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## An integrated high-throughput strategy enables the discovery of multifunctional ionic liquids for sustainable chemical processes<sup>†</sup>

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Development of new chemical processes with simplified reaction systems and work-up procedures is a challenging task. Although ionic liquids are a class of potential multifunctional compounds to simplify traditional chemical processes, their rational design is difficult due to complex interactions. In this work, a proof-of-concept strategy has been proposed to achieve an integration of high-throughput preparation of ionic liquids and *in situ* screening of their reaction-promoting performance in 96-well plates. The integrated approach then enables a facile identification of optimal ionic liquids from a 400-ionic liquid candidate pool to act as the solvent, the catalyst and the separating assistant, simultaneously, for carbonylazide cycloaddition reactions. Merits of the ionic liquids-based processes have been demonstrated not only in the convenient and efficient synthesis of 1,2,3-triazolyl compounds but also in the discovery of a new reaction for the chemical post-modification of free peptides.

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### Introduction

Typical chemical reactions usually require the use of solvents, catalysts and additives, such as acids, bases, catalyst ligands *etc.* The synergetic effect of these components are crucial for efficient chemical transformations. However, time and labor consuming procedures have to be used to optimize different reaction variables and to search for the optimal separation strategy for target compounds. At the same time, the chemical reaction, product separation and catalyst recovery are often considered as separate unit operations, which significantly add to the economic cost and the environmental impact of the process. Therefore, development of new chemical processes with simplified reaction systems and work-up procedures has been a long-term challenging task in academic researches and industrial applications.<sup>1–3</sup>

Ionic liquids (ILs) are composed of cations and anions and have been successfully applied in various chemical processes

as clean solvents,<sup>4</sup> organocatalysts,<sup>5</sup> catalyst ligands<sup>6</sup> and separation assistants.<sup>7,8</sup> Intriguingly, the easy combination of cations and anions leads to a number of ionic liquids up to 10<sup>18</sup>.<sup>9</sup> This huge number and their functional diversity make it possible to discover multifunctional ionic liquids that can replace traditional solvents, catalysts, additives and even separating materials, simultaneously. In this way, new, simple and efficient chemical processes can be developed only through the elaborate combination of the cation and the anion. However, there exist abundant interactions in ILs, such as electrostatic, hydrogen bond, van der Waals,  $\pi$ - $\pi$  stacking and hydrophobic-hydrophilic interactions.9 These complex interactions imply that it is very difficult to anticipate the relationship between the structure and properties of ionic liquids, thus, the rational design of multifunctional ILs for a specific chemical process is a great challenge.

High-throughput strategies have been recently used as an efficient tool to optimize the reaction conditions of known reactions and even to discover new chemical reactions.<sup>10–15</sup> The advances in the simplification of screening methods<sup>11</sup> and the miniaturization of screening substrates<sup>12</sup> greatly promote their practical applications in ordinary chemical laboratories. These strategies may offer a novel pathway to manipulate the multifunctional effects of ILs in the utilization of new chemical processes if the screening method is correct. In this work, we designed a fluorescence enhancing probe reaction for the high-throughput screening of ILs. With the assistance of this new screening method, it is possible to

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#### Paper

visualize the promoting performance of ILs for the reaction, identify the optimal ionic liquid candidates from a readily accessible plate reader and realize the integration of all highthroughput experiments on a 96-well plate.

#### **Results and discussion**

# One-plate integration of high-throughput preparation and screening of ILs

The intrinsic combinational properties of ILs imply the feasibility of generating  $m \times n$  ILs by mixing a set of n cation precursors and a set of m anion precursors (Fig. 1A). Neutralization reaction of amines and acids is a desirable means for the parallel synthesis of protic ILs.<sup>16</sup> Aprotic ILs, like choline-based liquid salts, can also be synthesized directly from the equivalent mixing of choline hydroxide and acids. According to the parallel orthotropic rules, 400 ILs have been prepared rapidly from 20 bases and 20 acids (Fig. 1B) possessing variant geometric shapes, acidities, sizes, and electronic and steric effects. In a typical procedure, equal stoichiometric amounts of acids and bases were added orderly into 400 mL × 1.5 mL Eppendorf tubes containing methanol as the solvent in ice bath; then, the 96-well plates bearing reaction mixtures were shaken in a water bath for 48 h. The solvents were evaporated under reduced pressure after the completion of the reactions. Considering the possible volatilization of acids and bases used for the building of the ILs pool, the samples were dried under reduced pressure at 50 °C for 12 h for ILs with non-volatile components or at 25 °C for 24 h for ILs with volatile components (Table S1 in the ESI†). The 1 : 1 stoichiometry of cations to anions in dried ILs was checked from <sup>1</sup>H NMR (the copies of <sup>1</sup>H NMR spectra are given in pages 36–90 in the ESI†). In addition, the water contents in ILs after drying were measured to be less than 0.5% (Table S5 in the ESI†). This level of water content did not affect the discovery of catalysts for carbonyl-azide cycloaddition reactions by high throughput screening (Scheme S2 in the ESI†).

