Parallel Glyco-SPOT Synthesis of Glycopeptide Libraries

Akul Y. Mehta, Ravi Kumar H. Veeraiah, Sucharita Dutta, Christoffer K. Goth, Melinda S. Hanes, Chao Gao, Kathrin Stavenhagen, Robert Kardish, Yasuyuki Matsumoto, Jamie Heimburg-Molinaro, Michael Boyce, Nicola L. B. Pohl, Richard D. Cummings

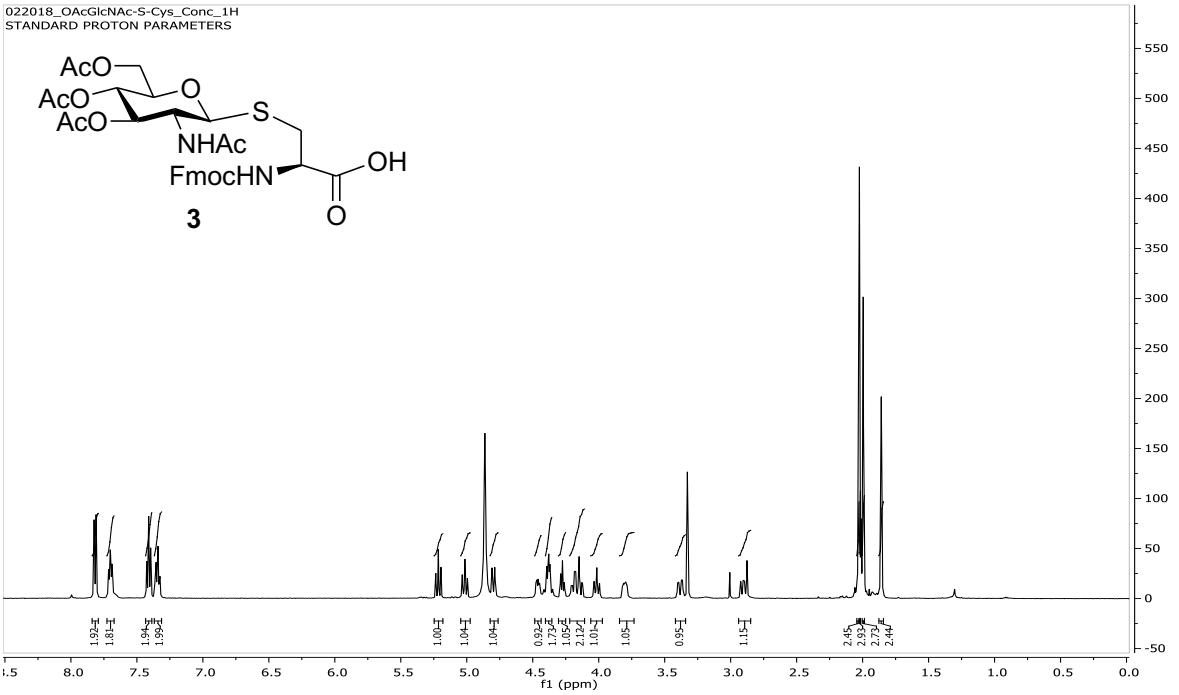
Data S1

Chemical Characterization- Related to Figure 7

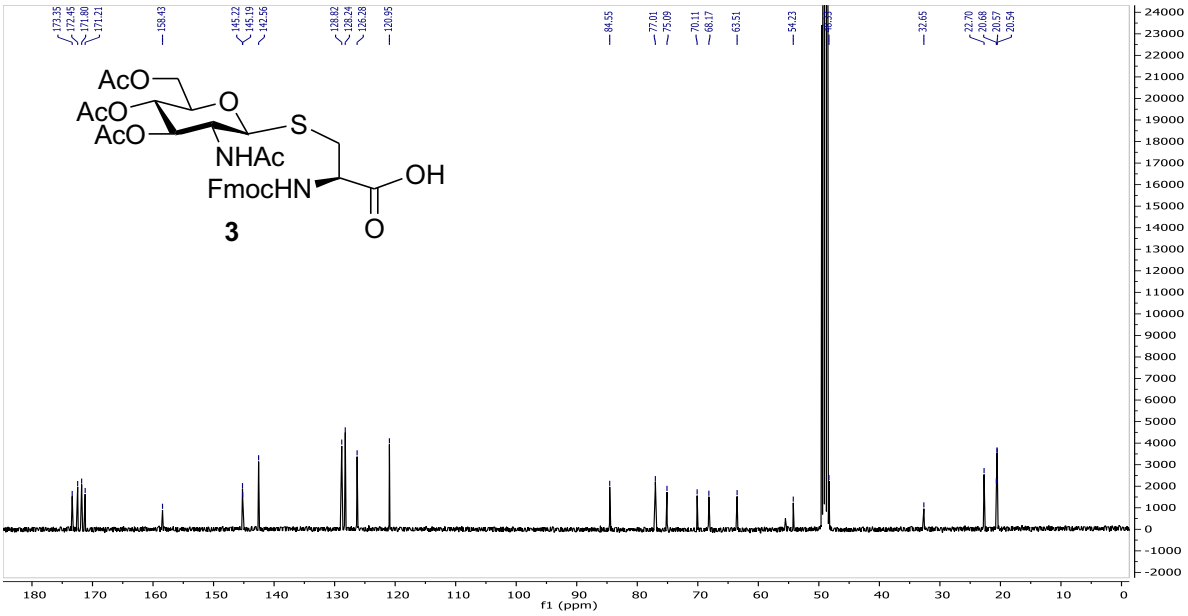
Contents

NMR Spectra for Synthesized Cys-linked FPGAs

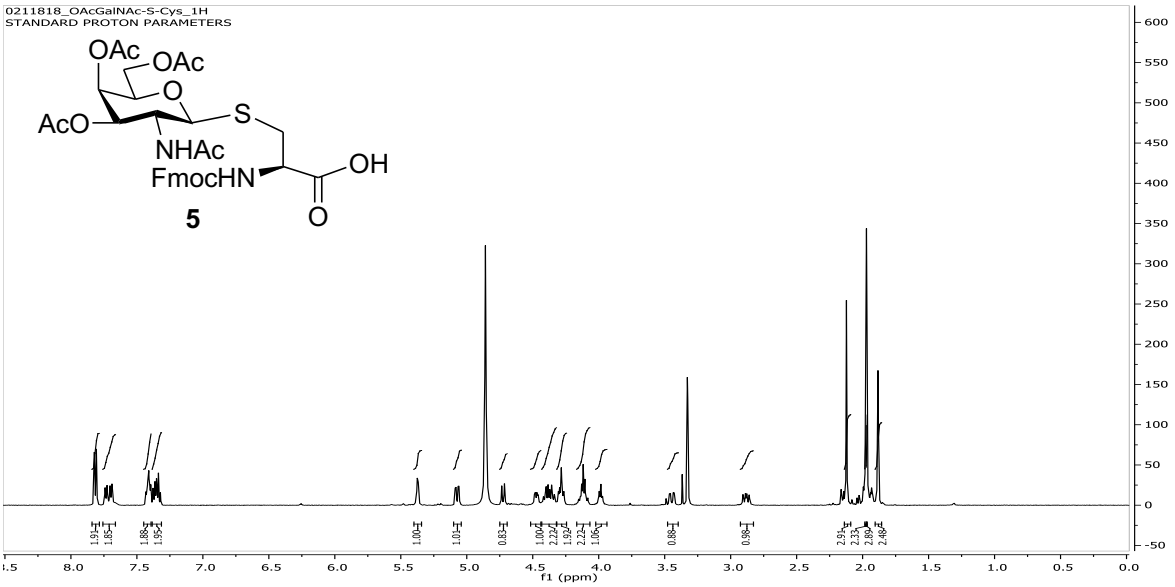
^{1}H NMR Spectrum of **3:**



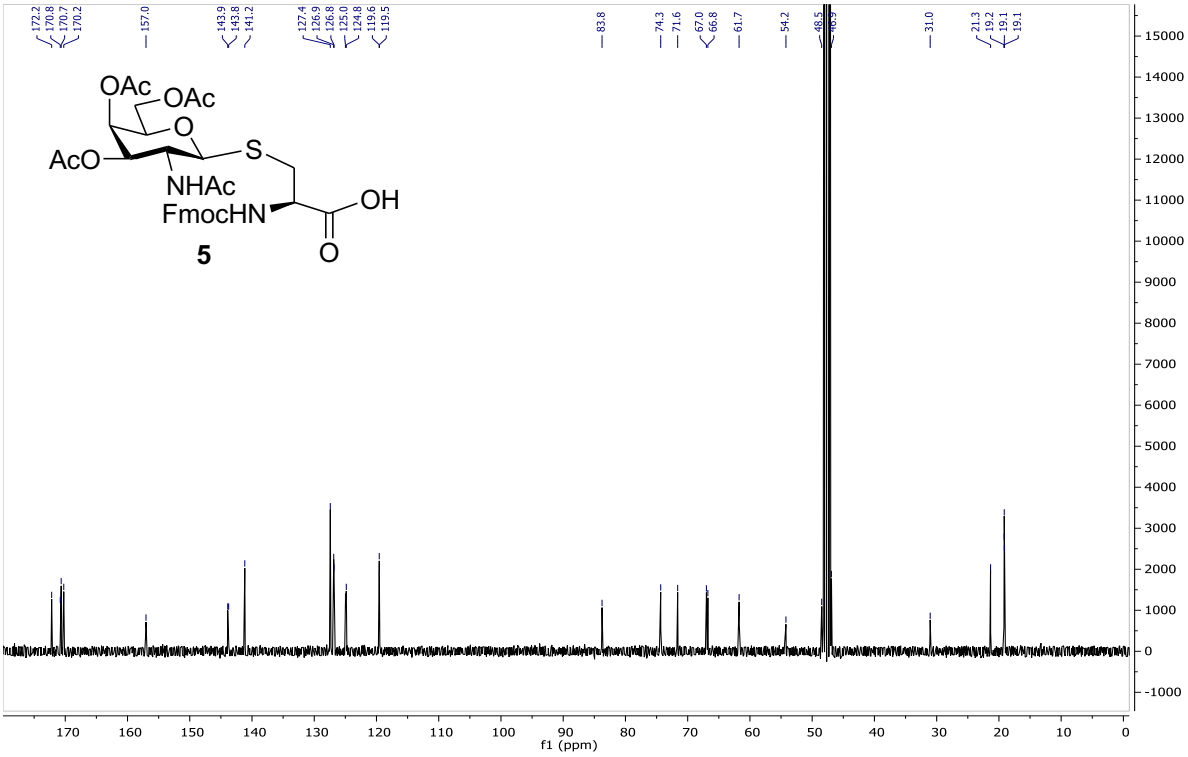
^{13}C NMR Spectrum of **3:**



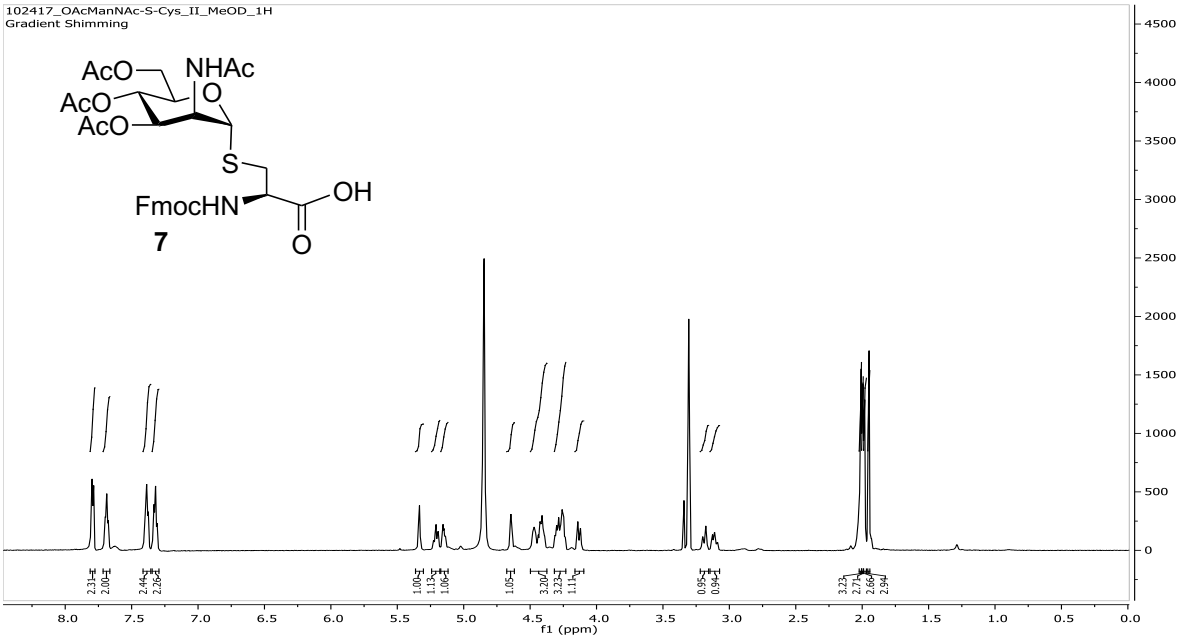
^{1}H NMR Spectrum of **5:**



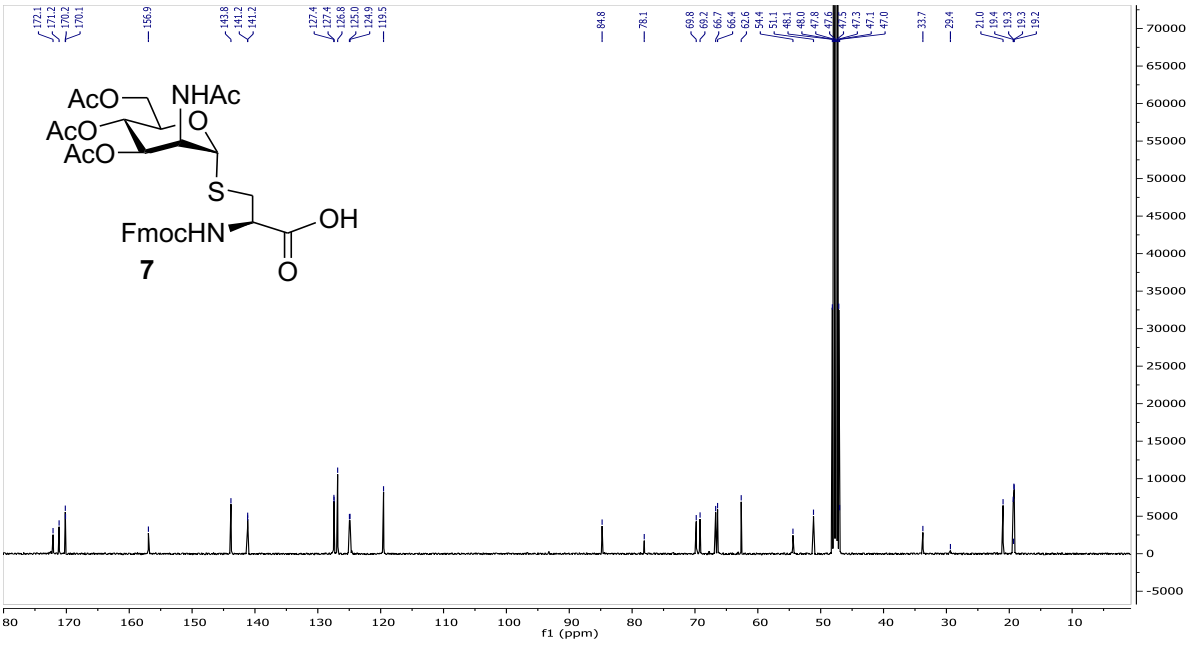
^{13}C NMR Spectrum of **5:**



^{1}H NMR Spectrum of **7:**



^{13}C NMR Spectrum of **7:**



MALDI Spectra For Glycopeptides

Notes About MALDI Spectra:

To perform MALDI experiments, 1 µL of glycopeptide was mixed with 1 µL of MALDI matrix solution of α-Cyano-4-hydroxycinnamic acid (HCCA) or 2,5-dihydroxybenzoic acid (for larger glycans). The matrix, once dried, was analyzed on a Bruker ultrafleXtreme MALDI TOF/TOF machine in positive mode. Only peaks with signal to noise ratio > 5 were annotated below.

In some cases a loss of 169 m/z was observed from the [M+H]+ peak as a result of metastable peak formed by the loss of HexNAc during time-of-flight as has been reported previously (Huberty et al., 1993). This is especially noticed in the case of O-linked glycans.

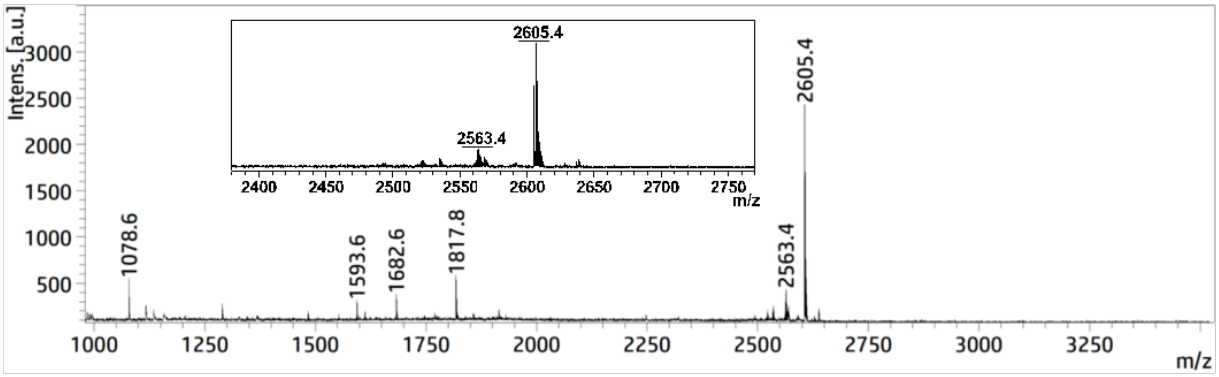
In case of GP1 library, which was released and deacetylated using sodium bicarbonate and water alone (no methanol), some peptides showed peaks of [M+126]+ peak. We suspect this is resistant peracetylated glycopeptide (where +126 Da would correspond to 3 Acetyl groups on the GlcNAc), which for some reason co-eluted in our HPLC semi-prep. We estimate this is <10% in most cases based on signal intensity. We did not further attempt purification or chemical treatment due to the small scale of synthesis, to avoid losses. This phenomenon was not observed when using methanol:water with sodium bicarbonate which is the preferred method of release used in the GP2 library. As we mentioned we used the GP1 library to learn and optimize the method.

In addition, where methanol:water method was used for release, a [M+14]+ peak was also observed due to the formation of methyl ester at the C-terminus of the peptide as discussed in Figure 3. Special cases for assignments are also highlighted along with the spectra in the list below.

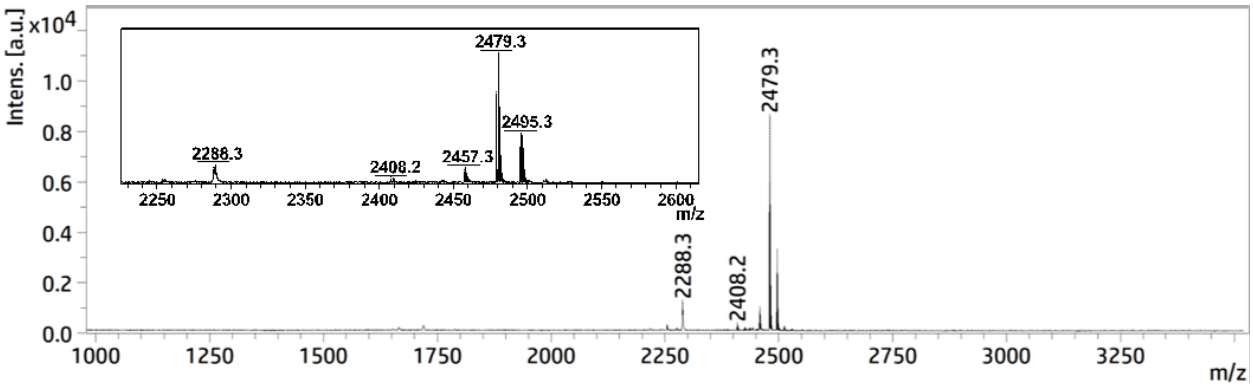
MALDI Spectra for all Peptides

GlycoPeptide 1 library (GP1)

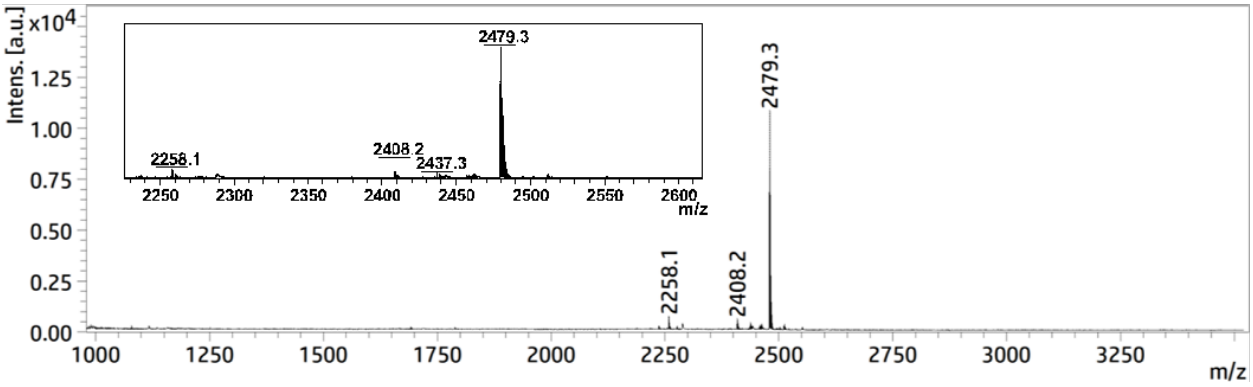
Spectrum: 1
Peptide ID: GP1.1 (Acetylated)
Internal ID: MBGP-04
Purification Level: Crude (initial release)
Sequence: AcNH-KTSTTATPPV{[3Ac][4Ac][6Ac]GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2605.5
Notes: Release with $NaHCO_3$ in water is very mild and can release the glycopeptide with the Acetyls intact.



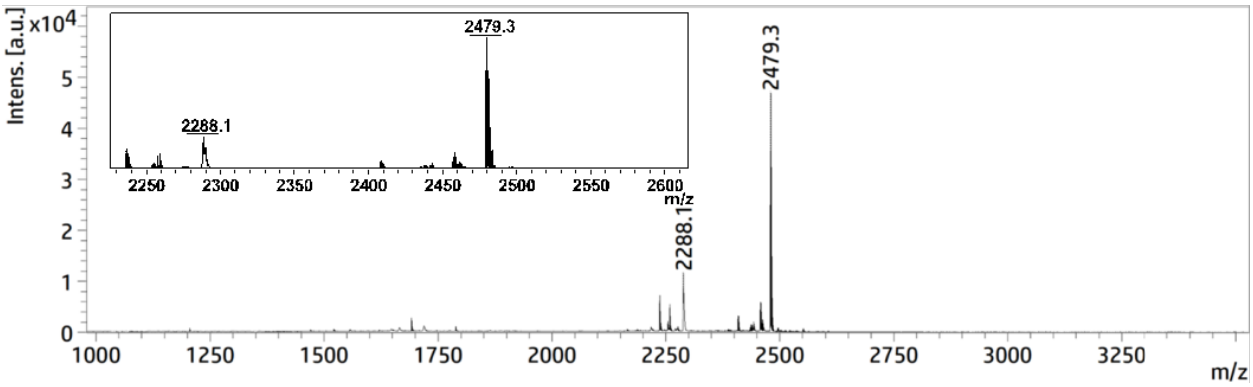
Spectrum: 2
Peptide ID: GP1.1 (Prototypical Peptide)
Internal ID: MBGP-04
Purification Level: HPLC Purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2479.3
Notes: After treatment with $NaHCO_3$ 2 times. Note in-source fragmentation shows a loss of m/z 169 (from [M+H] mass) in most spectra.



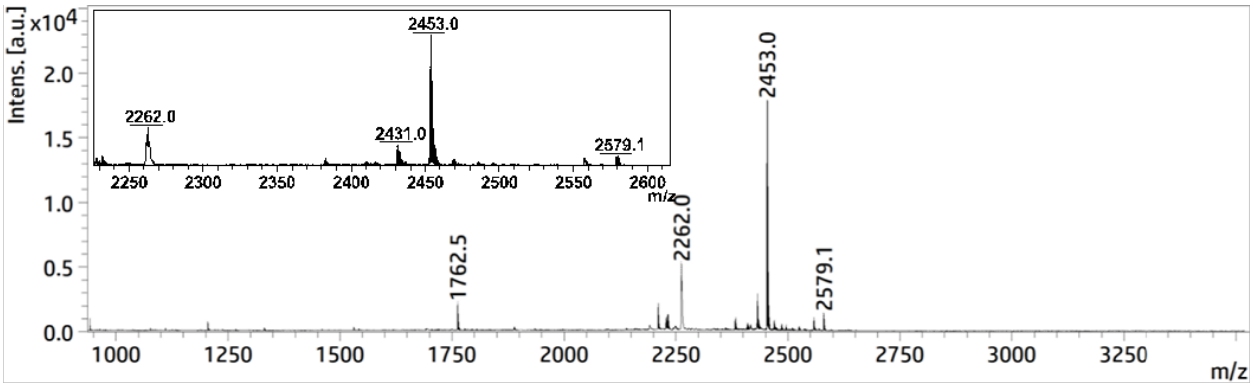
Spectrum: 3
Peptide ID: GP1.1HPLC
Internal ID: MBGP-05-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass [M+Na]+ = 2479.3



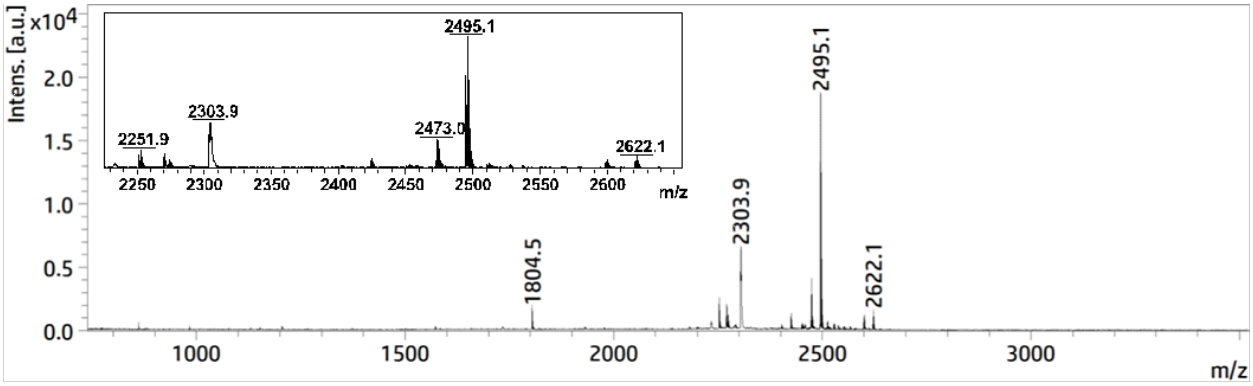
Spectrum: 4
Peptide ID: GP1.1SepPak
Internal ID: MBGP-05-SepPak
Purification Level: SepPak purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2479.3



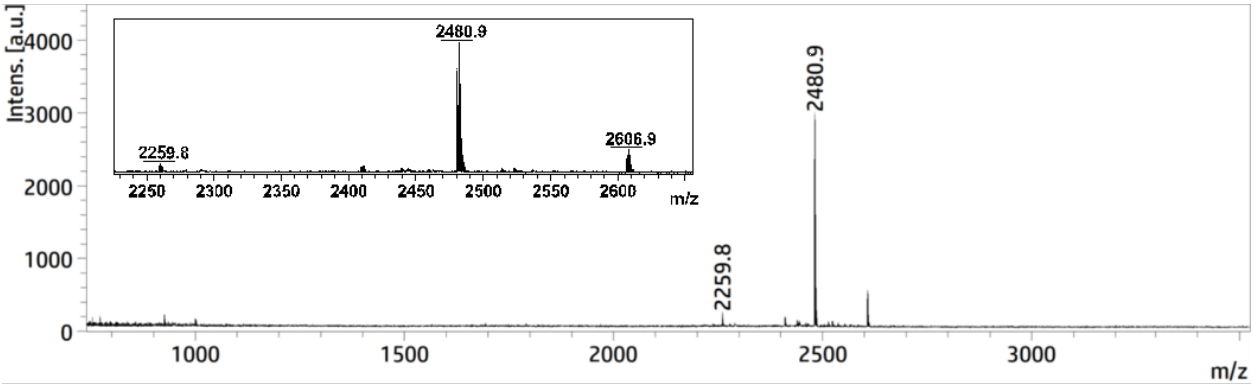
Spectrum: 5
Peptide ID: GP1.2
Internal ID: MBGP-08-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPAV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2453.5



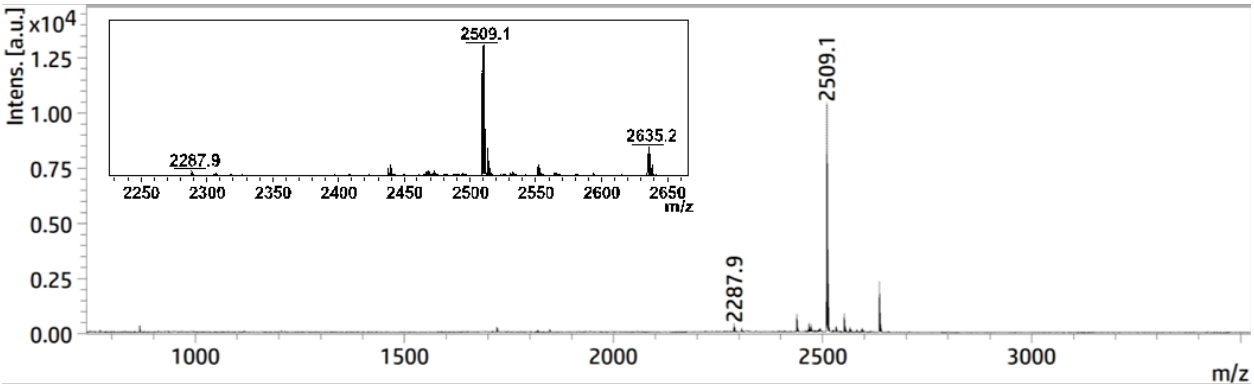
Spectrum: 6
Peptide ID: GP1.3
Internal ID: MBGP-09-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPLV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2495.3



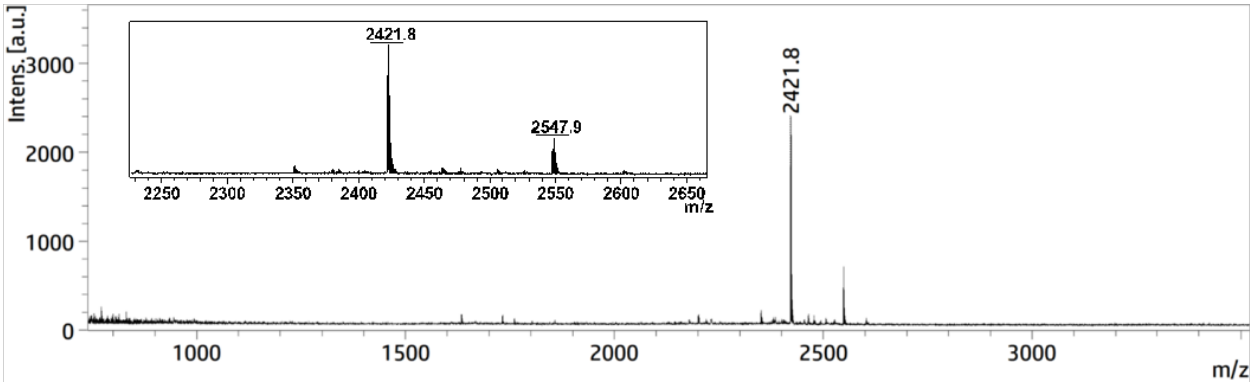
Spectrum: 7
Peptide ID: GP1.4
Internal ID: MBGP-10-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPT{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2481.2



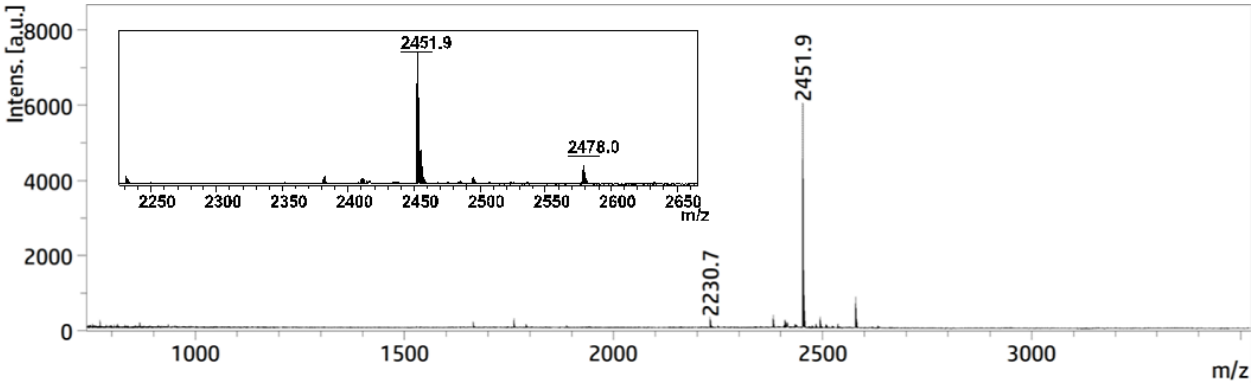
Spectrum: 8
Peptide ID: GP1.5
Internal ID: MBGP-11-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPE{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2509.3



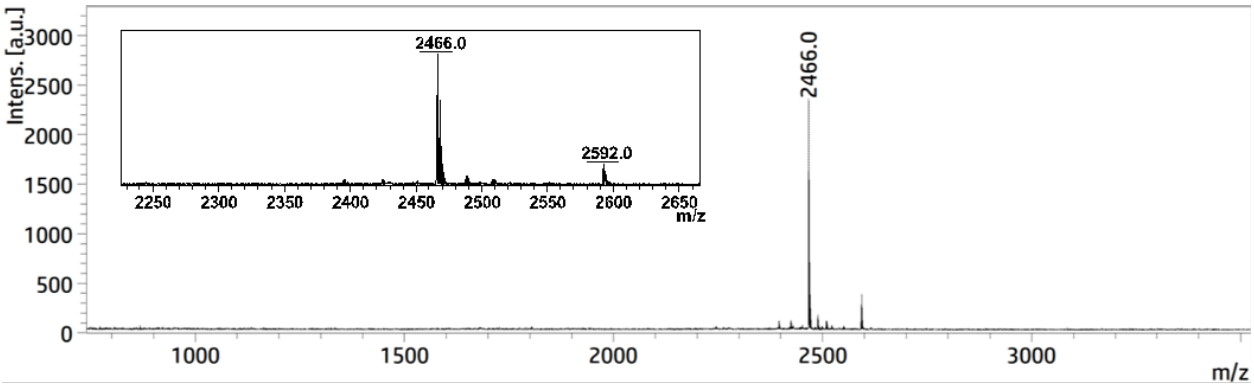
Spectrum: 9
Peptide ID: GP1.6
Internal ID: MBGP-12-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}AASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2422.0



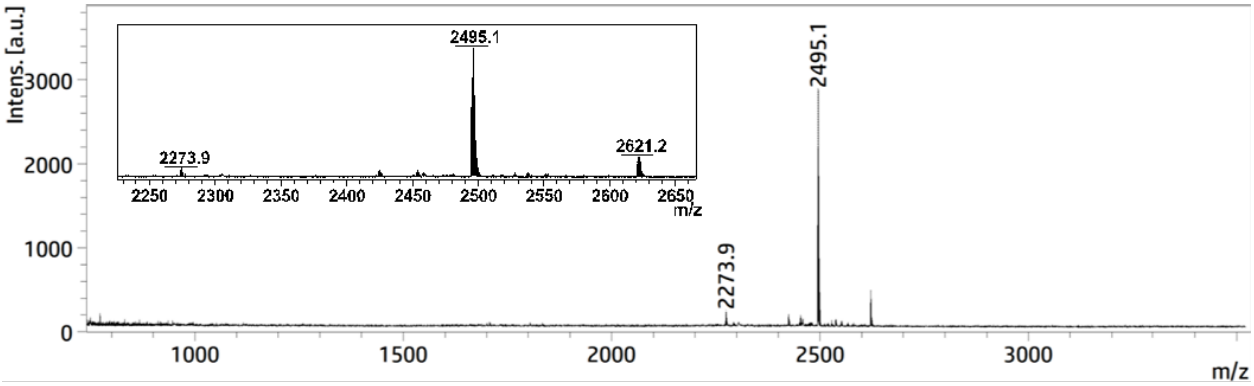
Spectrum: 10
Peptide ID: GP1.7
Internal ID: MBGP-13-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}TASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2452.0



