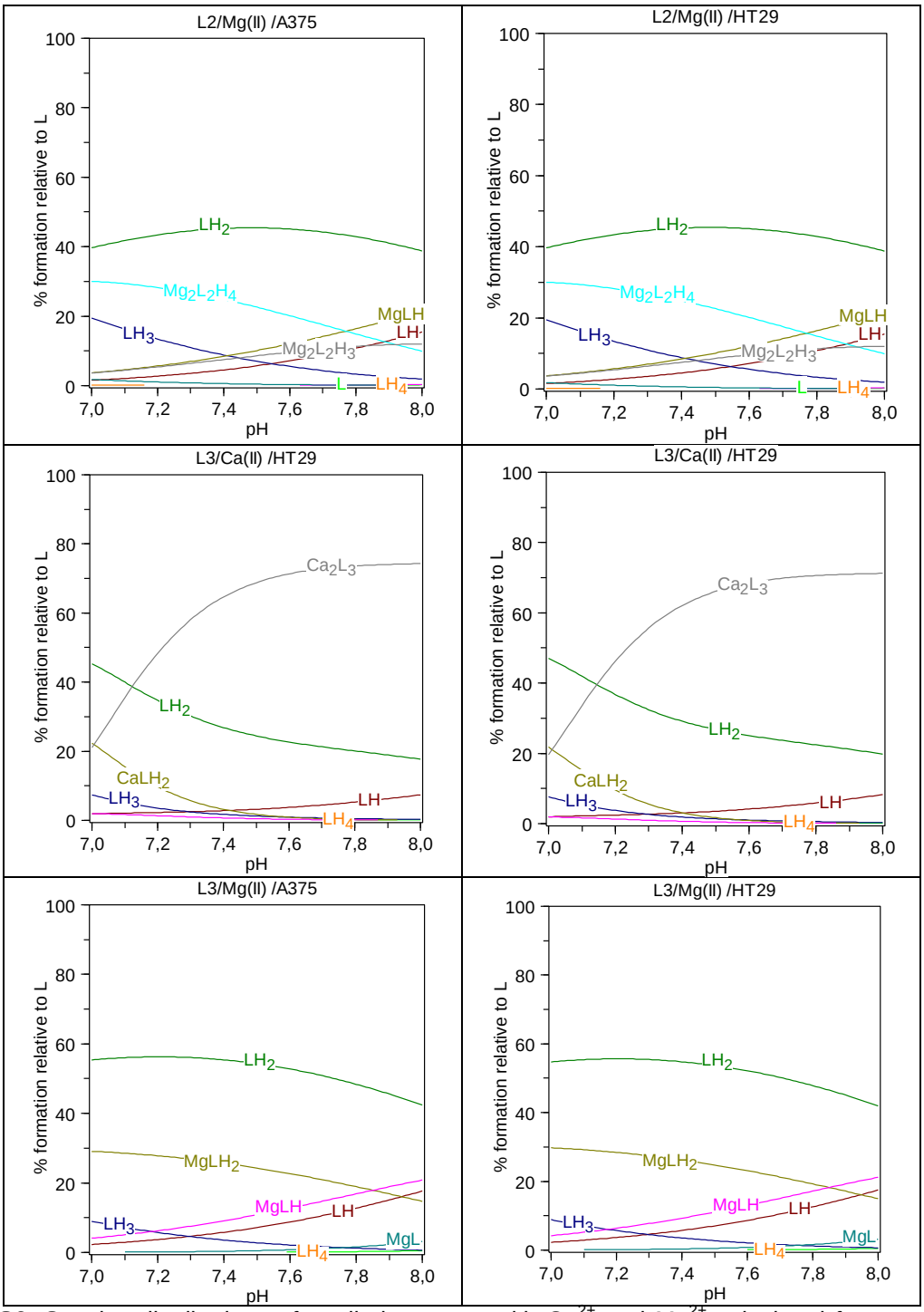
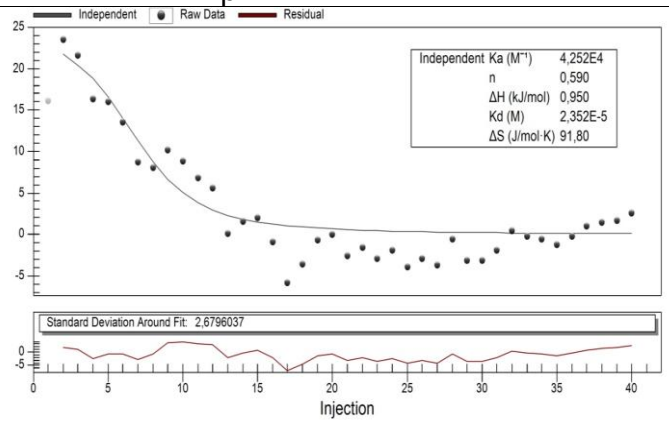
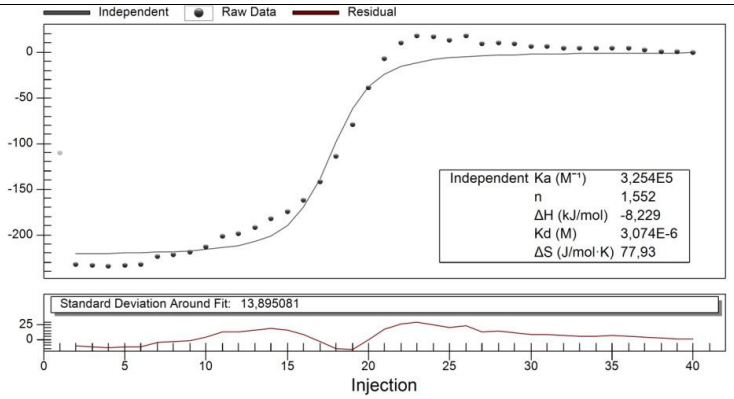
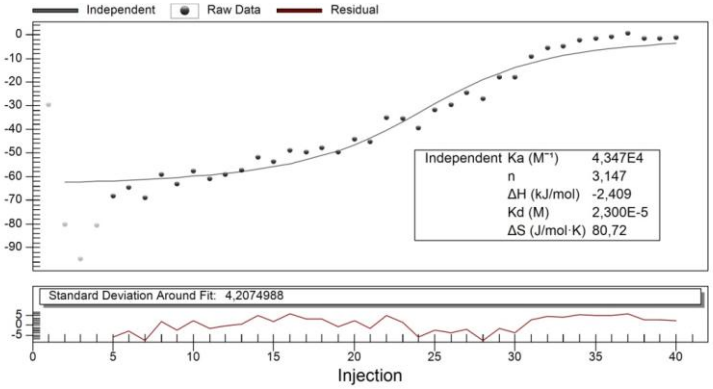
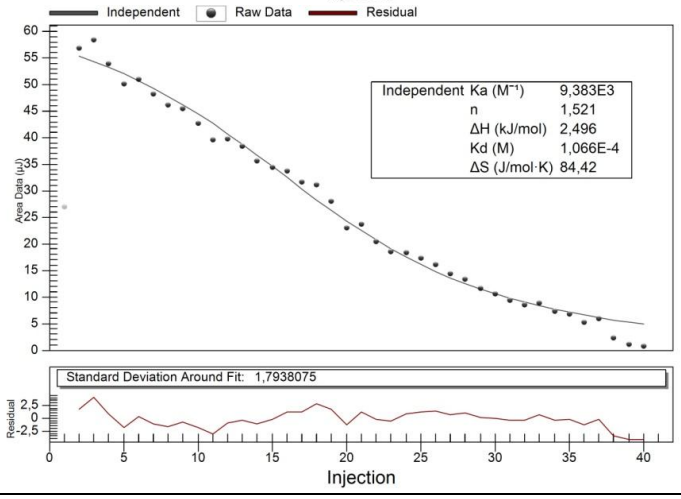
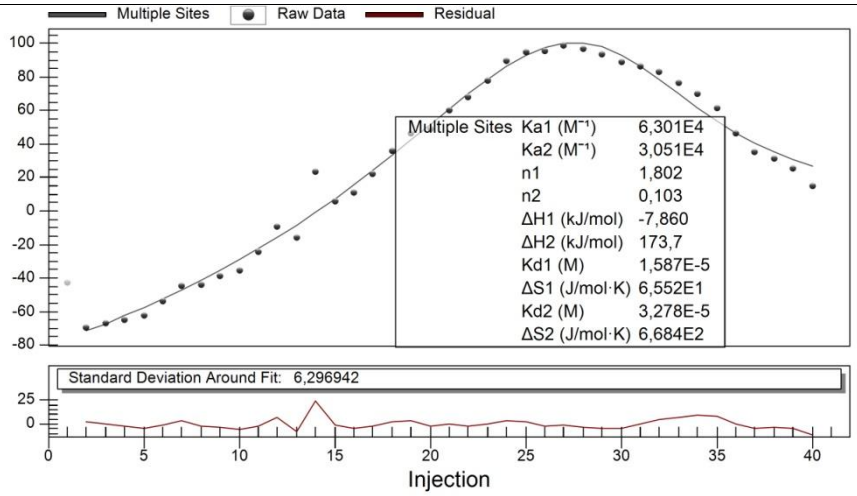
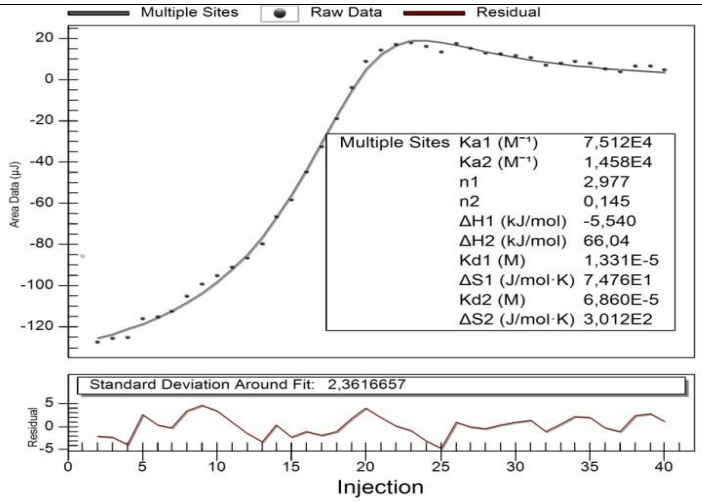
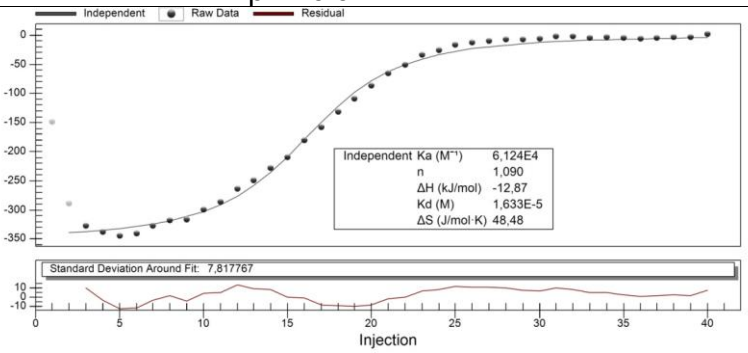
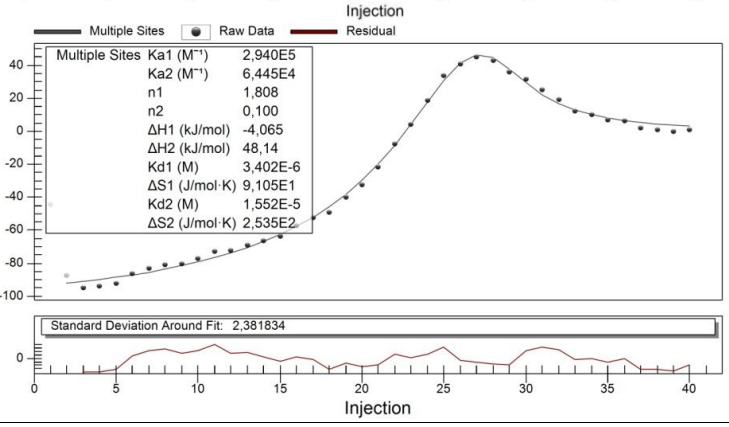


