SUPPORTING MATERIAL

Diasteroselective colloidal self-assembly affects the immunological response of the molecular adjuvant Sulfavant

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Dynamic Light Scattering (DLS)

Measurements were performed at (25.00 ± 0.05) °C with temperature controlled through the use of a thermostat bath. In DLS, the intensity autocorrelation function, $g^{(2)}(t)$, was measured for the instrument configuration corresponding to the scattering angle of 90°. The intensity autocorrelation function is related to the electric field autocorrelation function through the Siegert relation. The electric field autocorrelation function, $g^{(1)}(t)$ is defined as:

$$g^{(1)}(t) = \int_{-\infty}^{+\infty} \tau A(\tau) \exp\left(-\frac{t}{\tau}\right) \mathrm{d} \ln \tau \tag{1}$$

where $\tau = 1/\Gamma$ and q is the modulus of the scattering vector $q = 4\pi n_0/\lambda \sin(\theta/2)$, $n_0 = 1.33$ is the refractive index of the solvent, λ is the incident wavelength and θ represents the scattering angle. Evaluation of the relaxation rate Γ distribution allows calculating the translational diffusion coefficient: $D = \Gamma/q^2$: *(J. B. a. R. Pecora, Dynamic Light Scattering: With Applications to Chemistry, Biology, and Physics Couvire Dover Publications, 2003.)*

Inverse Laplace transforms were performed using a variation of CONTIN algorithm incorporated in Precision Deconvolve software. *A. Lomakin, D. Teplow and G. Benedek, in Amyloid Proteins, ed. E. Sigurdsson, Humana Press, 2005, vol. 299, ch. 10, pp. 153-174.*

For spheres diffusing in a continuum medium at infinite dilution, in the approximation of spherical objects, the diffusion coefficient is related to the hydrodynamic radius, R_h, through the Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta_0 D} \tag{2}$$

where k is the Boltzmann constant, T is the absolute temperature and $\eta_0 = 0.89cP$ is the solvent viscosity. For not spherical particles, R_h represents the radius of a spherical aggregate with the same diffusion coefficient measured. In the present system due to the high dilution it is possible to consider the approximation that $\eta \cong \eta_0$, where η represents the solution viscosity. In this hypothesis, equation (2) can be reasonably used to estimate the averaged hydrodynamic radius of the particles.

Human monocyte-dendritic cell differentation.

For each assay human peripheral blood mononuclear cells were isolated from two healthy donors by routine Ficoll density gradient centrifugation. Monocytes were purified from human peripheral blood mononuclear cells using MACS CD14 microbeads (Miltenyi Biotech, Auburn, CA) according to the manufacturer's recommendation. Purity was checked by staining with a FITC-conjugated anti-CD14 antibody (Milteny Biotech) and FACS analysis and was routinely found to be greater than 98%. Immature DCs were obtained by incubating monocytes at $7 \cdot 10^5$ cell/mL in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% L-glutamine 2mM , 1% penicillin and streptomycin, human IL-4 (5 ng/mL) and human GM-CSF (100 ng/mL) for five days.

Cells Staining and stimulation.

After five days in culture, surface staining was performed on monocyte-derived dendritic cells for flow cytometry analysis. moDCs were stained by using the following conjugated mAbs from BD-Biosciences: CD14Fitc, CD1a-BUV121, CD86 BV650, CD83BUV737, HLA-DRBV786, CD11c-BUV395, CD3 BV510, CD54Pe, CD1c Alexa Fluor 647, CD4- PeCys7, and analyzed by flow-cytometer (BD LSRFortessa X-20, BD Bioscience, Milano, Italy) according to standard protocol. moDCs were then incubated with synthetic compounds in 12-wells at concentration of 0.01-0.1-1-10 μg mL^{-1}. Stimulation with PAM2CSK4 1μg mL^{-1} (Invivogen) was used as positive control. After 24 hours expression of all surface markers was estimated again by fluorochrome-conjugated antibodies.

Real Time PCR analysis.

Total RNA was isolated using Trizol Reagent, according to the manufacturer's protocol. RNA quantity and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific). Sample purity was checked by A260/A280 ratios between 1.80 and 2.00. Extracted RNAs from all preparations were in this range. Cytokines mRNA expression was measured by quantitative Real

Time-PCR (QuantStudio 7 Flex Real-Time PCR System; Thermo Scientific). All data were analyzed by two way ANOVA followed by the Tukey test for multiple comparison test. A p-value less than 0.05 was considered as statistically significant. All analyses were performed using the GraphPad Prism 6.00 for Windows software (GraphPad Software, San Diego California, USA).

Cytotoxic assay on moDcs

Cells were incubated in 12-well plates ($7x10^5$ cells/well) wtih compounds at concentration of 0.01-0.1-1-10 µg mL^{-1}, vehicle and PAM2CSK4. After 24 hours, cells were collected and were stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions (Dead Cell Apoptosis Kit with Annexin V FITC and PI, for flow cytometry; Invitrogen). Untreated cells were used as the control for double staining. The cells were analyzed immediately after staining using a FACScan flow cytometer (BD Accuri C6). For each measurement, at least 10,000 cells were counted. After staining apoptotic cells show green fluorescence, dead cells (late apoptotic) show red and green fluorescence, and live cells show little or no fluorescence.

