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Novel α-Mannose-Functionalized Poly (β-amino ester) Nanoparticles as mRNA Vaccines with Increased Antigen Presenting Cells Selectivity in the Spleen

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Supplementary information

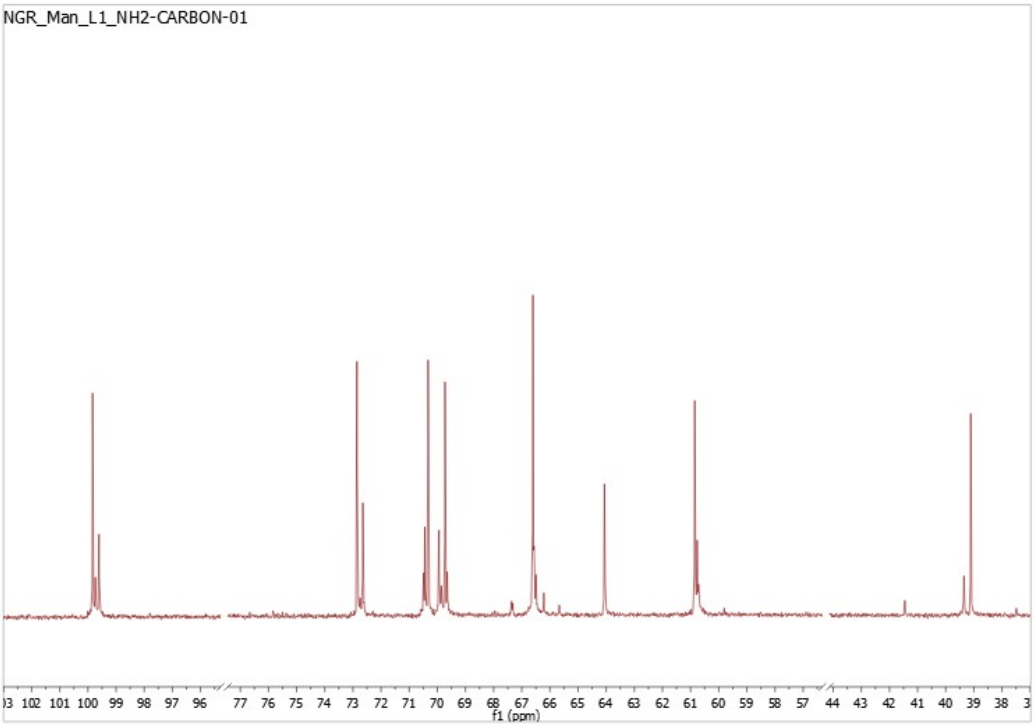

Figure S.1. ^{13}C of M0- 2-Aminoethyl α-D-mannopyranoside: ^{13}C-NMR (100 MHz; D2O) 99.83 (C-1), 72.86, 70.33, 69.73, 66.60 (C-2, -3, -4, -5), 64.60 (OCH2CH2NH2), 60.85 (C-6), 39.03 (OCH2- CH2NH2).

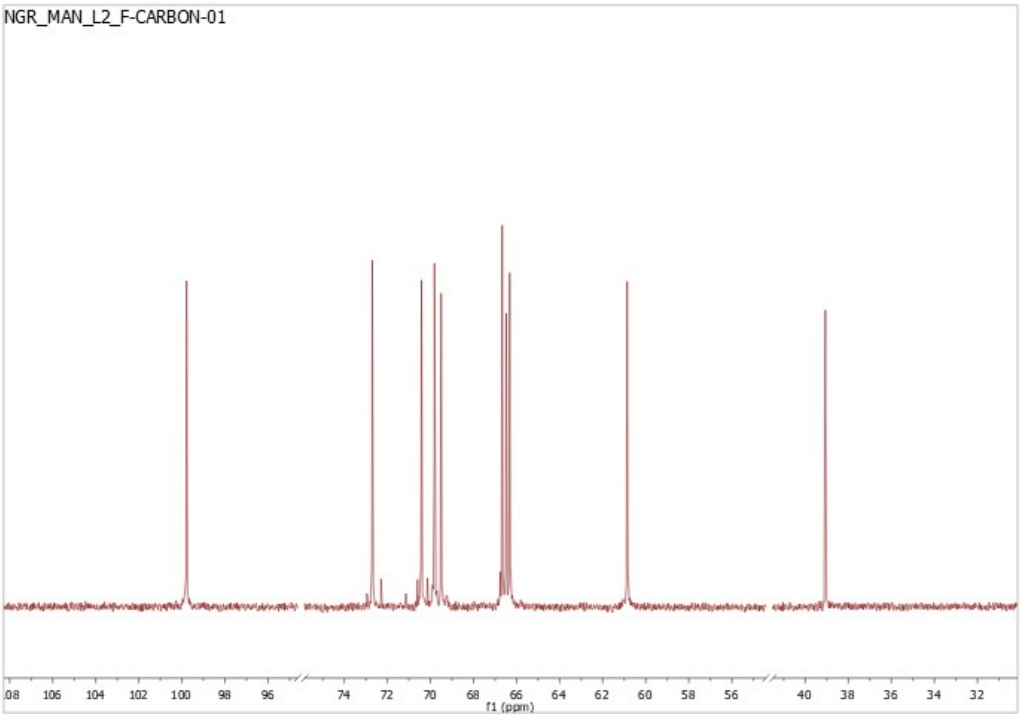

Figure S.2. ^{13}C of M1- 2-(2-Aminoethoxy) ethyl α-D-mannopyranoside: ^{13}C-NMR (100 MHz; D2O) 99.78 (C-1), 72.70, 69.81, 69.50, 66.48 (C-2, -3, -4, -5), 70.42 (OCH2CH2O), 66.67 (OCH2CH2NH2), 66.32 (OCH2CH2O), 60.87 (C-6), 39.06 (OCH2CH2NH2).

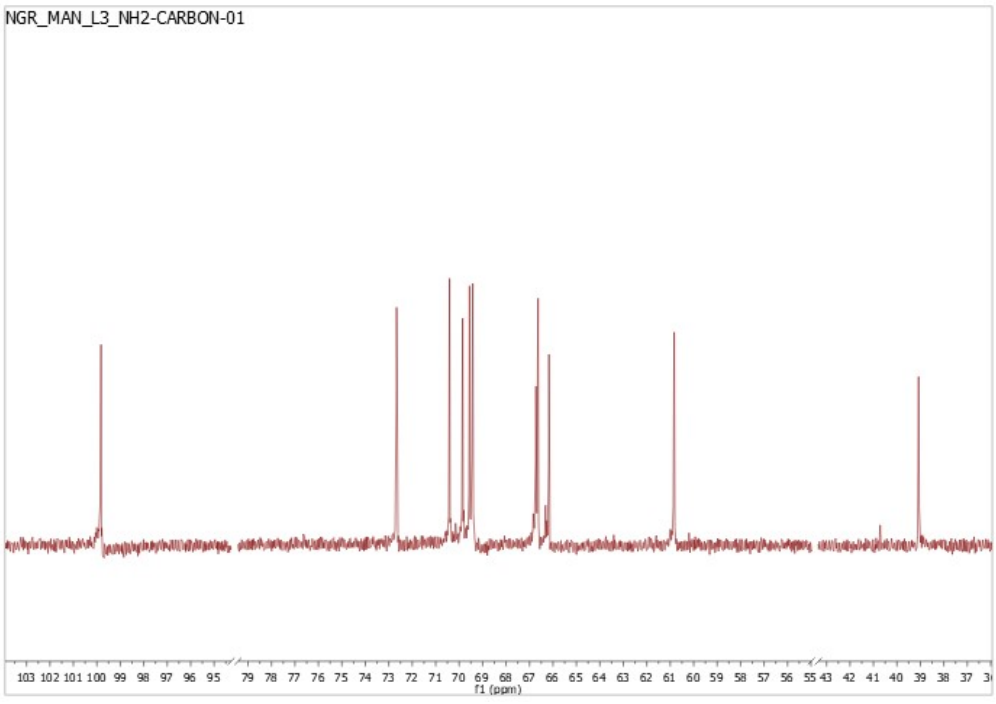

Figure S.3. ^{13}C of M2- 2-[2-(2-Aminoethoxy) ethoxy] ethyl α-D-mannopyranoside: ^{13}C-NMR (100 MHz; D2O) 99.82 (C-1), 72.66 (C-5), 70.41 (OCH2), 69.85 (C-2), 69.56, 69.44, 69.42,(3 × OCH2), 66.72 (C-3) 66.63 (C-4), 66.17 (OCH2CH2), 60.83 (C-6), 39.07 (CH2NH2).