Figure S6. Species distributions of studied systems with Ca^{2+} and Mg^{2+} calculated from equilibrium constants (Table 2, 3) using IC_{50} concentrations conditions in pH range 7.0-8.0

pH 7.4	
$Ca^{2+} \rightarrow L^1$	
$Ca^{2+} \rightarrow L^2$	
$Ca^{2+} \rightarrow L^3$	
$Mg^{2+} \rightarrow L^1$	

$Mg^{2+} \rightarrow L^2$	
$Mg^{2+} \rightarrow L^3$	
pH 10.0	
$Ca^{2+} \rightarrow L^1$	
$Ca^{2+} \rightarrow L^2$	

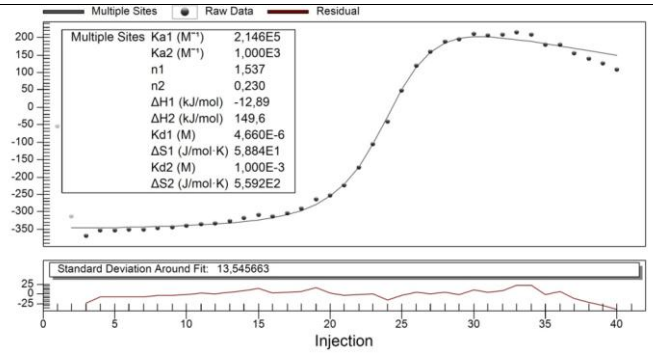
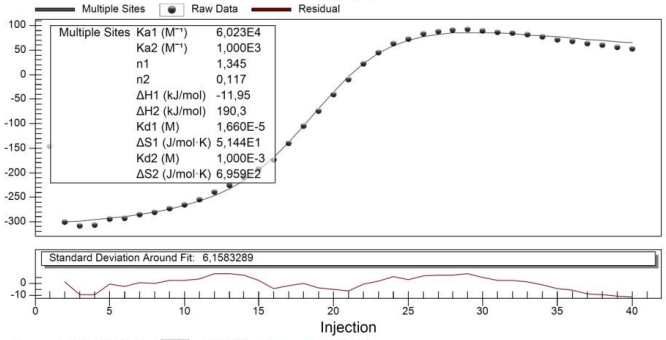
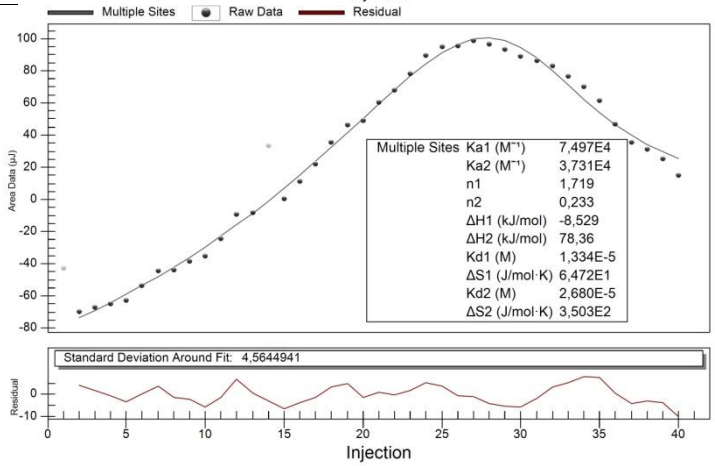
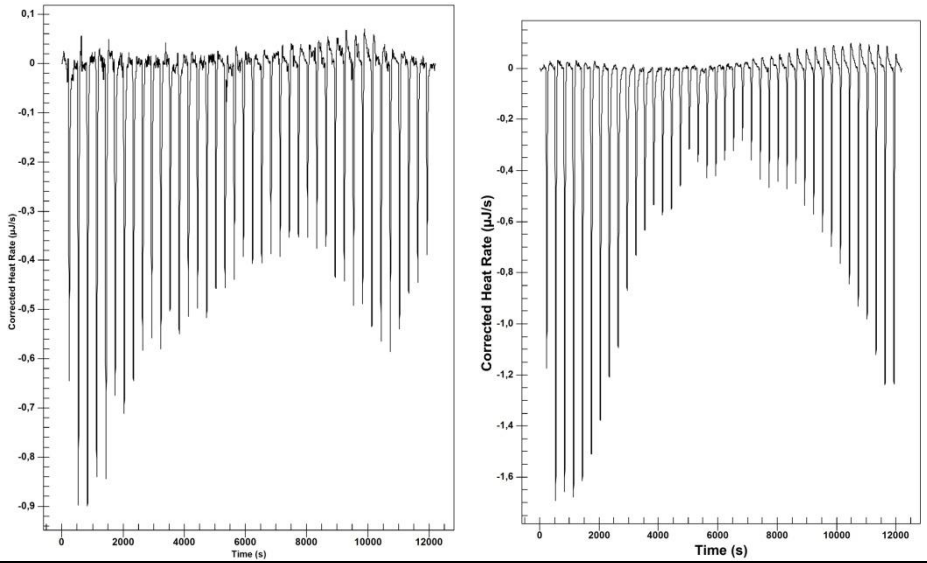
$Ca^{2+} \rightarrow L^3$	
$Mg^{2+} \rightarrow L^1$	
$Mg^{2+} \rightarrow L^2$	
$Mg^{2+} \rightarrow L^3$	

Figure S7. Standard deviation around ITC fit for studied systems. Obtained ITC data for titrations of $Mg^{2+} \rightarrow L^3$.

