Supplementary Information

Effects of Microvirin Mono- and Oligomers on Hepatitis C Virus

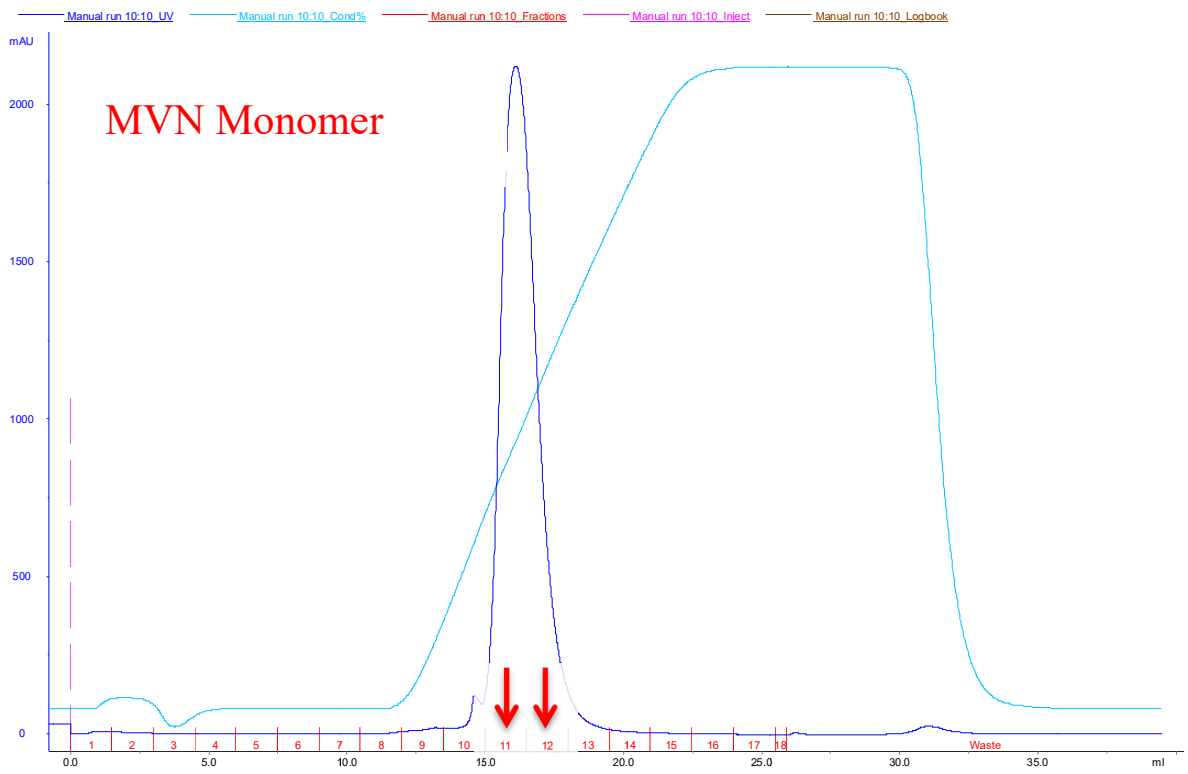
Yuan-Qin Min, Xu-Chu Duan, Yi-Dan Zhou, Anna Kulinich, Zhi-Peng Cai, Hong-Yu Ma, Li Liu, Xiao-Lian Zhang and Josef Voglmeir

Supplementary Table 1 Primers used for cloning MVN and its oligomers

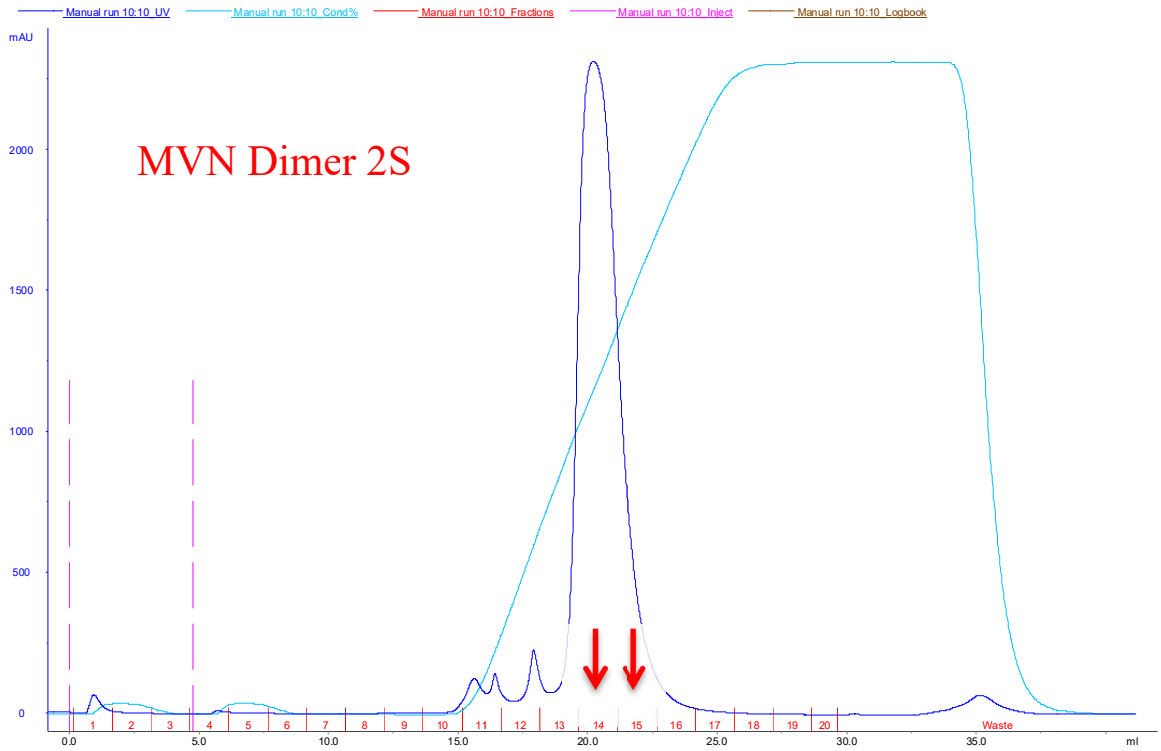
Primer names	Sequences (5' - 3')
MVN-NdeI For	GGAATTCCATATGCCGAACTTCTCTCACACCTG
MVN-XhoI Rev	CCGCTCGAGACCGATTTCCAGCTGAGAGTC
MVN-BamHI For	CGCGGATCCATGCCGAACTTCTCTCACACC
MVN-BamHI Rev	CGCGGATCCACCGATTTCCAGCTGAGAGTC
MVN-EcoRI For	CGCGAATTCATGCCGAACTTCTCTCACACC
MVN-EcoRI Rev	CGCGAATTCACCGATTTCCAGCTGAGAGTC
MVN-SacI For	CGCGAGCTCATGCCGAACTTCTCTCACACC
MVN-SacI Rev	CGCGAGCTCACCGATTTCCAGCTGAGAGTC
MVN-BamHI6 For	CGCGGATCC*GGCGGC*ATGCCGAACTTCTCTCACACC
MVN-BamHI6 Rev	CGCGGATCC*GGAGCC*ACCGATTTCCAGCTGAGAGTC
MVN-EcoRI6 For	CGCGAATTC*GGCGGC*ATGCCGAACTTCTCTCACACC
MVN-EcoRI6 Rev	CGCGAATTC*GGAGCC*ACCGATTTCCAGCTGAGAGTC
MVN-SacI6 For	CGCGAGCTC*GGCGGC*ATGCCGAACTTCTCTCACACC
MVN-SacI6 Rev	CGCGAGCTC*GGAGCC*ACCGATTTCCAGCTGAGAGTC
MVN-BamHI10 For	CGCGGATCC*GGCGGCTCCGGC*ATGCCGAACTTCTCTCACACC
MVN-BamHI10 Rev	CGCGGATCC*GCCGGAGCCGCC*ACCGATTTCCAGCTGAGAGTC
MVN-EcoRI10 For	CGCGAATTC*GGCGGCTCCGGC*ATGCCGAACTTCTCTCACACC
MVN-EcoRI10 Rev	CGCGAATTC*GCCGGAGCCGCC*ACCGATTTCCAGCTGAGAGTC
MVN-SacI10 For	CGCGAGCTC*GGCGGCTCCGGC*ATGCCGAACTTCTCTCACACC
MVN-SacI10 Rev	CGCGAGCTC*GCCGGAGCCGCC*ACCGATTTCCAGCTGAGAGTC
RT-HCV Fw	AGCCATGGCGTTAGTATGAGTGTC
RT-HCV Rv	ACAAGGCCTTTCGCAACCCAA
RT-GAPDH Fw	ACCACAGTCCATGCCATCAC
RT-GAPDH Rv	TCCACCACCCTGTTGCTGTA

