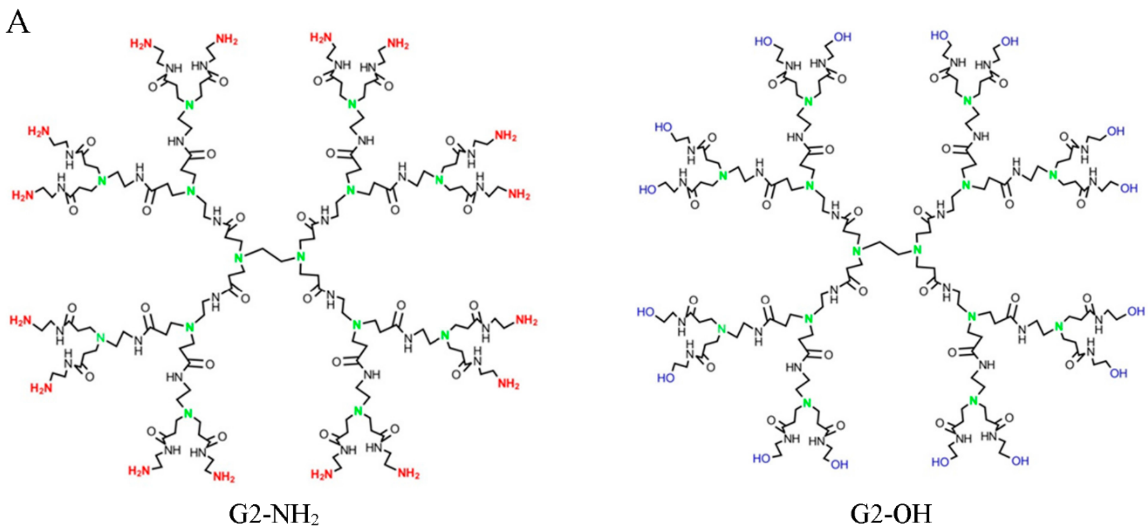
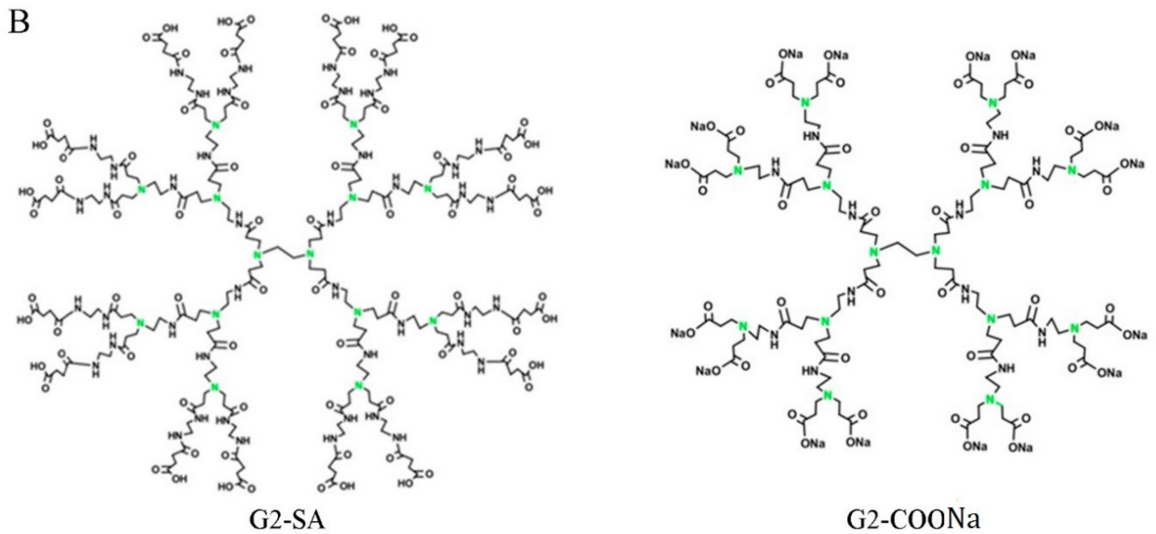
Supplementary Materials: Impact of Dendrimer Terminal Group Chemistry on Blockage of the Anthrax Toxin Channel: A Single Molecule Study

Goli Yamini, Nnanya Kalu and Ekaterina M. Nestorovich

Cationic PAMAM dendrimers



G2 PAMAM dendrimers with negatively charged terminal groups



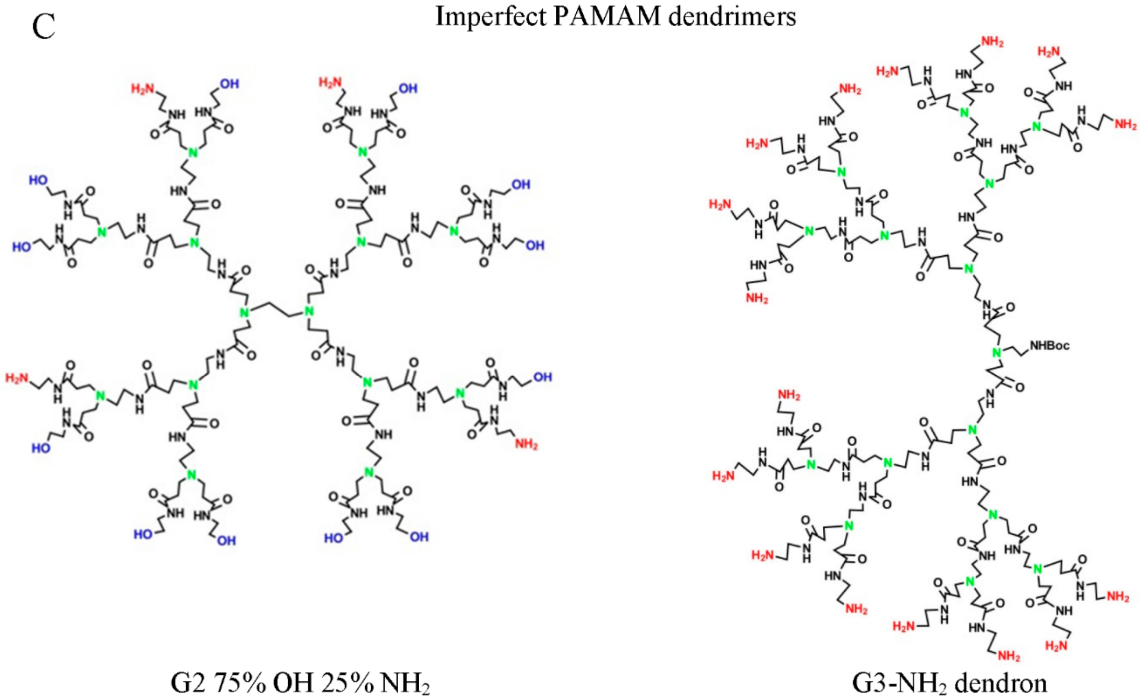


Figure S1. Chemical structures of the PAMAM dendrimers used in this study. (**A**) Cationic PAMAM dendrimers G2-NH2, with 16 positively charged terminal groups (left), G2-OH, with positively-charged PAMAM core and neutral OH terminal groups (right). (**B**) G2 PAMAM dendrimers with negatively charged succinamate (left) and carboxyl (right) terminal groups, G2-SA and G2-COONa respectively. (**C**) Imperfect G2 PAMAM dendrimers G2 75% OH 25 % NH2, with 12 neutral OH and 4 positively charged NH2 terminal groups on average (left), and G3-NH2 dendron, with a fractured more flexible structure and 16 positively charged terminal groups (right). Similar to the Figure 1B color coding, terminal primary amines are colored in red; core tertiary amines are colored in green; terminal hydroxyl groups are colored in blue. The images were created using chemical drawing software ChemDoodle 8.1.0, iChemLabs, LLC. Note that in contrast to all other dendrimers, G2 75% OH 25% NH2 is not monodisperse and contains 75% of terminal OH groups and 25% of terminal NH2 groups on average.

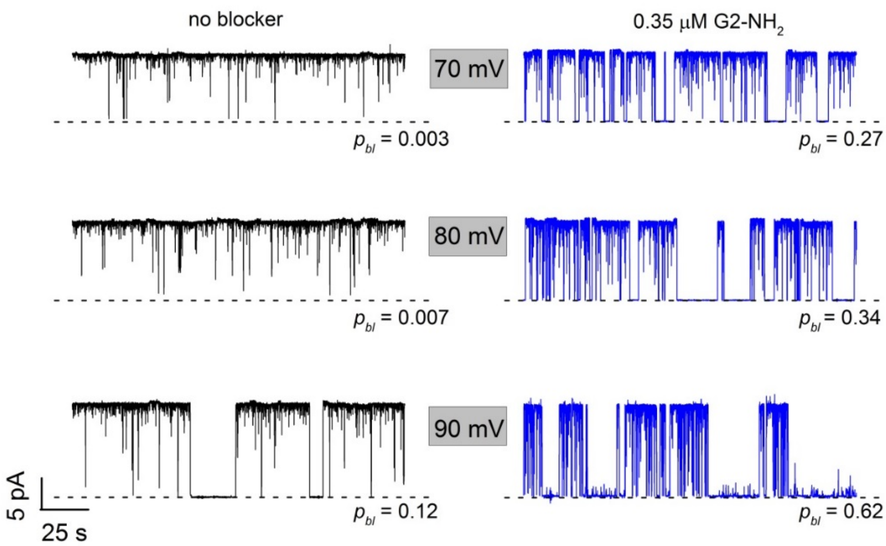


Figure S2. Second mode of G2-NH2-induced modulation of a single PA_{63} channel current. At the relatively low applied voltages (70 and 80 mV), PA_{63} mostly remains in an open state in the blocker-free solutions (left, two upper rows). Fast flickering between the open and closed states (the so-called

1/*f* noise) is mostly removed by averaging over a time interval of 100 ms. At 90 mV (left, low row), several pronounced voltage gating events are seen; p_{bl} = 0.12. In the presence of 0.35 μM of G2-NH_2 (right), the voltage gating of the channel is significantly increased. Multiple fast current blockages (first mode of dendrimer-induced current inhibition) are observed but they are partially filtered over a time interval of 100 ms.

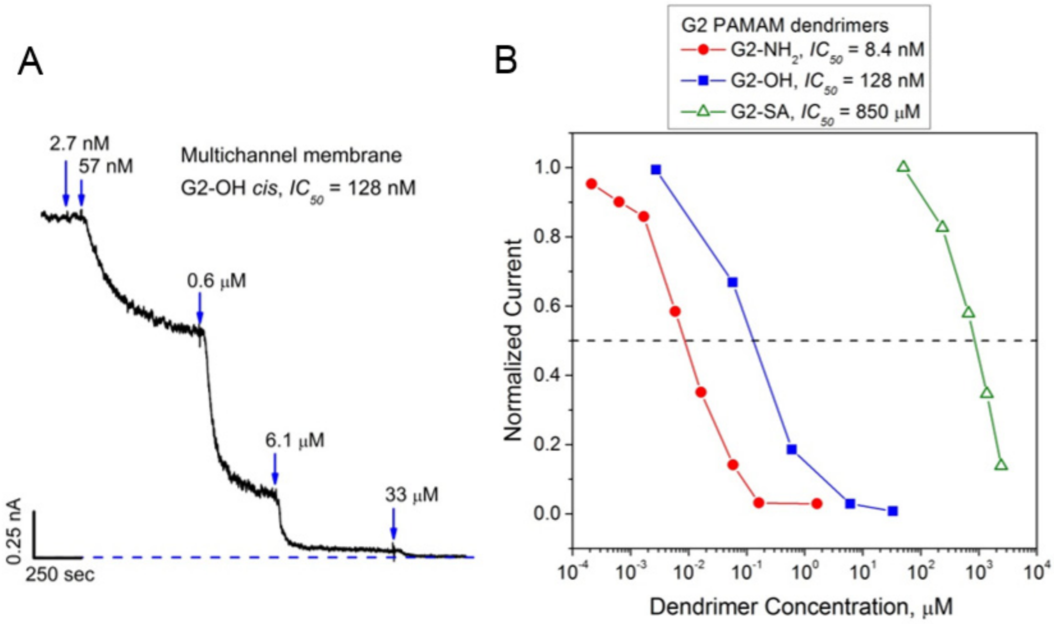


Figure S3. Influence of the PAMAM dendrimer terminal group chemistry on the PA_{63} channel inhibition studied on a multichannel level. (**A**) A typical dendrimer-induced PA_{63} inhibition curve (shown for G2-OH dendrimer). G2-OH additions are marked with the downward arrows; total bulk dendrimer concentration is indicated. The dashed line represents zero current level; (**B**) Typical multichannel titration curves of the PA_{63} channel inhibition by G2-NH_2, G2-OH, and G2-SA dendrimers. The dashed line represents 50% of the original current level. The recordings were taken in 0.1 M KCl solutions at pH 6 under 20 mV applied voltage.