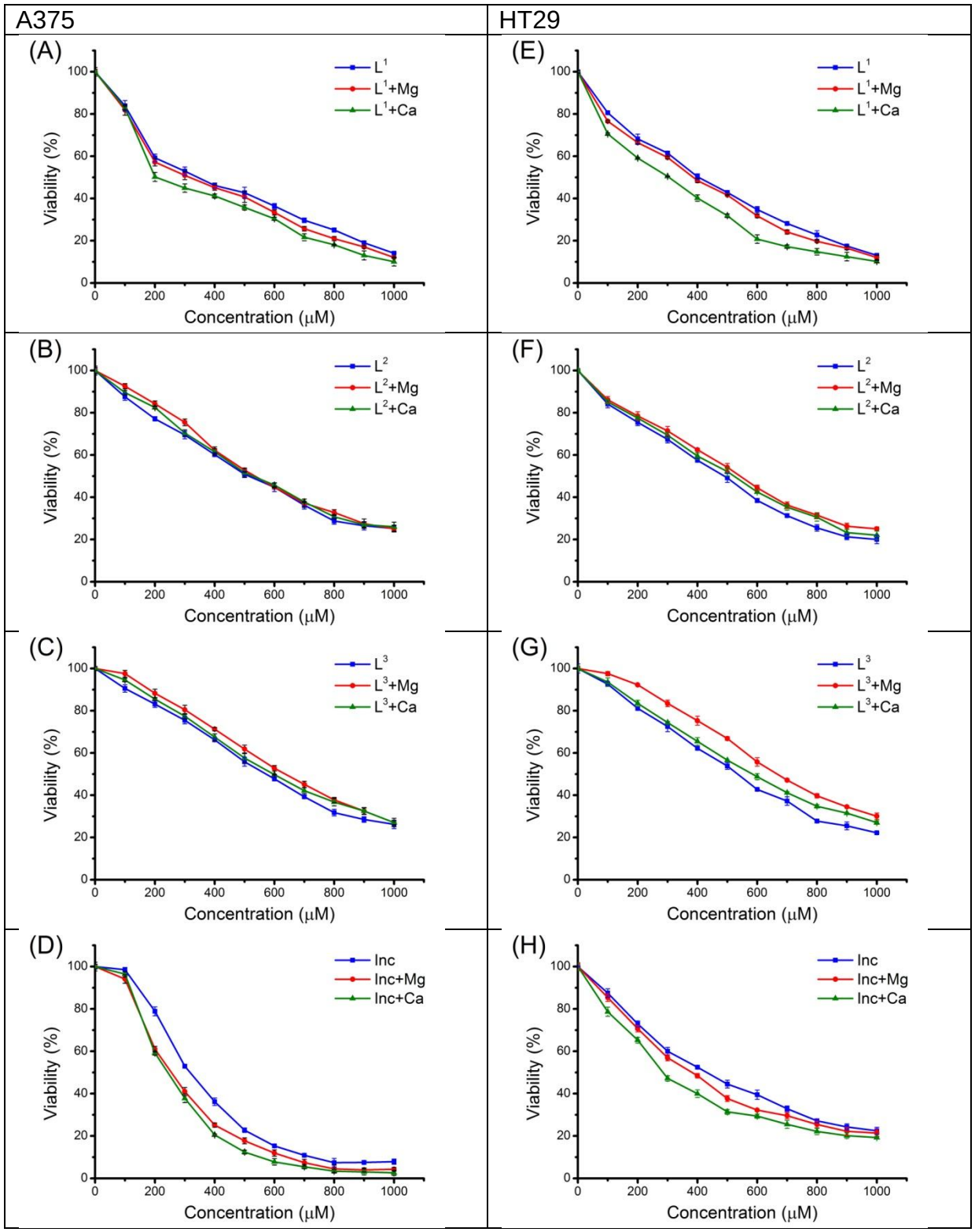
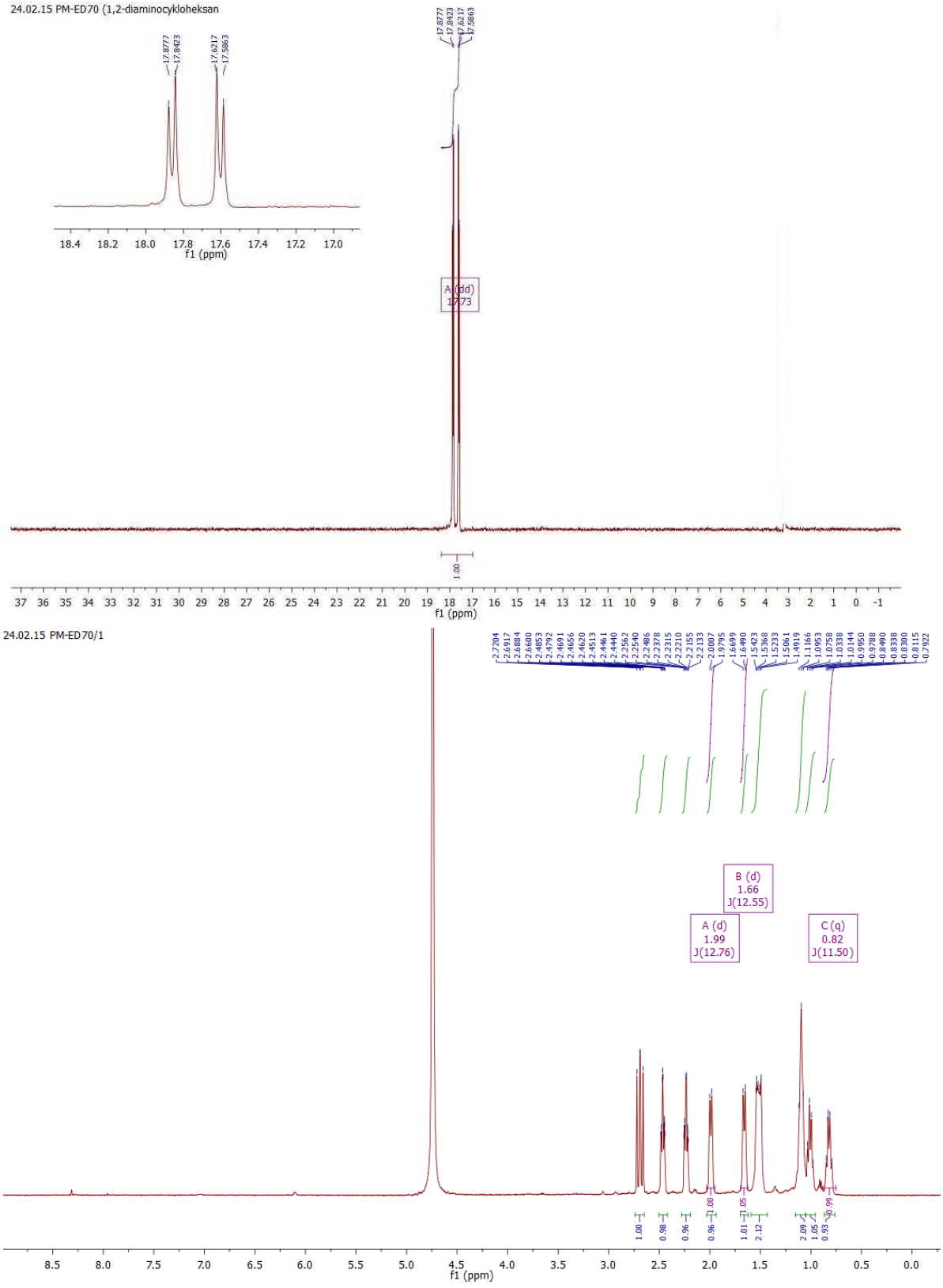
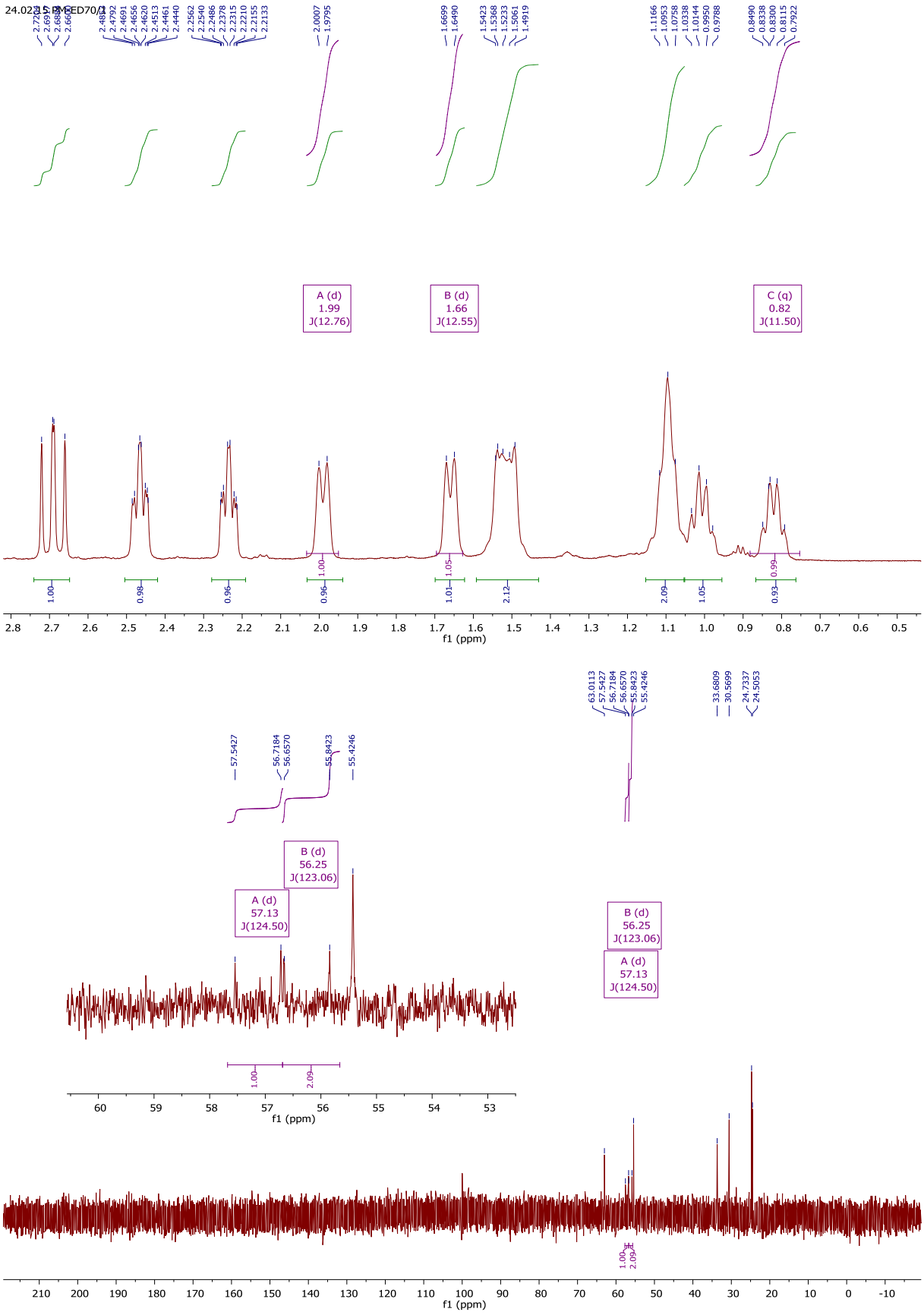
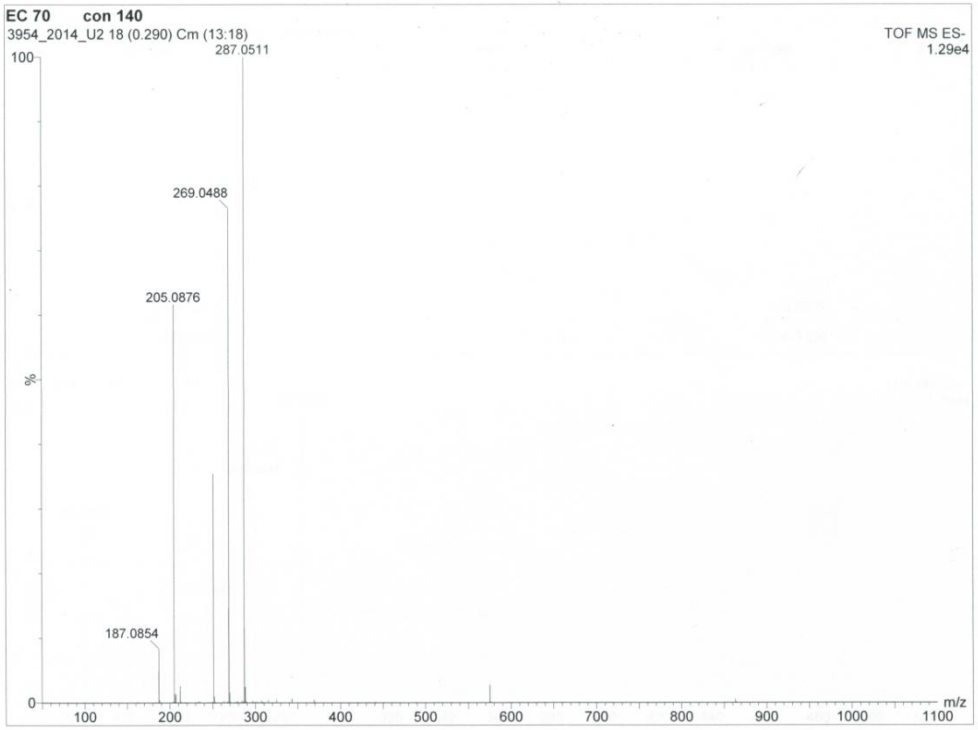


