Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Preparation and Evaluation of BBB-permeable Trehalose Derivatives as Potential Therapeutic Agents for Huntington's Disease

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Electronic Supplemental Information

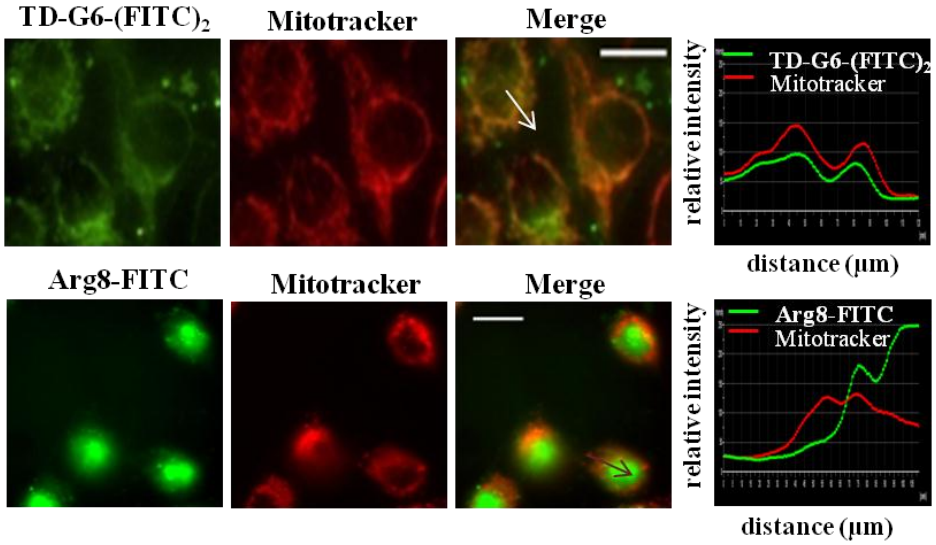
I. Supporting Results

I-1. Supporting Figures

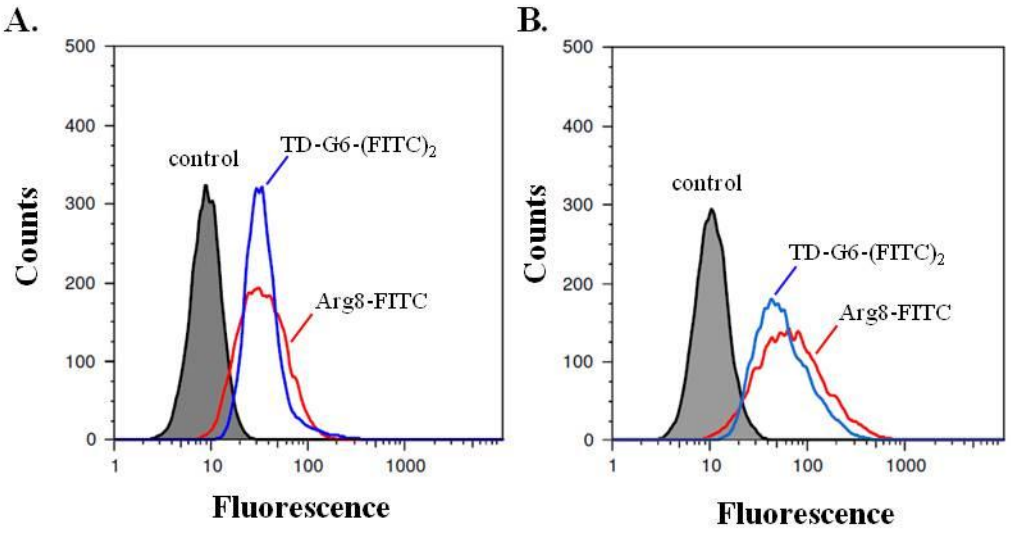
Cellular uptake study of TD-(FITC)$_2$

As a control the trehalose derivative without the guanidine residues, TD-(FITC)$_2$ (**16**) was examined for its cellular uptake. In contrast to TD-G6-(FITC)$_2$ (**7**), live HeLa cells incubated with TD-(FITC)$_2$ for 1 hr at 37 °C did not show negligible fluorescence. Incubation of the compound even at a higher concentration (100 μM) for 1 hr showed very low fluorescence intensity (Fig. S1).

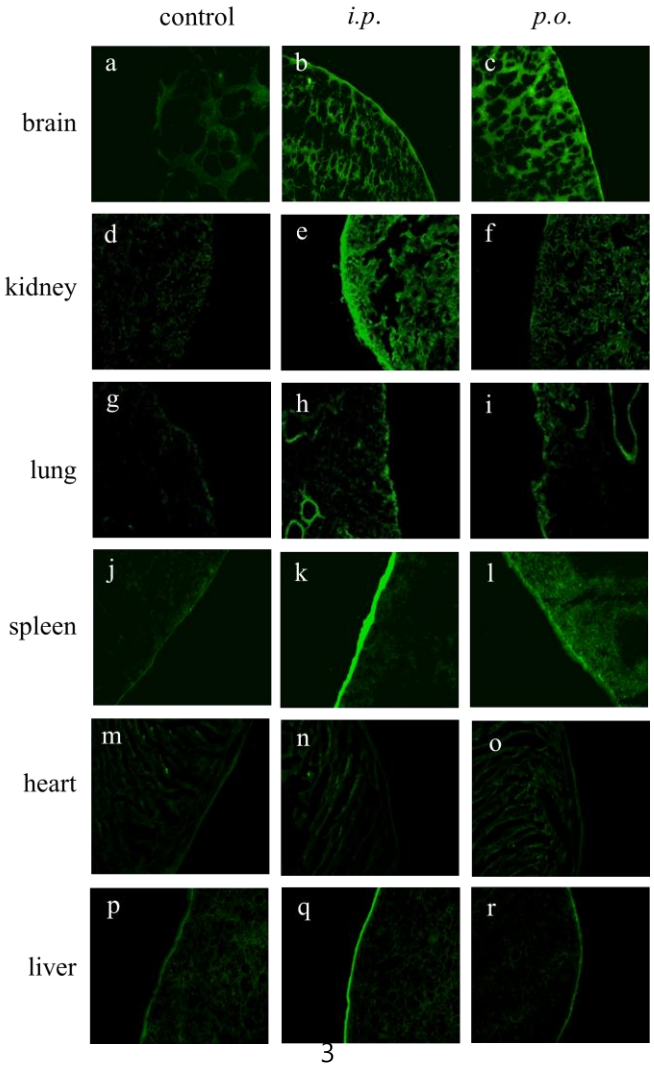
Supporting Figure S1. Extremely low cellular uptake of TD-(FITC)$_2$ (**16**) at 100 μM after 1 hr incubation in live HeLa cells.



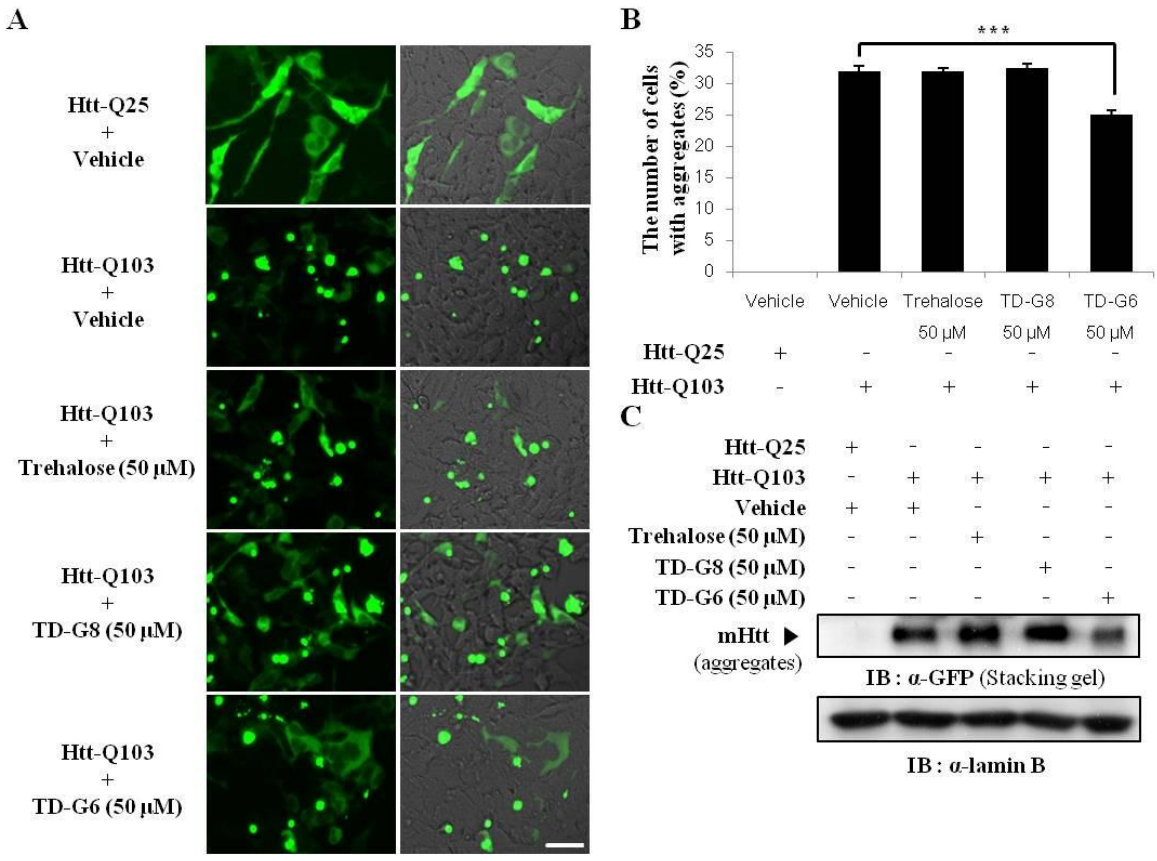
Supporting Figure S2. Confocal microscope images for the cellular localization of TD-G6-(FITC)$_2$ (10 μM) and Arg8-FITC (10 μM),each coincubated with Mitotracker (100 nM) for 10 min (scale bar : 20 μm). The intensity profiles of the fluorescence signals along the lines in the merge images are shown on the right.



