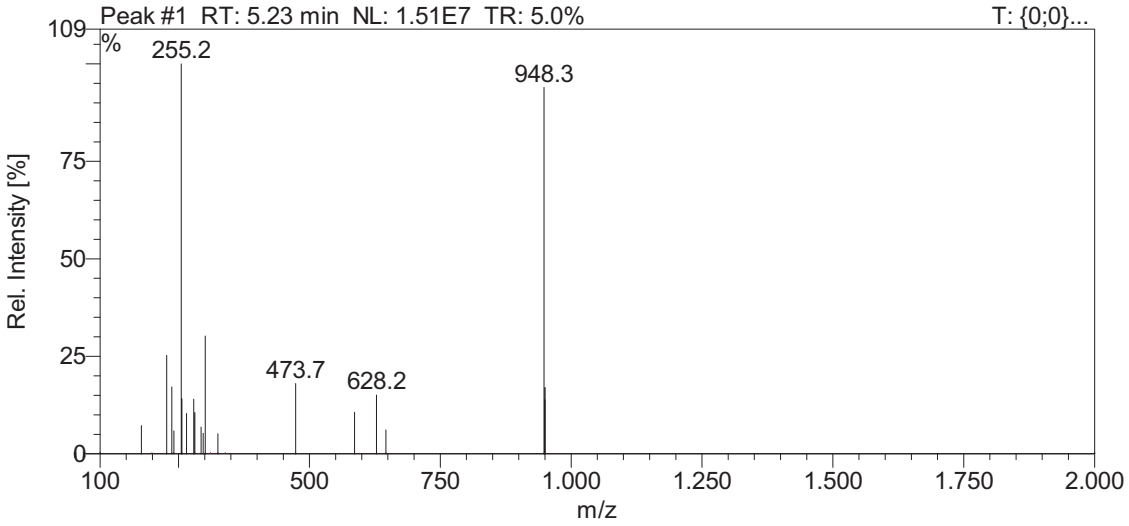
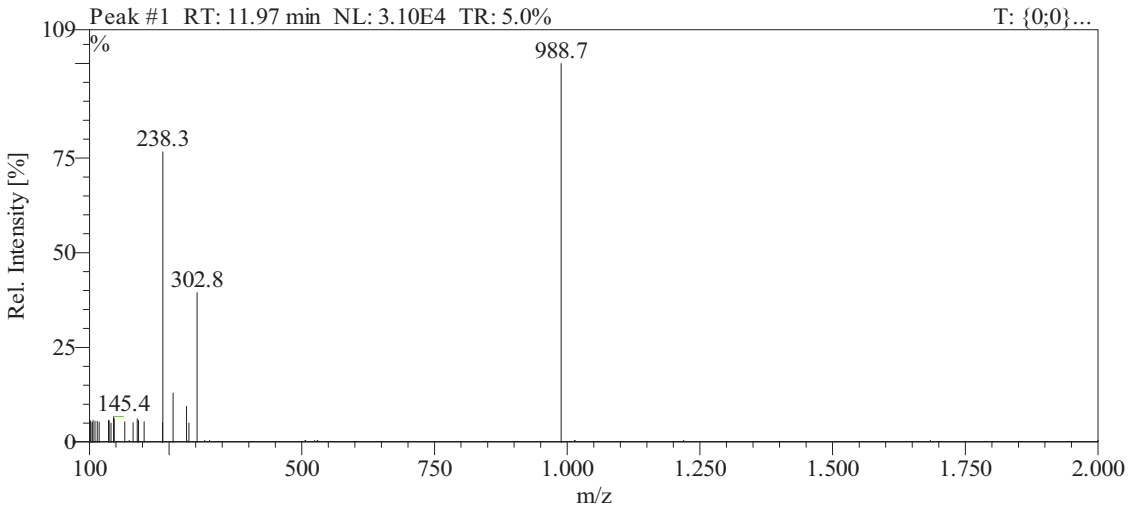
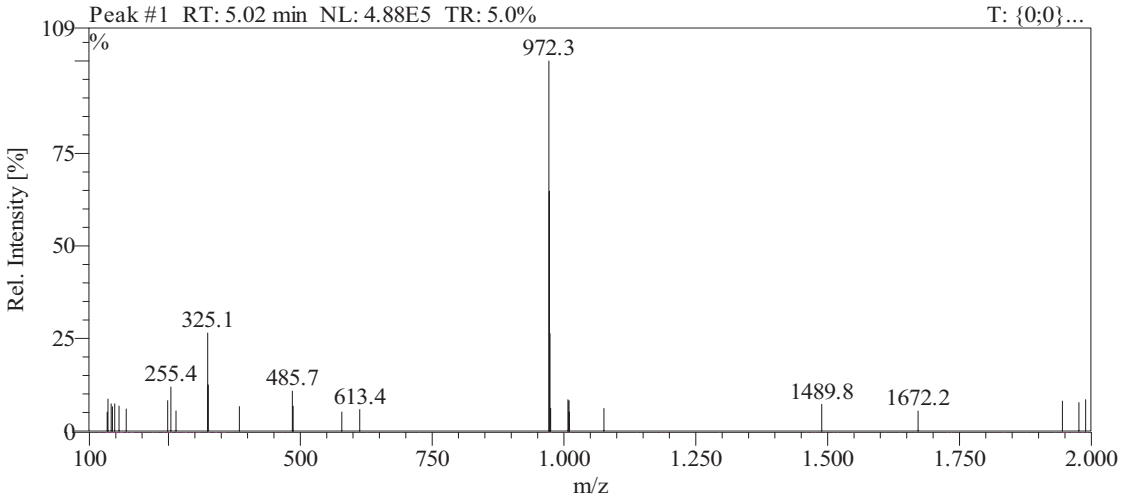
# Supplementary Information




