Cyclodextrin-based Complex Coacervate Core Micelles with Tuneable Supramolecular Host-Guest, Metal-to-Ligand and Charge interactions

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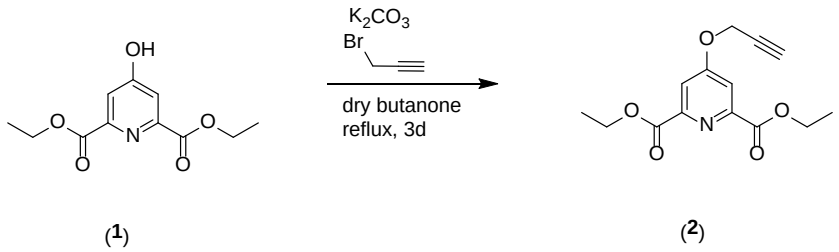
Synthesis of diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate (**2**)

Compound **1,** diethyl 4-hydroxypyridine-2,6-dicarboxylate, was prepared by protecting the chelidamic acid, following a standard procedure described in literature.[1,2] Compound **2,** diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate, was prepared following the procedure described in ref.S1.



(**1**) (**2**)

Scheme S1. Reaction scheme of the diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate synthesis.

Butanone (40 mL) was dried overnight using molecular sieves (4 Å). K_2CO_3 (2.0 g, 14.5 mmol), compound **1** (0.5 g, 2.0 mmol) and propargyl bromide (2 mL, 22 mmol) were added to the dry butanone. The reaction mixture was heated at 80 ^oC to reflux temperature, was refluxed for one night and was monitored with TLC. The reaction was cooled to RT and concentrated in vacuo. The residue was dissolved in DCM (50 mL) and extracted twice with water (50 mL) and once with brine. The organic phase was concentrated in vacuo and the product (**2**) was purified using a silica chromatography column (cyclohexane : ethylacetate from 2:1 to 1:2, as eluent). Yield 0.5 g (94%).

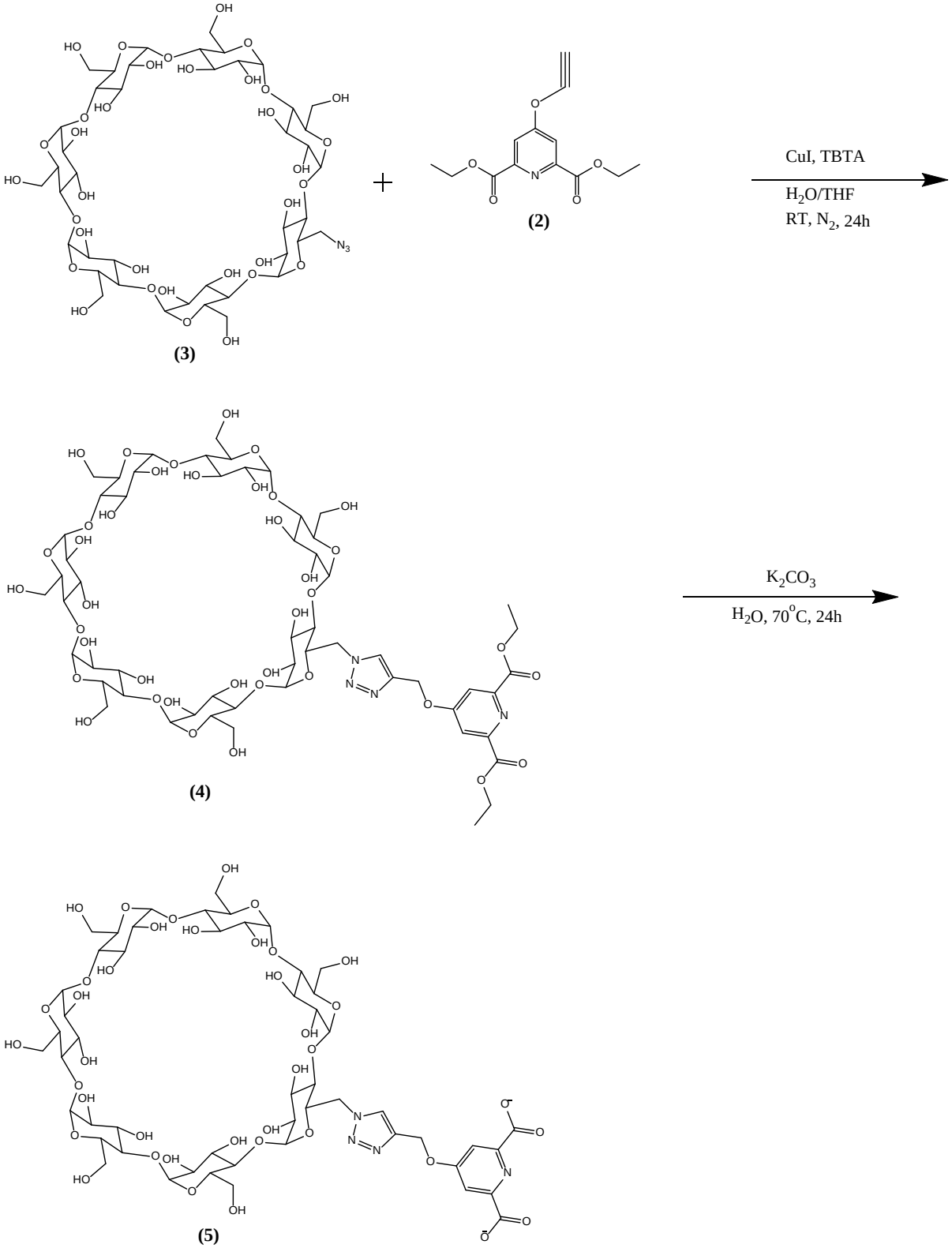
^1H NMR spectrum (400 MHz, $CDCl_3$): δ 7.87 (s, 2H, CH-Py), 4.87 (s, 2H, $CH_2C{\equiv}$ CH), 4.47 (q, *J*=7.2 Hz, 4H, CH_2 CH_3), 2.62 (s, 1H, C≡ CH), 1.44 (t, *J*=7.2 Hz, 6H, CH_3).

^{13}C NMR spectrum (100 MHz, $CDCl_3$): δ 165.48 (-C=O), 164.57 (C-Py), 150.34 (2,6-C-Py), 114.61 (3,5 CH-Py), 77.32 (-C≡ CH), 76.27 (-C≡ CH), 62.43 (CH_2CH_3), 56.34 (-CH_2C≡ CH), 14.18 (CH_3).

Calculated monoisotopic mass ($[M+H]^+$ and $[M+Na]^+$) for **2**, M= $C_{14}H_{15}NO_5$, is 278.10 and 300.08. The experimental mass is 278.10 and 300.08.

Synthesis of pyridine-2,6-dicarboxylate-modified β-cyclodextrin (**5**)

Compound **5**, pyridine-2,6-dicarboxylate-modified β-cyclodextrin, was prepared by adjusting the procedure in ref.S3**.** The synthesis was adjusted by using THF and water instead of MeOH, by leaving the reaction overnight instead of 10-30 min and by using CuI as copper catalyst.

