Supplementary Material (ESI) for Chemical Communications


**ELECTRONIC SUPPORTING INFORMATION FOR**

# Clickable Polyglycerol Hyperbranched Polymers and Their Application to Gold Nanoparticles and Acid-Labile Nanocarriers

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**General Methods, Materials, and Instrumentation**

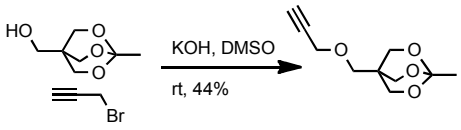
All reactions were performed under an atmosphere of $N_2$ unless otherwise stated. All chemicals were purchased from Sigma Aldrich unless otherwise stated and of reagent quality. Chemicals were used as received unless stated below. DMSO was dried over 4Å molecular Sieves. Diglyme was distilled under reduced pressure from NaH. Glycidol was twice distilled under reduced pressure. Propargyl alcohol was distilled under nitrogen. HOBT was purchased from Nova Biochem. EDCI was purchased from Advanced Chemtech. 11-Azido-3,6,9-trioxaundecan-1-amine (≥90%) was obtained from Fluka. Compound **11** was synthesized as described previously in our lab.[1] Benzoylated dialysis membranes (MWCO = 2000 Dalton) were purchased from Sigma Aldrich. Dialysis cassettes (Float-A-Lyzer dialysis tubes, MWCO=50,000 Dalton) were purchased from Spectra Pro. Reactions were monitored by TLC using silica gel 60 F254 on glass plates (Aldrich). Gravity and flash chromatography was performed with SiliaFlash® P60 silica gel (SiliCycle). TLC bands were visualized by UV or $I_2$ stain. BioWhittaker® 10X PBS (phosphate buffered saline) was obtained from Lonza. All solvent ratios are reported in % v/v unless otherwise noted. Aqueous SEC was performed using Bio-Rad Bio-Gel® p-10 Gel medium mesh eluting in 20% methanol in water.

Analytical SEC was performed using a Viscotek Viscogel I series LMW cutoff 2x, and HMW cutoff 1x columns in series with 0.05% (w/w) LiBr in DMF as the eluent with a column temperature of 50 °C and a flow rate of 1.0 mL/min or a Waters Ultrahydrogel™ 120 1x and Ultrahydrogel™ 250 1x columns in series with

0.01% (w/w) $NaN_3$ in water as the eluent with a flow rate of 1.0 mL/min. Peak detection was achieved by a Viscotek triple array 300 refractive index detector for the DMF column or a Hitachi L-4000H UV detector and Viscotek VE3580 refractive index detector for the aqueous column. SEC-derived molecular weights were based on calibration with linear polystyrene for the DMF column. $^1H$ NMR and $^{13}C$ NMR data was obtained on a 500 MHz Varian U500 instrument at the VOICE NMR laboratory at the University of Illinois. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in Hertz (Hz). $^1H$ NMR spectra obtained in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm, $CD_3OD$ spectra were referenced to residual $CD_2HOD$ at 3.31 ppm, and $D_2O$ were referenced to residual HDO at 4.79 ppm. $^{13}C$ NMR spectra obtained in $CDCl_3$ were referenced to 77.0 ppm and $CD_3OD$ spectra were referenced to 49.0 ppm. $^1H$ NMR end group analysis based on methylene peak at 4.18 ppm and polymer backbone 3.9 – 3.4 ppm assuming 74.01 g/mol per 5 H. MALDI MS spectra were obtained on an Applied Biosystems Voyager-DE STR mass spectrometer. Matrices employed in the collection of MALDI spectra were dithranol and 2-(4-hydroxyphenol-azo)-benzoic acid (HABA). Data was calibrated to an external standard solution of insulin and cytochrome C. High resolution ESI mass spectra were obtained using a Micromass Q-Tof Ultima. High resolution CI mass spectra were obtained on a Micromass 70-VSE. Fluorescence experiments were performed using a Horiba Jobin Yvon FluoroMax-3 in either the spectral emission or kinetics modes. IR spectra were collected on a Matson Galaxy Series FTIR 5000 with samples cast as thin films on NaCl plates.

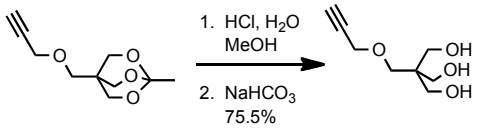
Absorption spectra were taken on a CARY 500 Scan UV-Vis-NIR spectrophotometer. Transmission electron microscopy (TEM) data were obtained with a JEOL 2100 Cryo-transmission electron microscope operating at 200 kV. TEM grids were prepared by drop casting 7.0 μL of the purified gold nanoparticle solution on the TEM grids and drying them in atmosphere. Zeta potential and dynamic light scattering measurements were performed on a Brookhaven Zeta PALS instrument. Melting points were measured with a Thomas Hoover melting point apparatus and are uncorrected.

## Synthesis and Characterization



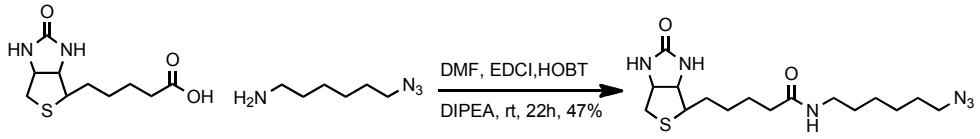

**1-Methyl-4-prop-2-ynyloxymethyl-2,6,7-trioxa-bicyclo[2.2.2]octane.** A mixture of 7.6 g (190 mmol, 2.8 equiv) crushed KOH in 40 mL DMSO was cooled in a 10 °C ice water bath before adding 10.8 g (67 mmol, 1 equiv) (1-Methyl-2,6,7-trioxa-bicyclo[2.2.2]oct-4-yl)-methanol.[2] The slurry was stirred for 5 minutes. A solution of 11.25 mL (163 mmol, 2.4 equiv) propargyl bromide (80% in toluene) was slowly added to the mixture dropwise. The solution was kept on ice for 5 minutes. The ice bath was removed and the reaction warmed due to the exothermic reaction. After 35 minutes the reaction cooled to room temperature. The reaction was diluted with 100 mL ethyl acetate and 20 mL water. The biphasic mixture was filtered and poured into a separatory funnel and the

aqueous layer was removed. The organic layer was washed three times with 50 mL portions of water. The organic layer was dried over sodium sulfate and concentrated and passed through a silica plug (5:1 petroleum ether:ethyl ecetate). The resulting orange solid was dissolved in a minimum of ethyl acetate and precipitated with petroleum ether to give 5.8 g (43%) of a white crystalline solid. Attempts to recrystallize by standard heating and cooling resulted in decomposition. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 4.09 (d, *J* = 1 Hz, 2H), 4.00 (s, 6H), 3.29 (s, 2H), 2.44 (t, *J* = 1 Hz, 1H), 1.45 (s, 3H). $^{13}$C NMR (500 MHz, $CDCl_3$) δ 108.5, 78.7, 75.1, 69.3, 67.9, 58.6, 34.6, 23.3. MS (HRCI) *m/z* 199.0973 (M + $H^+$), theoretical 199.0970. mp 80.5-82.5 °C.

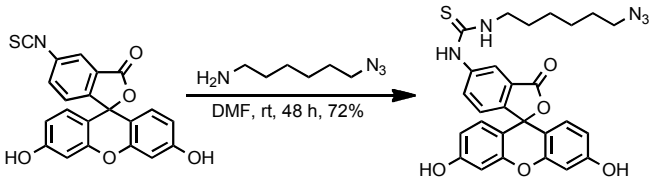



**2,2-Bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol (1).** A 1 mL solution of 1 M HCl was added to a 3.89 g (19.6 mmol) sample of 1-Methyl-4-prop-2ynyloxymethyl-2,6,7-trioxa-bicyclo[2.2.2]octane suspended in 20 mL 3:1 $H_2O$:MeOH. To the solution was added 10 mL of $H_2O$ and the clear solution was stirred overnight at room temperature. The solution was basified to pH 10 with a solution of saturated $NaHCO_3$. The solution was stirred an additional 24 hours. The reaction was filtered and the solvent was removed under vacuum. The resulting solid was suspended in methanol and the insoluble salts were removed by filtration. Solvent removal produced a free flowing oil which was purified by column chromatography using silica gel (1:4 petroleum ether:ethyl acetate) to

give 2.61 g (75%) of a clear oil. $^{1}$H NMR (500 MHz, $CD_3OD$) δ 4.14 (d, *J* = 2.5 Hz, 2H), 3.60, (s, 6H), 3.52, (s, 2H), 2.83 (t, *J* = 2.5 Hz, 1H). $^{13}$C NMR (500 MHz, $CD_3OD$) δ 79.8, 74.9, 69.4, 62.0, 58.4, 45.6. MS (HRES) *m/z* 175.0973 ($M^+$), theoretical 175.0970.

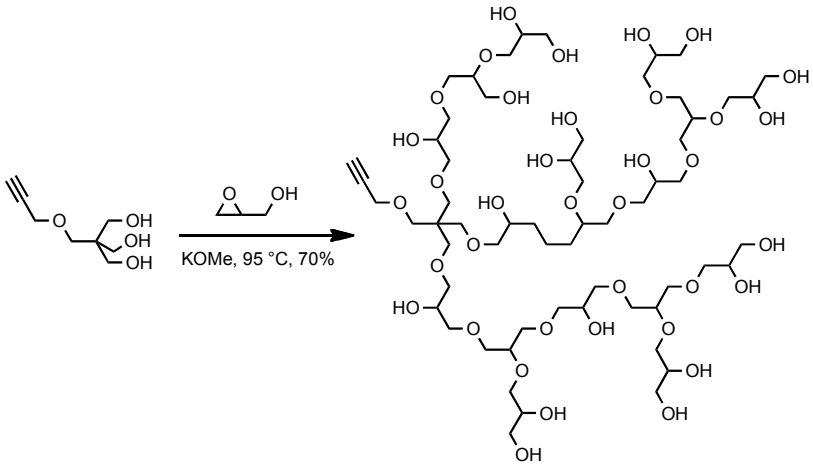



**N-Biotinyl-6-azido-1-hexylamine (3).** Compound prepared in an alternative procedure to that described by Inverarity.[3] To a solution of 100 mg (0.41 mmol, 1 equiv) of Biotin, 85 mg (0.61 mmol, 1.5 equiv) of HOBT, and 104 mg (0.49 mmol, 1.2 equiv) of EDCI, suspended in 4 mL DMF was added 85 mg, (0.49 mmol, 1.2 equiv) 6-azido-hexylamine.[4] The mixture was stirred for 36 h at room temperature. Silica gel was added to the reaction and DMF was removed under vacuum at 45 °C. The dry silica was loaded onto a silica column and the compound was purified by flash column chromatography (gradient 1:0 $CH_2Cl_2$:methanol to 10:1 $CH_2Cl_2$:methanol) to give 70.3 mg (47%) of the product as a pale yellow solid.



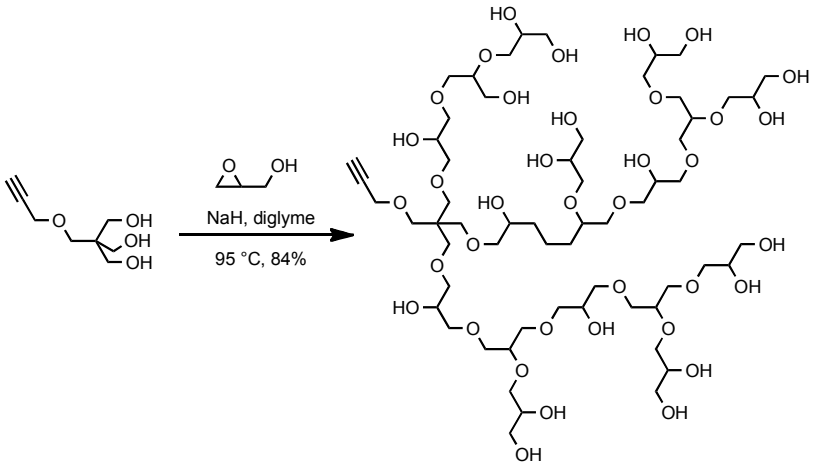

**Fluorescein-5-(6-Azido-hexyl)-thiourea (5)**. To a screw cap scintillation vial containing 100 mg fluorescein-5-Isothiocianate (0.26 mmol) and 54 mg 6-

azido-hexylamine[4] was added 1 mL DMF. The mixture was stirred for 48 h at room temperature. The reaction was dry loaded onto a silica column and purified by column chromatography using silica gel (2:1 ethyl acetate:hexanes) to give 98 mg (72%) of the product as a dark red solid. $^1$H NMR (500 MHz, $CD_3OD$) δ 8.20 (s, 1H), 7.81, (d, *J* = 7.3 Hz, 1H), 7.19, (d, *J* = 8 Hz, 1H), 6.83 (d, *J* = 9 Hz, 2H), 6.78, (d, *J* = 2 Hz, 2H), 6.65, (dd, J = 8.5, 2 Hz, 2H), 3.63 (br s, 2H), 1.75-1.60 (m, 4H), 1.5-1.4 (m, 4H). MS (HRES) *m/z* 532.1655 ($M^+$), theoretical 532.1655.



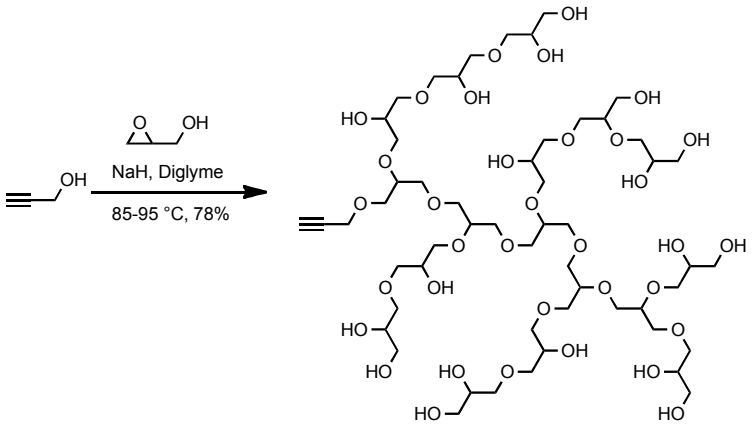

**Neat polymerization with 2,2-Bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol (1) as initiator (2a).** A 3.7 M KOMe solution in 150 uL MeOH (0.55 mmol, 0.1 equiv) was added to 1.0 g (5.7 mmol, 1 equiv) **1** in a three neck flask. The flask was equipped with a mechanical stirrer, rubber septum, and a schlenk line. The solution was stirred 30 minutes at room temperature and then the methanol was removed under vacuum at 65 °C for 4 hours. The temperature was increased to 95 °C the flask was backfilled with $N_2$. To the solution was added 13.1 mL (177 mmol, 32 equiv) Glycidol at a rate of 0.73 ml/h. The temperature was kept constant at 95 °C. After complete addition the temperature

was reduced to 60 °C and 25 mL MeOH was added. The solution was cooled to room temperature and quenched with 1 g of amberlite IR-120H ion exchange resin. The reaction mixture was filtered and the filtrate was precipitated with acetone twice. The thick oily residue was dried at 80 °C under vacuum 36 h to give 10.2 g (70 %) of a golden brown viscous oil. $^1$H NMR (500 MHz, $CD_3COD$) δ 4.92 (s, 62H), 4.16 (s, 2H), 3.90-3.45 (b, 207H), 2.92 (s, 1H) $^1$H NMR End group analysis $M_n$= 3000 g/mol.