(**a**)

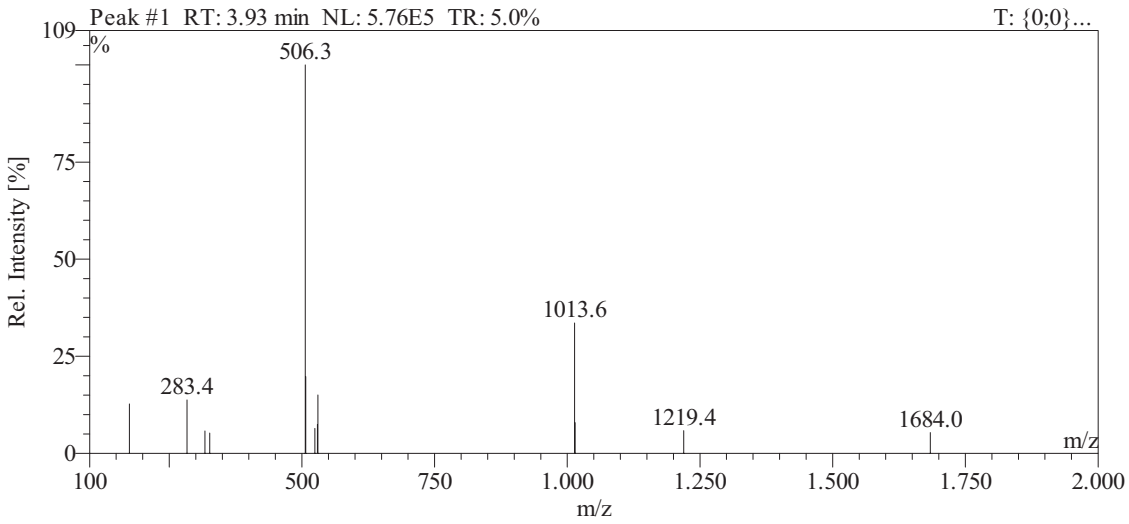



(**b**)