Supplementary Table 2 Absorbance values of the elution fractions with the highest protein contents using Ni-NTA purification.

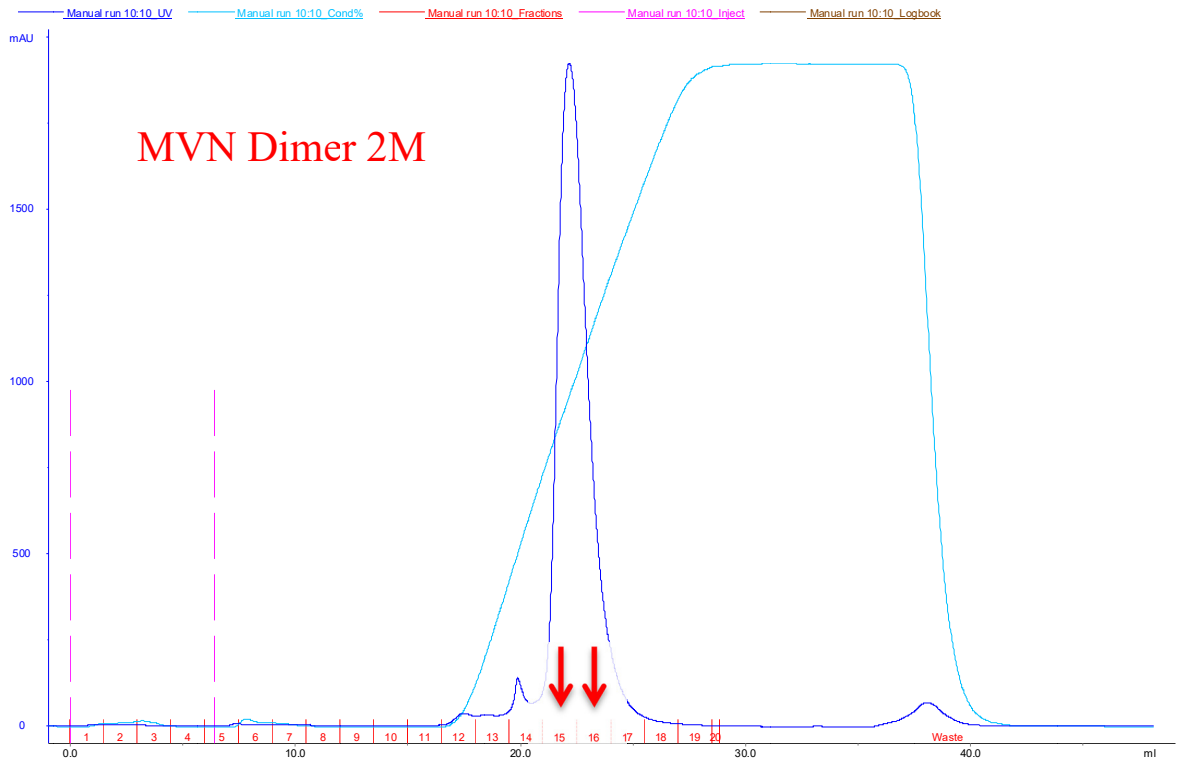
MVN type	Monomer	2S	2M	2L	3S	3M	3L	4S	4M	4L
UV Absorbance (280 nm)	0.393	0.460	0.390	0.424	0.426	0.387	0.209	0.198	0.158	0.550



