

Supporting Information

Antiviral Activity of Self-Assembled Glycodendro[60]fullerene Monoadducts

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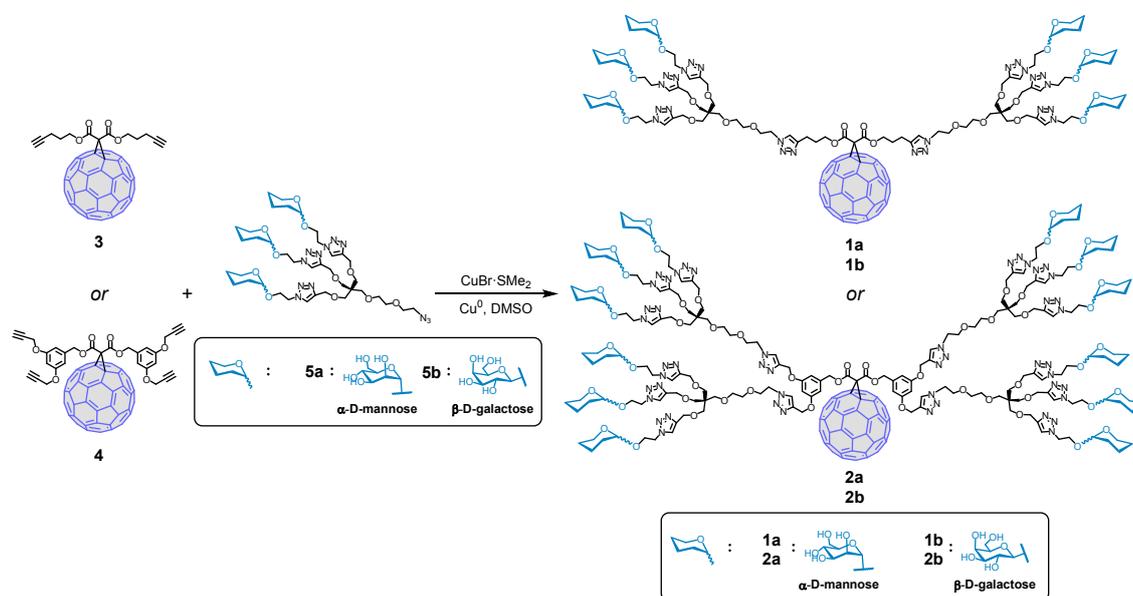
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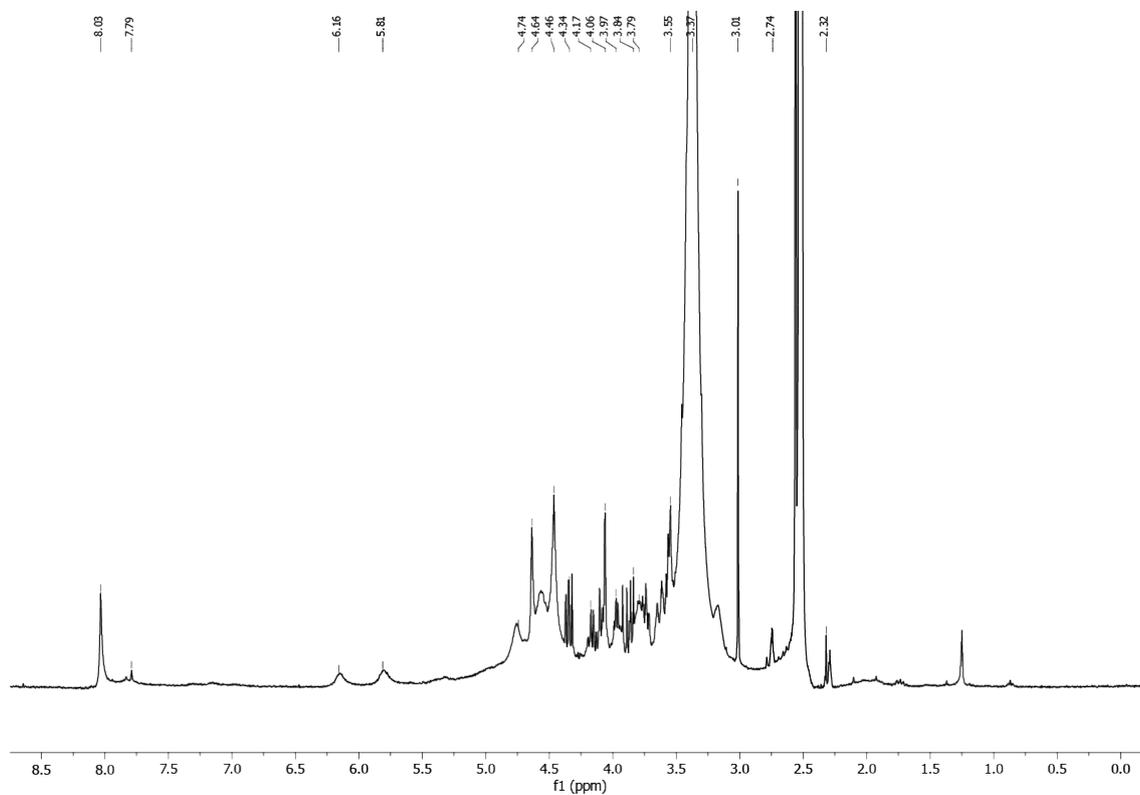
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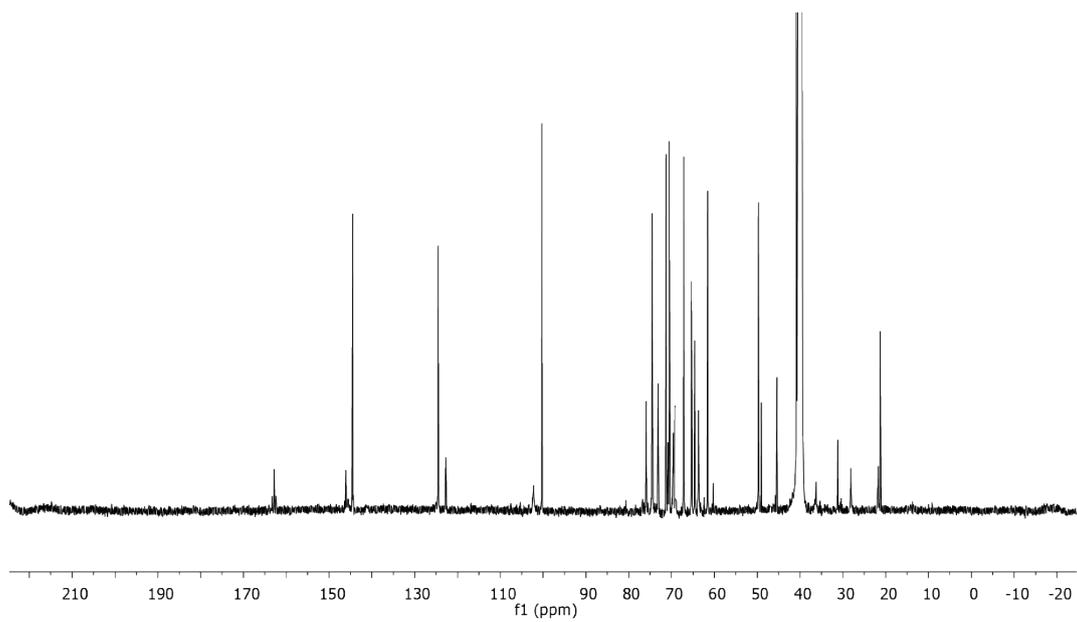
Synthesis and characterization.-



