Table S1. Summary of retention times, and accurate masses of the parent and daughter ions used for identification and quantification of the nine HMOs (the MS/MS spectra of individual HOMs).

| Analyte Name | RT, min | Atomic Composition | Origin of the parent ion | Accurate mass of the parent ion | Distinctive MS/MS fragment ions |
|---|---|---|---|---|---|
| 2-Fucosyllactose (2-FL) | 8.15 | $C_{18}H_{32}O_{15}$ | $[M+HCOO]^-$ | 533.1723 | 409.1346, 205.0713 |
| 3-Fucosyllactose (3-FL) | 8.29 | $C_{18}H_{32}O_{15}$ | $[M+HCOO]^-$ | 533.1723 | 179.0559 |
| 3'-Sialyllactose (3'-SL) | 9.23 | $C_{23}H_{39}NO_{19}$ | $[M-H]^-$ | 632.2044 | 290.0879 |
| 6'-Sialyllactose (6'-SL) | 9.53 | $C_{23}H_{39}NO_{19}$ | $[M-H]^-$ | 632.2044 | 290.0879, 470.1511, 572.1825 |
| Disialyl-lacto-N-tetralose (DSLNT) | 11.18 | $C_{48}H_{79}N_3O_{37}$ | $[M-2H]^{2-}$ | 643.7123 | 290.0877, 997.3387 |
| Lacto-N-tetraose (LNT) | 9.14 | $C_{26}H_{45}NO_{21}$ | $[M-H]^-$ | 706.2411 | 142.0506, 202.0717 |
| Lacto-N-fucopentaose I (LNFP I) | 9.64 | $C_{32}H_{55}NO_{25}$ | $[M+HCOO]^-$ | 898.3045 | 205.0713, 325.1137 |
| Lacto-N-fucopentaose II (LNFP II) | 9.75 | $C_{32}H_{55}NO_{25}$ | $[M+HCOO]^-$ | 898.3045 | 288.1086, 348.1298 |
| Lacto-N-fucopentaose III (LNFP III) | 9.75 | $C_{32}H_{55}NO_{25}$ | $[M+HCOO]^-$ | 898.3045 | 179.0559, 364.1244 |

Table S2: Absolute concentrations (nmol/mL) of individual HMO in all available HM samples

| | All Secretors samples | All Non-secretors samples | All HM samples |
|---|---|---|---|
| HMO | n=30 | n=23 | n=53 |
| 2'FL | 1788 ± 223.2 | 0 | 1012 ± 175.6 |
| 3FL | 182.9 ± 23.57 | 414.1 ± 57.84 | 283.3 ± 32.28 |
| 3'SL | 97.17 ± 10.51 | 81.03 ± 8.621 | 90.17 ± 7.054 |
| 6'SL | 192.2 ± 20.16 | 190.8 ± 25.06 | 191.6 ± 15.61 |
| DSLNT | 158.1 ± 14.01 | 191.2 ± 19.78 | 172.4 ± 11.79 |
| LNFP I | 819.4 ± 231.3 | 0 | 463.8 ± 141.6 |
| LNFPII | 85.56 ± 23.29 | 311.8 ± 88.59 | 183.7 ± 43.06 |
| LNFPIII | 63.49 ± 11.34 | 156.3 ± 34.79 | 103.8 ± 17.42 |
| LNT | 354.2 ± 42.39 | 630.6 ± 122.1 | 474.1 ± 60.54 |

Data are expressed as mean ± SEM

Figure S1:

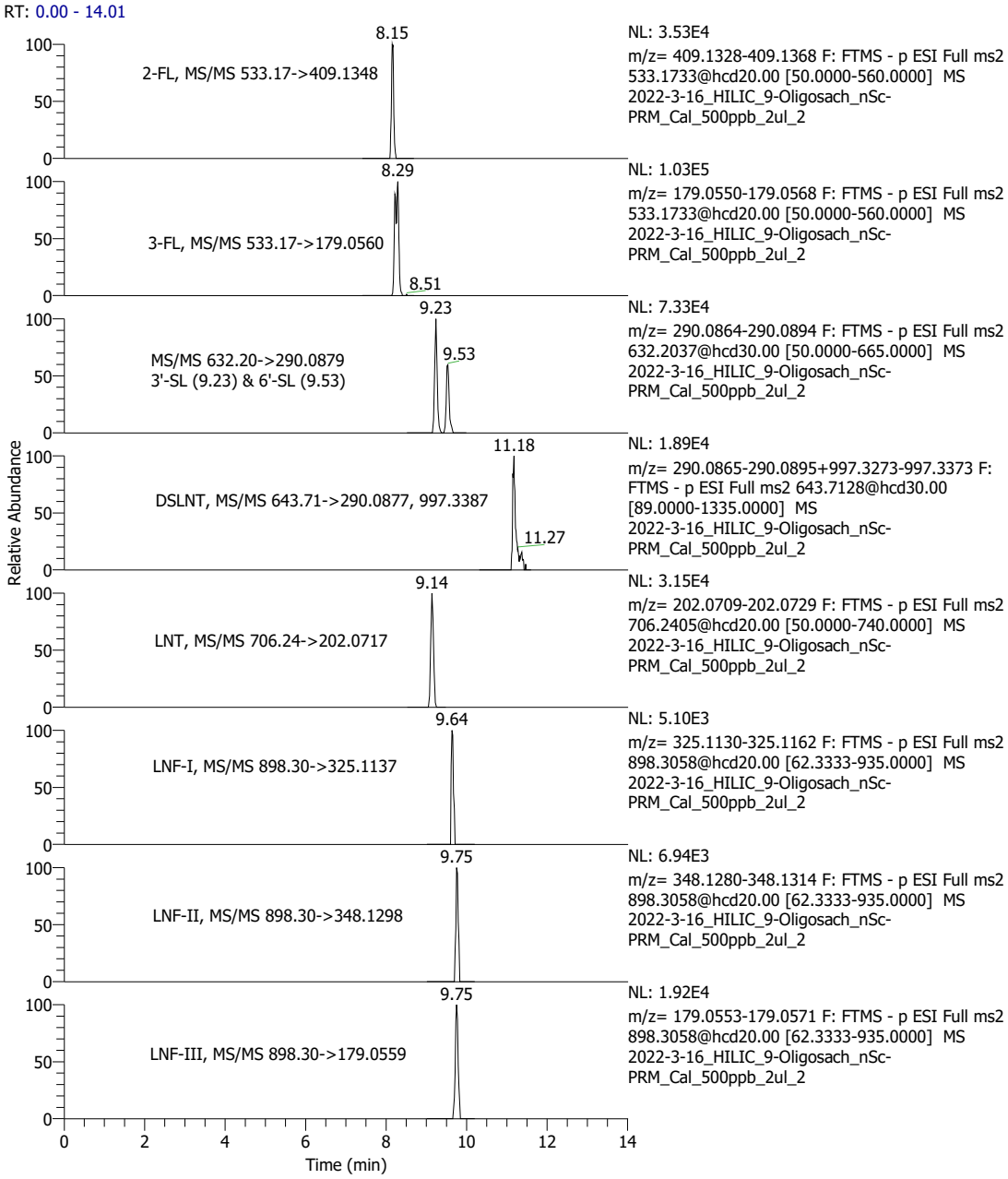


Figure S1: Negative ESI LC-MS/MS (PRM) extracted accurate mass ion chromatograms of nine analyzed HMOs. Co-eluted isomeric HOMs could be identified and distinguished by their characteristic MS/MS spectra.

