

Supplementary Information

Purity of the prepared Lipid 430 (1) by UHPLC-DAD-MS/MS

Figure S1 ESI– BPI chromatogram (A), Extracted Ion chromatogram of **1** (B) and A_{254 nm} chromatogram (C)

NMR Spectroscopic Data for Lipid 430 (1)

Figure S2 ¹H NMR (600 MHz, CD₃OD) spectrum of **1**

Figure S3 ¹³C NMR (151 MHz, CD₃OD) spectrum of **1**

Figure S4 HSQC + HMBC (600 MHz, CD₃OD) spectrum of **1**

Figure S5 COSY (600 MHz, CD₃OD) spectrum of **1**

Figure S6 H2BC (600 MHz, CD₃OD) spectrum of **1**

Results of the cytotoxicity assay

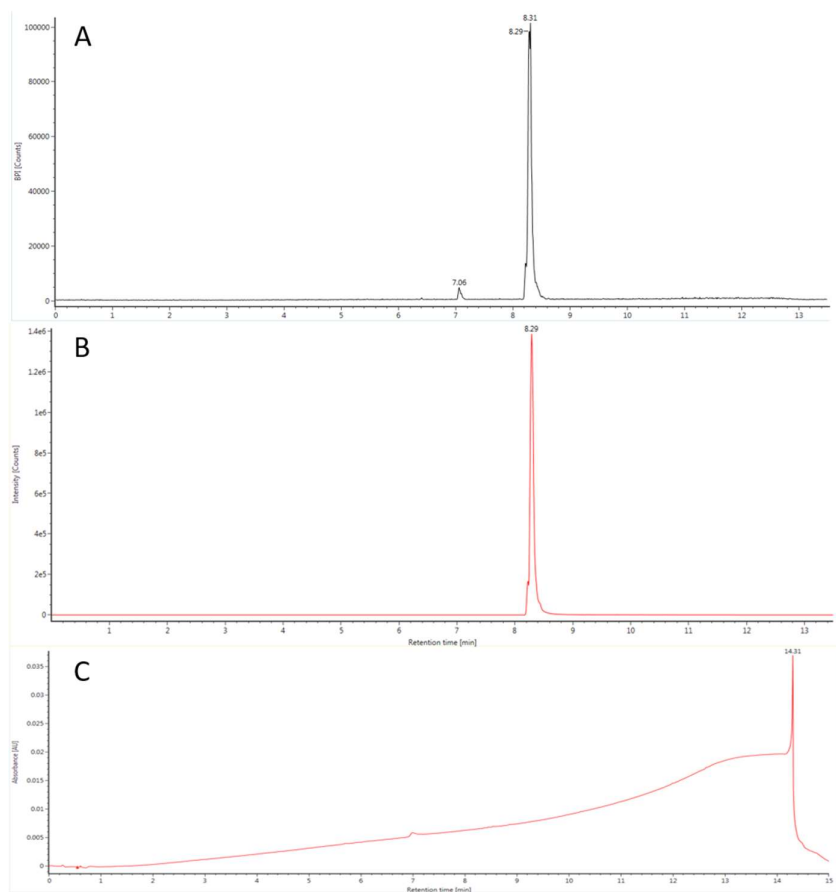
Figure S7 Results of the cytotoxicity assays for all tested cell lines

Results of the mode of action studies for all concentrations of Lipid 430 (1) and controls

Figure S8 Results of the flow cytometry experiments with propidium iodide staining

Figure S9 Pictures of the microscopic investigation

Figure S1. ESI– BPI chromatogram (A), Extracted Ion chromatogram of Lipid 430 (B) and $A_{254\text{ nm}}$ (C)



Chromatograms of the UHPLC analysis of the isolated Lipid 430 (1). In A the base peak intensity chromatogram of the ESI-MS/MS signal is depicted. In B the extracted ion chromatogram of the most abundant isotopic peak (m/z 429.2972, $[M-H]^-$) and in C the absorption at 254 nm. The signal at $RT = 7.06$ min is also visible when injecting the blank solution.

