

Synthesis of diglyceride conjugate of selectin ligand SiaLe^X as a vector for targeting of drug-loaded liposomes

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Spectral characteristics for polyethylene glycol containing compounds. MALDI-TOF mass spectra were obtained using a Bruker Ultraflex instrument (Bruker Daltonics, Germany). This system utilizes a pulsed nitrogen laser, emitting at 337 nm. Positive and negative ion spectra were recorded with dihydroxybenzoic acid (DHB) as matrix. DHB was applied as 0.5 mol/l solution in CH₃OH with 0.1 % trifluoroacetic acid. Matrix was mixed 1:1 (v/v) with the sample (1 mg/ml) and this mixture deposited onto the target. The extraction voltage was 20 kV and gated matrix suppression was applied to prevent the saturation of the detector by matrix ions. Spectra represent the average of 128 individual laser shots. ¹H NMR spectrum was recorded at 30°C with a Bruker WM-700 (USA) instrument.

1: Positive ion mass spectrum of diester **1** is shown in Figure 1S. Mass spectrum (MS), *m/z* (intensity, rel. u.): 597.24 [M + H]⁺ (1792; for n = 5 C₂₆H₃₂N₂O₁₄ [M + H]⁺ = 597.19); 641.28 [M + H]⁺ (1965; for n = 6 C₂₈H₃₆N₂O₁₅ [M + H]⁺ = 641.22); 685.33 [M + H]⁺ (2057; for n = 7 C₃₀H₄₀N₂O₁₆ [M + H]⁺ = 685.25); 729.36 (1954; for n = 8 C₃₂H₄₄N₂O₁₇ [M + H]⁺ = 729.27); 773.41 (1631; for n = 9 C₃₄H₄₈N₂O₁₈ [M + H]⁺ = 773.30); 817.45 (1136; for n = 10 C₃₆H₅₂N₂O₁₉ [M + H]⁺ = 817.32); 861.47 (769; for n = 11 C₃₈H₅₆N₂O₂₀ [M + H]⁺ = 861.35). According to MS data, product containing ethylene glycol oligomer with n = 7 (685 Da) was major one presented in the spectrum.

2: Positive ion mass spectrum of acid **2** is shown in Figure 2S. MS, *m/z* (intensity, rel. u.): 1243.16 [M + K]⁺ (2137; for n = 9 C₆₄H₁₁₇NO₁₉ [M + K]⁺ = 1242.79); 1287.19 [M + K]⁺ (3126; for n = 10 C₆₆H₁₂₁NO₂₀ [M + K]⁺ = 1286.81); 1331.24 [M + K]⁺ (3740; for n = 11 C₆₈H₁₂₅NO₂₁ [M + K]⁺ = 1330.84); 1375.28 [M + K]⁺ (3758; for n = 12 C₇₀H₁₂₉NO₂₂ [M + K]⁺ = 1374.86); 1419.31 [M + K]⁺ (3241; for n = 13 C₇₂H₁₃₃NO₂₃ [M + K]⁺ = 1418.89); 1463.34 [M + K]⁺ (2108; for n = 14 C₇₄H₁₃₇NO₂₄ [M + K]⁺ = 1462.92); 1507.39 [M + K]⁺ (1276; for n = 15 C₇₆H₁₄₁NO₂₅ [M + K]⁺ = 1506.94). Products containing ethylene glycol oligomers with n = 11 (1331 Da) and n = 12 (1375 Da) were major species presented in the spectrum as adducts of acid **2** with K⁺ ions. The Na⁺ adducts correspond to less intensive peaks shifted to lower masses by 16 Da, with maximum at 1315 Da (n = 11).

