

Electronic Supplementary Information (ESI)

Synthesis, binding and self-assembly properties of a well-defined Pillar[5]arene End Functionalised polydimethylacrylamide

N. Laggoune, F. Delattre, J.Lyskawa, F. Stoffelbach, J.M. Guigner, S. Ruellan, G. Cooke and P.Woisel

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Experimental Section

1. Materials

All reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. *N,N*-Dimethylacrylamide (DMAC) was purified by vacuum distillation under reduced pressure before use. The primary radical source used in all polymerizations was 2,2'-azobis(2-methylpropionitrile) (AIBN, > 98%, Fluka) and was used as received. Compounds **DiMePilla-NH₂**,¹ **CTA-NHS**,² **G1**³ and **G2**⁴ were prepared according to literature procedures.

2. Characterization Techniques

¹H NMR and ¹³C experiments were carried out on a Bruker Advance 300 or 500 MHz (for 2D-NOESY and 2D-DOSY studies) spectrometers.

2D-DOSY spectra have been performed using the bipolar longitudinal eddy current delay (BPPLIED – Bipolar Pulsed Field Gradient Longitudinal Eddy Delay) pulse sequence. The pulse gradients have been incremented in 16 steps from 2 to 98 % of the maximum gradient strength in a linear ramp. Diffusion times and gradient pulse durations were optimized for each experiment in order to achieve a 95 % decrease in resonance intensity at the largest gradient amplitude ; typically, diffusion time between 75 and 300 ms, gradient strength between 0.55 and 3 ms, spoil gradient strength of 0.6 ms, and longitudinal eddy current of 5 ms. After Fourier transformation, phase and baseline correction, the diffusion dimension of the 2D-DOSY spectra was processed by means of the Bruker Dynamics Center software (version 2.1.9). The diffusion constants are calculated by exponential fitting of the data belonging to individual columns of the 2D matrix.

Size exclusion chromatography (SEC): The number-average molar masses (M_n), the weight-average molar masses (M_w), and the molar mass distributions ($D = M_w/M_n$) were determined

by SEC. Measurements were performed in DMF (+ LiBr, 1g L⁻¹) at 50 °C, and at a flow rate of 0.3 mL min⁻¹, at a polymer concentration of 5 mg mL⁻¹ after filtration through a 0.22 µm pore-size membrane. The chromatography was carried out on three Waters styragel® HT3 columns (4.6 × 300 mm; separation limits: 300 to 500 kg mol⁻¹), one Waters styragel® HT4 (4.6 × 300 mm; separation limits: 5 to 600 kg mol⁻¹) and one Waters styragel® HR1 (4.6 × 300 mm; separation limits: 5 to 100 kg mol⁻¹) coupled with a differential refractive index (RI) detector (Waters 717plus Autosampler).

Dynamic light scattering (DLS): The average particle diameter (D_h) and the dispersity factor of samples (Poly) were measured by dynamic light scattering (DLS) using a Dynapro apparatus (Wyatt Instruments) at 90°.

The mass spectrum of **MePilla-CTA** was recorded by matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry using a Bruker autoflex III smartbeam mass spectrometer, equipped with the laser that produces pulses at 337 nm using dithranol as a matrix and NaI as cationizing agent. Spectra were recorded in linear mode at an accelerating potential of 20 kV.

Cryo-transmission electron microscopy (cryo-TEM) was used to determine the morphology and size of the polymer nanoparticles. Thin liquid films of particle dispersions, prepared at 298K for **MePilla-PDMAC** (3mg.L⁻¹) in water was flash frozen in liquid ethane and observed at -180°C on a JEOL JEM-2100 LaB6 microscope (Cs= 2.0 mm) operating at 200 kV under low-dose conditions (10 electrons Å⁻² s⁻¹). Digital images were recorded on a Gatan Ultrascan 1000 CCD camera

3. Experimental procedures and characterization

3.1. Synthesis of MePilla-CTA

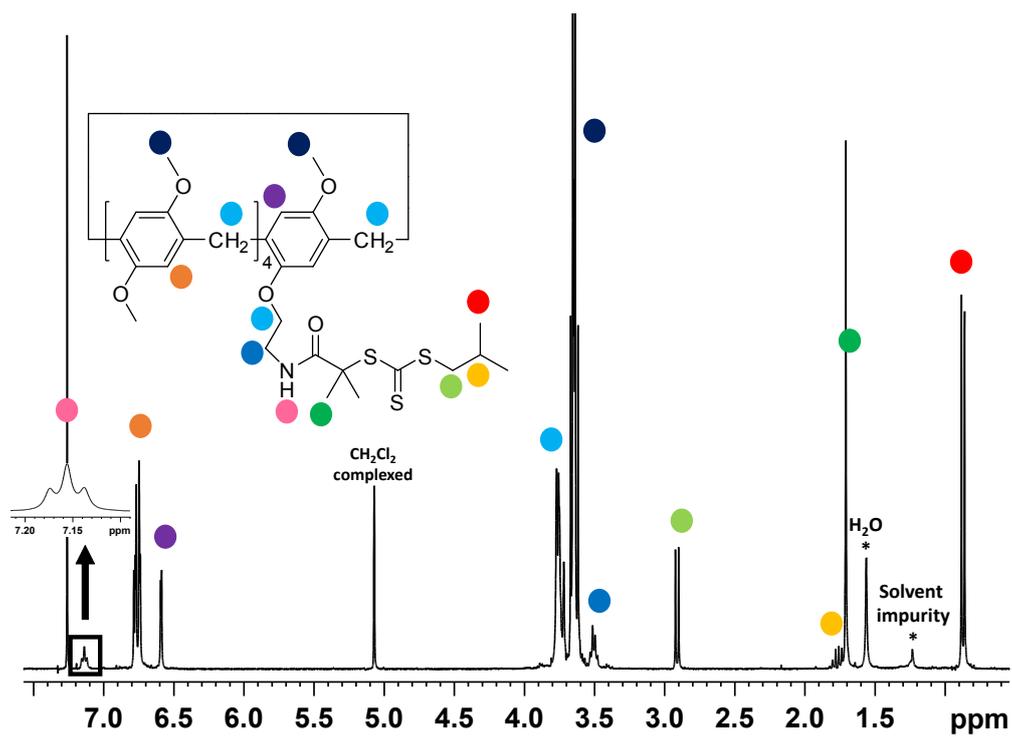
To a solution of **DiMePilla-NH₂** (0.267 g, 0.73 mmol) and Et₃N (0.50 ml, 3.6 mmol) in DCM (40 mL) was added, at 25°C and under N₂, a solution of **CTA-NHS** (215 mg, 0.62 mmol) in dry DCM (20 mL). The mixture was stirred for two days at 25°C. Then, water was added and the organic layer was dried over anhydrous Na₂SO₄. The solvent was evaporated and the residue was purified by column chromatography (SiO₂: DCM / MeOH: 40/1) afforded a yellow solid in 70 % yield.