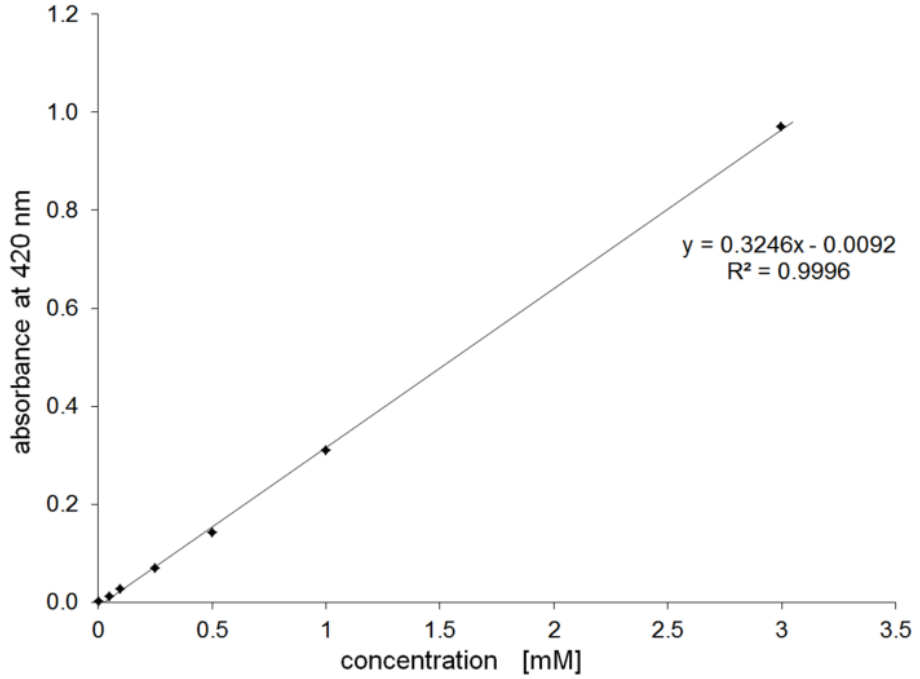

(**c**)

**Figure S1.** *Cont.*



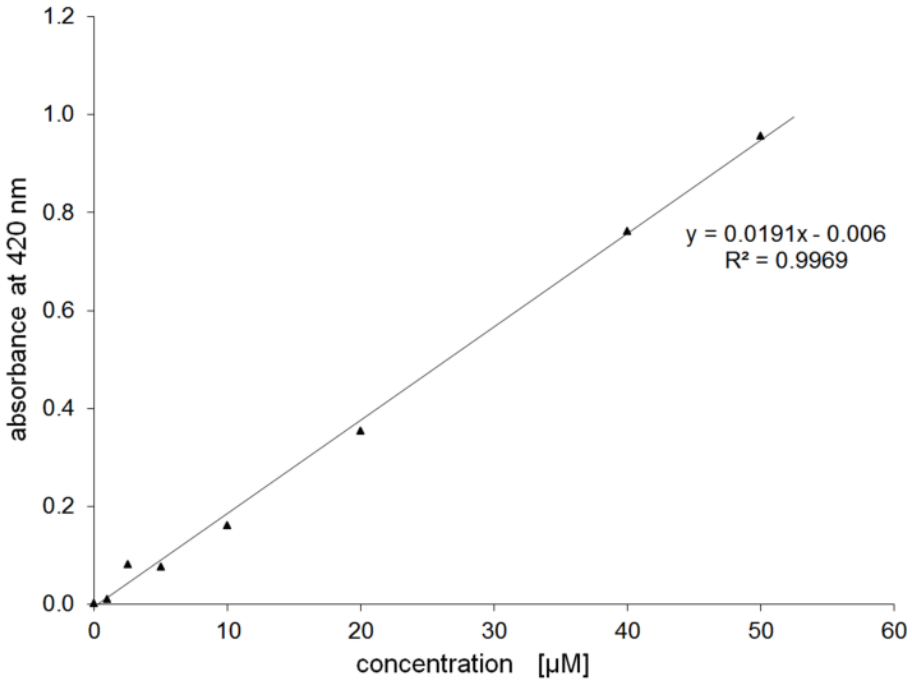

(**d**)

**Figure S1.** MS spectra of substances **4** (**a**), **5** (**b**), **9** (**c**) and **10** (**d**). Via LC-MS using electrospray ionization $[M-H]^-$ ions were analyzed using quadrupole mass analyzer. **4** $[M-H]^-$ = 949.97 *m*/*z*, **5** $[M-H]^-$ = 990.01 *m*/*z*, **9** $[M-H]^-$ = 973.95 *m*/*z* and **10** $[M-H]^-$ = 1013.99 *m*/*z* ($[M-2H]^{2-}$ = 506.3 *m*/*z*) could be verified ((**a**)–(**d**)).




