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Formation of Giant Amphiphiles by Post-functionalization of Hydrophilic Protein-Polymer Conjugates

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Supplementary Information

Experimental Section

Starting Materials. All chemicals were purchased from Fluka Chemica or Sigma-Aldrich (unless otherwise specified) and used without further purification. Cu(I)Br was purified as reported by Keller and Wycoff.[1] *N*-(*n*-propyl)-2-pyridylmethanimine[2] and 2-Methyl-acrylic acid 2,2-dimethyl-[1,3]dioxolan-4-ylmethyl ester [3] were prepared as described earlier and stored at 0 ºC. Protected maleimido initiator[4], protected alkyne monomer[5], fluorescent hostasol comonomer[6] and 1-azido-decane[7] and benzyl-azide were synthesized according to the literature. NEt_3 was dried over KOH pellets. Deuterated solvents were obtained from Cambridge Isotope Laboratories. Bovine Serum Albumin (BSA) was purchased from Sigma Aldrich. Polymerizations were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated. Yields of the reactions were not optimized.

Analytical techniques. GPC were measured on a Shimadzu VP HPLC system equipped with a Thermo Biobasic SEC-300 column eluting with a solvent mixture 70 % phosphate buffer 5 mM pH 7.4, 30 % acetonitrile (unless otherwise noticed). NMR spectra were recorded on a Bruker 300 MHz and a Bruker 400 MHz spectrometer system. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances (^{1}H and ^{13}C). The following abbreviations were used to explain the multiplicities: s=singlet, d=doublet, dd= doublet of doublets, t=triplet, m=multiplet. The molecular weights of the polymers M_n are calculated by comparing the integrals of the chain-end signals and appropriate peaks related to the polymer backbone. Infrared absorption spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (using a Golden Gate diamond or a NaCl cuvette). UV-vis spectra were recorded on a CARY 1 BIO UV-

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visible spectrophotometer. Solutions were sonicated in a Bandelin Sonorex RK 100 apparatus. Confocal microscopy experiments were performed with a Leica TCS SP2 AOBS confocal microscope using a 100x oil immersion objective. The Hostasol tag was excited with the 514 line of the Argon-Krypton laser. Unidirectional scanning was done at 400 Hz with an image format of 512x512 pixels. Transmission Electron microscopy experiments were performed using a FEI Tecnai G2 Electron Microscope. Micrographs were taken using a Tietz CCD camera at a 2048 by 2048 pixel resolution.

Initiator and Monomer Synthesis

4,10-Dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (1): Maleic anhydride (30.0 g, 306 mmol) was suspended in 150 mL of toluene and the mixture warmed to 80 °C. Furan (33.4 mL, 459 mmol) was added via syringe and the turbid solution stirred for 6 h. The mixture was then cooled to ambient temperature and the stirring stopped. After 1 h, the resulting white crystals were collected by filtration and washed with 2 x 30 mL of petroleum ether to obtain 44.4 g (267 mmol, 87% yield) of the product as small white needles. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 3.17 (s, 2H, CH), 5.45 (t, *J* =1.0Hz, 2H, CHO), 6.57 (t, *J* = 1.0 Hz, 2H, CH_{vinyl}).

4-(2-Hydroxyethyl)-10-oxa-4-azatricyclo[5.2.1.02,6]dec-8-ene-3,5- dione (2): The anhydride **1** (2.00 g, 12.0 mmol) was suspended in MeOH (50 mL) and the mixture cooled to 0 °C. A solution of ethanolamine (0.72 mL, 12.0 mmol) in 20 mL of MeOH was added dropwise (over ~ 10 min) and the resulting solution was stirred for 5 min at 0 °C, then 30 min at ambient temperature, and finally refluxed for 4 h. After cooling the mixture to ambient temperature, the solvent was removed under reduced pressure, and the white residue was dissolved in 150 mL of CH_2Cl_2 and washed with 3 x 100 mL of water. The organic layer was dried over $MgSO_4$ and filtered. Removal of the solvent under reduced pressure furnished an off-white residue that was purified by flash chromatography to give the product (1.04 g, 5.00 mmol, 42% yield) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.90 (bs, 1H, OH), 2.90 (s, 2H, CH), 3.69-3.72 (m, 2H, NCH_2), 3.76-3.78 (m, 2H, OCH_2), 5.28 (t, *J* = 0.9 Hz, 2H, CH), 6.52 (t, *J* = 0.9 Hz, 2H, CH_{vinyl}).

2-Bromo-2-methyl Propionic Acid 2-(3,5-Dioxo-10-oxa-4- azatricyclo[5.2.1.02,6]dec-8-en-4-yl) Ethyl Ester (II): A solution of the alcohol **2** (2.22 g, 10.6 mmol) and Et_3N (1.60 mL, 11.7 mmol) in 120 mL of THF (the solution remained slightly turbid) was cooled to 0 °C, and a solution of 2-bromo isobutyryl bromide (1.40 mL, 11.1 mmol) in 40 mL of THF was added dropwise (30 min). The white suspension was stirred for 3 h at 0 °C and subsequently at ambient temperature overnight. TLC revealed the complete disappearance of the starting material. The ammonium salt was filtered off and the solvent removed under reduced pressure to give a pale-yellow residue that was purified by flash chromatography (CC, SiO_2, petroleum ether/ethyl acetate 1:1). We obtained 3.54 g (9.88 mmol, 93% yield) of **II** as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.86 (s, 6H, CH_3), 2.84 (s, 2H, CH), 3.78 (t, *J* = 5.3 Hz, 2H, NCH_2), 4.30 (t, *J* = 5.3 Hz, 2H, OCH_2), 5.23 (t, *J* = 1.0 Hz, 2H, CHO), 6.49 (t, *J* =1.0 Hz, 2H, CH_{vinyl}).

2-Methyl-acrylic acid 2,2-dimethyl-[1,3]dioxolan-4-ylmethyl ester (III): In a three necked round-bottom flask, a mixture of (2,2-Dimethyl-[1,3]dioxolan-4-yl)-methanol (10.58 g, 53 mmol) and Et_3N (22. mL, 85 mmol) in 100 mL of anhydrous THF was cooled down to 0 ^oC under nitrogen atmosphere, and methacryloyl chloride (9.2 g, 88 mmol) was added dropwise. The mixture was allowed to warm to ambient temperature overnight and then stirred for 2 days at ambient temperature. Triethylamine hydrochloride salt was filtered off and the solvent removed under reduced pressure. The resulting viscous oil was dissolved in dichloromethane (100 mL), washed with a saturated $NaHCO_3$ solution (2 × 50 mL) and water (2 × 50 mL), and finally dried over $MgSO_4$. After filtration, removal of the solvent under reduced pressure gave a yellow viscous oil which was distilled under vacuum in presence of Galvinoxyl® (radical inhibitor) to give the pure solketal methacrylate (10.25 g, 64% yield) as a colourless oil.
IR (neat): $\tilde{\nu}$ = 2987, 2888, 1718, 1638, 1454, 1371, 1320, 1296, 1214, 1157, 1083, 1054, 941, 845, 814, 733, 649 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.38 and 1.45 (s, 3H, OC(C*H*$_3$)O), 1.97 (s, 3H, C*H*$_3$-C=CH_2) 3.81 (dd, 1H, *J* = 8.3, 6.1 Hz), 4.11 (dd, 1H, *J* = 8.3, 6.1 Hz), 4.22 (d, 2H, O=C-O-C*H*$_2$, *J* = 5.3 Hz), 4.37 (m, 1H, (CH_2)C*H*O), 5.61 (m,1H, C=C*H*H), 6.16 (m, 1H, C=CH*H*).
^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ = 18.31, 25.41, 26.68, 64.71, 66.36, 73.64, 109.77, 126.08, 135.93, 167.11

2-Methyl-acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester (IV): A solution of trimethylsilyl propyn-1-ol (10.0 g, 78.0 mmol) and Et_3N (14.2 mL, 101.3 mmol) in Et_2O (100 mL) was cooled to -20 °C and a solution of methacryloyl chloride (8.8 mL, 93 mmol) in Et_2O (50 mL) was added dropwise over ca. 1 h. The mixture was stirred at this temperature for 30 min, then at ambient temperature overnight; the ammonium salts were removed by filtration and the volatiles removed under reduced pressure. ^{1}H NMR analysis of the yellow oily residue did not reveal the presence of substantial amount of any impurity, but two additional faint spots were observed by TLC (petroleum ether/Et_2O 20:1) analysis, the crude product was therefore purified by flash chromatography (CC, SiO_2, petroleum ether/Et_2O 50:1; R_f = 0.67 in petroleum ether/Et_2O 20:1). 12.4 g (63.2 mmol, 81 %) of **IV** were obtained as colourless liquid.

