Dansyl acetyl trehalose: a novel tool to investigate the cellular fate of trehalose

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Electronic Supplementary Information

ESI 1. Materials and Methods

Chemistry.

The compounds prepared had a purity of at least 95%, as determined by combustion analysis. The melting point is uncorrected. TLC was performed on Fluka plates (art. 99577) and column chromatography on Silica gel 70-200 μ 60A (Fluorochem): the eluent was a mixture of petroleum ether/acetone in various proportions. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian MR 400 MHz (ATB PFG probe); the chemical shift (referenced to solvent signal, $CDCl_3$ = 7.26 ppm) is expressed in δ (ppm) and *J* in Hz. The following abbreviations were used in reporting spectra: s (singlet), d (doublet), m (multiplet).All solvents and reagents were supplied by Aldrich Chemical Co. Ltd. and were used as supplied. The trehalose dehydrate is commercially available. The compounds 1,[1] 2,[2] 3,[3] were prepared according to the literature.

*Synthesis of 2,2', 3,3', 4,4'-hexa-O-acethyl-6,6'- di-O-{[5,5'-(dimethylamino)-1,1'-naphtyl]sulfonyl}-α-D-trehalose (**4**).*

To the solution of diol **3** (0.55 mmol) in freshly distilled $CHCl_3$ 35 mL, were added triethylamine (TEA) 3 mL (21.5 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP, 0.35 mmol). The resulting solution was stirred at 0-10 °C for 10 min under a nitrogen atmosphere. DNS-Cl (1.4 mmol, 2.5 equiv) was then added and stirring was continued for 24 h at room temperature under a nitrogen atmosphere. DNS-Cl (1.4 mmol, 2.5 equiv) was then added and stirring was continued for 24 h at room temperature under a nitrogen atmosphere. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (100 mL) and extracted with $CHCl_3$ (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Petroleum ether/Acetone, from 100:0 to 80:20) to give 100 mg (17%) of **4** as a green powder.

^{1}H NMR ($CDCl_3$) 1.98 (6H, s), 1.98 (6H, s), 2.03 (6H, s), 2.86 (12 H, s), 3.95 – 4.03 (4H, m), 4.03 – 4.12 (2H, m), 4.60 (2H, d, J = 3.8), 4.78 – 4.90 (4H, m), 5.33 (2H, t, J = 9.7), 7.19 (2H, d, J = 7.6), 7.46 – 7.63 (4H, m), 8.15 (2H, d, J = 8.6), 8.21 (2H, d, J = 7.3), 8.60 (2H, d, J = 8.5). ^{13}C NMR ($CDCl_3$) 20.70, 20.77, 45.55, 68.10, 68.21, 68.78, 69.17, 70.07, 92.82, 115.83, 119.35, 123.09, 129.01, 129.94, 130.04, 130.64, 131.01, 132.06, 152.01, 169.53, 169.62, 170.05.

Elemental analysis calcd (%) for $C_{48}H_{56}N_2O_{21}S2$ (MW 1061.089) C 54.33, H 5.32, N 2.64. Found C 54.48, H 5.36, N 2.69.

Mp 98-100°C.

Fluorescence measurements.

Fluorescence spectra of DAT and DNS-Cl were measured in a 10 mm pathlength quartz cuvette using an Edinburgh Quanta Master spectrofluorimeter. DAT and DNS-Cl, dissolved at 10 mM concentration in dimethyl sulfoxide (DMSO), were aliquoted and kept at -20 °C in the dark. Fluorescence spectra were recorded after dilution of the thawed stock solutions in different solvents at 10 μM concentration.

Cell culture and trehalose treatment.

Three different colon cancer cell lines were employed in this study, namely primary colon carcinoma cells RKO and HCT116 and supraclavicular lymph node metastasis of human colon cancer cells LoVo. Cells were maintained in RPMI supplemented with 10% Foetal Bovine Serum (FBS), 2 mM glutamine, 100nunits/mL penicillin, and 100μg/mL streptomycin and grown at 37°C in an incubator with humidified atmosphere at 5% CO_2. For DAT treatment, $2x10^4$ cells were plated onto a glass-bottomed 36 mm dish and after 24 hours DAT was added at 1 μM concentration, incubated for 30 minutes and then examined by fluorescence microscopy at different time points (0, 2, 4 or 16 hours). For DAT and trehalose co-treatments, cells were treated overnight with 100 mM trehalose, then supplemented with 1μM DAT, and analysed by fluorescence microscopy after 30 min and after further 16 hours of incubation.