3: Negative ion mass spectrum of SiaLe^X-conjugate **3** is shown in Figure 3S (positive ion mode did not provide registration of the molecular ion). A set of peaks with maximum at 2152 Da (n = 11) represents molecular ion(s); MS, m/z (intensity, rel. u.): 2062.99 [M]⁻ (2031; for n = 9 C₉₈H₁₇₄N₄O₄₁ [M]⁻ = 2063.17); 2108.06 [M]⁻ (2740; for n = 10 C₁₀₀H₁₇₈N₄O₄₂ [M]⁻ = 2107.19); 2151.55 [M]⁻ (3158; for n = 11 C₁₀₂H₁₈₂N₄O₄₃ [M]⁻ = 2151.21); 2196.14 [M]⁻ (3074; for n = 12 C₁₀₄H₁₈₆N₄O₄₄ [M]⁻ = 2195.24); 2239.82 [M]⁻ (2878; for n = 13 C₁₀₆H₁₉₀N₄O₄₅ [M]⁻ = 2239.27). Another, equal in the intensity set of peaks with maximum at 1492 Da (n = 11) corresponds to product(s) of degradation by three glycoside bonds with cleavage of sialic acid (309 Da), fucose (164 Da), and galactose (180 Da). Using systematic nomenclature for carbohydrate fragmentation in FAB-MS/MS spectra of glycoconjugates [B. Domon and C. E. Costello, *Glycoconjugate J.*, 1988, **5**, 397] these ions should be assigned Z_{1α}Z_{1β}⁻, as species lost both oxygen atoms from interglycosidic bonds of GlcNAc-residue. The 3rd peak set of half intensity shifted as compared to previous one by 16 Da to higher masses, with maximum at 1464 Da (n = 10) corresponds to ions either Z_{1α}Y_{1β}⁻ or Y_{1α}Z_{1β}⁻, which retained one of the interglycosidic oxygens in GlcNAc-conjugate. And finally, the 4th peak set of much lower intensity with maximum at 1608 Da (n = 10) can be observed under preceding signals. This one represents product(s) of conjugate **3** fragmentation by loss of sialic acid and fucose along with both glycosidic oxygens (ions Z_{2α}Z_{1β}⁻). Cleavage of Neu5Ac- and Fuc-residues is very frequent type of oligosaccharide fragmentation in MALDI MS. Obviously, trifluoroacetic acid commonly added to the acidic matrix for promotion of ion formation from amide containing compounds is able to cause this deglycosylation. Besides that, maybe matrix 2,5-dihydroxybenzoic acid (pK=2.94) facilitates this reaction too. Acidic DHB is the matrix of choice to detect positive ions [B. Fuchs, C. Schober, G. Richter, R. Süß and J. Schiller, *J. Biochem. Biophys. Methods*, 2007, **70**, 689]. As we succeeded in registration of intensive molecular ion peak(s) in negative ion mode even with DHB, presumably, the use of *para*-nitroaniline (PNA) as matrix could permit to escape fragmentation since the basicity of PNA makes it an ideal matrix to record negative ion spectra [R. Estrada and M. C. Yappert. *J. Mass Spectrom.*, 2004, **39**, 412].

¹H NMR-spectrum, selected chemical shifts (700 MHz, CDCl₃/CD₃OD 2:1, 303 K, δ, ppm): 5.494 (m, 4H, 2 cis CH=CH), 5.422 (m, 1H, OCH₂-CH-CH₂O), 5.188 (d, 1H, J₂ = 3.9 Hz, H-1 Fuc), 4.876 (broadened q, 1H, H-5 Fuc), 4.629 (d, 1H, J₂ = 7.6 Hz, H-1 GlcNAc), 4.571 (d, 1H, J₂ = 7.6 Hz, H1 Gal), 4.50-3.55 (signals under CD₃OH, overlaid signals and 3.802 ppm of OCH₂CH₂O), 2.987 (dd, 1H, J_{3ax} = 12 J₄ = 4.5 Hz, H-3eq Neu5Ac), 2.760 (t, 2H, COCH₂CH₂NH), 2.486 (m, 4H, 2 COCH₂), 2.177 and 2.125 (s, 2 3H, 2 NCOCH₃ of Neu5Ac and GlcNAc), 2.171 (m, 8H, 2 CH₂CH=CHCH₂), 1.938 (t, 1H, J_{3eq} ~ J₄ ~ 12 Hz, H-3ax Neu5Ac),

1.916 (m, 2H, OCH₂CH₂CH₂N), 1.772 (m, 4H, 2 COCH₂CH₂), 1.459 (m, 40H, 20 CH₂), 1.336 (d, 3H, J = 6.5 Hz, H-6 Fuc), 1.034 (~t, 6H, J = 7.2 Hz, 2 CH₃).

