

Photoinduced Signal Amplification Through Controlled Externally Sensitized Fragmentation in Masked Sensitizer

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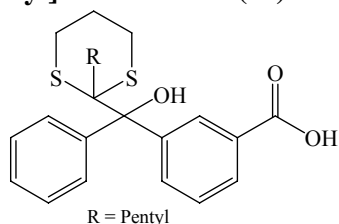
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Supporting Information

Common reagents were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. THF was refluxed over and distilled from potassium benzophenone ketyl prior to use. 5th generation PAMAM-NH₂ dendrimer was purchased from Sigma-Aldrich and TentaGel S-NH₂ beads (polyethylene glycol-grafted polystyrene, terminated by primary amino groups, 1.86 mmol/g loading) were purchased from Fluka. The ¹H and ¹³C NMR spectra were recorded at 25°C on a Varian Mercury 400 MHz instrument, CDCl₃ and DMSO-d₆ as solvents, and TMS was used as an internal standard. The diffusion coefficients were measured with a Varian Mercury Performa I pulsed field gradients module and a 4 nucleus autoswitchable PFG probe. Elemental analyses were conducted at Huffman Laboratories Inc., Denver. Column chromatography was performed on Silica Gel, 70-230 mesh ASTM. UV-Vis spectra were recorded on a Beckman DU-640 Spectrophotometer. Irradiations were carried out in a carousel Rayonet photo reactor (RPR-3500 lamps) with a 320 nm long pass solution filter. Gas chromatography was done using a Varian Saturn 2100 T Ion-Trap GCMS utilizing Electron Ionization (EI). Selective ion monitoring m/z 119, 74 was used to separate dithiane tags following fragmentation. The initial temperature was 70°C and a final temperature of 260°C was reached at the rate of 30°C/min. The inlet temperature was 100°C and the split ratio was 100. The flow rate was 1.0 mL/min with column dimensions of 30 m X 250 µm ID, as well as a 5% phenyl methyl siloxane fused silica bonded capillary.

Synthetic Procedures:

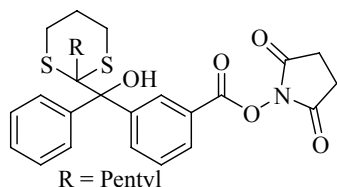
Typical procedure for the preparation of 3-[Hydroxy-(2-pentyl-[1,3]dithian-2-yl)-phenyl-methyl]-benzoic acid (2e):



2-pentyl-1,3-dithiane (4.46 mmol) was dissolved in 10ml of freshly distilled THF under a nitrogen atmosphere. Butyl lithium (1.6M solution in hexanes, 3.80 mmol) was added drop wise and stirred for 10 min at room temperature to generate the anion. 3-benzoylbenzoic acid (1.55 mmol) was added (in 10 ml of THF) and the reaction was stirred for 1.5 h. The reaction mixture was quenched with saturated aq. ammonium chloride, extracted twice with ether, and the organic layer was combined and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (silica gel) using ethyl acetate in hexane as the eluent to get pure

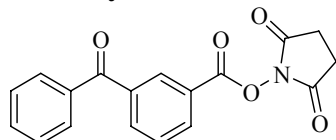
adduct (70% yield). ^1H NMR (CDCl_3 , 400): δ 8.83(s, 1H), 8.19-8.24(d, $J=8.49\text{Hz}$, 1H), 7.98-8.05 (d, $J=7.9\text{ Hz}$, 1H), 7.9-7.95 (d, $J=7.93\text{ Hz}$, 2H), 7.2-7.45 (m, 4H), 4.6 (s, 1H), 2.5(m, 2H), 2.1-2.3(m, 2H), 1.9(m, 2H), 1.51-1.53 (m, 4H), 1.1-1.3(m, 4H), 0.79-0.85(t, $J=7.35\text{Hz}$, 3H).

