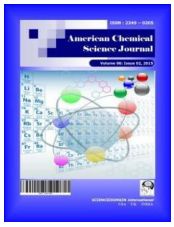

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Study on the Tunable LCST Properties of Phenylalaninate Derivative with Branching Oligoethyleneoxy Chains

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Authors' contributions

This work was carried out in collaboration between all authors. Author LL designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author PY undertook the experimental work, preformed the statistical analysis and managed the literature search with assistance from authors KH and ZY. All authors read and approved the final manuscript.

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ABSTRACT

Thermosensitive amino acid derivative with oligoethyleneoxy chains with tunable lower critical solution temperature (LCST) were prepared from L-methyl phenylalaninate and 3,5-diaminobenzoic acid derivative with oligoethyleneoxy chains. The solution properties and the effects of salt ion species on LCST of phenylalaninate derivative 11 were investigated by UV-vis. The results indicate that the LCST value of 11 could be controlled by adjusting the salt concentration with different cationic species.

Keywords: Metal ions; LCST; oligoethyleneoxy; L – phenylalanine.

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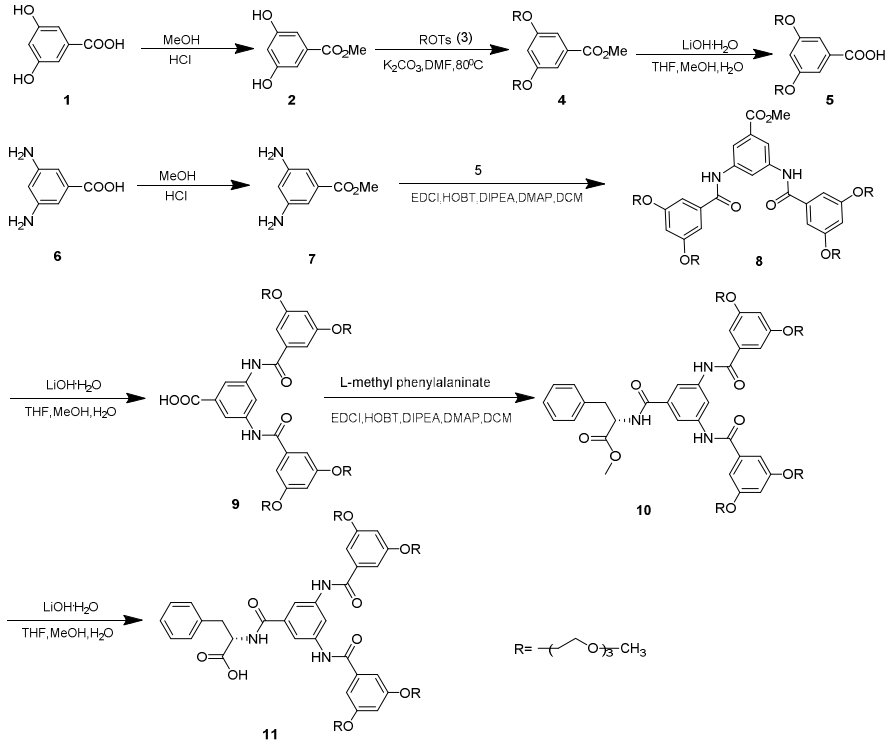
1. INTRODUCTION

Stimuli-responsive materials have attracted a great deal of attention for decades due to the potential applications in drug delivery, sensors, tissue engineering and disease diagnosis [1-2]. In particle, the researches about self-assembly liposomes and micelles, which can change their assembling morphology as the specific environment change, are quite popular. In the various stimuli-responsive terms, the temperature-responsive assemblies undergo a phase transition at a specific temperature, usually referred to lower critical solution temperature (LCST) [3-5]. These thermosensitive components have been used in drug delivery, cell culture and separation of bioactive molecules, due to the properties of phase transition (from soluble to insoluble) as the temperature of the solution is higher than LCST [6-8]. Among thermosensitive components, oligo(ethylene glycol) (OEG)- and poly(ethylene glycol) (PEG)-based systems are one of the hot spots in recent years [9].