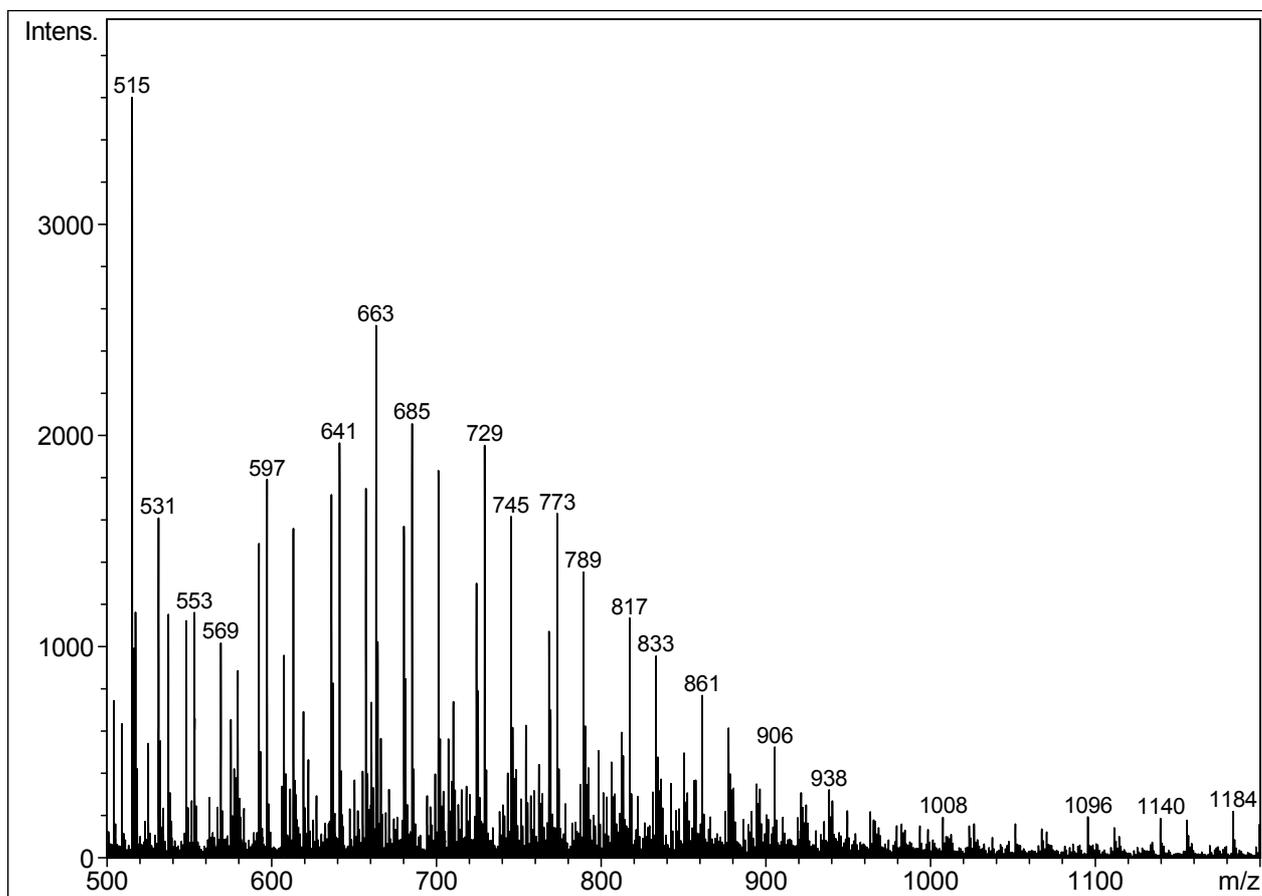


Figure 1S Mass spectrum MALDI TOF of di-*p*-nitrophenyl ester of bis(carboxymethyl) ether of PEG600 **1**. Detection of positive ions.

