Efficient synthesis of dendrimers via a thiol-yne chemistry

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1.0 General

1.1 Reagents

All reagents bought from Sigma-Aldrich except acetone, diethyl ether, ethyl acetate, *n*-hexane (Chemsupply, Crown), potassium carbonate, triethylamine (APS/AJAX, Crown), *O*-phenylenediamine (OPDA) (Hopkin and Williams) and silica gel (Scharlau, Crown). 4-oxo-4-(prop-2-ynyloxy)butanoic anhydride (**3**) was synthesized as previously described.[1] All chemicals were used as purchased unless otherwise specified.

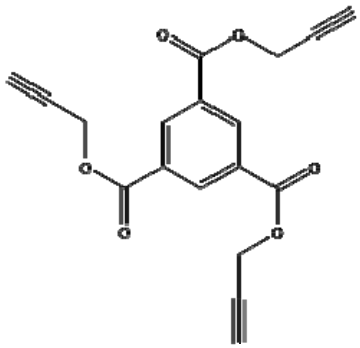
1.2 Analysis and equipments

Gel Permeation Chromatography (GPC) analysis of the polymers were performed in *N*,*N*-dimethylacetamide (DMAc) (0.05 % w/v LiBr, 0.05 % BHT) at 50 °C (1 mL min^{-1} flow rate) using a Shimadzu modular system comprising a DGU-12A solvent degasser, a LC-10AT pump, a CTO-10A column oven and a RID-10A refractive index detector. The system was equipped with a 5.0 μm bead-size guard column (50 × 7.8 mm) followed by four 300 × 7.8 mm linear Phenomenex columns (10^5, 10^4, 10^3 and 500 Å). The calibration curve was generated with narrow polydispersity polystyrene standards ranging from 500 to 10^6 g mol^{-1}. NMR spectra were acquired on a Bruker 300 MHz or 400 MHz spectrometer in deuterated solvents. Fourier-Transform Infrared (FT-IR) measurements were performed using a Bruker IFS 66/S Fourier transform spectrometer equipped with a tungsten halogen lamp, a KBr beam splitter and a DTGS detector. Each spectrum in the spectral region of 4000-800 cm^{-1} was calculated from the co-added interferograms of 32 scans with resolution of 4 cm^{-1}. The UV irradiation experiments were carried out using a UVP UV-38 3UV Lamp (365 nm) on top of a Ratek orbital mixer incubator (100 rpm). Quantitative determination of *cis*-dichlorodiammineplatinum (II) (CDDP) was performed

using a Varian Cary 300 UV-Vis spectrophotometer at 703 nm. MALDI-TOF spectra were collected using an Applied Biosystems Voyager DE STR MALDI reflectron TOFMS at the Bioanalytical Mass Spectrometry Facilities within the Analytical Centre of the University of New South Wales. Particle sizes were determined using a Malvern Nano-Zetasizer (laser: 4 mW, λ = 633 nm, angle: 173 º backscatter) and 1 mg/mL solutions in distilled water that were filtered through 0.45 μm filters before analysis. The mean diameter was obtained from the arithmetic mean using the number distributed diameter of each particle size.

2.0 Dendrimer synthesis

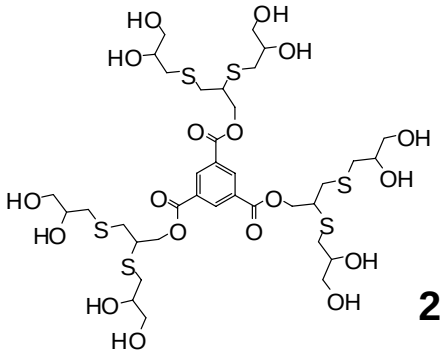
2.1 Synthesis of triprop-2-ynyl benzene-1,3,5-tricarboxylate (1)



Propargyl alcohol (6.33 g, 0.11 mol, 10 fold excess) and 1.00 g of triethylamine were placed in a round bottom flask with 40 mL of THF and chilled to 0 ˚C. 1,3,5-Benzenetricarbonyl trichloride (3.00 g, 0.011mol) was dissolved in 30 mL of THF and placed in a dripping funnel and introduced into the round bottom flask slowly over a period of 30 minutes. The mixture was allowed to stir at 0 ˚C for a further hour and then left to stir at 40 ˚C for 18 hours. The white precipitate was then removed via filtration and the solvent was evaporated under vacuum. The product was redissolved in

dichloromethane and washed with 10% HCl, aqueous $NaHCO_3$ and finally twice with distilled water. Dichloromethane was subsequently removed by rotary evaporation and the crude product was purified by column chromatography, using hexane to remove all the impurities before eluting the product through with ethyl acetate. Yield: 2.7 g, 75%. ^{1}H NMR ($CDCl_3$), 300MHz, δ (ppm): 3.32 (C*H*≡C-CH_2, 3H, t), 4.99 (C-C*H*$_2$-O, 6H, d), 8.49 (C*H* phenyl, 3H, s); ^{13}C NMR ($CDCl_3$), 300MHz, δ (ppm): 53.6 (C-*C*H_2-O), 76.0 (C≡*C*H), 77.5 (CH_2-*C*≡CH), 131.2 (*C*phenyl), 135.7 (*C*H phenyl), 164.4 (O=*C*-O)

