JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Reactions of Sulfur-Containing Organic Compounds and Peptides in 1-Ethyl-3-methyl-imidazolium Acetate

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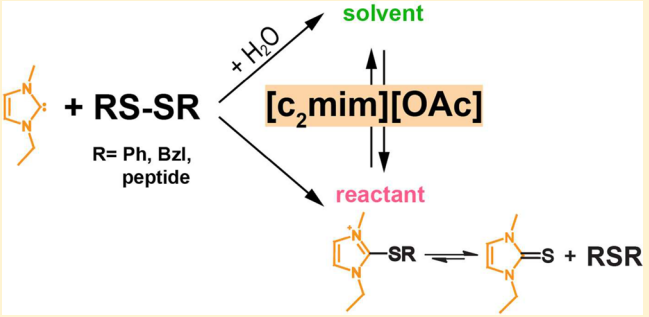
Supporting Information

ABSTRACT: The neat ionic liquid (IL) [C_2mim][OAc] is not just capable of dissolving thiol- and disulfide-containing compounds, but is able to chemically react with them without addition of any catalytic reagent. Through the analysis of four small organic molecules and a cysteine-containing peptide we could postulate a general reaction mechanism. Here, the imidazolium-carbenes preferentially react with the disulfide bond, but not thiol group. Moreover, the imidazole moiety was found to abstract the sulfur atom from the cysteine residue, providing an alternative way to transform Cys residues, which were artificially inserted into a peptide sequence in order to perform native chemical ligation (NCL) of two peptide fragments. Finally, the chemical reaction of [C_2mim][OAc] with a cysteine-containing biomolecules can be tuned or even suppressed through the addition of at least 30% of water to the reaction mixture.



INTRODUCTION

Ionic liquids (ILs), consist entirely of ionic species and offer a vast variety of physical and chemical properties primarily by the possibility of tailoring their structure, i.e., exchanging or modifying the cation or anion.[1−3] During the last decades, ILs attracted great attention as organic solvents, in reactions, such as cycloaddition, esterification, Wittig, and nucleophilic substitution reactions,[4−8] for the dissolution of cellulose,[9] for electrolytes, for lithium-ion batteries,[10] and other industrial applications.[11] On the other hand, they participate as reactants in various reaction processes, such as organic catalysis and oxidative addition.[12,13] Both the kinetics and thermodynamics of a reaction are dependent on the type of ionic liquid chosen, and even the reaction mechanism can be influenced. However, very little prescience currently exists on this topic.

In our previous studies we successfully applied ILs as solvents for reactions of bioactive peptides containing a high number of cysteine moieties in their sequence (>4), e.g., in oxidative folding and native chemical ligation.[14−16] The advantage in the use of IL was the good (up to 15 mM) solubility of hydrophobic peptides and selective formation of thermodynamically stable conformation (so-called "native fold") of peptides during oxidative folding. Further reports describe the advantages of using ILs for reactions involving biological macromolecules, i.e., enzyme catalysis, because of their structure-stabilizing capabilities or good solubilizing properties for hydrophobic peptides.[14,17,18] However, the possibility of chemical modifications through a proposed carbene-based chemical reaction are not fully understood yet and greatly limits their advantageous applicability for reactions involving biomolecules. This information is tremendously important for the knowledge-based application of ILs in a wide range of reactions involving biomolecules.

The *in situ* formation of N-heterocyclic carbenes (NHC)[19−22] in imidazolium-based ILs (e.g., 1-ethyl-3-methyl-imidazolium acetate) has long been suspected. NHCs in [C_2mim][OAc] were detected in the gas phase by infrared spectroscopy[23] and mass spectrometry.[24] A few reports suggest the presence of NHC in the neat IL.[23−26] However, so far, no direct spectroscopic observations of carbenes in ionic liquids were reported.[27]

Several chemical reactions were used as an indirect indication of formation or presence of carbenes in [C_2mim][OAc]. In 2011, Gurau et al. detected an acetic acid−acetate complex formed by chemisorption of carbon dioxide in [C_2mim][OAc] with the help of X-ray crystallography.[28] Rodriguez et al. was able to detect 1-ethyl-3-methylimidazole-2-thione as a result of chemical reaction of the neat [C_2mim][OAc] with elemental sulfur. Moreover, the authors indicated the inhibition of product formation in 50% IL/water solution.[26] One early literature example of a carbene-mediated reaction carried out in

Received: May 23, 2017
Published: June 22, 2017

DOI: 10.1021/acs.joc.7b01272
J. Org. Chem. 2017, 82, 7538−7545

related 4- and 5-methylthiazolium-based ionic liquids, albeit in the presence of small quantities of trimethylamine, was reported by Davis and Forrester.[29] Similarly, 1,3-dialkylimidazolium-based ionic liquids were activated by adding auxiliary base.[30,31] In 2011, Kelemen et al. found that the formation of the carbene takes place *in situ* if the anion of the ionic liquid is sufficiently basic, e.g., in acetate-based ionic liquids.[32]

The process of imidazol-2-ylidene formation in imidazolium-based ILs is reported to occur by a simple deprotonation of the carbon atom in-between the two nitrogens of the imidazolium ring.[24,25,33] For 1,3-dialkyl-substituted imidazolium ILs Hollóczki et al. reported that the equilibrium between the ion pair and the corresponding hydrogen-bonded complex of the free acid and NHC can be shifted toward NHC formation in the presence of the relatively basic anion of the IL.[24]

The hypothesis about the presence of trace amounts of highly reactive carbenes in the neat ILs with relatively basic anions might pave the way for a general facile use of *in situ* formed NHCs in catalytic reactions. Additionally, simple protonation of these carbenes (e.g., by addition of water) results in a switchable reactive solvent system, allowing the physicochemical properties to be tuned by the structure of the imidazolium cation.