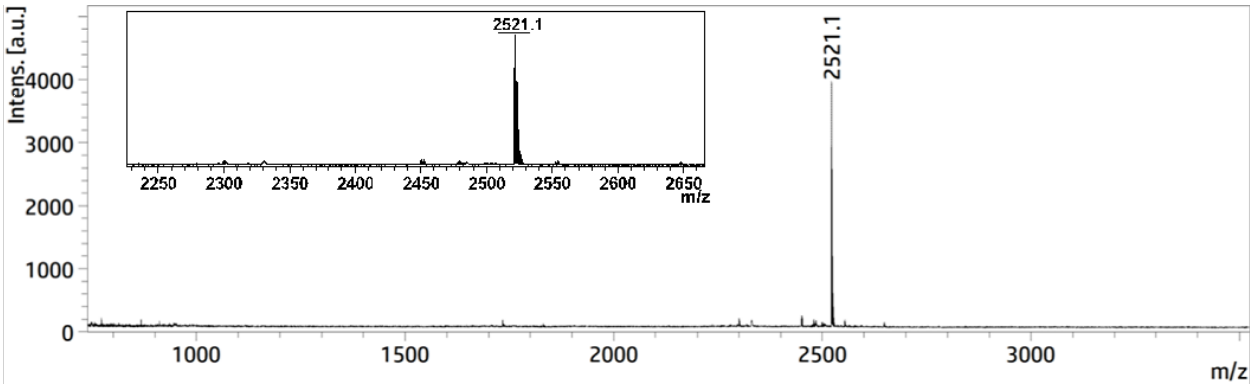
Spectrum: 11
Peptide ID: GP1.8
Internal ID: MBGP-14-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}DASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2466.0



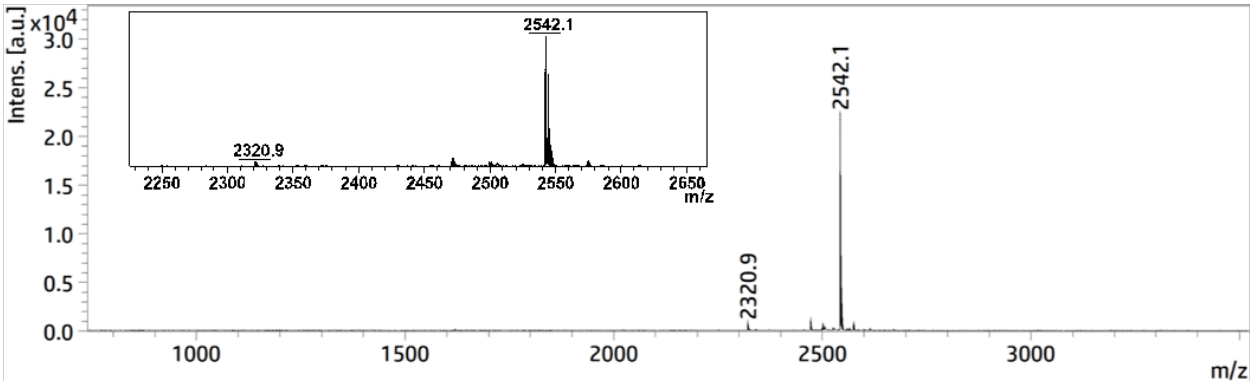
Spectrum: 12
Peptide ID: GP1.9
Internal ID: MBGP-15-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QSSSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2495.1



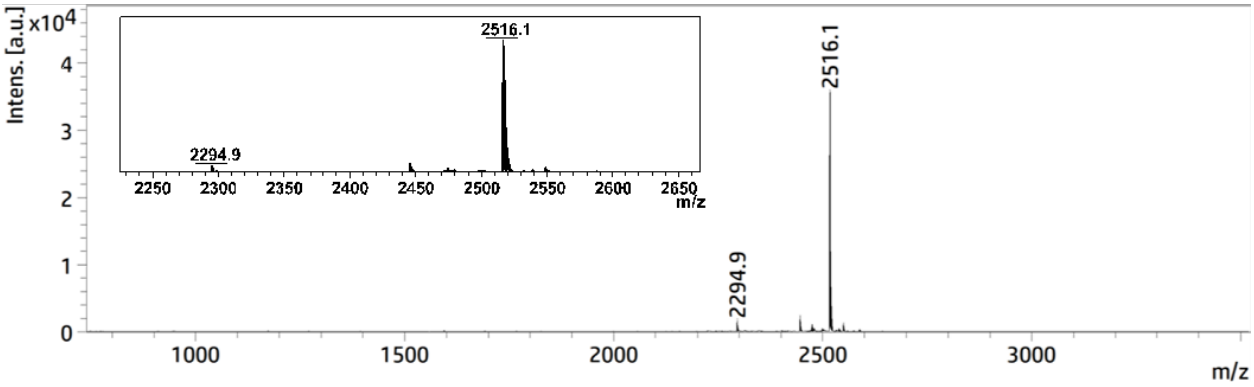
Spectrum: 13
Peptide ID: GP1.10
Internal ID: MBGP-16-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QLSSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2521.1



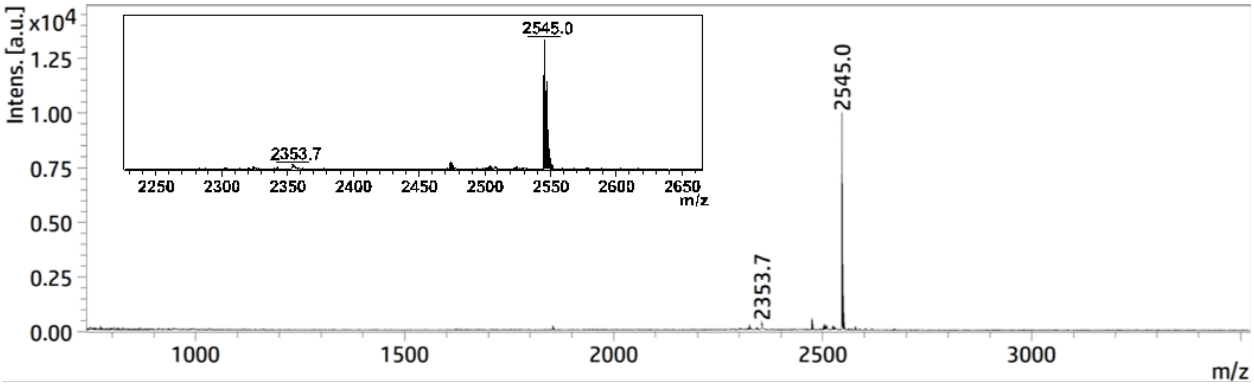
Spectrum: 14
Peptide ID: GP1.11
Internal ID: MBGP-17-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTRTPPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+H]^+$ = 2542.2



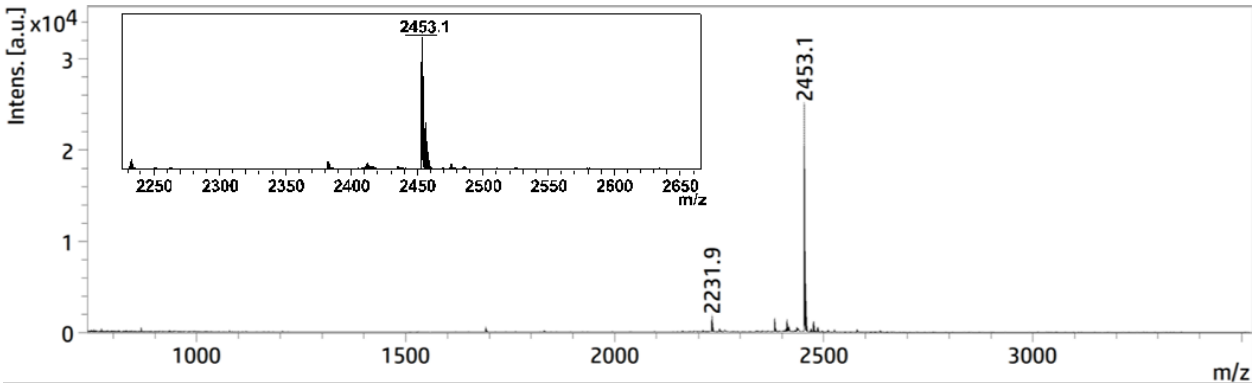
Spectrum: 15
Peptide ID: GP1.12
Internal ID: MBGP-18-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATRPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+H]^+$ = 2515.8



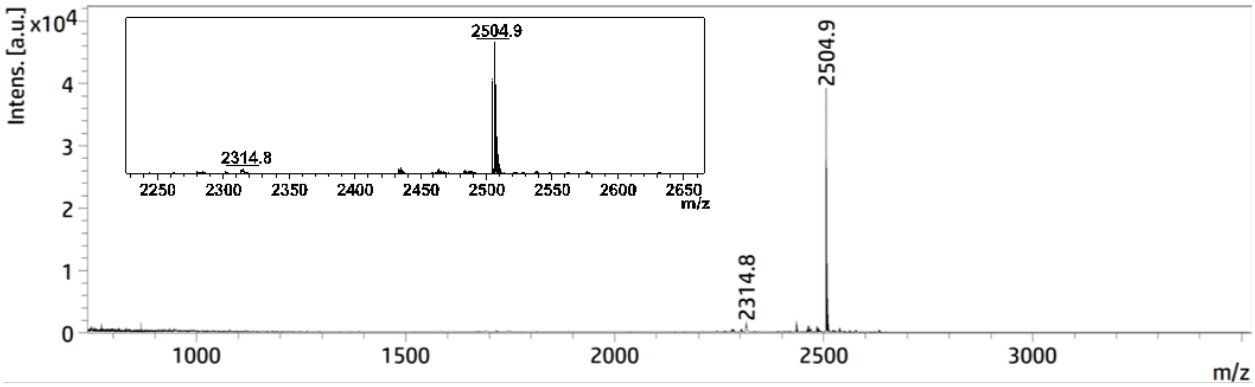
Spectrum: 16
Peptide ID: GP1.13
Internal ID: MBGP-19-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPYV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2545.1



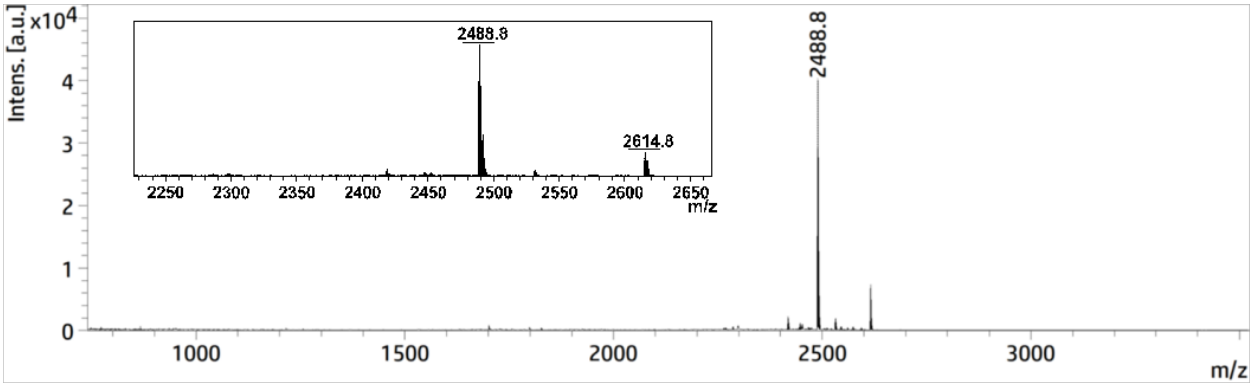
Spectrum: 17
Peptide ID: GP1.14
Internal ID: MBGP-20-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATAPV{GlcNAcb1-S}QASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2453.0



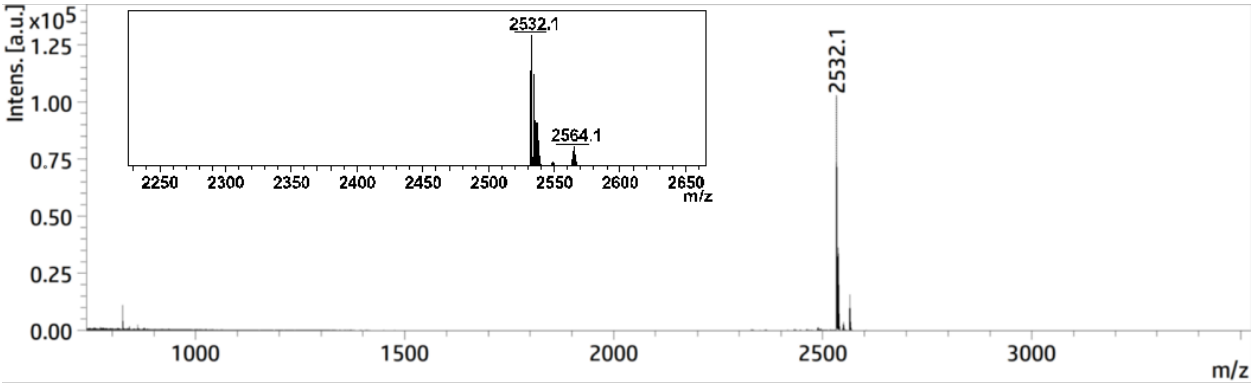
Spectrum: 18
Peptide ID: GP1.15
Internal ID: MBGP-21-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QPSSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2505.1



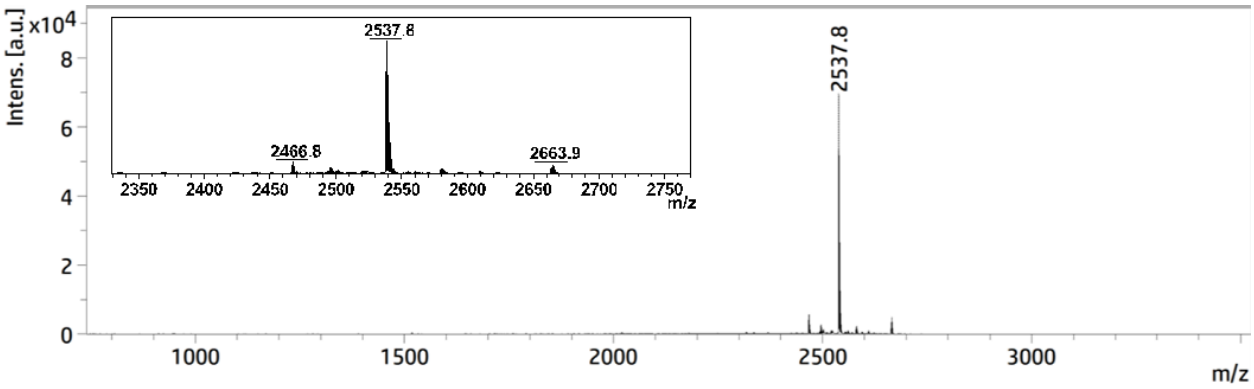
Spectrum: 19
Peptide ID: GP1.16
Internal ID: MBGP-22-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPV{GlcNAcb1-S}QAPSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2489.1



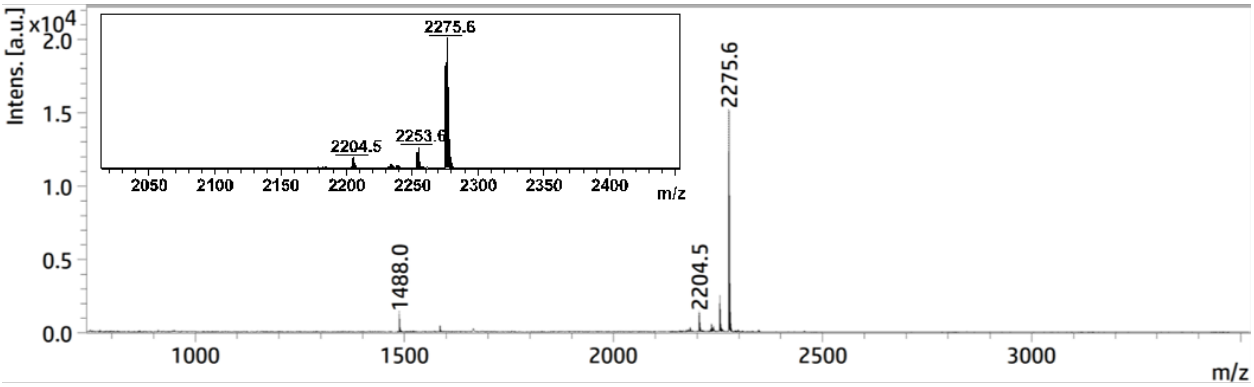
Spectrum: 20
Peptide ID: GP1.17
Internal ID: MBGP-23-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATRSV{GlcNAcb1-S}QPSSTTTSTWA-COOH
Calculated Mass $[M+H]^+$ = 2531.9



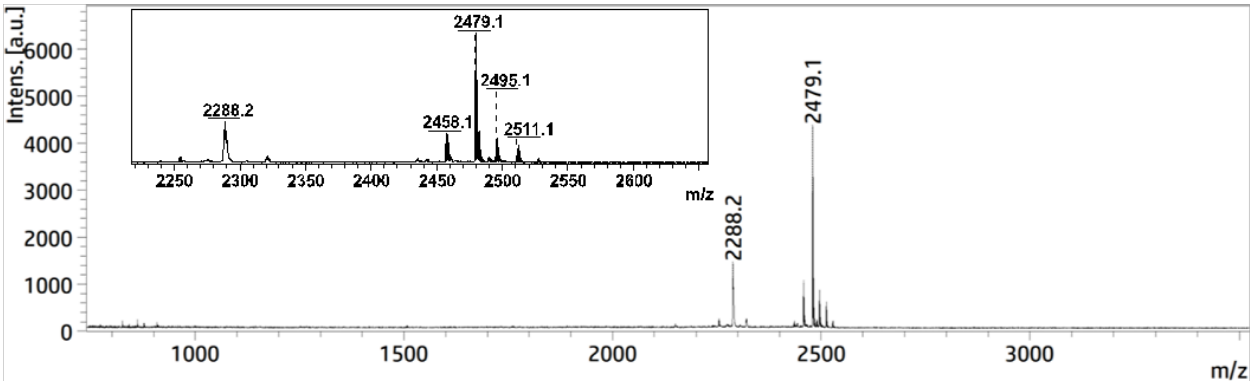
Spectrum: 21
Peptide ID: GP1.18
Internal ID: MBGP-24-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTARPPV{GlcNAcb1-S}QPSSTTTSTWA-COOH
Calculated Mass $[M+H]^+$ = 2537.9



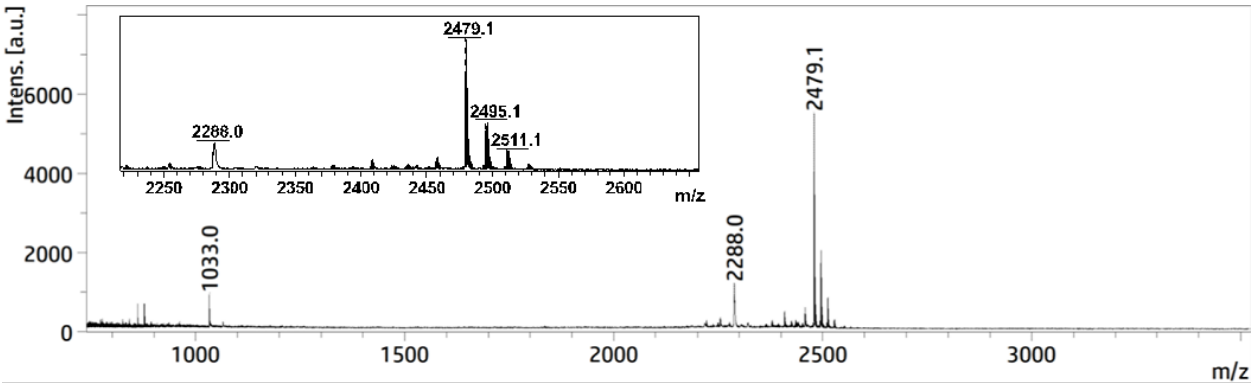
Spectrum: 22
Peptide ID: GP1.19 (non-glycosylated)
Internal ID: MBGP-25-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTATPPVSQASSTTTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2275.9



Spectrum: 23
Peptide ID: GP1.20 (Scrambled Sequence)
Internal ID: MBGP-26-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KPSTTASPTT{GlcNAcb1-S}QATSSTTVTWA-COOH
Calculated Mass $[M+Na]^+$ = 2479.1



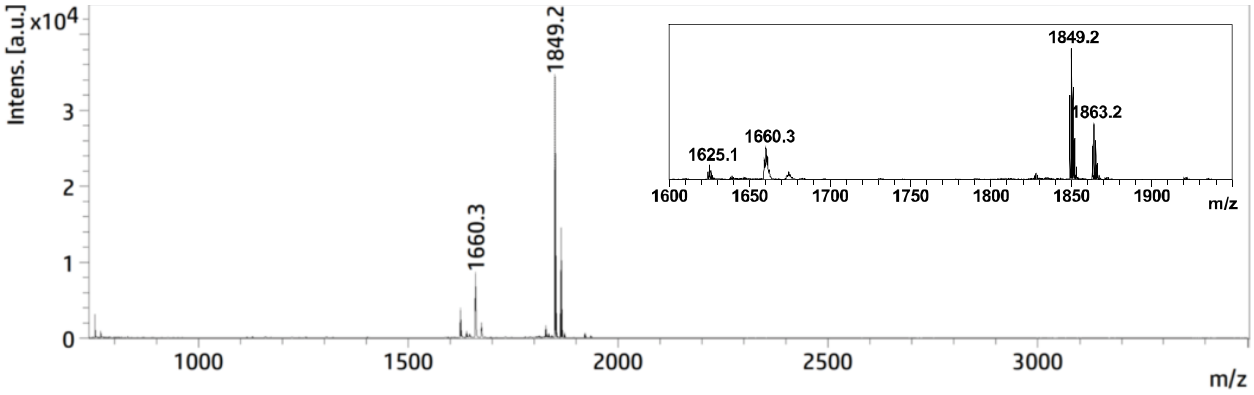
Spectrum: 24
Peptide ID: GP1.21 (Reversed Sequence)
Internal ID: MBGP-27-HPLC
Purification Level: HPLC purified
Sequence: AcNH-KTSTTTSSAQ{GlcNAcb1-S}VPPTATTSTWA-COOH
Calculated Mass $[M+Na]^+$ = 2479.1



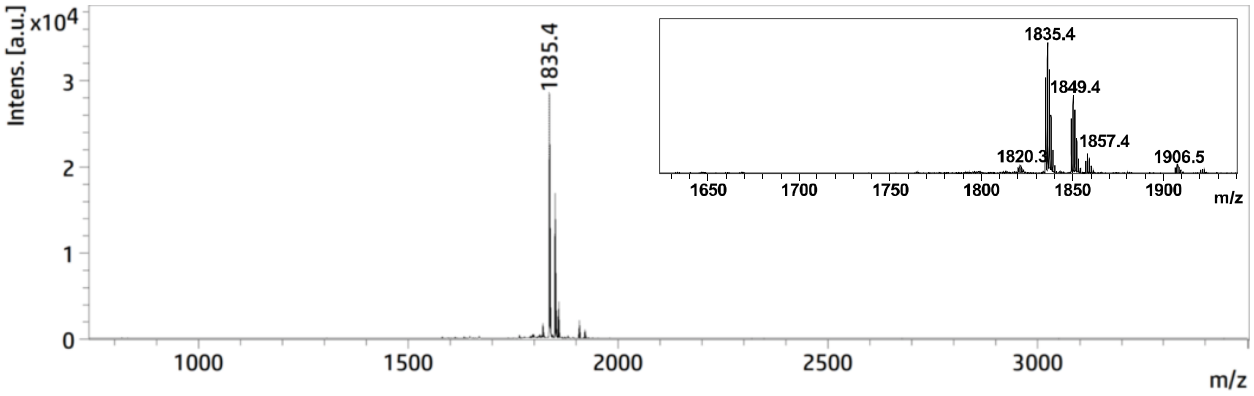
GlycoPeptide 2 library (GP2)

Note- minor peaks for peptides ending with $–OCH_3$ (-COOMe) C-terminus also observed with +14 Da caused due to using methanol:water mixture which results in better cleavage but forms ester at C-terminus.

Spectrum: 25
Peptide ID: GP2.1
Internal ID: GP2.1
Purification Level: SepPak C18 purified
Sequence: FINH-KVPS{GalNAca1-T}PPTPSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1849.4

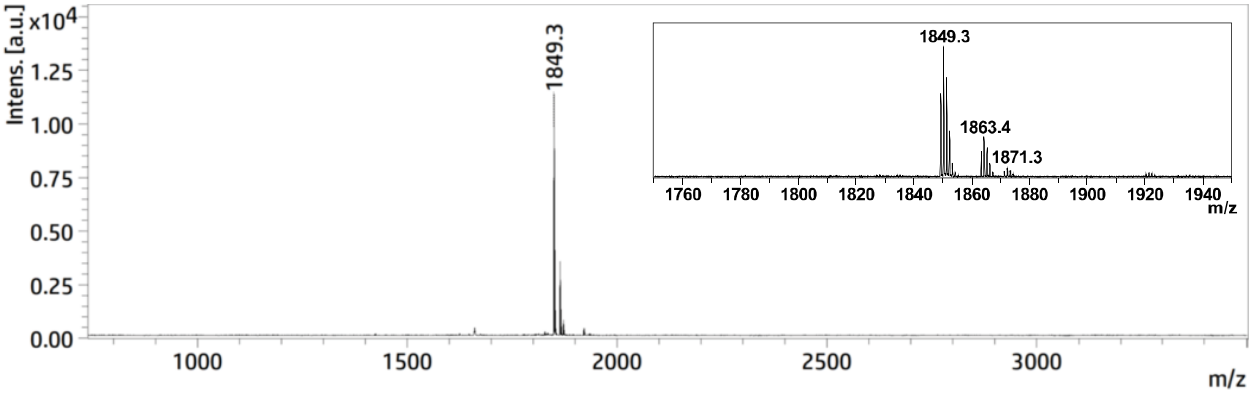


Spectrum: 26
Peptide ID: GP2.2
Internal ID: GP2.2
Purification Level: SepPak C18 purified
Sequence: FINH-KVPS{GalNAca1-S}PPTPSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1835.4

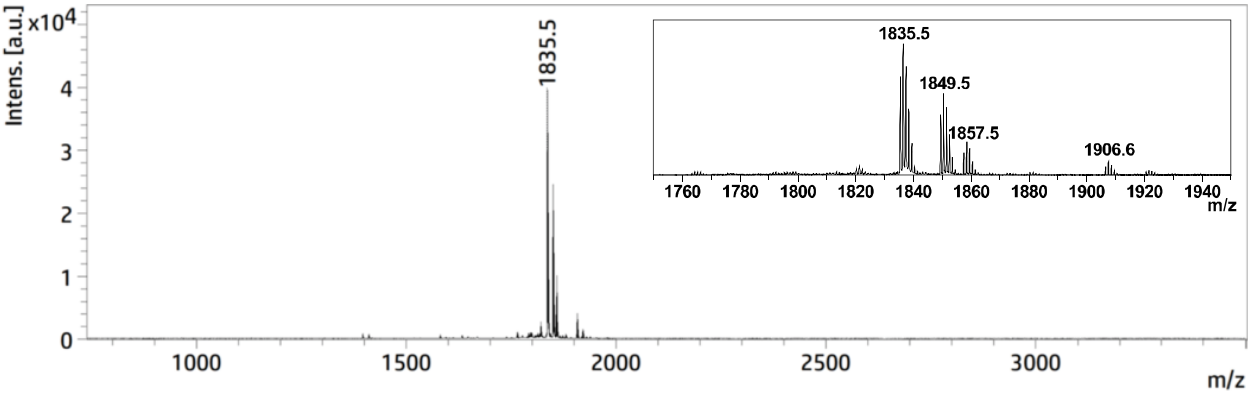


Spectrum: 27
Peptide ID: GP2.3
Internal ID: GP2.3

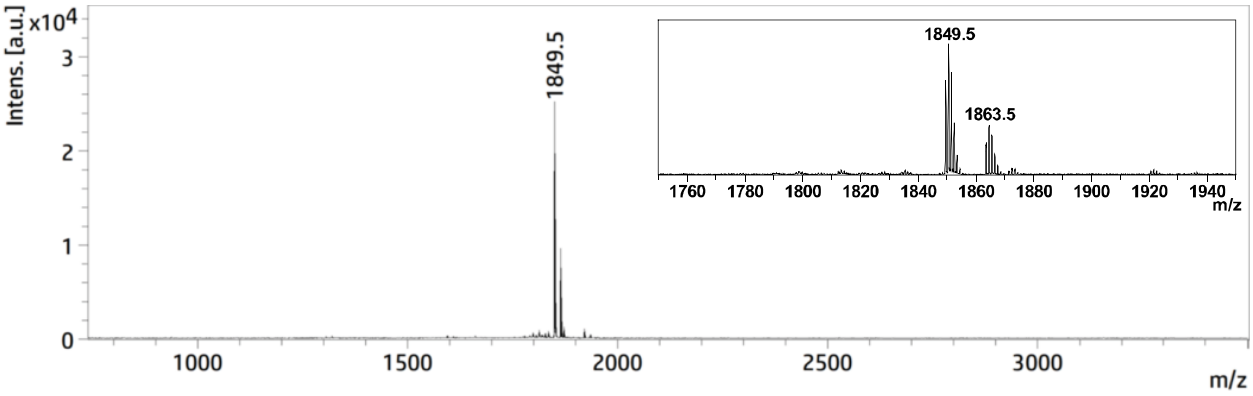
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1849.4



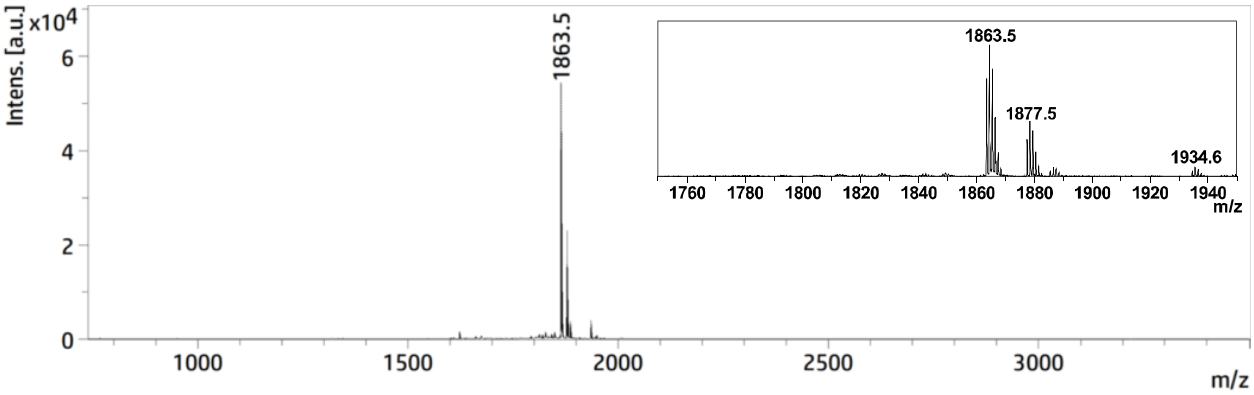
Spectrum: 28
Peptide ID: GP2.4
Internal ID: GP2.4
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAca1-S}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1835.4



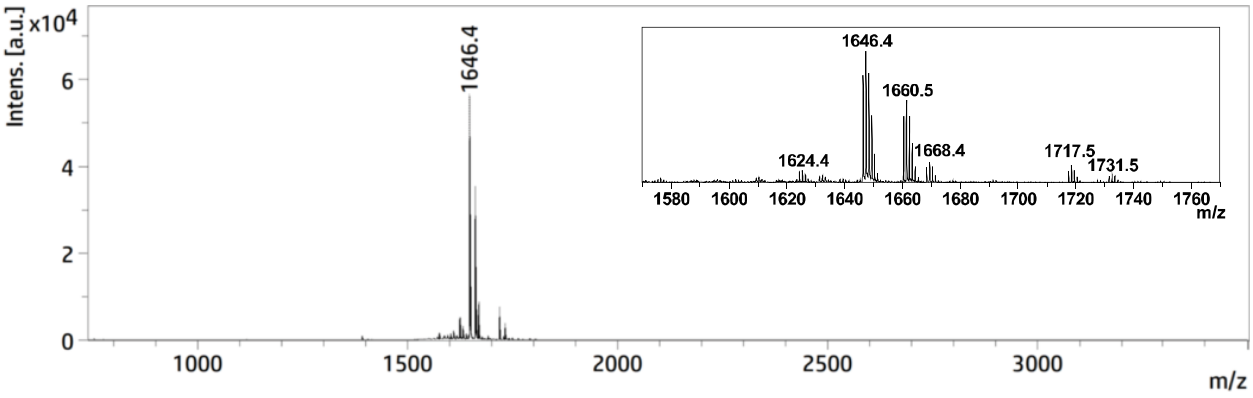
Spectrum: 29
Peptide ID: GP2.5
Internal ID: GP2.5
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPPTP{GalNAca1-S}PSA-COOH
Calculated Mass $[M+Na]^+$ = 1849.4



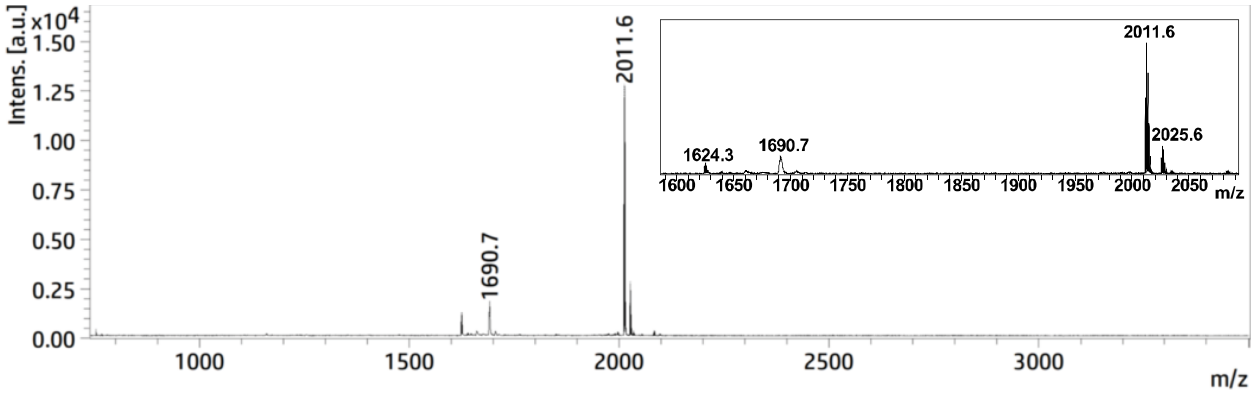
Spectrum: 30
Peptide ID: GP2.6
Internal ID: GP2.6
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPPTP{GalNAca1-T}PSA-COOH
Calculated Mass $[M+Na]^+$ = 1863.4



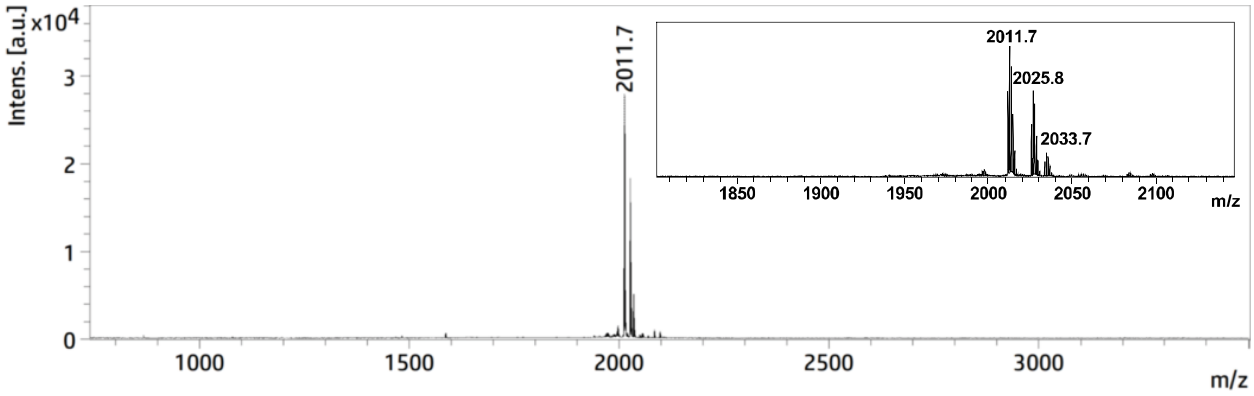
Spectrum: 31
Peptide ID: GP2.7
Internal ID: GP2.7
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPPTPSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1646.2



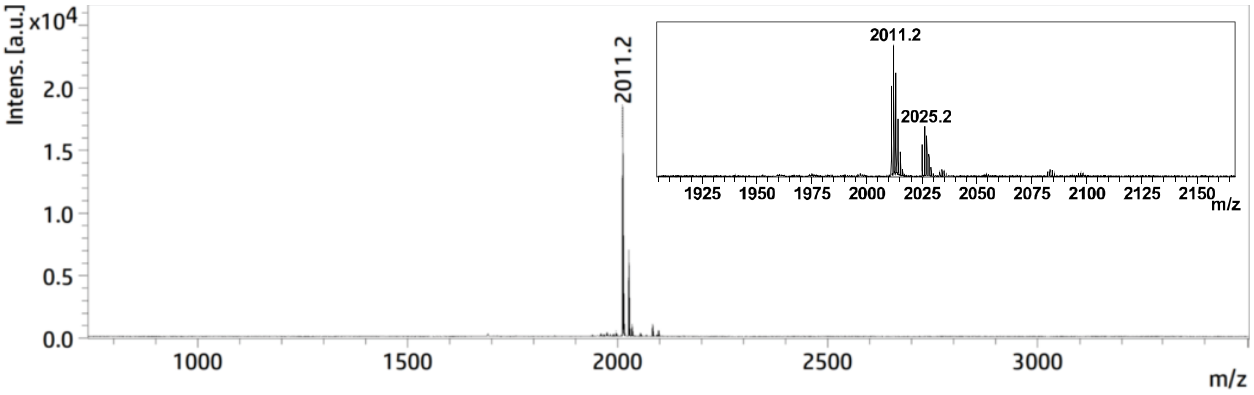
Spectrum: 32
Peptide ID: GP2.8
Internal ID: GP2.8
Purification Level: SepPak C18 purified
Sequence: FINH-KVPS{Galb1-3GalNAca1-T}PPTPSPSA-COOH
Calculated Mass $[M+Na]^+$ = 2011.6