IR (neat): $\tilde{\nu}$ =2960, 1723, 1638, 1452, 1366, 1314, 1292, 1251, 1147, 1035, 971, 942, 842, 813, 761 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K) δ = 0.16 (s, 9H, $Si(CH_3)_3$); 1.93-1.94 (m, 3H, $CH_3C=CH_2$); 4.73 (s, 2H, OCH_2); 5.58- 5.59 (m, 1H, C=CHH); 6.14 (m, 1H, C=CHH).

ATRP Polymerization – Synthesis of polymer VI.

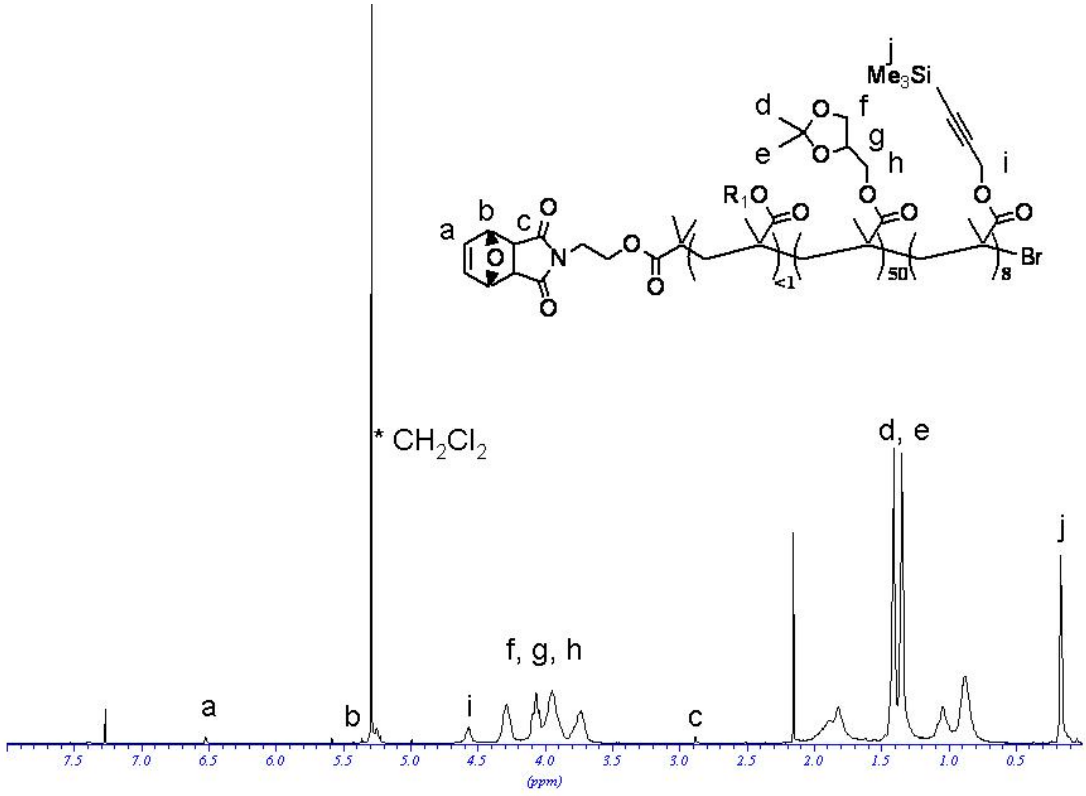
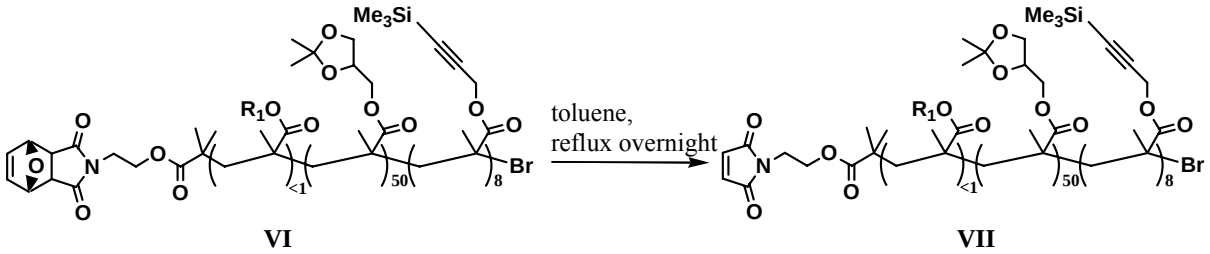


Figure 1: *^{1}H NMR* in $CDCl_3$ *of protected polymer* ***VI*** *(R^1=Hostasol fluorescent tag).*

Retro Diels-Alder reaction: synthesis of VII.



VI **VII**

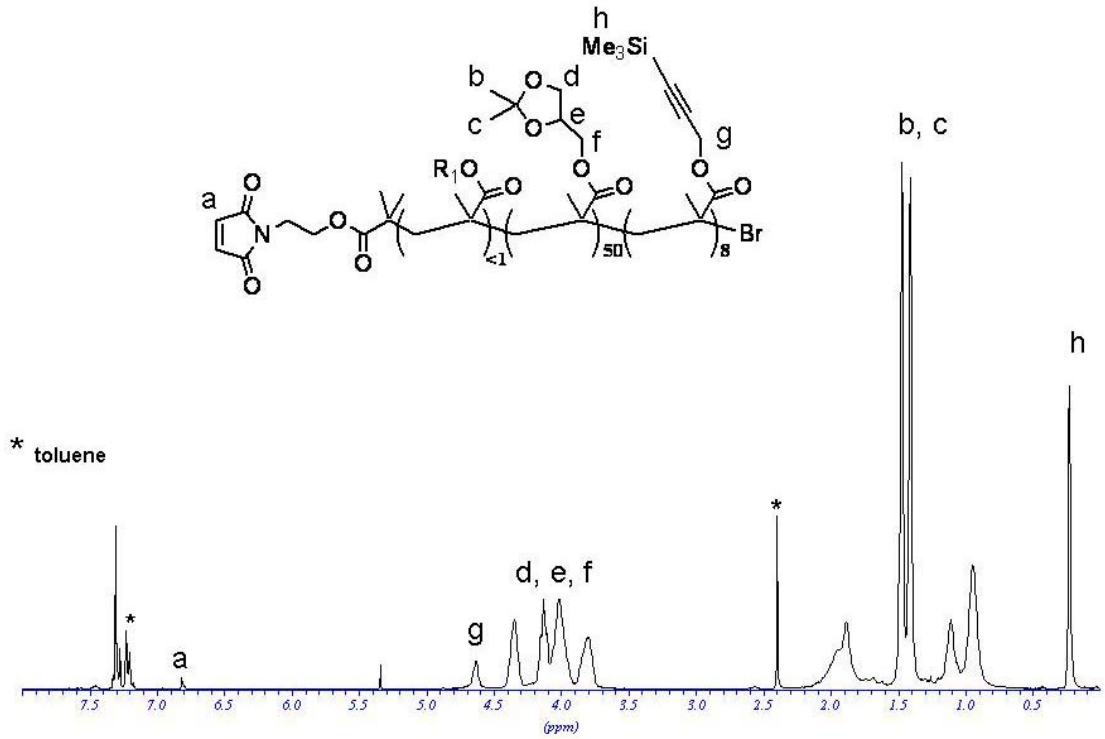
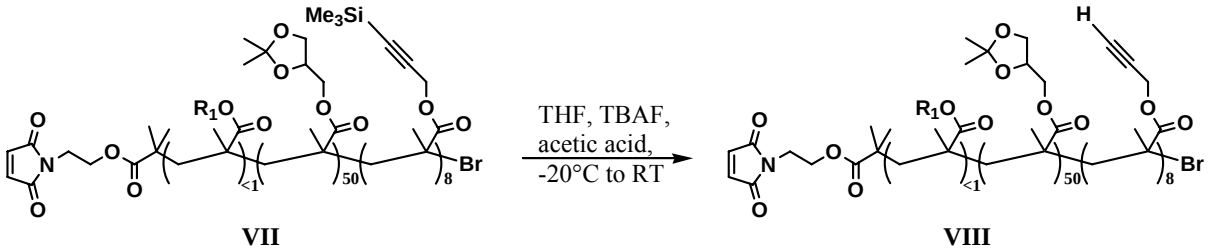


Figure 2: ^{1}H NMR in $CDCl_3$ of the polymer **VII** after retro-Diels-Alder reaction.