In biomolecules of different origin, e.g., neuropeptides, hormones, enzymes, and growth factors, disulfide-bonds play a crucial role in folding, bioactivity, function, and stabilization of their three-dimensional structure.[34] In general, disulfide-bond formation can be manipulated by its formation and reformation. This makes this area of chemistry both interesting and essential to a number of biochemical transformations.[35] It is well-known that phosphorus nucleophiles are able to react with disulfide bonds by a SN2-mechanism.[36,37] Banerjee et al. achieved disulfide-bond decomposition by a combination of tertiary phosphines and the IL 1-propyl-3-methylimidazolium bromide ([C_3mim]Br) that serves as catalytic system, which facilitates the thiolalkoxy-phosphonium cation and thiolate anion formation. A subsequent reaction with alkyl halides and activated alkenes leads to the formation of unsymmetrical sulfides and 1,4-addition products.[38]

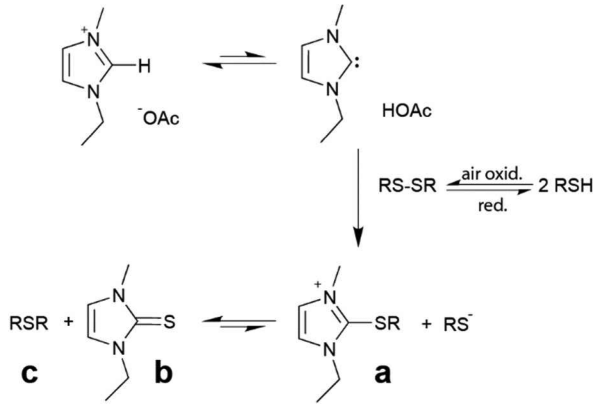
This report, in combination with the knowledge about similar nucleophilic properties of phosphines and imidazol-2-ylidenes inspired our studies to prove whether [C_2mim][OAc] is capable to react with selected sulfur-containing compounds, such as disulfides or thiols.[36,37] Selected organic compounds were meant to serve as model substances. Additionally, a cysteine bearing peptide, derived from the influenza virus B proton channel (residues 22−35) was analyzed whether [C_2mim][OAc] can chemically react with the thiol group of the cysteine side chain. This is especially of importance with respect to our recent approach which introduced [C_2mim]-[OAc] as a highly promising reaction medium for the native chemical ligation (NCL) of highly hydrophobic peptides.[16] Thus, we purse to identify reaction products of the IL with organic model compounds and with the cysteine side chain of the peptide fragment unveiling a general reaction mechanism. Additionally, we could show, that the addition of more than 30% of water to the IL was necessary to suppress the reaction with the peptide. Consequently, we found conditions where [C_2mim][OAc] can be used as a solvent only and is not involved in chemical transformations as a reactant. Furthermore, one can think of making use of carbene chemistry using ILs as *in situ* carbene generators for reactions without the use of additional reagent, and using ILs as an alternative solvent for highly hydrophobic peptides and their transformations, i.e., NCL.

RESULTS AND DISCUSSION

ILs were reported to be able to promote disulfide-bond formation on air.[14] However, it was observed that ILs not only build a network around biomolecules and therefore support the formation of thermodynamically stable disulfide-bond connectivity, but chemically interact with them. We supposed Cys-residues to be involved in this reaction. On the other hand, the cleavage of sulfur−sulfur bonds was reported by phosphorus nucleophiles following a carbene mechanism.[36,37] Moreover, the combination of IL and triphenylphosphin (PPh_3) was observed to serve as a catalyst which is able to cleave diphenyl disulfides facilitating the formation of a thiolalkoxy-phosphonium cation and a thiolate anion.[38] So far, *in situ* generated NHCs formed by the neat IL were not considered to be responsible for these processes. Therefore, we decided to analyze if the neat IL might react with the sulfur-containing compounds without the need of auxiliary catalytic compounds.

The reaction mechanism outlined behind (Scheme 1) is based on the analysis of the reaction of the small organic

Scheme 1. Reaction Processes of Sulfur-Containing Compounds with [C_2mim][OAc]



molecules, diphenyl disulfide (**1**), dibenzyl disulfide (**3**) and their reduced forms thiophenol (**2**) and benzyl mercaptan (**4**) with [C_2mim][OAc] (**5**). Here, the carbene-facilitated reaction between sulfur-containing molecules and the ionic liquid [C_2mim][OAc] is based on the deprotonation of the C2 atom of the imidazolium ring by the IL's anion acetate, formation of the singlet carbene, and subsequent nucleophilic substitution resulting in the intermediate imidazolium-thio adduct **a**, which is converted in a consecutive reaction to yield products **b** and **c** (Scheme 1). The molecules which contain the thiol-groups must be oxidized first to be able to react with the neat IL.

Compounds **1**−**4** were chosen as organic model molecules because of their simple structure in comparison to complex biomolecules, such as peptides, to interpret potential interactions between biomolecules and ionic liquids. After the underlying reaction mechanism of the IL with the organic compounds was understood we used a Cys-containing model peptide, in order to follow up the reaction of the biomolecule.

Complete dissolution of substances **1**−**4** occurred within 5 min by treatment in an ultrasonic bath and reaction monitoring was commenced immediately after dissolution. Reaction