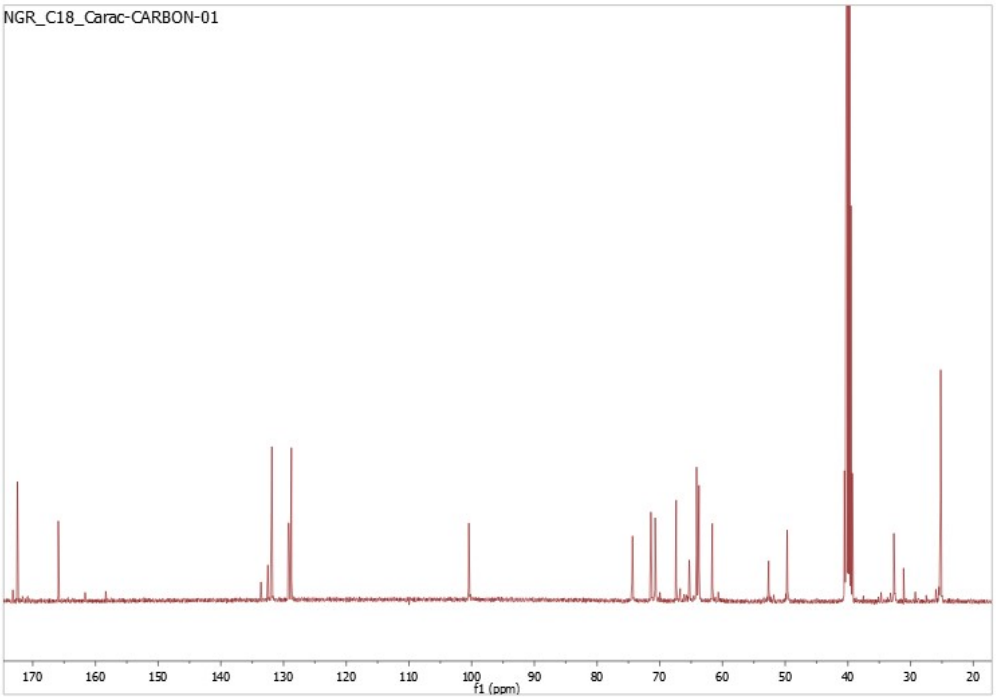

Figure S.4. ^{13}C of Man1: ^{13}C-NMR (100 MHz; DMSO-d) 172.45(O-(C=O)-C), 165.92 (CH2=CH-(C=O)-O), 131.86 (CH2=CH-(C=O)-O), 128.75 (CH2=CH-(C=O)-O), 100.45 (C-1), 74.34 (C-2), 71.42 (C-3), 70.74 (C-4), 67.79 (C-5), 65.29 (O-CH2-CH2-CH2-CH2-O), 63.74 (OCH2CH2NH2), 61.62 (C-6), 52.64 (OCH2CH2NH2), 49.68 ((C=O)-CH2-CH2-N), 32.57 ((C=O)-CH2-CH2-N), 25.22 (O-CH2-CH2-CH2-CH2-O).

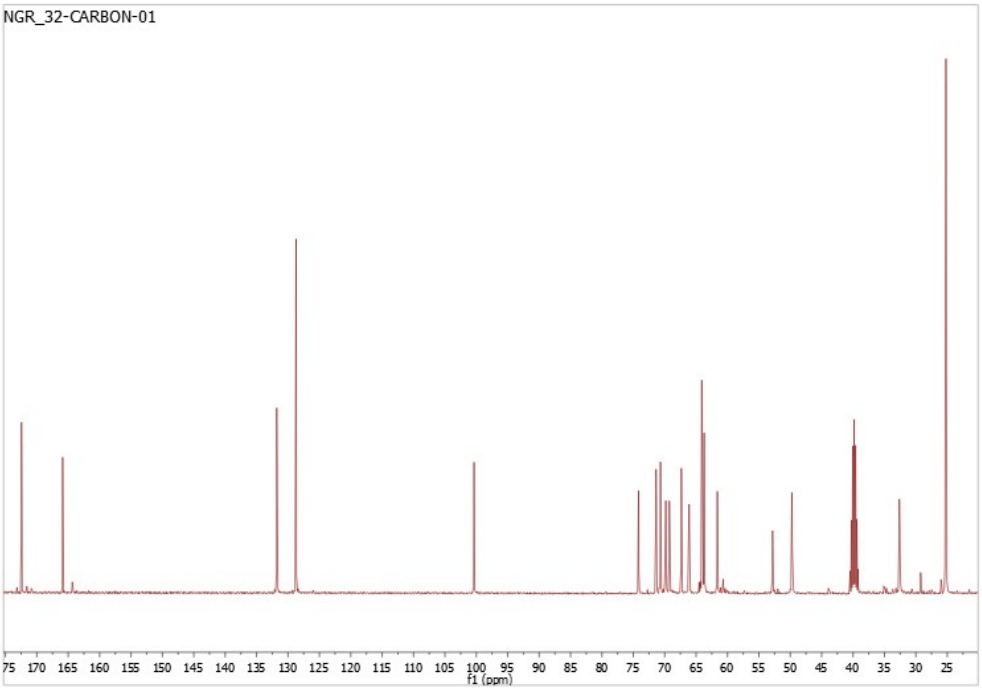

Figure S.5. ^{13}C of Man2: ^{13}C-NMR (100 MHz; DMSO-d) 172.45(O-(C=O)-C), 165.89 (CH2=CH-(C=O)-O), 131.79 (CH2=CH-(C=O)-O), 128.70 (CH2=CH-(C=O)-O), 100.36 (C-1), 74.16 (C-2), 71.38 (C-3), 70.67 (C-4), 69.82(C-OCH2CH2O), 69.26 (C-5), 67.35 (O-CH2-CH2-N), 66.12(C-OCH2CH2O), 64.11 & 63,72(O-CH2-CH2-CH2-CH2-O) 61.61 (C-6), 52.82 (OCH2CH2NH2), 49.74 ((C=O)-CH2-CH2-N), 32.57 ((C=O)-CH2-CH2-N), 25.22 (O-CH2-CH2-CH2-CH2-O).

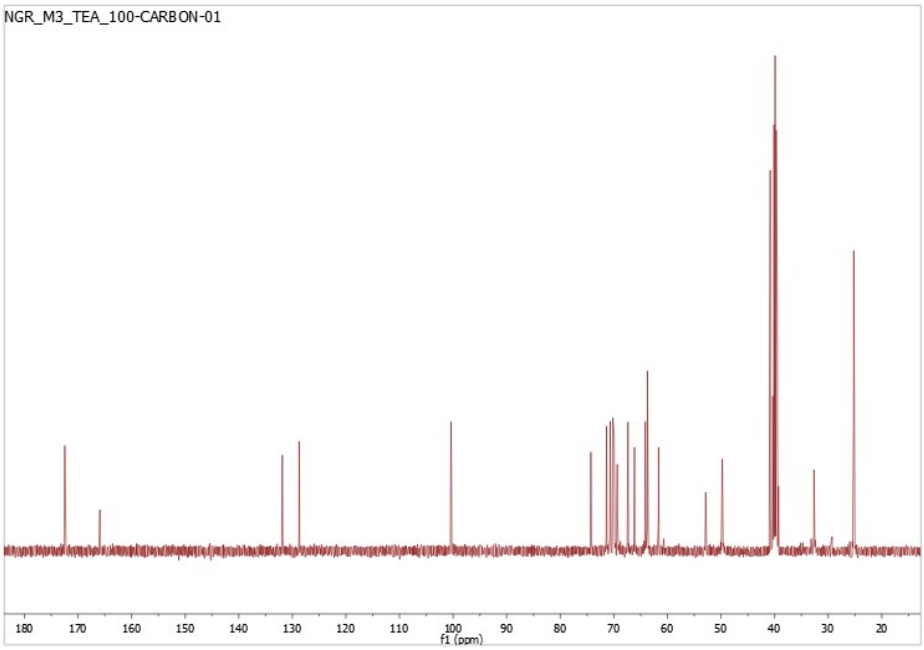

Figure S.6. ^{13}C of Man3: ^{13}C-NMR (100 MHz; DMSO-d) 172.49 (O-(C=O)-C), 165.92 (CH2=CH-(C=O)-O), 131.88 (CH2=CH-(C=O)-O), 128.74 (CH2=CH-(C=O)-O), 100.38 (C-1), 74.29 (C-2), 71.37 (C-3), 70.68 (C-4), 70.20(O-CH2-CH2-O), 70.13 (O-CH2-CH2-O), 69.94 (C-OCH2CH2O), 69.36 & 66.14 (O-CH2-CH2-CH2-CH2-O), 67.37(C-5), 66.14 (O-CH2-CH2-N), 64.13 (O-CH2CH2N), 63.73 (O-CH2CH2-OC-O) 61.65 (C-6), 52.86 (OCH2CH2NH2), 49.75 ((C=O)-CH2-CH2-N), 32.65 ((C=O)-CH2-CH2-N), 25.22 (O-CH2-CH2-CH2-CH2-O).

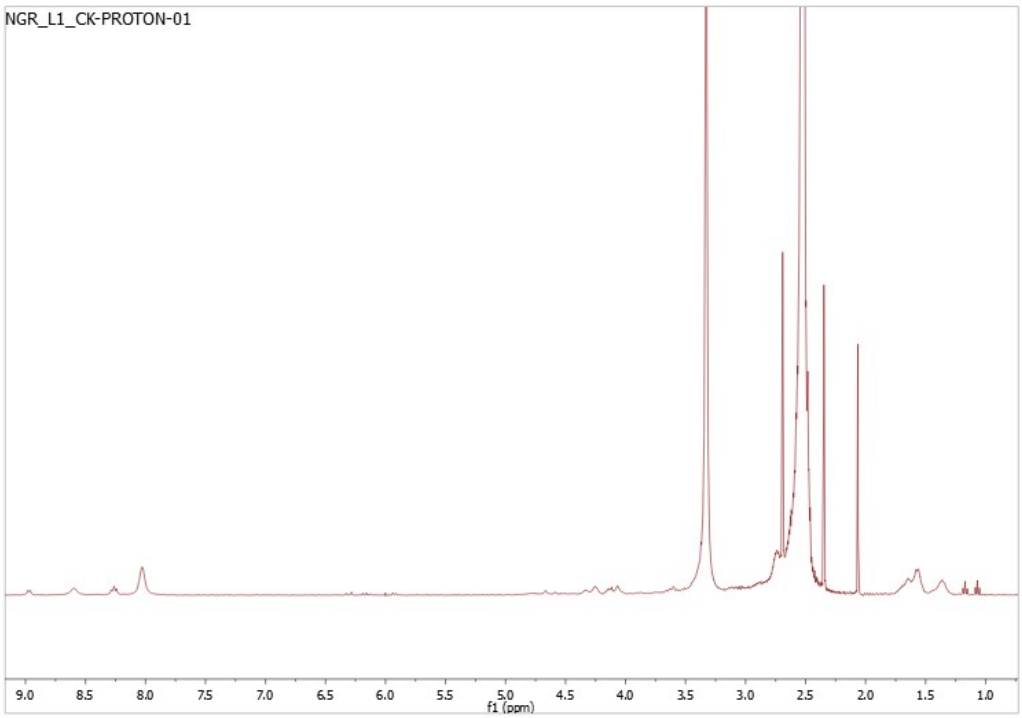

Figure S.7. ^{1}H of Man1- CK3: Acrylate signal removal. Further confirmation with LC-QTOF.

