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A versatile reagent to synthesize diverse ionic liquids ranging from small molecules and dendrimers to functionalized proteins†

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An ionic liquid "reagent" bearing a succinimidyl activated ester is reported that can be used to synthesize a variety of small molecule and macromolecular ionic liquids. In addition, the ionic liquid reagent was used to modify lysozyme, and the protein retained its structure and function after modification. This study describes a facile and reliable route to new ionic liquid compositions.

Ionic liquids are low melting salts held together through a pairing of noncovalent electrostatic interactions.[1–6] Phosphonium based ionic liquids such as tetradecyl(tributyl)phosphonium chloride, **1**, are of wide-spread interest for applications given their favorable properties, such as low vapor pressure and high thermal, mechanical, and electrochemical stability (Fig. 1).[7–9] These ionic liquids are synthesized by nucleophilic addition of a phosphine to a haloalkane (eqn (1)) and a number of tetraalkylphosphonium chloride ionic liquids have been prepared;[7] this procedure has also been used to synthesize dicationic bis-phosphonium ionic liquids.[10,11] To increase the diversity of ionic liquid structures known and to facilitate the synthesis of more complex materials (including biohybrids), additional synthetic transformations are needed that are well-defined, high yielding, thermodynamically favored, and user-friendly. Such types of coupling reactions have been recently classified as click reactions.[12] Of these various chemical transformations, amide bond formation *via* an active succinimidyl ester is a highly reliable, robust, and selective reaction that can be run in aqueous or nonaqueous solutions. Herein, we report the preparation of a phosphonium chloride ionic liquid (IL) bearing a succinimidyl activated ester (**2**, IL-OSu), and the use of this activated ester reagent to synthesize a series of new ionic liquids (eqn (2)).

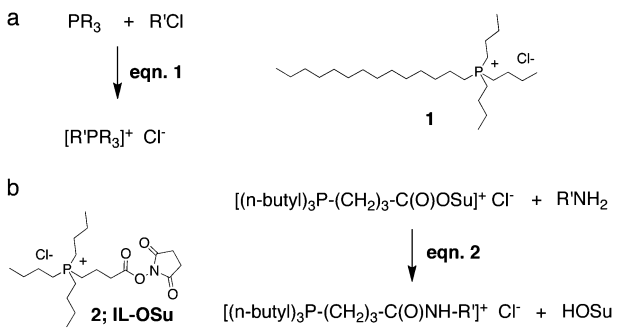

Fig. 1 (a) The conventional preparation route (eqn (1)) and chemical structure of tetradecyl(tributyl)-phosphonium chloride, **1**. (b) Chemical structure of butyric acid (tributyl)-phosphonium chloride bearing a *N*-hydroxysuccinimide activated ester (**2**, IL-OSu) and the activated ester preparation route to new ionic liquids.

Amide bond formation using *N*-hydroxysuccinimide ester chemistry was first reported in 1963 by Callahan *et al.* for peptide synthesis.[13,14] Since then, this active ester has been used extensively because it reacts with a primary amine to afford a stable amide bond in good yield (typically 60–99%).[15] This activated ester is relatively stable in weak basic solutions and the reaction rates depend on the basicity of the amine as well as sterics, with primary amines reacting faster than secondary amines. Moreover, NHS activated esters are stable enough to be purified and stored for future reactions facilitating the wide-spread use of succinimidyl activated ester "reagents" in many fields of chemistry and biochemistry. The success of this reaction is exemplified through its application in biomacromolecule modification and the impact this reaction has had on the bioconjugate field.[16] In fact, NHS activated ester derivatives of molecules such as chromophores, biotin, *etc.* can be readily and cost-effectively produced, and, today, are commercially available.

To demonstrate the generality of this synthetic approach for creating new ionic liquids, an ionic liquid bearing a succinimidyl activated ester (**2**, IL-OSu) was synthesized and reacted with a series of electronically and structurally different nucleophiles, including alkyl, benzyl, and aromatic amines, alkyl thiol, an amine terminated [G3]-PAMAM dendrimer, and lysozyme. As such, we were able to evaluate the effect of amine pK_a and amine *vs.* thiol reactivity, as well as extend the reaction to the modification of a protein. In addition, these new ionic liquids possessing ester, amide, or thioester linkages are likely to have different biodegradation profiles compared to the more common aliphatic ionic liquids. This is another area of active research and interest.[17,18] IL-OSu was prepared by