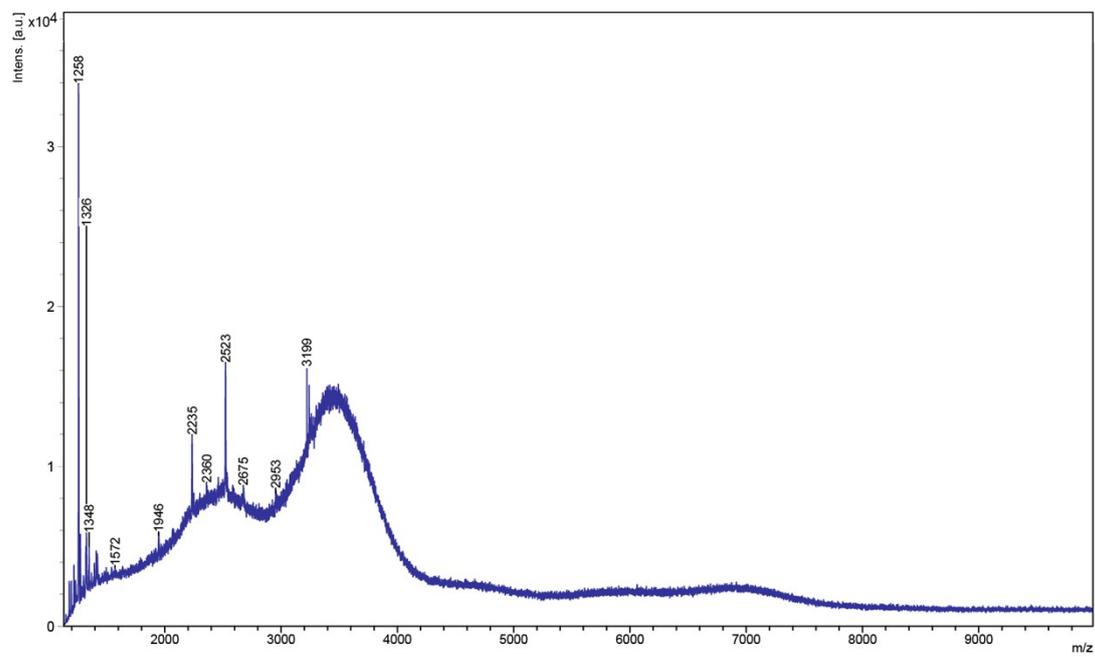
Scheme S1. Synthesis of glycodendrofullerenes **1a-b** and **2a-b**.



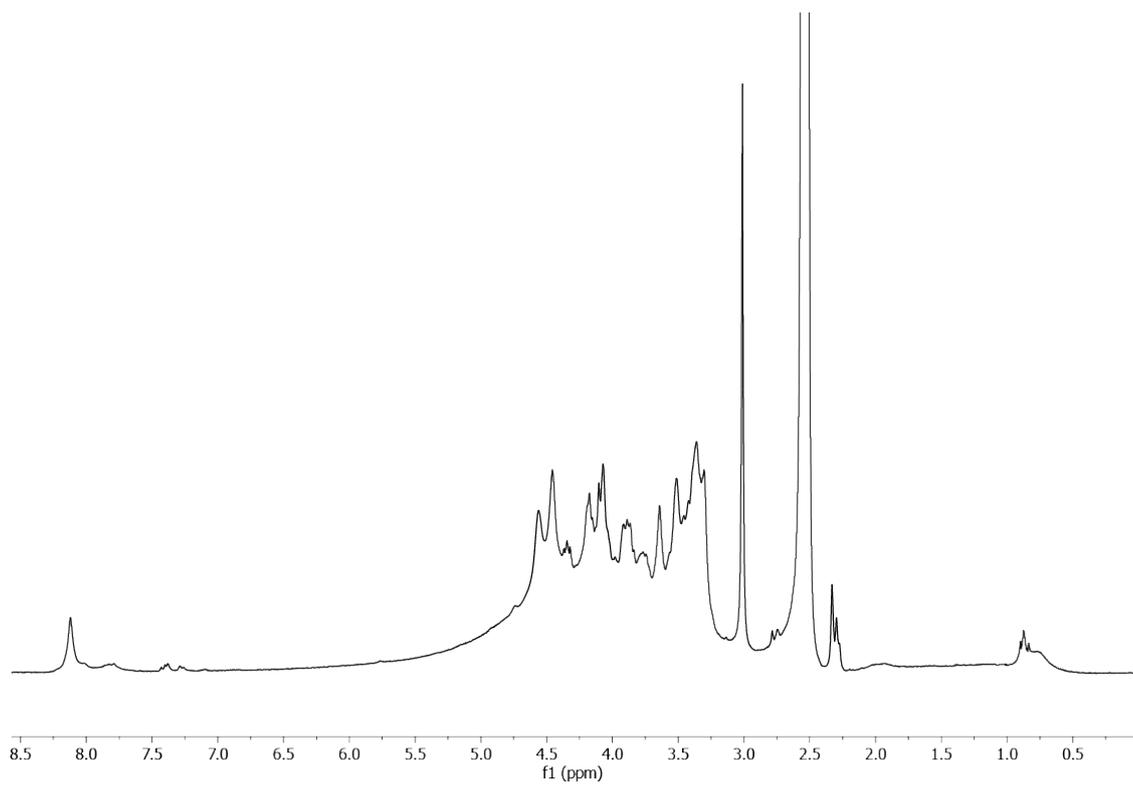
$^1\text{H-NMR}$ of **1a** (300 MHz, DMSO-d_6)