Surface tension measures

The surface tension of aqueous Sulfavant samples was measured with a Sigma 70 tensiometer (KSV, Stockholm, Sweden) using the Du Noüy ring method. Successive aliquots of a stock sulfavant mixture, freshly prepared in Millipore water, were added to the vessel with a known volume of water. The slope of the plot of surface tension *vs*. total concentration may be related to the area per adsorbed surfactant molecule at the solution-air interface (A_{min}) means of the Gibbs isotherm.

$$A_{min} = -\frac{1}{N_A}\left[\frac{1}{RT}\left(\frac{\partial \gamma}{\partial \ln C}\right)\right]^{-1}$$

As shown in figure S1 the Sulfavants R (**3**) and S (**2**) show a significant smaller area with respect to Sulfavant A (**1**).

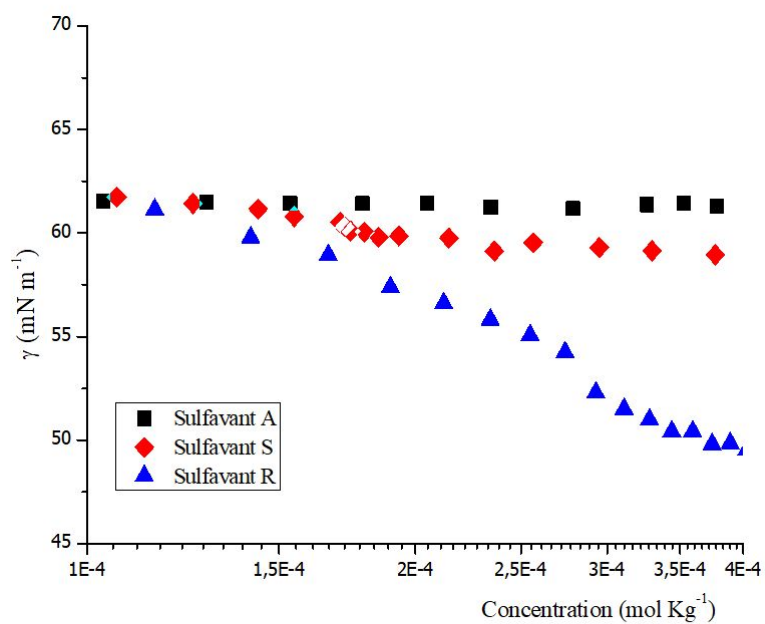

Figure S1. Surface tension *vs*. total concentration of Sulfavants-A (**1**), -S (**2**) and -R (**3**)

Figure S2. Flow-cytometry analysis of maturation phenotyping markers (HLA-DR, CD86, CD83) in moDCs stimulated with Sulfavant A (**1**) at concentrations of 0.01, 0.1, 1 and 10 µM.
Gray = isotype control; Dark gray = unstimulated cells; Orange = stimulated