Deprotection of 1-alkyne units : Synthesis of Polymer VIII



VII **VIII**

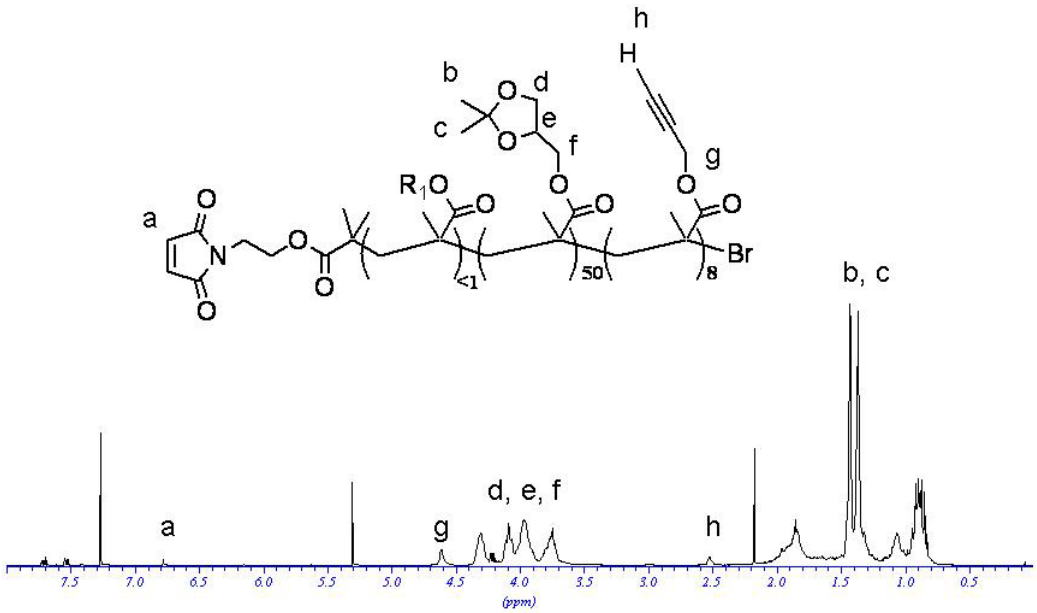


Figure 3: ^{1}H NMR in $CDCl_3$ of the polymer **VIII** after deprotection of the 1-alkyne units.

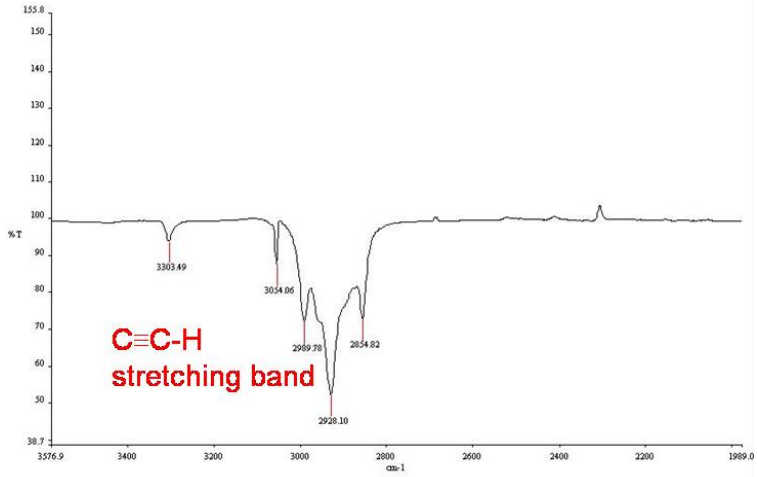


Figure 4: Partial FT-IR spectrum of the polymer **VIII** after deprotection of the 1-alkyne units.

Hydrolysis of ketal functions: Synthesis of Polymer I

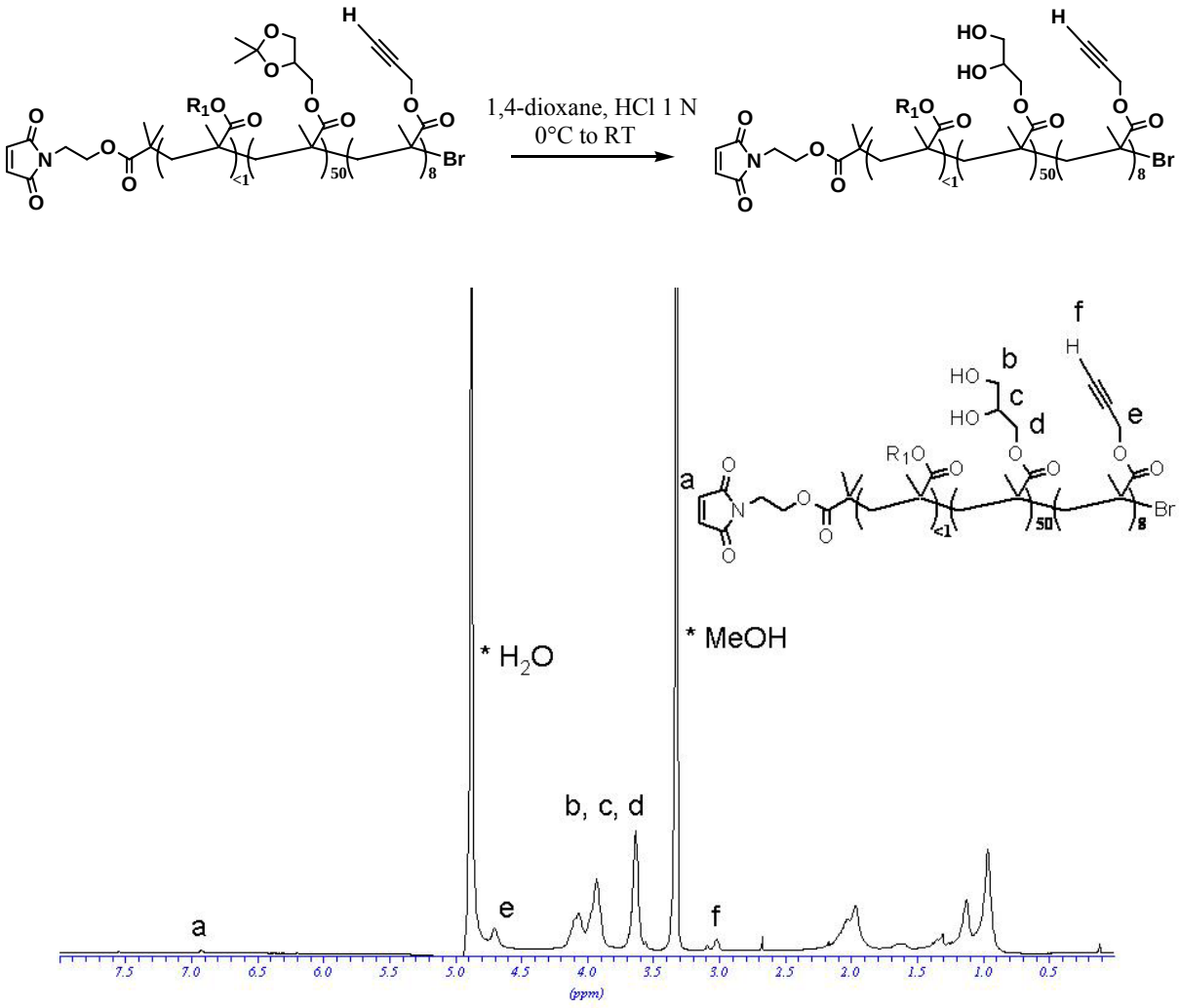


Figure 5: ^{1}H NMR spectrum in CD_3OH of the fully deprotected polymer **I**.

