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## Combinatorial Discovery of Thermoresponsive Cycloammonium **Ionic Liquids**

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This work demonstrated, for the first time, combinatorial discovery and rational identification of small-molecule cycloammoniumbased thermoresponsive ionic liquids that exhibit LCST phase transition and carry attractive T<sub>c</sub> values in water.

Based on heterocyclic amine cores (azepane, piperidine, and pyrrolidine), this communication reports the concise design and combinatorial synthesis of thermoresponsive ionic liquids (TILs) that exhibit lower critical solution temperature (LCST)-type phase transitions in water. Ionic liquids are composed fully of ions and, as anticipated, many of them are miscible with water.<sup>1,2</sup> Additionally, ionic liquids are known to display temperature-driven dynamic and reversible phase transitions; that is, upper critical solution temperature (UCST)- and LCSTtype phase behaviors.<sup>3-6</sup> This phase behavior of TILs in water is highly dependent on the component ions and has been imperative in various aspects of reaction catalysis, extraction and separation.<sup>3</sup> In this work, we are developing a combinatorial approach for the synthesis of cycloammoniumbased ionic liquids (ILs) (Figure 1) and demonstrate, for the first time, their value in temperature-switchable LCST phase transition.

Both LCST and UCST systems are two characteristic phase behaviors of thermoresponsive materials with solvents.<sup>3-6</sup> In UCST phase transition, miscibility of an aqueous two phase system increases upon heating and, for LCST system, two aqueous solutions homogeneously mix upon cooling. Ohno and coworkers were pioneers on the studies of TILs with LCST phase transitions.<sup>4-6</sup> This LCST-type reversible phase transitions were reported largely in non-ionic polymer solutions and seldom observed in mixtures of small-molecule ILs with water.4-6





⊖<sub>OTs</sub>



[N<sub>6CRR</sub>][OTMBS]

[N<sub>6CRR</sub>][OTs]

⊖<sub>OTs</sub>

[N<sub>4CRR</sub>][OTMBS]

OTs, 4-toulenesulfonate; OTMBS, 2,4,6-trimethylbenzenesulfonate R = n-butyl (4), n-pentyl (5), n-hexyl (6), n-heptyl (7), n-octyl (8) Figure 1. Structures of cycloammonium ionic liquids. [N<sub>4CRR</sub>], *N*,*N*-dialkylpyrrolidinium; [N<sub>5CRR</sub>], *N*,*N*-dialkylpiperidinium; [N<sub>6CRR</sub>], N,N-dialkylazepanium. [N<sub>4C66</sub>][OTs], for example, is an abbreviated form of N,N-dihexylpyrrolidinium 4toluenesulfonate.

[N<sub>5CRR</sub>][OTMBS]

Although some organic salts<sup>4-7</sup> were found LCST transition in water, no IL structures as shown in Figure 1 have been reported in literature and there was also no systematic study on rational design of ionic liquids to demonstrate LCST temperature responsiveness. Moreover, we were intrigued by the recent advances of TILs<sup>3-6</sup> and envisaged that ionic liquid library embedded with the core structure of heterocyclic amine (Figure 1) are easily tunable and should be of convenient candidates for study in thermoresponsiveness. Accordingly, we set out to develop a library synthesis for cycloammonium ionic liquids in short synthetic steps and aimed at the combinatorial discovery of new TILs with temperature-switchable phase separation.

Scheme 1 illustrates our synthesis of a small library of 15 ionic liquids [N<sub>4CRR</sub>][OTs], [N<sub>5CRR</sub>][OTs] and [N<sub>6CRR</sub>][OTs]. We commenced the synthesis from five commercial alcohols and three inexpensive heterocyclic amines, which underwent a twostep synthesis (i.e., alcohol tosylation and a subsequent amine alkylation with corresponding tosylate) to afford the desired ionic liquids. The ionic liquid syntheses were straightforward and the overall isolated yields for [N4CRR][OTs], [N5CRR][OTs] and

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 $[N_{6CRR}][OTs]$  were acceptable: 64-73%, 40-67% and 46-64%, respectively (Scheme 1). The assembly of the other library of 15 [OTMBS]-based ionic liquids followed identical synthetic route as depicted in Scheme 1 (see Scheme S1, ESI). In short, the overall isolated yields for these 2-step syntheses of  $[N_{4CRR}][OTMBS]$ ,  $[N_{5CRR}][OTMBS]$  and  $[N_{6CRR}][OTMBS]$  were moderate: 42-65%, 32-41% and 42-53%, respectively (Scheme S1, ESI). Detailed experimental procedures, H-1 and C-13 NMR, and high-resolution mass spectrometry (HRMS) spectra and data of 30 cycloammonium salts are summarized in the Supporting Information (ESI). Among them, ten were ionic liquids ( $T_m < 100$  °C) in their pure form (ESI). All 30 compounds synthesized could be classified as hydrated ionic liquids<sup>8,9</sup> upon associating with water.



