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Chiral Recognition in Amino Acid and Peptide Based Chiral Ionic Liquids

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Chiral Recognition in Amino Acid and Peptide Based Chiral Ionic Liquids

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Abbreviations

AA – amino acid
ATR-IR – attenuated total reflectance infrared (spectroscopy)
BOC - di-tertbutyldicarbonate
CD – circular dichroism
CPL – circularly polarized luminescence
D-AlaCl – D-Alanine methyl ester
dpa - 2,6-pyridinedicarboxylate dianion
Eu - europium
\([\text{Eu(dpa)}_3]^{3-}\) - europium(2,6-pyridinedicarboxylate)_3^{3-}
g_{em} – emission dissymmetry factor
IL – ionic liquid
I_L – left handed polarized light
I_R – right handed polarized light
IR - infrared
L-AlaCl – L-Alanine methyl ester
L-LeuC_l – L-Leucine methyl ester
L-ProCl – L-Proline methyl ester
NMR – nuclear magnetic resonance (spectroscopy)
TBA – tetrabutylammonium
[TF_2N] – bis(trifluoromethane)sulfonimide
TLC – thin layer chromatography
TMSCL - trimethylsilyl chloride
Abstract

In this study, amino acid and peptide based chiral ionic liquids (ILs) were prepared and their chiral recognition ability probed using circularly polarized luminescence (CPL) spectroscopy. Potential applications of amino acid and peptide based chiral ILs include asymmetric synthesis and chiral resolution. The amino acid based chiral ILs under study were prepared from amino acid methyl ester cations and bis(trifluoromethane)sulfonamide ([TF$_2$N]$^-$) anions, specifically L-alanine methyl ester, D-alanine methyl ester, L-leucine methyl ester, and L-proline methyl ester. Cationic peptide ILs were prepared from L-alanyl glycine methyl ester cations and [TF$_2$N]$^-$ anions and anionic peptide based ILs were prepared from tetrabutylammonium ([TBA]$^+$) cations and DL-alanyl glycine and DL-leucyl DL-alanine anions. Chiral recognition was quantified for each chiral IL by dissolving a racemic chiral luminescent probe, $\Lambda$ and $\Delta$ europium(2,6-pyridine dicarboxylate)$_3^{3+}$ ([Eu(dpa)$_3$]$^{3+}$), in the IL and measuring the ratios of left versus right-handed polarized light emitted from the sample using a custom-built CPL spectrometer. The role of amino acid stereochemistry and structural identity of IL components are discussed in terms of thermodynamically controlled chiral recognition in chiral IL solvents.
1 - Introduction

1.1 Overview

Overall, the goal of this study was to probe amino acid based chiral ILs for their chiral recognition ability using CPL spectroscopy and to develop new methods of increasing the chiral recognition ability of chiral IL solvents using peptide IL components. Amino acid and peptide based chiral ILs have the potential to serve as both solvent and chiral recognition agent for asymmetric processes with possible applications in asymmetric synthesis and as stationary phases for chiral chromatography. In this study, we've used a model CPL system to probe the chiral recognition ability of five amino acid based chiral ILs and two anionic peptide based chiral ILs using CPL spectroscopy. CPL spectroscopy is an ideal method for probing chiral recognition because it is a one-step optical measurement that does not require in-depth chemical work-ups. We have also optimized a multi-step synthetic procedure for the development of novel cationic based chiral ILs, which have the potential to open up many opportunities for future study.

1.2 Defining Chirality and Chiral Recognition

Chirality is the term used to describe an object that is non-superimposable with its mirror image. The classic example of chirality is the left and right human hand. No matter how hands are oriented in three dimensional space, an individual is never able to fully align one hand over the other. The same concept also occurs on the molecular level. Many molecules, such as amino acids, exhibit chirality. For amino acids, the difference in three-dimensional arrangement of functional groups bonded around a central carbon
atom determines its overall stereochemistry, which is illustrated in Figure 1.

Stereochemical designations can vary depending on the context used, but the three presented in this work are D/L, R/S, and A/Δ.

![Figure 1.1 D- and L- alanine amino acid stereoisomers exhibit chirality, which is similar to the left and right human hand.](image)

The selection, or preference, of one chiral object of the other is known as chiral recognition. Using the human hand analogy, a handshake is more comfortable when a right hand shakes another right hand rather than when a left hand shakes a right hand. For over a century, chemists have been interested in chiral recognition on the molecular level because chiral molecules often interact differently with other chiral molecules. In biology, chiral recognition is ubiquitous in enzyme-substrate interactions, so it is easy to understand the desire of pharmaceutical and agrochemical industries to control the chirality of their products. The methods most often used to control chirality are asymmetric catalysis and chiral resolution. Asymmetric catalysis occurs when one stereoisomer of a molecule is preferentially synthesized over another by means of a chiral catalytic pathway. Therefore, the resulting product will have an unequal ratio of chiral products. Chiral resolution is a post-synthetic procedure where a mixture of left and right handed molecules is separated into its chiral components. This is often performed using a chiral column.
Perhaps the best example of the need for simple and efficient methods of chiral recognition is that of the pharmaceutical drug thalidomide. First prescribed by a pharmaceutical company in Germany in 1956, the drug was supposed to treat morning sickness in pregnant women. It was later determined that while the (S)-stereoisomer had the desired therapeutic effect, the (R)-stereoisomer was a teratogen and caused birth defects in the children whose mother received the drug. Since then, the United States Food and Drug Administration has established policies that require drug developers to (1) quantitate the pharmacological and toxicological effects of each stereoisomer and (2) produce a single stereoisomer of the drug if one is found to be toxic or biologically harmful.¹ This has opened up the field for chemists to find the most efficient and economical means of performing chiral recognition for application in asymmetric catalysis and chiral resolution.

1.3 Defining Ionic Liquid

Molten salts have long attracted interest and curiosity, but applications have been limited due to the necessary high temperatures. The melting point of NaCl is 801°C, for example. Ionic liquids (ILs), though, are salts or ionic compounds with melting points of less than 100°C, which make them much easier to work with than molten salts. ILs are typically composed of organic salts with large levels of asymmetry and electron delocalization, thus inhibiting crystallization at room temperature.

ILs are advantageous due to their negligible volatility and high thermal stability range. Another key feature of ILs is that their physicochemical properties can be tailored by the selection of their cation and anion components.² Increasing the hydrophobicity of
the cation tends to decrease water solubility, while chemical and physical properties can readily be controlled by selection of the anion component. Some of these tailored physicochemical properties can include viscosity, polarity, conductivity, and chirality. Also, ILs can be tailored to dissolve nearly any chemical species.³

Controlling the chirality of ILs has been a topic of numerous recent studies for applications in chiral recognition.⁴,⁵,⁶ In fact, chiral ILs, or ILs in which the anion or cation component are chiral, have shown promise as chiral stationary phases for gas chromatography columns for chiral resolution applications.⁶ Amino acids have proven to be good targets for chiral IL preparation because they derive from the chiral pool and can be used as the cation or anion component of the chiral IL using relatively simple chemistry.⁷ Another important aspect of the use of amino acids for chiral ILs is the fact that there are twenty naturally occurring amino acids that differ only in the chemical identity of their functional group (R-group), therefore, the selection of which amino acid to include in chiral IL preparation adds yet another level of tailorability to the process. Table 1.1 shows the components of amino acids, peptides, anions, and cations used in the synthesis of ILs in this study.