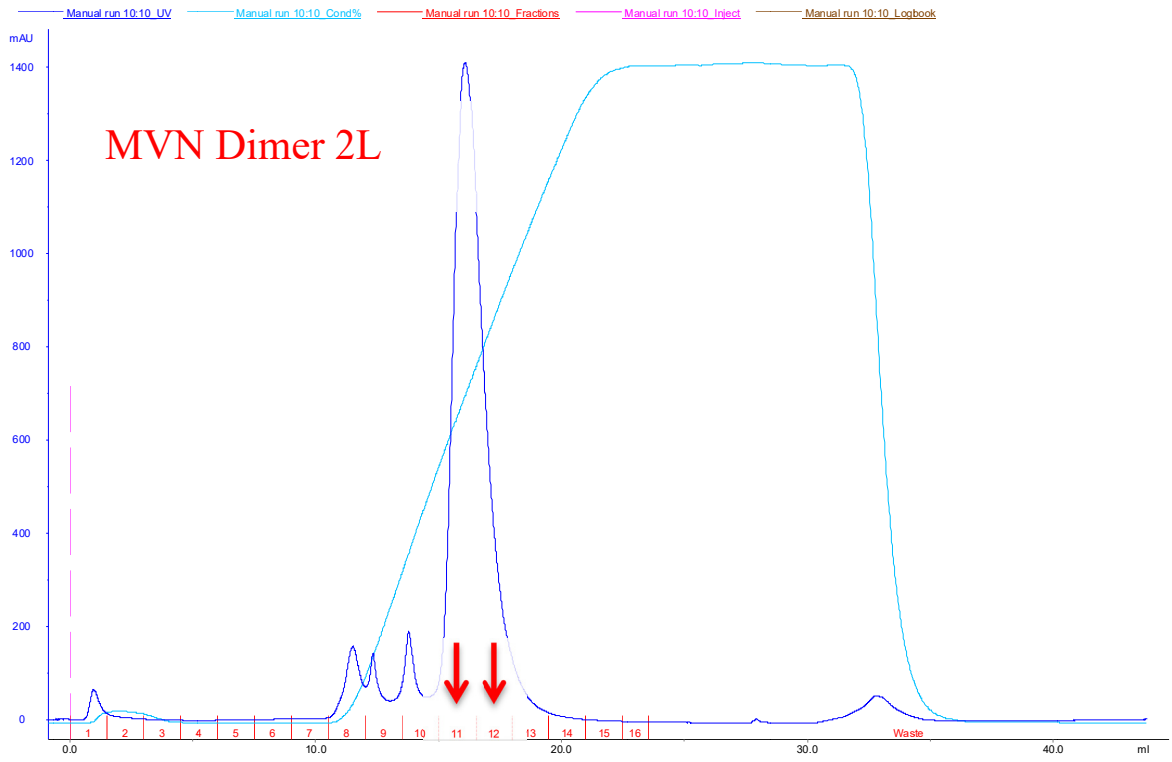
Supplementary Figure S1 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN monomer. Fractions 11 and 12 (red arrow) were collected and used for further experiments.



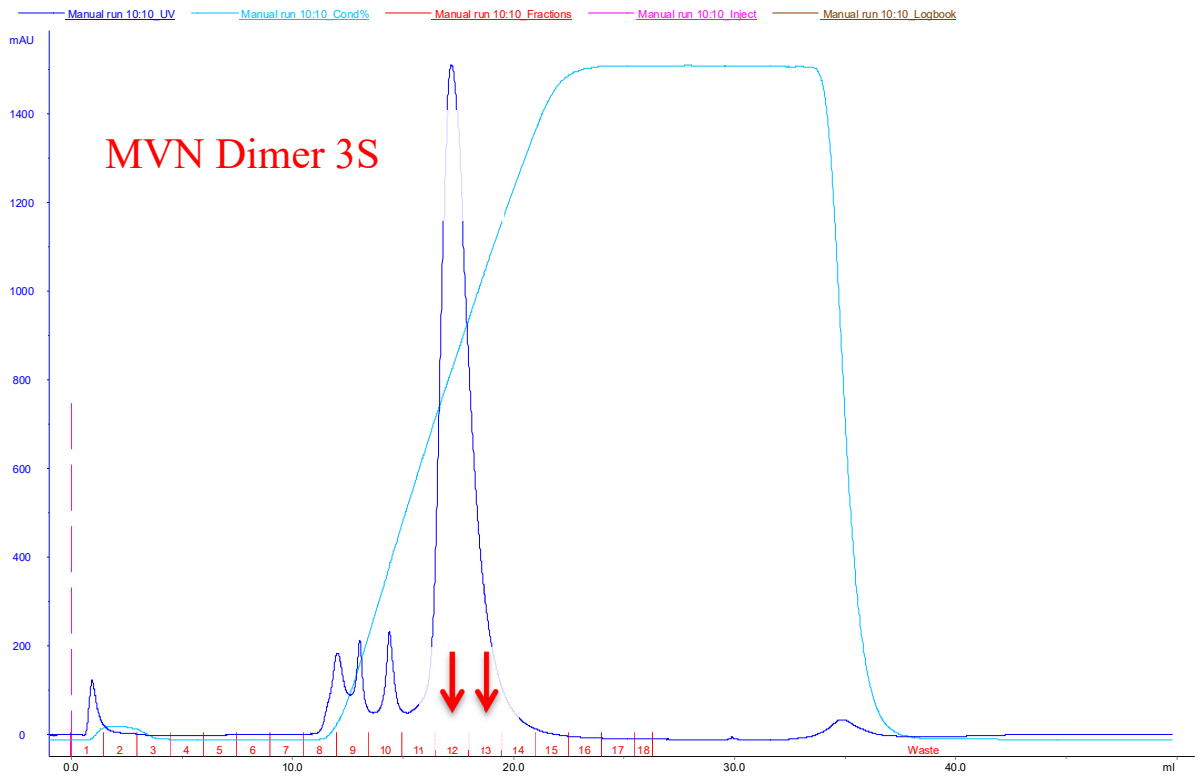
Supplementary Figure S2 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN dimer 2S. Fractions 14 and 15 (red arrow) were collected and used for further experiments.