¹H NMR (CDCl₃), δ (ppm from TMS): 0.88 (d, *J*=6.7Hz, 6H), 1.71 (s, 6H), 1.77 (m, 1H), 2.94 (d, *J*=6.9Hz 2H), 3.49 (m, 2H), 3.62 (m, 27H), 3.75 (m, 12H), 6.58 (s, 2H), 6.74 (m, 8H), 7.11 (t, *J*=5.4 Hz, 1H).

¹³C NMR (CDCl₃), δ (ppm from TMS): 22.10, 25.95, 27.66, 29.62, 29.66, 29.84, 40.14, 43.53, 52.81, 55.82, 55.93, 56.00, 56.21, 57.19, 66.71, 113.93, 114.05, 114.10, 114.14, 114.18, 114.58, 114.62, 128.17, 128.21, 128.4, 128.45, 128.59, 149.47, 105.86, 150.88, 151.05, 151.13, 173.00, 220.20.

MS(Maldi-TOF): *m/z* 1037 (M+Na)

A)



B)

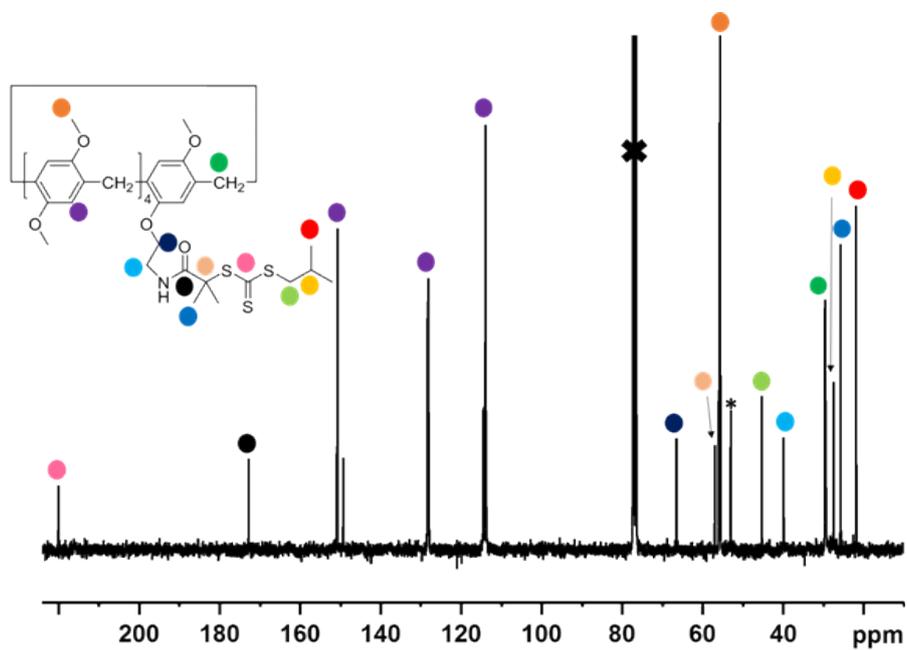


Figure S1: ¹H NMR (A) and ¹³C NMR (B) spectra of MePilla-CTA recorded in CDCl₃.

