

Functional Star Polymers with a Cholic Acid Core and Their Thermosensitive Properties

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Experimental Section

Materials. Cholic acid ($\geq 98\%$), ethyl chloroformate (97%), sodium borohydride ($\geq 98.5\%$), naphthalene ($\geq 99\%$), potassium chunks (98%, in mineral oil), cysteamine hydrochloride ($\geq 98\%$), 3-mercaptopropionic acid ($\geq 99\%$), 2,2'-azobisisobutyronitrile (AIBN, 98%), and trifluoroacetic anhydride ($> 99\%$) were purchased from Aldrich and used without further purification. Standardized solutions of hydrochloric acid and sodium hydroxide were purchased from Aldrich and titrated before use. Acetic anhydride ($> 98\%$, Fluka) was freshly distilled before use.

Preparation of 5 β -cholane-3 α ,7 α ,12 α ,24-tetrol (CA-OH). Cholic acid (10 g, 24.5 mmol) was dissolved in THF (40 mL/g). Dry triethylamine (13 mL, 93.3 mmol) and ethyl chloroformate (8 mL, 83.7 mmol) were then added to the solution. After stirring for 2 h, a solution of aqueous NaBH₄ (10.67 g in 107 mL of water, 282 mmol) was slowly added, the gas produced was evacuated, followed by stirring for 3 h. The mixture was diluted with water (200 mL) and concentrated HCl was added until all the white residue dissolved. (CAUTION! The acidification produces a lot of gas.) The solution was extracted with ethyl acetate (3 x 200 mL), the organic phase dried over anhydrous Na₂SO₄, then filtered and dried under vacuum.

Naphthalene radical anions were obtained by mixing naphthalene with potassium in anhydrous tetrahydrofuran (THF) at an approximate concentration of 0.40 M and further titrated with a standard 0.1 M hydrochloric acid aqueous solution. AGE ($\geq 99\%$) and dimethyl sulfoxide (DMSO, $\geq 99\%$) were obtained from Aldrich and dried over calcium hydride prior to reflux (2 hours) under reduced pressure followed by distillation.

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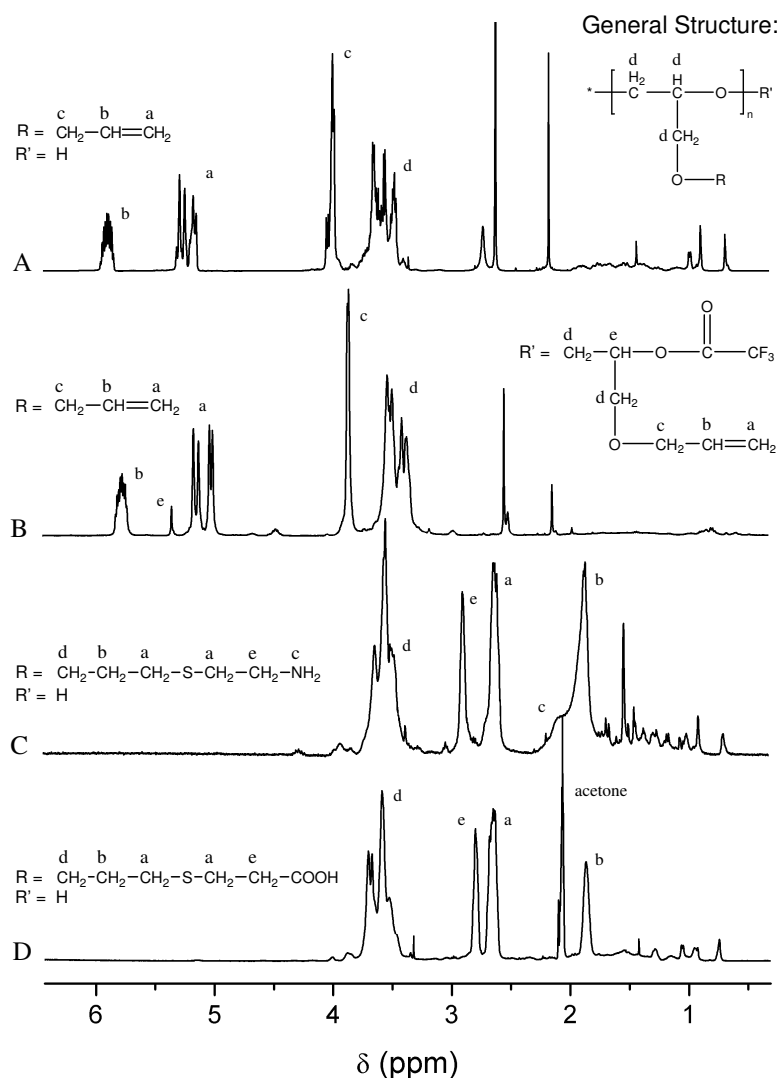


Figure S1. ^1H NMR spectra of A) $\text{CA}(\text{AGE}_5)_4$ in CDCl_3 , B) $\text{CA}(\text{AGE}_5)_4$ in $\text{DMSO } d_6$ after reaction with trifluoroacetic anhydride C) $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$ in CDCl_3 and D) $\text{CA}(\text{AGE}_5\text{-COOH})_4$ in $\text{acetone } d_6$ at room temperature. For spectrum B, the n in the general structure is replaced by $n-1$.

