Supporting Information

Heterogeneous Rupturing Dendrimers

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Experimental

Material and Methods

Material

2,2-bis(hydroxymethyl) propionic acid (bis-MPA) was obtained from Perstorp. Di-methyl amino pyridine (99%) (DMAP), 5, 5'-Dithiobis(2-nitrobenzoic acid) (DNTP), glutathione reductase (GR), glutathione (GSH), nicotinamide adenine dinucleotide phosphate (NADPH), *trifluoroacetic acid sodium salt (98%), 3-mercapto propionic acid (99%) were obtained from Sigma Aldrich. Chloroform-D (CDCl3) (99.8%) were acquired from Cil. Chloroform (HPCL grade) (CHCl3), diethylether (analytical reagent grade) (Ether), were acquired from Fisher Chemicals. Dichloromethane (analytical grade) (DCM), toluene-4-sulfonic acid (98%) (pTSA) were purchased from Merck.* F12 medium and glutamine was purchased from Lonza. Fetal calf serum was acquired from GT. Hydroethidine (HE) was obtained from Life Technologies. Glutaredoxin (hGrx) and thioredoxin (Trx) were purchased from IMCOcorp. Recombinant rat thioredoxin reductase (rrTrxR) was a kind gift from Prof. Elias Arnér, Karolinska Institutet. TMP-G4-OH was acquired from Polymer factory. *6-Azidohexanoic anhydride was synthesized according to previously published procedure.*[1] Other chemicals where purchased from common suppliers such as Sigma Aldrich or VWR and used as received.

Methods

Nuclear magnetic resonance (NMR) was performed on a Bruker AM NMR. ^{1}H-NMR and ^{13}C-NMR were recorded at 400 MHz and 101 MHz respectively. Spectra were acquired using a Bruker Avance instrument. ^{1}H-NMR spectra were acquired using a spectral window of 20 ppm, a relaxation delay of 1 second and 16 scans. ^{13}C-NMR spectra were acquired using a spectral window of 240 ppm, a relaxation delay of 2 seconds and 512 scans. Analyses of obtain spectra were performed using MestReNova version 7.1.1-9649 (Mestrelab Research S.L 2012).

SEC in dimethylformamide (DMF) was performed at a flowrate of 0.2 mL min^{-1} with 0.01 M LiBr as the mobile phase at 50 °C using a TOSOH EcoSEC HLC-8320GPC system equipped with an EcoSEC RI detector and three columns (PSS PFG 5µm; Microguard, 100Å, and 300Å) (MW resolving range: 300-100 000 Da) from PSS GmbH. Sample solutions with a concentration of 2.5 mg/ml were used. A conventional calibration method was created using narrow linear poly(methyl methacrylate) standards. Corrections for flow rate fluctuations were made using toluene as an internal standard. PSS WinGPC Unity software version 7.2 was used to process data.

SEC in tetrahydrofuran (THF) was performed at 50 °C with flow rate of 1 mL min^{-1} together with 25 ppm N,N-Dimethylacetamide on a GPCMAX and auto sampler from Malvern Instruments equipped with RI detector. Three columns where used one guard column (TGuard) and two linear mixed bead columns (LT4000L), a conventional calibration was created using linear polystyrene standards.

Matrix-assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF-MS) were performed on a Bruker UltraFlex MALDI-TOF MS with SCOUT-MTP Ion Source (Bruker Daltonics, Bremen)

with a gridless ion source and the N2-laser operating at 337nm. The intensity of the laser was set to the lowest possible for acquisition of high resolution spectra of the product and all spectra were acquired using a reflector-positive mode. The instrument was calibrated using SpheriCal^{TM} calibrants purchased from Polymer Factory Sweden AB. The received spectra were analyzed with FlexAnalysis Bruker Daltonics, Bremen, version 2.2. Matrixies were prepared by dissolution at a concentration of 10 mg/ml, salts at a concentration of 1mg/ml and analyte at a concentration of 1mg/ml all in THF. Samples were prepared at a ratio of 20:5:2.5 for the matrix, counter ion and analyte respectively. A 2 µl droplet was deposited on an MPT 284 Target ground steel TF Target plate purchased from Bruker Daltonics.

Column chromatography were performed using a Isolera 4 automated flash purification system from Biotage, LLC (Charlotte, NC, USA). Biotage® SNAP Ultra prepacked columns where used with 10 grams of silica together with samplets of 1 g where used. A method was developed for each sample; more details can be found where appropriate below.

Cell culturing

The lung cancer cell line A549 was cultured in F12 medium, supplemented with 10 % fetal calf serum and 1 % glutamine, in a humidified incubator at 37 ˚C, 5 % CO_2.

Activity measurements

To determine the reductase activity of hGrx1 on TMP-G4-(PEG750-MM)$_{48}$) and TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$, a coupled enzyme reaction system was used [2]. The system is composed of 0.2 mM NADPH, 6 µg/ml GR, 0.5 or 1 mM GSH, 1 µM of hGrx1, 25 µM TMP-G4-(PEG750-MM)$_{48}$ or TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$, in a 50 mM Tris-HCl pH 8.0 and 1 mM EDTA buffer. The activity of the reaction was determined by monitoring the decrease of absorbance at 340 nm caused by NADPH consumption. The same system was used in determination of the reaction rates of GSH (0.5-10 mM), and various concentrations of TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$ (5-25 µM), but without the presence of hGrx1.

The reducing activity of rrTrxR and Trx1 on TMP-G4-(PEG750-MM)$_{48}$ and TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$, was determined by a mixture containing 50 mM Tris-HCl pH 8.0, 2 mM EDTA, 0.2 mM NADPH, 75 nM rrTrxR, with or without 5 µM Trx1 and 5 or 10 µM TMP-G4-(PEG750-MM)$_{48}$ or TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$, as previously described [3]. Activity was determined by monitoring the change of absorbance at 340 nm.

Viability assay

For viability measurements, A549 cells were plated in 96-well plates at a density of 6 x 10^3 cells/well and cultured overnight. Cells were treated with indicated concentrations of TMP-G4-(PEG750-MM)$_{48}$ or TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$ for 24 h, before subjected to the MTT assay. 20 µl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 3 h. The medium was removed, and 150 µl DMSO was added to each well, and the plates were agitated on an orbital shaker for 15 min at room temperature. The cell viability was determined by measuring the absorbance at 550 nm using Spectra Max (SoftMax Pro 5.2).

Hydroethidine staining

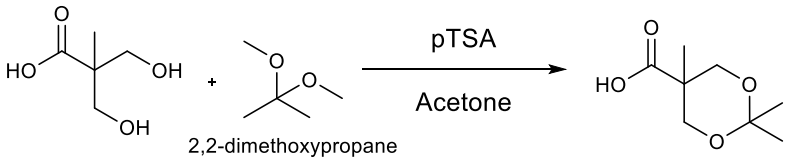
To determine production of reactive oxygen species (ROS), cells were seeded in 24-well plates with round cover glasses, at a density of 10 x 10^4 cells/ml, and incubated overnight. The cells were treated at the designed concentrations of TMP-$(S\text{-}S)_{45}$-G4-$(PEG750\text{-}MM)_{48}$ for 3 h. Hydroethidine was added with a final concentration of 5 µM and incubated for 10 min. Cells were mounted on slides, and visualization was performed using microscope Zeiss Axiovert 40 CFL, with images processed in Axiovision Rel 4.8.

Estimation of total thiols

After addition of TMP-$(S\text{-}S)_{45}$-G4-$(PEG750\text{-}MM)_{48}$ to the media, extracellular thiols were determined at given time points by DTNB reaction. Guanidinium hydrochloride in 200 mM Tris-HCl pH 8.0 was added to media to a final concentration of 2.7 M and DTNB to a final concentration of 1 mM. The reduction of DTNB was measured at an absorbance of 412 nm.

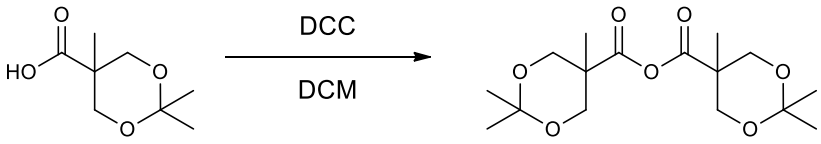
Synthesis

Acetonide protected bis-MPA (1)



Bis-MPA (300 g, 2.23 mol) was dissolved in 2.4 l acetone in an Erlenmeyer flask equipped with a magnetic stirbar. Upon dissolution pTSA (2.55 g, 13.4 mmol) and 2,2-dimethoxypropane (416 ml, 3.35 mol) was added. The reaction was allowed to proceed overnight and subsequently quenched for 2 hours by the addition of 18 ml of a 1:1 solution of NH_3:EtOH. The acetone was evaporated and replaced with 1.5 l of DCM. The organic phase was washed five times with 250 ml of deionized water, the organic phase collected and dried with $MgSO_4$, filtered and the solvent evaporated. Crude product was re dissolved in 600 ml of acetone and recrystallized at -20°C for 24 hours product was collected by filtration. Product was attained as milky crystals (313 g, 80.4 %). ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.17 (d, J = 11.8 Hz, 2H,C-C**H$_2$**-O), 3.65 (d, J = 11.8 Hz, 2H,C-C**H$_2$**-O), 1.41 (d, J = 13.2 Hz, 6H,CH_2-C**H$_3$**) 1.19, (s, 3H,C-C**H$_3$**). ^{13}C-NMR (101 MHz, $CDCl_3$) : δ(ppm) 180.03, 98.27, 65.81, 41.66, 25.30, 21.73, 18.33.

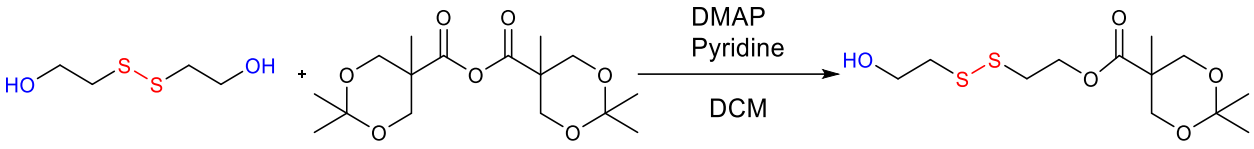
Acetonide protected bis-MPA anhydride (2)



1 (313 g, 1.80 mol) was dissolved in 500 ml DCM in a round bottom flask equipped with a magnetic stirrer. The reaction vessel was subsequently submerged in an ice bath. DCC (185 g, 0.90 mol) dissolved

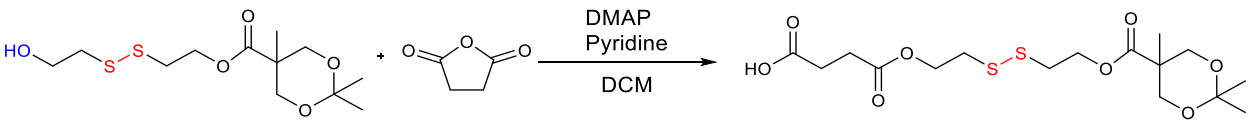
in DCM 200 ml was added drop wise using a dropping funnel over a period of 30 minutes. Once addition was done the dripping funnel was removed and a septum was used to seal the vessel, the reaction was allowed to proceed overnight. Raw reaction mixture was filtered through a pore 4 solid filter and the filtrate collected. The solvent was evaporated; resulting solid dissolved in minimal amount of ether and subsequently passed through a pore 4 filter and the filtrate collected. The ether was evaporated by rotary evaporation and the product collected as a white powder (249 g, 83.7 %). ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.22 (d, J = 11.9 Hz, 2H,C-C**H$_2$**-O), 3.71 (d, J = 11.9 Hz, 2H,C-C**H$_2$**-O), 1.43 (d, J = 18.3 Hz, 6H,CH_2-C**H$_3$**), 1.25, (s, 3H,C-C**H$_3$**). ^{13}C-NMR (101 MHz, $CDCl_3$): δ(ppm) δ 169.42, 98.31, 65.60, 43.59, 25.51, 21.48, 17.58.

Hydroxyethyl disulfide protected bis-MPA (3)



2-Hydroxyethyl disulfide (37.0 ml, 0.302 mol) were solubilized in 100ml DCM in a round bottom flask equipped with a magnetic stir bar, pyridine (70 ml, 0.91 mol) and DMAP (7.4 g, 60.5 mmol) was added. The solution was cooled to zero degrees using an ice bath. Subsequently **2** (70 g, 0.30 mol) dissolved in 200ml DCM was slowly added using a dripping funnel over a period of 2 hours, the reaction was allowed to proceed overnight. The crude reaction was diluted with 200 ml DCM and washed five times with 100 ml portions of a 10 w% aqueous solution of $NaHSO_4$ and five times with 100 ml portions of a 10 w% aqueous solution of Na_2CO_3. The organic phase was collected and dried with $MgSO_4$, filtered and the solvent evaporated. The resulting oily liquid was dissolved in DCM and absorbed on to silica (78 g) followed by purification by column chromatography. A column with a diameter of 10 cm and an effective length of 25 cm was used. The column was packed wet in a sequenced of sand, silica, sand, product and sand. A Heptane: EtOAc elution system was used starting from 100% heptane Δ = 2.5%, product was eluted at 65% EtOAc. The Solvent was evaporated and product collected yellow viscous liquid (34.2 g 36.4 %). ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.39 (t, J = 6.46 Hz, 2H, -S-CH_2-C**H$_2$**-COO-), 4.16 (d, J = 11.8 Hz, 2H,C-C**H$_2$**-O), 3.83 (q, *J* = 6.0 Hz, 2H, OH-C**H$_2$**-CH_2-S-), 3.65 (d, J = 12.0 Hz, 2H,C-C**H$_2$**-O), 2.91 (t, *J* = 6.5 Hz, 2H, -S-C**H$_2$**-CH_2-COO-), 2.83 (t, *J* = 5.9 Hz, 2H, OH- CH_2-C**H$_2$**-S-), 2.20 (t, *J* = 6.2 Hz, 1H, O**H**-CH_2-CH_2-S-), 1.41 (d, J = 13.2 Hz, 6H,CH_2-(C**H$_3$**)$_2$) 1.19, (s, 3H,C-C**H$_3$**). ^{13}C-NMR (101 MHz, $CDCl_3$) : δ(ppm) 174.28, 98.32, 66.14, 62.57, 60.51, 41.60, 37.01, 25.43, 22.12, 18.72.

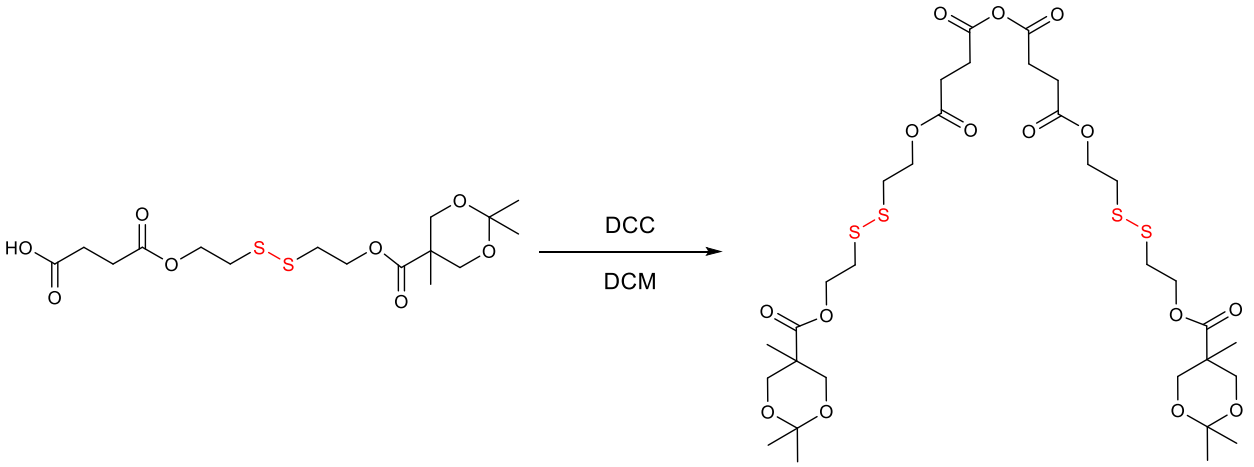
Carboxylic hydroxyethyl disulfide protected bis-MPA (4)



3 (34.2 g 0.11 mol) was dissolved in 100 ml DCM and pyridine (26.6 ml, 0.33 mol) in a round bottom flask equipped with magnetic stir bar. DMAP (2.69 g, 22.6 mmol) was added and reaction mixture cooled to

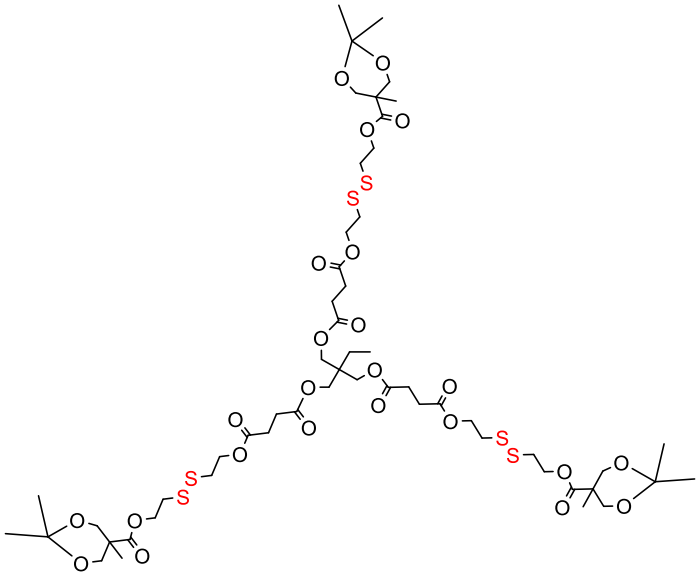
0°C. Succinic anhydride (16.53 g 0.16 mmol) was slowly added as a solid and the reaction left to precede overnight. The reaction was confirmed complete with ^{13}C-NMR, subsequently water (30 ml) was added to quench the reaction for five hours. The crude reaction was diluted with 400ml DCM, washed five times with 50 ml of a 10 w% aqueous solution of $NaHSO_4$, 3 times with 100 ml portions of deionized water, dried with $MgSO_4$, filtered and the solvent evaporated. Product was collected as orange viscous liquid (46.4 g, 98.0 %). 1H-NMR (400 MHz, $CDCl_3$): δ(ppm) 4.40 (t, *J* = 6.6 Hz, 2H, 2H, -S-CH_2-C**H**$_2$-COO), 4.36 (t, *J* = 6.5 Hz, 2H, COO-C**H**$_2$-CH_2-S-), 4.20 (d, *J* = 11.7 Hz, 2H,z C-C**H**$_2$-O), 3.65 (d, *J* = 11.7 Hz, 2H, C-C**H**$_2$-O), 2.93 (q, *J* = 6.3 Hz, 4H, -C**H**$_2$-S-S.C**H**$_2$), 2.76 – 2.58 (m, 4H, COOH-C**H**$_2$-C**H**$_2$-COO-), 1.41 (d, *J* = 18.6 Hz, 6H,CH_2-(C**H**$_3$)$_2$), 1.18 (s, Hz, 3H, CH_2-C**H**$_3$). ^{13}C-NMR (101 MHz, $CDCl_3$) : δ(ppm) 176.86, 174.00, 171.85, 98.18, 65.88, 62.48, 62.41, 41.90, 37.18, 37.02, 28.78, 28.70, 24.93, 22.19, 18.51.

Carboxylic hydroxyethyl disulfide protected bis-MPA anhydride (5)



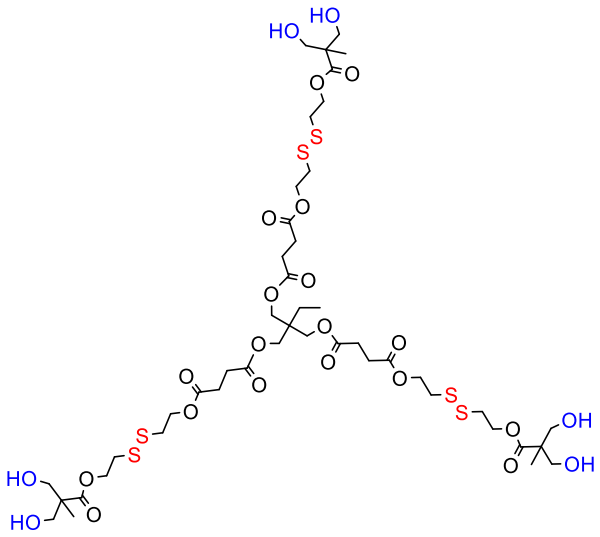
In a round bottom flask equipped with magnetic stir bar **4** (46.4 g, 0.11 mol) was dissolved in 200 ml DCM. The reaction vessel was cooled to 0°C and DCC (11.7 g, 56.6 mmol) dissolved in 50 ml DCM was drop wise added using a dripping funnel over a period of 20 minutes. The reaction was left to react overnight and then passed through a pour four filter. The DCM was evaporated and 50 ml of ether were added and the filtration repeated. The ether was evaporated and product collected as orange viscous liquid (44.4 g, 96.0 %). 1H-NMR (400 MHz, $CDCl_3$): δ(ppm) 4.35 (t, *J* = 6.5 Hz, 4H, 2H, -S-CH_2-C**H**$_2$-COO), 4.31 (t, *J* = 6.5 Hz, 4H, COO-C**H**$_2$-CH_2-S-), 4.13 (d, *J* = 11.7 Hz, 4H,z C-C**H**$_2$-O), 3.58 (d, *J* = 11.7 Hz, 4H, C-C**H**$_2$-O), 2.87 (q, *J* = 6.7 Hz, 8H, -C**H**$_2$-S-S.C**H**$_2$), 2.75 (t, J = 6.6 Hz 4H, COOOOC-CH_2-C**H**$_2$-COO-), 2.63 (t, J = 6.5 Hz H, COOOOC-C**H**$_2$-CH_2-COO-), 1.34 (d, *J* = 18.3 Hz, 6H,CH_2-(C**H**$_3$)$_2$), 1.13 (s Hz, 3H, CH_2-C**H**$_3$). ^{13}C-NMR (101 MHz, $CDCl_3$) : δ(ppm) 174.16, 171.55, 167.94, 98.24, 66.10, 62.84, 62.58, 42.08, 37.16, 30.29, 28.53, 25.00, 22.60, 18.76.

