Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2017

Supplementary Information

Polyvalent *C*-glycomimetics based on L-fucose or D-mannose as potent DC-SIGN antagonists

Benedetta Bertolotti[a], Ieva Sutkeviciute[b,c], Martino Ambrosini[d], Renato Ribeiro-Viana[e,f], Javier Rojo[e], Franck Fieschi[b], Hana Dvořáková[g], Martina Kašáková[a], Kamil Parkan[a], Martina Hlaváčková[a], Kateřina Nováková[h], and Jitka Moravcová*[a]

[a.] *Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague, Czech Republic.*
[b.] *Institut de Biologie Structurale (IBS), Univ. Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France.*
[c.] *Present address: Laboratory for G Protein-Coupled Receptor Biology, Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.*
[d.] *Department of Molecular Cell Biology and Immunology, VU University Medical Center (VUmc), Amsterdam, The Netherlands.*
[e.] *Glycosystems Laboratory, Instituto de Investigaciones Químicas (IIQ), CSIC - Universidad de Sevilla, Sevilla, Spain.*
[f.] *Present address: Departamento Academico de Química, Universidade Tecnologica Federal do Paraná. Av dos Pioneiros, 3131. Londrina - PR - Brazil.*
[g.] *Laboratory of NMR, Central Laboratories, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague, Czech Republic.*
[h.] *The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic*

*contact: Jitka.Moravcova@vscht.cz

Content

1. NMR and MS identification of new compounds

^{1}H, ^{13}C, COSY, HMQC and HMBC spectra were measured on a Bruker DPX-300, DRX-400, DRX-500, or Bruker Advance III 600 (Bruker Corporation, Germany) spectrometer. All spectra were acquired at 298 K. Chemical shifts are given in δ-units (ppm) and are referenced to TMS. Coupling constants (*J*) are reported in Hz. Numbering of atoms for NMR signal assignment is placed in figures.

Low resolution ESI-MS was carried out using an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. ESI high resolution mass spectra were measured with a LTQ Velos Orbitrap XL (Thermo Fisher Scientific, UK) instrument equipped with LockSpray in ES+ and ES- modes with mobile phase of 80% methanol. MALDI high resolution mass analysis was **carried** out in positive reflectron mode using a MALDI TOF UltrafleXtremeTM MALDI TOF/TOF (Bruker Daltonics, Germany) instrument equipped with 1 kHz smartbeam II laser.

General comment on NMR

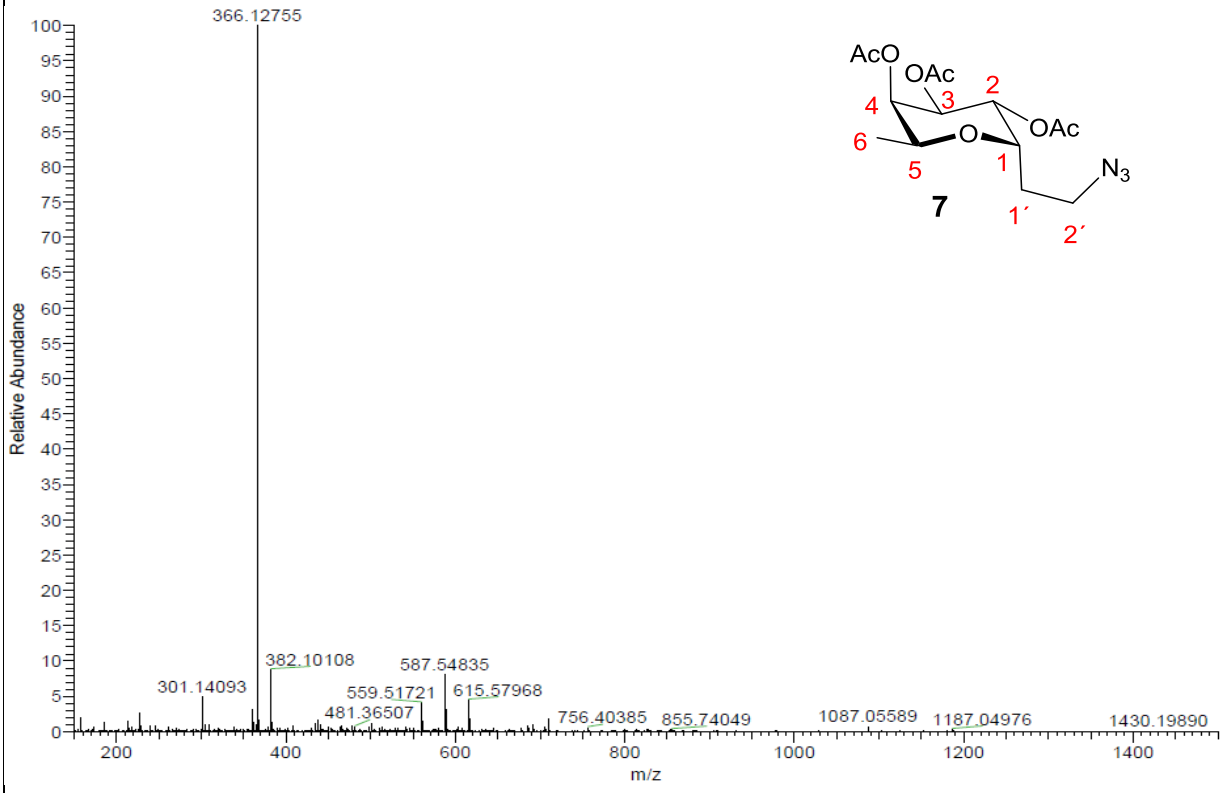
Due to the lack of triazolyl carbons signals in ^{13}C NMR spectrum of all dendrimers (**F-C-4**, **F-C-6**, **F-C-9**, **F-C-12**, **M-C-9**, and **M-C-12**) we suppose the existence of some dynamic process resulted from conformational behaviour of *C*-pseudoglycosidic dendrimers. In the case of **F-C-9**, most of the carbon and some of the proton resonances were doubled although MS revealed that **F-C-9** is a single compound with MW 3283. This observation could be explained by the presence of two **F-C-9** stereoisomers.

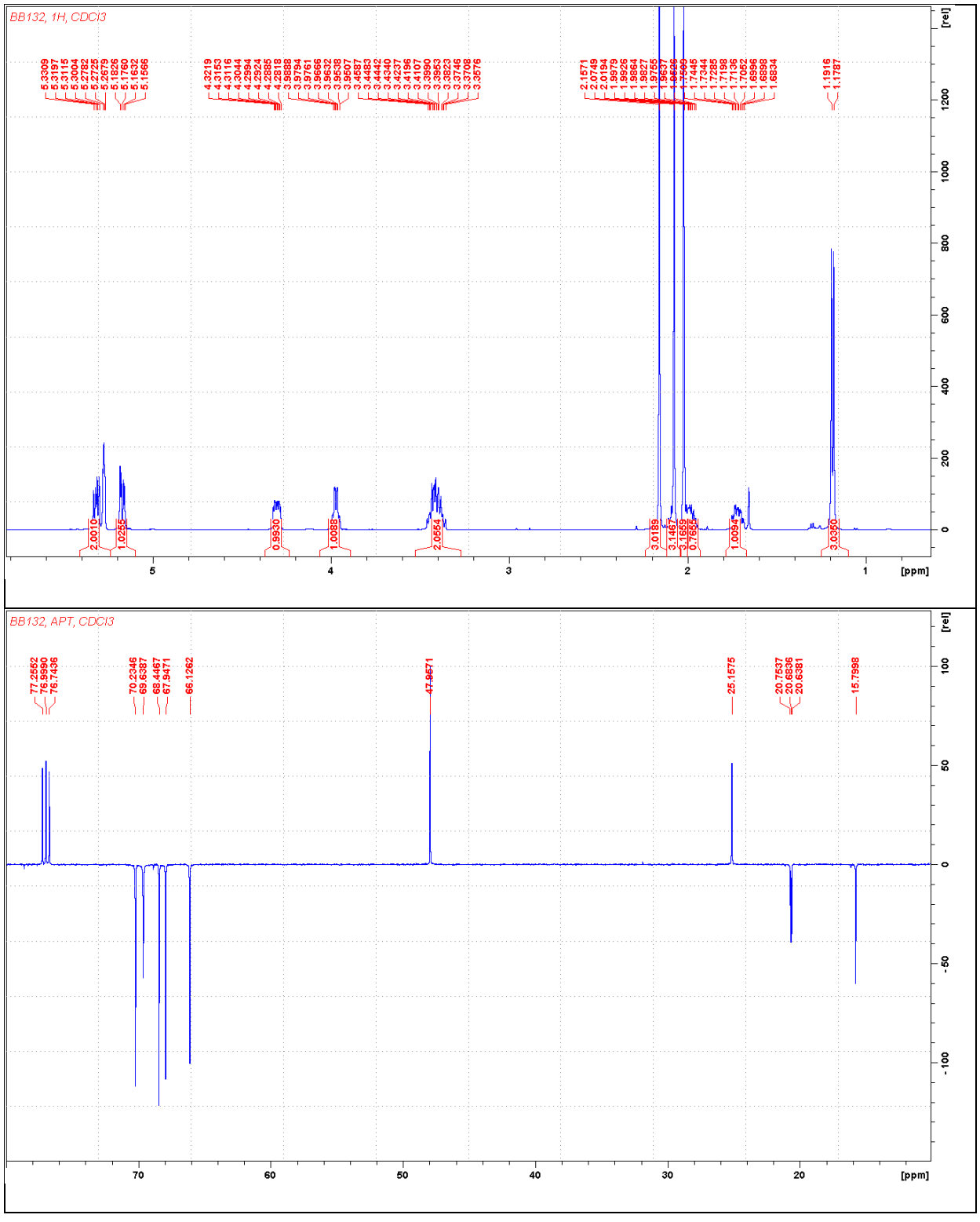
General comment on MS

A molecular associate with Na^+ was obviously the most abundant ion under both ESI and MALDI ionizations. Highly charged ions as well as associates of two molecules with H^+/Na^+ were formed frequently. The *C*-pseudoglycosidic dendrimers gave molecular ions, which were further fragmented by elimination of one arm generating the loss of 255 (**F-C-4**, **F-C-6**, **F-C-9**, and **F-C-12**) or 271 (**M-C-9**, and **M-C-12**) mass.

2-(2,3,4,6-Tetra-*O*-acetyl-α-L-fucopyranosyl)ethylazide (7)

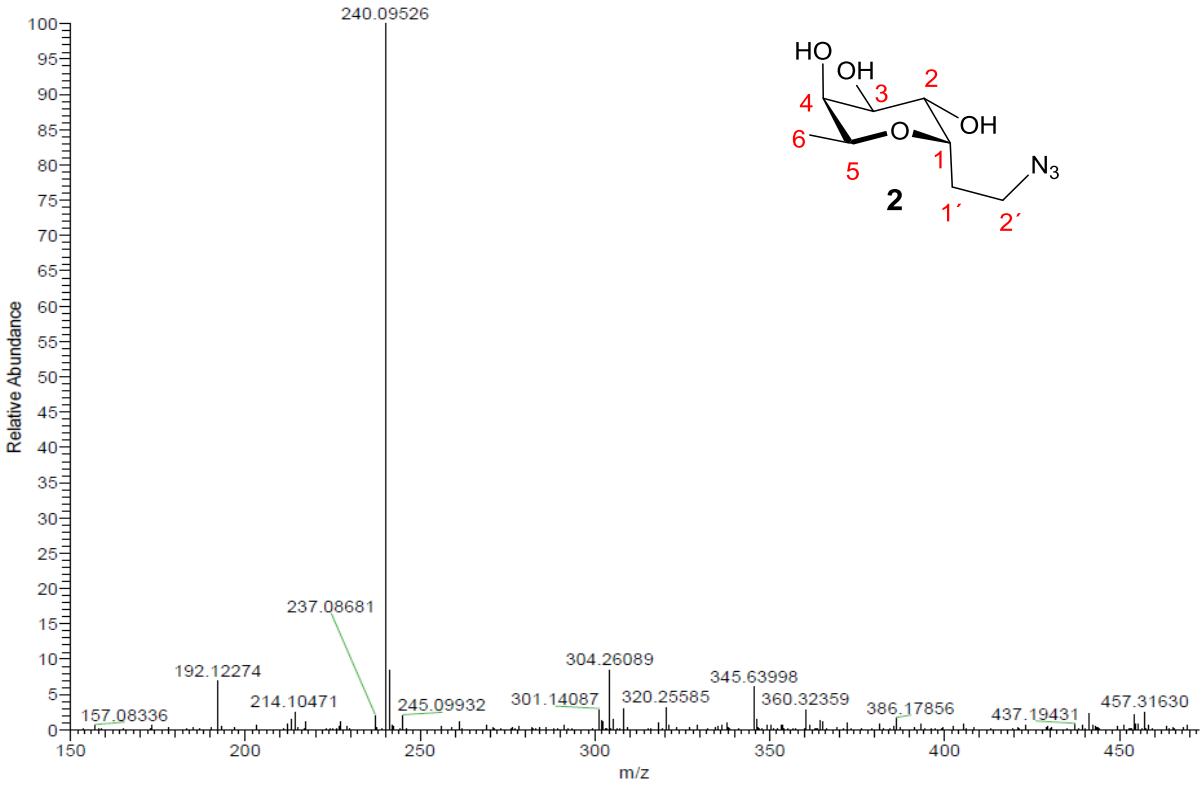
$C_{14}H_{21}N_3O_7$, MW 343; HRMS (ESI); ^{1}H NMR (600.1 MHz, $CDCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$)

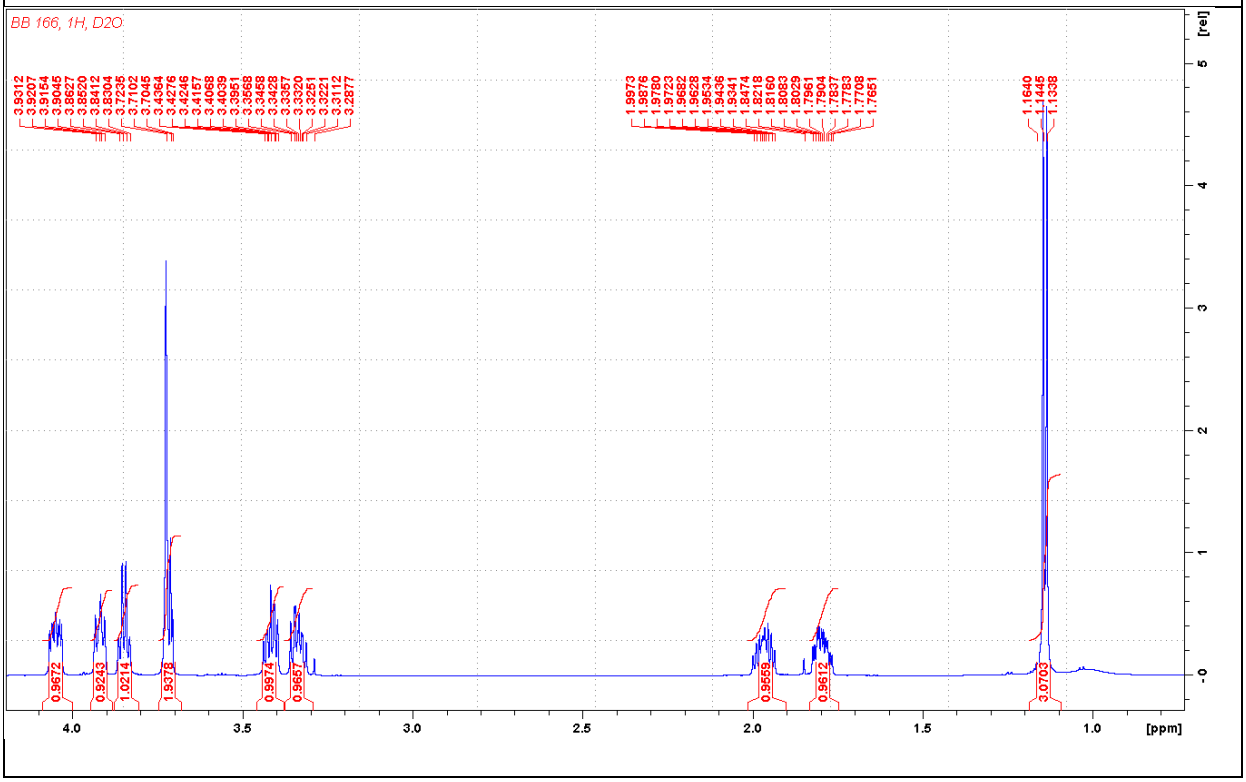


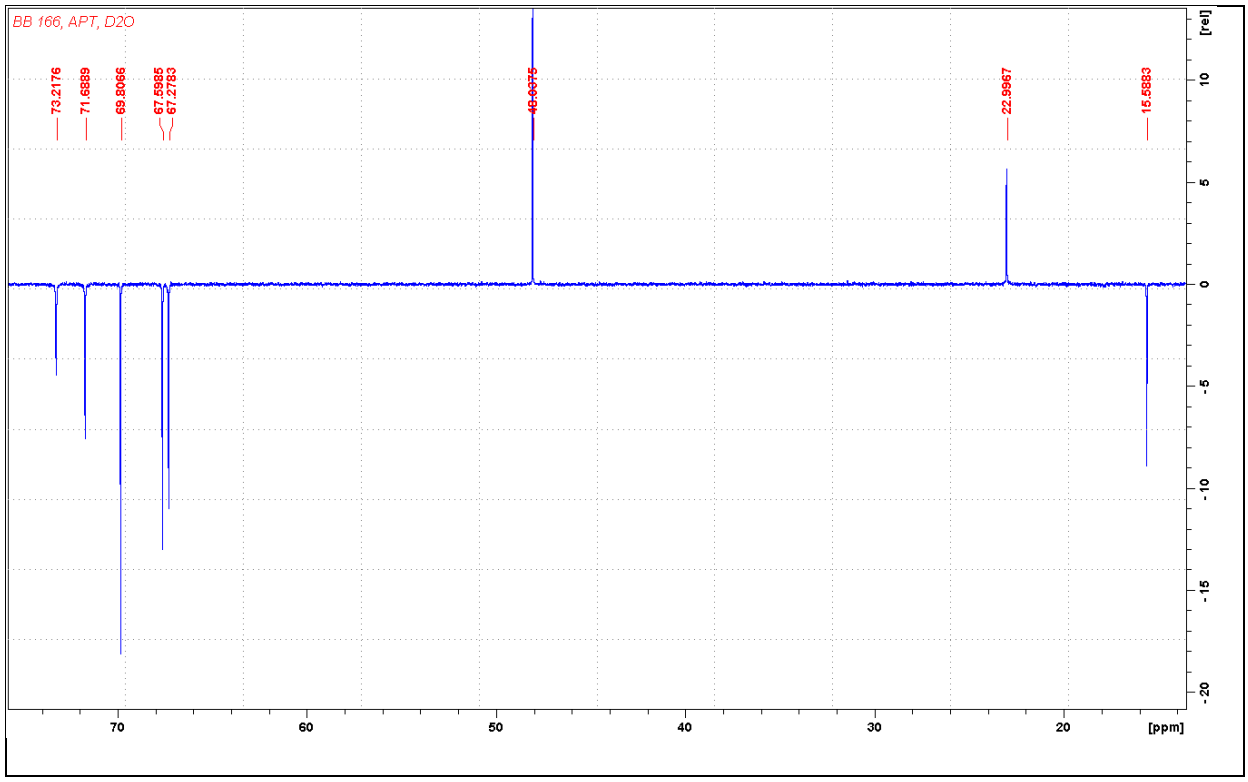

BB132, 1H, CDCl3
5.3309
5.3197
5.3115
5.3004
5.2782
5.2725
5.2679
5.1826
5.1760
5.1632
5.1566
4.3219
4.3153
4.3116
4.3044
4.2994
4.2924
4.2885
4.2818
3.9888
3.9794
3.9761
3.9666
3.9632
3.9538
3.9507
3.4587
3.4483
3.4442
3.4340
3.4237
3.4196
3.4107
3.3990
3.3953
3.3823
3.3746
3.3708
3.3576
2.1571
2.0749
2.0194
1.9973
1.9926
1.9864
1.9827
1.9755
1.9637
1.9626
1.7566
1.7445
1.7344
1.7285
1.7198
1.7136
1.7052
1.6996
1.6898
1.6834
1.1916
1.1787
2.0010
1.0255
0.9930
1.0088
2.0554
3.0189
3.1467
3.1665
0.7652
1.0094
3.0350
[ral]
[ppm]
BB132, APT, CDCl3
77.2552
76.9990
76.7436
70.2346
69.6387
68.4467
67.9471
66.1262
47.9671
25.1575
20.7537
20.6836
20.6381
15.7998
[ral]
[ppm]

2-(α-L-Fucopyranosyl)ethylazide (2)

$C_8H_{15}N_3O_4$, MW 217; HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

