Supplemental Information

Novel Reversible Fluorescent Glycan Linker for Functional Glycomics

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GENERAL METHODS

All purchased chemicals were used without further purification. HPLC solvents were purchased from Fisher Scientific. Details of the commercial substances are listed in the Key Resources Table. HPLC analysis of the F-MAPA- and AEAB-labeled glycans was performed on a Shimadzu HPLC CBM-20A system. It contained a UV detector SPD-20AV and a fluorescence detector RF-20A. Fluorescence at 265 nm excitation (Ex) and 315 nm emission (Em) were used to detect F-MAPA-labeled glycans and 330 nm excitation (Ex) and 420 nm emission (Em) were used to detect AEAB-labeled glycans. A 250 x 4.6 mm Zorbax NH2 column was used and the mobile phases were acetonitrile, water, and 250mM ammonium acetate. The concentration of water increased from 16% to 40% and the ratio of ammonium acetate from 4% to 50% over 60 min. An UltrafleXtreme MALDI-TOF/TOF system from Bruker was used for MALDI-TOF MS analysis of the glycans and their conjugates. 2,5-Dihydroxybenzoic acid (20 mg/ml in ACN:H_2O = 1:1) was used as the matrix. ESI-MS analysis was performed on an Orbitrap Fusion Lumos from Thermo Fisher Scientific. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$, MeOD or D_2O as the internal standard. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances); coupling constant are reported in Hertz (Hz). All NMR signals were assigned on the basis of ^{1}H ^{13}C NMR, APT, HSQC, TOCSY and COSY experiments.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data Quantitation for the Array Analysis
The signal intensities were quantified by the GenePix Pro 7 that is associated with the microarray scanner and processed by Excel (Microsoft). The results are shown as relative fluorescence units (RFUs) by averaging the background-subtracted fluorescence signals of four replicate spots with error bars representing the standard deviation among the four values.

DATA AND SOFTWARE AVAILABILITY

All of the glycan microarray datasets related to this work are shown in the Supplemental Information and are publicly available at the National Center for Functional Glycomics (NCFG) website: https://ncfg.hms.harvard.edu/ncfg-data

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Recombinant Human DC-SIGN/CD209 Fc Chimera Protein	R&D Systems	Cat#: 161-DC-050
Alexa488-labelled goat anti-human IgG	Life Technologies	Cat#: A-11013
Alexa594 anti-human CD15 Antibody	BioLegend	Cat#: 301917
Rat Anti-Human Cutaneous Lymphocyte Antigen	BD Pharmingen	Cat#: 555946

Alexa488-labeled goat anti-rat IgM	Molecular Probes	Cat#: A-21212
Blood Group Lewis a Antibody (7LE)	Santa Cruz Biotechnology, Inc.	Cat#: sc-51512
Alexa488-labeled goat anti-mouse IgG	Invitrogen	Cat#: A-11001
Lectins, Chemicals, Glycans		
AAL	Vector Labs	Cat#: B-1395
UEA-I	Vector Labs	Cat#: B-1065
GSL-I b4	Vector Labs	Cat#: B-1205
DBA	Vector Labs	Cat#: B-1035
Biotinylated hydrazide	Vector Labs	Cat#: SP-1100
Streptavidin-Cyanine 5	Invitrogen	Cat#: 434316
Nexterion H NHS functionalized slides	Schott AG	Cat#: 1070936
3-Aminopropionaldehyde diethylacetal	Sigma-Alderich	Cat#: A8597-25G
AEAB	Cummings lab	
di-*N*-hydroxysuccinimidyl suberate	Sigma-Alderich	Cat#: S1885
BSA	Boval BioSolutions, LLC	Cat#: LY-0081
Blood group glycans	ELICITYL	
LNnT	Cummings lab	
Crude egg yolk N-glycans	Cummings lab	

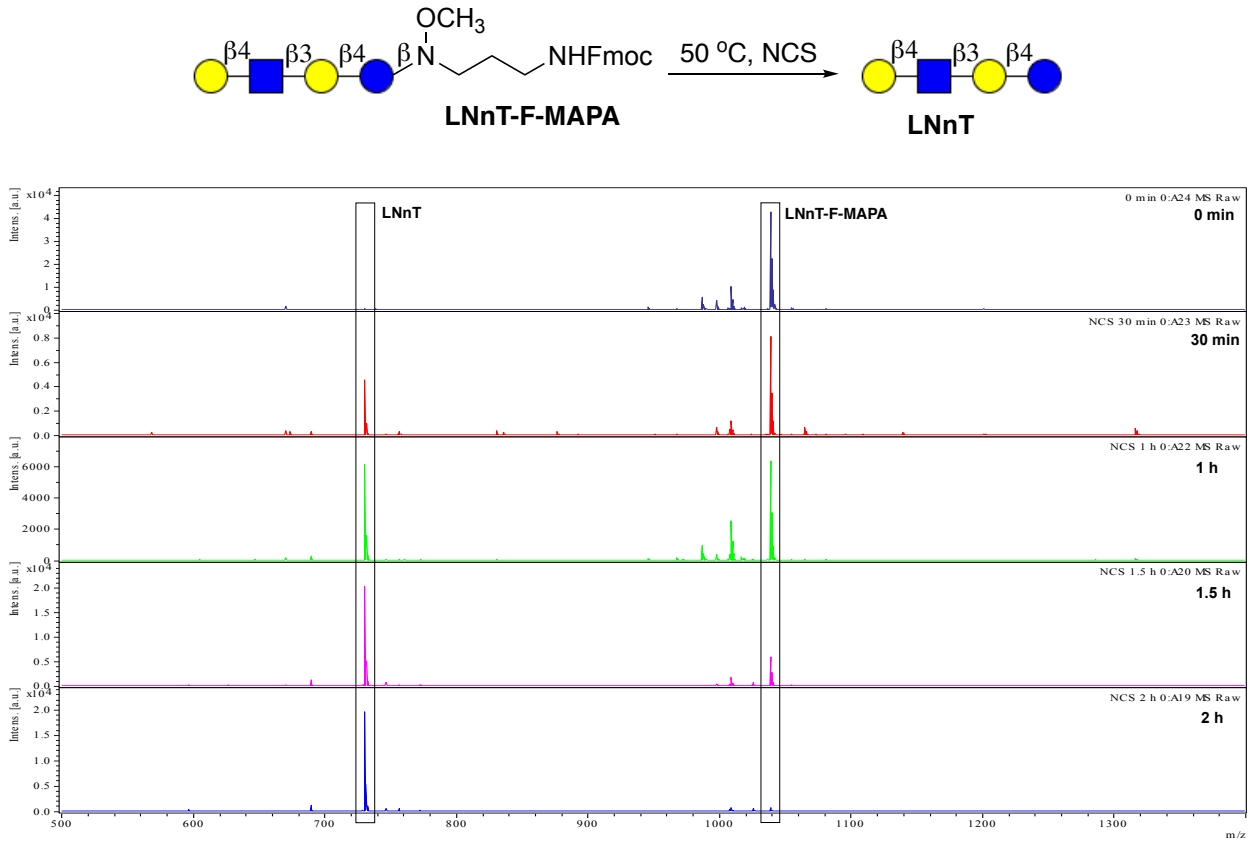


Figure S1. Cleavage of LNnT-F-MAPA by NCS to regenerate free reducing LNnT.

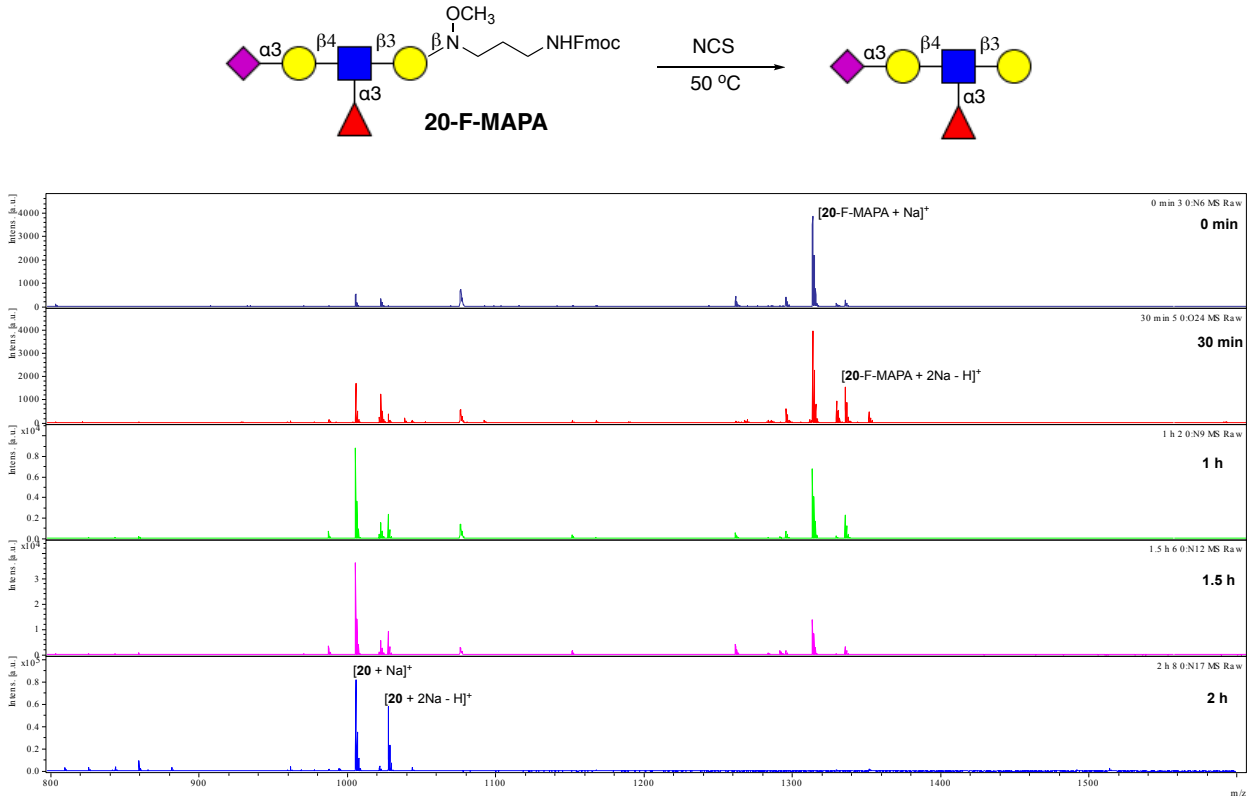
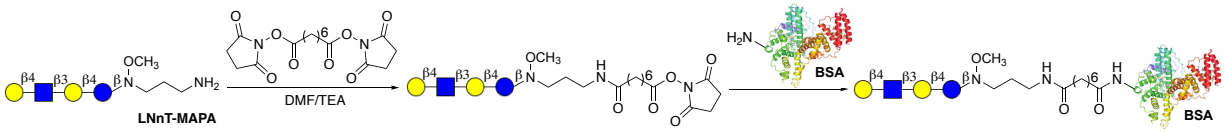
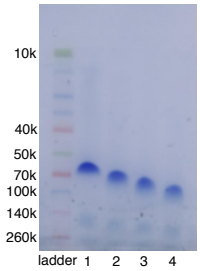


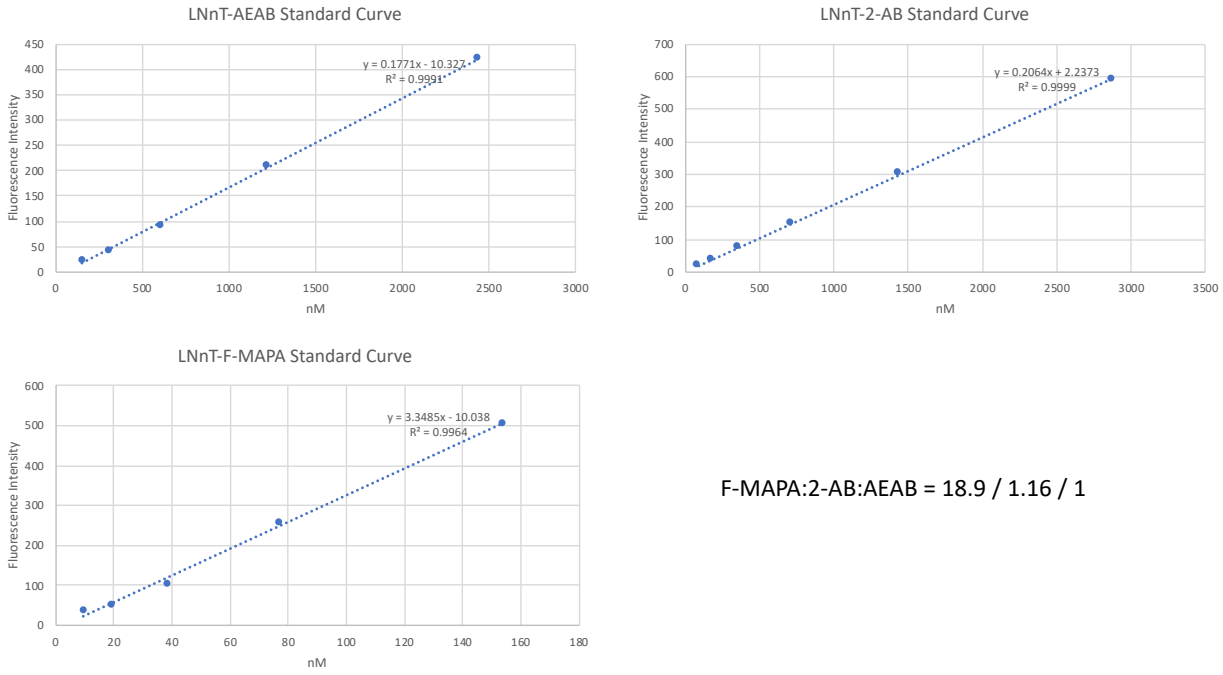
Figure S2. Cleavage of **20**-F-MAPA by NCS to regenerate free reducing **20**.





Lane	Starting molar ratio (LNnT-MAPA/BSA)	Product molar ratio (LNnT/BSA)
1	BSA only	NA
2	10	4.78
3	25	10.27
4	50	16.26

Figure S3. Synthesis of neoglycoprotein.



F-MAPA:2-AB:AEAB = 18.9 / 1.16 / 1

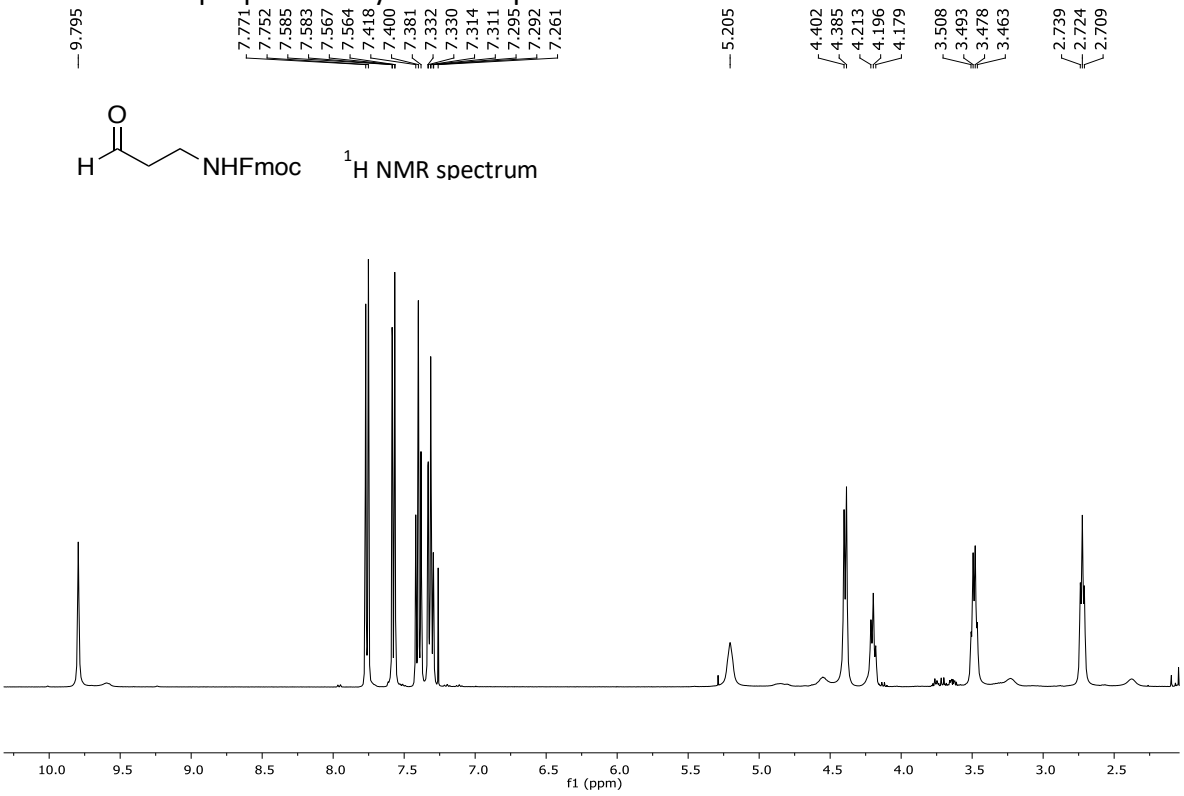
Figure S4. Relative fluorescence sensitivity of F-MAPA, 2-AB and AEAB.

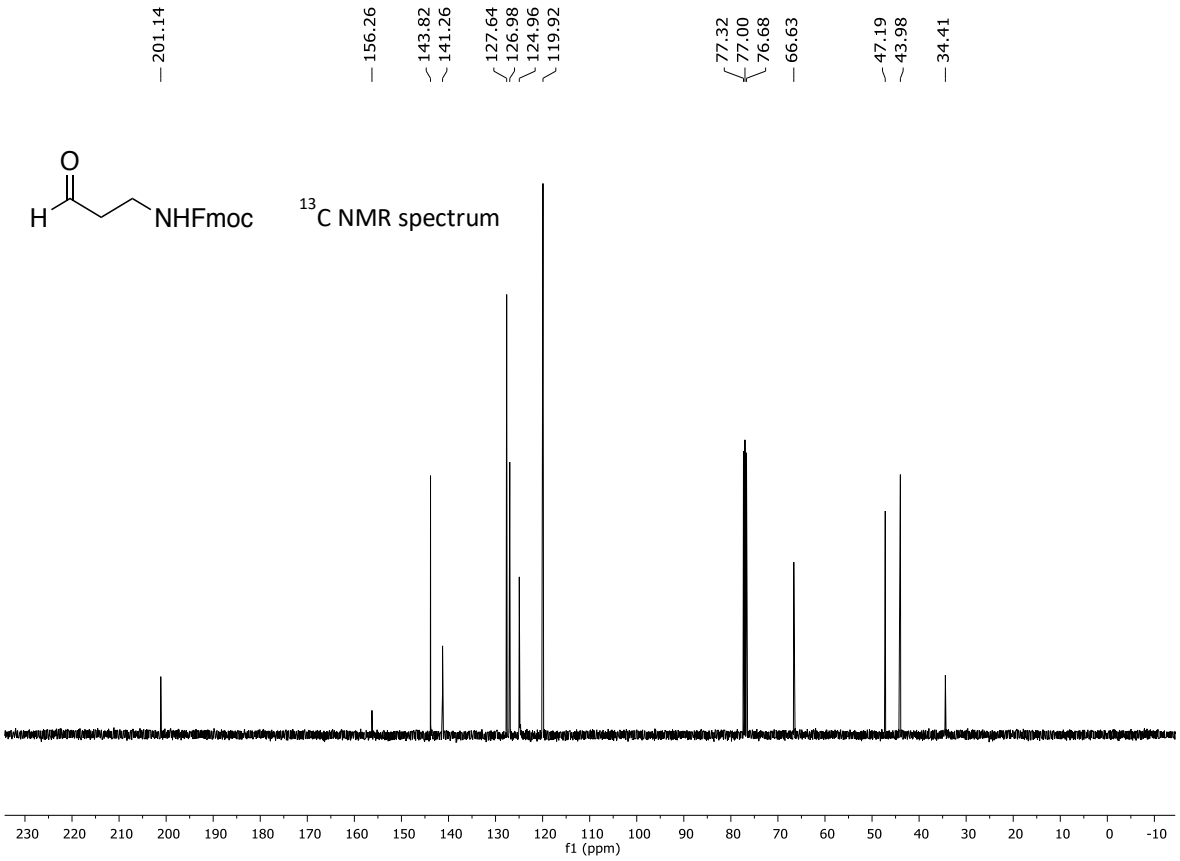
NMR data and Spectra

LNnT-MAPA: ^{1}H NMR (400 MHz, D_2O): δ 4.60 (d, *J* = 8.0 Hz, GlcNAc H1), 4.37 (d, *J* = 8.0 Hz, Gal-2 H1), 4.33 (d, *J* = 8.0 Hz, Gal-1 H1), 4.12 (d, *J* = 8.8 Hz, Glc H1), 4.07 (d, *J* = 3.2 Hz, 1 H), 3.88 (m, 2 H), 3.84 (m, 1 H), 3.77 (m, 2 H), 3.71 (m, 3 H), 3.68 (m, 2 H), 3.65 (m, 3 H), 3.64 (m, 2 H), 3.58 (m, 1 H), 3.57 (m, 1 H), 3.55 (s, 3 H), 3.54 (m, 1 H), 3.52 (m, 1 H), 3.51 (m, 2 H), 3.46 (m, 2 H), 3.07 (m, 1 H), 2.96 (t, *J* = 7.2 Hz, 2 H), 1.95 (s, 3 H), 1.85 (m, 2 H); ^{13}C NMR from HSQC (100 MHz, D_2O): δ 102.9, 102.8, 102.7, 91.8, 82.1, 78.2, 78.1, 76.4, 75.8, 75.4, 75.0, 74.5, 72.5, 72.1, 70.0,

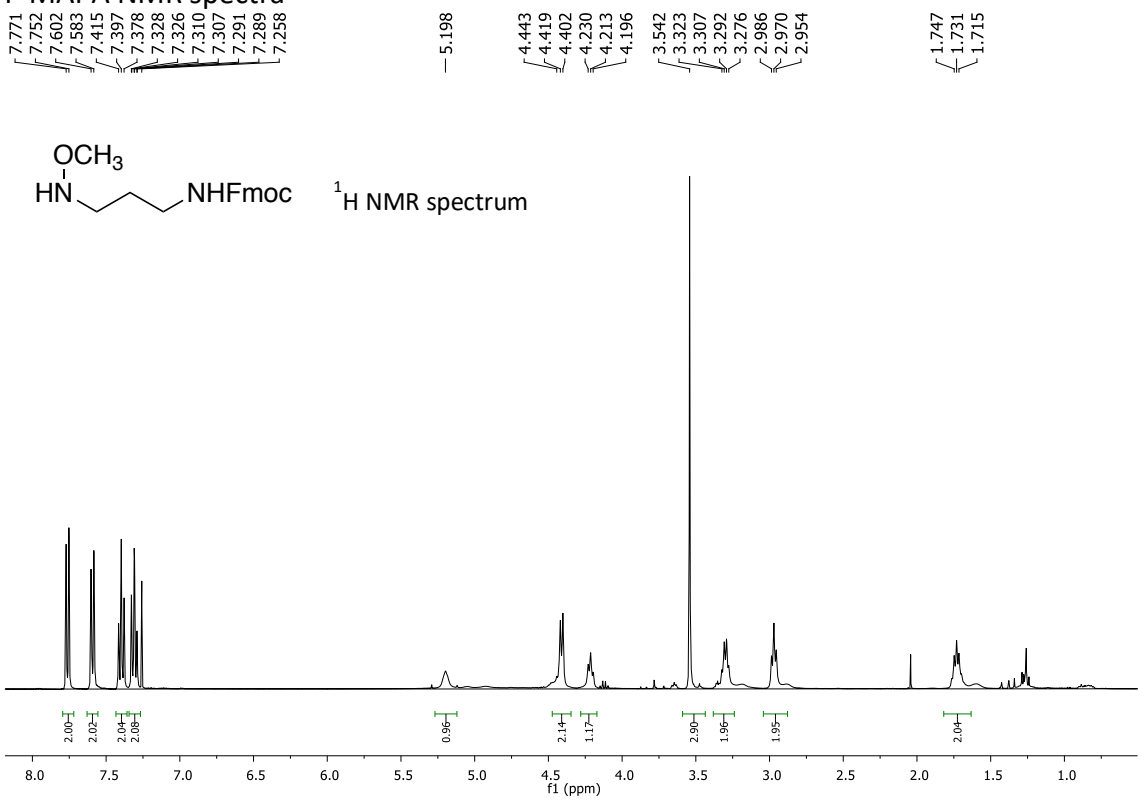
69.4, 68.5, 68.3, 62.1, 60.9, 60.2, 59.9, 59.7, 55.2, 49.9, 38.2, 25.5, 22.1. ESI HRMS calcd for $C_{30}H_{55}N_3NaO_{21}$ $[M+Na]^+$ 816.3220, found 816.3203.

