Supporting Information

A “turn-on” chemodosimeter for detection of Cu^{2+} in living cells

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Spectra

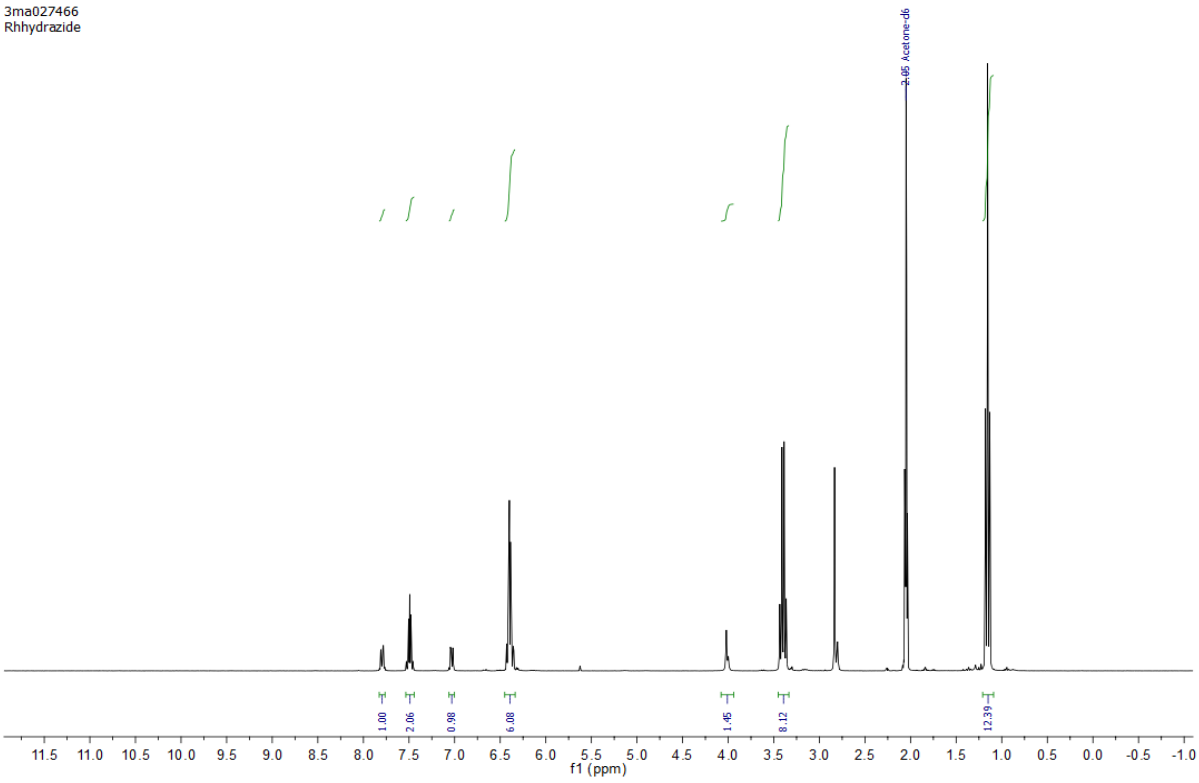

Figure S1. ^{1}H-NMR spectrum of rhodamine B hydrazide (**HCh**) in acetone-d6. The signal at 2.6 ppm corresponds to water.