*= CHCl₃ and CH₂Cl₂

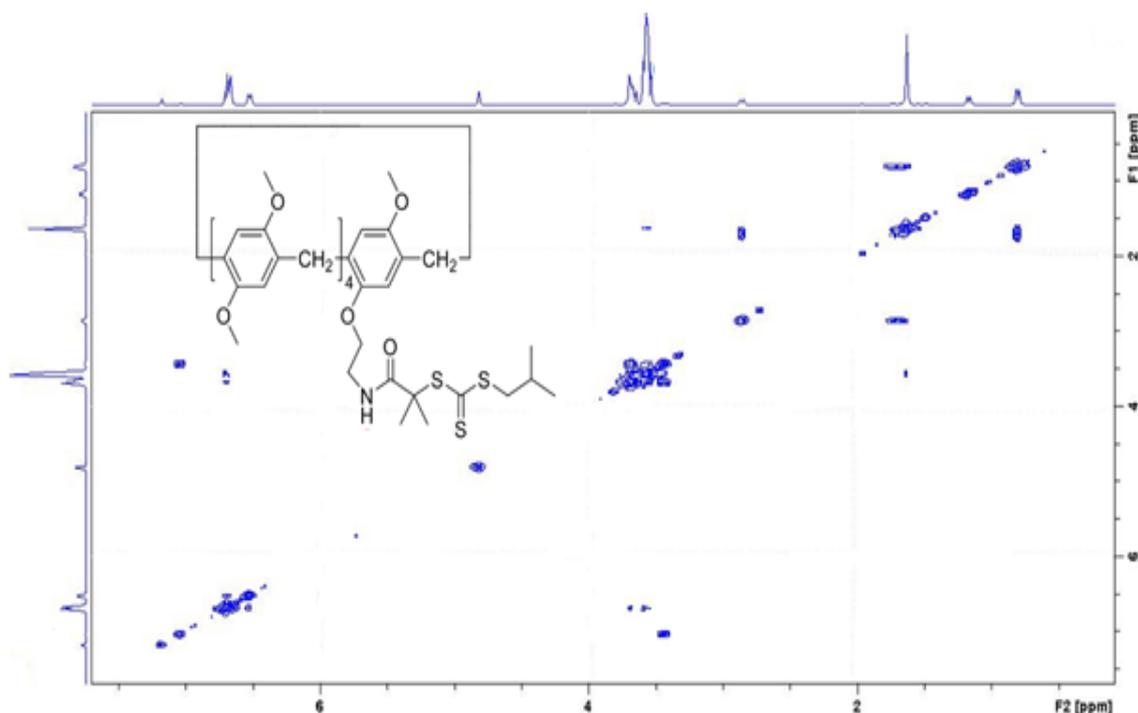


Figure S2: 2D-COSY NMR spectrum of **MePilla-CTA** recorded in CDCl_3 .

3.2. Synthesis of MePilla-PDMAC

MePilla-CTA (0.080 g, 0.079 mmol), AIBN (2.54 mg, 0.015 mmol) and DMAC (1.57 g, 15.8 mol) were dissolved in 2.016 g of DMF in a 25 ml round-bottom flask. The resulting mixture was purged with nitrogen for 20 minutes at 0°C . The sealed round bottom-flask was immersed in a thermostatically controlled oil bath at 70°C and the polymerization lasted for 30 minutes. Samples were periodically withdrawn: i) to measure the conversion by ^1H NMR spectroscopy in CDCl_3 by the relative integration of the vinylic proton peaks of DMAC and the CH protons peak of 1,3,5-trioxane (used as internal standard) and ii) to estimate the M_n by SEC (DMF) (Figure S4). The final **MePilla-PDMAC** was dissolved in acetone and purified by dialysis. Its number-average molar mass was determined by SEC (DMF), $M_n = 15000 \text{ g mol}^{-1}$, $D = 1.1$, and by ^1H NMR(Acetone- d_6), $M_n = 14500 \text{ g mol}^{-1}$ ($M_{n,\text{th}} = 15660 \text{ g mol}^{-1}$).

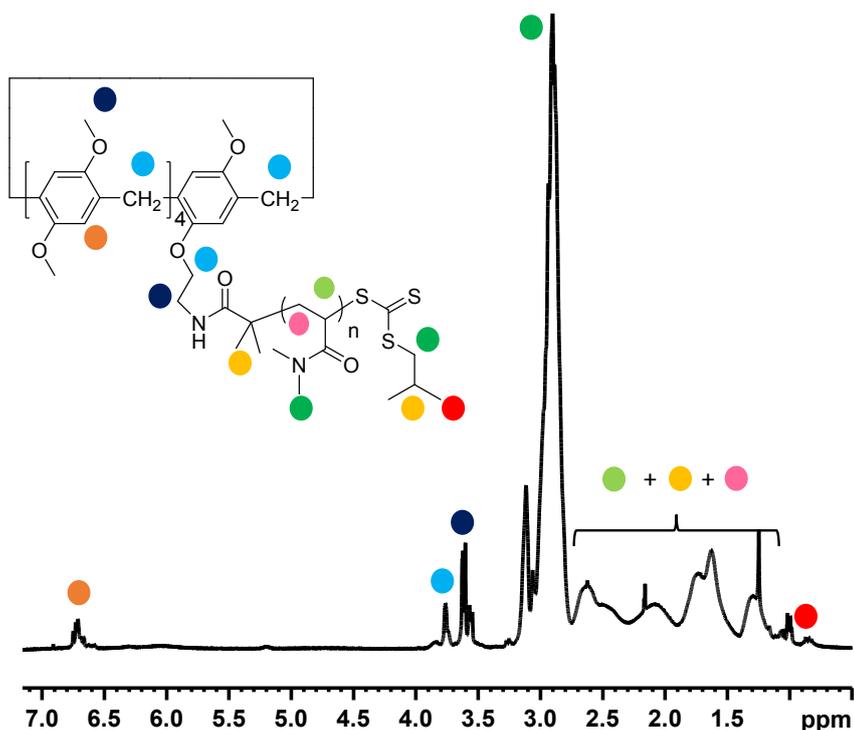


Figure S3: ^1H NMR spectrum of **MePilla-PDMAC** ($M_{n,NMR}=14500 \text{ g mol}^{-1}$) recorded in CDCl_3 at 298 K.

