

Two-component self-assembly of a Tetra-Guanidinio-carbonyl Pyrrole Cation and Na₄EDTA: Formation of pH switchable supramolecular networks

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1. General Remarks

Solvents were dried and distilled before use. All other reagents were used as obtained from Aldrich, Fluka or Alfa Aesar unless otherwise specified. All reactions were carried out in oven dried glassware. Reactions were monitored by TLC on silica gel plates (*Machery-Nagel POLYGRAM SIL G/UV254*). The IR spectra were recorded on a FT-IR 430 spectrometer from *Jasco* with a *Pike MIRacle ATR* sampling accessory. Bands are quoted in cm⁻¹. The ¹H NMR spectra were recorded at 300 MHz or 500 MHz and the ¹³C NMR spectra at 150 MHz from *Bruker* at ambient temperature. The chemical shifts are relative to the signals of the used solvent: DMSO-*d*₆ (δ ¹H = 2.50 and δ ¹³C = 39.52) and CDCl₃ (δ ¹H = 7.26 and δ ¹³C = 77.16). The apparent coupling constants are given in Hertz. The description of the fine structure means: s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet. All ESI-mass spectra were recorded by using a *Bruker BioTOF III* spectrometer. Melting points were obtained in open glass capillary tubes using an apparatus from *Büchi* and are quoted uncorrected. Determination of pH values was carried out with a pH-Meter 766 Calimatic from *Knick*. Fluorescence spectra were obtained with a *Varian Cary Eclipse* spectrometer. AFM imaging was carried out with an Innova Scanning Probe Microscope from *Veeco* and *Olympus* N-type silicon cantilevers AC-160TS in tapping mode on freshly cleaved mica surface from *Plano GmbH*. The analysis was done utilizing the software Gwyddion (Vers. 2.19). Dynamic Light Scattering (DLS) experiments were performed using a Zetasizer-Nano ZS from *Malvern* equipped with a 4 mW He-Ne laser (633 nm wavelength) at a fixed detector angle of 173° with an avalanche photodiode detector. All viscosity data were measured by using a Lovis 2000 M/ME microviscometer, device softwear version 2.21.

2. Experimental procedures and characterization

Synthesis of the Boc-protected tetra guanidiniocarbonyl pyrrole:

To a solution of guanidinio pyrrole carboxylic acid **3** (246 mg, 0.832 mmol) and HCTU (387 mg, 0.936 mmol) in dry DMF (10 mL), DIPEA (0.18 mL, 1.04 mmol) was added and the solution was stirred at room temperature for 45 min. First, the amine **2** (60 mg, 0.208 mmol) was dissolved in 300 μ L MeOH, then solution was added to the reaction mixture and it was stirred for 24 h at room temperature. After removing DMF from the reaction mixture, ethyl acetate (50 mL) was added. A light brown solid was precipitated out. The solid product was filtered and washed with ethyl acetate. Finally it was dried under vacuum to give desired compound as a light brown solid (140 mg, 48%). We proceeded next step without further purification. Mp: 264-266 °C; ^1H NMR (300MHz, DMSO- d_6): δ 11.33 (bs, 4H, NH), 9.32 (bs, 4H, NH), 8.57-8.44 (bs, 6H, NH), 6.80-6.74 (m, 8H, CH), 3.79-3.48 (m, 12H, CH_2), 3.28 (m, 8H, CH_2), 1.85-1.66 (m, 8H, CH_2), 1.43 (s, 36H, CH_3); ^{13}C NMR (150 MHz, DMSO- d_6): δ 170.36, 162.33, 161.48, 159.9, 158.43, 158.29, 131.91, 129.39, 128.27, 127.08, 125.97, 125.16, 120.84, 115.12, 113.81, 113.73, 111.78, 109.37, 79.18, 50.56, 41.86, 35.79, 30.78, 30.68, 27.75; FT-IR (KBr, cm^{-1}): 3156, 2981, 1619, 1317; MS (ESI): m/z (%): 1401 (5) [M + H] $^+$, 1301 (5) [M + H - Boc] $^+$, 1066 (100) [M + H - Boc-guanidino pyrrole carbonyl-C₃H₆N] $^+$, 966 (60) [M + H - Boc - Boc-guanidino pyrrole carbonyl-C₃H₆N] $^+$.