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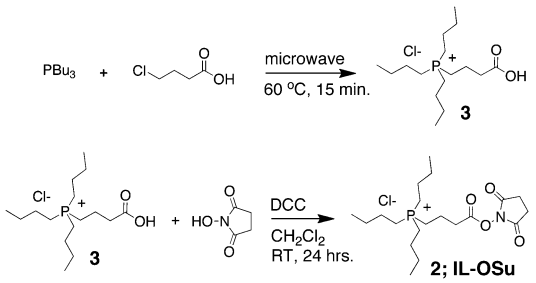


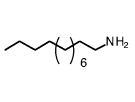
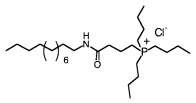
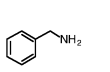
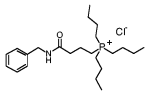
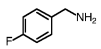
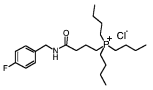
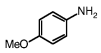
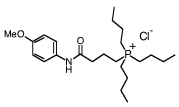
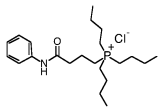
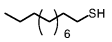
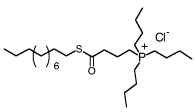
Scheme 1 Synthesis of a butyric acid (tributyl)phosphonium chloride bearing a succinimidyl activated ester (**2**, IL-OSu).

first reacting tributyl phosphine with chlorobutyric acid neat under microwave conditions at 60 °C for 15 minutes to obtain compound **3** (Scheme 1). The reaction was quantitative. As the phosphine requires atmospheric protection, the reactants were loaded into a microwave reactor chamber in a glove box. In comparison, a 40% yield was obtained after two weeks in refluxing toluene. The use of microwaves to accelerate and increase yields has been reported for imidazolium based ionic liquids.[19] Next, *N*-hydroxysuccinimide was reacted with **3** in the presence of DCC to afford **2** in 87% yield.

A series of different ionic liquids was synthesized, as shown in Table 1, by reacting **2** with the corresponding nucleophile. The general coupling procedure involved dissolving compound **2** (1.2 eq.) in dry dichloromethane followed by the addition of triethylamine (1.2 eq.) and the appropriate amine or thiol nucleophile (1 eq.) under nitrogen. The mixture was stirred for 12 hours at room temperature, concentrated, dried, and analyzed by 1H NMR spectrometry. The primary alkyl and benzyl amines reacted with **2** in good to high yield (> 80%). As expected, a lowering of the amine pK_a, either through *para* substitution of benzyl amine with fluorine or through the use of aniline instead of benzyl amine, reduced the coupling yield. This method was then used to synthesize an ionic liquid dendrimer by reacting **2** with a [G3]-PAMAM containing 32 terminal amines to afford the ionic liquid dendrimer. IL-dendritic structures are of interest and, recently, Percec *et al*. have reported the self-assembly of dendrons containing an ionic-liquid functionality at their apex.[20] In addition, the reaction was not limited to amines, for an alkyl thiol reacted readily with **2** in high yield to afford the thioester linkage and the corresponding ionic liquid (Table 1).

To extend this chemistry to ionic liquid functionalized proteins, we chose to study lysozyme as a prototypical enzyme. There is significant interest in using ionic liquids as a solvent or aqueous co-solvent for biocatalytic reactions with enzymes.[4,21–24] In general, dissolving enzymes in such solvents can lead to increased stability, prevent aggregation, and promote refolding, and such results have been reported for lysozyme in the presence of ammonium and *N*-methylimidazolium based ionic liquids.[25–29] With regards to ionic liquid modified enzymes, Blum and Doumèche *et al*. have reported recently the covalent labeling of formate dehydrogenase with multiple methylimidazolium cations.[30] This ionic liquid grafting procedure led to an increased half-life and shelf-life in aqueous solution as well as activity in a 70% ionic liquid/aqueous solution where the native enzyme exhibited essentially no activity.

Table 1 Screening of the substrates for coupling with **2**

Nucleophile	Product	%Yield[a]	T^c/°C
		80	100[d]
		99	−30[e]
		80	−35[e]
		79	−16[e]
		54	−44[e]
		95	18[f]
[G3]-PAMAM-$(NH_2)_{32}$	[G3]-PAMAM-$(NHC(O)\text{-IL})_{32}$	82	50[f]
Lysozyme[b]	Lysozyme$(IL)_7$	Quant.	