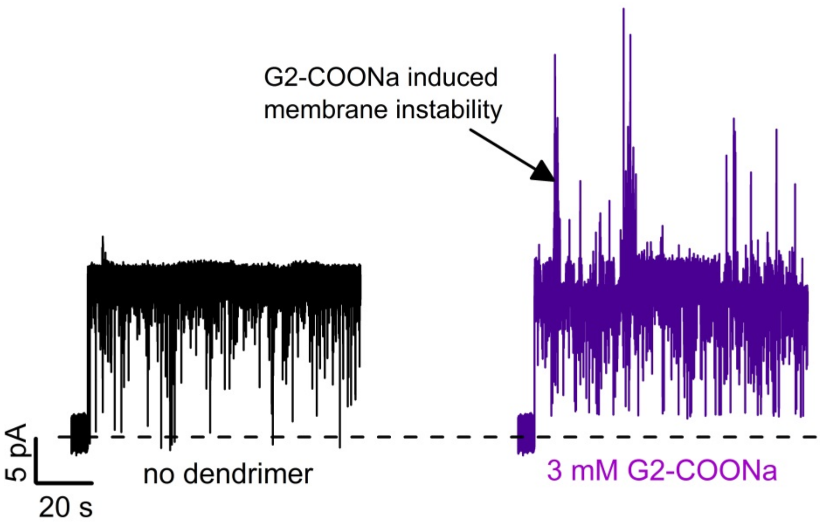
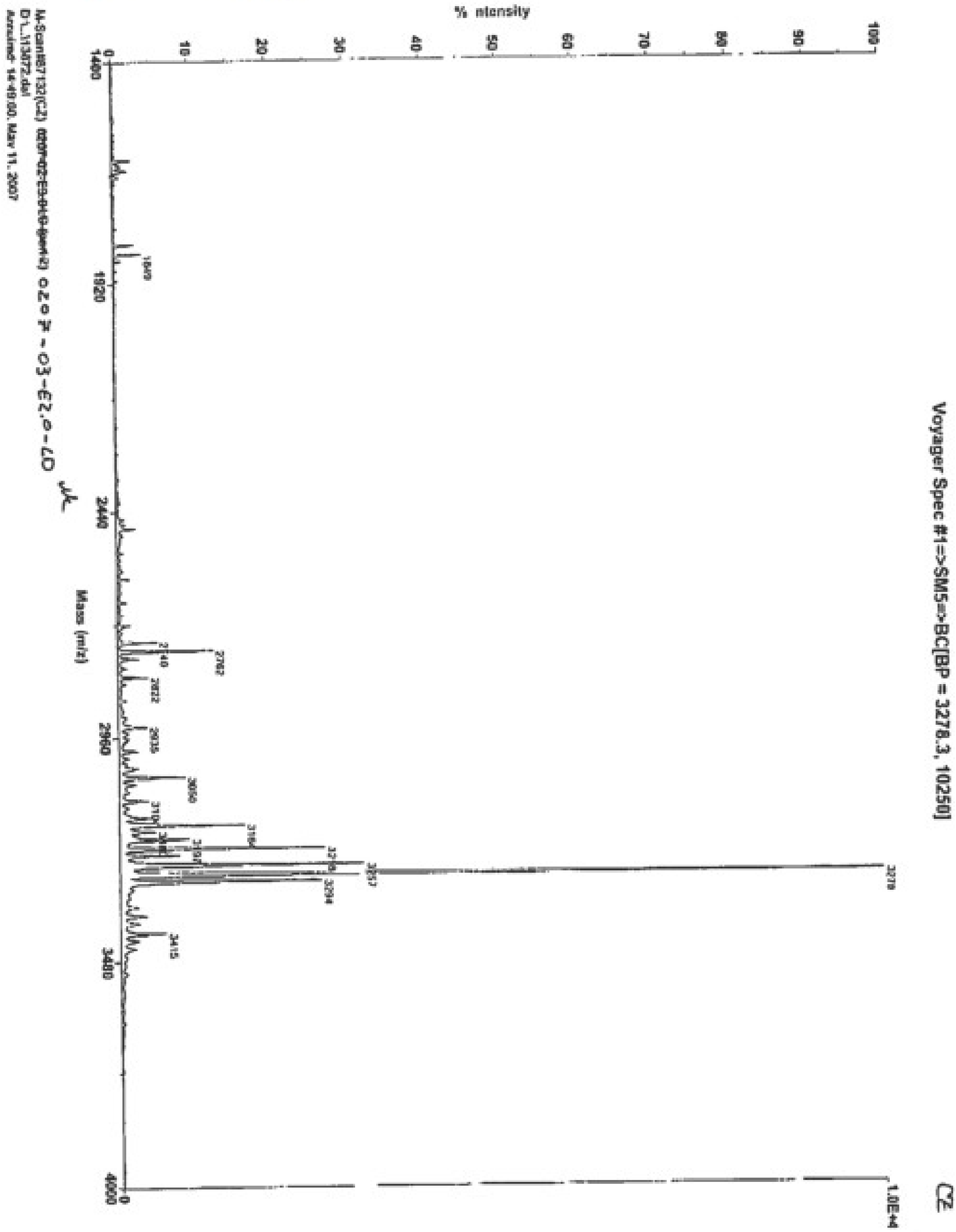
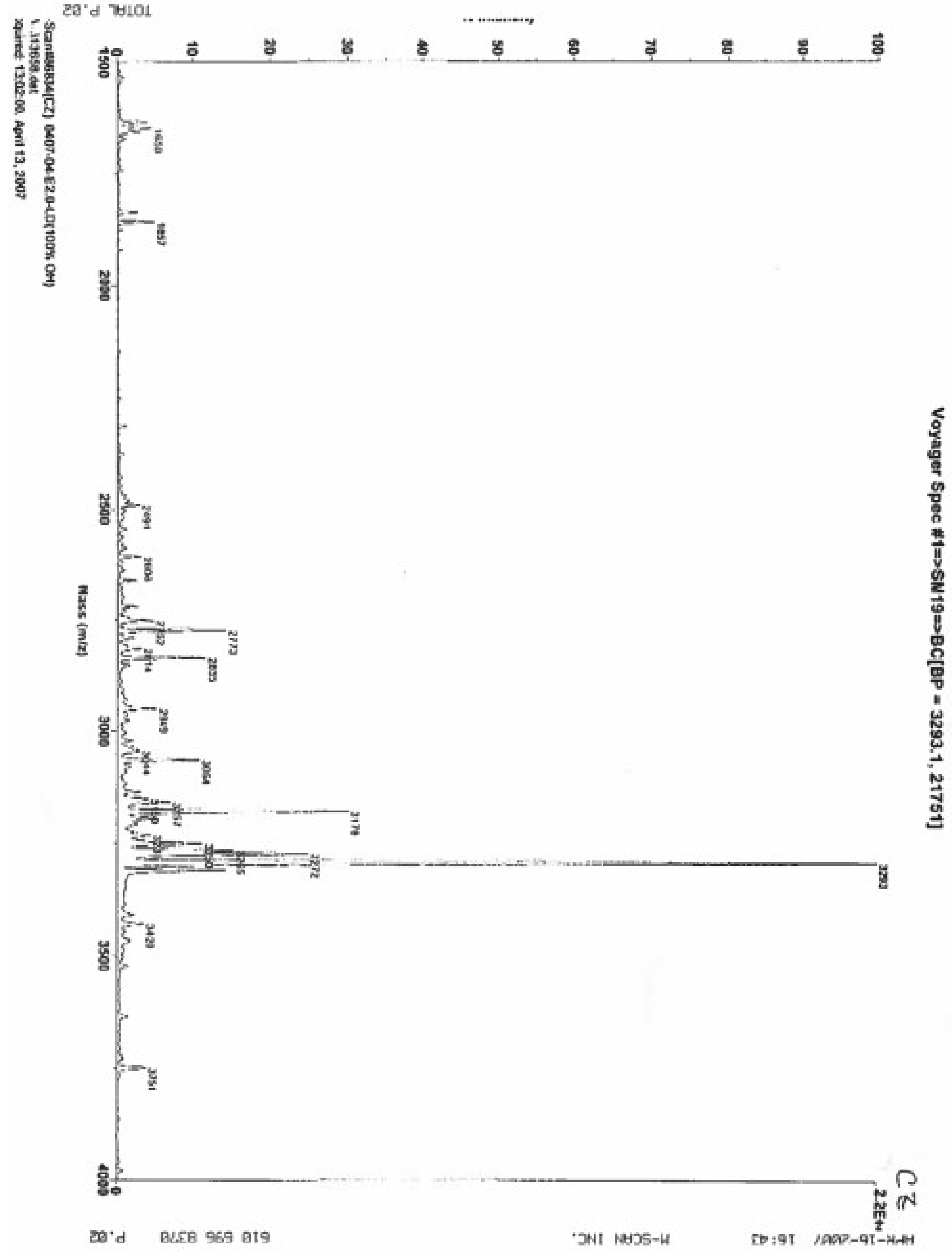


Figure S4. Effect of G2-COONa *cis*-side addition on a single PA_{63} channel. Both in blocker-free solution and in presence of 3 mM G2-COONa, PA_{63} mostly remains in an open state. The 1/*f* events are to a large extent removed by averaging over a time interval of 10 ms. G2-COONa addition causes lipid bilayer instability (upward events) and, eventually, breakage. Recordings were taken in 1 M KCl solutions at pH 6 and 100 mV applied voltage.