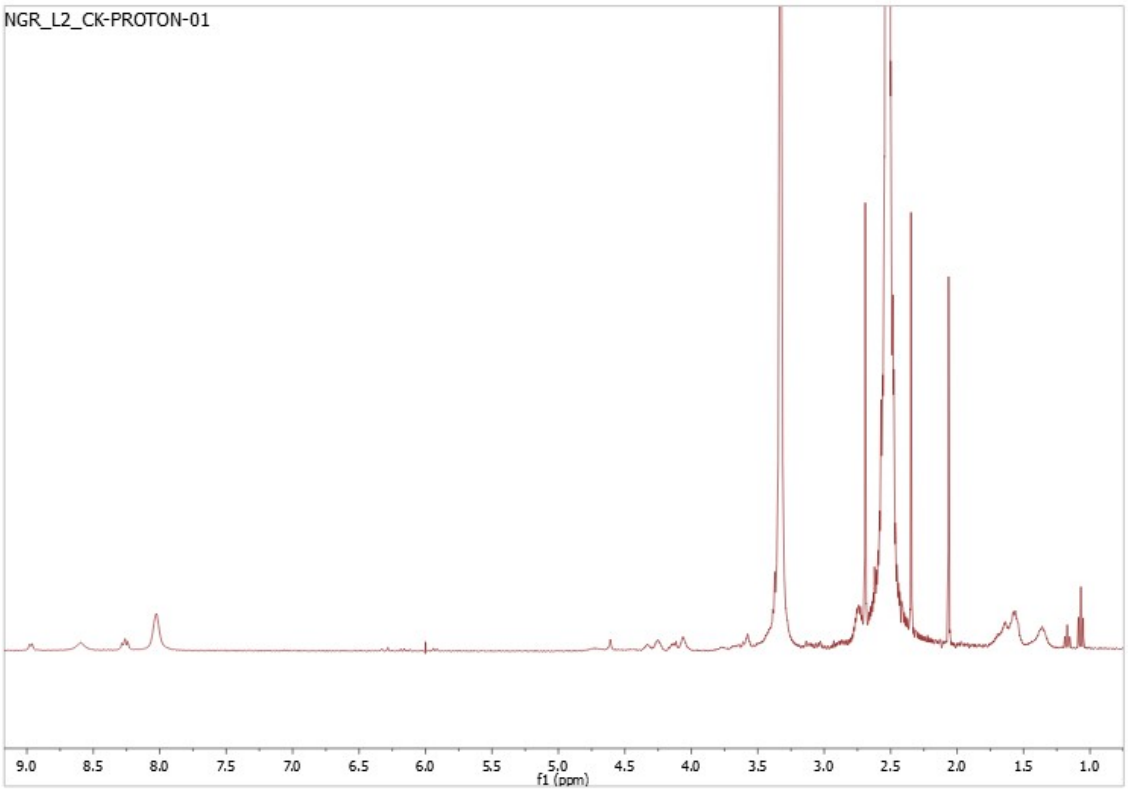

Figure S.8. ^{1}H of Man2- CK3: Acrylate signal removal. Further confirmation with LC-QTOF.

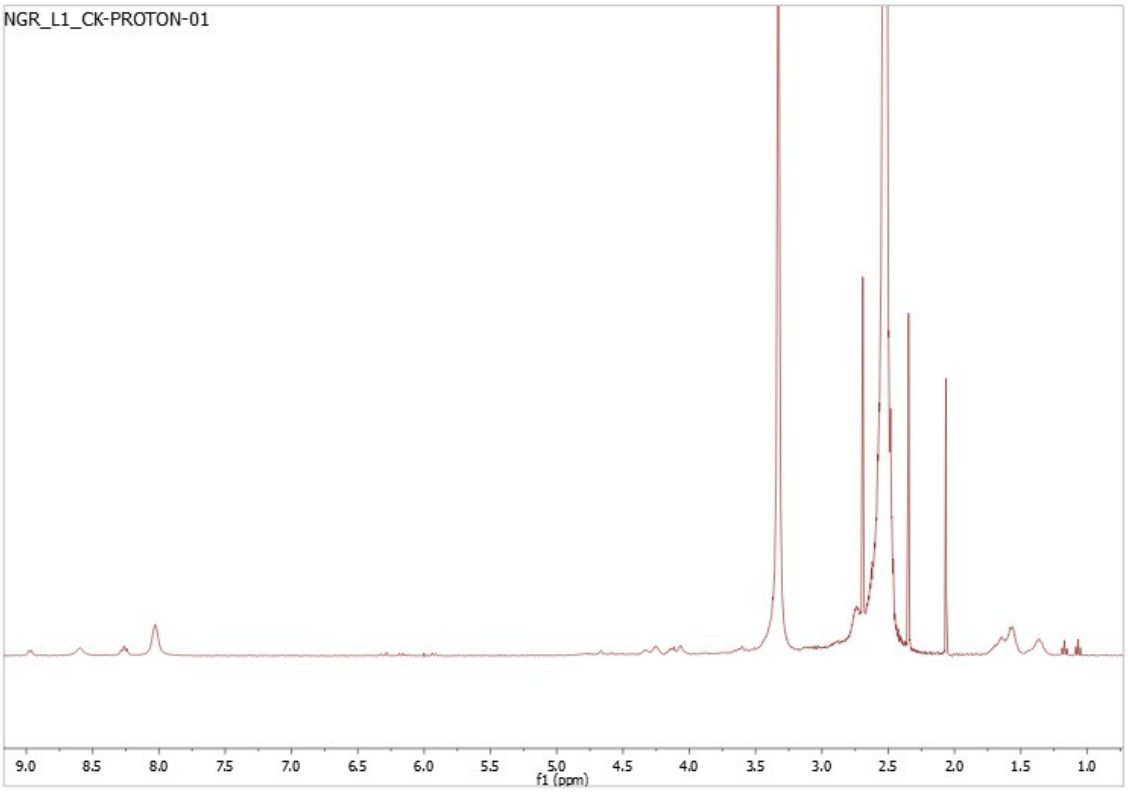

Figure S.9. ^{1}H of Man3- CK3: Acrylate signal removal. Further confirmation with LC-QTOF.

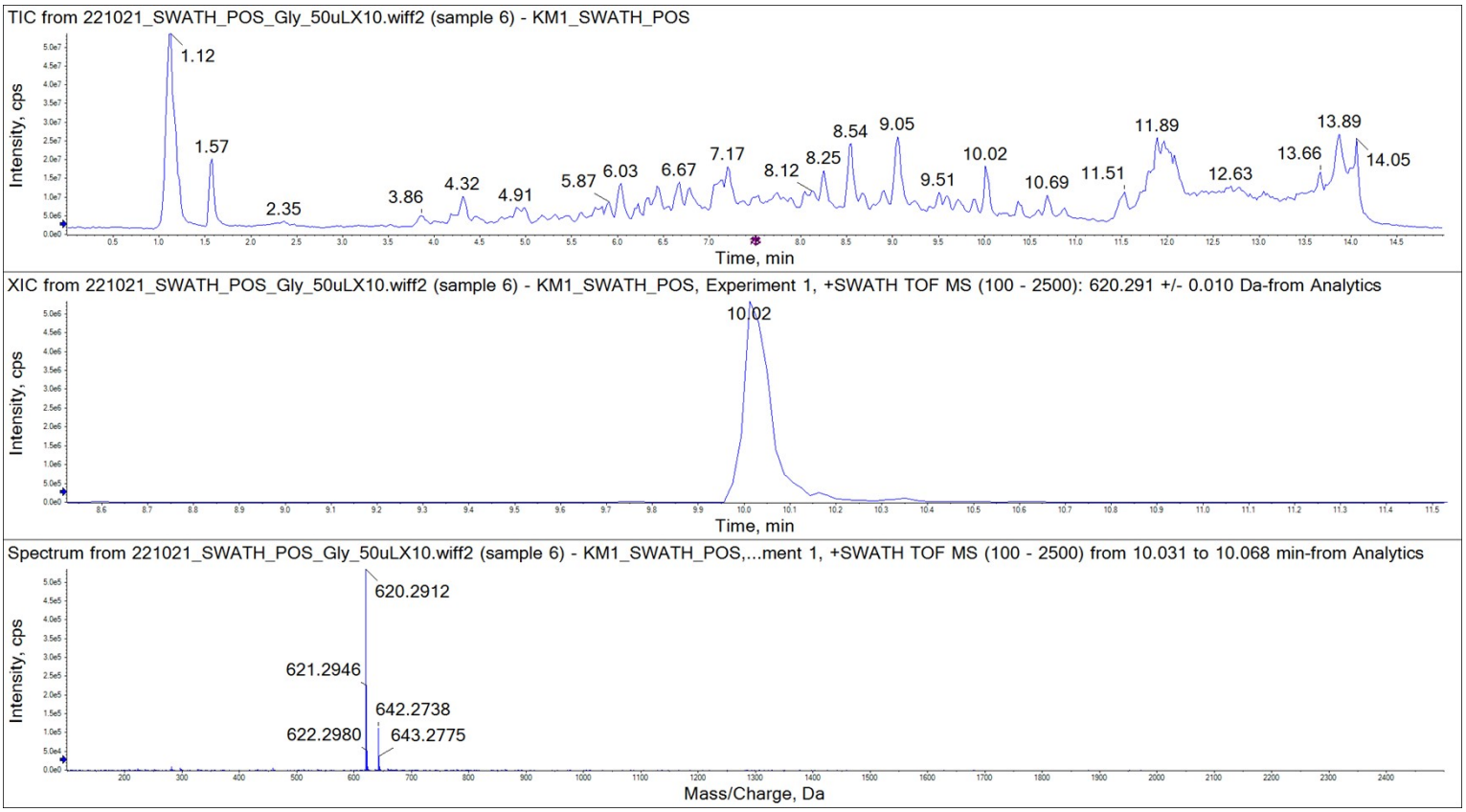

Figure S.10. UHPLC-QTOF analysis of Man1 n=1: Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing [M+H]+ and [M+Na]+ adducts.