Typical procedure for the preparation of 3-[Hydroxy-(2-pentyl-[1,3]dithian-2-yl)-phenyl-methyl]-benzoic acid succinamide ester (3e):



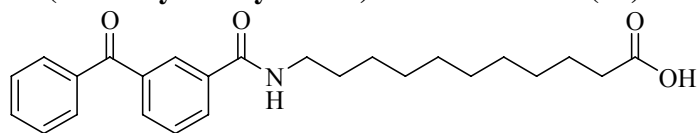
3-[Hydroxy-(2-pentyl-[1,3]dithian-2-yl)-phenyl-methyl]-benzoic acid (2.2 mmol), N-hydroxysuccinimide (3.5 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 2.6 mmol) were dissolved in CH_2Cl_2 (20 mL) and stirred overnight at room temperature. The solution was washed with 20 mL water, 20 mL of saturated aqueous NaHCO_3 , and 20 mL of brine. The organic layer was collected, dried over anhydrous Na_2SO_4 , and removed under vacuum to give a white solid which was purified by column chromatography (silica gel) using an eluent of 15% EtOAc-hexane (80 % yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.82 (s, 1H), 8.32-8.35 (d, 1H), 8.00-8.03 (d, 1H), 7.87-7.89 (d, 2H), 7.41-7.45 (t, 1H, $J=7.9\text{ Hz}$), 7.28-7.32 (t, 2H, $J=7.69\text{ Hz}$), 7.24-7.27 (m, 2H), 4.57 (s, 1H), 2.89 (s, 4H), 2.46-2.52 (td, 2H, $J=5.2\text{Hz}$, $J=9.9\text{Hz}$), 2.09-2.25 (tdd, 2H, $J=7.0\text{Hz}$, $J=14.0\text{Hz}$, $J=35.2\text{Hz}$), 1.82-1.90 (m, 2H), 1.61-1.72 (m, 4H), 1.09-1.25 (m, 4H), 0.80-0.83 (t, 3H, $J=7.20\text{ Hz}$).

3-benzoylbenzoic acid succinamide esters (S7):



3-benzoylbenzoic acid (2.2 mmol), N-hydroxysuccinimide (3.5 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 2.6 mmol) were dissolved in CH_2Cl_2 (20 mL) and then was stirred overnight at room temperature. The solution was washed with 20 mL water, 20 mL of saturated aqueous NaHCO_3 , and 20 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 , and removed under vacuum to give a white solid (95% yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.52 (s, 1H), 8.34-8.36 (d, 1H), 8.11-8.13 (d, 1H), 7.79-7.81 (d, 2H), 7.61-7.69 (m, 2H), 7.49-7.53 (t, 2H), 2.91 (s, 4H)

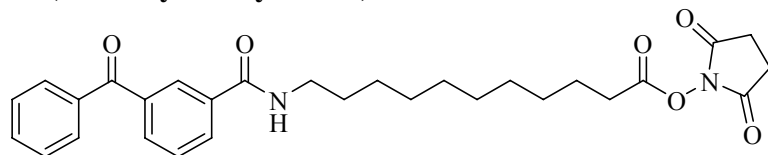
11-(3-benzoyl-benzylamino)undecanoic acid (S9):



3-benzoylbenzoic acid succinamide ester (3.09 mmol) and 11-aminoundecanoic acid (6.19 mmol) were combined in 20 mL DCM and 15 mL DMF, then stirred for 10 min at room temperature. Triethylamine (2 mL) was added and the reaction mixture was refluxed for 12 h. The organic layer was washed with 40 ml of 5% HCl solution, 15 ml of brine, then dried over anhydrous Na_2SO_4 , and removed under vacuum. The resulting solid was purified by column chromatography on silica gel using a 40% ethyl acetate in hexane as eluent (75% yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.35 (s, 1H), 8.03-8.05 (d, 1H), 7.79-7.81 (d, 2H), 7.67-7.69 (d, 1H), 7.22-7.36 (m, 4H), 6.21 (brt, 1H), 4.00 (brs, 1H), 3.38-3.43 (q,

2H), 2.71-2.85 (m, 2H), 2.48-2.56 (m, 4H), 2.30-2.34 (t, 4H), 1.79-1.97 (m, 5H), 1.54-1.63 (m, 5H), 1.27 (m, 8H);