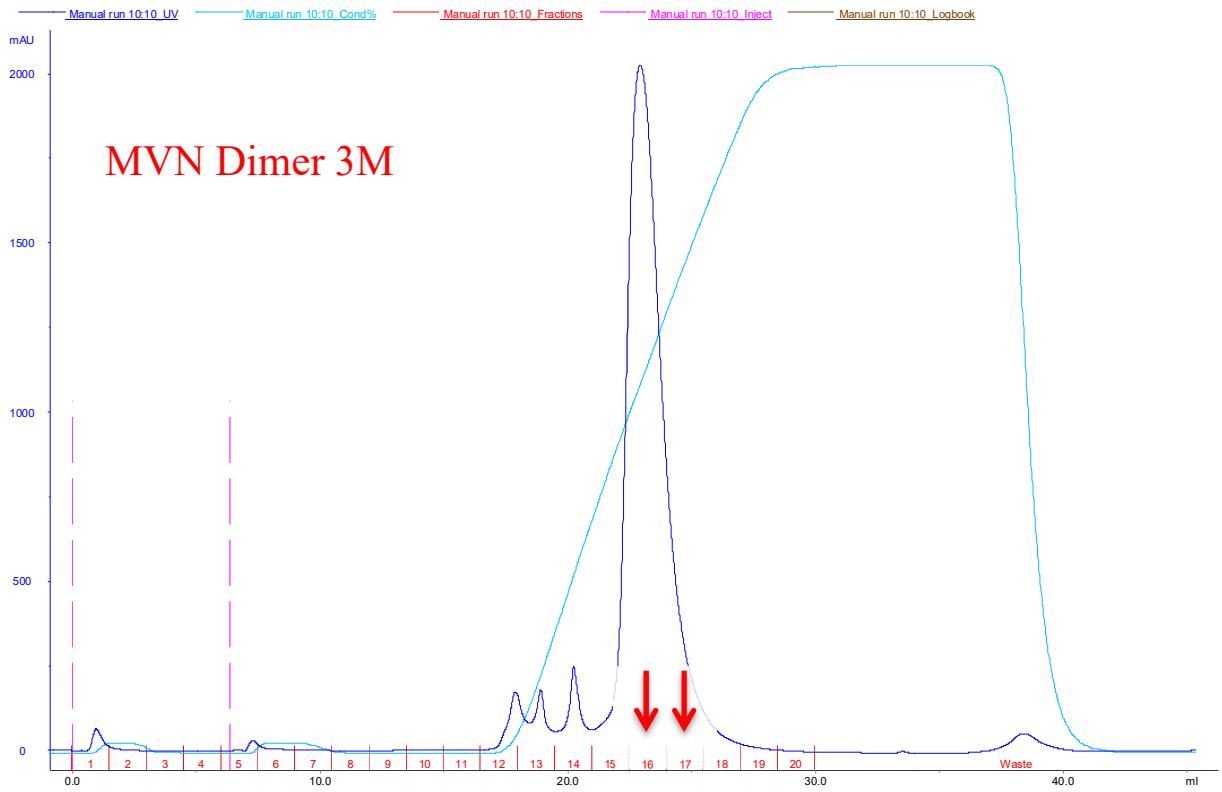
Supplementary Figure S3 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN dimer 2M. Fractions 15 and 16 (red arrow) were collected and used for further experiments.



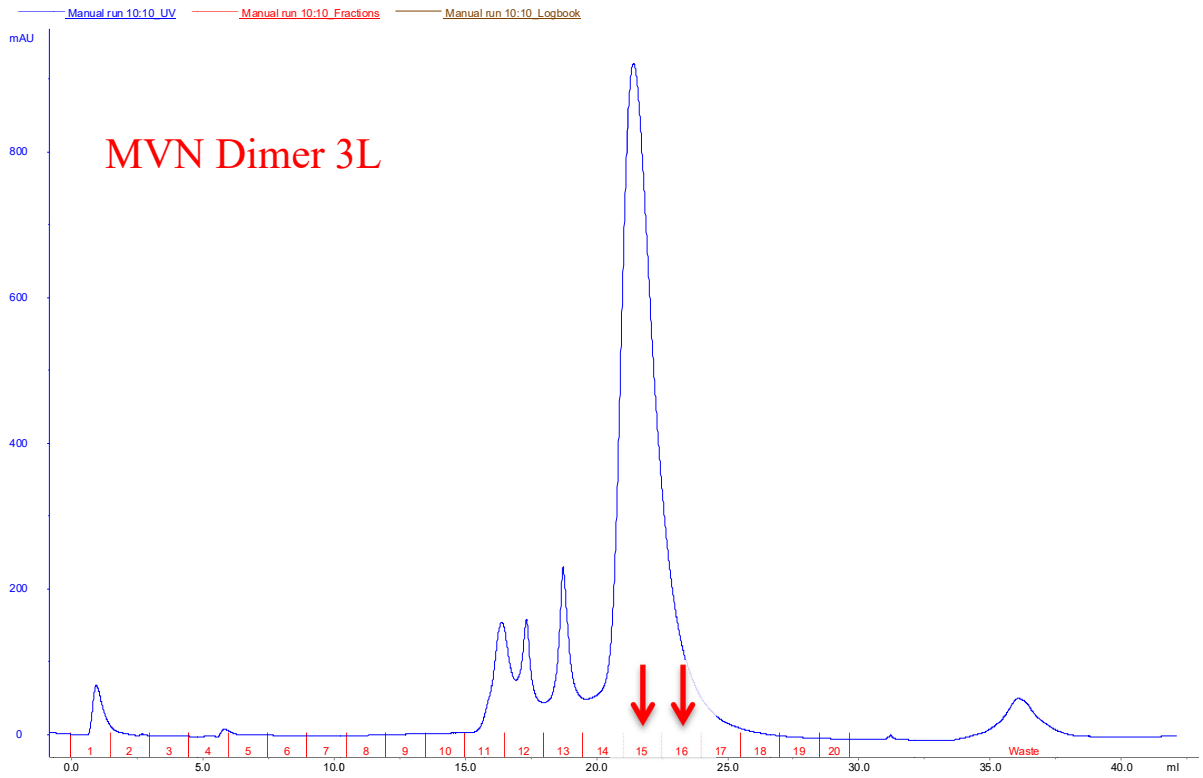
Supplementary Figure S4 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN dimer 2M. Fractions 11 and 12 (red arrow) were collected and used for further experiments.



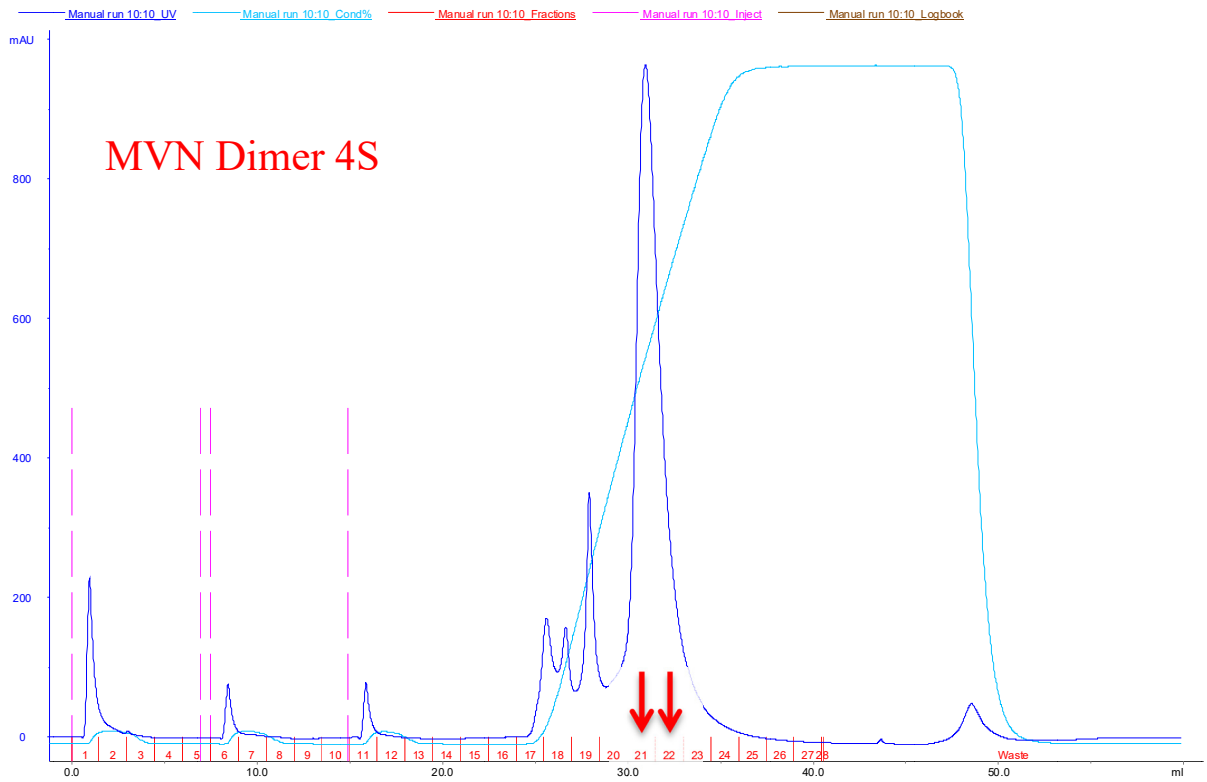
Supplementary Figure S5 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN trimer 3S. Fractions 12 and 13 (red arrow) were collected and used for further experiments.



