Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information for

Novel cyclodextrin-based pH-sensitive supramolecular host-guest assembly for staining acidic cellular organelles

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1. ESI-MS experiments for the investigation of the inclusion complex composition between compound 4 and β-CD in solution.

Mass spectrometry data were obtained using an Agilent 6520 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS. The LC system was connected directly to ionization source *via* mass spectrometer electrospray (ESI). The selected conditions were: ESI in a positive mode, drying gas debit (N2) 9L/min, gas temperature 325ºC; nebulizer pressure 25 psi, capillary voltage 4200 V; fragmentation voltage 200 V; compounds were investigated in a field of m/z 50–3000.

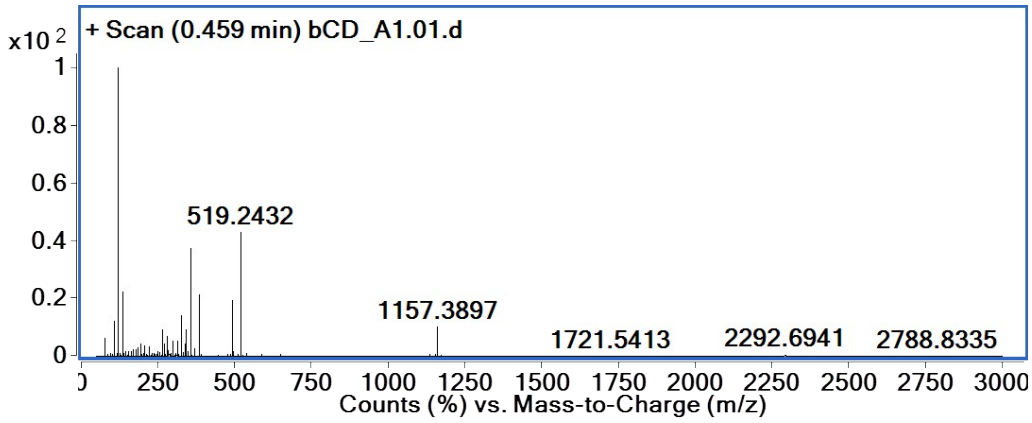

Figure S1. ESI-MS spectrum of the reaction mixture of compound **4** and β-CD at 1:1.5 ratio revealing peaks corresponding to: M^+-Br (519.2432), CD+Na^+ (1157.3897).

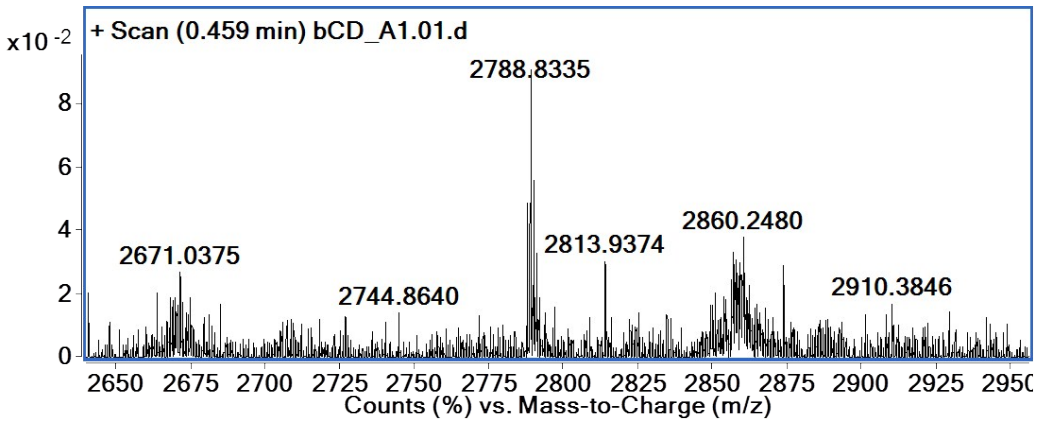

Figure S2. ESI-MS spectrum of the reaction mixture of compound **4** and β-CD at 1:1.5 ratio revealing peaks corresponding to the formation of 2:1 (2788.83 - M^+-Br+2CD).

