

Recognition Mediated Encapsulation and Isolation of Flavin Polymer Conjugates using Dendritic Guest Moieties

Chandramouleeswaran Subramani,^a Gulen Yesilbag,^b Brian J. Jordan,^a Xiaoning Li,^a Abraham Khorasani,^a Graeme Cooke,^c Amitav Sanyal,^{*b} and Vincent M. Rotello,^{*a}

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts

01003, and Department of Chemistry, Bogazici University, Bebek, 34342, Istanbul, Turkey

rotello@chem.umass.edu

Supporting Information

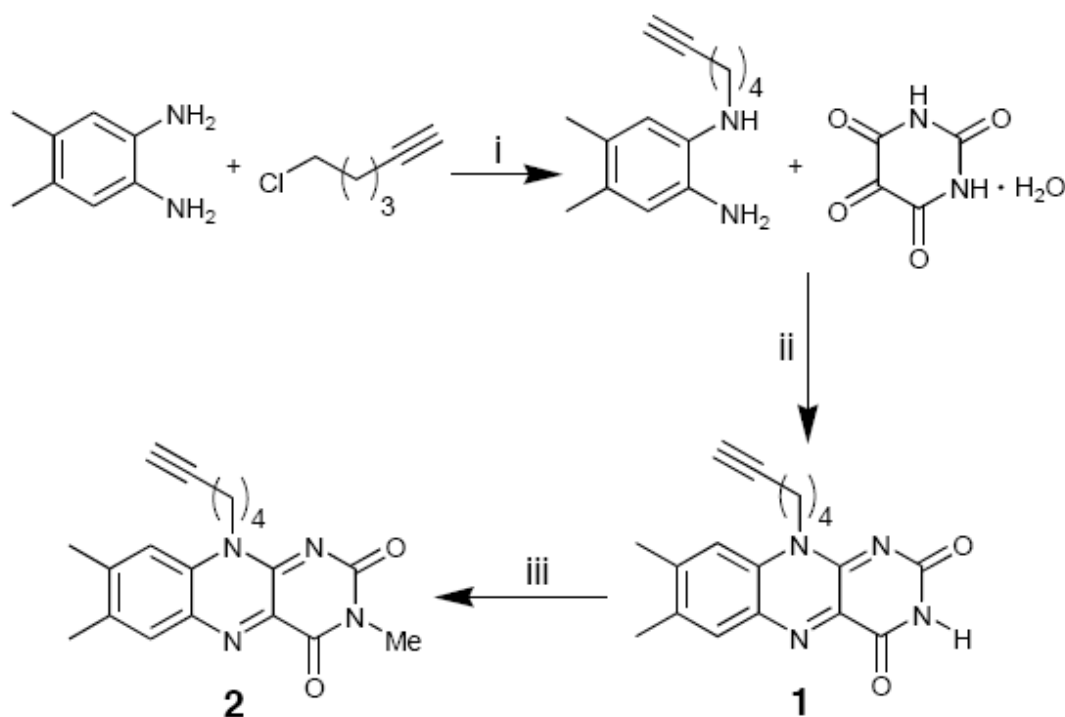
Materials and general methods

Solutions utilized in electrochemical experiments were prepared using reagent grade CH_2Cl_2 dried via distillation over CaH_2 . Tetrabutylammonium perchlorate (TBAP, obtained from SACHEM, electrometric grade) was dissolved in CHCl_3 , washed with distilled water, recrystallized twice from ethyl acetate and dried for several days under high vacuum. Triethylamine was distilled over CaH_2 . All chemicals were reagent grade, obtained from Aldrich and used without further purification unless otherwise noted.

Electrochemistry

All electrochemical experiments were carried out on a Cypress System potentiostat. A 1 mm platinum button and a gold-plated electrode were utilized as working and reference electrodes, respectively. A silver wire pseudo reference electrode was used and all potentials are referenced versus the ferrocene/ferrocenium couple. The sweep rate was 100 mV/s and the studies were run on an argon-purged temperature-controlled cell. Solutions of flavin/flavin polymer and the flavin/flavin polymer-DAP complexes were prepared maintaining a constant concentration of the cofactor, with the receptors added to

saturation. The solutions were degassed by bubbling argon through them for at least 10 minutes, at which time cyclic and square wave voltammograms were recorded.



Synthesis of alkyne functionalized flavin 1

To a solution of 4, 5-dimethyl-1, 2-phenylene diamine (7.3 mmol) and Et_3N (14.6 mmol) in THF (20 ml) at 60 °C was added 6-chlorohexyne (7.3 mmol) and an excess of NaI. The reaction was allowed to proceed overnight at 60 °C. Upon cooling the reaction mixture was washed with saturated NaCl solution, and the organic layer was then removed, dried with anhydrous Na_2SO_4 , filtered, and reduced under reverse pressure. The resulting mixture was then purified using flash column chromatography (hexanes/ethyl acetate

1:1), resulting in the mono alkylated, alkyne functionalized diamine derivative as a reddish oily product (4.4 mmol, 61% yield). In a round bottom flask was combined the purified alkyne functionalized diamine derivative (5.7 mmol), alloxan monohydrate (5.7 mmol) and boron oxide (11.5 mmol) in acetic acid (20 ml). The above mixture was then heated to 60 °C for 3 hours. The mixture was then cooled, and precipitated into H₂O. The mixture was then extracted with CH₂Cl₂ (3 x 25 ml), the organic layers were combined, washed with brine, anhydrous Na₂SO₄, filtered, and reduced under reverse pressure onto silica gel. The crude product was then purified using flash column chromatography (10:1 CH₂Cl₂/THF). The columned product was then recrystallized from absolute ethanol yielding flavin **1** (3.3 mmol, 59% yield after recrystallization, mp 279-282 °C).