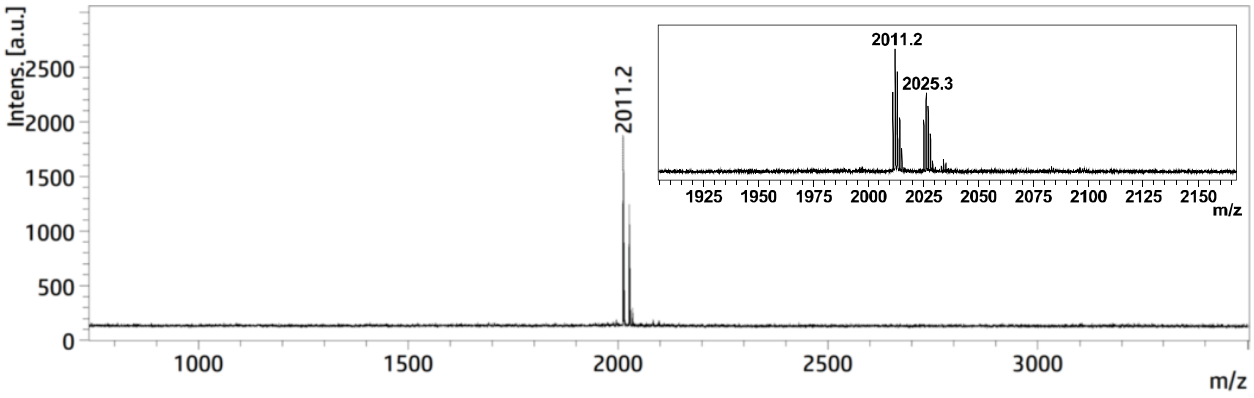
Spectrum: 33
Peptide ID: GP2.9
Internal ID: GP2.9
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{Galb1-3GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 2011.6



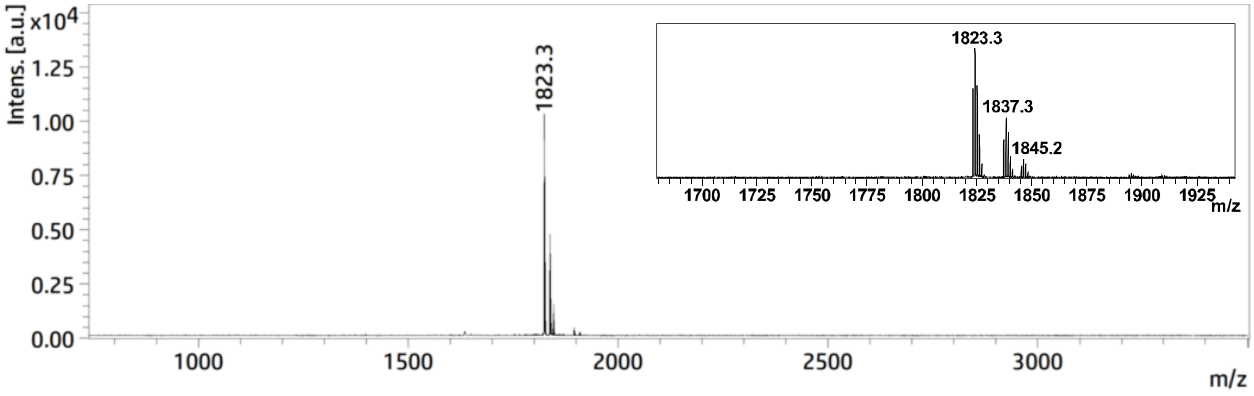
Spectrum: 34
Peptide ID: GP2.10
Internal ID: GP2.10
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPPTP{Galb1-3GalNAca1-S}PSA-COOH
Calculated Mass $[M+Na]^+$ = 2011.6



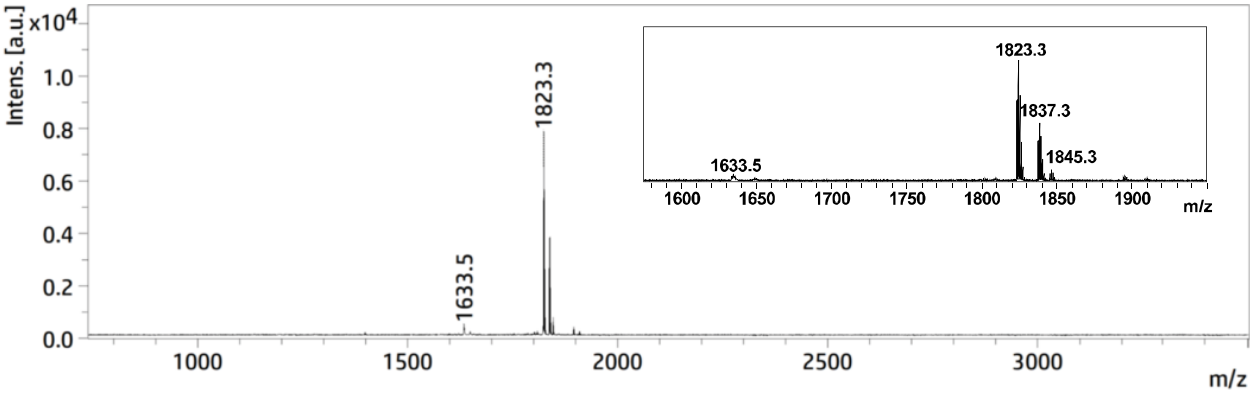
Spectrum: 35
Peptide ID: GP2.11
Internal ID: GP2.11
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{Galb1-3GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 2011.6



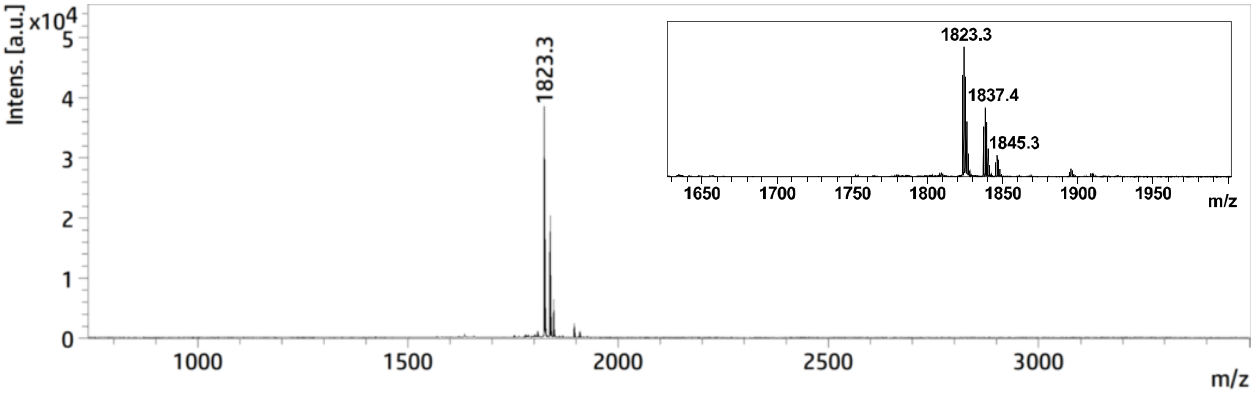
Spectrum: 36
Peptide ID: GP2.12
Internal ID: GP2.12
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTAP{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1822.9



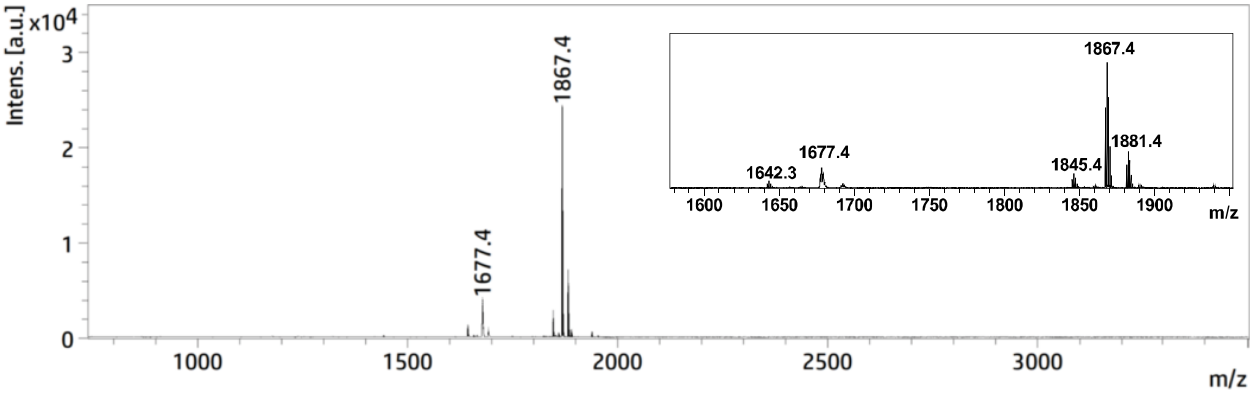
Spectrum: 37
Peptide ID: GP2.13
Internal ID: GP2.13
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPA{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1822.9



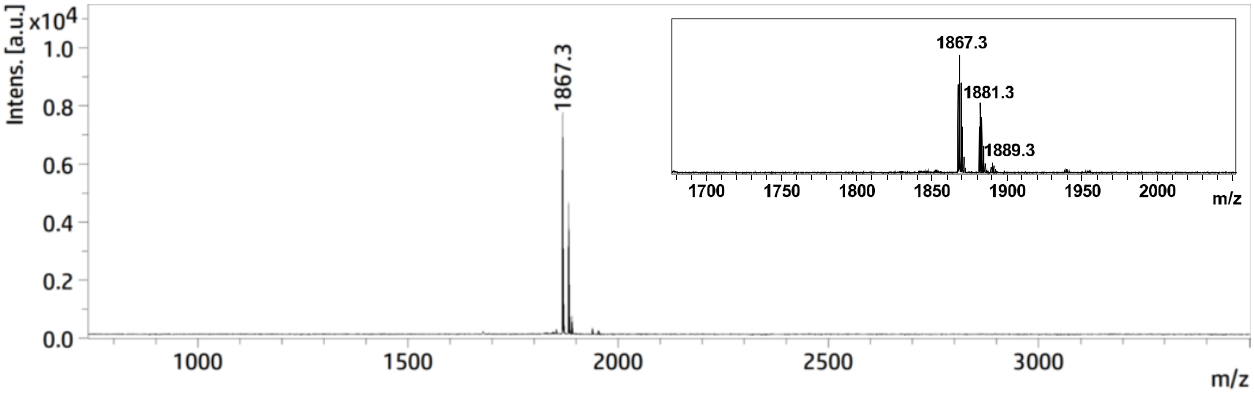
Spectrum: 38
Peptide ID: GP2.14
Internal ID: GP2.14
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAca1-T}ASPSA-COOH
Calculated Mass $[M+Na]^+$ = 1822.9



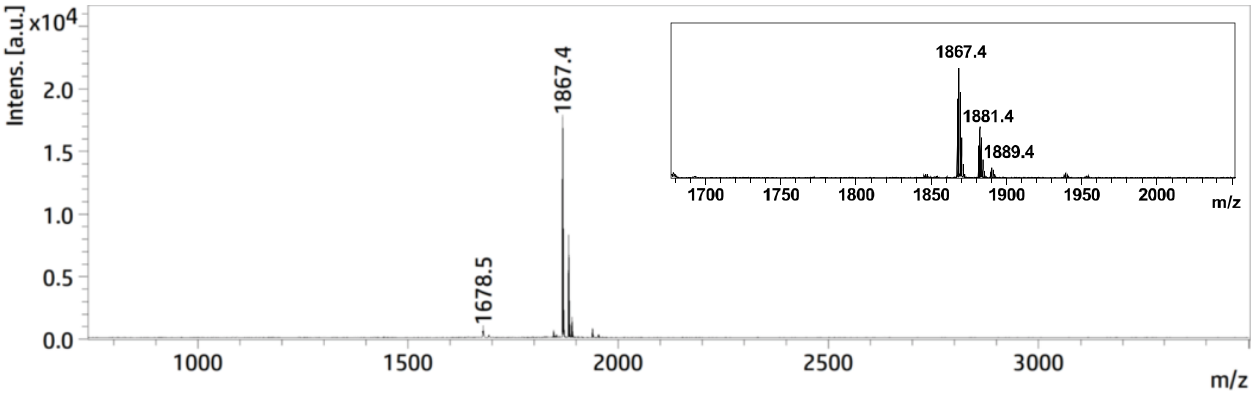
Spectrum: 39
Peptide ID: GP2.15
Internal ID: GP2.15
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTXP{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



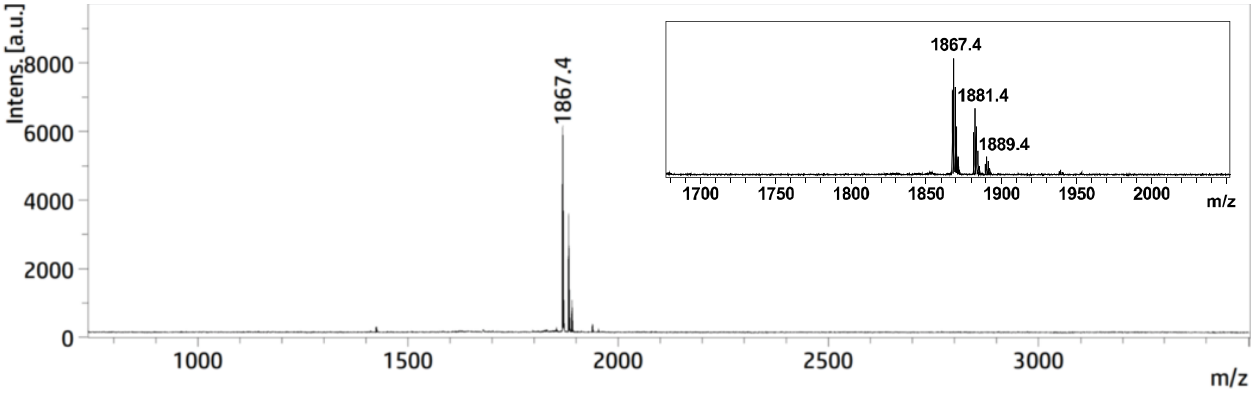
Spectrum: 40
Peptide ID: GP2.16
Internal ID: GP2.16
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPX{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



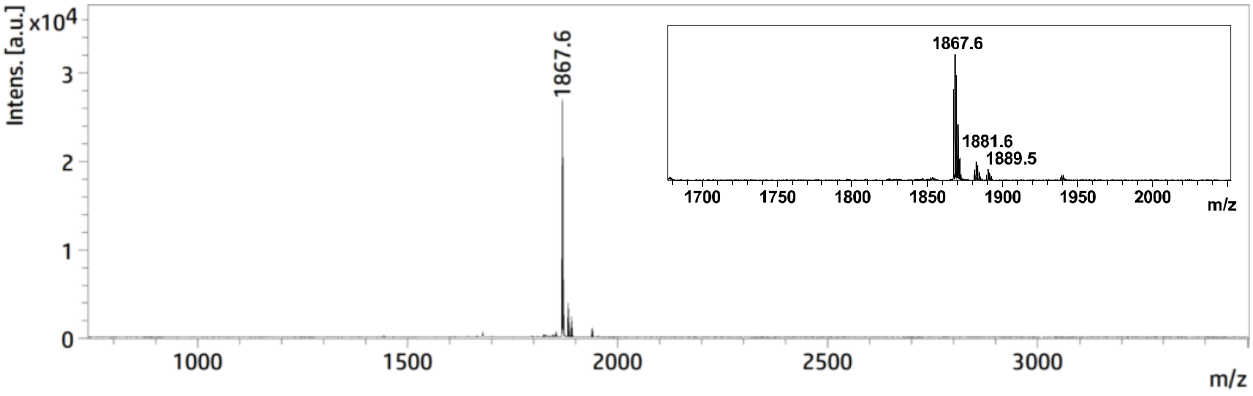
Spectrum: 41
Peptide ID: GP2.17
Internal ID: GP2.17
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAca1-T}XSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



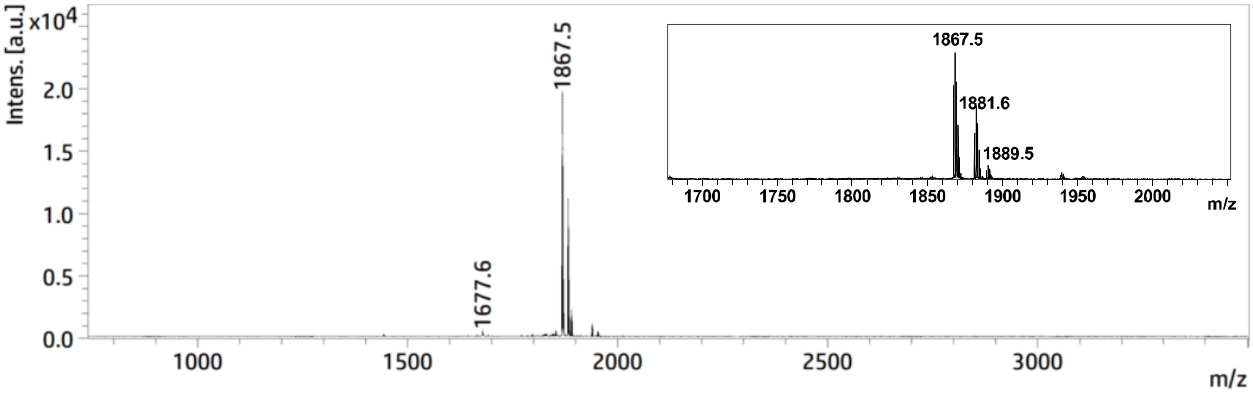
Spectrum: 42
Peptide ID: GP2.18
Internal ID: GP2.18
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTxP{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



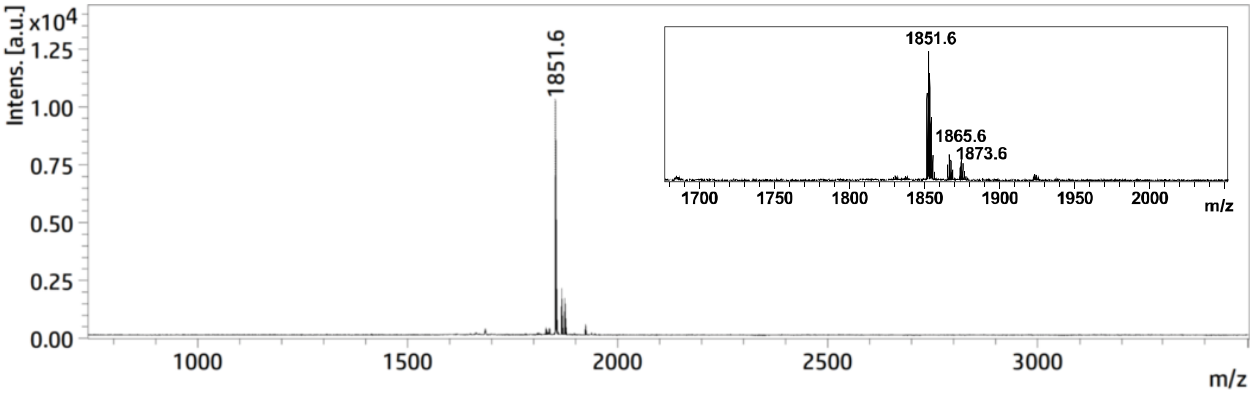
Spectrum: 43
Peptide ID: GP2.19
Internal ID: GP2.19
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPx{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



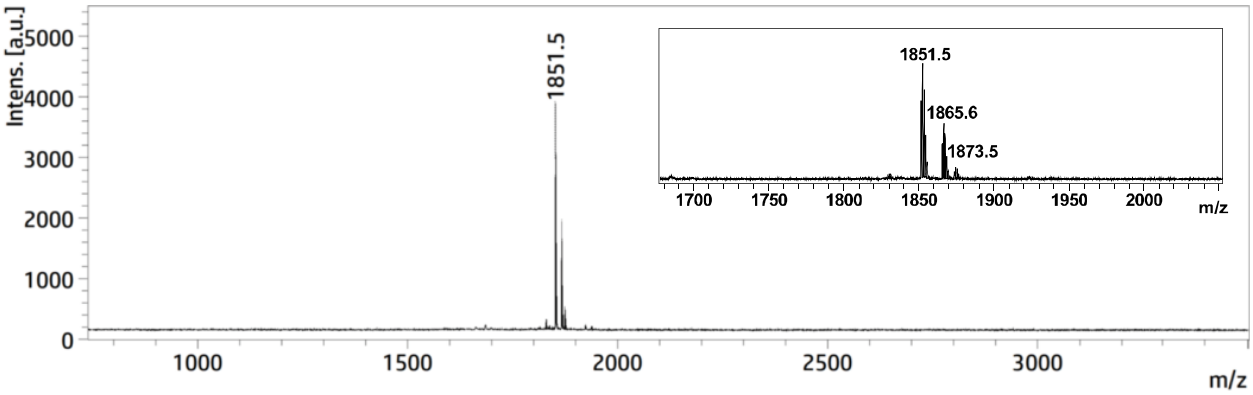
Spectrum: 44
Peptide ID: GP2.20
Internal ID: GP2.20
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAca1-T}xSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1866.9



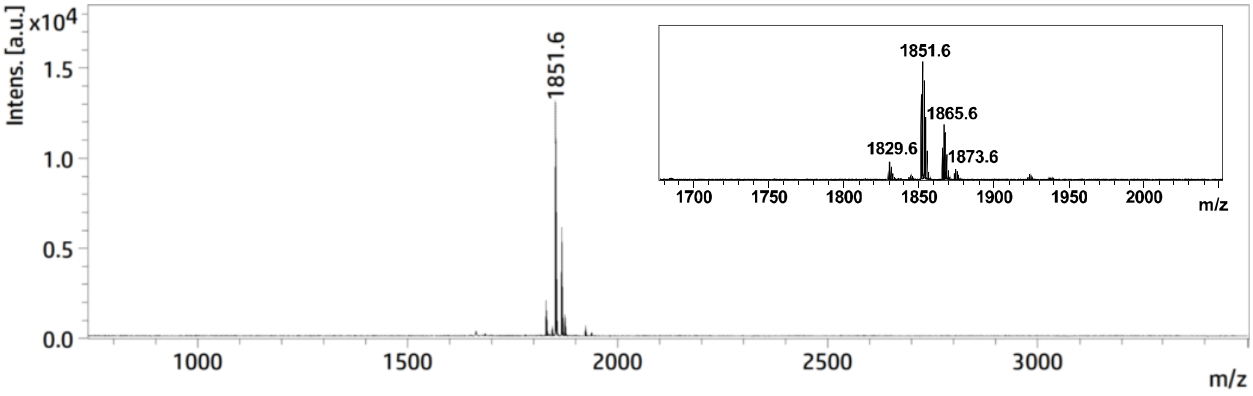
Spectrum: 45
Peptide ID: GP2.21
Internal ID: GP2.21
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAcb1-C}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1851.5



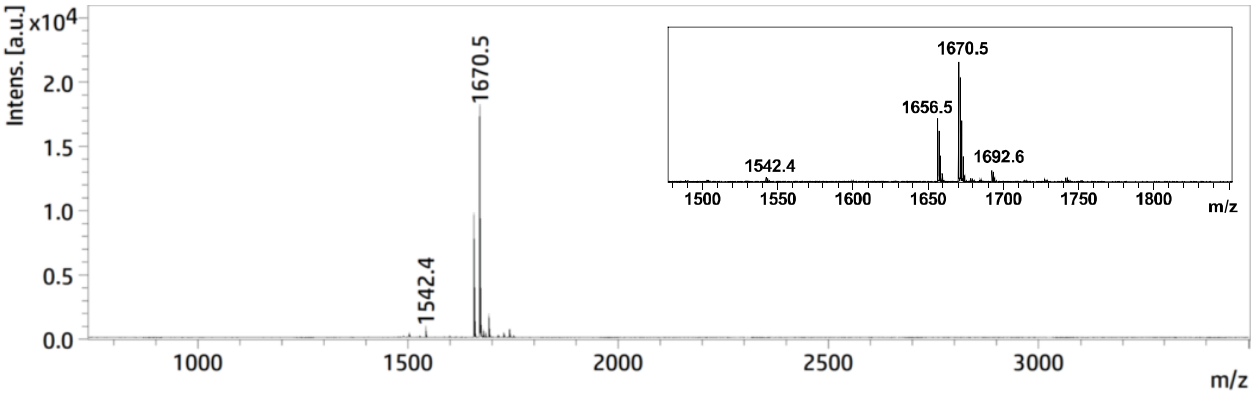
Spectrum: 46
Peptide ID: GP2.22
Internal ID: GP2.22
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GlcNAcb1-C}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1851.5



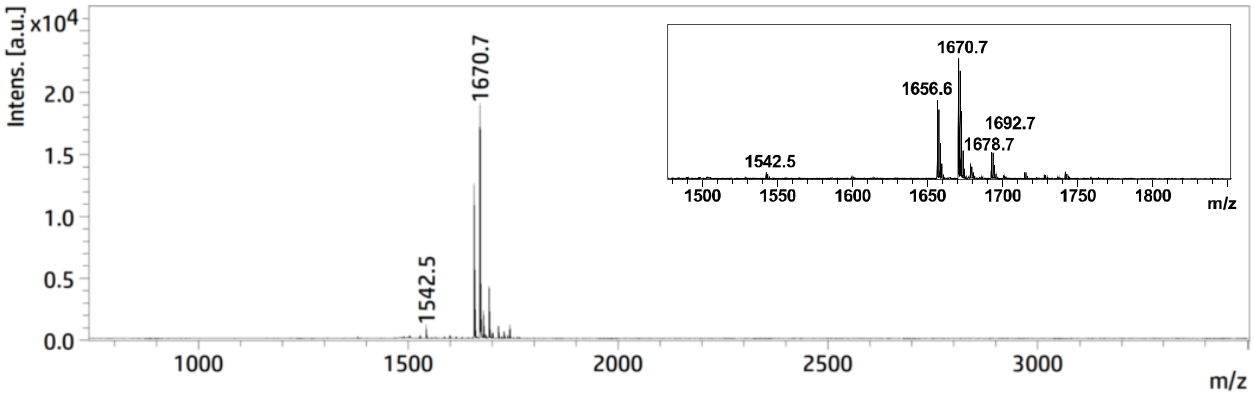
Spectrum: 47
Peptide ID: GP2.23
Internal ID: GP2.23
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{ManNAca1-C}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1851.5



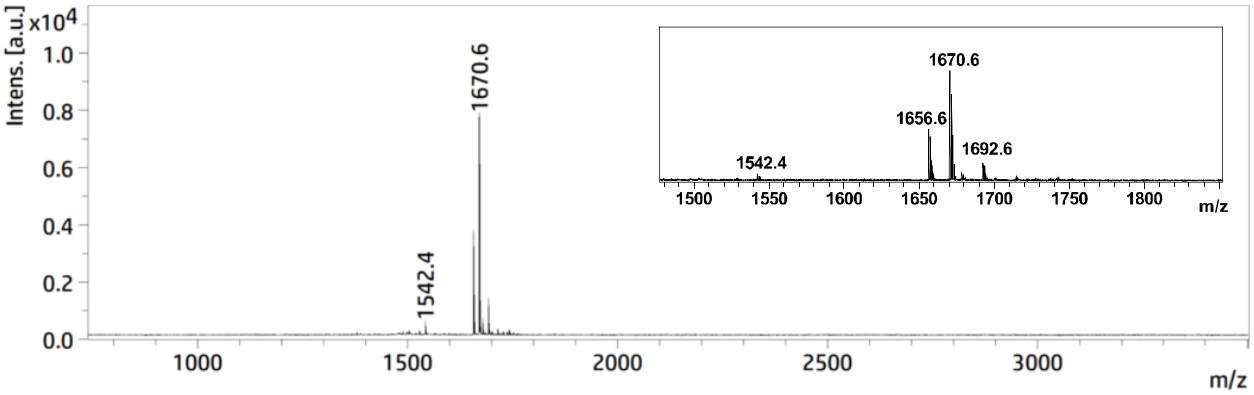
Spectrum: 48
Peptide ID: GP2.24
Internal ID: GP2.24
Purification Level: SepPak C18 purified
Sequence: AcNH-KYLAEVA{GalNAcb1-C}GDDRA-COOMe
Calculated Mass $[M+H]^+$ = 1670.8



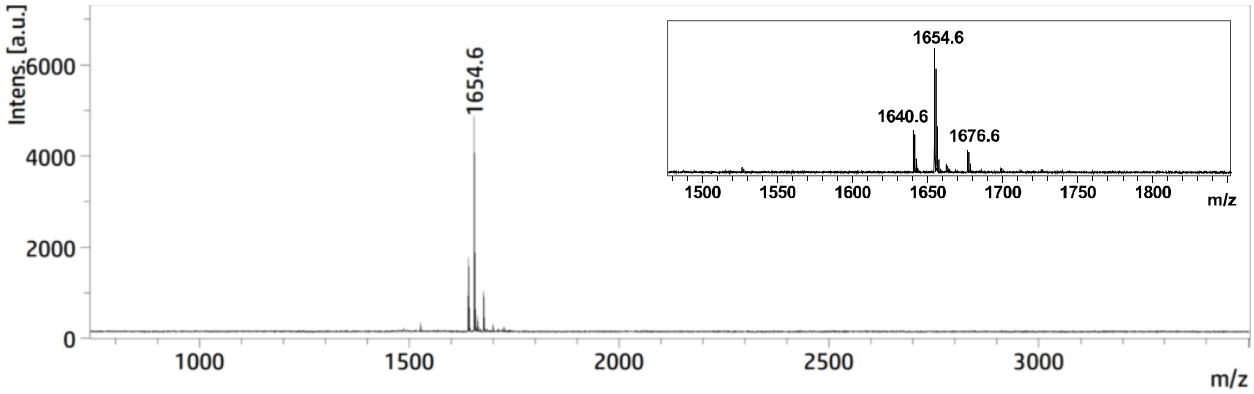
Spectrum: 49
Peptide ID: GP2.25
Internal ID: GP2.25
Purification Level: SepPak C18 purified
Sequence: AcNH-KYLAEVA{GlcNAcb1-C}GDDRA-COOMe
Calculated Mass $[M+H]^+$ = 1670.8



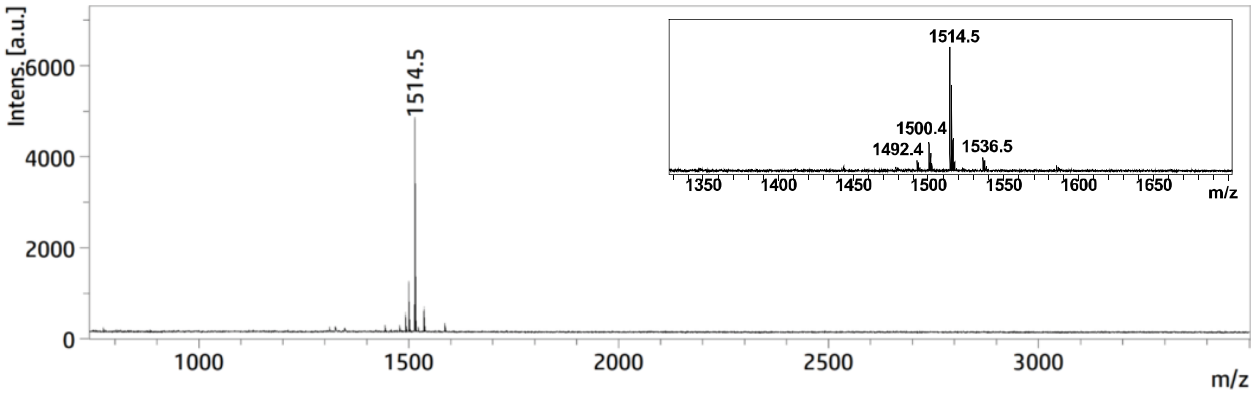
Spectrum: 50
Peptide ID: GP2.26
Internal ID: GP2.26
Purification Level: SepPak C18 purified
Sequence: AcNH-KYLAEVA{ManNAca1-C}GDDRA-COOMe
Calculated Mass $[M+H]^+$ = 1670.8



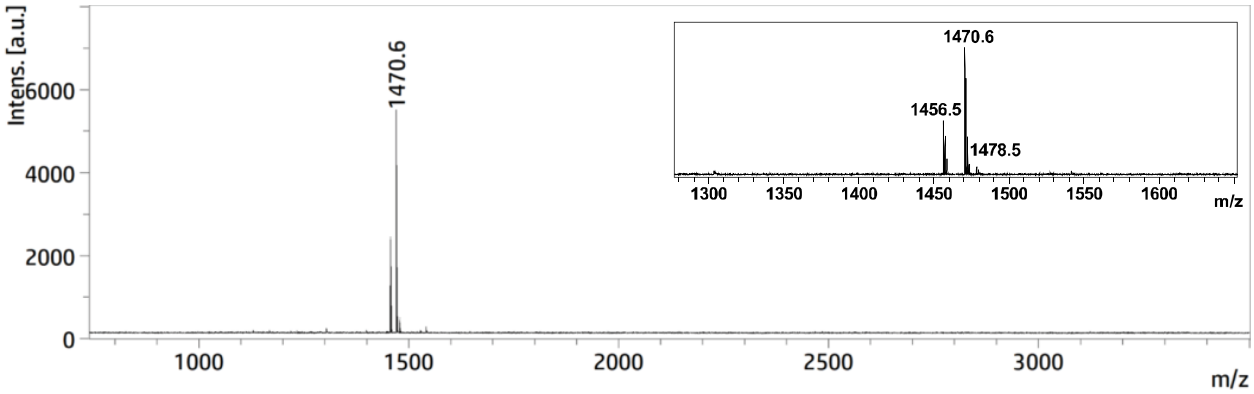
Spectrum: 51
Peptide ID: GP2.27
Internal ID: GP2.27
Purification Level: SepPak C18 purified
Sequence: AcNH-KYLAEVA{GlcNAcb1-S}GDDRA-COOMe
Calculated Mass $[M+H]^+$ = 1654.7



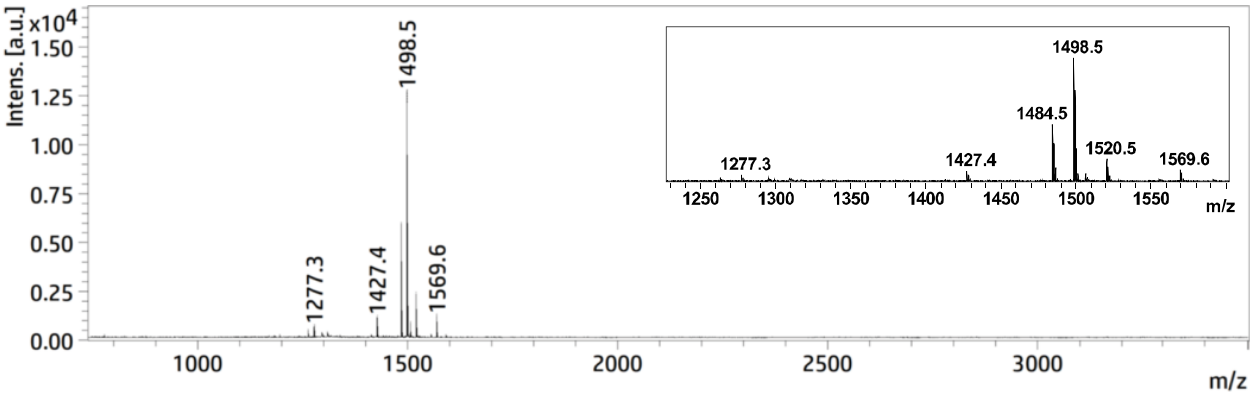
Spectrum: 52
Peptide ID: GP2.28
Internal ID:
Purification Level: SepPak C18 purified
Sequence: AcNH-KSSPVI{GlcNAcb1-C}AGGQDA-COOMe
Calculated Mass $[M+Na]^+$ = 1514.6



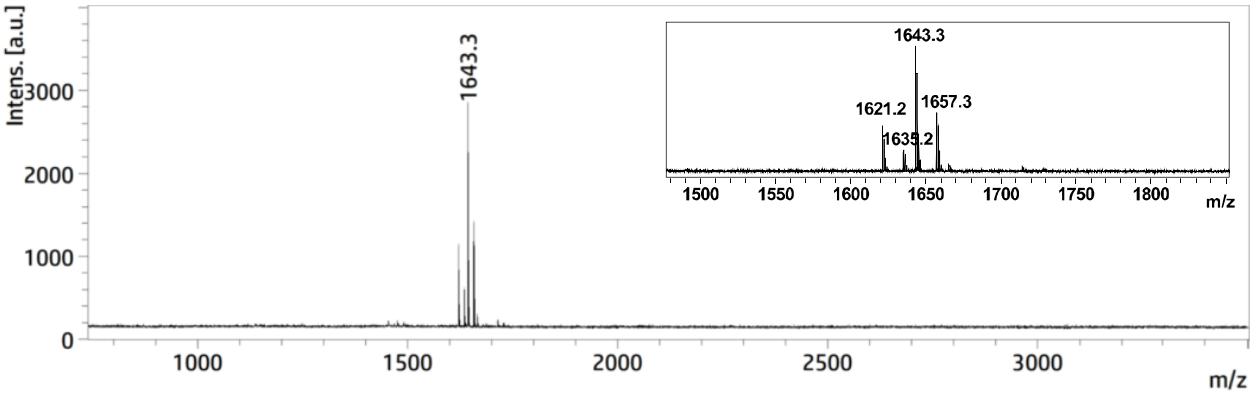
Spectrum: 53
Peptide ID: GP2.29
Internal ID: GP2.29
Purification Level: SepPak C18 purified
Sequence: AcNH-KSSPVI{GlcNAcb1-C}AGGQAA-COOMe
Calculated Mass $[M+Na]^+$ = 1470.6



