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Synthesis and toxicity studies of imidazolium-based ionic liquids

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Department:

SYNTHESIS AND TOXICITY STUDIES OF IMIDAZOLIUM-BASED IONIC LIQUIDS

by

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ABSTRACT

There is a considerable recent interest in the applications of room temperature ionic liquids (RTILs) as green solvents in organic synthesis and as nonvolatile electrolytes in lithium ion batteries. A variety of commercially available imidazolium based RTILs have been used for these applications, mainly due to their negligible vapor pressures, high thermal stabilities, wide liquid range, wide electrochemical windows and high anodic stability. The state of the art lithium ion batteries require the use of electrolytes that have relatively low viscosities at temperatures as low as -35 ^{o}C. This thesis describes the synthesis of novel oxygenated and fluorinated imidazolium based RTILs that maintain liquid range at as low as -35 ^{o}C, and their toxicity studies. The toxicities have been also measured as a function of counter-anions using human bronchoalveolar carcinoma cell line (A549), and determined their EC50 values. The toxicities of these newly synthesized ionic liquids are comparable to those of related imidazolium based RTILs, with concentration ranging from 0.2mM to 2.5 mM.

ACKNOWLEDGMENTS

I would like to thank my Advisors, Dr. V. Prakash Reddy and Dr. Yue Wern Huang, for their guidance, encouragement, inspiration, patience and suggestions for improving the quality of this thesis and also for providing this work a clear direction. I am also thankful to my committee members Dr. Ying Fa Ma and Dr. Katie Shannon for their time, encouragement and ideas. I would like to thank both Biology as well as Chemistry departments for providing all the support during my MS degree.

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1.1 INTRODUCTION

Room temperature ionic liquids (RTILs) have a wide range of applications as green solvents in organic synthesis,[1-2] as nonvolatile and thermally and electrochemically stable electrolytes in lithium ion batteries,[3] as proton exchange membranes in fuel cells,[4] as electrochemical sensors,[5] in homogeneous and heterogeneous catalysis,[6] and recently in asymmetric organic synthesis.[7] It is important to identify the factors that control the liquid range of these compounds. One of the main features of ionic liquids in synthesis and catalysis is that both the cationic and anionic components can be varied that are broadly applicable to specific applications[8] in Figure1.1.