The LCST of OEG-containing amphiphilic assemblies are the result of the fact that OEG units are hydrophilic groups. When the temperature of the solution is increased, the OEG units become hydrophobic because of the temperature-induced decrease in hydrogen-bonding [10]. This change in the hydrophilicity of the molecule is the reason for the observed LCST transitions. However, except for the thermosensitive polymers, there were less report about the dendrons and small molecules with LCST properities. We have recently reported a novel Benzyl ether dendrimer with Oligoethyleneoxy chains, which exhibited thermosensitivity and the turbidity decreased with increasing concentrations [11]. The reported researches have shown that the compounds with LCST properties can be used for an important uses for drug delivery. We also know that metal ions such as Ca, Na and Fe etc., exist everywhere within the body, which is probably the most important influencing factors for the drug delivery application [12-14]. Herein, we reported the solution properties and the effects of salt ion species on LCST of the derivatives containing oligoethyleneoxy chains (Scheme 1). The carboxylate group of **11** is a common site for the binding of a metal cation [15]. Furthermore, it is found from a variety of crystal structures that the carboxylate group may bind up to four metal cations or share the metal cation between both oxygen atoms [16-17]. Thus, adding the different concentration and spices of metal ions to the



Scheme 1. The synthesis pathway of 11

solution of **11** would control the LCST behavior due to the different self-assembly properties of **11**-ion complexes. For this purpose, we evaluated their effects on the solution of **11** for representative cations (eg. Fe, Cu, Na). The results showed that **11** exhibited **cation** controlled thermosensitivity and LCST properties.

2. METHODS

2.1 General Procedures

^{1}H NMR spectrum was recorded with a Bruker Avance 600 spectrometer in $CDCl_3$ or DMSO-d_6, and tetramethylsilane was used as an internal standard substance. FTIR spectra were recorded on a Spectrum one (Version BM) FTIR spectrometer at RT. UV/Vis spectra were recorded on UV5500PC spectrophotometer.

2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate(**3**), methyl 3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoate(**4**), methyl 3,5-diaminobenzoate (**7**) and were prepared as references. All other reagents obtained from commercial suppliers were used without further purification unless otherwise noted. Dichloromethane (DCM) was dried and distilled over calcium hydride.

2.2 Synthesis

2.2.1 Methyl 3,5-bis(3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzamido)benzoate (8)

To a solution of 3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoic acid **5** (1.8 g, 4 mmol) and 7 (664 mg, 4 mmol) in CH_2Cl_2 (30 mL), HOBt (570 mg, 4.2 mmol), EDCI (810 mg, 4.2 mmol) and DIPEA (1.06 g, 8 mmol) were successsively added at 0℃ and stirred at room temperature for 24 hours. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (60 mL) and washed with $NaHCO_3$ (sat.), 1 M HCl and brine. The organic layers were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EA/ CH_3OH=10:1, v/v) to give **8** (679 mg, 33%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (dt, *J* = 22.0, 2.3 Hz, 1H), 8.17 (d, *J* = 5.0 Hz, 1H), 7.19 (dt, *J* = 5.9, 2.1 Hz, 1H), 6.98 (dt, *J* = 8.0, 2.2 Hz, 1H), 6.60 (dd, *J* = 3.0, 1.9 Hz, 1H), 4.19 (t, *J* = 4.6 Hz, 4H), 3.96 (s, 2H), 3.82 (t, *J* = 5.6 Hz, 4H), 3.60 (dd, *J* = 5.9, 3.3 Hz, 2H), 3.58 (s, 10H), 3.50 (td, *J* = 6.0, 3.1 Hz, 4H), 3.31 (s, 6H).

2.2.2 3,5-bis(3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzamido)benzoic acid (9)

To a solution of **8** (0.98 g, 0.9 mmol) in THF/H_2O/MeOH (3:1:1, 25 mL) LiOH•H_2O (161 mg, 3.8 mmol) was added at 0℃. After stirred for 4 hours at rt, the reaction mixture was diluted by water. The aqueous solution was acidified by HCl (1M) to pH 2 and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated to give yellow oil **9** and used without further purification.