Figure S8. Effects of bisphosphonates and Mg^{2+} or Ca^{2+} bisphosphonates complexes on the viability of melanoma A375 cells (A-D) and HT29 cells (E-H). The cells were treated *in vitro* with various concentrations of bisphosphonates for 48 h. Each value represents the mean ± SD for three independent experiments.

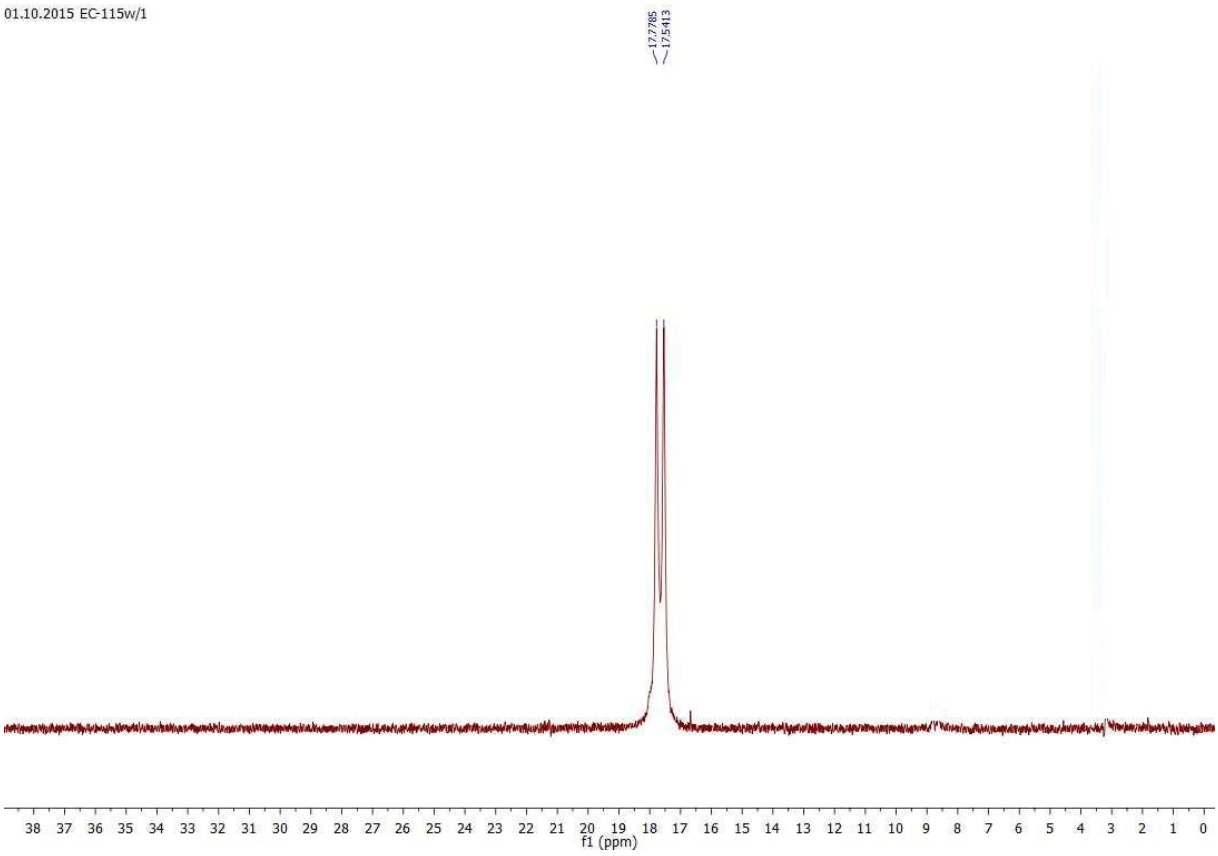
Cyclohexane-1-amino-2-aminomethylenebisphosphonic acid L^1:

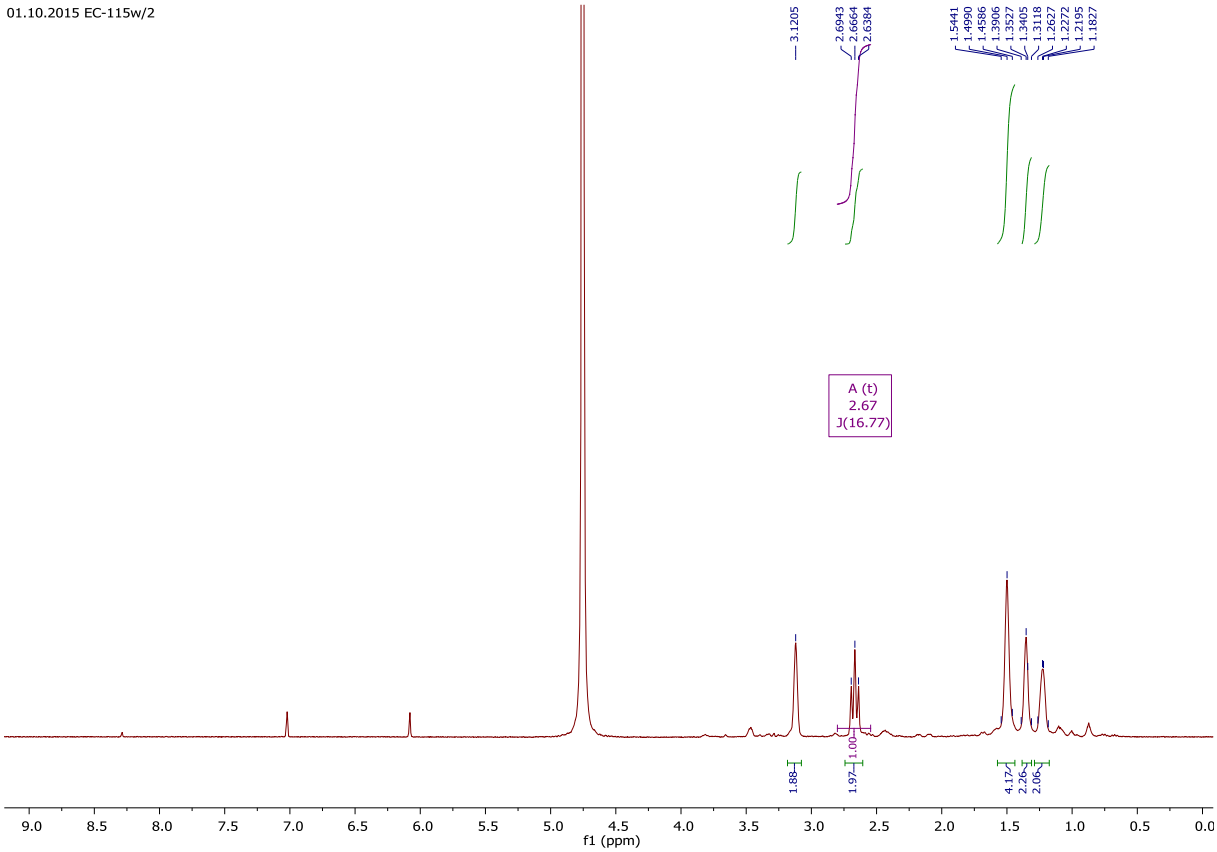


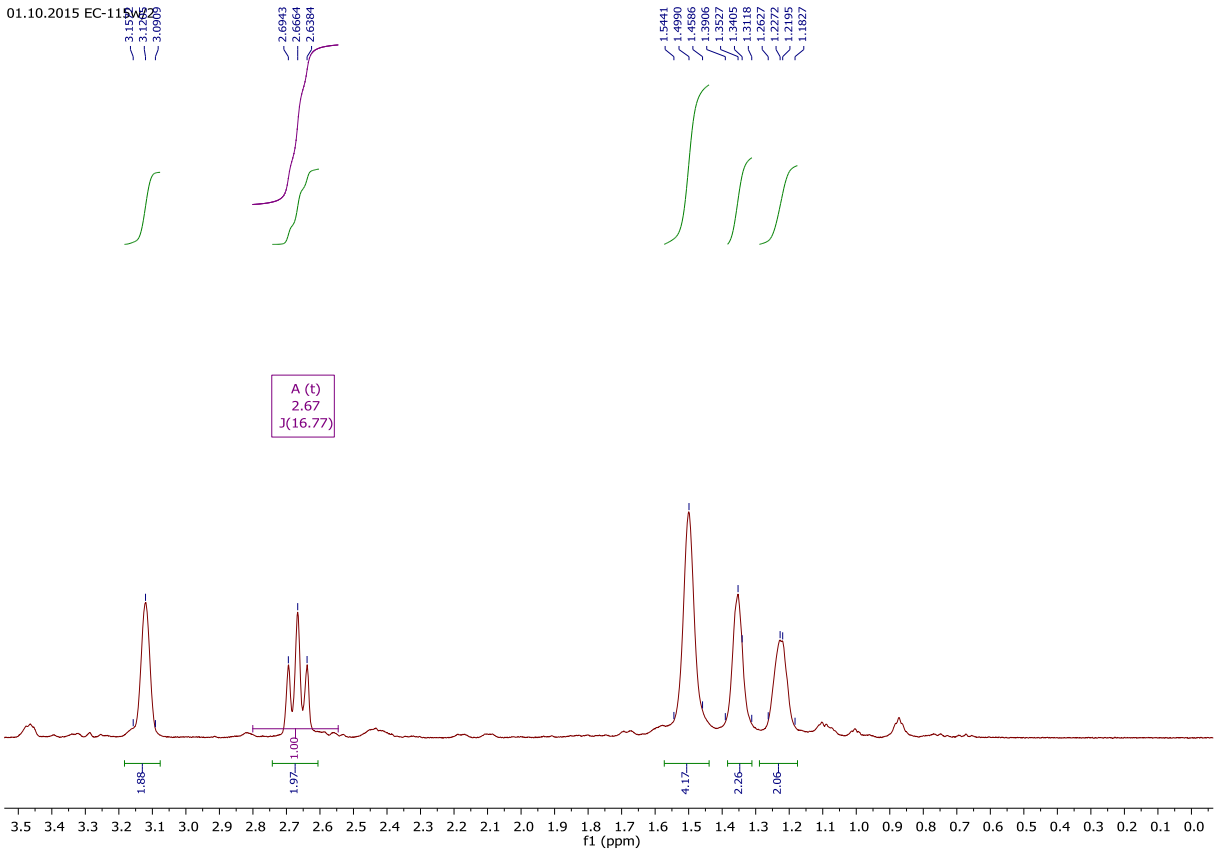

24.02.15 PM CED70 3
A (d)
1.99
J(12.76)
B (d)
1.66
J(12.55)
C (q)
0.82
J(11.50)
f1 (ppm)
B (d)
56.25
J(123.06)
A (d)
57.13
J(124.50)
f1 (ppm)

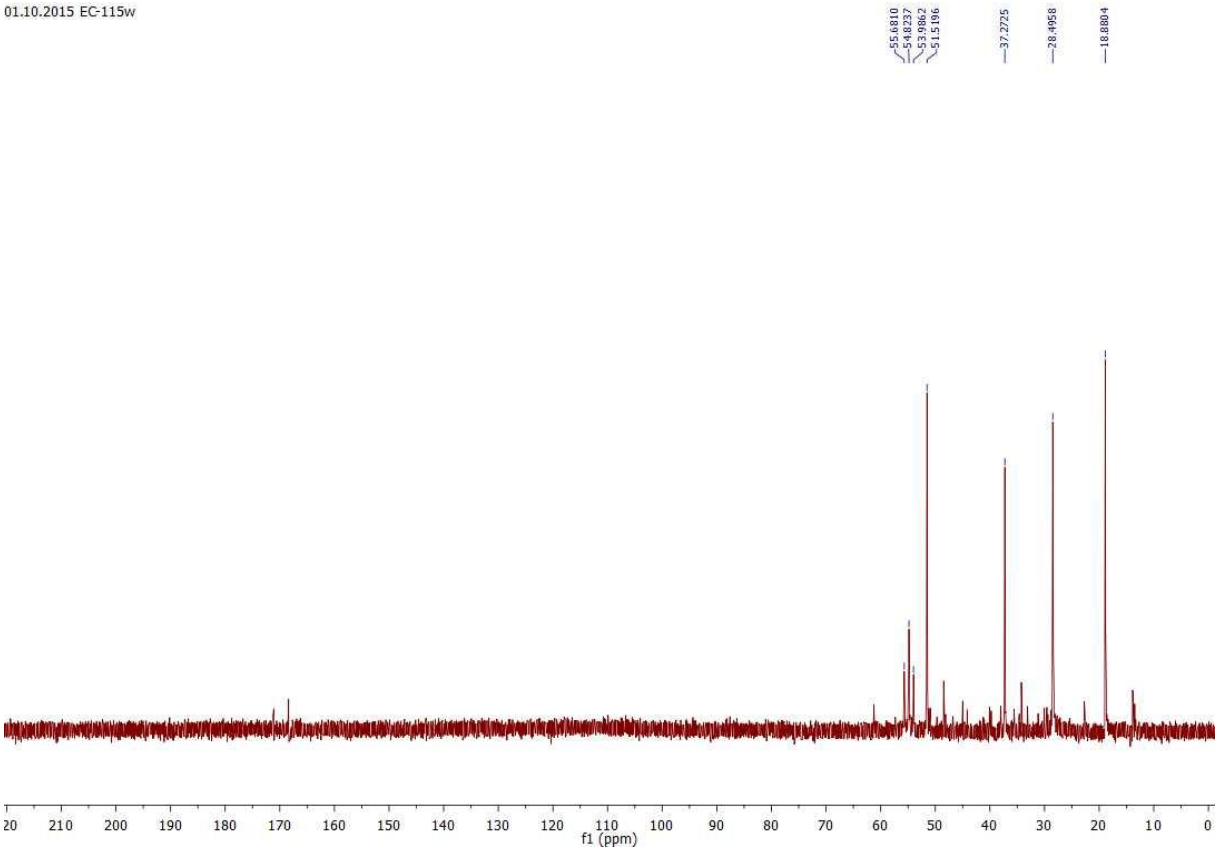

EC 70 con 140
3954_2014_U2 18 (0.290) Cm (13:18)
TOF MS ES-
1.29e4
287.0511
269.0488
205.0876
187.0854
100
%
0
100
200
300
400
500
600
700
800
900
1000
1100
m/z

Cyclohexane-1,3-di(aminomethylenebisphosphonic) acid L^2:



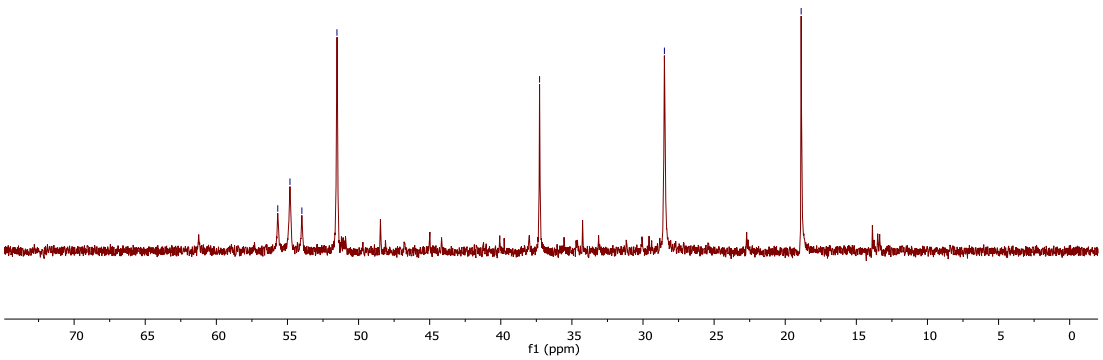


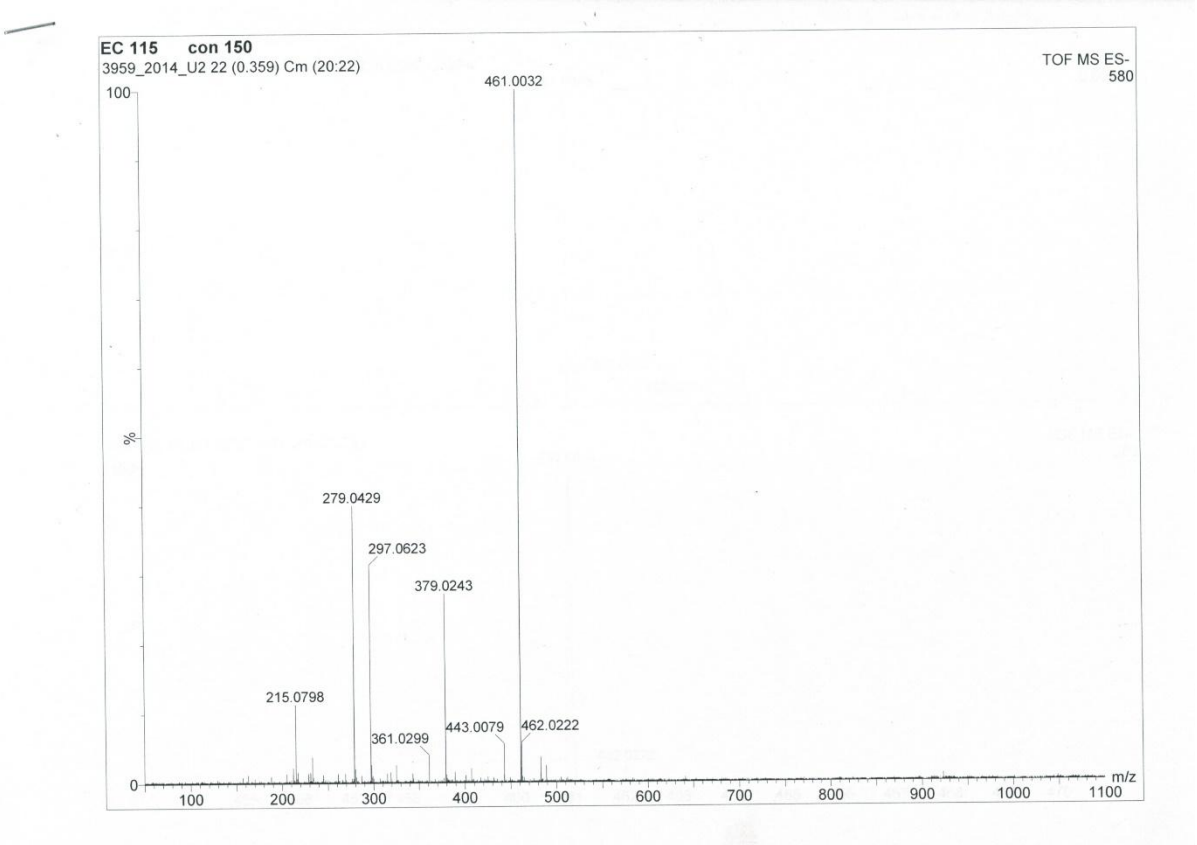

01.10.2015 EC-115w
3.1527
3.1262
3.0903
2.6943
2.6664
2.6384
1.5441
1.4990
1.4586
1.3906
1.3527
1.3405
1.3118
1.2627
1.2272
1.2195
1.1827
A (t)
2.67
J(16.77)
1.88
1.97
1.00
4.17
2.26
2.06
f1 (ppm)


01.10.2015 EC-115w
55.6810
54.8237
53.9862
51.5196
37.2725
28.4958
18.8804
f1 (ppm)

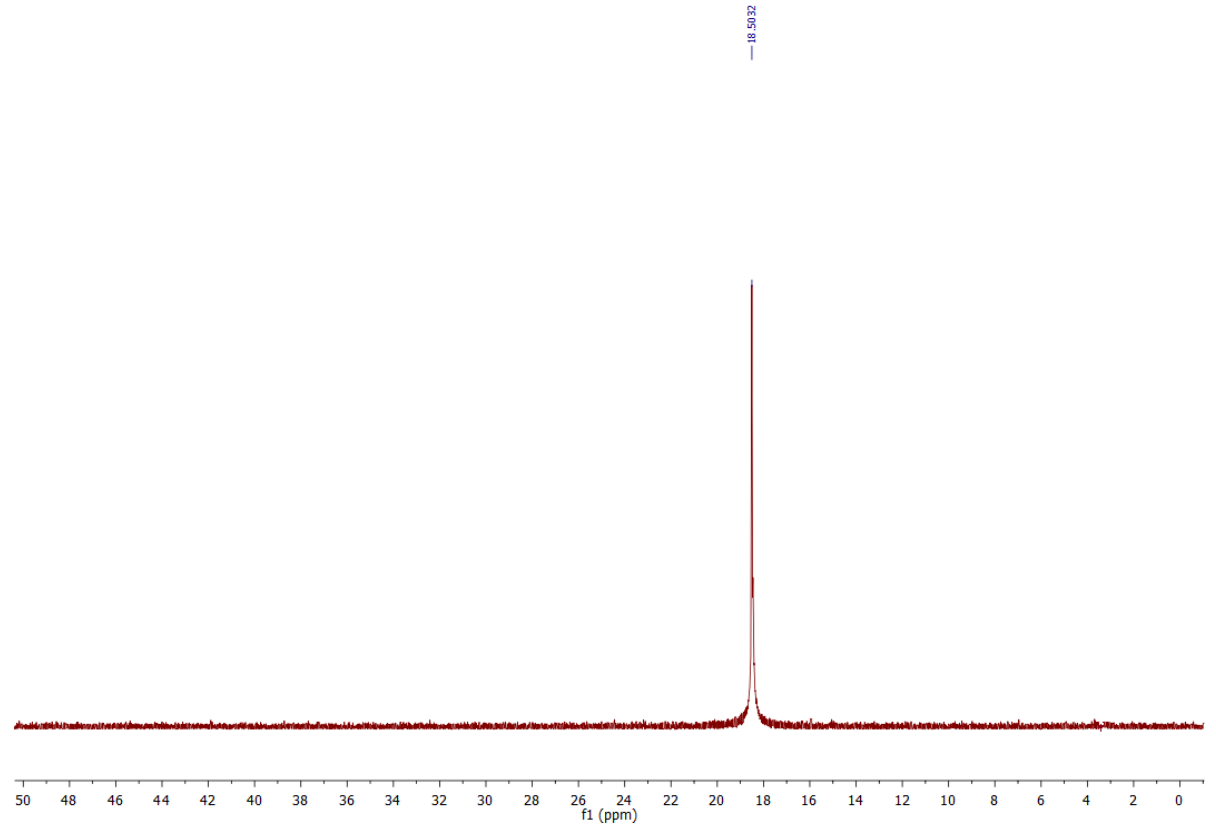
01.10.2015 EC-115w

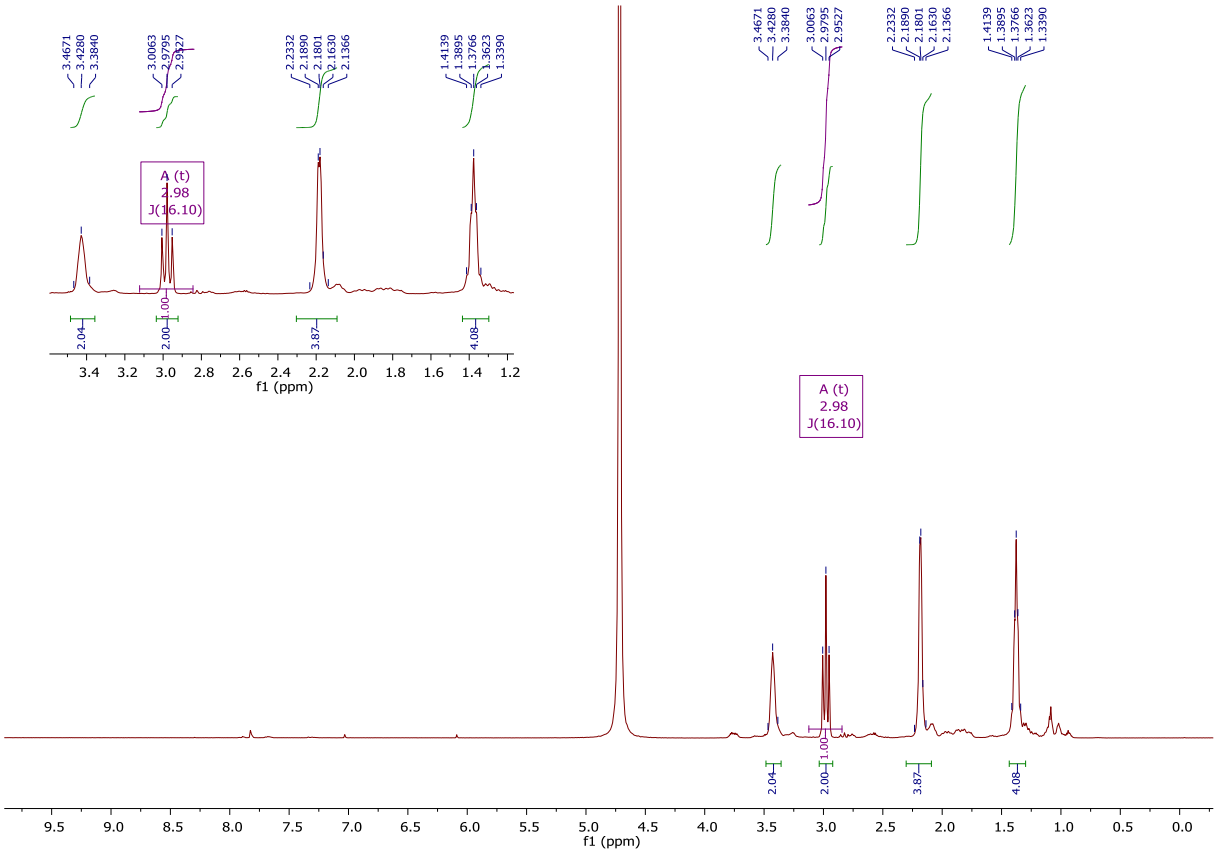
55.6810
54.8237
53.9862
51.5196
37.2725
28.4958
18.8804