DOI: 10.1021/acs.joc.7b01272
J. Org. Chem. 2017, 82, 7538−7545

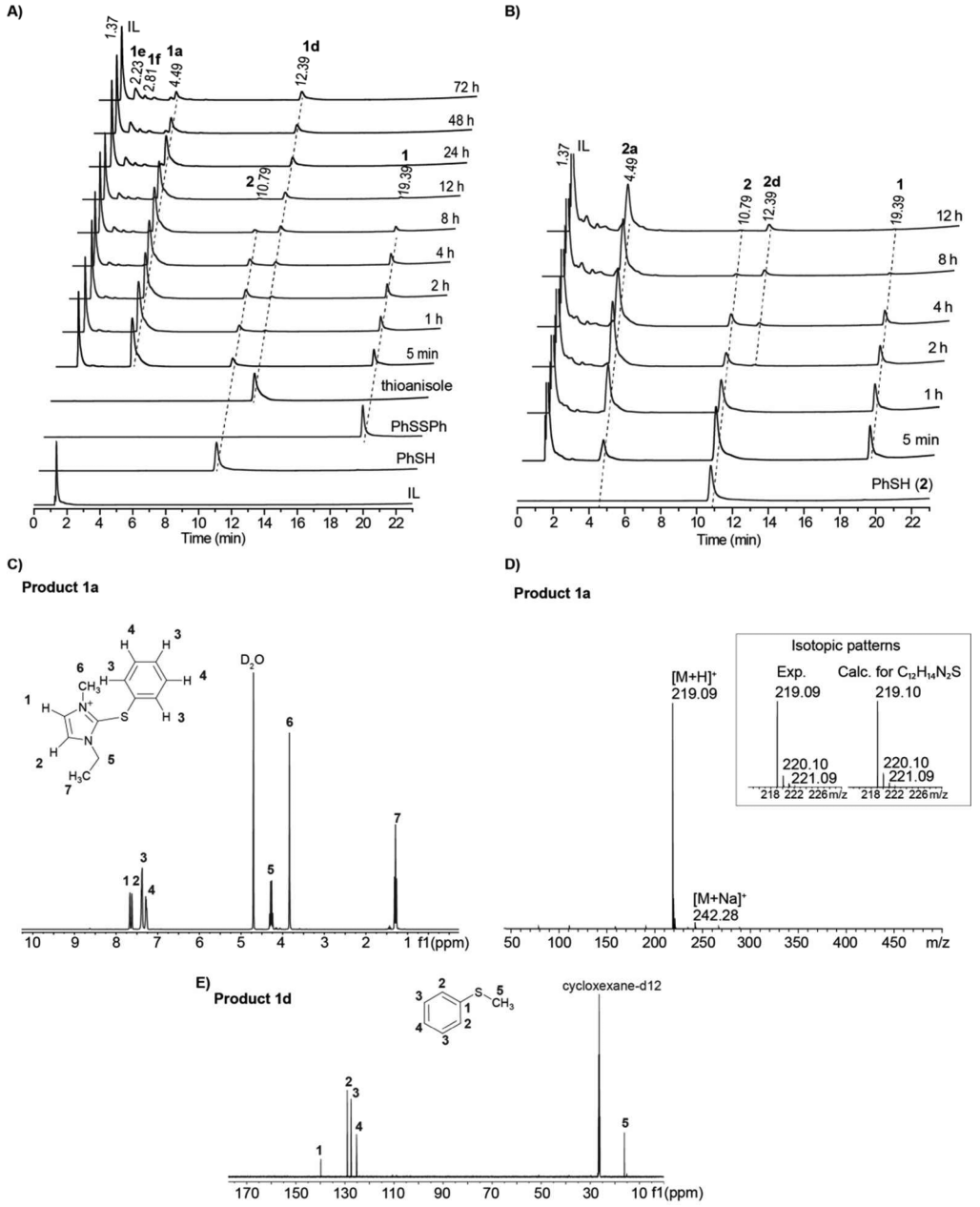


Figure 1. HPLC elution profiles of the reaction of diphenyl disulfide **1** (A) and thiophenol **2** (B) in [C_2mim][OAc] (molar ratio 1:50, 123 mM of **1** or **2**). (C) ^{1}H NMR spectrum and assignment of product **1a**. (D) ESI-MS spectrum and comparison of experimental and calculated isotopic patterns for product **1a**. (E) ^{13}C NMR spectrum of product **1d** in cyclohexane-d12. HPLC conditions: 20−55% eluent B in 15 min followed by a gradient of 55−95% eluent B for 7 min at a flow rate 1 mL/min and detection wavelength at 240 nm.

products were analyzed by liquid chromatography−mass spectrometry (LC-MS), electrospray ionization (ESI) MS, and NMR spectroscopy (Figure 1, Figure 2). Reactions were carried at room temperature and in the dark to exclude hemolysis of the sulfur−sulfur bond.[39]

Reaction of Diphenyl Disulfide (1) and Thiophenol (2) in [C_2mim][OAc] (5). During the reaction of diphenyl disulfide (**1**) (t_R= 19.39 min) in [C_2mim][OAc] (**5**) (t_R = 1.37 min) (Figure 1A), three different peaks appeared with retention times (t_R) of 4.49, 10.79, and 12.39 min. According to reference chromatogram the peak, eluting at t_R 10.79 min was assigned to thiophenol (**2**) (the reduced form of **1**), which is formed only in small amounts, being at a constant concentration during 2 h and disappearing after that time, indicating the equilibrium reaction between the reduced and oxidized forms of **1** (Figure 1A). Already at the starting point of the reaction the intense peak at t_R 4.39 min was observed corresponding to the main product. According to NMR (Figure 1C, Figure S1A), ESI-MS (Figure 1D) spectra, and thin-layer chromatography results (Figure S1), the product **a** (**1a**) was the main product formed during the reaction of **1** in **5** (Scheme 1). The experimental isotopic pattern of product **1a** was in a good agreement with the calculated isotopic pattern (Figure 1D). The concentration of product **1a** reached its maximum already at 5 min and stays constant for 12 h, since in the reaction mixture the initial compound **1** is still present to form this product **1a**. After 12 h compound **1** is completely transformed to product **1a**, which concentration then started to decrease in the ensuing time,

