

Supporting Information

Increasing phagocytosis of microglia through targeting CD33 with liposomes displaying glycan ligands

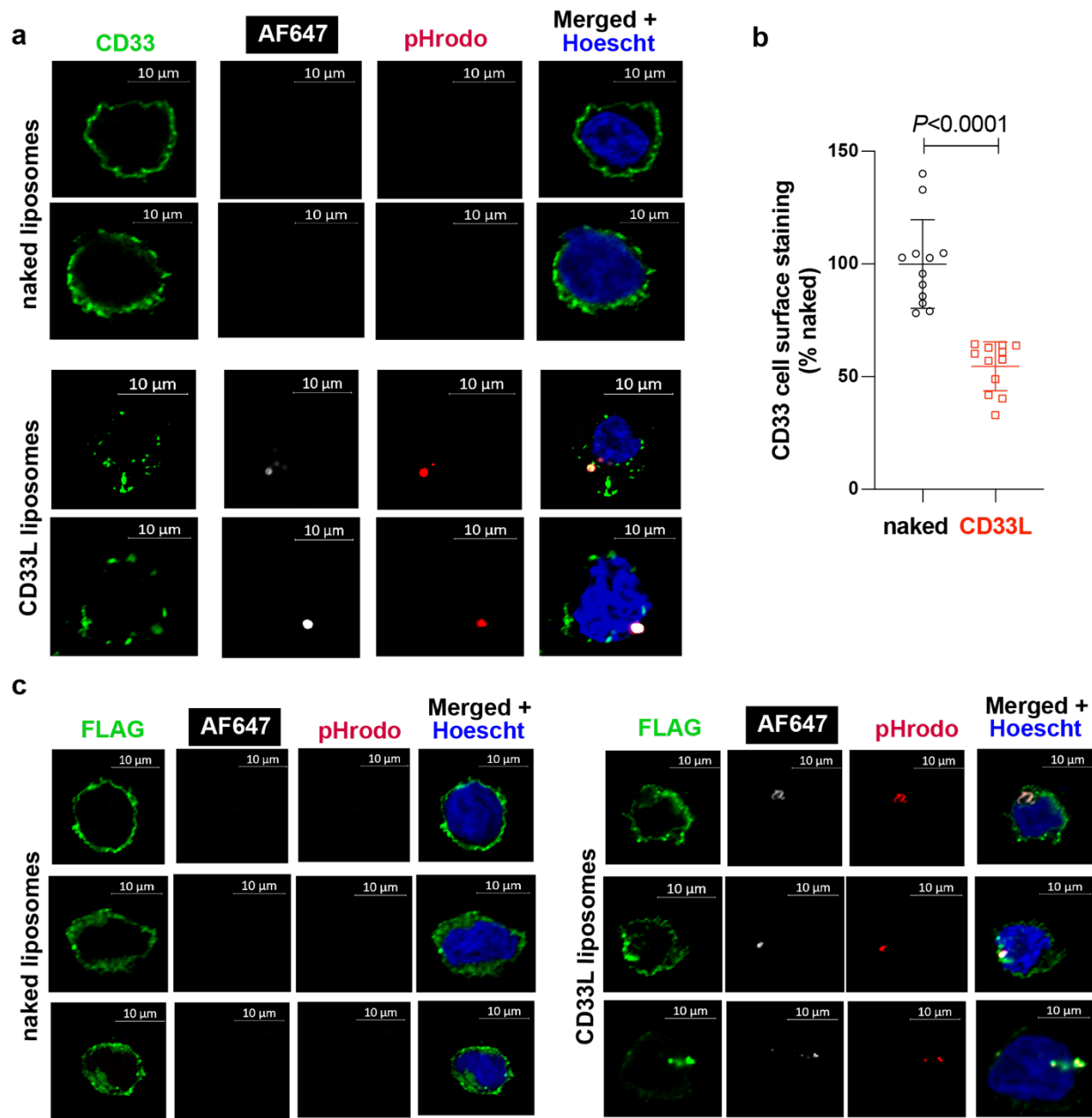
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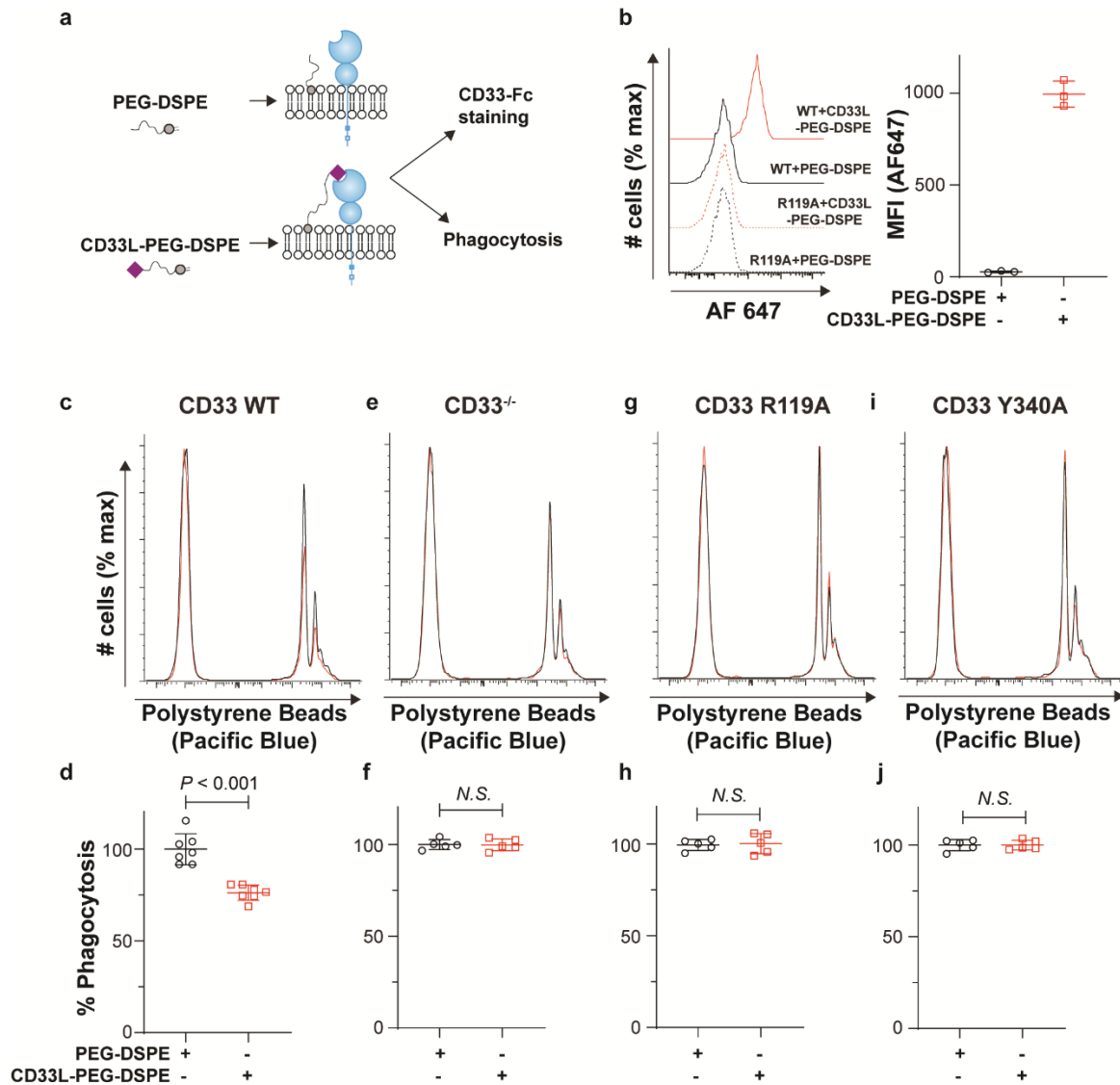
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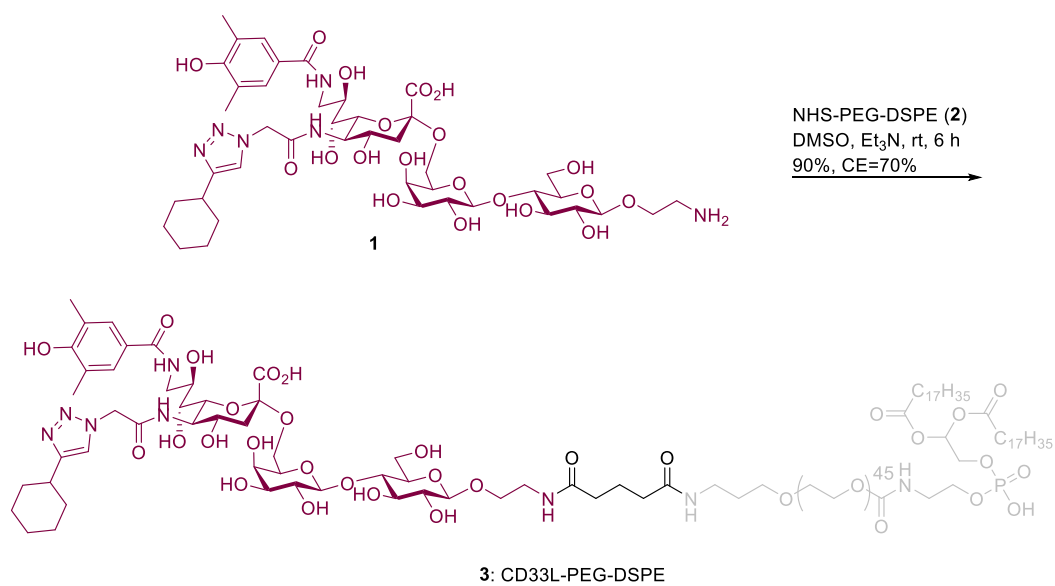
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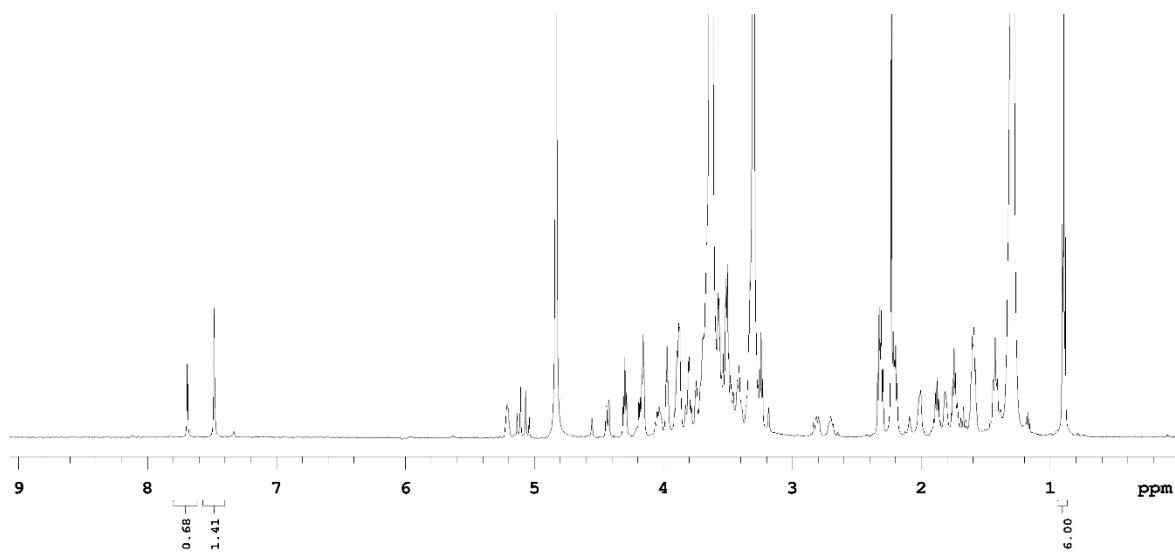
Supplementary Figure 1: Confocal microscopy demonstrates ligand-induced internalization of CD33 upon treatment with CD33L liposomes. (a) CD33^{-/-} U937 cells expressing hCD33-3xFlag were stained with anti-CD33 antibody clone HIM3-4 after treatment with naked or CD33L liposomes for 45 minutes. Following incubation, cells were washed and fixed, but not permeabilized. Two representative cells per group are presented. (b) Loss of CD33 signal from the cell surface was quantified from 12 cells per each group. Statistical analysis was performed using a two-tailed student's t-test. (c) To detect internalization of CD33, another set of cells treated under the same conditions with the liposomes (45 min) were fixed, permeabilized, and stained with anti-FLAG antibody. Three representative images are shown. No fluorescence signal (pHrodo or AF647) coming from internalized liposomes was observed in cells treated with naked liposomes.