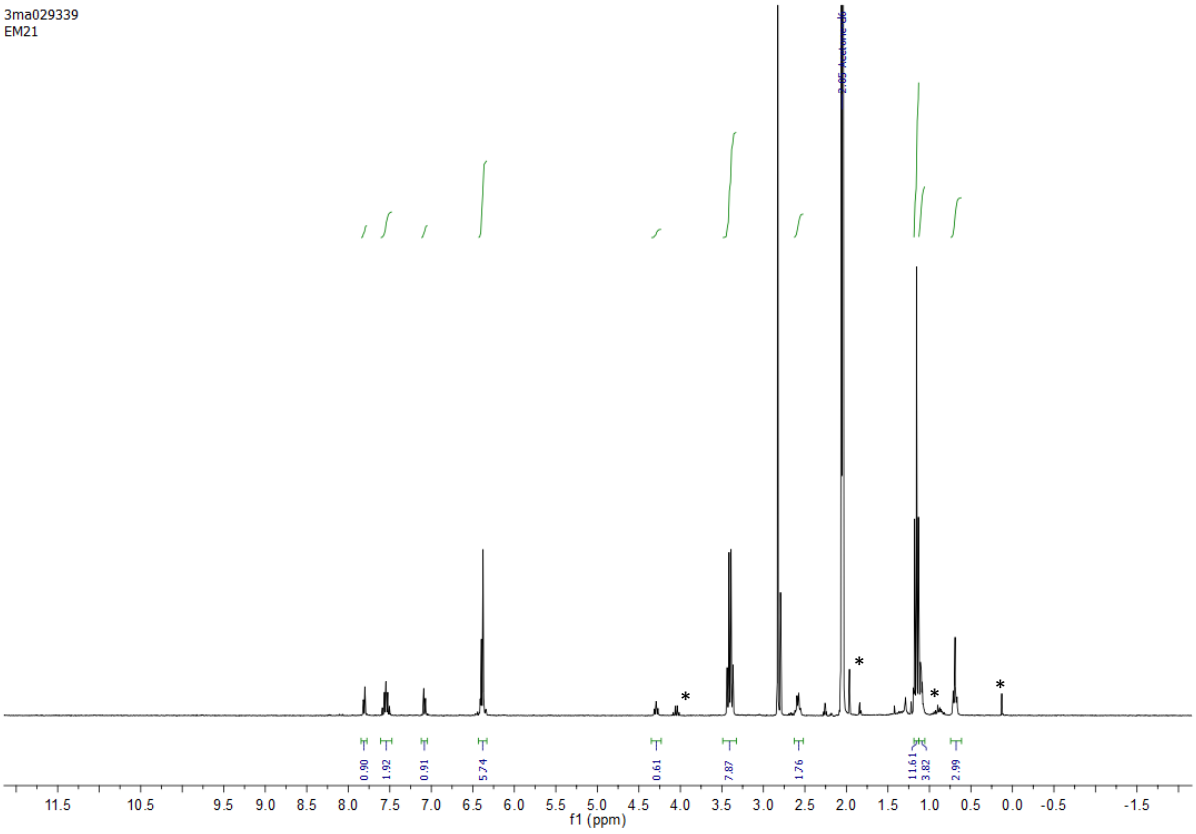

Figure S2. ^{1}H-NMR spectrum of **BuCh** in acetone-d6. The signal at 2.6 ppm corresponds to water. The signals indicated as "*" correspond to ethyl acetate and grease.

