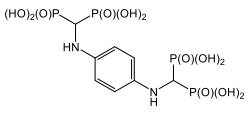
Three-component reaction of diamines with triethyl orthoformate and diethyl phosphite and antiproliferative and antiosteoporotic activities of the products

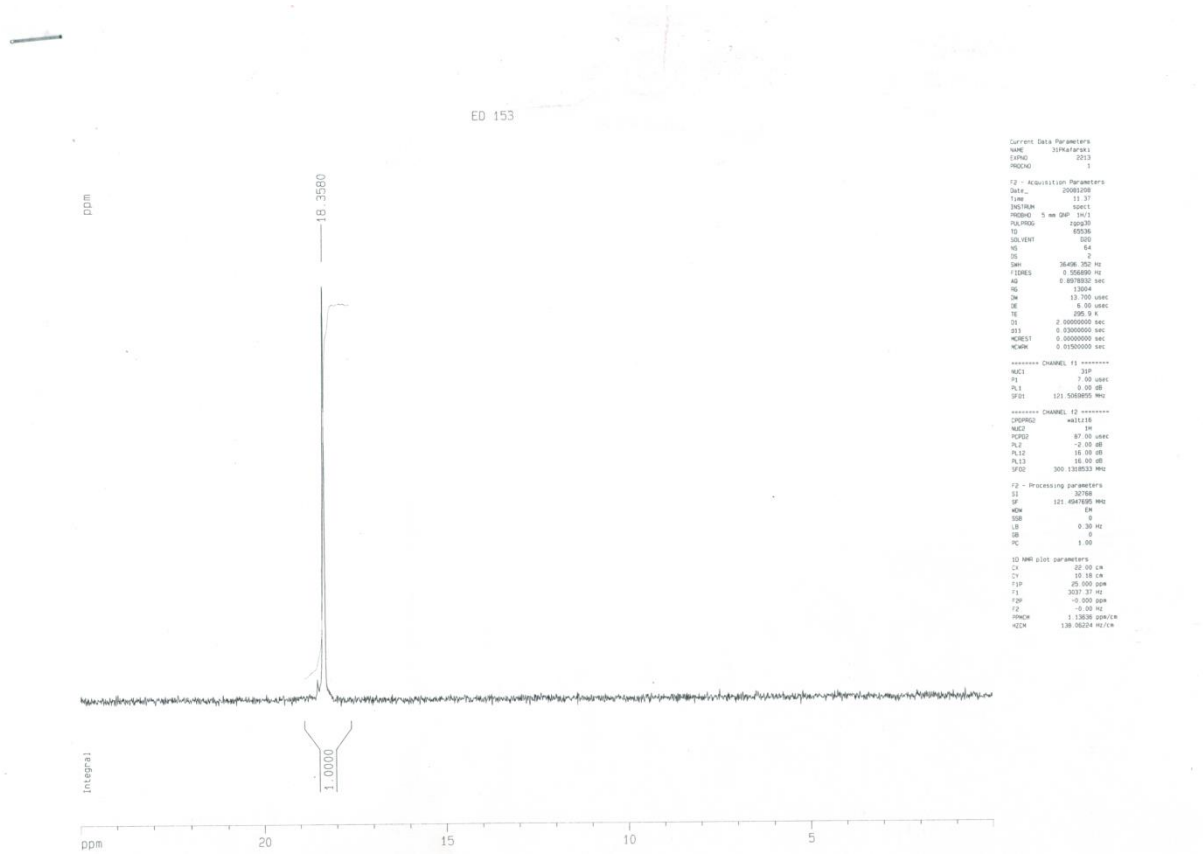
Patrycja Petruczynik[1], Paweł Kafarski[1], Mateusz Psurski[2], Joanna Wietrzyk[2], Zdzisław Kiełbowicz[3], Jan Kuryszko[4] and Ewa Chmielewska[1]*

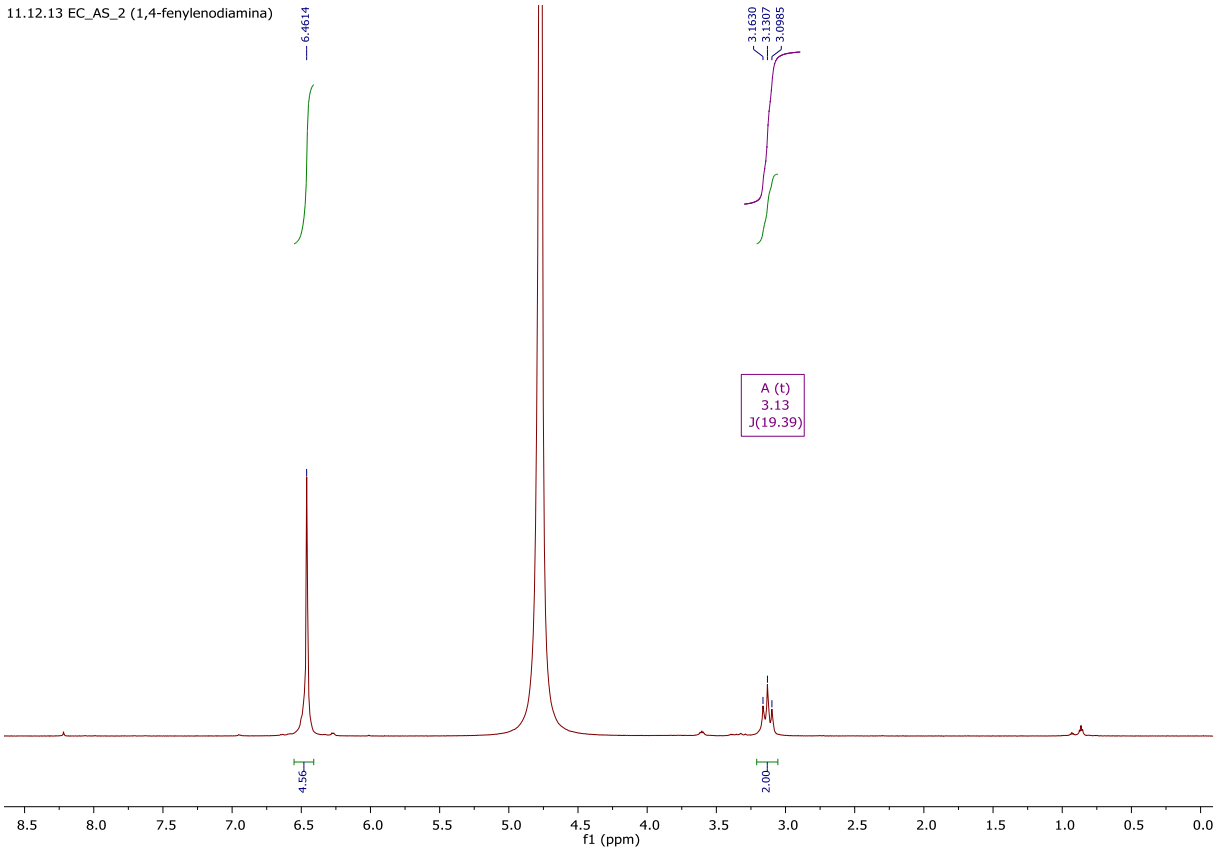
[1]Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; ewa.chmielewska@pwr.edu.pl
[2] Laboratory of Experimental Anticancer Therapy, Department Of Experimental Oncology, Ludwik Hirszfeld Institute of Immunology and Experimental TherapyPolish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocław, Poland;
[3] Department of Surgery, The Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland
[4] Division of Histology and Embryology, Department of Animal Physiology and Biostructure, The Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland
* Correspondence: ewa.chmielewska@pwr.edu.pl ; Tel.: +48-71 320 29 77

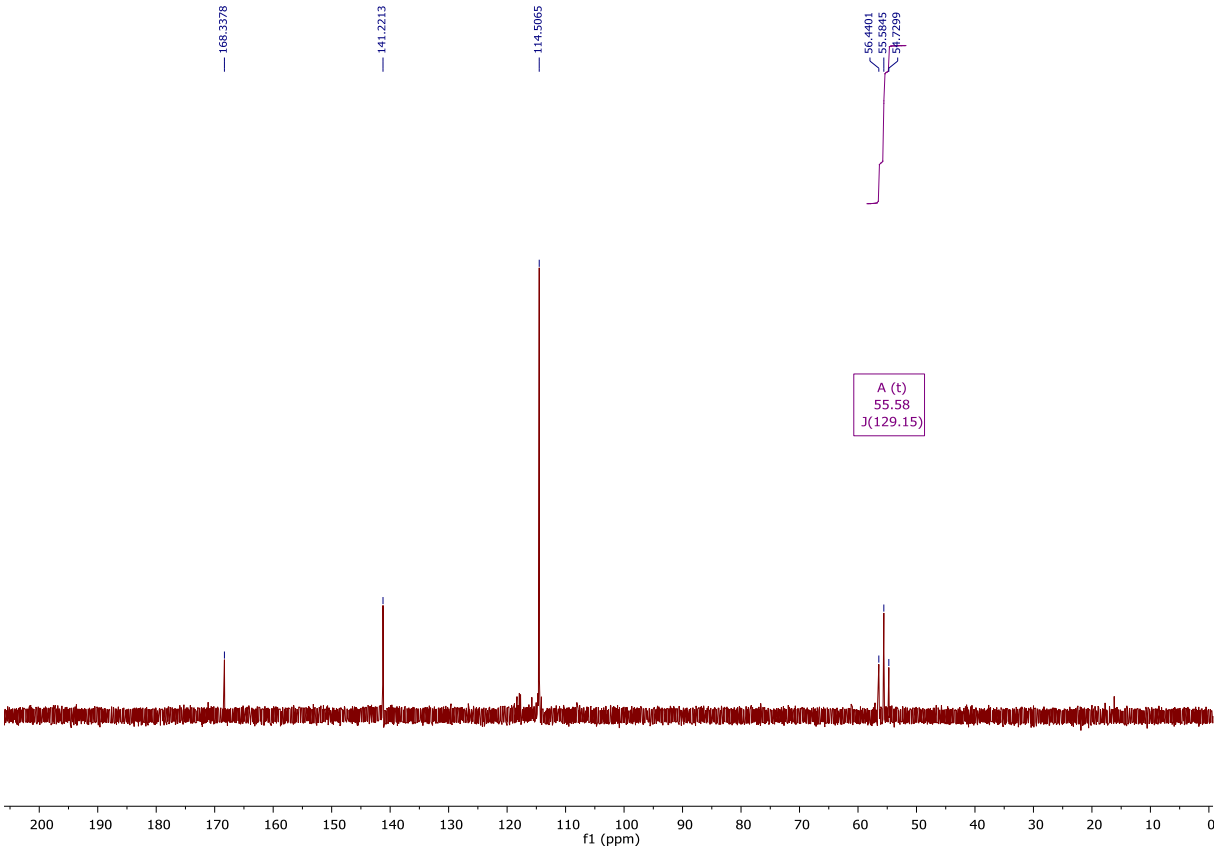
1. Spectroscopic Data

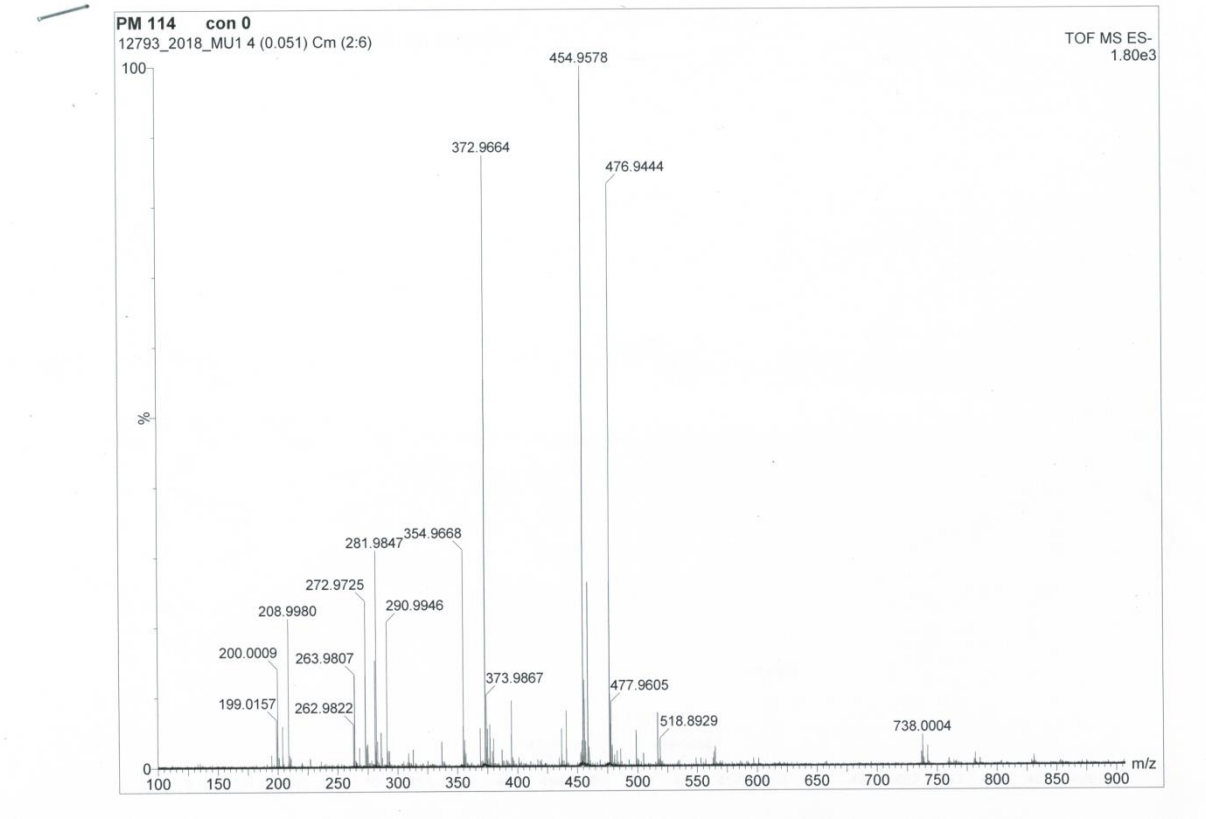


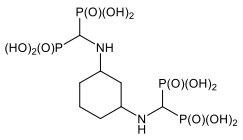
Phenylene-1,4-di(aminomethylenebisphosphonic) acid (**1**)



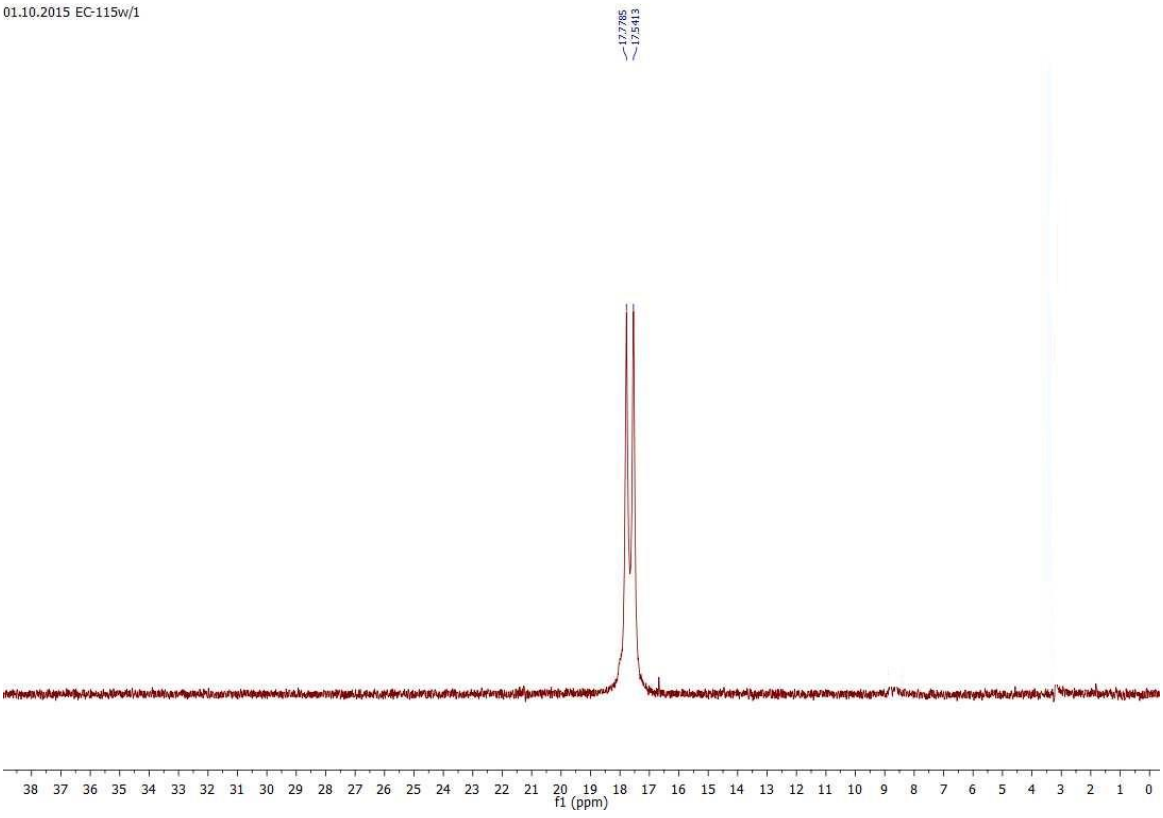

11.12.13 EC_AS_2 (1,4-fenylenodiamina)
6.4614
3.1630
3.1307
3.0985
A (t)
3.13
J(19.39)
4.56
2.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

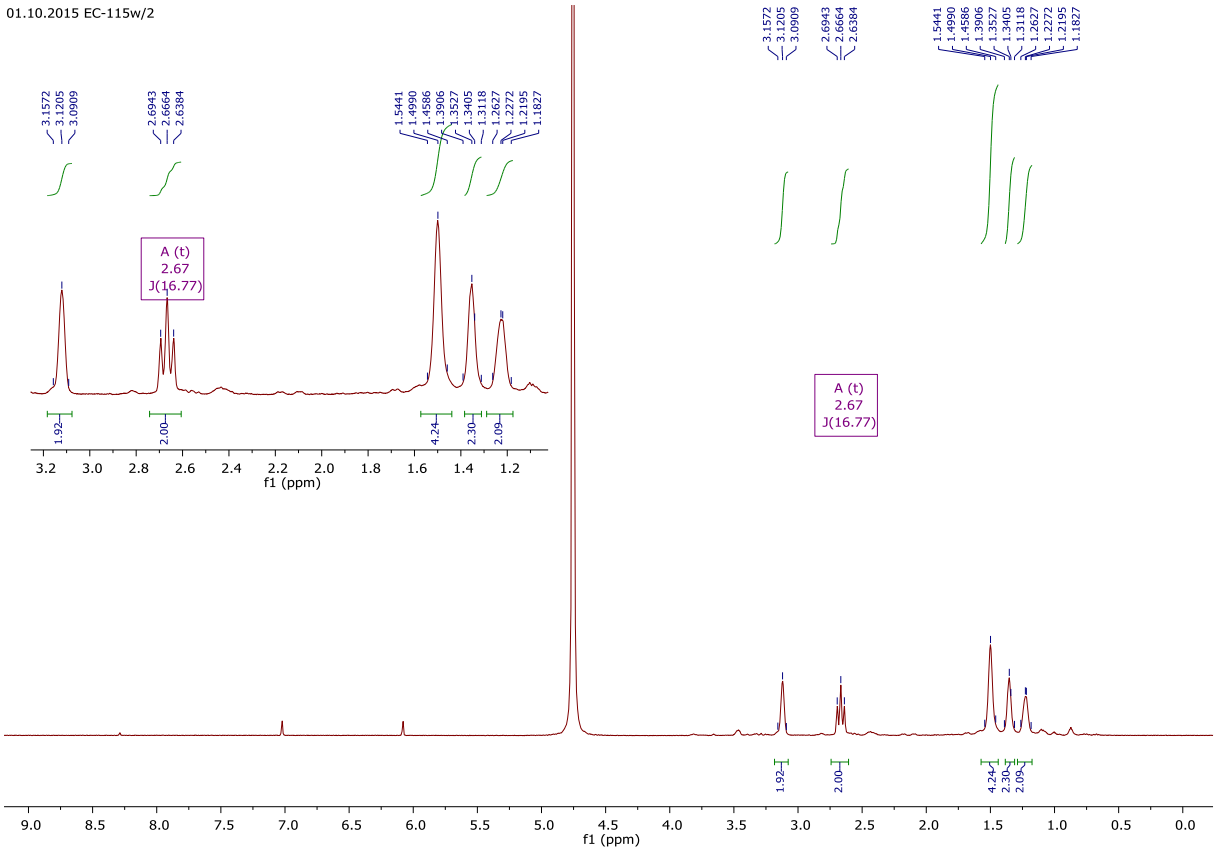

168.3378
141.2213
114.5065
56.4401
55.5845
54.7299
A (t)
55.58
J(129.15)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

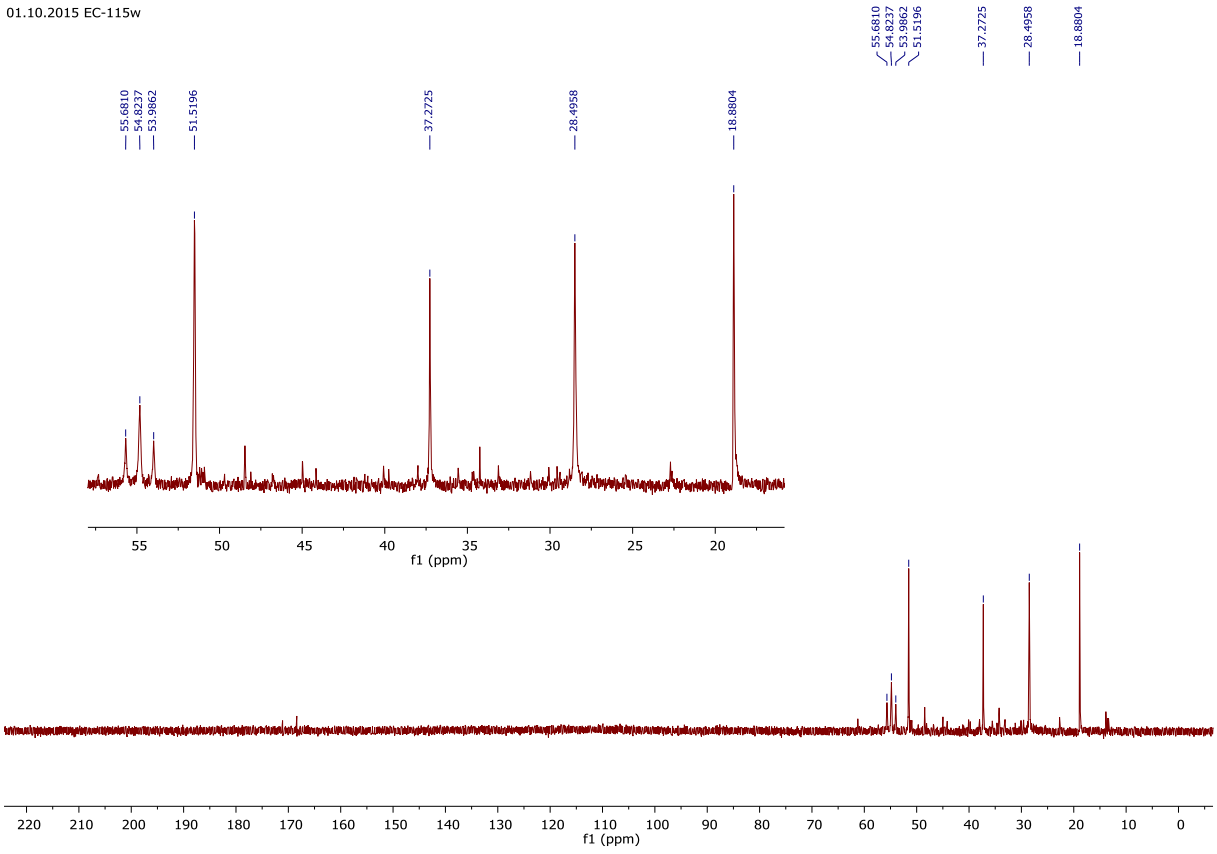


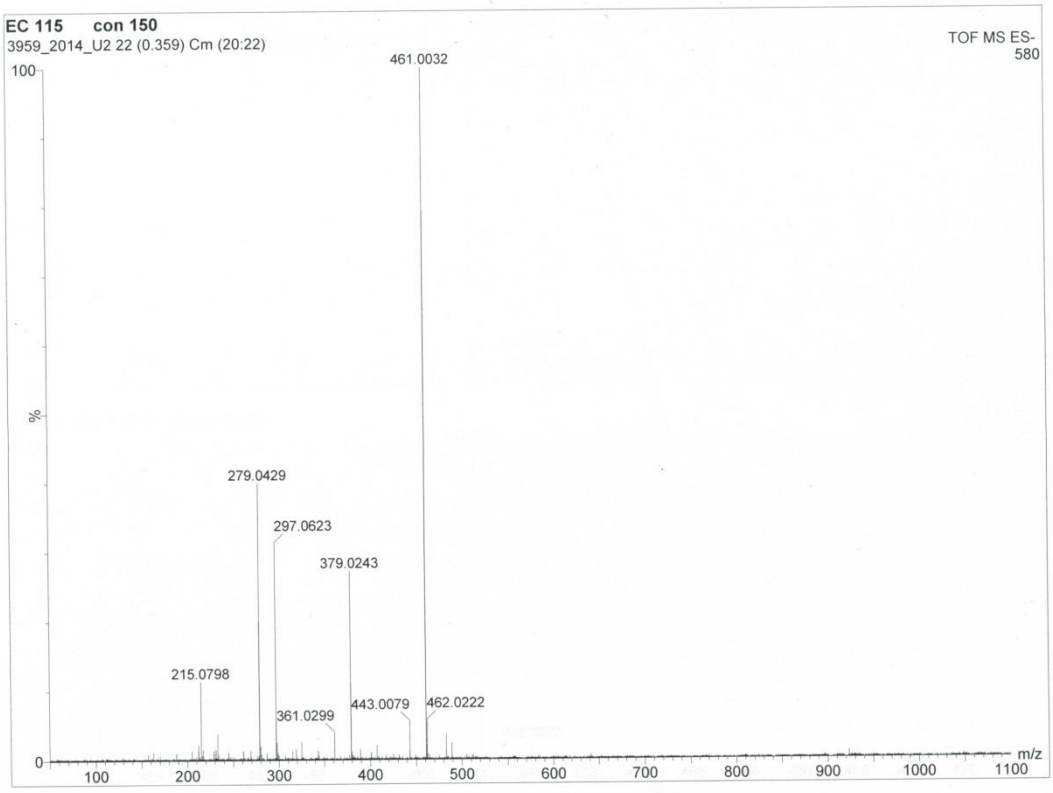


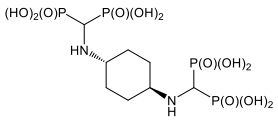
Cyclohexane-1,3-di(aminomethylenebisphosphonic) acid (**6**)



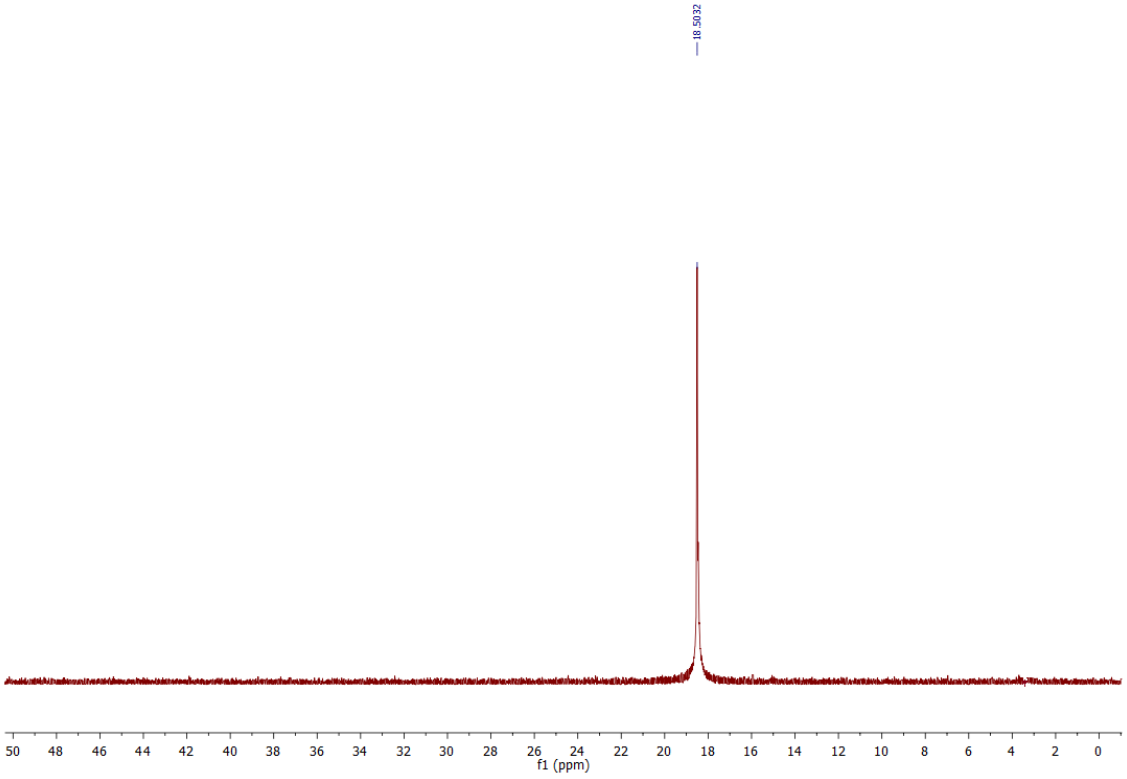

01.10.2015 EC-115w/2
3.1572
3.1205
3.0909
2.6943
2.6664
2.6384
1.5441
1.4990
1.4586
1.3906
1.3527
1.3405
1.3118
1.2627
1.2272
1.2195
1.1827
A (t)
2.67
J(16.77)
1.92
2.00
4.24
2.30
2.09
f1 (ppm)