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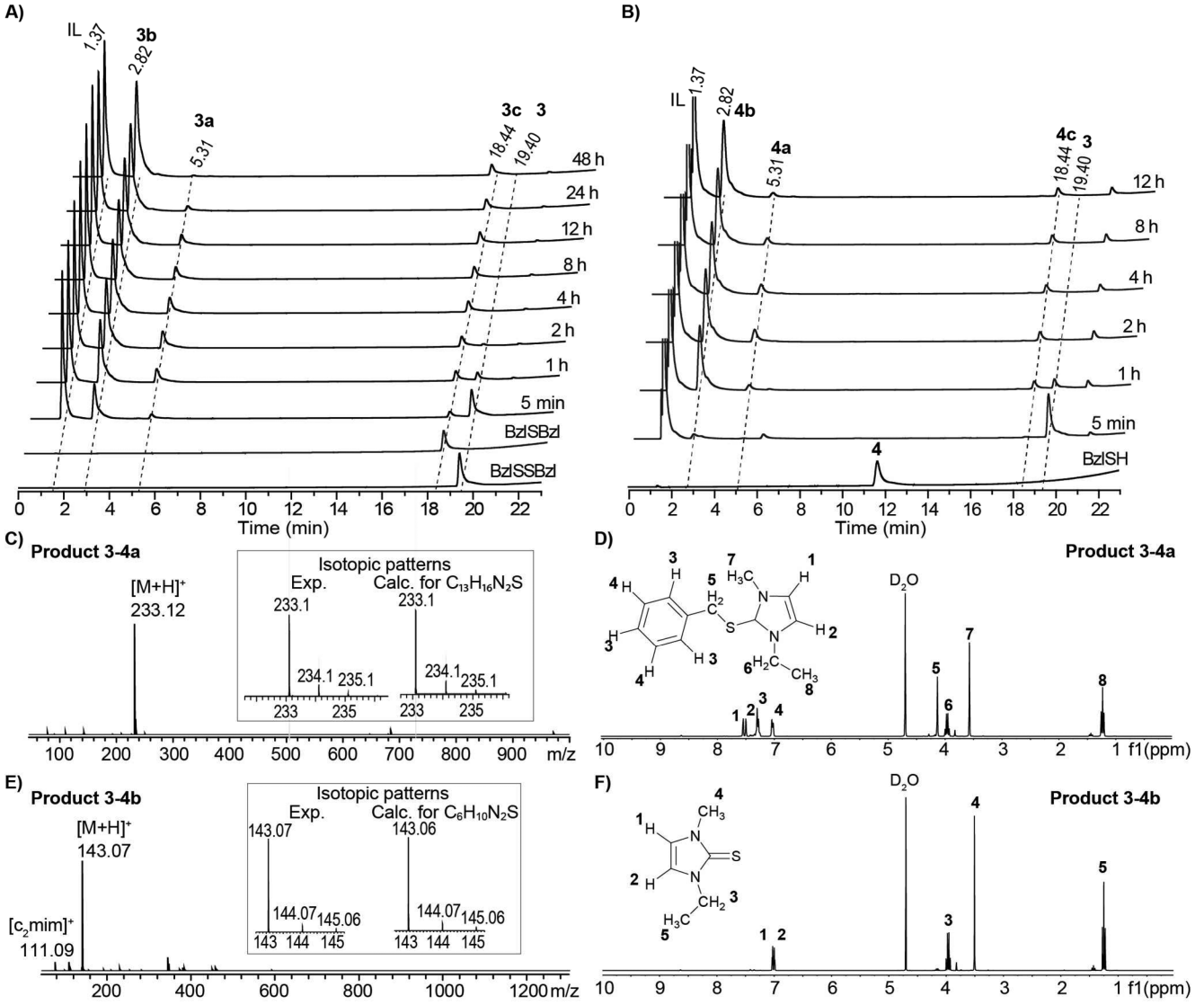

Figure 2. HPLC elution profiles of reaction of dibenzyl disulfide **3** (A) and benzyl mercaptan **4** (B) in $[C_2mim][OAc]$ (molar ratio 1:50, 123 mM of **3** or **4**). HPLC conditions: 20−55% eluent B in 15 min followed by a gradient of 55−95% eluent B for 7 min at flow rate 1 mL/min and detection wavelength at 240 nm. ESI-MS spectrum of products **3**−**4a** (C) and **3**−**4b** (E), and comparison of their experimental and calculated isotopic patterns. (D) ^{1}H NMR spectrum and assignment of the products **3**−**4a** (D) and **3**−**4b** (F) in D_2O.

indicating the instability of product **1a** in the ionic liquid. Thus, two new products **1e**, **1f** appeared as intense peaks in the chromatogram at t_R between 2−3 min (Figure S3).

The additional peak at 12.39 min (Figure 1A) was highly volatile during the work up procedure and difficult to obtain from organic solvents by vacuum evaporation. However, we were able to isolate product **1d** for NMR analysis by direct extraction with cyclohexane-d12 from the reaction mixture after 72 h. To our surprise, we identified thioanisole as product **1d** and confirmed this finding by comparison with the respective reference chromatogram and NMR data (Figure 1A,E, Figure S1B). Consequently, this indicates a second side-reaction between diphenyl disulfide (**1**) and the ionic liquid cation, in which $[C_2mim]^+$ was obviously capable to methylate the thiol group of thiophenol. Interestingly, only the methylated form **1d**, but not the ethylated form was found as a product in this reaction (Figure 1E, Figure S1B). An efficient reaction of thiol methylation was described by Xie et al., applying $[C_4mim]Cl$ as a catalyst using dimethyl carbonate as a source of the methyl group.[40] With respect to our data, $[C_2mim][OAc]$ seemed to perform methylation of aromatic thiols, i.e., thiophenol, without the need of an additional reactant.

During our investigation we observed that not only diphenyl disulfide (**1**) but also the reduced form thiophenol (**2**) was able to react with the ionic liquid forming the same products **1a**/**2a** and **1d**/**2d**. Moreover, we found that thiophenol is partially oxidized and diphenyl disulfide partially reduced in $[C_2mim]$-$[OAc]$, unveiling that both forms exist in an equilibrium. Nevertheless, the formation of product **a** is faster in diphenyl disulfide (**1**) than in thiophenol (**2**). This observation corresponds to our postulated mechanism in Scheme 1, where the thiol-compound must first be oxidized before it is attacked by an *in situ* formed imidazolium-carbene. In both reactions, the initial compounds diphenyl disulfide (**1**) and thiophenol (**2**) fully disappeared within 12 h, yielding the main products **1a**/**2a** and reaching its maximum concentration at 12 h. The second product **1d**/**2d** appears after 2 h of reaction time and reaches its maximum concentration at 24 h.