At present, although several multi-channel methods have been reported for the determination of chemical and physical properties of ILs,<sup>16–19</sup> high-throughput screening for the reaction-promoting performance of ILs is inherently difficult since its assessment requires the unambiguous detection of target products from a mixture of reactants, products and ILs. Mass spectrometry is usually used as a powerful tool for highthroughput screening of catalysts; however, the existence of lots of cations and anions in ILs interferes with the ionization of products. Therefore, at this stage, development of a practical



**Fig. 1** (A) Combinational preparation of the ILs library in 96-well plates. (B) The anions and cations used in the building of the ILs pool. (C) Fluorescence-enhancing probe reaction used in the present work. (D) Fluorescence intensities of compounds **2a** and **3a** under fluorescence excitation (Ex = 340 nm). (E) Conversion extents expressed by fluorescence yields (calculated from the standard curve) and isolated yields.

high-throughput detection strategy is vital for the successful application of high-throughput tools in the screening of reaction-promoting performance of multifunctional ILs. Fluorescence-enhancing probes have the advantages of quantitative and in situ detection of targets from complex surrounding environments,<sup>20</sup> which suggest that the possibility of direct detection of products in ILs involved systems by fluorescenceenhancing probe reactions. Following this clue, 5-N<sub>3</sub>-1,8naphthalimide 2a was found to have weak fluorescence by itself, but its potential cycloaddition product, 5-triazolyl-1,8naphthalimide exhibited strong fluorescence due to the chromophore effect of 1,2,3-triazole moieties in this kind of compounds. By using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the catalyst, we found for the first time that the donor of organic azide 2a could react with diacetone 1a to produce 3a, and the fluorescence intensity of 3a was about 300 times higher than that of 2a at its maximum excitation wavelength (Fig. 1C & D). This finding means that it is appropriate to use the reaction between 1a and 2a as a probe for the in situ detection of their [3 + 2] cycloaddition products only through the measurements of fluorescence intensity, without the need of any isolation and purification procedures.

Before the utilization of high throughput strategy, we evaluated the accuracy of the probe reaction by a panel of basic ILs bearing guanidinium cations (IL1-IL10 in Table S1 in ESI<sup>†</sup>). Guanidinium ILs were chosen here because they exhibited some catalytic performance in enamine/enolate mediated reactions.<sup>21</sup> In the blank control, a mixture of diacetone 1a and compound 2a without an IL was incubated at 50 °C for 2 hours. No fluorescence enhancement was detected, which was in agreement with the result (no produce of 3a) indicated by TLC. Then, ten ILs were used to test the model. Towards this end, 10 mg of azide 2a, 1.0 equivalent of diacetone 1a and 0.5 mL of an ionic liquid were mixed and incubated at 50 °C for 2 h. Subsequently, the diluted samples of reaction mixtures were analyzed by a plate reader. The products in reaction mixtures could also be isolated and purified by a silica gel column (Table S1 in ESI<sup>†</sup>). As shown in Fig. 1E, most of the conversion extents in this assay showed a high correlation to those from fluorescence yields, with an error in the range of  $\pm 7\%$  (Fig. S2 in the ESI<sup>†</sup>).

# Facile discovery of ILs to promote carbonyl-azide [3 + 2] cycloaddition reactions

The one-plate integrated high throughput strategy was next applied in the discovery of multifunctional catalytic ILs from 400-IL candidates to promote the reactions between azides and carbonyls. The [3 + 2] cycloaddition reaction of azides with carbonyls is known to be effective for the synthesis of 1,2,3-triazoles under metal-free conditions. Since the pioneering work in 1980s, a number of organic and/or inorganic bases, such as triethylamine,<sup>22</sup> NaOEt,<sup>23</sup> K<sub>2</sub>CO<sub>3</sub>,<sup>24</sup> Et<sub>2</sub>NH,<sup>25</sup> 1-butyl-3-methylimidazolium hydroxide ([Bmim]OH)<sup>26</sup> and DBU<sup>27,28</sup> have been actively investigated in the last 30 years. However, all the catalytic systems for carbonyl-azide cycloaddition reactions were found *via* trial-and-error experiments, which limited the current catalysts to organic bases and/or alkoxides. In addition, the harsh basic condition and the high reaction temperature make the carbonyl-azide [3 + 2] cycloaddition less competitive in applications compared to other click reactions.<sup>29,30</sup> Thus, we desired to find mild and effective ILs to promote a metal-free carbonyl-azide click reaction and demonstrate the use of our integrated high-throughput method to accelerate this discovery process.

