viruses

MDPI

*Communication – Supplementary material*

# Bacteriophage Sf6 tailspike protein for detection of *Shigella flexneri* pathogens

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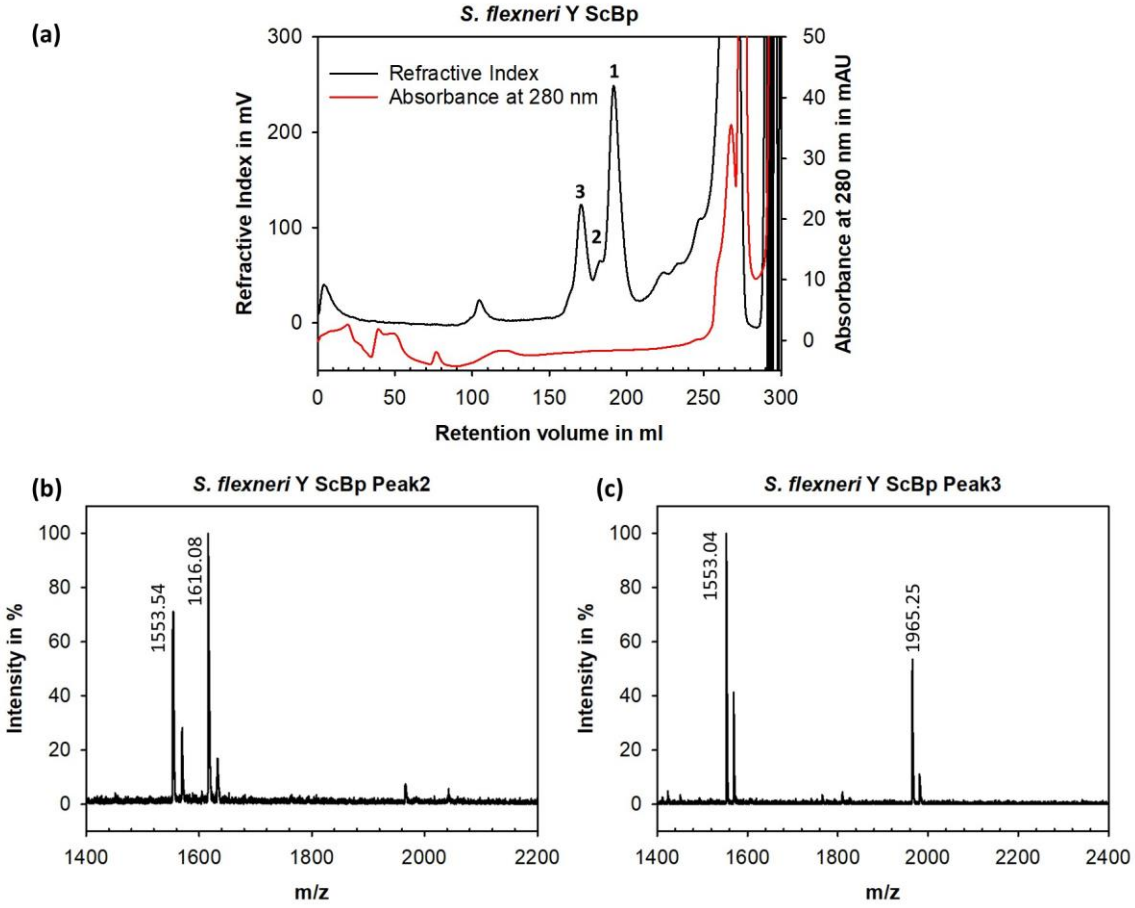


**Figure S1.** Chromatogram of digested SfY ScBp and MS data**. (a)** Chromatogram of digested *S. flexneri* polysaccharide Y ScBp with refractive index (black line) and absorption at 280 nm (red line). Numbers indicate the isolated peaks. **(b-c)** Mass spectrograms of the peak 2 and 3 from *S. flexneri* Y ScBp. Masses for non-acetylated peaks as sodium salt are indicated. Further peaks are listed in Table S1.

**Table S1.** Annotated masses from MALDI-TOF MS from digested SfY ScBp polysaccharide

| Oligosaccharide (Oligosac) | Molecular formula | Calculated mass | Annotated mass | Intensity %Peak1 | Intensity %Peak2 | Intensity %Peak3 |
|---|---|---|---|---|---|---|
| | C52O35H88N2 | 1300.52 | - | - | - | - |
| **Octasac (8)** | +Na+ | 1323.51 | 1323.79 | 100 | | |
| | +K+ | 1339.48 | - | - | | |
| **Unidentified** | | | 1553.05 | - | 66.0 | 100 |
| | | | 1569.14 | | 26.4 | 40.6 |
| **Decasac (10) (+2Rha)** | C64O43H108N2 | 1593.64 | - | - | - | - |
| | +Na+ | 1615.62 | 1616.08 | | 100 | |
| | +K+ | 1631.60 | 1631.99 | | 15.0 | |
| | C78O52H131N3 | 1943.87 | - | - | - | - |
| **Dodecasac (12)** | +Na+ | 1965.86 | 1965.25 | | | 50.2 |
| | +K+ | 1981.96 | 1981.22 | | | 10.8 |

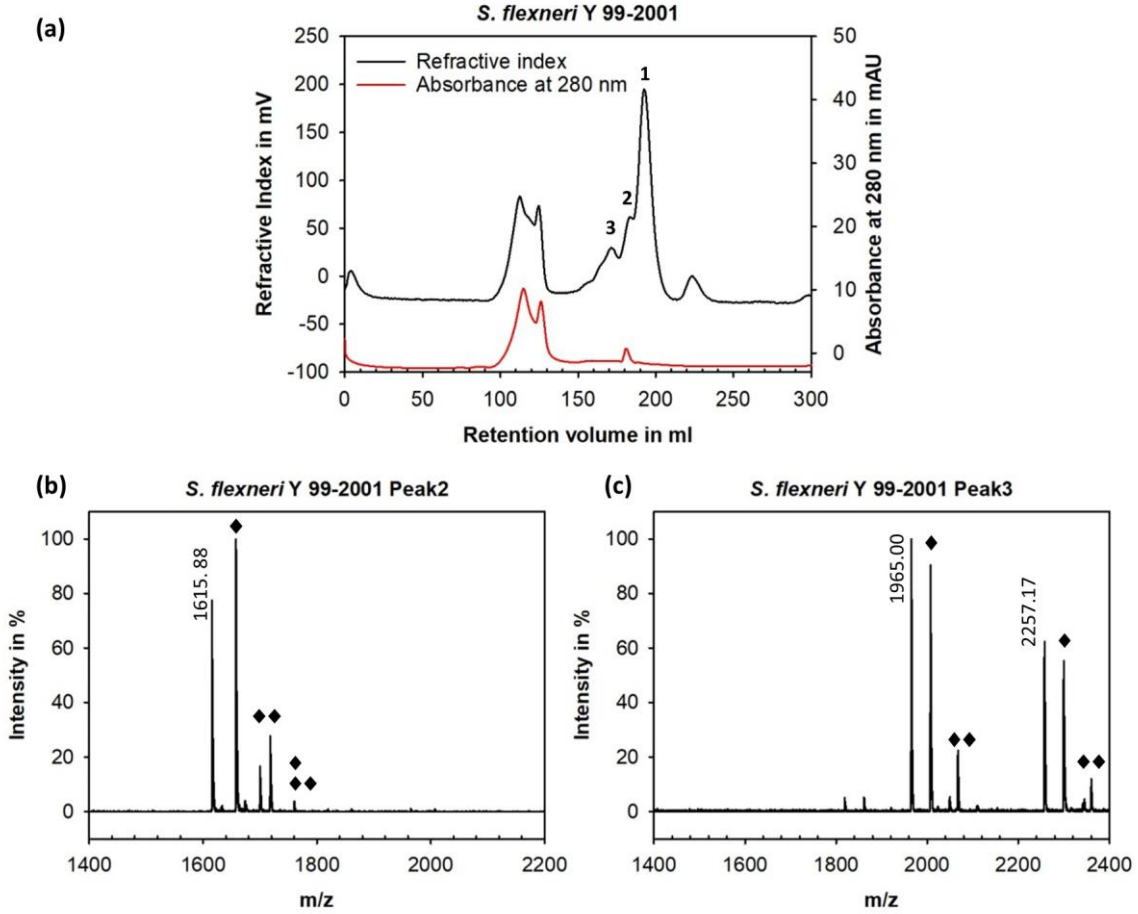


**Figure S2.** Chromatogram of digested SfY 99-2001 polysaccharide and MS data **(a)** Chromatogram of digested *S. flexneri* polysaccharide Y 99-2001 with refractive index (black line) and absorption at 280 nm (red line). Numbers indicate the isolated peaks. **(b-c)** Mass spectrograms of the peak 2 and 3 from *S. flexneri* Y 99-2001. Masses for non-acetylated peaks as sodium salt are indicated. Further peaks are listed in Table S2. Number of diamonds indicates the number of acetylations.