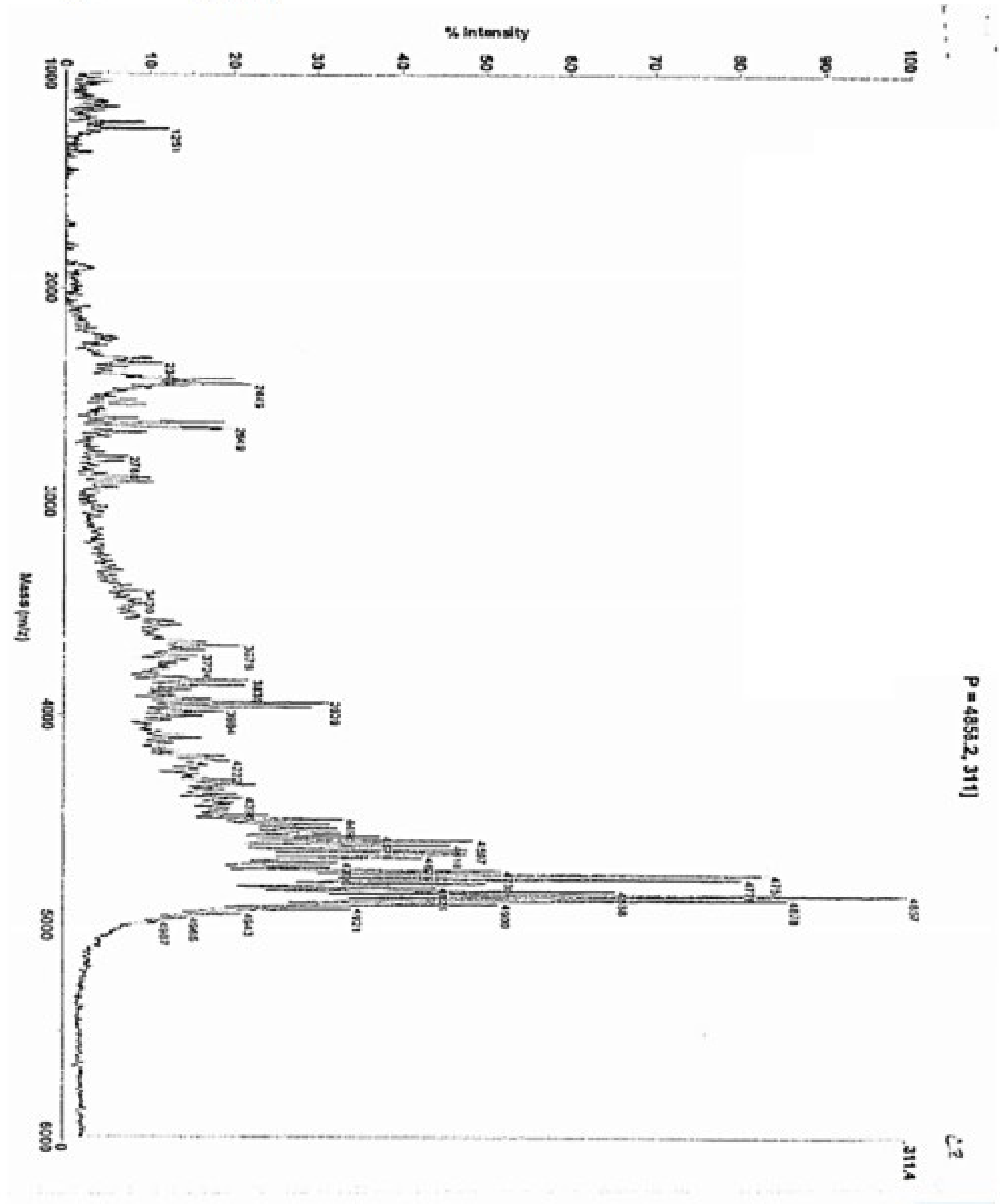
A G2-NH_2


Voyager Spec #1=>SM5=>BC[BP = 3278.3, 10250]
% Intensity
Mass (m/z)
1400
1920
2440
2960
3480
4000
1.0E+4
3279
3257
3294
3415
2762
3050
3184

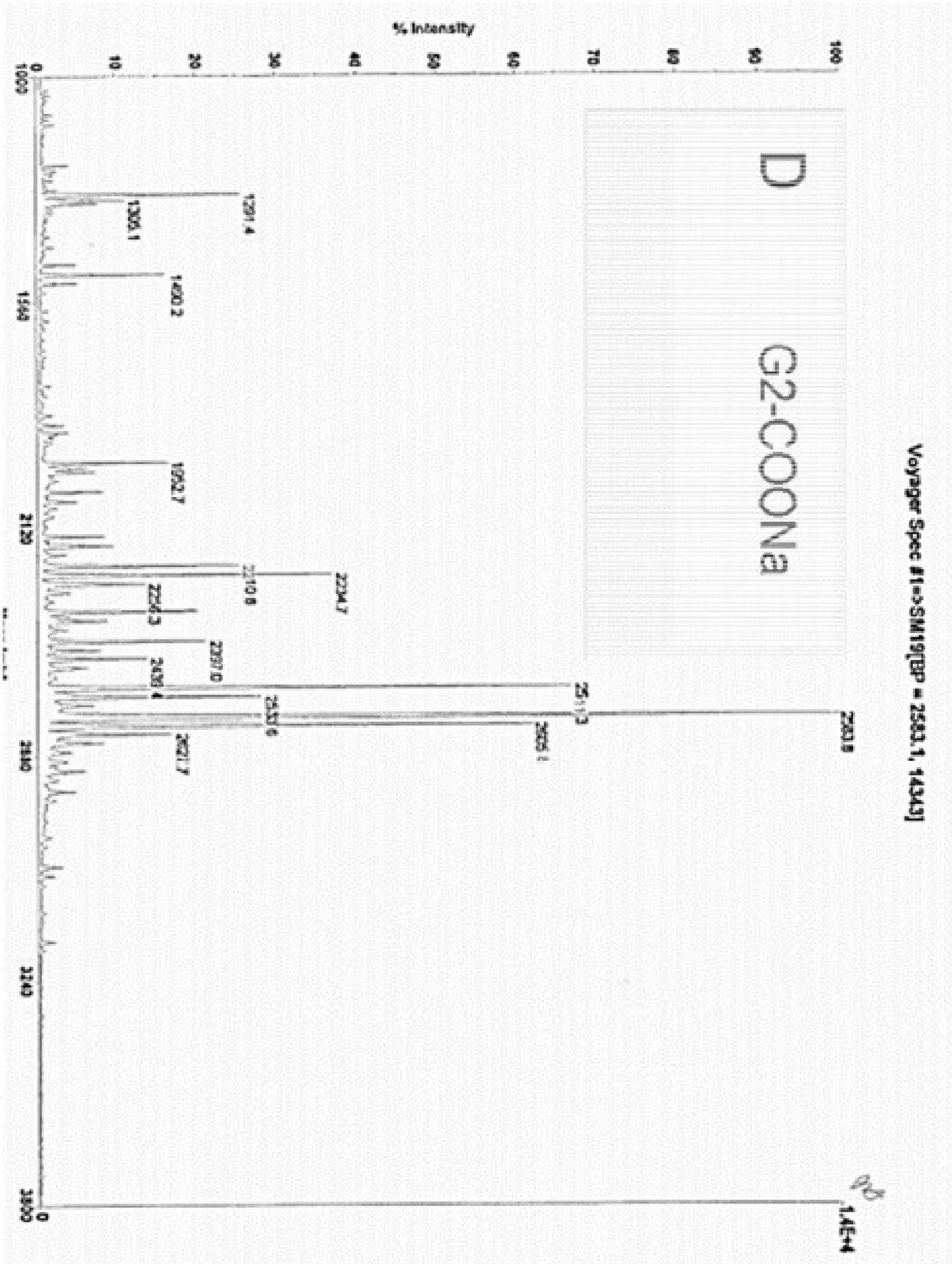
B G2-OH


Voyager Spec #1=>SN19=>BC[BP = 3293.1, 21751]
Mass (m/z)
1500
2000
2500
3000
3500
4000
0
10
20
30
40
50
60
70
80
90
100
1650
1857
2491
2773
2835
3054
3176
3293
3429
3751
2.2E+4