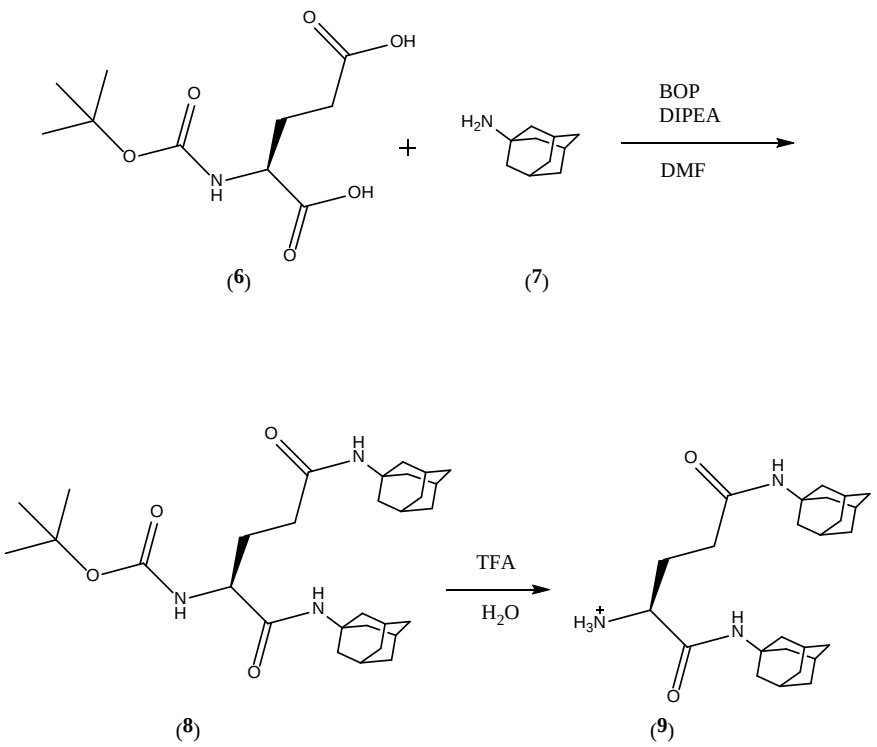


Scheme S2 . Reaction scheme of the pyridine-2,6-dicarboxylate-modified β-cyclodextrin synthesis.

THF and water were purged with nitrogen for two hours. Compound **3** (200 mg, 0.16 mmol) was mixed in a 100 mL round bottom flask with water (20 mL) and THF (10 mL). To this flask, **2** (80 mg, 0.28 mmol), TBTA (6 mg, 0.01 mmol) and CuI (38 mg, 0.20 mmol) were added. The reaction was left stirring under N_2-atmosphere at RT overnight and the reaction was monitored with TLC. The volume of the reaction mixture was reduced to 5 mL, by concentrating it in vacuo. The crude reaction mixture was added directly to a reversed phase silica column C18 column (water: methanol from 2:1 to 1:1). This yielded to 167 mg of **4**.

The deprotection of **4** was performed by dissolving it (167mg, 0.12 mmol) and K_2CO_3 (83.5 mg, 1.2 mmol) in water (20 mL). The reaction was heated to 70˚C and left stirring overnight. After concentration in vacuo, the product (**5**) was purified by dialysis (MW cut-off 500-1000 KDa) over three days, replacing the water twice a day. Water was evaporated. Yield 153 mg (55%).

Synthesis of 1,5-bis(((3s,5s,7s)-adamantan-1-yl)amino)-1,5-dioxopentan-2-aminium (**9**)



(**6**) (**7**) (**8**) (**9**)

Scheme S3. Reaction scheme of the 1,5-bis(((3s,5s,7s)-adamantan-1-yl)amino)-1,5-dioxopentan-2-aminium (Ad-Glu-Ad) synthesis.

Compound **6** (2.17 g, 10 mmol) was dissolved in DMF (25 mL). BOP (11.3 g, 25 mmol) was added. Amantadine HCl (**7)** (4.55 g, 24 mmol) and 10.5 mL of DIPEA (10.5 mL, 61 mmol) were dissolved in DMF (25 mL) and was added to the Boc-Glu-OH solution. This reaction mixture was stirred at RT for three days. The reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (200 mL). The organic phase was extracted with subsequently water, 0.2 M $KHSO_4$, water, 5% $NaHCO_3$, water and brine. After drying with Na_2SO_4 the organic phase was concentrated in vacuo. The product (**8**) was purified by a silica column (cyclohexane: ethyl acetate 4:1) resulting in 1.7 g (33 %) product as a clear oil.

Compound **8** was dissolved in 10 mL TFA:H2O (95/5) and stirred for 3 h. After thorough concentration in vacuo the product (**9**) was purified by silica column (DCM:MeOH 95:5 as eluent) resulting in 720 mg (41%) product as a white solid.

^{1}H NMR spectrum (400 MHz, DMSO) δ 8.09 (s, J , 3H, N+H_3), 7.91 (s, 1H, Ca C=0 NH), 7.48 (s, 1H Cg C=0 NH), 3.66 (m,1H, CaH), 2.14 (m, 2H, CgH_2), 2.02 (m, 4H, Ca C=0 Ad NH-C-CH-CH2-CH-), 1.99 (m, 4H, Cg C=0 Ad NH-C-CH-CH_2-CH-), 1.95 (m, 6H, Ca C=0 Ad NH-C-CH2-CH2-CH2), 1.91 (m, 6H, Cg C=0 Ad NH-C-CH_2-CH_2-CH_2), 1.84 (m, 2H, CβH_2), 1.63 (m, 5H, Ca C=0 Ad NH-C-CH_2-CH_1-CH_2), 1.60 (m, 5H, Cβ C=0 Ad NH-C-CH_2-CH_1-CH_2).

^{13}C NMR spectrum (100 MHz, DMSO): δ 170.55 (Cg C=0), 167.12 (Ca C=0), 52.09 (Ca), 51.39 (Ca C=0 C), 50.08 (Cg C=0 Ad C), 40.92 (Cg C=0 Ad C-CH_2), 39.49 (Ca C=0 Ad C-CH2), 36.00 (Cg C=0 Ad C-CH_2-CH-CH_2), 35.82 (Ca C=0 Ad C-CH_2-CH-CH_2), 31.32 (Cg), 28.72 (Cg C=0 Ad C-CH_2-CH), 28.34 (Ca C=0 Ad C-CH_2-CH).

Calculated monoisotopic mass ($[M+H]^+$) for **9**, M=$C_{25}H_{39}N_3O_2$, is 414.33. The experimental mass is 414.50.