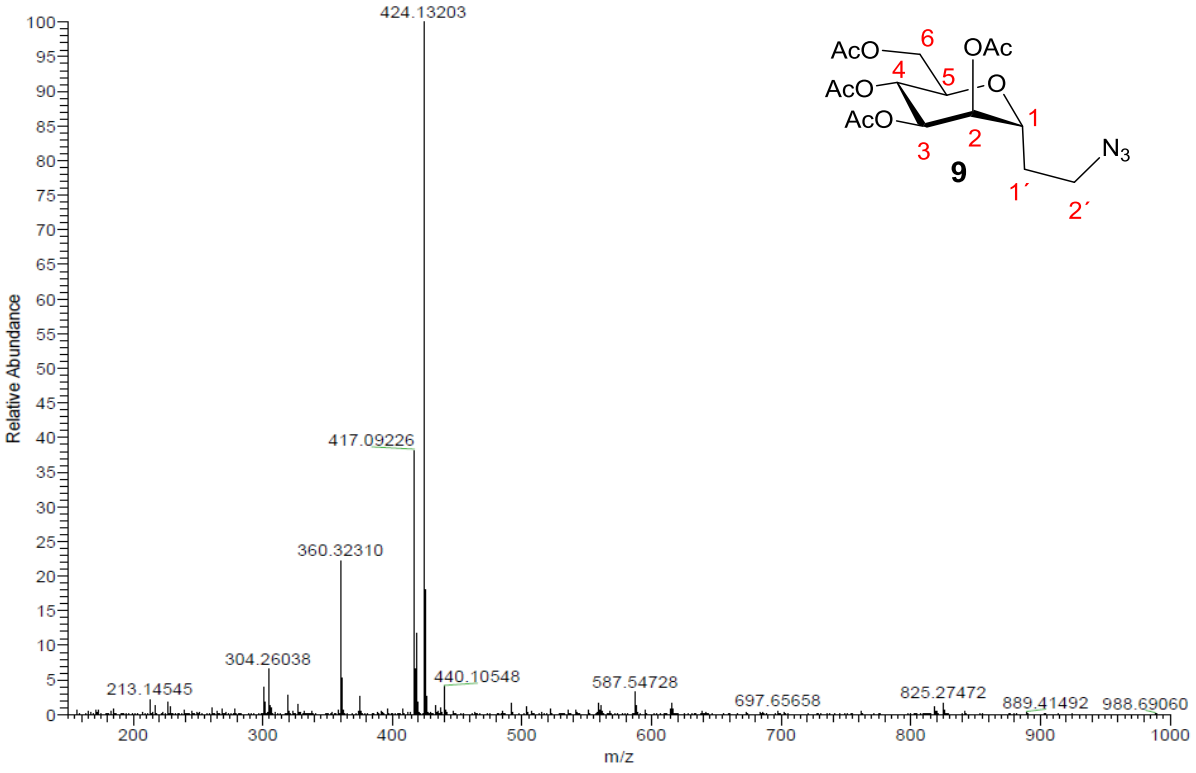


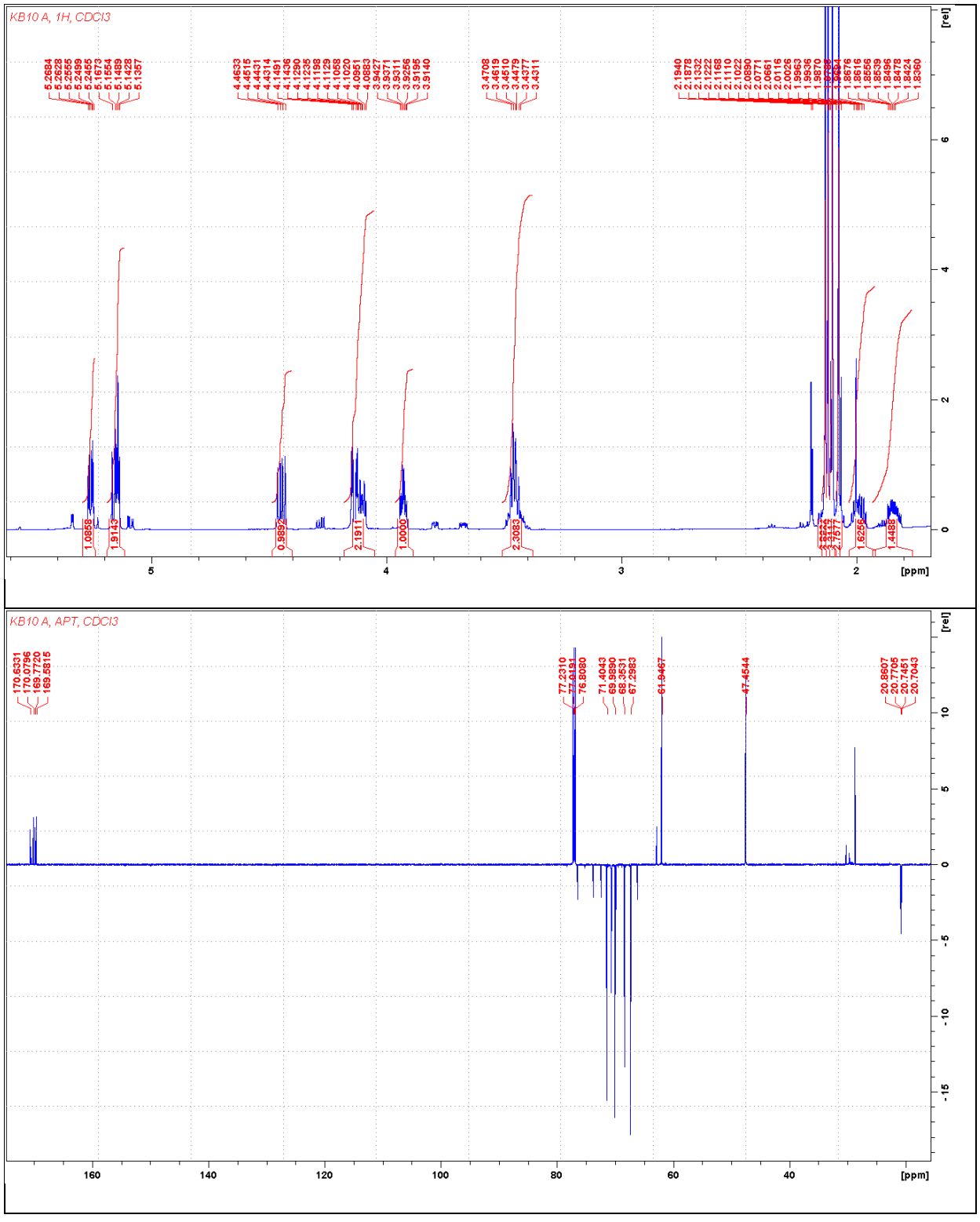




2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)ethylazide (9)

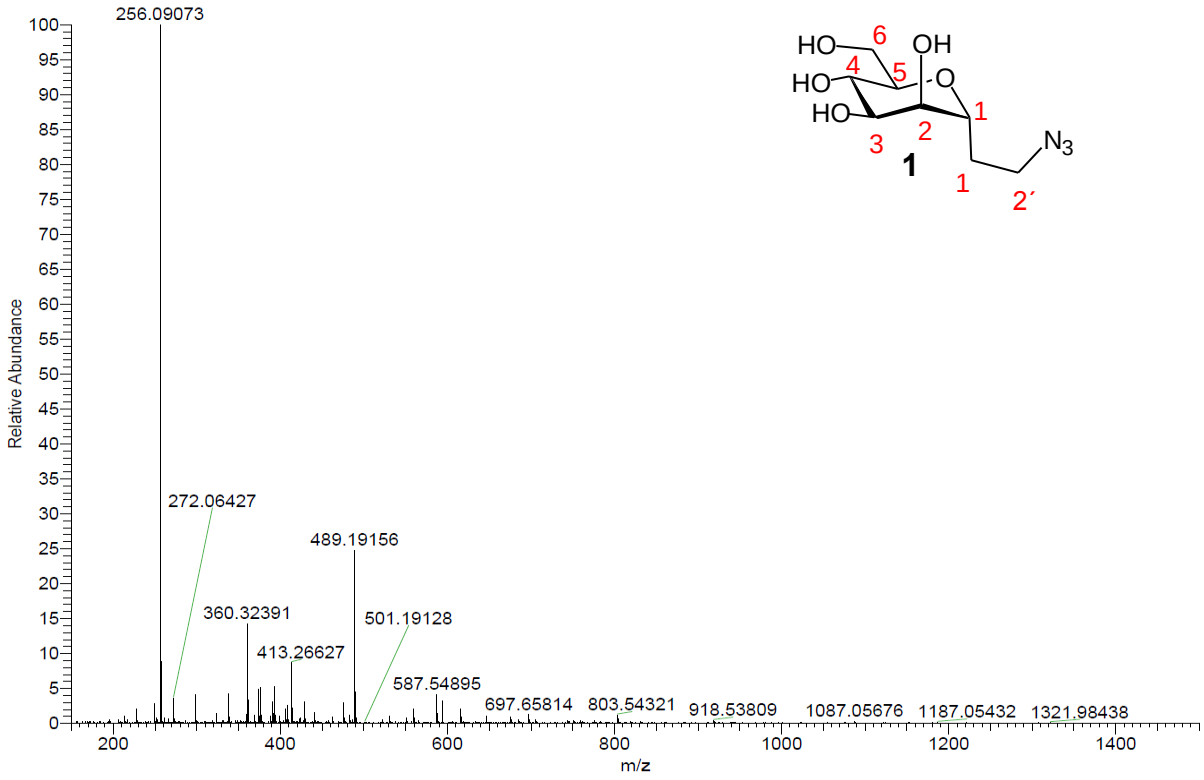
$C_{16}H_{23}O_9N_3$, MW 401; HRMS (ESI); ^{1}H NMR (600.1 MHz, $CDCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$)

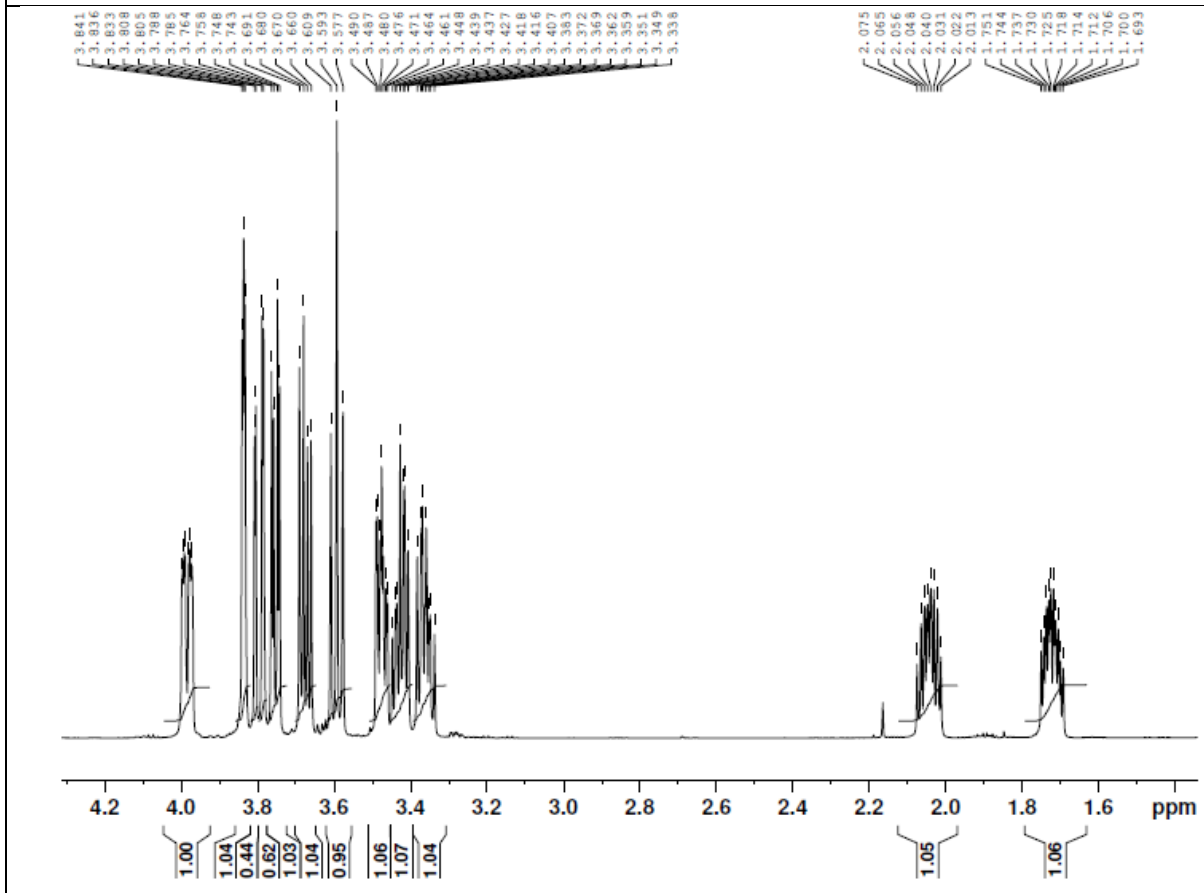


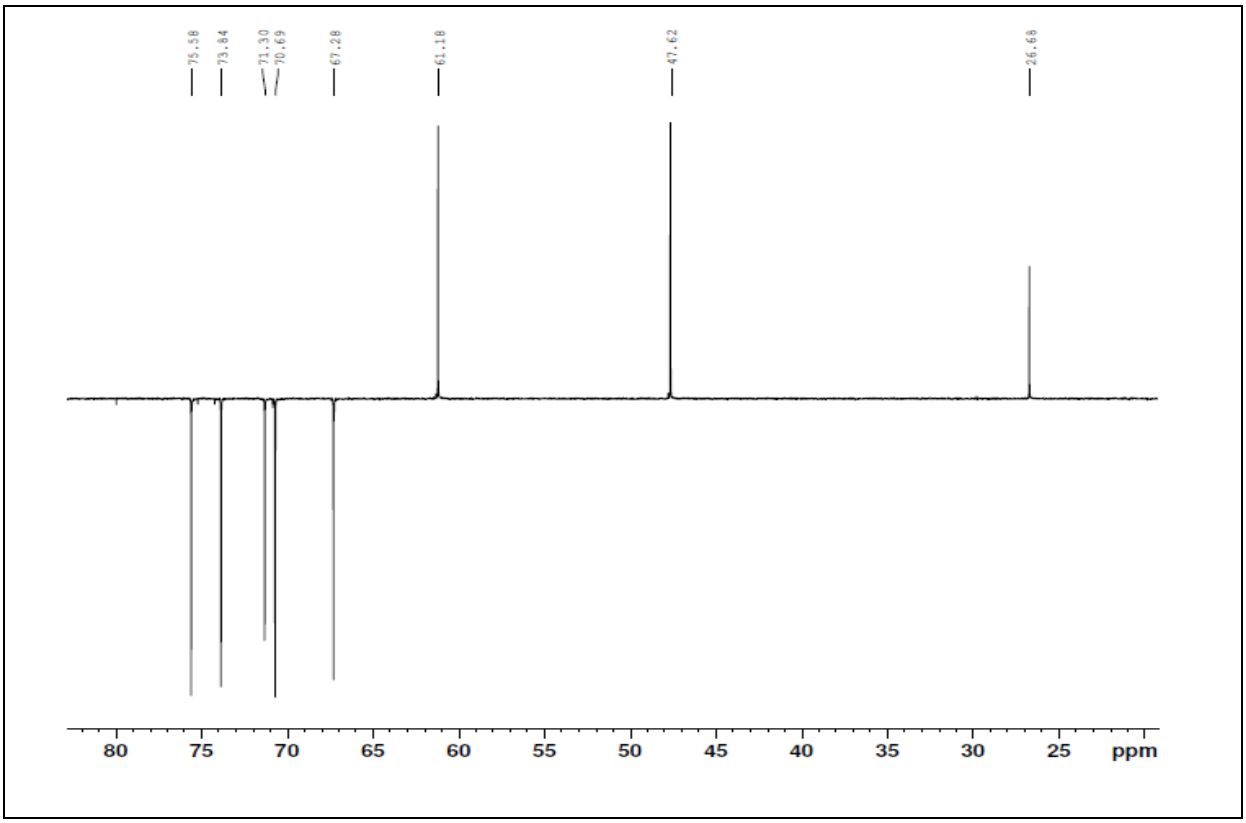

KB10 A, 1H, CDCl3
5.2684
5.2628
5.2555
5.2499
5.2455
5.1673
5.1554
5.1489
5.1428
5.1357
4.4633
4.4515
4.4431
4.4314
4.1491
4.1436
4.1290
4.1235
4.1198
4.1123
4.1058
4.1020
4.0961
4.0883
3.9427
3.9371
3.9311
3.9256
3.9195
3.9140
3.4708
3.4619
3.4510
3.4479
3.4377
3.4311
2.1940
2.1878
2.1332
2.1222
2.1168
2.1110
2.1022
2.0890
2.0771
2.0671
2.0116
2.0026
1.9963
1.9936
1.9870
1.8676
1.8616
1.8556
1.8496
1.8478
1.8424
1.8360
1.0858
1.9143
0.9892
2.1911
1.0000
2.3083
1.6256
1.4488
[rel]
[ppm]
KB10 A, APT, CDCl3
170.6331
170.0796
169.7720
169.5815
77.2310
77.0191
76.8080
71.4043
69.9890
68.3531
67.2983
61.9467
47.4544
20.8607
20.7705
20.7451
20.7043
[rel]
[ppm]

2-(α-D-Mannopyranosyl)ethylazide (1)

$C_8H_{15}N_3O_5$, MW 233; HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