C G2-SA



D G2-COONa



E G3-NH2 dendron

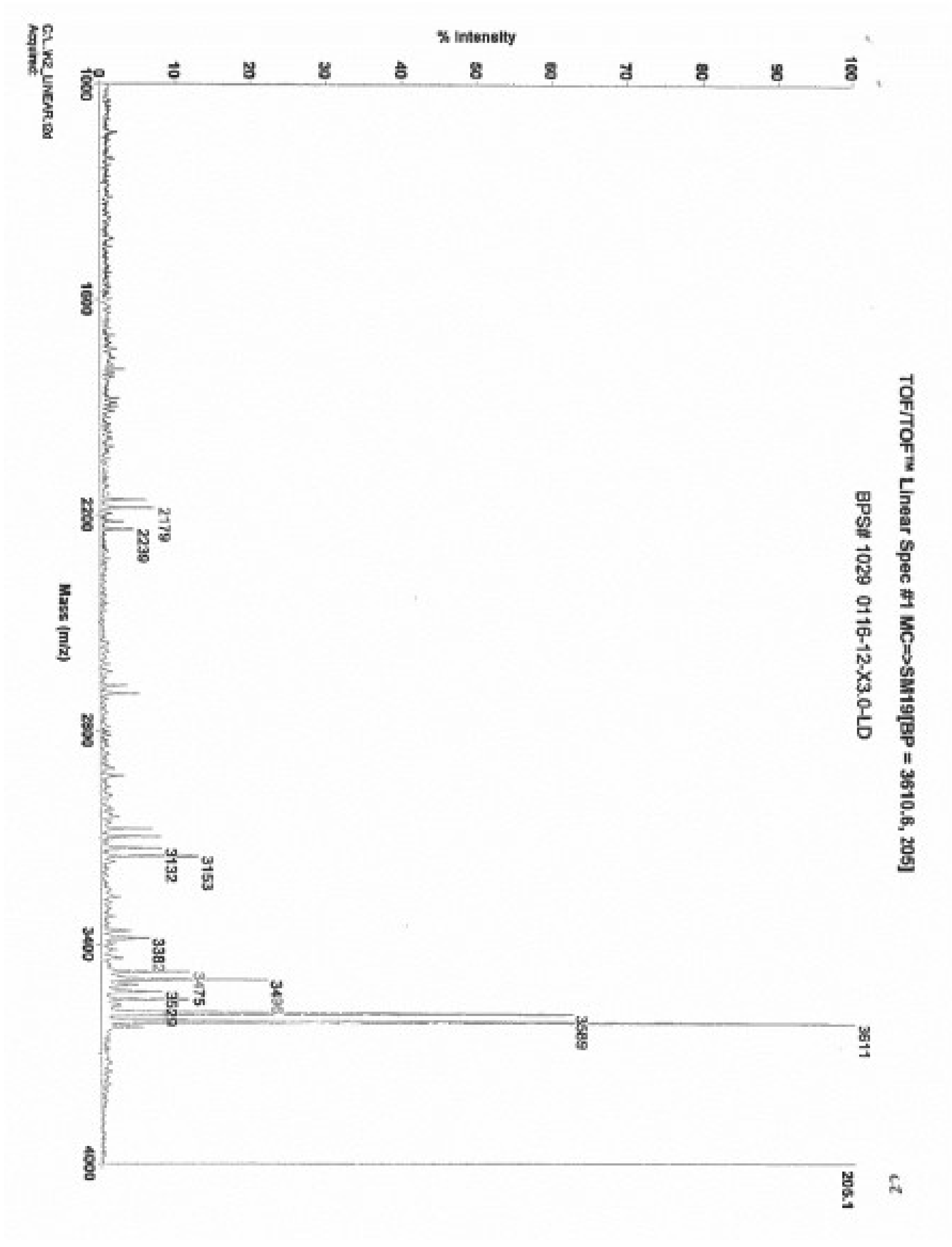
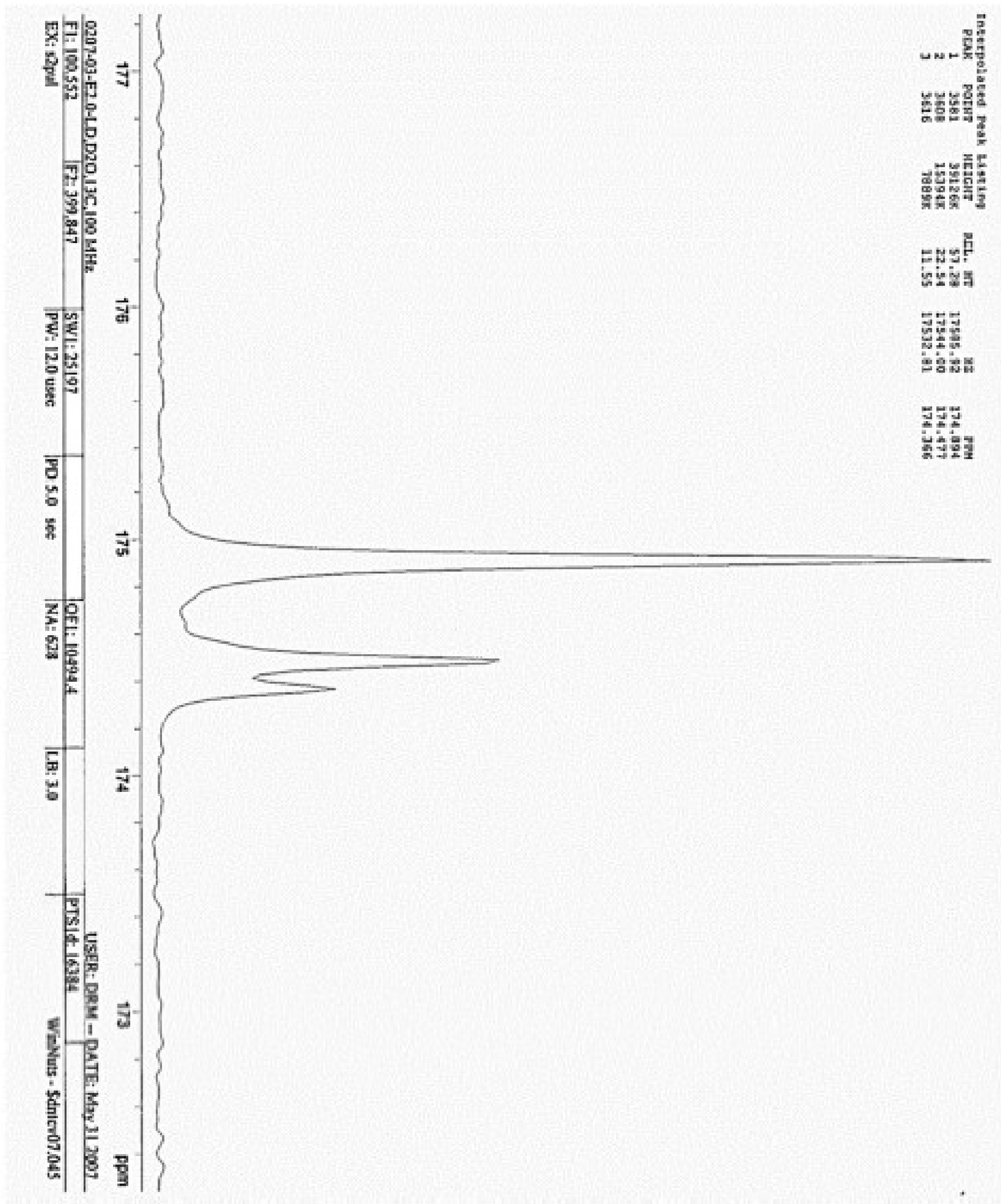
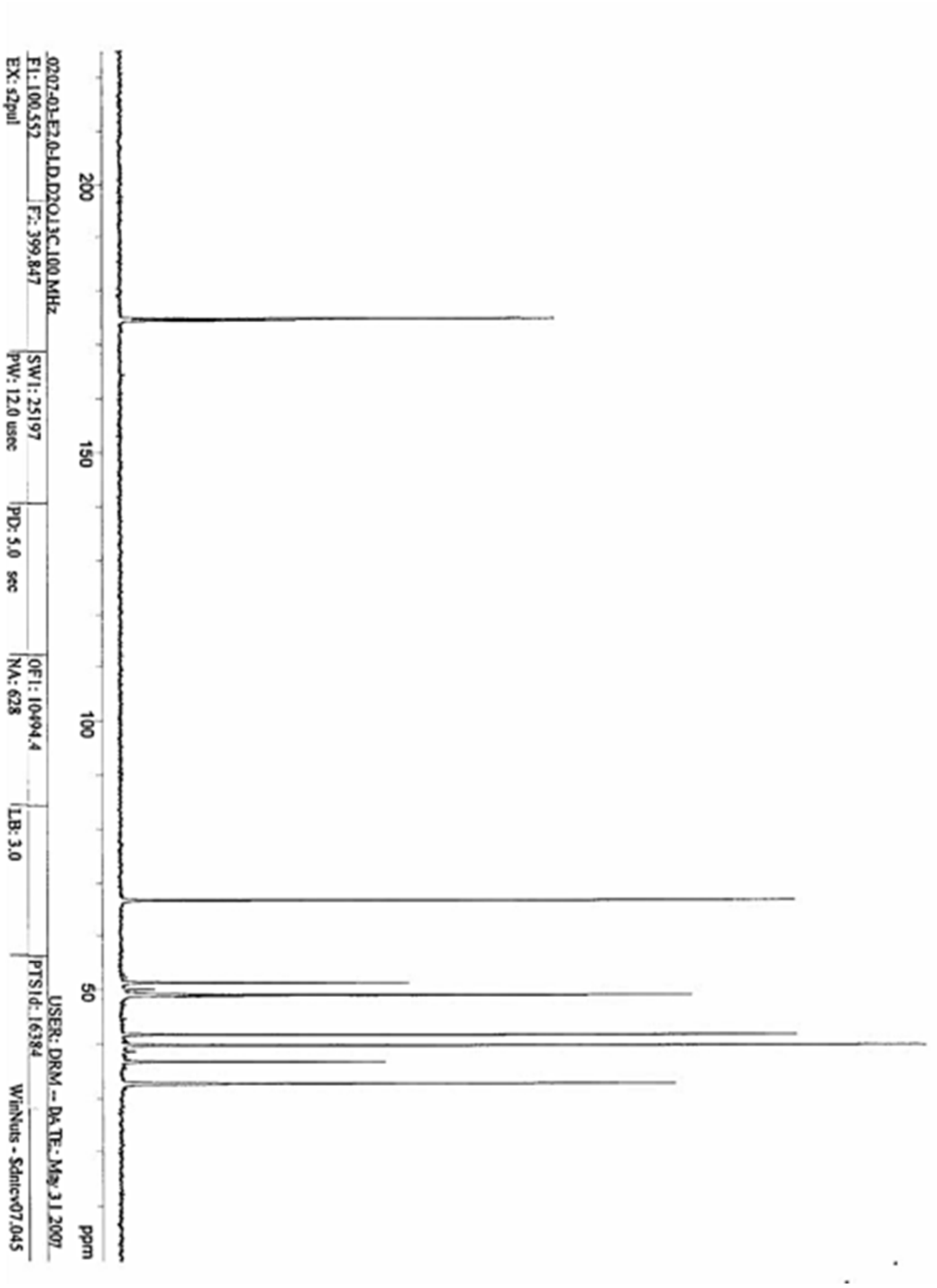
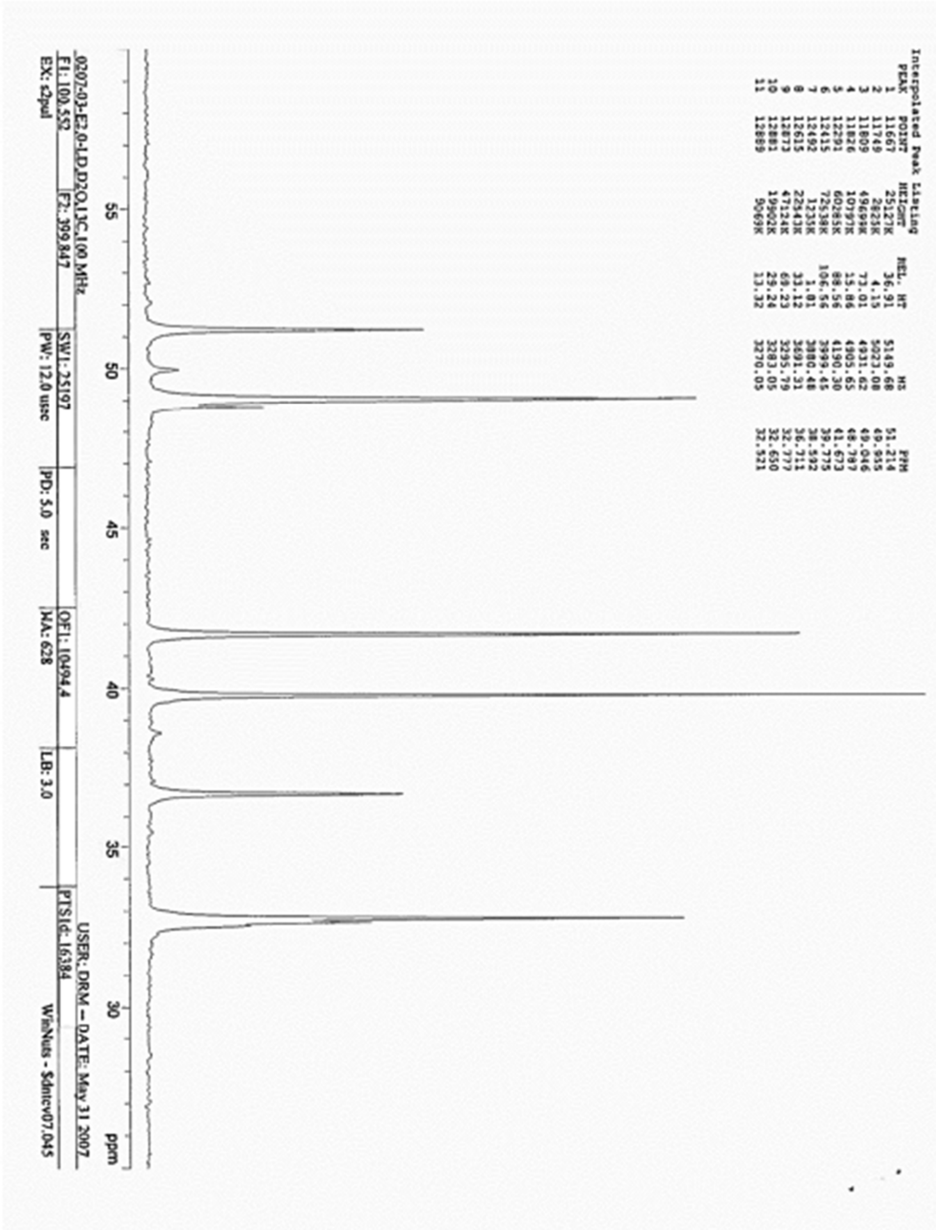


Figure S5. MALDI-TOF mass spectra of G2-NH2 (**A**); G2-OH (**B**); G2-SA (**C**); G2-COONa (**D**) dendrimers and G3-NH2 dendron (**E**). The data were provided by Dendritech, Inc (Midland, MI USA).