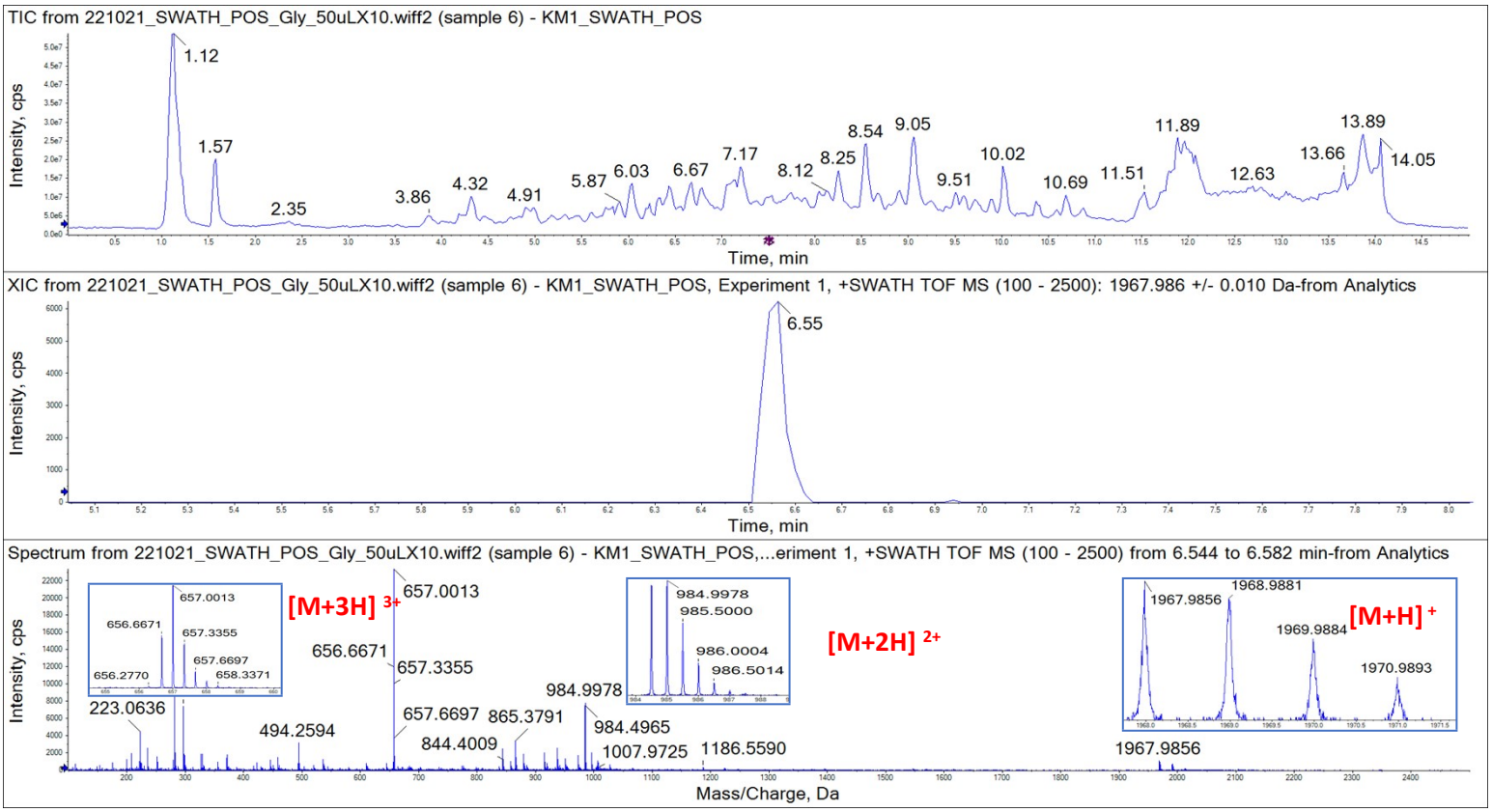

Figure S.11. UHPLC-QTOF analysis of S.11-Man1-CK3 n=3 1 peptide. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

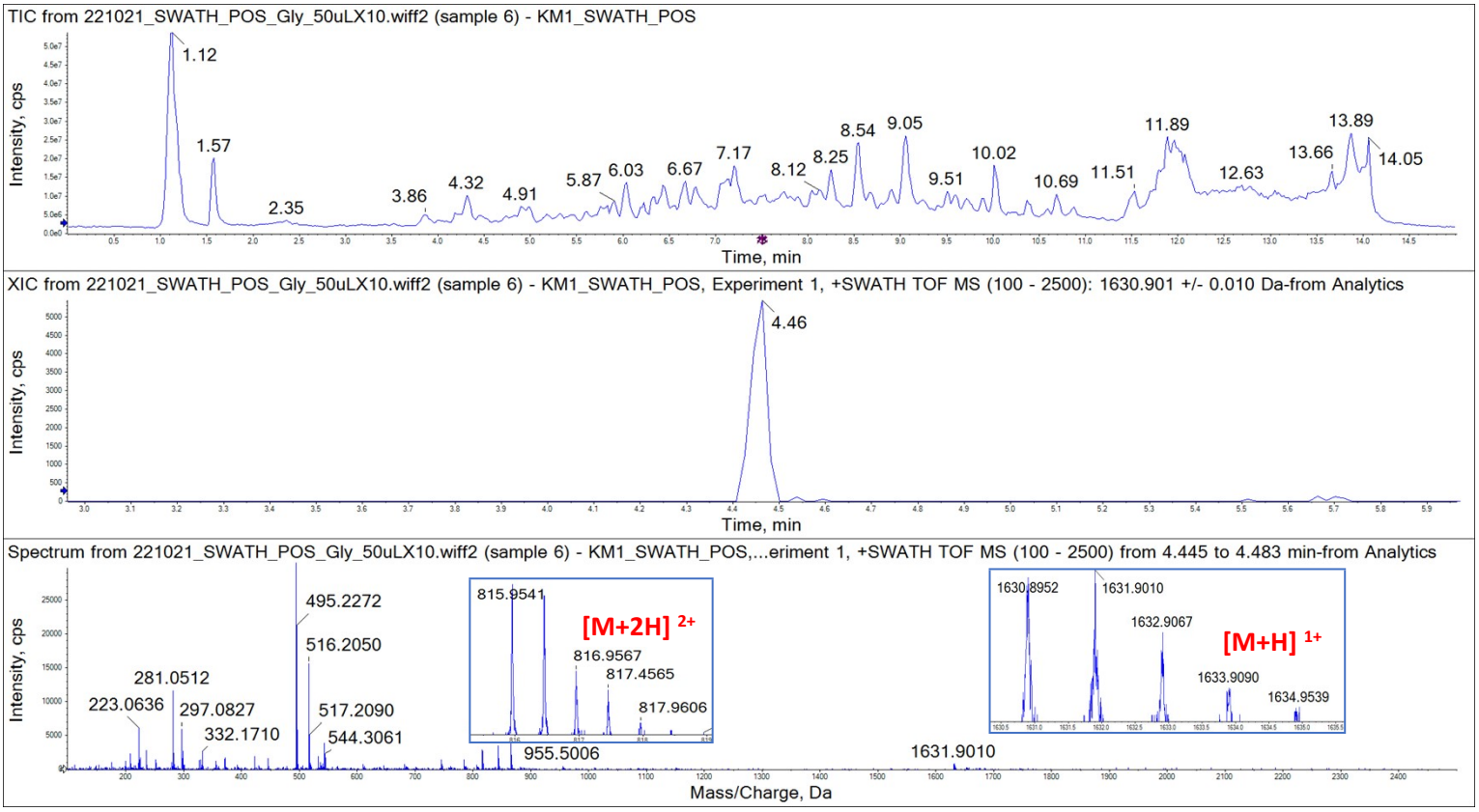

Figure S.12. UHPLC-QTOF analysis of Man1-CK3 n=1. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