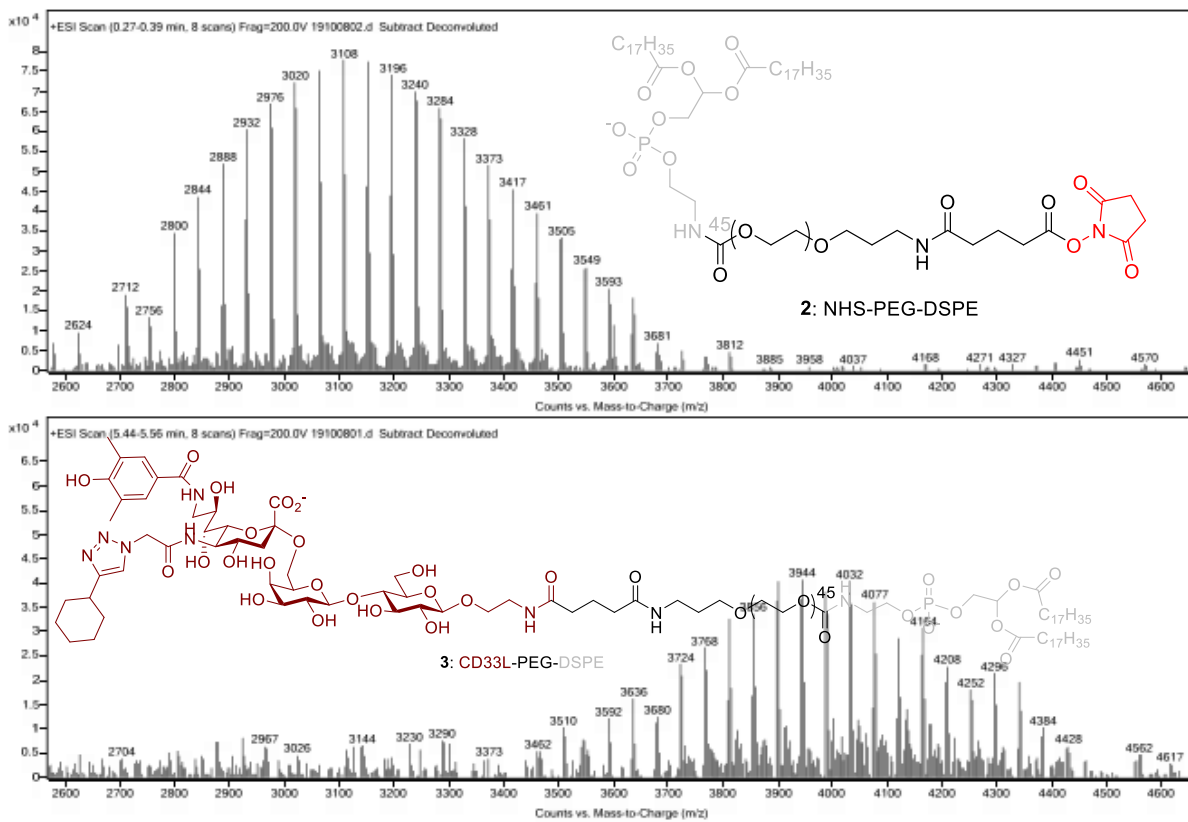
Supplementary Figure 2. Engaging CD33 with *cis* ligands shows a decrease in phagocytosis. (a) Experimental scheme for insertion of PEG-DSPE or CD33L-PEG-DSPE **3** into WT U937 cells following by probing with CD33-Fc or a phagocytosis assay. (b) Staining of U937 cells with WT and R119A CD33-Fc following insertion of PEG-DSPE or CD33L-PEG-DSPE **3**. WT (c,d), CD33^{-/-} (e,f), R119A CD33 (g,h), and Y340A CD33 (i,j) U937 cells were treated with either PEG-DSPE or CD33L-PEG-DSPE **3** prior to performing a phagocytosis assay. Raw flow cytometry plots (c,e,g,i) and summary plots of the % phagocytosis (d,f,h,j) are shown for each cell type. The assay was replicated five-seven times and Statistical significance was calculated based on an unpaired Student's T-test.