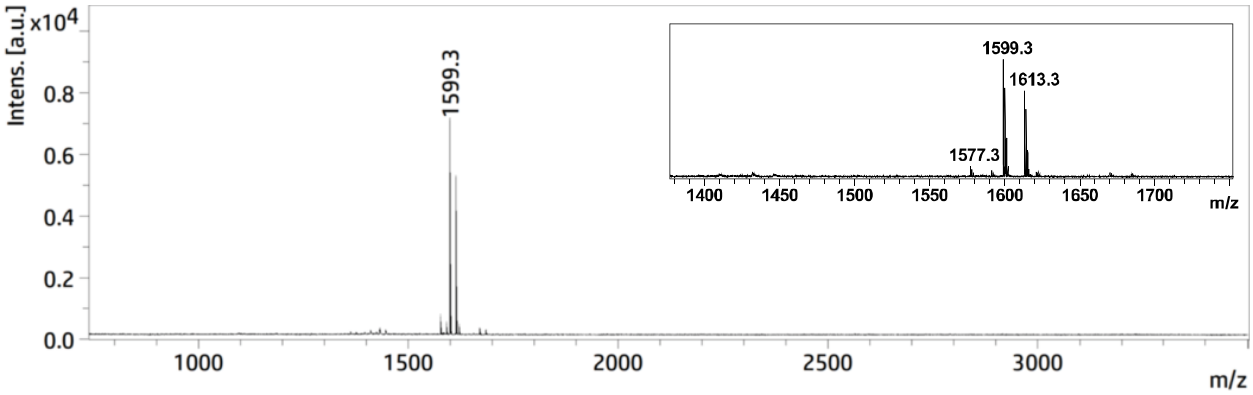
Spectrum: 54
Peptide ID: GP2.30
Internal ID: GP2.30
Purification Level: SepPak C18 purified
Sequence: AcNH-KSSPVI{GlcNAcb1-S}AGGQDA-COOMe
Calculated Mass $[M+Na]^+$ = 1498.6



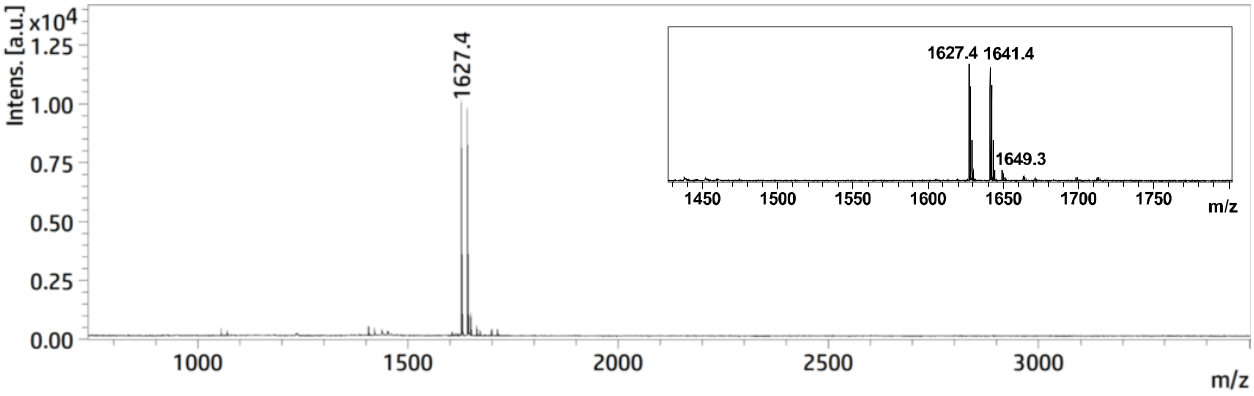
Spectrum: 55
Peptide ID: GP2.31
Internal ID: GP2.31
Purification Level: SepPak C18 purified
Sequence: AcNH-KTTKIP{GlcNAcb1-C}DSPQSA-COOH
Calculated Mass $[M+Na]^+$ = 1643.3



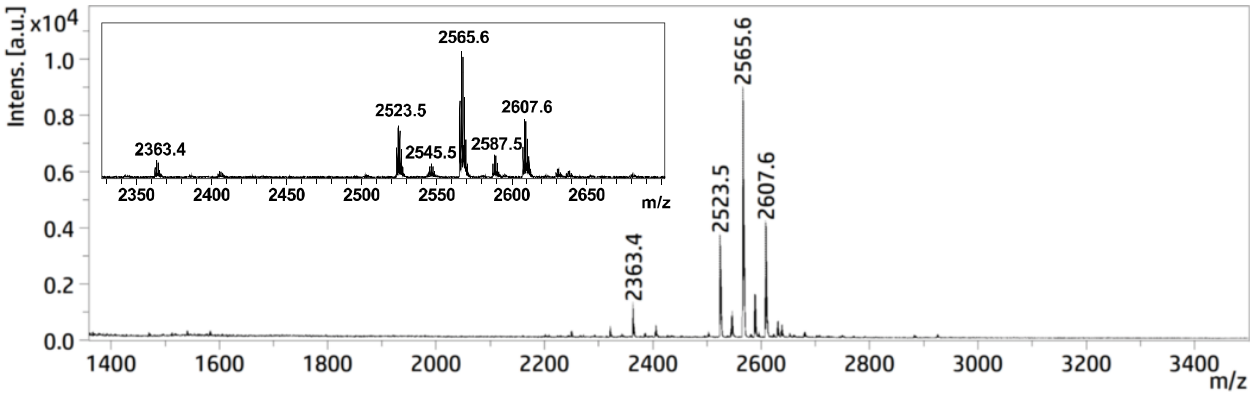
Spectrum: 56
Peptide ID: GP2.32
Internal ID: GP2.32
Purification Level: SepPak C18 purified
Sequence: AcNH-KTTKIP{GlcNAcb1-C}ASPQSA-COOH
Calculated Mass $[M+Na]^+$ = 1599.3



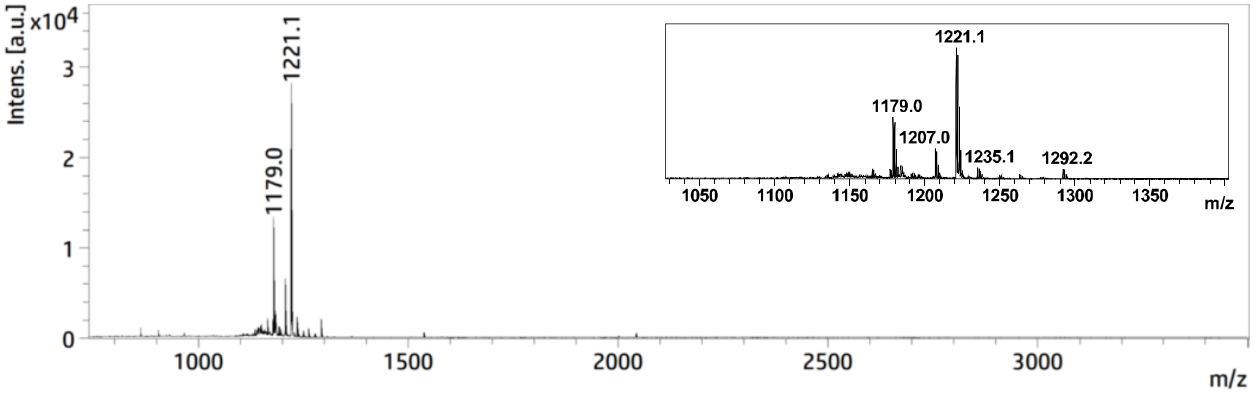
Spectrum: 57
Peptide ID: GP2.33
Internal ID: GP2.33
Purification Level: SepPak C18 purified
Sequence: AcNH-KTTKIP{GlcNAcb1-S}DSPQSA-COOH
Calculated Mass $[M+Na]^+$ = 1627.2



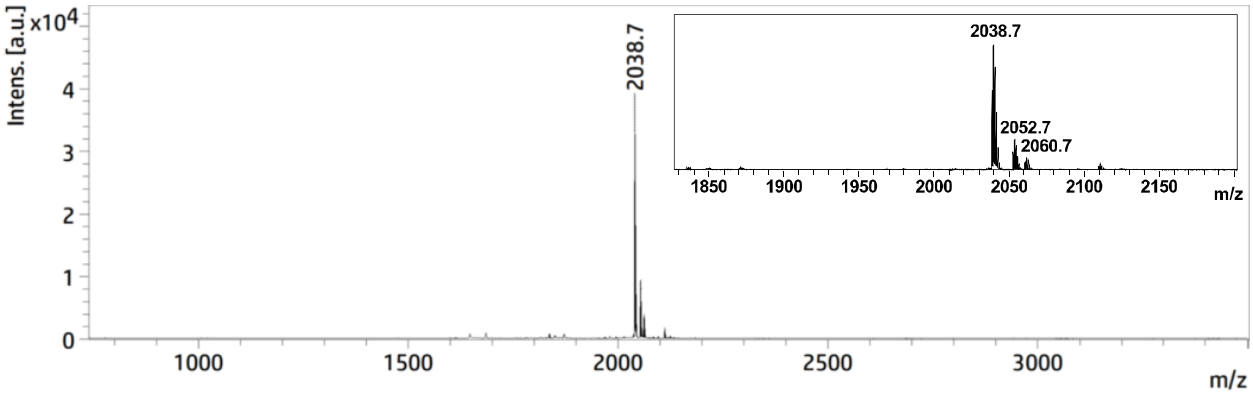
Spectrum: 58
Peptide ID: GP2.34
Internal ID: GP2.34
Purification Level: SepPak C18 purified
Sequence: FlNH-KVAL{GlcNAcb1-2Mana1-6(GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb1-N}KTA-COOH
Calculated Mass $[M+Na]^+$ = 2523.5 | $[M+Ac+Na]^+$ = 2565.5 | $[M+2Ac+Na]^+$ = 2607.5
Notes: The extra acetylation was caused as a result of N-capping step performed on the membrane while this spot was still on it. As a result the acetyls attached onto the fluorescein hydroxyl groups.



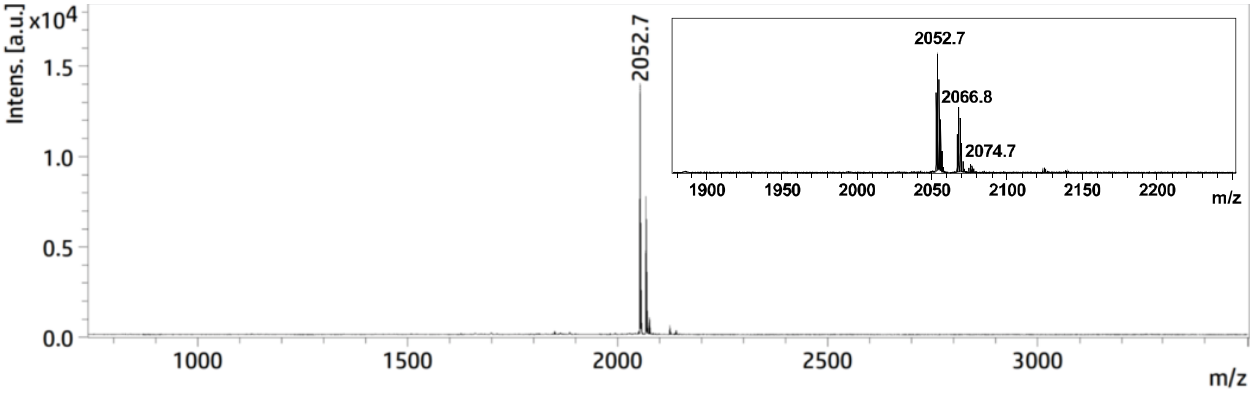
Spectrum: 59
Peptide ID: GP2.35
Internal ID: GP2.35
Purification Level: SepPak C18 purified
Sequence: FINH-KPTPSPSA-COOMe
Calculated Mass $[M+Na]^+$ = 1178.7 | $[M+Ac+Na]^+$ = 1220.7



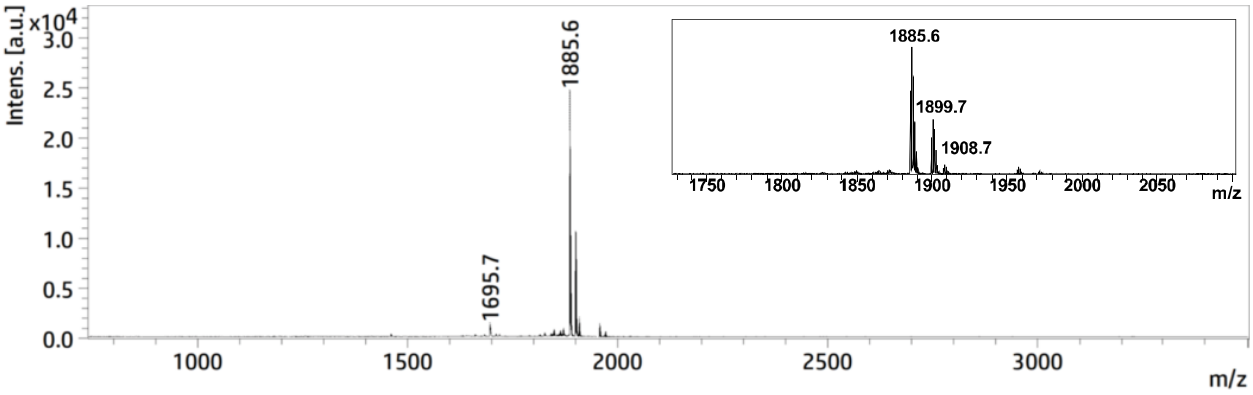
Spectrum: 60
Peptide ID: GP2.36
Internal ID: GP2.36
Purification Level: SepPak C18 purified
Sequence: FINH-KVPS{GalNAca1-T}PP{GalNAca1-S}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 2038.6



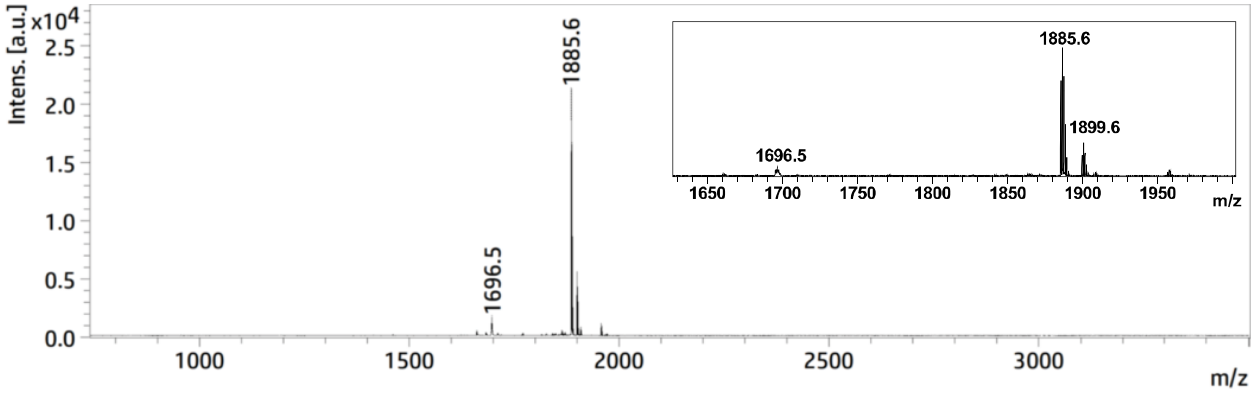
Spectrum: 61
Peptide ID: GP2.37
Internal ID: GP2.37
Purification Level: SepPak C18 purified
Sequence: FINH-KVPS{GalNAca1-T}PP{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 2052.6



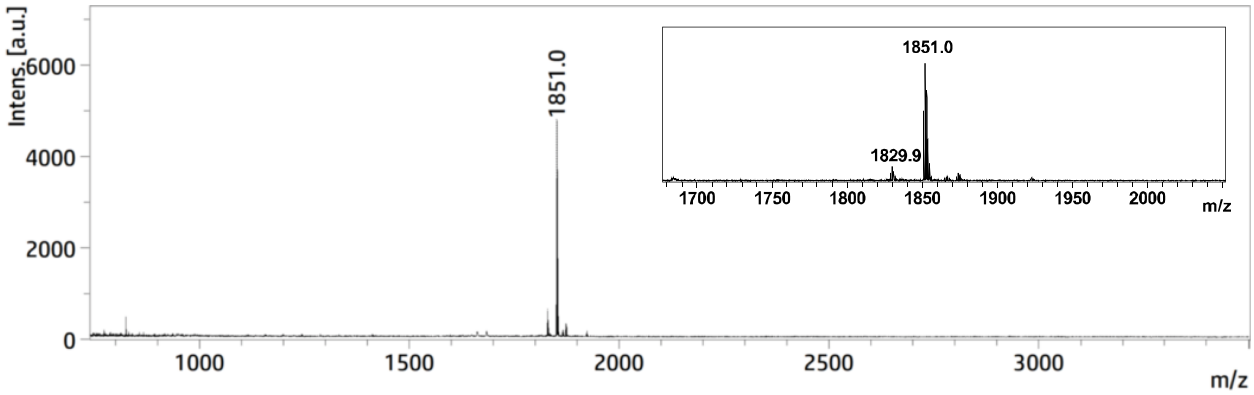
Spectrum: 62
Peptide ID: GP2.38
Internal ID: GP2.38
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTXX{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1885.6



Spectrum: 63
Peptide ID: GP2.39
Internal ID: GP2.39
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTxx{GalNAca1-T}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1885.6



Spectrum: 64
Peptide ID: GP2.40 (repeat of GP2.21)
Internal ID: GP2.40
Purification Level: SepPak C18 purified
Sequence: FINH-KVPSTPP{GalNAcb1-C}PSPSA-COOH
Calculated Mass $[M+Na]^+$ = 1851.5



HPLC Chromatography For Glycopeptides

GP1 Library HPLC Chromatography

Method 1: Semi-prep

Note- For this library semi-prep HPLC was performed on the crude material and hence there are a few minor peaks observed in the chromatograms. However, the major peak was found to be the product peak, which was isolated and confirmed to be >90% purity before use in other assays. The aim of showing these chromatograms is to let the reader judge the efficacy of synthesis straight out of the method.

Instrument: Shimadzu Prominence HPLC with SPD-20AV UV-Vis detector (Detector B) and RF-20A Fluorescence detector (Detector A)

Solvent A: 0.1% trifluoroacetic acid with 95:5 water:acetonitrile.

Solvent B: 0.1% trifluoroacetic acid with 95:5 acetonitrile:water.

Column: Phenomenex Luna® Omega 5µm PS C18 100Å 250 x 4.6 mm

Column Temperature: 40 °C

Flow rate: 1.25 ml/min

Total run time: 60 min

UV-Vis Detector: 220 nm

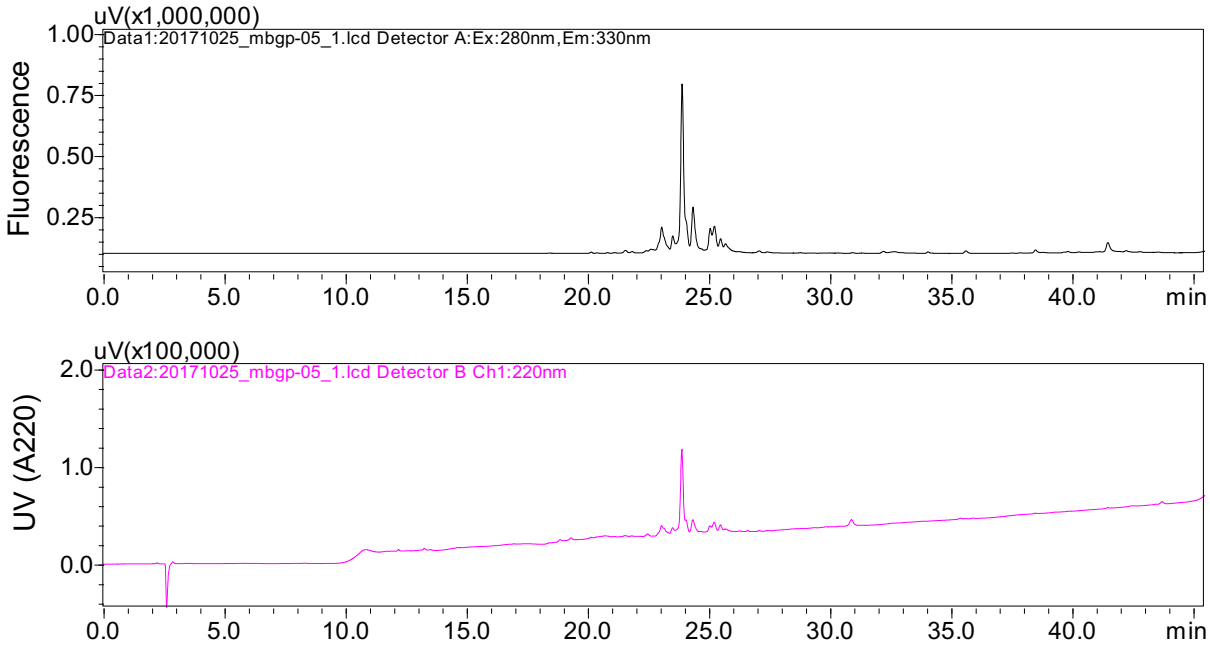
Fluorescence: Ex: 280 nm Em: 330 nm

Method: A sample of the glycopeptide was dissolved in 0.15 ml of solvent A. The sample was split into 3 injections of 50 µl each and injected onto the column. A 60 minute gradient was, in which the gradient gradually went from 0%-50% B over the first 40 minutes, followed by a wash cycle consisting of a sharp increase to 95% and back down (See details below). Fractions were collected with a vial volume set at 1.25 ml based on a slope of 100 µV/sec and level of 200000 µV. Fractions were analyzed by MALDI-MS for target mass. The fractions which contained the target mass were pooled and dried as final compounds.

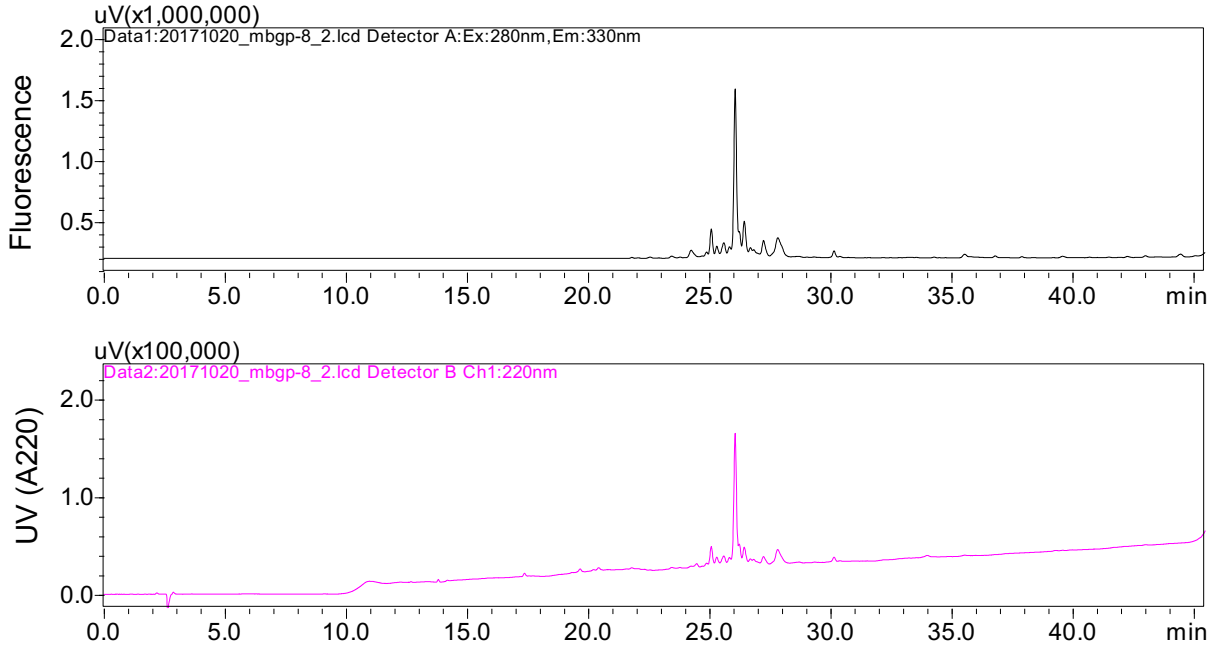
Gradient Details:

	Time	Module	Command	Value
1	0.01	Pumps	Total Pump A Flow	1.25
2	0.02	RF-20A(DET.A)	Zero	
3	0.02	SPD-20AV(DET.B)	Zero	
4	0.03	Pumps	Solvent B Conc.	0
5	3.00	Pumps	Solvent B Conc.	0
6	40.00	Pumps	Solvent B Conc.	50
7	42.00	Pumps	Solvent B Conc.	95
8	50.00	Pumps	Solvent B Conc.	95
9	51.00	Pumps	Solvent B Conc.	0
10	60.00	Controller	Stop	

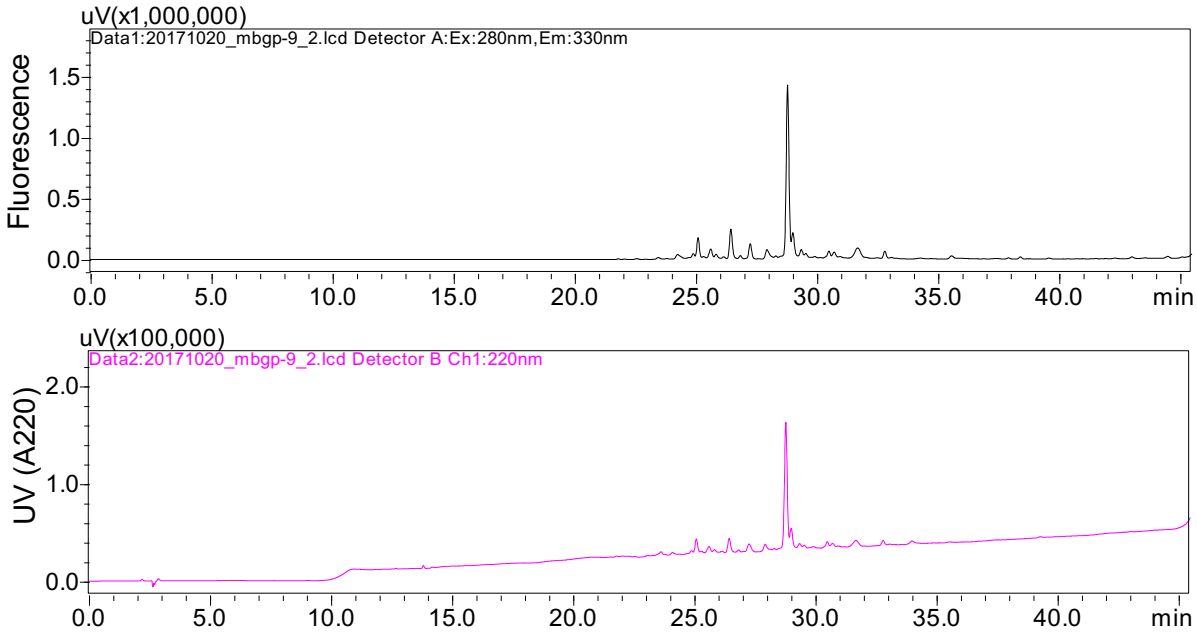
GP1.1


uV(x1,000,000)
Data1:20171025_mbgp-05_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.00
0.75
0.50
0.25
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171025_mbgp-05_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

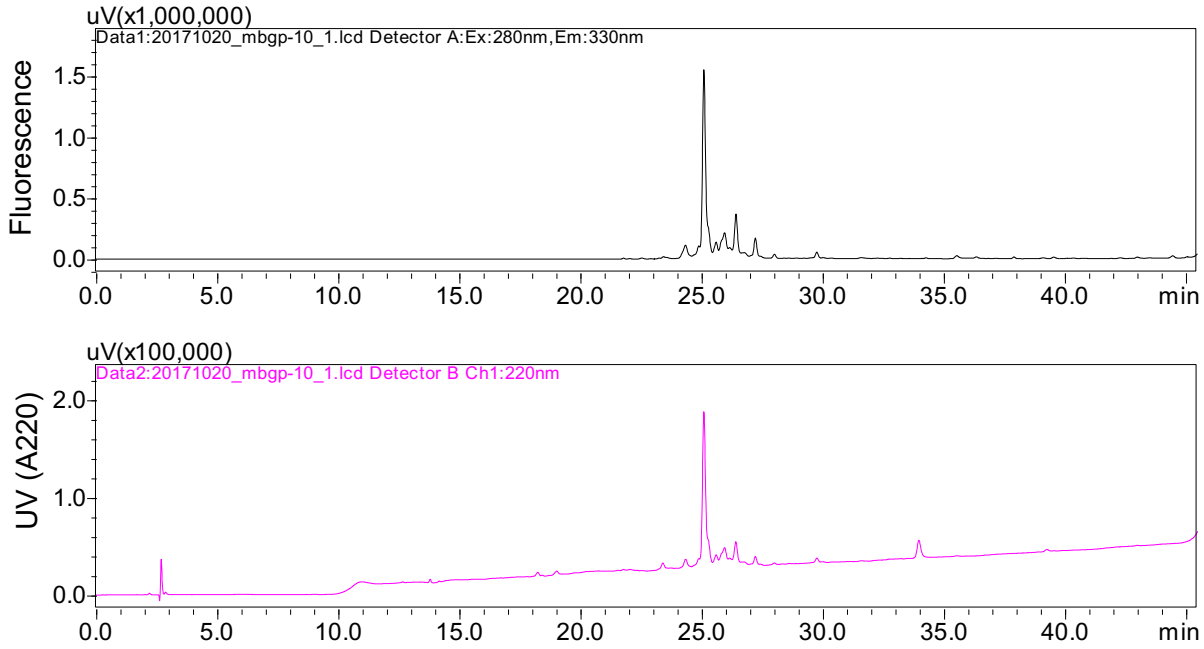
GP1.2


uV(x1,000,000)
Data1:20171020_mbgp-8_2.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
2.0
1.5
1.0
0.5
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171020_mbgp-8_2.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

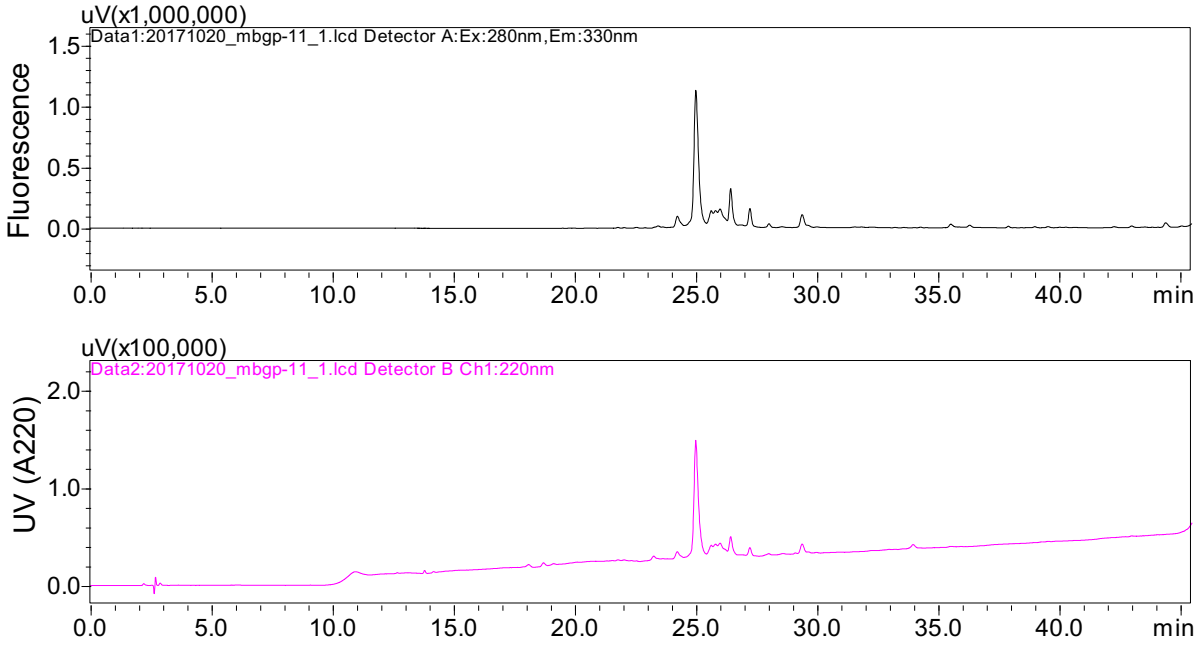
GP1.3


uV(x1,000,000)
Data1:20171020_mbgp-9_2.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171020_mbgp-9_2.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

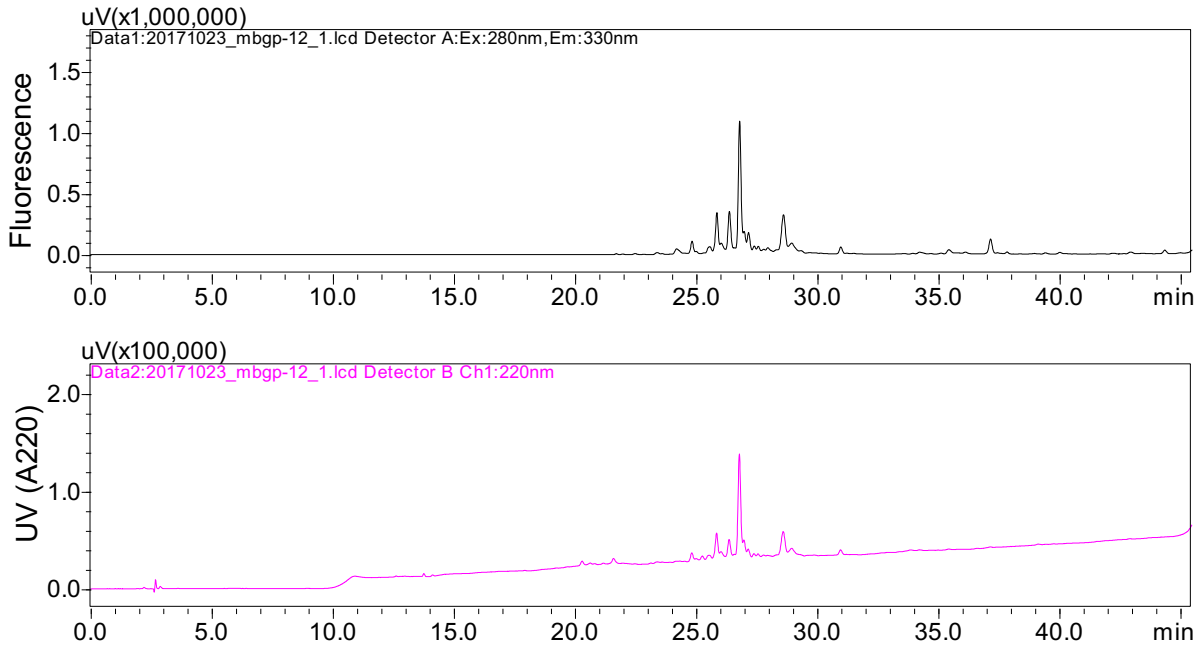
GP1.4


uV(x1,000,000)
Data1:20171020_mbgp-10_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171020_mbgp-10_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

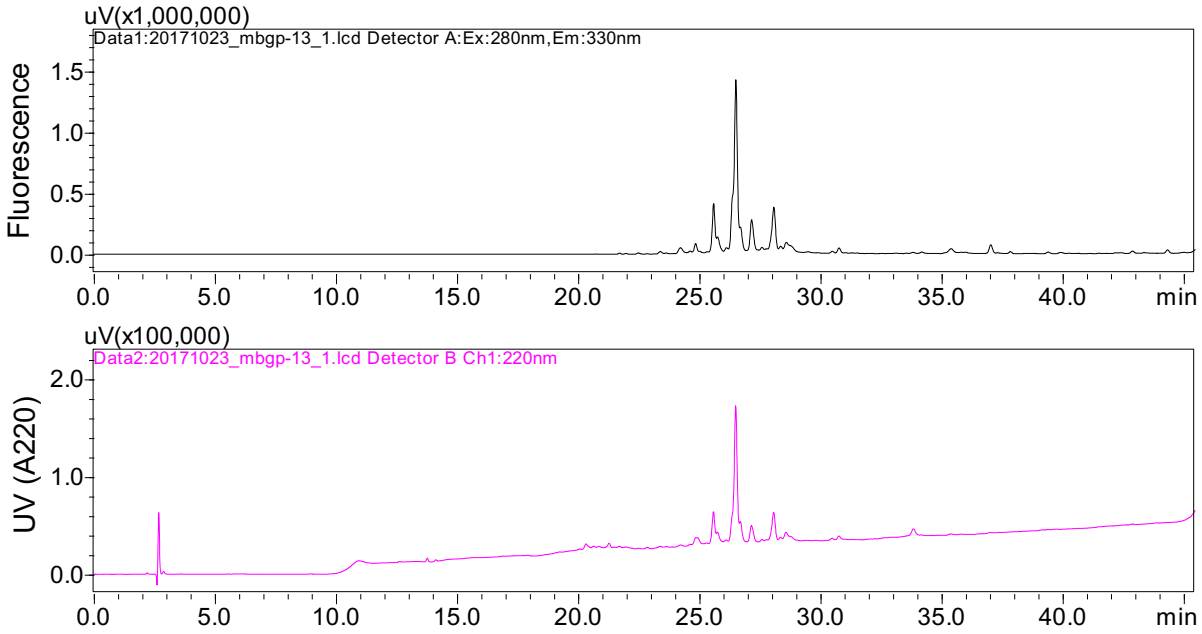
GP1.5


uV(x1,000,000)
Data1:20171020_mbgp-11_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171020_mbgp-11_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