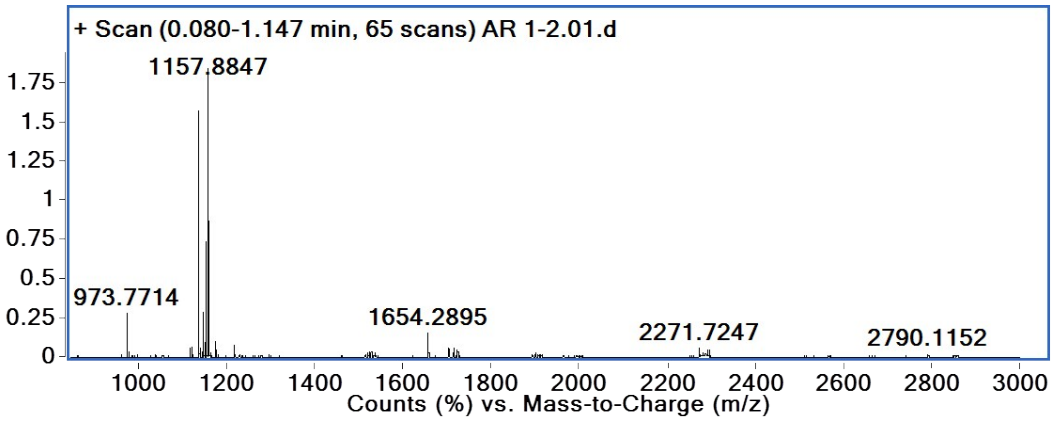

Figure S3. ESI-MS spectrum of the reaction mixture of compound **4** and β-CD at 1:2 ratio revealing peaks corresponding to CD+Na^+ (1157.8847), formation of 1:1 inclusion complex (1654.2895 – M^+-Br+CD) and 2:1 compound **4**:β-CD complex (2790.1152 - M^+-Br+2CD).

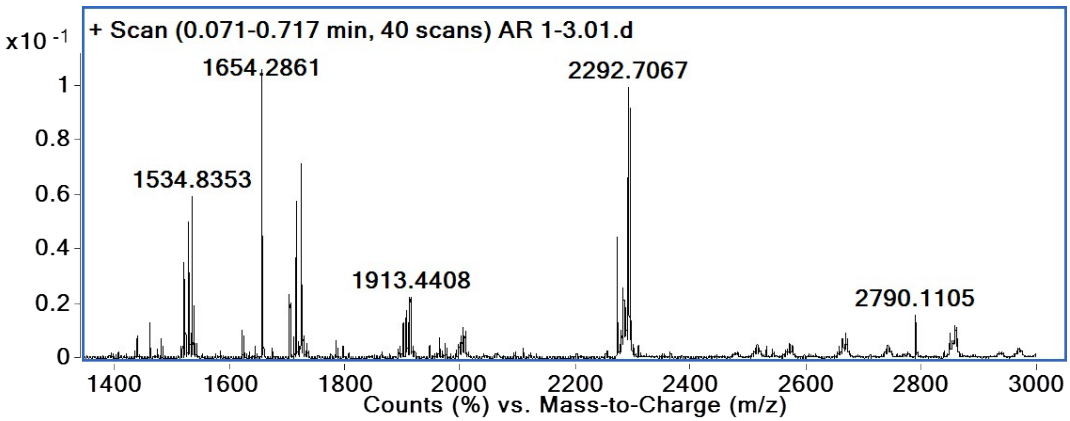

Figure S4. ESI-MS spectrum of the reaction mixture of compound **4** and β-CD at 1:3 ratio revealing peaks corresponding to the formation of 1:1 inclusion complex (1654.2861 – M^+-Br+CD) and 2:1 compound **4**:β-CD complex (2790.1105 - M^+-Br+2CD).

2. Transmission electron microscopy (TEM) analysis.

Transmission electron microscopy (TEM) analysis of the samples was performed on Hitachi HT7700 microscope operating at 100 kV in High Resolution Mode. TEM samples (3 µL) were deposited on 300 mesh carbon-coated copper grids and dried overnight before examination.