Chemical structure of the compound is shown above the spectrum. The structure is a long-chain fatty acid derivative with a terminal carboxylic acid group (labeled 1, 2, 3, 4) and a terminal hydroxyl group (labeled 23, 24). The spectrum shows peaks corresponding to the protons in the structure, with labels indicating the carbon numbers of the protons. The x-axis is labeled f1 (ppm) and ranges from 0 to 12. The y-axis represents intensity.

Peak assignments and integrations:

Peak Label	Integration
7	1.00
4	0.99
2	0.97
6b and 3a	2.04
3b	2.04
10 and 6a	1.03
9a	1.00
9b	1.17
22	4.32
12	19.01
11	2.27
21	5.96
13-20	1.00
23 and 24	1.00

Chemical structure of **1** is shown above the spectrum. The structure is a long-chain molecule with a terminal carboxylic acid group (C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24) and a terminal carboxylic acid group (C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24).

The ¹³C NMR spectrum (CDCl₃) shows the following chemical shifts (ppm):

Peak Number	Chemical Shift (ppm)
1	174.56
2	173.76
3	171.51
4	69.96
5	63.16
6	56.52
7	44.77
8	43.59
9	40.12
10	38.29
11	30.92
12	30.92
13-19	30.66
20	30.63
21	30.61
22	30.56
23 and 24	29.02
	28.41
	26.54
	22.91