Determination of M_n , NMR:

$$M_n, \text{ RMN} = \frac{I_{2.9 \text{ ppm}} / 6}{I_{6.7 \text{ ppm}} / 10} \times M_{\text{DMAc}} + M_{\text{CTA}}$$

M_n , NMR: Number-average molar mass, M_n , determined by NMR.

$I_{2.9 \text{ ppm}}$: integration area of $\text{N}(\text{CH}_3)_2$ methyl signal of **MePilla-PDMAC**

$I_{6.7 \text{ ppm}}$: integration area of H_1, H_2 signal of **MePilla-PDMAC**

M_{DMAc} : *N,N*-DiMethylAcrylamide molecular weight ($\text{g}\cdot\text{mol}^{-1}$).

M_{CTA} : Transfer (RAFT) agent molecular weight ($\text{g}\cdot\text{mol}^{-1}$)

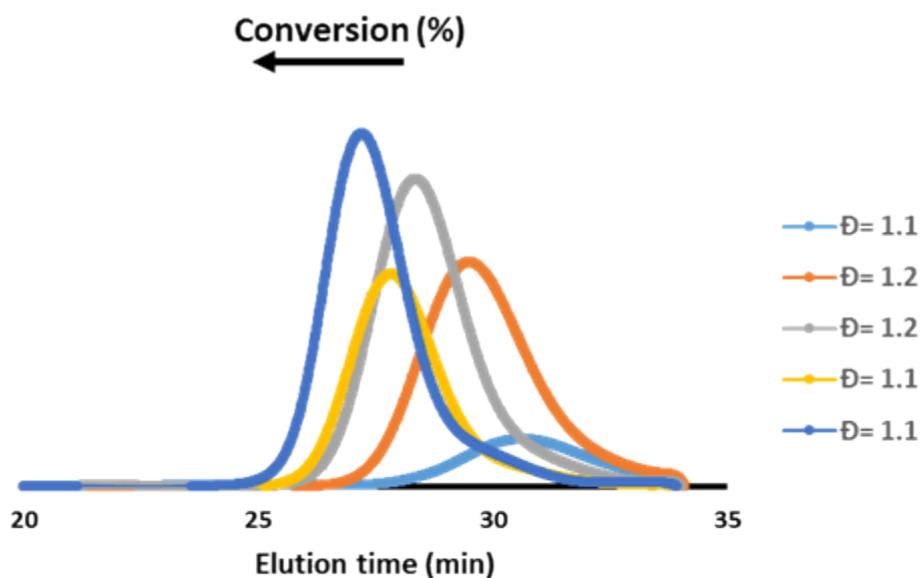


Figure S4: Evolution of SEC chromatograms for the polymerization of DMAC in the presence of **MePilla-CTA** initiated by AIBN. Recorded in DMF at 323K and at a flow rate of $0,3 \text{ mL min}^{-1}$

MePilla-PDMAC-2

MePilla-PDMAC-1

Figure S5: SEC traces for **MePilla-PDMAC-1** ($M_{n,SEC} = 22260 \text{ g mol}^{-1}$ and $\bar{D} = 1.19$) and **MePilla-PDMAC-2** ($M_{n,SEC} = 59500 \text{ g mol}^{-1}$ and $\bar{D} = 1.30$) prepared by chain extension of **MePilla-PDMAC-1** with DMAC