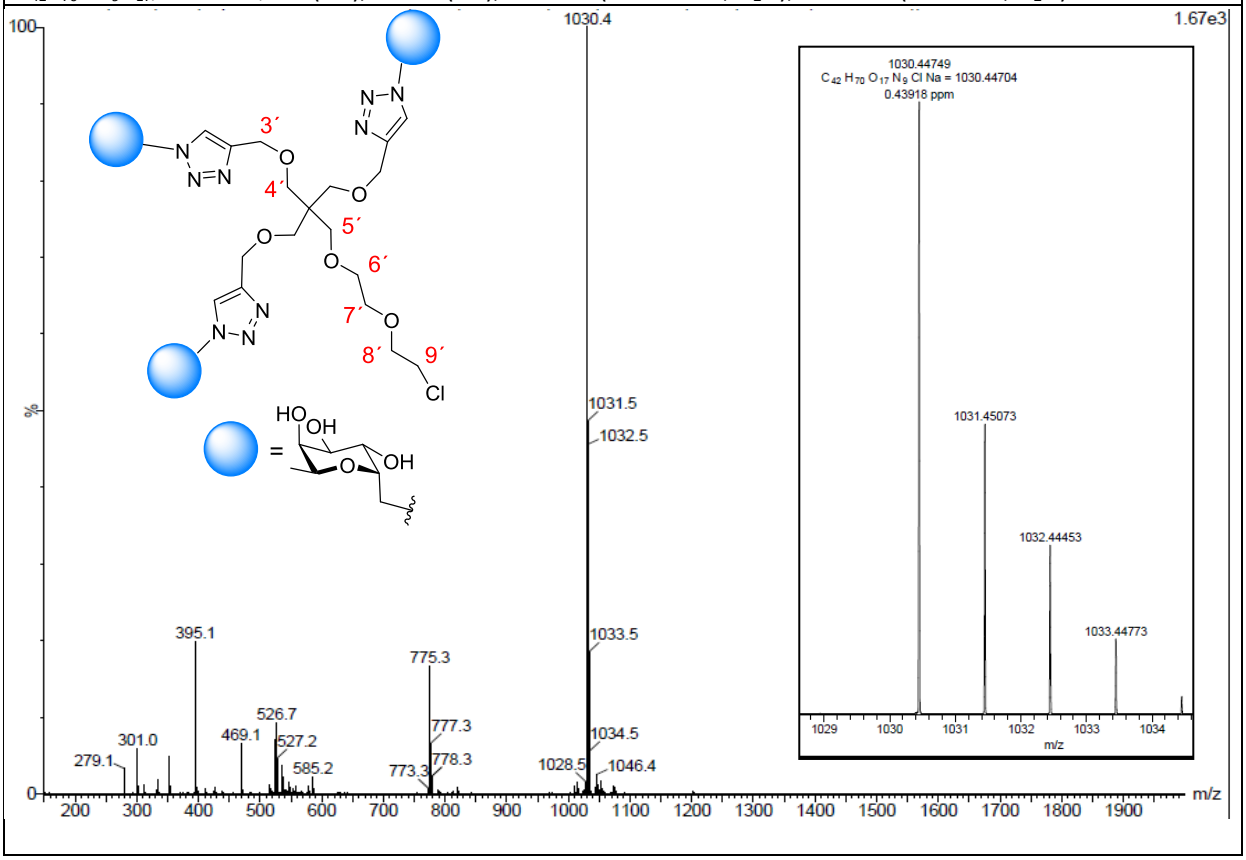


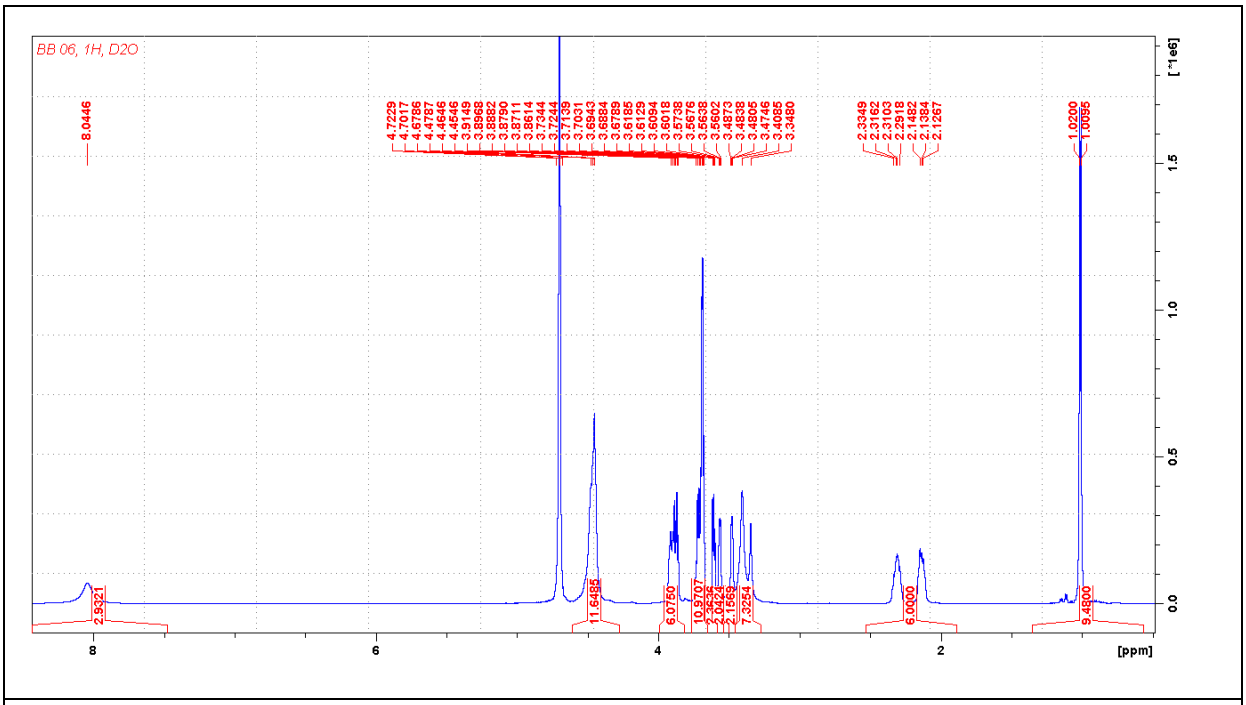


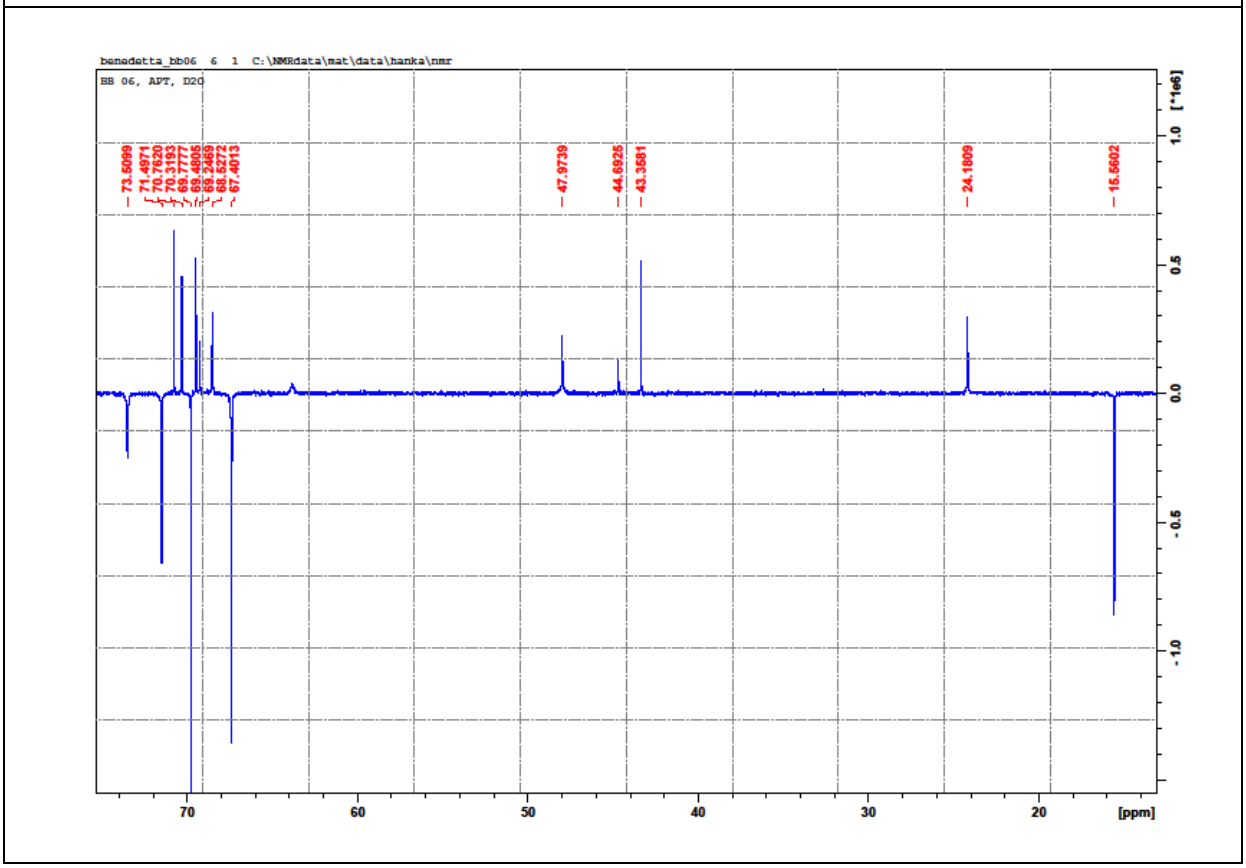


Dendron F-C-Cl

$C_{42}H_{70}ClN_9O_{17}$, MW 1007; MS (ESI); HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

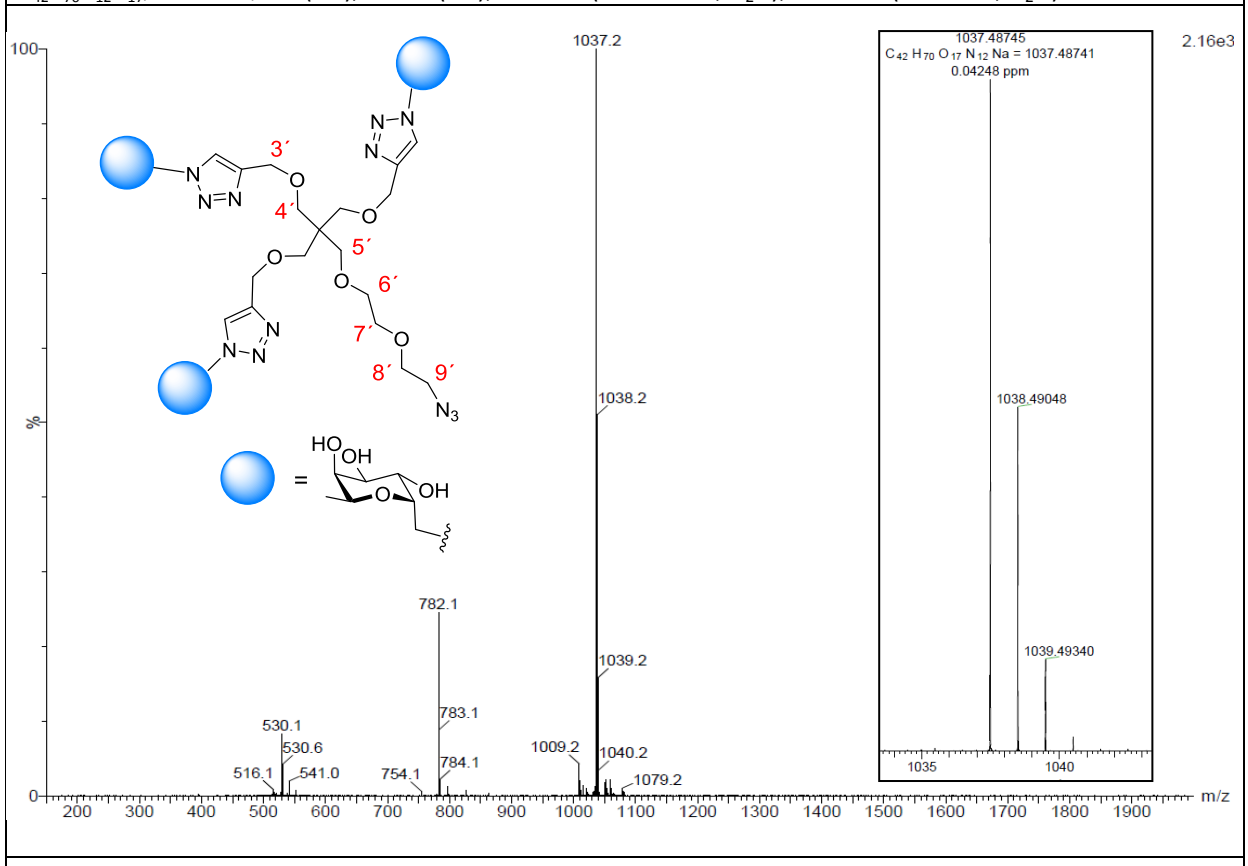


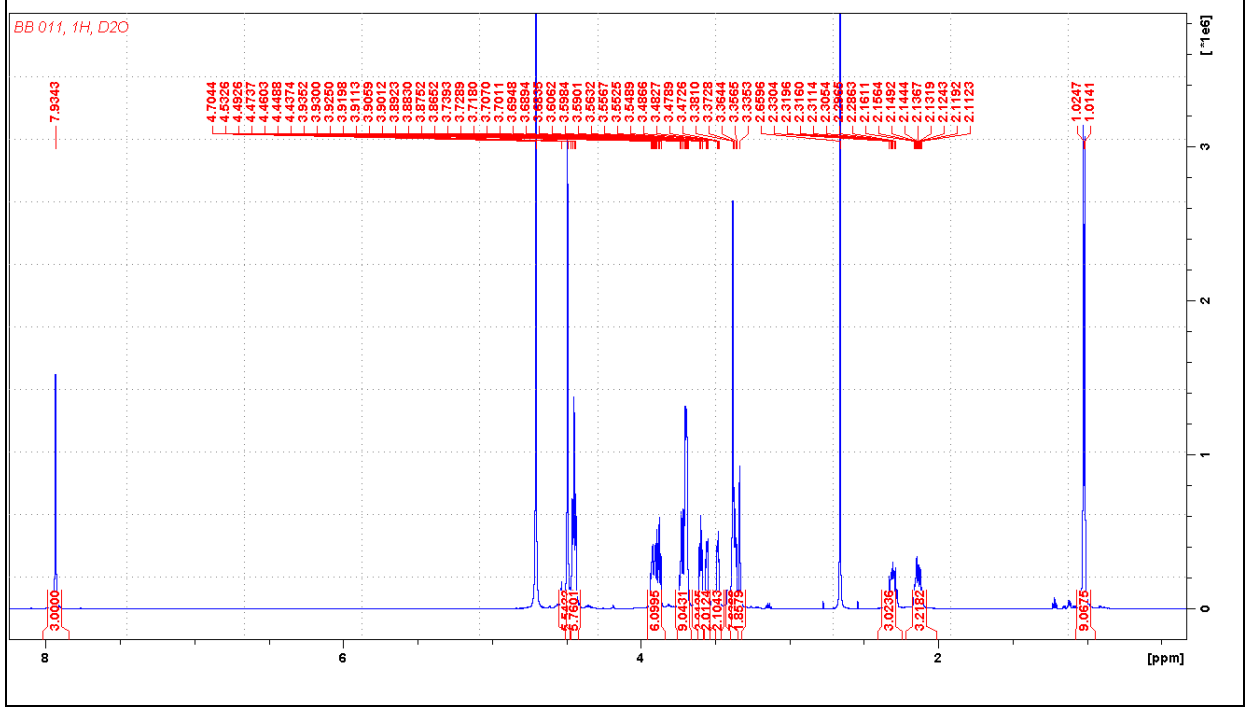

BB 06, 1H, D2O
8.0446
4.7229
4.7017
4.6795
4.4787
4.4646
4.4546
3.9149
3.8968
3.8882
3.8790
3.8711
3.8614
3.7344
3.7244
3.7139
3.7031
3.6943
3.6884
3.6789
3.6185
3.6123
3.6054
3.6018
3.5738
3.5676
3.5638
3.5602
3.4873
3.4838
3.4095
3.4746
3.4085
3.3480
2.3349
2.3162
2.3103
2.2918
2.1482
2.1364
2.1267
1.0200
1.0095
2.9321
11.6485
6.0750
7
10.3707
2.0424
2.1563
7.3254
6.0000
9.4800
[*1e6]
[ppm]

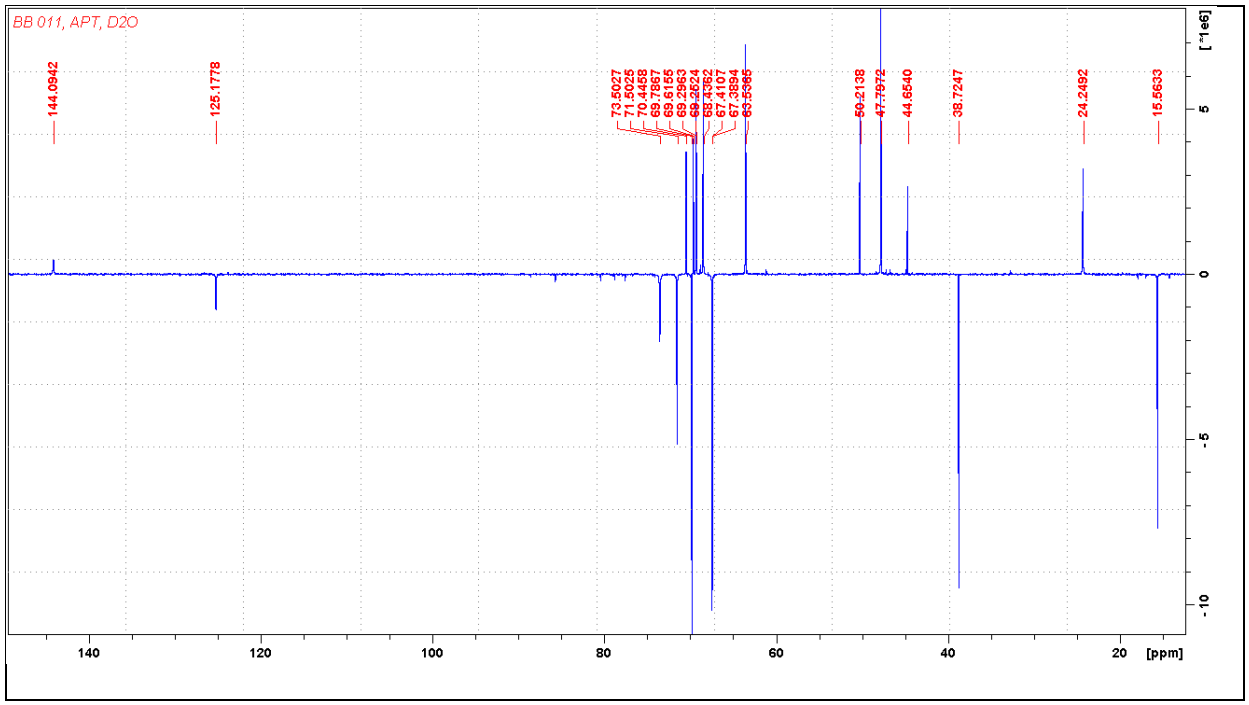

benedetta_bb06 6 1 C:\NMRdata\mat\data\hanka\nmr
BB 06, APT, D2O
73.5099
71.4971
70.7620
70.3193
69.7777
69.4806
69.2469
68.8272
67.4013
47.9739
44.6926
43.3581
24.1809
15.5602
[*1e6]
[ppm]

Dendron F-C-N

$C_{42}H_{70}N_{12}O_{17}$, MW 1015; MS (ESI); HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