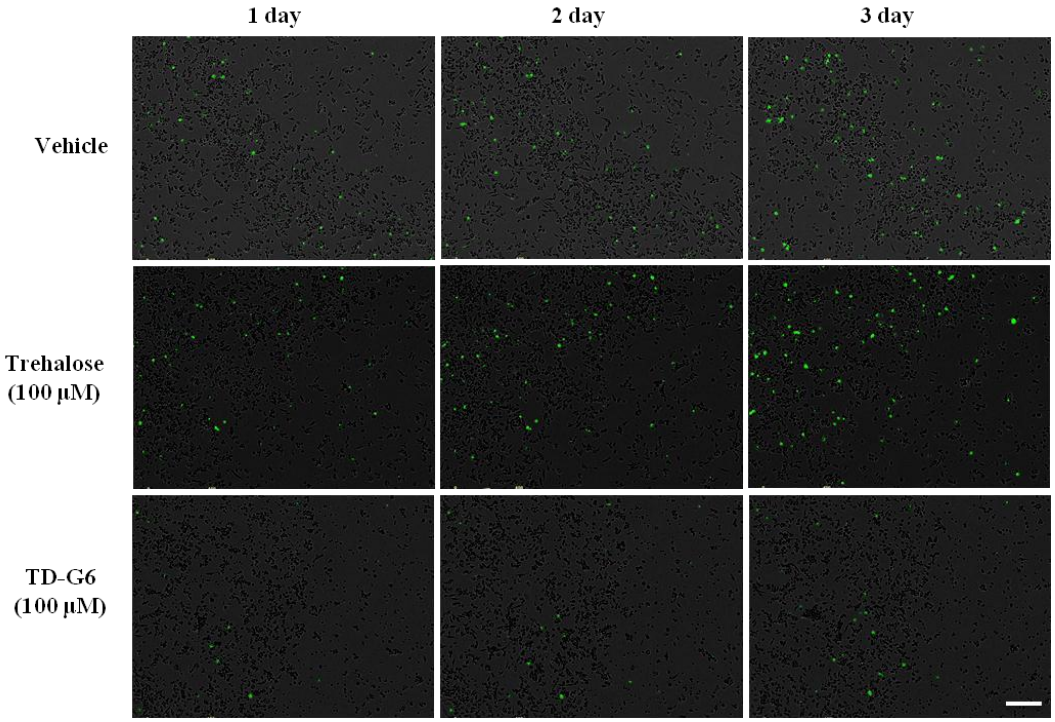
Supporting Figure S3. FACS analysis of Arg8-FITC and TD-G6-$(FITC)_2$ for the efficiency of the cellular uptake. Each compound was incubated for 10 min (A) at 5 μM. (B) at 10 μM.



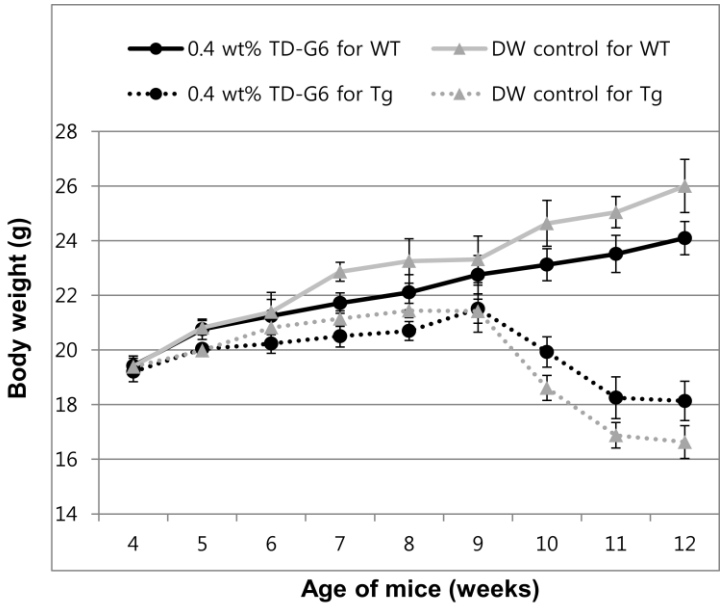
Supporting Figure S4. Distribution of TD-G6-$(FITC)_2$ in mouse tissues (left : control, middle : 90 mg/kg *i.p.*, right : 450mg/kg *p.o.*). Fluorescence micrographs of: [a-c] brain, [d-f] kidney, [g-i] lung, [j-l] spleen, [m-o] heart, and [p-r] liver tissue sections, isolated from mice 20 min after *i.p.* injection and 24 hr after *p.o.* injection, respectively. Exposure times (ms): [a-c] 8000, [d-f] 5000, [g-i] 6000, [j-l] 3000, [m-o] 1000, and [p-r] 3000.



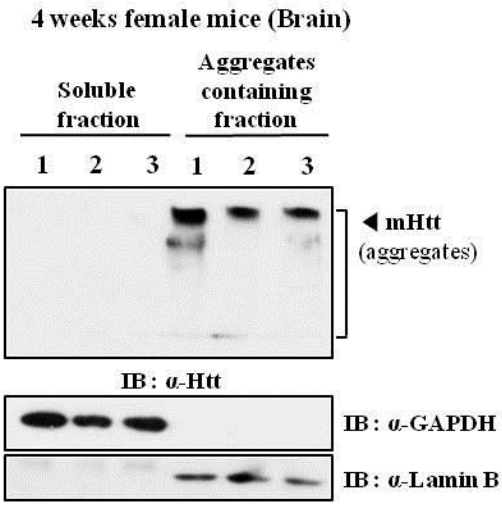
Supporting Figure S5. Inhibition effects of trehalose and trehalose derivatives toward polyglutamine aggregation in HEK293A cells. (A) Fluorescence images of Htt-exon1-Q25-GFP and Htt-exon1-Q103-GFP in HEK293A cells after three days of incubation with trehalose, TD-G8, TD-G6 (50 μM each) and vehicle. Shown are images of fluorescence microscopy (left) and merges of phase contrast (right). (scale bar: 50 μm). (B) The cells with punctate structures were counted by visual inspection. (C) Western blot analyses of Htt-Q103 aggregates in the cell lysates. The insoluble aggregates appeared at the stacking gel and were detected by anti-GFP antibody.



Supporting Figure S6. Time-course study of the formation of inclusions (green) in mouse neuroblastoma (Neuro2a) cells. The cells were transfected with Htt-exon1-Q103-GFP and then treated with vehicle, trehalose, and TD-G6 (100 µM each) for three days (scale bar: 20 µm). The inclusions were observed by a fluorescence microscope.



Supporting Figure S7. The body weight changes of wild type and R6/2 transgenic mice at 4-12 weeks age (n > 6) when distilled water or TD-G6 was administered. Error bars indicate mean ± SEM.



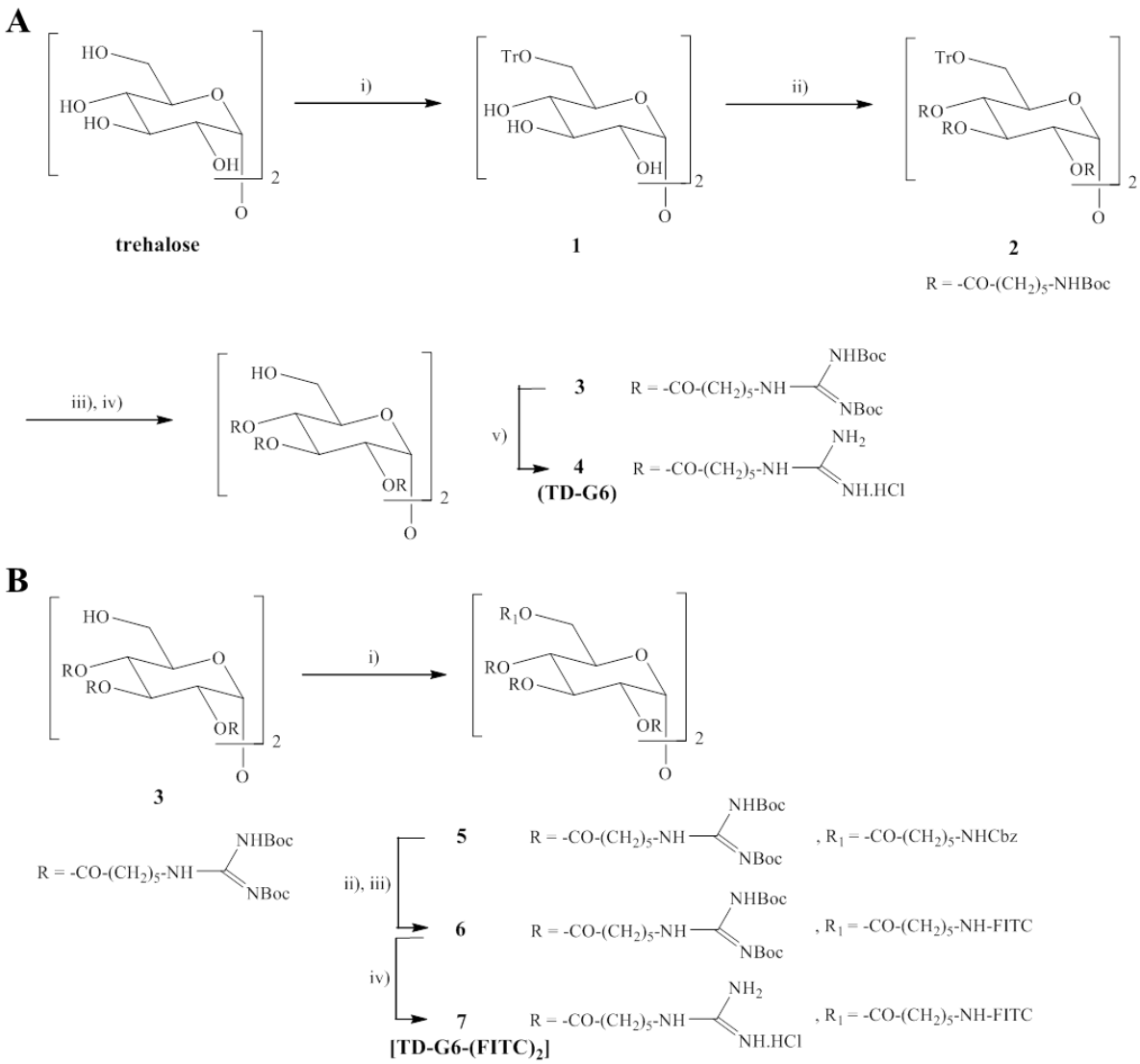
Supporting Figure S8. Analyses on brain homogenates from 4-week age R6/2 Tg female mice according to the treatment substance (1 = 0.4 wt% TD-G6, 2 = 1.0 wt% trehalose, 3 = DW control).