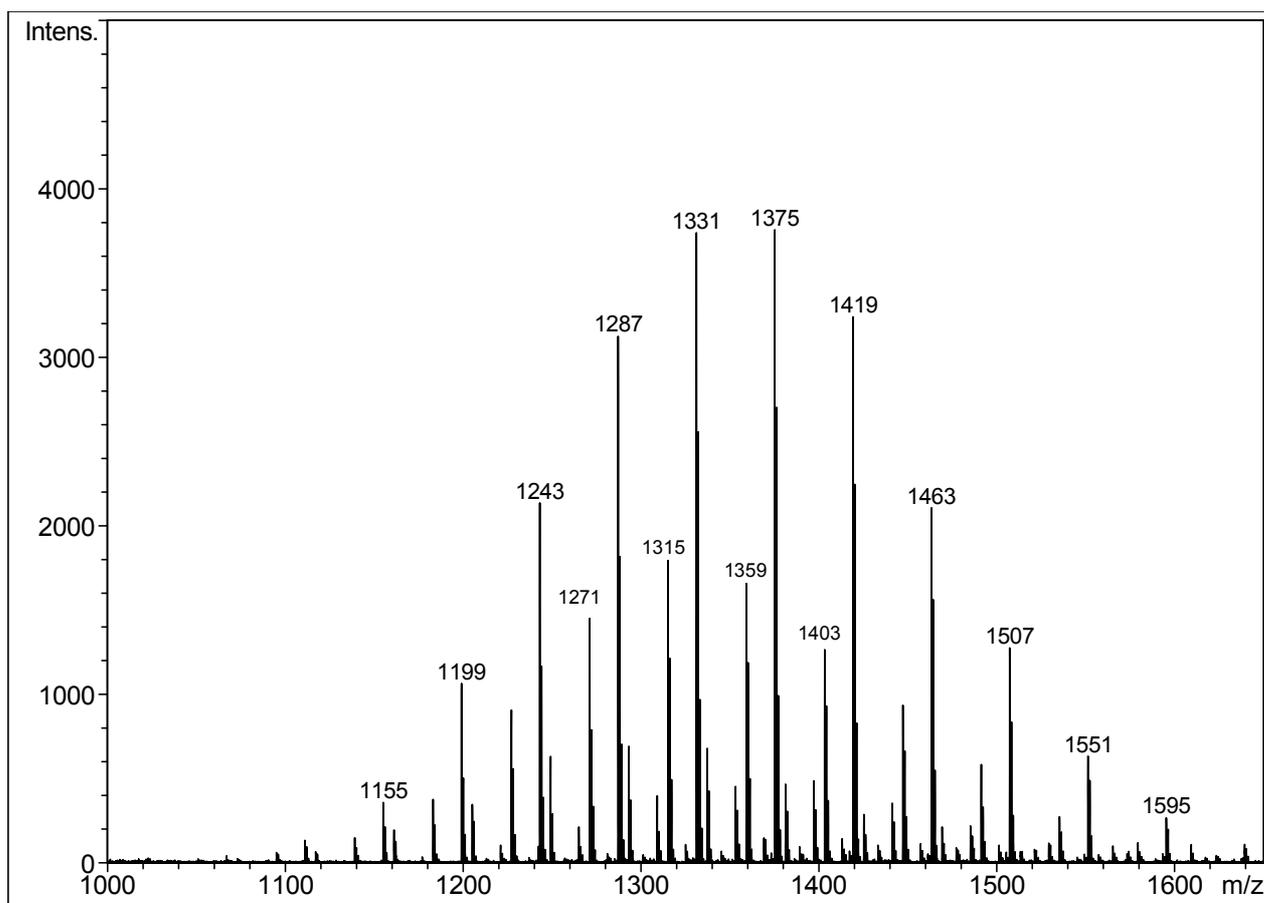


Figure 2S Mass spectrum MALDI TOF of *rac*-1,2-dioleoyl-3-[carboxymethylene-(polyoxyethylene)oxyacetyl-3-aminopropionyl]glycerol **2**. Detection of positive ions.