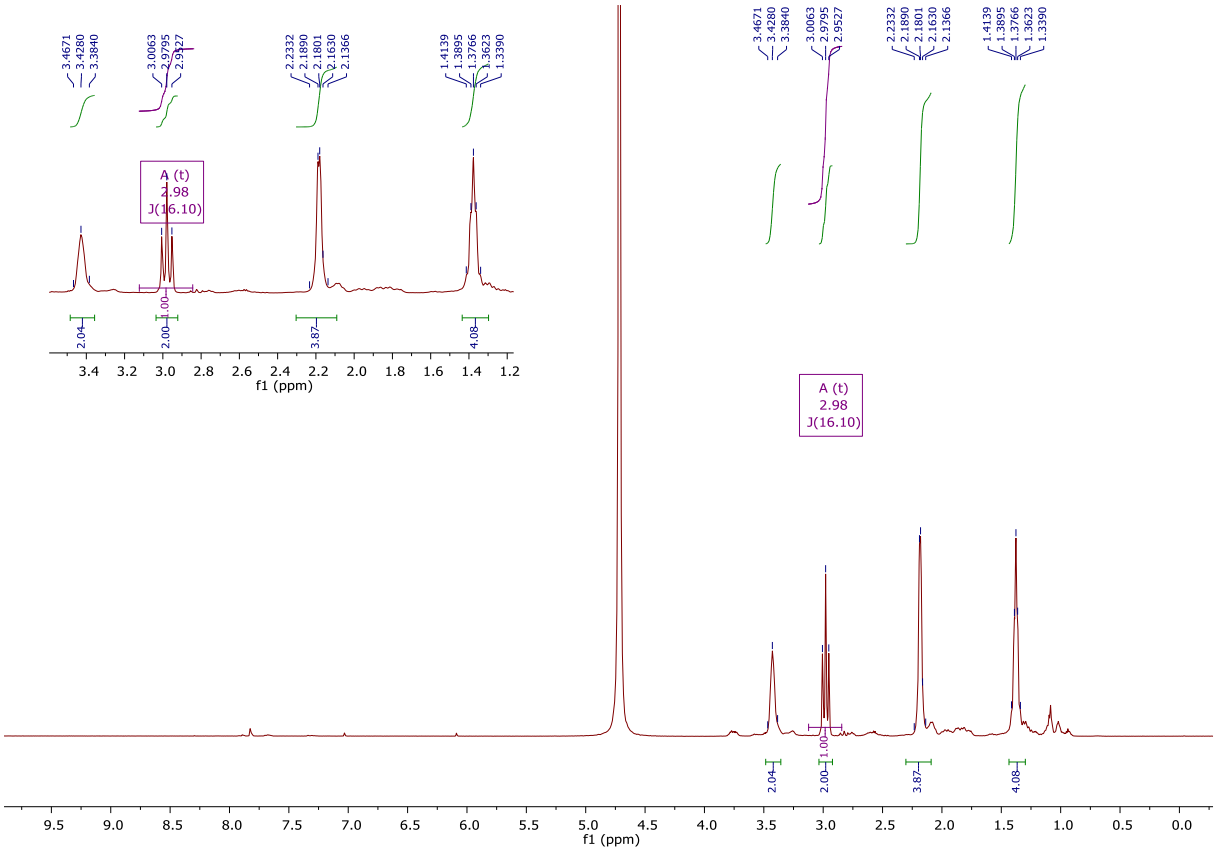

01.10.2015 EC-115w
55.6810
54.8237
53.9862
51.5196
37.2725
28.4958
18.8804
f1 (ppm)

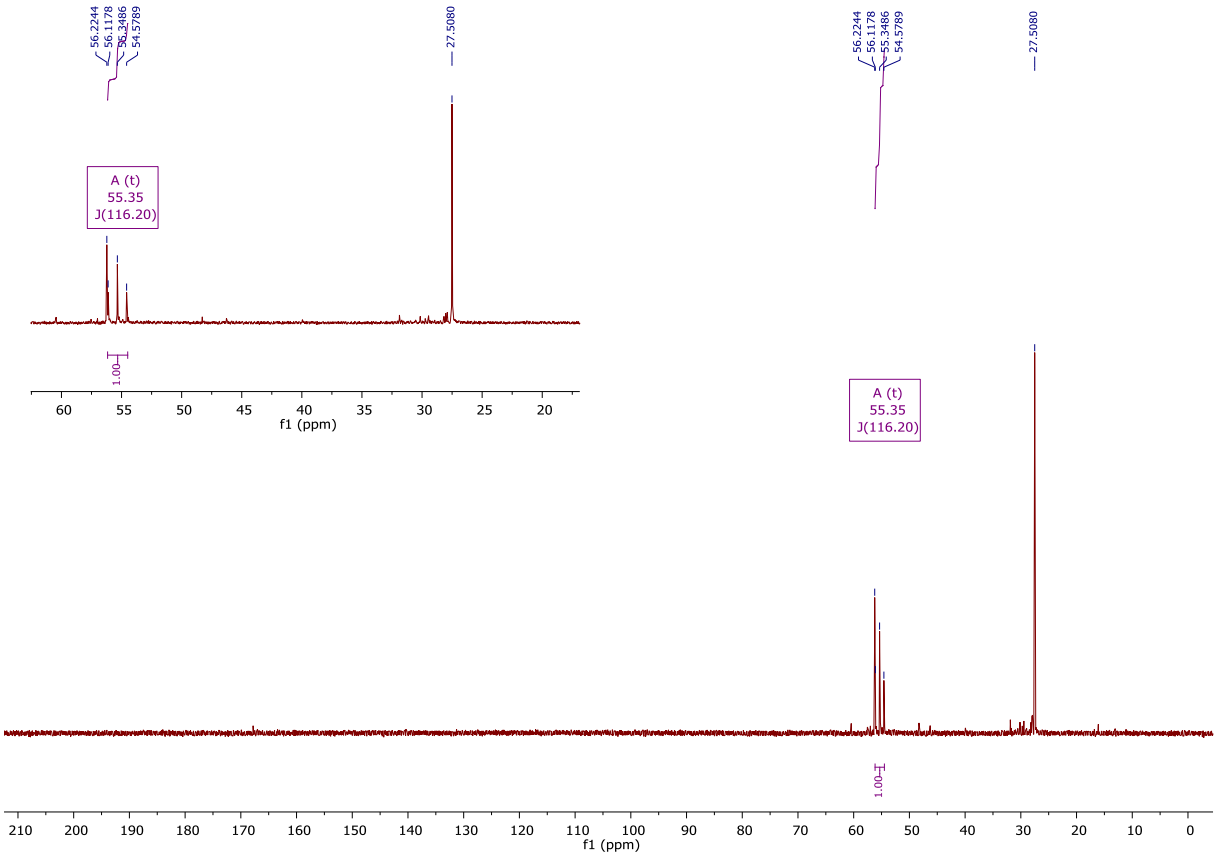


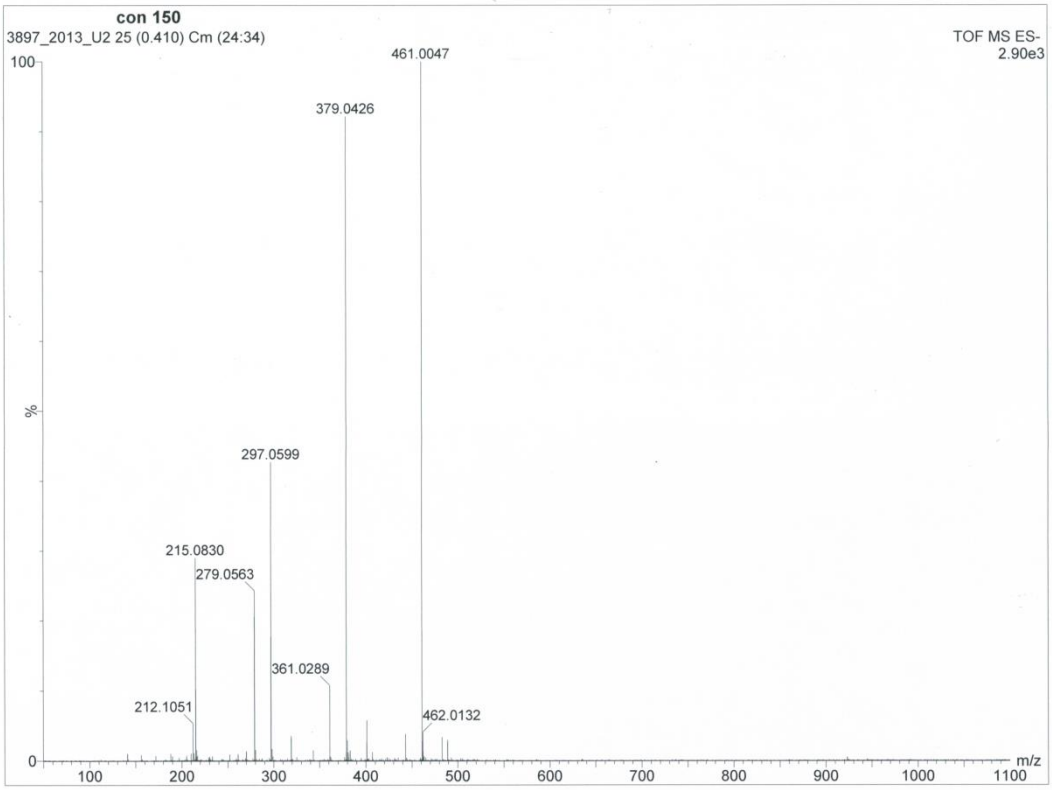


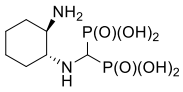
(trans)-Cyclohexane-1,4-di(aminomethylenebisphosphonic) acid (**7**)



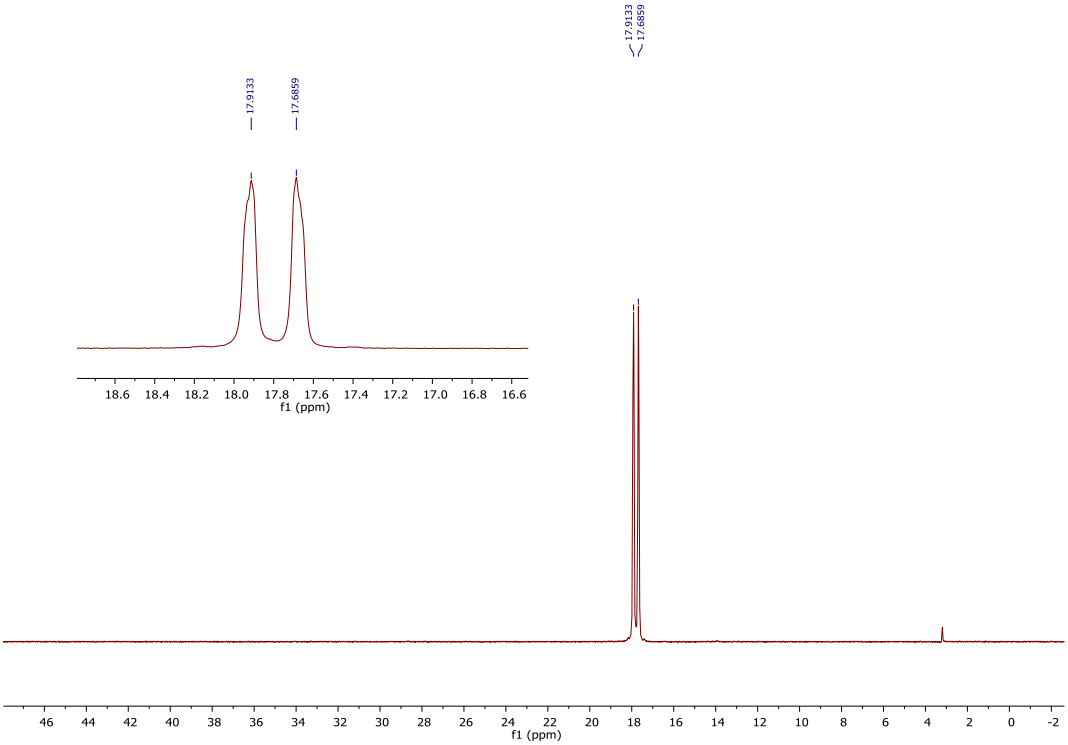

3.4671
3.4280
3.3840
3.0063
2.9795
2.9527
2.2332
2.1890
2.1801
2.1630
2.1366
1.4139
1.3895
1.3766
1.3623
1.3390
A (t)
2.98
J(16.10)
2.04
2.00
1.00
3.87
4.08
f1 (ppm)

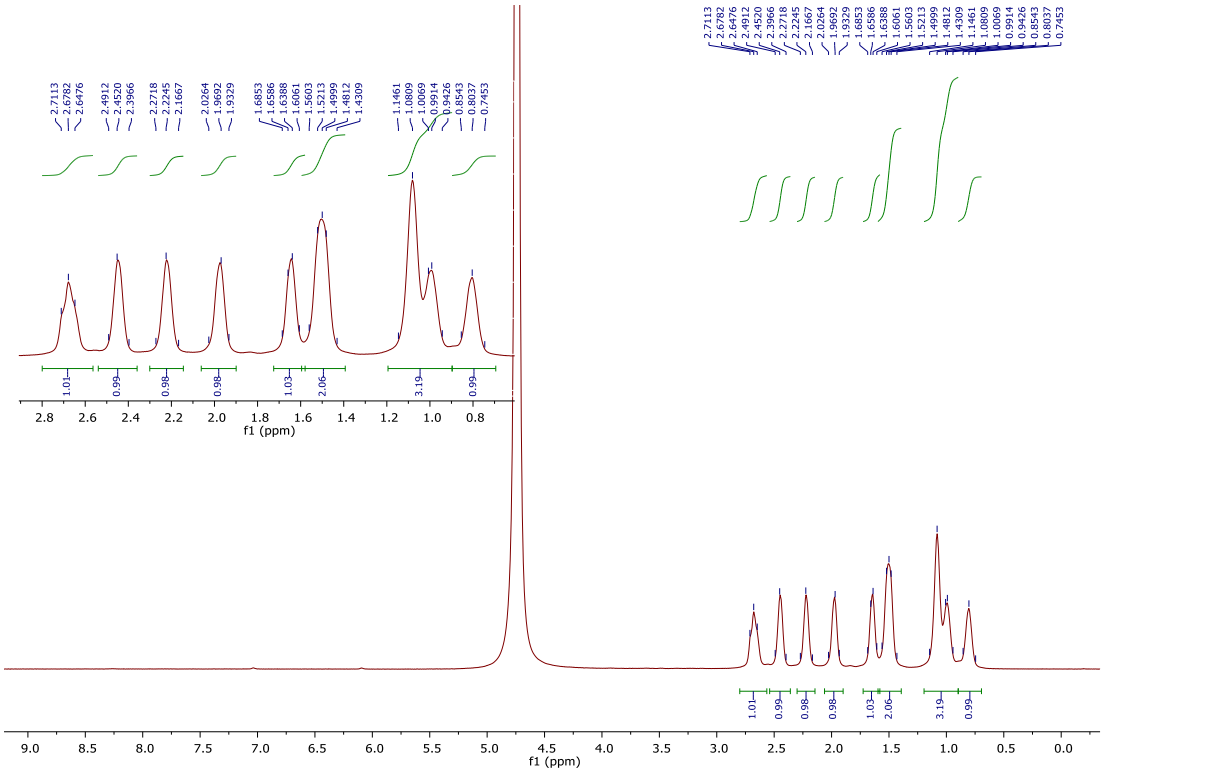

56.2244
56.1178
55.3486
54.5789
27.5080
A (t)
55.35
J(116.20)
1.00
f1 (ppm)

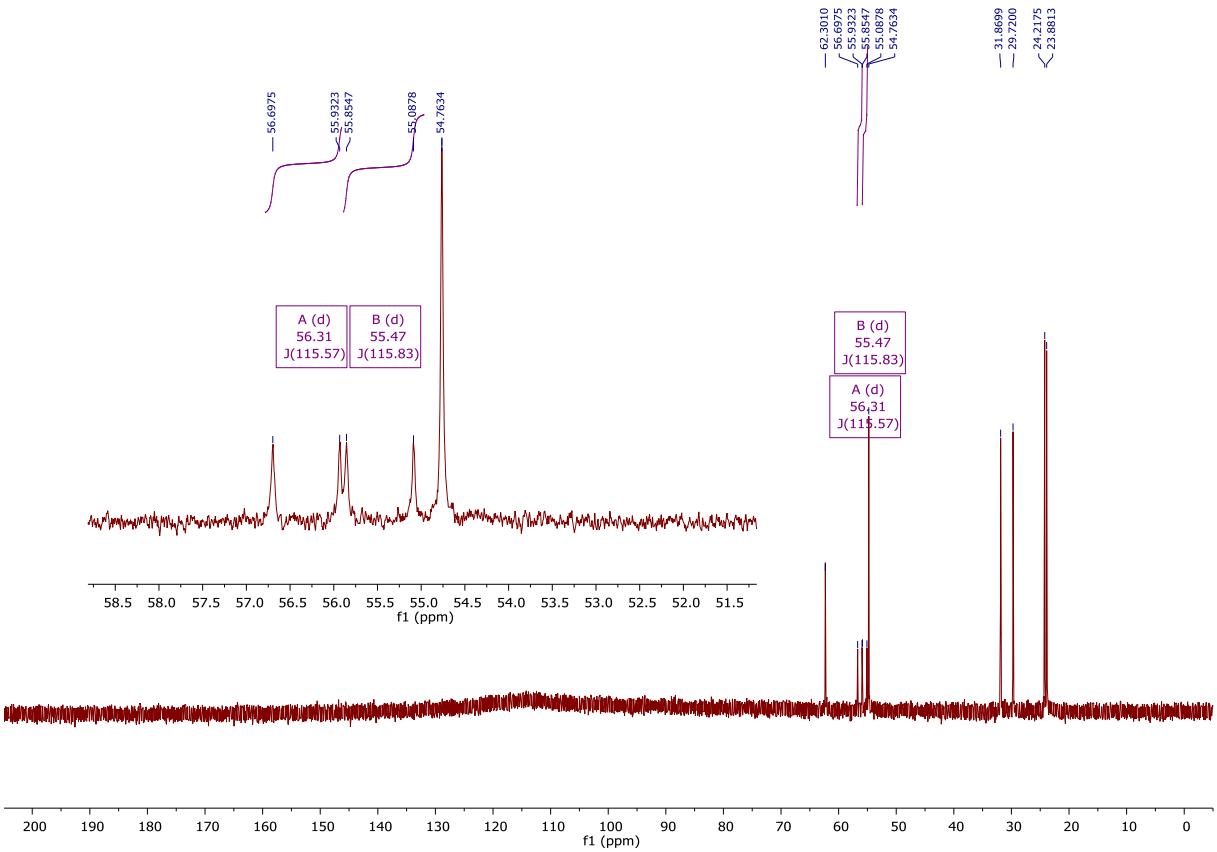


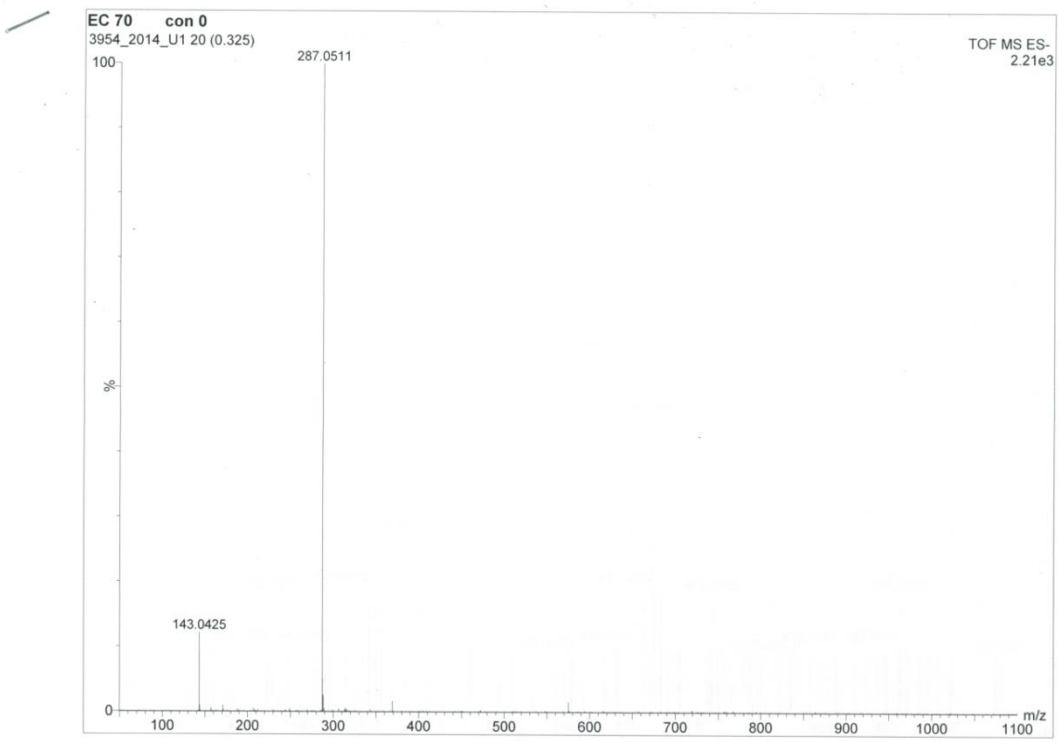


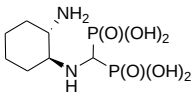
(1*S*,2*S*)-cyclohexane-1-amino-2-aminomethylenebisphosphonic acid [(1*S*,2*S*)-**8**]



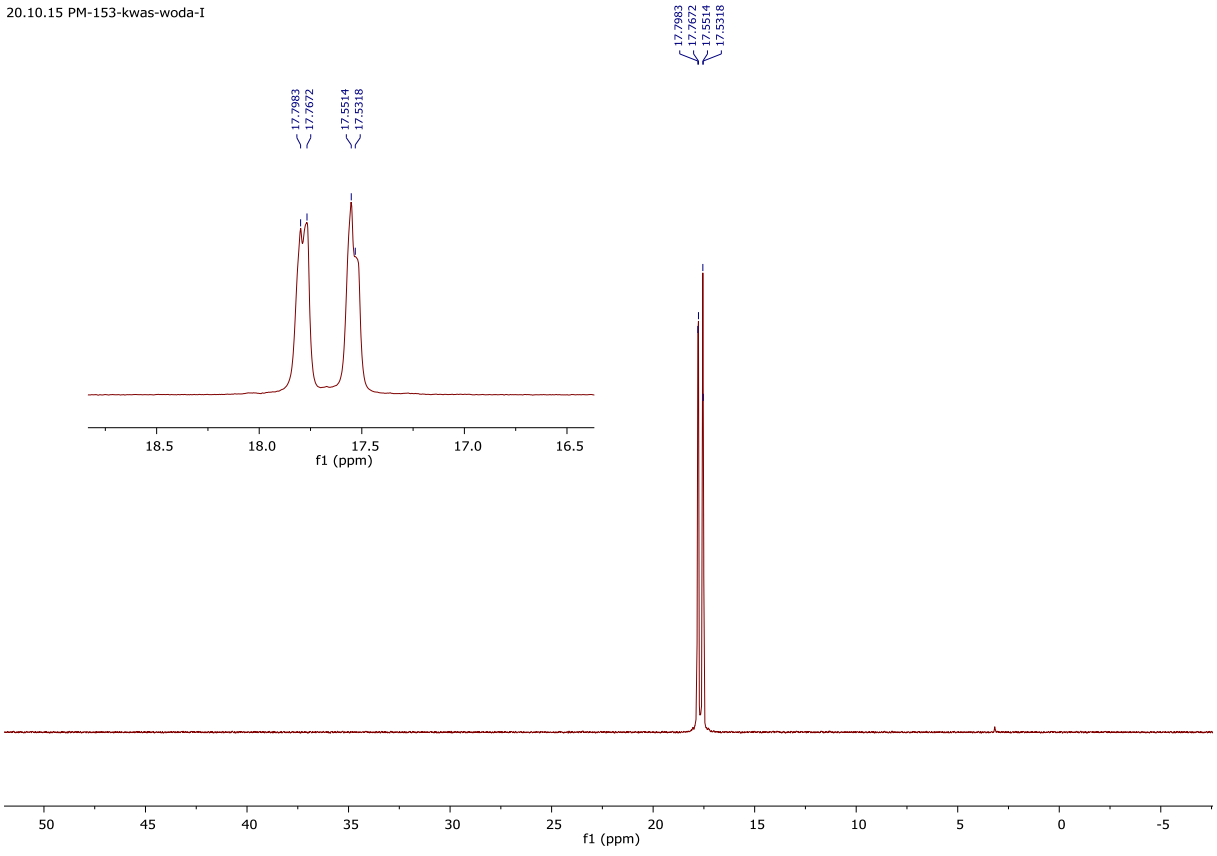

f1 (ppm)

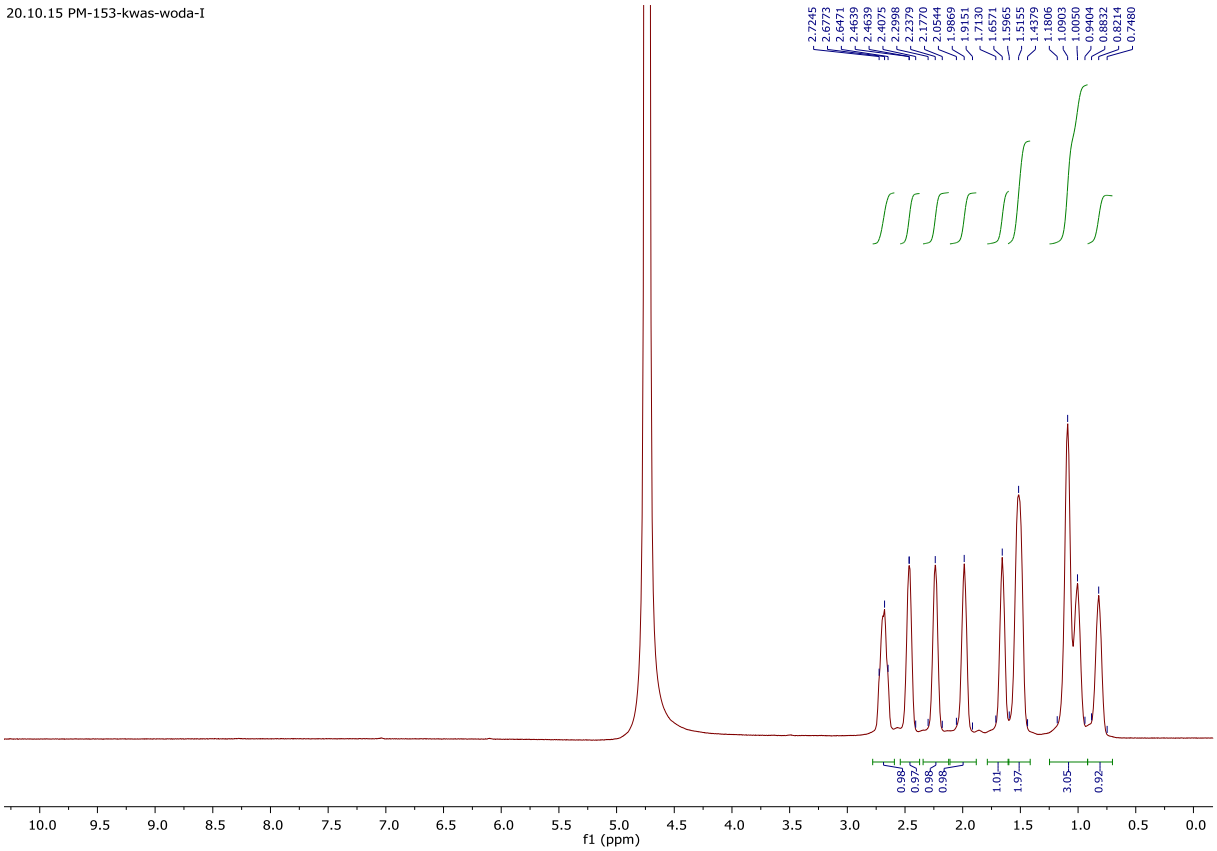

A (d)
56.31
J(115.57)
B (d)
55.47
J(115.83)
f1 (ppm)

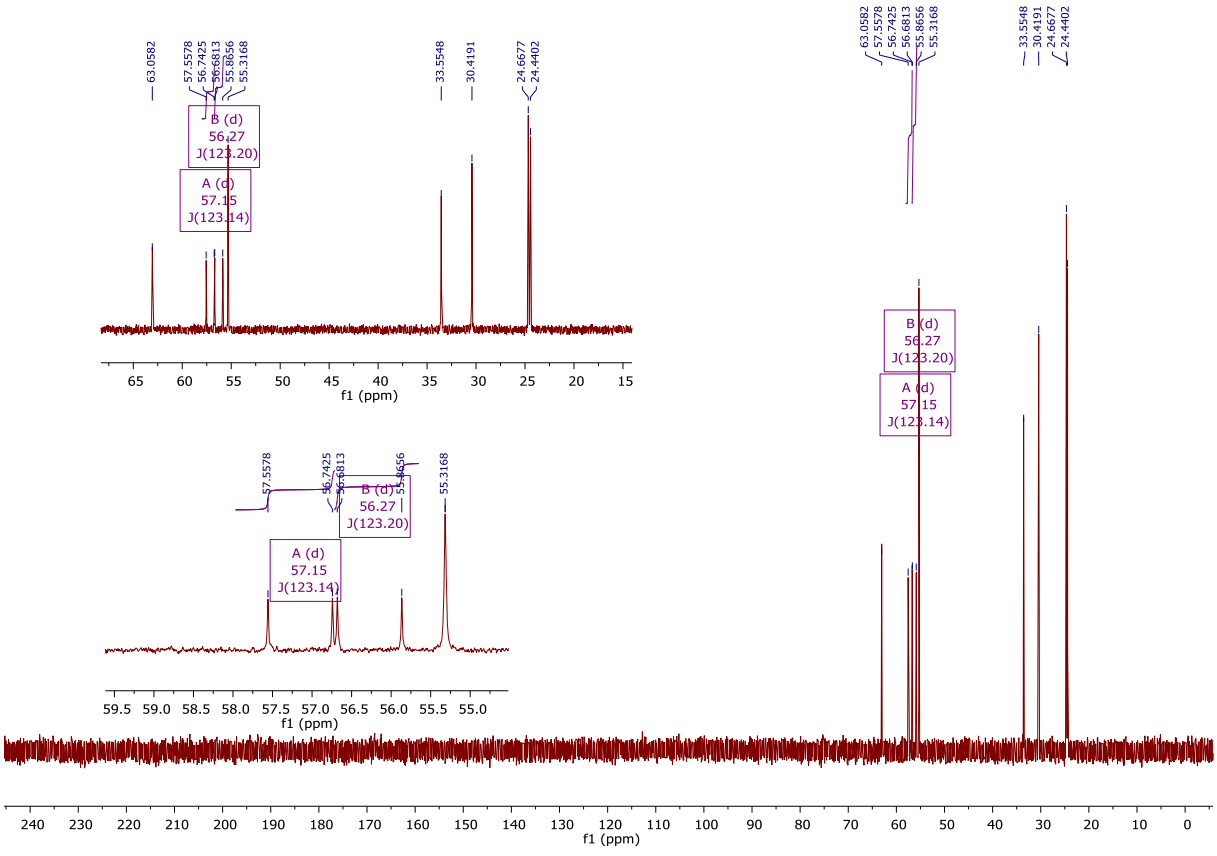


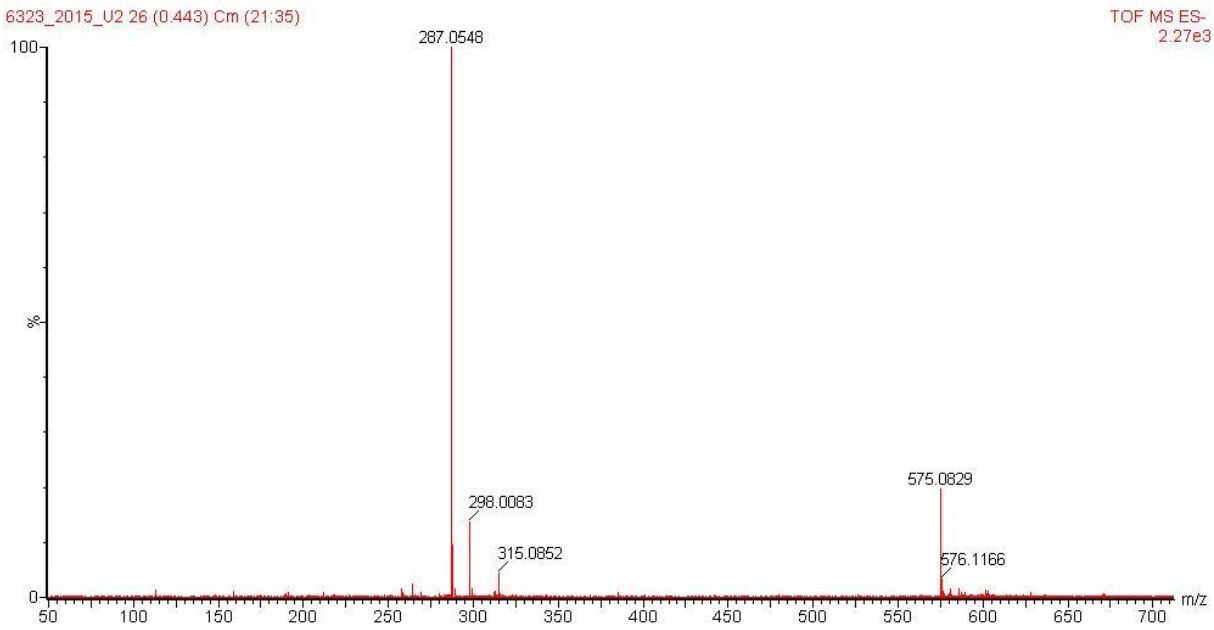


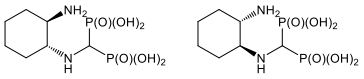
(1*R*, 2*R*)-(-)Cyclohexane-1-amino-2-aminomethylenebisphosphonic acid [(1*R*,2*R*)-**8**]



