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ARTICLE

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Solvation of Nucleobases in 1,3-Dialkylimidazolium Acetate Ionic Liquids: NMR Spectroscopy Insights into the Dissolution Mechanism

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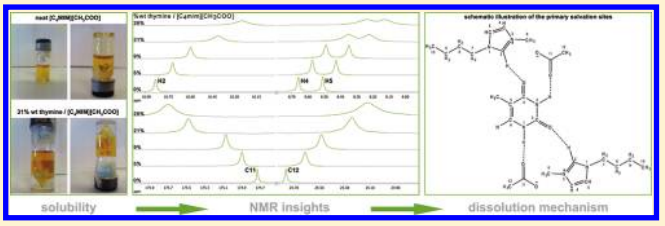
Supporting Information

ABSTRACT: NMR studies of uracil, thymine, and adenine dissolved in 1-ethyl-3-methyl-imidazolium acetate ($[C_2mim][CH_3COO]$) and 1-butyl-3-methyl-imidazolium acetate ($[C_4mim][CH_3COO]$) show that hydrogen bonds (HB) dictate the dissolution mechanism and that both cations and anions participate in the solvation process. For that, the 1,3-dialkylimidazolium acetate ionic liquids (ILs) were considered to be bifunctional solvation ionic liquids. In the solvation of uracil and thymine, the $[CH_3COO]^-$ anion favors the formation of hydrogen bonds with the hydrogen atoms of the N1−H and N3−H groups of the nucleobases, while the aromatic protons in the bulky cations ($[C_2mim]^+$ and $[C_4mim]^+$), especially the most acidic H2, interact with the oxygen atoms of the carbonyl groups. In the adenine solvation, while the $[CH_3COO]^-$ anion favors the formation of hydrogen bonds with the hydrogen atoms of the amino and N9−H groups of adenine, the aromatic protons in the bulky cations ($[C_2mim]^+$ and $[C_4mim]^+$), especially the most acidic H2, prefer to interact with the unprotonated nitrogen atoms (N1, N3, and N7) of adenine. It is clearly demonstrated that hydrogen bonding is the major driving force in the dissolution of nucleobases in 1,3-dialkylimidazolium acetate ILs. Our results show that the ionic liquid must be a good hydrogen bond acceptor and a moderate hydrogen bond donor to dissolve nucleic acid bases. To strengthen the evidence of the proposed mechanism, NMR studies in the absence of deuterated cosolvents have been used, because the use of deuterated solvents could seriously hinder the dissolving capability of the IL for nucleobases.



1. INTRODUCTION

Neoteric solvents engineering is one of the most promising areas in the evolving field of "green" sustainable technologies,[1] a category which includes supercritical CO_2,[2−4] aqueous biphasic systems,[5−7] and ionic liquids (ILs).[8−13] ILs are a class of novel liquid compounds offering a highly solvating medium in which a number of organic and inorganic solutes may be dissolved[4,14] as a result of their chemical nature combined with the formation of nanodomains.[15,16] They possess appealing features such as low melting points,[17] negligible vapor pressure at ambient conditions,[18] and generally nonflammability.[12,19−22] These physical properties result from the combined properties of the cations and anions, which can be tailored by selecting their nature and structure from a plethora of distinct possibilities. Such tunable properties enable one to widen the field of applications to, for example, organic synthesis and catalytic reaction,[23] electrochemistry,[24−26] biochemistry and dissolution of biomaterials,[27−30] and materials engineering.[31]

Nucleic acid bases, nitrogenous heterocyclic nucleic acids that form the structural units of DNA and RNA, are ubiquitous in nature, presenting a paramount importance as biochemical compounds. Their chemistry influences different synthetic pathways as well as enzyme systems and dictates the structures and properties of living cells and organisms. The interest in nucleobase solution chemistry[32,33] is growing as the knowledge of its biochemical importance and applications is becoming better known.[34−38] Nucleobases are involved in two qualitatively distinct mutual interactions: hydrogen bonding and aromatic base stacking. The hydrogen bonding capacity of the nucleobases is responsible for correct DNA base-pairing and structure stabilization. The specific interactions between the purine and pyrimidine bases are one of the cornerstones of molecular biology. For example, the Watson−Crick pairing scheme[39] involves two hydrogen bonds for the uracil−adenine (or thymine−adenine) base pair, with the oxygen atom of the C4−O group of the uracil being involved in a hydrogen bond with the amino group of the adenine and the hydrogen atom of the N3−H group of the uracil interacting with the heteroaromatic N of the adenine.[40,41] The practical importance of dissolving nucleobases in ionic liquids resides in the paramount importance of the solutes. For example, uracil is a "fascinating" molecule because of not only its biological significance but also its numerous therapeutic effects and applications in industrial chemistry and agriculture.[42] Also, it has been shown that DNA is soluble in a variety of ionic liquids with exceptional long-term stability.[43]

The key characteristics of a solvent are those that determine how it will interact with potential solutes. Polarity scales have been used to describe the affinity of solutes for molecular

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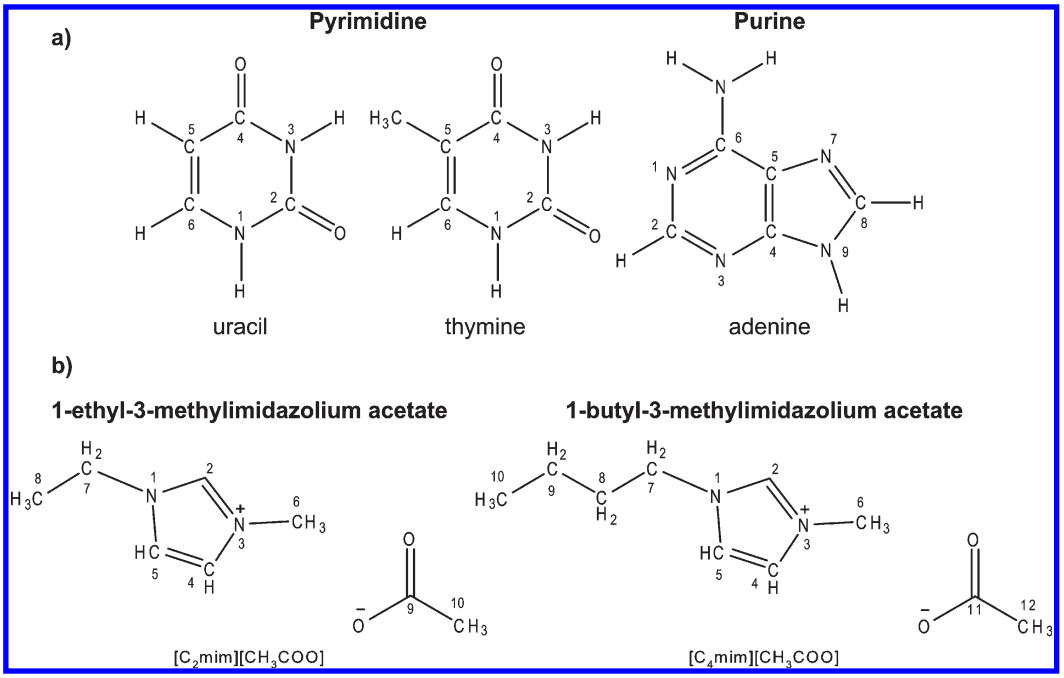

Figure 1. Structures and numbering: (a) purine (adenine) and pyrimidine (uracil and thymine) bases; (b) the two 1,3-dialkylimidazolium acetate ionic liquids considered in the present study, $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$.

solvents[44−47] as well as ionic liquids.[48] One of the most used polarity scales was devised by Kamlet and Taft and is based on the comparison of the effects of sets of dyes on the UV−vis spectra. Three polarity parameters have been proposed: the dipolarity polarizability effects (π^*); the hydrogen bond acidity (α), that is, hydrogen bond donation (HBD) ability; and the hydrogen bond basicity (β), that is, hydrogen bond acceptance (HBA) or electron pair donation ability to form a coordinative bond.

Good examples of the application of the polarity scales to selection of solvents for ILs are the studies focused on cellulose solubility in 1,3-dialkylimidazolium ILs that initially contained chloride as the anion,[49] which developed afterward to anions such as formate,[50] acetate, phosphate, or phosphonate,[51] with increased cellulose solubility. The concomitant measurement of Kamlet−Taft parameters showed that these ionic liquids are characterized by a high β parameter, indicating an increase in the hydrogen bonding accepting capability of the anion. Ionic liquid anions that can strongly coordinate with the hydrogen bond donator groups of cellulose are able to trigger solute−solvent interactions that are required for cellulose dissolution.