**Table S2.** Annotated masses from MALDI-TOF MS from digested SfY 99-2001 polysaccharide

| Oligosaccharide (Oligosac) | Molecular formula | Calculated mass | Annotated mass | Intensity %Peak1 | Intensity %Peak2 | Intensity %Peak3 |
|---|---|---|---|---|---|---|
| **Octasac (8)** | C52O35H88N2 | 1300.52 | - | - | - | - |
| | +Na⁺ | 1323.51 | 1323.70 | 100 | | |
| | +K⁺ | 1339.48 | 1339.68 | 14.5 | | |
| **Octasac -1Ac** | C54O36H90N2 | 1343.53 | - | - | - | - |
| | +Na⁺ | 1365.52 | 1365.70 | 14.4 | | |
| | +K⁺ | 1381.49 | - | - | | |
| **Decasac (10) (+2Rha)** | C64O43H108N2 | 1593.64 | - | - | - | - |
| | +Na⁺ | 1615.62 | 1615.83 | 8.41 | 77.8 | |
| | +K⁺ | 1631.60 | - | - | - | |
| **Decasac -1Ac** | C66O44H110N2 | 1635.65 | - | - | - | - |
| | +Na⁺ | 1657.63 | 1657.93 | | 100 | |
| | +K⁺ | 1673.61 | - | | - | |
| **Decasac -2Ac** | C68O45H112N2 | 1678.61 | - | - | - | - |
| | +Na⁺ | 1700.60 | 1699.90 | | 16.4 | |
| | +K⁺ | 1716.70 | 1717.93 | | 27.5 | |
| **Dodecasac (12)** | C78O52H131N3 | 1943.87 | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | +Na+ | 1965.86 | 1965.00 | | | 100 |
| | +K+ | 1981.96 | - | | | - |
| **Dodecasac -1Ac** | C80O53H133N3 | 1985.91 | - | - | - | - |
| | +Na+ | 2007.89 | 2007.07 | | | 91.44 |
| | +K+ | 2024.00 | - | | | - |
| **Dodecasac -2Ac** | C82O54H135N3 | 2027.95 | - | - | - | - |
| | +Na+ | 2049.93 | - | | | - |
| | +K+ | 2066.04 | 2067.08 | | | 20.9 |
| **Tetrakaidecasac (14) (+2 Rha)** | C90O60H151N3 | 2236.16 | - | - | - | - |
| | +Na+ | 2258.14 | 2257.17 | | | 56.9 |
| | +K+ | 2274.25 | - | | | - |
| **Tetrakaidecasac -1Ac** | C92O61H153N3 | 2278.19 | - | - | - | - |
| | +Na+ | 2300.18 | 2299.18 | | | 48.0 |
| | +K+ | 2316.28 | - | | | - |
| **Tetrakaidecasac -2Ac** | C94O62H155N3 | 2320.23 | - | - | - | - |
| | +Na+ | 2342.21 | - | | | - |
| | +K+ | 2358.32 | 2359.20 | | | 9.17 |

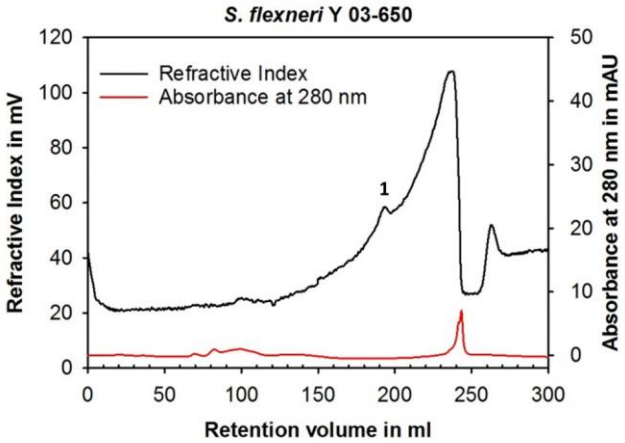


**Figure S3.** Chromatogram of digested *S. flexneri* polysaccharide Y 03-650. Refractive index (black line) and absorption at 280 nm (red line). Number indicates the isolated peak.

**Table S3.** Annotated masses from MALDI-TOF MS from digested SfY 03-650 polysaccharide

| Oligosaccharide (Oligosac) | Molecular formula | Calculated mass | Annotated mass | Intensity% Peak1 |
|---|---|---|---|---|
| **Heptasac (7) (2RU -1Rha)** | C46O31H78N2 | 1156.12 | - | - |
| | +Na+ | 1178.10 | 1177.69 | 8.50 |
| | +K+ | 1194.21 | - | - |
| **Heptasac -1Ac** | C48O32H80N2 | 1198.15 | - | - |
| | +Na+ | 1220.13 | 1219.51 | 10.3 |
| | +K+ | 1236.24 | - | - |
| **Octasac (8) (2RU)** | C52O35H88N2 | 1300.52 | - | - |
| | +Na+ | 1323.51 | 1323.60 | 100 |
| | +K+ | 1339.48 | 1339.96 | 28.6 |
| **Octasac -1Ac** | C54O36H90N2 | 1343.53 | - | - |
| | +Na+ | 1365.52 | 1365.96 | 60.5 |
| | +K+ | 1381.49 | 1382.26 | 21.2 |
| **Octasac -2Ac** | C56O37H92N2 | 1386.33 | - | - |
| | +Na+ | 1408.31 | 1408.09 | 32.4 |
| | +K+ | 1424.42 | 1424.96 | 6.62 |

| | | | | |
|---|---|---|---|---|
| **Nonasac (2RU +1Rha)** | C58O39H98N2 | 1448.40 | - | - |
| | +Na⁺ | 1470.38 | 1469.98 | 5.29 |
| | +K⁺ | 1486.49 | - | - |
| **Decasac (10) (2RU +2Rha)** | C64O43H108N2 | 1593.64 | - | - |
| | +Na⁺ | 1615.62 | 1616.36 | 27.2 |
| | +K⁺ | 1631.60 | 1632.31 | 5.89 |
| **Decasac -1Ac** | C66O44H110N2 | 1635.65 | - | - |
| | +Na⁺ | 1657.63 | 1658.41 | 11.0 |
| | +K⁺ | 1673.61 | - | - |
| **Undefined** | | | 1691.47 | 26.4 |

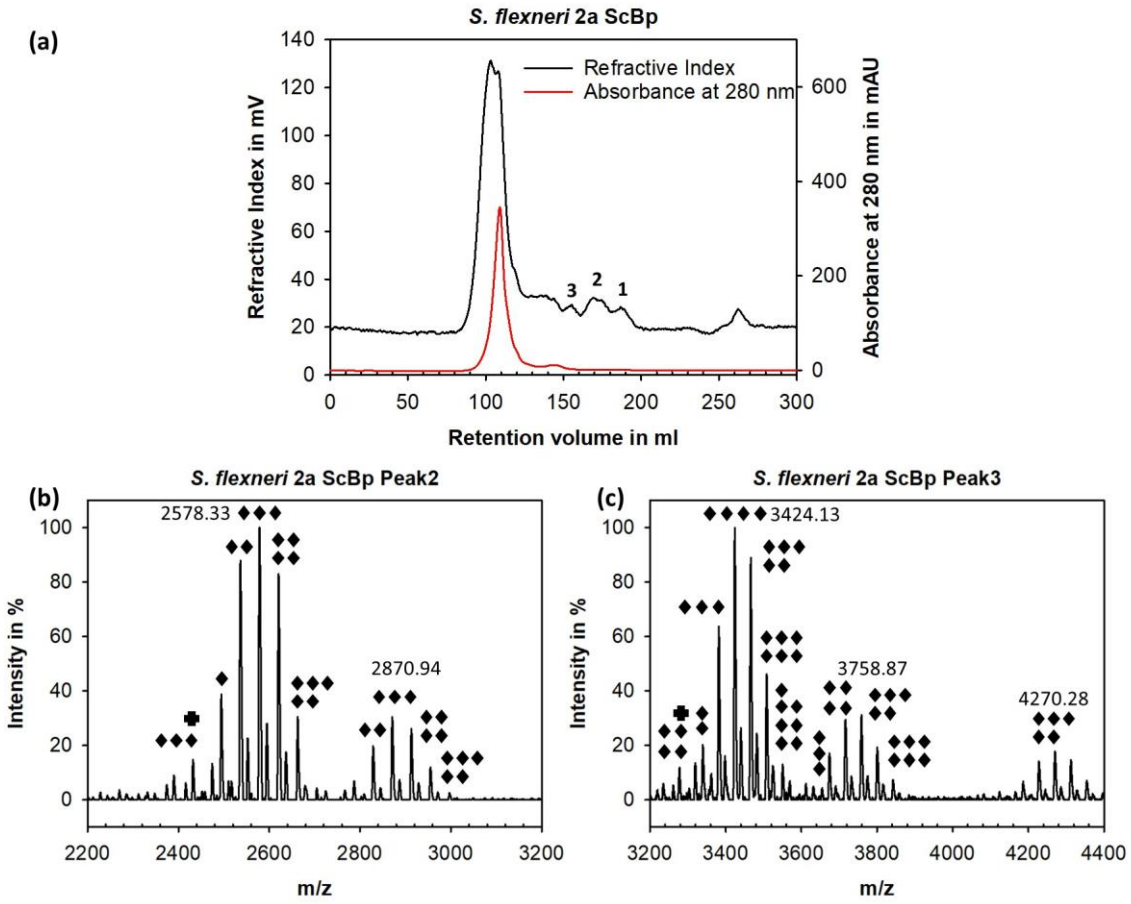


**Figure S4.** Chromatogram of digested Sf2a ScBp and MS data. **(a)** Chromatogram of digested *S. flexneri* polysaccharide 2a ScBp with refractive index (black line) and absorption at 280 nm (red line). Numbers indicate the isolated peaks. **(b-c)** Mass spectrograms of the peak 2 and 3 from *S. flexneri* 2a ScBp. Masses for the most populated acetylated peak as sodium salt are indicated. Further peaks are listed in Table S 4. Number of diamonds indicates the number of acetylation. Plus = Deglucosylated.