The experiments were performed by the following procedures. The probe reactants (400 nmol 2a in 400 µL of diacetone) were directly loaded into each of the 400 Eppendorf tubes containing freshly prepared ILs (20 µmol), and the reaction was then incubated in a shaking bath for 2 h at 50 °C. The resultant reaction mixtures were *in situ* detected by a plate reader, and all the data for 400 samples could be collected within 1.5 h. False positive results due to the existence of possible insoluble ILs and the reflection of UV light by them were removed by repeating the reactions. All the samples were tested and recorded in triplicate, and the result was an average of three determinations (Fig. 2A and Fig.S4-S23 in the ESI<sup>†</sup>). Distribution analysis of the acquired data is given in Fig. 2B. It was found that there were 55 kinds of ILs that could promote the reaction with the yields over 50%, which covered 5 cations and 15 anions. Interestingly, cations paired with different anions gave the optimal ILs for metal-free click reaction between 1a and 2a. For example, IL A1-B16 with the combination of guanidinium and 2-methylpentanoate could give the product with an 87% yield, IL A13-B2 consisting of 1,8-diazabicyclo[5.4.0]-7-undecenium and the acetate anion afforded a 95% yield, while IL A6-B6 composed of cholinium and 2,2,2trichloroacetate gave a 91% yield. These results indicate that the effect of an IL on a given reaction came from the synergistic actions of cations and anions as a whole, but not from the simple sum of the effects of the separated cation and anion. The huge number of ILs in this strategy was therefore preferable because this could avoid missing some useful combinations owing to subjective bias. Indeed, in the three optimal ILs, B16 and B6 anions were not frequently used in the existing task-specific ILs. In this context, the high-throughput method for the discovery of multifunctional ILs would be greatly valuable in the cases where the reaction mechanisms are not clear, and the relationship between the structure and activity of ILs is hardly predicted in advance.

After the optimal ILs were obtained for the effective promotion of probe reaction, we turned our attention to study the generality of ILs for a wider scope of substrates (Fig. 3). Taking 1-aryl-4-carbonyl-1,2,3-triazole scaffold as an example, we exhibited a series of merits of ILs-promoted chemical processes in Fig. 4. 1-Aryl-4-carbonyl-1,2,3-triazole moieties highlighted in Fig. 4A represent a useful scaffold with a wide scope of biological activities.<sup>31,32</sup> Using IL A6–B6 as an all-in-one catalyst, structurally diverse modifications on three parts of the scaffold, including aryl, carbonyl and triazolyl moieties could be accomplished smoothly from readily accessible starting materials. Much higher reaction efficiencies were achieved in the syntheses of 4-carbonyl-1,2,3-triazoles from azides-



Fig. 2 (A) The identified ILs for the efficient promotion of [3 + 2] cycloaddition reactions between azides and carbonyls. (B) Analysis of the distribution of cations and anions for the fluorescence-enhancing reaction with yields >50%. (C) Three optimal ILs for the efficient promotion of carbonyl-azide [3 + 2] cycloaddition reactions.



Fig. 3 (A) Azides used as the reactant in this work. (B) Dicarbonyls used as the reactant in this work.

bearing MeO- groups (**3i**, **3j**, **3k** in Fig. 4). We compared A6-B6 and ethylenediamine/DMSO systems<sup>25</sup> in three reactions of *o*-MeO-aryl, *m*-MeO-aryl and *p*-MeO-aryl with acetoacetic ester to prepare compounds **3i**, **3j** and **3k** (Fig. 4B). More than 93%

yields of target triazoles could be obtained in the A6-B6 system within 3 hours, while less than 32% yields were given in the ethylenediamine/DMSO system within the same reaction time. More intriguingly, the products of the ILs-promoted chemical processes could be separated automatically from the IL phase after the reaction. Through the extraction of cloudy reaction mixtures with ethyl acetate, clear ILs could be recovered (Fig. 4C). After the simple separation process, the recovered ILs were ready to be reused for the next run. All the recovered ILs, including A6-B6, A6-B10 and A13-B2 could be reused at least five times without additional activation (Fig. S24 in the ESI<sup>†</sup>). No decrease in catalytic efficiency was found during repeated cycles, and the IR spectra of the recovered ILs also indicated no significant differences from the fresh ILs (Fig. S25 in the ESI<sup>+</sup>). Therefore, this homogeneous reaction followed by the heterogeneous separation process had significant superiority in efficiency and energetic economy.