Synthesis of the tetra guanidiniocarbonyl pyrrole (**1**):

To a solution of the Boc-protected tetra guanidiniocarbonyl pyrrole (130 mg, 0.09 mmol) in CH₂Cl₂ (6 mL), TFA (6 mL) was added and the reaction mixture was stirred at room temperature for 2 h. After removal of CH₂Cl₂ and TFA under reduced pressure, the residual light brown gum was diluted with ethyl acetate (50 mL). A light brown solid was precipitated out. The resulting precipitate was filtered and washed thoroughly with ethyl acetate. Finally it was dried under reduced pressure to give **1** as a slightly brown powder (97 mg, 74 %). Mp: 236-238 °C; ^1H NMR (300MHz, DMSO- d_6): δ 12.30 (bs, 3H, NH), 11.25 (bs, 3H, NH), 8.57-8.39 (bs, 12H, NH), 7.10 (m, 4H, CH), 6.86 (m, 4H, CH), 3.77 (bs, 12H, CH_2), 3.05 (bs, 8H, CH_2), 1.84 (bs, 8H, CH_2); ^{13}C NMR (150 MHz, DMSO- d_6): δ 159.80, 159.50, 159.28, 159.07, 158.86, 158.64, 155.18, 132.50, 125.55, 119.87, 117.90, 115.93, 115.84, 113.96, 112.53, 50.66, 45.29, 36.21, 36.12, 35.96, 27.75; FT-IR (KBr, cm^{-1}): 3318, 1671, 1558, 1284, 1193; MS (ESI): m/z (%): 1001 (19) [M + H] $^+$, 766 (100) [M + H - guanidino pyrrole carbonyl-C₃H₆N] $^+$.

3. Spectra

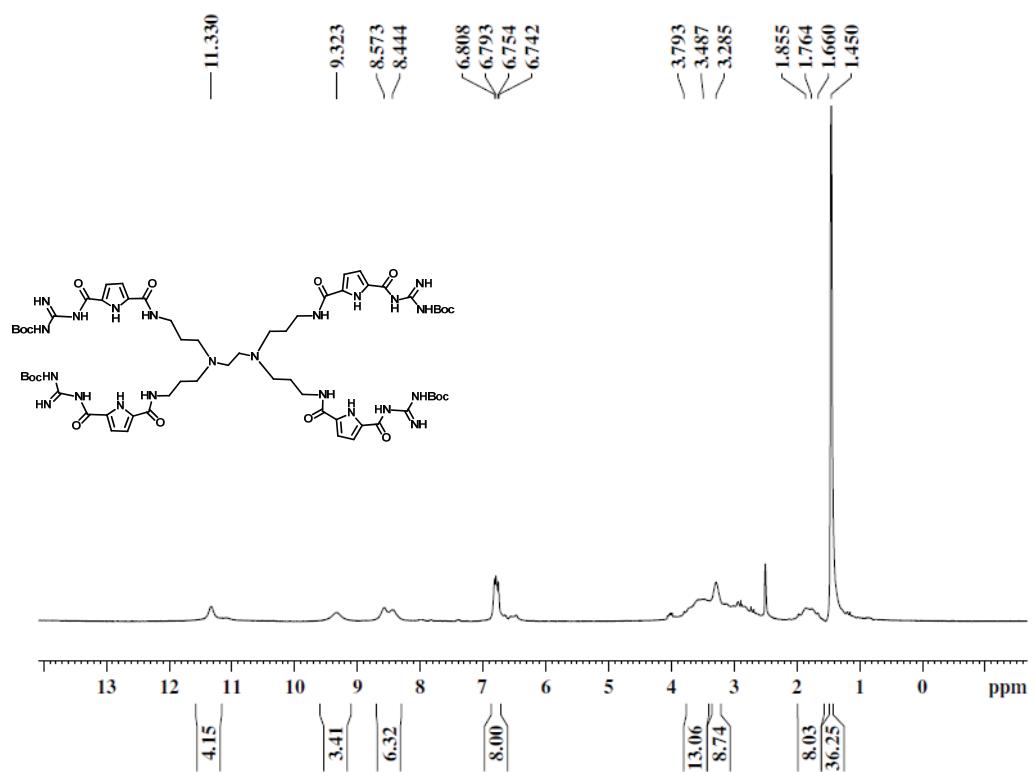


Figure S1: ^1H NMR spectrum of Boc-protected tetra guanidiniocarbonyl pyrrole in $\text{DMSO}-d_6$, 300 MHz.

