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Spectroscopic Studies on Tetracycline in Room-Temperature Ionic Liquids

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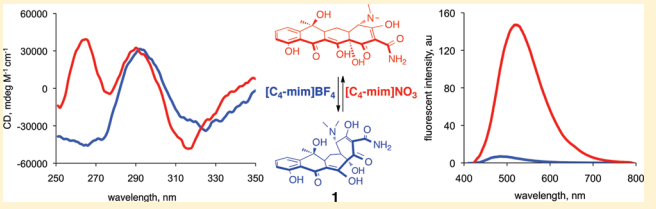
Supporting Information

ABSTRACT: Circular dichroism and steady-state fluorescence of tetracycline (**1**) were investigated in a series of 1-butyl-3-methylimidazolium room-temperature ionic liquids. The identity of the anion of the ionic liquids was found to modulate the conformation as well as the emission intensity of tetracycline over a wide range.



Tetracycline, **1** (Figure 1), is a widely used antibiotic with a broad spectrum of activity.[1] Recently, **1** was shown to act as an inhibitor of various amyloid oligomer- and fibril-formation processes, which are responsible for the progression of Alzheimer's and prion diseases.[2−7] Metal complexes of **1** have also been used as fluorescent probes for investigation of various biomolecular interactions.[8−12]

As a free base in solution, tetracycline exists in many tautomeric and conformationally distinct forms.[13,14] Numerous spectroscopic and theoretical studies have suggested that a primary equilibrium exists between the extended and twisted forms (Figure 1),[13−19] although each form is likely to be represented by a collection of tautomers. This equilibrium is controlled by the nature of the environment as well as by metal ions and/or biomolecules.[15−19] Since the two forms appear to have different spectroscopic features, it is possible to monitor this equilibrium using spectroscopic techniques. Specifically, circular dichroism (CD) spectroscopic studies have indicated that the movement of the A-ring (Figure 1) could be monitored by changes around the 260 nm region: a positive peak has been assigned to an extended conformation, whereas the negative peak has been attributed to the twisted conformation.[16] It has also been shown that binding of Ca^{2+} to **1** promotes the extended conformation.[16−19] In addition, organic solvents, such as dimethylsulfoxide (DMSO), promote the extended conformation. An interaction between the dimethylamino group and OH-12a in DMSO is believed to favor the extended conformation of **1**. Conceptually, the ability to control a conformation of **1** by simply changing the solvent constitutes an interesting paradigm that could be useful for materials, biochemical, and drug delivery applications.

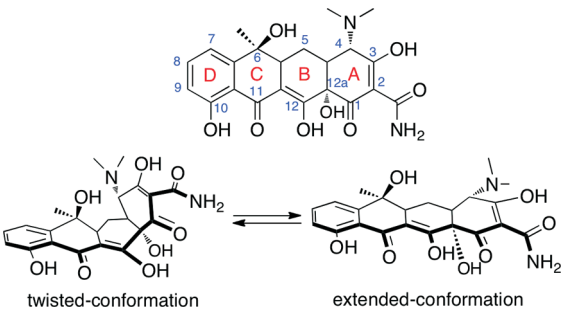


Figure 1. Structure of tetracycline (**1**) and a simplified conformational equilibrium.

Previous studies report the effect of medium on the emission intensity of **1**. It was shown that a pH-dependent extended-to-twisted conformational change of tetracycline is accompanied by an increase of the fluorescence intensity of the Ca^{2+}-tetracycline complex.[19] The effect of organic solvents on the emission intensity of tetracycline−metal complexes has received some attention as well.[20] Within a set of 14 molecular solvents, including water, alcohols, acetone, acetonitrile, DMF, DMSO, THF, and pyridine, ca. 2−6-fold increases in the emission intensity for a Li^{+}−tetracycline complex were observed.