Table 1.1. Amino acid and Peptide components of chiral ILs studied

<table>
<thead>
<tr>
<th>Cations</th>
<th>Anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Chemical Structure] D and L Alanine methyl ester (AlaCl)</td>
<td>![Chemical Structure] bis(trifluoromethane)sulfonimide (Tf₂N⁻)</td>
</tr>
</tbody>
</table>
Tetrabutylammonium ion (TBA$^+$)

L Leucine methyl ester (LeuC1)

OCH$_3$

L Proline methyl ester (ProC1)

L Alanyl L Proline methyl ester

(AlaProC1)

DL Alanyl Glycinate (AlaGly)

OCH$_3$

DL Leucyl DL Alanate (LeuAla)

OCH$_3$

Tetrabutylammonium ion (TBA$^+$)
1.4 Peptide Based Chiral Ionic Liquids

While amino acids have been extensively used as components in IL preparation, the use of peptides as chiral components in chiral ILs has remained relatively unstudied. The only mention of peptides in IL preparation is using a cationic histidine-based imidizolium IL component to couple with another amino acid, thus resulting in [(S)-His-AA][Tf$_2$N]. However, only one amino acid functional group (R-group) is available for differential diasteriomeric interactions with a racemic chiral solute, therefore, these peptidic ILs do not present an advantage over amino acid based ILs for chiral recognition purposes. Peptides are chains of amino acids, thus, the addition of each individual amino acid to a growing peptide chain also increases the number of chiral centers the peptide contains. At each chiral center is an R-group, so the level of possible differential diastereomeric interactions between the peptide IL and a racemic chiral solute increases with the addition of amino acid residues. Increasing the number of amino acid residues in the peptide will thus increase the chiral recognition ability of peptide based chiral ILs compared to amino acid based chiral ILs due to the potential for these increased interaction sites to have a greater specificity for one chiral conformer over the other. Altering the identity and position of amino acid residues that make up the peptide chain adds yet another level of tailorability to the properties of ILs. This lends millions of various dipeptide possibilities and billions of tripeptide possibilities.

Some small peptides are commercially available and can be used as received for making anionic peptide based chiral ILs, but in order to use peptides as chiral cationic components of chiral ILs the C-terminus (carboxylic acid) must be esterified. In the
literature, most amino acid esterification schemes result in racemization of the chiral center, which poses an obvious problem for use as a nonracemic chiral recognition agent in chiral ILs.\textsuperscript{9} In order to conserve stereochemistry and prevent side reactions at the N-terminus (free amine), the free amine must be BOC protected.\textsuperscript{9} Thus, a two-step synthetic procedure was reported for the esterification of peptides, which can be seen in Scheme 1.1.

Scheme 1.1. Two-step synthetic procedure for the esterification of a dipeptide.

1.5 Circularly Polarized Luminescence and CPL Spectroscopy

Circularly polarized light is classified as having either right-handed (\textit{I}_R) or left-handed (\textit{I}_L) polarization based on the direction that the electric field vector rotates observed from the point of view of the emission source. An illustration of right handed circularly polarized light can be seen in Figure 1.2.

Figure 1.2. Visual example of right handed circularly polarized light
The emission analog of circular dichroism (CD), circularly polarized luminescence (CPL) is the difference between left and right polarized luminescence (I_L - I_R). A major distinction between CPL and CD is that CPL is less likely to suffer from solvent background interference than CD because CPL is an emission measurement.

In CPL spectroscopy, the major task is to separate and measure I_L and I_R independently, which is often difficult because the majority of the luminescence is elliptically polarized. Thus, isolating and detecting the small differences between I_L and I_R is essential to making accurate CPL measurements.

Europium (Eu) complexes have shown to be good luminescent sources for CPL applications because of their long emission lifetimes (micro- to milliseconds) and sharp emission bands. Due to forbidden electronic transitions, Eu^{3+} ions tend to absorb very little incident radiation; however, coordination of certain ligands to the Eu^{3+} ion can more effectively transfer incident light energy to the central ion via the antenna effect.

In this study, a well characterized Eu complex, [Eu(dpa)_3]^{3-} (dpa=2,6-pyridinedicarboxylate dianion), serves as the CPL luminescent probe. Incident electromagnetic radiation in the UV range (~280nm) excites into the dpa ligands causing energy transfer to the Eu central atom. This energy transfer results in the excitation of a 4f electron, and upon relaxation, circularly polarized light can be emitted. In solution, [Eu(dpa)_3]^{3-} exists as a racemic mixture of interconverting A and Δ conformers, as seen in Figure 1.3, with a three bladed propeller structure and D_3 site symmetry.
Figure 1.3. Equilibrium between $\Lambda$ and $\Delta$ [Eu(dpa)${}_3$]$^{3-}$ conformers

For a sample in which there is an equal population of $\Lambda$ and $\Delta$ conformers, [Eu(dpa)${}_3$]$^{3-}$ emits equal amounts of left and right handed polarized light giving an overall CPL signal of zero. However, when the [Eu(dpa)${}_3$]$^{3-}$ complex is subjected to a chiral environment, such as a chiral IL, differential diastereomeric interactions between the [Eu(dpa)${}_3$]$^{3-}$ complex and the chiral perturbation can create a non-racemic equilibrium in the sample—a phenomenon known as the Pfeiffer effect.\(^{11}\) This non-racemic equilibrium between $\Lambda$ and $\Delta$ conformers result in overall unequal amounts of left and right handed polarized light emission from the sample, which can be measured and quantified as a CPL signal using a CPL spectrometer. The CPL signal can be utilized as a direct probe of chiral recognition in chiral solvents. This information can be related to the use of chiral ILs in asymmetric catalysis and chiral resolution. Overall, CPL spectroscopy is an ideal method of probing chiral recognition because, unlike other chiral recognition measurement methods, it is a one step optical measurement that does not require product separation or isolation, enantiomeric excess determination, catalyst recovery steps, or co-solvent additions.\(^{15,16}\)
In order to quantify the chiral recognition ability of the IL samples for \([\text{Eu(dpa)}_3]^3\), and to compare chiral recognition across samples, an emission dissymmetry factor \((g_{em})\) can be calculated from the measured CPL data for each sample using the following equation:\(^{17}\)

\[
ge_{em} = \frac{2(I_L - I_R)}{I_L + I_R}
\]

With values ranging from -2 to +2, dissymmetry factors yield the handedness of discrimination determined by the sign of the value, as well as the degree of chiral recognition determined by the magnitude of the value. In the event that no chiral recognition occurs between solvent and solute, both the CPL signal \((I_L - I_R)\) and overall \(g_{em}\) value will be zero. Therefore, any non-zero dissymmetry value is evidence of chiral recognition because the chiral IL solvent is creating a non-racemic population of \(\Lambda\) and \(\Delta\) \([\text{Eu(dpa)}_3]^3\) conformers.