I-2. Supporting Schemes

Syntheses of TD-G6 (4) and TD-G6-(FITC)$_2$ (7)

The primary hydroxyl groups of trehalose were selectively protected by treatment with TrCl in pyridine. The crude product was washed with diethyl ether several times to give **1** as a homogeneous solid in 91% yield. *N*-Boc-protected aminohexanoic acid was coupled to **1** by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) as the coupling reagents to give **2**. The trityl groups and the *N*-Boc protecting groups in **2** were simultaneously removed by treatment with gaseous HCl saturated in ethyl acetate for 2 days, and the six available primary amino groups were completely guanidinylated with *N*,*N*´-bis-Boc-*N*´´-trifluoromethanesulfonylguanidine and triethylamine in 1,4-dioxane and water. Completeness of the guanidinylation was confirmed by the disappearance of the α–CH_2 peaks to the primary amine functionality at δ 3.37 ppm in its ^{1}H NMR spectrum. After purification by flash column chromatography, product **3** was obtained in 80% yield. All *N*-Boc protecting groups in **3** were removed in ethyl acetate saturated with gaseous HCl to provide the target compound **4** (TD-G6) in 87% yield after purification by preparative RP-HPLC on a C18 column (Scheme S1a).

For the FITC attachment, *N*-Cbz-protected aminohexanoic acid was coupled to **3** by using the EDC and DMAP coupling conditions to give **5** in 66% yield. Hydrogenolysis of **5** over Pd/C, followed by treatment of with FITC-I (with isothiocyanate functionality) and triethylamine in THF/EtOH/MeOH (6 : 4 : 1) for 24 hr provided **6** in 57% after purification on SiO_2. Removal of the Boc protecting groups of **6** with gaseous HCl in ethyl acetate gave **7** [TD-G6-(FITC)$_2$], which was purified by preparative RP-HPLC on a C18 column in 64% yield (Scheme S1b).



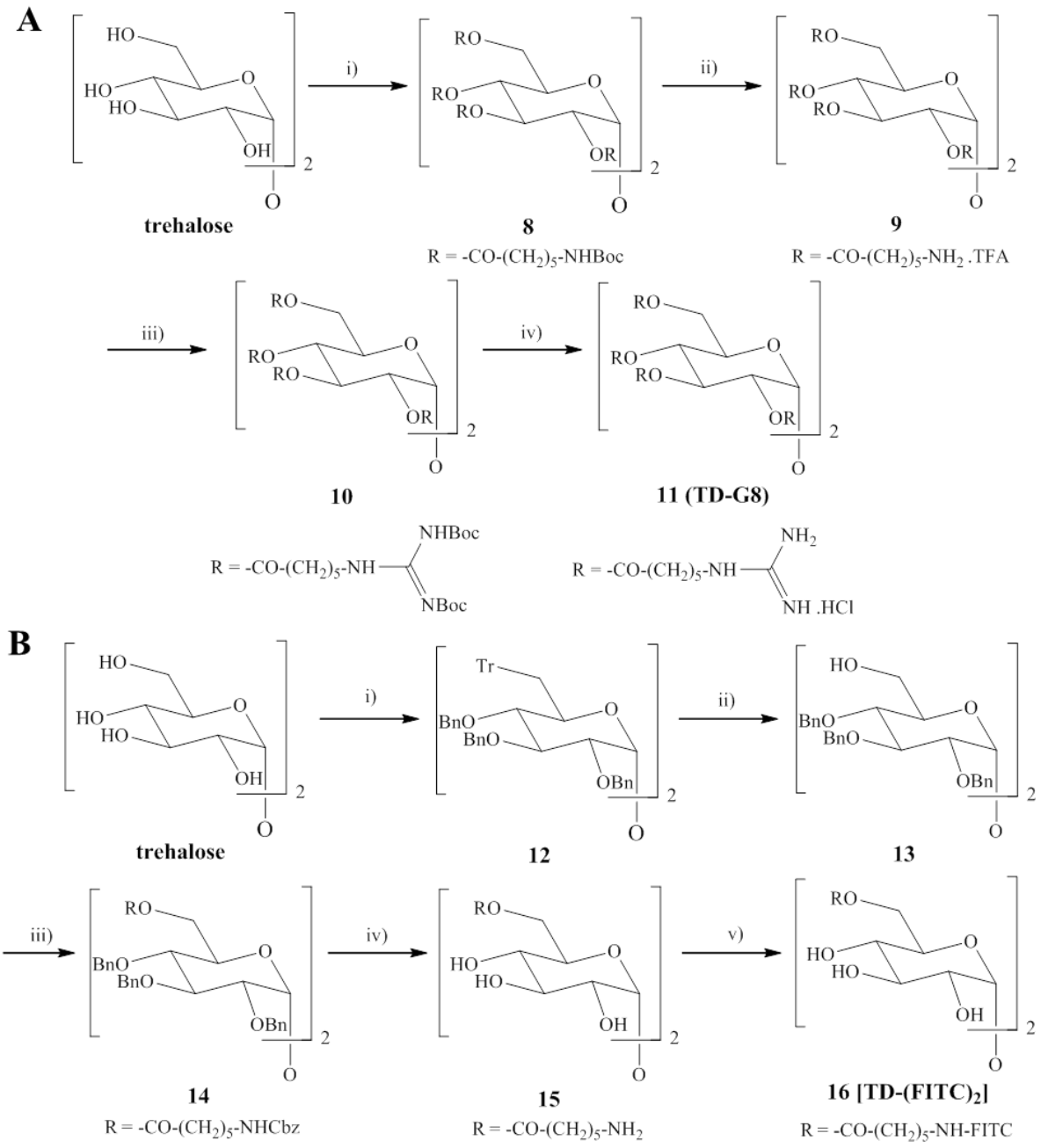
Supporting Scheme S1. Reagents and conditions for the syntheses of trehalose derivatives. (A) i) TrCl, pyridine, 60 °C, 5 hr, 91%; ii) HOOC-$(CH_2)_5$-NHBoc, EDC, DMAP, DMF, 60 °C, 2 days, 55%; iii) HCl (g) in EtOAc, RT, 2 days, quantitative yield; iv) Et_3N, *N,N´*-bis-Boc-*N´´*-trifluoromethanesulfonylguanidine, 1,4-dioxane/H_2O (5 : 1), RT, 80%; v) HCl (g) in EtOAc, RT, 2 days, 87%. (B) i) HOOC-$(CH_2)_5$-NHCbz, EDC, DMAP, DMF, RT, 2 days, 66%; ii) Pd/C 10%, CH_2Cl_2/MeOH (1 : 9), H_2 (g) 50 psi, 16 hr, 90%; iii) FITC, Et_3N, THF/EtOH/MeOH (6 : 4 : 1), RT, 24 hr, 57%; iv) HCl (g) in EtOAc, RT, 2 days, 64%.

Synthesis of TD-G8 (11)

Trehalose was exhaustively acylated to give **8** in 86% yieldby reacting with *N*-Boc-protected aminohexanoic acids in the presence of EDC, DMAP in DMF for 2 days. Completeness of the acylation was confirmed by counting the number of protons in ^{1}H NMR. The *N*-Boc protecting groups of **8** were removed by treatment with a mixture of TFA and CH_2Cl_2 (1 : 1) for 2 hr. The resulting eight primary amino groups of **9** were subjected to guanidinylation with triflylguanidineand Et_3N in aqueous 1,4-dioxane for 3 days. The product (**10)** was obtained in 64% yield after purification by flash column chromatography. Compound (**10**) was dissolved in EtOAc saturated with gaseous HCl to give TD-G8 (**11**), which was purified by preparative RP-HPLC on a C18 column (Scheme S2a).