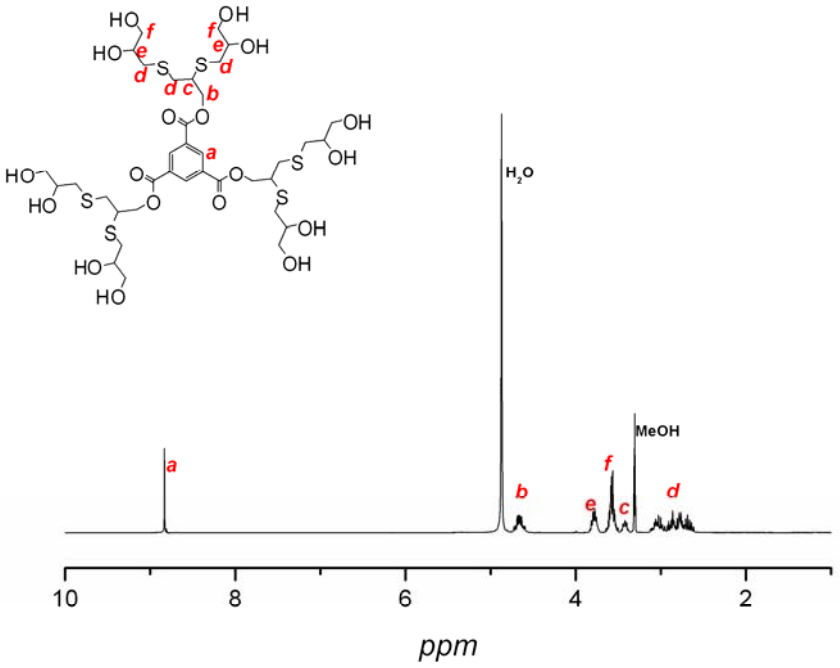
2.2 Synthesis of [G1]-OH_{12} (2)



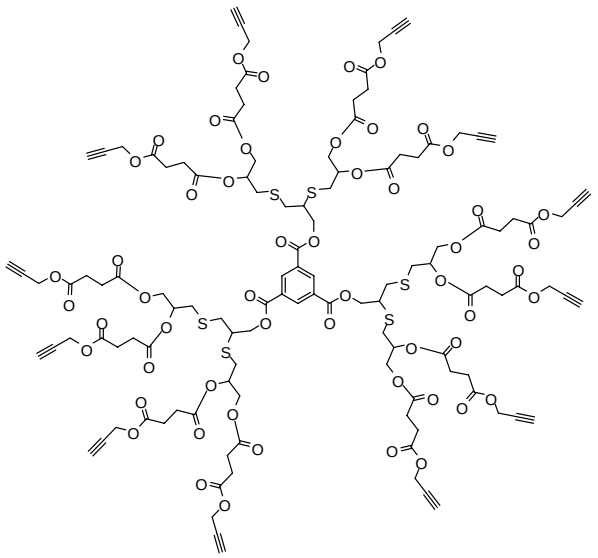
2

Compound (**1**) (480 mg, 1.50 mmol), 5% weight 2,2'-dimethoxy-2-phenylacetophonone (DMPA), 0.3 mL DMF and finally thiolglycerol (4.86 g, 45 mmol) were all placed in a vial. The vial was then purged with N_2 for 10 min and irradiated for 10 min with 365 nm light at room temperature, within a Ratek orbital mixer incubator (100 rpm). The contents were subsequently precipitated three times in diethyl ether to afford a clear viscous liquid and was further purified by column chromatography using a mixture of DCM and methanol (10 % methanol, R_f = 0.1). (Yield: 1.3 g, 89%) ^{1}H-NMR (D_2O, 300 MHz) δ 2.70 – 3.10 (m, 18H, -C*H*$_2$S-), 3.37 (m, 3H, -(CH_2)$_2$C*H*S-), 3.62 (m, 12H, -C*H*$_2$OH), 3.89 (m, 6H, -(CH_2)$_2$C*H*OH), 4.59 (m, 6H, -OC*H*$_2$-), 8.18 (s, 3H, benzene); ^{13}C-NMR

(MeOH-d_4, 75 MHz) δ 35.75 (-*C*H_2SCH-), 37.10 (-*C*H_2S-), 37.19 (*C*H_2S-), 46.39 (-(CH_2)$_2$*C*HS-), 65.91 (-*C*H_2OH), 67.51 (-O*C*H_2-), 73.19 (-(CH_2)$_2$*C*HOH), 132.64 (*C*, benzene), 135.40 (*C*H, benzene), 165.86 (C=O). MALDI: calculated [M+Na^+] = 996.23 g/mol, found [M+Na^+] = 996.19 g/mol. IR: 3270 (br), 2920, 1730, 1390, 1230, 1100, 1030, 740, 630, 540 cm^{-1}.