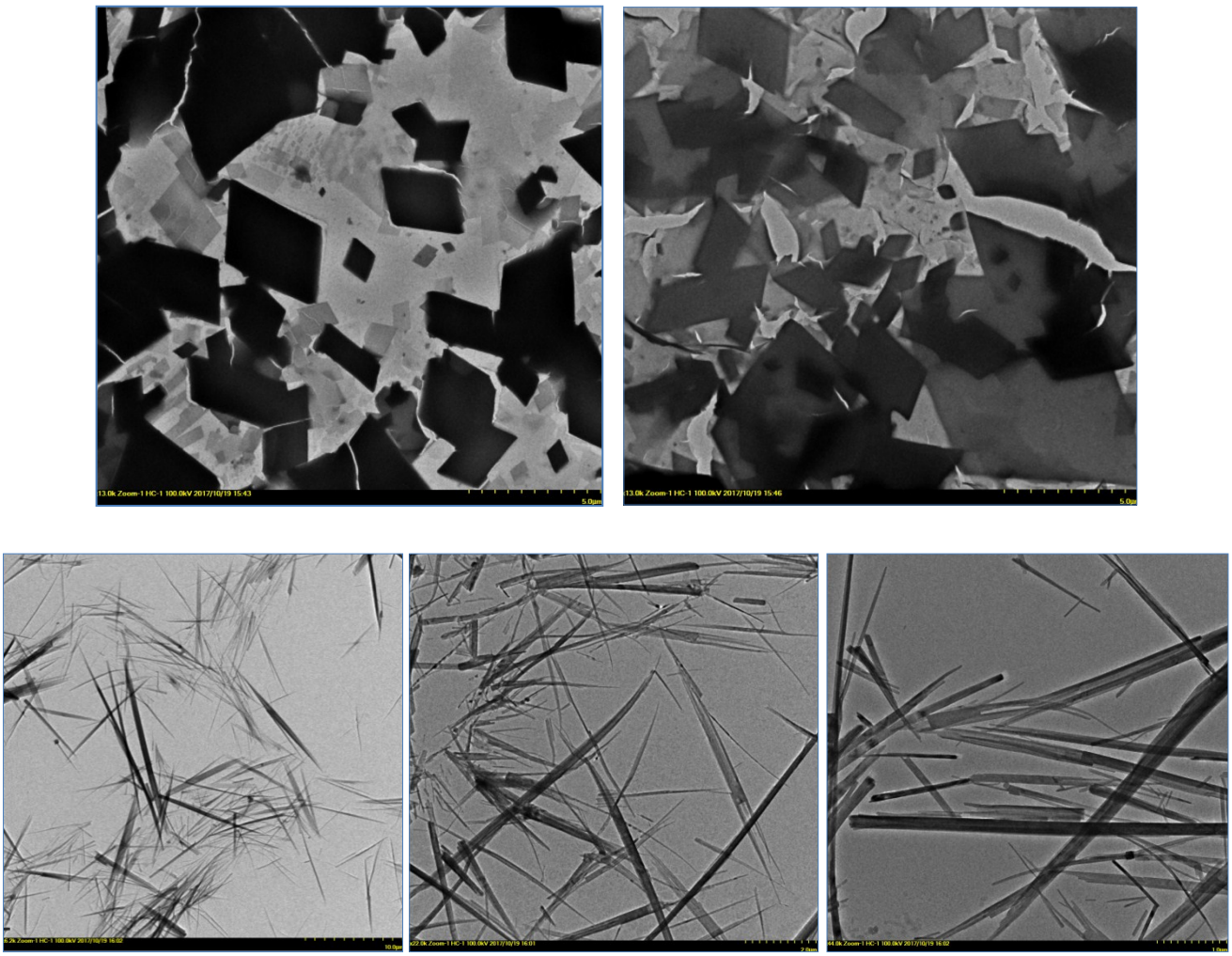

Figure S5. Examples of TEM images of β-cyclodextrin (up, scale bar – 5 µm) and **4_CD** (down, scale bars from left to right – 10 µm, 2 µm, 1 µm).

3. Competitive fluorescence experiment between 4_CD and 1-adamantanecarboxylic acid.

5 µL of **4_CD** stock solution (4 mM of compound **4** in the 1:1.5 inclusion complex with β-cyclodextrin) were diluted in 2995 µL of HCl (0.1 M, pH = 1) and the emission of the solution was measured at λ_{ex}=415 nm (figure S6, black curve). Next, 25 µL of 1-adamantanecarboxylic acid (15 mM), representing an x20 excess in comparison to compound **4** was added and the emission of the solution measured at λ_{ex}=415 nm (figure S6, red curve) after 30 min of incubation in the dark at room temperature. A slight increase in fluorescence together with a shift toward the lower wavelength was observed indicating the partial elimination of compound **4** from the **4_CD**.