Figure S4. HSQC + HMBC (600 MHz, CD₃OD) spectrum of **1**

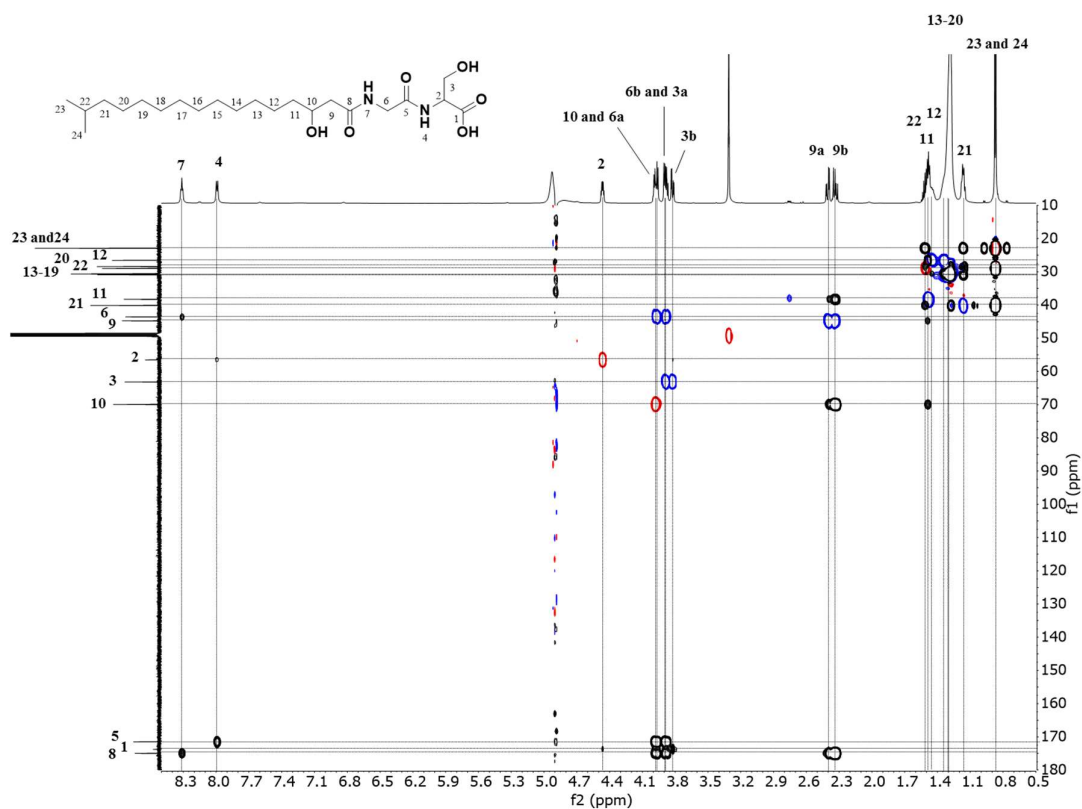


Figure S5. COSY (600 MHz, CD₃OD) spectrum of **1**

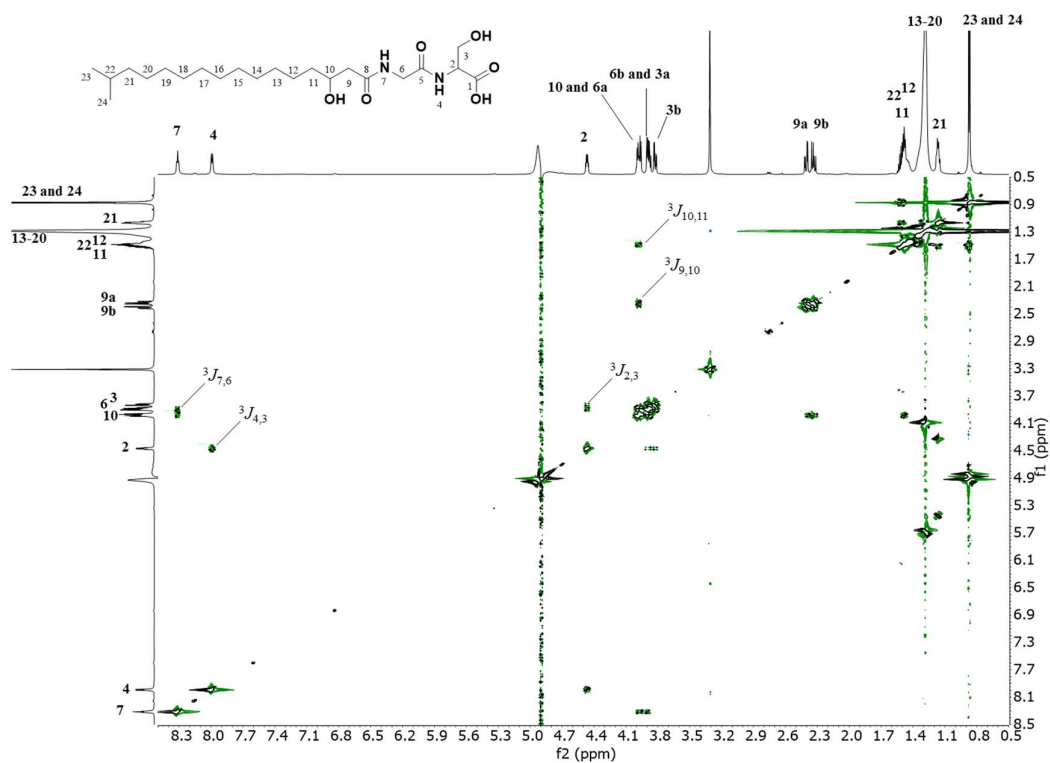