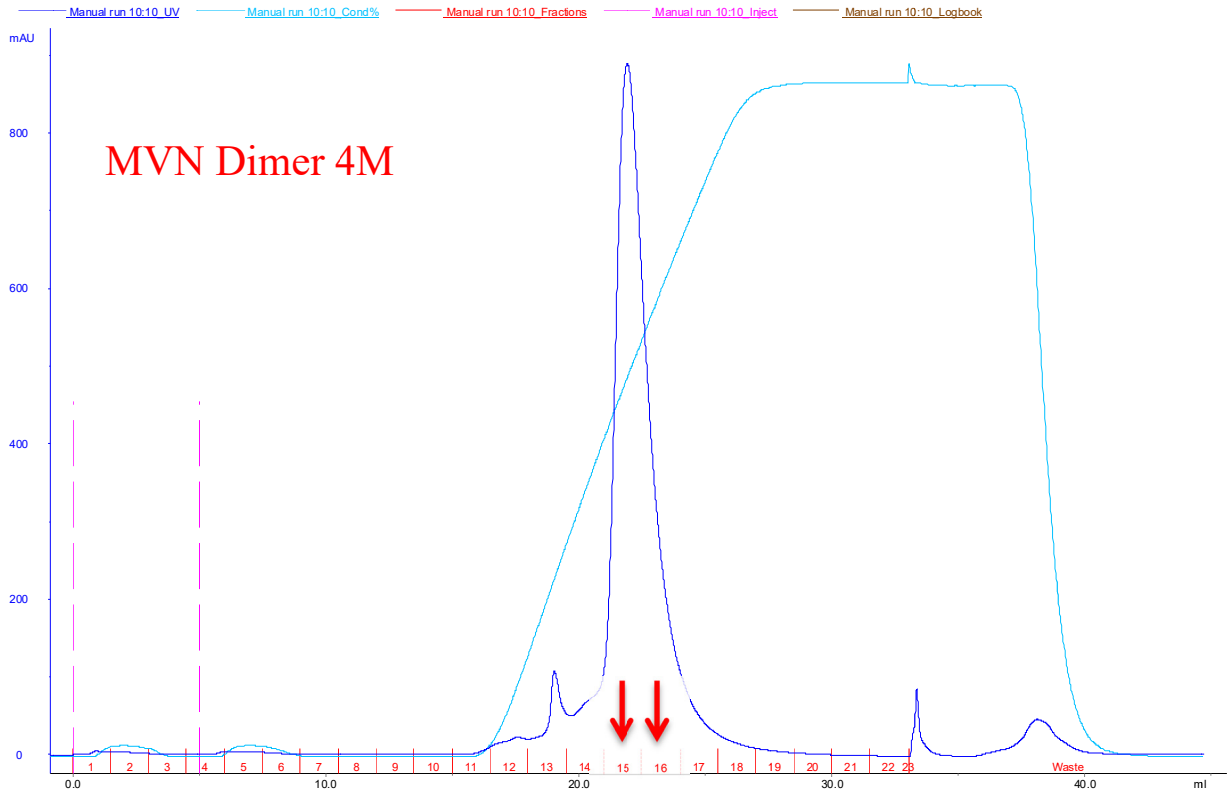
Supplementary Figure S6 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN trimer 3M. Fractions 16 and 17 (red arrow) were collected and used for further experiments.



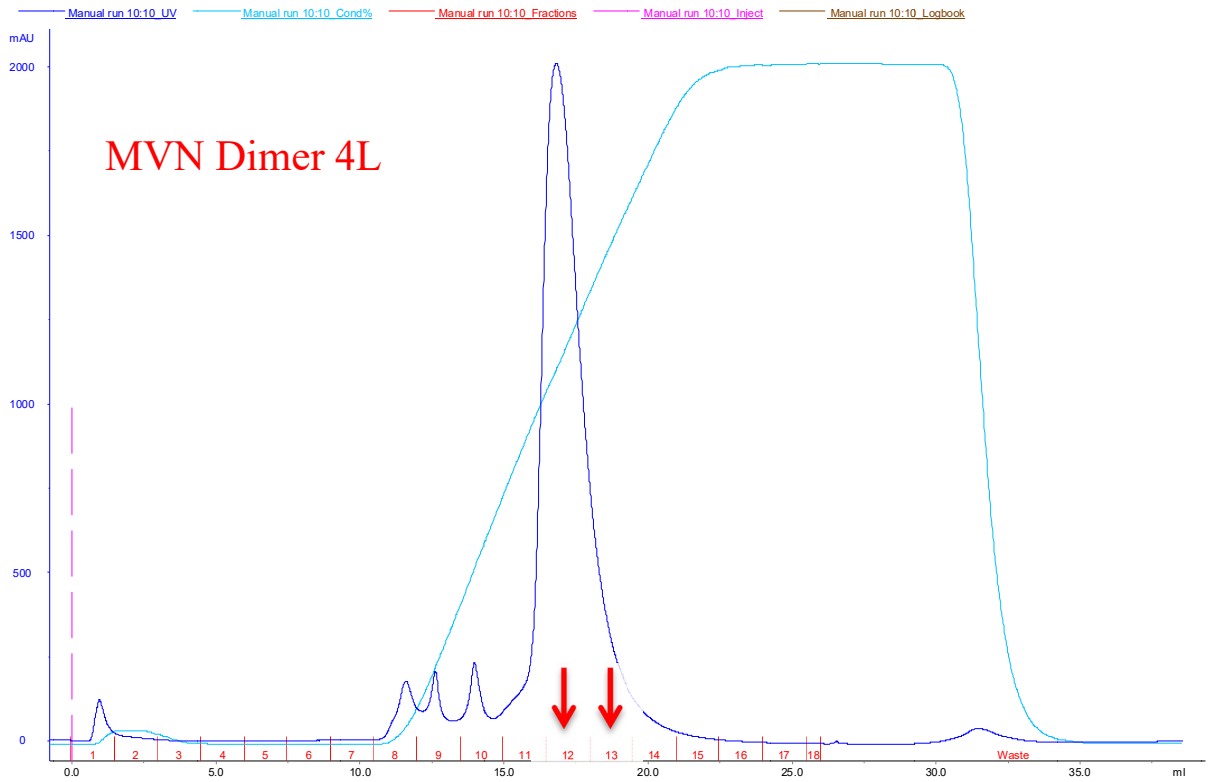
Supplementary Figure S7 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN trimer 3L. Fractions 15 and 16 (red arrow) were collected and used for further experiments.