Synthesis of control *N*-methylated, alkyne functionalized flavin **2**

To a solution of **1** (0.462 mmol) and K₂CO₃ (1.84 mmol) in DMF (5 ml) at 60 °C was added MeI (0.924 mmol) and the reaction was allowed to proceed overnight. Upon cooling, the reaction was quenched with H₂O and extracted with CHCl₃ (3 x 25 ml). The organic layers were combined, washed with brine, anhydrous Na₂SO₄, filtered, and reduced under reverse pressure onto silica gel. The crude product was then purified using flash column chromatography (15:1 CH₂Cl₂/THF) yielding control *N*-methylated, alkyne functionalized flavin **2** (0.420 mmol, 91% yield, mp 260-263 °C).

Flavin functionalized copolymers

For the synthesis of chloromethylstyrene/styrene copolymer, please see to: Ilhan, F.; Gray, M.; Rotello, V. M. *Macromolecules* 2001, *34*, 2597-2601.

Azide functionalized polystyrene polymer **3.** To a solution of chloromethylstyrene copolymer (0.2 mmol) in DMSO (10 ml) was added an excess of NaN₃ (2 mmol, ~10 eq.

azide per polymer chain). The reaction was allowed to proceed overnight. The polymer was then precipitated from H₂O. The crude polymer was then filtered, washed with copious amounts of H₂O, and allowed to dry yielding azide functionalized polystyrene polymer **3** (0.196 mmol, 98% yield).

Flavin functionalized polystyrene polymer 4. To a solution of polymer **3** (40 μ mol) and flavin **1** (0.24 mmol) in DMSO (5 ml) was added CuI (cat.). The reaction was allowed to proceed for 3 hours whereupon the polymer was precipitated into a stirring solution of saturated NH₄⁺Cl⁻. The dark, reddish brown DMSO mixture instantaneously yielded highly fluorescent polymer **4**. The polymer was then filtered, washed with copious amounts of H₂O, and dried resulting in flavin functionalized polystyrene polymer **4** (36 μ mol, 90% yield).

Control *N*-methylated flavin functionalized polystyrene polymer 5. To a solution of polymer **3** (40 μ mol) and control flavin **2** (0.24 mmol) in DMSO (2 ml) was added CuI (cat.). The reaction was allowed proceed for 3 hours whereupon the polymer was precipitated into a stirring solution of saturated NH₄⁺Cl⁻. The dark, reddish brown DMSO mixture instantaneously yielded highly fluorescent polymer **5**. The polymer was then filtered, washed with copious amounts of H₂O, and dried resulting in control *N*methylated flavin functionalized polystyrene polymer **5** (37 μ mol, 93% yield).

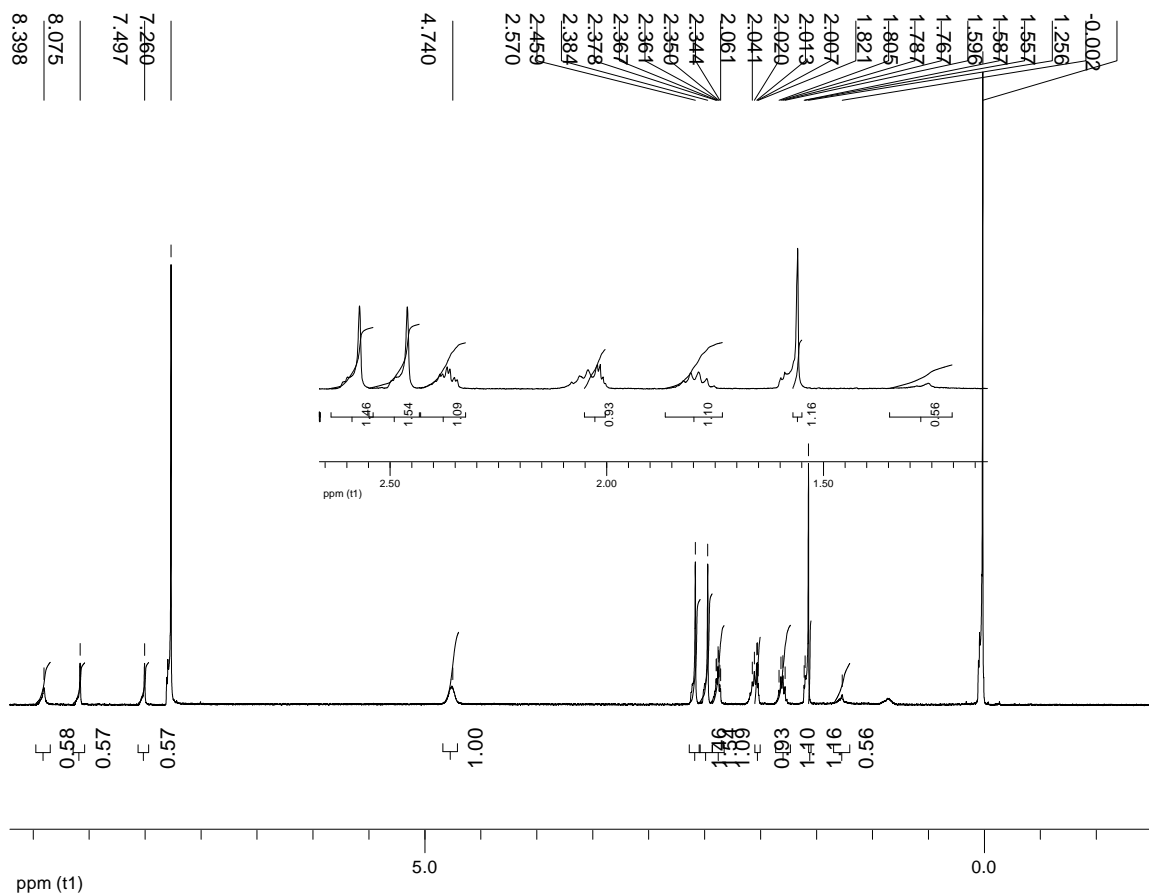
Synthesis of DAP Dendrons

The poly (aryl ether) bromide dendrons reported here were synthesized by the adaptation of the procedures used by Fréchet and Hawker [1]. The poly (aryl ether) azide dendrons were synthesized according to literature [2]. DAP-OH was prepared according to reported literature [3].