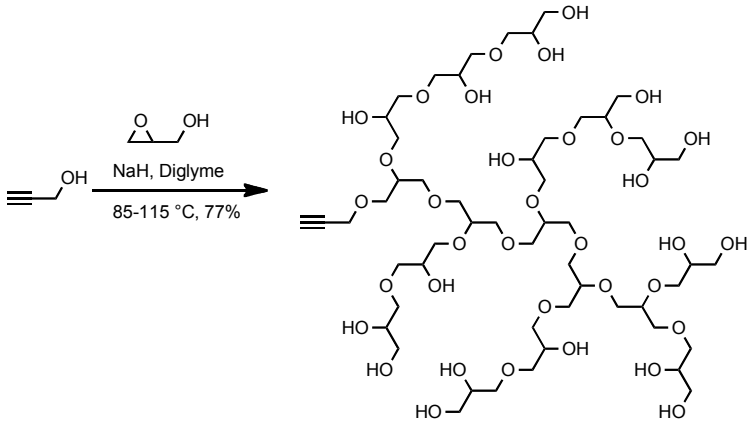

**Polymerization of HPG with 2,2-Bis-hydroxymethyl-3-prop-2-ynyloxy-propan-1-ol (1) as initiator in dilgyme solvent (2a).** A solution of 400 mg (2.27 mmol, 1 equiv) of **1** in 800 μL diglyme (dried over sieves), was added to an oven dried three neck flask equipped with a mechanical stirring mechanism containing 22.7 mg (0.568 mmol, 0.25 equiv) NaH (60% suspension in mineral oil). The solution was degassed by a series of three freeze pump thaw cycles. The solution was heated to 95 °C and 15.2 mL (227 mmol, 100 equiv) of glycidol was slowly added to the reaction over 64 h. After complete addition the reaction mixture was cooled to 55 °C and 20 mL methanol was added. The mixture was

stirred for 1 hour and allowed to cool to room temperature. Amberlite ion exchange resin, 1 g, was added and the mixture was stirred for an additional 5 hours. The mixture was filtered and purified by three rounds of acetone precipitation to give 14.1 g 84% of a clear colorless thick syrup. SEC (DMF) $M_n$ = 14,500 g/mol, $M_w/M_n$ = *1.54*.



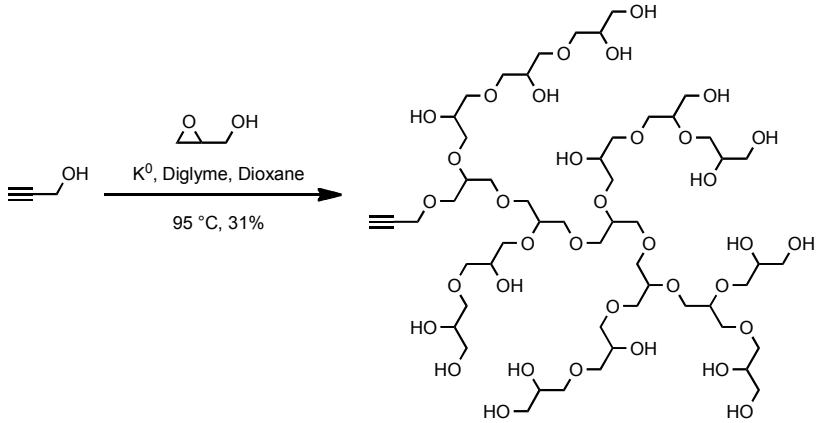

**Small scale polymerization with propargyl alcohol as initiator (2b).** A solution of 1 mL (17 mmol, 1 equiv) propargyl alcohol in 4.0 mL diglyme was added to an oven dried three neck flask equipped with a mechanical stirrer containing 67 mg (1.7 mmol, 0.1 equiv) NaH (60% suspension in mineral oil) in liquid nitrogen. The solution was degassed by a series of three freeze pump thaw cycles. The solution was heated to 75 °C and 25 mL (371 mmol, 22 equiv) of glycidol was slowly added to the vessel at a rate of 0.7 mL per hour. The temperature of the reaction was increased to 85 °C after 3 mL of glycidol was added and increased to 95 °C after 6 mL glycidol was added. After complete addition the reaction was stirred at 95 °C for 1 h. The reaction was cooled to 60 °C and 50 mL methanol was added. The dissolved polymer was cooled to room

temperature and 1 g of amberlite IR-120H ion exchange resin was added. This mixture was stirred for 8 h. The mixture was filtered, reduced in volume to obtain a thick viscous oil which was precipitated with acetone (x3). The viscous white oil was dissolved in methanol and dried under vacuum at 70 °C for 12 h to give 22 g (78%) of a golden yellow viscous oil. $^1$H NMR (500 MHz, $CD_3COD$) δ 4.92 (s, 66H), 4.20 (s, 2H), 3.90-3.45 (b, 172H), 2.95 (b, 1H), $^1$H NMR End group analysis $M_n$ = 2500 g/mol. SEC (DMF) $M_n$ = 6700 g/mol, $M_w/M_n$ = *1.17*. MS (MALDI) *m/z* 894 (M + $Na^+$), peak separation 74.

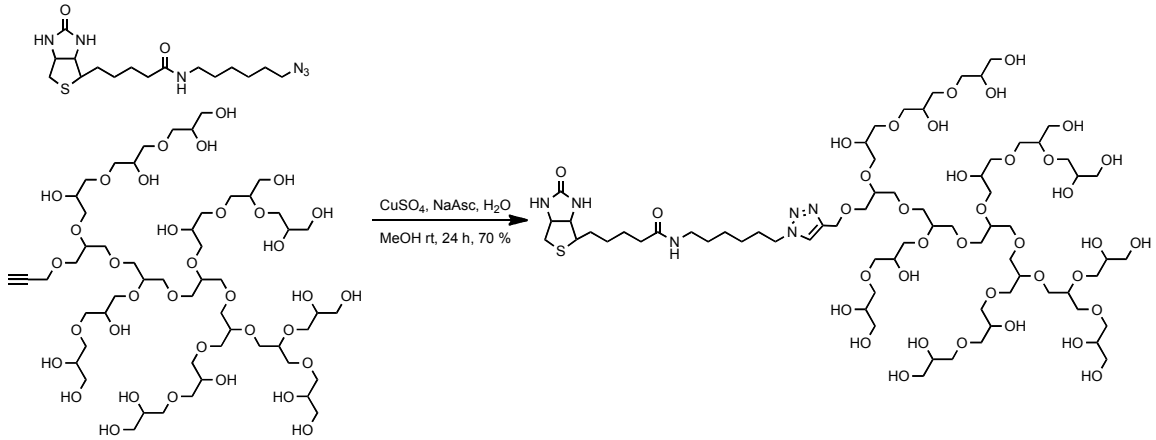



**Large scale polymerization and fractionation of HPG with propargyl alcohol as initiator (2b).** A solution of 3 mL (52 mmol, 1 equiv) proparyl alcohol in 15 mL diglyme (dried over sieves), was added to an oven dried three neck flask equipped with a mechanical stirring mechanism containing 201 mg (5.2 mmol, 0.1 equiv) NaH (60% suspension in mineral oil). The solution was degassed by a series of three freeze pump thaw cycles. The solution was heated to 85 °C and 94 mL (1.43 mol, 27 equiv) of glycidol was slowly added to the reaction over 50 h. The temperature was slowly increased to 115 °C and the addition rate increase from 1 mL/h to 2.7 mL/h over the course of the reaction.

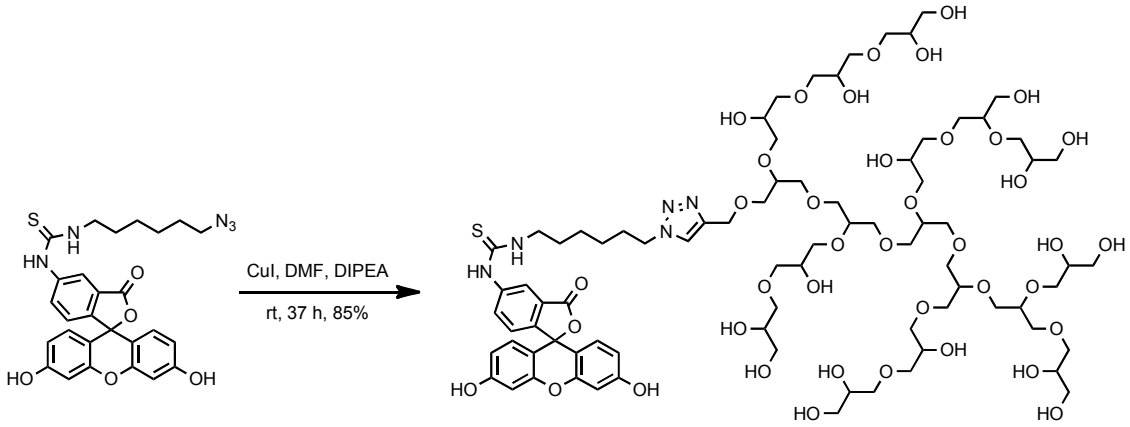
After complete addition the reaction mixture was cooled to 55 °C and 50 mL methanol was added. The mixture was stirred for 1 hour and allowed to cool to room temperature. Amberlite ion exchange resin, 5 g, was added and the mixture was stirred for an additional 5 hours. The mixture was filtered and purified by three rounds of acetone precipitation to give 90 g of crude polymer. The polymer was fractionated by dissolving it in 1 L of MeOH and rapidly stirring while acetone was added dropwise. The polymer composition in the supernatant was checked periodically by SEC. The solution was allowed to settle for 36 hours and the supernatant was removed to leave behind 20.1 g (18%) of polymer fraction 1. This process was repeated twice more to give 29.0 g (27%) polymer fraction 2, and 34.5 g (32%) polymer fraction 3. Total mass, 83.6 g (77 %). SEC (DMF) Crude polymer $M_n$ = 8200, $M_w/M_n$ = 1.50. Fraction 1 $M_n$ = 14,000, $M_w/M_n$ = 1.35. Fraction 2 $M_n$ = 11,600, $M_w/M_n$ = 1.26. Fraction 3 $M_n$ = 7500, $M_w/M_n$ = 1.25. $^1$H NMR End group analysis Fraction 1 $M_n$ = 7000. Fraction 2 $M_n$ = 6000. Fraction 3 $M_n$ = 3000. MS (MALDI) Fraction 3 *m/z* (peak) 1855 (M + $Na^+$ ), peak separation 74, $M_n$ =2100 g/mol, $M_w/M_n$ = 1.31.



**High molecular weight alkyne functional HPG synthesized via Brooks method (S13).** A solution of 120 mg (2.15 mmol, 1 equiv) propargyl alcohol in 700 μL diglyme (dried over sieves), was added to an oven dried three neck flask equipped with a mechanical stirring mechanism containing 8.5 mg (0.215 mmol, 0.1 equiv) $K^0$. The solution was stirred until the $K^0$ had completely dissolved. The solution was heated to 95 °C as 1 mL (15 mmol 7.2 equiv) glycidol was added over a 2 hour period. To the reaction was added 12 mL dry dioxane. An additional 19 mL (295 mmol, 137 equiv) glycidol was added over a 24 h period. The solution was cooled to 50 °C and 2 g amberlite exchange resin was added. The mixture was slowly stirred for 1 hour and filtered, precipitated three times with acetone and dried to reveal 6.56 g (31%) of a white sticky foam. $^1$H NMR (500 MHz, $D_2O$) δ 3.90-3.45 (b). Inverse gated $^{13}$C NMR (500 MHz, $CD_3OD$:$D_2O$ 1:1) δ 80.0 (1C), 78.5 (2C), 72.6 (5C), 71.5-70.5 (9.4C), 69.9-69.2 (3.9C), 63.2 (3C), 61.3 (1C) Degree of branching 53%. Dynamic light scattering, hydrodynamic diameter 14.0 nm, polydispersity 0.110. SEC (DMF) $M_n$ > 50,000 g/mol column exclusion limit.

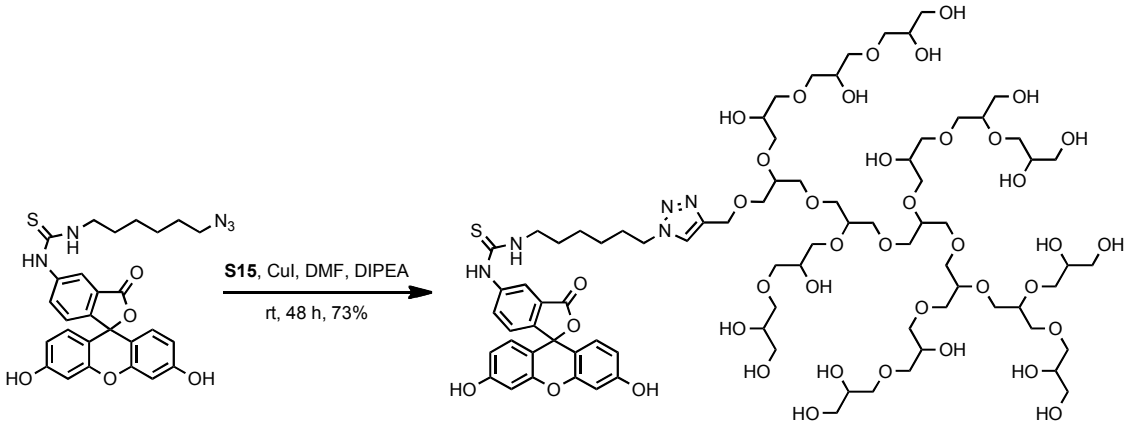


**HPG biotin conjugate (4).** A solution of 11.2 mg (0.045 mmol, 1 equiv) $CuSO_4$ and 17.8 mg (0.09 mmol, 2 equiv) sodium ascorbate in 500 µL DI water was added to a solution of 25 mg (0.07 mmol, 1.5 equiv) biotin azide **3**, and 90 mg (0.045 mmol, 1 equiv) polymer **2b**, (small scale polymerization, $^1$H NMR End group analysis $M_n$ = 2500 g/mol) in 1 mL MeOH. The solution was stirred under nitrogen for 24 h. The reaction mixture was precipitated with methanol to remove salts and filtered through a 0.2 micron nylon filter. The filtrates were dialyzed (benzoylated dialysis membrane) against water followed by methanol. The dialyzed solution was dried under vacuum to give 73 mg (70%) of a slightly green transparent film. $^1$H NMR (500 MHz, $CD_3COD$) δ 8.08 (s, 1H), 4.66 (bs, 2H), 4.52 (bs, 1H), 4.44 (bs, 2H), 4.33 (bs, 1H) 3.9-3.5 (b, 185H) 3.25-3.15 (b, 3H), 2.94 (bd, *J* = 12.5 Hz, 1H), 2.73 (bd, *J* = 12.5 Hz, 1H) 2.21 (bt, 2H), 1.95 (bt, 2H), 1.8-1.3 (bm, 12H). $^1$H NMR End group analysis $M_n$ = 2500 g/mol. SEC (DMF) $M_n$ = 7800 g/mol, $M_w/M_n$ = *1.25*.