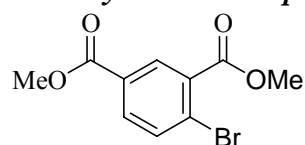
11-(3-benzoyl-benzylamino)undecanoic acid succinamide ester (S10):



A mixture of 11-(3-benzoyl-benzylamino)undecanoic acid (1.39 mmol), N-hydroxysuccinimide (2.23 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 1.68 mmol) were dissolved in CH_2Cl_2 (20 mL) and stirred overnight at room temperature. The solution was washed with 20 mL water, 20 mL of saturated aqueous NaHCO_3 , and 20 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 , and removed under vacuum to give a white solid which was purified by column chromatography (silica gel) using 1:1 EtOAc-hexane as eluent (85% yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.18 (s, 1H), 8.06-8.08 (d, 1H), 7.86-7.88 (d, 1H), 7.78-7.80 (d, 2H), 7.48-7.63 (m, 4H), 6.37 (brt, 1H), 3.43-3.48 (q, 2H), 2.33-2.36 (t, 2H), 1.61-1.64 (m, 4H), 1.9 (m, 12H)

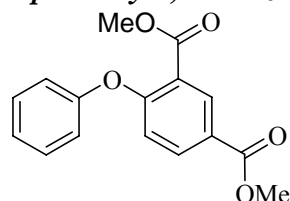
Xanthone-2-carboxylic acid (S11)

Dimethyl 4-bromoisophthalate:



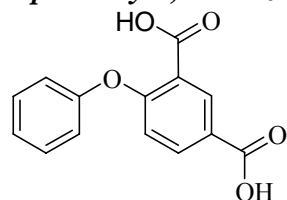
To a suspension of 4-bromoisophthalic acid (41 mmol) in MeOH (150 mL) at 0°C was added thionyl chloride (140 mmol) drop wise. The reaction was stirred for 24 h, then filtered and concentrated. The resulting oil was put into CH_2Cl_2 (250 mL), and washed with saturated NaHCO_3 solution (2 X 35 mL). The organic solvent was collected, dried over anhydrous Na_2SO_4 , and removed under vacuum to give a white, oily solid, which upon trituration with hexanes gave a white powder (90% yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.42 (d, $J=2.0$ Hz, 1H), 7.94-7.96 (dd, $J=8.3, 2.1$ Hz, 1H), 7.73-7.75 (dd, $J=8.2$ Hz, 1H), 3.95 (s, 3H), 3.92 (s, 3H)

4-phenoxy-1,3- benzenedicarboxylic acid dimethyl ester:



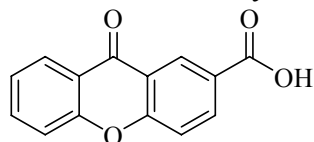
Dimethyl 4-bromoisophthalate (34 mmol), phenol (51 mmol), and cuprous oxide (21 mmol) were put into N,N-dimethylacetamide and heated at 165°C with stirring for 20 h under a nitrogen atmosphere. The mixture was diluted with large quantities of water and extracted with ether. The extracts were dried and evaporated to give the product (70% yield). ^1H NMR (CDCl_3 , 400 MHz): δ 8.71 (d, 1H), 8.05-8.07 (dd, 1H), 7.16-7.22 (m, 2H), 7.03-7.05 (d, 2H), 6.83-6.91 (dd, 2H).

4-phenoxy-1,3- benzenedicarboxylic acid:



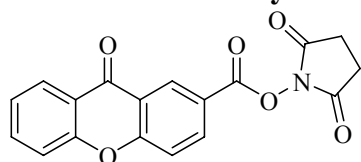
4-phenoxy-1,3- benzenedicarboxylic acid dimethyl ester (18 mmol) was dissolved in ethanol and the mixture was treated with aqueous KOH (63 mmol in 5-10 mL water). The reaction mixture was refluxed for 30 minutes, concentrated under reduced pressure, and acidified with dilute hydrochloric acid. The solid collected upon filtration was washed with water and dried to yield the product (75% yield). ¹H NMR (DMSO-d₆, 400 MHz): δ 8.35 (s, 1H), 8.01-8.05 (dd, 1H), 7.39-7.43 (t, 2H, J=7.36 Hz), 7.16-7.20 (dt, 1H, J=1.1Hz, J=7.3Hz), 7.02-7.05 (dd, 2H, J=1.1Hz, J=7.6Hz), 6.94-6.96 (dd, 1H, J=1.1Hz, J=8.6Hz).

