Supporting information

A Lysosome- Targeted Drug Delivery System Based on Sorbitol Backbone towards Efficient Cancer Therapy

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1. General Synthetic Chemistry Methods.

Unless otherwise noted, all non-hydrolytic reactions were carried out in oven-dried glassware under an inert atmosphere of dry argon or nitrogen. All commercial chemicals were used as received, except for solvents, which were purified and dried by standard methods, prior to use. Analytical TLC was performed on a Merck 60 F254 silica gel plate (0.25mm thickness), and visualization was done with UV light (254nm and 365nm), or by spraying with a 5% solution of phosphomolybdic acid or ninhydrine solution, followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (60-120 or 100-200 mesh), and HPLC

was performed on supeclean LC-C18-reversed phase silica gel. NMR spectra were recorded on Bruker AMX 300 (^{1}HNMR at 300MHz; ^{13}C-NMR at 75 MHz) and Bruker AMX 500 (^{1}H-NMR at 500MHz; ^{13}C-NMR at 125MHz) spectrometers. Tetramethysilane was used as reference for ^{1}H NMR, and the chemical shifts were reported in ppm and the coupling constant in Hz. Analytical and preparative HPLC were performed using Shimadzu HPLC system consisting of SCL-10Avp system controller, two LC-8A solvent delivery units , SPD-M20A UV-vis photo diode array (PDA) detector, equipped with Multi PDA- LC solution (software) on a 250 mm x 4.6 mm i.d, 5µm, YMC-Pack R&D ODS analytical column (9YMC Co., Ltd. Japan). High resolution mass spectra were determined on a HR- EMI analysis of Thermo Scientific Exactive system, and MALDI-TOF mass spectra on a Shimadzu Biotech, AXIMA-CFR PLUS system. The standard extractive work-up procedure consisted of pouring into a large amount of water, extracting with organic solvent indicated, washing the combined extracts successively with water and brine, drying the extract over anhydrous Na_2SO_4 or $MgSO_4$, and evaporating the solvent.

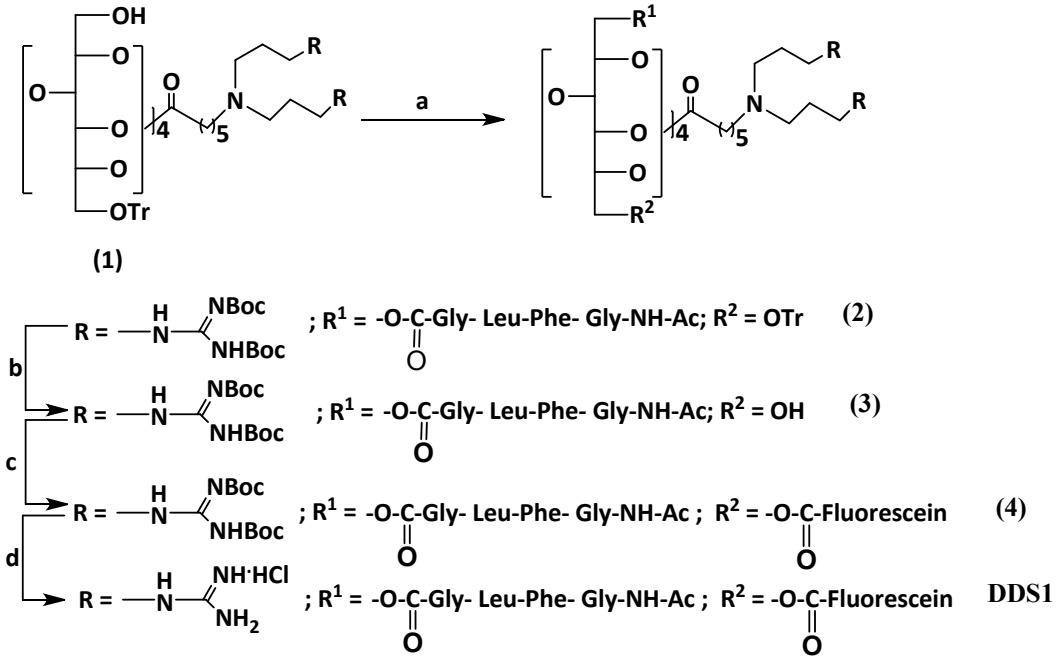
1.1 Synthesis of Cathepsin B peptide sequence (GLPG & GLPGC)

Preparation of GLPG and GLPGC was initiated with Fmoc- Gly-OH (337.6mg, 0.028 mmol) which was taken in dry dichloromethane (10mL) and DIC (0.218mL, 0.071mmol) was added. This was stirred at 0-5 °C for 1h under N_2 atmosphere. The Fmoc- Gly-OH activated DIC complex was concentrated and then dissolved in DMF along with 2-3 drops of DIPEA and charged into the resin bed of HMPB-MBHA resin (200mg, 0.0142mmol) which was swelled up in dry dichloromethane (6mL) for 30 min. The reaction was continued for 8 h with shaking. The progress of the reaction was monitored by the Kaiser test. After completion of the coupling, the resin was washed with DMF (3 x 3 mL), and the Fmoc protection group was removed by treatment with piperidine in DMF (20%, 3 x 2 ml, 3 x 15 min). The reaction cycle was continued in a similar manner with Fmoc- Phe-OH (165mg, 0.028mmol), Fmoc-Gly-OH

(337.6mg, 0.028 mmol) and Fmoc -Cys-OH (249mg, 0.042mmol) amino acids charged to the resin. The resulting resin-bound tetrapeptide was washed with DMF (3 x 5 ml), dichloromethane (7 x 3 mL) and methanol (3 x 3 mL), dried *in vacuo* (10 h), re-swelled in dichloromethane (5 ml), and filtered. Finally desired peptide sequence was released from the resin by treatment with 2% trifluoroacetic acid in dichloromethane (10 x 2 mL). The resin washing was combined and concentrated under reduced pressure, and the residue co-evaporated with toluene. The residue was precipated with cold ether (3 mL) and filtered the residue peptide that afforded of white solid (74 mg, 89%). ^{1}H-NMR (500MHz, $CDCl_3$) : δ 1.84 (t, 2H), 2.16 (s, 3H),7.28-7.38 (m, 5H), 8.56 ppm (s, 5H) ; MALDI-TOF-MS: m/z calcd for $C_{24}H_{35}N_5O_7SNa$: 559.1452, found 559.1481 $[M+Na]^+$.

1.2 Synthesis of DDS1

Compound 1 has been synthesized as per our previously reported synthetic route [1]



Scheme1. Reagent and conditions: a) HO_2C-Gly-Leu-Phe-Gly-NH-Ac (Cathepsin- B peptide sequence),EDC, DMAP, DIPEA, CH_2Cl_2, RT,16h, 62%; b) 2%TFA in CH_2Cl_2,RT,4h,76%; c) Fluorescein, EDC, DMAP,TEA,RT, dark,12h, 64%; d) HCl (gas), EtOAc, RT, 4h, 65%.

Synthesis of compound (2)

Cathepsin B peptide sequence (HO_2C-Gly-Leu-Phe-Gly-NH-Ac, 11.15mg, 0.025mmol), EDC (5.7mg, 0.029mmol), N, N'- dimethyl amino pyridine (1.3mg, 0.011mmol), and DIPEA (1.8mL, 0.010mmol) were added to a solution of **1** (70mg, 0.021mmol) in dry CH_2Cl_2 (3mL). The mixture was stirred at RT for 16h under N_2 atmosphere. The crude product was purified using column chromatography on silica gel to afford the product **2** as a white foamy solid (49.87 mg, 62%).

^{1}H-NMR (500MHz, $CDCl_3$) : δ 0.84-0.95 (m, 6H), 1.45-1.67 (m, 186H), 2.01-2**.**76 (m, 19H), 3.14-3.66 (m, 10H), 7.23-7.25 (m, 4H), 7.23-7.42 (m, 20H), 8.23 (br s, 8H), 11.42 ppm (br s, 8H); MALDI-TOF-MS: m/z calcd for $C_{183}H_{296}N_{32}O_{51}Na$: 3781.1452, found 3781.3145 $[M+Na]^+$

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Synthesis of compound (3)

Trifluoroacetic acid (0.15mL, 0.002mmol) was added to a solution of **2** (40mg, 0.011mmol) in dry CH_2Cl_2 (2ml), and the solution was stirred at RT for 4h. The result mixture was concentrated to give the crude product. This was purified using column chromatography on silica gel to afford the product **3** as a white foamy solid (27.85mg, 76%).

^{1}H-NMR (500MHz, $CDCl_3$): δ 0.73-0.82 (m, 6H), 1.18-1.50 (m, 184H), 2.96-3.57 (m, 32H), 4.09-4.14 (m, 4H), 7.18-7.26 (m, 5H), 7.21 ppm (s, 4H); MALDI-TOF-MS: m/z calcd for $C_{163}H_{286}N_{32}O_{47}Na$: 3467.0873, found 3467.4160 $[M+Na]^+$.