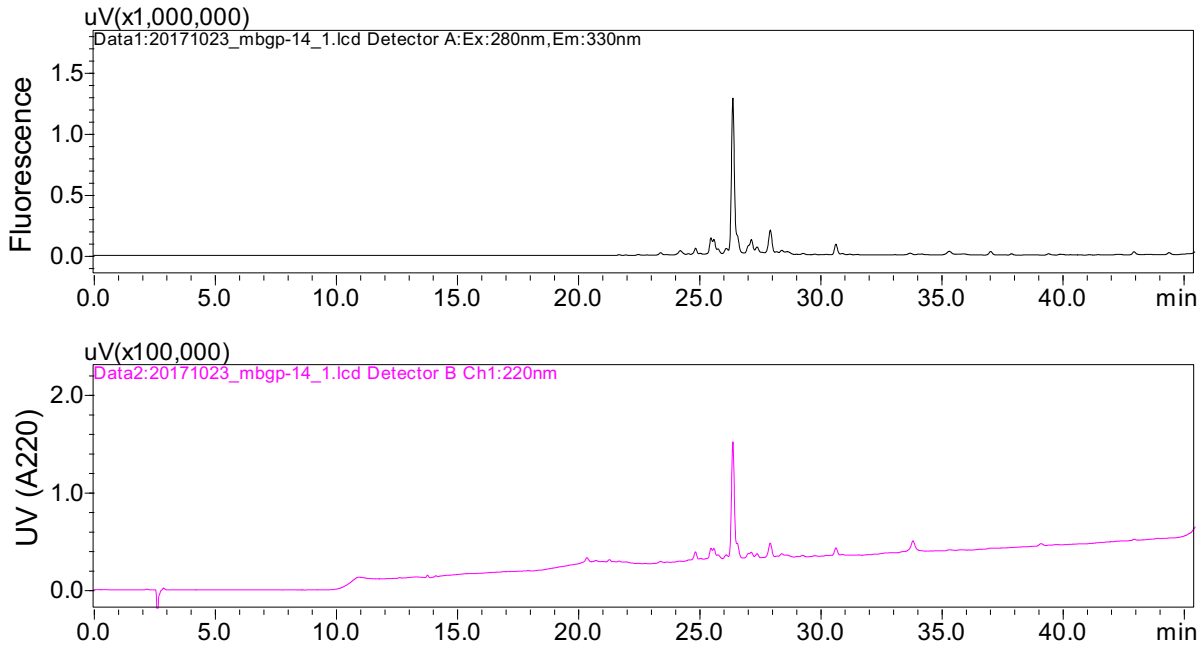
GP1.6


uV(x1,000,000)
Data1:20171023_mbgp-12_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171023_mbgp-12_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

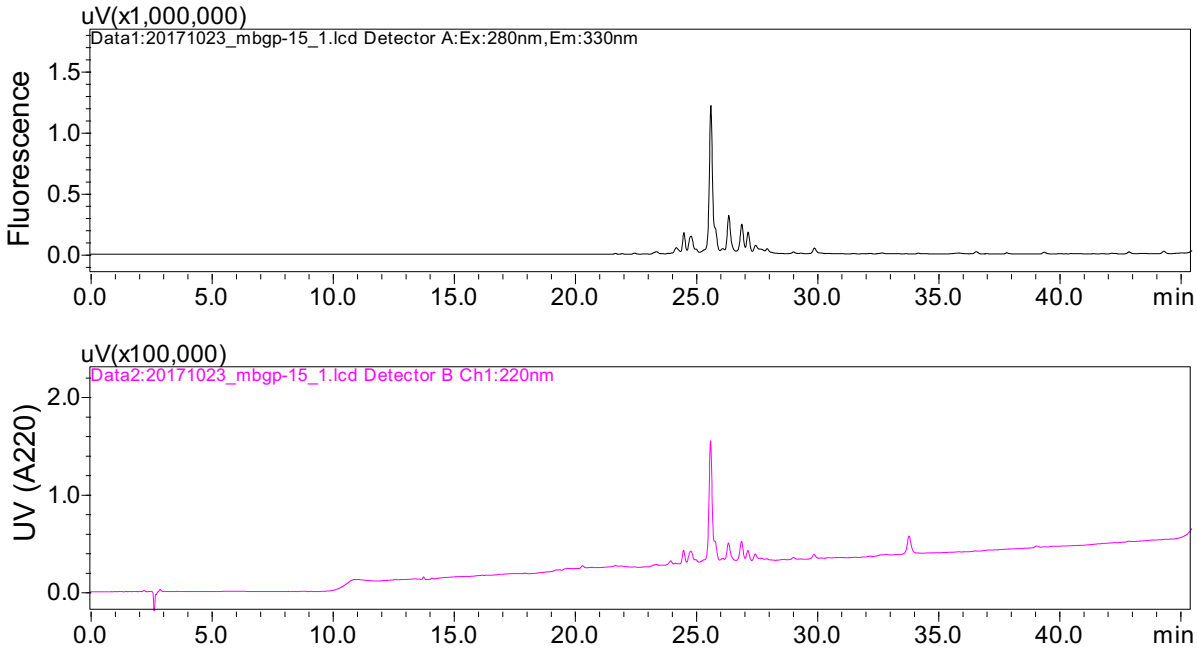
GP1.7


uV(x1,000,000)
Data1:20171023_mbgp-13_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171023_mbgp-13_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

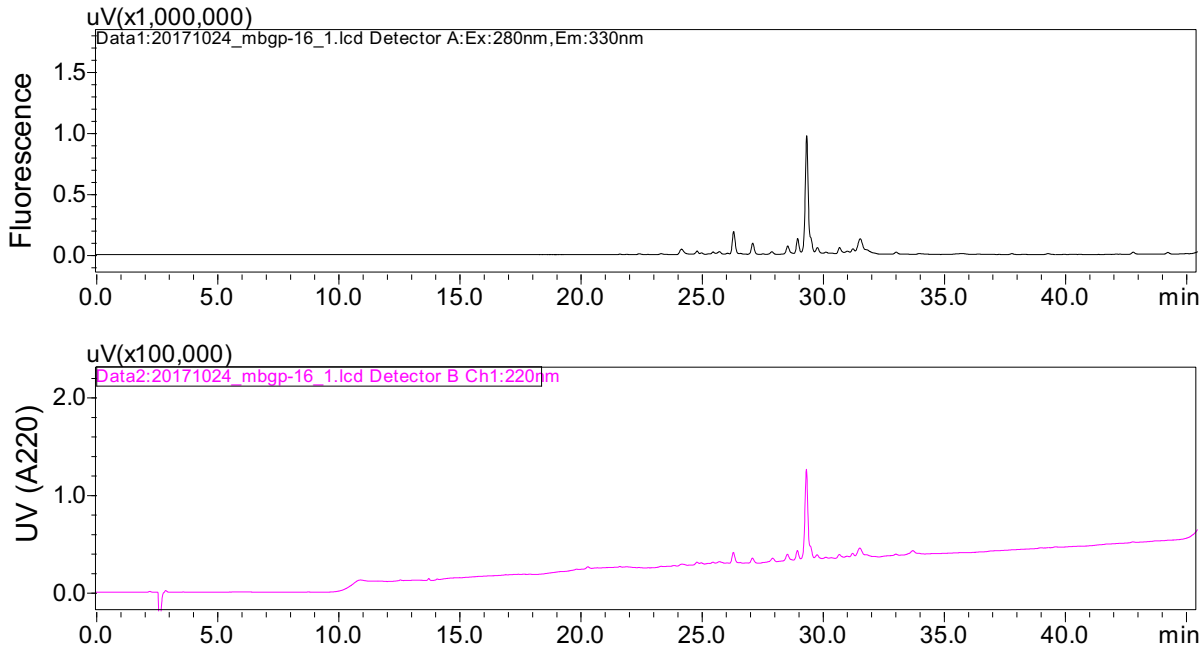
GP1.8


uV(x1,000,000)
Data1:20171023_mbgp-14_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171023_mbgp-14_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

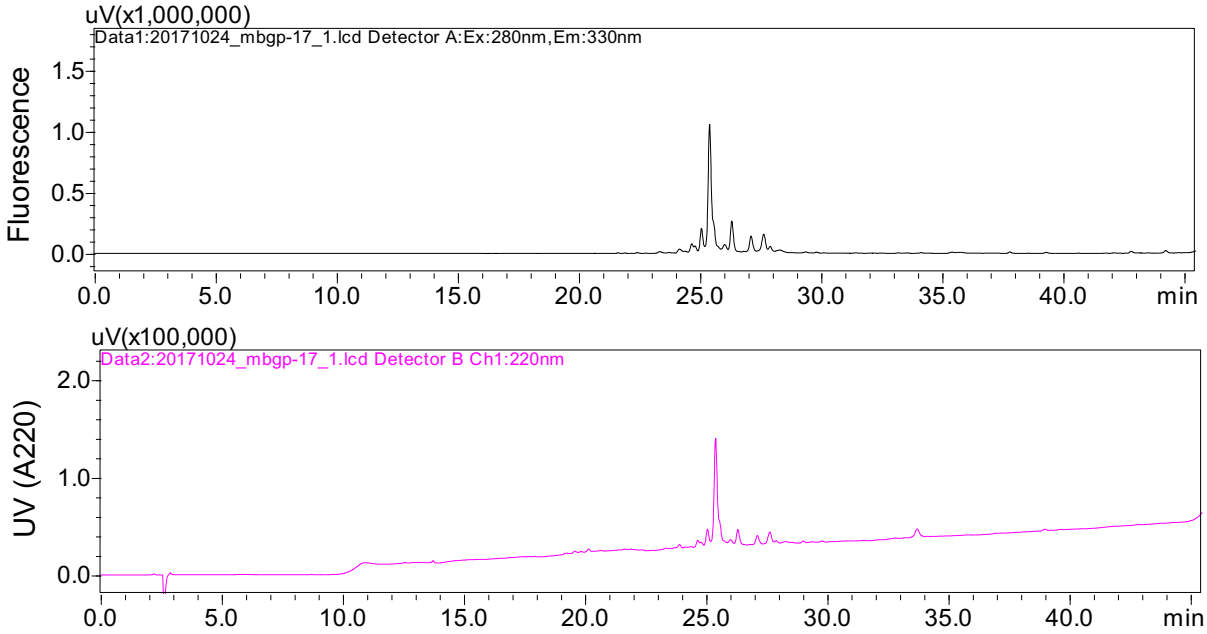
GP1.9


uV(x1,000,000)
Data1:20171023_mbgp-15_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171023_mbgp-15_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

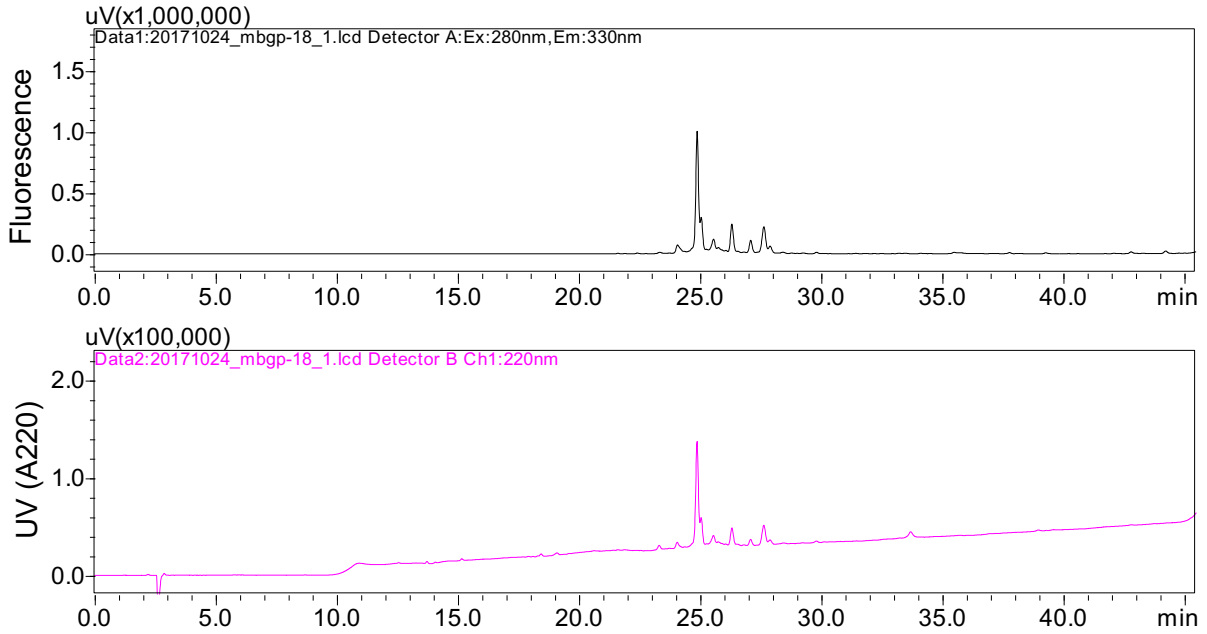
GP1.10


uV(x1,000,000)
Data1:20171024_mbgp-16_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171024_mbgp-16_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

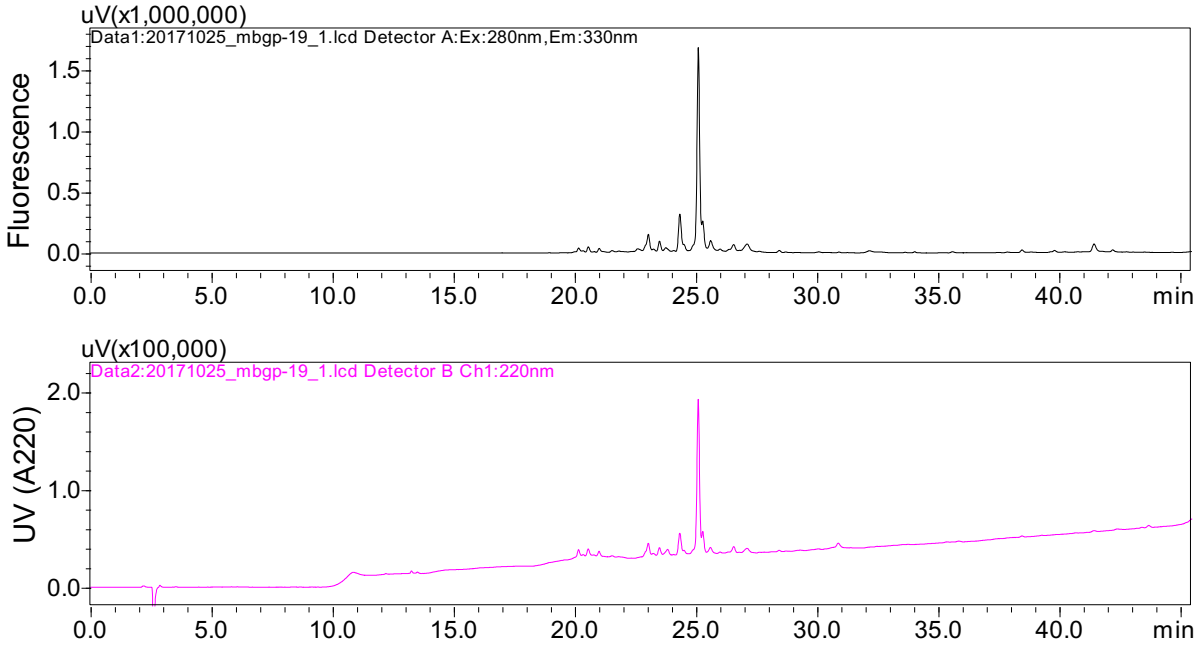
GP1.11


uV(x1,000,000)
Data1:20171024_mbgp-17_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171024_mbgp-17_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

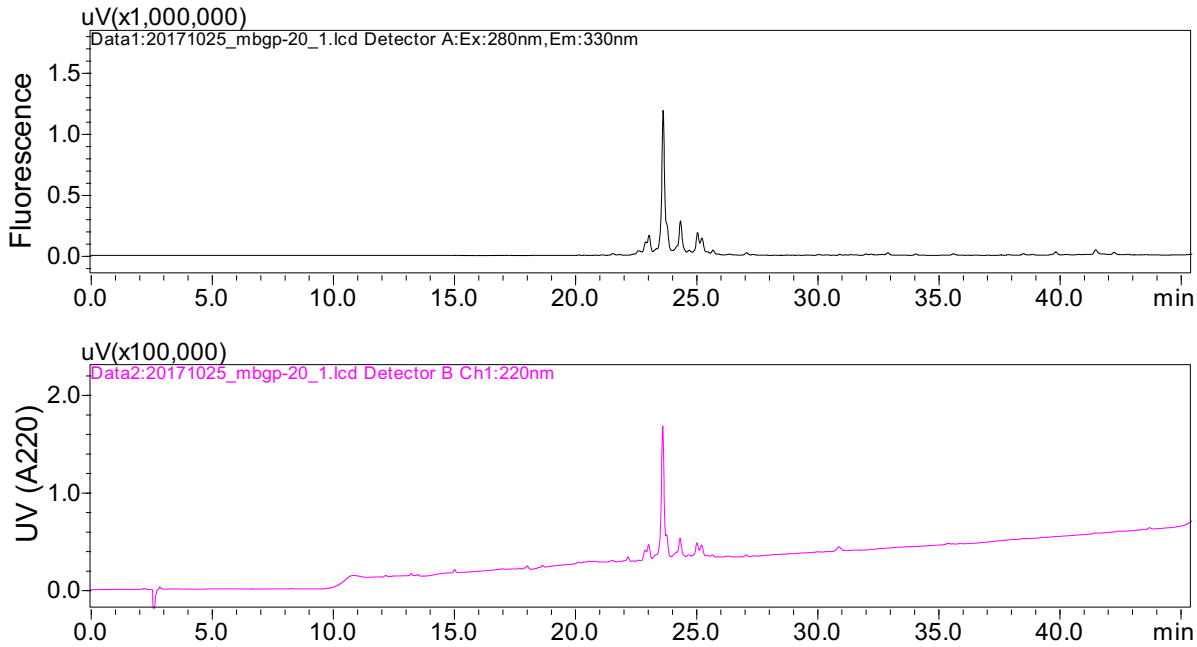
GP1.12


uV(x1,000,000)
Data1:20171024_mbgp-18_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171024_mbgp-18_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

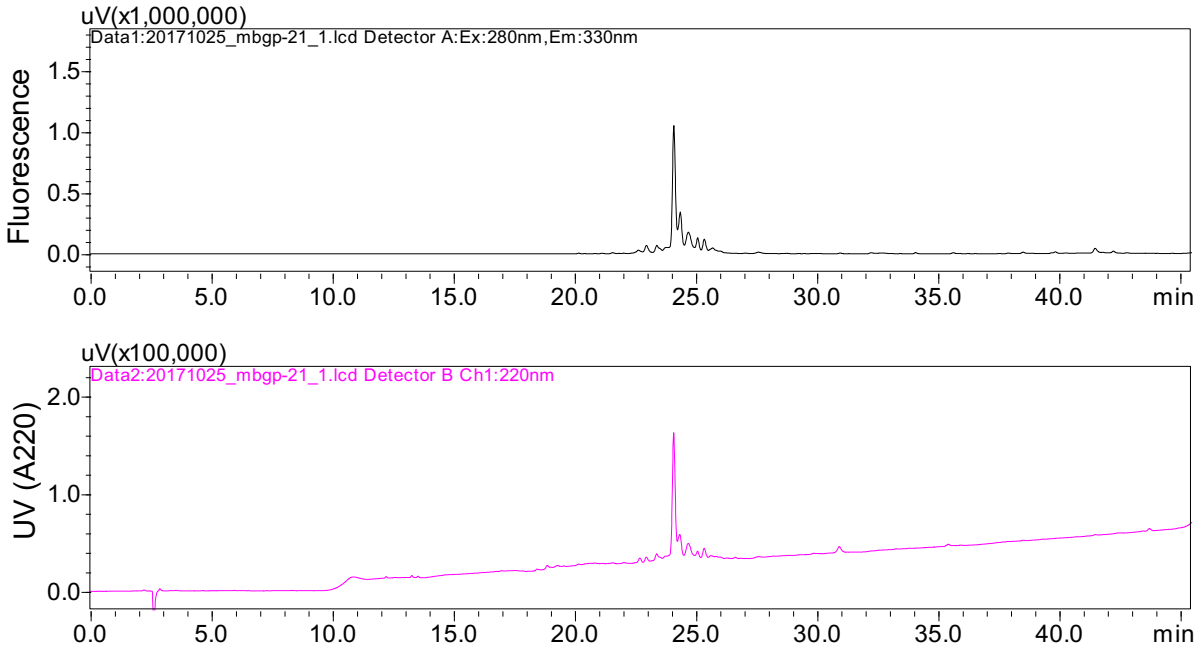
GP1.13


uV(x1,000,000)
Data1:20171025_mbgp-19_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171025_mbgp-19_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

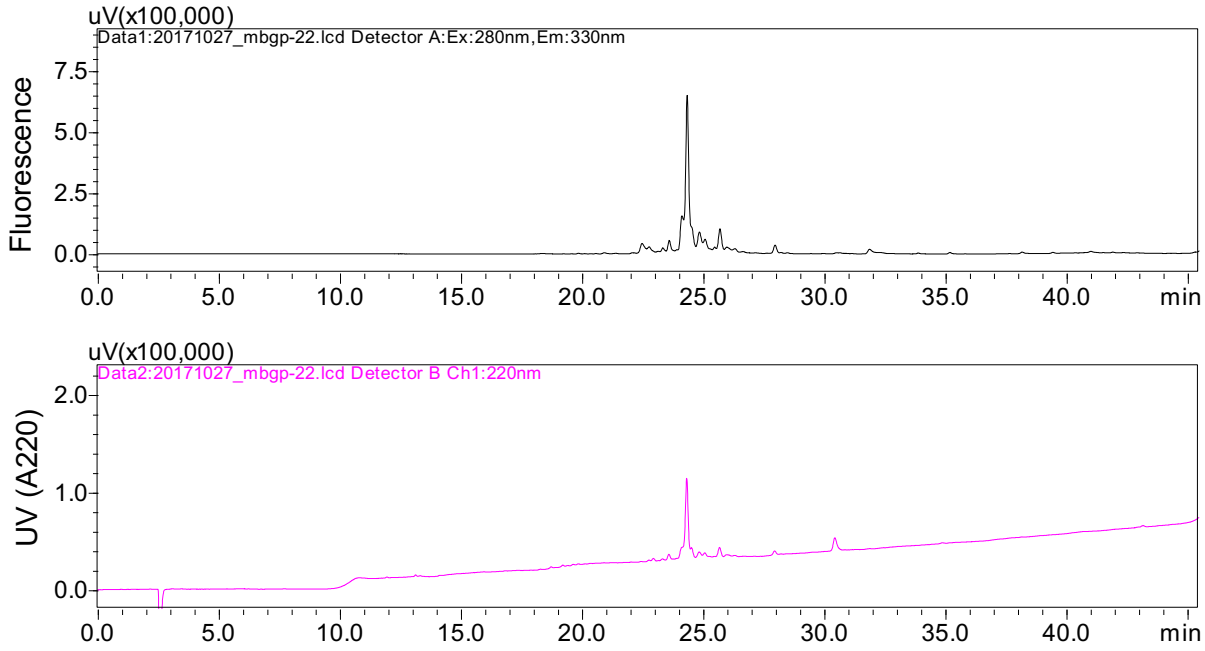
GP1.14


uV(x1,000,000)
Data1:20171025_mbgp-20_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171025_mbgp-20_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

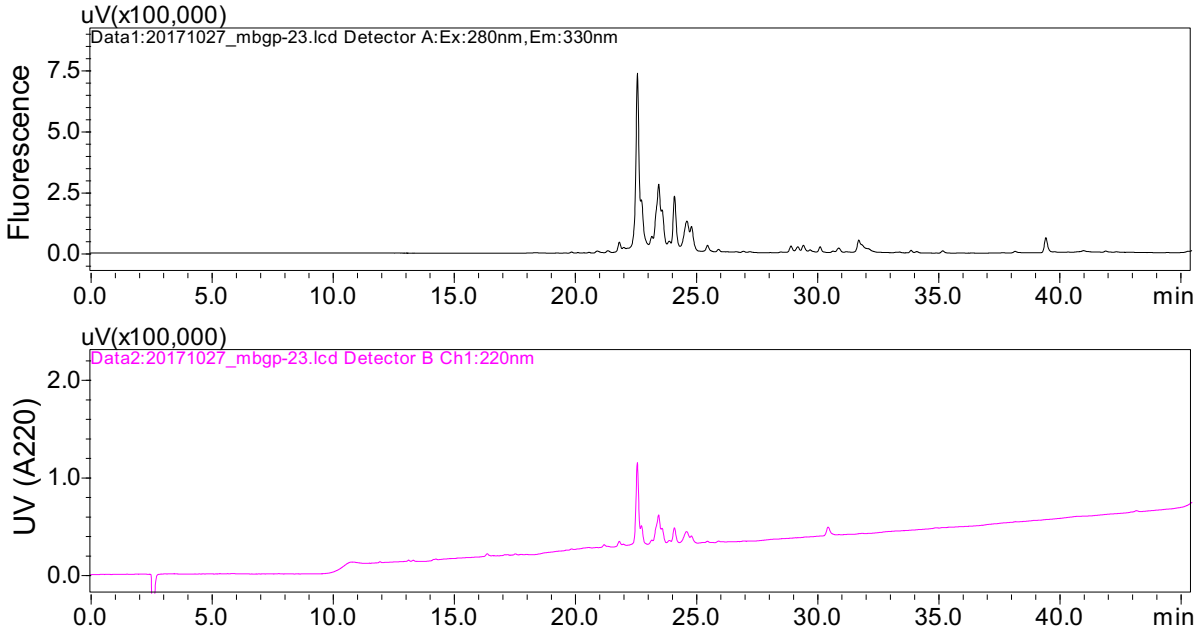
GP1.15


uV(x1,000,000)
Data1:20171025_mbgp-21_1.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171025_mbgp-21_1.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

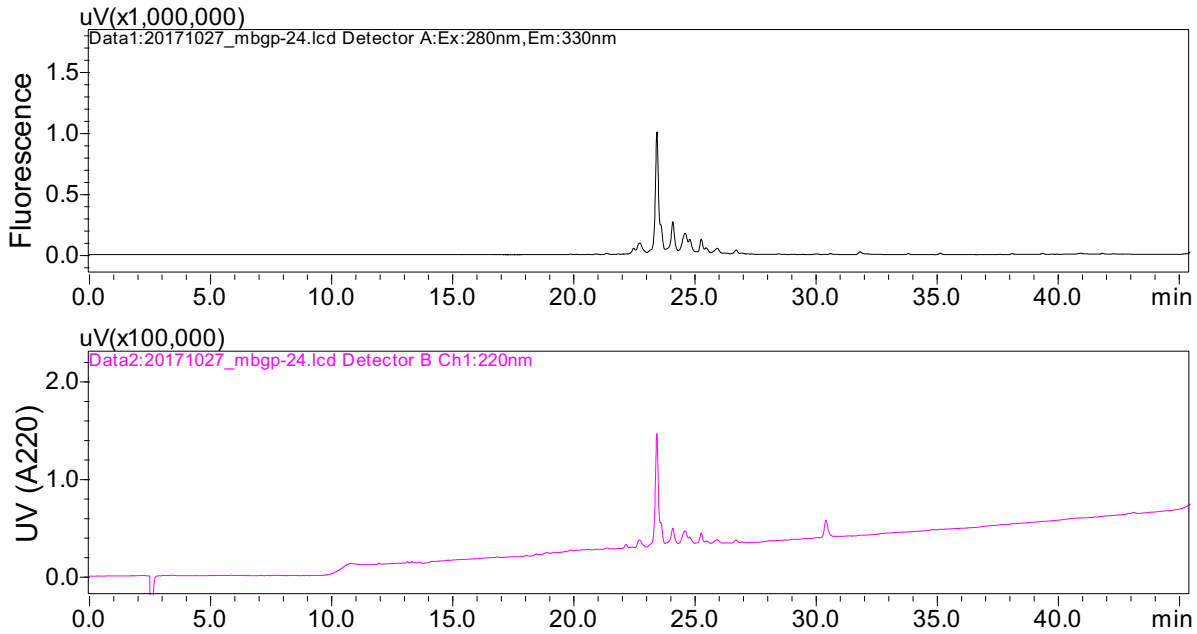
GP1.16


uV(x100,000)
Data1:20171027_mbgp-22.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
7.5
5.0
2.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171027_mbgp-22.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

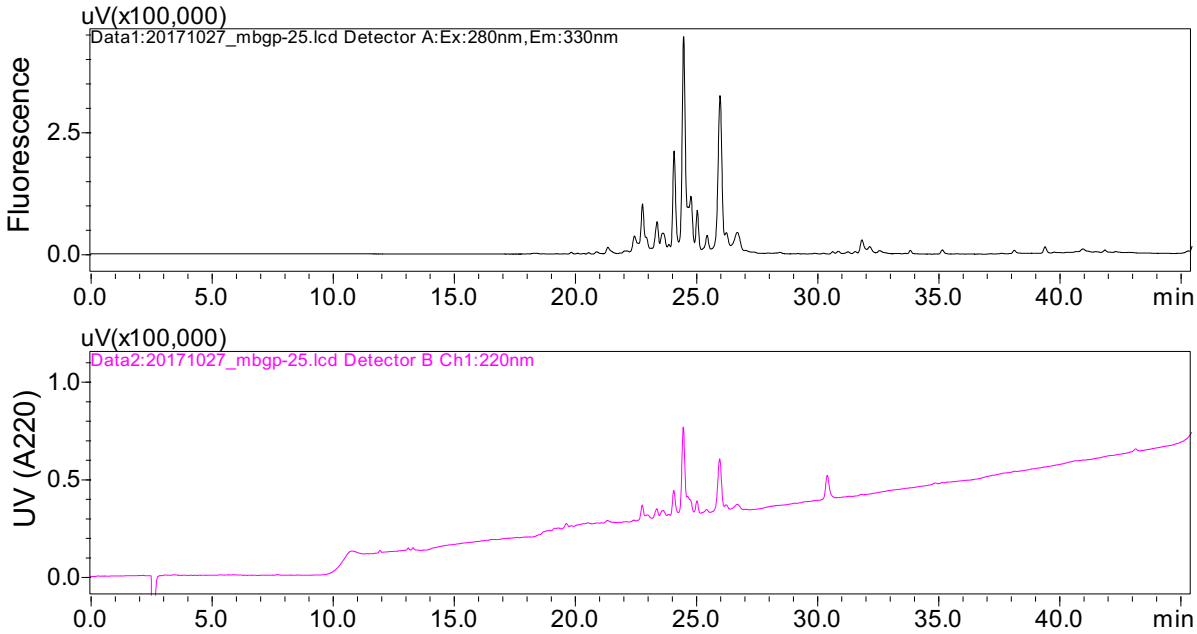
GP1.17


uV(x100,000)
Data1:20171027_mbgp-23.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
7.5
5.0
2.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171027_mbgp-23.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

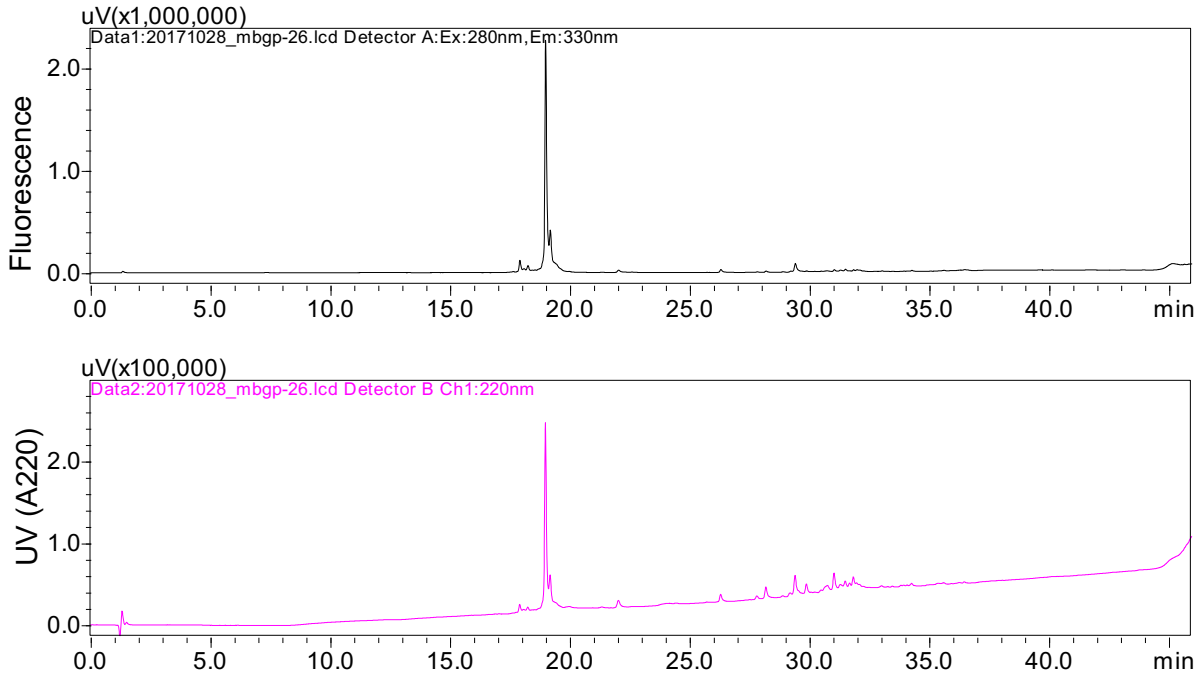
GP1.18


uV(x1,000,000)
Data1:20171027_mbgp-24.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
1.5
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171027_mbgp-24.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

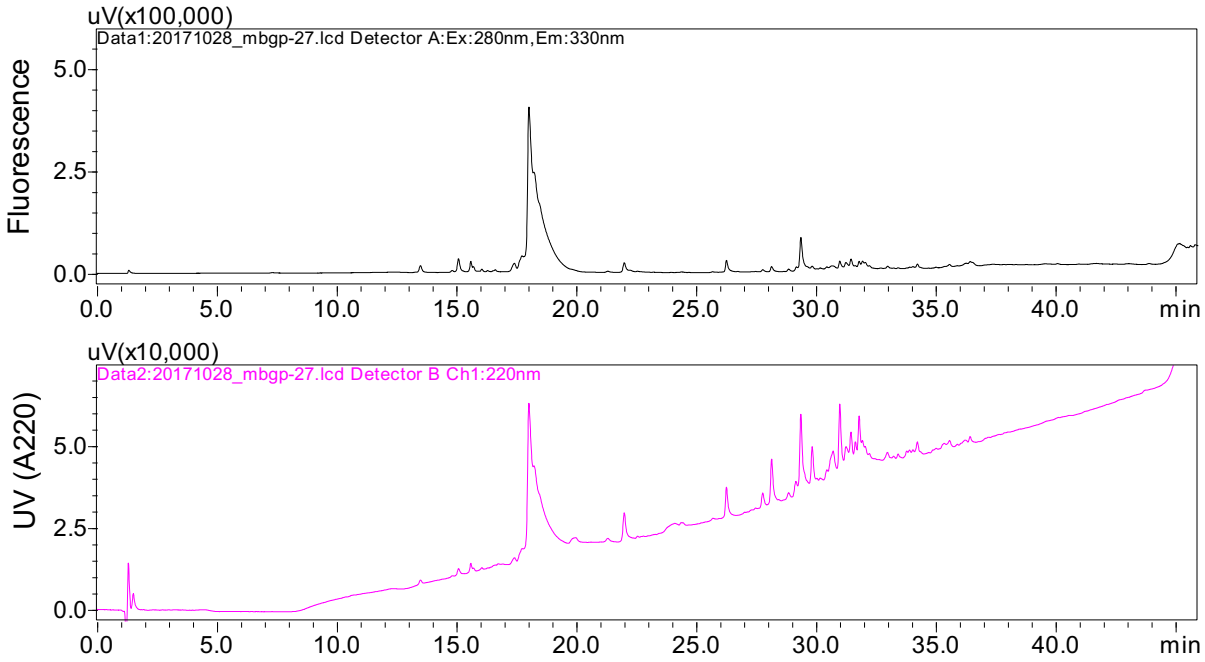
GP1.19


uV(x100,000)
Data1:20171027_mbgp-25.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
2.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171027_mbgp-25.lcd Detector B Ch1:220nm
UV (A220)
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

GP1.20


uV(x1,000,000)
Data1:20171028_mbgp-26.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x100,000)
Data2:20171028_mbgp-26.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

GP1.21


uV(x100,000)
Data1:20171028_mbgp-27.lcd Detector A:Ex:280nm,Em:330nm
Fluorescence
5.0
2.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min
uV(x10,000)
Data2:20171028_mbgp-27.lcd Detector B Ch1:220nm
UV (A220)
5.0
2.5
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
min

GP2 Library HPLC Chromatography

Method 2: Analytical

Note- For this series the peptides were first purified over SepPak C18 and then checked on analytical HPLC. The shoulder peak is for C-terminus -OMe/-OH transesterification due to the use of methanol (See Figure 3). The shoulder peak was isolated for most cases and checked using MALDI to confirm this observation. For our purposes, the difference in C-terminus group does not have dramatic effect and hence we consider the two species together for the purity. All compounds were confirmed to be >90% pure after isolation before use in other assays.

Instrument: Shimadzu Prominence HPLC with SPD-20AV UV-Vis detector (Detector B) and RF-20A Fluorescence detector (Detector A)

Solvent A: 0.1% trifluoroacetic acid with 95:5 water:acetonitrile.

Solvent B: 0.1% trifluoroacetic acid with 95:5 acetonitrile:water.