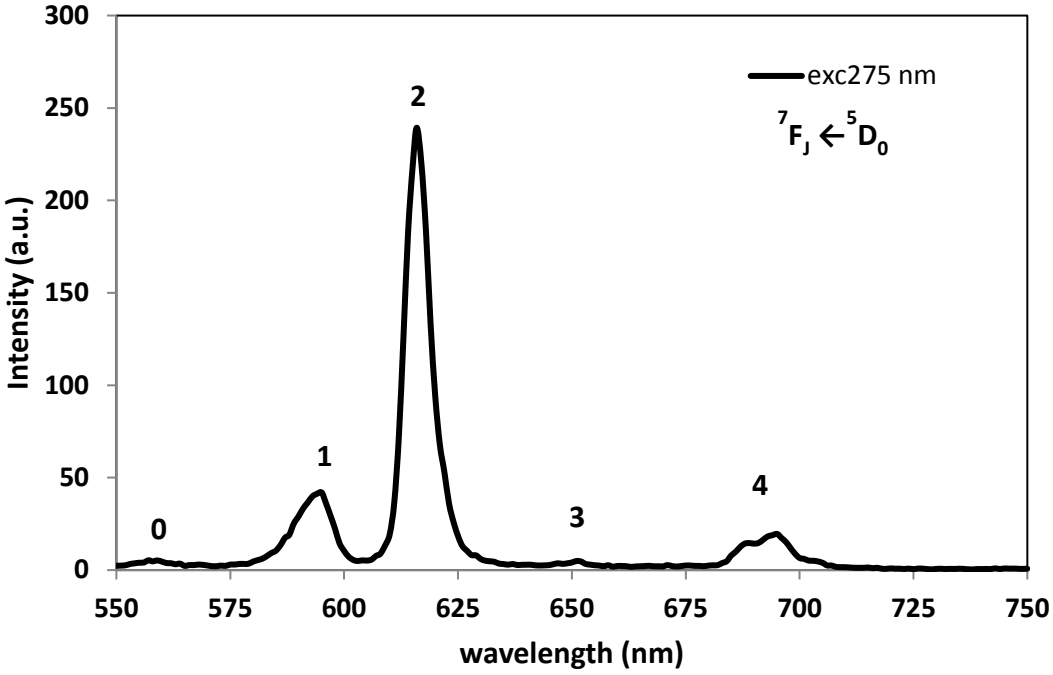


Figure S1. Fluorescence emission spectrum of core-unit^{9-} C4Ms, exciting at 275nm. (micelles are prepared at pH 7, final europium concentration is fixed at 0.2 mM)

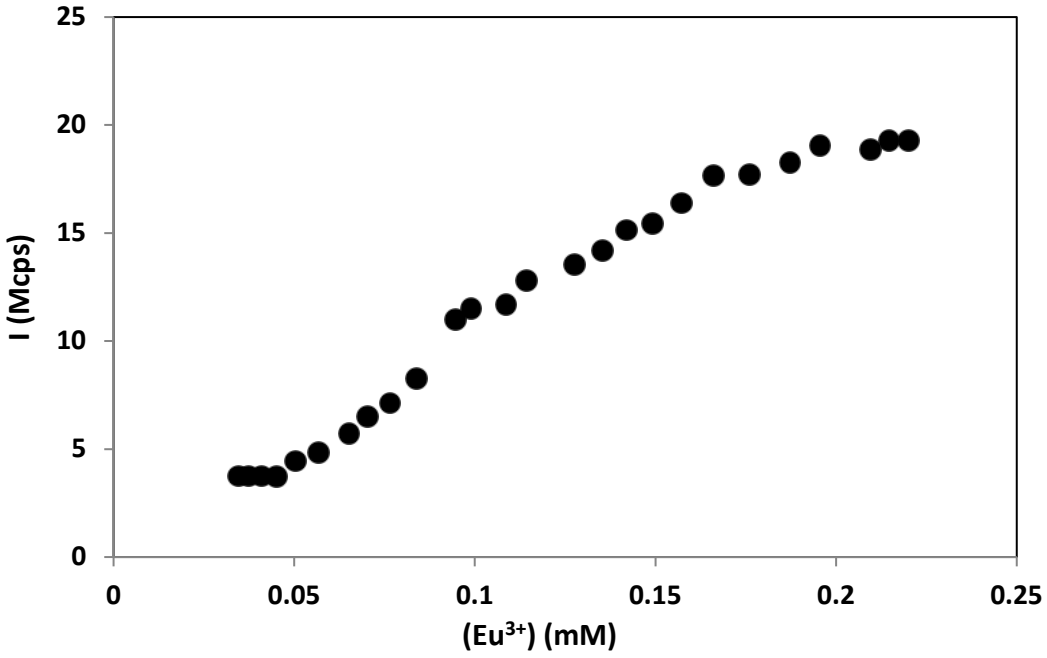


Figure S2. Critical Micelle Concentration determination of core-unit^{9-} depicted as concentration of europium ions. Scattering intensity was monitored after dilution with water.

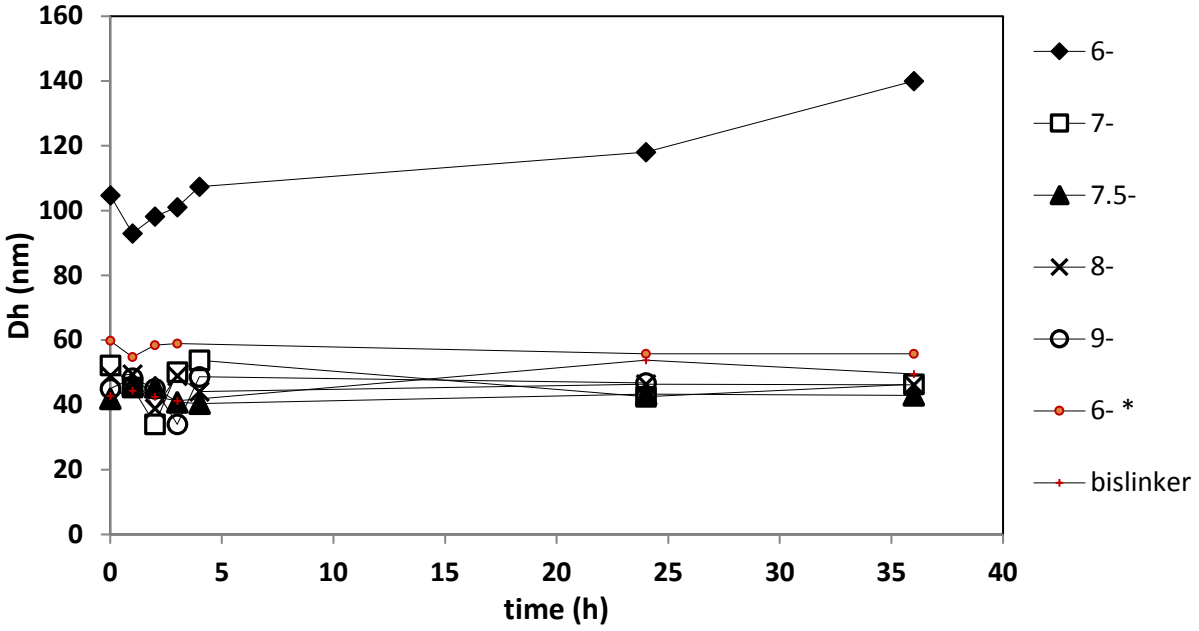


Figure S3. Effect of time on the hydrodynamic diameter, at different monomeric core-unit charges C4Ms, dimeric unit^{6-*} C4Ms and polymeric unit C4Ms based on the bislinker. (micelle are prepared at pH 7, final europium concentration is fixed at 0.2 mM)