Figure S2

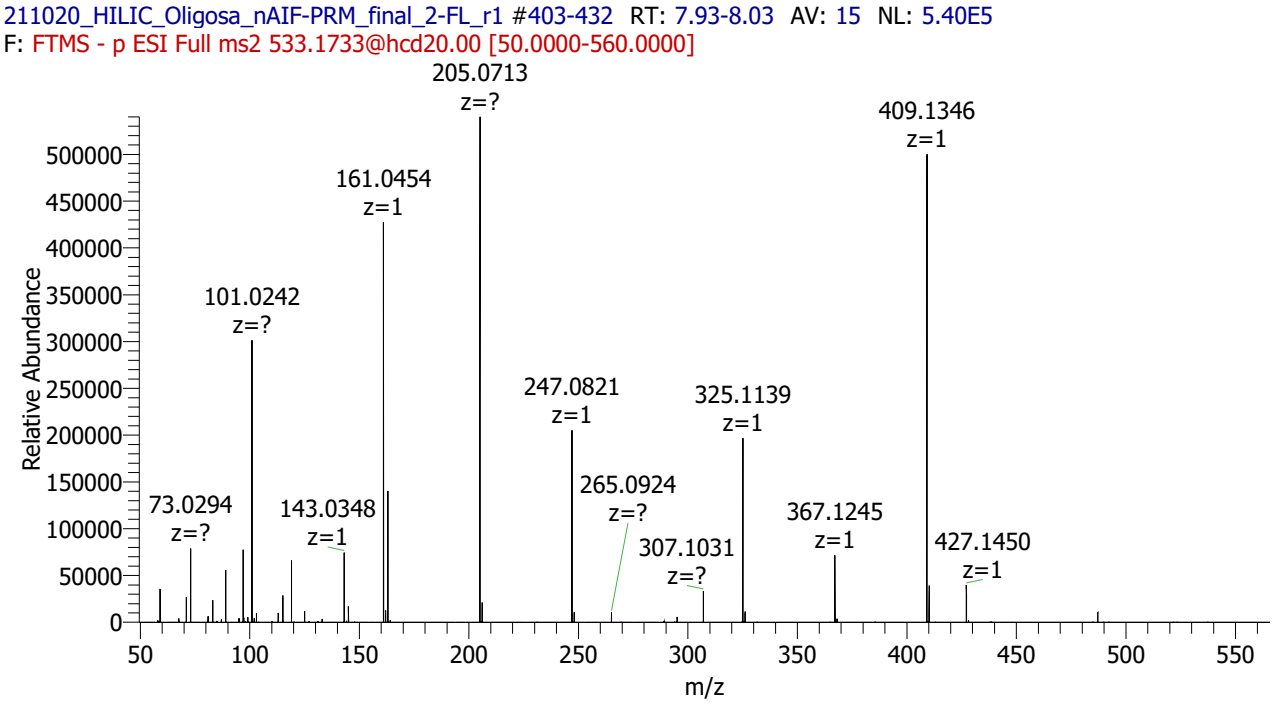


Figure S2A: Negative ESI CID mass spectrum of 2-FL [M+HCOO]⁻ ion.