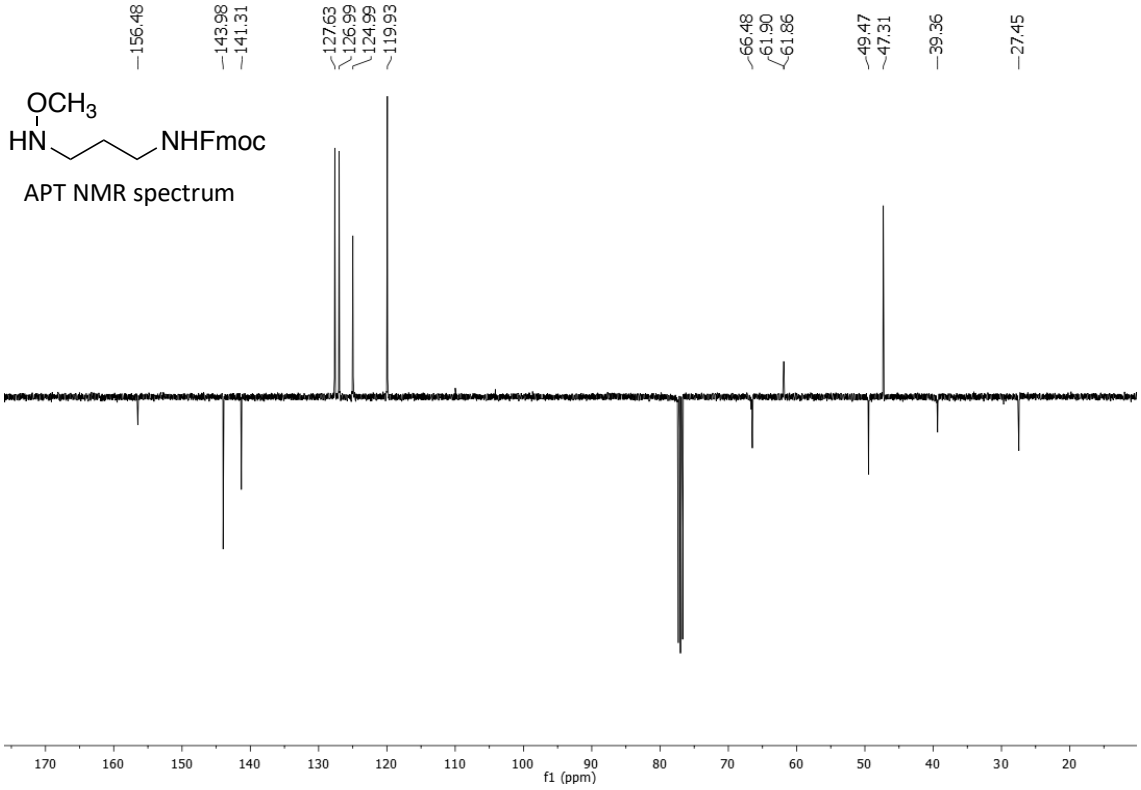
Fmoc-3-amino propionaldehyde NMR spectra

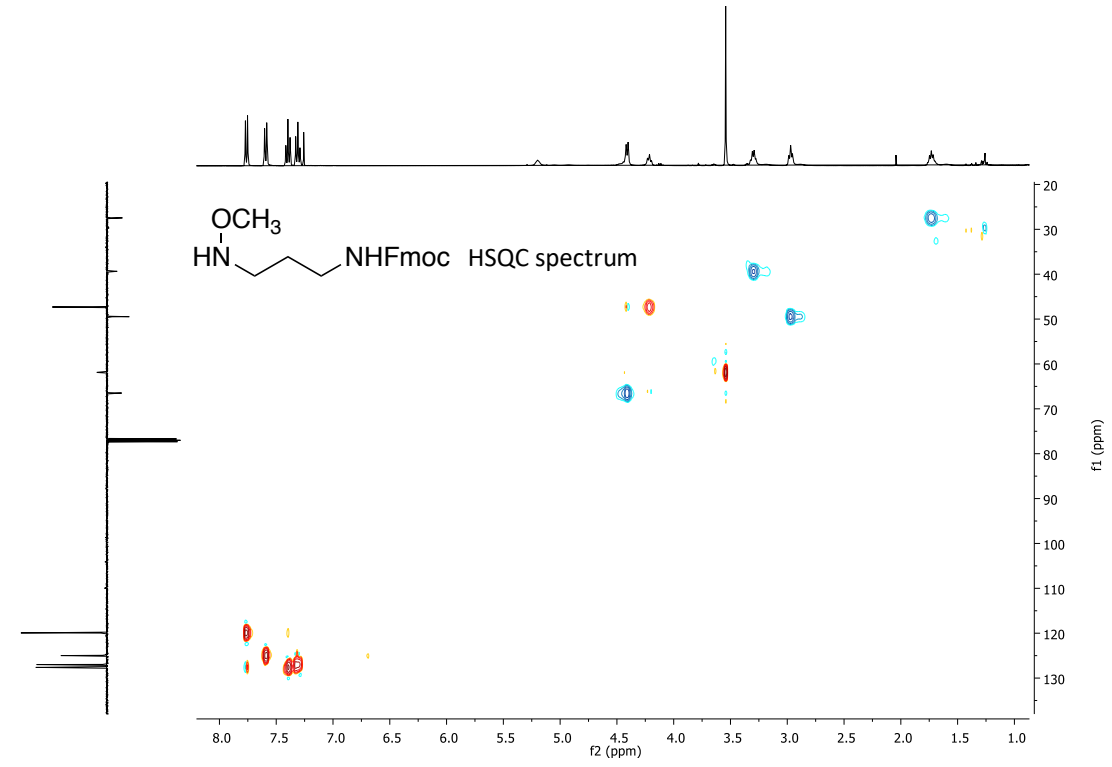




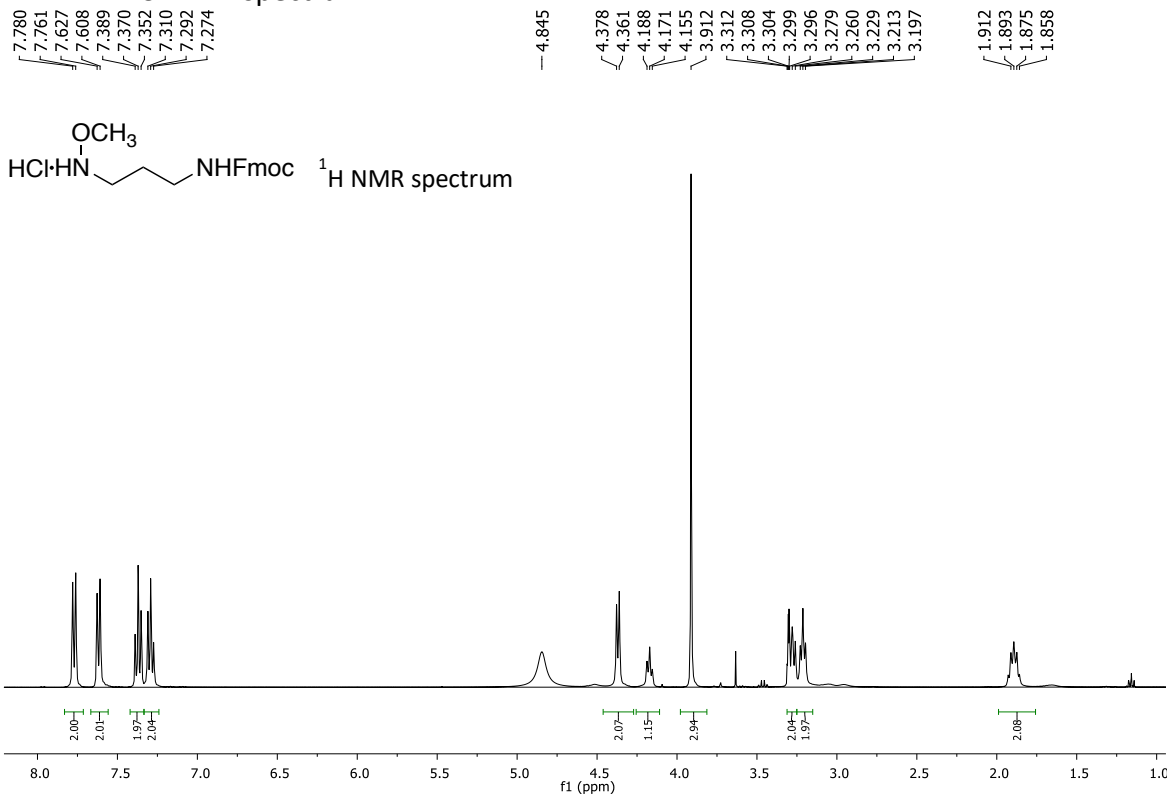
F-MAPA NMR spectra

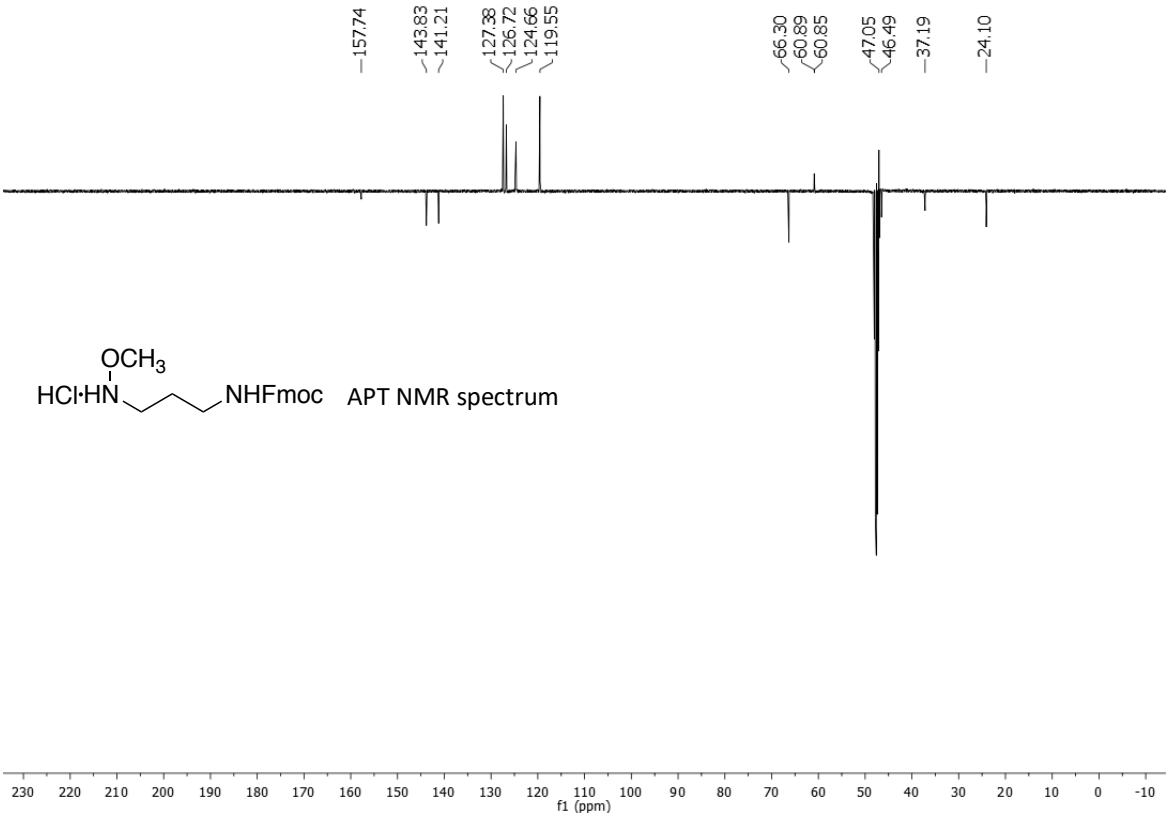



156.48
143.98
141.31
127.63
126.99
124.99
119.93
66.48
61.90
61.86
49.47
47.31
39.36
27.45
OCH3
HN
NHFmoc
APT NMR spectrum
f1 (ppm)

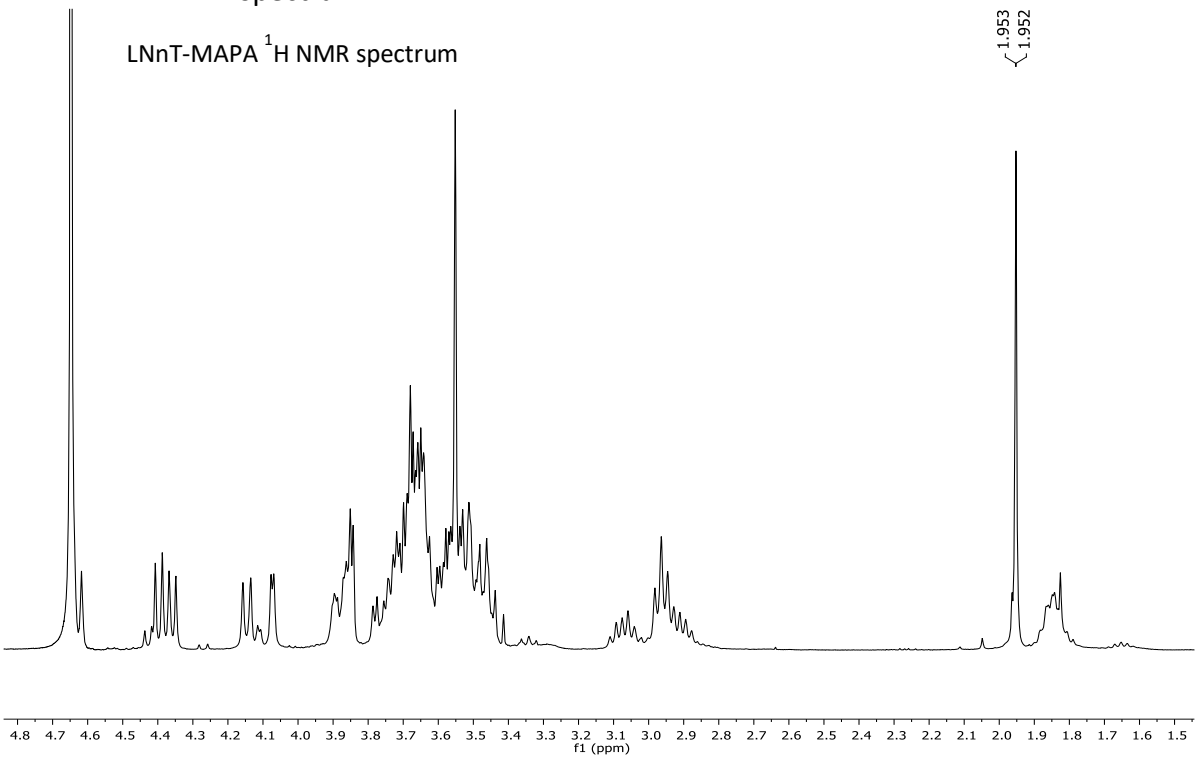

OCH3
HN
NHFmoc
HSQC spectrum
f2 (ppm)
f1 (ppm)

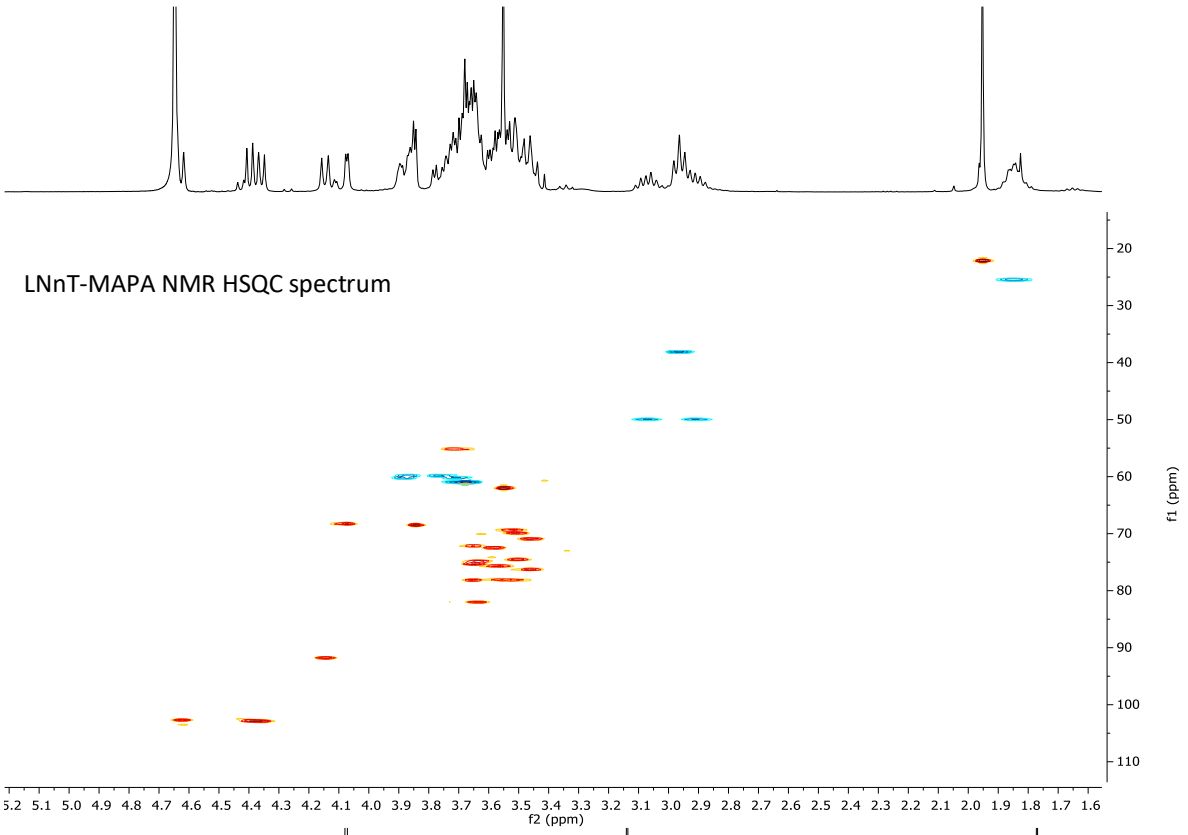
F-MAPA-HCl NMR spectra

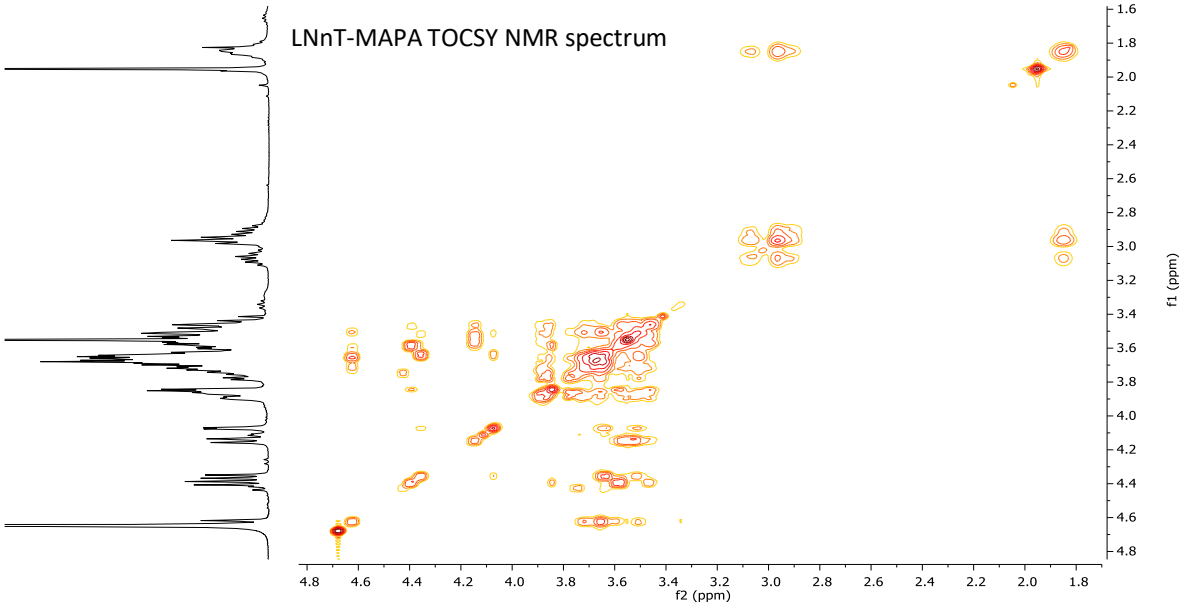


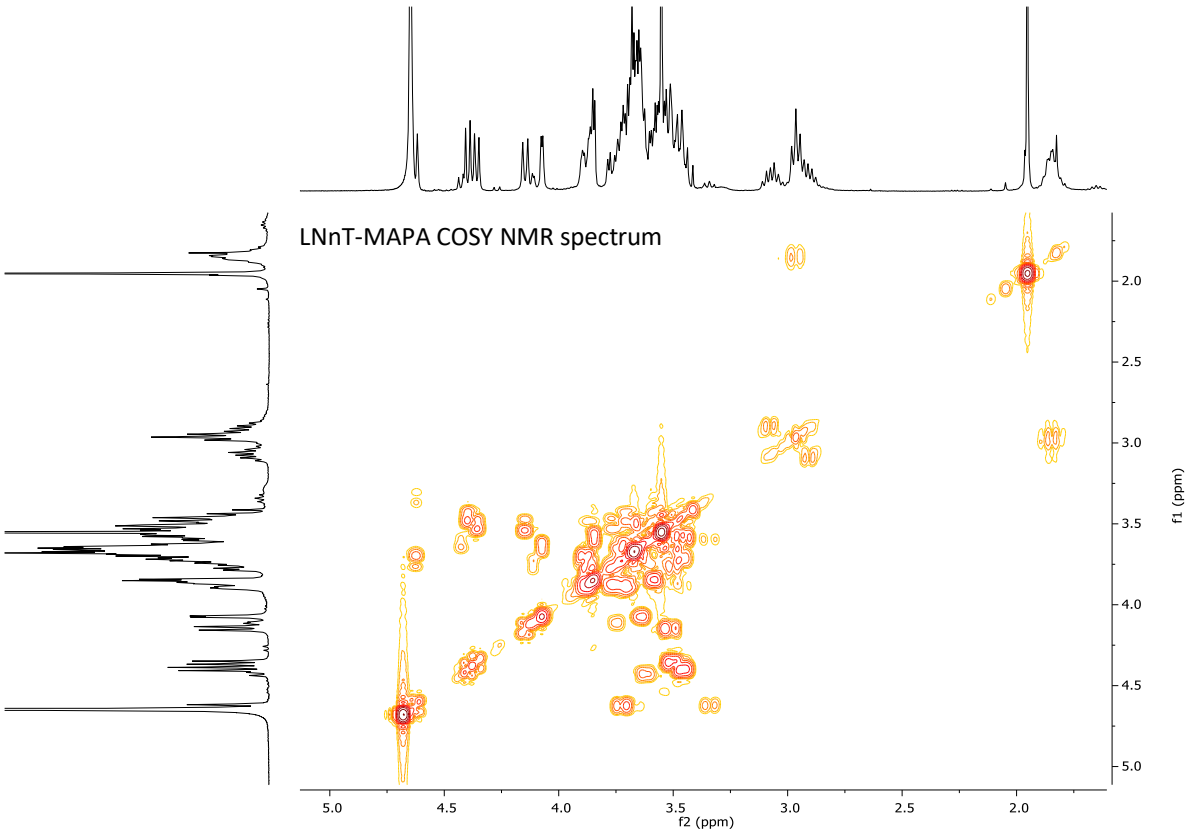


LNnT-MAPA NMR spectra

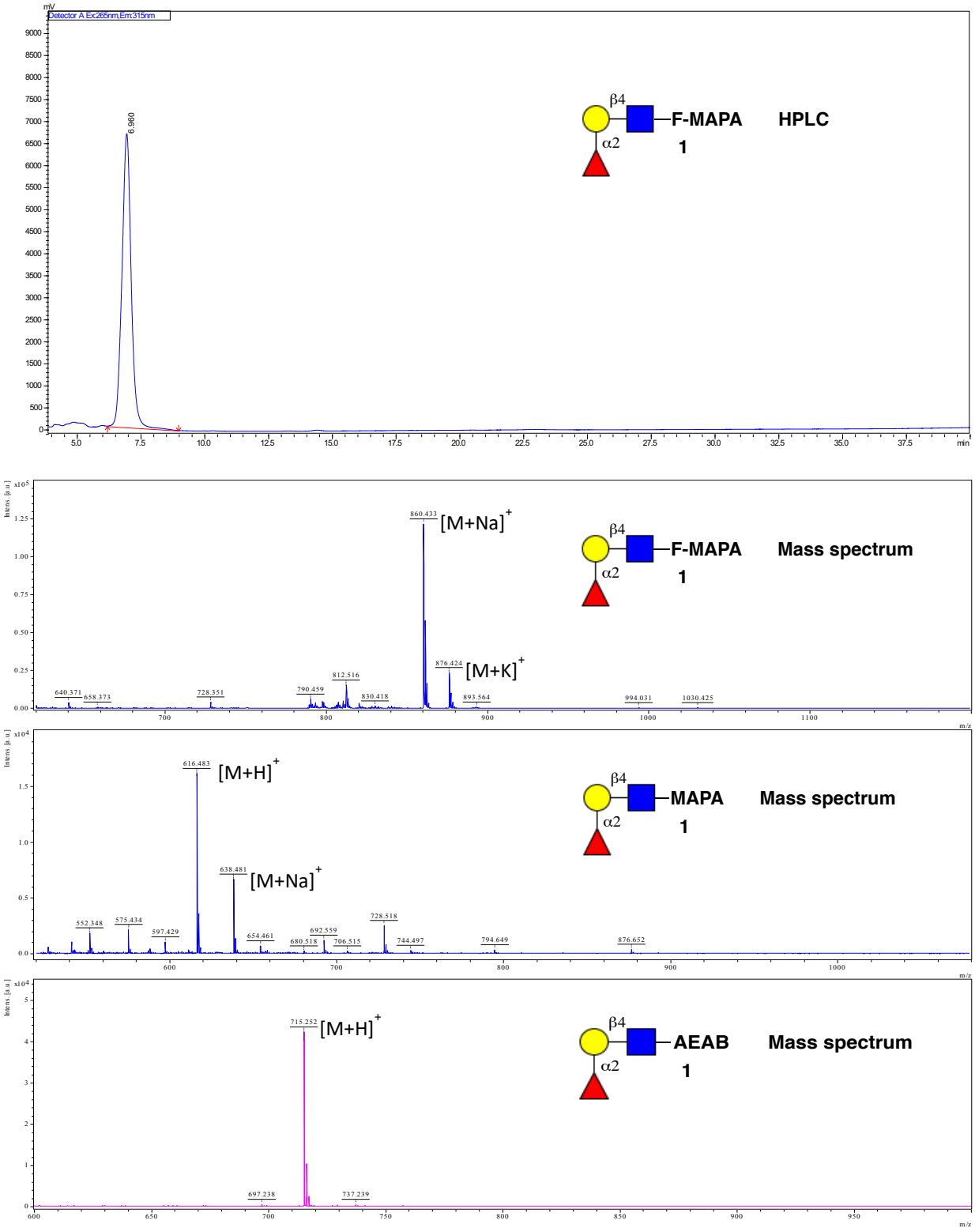


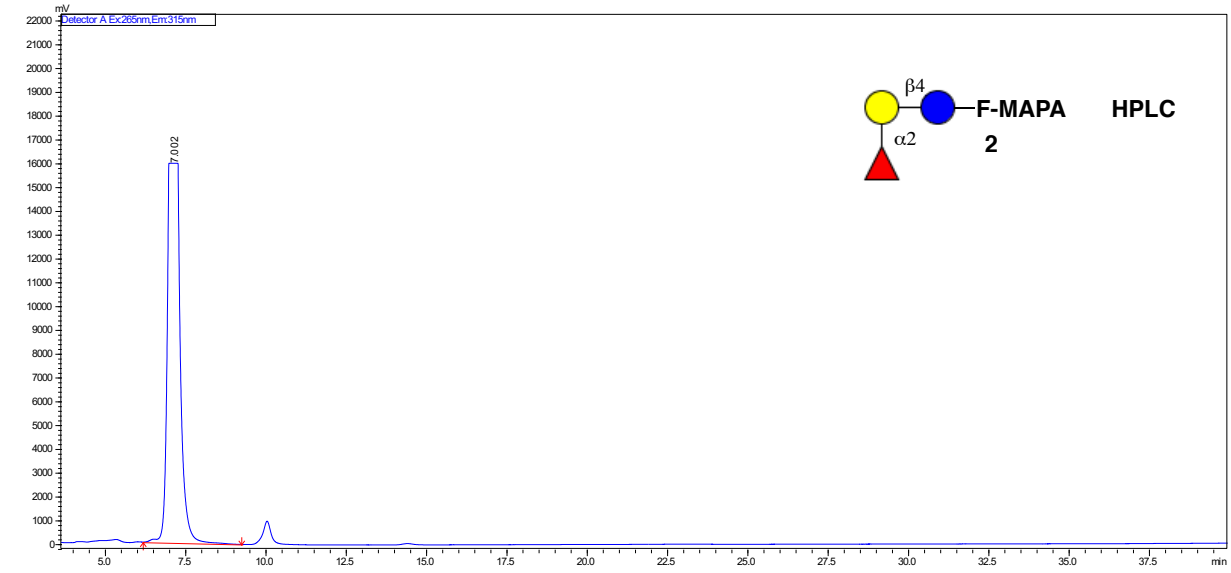

LNnT-MAPA NMR HSQC spectrum
f2 (ppm)
f1 (ppm)