62 **Table S4.** Annotated masses from MALDI-TOF MS from digested Sf2a ScBp polysaccharide

| Oligosaccharide (Oligosac) | Molecular formula | Mass (calc.) | Mass (exp.) | Intensity% Peak1 | Intensity% Peak2 | Intensity% Peak3 |
|---|---|---|---|---|---|---|
| **Decasac (10) (2RU)** | $C_{64}O_{45}H_{108}N_2$ | 1626.54 | - | - | - | - |
| | $+Na^+$ | 1648.52 | 1648.03 | 34.8 | | |
| | $+K^+$ | 1664.63 | 1664.17 | 7.17 | | |
| **Decasac -1Ac** | $C_{66}O_{46}H_{110}N_2$ | 1668.58 | - | - | - | - |
| | $+Na^+$ | 1690.57 | 1689.68 | 92.6 | | |
| | $+K^+$ | 1706.67 | 1706.14 | 23.0 | | |
| **Decasac -2Ac** | $C_{68}O_{47}H_{112}N_2$ | 1710.61 | - | - | - | |
| | $+Na^+$ | 1732.59 | 1731.69 | 100 | | |
| | $+K^+$ | 1748.70 | 1748.40 | 25.3 | | |
| **Decasac -3Ac** | $C_{70}O_{48}H_{114}N_2$ | 1752.65 | - | - | - | |
| | $+Na^+$ | 1774.63 | 1774.30 | 53.8 | 5.12 | |
| | $+K^+$ | 1790.74 | 1790.14 | 11.2 | - | |
| **Decasac -4Ac** | $C_{72}O_{49}H_{116}N_2$ | 1794.69 | - | - | - | |
| | $+Na^+$ | 1816.67 | 1816.63 | 10.3 | | |
| | $+K^+$ | 1832.78 | - | - | | |
| **Dodecasac (12) -1Ac (2RU +2Rha)** | $C_{78}O_{54}H_{130}N_2$ | 1960.86 | - | - | - | |
| | $+Na^+$ | 1982.84 | 1982.52 | 20.9 | | |
| | $+K^+$ | 1998.95 | 1998.51 | 5.17 | | |
| **Dodecasac -2Ac** | $C_{80}O_{55}H_{132}N_2$ | 2002.90 | - | - | - | |
| | $+Na^+$ | 2024.88 | 2024.52 | 31.0 | 13.4 | |
| | $+K^+$ | 2040.99 | 2040.42 | 7.99 | - | |
| **Dodecasac -3Ac** | $C_{82}O_{56}H_{134}N_2$ | 2044.93 | - | - | - | |
| | $+Na^+$ | 2066.91 | 2066.62 | 18.4 | 21.1 | |
| | $+K^+$ | 2083.02 | - | - | 6.09 | |
| **Dodecasac -4Ac** | $C_{84}O_{57}H_{136}N_2$ | 2086.97 | - | - | - | |
| | $+Na^+$ | 2108.95 | 2108.72 | | 12.52 | |
| | $+K^+$ | 2125.06 | - | | - | |
| **Tetrakaidecasac (14)-2Ac (3RU -1Glc)** | $C_{94}O_{64}H_{155}N_3$ | 2352.23 | - | - | - | - |
| | $+Na^+$ | 2374.21 | 2374.43 | | 5.55 | |
| | $+K^+$ | 2390.32 | 2390.15 | | 9.01 | |
| **Tetrakaidecasac -3Ac** | $C_{96}O_{65}H_{157}N_3$ | 2394.27 | - | | - | |
| | $+Na^+$ | 2416.25 | 2416.14 | | 6.24 | |
| | $+K^+$ | 2432.36 | 2431.86 | | 14.8 | |
| **Tetrakaidecasac -4Ac** | $C_{98}O_{66}H_{159}N_3$ | 2436.30 | - | - | - | |
| | $+Na^+$ | 2458.28 | - | | - | |
| | $+K^+$ | 2474.39 | 2474.30 | | 13.3 | |
| **Tetrakaidecasac -5Ac** | $C_{100}O_{67}H_{161}N_3$ | 2478.34 | - | - | - | |
| | $+Na^+$ | 2500.32 | - | | - | |
| | $+K^+$ | 2516.43 | 2516.83 | | 6.78 | |
| **Pentakaidecasac (15)-1Ac (3RU)** | $C_{98}O_{68}H_{163}N_3$ | 2472.33 | - | - | - | - |
| | $+Na^+$ | 2494.32 | 2494.61 | | 38.7 | |
| | $+K^+$ | 2510.42 | 2510.48 | | 6.84 | |
| **Pentakaidecasac -2Ac** | $C_{100}O_{69}H_{165}N_3$ | 2514.37 | - | - | - | - |
| | $+Na^+$ | 2536.35 | 2536.41 | | 87.9 | |
| | $+K^+$ | 2552.46 | 2552.28 | | 22.6 | |
| **Pentakaidecasac -3Ac** | $C_{102}O_{70}H_{167}N_3$ | 2556.41 | - | - | - | - |
| | $+Na^+$ | 2578.39 | 2578.33 | | 100 | |
| | $+K^+$ | 2594.50 | 2594.47 | | 28.0 | |
| **Pentakaidecasac** | $C_{104}O_{71}H_{169}N_3$ | 2598.44 | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| **-4Ac** | +Na+ | 2620.43 | 2620.45 | | 83.0 | |
| | +K+ | 2636.53 | 2636.72 | | 17.6 | |
| **Pentakaidecasac -5Ac** | C106O72H171N3 | 2640.48 | - | - | - | |
| | +Na+ | 2662.46 | 2662.62 | | 30.4 | |
| | +K+ | 2678.57 | 2678.68 | | 5.20 | |
| **Heptakaidecasac (17)-1Ac (3RU +2Rha)** | C110O76H183N3 | 2764.62 | - | - | - | |
| | +Na+ | 2786.60 | 2786.65 | | 6.79 | |
| | +K+ | 2802.71 | - | | - | |
| **Heptakaidecasac -2Ac** | C112O77H185N3 | 2806.65 | - | - | - | |
| | +Na+ | 2828.64 | 2829.04 | | 19.76 | |
| | +K+ | 2844.74 | | | - | |
| **Heptakaidecasac -3Ac** | C114O78H187N3 | 2848.69 | - | - | - | |
| | +Na+ | 2870.67 | 2870.94 | | 30.5 | |
| | +K+ | 2886.78 | 2887.27 | | 7.43 | |
| **Heptakaidecasac -4Ac** | C116O79H189N3 | 2890.73 | - | - | - | |
| | +Na+ | 2912.71 | 2913.11 | | 26.1 | |
| | +K+ | 2928.82 | 2928.99 | | 6.12 | |
| **Heptakaidecasac -5Ac** | C118O80H191N3 | 2932.76 | - | - | - | - |
| | +Na+ | 2954.75 | 2955.17 | | 11.9 | 5.06 |
| | +K+ | 2970.85 | - | | - | - |
| **Nonakaidecasac (19)-4Ac (4RU -1Glc)** | C130O88H212N4 | 3240.06 | - | - | - | - |
| | +Na+ | 3262.04 | - | | | - |
| | +K+ | 3278.15 | 3278.14 | | | 11.6 |
| **Nonakaidecasac -5Ac** | C132O89H214N4 | 3282.10 | - | - | - | - |
| | +Na+ | 3304.08 | - | | | - |
| | +K+ | 3320.19 | 3319.52 | | | 13.4 |
| **Nonakaidecasac -6Ac** | C134O90H216N4 | 3324.13 | - | - | - | - |
| | +Na+ | 3346.12 | - | | | - |
| | +K+ | 3362.23 | 3362.46 | | | 9.44 |
| **Icosasac (20)-2Ac (4RU)** | C132O91H218N4 | 3318.13 | - | - | - | - |
| | +Na+ | 3340.11 | 3340.01 | | | 20.0 |
| | +K+ | 3356.22 | - | | | - |
| **Icosasac -3Ac** | C134O92H220N4 | 3360.16 | - | - | - | - |
| | +Na+ | 3382.15 | 3381.99 | | 9.10 | 63.7 |
| | +K+ | 3398.26 | 3398.18 | | - | 15.9 |
| **Icosasac -4Ac** | C136O93H222N4 | 3402.20 | - | - | - | - |
| | +Na+ | 3424.18 | 3424.13 | | 9.92 | 100 |
| | +K+ | 3440.29 | 3440.37 | | - | 26.3 |
| **Icosasac -5Ac** | C138O94H224N4 | 3444.24 | - | - | - | - |
| | +Na+ | 3466.22 | 3466.42 | | 6.16 | 89.0 |
| | +K+ | 3482.33 | 3482.32 | | - | 24.2 |
| **Icosasac -6Ac** | C140O95H226N4 | 3486.28 | - | - | - | - |
| | +Na+ | 3508.26 | 3508.20 | | | 46.1 |
| | +K+ | 3524.37 | 3524.63 | | | 12.3 |
| **Icosasac -7Ac** | C142O96H228N4 | 3528.31 | | - | - | - |
| | +Na+ | 3550.29 | 3551.06 | | | 12.8 |
| | +K+ | 3566.40 | - | | | - |
| **Doicosasac (22)-3Ac (4RU +2Rha)** | C146O100H240N4 | 3652.45 | - | - | - | - |
| | +Na+ | 3674.43 | 3674.44 | | | 16.9 |
| | +K+ | 3690.54 | - | | | - |
| **Doicosasac -4Ac** | C148O101H242N4 | 3694.48 | - | - | - | - |
| | +Na+ | 3716.47 | 3717.17 | | | 29.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | $+K^+$ | 3732.58 | 3732.42 | | | 8.44 |
| **Doicosasac -5Ac** | C150O102H244N4 | 3736.52 | - | - | - | - |
| | $+Na^+$ | 3758.50 | 3758.87 | | | 31.1 |
| | $+K^+$ | 3774.61 | 3775.25 | | | 8.62 |
| **Doicosasac -6Ac** | C152O103H246N4 | 3778.56 | - | - | - | - |
| | $+Na^+$ | 3800.54 | 3800.58 | | | 19.2 |
| | $+K^+$ | 3816.65 | 3816.12 | | | 5.65 |
| **Doicosasac -7Ac** | C154O104H248N4 | 3820.60 | - | - | - | - |
| | $+Na^+$ | 3842.58 | 3842.00 | | | 7.19 |
| | $+K^+$ | 3858.69 | - | | | - |
| **Pentaicosasac (25)-3Ac (5RU)** | C166O114H272N5 | 4162.92 | - | - | - | - |
| | $+Na^+$ | 4184.90 | 4186.37 | | | 7.19 |
| | $+K^+$ | 4201.01 | - | | | - |
| **Pentaicosasac -4Ac** | C168O115H274N5 | 4204.95 | - | - | - | - |
| | $+Na^+$ | 4226.93 | 4228.07 | | | 14.0 |
| | $+K^+$ | 4243.04 | - | | | - |
| **Pentaicosasac -5Ac** | C170O116H276N5 | 4246.99 | - | - | - | - |
| | $+Na^+$ | 4268.97 | 4270.28 | | | 17.8 |
| | $+K^+$ | 4285.08 | - | | | - |
| **Pentaicosasac -6Ac** | C172O117H278N5 | 4289.03 | - | - | - | - |
| | $+Na^+$ | 4311.01 | 4312.89 | | | 14.6 |
| | $+K^+$ | 4327.12 | - | | | - |
| **Pentaicosasac -7Ac** | C174O118H280N5 | 4331.06 | - | - | - | - |
| | $+Na^+$ | 4353.04 | 4354.66 | | | 7.00 |
| | $+K^+$ | 4369.15 | - | | | - |