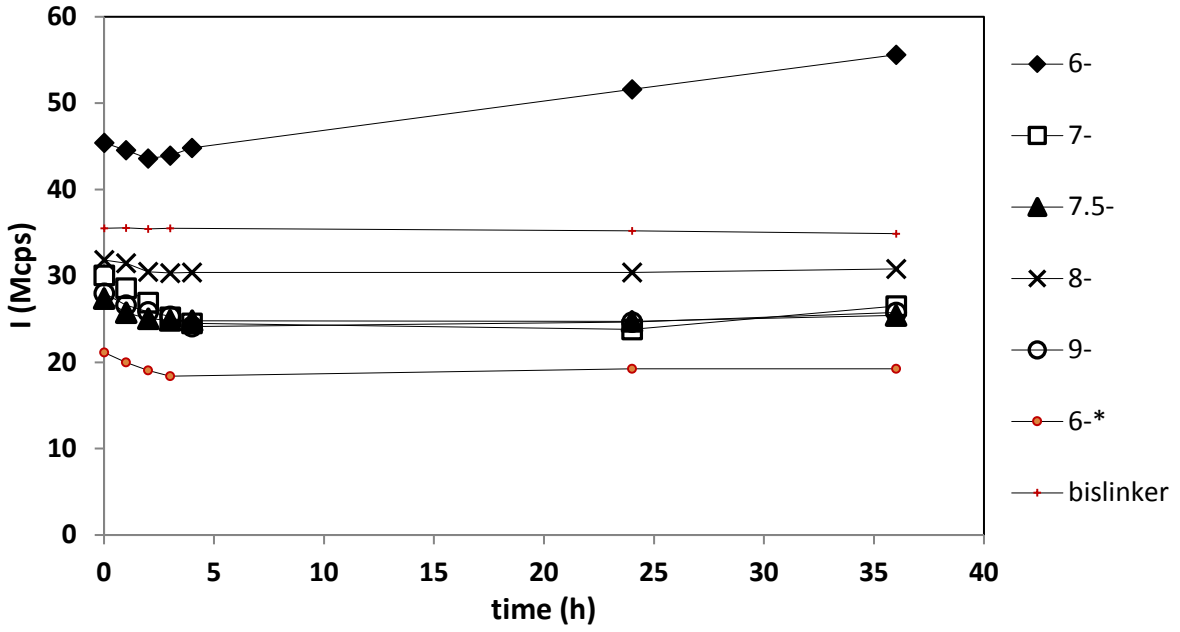


Figure S4. Effect of time on the scattered intensity, at different monomeric core-unit charges C4Ms, dimeric unit^{6-*} C4Ms and polymeric unit C4Ms based on the bislinker (micelle are prepared at pH 7, final metal concentration is fixed at 0.2 mM)

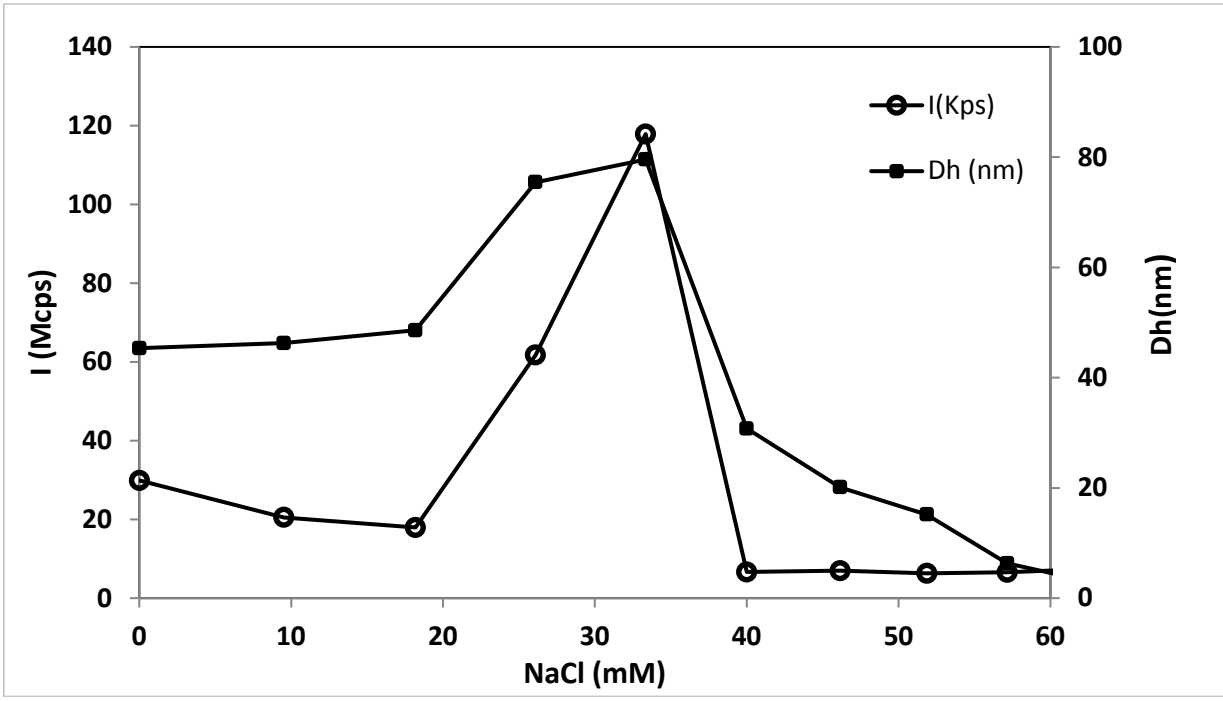


Figure S5. The effect of salt concentration (NaCl) on scattering intensity and hydrodynamic diameter of monomeric unit^{9-} C4Ms. (micelles are prepared at pH 7, final europium concentration is fixed at 0.2 mM)

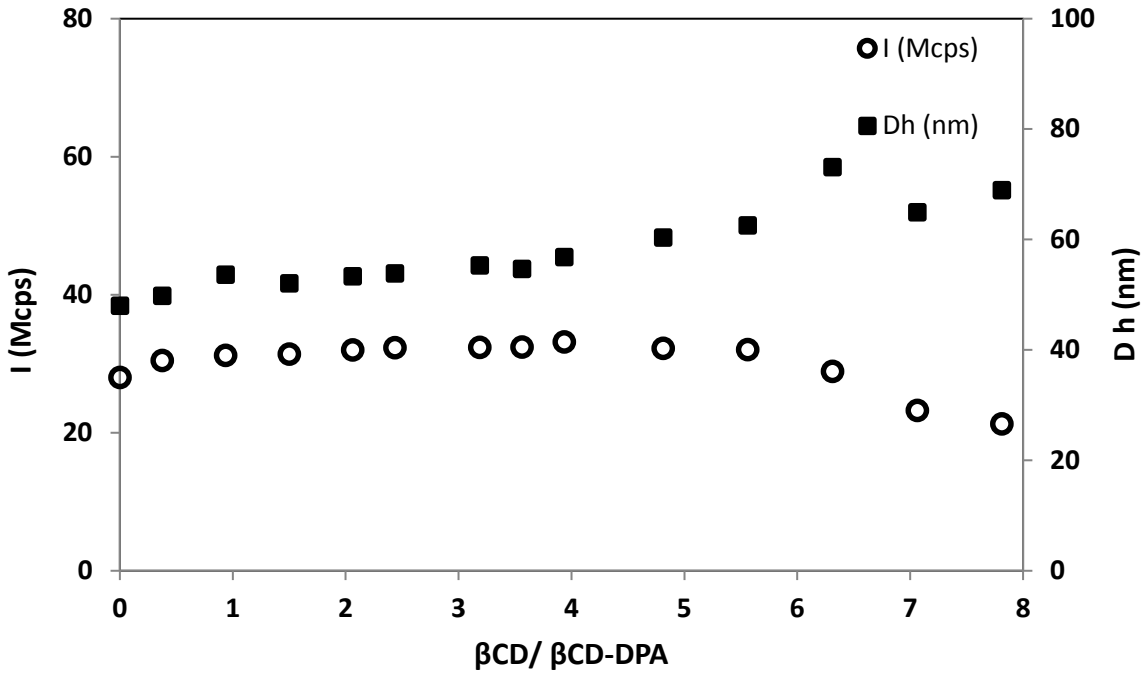


Figure S6. The effect of competing β-cyclodextrin on the scattering intensity and on the hydrodynamic diameter of monomeric unit^{9-} C4Ms. (micelles are prepared at pH 7, final europium concentration is fixed at 0.2 mM)