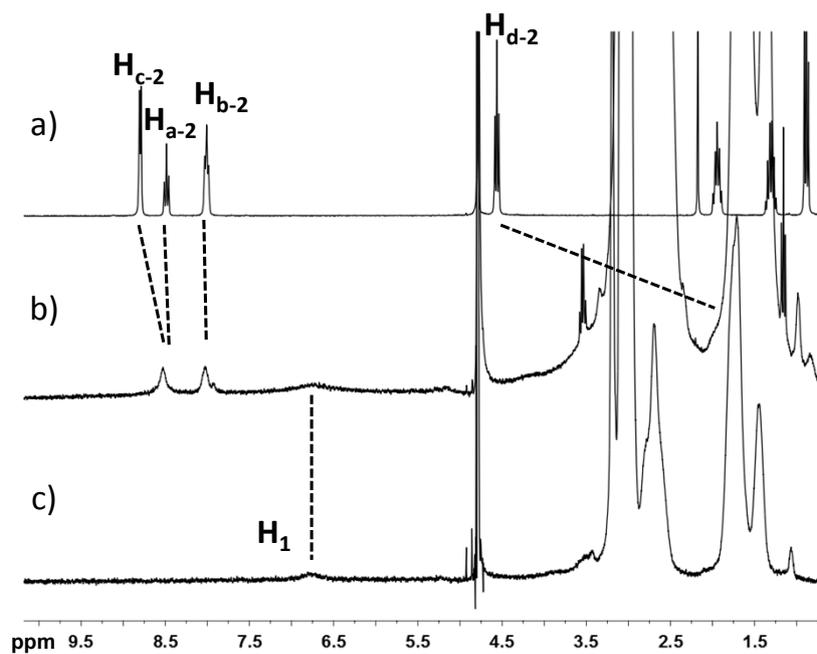
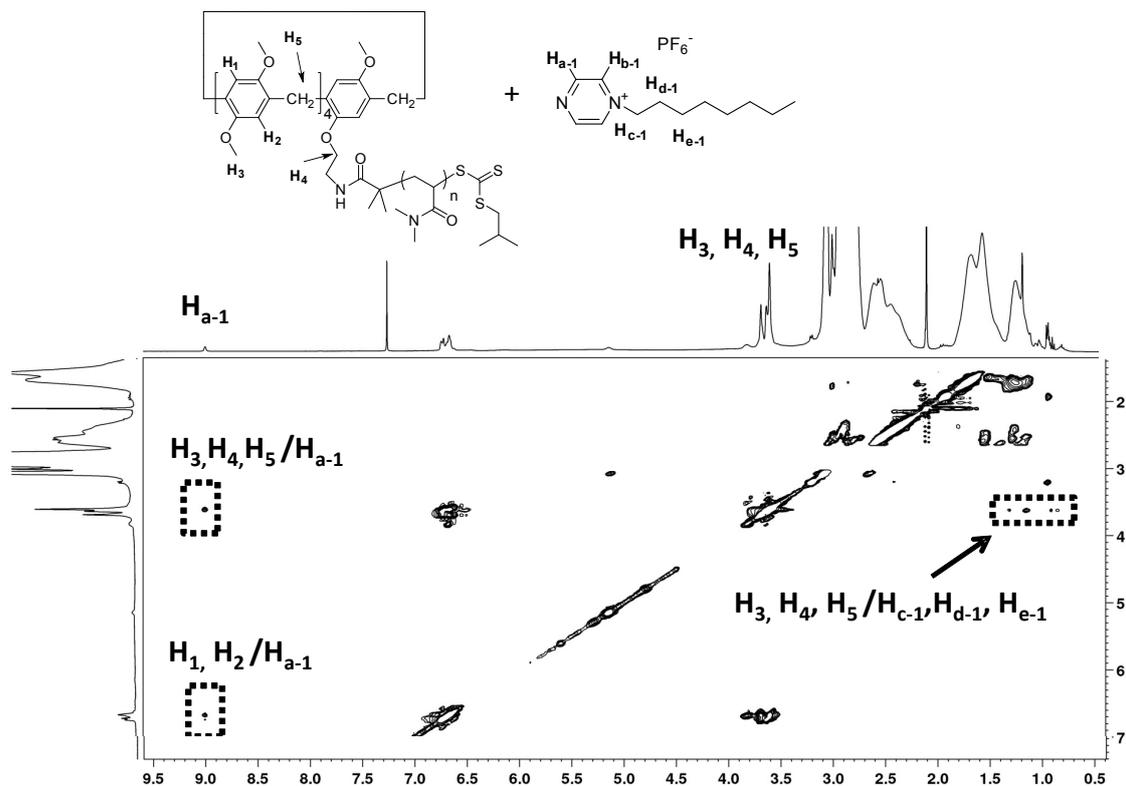


Figure S6: ^1H NMR spectra (300 MHz, D_2O , 300K) of a) G2 (3.7 mM), b) a 1:1 mixture of G2 and MePilla-PDMAC and c) MePilla-PDMAC (3.7mM)

A)



B)

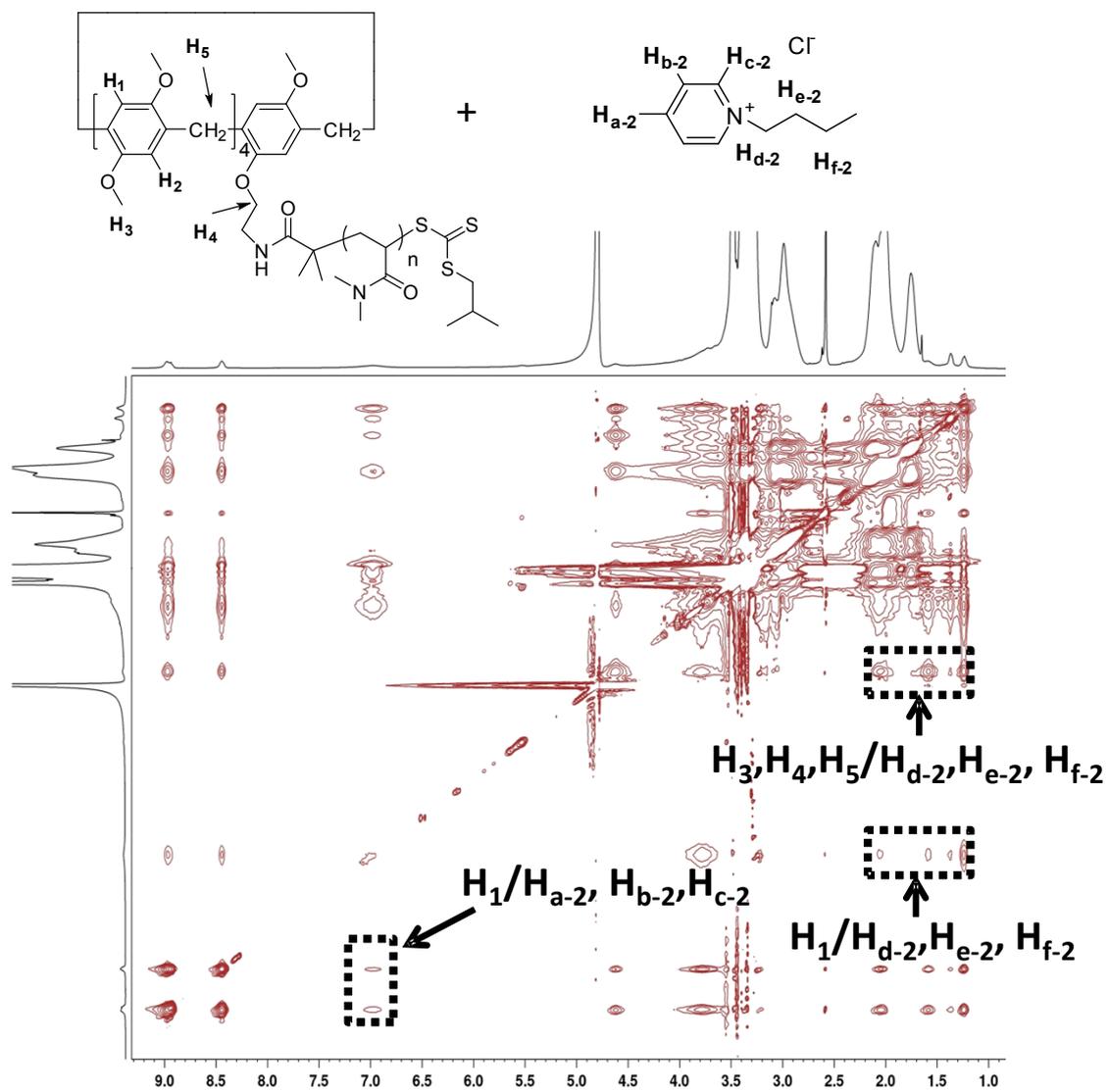
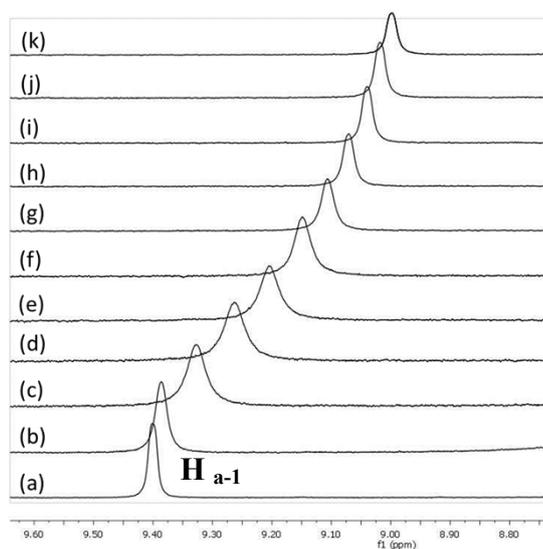