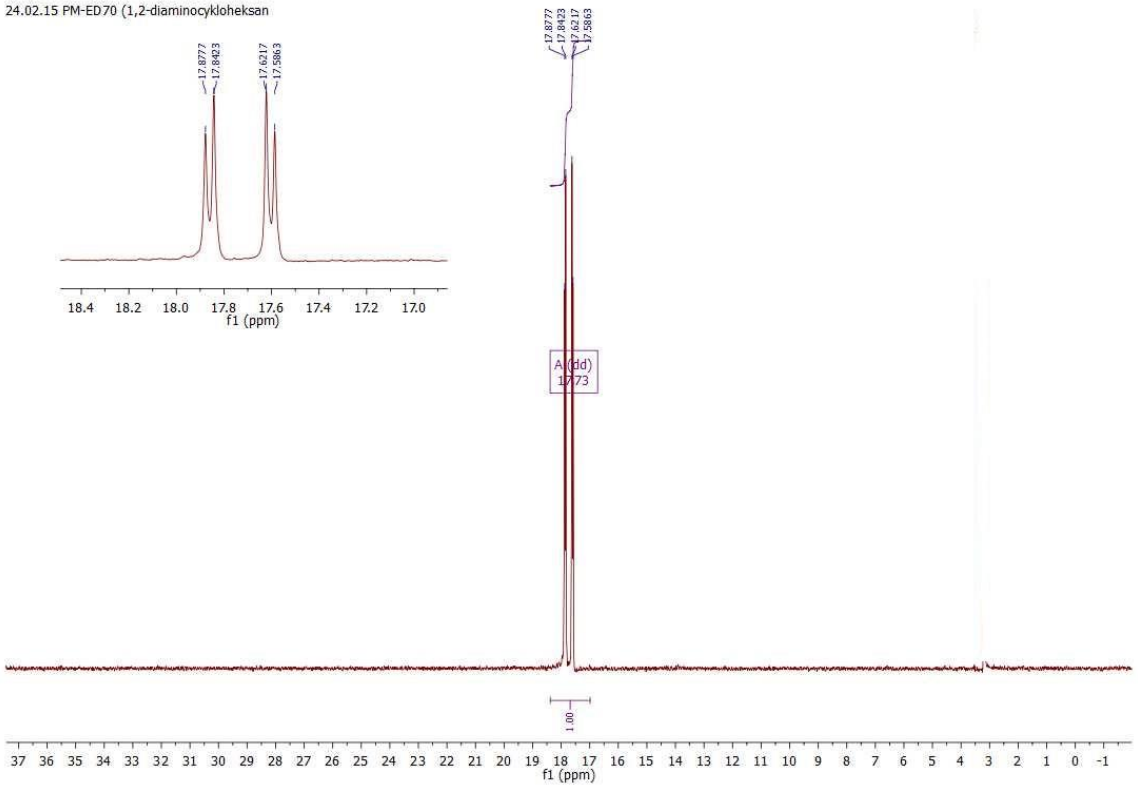

20.10.15 PM-153-kwas-woda-I
2.7245
2.6773
2.6471
2.4639
2.4639
2.4075
2.2998
2.2379
2.1770
2.0544
1.9869
1.9151
1.7130
1.6571
1.5965
1.5155
1.4379
1.1806
1.0903
1.0050
0.9404
0.8832
0.8214
0.7480
0.98
0.97
0.98
0.98
1.01
1.97
3.05
0.92
f1 (ppm)

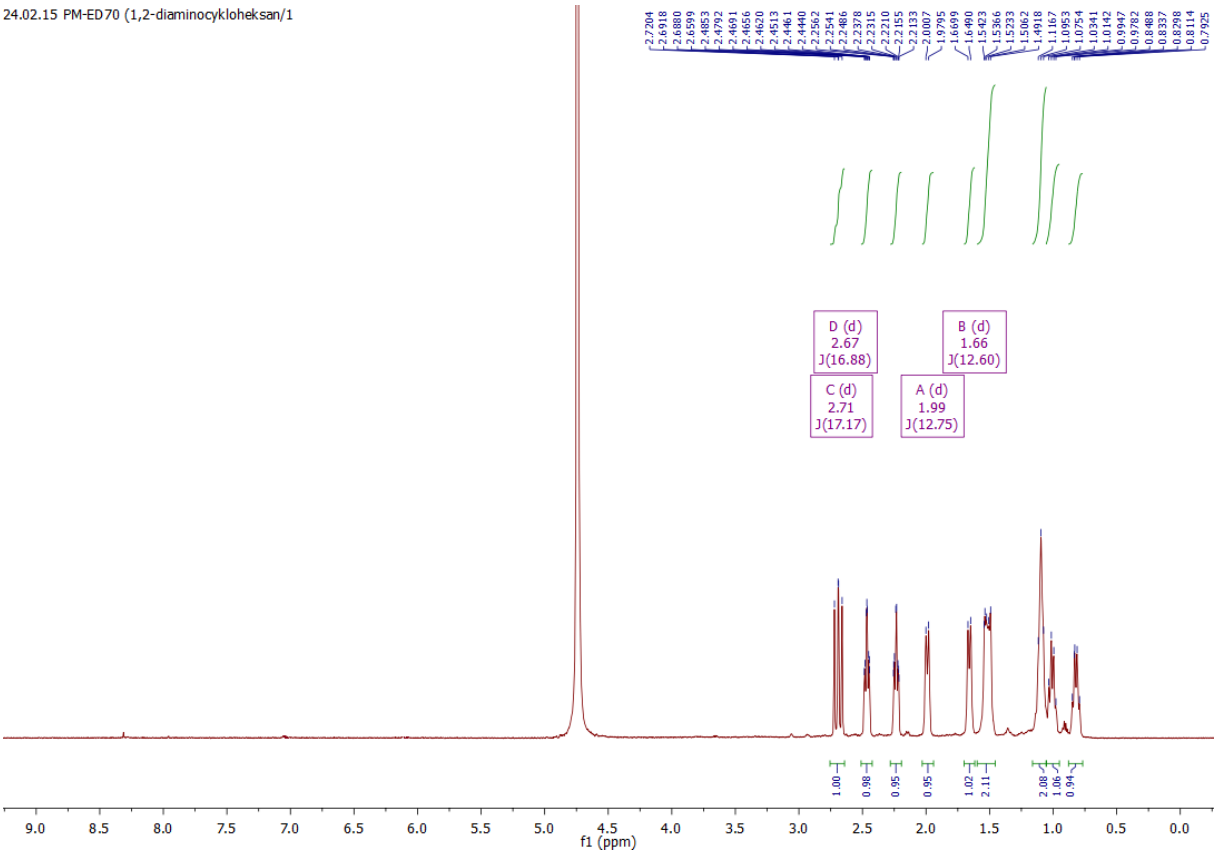

63.0582
57.5578
56.7425
56.6813
55.8656
55.3168
33.5548
30.4191
24.6677
24.4402
B (d)
56.27
J(123.20)
A (d)
57.15
J(123.14)
f1 (ppm)

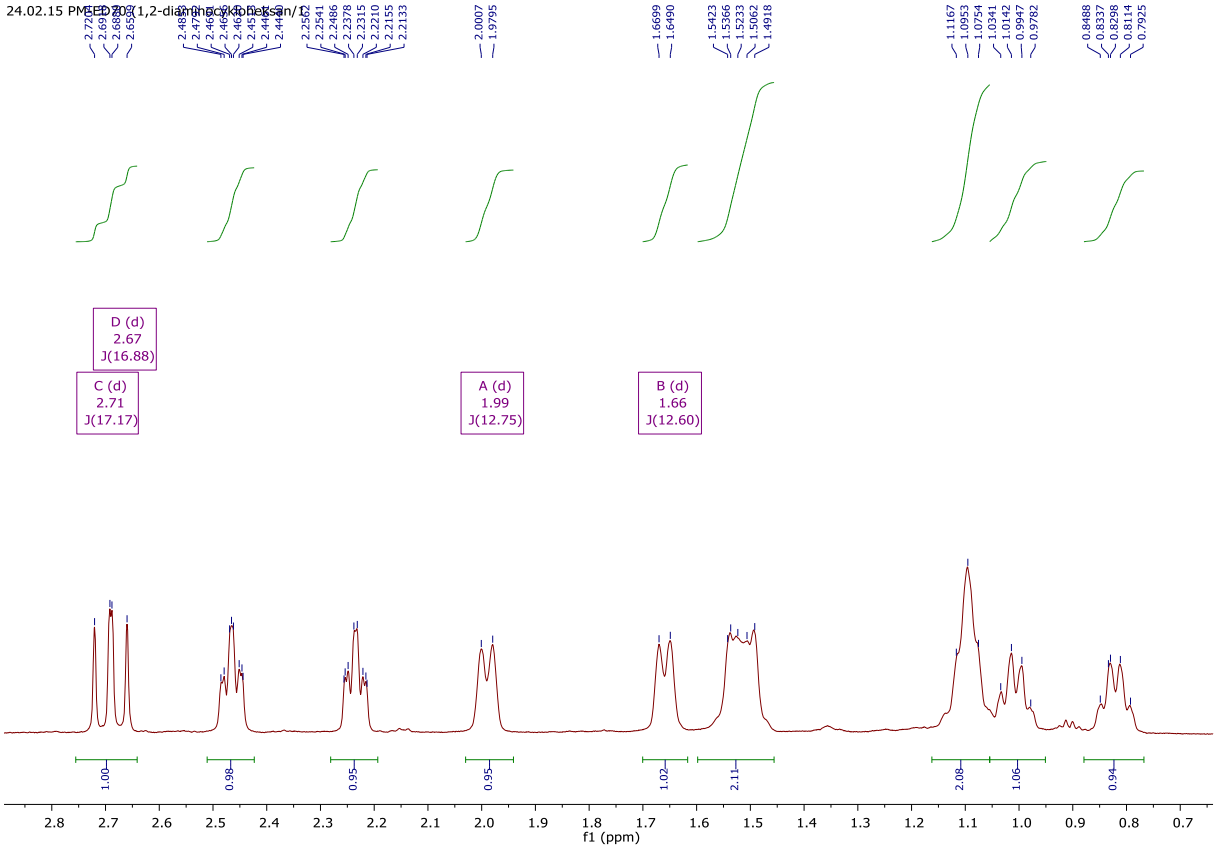




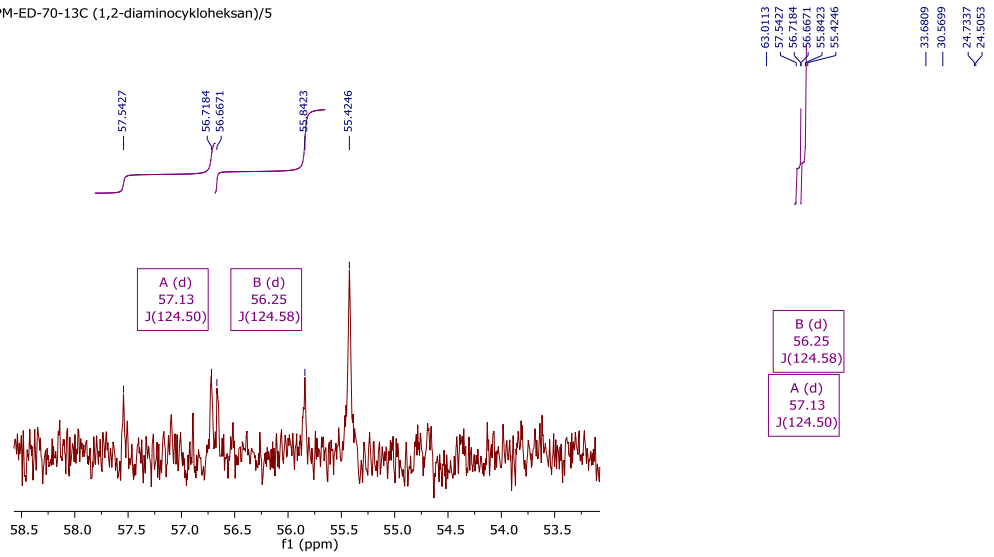
(±)-(trans)-Cyclohexane-1-amino-2-aminomethylenebisphosphonic acid (*trans*-**8**)

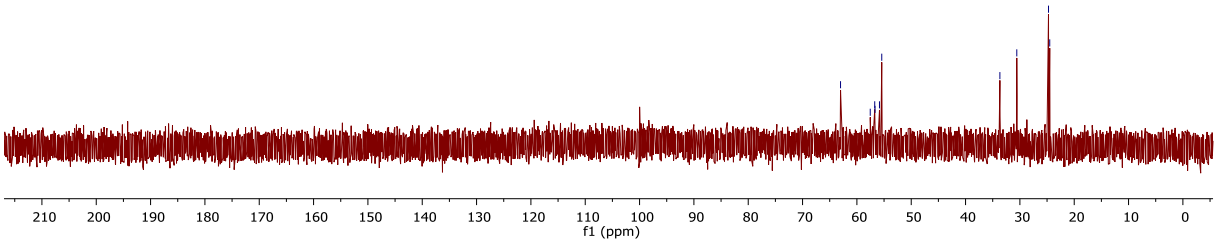


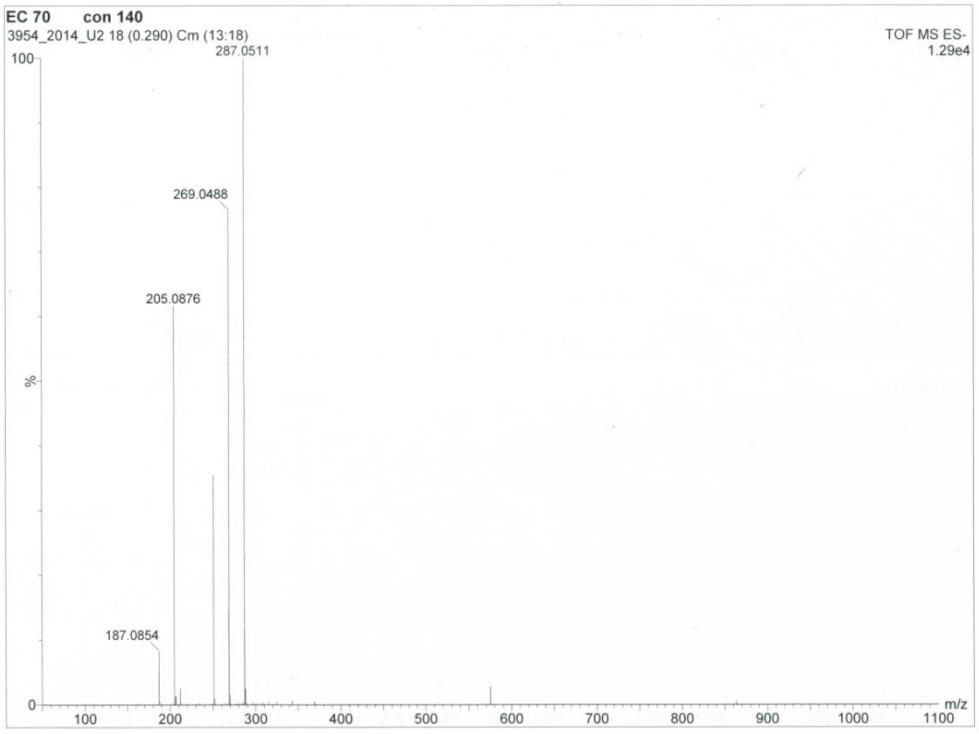

24.02.15 PM-ED70 (1,2-diaminocykloheksan/1
D (d)
2.67
J(16.88)
C (d)
2.71
J(17.17)
A (d)
1.99
J(12.75)
B (d)
1.66
J(12.60)
f1 (ppm)

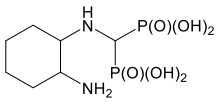

D (d)
2.67
J(16.88)
C (d)
2.71
J(17.17)
A (d)
1.99
J(12.75)
B (d)
1.66
J(12.60)
f1 (ppm)

03.03.15 PM-ED-70-13C (1,2-diaminocykloheksan)/5

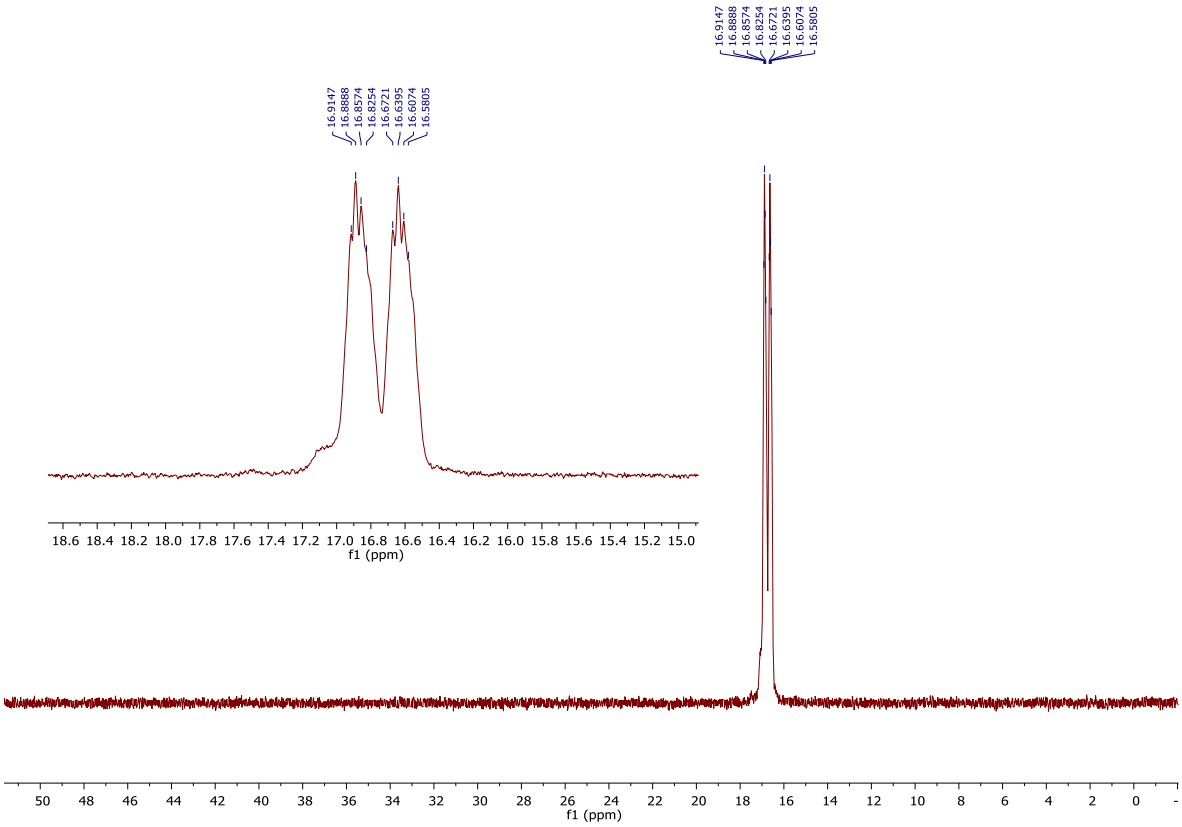


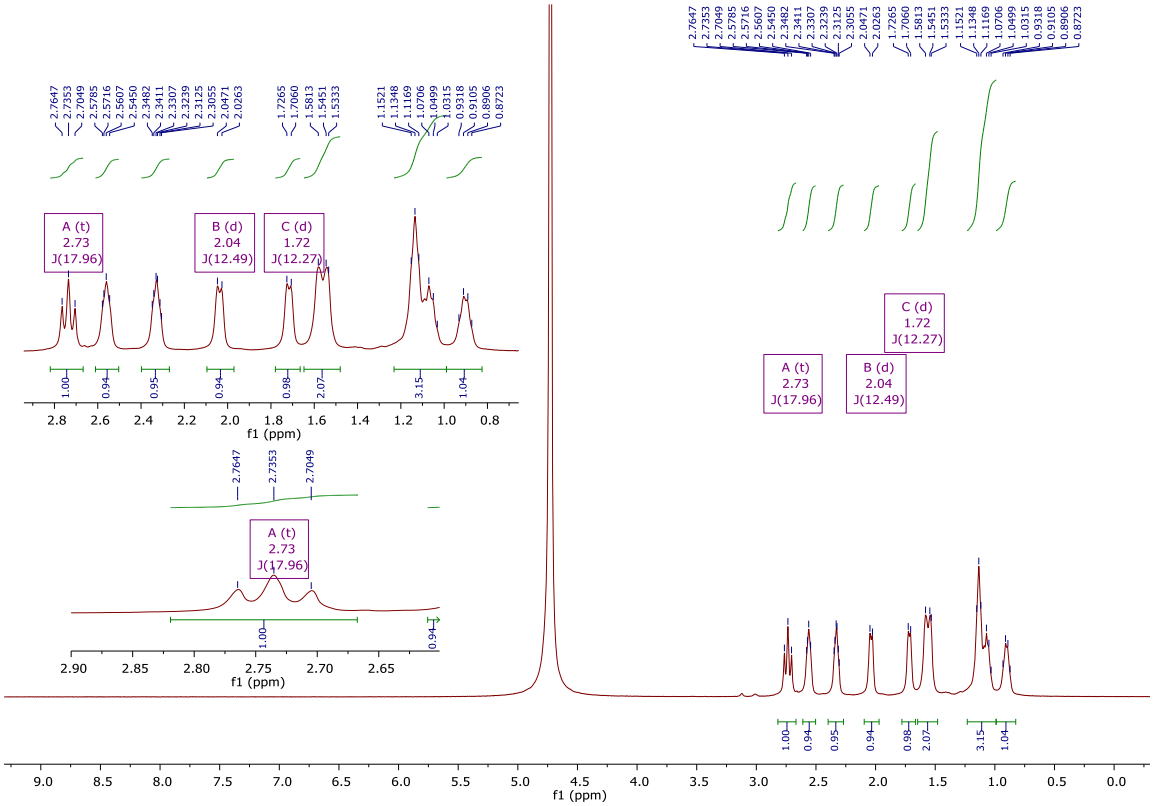


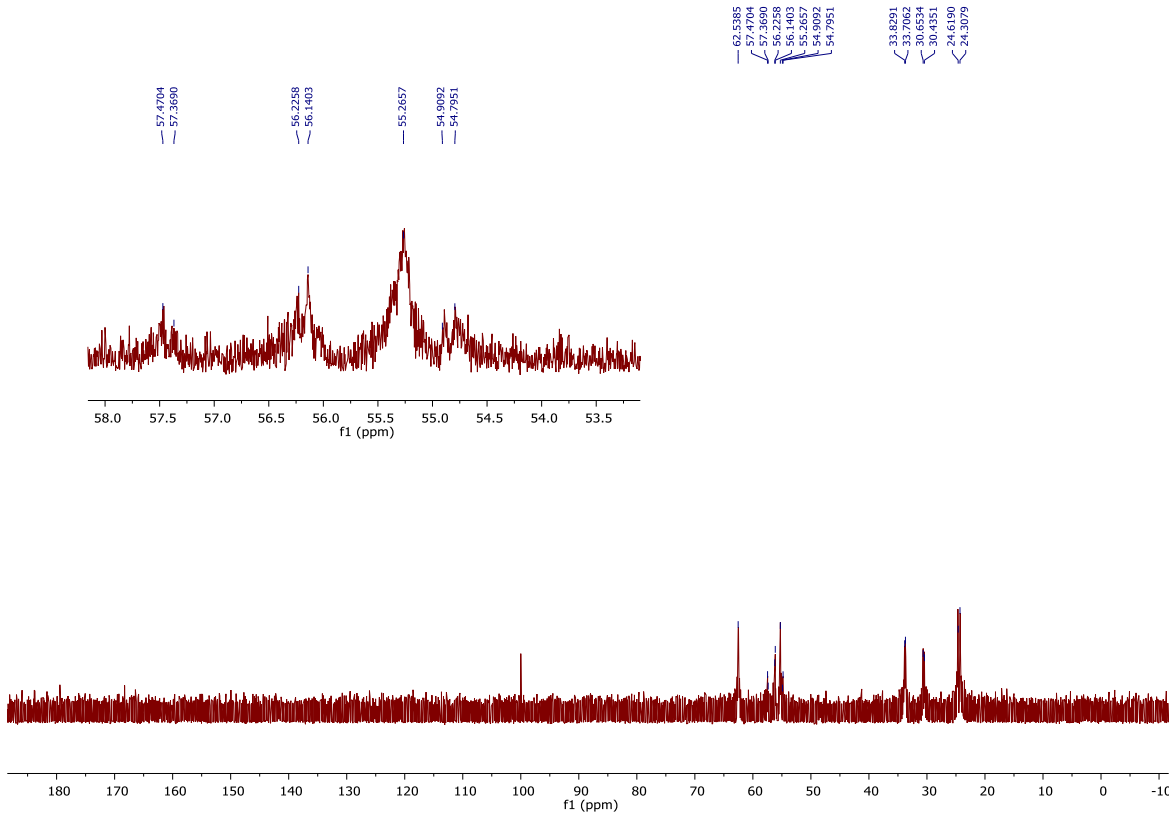


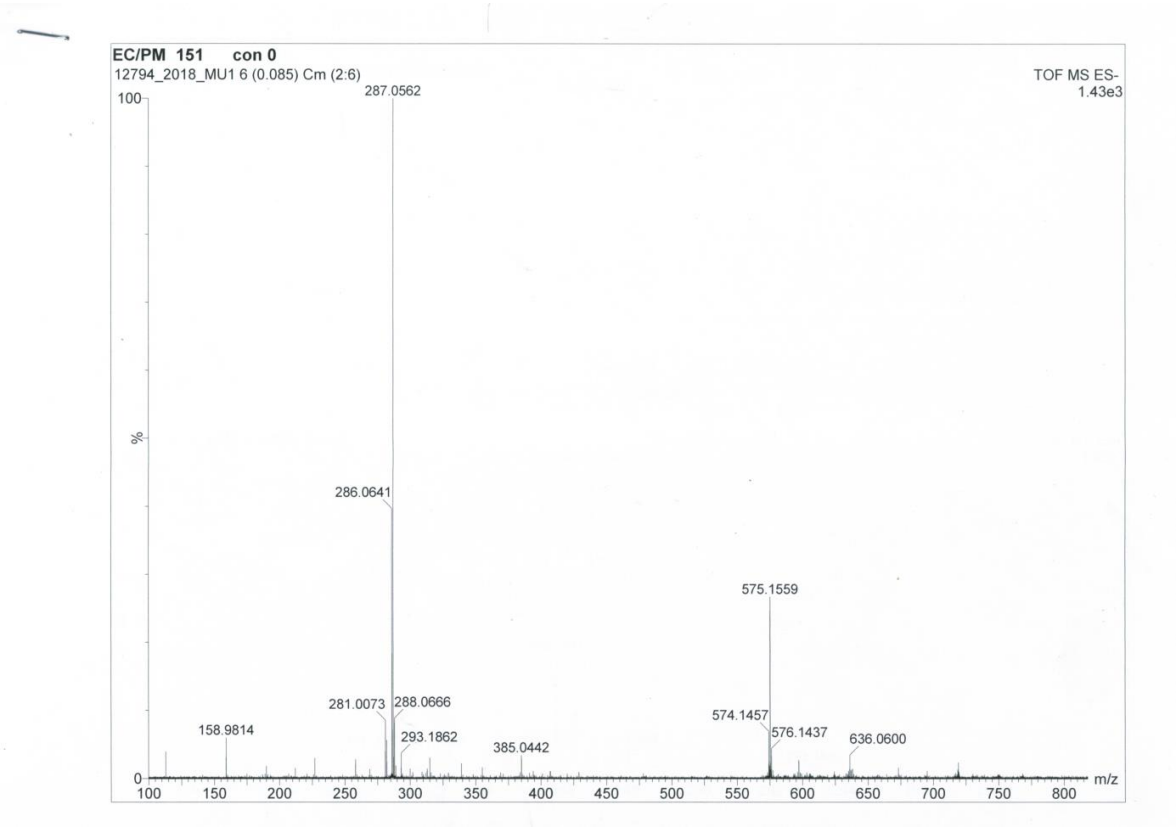


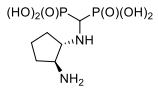
Racemic cyclohexane-1-amino-2-aminomethylenebisphosphonic acid [(rac)-**8**]