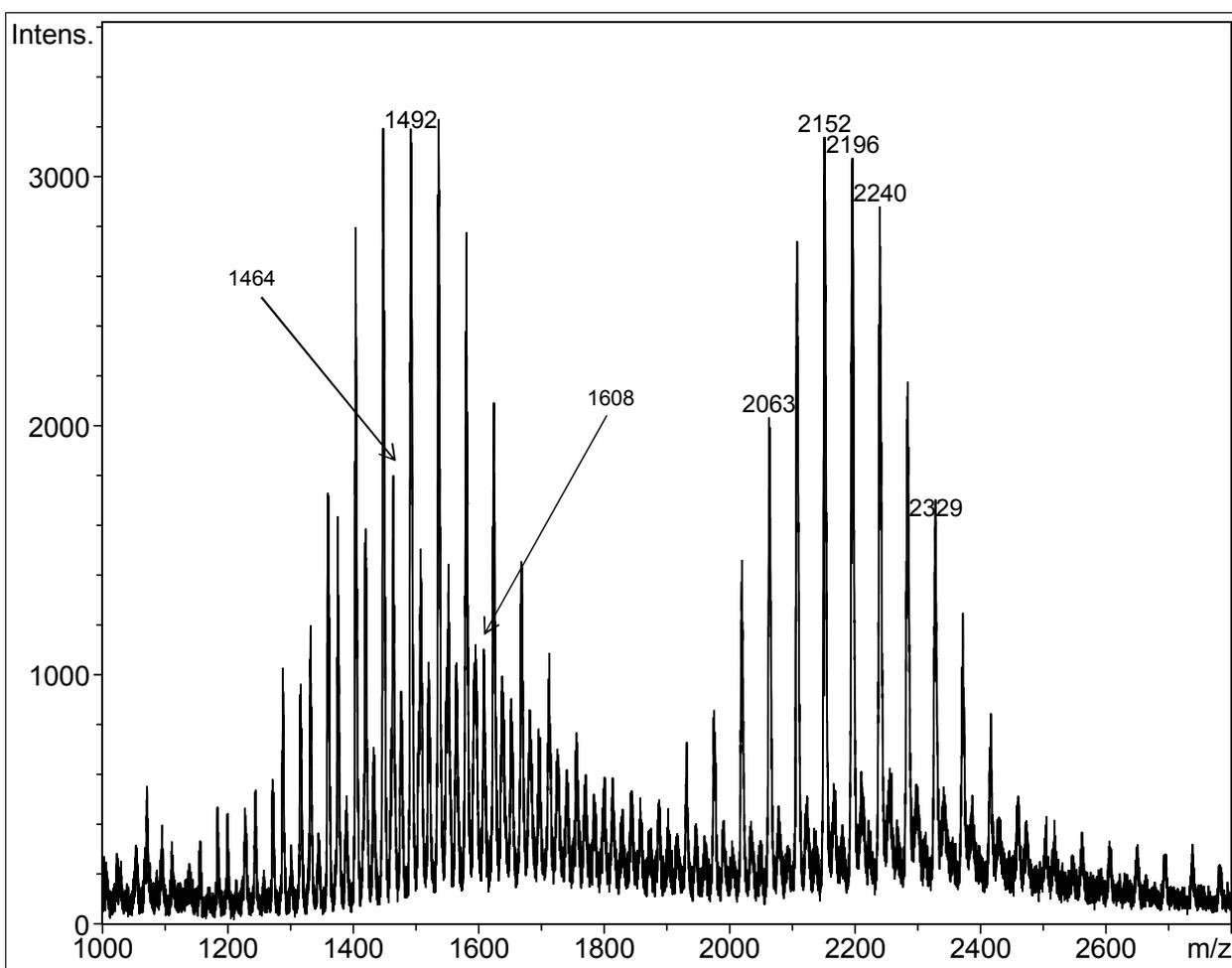


Figure 3S Mass spectrum MALDI TOF of PEG-diglyceride SiaLe^X conjugate **3**. Detection of negative ions.