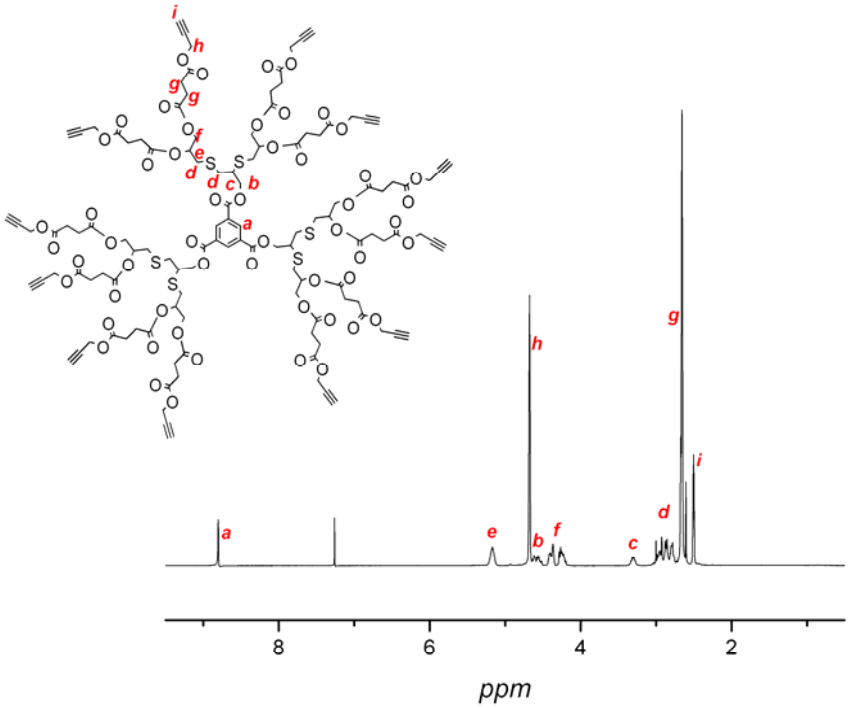


2.3 Synthesis of [G1]-yne$_{12}$ (4)

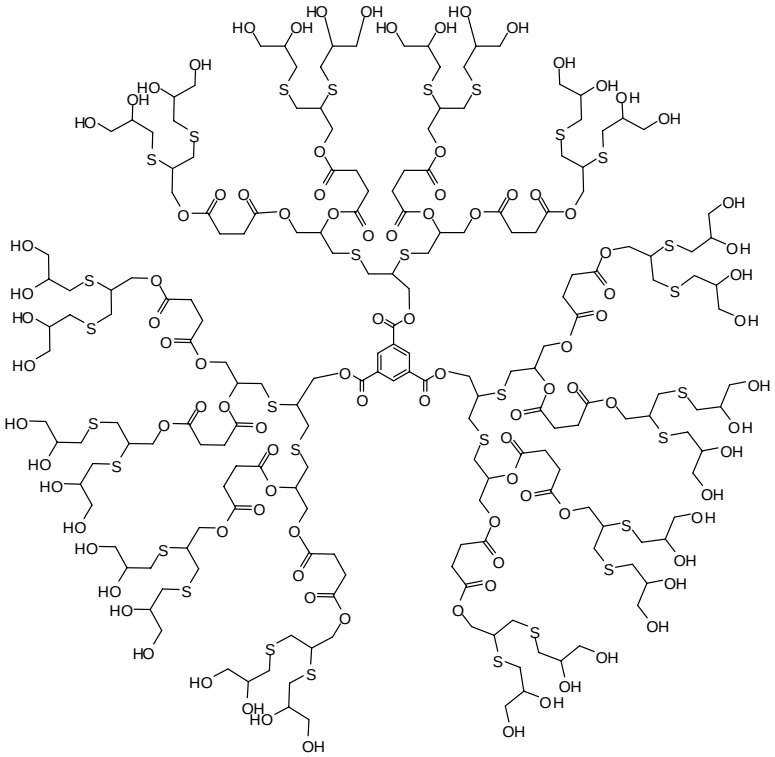


4

Hydoxyl-terminal dendrimer (**2**) (243 mg, 0.25 mmol), acetylene anhydride (**3**) (2.6 g, 9 mmol), 4-(dimethylamino)pyridine (DMAP) (55 mg, 0.45 mmol), TEA (1.26 mL, 9 mmol) and 10 mL DMF were placed in a small vial. The reaction was left overnight and the contents were subsequently precipitated in diethyl ether and then purified by column chromatography (DCM/methanol 9:1, R_f = 0.59). (Yield: 0.49 g, 76%) ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.50 (s, 12H, -C≡C*H*), 2.66 (m, 48H, -COCH_2CH_2CO-), 2.67-3.0 (m, 18H, -SCH_2-), 3.31 (m, 3H, -$(CH_2)_2$C*H*S-), 4.20-4.65 (m, 18H, -OCH_2CH-), 4.66 (s, 24H, -CH_2C≡CH), 5.17 (m, 6H, -$(CH_2)_2$C*H*O-), 8.80 (s, 3H, benzene); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 28.86 (-*C*H_2CO-), 29.06 (-*C*H_2CO-), 31.61 (-*C*H_2SCH-), 33.02 (-*C*H_2S-), 34.29 (*C*H_2S-), 41.09 (-$(CH_2)_2$*C*HS-), 52.36 (-*C*H_2C≡CH), 63.89 (-O*C*H_2CH-), 66.05 (-O*C*H_2-), 70.93 (-C≡*C*H), 71.17 (-$(CH_2)_2$*C*HO-), 75.28 (-*C*≡CH), 131.19 (*C*,benzene), 135.01 (*C*H, benzene), 164.52 (benzene-*C*=O), 171.47 (*C*=O), 171.53 (*C*=O). MALDI: calculated [M+Na^+] = 2653.68 g/mol, found [M+Na^+] = 2653.54 g/mol. IR: 3270, 2950, 2130, 1730, 1410, 1380, 1230, 1150, 1030, 990, 830, 740, 630, 540 cm^{-1}.