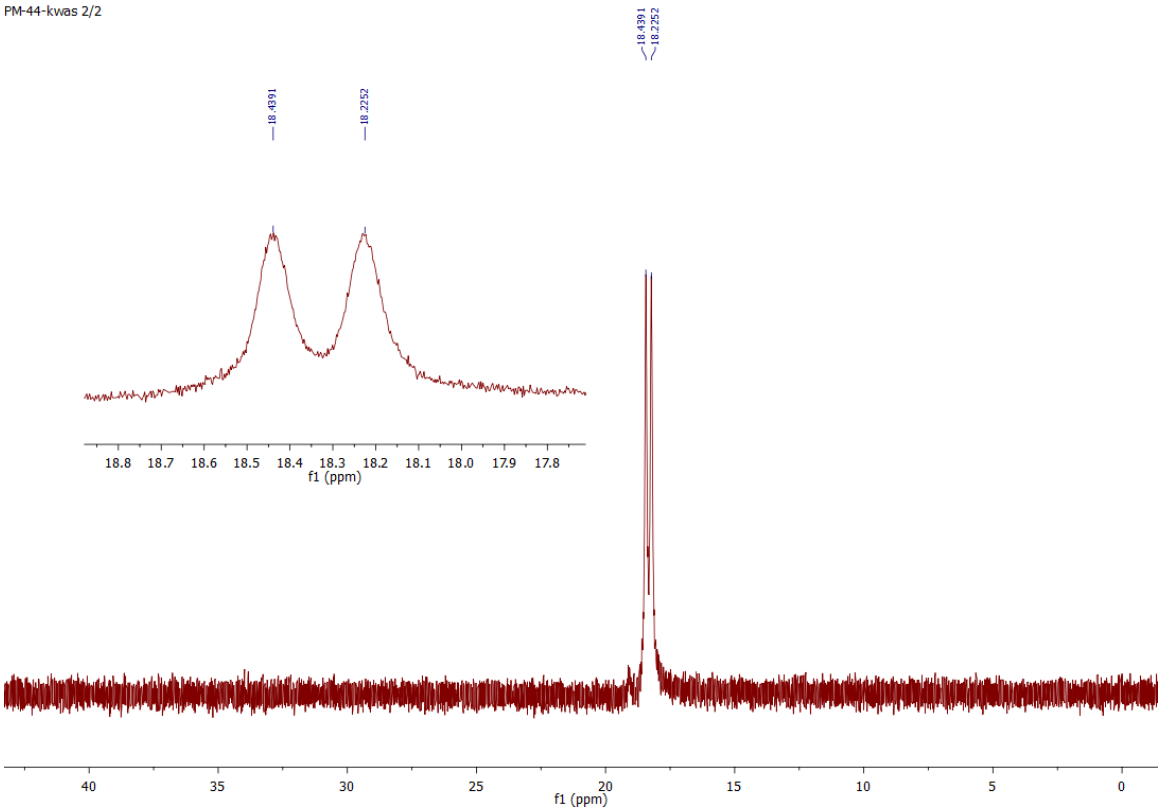


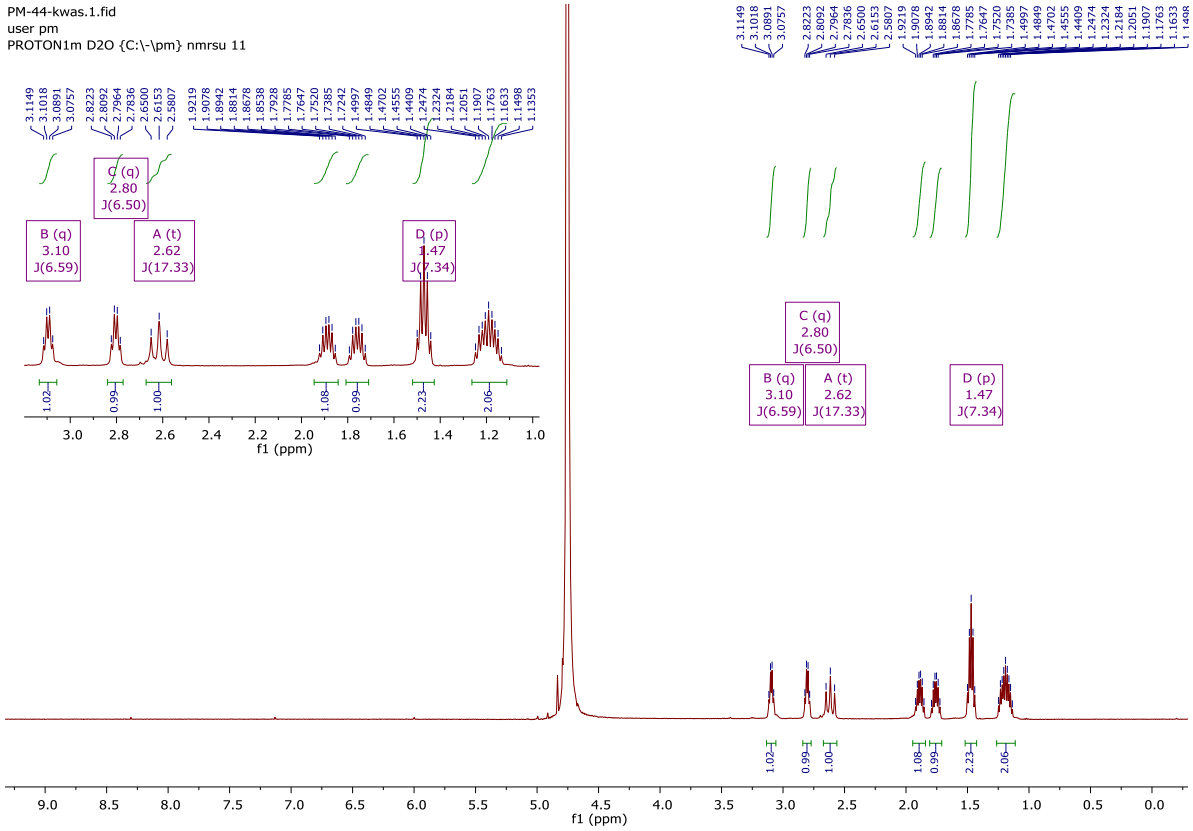

62.5385
57.4704
57.3690
56.2258
56.1403
55.2657
54.9092
54.7951
33.8291
33.7062
30.6534
30.4351
24.6190
24.3079
57.4704
57.3690
56.2258
56.1403
55.2657
54.9092
54.7951
58.0
57.5
57.0
56.5
56.0
55.5
55.0
54.5
54.0
53.5
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

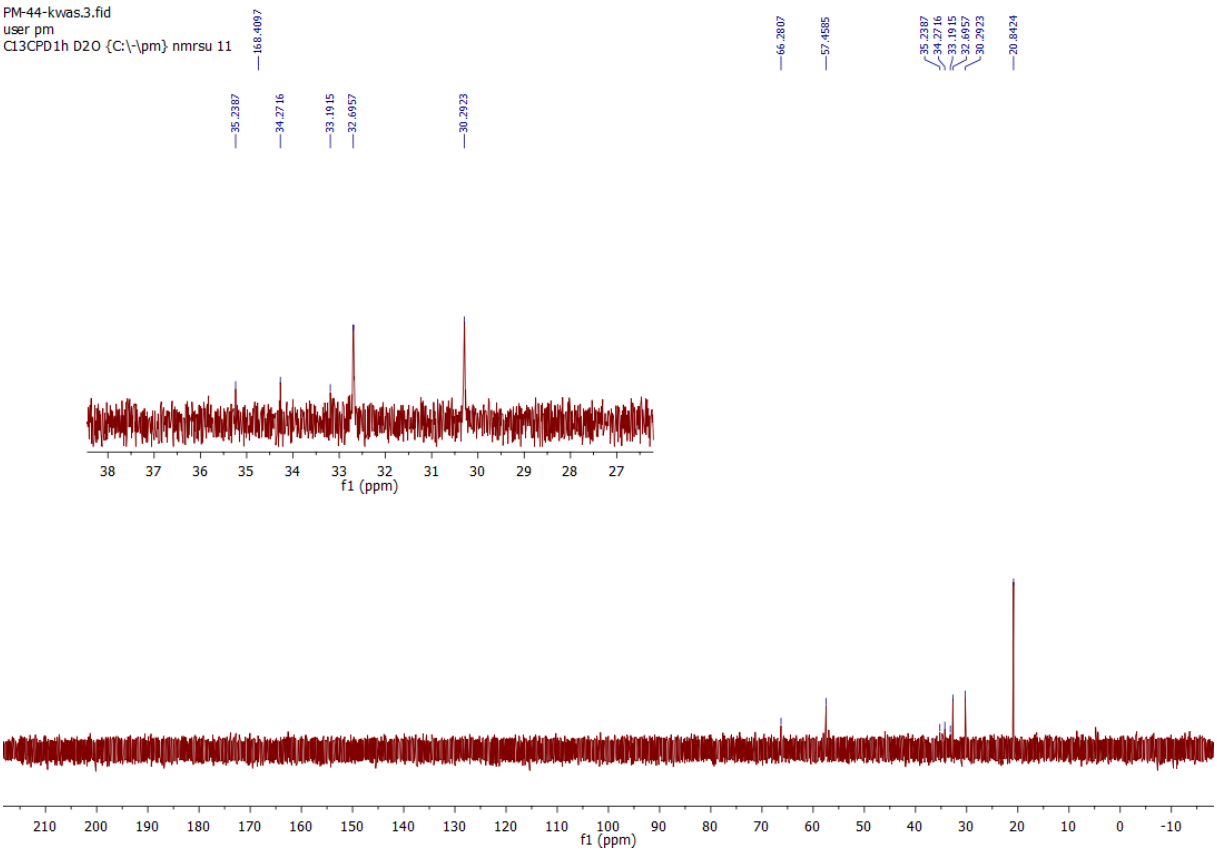

EC/PM 151 con 0
12794_2018_MU1 6 (0.085) Cm (2:6)
TOF MS ES-
1.43e3
100
%
0
287.0562
286.0641
575.1559
281.0073
288.0666
158.9814
293.1862
385.0442
574.1457
576.1437
636.0600
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z

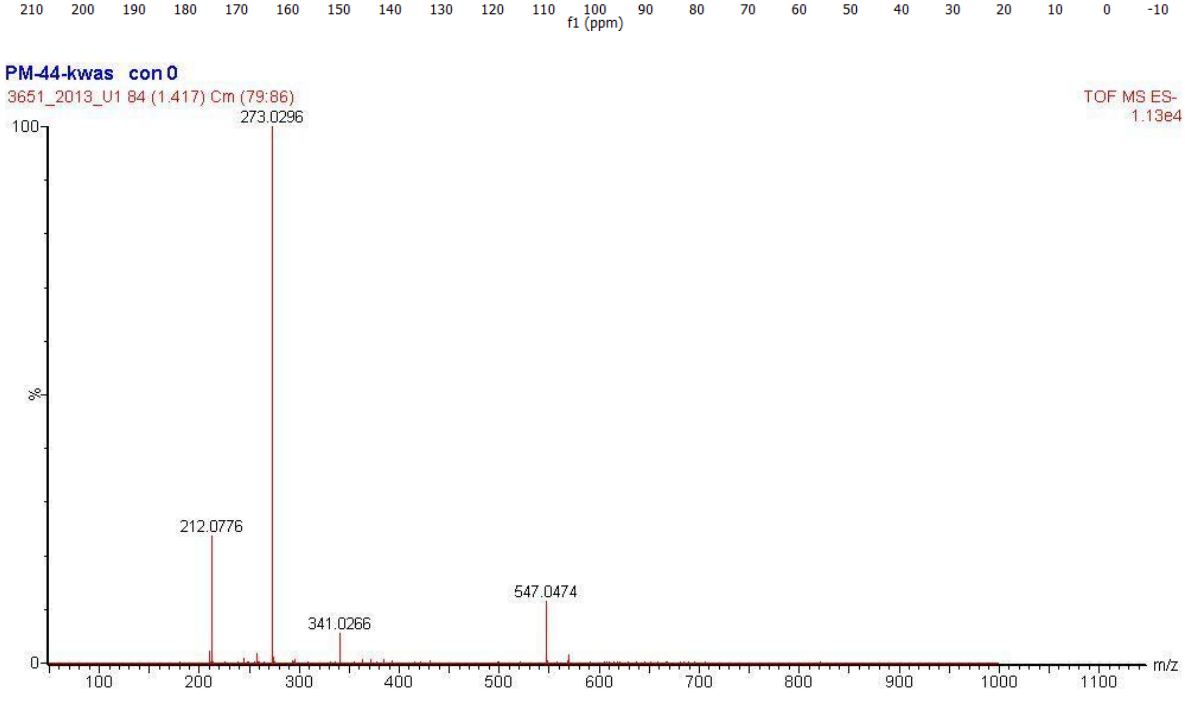


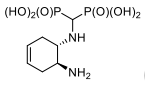
(*1S,2S*)-Cyclopentane-1-amino-2-aminomethylenebisphosphonic acid [(1S,2S)-**9]**



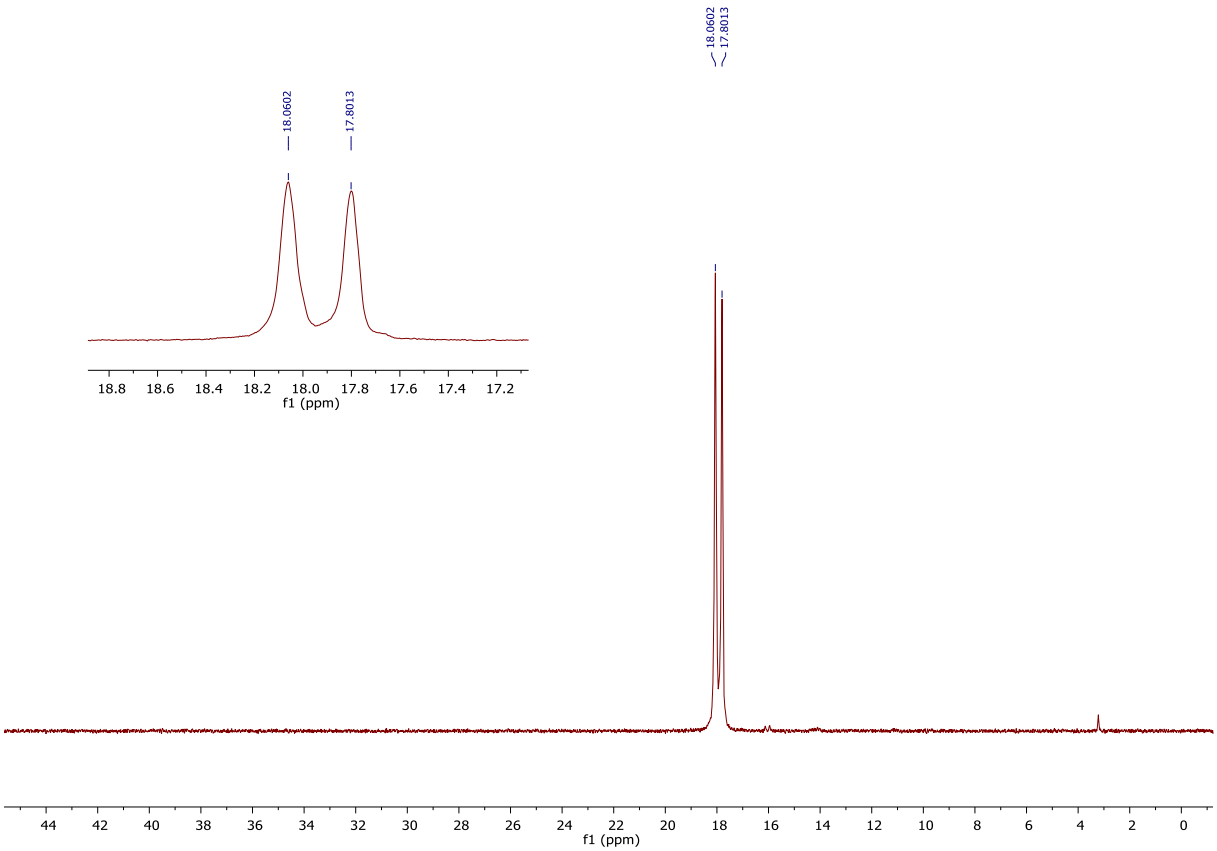


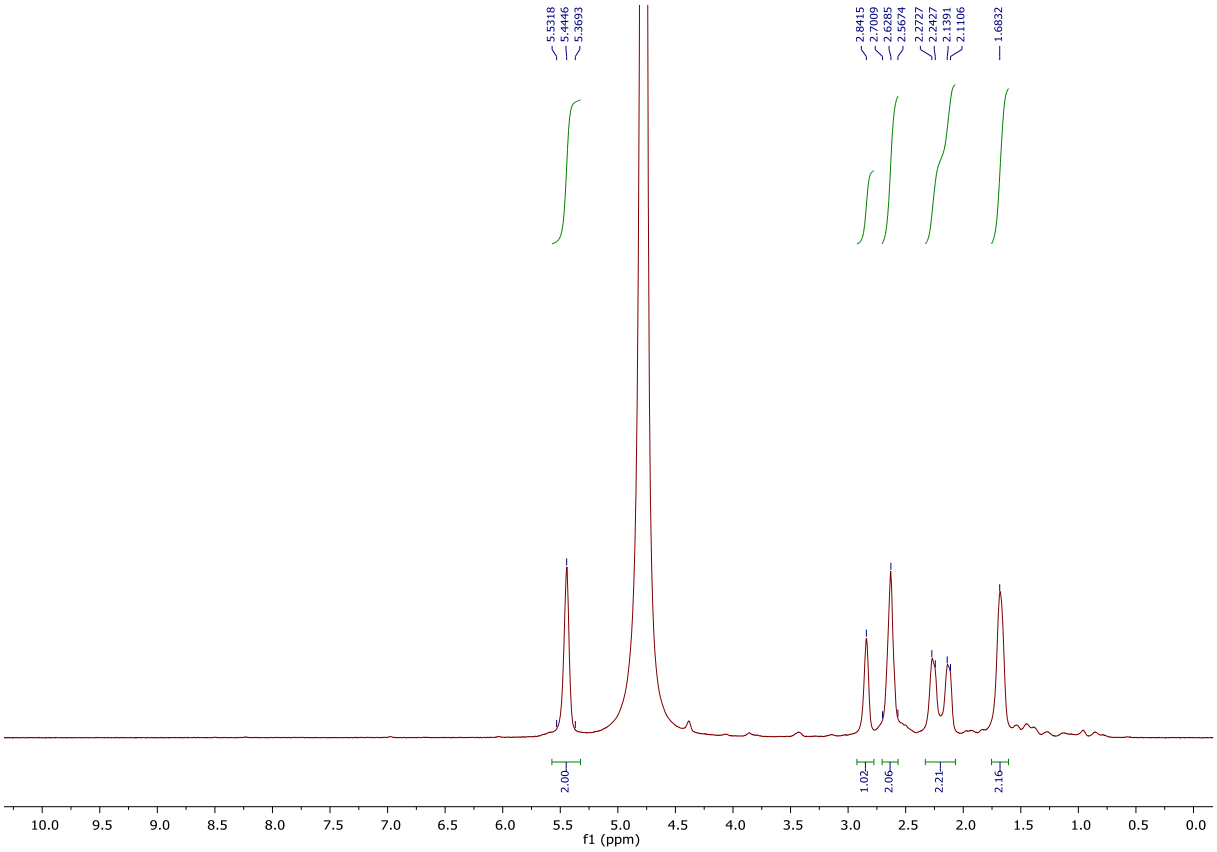

PM-44-kwas.3.fid
user pm
C13CPD1h D2O {C:\~\pm} nmrsu 11
168.4097
66.2807
57.4585
35.2387
34.2716
33.1915
32.6957
30.2923
20.8424
f1 (ppm)
f1 (ppm)

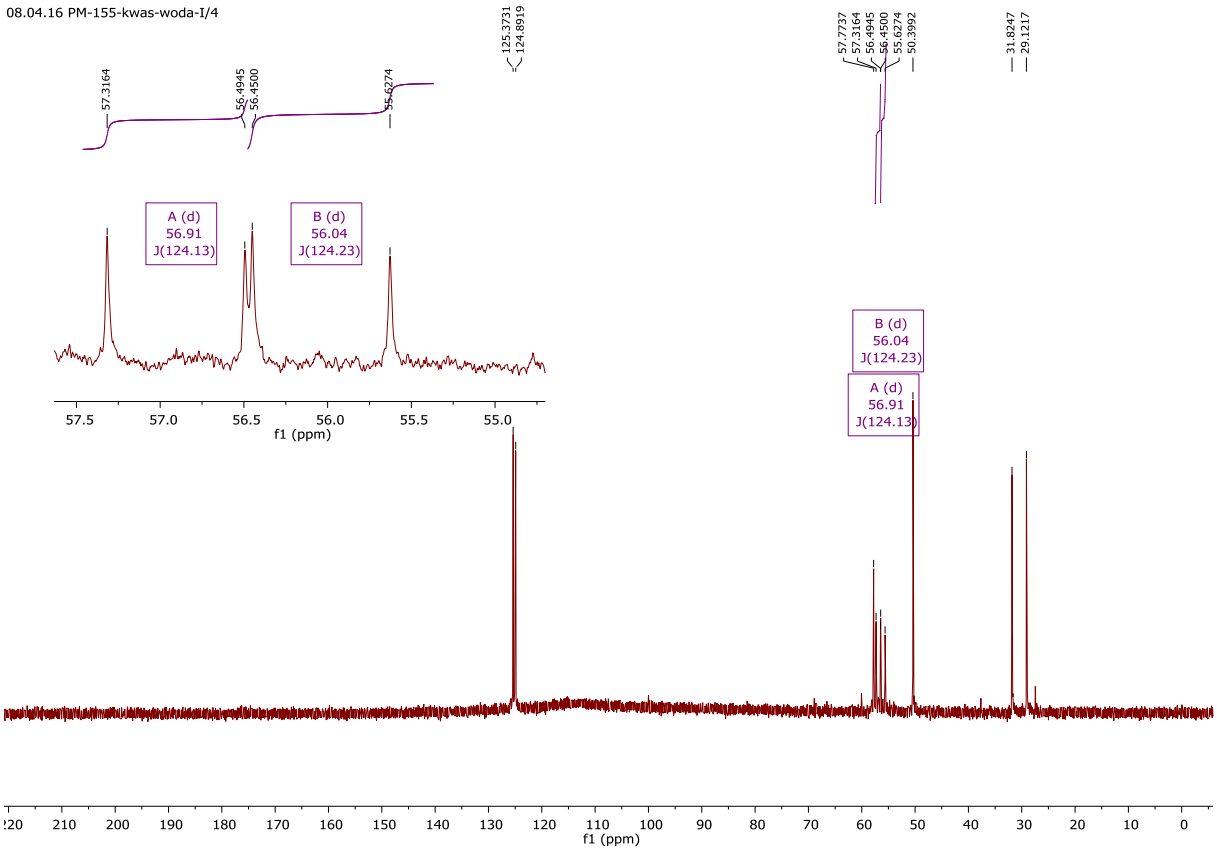

PM-44-kwas con 0
3651_2013_U1 84 (1.417) Cm (79:86)
TOF MS ES-
1.13e4
273.0296
212.0776
547.0474
341.0266
%
m/z

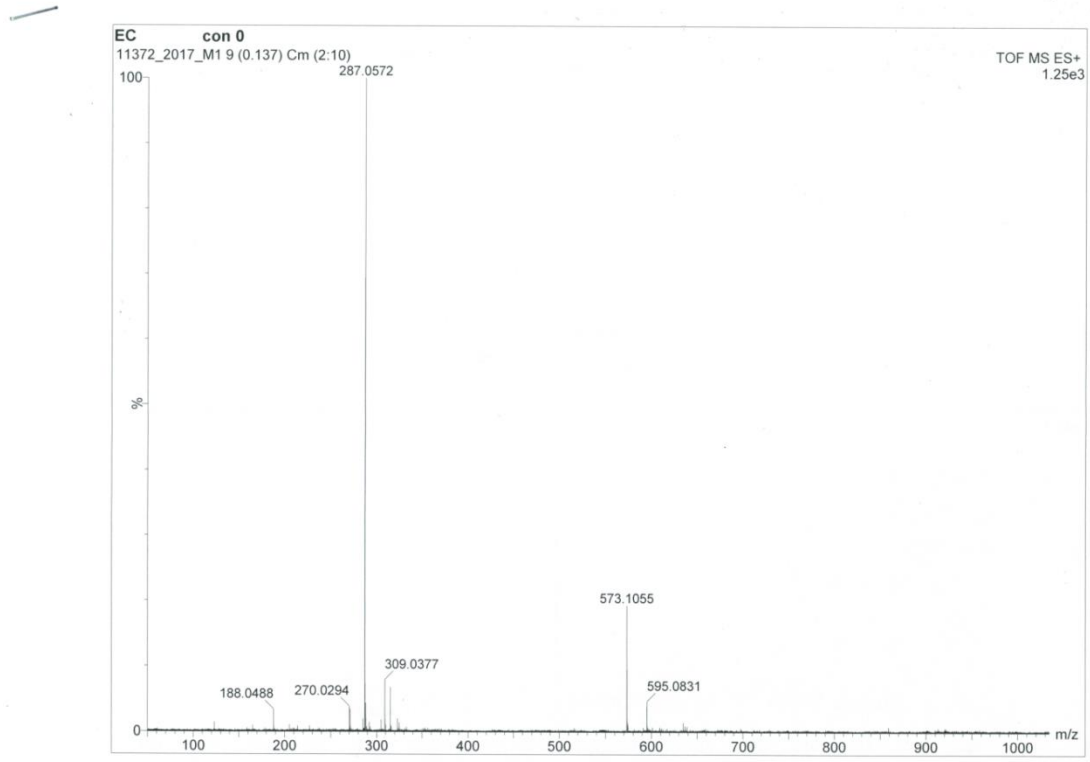


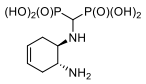
(*1S,2S*)(+)- 4-Cyclohexene-1-amino-2-aminomethylenebisphosphonic acid [(1S,2S)-**10]**



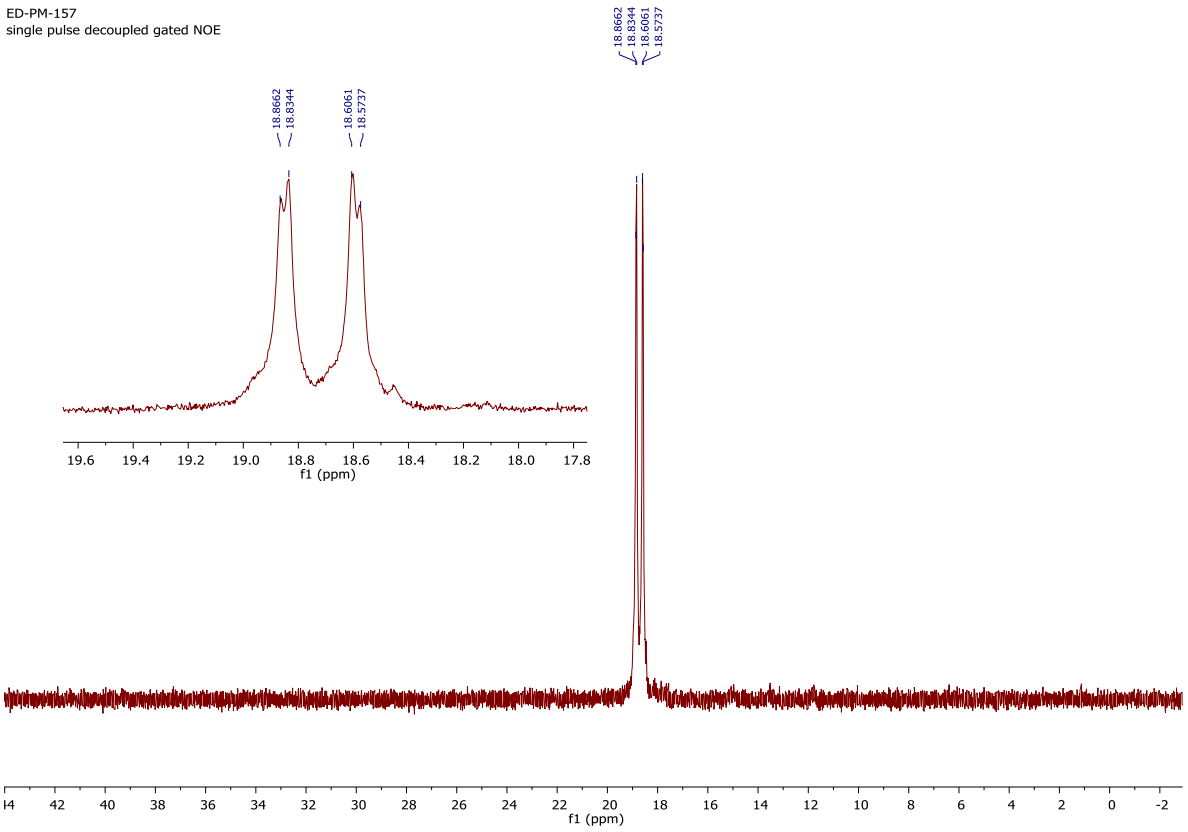


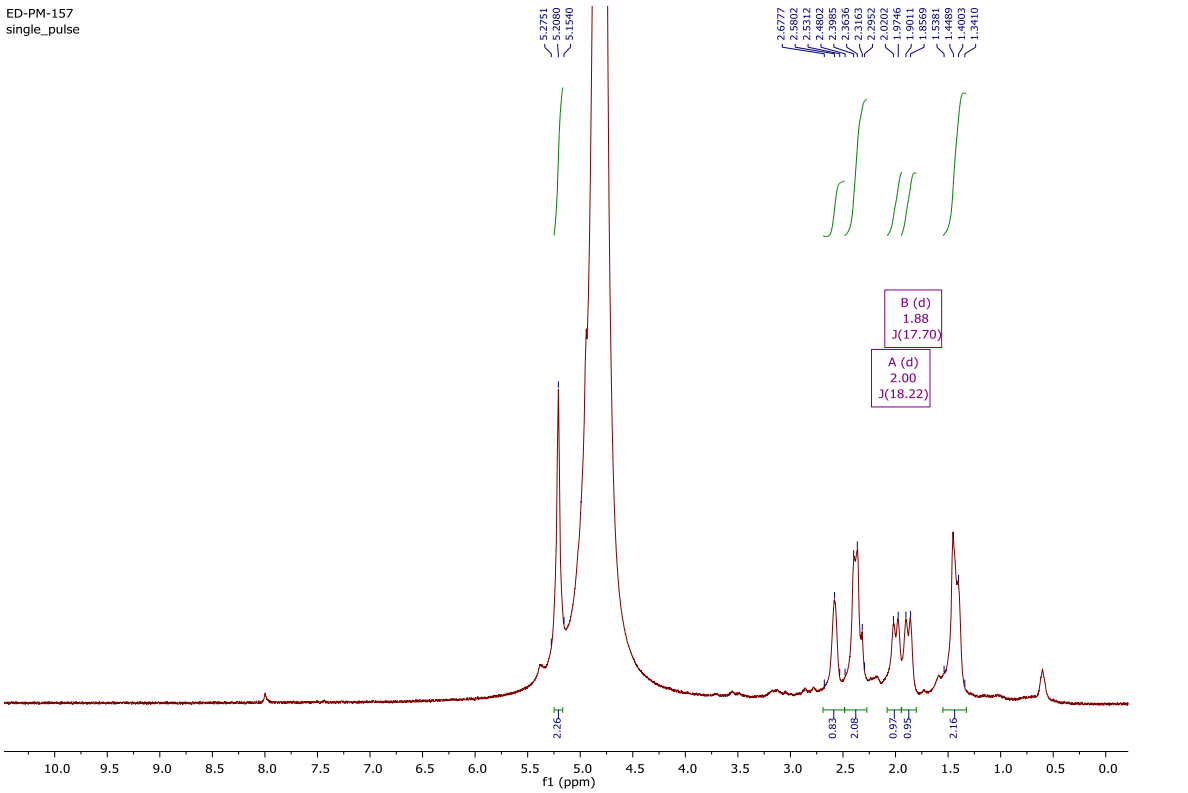

08.04.16 PM-155-kwas-woda-I/4
125.3731
124.8919
57.7737
57.3164
56.4945
56.4500
55.6274
50.3992
31.8247
29.1217
57.3164
56.4945
56.4500
55.6274
A (d)
56.91
J(124.13)
B (d)
56.04
J(124.23)
57.5
57.0
56.5
56.0
55.5
55.0
f1 (ppm)
B (d)
56.04
J(124.23)
A (d)
56.91
J(124.13)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