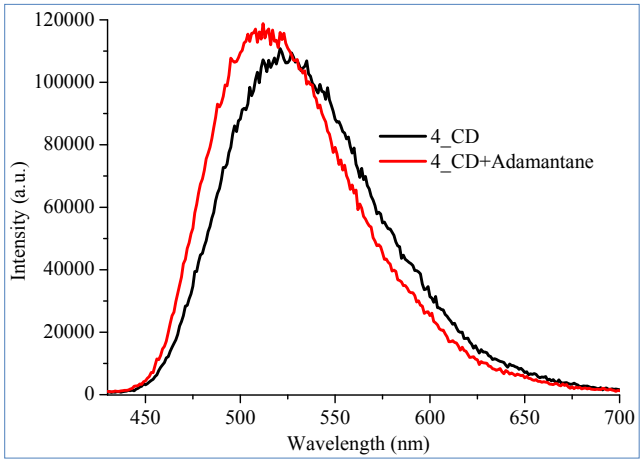

Figure S6. Fluorescence spectra of compound **4_CD** at pH = 1 before (black curve) and after (red curve) the addition of an excess of adamantine carboxylic acid.

4. Job Plot investigations of the inclusion complexes stoichiometry.

Job's method using fluorescence spectroscopy to recognize the stoichiometry of the host-guest inclusion complexes has been applied for the compound **4** and β –CD. A set of solutions for indolizinyl-pyridinium salt **4** and β –CD was prepared varying the mole fraction of the guest in the range 0–1. Stock solution: 10 mM (6 mg of indolizinyl-pyridinium salt **4** in 10 mL of pure water and 11.3 mg of β-CD in 10 mL of pure water). For measurements 10 µL of each stock solution was diluted in 990 µL of water. Job's plot was generated by plotting $\Delta I \times R$ against R, where ΔI is the difference in fluorescence intensity of the indolizinyl-pyridinium salt **4** without and with β-CD and $R = [indolizinyl - pyridinium\ salt\ 4\ ]/([indolizinyl - pyridinium\ salt\ 4\ ] + [\beta - CD])$.

Fluorescence spectra were measured at λ_{ex}=415 nm for each solution at room temperature. The maximum was found at R = 0.36, which suggest the presence of a mixture between 2:1 and 1:1 stoichiometry of the host-guest inclusion complexes.

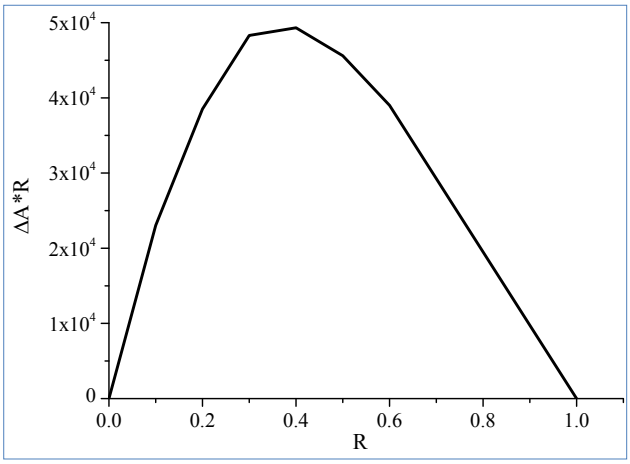

Figure S7. Job Plot for the determination of the stoichiometry of the compound 4 : β-cyclodextrin. The maximum was found at R = 0.36, which suggest the presence of a mixture between 2:1 and 1:1 stoichiometry of the host-guest inclusion complexes.