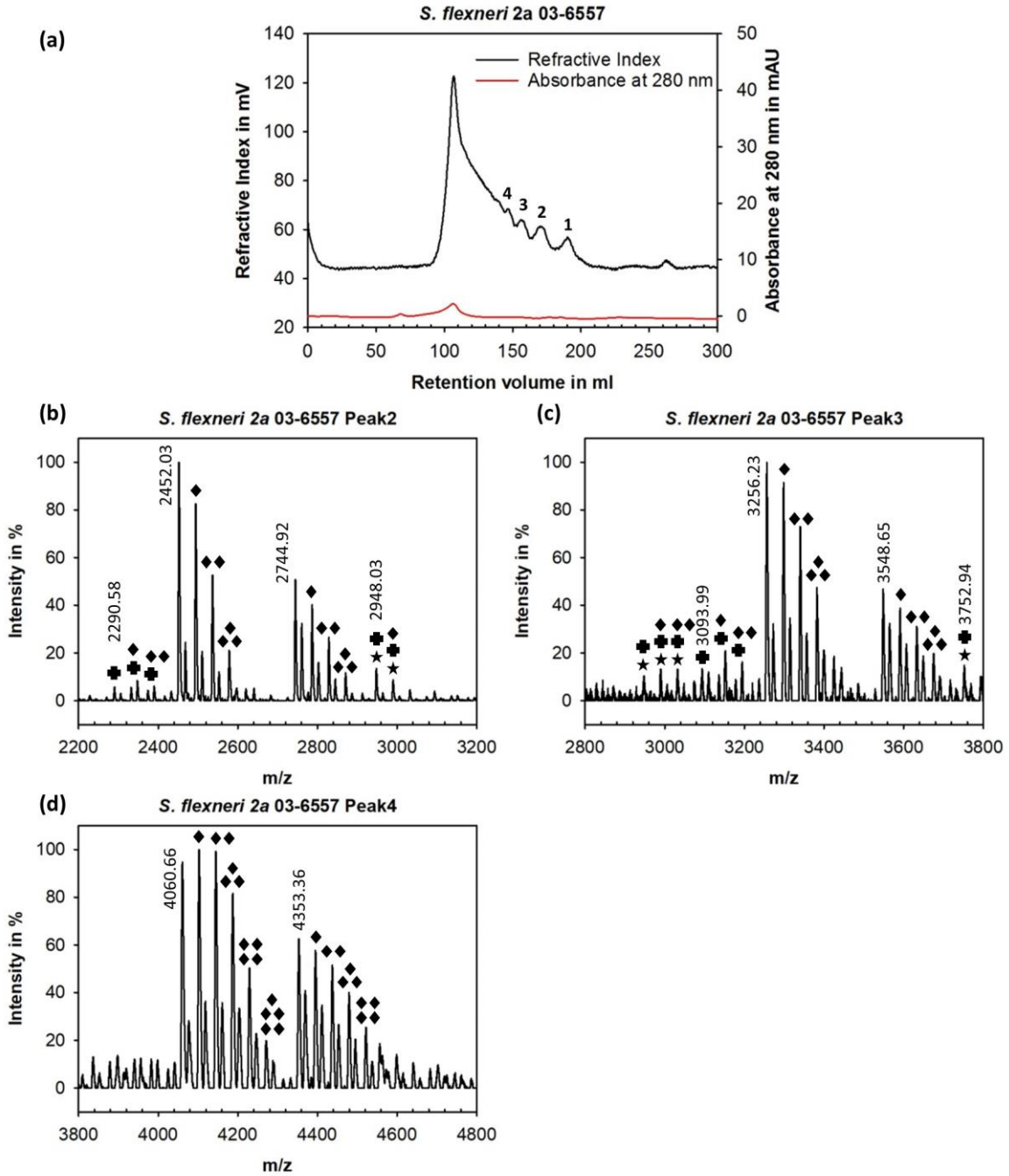


**Figure S5.** Chromatogram of digested Sf2a 03-6557 polysaccharide and MS data **(a)** Chromatogram with refractive index (black line) and absorption at 280 nm (red line). Numbers indicate the isolated peaks. **(b-d)** Mass spectrograms of *S. flexneri* 2a 03-6557. Masses for non-acetylated peaks as sodium salt are indicated. Further peaks are listed in Table S5. Diamond=Acetylation. Plus=Loss of Glc. Star=Loss of Rha.