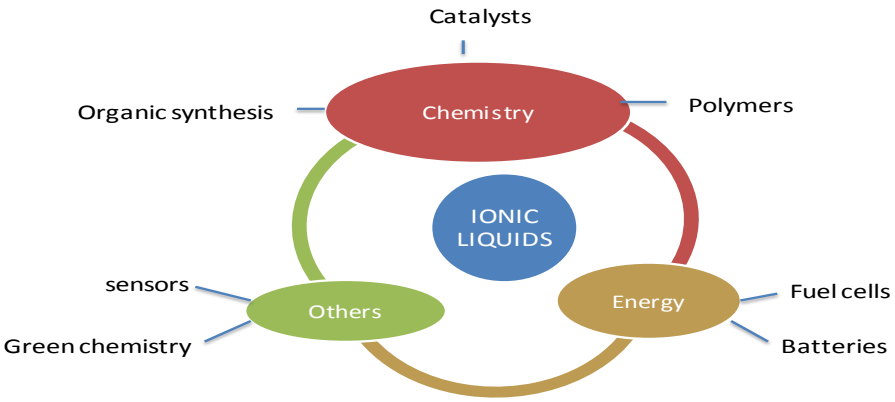
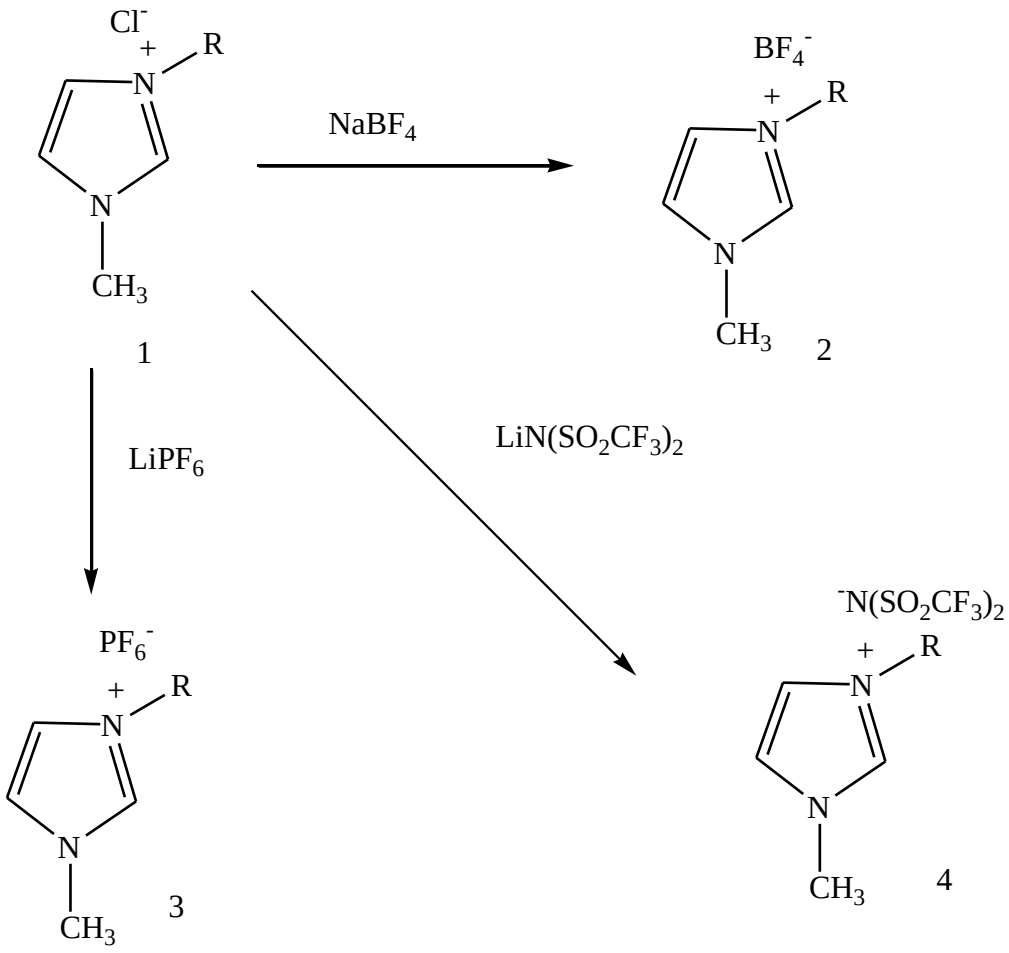


Figure1.1.Application of Ionic Liquids

1.2. IMIDAZOLIUM -BASED IONIC LIQUID

All the ionic liquids explored, 1, 3-dialkylimidazolium salts have found numerous applications as many of them exist as liquids at ambient temperature.[9] It is believed that the asymmetry of cations and anion plays an important role in controlling their melting points. There is increasing focus on extending the liquid range of these compounds, and by appropriate choice of the side chain the melting points and viscosities of the ionic liquids can be modulated. For example, 1, 3-dialkylimidaolium chlorides in Figure1.2 are solids at room temperature, whereas metathesis of the anion to tetrafluoroborates or hexafluorophosphates converts them to relatively low melting salts, 2 and 3, existing as liquids at ambient and subambient temperatures. Anion metathesis using lithium bis (trifluoromethanesulfonyl) imide gives ionic liquids, 4, that have even more liquid range. Nature of the side chains on the imidazolium ring also has significant effect on the melting points of ionic liquids. The branched chain alkyl groups usually give low melting ionic liquids.