All reactions were performed at 25 °C for 12 hours in dichloromethane with 1.2 equivalents of **2** except for the mercapto derivative (3 eq.). Amines are listed in decreasing order of basicity.[a] Yields determined by ^{1}H NMR. [b] Reaction performed in carbonate buffer at pH = 8.0 at 25 °C for 24 hours. [c] DSC measurements. Melting temperature for entries 1, 6, and 7. [d] The physical state of the materials at room temperature is solid. [e] The physical state of the materials at room temperature is viscous liquid. [f] The physical state of the materials at room temperature is waxy.

Lysozyme, a glycosidase, is a relatively small compact enzyme composed of 129 amino acids. Lysozyme has six lysines and a terminal amine available for reaction with the IL-OSu, **2**. Specifically, lysozyme was dissolved in a carbonate buffer (pH = 8) and **2** (3 eq. per amine) was added. Following the addition, the mixture was stirred for another six hours at room temperature. MALDI mass spectrometry analysis of the product using a CHCA matrix was then performed, and as shown in Fig. 2, the peak for the native lysozyme shifted to higher mass with two peaks corresponding to the lysozyme modified with six or seven phosphonium chlorides, respectively. The reaction was subsequently repeated, but this time **2** (3 eq.) was added to the lysozyme solution every two hours over a six-hour period. MALDI analysis of the subsequent product confirmed formation of only the completely modified product possessing seven phosphonium chloride ionic liquid moieties (lysozyme(IL)7; Fig. 2). The circular dichroism (CD) spectrum of the modified lysozyme between 200 and 240 nm matched previously published data suggesting that no gross changes in secondary structure occurred upon modification.[31]

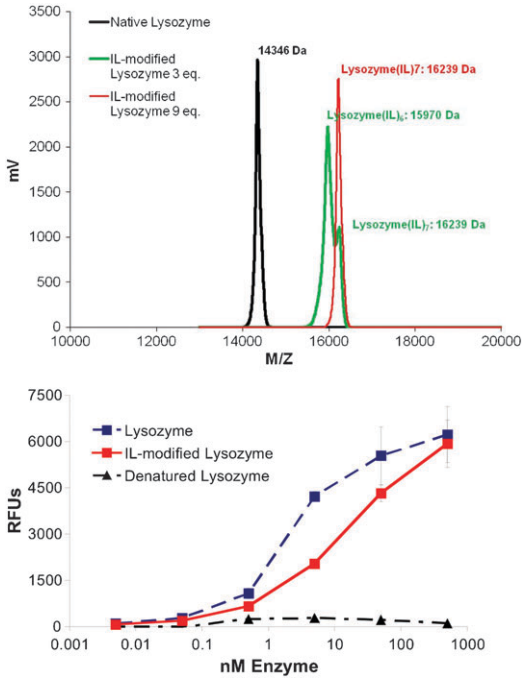


Fig. 2 (top) MALDI traces of lysozyme and IL-modified lysozyme. (bottom) Enzymatic activity of lysozyme, IL-modified lysozyme, and denatured lysozyme. $N = 3$; avg $\pm$ SD.

Finally, we evaluated the enzymatic activity of the lysozyme(IL)7 using a well-characterized fluorescence-based assay that involves *Micrococcus lysodeikticus* cell walls which have been extensively modified with fluorescein such that the fluorescence is quenched. Subsequent lysozyme cleavage of the β-(1–4)-glucosidic linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine of the cell wall affords an increase in fluorescence and thus a measure of enzymatic activity. As shown in Fig. 2, an increase in fluorescence as a function of enzyme concentration was observed for both lysozyme and IL modified lysozyme but not for denatured lysozyme, demonstrating that such ionic liquid modifications of protein structure can retain enzymatic activity. Though the IL modified lysozyme did show a decrease in activity, it was still quite active despite this high degree of protein modification.

In summary, an IL reagent—an ionic liquid succinimidyl activated ester—was synthesized in two steps from commercially available materials in high yield. Using this reagent, several ionic liquids were prepared in good to high yields. The generality of this approach will facilitate the preparation of new ionic liquid materials from small molecules to macromolecules. The ionic liquids reported herein highlight this point as the small molecule compounds possess aromatic groups or amide linkages that are capable of participating in hydrogen bonding or aromatic substitution reactions. The protein study showed that an ionic liquid modified lysozyme retained its structure and enzymatic activity, and thus encourages the study of new biohybrid compositions. Given the diversity of applications being explored with current ionic liquids, additional synthetic transformations that can be used by everyone, such as the one described, are needed to meet the ever-growing interest, curiosity, and demand for these unique materials and their properties.

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