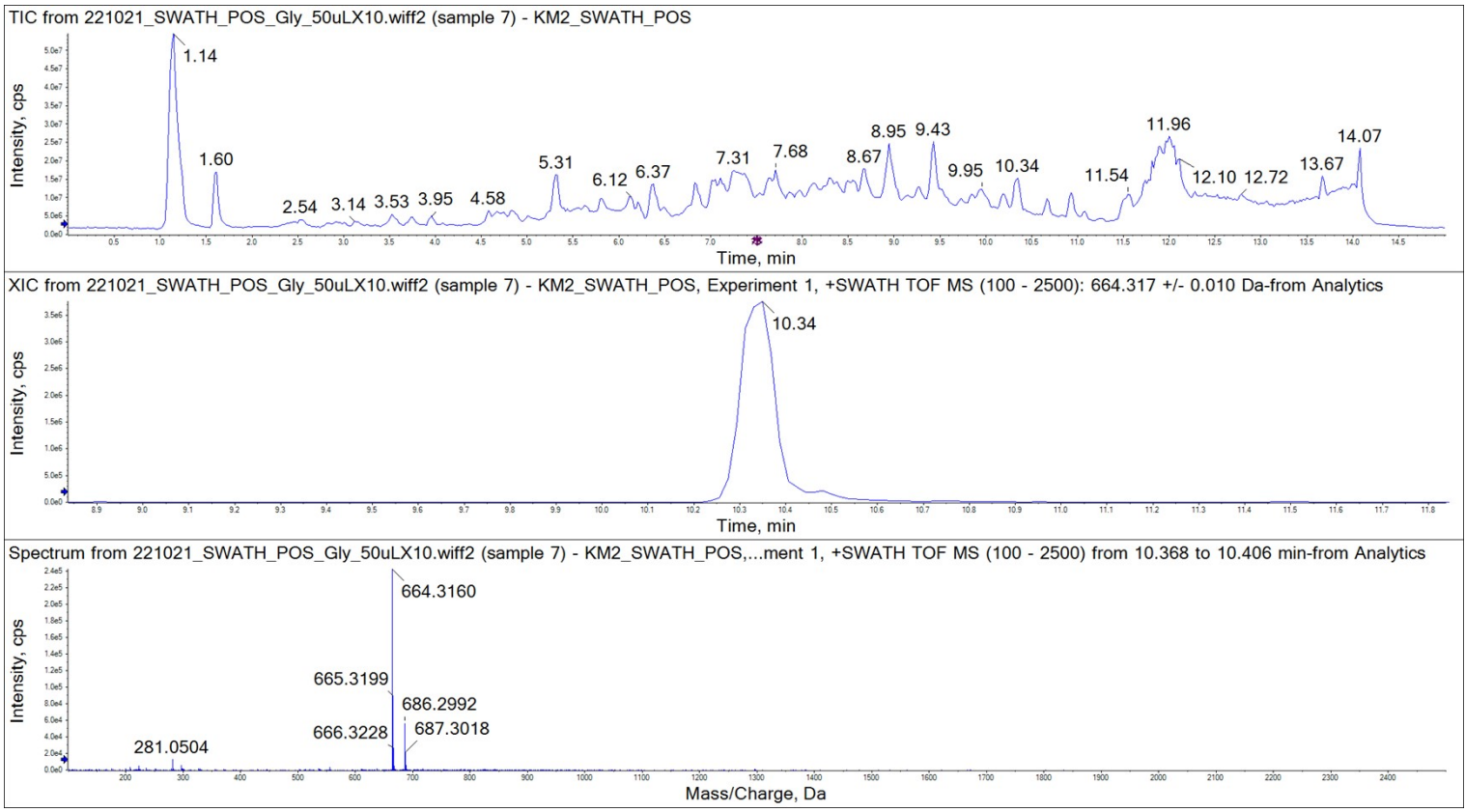

Figure S.13. UHPLC-QTOF analysis of Man2 n=1. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing [M+H]+ and [M+Na]+ adducts.

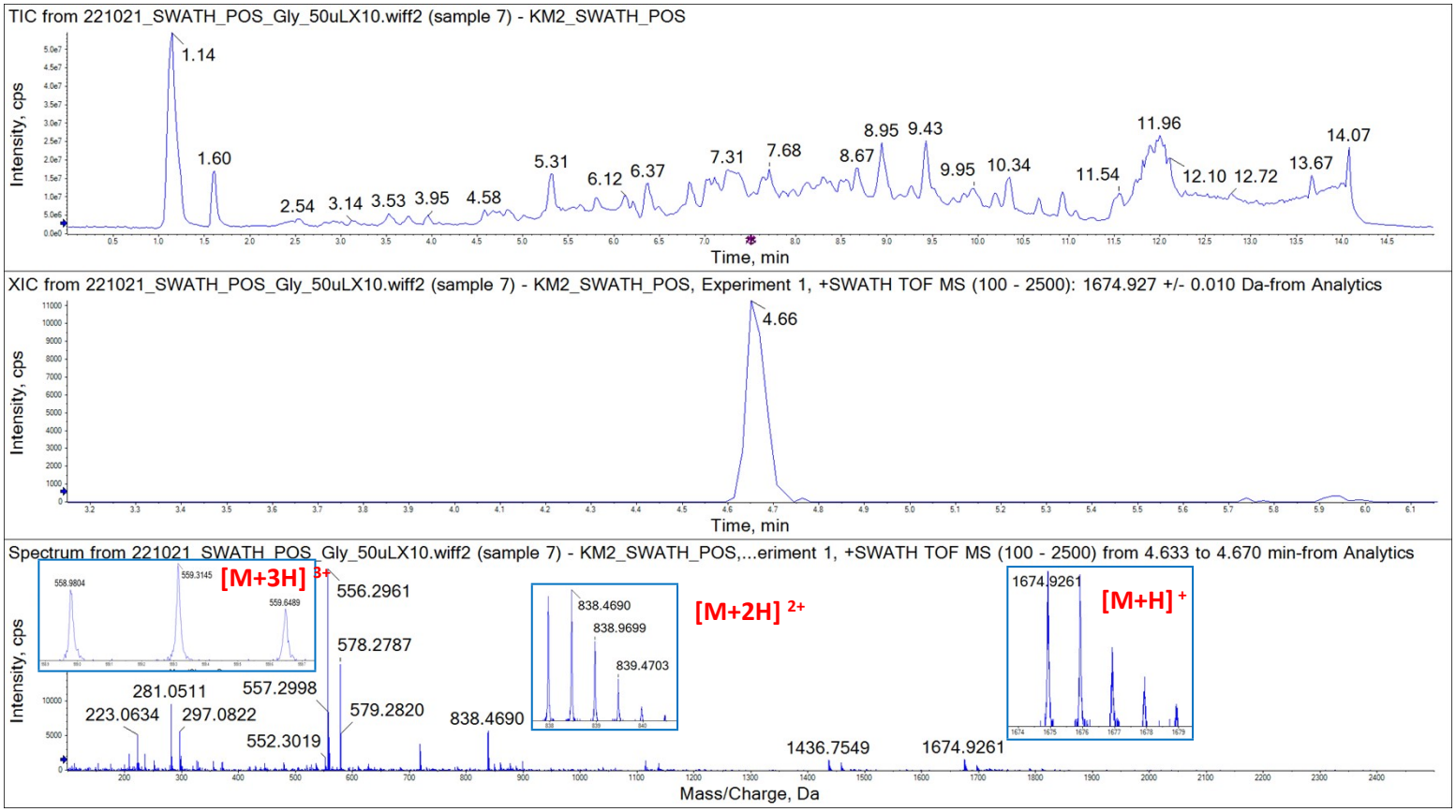

Figure S.14. UHPLC-QTOF analysis of Man2-CK3 n=1. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

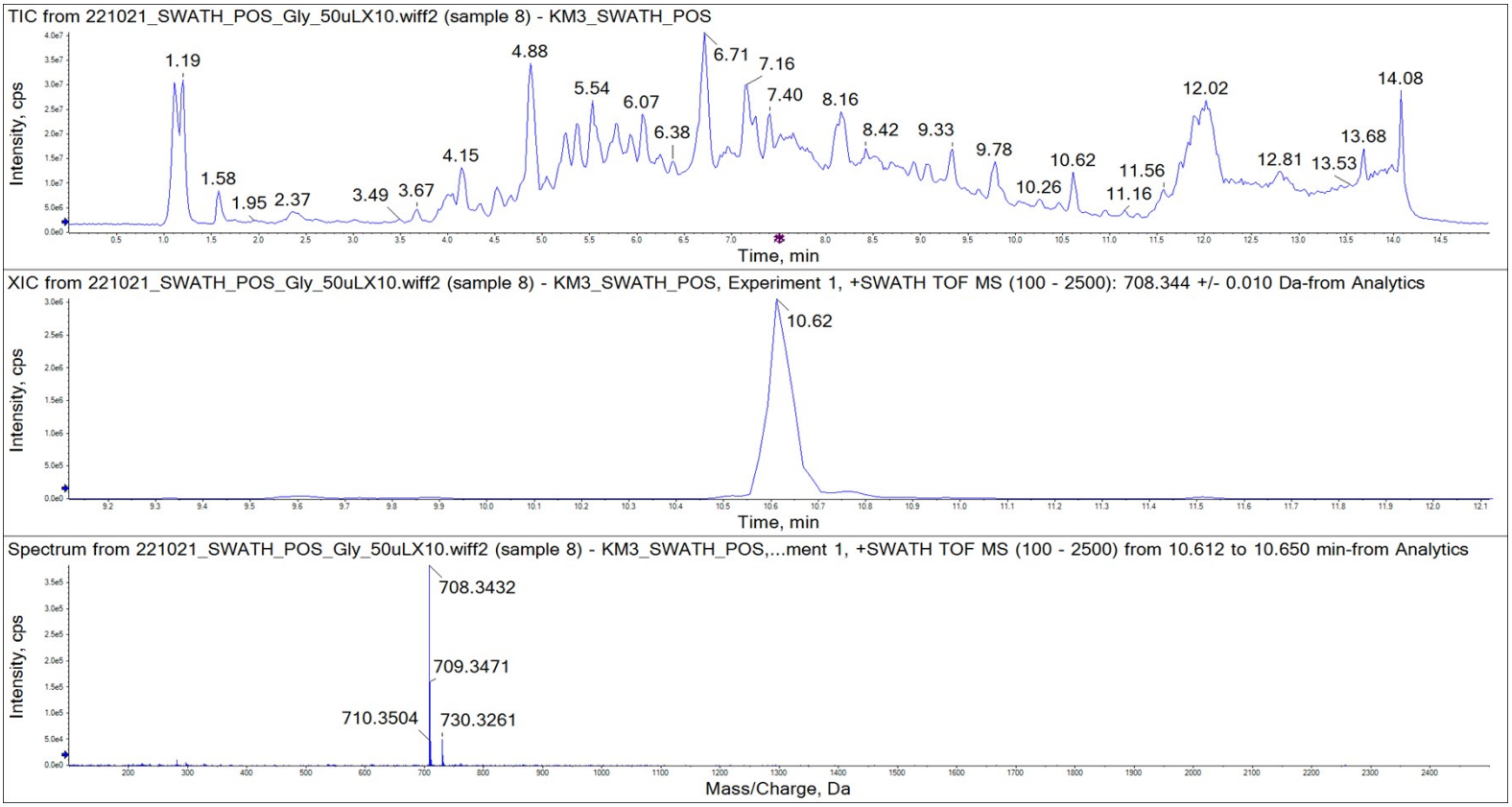

Figure S.15. UHPLC-QTOF analysis of Man2 n=4. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing [M+H]+ and [M+Na]+ adducts.