70 **Table S5.** Annotated masses from MALDI-TOF MS from digested Sf2a 03-6557 polysaccharide

| Oligosaccharide (Oligosac) | Molecular formula | Mass (calc.) | Mass (exp.) | Int.% Peak1 | Int.% Peak2 | Int.% Peak3 | Int.% Peak4 |
|---|---|---|---|---|---|---|---|
| **Nonasac (9) (2RU- 1Glc)** | C58O40H98N2 | 1464.40 | - | - | - | - | - |
| | +Na+ | 1486.38 | 1486.29 | 11.9 | | | |
| | +K+ | 1502.49 | - | - | | | |
| **Nonasac -1Ac** | C60O41H100N2 | 1506.43 | - | - | - | - | - |
| | +Na+ | 1528.42 | 1528.30 | 10.4 | | | |
| | +K+ | 1544.53 | 1543.96 | 12.1 | | | |
| **Nonasac -2Ac** | C62O42H102N2 | 1548.47 | - | - | - | - | - |
| | +Na+ | 1570.45 | 1570.16 | 5.47 | | | |
| | +K+ | 1586.56 | 1586.18 | 6.56 | | | |
| **Decasac (10) (2RU)** | C64O45H108N2 | 1626.54 | - | - | - | - | - |
| | +Na+ | 1648.52 | 1647.91 | 100 | | | |
| | +K+ | 1664.63 | 1664.17 | 23.43 | | | |
| **Decasac -1Ac** | C66O46H110N2 | 1668.58 | - | - | - | - | - |
| | +Na+ | 1690.57 | 1690.37 | 71.5 | | | |
| | +K+ | 1706.67 | 1706.22 | 14.2 | | | |
| **Decasac -2Ac** | C68O47H112N2 | 1710.61 | - | - | - | - | - |
| | +Na+ | 1732.59 | 1732.51 | 32.8 | | | |
| | +K+ | 1748.70 | - | - | | | |
| **Decasac -3Ac** | C70O48H114N2 | 1752.65 | - | - | - | - | - |
| | +Na+ | 1774.63 | 1774.62 | 7.20 | | | |
| | +K+ | 1790.74 | - | - | | | |
| **Dodecasac (12) (2RU +2Rha)** | C76O53H128N2 | 1918.82 | - | - | - | - | - |
| | +Na+ | 1940.80 | 1940.64 | 38.59 | | | |
| | +K+ | 1956.91 | 1956.58 | 22.78 | | | |
| **Dodecasac -1Ac** | C78O54H130N2 | 1960.86 | - | - | - | - | - |
| | +Na+ | 1982.84 | 1982.56 | 27.1 | | | |
| | +K+ | 1998.95 | 1998.60 | 10.6 | | | |
| **Dodecasac -2Ac** | C80O55H132N2 | 2002.90 | - | - | - | - | - |
| | +Na+ | 2024.88 | 2024.73 | 13.8 | | | |
| | +K+ | 2040.99 | - | - | | | |
| **Trikaidecasac (13) (3RU -1Glc -Rha)** | C84O58H141N3 | 2122.01 | - | - | - | - | - |
| | +Na+ | 2144.00 | 2144.07 | 9.64 | | | |
| | +K+ | 2160.10 | - | - | | | |
| **Tetrakaidecasac (14) (3RU –Glc)** | C90O62H151N3 | 2268.16 | - | - | - | - | - |
| | +Na+ | 2290.14 | 2290.58 | | 5.70 | | |
| | +K+ | 2306.25 | - | | - | | |
| **Tetrakaidecasac -1Ac** | C92O63H153N3 | 2310.19 | - | - | - | - | - |
| | +Na+ | 2332.17 | 2332.36 | | 5.50 | | |
| | +K+ | 2348.28 | 2347.76 | | 8.32 | | |
| **Tetrakaidecasac -2Ac** | C94O64H155N3 | 2352.23 | - | - | - | - | - |
| | +Na+ | 2374.21 | - | | - | | |
| | +K+ | 2390.32 | 2390.10 | | 6.02 | | |
| **Pentakaidecasac (15) (3RU)** | C96O67H161N3 | 2430.30 | - | - | - | - | - |
| | +Na+ | 2452.28 | 2452.03 | 14.66 | 100 | | |
| | +K+ | 2468.39 | 2468.56 | - | 24.4 | | |
| **Pentakaidecasac -1Ac** | C98O68H163N3 | 2472.33 | - | - | - | - | - |
| | +Na+ | 2494.32 | 2494.18 | 6.99 | 82.6 | | |
| | +K+ | 2510.42 | 2510.71 | - | 20.5 | | |
| **Pentakaidecasac** | C100O69H165N3 | 2514.37 | - | - | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **-2Ac** | +Na+ | 2536.35 | 2536.65 | | 52.6 | | |
| | +K+ | 2552.46 | 2552.70 | | 12.0 | | |
| **Pentakaidecasac -3Ac** | C102O70H167N3 | 2556.41 | - | - | - | - | - |
| | +Na+ | 2578.39 | 2578.71 | | 21.0 | | |
| | +K+ | 2594.50 | - | | - | | |
| **Heptakaidecasac (17) (3RU +2Rha)** | C108O75H181N3 | 2722.58 | - | - | - | - | - |
| | +Na+ | 2744.56 | 2744.92 | | 50.9 | | |
| | +K+ | 2760.67 | 2760.69 | | 32.3 | | |
| **Heptakaidecasac -1Ac** | C110O76H183N3 | 2764.62 | - | - | - | - | - |
| | +Na+ | 2786.60 | 2786.70 | | 40.1 | | |
| | +K+ | 2802.71 | 2802.78 | | 16.0 | | |
| **Heptakaidecasac -2Ac** | C112O77H185N3 | 2806.65 | | - | - | - | - |
| | +Na+ | 2828.64 | 2829.04 | | 26.5 | | |
| | +K+ | 2844.74 | 2844.89 | | 9.00 | | |
| **Heptakaidecasac -3Ac** | C114O78H187N3 | 2848.69 | - | - | - | - | - |
| | +Na+ | 2870.67 | 2871.19 | | 11.7 | | |
| | +K+ | 2886.78 | - | | - | | |
| **Octakaidecasac (18) (4RU -1Glc -1Rha)** | C116O80H194N4 | 2925.77 | - | - | - | - | - |
| | +Na+ | 2947.75 | 2948.03 | | 13.4 | | |
| | +K+ | 2963.86 | - | | - | | |
| **Octakaidecasac -1Ac** | C118O81H196N4 | 2967.81 | - | - | - | - | - |
| | +Na+ | 2989.79 | 2989.63 | | 8.69 | 13.2 | |
| | +K+ | 3005.90 | - | | - | - | |
| **Octakaidecasac -2Ac** | C120O82H198N4 | 3009.85 | - | - | - | - | - |
| | +Na+ | 3031.83 | 3032.24 | | | 13.1 | |
| | +K+ | 3047.94 | - | | | - | |
| **Nonakaidecasac (19) (4RU -1Glc)** | C122O84H204N4 | 3071.91 | - | - | - | - | - |
| | +Na+ | 3093.90 | 3093.99 | | | 13.4 | |
| | +K+ | 3110.00 | 3109.73 | | | 12.0 | |
| **Nonakaidecasac -1Ac** | C124O85H206N4 | 3113.95 | - | - | - | - | - |
| | +Na+ | 3135.93 | 3136.45 | | | 10.9 | |
| | +K+ | 3152.04 | 3152.25 | | | 21.1 | |
| **Nonakaidecasac -2Ac** | C126O86H208N4 | 3155.99 | - | - | - | - | - |
| | +Na+ | 3177.97 | - | | | - | |
| | +K+ | 3194.08 | 3194.47 | | | 16.2 | |
| **Icosasac (20) (4RU)** | C128O89H214N4 | 3234.05 | - | - | - | - | - |
| | +Na+ | 3256.04 | 3256.23 | | 27.8 | 100 | |
| | +K+ | 3273.14 | 3272.49 | | - | 31.7 | |
| **Icosasac -1Ac** | C130O90H216N4 | 3276.09 | - | - | - | - | - |
| | +Na+ | 3298.07 | 3298.88 | | 16.1 | 91.3 | |
| | +K+ | 3314.18 | 3314.86 | | - | 34.4 | |
| **Icosasac -2Ac** | C132O91H218N4 | 3318.13 | - | - | - | - | - |
| | +Na+ | 3340.11 | 3340.39 | | 8.72 | 73.1 | |
| | +K+ | 3356.22 | 3356.58 | | - | 28.3 | |
| **Icosasac -3Ac** | C134O92H220N4 | 3360.16 | - | - | - | - | - |
| | +Na+ | 3382.15 | 3382.65 | | | 47.59 | |
| | +K+ | 3398.26 | - | | | - | |
| **Doicosasac (22) (4RU +2Rha)** | C140O97H234N4 | 3526.34 | - | - | - | - | - |
| | +Na+ | 3548.32 | 3548.65 | | | 46.8 | |
| | +K+ | 3564.43 | 3565.24 | | | 32.1 | |
| **Doicosasac -1Ac** | C142O98H236N4 | 3568.37 | - | - | - | - | - |
| | +Na+ | 3590.36 | 3591.32 | | | 38.5 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | $+K^+$ | 3606.46 | 3607.32 | | | 23.5 | |
| **Doicosasac -2Ac** | C144O99H238N4 | 3610.41 | - | - | - | - | - |
| | $+Na^+$ | 3632.39 | 3632.87 | | | 31.0 | |
| | $+K^+$ | 3648.50 | 3649.43 | | | 18.7 | |
| **Doicosasac -3Ac** | C146O100H240N4 | 3652.45 | - | - | - | - | - |
| | $+Na^+$ | 3674.43 | 3675.53 | | | 19.8 | |
| | $+K^+$ | 3690.54 | 3690.64 | | | 10.3 | |
| **Triicosasac (23) (5RU -1Glc -1Rha)** | C148O102H247N5 | 3729.53 | - | - | - | - | - |
| | $+Na^+$ | 3751.51 | 3752.94 | | | 14.7 | |
| | $+K^+$ | 3767.62 | | | | - | |
| **Triicosasac -1Ac** | C150O103H249N5 | 3771.57 | - | - | - | - | - |
| | $+Na^+$ | 3793.55 | 3795.31 | | | 10.5 | |
| | $+K^+$ | 3809.66 | - | | | - | |
| **Pentaicosasac (25) (5RU)** | C160O111H267N5 | 4037.81 | - | - | - | - | - |
| | $+Na^+$ | 4059.79 | 4060.66 | | | 38.2 | 97.2 |
| | $+K^+$ | 4075.90 | 4077.26 | | | 14.3 | 24.5 |
| **Pentaicosasac -1Ac** | C162O112H268N5 | 4078.84 | - | - | - | - | - |
| | $+Na^+$ | 4100.82 | 4102.69 | | | 27.7 | 100 |
| | $+K^+$ | 4116.93 | 4118.29 | | | 11.0 | 32.0 |
| **Pentaicosasac -2Ac** | C164O113H270N5 | 4120.88 | - | - | - | - | - |
| | $+Na^+$ | 4142.86 | 4144.81 | | | 19.0 | 99.8 |
| | $+K^+$ | 4158.97 | 4160.87 | | | - | 32.2 |
| **Pentaicosasac -3Ac** | C166O114H272N5 | 4162.92 | - | - | - | - | - |
| | $+Na^+$ | 4184.90 | 4186.98 | | | 10.7 | 82.1 |
| | $+K^+$ | 4201.01 | 4203.11 | | | - | 31.0 |
| **Pentaicosasac -4Ac** | C168O115H274N5 | 4204.95 | - | - | - | - | - |
| | $+Na^+$ | 4226.93 | 4229.35 | | | | 50.2 |
| | $+K^+$ | 4243.04 | 4246.30 | | | | 21.4 |
| **Pentaicosasac -5Ac** | C170O116H276N5 | 4246.99 | | - | - | - | - |
| | $+Na^+$ | 4268.97 | 4271.94 | | | | 19.1 |
| | $+K^+$ | 4285.08 | - | | | | - |
| **Heptaicosasac (27) (5RU +2Rha)** | C172O119H287N5 | 4330.10 | - | - | - | - | - |
| | $+Na^+$ | 4352.08 | 4353.36 | | | 9.47 | 64.4 |
| | $+K^+$ | 4368.19 | 4368.94 | | | 8.05 | 40.0 |
| **Hepaticosasac -1Ac** | C174O120H289N5 | 4372.13 | - | - | - | - | - |
| | $+Na^+$ | 4394.11 | 4395.51 | | | | 56.7 |
| | $+K^+$ | 4410.22 | 4411.36 | | | | 32.1 |
| **Heptaicosasac -2Ac** | C176O121H291N5 | 4414.17 | - | - | - | - | - |
| | $+Na^+$ | 4436.15 | 4437.68 | | | | 50.8 |
| | $+K^+$ | 4452.26 | 4453.10 | | | | 24.7 |
| **Heptaicosasac -3Ac** | C178O122H293N5 | 4456.21 | - | - | - | - | - |
| | $+Na^+$ | 4478.19 | 4479.61 | | | | 40.08 |
| | $+K^+$ | 4494.30 | 4495.54 | | | | 19.76 |
| **Heptaicosasac -4Ac** | C180O123H295N5 | 4498.24 | - | - | - | - | - |
| | $+Na^+$ | 4520.22 | 4522.24 | | | | 24.9 |
| | $+K^+$ | 4536.33 | 4537.49 | | | | 9.63 |
| **Triakonsac (30) (6RU)** | C192O133H320N6 | 4841.57 | - | - | - | - | - |
| | $+Na^+$ | 4863.55 | 4865.78 | | | | 44.1 |
| | $+K^+$ | 4879.66 | 4881.67 | | | | 13.6 |
| **Triakonsac -1Ac** | C194O134H322N6 | 4883.61 | - | - | - | - | - |
| | $+Na^+$ | 4905.59 | 4907.45 | | | | 32.0 |
| | $+K^+$ | 4921.70 | 4932.87 | | | | 9.78 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Triakonsac -2Ac** | C196O135H324N6 | 4925.64 | - | - | - | - | - |
| | +Na+ | 4947.63 | 4949.37 | | | | 24.3 |
| | +K+ | 4963.73 | 4964.93 | | | | 6.00 |
| **Triakonsac -3Ac** | C198O136H326N6 | 4967.68 | - | - | - | - | - |
| | +Na+ | 4989.66 | 4992.33 | | | | 16.5 |
| | +K+ | 5005.77 | - | | | | - |