Figure S7: Partial 2D-NOESY NMR spectrum of A) **MePilla-PDMAC .G1** in CDCl₃ and B) **MePilla-PDMAC.G2** in D₂O. Recorded at 298K with a mixing time of 600 ms.

A)



B)

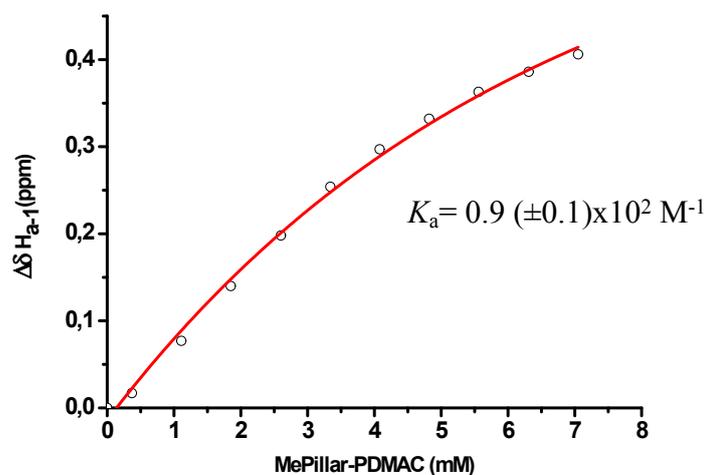
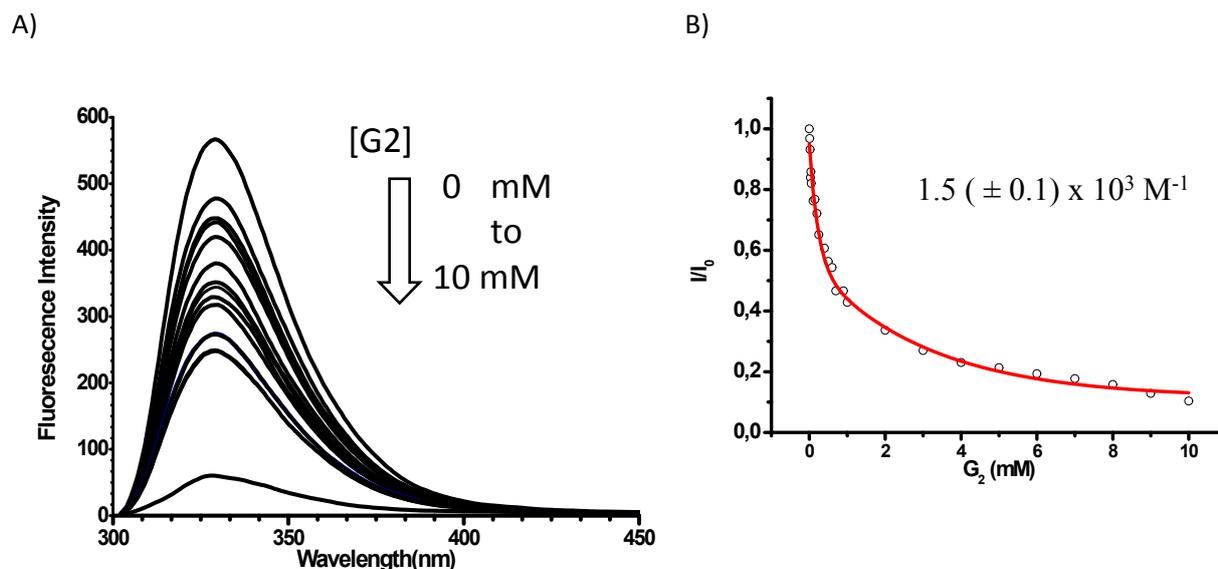