Synthesis of compound DDS1

Fluorescein (2.3mg, 0.0070mmol), EDC (1.7mg, 0.0088mmol), triethylamine (0.4117mL, 0.0029mmol) and DMAP (0.21mg, 0.0017mmol) were to a solution of **3** (20mg, 0.0058mmol) in dry CH_2Cl_2. The reaction mixture was stirred in dark conditions at RT for 12h. The resultant

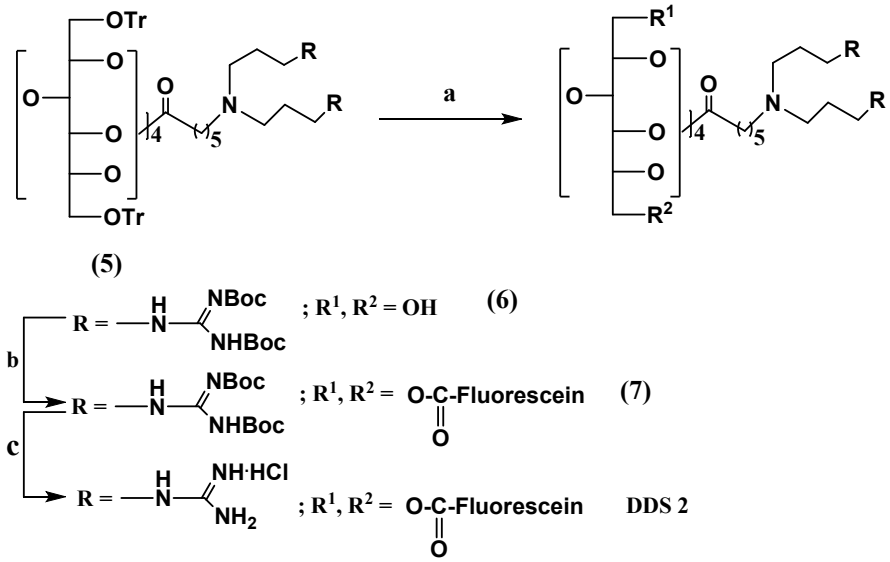
mixture was concentrated to give the crude product and was purified using column chromatography on silica gel to afford the coupled product **4** as an orange colored foamy solid.

Ethyl acetate (4mL) saturated with gaseous HCl was added to a solution of **4** (12mg, 0.0032mmol) in ethyl acetate (1mL). The reaction mixture was stirred for 4h. Then the solution was concentrated and the residue was washed with dichloromethane to remove less polar impurities. The residue was dried and purified using MPLC on supelclean LC-18 reverse - phase silica gel (CH_3CN / H_2O 9: 1 to 4: 1). The purified product was dissolved in de-ionized water, filtered through a PTGE syringe filter, and lyophilized to give DDS1 as a light-greenish-yellow foamy solid (HCl salt, 6.0mg, 65%).

^{1}H-NMR (500MHz, CD_3OD): δ 0.75-0.81 (m, 3H), 1.12-1.28 (m, 42H), 1.92 (s, 3H), 1.92-2.17 (m, 32H), 3.98-4.02 (m,4H), 6.73-6.8 (m, 4H), 7.10-7.84 (m, 15H), 7.62 (br,s.; 4H); MALDI-TOF-MS: m/z calcd for $C_{103}H_{176}N_{32}O_{19}Na$: 2188.3690, found 2188.5821 [M+Na]+ ; Analytical HPLC (Shimadzu-ODS): R_t = 4.24 min (Flow rate = 1ml / min, UV 480nm, CH_3CN: H_2O = 90:10).

1.3. Synthesis of DDS2

(Compound **5** has been synthesized as per our previously reported synthetic route [1] **)**



Scheme 2. Synthesis of transporters. a) 2%TFA in CH_2Cl_2, RT,4h,76%; b) Fluorescein, EDC, DMAP, TEA, CH_2Cl_2, RT,dark,72%; c) HCl (g), EtOAc, RT, 4h, 62%.

Synthesis of compound (6)

Trifluoroacetic acid (0.89mL, 0.011mmol) was added to a solution of **5** (100mg, 0.029mmol), in dry CH_2Cl_2 and stirred at RT for 4h under nitrogen atmosphere. The resultant mixture was concentrated to give the crude product, which was purified by column chromatography on silica gel to afford the product **6** (65.2mg, 76%) as a white foamy solid.

^{1}H-NMR (500MHz, $CDCl_3$): δ 1.26-1.96 (m, 184H), 2.01-2.32 (m, 16H), 3.05-3.38 (m, 32H), 8.22-8.23 (m, 8H); MALDI-TOF-MS: m/z calcd for $C_{142}H_{258}N_{28}O_{42}Na$: 3050.8813, found 3050.8472 $[M+Na]^+$.

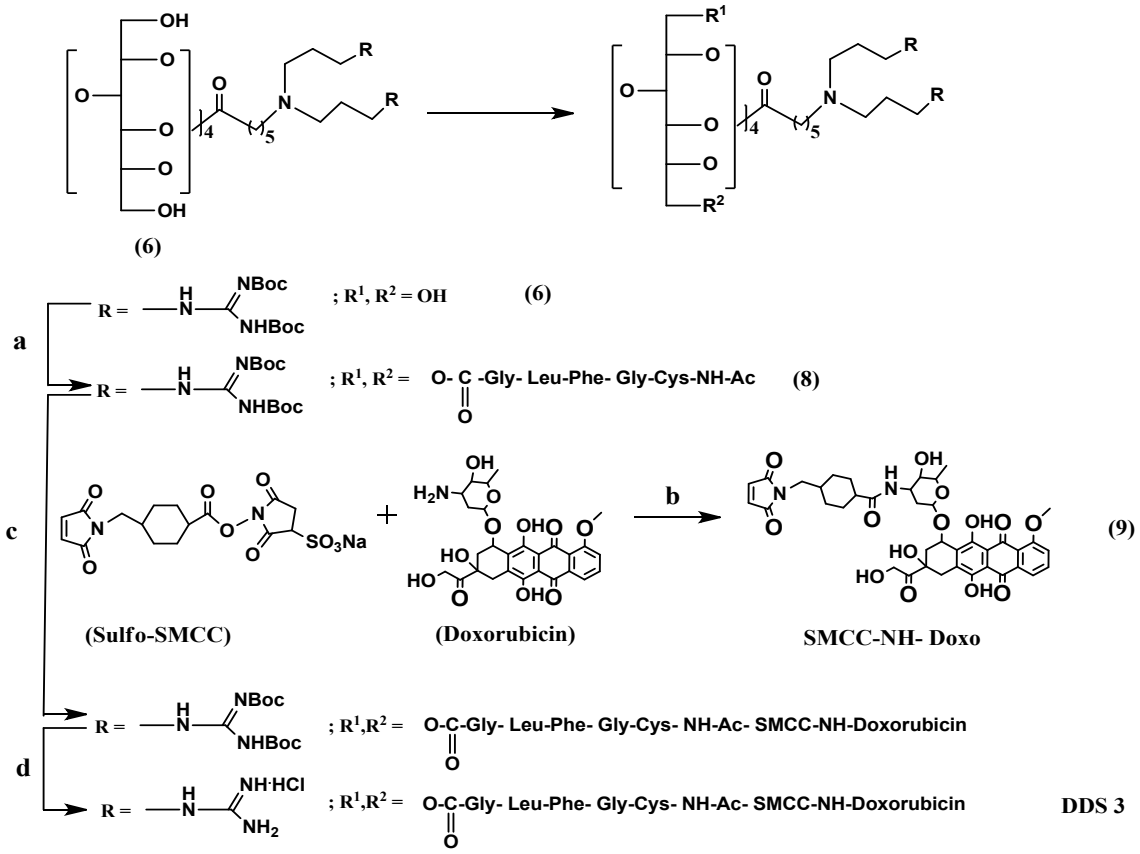
Synthesis of compound DDS2

Fluorescein (7.9mg, 0.002mmol) was added to a solution of **6** (60mg, 0.020mmol), EDC (9.5mg, 0.050mmol), triethylamine (1.11mL, 0.008mmol) and DMAP (1.2mg, 0.010mmol) in dry CH_2Cl_2. This was stirred at RT for 12h. The reaction mixture was concentrated to give the crude product and which was purified by column chromatography on silica gel to afford the compound **7** as an orange colored foamy solid. Ethyl acetate (5mL) saturated with gaseous HCl was added to a solution of **7** (30mg, 0.008mmol) in ethyl acetate (1mL) at RT. After 4h of stirring, the solution was concentrated and the residue was washed with dichloromethane to remove less polar impurities. The residue was dried and purified using MPLC on supelclean LC-18 reverse - phase silica gel (CH_3CN / H_2O 9: 1 to 3: 1). The purified product was dissolved in deionized water, filtered through a PTGE syringe filter, and lyophilized to give DDS2 (HCl salt, 10 mg, 62%) as a light- yellow foamy solid.

^{1}H-NMR (500MHz, CD_3OD): δ 0.90-1.45 (m, 40H), 3.22-3.27 (m, 16H), 3.72-3.77 (m, 4H), 6.69 (br,s.; 8H), 6.95-7.26ppm (m, 14H); MALDI-TOF-MS: m/z calcd for $C_{102}H_{158}N_{28}O_{18}Na$:

2086.2209, found: 2086.1063[M+Na] $^{+}$; Analytical HPLC (Shimadzu-ODS): R_t = 3.50 min (Flow rate = 1ml / min, UV 480nm, CH_3CN: H_2O = 90:10).