Volatility and limited structural variations within common molecular solvents do not allow for extensive investigation of solvent effects on the spectroscopic properties of **1**. Hence, it was decided to examine the effect of ionic solvents, namely, room-temperature ionic liquids (often referred to as ionic liquids)[21,22] on the spectroscopic properties of tetracycline. From the molecular point of view, ionic liquids are composed exclusively of ions and have phase-transition temperatures below or around room temperature. The physicochemical properties of ionic liquids can be controlled by variation of the structure and identity of both the cationic and anionic counterparts. Ease of synthesis and a high degree of structural and functional diversity of the ions allow for facile access to a large number of ionic liquids with a wide range of properties, a feature that is inaccessible with the

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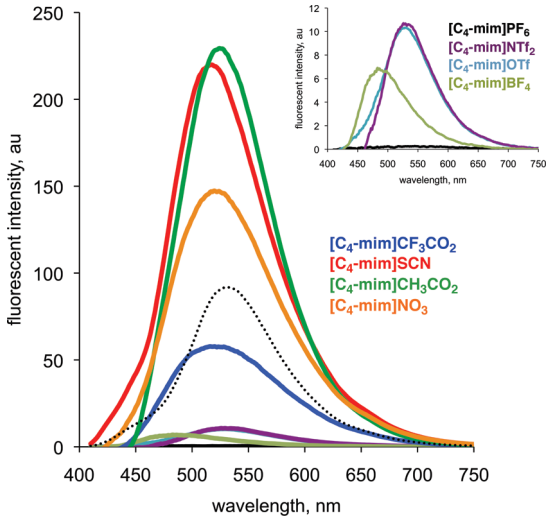


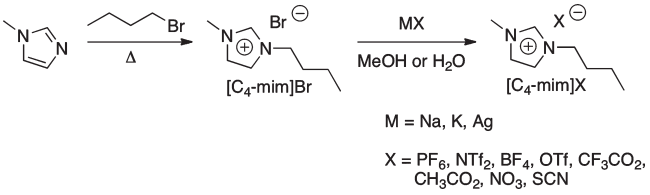
Figure 2. Emission spectra of tetracycline (**1**, 20 μM) in ionic liquids (DMSO content 1% v/v); λ_{ex} = 400 nm. Inset shows the expanded region for [C_4-mim]BF_4, [C_4-mim]PF_6, [C_4-mim]NTf_2, and [C_4-mim]OTf. The emission of **1** in DMSO (dotted line) is given for comparison purposes.

molecular organic solvents.[21,22] Due to this property, ionic liquids are often referred to as "designer solvents".[21,23] The applications of ionic liquids span a large number of fields and have become particularly attractive as a complement to molecular organic solvents for various synthetic, analytical, electrochemical, and biochemical processes.[21−25] Recent accounts on the use of ionic liquids for solubilization of biomolecules, protein formulation, and drug delivery systems have demonstrated their feasibility for pharmaceutical applications.[26−29] Since natural products and natural product-based compounds constitute a significant proportion of available modern drugs, it is imperative to gain an understanding on how the structure of ionic liquids could affect properties of drug molecules. Furthermore, the use of ionic liquids for natural product oriented research has received limited attention. Primarily, the utilization of ionic liquids for the isolation and/or separation of several classes of natural products has been reported.[30−35] Some natural product-based ionic liquids have also been developed.[36,37] However, spectroscopic studies on natural products in ionic liquids is an underexplored area of research. Considering the great structural and functional diversity of natural products, along with tunable physical properties of ionic liquids, the development of interesting and potentially useful natural product−ionic liquid systems can be anticipated. Herein, we report initial findings on the ability of ionic liquids to modulate the conformational equilibrium of tetracycline.