TMP-(S-S)$_3$-G1-(acet)$_3$ (6)



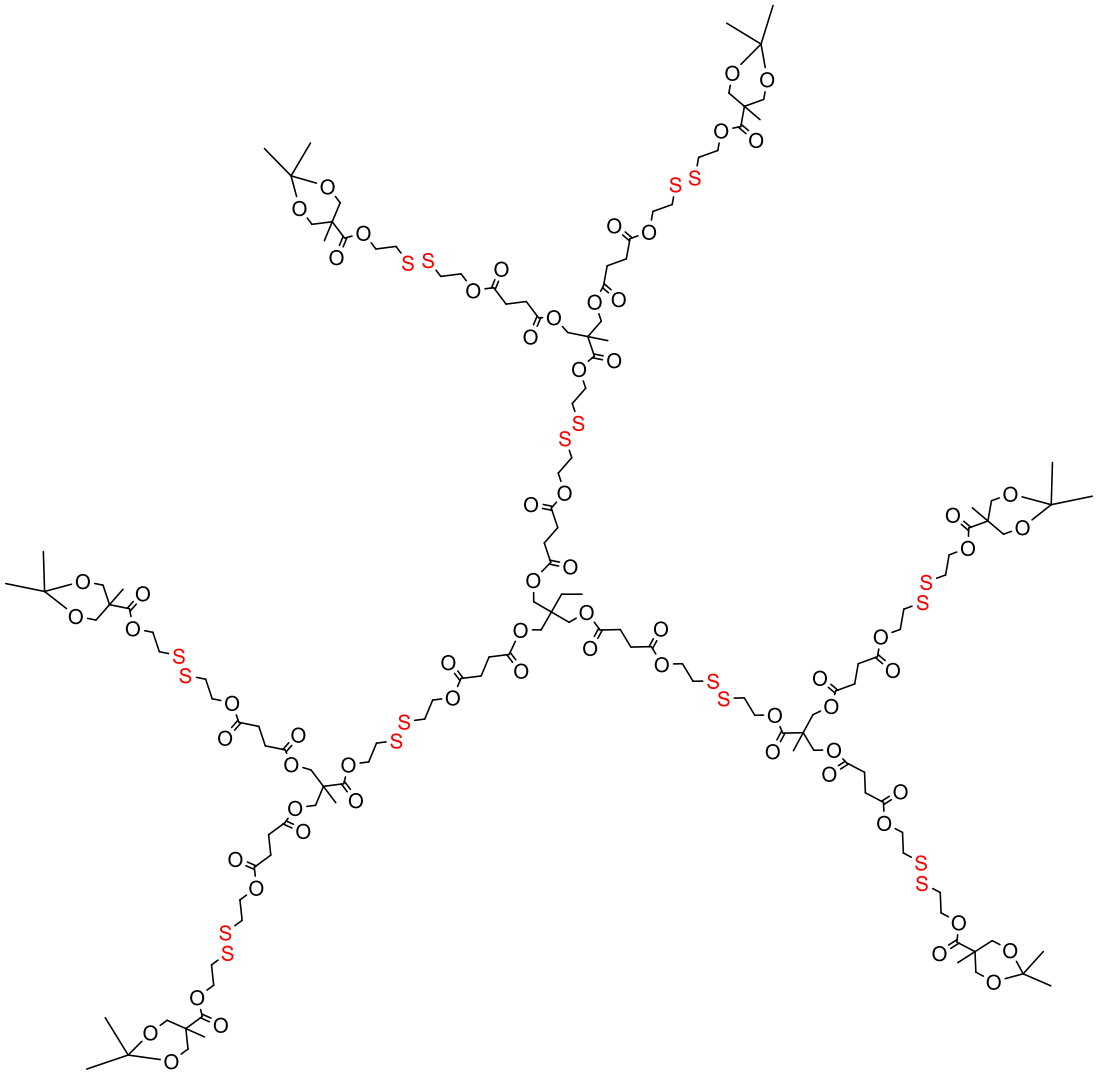
In a round bottom flask with a magnetic stir bar lyophilized TMP (255 mg, 1.90 mmol), DAMP (139 mg, 1.15 mmol) was dissolved in pyridine (1.38 ml, 17.1 mmol) and 1 ml DCM. The reaction mixture was cooled to 0ºC and **5** (5.60 g, 6.84 mmol) dissolved in 5 ml DCM was drop wise added using a dropping funnel. The reaction was left to react overnight and the completion was confirmed using MALDI-TOF-MS (HABA:DCTB, 1:1). The residual anhydride was quenched during four hours using 1 ml of deionized water. Subsequently the reaction mixture was diluted with 50 ml DCM and the organic phase washed five times with 10 ml portions of a 10 w% aqueous solution of $NaHSO_4$, dried with $MgSO_4$, filtered and the solvent evaporated. Resulting orange waxy solid was absorbed onto silica and purified using column chromatography. A column with a diameter of 5 cm was used with an effective length of 20 cm. A heptane:EtOAc solvent system was used and product eluted at 6:4 EtOAc:Heptane. Solvent was evaporated and product collected as colorless liquid (2.40 g, 95.1 %). MALDI calc [M+Na$^+$] = 1333.4 Da, Obtained [M+Na$^+$] = 1333.1 Da. M_w (theoretical) = 1311.6 g mol^{-1}, SEC (THF) M_n =1577 g mol^{-1}, Đ = 1.025. ^{1}H-NMR (400 MHz, CDCl3): **δ**(ppm) 4.41 (t, J = 6,5 Hz, 6H, -CH_2-C**H$_2$**-COO(disulfide)), 4.34 (t, J = 6.6 Hz, 6H, -COO-C**H$_2$**-CH_2-(disulfide)), 4.19 (d, J = 11.8 Hz, 6H, -C-C**H$_2$**-O-(Bis-MPA)), 4.03 (s, 6H, -C-C**H$_2$**-COO-(TMP)) , 3.64 (d, J = 11.8 Hz, 4H, -C-C**H$_2$**-O-(Bis-MPA)), 2.93 (q, *J* = 8.2, 6.6 Hz,, 12H, -CH_2-C**H$_2$**-S-S-C**H$_2$**-CH_2-(disulfide)), 2.64 (s, 12H, COO-C**H$_2$**-CH_2-COO-(succinic)), 1.48 (q, *J* = 7.8 Hz, 2H, CH_3-C**H$_2$**-C-(TMP)) 1.40 (d, J = 7.8 Hz, 18H,-CH_2-C**H$_3$** (bis-MPA)), 1.19 (s, 9H,-C-C**H$_3$**(bis-MPA)), 0.88 (t, J = 7.56 Hz, 3H, C-C**H$_3$** (TMP)).

TMP-(S-S)$_3$-G1-(OH)$_6$ (7)



In a round bottom flask with magnetic stir bar **6** (2.31 g, 1.72 mmol) was dissolved in 300 ml MeOH, Dowex™ (3g) was added and reaction was allowed to proceed one hour. Progress was checked with MALDI and protected dendrimer was still observed, Dowex™ was filtered off and MeOH was evaporated. The product was re dissolved in MeOH and an additional 3 g of Dowex™ was added. The procedure was repeated until no residual protective groups could be observed on MALDI. Solvent was evaporated and product collected as lightly yellow liquid (1.68 g, 81.5 %) MALDI calc [M+Na$^+$] = 1213.3 Da, Obtained [M+Na$^+$] = 1213.9 Da. M_w (theoretical) = 1190.3 g mol^{-1}, SEC (THF) M_n = 1300.1 g mol^{-1}, Đ = 1.027. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.41 (t, J = 6.4 Hz, 6H, -CH_2-C**H$_2$**-COO(disulfide)), 4.33 (t, J = 6.5 Hz, 6H, -COO-C**H$_2$**-CH_2-(disulfide)), 4.02 (s, 6H, -C-C**H$_2$**-COO-(TMP)) ,3.86 (d, J 11.25 Hz, 6H, -C-C**H$_2$**-O-(Bis-MPA)), 3.70 (d, J = 11.23 Hz, 6H, -C-C**H$_2$**-O-(Bis-MPA)), 2.95 (q, J = 5.4 Hz, 12H, -CH_2-CH_2-S-S-C**H$_2$**-CH_2-(disulfide)), 2.92 (t, J = 5.6 Hz, 6H, -CH_2-CH_2-S-S-C**H$_2$**-CH_2-(disulfide)), 2.63 (s, 6H, COO-C**H$_2$**-CH_2-COO-(succinic)), 1.47 (t, J = 7.52 Hz, 2H, CH_3-C**H$_2$**-C-(TMP)) 1.08 (s, 9H,-C-C**H$_3$**(bis-MPA)), 0.87 (t, J = 7.52 Hz, 3H, C-C**H$_3$** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.62, 172.13, 172.07, 67.77, 64.17, 62.68, 62.44, 49.55, 40.86, 37.29, 37.20, 29.03, 17.28, 7.49.

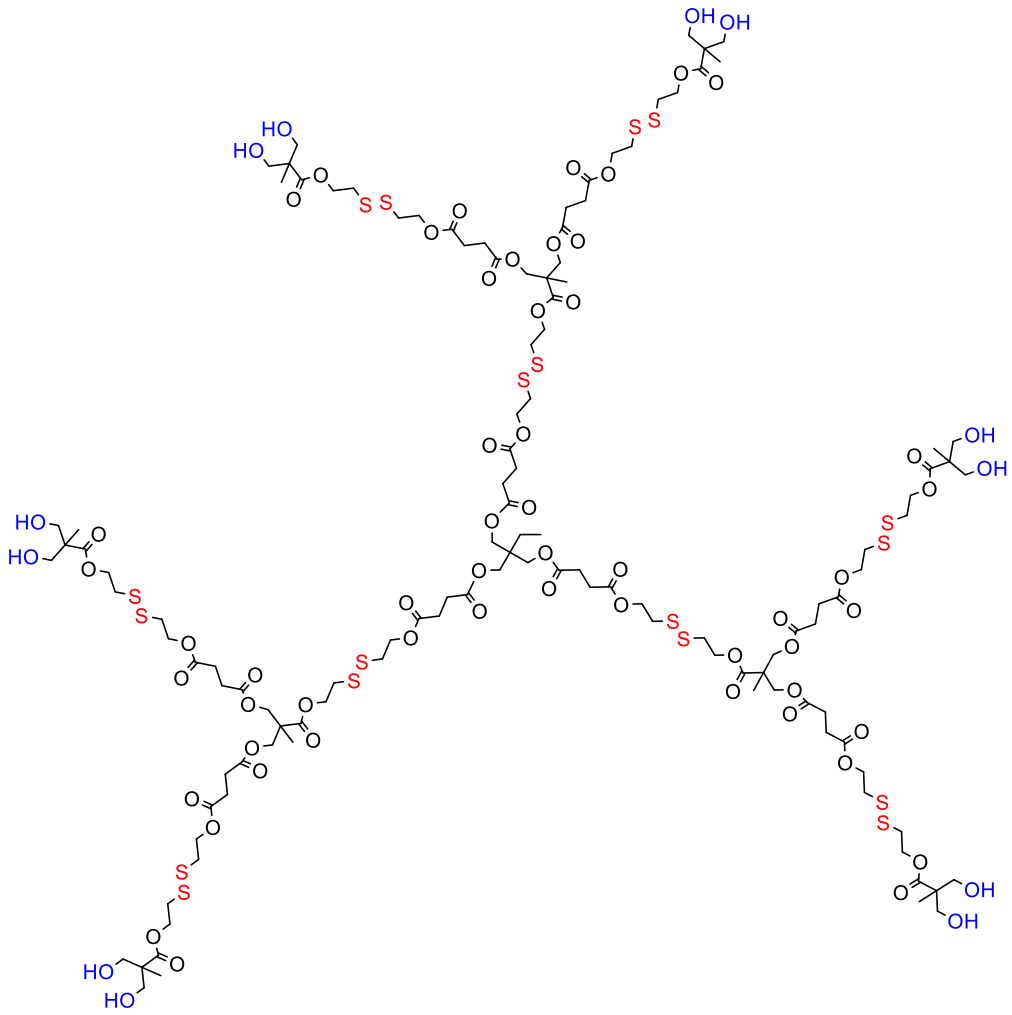
TMP-(S-S)$_9$-G2-(Acet)$_6$ (8)



In a round bottom flask with a magnetic stir bar, **7** (1.38 g, 1.14 mmol) were solubilized in pyridine (1.65 ml, 20.5 mmol) and 1 ml DCM. DMAP (167 mg, 1.36 mmol) was added and the reaction vessel was cooled to zero degrees Celsius. **5** (7.82 g, 9.56 mmol) dissolved in 5 ml DCM was added drop wise using a dripping funnel over a period of 30 minutes and left to react overnight. Completion was confirmed using MALDI-TOF-MS (HABA:DCTB, 1:1) and residual anhydride quenched by the addition of 3 ml of deionized water. The organic phase was diluted with 50ml DCM and washed five times with 10 ml portions of a 10 w% aqueous solution of $NaHSO_4$, dried with $MgSO_4$, filtered and the solvent evaporated. Resulting orange waxy solid was absorbed onto silica and purified using column chromatography. A column with a diameter of 5 cm was used with an effective length of 20 cm. A heptane:EtOAc.MeOH solvent system was used and product eluted at 90:10 EtOAc:MeOH. Solvent was evaporated and product collected as colorless liquid (2.44 g, 60.0 %). MALDI calc [M+Na$^+$] = 3565.8 Da, Obtained [M+Na$^+$] = 3565.8 Da. M_w (theoretical) = 3546.4 g mol^{-1}, SEC (THF) M_n 4116 g mol^{-1}, Đ = 1.070. ^{1}H-NMR (400 MHz, CDCl3): **δ**(ppm) 4.41 (t, J = 4.4 Hz, 18H, -CH_2-C**H$_2$**-COO(disulfide)), 4.35 (t, J = 4.3 Hz ,18H, -COO-C**H$_2$**-CH_2-(disulfide)), 4.25

(dd, *J* = 27.1, 16.4 Hz, 12H, -C-C**H₂**-O-(Bis-MPA dendritic)), 4.19 (d, J = 11.8 Hz, 12H, -C-C**H₂**-O-(Bis-MPA terminal)), 4.04 (s, 6H, -C-(C**H₂**)₃-COO-(TMP)) , 3.64 (d, J = 11.9 Hz 12H, -C-C**H₂**-O-(Bis-MPA terminal)), 2.96- 2.90 (m, 36H, -CH_2-C**H₂**-S-S-C**H₂**-CH_2-(disulfide)), 2.64 (s, 36H, COO-C**H₂**-C**H₂**-COO-(succinic)), 1.47 (m, 2H, CH_3-C**H₂**-C-(TMP)) 1.41 (d, J = 17.7 Hz, 36H,-C-(C**H₃**)₂(bis-MPA acetonide)), 1.26 (s, 9H,-C-C**H₃**(bis-MPA dendritic)), 1.20 (s, 18H,-C-C**H₃**(bis-MPA terminal)), 0.88 (t,J = 7.49 Hz, 3H, $(CH_2)_3$-C-C**H₃** (TMP)).

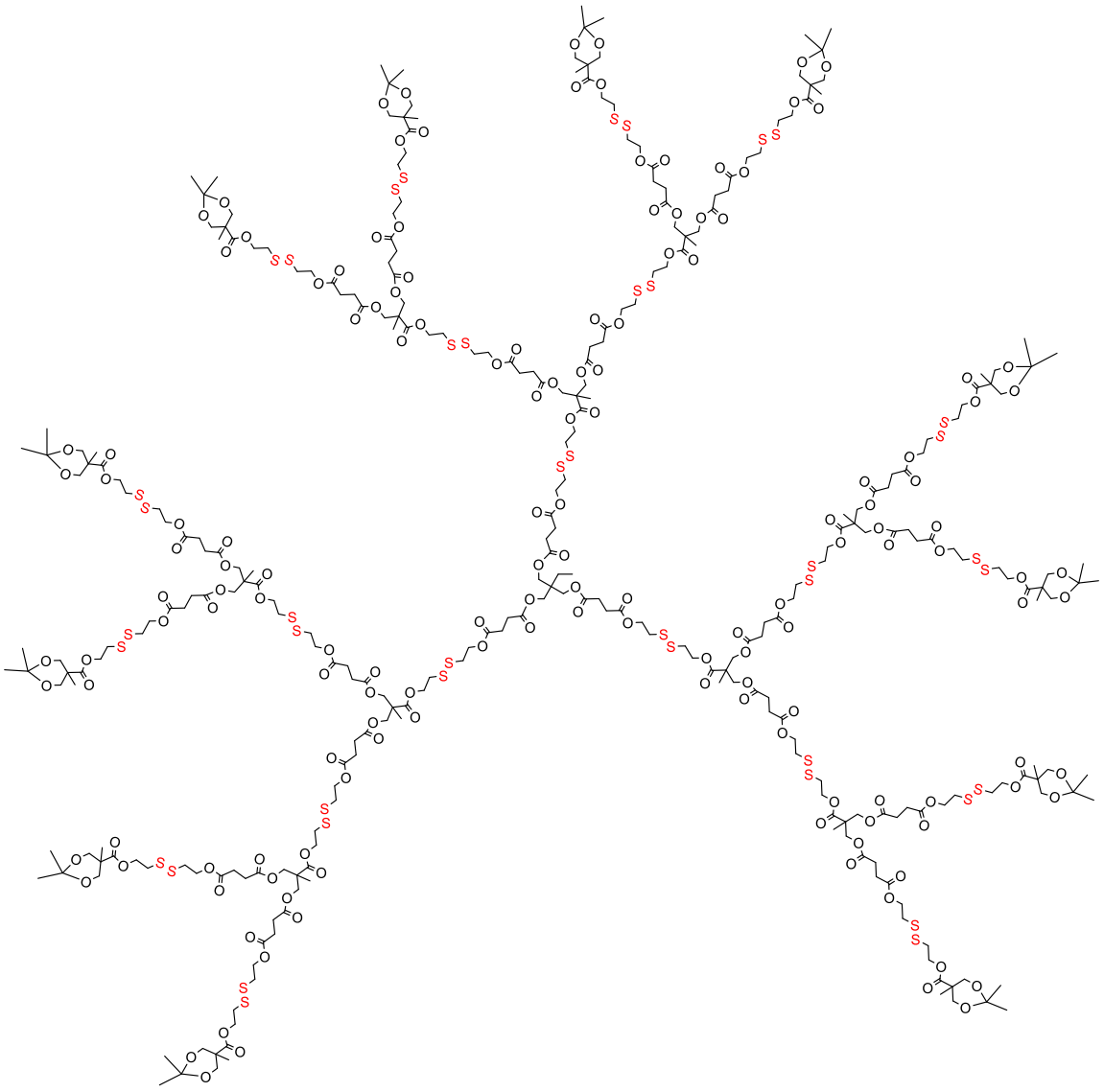
TMP-(S-S)₉-G2-(OH)₁₂ (9)



In a round bottom flask equipped with a magnetic stir bar **8** (2.30 g, 0.64 mmol) was dissolved in a mixture 60:40 by volume DCM:MeOH, Dowex™ (2.3g) was added and reaction was allowed to proceed one hour. Solvent was evaporated and product collected as lightly yellow liquid (1.92 g, 89.2 %) MALDI calc [M+Na^+] = 3325.7 Da, Obtained [M+Na^+] = 3325.6 Da. M_w (theoretical) = 3329.0 mol^{-1}, SEC (THF) M_n 4129 g mol^{-1}, Đ = 1.069. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.41 (m, 18H, -CH_2-C**H₂**-COO(disulfide)), 4.35 (t, J = 6.6 Hz ,18H, -COO-C**H₂**-CH_2-(disulfide)), 4.26 (dd, *J* = 27.1, 16.4 Hz, 12H, -C-C**H₂**-O-(Bis-MPA dendritic)), 4.04 (s, 6H, -C-(C**H₂**)₃-COO-(TMP)) , 3.88 (d, J = 11.2 Hz, 12H, -C-C**H₂**-O-(Bis-MPA terminal)), 3.72 (d, J = 11.3 Hz 12H, -C-C**H₂**-O-(Bis-MPA terminal)), 2.98- 2.87 (m, 36H, -CH_2-C**H₂**-S-S-C**H₂**-CH_2-(disulfide)), 2.64 (s, 36H, COO-C**H₂**-C**H₂**-COO-(succinic)), 1.48 (q, J = 7.50, 2H, CH_3-C**H₂**-C-(TMP)) 1.26 (s,

9H,-C-C**H$_3$**(bis-MPA dendritic)), 1.09 (s, 18H,-C-C**H$_3$**(bis-MPA terminal)), 0.88 (t,J = 7.5 Hz, 3H, $(CH_2)_3$-C-C**H$_3$** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$): **δ**(ppm) 175.68, 172.13, 171.88, 68.01, 65.65, 62.94, 62.71, 62.47, 49.56, 46.49, 40.89, 37.31, 37.23, 37.15, 36.94, 29.02, 17.94, 17.31.

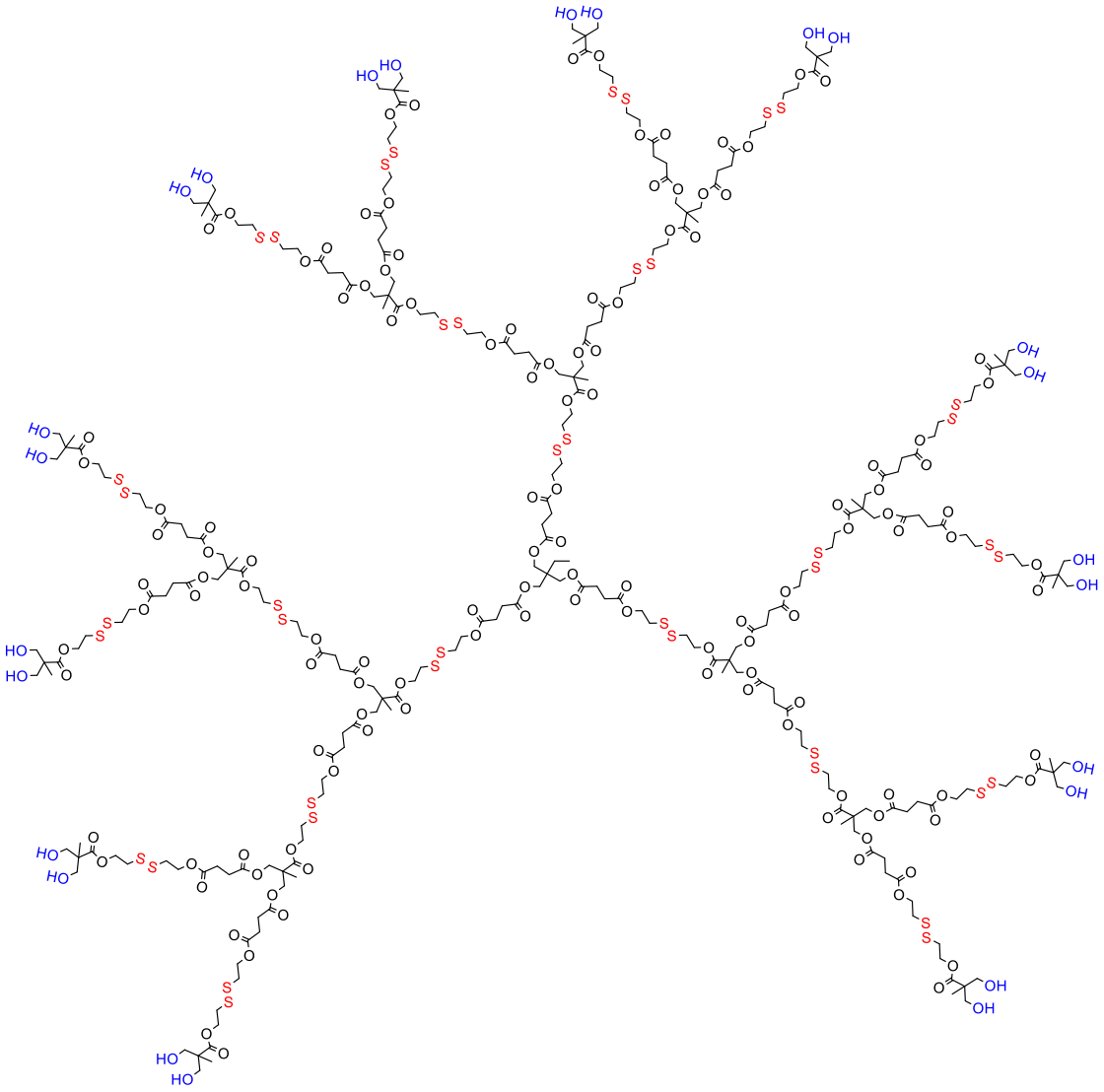
TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)



In a round bottom flask with a magnetic stir bar, **9** (1.23 g, 0.37 mmol) were solubilized in pyridine (1.07 ml, 13.2 mmol) and 50 ml DCM. DMAP (107 mg, 0.88 mmol) was added and the reaction vessel was cooled to zero degrees Celsius. **5** (5.05 g, 6.18 mmol) dissolved in DCM was added drop wise using a dripping funnel and the reaction left overnight. Completion was confirmed using MALDI-TOF-MS (HABA:DCTB, 1:1 and residual anhydride quenched by the addition of 15 ml of deionized water. The organic phase was diluted with DCM and washed five times with a 10 w% aqueous solution of $NaHSO_4$, dried with $MgSO_4$, filtered and the solvent evaporated. Resulting orange waxy solid was absorbed onto silica and purified using column chromatography. A column with a diameter of 5 cm was used with an

effective length of 20 cm. A heptane:EtOAc.MeOH solvent system was used and product eluted at 90:10 EtOAc:MeOH. Solvent was evaporated and product collected as a colorless liquid (2.68 g, 91.0 %). MALDI calc [M+Na$^+$] = 8039.8 Da, Obtained [M+Na$^+$] = 8030.9 Da. M_w (theoretical) = 8038.83 g mol^{-1}, SEC (THF) M_n = 8343 g mol^{-1}, Đ = 1.056. ^{1}H-NMR (400 MHz, CDCl3): **δ**(ppm) 4.40 (q, J = 6.3 Hz, 42H, -CH_2-C**H$_2$**-COO(disulfide)), 4.35 (t, J = 6.5 Hz ,42H, -COO-C**H$_2$**-CH_2-(disulfide)), 4.25 (dd, *J* = 26.8, 16.2 Hz, 36H, -C-C**H$_2$**-O-(Bis-MPA dendritic)), 4.19 (d, J = 11.1 Hz, 24H, -C-C**H$_2$**-O-(Bis-MPA terminal)), 4.04 (s, 6H, -C-(C**H$_2$**)$_3$-COO-(TMP)), 3.64 (d, J = 11.1 Hz 24H, -C-C**H$_2$**-O-(Bis-MPA terminal)), 2.96- 2.90 (m, 84H, -CH_2-C**H$_2$**-S-S-C**H$_2$**-CH_2-(disulfide)), 2.64 (s, 84H, COO-C**H$_2$**-C**H$_2$**-COO-(succinic)), 1.40 (d, J = 18.2 Hz, 72H,-C-(C**H$_3$**)$_2$(bis-MPA acetonide)), 1.26 (s, 27H,-C-C**H$_3$**(bis-MPA dendritic)), 1.20 (s, 36H,-C-C**H$_3$**(bis-MPA terminal)), 0.88 (t, J = 6.4 Hz, 3H, $(CH_2)_3$-C-C**H$_3$** (TMP)).