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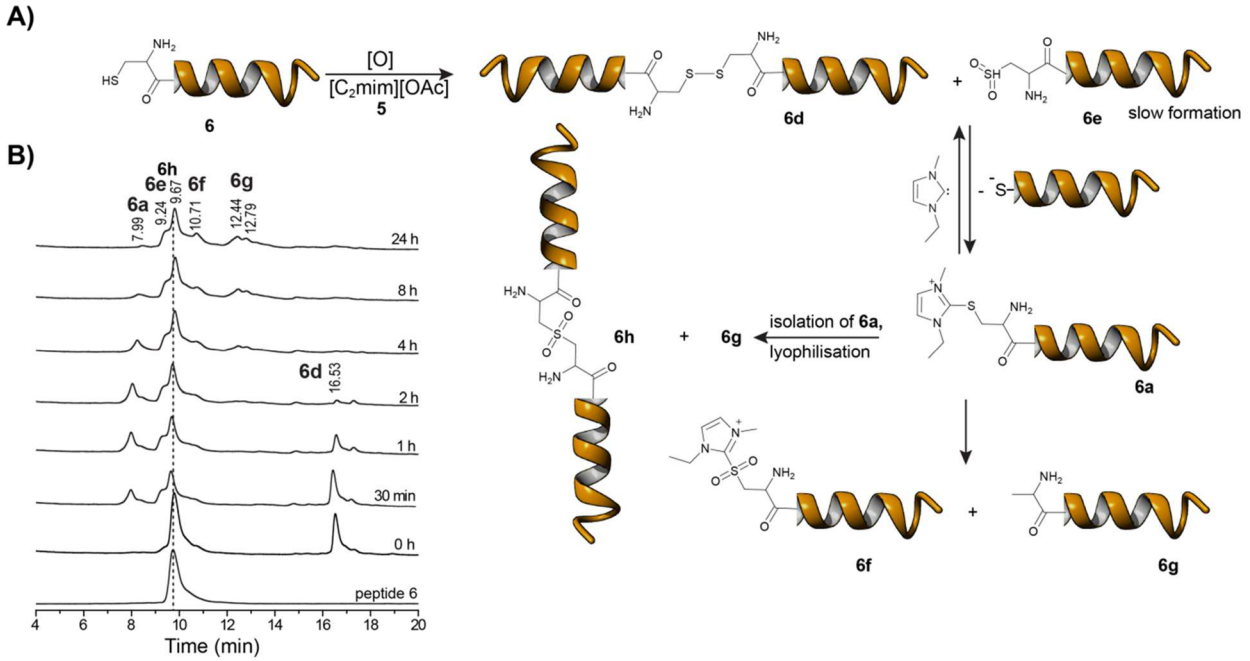

Figure 3. Reaction of Cys-containing peptide in [C_2mim][OAc] and products formed during the reaction.

Reaction of Dibenzyl Disulfide (3) and Benzyl Mercaptan (4) in [C_2mim][OAc] (5). For the reaction of dibenzyl disulfide (**3**) and benzyl mercaptan (**4**) in [C_2mim]-[OAc] (**5**) the formation of the products **a**–**c**, according to the Scheme 1, was monitored by HPLC. The product **a** (**3a** and **4a**), eluting at t_R 5.31 min, was already formed at the beginning of reaction (5 min) as indicated by a small peak in the chromatogram (Figure 2A,B). Its concentration constantly increased until 2 h and then began to decrease completely disappearing after 48 h. This product was isolated and identified by ESI-MS and NMR analysis (Figure 2C,D, Figure S2C). Also product **b** (**3b** and **4b**), eluting at t_R 2.82 min, and product **c** (**3c** and **4c**), eluting at t_R 18.44 min, were already present immediately after the reaction was started (Figure 2A,B,E,F, Figure S2D). The concentration of both products **3b** and **4b** slightly increased during the reaction time, while the concentration of **3c** and **4c** remained constant. Interestingly, no benzyl mercaptan (**4**) was observed during the reaction of **3** in [C_2mim][OAc], in comparison to diphenyl disulfide (**1**) (Figure 1A, Figure 2A). Moreover, for the reaction of **4** in [C_2mim][OAc] the initial compound was completely expended already at the beginning of the reaction yielding its oxidized form dibenzyl disulfide (**3**), indicating a much faster oxidation of benzyl mercaptan (**4**) in comparison to thiophenol (**2**). Furthermore, this is an evidence that the reduce compounds indeed oxidize first to be able to react with the imidazole-carbene. The products **3b**/**4b** were isolated and assigned according to ESI-MS and solution NMR analysis (Figure 2E,F) and **3c**/**4c** according to reference elution of dibenzyl sulfide (Figure 2A).

In summary, the obtained data for compounds **1**–**4** clearly support our initial hypothesis (Scheme 1) that constantly formed carbenes in [C_2mim][OAc] are capable to participate in nucleophilic substitution of disulfides forming product **a**. For diphenyl disulfide/thiophenol product **1a**/**2a** was formed first and then subsequently transformed to products **1e** and **1f**. Moreover, [C_2mim][OAc] seemed to perform methylation of aromatic thiols, i.e., thiophenol, by forming product **1d** without the need of an additional reactant. In contrast, for dibenzyl disulfide/benzyl mercaptan we observed two additional products **b** and **c**. Most likely, the additional CH2-group between the phenyl ring and [C_2mim]S in product **3a**/**4a** allows for the nucleophilic attack of the RS^- intermediate at this position. Moreover, thiophenol (**2**) and benzyl mercaptan (**4**) dimerized first forming diphenyl disulfide (**1**) and dibenzyl disulfide (**3**) before they were reacting with the NHC of [C_2mim][OAc]. Thus, we concluded that the carbene itself is able to "break" the disulfide bond, without the need of further catalytic compounds. Consequently, this type of reaction must be taken into consideration when discussing increased m/z peaks of proteins derived from IL mixtures, or the highly selective oxidative folding (disulfide bond formation) to a single thermodynamically stable isomer in ILs.[14] Finally, the chemical reactions observed in this study are clearly indicative for the presence of a small carbene fraction in neat [C_2mim][OAc].

Reaction of Model Peptide CWTIGHLNQIKRGI in [C_2mim][OAc] (5). With respect to the chemical reaction of [C_2mim][OAc] (**5**) with the disulfide bonds of the small organic compounds **1** and **3** and their reduced forms, compounds **2** and **4**, a small cysteine containing peptide with the sequence CWTIGHLNQIKRGI (**6**) was investigated.