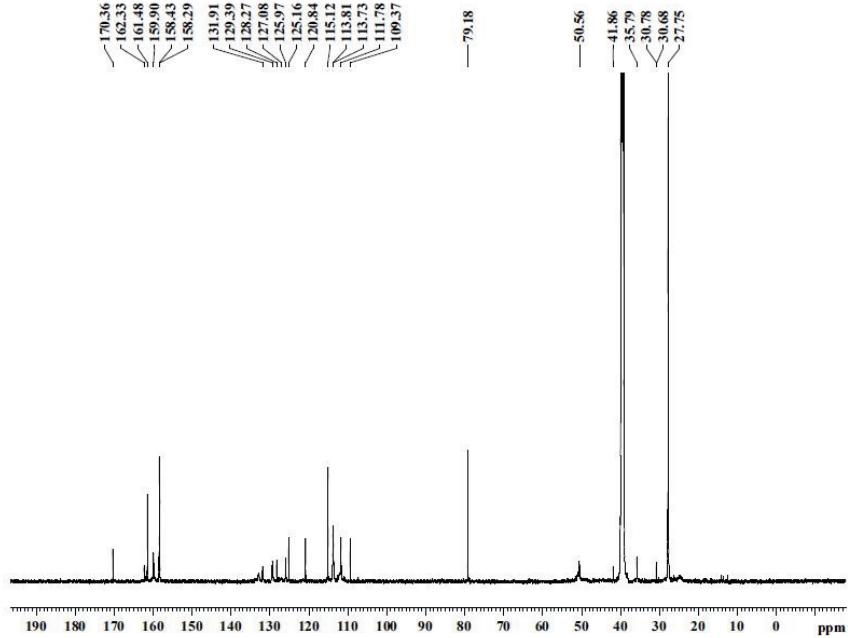
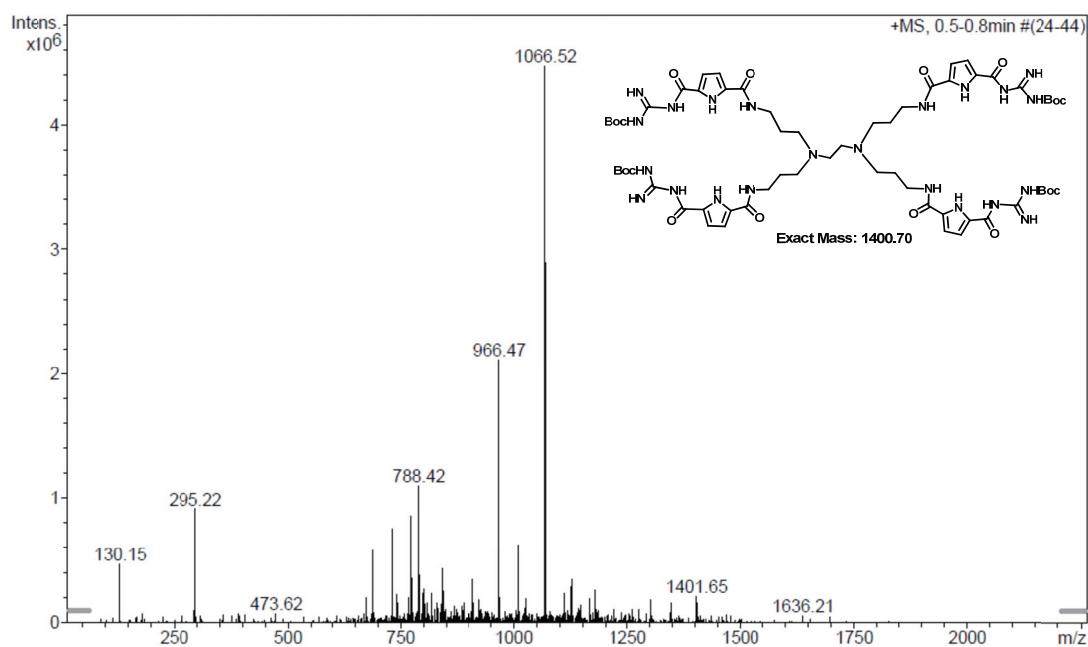


Figure S2: ^{13}C NMR spectrum of Boc-protected tetra guanidiniocarbonyl pyrrole in $\text{DMSO}-d_6$, 150 MHz.



Bruker Compass DataAnalysis 4.0

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Figure S3: ESI mass spectrum of Boc-protected tetra guanidiniocarbonyl pyrrole in 10% DMSO/MeOH.