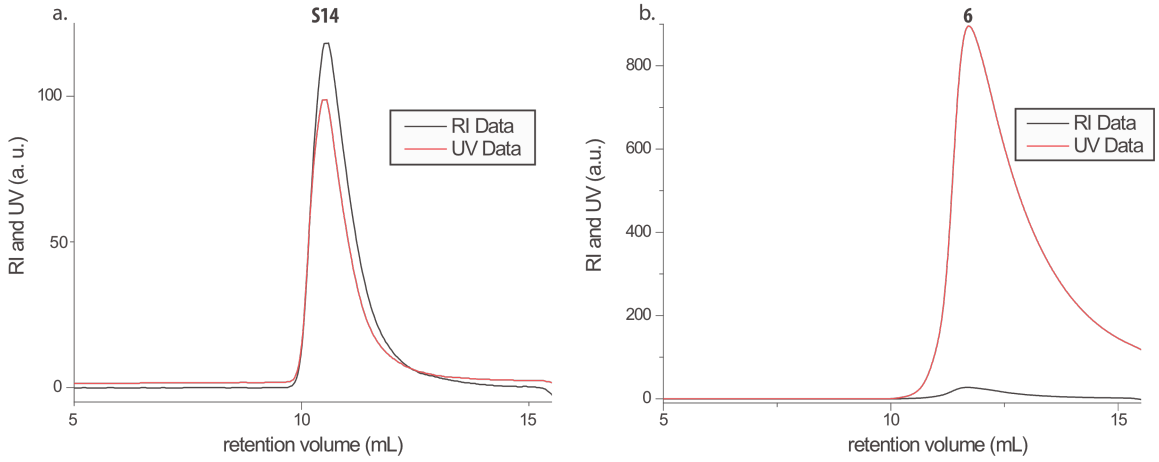

**HPG fluorescein conjugate (6).** A 0.500 mL solution of DMF containing 50 mg HPG **2b** (fraction 2, $^1$H NMR End group analysis $M_n$ = 6000, 0.0083 mmol, 1 equiv), 6.6 mg (0.012 mmol, 1.4 equiv) fluorescein-5-(6-Azido-hexyl)-thiourea

**5,** and 50 μL DIPEA was degassed with a nitrogen stream for 1 hour. To this solution was added 2.7 mg (0.014 mmol 1.7 equiv) CuI. The solution was sealed under a nitrogen atmosphere and stirred overnight. The reaction was monitored by SEC using UV and RI detectors. After 14 hours an additional 6.6 mg (0.012 mmol, 1.4 equiv) **5**, and 2 mg (0.0105 mmol, 1.27 equiv) CuI was added. The reaction was stirred for an additional 23 hours. To the reaction was added 1 mL pH 7 sodium phosphate buffer (10:1 water:85% phosphoric acid solution pH adjusted using concentrated NaOH solution) followed by 10 mL methanol. The solution was filtered and the solvent was removed. The polymer obtained was dissolved in methanol and precipitated in ether. The precipitated polymer was purified on an aqueous SEC column (5:1 water:methanol), collecting the first orange colored band. Solvent removal gave 47 mg (85%) of a dark red film. $\lambda_{max}$(sodium phosphate buffer, pH = 8.1) = 495 nm, $\varepsilon_{max}$(sodium phosphate buffer, pH = 8.1, assuming $M_n$ = 6000 based on $^1$H NMR end group analysis) = 68,000 $M^{-1}$ $cm^{-1}$.

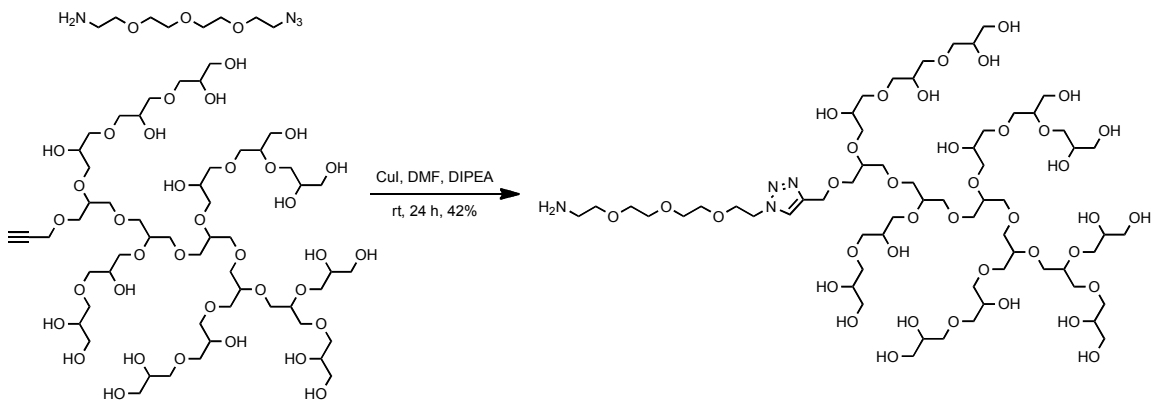



**High Molecular Weight HPG Fluorescein Conjugate (S14).** A 2 mL solution of 250 mg high molecular weight HPG **S13**, 100 μL DIPEA and 2 mg

fluorescein-5-(6-Azido-hexyl)-thiourea **5**, and 40 mg CuI was degassed by 3 freeze pump thaw cycles. The solution was warmed to room temperature and stirred under nitrogen for 48 hours. The solution was treated with 12mL pH 7 sodium phosphate buffer and stirred for 1 hour and filtered to remove a blue solid. The filtrates were precipitated with acetone and dissolved in 3 mL water and 5 mL pH 7 sodium phosphate buffer. This solution was treated with 5 mL methanol and a white solid was removed by filtration. The filtrates were further purified by dialysis (spectra/por MWCO = 12,000) against water. The dialyzed polymer was lyophilized to reveal 182 mg (73%) of a yellowish brown film. SEC (DMF) $M_n$ > 50,000 g/mol column exclusion limit.




**S1.** Unnormalized SEC curves of (a) high and (b) low molecular weight HPG functionalized with fluorescein using a UV and RI detector. The ratio of the UV and RI area ($UV_{area}/RI_{area}$) was 0.95 for **S14** and 38 for **6**.



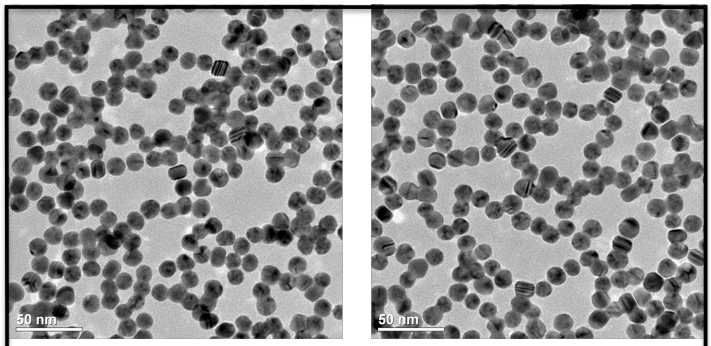
**HPG Amino PEG conjugate (8).** To a solution of 100 mg HPG **2b** (fraction 3, $^1$H NMR End group analysis $M_n$ = 3000 g/mol, 0.033 mmol, 1 equiv) was added 25 mg 11-Azido-3,6,9-trioxaundecan-1-amine **9** (0.114 mmol, 3.5 equiv) and 100 µL DIPEA. The solution was degassed with a nitrogen stream for 15 minutes. To the solution was added 4.5 mg CuI (0.023 mmol, 0.72 equiv) and the solution was degassed an additional 20 minutes. The reaction was stirred under nitrogen 24 hours. To the reaction was added 1 mL deionized water and stirred while 2 mL pH 7 sodium phosphate buffer (10:1 water-85% phosphoric acid solution pH adjusted using concentrated NaOH solution) was slowly added. An additional 1 mL DMF was added and the solution was filtered washed with ether and ethyl acetate and precipitated with THF. The polymer was collected by centrifugation dissolved in methanol, precipitated in THF twice more dissolved in methanol and filtered before drying under vacuum to give 96 mg of a clear film. Polymer was further purified by dialysis (benzoylated dialysis membrane) against de-ionized water to give 42 mg (42%) of a clear film. $^1$H NMR (500 MHz, $H_2O$) δ 8.12 (s, 1H), 4.71 (bs, 2H), 4.67 (bs, 1H), 4.05-3.50 (b, 370H) 3.21 (t, $J$ = 5.5, 1H), $^1$H NMR End group analysis $M_n$ = 5500g/mol. SEC (DMF) $M_n$ = 6680 g/mol, $M_w/M_n$ = *1.25*. MS (MALDI) *m/z* 2831 (M + $K^+$), peak separation 74.

**Gold nanoparticles synthesis (10).** Citrate-capped gold nanoparticles were synthesized using the Frens method.[5] An aqueous solution of 0.25 mM $HAuCl_4$, 100 mL, was heated in a conical flask to boiling. To the boiling solution,

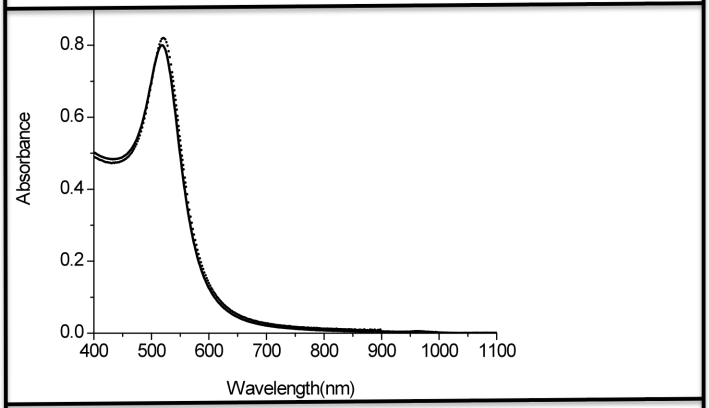
3.0 mL of an aqueous solution of 1% (w/w) sodium citrate was added. The heating was maintained until a deep ruby red color appeared (10 minutes), indicating the formation of gold nanoparticles. Transmission electron microscopy measurements indicated that the nanoparticles were spherical with a diameter of 13.5 ±1.1 nm. Zeta potential analysis indicated that the particles bore a net negative charge of -30.2 mV, due to adsorbed citrate.

**Attachment of amino-HPG to citrate-capped nanoparticles (7).** The as-prepared solution of the citrate-capped nanoparticles (4.0 mL) were centrifuged at 5000 rcf for 10 minutes using 1.5 mL Eppendorf microcentrifuge tubes. The supernatant was withdrawn and the resulted pellets were collected into 1.0 mL DMF and transferred into a 2.0 mL glass vial. To this vial, 70 µL of either aqueous HPG solution (42 mg/mL) or DI water (control) was added and mixed gently with a pipette. The reaction was left for 48 hours before purification. The nanoparticle solutions were centrifuged at 5000 rcf for 10 minutes and the resulted pellets were resuspended in DI water. The gold nanoparticles were dialyzed (Float-A-Layzer, MWCO=50,000 Daltons) at 25˚ C against DI water (1.0 L) for 12 hours to get rid of any left over reactants and non-attached polymer. Zeta potential analysis indicated that the particles bore a net negative charge of –0.01 mV ± 4.