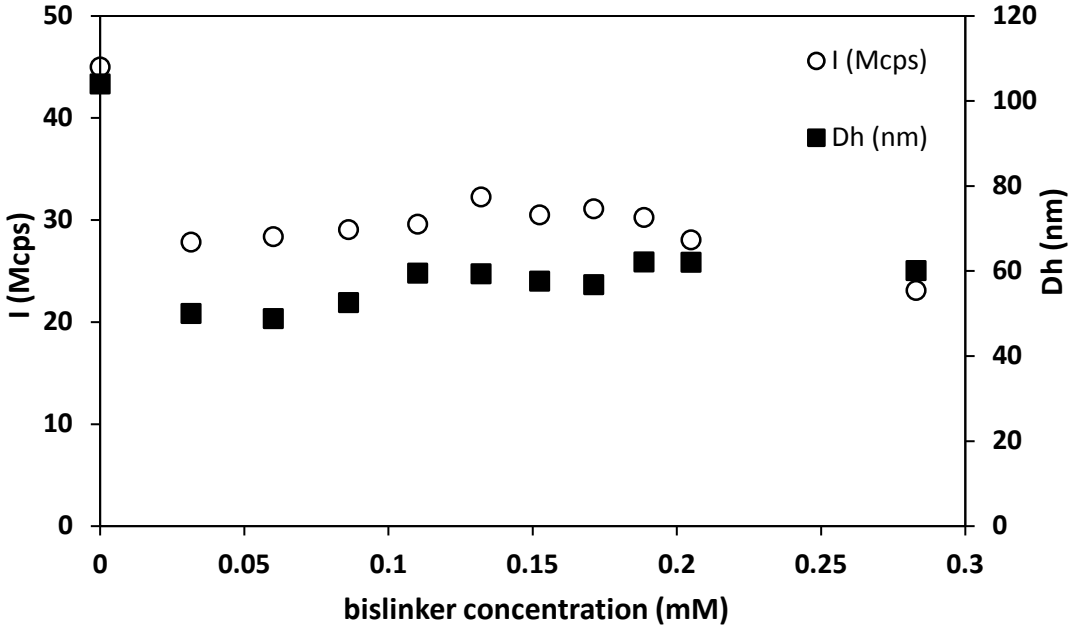


Figure S7. Effect of Ad-Glu-Ad bislinker (S9) titration on the scattered intensity and hydrodynamic diameter of monomeric unit^{6-} C4Ms. 0.1 mM of bislinker corresponds to 16% of bislinker/Ad-ma concentration ratio (final βCD-DPA concentration is fixed at 0.6 mM).

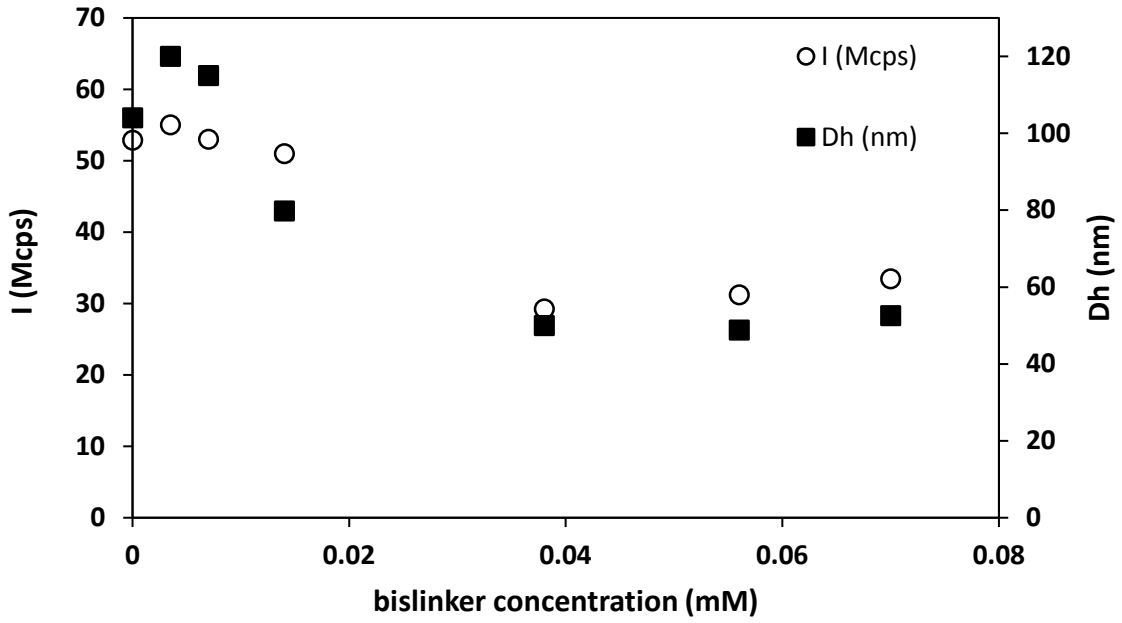


Figure S8. Effect of Ad-Glu-Ad bislinker (S9) pre-mixing on the scattered intensity and hydrodynamic diameter of monomeric unit^{6-} C4Ms. 0.04 mM of bislinker corresponds to 5% of bislinker/Ad-ma concentration ratio (final βCD-DPA concentration is fixed at 0.6 mM).

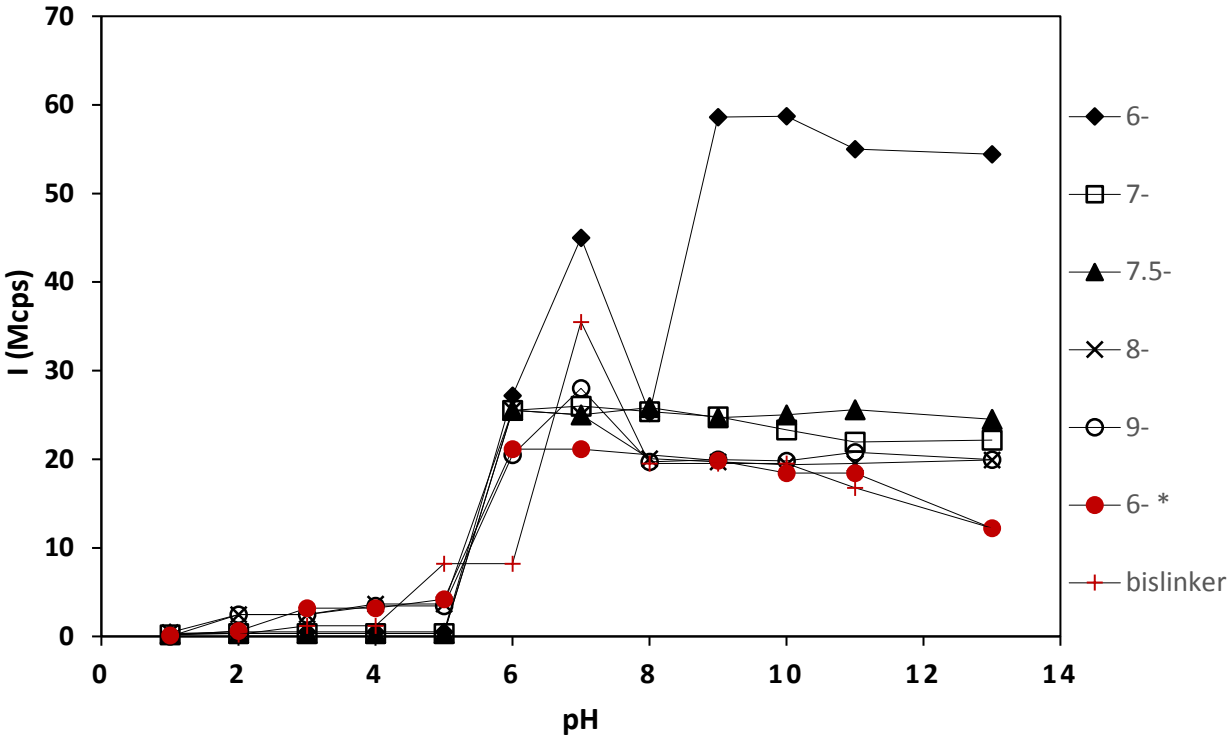


Figure S9. Effect of pH on the scattered intensity, at different C4Ms core-unit charges, dimeric unit^{6-*} C4Ms and polymeric unit C4Ms (final metal concentration is fixed at 0.2 mM)