Synthesis of TD-(FITC)$_2$ (16)

The two primary OH groups in trehalose was tritylated with TrCl in pyridine as described before, and the secondary hydroxyl groups were benzylated with NaH and BnBr to give **12** in 51% yield after flash column chromatography. Removal of the trityl groups in **12** was performed with *p*TSA monohydrate in CH_2Cl_2/MeOH mixture (1 : 1) to give **13** in 65% yield. (This condition gave a better yield than other acidic conditions such as TFA/ CH_2Cl_2 or gaseous HCl in EtOAc.) For the FITC attachment, *N*-Cbz-protected aminohexanoic acid was coupled to **13** under the EDC coupling conditions to afford **14** in 95% yield. All protecting groups in **14** were removed by hydrogenolysis over Pd/$(OH)_2$ to afford **15** in 97% yield. During the hydrogenolysis, the reaction progress was monitored by ^{1}H NMR to ensure the completeness of the reaction. It was found that two Cbz groups were removed first within a day and the removal of the remaining Bn groups required additional reaction time. Upon treatment with FITC and Et_3N in THF/MeOH, **15** gave the target compound, TD-(FITC)$_2$ (**16**) in 32% yield after purification by flash column chromatography (Scheme S2b).



Supporting Scheme S2. Reagents and conditions: (A) i) HOOC-$(CH_2)_5$-NHBoc, EDC, DMAP, DMF, RT, 2 days, 86%; ii) TFA/CH_2Cl_2 (1 : 1), 2 hr, RT, 93%; iii) Et_3N, *N*,*N´*-bis-Boc-*N´´*-trifluoromethanesulfonylguanidine, 1,4-dioxane/H_2O (5 : 1), RT, 3 days, 64%; iv) HCl(g) saturated in EtOAc, RT, 2 days, 81%. (B) i) TrCl, pyridine, 75 °C, 8 hr, ii) NaH, BnBr, DMF, 0 °C → RT, overnight, 51%; b) *p*TSA·H_2O, CH_2Cl_2/MeOH (1 : 1), RT, 3 hr, 65%; c) HOOC-$(CH_2)_5$-NHCbz, EDC, DMAP, DMF, RT, 24 hr, 95%; d) $Pd(OH)_2$/C 20%, CH_2Cl_2/MeOH (2 : 3), H_2(g) 55 psi, 4 days, 97%; e) FITC, Et_3N, THF/MeOH (1 : 3), RT, 24 hr, 32%.

II. Experimental

II-1. Biological Experiments

Materials: The FITC labeled arginine octamer (Arg8-FITC) was synthesized by Peptron (Daejeon, Korea). High glucose Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (PBS, pH 7.4), fetal bovine serum (FBS), and trypsin/EDTA were obtained from Invitrogen.

Cellular uptake experiments: For each assay, HeLa cells (1 X 10^5 cells per well) were seeded into a 35-mm coverglass bottomed dish (SPL Ltd., Korea) and cultured for 24 hr. After removing the medium, HeLa cells were washed with PBS. The cells were incubated for 30 min at 37°C in 2 ml of DMEM containing TD-G6-$(FITC)_2$. For kinetic assay, live HeLa cells were incubated with 1 μM of TD-G6-$(FITC)_2$ for 5, 10, 30, and 60 min. TD-$(FITC)_2$ was also incubated for 1 hr as a control. For comparison, Arg8-FITC was subjected to the same conditions described for TD-G6-$(FITC)_2$.

Confocal laser scanning microscopy (CLSM):After cellular incubation, the culture medium was removed from each well. HeLa cells were washed five times with cold PBS, placed in cold PBS, and then imaged directly on the coverglass bottomed dish. CLSM was performed by using an Olympus Fluoview FV1000 equipped with an N.A. 1.30, 40X, planApo, oil immersion lens. Fluorescence was analyzed and collected using the following excitation and emission bands: FITC, 488 nm (ex), 500-530 nm (em); Mitotracker, 543 nm (ex), 600-680 nm (em). In order to prevent the crosstalk between different colors of fluorophores, the excitation and the collection of emission were performed sequentially from the individual fluorophores. Merged images and intensity profiles were obtained by the Olympus Fluoview Viewer.

Fluorescence-activated cell-sorter (FACS): To compare the cellular uptake efficiency between TD-G6-$(FITC)_2$ and Arg8-FITC, HeLa cells were incubated with either 5 μM or 10 μM of the compounds for 10 min at 37 °C. After PBS washing, cells were fixed by 70% ethanol with 0.4% Tween-20. After 1 hr incubation at room temperature, samples containing 10,000 cells were analyzed for the fluorescent signals on a FACSCalibur system (Becton Dickinson, San Jose, CA).

Mouse tissue biodistribution study: For *i.p.* administration, TD-G6-$(FITC)_2$ (90.9 mg kg^{-1}) was dissolved in sterile distilled water with 5% (v/v) DMSO, and the solution was injected into an eight-week-old mouse (C57BL/6, 22 g). After 60 min, the administered mice were perfused with paraformaldehyde (PFA) (4%) in phosphate buffered saline (PBS) (pH 7.4), and the major organs (brain, heart, lung, kidney, spleen, and liver) were incubated overnight in a solution of sucrose (0.5 M) in PBS. Placed in cryoprotectant, they were cut into 15 μm sections with a cryostat and analyzed with an Axioplan2 fluorescence imaging microscope. For neuron staining, the sections were incubated with NeuN (Millipore, Billerica, MA) primary antibody solution (1% normal goat serum, 0.3% Triton X-100, PBS). As a control, triple distilled water (500 μL) was also injected into the mouse and treated in the same process. For per os (*p.o.*) administration, TD-G6-$(FITC)_2$ (454 mg kg^{-1}) was dissolved in sterile distilled water with 5% (v/v) DMSO, and the solution was injected into an eight-week-old mouse (C57BL/6, 22 g). After 24 hr, the administered mice were perfused with paraformaldehyde (4%) in PBS (pH 7.4), and the remaining protocols for specimen preparation and fluorescence imaging were the same as described above. Both *i.p.* and *p.o.* administration was conducted on the same day, and fluorescence signals from the specimens were compared.

Cell culture and in vitro experiments (inhibition of polyQ aggregation): HEK293A and Neuro2a cells

were grown in DMEM supplemented with 10% FBS, 100 U ml^{-1} each of penicillin G and streptomycin. Transient transfection was carried out with Metafectene reagent (Biontex, Munich, Germany) for HEK293A and Microporator (Invitrogen, Carlsbad, CA) for Neuro2a as described in the manufacturer's protocol. For the *in vitro* polyQ aggregation assay, HEK293A and Neuro2a cells were transfected with Htt-exon1-GFP-pcDNA3.1 (n = length of polyQ tract = 25 or 103) (a kind gift from Judith Frydman, Stanford University), and then compounds were added to culture media. After 3 days, cells with aggregates were counted using Olympus IX71 inverted fluorescence microscope (Olympus, Center Valley, PA). For the live cell imaging of polyQ aggregates, mouse neuroblastoma (Neuro2a) cells were monitored every 30 min for 3 days using IncuCyte FLR (Essen Bioscience, Ann Arbor, Michigan). To detect amounts of aggregates, HEK293A cells were lysed in buffer containing 10 mM HEPES (pH 7.6), 3 mM $MgCl_2$, 40 mM KCl, 5% glycerol, 0.5% NP-40, 2 mM DTT and 0.5 mM PMSF, and then centrifuged at 3,500 rpm for 5 min. The supernatant (soluble fraction) was discarded and then pellet (aggregates containing fraction) was further lysed in RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1 mM PMSF. Western blotting experiments with the indicated primary antibody were performed as described previously (1).

Filter trap assay: Cells were lysed in RIPA buffer, and then protein concentrations were measured with Bradford reagent (Amresco, OH). Lysates were diluted in 1% SDS-PBS and boiled for 5 min. Immediately after cooling, 10 μg of total proteins were loaded onto nitrocellulose membrane (0.2 μm) settled on a dot blotter (Bio-Rad, Hercules, CA), and anti-GFP antibody (Santa Cruz, CA) was used for detection.

R6/2 transgenic and wild-type mice: Breeding pairs of R6/2 transgenic mice (bearing the 5' end of the human HD gene carrying approximately 160 +/- 10 CAG repeats) were obtained from the Jackson Laboratory. For the genotyping, mice were determined at 3 weeks of age by standard PCR measuring CAG repeat lengths. Compounds (either 1.0 wt% trehalose or 0.4 wt% TD-G6) in water were given to R6/2 transgenic and wild-type mice as previously described (2). Each behavioral test was performed every week (from 4 weeks to 12 weeks) and mice were sacrificed at the age of indicated week for Western blotting and immunohistochemistry. All the mouse experiments were performed in the POSTECH animal facility and were approved by the POSTECH Institutional Animal Care and Use Committee (approval number: 2010-03-13).

Imunohistochemistry and Western blotting: Mice (n = 4 for 8-week-old mice) were perfusion fixed through the heart using 4% PFA/PBS. The paraffin-embedded tissues were cut at 4 μm on a rotary microtome (Shandon, Pittsburgh, PA). For immunostaining, the sections were blocked with 1% normal goat serum (0.3% Triton X-100, PBS) for 2 hr at room temperature. The sections were incubated with expanded polyglutamine (1C2) (Millipore, Billerica, MA) primary antibody solution (1% normal goat serum, 0.3% Triton X-100, PBS). Nucleus was then stained with Hoechst33342 (2 μg ml^{-1}) for 15 min at room temperature. The samples were analyzed with confocal microscope Fluoview FV1000 (Olympus). For the Western blotting, mice (n = 4 for 4-week old mice, n = 4 for 8-week-old mice) brain and liver were lysed in buffer as previously described in the *in vitro* experiments. Insoluble aggregates were detected by primary antibody to huntingtin (EM48) (Millipore), and primary antibodies to GAPDH (Santa Cruz) and Lamin B (Santa Cruz) were used for loading control.