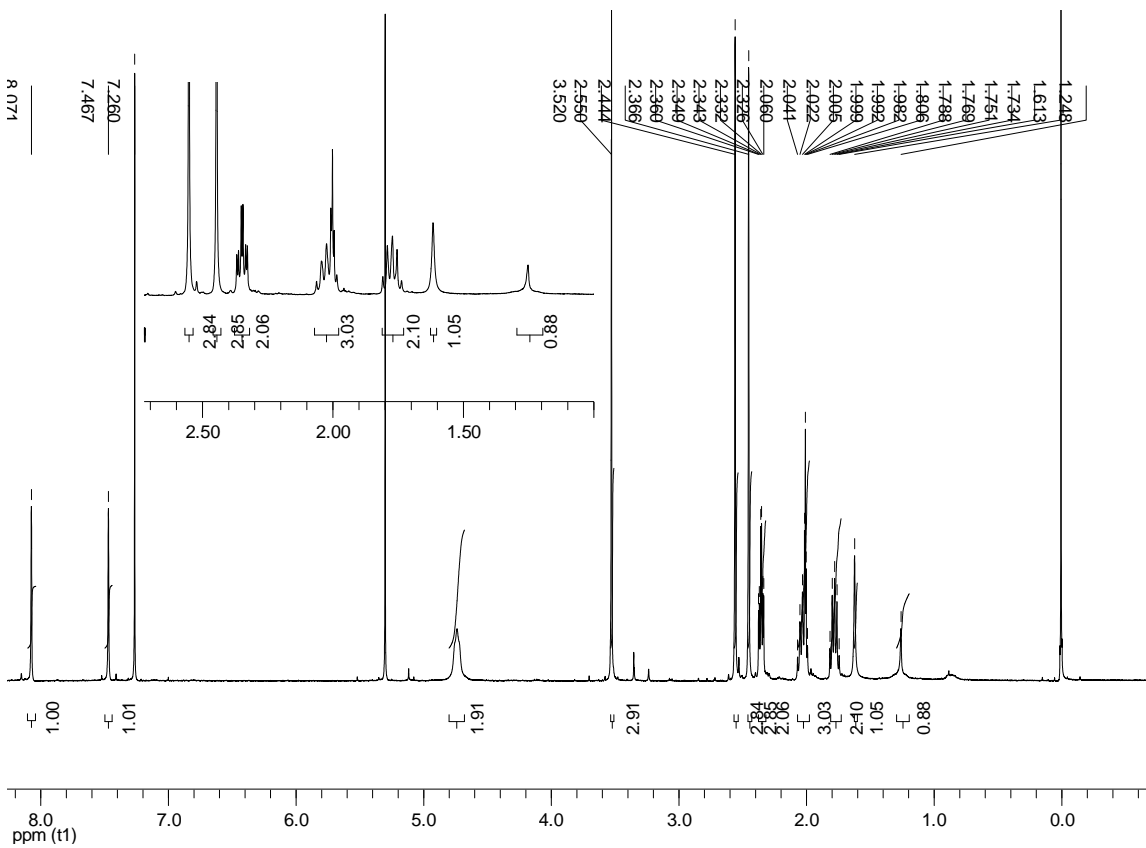
Representative procedure for G2-DAP-dendron :

A solution of G₂-bromide (0.131 g, 0.16 mmol), DAP-OH (0.040 g, 0.18 mmol), 18-crown-6 (0.002 g, 0.008 mmol) and anhydrous K₂CO₃ (0.033 g, 0.24 mmol) in dry acetone (6 mL) was refluxed under N₂ for 18 h. Then, the reaction mixture was filtered to remove K₂CO₃, and the filtrate evaporated under *vacuo*. The residue was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate (2 x 25 mL). The combined organic layers were dried over Na₂SO₄, filtered and the volatiles were removed under *vacuo*. The crude product was filtered through a plug of silica with ethyl acetate/ hexane (1:1) as eluent to obtain DAP G₂-dendron as a waxy solid. (0.127 g, 83 % yield).