 $\label{eq:scheme 1. Synthesis of cycloammonium ionic liquids} $ [N_{4CRR}][OTs], [N_{5CRR}][OTs] $ and [N_{6CRR}][OTs]. $ \end{tabular}$ 

Figure 2 shows a library of 30 cycloammonium salts and their phase transitions toward temperature changes with water. To discover thermoresponsive ionic liquids, each organic salt was mixed with water in a mass ratio of 1:2 (w/w) and the mixture was then placed in an ice bath (0 °C) followed by gradual heating until it reached 95 °C. Phase transition temperature  $(T_c)$  for LCST was determined at the temperature point when the aqueous solution turned cloudy during heating by naked eyes. We were pleased that, among 30 organic salts tested, six hydrated ionic liquids (labeled in green) were found exhibiting LCST phase transitions (Figure 2): [N<sub>4C66</sub>][OTs],  $[N_{5C66}][OTs], [N_{6C66}][OTs], [N_{5C55}][OTMBS], [N_{5C66}][OTMBS], and$ [N6C55][OTMBS]. Phase transition results from those organic salts labeled in red and blue (Figure 2) indicate entirely homogeneous (one-phase) solution and heterogeneous (twophase) mixture between 0 °C and 95 °C, respectively. Not surprisingly, more blues were observed in more hydrophobic [OTMBS]- than [OTs]-based ionic liquid library: 8 (Figure 2B) vs. 6 (Figure 2A), respectively. Since it has been disclosed in literature<sup>4-6</sup> that phase behavior of a TIL is a fine balance between hydrophobicity and hydrophilicity of the ionic liquid investigated, our results concurred that six TILs obtained were all identified to reside on the rim between being totally hydrophilic (red) and totally hydrophobic (blue) in Figure 2. In

LCST system, the increase of carbon numbers in structures increases the hydrophobicity of ILs that typically to dower  $T_c$  values. Our results for TILs with hydrophobic [OTMBS] anion correlate well with such principle: [N<sub>5C55</sub>][OTMBS] ( $T_c = 56 \circ$ C), [N<sub>6C55</sub>][OTMBS] ( $T_c = 37 \circ$ C), and [N<sub>5C66</sub>][OTMBS] ( $T_c = 1 \circ$ C), respectively (Figure 2B). For ILs with less hydrophobic [OTS] anion, ring size matters; that is, larger ring potentially inhibits its association with [OTs] anion and conversely promotes its solvation with small-molecule water, eventually resulting in higher  $T_c$  values: [N<sub>4C66</sub>][OTs] ( $T_c = 8 \circ$ C), [N<sub>5C66</sub>][OTs] ( $T_c = 15 \circ$ C), [N<sub>6C66</sub>][OTs] ( $T_c = 23 \circ$ C), respectively (Figure 2A). Most significantly, for all six TILs discovered, five carry attractive  $T_c$ values (0-37 °C) that should be well suited for biomolecules study (Figure 2).



**Figure 2.** Phase transitions of a library of 30 ionic liquids upon mixing with water (1:2, w/w) at temperatures between 0 °C and 95 °C: (A) [OTs]-based ionic liquids and (B) [OTMBS][-based ionic liquids. Phase transition results shown in red and blue indicate entirely homogeneous (one-phase) solution and heterogeneous (two-phase) mixture, respectively, between 0 °C and 95 °C. In the library, 6 ionic liquids show phase transitions: [N<sub>4C66</sub>][OTs] ( $T_c = 8$  °C), [N<sub>5C66</sub>][OTs] ( $T_c = 15$  °C), [N<sub>6C66</sub>][OTs] ( $T_c = 23$  °C), [N<sub>5C55</sub>][OTMBS] ( $T_c = 56$  °C), [N<sub>5C66</sub>][OTMBS] ( $T_c = 1$  °C), and [N<sub>6C55</sub>][OTMBS] ( $T_c = 37$  °C).