1.4. Synthesis of DDS3



Scheme 3. Reagent and conditions: a) HO_2C-Gly-Leu-Phe-Gly-Cys-NH-Ac (Cathepsin- B peptide sequence), EDC, DMAP, TEA, CH_2Cl_2, 12h, 94%; b) Sulfo-SMCC, Doxorubicin, TEA, DMSO, RT, dark, 12h, 90%; c) **8**, SMCC-Doxo, RT, DMSO, 15h, d) HCl(g) l(g), EtOAc,RT,4h,65%. EDC=3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; DMAP=4-(dimethylamino) pyridine; TBAF=Tetrabutylamonium fluoride; Sulfo-SMCC= 4-(maleimidomethyl) cyclohexane -1-carboxylic acid 3-sulfo-N-hydroxy- succinimide ester sodium salt. Boc= tert-butoxycarbonyl Sulfo-SMCC=Sulfosuccinimidyl-4-[N-maleimidomethyl] Cyclohexane-1-carboxylate; Boc=tert-butoxycarbonyl.

Synthesis of compound (8)

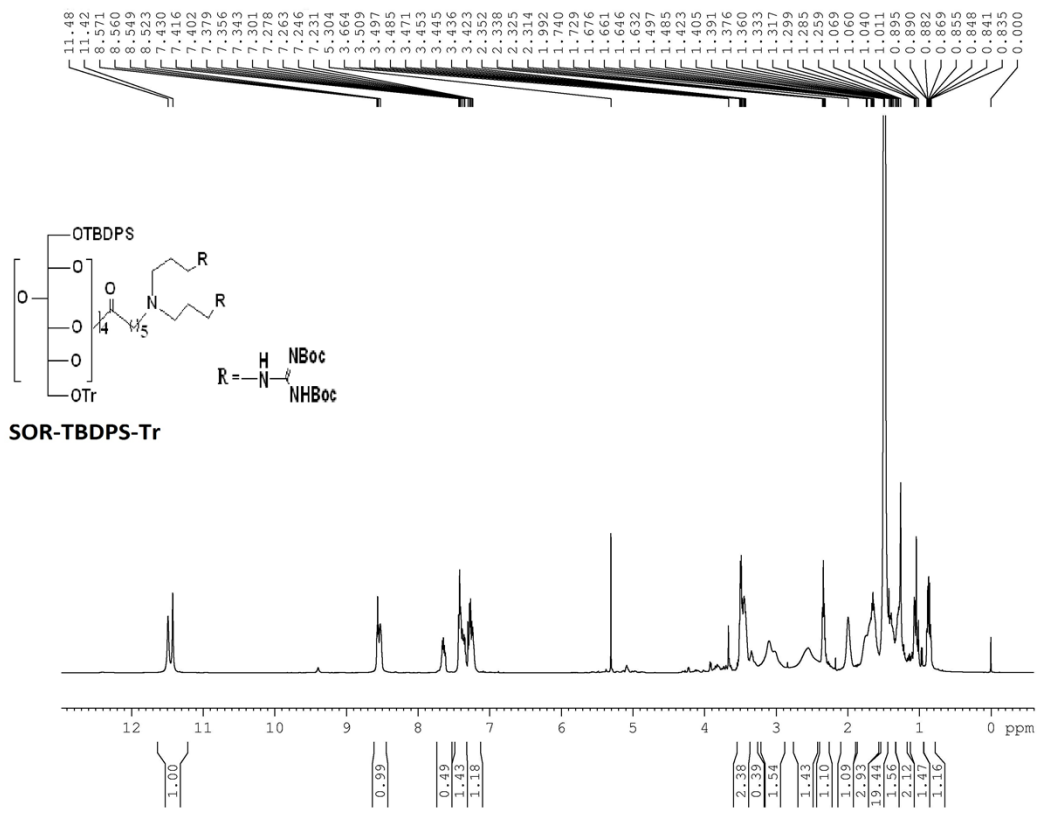
Cathepsin-B peptide sequence (HO_2C-Gly-Leu-Phe-Gly-Cys-NH-Ac) (31.93mg, 0.041mmol) was added to a solution of sobitol carrier **6** (56mg, 0.019mmol), EDC (8.9mg, 0.046mmol), triethylamine (1.06mL, 0.007mmol) and DMAP (1.16mg, 0.009mmol) in dry CH_2Cl_2. This was stirred at RT for 12h under N_2 atmosphere. The reaction mixture was concentrated to give the crude product and was purified by column chromatography on silica gel to afford the compound **8** as a white foamy solid (71.2 mg, 84%).

^{1}H-NMR (500MHz, $CDCl_3$): 1.28-1.31 (m, 188H), 2.84-2.92 (m, 32H), 3.10 (br, s; 14H), 7.18-7.98 (m, 20H), 12.04 (br, s, 8H); **MALDI-TOF-MS**: m/z calcd for $C_{190}H_{324}N_{38}O_{56}Na$: 4121.3015, found: 4121.5228 [M+Na]+ .

Synthesis of compound DDS3

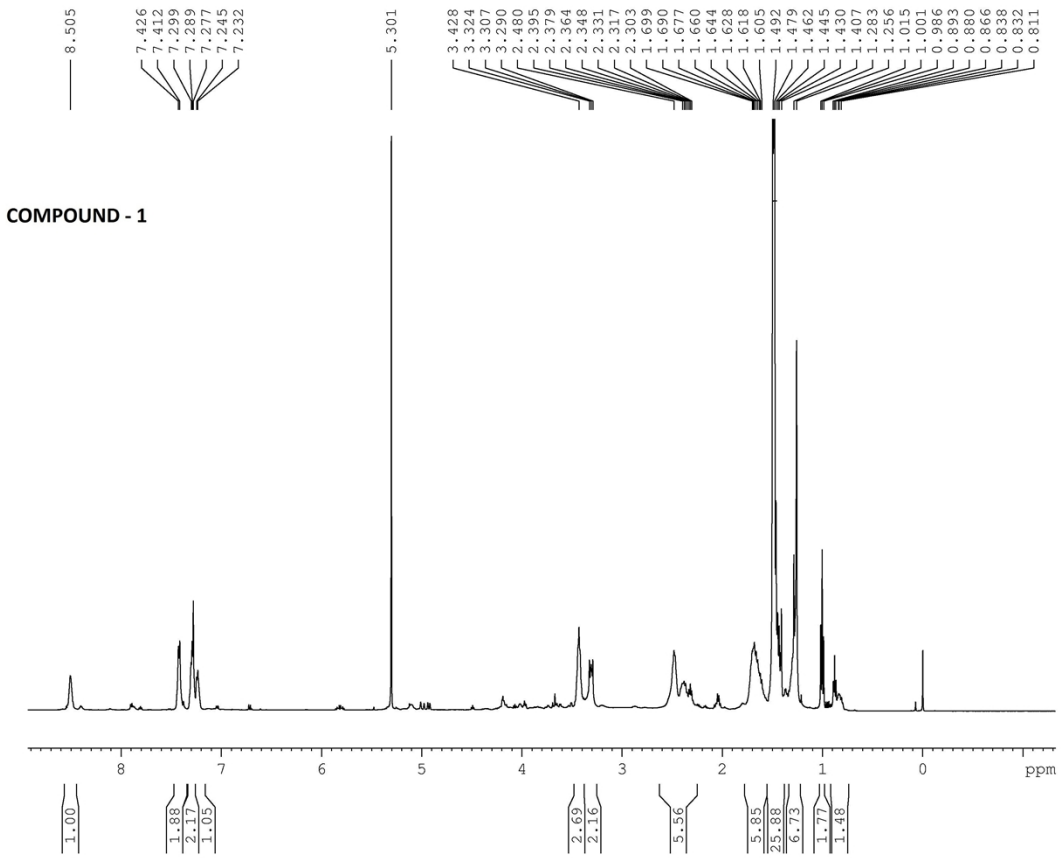
Doxorubicin (2.0mg, 0.003mmol) was taken in DMSO (2 ml) and stirred with triethylamine (200 µL) for 10 min followed by addition of Sulfo-SMCC (2.6mg, 0.006mmol) to the stirred solution and continued stirring at room temperature for 12 h under N_2 atmosphere. The resultant mixture was concentrated under reduced pressure and the crude red colored sticky solid **9** (SMCC-NH-Dox) was added to a solution of **8** (10mg, 0.002mmol) in DMSO (4 ml) at RT under N_2 atmosphere. After 15 h of stirring, the reaction mixture was concentrated again under reduced pressure and dried thoroughly using high vacuum pump. The crude Dox- conjugated compound 10 (6. 2 mg) was taken in EtOAc (1mL), and ethyl acetate (4mL) saturated with gaseous HCl was added gently into it. After 4h of stirring at RT, the solution was concentrated and the residue was washed thoroughly with dichloromethane to remove less polar impurities. The residue was dried and purified using MPLC on supelclean LC-18 reverse - phase silica gel (CH_3CN / H_2O 9: 1 to 5: 1). The purified product was dissolved in de-ionized water, filtered through a PTGE syringe filter, and lyophilized to afford DDS3 as a light red foamy solid (HCl salt, 4.2mg, 45%).

^{1}H-NMR (500MHz, CD_3OD): δ 1.17-1.35 (m, 58H), 2.02-2.04 (m, 3H), 3.21-3.32 (m, 18H), 4.09-4.13 (m, 14H), 4.25-4.61 (m, 4H), 6.82 (m, 8H), 7.23-7.43 (m, 16H), 8.13ppm (br s, 10H); MALDI-TOF-MS: m/z calcd for $C_{188}H_{288}N_{42}O_{52}S_2Na$: 4053.0524, found: 4053.3603 $[M+Na]^+$; Analytical HPLC (Shimadzu-ODS): R_t = 4.37 min (Flow rate = 1ml / min, UV 480nm, CH_3CN: H_2O = 90:10).