1.6 Introduction Summary

In this study, \([\text{L-AlaCl}]\text{[Tf}_2\text{N}]\), \([\text{D-AlaCl}]\text{[Tf}_2\text{N}]\), \([\text{L-ProCl}]\text{[Tf}_2\text{N}]\), \([\text{L-LeuCl}]\text{[Tf}_2\text{N}]\), \([\text{TBA}]\text{[DL-Ala Gly]}\), and \([\text{TBA}]\text{[DL-Leu DL-Ala]}\) chiral ILs were prepared and their chiral recognition ability probed using CPL spectroscopy. CPL spectroscopy is an ideal screen for chiral recognition because it is a one step optical measurement. \([\text{Eu(dpa)}_3]^3\) was used as a chiral luminescent CPL probe dissolved in the ILs, and emission spectra and luminescent lifetime spectra were recorded in order to
ensure the stability of the $[\text{Eu(dpa)}_3]^3$ complex in the ILs. Chiral recognition measurements of the IL samples were recorded over a temperature range of 283-323K to observe the thermodynamics of chiral recognition. One hypothesis is that peptide based ILs could increase chiral recognition of $[\text{Eu(dpa)}_3]^3$ due to increased levels of differential diastereomeric interactions, however, there is very little in the literature pertaining to the preparation of peptide based ILs. Therefore, [L-Ala L-ProCl][Tf$_2$N] was prepared as a proof of principle experiment, which opens up many avenues for future work.
2 - Experimental

2.1 General Methods

All reagents and solvents were purchased from commercial sources and were used without further purification unless otherwise noted. Trace amounts of water were removed from the ILs under vacuum with a liquid nitrogen cold trap. Water content was determined using a Metrohm 870 KF Titrino Plus, $^1$H and $^{13}$C NMR spectra were recorded using a Bruker AVANCE 250 MHz NMR, and IR spectra were recorded using a BioRad FTS700 FTIR.

The general metathesis procedure for cationic amino acid based chiral IL preparation can be seen in Scheme 2.1

![Scheme 2.1. General metathesis procedure for cationic amino acid based IL preparation](image)

For cationic peptide based chiral IL preparation, the [AA] component can simply be replaced with a [peptide] component. The two-step synthetic procedure for peptide esterification can be seen in Scheme 1.1. The general metathesis procedure for anionic peptide based chiral IL preparation can be seen in Scheme 2.2.

![Scheme 2.2. General metathesis procedure for anionic peptide based IL preparation](image)
2.2 Amino Acid Based Chiral IL Metathesis

2.2.1 L-AlaC1/TF2N

A 50 mL beaker was charged with 1.53 g (0.0110 mols; 1 eq) L-alanine methyl ester hydrochloride and a magnetic stir bar. Then, the solid was dissolved in 1.5 mL of DI water with stirring. To the beaker was added 5.16 g bis(trifluoromethane)sulfonimide lithium salt (0.0110 mols; 1 eq) with stirring. The mixture was stirred for 3 hours at room temperature and transferred to a 30 mL separatory funnel. The mixture was allowed to sit in the separatory funnel overnight to ensure complete separation of the ionic liquid layer from the aqueous layer. The two layers were subsequently separated and the ionic liquid layer was transferred to a 50 mL round-bottomed flask equipped with a stir bar. To remove trace water, the ionic liquid was put under vacuum with stirring and placed in a hot water bath not to exceed 60°C. The product was a colorless, transparent viscous liquid at room temperature. ¹H NMR (D₂O, 250MHz): δ = 8.46 (s, 3H, NH₃), 4.24 (q, J=1.75 Hz, 1H, CH), 3.87 (s, 3H, CH₃), 1.58 (d, J=7 Hz, 3H, CH₃) ppm.

2.2.2 D-Ala C1/TF2N

A 50 mL beaker was charged with 1.52 g (0.0109 mols; 1 eq) D-alanine methyl ester hydrochloride and a magnetic stir bar. Then, the solid was dissolved in 1.5 mL of DI water with stirring. To the beaker was added 3.14 g bis(trifluoromethane)sulfonimide lithium salt (0.0109 mols; 1 eq) with stirring. The mixture was stirred for 3 hours at room temperature and transferred to a 30 mL separatory funnel. The mixture was allowed to sit in the separatory funnel overnight to ensure complete separation of the ionic liquid layer from the aqueous layer. The two layers were subsequently separated
and the ionic liquid layer was transferred to a 50 mL round-bottomed flask equipped with a stir bar. To remove trace water, the ionic liquid was put under vacuum with stirring and placed in a hot water bath not to exceed 60°C. The product was a slightly yellow, transparent viscous liquid at room temperature. $^1$H NMR ((CD$_3$)$_2$CO, 250MHz): $\delta = 8.78$ (s, 3H, NH$_3$) 4.19 (q, $J=1.75$ Hz, 1H, CH), 3.84 (s, 3H, CH$_3$), 1.78 (d, $J=7$ Hz, 3H, CH$_3$) ppm.

2.2.3 L-ProC$_t$/TF$_2$N

A 50 mL beaker was charged with 6.89g (0.0416 mols; 1 eq) L-proline methyl ester hydrochloride and a magnetic stir bar. Then, the solid was dissolved in 4.8 mL of DI water with stirring. To the beaker was added 12.06 g bis(trifluoromethane)sulfonimide lithium salt (0.0420 mols; 1 eq) with stirring. The mixture was stirred for 3 hours at room temperature and transferred to a 30 mL separatory funnel. The mixture was allowed to sit in the separatory funnel overnight to ensure complete separation of the ionic liquid layer from the aqueous layer. The two layers were subsequently separated and the ionic liquid layer was transferred to a 50 mL round-bottomed flask equipped with a stir bar. To remove trace water, the ionic liquid was put under vacuum with stirring and placed in a hot water bath not to exceed 60°C. The product was a colorless, transparent viscous liquid at room temperature. $^1$H NMR (CDCl$_3$, 250MHz): $\delta = 7.97$ (s, 1H, NH$_2$), 7.26 (s, 1H, NH$_2$), 4.53 (m, 1H, CH), 3.90 (s, 3H), 3.59 (m, 2H, CH$_2$), 2.41-2.59 (m, 1H, CH$_2$), 2.00-2.29 (m, 3H, CH$_2$ CH$_2$) ppm
2.2.4 L-LeuC₁/TF₂N

A 50 mL beaker was charged with 10.36 g (0.0570 mols; 1 eq) L-leucine methyl ester hydrochloride and a magnetic stir bar. Then, the solid was dissolved in 1.5 mL of DI water with stirring. To the beaker was added 16.37 g bis(trifluoromethane)sulfonimide lithium salt (0.0570 mols; 1 eq) with stirring. The mixture was stirred for 3 hours at room temperature and transferred to a 30 mL separatory funnel. The mixture was allowed to sit in the separatory funnel overnight to ensure complete separation of the ionic liquid layer from the aqueous layer. The two layers were subsequently separated and the ionic liquid layer was transferred to a 50 mL round-bottomed flask equipped with a stir bar. To remove trace water, the ionic liquid was put under vacuum with stirring and placed in a hot water bath not to exceed 60°C. The product was a slightly yellow, transparent viscous liquid at room temperature. \(^1\)H NMR ((D₂O, 250MHz): \(\delta = 4.13\) (t, J=7.5Hz, CH), 3.83 (s, 3H, CH₃), 1.68-1.89 (m, 3H, CH-CH₂), 0.96 (m, 6H, (CH₃)₂) ppm

2.3 Peptide Esterification Procedure

2.3.1 Peptide Esterification Introduction

Chen et al. have developed an easy method of esterifying BOC-protected amino acids using trimethylsilyl chloride as a catalyst while maintaining >97% enantiomeric excess. Most commercially available peptides are not BOC-protected, but there are many examples of simple amino acid BOC-protection methods in the literature. While these methods were developed for amino acid chemistry, they can be easily adapted for peptide chemistry if certain considerations are taken into account to ensure the integrity
of the peptide bond. The two-step peptide esterification procedure can be seen in Scheme 1.1.