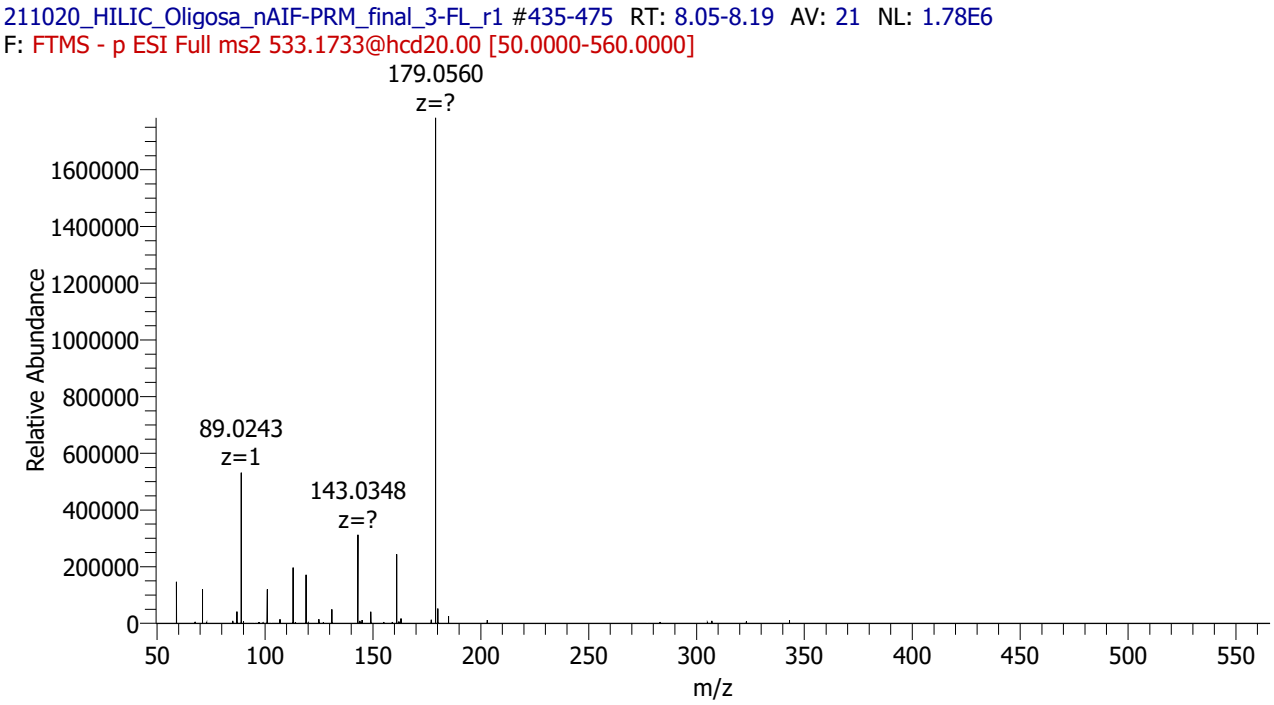


Figure S2B: Negative ESI CID mass spectrum of 3-FL [M+HCOO]⁻

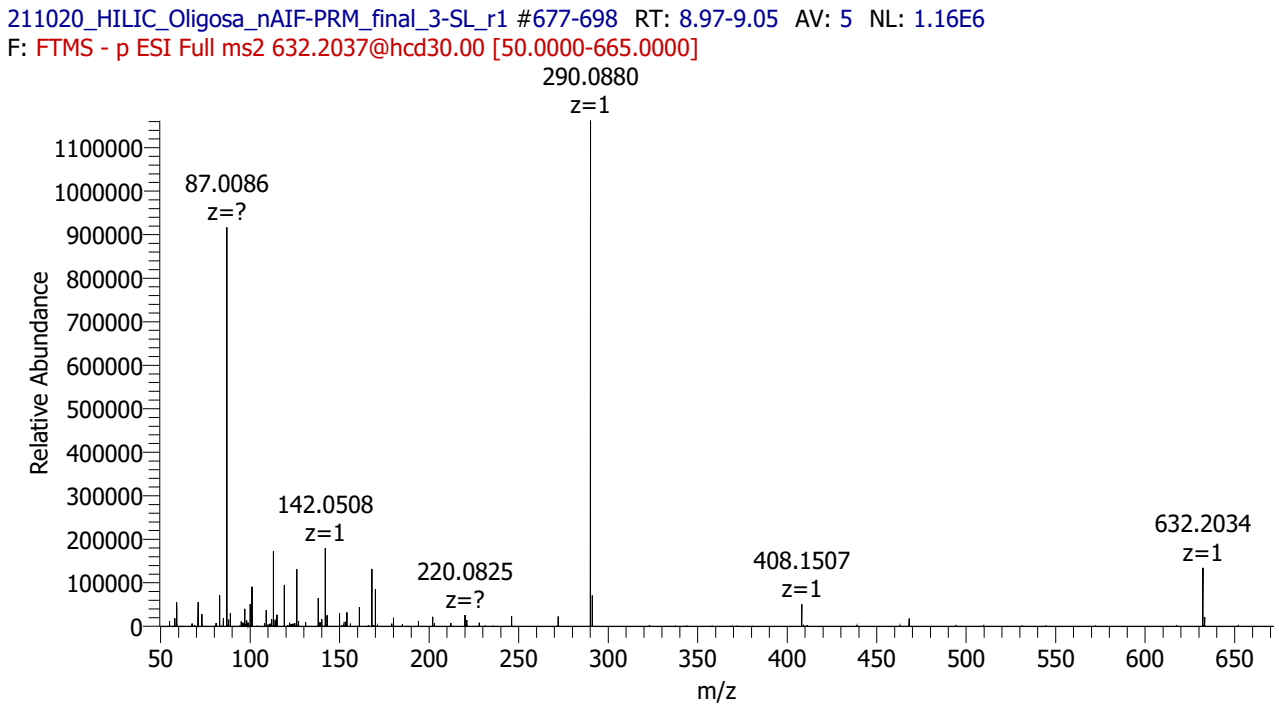


Figure S2C: Negative ESI CID mass spectrum of 3'-SL [M-H]⁻ ion.