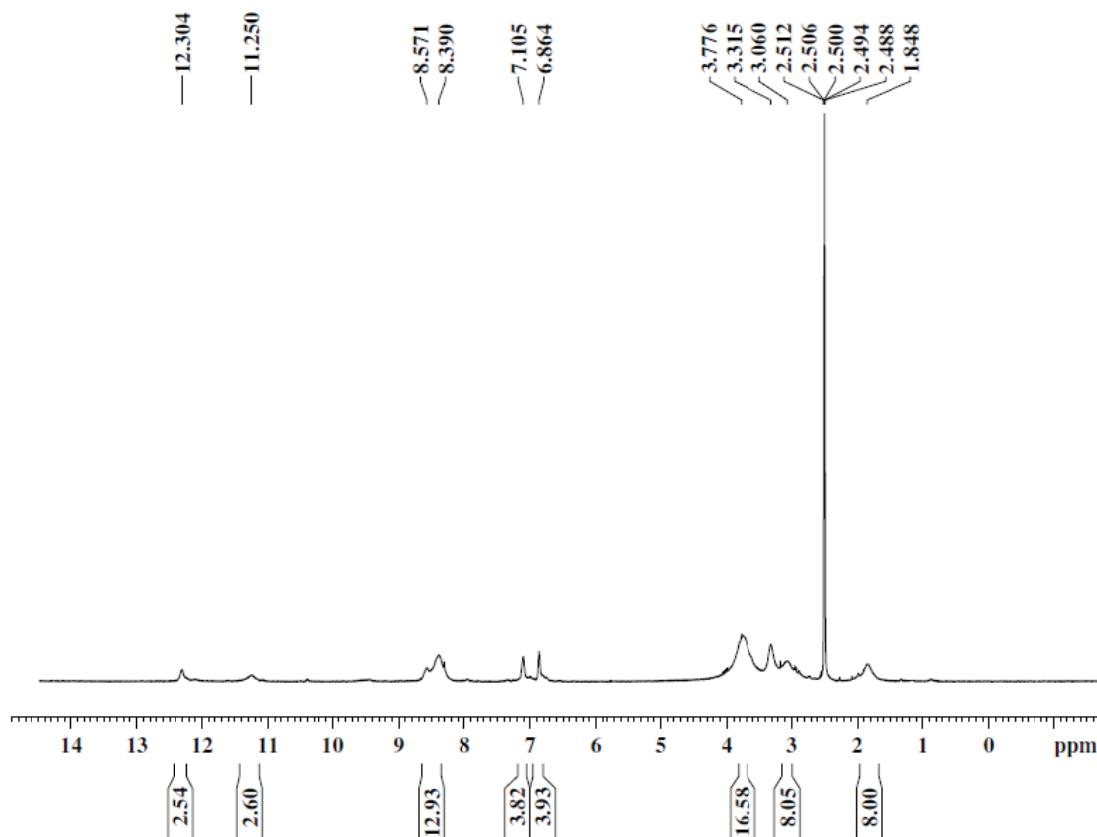


Figure S4: ^1H NMR spectrum of **1** in $\text{DMSO}-d_6$, 300 MHz.

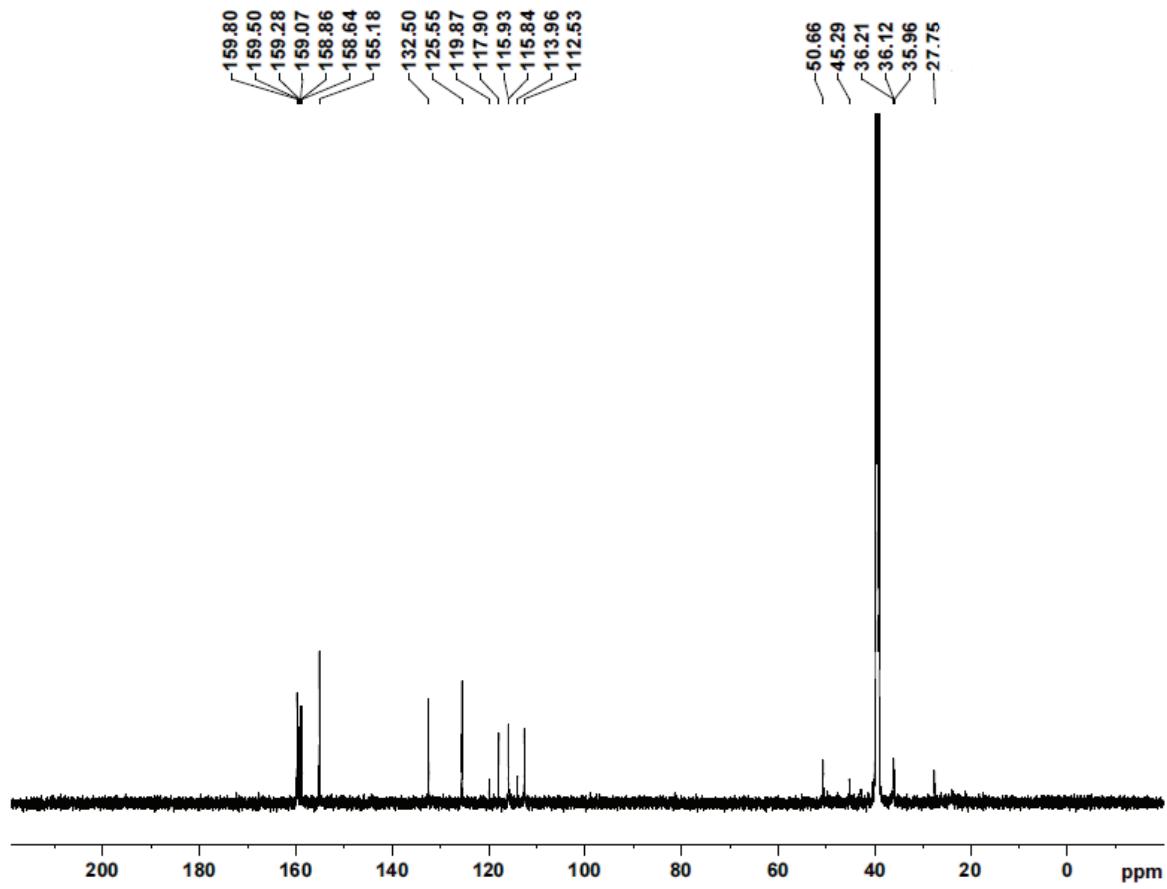


Figure S5: ^{13}C NMR spectrum of **1** in $\text{DMSO}-d_6$, 150 MHz.