In this work, we investigate the dissolution mechanism of uracil, thymine, and adenine, in 1-ethyl-3-methylimidazolium and 1-butyl-3-methylimidazolium acetate ILs using NMR spectroscopy. Nucleobases and ILs are depicted in Figure 1. The selection of 1,3-dialkylimidazolium acetate ILs to accomplish this study was based on the Kamlet−Taft parameters listed in Table S1 in the Supporting Information. The Kamlet−Taft parameters explain the superior solubility of nucleobases in the 1,3-dialkylimidazolium acetates, when compared to the very low aqueous solubility of nucleobases.[52] For example, we could dissolve up to 30 wt % of uracil in $[C_4mim][CH_3COO]$ (see Table S4 in the Supporting Information) while its water solubility is ca. 0.3 wt %[52] —a 100-fold increase.

Generally, the HBD ability, the α value, is largely determined by the availability of hydrogen bond donor sites on the cation. For the imidazolium-based salts, α values are in the 0.3−0.8 interval,[53] with the lower values corresponding to the more basic anions. Within these salts, in the case of 1,3-dialkyl substituted cation, the acidity of the proton that is attached to the carbon between the two ring nitrogen atoms is responsible for the α value. On the other hand, the anion plays a determinant role in the β parameter,[48] increasing its value with the basicity of the anion, that is, the availability of hydrogen bond acceptance. The ability of the IL anion to hydrogen bond is illustrated in Table S1 in the Supporting Information. Their increased nucleophilicity is the following: $[PF_6]^- \approx [Tf_2N]^- < [BF_4]^- \approx [TfO]^- < [Cl]^- < [(CH_3)_2PO_4]^- \approx [CH_3COO]^-$. For 1,3-dialkylimidazolium ILs containing these anions, the α values range 0.4−0.63[48] and the β values range 0.21−1.20. $[C_4mim][PF_6]$ is at the lower end of the β scale, while $[C_4mim][(CH_3)_2PO_4]$, $[C_2mim][CH_3COO]$, and $[C_4mim][CH_3COO]$ exhibited very high β values. From the analysis of these values, $[C_4mim][CH_3COO]$ should be the most suitable ionic liquid to participate in hydrogen bonding. However, the high viscosity already presented by $[C_4mim][CH_3COO]$ at room temperature (i) prevented us from using even predictably better candidates such as those based on $[C_nmim]^+$, with $n > 4$, and (ii) guided us to also test $[C_2mim][CH_3COO]$ as a lower-viscosity alternative.

2. EXPERIMENTAL SECTION

2.1. Material. Adenine (6-aminopurine, ≥99%), uracil (2,4-dihydroxypyrimidine, ≥99%), and thymine (2,4-dihydroxy-5-methylpyrimidine, ≥99%) were purchased from Sigma-Aldrich (Sheinheim, Germany) and were used without further purification. $[C_2mim][CH_3COO]$ (1-ethyl-3-methyl-imidazolium acetate, >95%) and $[C_4mim][CH_3COO]$ (1-butyl-3-methyl-imidazolium acetate, ≥95%) were supplied by IoLiTec GmbH and BASF Corporation, respectively. Both 1,3-dialkylimidazolium acetate ILs were dried *in vacuo* (3×10^{-2} Torr) under

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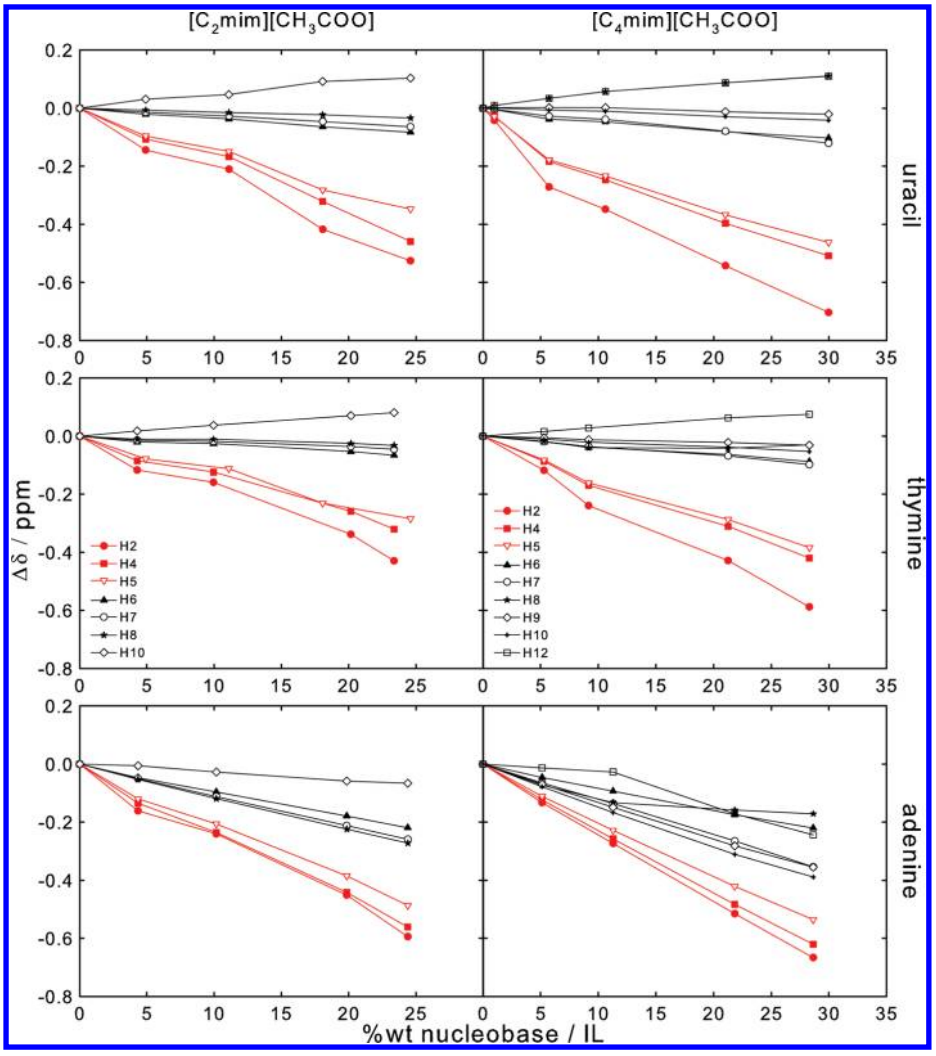

Figure 2. Trend of the chemical shift difference of protons in ^{1}H NMR of $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ with increasing nucleobase concentration ($\Delta\delta = \delta - \delta_{neat}$). The ring protons in the imidazolium cation (red) show a marked upfield shift.

vigorous stirring at 45 °C for at least 4 days and were kept under vacuum until used. The water content, determined by Karl Fischer titration, was less than 1500 ppm (0.15 water mass %) for both 1,3-dialkylimidazolium acetate ILs.

2.2. NMR Studies. Solutions of adenine, uracil, and thymine in $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ were prepared gravimetrically by heating a mixture of nucleobase in the IL to 50 °C with constant stirring. Upon complete dissolution, the samples were transferred to capillary tubes that were subsequently inserted coaxially into a 5 mm NMR tubing containing D_2O, a procedure that was required for field-frequency lock and NMR external standards. Samples of the neat ILs were prepared similarly. It is important to remark that all the NMR data were acquired *in situ* to strengthen the evidence of the proposed mechanism. With this technique, the contact between the deuterated solvent and the IL is avoided, preventing several experimental phenomena that could seriously hinder the dissolving capability of the IL for nucleobases. Another advantage of the outlined procedure when compared with the more conventional one, where the solution is inserted in NMR tubes with coaxial capillary inserts containing deuterated solvents, is the IL saving. It is important to notice that the achieved results are alike.

All experiments were carried out on a Bruker Avance 500 spectrometer operated at room temperature with 1−4 scans for ^{1}H NMR and 128−768 scans for ^{13}C NMR.