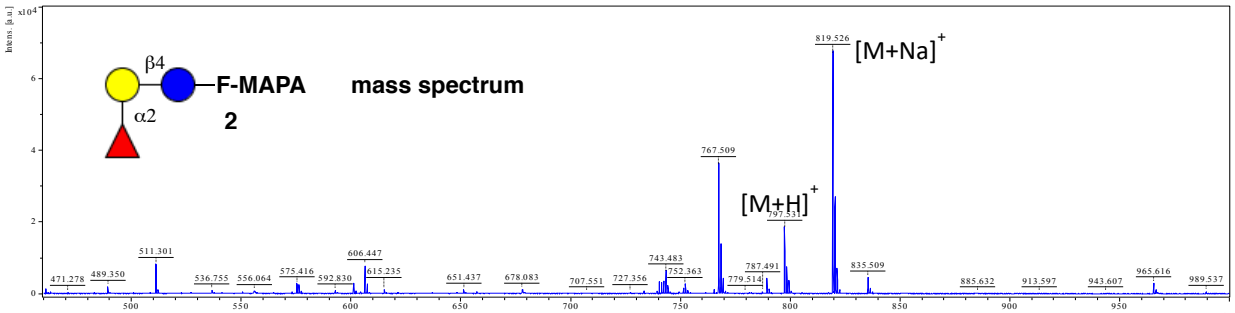

LNnT-MAPA TOCSY NMR spectrum
f2 (ppm)
f1 (ppm)

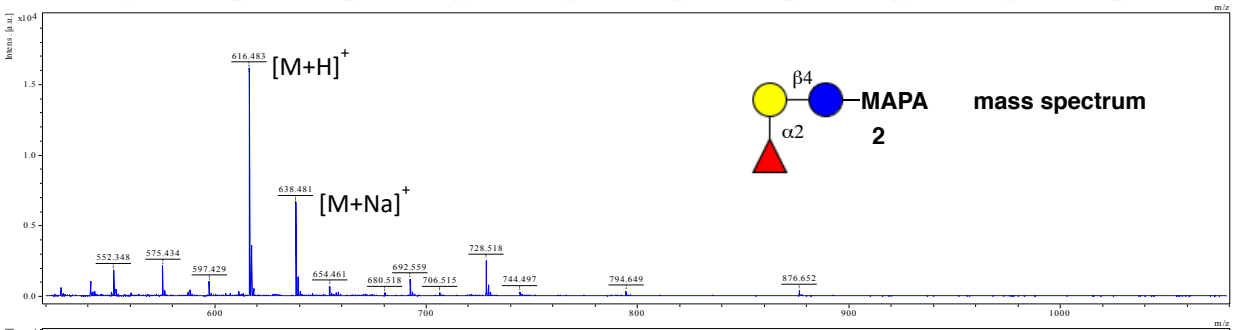

LNnT-MAPA COSY NMR spectrum
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
f1 (ppm)

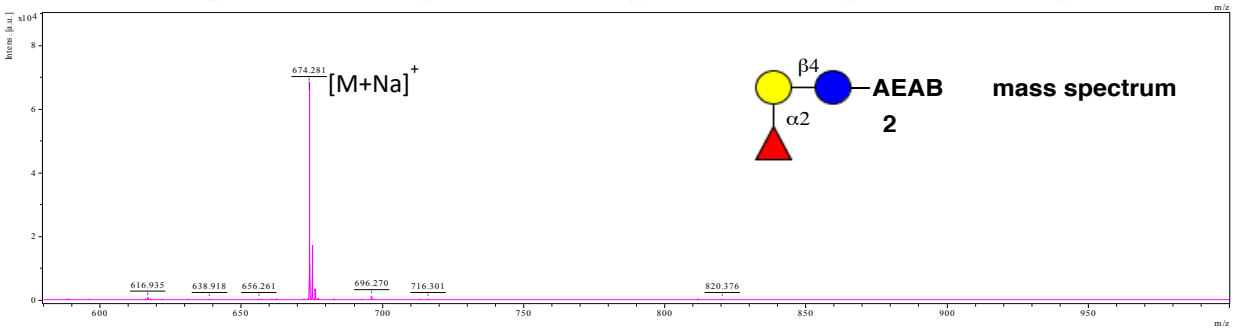
HPLC profiles of Glycan-F-MAPA, and Mass spectra of Glycan-F-MAPA, Glycan-MAPA and Glycan-AEAB.

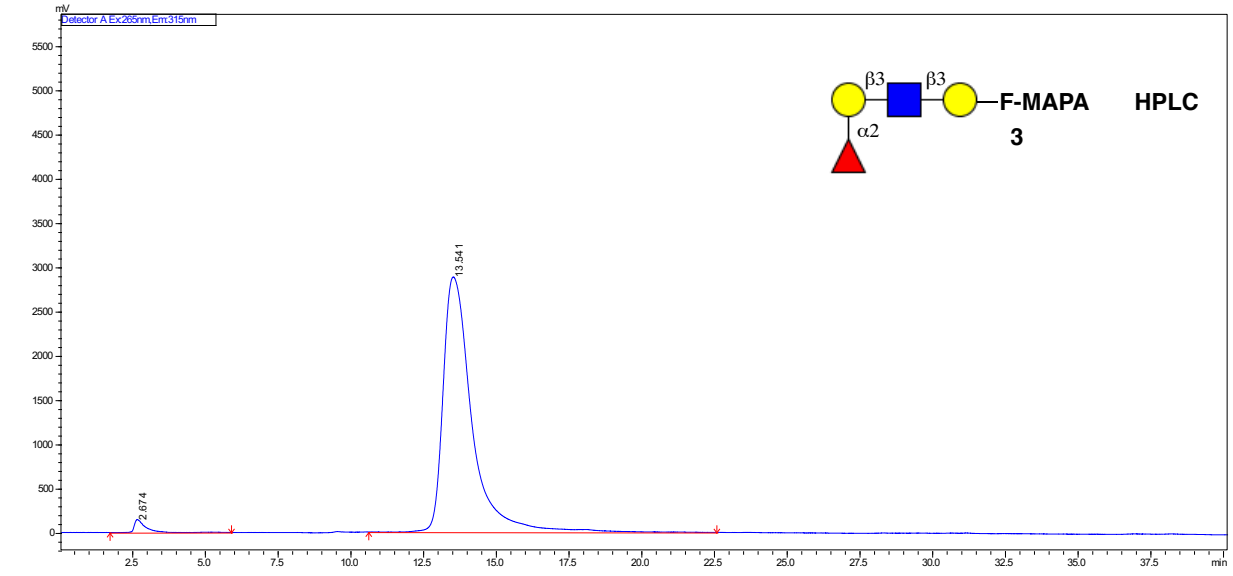


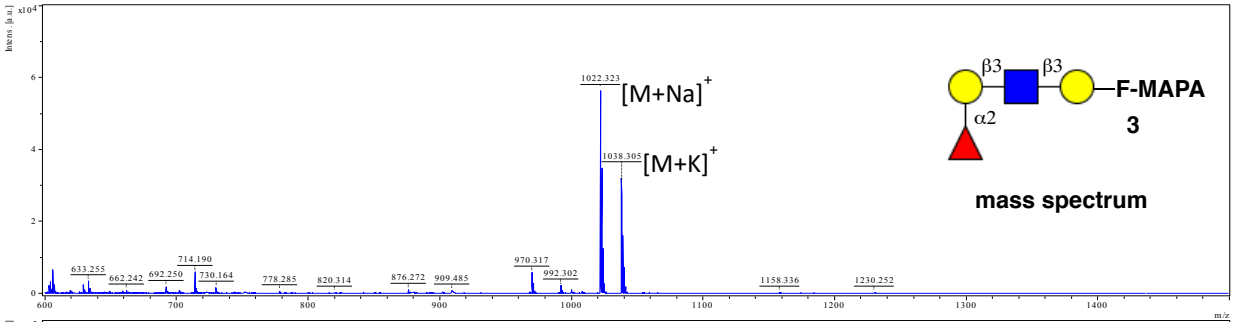

mV
Detector A Ex:265nm,Em:315nm
7.002
β4
α2
F-MAPA
2
HPLC
min

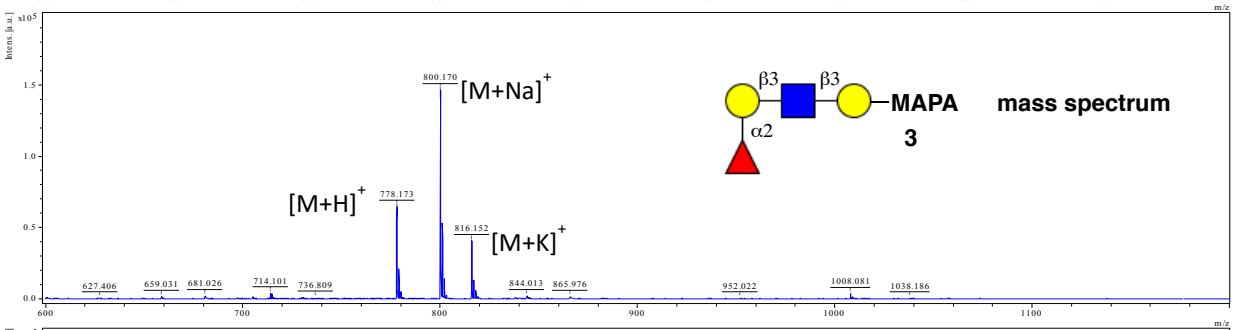

β4
α2
F-MAPA
2
mass spectrum
819.526 [M+Na]+
767.509
[M+H]+
511.301
606.447
743.483
787.491
835.509
m/z

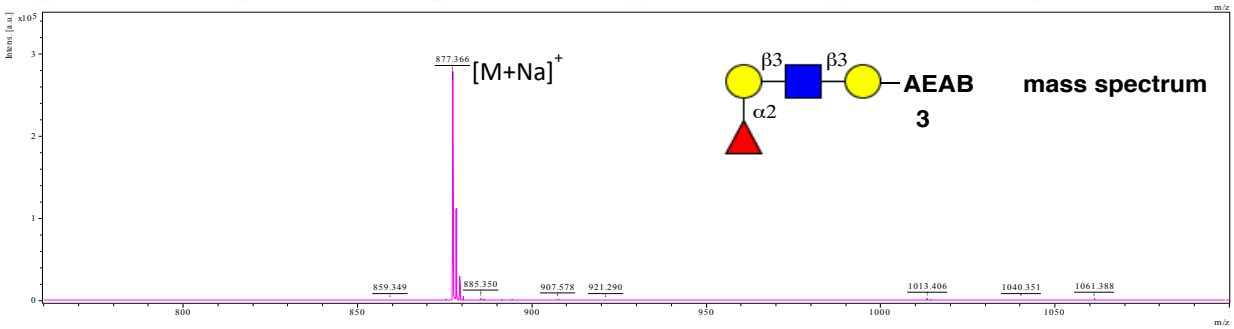

616.483 [M+H]+
638.481 [M+Na]+
β4
α2
MAPA
2
mass spectrum
552.348
575.434
597.429
654.461
692.559
680.518
706.515
728.518
744.497
794.649
876.652
m/z

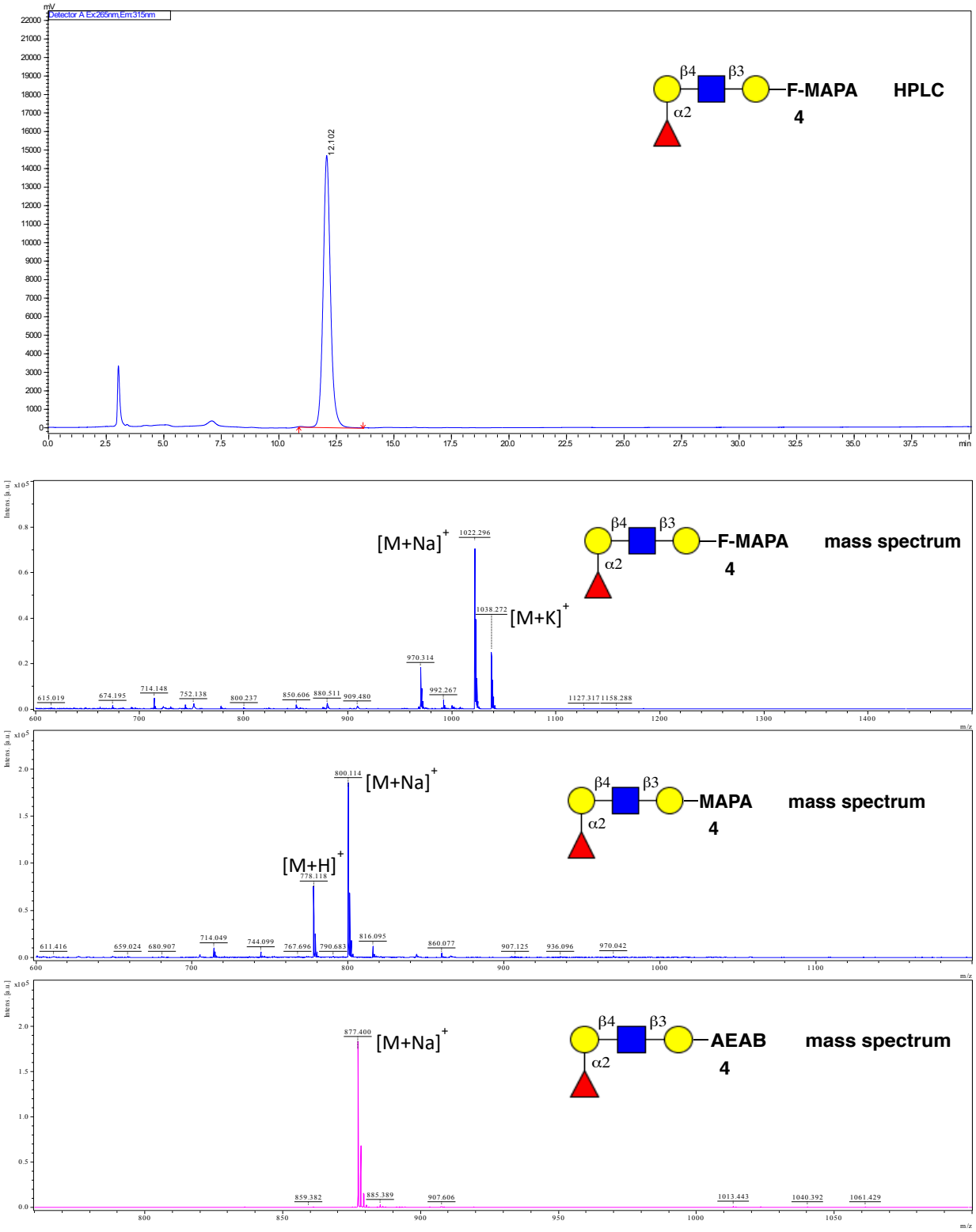

674.281 [M+Na]+
β4
α2
AEAB
2
mass spectrum
616.935
638.918
656.261
696.270
716.301
820.376
m/z

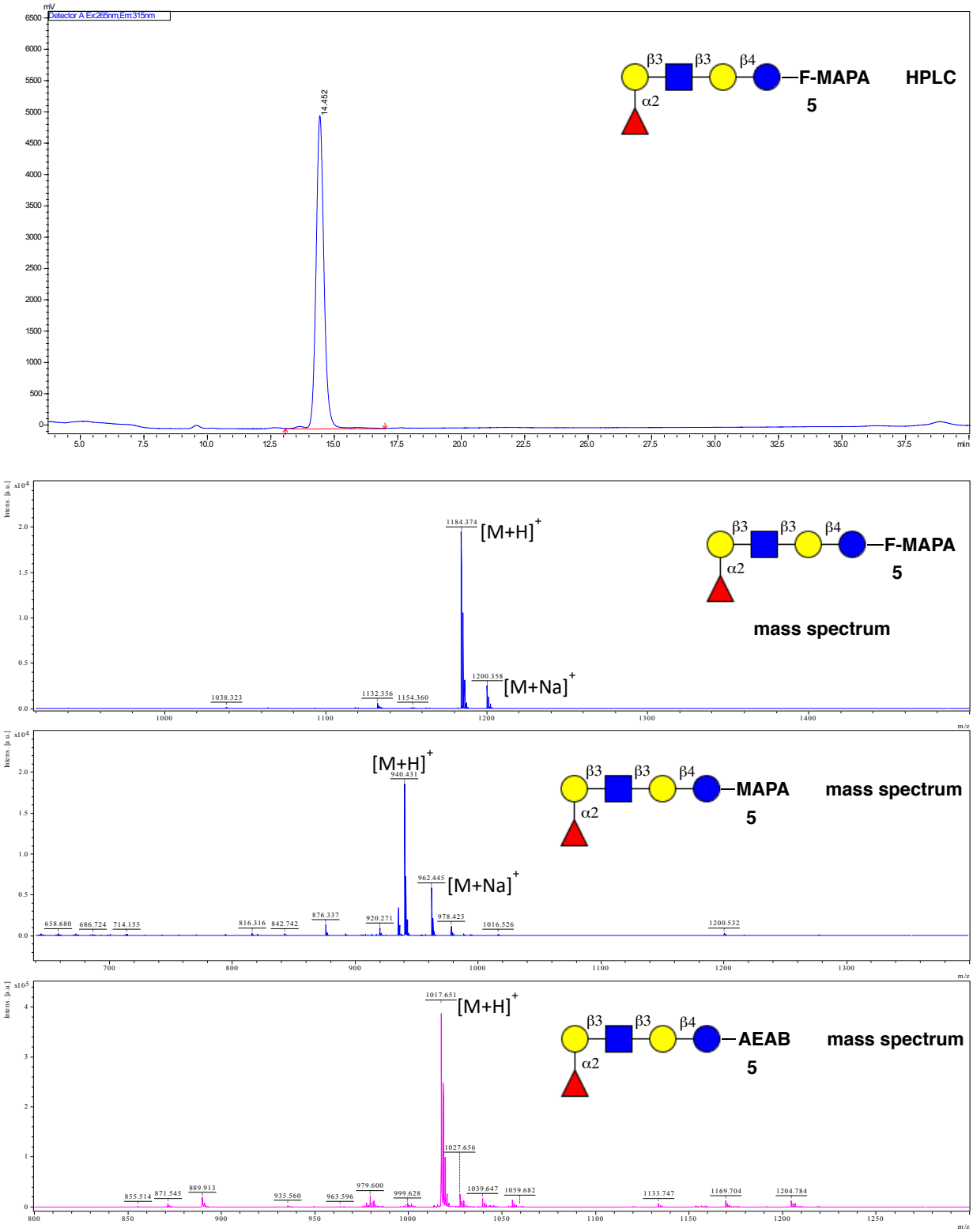

mV
Detector A Ex:265nm,Em:315nm
2.674
13.541
min
β3
β3
α2
F-MAPA
3
HPLC

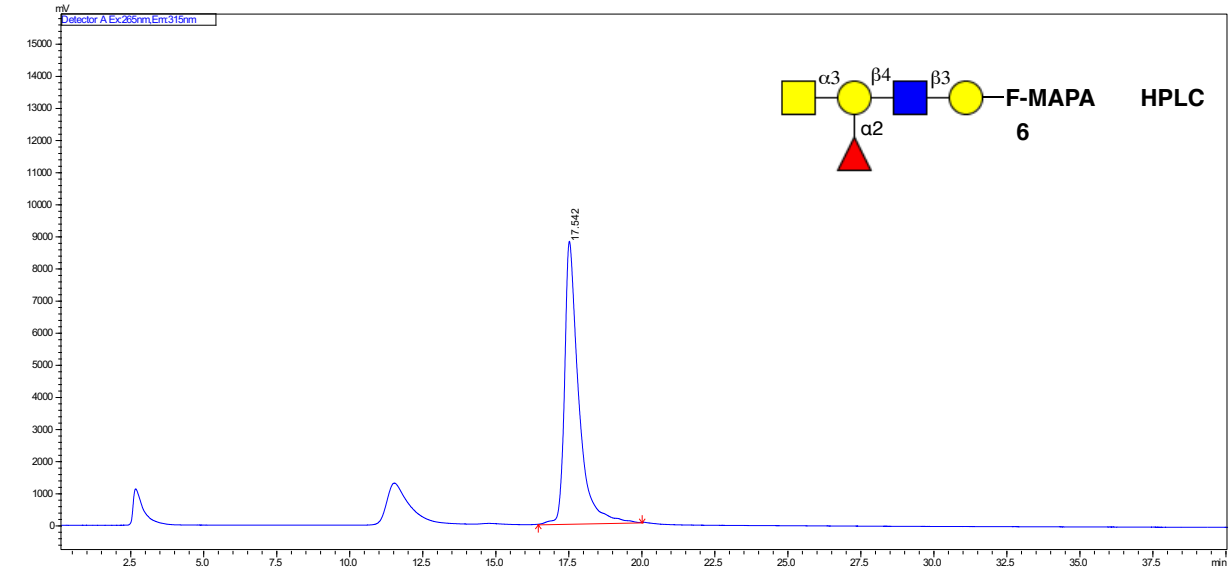

1022.323
[M+Na]+
1038.305
[M+K]+
β3
β3
α2
F-MAPA
3
mass spectrum
m/z

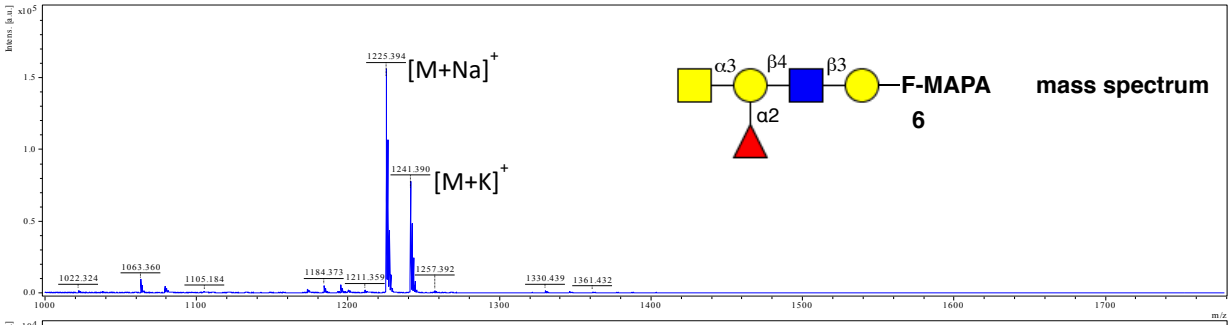

800.170
[M+Na]+
[M+H]+
778.173
816.152
[M+K]+
β3
β3
α2
MAPA
3
mass spectrum
m/z

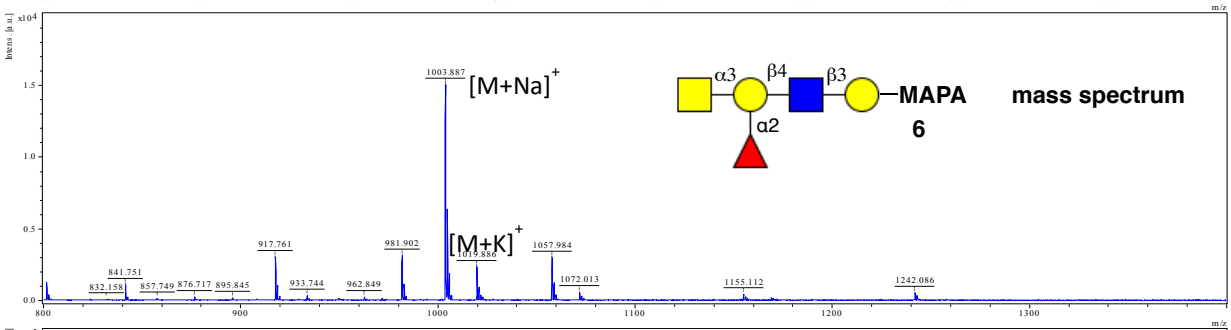

877.366
[M+Na]+
β3
β3
α2
AEAB
3
mass spectrum
m/z

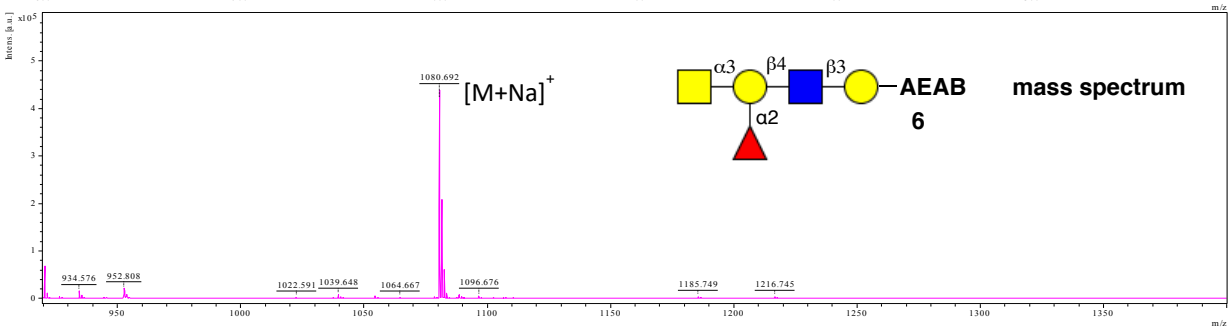

mV
Detector A Ex:265nm,Em:315nm
12.102
min
β4
β3
α2
F-MAPA
4
HPLC
[M+Na]+
1022.296
[M+K]+
1038.272
970.314
615.019
674.195
714.148
752.138
800.237
850.606
880.511
909.480
992.267
1127.317
1158.288
mass spectrum
[M+Na]+
800.114
[M+H]+
778.118
611.416
659.024
680.907
714.049
744.099
767.696
790.683
816.095
860.077
907.125
936.096
970.042
MAPA
[M+Na]+
877.400
859.382
885.389
907.606
1013.443
1040.392
1061.429
AEAB
Intens. [a.u.]
m/z