a



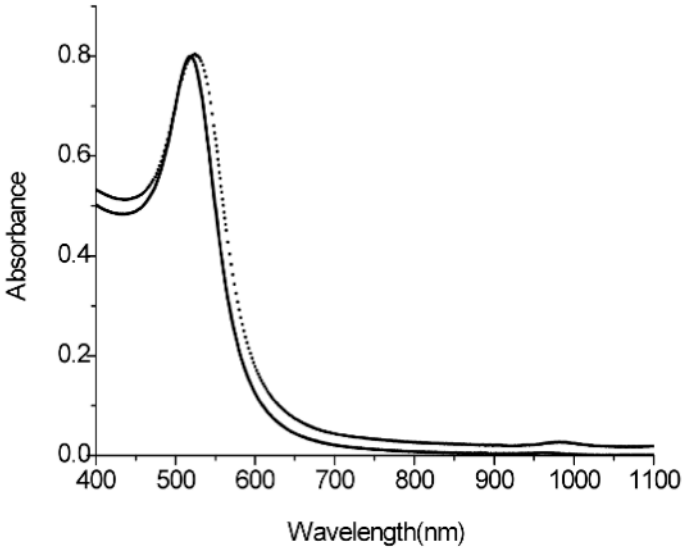

b

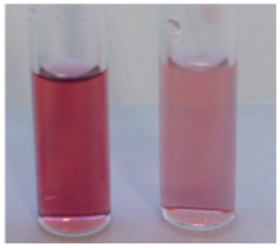



c

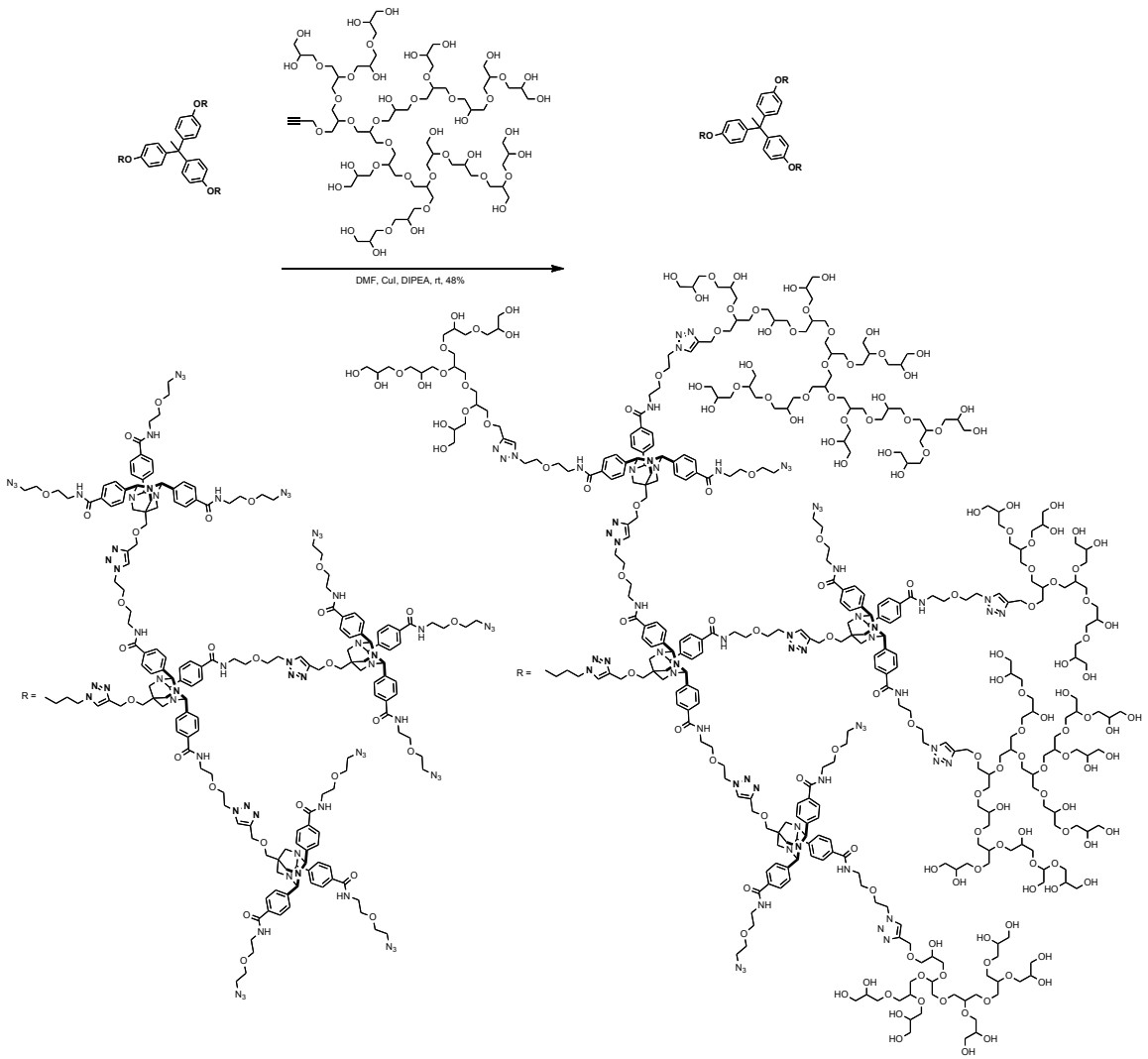
| | Zeta potential (mV) | Hydrodynamic diameter (nm) |
|---|---|---|
| Citrate-NP | -30.19±0.6 | 28.2±0.4 |
| HPG-NP | -0.01±4.11 | 30.9±0.7 |

**S2.** a) Transmission electron micrographs of citrate-capped gold nanoparticles (left) and HPG-capped nanoparticles (right). Scale bar=50 nm. b) UV-Vis spectra of citrate-capped gold nanoparticles (solid line) and HPG-capped nanoparticles (dashed line). c) Zeta potential and hydrodynamic diameter values of citrate-capped gold nanoparticles and HPG-capped nanoparticles.



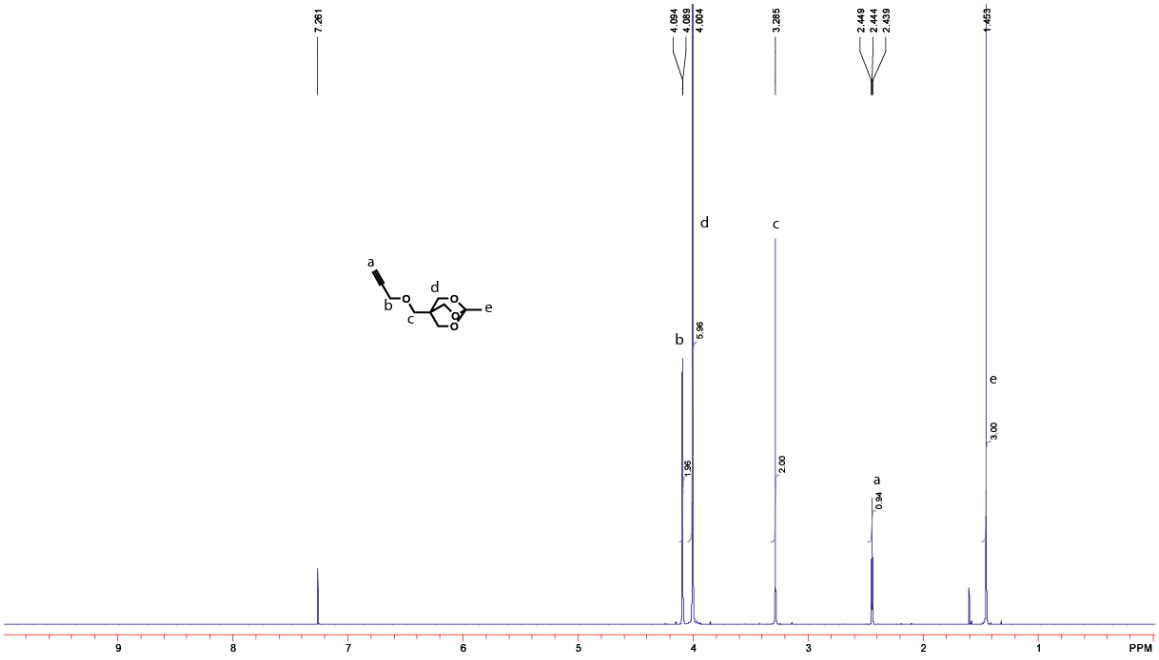



**S3**. Left) UV-vis spectra of Citrate-capped gold nanoparticles (solid line) in water and same nanoparticles suspended in DMF (dashed line). Right) Optical photographs of citrate capped nanoparticles in DMF (left) and water (right).




**TAA dendrimer polymer conjugation (12).** A solution of 33 mg (2.9 μmol, 78 μmol azide, 1 equiv $N_3$) second generation TAA dendrimer **5,**[1] 195 mg (78 μmol, 1 equiv) polymer **2b** (fraction 3 $^1$H NMR End group analysis $M_n$ = 3000 g/mol, 0.033 mmol), and 110 μL (632 μmol 8.1 equiv) DIPEA in 1 mL DMF was degassed under a nitrogen stream. To the solution was added 0.15 mg (0.8 μmol) CuI. The reaction mixture was stirred 24 hours at room temperature. An additional 2 mg (10 μmol) CuI was added and the reaction was stirred another 24 hours. The reaction mixture was diluted with 20 mL $H_2O$ and 4 mL of a pH 9.2 100 mM $NH_4OH$/Acetic Acid solution. The solution was filtered and the filtrate