On the basis of our experimental results and previous reports in literature,<sup>5,25</sup> a possible reaction mechanism is proposed in Scheme 1. First, the IL would activate the alpha-H and the carbonyl of the acetoacetic ester through the formation of H-bonds (intermediate 4), which can simultaneously increase the nucleophilicity of the alpha-carbon and the electrophilicity of the carbonyl-carbon. The activated alpha-carbon then attacks the gamma-nitrogen of azide, while the alpha-nitrogen of azide attacks the carbonyl-carbon to complete a dipolar cycloaddition for the formation of intermediate **6**.



Fig. 4 (A) 1-Aryl-4-carbonyl-1,2,3-triazole parent core in bioactive molecules. (B) Structurally diverse 1-aryl-4-carbonyl-1,2,3-triazole compounds prepared by the ILs-promoted click reaction. (C) Simple recovery and the reuse of ILs and the purification of products.



Scheme 1 Plausible reaction mechanism (BH<sup>+</sup> is the cation of ILs).

Furthermore, another activation by the formation of H-bonds between the cations and the hydroxyl of intermediate **6** can promote the subsequent dehydration reaction. Finally, the 1,2,3-triazole product is given. The existence of H-bond activation of the ILs on the dicarbonyls was suggested by the analysis of <sup>1</sup>H NMR spectra of the mixture of ILs (A13–B2 and A1–B16) and the acetoacetic ester, where the unambiguous broad peaks for the alpha-carbon of dicarbonyl could be observed (Fig. S26 in the ESI†).

#### Application in the chemical modification of free peptides

Chemical modification of free peptides is of great importance in the synthesis of antibody drugs and bio-materials.<sup>33,34</sup> However, it is usually difficult to modify free peptides due to their structural complexity, unique solubility and binding ability to some metal ions. Thus, high selectivity, insensitivity to water and mild reaction conditions are required for candidate reactions. It is known that the reactions that can proceed smoothly under neutral or closed to neutral conditions without the addition of metal catalysts and ligands are highly desirable for the direct modification of biomolecules.<sup>30</sup> In this regard, we determined the pH values of the aqueous solutions of the optimal ILs (1 M) obtained by the high throughput screening. The ILs, A6–B6, A6–B10 and A13–B2 (Fig. 2B) showed the pH values of 7.2, 7.3 and 7.8 in aqueous solutions, respectively. In addition, the unique solvent structures of ILs may stabilize the biomacromolecules.<sup>35</sup> We were thus inspired to apply our all-in-one ILs for the chemical post-modification of free peptides. From the screening, the peptide-I was found to effectively react with the  $\beta$ -carbonyl amide of the IL A13–B2 at 50 °C, producing modified peptide-II in a 76% yield (Scheme 2). Furthermore, this IL-promoted metal-free click



Scheme 2 Application of ILs-promoted chemical processes in the modification of free peptides.

reaction demonstrated good tolerance to water and air. In the system of IL-water (5:1 mol mol<sup>-1</sup>), the cycloaddition of peptide-I and the  $\beta$ -carbonyl amide kept the same level of isolated yield (75%) after 24 hours. However, a higher content of water (*e.g.* IL-water (1:1 mol mol<sup>-1</sup>)) would result in a dramatic decrease of the reaction yield. The discovery that IL A13–B2 could promote the cycloaddition of free peptides might be intriguing to develop new bioconjugation reactions on complex biomolecules.

## Conclusion

In this work, we report an integrated high-throughput strategy to discover multifunctional ILs toward the development of new, simple and effective chemical processes. By using this integrated high-throughput strategy, a facile discovery of multifunctional catalytic ILs for cycloaddition reactions between dicarbonyls and azides (a kind of metal-free click reaction) could be achieved directly from a 400-IL pool containing simple and inexpensive cations and anions. Then, the ionic liquids-based processes have been demonstrated not only in the more convenient and efficient synthesis of small molecular 1,2,3triazolyl compounds but also in the discovery of a new method for the post-modification of free peptides. In all these processes, ILs played the roles of the solvent, the catalyst and the additives; thus, these systems are particularly simple, and only the reactants and an IL are required for each system without any other chemicals. The pool of potential ILs is huge and contains plenty of structurally and functionally diverse candidates. Therefore, probe reactions can be designed based on different chemical transformations, and the equipment involved in this strategy are commonly available in a chemistry laboratory. Together with the other intrinsic advantages of ILs, the high-throughput methods developed in this work would provide a general platform to discover various new chemical processes associated with simplified reaction systems and work-up procedures.

## Conflicts of interest

There are no conflicts to declare.

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