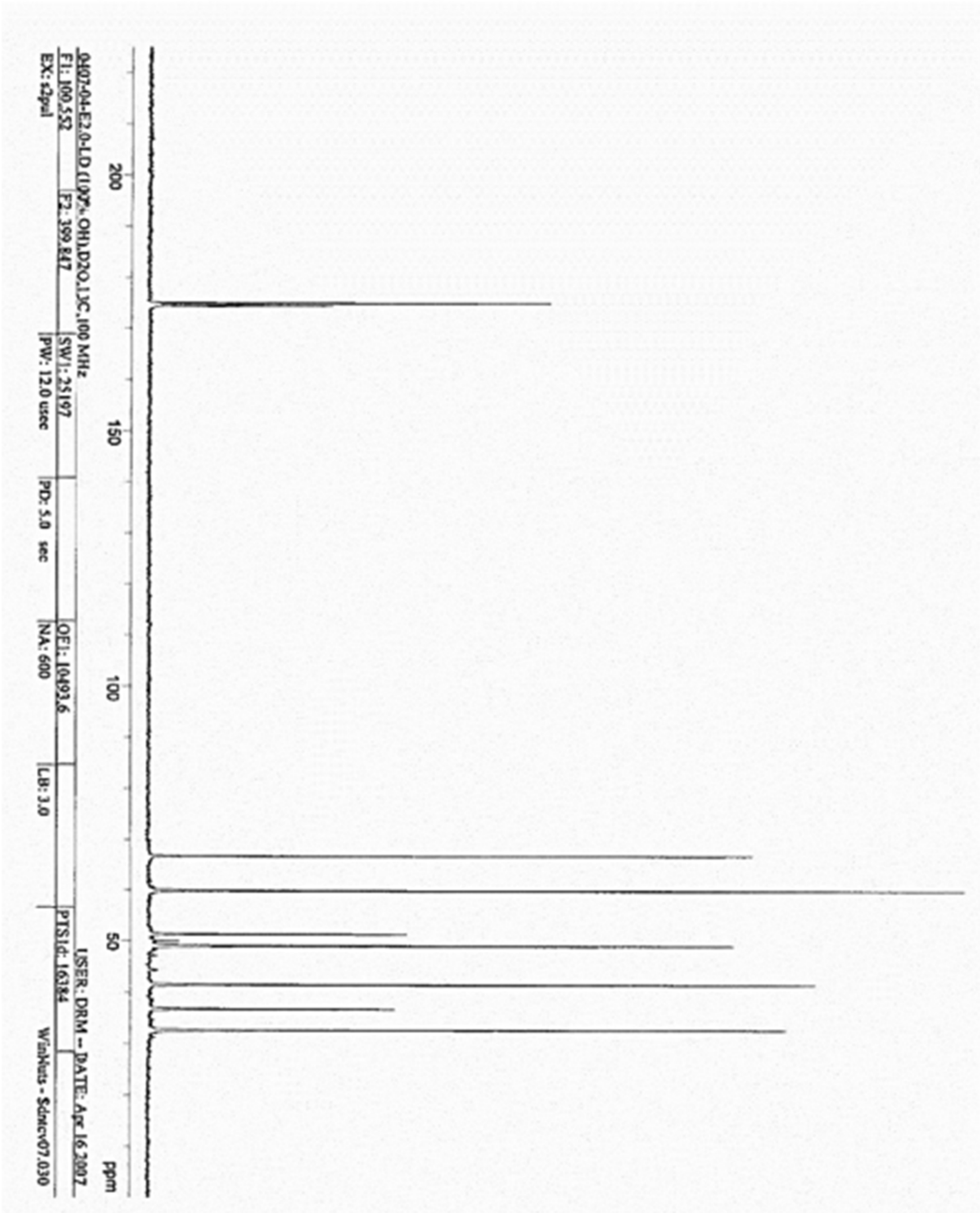
A G2-NH_2

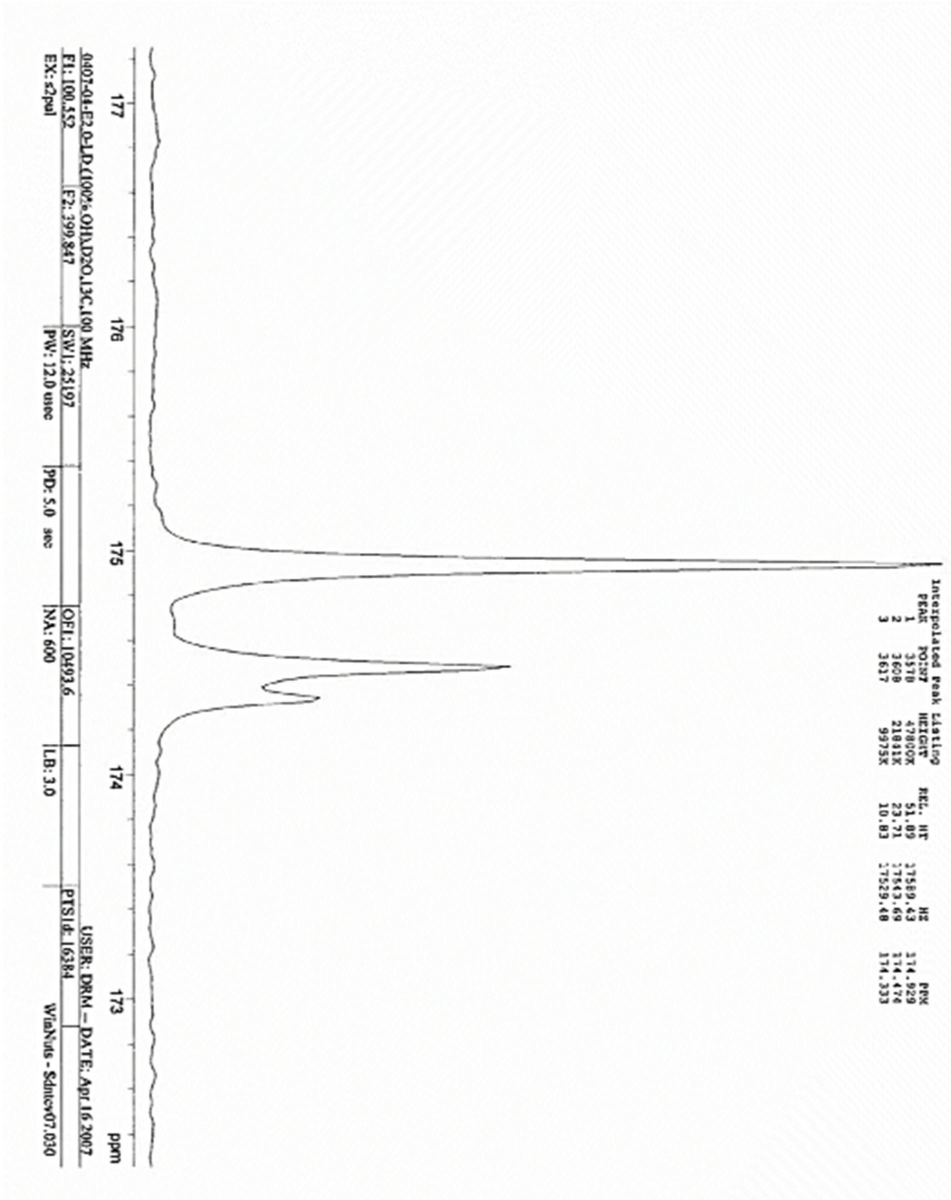



0207-03-E2.0-LD.D2O.13C.100.MHz
F1: 100.552
F2: 399.847
SW1: 25197
PW: 12.0 usec
PD: 5.0 sec
OF1: 10494.4
NA: 628
LB: 3.0
USER: DRM -- DATE: May 31 2007
PTS1d: 16384
WinNuts - Sdnmcv07.045
200
150
100
50
ppm

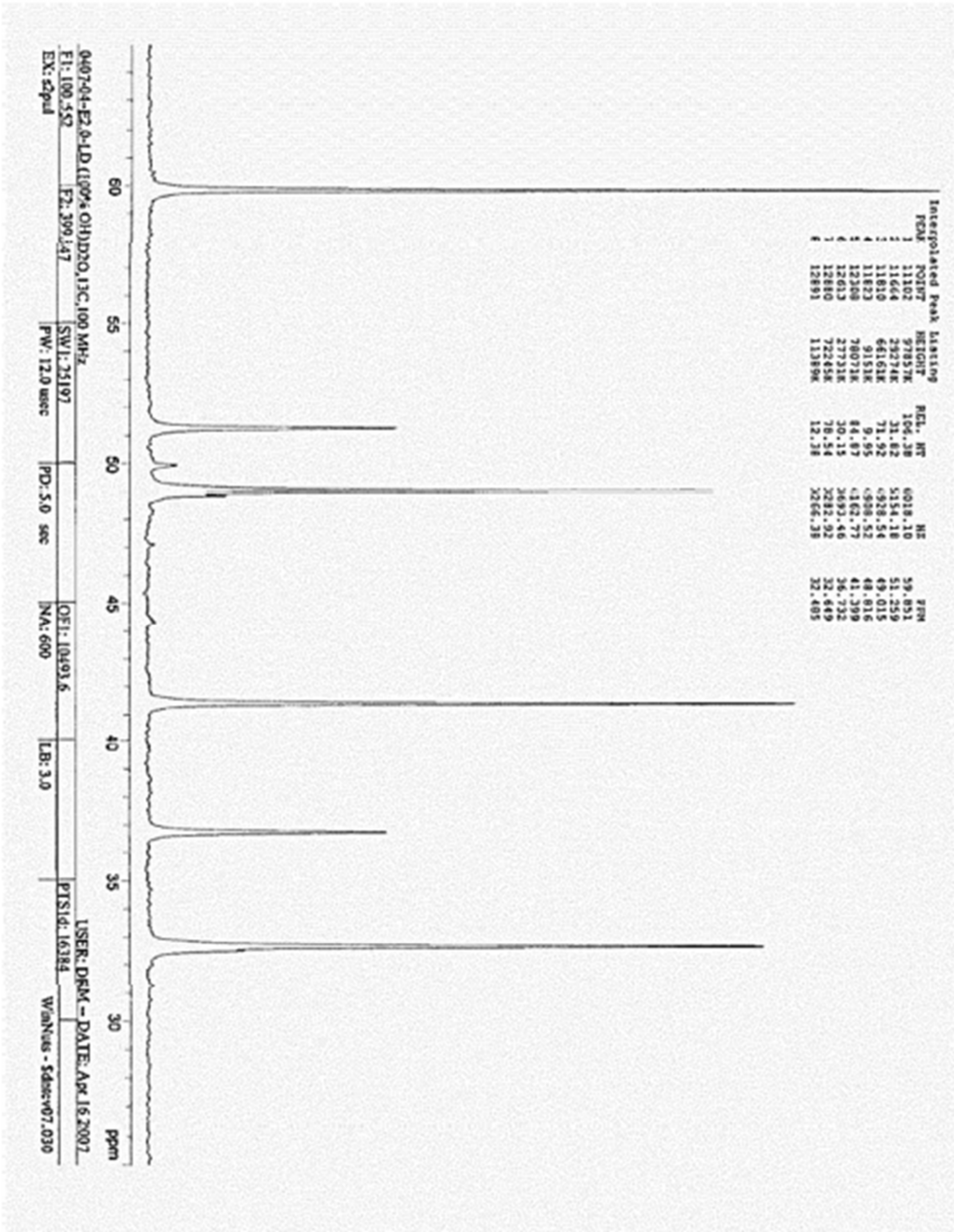

55
50
45
40
35
30
ppm
0207-03-E2.0-LD D2O L13C.100 MHz
F1: 100.552
F2: 399.847
SW1: 25197
OF1: 10494.4
USER: DRM -- DATE: May 31 2007
EX: s2pul
PW: 12.0 usec
PD: 5.0 sec
NA: 628
LB: 3.0
PTS1d: 16384
WinNuts - $dntcv07.045