EC
con 0
11372_2017_M1 9 (0.137) Cm (2:10)
TOF MS ES+
1.25e3
100
%
0
287.0572
573.1055
309.0377
188.0488
270.0294
595.0831
100
200
300
400
500
600
700
800
900
1000
m/z

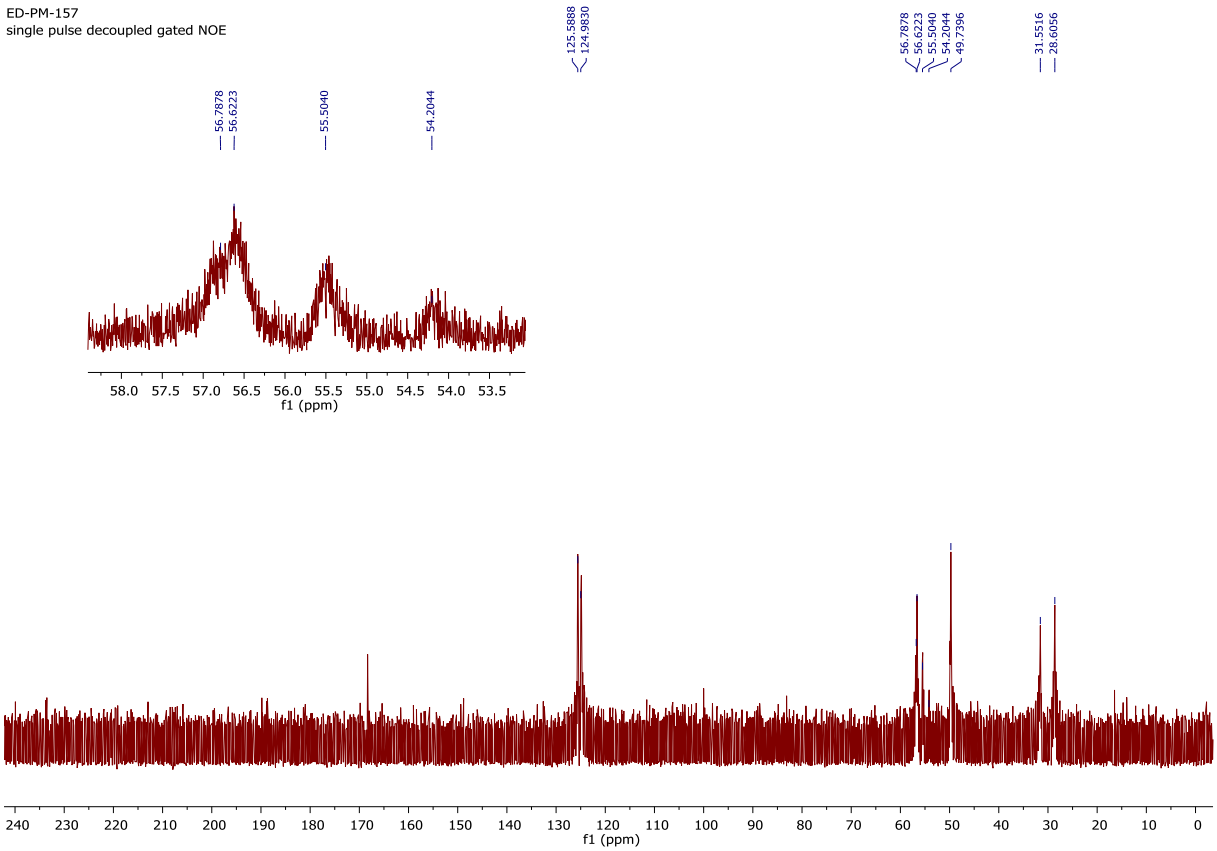


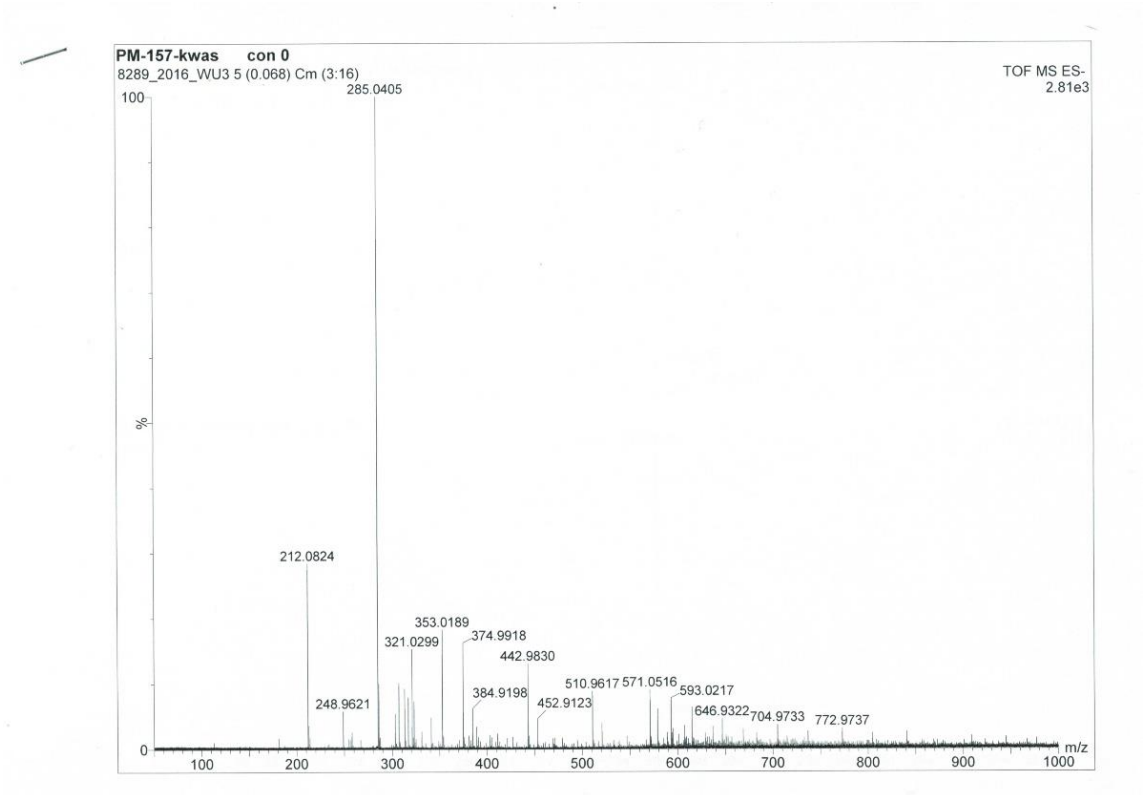
(1*R*,2*R*)- 4-Cyclohexene-1-amino-2-aminomethylenebisphosphonic acid [(1*R*,2*R*)-**10**]

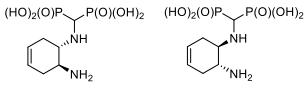




ED-PM-157
single pulse decoupled gated NOE

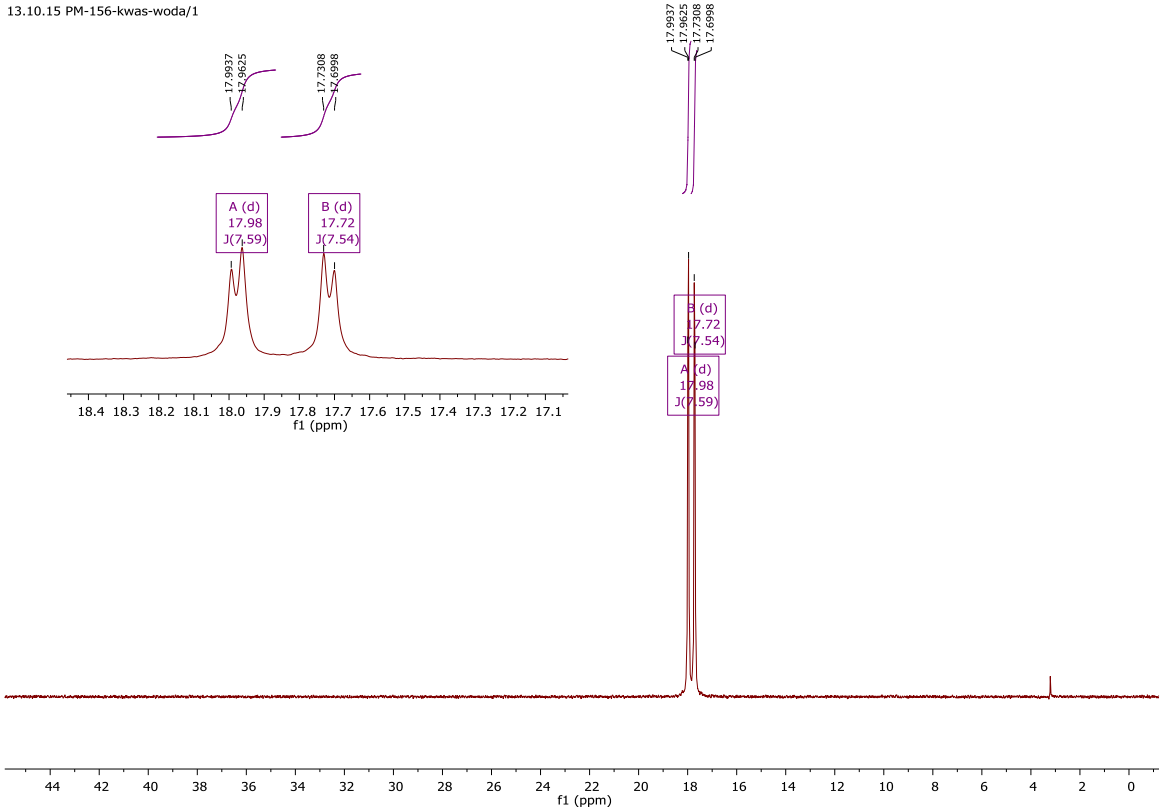


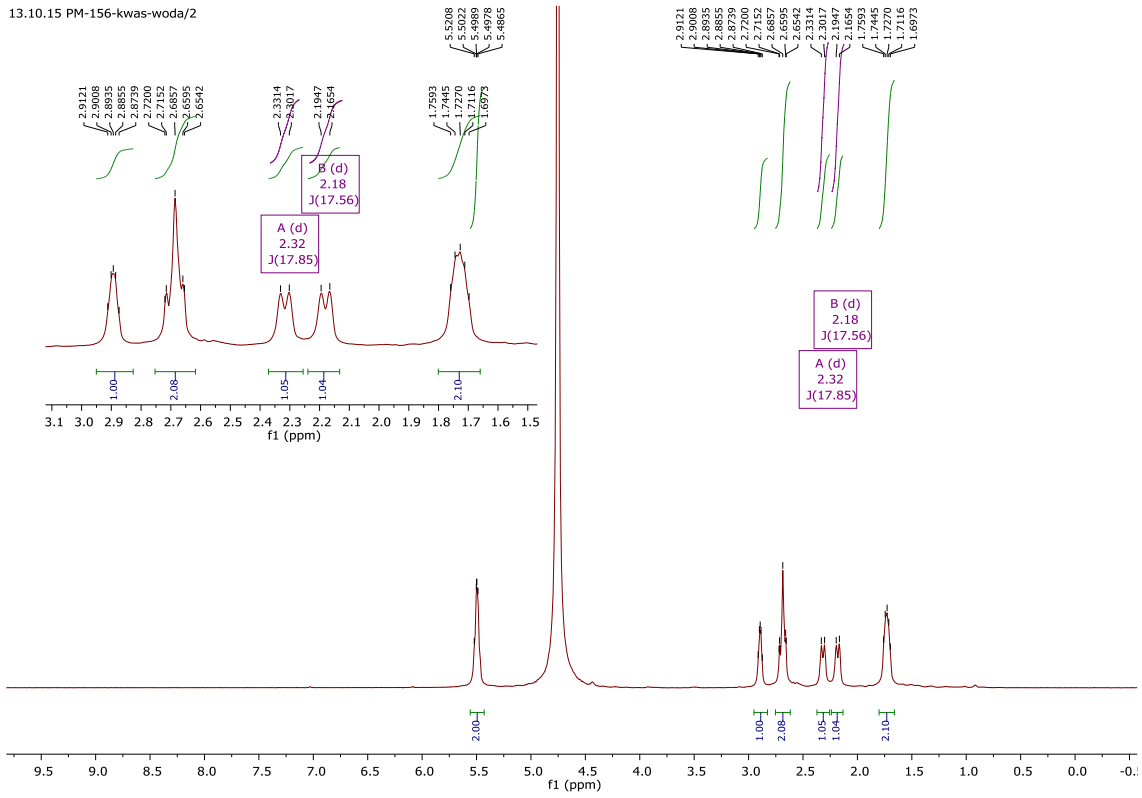


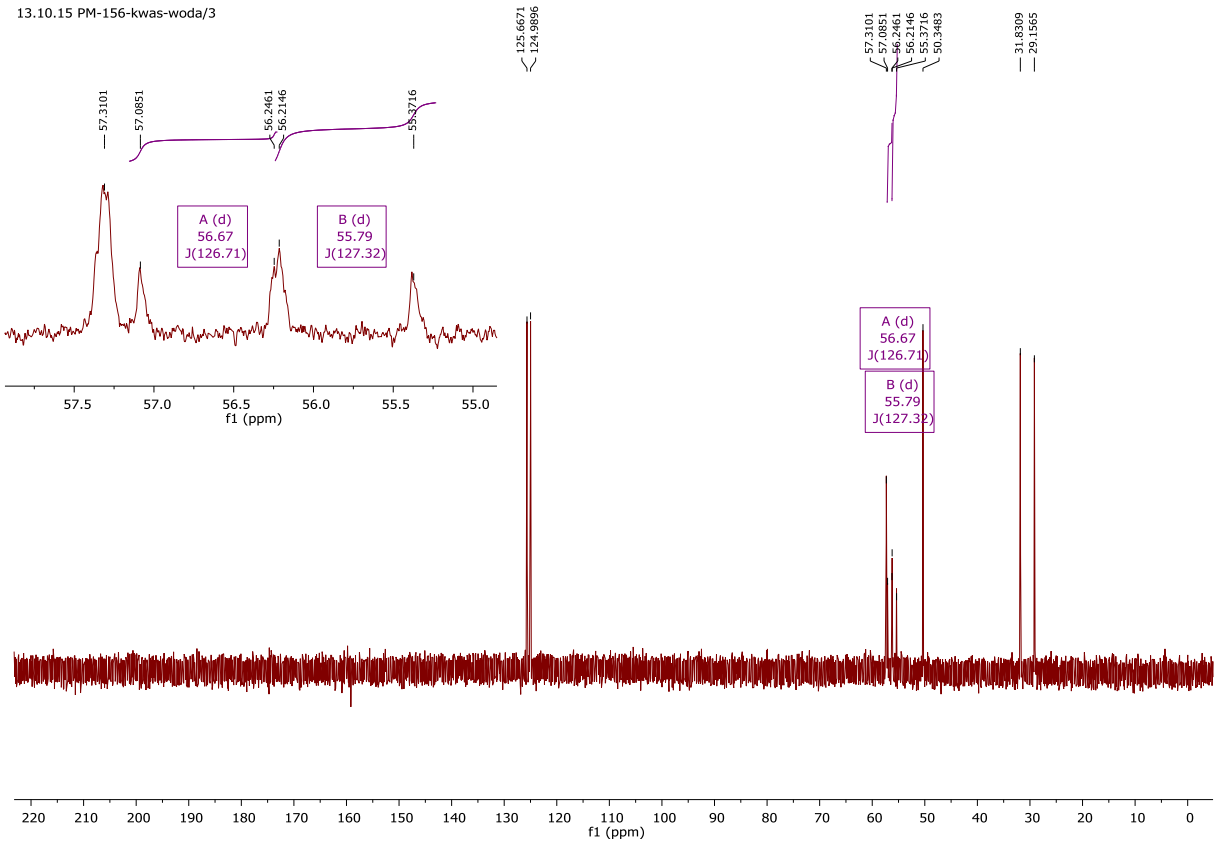


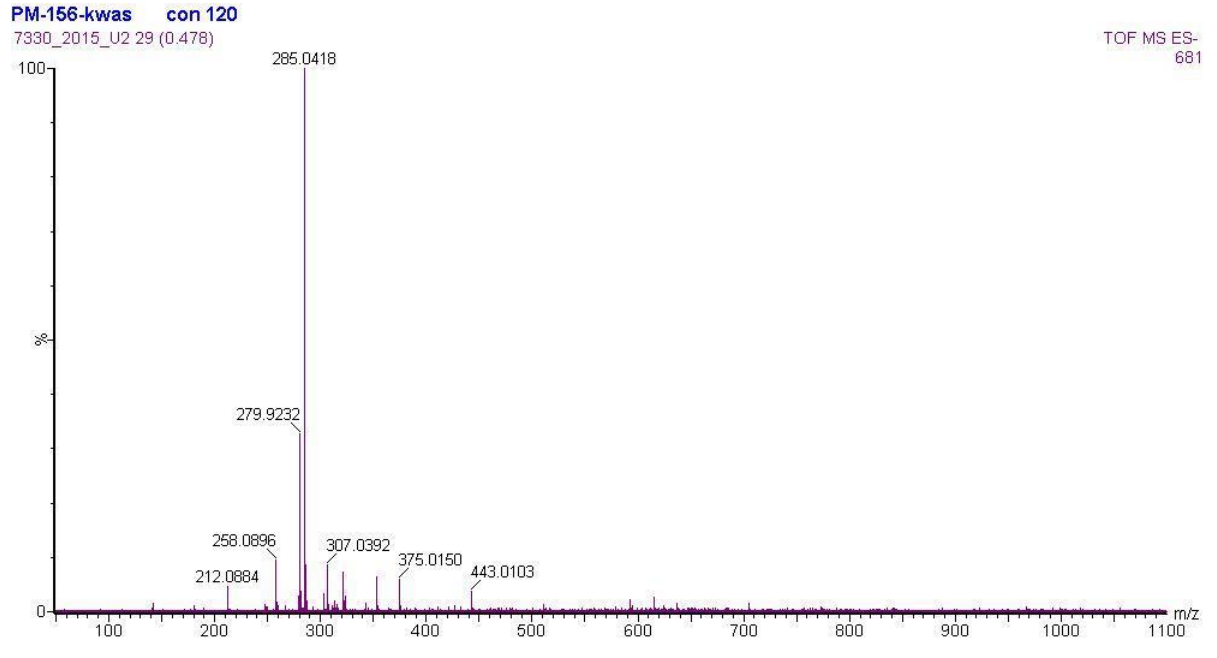
(±)-(trans)-4-Cyclohexene-1-amino-2-aminomethylenebisphosphonic acid

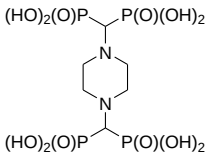
[(*trans*)-**10**]



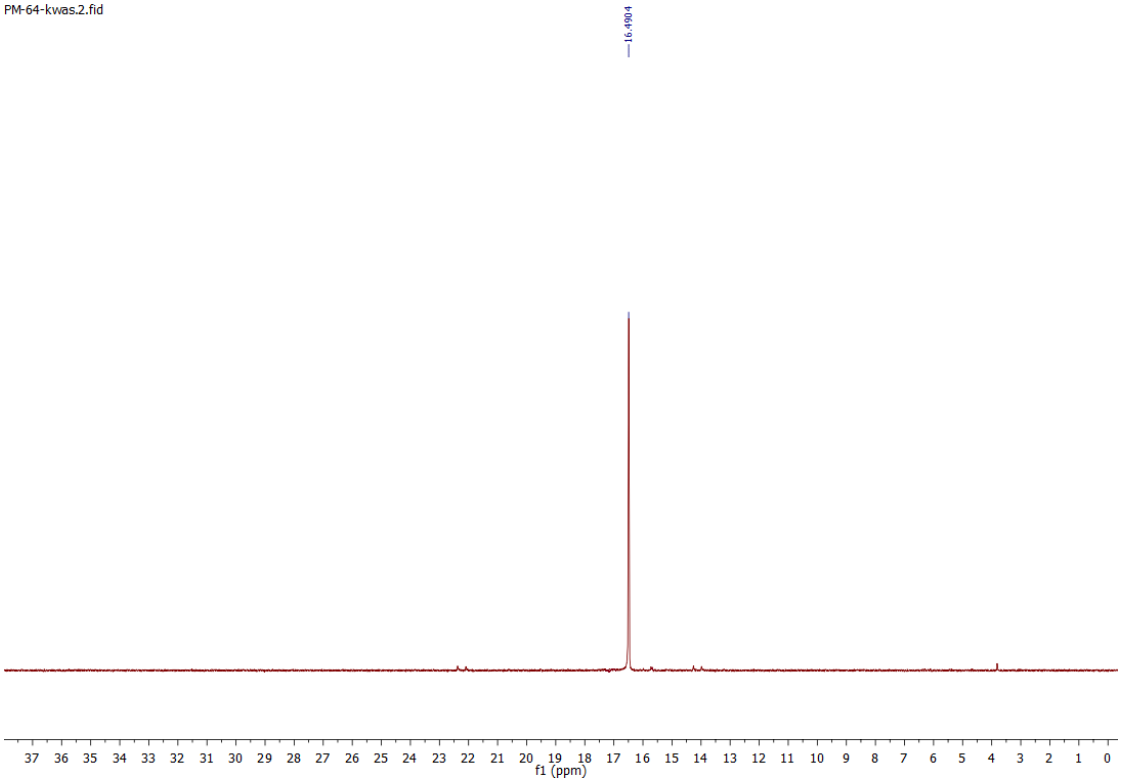


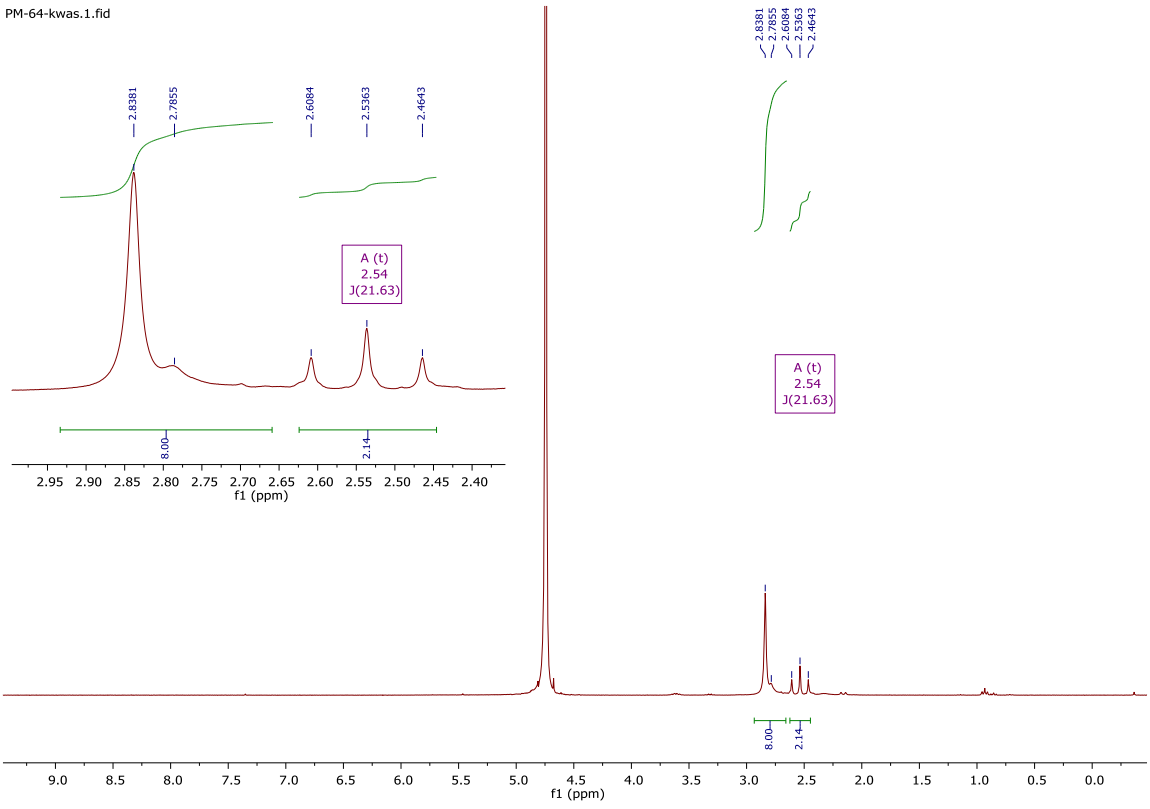

13.10.15 PM-156-kwas-woda/3
125.6671
124.9896
57.3101
57.0851
56.2461
56.2146
55.3716
50.3483
31.8309
29.1565
A (d)
56.67
J(126.71)
B (d)
55.79
J(127.32)
f1 (ppm)


PM-156-kwas con 120
7330_2015_U2 29 (0.478)
TOF MS ES-
681
285.0418
279.9232
258.0896
307.0392
375.0150
443.0103
212.0884
%
m/z

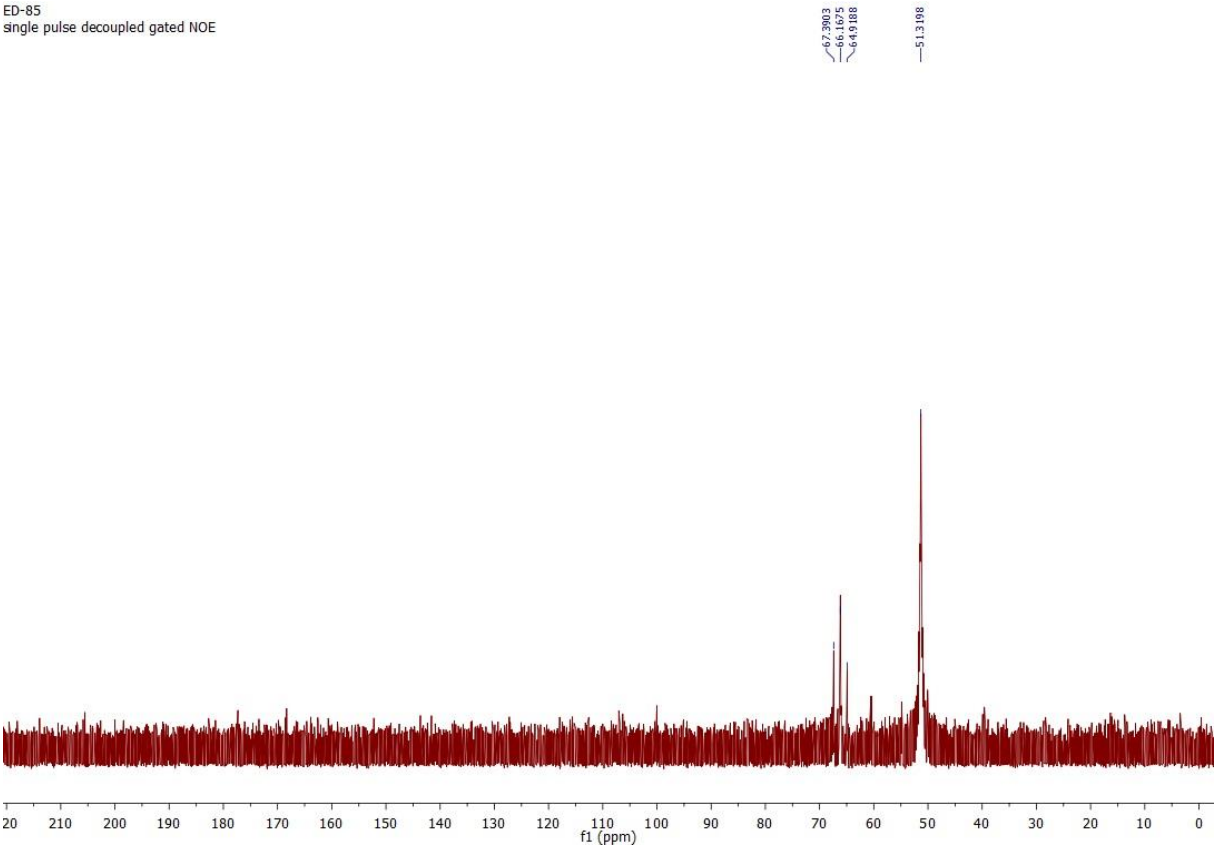


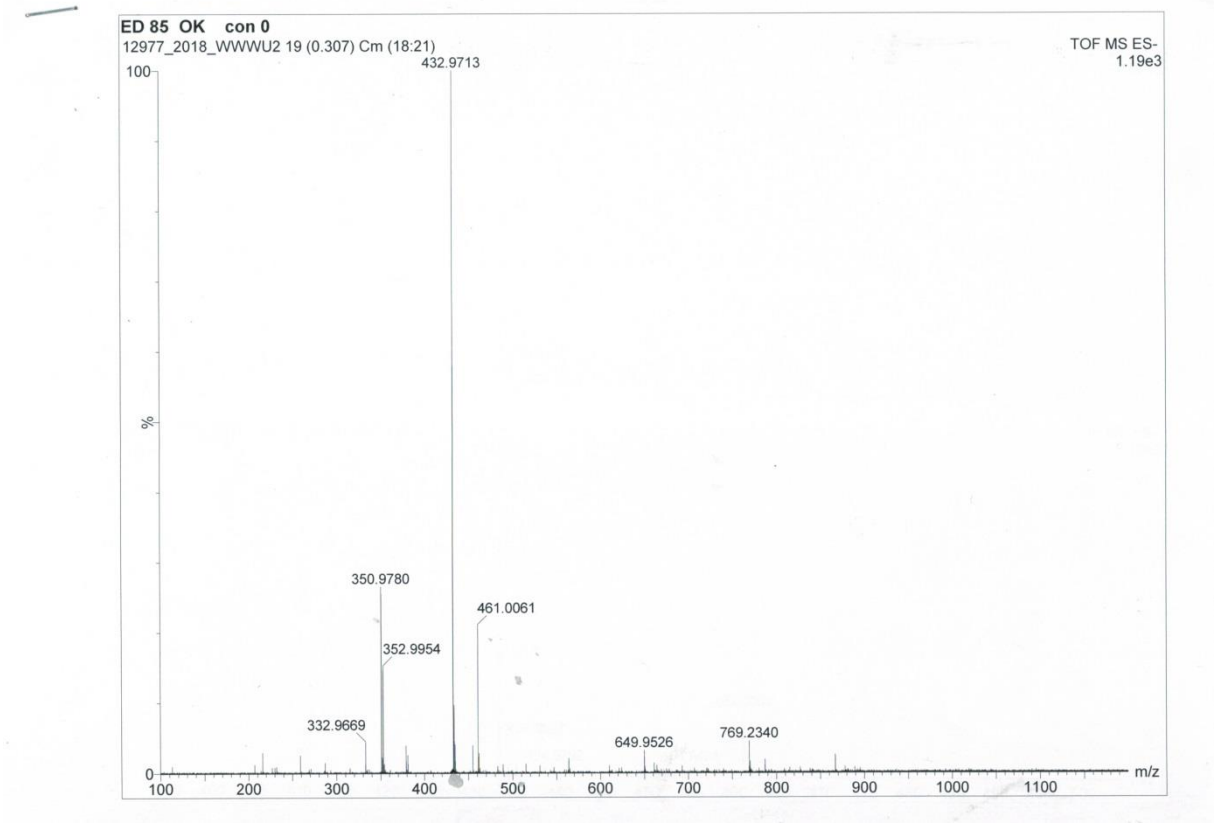
Piperaz-1,4-diylmethylenebisphosphonic acid (**11**)