Behavioral tests of mice: For the rotarod test, mice were trained before record for 5 min at 4 rpm on

the rotarod apparatus (Panlab, Barcelona, Spain). Mice were placed on a rotating rod (4 rpm) and then were tested for the accelerating trial of 5 min with the speed changing from 4 to 40 rpm. The latency to fall from the rod was recorded and data were analyzed using the program SeDaCom32 (Bioseb, Chaville, France). For the open field test, a chamber open field with 60 cm by 40 cm was used to evaluate spontaneous locomotor activity of the mice. Mice were placed on the chamber and their traveled distances were recorded for 20 min using video-tracking system with a Smart v2.5 program (Panlab). To elicit limbs clasping, mice were suspended from the tail for 1 min. It was scored on a scale from 0 to 3 (0 = meaning no clasping, 1 = clasping of the forelimbs only, 2 = clasping of both fore and hind limbs below three times, 3 = clasping of both fore and hind limbs more than three times or more than 5 sec). All the mice were observed daily in order to determine lifespan, and mice were taken as dead when they no longer had movement.

Statistical analysis: All statistical analyses were performed with one-way ANOVA, and post hoc Tukey's honestly significant difference test and survival curves were analyzed by Kaplan-Meier method and log-rank test with GraphPad Prism 5.0

II-2. Synthetic Chemistry

General methods: Laboratory glassware and all the equipments were cleaned according to the standard protocols. Analytical TLC was performed on Merck 60 F254 silica gel plate (0.25 mm thickness) and visualized by using UV light, and/or by spraying with one of three solutions: a 5% solution of phosphomolybdic acid (PMA) in ethanol, ninhydrin specific for amino compounds, and 7.4% of sulfuric acid in ethanol, then followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel [70-230 or 230-400 mesh (flash)]. All NMR spectra were recorded on a Bruker DPX 300 instrument operating at 300 MHz for ^{1}H, and 75 MHz for ^{13}C, unless otherwise stated. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) or deuterium oxide (D_2O), which were used as internal and external standard for the ^{1}H NMR. High resolution mass spectral data (HR-FABMS) were obtained on a Jeol JMS 700 high resolution mass spectrometer at the Korea Basic Science Institute (Daegu), and MALDI-TOF-MS on a Micromass M@DI at the Biomolecular Diversity Core Facility (POSTECH). Analytical HPLC was performed on Agilent 1100-HPLC Chemstation with an analytical column ZORBAX C8-monomeric (BU-300, 5 μm, 300Å , 4.6 X 250 mm), and preparative HPLC on Agilent 1100-HPLC Chemstation with a semi-preparative column GRACEVYDAC C18 (5 μm, 300 Å , 10 X 250 mm).

6,6´-di-*O*-trityl-α-D-trehalose (1)

To a solution of trehalose dihydrate (2.2 g, 5.82 mmol) in pyridine (40 ml) at RT, was added trityl chloride (7.7 g, 27.21 mmol). The solution was stirred at 60 °C for 5 hr., diluted with EtOAc, washed twice with aq. HCl (1N) and aq. $NaHCO_3$. Brine was added to the organic layer and the resulting suspension was filtered through a Büchner funnel. The precipitate was washed with diethyl ether several times and dried in vacuo to yield **1** (4.37 g, 91%) as a white solid.

R_f : 0.6 (CH_2Cl_2 : MeOH = 5 : 1); m.p. 275-276 °C (dec.) [m.p. 276-281°C]; $[\alpha]_D^{24}$ 70.13° (c 0.51, pyridine) [$[\alpha]_D^{24}$ 64° (c 0.87, pyridine)]; ^{1}H NMR (MeOD) δ ppm 3.37 (app. t, 6H, J = 3.8 Hz), 3.57 (dd, 2H, J = 9.7 and 3.8 Hz), 3.79 (app. t, 2H, J = 9.3 Hz), 4.02-4.09 (m, 2H), 5.36 (d, 2H, J = 3.8 Hz, anomeric protons), 7.14-7.25 (m, 18H, trityl protons), 7.41-7.52 (m, 12H, trityl protons); ^{13}C NMR (MeOD) δ ppm 64.88, 72.59, 73.07, 73.62, 75.38, 87.85, 94.72, 128.12, 128.89, 130.19, 145.76; HR-FABMS $[M+Na]^+$ calcd for $C_{50}H_{50}O_{11}Na$ m/z 849.3251, found 849.3254.

2,2´,3,3´,4,4´-hexa-*O*-(*N*-Boc-6-aminohexanoyl)-6,6´-di-*O*-trityl-α-D-trehalose (2)

To a solution of **1** (2.19 g, 2.65 mmol) in DMF (60 ml) at RT, were added *N*-Boc-protected aminohexanoic acid (5.52 g, 23.87 mmol), EDC (4.58 g, 23.86 mmol), and DMAP (0.97 g, 7.96 mmol). After stirring for 2 days at 60 °C, the solution was concentrated at 60 °C with azeotropic removal with toluene. The residue was diluted with aq. LiCl (5%) and then extracted with EtOAc three times. The organic layer was washed with aq. $NaHCO_3$, dried over Na_2SO_4, filtered and condensed in vacuo to give the crude product, which was purified by column chromatography on silica gel to give **2** (3.05 g, 55%) as yellow oil.

R_f : 0.6 (EtOAc : Hex = 1 : 1); $[\alpha]_D^{23}$ 49.36° (c 0.57, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.08-1.75 (m, 90H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 1.85-2.30 (m, 12H, CO-CH_2), 2.97-3.03 (m, 4H), 3.05-3.14 (m, 12H, NCH_2), 4.08-4.16 (m, 2H), 4.73-4.89 (m, 6H, NH), 5.10 (app. t, 2H, J = 10.1 Hz), 5.23 (dd, 2H, J = 10.2 and 3.8 Hz), 5.43-5.50 (m, 4H), 7.20-7.42 (m, 30H, trityl protons); ^{13}C NMR ($CDCl_3$) δ ppm 24.43, 24.70, 26.49, 28.68, 29.97, 33.81, 33.95, 34.18, 40.56, 62.31, 68.94, 70.01, 70.68, 79.22, 86.79, 92.19, 127.35, 128.12, 172.33, 172.67; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{116}H_{164}N_6O_{29}Na$ m/z 2128.1440, found 2128.1402.

2,2´,3,3´,4,4´-hexa-*O*-[6-(*N*,*N*´-bis-Boc-guanidino)-hexanoyl]-α-D-trehalose (3)

Compound **2** (625 mg, 0.30 mmol) dissolved in EtOAc (17 ml) saturated with HCl(g) at RT was stirred for 2 days. After evaporation of solvent, the precipitate (368 mg, quantitative) was obtained as a white foamy solid, and it was used in the next step without further purification.

$[\alpha]_D^{20}$ 62.59° (c 0.94, MeOH); ^{1}H NMR (MeOD) δ ppm 1.39-1.72 (m, 36H, $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.29-2.49 (m, 12H, CO-CH_2), 2.91-3.11 (m, 12H, NCH_2), 3.56-3.69 (m, 3H), 3.80-3.95 (m, 2H), 4.16-4.42 (m, 2H), 4.90-5.10 (m, 3H), 5.24-5.54 (m, 4H); ^{13}C NMR (MeOD) δ ppm 25.33, 25.42, 25.47, 25.55, 27.04, 27.12, 28.37, 34.61, 34.76, 34.92, 40.64, 61.66, 67.03, 69.84, 71.92, 73.66, 92.46, 173.86, 174.64, 174.91; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{48}H_{88}N_6O_{17}Na$ m/z 1043.6104, found 1043.6043, $[M+H]^+$ calcd for $C_{48}H_{89}N_6O_{17}$ m/z 1021.6284, found 1021.6246.

To a solution of the above product (358 mg, 0.29 mmol) in 1,4-dioxane/ H_2O (5 : 1, 5 ml) at RT, were sequentially added Et_3N (0.8 ml, 4.22 mmol) and *N,N'*-bis-Boc-*N''*-trifluoromethanesulfonyguanidine (1.63 g, 4.16 mmol). After stirring for 3 days, the solution was concentrated under reduced pressure and diluted with EtOAc. The solution was washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered and condensed in vacuo to give a crude product, which was purified by column chromatography on silica gel to give **3** (564 mg, 80%) as a colorless foamy solid.

R_f : 0.3 (EtOAc : Hex = 2 : 3); $[\alpha]_D^{24}$ 37.73° (c 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.36-1.75 (m, 144H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.25-2.38 (m, 12H, CO-CH_2), 3.30-3.45 (m, 12H, NCH_2), 3.62-3.70 (m, 4H), 4.25 (dd, 2H, J = 11.8 and 5.0 Hz), 4.38-4.44 (m, 2H), 4.88 (dd, 2H, J=10.1 and 3.7 Hz), 5.31 (d, 2H, J = 3.7 Hz, anomeric protons), 5.39 (app. t, 2H, J = 9.9 Hz), 8.31 (brs, 6H), 11.49 (brs, 6H); ^{13}C NMR ($CDCl_3$) δ ppm 24.27, 24.47, 24.53, 26.41, 26.87, 28.08, 28.32, 28.40, 28.79, 33.76, 33.93, 34.67, 40.69, 40.74, 40.85, 62.76, 68.76, 70.34, 70.69, 72.99, 79.20, 79.38, 83.00, 83.04, 83.18, 89.83, 153.20, 153.31, 156.12, 163.28, 163.64, 172.41, 173.22, 173.38; MALDI-TOF-MS $[M+H]^+$ calcd for $C_{114}H_{197}N_{18}O_{41}$ m/z 2474.3884, found 2474.3831.

