Supplementary Information

Nanoporous silica nanoparticles functionalized with a fluorescent turn-on spirorhodamineamide as pH indicators.

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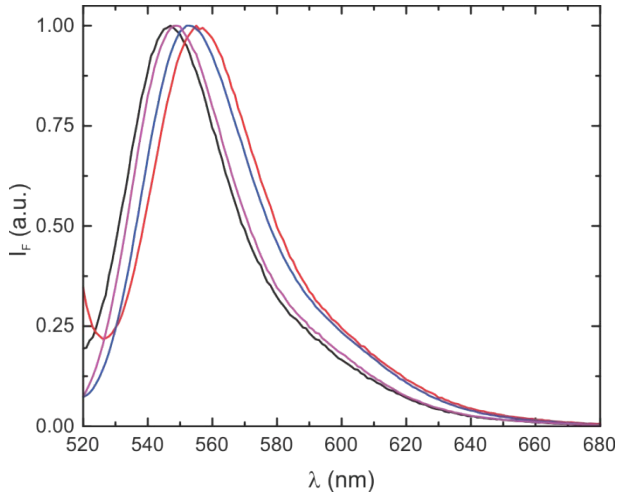


Figure S1. Normalized emission spectra of the NPs equilibrated at pH = 3. Color code: *S*-NPs (blue line), *CC*-NPs (black line), *PG*-NPs (red line), and the free dye **2** (purple line).

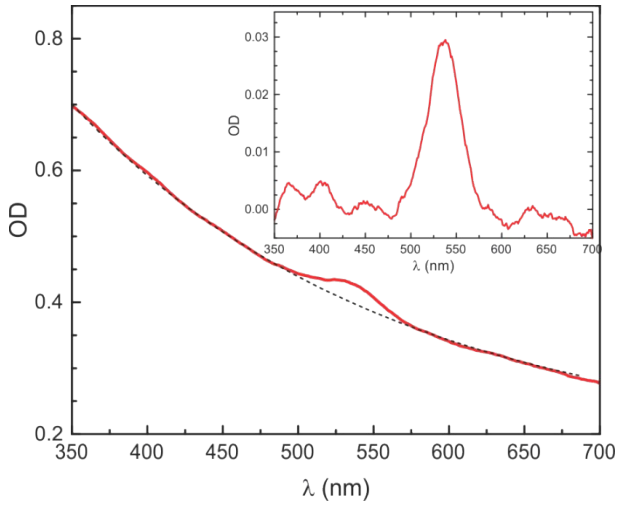


Figure S2. Transmission spectrum of a concentrated dispersion of *S*-NPs equilibrated at pH 3, where most of the SRA is on the protonated open isomer. A baseline corresponding to a λ^{-4} function (dotted line) was subtracted to yield the data shown in the inset.