Xanthone-2-carboxylic acid:



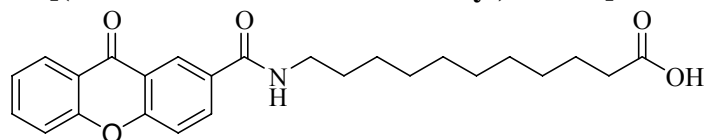
A solution of 4-phenoxy-1,3- benzenedicarboxylic acid (13 mmol) in concentrated H₂SO₄ (15 mL) was warmed to 80°C. The mixture was then cooled to, and stirred, at room temperature for 18 h, poured into ice water, filtered, washed with water and dried to give crude white solid which is recrystallised from THF-ethanol (4:1) (80% yield). ¹H NMR (DMSO-d₆, 400 MHz): δ 8.71 (d, 1H), 8.31-8.33 (dd, 1H), 8.19-8.21 (dd, 1H), 7.87-7.91 (t, 1H), 7.67-7.74 (dd, 2H), 7.48-7.52 (t, 1H)

Xanthone-2-carboxylic acid succinamide ester (S12):

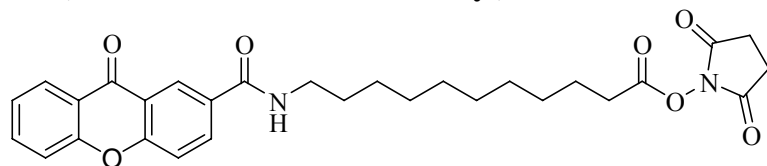


Xanthone-2-carboxylic acid (3.4 mmol), N-hydroxysuccinimide (5.3 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 4.0 mmol) were placed in a solution of THF:CH₂Cl₂ (2:1, 50 mL) in this order and stirred overnight. The solution was washed with 20 mL water, 20 mL saturated aqueous NaHCO₃, and 20 mL brine. The organic layer was dried over anhydrous sodium sulfate and removed under vacuum to yield a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 9.17–9.18 (d, 1H), 8.41-8.44 (dd, 1H), 8.35-8.37 (dd, 1H), 7.77-7.82 (dt, 1H), 7.61-7.63 (dd, 1H), 7.54-7.56 (dd, 1H), 7.44-7.48 (dt, 1H), 2.94 (s, 2H).

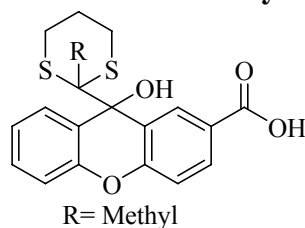
11-[(9-Oxo-9H-xanthene-2-carbonyl)-amino]-undecanoic acid (S13):



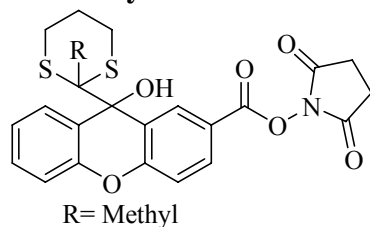
To a solution of xanthone-2-carboxylic acid succinamide ester (1.48 mmol) in DMF (20 mL) was added 11-aminoundecanoic acid (5.9 mmols) and stirred for 10 min at ambient temperature. Triethylamine (2 mL) was added, and the reaction was refluxed for 12 h. The organic layer was washed with 40 ml of 5% HCl solution, 15 ml of brine, dried over Na₂SO₄, and removed under vacuum. The resulting solid was separated by column chromatography on silica gel (EtOAc/hexanes = 1:1 to 2:1). (Yield =70%). ¹H NMR (DMSO-d₆, 400 MHz): δ 8.74-8.77 (t, 1H, J=5.4Hz), 8.69 (d, 1H, J=2.2Hz), 8.27-8.30 (dd, 1H, J=2.3Hz, J=8.8Hz), 8.18-8.20 (dd, 1H, J=1.6Hz, J=7.9Hz), 7.85-7.89 (ddd, 1H, J=1.7Hz, J=7.2Hz, J=8.7Hz), 7.69-7.71 (d, 1H, J=8.8Hz), 7.64-7.66 (d, 1H, J=8.4Hz), 7.46-7.50 (t, 1H, J=7.5Hz), 3.24-3.29 (dd, 1H, J=6.7Hz, J=12.8Hz), 2.13-2.17 (t, 1H, J=7.4Hz), 1.43-1.54 (m, 4H), 1.15-1.33 (m, 12H).