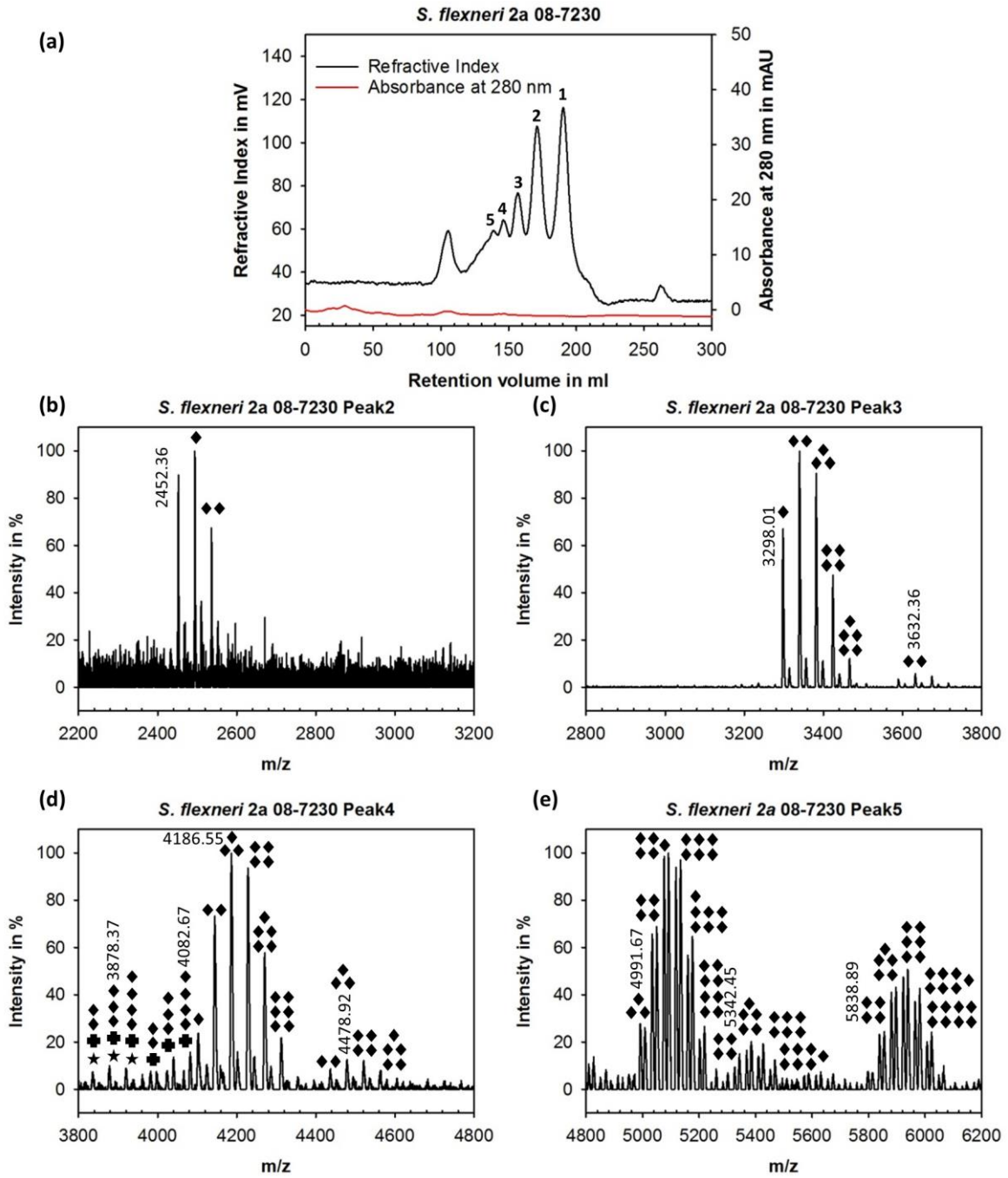


**Figure S6.** Chromatogram of Sf2a 08-7230 polysaccharide and MS data. **(a)** Chromatogram of digested *S. flexneri* polysaccharide 2a 08-7230 with refractive index (black line) and absorption at 280 nm (red line). Numbers indicate the isolated peaks. **(b-e)** Mass spectrograms of the peak 2-5 from *S. flexneri* 2a 08-7230. Masses for least acetylated oligosaccharide peaks as sodium salt are indicated. Further peaks are listed in Table S6. Number of diamonds indicates the number of acetylation. Plus = Loss of one glucose. Star = Loss of one rhamnose.