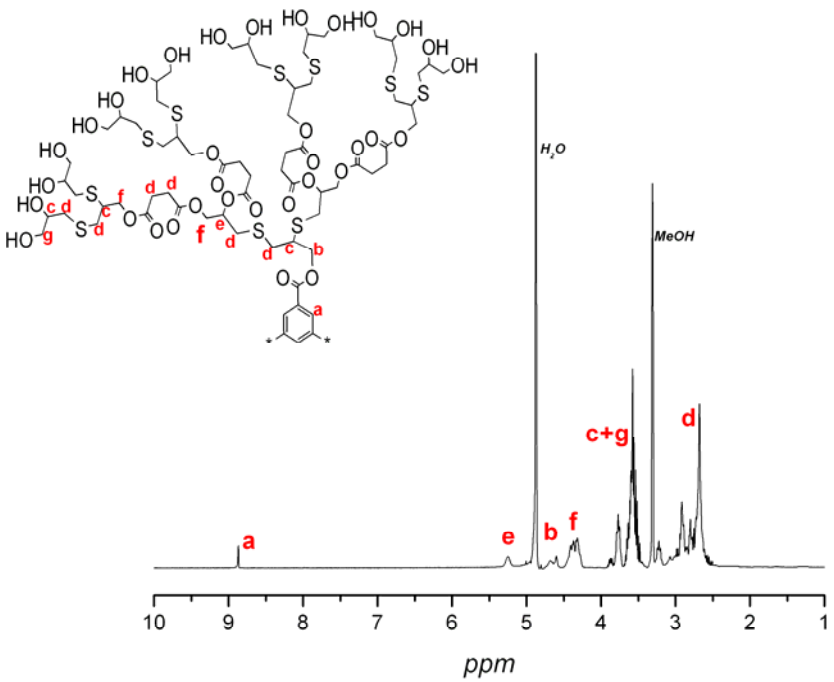


2.4 Synthesis of [G2]-OH_{48} (5)

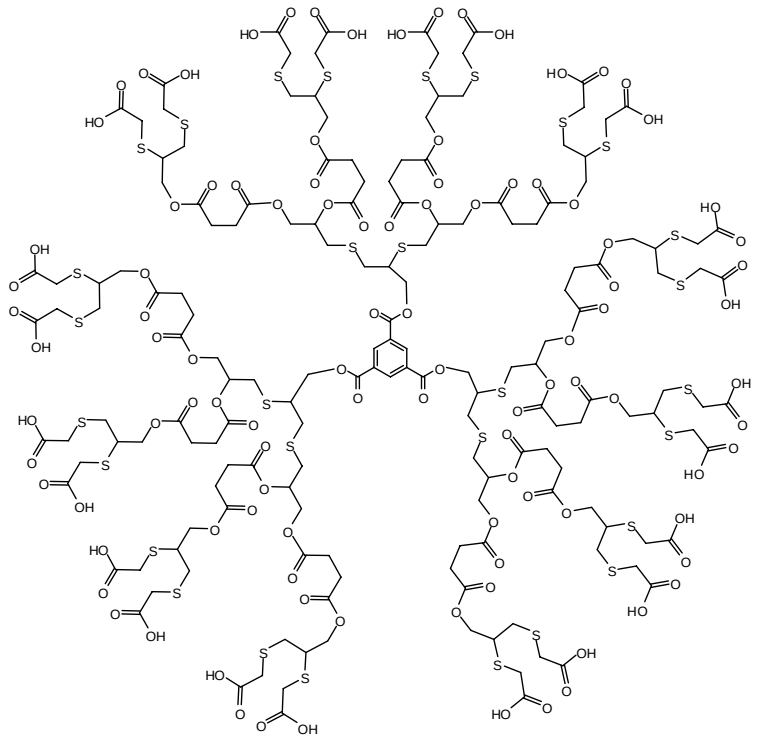


5

The following were placed in a vial: (**4**) (26 mg, 0.01 mmol), 5% weight 2,2'-dimethoxy-2-phenylacetophonone (DMPA), 0.03 mL DMF and finally thiolglycerol (260 mg, 240 mmol). The contents of the vial were then purged with N_2 for 10 min and irradiated for 10 min with 365 nm light at room temperature within a Ratek orbital mixer incubator (100 rpm). The contents were subsequently precipitated three times in diethyl ether to afford a clear viscous liquid before undergoing ultra-filtration using Sartorius Vivaspin 6 centrifugal filter devices with a molecular weight cut off of 5k before finally being freeze-dried. (Yield: 41 mg, 78%) ^{1}H-NMR (MeOH-d4, 300 MHz) δ 2.51-3.22 (m, 138H, -SC*H*$_2$-), 3.4-3.9 (m, 87H, -(CH$_2$)$_2$C*H*S-, -C*H*$_2$OH, -(CH$_2$)$_2$C*H*OH-), 4.20-4.5 (m, 36H, -OC*H*$_2$-), 4.5-4.8 (m, 6H, -CHC*H*$_2$O-), 5.25 (m, 6H, -(CH$_2$)$_2$C*H*O-), 8.87 (s, 3H, benzene). MALDI: calculated [M+Na$^+$] = 5249.50 g/mol, found [M+Na$^+$] = 5249.05 g/mol. IR: 3340 (br), 2920, 1730, 1410, 1230, 1150, 1030, 630, 530 cm^{-1}.