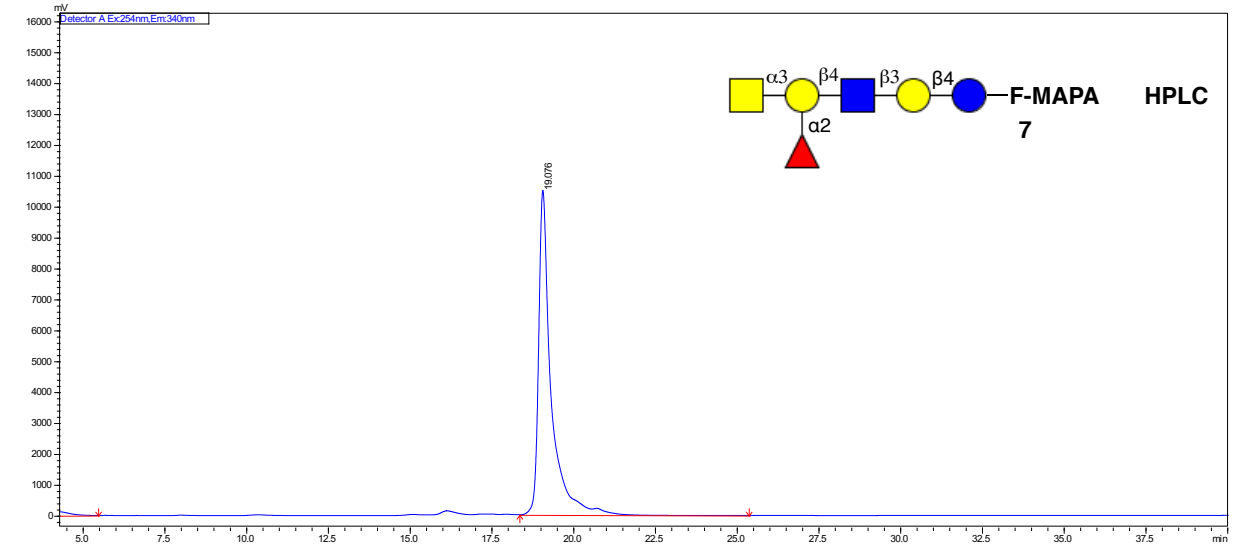

mV
Detector A Ex:265nm,Em:315nm
14.452
min
β3 β3 β4
α2
F-MAPA
5
HPLC
Intens. [a.u.]
1184.374
[M+H]+
1200.358
[M+Na]+
1038.323
1132.356
1154.360
mass spectrum
m/z
940.431
962.445
658.680
686.724
714.155
816.316
842.742
876.337
920.271
978.425
1016.526
1200.532
MAPA
1017.651
1027.656
855.514
871.545
889.913
935.560
963.596
979.600
999.628
1039.647
1059.682
1133.747
1169.704
1204.784
AEAB

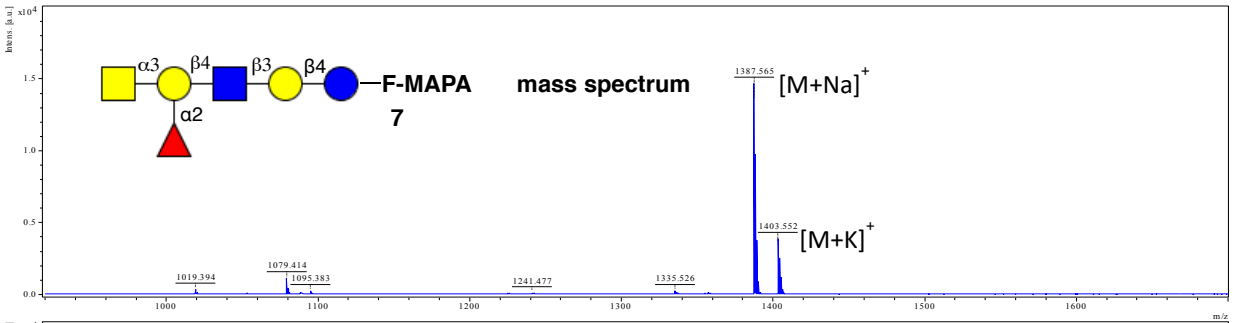

mV
Detector A Ex:265nm,Em:315nm
17.542
min
α3
β4
β3
α2
F-MAPA
6
HPLC

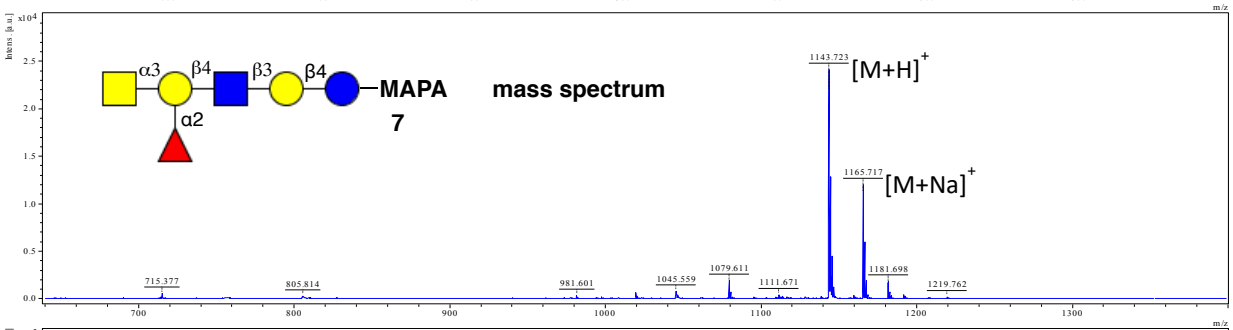

Intens. [a.u.]
1225.394
[M+Na]+
1241.390
[M+K]+
1022.324
1063.360
1105.184
1184.373
1211.359
1257.392
1330.439
1361.432
m/z
α3
β4
β3
α2
F-MAPA
6
mass spectrum

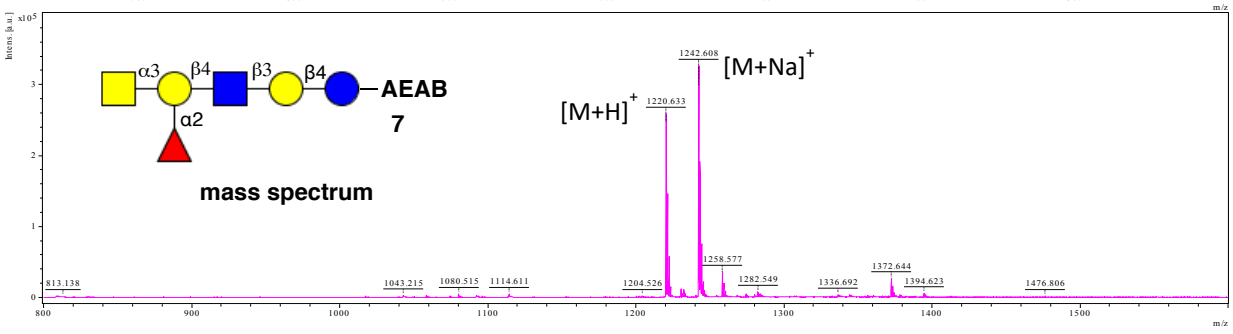

Intens. [a.u.]
1003.887
[M+Na]+
[M+K]+
841.751
832.158
857.749
876.717
895.845
917.761
933.744
962.849
981.902
1057.984
1072.013
1155.112
1242.086
m/z
α3
β4
β3
α2
MAPA
6
mass spectrum

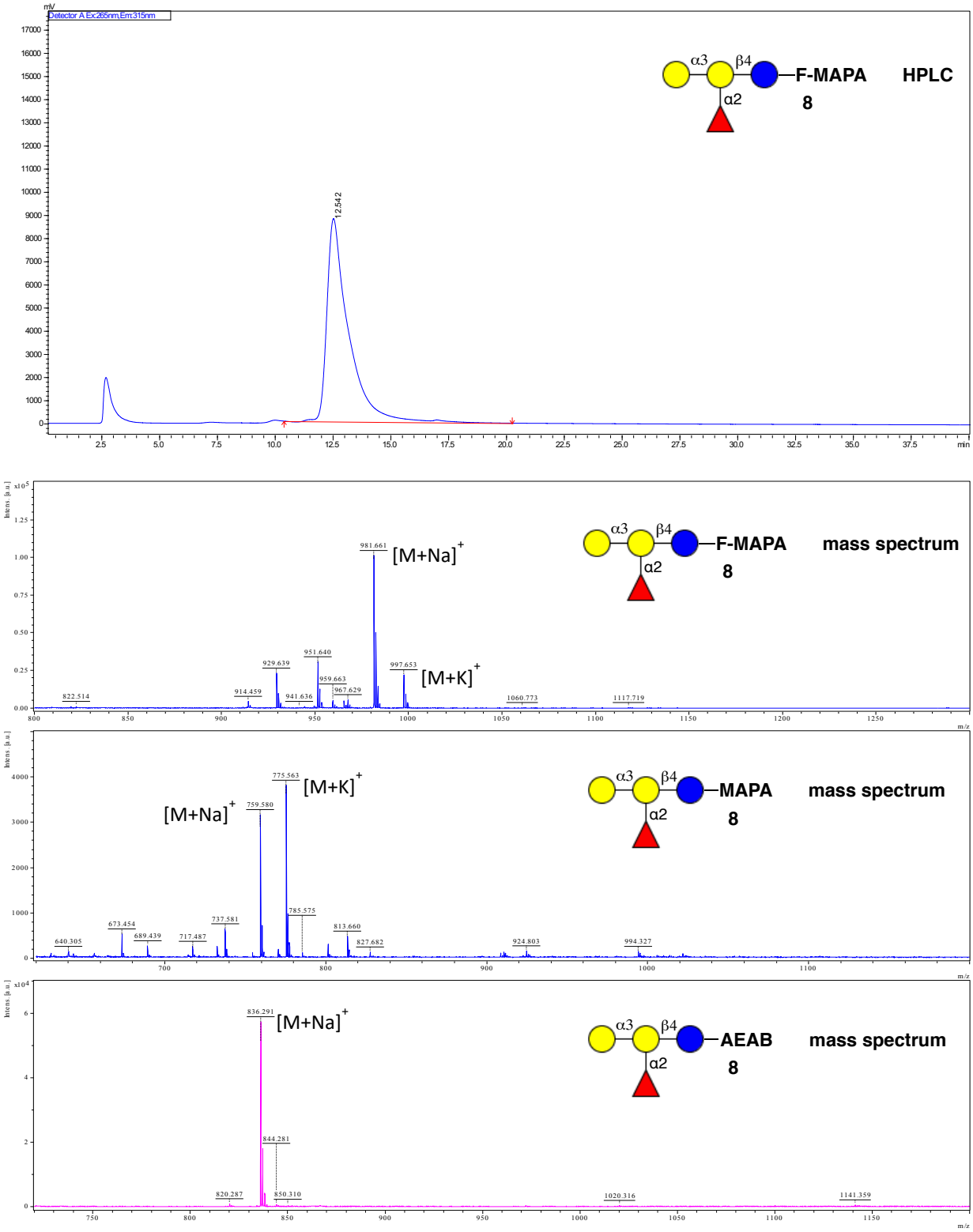

Intens. [a.u.]
1080.692
[M+Na]+
934.576
952.808
1022.591
1039.648
1064.667
1096.676
1185.749
1216.745
m/z
α3
β4
β3
α2
AEAB
6
mass spectrum

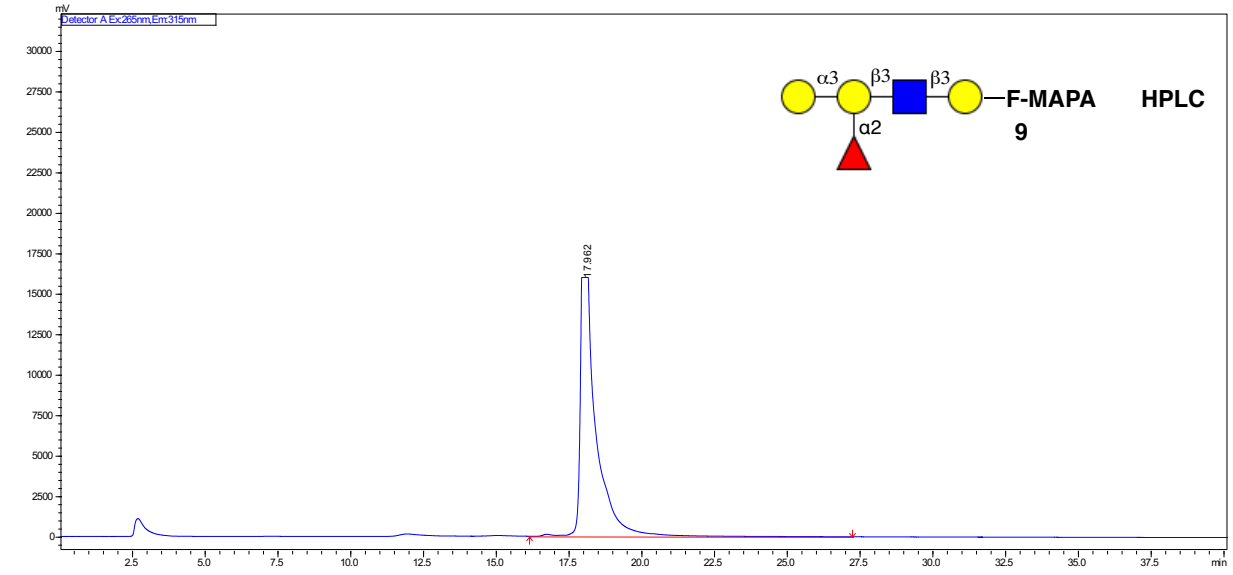

mV
Detector A Ex:254nm,Em:340nm
19.076
α3 β4 β3 β4
α2
F-MAPA
7
HPLC
min

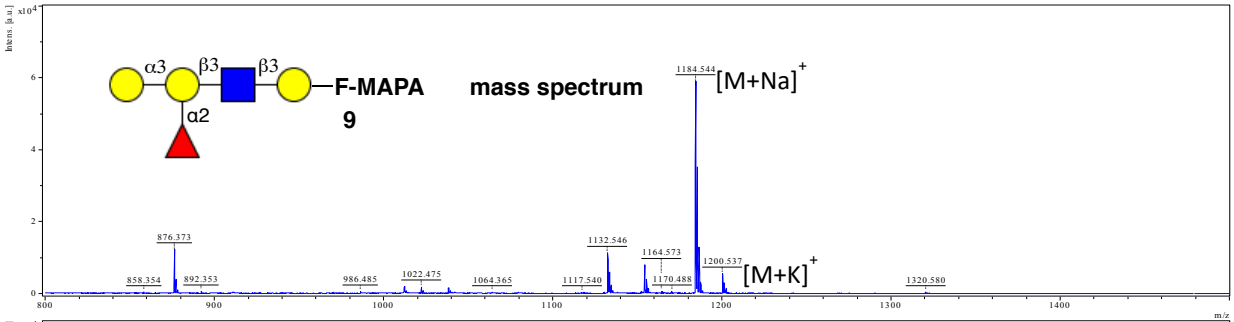

α3 β4 β3 β4
α2
F-MAPA
7
mass spectrum
1387.565 [M+Na]+
1403.552 [M+K]+
1019.394
1079.414
1095.383
1241.477
1335.526
m/z

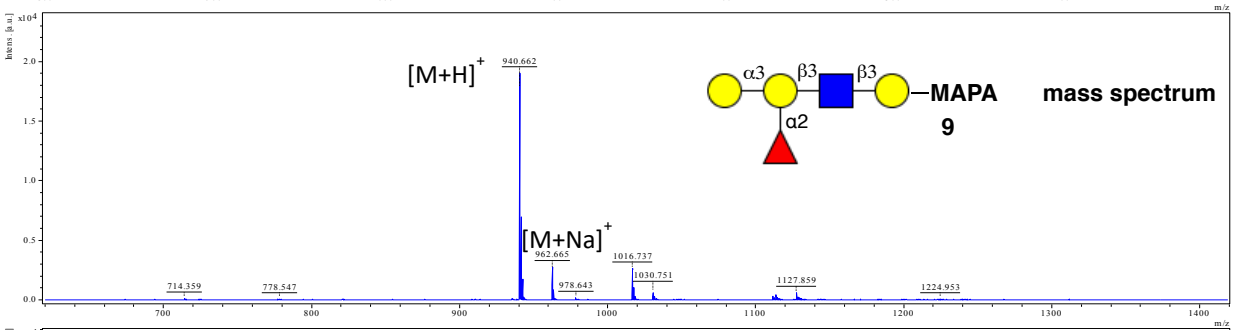

α3 β4 β3 β4
α2
MAPA
7
mass spectrum
1143.723 [M+H]+
1165.717 [M+Na]+
715.377
805.814
981.601
1045.559
1079.611
1111.671
1181.698
1219.762
m/z

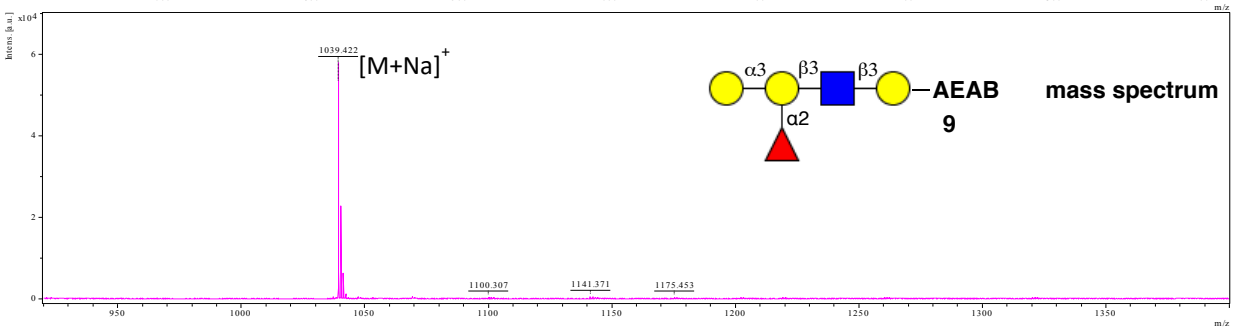

α3 β4 β3 β4
α2
AEAB
7
mass spectrum
1242.608 [M+Na]+
[M+H]+ 1220.633
1258.577
813.138
1043.215
1080.515
1114.611
1204.526
1282.549
1336.692
1372.644
1394.623
1476.806
m/z

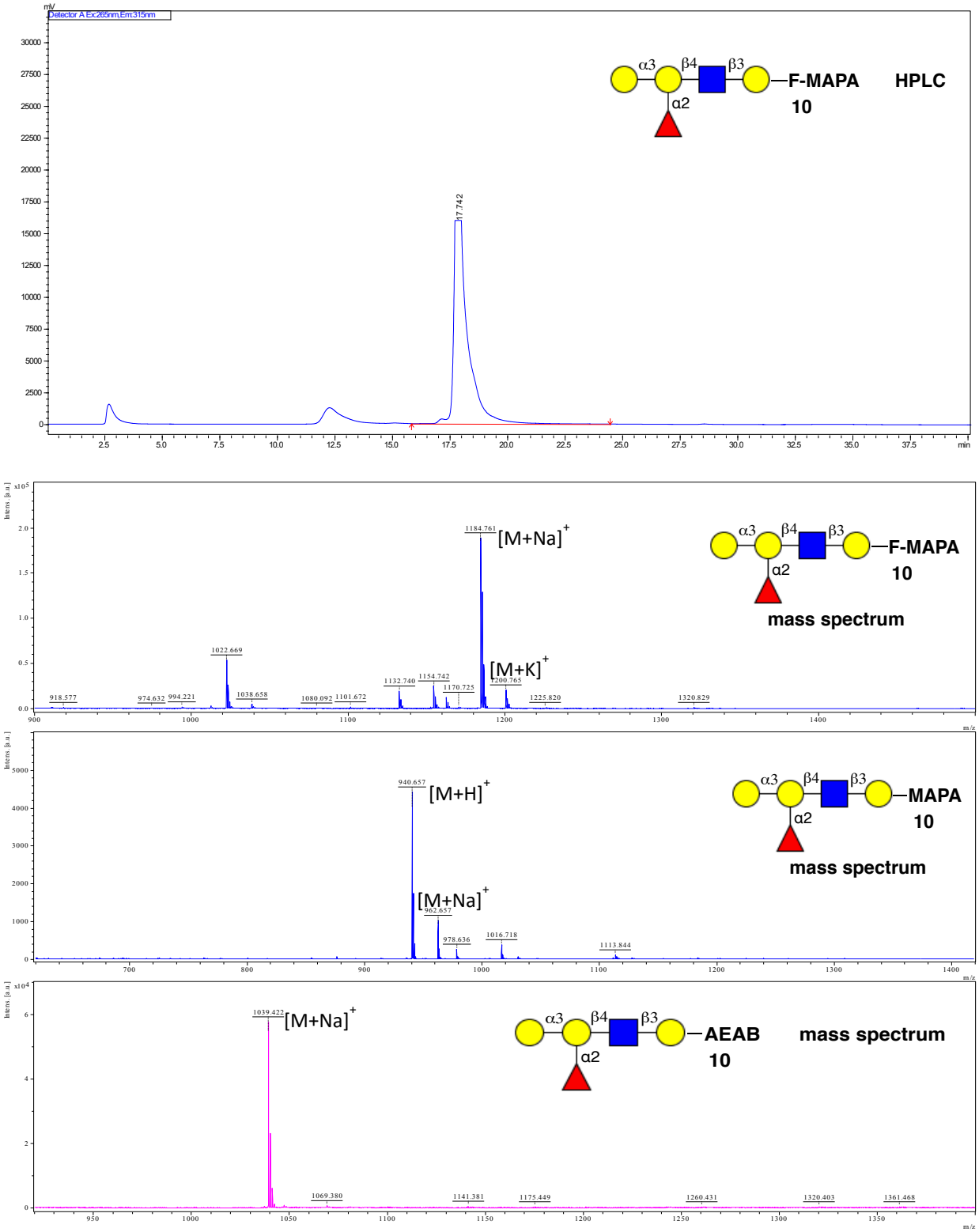

mV
Detector A Ex:265nm,Em:315nm
12.542
min
α3
β4
α2
F-MAPA
8
HPLC
981.661
[M+Na]+
997.653
[M+K]+
929.639
951.640
959.663
967.629
914.459
941.636
822.514
1060.773
1117.719
mass spectrum
F-MAPA
8
m/z
775.563
[M+K]+
[M+Na]+
759.580
785.575
673.454
689.439
737.581
717.487
813.660
827.682
640.305
924.803
994.327
MAPA
8
836.291
[M+Na]+
844.281
820.287
850.310
1020.316
1141.359
AEAB
8