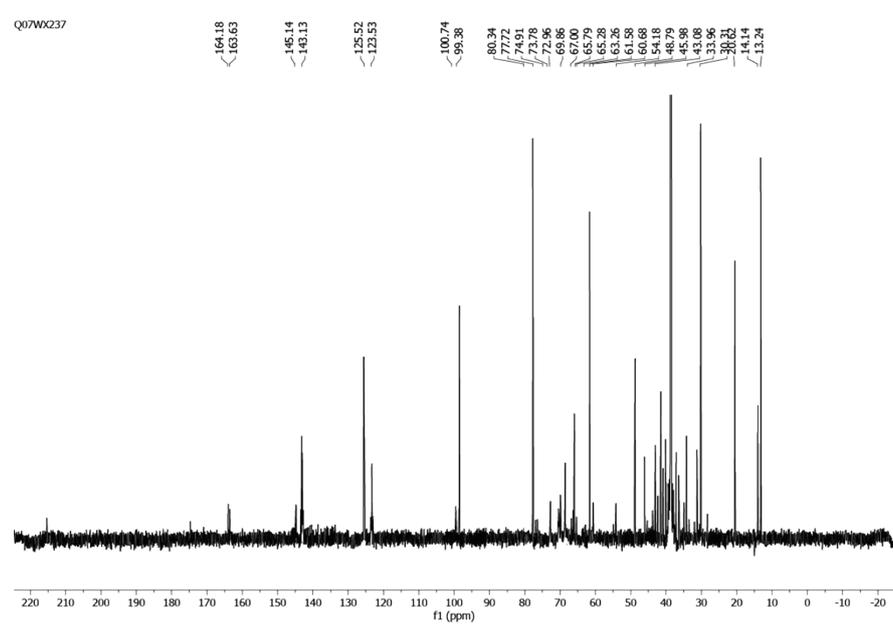
^{13}C NMR of **1a** (175 MHz, DMSO-d_6)



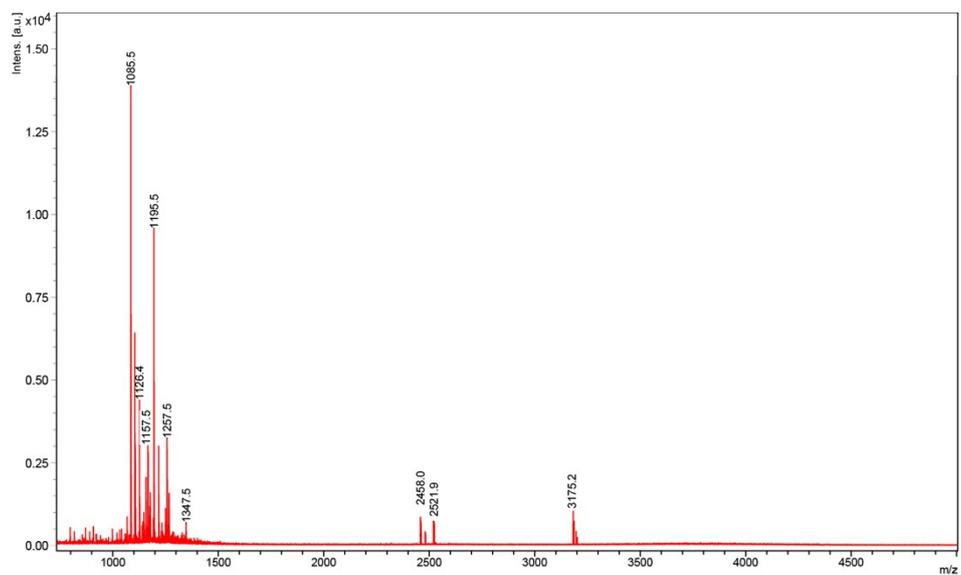
MS of **1a** (MALDI-TOF) Lineal Mode



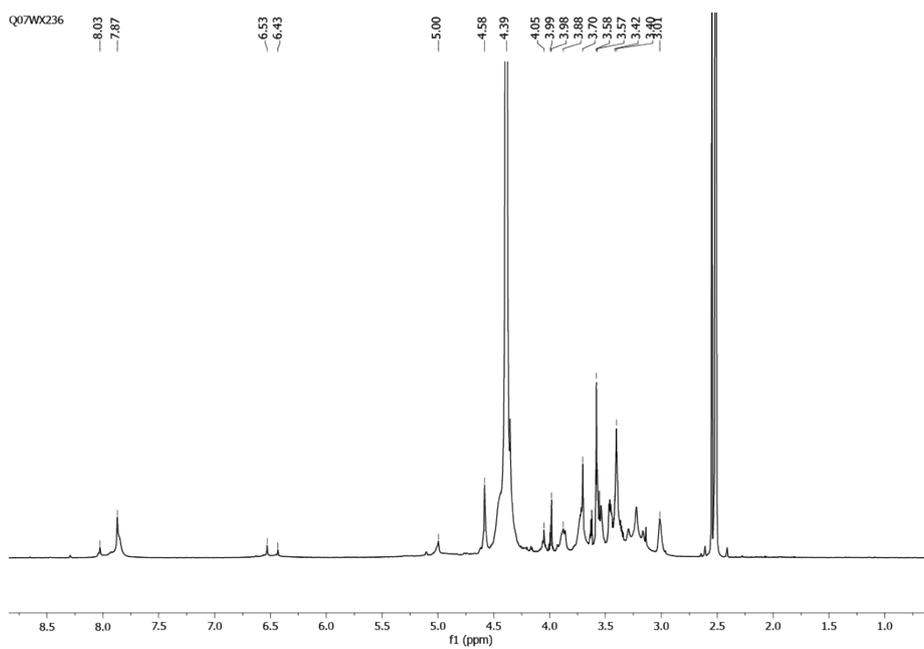
¹H-NMR of 1b (300 MHz, DMSO-d⁶)