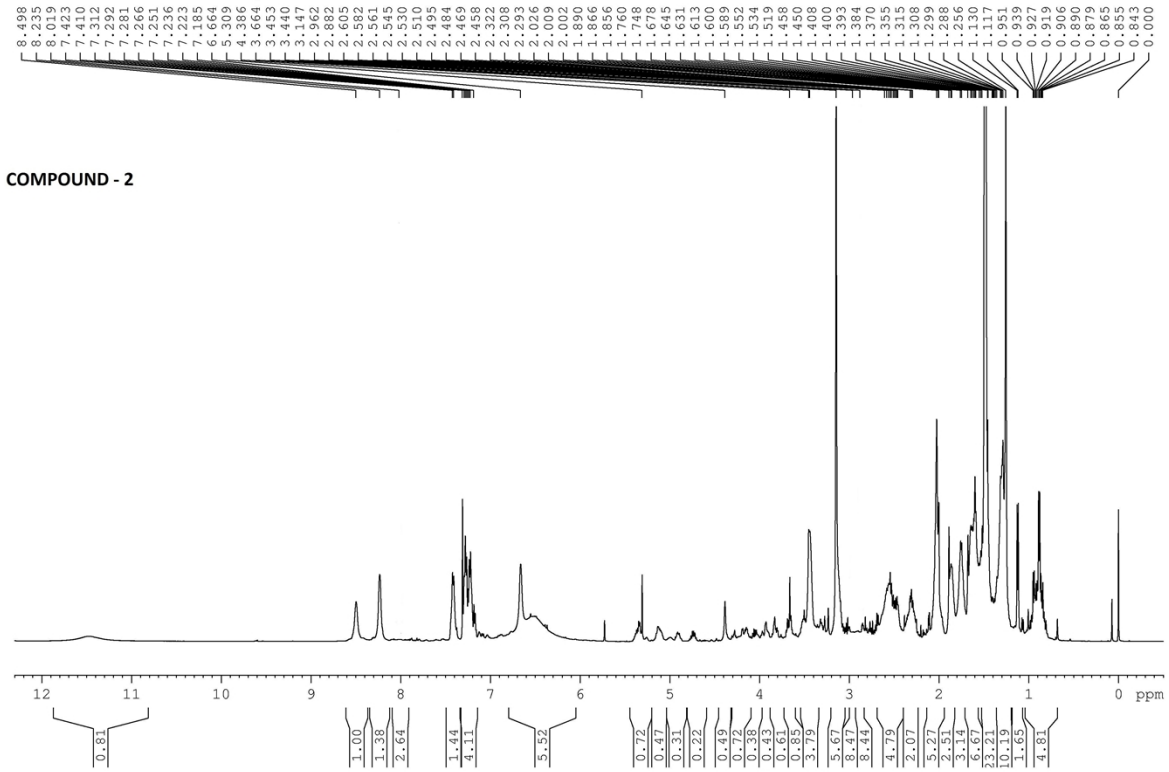


SOR-TBDPS-Tr

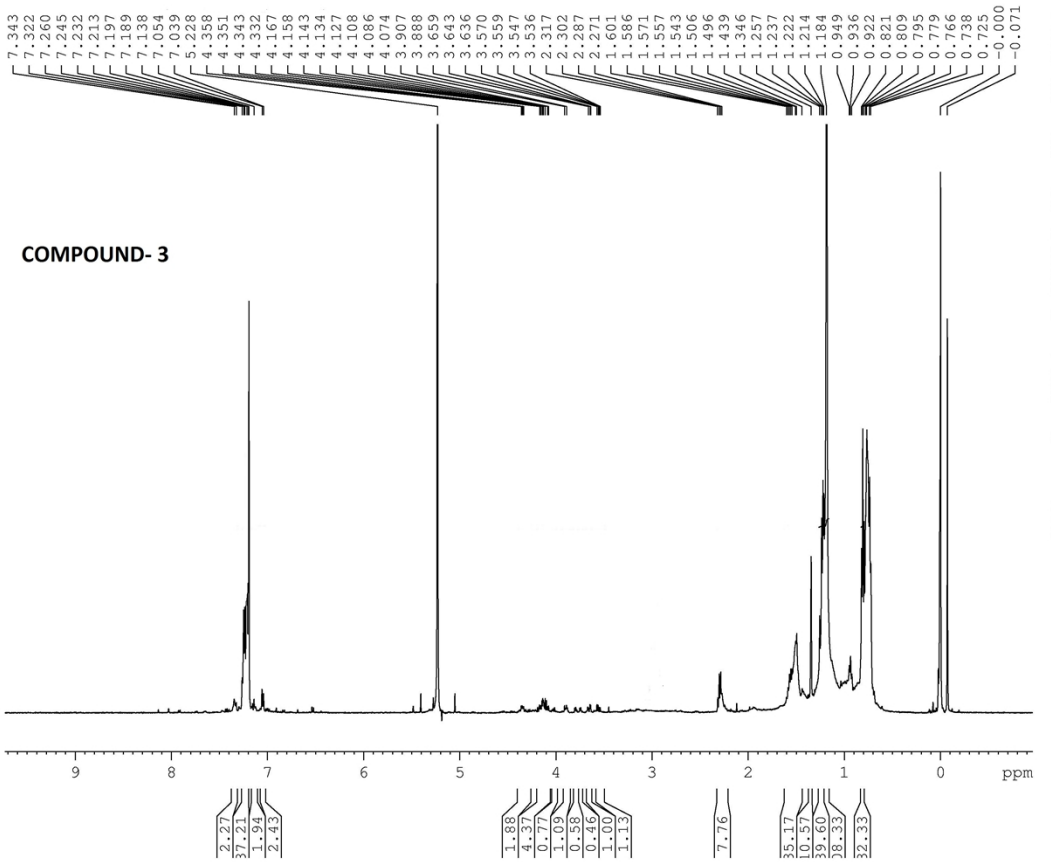
SS1. H[1] NMR SPECTRA OF COMPOUND- SOR-TBDPS-Tr



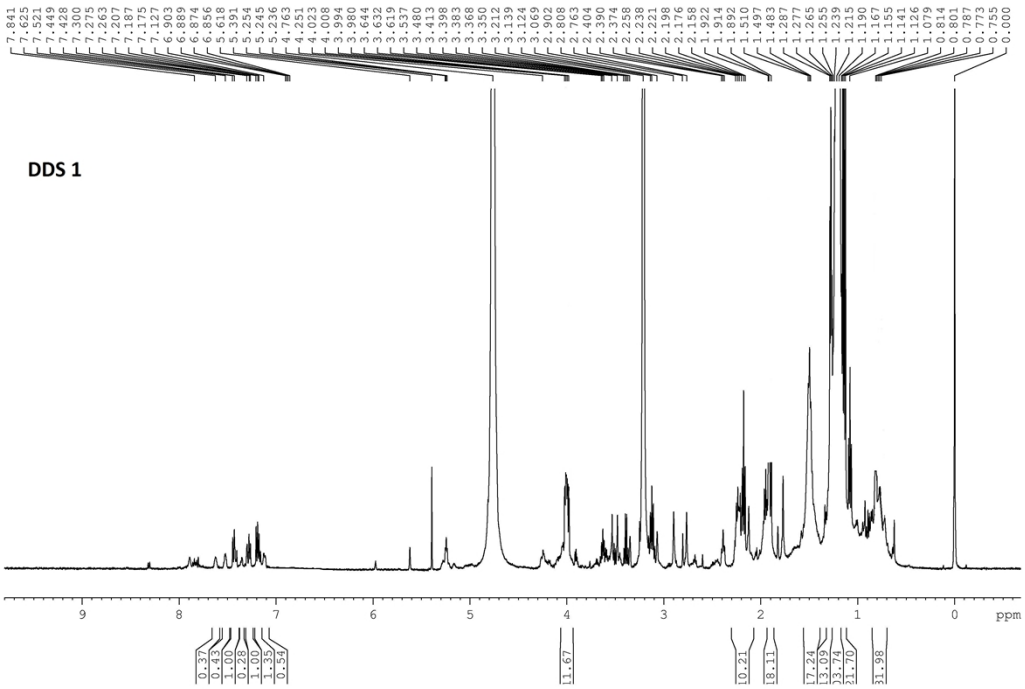
SS2. H^1- NMR spectrum of compound-1 (500MHz, $CDCl_3$)