79 **Table S6.** Annotated masses from MALDI-TOF MS from digested Sf2a 08-7230 polysaccharide

| Oligosac | Molecular formula | Mass (calc.) | Mass (exp.) | Int.% Peak 1 | Int.% Peak 2 | Int.% Peak 3 | Int.% Peak 4 | Int.% Peak 5 |
|---|---|---|---|---|---|---|---|---|
| **Nonasac-1 Ac (9) (2RU-1Glc)** | C60O41H100N2 | 1506.43 | - | - | - | - | - | - |
| | +Na+ | 1528.42 | - | - | | | | |
| | +K+ | 1544.53 | 1544.47 | 10.0 | | | | |
| **Decasac (10) (2RU)** | C64O45H108N2 | 1626.54 | - | - | - | - | - | - |
| | +Na+ | 1648.52 | 1647.61 | 100 | | | | |
| | +K+ | 1664.63 | 1664.44 | 8.68 | | | | |
| **Decasac -1Ac** | C66O46H110N2 | 1668.58 | - | - | - | - | - | - |
| | +Na+ | 1690.57 | 1689.72 | 51.2 | | | | |
| | +K+ | 1706.67 | 1706.22 | 5.87 | | | | |
| **Decasac -2Ac** | C68O47H112N2 | 1710.61 | - | - | - | - | - | - |
| | +Na+ | 1732.59 | 1732.36 | 23.4 | | | | |
| | +K+ | 1748.70 | - | - | | | | |
| **Decasac -3Ac** | C70O48H114N2 | 1752.65 | - | - | - | - | - | - |
| | +Na+ | 1774.63 | 1775.41 | 7.09 | | | | |
| | +K+ | 1790.74 | - | - | | | | |
| **Pentakaide casac (15) (3RU)** | C96O67H161N3 | 2430.30 | - | - | - | - | - | - |
| | +Na+ | 2452.28 | 2452.36 | | 89.9 | | | |
| | +K+ | 2468.39 | 2469.59 | | 27.4 | | | |
| **Pentakaide casac -1Ac** | C98O68H163N3 | 2472.33 | - | - | - | - | - | - |
| | +Na+ | 2494.32 | 2493.62 | | 100 | | | |
| | +K+ | 2510.42 | 2510.62 | | 36.4 | | | |
| **Pentakaide casac -2Ac** | C100O69H165N3 | 2514.37 | - | - | - | - | - | - |
| | +Na+ | 2536.35 | 2536.46 | | 67.5 | | | |
| | +K+ | 2552.46 | 2552.70 | | 28.1 | | | |
| **Icosasac (20)-1Ac (4RU)** | C130O90H216N4 | 3276.09 | - | - | - | - | - | - |
| | +Na+ | 3298.07 | 3298.01 | | | 67.1 | | |
| | +K+ | 3314.18 | 3314.11 | | | 8.32 | | |
| **Icosasac -2Ac** | C132O91H218N4 | 3318.13 | - | - | - | - | - | - |
| | +Na+ | 3340.11 | 3340.17 | | | 100 | 11.6 | |
| | +K+ | 3356.22 | 3356.09 | | | 12.4 | - | |
| **Icosasac -3Ac** | C134O92H220N4 | 3360.16 | - | - | - | - | - | - |
| | +Na+ | 3382.15 | 3382.16 | | | 90.5 | 14.1 | |
| | +K+ | 3398.26 | 3398.84 | | | 11.3 | - | |
| **Icosasac -4Ac** | C136O93H222N4 | 3402.20 | - | - | - | - | - | - |
| | +Na+ | 3424.18 | 3424.19 | | | 47.6 | 14.6 | |
| | +K+ | 3440.29 | 3440.47 | | | 5.65 | - | |
| **Icosasac -5Ac** | C138O94H224N4 | 3444.24 | - | - | - | - | - | - |
| | +Na+ | 3466.22 | 3466.42 | | | 12.3 | 9.52 | |
| | +K+ | 3482.33 | - | | | - | - | |
| **Doicosasac (22)-2Ac 4RU+2Rha** | C144O99H238N4 | 3610.41 | - | - | - | - | - | - |
| | +Na+ | 3632.39 | 3632.36 | | | 5.87 | 8.67 | |
| | +K+ | 3648.50 | - | | | - | - | |
| **Doicosasac -3Ac** | C146O100H240N4 | 3652.45 | - | - | - | - | - | - |
| | +Na+ | 3674.43 | 3674.39 | | | | 11.8 | |
| | +K+ | 3690.54 | - | | | | - | |
| **Doicosasac -4Ac** | C148O101H242N4 | 3694.48 | - | - | - | - | - | - |
| | +Na+ | 3716.47 | 3716.60 | | | | 12.2 | |
| | +K+ | 3732.58 | - | | | | - | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Triicosasac (23)-2Ac 5RU-1Glc-1Rha** | C152O104H251N5 | 3813.60 | - | - | - | - | - | - |
| | +Na+ | 3835.59 | 3836.35 | | | | 7.48 | |
| | +K+ | 3851.69 | - | | | | - | |
| **Triicosasac -3Ac** | C154O105H253N5 | 3855.64 | - | - | - | - | - | - |
| | +Na+ | 3877.62 | 3878.37 | | | | 10.1 | |
| | +K+ | 3893.73 | - | | | | - | |
| **Triicosasac -4Ac** | C156O106H255N5 | 3897.68 | - | - | - | - | - | - |
| | +Na+ | 3919.66 | 3919.62 | | | | 9.00 | |
| | +K+ | 3935.77 | - | | | | - | |
| **Tetraicosasac (24)-2Ac (5RU-1Glc)** | C158O108H261N5 | 3959.75 | - | - | - | - | - | - |
| | +Na+ | 3981.73 | 3982.53 | | | | 7.78 | |
| | +K+ | 3997.84 | 3996.95 | | | | 7.64 | |
| **Tetraicosasac-3Ac** | C160O109H263N5 | 4001.78 | - | - | - | - | - | - |
| | +Na+ | 4023.76 | 4024.75 | | | | 7.88 | |
| | +K+ | 4039.87 | 4040.62 | | | | 13.72 | |
| **Tetraicosasac-4Ac** | C162O110H265N5 | 4043.82 | - | - | - | - | - | - |
| | +Na+ | 4065.80 | 4066.23 | | | | 8.00 | |
| | +K+ | 4081.91 | 4082.67 | | | | 15.8 | |
| **Pentaicosasac(25)-1Ac (5RU)** | C162O112H268N5 | 4078.84 | - | - | - | - | - | - |
| | +Na+ | 4100.82 | 4101.91 | | | | 23.7 | |
| | +K+ | 4116.93 | - | | | | - | |
| **Pentaicosasac -2Ac** | C164O113H270N5 | 4120.88 | - | - | - | - | - | - |
| | +Na+ | 4142.86 | 4144.15 | | | | 73.33 | |
| | +K+ | 4158.97 | - | | | | - | |
| **Pentaicosasac-3Ac** | C166O114H272N5 | 4162.92 | - | - | - | - | - | - |
| | +Na+ | 4184.90 | 4186.55 | | | | 100 | |
| | +K+ | 4201.01 | 4201.95 | | | | 15.84 | |
| **Pentaicosasac -4Ac** | C168O115H274N5 | 4204.95 | - | - | - | - | - | - |
| | +Na+ | 4226.93 | 4228.19 | | | | 93.7 | |
| | +K+ | 4243.04 | 4244.83 | | | | 14.0 | |
| **Pentaicosasac -5Ac** | C170O116H276N5 | 4246.99 | - | - | - | - | - | - |
| | +Na+ | 4268.97 | 4270.96 | | | | 57.8 | |
| | +K+ | 4285.08 | 4286.14 | | | | 9.75 | |
| **Pentaicosasac-6Ac** | C172O117H278N5 | 4289.03 | - | - | - | - | - | - |
| | +Na+ | 4311.01 | 4312.83 | | | | 21.8 | |
| | +K+ | 4327.12 | - | | | | - | |
| **Heptaicosasac(27)-2Ac 5RU+2Rha** | C176O121H291N5 | 4414.17 | - | - | - | - | - | - |
| | +Na+ | 4436.15 | 4437.55 | | | | 8.85 | |
| | +K+ | 4452.26 | - | | | | - | |
| **Heptaicosasac -3Ac** | C178O122H293N5 | 4456.21 | - | - | - | - | - | - |
| | +Na+ | 4478.19 | 4478.92 | | | | 12.6 | |
| | +K+ | 4494.30 | - | | | | - | |
| **Heptaicosasac -4Ac** | C180O123H295N5 | 4498.24 | - | - | - | - | - | - |
| | +Na+ | 4520.22 | 4521.30 | | | | 12.3 | |
| | +K+ | 4536.33 | - | | | | - | |
| **Heptaicosasac-5Ac** | C182O124H297N5 | 4540.28 | - | - | - | - | - | - |
| | +Na+ | 4562.26 | 4563.11 | | | | 7.99 | |
| | +K+ | 4578.37 | - | | | | - | |
| **Triakonsac (30)-2Ac (6RU)** | C196O135H324N6 | 4925.64 | - | - | - | - | - | - |
| | +Na+ | 4947.63 | 4948.51 | | | | 7.99 | |
| | +K+ | 4963.73 | - | | | | - | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Triakonsac-3Ac** | C198O136H326N6 | 4967.68 | - | - | - | - | - | - |
| | +Na+ | 4989.66 | 4991.67 | | | | 16.5 | 27.3 |
| | +K+ | 5005.77 | - | | | | - | 24.2 |
| **Triakonsac-4Ac** | C200O137H328N6 | 5009.72 | - | - | - | - | - | - |
| | +Na+ | 5031.70 | 5033.35 | | | | 18.7 | 66.6 |
| | +K+ | 5047.81 | - | | | | - | 67.9 |
| **Triakonsac-5Ac** | C202O138H330N6 | 5051.75 | - | - | - | - | - | - |
| | +Na+ | 5073.74 | 5075.94 | | | | 15.1 | 99.9 |
| | +K+ | 5089.85 | - | | | | - | 100 |
| **Triakonsac-6Ac** | C204O139H332N6 | 5093.79 | - | - | - | - | - | - |
| | +Na+ | 5115.77 | 5117.76 | | | | 9.01 | 94.7 |
| | +K+ | 5131.88 | - | | | | - | 96.7 |
| **Triakonsac-7Ac** | C206O140H334N6 | 5135.83 | - | - | - | - | - | - |
| | +Na+ | 5157.81 | 5160.32 | | | | | 57.8 |
| | +K+ | 5173.92 | 5176.66 | | | | | 65.4 |
| **Triakonsac-8Ac** | C208O141H336N6 | 5177.87 | - | - | - | - | - | - |
| | +Na+ | 5199.85 | 5202.13 | | | | | 21.6 |
| | +K+ | 5215.96 | 5219.01 | | | | | 26.7 |
| **Dotriakonsac(32)-4Ac 6RU+2Rha** | C212O145H347N6 | 5300.99 | - | - | - | - | - | - |
| | +Na+ | 5322.97 | - | | | | | - |
| | +K+ | 5339.08 | 5342.45 | | | | | 14.2 |
| **Dotriakonsac-5Ac** | C214O146H349N6 | 5343.03 | - | - | - | - | - | - |
| | +Na+ | 5365.01 | 5368.01 | | | | | 17.0 |
| | +K+ | 5381.12 | 5384.94 | | | | | 19.5 |
| **Dotriakonsac-6Ac** | C216O147H351N6 | 5385.07 | - | - | - | - | - | - |
| | +Na+ | 5407.05 | 5410.98 | | | | | 14.0 |
| | +K+ | 5423.16 | 5427.03 | | | | | 16.7 |
| **Dotriakonsac-7Ac** | C218O148H353N6 | 5427.10 | - | - | - | - | - | - |
| | +Na+ | 5449.09 | - | | | | | - |
| | +K+ | 5465.19 | 5468.52 | | | | | 10.0 |
| **Pentatriakonsac(35)-4Ac (7RU)** | C232O159H380N7 | 5812.47 | - | - | - | - | - | - |
| | +Na+ | 5834.45 | 5838.89 | | | | | 21.8 |
| | +K+ | 5850.56 | 5855.10 | | | | | 23.8 |
| **Pentatriakonsac-5Ac** | C234O160H382N7 | 5854.51 | - | - | - | - | - | - |
| | +Na+ | 5876.49 | 5880.90 | | | | | 40.2 |
| | +K+ | 5892.60 | 5897.40 | | | | | 42.9 |
| **Pentatriakonsac-6Ac** | C236O161H384N7 | 5896.54 | - | - | - | - | - | - |
| | +Na+ | 5918.52 | 5923.05 | | | | | 47.6 |
| | +K+ | 5934.63 | 5939.30 | | | | | 51.9 |
| **Pentatriakonsac-7Ac** | C238O162H386N7 | 5938.58 | - | - | - | - | - | - |
| | +Na+ | 5960.56 | 5965.13 | | | | | 36.7 |
| | +K+ | 5976.67 | 5981.45 | | | | | 39.9 |
| **Pentatriakonsac-8Ac** | C240O163H388N7 | 5980.62 | - | - | - | - | - | - |
| | +Na+ | 6002.60 | 6007.02 | | | | | 18.1 |
| | +K+ | 6018.71 | 6023.12 | | | | | 22.9 |
| **Pentatriakonsac-9Ac** | C242O164H390N7 | 6022.65 | - | - | - | - | - | - |
| | +Na+ | 6044.63 | - | | | | | - |
| | +K+ | 6060.74 | 6065.63 | | | | | 10.3 |