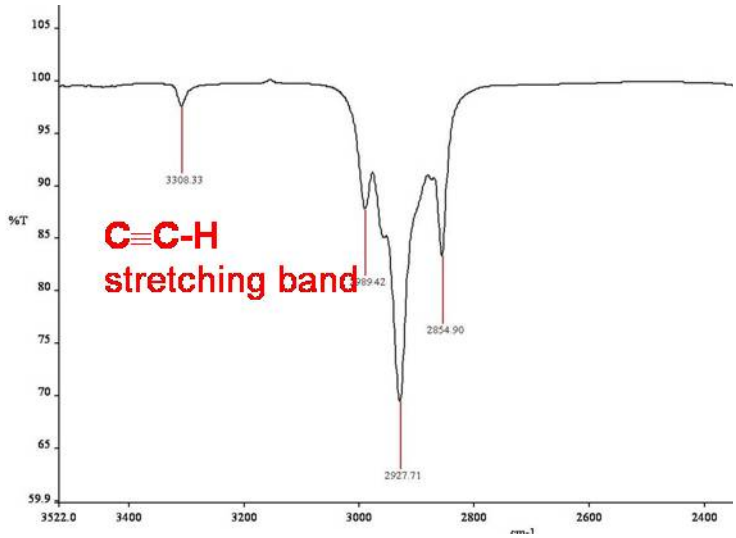
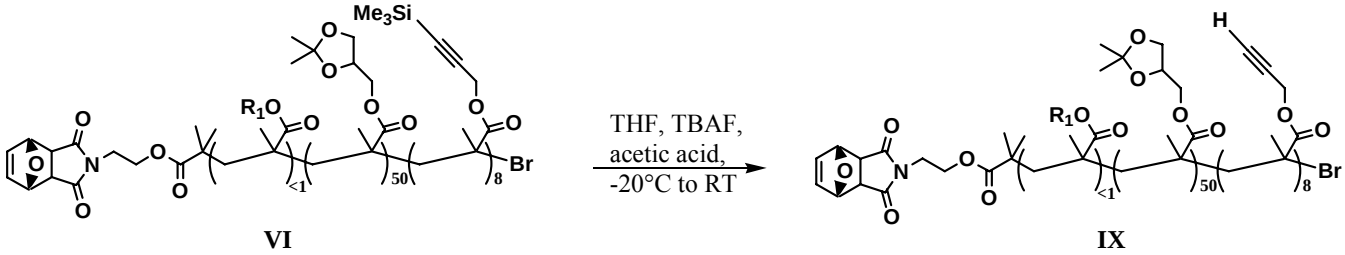


Figure 6: Partial FT-IR spectrum of polymer **I**.

Deprotection of the 1-alkyne functionalities of polymer VI.



VI **IX**

The reaction conditions employed were analogous to those used for the deprotection of **VII**.

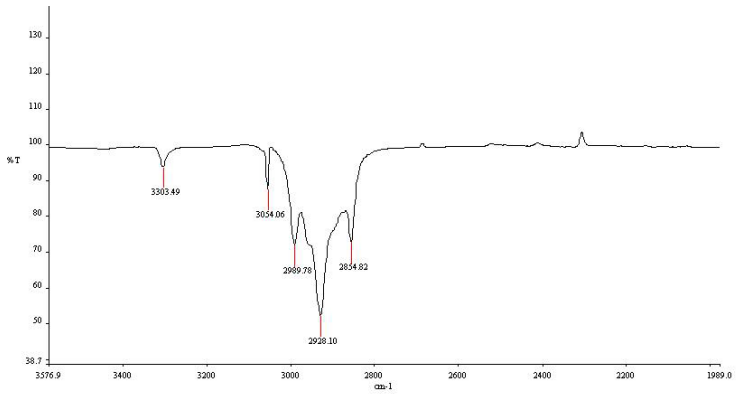


Figure 7: Partial FT-IR spectrum of the 1-alkyne deprotected polymer **IX**.

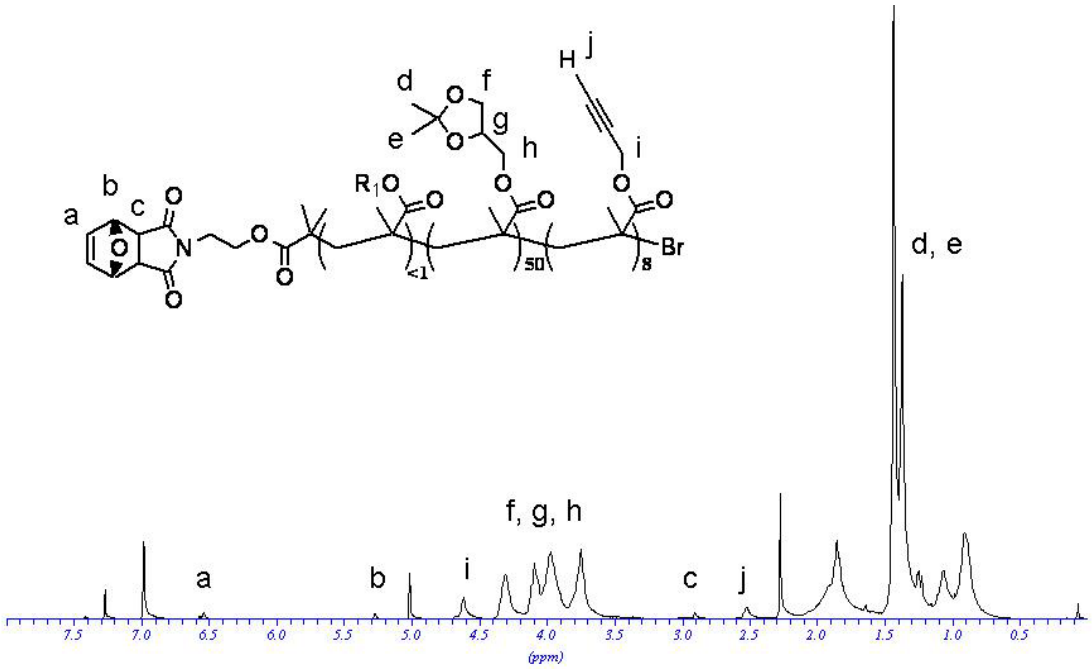
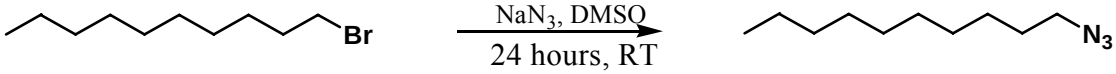


Figure 8: ^{1}H NMR in $CDCl_3$ of the polymer **IX** before click chemistry reaction with 1-azido-decane

Azidation of 1-bromo-decane



In a round bottom flask, to a 0.5 M solution of NaN_3 (0.325 g, 5.00 mmol) in DMSO (10 mL) was added 1-bromo-decane (0.44 g, 2.0 mmol) at ambient temperature. The solution was stirred for 24 hours at the same temperature, then 10 mL H_2O were added (the mixing was slightly exothermic). After cooling down to ambient temperature, the mixture was extracted with Et_2O. The organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure to afford the pure product (1-Azido-decane, yield = 90 %) as a colorless oil.

IR (neat): $\tilde{\nu}$ = 2924, 2854, 2091 (C-N_3 absorption band), 1466, 1348, 1259, 893, 721 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 0.89 (t, *J* =6.8 Hz, 3H, CH_3-), 1.2-1.4 (m, 14H, -CH_2-), 1.65-1.56 (m, 2H, N_3-CH_2-C*H*$_2$), 3.26 (t, *J* =7.0 Hz, 2H, N_3-CH_2-).

^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ = 14.11, 22.70, 26.75, 26.94, 28.87, 29.19, 29.32, 29.53, 31.91, 51.51.

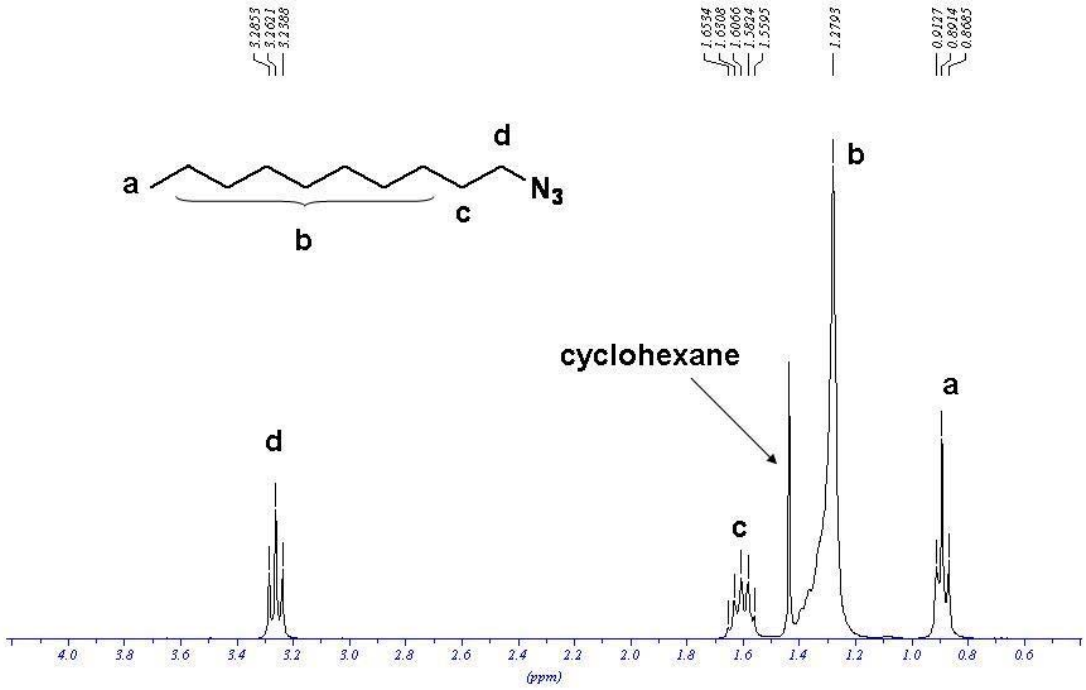
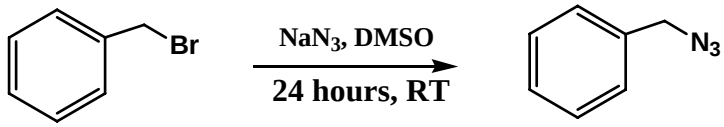


Figure 9: ^{1}H NMR spectrum in $CDCl_3$ of 1-azido-decane.