was freeze-dried to reduce its volume. This solution was dialyzed against methanol. The dialyzed solution was passed through a 0.2 micron nylon filter. Solvent was removed to give 135 mg (60 %) of a clear oil. $^{1}$H NMR (500 MHz, $CD_3COD$) δ 8.1-7.6 (b, 1H), 4.61 (bs, 1H), 3.9-3.3 (b, 19H). SEC (DMF) $M_n$ < 50,000 g/mol column exclusion limit. IR (thin film): 3370, 2920, 2105, 1641, 1112 $cm^{-1}$.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4.004
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2.439
1.453
a
b
c
d
e
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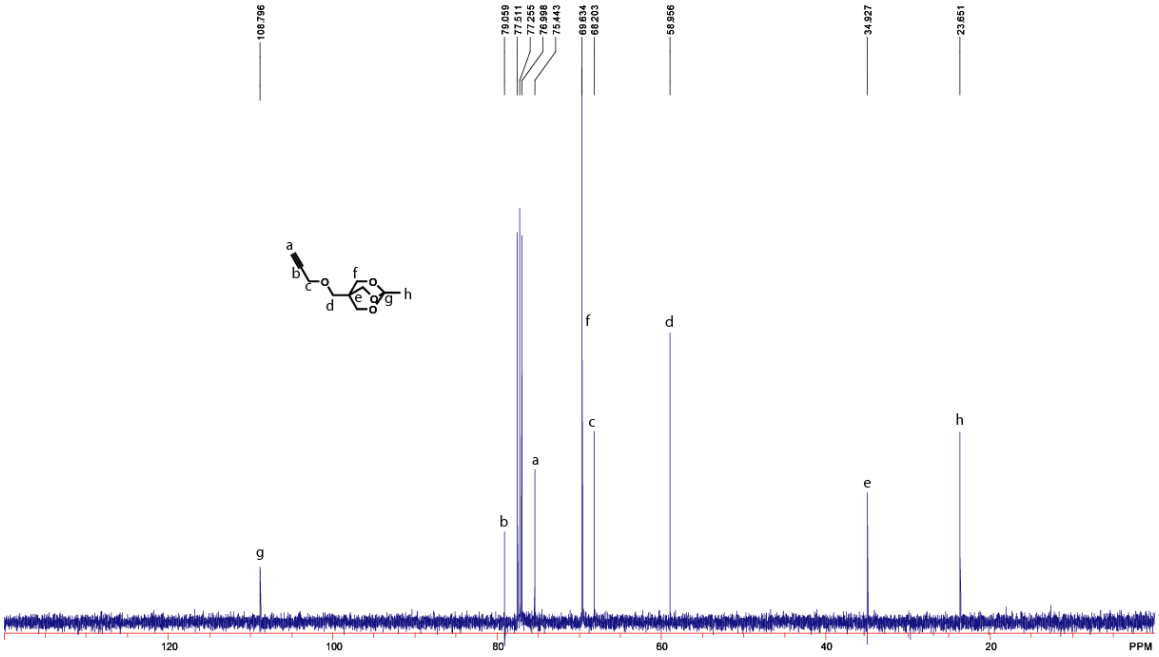
1.96
2.00
0.94
3.00
9
8
7
6
5
4
3
2
1
PPM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69.834
68.203
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a
b
c
d
e
f
g
h
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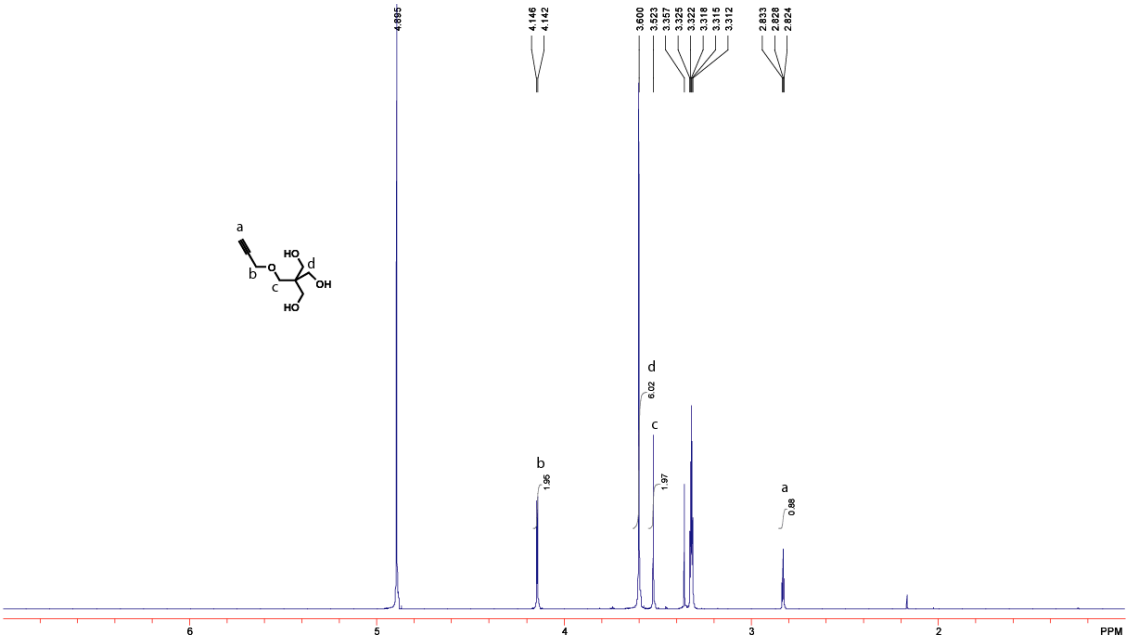
100
80
60
40
20
PPM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4.142
3.600
3.523
3.357
3.325
3.322
3.318
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3.312
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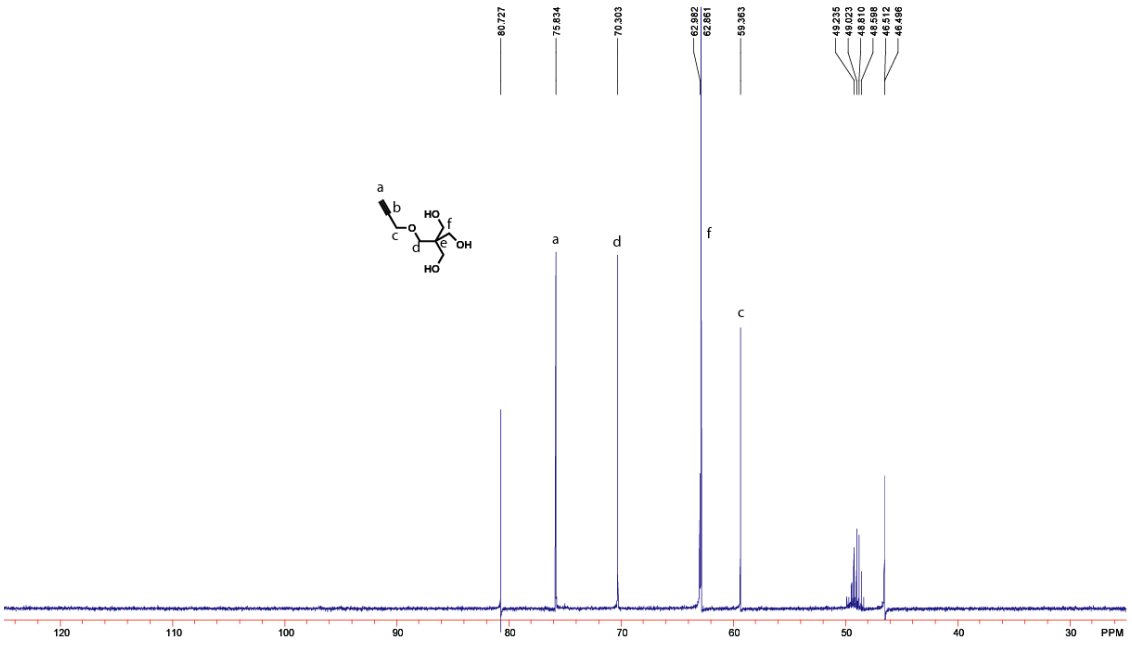
2.828
2.824
a
b
c
d
HO
OH
HO
d
6.02
c
1.97
b
1.95
a
0.88
6
5
4
3
2
PPM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49.023
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48.598
48.512
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b
c
d
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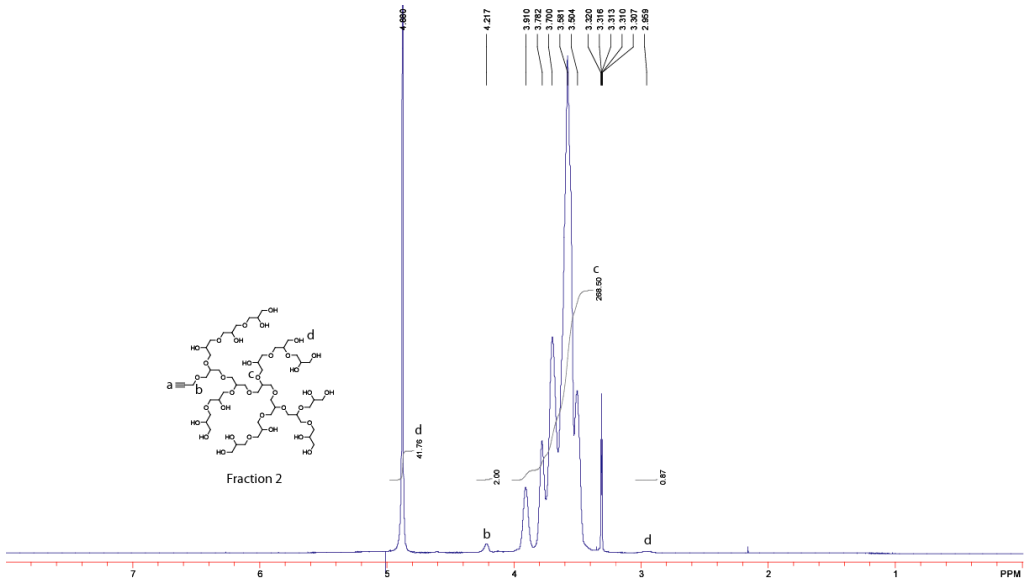
100
90
80
70
60
50
40
30
PPM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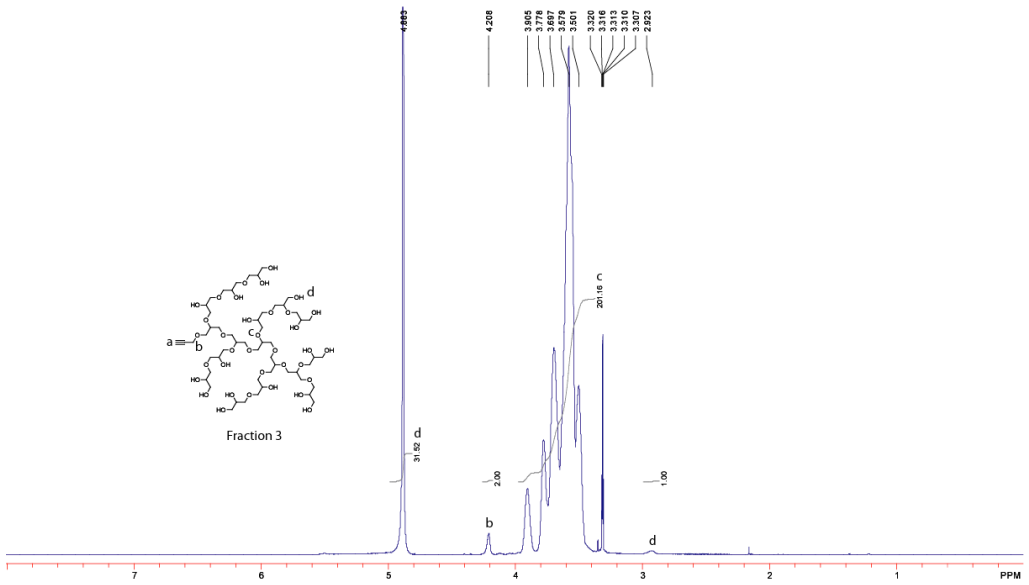
3.810
3.792
3.700
3.581
3.504
3.320
3.316
3.313
3.310
3.307
2.859
c
288.50
d
41.76
2.00
0.67
b
d
a
b
c
d
Fraction 2
7
6
5
4
3
2
1
PPM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3.316
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3.310
3.307
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201.16
d
31.52
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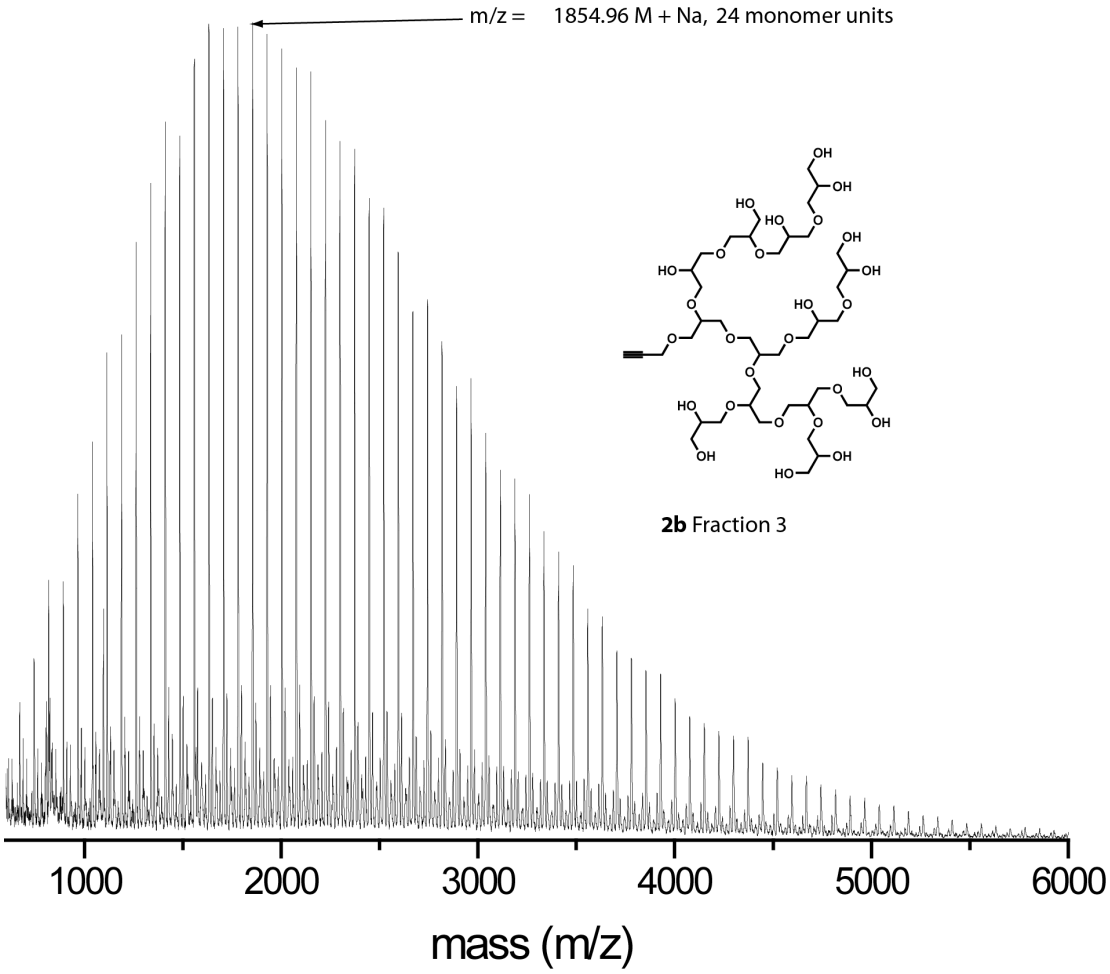
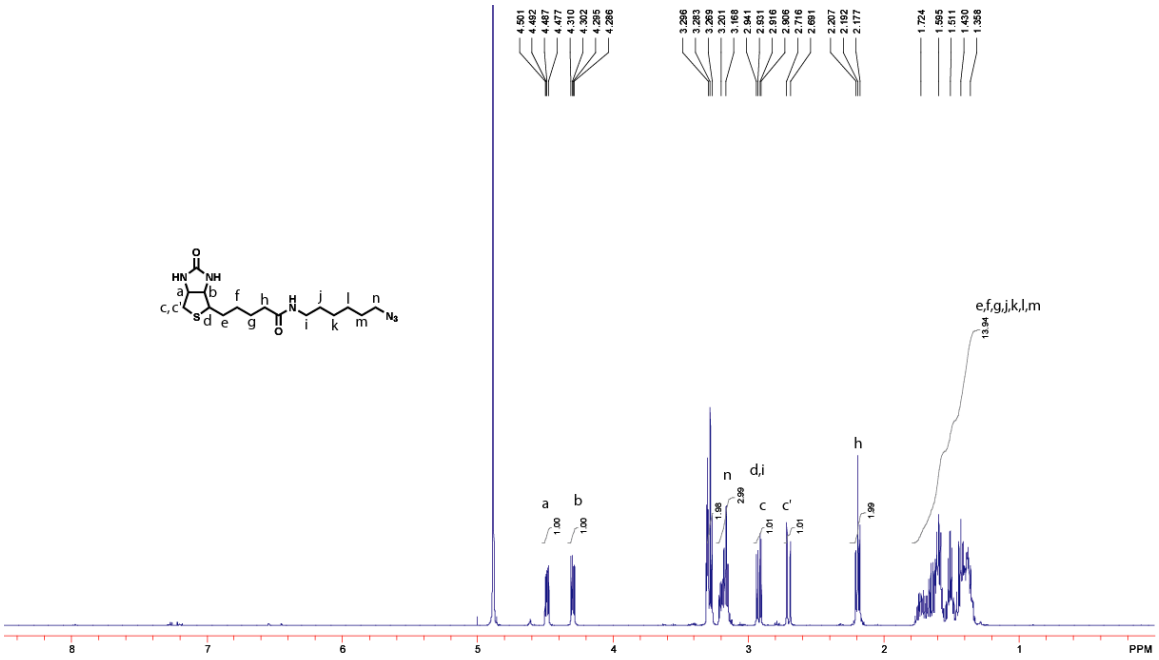
1.00
b
d
a
b
c
d
Fraction 3
7
6
5
4
3
2
1
PPM