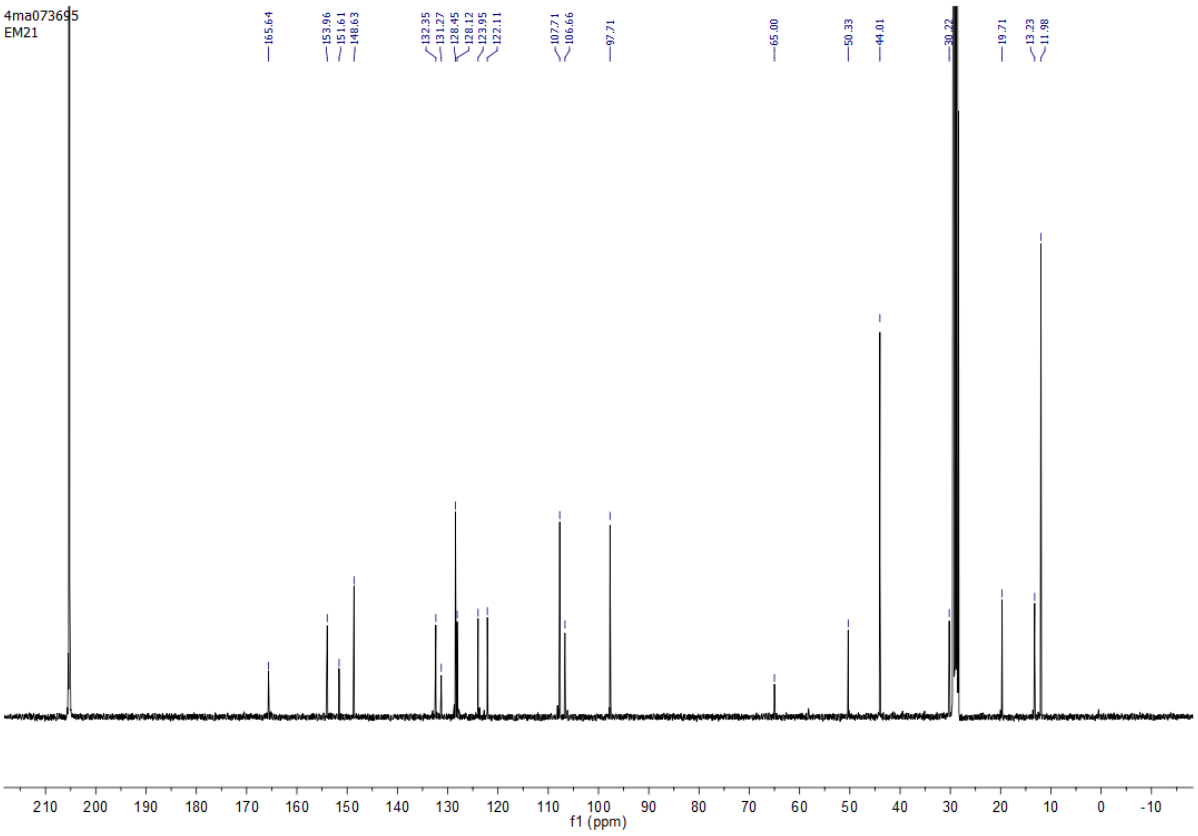

Figure S3. ^{13}C-NMR spectrum of **BuCh** in acetone-d6.