3. RESULTS AND DISCUSSION

3.1. NMR vs HB Interactions. Figures S1 and S2 in the Supporting Information show the ^{1}H NMR and ^{13}C NMR spectra of the pure $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$, respectively. The assignments were performed with 2D COSY and HSQC experiments. To achieve a better understanding of the interactions between purine or pyrimidine nucleic acid bases and $[C_2mim][CH_3COO]$ or $[C_4mim][CH_3COO]$, the effect of nucleobases on the proton and carbon chemical shifts of both ILs has been investigated. In this way, the sites of the imidazolium ring participating in the cation−nucleobase interactions and the sites of $[CH_3COO]^-$ anion involved in the anion−nucleobase interactions can be identified. It is important to remark that the analysis of the proton and the carbon chemical shifts deviations depicted throughout the paper was plotted to illustrate a specific trend, that is, an analysis to better summarize the effect of the nucleobase concentration in

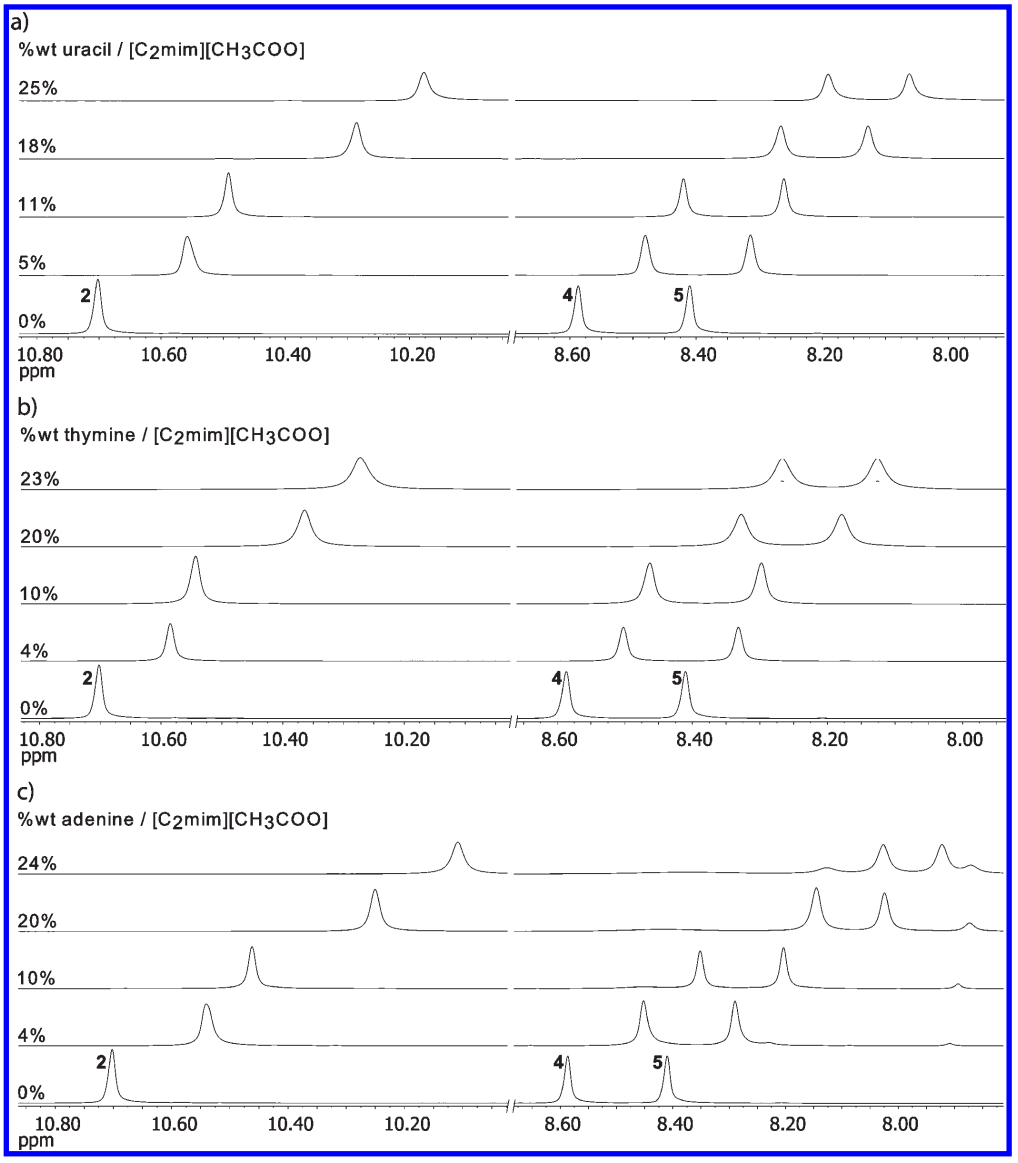

Figure 3. Effect of nucleobase concentration on the ^{1}H NMR of $[C_2mim][CH_3COO]$ spectra: (a) uracil; (b) thymine; (c) adenine. Only the ring protons (H2, H4, and H5) of the imidazolium cation are depicted, showing a marked upfield shift. The bottom spectrum of each group is the neat $[C_2mim][CH_3COO]$.

the "shielding" or "deshielding" effect on a nucleus of a specific chemical group of the ionic liquid.

The relative changes of proton chemical shifts in the $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ ^{1}H NMR spectra with increasing nucleobase (uracil, thymine, and adenine) concentration are depicted in Figure 2, and the respective ^{1}H NMR spectra of the imidazolium ring protons are presented in Figure 3 for $[C_2mim][CH_3COO]$ and Figure S3 (see the Supporting Information) for $[C_4mim][CH_3COO]$. The ring protons in the imidazolium cation show a marked upfield shift, while a negligible change is observed in methyl and ethyl protons of the cation and anion, with increasing nucleobase concentration. The most acidic H2 proton in the imidazolium cation presents the greatest upfield shift as compared to the H4 and H5 protons. At first sight, this seems contrary to what would be expected because the formation of a hydrogen bond should cause a downfield shift of the proton resonance. However, one should recall that these protons also form hydrogen bonds with the anion and these latter bonds are more stable than those between the bases and the cation.

The hydrogen bonding that occurs between cations and anions of the ionic liquids has been proven by experimental measurements and computer simulations.[54−59] The cation and anion of pure 1,3-dialkylimidazolium IL establish an interionic hydrogen-bonded network, in which the most acidic H2 proton of the imidazolium ring is involved in the strongest hydrogen bond (pK_a = 22.1 for the 1,3-dimethylimidazolium cation), followed by the other two protons (H4 and H5) of the imidazolium cation.[58−60] The partial disruption of this interionic hydrogen bond network can be observed by inserting molecular solvents such as water and dichloromethane, leading to the formation of new intermolecular hydrogen bonds.[54,57]

The effect of nucleobase concentration on the ^{13}C NMR spectra of $[C_2mim][CH_3COO]$ is summarized in Figure S4 in the Supporting Information for the ring carbons (C2, C4, and C5) of the imidazolium cation, and the distinct behavior of the