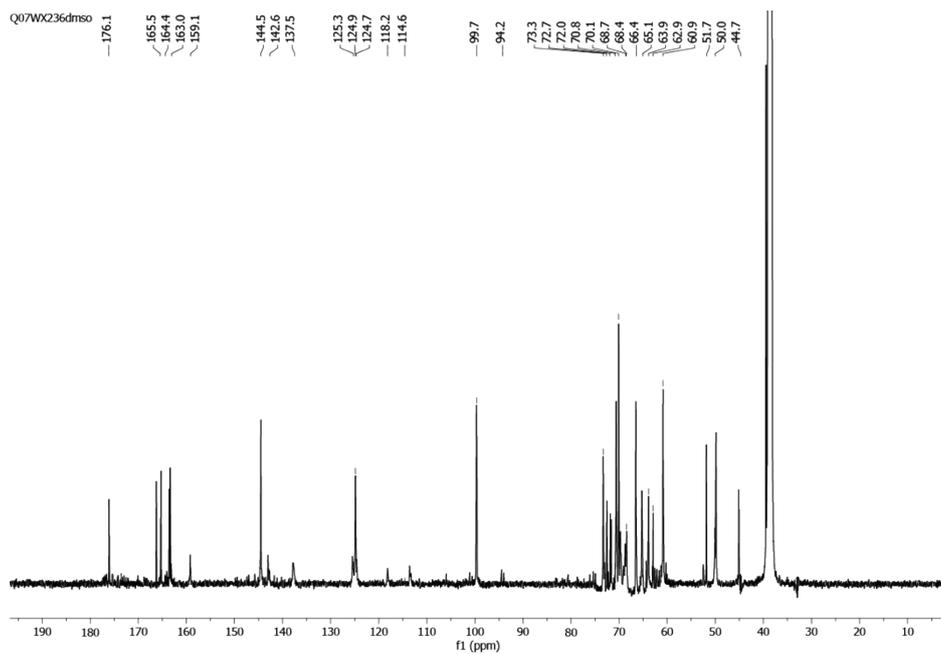
¹³C NMR of 1b (175 MHz, DMSO-d⁶)



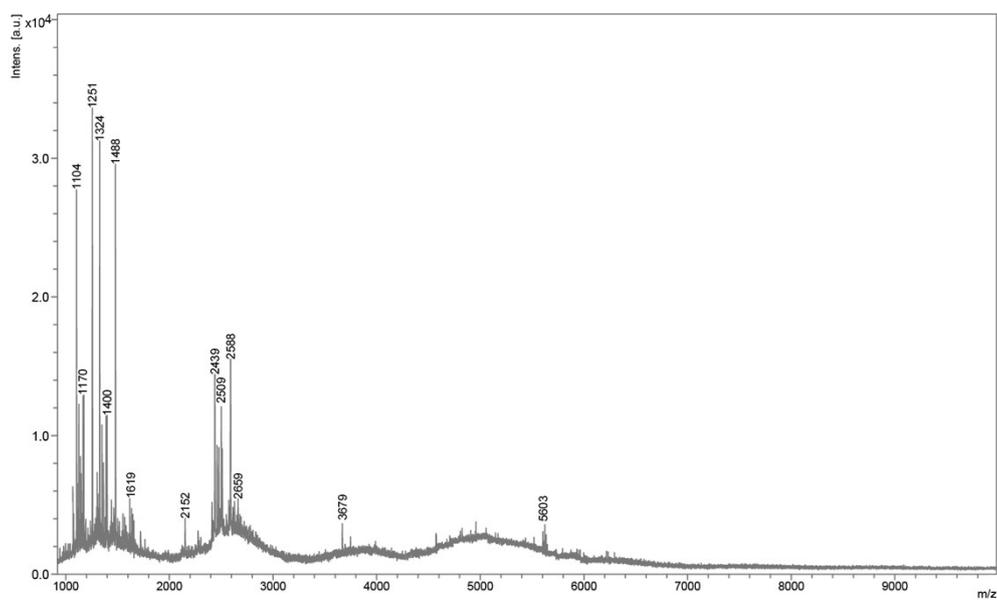
MS of **1b** (MALDI-TOF) Reflector Mode



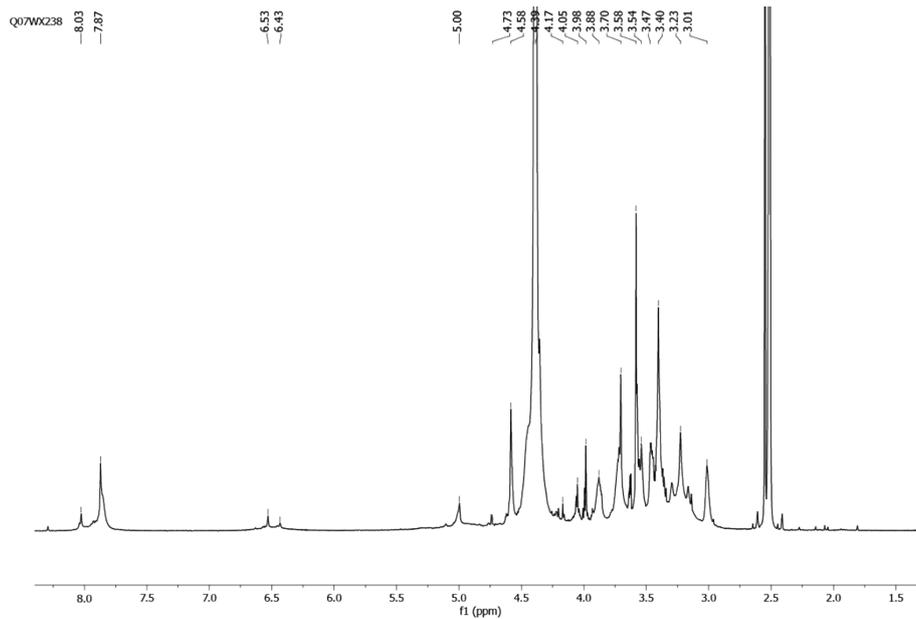
^1H NMR of **2a** (700 MHz, DMSO-d_6)