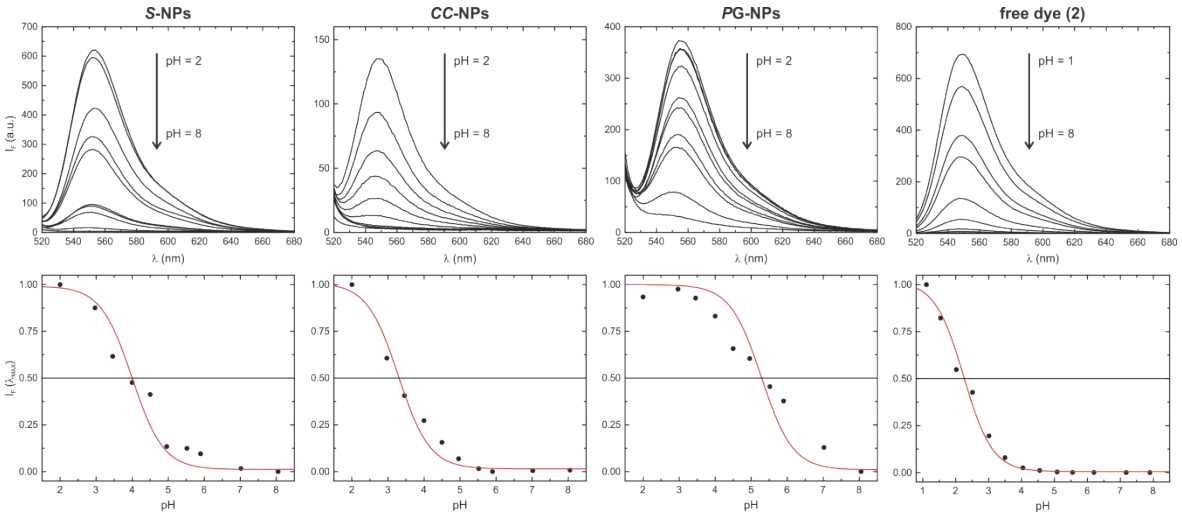


Figure S3. Emission of NPs and the free dye stabilized on buffered aqueous solutions (except for the free dye where 1:1 mixtures of MeOH/buffer solutions were used) at the indicated pH. The emission spectra are shown in the top panels, and the fluorescence intensity at the maximum as a function of the pH, in the lower panel. Red lines show the expected dependence of the fluorescence intensity with pH corresponding to a single pKa value. All the curves in the lower panel are shown together in Figure 4 on the main text.

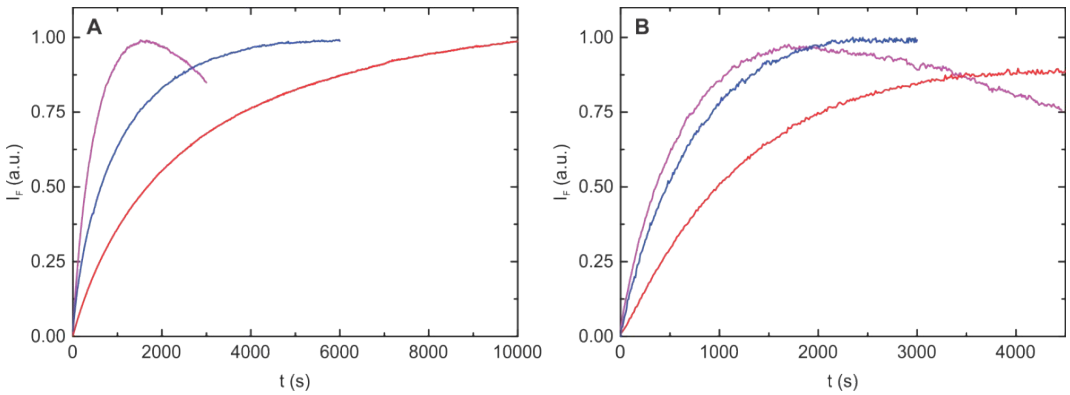


Figure S4. Ensemble kinetics experiments. Normalized fluorescence signal of *S*-NPs (purple lines), *CC*-NPs (red lines) and *PG*-NPs (blue lines) after a pH jump from pH 8 to a final pH of 2 (A) and 4 (B). The drop of the signal in *S*-NPs after reaching the maximum was assigned to aggregation or agglomeration of the NPs, followed by precipitation, caused by the change in the surface charge induced by the presence of positively charged open (protonated) isomer of the probe.

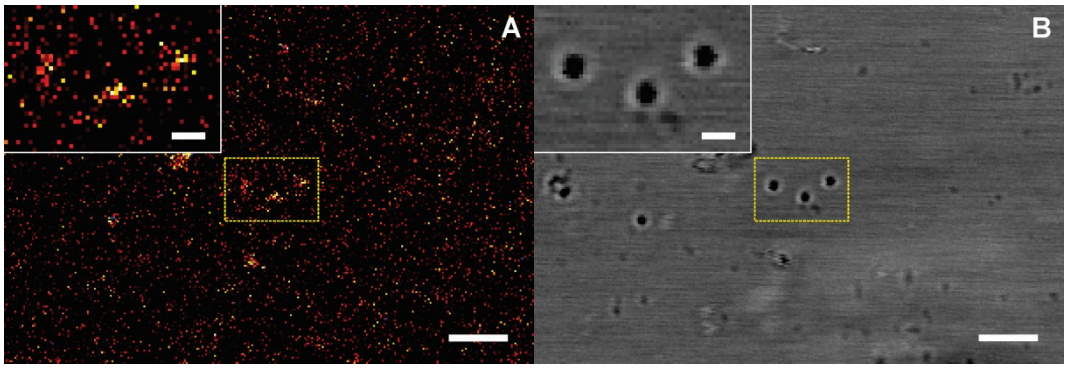


Figure S5. Confocal images of *CC*-NPs on a coverslide (not internalized by a cell), imaged under identical conditions as those described in Figure 7. NPs are observed with a size of around 430 nm, in good agreement with the size determined by SEM. Scale bars: 3 μm and 300 nm (zoomed area).