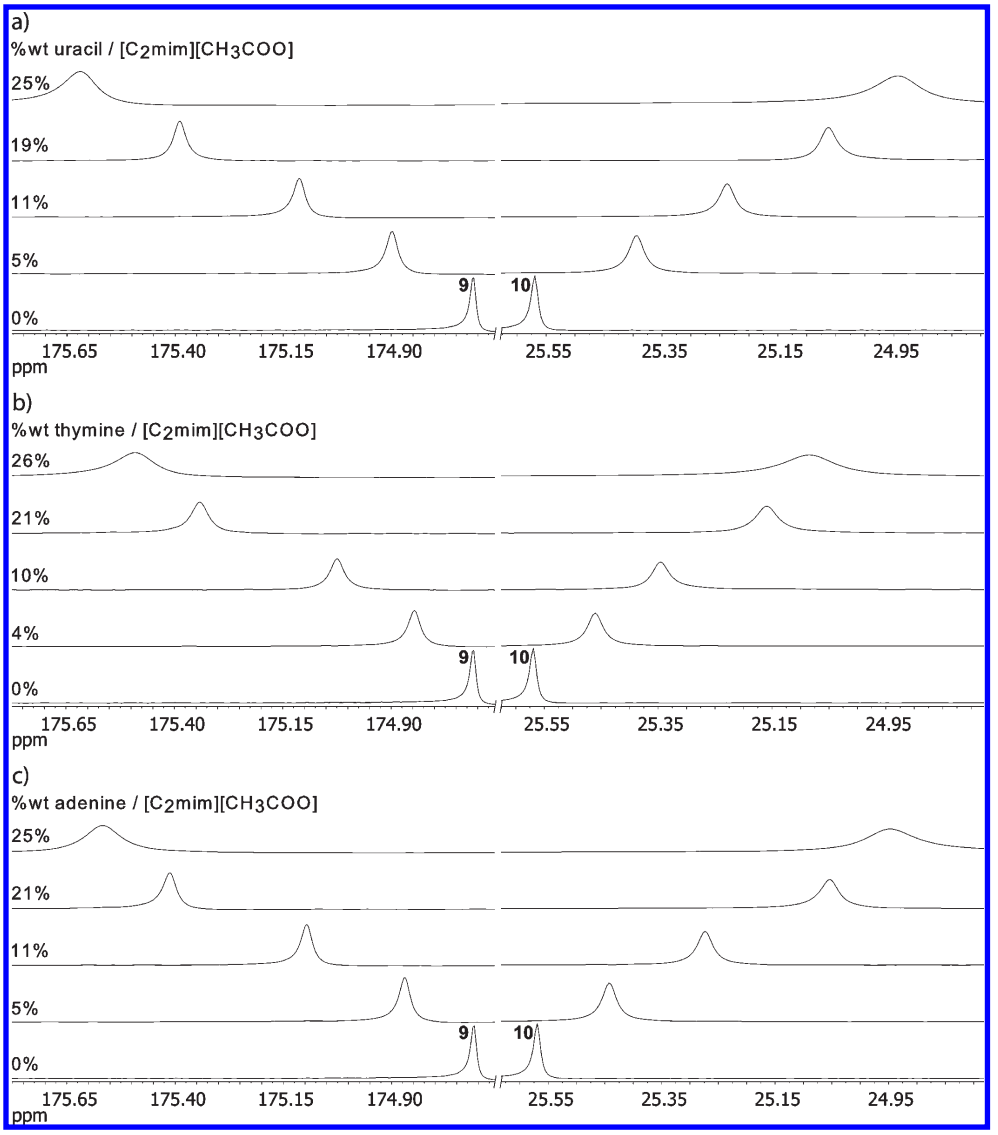

Figure 4. Effect of nucleobase concentration on the ^{13}C NMR of $[C_2mim][CH_3COO]$ spectra: (a) uracil; (b) thymine; (c) adenine. Only the anion carbons are depicted, disclosing distinct behaviors. The signal of the carboxyl (C9) moves downfield significantly, and an upfield shift of the methyl carbon (C10) is verified. The bottom spectrum of each group is the neat $[C_2mim][CH_3COO]$.

anion carbons is outlined in Figure 4. The $[C_4mim][CH_3COO]$ counterparts are depicted in Figures S5 and S6 in the Supporting Information, respectively. A summary of these results is outlined in Figure 5. The anion carbons (blue) show distinct behaviors: the signal of the carbon in the carboxyl group (C9 in $[C_2mim][CH_3COO]$ and C11 in $[C_4mim][CH_3COO]$) moves downfield significantly, while the methyl carbon (C10 in $[C_2mim][CH_3COO]$ and C12 in $[C_4mim][CH_3COO]$) presents an upfield change. The significant downfield variation on the carboxyl signal indicates that the acetate anions create stronger hydrogen bonding with nucleobases[61] than those previously observed in the pure IL between the cation and anion.

An important broadening is observed on the peaks of all the spectra depicted in the above-mentioned figures, when the nucleobase concentration is increased, that is related to the tremendous increase in the solution viscosity. At room temperature, the viscosity of the solutions of nucleobases in 1,3-dialkylimidazolium acetate ionic liquids presents, at minimum, a 15-fold increase. For example, $[C_4mim][CH_3COO]$ is already a viscous IL; its viscosity at room temperature is 485 mPa·s (measured experimentally @ SVM 3000 stabinger viscometer from Anton Paar; 440 mPa·s[62]). However, the viscosity of a 31 wt % uracil $[C_4mim][CH_3COO]$ solution at room temperature is 7402 mPa·s (measured experimentally @ SVM 3000 stabinger viscometer from Anton Paar). This trend in the viscosity increment of the nucleobases IL solution is verified for all nucleic acid bases, which is illustrated by the image of the neat $[C_4mim][CH_3COO]$ against a solution of 31 wt % thymine $[C_4mim][CH_3COO]$ depicted in Figure 6. In these pictures, the solvation process of the nucleic acid bases in 1,3-dialkylimidazolium ILs is also disclosed, showing a stable solution of thymine in $[C_4mim][CH_3COO]$ near the saturation limit and also the neat $[C_4mim][CH_3COO]$ counterpart.

The primary solvation sites of the nucleobases in 1,3-dialkylimidazolium acetate ILs can be inferred from previous experimental and theoretical studies on hydration sites of nucleic acid bases,[63,64] DFT studies on protonation, and calculations of pK_a of nucleobases,[65,66] as well as from spectroscopic studies to probe

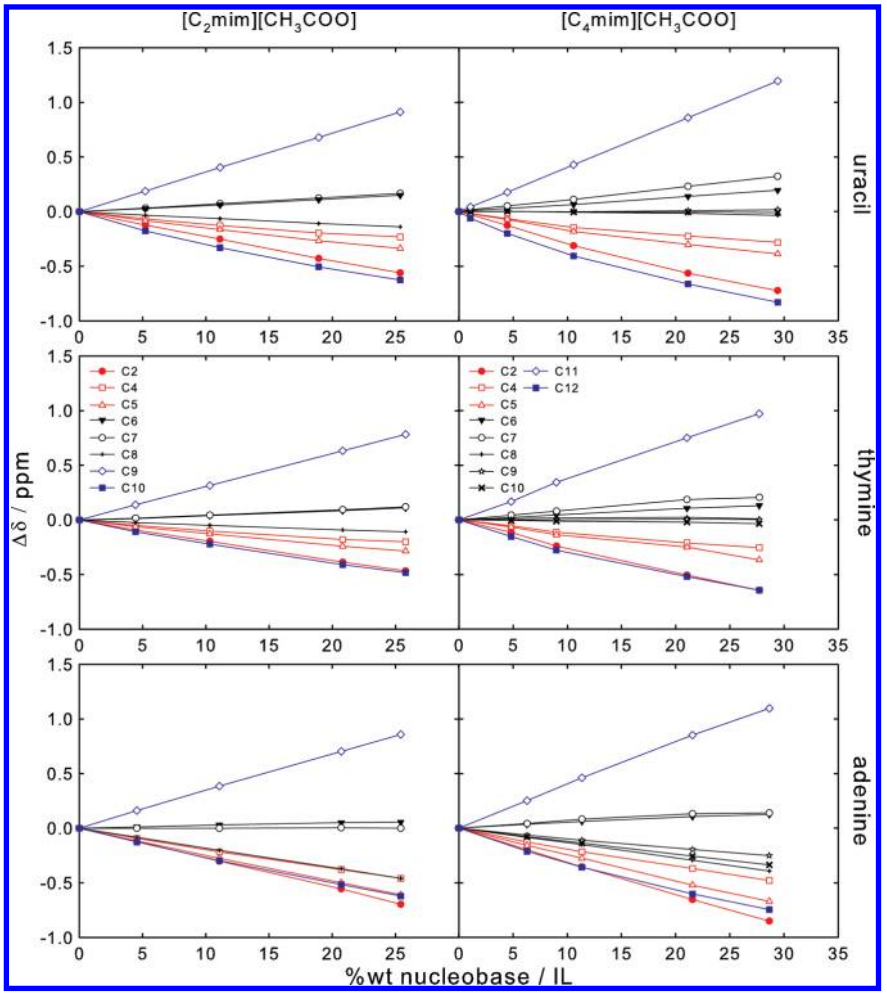

Figure 5. Trend of the chemical shift difference of carbons in ^{13}C NMR of $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ with increasing nucleobase concentration ($\Delta\delta = \delta - \delta_{neat}$). The ring carbons in the imidazolium cation (red) show a marked upfield shift. The anion carbons (blue) show distinct behaviors: the signal of the carboxyl (C9 for $[C_2mim][CH_3COO]$ and C11 for $[C_4mim][CH_3COO]$) moves downfield significantly, and the methyl carbon (C10 for $[C_2mim][CH_3COO]$ and C12 for $[C_4mim][CH_3COO]$) presents an upfield change.