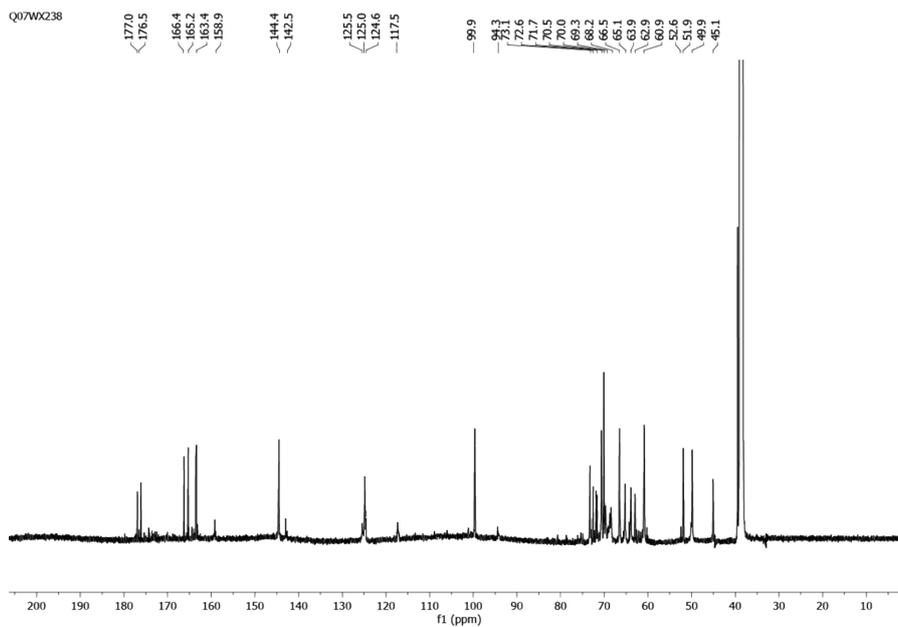
^{13}C NMR of **2a** (175 MHz, DMSO-d_6)



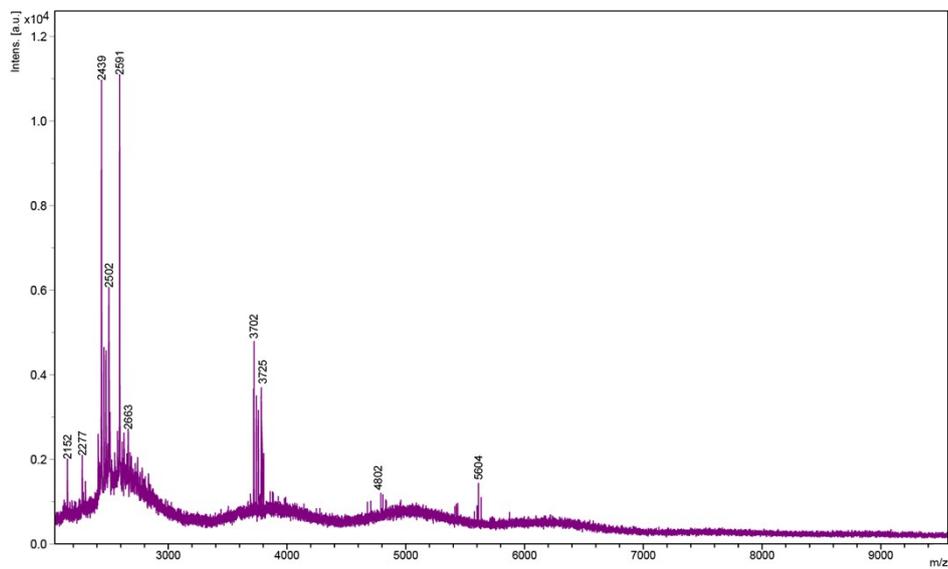
MS of **2a** (MALDI-TOF) Lineal Mode



^1H NMR of **2b** (700 MHz, DMSO-d_6)



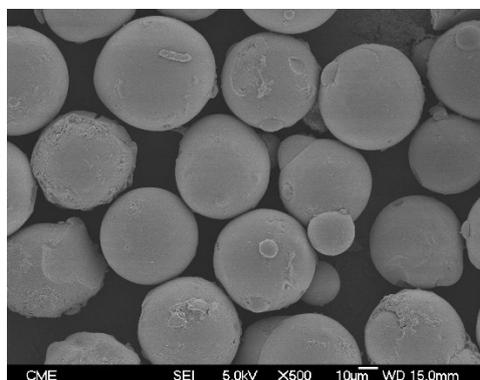
^{13}C NMR of **2b** (175 MHz, DMSO-d_6)



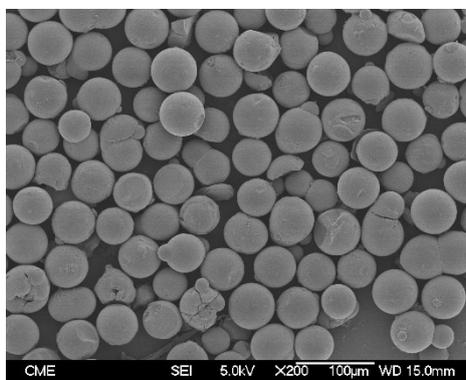
MS of 2b (MALDI-TOF) Lineal Mode

SEM images.-

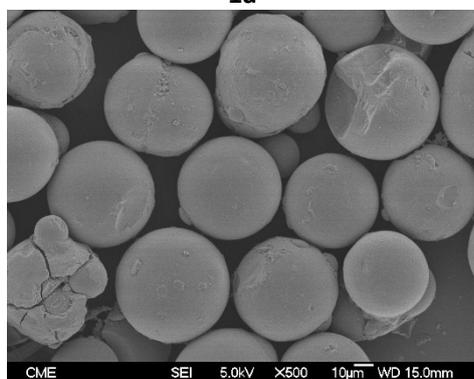
General Procedure.- The SEM-FEG samples were prepared by depositing a water solution at a concentration of 10^{-6} M. The samples were deposited by drop casting and dried under vacuum overnight at room temperature. The samples were metallized with gold atoms in a Sputter Coater in high vacuum. SEM images were obtained from a JEOL JSM 6335F microscope working at 5kV or 10kV.