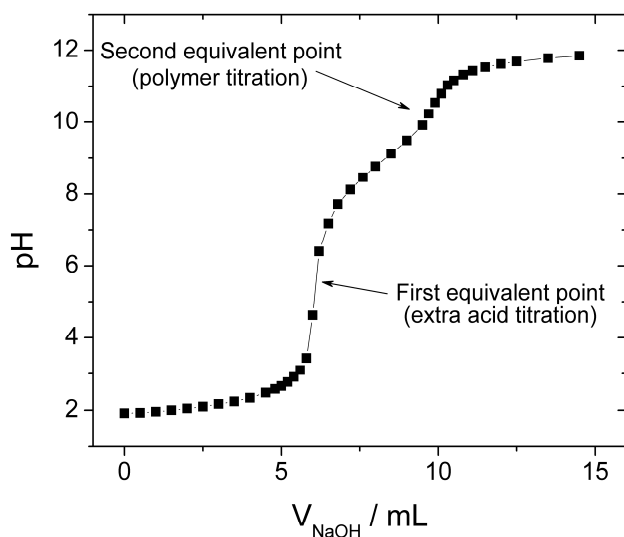


Figure S2. Example of a back titration for $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$ with NaOH (0.1 M). The first equivalent point corresponds to the neutralization of the extra acid (HCl) added in the system, and the second equivalent point corresponds to the neutralization of the protonated amine groups on the polymer. Both are used to calculate the substitution on the polymer.

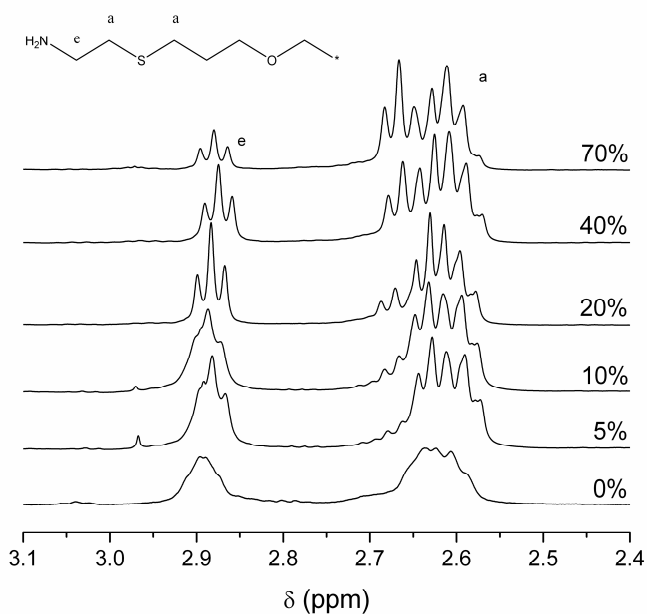


Figure S3. ^1H NMR spectra of acetylated samples of $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$ in CDCl_3 . The expected percentages of the NH_2 groups of $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$ reacted with acetic anhydride are indicated next to the spectra. The chemical shift of the methylene groups **a** changes from 2.9 to 3.55 ppm (PEG protons region) upon acetylation. The integrations of the proton signals **a** and **e** are used to calculate the experimental percentage of reacted NH_2 groups on the polymer.

Table S1. The targeted degrees of acetylation and the experimentally determined values for $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$, showing the quantitative reaction process.

Expected acetylation ratio (%)	^1H NMR acetylation ratio (%) ^a
5	5.8
10	11
20	24
40	45
70	74

^a calculated with the ratio of the protons **e** / **a** (see Figure S3) with $\pm 2\%$