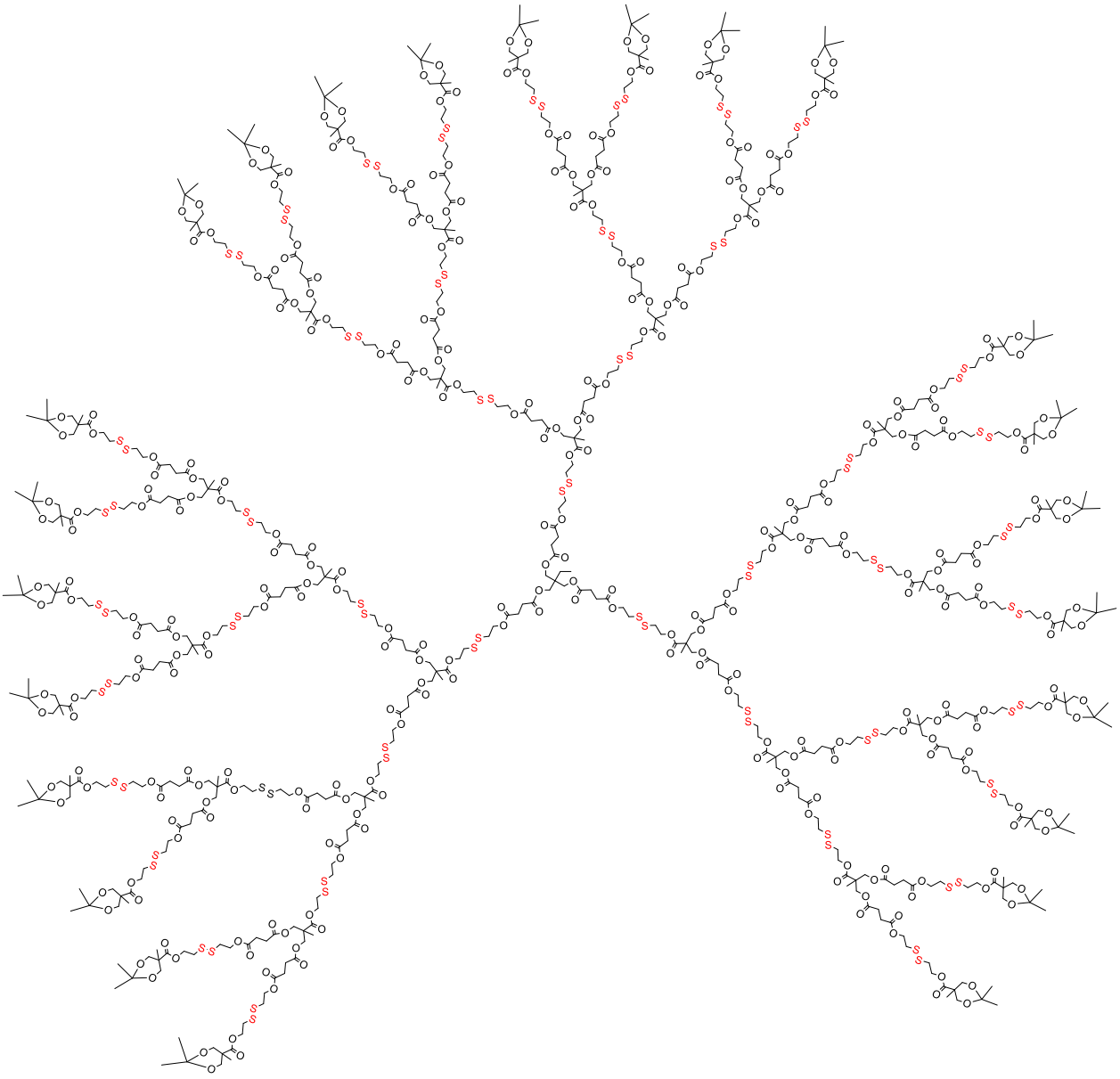
TMP-(S-S)$_{21}$-G3-(OH)$_{24}$ (11)



In a round bottom flask equipped with a magnetic stir bar **10** (2.65 g, 0.33 mmol) was dissolved in a mixture 60:40 by volume DCM:MeOH, Dowex™ (2.7g) was added and reaction was allowed to proceed

one hour. Solvent was evaporated and product collected as lightly yellow viscous liquid (2.22 g, 88.1 %) MALDI calc [M+Na^+] = 7550.4 Da, Obtained [M+Na^+] = 7550.7 Da. M_w (theoretical) = 7550 mol^{-1}, SEC (THF) M_n 8284 g mol^{-1}, Đ = 1.082. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.41 (m, 42H, -CH_2-C**H₂**-COO(disulfide)), 4.35 (t, J = 6.5 Hz ,42H, -COO-C**H₂**-CH_2-(disulfide)), 4.23 (dd, *J* = 28.7, 16.6 Hz, 36H, -C-C**H₂**-O-(Bis-MPA dendritic)), 4.04 (s, 6H, -C-(C**H₂**)$_3$-COO-(TMP)) , 3.87 (d, J = 11.1 Hz, 24H, -C-C**H₂**-O-(Bis-MPA terminal)), 3.72 (d, J = 11.3 Hz 24H, -C-C**H₂**-O-(Bis-MPA terminal)), 2.98- 2.91 (m, 84H, -CH_2-C**H₂**-S-S-C**H₂**-CH_2-(disulfide)), 2.64 (s, 84H, COO-C**H₂**-C**H**$_2$-COO-(succinic)), 1.48 (q, J = 7.38, 2H, CH_3-C**H₂**-C-(TMP)) 1.26 (s, 9H,-C-C**H₃**(bis-MPA dendritic)), 1.09 (s, 18H,-C-C**H₃**(bis-MPA terminal)), 0.88 (t, J = 7.5 Hz, 3H, $(CH_2)_3$-C-C**H₃** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$): **δ**(ppm) 192.06, 175.66, 172.13, 171.88, 68.36, 67.83, 65.64, 62.70, 62.47, 52.08, 49.57, 49.49, 49.42, 46.49, 37.30, 37.23, 37.13, 36.94, 29.02, 17.93, 17.31.

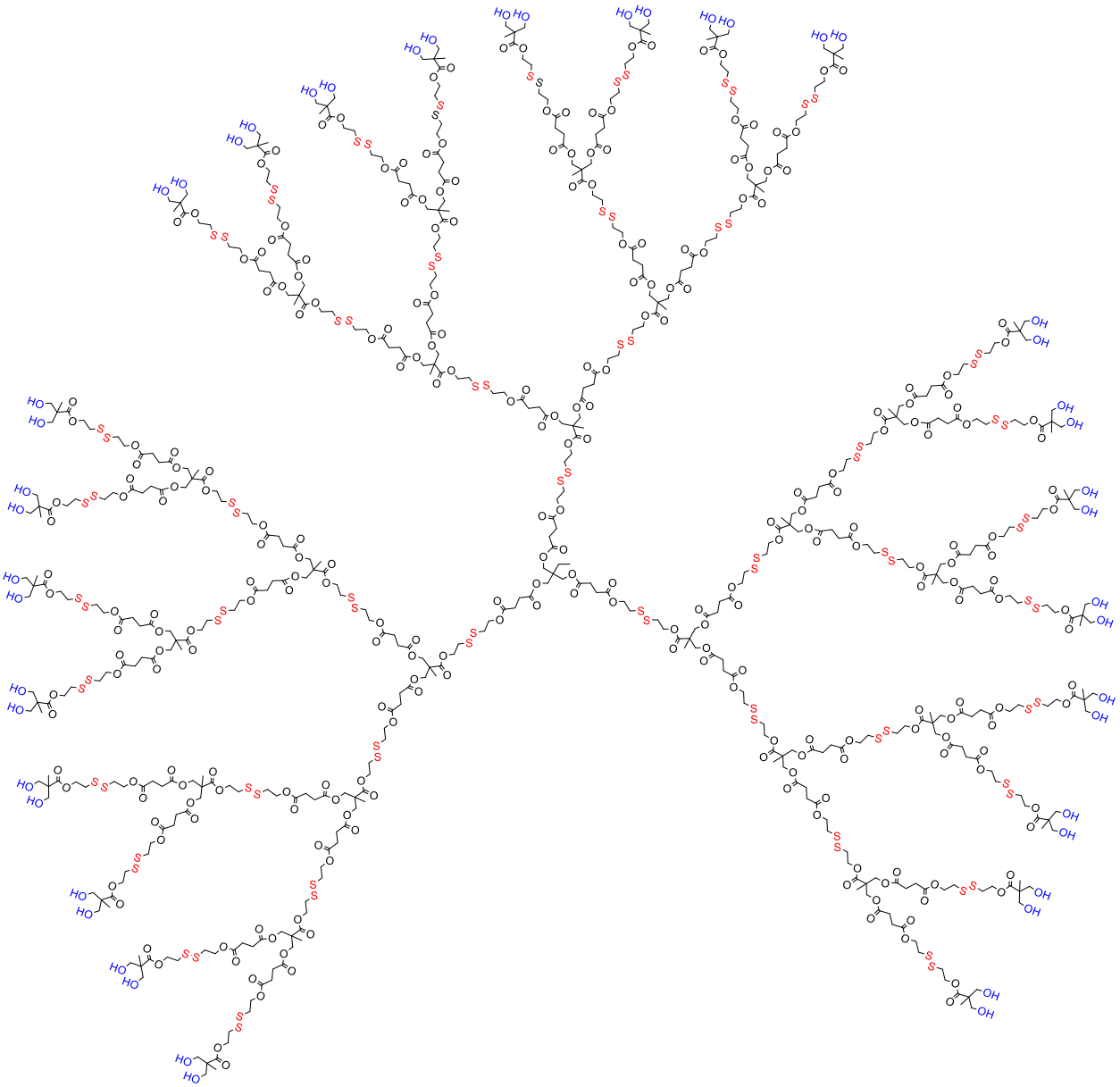
TMP-(S-S)$_{45}$-G4-(Acet)$_{24}$ (12)



In a round bottom flask with a magnetic stir bar, **11** (1.33 g, 0.17 mmol) were solubilized in pyridine (1.02 ml, 12.7 mmol) and 50 ml DCM. DMAP (103 mg, 0.85 mmol) was added and the reaction vessel was cooled to zero degrees Celsius. **5** (4.78 g, 4.96 mmol) dissolved in DCM was added drop wise using a dripping funnel and the reaction left overnight. Completion was confirmed using MALDI-TOF-MS (HABA:DCTB, 1:1) and residual anhydride quenched by the addition of 15 ml of deionized water. The organic phase was diluted with DCM and washed five times with a 10 w% aqueous solution of $NaHSO_4$, dried with $MgSO_4$, filtered and the solvent evaporated. Resulting orange waxy solid was absorbed onto silica and purified using column chromatography. A column with a diameter of 5 cm was used with an effective length of 20 cm. A heptane:EtOAc.MeOH solvent system was used and product eluted at 90:10

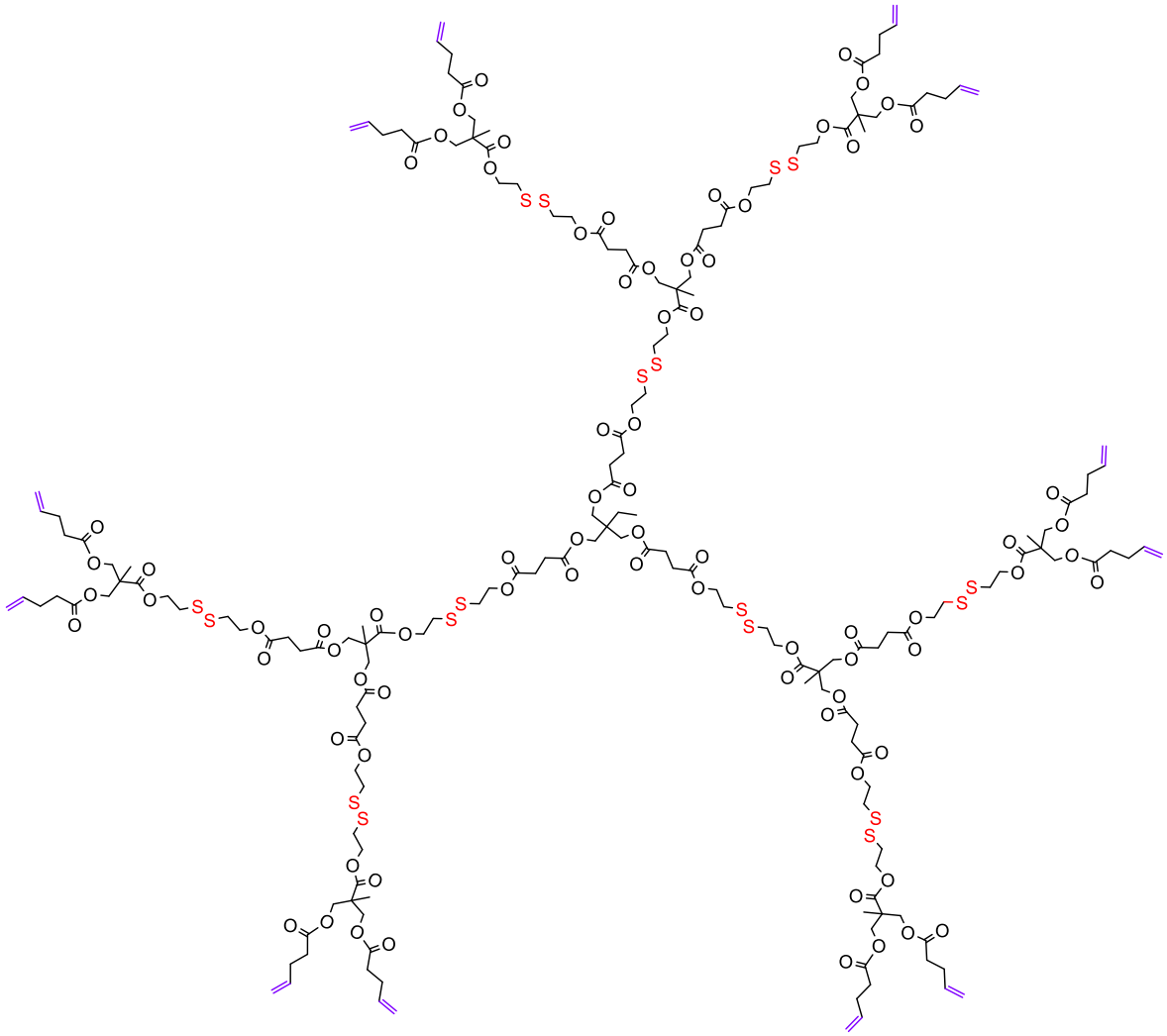
EtOAc:MeOH. Solvent was evaporated and product collected as a colorless liquid (1.75 g, 58.0 %). MALDI calc [M+Na^+] = 16960.8 Da, Obtained [M+Na^+] = 16968.0 Da. M_w (theoretical) = 16977.7 g mol^{-1}, SEC (THF) M_n 14883 g mol^{-1}, Đ = 1.128. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.40 (q, J = 6.3 Hz, 90H, -CH_2-C**H₂**-COO(disulfide)), 4.34 (t, J = 6.6 Hz ,90H, -COO-C**H₂**-CH_2-(disulfide)), 4.25 (dd, *J* = 27.6, 16.6 Hz, 84H, -C-C**H₂**-O-(Bis-MPA dendritic)), 4.19 (d, J = 11.7 Hz, 48H, -C-C**H₂**-O-(Bis-MPA terminal)), 4.04 (s, 6H, -C-(C**H₂**)$_3$-COO-(TMP)), 3.64 (d, J = 11.8 Hz 48H, -C-C**H₂**-O-(Bis-MPA terminal)), 2.96- 2.91 (m, 180H, -CH_2-C**H₂**-S-S-C**H₂**-CH_2-(disulfide)), 2.63 (s, 180H, COO-C**H₂**-C**H**$_2$-COO-(succinic)), 1.40 (d, J = 17.9 Hz, 144H,-C-(C**H₃**)$_2$(bis-MPA acetonide)), 1.26 (s, 63H,-C-C**H₃**(bis-MPA dendritic)), 1.20 (s, 72H,-C-C**H₃**(bis-MPA terminal)), 0.88 (t, J = 7.5 Hz, 3H, $(CH_2)_3$-C-C**H₃** (TMP)).

TMP-(S-S)$_{45}$-G4-(OH)$_{48}$ (13)



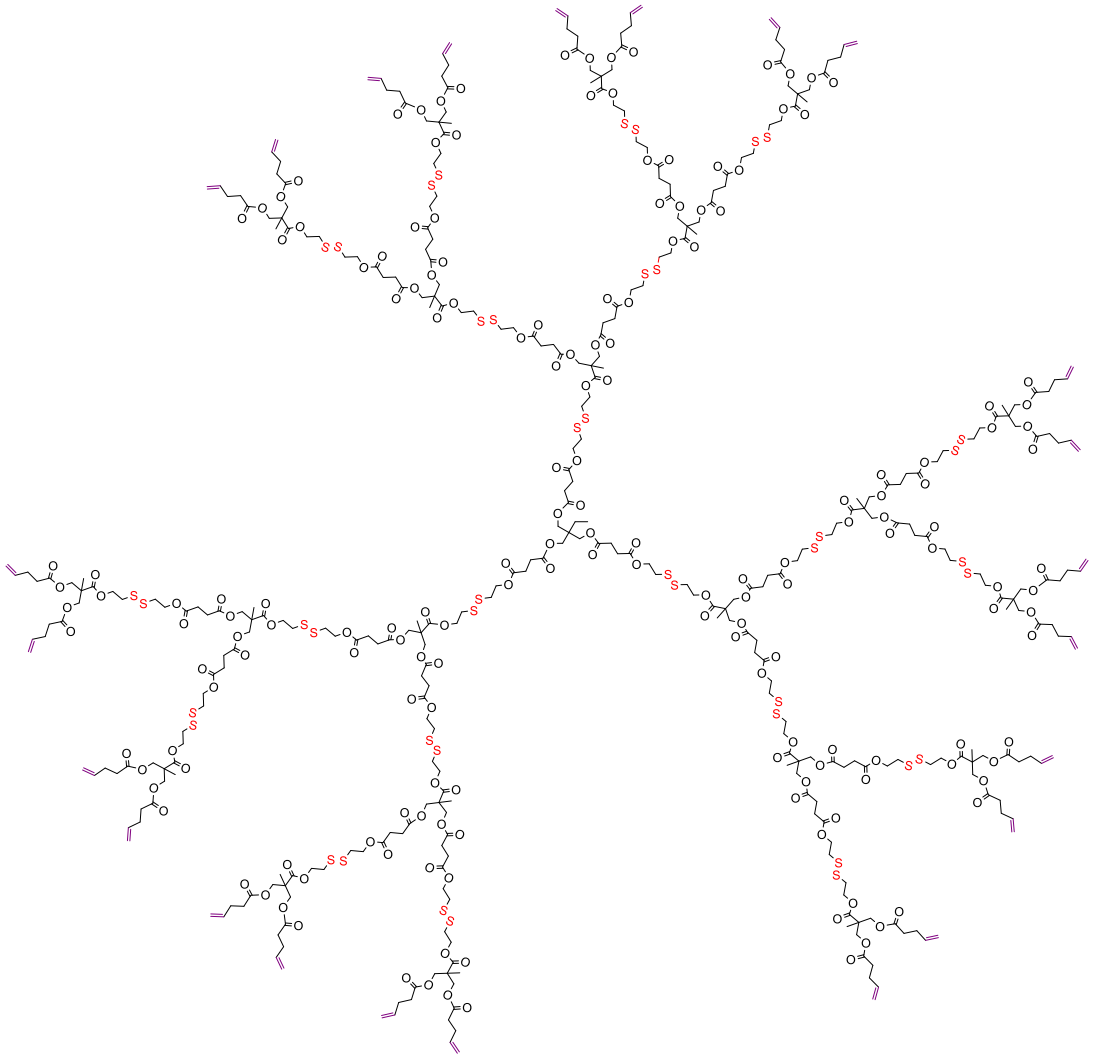
In a round bottom flask equipped with a magnetic stir bar **12** (1.55 g, 0.09 mmol) was dissolved in a mixture 60:40 by volume DCM:MeOH, Dowex™ (1.6 g) was added and reaction was allowed to proceed one hour. Solvent was evaporated and product collected as lightly yellow viscous liquid (1.00 g, 68.4 %). MALDI calc [M+Na^+] = 16000,0 Da, Obtained [M+Na^+] = 16005.2 Da. M_w (theoretical) = 16016.2 mol^{-1}, SEC (THF) M_n 14441.1 g mol^{-1}, Đ = 1.047. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.42 (m, 90H, -CH_2-C**H₂**-COO(disulfide)), 4.35 (t, J = 6.6 Hz ,90H, -COO-C**H₂**-CH_2-(disulfide)), 4.26 (dd, *J* = 28.7, 16.6 Hz, 84H, -C-C**H₂**-O-(Bis-MPA dendritic)), 4.04 (s, 6H, -C-(C**H₂**)$_3$-COO-(TMP)) , 3.73 (d, J = 8.4 Hz, 48H, -C-C**H₂**-O-(Bis-MPA terminal)), 3.72 (d, J = 11.1 Hz 48H, -C-C**H₂**-O-(Bis-MPA terminal)), 2.94- 2.91 (m, 180H, -CH_2-C**H₂**-S-S-C**H₂**-CH_2-(disulfide)), 2.64 (s, 180H, COO-C**H₂**-C**H₂**-COO-(succinic)), 1.48 (q, J = 7.38, 2H, CH_3-C**H₂**-C-(TMP)) 1.26 (s, 63H,-C-C**H₃**(bis-MPA dendritic)), 1.10 (s, 72H,-C-C**H₃**(bis-MPA terminal)), 0.88 (m, 3H, $(CH_2)_3$-C-C**H₃** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.68, 172.14, 171.89, 67.92, 65.65, 62.94, 62.71, 62.47, 49.58, 46.50, 37.31, 37.24, 37.14, 36.94, 29.03, 17.95, 17.33.