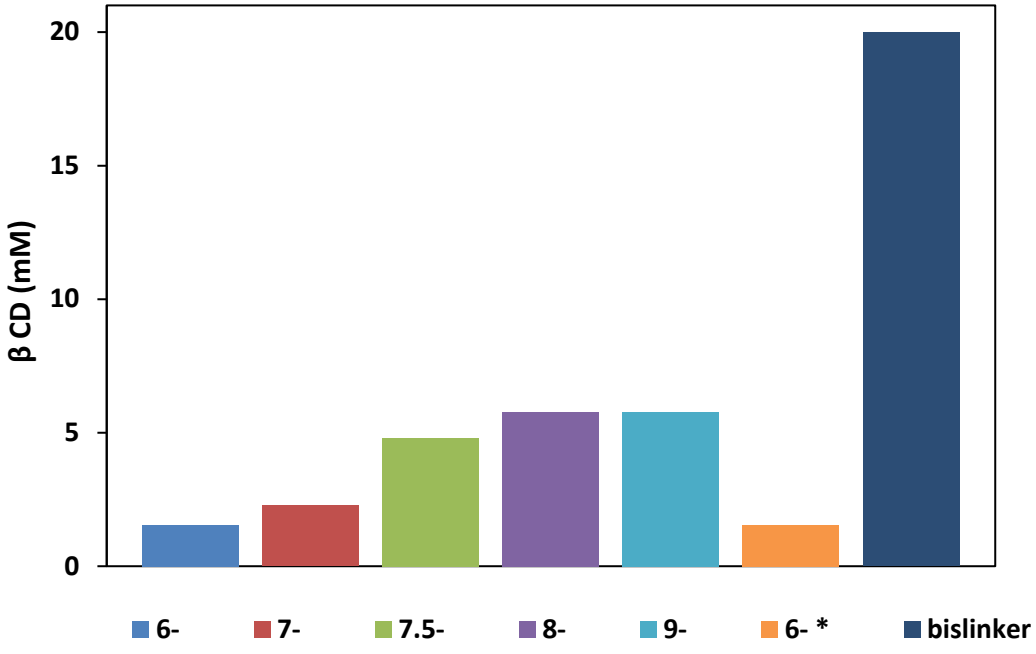


Figure S10. Competing β-cyclodextrin stability at different C4Ms monomeric core-unit charges, dimeric unit^{6-*} C4Ms and polymeric unit C4Ms based on the bislinker. The β-cyclodextrin stability was calculated as the maximum β-cyclodextrin concentration that C4Ms can tolerate, before the DLS scattered intensity and the size drop, as in figure S 5. (micelles are prepared at pH 7, final europium concentration is fixed at 0.2 mM)

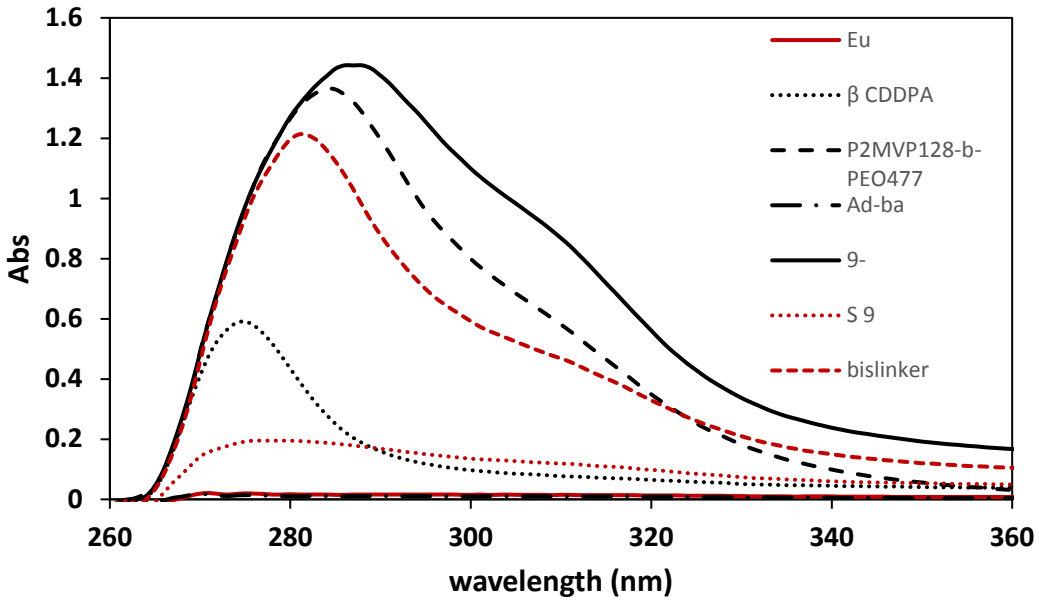


Figure S11. UV-Vis spectra of building blocks, Eu, βCD-DPA, $PMVP_{128}$-PEO_{477}, Ad-ba, monomeric unit^{9-} C4Ms, S 9 and polymeric C4Ms.

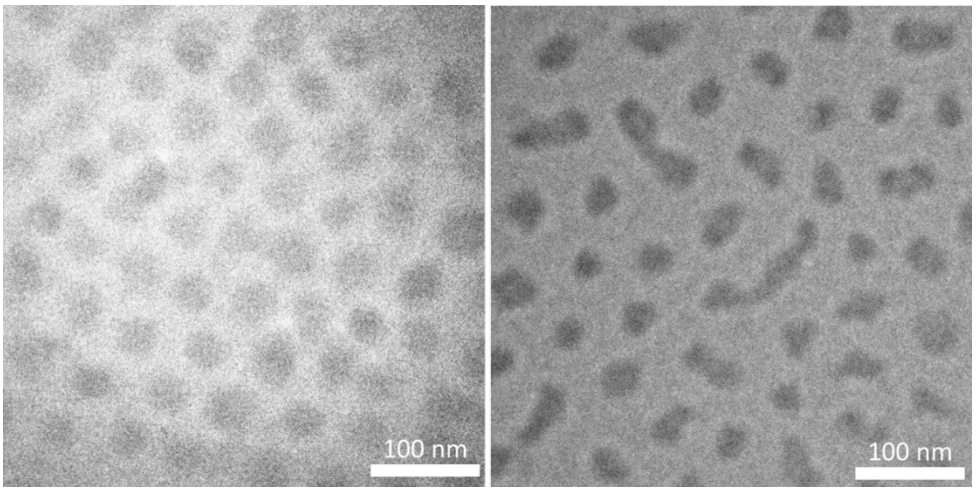


Figure S12. Original size and shape characterization at Cryo-TEM of C4Ms based on core-unit charge $^{9-}$ (left) and C4Ms based on core-unit charge $^{6-}$ (right). The highest core-unit charge C4Ms revealed homogeneously distributed spherical micelles, while the revealed core-unit charge C4Ms showed elongated micelles.