5. Imaging of living cells using 4_CD solution.

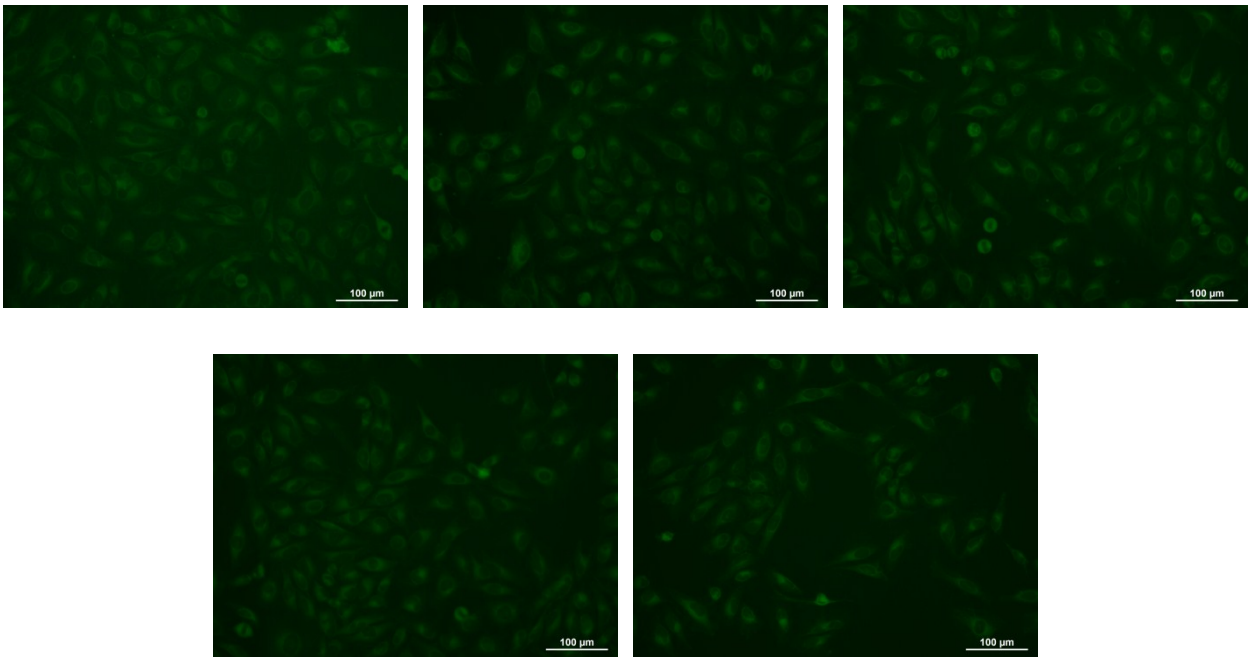

Figure S8. Compound **4_CD** uptake into HeLa cell line after 15 min incubation at concentrations: 17.4 µM (up left), 34.8 µM (up middle), 52.2 µM (up right), 69.6 µM (down left) and 86.9 µM (down right).

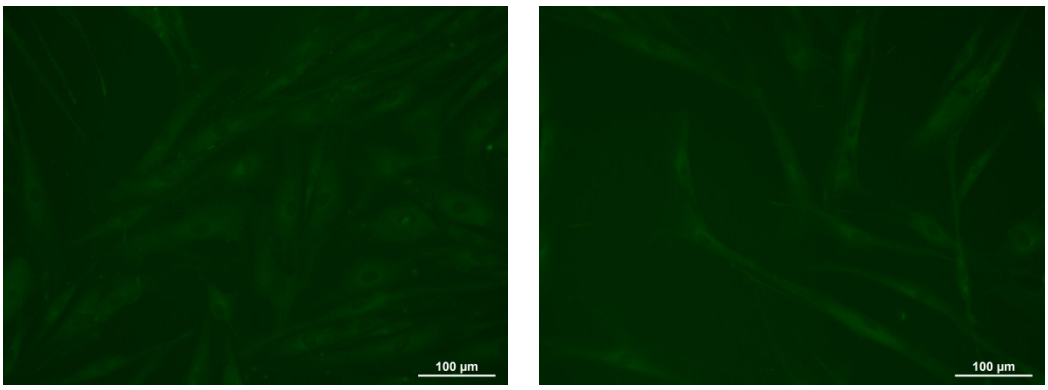

Figure S9. Compound **4_CD** uptake into NHDF cell line after 15 min incubation at 69.6 µM.