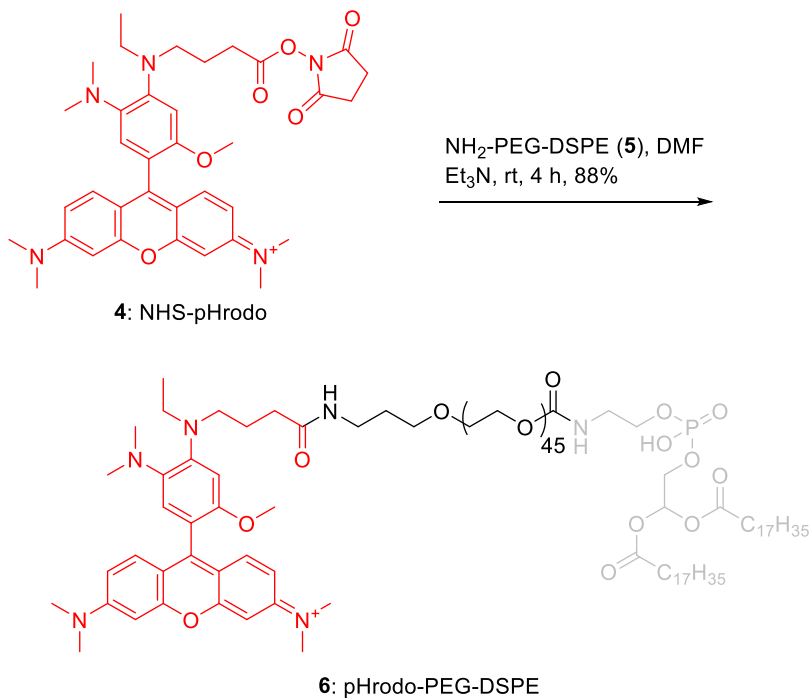
Supplementary Figure 3. Reaction synthesis scheme showing experimental conditions required for CD33L-PEG-DSPE **3** production.