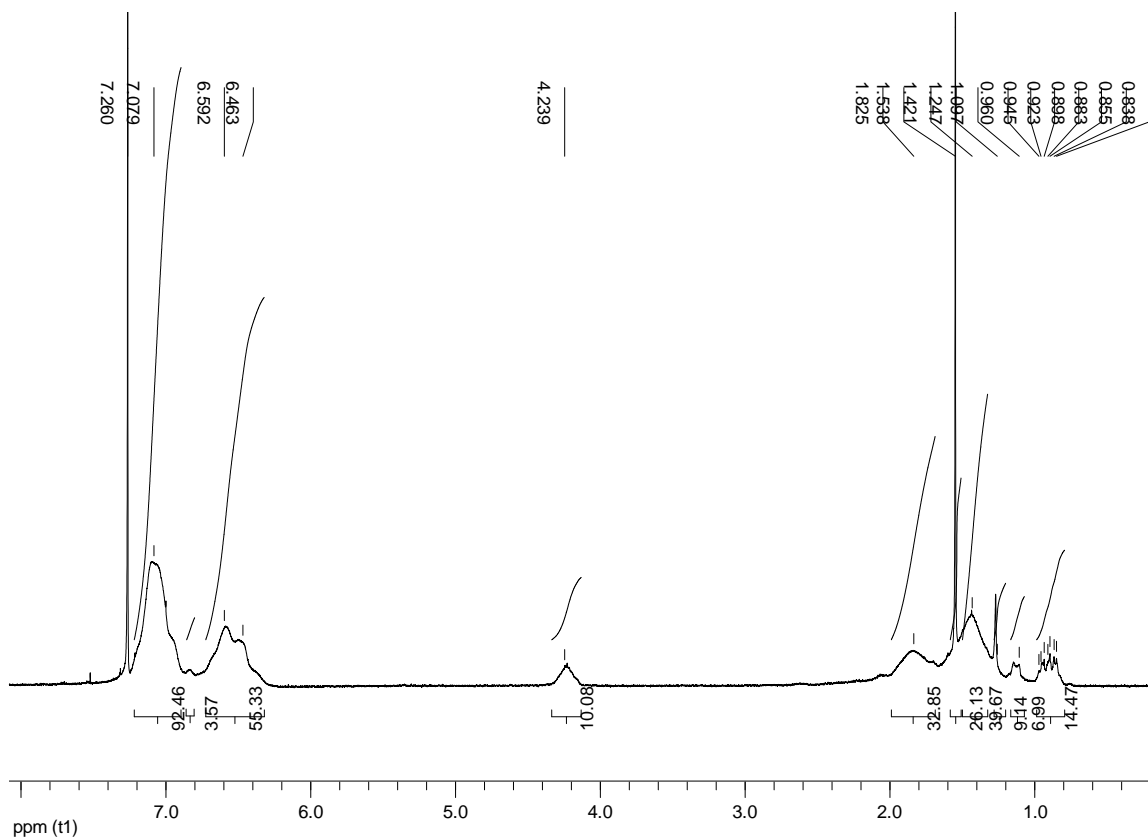
¹H NMR spectra of flavin alkyne 1



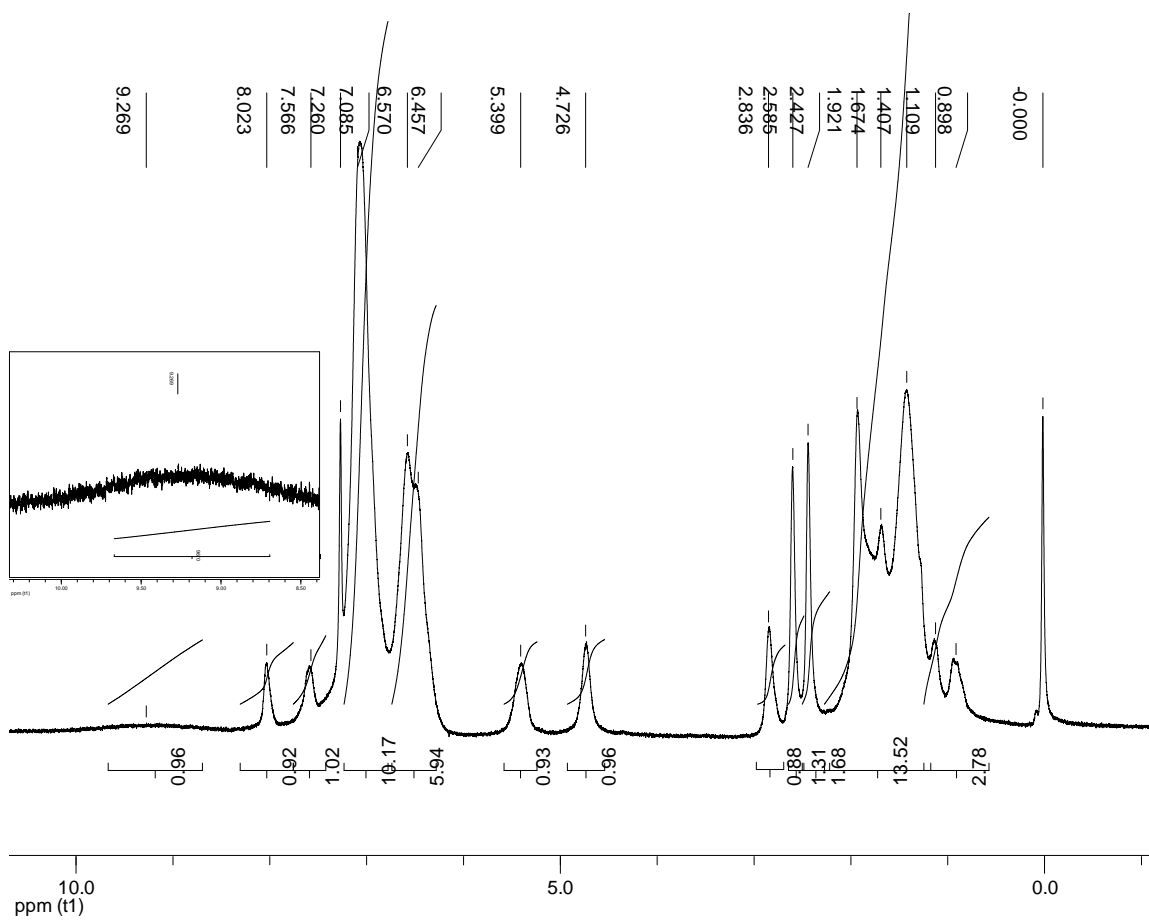
¹H NMR spectra of NMe-flavin 2



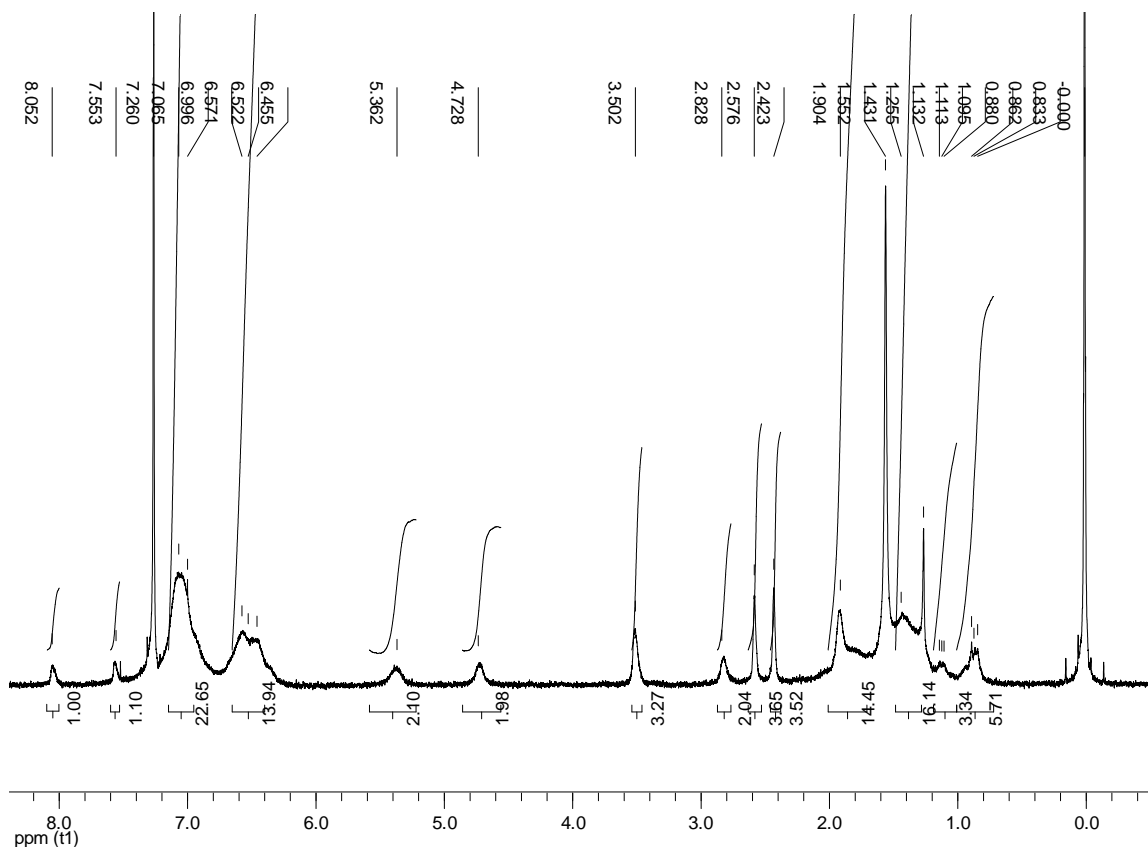
^1H NMR spectra of azide functionalized polystyrene polymer 3