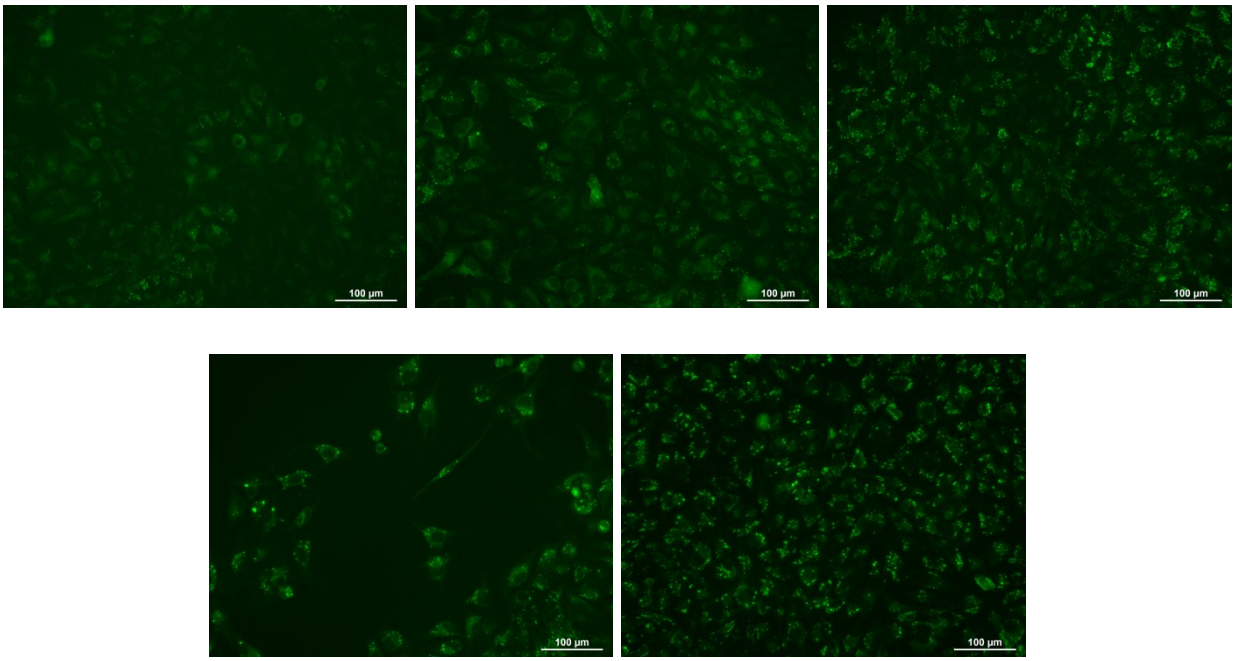

Figure S10. Compound **4_CD** uptake into HeLa cell line after 24 hours incubation at concentrations: 17.4 µM (up left), 34.8 µM (up middle), 52.2 µM (up right), 69.6 µM (down left) and 86.9 µM (down right).

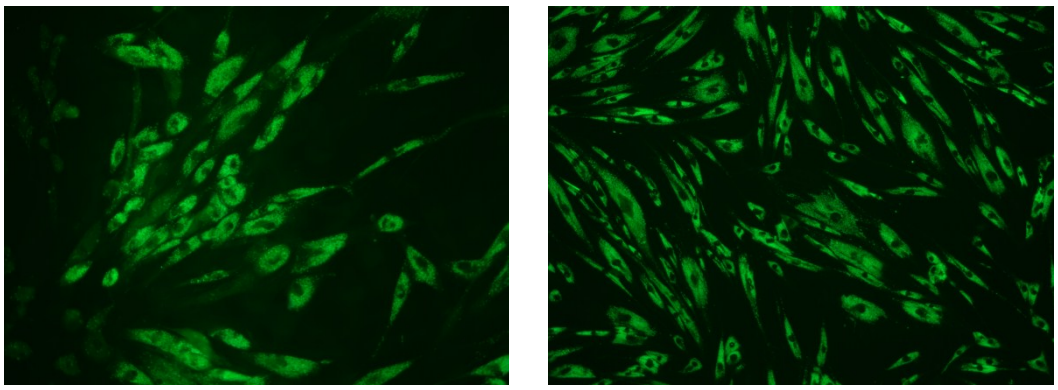

Figure S11. Compound **4_CD** uptake into NHDF cell line after 24 hours incubation at 69.6 μM.