**2b** Fraction 3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4.302
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4.286
3.286
3.283
3.269
3.201
3.168
2.841
2.831
2.816
2.806
2.716
2.681
2.207
2.192
2.177
1.724
1.595
1.511
1.430
1.358
e,f,g,j,k,l,m
13.94
h
n
d,i
a
b
c
c'
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1.01
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8
7
6
5
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3
2
1
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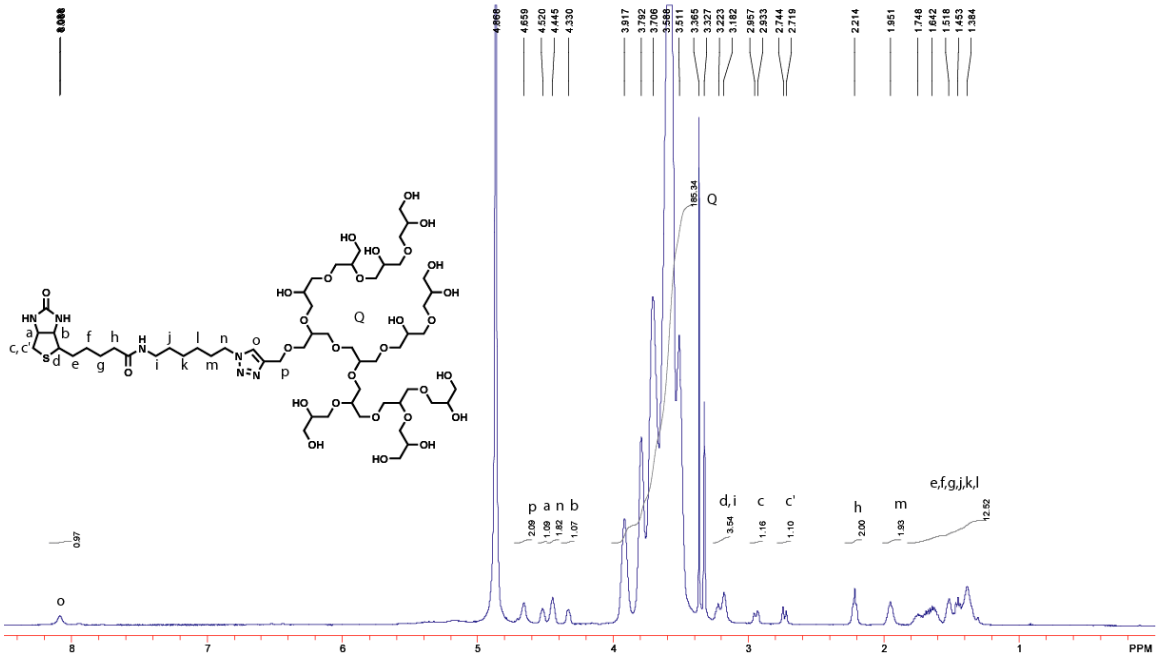
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3.706
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3.182
2.957
2.933
2.744
2.719
2.214
1.951
1.748
1.642
1.518
1.453
1.384
185.34
Q
e,f,g,j,k,l
12.92
p a n b
d, i
c
c'
h
m
2.09
1.09
1.82
1.07
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1.10
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o
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7
6
5
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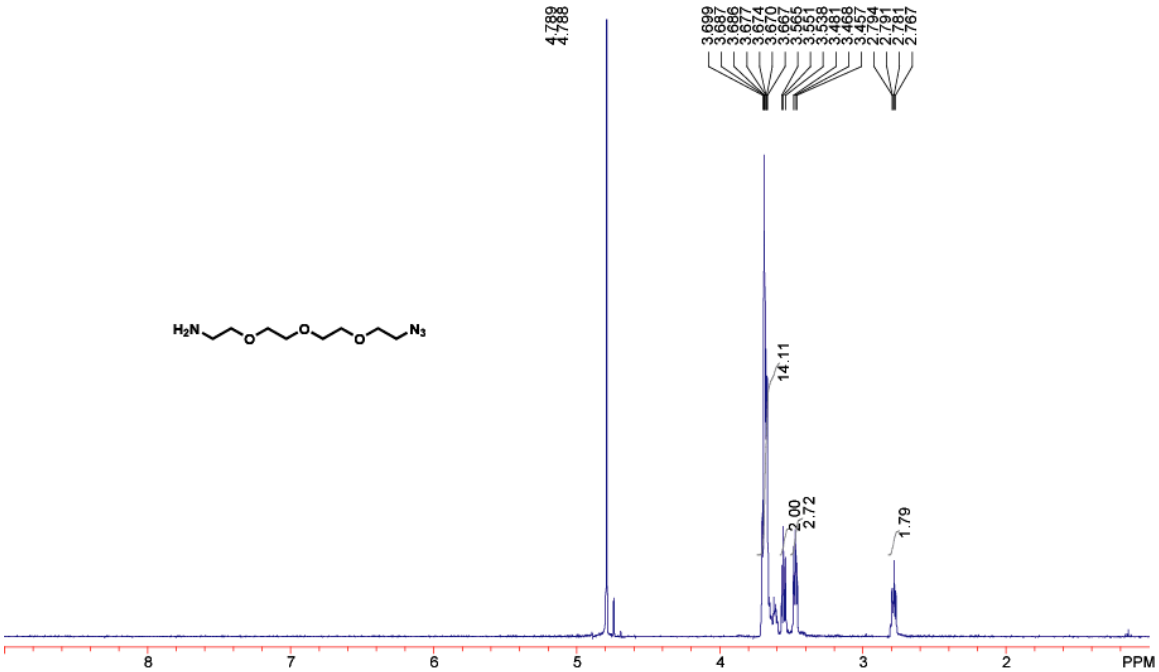
3
2
1
PPM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3.468
3.457
2.794
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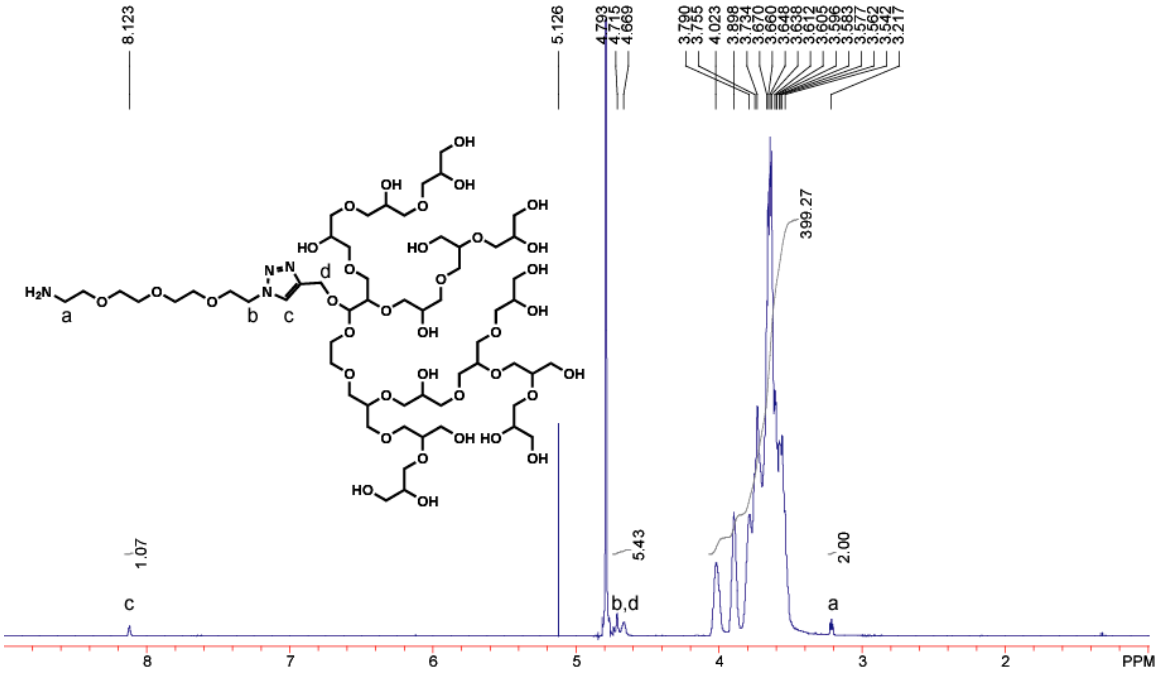
N3
8
7
6
5
4
3
2
PPM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3.755
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3.734
3.670
3.660
3.648
3.638
3.612
3.605
3.596
3.583
3.577
3.562
3.542
3.217
399.27
1.07
5.43
2.00
c
b,d
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H2N
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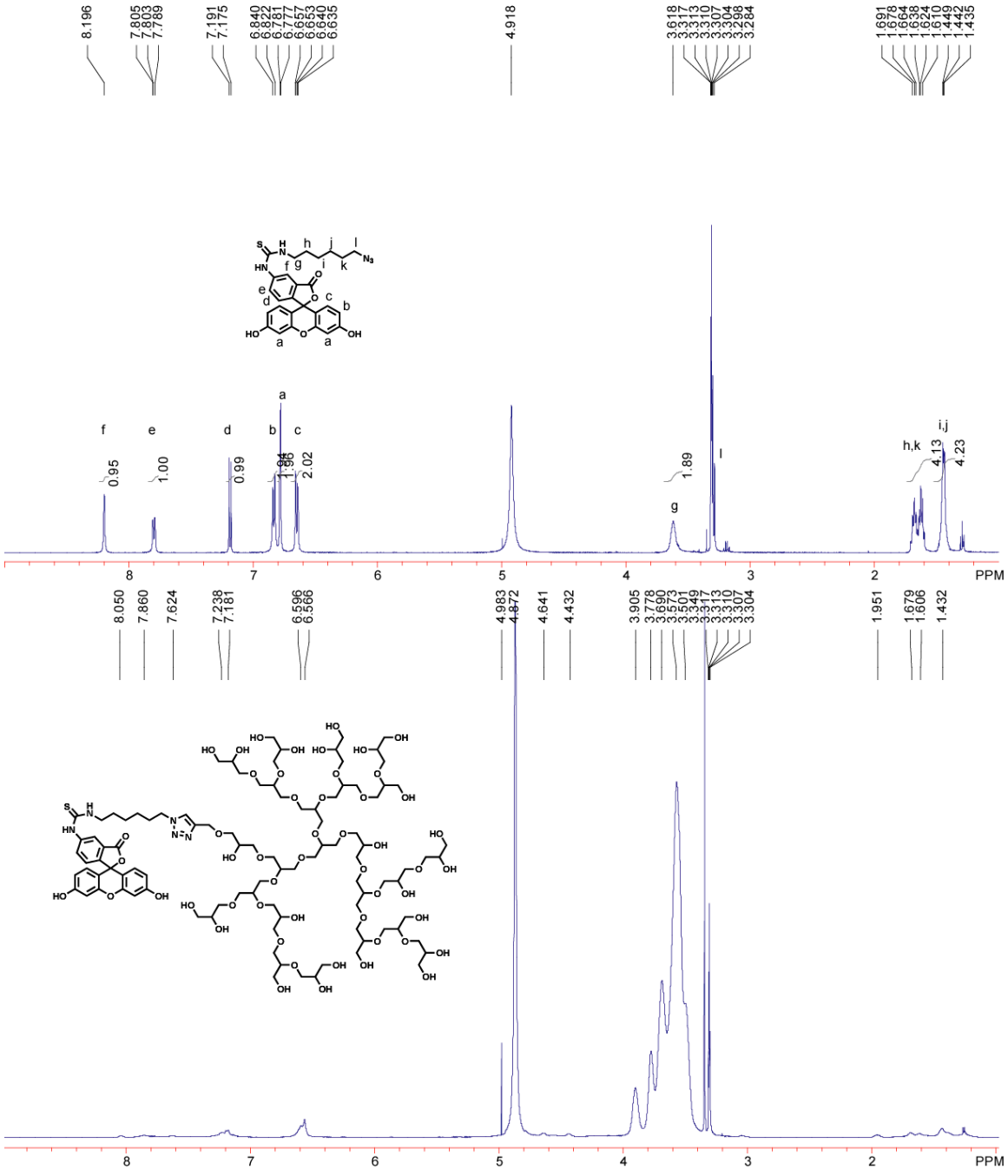
OH
HO
8
7
6
5
4
3
2
PPM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6.635
4.918
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3.310
3.307
3.304
3.298
3.284
1.691
1.678
1.664
1.638
1.624
1.610
1.449
1.442
1.435
f
e
d
b
a
c
0.95
1.00
0.99
1.94
1.96
2.02
g
1.89
l
h,k
i,j
4.13
4.23
PPM
8.050
7.860
7.624
7.238
7.181
6.596
6.566
4.983
4.872
4.641
4.432
3.905
3.778
3.690
3.573
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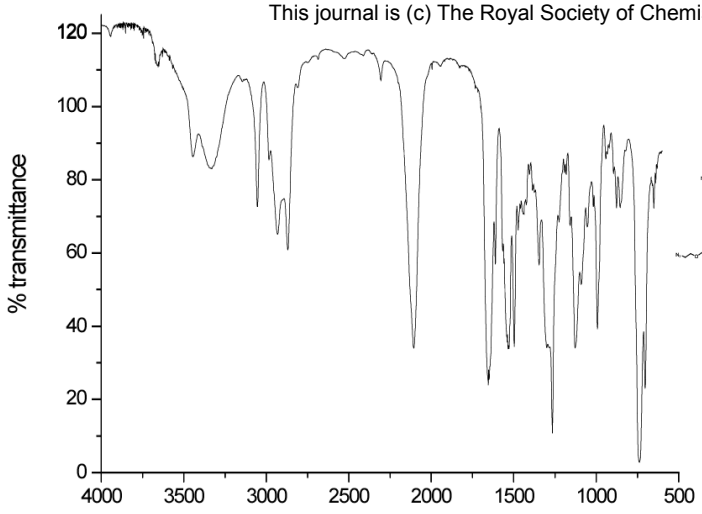
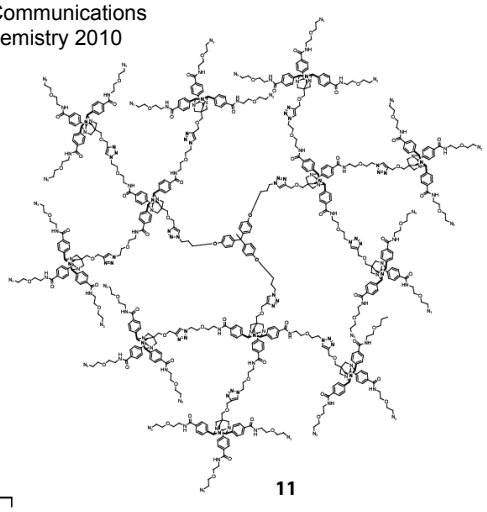
1.606
1.432
PPM