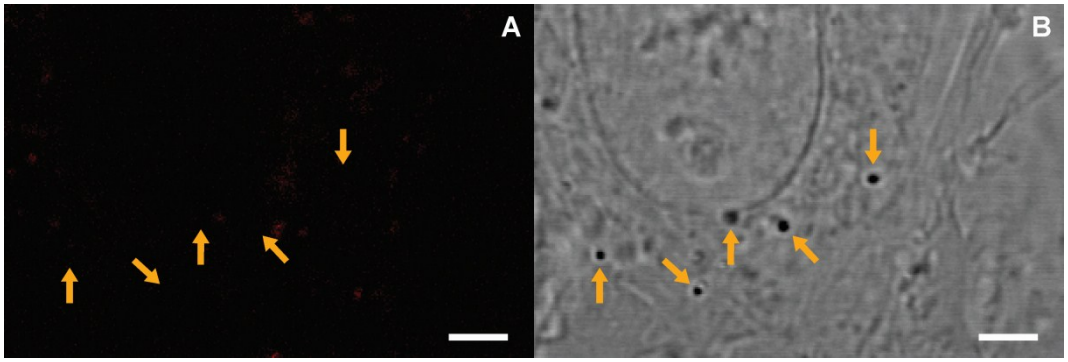


Figure S6. Confocal (A) and transmission (B) images of control cells were no NPs were fed, treated and imaged in the same conditions as those on image 7. Some high-contrast organelles are indicated with orange arrows in the transmission image. However, no significant signal is observed in the fluorescence counterpart. In the latter, only some low-counts autofluorescence is observed, and no evident structures are distinguishable. Scale bars: 3 μm.

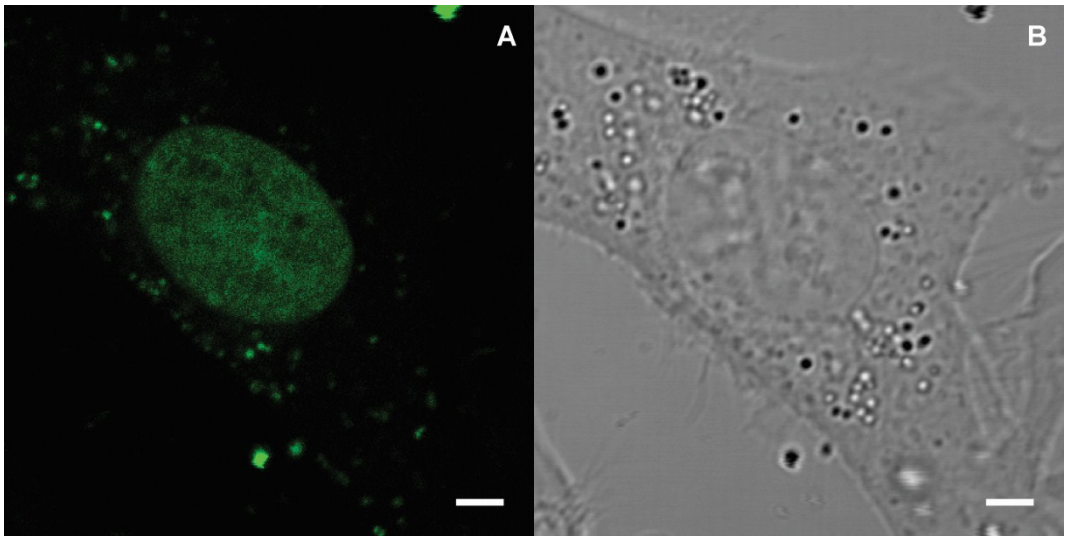


Figure S7. An overview of the cell on Figure 8, with cells endocyted with *S*-NPs. This image was recorded before the lysotracker solution was added to the cells. (A) Fluorescence channel of the sensor (λ_{EX} = 514 nm; λ_{EM} = 525-578 nm); (B) Transmission image. Live cells incorporated the *S*-NPs by endocytosis and lysotracker red was applied to detect lysosomes in a separate emission channel. The cells expressed GFP in the nucleus, whose signal shows partial bleed-through to the sensor channel (A) Scale bars: 3 μm.