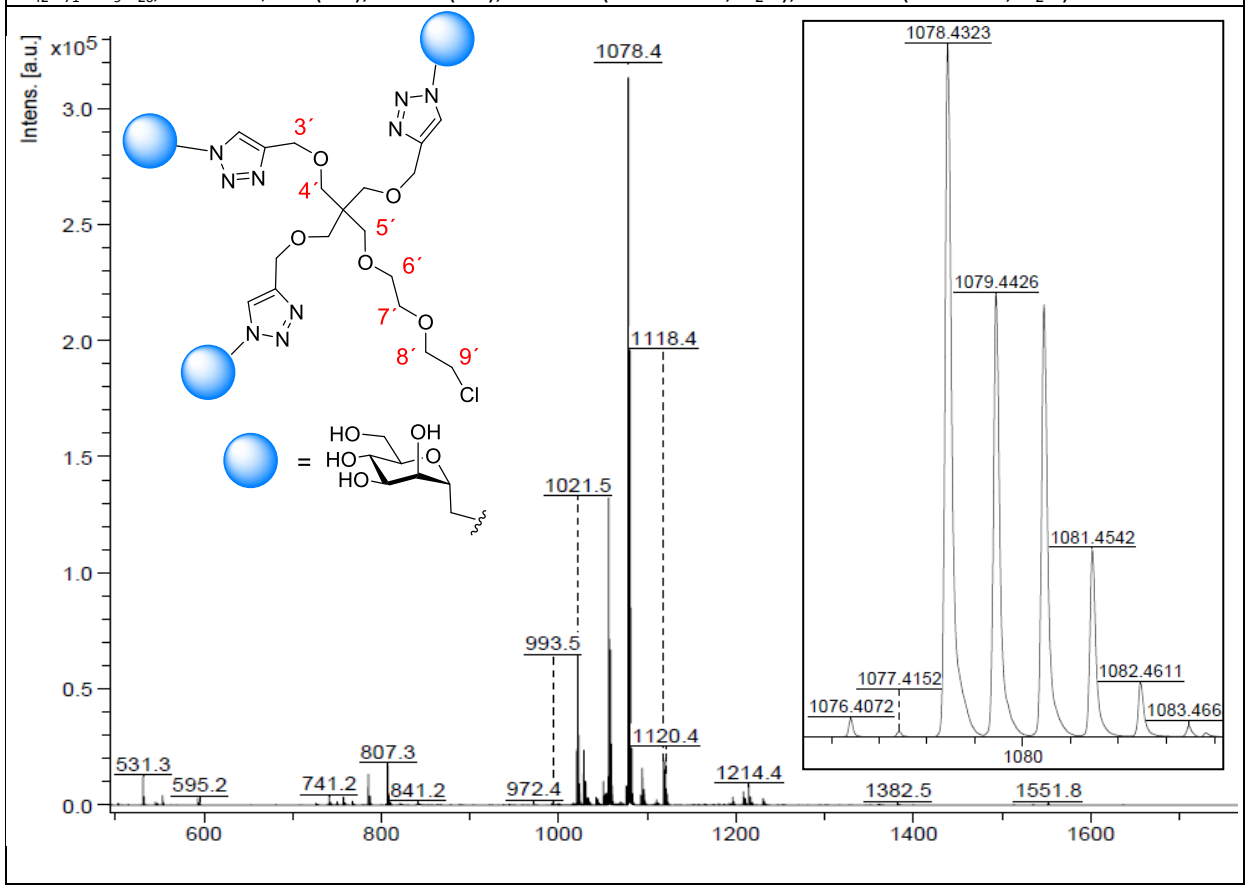


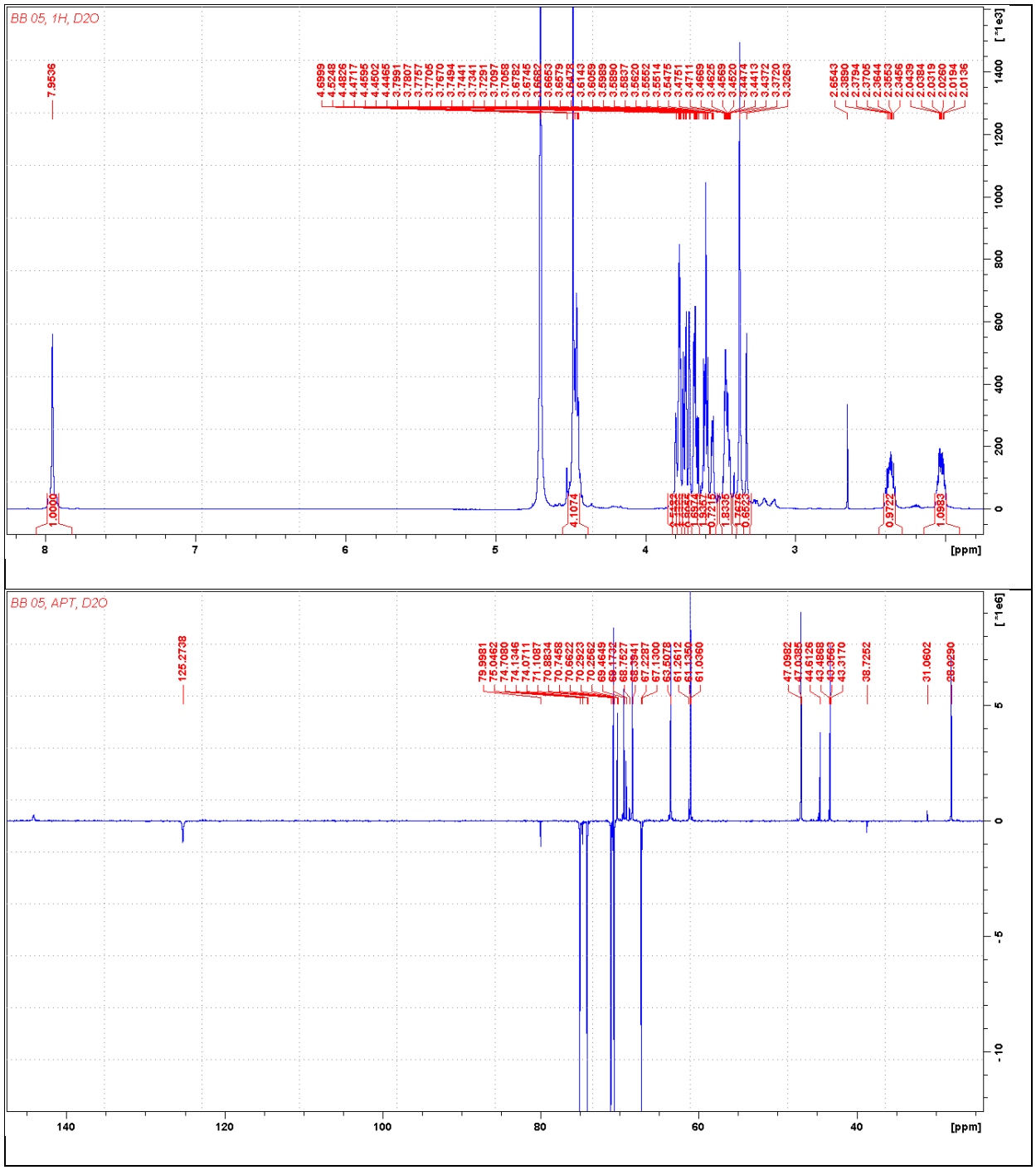




Dendron M-C-Cl

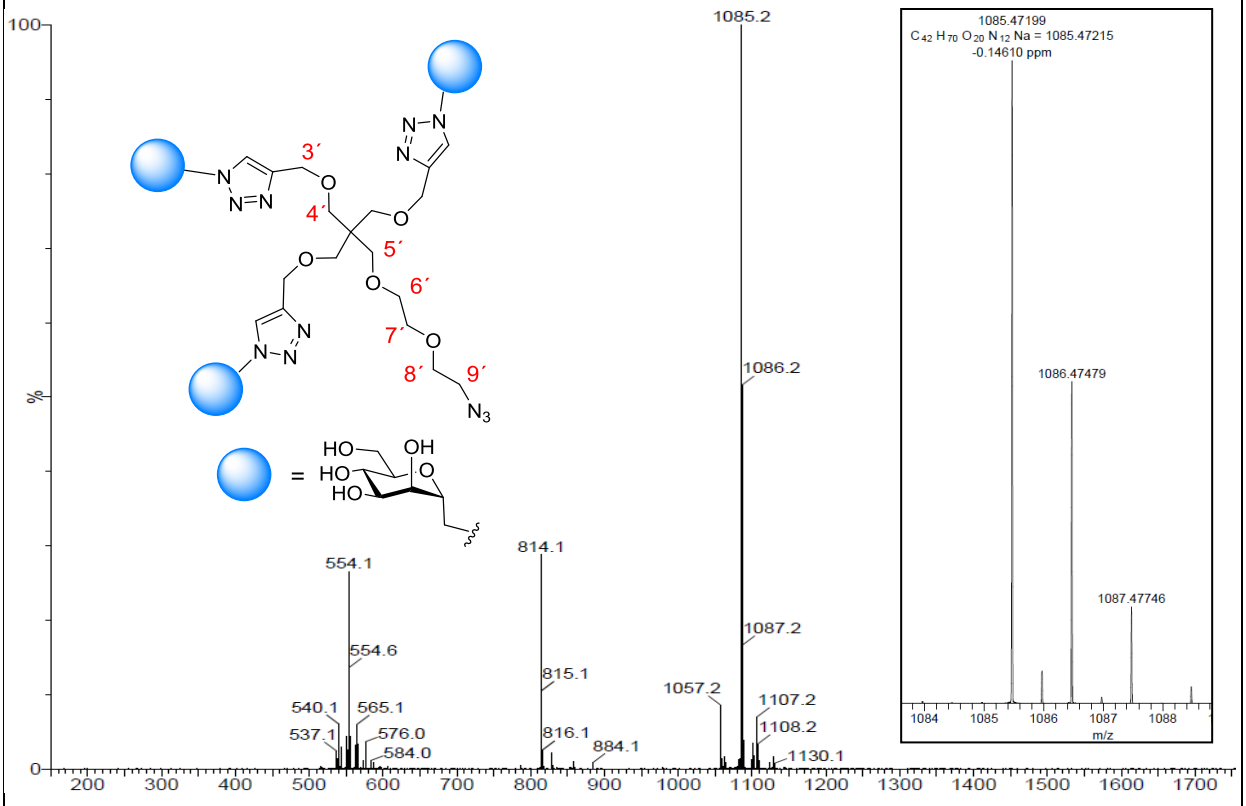
$C_{42}H_{71}ClN_9O_{20}$, MW 1056; MS (ESI); HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

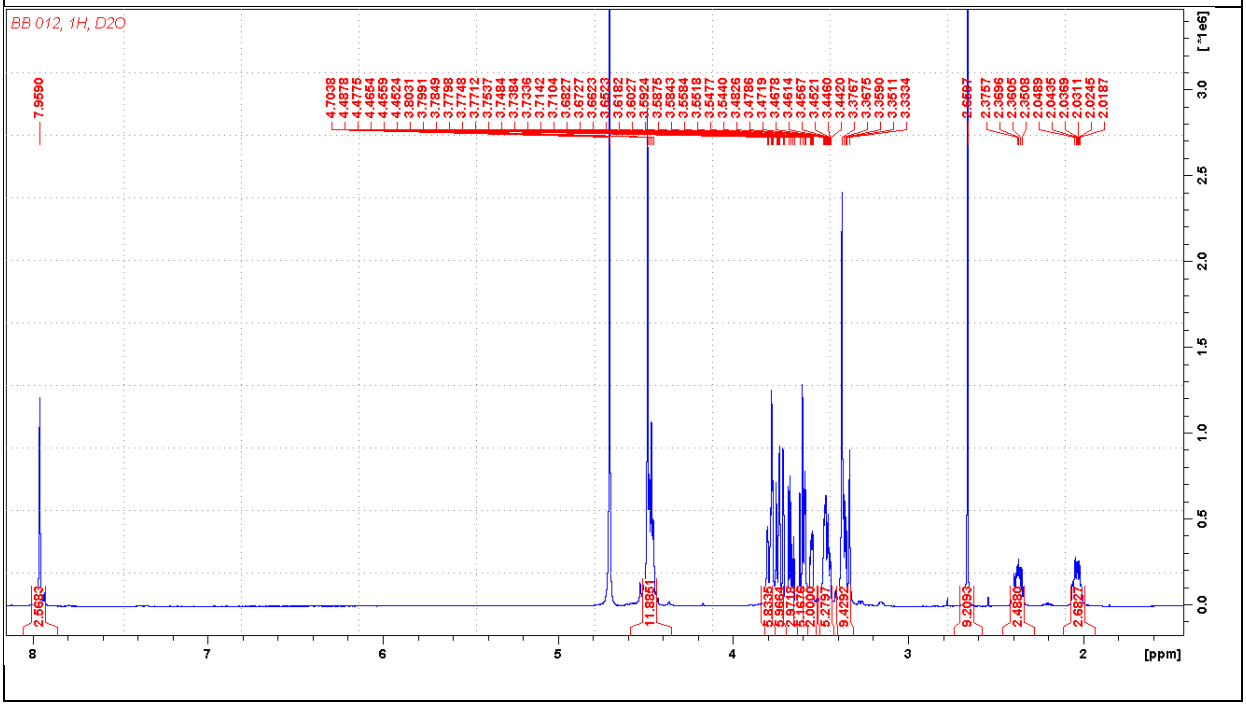


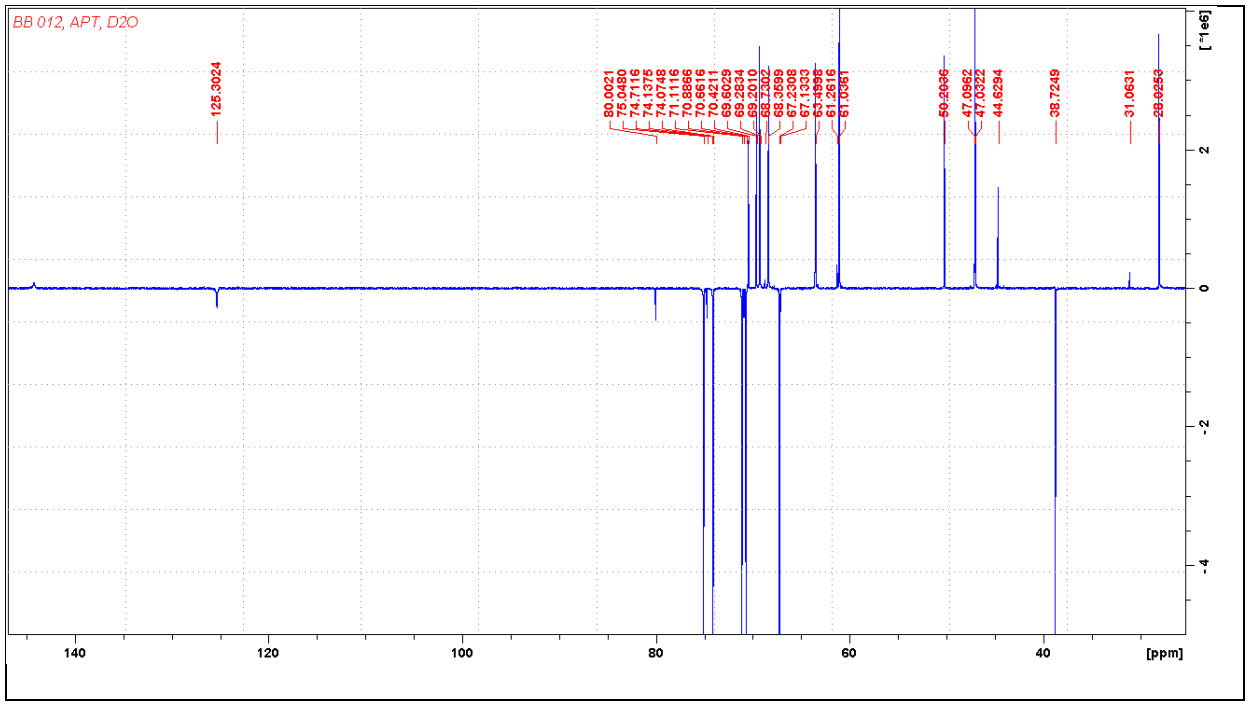

BB 05, 1H, D2O
BB 05, APT, D2O
[ppm]

Dendron M-C-N

$C_{42}H_{70}N_{12}O_{20}$, MW 1063; MS (ESI); HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)





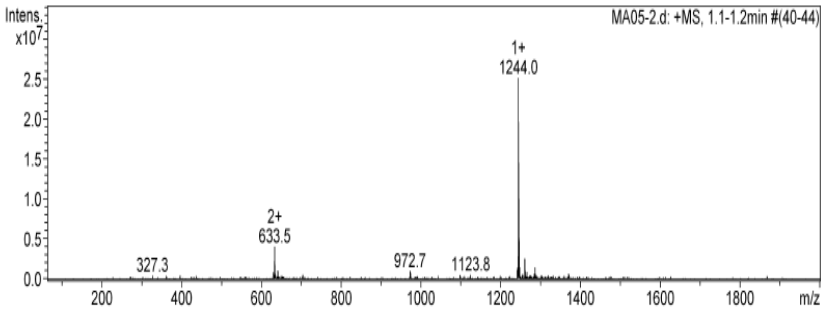


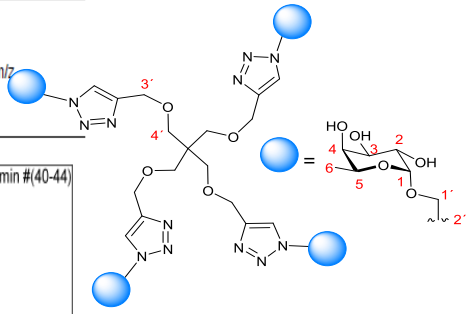
Dendrimer F-O-4

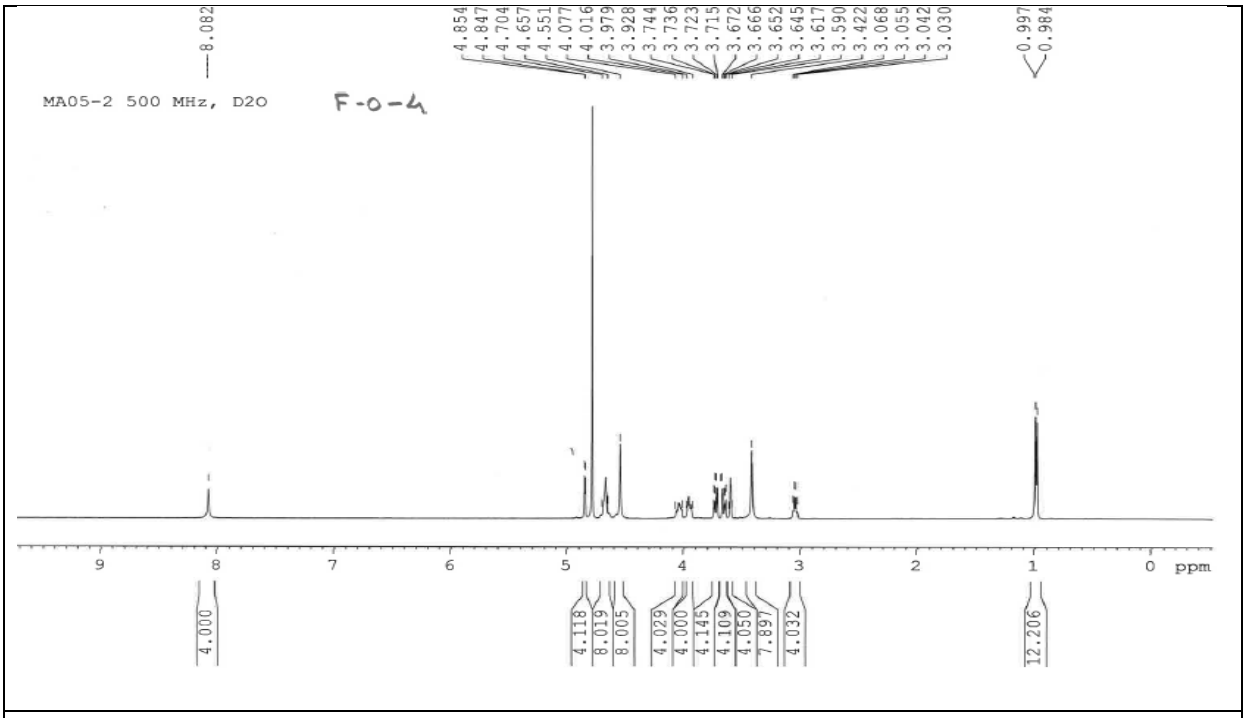
$C_{49}H_{80}N_{12}O_{24}$, MW 1220; LRMS (ESI); ^{1}H NMR (500 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

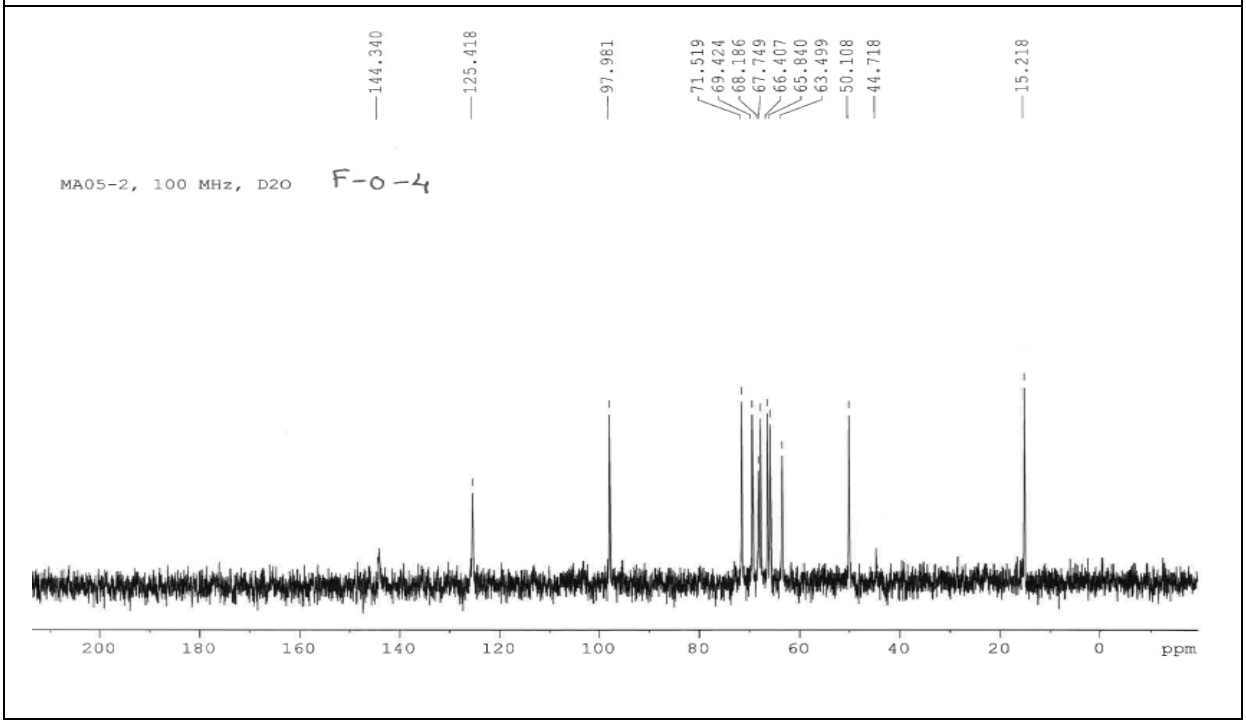
Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	65 m/z	Scan End	2000 m/z
Capillary Exit	175.1 Volt	Skim 1	40.0 Volt	Trap Drive	114.2
Accumulation Time	200 µs	Averages	8 Spectra	Auto MS/MS	off






8.082
4.854
4.847
4.704
4.657
4.551
4.077
4.016
3.979
3.928
3.744
3.736
3.723
3.715
3.672
3.666
3.652
3.645
3.617
3.590
3.422
3.068
3.055
3.042
3.030
0.997
0.984
MA05-2 500 MHz, D2O
F-O-4
9
8
7
6
5
4
3
2
1
0
ppm
4.000
4.118
8.019
8.005
4.029
4.000
4.145
4.109
4.050
7.897
4.032
12.206

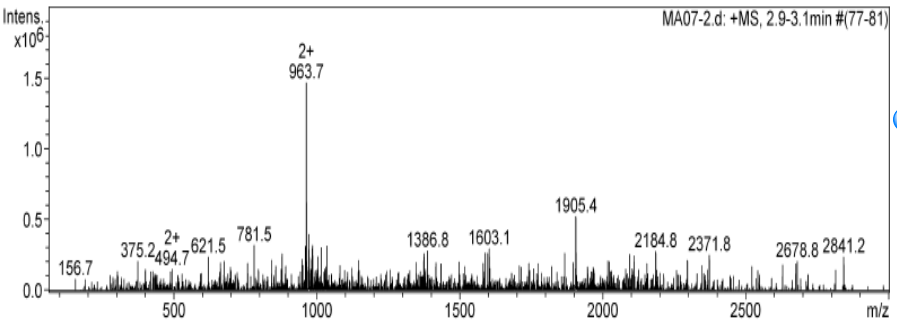

144.340
125.418
97.981
71.519
69.424
68.186
67.749
66.407
65.840
63.499
50.108
44.718
15.218
MA05-2, 100 MHz, D2O
F-O-4
200
180
160
140
120
100
80
60
40
20
0
ppm