% transmittance
wavenumbers cm-1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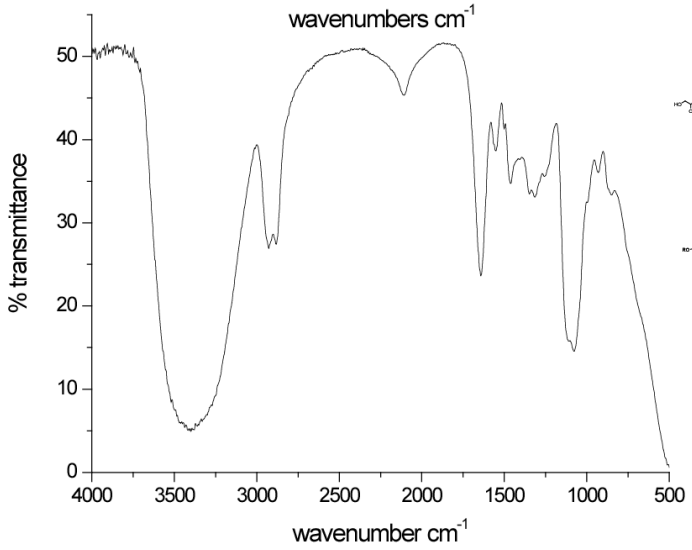
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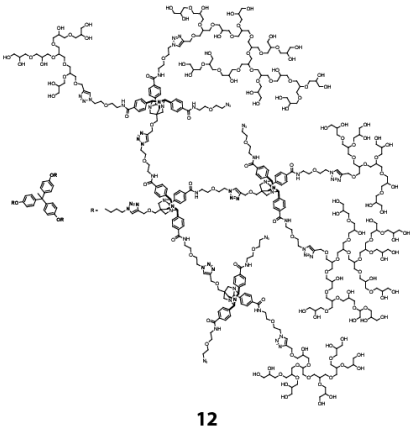
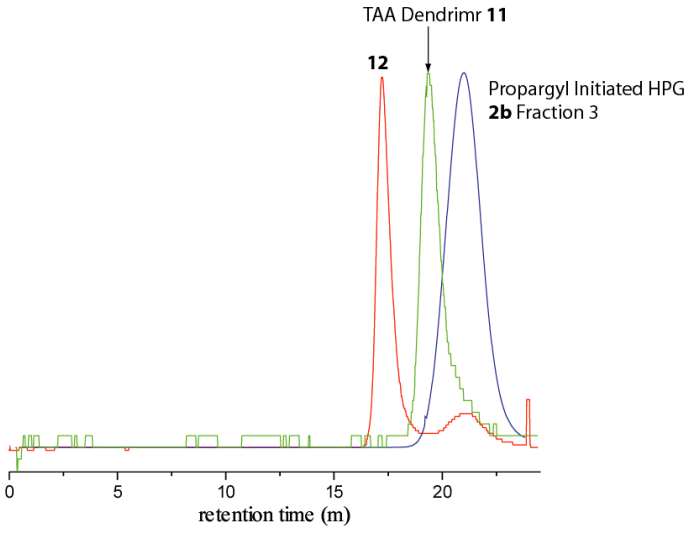
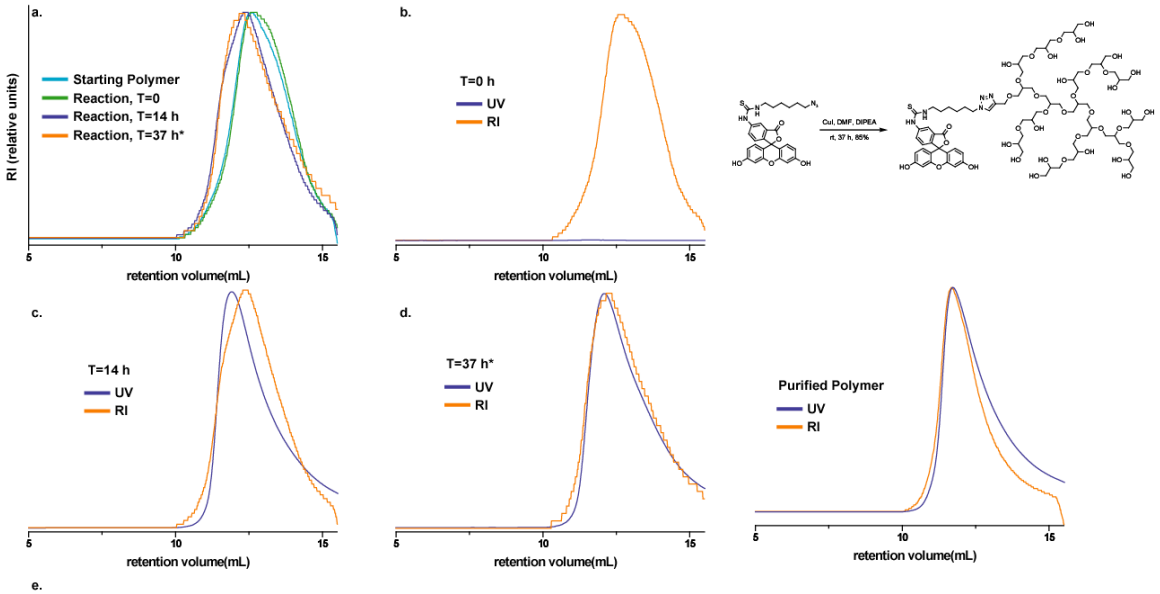

12


TAA Dendrimr 11
12
Propargyl Initiated HPG
2b Fraction 3
retention time (m)

## Analytical Techniques

**SEC analysis of HPG click reaction for product 6.** Polymer samples were prepared by removing 20 μL samples from reaction mixture and diluting with 750 μL of DI water. Aqueous samples were then filtered through a 0.2 micron nylon filter. Samples were injected directly onto an aqueous SEC column with UV-Vis peak detection set at 488 nm.

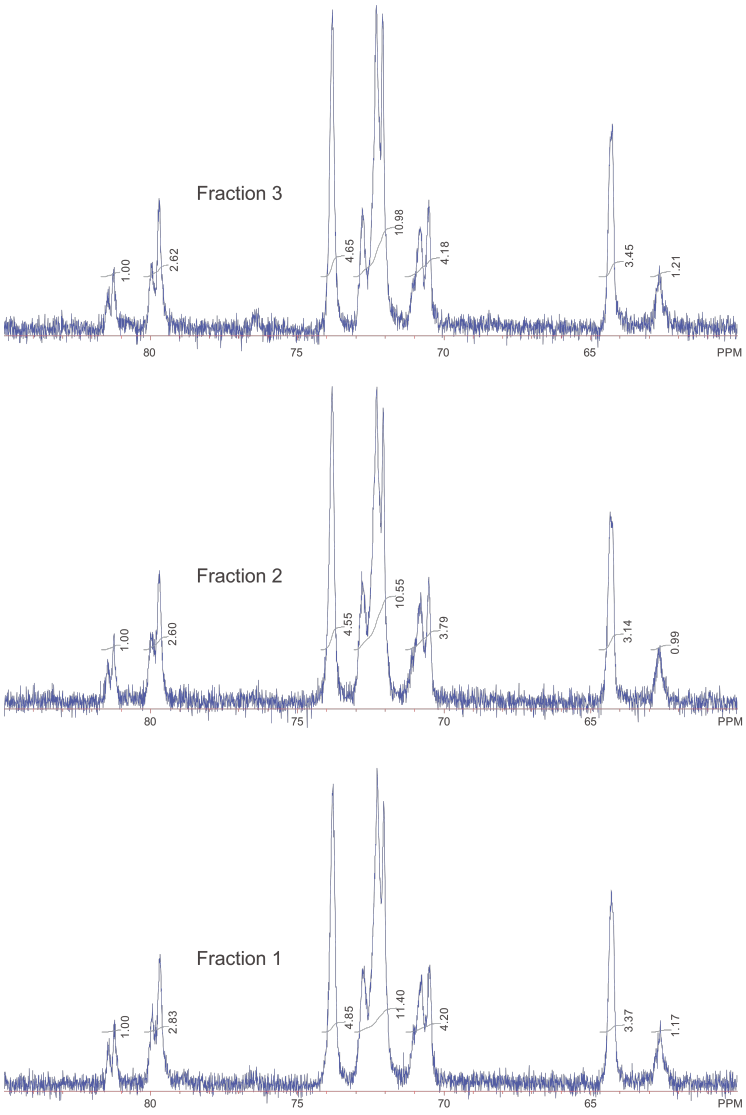



**S4.** SEC traces obtained using UV-Vis and RI detectors. Each peak is normalized to 1. a) RI signals for each sample including starting polymer showing the shift in retention volume upon coupling. b) Overlay of RI and UV signal at time 0. The fluorescein starting material is completely removed during the filtration. c) After 14 h a UV signal is observed which is monomodal and corresponds to the shoulder observed in the RI signal indicating only partial reaction. d) After 37 h and an additional aliquot of fluorescein and CuI the UV and RI signals overlap and there is no shoulder observed in the RI signal.

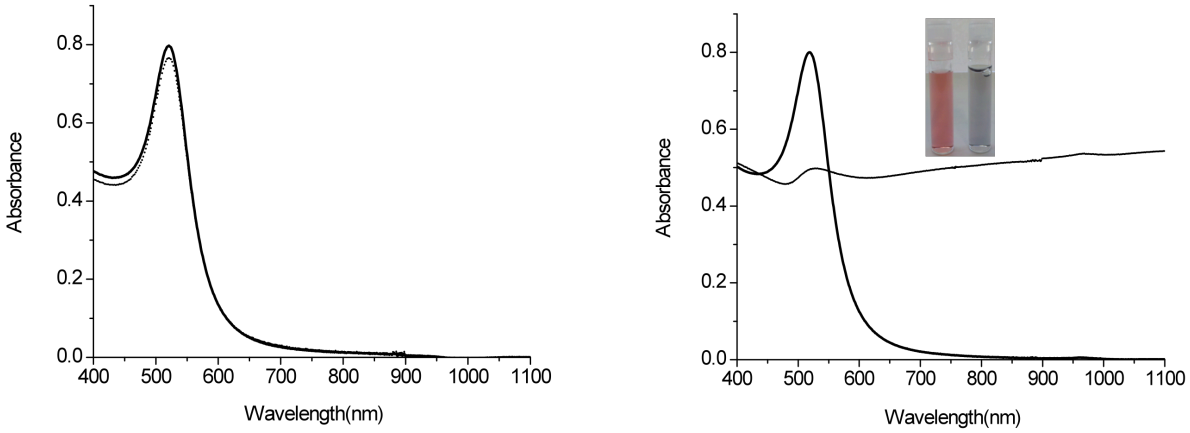
**Degree of branching measurements of fractionated HPG 2b.** HPG samples were prepared by dissolving 250 mg **2b** in 700 uL $CD_3OD$. Each sample was analyzed using IG gated $^{13}C$ NMR with a 2.5 second total run (1.5 second delay and 1 second run time.) Degree of branching (DB) was determined based on assignments given in reference 6 using the equation.

$DB = 2D/(2D + L_{13} + L_{14})$

With D, $L_{13}$, and $L_{14}$ representing dendritic, linear 1,3-units and linear 1,4-units obtained from the NMR spectrum.



**Stability of nanoparticles against aggregation.** To 1.0 mL of either citrate or HPG-capped gold nanoparticle in DI water (optical density of 1.0), was added 100 µL of a 10X PBS buffer solution was added, resulting in a final solution containing 1X PBS. After buffer addition, UV-Vis spectra were obtained for both citrate-capped gold nanoparticles and HPG-capped nanoparticles.



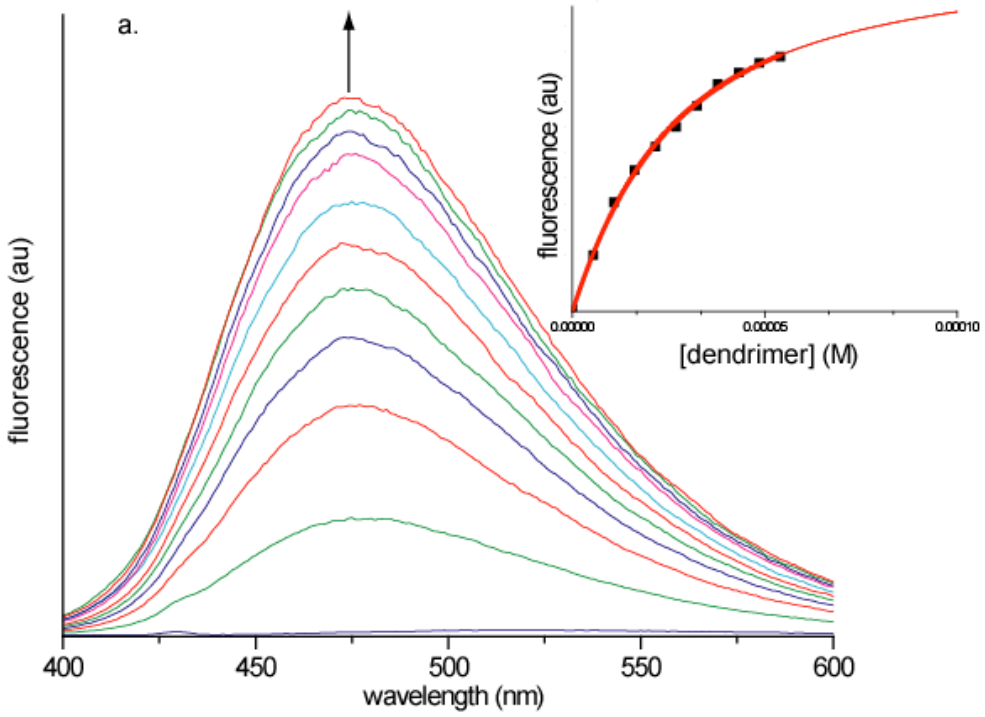

**S5.** Left) UV-Vis spectra of HPG-capped gold nanoparticles in water (solid line) and in 1X PBS buffer solution (dashed line). Right) Citrate-capped nanoparticles suspended in water (solid line) and in 1X PBS buffer solution (dashed line). Insertion in the right figure) Optical photographs of citrate-capped nanoparticles in water (red solution) and in 1X PBS buffer solution (blue vial).

**Titration of 1,8 ANS with polymer 12.** A 500 µL solution of 11 µM ANS in 100 mM PBS adjusted to pH 7.4 was incubated at 37 °C. The solution was titrated with 2 µL aliquots of a 67 mg/mL (1.35 mM) solution of **12** representing 2.7 nmols/aliquot or 0.5 equivalents/aliquot. The solution was excited at 375 nm and the fluorescence was observed between 400 and 600 nm. A binding constant was obtained by plotting fluorescence increase verse concentration of

**12** using a 1:1 binding isotherm as described by Pilch[7] and by Stootman[8] using the following equation.

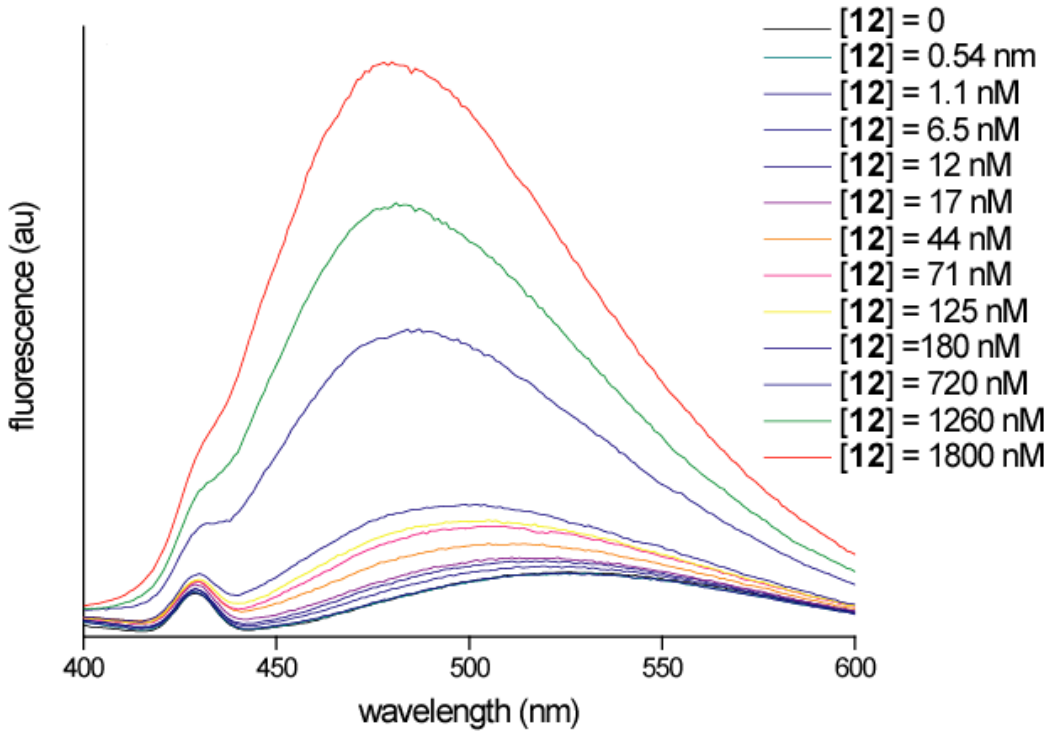
$F = F_{\infty}(1/2)\{([\mathbf{12}] + [ANS] + 1/K^{fit}) - (([\mathbf{12}] + [ANS] + 1/K^{fit})^{\wedge}2 - 4[\mathbf{12}][ANS])^{\wedge(1/2)}\}$

Where F = the observed fluorescence, and $F_{\infty}$ is the theoretical maximum fluorescence at saturation. Fitting was performed using the origin 7 graphing software. $K^{fit}$ = 51000 ± 4000 $M^{-1}$.