2,2´,3,3´,4,4´-hexa-*O*-(6-guanidinohexanoyl)-α-D-trehalose·6HCl (4, TD-G6)

Compound **3** (26 mg, 10.59 μmol) was added to EtOAc (2 ml) saturated with HCl(g) at RT, and the resulting solution was stirred for 2 days. After evaporation, the precipitate was dissolved in deionized

water and lyophilized to give the crude product, which was purified by using preparative RP-HPLC (GRACEVYDAC, C18), (2.5 ml min^{-1}, 25% CH_3CN in H_2O, 220 nm) to give **TD-G6** (**4**, 13.70 mg, 87%) as a white sticky solid.

$[\alpha]_D^{23}$ 49.27° (c 1.17, MeOH); ^1H NMR (D_2O) δ ppm 1.31-1.73 (m, 36H, $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.39-2.62 (m, 12H, $CO\text{-}CH_2$), 3.11-3.28 (m, 12H, NCH_2), 3.81 (app. t, 1H, J = 9.12 Hz), 3.95-4.11 (m, 2H), 4.15-4.52 (m, 5H), 4.89-5.12 (m, 2H), 5.19-5.55 (m, 4H); ^{13}C NMR (D_2O) δ ppm 23.33, 23.37, 24.85, 24.94, 25.03, 25.14, 33.03, 33.13, 40.45, 62.54, 67.09, 68.04, 70.10, 71.75, 90.66, 156.19, 174.28, 175.28, 175.55; MALDI-TOF-MS $[M+H]^+$calcd for $C_{54}H_{101}N_{18}O_{17}$ m/z 1273.7592, found 1273.7263; analytical HPLC (BU-300, 220 nm, 1 ml min^{-1}, 30% CH_3CN in H_2O during 15 min, t_R = 2.14 min), purity 99+ %.

6,6´-di-*O*-(*N*-benzyloxylcarbonyl-6-aminohexanoyl)-2,2´,3,3´,4,4´-hexa-*O*-[6-(*N*,*N*´-bis-Boc-guanidino)-hexanoyl]-α-D-trehalose (5)

To a solution of **3** (397 mg, 0.16 mmol) in DMF (10ml) at RT, were added *N*-Cbz-protected aminohexanoic acid (512 mg, 1.92 mmol), EDC (370 mg, 1.93 mmol), and DMAP (47 mg, 0.38 mmol). After stirring for 2 days, the solution was diluted with EtOAc, washed with aq. LiCl (5%) and brine. The organic layer was dried over Na_2SO_4, filtered and condensed in vacuo to give the crude product, which was purified by column chromatography on silica gel to give **5** (313 mg, 66%) as a colorless foamy solid.

R_f : 0.4 (CH_2Cl_2 : MeOH = 50 : 1); $[\alpha]_D^{22}$ 31.76° (c 0.96, $CHCl_3$); ^1H NMR ($CDCl_3$) δ ppm 1.23-1.73 (m, 156H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.21-2.39 (m, 16H, $CO\text{-}CH_2$), 3.10-3.24 (m, 4H, $C\underline{H}_2NH\text{-}CO$), 3.35-3.45 (m, 12H, $C\underline{H}_2NHCN$), 3.90-4.05 (m, 4H), 4.17-4.29 (m, 2H), 4.97-5.18 (m, 8H), 5.24-5.37 (m, 4H), 5.47 (app. t, 2H, J = 9.7 Hz), 7.28-7.39 (m, 10H, benzene protons), 8.30 (brs, 6H), 11.50 (brs, 6H); ^{13}C NMR ($CDCl_3$) δ ppm 24.51, 24.62, 24.69, 26.38, 26.53, 26.60, 28.22, 28.45, 28.90, 33.79, 33.85, 33.91, 34.03, 40.79, 40.83, 40.89, 40.97, 61.31, 66.52, 68.09, 68.41, 70.01, 77.43, 79.35, 83.15, 83.19, 91.87, 128.11, 128.15, 128.59, 136.95, 153.44, 156.63, 163.73, 163.78, 172.03, 172.24, 172.35, 173.19; MALDI-TOF-MS $[M+Na]^+$calcd for $C_{142}H_{230}N_{20}O_{47}Na$ m/z 2990.6120, found 2990.4188.

2,2´,3,3´,4,4´-hexa-*O*-[6-(*N*,*N*´-bis-Boc-guanidino)-hexanoyl]-6,6´-di-*O*-[6-(fluoresceinyl-5-thioureido)-hexanoyl]-α-D-trehalose (6)

A mixture of **5** (159 mg, 54 μmol) and Pd/C 10% (120 mg) in CH_2Cl_2/MeOH (1 : 9, 19 ml) was stirred under H_2 (50 psi) for 16 hr. The reaction mixture was filtered through a short pad of celite and washed with MeOH. Evaporation gave the amino compound (130 mg, 90%) as a colorless foamy solid, which was used in the next step without further purification.

^1H NMR (MeOD) δ ppm 1.39-1.78 (m, 156H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.25-2.45 (m, 16H, $CO\text{-}CH_2$), 2.85-2.99 (m, 4H, $C\underline{H}_2NH_2$), 3.32-3.39 (m, 12H, $C\underline{H}_2NH$), 3.94-4.11 (m, 2H), 4.09-4.28 (m, 4H), 5.05-5.14 (m, 4H), 5.36 (d, 2H, J = 3.6 Hz, anomeric protons), 5.53 (app. t, 2H, J = 9.7 Hz); ^{13}C NMR (MeOD, 125 MHz) δ ppm 25.47, 25.66, 25.75, 27.08, 27.45, 27.53, 27.62, 28.49, 28.52, 28.82, 29.90, 30.00, 34.69, 34.81, 34.89, 35.02, 40.75, 41.80, 41.84, 41.87, 63.10, 69.95, 70.09, 71.40, 71.50, 80.67, 80.71, 80.75, 84.72, 84.75, 92.30, 154.39, 157.55, 157.57, 157.60, 164.43, 164.49, 173.47, 173.68, 174.17, 174.78; MALDI-TOF-MS $[M]^+$ calcd for $C_{126}H_{218}N_{20}O_{43}$ m/z 2699.5487, found 2699.7160.

To a solution of the above product (129 mg, 47.83 μmol) in THF/EtOH/MeOH (6 : 4 : 1, 4 ml) at RT, were added Et_3N (65 μl, 0.34 mmol) and FITC-I (55 mg, 0.13 mmol). After stirring for 24 hr in the dark, the solution was concentrated to give the crude product, which was purified by a long column

chromatography on flash silica gel to yield **6** (95 mg, 57%) as a sticky orange solid.
R_f : 0.6 (CH_2Cl_2 : MeOH = 10 : 1); $[\alpha]_D^{24}$ 5.76° (c 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.23-1.66 (m, 156H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.11-2.30 (m, 12H, CO-CH_2), 3.30-3.48 (m, 12H, $C\underline{H}_2NHC{=}N$), 3.51-3.69 (m, 4H, $C\underline{H}_2NHC{=}S$), 3.91-4.01 (m, 4H), 4.10-4.20 (m, 2H), 4.92-5.11 (m, 4H), 5.21-5.31 (m, 2H), 5.49 (app. t, 2H, J = 9.3 Hz), 6.57-6.79 (m, 10H), 7.10-7.18 (m, 2H), 7.91-8.11 (m, 2H), 8.31-8.49 (m, 4H), 9.04 (brs, 2H), 11.49 (brs, 6H); ^{13}C NMR ($CDCl_3$) δ ppm 24.49, 26.50, 28.23, 28.40, 28.43, 28.94, 33.82, 41.01, 44.38, 61.51, 68.24, 68.41, 70.13, 70.14, 79.70, 80.11, 83.39, 83.66, 92.09, 103.27, 110.82, 118.89, 129.58, 140.83, 153.35, 153.43, 156.41, 156.56, 163.16, 163.53, 171.17, 172.13, 172.32, 172.65, 173.33, 181.16; MALDI-TOF-MS $[M]^+$ calcd for $C_{168}H_{240}N_{22}O_{53}S_2$ m/z 3477.6203, found 3477.6478.