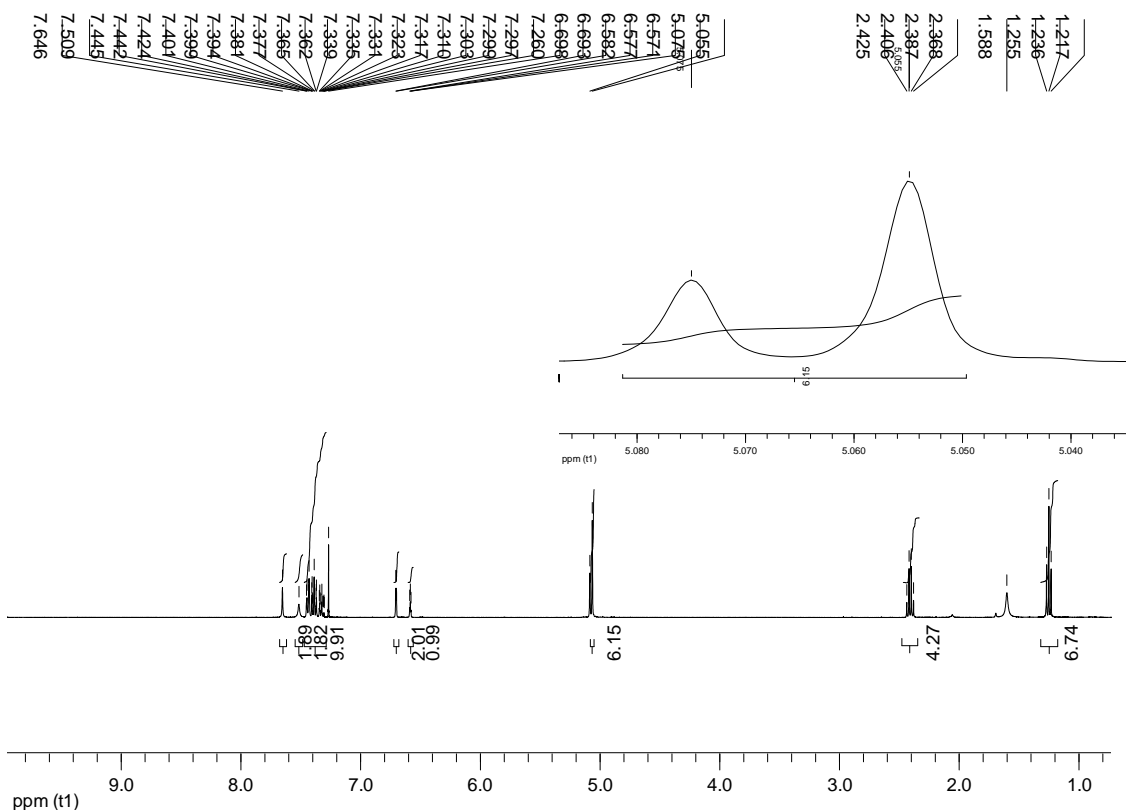
¹H NMR spectra of flavin polymer 4



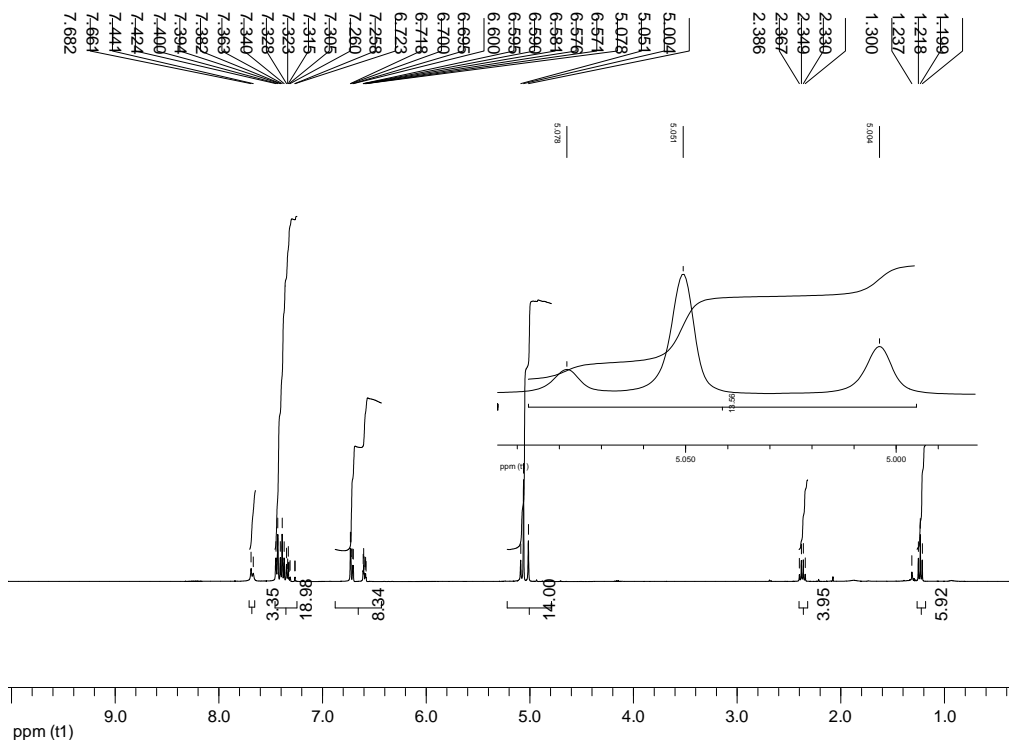
^1H NMR spectra of NMe-flavin polymer 5



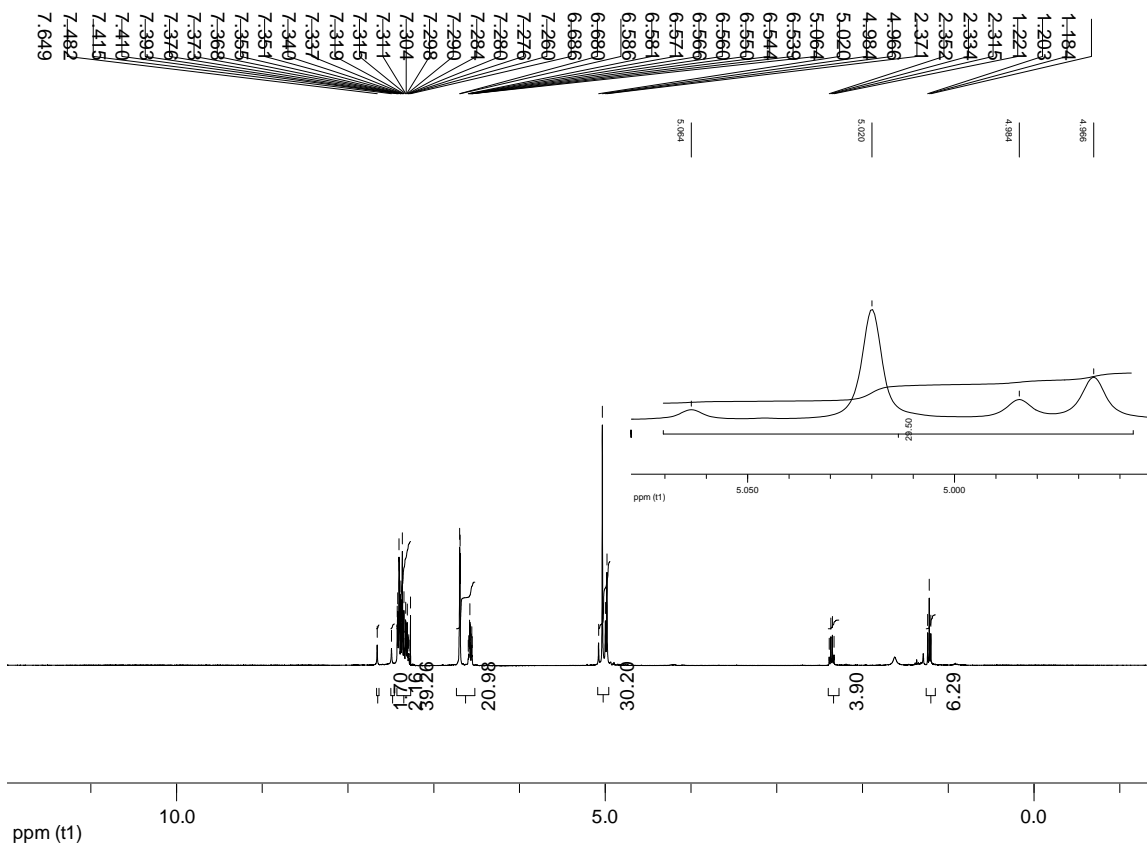
¹H NMR spectra of G1-DAP



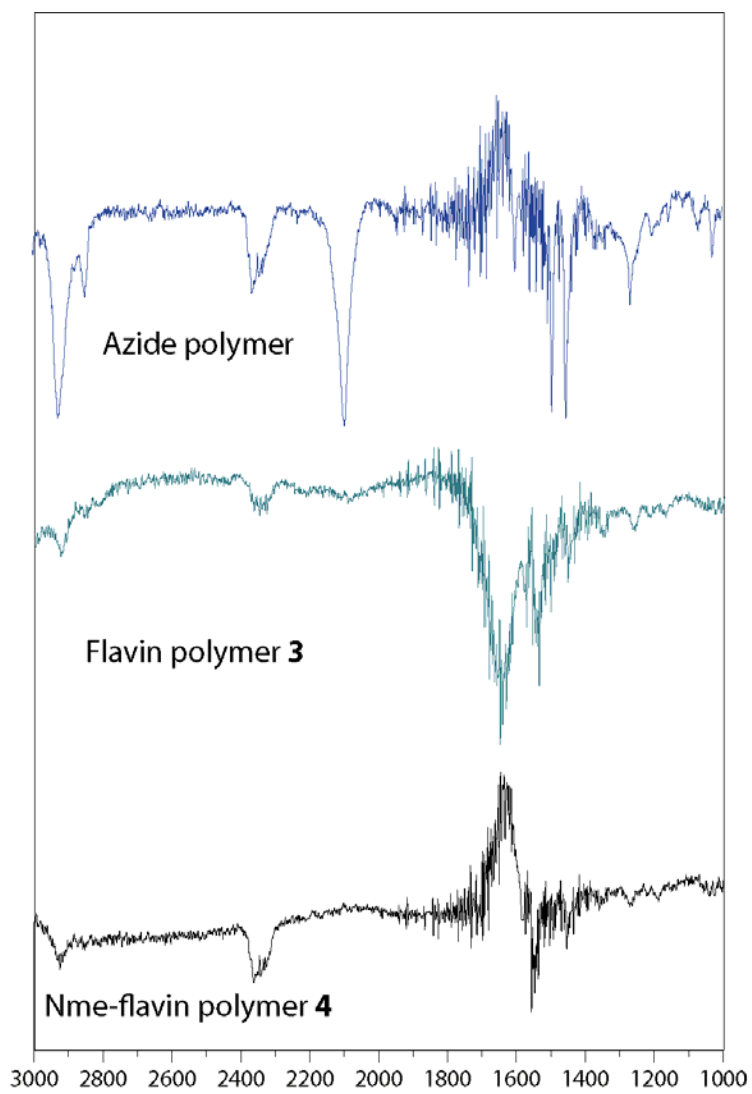
¹H NMR spectra of G2-DAP



¹H NMR spectra of G3-DAP



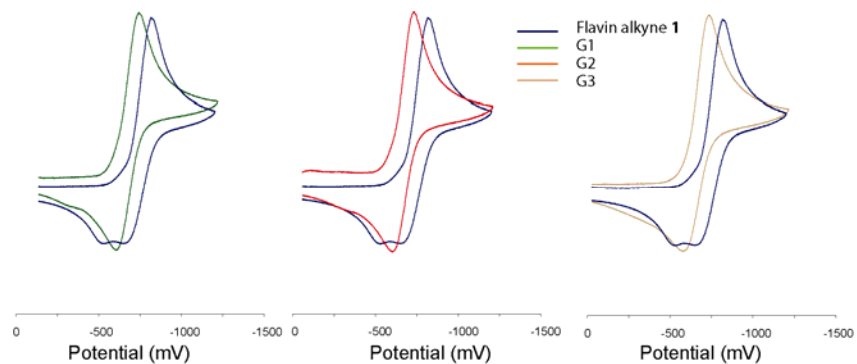
Infrared Spectroscopy



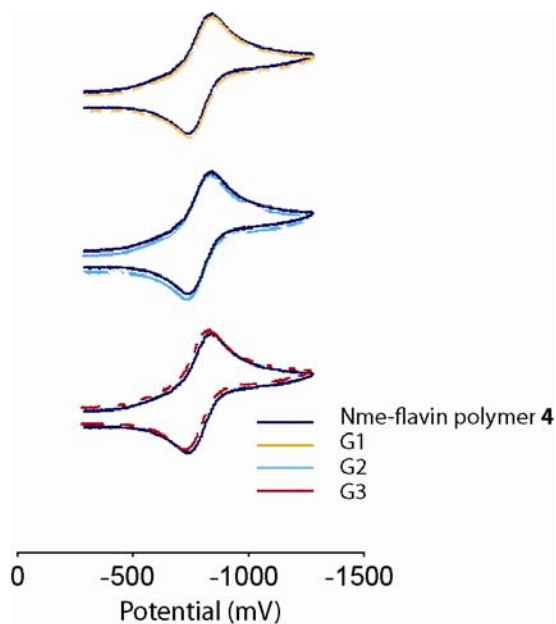
S1. FTIR spectrums for following the click reaction

Cyclic Voltammetry:

CV traces were recorded for a CH_2Cl_2 blank, then for a solution of the flavin or flavin polymer and ferrocene (as a reference), and finally for the same samples containing excess DAP. Differences in halfwave potentials were determined using square-wave voltammetry. All scans were recorded at a sweep rate of 100 mV S^{-1} .



S2. CV traces of flavin alkyne 1 with G1,G2 and G3 DAP dendrons at 4 equivalents



S3. CV traces of NMe flavin polymer 4 with G1,G2 and G3 DAP dendrons at 2 equivalents

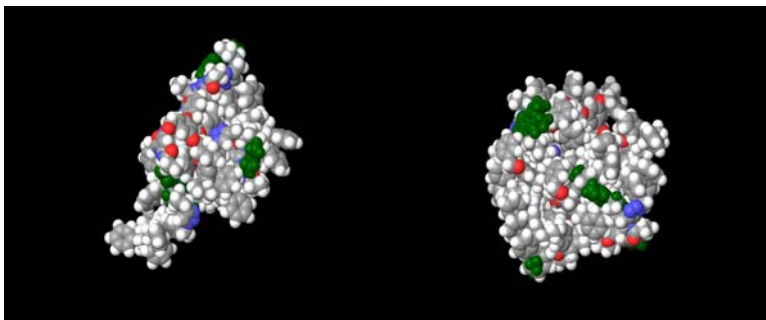