TMP-(S-S)$_{21}$-G2-(Allyl)$_{24}$ (14)



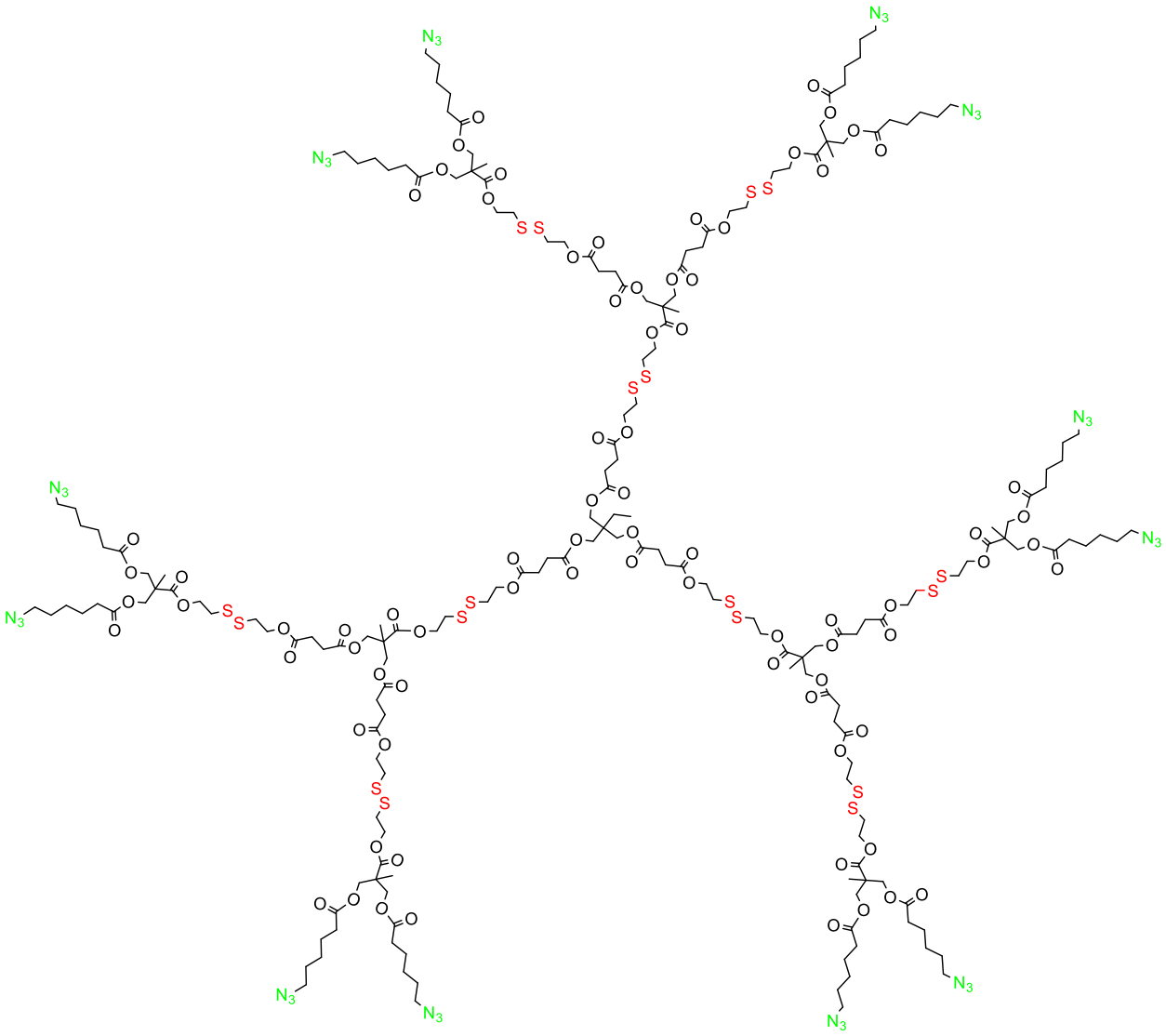
In a round bottom flask quipped with a magnetic stir bar **9** (100 mg, 30,2 µmol) was dissolved in 600µl DCM. DMAP (8.90 mg, 72.9 µmol) and pyridine (88.0 µl, 1,09 mmol) was added. The reaction was cooled to 0 °C in an ice bath and 4-penthanoic anhydride (100 µL, 544 µmol) was added to the reaction. The reaction was allowed to proceed overnight and was monitored using MALDI-TOF-Ms. Upon completion residual anhydride was quenched by the addition of 500 µl of deionized water. The organic phase was diluted with DCM to a volume of 10 ml and washed tree times with a 10 w% aqueous solution of $NaHSO_4$ followed by three times with a 10 w% aqueous solution of $NaHCO_3$, dried with MgSO4, filtered and the solvent evaporated. The resulting yellow viscous solid was dissolved in 1 mL of DCM and adsorbed onto a 1 g silica samplet and subsequently purified by column chromatography using Biotage Isolera 4 automatic column system in EtOAc: Heptane. A SNAP Ultra 10g column was used at a flow rate of 36 mL/min, a linear gradient from 1:0 to 1:1 Heptane EtOAC was run over 7 column volumes (CV) followed by from 1:1 to 0.1 Heptane:EtoAc over 2 column volumes with a final length of 1 CV at 0:1. Subequently from 1:0 to 0.8:0.2 EtoAc: MeOH over 1.5 CV followed by static 0.8:0.2 EtoAc:MeOH for 2.5 CV. Product was attained as a clear viscous liquid (116,65 mg, 75.1%). (MALDI calc $[M+Na^+]$ = 4310.17 Da, Obtained $[M+Na^+]$ = 4311.5 Da. M_w (theoretical) = 9544.5 g mol^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 5.82 (ddt, *J* = 16.2, 10.2, 6.1 Hz, 12H, CH_2=C**H**-CH_2-(Allyl)), 5.14 – 4.93 (m, 22H, C**H**$_2$=CH-CH_2-(Allyl)),), 4.49 – 4.14 (m, 72H, -CH_2-C**H$_2$**-COO (disulfide), -COO-C**H$_2$**-CH_2-(disulfide), -C-C**H$_2$**-O-(Bis-MPA)), 4.06 (s, 6H, -C-(C**H$_2$**)$_3$-COO-(TMP), 2.94 (tt, *J* = 6.5, 2.0 Hz, 35H, -CH_2-C**H$_2$**-S-S-C**H$_2$**-CH_2-(disulfide)), 2.66 (d, *J* = 3.0 Hz, 36H, COO-C**H$_2$**-C**H**$_2$-COO-(succinic)), 2.49 – 2.31 (m, 46H, C**H**$_2$-C**H**$_2$-CH=CH_2), 1.62 (s, 10H,-C-C**H$_3$**(bis-MPA)), 1.50 (q, *J* = 7.5 Hz, 2H, $(CH_2)_3$-C-C**H$_2$**-O- (TMP)).), 1.28 (s, 28H,-C-C**H$_3$**(bis-MPA)), 0.90 (t, *J* = 7.5 Hz, 3H, $(CH_2)_3$-C-C**H$_3$** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.68, 172.59, 172.07, 172.02, 171.77, 136.58, 115.79, 65.61, 65.41, 62.85, 62.68, 46.48, 37.16, 37.02, 36.94, 33.47, 28.98, 28.86, 17.99, 17.93.

TMP-(S-S)$_{21}$-G3-(Allyl)$_{24}$ (15)



In a round bottom flask quipped with a magnetic stir bar **11** (100 mg, 13,3 µmol) was dissolved in 400µl DCM. DMAP (7.8 mg, 68.7 µmol) and pyridine (77.1 µl, 955µmol) was added. The reaction was cooled to 0 °C in an ice bath and 4-penthanoic anhydride (81.5 µl, 446 µmol) was added to the reaction. The reaction was allowed to proceed overnight and was monitored using MALDI-TOF-Ms. Upon completion residual anhydride was quenched by the addition of 500 µl of deionized water. The organic phase was diluted with DCM to a volume of 10 ml and washed tree times with a 10 w% aqueous solution of $NaHSO_4$ followed by three times with a 10 w% aqueous solution of $NaHCO_3$, dried with MgSO4, filtered and the solvent evaporated. Product was attained as a lightly yellow viscous liquid (120 mg, 95.3%). (MALDI calc [M+K$^+$] = 9535.4 Da, Obtained [M+Na$^+$] = 9530.1 Da. M_w (theoretical) = 9544.5 g mol^{-1}, SEC (THF) M_n 9842 g mol^{-1}, Đ = 1.091. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 5.80 (ddt, *J* = 16.5, 10.3, 5.9 Hz, 24H, CH_2=C**H**-CH_2-(Allyl)), 5.10 – 4.96 (m, 48H, C**H$_2$**=CH-CH_2-(Allyl)), 4.32 (m, J = 6.3 Hz, 168H, -CH_2-C**H$_2$**-COO(disulfide), -COO-C**H$_2$**-CH_2-(disulfide), -C-C**H$_2$**-O-(Bis-MPA)), 4.03 (s, 6H, -C-(C**H$_2$**)$_3$-COO-(TMP), 2.94- 2.89 (m, 84H, -CH_2-C**H$_2$**-S-S-C**H$_2$**-CH_2-(disulfide)), 2.63 (s, 84H, COO-C**H$_2$**-C**H$_2$**-COO-(succinic)), 2.46 – 2.29 (m, 96H, C**H$_2$**-C**H$_2$**-CH=CH_2), 1.25 (s, 27H,-C-C**H$_3$**(bis-MPA)), 0.88 (t, J = 7.6 Hz, 3H, $(CH_2)_3$-C-C**H$_3$** (TMP)).

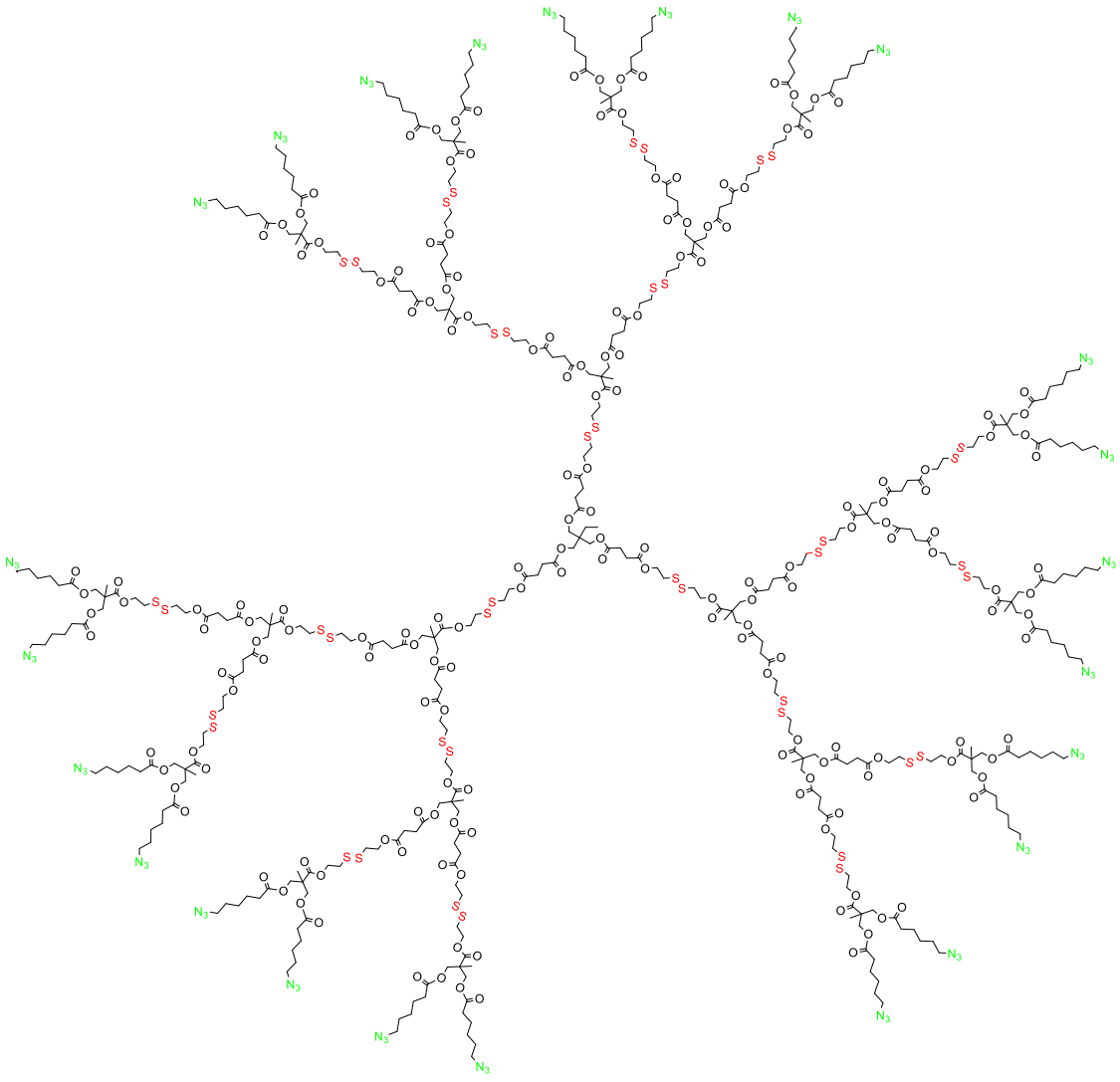
TMP-(S-S)$_{21}$-G2-(Azide)$_{24}$ (16)



In a round bottom flask quipped with a magnetic stir bar **9** (100 mg, 30,2 µmol) was dissolved in 600µl DCM. DMAP (8.90 mg, 72.9 µmol) and pyridine (88.0 µl, 1,09 mmol) was added. The reaction was cooled to 0 °C in an ice bath and 6-Azidohexanoic anhydride (161,32 mg, 544 µmol) was added to the reaction. The reaction was allowed to proceed overnight and was monitored using MALDI-TOF-Ms. Upon completion residual anhydride were quenched by the addition of 500 µl of deionized water. The organic phase was diluted with DCM to a total volume of 10 ml and washed tree times with a 10 w% aqueous solution of $NaHSO_4$ followed by three times with a 10 w% aqueous solution of $NaHCO_3$, dried with MgSO4, filtered and the solvent evaporated. The resulting yellow viscous solid was dissolved in 1 mL of DCM and adsorbed onto a 1 g silica samplet and subsequently purified by column chromatography using Biotage Isolera 4 automatic column system in EtOAc: Heptane. A SNAP Ultra 10g column was used at a flow rate of 36 mL/min, a linear gradient from 1:0 to 1:1 Heptane EtOAC was run over 7 column volumes

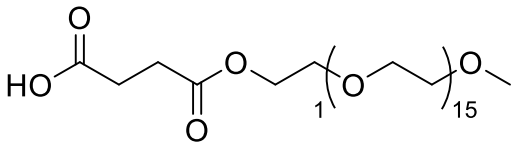
(CV) followed by from 1:1 to 0.1 Heptane:EtoAc over 2 column volumes with a final length of 1 CV at 0:1. Subequently from 1:0 to 0.8:0.2 EtoAc: MeOH over 1.5 CV followed by static 0.8:0.2 EtoAc:MeOH for 2.5 CV. The progression was monitored at 254 and 280 nm with a collection threshold of 10 mAU. Product was attained as a transparent viscous liquid (115 mg, 76.0 %). (MALDI calc [M-3N+Na$^+$] = 4955.6 Da, Obtained [M-3N+nnNa$^+$] = 4955.3 Da ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 (dt, *J* = 18.3, 6.5 Hz, 36H, -CH_2-C**H$_2$**-COO(disulfide), -COO-C**H$_2$**-CH_2-(disulfide)), 4.32 – 4.20 (m, 34H, -C-C**H$_2$**-O-(Bis-MPA)), 4.06 (s, 6H, -C-(C**H$_2$**)$_3$-COO-(TMP), 3.30 (t, *J* = 6.8 Hz, 23H, -C**H$_2$**-CH_2-CH_2-N_3 (azide)), 2.94 (td, *J* = 6.5, 2.4 Hz, 36H, -CH_2-C**H$_2$**-S-S-C**H$_2$**-CH_2-(disulfide)), 2.66 (d, *J* = 2.7 Hz, 36H, COO-C**H$_2$**-C**H$_2$**-COO-(succinic)), 2.35 (t, *J* = 7.4 Hz, 22H COO-C**H$_2$**-C**H$_2$**-(azide)), 1.48 – 1.35 (m, 23H-CH_2-C**H$_2$**-N_3 (azide)), 1.28 (s, 30H-C-C**H$_3$**(bis-MPA)), 0.91 (t, *J* = 7.6 Hz, 3H, $(CH_2)_3$-C-C**H$_3$** (TMP)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.98, 172.03, 171.78, 170.64, 169.62, 65.35, 62.83, 62.69, 51.35, 46.53, 37.16, 37.07, 34.01, 28.99, 28.69, 26.35, 24.51, 17.97.

TMP-(S-S)$_{21}$-G3-(Azide)$_{24}$ (17)



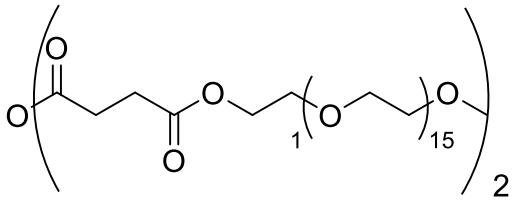
In a round bottom flask quipped with a magnetic stir bar **11** (100 mg, 13,3 µmol) was dissolved in 400µl DCM. DMAP (7.8 mg, 68.7 µmol) and pyridine (77.1 µl, 955µmol) was added. The reaction was cooled to 0 °C in an ice bath and 6-Azidohexanoic anhydride (120 mg, 446 µmol) was added to the reaction. The reaction was allowed to proceed overnight and was monitored using MALDI-TOF-Ms. Upon completion residual anhydride were quenched by the addition of 500 µl of deionized water. The organic phase was diluted with DCM to a total volume of 10 ml and washed tree times with a 10 w% aqueous solution of $NaHSO_4$ followed by three times with a 10 w% aqueous solution of $NaHCO_3$, dried with MgSO4, filtered and the solvent evaporated. Product was attained as a lightly yellow viscous liquid (137 mg, 95.5%). M_w (theoretical) = 10874.7 mol^{-1}, SEC (THF) M_n 10232 g mol^{-1}, Đ = 1.136. ^{1}H-NMR (400 MHz, $CDCl_3$): **δ**(ppm) 4.37 (dt, *J* = 18.5, 6.6 Hz, 85H, -CH_2-C**H_2**-COO(disulfide), -COO-C**H_2**-CH_2-(disulfide)), 4.31 – 4.17 (m, 84H, -C-C**H_2**-O-(Bis-MPA)), 4.04 (s, 6H, -C-(C**H_2**)$_3$-COO-(TMP), 3.28 (t, *J* = 6.8 Hz, 48H, -C**H_2**-CH_2-CH_2-N_3 (azide)), 2.93 (t, J = 6.6 Hz, 84H, -CH_2-C**H_2**-S-S-C**H_2**-CH_2-(disulfide)), 2.64 (s, 84H, COO-C**H_2**-C**H_2**-COO-(succinic)), 2.33 (t, *J* = 7.4 Hz, 96H, COO-C**H_2**-C**H_2**-(azide)), 1.40 (ttd, *J* = 10.1, 7.0, 6.5, 3.5 Hz, 48H - --CH_2-C**H_2**-N_3 (azide)), 1.26 (s, 27H,-C-C**H_3**(bis-MPA)), 0.88 (t, J = 7.2 Hz, 3H, $(CH_2)_3$-C-C**H_3** (TMP)).

MM-PEG750-COOH (18)



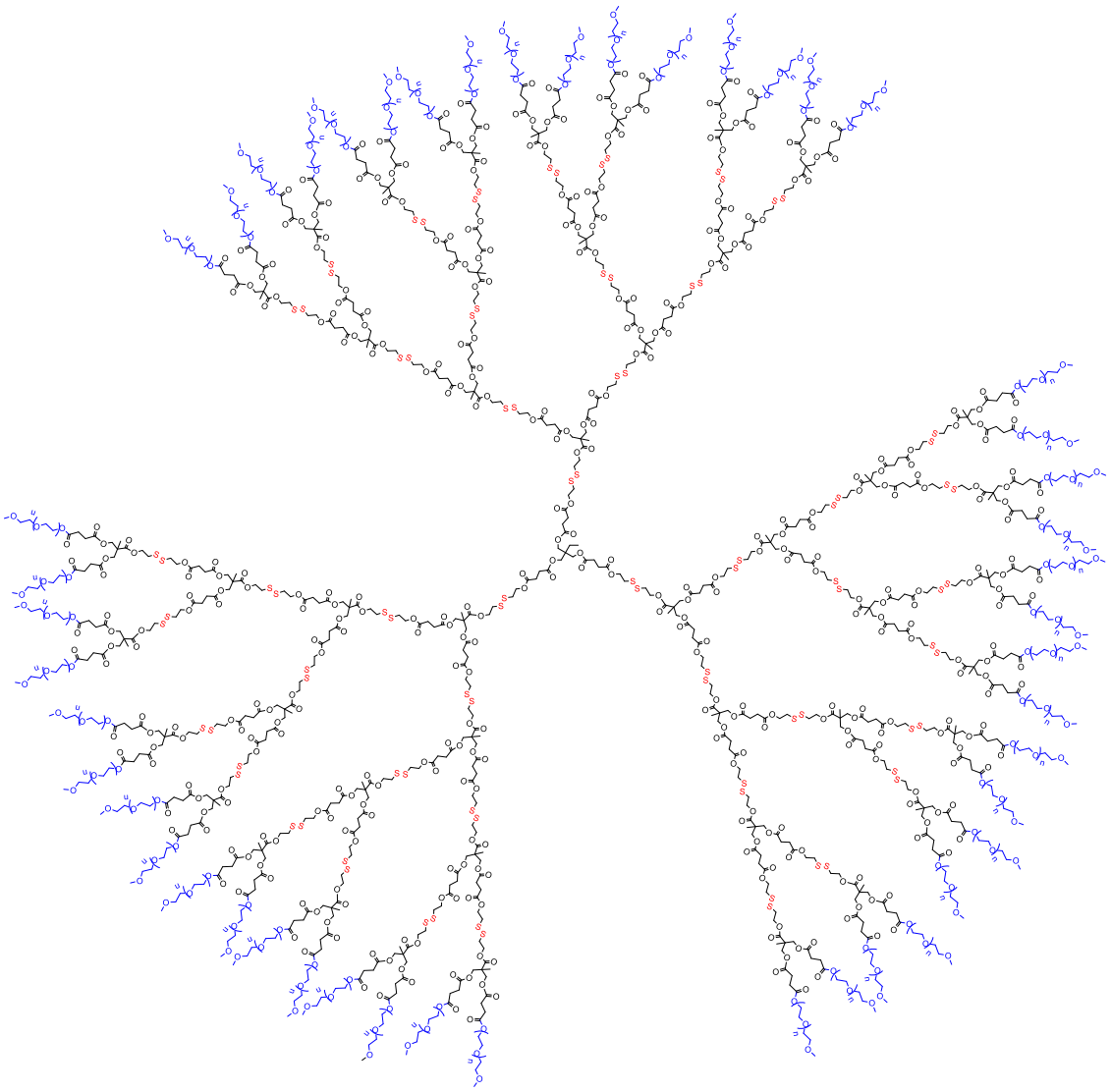
MM-PEG750-OH (15 g, 20.0 mmol) was dissolved in pyridine (4.84 mL, 60.0 mmol) and DCM (10 mL) in a round bottom flask equipped with magnetic stir bar. DMAP (489 mg,4.00 mmol) was added and the reaction mixture cooled to 0°C. Succinic anhydride (3.00 g, 30.0 mmol) was added as a powder and the reaction was stirred vigorously for 12 hours. Water (2 mL) was added and the reaction allowed to stir for 6 hours. Subsequently the reaction mixture was transferred and diluted to 200 mL in to a separator funnel. The organic phase was washed trice with 10 wt% aqueous $NaHSO_4$, dried with $MgSO_4$ and evaporated to dryness. the resulting viscous liquid with diluted with 5 mL DCM and precipitated twice in cold di ethyl ether (300 mL). The product was collected as a transparent waxy solid. (13.7 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 – 4.09 (m, 3H), 3.58 (d, *J* = 4.8 Hz, 80H), 3.48 (dd, *J* = 5.9, 3.5 Hz, 3H), 3.31 (s, 3H), 2.57 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.63, 172.10, 71.84, 70.55, 70.51, 70.47, 70.40, 68.93, 63.75, 58.94, 29.14, 28.80.

MM-PEG750-Anhydride (19)



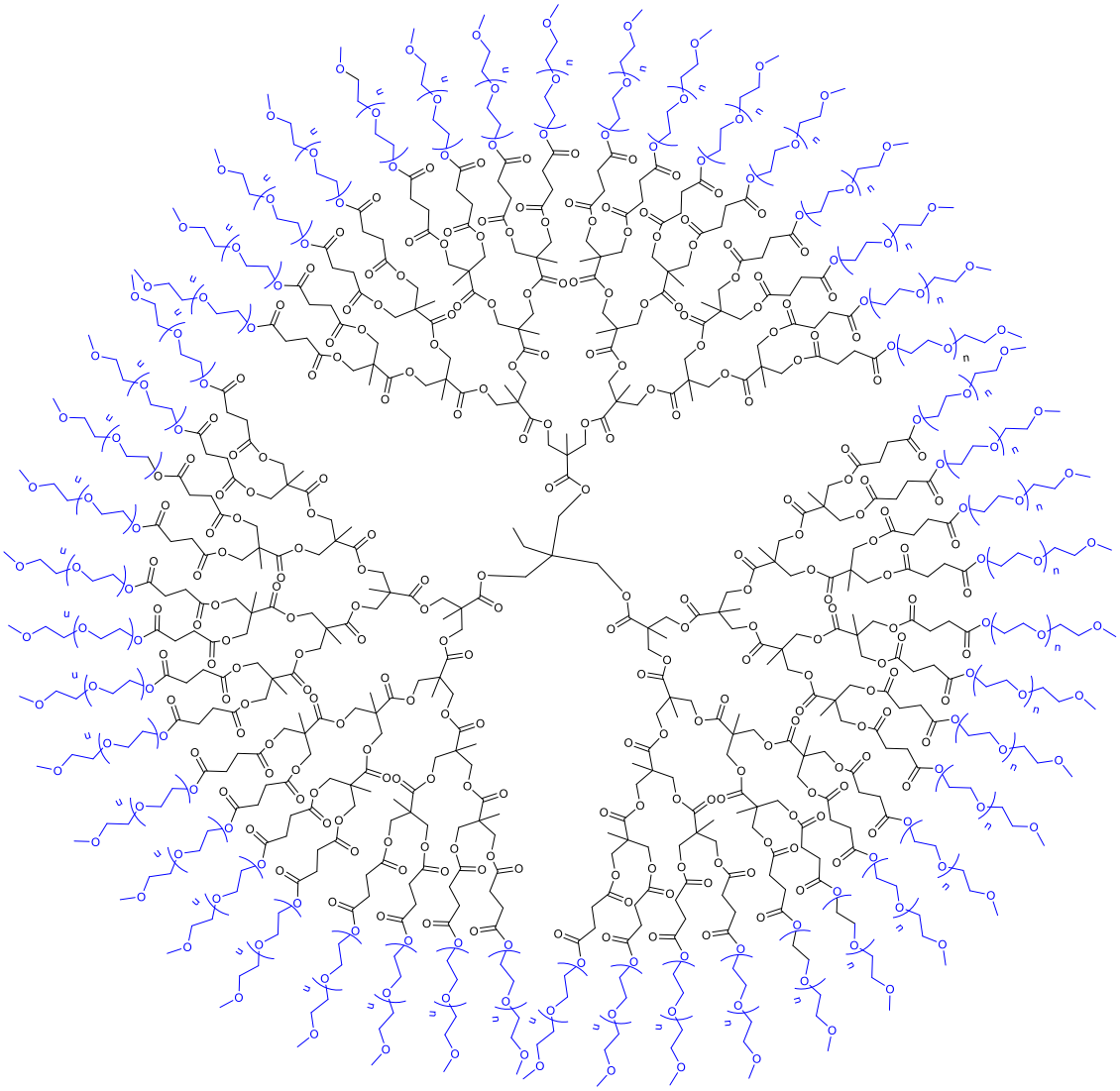
MM-PEG750-COOH (2.5 g, 2.82 mmol) was dissolved in DCM (10 mL) in a round bottom flask equipped with magnetic stir bar. The reaction mixture cooled to 0°C and DCC (300 mg,1.41 mmol) was added. The reaction was stirred vigorously for 12 hours. The crude reaction mixture was filtered through a pour 4 solid filter and the filtrate collected and evaporated to dryness. The resulting viscous liquid with diluted with 5 mL DCM and precipitated twice in cold di ethyl ether (300 mL). The product was collected as a transparent waxy solid. (2.28 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$)) δ 4.21 – 4.14 (m, 4H), 3.65 – 3.61 (m, 3H), 3.57 (d, *J* = 2.1 Hz, 112H), 3.50 – 3.45 (m, 4H), 3.30 (s, 6H), 2.72 (td, *J* = 6.5, 5.9, 1.1 Hz, 3H), 2.65 – 2.60 (m, 3H), 2.56 (td, *J* = 4.7, 1.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.13, 171.55, 167.78, 71.84, 70.56, 70.52, 70.48, 70.42, 68.92, 68.89, 65.75, 64.00, 63.70, 58.94, 53.49, 30.09, 29.27, 28.34, 15.21.

TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$ (20)



TMP-$(S\text{-}S)_{45}$-G4-$(OH)_{48}$ (**13**) (50.0 mg, 2.95 µmol) was dissolved in pyridine (33.0 µL, 0.41 mmol) and $CHCl_3$ (600 µL) in a round bottom flask equipped with magnetic stir bar. DMAP (3.46 mg, 28,3 µmol) was added and the reaction mixture was cooled to 0°C. MM-PEG750-Anhydride (476 m, 283 µmol) was slowly added subsequently the reaction was allowed to proceed for 12 hours under vigorous stirring. Water (200µL) was added and the reaction vigorously stirred for 6 hours. The crude reaction mixture was deposited in a Spetra/por 4 dialysis membrane with a molecular weight cut off 12-14 kDa and dialyzed against 1 L of Milli-Q water with hourly exchanges over 6 hours. The contents of the membrane were lyophilized and dissolved in minimal amount of EtOH and eluted in EtOH on a Sephadex LH-20 column (L: 60 cm D: 2 cm). The product was collected as a slightly yellow waxy solid (149 mg, 88%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.44 – 4.30 (m, 110H), 4.31 – 4.18 (m, 257H), 4.12 (q, *J* = 7.1 Hz, 122H), 3.64 (d, *J* = 1.8 Hz, 3723H), 3.58 – 3.51 (m, 153H), 3.38 (s, 198H), 2.92 (t, *J* = 6.7 Hz, 121H), 2.71 – 2.57 (m, 372H), 1.30 – 1.21 (m, 329H), 0.92 – 0.83 (m, 4H). $Mw_{\text{Theoretical}}$= 56938.77 g mol^{-1}, $Mw_{\text{DMF-SEC}}$ = 20675 g mol^{-1}, Đ = 1.158.

MP-G4-(PEG750-MM)$_{48}$ (21)



TMP-G4-(OH)$_{48}$ (50.0 mg, 9.33 µmol) was dissolved in pyridine (108 µL, 895 µmol) and $CHCl_3$ (800 µL) in a round bottom flask equipped with magnetic stir bar. DMAP (10.9 mg, 89,6 µmol) was added and the reaction mixture was cooled to 0°C. MM-PEG750-Anhydride (1.51 g, 895 µmol) was slowly added subsequently the reaction was allowed to proceed for 12 hours under vigorous stirring. Water (400µL) was added and the reaction vigorously stirred for 6 hours. The crude reaction mixture was deposited in a Spectra/por 4 dialysis membrane with a molecular weight cut off 12-14 kDa and dialyzed against 1 L of Milli-Q water with hourly exchanges over 6 hours. The contents of the membrane were lyophilized and dissolved in minimal amount of EtOH and eluted in EtOH on a Sephadex LH-20 column (L: 60 cm D: 2 cm). The product was collected as a slightly yellow waxy solid (387 mg, 91%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 4.11 (m, 379H), 3.63 (d, *J* = 2.0 Hz, 3638H), 3.58 – 3.50 (m, 108H), 3.37 (s, 144H), 2.79 – 2.48 (m, 203H), 1.22 (d, *J* = 14.0 Hz, 133H), 0.94 – 0.76 (m, 5H). Mw $_{Theoretical}$= 45343.4 g mol^{-1}, Mw $_{DMF\text{-}SEC}$ = 19728 g mol^{-1}, Đ = 1.016.

General Fragmentation procedure using DTT

Disulfide containing dendrimer was dissolved in $CHCl_3$, in a round bottom flask equipped with magnetic stirrbar. DTT (two equivalents for every disulfide in the dendrimer) was added along with 1.1 equivalents of TEA per DTT. The reaction mixture was frozen in liquid nitrogen and allowed to thaw under high vacuum, upon melting the reaction vessel was flushed with argon for three minutes. The reaction was allowed to proceed for fifteen minutes whereupon it was characterized with ^{1}H-NMR and MALDI-TOF-MS.

Fragmentation TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)

Disulfide containing dendrimer **10** (150 mg, 452 µmol) was dissolved in $CHCl_3$ (1 ml) in a small vial equipped with magnetic stir bar. DTT (125 mg 8.12 µmol) and TEA (125.5 µL, 902 µmol) was added to the vial, the vial was sealed and frozen in liquid nitrogen and allowed to thaw under high vacuum, upon melting the vessel was flushed with argon for three minutes and subsequently sealed. The reaction was allowed to proceed for fifteen minutes after which it was exposed to the atmosphere and analyzed with ^{1}H-NMR and MALDI-TOF-MS. The crude reaction mixture was evaporated and resulting yellow viscous solid was dissolved in 1 mL of DCM and adsorbed onto a 1 g silica samplet and subsequently purified by column chromatography using Biotage Isolera 4 automatic column system in EtOAc: Heptane. A SNAP Ultra 10g column was used at a flow rate of 36 mL/min, a linear gradient from 1:0 to 0:1 Heptane EtOAC was run over 15 column volumes (CV) with a final length of 2 CV at 0:1. The progression was monitored at 254 and 280 nm with a collection threshold of 20 mAU. Products: 2-mercaptoethyl 2,2,5-trimethyl-1,3-dioxane-5-carboxylate (**DB$_2$**), O,O'-(2-((2-mercaptoethoxy)carbonyl)-2-methylpropane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**C$_3$**), O,O'-(2-ethyl-2-(((4-(2-mercaptoethoxy)-4-oxobutanoyl)oxy)methyl)propane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**CD$_2$**) where collected in that order as transparent oils. C_3 (6,47 mg, 66%), CD_2 (37.2 mg, 50%) and BC_2 (27.4 mg, 61 %). **C$_3$:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (t, *J* = 6.6 Hz, 6H), 4.04 (s, 6H), 2.75 (dt, *J* = 8.5, 6.6 Hz, 6H), 2.64 (d, *J* = 1.5 Hz, 13H), 1.57 – 1.42 (m, 5H), 0.88 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.01, 66.15, 64.15, 40.90, 29.10, 29.04, 23.36, 23.08, 7.51. **DB$_{2\ acet}$:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 4.23 (m, 6H), 4.21 (t, *J* = 6.7 Hz, 4H), 2.80 – 2.71 (m, 6H), 2.64 (s, 8H), 1.51 (t, *J* = 8.5 Hz, 2H), 1.48 (t, *J* = 8.5 Hz, 1H), 1.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.44, 171.93, 171.79, 66.48, 66.15, 65.67, 46.54, 29.04, 28.99, 28.84, 23.34, 23.26, 17.96. **CD$_2$:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (t, *J* = 6.5 Hz, 2H), 4.24 – 4.14 (m, 2H), 3.68 – 3.61 (m, 2H), 2.78 (dt, *J* = 8.5, 6.5 Hz, 2H), 1.60 (t, *J* = 8.5 Hz, 1H), 1.47 – 1.36 (m, 6H), 1.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.10, 98.29, 66.22, 66.10, 42.17, 25.43, 23.51, 22.17, 18.68.

Fragmentation TMP-(S-S)$_{21}$-G2-(Allyl)$_{24}$ (14)

Disulfide containing dendrimer **14** (97.5 mg, 22.7 µmol) was dissolved in $CHCl_3$ (0.5 ml) in a small vial equipped with magnetic stir bar. DTT (35.0 mg 227 µmol) and TEA (30 µL, 250 µmol) was added to the vial, the vial was sealed and frozen in liquid nitrogen and allowed to thaw under high vacuum, upon melting the vessel was flushed with argon for three minutes and subsequently sealed. The reaction was allowed to proceed for fifteen minutes after which it was exposed to the atmosphere and analyzed with ^{1}H-NMR and MALDI-TOF-MS. The crude reaction mixture was evaporated and resulting yellow viscous solid was dissolved in 1 mL of DCM and adsorbed onto a 1 g silica samplet and subsequently purified by column chromatography using Biotage Isolera 4 automatic column system in EtOAc: Heptane. A SNAP

Ultra 25g column was used at a flow rate of 36 mL/min, a linear gradient from 1:0 to 0:1 Heptane EtOAC was run over 15 column volumes (CV) with a final length of 2 CV at 0:1. The progression was monitored at 254 and 280 nm with a collection threshold of 20 mAU. Products: 2-((2-mercaptoethoxy)carbonyl)-2-methylpropane-1,3-diyl bis(pent-4-enoate) (**DB_2**), O,O'-(2-((2-mercaptoethoxy)carbonyl)-2-methylpropane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**C_3**), O,O'-(2-ethyl-2-(((4-(2-mercaptoethoxy)-4-oxobutanoyl)oxy)methyl)propane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**CD_2**) where collected in that order as transparent oils. C_3 (6.08 mg, 44 %), CD_2 (35.0 mg, 50 %) and BC_2 (48.4 mg, 66 %). **C_3 :** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (t, *J* = 6.6 Hz, 6H), 4.04 (s, 6H), 2.75 (dt, *J* = 8.5, 6.6 Hz, 6H), 2.64 (d, *J* = 1.5 Hz, 13H), 1.57 – 1.42 (m, 5H), 0.88 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.01, 66.15, 64.15, 40.90, 29.10, 29.04, 23.36, 23.08, 7.51. **$DB_{2\ allyl}$:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.4, 10.2, 6.1 Hz, 2H), 5.22 – 4.85 (m, 4H), 4.30 – 4.16 (m, 6H), 2.75 (dt, *J* = 8.5, 6.5 Hz, 2H), 2.52 – 2.37 (m, 4H), 2.35 (tp, *J* = 6.6, 1.5 Hz, 4H), 1.46 (t, *J* = 8.5 Hz, 1H), 1.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.58, 136.52, 115.80, 66.42, 65.46, 46.51, 33.48, 28.84, 23.28, 18.00. **CD_2 :** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (t, *J* = 6.5 Hz, 2H), 4.24 – 4.14 (m, 2H), 3.68 – 3.61 (m, 2H), 2.78 (dt, *J* = 8.5, 6.5 Hz, 2H), 1.60 (t, *J* = 8.5 Hz, 1H), 1.47 – 1.36 (m, 6H), 1.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.10, 98.29, 66.22, 66.10, 42.17, 25.43, 23.51, 22.17, 18.68.

Fragmentation TMP-$(S\text{-}S)_{21}$-G2-$(Azide)_{24}$ (16)

Disulfide containing dendrimer **16** (86.0 mg, 17.3 µmol) was dissolved in $CHCl_3$ (1 ml) in a small vial equipped with magnetic stir bar. DTT (27.0 mg 173 µmol) and TEA (21.0 µL, 190 µmol) was added to the vial, the vial was sealed and frozen in liquid nitrogen and allowed to thaw under high vacuum, upon melting the vessel was flushed with argon for three minutes and subsequently sealed. The reaction was allowed to proceed for fifteen minutes after which it was exposed to the atmosphere and analyzed with ^{1}H-NMR and MALDI-TOF-MS. The crude reaction mixture was evaporated and resulting yellow viscous solid was dissolved in 1 mL of DCM and adsorbed onto a 1 g silica samplet and subsequently purified by column chromatography using Biotage Isolera 4 automatic column system in EtOAc: Heptane. A SNAP Ultra 25g column was used at a flow rate of 36 mL/min, a linear gradient from 1:0 to 0:1 Heptane EtOAC was run over 15 column volumes (CV) with a final length of 2 CV at 0:1. The progression was monitored at 254 and 280 nm with a collection threshold of 20 mAU. Products: 2-(((5-azidopentanoyl)oxy)methyl)-3-(2-mercaptoethoxy)-2-methyl-3-oxopropyl 6-azidohexanoate (**DB_2**), O,O'-(2-((2-mercaptoethoxy)carbonyl)-2-methylpropane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**C_3**), O,O'-(2-ethyl-2-(((4-(2-mercaptoethoxy)-4-oxobutanoyl)oxy)methyl)propane-1,3-diyl) bis(2-mercaptoethyl) disuccinate (**CD_2**) where collected in that order as transparent oils. C_3 (7.07 mg, 66%), CD_2 (38.3 mg, 71%) and BC_2 (47.3 mg, 66 %). **C_3 :** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (t, *J* = 6.6 Hz, 6H), 4.04 (s, 6H), 2.75 (dt, *J* = 8.5, 6.6 Hz, 6H), 2.64 (d, *J* = 1.5 Hz, 13H), 1.57 – 1.42 (m, 5H), 0.88 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.01, 66.15, 64.15, 40.90, 29.10, 29.04, 23.36, 23.08, 7.51. **$DB_{2\ azide}$:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.42 – 4.07 (m, 6H), 3.27 (t, *J* = 6.8 Hz, 4H), 2.75 (dt, *J* = 8.6, 6.5 Hz, 2H), 2.33 (t, *J* = 7.5 Hz, 4H), 1.62 (ddd, *J* = 12.4, 7.8, 6.3 Hz, 8H), 1.47 (t, *J* = 8.5 Hz, 1H), 1.46 – 1.33 (m, 4H), 1.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.98, 172.58, 66.43, 65.41, 51.33, 46.56, 34.00, 28.67, 26.34, 24.49, 23.32, 17.97, 1.16. **CD_2 :** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (t, *J* = 6.5 Hz, 2H), 4.24 – 4.14 (m, 2H), 3.68 – 3.61 (m, 2H), 2.78 (dt, *J* =

8.5, 6.5 Hz, 2H), 1.60 (t, *J* = 8.5 Hz, 1H), 1.47 – 1.36 (m, 6H), 1.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.10, 98.29, 66.22, 66.10, 42.17, 25.43, 23.51, 22.17, 18.68.

Nuclear magnetic resonance (NMR)

Acetonide protected bis-MPA (1)

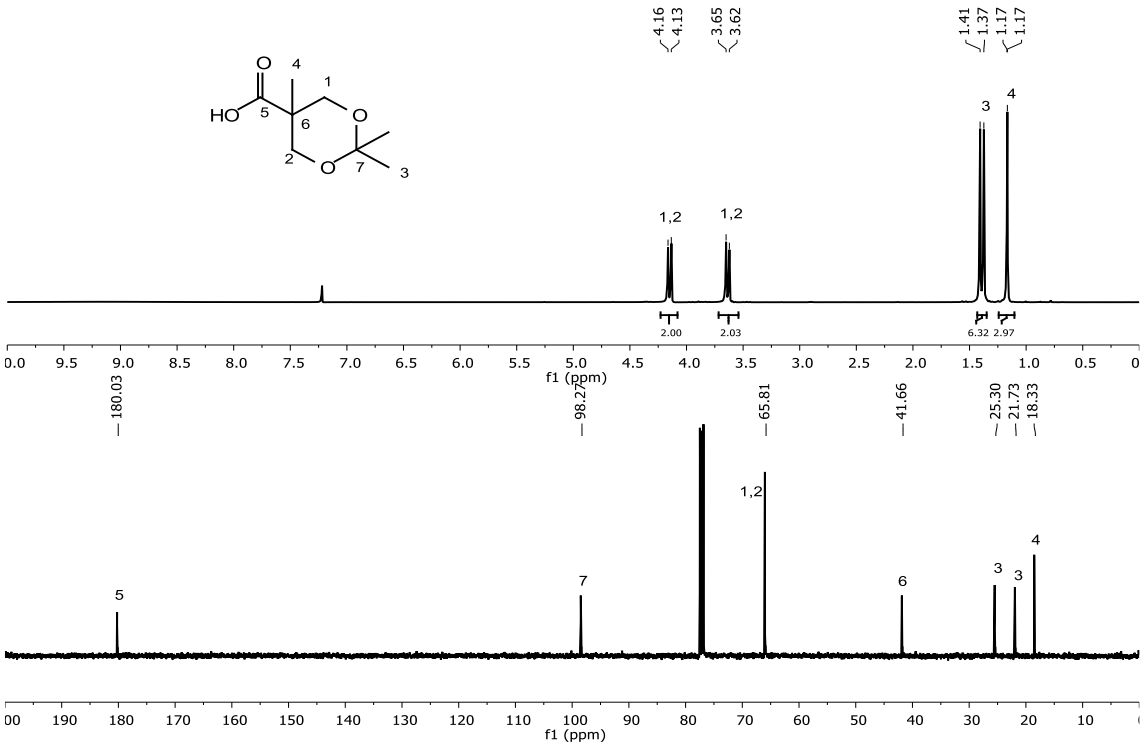


Figure S1 ^{1}H, ^{13}C-NMR of acetonide protected bis-MPA **(1)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

Acetonide protected bis-MPA Anhydride (2)

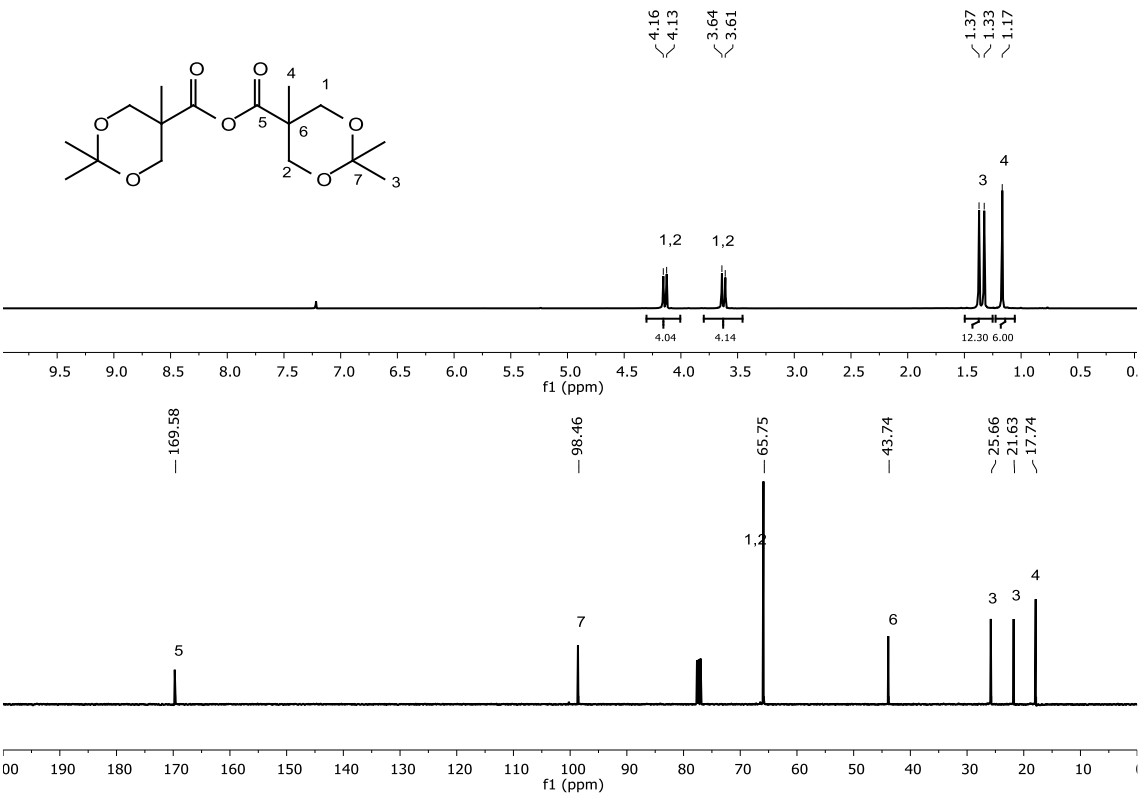


Figure S2 ^{1}H, ^{13}C-NMR of bis-MPA anhydride **(2)** recorded in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

Hydroxyethyl disulfide-protected bis-MPA (3)

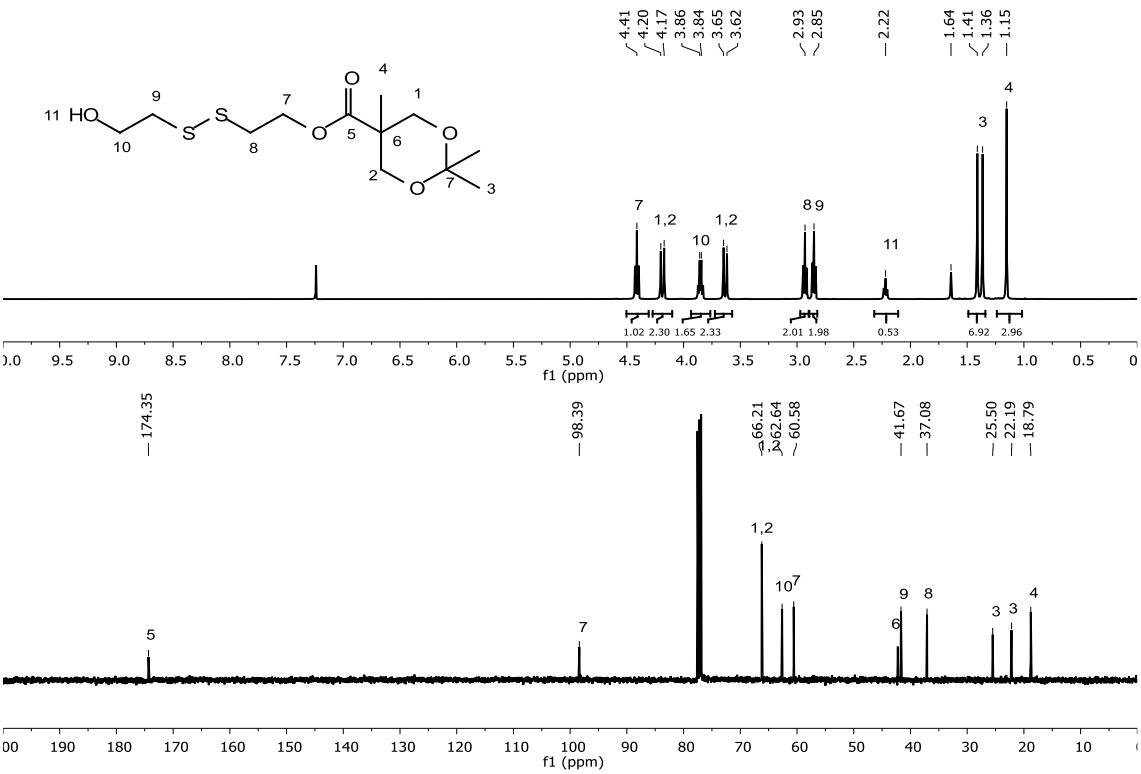


Figure S3 ^{1}H, ^{13}C-NMR of hydroxyethyl disulfide-protected bis-MPA (3) recorded in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

Carboxylic hydroxyethyl disulfide protected bis-MPA (4)