B G2-OH

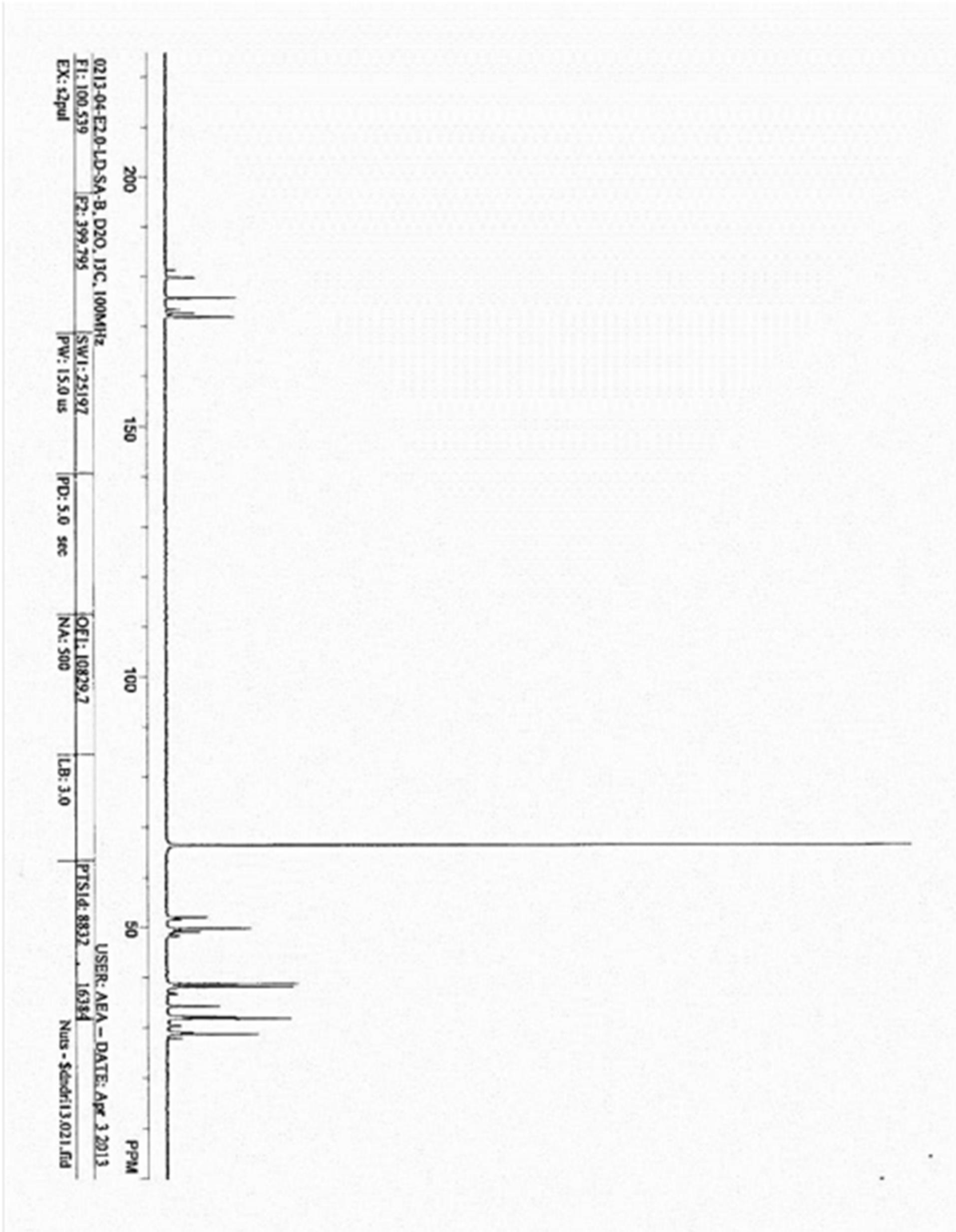


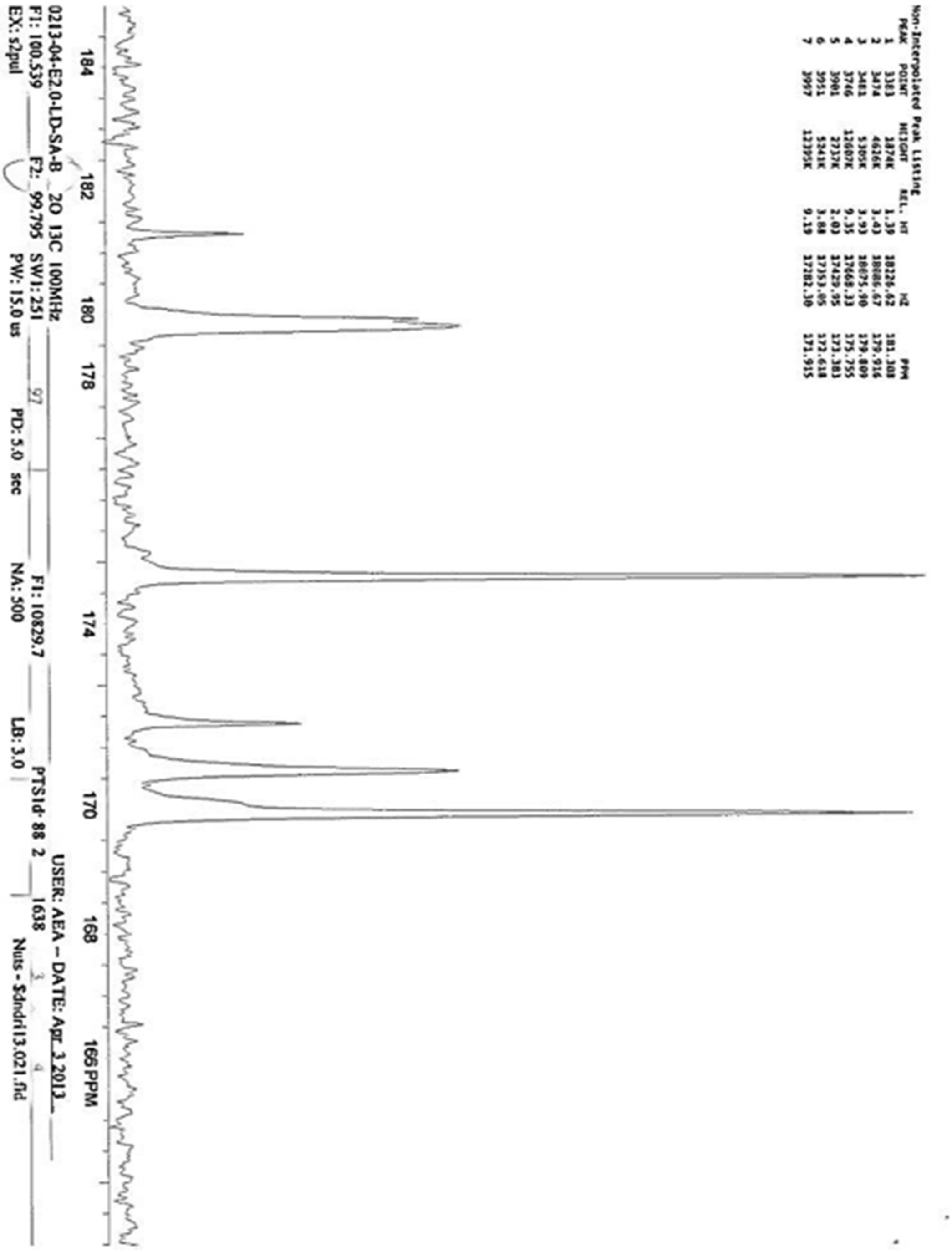


Interpolated Peak Listing
PEAK POINT HEIGHT REL. HT HZ PPM
1 3578 47800K 51.09 17589.43 174.929
2 3608 21841K 23.71 17543.69 174.474
3 3617 9975K 10.83 17529.48 174.333
177
176
175
174
173
ppm
F1: 100.552
F2: 399.847
SW1: 25197
PW: 12.0 usec
PD: 5.0 sec
OF1: 10493.6
NA: 600
LB: 3.0
PTS1d: 16384
EX: s2pul
USER: DRM -- DATE: Apr 16 2007
WinNuts - Sdntcv07.030