Azidation of Benzyl bromide



In a round bottom flask, to a 0.5 M solution of NaN_3 (0.25 g, 3.8 mmol) in DMSO (8 mL) was added benzyl bromide (0.51 g, 3.0 mmol) at ambient temperature. The solution was stirred for 24 hours at ambient temperature and then quenched with 10 mL H_2O (reaction slightly exothermic). After cooling down to ambient temperature, the mixture was extracted with Et_2O. The organic layers were washed with brine, dried over $MgSO_4$, filtered off and the solvent removed under reduced pressure to afford the benzyl azide pure product as pale yellow oil that was used for the click step without further purification.

IR (neat): $\tilde{\nu}$ = 3032, 2929, 2089 (C-N_3 absorption band), 1738, 1496, 1455, 1349, 1252, 1202, 1078, 1029, 875, 735, 695 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K) δ = 4.36 (s, 2H, CH_2-N_3), 7.34-7.40 (m, 5H, $CH_{aromatic}$).

^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ = 54.85, 128.28, 128.37, 128.89

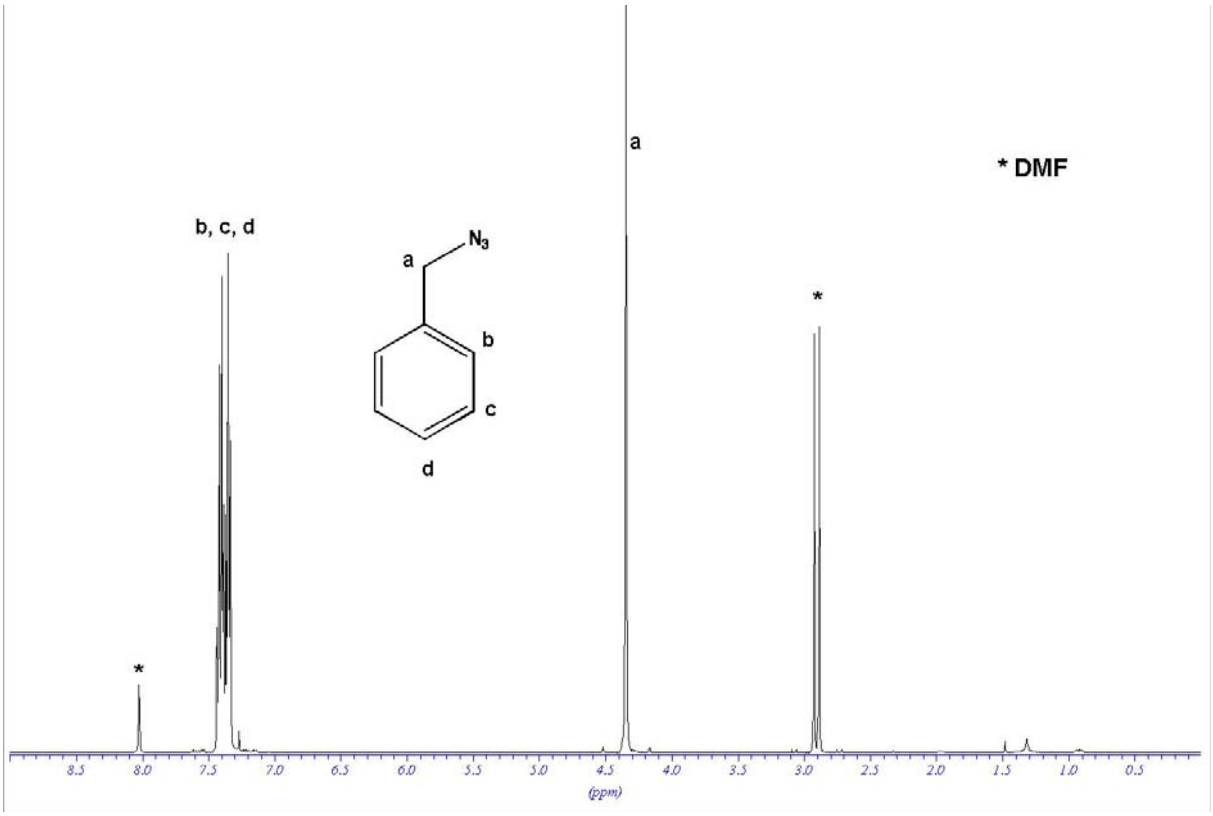


Figure 10: ^1H NMR spectrum in $CDCl_3$ of benzyl azide.

[3+2] Huisgen cycloaddition ("Click" chemistry reaction) of 1-alkyne-deprotected polymer with 1-azido-decane

A solution of polymer **IX** (22 mg, $1.3.10^{-3}$ mmol) in 100 µL of dimethylsulfoxyde was added to 900 µL of a 20 mM phosphate buffer (pH 7.4). The yellowish suspension was sonicated before the addition of 1-azido-decane (9.7 mg, $5.3.10^{-2}$ mmol). Sodium ascorbate (2.5 mg, $1.3.10^{-2}$ mmol) and $CuSO_4$ (1 mg, 6.10^{-3} mmol) were sequentially added. The mixture was stirred at ambient temperature for 24 hours in the dark and the resulting greenish slurry was extracted with dichloromethane (3 × 3 mL). The organic fractions, collected, were passed through a pad of neutral alumina that was subsequently eluted with dichloromethane and then with THF. The final product was isolated by removing solvents were under reduced pressure.

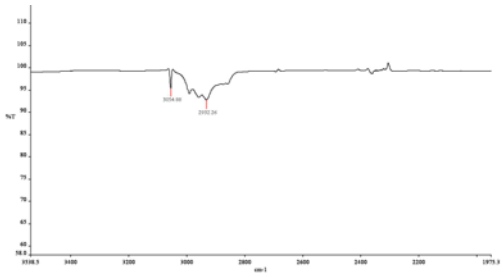


Figure 11: Partial FT-IR spectrum of polymer **IX** after click chemistry reaction with 1-azido-decane. The 1-alkyne C-H stretching was no longer present, indicating a virtually quantitative conversion of the alkyne groups into triazole moieties.

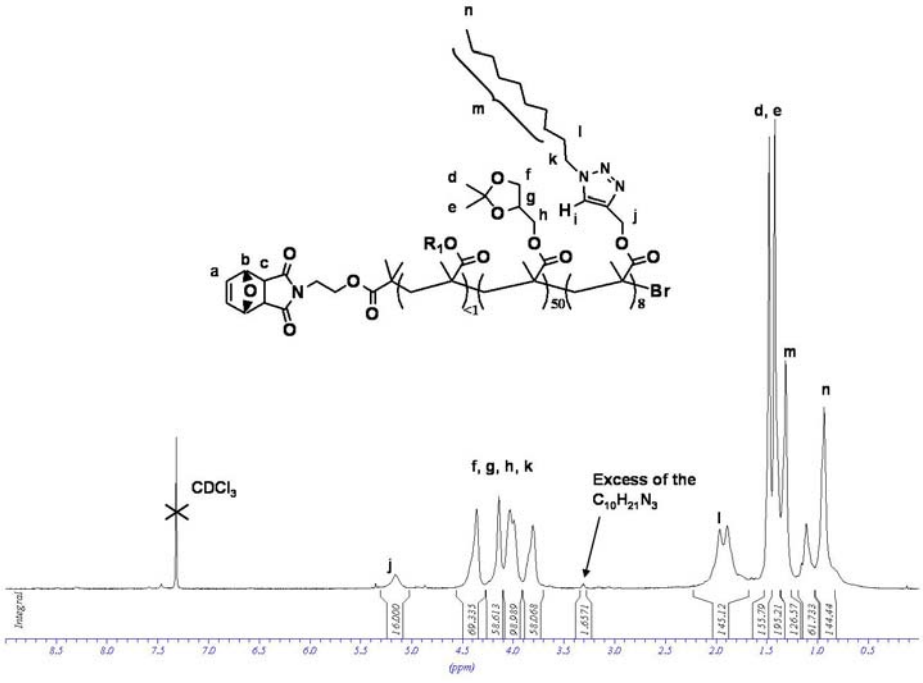


Figure 12: ^{1}H NMR of the polymer **IX** after click chemistry reaction with 1-azido-decane.