11-[(9-Oxo-9H-xanthene-2-carbonyl)-amino]-undecanoic acid succinimide ester (S14):

11-[(9-Oxo-9H-xanthene-2-carbonyl)-amino]-undecanoic acid (1.22 mmol), N-hydroxysuccinimide (1.83 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 1.46 mmol) were placed in a solution of THF:CH₂Cl₂ (2:1, 50 mL) in this order and stirred overnight. The solution was washed with 20 mL water, 20 mL saturated aqueous NaHCO₃, and 20 mL brine. The organic layer was dried over anhydrous sodium sulfate and removed under vacuum to yield a white solid. ¹H NMR (DMSO-d₆, 400 MHz): δ 8.75-8.77 (t, 1H, J=5.4Hz), 8.69 (d, 1H, J=2.2Hz), 8.27-8.30 (dd, 1H, J=2.3Hz, J=8.8Hz), 8.19-8.21 (dd, 1H, J=1.6Hz, J=7.9Hz), 7.88-7.93 (ddd, 1H, J=1.7Hz, J=7.2Hz, J=8.8Hz), 7.73-7.75 (d, 1H, J=8.8Hz), 7.69-7.71 (d, 1H, J=8.5Hz), 7.49-7.53 (t, 1H, J=7.5Hz), 3.26-3.30 (dd, 2H, J=5.9Hz, J=11.9Hz), 2.80 (s, 4H), 2.61-2.65 (t, 2H, J=7.2Hz), 1.51-1.63 (m, 4H), 1.20-1.38 (m, 12H).

Typical procedure for the preparation of 9-Hydroxy-9-(2-methyl-[1,3]dithian-2-yl)-9H-xanthene-2-carboxylic acid (6a):

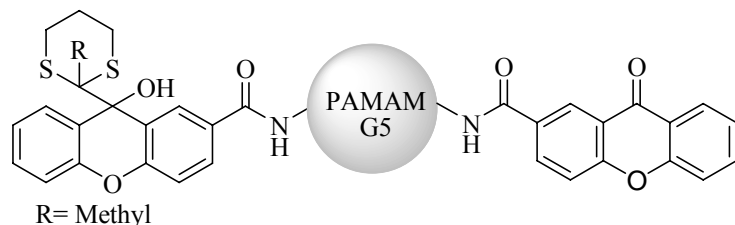
To a solution of 2-methyl-1,3-dithiane (6.2 mmol) in freshly distilled THF (30 mL) was added n-butyl lithium (1.6M solution in hexanes, 7.5 mmol) and stirred for 10 minutes at room temperature to generate the anion. Xanthone-2-carboxylic acid (1.3 mmol) was added to the dithiane mixture and stirred 1 hr at room temperature. The reaction was quenched with a saturated solution of ammonium chloride (20 mL), extracted once with ethyl acetate (50 mL) and dried over anhydrous NaSO₄. The solvent was removed under vacuum and the residue was purified on a silica gel column eluted with ethyl acetate in hexanes to give the product (90 % yield). ¹H NMR (CDCl₃, 400 MHz): δ 8.71 (d, 1H), 8.10-8.13 (dd, 1H), 7.94-7.96 (dd, 1H), 7.38-7.42 (m, 2H), 7.19-7.27 (m, 3H), 2.89-3.98 (m, 2H), 2.63-2.70 (m, 2H), 1.85-1.94 (m, 2H), 1.27 (s, 3H).