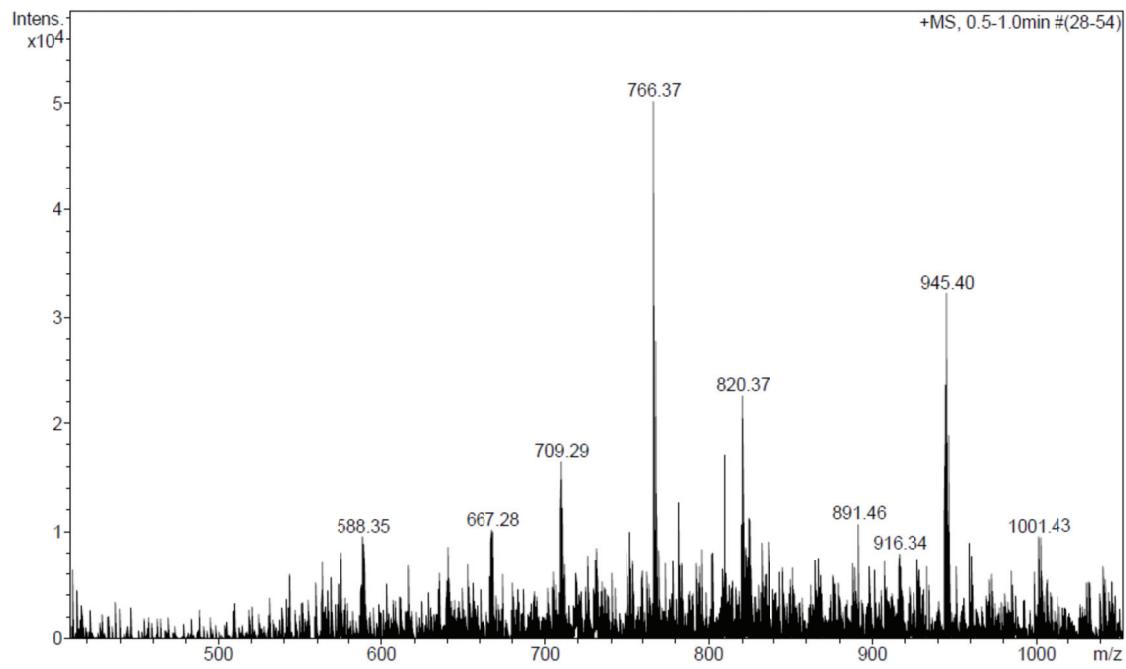


Figure S6: ESI mass spectrum of **1** in 10 % DMSO/MeOH.

4. Dynamic light scattering (DLS) experiments

First $c = 1 \times 10^{-3}$ M solution of both tetra cationic guanidiniocarbonyl pyrrole (**1**) and Na₄EDTA were prepared in pure DMSO and 10% H₂O/DMSO respectively. The two solutions were mixed by equal volume (v/v, 50/50) to prepare $c = 5 \times 10^{-4}$ M solution and checked the pH (6.95 ± 0.01) without adding either acid or base. All measurements were carried out at 25° C in UV-transparent microcuvettes (1 cm) equipped with a stopper. The solution was filtered prior to measure *via* 0.20 µm nylon filters. The autocorrelation functions of the backscattered light fluctuations were analyzed with the DTS 6.20 software from Malvern providing the hydrodynamic diameter (Z-average), polydispersity and size distribution (NNLS analysis).

Time dependent DLS study

Sample name	Z-average (d.nm)	Size (d.nm) (%)	PDI
Compound 1	14.31	0.72 (100)	0.842
Na₄EDTA	13.25	0.67 (100)	0.914
mix 0 h	177.6	0.66 (50), 72.63 (50)	0.409
mix 2 h	324	257.1 (100)	0.235
mix 4 h	677.2	677 (100)	0.177
mix 6 h	952.2	920.3 (100)	0.101
mix 30 h	2016	1726 (100)	0.509
mix 57 h	4416	1360 (35.1), 3802 (64.9)	0.287

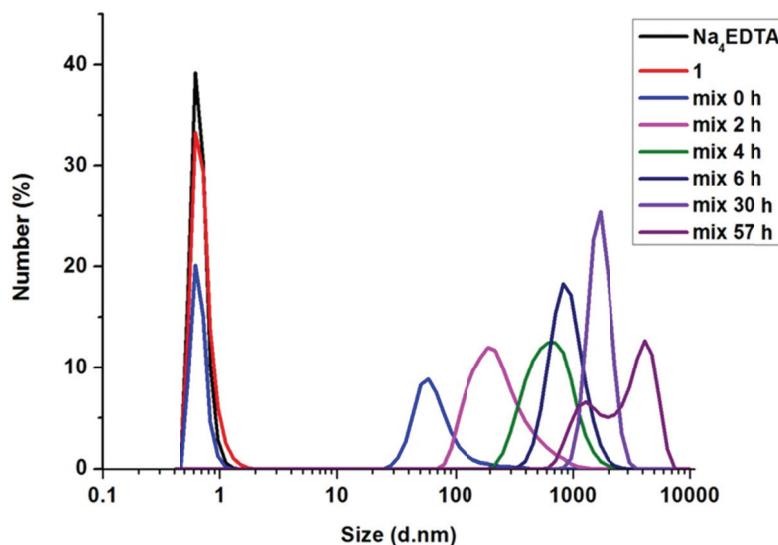


Figure S7: Time dependent DLS study in aqueous DMSO.