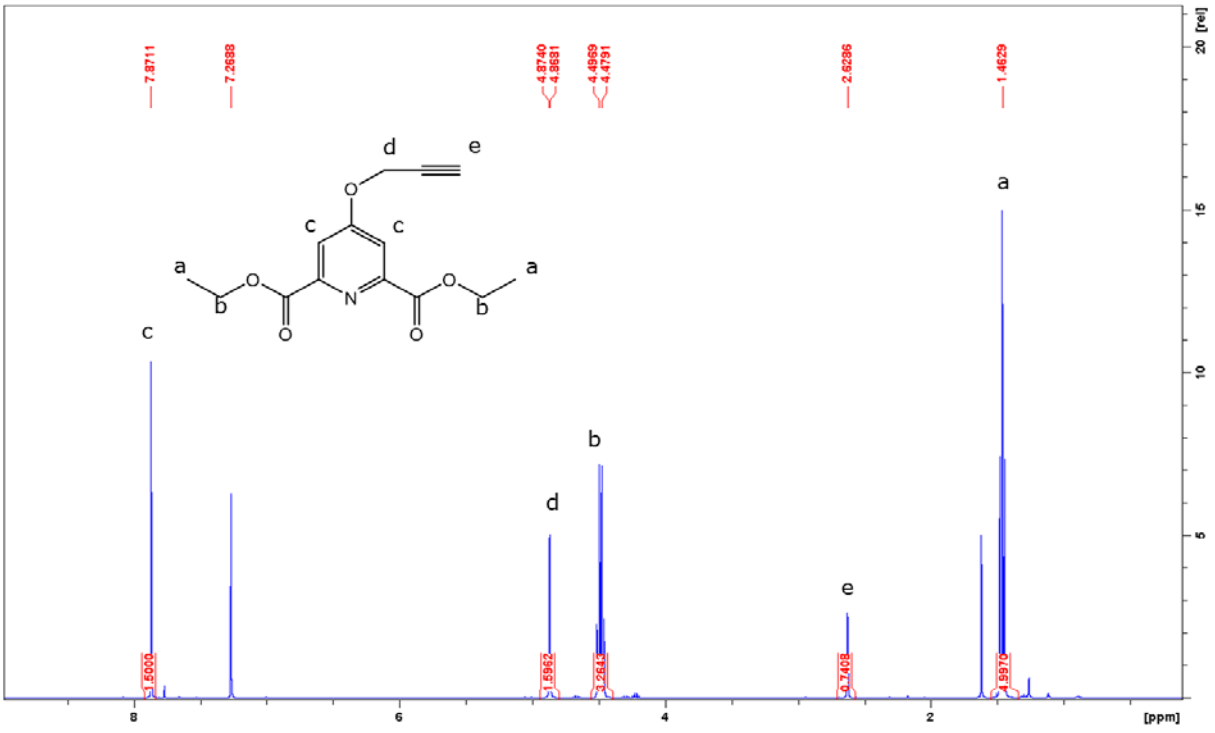


Figure S13. ^{1}H-NMR spectrum of diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate (2) ($CDCl_3$, 400 MHz).

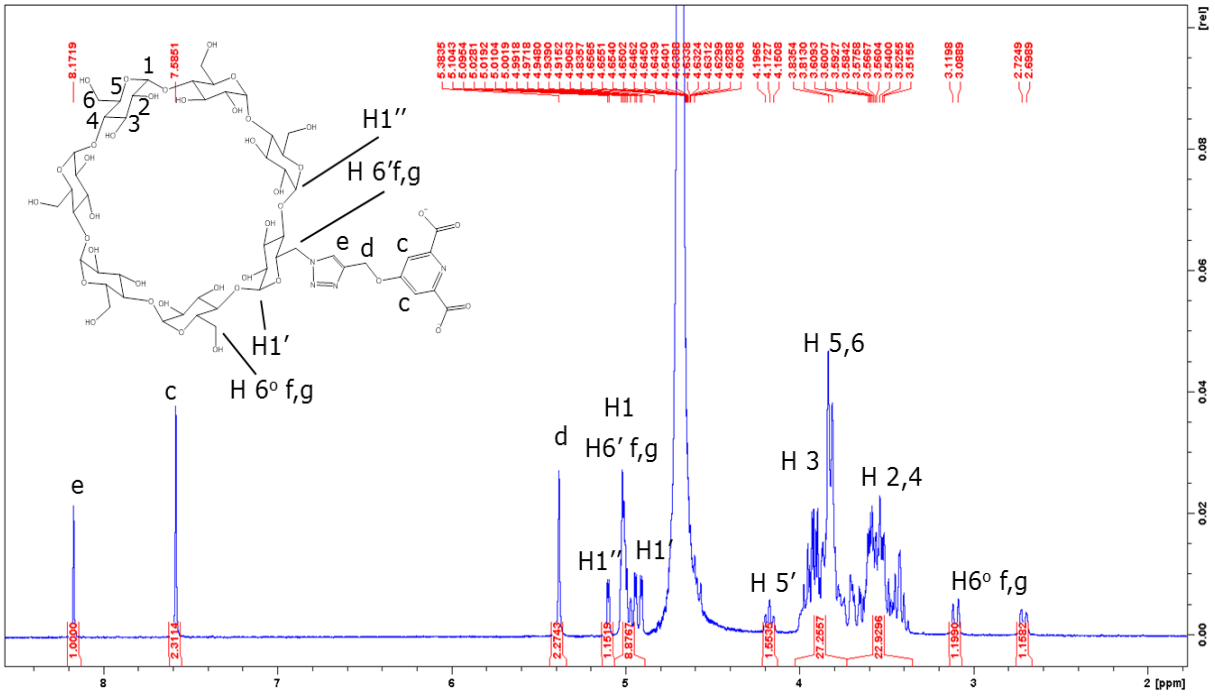


Figure S14. ^{1}H-NMR spectrum of pyridine-2,6-dicarboxylate modified β-cyclodextrin (5) (D_2O, 600 MHz).