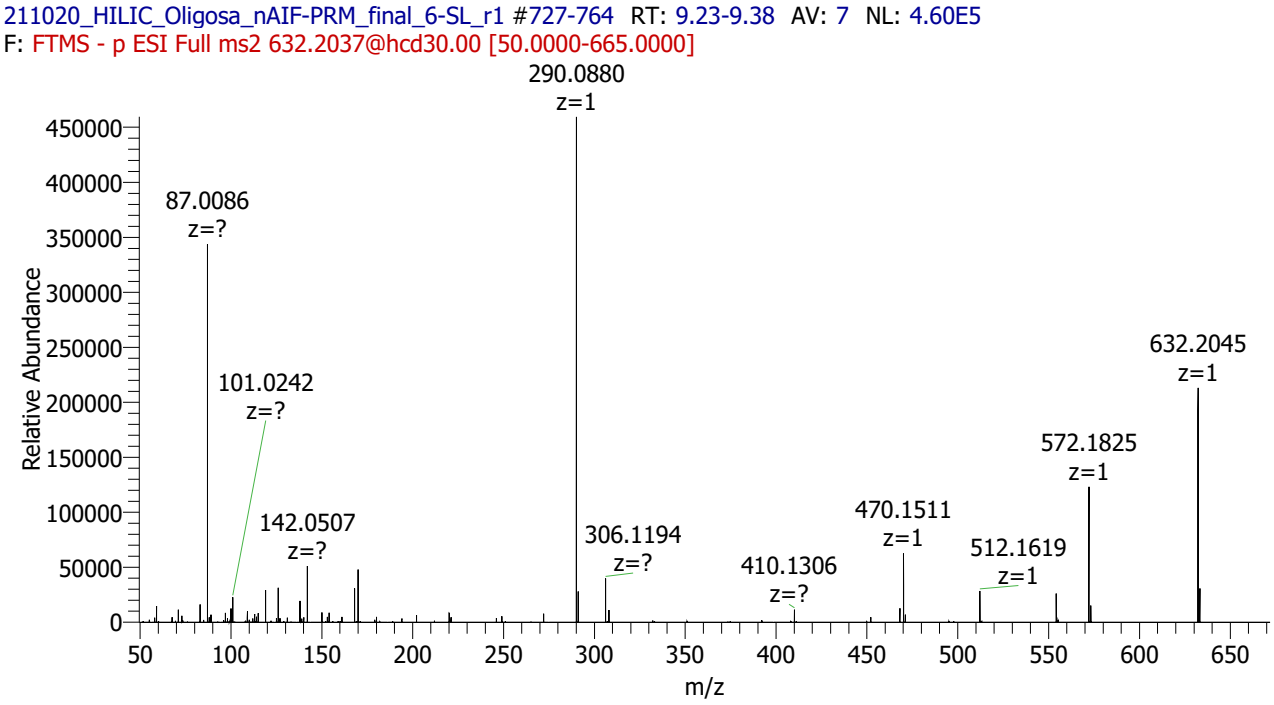


Figure S2D: Negative ESI CID mass spectrum of 6'-SL [M-H]$^-$ ion.