From the results discussed above, one would expect for this reaction the disulfide bond formation, followed by nucleophilic substitution and scission of the S–S bond by the *in situ* formed NHC carbene from IL according to Scheme 1. The reaction was monitored by RP-HPLC and all peaks were characterized by LC-MS (Figure 3). Product peaks were isolated and reinjected to prove their stability. The LC-MS analysis was performed for two reaction times, at 1 and 24 h (Figure S4). The reaction products are depicted in Figure 3A. Directly after dissolving the peptide (**6**) in [C_2mim][OAc] (**5**), an intensive peak was observed (t_R 16.52 min) in the RP-HPLC chromatogram (Figure 3B), indicating peptide oxidation and formation of the peptide dimer (IGRKIQNLHGITW**CC**W-TIGHLNQIKRGI, **6d**) through a disulfide bond (Figures S4A and S6). Disulfide bond formation typically occurs through

DOI: 10.1021/acs.joc.7b01272
J. Org. Chem. 2017, 82, 7538–7545

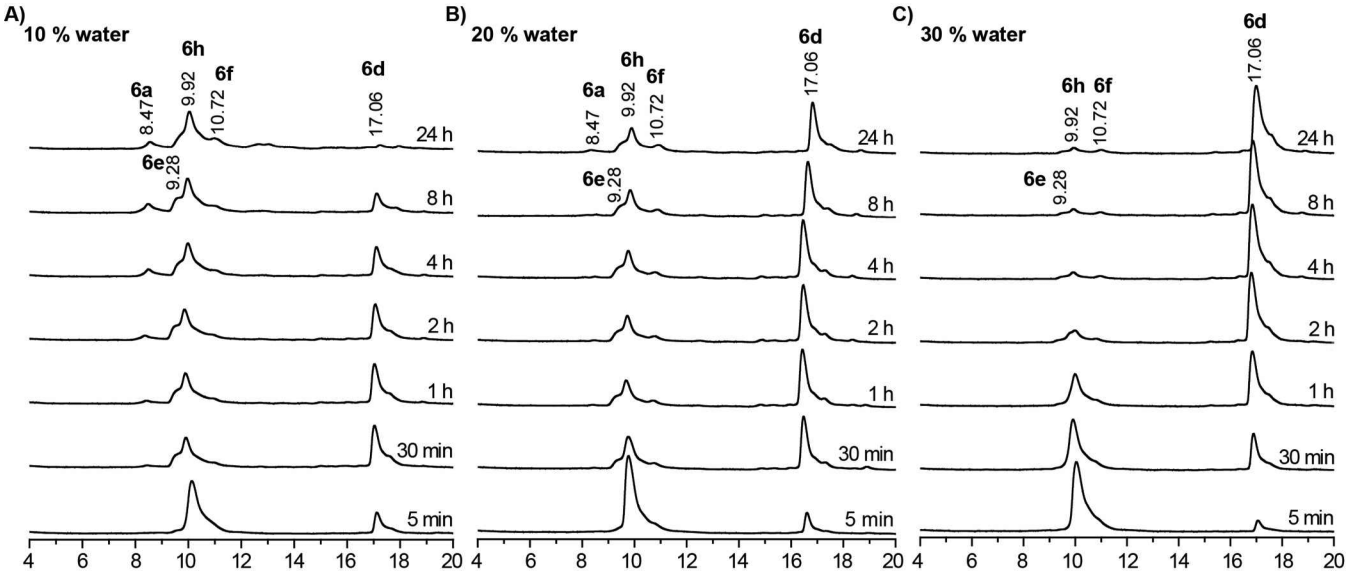

Figure 4. Effect of water on product formation during reaction of peptide **6** in [C_2mim][OAc] **5**.

peptide oxidation in the presence of oxygen from the air.[13] Within 2 h, this peak completely disappeared, while three other main peaks at t_R 7.99 and 9.24 and 10.71 min built up reaching their maximum concentration at 2 h. According to the LC-MS analysis and observations made for the small organic model compounds in this study the peak at t_R 7.99 min corresponded to the reaction product **a** (**6a**) of Scheme 1, indicating the chemical reaction of peptide (**6**) with **5** (Figures S4A and S6). The peak at t_R 9.24 min had a corresponding *m/z*, which was 32 *m/z* higher than for the nonaltered peptide (**6**) and most likely corresponded to peptide Cys-sulfone (product **6e**) (Figure 3B, Figures S4A and S6). The peak at t_R 10.71 min increased until 24 h and was assigned to product **6f** (Figures S4B and S6), assuming further oxidation of product **6a** by forming the sulfone product. After 2 h product **6a** (t_R 7.99 min) continuously decreased and in parallel peaks at t_R 12.44/12.79 min were observed. The analysis of the isotopic pattern of this product indicated the desulfurization of the Cys-residue of the peptide yielding product AWTIGHLNQIKRGI (**6g**), where Cys was transformed to Ala (Figures S4B and S6). Interestingly, our data suggest that the desulfurization process observed herein might proceed via a very similar mechanism as reported for the well-described reaction of desulfurization by tris(2-carboxyethyl)phosphine (TCEP).[41] So far, [C_2mim]-[OAc] had not been described to be involved in such kind of reactions and further investigations are needed whether this reaction is an alternative procedure for the desulfurization of cysteine residues, which were artificially inserted into a peptide sequence for NCL purposes.

Product **6a** could not be isolated most likely because it was unstable upon the workup procedure. Instead, from the respective HPLC peak we isolated the desulfurized peptide **6e** and product **6h**, which was the oxidized form of **6c** (Figure S5). Conclusively, the Cys-containing peptide underwent the same reactions in neat [C_2mim][OAc] as observed for the small organic model compounds. But unlike for the model compounds peptide products **6a** and **6c** were not stable and further oxidized yielding products **6f** and **6h** (Figure 3) confirming the reactions outlined in Scheme 1.

Effect of Water on Product Formation during Reaction of Peptide 6 in 5. From the observations made for the chemical transformation of the small organic model compounds and the Cys-containing peptide fragment induced by small fractions of NHCs in neat [C_2mim][OAc] we concluded that the addition of water might prevent NHC formation. Since, peptide **6** was water-soluble, we investigated if water can prevent the reactions observed in [C_2mim][OAc]. Indeed, HPLC analysis revealed that at water:IL ratios ranging from 10 to 30% (v/v) the reaction with imidazole-carbene was slowed down and fewer products were formed in comparison to reaction in the neat IL (Figures 3B and 4).