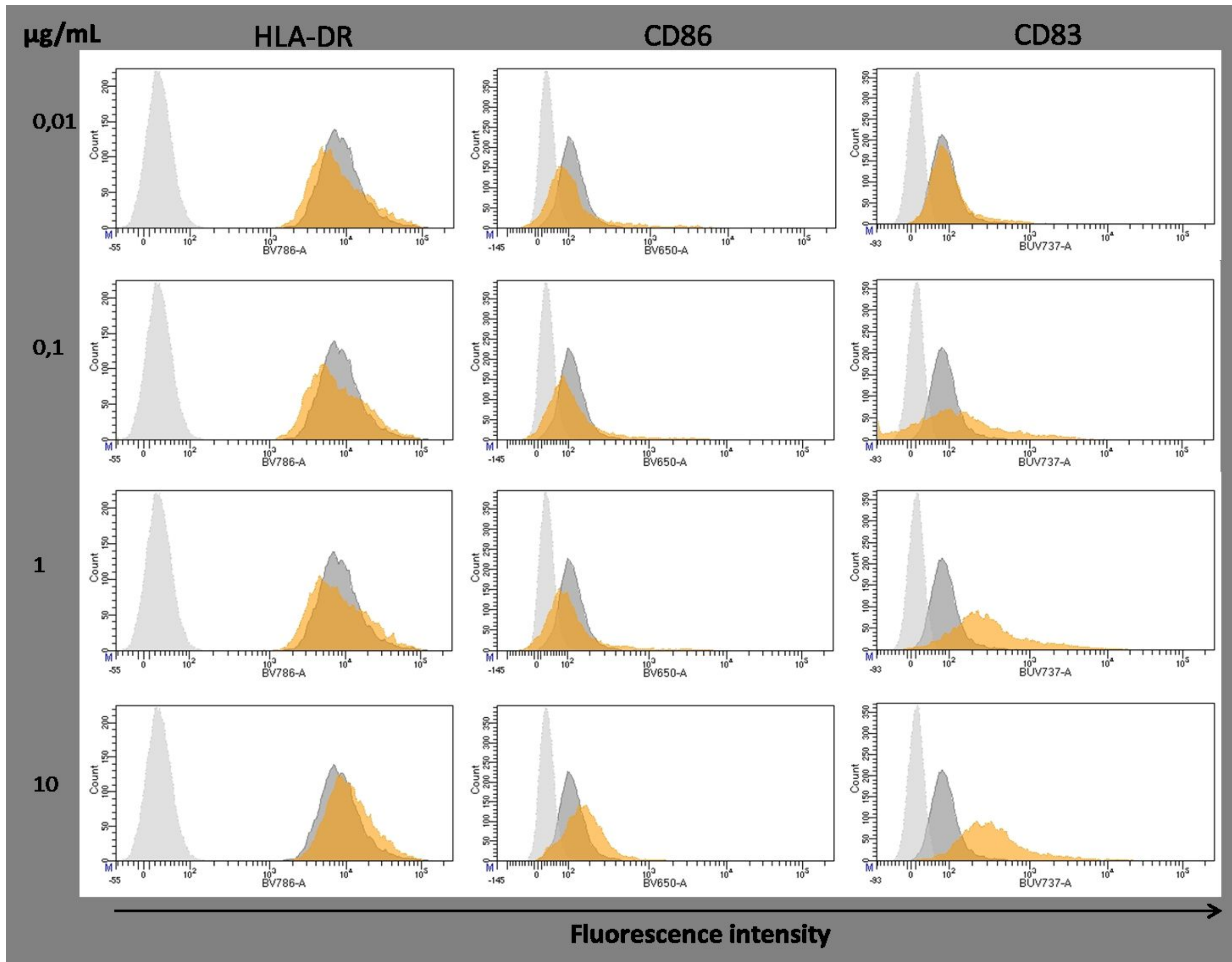

Figure S3. Flow-cytometry analysis of maturation phenotyping markers (HLA-DR, CD86, CD83) in moDCs stimulated with Sulfavant S (**2**) at concentrations of 0.01, 0.1, 1 and 10 µM.
Gray = isotype control; Dark gray = unstimulated cells; Orange = stimulated

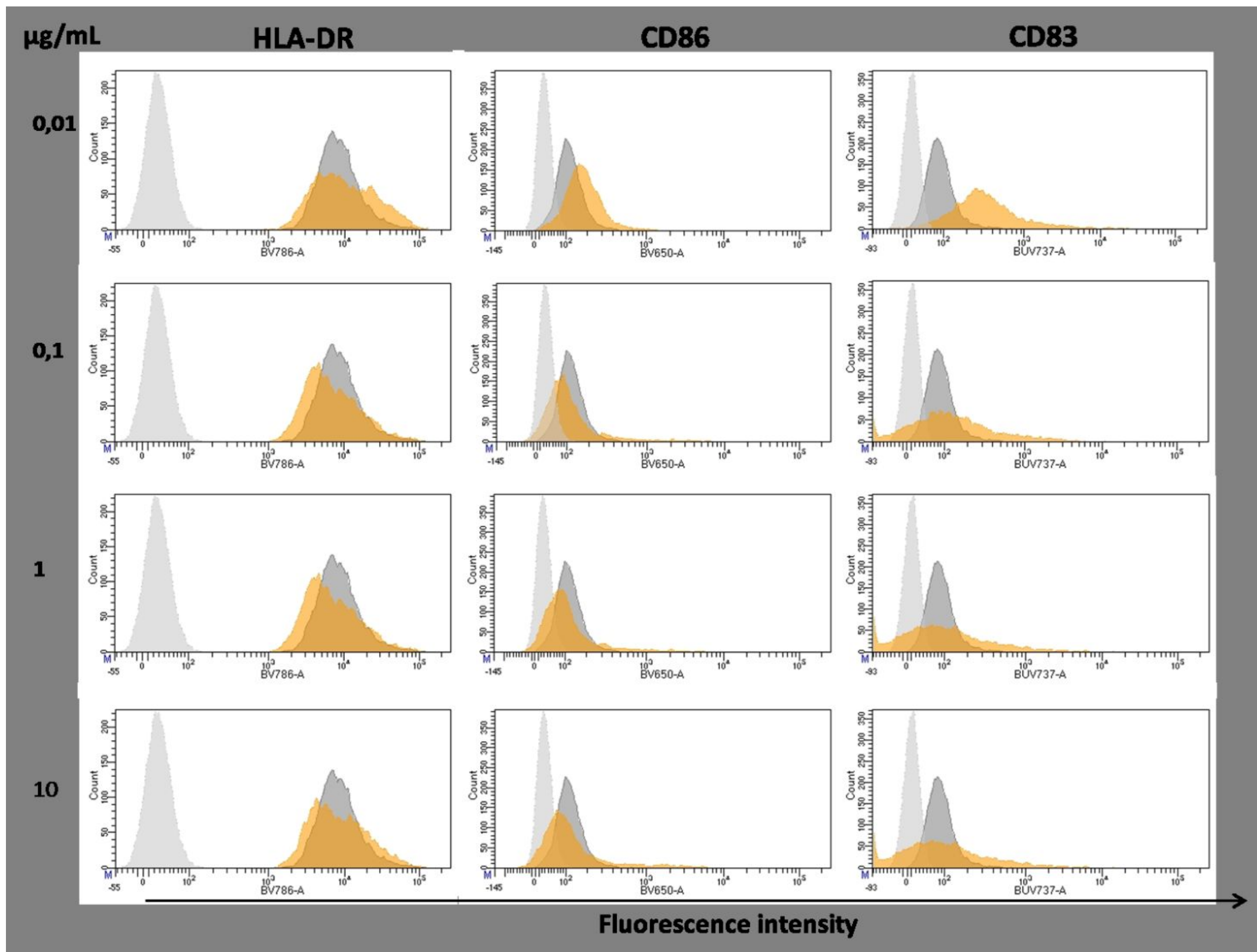

Figure S4. Flow-cytometry analysis of maturation phenotyping markers (HLA-DR, CD86, CD83) in moDCs stimulated with Sulfavant R (**3**) at concentrations of 0.01, 0.1, 1 and 10 µM.
Gray = isotype control; Dark gray = unstimulated cells; Orange = stimulated