2.2.3 Methyl (3,5-bis(3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzamido)benzoyl)- L-phenylalaninate (10)

To a solution of **9** (3.28 g, 3.2 mmol) and L-methyl phenylalaninate (692 mg, 3.9 mmol) in CH_2Cl_2 (40 mL), HOBt (434 mg, 3.2 mmol), EDCI (616 mg, 3.2 mmol) and DIPEA (1.66 g, 8 mmol) were successsively added at 0℃ and stirred at room temperature for 36 hours. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (60 mL) and washed with $NaHCO_3$ (sat.), 1 M HCl and brine. The organic layers were dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EA/ CH_3OH=10:1, v/v) to give **10** (2.46 g, 36.8%) as a yellow oil. ^{1}H NMR (600 MHz, CDCl3) δ 9.48 (s, 2H), 8.77 (d, *J* = 2.0 Hz, 1H), 8.11 (s, 2H), 7.28-7.23 (m, 2H), 7.19 (dd, *J* = 8.6, 7.0 Hz, 3H), 7.05 (d, *J* = 7.9 Hz, 1H), 6.81 (d, *J* = 2.2 Hz, 4H), 6.40 (d, *J* = 2.3 Hz, 2H), 5.04 (dt, *J* = 8.0, 6.2 Hz, 1H), 4.01 (t, *J* = 4.6 Hz, 8H), 3.82 (dd, *J* = 5.6, 3.6 Hz, 8H), 3.74 (dd, *J* = 5.9, 3.6 Hz, 8H), 3.71 (s, 3H), 3.68 (dd, *J* = 5.8, 3.7 Hz, 8H), 3.65 – 3.61 (m, 8H), 3.51 (dd, *J* = 5.8, 3.6 Hz, 8H), 3.31 (s, 12H), 3.30 – 3.22 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 165.9, 165.4, 158.6, 138.5, 135.8, 135.0, 133.7, 128.2, 128.2, 127.6, 126.0, 114.4, 113.9, 105.0, 103.6, 70.9, 70.8, 69.7, 69.5, 69.4, 68.6, 66.4, 57.9, 52.7, 51.2, 37.0, 28.6. ESI-HR-MS m/z: Calcd for $C_{59}H_{83}N_3O_{21}Na$ ($[M+Na]^+$) 1192.5421, found 1192.5417.

2.2.4 (3,5-bis(3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzamido)benzoyl)-L-phenyl- alanine (11)

To a solution of **10** (1.3 g, 1.1 mmol) in THF/H_2O/MeOH (3:1:1, 25 mL) LiOH•H_2O (1.37 g, 32.6 mmol) was added at 0℃. After stirred for 4 hours at rt, the reaction mixture was diluted by water. The aqueous solution was acidified by HCl

(1M) to pH 2 and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated to give **11** (1.24 g, 84.7%) as yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.47 (s, 2H), 8.48 (s, 1H), 7.95 (s, 2H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.30 – 7.16 (m, 4H), 7.16 – 7.07 (m, 1H), 6.88 (s, 4H), 6.45 (s, 2H), 4.91 (d, *J* = 6.6 Hz, 1H), 4.07 – 3.99 (m, 8H), 3.88 – 3.74 (m, 16H), 3.75 – 3.61 (m, 8H), 3.64 – 3.57 (m, 8H), 3.56 – 3.48 (m, 8H), 3.31 (s, 12H), 3.34 – 3.24 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.2, 167.8, 166.1, 161.1, 138.4, 138.2, 137.6, 133.8, 129.5, 128.7, 126.1, 122.3, 117.3, 117.1, 109.7, 106.9, 72.2, 71.7, 70.6, 70.5, 70.4, 70.3, 70.1, 69.1, 68.4, 57.8, 52.1, 37.8.

2.3 The LCST Measurements

The LCST measurements were carried out with a UV5500PC UV/VIS spectrometer equipped with a thermo-regulated cell-holder. The temperature of the solution of derivative **11** (1 mg/mL) was elevated from 20 to 60℃ in increments of 0.2 ℃ /min and the transmittance was monitored at 500 nm wavelength. The LCST was approximated as the temperature whereby the transmittance had decreased by 5% from the initial baseline reading at 20℃. All solutions were allowed to equilibrate in the prescribed temperature for at least 10 min prior to the measurement. The samples were dissolved in water or the desired salt solution prepared with deionized water made with the Milli-Q system.