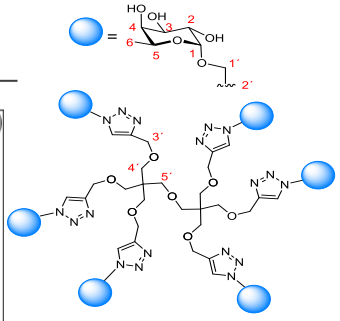
Dendrimer F-O-6

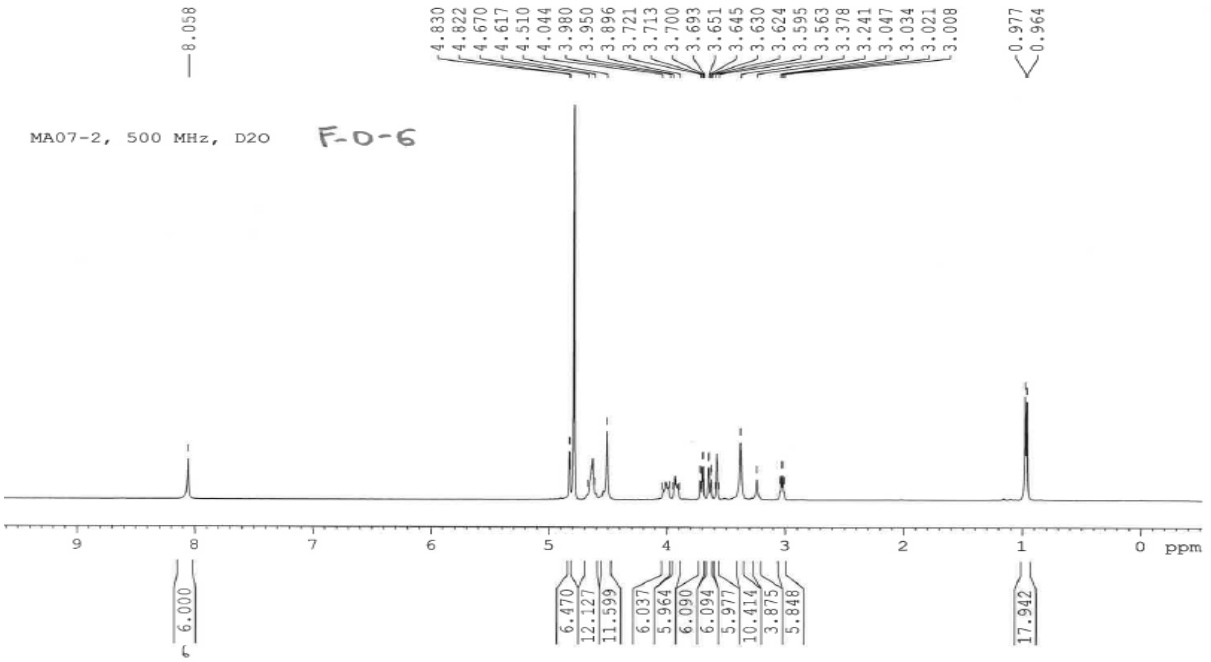
$C_{76}H_{122}N_{18}O_{37}$, MW 1878; LRMS (ESI); ^{1}H NMR (500 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

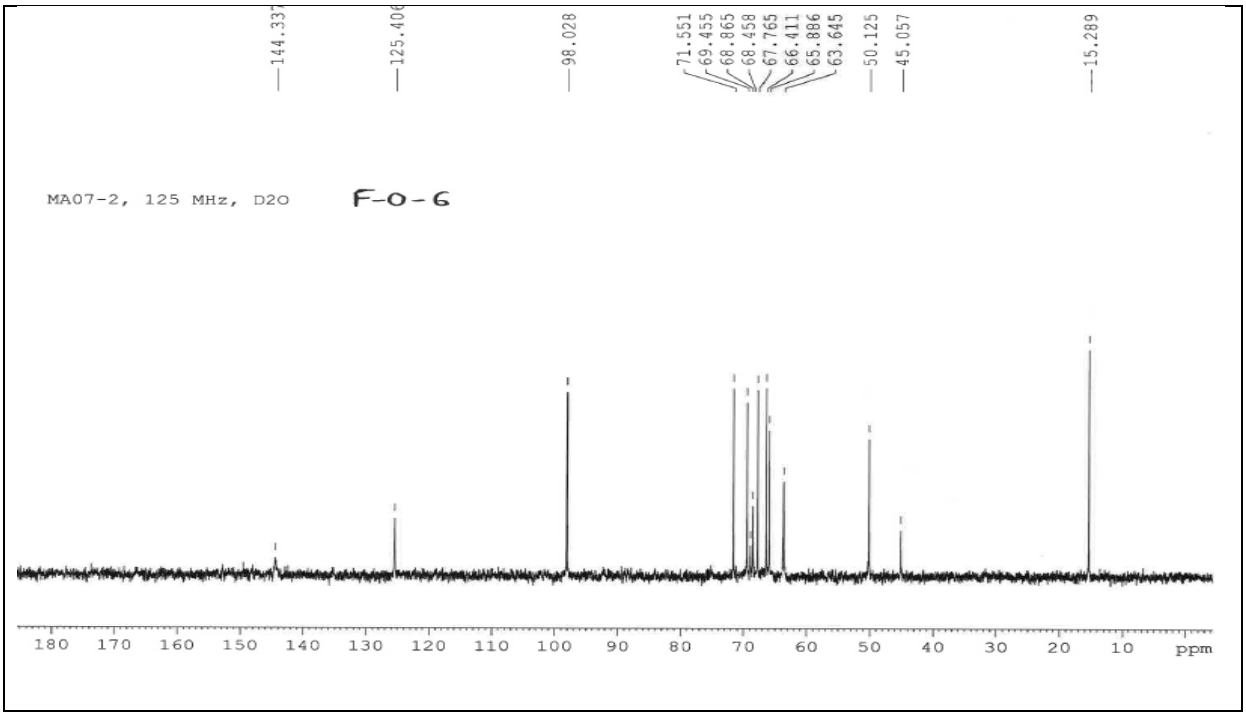
Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	65 m/z	Scan End	3000 m/z
Capillary Exit	232.1 Volt	Skim 1	40.0 Volt	Trap Drive	167.8
Accumulation Time	275 µs	Averages	8 Spectra	Auto MS/MS	off



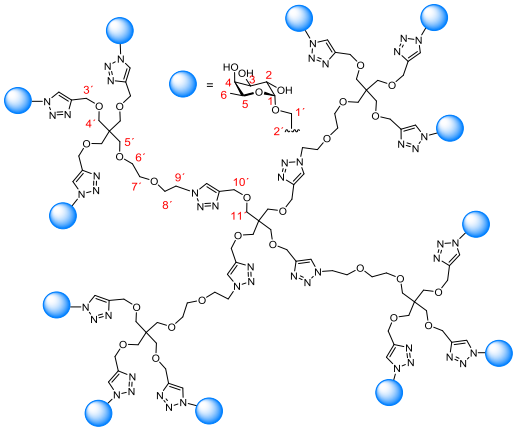




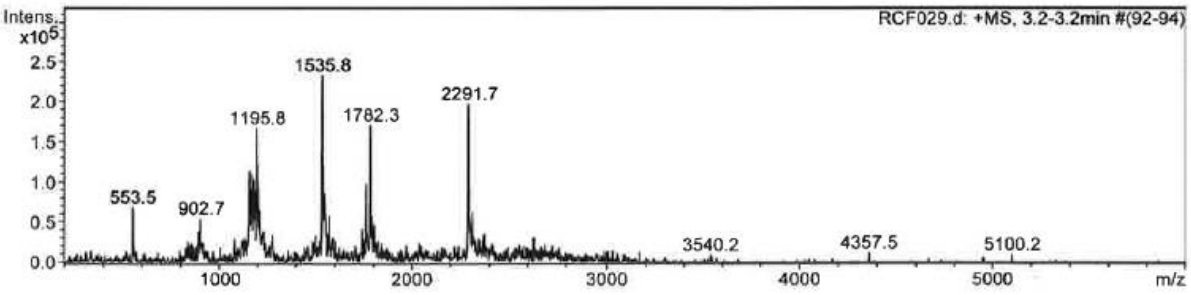


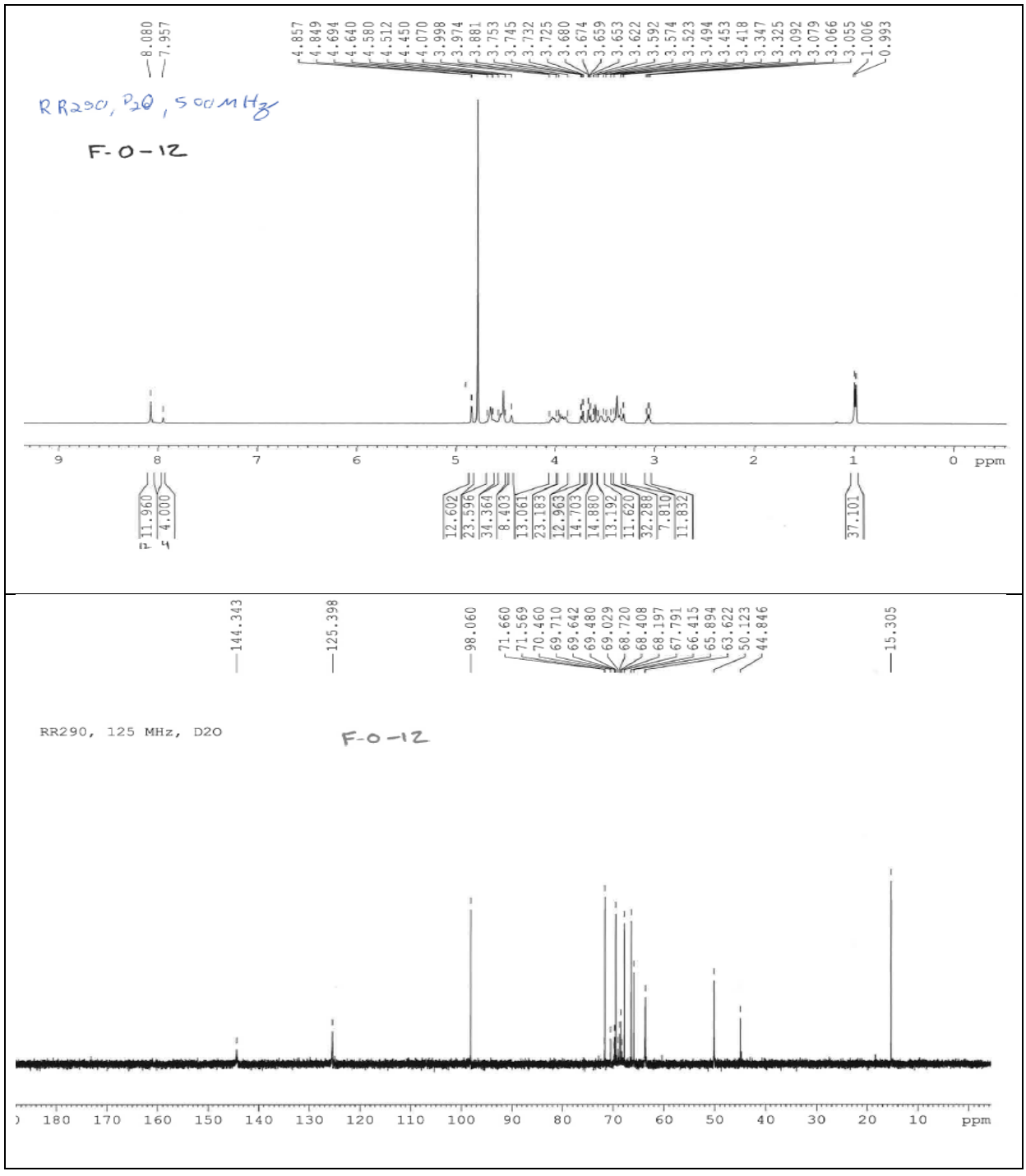
Dendrimer F-O-12

$C_{185}H_{300}N_{48}O_{84}$, MW 4536; LRMS (ESI); ^{1}H NMR (500 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)



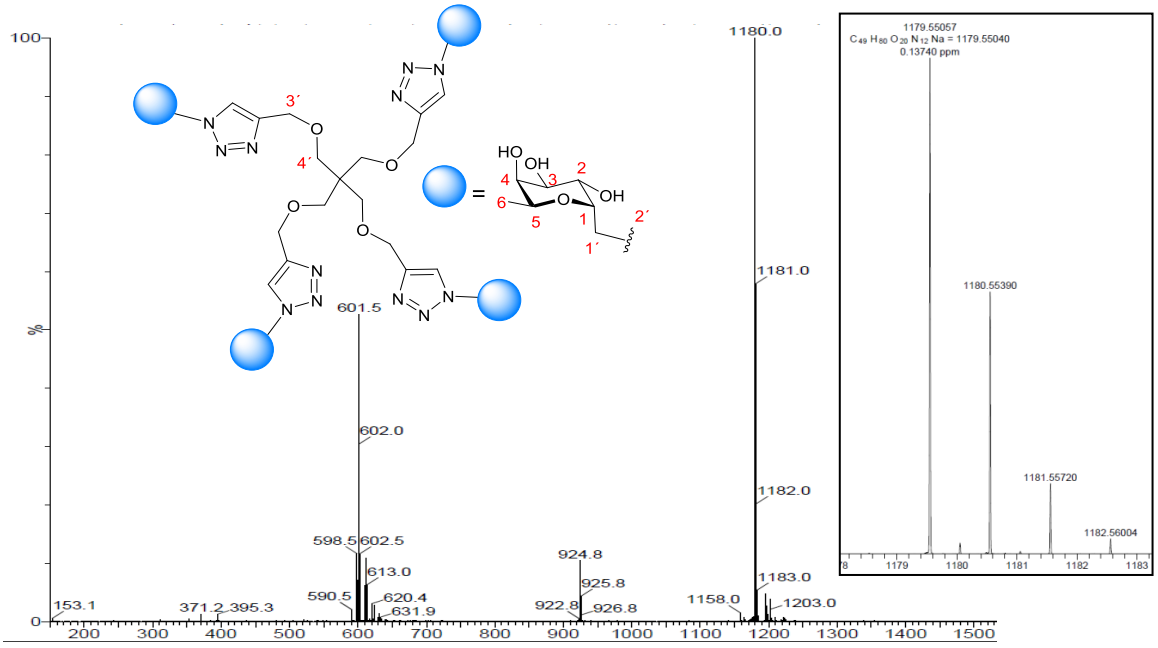
2291.7 $[M+2Na]^{2+}$, 1540.7 $[M+3Na]^{3+}$, 1157.4 $[M+4Na]^{4+}$

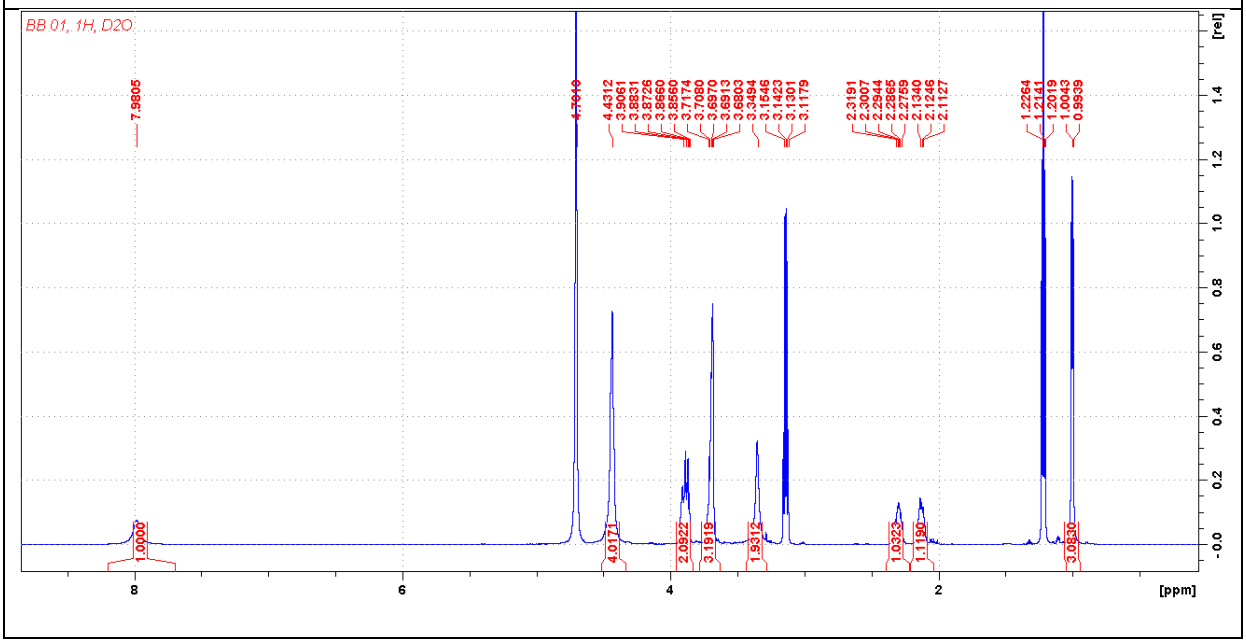


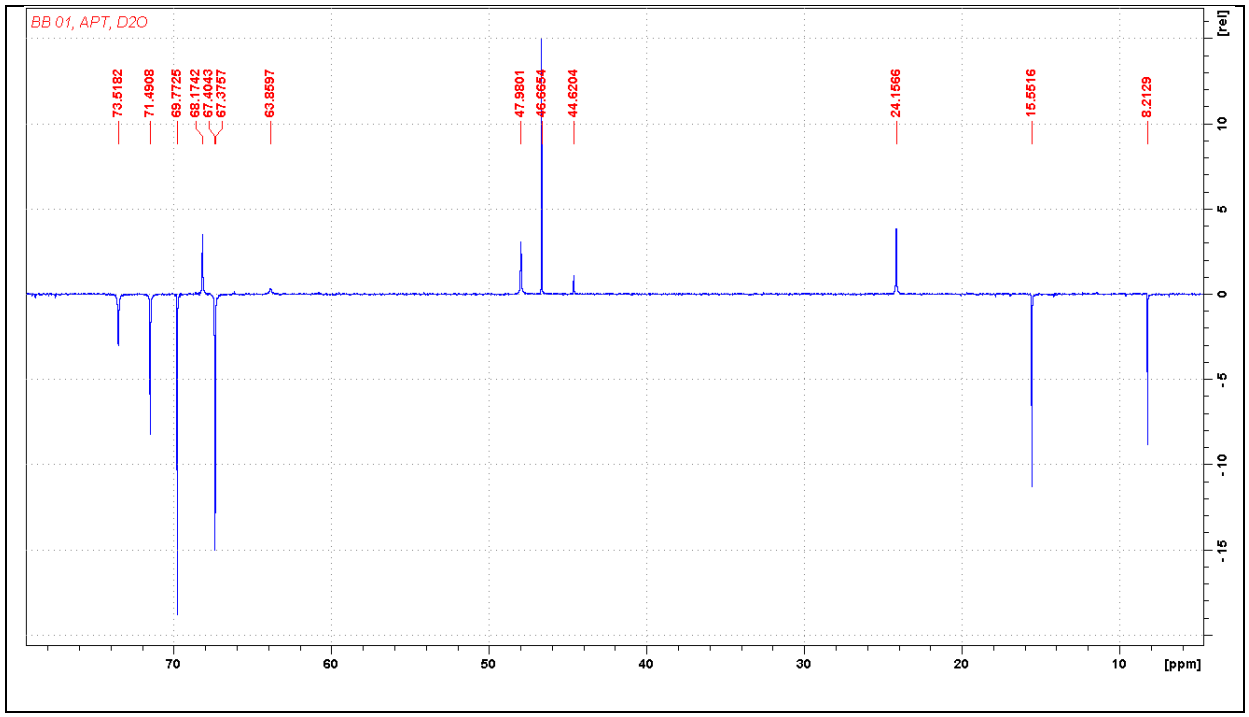

8.080
7.957
4.857
4.849
4.694
4.640
4.580
4.512
4.450
4.070
3.998
3.974
3.881
3.753
3.745
3.732
3.725
3.680
3.674
3.659
3.653
3.622
3.592
3.574
3.523
3.494
3.453
3.418
3.347
3.325
3.092
3.079
3.066
3.055
1.006
0.993
RR290, D2O, 500 MHz
F-O-12
9
8
7
6
5
4
3
2
1
0
ppm
11.960
4.000
12.602
23.596
34.364
8.403
13.061
23.183
12.963
14.703
14.880
13.192
11.620
32.288
7.810
11.832
37.101
144.343
125.398
98.060
71.660
71.569
70.460
69.710
69.642
69.480
69.029
68.720
68.408
68.197
67.791
66.415
65.894
63.622
50.123
44.846
15.305
RR290, 125 MHz, D2O
F-O-12
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Dendrimer F-C-4

$C_{49}H_{80}N_{12}O_{20}$, MW 1157; MS (ESI); HRMS (ESI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