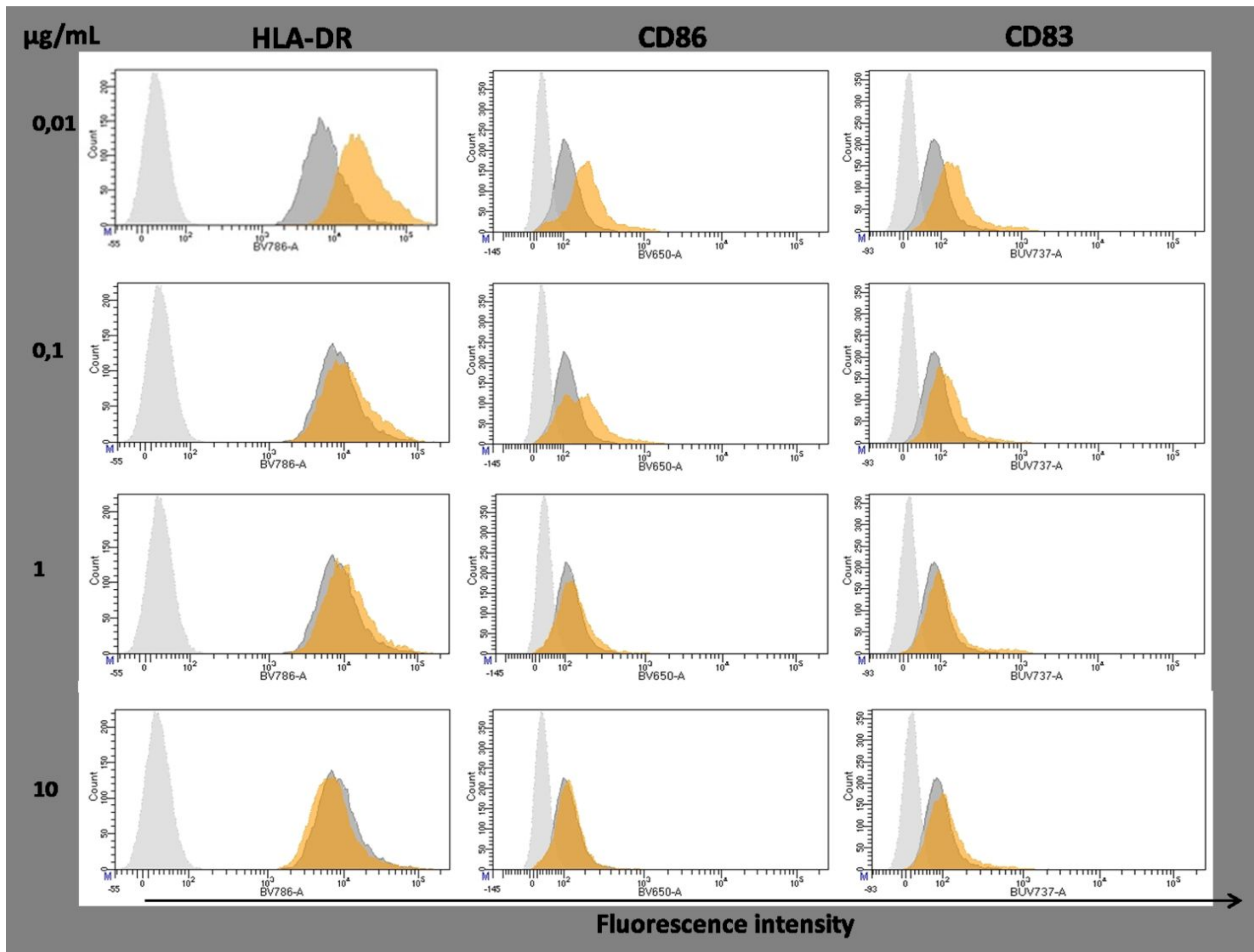

Figure S5. Cytotoxic assay on moDcs. Percentage of viable, early apoptotic and late apoptotic cells stimulated with Sulfavant A (**1**), Sulfavant S (**2**) and Sulfavant R (**3**). Data are expressed as mean and standard deviation from a duplicate of two independent experiments and compared to cells treated only with vehicle (DMSO, ctrl), . ****P<0.0001 vs. control. PAM2CSK4 is used as positive control for moDCs stimulation.