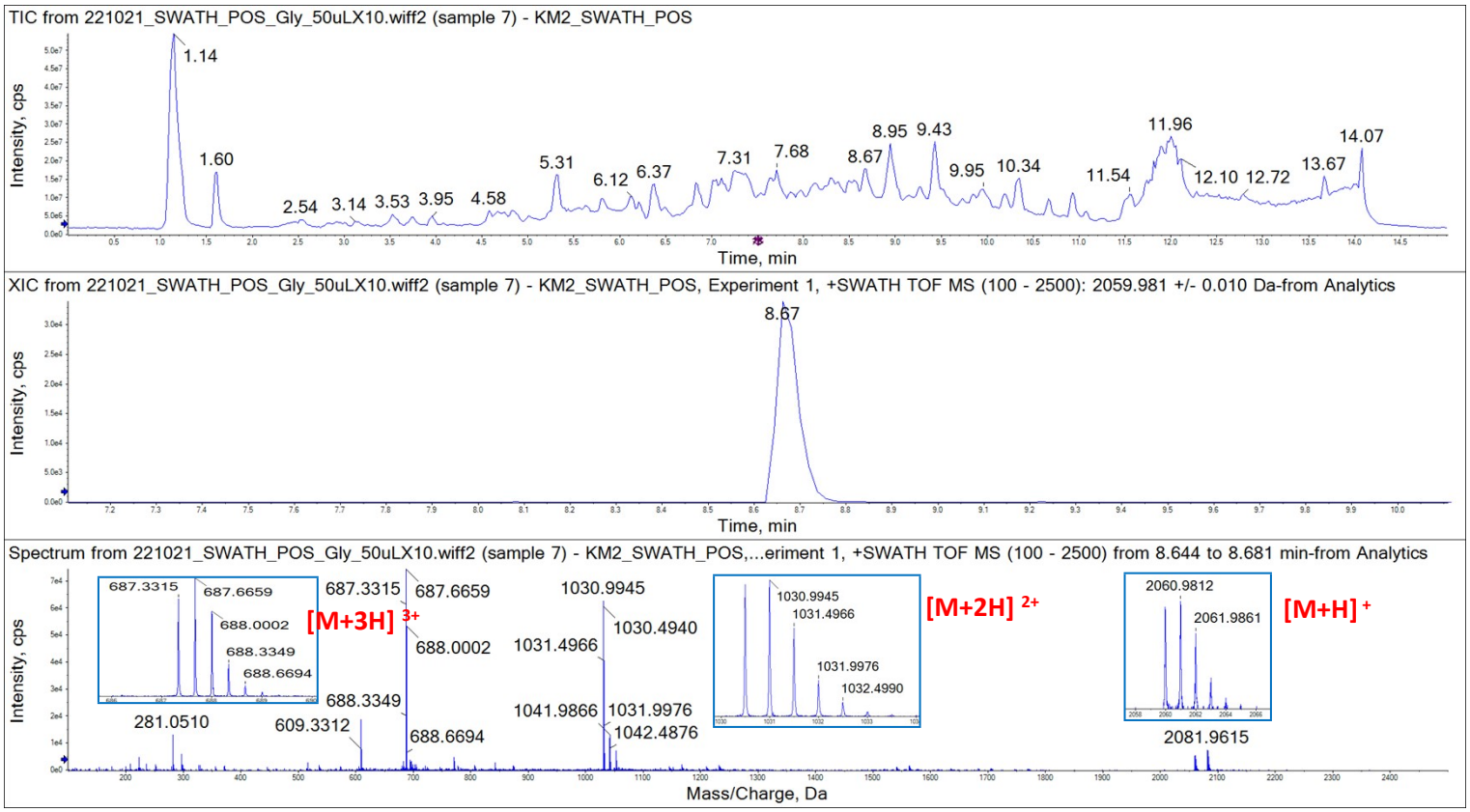

Figure S.16. LC-QTOF analysis of Man3 n=1. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

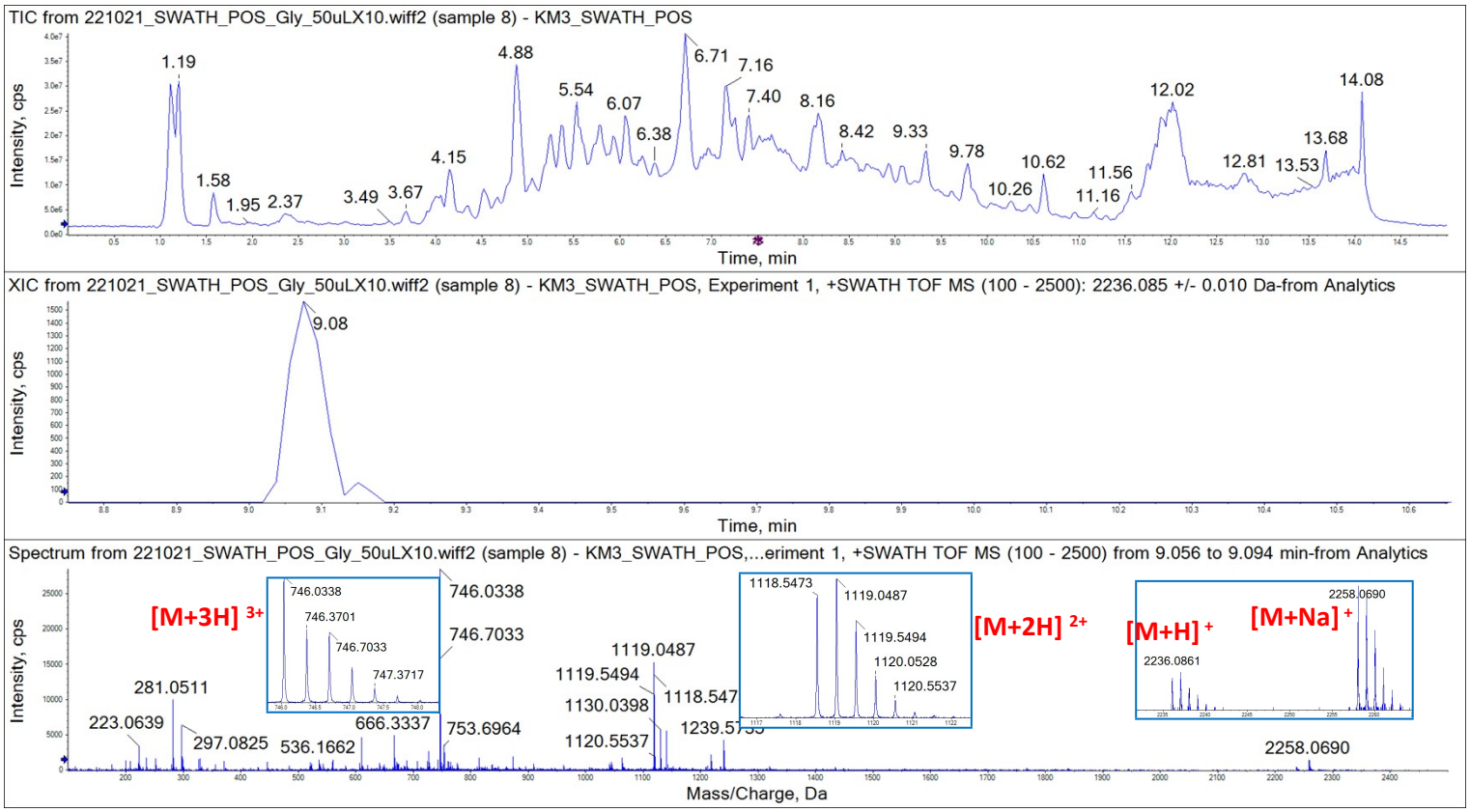

Figure S.17. UHPLC-QTOF analysis of Man3 n=4. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

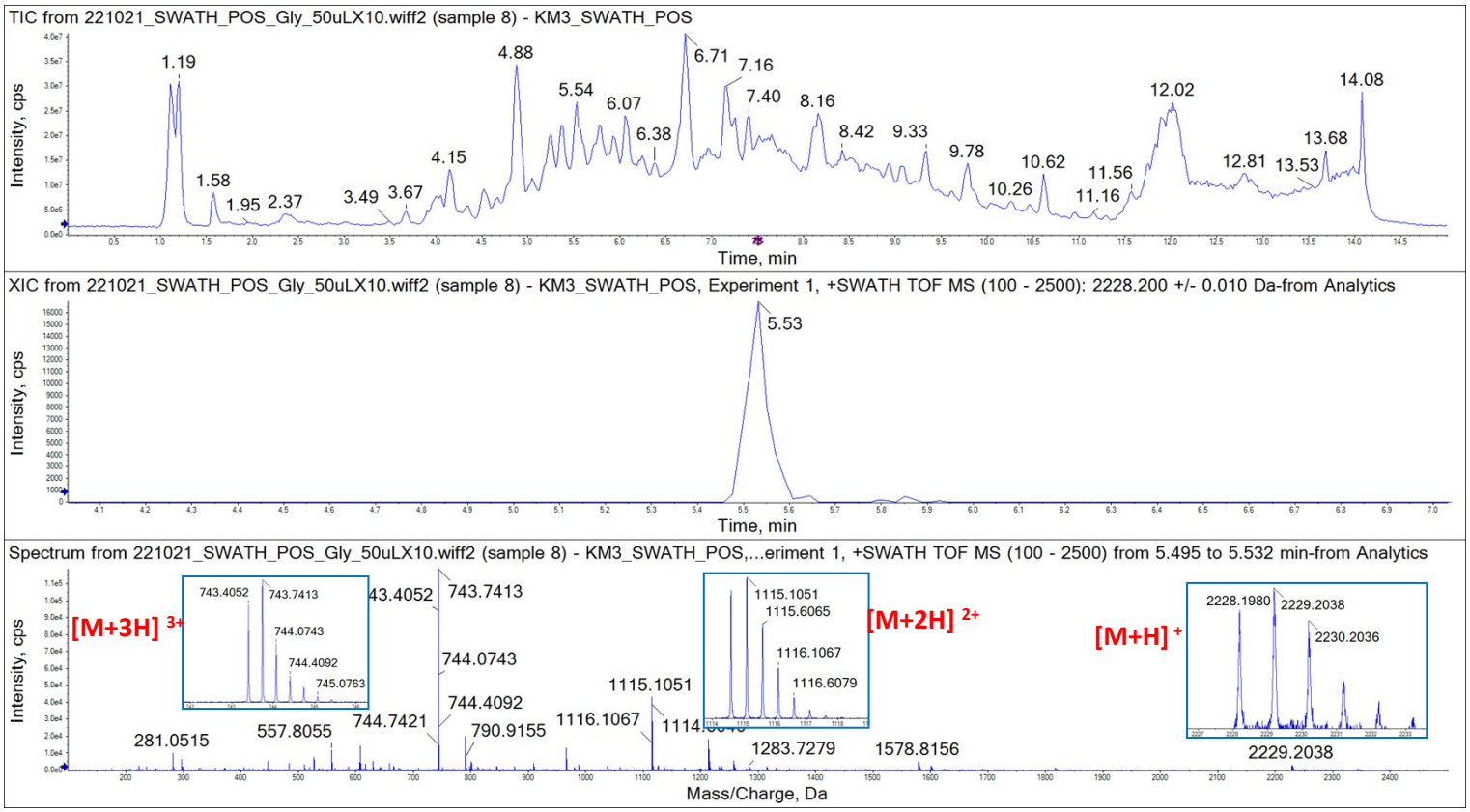

Figure S.18. UHPLC-QTOF analysis of Man3-CK3 n=2. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