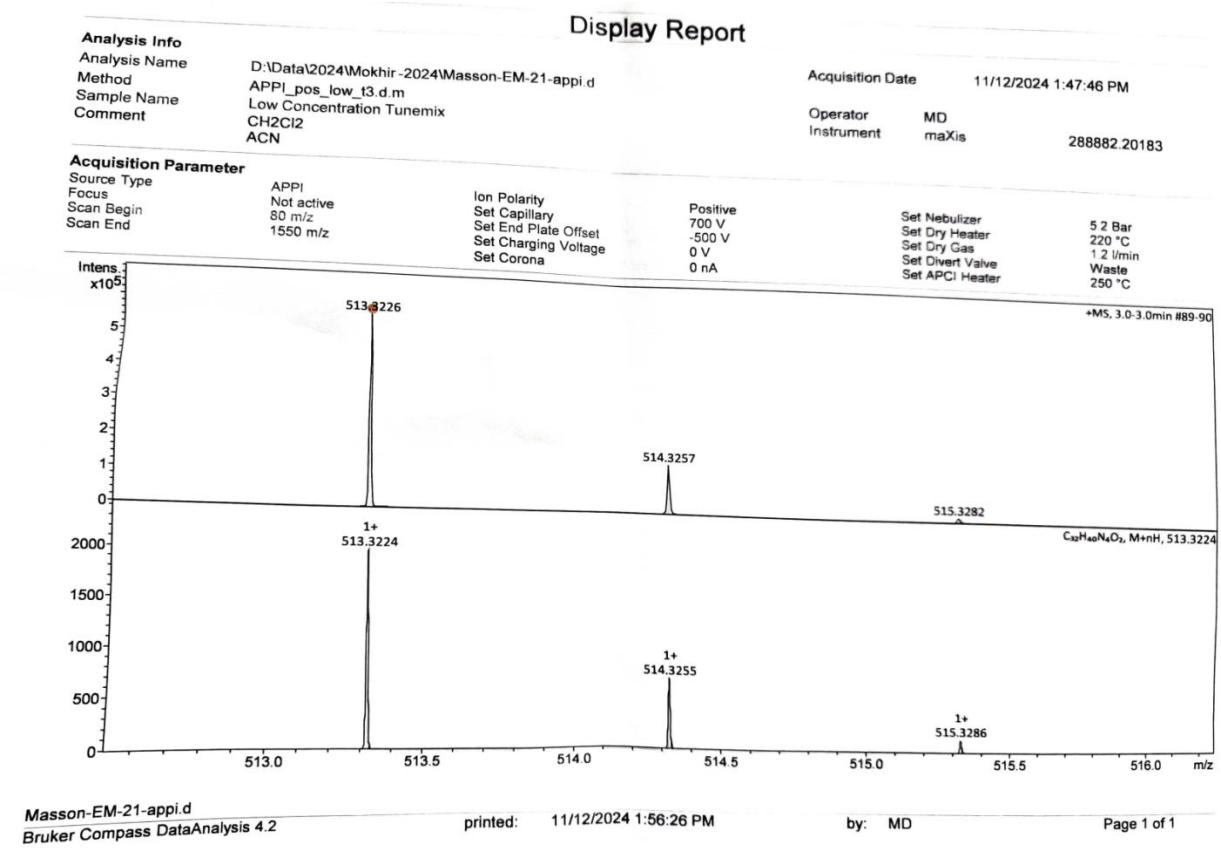

Figure S4. Mass spectrum (APPI, pos. mode) of **BuCh**. Lower plot: calculated mass *m/z* for $C_{32}H_{40}N_4O_2$ $[M+H]^+$ = 513.3224; upper plot: measured *m/z* for $C_{32}H_{40}N_4O_2$ $[M+H]^+$ = 513.3226.

Log P (octanol-water partition coefficient)

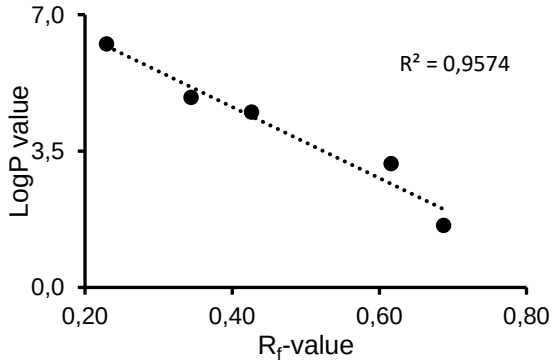

Figure S5. shows an example measurement of reference samples with known LogP values against the measured R_f - values in the eluent system 3-(N-morpholino)propanesulfonic acid (MOPS)/acetonitrile 2/3, v/v.

Table S1. shows the measured logP of BuCh and its p-value.

	logP ± p-value
BuCh	6.24 ± 0.06

Solubility in aqueous solutions

Figure S6. shows an image taken of 1ml UV/vis quarz cuvettes containing BuCh (left: 10 µM, right: 25 µM) in PBS (10 mM, pH 7.4, 1% EtOH, v/v). The observed turbidity in the right cuvette is an indication for a suspension rather than a solution.

Table S2. shows the solubility limit of BuCh in aq. solution: PBS (10 mM, pH 7.4) with 1% EtOH (v/v).