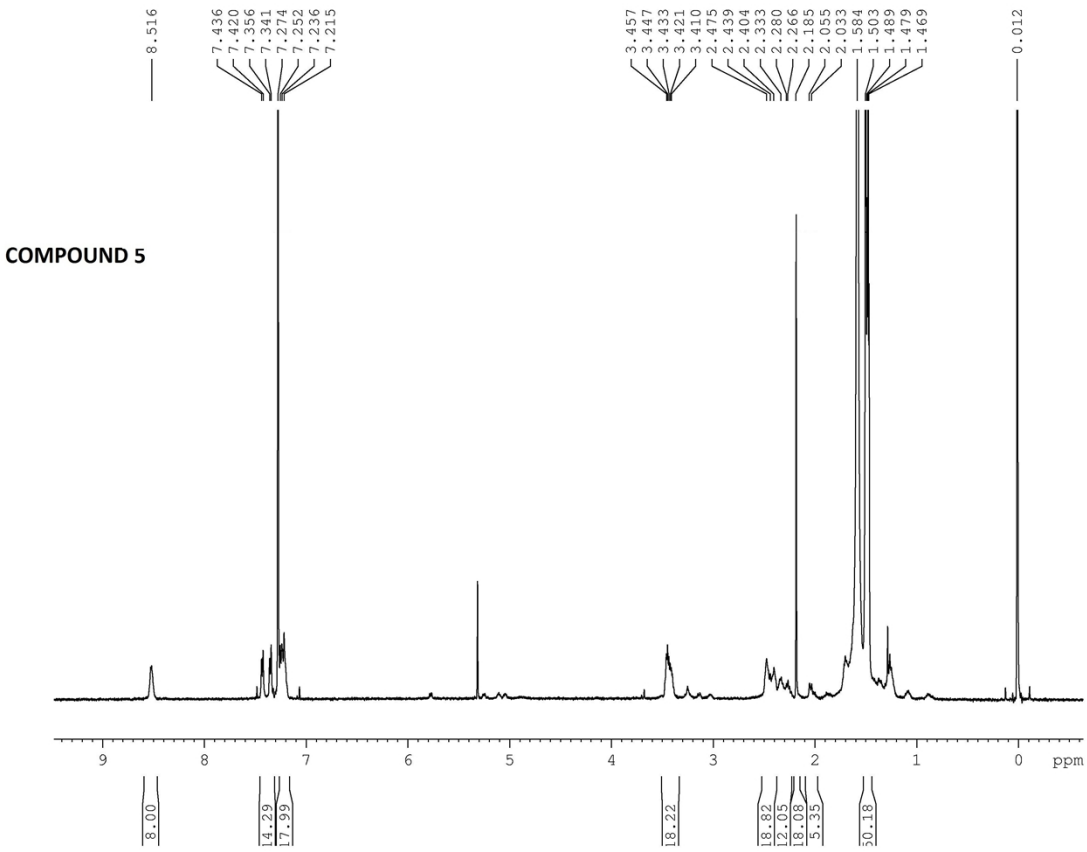
SS3. H^1- NMR spectrum of compound-2 (500MHz, $CDCl_3$)



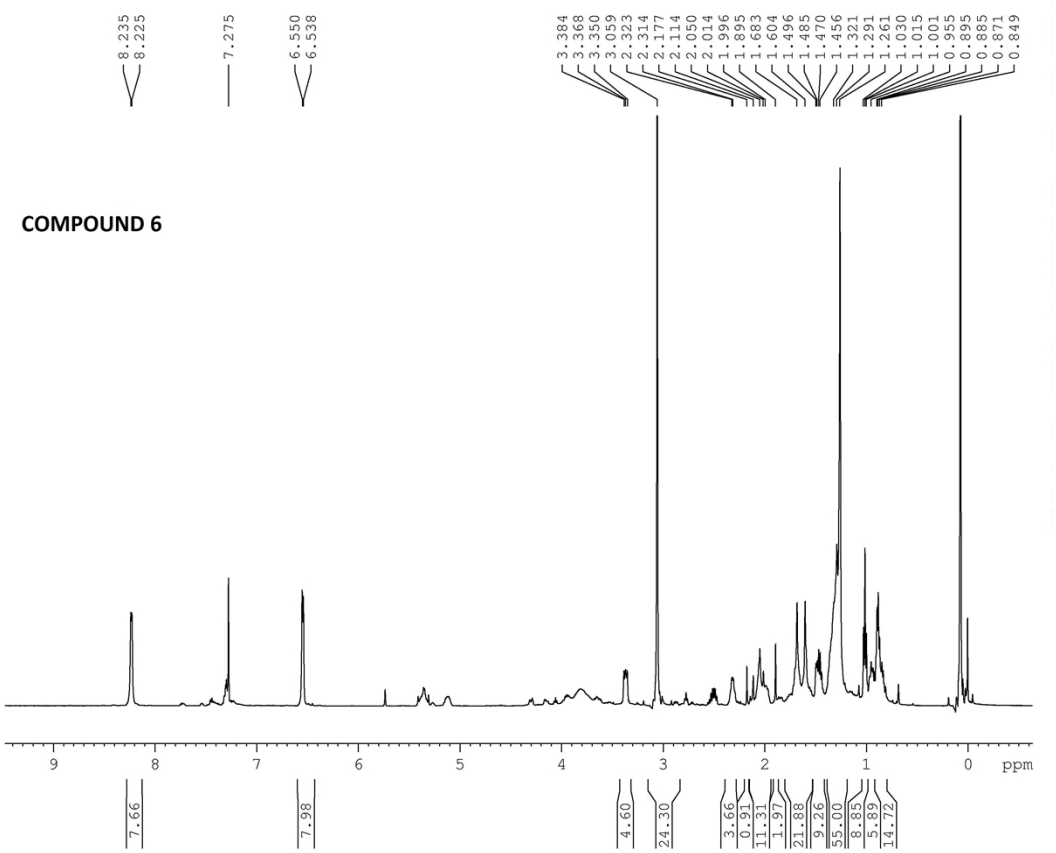
SS4. H[1]- NMR spectrum of Compound-3 (500MHz, $CDCl_3$)



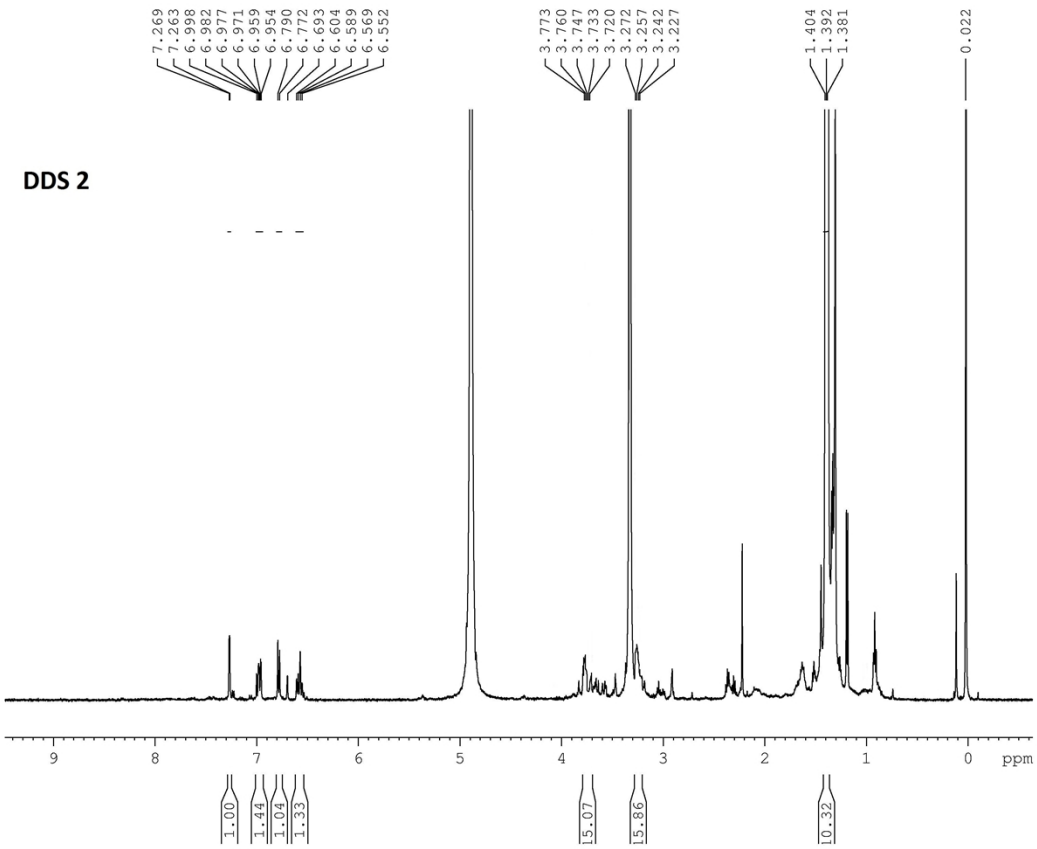
SS5. H^1- NMR spectrum of DDS1 (500MHz, CD_3OD)