Supplementary Figure S8 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN tetramer 4S. Fractions 21 and 22 (red arrow) were collected and used for further experiments.



Supplementary Figure S9 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN tetramer 4M. Fractions 15 and 16 (red arrow) were collected and used for further experiments.



Supplementary Figure S10 FPLC chromatogram of the Q Sepharose XL ion exchange purification of the MVN tetramer 4L. Fractions 12 and 13 (red arrow) were collected and used for further experiments.

Supplementary Table 3 HCV N-glycan composition and possible isomers

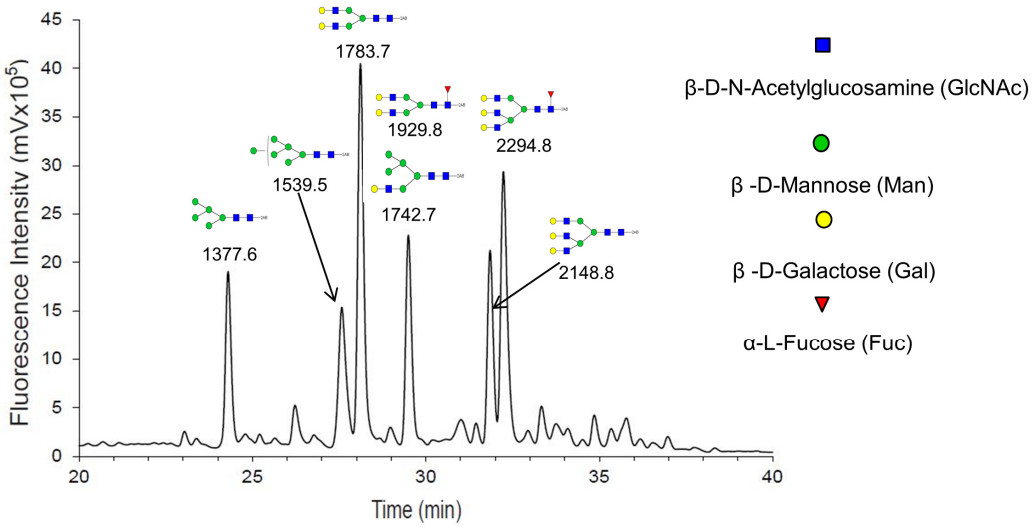
Retention time (min)[a]	GU[a]	GU (NIBRT)[b]	Hex	HexNAc	Fuc	m/z[c]
24.3	6.11	6.18±0.02	5	2	-	1377.6
27.6	6.93	7.02±0.05	6	2	-	1539.5
28.1	7.08	7.20±0.02	5	4	-	1783.7
29.5	7.47	7.61±0.02	6	3	-	1742.7
29.5	7.47	7.61±0.02	5	4	1	1929.8
31.9	8.15	8.38±0.04	6	5	-	2148.8
32.2	8.28	8.33±0.00	6	4	1	2091.8

[a] Retention time and glucose unit (GU) values of 2-AB-labeled glycan species obtained by HILIC-UPLC

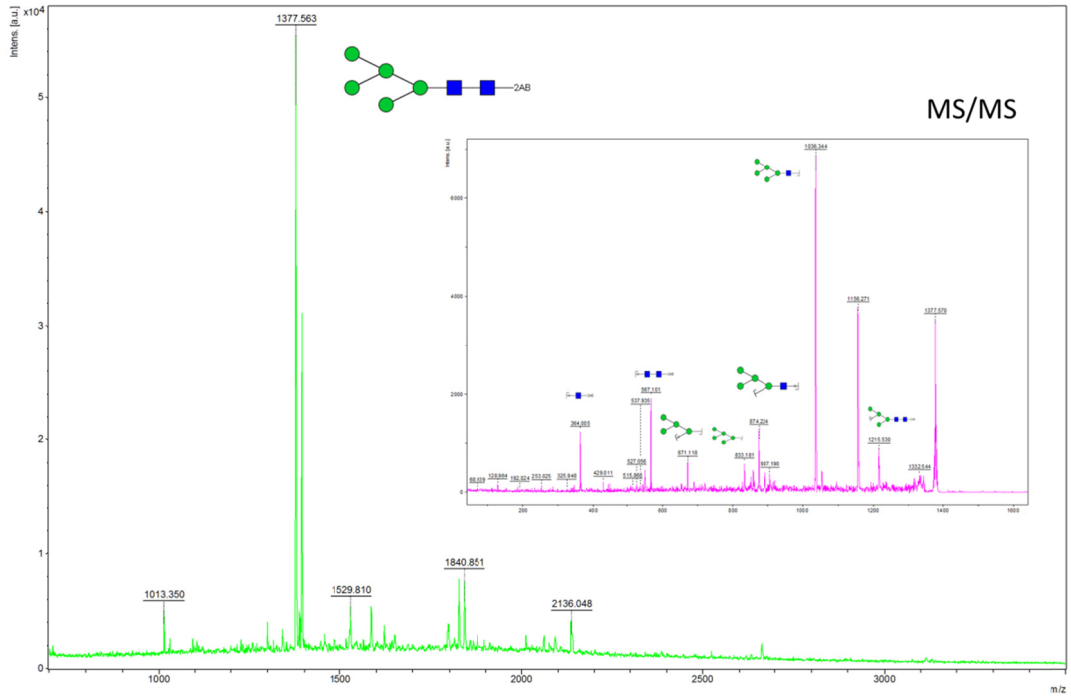
[b] The reference GU values from the National Institute for Bioprocessing Research and Training