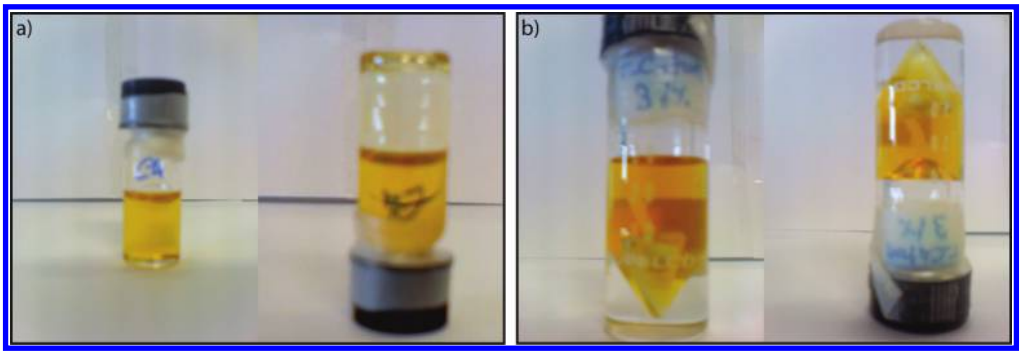

Figure 6. Image of (a) the neat $[C_4mim][CH_3COO]$ and (b) the stable solution near the saturation limit of thymine in $[C_4mim][CH_3COO]$ (31 wt % thymine $[C_4mim][CH_3COO]$). By comparing the two sets of pictures, the perception of the enormous increase in the solution viscosity with the increase of nucleobase concentration, as well as the dissolution process of the nucleic acid bases in 1,3-dialkylimidazolium ILs, is attained.

the structure and interactions of nucleobases.[67−77] However, the main evidence for the primary solvation sites arises from the Watson−Crick, reverse Watson−Crick, Hoogsteen, and reverse Hoogsteen hydrogen-bonding schemes that stabilize various nucleic acid structures. The remarkable hydrogen bonding capacity of nucleobases, evidenced through the canonical purine−pyrimidine base pairs, the key to the complementary hydrogen bonding in nucleic acids, undoubtedly highlights the solvation sites of the nucleobases in 1,3-dialkylimidazolium acetate ILs. On the basis of the aforementioned studies, it is

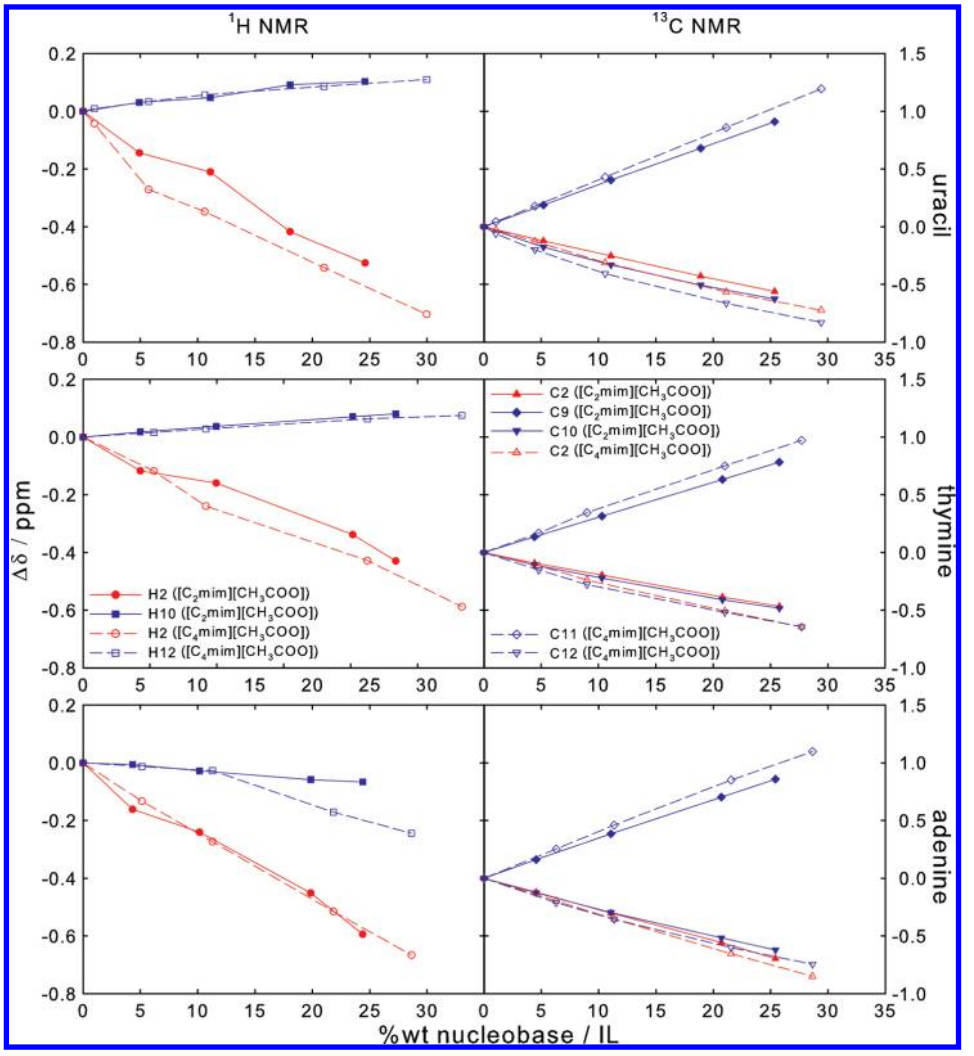

Figure 7. Trend of the chemical shift difference of protons and carbons in ^{1}H NMR and ^{13}C NMR, respectively, of $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ with increasing nucleobase concentration ($\Delta\delta = \delta - \delta_{neat}$). Only the sites of the 1,3-dialkylimidazolium acetate ILs involved in the dissolution mechanism are highlighted. The most acidic ring proton (H2) and the attached ring carbon (C2) in the imidazolium cation (red) show a marked upfield shift. The acetate protons are also depicted to indicate the small effect of nucleobase concentration on its chemical shifts. The anion carbons (blue) show distinct behavior: the signal of the carboxyl (C9 for $[C_2mim][CH_3COO]$ and C11 for $[C_4mim][CH_3COO]$) moves downfield significantly, and the methyl carbon (C10 for $[C_2mim][CH_3COO]$ and C12 for $[C_4mim][CH_3COO]$) presents an upfield change.

reasonable to assume that the acetate anions create hydrogen bonds with the hydrogen atoms of the N1−H and N3−H groups in uracil and thymine and of the amino and N9−H groups in adenine. The upfield change of the methyl carbon is mostly attributed to the increase of electron density around the C10 ($[C_2mim][CH_3COO]$) and C12 ($[C_4mim][CH_3COO]$) nuclei, because the strong hydrogen bonding between the carboxyl and the N1−H and N3−H groups in uracil and thymine and the amino and N9−H groups in adenine causes electronic redistribution of the acetate anion. The ring carbons of the imidazolium cation (C2, C4, and C5) show a marked upfield shift,[54] as depicted in Figure 5 (red); hence, the carbonyl groups in uracil and thymine and the unprotonated nitrogen atoms (N1, N3, and N7) in adenine replace the acetate anions and form weaker hydrogen bonds with the aromatic protons of the imidazolium cation.