2,2´,3,3´,4,4´-hexa-*O*-(6-guanidinohexanoyl)-6,6´-di-*O*-[6-(fluoresceinyl-5-thioureido)-hexanoyl]-α-D-trehalose·6HCl [7, TD-G6-(FITC)$_2$]
Compound **6** (92 mg, 26.52 μmol) was added to EtOAc (3.5 ml) saturated with HCl(g) at RT, and the solution was stirred for 2 days. After evaporation, the precipitate was dissolved in deionized water and lyophilized to give a crude product, which was purified by preparative RP-HPLC (GRACEVYDAC, C18), (2.5 ml min^{-1}, 25% CH_3CN in H_2O, 220 nm) to give **TD-G6-(FITC)$_2$** (**7**, 42 mg, 64%) as an orange sticky solid.
$[\alpha]_D^{23}$ -11.75° (c 0.56, MeOH); UV (MeOH): λ_{max} = 483 nm, ε = 21537 cm^{-1}M^{-1}; ^{1}H NMR (MeOD) δ ppm 1.30-1.75 (m, 48H, $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.24-2.49 (m, 16H, CO-CH_2), 3.11-3.28 (m, 12H, $C\underline{H}_2NHC{=}N$), 3.59-3.66 (m, 4H, $C\underline{H}_2NHC{=}S$), 4.00-4.09 (m, 2H), 4.10-4.28 (m, 4H), 5.04-5.18 (m, 4H), 5.35 (d, 2H, J = 3.4 Hz, anomeric protons), 5.54 (app. t, 2H, J = 9.8 Hz), 6.55-6.73 (m, 12H), 7.15-7.18 (m, 2H), 7.73-7.80 (m, 2H), 8.32 (brs, 2H); ^{13}C NMR (MeOD) δ ppm 23.55, 23.67, 23.74, 25.29, 25.37, 25.48, 25.53, 27.70, 27.80, 32.73, 32.91, 32.06, 40.50, 40.59, 43.30, 61.05, 67.66, 67.93, 69.44, 69.54, 90.66, 101.64, 112.30, 124.20, 128.73, 140.85, 152.80, 156.68, 169.11, 171.76, 172.38, 172.80, 181.18; MALDI-TOF-MS $[M+H]^+$ calcd for $C_{108}H_{145}N_{22}O_{29}S_2$ m/z 2277.9989, found 2278.0258; analytical HPLC (BU-300, 220 nm, 1 ml min^{-1}, 30% CH_3CN in H_2O during 10 min, t_R = 2.30 min), purity 99+ %.

2,2´,3,3´,4,4´,6,6´-octa-*O*-(*N*-Boc-6-aminohexanoyl)-α-D-trehalose (8)
To a solution of trehalose dihydrate (0.87 g, 2.30 mmol) in DMF (24 ml) at RT, were added *N*-Boc-protected aminohexanoic acid (6.37 g, 27.55 mmol), EDC (5.81 g, 30.30 mmol), and DMAP (0.84 g, 6.89 mmol). After stirring for 2 days, the solution was concentrated at 60 °C under reduced pressure by azeotropic distillation with toluene. The residue was diluted with EtOAc and washed with aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and condensed in vacuo to give the crude product, which was purified by column chromatography on silica gel to give **8** (4.03 g, 86%) as a colorless foamy solid.
R_f : 0.4 (EtOAc : Hex = 1 : 1); $[\alpha]_D^{24}$ 54.07° (c 1.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.24-1.66 (m, 120H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.20-2.37 (m, 16H, CO-CH_2), 3.08-3.21 (m, 16H, $NC\underline{H}_2$), 3.83-4.03 (m, 4H), 4.20-4.25 (m, 2H), 4.65-5.00 (m, 7H, NH), 5.01-5.09 (m, 4H), 5.29 (d, 2H, J = 3.7 Hz, anomeric protons), 5.48 (app. t, 2H, J = 9.7 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 24.56, 24.70, 26.49, 28.67, 29.99, 33.99, 34.15, 40.56, 61.52, 68.25, 68.50, 79.24, 91.89, 156.28, 172.20, 172.41, 172.58, 173.35; MALDI-TOF-MS $[M+Na]^+$calcd for $C_{100}H_{174}N_8O_{35}Na$ m/z 2070.1979, found 2070.1929.

2,2´,3,3´,4,4´,6,6´-octa-*O*-(6-aminohexanoyl)-α-D-trehalose·8TFA (9)

Compound **8** (1.94 g, 0.95 mmol) was added to a TFA/CH_2Cl_2 mixture (1 : 1, 20 ml) at RT and the solution was stirred for 6 hr. Progress of the reaction was monitored by TLC. After the starting material disappeared, the solution was concentrated. The residue was washed with a mixture of diethyl ether and MeOH (20 : 1), and thoroughly dried under vacuum. The residue was dissolved in deionized water, filtered through a polytetrafluoroethylene (PTFE) syringe filter, and lyophilized to give the TFA salt **9** (3.17 g, 93%) as a sticky brown solid.
^{1}H NMR (MeOD) δ ppm 1.25-1.39 (m, 16H, $NCH_2CH_2C\underline{H}_2$), 1.42-1.66 (m, 32H, $NCH_2C\underline{H}_2CH_2C\underline{H}_2$), 2.10-2.35 (m, 16H, CO-CH_2), 2.71-2.89 (m, 16H, NCH_2), 3.81-3.93 (m, 2H), 4.00-4.15 (m, 4H), 4.91-5.03 (m, 4H), 5.23 (d, 2H, J = 3.6 Hz, anomeric protons), 5.35 (app. t, 2H, J = 9.7 Hz); ^{13}C NMR (MeOD) δ ppm 25.43, 25.47, 25.59, 27.04, 27.13, 27.23, 28.42, 34.58, 34.90, 40.63, 40.69, 62.83, 69.64, 69.95, 71.49, 71.58, 92.97, 160.02, 160.59, 161.07, 161.60, 173.58, 173.66, 174.21, 174.70; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{60}H_{110}N_8O_{19}Na$ m/z 1269.7785, found 1269.6978.

2,2´,3,3´,4,4´,6,6´-octa-*O*-[6-(*N,N´*-bis-Boc-guanidino)-hexanoyl]-α-D-trehalose (10)
To a solution of **9** (86 mg, 39.59 μmol) in 1,4-dioxane/ H_2O (5 : 1, 2 ml) at RT, were sequentially added Et_3N (0.5 ml, 2.64 mmol) and triflylguanidine (187 mg, 0.48 mmol). After stirring for 3 days, the solution was concentrated under reduced pressure and was diluted with EtOAc. The solution was washed with H_2O, and brine. The organic layer was dried over Na_2SO_4, filtered and condensed in vacuo to give a crude product, which was purified by column chromatography on silica gel to give **10** (81 mg, 64%) as a colorless foamy solid.
R_f : 0.8 (EtOAc : Hex = 2 : 3); $[\alpha]_D^{24}$ 28.39° (c 0.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.28-1.71 (m, 192H, CH_3 and $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.20-2.39 (m, 16H, CO-CH_2), 3.33-3.46 (m, 16H, NCH_2), 3.94-4.01 (m, 4H), 4.16-4.24 (m, 2H), 4.97-5.10 (m, 4H), 5.27 (d, 2H, J = 3.7 Hz, anomeric protons), 5.45 (app. t, 2H, J = 9.7 Hz), 8.31 (brs, 8H), 11.50 (brs, 8H); ^{13}C NMR ($CDCl_3$) δ ppm 24.62, 24.80, 26.61, 26.69, 28.28, 28.51, 29.00, 33.85, 34.14, 40.80, 40.90, 40.95, 61.30, 68.14, 68.47, 70.07, 79.37, 83.19, 92.41, 153.48, 156.31, 163.82, 172.08, 172.32, 172.41, 173.13; MALDI-TOF-MS $[M+H]^+$ calcd for $C_{148}H_{255}N_{24}O_{51}$ m/z 3184.8098, found 3184.8136.

2,2´,3,3´,4,4´,6,6´-octa-*O*-(6-guanidinohexanoyl)-α-D-trehalose·8HCl (11, TD-G8)
Compound **10** (20 mg, 6.28 μmol) was added to EtOAc (3 ml) saturated with HCl(g) at RT and the solution was stirred for 2 days. After evaporation, the precipitate was dissolved in deionized water and lyophilized to give the crude product, which was purified by preparative RP-HPLC (GRACEVYDAC, C18), (2.5 ml min^{-1}, 25% CH_3CN in H_2O, 220 nm) to give **TD-G8** (**11**, 9.5 mg, 81%) as a white sticky solid.
$[\alpha]_D^{23}$ 49.02° (c 1.27, MeOH); ^{1}H NMR (D_2O) δ ppm 1.29-1.73 (m, 48H, $NCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.31-2.49 (m, 16H, CO-CH_2), 3.12- 3.22 (m, 16H, NCH_2), 4.02-4.13 (m, 2H), 4.21-4.40 (m, 4H), 5.15-5.27 (m, 4H), 5.45 (d, 2H, J = 3.5 Hz, anomeric protons), 5.58 (app. t, 2H, J = 9.7 Hz); ^{13}C NMR (D_2O) δ ppm 24.50, 24.59, 26.05, 26.13, 26.38, 28.20, 28.23, 28.32, 28.40, 34.13, 34.21, 34.30, 41.65, 41.74, 62.33, 68.65, 69.03, 70.45, 71.17, 91.39, 157.40, 174.84, 175.13, 175.92, 176.44; MALDI-TOF-MS $[M+H]^+$ calcd for $C_{68}H_{127}N_{24}O_{19}$ m/z 1583.9709, found 1583.9279; analytical HPLC (BU-300, 220 nm, 1 ml min^{-1}, 0 to 40% CH_3CN in H_2O during 15 min, t_R = 2.40 min), purity 99+ %.

2,2´,3,3´,4,4´-hexa-*O*-benzyl-6,6´-di-*O*-trityl-α-D-trehalose (12)
To a solution of trehalose dihydrate (637 mg, 1.68 mmol) in pyridine (30 ml) at RT, was added trityl chloride (2.86 g, 10.05 mmol). After stirring for 8 hr at 75 °C, the solution was cooled down to RT

and MeOH (10 ml) was added. The solution was concentrated by azeotropic distillation with toluene and the resulting mixture was dried in vacuum for 4 hr. The residue was dissolved in DMF (20 ml) and cooled to 0 °C. To the solution at 0 °C, were added NaH (60%, 1.21 g, 30.25 mmol) and then BnBr (3.06 ml, 25.21 mmol). The temperature was slowly increased to RT. After stirring vigorously overnight, aq. $NaHCO_3$ (10 ml) and aq. LiCl (5%) were added. The solution was extracted with EtOAc three times. The organic layer was dried over Na_2SO_4, filtered and evaporated to give a crude product, which was purified by column chromatography on flash silica gel to yield **12** (1.17 g, 51%) as a yellow foamy solid.