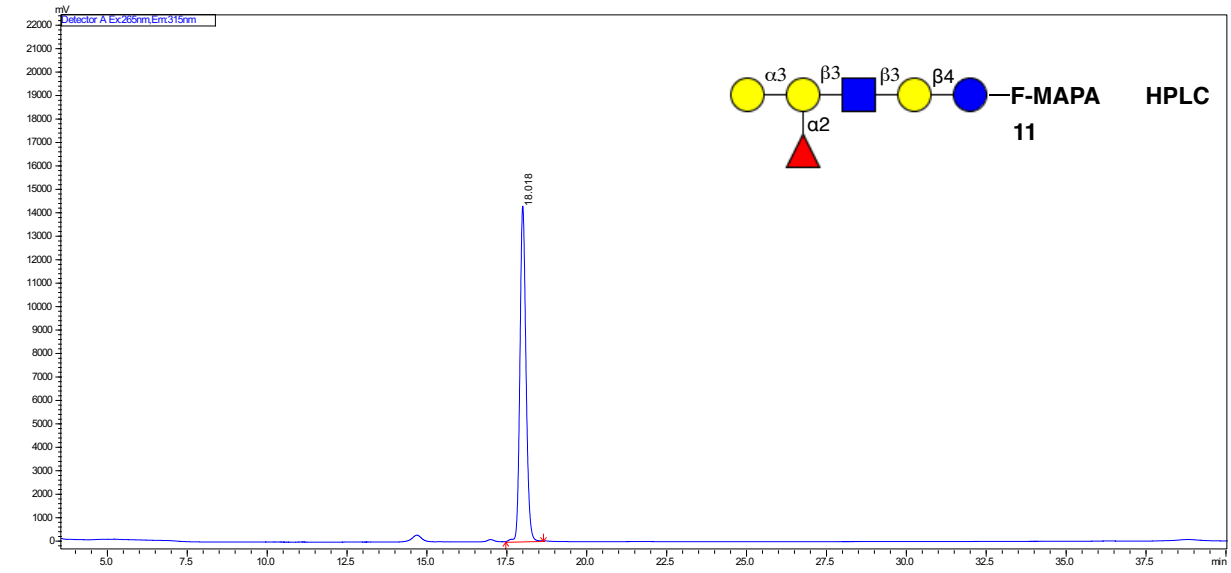

mV
Detector A Ex:265nm,Em:315nm
17.962
min
α3 β3 β3
α2
F-MAPA
9
HPLC

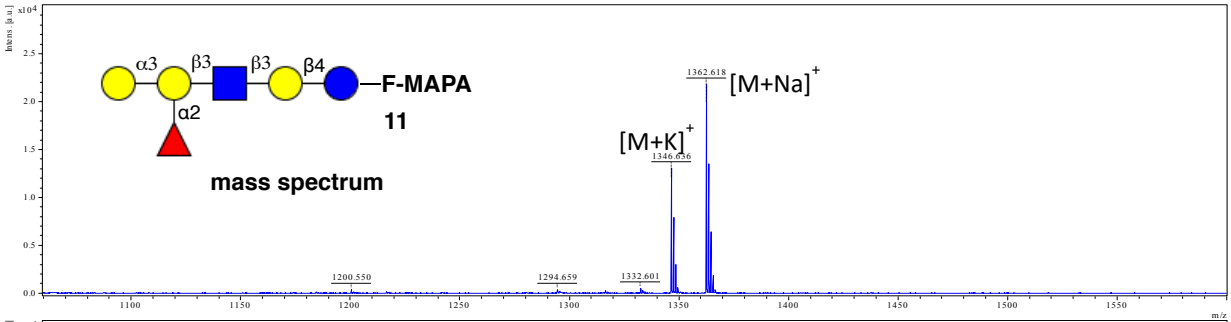

α3 β3 β3
α2
F-MAPA
9
mass spectrum
1184.544 [M+Na]+
[M+K]+
876.373
858.354
892.353
986.485
1022.475
1064.365
1117.540
1132.546
1164.573
1170.488
1200.537
1320.580
m/z

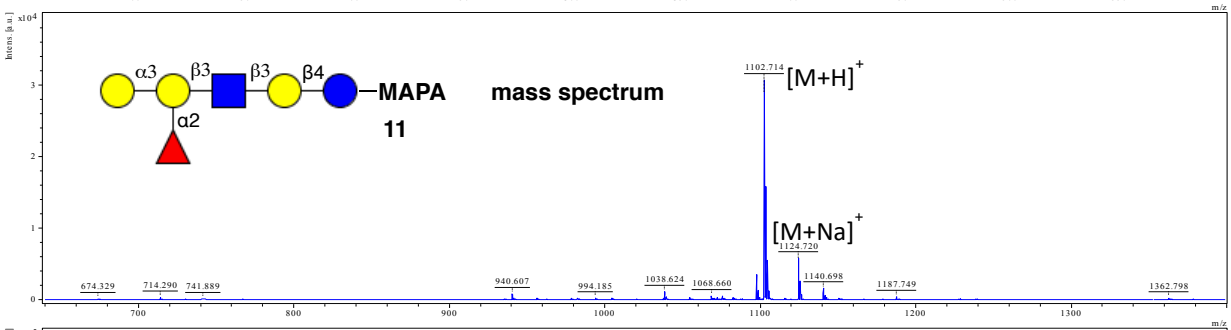

[M+H]+
940.662
[M+Na]+
962.665
α3 β3 β3
α2
MAPA
9
mass spectrum
714.359
778.547
978.643
1016.737
1030.751
1127.859
1224.953
m/z

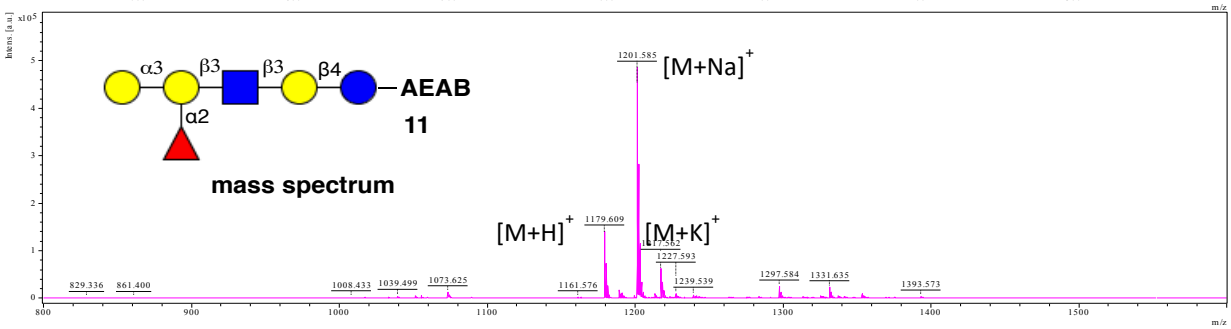

1039.422
[M+Na]+
α3 β3 β3
α2
AEAB
9
mass spectrum
1100.307
1141.371
1175.453
m/z

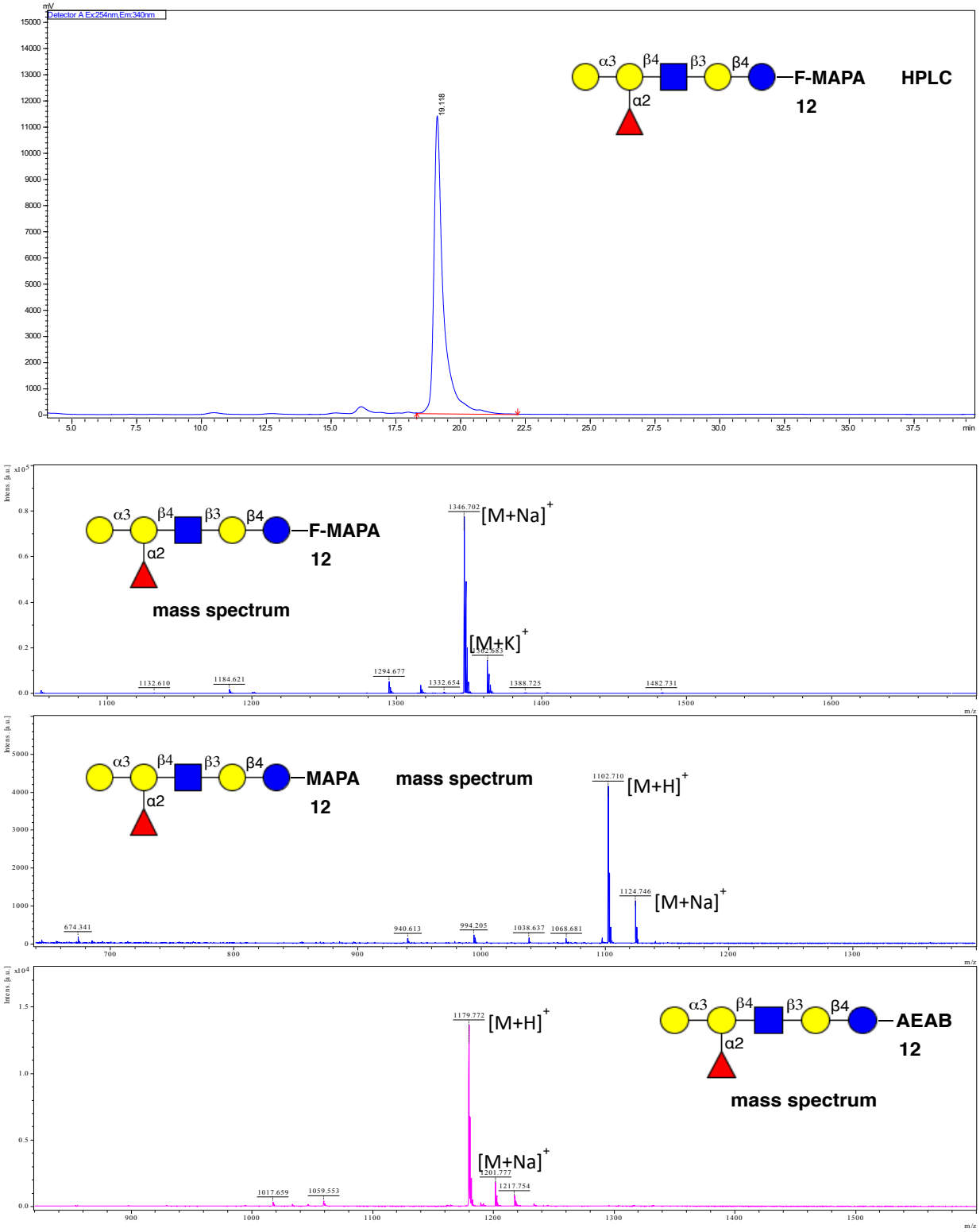

mV
Detector A Ex:265nm,Em:315nm
17.742
min
α3
β4
β3
α2
F-MAPA
10
HPLC
[M+Na]+
[M+K]+
mass spectrum
[M+H]+
MAPA
AEAB

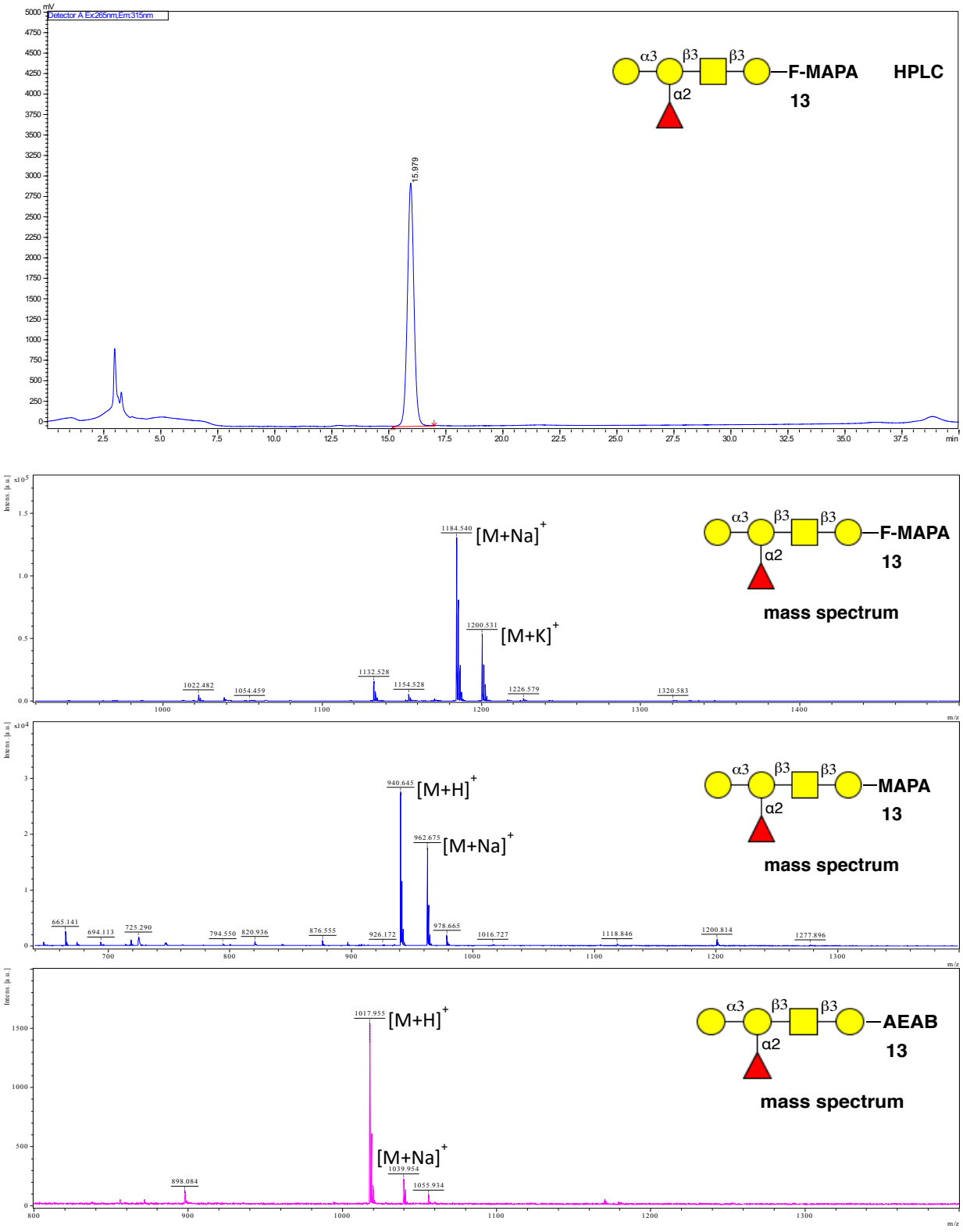

mV
Detector A Ex:265nm,Em:315nm
18.018
α3 β3 β3 β4
α2
F-MAPA
11
HPLC
min

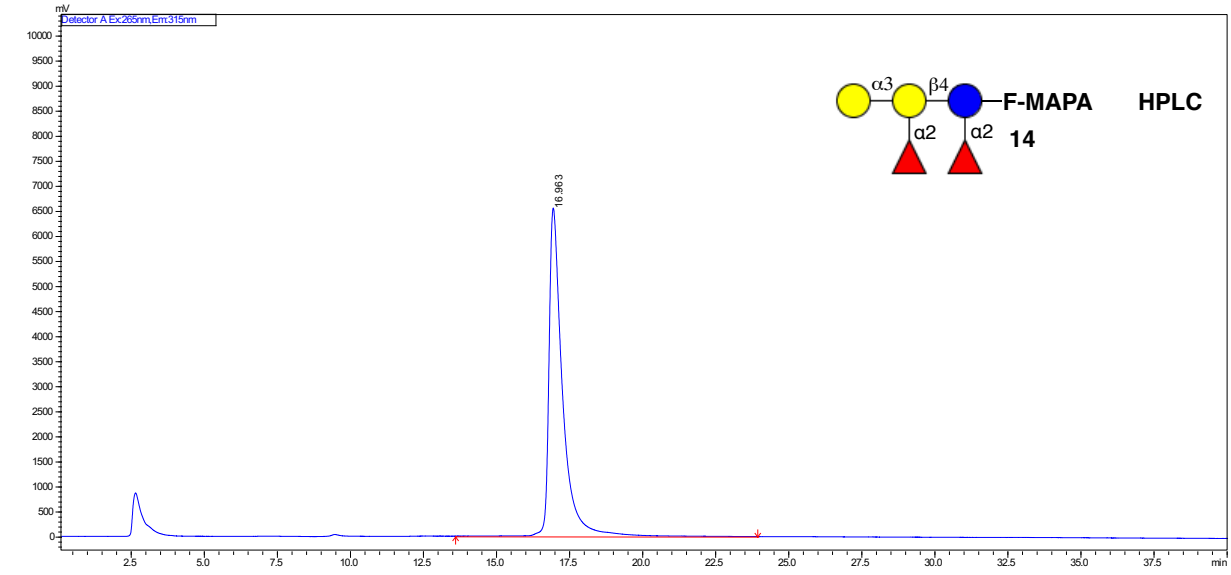

α3 β3 β3 β4
α2
F-MAPA
11
mass spectrum
1362.618 [M+Na]+
[M+K]+
1346.636
1200.550
1294.659
1332.601
Intens. [a.u.]
m/z

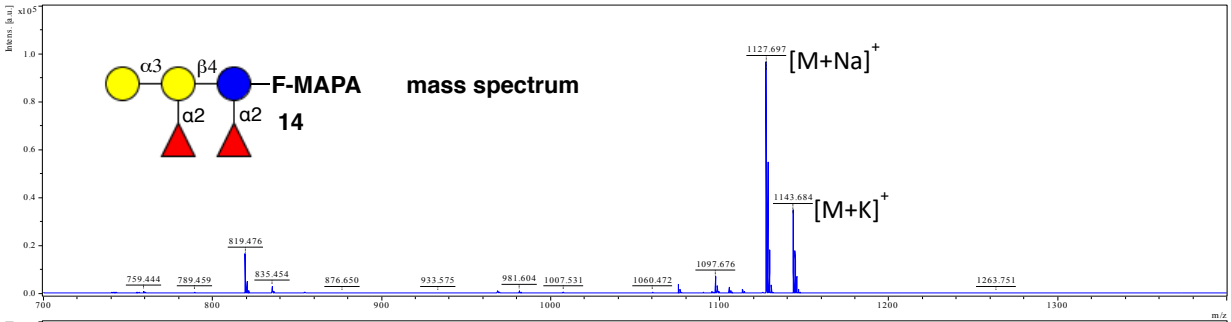

α3 β3 β3 β4
α2
MAPA
11
mass spectrum
1102.714 [M+H]+
[M+Na]+
1124.720
674.329
714.290
741.889
940.607
994.185
1038.624
1068.660
1140.698
1187.749
1362.798
Intens. [a.u.]
m/z

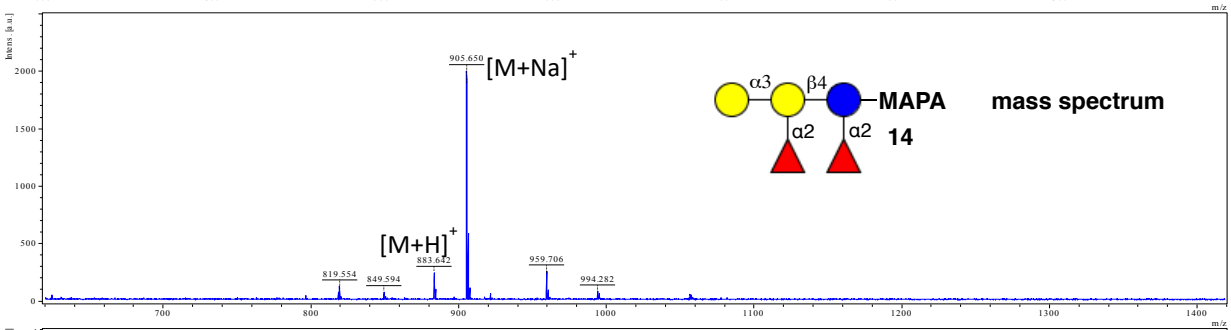

α3 β3 β3 β4
α2
AEAB
11
mass spectrum
1201.585 [M+Na]+
[M+H]+
1179.609
[M+K]+
1177.562
1227.593
829.336
861.400
1008.433
1039.499
1073.625
1161.576
1239.539
1297.584
1331.635
1393.573
Intens. [a.u.]
m/z

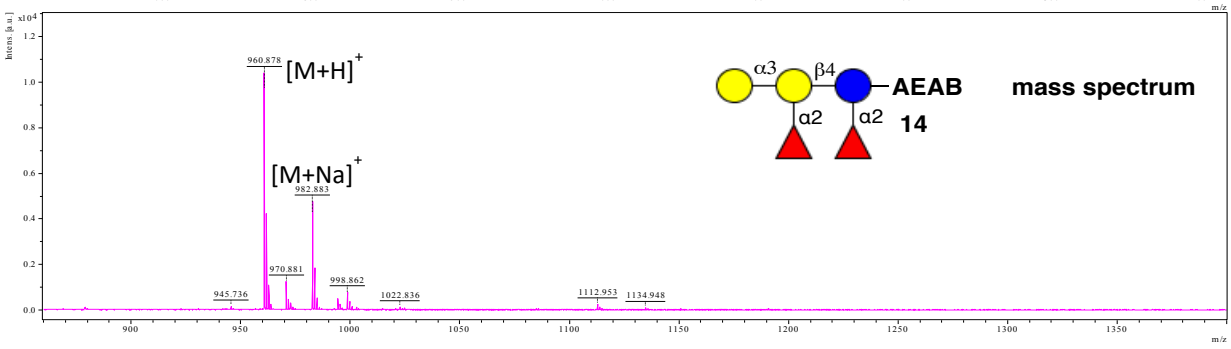

Detector A Ex:254nm,Em:340nm
19.118
α3 β4 β3 β4
α2
F-MAPA
12
HPLC
mV
min
mass spectrum
1346.702 [M+Na]+
[M+K]+
1132.610
1184.621
1294.677
1332.654
1388.725
1482.731
MAPA
12
1102.710 [M+H]+
1124.746 [M+Na]+
674.341
940.613
994.205
1038.637
1068.681
AEAB
1179.772 [M+H]+
[M+Na]+
1201.777
1217.754
1017.659
1059.553
m/z
Intens. [a.u.]

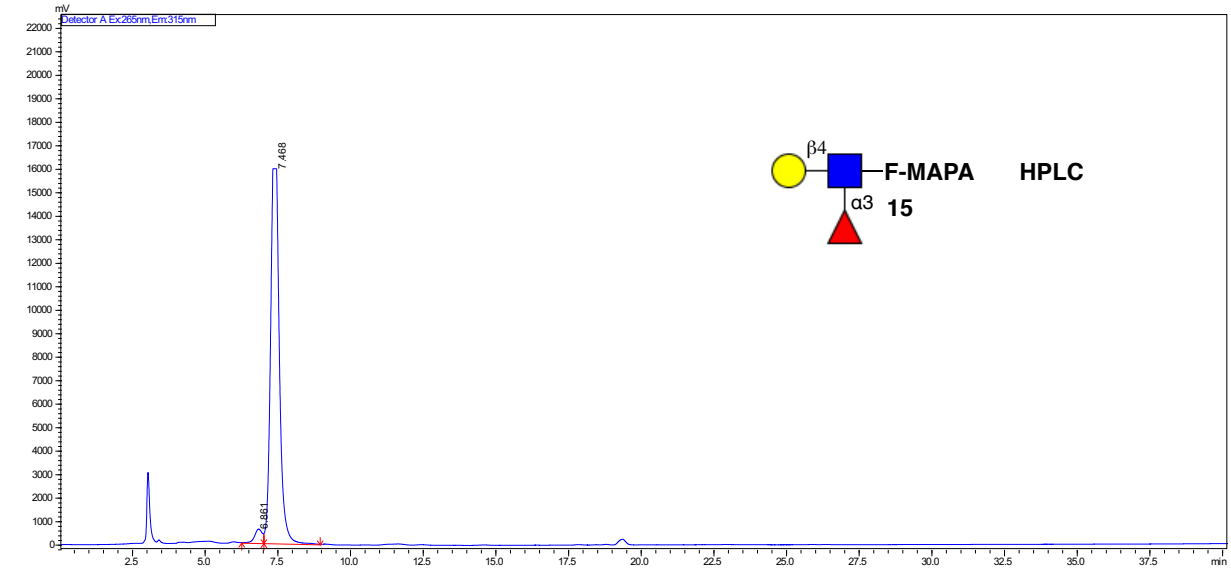

mV
Detector A Ex:265nm,Em:315nm
15.979
min
α3 β3 β3
α2
F-MAPA
13
HPLC
Intens. [a.u.]
1184.540 [M+Na]+
1200.531 [M+K]+
1022.482
1054.459
1132.528
1154.528
1226.579
1320.583
m/z
F-MAPA
13
mass spectrum
940.645 [M+H]+
962.675 [M+Na]+
665.141
694.113
725.290
794.550
820.936
876.555
926.172
978.665
1016.727
1118.846
1200.814
1277.896
MAPA
13
mass spectrum
1017.955 [M+H]+
[M+Na]+
898.084
1039.954
1055.934
AEAB
13
mass spectrum