The relatively small $[CH_3COO]^-$ anion is a good hydrogen bond acceptor and exhibits a great capability of forming hydrogen bonds, thus favoring the attack of the hydrogen atoms of the N1−H and N3−H groups in uracil and thymine and of the amino and N9−H groups in adenine. The acetate anion triggers the nucleobase−IL solvation mechanism, forming stronger hydrogen bonds with the refereed hydrogen atoms of the nucleobases, because these hydrogen atoms are stronger hydrogen bonding donors than the protons of the imidazolium cation.[78] Consequently, the interaction between the anion and the ring protons of the cation is disrupted. Subsequently, the aromatic ring protons in the bulky cations ($[C_2mim]^+$ and $[C_4mim]^+$), especially the most acidic H2 proton, establish hydrogen bonding with the oxygen atoms of carbonyl groups in uracil and thymine and with the unprotonated nitrogen atoms (N1, N3, and N7) in adenine, replacing the acetate anions. The weaker hydrogen bond acceptor ability of the mentioned groups, in comparison with that of the acetate anion, causes an upfield shift of the imidazolium ring protons.[79,80] From the foregoing discussion, it is acutely shown that the formation of hydrogen bonds between the nucleobases and 1,3-dialkylimidazolium acetate ILs affects the hydrogen atoms involved as well as the

carbon atoms around the involved groups of [C_2mim][CH_3COO] and [C_4mim][CH_3COO]. Hence, the effect of nucleobase concentration on the chemical shift difference of the aforementioned hydrogen and carbon atoms in the ^{1}H and ^{13}C NMR spectra, respectively, of both 1,3-dialkylimidazolium acetate ILs is an unequivocal method to track the suggested hydrogen bonding. In fact, the detection and analysis of the chemical shift perturbation ($\Delta\delta$) have been extensively applied to prove the existence of hydrogen bond interactions.[80−83]

As a final remark, while the nucleobase concentration increases in the nucleobase IL solutions, only the ring protons of the imidazolium cation (especially H2) show a marked shift, while the other protons of the imidazolium cation present negligible change. Obviously, this fact implies that only the aromatic hydrogens in the imidazolium cation (H2, H4, and H5), especially H2, form hydrogen bonds with the oxygen atoms of carbonyl groups in uracil and thymine and with the unprotonated nitrogen atoms (N1, N3, and N7) in adenine. It has been reported that because the aromatic hydrogens are the most acidic protons in the IL (especially H2), they can form hydrogen bonds with oxygen atoms in acetone,[84] alcohols,[85] water,[86,87] etc. Therefore, to attribute the upfield shift of the imidazolium ring protons to the disruption of the interionic hydrogen bond between the IL cation and anion and the formation of weaker ones involving the IL cation and the activated nucleobase sites, is a reasonable assumption. Moreover, as Barthel et al.[88] observed in their studies concerning the application of IL as reaction medium for cellulose functionalization, if the H2 is substituted by other groups, the dissolving capability of ILs for nucleobases will decrease. In addition, the molecular dynamics (MD) data from Youngs et al.[89] have shown that weak but non-negligible hydrogen bonding interactions between glucose and 1,3-dimethylimidazolium IL cation are observable. These results prove that the activated protons in the IL cation hydrogen bond with the activated sites of the nucleobases and that the effect of the IL cation on nucleobases solubility cannot be ignored.

Other interactions, such as $\pi-\pi$ interactions and C−H−π interactions, given the similarity of the imidazolium cation to the nucleobases ring, may be a hypothesis of interaction with this type of ionic liquid cation, but they cannot explain all the aforementioned results. Additionally, both experimental and theoretical results from Leist et al.[90] show that π stacking is not the dominant binary intermolecular interaction of fluorobenzene with the nucleobase; as long as hydrogen bonds can be formed, the hydrogen bonding mode is always utilized. This result, hydrogen bonding wins over π stacking, is in concordance with our observations and proves that the principal nucleobase−ionic liquid interactions occurring in the system are hydrogen bonds.

As pointed out in the Introduction, according to the α (the HBD ability) and β (the HBA ability) values of the Kamlet−Taft parameters (Table S1 in the Supporting Information), [C_4mim][CH_3COO] should be the most suitable ionic liquid to participate in hydrogen bonding. This statement is unequivocally corroborated by the analysis illustrated in Figure 7, where the relative changes of proton and carbon chemical shifts in the ^{1}H NMR and ^{13}C NMR spectra of both 1,3-dialkylimidazolium acetate ILs, respectively, with increasing nucleobase concentration are depicted. By comparing the variation trend of the chemical shift difference of the sites involved in the dissolution mechanism for [C_2mim][CH_3COO] and [C_4mim][CH_3COO], it is straightforwardly concluded that [C_4mim][CH_3COO] presents the higher dissolution capacity (i.e. the ability to form stronger hydrogen bonds) due to the steeper slope of the variation trend.

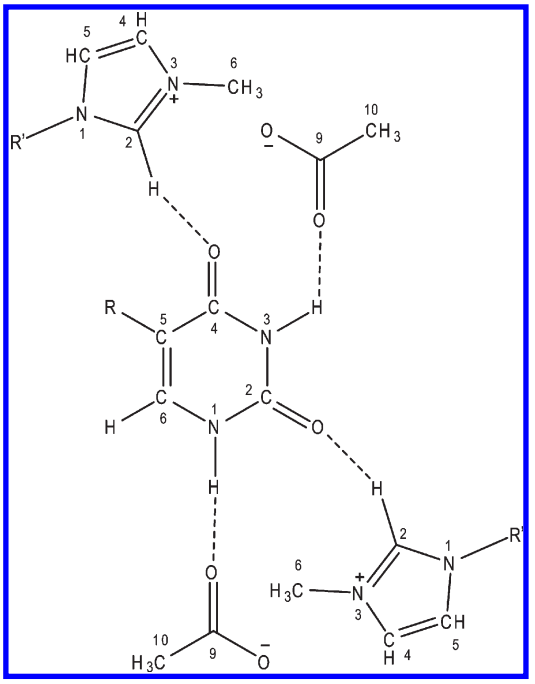

Figure 8. Schematic illustration of the primary solvation sites of uracil (R = H) and thymine (R = CH_3) with [C_2mim][CH_3COO] (R′ = C_2H_5) or [C_4mim][CH_3COO] (R′ = C_4H_9). Hydrogen bonding between the hydrogen atoms of the N1−H and N3−H groups of uracil and thymine and the $[CH_3COO]^-$ anion and between the most acidic hydrogen (H2) of the imidazolium cation and the oxygen atoms of the carbonyl groups of uracil and thymine causes the solvation.

3.2. Dissolution Mechanism. On the basis of the NMR studies discussed in the previous section, we conclude that the hydrogen bonding between the hydrogen atoms of the N1−H or N3−H groups in uracil or thymine and of the amino and N9−H groups in adenine and the acetate anion is the main driving force for nucleic acid base dissolution into 1,3-dialkylimidazolium acetate ILs. However, the hydrogen bonding between the oxygen atoms of carbonyl groups in uracil or thymine and between the unprotonated nitrogen atoms (N1, N3, and N7) in adenine and the ring protons of the imidazolium cation cannot be neglected. On the basis of the canonical purine−pyrimidine base pairs, where the considered purine nucleobase, adenine, binds to thymine or uracil (studied pyrimide nucleobases) with the help of two hydrogen bonds, while guanine specifically recognizes cytosine with the help of three hydrogen bonds, it is also reasonable to conclude that guanine and cytosine can be solubilized in 1,3-dialkylimidazolium acetate ILs and that hydrogen bonding between guanine and cytosine and both the acetate anion and imidazolium cation participate in the nucleobases dissolution into ILs.

It is worthwhile now to go a step forward and explore the saturation molar ratios of nucleobase/IL, obtained using the solubility[91] of nucleobases in 1,3-dialkylimidazolium acetate ILs at 25 °C. The stoichiometric ratio of nucleobase to [C_2mim][CH_3COO] and nucleobase to [C_4mim][CH_3COO] is 1:2 for uracil and thymine and 1:3 for adenine. A concise depiction of the dissolution process of the nucleobases in

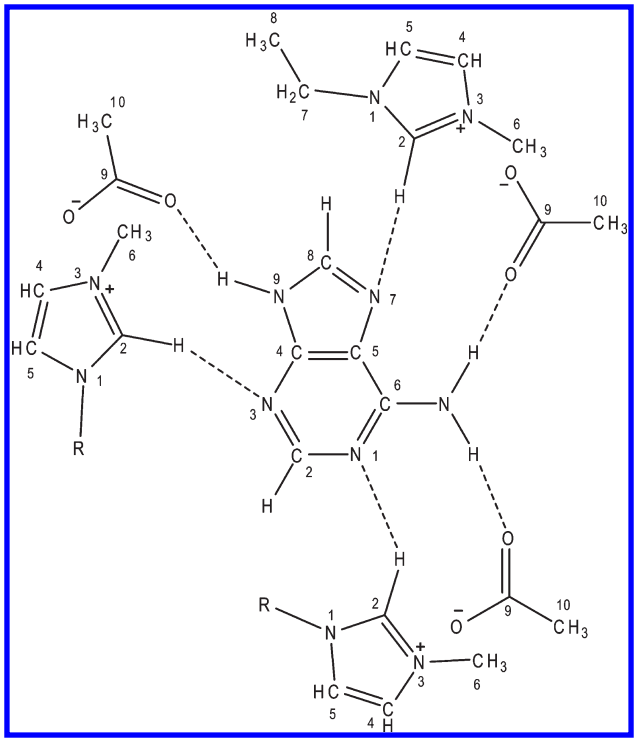

Figure 9. Schematic illustration of the primary solvation sites of adenine with $[C_2mim][CH_3COO]$ (R = C_2H_5) or $[C_4mim][CH_3COO]$ (R = C_4H_9). Hydrogen bonding between the hydrogen atoms of the amino and N9−H groups of adenine and the $[CH_3COO]^-$ anion and between the most acidic hydrogen (H2) of the imidazolium cation and the unprotonated nitrogen atoms (N1, N3, and N7) of adenine causes the solvation.