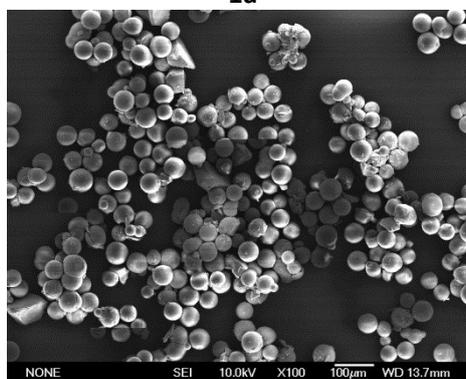
1a



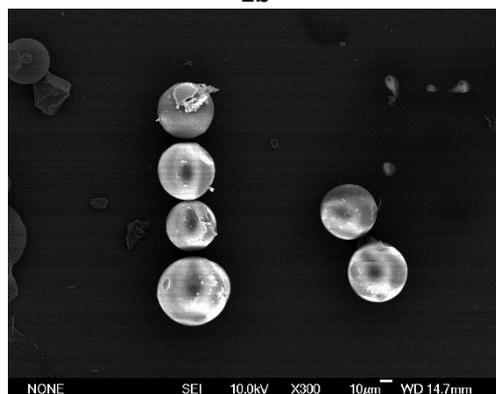
1a



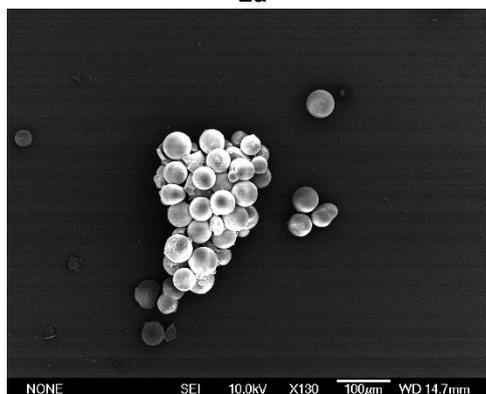
1b



2a



2a



2b

Figure S1.- SEM images of 1a-b and 2a-b.

DLS analysis.-

Dynamic Light Scattering measurements were carried out on an ALV GSC08 correlator working in a cross correlation mode with an Ar⁺ laser operating at $\lambda = 514.5$ nm. The output signals were obtained with backscatter detection at an angle of 90° and processed with a digital correlator that computed intensity-intensity autocorrelation of the scattered light. Measurements were made in a 1 cm path length round quartz cell maintained at 298 K. Solution samples were filtered through nylon Acrodisc syringe filters (Pall Life Sciences) with 0.2 μm pore size.

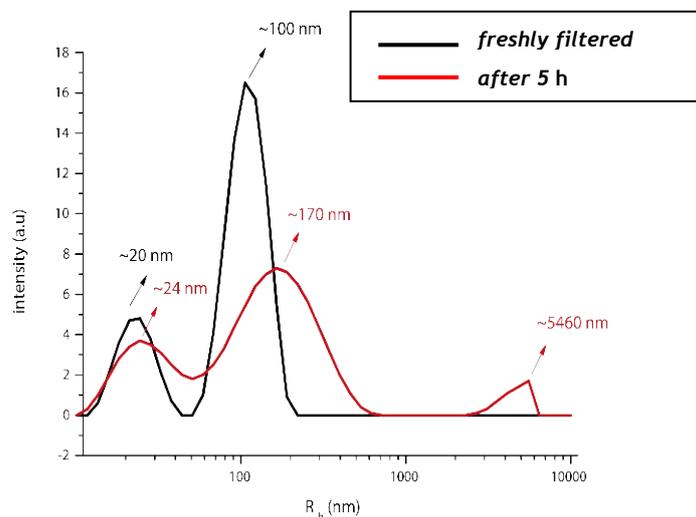


Figure S2.- DLS measurements of **1a** recently filtered (in black) and the same solution after 5h (in red).

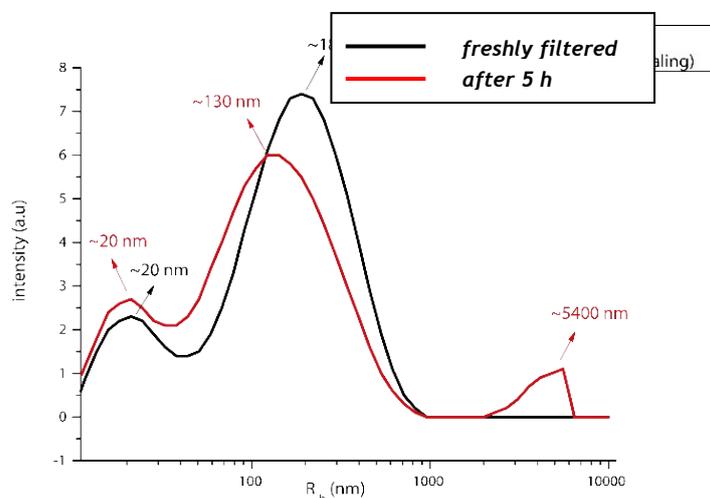
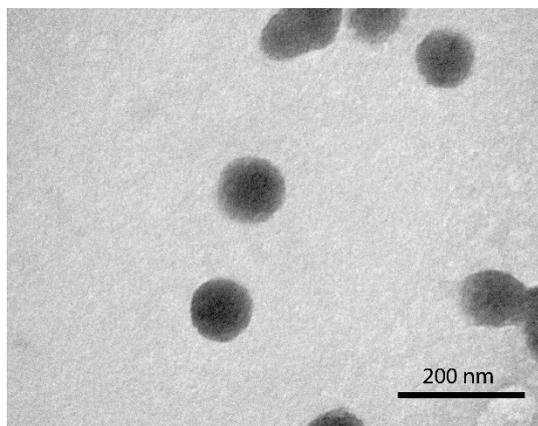
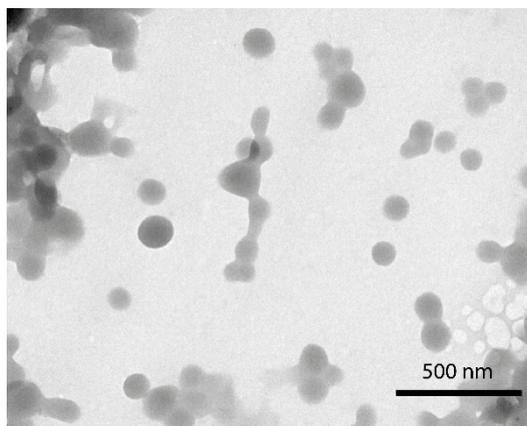


Figure S3.- DLS measurements of **2a** recently filtered (in black) and the same solution after 5h (in red).