	PBS
BuCh	≤ 20 µM

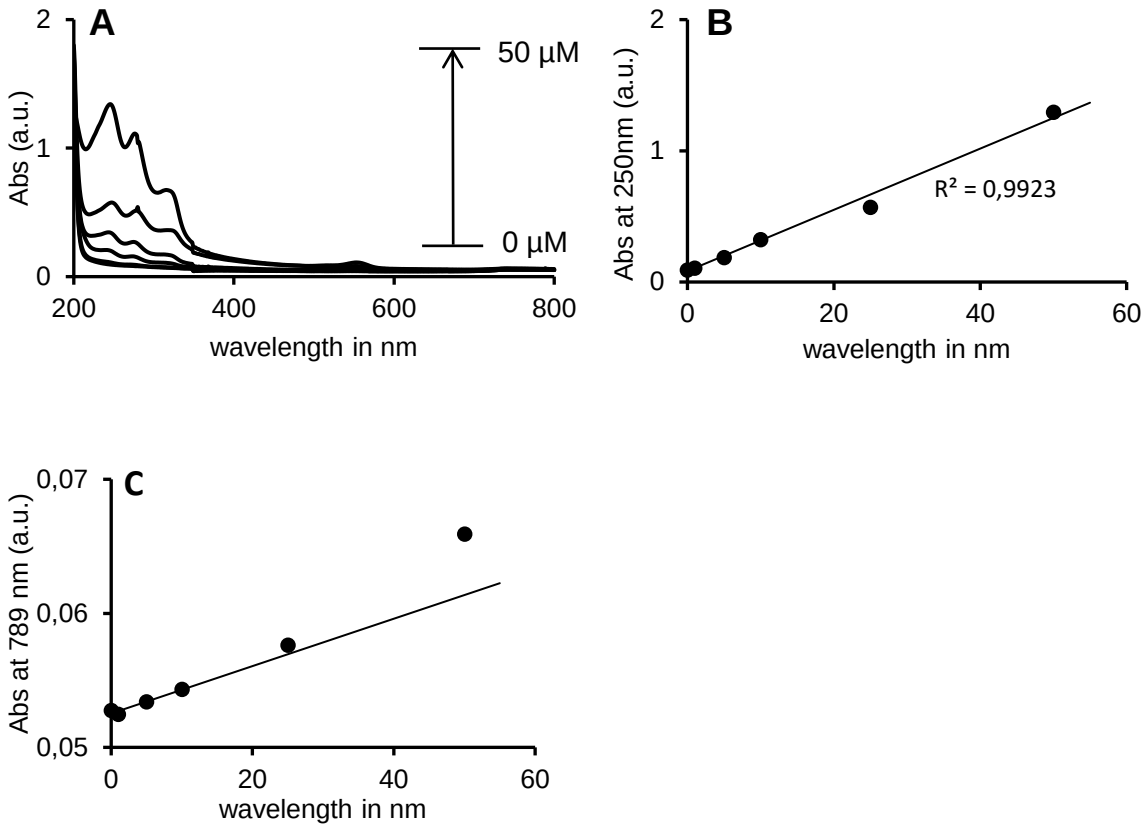

Figure S7. A: shows UV/vis spectra of BuCh in increasing concentrations (1, 5, 10, 25, and 50 µM) in PBS (10 mM, pH 7.4, 1% EtOH, v/v), B: shows the absorption at 250 nm of the corresponding concentrations of BuCh from the measurement in A and reveals a linear dependence according to Lambert-Beer's law, C: shows the absorption at 789 nm (baseline) of the increasing BuCh concentrations of A.

Cu^{2+} detection in different human cell lines

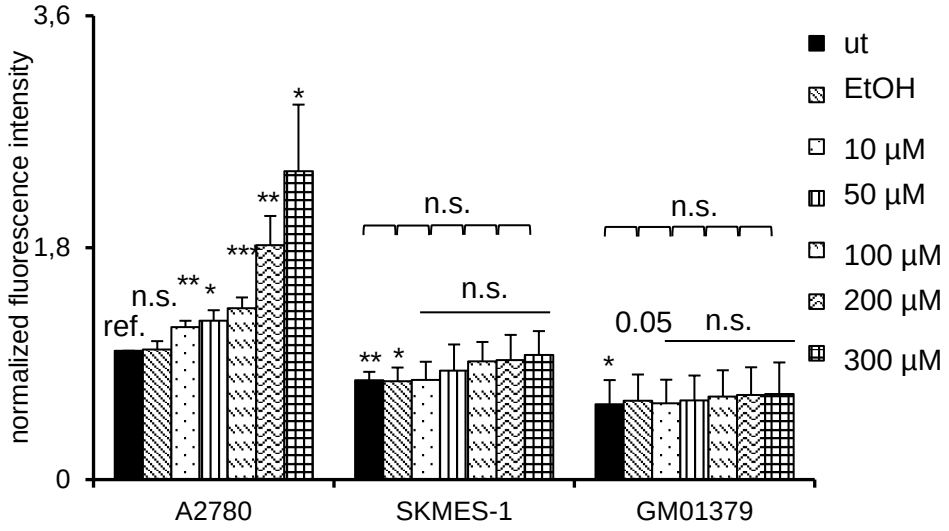

Figure S8. Shows the change of fluorescence ($\lambda_{ex.}$ = 488 nm and $\lambda_{em.}$ = 585 ± 42 nm) of BuCh (20 µM)-loaded cells (A2780 in RPMI 1640 medium, SK-MES-1, and GM013789 both in DMEM medium) that were either untreated (ut.), pre-treated with the carrier ethanol (EtOH, 1%, v/v), or $Cu(OAc)_2$ (10, 50 100, 200 and 300 µM, 1% EtOH, v/v) for 24 h. Untreated, probe-loaded A2780 cells were used as a reference. Their fluorescence was set to 1. Statistical significance was determined using the Student's t-test, where * - $p < 0.05$, ** - $p < 0.01$, *** - $p<0.005$ and not significant (n.s.) – $p \geq 0.05$.