Figure S6. H2BC (600 MHz, CD₃OD) spectrum of **1**

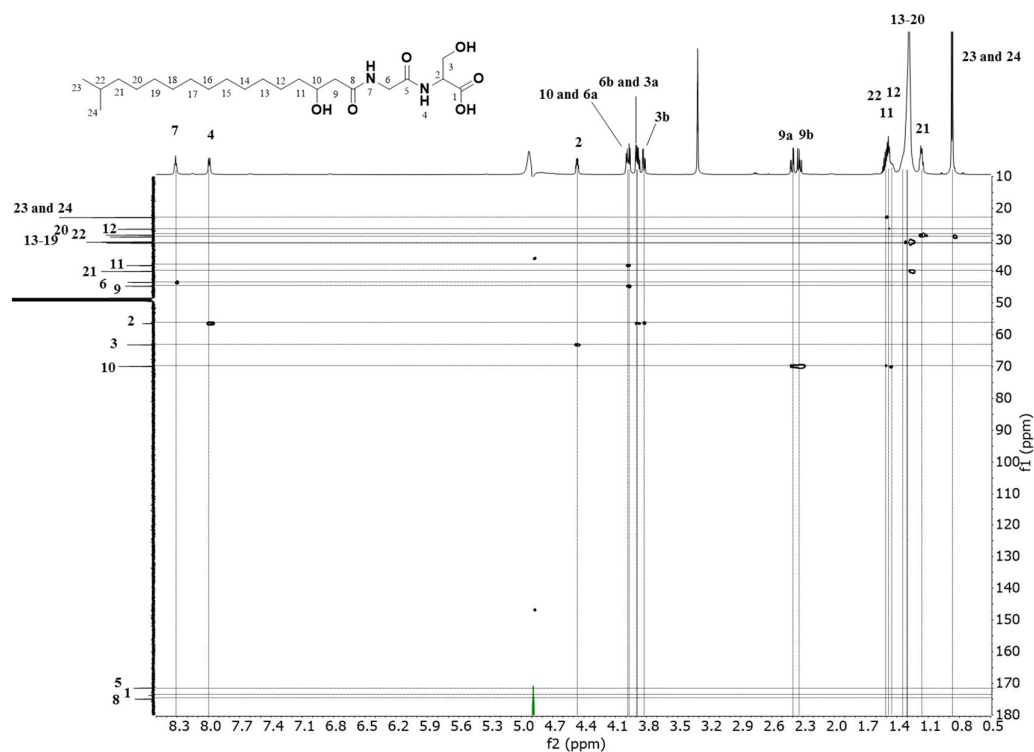
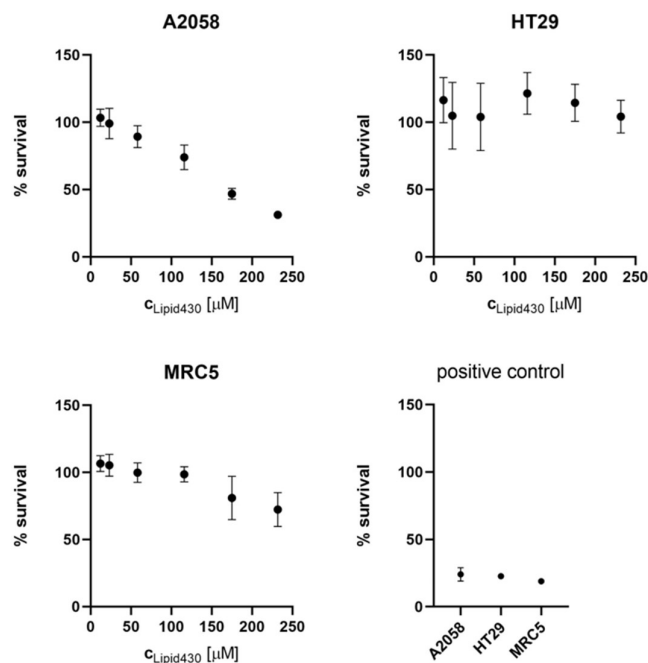
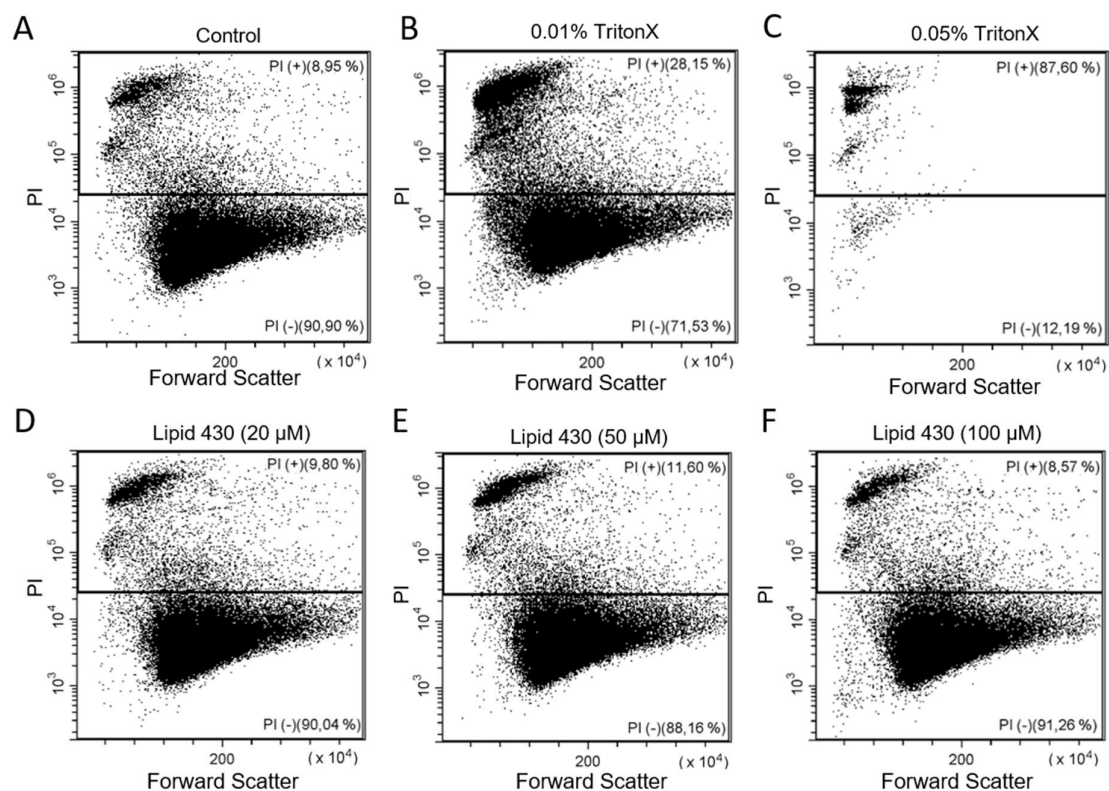