Typical procedure for the preparation 9-Hydroxy-9-(2-methyl-[1,3]dithian-2-yl)-9H-xanthene-2-carboxylic acid succinimide ester (7a):

9-Hydroxy-9-(2-methyl-[1,3]dithian-2-yl)-9H-xanthene-2-carboxylic acid (0.83 mmol), N-hydroxysuccinimide (1.24 mmol), and EDC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 0.99 mmol) were placed in a solution of THF:CH₂Cl₂ (2:1, 50 mL) in this order and stirred overnight. The solution was washed with 20 mL water and 20 mL of saturated aqueous NaHCO₃, and 20 mL of brine. The organic layer was dried over anhydrous Na₂SO₄ removed vacuum to

yield the product (95% yield). ^1H NMR (CD_3OD , 400 MHz): δ 8.72 (d, 1H), 8.12 (dd, 1H), 7.92 (dd, 1H), 7.38 (dt, 1H), 7.19-7.27 (m, 3H), 3.58 (brs, 1H), 2.95-2.97 (m, 6H), 1.83-1.91 (m, 2H), 1.24 (s, 3H)

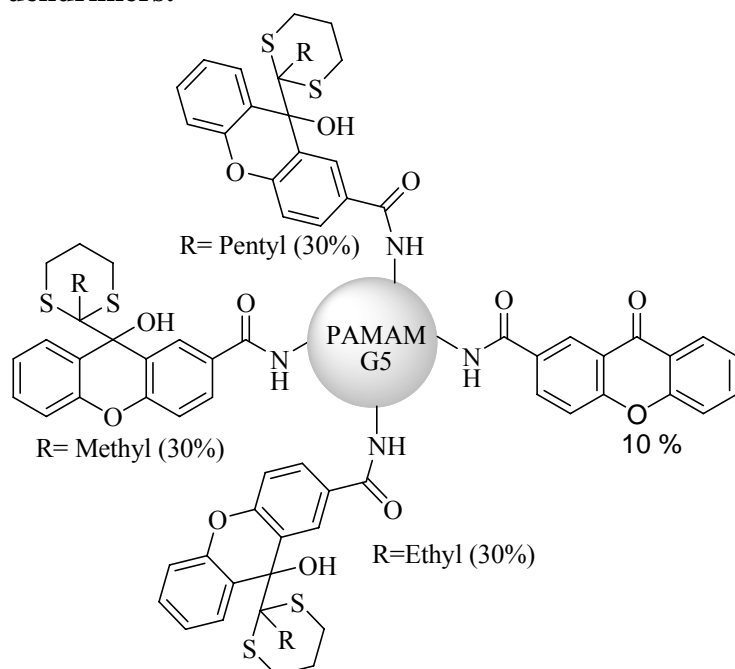
General procedure for the immobilization of an adduct and sensitizer on PAMAM G5 dendrimers:



To 10 mL of CH_2Cl_2 was added xanthone-2-carboxylic acid succinamide ester with desired linker length (0.012 mmol), and 1.0 mL of starburst dendrimer solution (generation 5, 5 wt% in methyl alcohol). The reaction mixture was shaken, on an orbital shaker, for 24 h. The desired 2-alkyl-1,3-dithiane NHS ester (0.24 mmole) was added and the reaction was shaken for another 24 h. The solvent was removed under vacuum, 20mL of 1 N NaOH solution was added, and the suspension was shaken for 3 h. The solid was collected by vacuum filtration, washed with 1 N NaOH solution, distilled water, and demineralized water. After being vacuum-dried, the product obtained was a pale yellow solid (85% yield)

According to the analysis of sulfur for multiple dendrimer immobilizations there were between 86 and 99 NH_2 groups, out of 128, for which immobilization of adduct occurred.