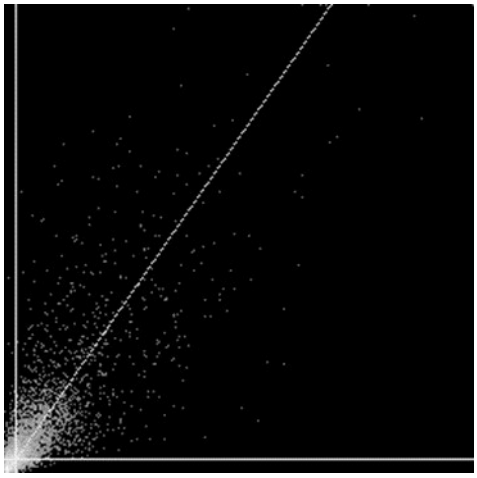
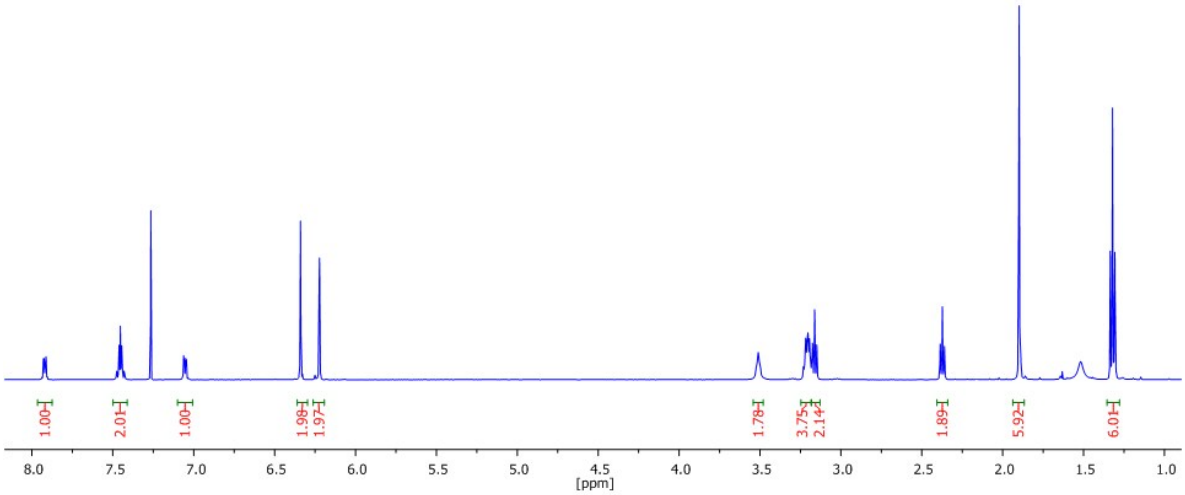