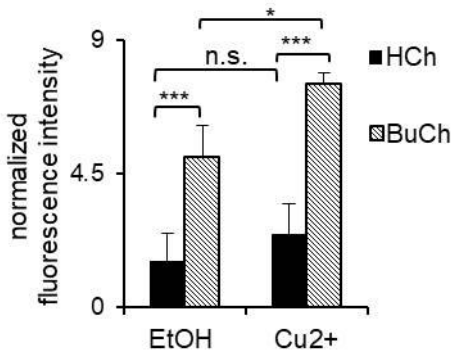

Figure S9. shows the change of fluorescence intensity ($\lambda_{ex.}$ = 488 nm and $\lambda_{em.}$ = 585 ± 42 nm) of BuCh (20 µM)-loaded and HCh (20 µM)-loaded A2780 cells which were either pretreated with ethanol as a carrier (1% EtOH, v/v) or with $Cu(OAc)_2$ (300 µM, 1% EtOH, v/v) for 24h. Statistical significance was determined using the Student's t-test, where * - $p < 0.05$, *** - $p<0.005$ and not significant (n.s.) – $p \geq 0.05$.

Confocal microscopy experiment

Determination of subcellular distribution:

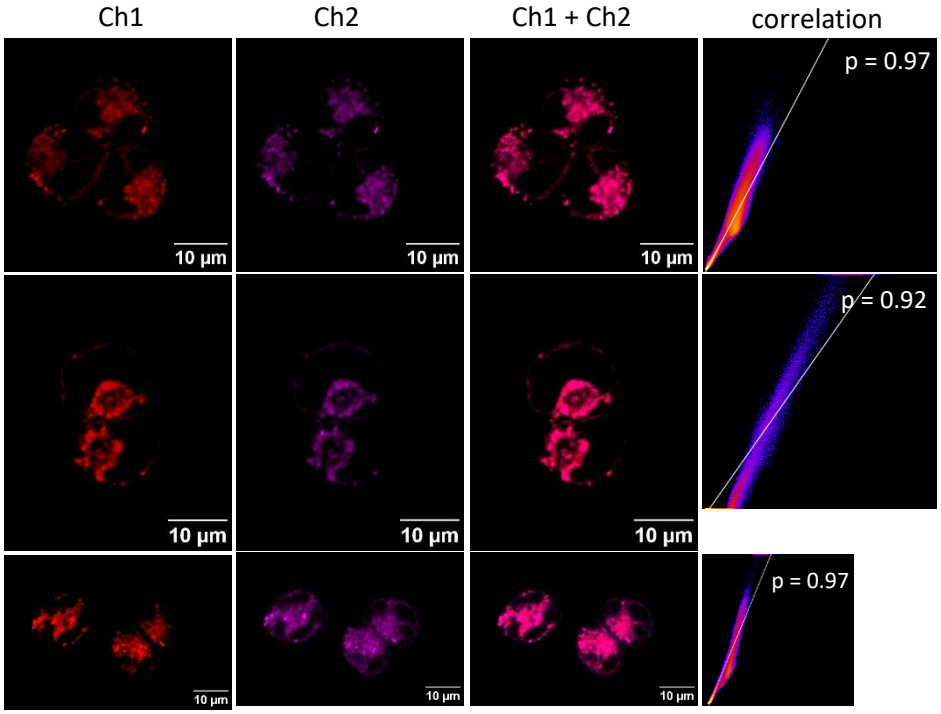

Figure S10: shows confocal microscopy images of A2780 cells co-incubated with BuCh (20 µM; $\lambda_{ex.}$ = 561 nm, $\lambda_{em.}$ = 629 ± 62 nm Ch1 (red) channel) and Cy5Azide (10 µM; $\lambda_{ex.}$ = 635 nm, $\lambda_{em.}$ = 690 ± 50 nm, Ch2 (magenta) channel) for 30 min in HBSS (1% EtOH, v/v) and their merged images with the calculated Pearson coefficient p (correlation).