Table 1. Half-Wave Potential (mV) and Association Constants(M^{-1}) Associated with NMe flavin Systems

Flavin system	K_a (ox) ^b	$E_{1/2}(u)$ ^c	$E_{1/2}(b)$ ^d	$\Delta E_{1/2}$	K_a (red)
NMe flavin 2	0	-1350	0	0	0
	0	-1350	0	0	0
	0	-1350	0	0	0
Polymer 4	0	-1350	0	0	0
	0	-1350	0	0	0
	0	-1350	0	0	0

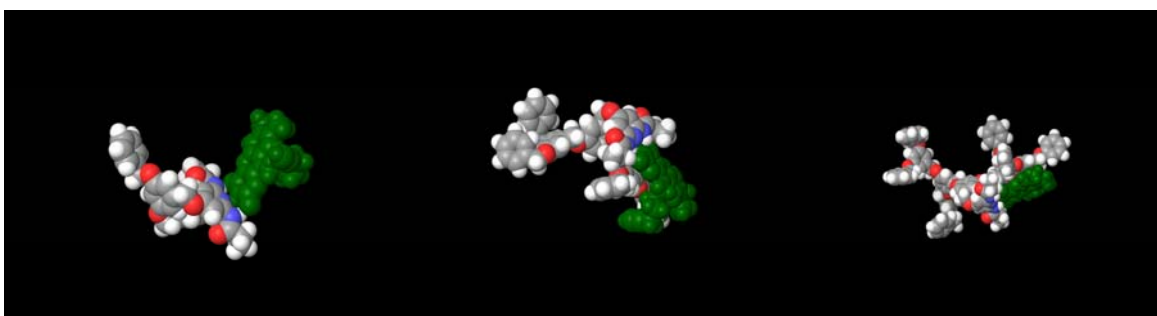
Molecular dynamics simulation(Amber force field) of a. flavin polymer 4, b.flavin polymer 4-G1 DAP, G2 DAP and G3 DAP dendron showing partial shielding of the flavin moiety(highlighted in green).