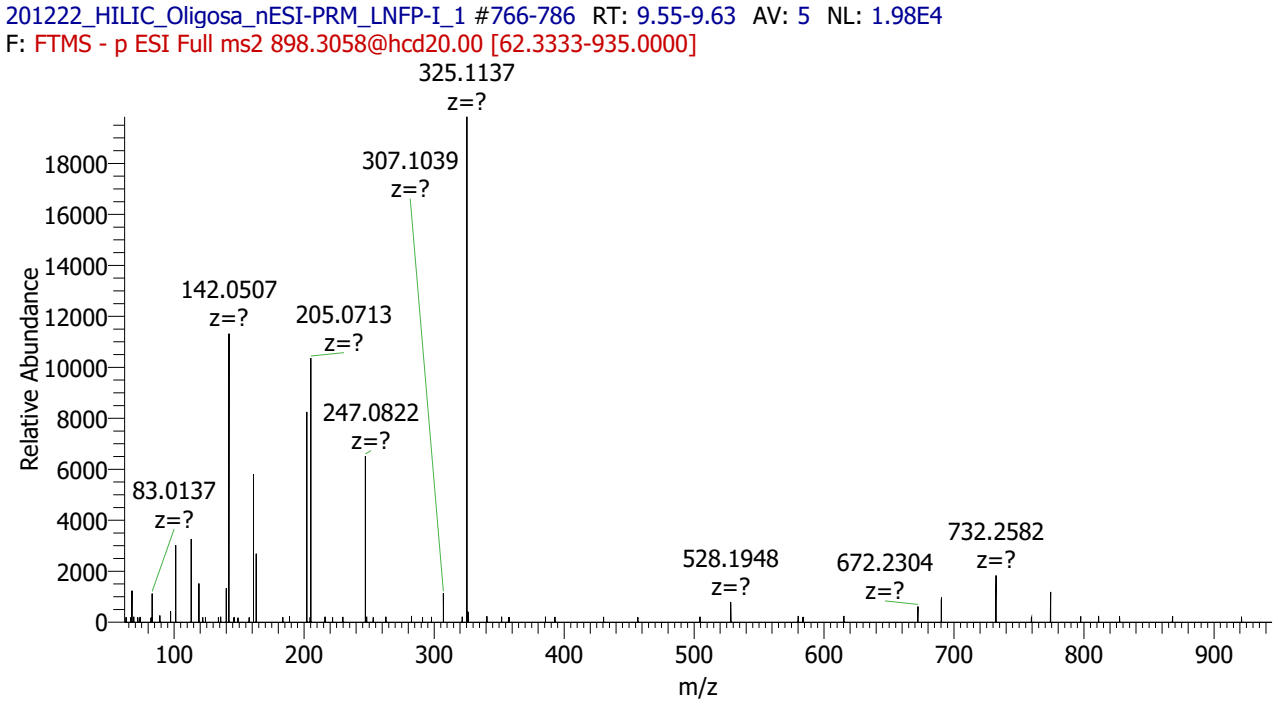


Figure S2E: Negative ESI CID mass spectrum of LNF I [M+HCOO]⁻ ion.

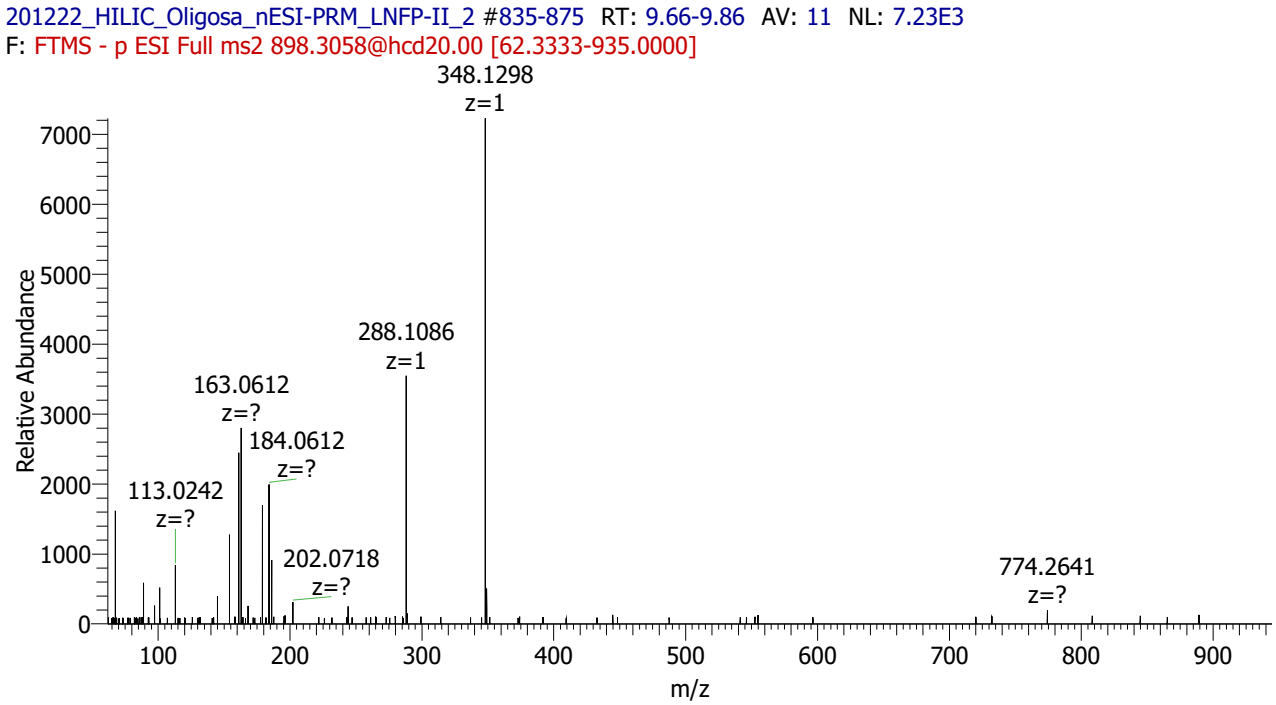


Figure S2F: Negative ESI CID mass spectrum of LNF II [M+HCOO]⁻ ion.