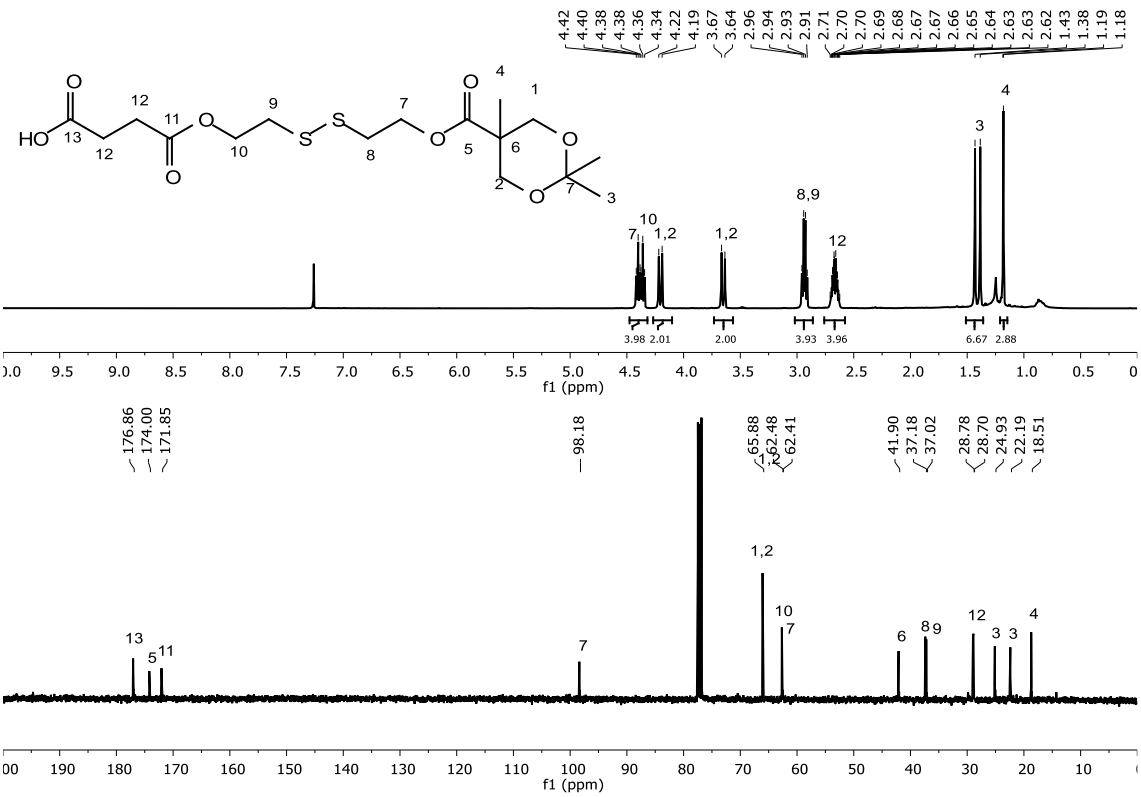


Figure S4 ^{1}H, ^{13}C-NMR of Carboxylic hydroxyethyl disulfide protected bis-MPA **(4)** recorded in $CDCl_3$ recorded at 400 MHz and 101 MHz

Carboxylic hydroxyethyl disulfide protected bis-MPA anhydride (5)

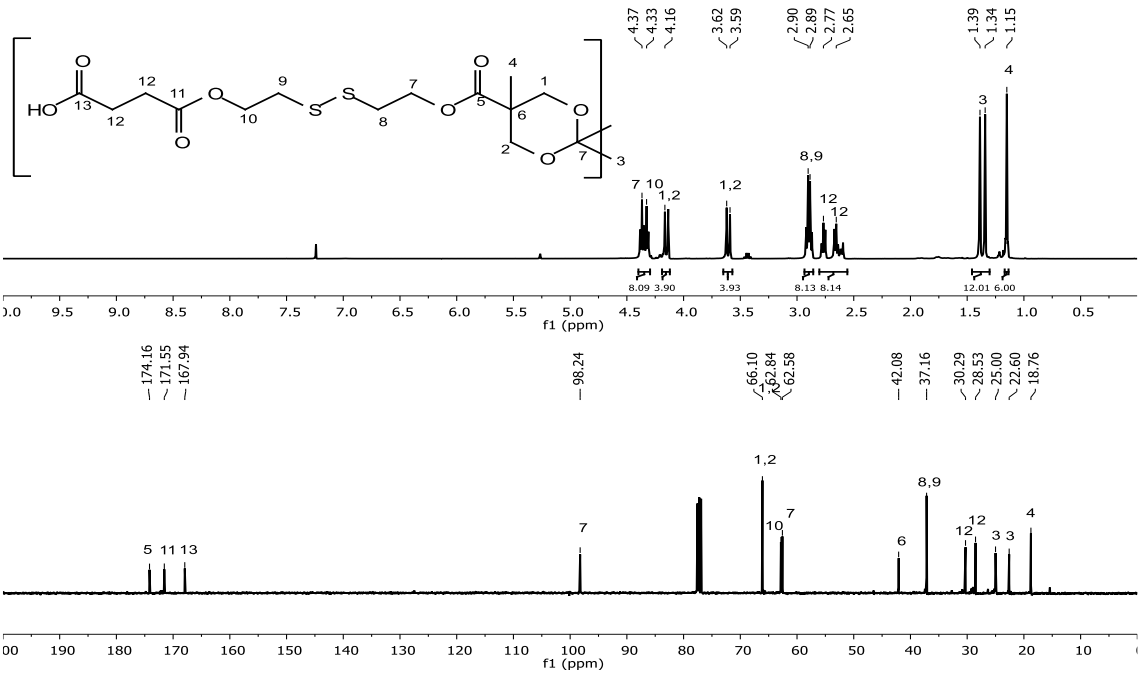


Figure S5 ^{1}H, ^{13}C-NMR of carboxylic hydroxyethyl disulfide protected bis-MPA anhydride **(5)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

TMP-$(S\text{-}S)_3$-G1-$(acet)_3$ (6)

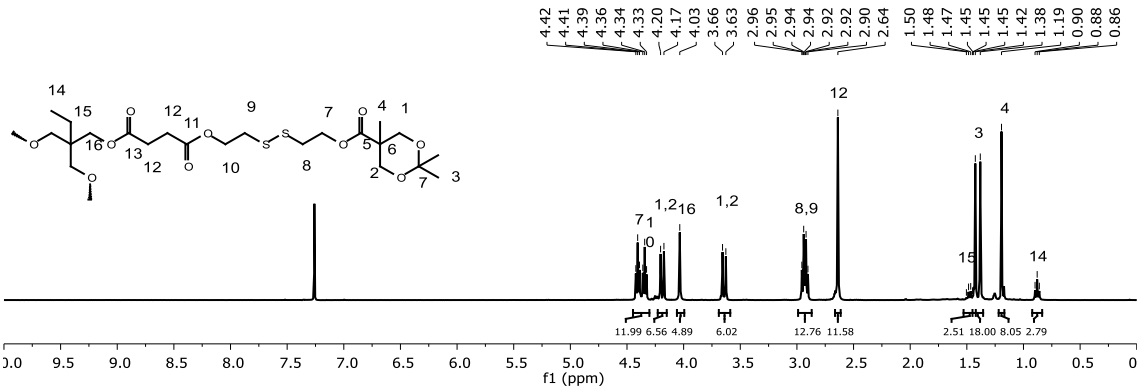


Figure S6 ^{1}H-NMR TMP-$(S\text{-}S)_3$-G1-$(acet)_3$ **(6)** in $CDCl_3$ recorded at 400 MHz.

TMP-(S-S)$_3$-G1-(OH)$_6$ (7)

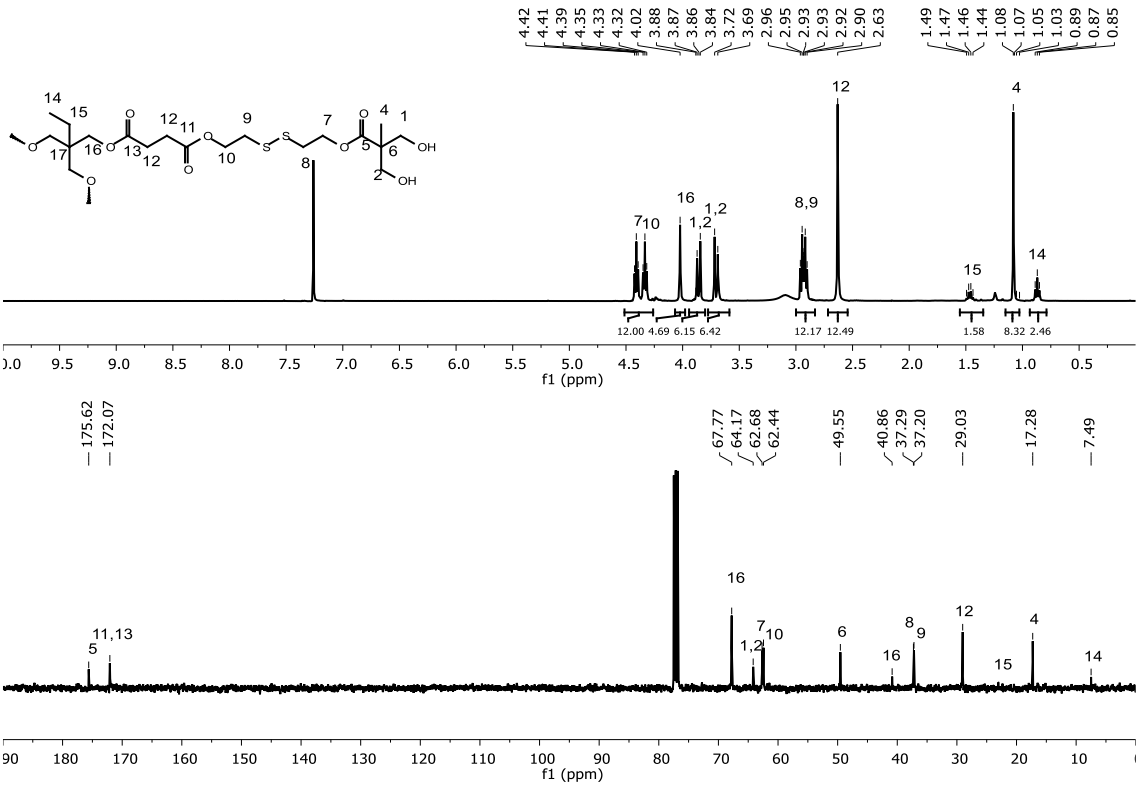


Figure S7 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_3$-G1-(OH)$_6$ **(7)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively**.**

TMP-(S-S)$_9$-G2-(Acet)$_6$ (8)

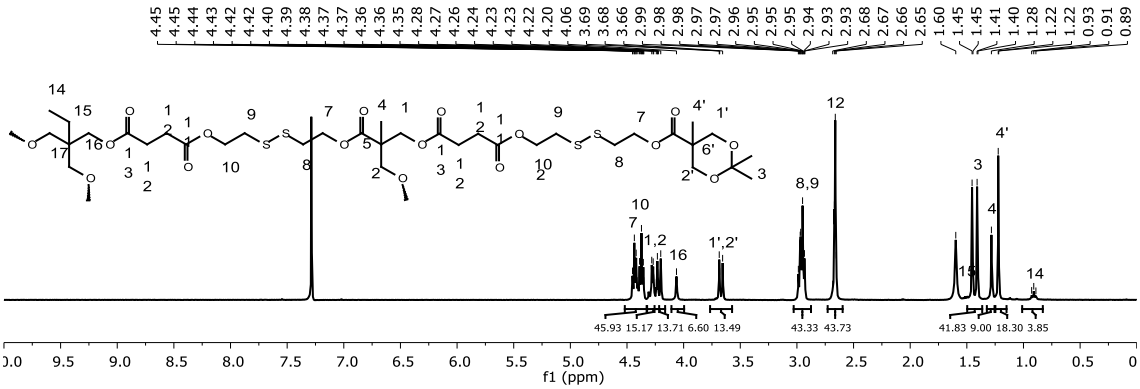


Figure S8 ^{1}H-NMR of TMP-(S-S)$_9$-G2-(Acet)$_6$ **(8)** in $CDCl_3$ recorded at 400 MHz.

TMP-(S-S)$_9$-G2-(OH)$_{12}$ (9)

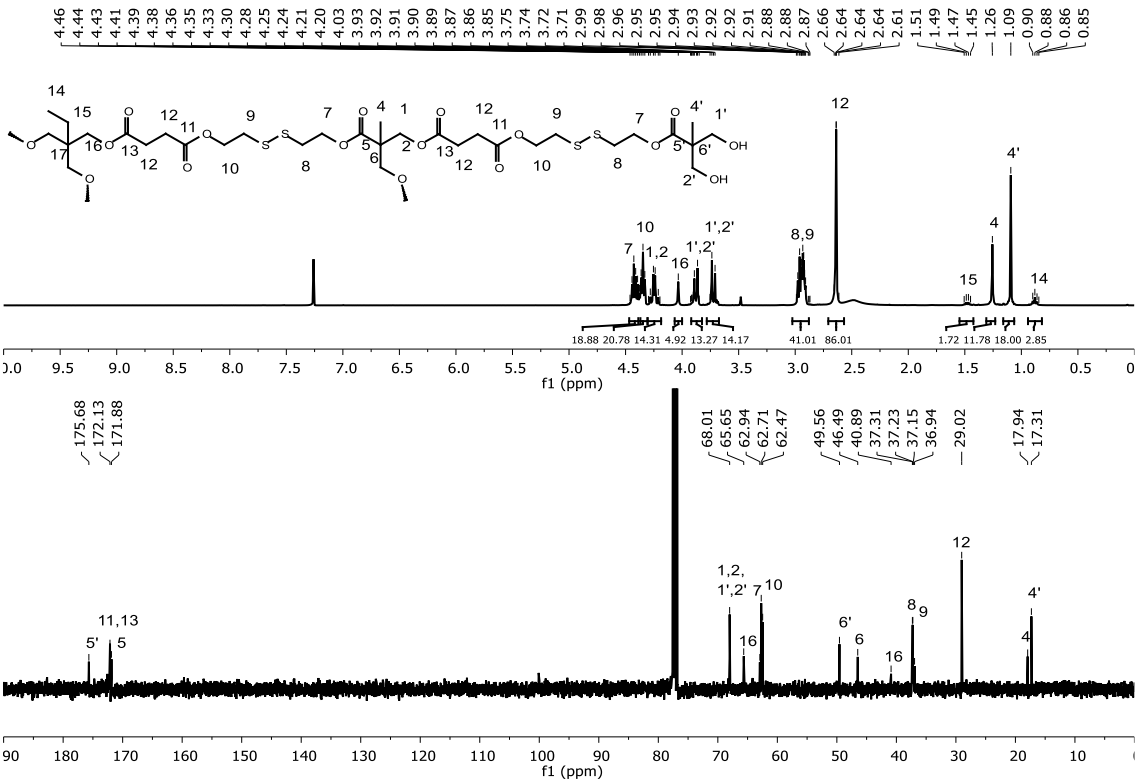


Figure S9 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_9$-G2-(OH)$_{12}$ **(9)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)

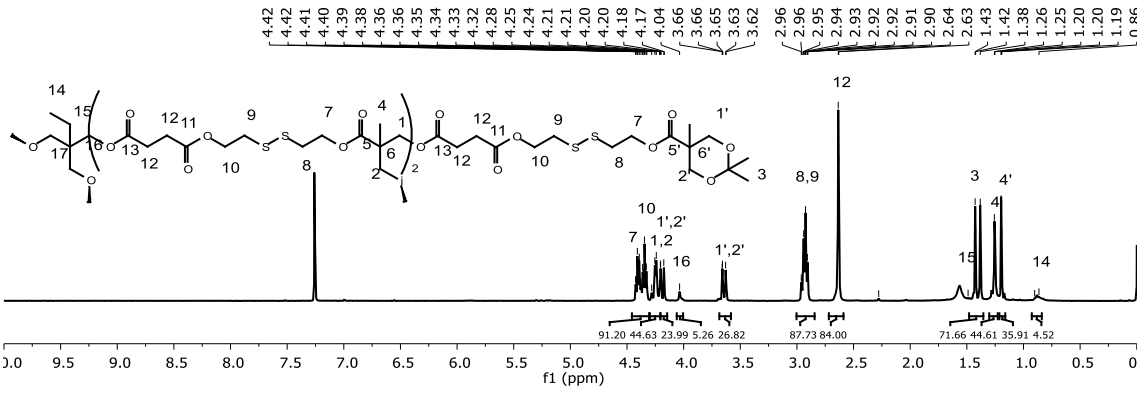


Figure S10 ^{1}H-NMR of TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ **(10)** in $CDCl_3$ recorded at 400 MHz.

TMP-(S-S)$_{21}$-G3-(OH)$_{24}$ (11)

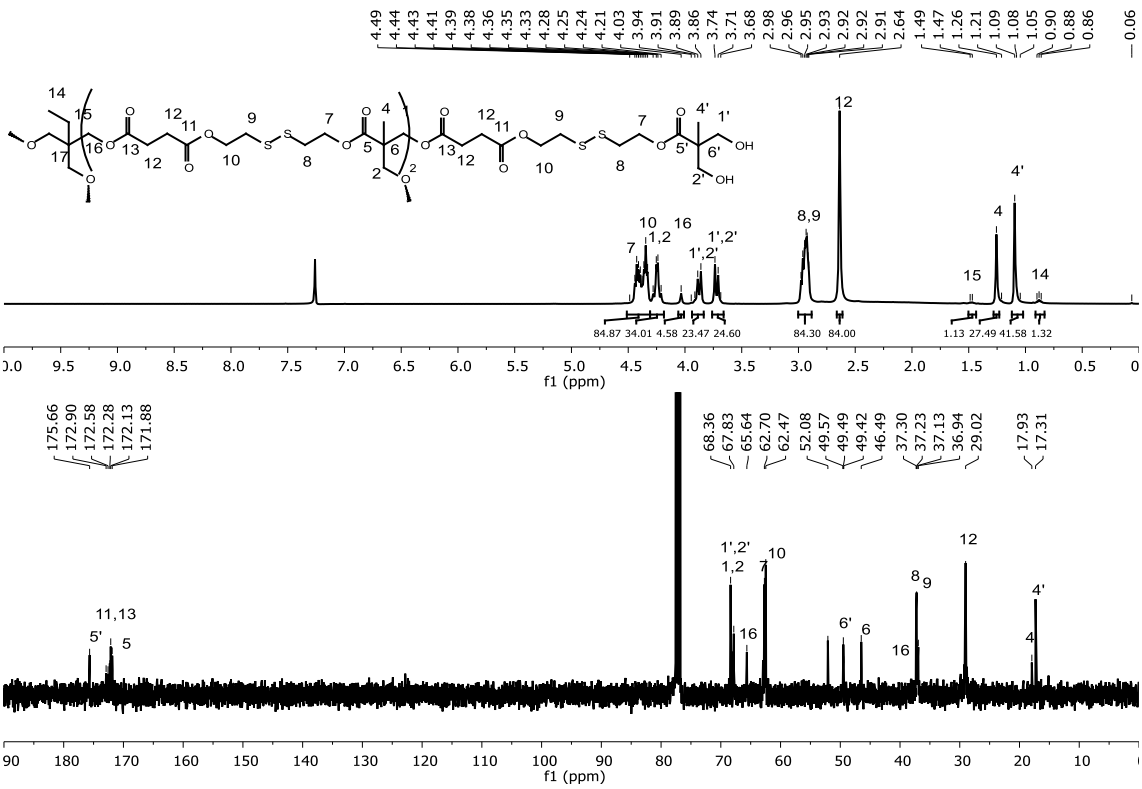


Figure S11 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_{21}$-G3-(OH)$_{24}$ **(11)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

TMP-(S-S)$_{45}$-G4-(Acet)$_{24}$ (12)

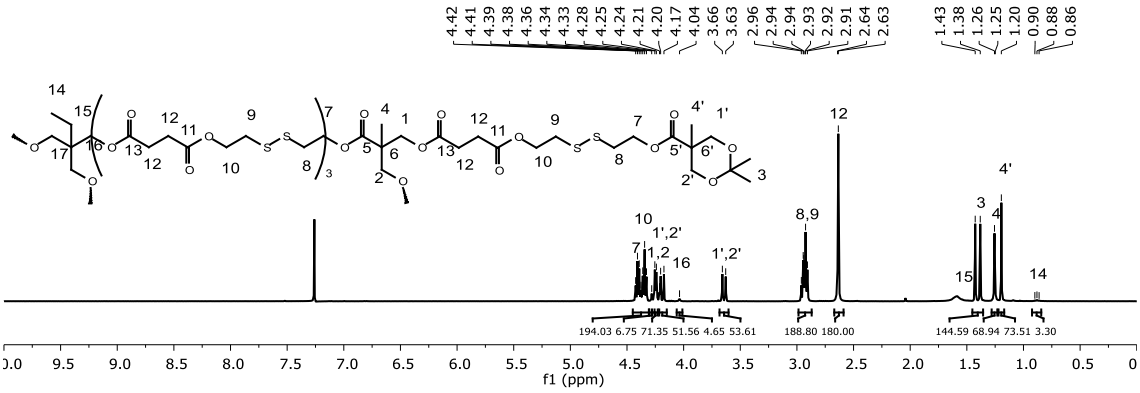


Figure S12 ^{1}H-NMR of TMP-(S-S)$_{45}$-G4-(Acet)$_{24}$ **(12)**in $CDCl_3$ recorded at 400.

TMP-(S-S)$_{45}$-G4-(OH)$_{48}$ (13)

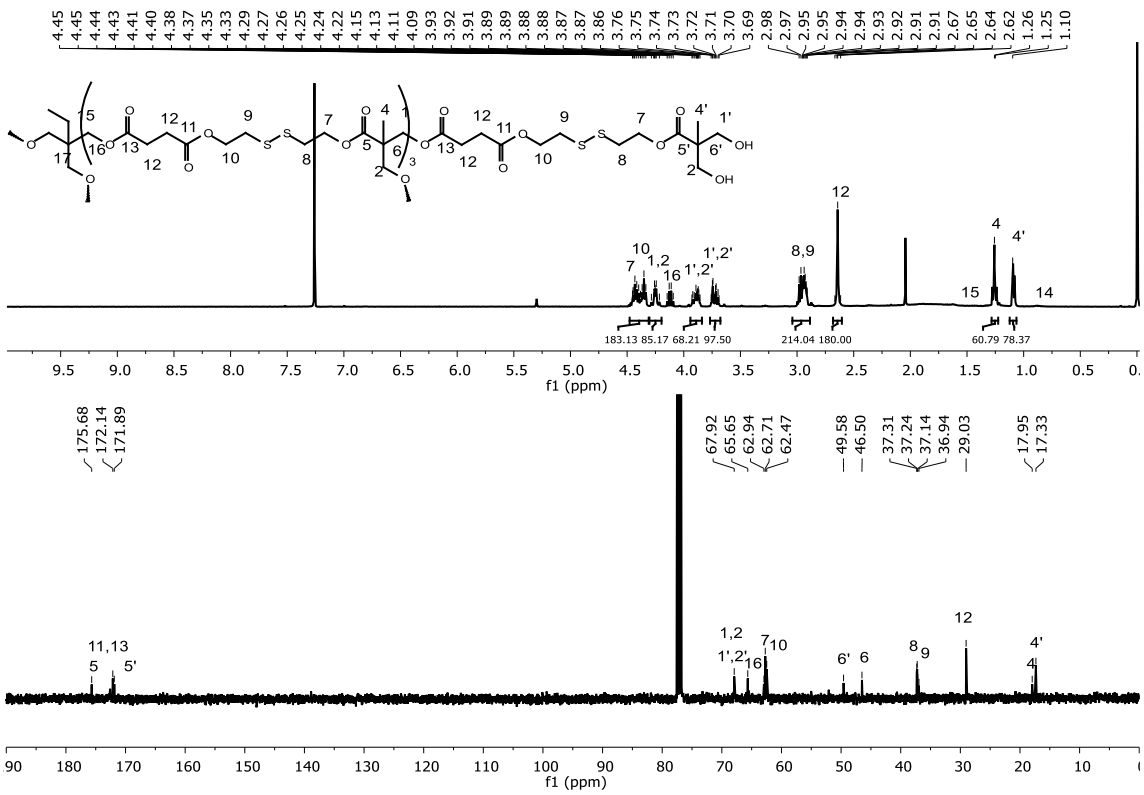


Figure S13 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_{45}$-G4-(OH)$_{48}$ **(13)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

TMP-(S-S)$_{21}$-G3-(Allyl)$_{12}$ (15)

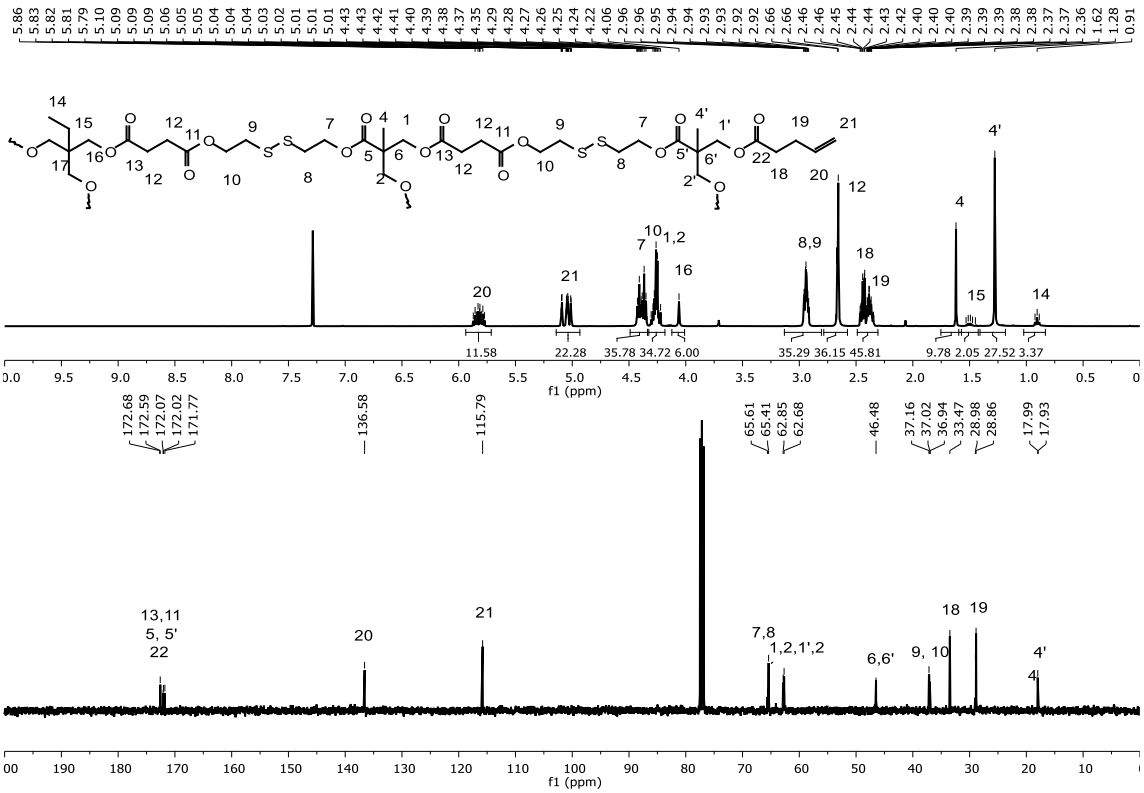


Figure S14 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_{21}$-G2-(Allyl)$_{12}$ **(14)** in $CDCl_3$ recorded at 400 and 101 MHz respectively.