Due to the unexplored nature of this topic, it was decided to use a set of ionic liquids, based on one of the most commonly used cationic components, i.e., 1-butyl-3-methylimidazolium, [C_4-mim]. Toward this end, we prepared several [C_4-mim]X ionic liquids (Scheme 1), using literature protocols.[38−41]

It was reasoned that if similar spectroscopic trends are observed in ionic liquids and molecular solvents, one could suggest that **1** undergoes a similar conformational change in ionic liquids as in molecular solvents. Specifically, CD studies have indicated that the presence of a negative transition around 260 nm range could be due to the bending of the ring A over the BCD-ring system, i.e., adopting a twisted conformation.[15−19] Therefore, the presence of a positive peak at 260 nm in the CD spectrum of **1**

Scheme 1. Synthesis of Ionic Liquids



in an ionic liquid would be indicative of an extended conformation of **1** in that ionic liquid. Conversely, a negative transition at 260 nm would indicate that **1** predominantly adopts a twisted conformation in that particular ionic liquid. In accord with this rationale, the CD spectrum of tetracycline in DMSO (a solvent that promotes an extended conformation of **1**) and water (a solvent that promotes a twisted conformation of **1**) exhibited opposite trends (Supporting Information, Figure S1). With respect to fluorescence, **1** showed a weak emission in water, whereas in DMSO the emission intensity was increased significantly (Supporting Information, Figure S1). On the basis of literature accounts, the correlations between the conformation of **1** and the corresponding emission properties are not straightforward and might depend on the nature and amounts of the additives, such as metal ions.[15−19]

First, it was decided to examine whether the ionic liquid environment could modulate the emission intensity of **1**. Since **1** (as a free base) does not possess sufficient solubility in all of the ionic liquids, utilization of a co-solvent was necessary. Toward this end, we prepared a stock solution of **1** in DMSO (a solvent that is miscible with all of the above ionic liquids), which was introduced into the ionic liquid. The final concentration of DMSO was ca. 1% v/v, which arguably should not interfere with the effects of the ionic liquids.

The emission spectra of **1** (Figure 2) revealed that ionic liquids might be differentiated into two distinct categories. As compared to DMSO, the emission of **1** was significantly quenched in [C_4-mim]BF_4, [C_4-mim]PF_6, [C_4-mim]NTf_2, and [C_4-mim]OTf ionic liquids. However, a drastically enhanced emission was observed in [C_4-mim]NO_3, [C_4-mim]CF_3CO_2, [C_4-mim]CH_3CO_2, and [C_4-mim]SCN (Figure 2).

It should be pointed out that ionic liquids are fluorescent. The origin of the intrinsic fluorescence of ionic liquids remains to be clarified, and it has been attributed to the presence of nanoscale aggregates[42,43] as well as impurities.[44] Thus, the ionic liquids utilized here were purified according to established protocols.[42−44] The emission spectra of tetracycline in ionic liquids were background-subtracted to eliminate any potential interference from the solvent.

Polarity and viscosity of the medium are among the main physical properties of a solvent that could control the emission of a dissolved molecule. However, there appears to be no correlation between these physical properties of ionic liquids and the emission intensity of **1** (Supporting Information, Table S1).

Next, CD spectroscopy was used to probe whether the ionic liquids could modulate the conformational equilibrium of **1** and favor either a twisted or an extended form. Notably, ionic liquids possess significant absorbance in the far-UV region, which led to the appearance of artifacts in the CD spectra of ionic liquids. Arguably, this is due to the presence of the imidazolium chromophore and/or the nature of the anion. In addition, ionic liquids

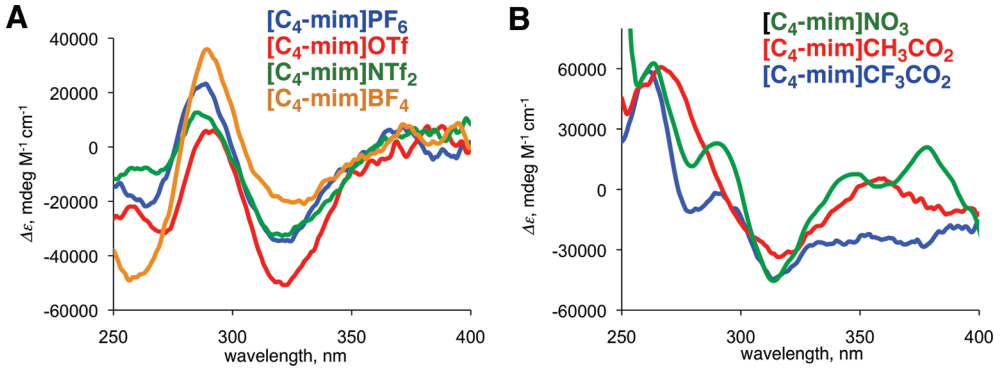


Figure 3. CD spectra of tetracycline (**1**, 200 μM) in ionic liquids (DMSO content 1% v/v).