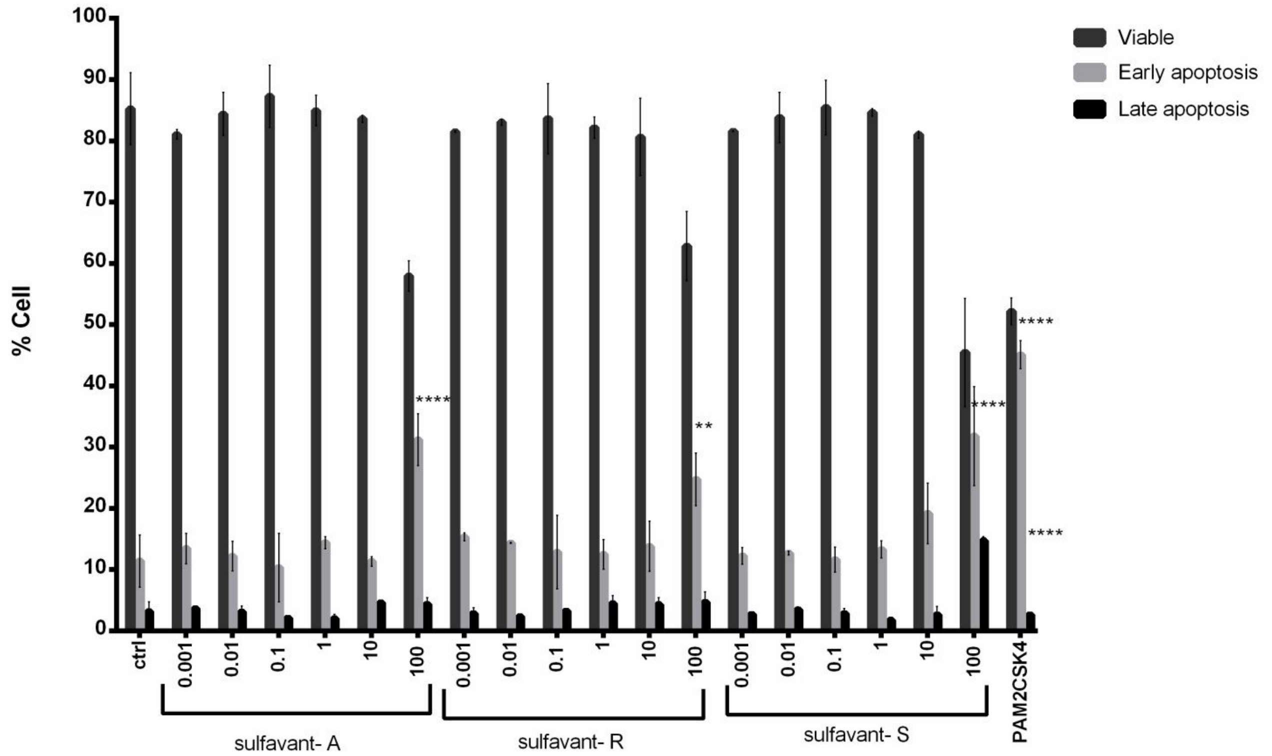

Figure S6.^{1}H-NMR (400 MHz, $CDCl_3$) spectra of compound **4**.