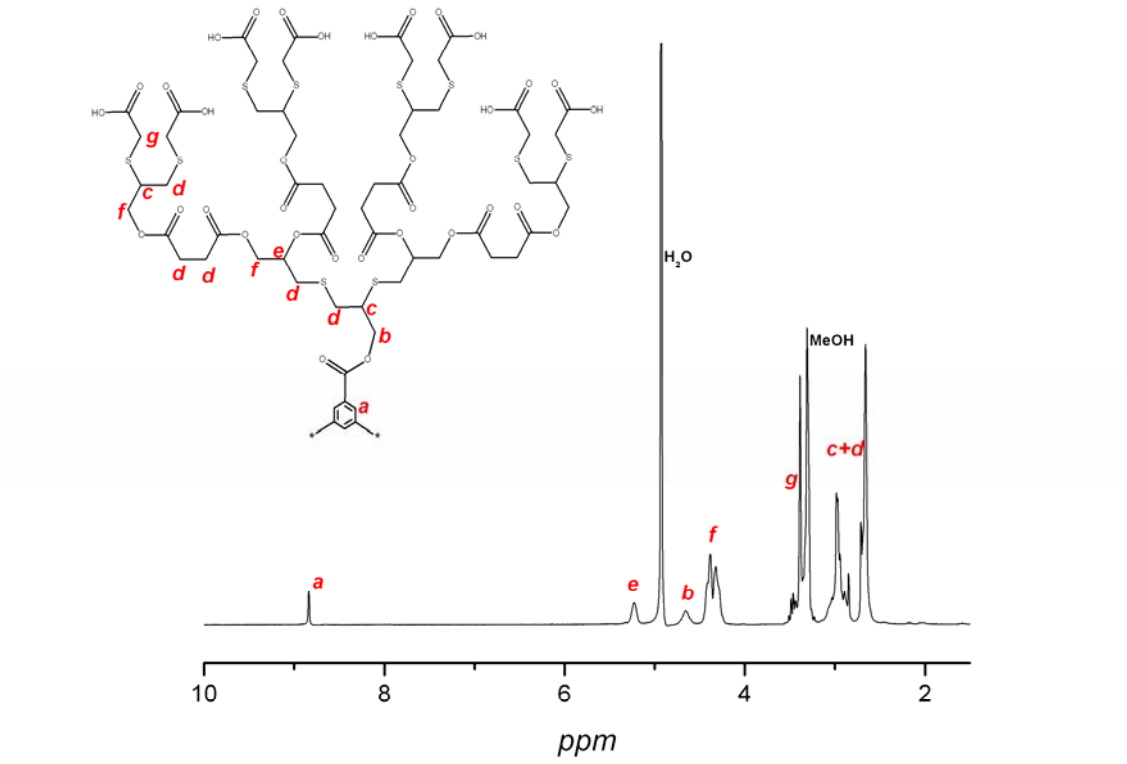


2.5 Synthesis of [G2]-1-thioglycolic acid adduct (6)

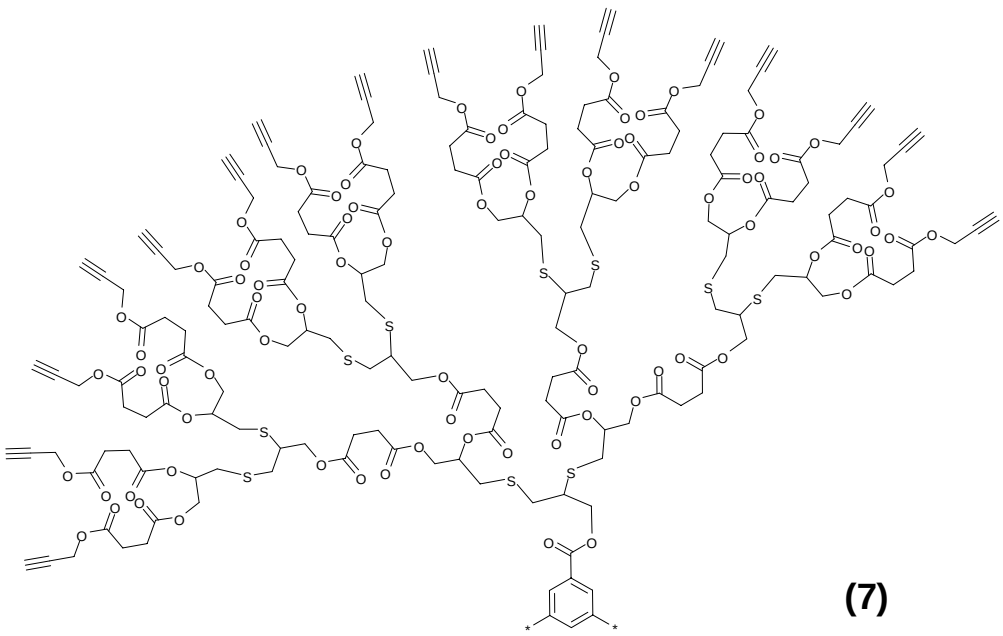


6

Compound (**4**) (26 mg, 0.01 mmol), 5% weight 2,2'-dimethoxy-2-phenylacetophonone (DMPA), 0.03 mL DMF and finally thiolglycerol (221 mg, 240 mmol) were placed in a vial. The contents of the vial were then purged with N_2 for 10 min and irradiated for 10 min with 365 nm light at room temperature within a Ratek orbital mixer incubator (100 rpm). The contents were subsequently precipitated three times in diethyl ether to afford a clear viscous liquid before undergoing ultra-filtration using Sartorius Vivaspin 6 centrifugal filter devices with a molecular weight cut off of 3k before being freeze-dried. (Yield: 44 mg, 91%) ^{1}H-NMR (MeOH-d4, 300 MHz) δ 2.51-3.19 (m, 105H, -C*H*$_2$-, -(CH$_2$)$_2$C*H*S-), 3.38-3.51 (m, 48H, , -C*H*$_2$COOH), 4.19-4.51 (m, 36H, -C*H*$_2$O-), 4.66 (m, 6H, -OC*H*$_2$CH-), 5.23 (m, 6H, -(CH$_2$)$_2$C*H*O-), 8.84 (s, 3H, benzene). MALDI: calculated [M+K$^+$] = 4880.59 g/mol, Found [M+K$^+$] = 4880.88 g/mol. IR: 3350 (br), 2920, 1720, 1380, 1230, 1150, 1030, 880, 630, 560 cm^{-1}.