R_f : 0.4 (EtOAc : Hex = 1 : 4); $[\alpha]_D^{20}$ 78.26° (c 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 3.20 (dd, 4H, J = 79.1 and 8.8 Hz), 3.72 (dd, 2H, J = 9.4 and 3.6 Hz), 3.80-4.01 (m, 4H), 4.60-4.75 (m, 6H), 4.87 (dd, 4H, J = 4.1 and 10.6 Hz), 5.46 (d, 2H, J = 3.6 Hz, anomeric protons), 6.75-7.59 (m, 60H, benzene protons); ^{13}C NMR ($CDCl_3$) δ ppm 61.68, 70.87, 72.70, 75.26, 76.17, 78.07, 80.54, 82.02, 86.37, 95.12, 126.95, 127.13, 127.13, 127.29, 127.47, 127.73, 127.83, 127.96, 128.22, 128.38, 128.62, 129.09, 129.64, 138.18, 138.42, 138.95, 144.03; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{92}H_{86}O_{11}Na$ m/z 1389.6068, found 1389.6056.

2,2´,3,3´,4,4´-hexa-*O*-benzyl-α-D-trehalose (13)

To the solution of **12** (818 mg, 0.60 mmol) in CH_2Cl_2/MeOH (1 : 1, 12 ml) at RT, was added *p*TSA monohydrate (340 mg, 1.76 mmol). After stirring for 3 hr, Et_3N (3 ml, 15.81 mmol) was added. The solution was evaporated to give a crude product, which was purified by column chromatography on flash silica gel to yield **13** (345 mg, 65%) as a colorless foamy solid.

R_f : 0.5 (CH_2Cl_2 : MeOH = 20 : 1); $[\alpha]_D^{22}$ 39.99° (c 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 3.51-3.59 (m, 8H, C<u>H</u>$_2$Bn), 4.01-4.14 (m, 4H, C<u>H</u>$_2$Bn), 4.61-4.75 (m, 6H), 4.86-4.95 (m, 4H), 4.94-5.12 (m, 2H), 5.14 (d, 2H, J = 3.0 Hz, anomeric protons), 7.19-7.47 (m, 30H, benzene protons); ^{13}C NMR ($CDCl_3$) δ ppm 61.80, 71.52, 73.26, 75.28, 75.82, 77.44, 79.74, 81.83, 94.27, 127.74, 127.79, 127.90, 128.11, 128.33, 128.63, 128.70, 138.28, 138.46, 139.01; HR-FABMS $[M+Na]^+$ calcd for $C_{54}H_{58}O_{11}Na$ m/z 905.3877, found 905.3882.

6,6´-di-*O*-(*N*-benzyloxylcarbonyl-6-aminohexanoyl)-2,2´,3,3´,4,4´-hexa-*O*-benzyl-α-D-trehalose (14)

To a solution of **13** (342 mg, 0.39 mmol) in DMF (8 ml) at RT, were added *N*-Cbz-protected aminohexanoic acid (308 mg, 1.16 mmol), EDC (193 mg, 1.01 mmol), and DMAP (24 mg, 0.20 mmol). After stirring for 1 day, the solution was diluted with aq. LiCl (5%), extracted with EtOAc three times. The organic layer was washed with aq. $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography on flash silica gel to yield **14** (505 mg, 95%) as a colorless oil.

R_f : 0.5 (EtOAc : Hex = 2 : 3); $[\alpha]_D^{22}$ 57.28° (c 1.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ ppm 1.21-1.62 (m, 12H, NCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 2.23 (t, 4H, J = 7.2 Hz, CO-CH$_2$), 3.12 (q, 4H, J = 6.4 Hz, NCH$_2$), 3.48-3.59 (m, 4H, HCOC<u>H</u>$_2$Bn), 4.01-4.28 (m, 8H, HCOC<u>H</u>$_2$Bn), 4.48-4.53 (m, 2H), 4.64-4.74 (m, 4H), 4.78 (brs, 2H, NH), 4.83-4.89 (m, 4H), 4.95-5.03 (m, 2H), 5.07 (s, 2H, CO-OC<u>H</u>$_2$Bn), 5.17 (d, 2H, J = 3.5 Hz, anomeric protons), 7.21-7.39 (m, 40H, benzene protons); ^{13}C NMR ($CDCl_3$) δ ppm 24.55, 26.30, 29.74, 34.01, 40.95, 60.53, 62.79, 66.72, 69.31, 73.09, 75.30, 75.84, 79.51, 81.75, 94.05, 127.61, 127.83, 127.97, 128.10, 128.23, 128.60, 128.66, 136.82, 137.97, 138.07, 138.74, 156.51, 173.24; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{82}H_{92}N_2O_{17}Na$ m/z 1399.6294, found 1399.58901.

6,6´-di-*O*-(6-aminohexanoyl)-α-D-trehalose (15)

A mixture of **14** (477 mg, 0.35 mmol) and $Pd(OH)_2/C$ 20% (120 mg) in CH_2Cl_2/EtOH (2 : 3, 24 ml) was stirred under H_2 (55 psi) for 4 days. The reaction mixture was filtered through a short pad of celite and washed with MeOH. Evaporation of solvents gave the amino-alcohol compound **15** (192 mg, 97%) as a white foamy solid.

$[\alpha]_D^{22}$ 57.09° (c 1.17, MeOH); ^{1}H NMR (MeOD) δ ppm 1.41-1.79 (m, 12H, $NCH_2C\underline{H_2}C\underline{H_2}C\underline{H_2}$), 2.41 (t, 4H, J = 6.8 Hz, $CO\text{-}CH_2$), 2.95 (t, 4H, J = 7.0 Hz, NCH_2), 3.35-3.39 (m, 2H), 3.43-3.54 (m, 2H), 3.82 (app. t, 2H, J = 8.9 Hz), 4.00-4.04 (m, 2H), 4.16-4.34 (m, 2H), 4.35-4.45 (m, 2H), 5.04 (d, 2H, J = 2.6 Hz, anomeric protons); ^{13}C NMR (MeOD) δ ppm 25.51, 26.96, 28.31, 30.84, 34.72, 40.75, 64.67, 71.68, 72.05, 73.26, 74.01, 74.62, 95.48, 110.95, 175.20; HR-FABMS $[M+H]^+$ calcd for $C_{24}H_{45}N_2O_{13}$ m/z 569.2926, found 569.2922.

6,6´-di-*O*-[6-(fluoresceinyl-5-thioureido)-hexanoyl]-α-D-trehalose [16, TD-(FITC)$_2$]

To a solution of **15** (75 mg, 0.13 mol) in THF/MeOH (1 : 3, 4 ml) at RT, were added Et_3N (0.17 ml, 0.90 mmol) and FITC-I (147 mg, 0.34 mmol). After stirring for 24 hr in the dark, the solution was concentrated to give a crude product, which was purified by column chromatography on flash silica gel to yield **TD-(FITC)$_2$** (**16**, 57 mg, 32%) as an orange sticky solid.

R_f : 0.6 (EtOAc : MeOH : H_2O = 6 : 1 : 1); $[\alpha]_D^{23}$ 8.04° (c 0.68, MeOH); UV (MeOH): λ_{max} = 454 nm, ε = 11917 $cm^{-1}M^{-1}$; ^{1}H NMR (MeOD, 500 MHz) δ ppm 1.40-1.75 (m, 12H, $NCH_2C\underline{H_2}C\underline{H_2}C\underline{H_2}$), 2.41 (t, 4H, J = 7.5 Hz, $CO\text{-}CH_2$), 3.35-3.40 (m, 2H), 3.52 (dd, 2H, J = 9.5 and 3.5 Hz), 3.58-3.69 (m, 4H, NCH_2), 3.80 (app. t, 2H, J = 9.0 Hz), 4.00-4.09 (m, 2H), 4.22 (dd, 2H, J = 12.0 and 5.5 Hz), 4.41 (dd, 2H, J = 11.5 and 2.0 Hz), 5.08 (d, 2H, J = 4.0 Hz, anomeric protons), 6.53-6.70 (m, 8H), 6.90-6.93 (m, 4H), 7.15-7.19 (m, 2H), 7.72-7.74 (m, 2H), 8.08-8.10 (m, 2H); ^{13}C NMR (MeOD, 125 MHz) δ ppm 24.39, 26.04, 28.18, 33.58, 44.13, 63.08, 70.20, 70.59, 71.77, 73.24, 93.82, 102.32, 112.02, 126.29, 129.90, 140.70, 154.68, 170.56, 173.96, 181.10; MALDI-TOF-MS $[M+Na]^+$ calcd for $C_{66}H_{66}N_4O_{23}S_2Na$ m/z 1369.3457, found 1369.3112, $[M+H]^+$ calcd for $C_{66}H_{67}N_4O_{23}S_2$ m/z 1347.3638, found 1347.3257; analytical HPLC (BU-300, 220 nm, 1 ml min^{-1}, 30% to 80% CH_3CN gradient in H_2O over 20 min, t_R = 10.21 min), purity 97+ %.

III. Supporting Reference

1. Kim, W. *et al*. (2012) Macro Histone H2A1.2 (MacroH2A1) Protein Suppresses Mitotic Kinase VRK1 during Interphase. *J. Biol. Chem*. 287(8): 5278-5289.
2. Tanaka M, et al. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.* 10(2):148-154.