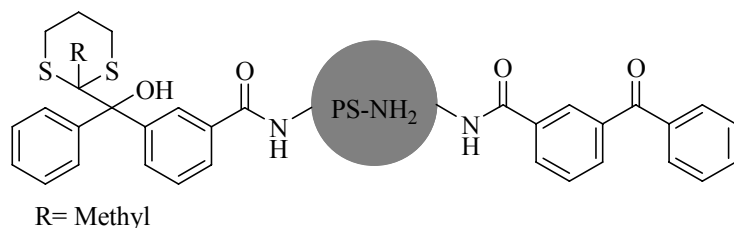
General procedure for the immobilization of multiple adducts and sensitizer on PAMAM G5 dendrimers:



To 10 mL of CH_2Cl_2 was added xanthone-2-carboxylic acid succinamide ester with desired linker length (0.02 mmol), and 1.0 mL of starburst dendrimer solution (generation 5, 5 wt% in methyl alcohol). The reaction mixture was shaken, on an orbital shaker, for 12 h. To this was added the appropriate 2-alkyl-1,3-dithiane NHS esters (3 different adducts at a concentration of 0.06 mmole each). The reaction was then shaken for an additional 24 h. The solvent was removed under vacuum, 20mL of 1 N NaOH solution was added and the suspension was shaken for 3 h. The solid was collected by vacuum filtration,

washed with 1 N NaOH solution, distilled water, and demineralized water. After being vacuum-dried, the product obtained was a pale yellow solid (85% yield)

General procedure for the immobilization of an adduct and sensitizer on Polystyrene-NH₂ beads:



A mixture of Polystyrene-NH₂ beads and the appropriate sensitizer (5 molar % calculated based on 1.86 mmol/g loading) in 2 mL CH₂Cl₂ were shaken, on an orbital shaker, for 24 h. The desired 2-alkyl-1,3-dithiane adduct (110%) was added and the reaction was shaken for an additional 24 h. The contents of the vial were washed with EtOAc (2 mL) six times, and CH₃CN (2 mL) two times. Each vial was filled with CH₃CN (2 mL) and shaken for 1 h after which the solvent was decanted. This was repeated 3 times. The contents of the vial were then dried under vacuum at 50 °C overnight giving the desired beads.

Anal.: Sulfur, Found: 5.24 %, which corresponds to 1.17 mmol/g loading. The ketone loading is assumed to be 5 molar % (i.e. 0.09 mmol/g quantitative)

Photochemical procedure for immobilized dendrimers:

8mg of immobilized dendrimer was put into in CH₂Cl₂ and degassed under Ar for 45 min. 2 μL of the solution was removed and analyzed by GC-MS to verify that none of the dithiane tags were present. The sample was then irradiated in a carousel Rayonet photo reactor (RPR-3500 lamps) with a 320 nm long pass solution filter. Every 10 min, 2 μL of the reaction mixture were removed and analyzed by GC-MS to determine the progress of dithiane cleavage. The areas were recorded and used to calculate the amount of released dithiane (based on a calibration curve).

Formation of micelles and Photochemical procedure for dendrimers in micelles:

Immobilized dendrimer (10mg) and SDS (10mg) were put into in 0.6 mL D₂O and stirred at RT for 12 h. After a clear solution was obtained 0.5mM of dodecane solution (1mL) and 1 mL of hexane were added and the sample was degassed under Ar for 45 min. 2 μL of the solution was removed and analyzed by GC-MS to verify that none of the dithiane tags were present. The solution was then irradiated in a carousel Rayonet photo reactor (RPR-3500 lamps) with a 320 nm long pass solution filter for 1 hr. After irradiation 2 μL of hexane were analyzed by GC-MS to detect released dithiane tags. The areas were recorded and used to calculate the amount of released dithiane (based on a calibration curve).

Photochemical procedure for immobilized polystyrene beads:

50 mg of immobilized beads were weighed into a vial (capacity 5 mL) and a 1mM dodecane solution (1mL) was added. Photolysis were carried out in intervals of 10 min using a carousel Rayonet photo reactor (RPR-3500 lamps) with a 320 nm long pass solution filter. After each irradiation 1 uL of the sample was withdrawn and analyzed by GC-MS to detect cleaved dithiane tags. The areas were recorded and used to calculate the amount of released dithiane (based on a calibration curve). These values were plotted as shown in Figure 3.