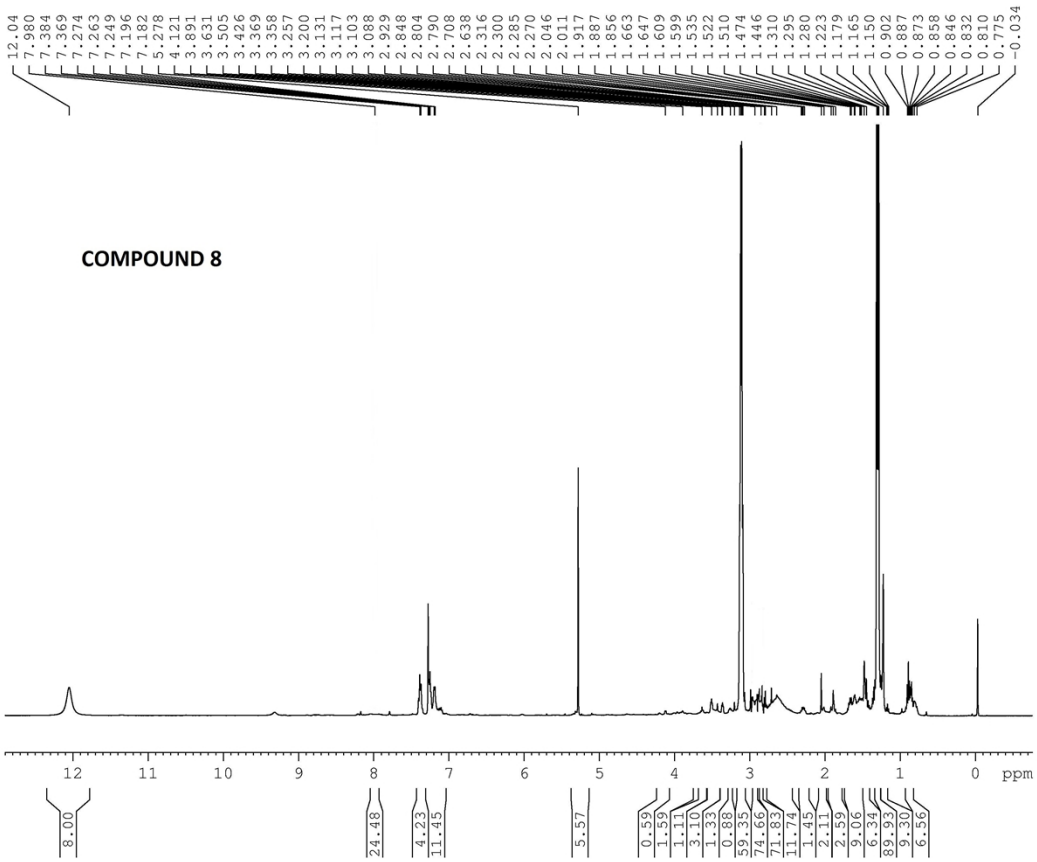
SS6. H^1- NMR spectrum of compound-5 (500MHz, $CDCl_3$)



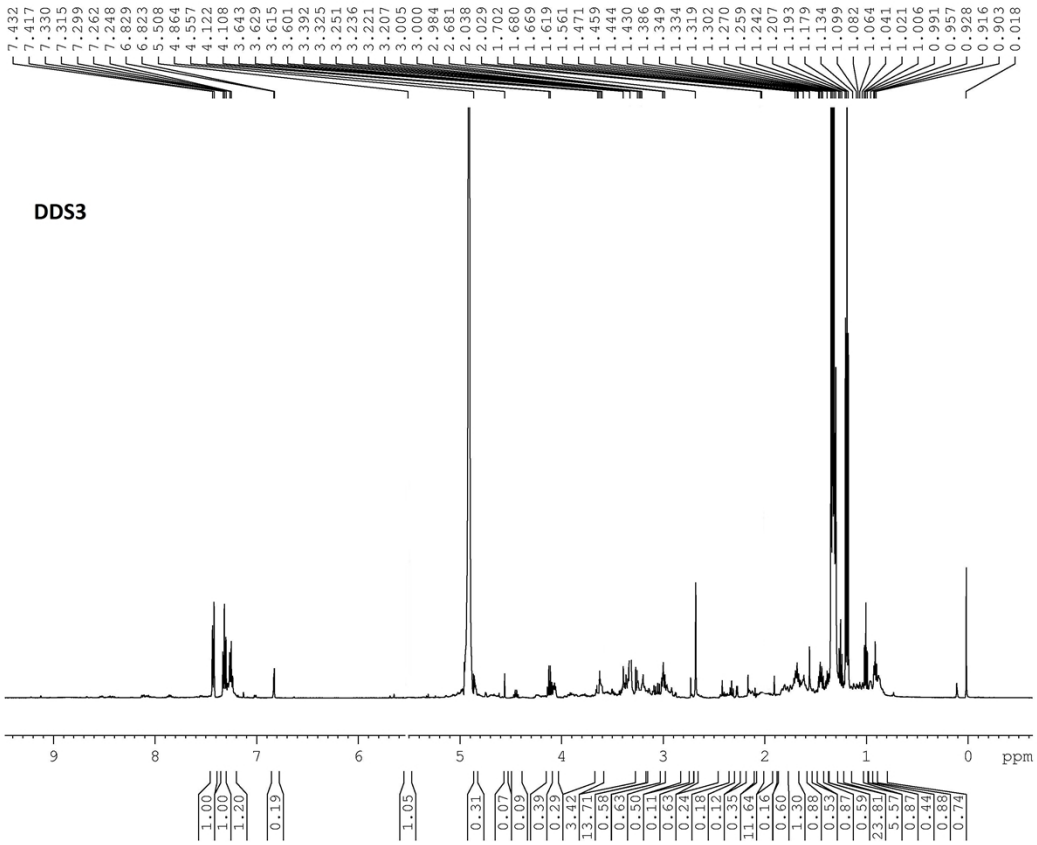
SS7. H^1- NMR spectrum of compound-6 (500MHz, $CDCl_3$)



SS8. H^1- NMR spectrum of DDS2 (500MHz, CD_3OD)



SS9. H^1- NMR spectrum of compound-8 (500MHz, $CDCl_3$)



SS10. H^1- NMR spectrum of DDS3 (500MHz, CD_3OD)

1.5 HPLC purification of DDS1, DDS2 and DDS3

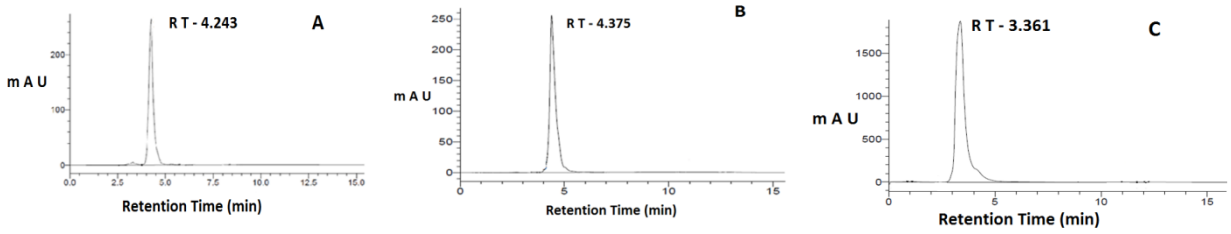
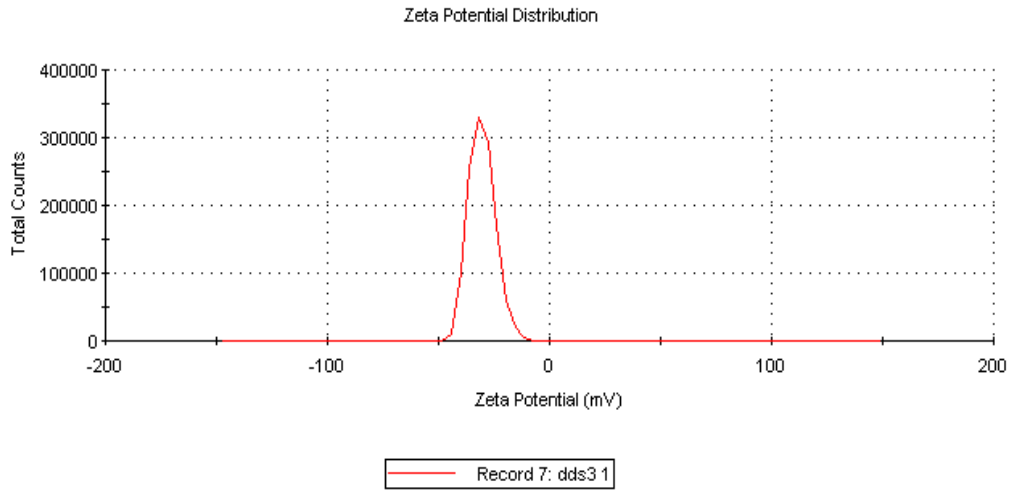


Fig. S1a The compounds DDS1 (A), DDS2 (B) and DDS3(C) analyzed by HPLC.

The HPLC analysis was conducted using Shimadzu RP- HPLC ODS column with mobile phase consisting of 0.1% TFA in acetonitrile (A) and 0.1% TFA in water (B).The gradient was linearly increased from 0% to 80% B for 15 minutes at the flow rate of 1mL/min at ambient temperature. UV-VIS detection was monitored simultaneously at 254nm and 480nm wavelengths. The synthesized DDS1, DDS2 and DDS3 compounds were dissolved in 50mM Tris HCl buffer (pH 7.4) at 2mg/ml concentration and were purified by analytical HPLC. [2] The purity of compounds DDS1, DDS2 and DDS3 is as shown in **Fig. S1**.

1.6 Zeta potential of DDS3

Zeta-potential of DDS3 was measured using a *Malvern Zeta Sizer Nano-ZS ZEN 3600*. 0.5mM of DDS3 was dissolved in milli Q water for evaluation.



Zeta potential (mV): - 30.4

Fig:**S1b**: Zeta potential of DDS-3

Zeta potential was found to be -30.4mV. This is due to positive net charge on the guanidine moieties on the sorbitol backbone.