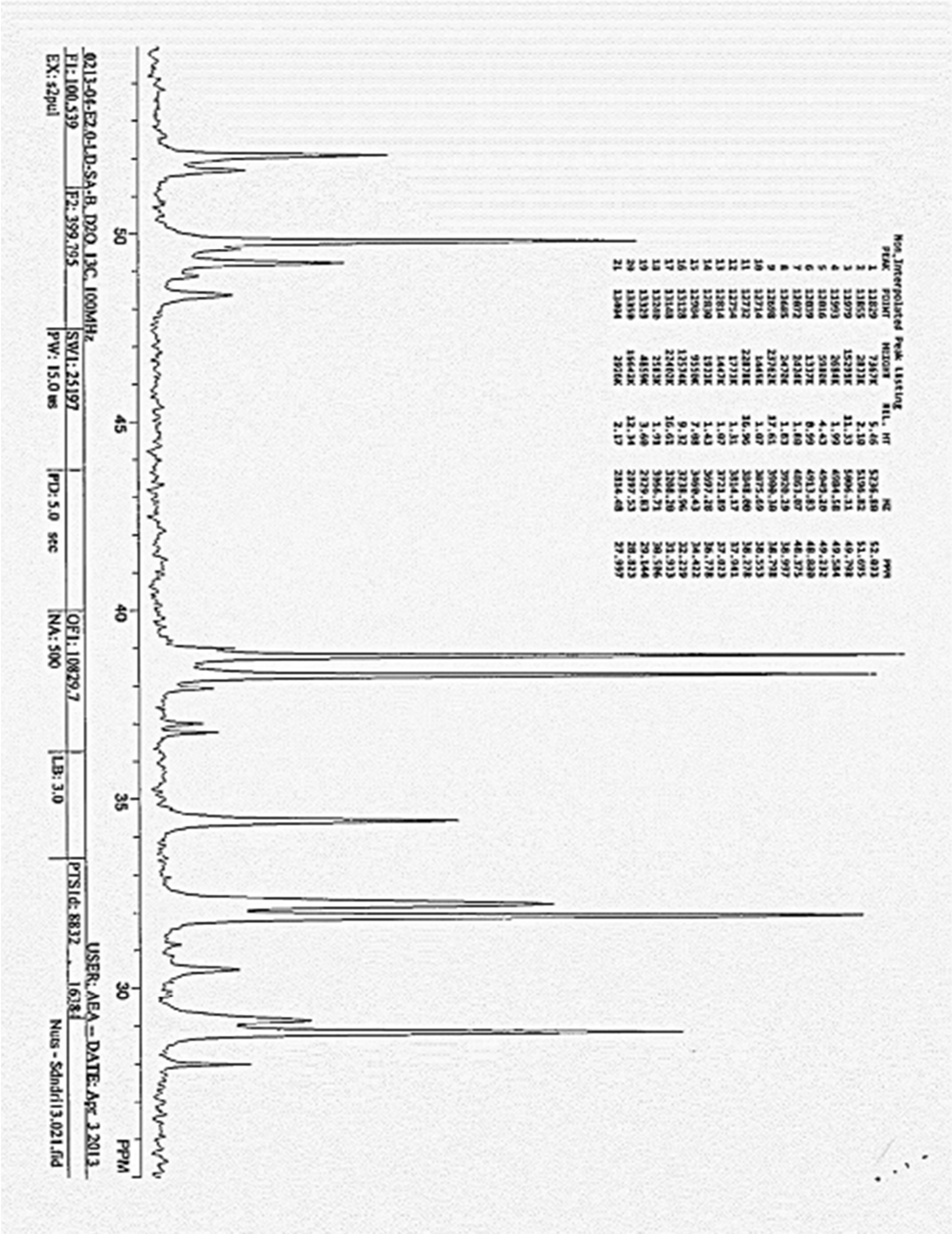


C G2-SA

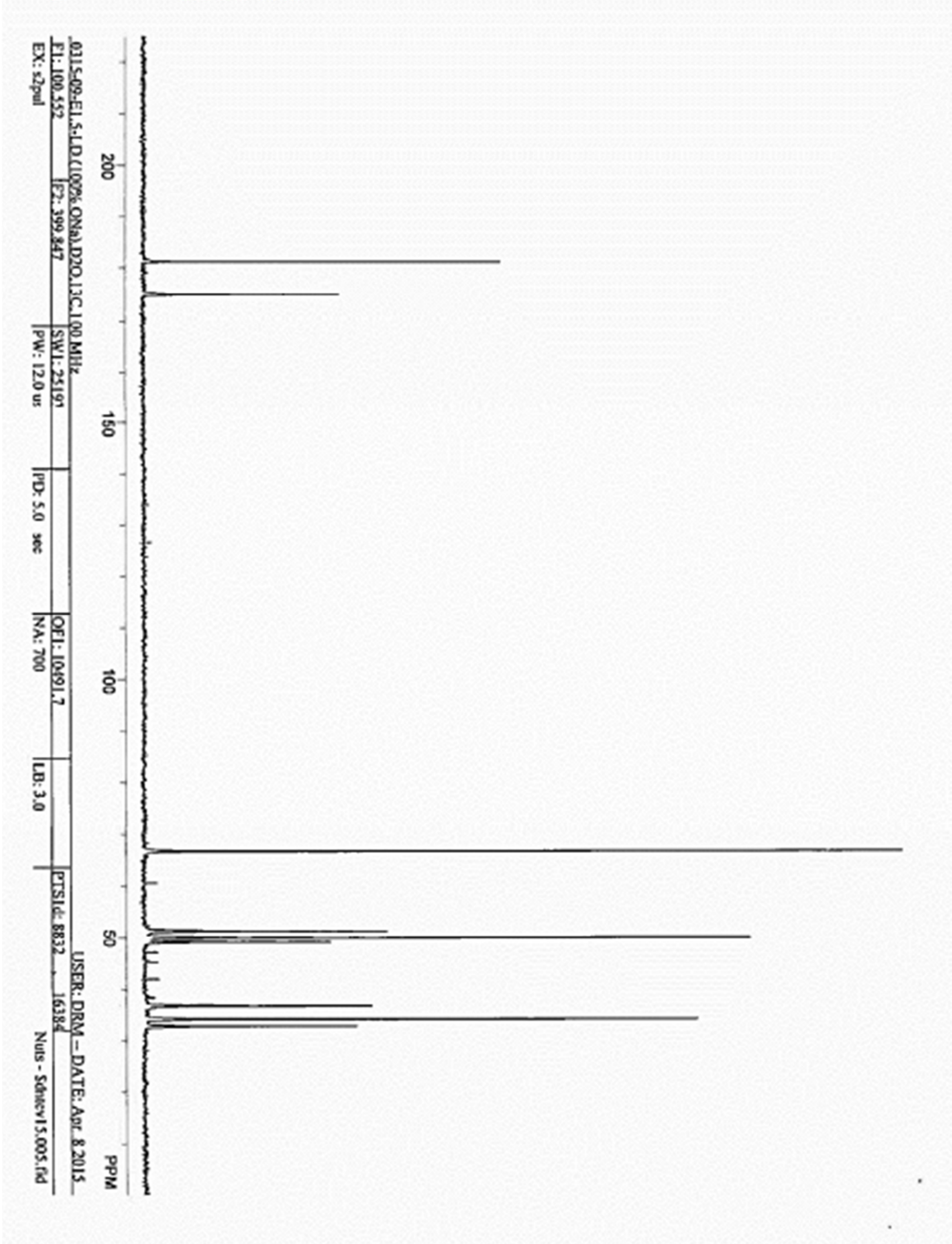


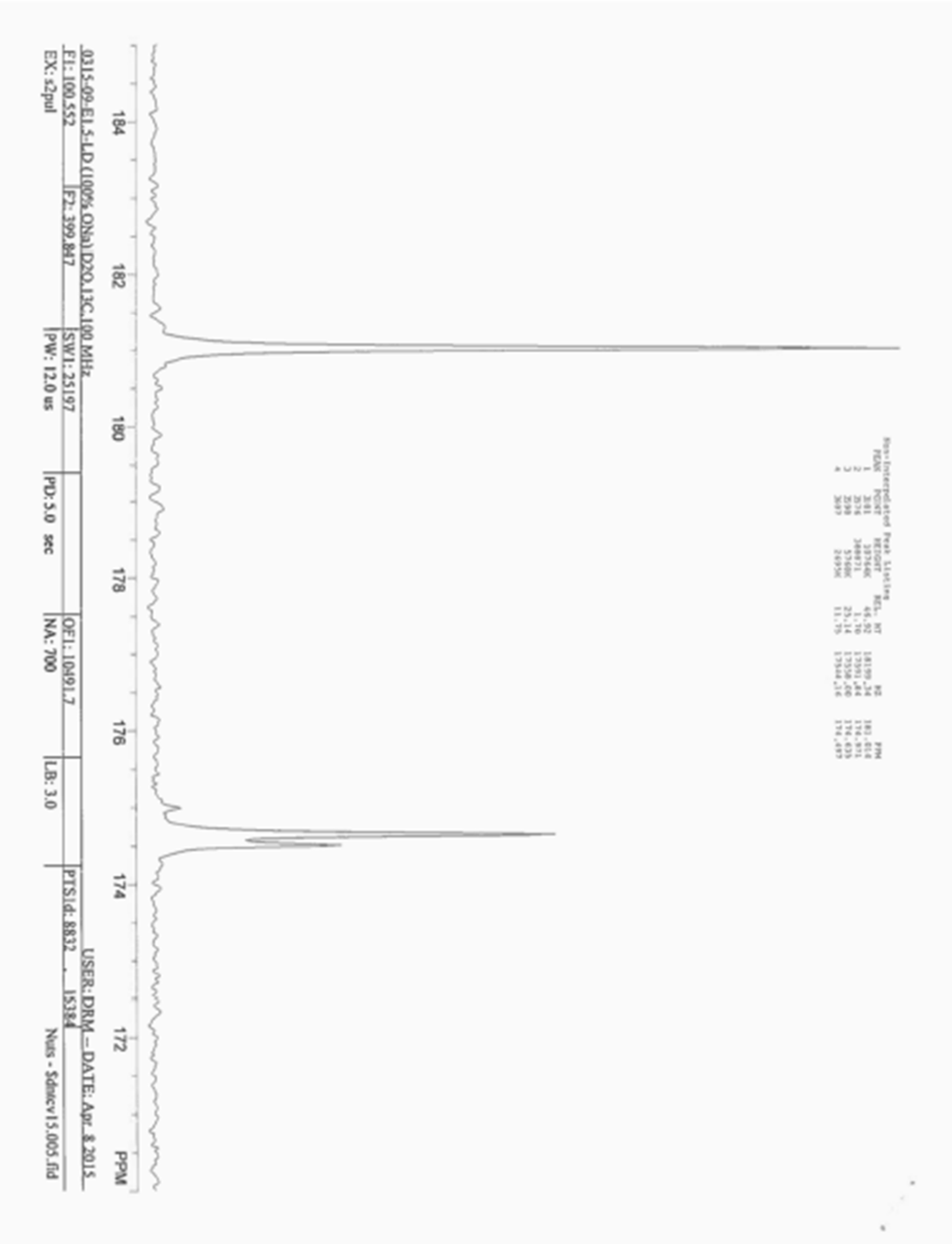


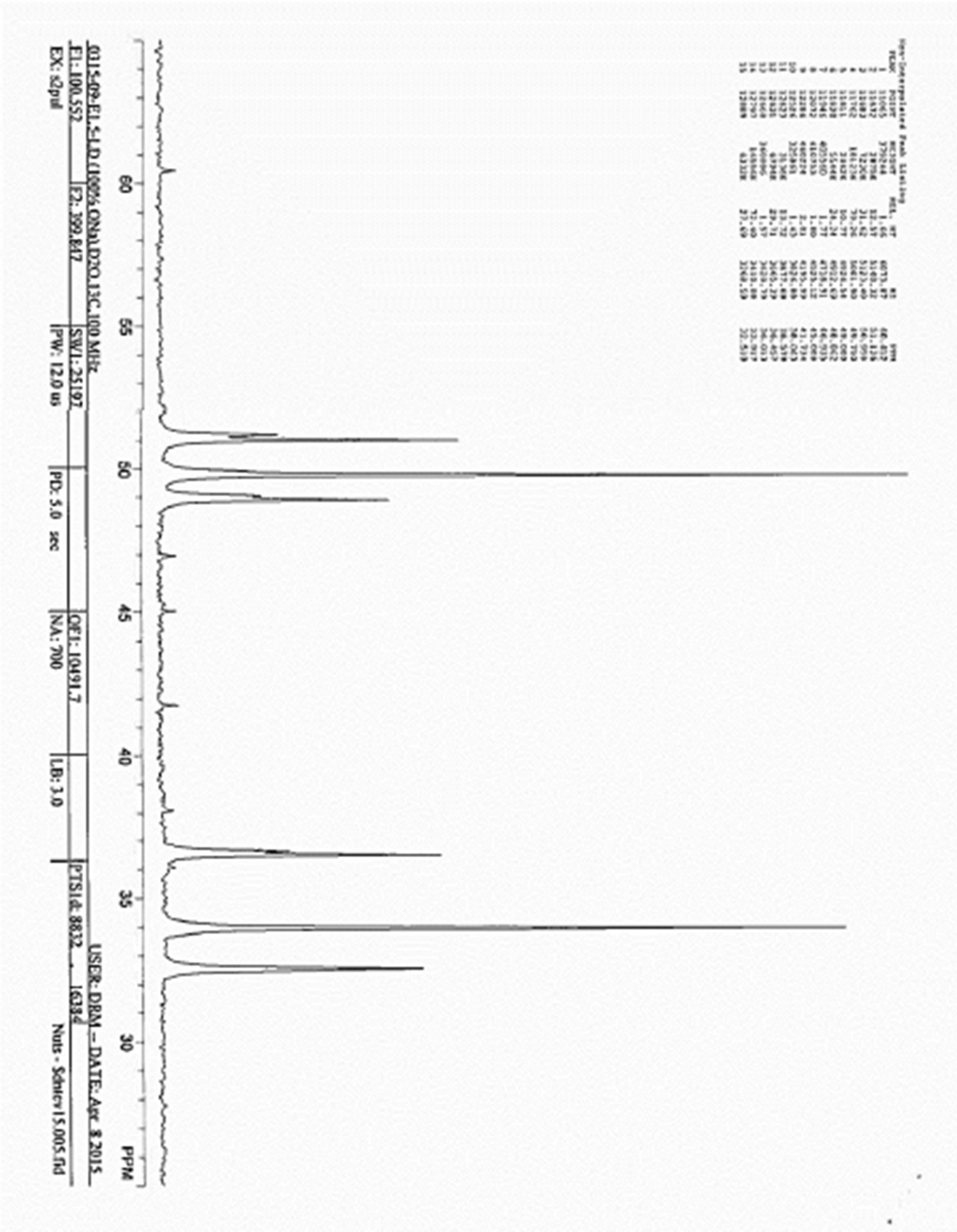
184
182
180
178
174
170
168
166 PPM
0213-04-E2.0-LD-SA-B 2O 13C 100MHz
FI: 100.539 F2: 99.795 SW1: 251
EX: s2pul PW: 15.0 us
PD: 5.0 sec
FI: 10829.7
NA: 500
LB: 3.0
PTS1d: 88 2
1638
USER: AEA – DATE: Apr 3 2013
Nuts - $dndr113.021.fid
Non-Interpolated Peak Listing