(**a**)

**Figure S2.** *Cont.*




(**b**)

**Figure S2.** TNBSA-assay calibration curves with lysine hydrochloride (**a**) and bovine serum albumin (**b**). Chromogenic products (*N*-trinitrophenylamine) resulting from reaction of trinitrobenzene sulfonic acid (TNBSA) with primary amine (ε-amino group of lysine) were quantified in microplate reader at 420 nm. For calculating the modification degree of the neo-glycoproteins, calibration with bovine serum albumin (**b**) was used.

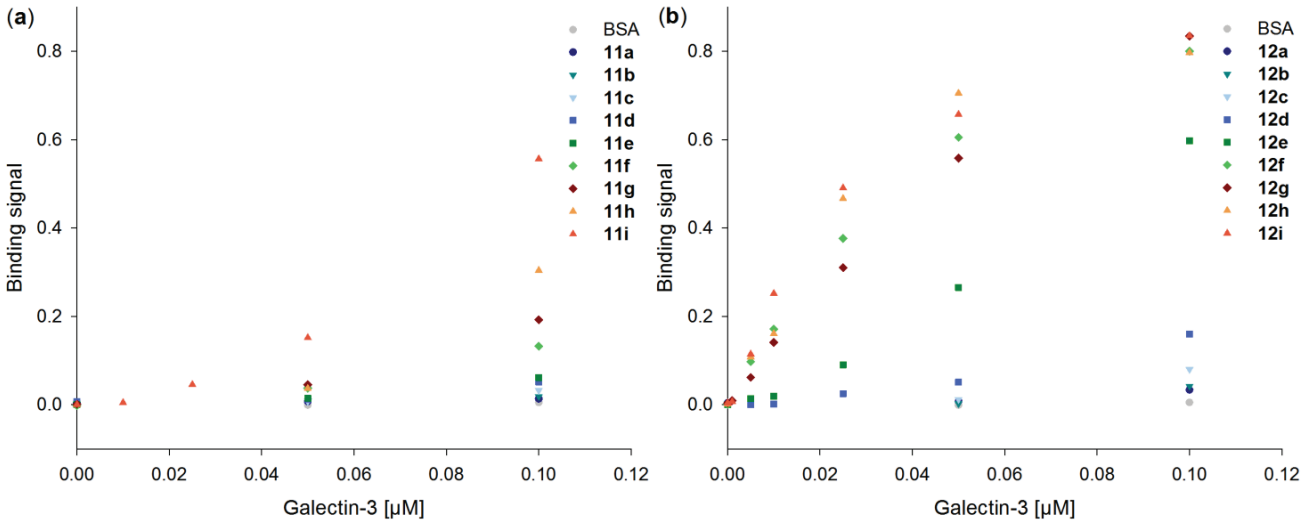



**Figure S3.** Binding signals of galectin-3 for concentrations of 0 to 0.1 µM to (**a**) LacNAc-LacNAc conjungated BSA (**11a**–**i**) and (**b**) LacDiNAc-LacNAc conjugated BSA (**12a**–**i**). Data were obtained for all neo-glycoproteins (**11a**–**i** and **12a**–**i**) in the galectin-3 concentration range of 0.05 to 10 µM. Moreover, binding signals of galectin-3 at concentrations below 0.05 µM were detectable for **11i** and **12d**–**i**.

**Table S1.** Binding signals of galectin-1 and galectin-3 to immobilized neo-glycoproteins **11a**–**i** and **12a**–**i**, ASF and BSA. Comparison of galectin-1 and galectin-3 binding is shown with standard deviation of at least 11 measured values. Significant higher binding of galectin-3 compared to galectin-1 is observed for neo-glycoproteins presenting LacNAc-LacNAc and LacDiNAc-LacNAc, respectively.