1.7 *In vitro* drug release of DDS3

Dox release from DDS3 was measured after incubation of cathepsin B protease enzyme at 37°C. 10µl (concentration= 62ng/µL) of enzymatically active cathepsin B substrate was added to 90 µl of 0.1mM DDS3 compound in buffer (50mM of sodium acetate and 1mM of EDTA , in the ratio 9:1) . A blank was carried out without cathepsin B enzyme, using 100µl of DDS3 buffer solution. All measurements were carried out at different pH conditions (5.1, 7, 7.4 and 9) at specific time intervals from 0 to 24 hrs. [3] Fluorescence was measured at different time intervals using *BioTec Synergy 4* spectrophotometer at 590nm , which has been reflected as % drug release (plotted in Y- axis) as shown in **Fig. S2.**

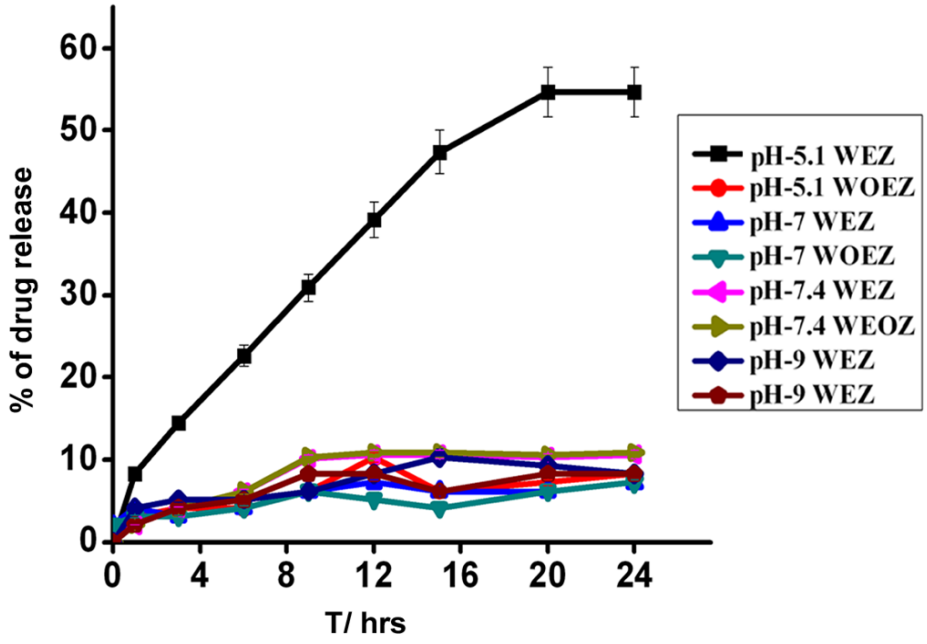


Fig. S2 Release of doxorubicin from DDS3 in the presence of cathepsin B enzyme at pH 5.1, 7, 7.4 and 9. WEZ stands for *with enzyme* and WOEZ for *without enzyme*. T stands for *time*. Data represented as mean± standard deviation (SD), n=3.

CELL CULTURE STUDIES:

HeLa cell lines were obtained from National Centre for Cell Science, Pune, India and also from Prof S. Murty Srinivasula of Indian Institute for Science Education and Research (IISER), Thiruvananthapuram, India. For maintenance of cell lines, Dulbeccos Modified Eagle's Medium (DMEM) *(Sigma)* containing 10% fetal bovine serum (FBS) *(Gibco),* antibiotics (100U/ml Penicillin and 100µg/ml streptomycin) and amphotericin (0.25µg/ml) *(HiMedia)* was employed. The cells were maintained in CO_2 incubators at 37°C with 5% CO_2 in air and 99% humidity. Passaging of cells when confluent was carried out using 0.25% trypsin and 0.02% EDTA

(HiMedia) in phosphate buffered saline (PBS). Experiments were carried out after 36 h of seeding the cells at appropriate density in suitable well plates.

1.8 MTT assay:

Cell viability after incubating the cells with different concentrations of DDS1 was determined by methyl thiazolyl tetrazolium (MTT) assay. It is a colorimetric assay based on the ability of live, but not dead cells to reduce MTT (yellow) to a purple formazan product. The cells were spread in 96-well plates at $5x10^3$ cells/well. After incubating with DDS1 for 24 h, the cells were exposed to MTT at a concentration of 50µg/well for 2.5 to 3 hrs at 37^{o}C in CO_2 incubator. The working solution of MTT was prepared in Hanks balanced salt solution (HBSS). After viewing formazan crystals under the microscope, the crystals were solubilized by treating the cells with DMSO: isopropanol at a ratio of 1:1 for 20 min at 37^o C. Plate was read at an absorbance of 570nm. The relative cell viability in percent was calculated as: Absorbance of treated /Absorbance of control x100. Control samples used were cells without DDS1.

Fig.S3 shows the results of MTT assay carried out after incubation of HeLa cells with different concentrations of DDS1 for 24h.

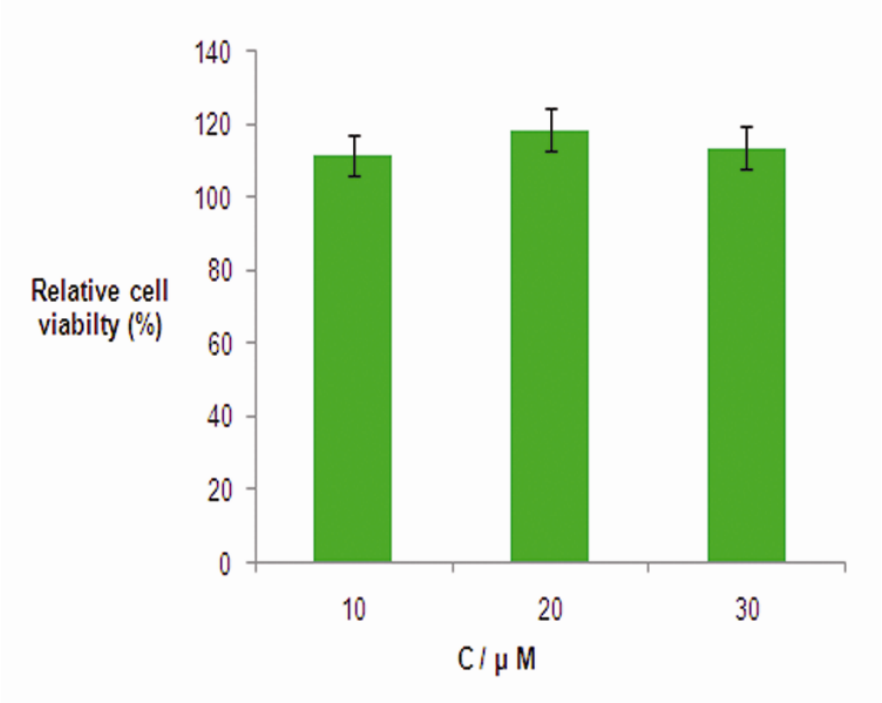


Fig. S3 MTT assay demonstrating relative viability of HeLa cells on incubation with different concentrations of DDS1 for 24h. *C* stands for concentration. Data expressed as mean±SD, n=3.

1.9 Cellular uptake studies of DDS1, DDS2 and DDS3 by fluorescence imaging:

Cellular uptake studies of DDS1, 2 and 3 were executed by fluorescence imaging of adherent cells. The cells were seeded at a density of 5x 10^3 cells/ well of 96 well black plates *(BD Biosciences, USA)* for the purpose. The cells were incubated with DDS1, DDS2 and DDS3 in HBSS individually for 1 h; at a concentration of 30µM. Images of the cells were collected by high-content spinning disk facility *(BD Pathway 855; BD Biosciences)* using *AttoVision 1.5.3 software.* For imaging DDS1 and DDS2, A488/10nm excitation filter and 515nm LP emission filter was used. For DDS3, B548/20nm excitation filter and 570 nm LP emission filter was used.

Fig. S4 represents fluorescent microscopic images revealing cellular uptake of DDS1, 2 and 3

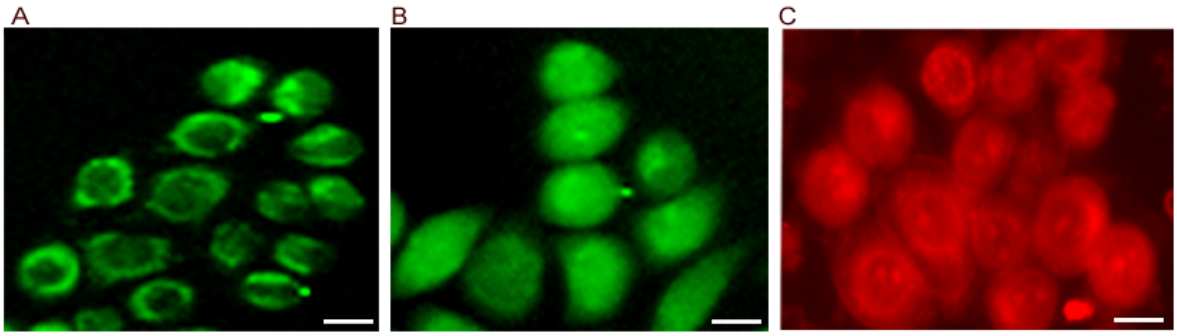


Fig. S4 Cellular uptake of A.DDS1 B. DDS2 and C.DDS3 demonstrated by fluorescence imaging. Scale bar: 25µm

2.0 Specific localization of DDS1, DDS2 and DDS3 in intracellular organelles:

Specific localization of DDS1, 2 and 3 in intracellular organelles was determined by selective permeabilization of plasma membrane by digitonin. Digitonin is a cholesterol- solubilizing agent; low concentrations of digitonin permeabilize cholesterol-rich membranes, such as the plasma membrane, but not cholesterol-poor lysosomal or mitochondrial membranes. Digitonin *(Sigma)* used at a concentration of 40µM selectively permeabilizes the plasma membrane, releasing the cytosolic dye, calcein AM (1µM) *(Invitrogen)* from the cells without altering the lysosomes. The localization of DDS1/2/3 in the lysosomes has been validated by this approach.[4] There were 2 groups of treatments: one group of cells was incubated with DDS1/2/3 individually for 1 hour, followed by incubation with the cytoplasmic probe, calcein (1µM) for 30 min at 37 ^{o}C. Cells immediately after incubation with DDS1/2/3 as well as after incubating with calcein were washed with HBSS to remove unbound DDS1/2/3 and calcein respectively. Another group of cells was incubated only with calcein and not DDS1/2/3. This group formed the control. Both the groups were then treated with digitonin. Cell images were collected prior to and post-digitonin treatment. **Fig.S5** represents selective permeabilization of cells incubated with DDS2 / DDS3 along with calcein.