The results demonstrated that the size of the polymers increased with increasing time for (1:1) solutions (from 72.63 nm diameter to up to 3802 nm diameter in 57 h).

pH dependent DLS study

Sample name	Z-average (d.nm)	Size (d.nm) (%)	PDI
pH 2.65	21.26	4.12 (100)	0.971
pH 9.95	6.51	0.65 (100)	0.247
pH 6.99	678.6	148.5 (19.3), 519.1 (80.7)	0.561

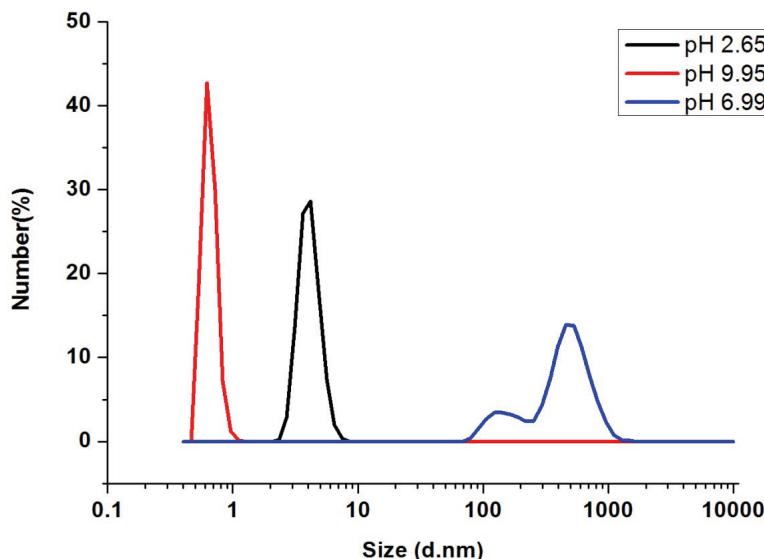


Figure S8: pH dependent DLS study in aqueous DMSO.

The polymers can be switched between the monomers and polymer reversibly using external stimuli like pH-alteration.

5. Fluorescence experiments

All fluorescence spectra were recorded using a VARIAN Fluorescence at 25 °C. The samples were each excited at the wavelength $\lambda = 297$ nm appropriate for the fluorescence of **1**. The slit widths were set to 5 nm for excitation and emission. All spectra were corrected for intensity using the manufacturer-supplied correction factors and corrected for background fluorescence.

Fluorescence titration of **1** ($c = 1 \times 10^{-3}$ M) with Na₄EDTA ($c = 6 \times 10^{-3}$ M) in aqueous DMSO.

Calculation

Na ₄ EDTA added (μL)	Fluorescence (nM)	concentration (M)	Molar fraction	I0/Ii
0 intensity	0	0	0	#VALUE!
5	485	8.95522E-05	0.083333333	1
10	436	0.000176471	0.153846154	1.112385321
15	392	0.00026087	0.214285714	1.237244898
20	376	0.000342857	0.266666667	1.289893617
25	358	0.000422535	0.3125	1.354748603
30	353	0.0005	0.352941176	1.373937677
35	348	0.000575342	0.388888889	1.393678161
40	344	0.000648649	0.421052632	1.409883721
45	343	0.00072	0.45	1.413994169
55	342	0.000857143	0.5	1.418128655
65	340	0.000987342	0.541666667	1.426470588
75	338	0.001111111	0.576923077	1.434911243
85	336	0.001228916	0.607142857	1.443452381
95	335	0.001341176	0.633333333	1.447761194
105	333	0.001448276	0.65625	1.456456456
115	334	0.001550562	0.676470588	1.452095808
125	332	0.001648352	0.694444444	1.460843373
135	330	0.001741935	0.710526316	1.46969697
145	332	0.001831579	0.725	1.460843373
155	331	0.001917526	0.738095238	1.465256798
165	329	0.002	0.75	1.474164134
175	329	0.002079208	0.760869565	1.474164134
195	328	0.002228571	0.78	1.478658537
225	328	0.002432432	0.803571429	1.478658537
255	329	0.002615385	0.822580645	1.474164134

These above data demonstrated the calculation of the whole titration experiments

6. NMR-Titration

Aliquots (10-160 μL) of a stock solution of the corresponding Na₄EDTA ($c = 6 \times 10^{-3}$ M) in 10 % H₂O/DMSO-*d*₆ were added to a solution (500 μL) of the tetra cationic guanidiniocarbonyl pyrrole ($c = 1 \times 10^{-3}$ M) in DMSO-*d*₆. The complexation induced shift changes of the pyrrole CHs and NHs were recorded without presaturation of the water signal (Bruker Avance 600 spectrometer). The chemical shifts are calibrated relative to the residual DMSO solvent peak.