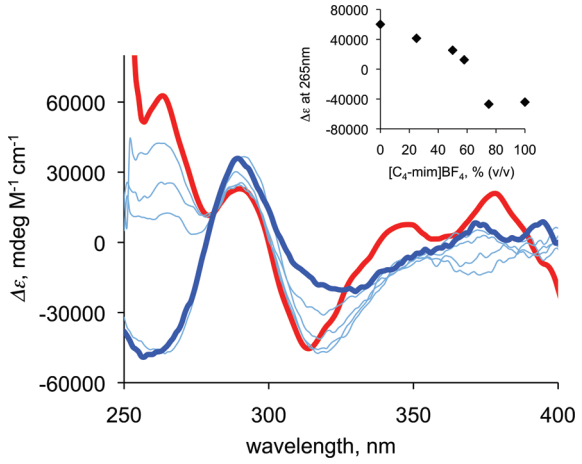


Figure 4. CD spectra of tetracycline (**1**) in $[C_4\text{-mim}]NO_3$–$[C_4\text{-mim}]BF_4$ mixtures (DMSO content 1% v/v): 100% $[C_4\text{-mim}]BF_4$, blue line; 100% $[C_4\text{-mim}]NO_3$, red line; intermediate compositions, light blue thin lines; inset shows the change of the 265 nm intensity as a function of the increasing concentration of $[C_4\text{-mim}]BF_4$.

are known to form nanoscale aggregates[42,43] that are likely to scatter light. The use of a 0.01 cm path length cell resulted in artifact-free baseline spectra for all of the ionic liquids.

The CD spectra of **1** in ionic liquids indicated that $[C_4\text{-mim}]PF_6$, $[C_4\text{-mim}]NTf_2$, and $[C_4\text{-mim}]OTf$ promoted a twisted conformation of **1**, as judged by the presence of a negative transition at 260 nm (Figure 3A). Notably, a similar spectrum was obtained in water (Supporting Information, Figure S1), a solvent that promotes the twisted conformation of **1**.

On the contrary, in $[C_4\text{-mim}]NO_3$, $[C_4\text{-mim}]CF_3CO_2$, and $[C_4\text{-mim}]CH_3CO_2$, the CD spectra exhibited maxima in the 260–270 nm range (Figure 3B). Due to a high absorbance of $[C_4\text{-mim}]SCN$ ionic liquid in the far-UV region (below 270 nm), we were unable to obtain the corresponding CD spectrum. It should be pointed out that CD spectroscopic features above 280 nm are somewhat similar in all ionic liquids, as well as in DMSO and water, which probably indicates that the BCD-ring system is largely unaffected by the nature of each medium.

The absorption spectra of **1** in all of the ionic liquids produced qualitatively similar results to the CD investigation (Supporting Information, Figure S2). In $[C_4\text{-mim}]PF_6$, $[C_4\text{-mim}]BF_4$, $[C_4\text{-mim}]NTf_2$, and $[C_4\text{-mim}]OTf$ a transition at ca. 360 nm was noted, which is consistent with the twisted conformation of tetracycline. On the other hand, a 380 nm transition in $[C_4\text{-mim}]NO_3$, $[C_4\text{-mim}]CH_3CO_2$, $[C_4\text{-mim}]CF_3CO_2$, and $[C_4\text{-mim}]SCN$ indicated the presence of an extended conformation of **1**.