| Ligand | Binding Signal | | Ligand | Binding Signal | |
|---|---|---|---|---|---|
| | Galectin-1 | Galectin-3 | | Galectin-1 | Galectin-3 |
| BSA | 0.00 ± 0.01 | 0.00 ± 0.01 | ASF | 0.32 ± 0.05 | 0.28 ± 0.12 |
| **11a** | 0.02 ± 0.02 | 0.23 ± 0.04 | **12a** | 0.01 ± 0.01 | 0.21 ± 0.05 |
| **11b** | 0.02 ± 0.03 | 0.20 ± 0.04 | **12b** | 0.01 ± 0.01 | 0.39 ± 0.09 |
| **11c** | 0.06 ± 0.02 | 0.49 ± 0.04 | **12c** | 0.01 ± 0.02 | 0.54 ± 0.08 |
| **11d** | 0.13 ± 0.04 | 0.57 ± 0.05 | **12d** | 0.02 ± 0.02 | 0.68 ± 0.05 |
| **11e** | 0.23 ± 0.06 | 0.62 ± 0.05 | **12e** | 0.05 ± 0.03 | 0.74 ± 0.07 |
| **11f** | 0.31 ± 0.07 | 0.68 ± 0.04 | **12f** | 0.13 ± 0.05 | 0.85 ± 0.05 |
| **11g** | 0.34 ± 0.07 | 0.75 ± 0.06 | **12g** | 0.14 ± 0.06 | 0.95 ± 0.04 |
| **11h** | 0.34 ± 0.08 | 0.79 ± 0.05 | **12h** | 0.11 ± 0.06 | 0.90 ± 0.05 |
| **11i** | 0.40 ± 0.08 | 0.89 ± 0.05 | **12i** | 0.15 ± 0.06 | 0.95 ± 0.05 |

**Table S2.** $K_d$ values of galectin-3 bound to neo-glycoproteins **11a**–**i** and **12a**–**i**. $K_d$ in [µM] galectin-3 in ELISA-type binding assay to immobilized neo-glycoproteins (5 pmol) and respective standard deviations of at least 11 measured data are shown. Values were calculated by data fitting using equation for one site saturation ($y = \frac{B_{max} \cdot x}{K_d + x}$). Binding affinity of galectin-3 increases with increasing modification densities of neo-glycoproteins, more pronounced for LacDiNAc-LacNAc conjugated BSA (**12a**–**i**).

| Compound | $K_d$ [µM] | Compound | $K_d$ [µM] |
|---|---|---|---|
| **11a** | 0.86 ± 0.20 | **12a** | 0.33 ± 0.11 |
| **11b** | 0.97 ± 0.18 | **12b** | 0.46 ± 0.13 |
| **11c** | 0.69 ± 0.17 | **12c** | 0.45 ± 0.09 |
| **11d** | 0.63 ± 0.13 | **12d** | 0.23 ± 0.06 |
| **11e** | 0.52 ± 0.11 | **12e** | 0.08 ± 0.02 |
| **11f** | 0.30 ± 0.06 | **12f** | 0.03 ± 0.00 |
| **11g** | 0.21 ± 0.06 | **12g** | 0.04 ± 0.01 |
| **11h** | 0.18 ± 0.05 | **12h** | 0.03 ± 0.00 |
| **11i** | 0.11 ± 0.03 | **12i** | 0.03 ± 0.00 |

**Table S3.** Binding signals of galectin-3 per glycan of immobilized neo-glycoproteins **11a**–**i** (**a**) and **12a**–**i** (**b**) in relation to **11d** and **12d**, respectively. Binding signals at different galectin-3 concentrations are related to one binding site of the neo-glycoproteins and the relative potencies to **11d** or **12d** are given with standard deviations. For galectin-3 concentrations below 0.05 µM, binding to **12a**–**c** was not detectable (n.d.), setting binding signal per ligand for **12d** (and **11d**) to 1.00.

(a)