Column: Phenomenex Kinetex® 2.6 µm Polar C18 100Å 150 x 4.6 mm

Column Temperature: 40 °C

Flow rate: 1 ml/min

Total run time: 35 min

UV-Vis Detector: 220 nm

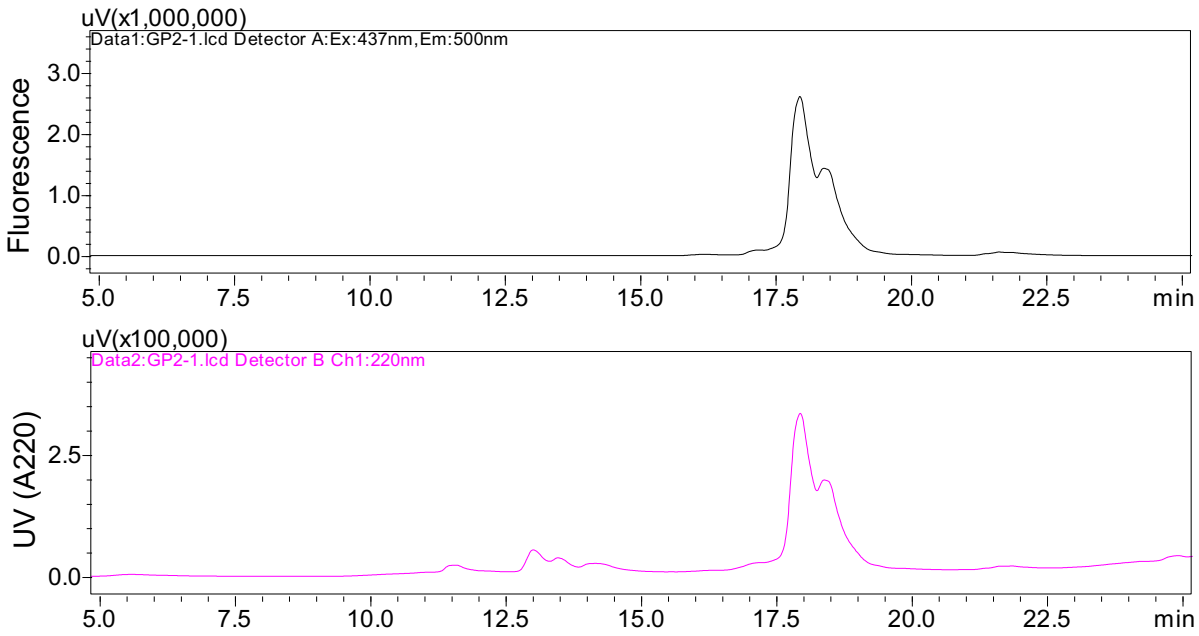
Fluorescence: Ex: 437 nm Em: 500 nm (Fluorescein at low pH)

Method: A 5µL sample of the stock glycopeptide (100 – 440 µM) obtained after SepPak C18, was dissolved in 45 µl of solvent A. 40 µl of this was injected onto the column. A 35 minute gradient was, in which the gradient gradually went from 0%-50% B over the first 20 minutes, followed by a wash cycle consisting of a sharp increase to 95% and back down (See details below). Fractions were collected with a vial volume set at 1 ml based on a slope of 1000 µV/sec and level of 20000 µV. Fractions were analyzed by MALDI-MS for target mass.

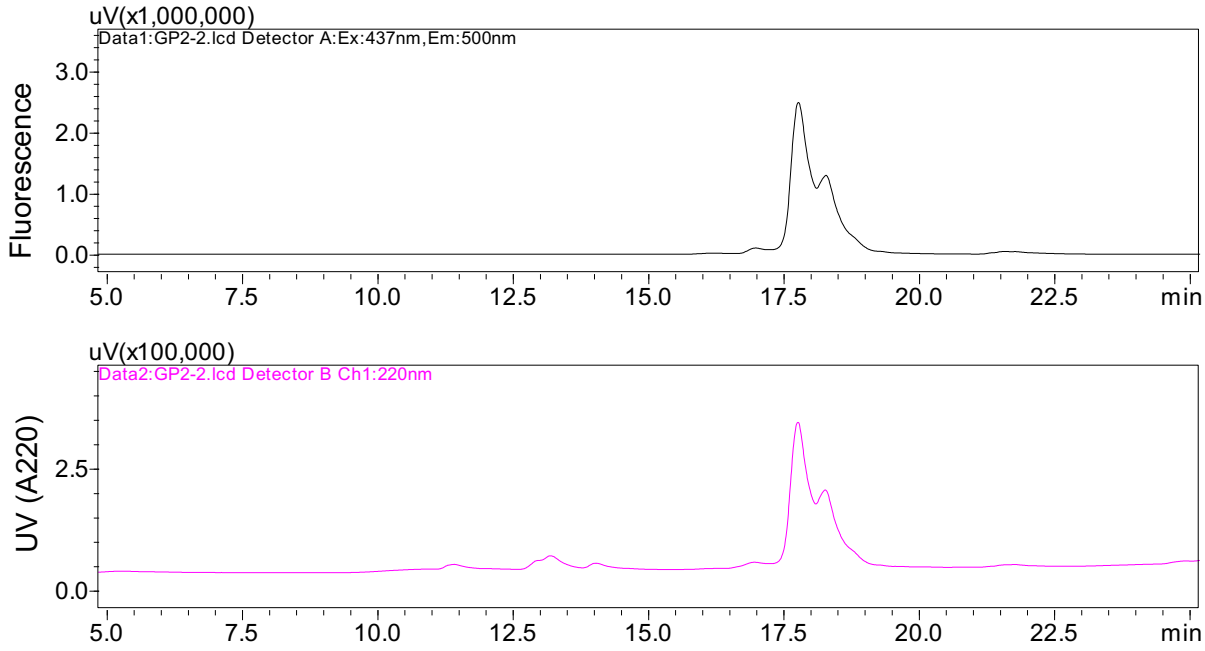
Gradient Details:

	Time	Module	Command	Value
1	0.01	Pumps	Total Pump A Flow	1
2	0.02	RF-20A(DET.A)	Zero	
3	0.02	SPD-20AV(DET.B)	Zero	
4	0.03	Pumps	Solvent B Conc.	0
5	3.00	Pumps	Solvent B Conc.	0
6	20.00	Pumps	Solvent B Conc.	50
7	22.00	Pumps	Solvent B Conc.	95
8	28.00	Pumps	Solvent B Conc.	95
9	29.00	Pumps	Solvent B Conc.	0
10	35.00	Controller	Stop	

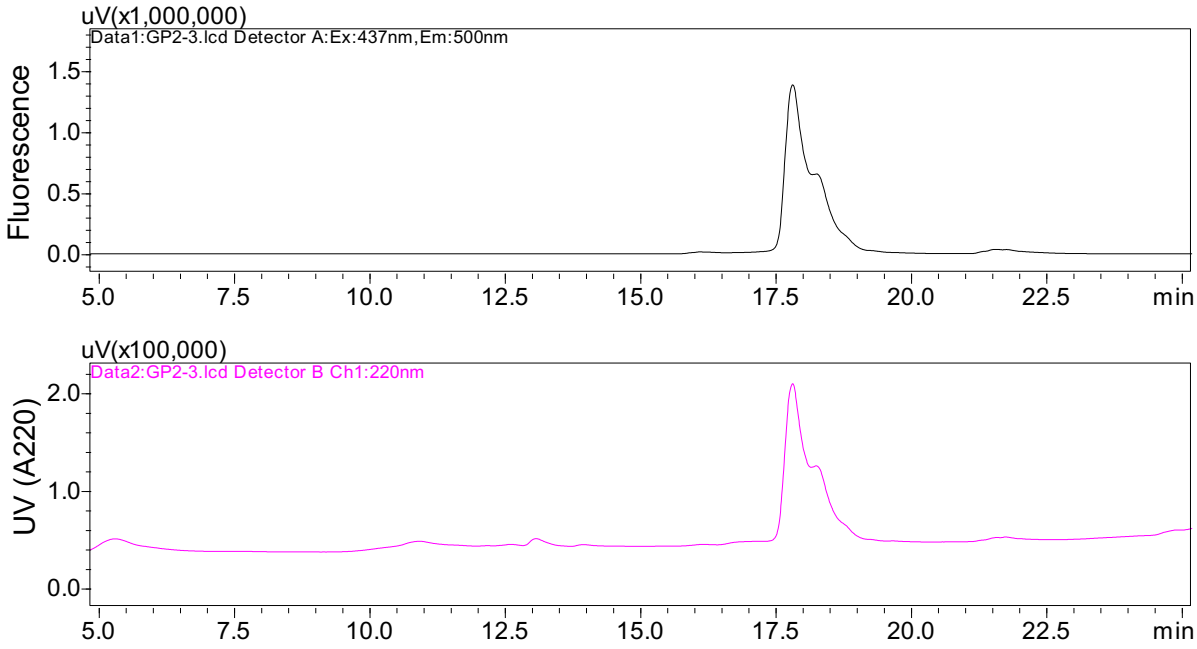
GP2.1


uV(x1,000,000)
Data1:GP2-1.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-1.lcd Detector B Ch1:220nm
UV (A220)
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

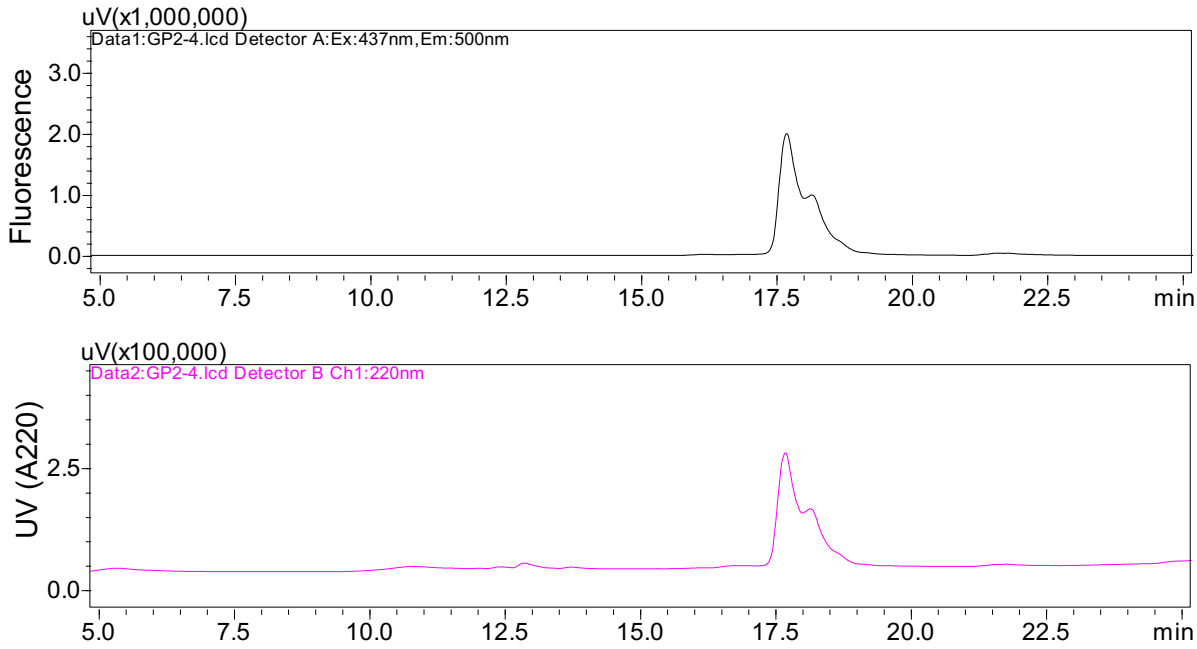
GP2.2


uV(x1,000,000)
Data1:GP2-2.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-2.lcd Detector B Ch1:220nm
UV (A220)
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

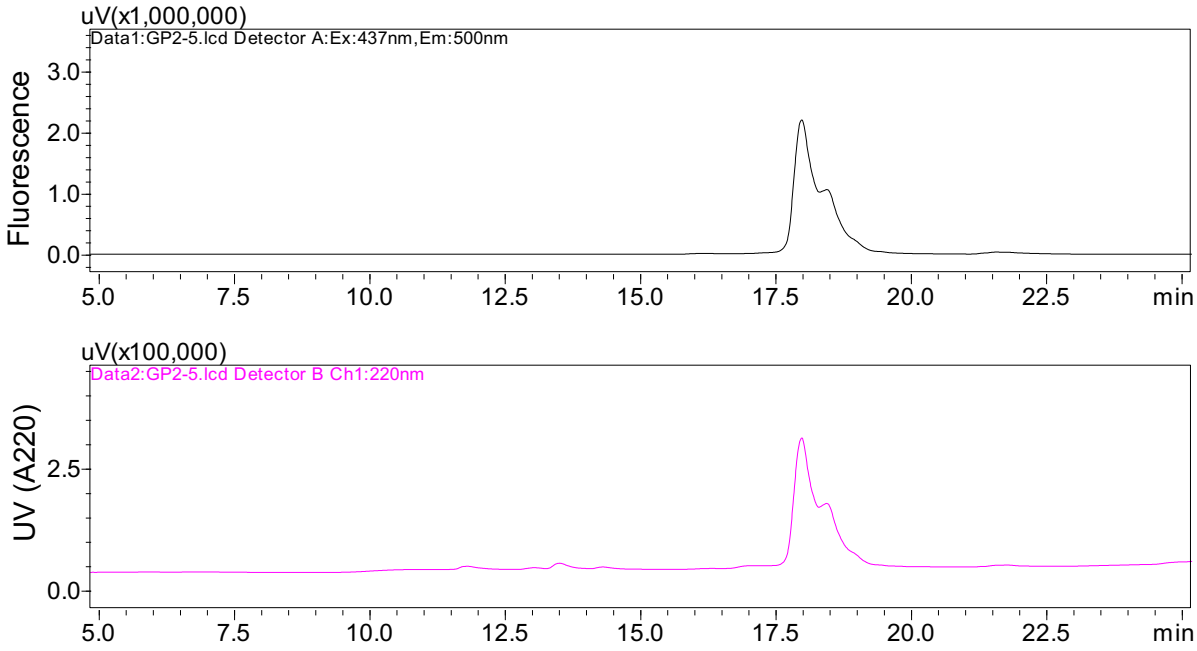
GP2.3


uV(x1,000,000)
Data1:GP2-3.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-3.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

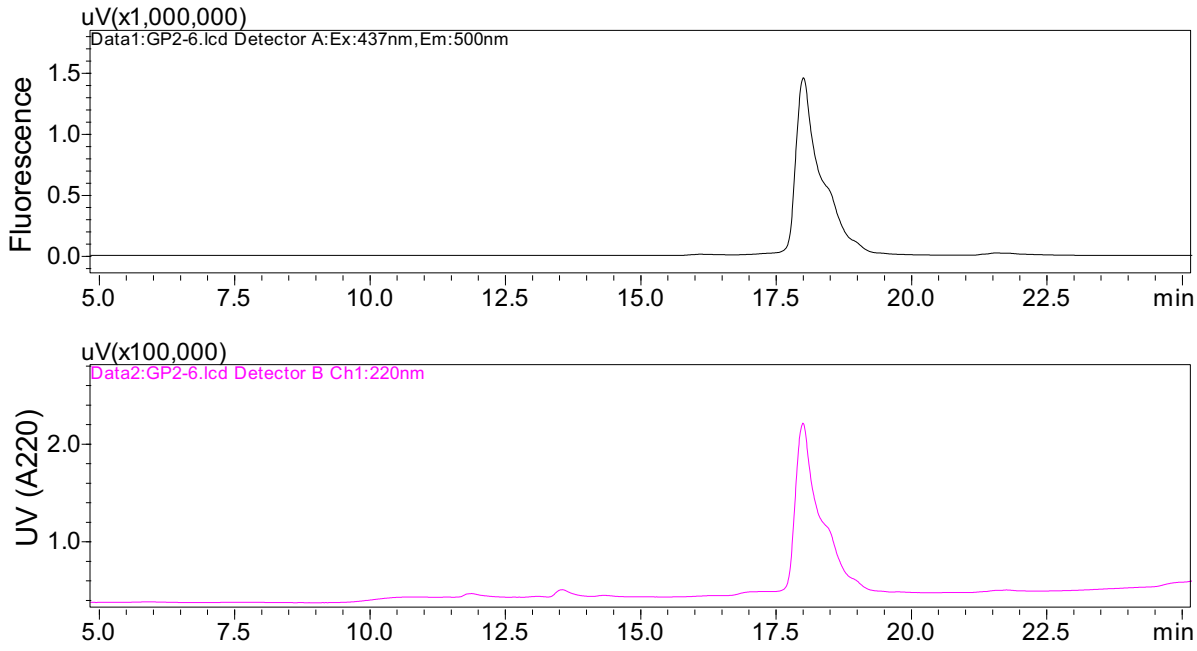
GP2.4


uV(x1,000,000)
Data1:GP2-4.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-4.lcd Detector B Ch1:220nm
UV (A220)
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

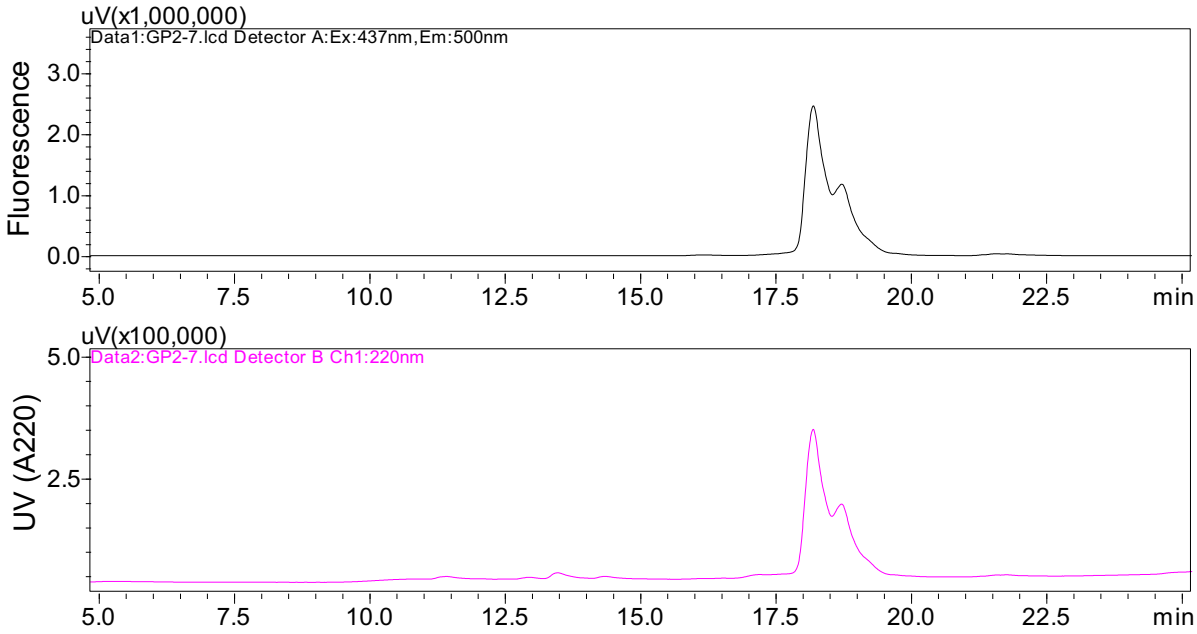
GP2.5


uV(x1,000,000)
Data1:GP2-5.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-5.lcd Detector B Ch1:220nm
UV (A220)
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

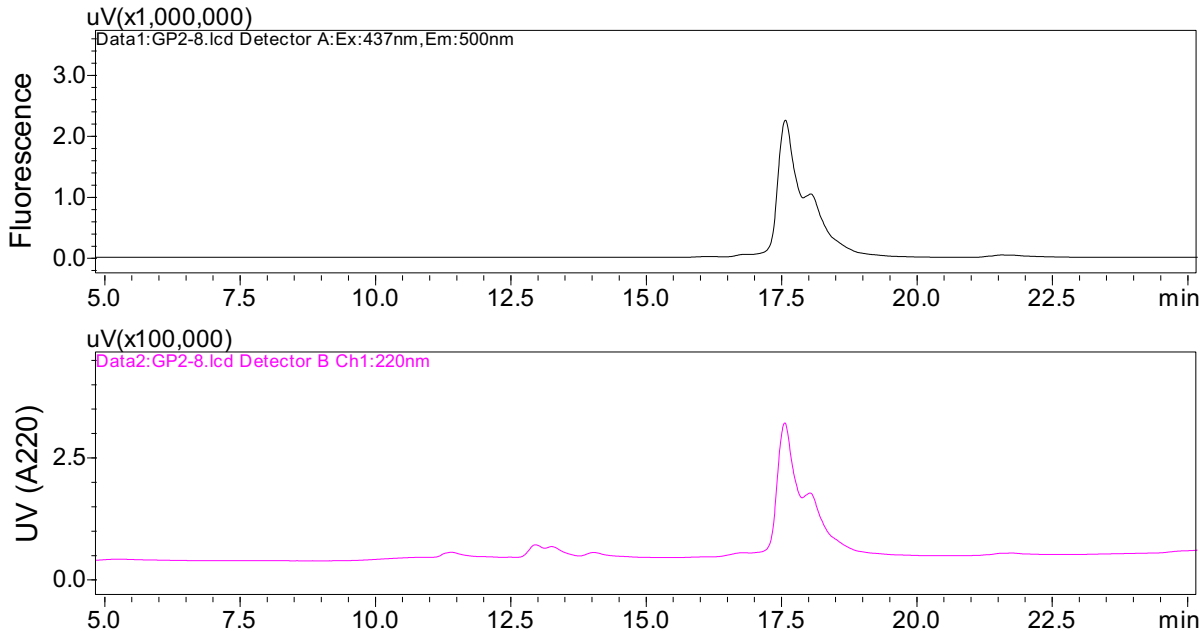
GP2.6


uV(x1,000,000)
Data1:GP2-6.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-6.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

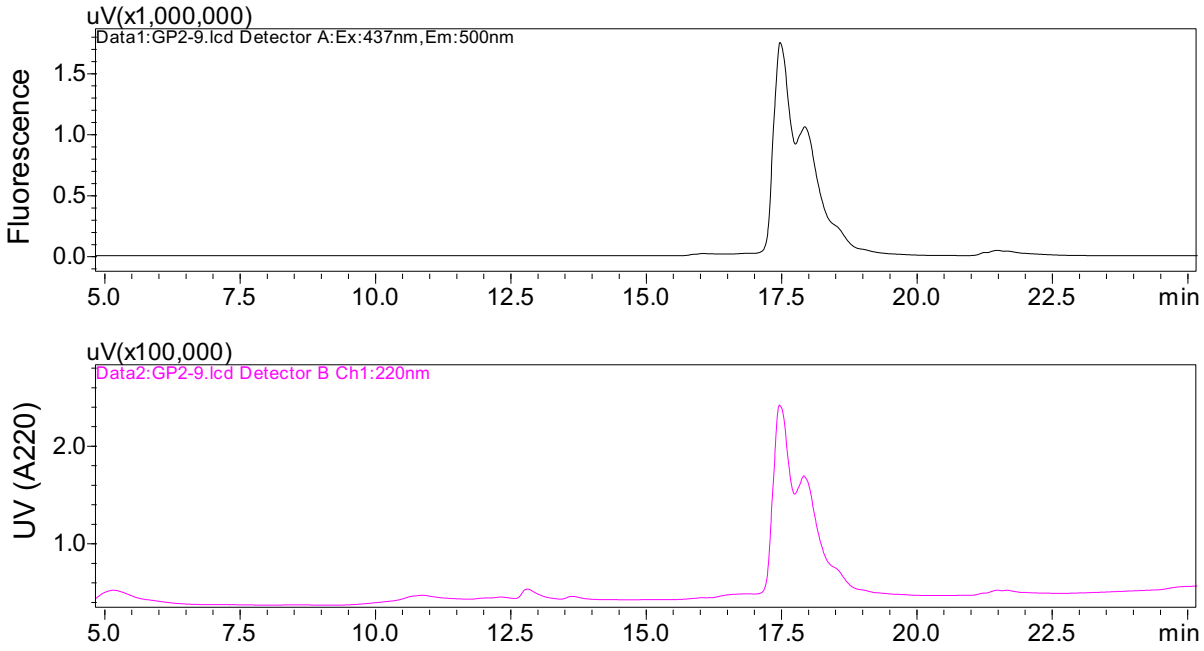
GP2.7


uV(x1,000,000)
Data1:GP2-7.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-7.lcd Detector B Ch1:220nm
UV (A220)
5.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

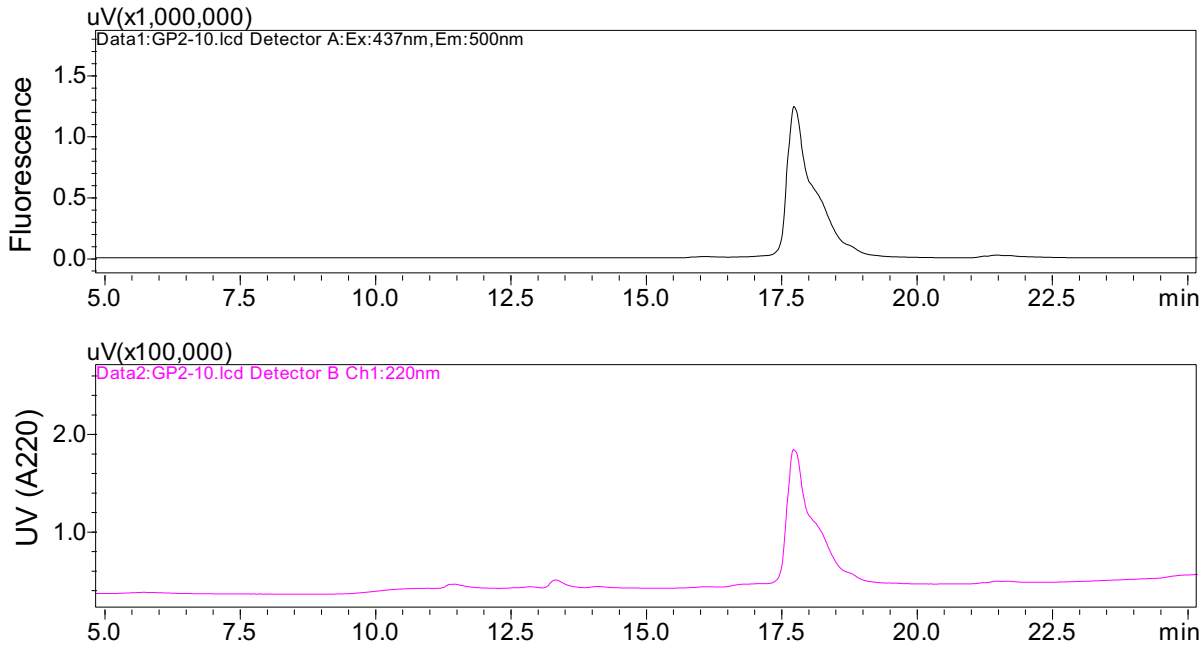
GP2.8


uV(x1,000,000)
Data1:GP2-8.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-8.lcd Detector B Ch1:220nm
UV (A220)
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

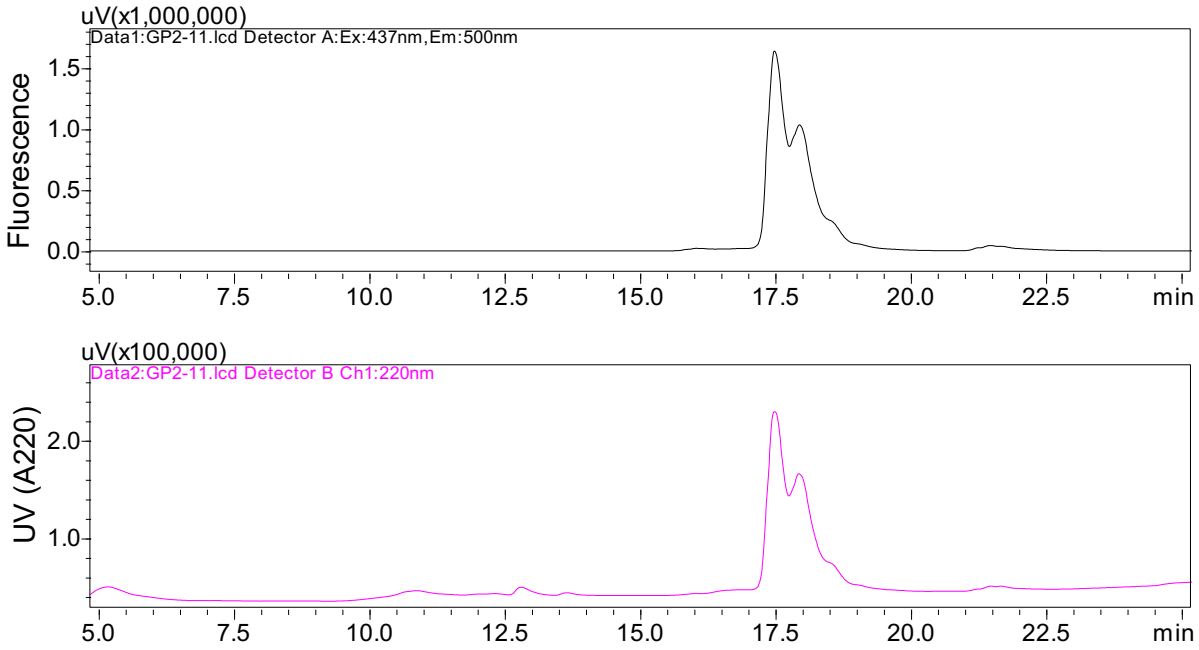
GP2.9


uV(x1,000,000)
Data1:GP2-9.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-9.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

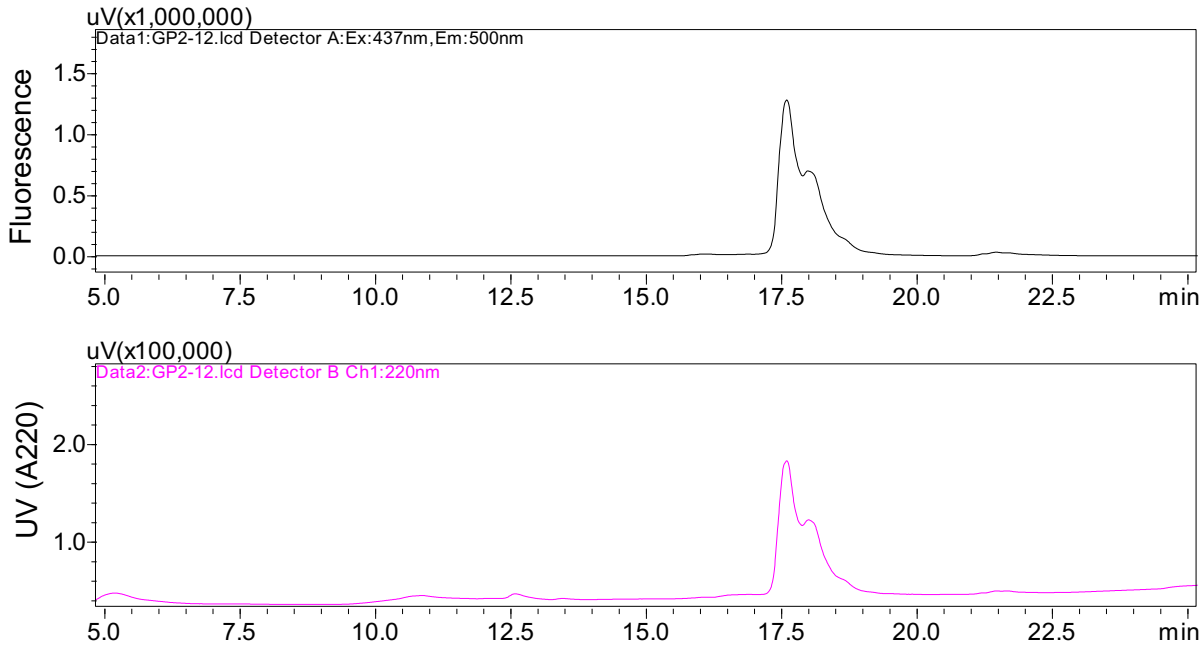
GP2.10


uV(x1,000,000)
Data1:GP2-10.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-10.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

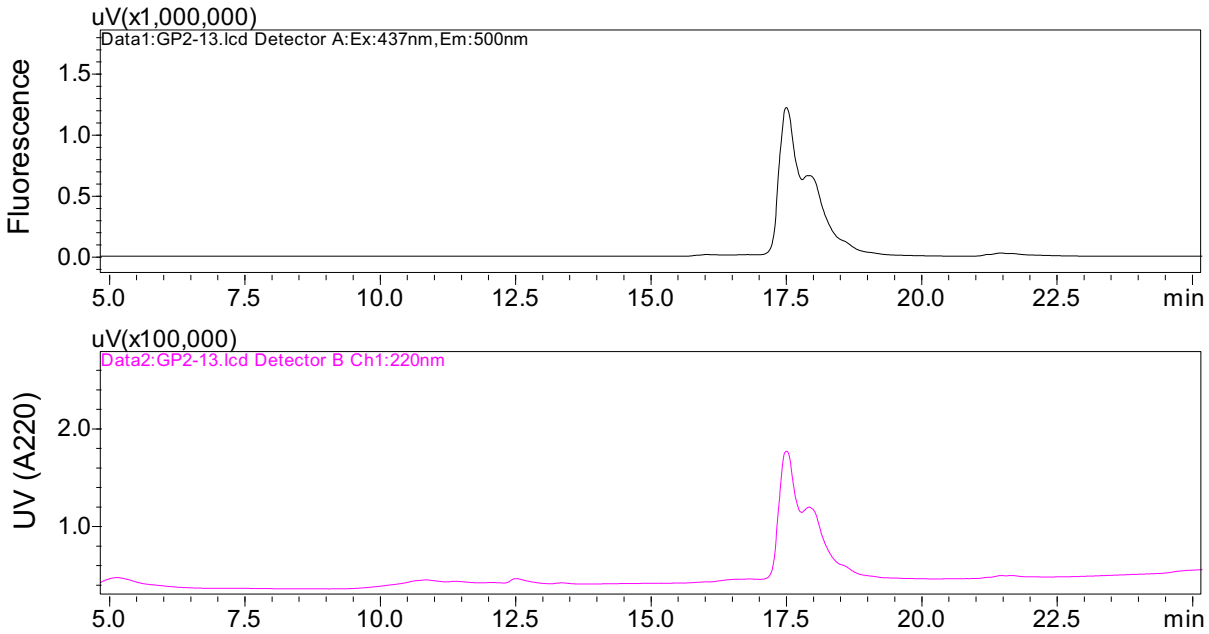
GP2.11


uV(x1,000,000)
Data1:GP2-11.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-11.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

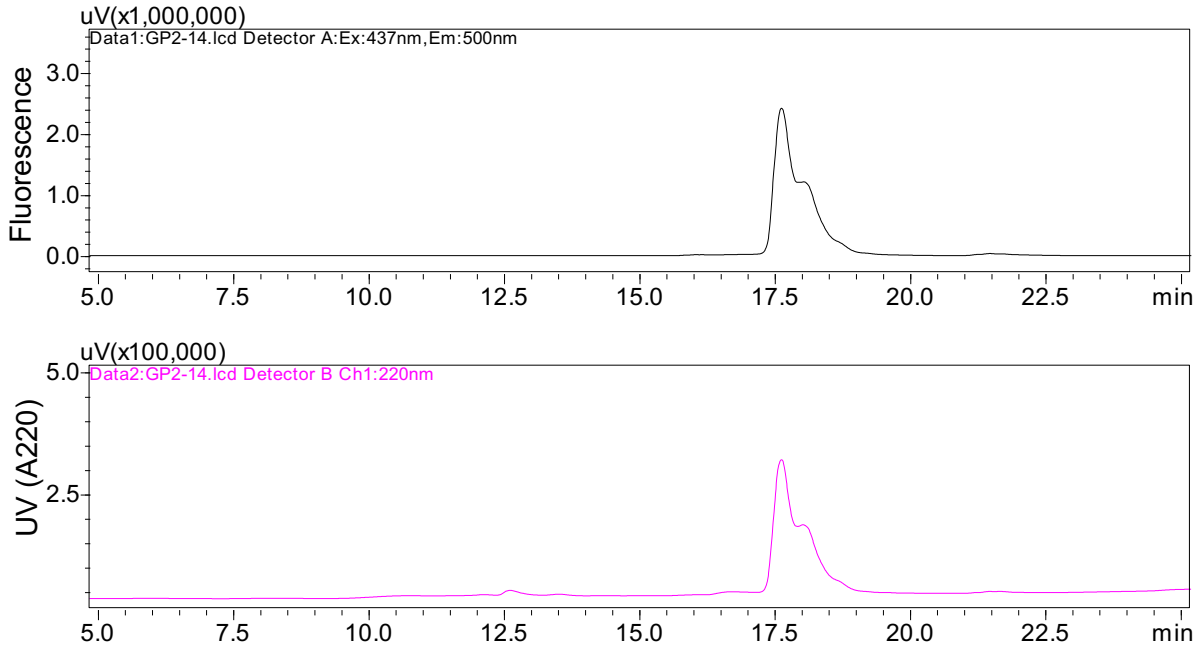
GP2.12


uV(x1,000,000)
Data1:GP2-12.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-12.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