Over time, the concentration of dimerized peptide **6d** increased and reached its maximum at 2 h staying at a constant concentration for the rest of the monitored reaction time (24 h) when the water content was 20 or 30% (Figure 4B,C), respectively. At a water content of 10% product **6a** was formed after 2 h, whereas at 20% water only small traces of product **6a** were observed in the HPLC chromatogram after 24 h (Figure 4B). Also, only small amounts of the oxidation products **6e** and **6f** were formed within the whole reaction process (Figure 4B). In contrast, at water concentration of at least 30% (Figure 4C) product **6a** was not observed within 24 h of reaction time, but minimal concentrations of products **6h** and **6f**. So far, our results support previous findings, which described the inhibition of carbene formation (in reaction with elemental sulfur) with water at concentrations higher than 50%[42,43] due to the disruption of interaction between the anion and the cation of the IL through water.[26,44]

As a result, water reduces the reactivity of imidazolium-carbenes toward sulfur-rich peptides and small amounts of carbene present in neat IL can be protonated or even decomposed in water.[41,42] This finding explains the absence of product **6a** in the RP-HPLC chromatogram for water contents ≥30% (Figure 4).

■ CONCLUSIONS

In this work we demonstrated, that the ionic liquid [C_2mim]-[OAc] is not just capable of dissolving thiol- and disulfide-containing model compounds, such as diphenyl disulfide, dibenzyl disulfide, thiophenol, and benzyl mercaptan, but also are able to chemically react with them without addition of any catalytic reagents. Due to the anion's basicity (acetate) the

DOI: 10.1021/acs.joc.7b01272
J. Org. Chem. 2017, 82, 7538−7545

equilibrium shifts toward the deprotonated C2 atom of the imidazolium cation allowing for a reaction with thiol- or disulfide- containing compound. Time-resolved RP-HPLC analysis indicated the formation of a various products. By ESI-MS, NMR spectroscopy, and TLC analysis, we could unambiguously identify and characterize all formed products. Consequently, our data fully supports the reaction scheme as outlined in Scheme 1 hypothesizing that the imidazolium-carbene can react with a disulfide bond, but not with a thiol group. In turn, thiols must be oxidized first in order to be able to form products **a**, **b**, and **c** (Scheme 1). Further, the structure of the disulfide played an important role. In order to allow the formation of products **b** and **c** from **a**, the thiol group must have an adjacent methylene group, allowing $[C_2mim]S^+$ to cleave off. If there is no adjacent methylene group, the reaction stops after the formation of product **a**. However, several other products were formed, namely thioanisole (**1d**/**2d**), in the reaction of **1**/**2** in **5** indicating that **5** is capable to methylate the thiol-group without the help of additional methylation reagents.

Moreover, we could show that a cysteine-containing peptide results into similar products as observed for the organic model compounds in $[C_2mim][OAc]$ which also had to be oxidized first to react with imidazole-carbenes forming product **a** (Figure 3A). Additionally, the spontaneous elimination of $[C_2mim]S^+$ from the peptide leads to the desulfurization of the respective Cys residue which might provide an alternative way for Cys transformation, e.g., after NCL of two peptide fragments.

Despite the interesting ability of neat $[C_2mim][OAc]$ to react with sulfur-containing molecules, there are some reactions where IL is preferred to promote its features as a solvent, i.e., NCL, where such reactivity is undesired. Thus, the addition of at least 30% of water to $[C_2mim][OAc]$ was sufficient to suppress any chemical reaction of the IL with the peptide.

Finally, our results allow for making use of a tailored combination of carbene chemistry in the field of organic and biochemical reactions, with the beneficial use of the environmental friendly ILs, but also overcomes the problematic use of ILs in the presence of cystine-rich peptides and proteins.

Our study has a major impact on further IL application for sulfur-containing compounds, e.g., peptides and proteins or small organic molecules. To investigate the border case of basicity of anion in ionic liquid to be able to deprotonate IL cation, ILs with anions of different basicity, according to Hofmeister series, will be studied.[45,46] Optimal reaction conditions (choice of IL, water amount, temperature, reactant concentration) and selectivity for the formation of a single reaction product for specific applications will be determined in future work.

EXPERIMENTAL SECTION

General Experimental. For the reaction diphenyl disulfide **1** (39.3 mg), thiophenol **2** (19.8 mg, 1.83 μL), dibezyl disulfide **3** (44.4 mg), and benzyl mercaptan **4** (22.3 mg, 2.11 μL) were dissolved in 1.5 mL of $[C_2mim][OAc]$ (molar ratio 1:50, 123 mM) respectively. The reaction mixtures were left to react while agitating at room temperature until the reaction was completed. For reaction monitoring of compounds **1**–**4** in $[C_2mim][OAc]$ aliquots of the mixtures (5 μL) were diluted with 250 μL acetonitrile with 0.1% TFA and 5 μL of the resulting solution was injected for analytical RP-HPLC. The reaction was monitored at 0, 1, 2, 4, 8, 12, 24, 48, and 72 h. Products **1a**/**2a**, **3a**/**4a**, and **3b**/**4b** were isolated by column chromatography, applying a mixture of acetonitile/water (9:1) as a mobile phase. After lyophilization, a light yellow, viscous liquid was obtained (product **1**–**2a**: 31.6 mg (yield: 80%), product **3**–**4a**: 11.2 mg (yield: 26%)). The fraction, containing the product **3**–**4b** was evaporated under reduced pressure and finally 15.4 mg (yield: 60%) of a dark orange liquid was obtained.

*Synthesis of Peptide CWTIGHLNQIKRGI (**6**).* Peptide **6** was synthesized on an automated peptide synthesizer (Liberty, CEM) according to a standard Fmoc-protocol on AmphiSpheres RAM resin (0.37 mmol/g loading size). Coupling reactions (15 min, double coupling) were performed using Fmoc-amino acids (4 equiv), activated with 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-*b*]pyridinium 3 oxide hexafluorophosphate (HATU, 3.9 equiv) and *N*-ethyl-*N*-(propan-2-yl)propan-2-amine (DIEA, 8 equiv) in *N*,*N*-dimethylformamide (DMF) under microwave irradiation (50 °C, 35W). Fmoc-protecting group was removed by treating the peptide-resin with 20% piperidine in DMF under microwave irradiation (50 °C, 35W), twice. All deprotection and coupling steps were followed by intensive washings using DMF and dichloromethane (DCM).