2.3.2 BOC protected L-Alanyl L-Proline Preparation

A 250 mL round bottom flask was charged with 1.089g L-alanyl L-proline (0.005851 mols; 1 eq) and was put in an ice bath. To the flask was also added a stir bar and 15 mL of 1.0M NaHCO₃ solution (0.015 mols; 2.6 eq). The reaction mixture was allowed to stir until all of the solid dissolved so that the pH of the solution was 8-10 monitored by pH paper. To a separate 50 mL beaker was added 3.944g di-tertbutyldicarbonate (0.01807 mols; 3.09 eq), which was dissolved in 15 mL of para-dioxane. The contents of the beaker were manually stirred until the di-tertbutyldicarbonate dissolved, and was then placed in an ice bath. The contents of the 50mL beaker was then added dropwise to the 250 mL round bottom flask, which was being stirred in an ice bath. The reaction mixture was put under nitrogen and allowed to stir in an ice bath for 90 minutes. Throughout the 90 minute reaction period, the pH of the aqueous layer was monitored to ensure that it stayed within the desired 8-10 range. After 90 minutes, the pH was found to have dropped to ~7, therefore, two additional microscoopulas of NaHCO₃ was added to the reaction flask to adjust the pH. Once the 90 minute reaction period has been reached, the reaction flask was removed from the ice bath and allowed to warm to room temperature. The reaction was monitored via TLC. The plate was eluted with a 6:2:1 mixture of n-butanol:glacial acetic acid:water, stained with a ninhydrin:n-butanol mixture, and developed using a heat gun. After 12 hours the reaction was complete and the reaction flask was diluted with 20 mL of water and
quickly transferred to a large separatory funnel charged with 40 mL of ethyl acetate. The reaction mixture was then washed and separated. The aqueous layer was washed again with 20 mL of ethyl acetate, and the resulting organic layers were extracted with 25 mL of 1M NaHCO₃ solution. The aqueous layers were monitored by TLC to ensure the product was present and the layers were combined in a 250mL round bottom flask already charged with 10 mL of ethyl acetate. The reaction mixture was put in an ice bath with stirring and the aqueous layer was subsequently acidified to a pH of 2 using slow dropwise additions of 3.0M HCl. The acidified reaction mixture was then quickly added to a large separatory funnel charged with 60 mL of ethyl acetate. The aqueous layer was extracted with the ethyl acetate and both layers were kept. The organic layer was then washed with 30 mL of water and both layers were kept. Subsequently, the two aqueous layers were then extracted with 30 mL of ethyl acetate. TLC was then used to ensure that the product was in the organic layers, and the aqueous layers were stored at low temperature. The organic layers were combined in a large beaker and dried with magnesium sulfate. The magnesium sulfate was removed using vacuum filtration and the reaction mixture was transferred to a 500 mL round bottom flask. The ethyl acetate was removed via rotary evaporation at 45°C to yield a white solid. To the round bottom flask was added 15 mL of toluene with stirring. Once all of the solid was dissolved, toluene was removed under vacuum with a liquid nitrogen trap to yield a white solid. The solid was subsequently dissolved in 10 mL of dichloromethane and the solvent removed via rotary evaporation. Anhydrous diethyl ether (10mL) was added to the flask with stirring to give a slurry. The ether was removed via rotary evaporation and the ether wash was performed a second time. The reaction flask was then put under vacuum for eight hours
to remove trace solvent to yield 0.8355g of BOC- L-alanyl L-proline (0.002918 mols) for a 65% yield. $^1$H NMR analysis of product is shown in Figure 2.1

Figure 2.1. $^1$H NMR analysis of BOC protected L-alanyl L-proline in CDCl$_3$.

2.3.3 BOC-L-Alanyl L-Proline Esterification Procedure

To a 100 mL round bottom flask was added 1.014g BOC- L-alanyl L-proline (0.003542 mols; 1 eq) and 11 mL of methanol. The mixture was stirred at room temperature using a magnetic stir bar until the solid was dissolved. Once dissolved, the
round bottom flask was put into an ice bath and placed under nitrogen. To the flask was slowly added 1.8 mL of trimethylsilyl chloride (0.010 mols; 2.8 eq) and the reaction was allowed to proceed for approximately 24 hours. The reaction mixture was allowed to warm to room temperature, and the reaction was monitored for completion by TLC. After the reaction had reached completion, the solvent was removed under vacuum with a liquid nitrogen cold trap to give a white/yellow solid. To the reaction flask was added 20 mL of anhydrous diethyl ether with stirring, which resulted in a slurry. The solid white/yellow product was isolated using vacuum filtration and the product was washed twice with 5 mL of anhydrous diethyl ether to yield 0.5810g L-alanyl L-prolineCl (0.002901 mols) for an 81.90% yield. $^1$H NMR and IR analysis of product is shown in Figure 2.2 and Figure 2.3 respectively.

![Figure 2.2. $^1$H NMR analysis of L-alanyl L-proline methyl ester in CDCl₃.](image)
2.4 Peptide Based Chiral IL Metathesis

2.4.1 TBA / AlaGly

A 50 mL beaker was charged with 0.9937g DL-alanyl glycine (0.006797 mols; 1 eq), which was dissolved in 2 mL of water with stirring via a magnetic stirbar. To the beaker was added 5.475g tetrabutylammonium hydroxide (0.006844 mols; 1.007 eq) with continuous stirring. After heating to 55°C, a white solid remained in the mixture. The mixture was titrated with aqueous tetrabutylammonium hydroxide until the white solid disappeared. The temperature was gradually increased to ~ 80°C to remove water from
the ionic liquid. The resulting substance was an extremely viscous, slightly yellow, and clear liquid. Spectroscopic analysis was not performed due to small quantities made.

2.4.2 TBA / LeuAla

A 50 mL beaker was charged with 0.5089g DL-leucyl DL-alanine (0.002516 mols; 1eq), which was added to 5 mL of water. Stirring using a magnetic stirbar resulted in a thick, white paste suggesting the peptide does not dissolve in water. To the beaker was incrementally added 2.025g tetrabutylammonium hydroxide (0.002531mols; 1.007 eq) and gentle heat was applied with stirring to raise the temperature to 55°C. The resulting mixture was a clear liquid with small white solid particles. The mixture was titrated with aqueous tetrabutylammonium hydroxide, but solid still remained. The mixture was allowed to cool. After ten hours, the beaker was reheated to 80°C to remove water from the ionic liquid. With the applied heat, the solid particles disappeared to yield an extremely viscous, slightly yellow, and clear liquid. Spectroscopic analysis was not performed due to small quantities made.

2.4.3 AlaProC1 / Tf2N

A 50 mL beaker was charged with 0.5116g L-alanyl L-proline methyl ester hydrochloride (0.002555 mols; 1 eq) and a magnetic stir bar. The solid was dissolved in 1.5 mL of water with stirring. The resulting solution was yellow in color with small, solid particles. The solution was vacuum filtered to remove the solid impurities. To the solution was added 0.7132g lithium bis(trifluoromethane)sulfonimide (0.002484mols; 0.9726 eq) with stirring. Two distinct layers did not spontaneously form upon settling. The solution was lightly heated with stirring to remove the excess water. After heating,
two layers formed and the heterogeneous mixture was transferred to a small separatory funnel and allowed to for 10 hours for full separation. The next morning, the layers were separated and the IL layer was transferred to a 50 mL round bottom flask. Trace water was removed from the IL under vacuum with a liquid nitrogen cold trap while heated in a hot water bath not to exceed 80°C. The product was a clear, slightly yellow viscous liquid. $^1$H NMR and IR analysis of the IL is shown in Figure 2.4 and Figure 2.5 respectively.

Figure 2.4. $^1$H NMR analysis of [AlaProCl][Tf$_2$N] in CDCl$_3$. 
Figure 2.5. IR Analysis of [AlaProC1][Tf2N] using NaCl disks.