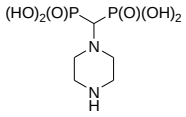




ED-85
single pulse decoupled gated NOE

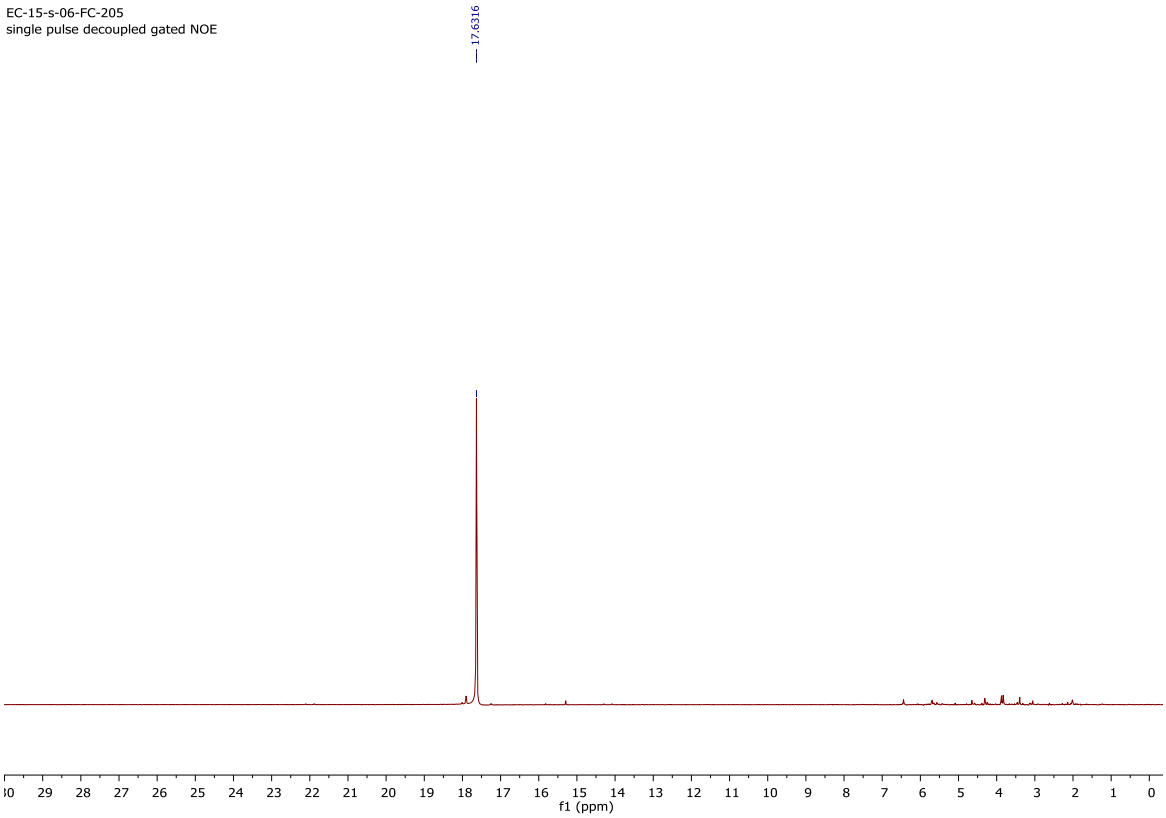




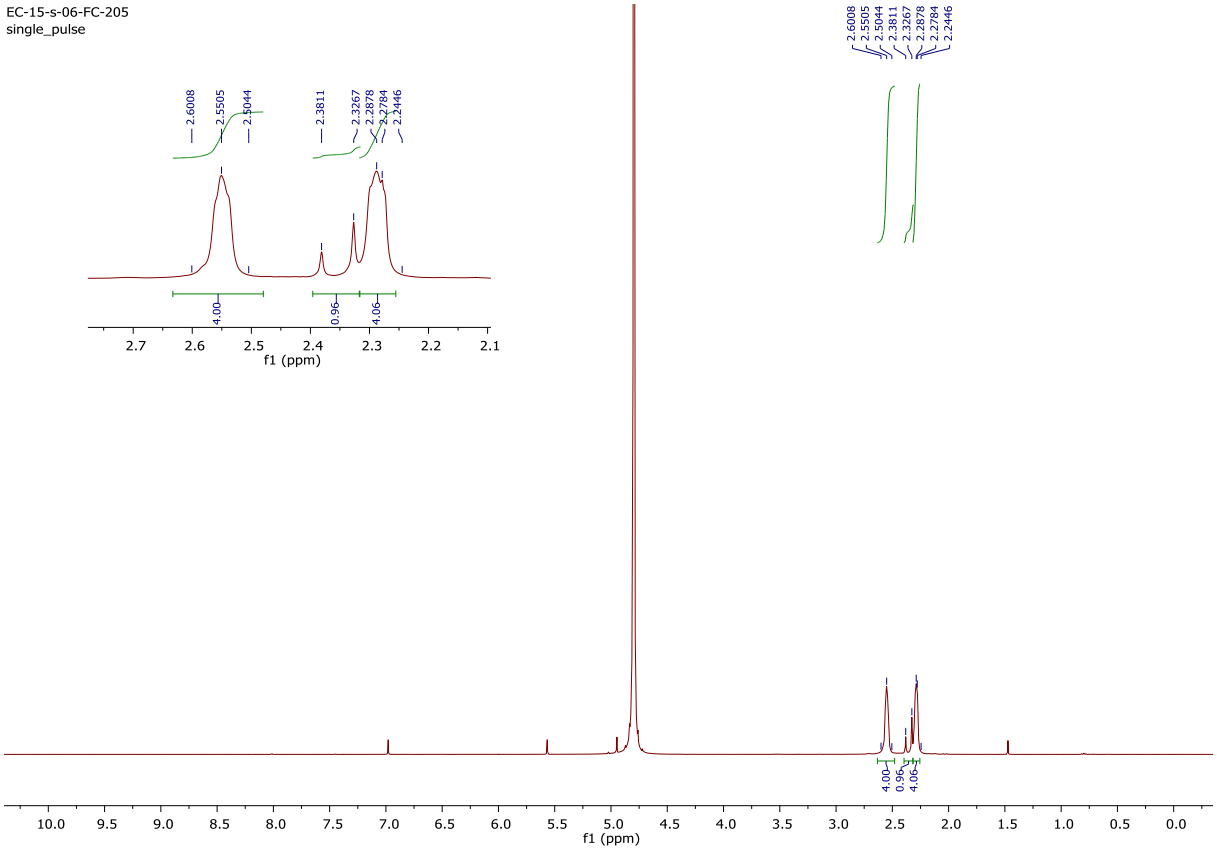


Piperaz-1-ylmethylenebisphosphonic acid (**12**)

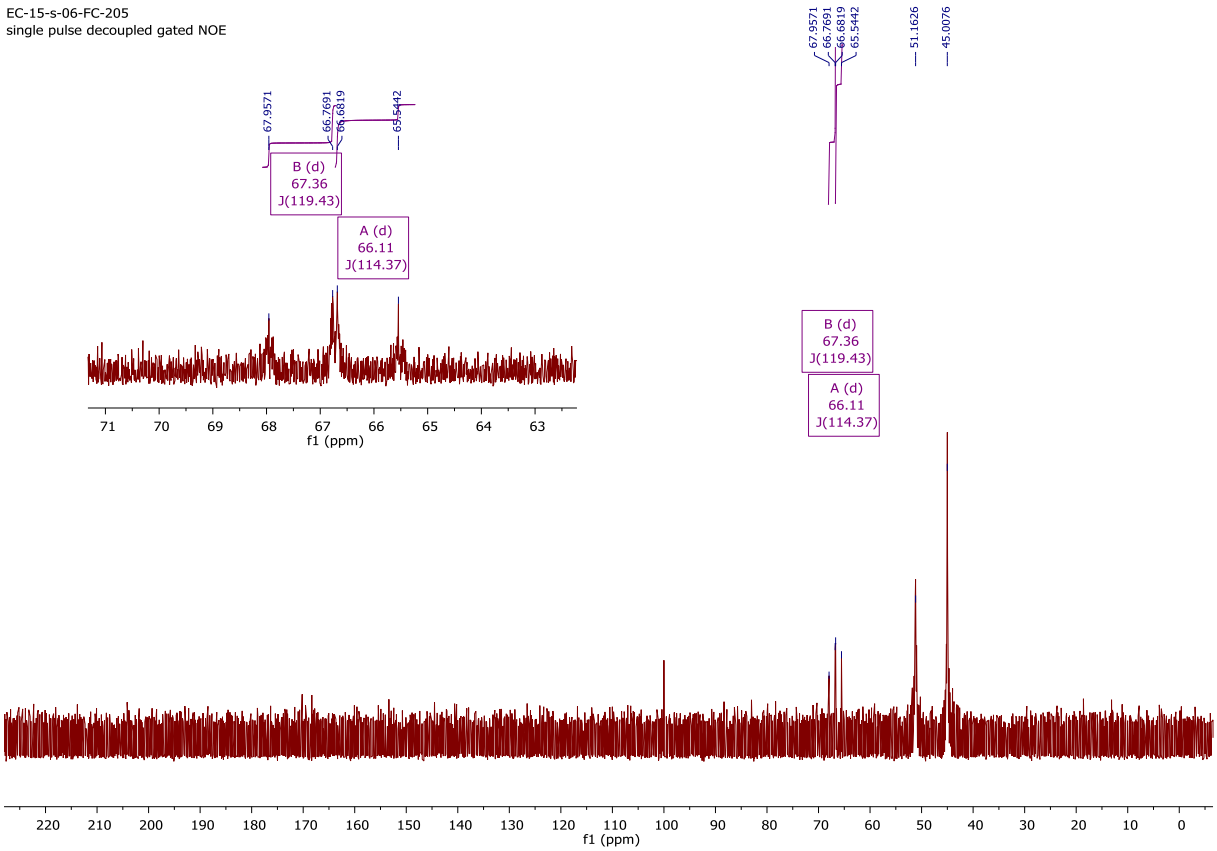
EC-15-s-06-FC-205
single pulse decoupled gated NOE

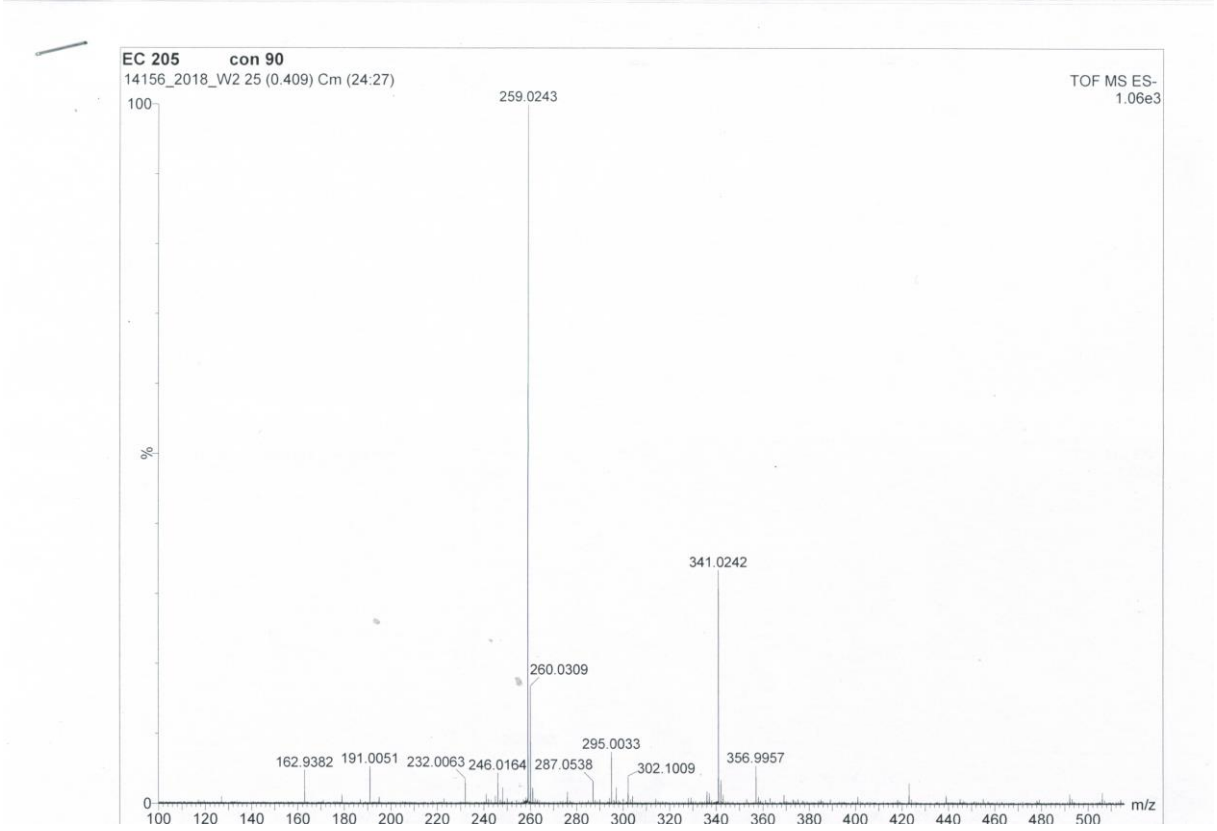


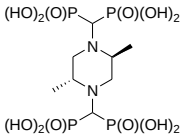
EC-15-s-06-FC-205
single_pulse



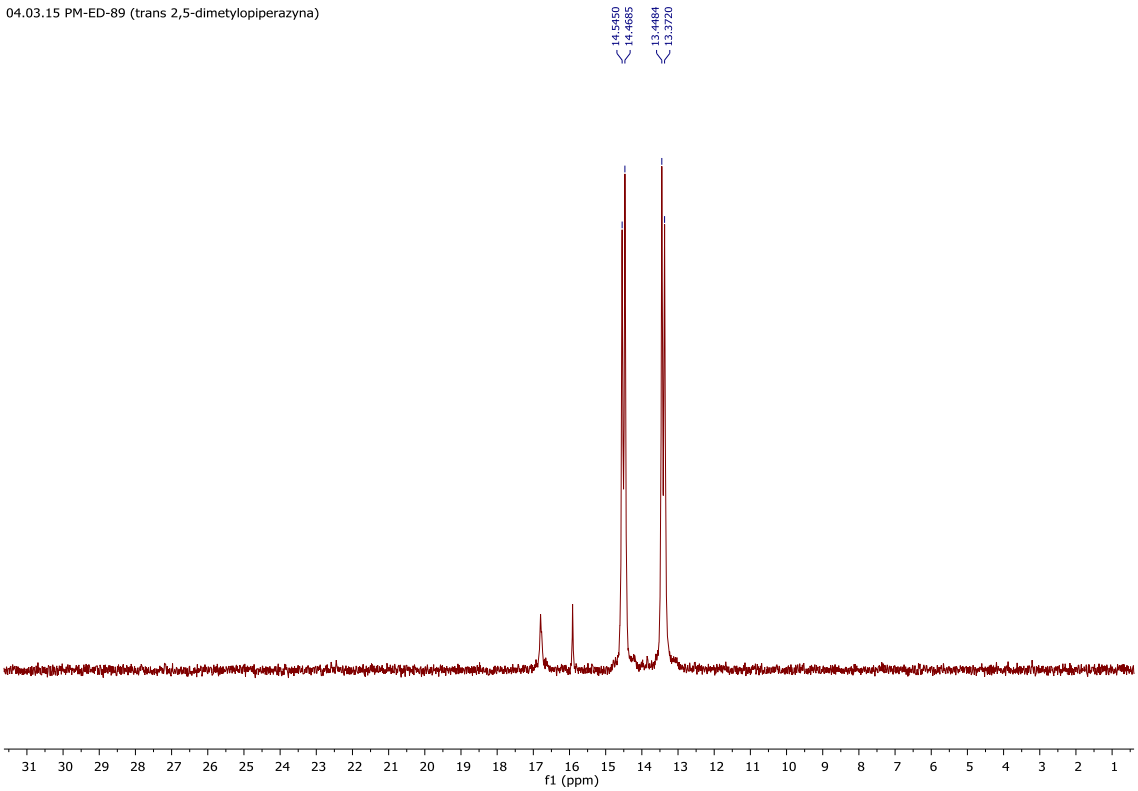
EC-15-s-06-FC-205
single pulse decoupled gated NOE

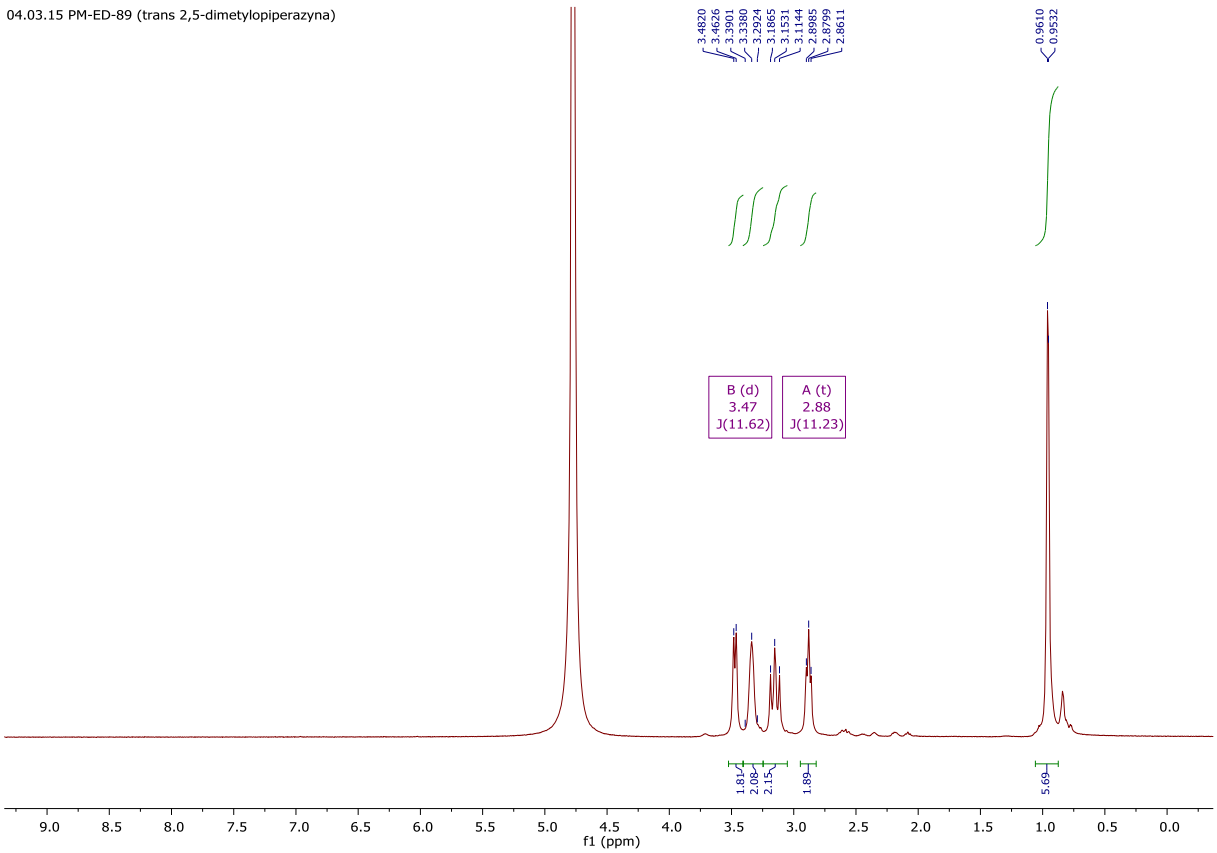




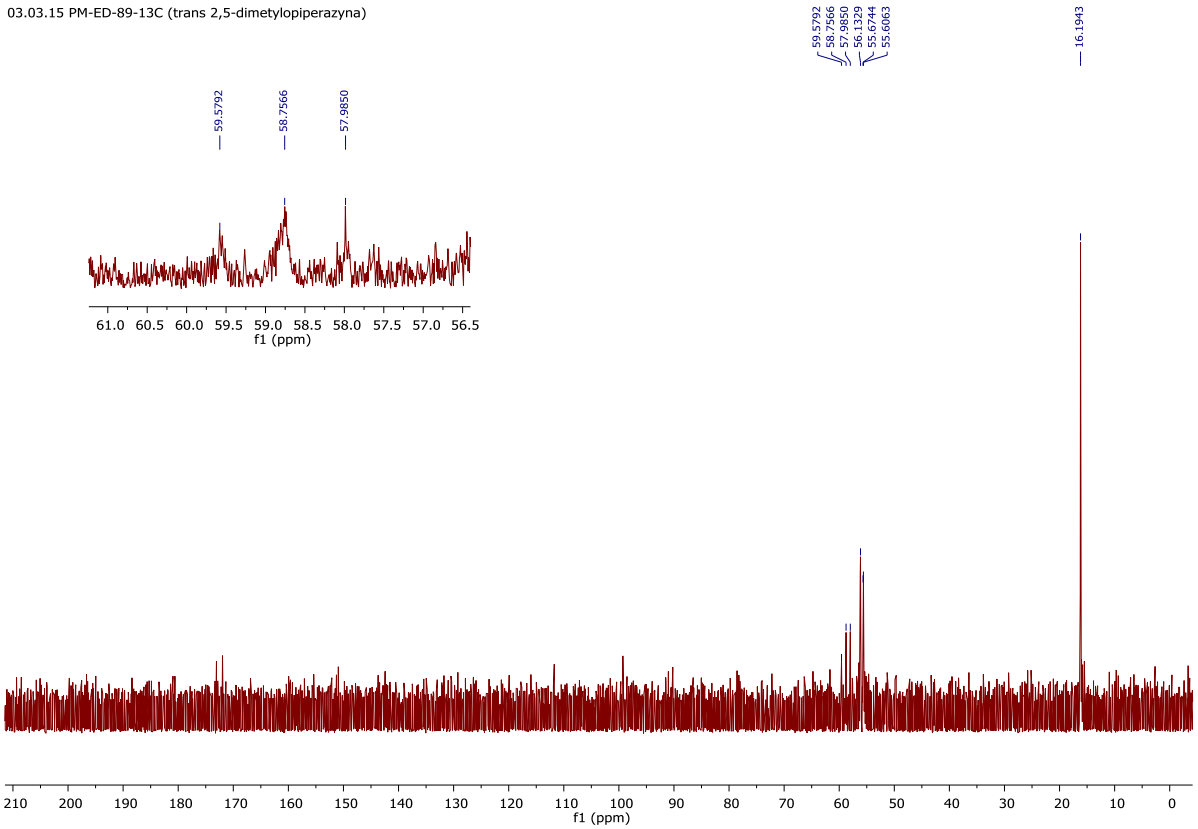


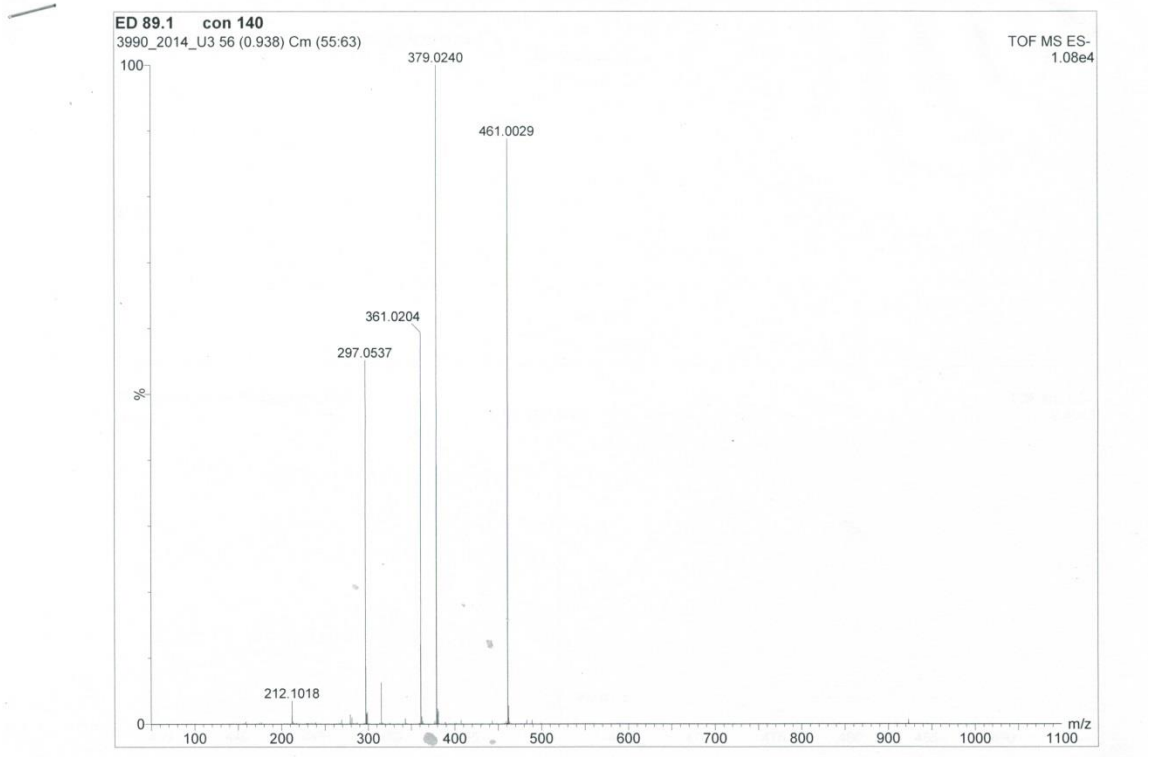
2,5-trans-Dimethylpiperaz-1,4-diylmethylenebisphosphonic acid [(*trans*)-**13]**

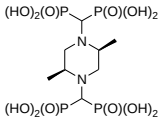




03.03.15 PM-ED-89-13C (trans 2,5-dimetylopiperazyna)



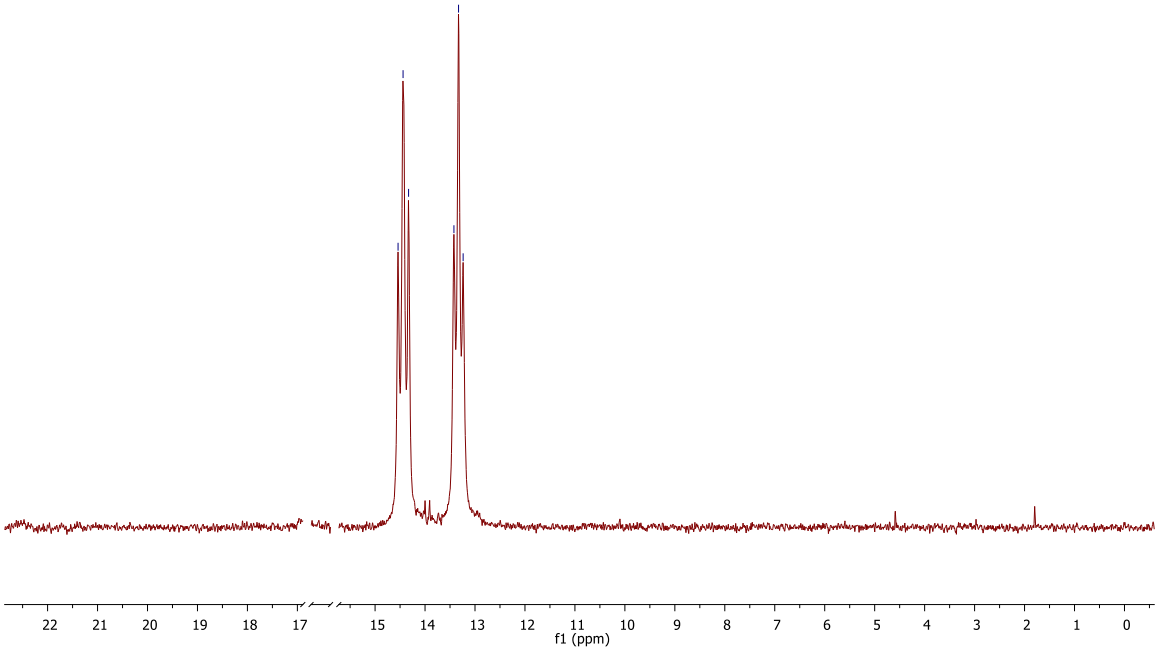




2,5-cis-Dimethylpiperaz-1,4-diylmethylenebisphosphonic acid [(*cis*)-**13**]

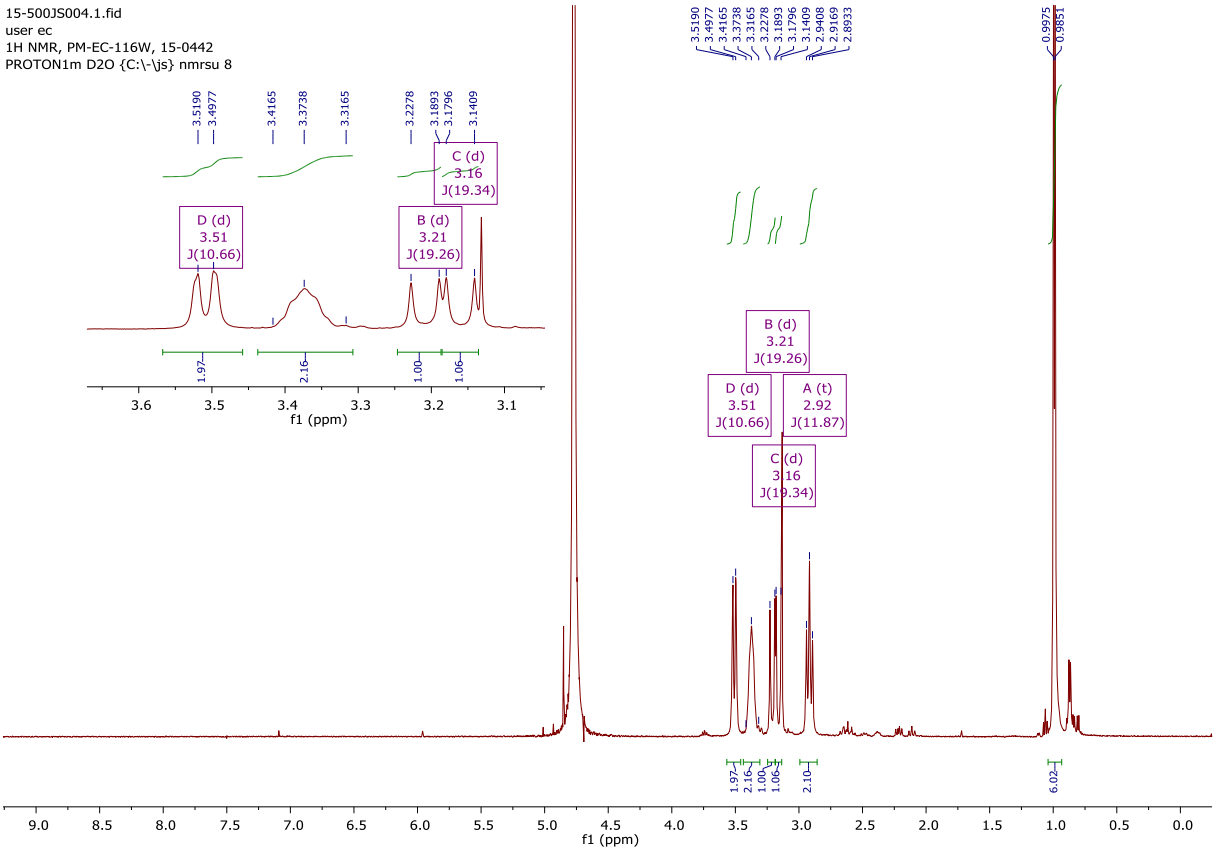
15-500JS004
user ec
31P NMR, PM-EC-116W, 15-0442
P3164 D2O {C:\-\js} nmrsu 8