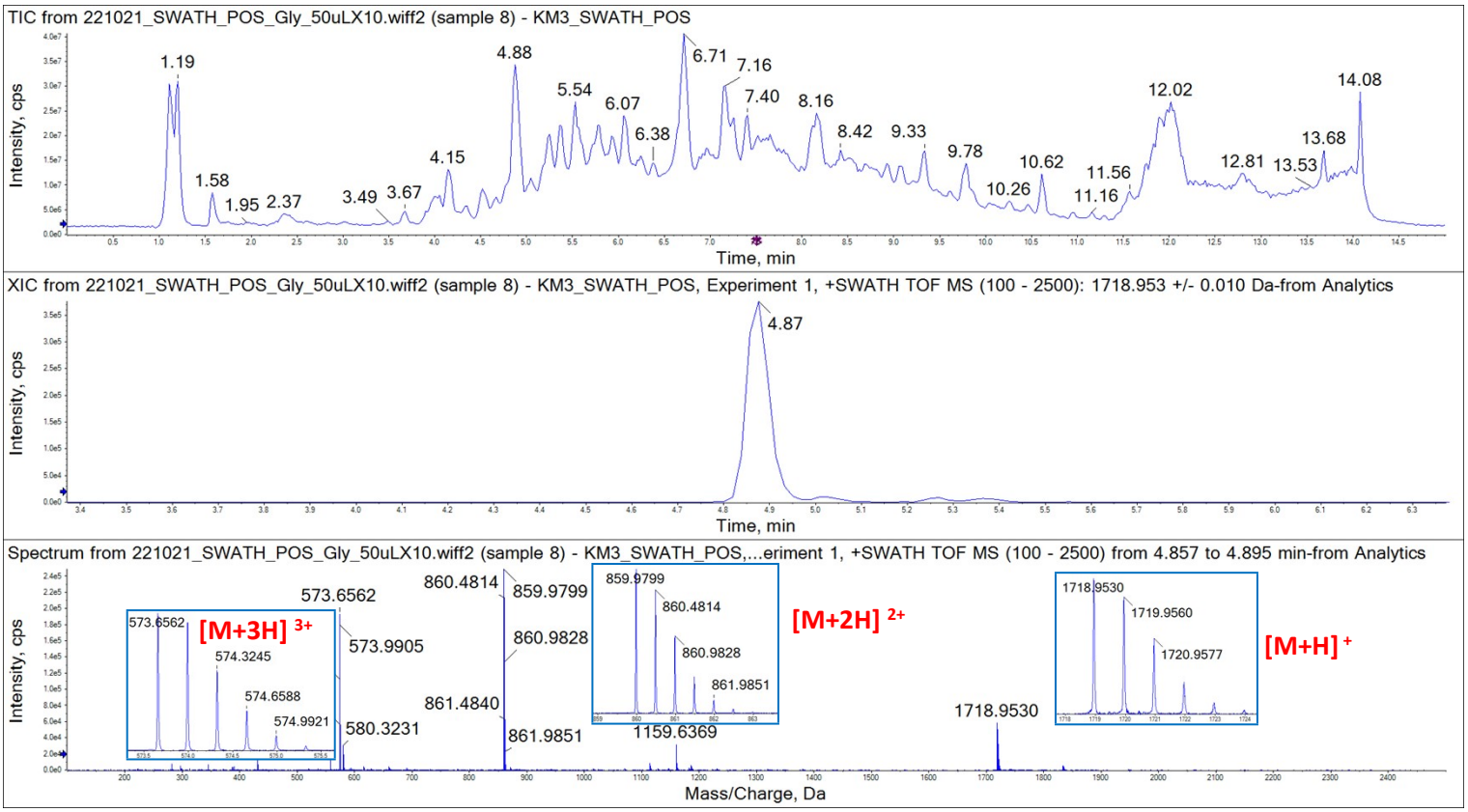

Figure S.19. UHPLC-QTOF analysis of Man3-CK3 n=1. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

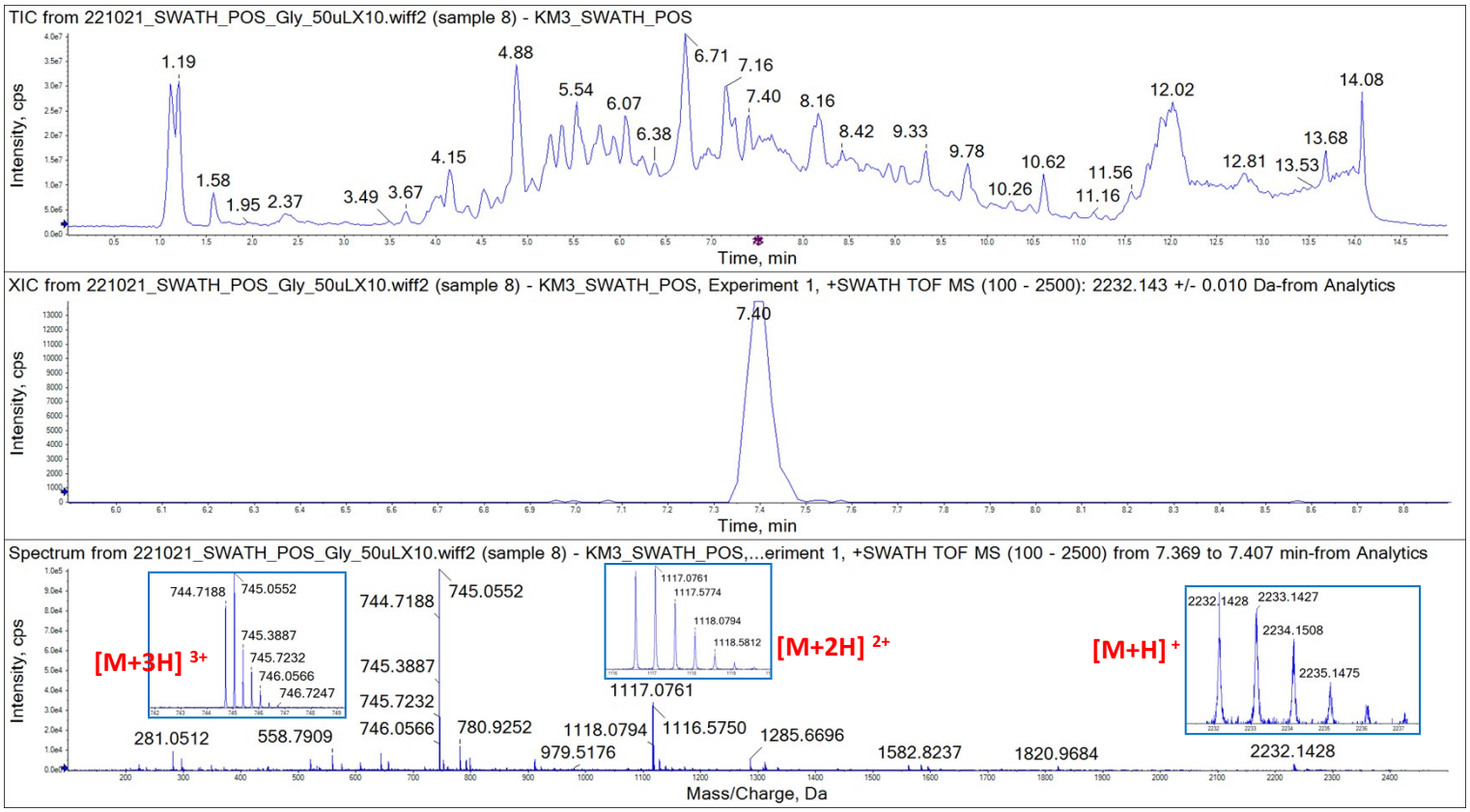

Figure S.20. UHPLC-QTOF analysis of Man3-CK3 n=3 1 peptide. Upper: Total Ion Chromatograms (TIC) in ESI positive TOFMS acquisition mode. Middle: Extracted Ion Chromatograms (XIC). Lower: MS spectra showing different [M+H]+ adducts.