TMP-(S-S)$_{21}$-G3-(Allyl)$_{24}$ (15)

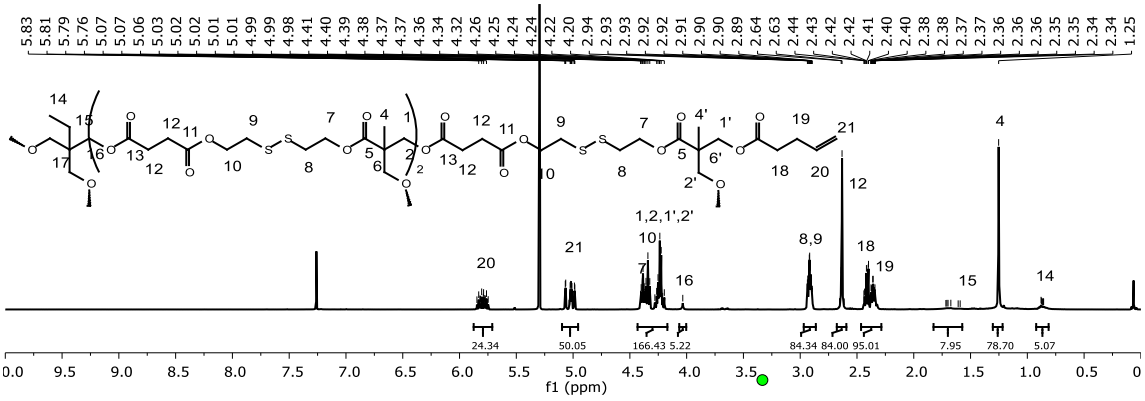


Figure S15 ^{1}H-NMR of TMP-(S-S)$_{21}$-G3-(Allyl)$_{24}$ **(15)** in $CDCl_3$ recorded at 400.

TMP-(S-S)$_{21}$-G2-(Azide)$_{12}$ (16)

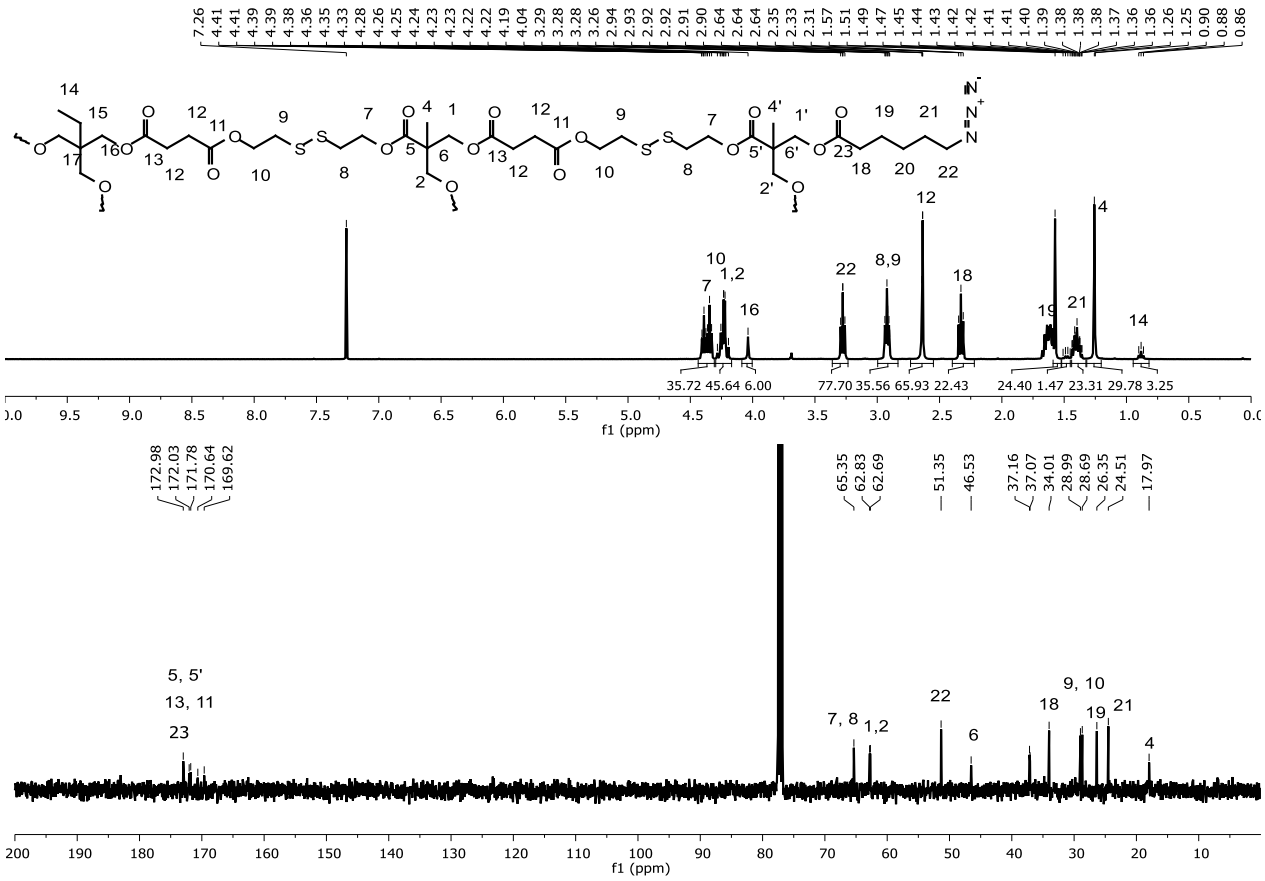


Figure S16 ^{1}H, ^{13}C-NMR of TMP-(S-S)$_{21}$-G2-(Azide)$_{24}$ **(16)** in $CDCl_3$ recorded at 400 and 101 MHz respectively.

TMP-(S-S)$_{21}$-G3-(Azide)$_{24}$ (17)

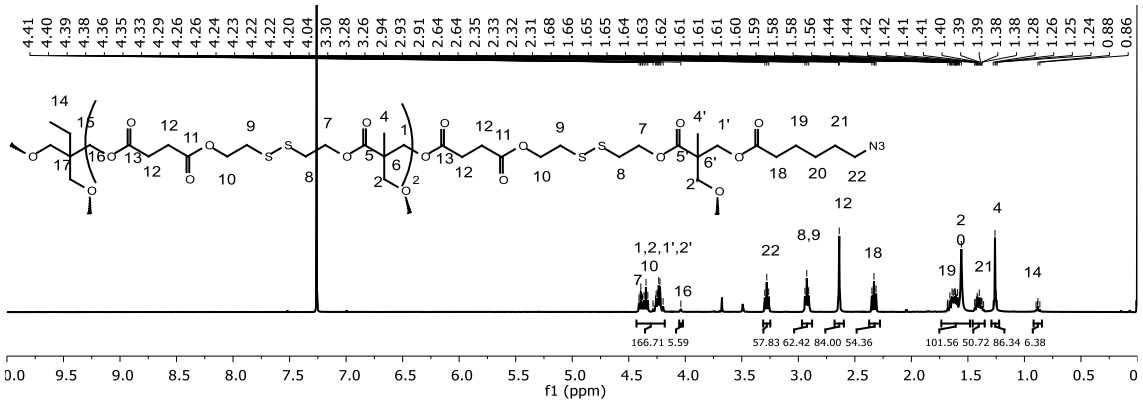


Figure S17 ^{1}H-NMR of TMP-(S-S)$_{21}$-G3-(Azide)$_{24}$ **(17)** in $CDCl_3$ recorded at 400.

TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$ (20)

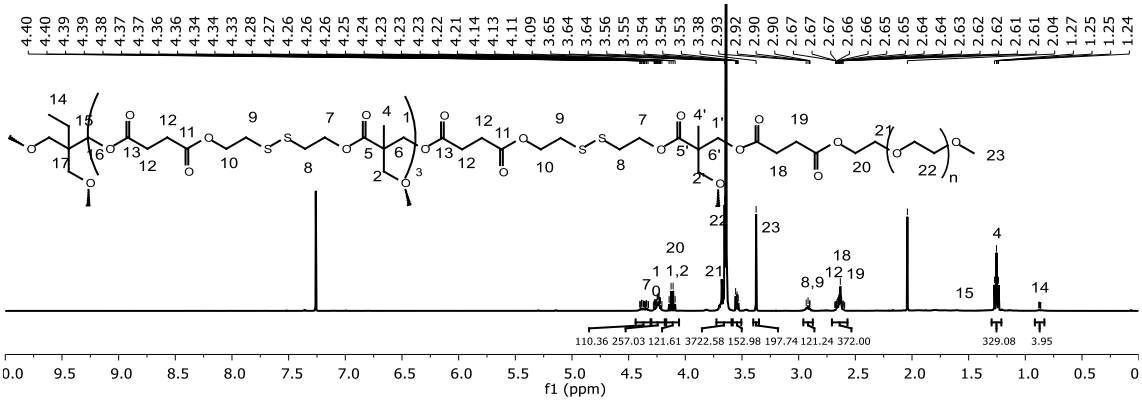


Figure S18 ^{1}H-NMR of TMP-(S-S)$_{45}$-G4-(PEG750-MM)$_{48}$ **(20)** in $CDCl_3$ recorded at 400.

TMP-G4-(PEG750-MM)$_{48}$ (21)

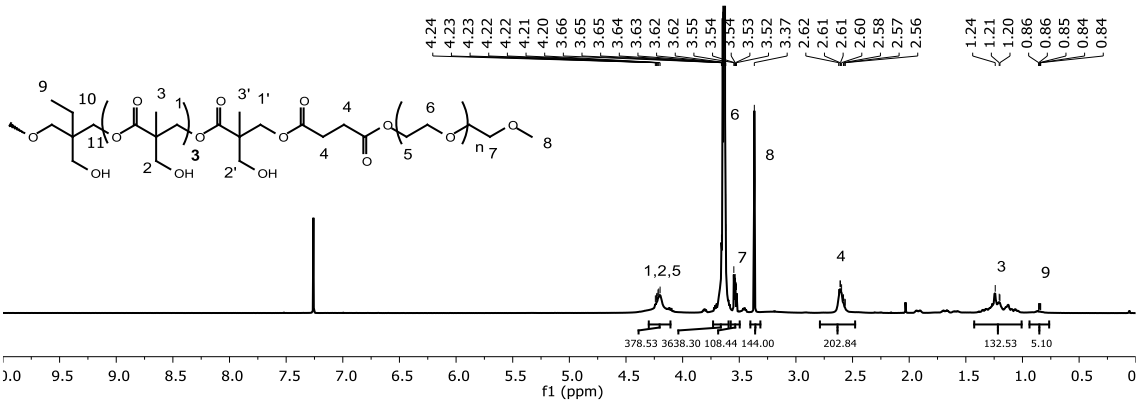


Figure S19 ^{1}H-NMR of TMP-G4-(PEG750-MM)$_{48}$ **(21)** in $CDCl_3$ recorded at 400.

Fragmentation TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)

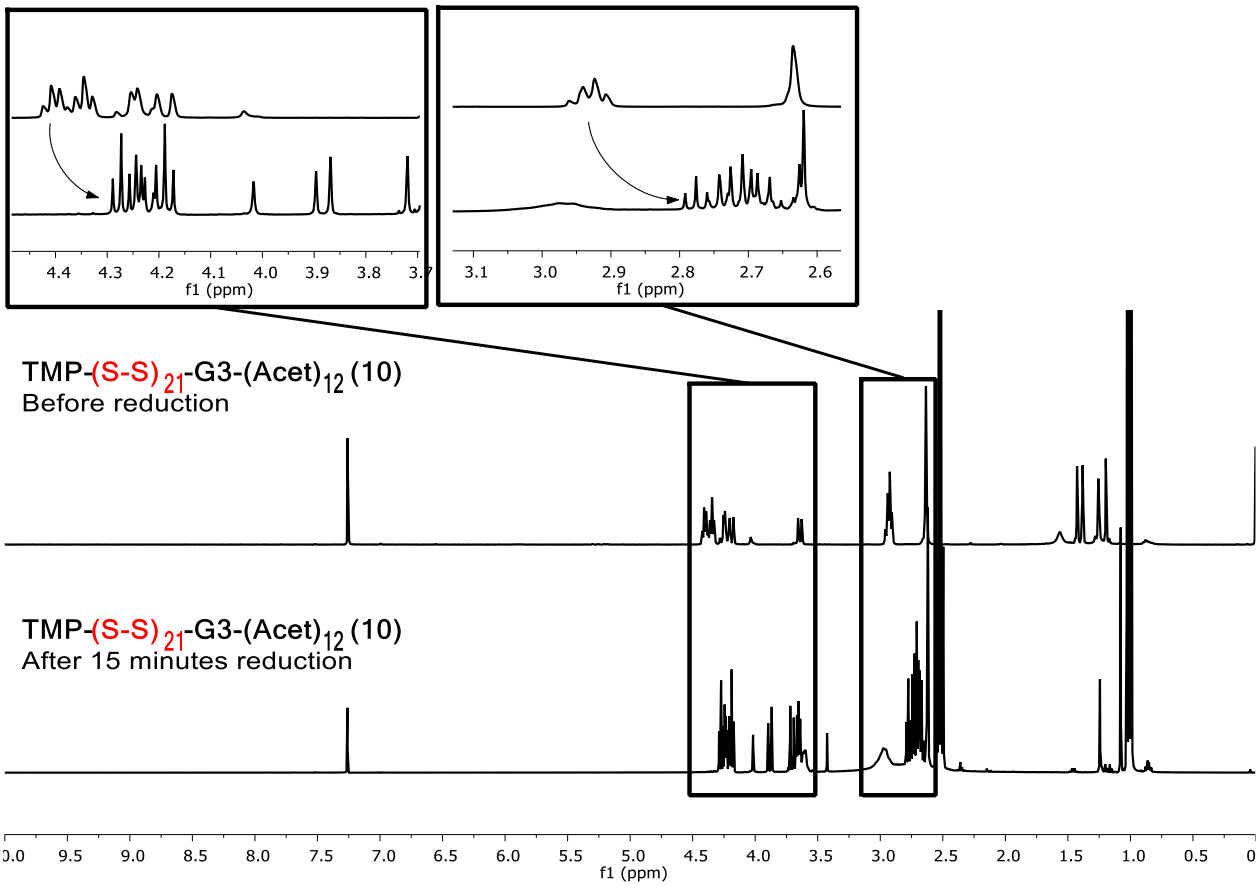


Figure S20 ^{1}H-NMR before and after fractionation of **TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)** in $CDCl_3$ recorded at 400MHz.

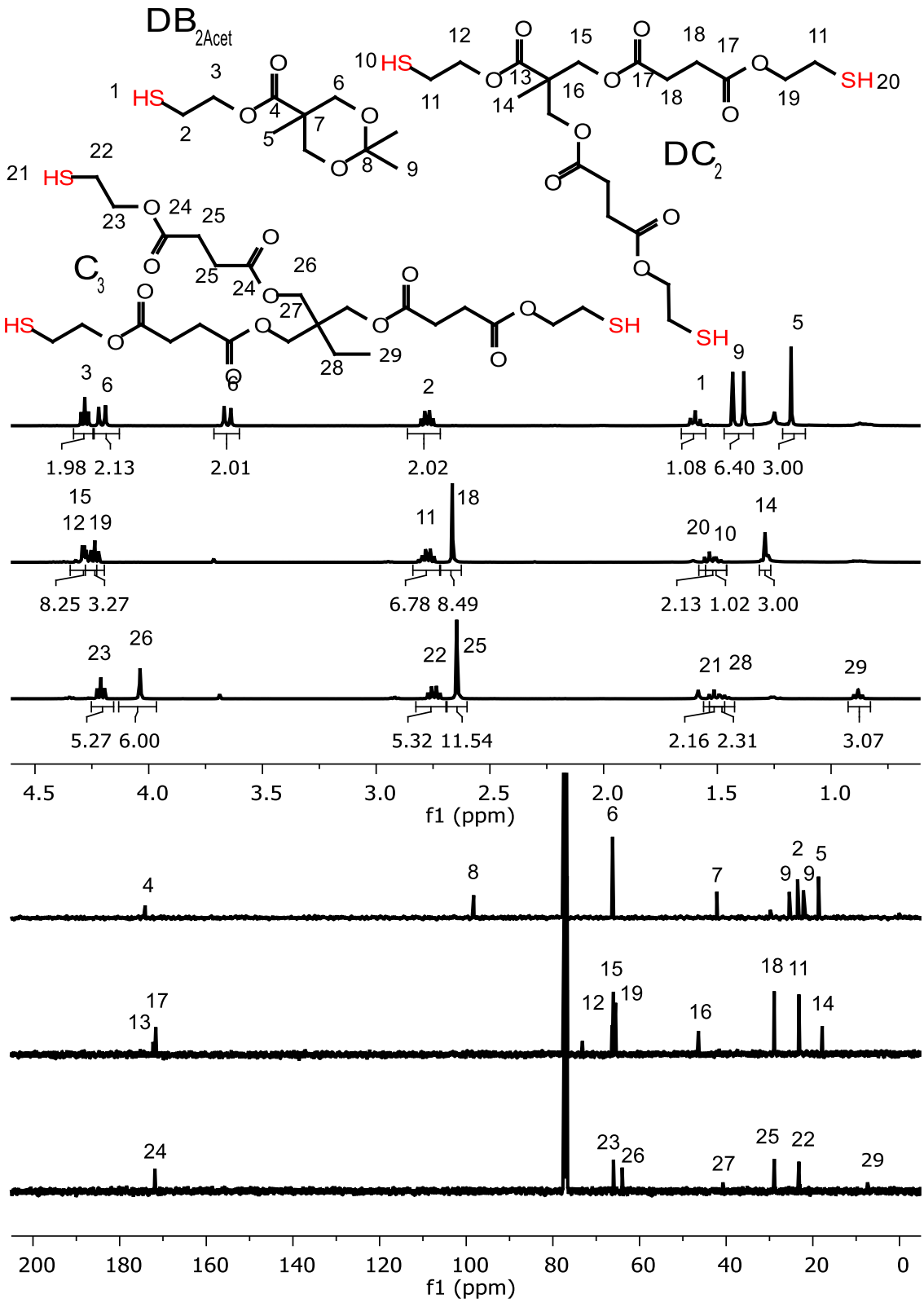


Figure S21 ^{1}H and ^{13}C-NMR of separated unique fragments of **TMP-(S-S)$_{21}$-G3-(Acet)$_{12}$ (10)** in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

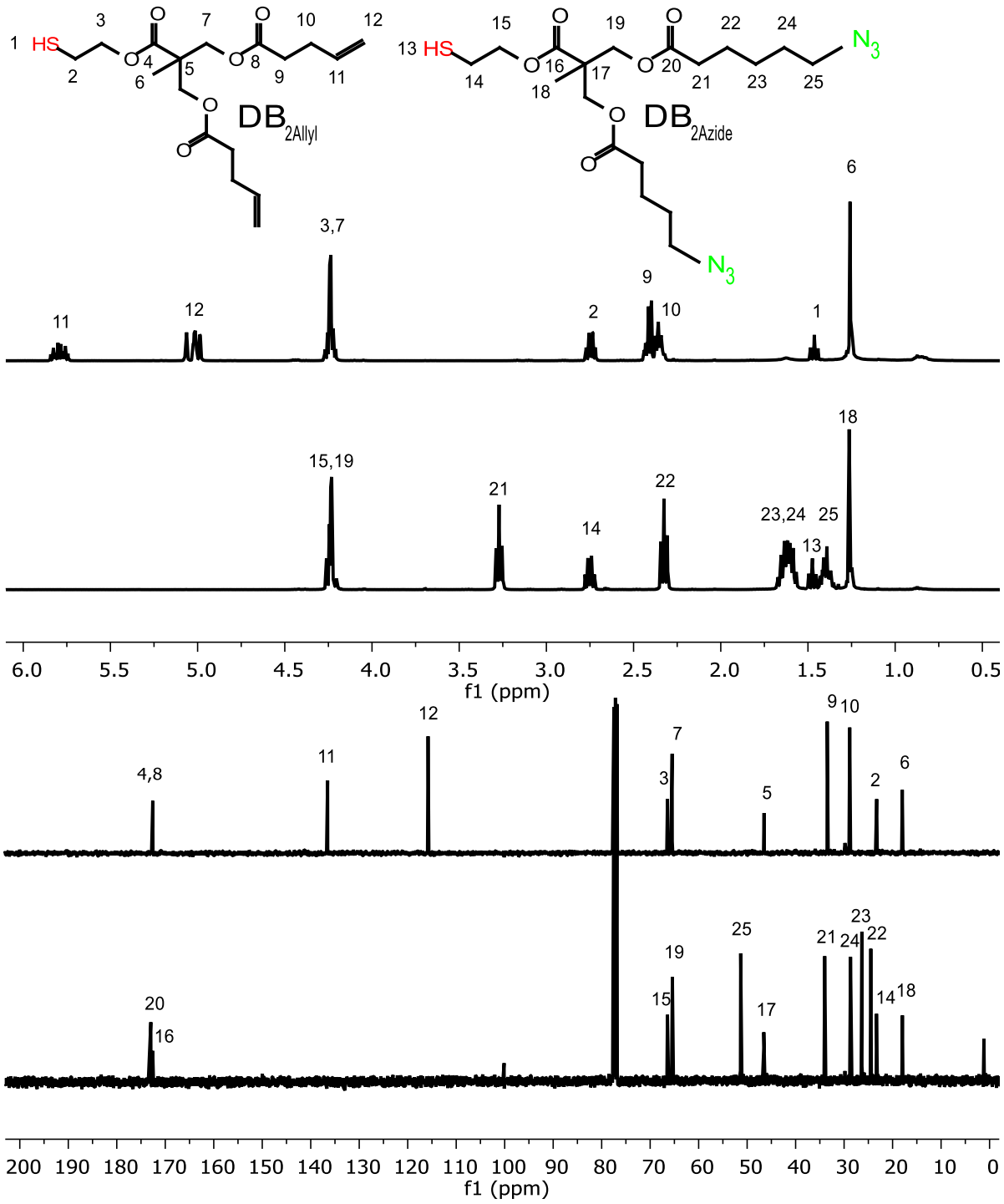


Figure S22 ^{1}H and ^{13}C-NMR of additional DB_2 Fragments provided from **TMP-(S-S)$_9$-G3-(Allyl)$_{12}$ (15)** and **TMP-(S-S)$_9$-G3-(Azide)$_{12}$ (16)-**in $CDCl_3$ recorded at 400 MHz and 101 MHz respectively.