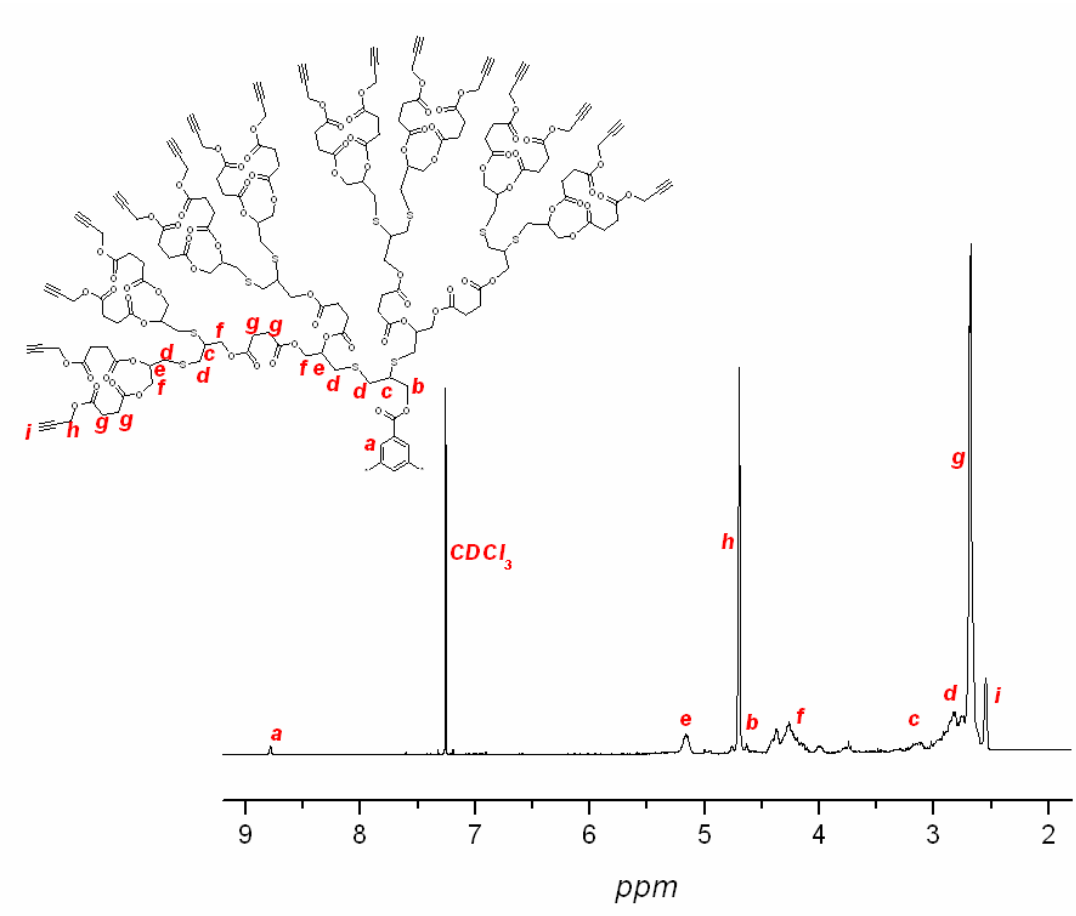


2.6 Synthesis of [G2]-yne$_{48}$ (7)

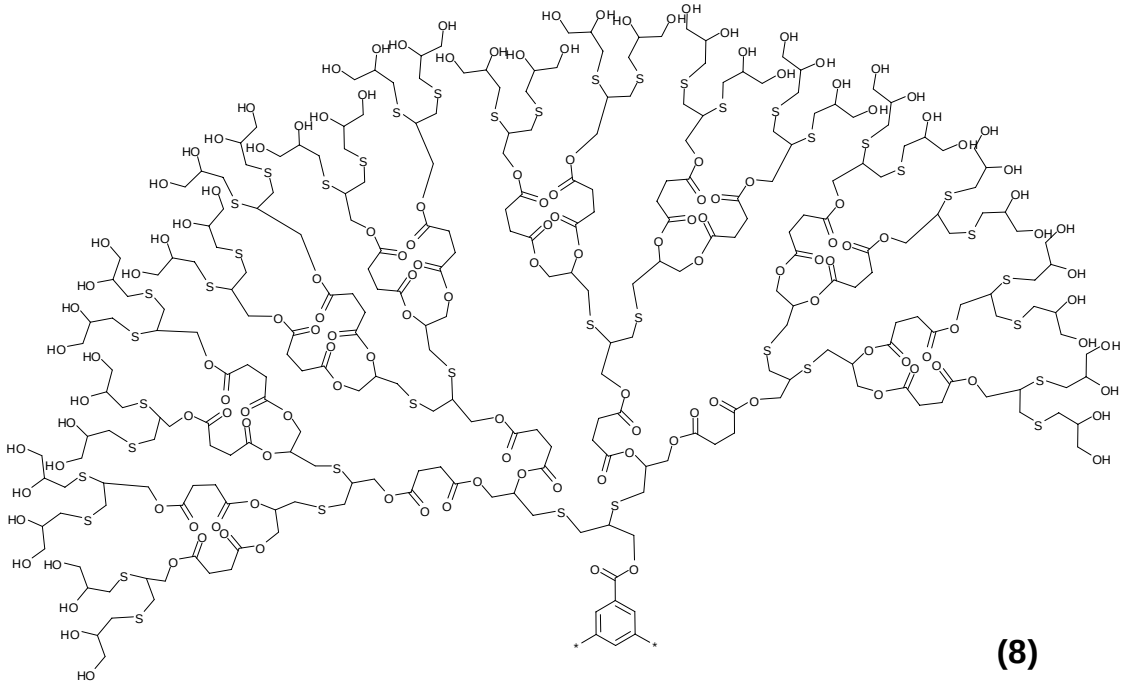


(7)

Hydoxyl-terminal dendrimer (**5**) (26 mg, 5 μmol), acetylene anhydride (**3**) (212 mg, 0.72 mmol), 4-(dimethylamino)pyridine (DMAP) (4.4 mg, 0.036 mmol), TEA (0.1 mL, 0.72 mmol) and 5 mL DMF were placed in a small vial. The reaction was left overnight at 40 ^{o}C and the contents were subsequently precipitated in diethyl ether and then further purified by column chromatography. (Yield: 46 mg, 78%) ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.55 (s, 78H, -C≡C*H*), 2.68 (m, 240H, -COC*H*$_2$C*H*$_2$CO-), 2.7-3.0 (m, 90H, -SC*H*$_2$-), 3.13 (m, 15H, -(CH$_2$)$_2$C*H*S-), 3.75-4.63 (m, 108H, -OC*H*$_2$CH-), 4.7 (t, 96H, -C*H*$_2$C≡CH), 5.17 (m, 30H, -(CH$_2$)$_2$C*H*O-), 8.80 (s, 3H, benzene). IR: 3280, 2930, 2130, 1730, 1410, 1380, 1210, 1150, 1030, 990, 700, 630, 550 cm^{-1}.