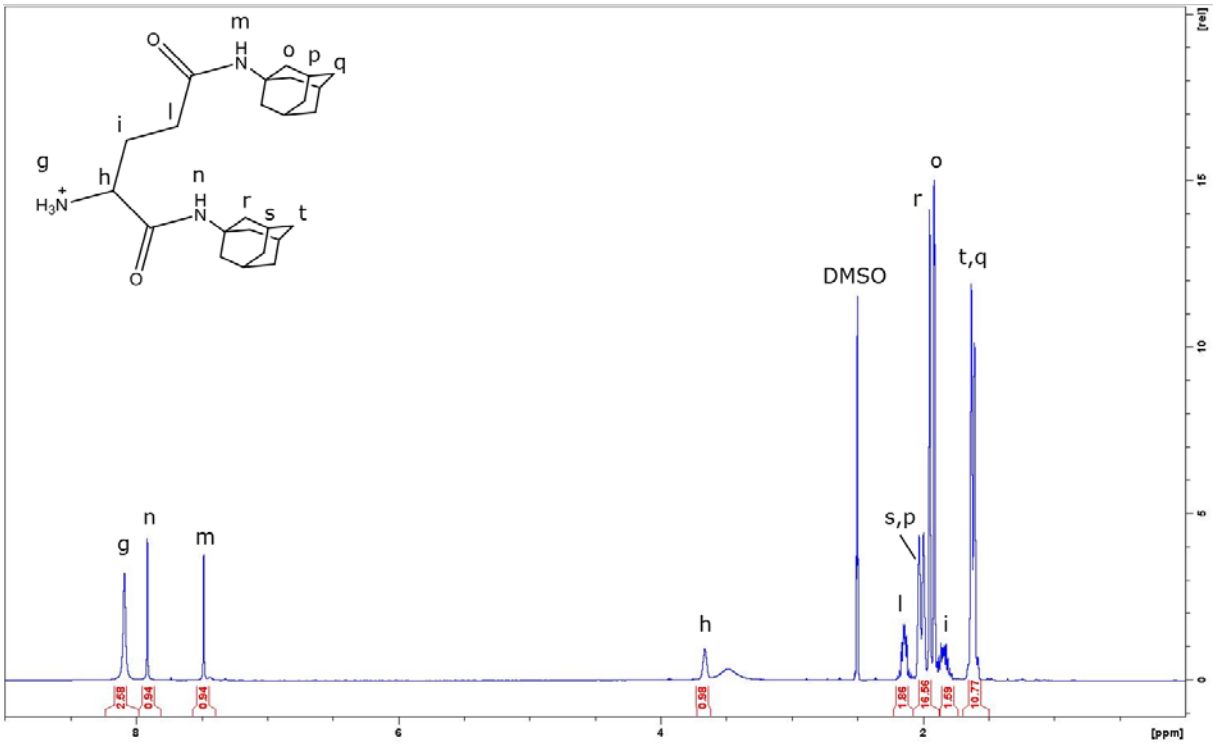


Figure S15. ^{1}H-NMR spectrum of 1,5-bis(((3s,5s,7s)-adamantan-1-yl)amino)-1,5-dioxopentan-2-aminium (9) (DMSO, 500 MHz).

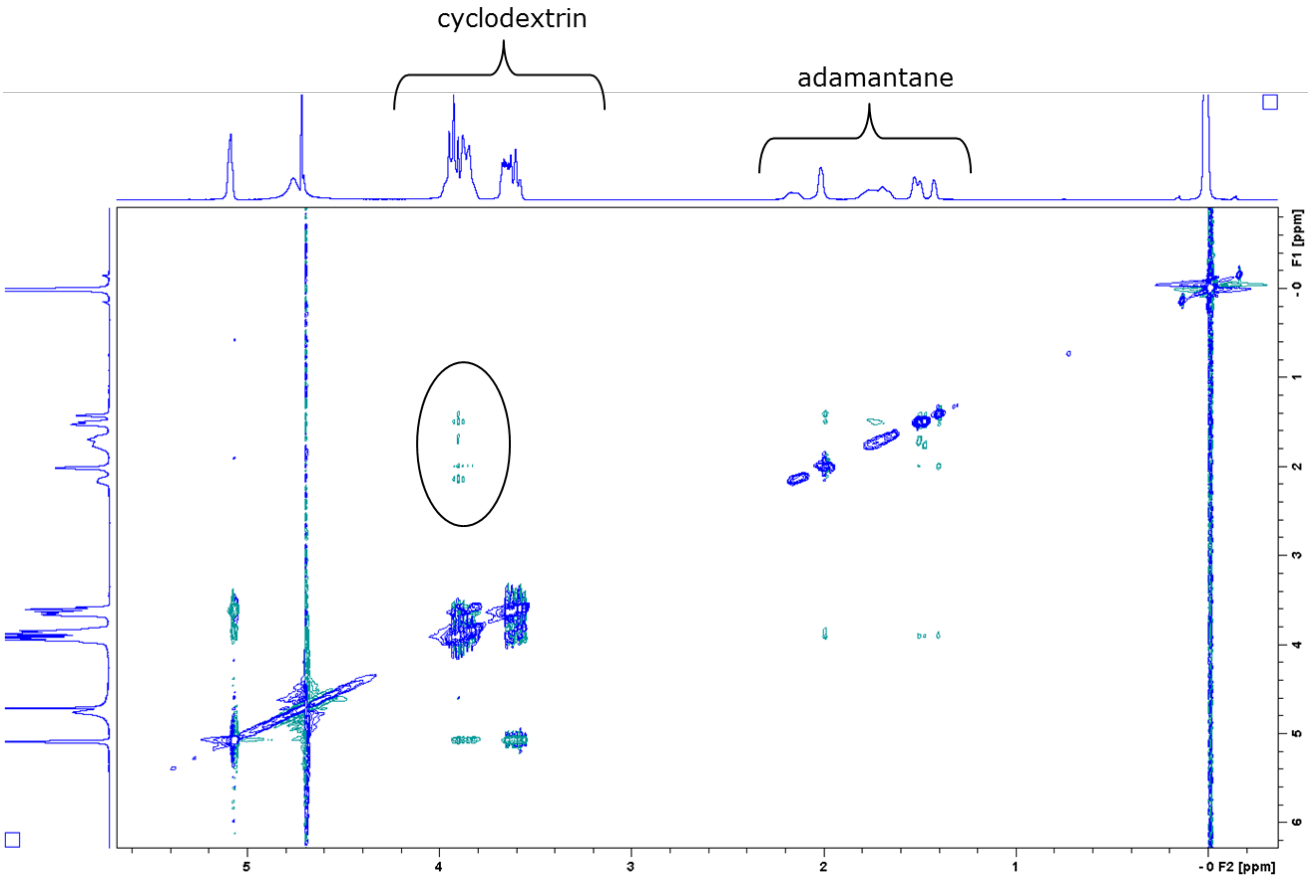


Figure S16. ROESY spectrum of pyridine-2,6-dicarboxylate modified β-cyclodextrin and 1,3 adamantanediacetic acid (D2O, 400 MHz). Ref. 5

Table S1. DLS intensity for individual building blocks in comparison to the C4Ms. Europium ions (Eu), adamantine mono-acid and bis-acid (Ad-ma and Ad-ba), block copolymer (BP), pyridine-2,6-dicarboxylate-modified β-cyclodextrin (bCD-DPA) and combinations of the components.

Sample name	*Intensity (Mcps)*
Eu	*0.2*
bCD-DPA	*0.4*
AD-ma	*0.9*
AD-ba	*0.9*
BP	*0.3*
Eu+bCD-DPA	*1.0*
Eu+Ad-ma	*2.8*
Eu+Ad-ba	*2.8*
Eu+bCD-DPA+Ad-ma	*0.8*
Eu+bCD-DPA+Ad-ba	*0.6*
Eu+bCD-DPA+BP	*1.1*
Eu+BP	*3.0*
C4Ms $^{9-}$	*29.0*

** All the controls were investigated at the DLS, keeping the concentrations of the single components the same as the in the final C4Ms.*

References

S1. Vermonden, T., et al., *Synthesis of 4-functionalized terdendate pyridine-based ligands.* Tetrahedron, 2003. **59**(27): p. 5039-5045.

S2. Kim, I., et al., *Allyloxy- and Benzyloxy-Substituted Pyridine-bis-imine Iron(II) and Cobalt(II) Complexes for Ethylene Polymerization.* Macromolecular Research, 2005. **13**(1): p. 2-7.

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S4. Vrettos, E.I., et al., *Unveiling and tackling guanidinium peptide coupling reagent side reactions towards the development of peptide-drug conjugates.* RSC Adv., 2017. **7**(80): p. 50519-50526.

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