Live imaging experiments

Lysosomes and lipid droplets were stained by using the fluorescent dies LysoTracker Red and HCS LipidTOX Red (Life Technologies), respectively. Briefly, $2x10^4$ cells were seeded onto a glass-bottomed 36 mm dish cells and grown in complete medium. For lysosomal staining, after 24h cells were incubated with 10 nM LysoTracker Red in DMEM without Red Phenol for 30 minutes at 37°C. For lipid droplets staining, after 24h cells were fixed with 4% PFA in PBS for 30 minutes at room temperature and then washed twice with PBS. Subsequently, cells were stained with 1X LipidTOX for 30 minutes at room temperature. Cells were visualized with a digital imaging system,

using an inverted epifluorescence microscope with x63/1.4 oil objective (Nikon Eclipse Ti-U; Nikon) at 488nm. Images were captured with a back-illuminated Photometrics Cascade CCD camera system (Roper Scientific) and elaborated with Metamorph Acquisition/Analysis Software (Universal Imaging Corp.).

References

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ESI 2. DAT ^{1}H and ^{13}C NMR spectra

Figure ESI 2.1. ^{1}H NMR spectra

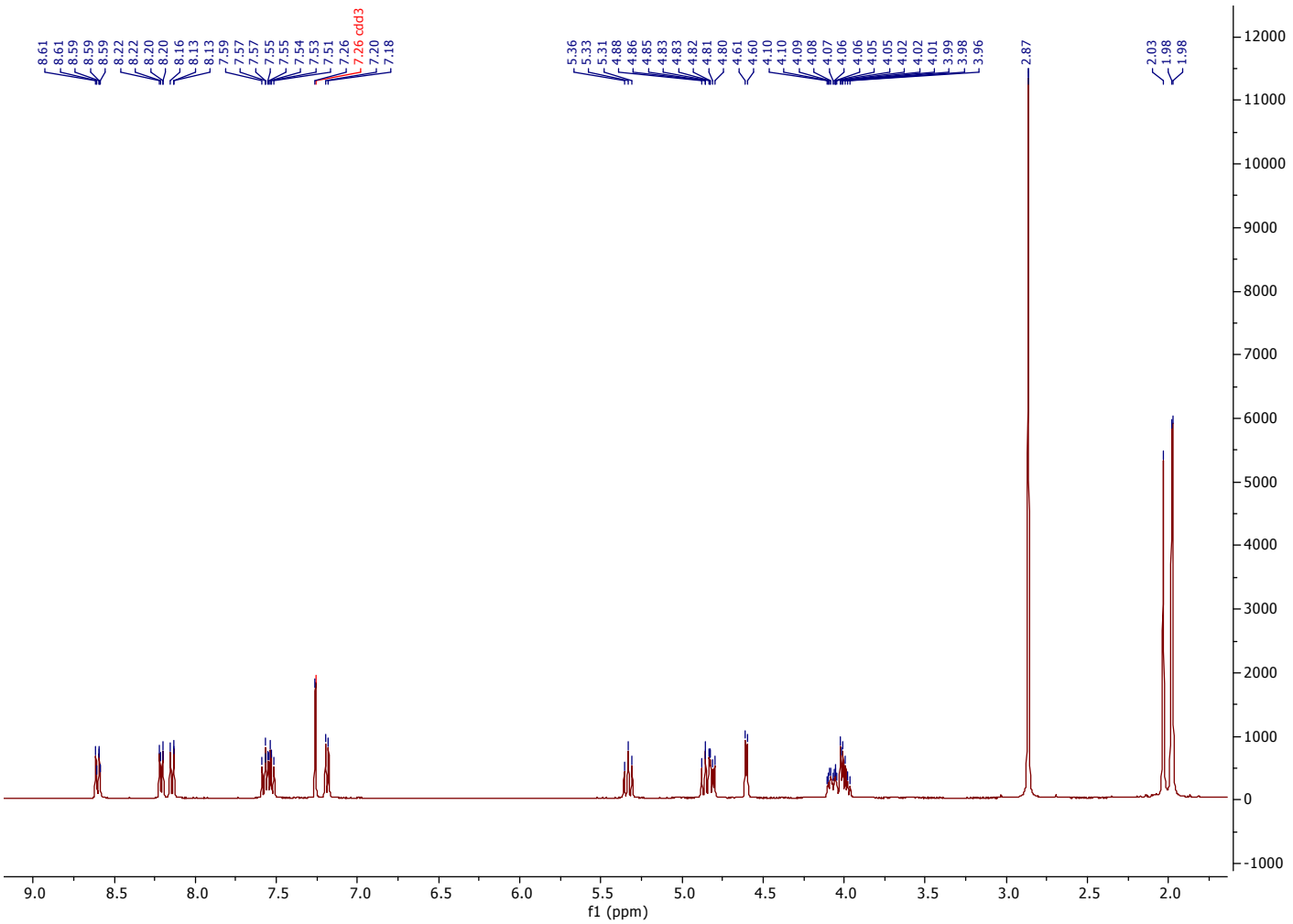

Figure ESI 2.2. ^{13}C NMR spectra