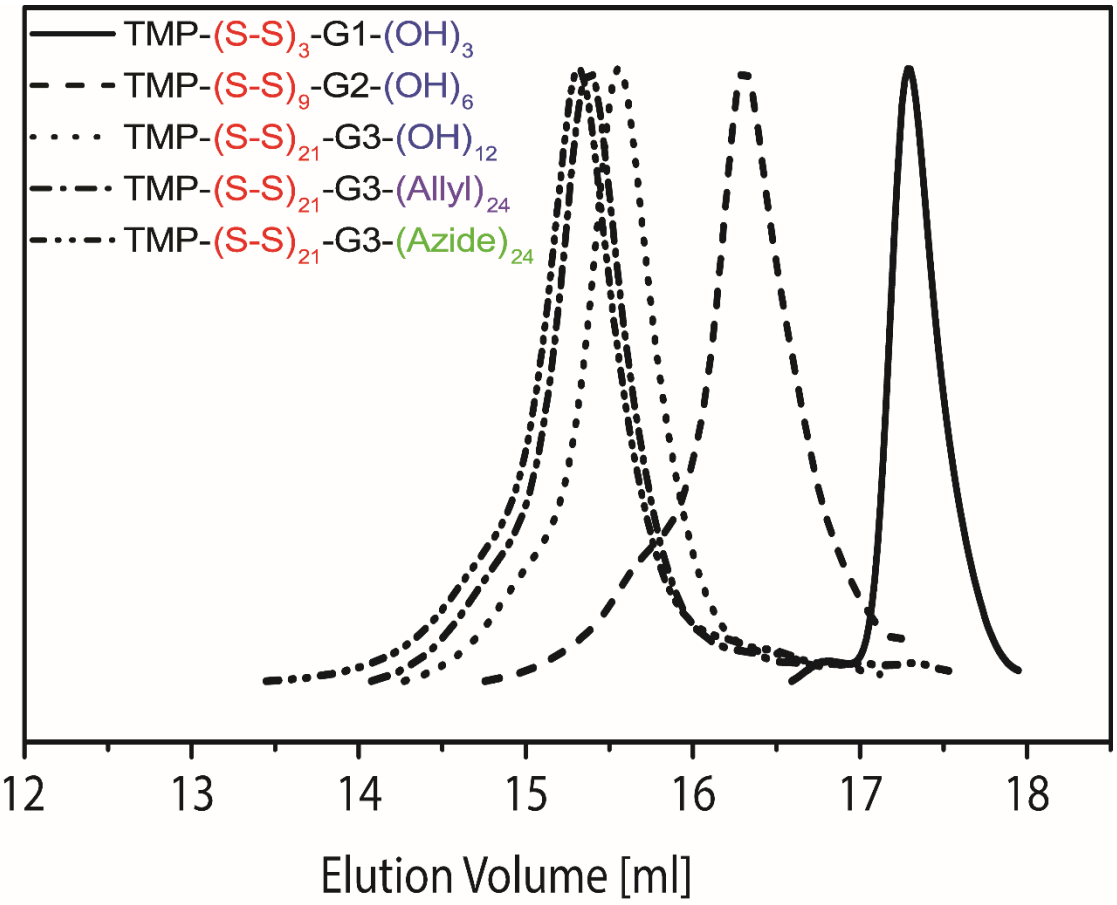


Figure S23 THF SEC of **TMP-$(S\text{-}S)_{45}$-G1,2,3-$(OH)_{48}$ (6, 7, 9, 11) and TMP-$(S\text{-}S)_{21}$-G3-$(Allyl)_{24}$ (15) TMP-$(S\text{-}S)_{21}$-G3-$(Azide)_{24}$ (17) run at 1 ml/min in THF .**

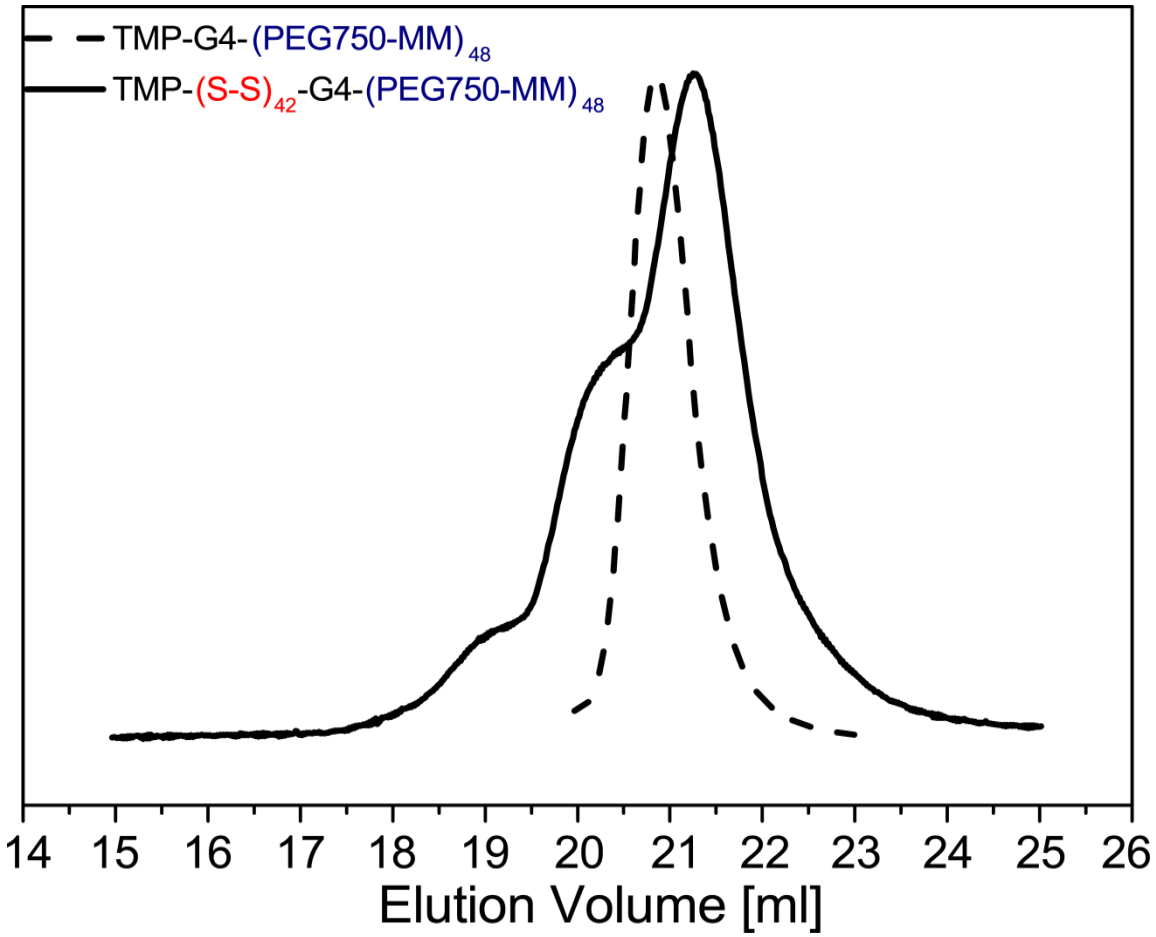


Figure S24 SEC traces of TMP-$(S\text{-}S)_{45}$-G4-$(PEG750\text{-}MM)_{48}$ **(20)** and TMP-G4-$(PEG750\text{-}MM)_{48}$ **(21)** In DMP 0.01 M LiBr mobile phase at 0.2 ml/min at 50 °C. Degradation associated with the conditions can be seen for **20** a phenomena observed for all samples of disulphide dendimer run in the DMF SEC that does not appear in the THF SEC, however poor solubility prevents samples from being run in THF:

MALDI-TOF-MS

Degradation associated with MALDI lazer of TMP-$(S\text{-}S)_{21}$-G3-$(OH)_{24}$ (11)

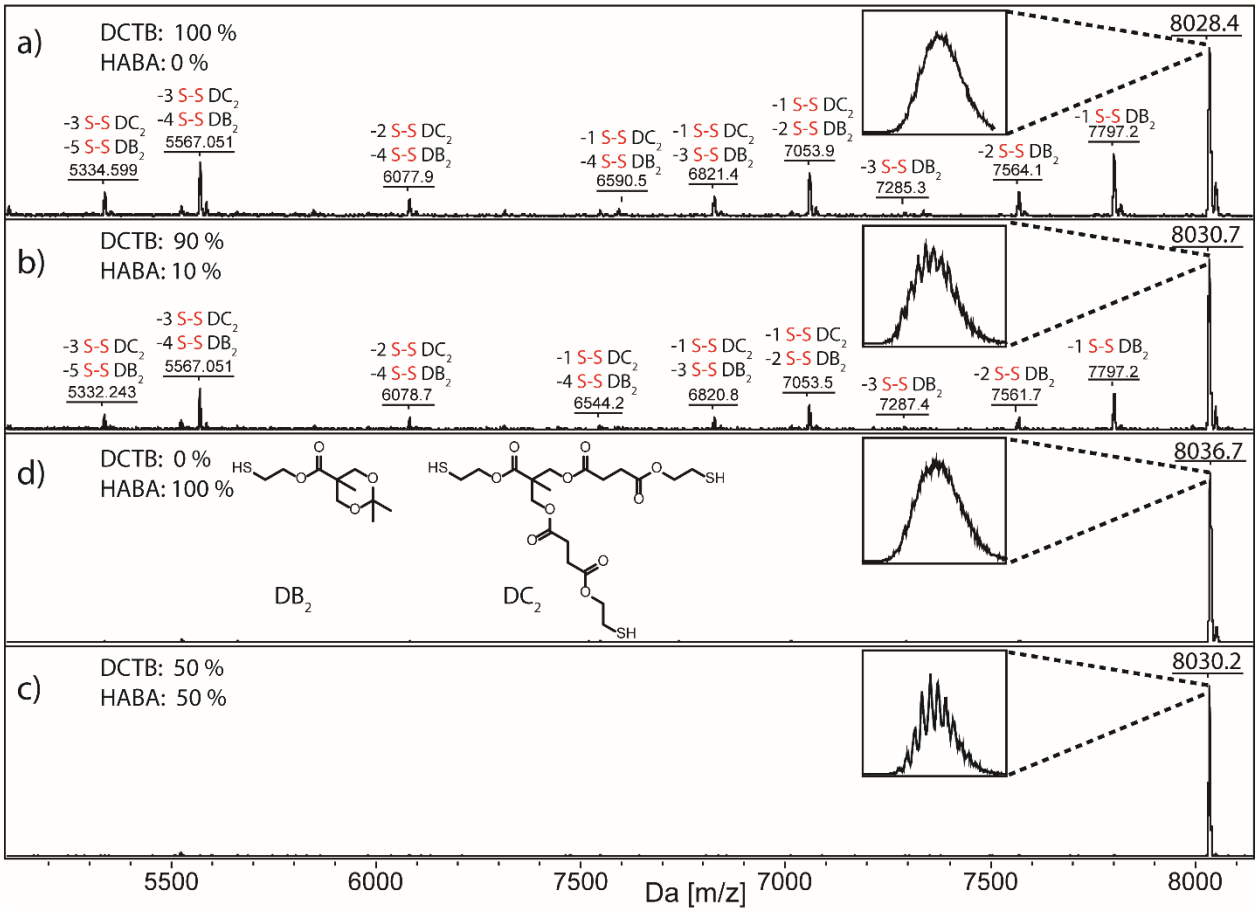


Figure S25 Initially degradation was observed associated with the N_2 laser, operating at 337 nm, causing radical cleavage of the disulfide bond. The phenomenon is well discussed in the literature associated to the characterization of disulfide containing peptides.[4-5] Consequently, a matrix mixture containing 2-(4'-Hydroxybenzeneazo) benzoic acid (HABA) that acted as radical scavenger[6] and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DcTB) for desorption and ionization was successfully used to accurately determine monodispersity of the dendrimers. In the figure degradation caused by the MALDI laser operating at 337 nm for TMP-$(S\text{-}S)_{21}$-G3-$(OH)_{24}$ (**11**) causing fragments denoted DC_2 and DB_2 cleave of the dendrimer. All spectra were collected at 50% laser power and with 1000 shots; samples were prepared at a concentration of 1 mg /ml and diluted 5:20 in matrix or matrix mixture. a) Degradation in DcTb where various populations can be seen, b) addition of 10% HABA with 90% DcTb caused a decreased intensity of degradation products, c) 100% HABA caused low resolution spectra, d) a mixture of 50:50 Haba:DcTb was found to be optimal to maintain resolution and suppress degradation.

TMP-$(S\text{-}S)_{21}$-G3-$(Allyl)_{24}$ (15)

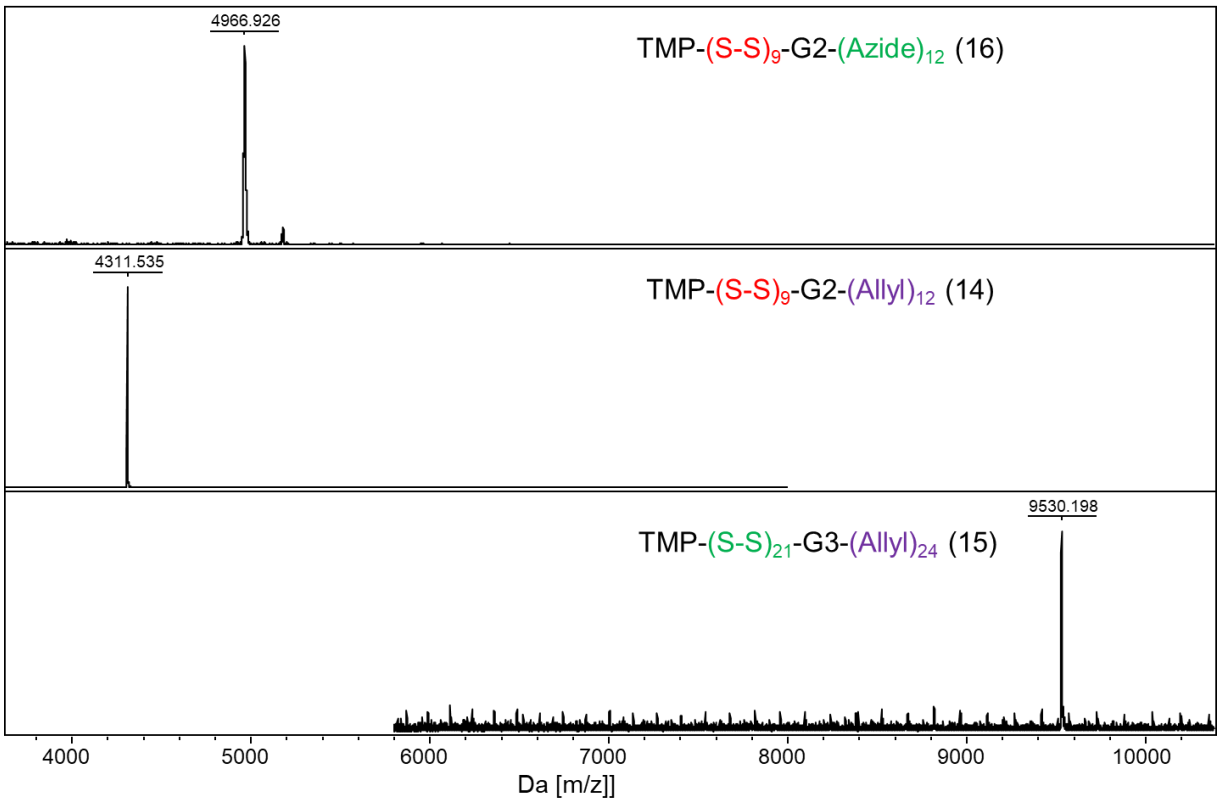


Figure S26 MALDI-TOF-MS of TMP-$(S\text{-}S)_9$-G2-(Allyl) (**14**), TMP-$(S\text{-}S)_{21}$-G3-$(Allyl)_{24}$ (**15**) with DcTB/HABA (1:1) as matrix in reflector mode . And TMP-$(S\text{-}S)_9$-G2-$(Allyl)_{12}$ (**16**) with DcTB/HABA (1:1) as matrix in linear mode.

TMP-G4-$(PEG750\text{-}MM)_{48}$ (21)

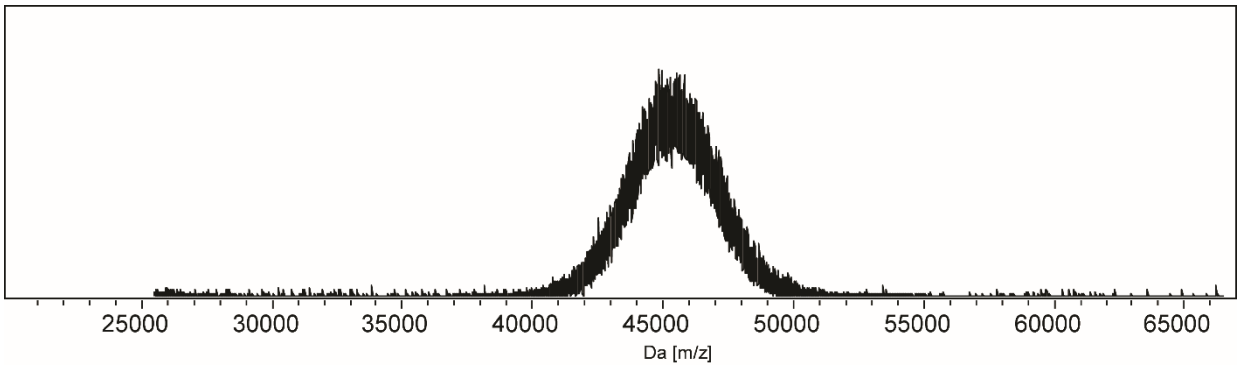


Figure S27 MALDI TOF-MS for TMP-G4-$(PEG750\text{-}MM)_{48}$ (**21**) with DcTB as matrix in linear mode outside of calibration range.

TMP-$(S\text{-}S)_{45}$-G4-$(PEG750\text{-}MM)_{48}$ (20)

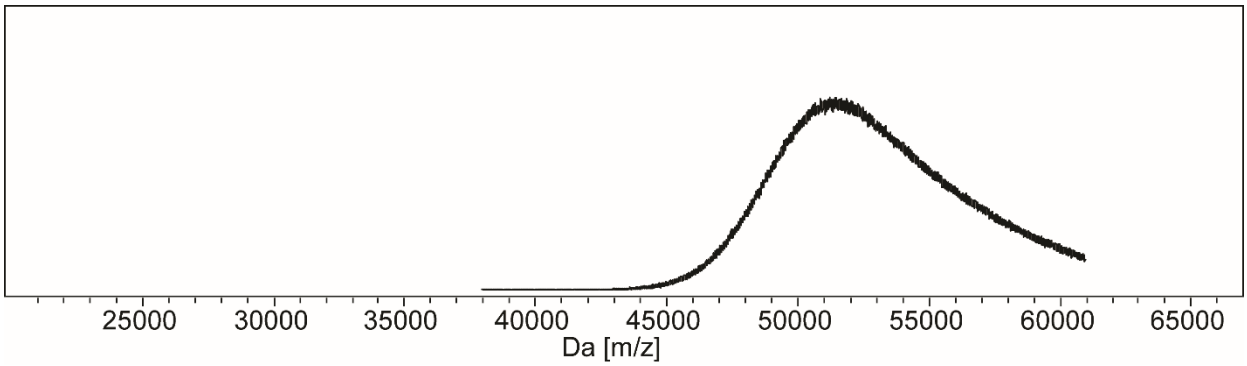


Figure S28 MALDI TOF-MS for TMP-$(S\text{-}S)_{45}$-G4-$(PEG750\text{-}MM)_{48}$ (**20**) with DcTB as matrix in linear mode outside of calibration range.

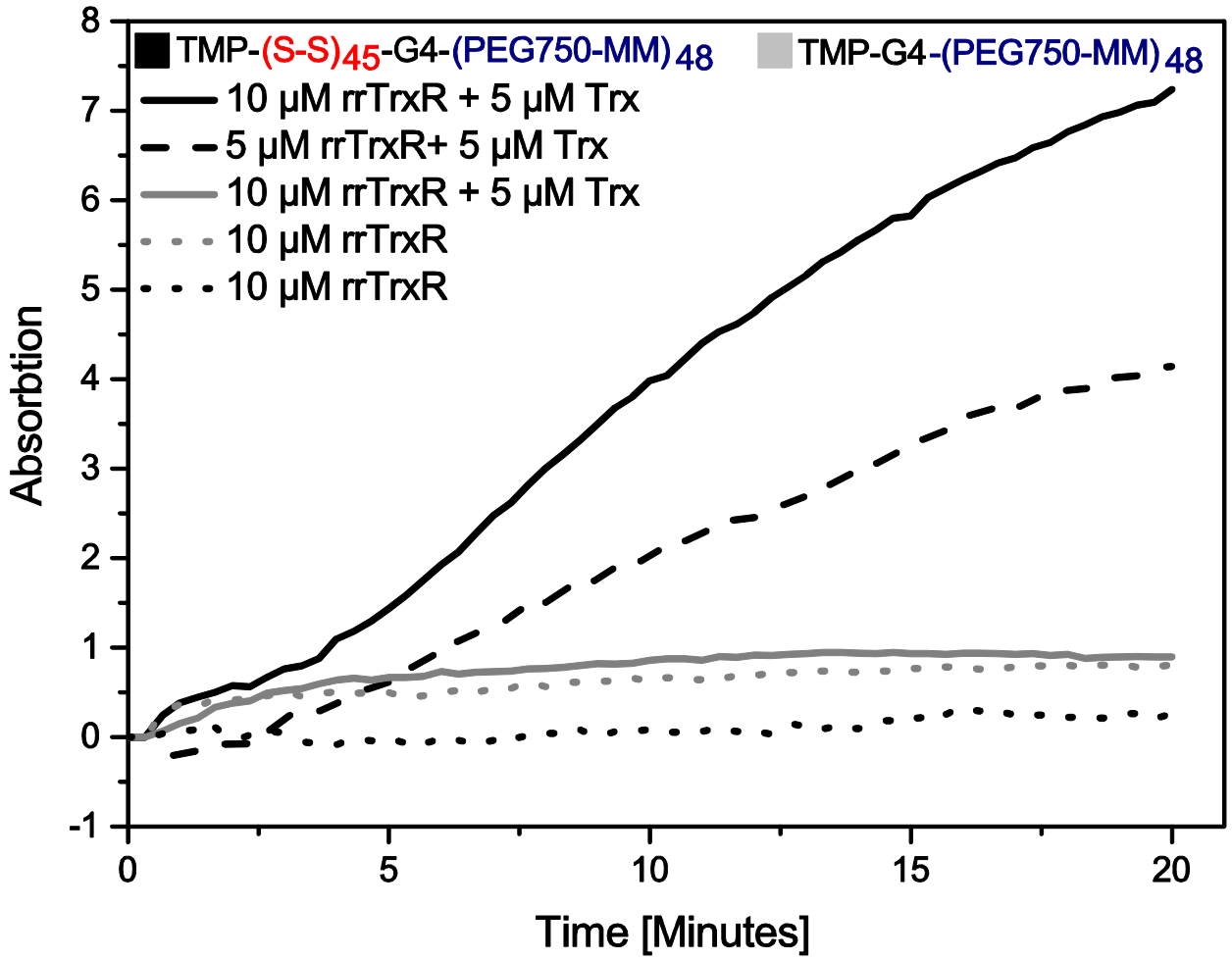


Figure S29 rrTrxR and rrTrxR-Trx reduction of **20** and **21** where no evidence of degradation can be seen for inactive compound **21** and Trx alone cannot fragment **20**. A mixture of both rrTrxR and Trx causes fragmentation of active compound **20** with a dose dependence.

References

1. Antoni, P.; Nystrom, D.; Hawker, C. J.; Hult, A.; Malkoch, M., Chem Commun, 2007, 2249-2251
2. Holmgren, A.; Aslund, F., Methods Enzymol, 1995, 252, 283-292
3. Arner, E. S.; Holmgren, A. Current protocols in toxicology; John Wiley and Sons, Inc.: New Jersey, 2001
4. Chait, B. T.; Kent, S. B. H., Science, 1992, 257, 1885-1894
5. Crimmins, D. L.; Saylor, M.; Rush, J.; Thoma, R. S., Anal Biochem, 1995, 226, 355-361
6. Huwiler, K. G.; Mosher, D. F.; Vestling, M. M., J Biomol Tech, 2003, 14, 289-297