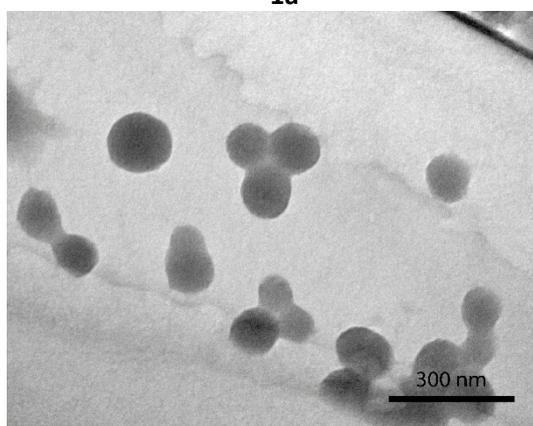
TEM images.-



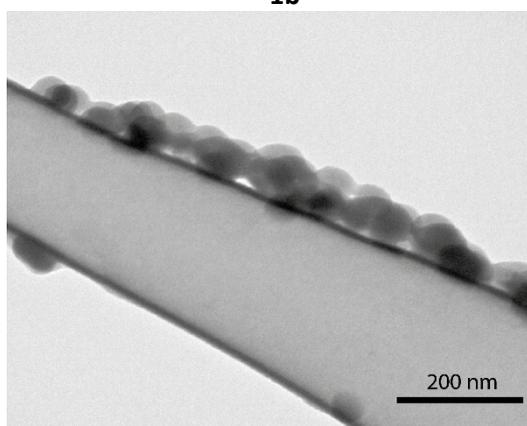
1a



1b



2a



2b

Figure S4. TEM images obtained from 10^{-6} M water solutions of **1a-b** and **2a-b**.

Small Angle X-Ray Scattering (SAXS) characterization.-

Measurements were performed in water solution at a concentration of 10^{-6} . The blank was registered with water and this measurement was subtracted to the signal of the sample. The logarithm of the intensity was multiplied by form factor $I(Q)$ corresponding to a spherical shape and was represented versus the parameter q expressed in \AA^{-1} . The spacing can be obtained from $d=2\pi/q$.

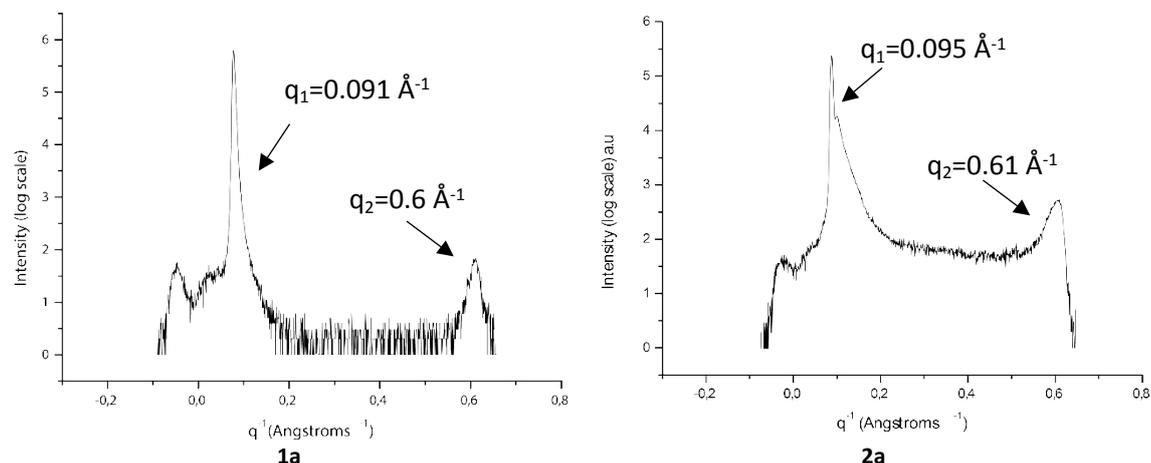


Figure S5. Scattering SAXS corresponding to the spacings of ~ 7 nm and ~ 1 nm in the supramolecular structure of **1a** and **2a**.

XRD Characterization.-

X-ray diffraction was performed in a Panalytical X'Pert PRO diffractometer with Cu ($\lambda=1.54 \text{ \AA}$) tube operated at 45 kV, 40 mA, Ni beta filter, programmable divergence and anti-scatter slits working in fixed mode, and fast linear detector (X'Celerator) working in scanning mode. Samples were deposited on "zero background" silicon sample holders and measured in reflection geometry.

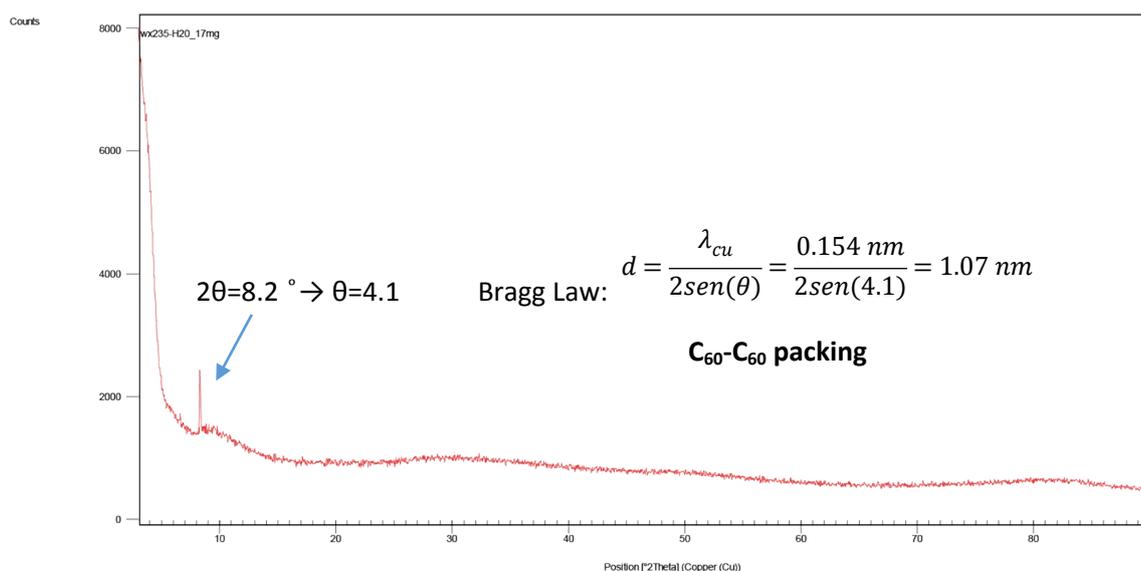


Figure S6. X-ray diffraction patterns of **1a** at room temperature.