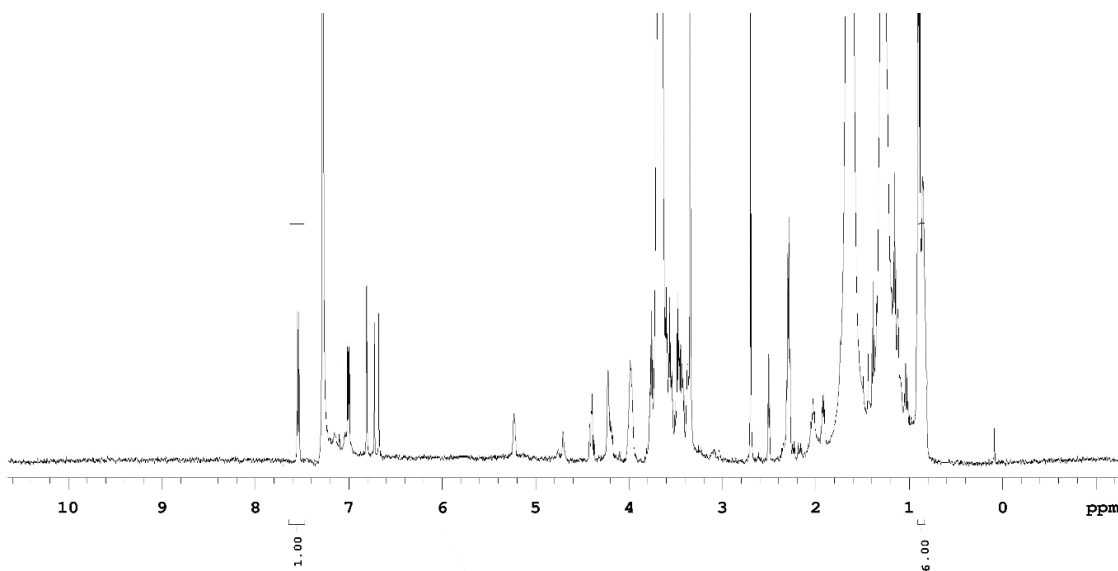
Supplementary Figure 4. Purified CD33L-PEG-DSPE **3** is characterized by ¹H NMR (600 MHz, D₂O).



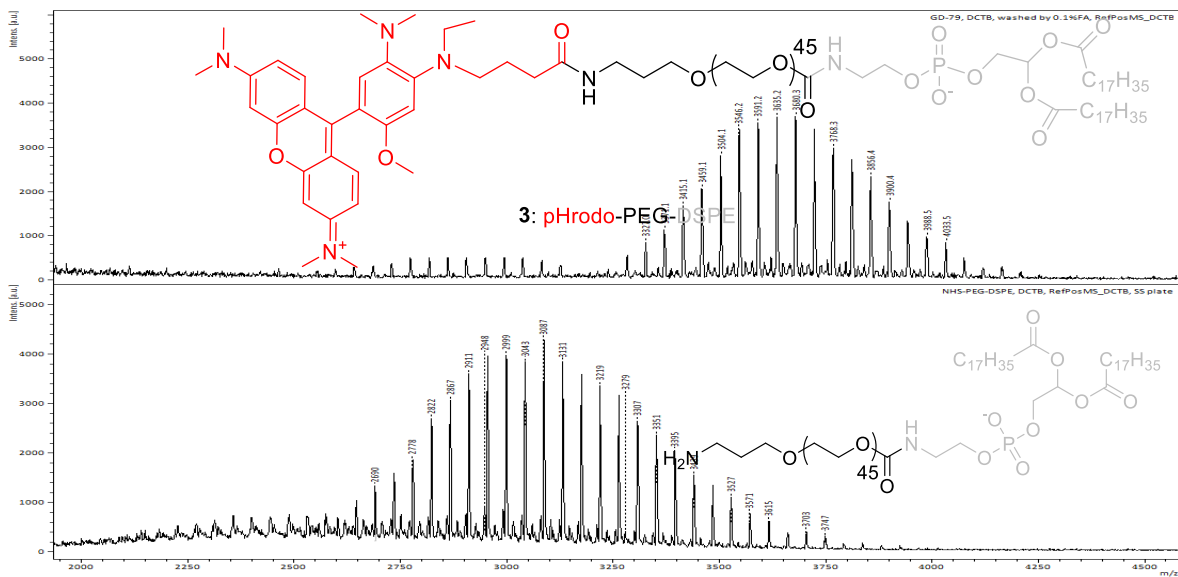
Supplementary Figure 5. Molecular weight of CD33L-PEG-DSPE **3** was determined by MALDI-TOF-MS.



Supplementary Figure 6. Reaction synthesis scheme showing experimental conditions required for pHrodo-PEG-DSPE **6** production.



Supplementary Figure 7. Purified pHrodo-PEG-DSPE **6** is characterized by ^1H NMR (600 MHz, D_2O).



Supplementary Figure 8. Molecular weight of pHrodo-PEG-DSPE **6** was determined by MALDI-TOF-MS.