Figure S8: A) Partial ^1H NMR spectra (400 MHz, CDCl_3 , room temperature) of **G1** at a concentration of 3.71 mM upon addition of **MePilla-PDMAC**: (a) 0.00 mM, (b) 0.37 mM, (c) 1.11 mM, (d) 1.85 mM, (e) 2.60 mM, (f) 3.34 mM, (g) 4.08 mM, (h) 4.82 mM, (i) 5.56 mM, (j) 6.31 mM, (k) 7.05 mM, B) Chemical shift changes of H_{a-1} of **G1** upon addition of **MePilla-PDMAC**. The solid line (-) was obtained from the nonlinear curve-fitting using Eq. 1. The K_a value was determined to be $0.9 (\pm 0.1) \times 10^2 \text{ M}^{-1}$ for a 1:1 stoichiometry.⁵

The non-linear curve-fitting was based on the following equation:

Equation 1 $\Delta\delta = (\Delta\delta_\infty/[G1]_0) (0.5[MePilla-PDMAC]_0 + 0.5([G1]_0 + 1/Ka) - (0.5 ([MePilla-PDMAC]_0)^2 + (2[MePilla-PDMAC]_0 (1/Ka - [MePilla-PDMAC]_0)) + (1/Ka + [MePilla-PDMAC]_0)^{2.0.5})).$

Where $\Delta\delta$ is the chemical shift change of H_{a-1} on G1 at $[MePilla-PDMAC]_0$, $\Delta\delta_\infty$ is the chemical shift change of H_{a-1} when the guest is completely complexed, $[MePilla-PDMAC]_0$ is the fixed initial concentration of the guest, and $[G1]_0$ is the varying concentrations of MePilla-PDMAC.



Equation 2
$$\frac{I}{I_0} = \frac{(1 + aK[G2])}{(1 + K[G2])}$$

Figure S9: A) Emission fluorescence spectra of **MePilla-PDMAC** (1.10^{-2} mM) in presence of different amounts of **G2** (from 0 to 10 mM) in water (excited at 291 nm, measured at 298K). Addition of **G2** resulted in a quench of the fluorescence emission of **MePilla-PDMAC**, there indicating the formation of the MePilla-PDMAC complex. B) Plot of the relative fluorescence intensity (I/I_0) of **MePilla-PDMAC** versus the concentration of **G2**. The K_a value was determined to be $1.5 (\pm 0.1) \times 10^3 M^{-1}$ by fitting data for a 1:1 stoichiometry with Eq. 2 (-), where a is a constant.

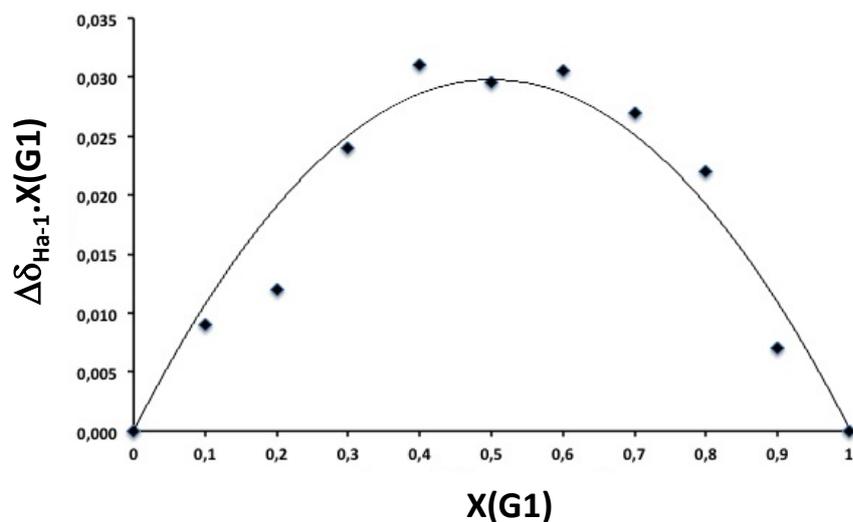


Figure S10: Job plot showing the 1:1 stoichiometry of the complex between **MePilla-PDMAC** and **G1** using ^1H NMR peak shifts of H_{a-1} . ($[\text{MePilla-PDMAC}] + [\text{G1}] = 2 \text{ mM}$). The dark curve acts a visual guide.