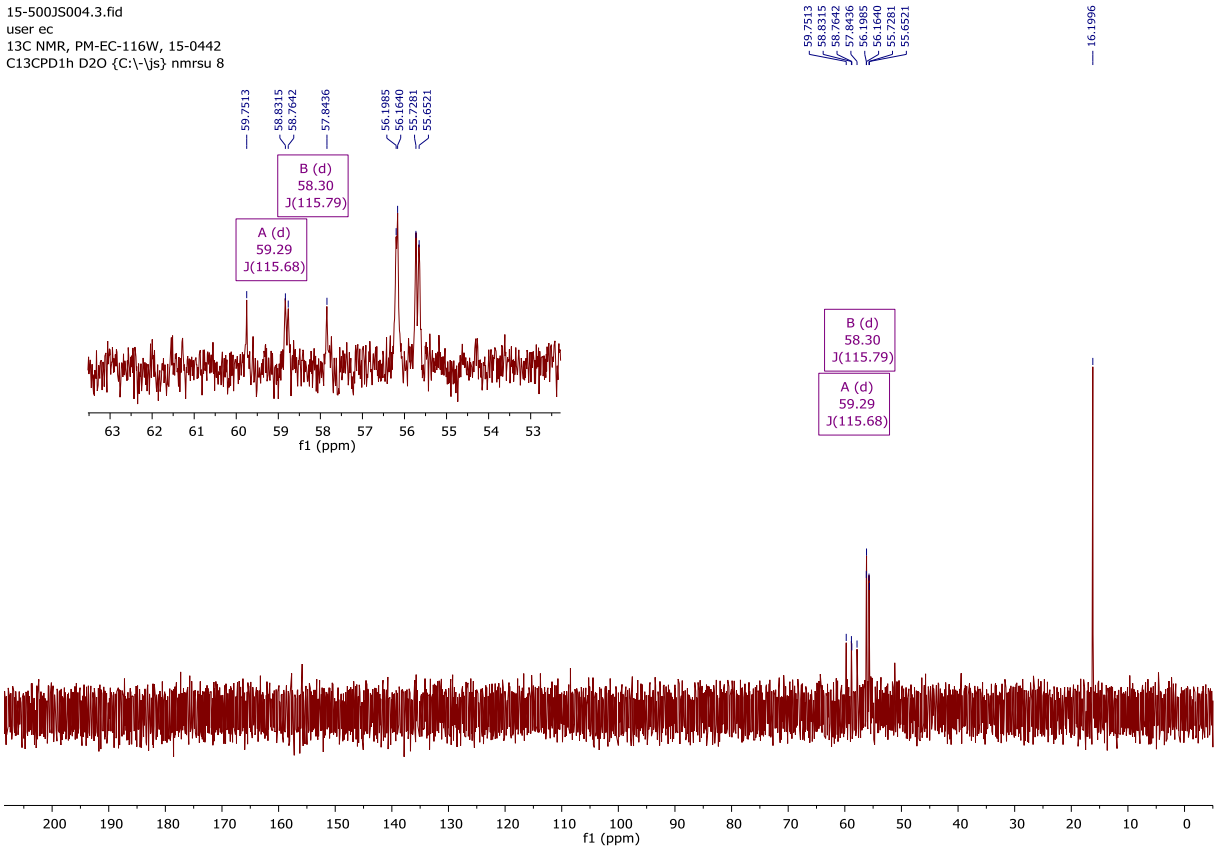
14.5402
14.4398
14.3294
13.4219
13.3294
13.2376

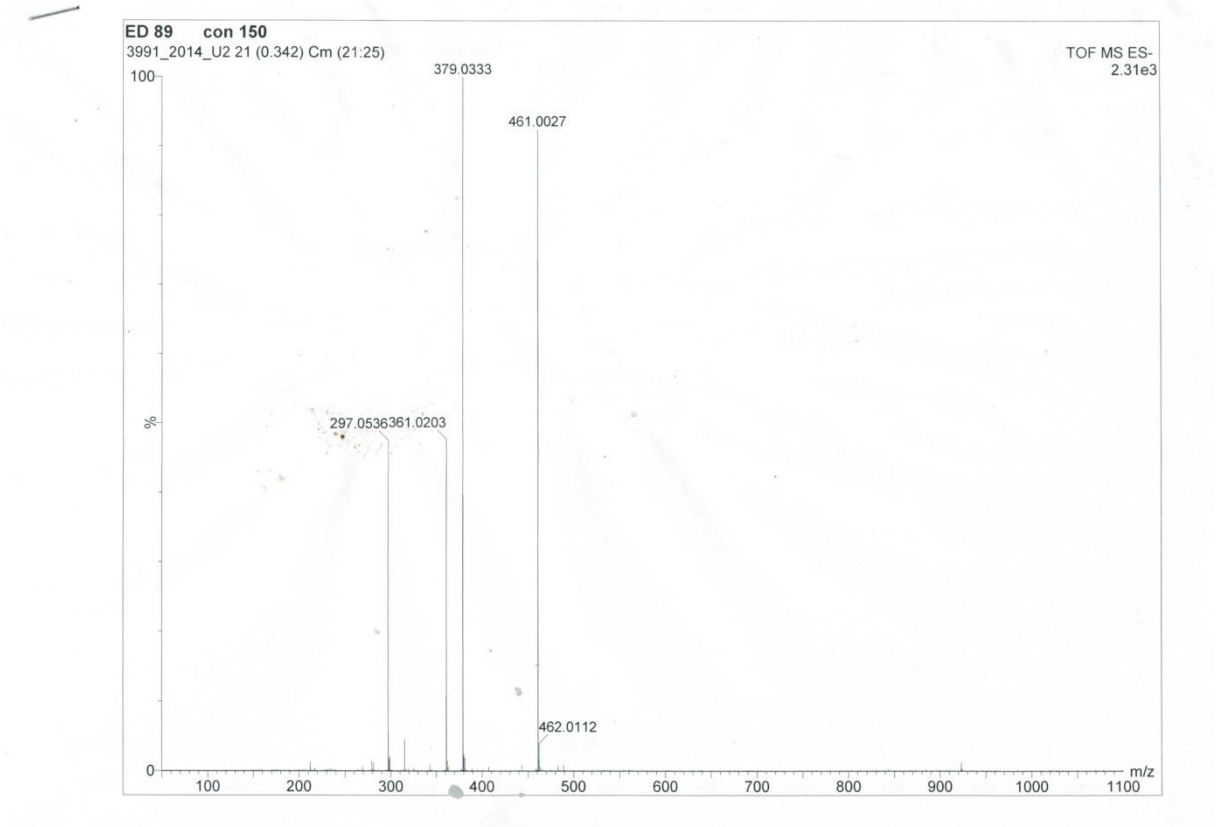


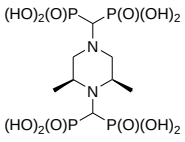
15-500JS004.1.fid
user ec
1H NMR, PM-EC-116W, 15-0442
PROTON1m D2O {C:\-\js} nmrsu 8

3.5190
3.4977
3.4165
3.3738
3.3165
3.2278
3.1893
3.1796
3.1409
2.9408
2.9169
2.8933
0.9975
0.9851

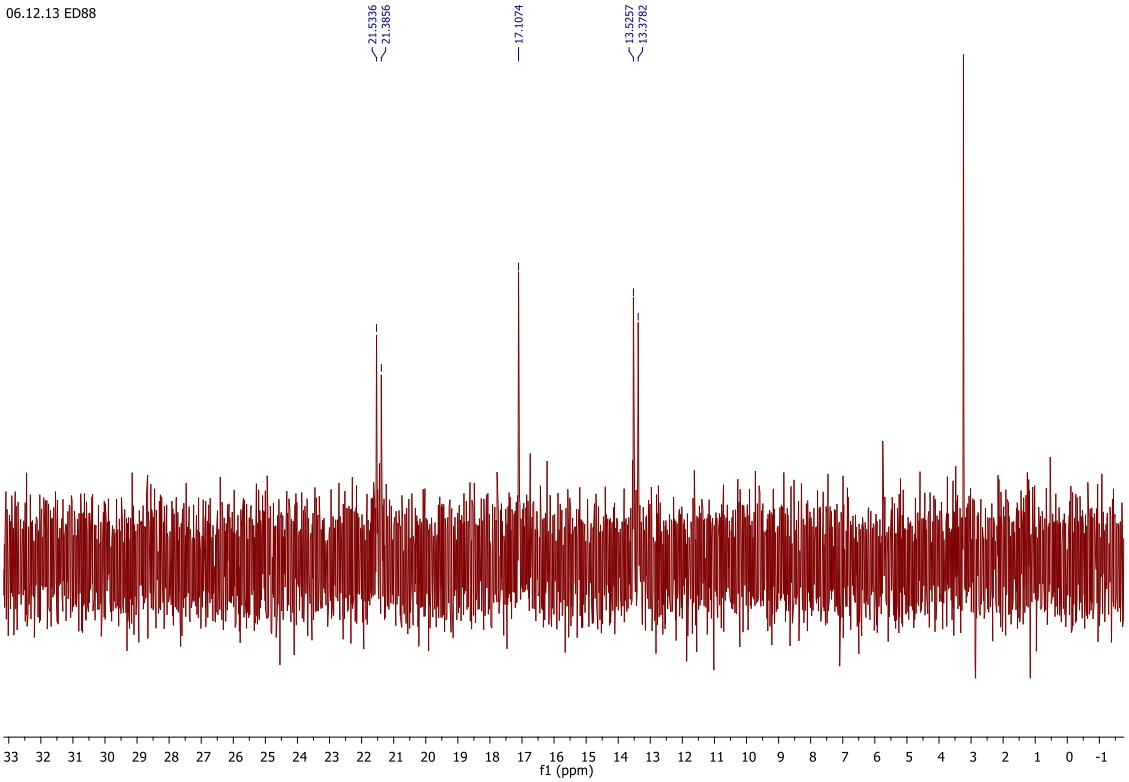


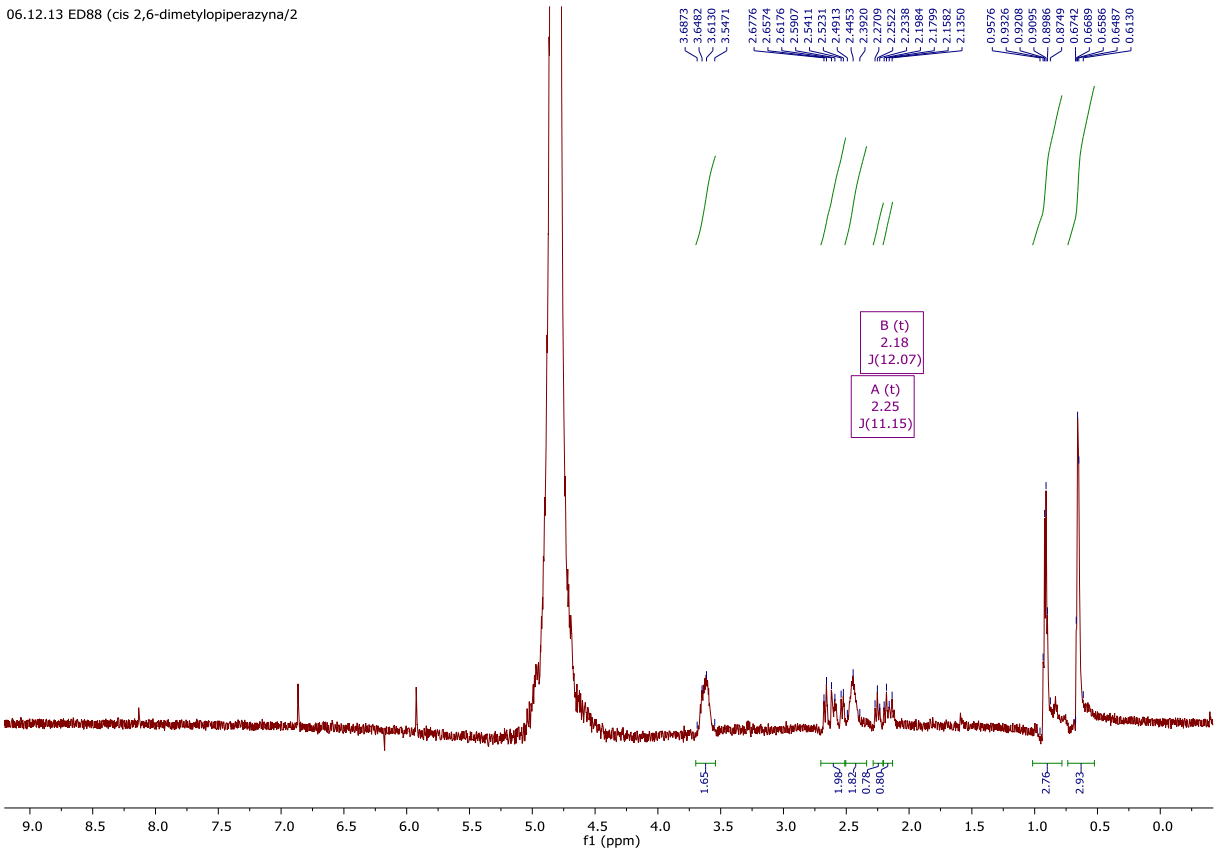

15-500JS004.3.fid
user ec
13C NMR, PM-EC-116W, 15-0442
C13CPD1h D2O {C:\-\js} nmrsu 8
59.7513
58.8315
58.7642
57.8436
56.1985
56.1640
55.7281
55.6521
16.1996
B (d)
58.30
J(115.79)
A (d)
59.29
J(115.68)
f1 (ppm)

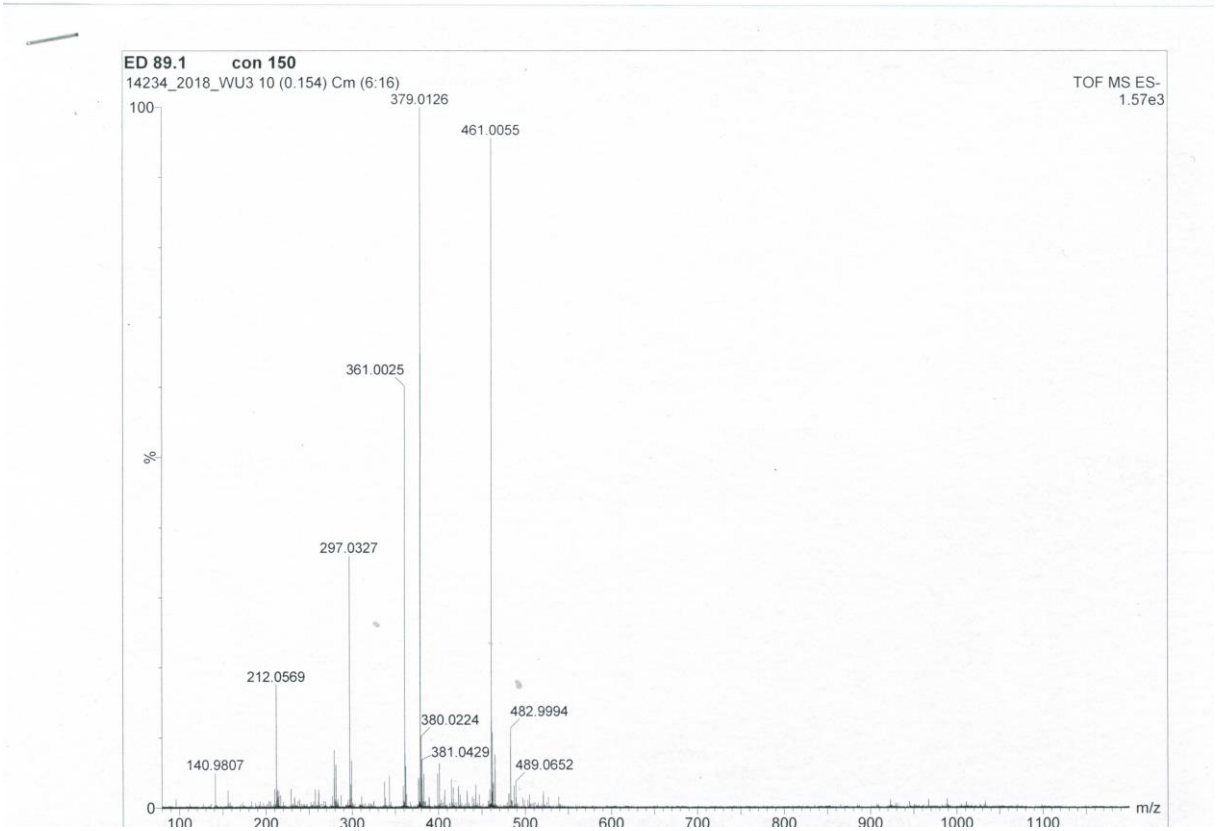

ED 89 con 150
3991_2014_U2 21 (0.342) Cm (21:25)
TOF MS ES-
2.31e3
379.0333
461.0027
297.0536
361.0203
462.0112
%
m/z

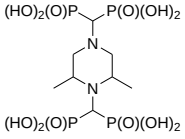


2,6-cis-Dimethylpiperaz-1,4-diylmethylenebisphosphonic acid [(*cis*)-**14]**



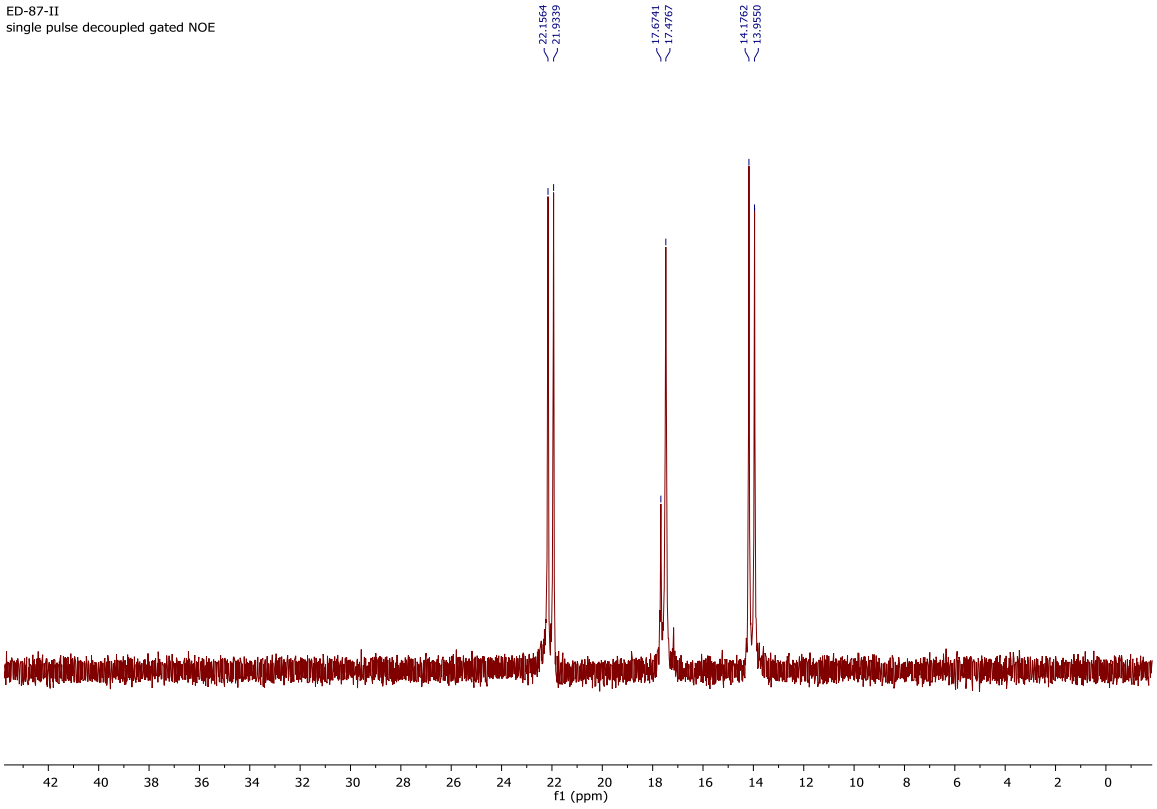




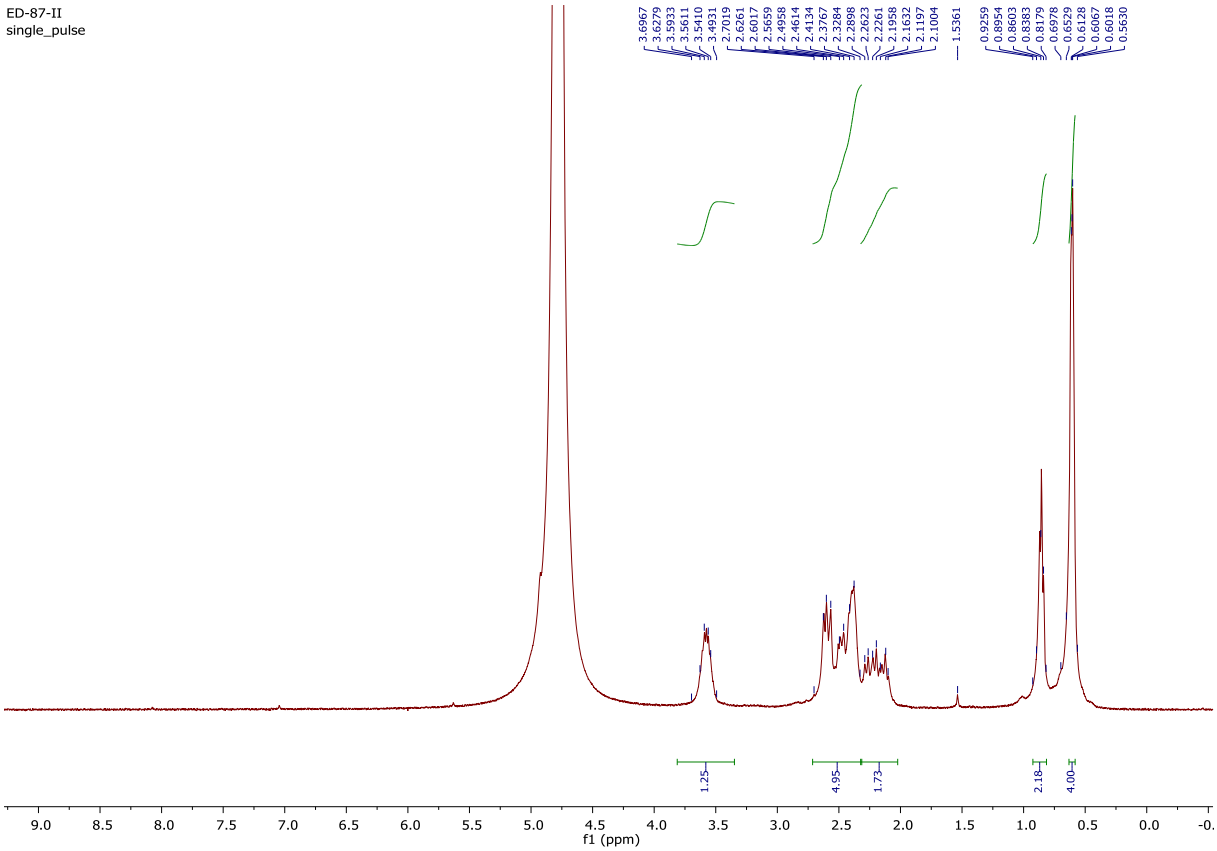


2,6-Dimethylpiperaz-1,4-diylmethylenebisphosphonic acid [(*rac*)-**14]**

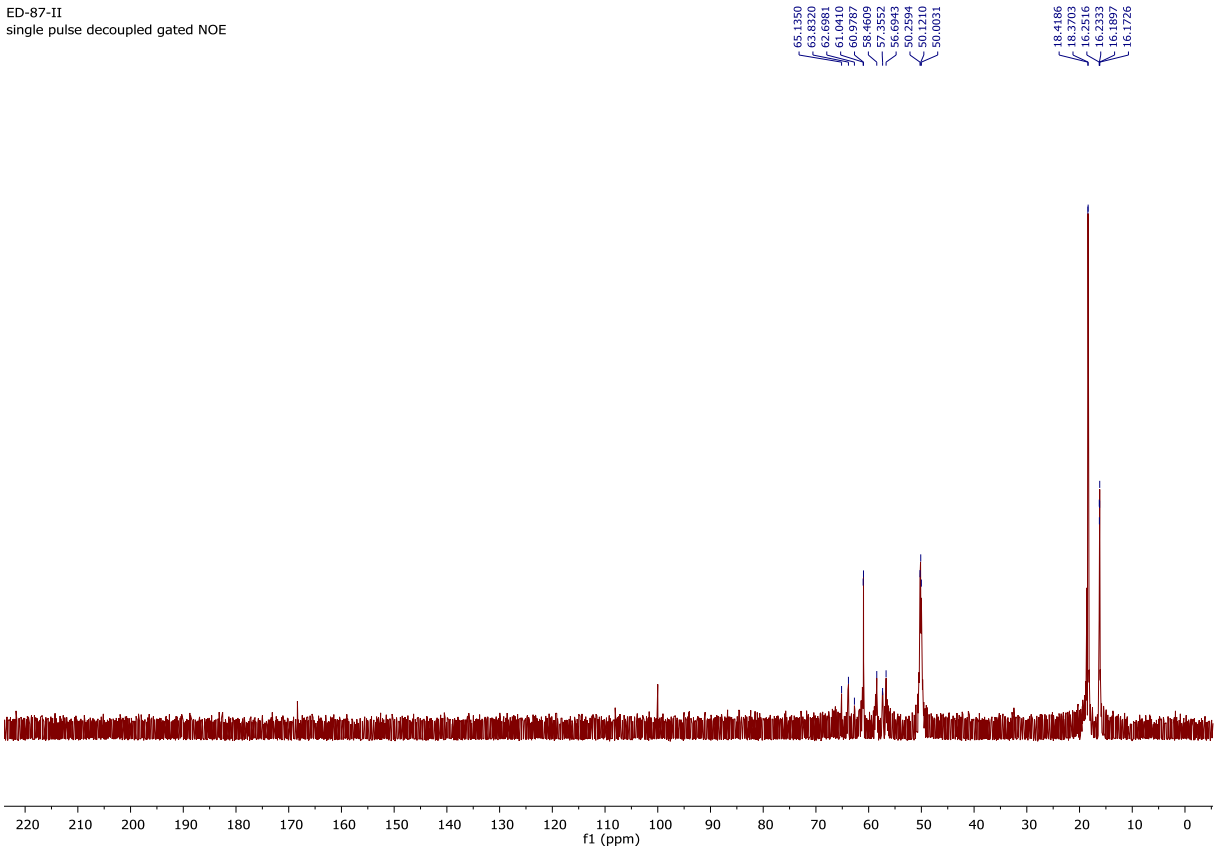
ED-87-II
single pulse decoupled gated NOE

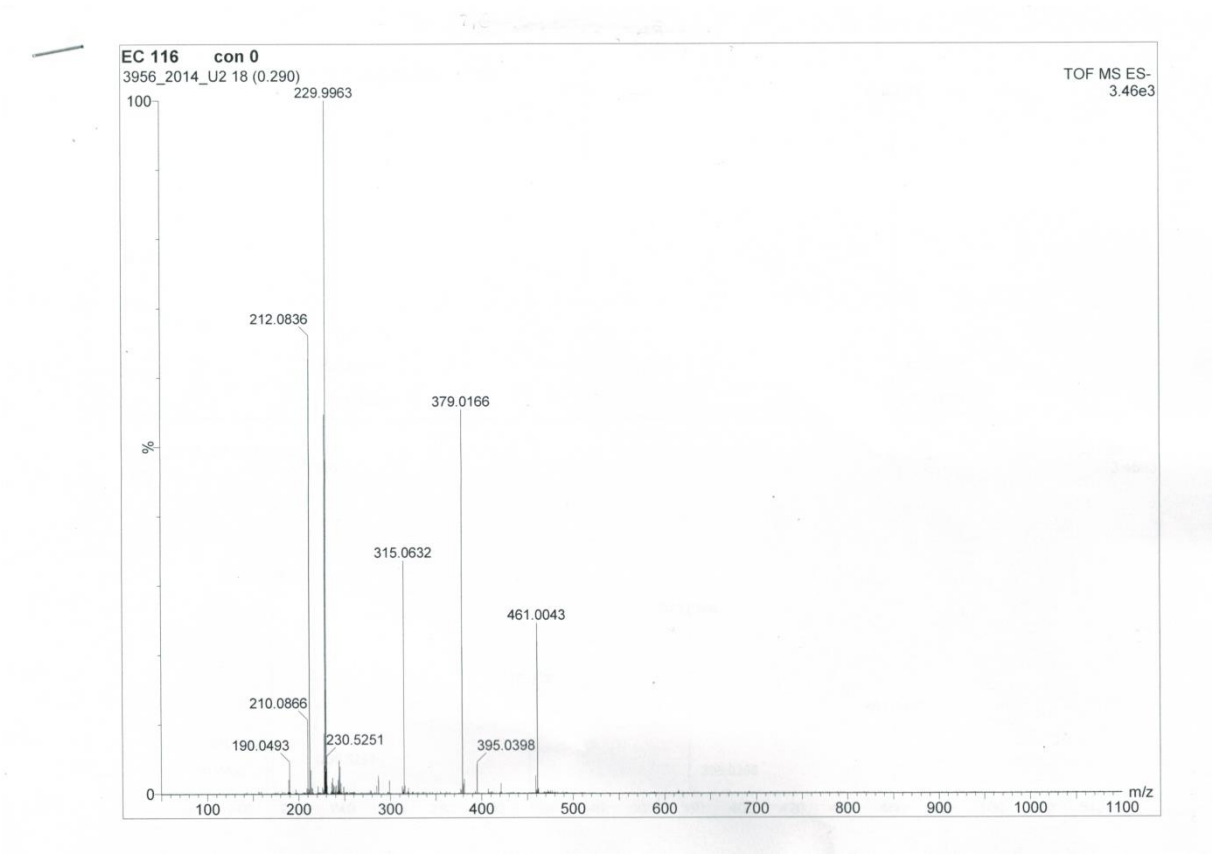


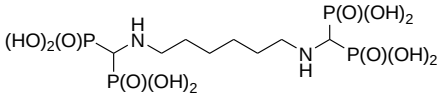
ED-87-II
single_pulse



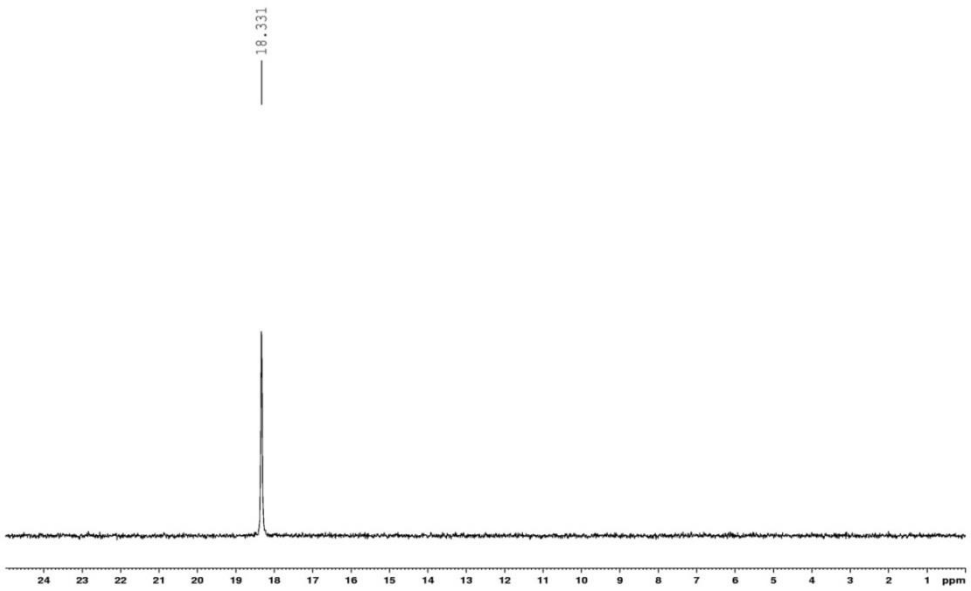
ED-87-II
single pulse decoupled gated NOE