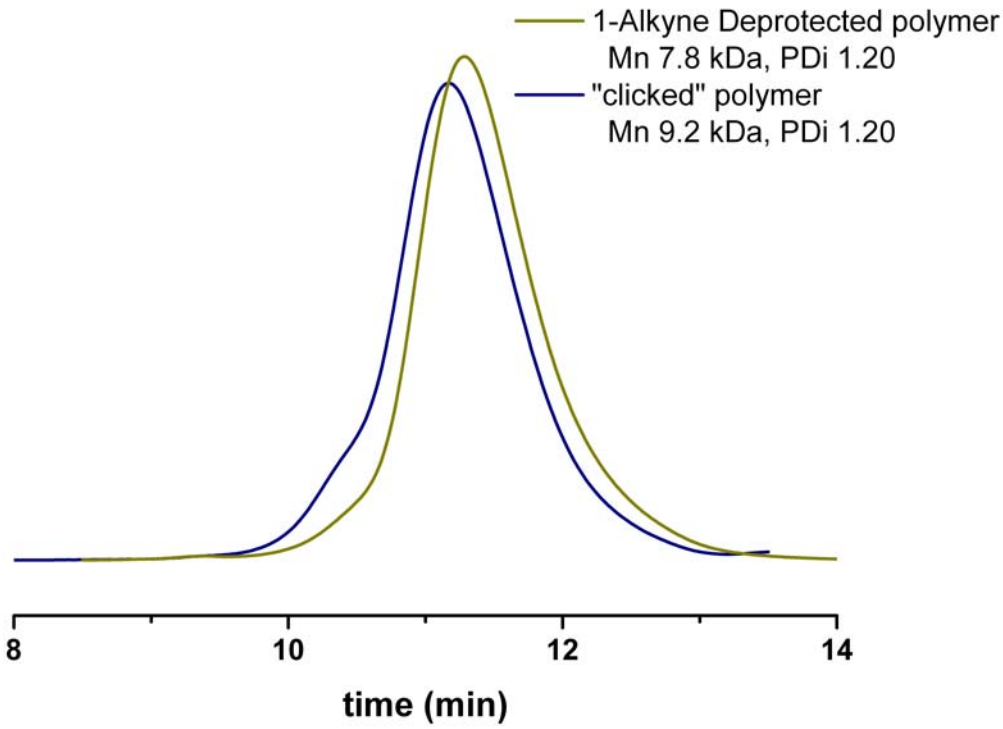


Figure 13: SEC traces of the 1-Alkyne deprotected **IV** polymer (green) and the product of the "clicking" reaction with 1-azido decane (blue). (The measurements were performed in a system equipped with two PL gel 3 µm mixed E-columns (300x7.5 mm^2) and one PL gel 3 µm guard column (50x7.5 mm^2) (Polymer Laboratories) with differential refractive index detection using THF/Et_3N 95:5 (vol/vol) at 1.0 mL min^{-1} as the eluent. Poly(MMA) standards ($3x10^5$–200 g mol^{-1}) were used for calibration. The analysed samples contained toluene (0.2% vol/vol) as the flow marker.

Huisgen [3+2] cycloaddition ("Click" chemistry reaction) of 1-alkyne deoprotected polymer with benzyl-azide

This reaction was carried out under reaction conditions identical to those employed for 1-azido-decane.

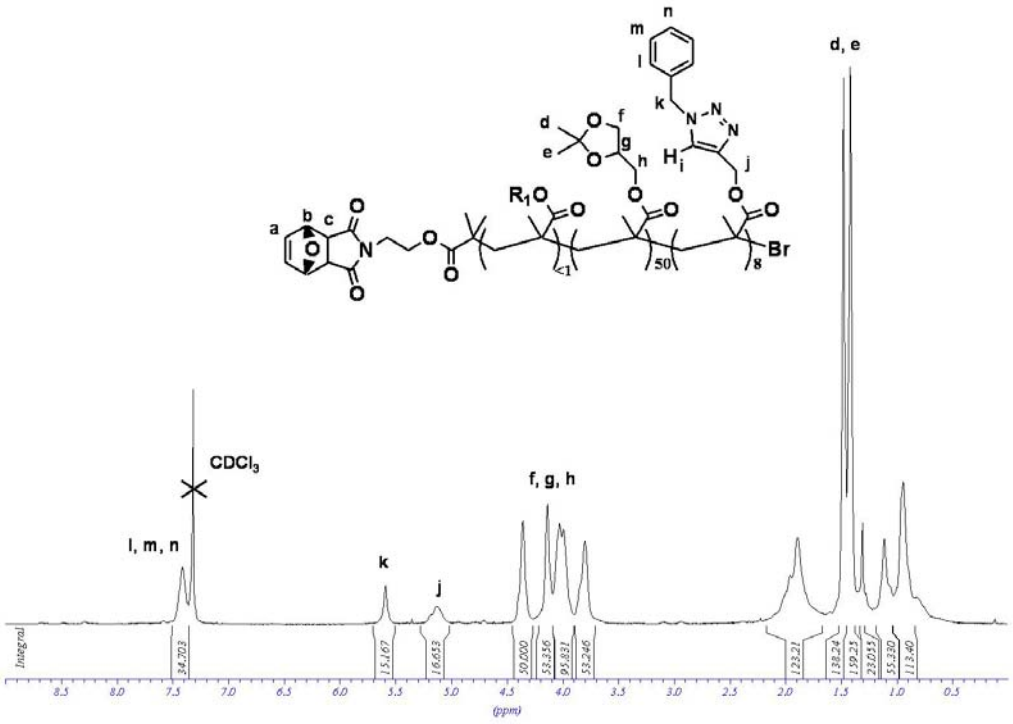


Figure 14: ^{1}H NMR spectrum of the polymer **IX** after click chemistry reaction with benzyl-bromide.

Coupling of BSA to the maleimide appended polymer I : Synthesis of the BSA-PA(I) Biohybrid IX.

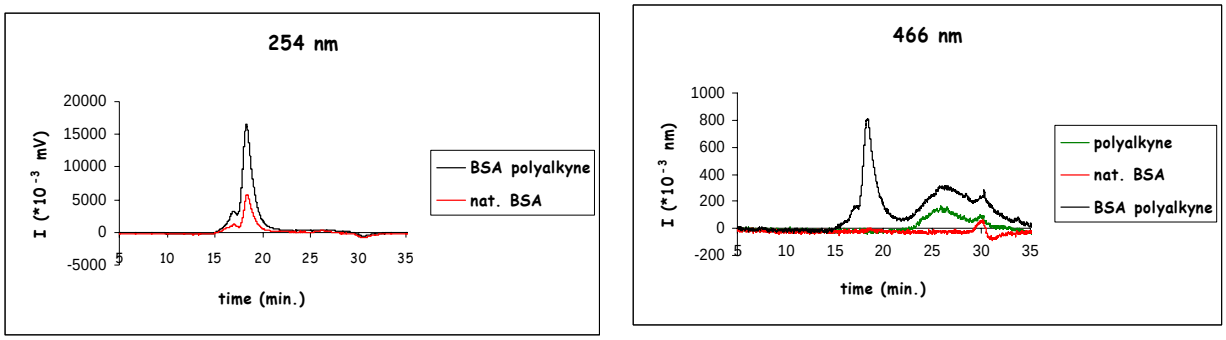


Figure 15: GPC traces of BSA (red), BSA-polyalkyne **I** (black, after dialysis) and polyalkyne **I** (green) recorded at 254 and 466 nm.