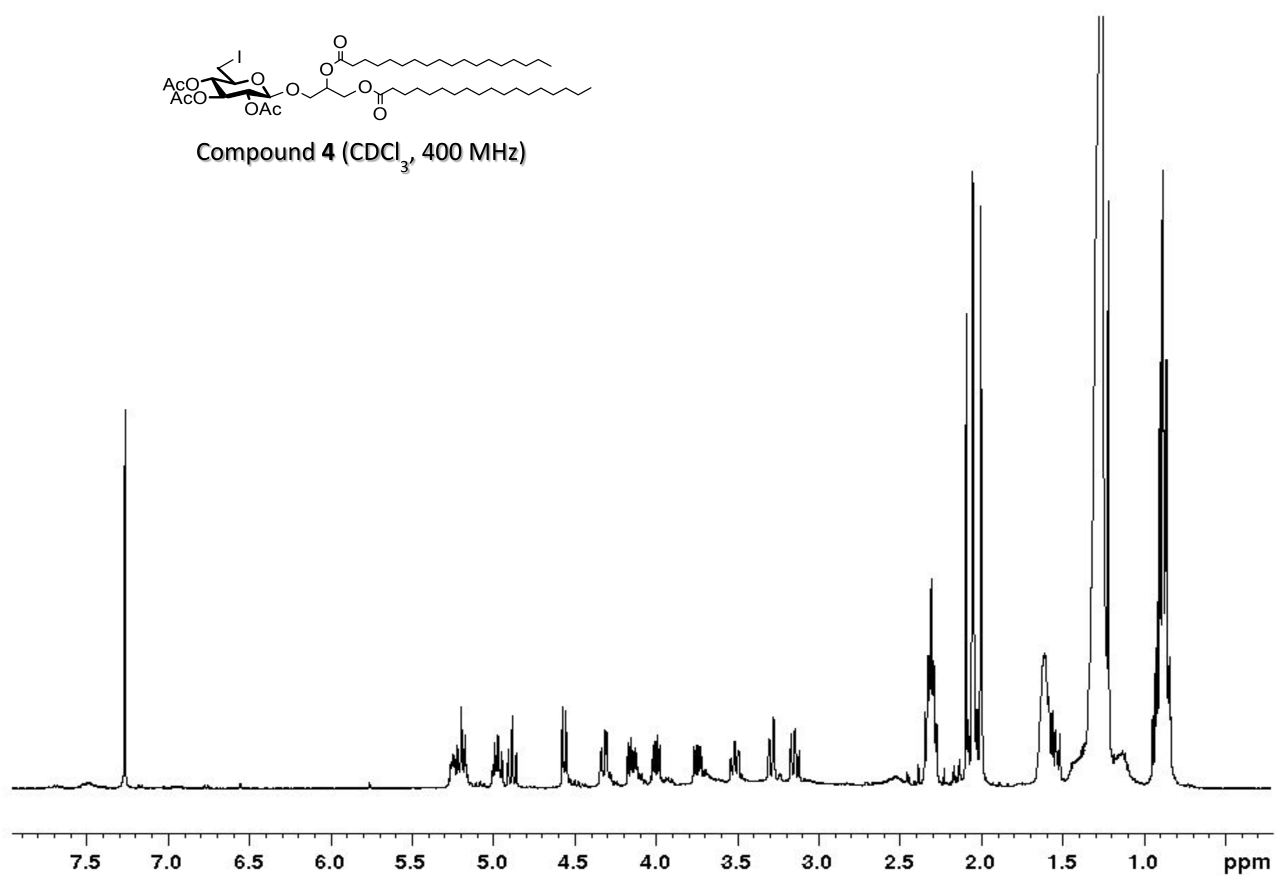

Figure S7.^{1}H-NMR (400 MHz, $CDCl_3$) spectra of compound **5**.

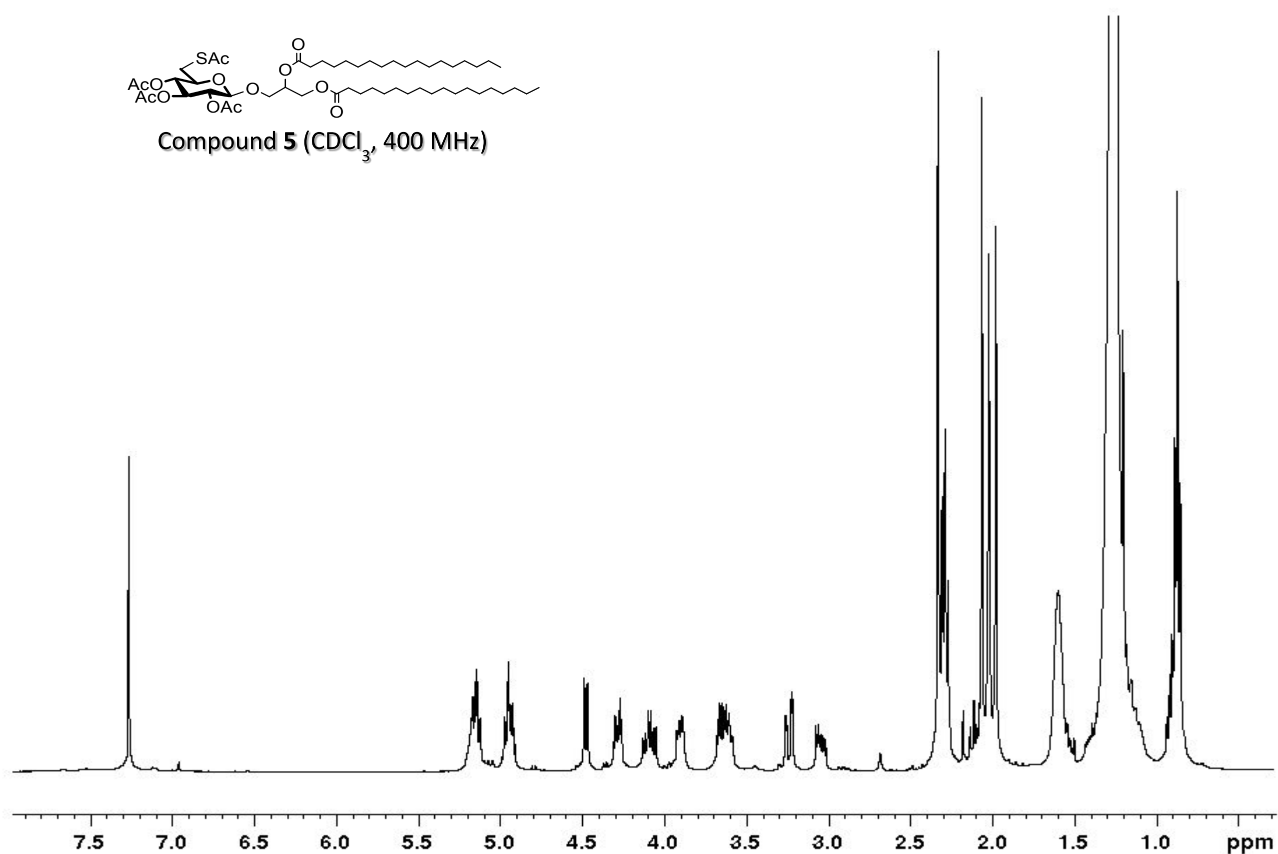

Figure S8.^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 1/1) spectra of compound **2**.