Contrasting effects exhibited by $[C_4\text{-mim}]BF_4$ and $[C_4\text{-mim}]NO_3$ ionic liquids prompted us to examine the effect of composition of $[C_4\text{-mim}]NO_3$–$[C_4\text{-mim}]BF_4$ mixtures on the conformation of **1** (Figure 4). The CD spectra showed a decrease of the 260 nm peak as the concentration of $[C_4\text{-mim}]BF_4$ was increased. This trend indicated that a change from an extended conformation to the twisted one was in place. An apparent isosbestic point at ca. 280 nm might be indicative of the two-species system, i.e., extended–twisted conformations. Somewhat dissimilar features above 290 nm might be attributed to the presence of distinct tautomeric forms of **1**.

In conclusion, ionic liquids appear to control the conformational bias of tetracycline (**1**) as judged by the changes in their respective CD spectra. The emission of **1** is strongly dependent on the identity of the anion within 1-butyl-3-methylimidazolium-containing ionic liquids. This anion effect can be tentatively explained in terms of the Hofmeister series.[47,48] It should be pointed out, however, that the Hofmeister series is not a universal paradigm, and the order could be affected by the specific nature of the interactions present in a given system. Furthermore, only a limited number of ionic liquids have been tested with the aim of establishing Hofmeister's correlations, which might not allow for establishing unambiguous correlations.[49,50] Here, in accord with the chaotropic nature of SCN^- and NO_3^- anions (species that are known to impede intramolecular interactions mediated by noncovalent forces),[47−50] the $[C_4\text{-mim}]SCN$ and $[C_4\text{-mim}]NO_3$ demonstrated similar effects on the conformation of **1**. Kosmotropic BF_4^- and PF_6^- anions (species that are known to promote intramolecular interactions mediated by noncovalent forces)[47−50] were responsible for the effects exhibited by the $[C_4\text{-mim}]BF_4$ and $[C_4\text{-mim}]PF_6$ ionic liquids. However, the $CH_3CO_2^-$ anion, which is known to be kosmotropic, should have shown a correlation between $[C_4\text{-mim}]CH_3CO_2$ and $[C_4\text{-mim}]BF_4$. Yet in this particular case, $[C_4\text{-mim}]CH_3CO_2$ tended to behave more like $[C_4\text{-mim}]SCN$ and $[C_4\text{-mim}]NO_3$. A larger scope of ionic liquids is definitely required to elucidate the effect of the ionic liquids on the conformations of **1**. These studies are in progress in our laboratory.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents and solvents were from commercial sources (Sigma-Aldrich, Acros, Alfa Aesar) and were used as received. Tetracycline (**1**) was purchased as a free base from Fluka and used without further purification. A stock solution of **1** in

DMSO was prepared fresh prior to experiments and used within 12 h. Ionic liquids were synthesized and purified according to literature procedures.[38−41] All solutions of **1** in ionic liquids were prepared by addition of the DMSO stock solution of **1** to the ionic liquid, followed by vortexing at 3000 rpm for 10−30 s. Absorbance measurements were performed on an Agilent 8453 UV−visible instrument with a resolution of 1 nm using 0.01 or 1.0 cm quartz cells. Fluorescence measurements were performed using a Shimadzu RF-5301PC; the measurements were carried out as follows: λ_{ex} = 400 nm; excitation and emission slit widths were 3 and 3 nm or 5 and 3 nm, respectively; intensity, high; 1.0 cm quartz cells. All spectra were background-subtracted using appropriate blanks and subsequently smoothed using manufacturer-provided software. CD spectra were acquired on a JASCO J-815 instument using 0.01 or 1.0 cm quartz cells; spectra were recorded at room temperature and 1 nm resolution with a scan rate of 100 nm/min; four scans were acquired and averaged for each sample, and raw data were manipulated by subtraction of appropriate background spectra, followed by smoothing using manufacturer-provided software.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis and characterization of ionic liquids; CD, absorbance, and fluoresecence spectra of **1** in molecular and ionic solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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