Figure 3 shows photos of phase behavior for a set of three representative ionic liquids,  $[N_{5C55}][OTs]$ ,  $[N_{5C66}][OTs]$ , and  $[N_{5C77}][OTs]$ . The result unambiguously demonstrated that the R sidechain engineering of  $[N_{5CRR}][OTs]$  from *n*-pentyl (Pent) group in  $[N_{5C55}][OTs]$  to *n*-heptyl (Hept) group in  $[N_{5C77}][OTs]$ 

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completely changed their phase behavior from being totally hydrophilic to completely hydrophobic. Most notably, the incorporation of *n*-hexyl (Hex) group completely changed phase behavior and made [ $N_{5C66}$ ][OTs] a TIL with LCST phase transition in water (Figure 3). This discovery of [ $N_{5C66}$ ][OTs] as a new TIL clearly highlighted the value of fine-tunability of ionic liquid structures.



**Figure 3.** Temperature-dependent phase transitions of binary mixtures (3:1, w/w) of water with  $[N_{5C55}][OTs]$ ,  $[N_{5C66}][OTs]$ , and  $[N_{5C77}][OTs]$ , respectively. The coomassie brilliant blue R-250 (0.006 wt% in water) was added to accentuate the phase separation. Only  $[N_{5C66}][OTs]$  ionic liquid shows phase transition ( $T_c = 17 \text{ °C}$ ), whereas  $[N_{5C55}][OTs]$  and  $[N_{5C77}][OTs]$  are totally miscible and immiscible, respectively, with water at temperatures between 0 °C and 95 °C.

In LCST systems, the phase transition temperatures depend on the mass fraction of ionic liquids in water. Figure 4 shows representative phase diagrams of a mixture of water with  $[N_{5C66}][OTs]$ . As expected, its phase diagrams are of a concave curve with the lowest critical temperature near its mass ratio of 1:4 and mole ratio of 1:99 ( $[N_{5C66}][OTs]/H_2O$ ), respectively.



Figure 4. Phase diagram of a mixture of  $[N_{5C66}][OTs]$  and water. Solid line is a guide for the eye.

For results shown in Figure 2, we went further to test whether mixtures of a hydrophilic ionic liquid and a neighboring hydrophobic ionic liquid might possibly change their overall phase behavior in water. As demonstrated in Figure 2B,  $[N_{4C55}][OTMBS]$  is totally miscible with waterale obut  $[N_{4C66}][OTMBS]$  is entirely water-immiscible total the peratures between 0 °C and 95 °C; that is, both are not TILs. We demonstrated here that a simple mixing of  $[N_{4C55}][OTMBS]$  with  $[N_{4C66}][OTMBS]$  in water was a convenient way to regulate total hydrophobicity toward phase transition and, using this example, an equal mass mixture in water readily formed a clear phase separation at 50 °C (Figure 5a). In addition, this mixture reversibly returned to a homogeneous solution when cooled to 4 °C, confirming the successful development of a three-component LCST system.



**Figure 5.** Temperature dependence of phase behavior of mixtures (3:1, w/w) of water with (a)  $[N_{4C55}][OTMBS]$ ,  $[N_{4C66}][OTMBS]$ , and a binary mixture (1:1, w/w) of  $[N_{4C55}][OTMBS]$  and  $[N_{4C66}][OTMBS]$  with  $T_c = 21$  °C (labeled in red), (b)  $[N_{4C56}][OTMBS]$  ( $T_c = 19$  °C) and  $[N_{4C47}][OTMBS]$  ( $T_c = 37$  °C), and (c)  $[N_{4C57}][OTS]$  ( $T_c = 22$  °C).

For results shown in Figure 2, we also reasoned that asymmetric  $[N_{4CR}^{1}R^{2}][OTMBS]$  and  $[N_{4CR}^{1}R^{2}][OTs]$ , of which one R<sup>1</sup> is from a red-labeled alkyl group and the other R<sup>2</sup> is of a bluelabeled alkyl group, once synthesized the phase transition might be experimentally observed. Gratifyingly, we were pleased that both [N<sub>4C56</sub>][OTMBS] and [N<sub>4C47</sub>][OTMBS] prepared indeed showed LCST phase separation with biologically attractive  $T_c$ values: 19 °C and 37 °C, respectively (Figure 5b). This result clearly demonstrated that, albeit none of [N<sub>4CRR</sub>][OTMBS] showed successful phase transition (Figure 2B), rational incorporation of red- and blue-labeled alkyl substituents in [N<sub>4CR<sup>1</sup>R<sup>2</sup></sub>][OTMBS] fruitfully produced TILs (Figure 5b). Most interestingly, as shown in Figure 2A, one TIL (i.e., [N<sub>4C66</sub>][OTs]) was resulted and, using the aforementioned rational approach, an additional asymmetric [N4C57][OTs] could be discovered as a new TIL ( $T_c = 22 \text{ °C}$ , Figure 5c). The synthesis of [N<sub>4C56</sub>][OTMBS],  $[N_{4C47}][OTMBS]$ , and  $[N_{4C57}][OTs]$  was summarized in Scheme S2 (ESI), and detailed experimental procedures, NMR and HRMS spectra and data are provided in the Supporting Information (ESI). Using thermogravimetric analysis (TGA), Figure S1 shows that all six TILs identified from library screening exhibiting thermoresponsiveness have similar high temperature stability