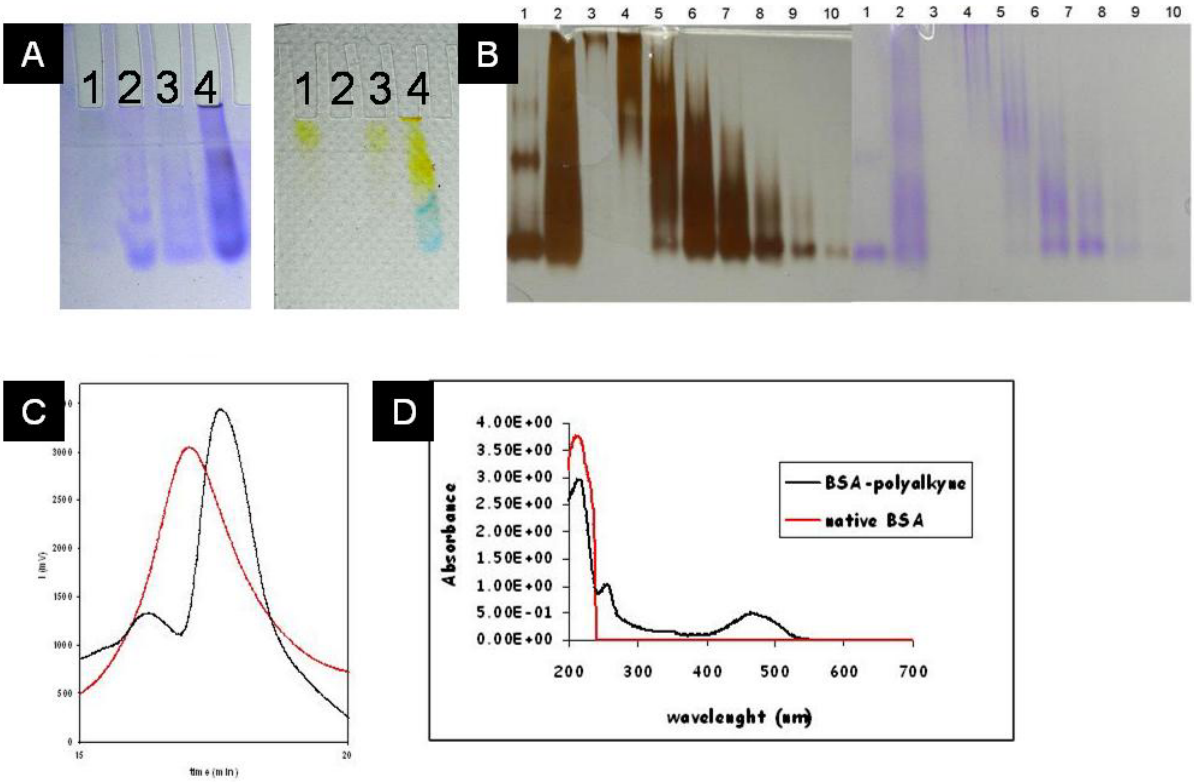


Figure 16: A. SDS-PAGE electrophoresis (left Coomassie Blue, right visualization at 366 nm), Lane 1: Poly-1-alkyne **I**, Lane 2: native BSA, Lane 3: BSA and **I** blank, Lane 4: BSA-PA reaction mixture **B.** Native gel electrophoresis (left Silver Staining, right Commassie Blue), Lane 1: Native BSA, lane 2: BSA-PA reaction mixture, lane 3: BSAPA@C10, lane 4: BSA-PA fraction 1, lane 5: BSA-PA fraction 3, lane 6: BSA-PA fraction 5, lane 7: BSA-PA fraction 7, lane 8: BSA-PA fraction 9, lane 9: BSA-PA fraction 11, lane 10: BSA-PA fraction 13. **C**. GPC traces at 280 nm of BSA (black) and BSA-polyalkyne **I** biohybrid (red) after Superdex 75 column (Thermo Biobasic SEC-300, 70% 5mM phosphate buffer pH 7.4, 30 % acetonitrile). **D.** UV-vis spectra of native BSA and BSA-polyakyne **I**.

Huisgen [3+2] cycloaddition ("Click" chemistry reaction) of 1-azido-decane on the BSA-polyalkyne adducts – Formation of the Giant Amphiphiles.

A ~0.1 mM sample of compound in milliQ was diluted 100 times before depositing on a TEM formvar grid (waiting period before drying 5 min) or the glass slide for the confocal microscopy. In the latter case, the samples were sealed prior to measurements.

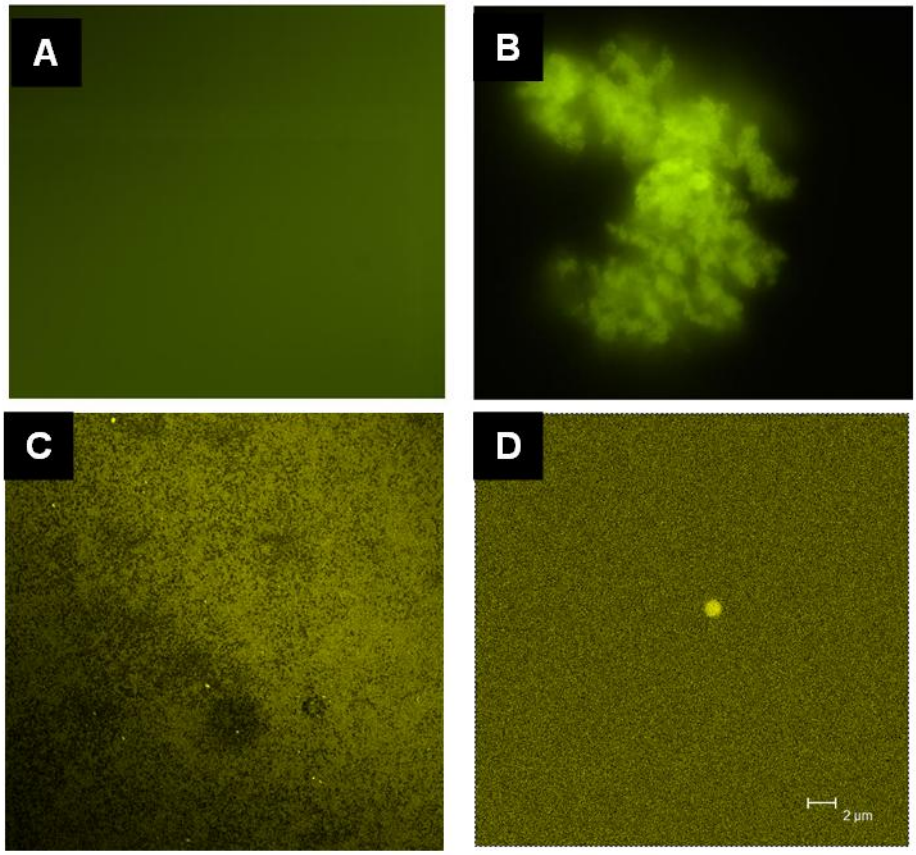


Figure 17: Images from the fluorescence microscope. Both the PA and the BSA-PA did not reveal any fluorescent aggregates in solution (A, C, D). The BSA-PA@C_{10} (**X**) aggregates were highly fluorescent when dispersed in water due to the Hostasol tag allowing for the observation of the aggregates (B).