2.5 (TBA)$_3$[Eu(dpa)$_3$] Synthesis

To a 50 mL beaker equipped with a magnetic stir bar was added 0.3690g EuCl$_3$ (0.001007 mols, 1 eq) and was subsequently dissolved in 10 mL of water with vigorous stirring. The aqueous solution was titrated with 6.0 M NaOH to drive basify the solution (pH > 10). The resulting precipitate was collected by vacuum filtration. The wet precipitate was transferred to a new 50 mL beaker and 10 mL of water was added. To the solution was added 0.5216g of 2,6 pyridinedicarboxylic acid (0.003122 mols; 3.100 eq) followed by stirring with a magnetic stir bar. Subsequently, 2.4947g of
tetrabutylammonium hydroxide (0.003050 mols; 3.0288 eq) was added to the beaker. An excess of tetrabutylammonium hydroxide was added dropwise until all of the solid was gone and the solution was somewhat opaque. The pH of the solution was adjusted to 4 with dropwise addition of 6M HCl, upon which the solution became a cloudy white color. The pH was then brought back to 6 with dropwise addition of tetrabutylammonium hydroxide solution. The beaker was heated at 70°C to remove excess water, and the resulting product was a cloudy white, viscous oil.

2.6 Sample Preparation

Samples were prepared by adding approximately 0.25 mg of TBA₃[Eu(dpa)₃] to 1g of chiral IL. The mixture was heated to temperatures <60°C with vigorous stirring to achieve full solvation of the europium complex. Emission spectra of the europium complexes in the IL were recorded on a Perkin-Elmer LS-55 Luminescence spectrometer to ensure that the complexes remained intact when solvated by the chiral IL. Luminescent lifetime measurements were also recorded to monitor changes in the sample over time using a custom-built CPL spectrometer with the output of the PMT sent to a digitizing oscilloscope to record intensity via analog capture.

2.7 Measuring Chiral Recognition Using CPL Spectroscopy

Using [Eu(dpa)₃]³⁻ as a chiral luminescent probe for chiral recognition in chiral ILs is completely reliant upon the ability to independently measure the intensity of emitted right handed and left handed polarized light from a sample. CPL instrumentation of this kind is not commercially available; therefore, a custom-built CPL spectrometer
was built in house to make the measurements necessary to probe chiral recognition. The schematic for our custom-built CPL spectrometer can be seen in figure 1.3.

![Schematic of custom built CPL spectrometer](image)

**Figure 1.4.** Schematic of custom built CPL spectrometer

This instrument consists of numerous components. First is a 450W Xenon arc lamp used as an excitation source, which is passed through a pre-sample monochromator to select the desired wavelength of electromagnetic radiation necessary (~280nm) to excite the \([\text{Eu}(\text{dpa})_3]^3\) complex in the sample. The sample is contained in a fluorescence quartz cuvette, which is encased in a temperature controlled water jacket connected to a circulating water bath (Brinkman RMS Lauda). CPL emission from the sample is then passed through a photoelastic modulator (Hinds PEM-80) operating at 50 kHz to select which handed polarized light is allowed to pass through. The radiation allowed to pass through the photoelastic modulator is subsequently linearly polarized using a Glan-Thomson linear polarizer. This light then passes through a high resolution 0.32 m monochromator (Horiba Jobin Yvon iHR320) to select the emission wavelength and finally detected by a red-sensitive photomultiplier tube (Hammamatsu R928). For the amino acid based IL studies, the output signal from the photomultiplier tube is sent to a lock-in amplifier that reduces background electrical noise. For the peptide based IL
studies, the photomultiplier tube output is sent to a digitizing oscilloscope to record the luminescence intensity (analog capture), which requires signal averaging to reduce background noise. After the data is recorded, it is sent to a computer that stores the raw left and right handed data, $I_L$ and $I_R$, for CPL determination.
3 – Results and Discussion

3.1 IL Preparation

3.1.1 General Remarks for IL Preparation

In these experiments, the chiral ILs studied were [L-ProC1][Tf2N], [L-AlaC1][Tf2N], [D-AlaC1][Tf2N], [L-LeuC1][Tf2N], [TBA][AlaGly], [TBA][LeuAla], and [L-Ala-L-ProC1][Tf2N]. All of the ILs were clear directly after preparation, but in certain cases solid precipitated out of the IL with time. [TBA][AlaGly] and [TBA][LeuAla] were the two ILs that had significant levels of solid precipitation after sample preparation.

All of the amino acid based ILs maintained a syrup-like viscosity while the peptide based ILs were even more viscous, nearing solidification at room temperature. After initial counter-ion metathesis, all of the ILs, with the exception of [D-AlaC1][Tf2N] and [L-Ala-L-ProC1][Tf2N], were clear with water contents near 33%. [D-AlaC1][Tf2N] and [L-Ala-L-ProC1][Tf2N] had a slight yellow color after metathesis. After subsequent heating to remove water and dissolve the europium complex in the ILs, all of the ILs displayed slight yellow coloration but remained clear.

3.1.2 Water Content Determination

Water concentration of the prepared ILs was determined using a Karl Fischer Titrator. Water concentrations of the ILs used to make samples are shown in Table 3.1.
Table 3.1. Water content of amino acid based chiral ILs.

<table>
<thead>
<tr>
<th></th>
<th>[L-AlaC1][Tf2N]</th>
<th>[D-AlaC1][Tf2N]</th>
<th>[L-ProC1][Tf2N]</th>
<th>[L-LeuC1][Tf2N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>&lt;0.10%</td>
<td>0.40%</td>
<td>0.54%</td>
<td>0.42%</td>
</tr>
</tbody>
</table>

Water content of the peptide based ILs was not determined due to the small amount of ILs prepared. Since water content measurement is destructive to the IL, in order to have enough IL for sample preparation the water content was not measured. After removing water from the ILs on vacuum at elevated temperature (<60°C), the ILs became much more viscous, which suggests a relationship between water content and IL viscosity.

An observation made throughout the course of these experiments was that [L-AlaC1][Tf2N] and [D-AlaC1][Tf2N] became less viscous with time when sitting out on the bench top. This suggests that these ILs easily pick up water from the atmosphere and are slightly hygroscopic. L- and D-alanine have a methyl side chain, which is less hydrophobic than the R-groups of proline and leucine. Therefore, the Alanine based ILs are more hydrophilic relative to the other ILs of study, and could more easily pick up water from the surrounding environment resulting in decreased viscosity with prolonged exposure to the ambient atmosphere.

3.1.3 Peptide Based IL Preparation

Peptides have remained a relatively unstudied approach to IL preparation, especially in applications such as chiral recognition; therefore, one of the major thrusts of this study was to determine an effective way of incorporating peptides as IL components. To ensure that peptides could in fact be incorporated into an IL preparation scheme,
dipeptide based ILs in which the dipeptide served as the anion were first prepared as a proof of concept. With the successful preparation of [TBA][AlaGly] and [TBA][LeuAla], it seemed that dipeptide based room temperature ILs were indeed a possibility. However, the use of [TBA][AlaGly] and [TBA][LeuAla] as solvents for chiral recognition in the experimental setup is not ideal for two reasons. One, the peptides utilized, DL-alanyl glycine and DL-leucyl-DL-alanine, are racemic mixtures and would show equal and opposite specificity for a chiral solute within the same solvent. This would result in an equal population of \( \Lambda \) and \( \Delta \) [Eu(dpa)\(_3\)]\(^{3-}\) (no chiral recognition). Secondly, the chiral component of these ILs, the peptide, is negatively charged and would not likely interact with the negatively charged [Eu(dpa)\(_3\)]\(^{3-}\) complex due to unfavorable coulombic interactions/charge repulsion. For the best chance of increasing the chiral recognition potential of peptide-based ILs, the peptide must have only one stereoisomer per stereocenter and the charge of the solute relative to the chiral component of the IL must also be taken into account. For this work, a fully resolved dipeptide was selected to serve as the cationic component of the synthesized ILs.