50
45
40
35
30
PPM

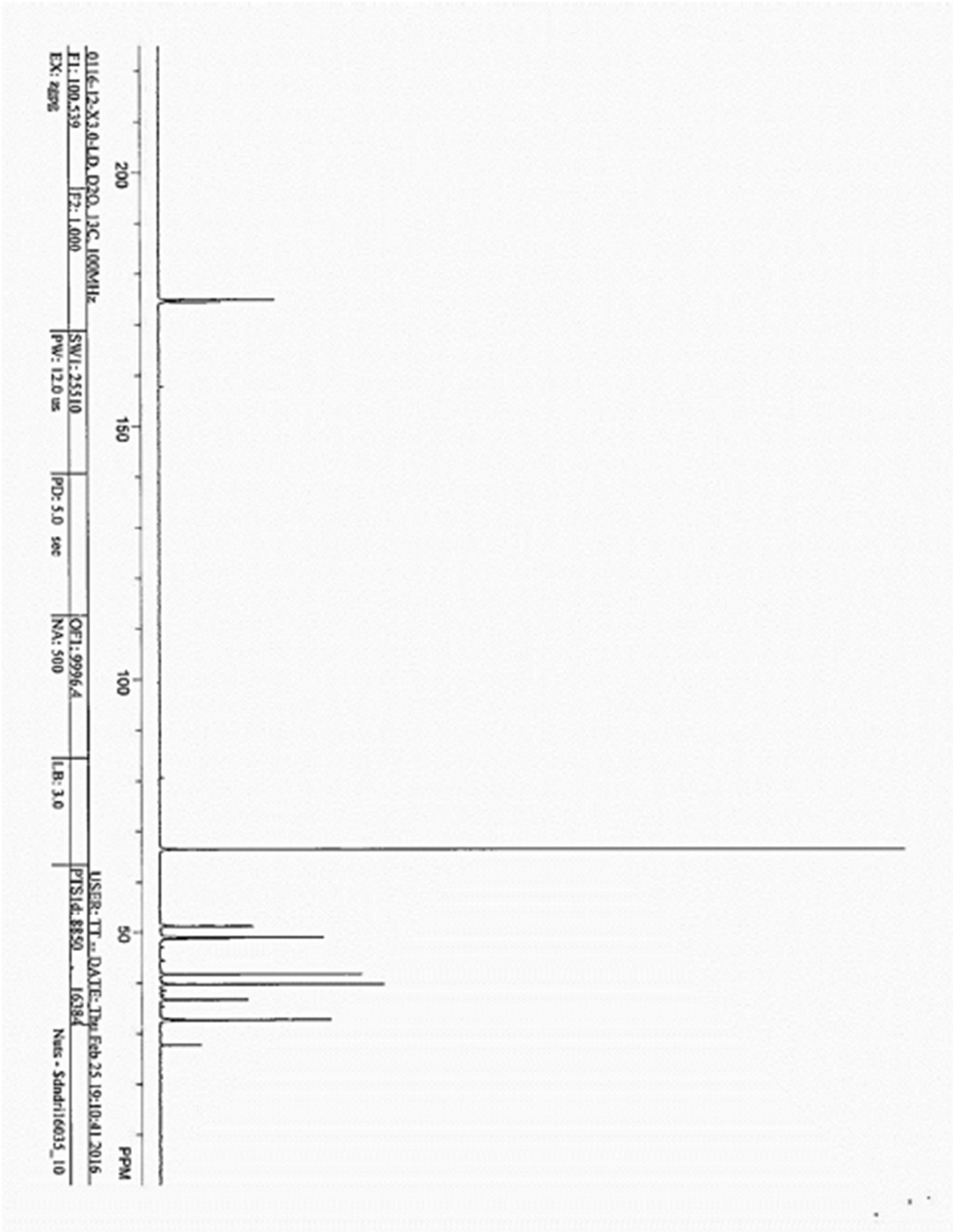
D G2-COONa

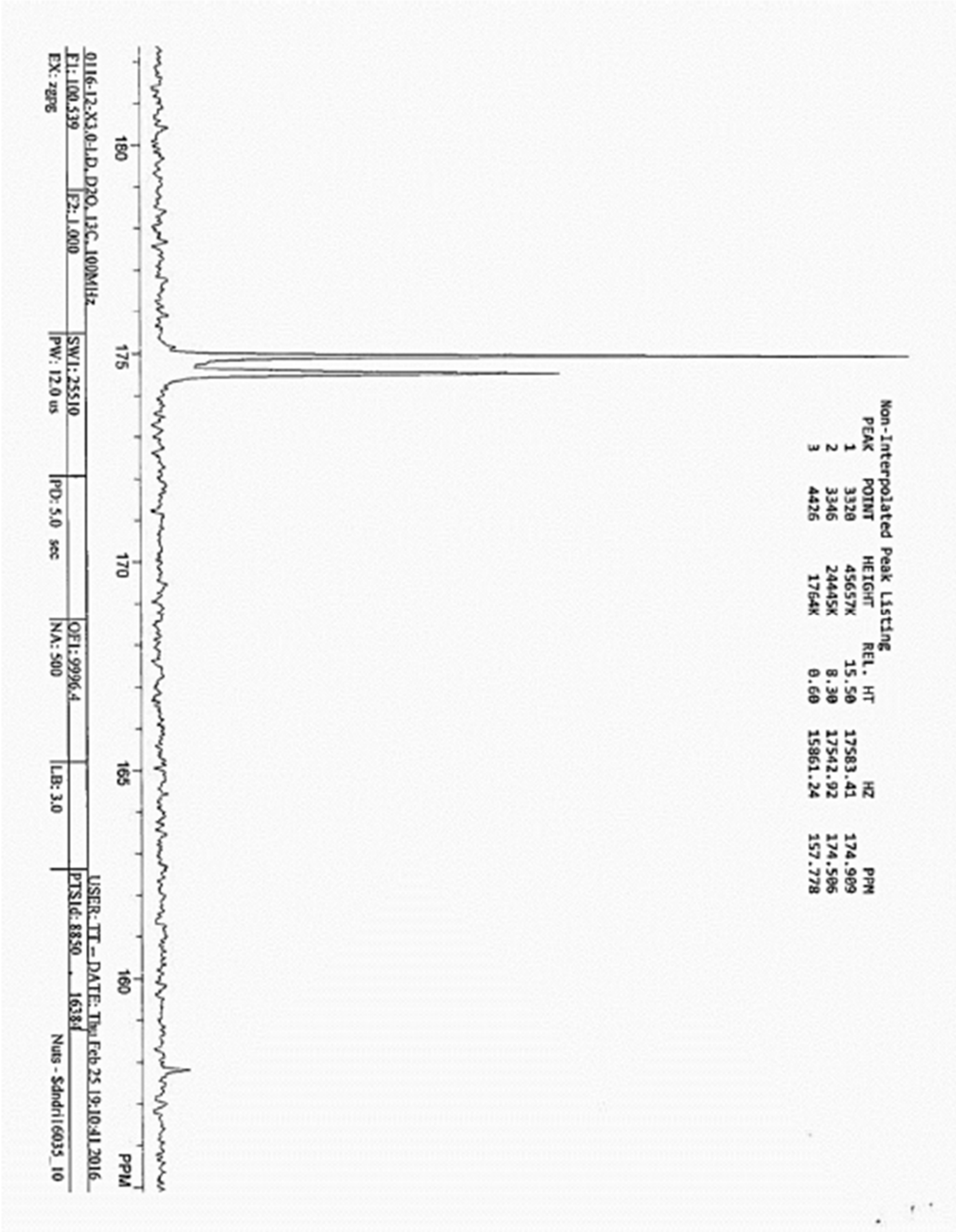



0315-09-EL5-LD (100% ONa) D2O 13C 100 MHz
F1: 100.552
F2: 399.847
SW1: 25197
PW: 12.0 us
PD: 5.0 sec
OF1: 10491.7
NA: 700
LB: 3.0
PTS1d: 8832
15384
USER: DRM -- DATE: Apr. 8 2015
Nuts - Sdnicv15.005.fid
EX: s2pul
184
182
180
178
176
174
172
PPM



E G3-NH₂ dendron




Non-Interpolated Peak Listing
PEAK POINT HEIGHT REL. HT HZ PPM
1 3320 45657K 15.50 17583.41 174.909
2 3346 24445K 8.30 17542.92 174.506
3 4426 1764K 0.60 15861.24 157.778
180
175
170
165
160
PPM
0116-12-X3.0-L.D. D2O. 13C. 100MHz
F1: 100.539
F2: 1.000
SW1: 25510
OF1: 9996.4
USER: TT DATE: Thu Feb 25 19:10:41 2016
EX: zgpg
PW: 12.0 us
PD: 5.0 sec
NA: 500
LB: 3.0
PTS1d: 8850 16384

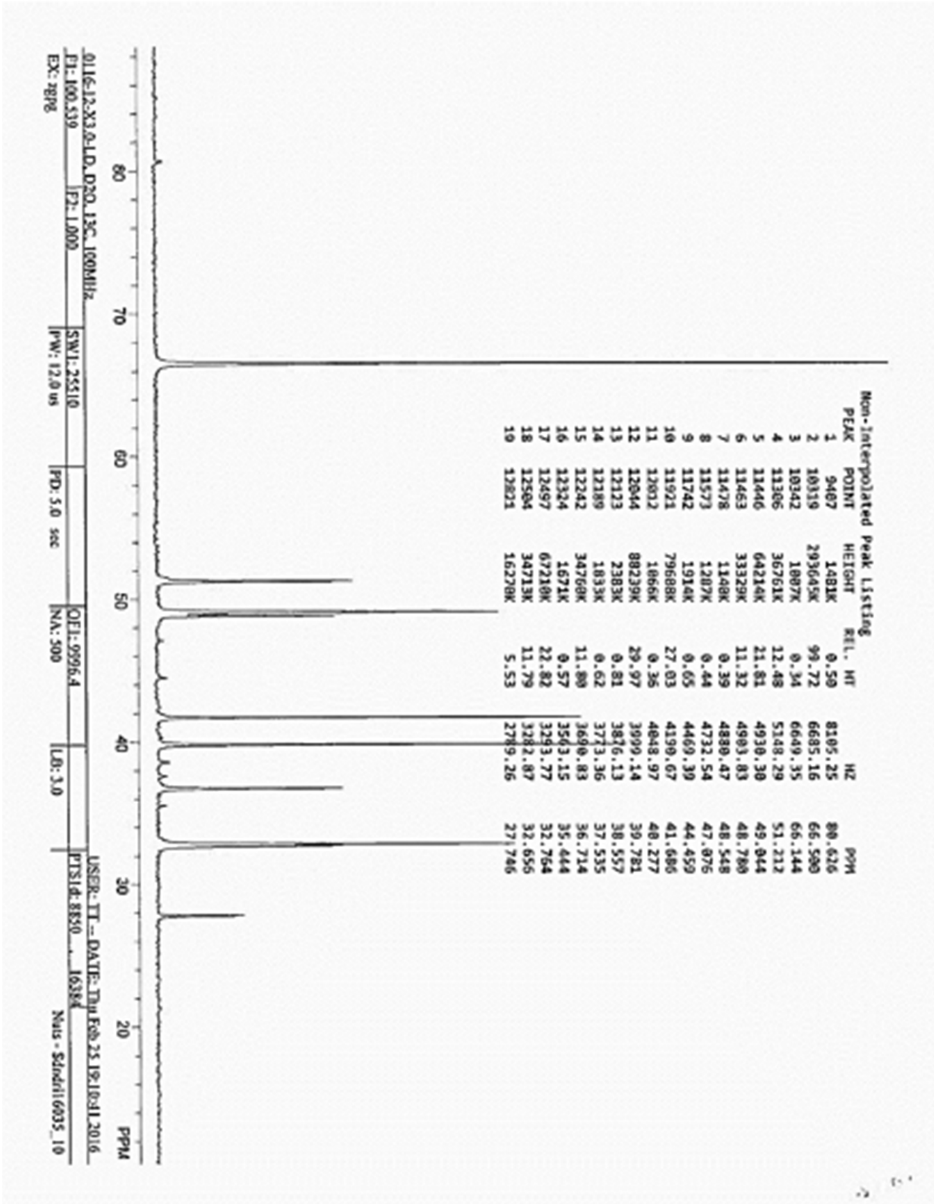


Figure S6. Characterization of generation 2 PAMAM dendrimers and generation 3 PAMAM-NH_2 dendron by 13 C NMR. The data were provided by Dendritech, Inc.(Midland, MI USA), (**A**) 13 C NMR of G2-NH_2. (**B**) 13 C NMR of G2-OH; (**C**) 13 C NMR of G2-SA; (**D**) 13 C NMR of G2-COONa. (**E**) 13 C NMR G3-NH_2 dendron.