| Galectin-3 [µM] | Relative binding signal per glycan | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **11a** | **11b** | **11c** | **11d** | **11e** | **11f** | **11g** | **11h** | **11i** |
| 10 | 2.76 ± 0.35 | 1.13 ± 0.13 | 1.37 ± 0.11 | 1.00 ± 0.08 | 0.73 ± 0.04 | 0.57 ± 0.03 | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.43 ± 0.02 |
| 5 | 2.80 ± 0.39 | 1.15 ± 0.09 | 1.38 ± 0.10 | 1.00 ± 0.08 | 0.74 ± 0.04 | 0.56 ± 0.03 | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.42 ± 0.02 |
| 1 | 2.53 ± 0.42 | 0.95 ± 0.18 | 1.41 ± 0.12 | 1.00 ± 0.10 | 0.76 ± 0.06 | 0.62 ± 0.04 | 0.55 ± 0.04 | 0.53 ± 0.03 | 0.54 ± 0.03 |
| 0.5 | 2.34 ± 0.39 | 0.88 ± 0.16 | 1.27 ± 0.19 | 1.00 ± 0.12 | 0.82 ± 0.08 | 0.77 ± 0.05 | 0.72 ± 0.05 | 0.73 ± 0.05 | 0.72 ± 0.04 |
| 0.25 | 1.60 ± 0.70 | 0.67 ± 0.25 | 1.10 ± 0.47 | 1.00 ± 0.31 | 0.96 ± 0.17 | 1.27 ± 0.09 | 1.42 ± 0.11 | 1.44 ± 0.11 | 1.56 ± 0.07 |
| 0.1 | 1.62 ± 0.96 | 0.94 ± 0.42 | 1.03 ± 0.62 | 1.00 ± 0.58 | 0.83 ± 0.36 | 1.32 ± 0.59 | 1.53 ± 0.98 | 2.26 ± 1.24 | 3.70 ± 0.64 |
| 0.05 | 3.58 ± 2.87 | 1.70 ± 1.78 | 0.94 ± 1.49 | 1.00 ± 0.97 | 0.76 ± 0.46 | 1.48 ± 0.72 | 1.43 ± 0.70 | 1.09 ± 0.81 | 3.98 ± 2.86 |

(b)

| Galectin-3 [µM] | Relative binding signal per glycan | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **12a** | **12b** | **12c** | **12d** | **12e** | **12f** | **12g** | **12h** | **12i** |
| 10 | 1.35 ± 0.21 | 2.14 ± 0.28 | 1.18 ± 0.06 | 1.00 ± 0.06 | 0.68 ± 0.04 | 0.48 ± 0.02 | 0.45 ± 0.01 | 0.44 ± 0.02 | 0.40 ± 0.01 |
| 5 | 1.41 ± 0.26 | 2.18 ± 0.37 | 1.16 ± 0.10 | 1.00 ± 0.06 | 0.66 ± 0.04 | 0.47 ± 0.03 | 0.45 ± 0.02 | 0.42 ± 0.02 | 0.40 ± 0.01 |
| 1 | 1.62 ± 0.36 | 2.27 ± 0.52 | 1.18 ± 0.10 | 1.00 ± 0.07 | 0.68 ± 0.07 | 0.52 ± 0.03 | 0.51 ± 0.02 | 0.47 ± 0.03 | 0.45 ± 0.02 |
| 0.5 | 1.33 ± 0.40 | 1.62 ± 0.49 | 0.86 ± 0.10 | 1.00 ± 0.11 | 0.70 ± 0.09 | 0.55 ± 0.03 | 0.52 ± 0.02 | 0.48 ± 0.02 | 0.47 ± 0.01 |
| 0.25 | 0.75 ± 0.33 | 0.99 ± 0.36 | 0.59 ± 0.08 | 1.00 ± 0.15 | 0.86 ± 0.09 | 0.65 ± 0.04 | 0.63 ± 0.02 | 0.58 ± 0.04 | 0.56 ± 0.02 |
| 0.1 | 1.09 ± 0.62 | 1.05 ± 0.69 | 0.75 ± 0.23 | 1.00 ± 0.48 | 2.34 ± 0.25 | 2.07 ± 0.15 | 1.89 ± 0.15 | 1.76 ± 0.11 | 1.67 ± 0.09 |
| 0.05 | 0.77 ± 0.75 | 0.20 ± 0.93 | 0.35 ± 0.18 | 1.00 ± 0.58 | 3.26 ± 0.92 | 4.92 ± 0.85 | 3.96 ± 0.61 | 4.89 ± 0.55 | 4.14 ± 0.32 |
| 0.025 | n.d. | n.d. | n.d. | 1.00 ± 0.70 | 2.24 ± 0.90 | 6.16 ± 0.43 | 4.43 ± 1.48 | 6.50 ± 0.74 | 6.21 ± 2.31 |
| 0.01 | n.d. | n.d. | n.d. | 1.00 ± 5.58 | 10.01 ± 14.78 | 58.74 ± 15.82 | 42.18 ± 20.69 | 46.97 ± 15.05 | 66.87 ± 18.31 |
| 0.005 | n.d. | n.d. | n.d. | 1.00 ± 4.58 | 24.34 ± 13.11 | 115.27 ± 8.94 | 63.84 ± 7.09 | 109.12 ± 15.02 | 104.96 ± 11.61 |