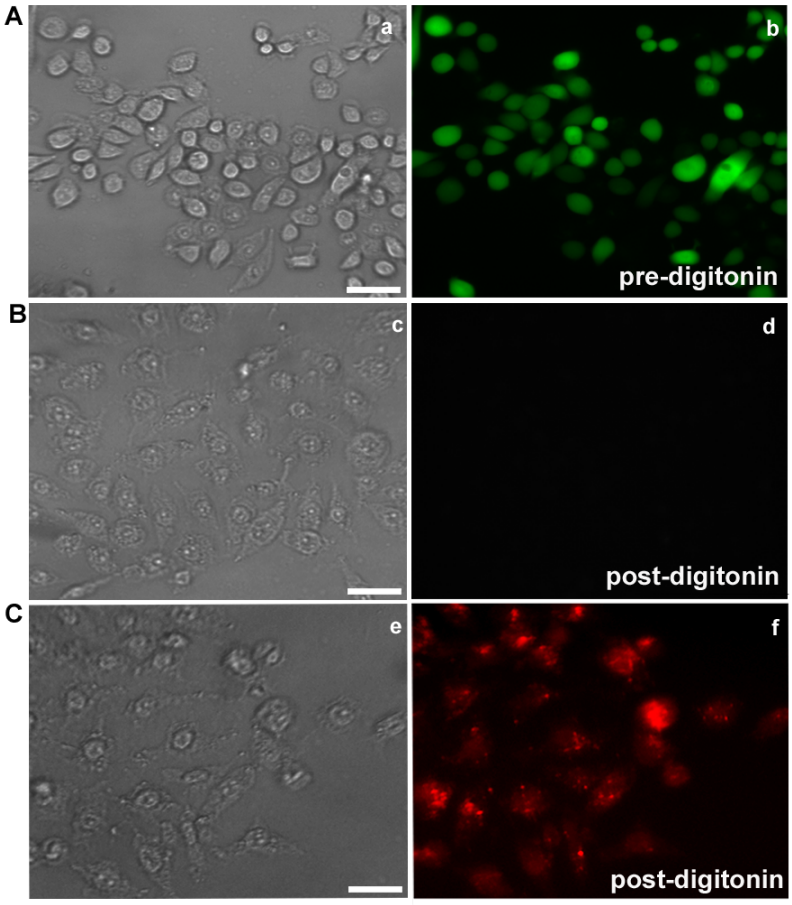


Fig.S5 Selective permeabilization of plasma membrane reveals no localization of DDS2 in intracellular organelles, but specific localization of DDS3 in the organelles. A-b denotes DDS2 and calcein (515 nm emission) incorporated cells, prior to permeabilization by digitonin. B-d denotes loss of fluorescence within 10min of digitonin treatment in cells loaded with DDS2 and calcein. No punctiform green fluorescence similar to DDS1 was retained, demonstrating that DDS2 was confined to the cytosol alone and had escaped along with calcein after permeabilization of the plasma membrane. A-a and B-c show the transmitted light images of A-b and B-d respectively. C-f shows the punctiform red fluorescence of DDS3 (570nm emission) retained even after digitonin treatment in cells loaded with both DDS3 and calcein. Calcein escaped from the cells on digitonin treatment. C-e shows the transmitted light image of C-f. Scale bar: 25μm

2.1 Co localization studies of DDS1, DDS2 and DDS3:

Co localization studies were carried out to gather information on the subcellular localization of DDS1, 2 and 3. Commercially available Mitotracker deep red *(Invitrogen)*, Lysotracker deep red *(Invitrogen)* and Hoechst *(Invitrogen)* were used for the experiments. The cells were seeded at a density of 5x 10^3 cells/ well of 96 well black plates for the purpose. They were incubated with Mitotracker and lysotracker in HBSS individually for 30 min at 37^{o}C. This was followed by

washing the cells twice with HBSS to remove unbound dye. The cells were then exposed to DDS1, 2 and 3 in HBSS individually for 1 hour. Subsequent to washing with HBSS, nuclear staining was carried out with Hoechst for 20 min at room temperature. Images of the cells were collected using high-content spinning disk facility as described earlier. **Fig.S6** represents colocalization studies of DDS1 and 3 individually with Hoechst.

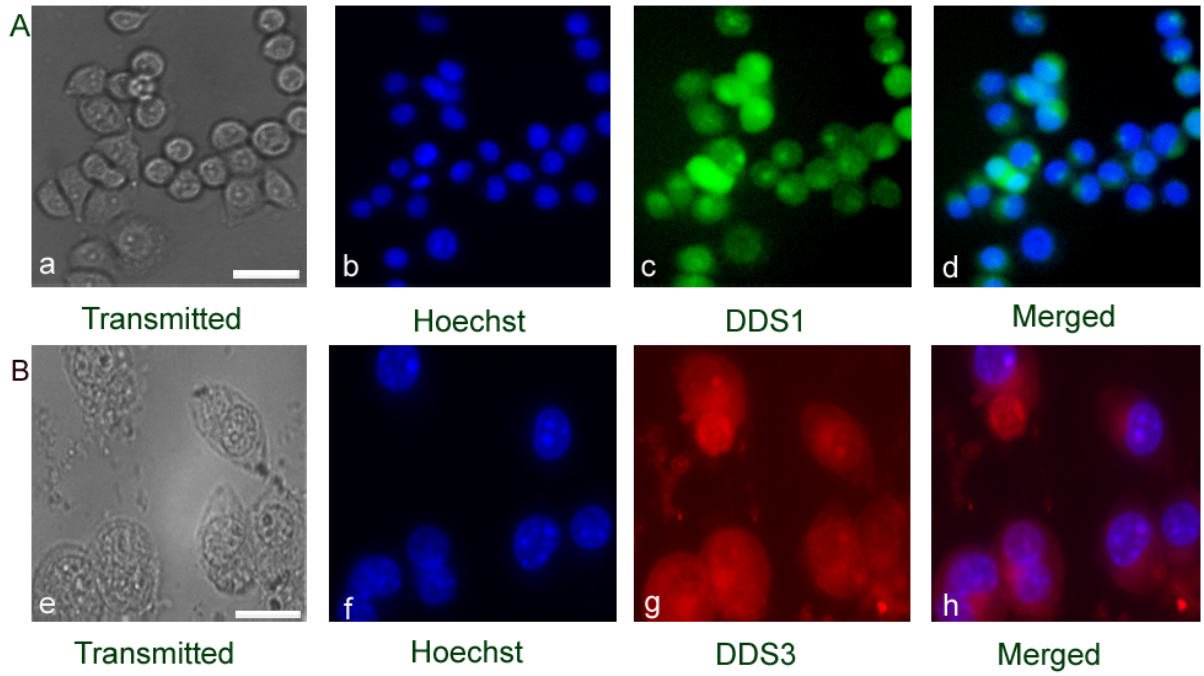


Fig.S6 Co localization studies: No significant localization of DDS1 and DDS3 in the nucleus. Merged image (A-d) of Hoechst (A-b) & DDS1 (A-c) and the merged image (B-h) of Hoechst (B-f) & DDS3 (B-g) demonstrates the fact. A-a and B-e represent corresponding transmitted light images. Scale bar: 25µm.

2.2 Flow cytometry for demonstration of cellular uptake of DDS1, DDS2 and DDS3:

Cellular uptake of DDS1, 2 and 3 was evaluated by flow cytometric analysis using *BD FACS Aria II flow cytometer (BD Biosciences).* Acquisition and analysis of flow cytometric data were carried out using *BD FACS Diva software*. The cells were seeded at a density of 5 x 10^4 cells per well of 24-well plates. Subsequent to incubation of the cells with DDS1, DDS2 and DDS3 individually for 1 hour in HBSS, trypsinization was done to detach the cells from the well plate.

Trypsin was inactivated with 10% FBS containing medium, followed by centrifugation at 2300 rpm for 1 minute. The pellets were resuspended in HBSS. They were again pelleted at 400g for 5 min at 4^{o}C. They were further resuspended in cold HBSS without calcium and magnesium for flow cytometric analysis. The whole procedure was executed under dark conditions. The flow cytometric data for DDS1 and DDS2 was recorded in the green channel (530nm emission), whereas data for DDS3 was recorded in the red channel (570nm emission).

2.3 References

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