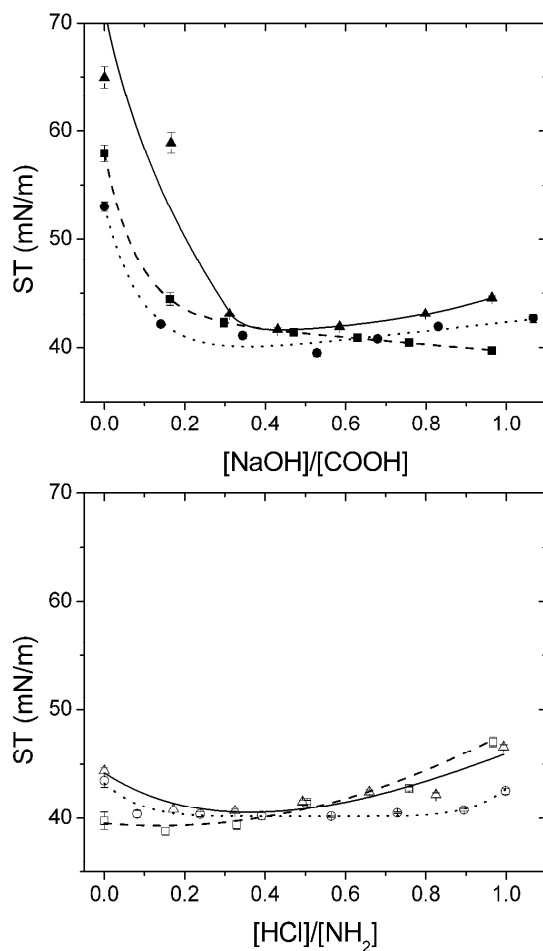


Figure S4. Surface tension of samples $\text{CA}(\text{AGE}_5\text{-COOH})_4$ (●), $\text{CA}(\text{AGE}_{10}\text{-COOH})_4$ (■), $\text{CA}(\text{AGE}_{15}\text{-COOH})_4$ (▲), $\text{CA}(\text{AGE}_5\text{-NH}_2)_4$ (○), $\text{CA}(\text{AGE}_{10}\text{-NH}_2)_4$ (□) and $\text{CA}(\text{AGE}_{15}\text{-NH}_2)_4$ (△) when HCl (for the amine $\text{CA}(\text{AGE}_n\text{-NH}_2)_4$ series) or NaOH (for the acid $\text{CA}(\text{AGE}_n\text{-COOH})_4$ series) is added.

CMC and Thermodynamic Consideration. Changes in the polar and/or non-polar groups usually affect the CMC. It is possible to calculate the CMC of a surfactant according to the hydrophilic and hydrophobic segments in the molecule.¹ The influence of the hydrophobic part is greater than the hydrophilic one as described by the Klevens rule:²

$$\log(\text{CMC}) = A - B \cdot N_c$$

where A depends on the surfactant head group, temperature, and the addition of inert electrolytes, B represents the contribution of each methylene group in the lowering of the CMC by the tail and N_c the number of hydrocarbons in the chain.

In terms of thermodynamics, adding an extra methylene unit to the paraffin chain will decrease the Gibbs energy of micellization by about 1.08 kT.³ In the field of amphiphilic block copolymers, propylene oxide and butylene oxide are among the oxirane monomers often used to form the hydrophobic segment. For amphiphilic block copolymers with hydrophobic oxirane monomer, the ΔG of micellization changes by ca -150 J/mol for every extra propylene oxide unit and ca -960 J/mol for every extra butylene oxide unit.⁴ The smaller changes in ΔG for the oxirane monomers reflect their greater hydrophilic character than that of a methylene unit. In this study, we have roughly determined the change in ΔG as being -60 J/mol (see Figure S5 for details) assuming that $\Delta G \approx RT \cdot \ln(\text{CMC})$.¹ This small change in the Gibbs energy shows the moderate hydrophobicity of the modified AGE polymeric units. The result indicates that long polymer chains made of modified AGE can be attached to an amphiphilic molecule such as bile acid while the aggregation properties of the molecule is preserved while obtaining the advantages of polymers for their specific applications.

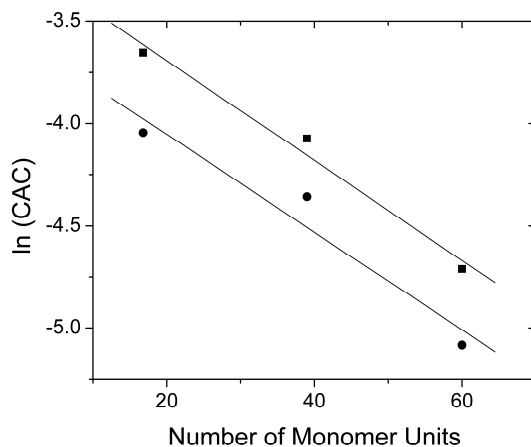


Figure S5. Gibbs energy of micellization involved per monomer unit ($\Delta G_m = RT(\partial \ln[\text{CAC}]/\partial(\text{number of monomer units}))$; star polymers bearing COOH groups **CA(AGE_n-COOH)₄** (circles), and amino groups **CA(AGE_n-NH₂)₄** (squares).

References:

1. Shinoda, K.; Nakagawa, T.; Tamamushi, B.; Isemura, T., Colloidal Surfactants: Some Physicochemical Properties. Physical Chemistry, Eds Hutchinsonvan, E.; van Rysselberghe, P., Academic Press: New York, 1963; Vol. XII, p 310.
2. Klevens, H. B., *J. Am. Oil Chem. Soc.* **1953**, 30, 74-80.
3. Shinoda, K., *J. Phys. Chem.* **1955**, 59, 432-435.
4. Altinok, H.; Nixon, S. K.; Gorry, P. A.; Attwood, D.; Booth, C.; Kellarakis, A.; Havredaki, V., *Colloid Surf. B-Biointerfaces* **1999**, 16, 73-91