$[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$ is summarized in Figures 8 and 9.

Accordingly, to maximize the solubility of nucleic acid bases, the IL should fulfill at least three conditions: (a) the anion must be a good hydrogen bond acceptor; (b) the cation should be a moderate hydrogen bond donor with a sufficiently high dissociation degree; and (c) the cation size should be relatively small.

4. CONCLUSIONS

The results established in the current study provide straightforward evidence that hydrogen bonding between nucleobases and the acetate anion is the major driving force for nucleic acid bases dissolution into 1,3-dialkylimidazolium acetate ILs. However, hydrogen bonding between nucleobases and the imidazolium cation also participates in the dissolution process, and the effect of the IL cation on nucleobases solubility cannot be neglected. The relatively small $[CH_3COO]^-$ anion, a good hydrogen bond acceptor exhibiting a great capability of forming hydrogen bonds, pairs with the hydrogen atoms of the N1−H and N3−H groups in uracil and thymine and with the amino and N9−H groups in adenine. The ring protons of the bulky imidazolium cations ($[C_2mim]^+$ and $[C_4mim]^+$), especially the H2 proton, prefer to pair with the oxygen atoms of the carbonyl groups in uracil and thymine and with the unprotonated nitrogen atoms (N1, N3, and N7) in adenine. Accordingly, the ionic liquid must be a good hydrogen bond acceptor and a moderate hydrogen bond donor with a dissociation degree sufficiently high to dissolve the bases.

The possibilities of increasing the solvation capacity of the desired IL are outstanding, merely by using their intrinsic modular nature. This can be achieved by fine-tuning the nature of the anion and/or cation. This issue will be address in future work, as well as the analysis of the solvation sites of purine (adenine) and pyrimidines (uracil and thymine) in 1,3-dialkylimidazolium acetate ILs by DFT methods.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^1H NMR and ^{13}C NMR spectra and chemical shifts (ppm) of $[C_2mim][CH_3COO]$ and $[C_4mim][CH_3COO]$, and the effect of nucleobases concentration upon the chemical shifts in D_2O at 25 °C . This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Freemantle, M. *Chem. Eng. News* **1998**, *3*, 32.
(2) Blanchard, L. A.; Hancu, D.; Beckman, E. J.; Brennecke, J. F. *Nature* **1999**, *399*, 28.
(3) Kazarian, S. G.; Briscoe, B. J.; Welton, T. *Chem. Commun.* **2000**, 2047.
(4) Blanchard, L. A.; Brennecke, J. F. *Ind. Eng. Chem. Res.* **2001**, *40*, 287.
(5) Myasoedov, B. F.; Molochnikova, N. P.; Shkinev, V. M.; Spivakov, B. Y. *The Behavior of Actinides in Two-Phase Aqueous Systems Based on Polyethylene Glycol*; Plenum: New York, 1995.
(6) Rogers, R. D.; Bond, A. H.; Bauer, C. B.; Zhang, J.; Rein, S. D.; Chomko, R. R.; Roden, D. M. *Solvent Extr. Ion Exch.* **1995**, *13*, 689.
(7) Willauer, H. D.; Huddleston, J. G.; Griffin, S. T.; Rogers, R. D. *Sep. Sci. Technol.* **1999**, *34*, 1069.
(8) Huddleston, J. G.; Willauer, H. D.; Swatloski, R. P.; Visser, A. E.; Rogers, R. D. *Chem. Commun.* **1998**, 1765.
(9) Rogers, R. D.; Visser, A. E.; Swatloski, R. P.; Hartman, D. H. In *Metal Ion Separations Beyond 2000: Integrating Novel Chemistry With Processing*; Liddell, K. C., Chaiko, D. J. Eds.; The Minerals, Metals, and Materials Society: Warrendale, PA, 1999.
(10) Visser, A. E.; Swatloski, R. P.; Rogers, R. D. *Green Chem.* **2000**, *1*, 1.
(11) Visser, A. E.; Swatloski, R. P.; Reichert, W. M.; Griffin, S. T.; Rogers, R. D. *Ind. Eng. Chem. Res.* **2000**, *39*, 3596.
(12) Welton, T. *Chem. Rev.* **1999**, *99*, 2071.
(13) Plechkova, N. V.; Seddon, K. R. *Chem. Soc. Rev.* **2008**, *37*, 123.