Addition of Na ₄ EDTA (6mM) in μL	Concentration in mM
0	0
10	0.118
30	0.34
70	0.7
130	1.2
160	1.4

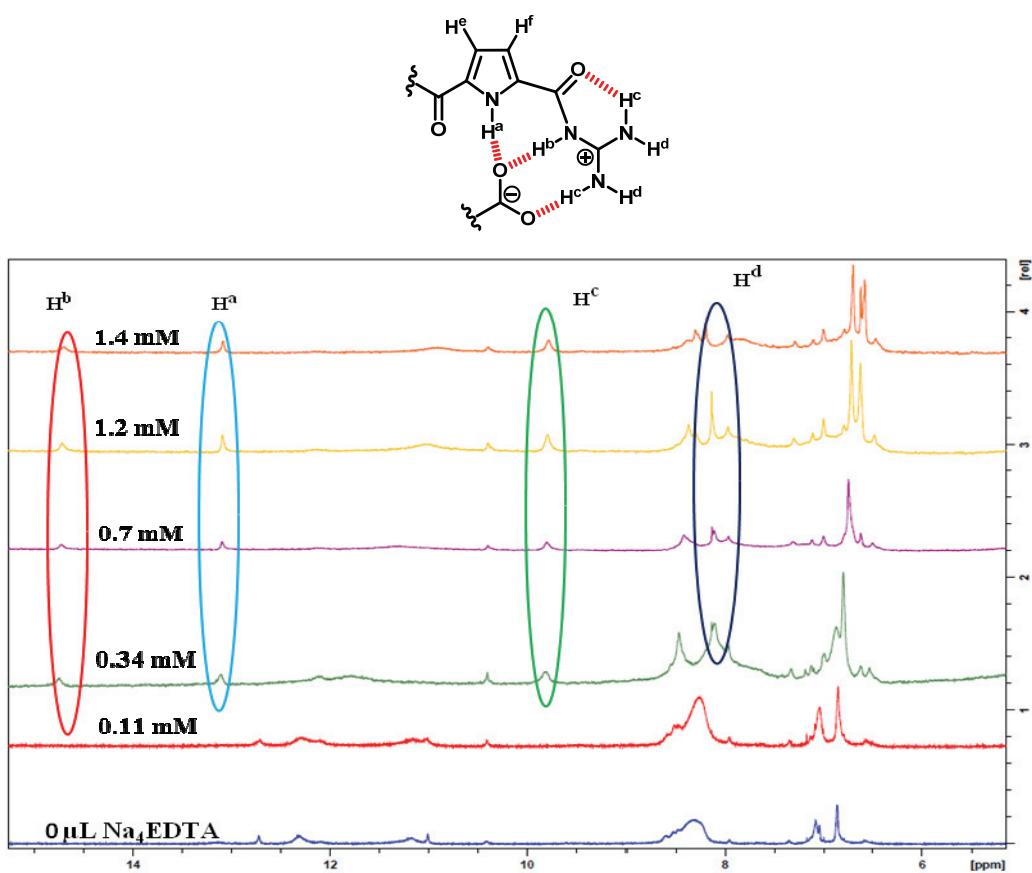


Figure S9. NMR shifts for the titration of **1** ($c = 1 \times 10^{-3}$ M) with Na₄EDTA ($c = 6 \times 10^{-3}$ M) in 95% DMSO- d_6 /H₂O.

Four new peaks appeared at $\delta = 14.71$ for H^b , 13.09 (H^a), 9.78 (H^c) and 8.19 for H^d respectively, as expected for the complexation of a carboxylate by a guanidiniocarbonyl pyrrole cation.¹

7. Viscosity

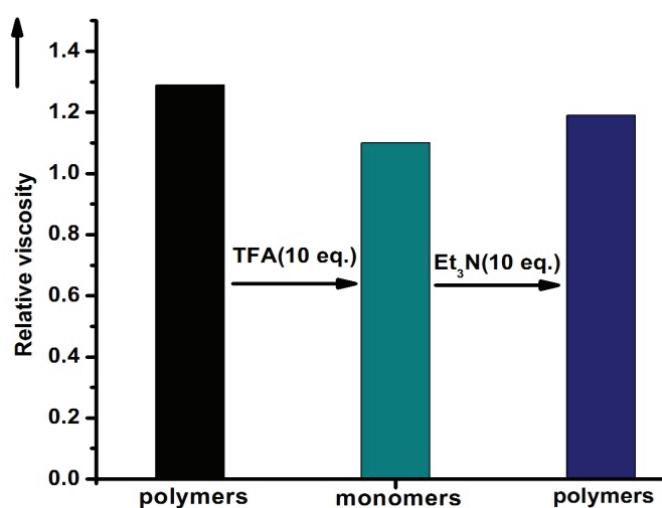


Figure S10: Variation in relative viscosity of a $c = 4 \times 10^{-4}$ M solution of (1:1) mixtures of both in aq. DMSO upon sequential addition of trifluoroacetic acid (TFA) and triethyl amine.