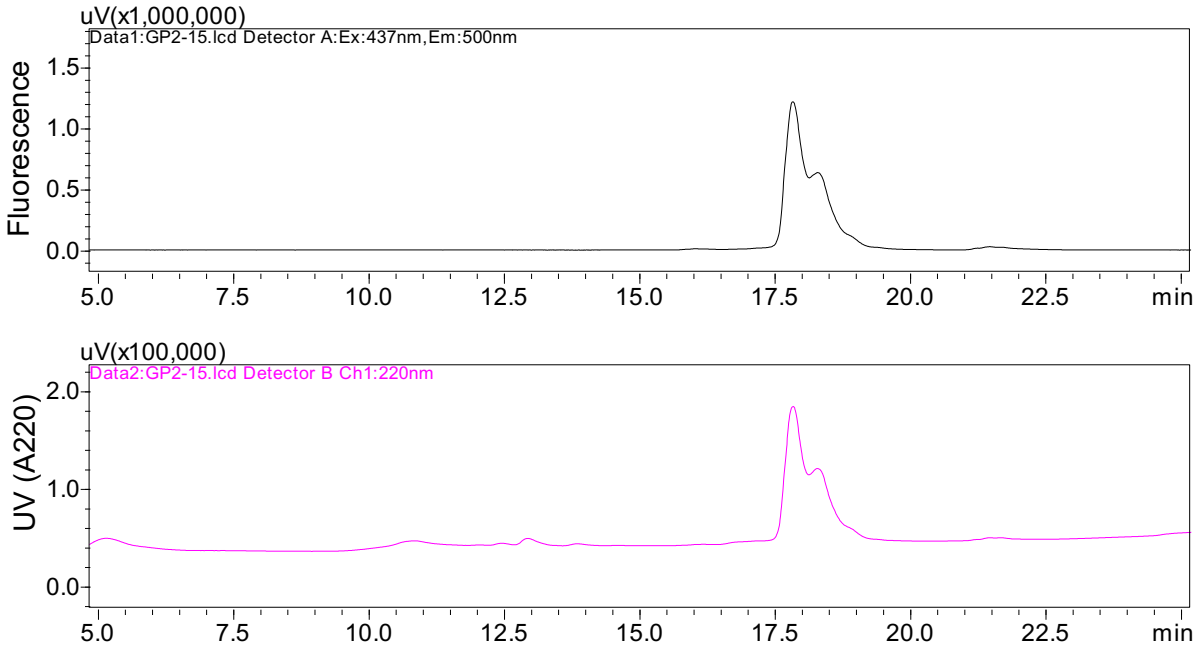
GP2.13


uV(x1,000,000)
Data1:GP2-13.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-13.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

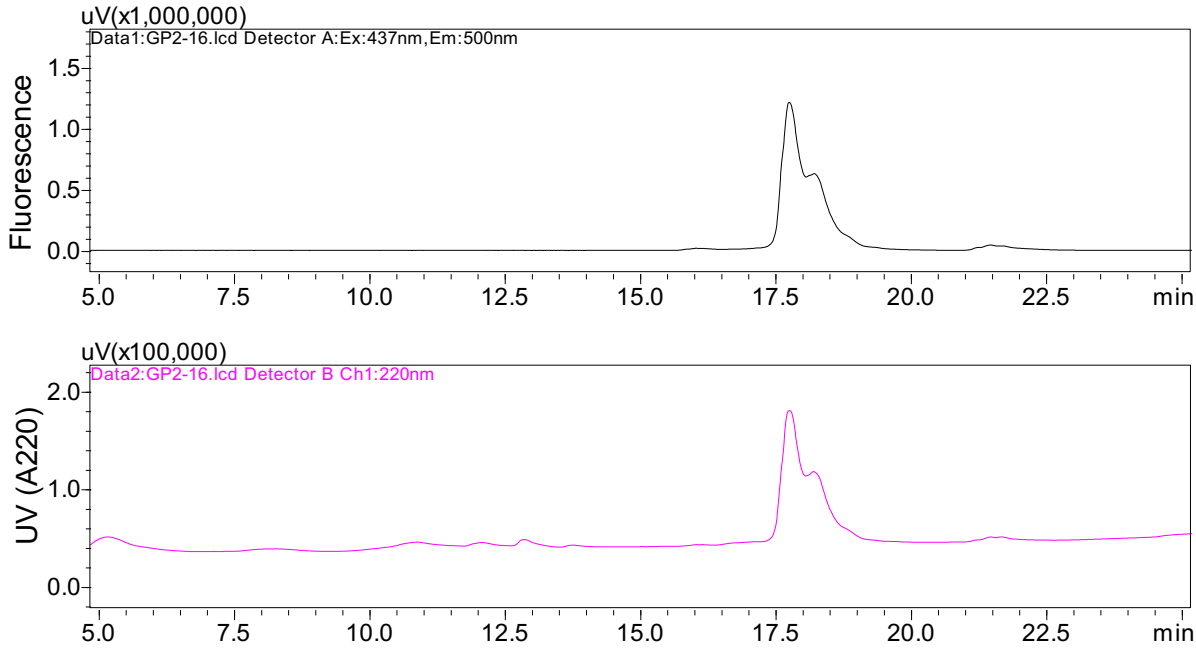
GP2.14


uV(x1,000,000)
Data1:GP2-14.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
3.0
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-14.lcd Detector B Ch1:220nm
UV (A220)
5.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

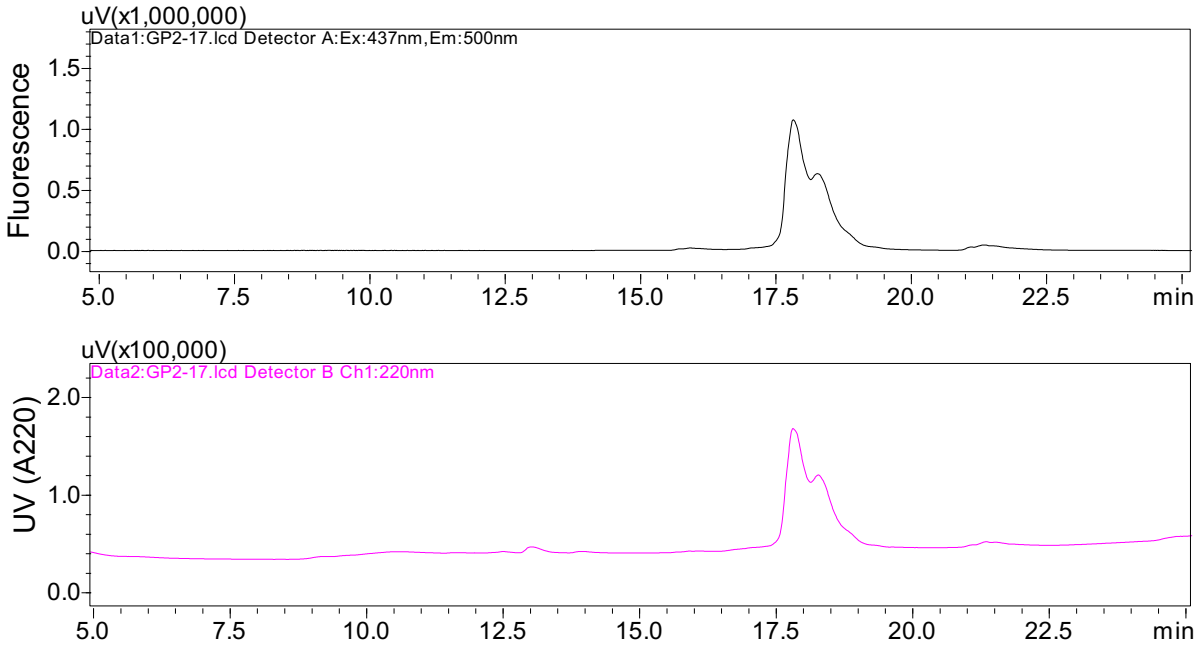
GP2.15


uV(x1,000,000)
Data1:GP2-15.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-15.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

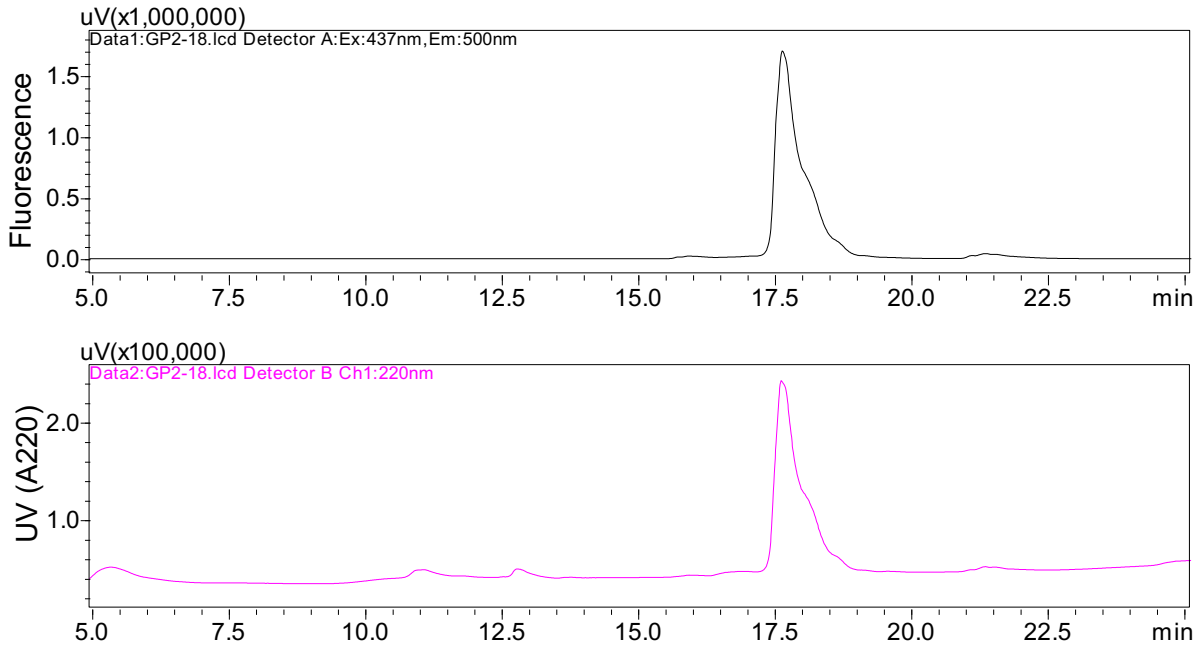
GP2.16


uV(x1,000,000)
Data1:GP2-16.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-16.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

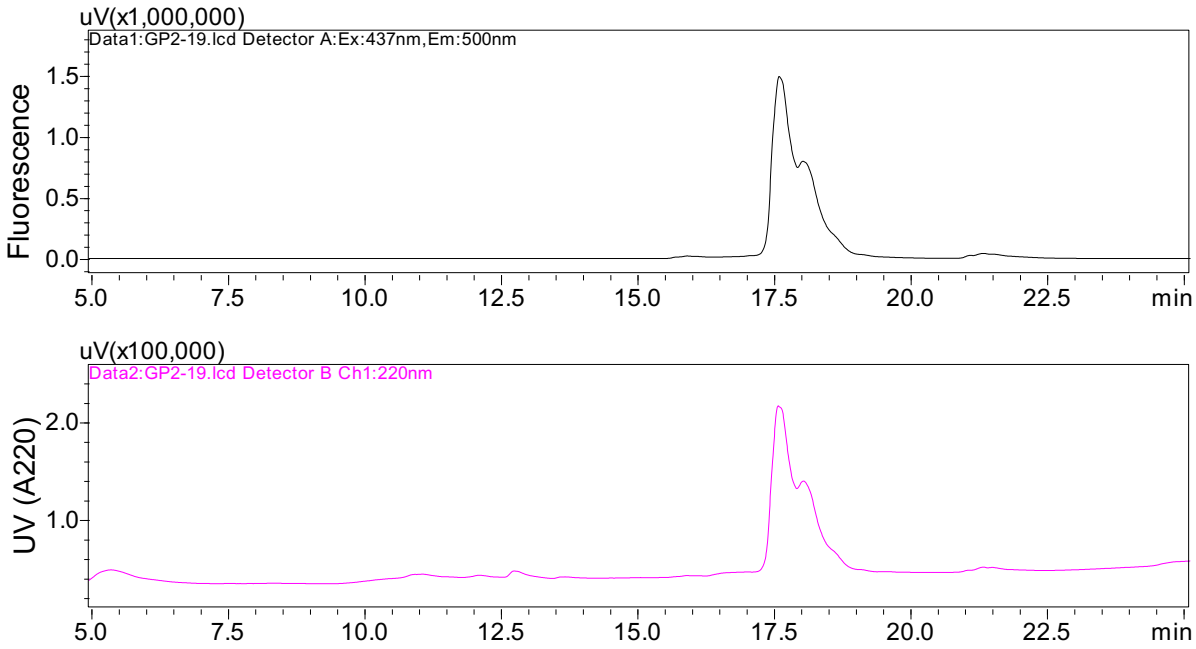
GP2.17


uV(x1,000,000)
Data1:GP2-17.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-17.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

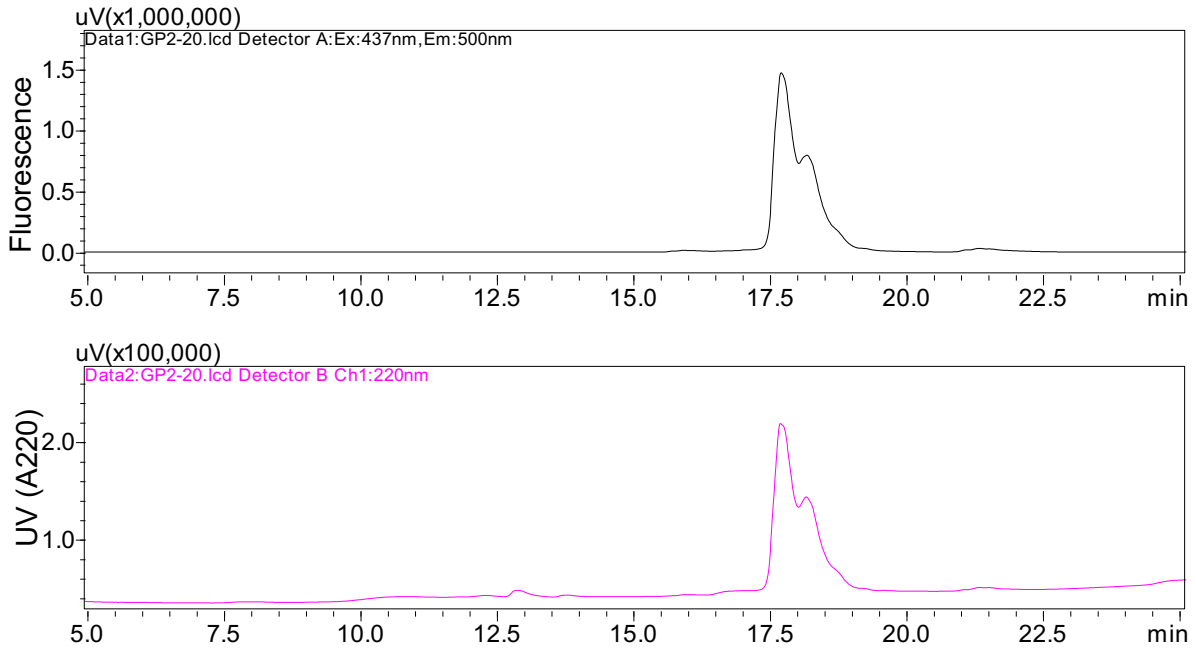
GP2.18


uV(x1,000,000)
Data1:GP2-18.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-18.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

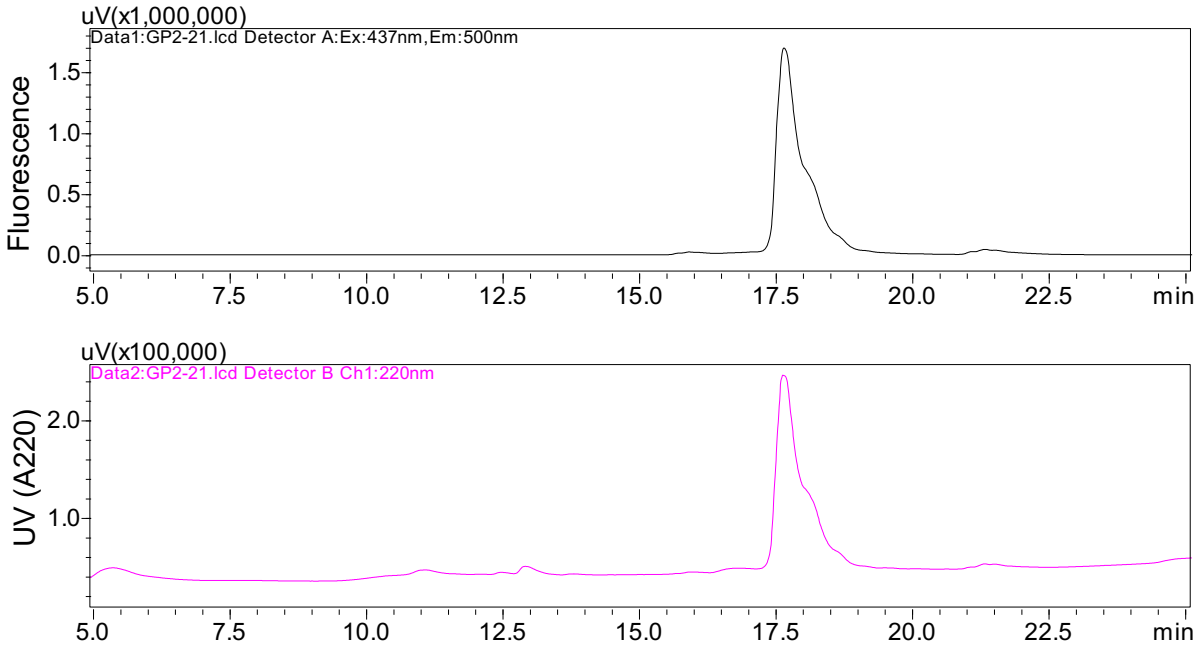
GP2.19


uV(x1,000,000)
Data1:GP2-19.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-19.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

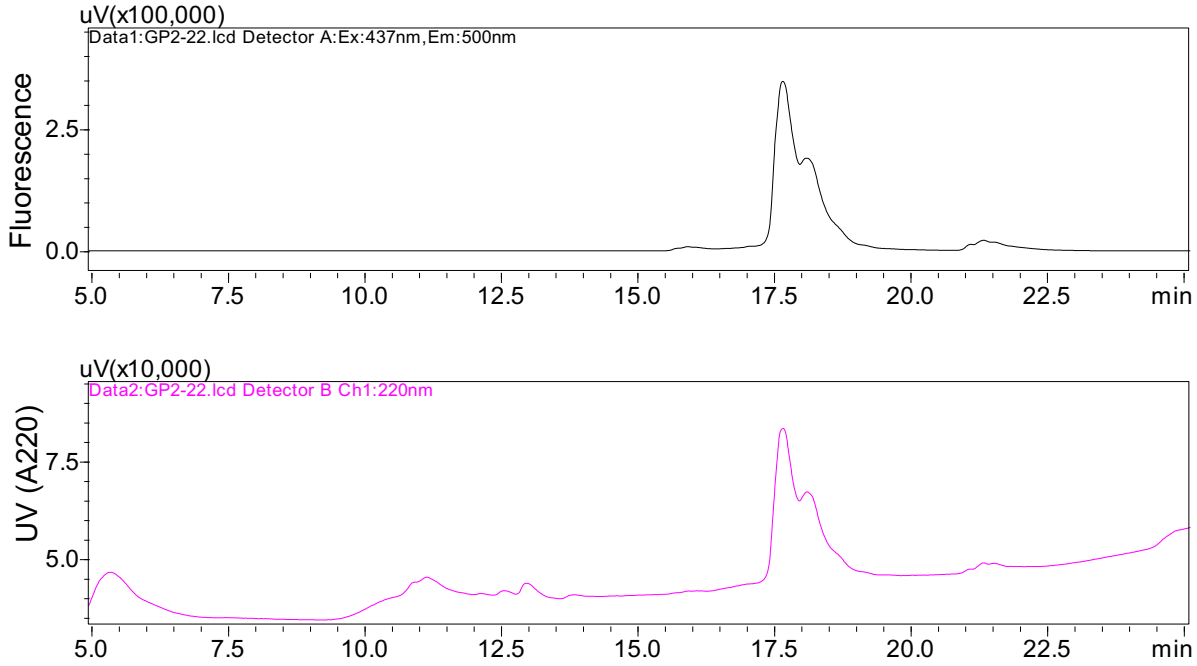
GP2.20


uV(x1,000,000)
Data1:GP2-20.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-20.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

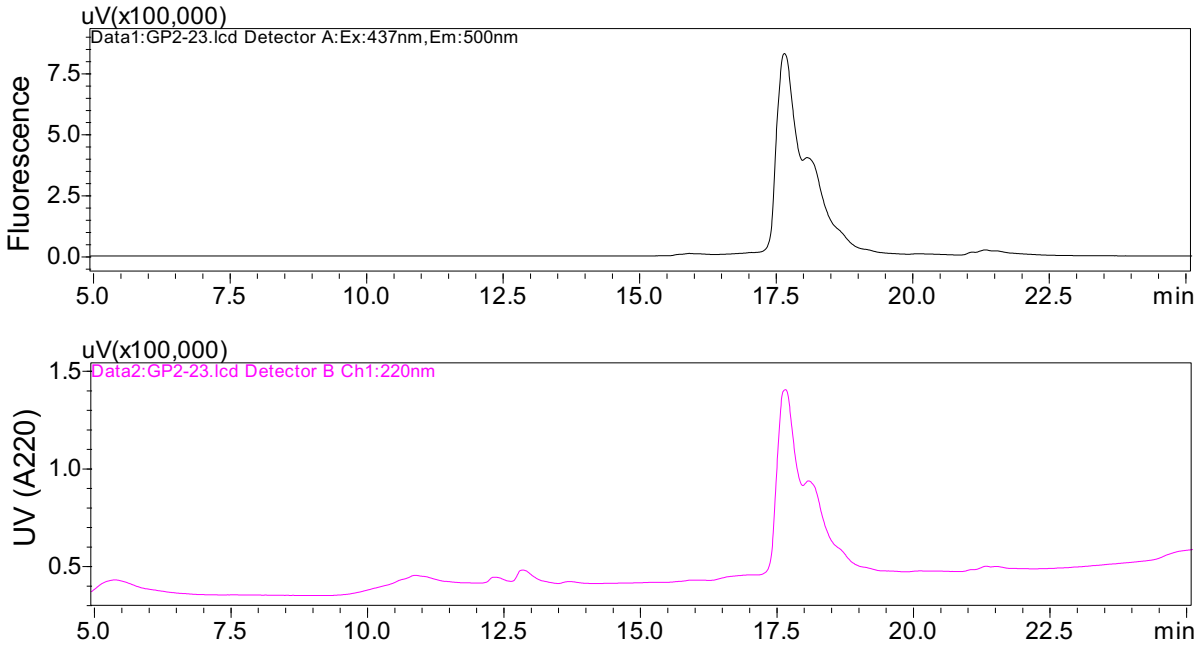
GP2.21


uV(x1,000,000)
Data1:GP2-21.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-21.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

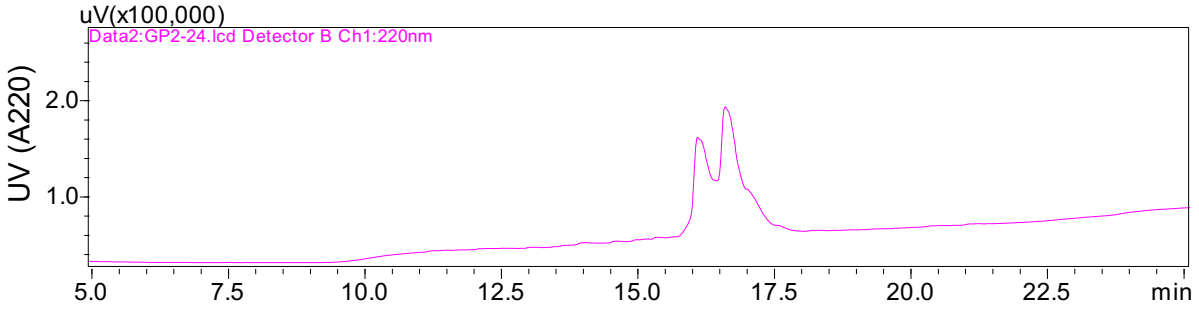
GP2.22


uV(x100,000)
Data1:GP2-22.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x10,000)
Data2:GP2-22.lcd Detector B Ch1:220nm
UV (A220)
7.5
5.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

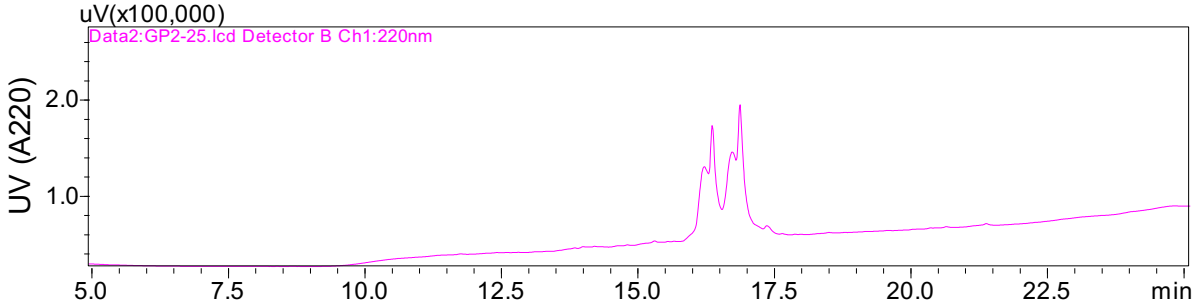
GP2.23



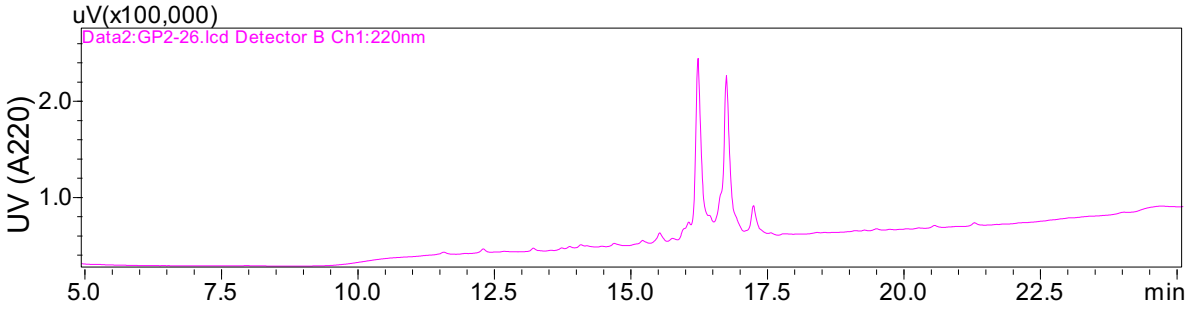
GP2.24 (no fluorophore)



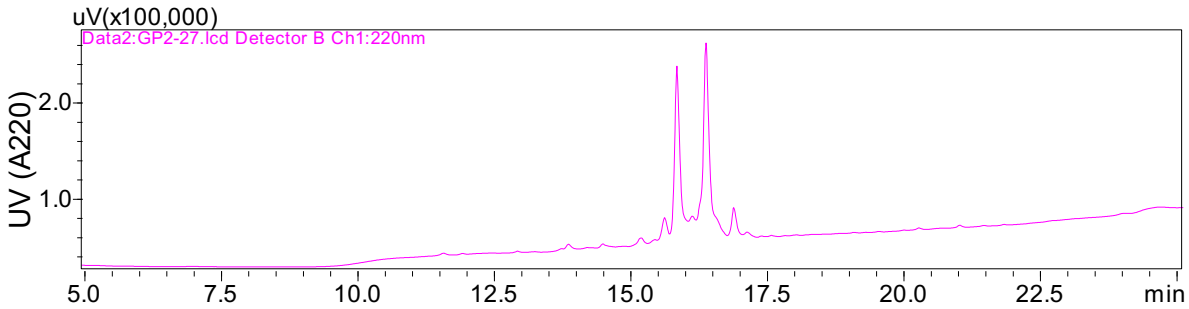
GP2.25 (no fluorophore)



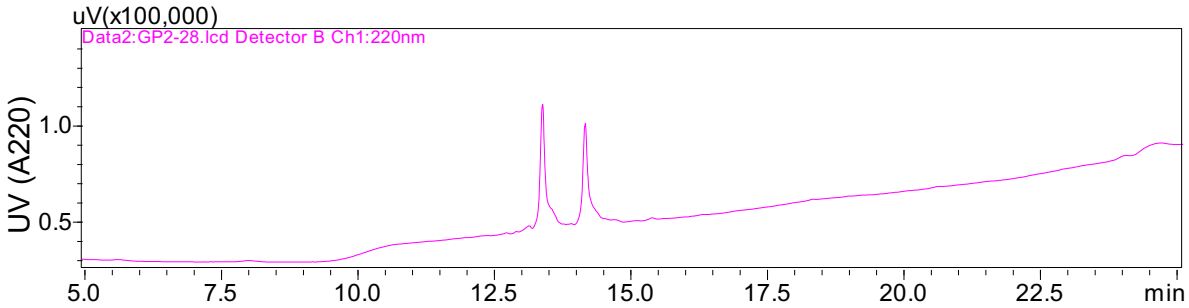
GP2.26 (no fluorophore)



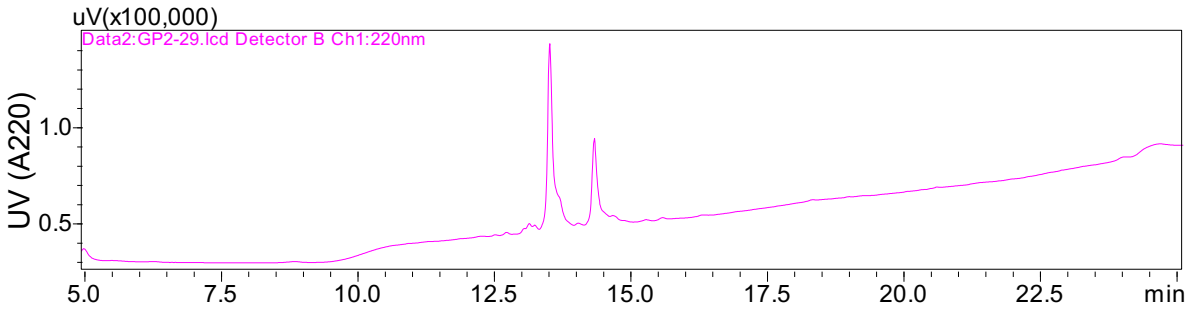
GP2.27 (no fluorophore)



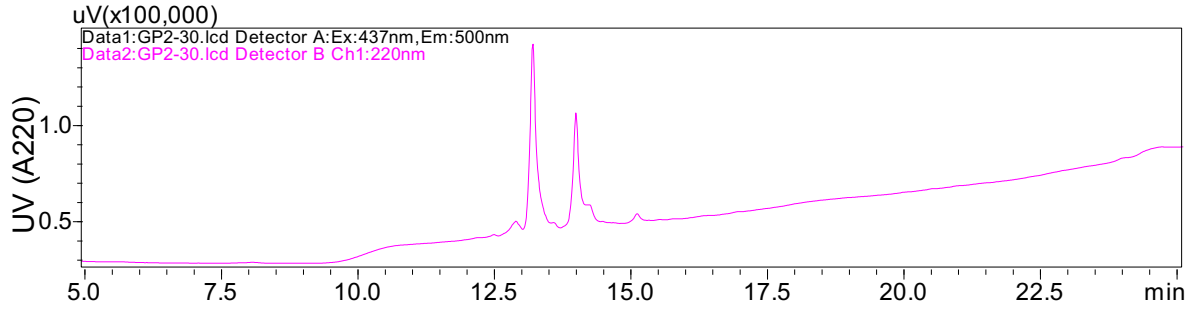
GP2.28 (no fluorophore)



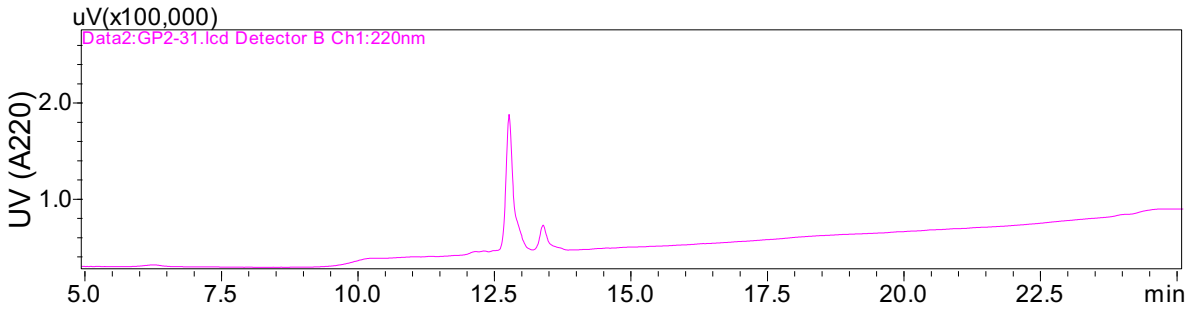
GP2.29 (no fluorophore)



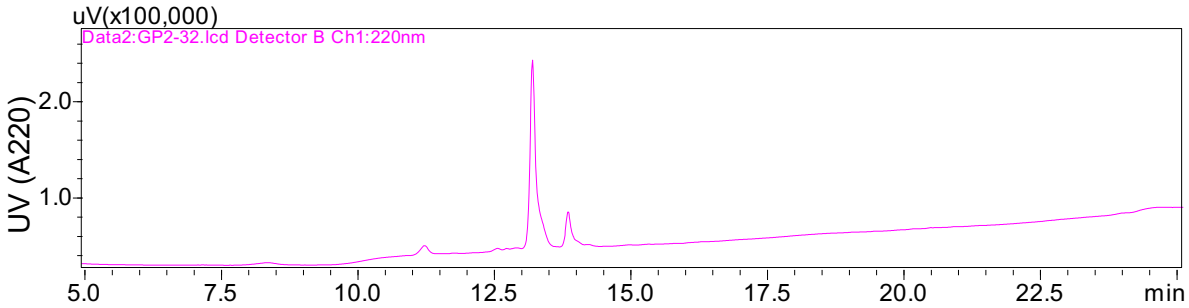
GP2.30 (no fluorophore)



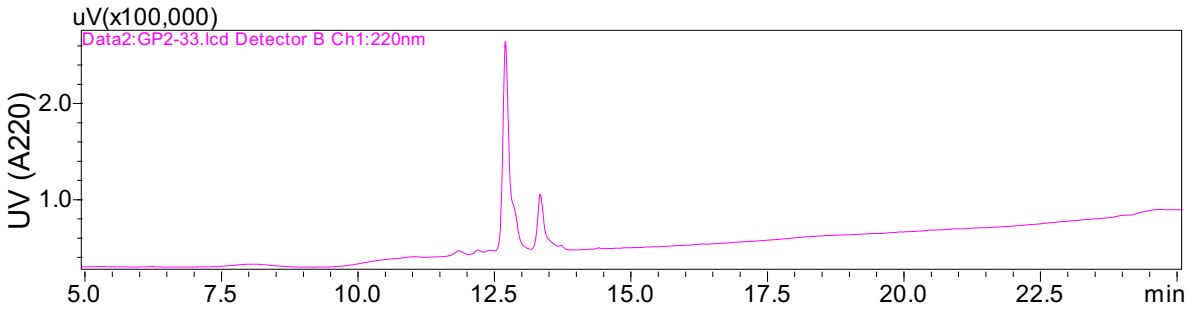
GP2.31 (no fluorophore)



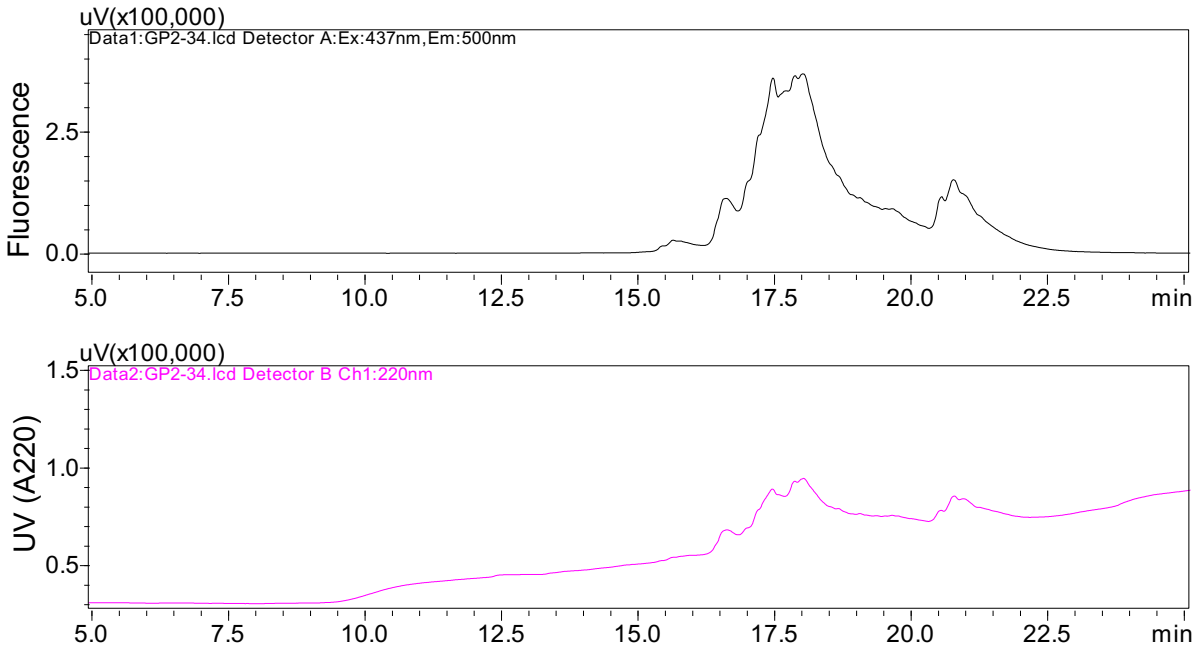
GP2.32 (no fluorophore)