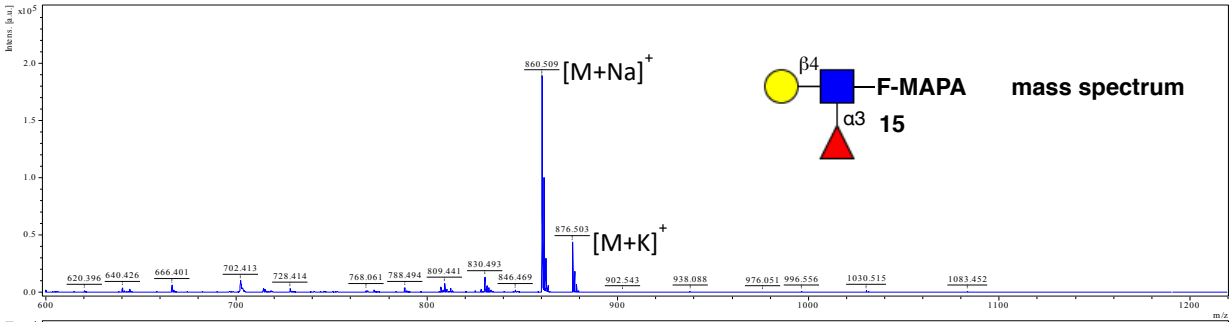

mV
Detector A Ex:265nm,Em:315nm
16.963
α3
β4
α2
α2
F-MAPA
14
HPLC
min

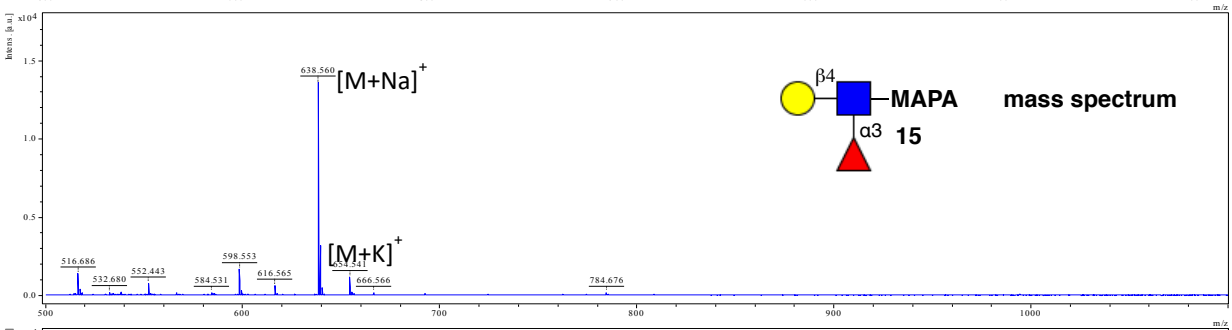

α3
β4
α2
α2
F-MAPA
14
mass spectrum
1127.697
[M+Na]+
1143.684
[M+K]+
819.476
759.444
789.459
835.454
876.650
933.575
981.604
1007.531
1060.472
1097.676
1263.751
m/z

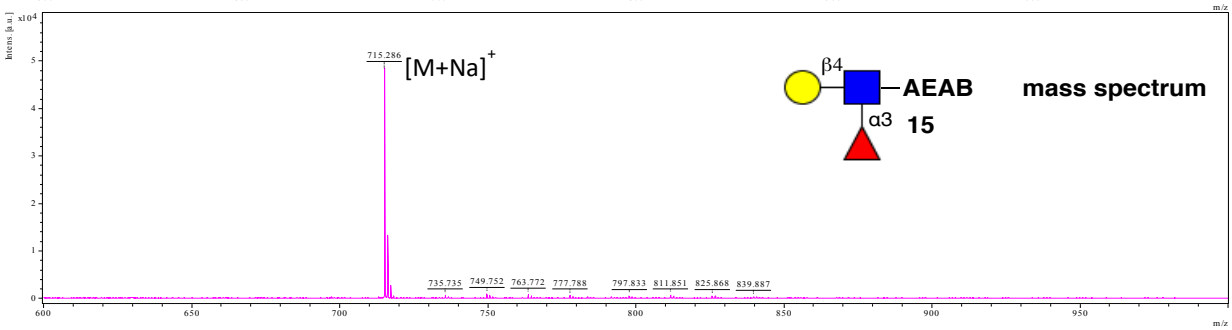

905.650
[M+Na]+
[M+H]+
883.642
819.554
849.594
959.706
994.282
α3
β4
α2
α2
MAPA
14
mass spectrum
m/z

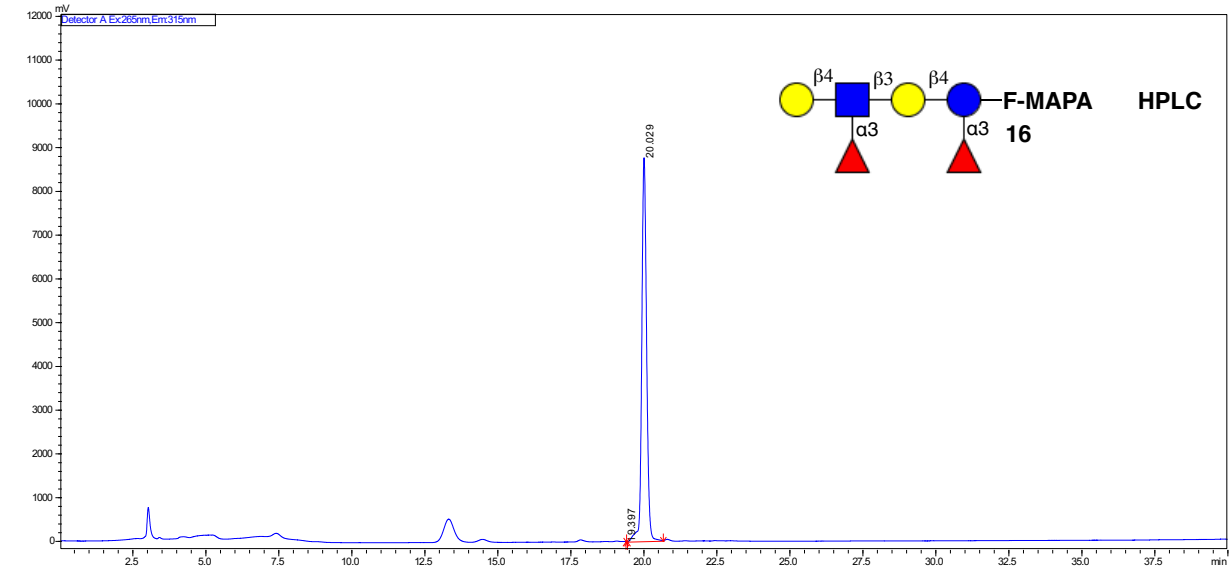

960.878
[M+H]+
[M+Na]+
982.883
970.881
998.862
945.736
1022.836
1112.953
1134.948
α3
β4
α2
α2
AEAB
14
mass spectrum
m/z

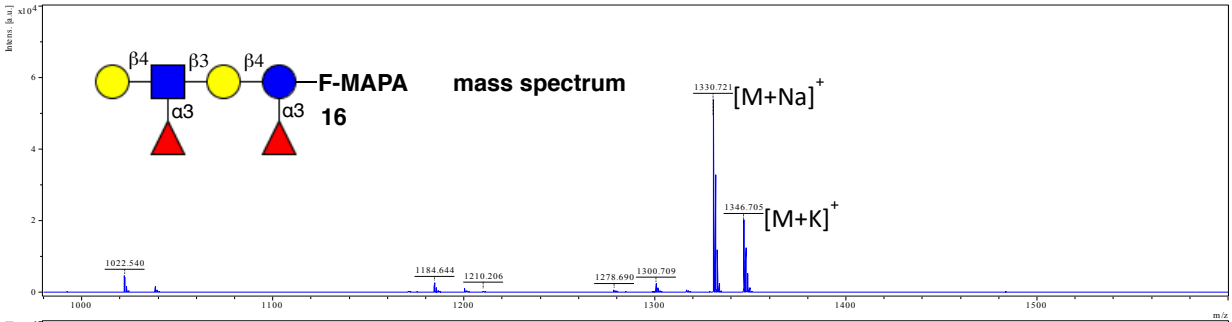

mV
Detector A Ex:265nm,Em:315nm
7.468
6.861
β4
F-MAPA
α3 15
HPLC
min

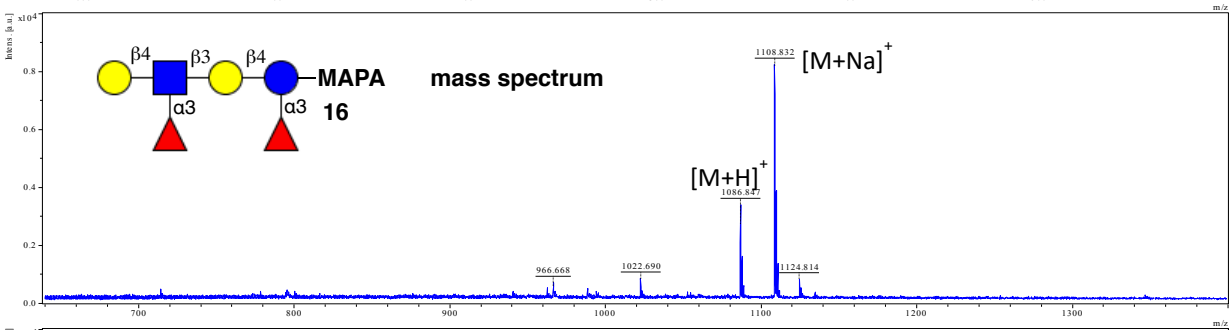

Intens. [a.u.]
860.509 [M+Na]+
876.503 [M+K]+
β4
F-MAPA
α3 15
mass spectrum
m/z

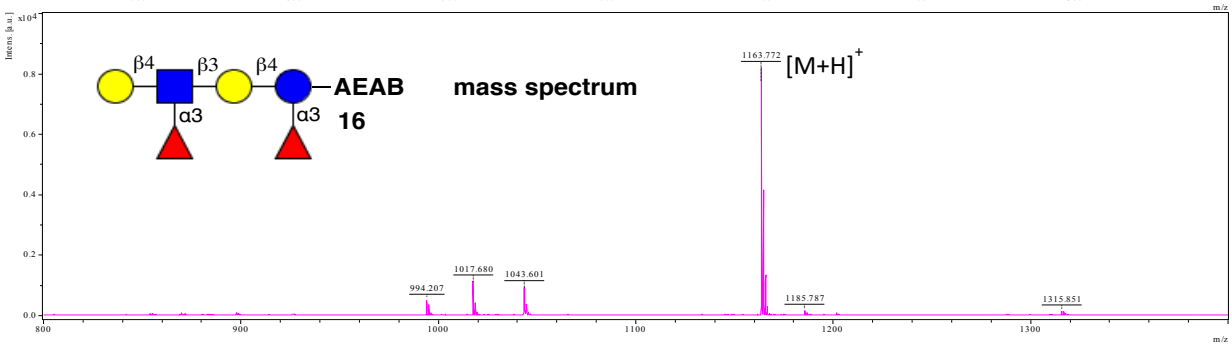

Intens. [a.u.]
638.560 [M+Na]+
[M+K]+
β4
MAPA
α3 15
mass spectrum
m/z

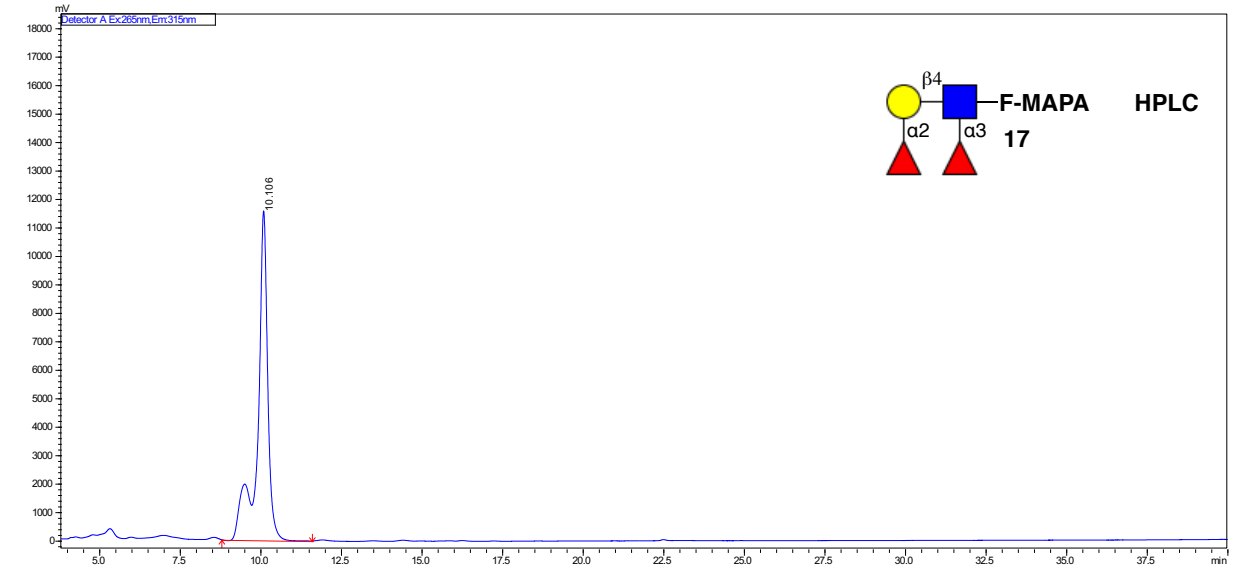

Intens. [a.u.]
715.286 [M+Na]+
β4
AEAB
α3 15
mass spectrum
m/z

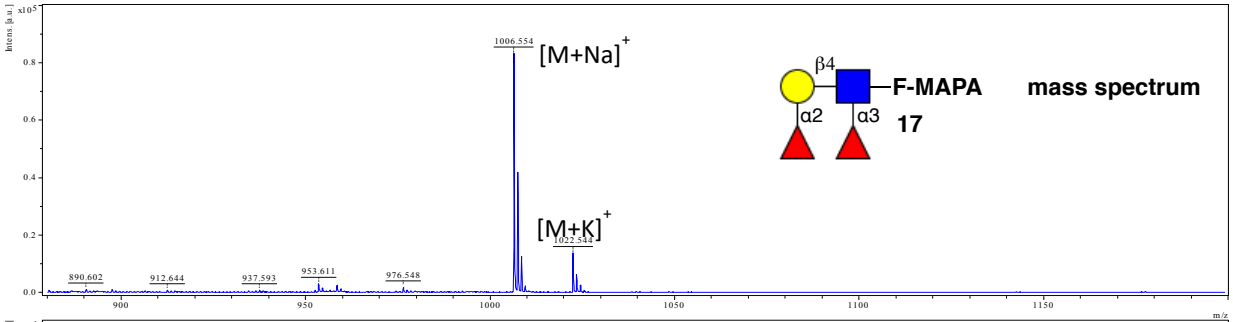

mV
Detector A Ex:265nm,Em:315nm
20.029
19.397
β4 β3 β4
α3 α3
F-MAPA
16
HPLC
min

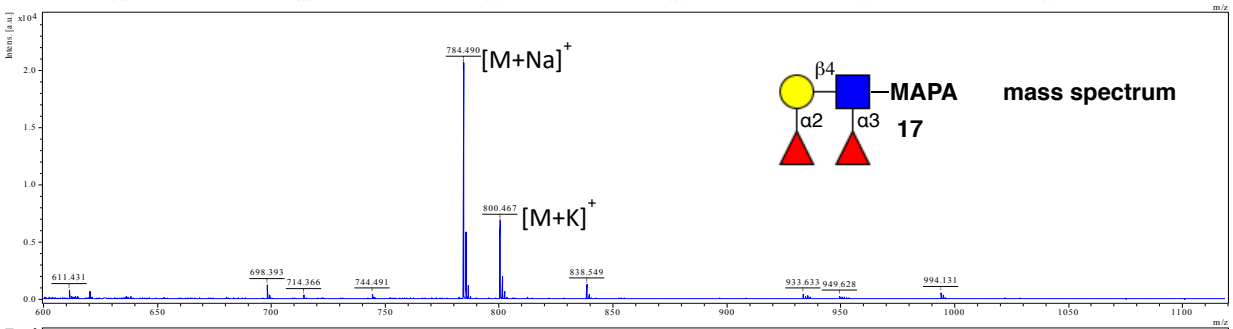

β4 β3 β4
α3 α3
F-MAPA
16
mass spectrum
1330.721 [M+Na]+
1346.705 [M+K]+
1022.540
1184.644
1210.206
1278.690
1300.709
Intens. [a.u.]
m/z

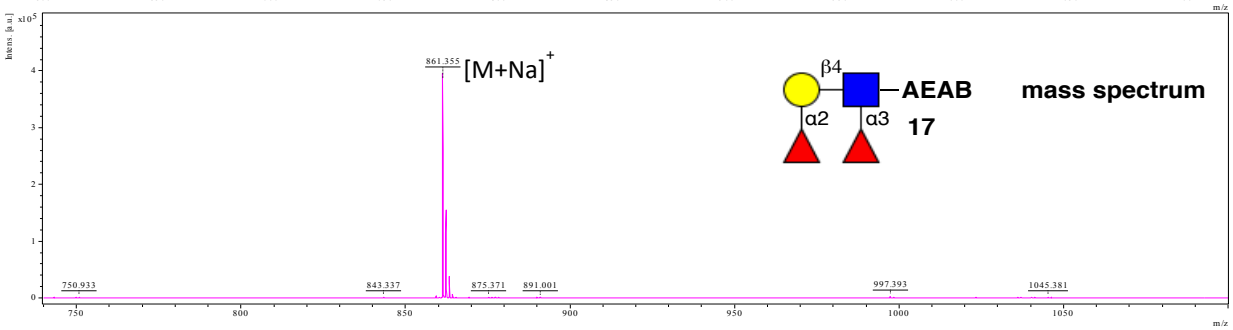

β4 β3 β4
α3 α3
MAPA
16
mass spectrum
1108.832 [M+Na]+
[M+H]+
1086.847
966.668
1022.690
1124.814
Intens. [a.u.]
m/z

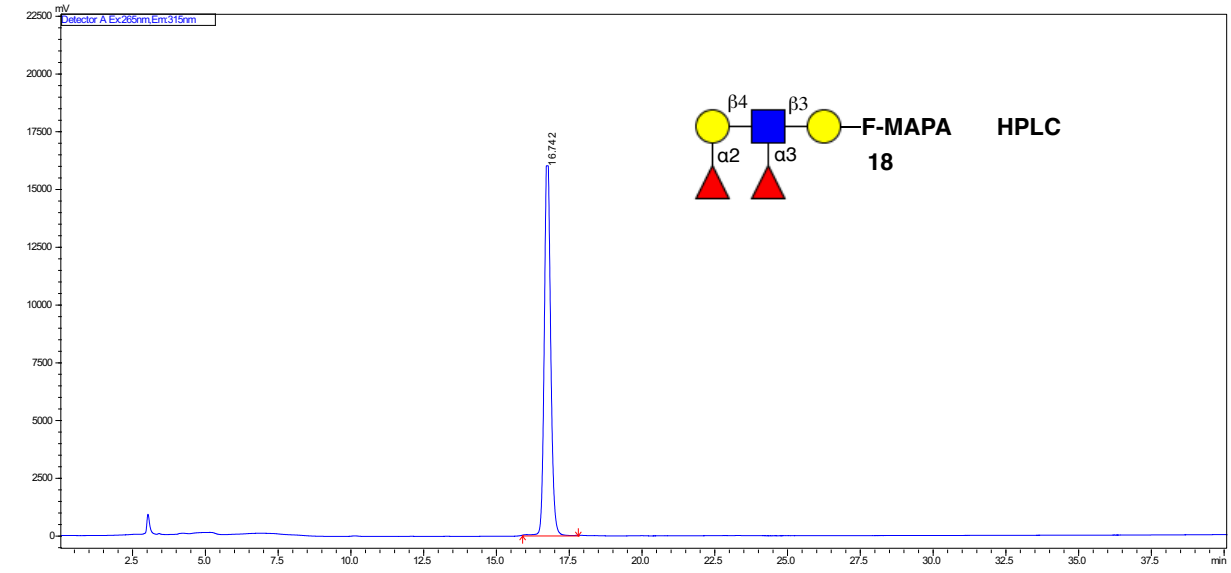

β4 β3 β4
α3 α3
AEAB
16
mass spectrum
1163.772 [M+H]+
994.207
1017.680
1043.601
1185.787
1315.851
Intens. [a.u.]
m/z

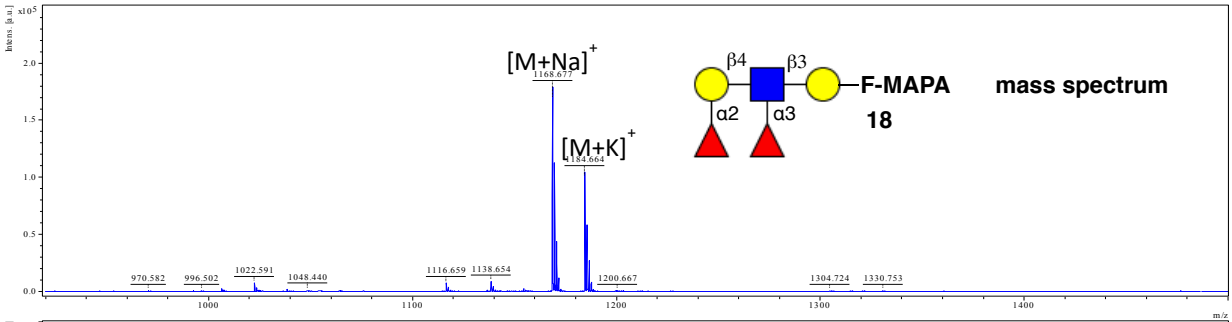

mV
Detector A Ex:265nm,Em:315nm
10.106
min
β4
α2
α3
F-MAPA
17
HPLC

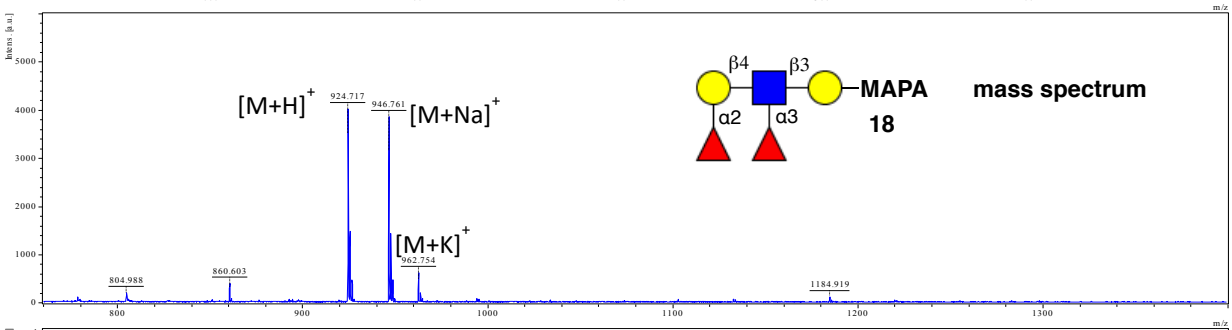

Intens. [a.u.]
1006.554
[M+Na]+
1022.544
[M+K]+
890.602
912.644
937.593
953.611
976.548
m/z
β4
α2
α3
F-MAPA
17
mass spectrum

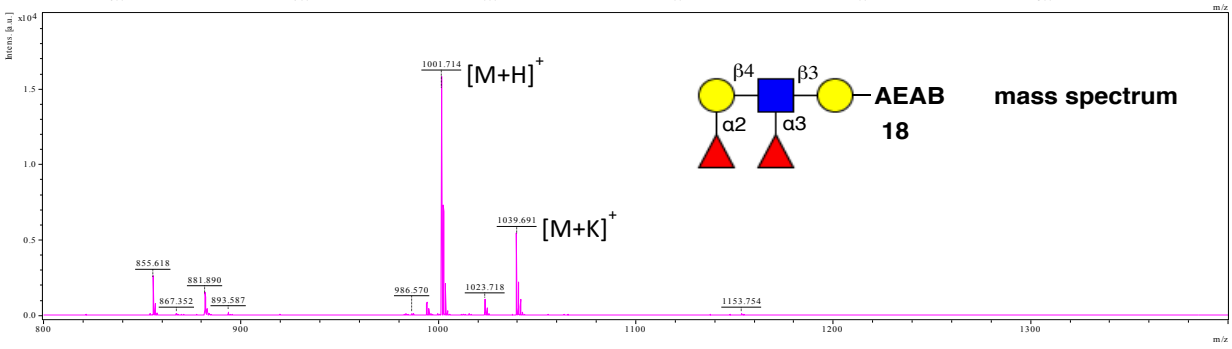

Intens. [a.u.]
784.490
[M+Na]+
800.467
[M+K]+
611.431
698.393
714.366
744.491
838.549
933.633
949.628
994.131
m/z
β4
α2
α3
MAPA
17
mass spectrum

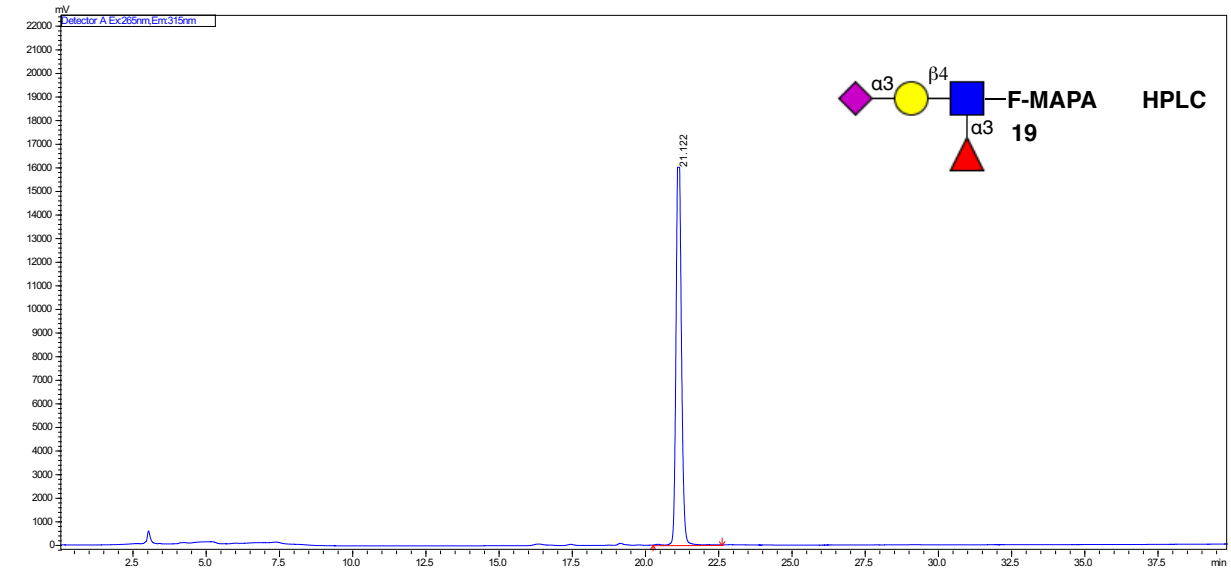

Intens. [a.u.]
861.355
[M+Na]+
750.933
843.337
875.371
891.001
997.393
1045.381
m/z
β4
α2
α3
AEAB
17
mass spectrum