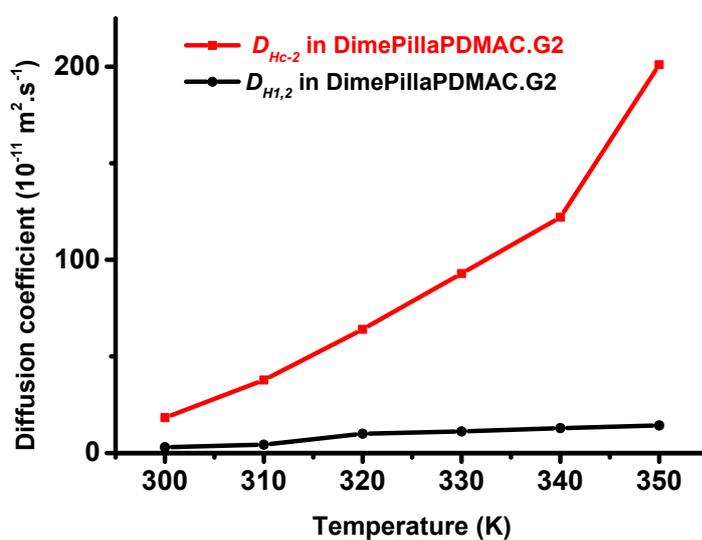


Figure S11: Evolution of diffusion coefficients H_{c-2} of **G2** and $\text{H}_{1,2}$ of **MePilla-PDMAC** versus temperature for **G2.MePilla-PDMAC** complex

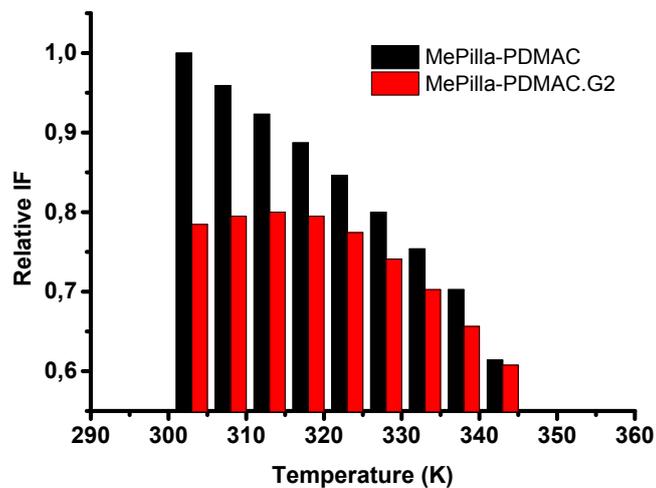


Figure S12: Evolution of the relative fluorescence intensity of **MePilla-PDMAC** (10^{-5}M) and **MePilla-PDMAC.G2** (10^{-5}M) *versus* temperature.

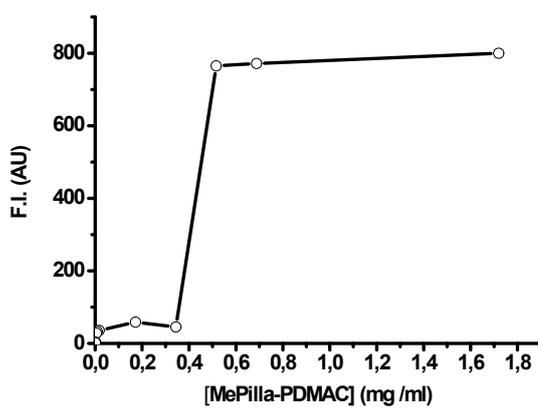


Figure S13: Plot of the fluorescence intensity as a function of the concentration of **MePilla-PDMAC** ($\lambda_{\text{exc}} = 291 \text{ nm}$, $T = 298\text{K}$, in water).

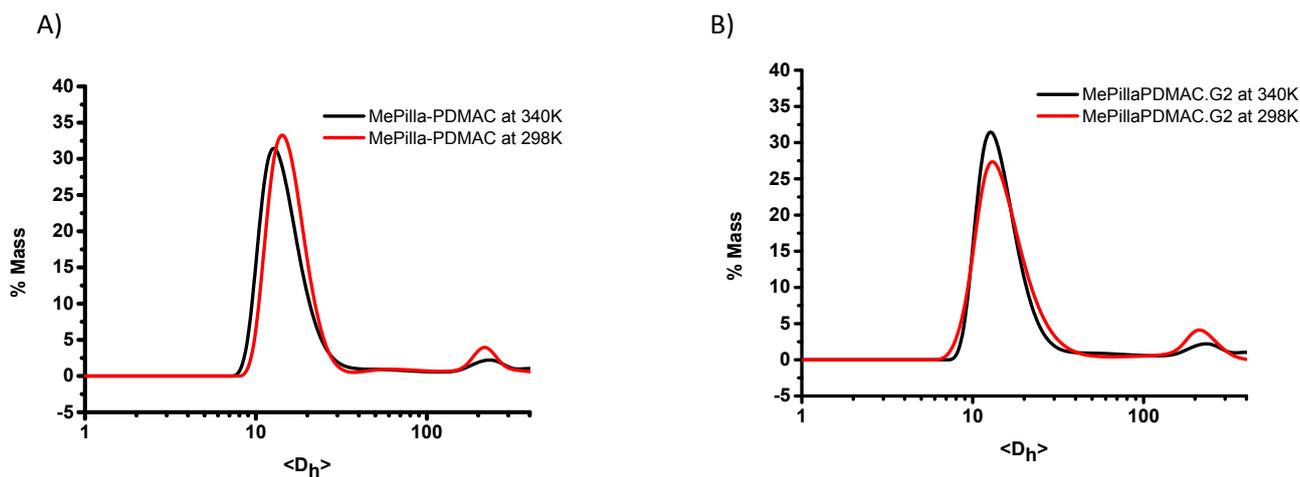


Figure S14: A) DLS data for **MePilla-PDMAC** (4.5 mM) in water recorded at 298 K (–) and 340K (–), B) DLS data for **MePilla-PDMAC.G2** in water recorded at 298 K (–) and 340K (–)

References

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