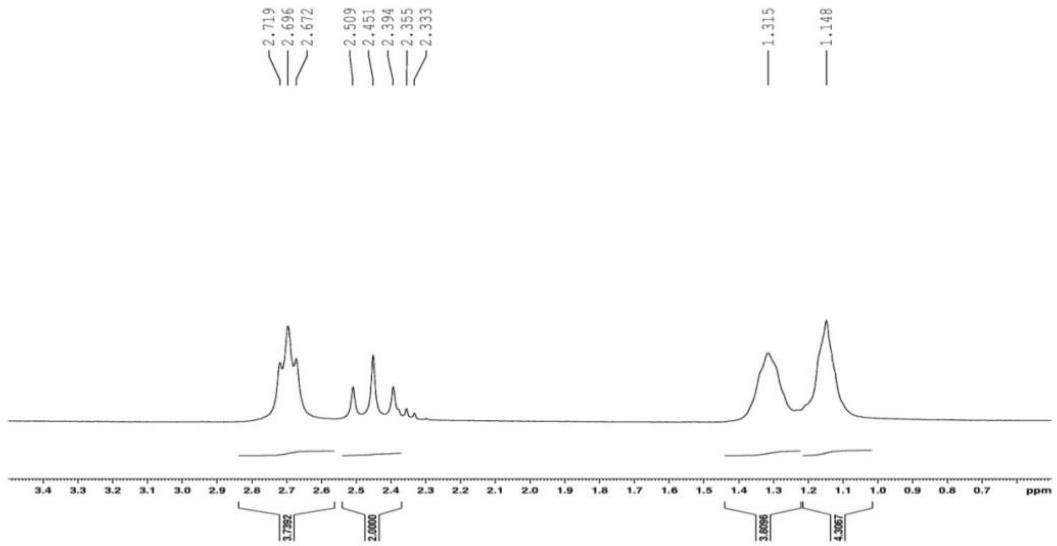




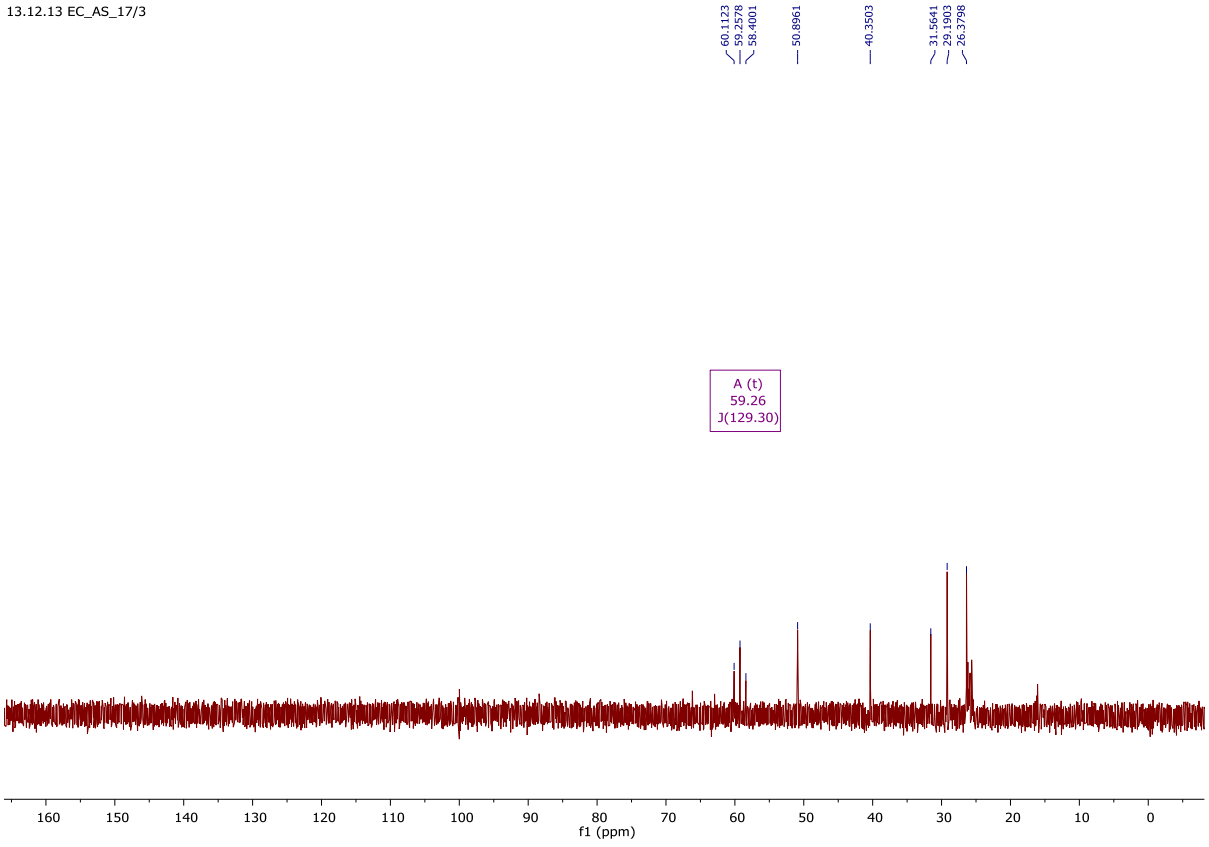


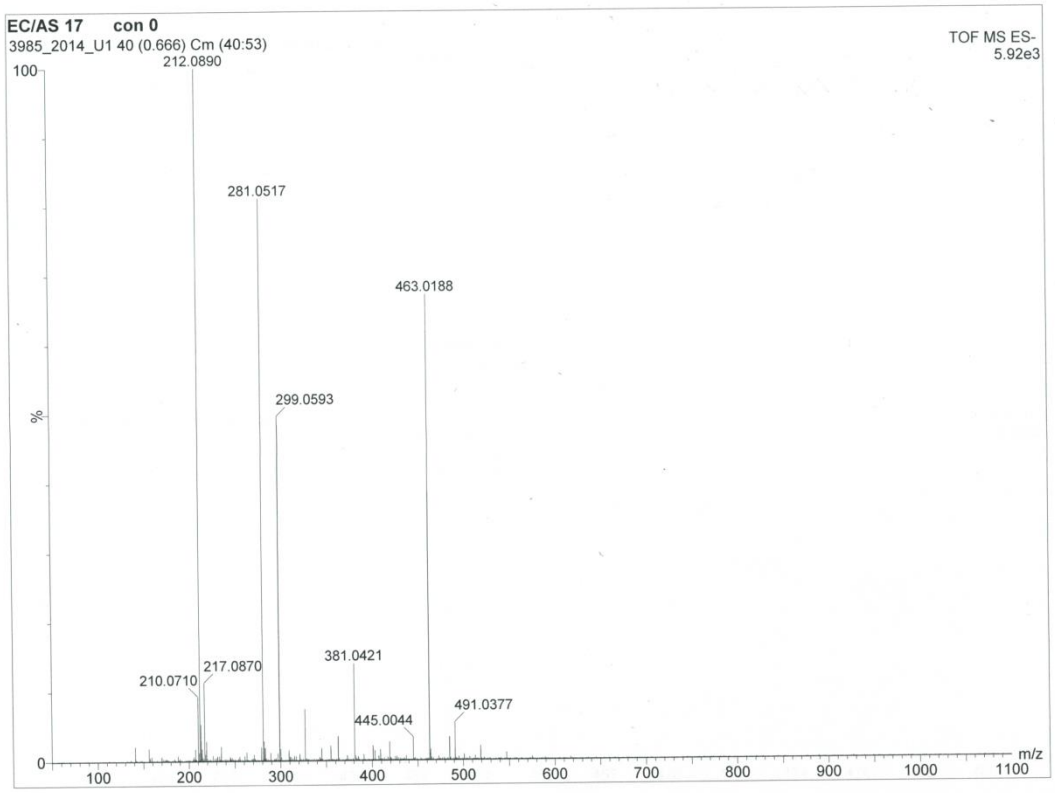
Heksylene-di(aminomethylenebisphosphonic acid) (**16)**

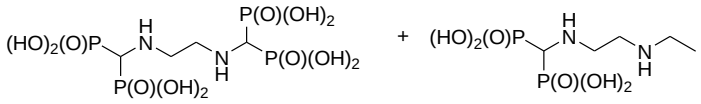




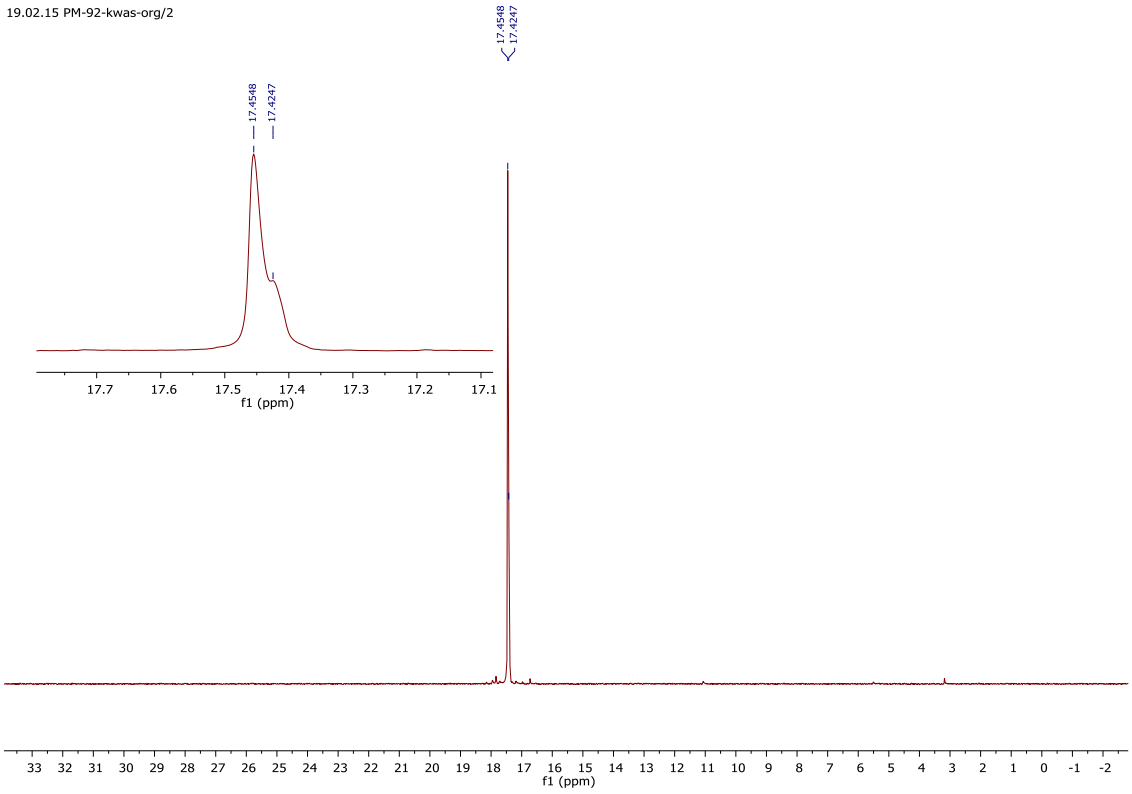
13.12.13 EC_AS_17/3

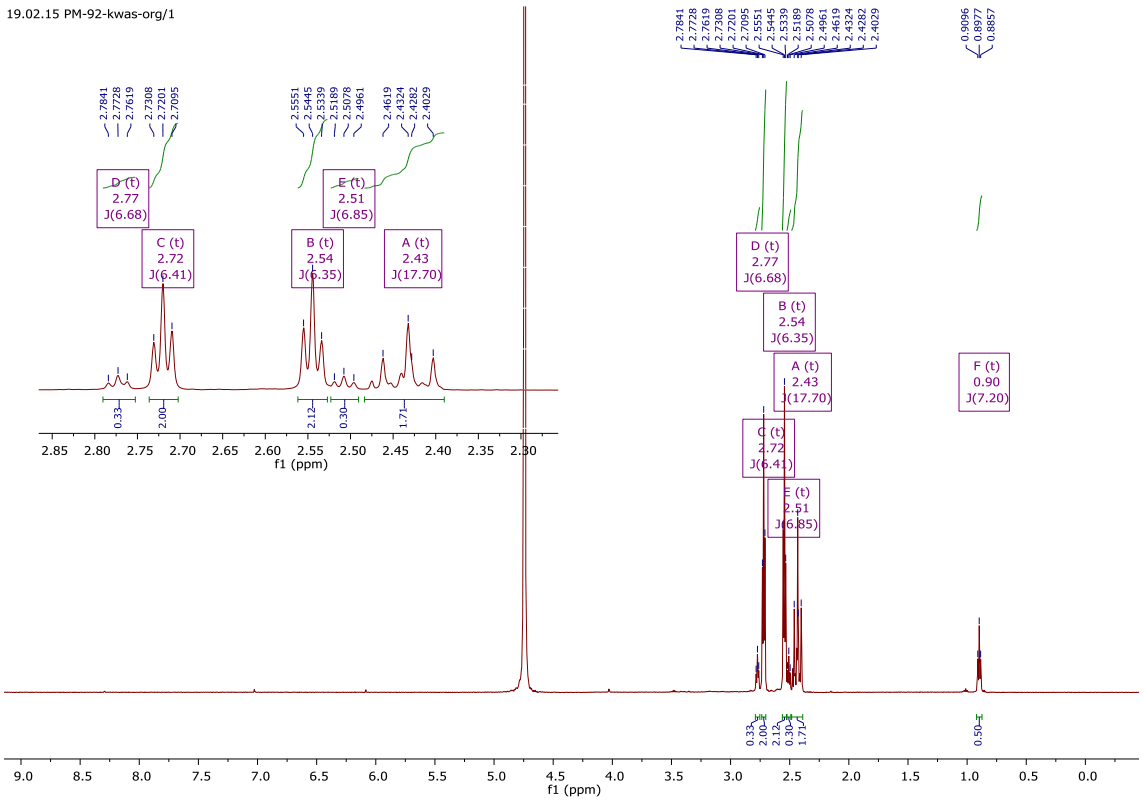


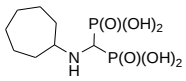

EC/AS 17 con 0
3985_2014_U1 40 (0.666) Cm (40:53)
TOF MS ES-
5.92e3
100
%
0
212.0890
281.0517
463.0188
299.0593
381.0421
217.0870
210.0710
491.0377
445.0044
m/z
100
200
300
400
500
600
700
800
900
1000
1100



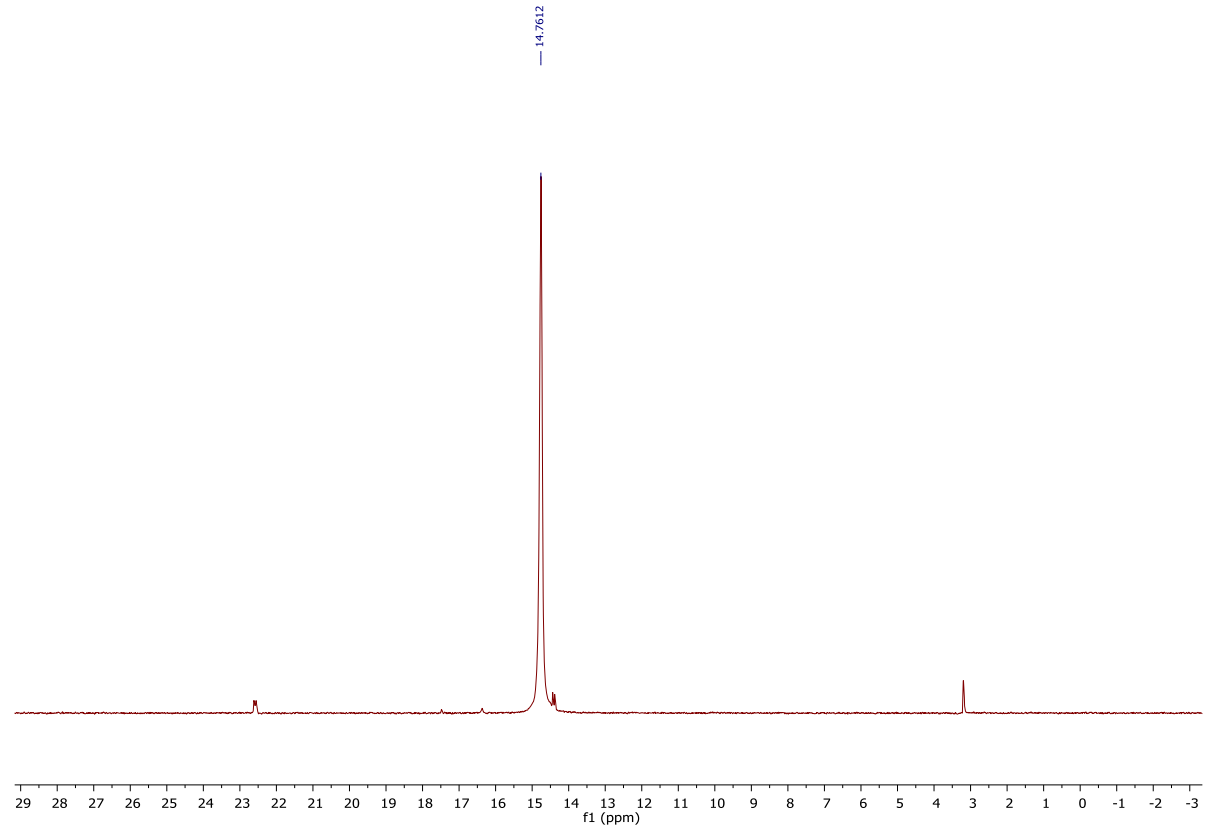
micture of 15 and**17**

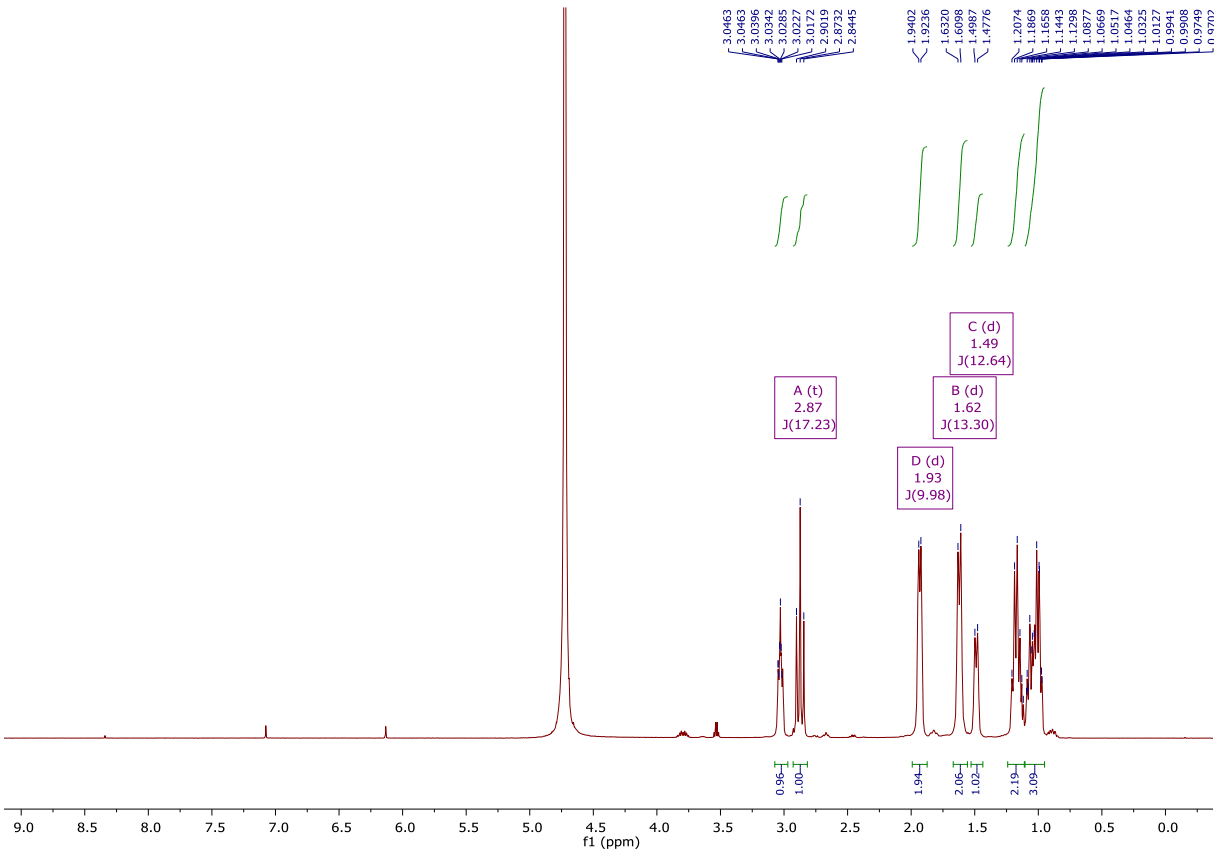






Incadronate





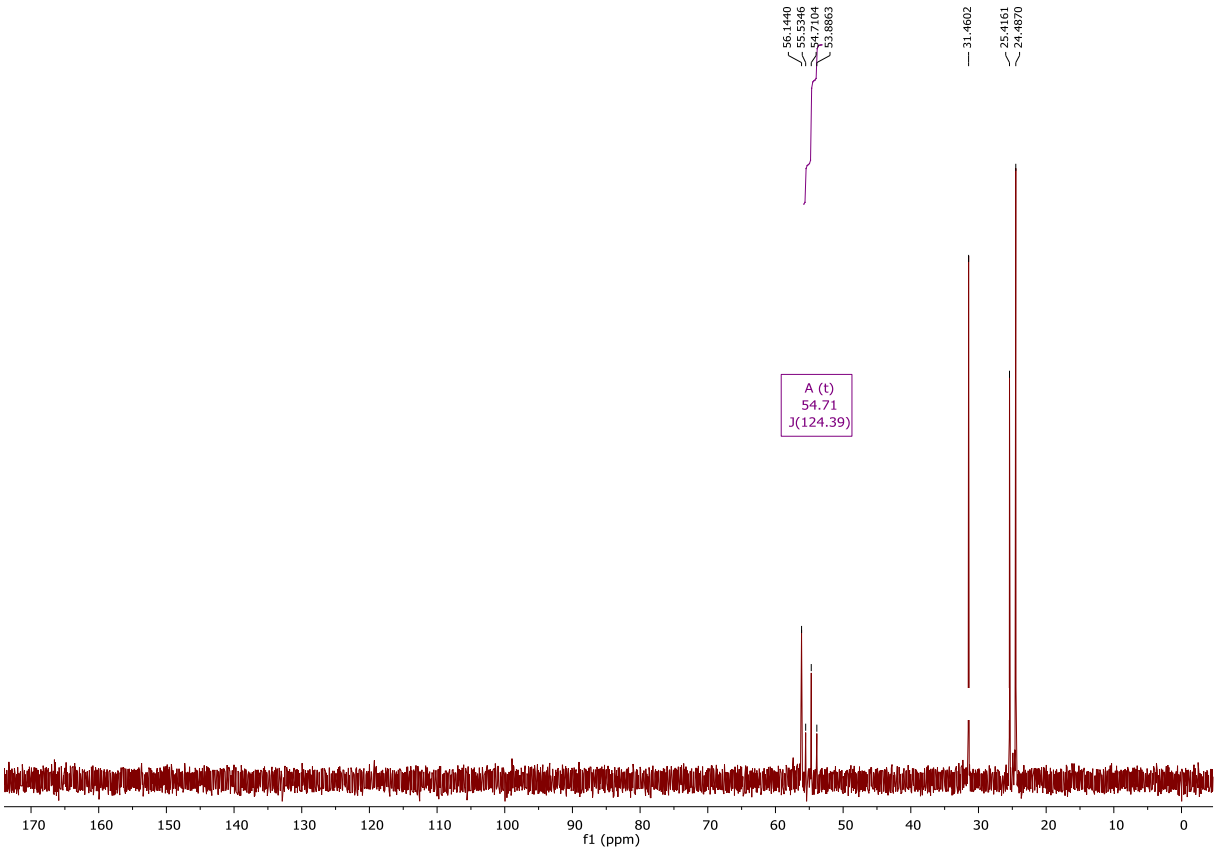
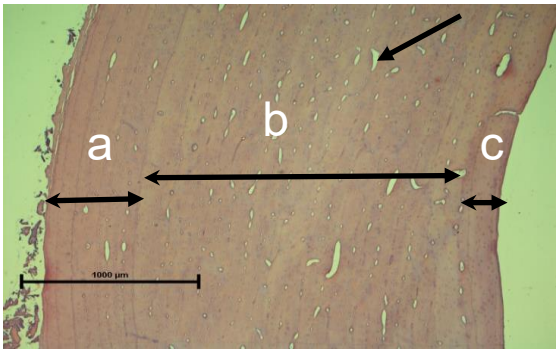
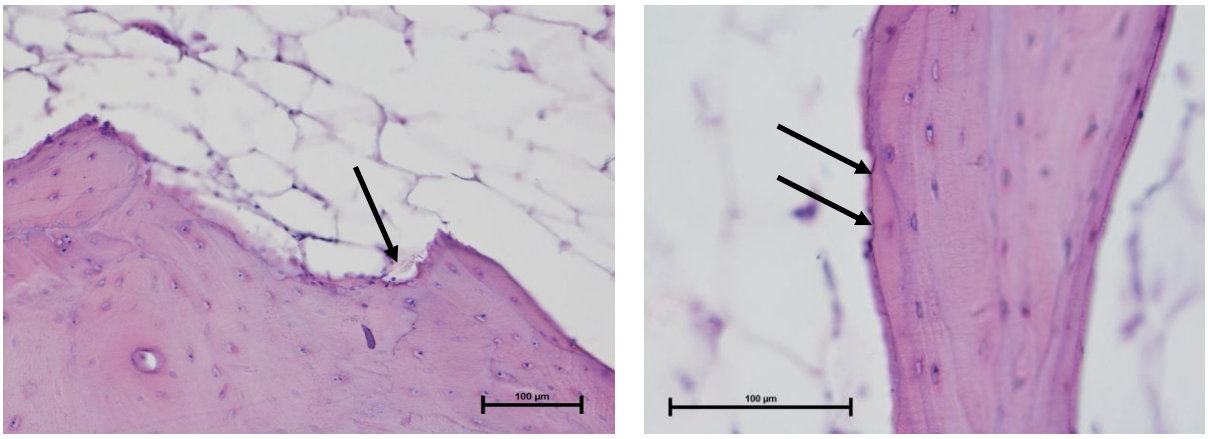


Figure S1. Histology (Delafield staining)



Fragment of femur shaft. From left to right: (a) area of osteogenesis at periosteum side, (b) central area, (c) bone lamella at endosteum site. Arrow shows micropore resulting from osteoresorption.

Figure S2. Examples of erosion bays



Total hip of femour (magnification 200x)

Proximal tibial epiphysis (magnification 400x)

Figure S3. Dysfunctional course of collagen fibres of tibia (magnification 600x)

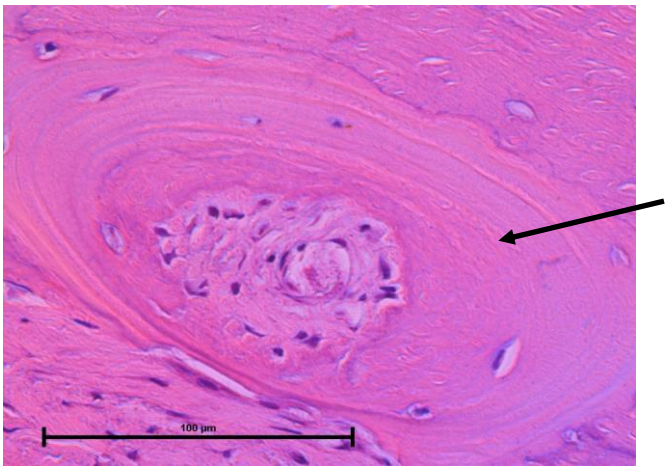
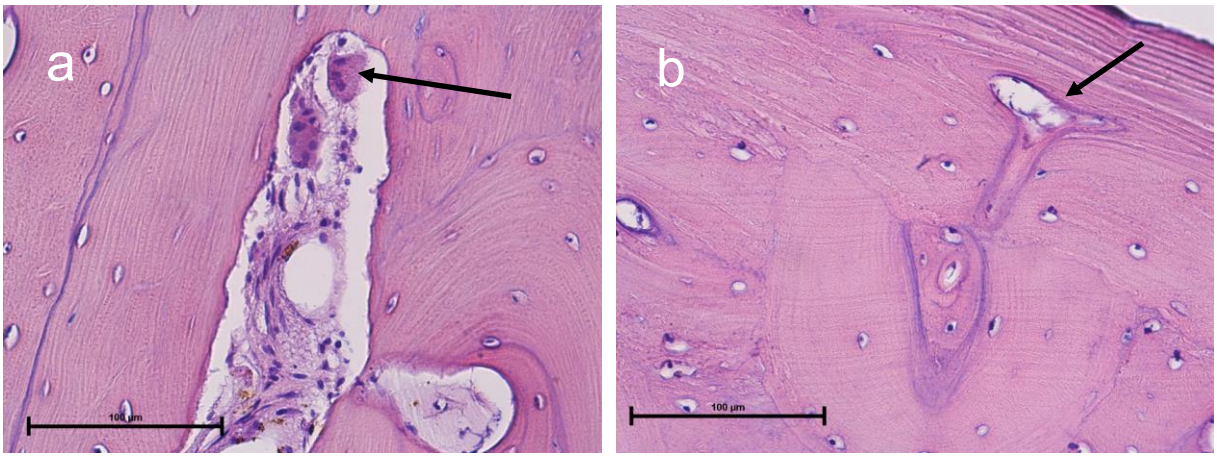


Figure S4. Bone repair (magnification 400x)



(a) Femur, fragment of intensive reconstruction of bone issue. Arrows indicates multinuclar osteoclast. Osteoclasts are accompanied by angiogenic and ostogenic cells. (b) Tibial bone at endosteum site. Arrow indicates intensive formation of bone lamella.