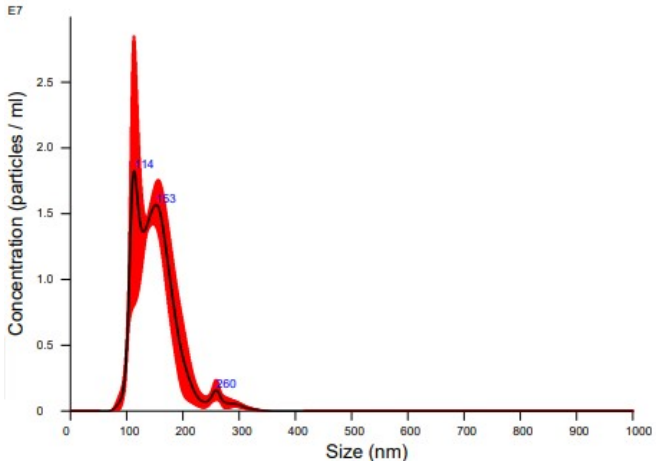

Figure S.21. NTA dry-freeze KHM2 15% NPs with 0%Sucrose

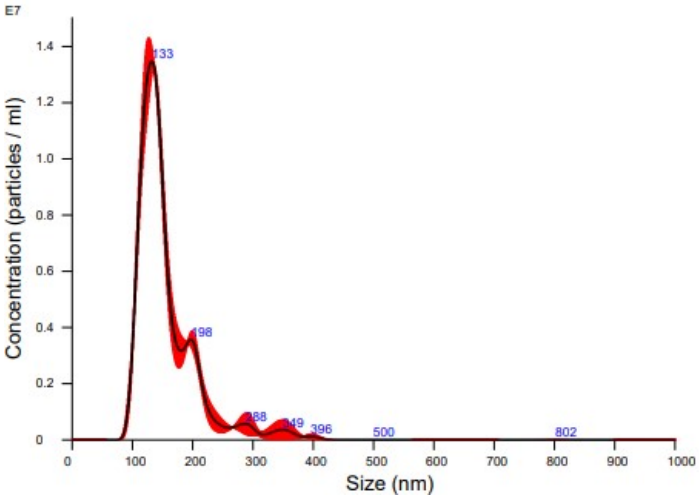

Figure S.22. NTA dry-freeze KHM2 15% with 2%Sucrose

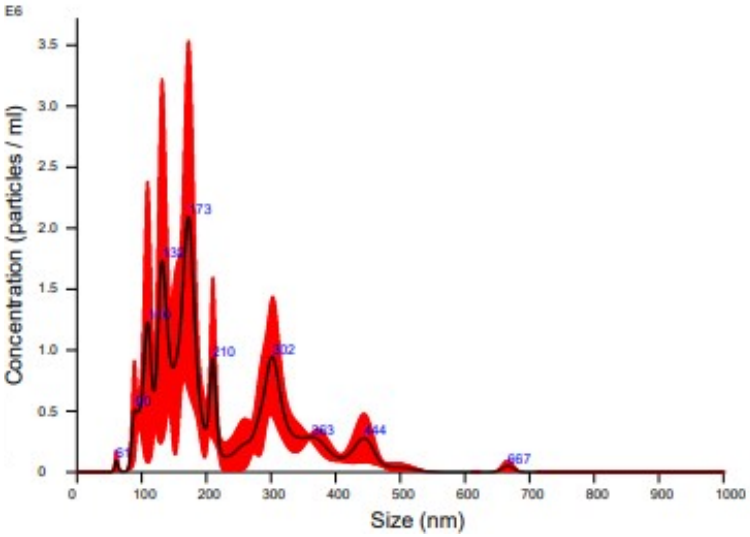

Figure S.23. NTA dry-freeze KHM2 15% with 4%Sucrose

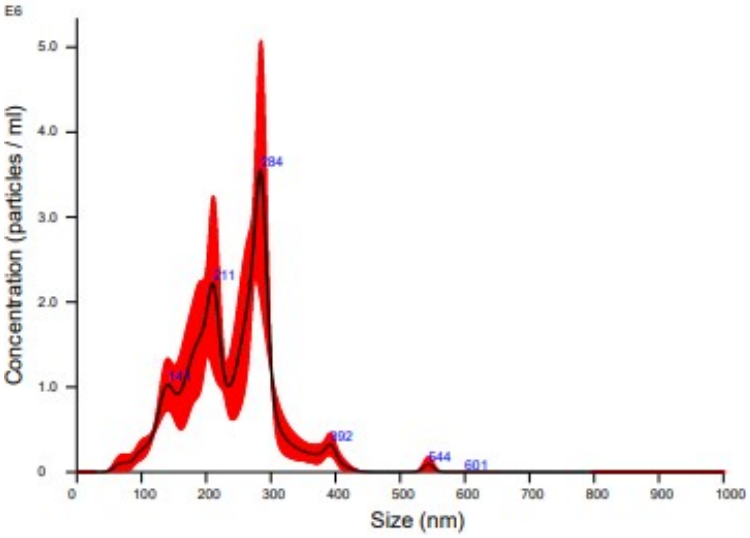

Figure S.24. NTA dry-freeze KHM2 15% with 6%Sucrose

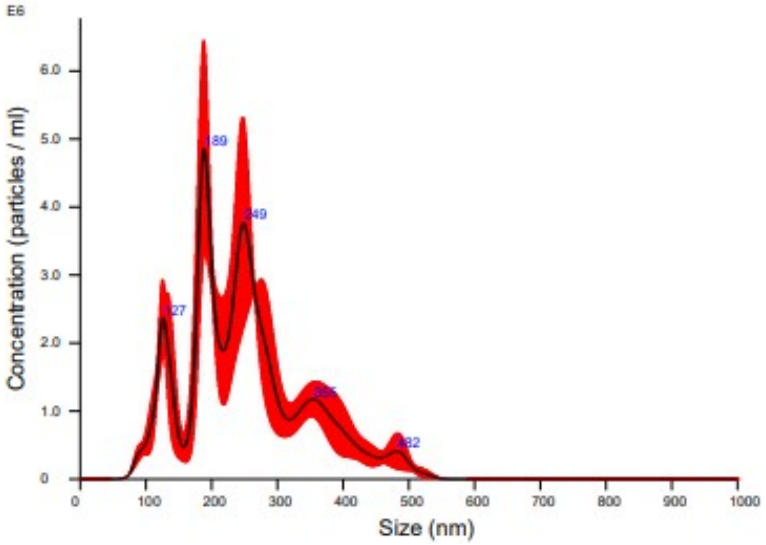

Figure S.25. NTA dry-freeze KHM2 15% with 8%Sucrose

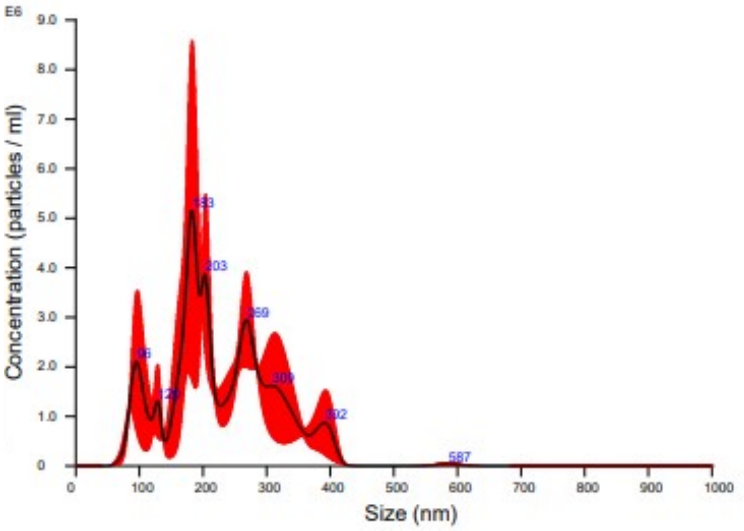

Figure S.26. NTA dry-freeze KHM2 15% with 10%Sucrose

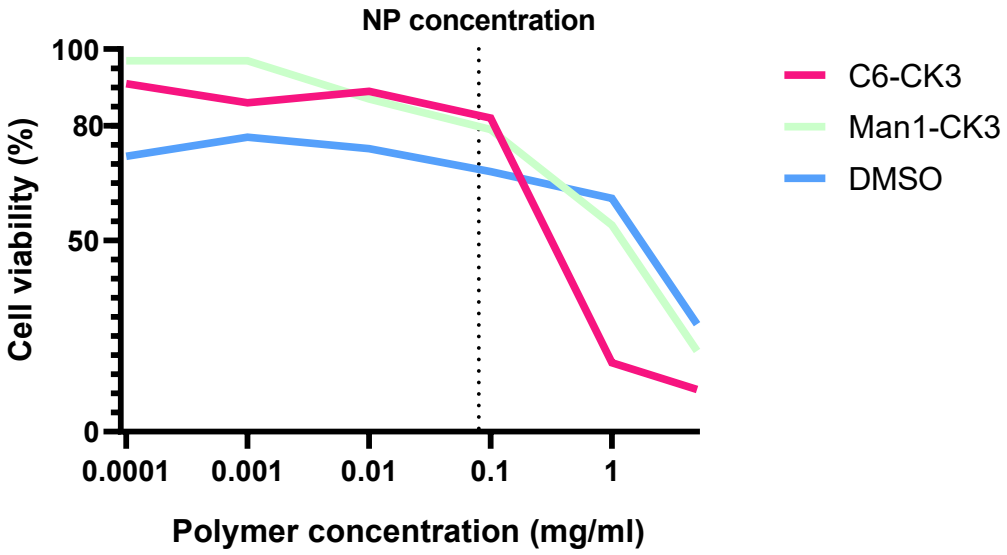

Figure S.27. Analysis of the toxicity of the non-formulated polymers and the vehicle (DMSO) used to dissolve them, performed incubating them for 24h, with JAWSII model cells.

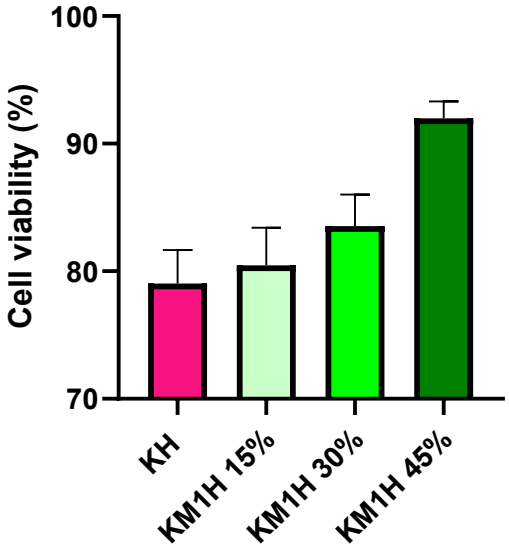

Figure S. 28. Analysis of the toxicity of the polyplexes, incubated at 0,08 μg/μL, for 24h, with JAWSII cells.