The initial viscosity was not completely restored probably owing to the fact that the sample now also contains a substantial amount of the salt generated by neutralizing TFA by Et_3N^+ .²

8. AFM sample preparation and results

$c = 5 \times 10^{-4}$ M solution was prepared as described in the DLS measurement section. For each measurement 10 μL of the mixture was dropped onto a freshly cleaved mica surface and dried by spin coating (50 rps for 10 min) Figure D, E and by drop casting, keep it for 24 h at room temperature.

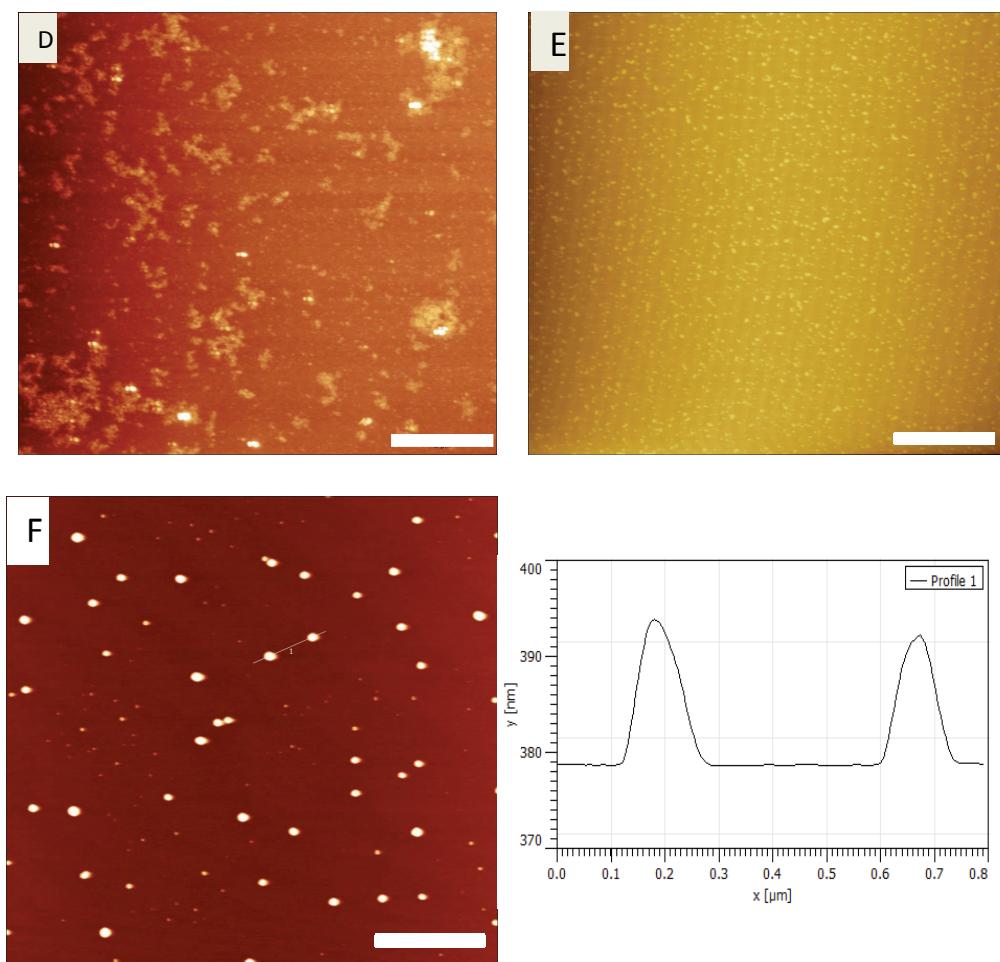


Figure S11: (D) compound **1** ($c = 1 \times 10^{-3}$ M); (E) Na_4EDTA ($c = 1 \times 10^{-3}$ M); (F) Height images of the particles; white scale bar = 2 μm .

9. References

- (1) (a) Schmuck, C. *Chem. Eur. J.* **2000**, *6*, 709-718; (b) Schmuck, C.; Wienand, W. *J. Am. Chem. Soc.* **2003**, *125*, 452.
- (2) Hisamatsu, Y.; Banerjee, S.; Avinash, M. B.; Govindaraju, T.; Schmuck, C. *Angew. Chem., Int. Ed.* **2013**, *52*, 12550-12554.