Dipeptide methyl esters are either not commercially available or cost prohibitive on the scale needed for IL preparation. It was necessary to synthesize dipeptide methyl esters starting from dipeptides. Esterification schemes are well-studied, but certain considerations must be taken into account in order to minimize \( \alpha \)-carbon racemization and prevent peptide bond hydrolysis. This includes adding a BOC protection group to the N-terminus of the peptide to decrease the free amine reactivity as well as limiting exposure to harsh pH conditions, which could catalyze peptide bond hydrolysis.
In this study, [L-Ala-L-Pro][Tf₂N] was successfully metathesized from synthesized L-alanyl L-proline methyl ester and its identity confirmed using IR spectroscopy and NMR spectroscopy. Further characterization is required to understand the chiral nature of the IL. In order to maximize yields of esterified peptide, the esterification scheme could be improved by changing extraction solvents or optimizing reaction times. The elution solvent for TLC analysis could be optimized in order to decrease the BOC-peptide elution distance and ensure complete separation between reaction components at large Rf values. Another improvement in the overall process would be with the purification of esterified peptide so that impurities during IL metathesis would be minimized. Overall, as detailed in the experimental section the esterification scheme has had yields at 24%, 53%, and 68% of desired product. The general route could be applicable to other di or tripeptides that do not contain amino acid residues with amino containing or carboxylic acid containing R-groups.

3.2 Sample Preparation

In order to measure the chiral recognition ability of the ILs, the chiral luminescent probe [TBA]₃[Eu(dpa)₃] was dissolved in each IL with a approximate ratio of 0.25 mg of [TBA]₃[Eu(dpa)₃] to 1 g of chiral IL. The molalities of the probe dissolved in the amino acid based ILs are presented in Table 3.2.
Table 3.2. Molality of [TBA]$_3$[Eu(dpa)$_3$] dissolved in studied IL samples.

<table>
<thead>
<tr>
<th>[L-AlaCl][Tf$_2$N]</th>
<th>[D-AlaCl][Tf$_2$N]</th>
<th>[L-ProC1][Tf$_2$N]</th>
<th>[L-LeuCl][Tf$_2$N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.11x$10^{-4}$ m</td>
<td>2.23x$10^{-4}$ m</td>
<td>3.57x$10^{-4}$ m</td>
<td>4.36x$10^{-4}$ m</td>
</tr>
</tbody>
</table>

It is interesting to note that [TBA]$_3$[Eu(dpa)$_3$] more quickly dissolved in [L-ProCl][Tf$_2$N] than the other three amino acid based ILs studied. This is likely a function of IL viscosity and the transport properties of the [Eu(dpa)$_3$]$^{3-}$ in the IL.

It was important to monitor that, once dissolved, the [Eu(dpa)$_3$]$^{3-}$ remains stable in the IL solvation environment. This is accomplished through a twofold approach. The first method was recording emission spectra and the second was by monitoring the luminescence lifetimes of the [Eu(dpa)$_3$]$^{3-}$ dissolved in the ILs. The emission spectra of [Eu(dpa)$_3$]$^{3-}$ dissolved in amino acid based ILs and water are shown in Figure 3.1.
Table 3.2. Molality of $[\text{TBA}_3][\text{Eu(dpa)}_3]$ dissolved in studied IL samples.

<table>
<thead>
<tr>
<th></th>
<th>[L-AlaCl][Tf$_2$N]</th>
<th>[D-AlaCl][Tf$_2$N]</th>
<th>[L-ProCl][Tf$_2$N]</th>
<th>[L-LeuCl][Tf$_2$N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>molality</td>
<td>$3.11 \times 10^{-4}$ m</td>
<td>$2.23 \times 10^{-4}$ m</td>
<td>$3.57 \times 10^{-4}$ m</td>
<td>$4.36 \times 10^{-4}$ m</td>
</tr>
</tbody>
</table>

It is interesting to note that $[\text{TBA}_3][\text{Eu(dpa)}_3]$ more quickly dissolved in [L-ProCl][Tf$_2$N] than the other three amino acid based ILs studied. This is likely a function of IL viscosity and the transport properties of the $[\text{Eu(dpa)}_3]^{3-}$ in the IL.

It was important to monitor that, once dissolved, the $[\text{Eu(dpa)}_3]^{3-}$ remains stable in the IL solvation environment. This is accomplished through a twofold approach. The first method was recording emission spectra and the second was by monitoring the luminescence lifetimes of the $[\text{Eu(dpa)}_3]^{3-}$ dissolved in the ILs. The emission spectra of $[\text{Eu(dpa)}_3]^{3-}$ dissolved in amino acid based ILs and water are shown in Figure 3.1.
From the emission spectra in Figure 3.1, it is clear that \([\text{Eu(dpa)}_3]^{3-}\) shows similar transitions around 594nm and 614nm in both the ILs and water. This suggests that the dpa ligands are arranged in the same site symmetry (D₃) around the Eu³⁺ central atom in all of the solvents, therefore, the \([\text{Eu(dpa)}_3]^{3-}\) remains intact when solvated by the ILs. However, there is a notable difference in the resolution of the two peaks around the 594 transition between \([\text{Eu(dpa)}_3]^{3-}\) in the various spectra, which suggests that there is variation in an outer sphere solvation interaction between the IL solvents as well as
water. This is necessary if we expect to see differential levels of interaction between solvated \([\text{Eu(dpa)}_3]^{3-}\) and the solvent molecules due to the varying chemical identity of those solvent molecules. Therefore, the difference in resolution can be attributed to outer sphere interactions rather than a change in the complex’s coordination environment. There is also a notable difference in the peak broadness amongst the emission spectra of \([\text{Eu(dpa)}_3]^{3-}\) in the various solvents. The source of peak broadening is differing monochromator slit widths in the instrument that were necessary to allow for the instrument signal to stay within detection limits.

Luminescence lifetimes were also measured to ensure the stability of the \([\text{Eu(dpa)}_3]^{3-}\) in our IL solvents. An example luminescence lifetime spectra can be seen in Figure 3.2.
The luminescent lifetime measurements of [Eu(dpa)_3]^{3+} dissolved in the ILs were constant over the 282-323K temperature range of this study, and indicate that there is a single, dominant emitting species due to mono-exponential decay fits of the data. The lifetime data also suggests that the solvated [Eu(dpa)_3]^{3+} is not significantly quenched by the IL or water present due to the fact that the observed lifetimes are longer than those observed in aqueous solution. Luminescent lifetimes ranged from 1.91 ms to 2.04 ms, which can be seen in Table 3.3.
Table 3.3. Luminescent lifetimes of \([\text{Eu(dpa)}_3]^{3-}\) dissolved in ILs of study.