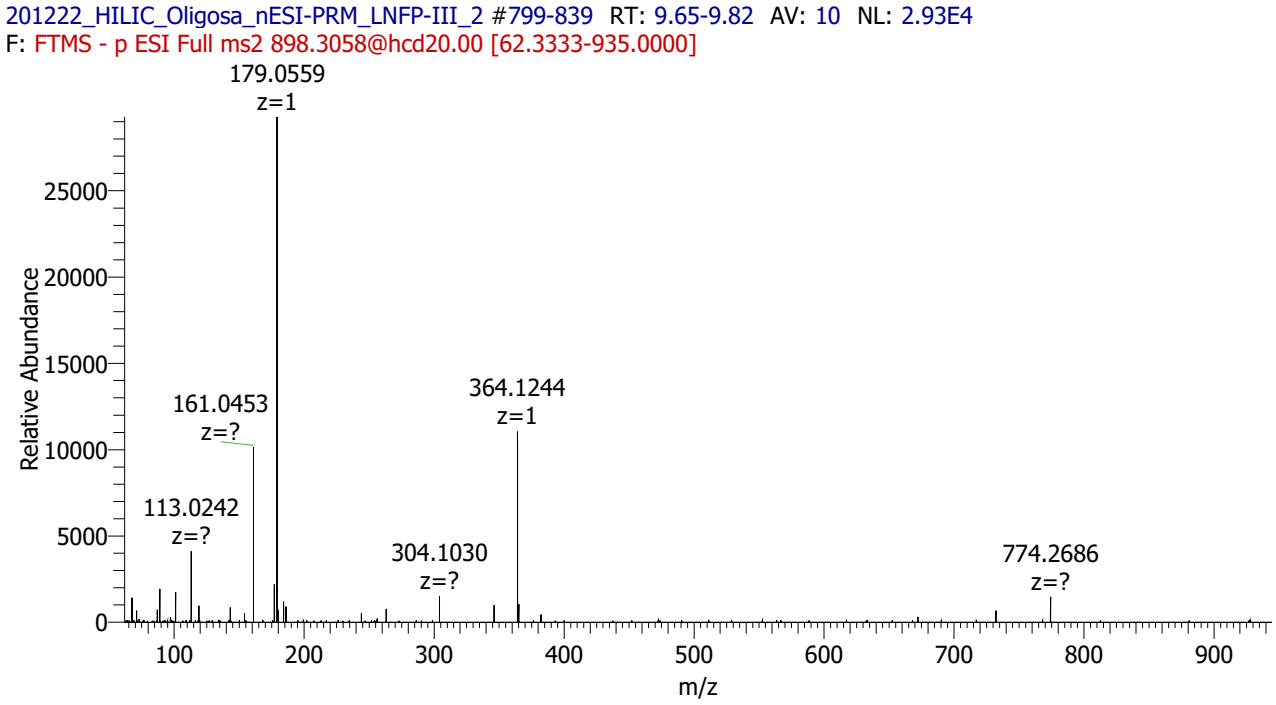


Figure S2G: Negative ESI CID mass spectrum of LNF III $[M+HCOO]^-$ ion.