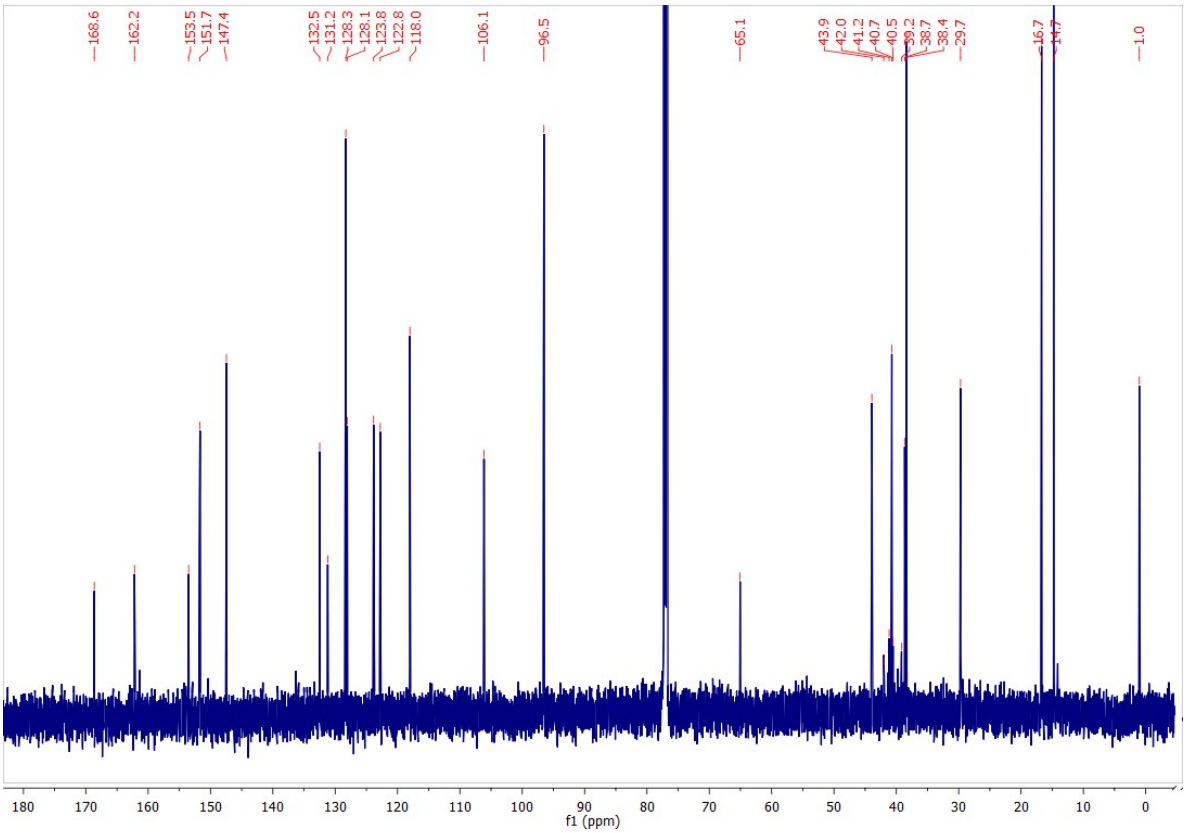
Figure S8. 2D-histogram of pixel intensity from a colocalization analysis performed on the image in Figure 8. A mask was used in the green channel to exclude the area of the nucleus. The colocalization test was performed with the "coloc2" ImageJ [1] plugin (http://imagej.net/Coloc_2).