Peptide cleavage and deprotection was accomplished with a cleavage cocktail of a 90% TFA, 5% water, 2.5% TIPS, and 2.5% anisole for 3 h at room temperature. The crude peptides were precipitated in cold diethyl ether, centrifuged, and washed with diethyl ether. The crude peptide was purified by preparative RP-HPLC using a C18 column (MultoKrom 100–5, 10 nm, 250 × 20 mm,), eluent A: water (0.1% TFA) and eluent B: acetonitrile (0.1% TFA). Preparative HPLC conditions: 20–80% acetonitrile in 40 min using a flow rate of 10 mL/min.

*Peptide CWTIGHLNQIKRGI (**6**) Reaction in $[C_2mim][OAc]$ (**5**).* For the reaction 0,825 mg of peptide **6** were dissolved in 43 μL of $[C_2mim][OAc]$ (molar ratio 1:500, 12 mM). The reaction mixture was left to react while agitating at room temperature until the reaction was completed.

The effect of the water content on the reaction progress was determined for the peptide BM2(22–35). A series containing different amounts of water (10, 20, and 30% (v/v)) were prepared and the reaction progress was followed by RP-HPLC at 0 min, 30 min, 1, 2, 4, 8, and 24 h.

Reversed-Phase High Performance Liquid Chromatography (RP-HPLC). Reactions were monitored by analytical RP-HPLC on a Prontosil 120–5-C8-SH 5.0 μm analytical column. The gradient elution system was 0.1% TFA in water (eluent A) and 0.1% TFA in acetonitrile (eluent B). Organic compounds **1**–**4** were eluted with a gradient of 20–55% eluent B in 15 min followed by 55–95% eluent B in 22 min and a flow rate of 1 mL/min. The peptide BM2(22–35) was eluted with a gradient of 20–30% eluent B in 20 min and a flow rate of 1 mL/min. The detector was operated at 240 nm for organic compounds **1**–**4** and at 214 nm for the peptide BM2(22–35).

Thin-Layer Chromatography (TLC). Thin layer chromatography was performed on silica gel 60 F 254 coated glass plates. Two solvent systems were applied for each compound TLC *n*-hexane or *n*-hexane/ethyl acetate (59:1, v/v) and acetonitrile/water (9:1, v/v). The R_f values are summarized in Table 1 in Supporting Information.

NMR Spectroscopy. Solution NMR experiments were performed on a Bruker Avance III spectrometer or on a Bruker DRX 500 at proton frequencies of 300 or 500 MHz, respectively. The products **1a**, **3a**, **3b** were dissolved in D_2O and **1d** in cyclohexane-d12.

1a/2a: ^{1}H NMR (300 MHz, D_2O) δ = 7.7 (d, J = 2.2 Hz, 1-H), 7.6 (d, J = 2.2 Hz, 1-H), 7.4 (m, 3-H, Ar), 7.3 (m, 2-H, Ar), 4.3 (q, J = 7.3 Hz, 2-H), 3.8 (s, 3-H), 1.3 (t, J = 7.3 Hz, 3-H) ppm. ^{13}C NMR (D_2O) δ = 130.3, 130.0, 129.2, 128.6, 125.6, 123.5, 45.17, 36.1, 14.3 ppm.

1d/2d: ^{1}H NMR (500 MHz, cyclohexane-d_{12}) δ 7.88–7.80 (m, 4-H, Ar), 7.70–7.67 (m,1-H, Ar), 3.04 (s, 3-H) ppm. ^{13}C NMR (cyclohexane-d_{12}) δ = 139.8, 129.0, 127.5, 125.3, 16.1 ppm.

3a/4a: ^{1}H NMR (300 MHz, D_2O) δ = 7.5 (d, J = 2.1 Hz, 1-H), 7.5 (d, J = 2.2 Hz, 1-H), 7.3–7.2 (m, 3-H), 7.1–7.0 (m, 2-H), 4.1 (s, 2-H), 4.0 (q, J = 7.3 Hz, 2-H), 3.6 (s, 3-H), 1.2 (t, J = 7.4 Hz, 3-H) ppm. ^{13}C NMR (D_2O) δ = 136.0, 129.2, 128.7, 128.5, 125.2, 123.0, 44.6, 39.8, 35.5, 14.2 ppm.

3b/4b: ^{1}H NMR (300 MHz, D_2O) δ = 7.0 (d, J = 2.3 Hz, 1-H), 7.0 (d, J = 2.3 Hz, 1-H), 4.0 (q, J = 7.3 Hz, 2-H), 3.5 (s, 3-H), 1.2 (t, J = 7.3 Hz, 3-H) ppm. ^{13}C NMR (D_2O) δ = 119.8, 117.8, 43.0, 34.7, 13.6 ppm.

DOI: 10.1021/acs.joc.7b01272
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LC-MS. The LC-MS of the reaction of peptide CWTIGHLNQIKRGI (**6**) with [C_2mim][OAc] (**5**) was performed using a gradient of 20−30% eluent B in 20 min and a flow rate of 0.2 mL/min. The gradient elution system was 0.1% TFA in water (eluent A) and 0.1% TFA in acetonitrile (eluent B).

Characterization of starting materials and products, including retention times and response factors, is given in the Supporting Information along with selected chromatograms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b01272.

NMR spectra, LC-MS analysis, RP-HPLC chromatograms, MS spectra (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The Liebig Fellowship of the Fonds der Chemischen Industrie and the LOEWE iNAPO funded by the Ministry of Higher Education, Research and the Arts (HMWK) of the Hessen state are gratefully acknowledged (A.A.T.). The authors thank the MS-Service team of the Chemistry Department, especially C. Rudolph and Dr. A. Schiesser (TU Darmstadt). We thank Diana Imhof (University of Bonn) and members of the COST Action CM1206 Exchange on Ionic Liquids for useful scientific discussions.

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DOI: 10.1021/acs.joc.7b01272
J. Org. Chem. 2017, 82, 7538−7545