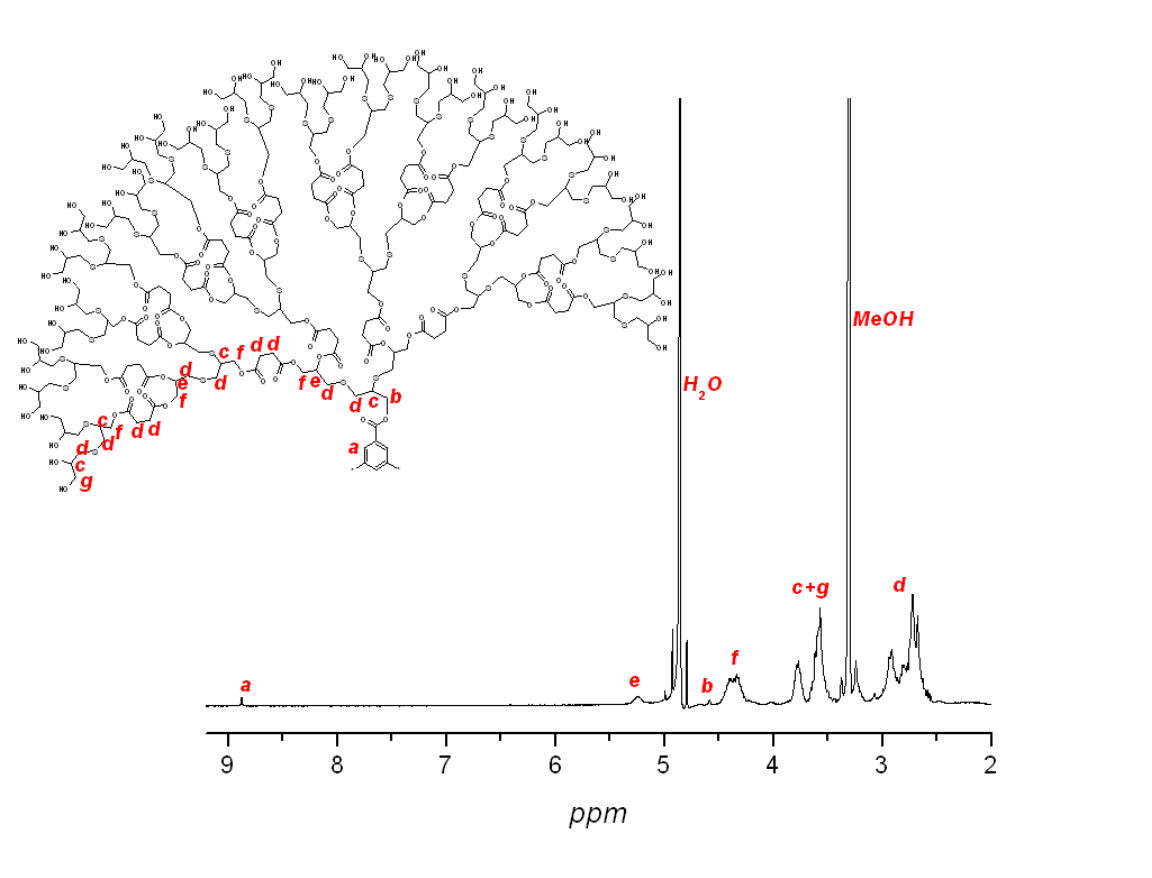


2.4 Synthesis of [G3]-OH_{192} (8)



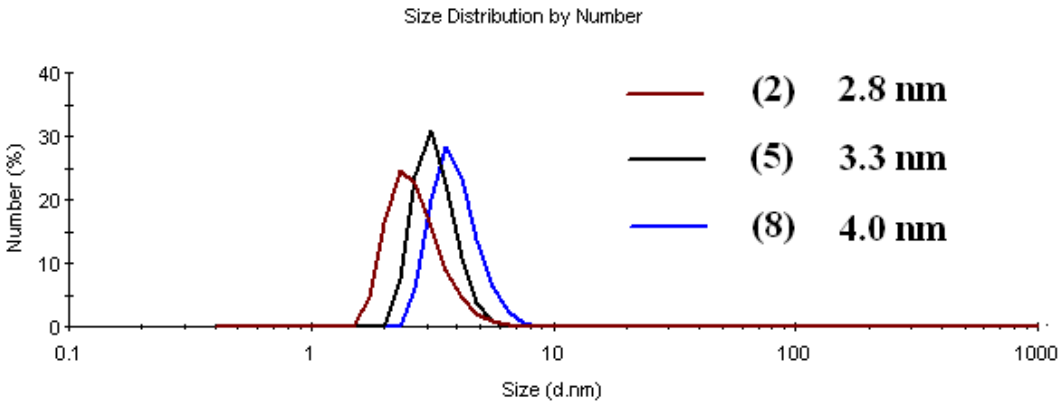
(8)

The following were placed in a vial: **(7)** (10 mg, 0.84 μmol), 4 mg 2,2'-dimethoxy-2-phenylacetophonone (DMPA), 0.02 mL DMF and finally thiolglycerol (42 mg, 0.4 mmol). The contents of the vial were then purged with N_2 for 10 min and irradiated for 10 min with 365 nm light at room temperature within a Ratek orbital mixer incubator (100 rpm). The contents were subsequently precipitated three times in diethyl ether to afford the final product. (Yield: 17 mg, 89%) ^{1}H-NMR (MeOH-d4, 300 MHz) δ 2.51-3.24 (m, 618H, -SC*H*$_2$-), 3.4-3.9 (m, 351H, -(CH$_2$)$_2$C*H*S-, -C*H*$_2$OH, -(CH$_2$)$_2$C*H*OH-), 4.20-4.5 (m, 198H, -OC*H*$_2$-), 4.5-4.8 (m, 6H, -CHC*H*$_2$O-), 5.24 (m, 30H, -(CH$_2$)$_2$C*H*O-), 8.88 (s, 3H, benzene). MALDI: calculated [M+H$^+$] = 22240.62 g/mol, Found [M+H$^+$] = 22241.17 g/mol. IR: 3360 (br), 2920, 1730, 1630, 1410, 1160, 1070, 1030, 760, 630, 550 cm^{-1}.



2.7 Size of dendrimers

Dynamic Light Scattering (DLS) was used to determine the approximate diameters of the hydroxyl-functionalized dendrimers. The results are summarized in the following figure:



3.0 Synthesis of dendrimer-platinate

3.1 Synthesis of (6)-CDDP conjugate

A procedure similar to one previously described[2, 3] was used for the conjugation reaction. *cis*-Dichlorodiammineplatinum (CDDP) (10 mg) was suspended in 10 ml distilled water and mixed with silver nitrate ([$AgNO_3$]/[CDDP] = 2) to form the aqueous complex. The solution was kept in the dark at room temperature for 4 hrs. AgCl precipitates were found