[c] 2-AB-labeled glycan species ($[M+Na]^+$) analyzed by MALDI-TOF MS/MS

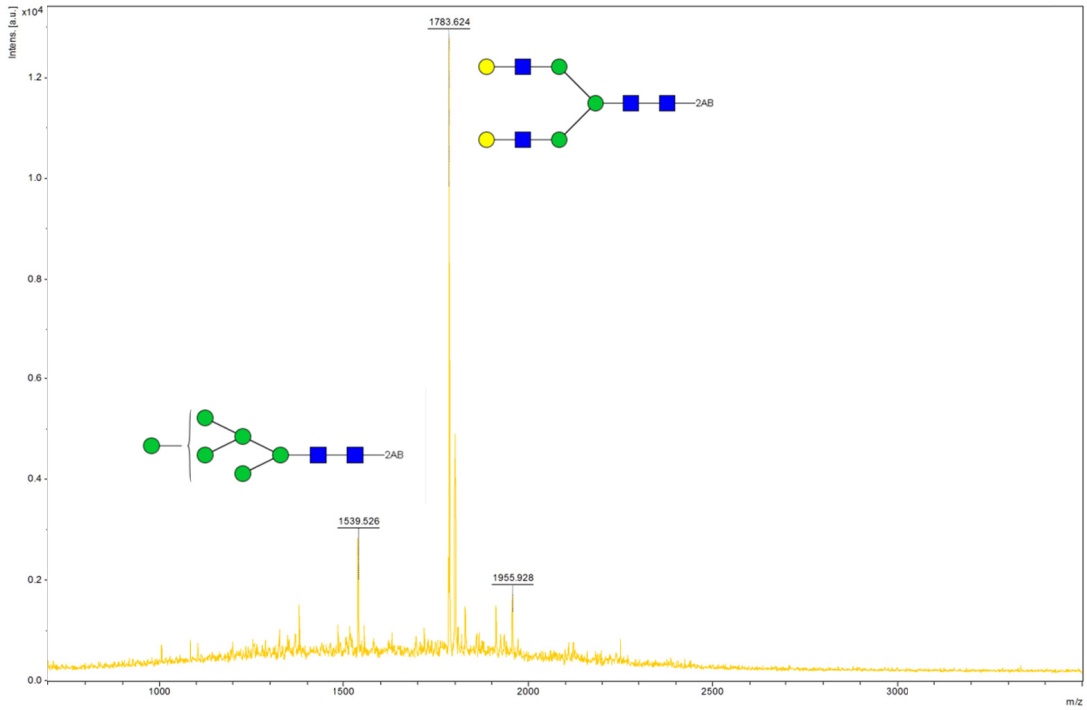
Hex= hexose; HexNAc=N-acetylhexosamine; Fuc=fucose



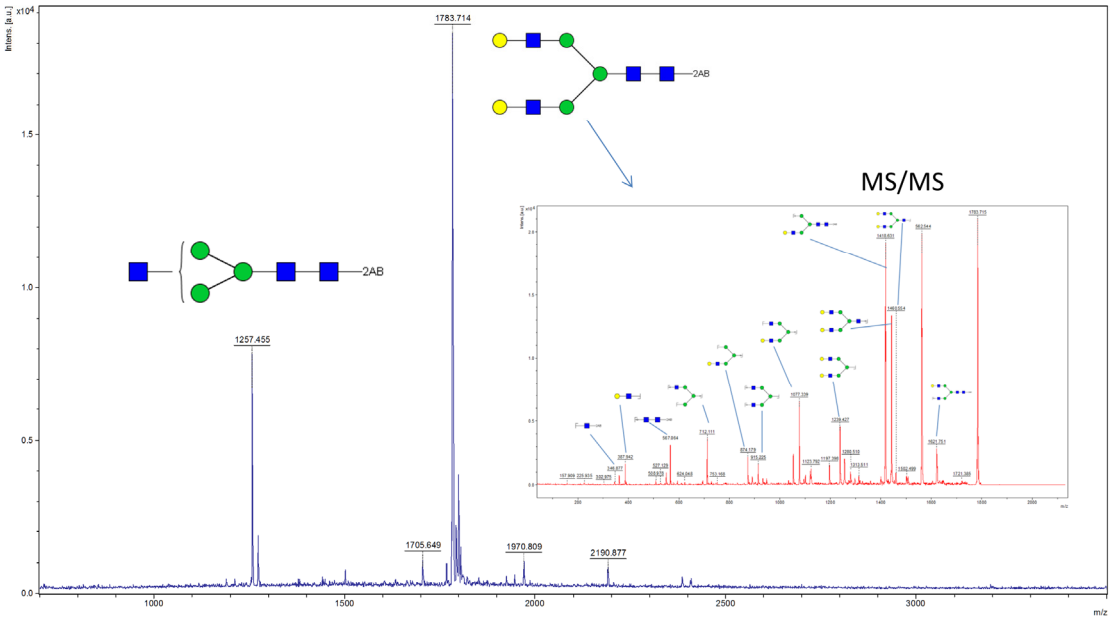
Supplementary Figure 11 UPLC chromatogram of HCV-derived N-glycans.



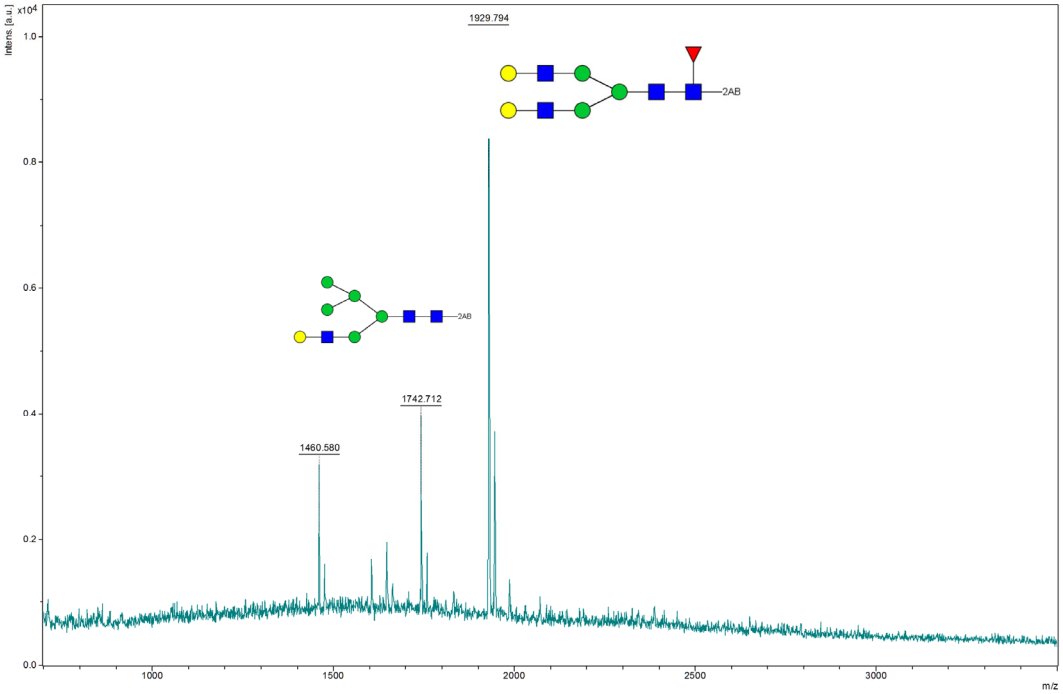
Supplementary Figure 12 Mass spectrometric analysis of UPLC elution peak at 24.3 min.