**Figure S7: Organic solvent stability of Sf6TSP**

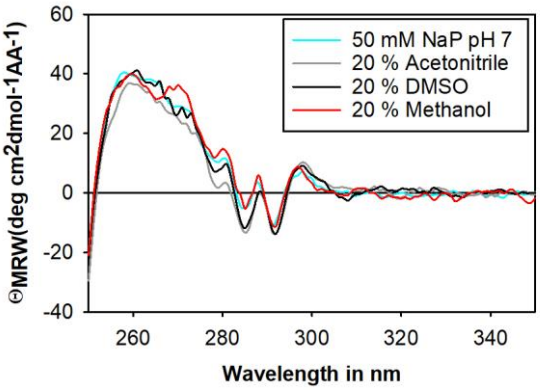


**Figure S7.** Organic solvent stability of Sf6TSP. Near-UV circular dichroism spectra monitored between 350-250 nm of 36 µM Sf6TSP in 50 mM sodium phosphate buffer pH 7 or with 20 % volume percent of organic solvent in the respective buffer.

**Figure S8: Binding kinetics of Sf6TSP N340C labeled with NBD to SfY polysaccharide**

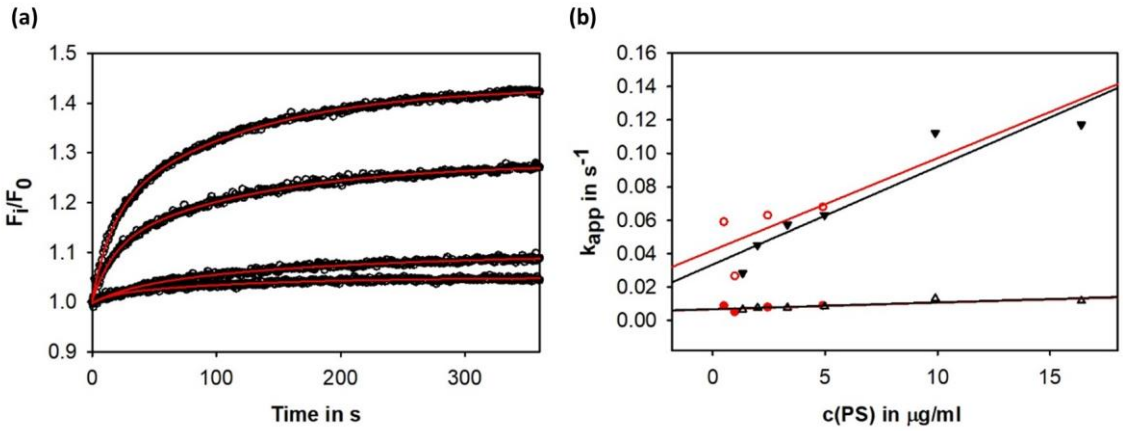


**Figure S8.** Binding kinetics of Sf6TSP N340C labeled with NBD to SfY polysaccharide. **(a)** Sf6TSP N340C-NBD (184 µM) excited at 492 nm and emission measured as kinetic traces of binding relaxations monitored at 540 nm between with the respective SfY polysaccharide concentrations in µg/ml were fitted by a biexponential equation, which resulted in $k_{app}$. **(b)** Rate constants from kinetic binding traces of Sf6TSP probed by intrinsic fluorescence excited at 280 nm (black) [1]. Rate constants from kinetic traces of Sf6TSP N340C NBD as shown in a (red). Dissociation rates were calculated after linear fitting by $k_{app}=k_{diss}+k_{ass}$ ([P]+[L]): $k_{d1}$(Sf6TSP N340C NBD)=0.0068/s, $k_{d1}$(Sf6TSP)=0.0059/s, $k_{d2}$(Sf6TSP N340C NBD)=0.0419/s, $k_{d2}$(Sf6TSP)=0.0335/s.

1. Kang, Y.; Gohlke, U.; Engström, O.; Hamark, C.; Scheidt, T.; Kunstmann, S.; Heinemann, U.; Widmalm, G.; Santer, M.; Barbirz, S. Bacteriophage Tailspikes and Bacterial O-Antigens as a Model System to Study Weak-Affinity Protein–Polysaccharide Interactions. *J. Am. Chem. Soc.* **2016**, *138*, 9109–9118, doi:10.1021/jacs.6b00240.

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