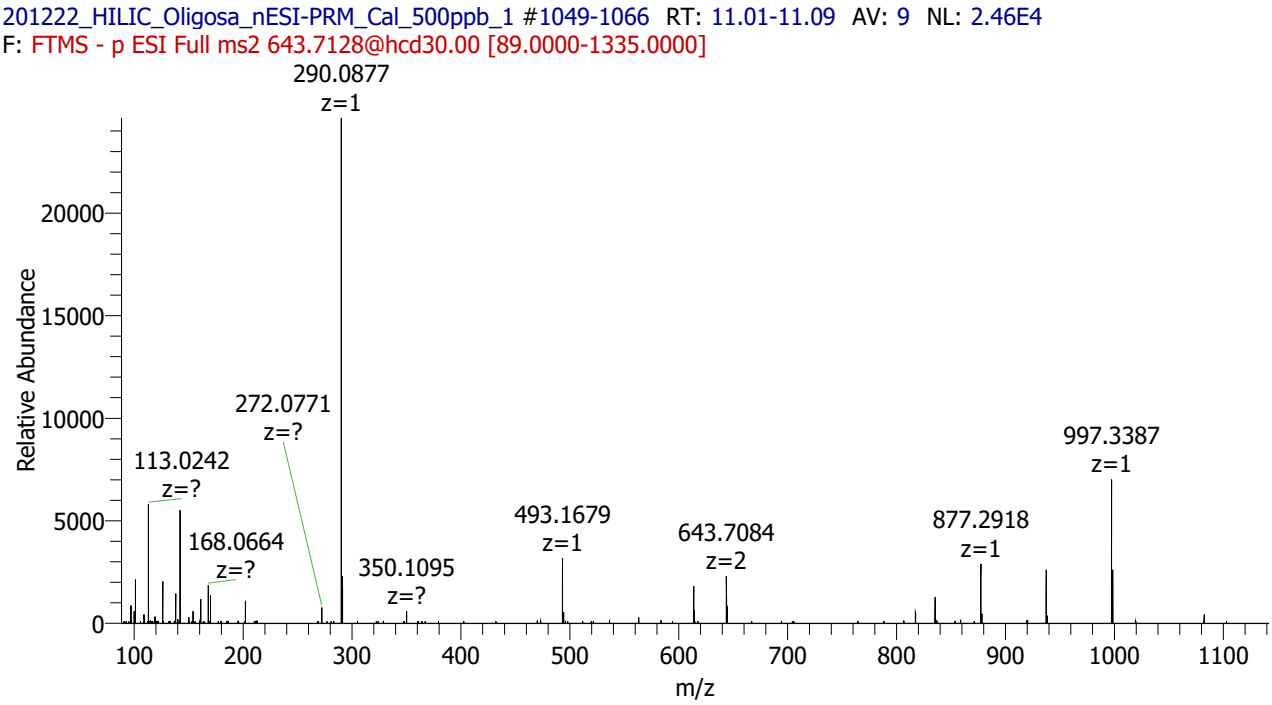


Figure S2H: Negative ESI CID mass spectrum of DSLNT $[M-2H]^{2-}$ ion.

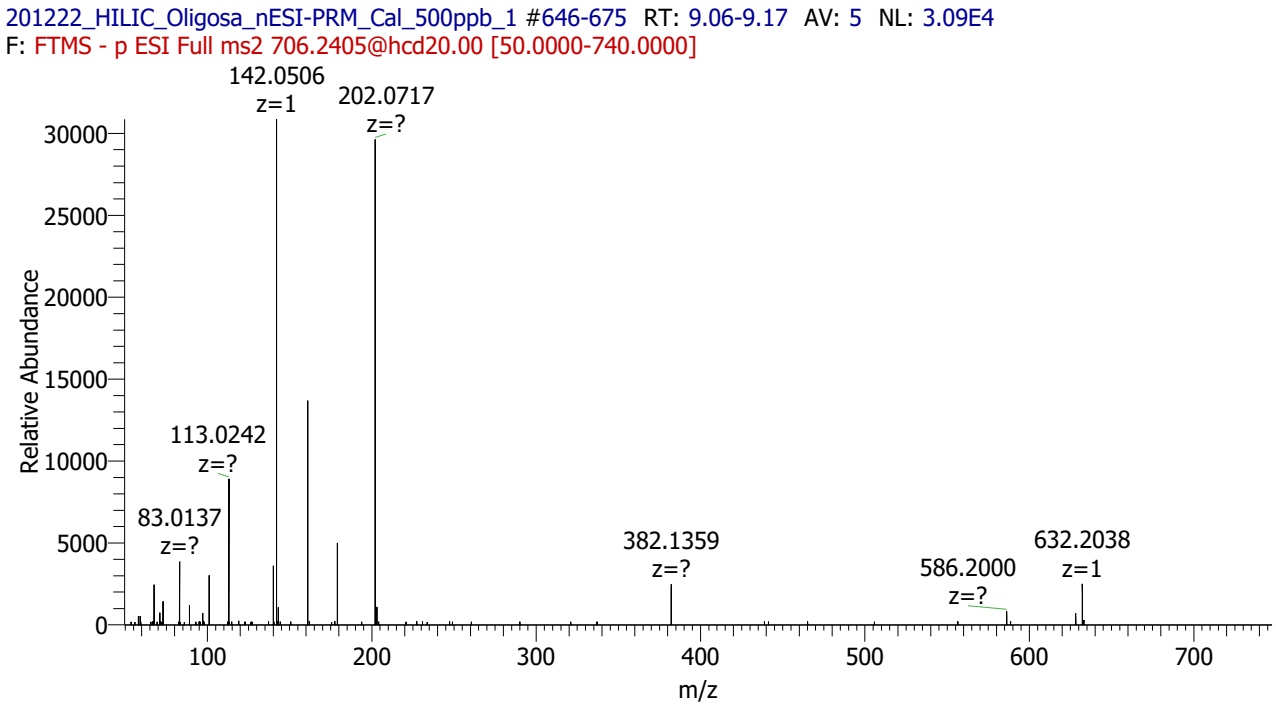


Figure S2I: Negative ESI CID mass spectrum of LNT $[M-H]^-$ ion.