Molecular dynamic simulations were performed on the flavin/flavin polymer with DAP dendrons using MacroModel (Maestro v8.0) from Schrodinger, Inc. Amber* force field was chosen from the subsystem “Dynamics” and the calculations were performed in a continuum of chloroform as the solvent. Electrostatic treatment was set to the constant dielectric setting of 1.0. The external cutoffs were automatically set to 8.0 (Van der Waals), 20.0 (electrostatics), and 4.0 (hydrogen bonding). There were no constraints placed on the molecule. The method used was TNCG (Truncated Newton linear Conjugate Gradient), with the maximum number of iterations at 500. Convergence was based on a gradient and the threshold was set to 0.0500. Stochastic dynamics were used, and all the bonds were shaken during the calculation. The simulation temperature was performed over the range of 1000K-100K (decreasing). The time steps (fs) were set to 1.5, the equilibrium time (ps) set to 1.0, and the simulation time (ps) set to 10.0.

S4. Molecular dynamics simulation(Amber force field)

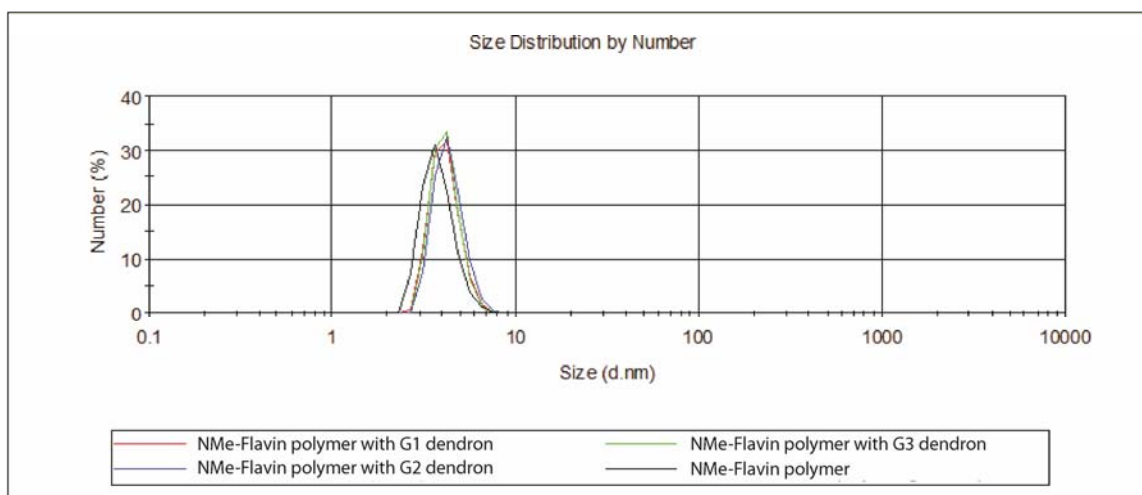
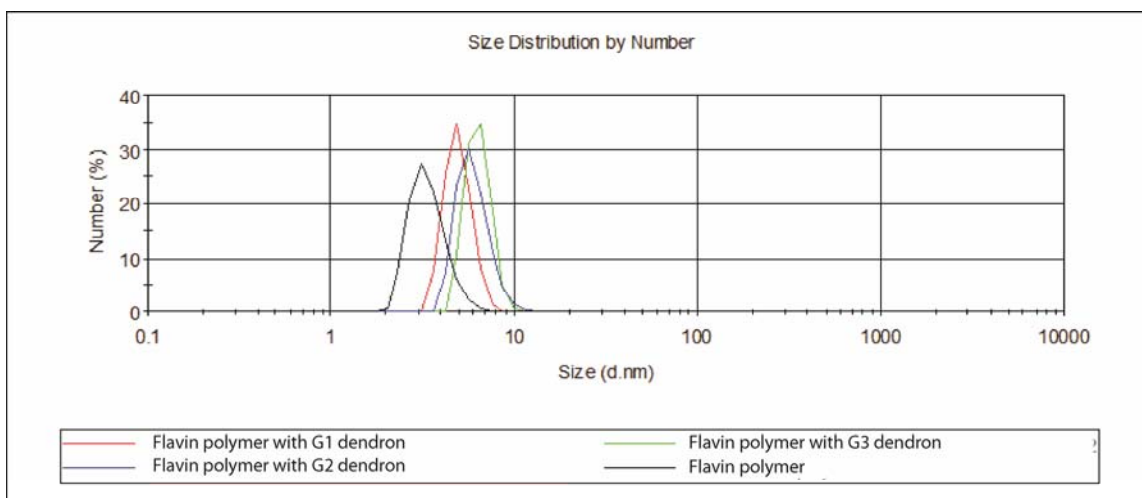


a. Polymer Flavin 3 – with G1, and G2 DAP dendron respectively



b. Flavin alkyne 1- G1 DAP, G2 DAP and G3 DAP dendron do not show shielding of flavin moiety (highlighted in green).

S5. Dynamic light scattering studies:



References

- [1] Hawker, C. J. and J. M. J. Fréchet, *J. Am. Chem. Soc.* **1990**, *112*, 7638.
- [2] Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules*, **2005**, *38*, 3663.
- [3] Ilhan, F.; Galow, T. H.; Gray, M.; Clavier, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 5895.