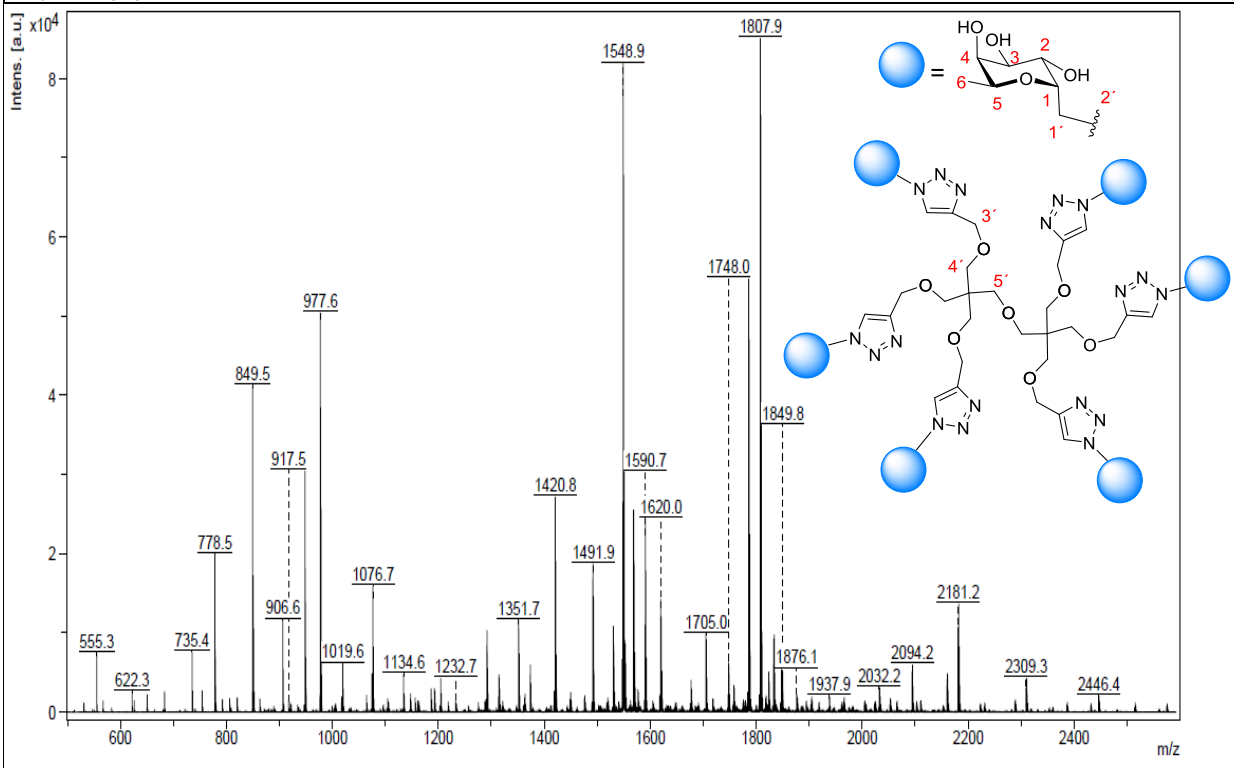


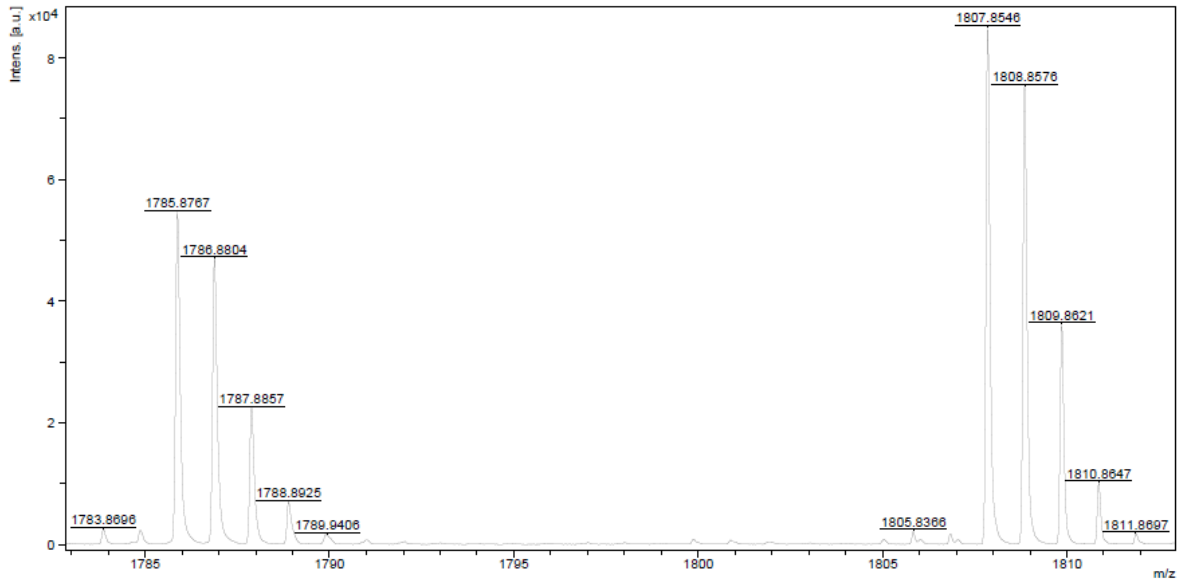


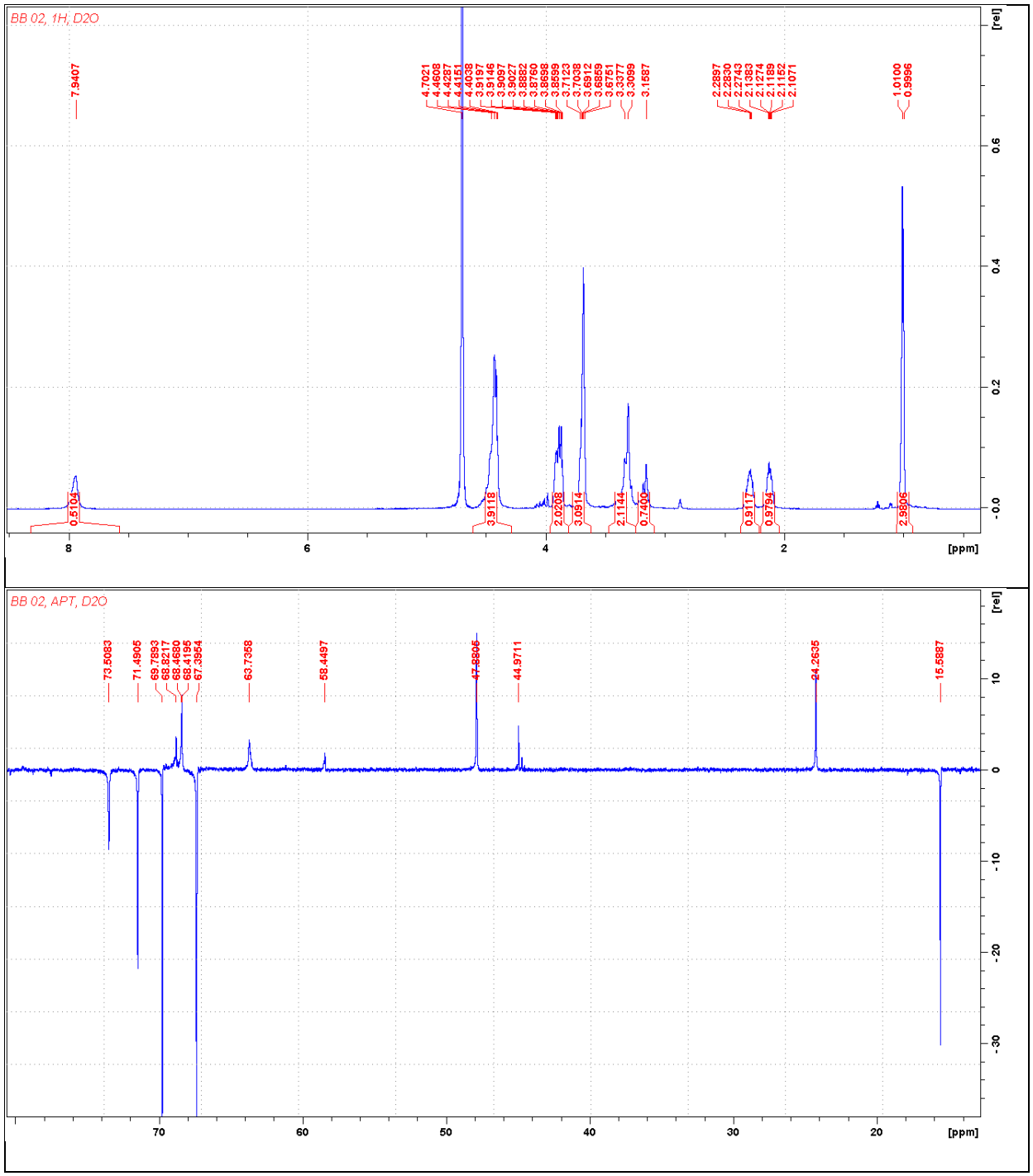

BB 01, APT, D2O
73.5182
71.4908
69.7725
68.1742
67.4043
67.3757
63.8597
47.9801
46.6654
44.6204
24.1566
15.5516
8.2129
[rel]
10
5
0
-5
-10
-15
70
60
50
40
30
20
10
[ppm]

Dendrimer F-C-6

$C_{76}H_{124}N_{18}O_{31}$, MW 1785; MS (MALDI); HRMS (MALDI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

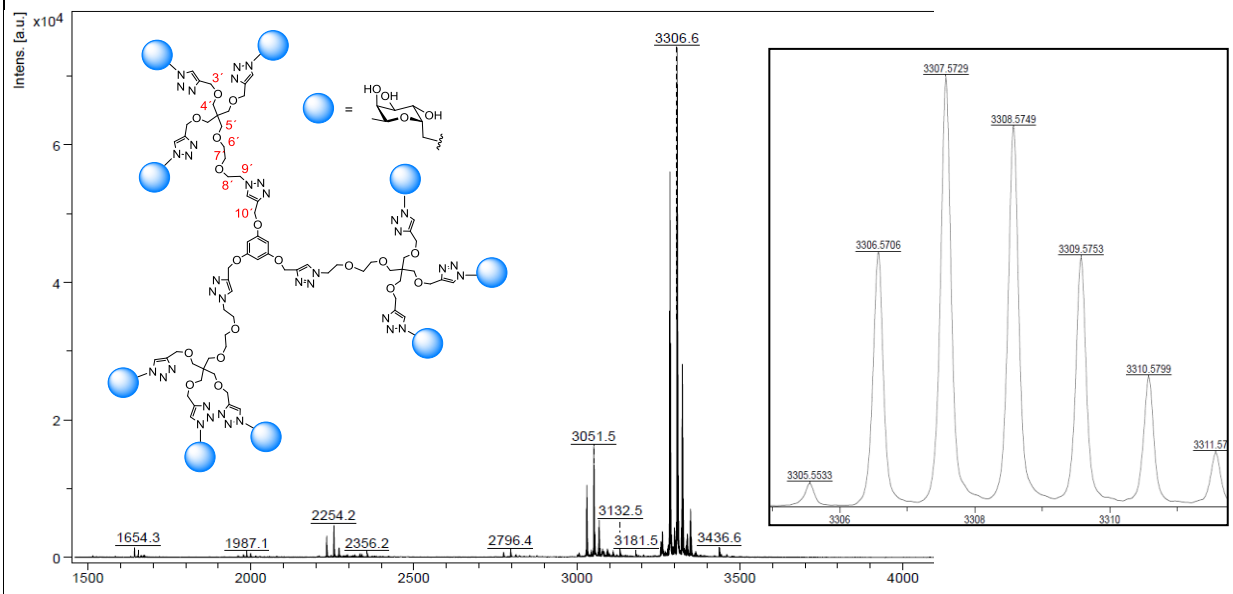


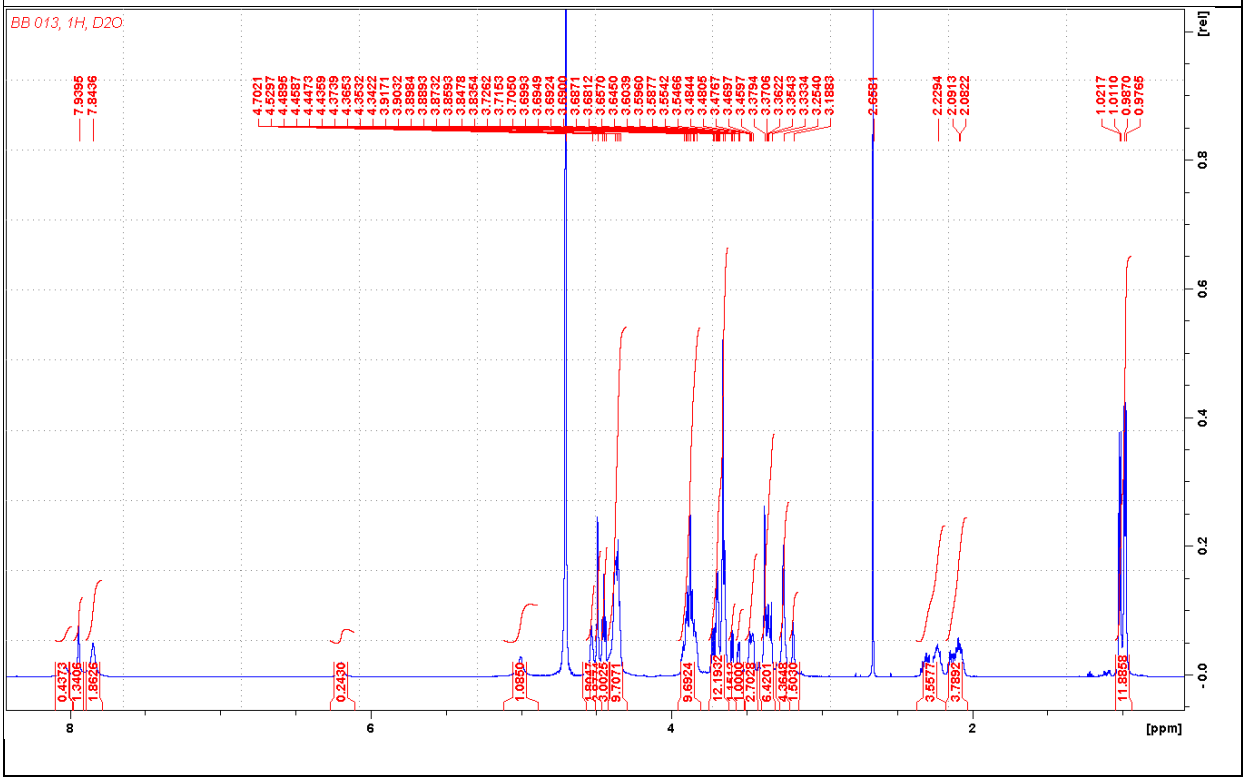


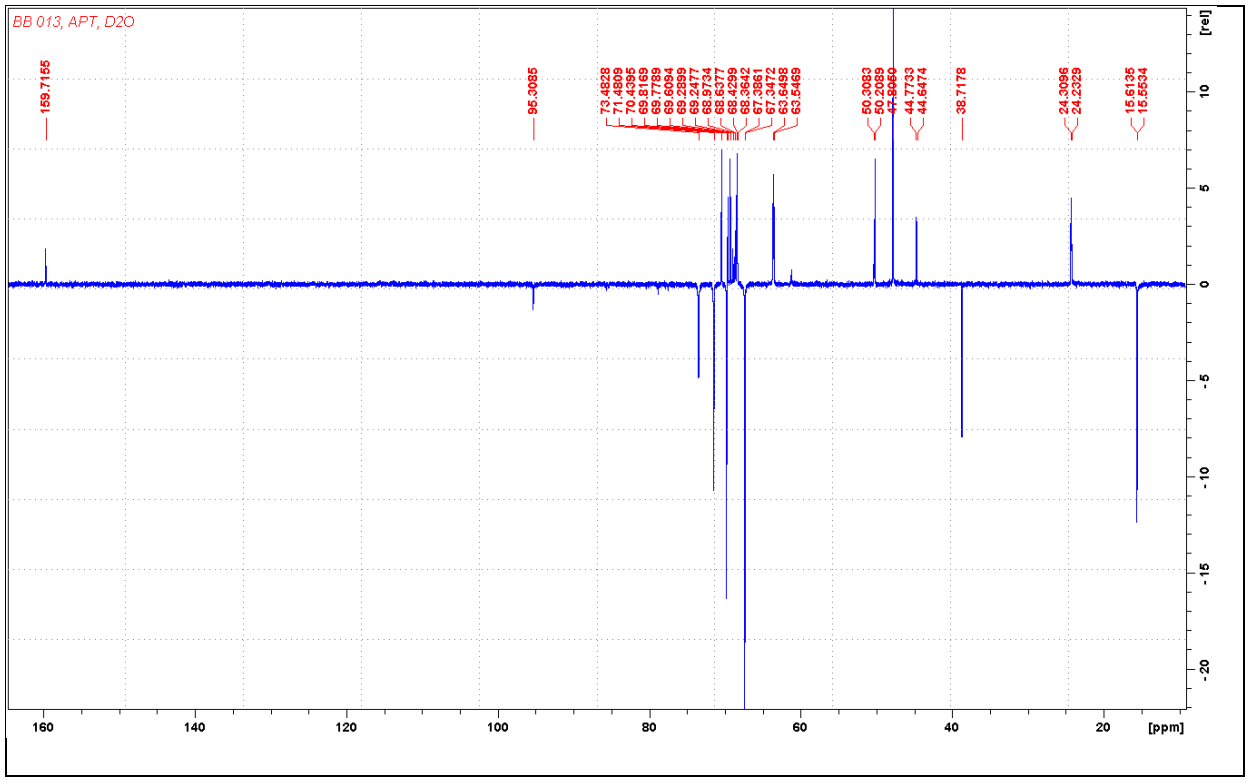

BB 02, 1H, D2O
7.9407
4.7021
4.4608
4.4287
4.4151
4.4038
3.9197
3.9146
3.9097
3.9027
3.8882
3.8760
3.8698
3.8599
3.7123
3.7038
3.6912
3.6859
3.6751
3.3377
3.3099
3.1587
2.2897
2.2830
2.2743
2.1383
2.1274
2.1189
2.1152
2.1071
1.0100
0.9996
0.5104
3.9118
2.0208
3.0914
2.1144
0.7405
0.9117
0.9794
2.9996
[rel]
[ppm]
BB 02, APT, D2O
73.5083
71.4905
69.7893
68.8217
68.4680
68.4195
67.3954
63.7358
58.4497
47.8805
44.9711
24.2635
15.5887
[rel]
[ppm]

Dendrimer F-C-9

$C_{141}H_{222}N_{36}O_{54}$, MW 3283; MS (MALDI); HRMS (MALDI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