after the reaction was complete. The mixture was then centrifuged at 8000 rpm for 10 min to eliminate the AgCl precipitates and the supernatant was purified by passing through a 0.22 μm filter. Dendrimer (**6**) (4.8 mg, dissolved in 1 ml 1 mg/ml NaOH solution) was added to the above CDDP aqueous solution and left to react for 48 h at 37 ^{o}C with gentle shaking to prepare the (**6**)-CDDP conjugate. The prepared conjugate was purified by ultra-filtration[4] using Sartorius Vivaspin 6 centrifugal filter devices with a molecular weight cut off of 3k and then freeze-dryed.

3.2 Determination of the loading of CDDP

Quantitative determination of CDDP was performed using a previously described method using OPDA.[5] The conjugated species, containing an estimated 3-12 mg CDDP was dissolved in 0.6 ml water and mixed with 0.6 ml OPDA which had been dissolved in DMF (1.2 mg/ml). The reaction mixture was placed in a 100 ^{o}C oil bath for 10 min and the absorbance of the solutions was measured at 703 nm. The amount of CDDP in the conjugate was calculated in reference to standard solutions of free CDDP.

4.0 References

1. P. Antoni, Y. Hed, A. Nordberg, D. Nystrom, H. von Holst, A. Hult and M. Malkoch, *Angew. Chem. Int. Ed.*, 2009, **48**, 2126-2130.
2. K. J. Haxton and H. M. Burt, *Dalton Transactions*, 2008, 5872-5875.
3. H. Ye, L. Jin, R. Hu, Z. Yi, J. Li, Y. Wu, X. Xi and Z. Wu, *Biomaterials*, 2006, **27**, 5958-5965.
4. M. Yokoyama, T. Okano, Y. Sakurai, S. Suwa and K. Kataoka, *J. Controlled Release*, 1996, **39**, 351-356.
5. B. Schechter, M. A. Rosing, M. Wilchek and R. Arnon, *Cancer Chemoth Pharm*, 1989, **24**, 161-166.