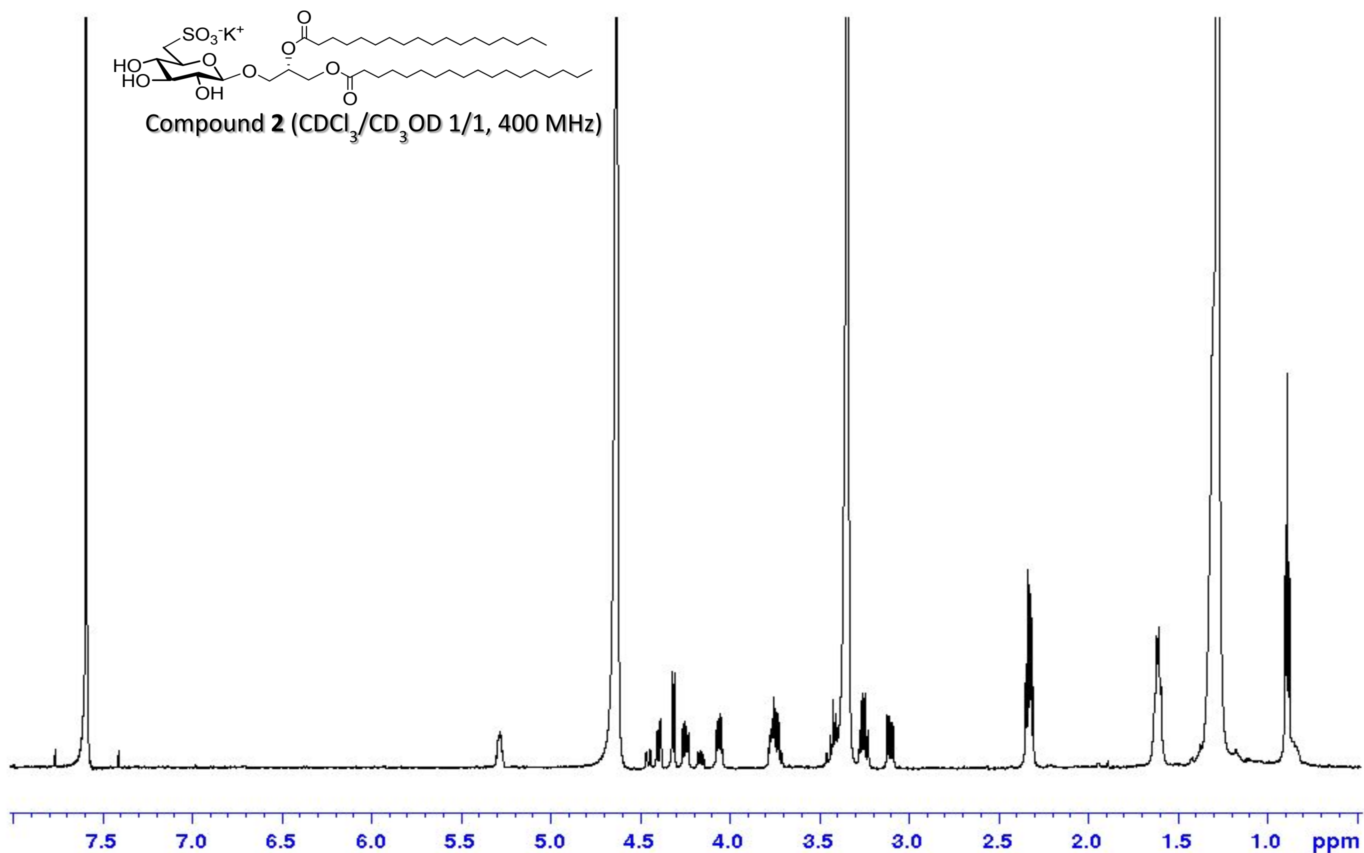

Figure S9.^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 1/1) spectra of compound **3**.