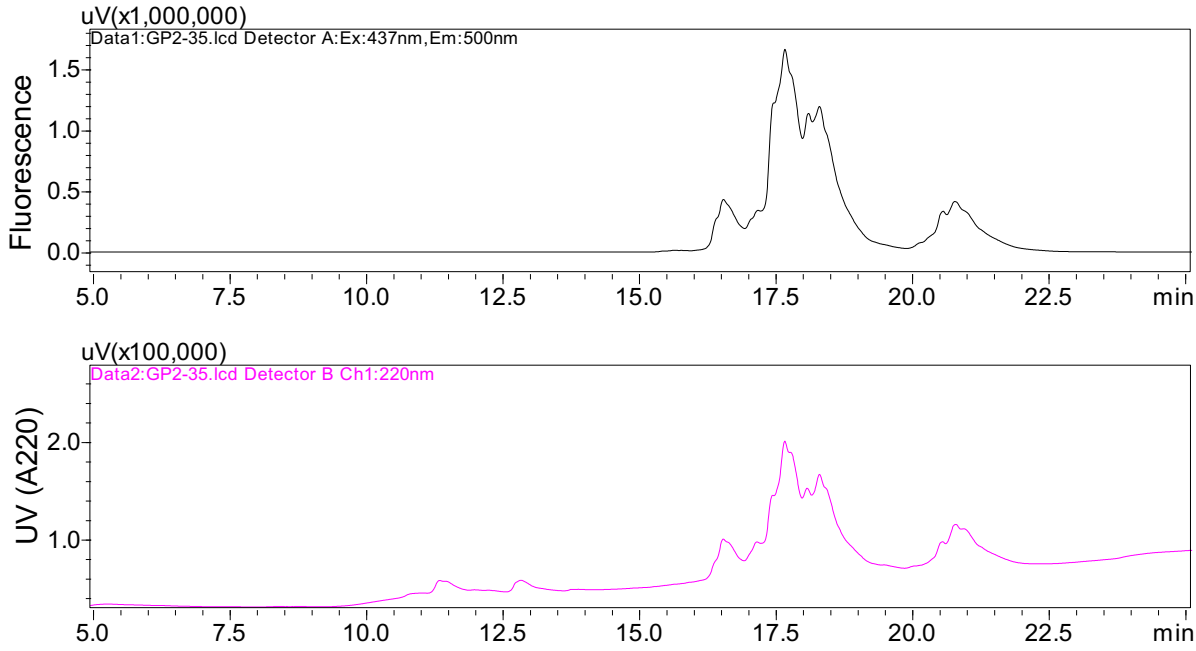
GP2.33 (no fluorophore)



GP2.34*

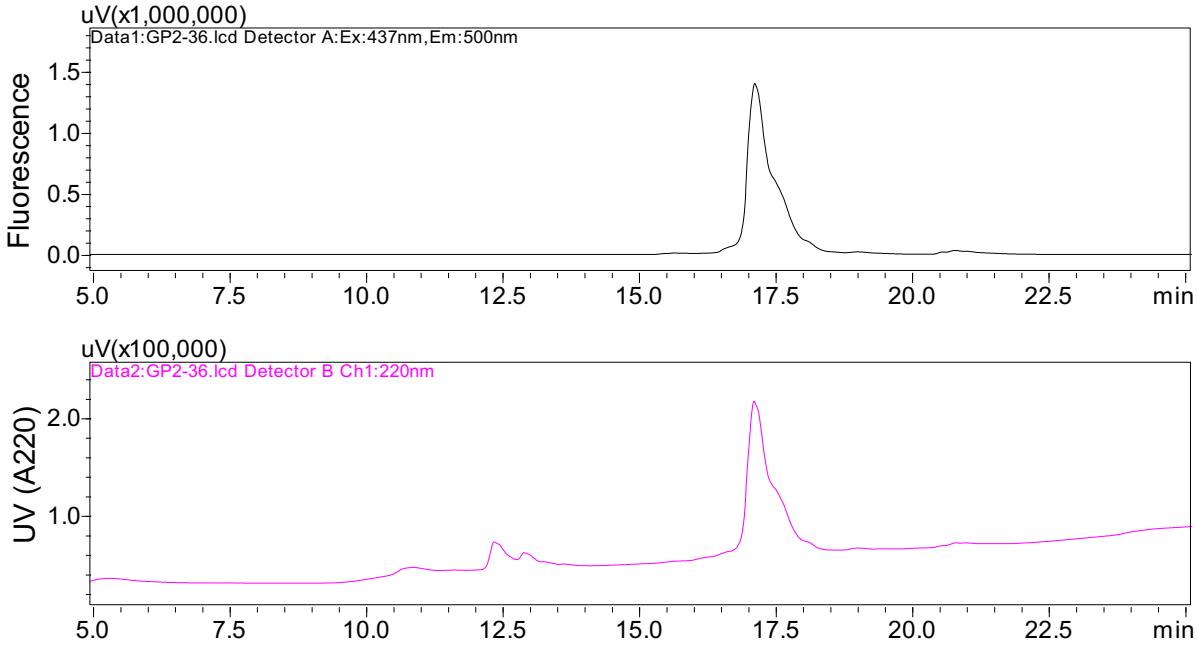


GP2.35*

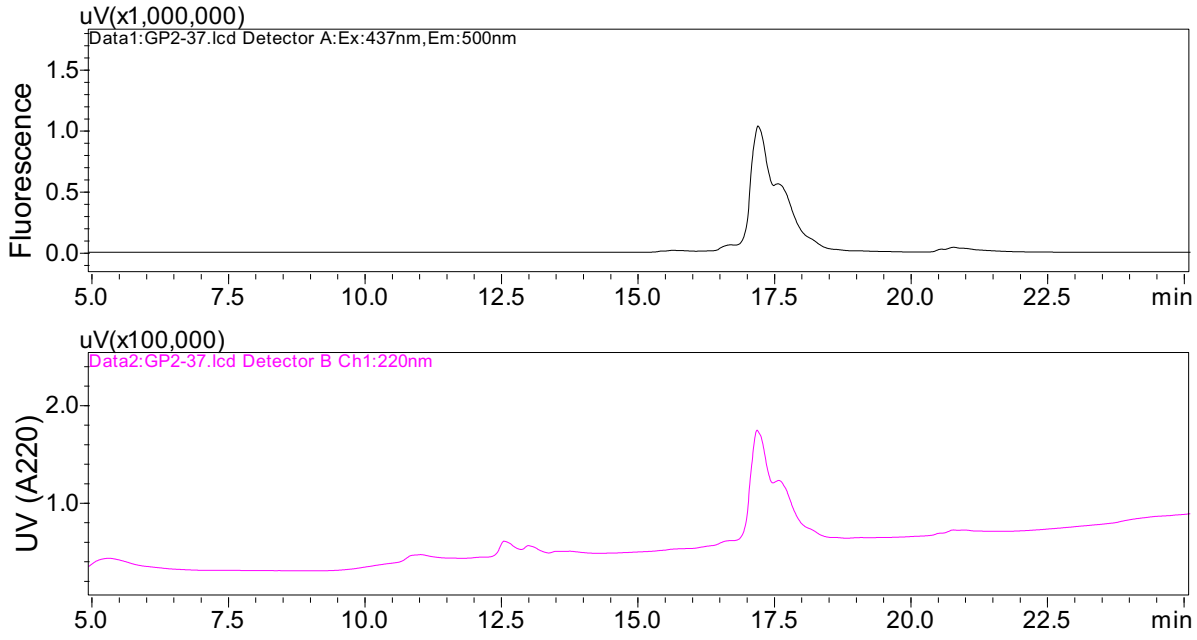


* **Note-** For GP2.34 and GP2.35 we noticed some acetylation side reaction as the spots were still on the membrane during the N-capping step. Since the side-chains are protected during this stage, the acetylation most-likely occurs on the hydroxyl groups of the fluorescein. As a result, the mixture is a bit more complex with acetylated, -COOH and -COOMe terminal peptides causing broader peaks.

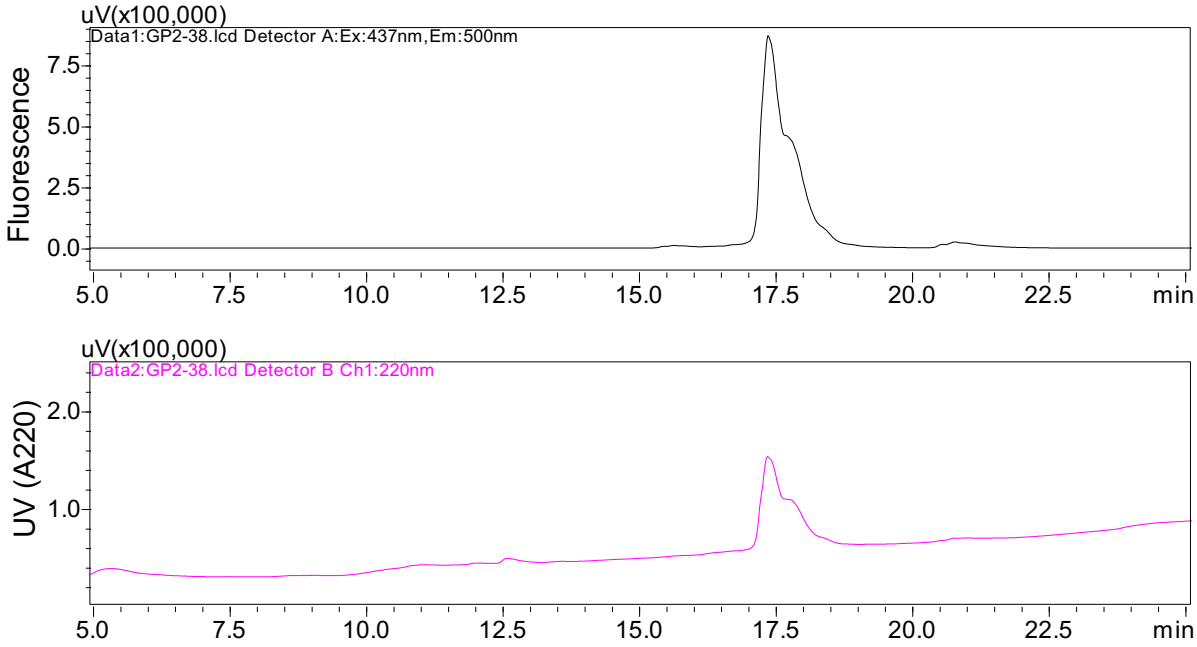
GP2.36


uV(x1,000,000)
Data1:GP2-36.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-36.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

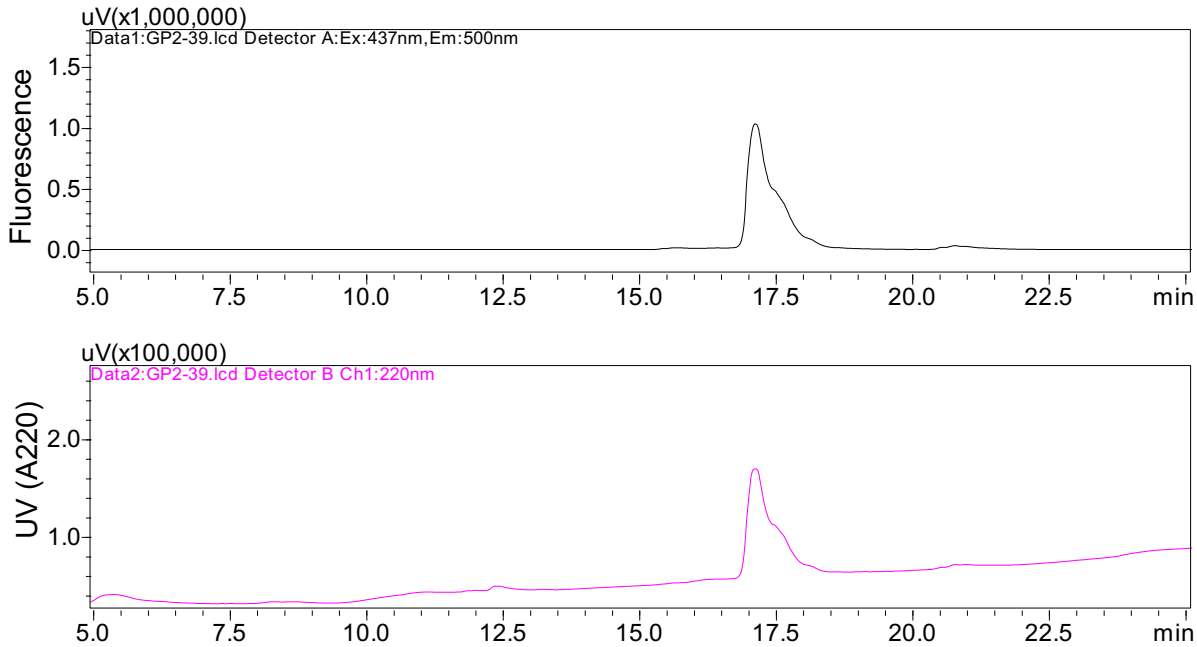
GP2.37


uV(x1,000,000)
Data1:GP2-37.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-37.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

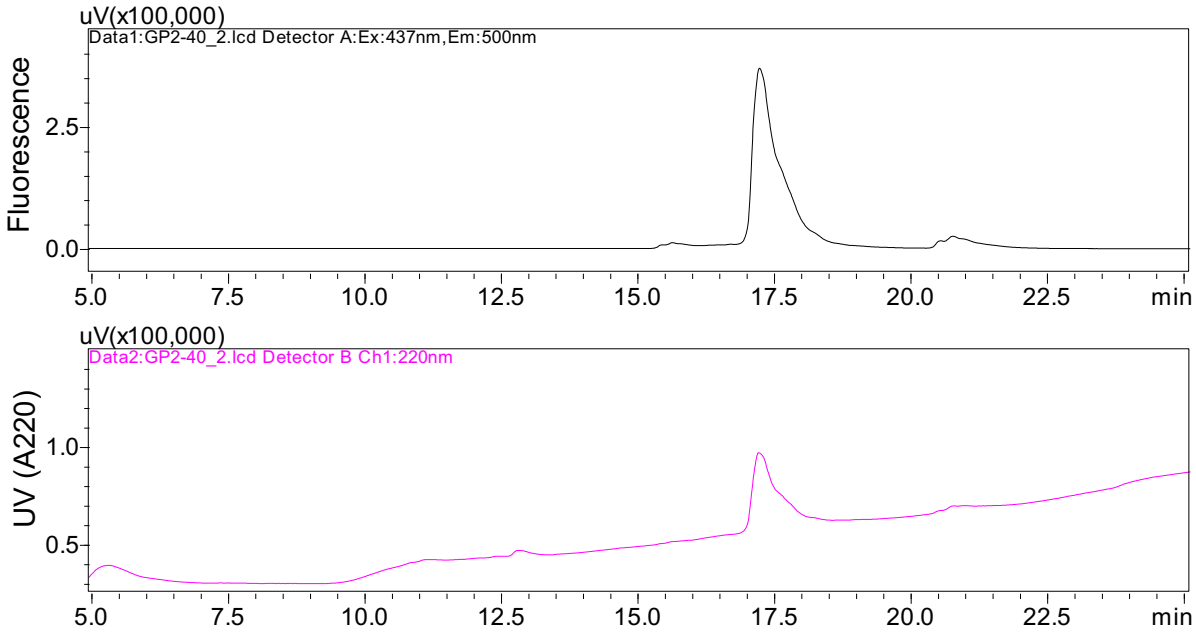
GP2.38


uV(x100,000)
Data1:GP2-38.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
7.5
5.0
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-38.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

GP2.39


uV(x1,000,000)
Data1:GP2-39.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
1.5
1.0
0.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-39.lcd Detector B Ch1:220nm
UV (A220)
2.0
1.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

GP2.40

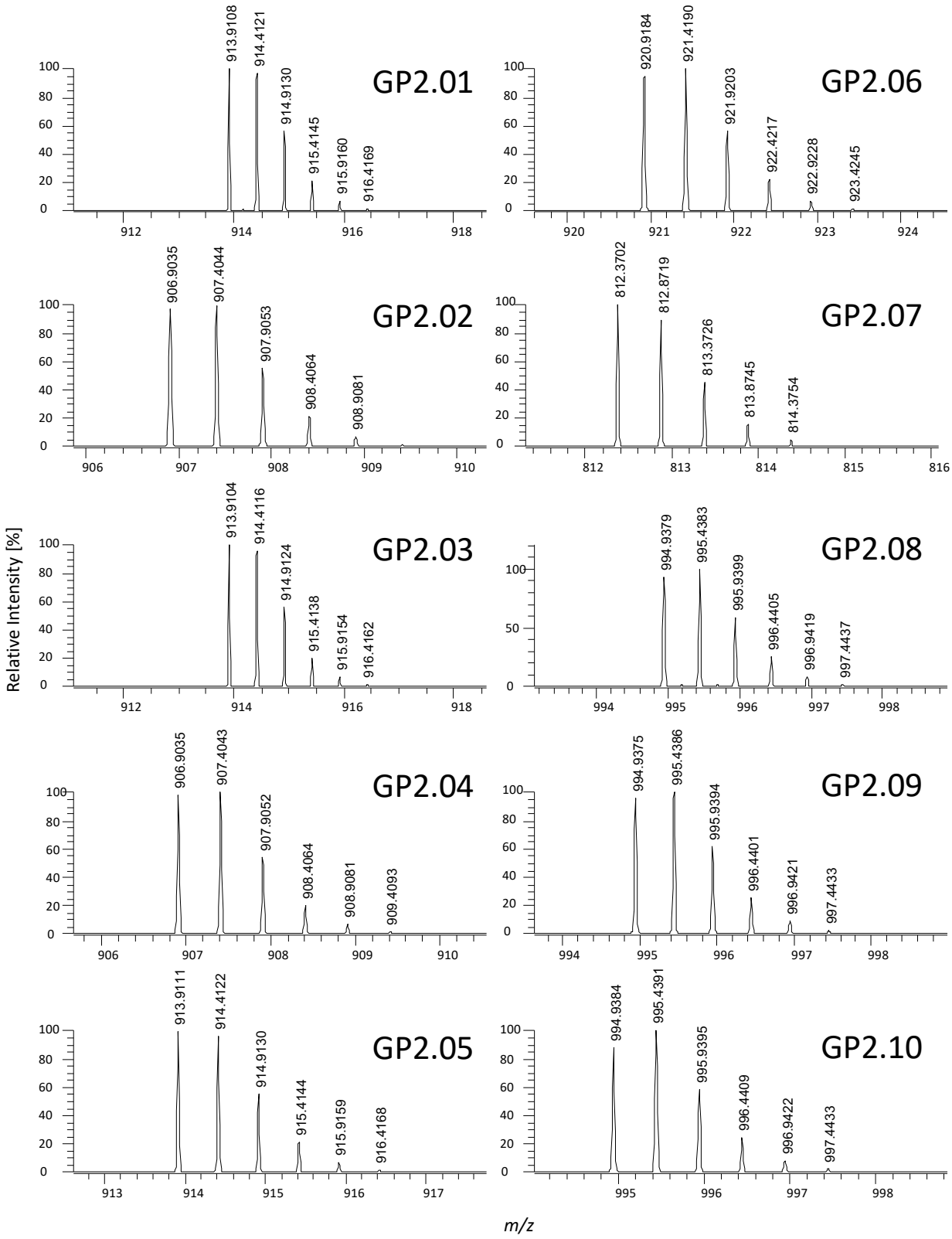

uV(x100,000)
Data1:GP2-40_2.lcd Detector A:Ex:437nm,Em:500nm
Fluorescence
2.5
0.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min
uV(x100,000)
Data2:GP2-40_2.lcd Detector B Ch1:220nm
UV (A220)
1.0
0.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

Orbitrap Characterization of GP2 Library

MS spectra of GP2.01-GP.40 by high-resolution C18-RP-LC-MS

Summary of calculated and observed masses

	[M]	[M+2H]2+ theor	[M+2H]2+ observed	ppm error	[M+3H]3+ theor	[M+3H]3+ observed	ppm error
GP2.01	1825.7935	913.9040	913.9108	7.4	609.6051	609.6094	7.0
GP2.02	1811.7779	906.8962	906.9035	8.1	604.9332	604.9373	6.7
GP2.03	1825.7935	913.9040	913.9104	7.0	609.6051	609.6090	6.4
GP2.04	1811.7779	906.8962	906.9035	8.1	604.9332	604.9372	6.6
GP2.05	1825.7935	913.9040	913.9111	7.7	609.6051	609.6093	6.9
GP2.06	1839.8092	920.9118	920.9184	7.1	614.2770	614.2812	6.9
GP2.07	1622.7141	812.3643	812.3702	7.2	541.9120	541.9155	6.5
GP2.08	1987.8463	994.9304	994.9379	7.5	663.6227	663.6280	8.0
GP2.09	1987.8463	994.9304	994.9375	7.1	663.6227	663.6276	7.4
GP2.10	1987.8463	994.9304	994.9384	8.0	663.6227	663.6280	8.0
GP2.11	1987.8463	994.9304	994.9378	7.4	663.6227	663.6282	8.3
GP2.12	1799.7779	900.8962	900.9030	7.6	600.9332	600.9375	7.1
GP2.13	1799.7779	900.8962	900.9028	7.3	600.9332	600.9375	7.1
GP2.14	1799.7779	900.8962	900.9031	7.7	600.9332	600.9374	7.0
GP2.15	1843.7841	922.8993	922.9064	7.7	615.6020	615.6065	7.4
GP2.16	1843.7841	922.8993	922.9060	7.2	615.6020	615.6062	6.9
GP2.17	1843.7841	922.8993	922.9057	6.9	615.6020	615.6063	7.0
GP2.18	1843.7841	922.8993	922.9068	8.1	615.6020	615.6064	7.2
GP2.19	1843.7841	922.8993	922.9063	7.6	615.6020	615.6066	7.5
GP2.20	1843.7841	922.8993	922.9057	6.9	615.6020	615.6059	6.4
GP2.21	1827.7550	914.8848	914.8916	7.5	610.2589	610.2632	7.0
GP2.22	1827.7550	914.8848	914.8917	7.6	610.2589	610.2633	7.1
GP2.23	1827.7550	914.8848	914.8955	11.7	610.2589	610.2657	11.1
GP2.24	1654.7509	828.3827	828.3929	12.3	552.5909	N/A	N/A
GP2.25	1654.7509	828.3827	828.3932	12.6	552.5909	N/A	N/A
GP2.26	1654.7509	828.3827	828.3934	12.9	552.5909	N/A	N/A
GP2.27	1638.7738	820.3942	820.4047	12.9	547.2652	N/A	N/A
GP2.28	1476.6767	739.3456	N/A	N/A	493.2328	N/A	N/A
GP2.29	1432.6869	717.3507	717.3592	11.8	478.5696	N/A	N/A
GP2.30	1460.6995	731.3570	731.3658	12.0	487.9071	N/A	N/A
GP2.31	1619.7713	810.8929	810.9038	13.4	540.9310	N/A	N/A
GP2.32	1575.7815	788.8980	788.9089	13.8	526.2678	N/A	N/A
GP2.33	1603.7942	802.9044	802.9147	12.9	535.6053	N/A	N/A
GP2.34	2500.0416	1251.0281	1251.0440	12.7	834.3545	834.3669	14.9
GP2.35	1141.4605	571.7375	571.7451	13.3	381.4941	N/A	N/A
GP2.36	2014.8573	1008.4359	1008.4499	13.9	672.6264	672.6359	14.2
GP2.37	2028.8729	1015.4437	1015.4573	13.4	677.2982	677.3074	13.5
GP2.38	1861.7747	931.8946	931.9067	13.0	621.5988	621.6066	12.5
GP2.39	1861.7747	931.8946	931.9073	13.6	621.5988	621.6070	13.1
GP2.40	1827.7550	914.8848	914.8993	15.9	610.2589	610.2680	14.8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921.9203
922.4217
922.9228
923.4245
GP2.02
906.9035
907.4044
907.9053
908.4064
908.9081
GP2.07
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813.8745
814.3754
GP2.03
913.9104
914.4116
914.9124
915.4138
915.9154
916.4162
GP2.08
994.9379
995.4383
995.9399
996.4405
996.9419
997.4437
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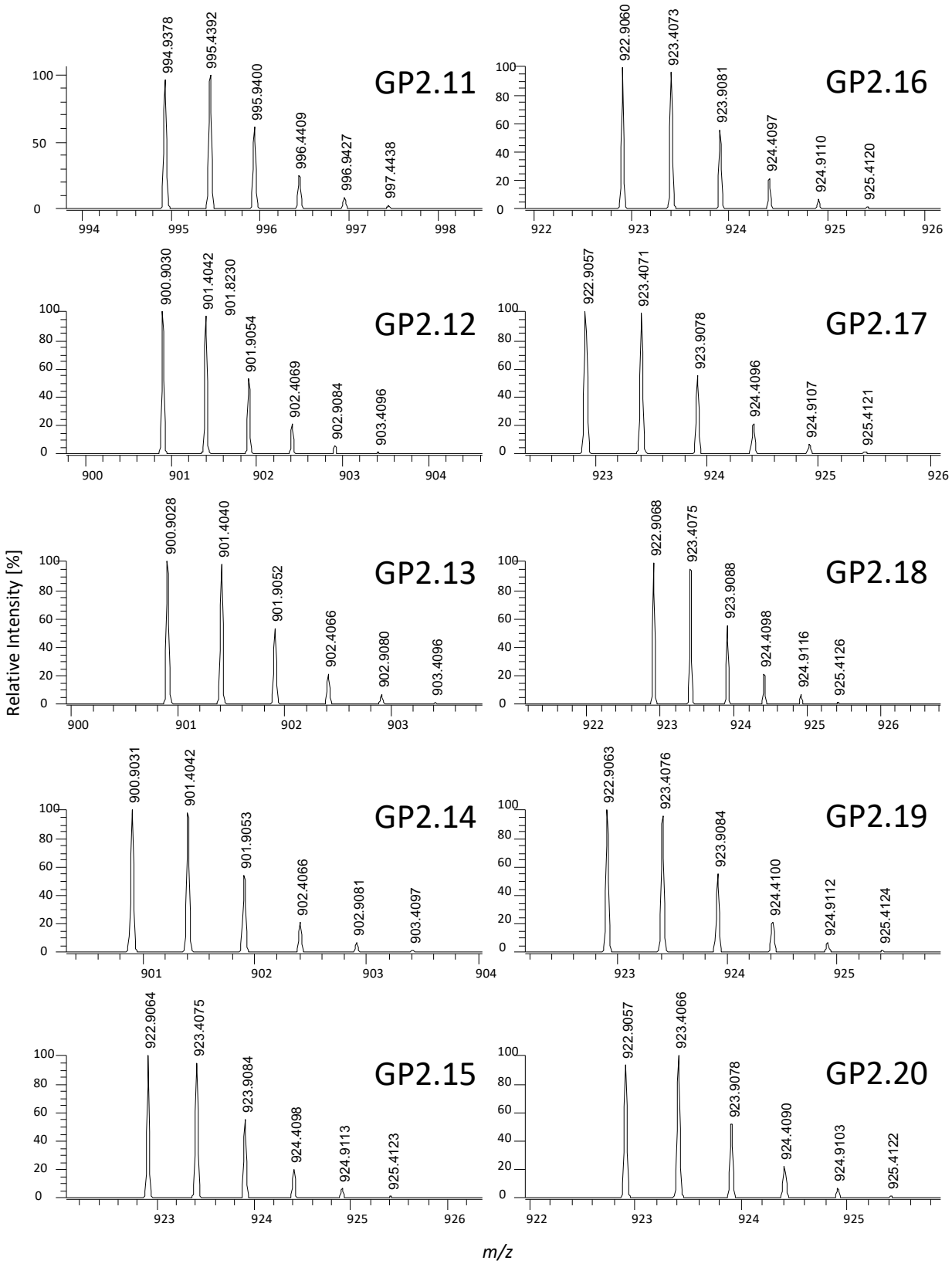
906.9035
907.4043
907.9052
908.4064
908.9081
909.4093
GP2.09
994.9375
995.4386
995.9394
996.4401
996.9421
997.4433
GP2.05
913.9111
914.4122
914.9130
915.4144
915.9159
916.4168
GP2.10
994.9384
995.4391
995.9395
996.4409
996.9422
997.4433
Relative Intensity [%]
m/z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923.9081
924.4097
924.9110
925.4120
GP2.12
900.9030
901.4042
901.8230
901.9054
902.4069
902.9084
903.4096
GP2.17
922.9057
923.4071
923.9078
924.4096
924.9107
925.4121
GP2.13
900.9028
901.4040
901.9052
902.4066
902.9080
903.4096
GP2.18
922.9068
923.4075
923.9088
924.4098
924.9116
925.4126
GP2.14
900.9031
901.4042
901.9053
902.4066
902.9081
903.4097
GP2.19
922.9063
923.4076
923.9084
924.4100
924.9112
925.4124
GP2.15
922.9064
923.4075
923.9084
924.4098
924.9113
925.4123
GP2.20
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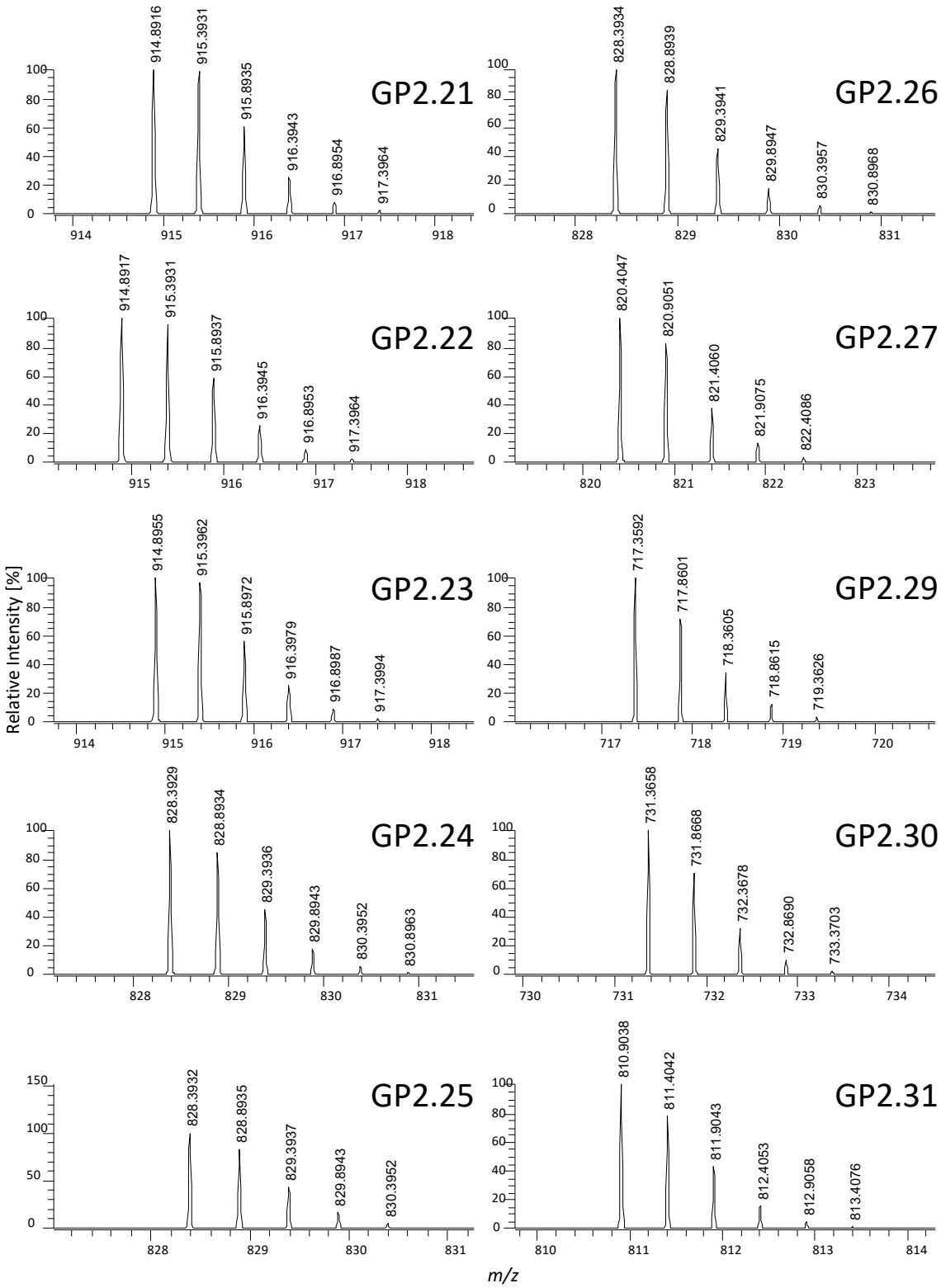
923.4066
923.9078
924.4090
924.9103
925.4122
Relative Intensity [%]
m/z


GP2.21
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GP2.26
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828.8939
829.3941
829.8947
830.3957
830.8968
GP2.22
914.8917
915.3931
915.8937
916.3945
916.8953
917.3964
GP2.27
820.4047
820.9051
821.4060
821.9075
822.4086
GP2.23
914.8955
915.3962
915.8972
916.3979
916.8987
917.3994
GP2.29
717.3592
717.8601
718.3605
718.8615
719.3626
GP2.24
828.3929
828.8934
829.3936
829.8943
830.3952
830.8963
GP2.30
731.3658
731.8668
732.3678
732.8690
733.3703
GP2.25
828.3932
828.8935
829.3937
829.8943
830.3952
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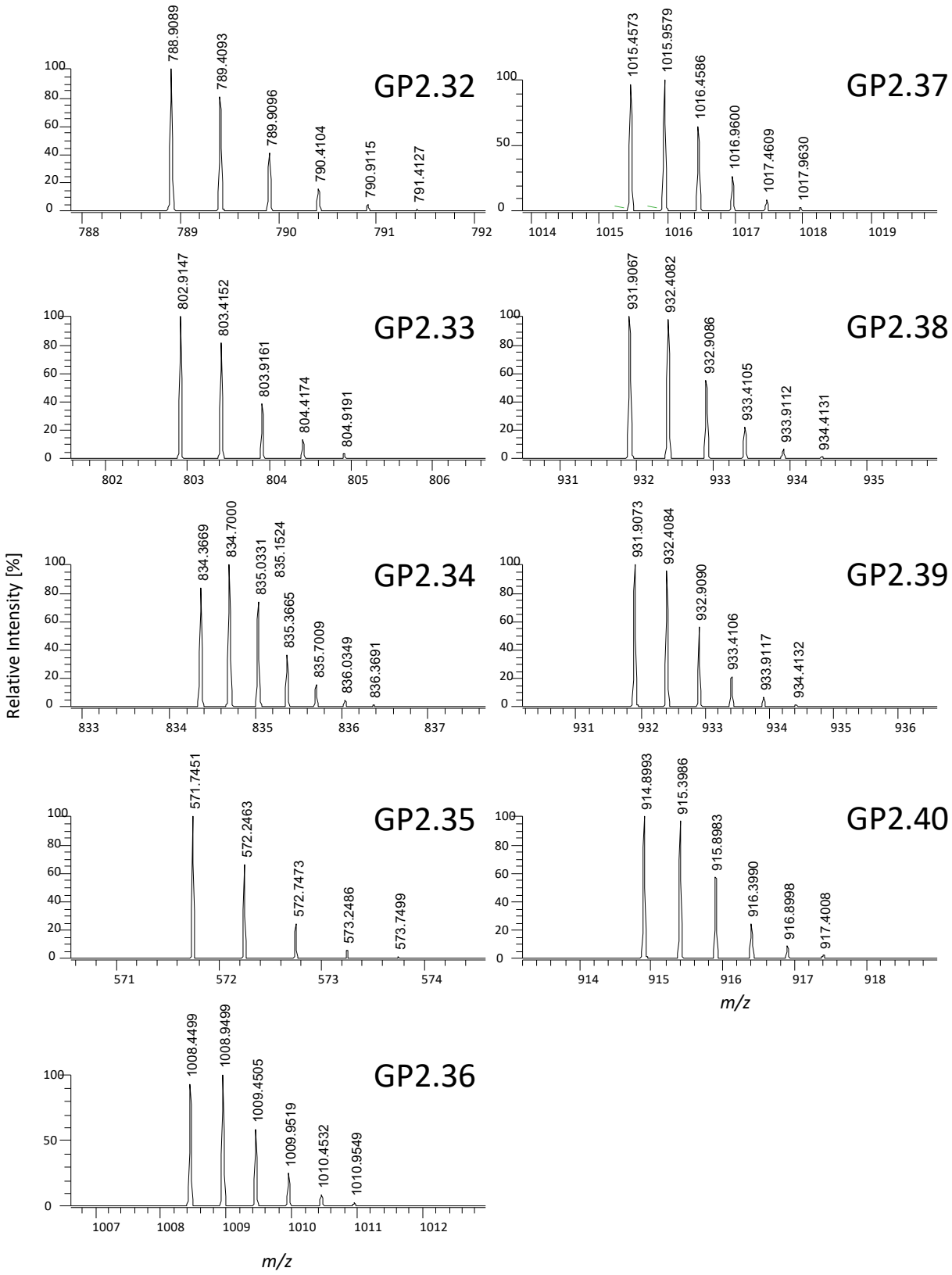
811.9043
812.4053
812.9058
813.4076
Relative Intensity [%]
m/z


GP2.32
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GP2.37
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1016.4586
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1017.4609
1017.9630
GP2.33
802.9147
803.4152
803.9161
804.4174
804.9191
GP2.38
931.9067
932.4082
932.9086
933.4105
933.9112
934.4131
GP2.34
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835.1524
835.3665
835.7009
836.0349
836.3691
GP2.39
931.9073
932.4084
932.9090
933.4106
933.9117
934.4132
GP2.35
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572.7473
573.2486
573.7499
GP2.40
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915.3986
915.8983
916.3990
916.8998
917.4008
GP2.36
1008.4499
1008.9499
1009.4505
1009.9519
1010.4532
1010.9549
Relative Intensity [%]
m/z
m/z

References

Huberty, M.C., Vath, J.E., Yu, W., and Martin, S.A. (1993). Site-specific carbohydrate identification in recombinant proteins using MALD-TOF MS. Anal Chem *65*, 2791-2800.