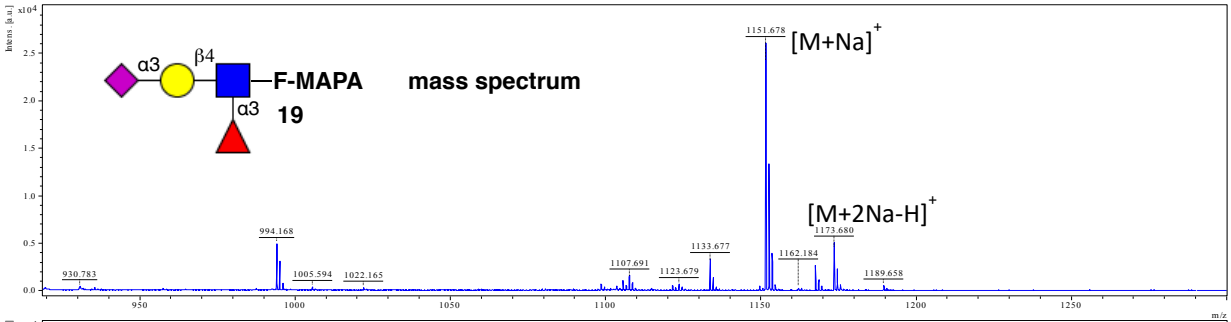

mV
Detector A Ex:265nm,Em:315nm
16.742
β4
β3
α2
α3
F-MAPA
18
HPLC
min

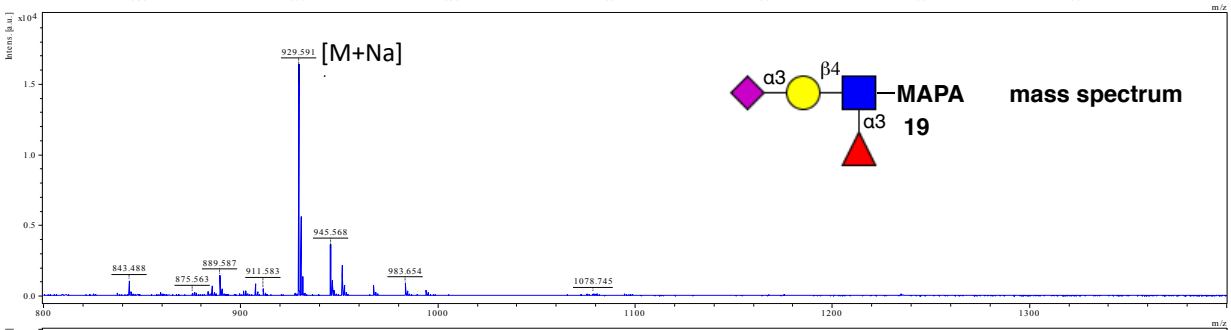

[M+Na]+
1168.677
[M+K]+
1184.664
970.582
996.502
1022.591
1048.440
1116.659
1138.654
1200.667
1304.724
1330.753
β4
β3
α2
α3
F-MAPA
18
mass spectrum
m/z

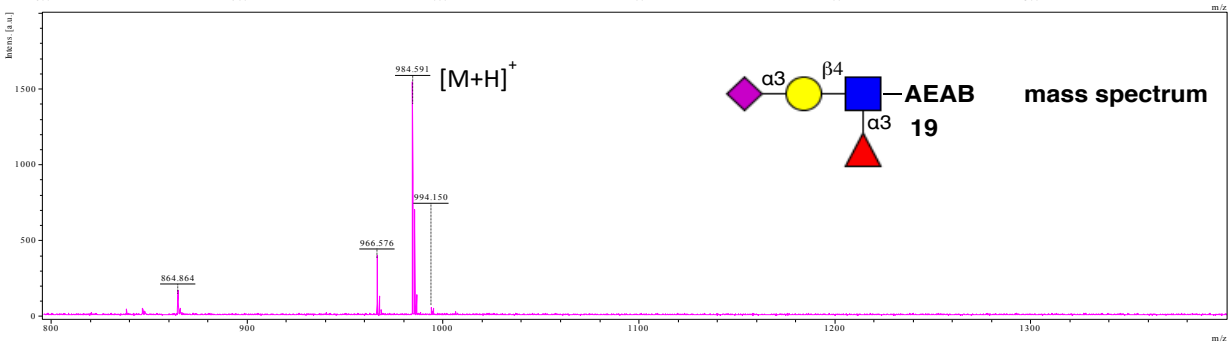

[M+H]+
924.717
946.761
[M+Na]+
[M+K]+
962.754
804.988
860.603
1184.919
β4
β3
α2
α3
MAPA
18
mass spectrum
m/z

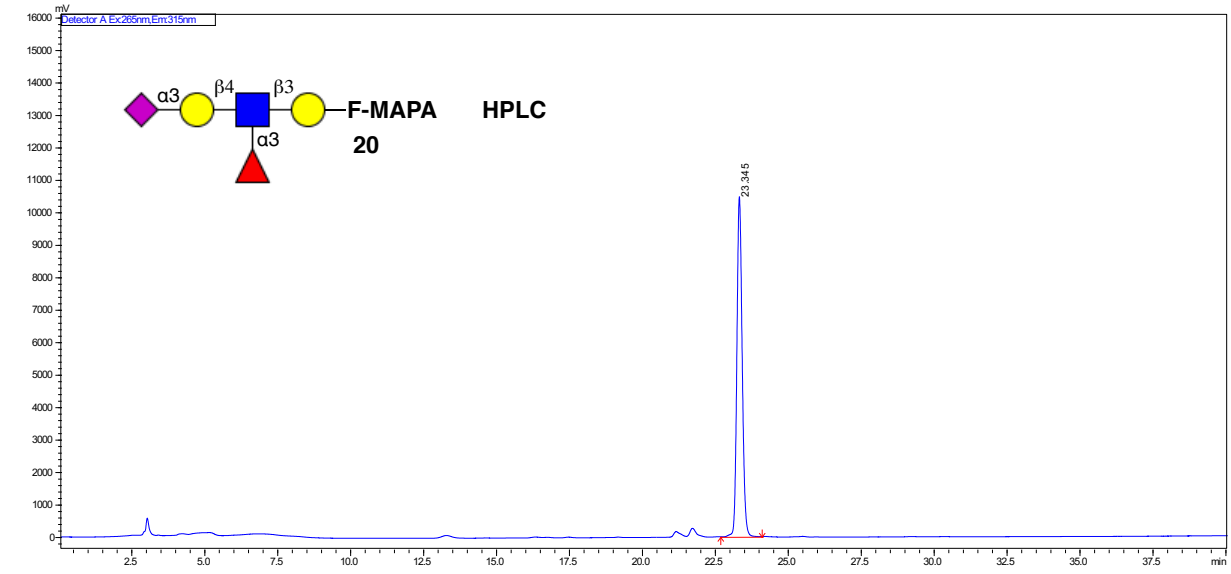

1001.714
[M+H]+
1039.691
[M+K]+
855.618
881.890
867.352
893.587
986.570
1023.718
1153.754
β4
β3
α2
α3
AEAB
18
mass spectrum
m/z

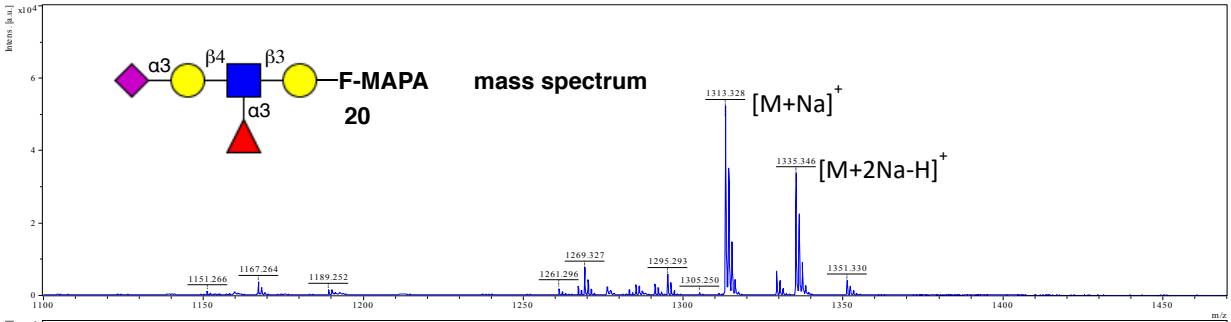

mV
Detector A Ex:285nm,Em:315nm
21.122
α3
β4
α3
F-MAPA
19
HPLC
min

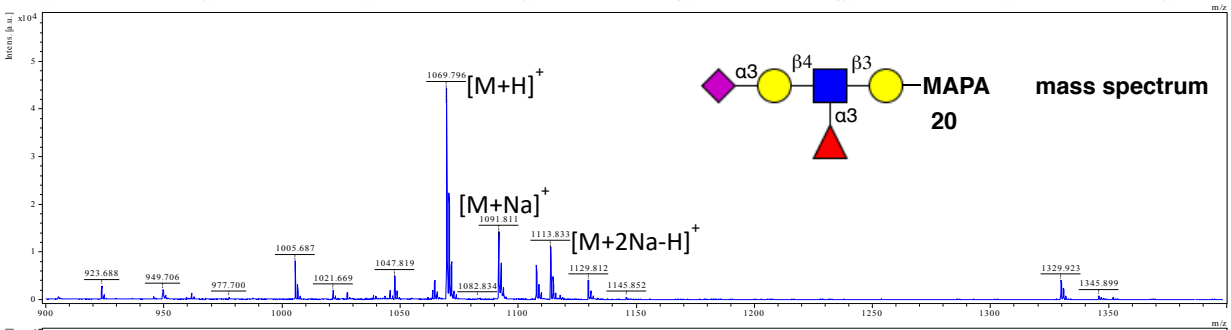

α3
β4
α3
F-MAPA
19
mass spectrum
1151.678
[M+Na]+
[M+2Na-H]+
994.168
930.783
1005.594
1022.165
1107.691
1123.679
1133.677
1162.184
1173.680
1189.658
m/z

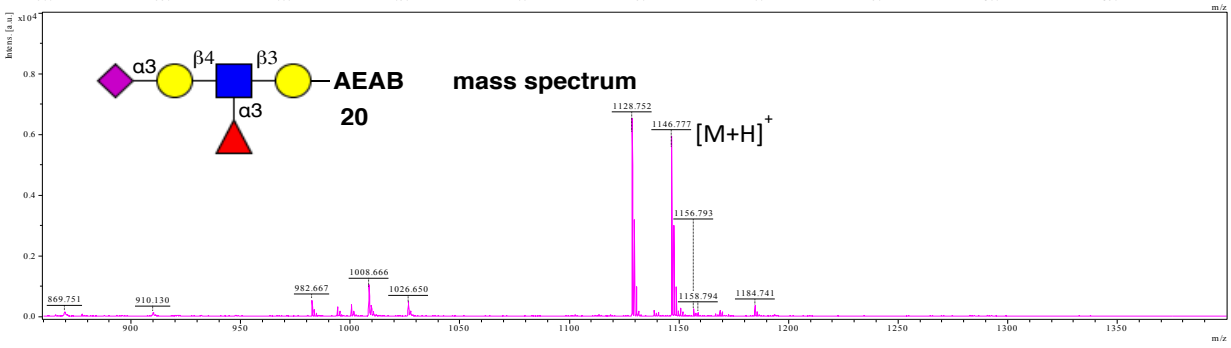

929.591
[M+Na]
α3
β4
α3
MAPA
19
mass spectrum
843.488
875.563
889.587
911.583
945.568
983.654
1078.745
m/z

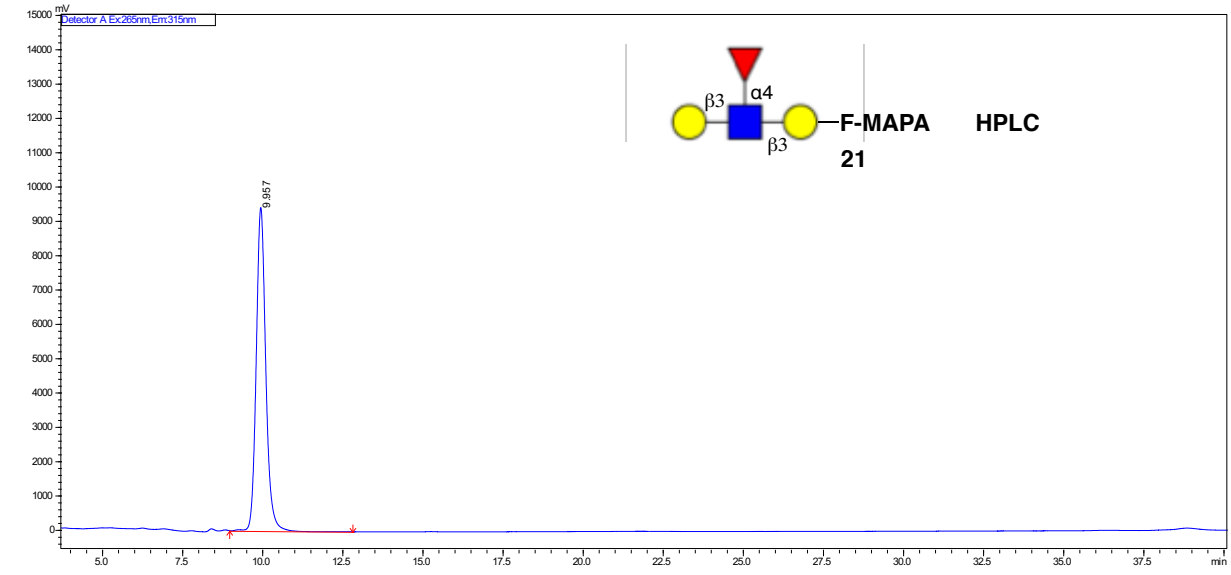

984.591
[M+H]+
α3
β4
α3
AEAB
19
mass spectrum
994.150
966.576
864.864
m/z

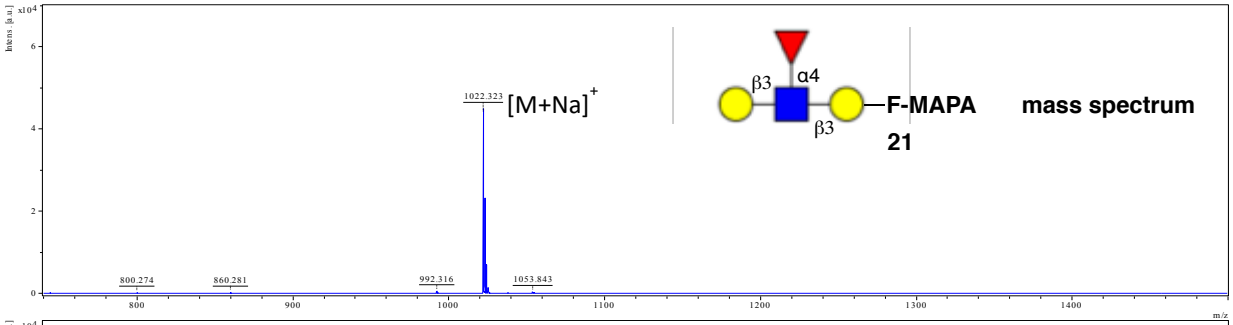

mV
Detector A Ex:285nm,Em:315nm
α3 β4 β3 α3
F-MAPA
20
HPLC
23.345
min

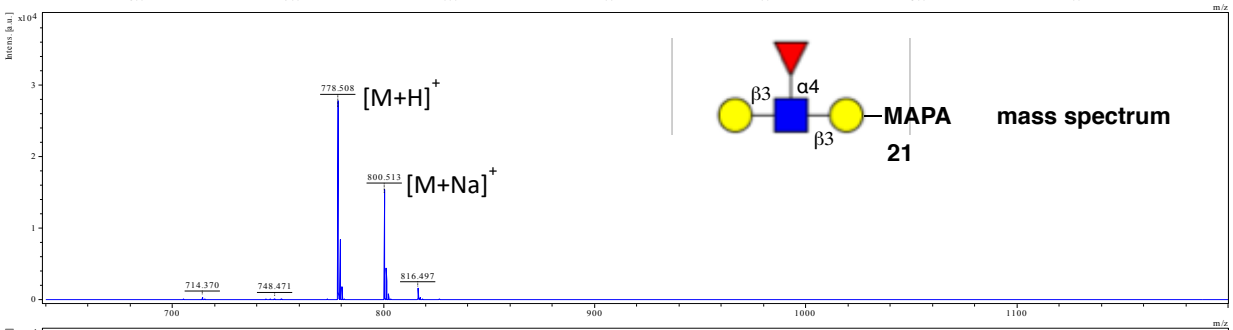

α3 β4 β3 α3
F-MAPA
20
mass spectrum
1313.328 [M+Na]+
1335.346 [M+2Na-H]+
m/z

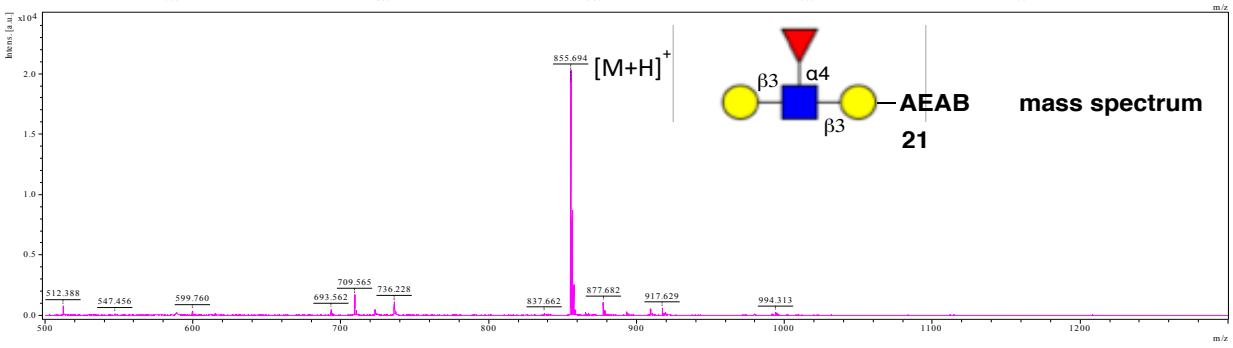

α3 β4 β3 α3
MAPA
20
mass spectrum
1069.796 [M+H]+
[M+Na]+
1091.811
1113.833 [M+2Na-H]+
m/z

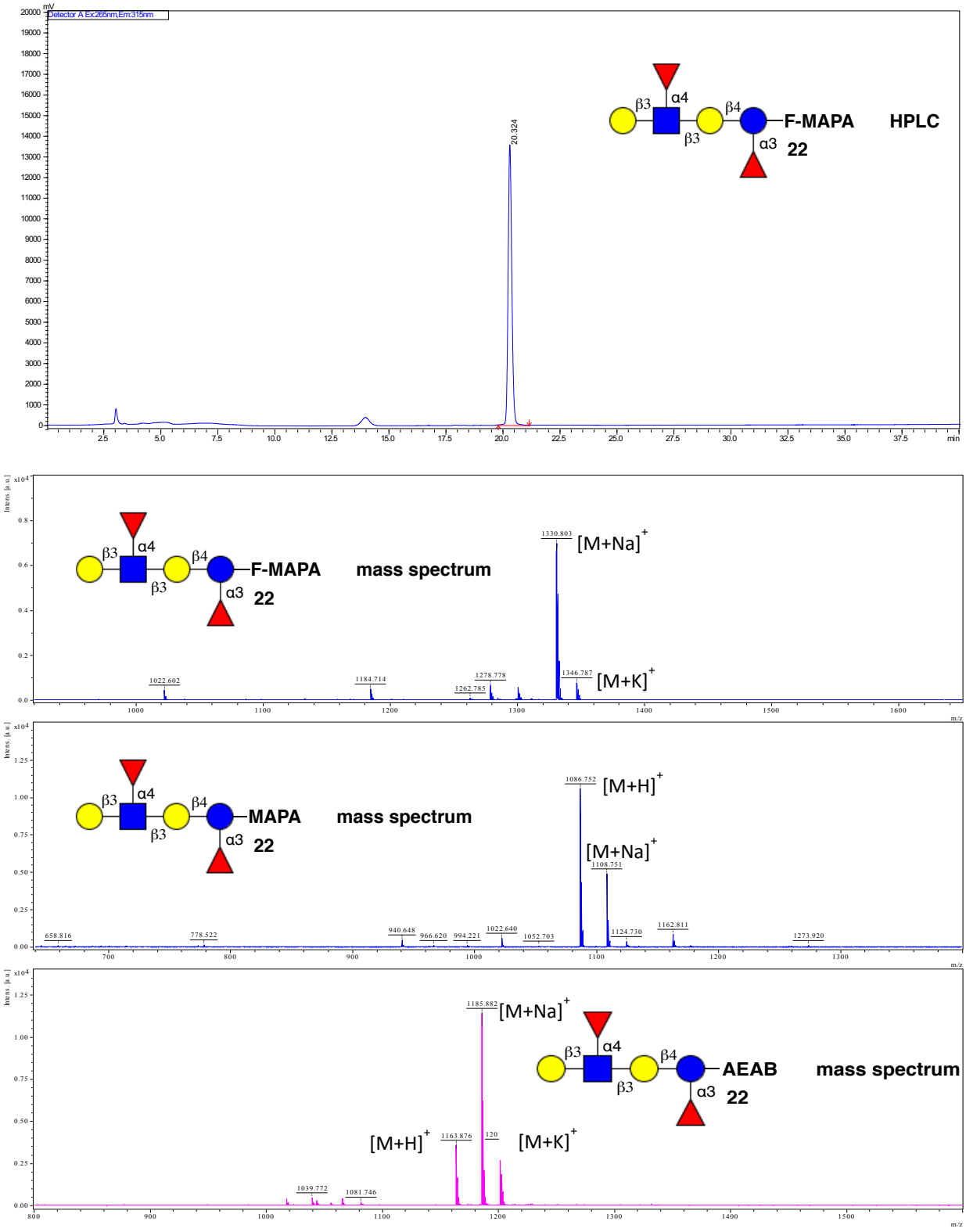

α3 β4 β3 α3
AEAB
20
mass spectrum
1128.752
1146.777 [M+H]+
1156.793
1008.666
m/z

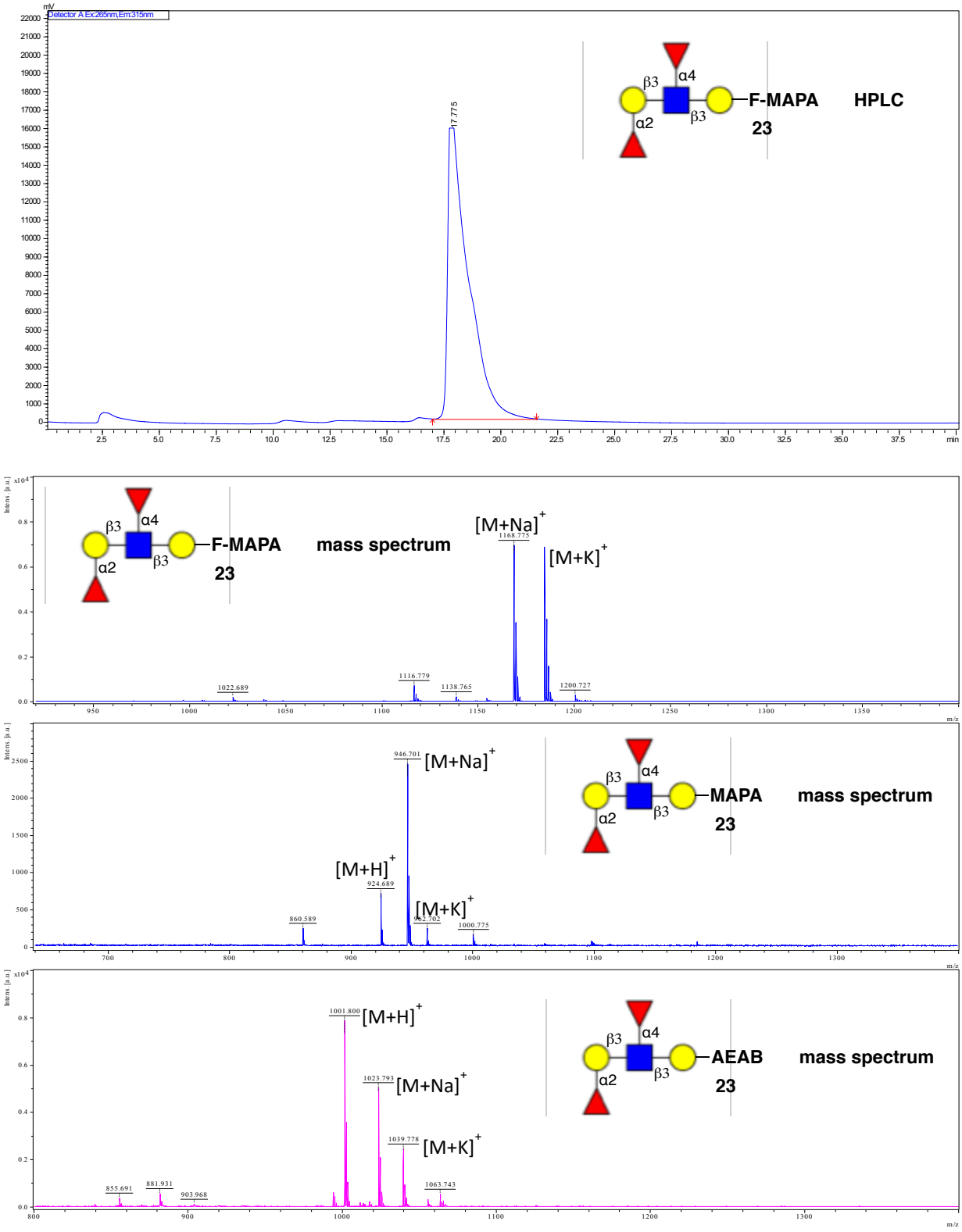

mV
Detector A Ex:265nm,Em:315nm
9.957
β3
α4
β3
F-MAPA
21
HPLC
min

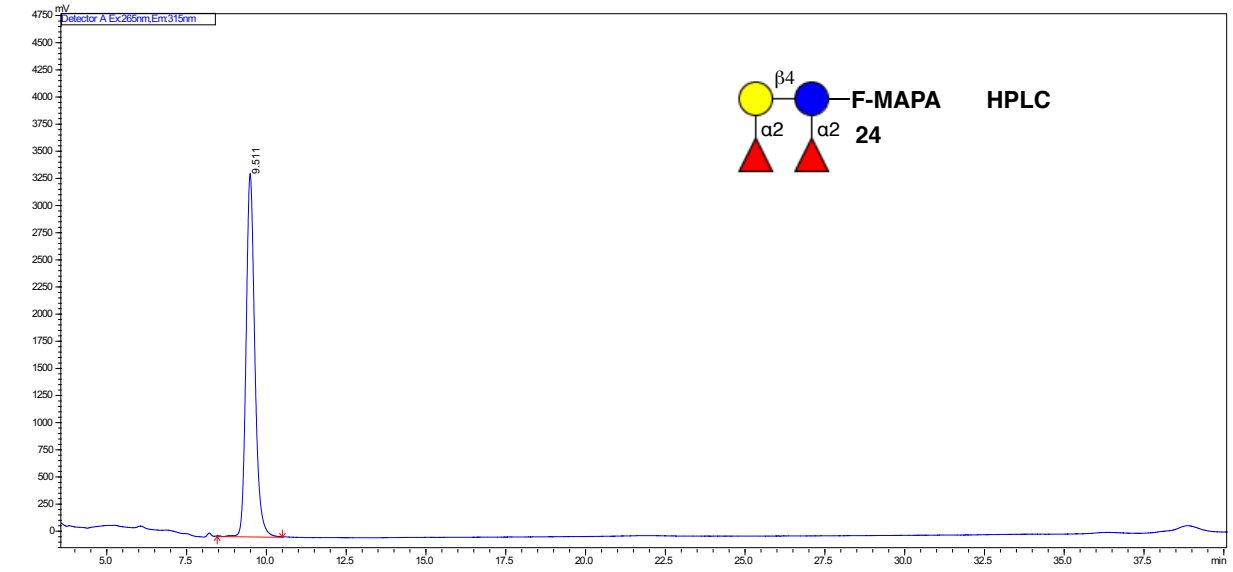

Intens. [a.u.]
1022.323
[M+Na]+
800.274
860.281
992.316
1053.843
β3
α4
β3
F-MAPA
21
mass spectrum
m/z