Figure S7. Results of the cytotoxicity assays for all tested cell lines



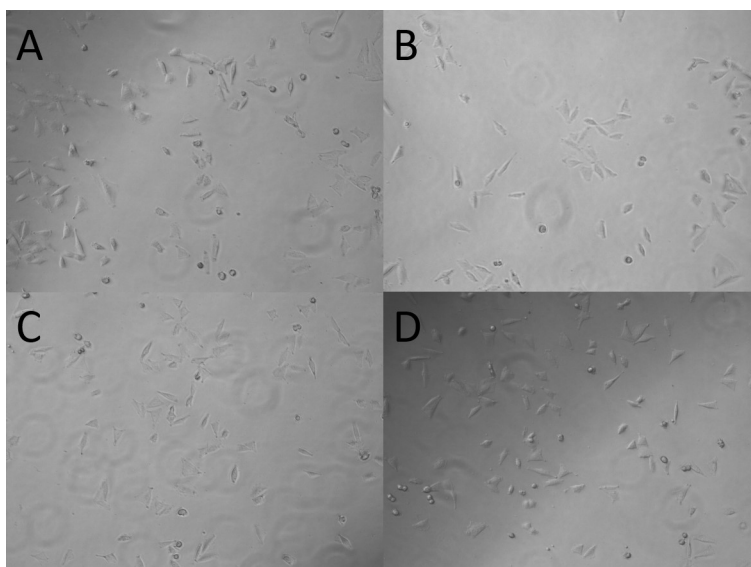
Cytotoxicity assay results for the melanoma (A2058), colon carcinoma (HT29) and lung fibroblast (MRC5) cells. The assay result is given as % survival on the y-axis and the concentrations of Lipid 430 on the x-axis. The exact tested concentrations were of 233, 175, 116, 58, 23 and 12 μM or 100, 75, 50, 25, 10 and 5 μg/mL respectively. 0.5% Triton™ X-100 was used as positive control.

Figure S8. Results of the flow cytometry experiments with propidium iodide staining



DotPlot graphs of the flow cytometry experiments with melanoma cell line A2058. In the upper sections the propidiumiodide positive (PI+) events (cell integrity destroyed/ affected) and in the lower the propidiumiodide negative events (PI-, physiologic cells). Forward scatter is displayed on the x-axis and propidiumiodide absorption on the y-axis. The relative ratio of events is given in %. A: stained control, 8.95% PI+; B: 0.01% TritonX, 28.15% PI+; C: 0.05% TritonX, 87.60% PI+; D: 20 μ M Lipid 430, 9.80% PI+; E: 50 μ M Lipid 430, 11.60% PI+; F: 100 μ M Lipid 430, 8.57% PI+.

Figure S9. Results of the microscopic investigation



Microscopic investigation of Melanoma cells (A2058) after 1h of incubation with test solution. Inspection was done at 100× magnification. A: PBS-control; B: 1% (v/v) DMSO; C: Lipid 430, 100 µg/mL; D: Lipid 430, 500 µg/mL.