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close to 300 °C, which are far above the temperatures used in  $T_c$  measurements (ESI).

As we successfully developed cycloammonium sulfonates as TILs carrying LCST phase behavior, we turned our attention to undertake a preliminary study to see if these TILs are compatible with proteins with the hope that, upon temperature-triggered phase separation, proteins may preferentially partition in ionic liquid layer, which would ultimately make biomolecular interaction analysis possible. Isolation, separation and purification of proteins are usually performed using various forms of chromatographic, electrophoretic, ultrafiltration, precipitation and dialysis procedures, and used in almost all branches of biosciences and biotechnologies.<sup>10</sup> New methods capable of treating dilute solutions or solutions containing only minute amounts of target biomolecules should be of indispensable need in studies of precious proteins. In this work, TILs were considered and used as an immediate application for enriching dilute protein solutions. Here,  $[N_{5C66}][OTs]$  was selected as a model TIL, primarily because it undergoes a LCST phase transition below room temperature with water. We chose cytochrome c from equine heart and green fluorescent protein (GFP) of the jellyfish Aequorea victoria for their ease of visualization by naked eyes and with light box, respectively, to test to see if their dilute concentration enrichments could be realized by TILs. Pleasingly, as shown in Figure 6, both proteins were readily concentrated by adding  $[N_{\rm 5C66}][OTs]$  into and mixing with dilute protein solutions. In our hand, cytochrome c and GFP were enriched 39and 11-fold, respectively. From spectra shown in Figure 6, upper aqueous phases (blue spectra) contained essentially free of proteins; that is, most, if not all, of proteins were preferentially partitioned and concentrated in ionic liquid-rich bottom phases.



**Figure 6.** Photos and spectra of temperature-dependent phase transition were exploited for demonstrating protein enrichment from its aqueous mixtures (1:15, w/w) of  $[N_{5C66}][OTs]$  with (a) a solution of equine heart cytochrome c (4 mg/mL, H<sub>2</sub>O) and (b) a solution of jellyfish *Aequorea victoria* green fluorescent protein (GFP, 16 µg/75 µL, 3 mM tris buffer). UV-vis spectrometry and fluorometry were used for quantitatively determining degree of enrichment in cytochrome c (39-fold increase, Soret band at 406 nm) and GFP (11-fold increase, emission band at 511 nm), respectively.

It is also of worth highlighting in this preliminary study, that the  $[N_{5C66}][OTs]$  appeared to be capable of stabilizing protein, that is, after concentration, the resulting TIL-GFP solution stayed bright green for over one week at room temperature.

In summary, we reported in this work the combinatorial discovery of new cycloammonium TILs that unambiguously illustrated and highlighted the real value of fine structural tunability of ionic liquids. All TILs discovered were identified to situate on the rim of totally hydrophilic and hydrophobic ionic liquids (Figure 2). Albeit no single TIL was found from the [N<sub>4CBB</sub>][OTMBS] sub-library, rational incorporation appropriate but asymmetric alkyl groups on pyrrolidine made rational identification of new TILs possible (Figure 5c). All TILs are eminently capable of performing LCST phase transitions with low  $T_c$  values in water. Combinatorial chemistry allows the synthesis of compounds simultaneously and has been an important tool in many areas of research, including ionic liquid synthesis.<sup>11,12</sup> To our knowledge, this work is the first combinatorial approach of small-molecule cycloammonium ionic liquids that exhibit LCST property with water. The results presented in this work hold compelling possibilities of the use of TILs as functional materials for advancing biomolecule separation and potential interaction analysis.

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### **Conflicts of interest**

There are no conflicts to declare.

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