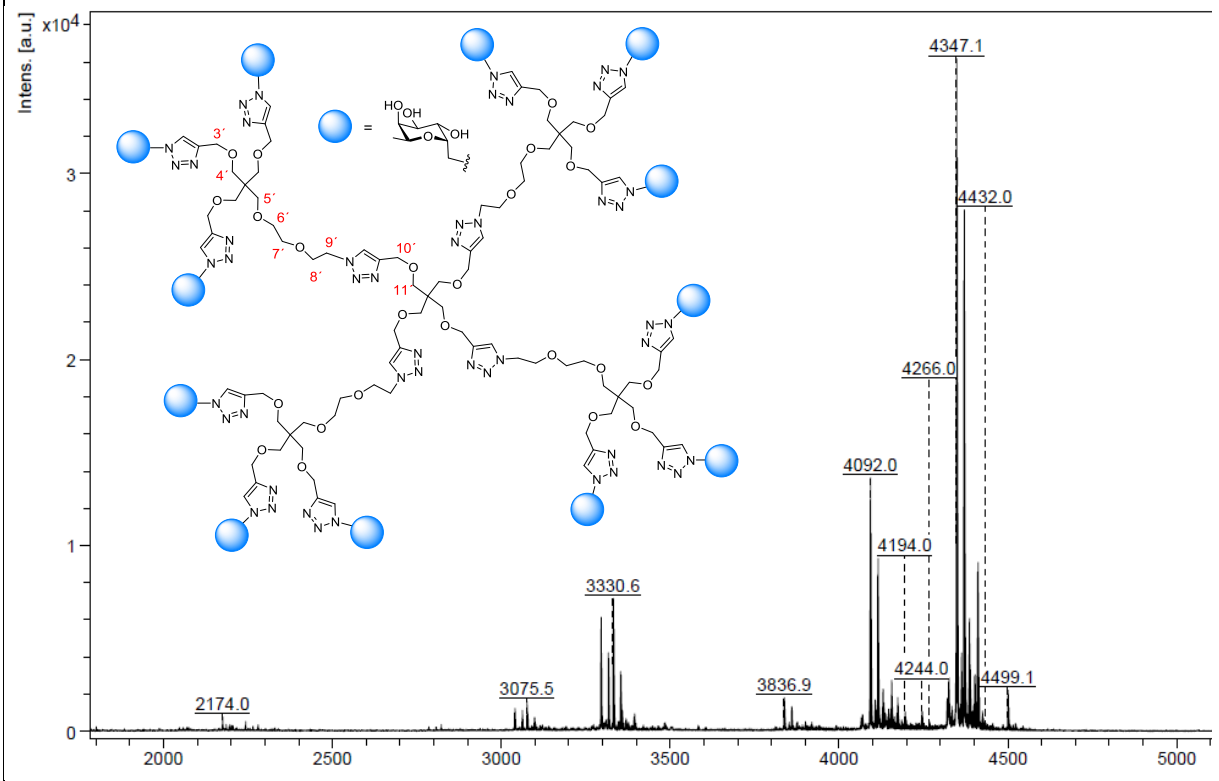


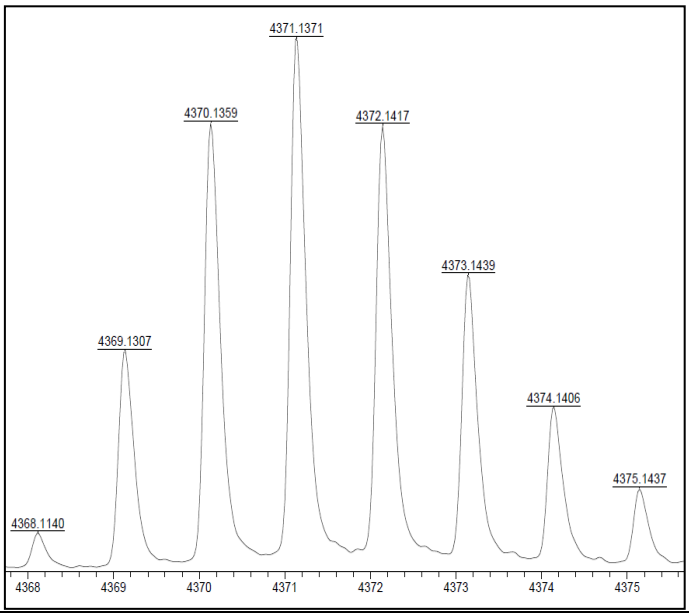


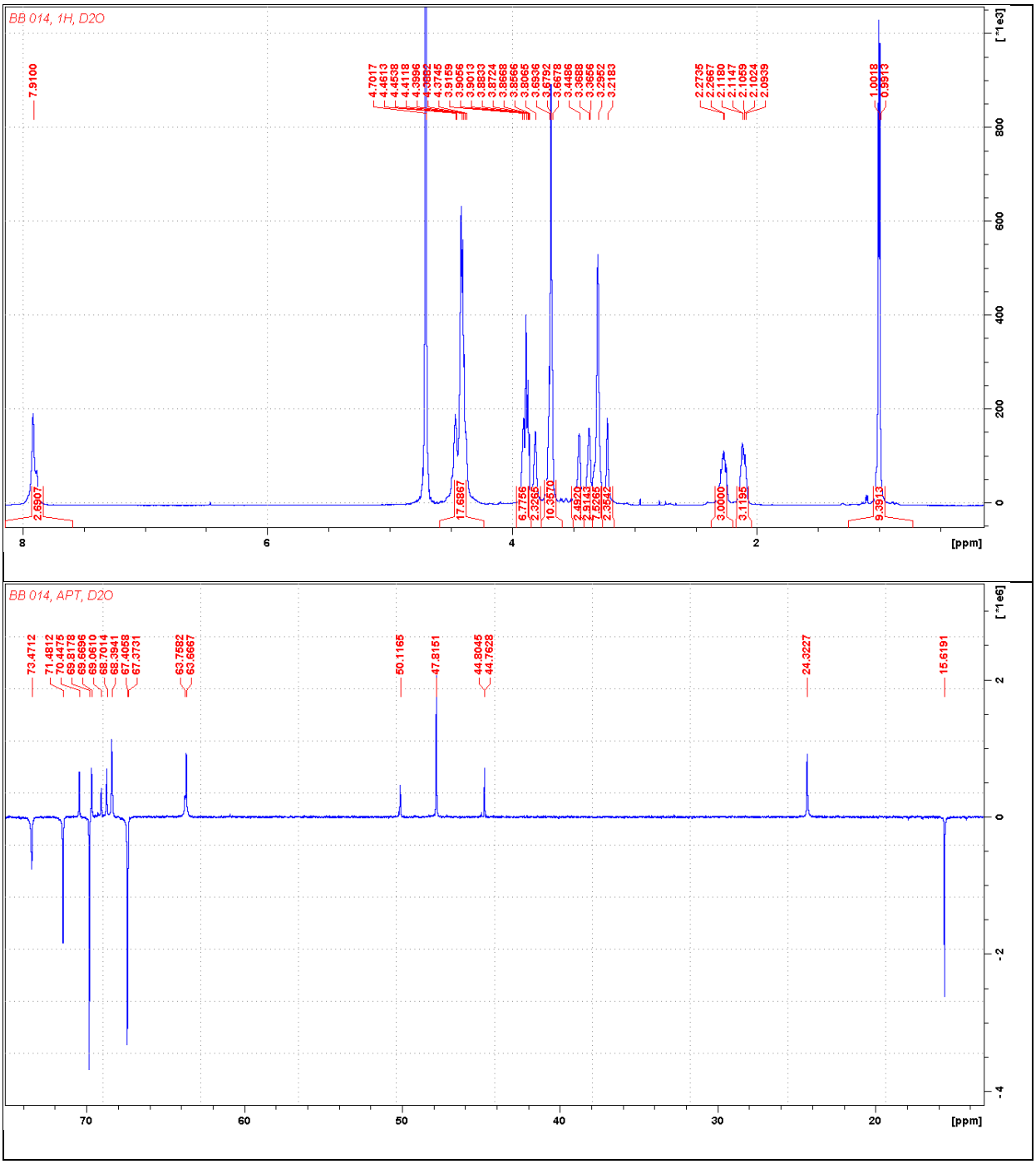

BB 013, APT, D2O
159.7155
95.3085
73.4828
71.4809
70.4395
69.5189
69.2799
69.0094
69.2399
69.2477
68.9734
68.6377
68.4299
68.3642
67.3861
67.3472
63.6488
63.5469
50.3083
50.2089
47.9080
44.7733
44.6474
38.7178
24.3096
24.2329
15.6135
15.5534
[rel]
10
5
0
-5
-10
-15
-20
160
140
120
100
80
60
40
20
[ppm]

Dendrimer F-C-12

$C_{185}H_{300}N_{48}O_{72}$, MW 4346; MS (MALDI); HRMS (MALDI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

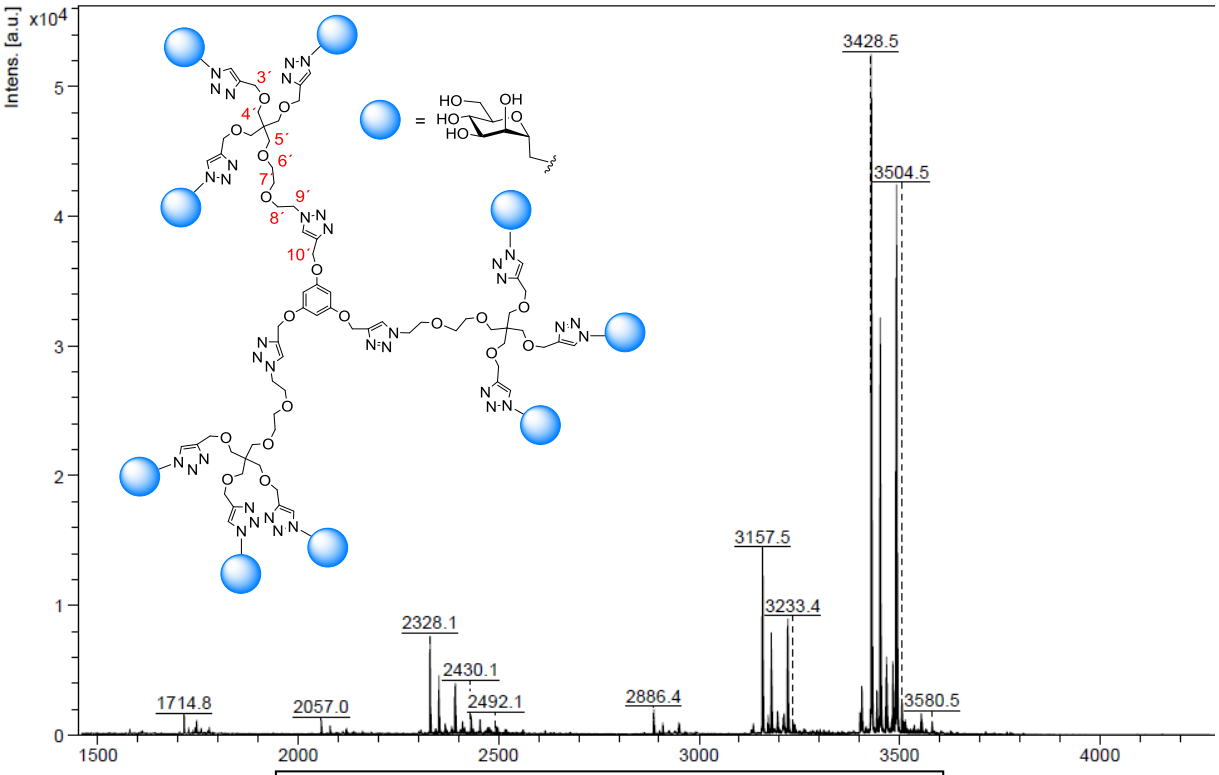


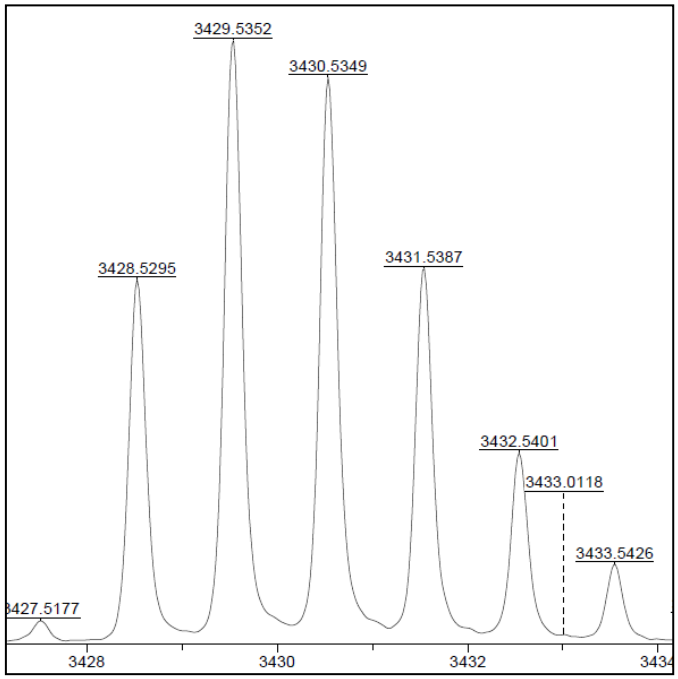


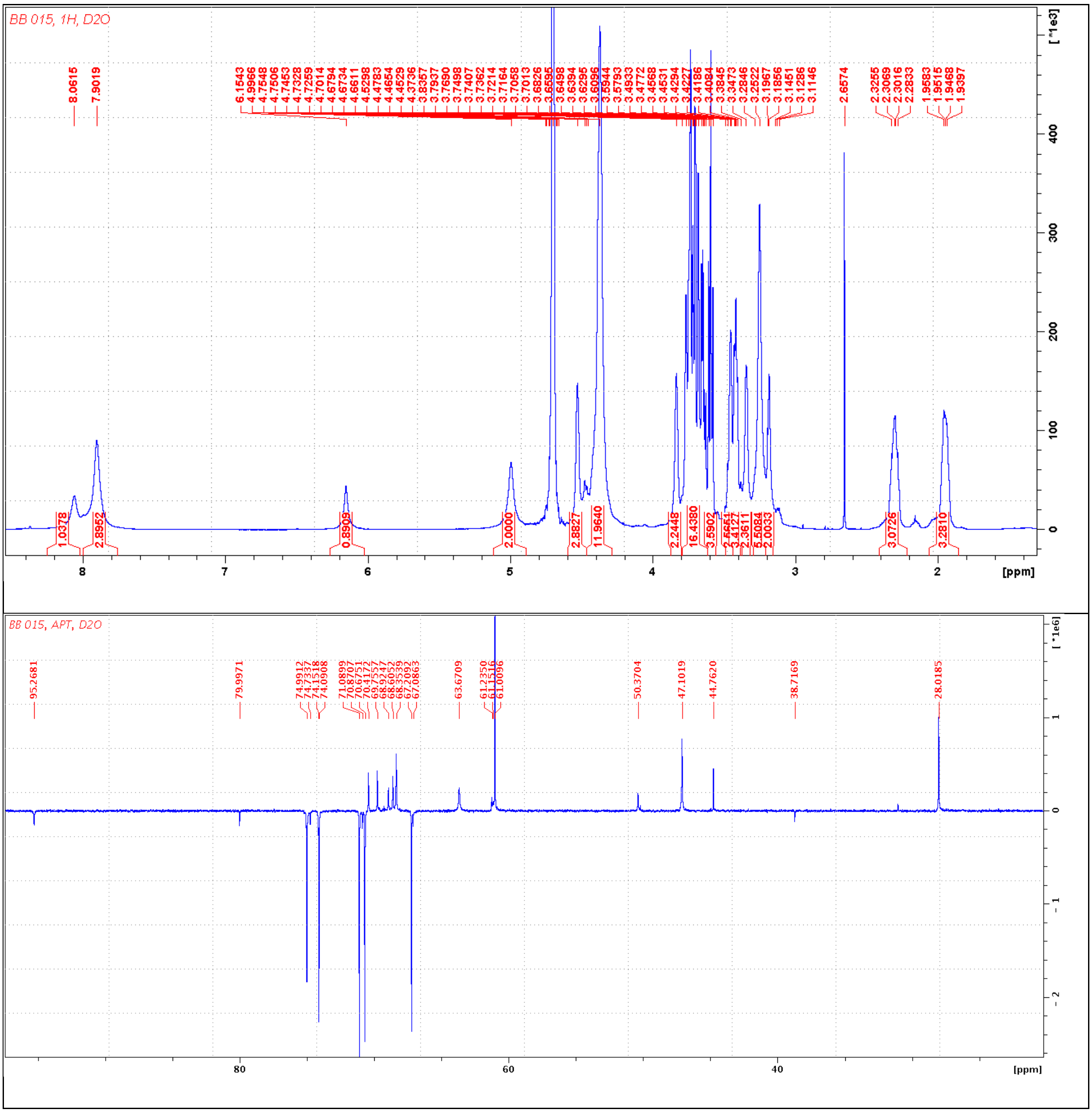

BB 014, 1H, D2O
7.9100
4.7017
4.4613
4.4538
4.4118
4.3982
4.3745
3.9159
3.9056
3.9013
3.8833
3.8724
3.8668
3.8566
3.8065
3.6936
3.6792
3.6678
3.4486
3.4368
3.3656
3.2952
3.2183
2.2735
2.2667
2.1180
2.1147
2.1059
2.1024
2.0539
1.0018
0.9913
2.6907
17.6867
6.7756
2.3258
10.3570
2.4920
2.9143
7.5265
2.3542
3.0000
3.1195
9.3913
[ppm]
BB 014, APT, D2O
73.4712
71.4812
70.4475
69.8178
69.6696
69.0610
68.7014
68.3941
67.4458
67.3731
63.7582
63.6667
50.1165
47.8151
44.8045
44.7628
24.3227
15.6191
[ppm]

Dendrimer M-C-9

$C_{141}H_{222}N_{36}O_{63}$, MW 3426; MS (MALDI); HRMS (MALDI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)