3. RESULTS AND DISCUSSION

The LCST of **11** was determined by UV measurement, and the LCST was approximated as the temperature whereby the transmittance had decreased by 5% from the initial baseline reading at 20℃. We can find out that the compound **11** exhibited thermosensitivity and LCST properties [18]. Figs. 1-3 show the effects of Na^+, Fe^{3+} and Cu^{2+} as the cations but with different concentrations on the LCST of **11** (1mg/mL). In principle, the increase in salt concentration lowers the LCST, which is a well-documented effect. Furthermore, it is obvious that the variations in LCST characteristics were dependent characteristically on the cationic species of added salt. For Na^+, reduced *LCSTs* and a *narrow* transition *range* were observed with the increase of concentration, whereby it was suggested that the broad LCST may be a result of the hydrophilic side chains preventing the self-assembly aggregation of **11** and more ions were incorporated into the system [19]. For Fe^{3+}, almost the same results were found except for the higher LCST and narrower transition range, suggesting the Fe-based complex had stronger self-assembly aggregation properties. For Cu^{2+}, we found that the increasing concentration did not influence the LCST very much as other cations done, which changed only 5℃ while the others changed more than 10℃ under the same condition, implying that the excess Cu^{2+} ions were not incorporated into the system. Generally, we can conclude that the influence on the LCST of compound **11** is Fe^{3+} > Cu^{2+} >Na^+, and with the increase of ion concentration, the LCST of **11** varied more obvious, which may relate to the ability of ions-**11** complexes formation. Besides, according to theory of Hofmeister research theory, the salting out effect of trivalent ions is stronger than that of bivalent ions [20].

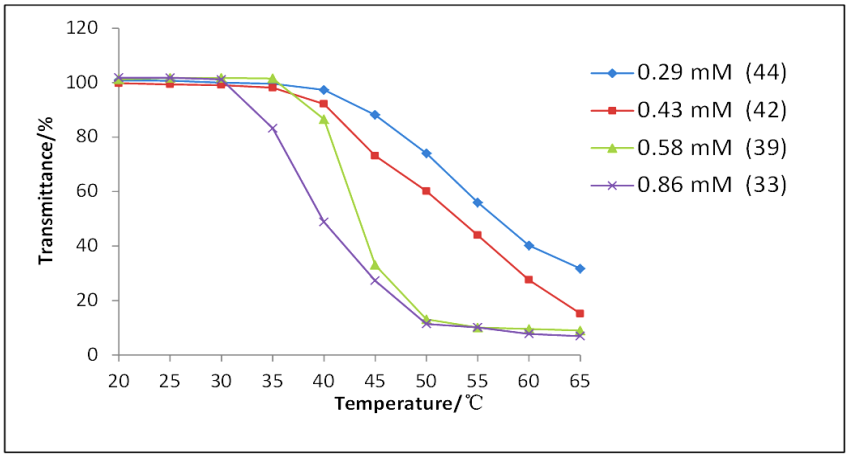


Fig. 1. The influence of NaCl to compound 11. (The corresponding LCST is showed in parentheses, respectively)

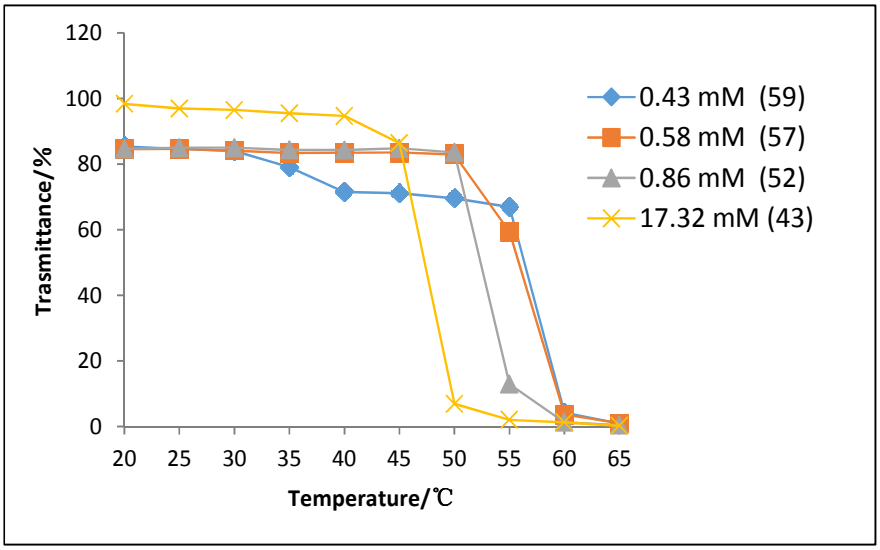


Fig. 2. The influence of $FeCl_3$ to compound 11. (The corresponding LCST is showed in parentheses, respectively)