**S6.** A concentrated stock solution of dendrimer **12** was titrated into an ANS solution. Each curve represents 0.48 equilvilents **12** added. Inset, fluorescence max verse concentration fit to a 1:1 binding isotherm.

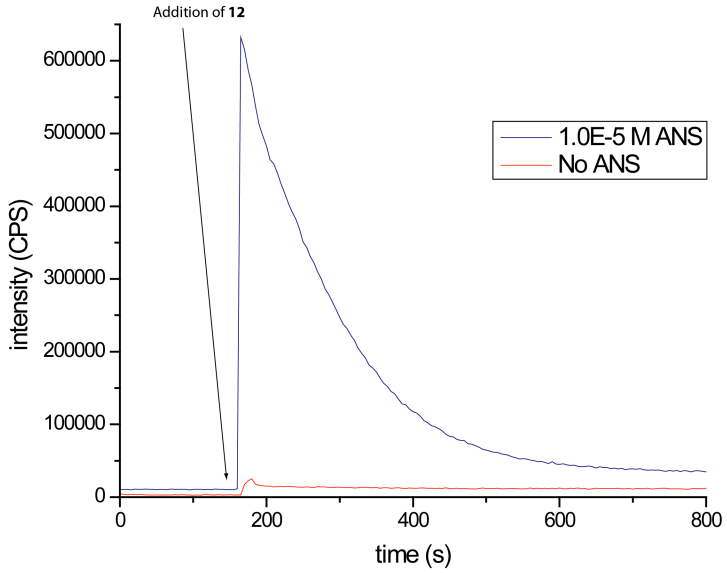
**Evaluation of CMC of polymer 12.** A 1.35 mM stock solution of polymer **12** was diluted 10, 100, 1000, and 10,000 fold in water. These solutions were used to titrate an 11 μM ANS solution under the same conditions as above. The first perceptible change was observed at 6.5 nM.




**S7.** a) Relative fluorescence of ANS in the presence of polymer **12** at low concentration.

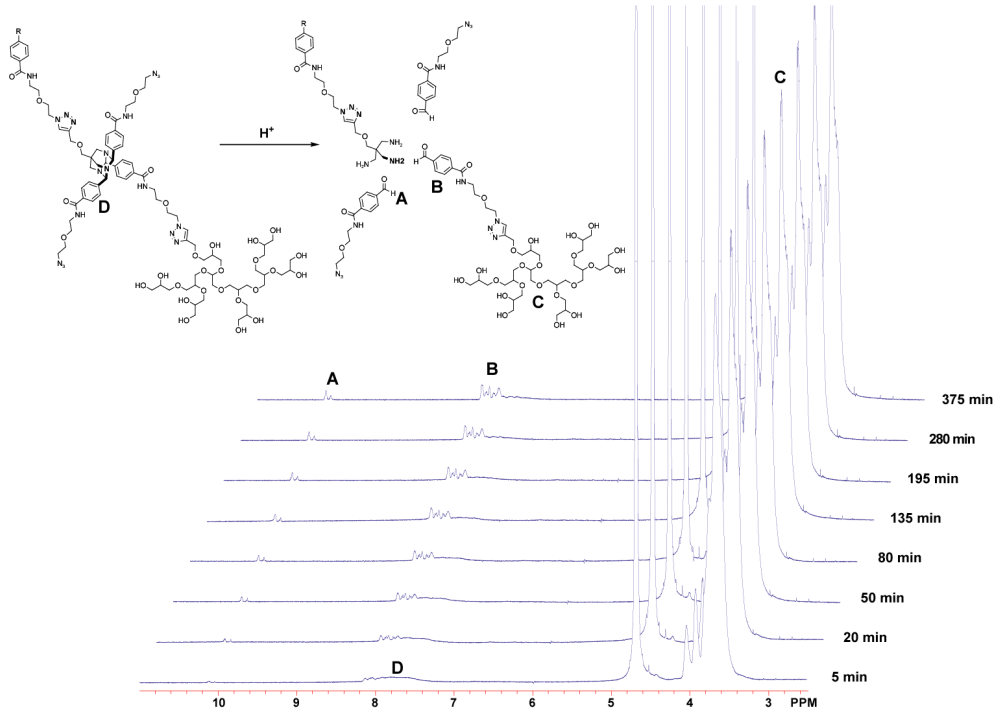
**Fluorescence studies of dendrimer degradation.** The fluorescence of an 11 μM ANS solution in 100 mM PBS adjusted to the appropriate pH with 10% phosphoric acid was excited at 375 nm and monitored every 5 seconds at 475 nm. After 2 minutes a 10 μL aliquot of a 1.35 mM solution (2.45 equiv) of **12** was added. The solution was monitored for up to 8 hours or until a steady state was reached. The degradation process was fit to a first order decay using the equation $F = F_\infty + F_0 e^{\wedge(-t/\tau)}$ where F is the observed fluorescence at time t, $F_\infty$ is the final fluorescence, $F_0$ is the initial fluorescence, and τ is a time constant. The data was fit using the Origin 7 graphing software. Half lives for the fluorescence degradation are, $T_{(1/2)pH\ 1.7}$ = 0.65 m, $T_{(1/2)pH\ 2.5}$ = 2.1 m, $T_{(1/2)pH\ 4}$ = 86 m.

**Fluorescence control study in the absence of ANS.** The fluorescence of a 100 mM PBS buffer adjusted to pH 2.5 containing 10 μM ANS or no ANS was excited at 375 nm and monitored every 5 seconds at 475 nm. After 165 seconds a 10 μL aliquot of a 1.35 mM solution of **12**, 2.45 Eq, was added. Both solutions were monitored using identical instrumental setups until a steady state was reached.

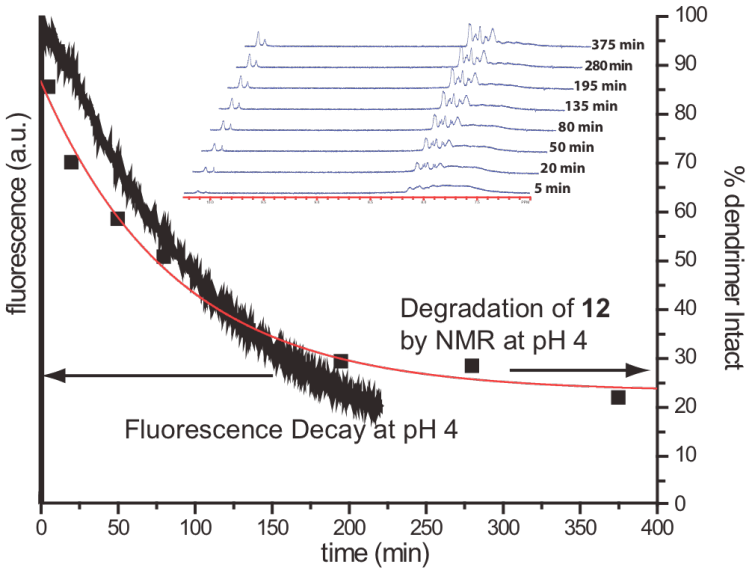



**S8.** Fluorescence degradation with and without ANS upon addition of **12** at pH 2.5.

**NMR studies of dendrimer degradation.** A 100 mM sodium phosphate and 100 mM NaCl solution in $D_2O$ was adjusted to pH 4.0 using a 35% DCl solution. To 700 μL of this solution was added 10 mg of **12**. The sample was placed in an NMR tube, loaded into an NMR probe (U500NB), and heated to 37 °C. NMR measurements were taken at roughly 30 minute intervals. The degree of degradation was determined by comparing the aldehyde peaks at 10.5 ppm and the total integration of the aromatic region.

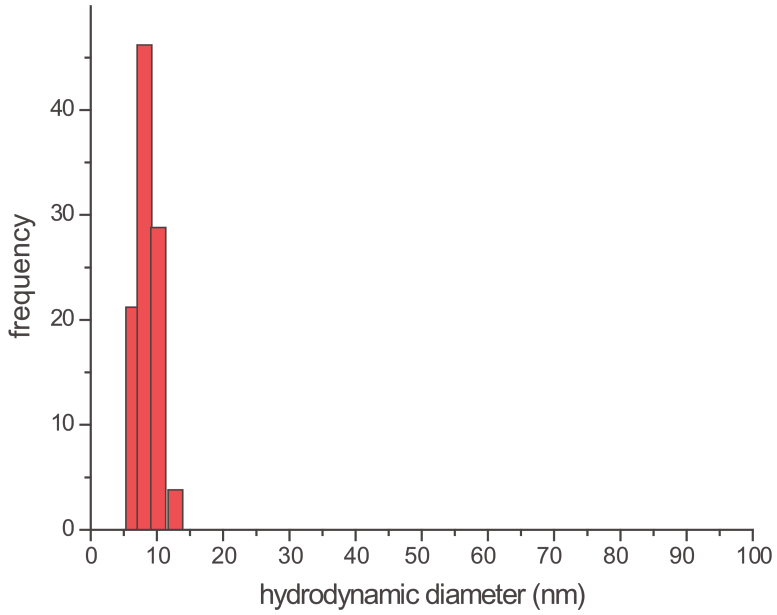



**S9.** Full NMR spectra of **12** in pH 4 PBS buffer. Showing the formation of aldehyde groups over time upon degradation and the sharpening of the aromatic peaks.




**S10.** Comparison of fluorescence and NMR degradation at pH 4 in sodium phosphate buffer. Red and black lines represent the degradation determined by NMR and fluorescence, respectively.

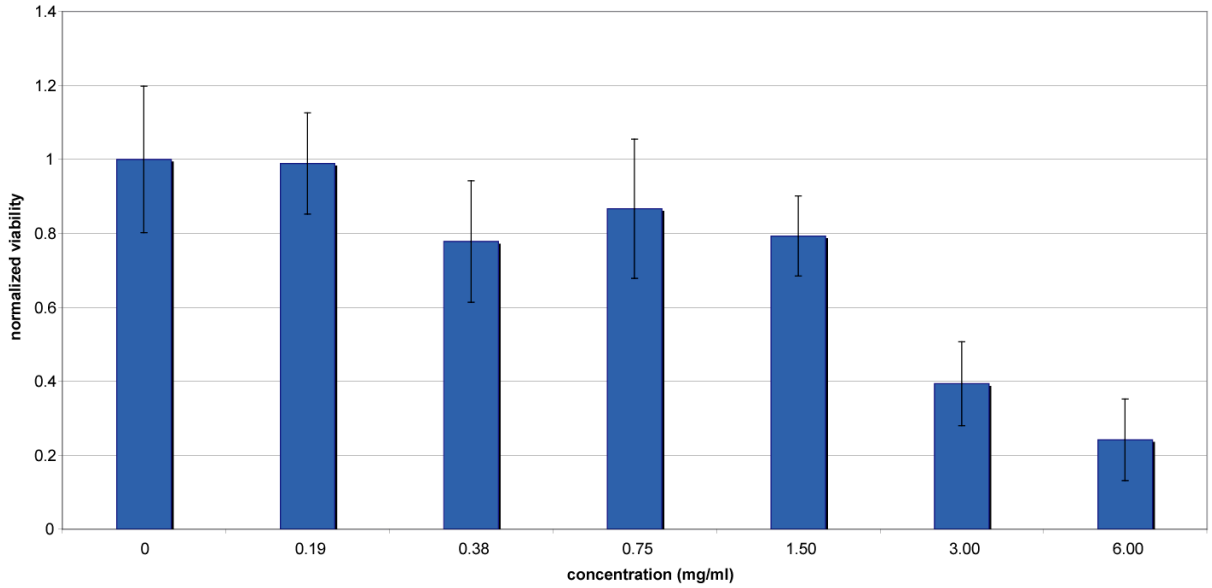
**Dynamic light scattering.** A 1 mL solution of polymer **12**, 6.7 mg/mL, was filtered directly into a 2 mL 100 mM PBS buffer solution, to give a final concentration 2.23 mg/mL. This concentration is equivalent to the highest concentration used in the ANS titration. These were analyzed as an average of 10 runs lasting 1 minute each.




**S11.** Dynamic light scattering study of a 2.2 mg/mL solution of **12** in 100 mM Phosphate Buffer pH 7.4.

**Cytotoxicity measurement.** Cytotoxicity was characterized as a decrease in metabolic rate using an MTT assay. D1 cells were plated in a 96 well plate at an initial density of 10,000 cells per well in 200 µL of DMEM. After 24 h, medium was replaced with fresh medium containing the polymer of interest. After 3 days the medium was removed, cells were washed with 200 µL PBS. The PBS was removed, and 100 µL medium followed by 10 µL MTT solution was added. After 4 h, 100 µL stop solution (20 % SDS in $H_2O$/DMF(50:50)) was

added. After 2 h, the absorbance at 570 nm was measured relative to blank wells prepared without cells and normalized to cells prepared without any polymer added.




**S12**. Cytotoxicity studies of **12**.

1. R. E. Kohman, S. C. Zimmerman, *Chem. Commun.* **2009**, 794-796.
2. T. J. Dunn, W. L. Neumann, M. M. Rogic, S. R. Woulfe, *J. Org. Chem.* **1990**, *55*, 6368-6373.
3. I. A. Inverarity, R. F. H. Viguier, P. Cohen, A. N. Hulme, *Bioconjugate Chem.* **2007**, *18*, 1593-1603.
4. J. W. Lee, S. I. Jun, K. Kim, *Tetrahedron Lett.* **2001**, *42*, 2709-2711.
5. G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20.
6. A. Sunder, R. Hanselmann, H. Frey and R. Mülhaupt, *Macromolecules*, 1999, **32**, 4240.

7. D. S. Pilch, N. Poklar, E. E. Baird, P. B. Dervan, K. J. Breslauer, *Biochemistry* **1999**, *38*, 2143-2151.
8. F. H. Stootman, D. M. Fisher, A. Rodger, J. R. Aldrich-Wright, *Analyst* **2006**, *131*, 1145-1151.