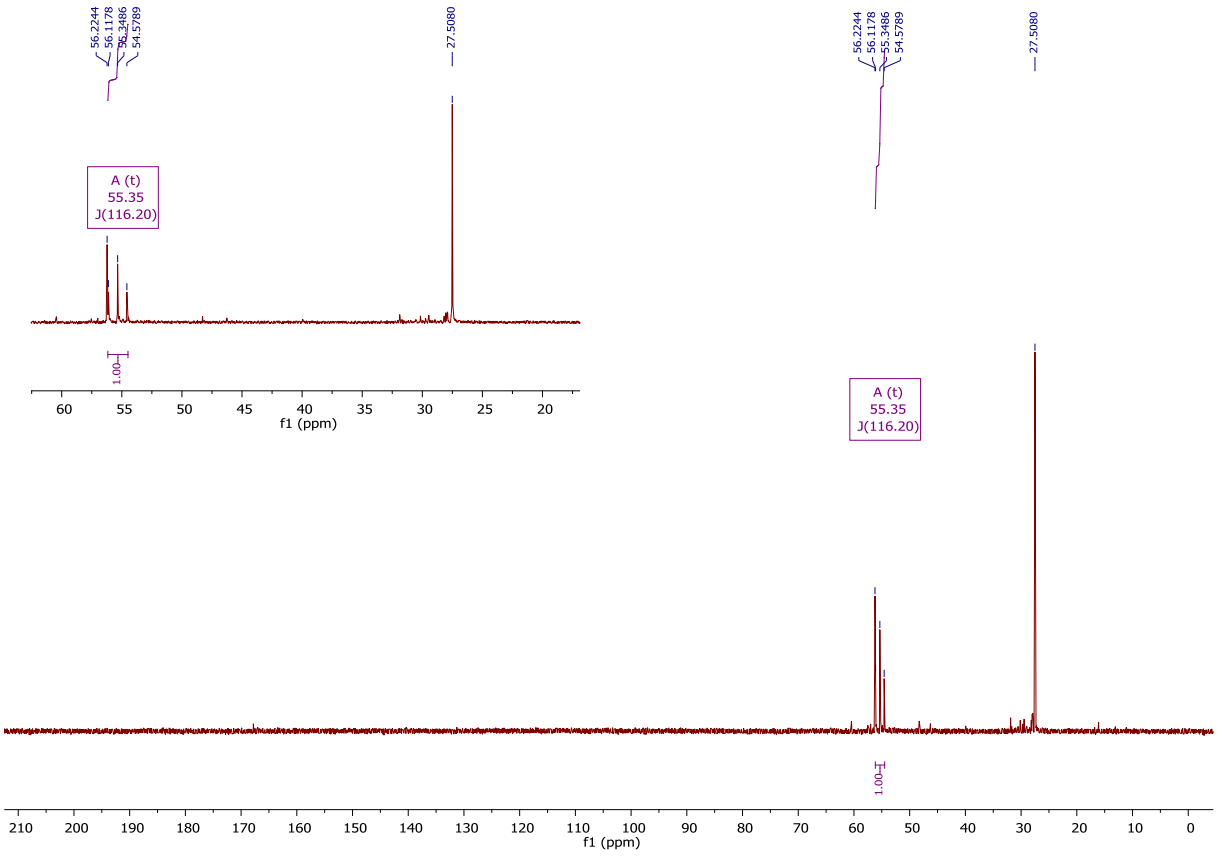


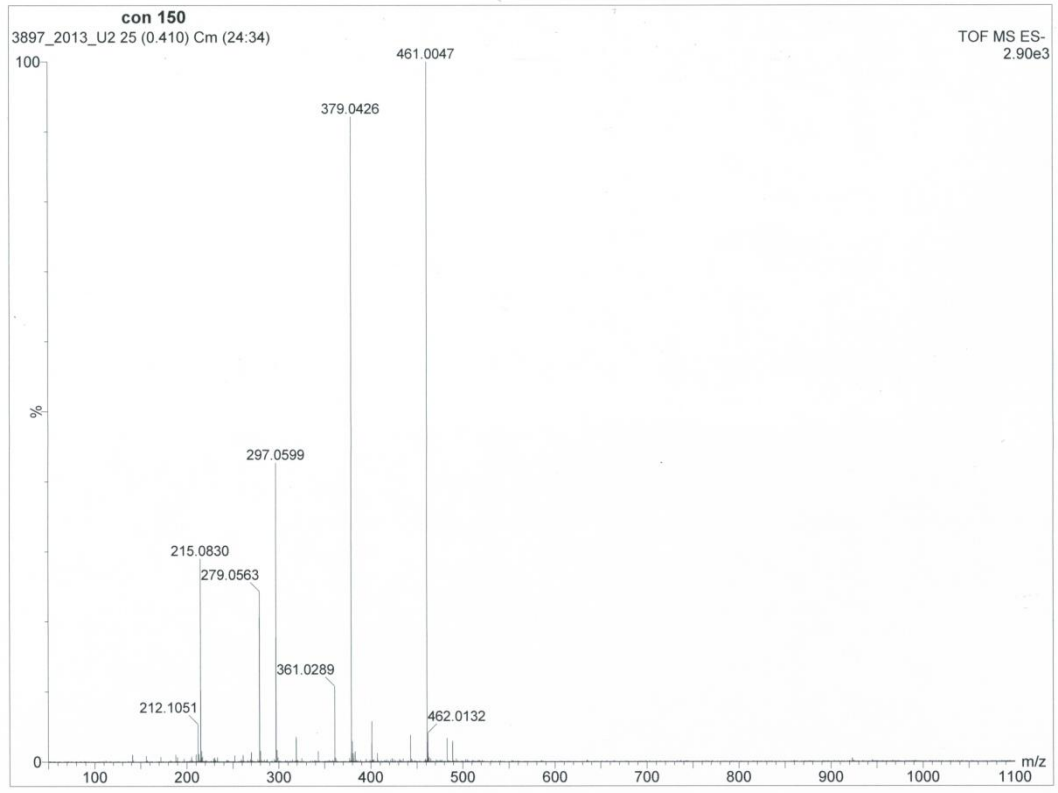


(trans)-Cyclohexane-1,4-di(aminomethylenebisphosphonic) acid L^3

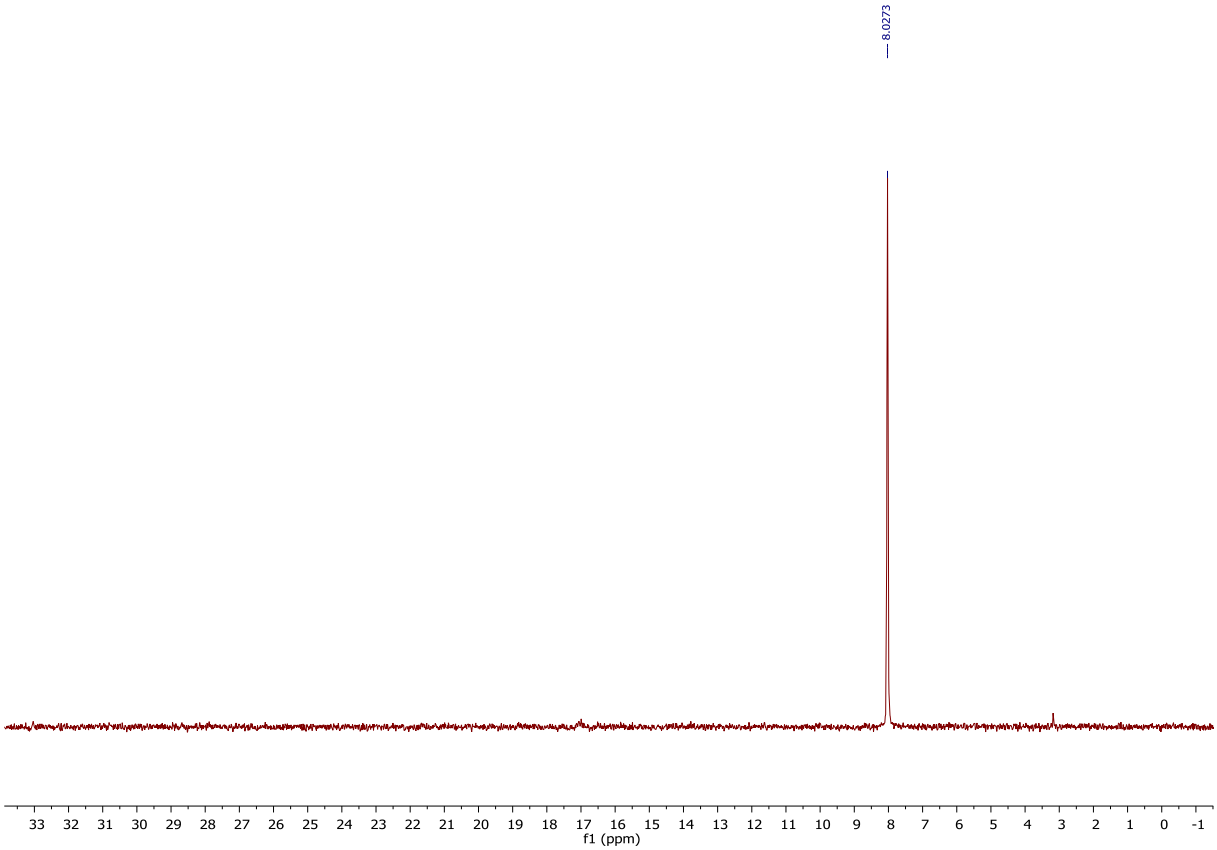


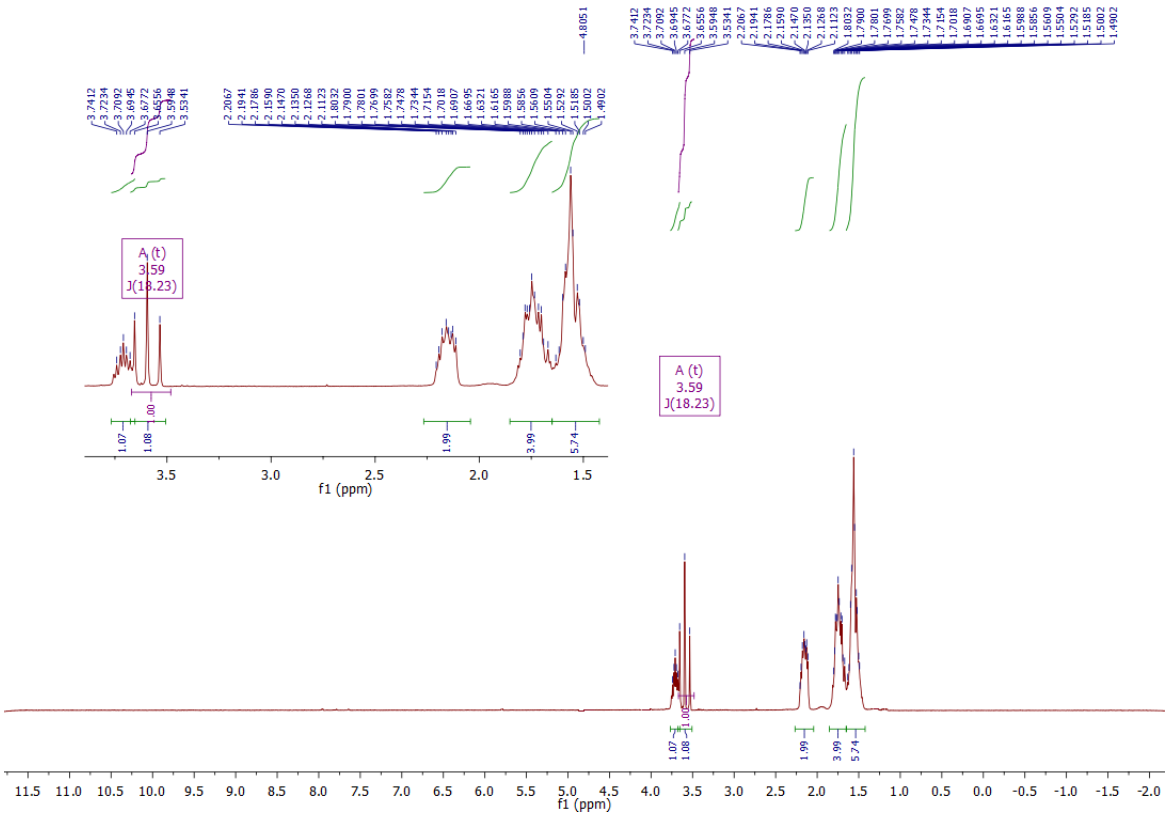


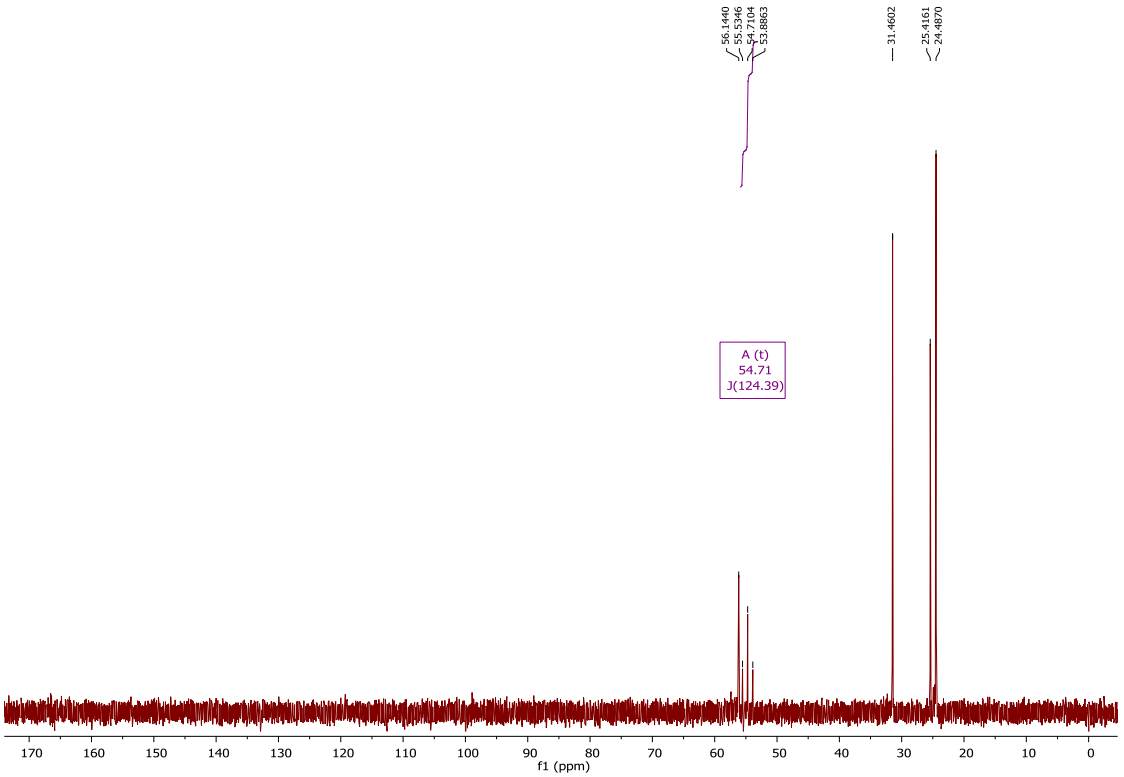

56.2244
56.1178
55.3486
54.5789
27.5080
A (t)
55.35
J(116.20)
1.00
f1 (ppm)

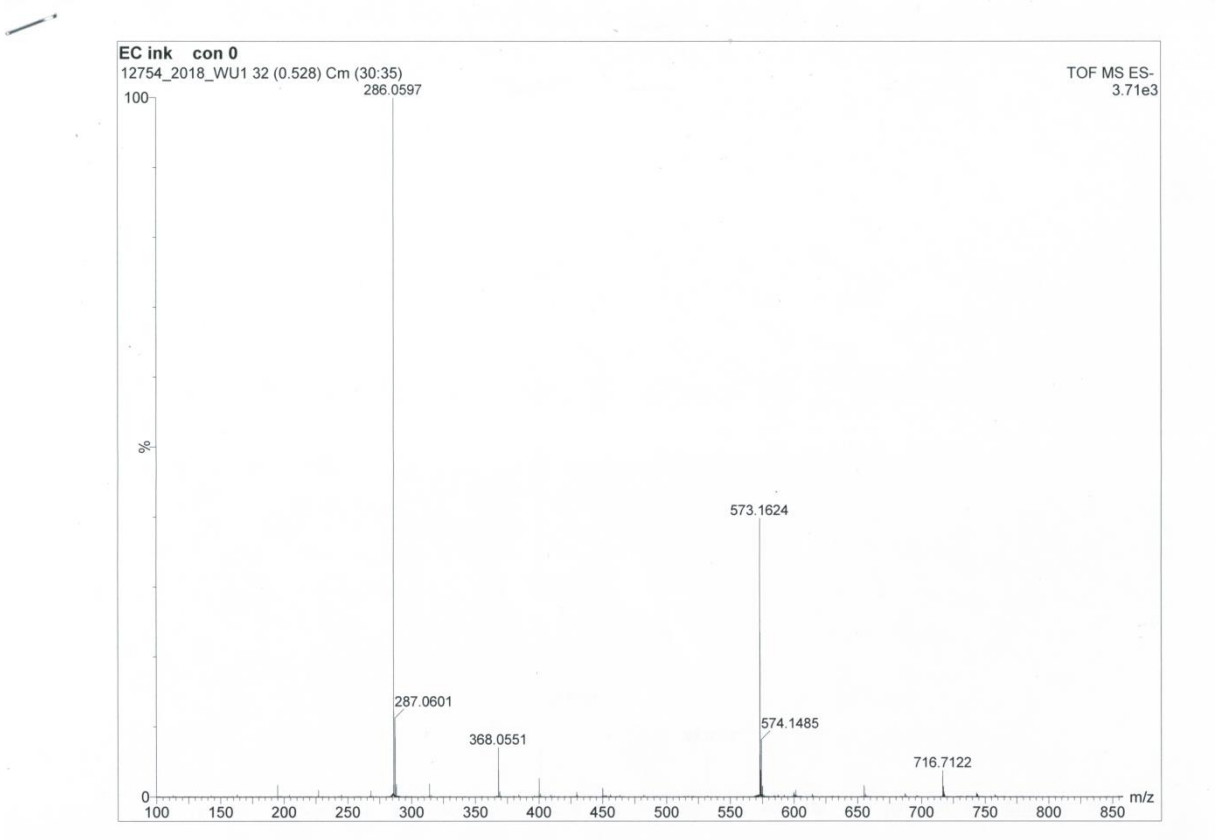

con 150
3897_2013_U2 25 (0.410) Cm (24:34)
TOF MS ES-
2.90e3
461.0047
379.0426
297.0599
215.0830
279.0563
361.0289
212.1051
462.0132
%
m/z

***N*-(cycloheptyl)aminomethylenebisphosphonic acid (Incadronate):**






56.1440
55.5346
54.7104
53.8863
31.4602
25.4161
24.4870
A (t)
54.71
J(124.39)
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


EC ink con 0
12754_2018_WU1 32 (0.528) Cm (30:35)
TOF MS ES-
3.71e3
100
%
0
286.0597
573.1624
287.0601
574.1485
368.0551
716.7122
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850
m/z