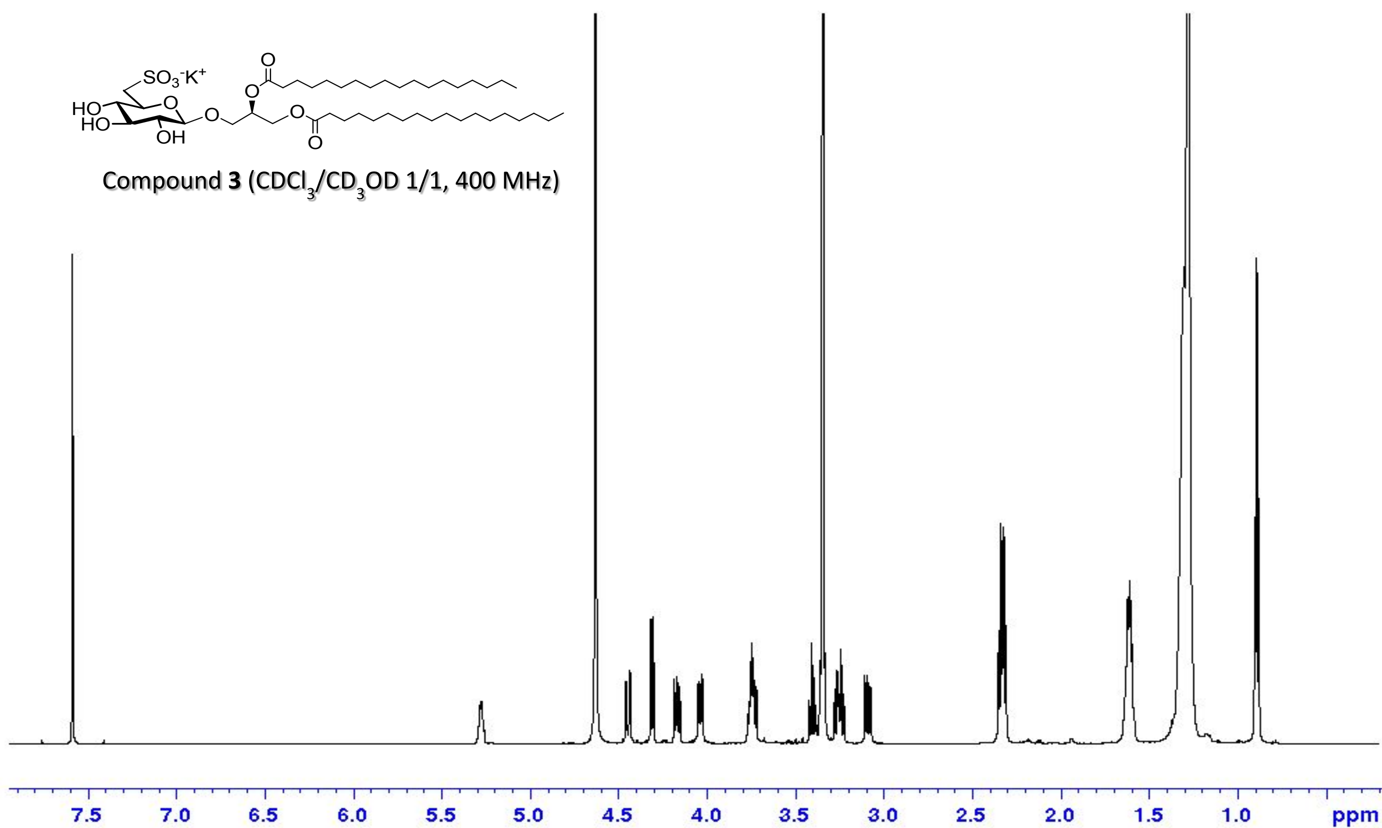

Figure S10.^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 1/1) spectra of compound **2**.

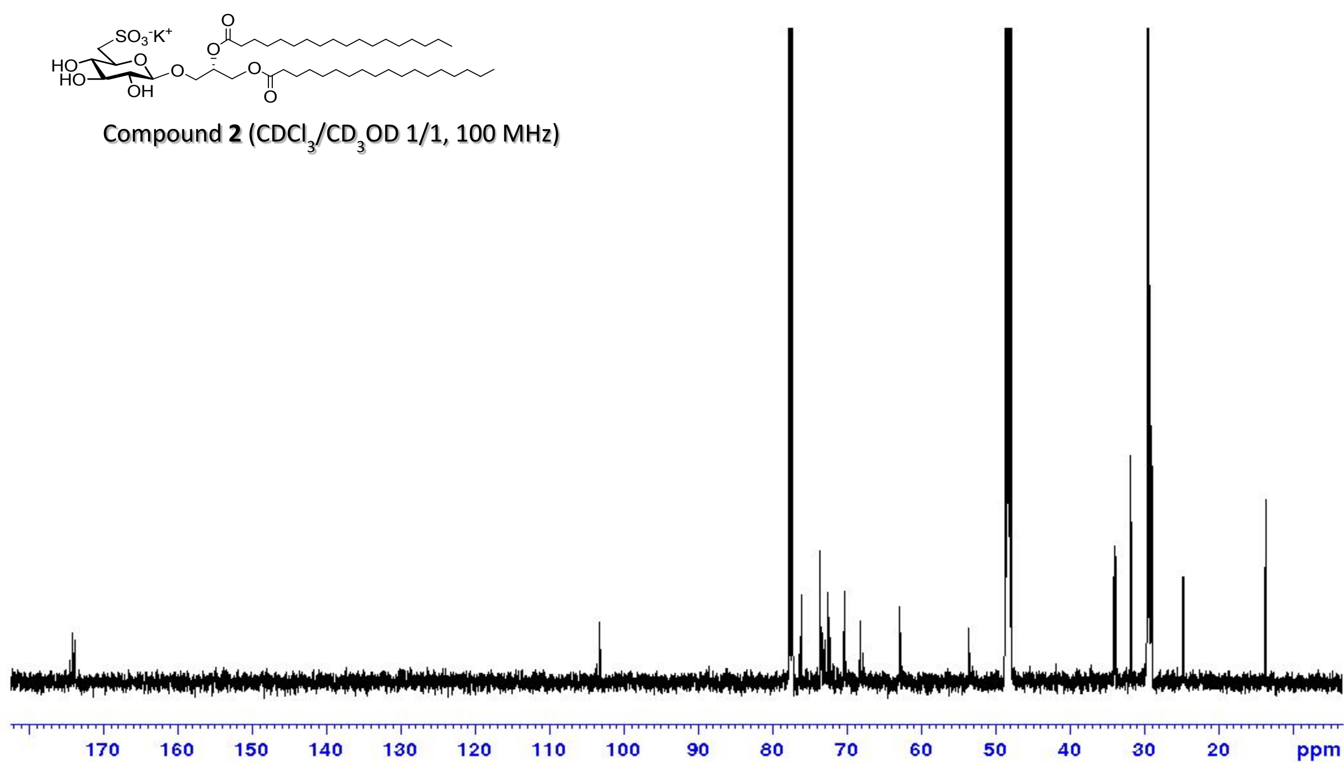

Figure S11.^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 1/1) spectra of compound **3**.