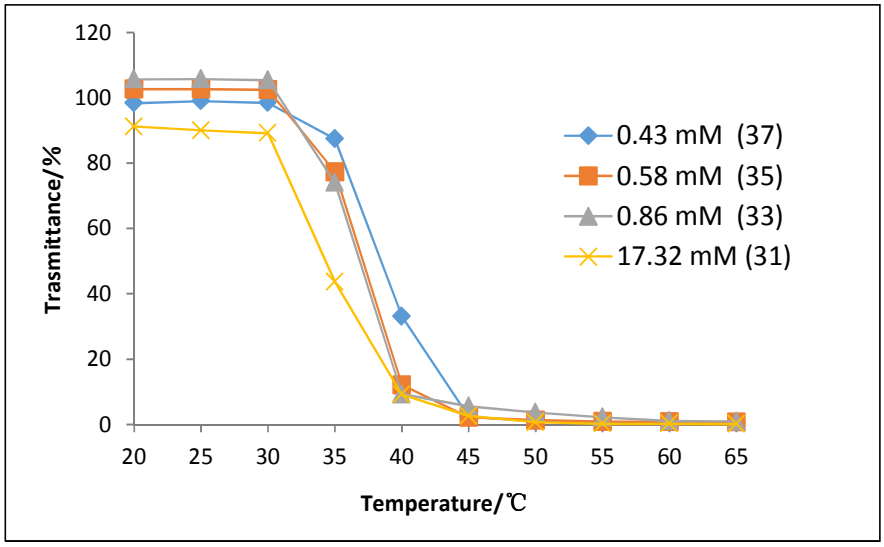


Fig. 3. The influence of $CuCl_2$ to compound 11. (The corresponding LCST is showed in parentheses, respectively)

We also evaluated the effects of representative anions such as SO_4^{2-}, F^- and NO_2^{2-} with same Na^+, (Fig. 4) at a compound **11** concentration of 1 mg/mL. From the results, it could be concluded that anions have less impact on the LCST of compound **11** as compared to cations due to the anions have less influences on the metal complex formation and the later aggregations. The transition ranges with addition of F^- and NO_3^- were slightly narrower than that of Cl^- and $SO4^{2-}$, which recorded almost the same LCST of 40℃ for **11**.

Ionic concentration gradients exist among the blood, interstitial and intracellular compartments, for example, in Intracellular fluid the concentration of Na^+ is about 10 mM, but would increase to 140 mM in Plasma [20]. Thus, compound **11** would be potential to use in drug delivery systems, which showed special LCST at the body temperature (about 35-40℃) in low concentration of Na^+.

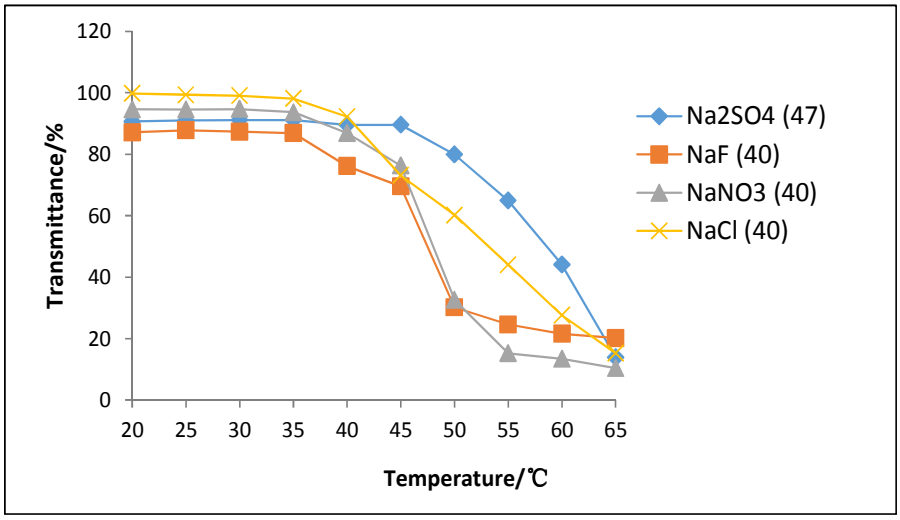


Fig. 4. The influence of anion to compound 11. (The corresponding LCST is showed in parentheses, respectively)

4. CONCLUSIONS

In summary, a thermosensitive amino acid derivative with oligoethyleneoxy chains, was synthesized from L-methyl phenylalaninate and 3,5-diaminobenzoic acid derivative with oligoethyleneoxy chains. The solution properties of phenylalaninate derivative **11** and the effects of salt ion species on LCST were investigated by UV-vis. The results showed that the influence on the LCST of compound **11** is $Fe3^{+}$ > Cu^{2+} >Na^{+} and anions have less impact. The results indicate that the LCST value of **11** could be controlled by adjusting the salt concentration with different cationic species, which would be potential to use in drug delivery systems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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