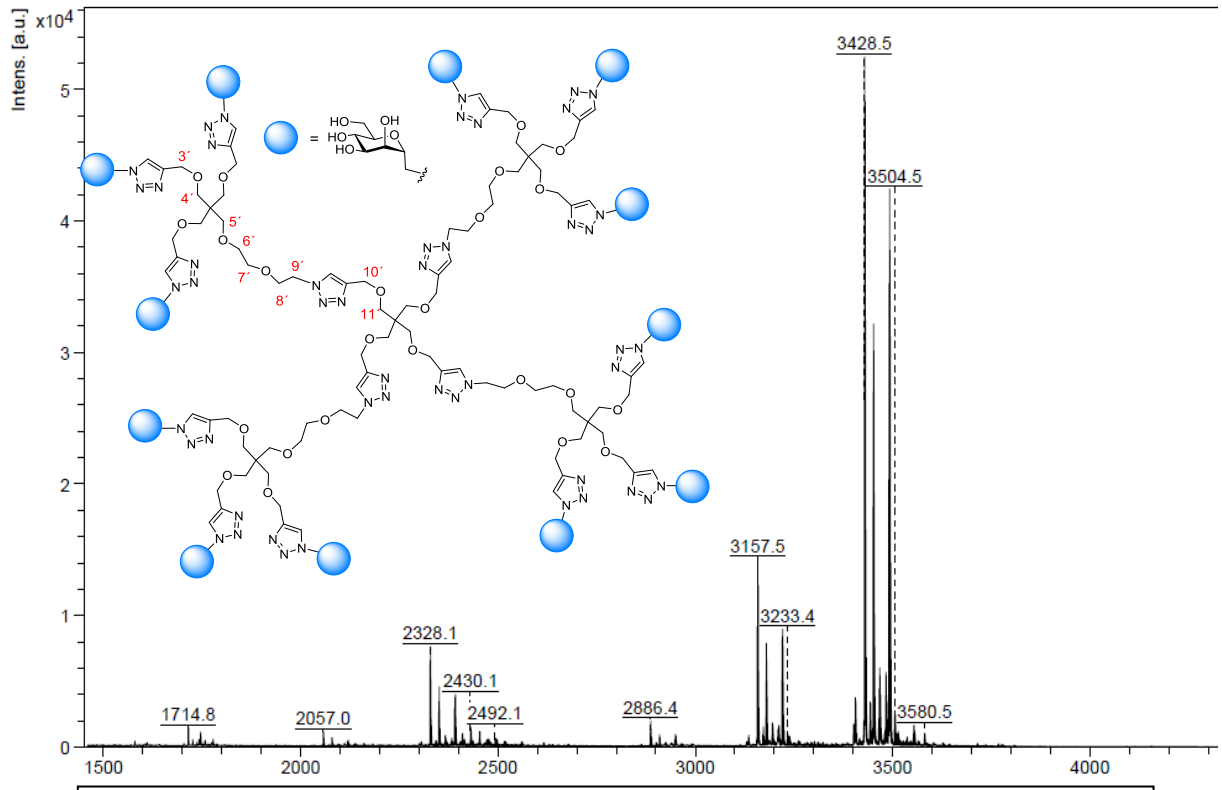


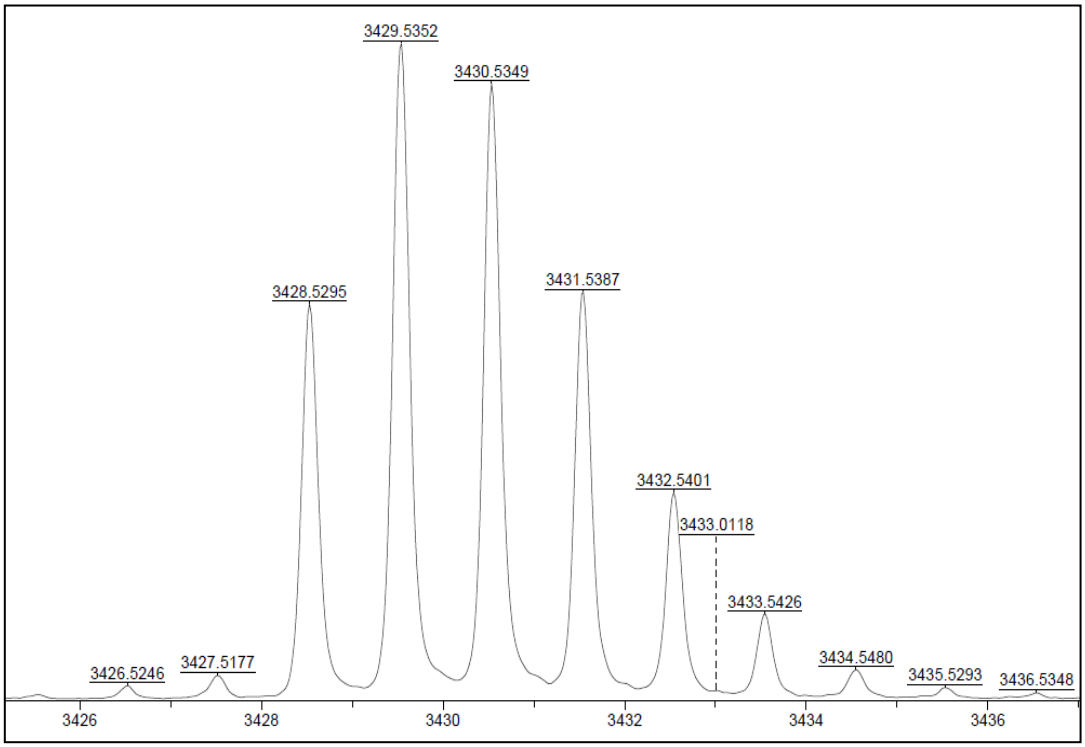


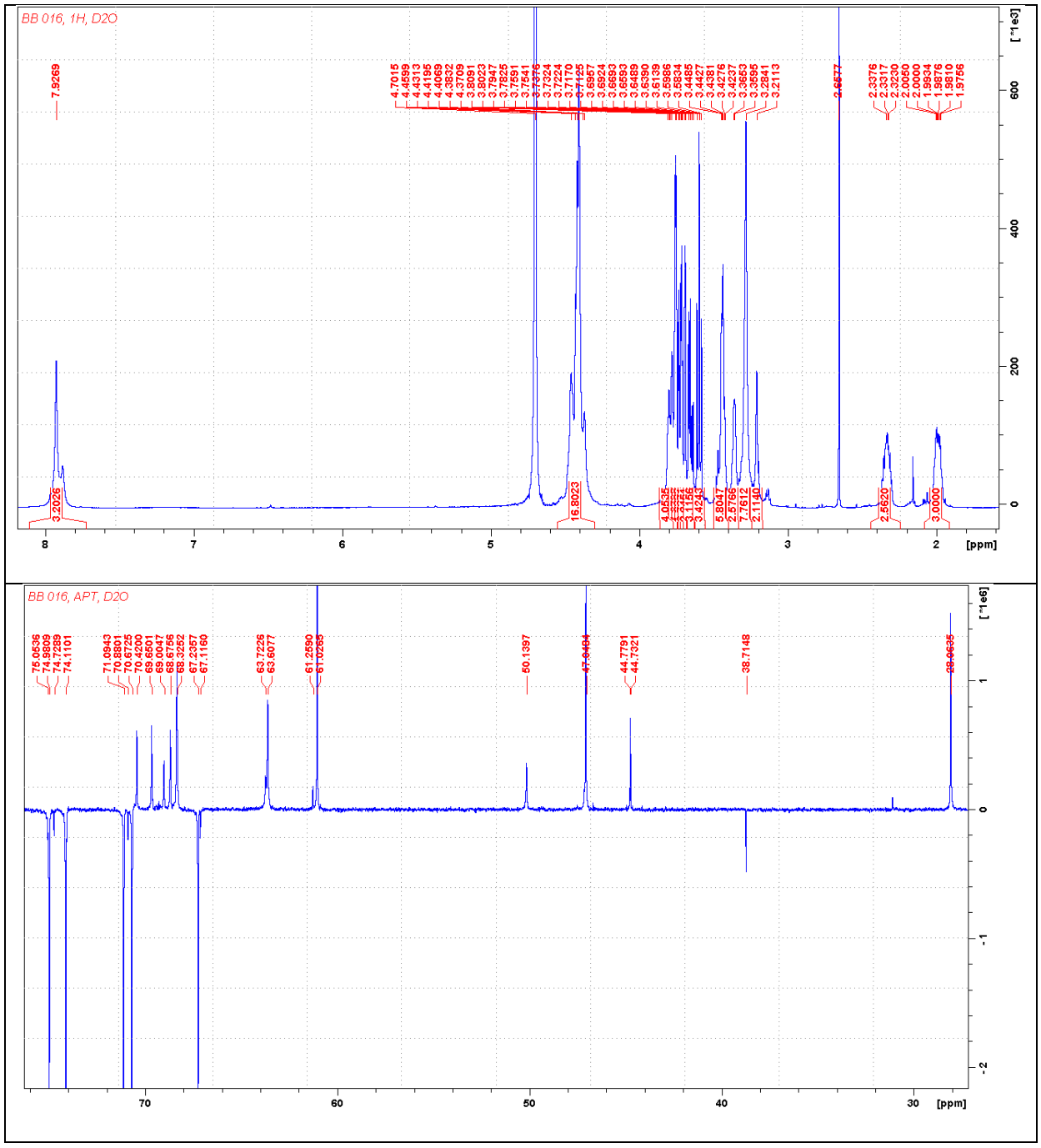

BB 015, 1H, D2O
[ppm]
[*1e3]
BB 015, APT, D2O
[ppm]
[*1e6]

Dendrimer M-C-12

$C_{185}H_{300}N_{48}O_{84}$, MW 4536; MS (MALDI); HRMS (MALDI); ^{1}H NMR (600.1 MHz, D_2O); ^{13}C NMR (125 MHz, D_2O)





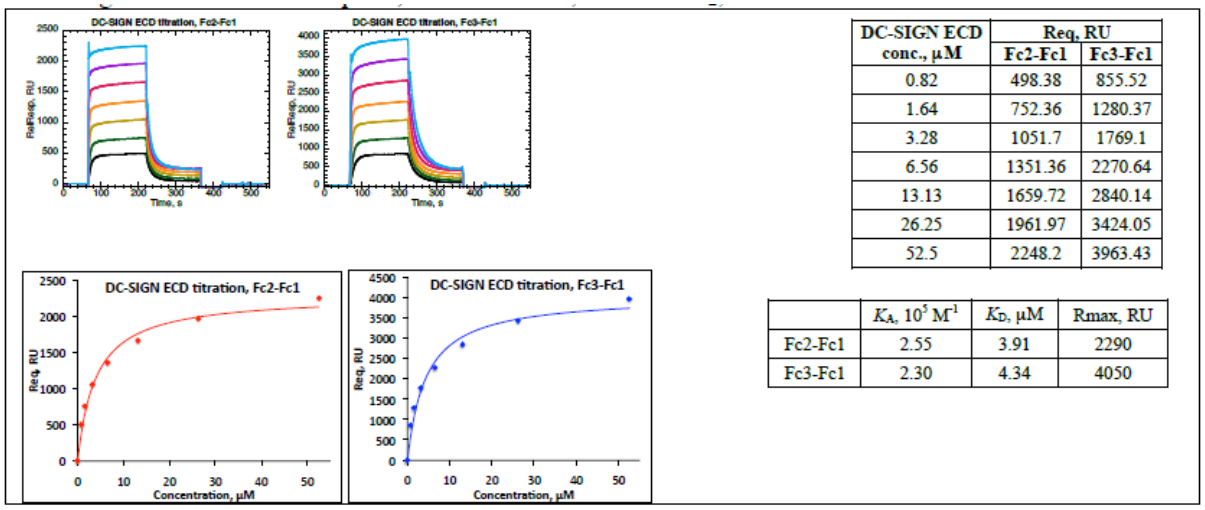

BB 016, 1H, D2O
7.9269
4.7015
4.4599
4.4313
4.4195
4.4069
4.3832
4.3709
4.0091
3.8023
3.7947
3.7825
3.7591
3.7541
3.7224
3.7170
3.6957
3.6924
3.6693
3.6593
3.6489
3.6390
3.6139
3.5986
3.5834
3.4485
3.4427
3.4276
3.4237
3.3853
3.3695
3.2841
3.2113
2.6677
2.3376
2.3317
2.3230
2.0050
2.0000
1.9934
1.9876
1.9810
1.9766
3.2026
16.8023
4.0535
5.8047
2.5766
7.7612
2.1140
2.5620
3.0000
[*1e3]
[ppm]
BB 016, APT, D2O
75.0536
74.9809
74.7289
74.1101
71.0943
70.8801
70.6725
70.4700
69.6501
69.0047
68.6756
68.3252
67.2357
67.1160
63.7226
63.6077
61.2590
61.0265
50.1397
47.0484
44.7791
44.7321
38.7148
28.0635
[*1e6]
[ppm]

2. SPR competition assay

2.1 General procedure

DC-SIGN extracellular domain (ECD) was expressed in *E.coli* as inclusion bodies, refolded and purified as described previously.[1] SPR competition experiments were performed using Biacore 3000 instrument, at 25 °C as described previously.[2,3] Briefly, using Biacore amino coupling kit and standard amino coupling procedure, interaction flow cell of sensor chip CM4 was covalently functionalized with BSA-Manα1-3[Manα1-6]Man (Man-BSA), while reference flow cell was EDC/NHS-activated and ethanolamine-deactivated carboxymethyldextran. DC-SIGN ECD alone (20 µM) or in presence of increasing concentrations of test compounds was injected over reference (Fc1) and interaction surfaces (Fc2 and Fc3) at 20 µL/min flow rate. All samples were prepared in running buffer consisting of 25 mM Tris-HCl pH 8, 150 mM NaCl, 4 mM $CaCl_2$ and 0.005% surfactant P20. Binding of DC-SIGN ECD to Man-BSA surface was recorded in sensorgrams. After reference surface correction (Fc1 - reference surface, activated/deactivated CM-dextran), DC-SIGN ECD binding responses for each injection were extracted and normalized to DC-SIGN ECD alone binding response. Normalized binding responses were plotted against compound concentration and IC_{50} values were calculated from resulting competition curves using 4-parameter logistic model.[2,3] Each run was repeated twice using two different ManBSA surfaces Fc2 (1912 RU) and Fc3 (1612 RU) within two days. ManBSA densities on Fc2 and Fc3 were different with higher density being on Fc3, however, the overall affinity of DC-SIGN to both Man-BSA surfaces was unchanged as K_A and K_D values were comparable (Fig S1).

1 G. Tabarani, M. Thépaut, D. Stroebel, C. Ebel, C. Vivès, P. Vachette, D. Durand and F. Fieschi, *J. Biol. Chem*., 2009, **284**, 21229.
2 N. Varga, I. Sutkeviciute, R. Ribeiro-Viana, A. Berzi, R. Ramdasi, A. Daghetti, G. Vettoretti, A. Amara, M. Clerici, J. Rojo, F. Fieschi and A. Bernardi, *Biomaterials*, 2014, **35**, 4175.
3 M. Andreini, D. Doknic, I. Sutkeviciute, J. J. Reina, J. Duan, E. Chabrol, M. Thepaut, E. Moroni, F. Doro, L. Belvisi, J. Weiser, J. Rojo, F. Fieschi and A. Bernardi, *Org. Biomol. Chem*., 2011, **9**, 5778.



DC-SIGN ECD conc., µM	Req, RU	
	Fc2-Fc1	Fc3-Fc1
0.82	498.38	855.52
1.64	752.36	1280.37
3.28	1051.7	1769.1
6.56	1351.36	2270.64
13.13	1659.72	2840.14
26.25	1961.97	3424.05
52.5	2248.2	3963.43

	K_A, 10^5 M^{-1}	K_D, µM	Rmax, RU
Fc2-Fc1	2.55	3.91	2290
Fc3-Fc1	2.30	4.34	4050

Fig. S1 First titration of Man-BSA surfaces with DC-SIGN ECD. Running buffer 25 mM TRIS pH 8, 150 mM NaCl, 4 mM $CaCl_2$, 0.005% P20

2.2 IC_{50} values

Table S1

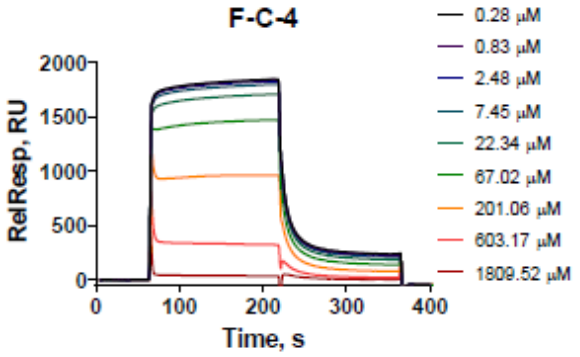
IC_{50} values (μM) obtained in DC-SIGN inhibition assays (SPR) and valency-corrected factors β†

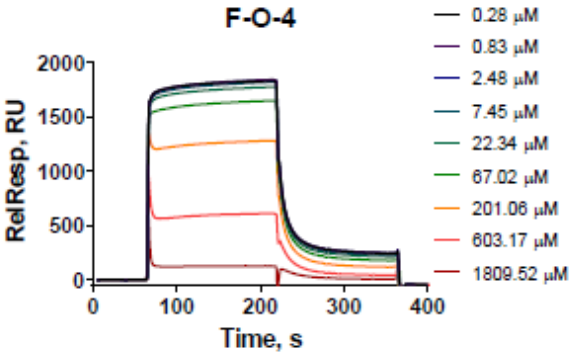
Acronym	Valency	IC_{50} (μM)	SD (%)	Factor β
F-C-4	4	212	5.22	2.4
F-C-6	6	97	2.69	3.5
F-C-9	9	41	2.65	5.6
F-C-12	12	17	4.95	10.1
F-O-4	4	375	3.28	1.4
F-O-6	6	392	3.19	0.9
F-O-12	12	4.7	2.02	36.5
M-C-9	9	24	2.41	15.7
M-C-12	12	12	2.60	20.2
M-O-9*	9	128	4.90	2.9
M-O-12*	12	67	2.50	4.2

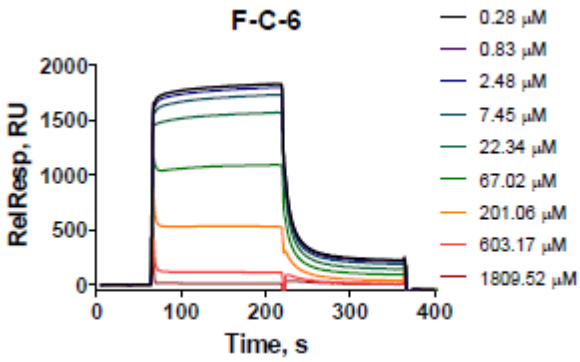
†L-fucose: IC_{50} = 2,061 μM; D-mannose: IC_{50} = 3,392 μM

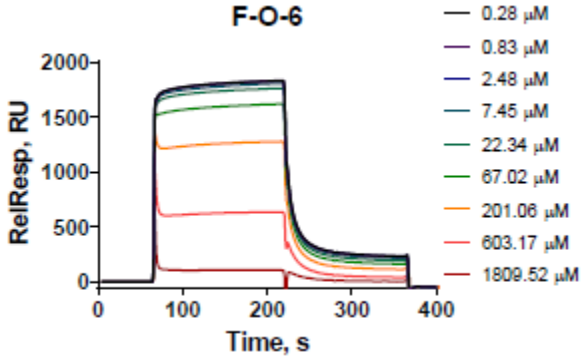
***N. Varga, I. Sutkeviciute, R. Ribeiro-Viana, A. Berzi, R. Ramdasi, A. Daghetti, G. Vettoretti, A. Amara, M. Clerici, J. Rojo, F. Fieschi and A. Bernardi, *Biomaterials,* 2014, **35**, 4175.

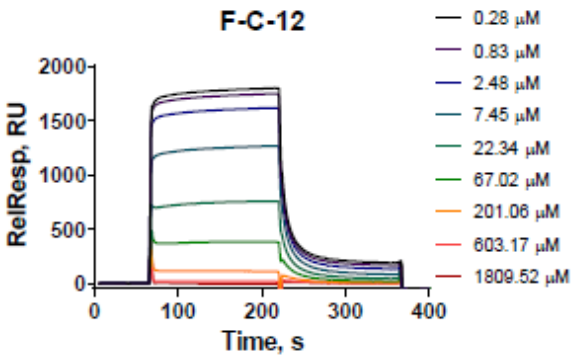
2.4. Sensorgrams and inhibition curves

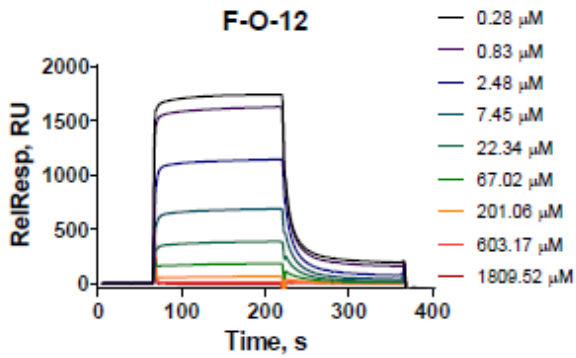


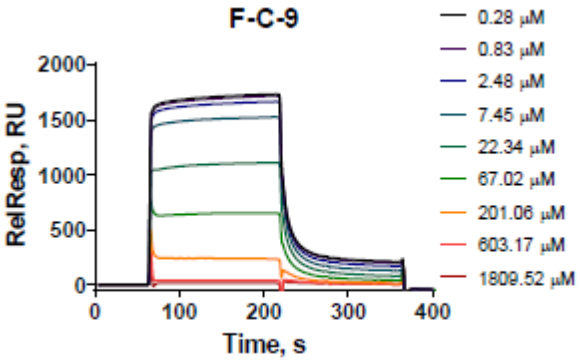












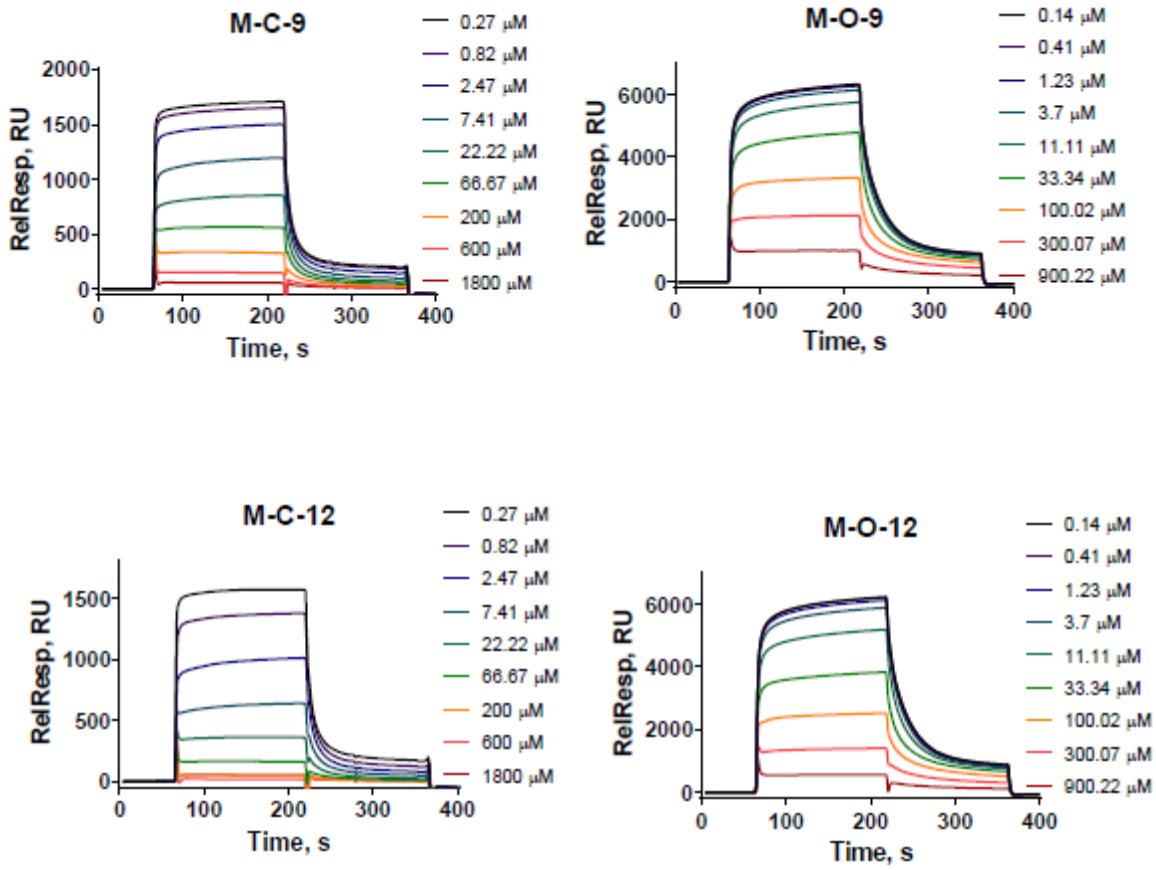


Fig. S2 Reference surface corrected **s**ensorgrams

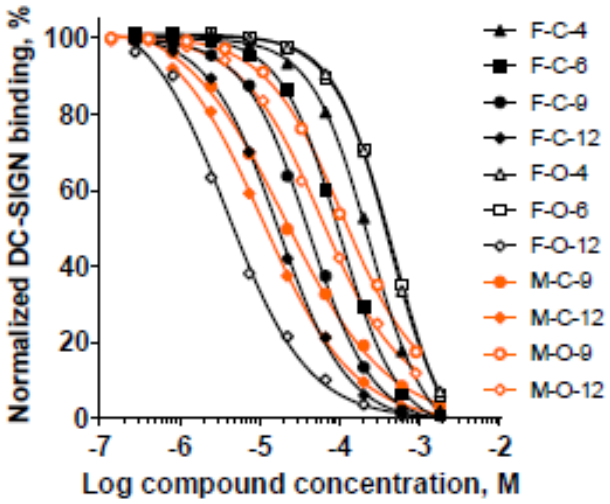


Fig. S3 Inhibition curves