<table>
<thead>
<tr>
<th></th>
<th>[L-AlaC1][Tf$_2$N]</th>
<th>[D-AlaC1][Tf$_2$N]</th>
<th>[L-ProC1][Tf$_2$N]</th>
<th>[L-LeuC1][Tf$_2$N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime (ms)</td>
<td>$1.91 \pm 0.04$</td>
<td>$1.99 \pm 0.05$</td>
<td>$1.89 \pm 0.19$</td>
<td>$2.04 \pm 0.08$</td>
</tr>
</tbody>
</table>

From both the emission spectra and the luminescent lifetime measurements, it can be concluded that the \([\text{Eu(dpa)}_3]^{3-}\) complex remains intact and in $D_3$ symmetry when dissolved in both aqueous and IL solvation environments. Also, it is apparent that the outer-sphere interactions between solvent and the \([\text{Eu(dpa)}_3]^{3-}\) complex differ between aqueous and IL solvation environment.

3.3 Chiral Recognition

The chiral recognition ability of the ILs was measured using CPL spectroscopy over a temperature range of 283-323K. Example CPL spectra of amino acid based ILs studied are displayed in Figures 3.3 - 3.6 and example CPL spectra of peptide based IL studied are displayed in Figures 3.7-1.8.
Figure 3.3. Total Luminescence and CPL spectra for the 594nm region excitation of $3.11 \times 10^{-3}$ m $[\text{Eu(dpa)}_3]^{3-}$ in $[\text{L-AlaC1][Tf}_2\text{N}]$ at 293K.

Figure 3.4. Total Luminescence and CPL spectra for the 594nm region excitation of $2.23 \times 10^{-3}$ m $[\text{Eu(dpa)}_3]^{3-}$ in $[\text{D-AlaC1][Tf}_2\text{N}]$ at 293K.
Figure 3.5. Total Luminescence and CPL spectra for the 594nm region excitation of $3.57 \times 10^{-4}$ m [Eu(dpa)$_3$]$^{3-}$ in [L-ProCl][Tf$_2$N] at 293K.

Figure 3.6. Total Luminescence and CPL spectra for the 594nm region excitation of $4.36 \times 10^{-4}$ m [Eu(dpa)$_3$]$^{3-}$ in [L-LeuCl][Tf$_2$N] at 293K.
Figure 3.7. Total Luminescence and CPL spectra for the 594nm region excitation of [Eu(dpa)$_3$]$^{3-}$ in [TBA][DL-Ala Gly] at 293K recorded using analog capture.

Figure 3.8. Total Luminescence and CPL spectra for the 594nm region excitation of [Eu(dpa)$_3$]$^{3-}$ in [TBA][DL-Leu DL-Ala] at 293K recorded using analog capture.
Figures 3.3-3.8 plot total luminescence ($I_L + I_R$) versus emission wavelength in red and CPL ($I_L - I_R$) versus emission wavelength in blue. The CPL value for each spectra can be determined from the maximum value of the blue curve and the total luminescence from the maximum value of the red curve. These values can then be plugged into equation 1.1 to calculate the dissymmetry factor. Using this method, the dissymmetry factor for [L-AlaCl][Tf$_2$N] at 293K is +0.013 and is -0.013 for [D-AlaCl][Tf$_2$N] at 293K, while the dissymmetry factor for [L-ProCl][Tf$_2$N] at 293K is +0.012 and is +0.011 for [L-LeuCl][Tf$_2$N] at 293K. Dissymmetry factors for all of the amino acid based ILs across the studied temperature range (283-323K) were calculated from spectra in the same manner, and are presented in Table 3.4. For the peptide based IL spectra represented in Figures 3.7 and 3.8, both the total luminescence and CPL data are much noisier than those in the amino acid based IL spectra, which can be attributed to the method in which the data was recorded. Peptide based IL data was recorded using analog capture, an inherently noisier data collection method than the lock-in-amplifier method used for amino acid based IL data collection, because the lock-in amplifier was not fully functional at the time the data was taken. Both peptide based ILs studied gave a CPL signal of 0 at 293K, and thus, calculated dissymmetry factors for both peptide based ILs were 0 (shown in Table 3.4) and chiral recognition of these samples was not screened at any other temperatures.
Table 3.4. Temperature dependent \( g_{\text{em}} \) values for \([\text{Eu(dpa)}_3]^3-\) dissolved in ILs over 283-323 K range.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>( g_{\text{em}} ) [L-AlaCl][Tf₂N]</th>
<th>( g_{\text{em}} ) [D-AlaCl][Tf₂N]</th>
<th>( g_{\text{em}} ) [L-ProCl][Tf₂N]</th>
<th>( g_{\text{em}} ) [L-LeuCl][Tf₂N]</th>
<th>( g_{\text{em}} ) [TBA][L-Ala]</th>
<th>( g_{\text{em}} ) [TBA][AlaGly]</th>
<th>( g_{\text{em}} ) [TBA][LeuAla]</th>
</tr>
</thead>
<tbody>
<tr>
<td>283</td>
<td>+0.018</td>
<td>-0.015</td>
<td>+0.016</td>
<td>+0.011</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>293</td>
<td>+0.013</td>
<td>-0.013</td>
<td>+0.012</td>
<td>+0.011</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>298</td>
<td>+0.012</td>
<td>-0.008</td>
<td>+0.011</td>
<td>+0.010</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>303</td>
<td>+0.011</td>
<td>-0.006</td>
<td>+0.011</td>
<td>+0.009</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>308</td>
<td>+0.011</td>
<td>-0.006</td>
<td>+0.011</td>
<td>+0.010</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>313</td>
<td>+0.009</td>
<td>-0.005</td>
<td>+0.010</td>
<td>+0.010</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>318</td>
<td>+0.009</td>
<td>-0.004</td>
<td>+0.010</td>
<td>+0.011</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>323</td>
<td>+0.009</td>
<td>-0.005</td>
<td>+0.007</td>
<td>+0.010</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Experiment performed by Katie Cox

From Table 3.4, it is clear that [L-AlaCl][Tf₂N], [D-AlaCl][Tf₂N], [L-ProCl][Tf₂N], and [L-LeuCl][Tf₂N] exhibit chiral recognition due to their non-zero dissymmetry factors, which is evidence of a nonracemic population of \( \Lambda \) and \( \Delta [\text{Eu(dpa)}_3]^3-\) conformers dissolved in the ILs. It is significant that the L-alanine methyl ester stereoisomer gave a \( g_{\text{em}} > 0 \) while the D-alanine methyl ester stereoisomer gave a \( g_{\text{em}} < 0 \), which provides evidence for a relationship between IL stereochemistry of the methyl ester cation and the handedness of recognition. Like the L-alanine methyl ester stereoisomer, both the L-proline methyl ester stereoisomer and L-leucine methyl ester
stereoisomer gave $g_{en} > 0$, providing further evidence for the relationship between IL stereochemistry of the methyl ester cation and the handedness of recognition.

[TBA][DL-Ala Gly], [TBA][DL-Leu DL-Ala], and a previously studied IL, [TBA][L-Ala], did not show chiral recognition of the luminescent probe, which is evident from a calculated $g_{en}$ of 0. For the first two peptide based ILs, this could be due to the racemic mixtures of amino acid residues that make up the ILs, but this is not the case for [TBA][L-Ala]. [TBA][L-Ala] likely shows no level of chiral recognition because charge repulsion between the negatively charged [Eu(dpa)$_3$]$^{3-}$ and [L-Ala]$^-$ would limit interaction between the two ions; thus, having a limited affect upon a chiral perturbation of the $\Lambda$ and $\Delta$ [Eu(dpa)$_3$]$^{3-}$ equilibrium. From this it can be concluded that in order to find useful IL solvents for applications in asymmetric catalysis and chiral resolution, the charges of the chiral IL component and of the chiral product/analyte must be taken into account.