Biological assays.-

Production of recombinant viruses

Recombinant viruses were produced in 293T cells. The viral construction was pseudotyped with Zaire Ebola virus (ZEBOV) envelope glycoprotein (GP) or vesicular stomatitis virus envelope GP (VSV-G) and expressed luciferase as a reporter of the infection.^{3, 4} One day (18-24 h) before transfection, 5×10^6 293T were seeded onto 10 cm plates. Cells were cultured in DMEM medium supplemented with 10% heat-inactivated FBS, 25 mg Gentamycin, 2 mM L-glutamine. Few minutes before transfection, the medium on transfection plates was changed to 9 ml DMEM and chloroquine was added to 25 μ M final concentration. Transfection reaction with all reagents at room temperature (RT) was prepared in 15 ml tubes: 183 μ l of 2M CaCl₂, 500 ng of EBOV-GP or 2 μ g of VSV-G, 21 μ g of pNL4-3 luc⁵, 1300 μ l of H₂O. Next, 1.5 ml of 2xHBS (Hepes Buffer Saline) pH 7.00 was added quickly to the tubes and bubbled for 30 seconds. HBS/DNA solution was gently dropped onto medium. After 8 hours of incubation at 37^o C with 5% CO₂, medium on transfection plates was changed to 10 ml DMEM and once again one day after transfection to 7 ml DMEM. Transfection supernatants were harvested after 48 h, centrifuged at 1200 rpm for 10 minutes at RT to remove cell debris, and stored frozen at -80^o C.^{2, 6}

Ebola virus infection experiments

Infection was performed on Jurkat cells (T-lymphocyte cell line) expressing receptor DC-SIGN on its surface. Since Ebola virus does not infect T-lymphocytes, its entry is absolutely dependent on DC-SIGN for infection of Jurkat cells.^{4, 7}

Jurkat-DC-SIGN cells (2.5×10^5) were plated into each well of 96-well plate. Cells were incubated at RT for 20 minutes with the carbohydrate-based compounds and then challenged with 5000 TCID (Tissue Culture Infective Dose) of recombinant viruses. After 48 h of incubation cells were washed twice with PBS and assayed with the Luciferase Assay System (Promega, Madison, WI).

The range of concentrations tested for compounds **1a-b** and **2a-b** was 1 pM – 10 μ M. As a control, experiment of infection with VSV-G pseudoviruses was performed in the same conditions. Infection with VSV-G is independent of the presence of DC-SIGN receptor.

Statistical analysis

The values of percentage of inhibition of the infection presented on the graph correspond to the mean of 6 independent experiments with error bars corresponding to the standard errors of the mean. The IC₅₀s values were estimated using GraphPad Prism v6.0 with a 95% confidence interval and settings for normalize dose-response curves.

Toxicity analysis of compounds 1a and 2a.-

Hela and Jurkat DC-SIGN cells (2×10^4 and 1×10^5 /well respectively) were cultured in a 96-well plate with or without (control) 1a or 2a compounds respectively at the indicated concentrations. After 24 hours, cells were washed with culture medium and viability was measured by a colorimetric method (Cell Titer 96 AQueous Non-Radioactive Cell Proliferation Assay, Promega).

In brief, 20 μ l of the combined MTS/PMS solution (reagent) was combined with 100 μ l of cells in culture medium and further incubated for 2 hours at 37°C in a humidified, 5% CO₂ atmosphere. Absorbance was recorded at 490 nm using an ELISA plate reader.

Cell viability was reported as the percentage of absorbance in treated cells relative to non-treated cells.

Schematic Ebola infection model

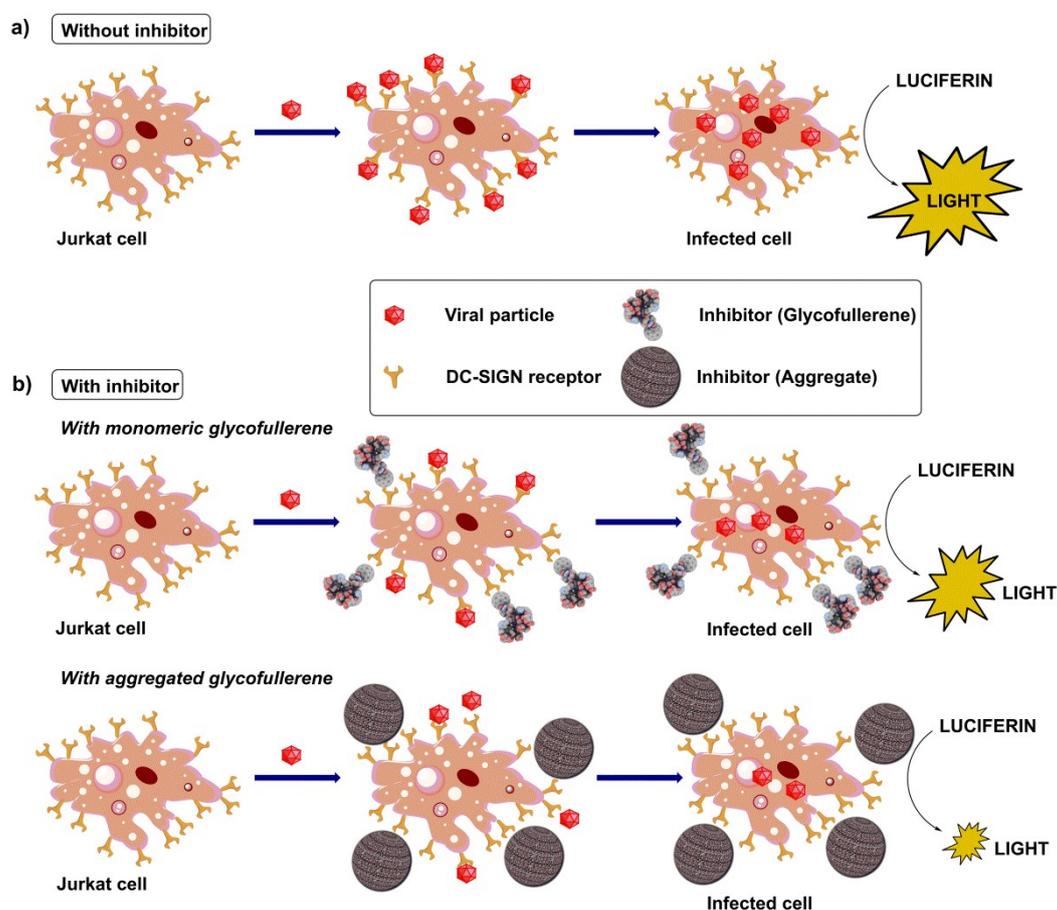


Figure S7. Schematic Ebola (shown in red) infection model and its competitive inhibition with glycofullerenes. a) Without inhibitor. b) In the presence of an inhibitor.

References.-

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