(14) Dutta, L. M. M. Phil. Thesis; School of Chemistry and Molecular Sciences, University of Sussex: Brighton, 1994.
(15) Rebelo, L. P. N.; Lopes, J. N.; Esperança, J. M. S. S.; Guedes, H. J. R.; Lachwa, J.; Najdanovic-Visak, V.; Visak, Z. P. *Acc. Chem. Res.* **2007**, *40*, 1114.
(16) Shimizu, K.; Gomes, M. F. C.; Pádua, A. A. H.; Rebelo, L. P. N.; Lopes, J. N. C. *THEOCHEM* **2010**, *946*, 70.
(17) Lopez-Martin, I.; Burello, E.; Davey, P. N.; Seddon, K. R.; Rothenberg, G. *ChemPhysChem* **2007**, *8*, 690.
(18) Earle, M. J.; Esperança, J. M. S. S.; Gilea, M. A.; Lopes, J. N. C.; Rebelo, L. P. N.; Magee, J. W.; Seddon, K. R.; Widegren, J. A. *Nature* **2006**, *439*, 831.
(19) Wasserscheid, P.; Keim, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 3772.
(20) Seddon, K. R. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351.
(21) Hagiwara, R.; Ito, Y. *J. Fluorine Chem.* **2000**, *105*, 221.
(22) Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D. *Green Chem.* **2001**, *3*, 156.
(23) Baudequin, C.; Baudoux, J.; Levillain, J.; Cahard, D.; Gaumont, A.-C.; Plaquevent, J.-C. *Tetrahedron: Asymmetry* **2003**, *5*, 3081.
(24) De Souza, R. F.; Padilha, J. C.; Gonçalves, R. S.; Dupont, J. *Electrochem. Commun.* **2003**, *5*, 728.
(25) Wang, P.; Zakeeruddin, S. M.; Comte, P.; Exnar, I.; Gratzel, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 1166.
(26) Armand, M.; Endres, F.; MacFarlane, D. R.; Ohno, H.; Scrosati, B. *Nat. Mater.* **2009**, *8*, 621.
(27) Dyson, P. J.; McIndoe, J. S.; Zhao, D. B. *Chem. Commun.* **2003**, 508.
(28) Lozano, P.; de Diego, T.; Carrie, D.; Vaultier, M.; Iborra, J. L. *Biotechnol. Prog.* **2003**, *19*, 380.
(29) Abraham, M. H.; Zissimos, A. M.; Huddleston, J. G.; Willauer, H. D.; Rogers, R. D.; Acree, W. E. *Ind. Eng. Chem. Res.* **2003**, *42*, 413.
(30) Garcia, H.; Ferreira, R.; Petkovic, M.; Ferguson, J. L.; Leitão, M. C.; Gunaratne, H. Q. N.; Seddon, K. R.; Rebelo, L. P. N.; Pereira, C. S. *Green Chem.* **2010**, *12*, 367.
(31) Mjewski, P.; Pernak, A.; Grzymislawski, M.; Iwanik, K.; Pernak, J. *Acta Histochem.* **2003**, *105*, 35.
(32) Banks, J. F., Jr.; Whitehouse, C. M. *Int. J. Mass Spectrom. Ion Processes* **1997**, *162*, 163.
(33) Davies, R. G.; Gibson, V. C.; Hursthouse, M. B.; Ligth, M. E.; Marshall, E. L.; North, M.; Robson, D. A.; Thompson, I.; White, A. J. P.; Williams, D. J.; Williams, P. J. *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 3365.
(34) Baker, E. A.; Hayes, A. L.; Butler, R. C. *Pestic. Sci.* **1992**, *34*, 167.
(35) Zielenkiewicz, W.; Poznanski, J.; Zielenkiewicz, A. *J. Solution Chem.* **2000**, *29*, 757.
(36) Ganguly, S.; Kundu, K. K. *J. Phys. Chem.* **1993**, *97*, 10862.
(37) Ganguly, S.; Kundu, K. K. *Indian J. Chem., Sect. A* **1995**, *34*, 857.
(38) Ganguly, S.; Kundu, K. K. *Indian J. Chem., Sect. A* **1996**, *34*, 423.
(39) Watson, J. D.; Crick, F. H. *Nature* **1953**, *171*, 737.
(40) Saenger, W. *Principals of Nucleic Acid Acid Structure*; Springer-Verlag: New York, 1984.
(41) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, Heidelberg, New York, 1991.
(42) De Pascuale, R. J. *Ind. Eng. Chem. Prod. Res. Dev.* **1978**, *17*, 278.
(43) Vijayaraghavan, R.; Izgorodin, A.; Ganesh, V.; Surianarayanan, M.; MacFarlane, D. *Angew. Chem., Int. Ed.* **2010**, *49*, 1631.
(44) Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877.
(45) Kamlet, M. J.; Abboud, J. L. M.; Taft, R. W. *J. Am. Chem. Soc.* **1977**, *99*, 6027.
(46) Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* **1976**, *98*, 377.
(47) Taft, R. W.; Kamlet, M. J. *J. Am. Chem. Soc.* **1976**, *98*, 2886.
(48) Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T. *Phys. Chem. Chem. Phys.* **2003**, *5*, 2790.
(49) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. *J. Am. Chem. Soc.* **2002**, *124*, 4974.
(50) Fukaya, Y.; Sugimoto, A.; Ohno, H. *Biomacromolecules* **2006**, *7*, 3295.
(51) Fukaya, Y.; Hayashi, K.; Wada, M.; Ohno, H. *Green Chem.* **2008**, *10*, 44.
(52) *CRC Handbook of Chemistry and Physics*, 89th ed.; Lide, D. R., Ed.; 2008−2009.
(53) *Ionic Liquids in Synthesis*; Wasserscheid, P., Welton, T., Eds.; Wiley-VCH: Weinheim, Germany, 2003.
(54) Avent, A. G.; Chaloner, P. A.; Day, M. P.; Seddon, K. R.; Welton, T. *J. Chem. Soc., Dalton Trans.* **1994**, 3405.
(55) Dong, K.; Zhang, S. J.; Wang, D.; Yao, X. Q. *J. Phys. Chem. A* **2006**, *110*, 9775.
(56) Dieter, K. M.; Dymek, C. J.; Heimer, N. E.; Rovang, J. W.; Wilkes, J. S. *J. Am. Chem. Soc.* **1988**, *110*, 2722.
(57) Singh, T.; Kumar, A. *J. Phys. Chem. B* **2007**, *111*, 7843.
(58) Dupont, J. *J. Braz. Chem. Soc.* **2004**, 15.
(59) Headley, A. D.; Jackson, N. M. *J. Phys. Org. Chem.* **2002**, *15*, 52.
(60) Chu, Y.; Deng, H.; Cheng, J. P. *J. Org. Chem.* **2007**, *72*, 7790.
(61) Hagen, R.; Roberts, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 4504.
(62) Crosthwaite, J. M.; Muldoon, M. J.; Dixon, J. K.; Anderson, J. L.; Brennecke, J. F. *J. Chem. Thermodyn.* **2005**, *37*, 559.
(63) Bencivenni, L.; Ramondo, F.; Pieretti, A.; Sanna, N. *J. Chem. Soc., Perkin Trans.* **2000**, *2*, 1685.
(64) Nosenko, Y.; Kunitski, M.; Riehn, C.; Harbach, P. H. P.; Dreuw, A.; Brutschy, B. *Phys. Chem. Chem. Phys.* **2010**, *12*, 863.
(65) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2000**, *104*, 7346.
(66) Verdolino, V.; Cammi, R.; Munk, B. H.; Schlegel, H. B. *J. Phys. Chem. B* **2008**, *112*, 16860.
(67) Billinghurst, B. E.; Oladepo, S. A.; Loppnow, G. R. *J. Phys. Chem. B* **2009**, *113*, 7392.
(68) Yarasi, S.; Brost, P.; Loppnow, G. R. *J. Phys. Chem. A* **2007**, *111*, 5130.
(69) Billinghurst, B. E.; Yeung, R.; Loppnow, G. R. *J. Phys. Chem. A* **2006**, *110*, 6185.
(70) Billinghurst, B. E.; Loppnow, G. R. *J. Phys. Chem. A* **2006**, *110*, 2353.
(71) Fodor, S. P. A.; Rava, R. P.; Hays, T. R.; Spiro, T. G. *J. Am. Chem. Soc.* **1985**, *107*, 1520.
(72) Fodor, S. P. A.; Spiro, T. G. *J. Am. Chem. Soc.* **1986**, *108*, 3198.
(73) Perno, J. R.; Grygon, C. A.; Spiro, T. G. *J. Phys. Chem.* **1989**, *93*, 5672.
(74) Kubasek, W. L.; Hudson, B.; Peticolas, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2369.
(75) Myers, A. B.; Mathies, R. A. In *Biological Applications of Ramam Spectroscopy, Resonance Ramam Spectra of Polyenes and Aromatics*, Vol. 2; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1987.
(76) Myers, A. B. Excited Electronic State Properties from Ground-State Resonance Raman Intensities. In *Laser Techniques in Chemistry*; Myers, A.B., Rizzo, T. R., Eds.; Wiley: New York, 1995.
(77) Kelley, A. M. *J. Phys. Chem. A* **1999**, *103*, 6891.
(78) Handy, S. T.; Okello, M. *J. Org. Chem.* **2005**, *70*, 1915.
(79) Shimura, H.; Yoshio, M.; Hoshino, K.; Mukai, T.; Ohno, H.; Kato, T. *J. Am. Chem. Soc.* **2008**, *130*, 1759.
(80) Chierotti, M. R.; Gobetto, R. *Chem. Commun.* **2008**, 1621.
(81) Avent, A. G.; Chaloner, P. A.; Day, M. P.; Seddon, K. R.; Welton, T. *J. Chem. Soc., Dalton Trans.* **1994**, 3405.
(82) Yashima, E.; Yamamoto, C.; Okamoto, Y. *J. Am. Chem. Soc.* **1996**, *118*, 4036.
(83) McCormick, C. L.; Callais, P. A.; Hutchinson, B. H. *Macromolecules* **1985**, *18*, 2394.
(84) Zhai, C. P.; Wang, J. J.; Xuan, X. P.; Wang, H. Q. *Acta Phys.—Chim. Sin.* **2006**, *22*, 456.
(85) Crosthwaite, J. M.; Aki, S. N. V. K.; Maginn, E. J.; Brennecke, J. F. *J. Phys. Chem. B* **2004**, *108*, 5113.
(86) Mele, A.; Tran, C. D.; De Paoli Lacerda, S. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4364.
(87) Wu, B.; Liu, Y.; Zhang, Y. M.; Wang, H. P. *Chem.—Eur. J.* **2009**, *15*, 6889.
(88) Barthel, S.; Heinze, T. *Green Chem.* **2006**, *8*, 301.

(89) Youngs, T. G. A.; Hardacre, C.; Holbrey, J. D. *J. Phys. Chem. B* **2007**, *111*, 13765.
(90) Leist, R.; Frey, J. A.; Ottiger, P.; Frey, H.-M.; Leutwyler, S.; Bachorz, R. A.; Klopper, W. *Angew. Chem., Int. Ed.* **2007**, *46*, 7449.
(91) Araújo, J. M. M.; Veiga, H. I. M.; Esperança, J. M. S. S.; Marrucho, I. M.; Rebelo, L. P. N. *J. Chem. Eng. Data* **2011**, submitted for publication.