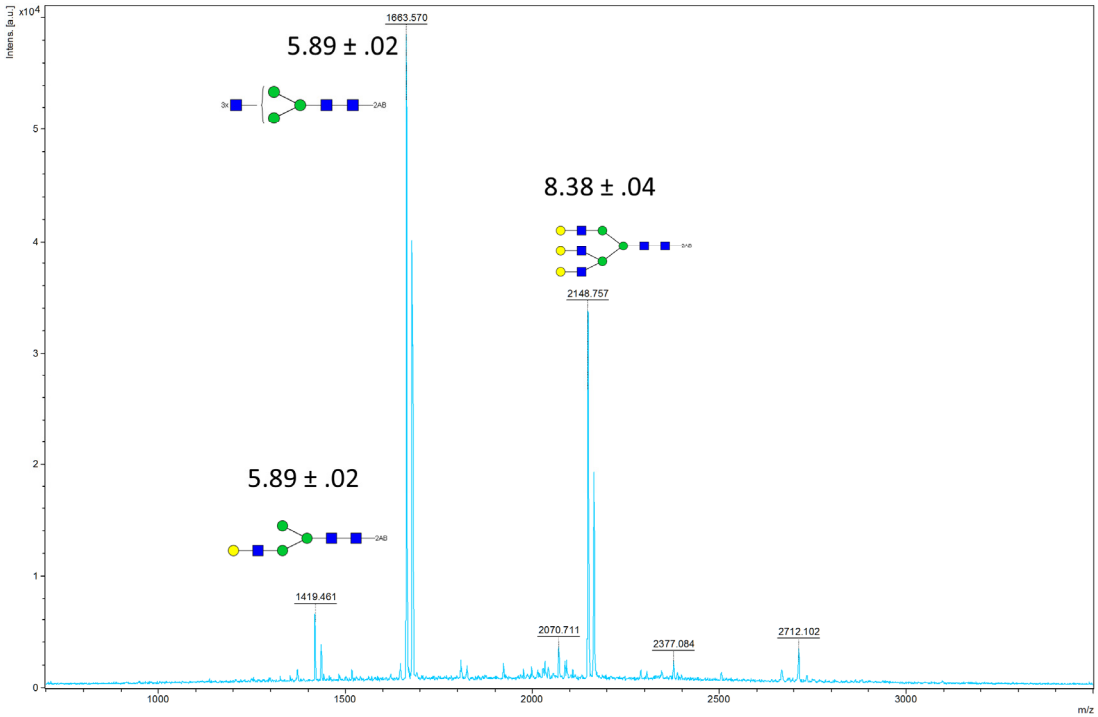
Supplementary Figure 13 Mass spectrometric analysis of UPLC elution peak at 27.6 min.



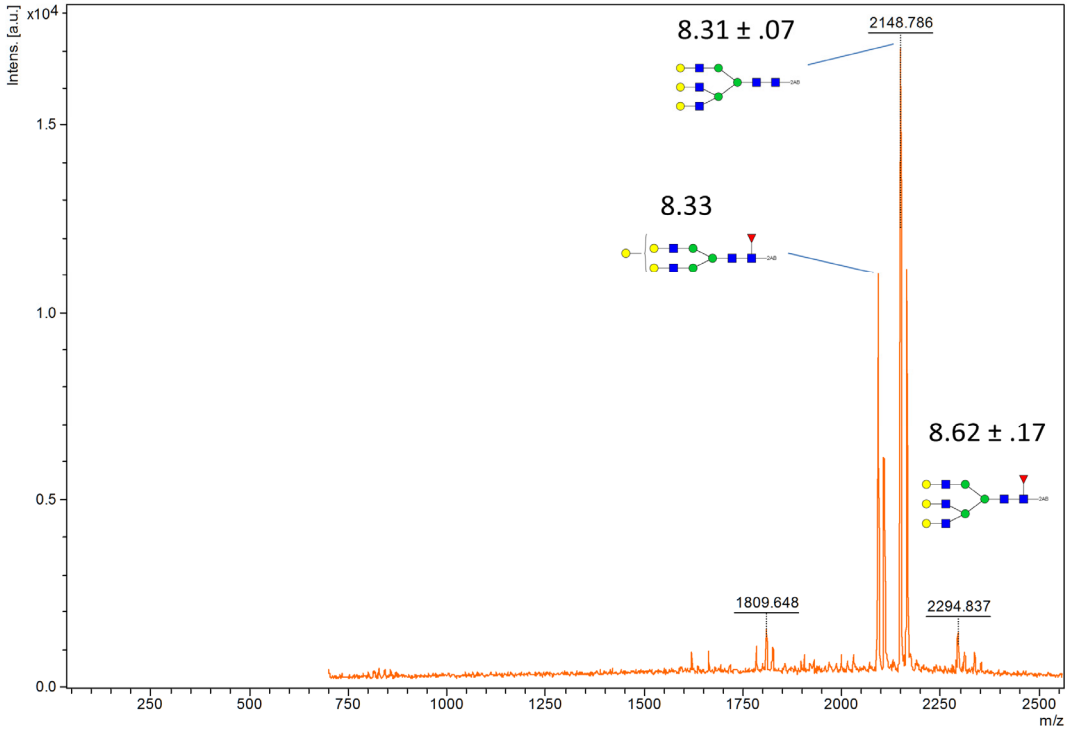
Supplementary Figure 14 Mass spectrometric analysis of UPLC elution peak at 28.1 min.



Supplementary Figure 15 Mass spectrometric analysis of UPLC elution peak at 29.5 min.



Supplementary Figure 16 Mass spectrometric analysis of UPLC elution peak at 31.9 min.



Supplementary Figure 17 Mass spectrometric analysis of UPLC elution peak at 32.2 min.