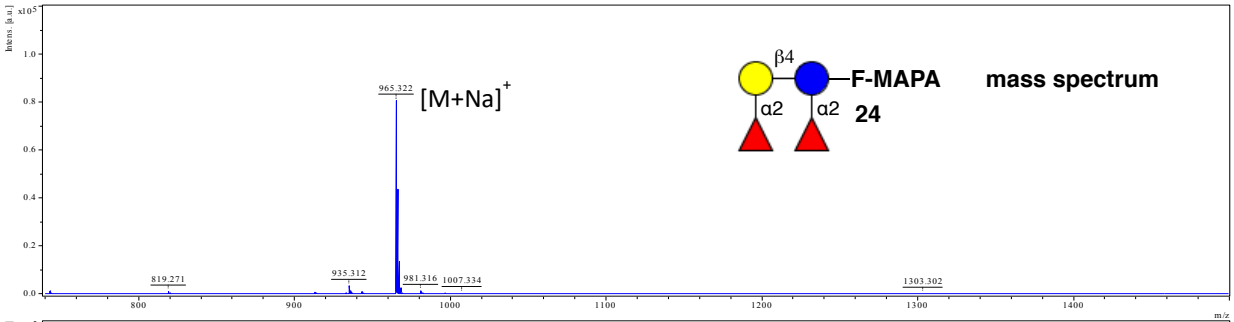

Intens. [a.u.]
778.508
[M+H]+
800.513
[M+Na]+
714.370
748.471
816.497
β3
α4
β3
MAPA
21
mass spectrum
m/z

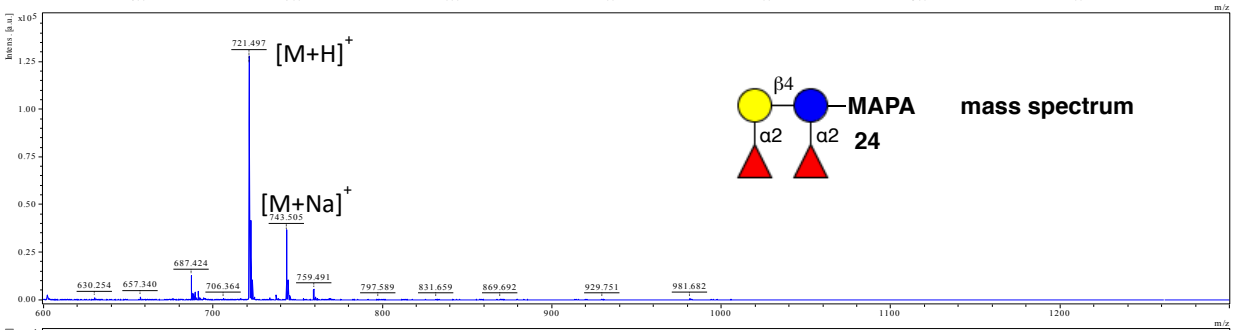

Intens. [a.u.]
855.694
[M+H]+
512.388
547.456
599.760
693.562
709.565
736.228
837.662
877.682
917.629
994.313
β3
α4
β3
AEAB
21
mass spectrum
m/z

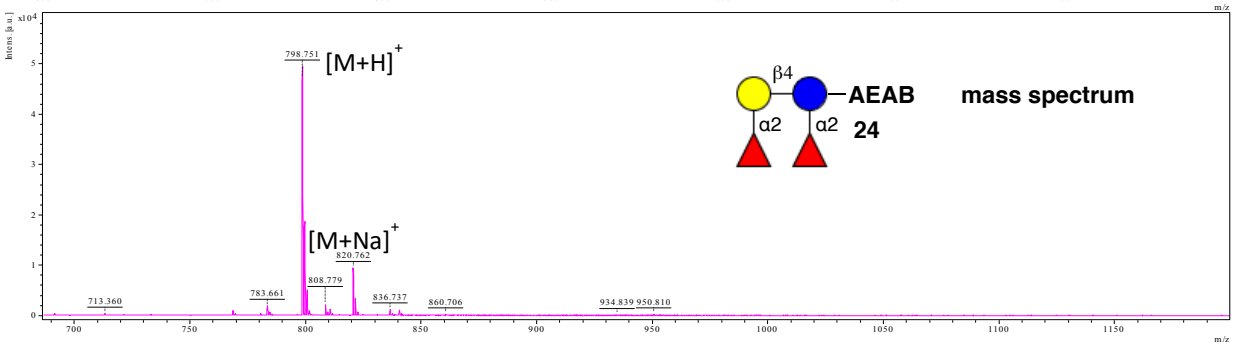

mV
Detector A Ex:265nm,Em:315nm
20.324
β3 α4 β4
β3 α3
F-MAPA
22
HPLC
min
Intens. [a.u.]
x10⁴
1330.803
[M+Na]⁺
1346.787
[M+K]⁺
1022.602
1184.714
1278.778
1262.785
mass spectrum
MAPA
1086.752
[M+H]⁺
1108.751
658.816
778.522
940.648
966.620
994.221
1022.640
1052.703
1124.730
1162.811
1273.920
m/z
1185.882
[M+Na]⁺
AEAB
[M+H]⁺
1163.876
120
[M+K]⁺
1039.772
1081.746

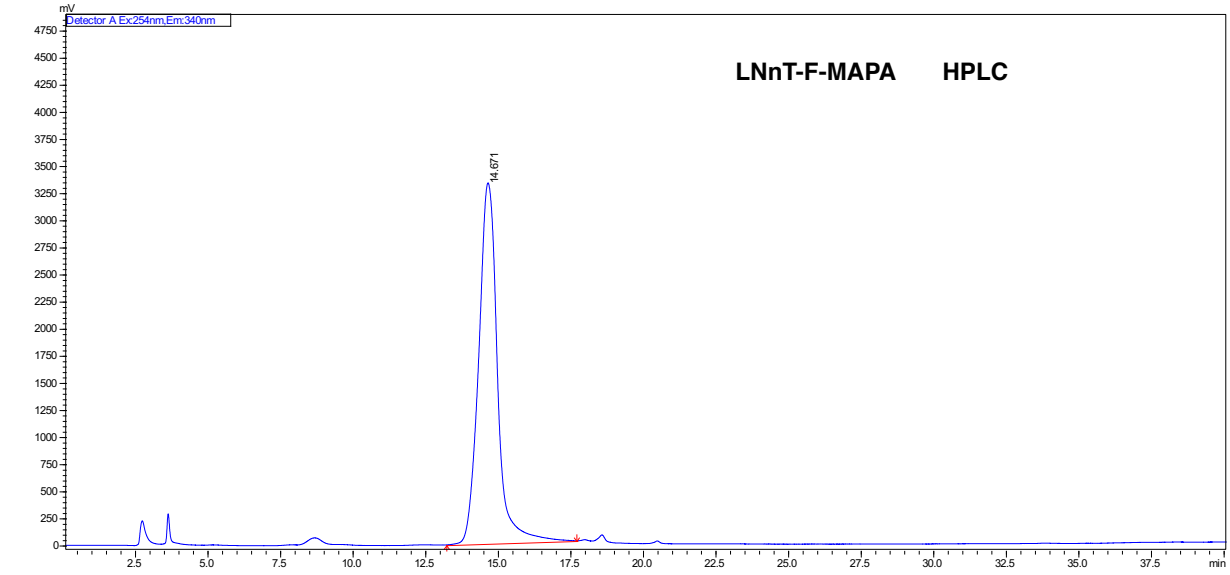

mV
Detector A Ex:265nm,Em:315nm
17.775
min
β3
α4
α2
β3
F-MAPA
23
HPLC
mass spectrum
[M+Na]+
[M+K]+
1168.775
1022.689
1116.779
1138.765
1200.727
m/z
946.701
[M+H]+
924.689
860.589
962.702
1000.775
MAPA
mass spectrum
1001.800
1023.793
1039.778
1063.743
855.691
881.931
903.968
AEAB
mass spectrum

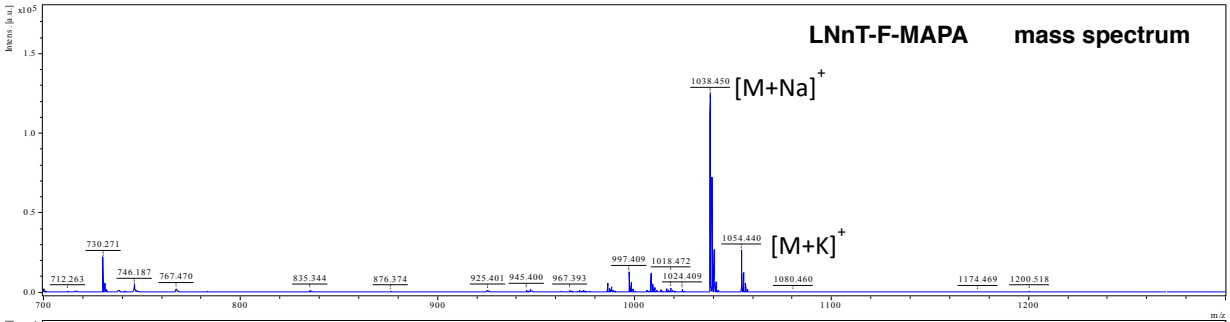

mV
Detector A Ex:265nm,Em:315nm
9.511
min
β4
α2
α2
F-MAPA
24
HPLC

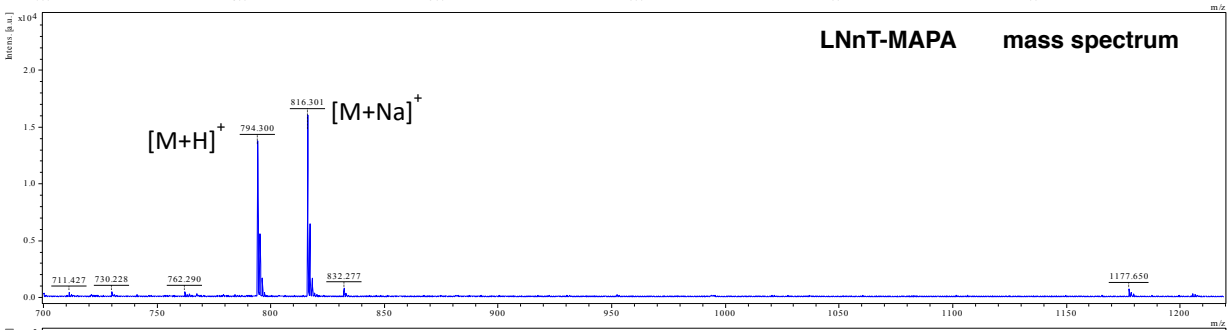

Intens. [a.u.]
965.322
[M+Na]+
819.271
935.312
981.316
1007.334
1303.302
m/z
β4
α2
α2
F-MAPA
24
mass spectrum

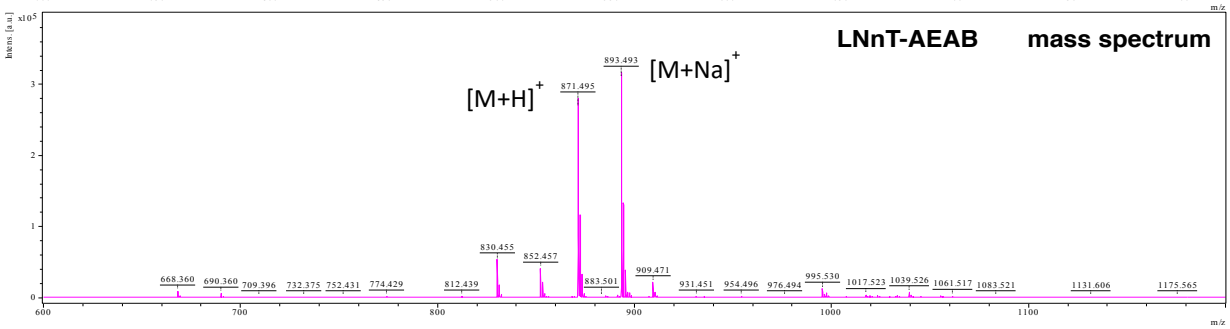

Intens. [a.u.]
721.497
[M+H]+
[M+Na]+
743.505
687.424
630.254
657.340
706.364
759.491
797.589
831.659
869.692
929.751
981.682
m/z
β4
α2
α2
MAPA
24
mass spectrum


Intens. [a.u.]
798.751
[M+H]+
[M+Na]+
820.762
808.779
713.360
783.661
836.737
860.706
934.839
950.810
m/z
β4
α2
α2
AEAB
24
mass spectrum


mV
Detector A Ex:254nm,Em:340nm
LNnT-F-MAPA HPLC
14.671
min


LNnT-F-MAPA mass spectrum
1038.450 [M+Na]+
1054.440 [M+K]+
730.271
746.187
767.470
712.263
835.344
876.374
925.401
945.400
967.393
997.409
1018.472
1024.409
1080.460
1174.469
1200.518
m/z


LNnT-MAPA mass spectrum
[M+H]+
794.300
816.301 [M+Na]+
711.427
730.228
762.290
832.277
1177.650
m/z


LNnT-AEAB mass spectrum
[M+H]+
871.495
893.493 [M+Na]+
668.360
690.360
709.396
732.375
752.431
774.429
812.439
830.455
852.457
883.501
909.471
931.451
954.496
976.494
995.530
1017.523
1039.526
1061.517
1083.521
1131.606
1175.565
m/z

The original binding signals (RFUs) elicited with lectins.

Probe ID	Glycan structure	Blood type	AAL		UEA-I		GSL-I b4		DBA	
			Average RFU	Std Dev	Average RFU	Std Dev	Average RFU	Std Dev	Average RFU	Std Dev
1a	Fucα1-2Galβ1-4GlcNAc-AEAB	H2	15926	638	13025	612	1	0	14	1
2a	Fucα1-2Galβ1-4Glc-AEAB	H5	17722	1230	10925	481	1	1	14	3
3a	Fucα1-2Galβ1-3GlcNAcβ1-3Gal-AEAB	H1	13508	543	6	4	0	1	14	1
4a	Fucα1-2Galβ1-4GlcNAcβ1-3Gal-AEAB	H2	11321	499	6735	307	2	2	40	50
5a	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AEAB	H1	18637	553	9	1	1	0	13	1
6a	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-AEAB	A2	229	9	2	1	0	1	1439	117
7a	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AEAB	A2	3	2	2	1	1	1	7592	408
8a	Galα1-3(Fucα1-2)Galβ1-4Glc-AEAB	B5	10611	266	2	1	12310	529	14	4
9a	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-AEAB	B1	94	14	2	0	12312	498	16	2
10a	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-AEAB	B2	399	31	2	1	12639	683	15	1
11a	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AEAB	B1	2	1	7	10	9519	820	17	2
12a	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AEAB	B2	244	22	2	0	23760	828	22	18
13a	Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-AEAB	B4	1	1	3	1	9649	2740	10	3
14a	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-2)Glc-AEAB	B5	15731	1782	2	1	16725	2360	10	1
15a	Galβ1-4(Fucα1-3)GlcNAc-AEAB	Lex	13431	641	12	1	1	1	16	3
16a	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-AEAB	Lex	16030	707	26	6	2	2	30	34
17a	Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-AEAB	Ley	18498	907	1161	103	3	1	14	1
18a	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-AEAB	Ley	15442	429	12365	552	3	1	9	1
19a	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc-AEAB	Slex	17269	884	2	0	1	1	40	43
20a	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-AEAB	Slex	18846	1858	2	0	3	1	9	2
21a	Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-AEAB	Lea	15304	1900	35	6	3	1	10	2
22a	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc-AEAB	Lea	16011	1221	50	10	3	1	9	3
23a	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-AEAB	Leb	15071	1776	2	1	3	1	8	3
24a	Fucα1-2Galβ1-4(Fucα1-2)Glc-AEAB		16950	2048	8169	1466	-1	1	11	3
25a	Streptavidin Cy5 / A488 Hydrazide landing light		580	403	1013	444	1280	464	904	396
1b	Fucα1-2Galβ1-4GlcNAc-MAPA	H2	14565	878	10731	269	0	1	11	2
2b	Fucα1-2Galβ1-4Glc-MAPA	H5	17484	363	14333	254	0	1	13	2
3b	Fucα1-2Galβ1-3GlcNAcβ1-3Gal-MAPA	H1	7196	233	3	1	0	1	12	1
4b	Fucα1-2Galβ1-4GlcNAcβ1-3Gal-MAPA	H2	21015	299	15775	283	1	1	13	4
5b	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-MAPA	H1	12335	296	2	0	1	1	14	2
6b	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-MAPA	A2	5	1	2	1	1	1	971	90
7b	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-MAPA	A2	3	1	2	1	1	1	1204	112
8b	Galα1-3(Fucα1-2)Galβ1-4Glc-MAPA	B5	2201	152	2	0	9127	660	15	1
9b	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-MAPA	B1	4	1	2	0	6686	373	16	2

10b	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-MAPA	B2	4	1	2	0	7009	385	15	3
11b	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-MAPA	B1	3	1	3	1	7463	596	15	3
12b	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-MAPA	B2	2	1	2	1	14884	617	15	1
13b	Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-MAPA	B4	10	1	5	1	6520	2564	13	3
14b	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-2)Glc-MAPA	B5	2052	384	63	12	11609	3584	13	1
15b	Galβ1-4(Fucα1-3)GlcNAc-MAPA	Lex	15578	559	2	1	2	1	16	2
16b	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-MAPA	Lex	9509	343	231	17	125	5	12	2
17b	Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-MAPA	Ley	19884	819	13101	625	3	1	22	14
18b	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-MAPA	Ley	16962	337	4574	133	3	1	22	19
19b	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc-MAPA	Slex	13801	532	2	0	3	0	14	3
20b	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-MAPA	Slex	13067	1581	2	1	3	1	9	2
21b	Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-MAPA	Lea	12611	2190	36	6	3	1	9	3
22b	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc-MAPA	Lea	14012	1777	42	9	0	1	9	1
23b	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-MAPA	Leb	11558	1476	22	39	0	1	7	2
24b	Fucα1-2Galβ1-4(Fucα1-2)Glc-MAPA		17507	1561	11259	2077	-1	1	9	2
25b	Negative Control		9	2	1	1	-4	1	8	2

The original binding signals (RFUs) elicited with anti-blood group antibodies.

Probe ID	Glycan structure	Blood type	Human DC-SIGN		anti-CD15		anti-SLe^x/SLe^a		anti-Le^a	
			Average RFU	Std Dev	Average RFU	Std Dev	Average RFU	Std Dev	Average RFU	Std Dev
1a	Fucα1-2Galβ1-4GlcNAc-AEAB	H2	20163	7232	3	5	5	4	5	3
2a	Fucα1-2Galβ1-4Glc-AEAB	H5	17918	751	6	3	6	3	4	3
3a	Fucα1-2Galβ1-3GlcNAcβ1-3Gal-AEAB	H1	0	26	4	1	5	6	6	3
4a	Fucα1-2Galβ1-4GlcNAcβ1-3Gal-AEAB	H2	3923	1985	4	10	260	35	237	29
5a	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AEAB	H1	1403	319	7	3	10	6	8	5
6a	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-AEAB	A2	0	8	4	1	15	12	4	1
7a	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AEAB	A2	0	153	5	1	15	4	7	2
8a	Galα1-3(Fucα1-2)Galβ1-4Glc-AEAB	B5	1346	162	4	5	4	2	2	1
9a	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-AEAB	B1	0	13	3	1	6	3	3	3
10a	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-AEAB	B2	0	18	5	2	4	2	2	2
11a	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AEAB	B1	0	25	5	2	5	5	2	1
12a	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AEAB	B2	31	18	4	1	0	4	1	1
13a	Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-AEAB	B4	0	28	4	1	4	3	1	2
14a	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-2)Glc-AEAB	B5	21224	2408	6	2	4	3	1	1
15a	Galβ1-4(Fucα1-3)GlcNAc-AEAB	Lex	191	74	6	1	9	7	1	1
16a	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-AEAB	Lex	42192	5536	26199	2149	2	2	1	2
17a	Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-AEAB	Ley	26822	3232	7	2	10	6	2	3

18a	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-AEAB	Ley	41164	2729	4	3	5	5	1	1
19a	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc-AEAB	Slex	15	23	3	1	4	5	1	1
20a	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-AEAB	Slex	27	13	5	1	12530	1257	8	3
21a	Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-AEAB	Lea	21052	3352	5	2	0	1	1395	140
22a	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc-AEAB	Lea	43218	5679	5	2	1	1	25744	1059
23a	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-AEAB	Leb	20689	2748	7	4	4	4	2	1
24a	Fucα1-2Galβ1-4(Fucα1-2)Glc-AEAB		34828	3431	6	2	2	1	1	1
25a	Streptavidin Cy5 / A488 Hydrazide landing light		2194	335	35	28	527	155	348	73
1b	Fucα1-2Galβ1-4GlcNAc-MAPA	H2	6735	5844	7	4	3	3	2	1
2b	Fucα1-2Galβ1-4Glc-MAPA	H5	7532	386	8	2	10	8	5	2
3b	Fucα1-2Galβ1-3GlcNAcβ1-3Gal-MAPA	H1	0	30	4	1	5	6	7	4
4b	Fucα1-2Galβ1-4GlcNAcβ1-3Gal-MAPA	H2	27234	3608	4	2	3	3	3	1
5b	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-MAPA	H1	33	10	5	2	7	4	3	2
6b	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-MAPA	A2	0	14	3	1	5	5	1	1
7b	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-MAPA	A2	0	27	4	1	6	3	1	1
8b	Galα1-3(Fucα1-2)Galβ1-4Glc-MAPA	B5	0	11	3	1	3	1	1	1
9b	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-MAPA	B1	15	18	3	1	8	4	2	2
10b	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-MAPA	B2	15	23	4	1	2	3	2	3
11b	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-MAPA	B1	0	20	4	2	3	1	2	1
12b	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-MAPA	B2	19	7	5	1	0	1	1	1
13b	Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-MAPA	B4	0	14	3	1	9	2	2	1
14b	Galα1-3(Fucα1-2)Galβ1-4(Fucα1-2)Glc-MAPA	B5	18225	2637	3	4	70	5	1	0
15b	Galβ1-4(Fucα1-3)GlcNAc-MAPA	Lex	14087	1417	2935	1684	2	1	1	1
16b	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-MAPA	Lex	23756	1020	17498	1060	540	69	1	1
17b	Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-MAPA	Ley	27759	1658	5	1	3	4	0	1
18b	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-MAPA	Ley	29569	481	3	3	13	7	1	1
19b	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc-MAPA	Slex	0	30	5	1	9520	2118	0	1
20b	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-MAPA	Slex	32	20	5	1	10785	1049	2	1
21b	Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-MAPA	Lea	18606	5528	4	2	0	1	19027	617
22b	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc-MAPA	Lea	35680	6528	6	2	1	1	36835	1787
23b	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-MAPA	Leb	13930	3218	5	1	1	1	2	1
24b	Fucα1-2Galβ1-4(Fucα1-2)Glc-MAPA		29794	814	4	1	6	5	1	1
25b	Negative Control		0	21	1	1	0	1	1	1