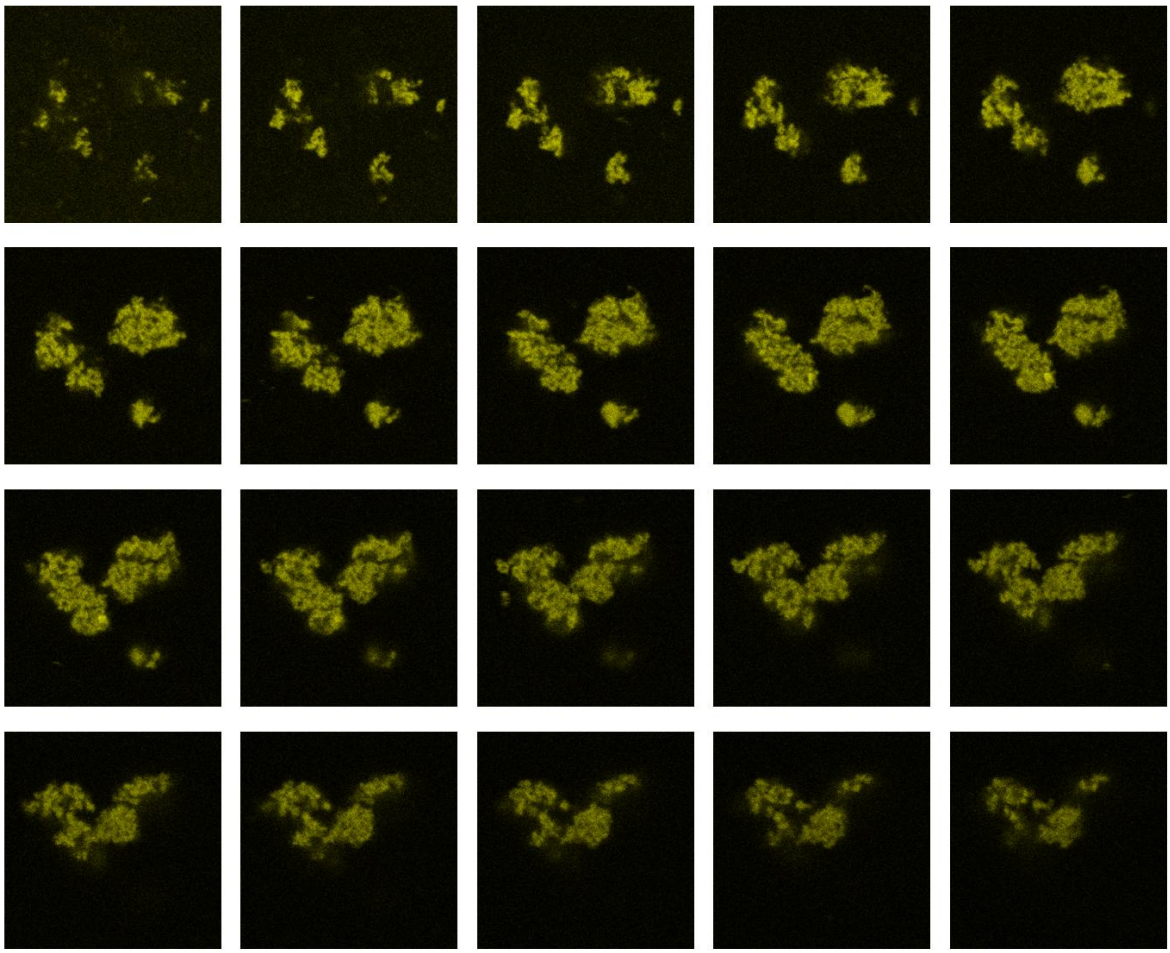


Figure 18: Confocal microscopy images if the unidirectional scanning (400 Hz) of BSA-PA@C_{10} (X) aggregates for a total height of 4µm.

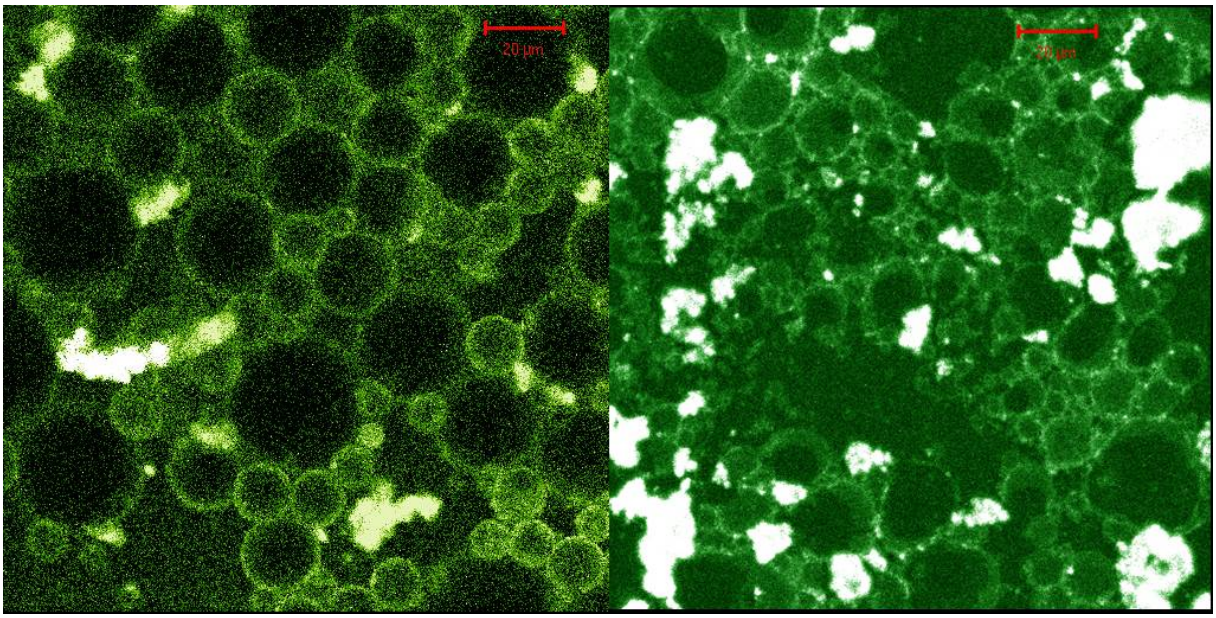


Figure 19: 0.6 mg of the conjugate **X** were suspended in 50 ml of water and 50 ml of ethanol. 2 drops of decane were then added and the mixture was shaken vigorously for 10 seconds. The resulting milky emulsion was analysed by confocal microscopy (left). The right picture

represents the confocal image of the same sample after almost complete evaporation of the solvent mixture.

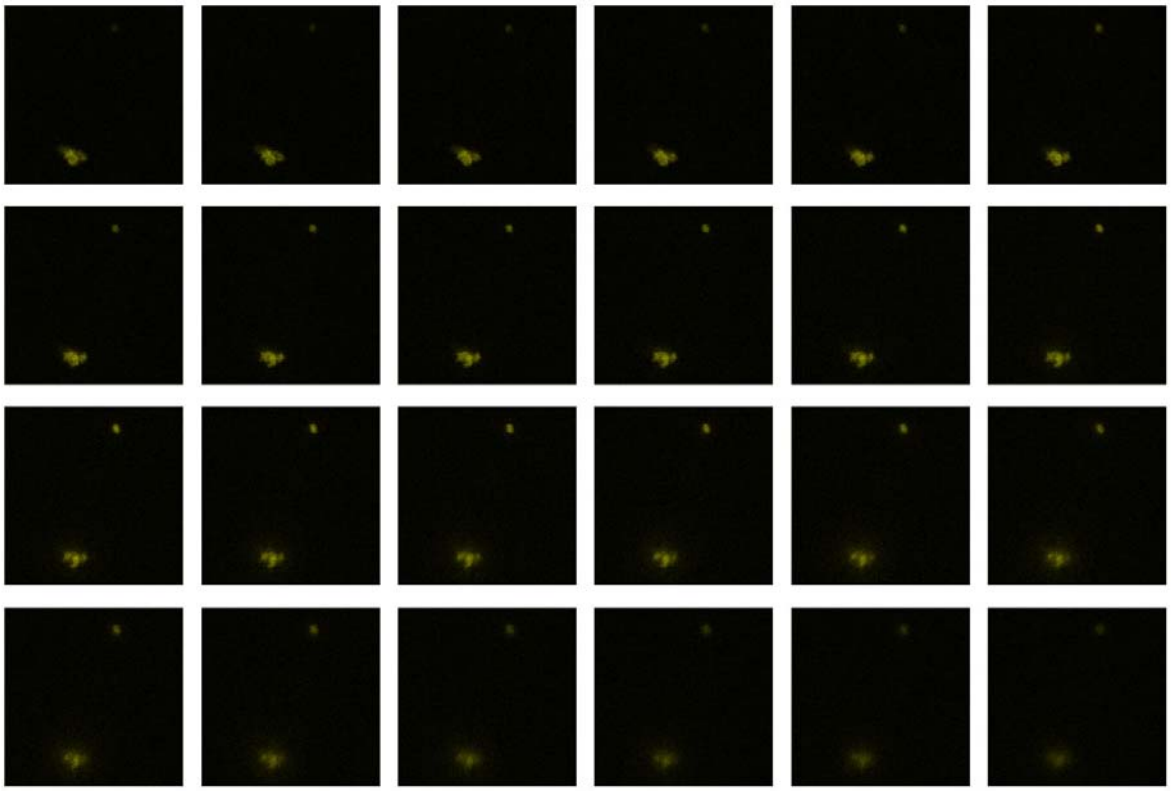


Figure 20: Confocal microscopy images if the unidirectional scanning (400 Hz) of BSA-PA@C_7 (**XI**) aggregates for a total height of 2μm.

References:

(1) R. N. Keller, and H. D. Wycoff, *Inorganic Syntheses*, 1946, pp 1-4.
(2) D. M.Haddleton, M. C. Crossman, B. H. Dana, D. J. Duncalf, A. M. Heming, , D. Kukulj, and A. J. Shooter, *Macromolecules* 1999, **32**, 2110-2119.
(3) S. Perrier,; S. P. Armes, X. S. Wang,; F. Malet, and D. M. Haddleton, *Journal of Polymer Science, Part A: Polymer Chemistry* 2001, **39**, 1696-1707.
(4) G. Mantovani, , F. Lecolley, L. Tao, D. M. Haddleton, J. Clerx, J. J. L. M. Cornelissen, and K. Velonia, *J. Am. Chem. Soc.* 2005, **127(9)**, 2966-2973.
(5) V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin, and D. M. Haddleton, *J. Am. Chem. Soc.* 2006, **128**, 4823-4830.
(6) F. Tronc, M. Li, J. Lu, M. A. Winnik, B. L. Kaul and J.-C. Graciet *J. Polym. Sci.*, *Part A: Polym. Chem.* 2003, **41**, 766–778.
(7) S. G. Alvarez and M. T. Alvarez,*Synthesis* 1997, **5**, 413-414.