Figure S9: NMR Spectra

Compound **2:** ^{1}H



Compound **2:** ^{13}C



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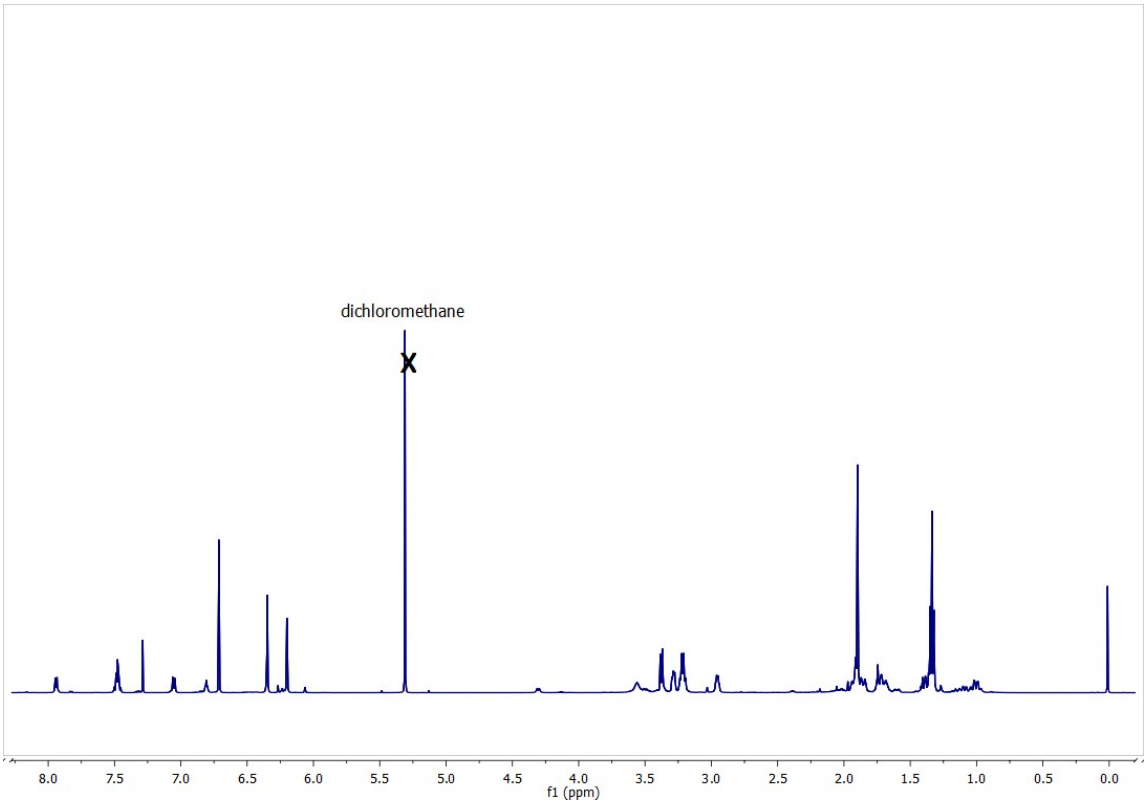
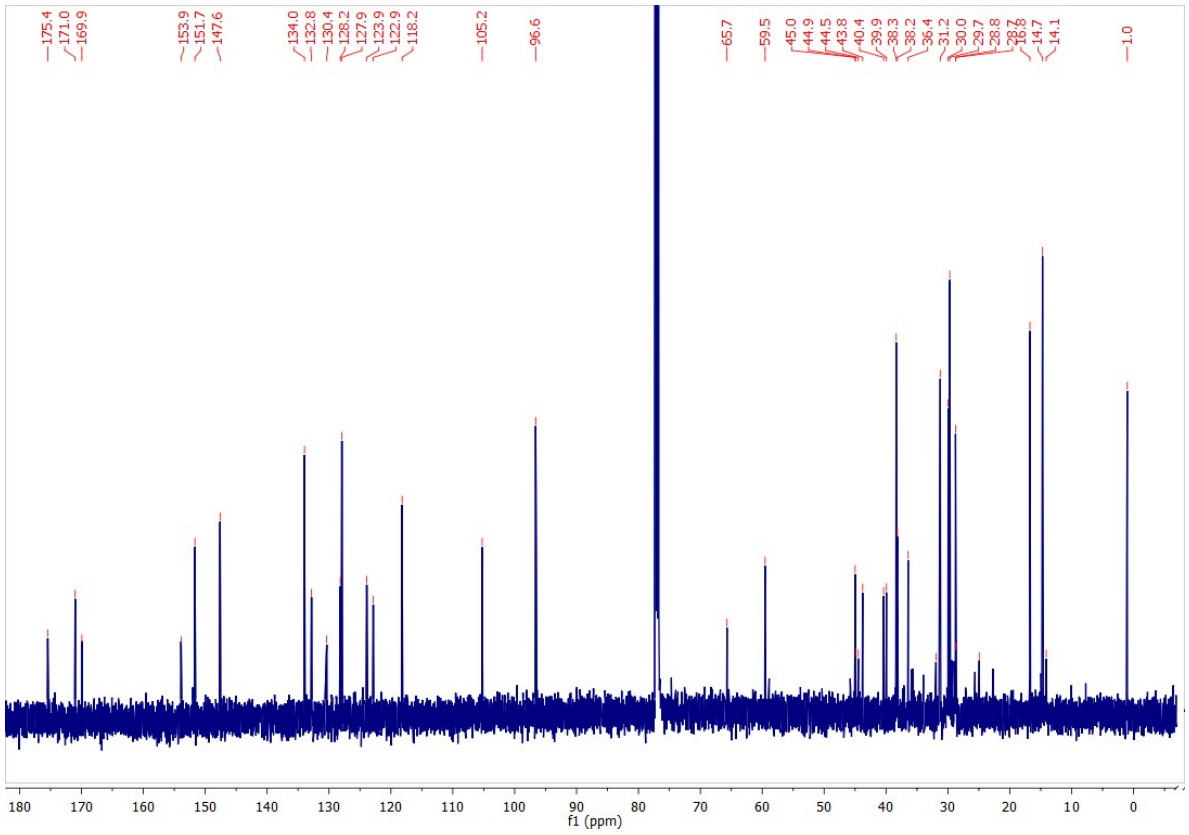


Compound **4:** ^{13}C



References

[1] Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016.