It is also clear from the data that the structural identity of the amino acid methyl ester cations plays a role in chiral recognition. By taking the absolute value of the dissymmetry factor, $|g_{en}|$, at any given temperature interval we see relatively equal magnitudes of chiral recognition between the [AlaCl]$^+$ and [ProCl]$^+$ based ILs. This means that the structural differences between the methyl R-group of [AlaCl]$^+$ and the rigid five-membered ring of [ProCl]$^+$ are not great enough to affect differences in chiral recognition. This is not the case with the long, flexible isobutyl R-group of the [LeuCl]$^+$ based IL, which suggests that the structural differences between [AlaCl]$^+$/[ProCl]$^+$ and [LeuCl]$^+$ are great enough to cause varying levels of chiral recognition. Thus, the difference in chiral recognition between [AlaCl]$^+$/[ProCl]$^+$ and [LeuCl]$^+$ suggest
differing modes of chiral recognition based on their structural differences, further emphasized by the results of the thermodynamically controlled chiral recognition studies.

[L-AlaCl][Tf₂N], [D-AlaCl][Tf₂N], and [L-ProCl][Tf₂N] show a clear decrease in the magnitude of the dissymmetry factor with an increase in temperature, which suggests an enthalpically driven mode of chiral recognition between these chiral solvents and the [Eu(dpa)₃]³⁻ solute. This means that the chiral recognition likely arises from more energetically stable differential diastereomeric intermolecular interactions between the amino acid methyl ester components of the ILs and the [Eu(dpa)₃]³⁻ conformers, which decreases with increased temperature. On the other hand, [L-LeuCl][Tf₂N] displays a relatively constant dissymmetry factor with a change in temperature. This suggests that [L-LeuCl][Tf₂N] has a different mode chiral recognition other than enthalpically driven recognition. With this data alone, the mode of chiral recognition in [L-LeuCl][Tf₂N] cannot be fully understood; however, recent studies performed by Seddon et. al. and Welton et. al. have showed entropically driven ideal mixing behavior in ILs based on ionic size matching that might have implications to this study’s experimental results. The isobutyl chain of [L-LeuC]⁺ is larger than the R-groups of [L-AlaC]⁺, [D-AlaC]⁺, and [L-ProC]⁺, and is more similar in size to the [TBA]⁺ cation from the [TBA]₃[Eu(dpa)₃] salt. Therefore, entropically driven, temperature independent ionic size matching between [L-LeuC]⁺ and [TBA]⁺ could be a possible mode of chiral recognition. This could explain the experimental observations of temperature independent chiral recognition in the [L-LeuC][Tf₂N] solvent compared to the temperature dependent chiral recognition in the [L-AlaC][Tf₂N], [D-AlaC][Tf₂N], and [L-ProC][Tf₂N] solvents.
4 – Conclusion

In this study, the chiral recognition ability of four amino acid based chiral ILs ([L-AlaCl][Tf$_2$N], [D-AlaCl][Tf$_2$N], [L-ProCl][Tf$_2$N], and [L-LeuCl][Tf$_2$N]) was probed using CPL spectroscopy. Three dipeptide based chiral ILs were prepared, and two ([TBA][AlaGly] and [TBA][LeuAla]) were probed for their chiral recognition ability. The third peptide based IL ([L-Ala L-ProCl][Tf$_2$N]) was made as a proof of principle experiment in an attempt to explore novel chiral ILs. With a larger scale synthetic approach, cationic dipeptide based chiral ILs have the potential to increase the chiral recognition ability of chiral IL solvents, opening up many avenues for future experiments.

The ILs were prepared using a simple metathesis procedure and final samples contained <0.5% water. The luminescent CPL probe [Eu(dpa)$_3$]$^{3-}$ was dissolved in each of the ILs and the complex’s stability was ensured using emission spectroscopy and luminescence lifetime measurements. Results show that the site symmetry around [Eu(dpa)$_3$]$^{3-}$ remains intact when solvated by the ILs. However, outer-sphere interactions between [Eu(dpa)$_3$]$^{3-}$ and the IL differ between samples, which is likely a result of the difference in structural identity of the R-groups of the amino acid methyl ester cation between ILs. The chiral recognition ability of the ILs was measured using a custom-built CPL spectrometer. All of the amino acid based ILs displayed chiral recognition, but the anionic peptide based ILs showed no recognition. Within the amino acid based ILs, it was observed that the preference of chiral recognition was dictated by the stereochemistry of the amino acid methyl ester, while the relationship between amino
acid structure and chiral recognition was more complex. It was observed that the alanine methyl esters structure did not have much of a difference on the perturbation of the [Eu(dpa)$_3$]$^3^-$ equilibrium than the proline methyl ester structure. However, both displayed a different structure relationship with chiral recognition than the leucine methyl ester IL, as seen in the temperature dependent data. The temperature independent relationship of chiral recognition in [L-LeuC1][Tf$_2$N] could be a result of entropic chiral ordering due to ionic size matching observed in recent studies. Future experiments with other bulky amino acid ILs such as an isoleucine methyl ester based IL, as well as using less bulky counter cations than [TBA]$^+$ such as tetramethylammonium, could shed more light on the cause of the observed temperature independent chiral recognition in [L-LeuC1][Tf$_2$N].

Overall, amino acid based chiral ILs show promise as solvents for asymmetric synthesis and as stationary phases in chiral resolution applications due to the observed dissymmetry factors shown in this study. An advantage of amino acid based ILs elucidated from this experiment is the ability to tailor, or control, the chiral recognition ability of the solvents through the choice of anion and cation used in IL preparation. Peptide based ILs have the potential to offer even more control over chiral recognition due to the extremely large amount of combinations of amino acid residues that could make up a peptide IL component. Finally, CPL spectroscopy has proven to be an ideal tool for the study of chiral recognition due to the relative ease of taking a one step optical measurement.
References


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Appendix I: CPL Spectra

L-AlaCl / Tf₂N CPL Spectra

283 K 293 K

298 K 303 K

308 K 313 K

318 K 232 K1
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<tr>
<td>232 K1</td>
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</tbody>
</table>
L-LeuC1 / Tf2N CPL Spectra

283 K

293 K

298 K

303 K

308 K

313 K

318 K

232 K1
Appendix II: 2012 Spring ACS National Meeting – San Diego

Abstract: In this study, amino acid (AA) based chiral ionic liquids (ILs) were prepared and their chiral recognition ability probed using a model system. Potential applications of amino acid based chiral ionic liquids include enatioselective catalysis and chiral resolution. The chiral ionic liquids under study were prepared from amino acid methyl ester cations and bis(trifluoromethane)sulfonamide (TF$_2$N) anions, specifically L-alanine methyl ester (L-AlaCl), D-alanine methyl ester (D-AlaCl), L-leucine methyl ester (L-LeuCl), and L-proline methyl ester (L-ProCl). The model system used to quantify chiral recognition of each chiral ionic liquid consisted of dissolving a chiral luminescent probe, racemic Eu(2,6-pyridine dicarboxylate)$_3$- [Eu(dpa)$_3$]$^{3-}$ in the ionic liquid and measuring the ratios of left versus right-handed circularly polarized luminescence (CPL) emitted from the sample. The role of stereochemistry of the amino acids in chiral recognition of the luminescent probe is discussed.

Study of chiral recognition in amino acid based chiral ionic liquid solvents
Daniel Kroupa, Laurel Heckman, Chris Brown, and Todd Hopkins
Butler University, ACS National Meeting 2012

Abstract

Goals

Experimental

Results and Discussion

Conclusion

Acknowledgements

Figure Appendix II: ACS Poster presented at Spring National Meeting in San Diego CA