R = e.g., Me, Et, Pr^n, Bu^n

Figure1.2. Metathesis reaction of imidazolium salts

1.3. GENERAL SYNTHETIC METHODS OF IONIC LIQUIDS

A vast majority of ionic liquids currently employed in a variety of applications include 1-methyl-3-alkyimidazolium compounds. It is convenient to synthesize these compounds by alkylation of 1-methylimidazole using alkyl halides or alkyl tosylates. These reactions proceed by bimolecular nucleophilic substitution (S_N2) pathway, and these are usually restricted to primary alkyl substrates. In general, alkyl chlorides react relatively slower than the corresponding bromides or tosylates, and normally refluxing

the neat solutions (i.e., without adding any further solvents) for several hours, whereas the use of primary bromides or tosylates makes it possible for the reactions to proceed under milder conditions, even at ambient temperatures, as expected based on the S_N2 reaction pathway. These reactions cannot be used for primary alkyl fluorides as the fluoride anion is relatively a poor leaving group in Figure1.3.

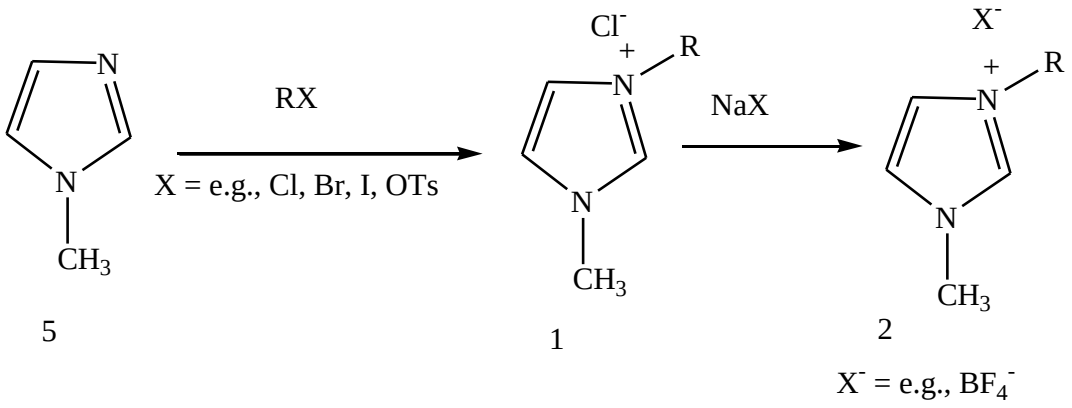


Figure1.3. Alkylation reaction of 1-methyl imidazole

1.4. IONIC LIQUIDS AS GREEN SOLVENTS

Due to negligible vapor pressures and relatively nontoxicity, RTILs have been increasingly adopted to industrial scale synthesis as green solvents. Many of the conventional organic reactions such as Friedel-Crafts reactions have been achieved using RTILs as solvents.[10] Nucleoside based anti-viral drug candidates have been synthesized using RTILs as solvents.[11] The RTILs, in these reactions were reported to be better than the conventional molecular solvents in terms of solubility and reaction rates. Recently, there has been some progress in the applications of ionic liquid based compounds as chiral catalysts in organic synthesis. Thus, ionic liquids possessing chirality have been explored as chiral catalysts for organic transformations.[12-17] Ionic liquids derived from,

for example, proline has been used as chiral catalysts in asymmetric Mukaiyama aldol reactions.[15, 18-19] However, recent studies show that some of the RTILs may not be as environmentally benign as originally considered.[20] Thus development of novel type of RTILs without adverse environmental impact is crucial for the successful application of these solvents for industrial scale processes.

ILs based on the Imidazolium cations especially 1,3-dimethylimidazolium cations and their coordinating anions (such as $(CF_3SO_2)_2N^-$, BF^-_4) have been demonstrated to have low viscosity. RTILs are thermally stable at relatively high temperatures (stable over 300 ^oC) and they are also relatively non-flammable compounds. Further, these properties can be tailored by varying the nature of the cations and counter anions.

In spite of the demonstrated toxic properties of some of the ionic liquids, the RTILs are relatively environmentally benign in comparison to related organic solvents as they are essentially thermally stable compounds towards degradation. Further, RTILs can be biodegraded to simpler non-toxic products. It has been shown that the ionic liquids can be degraded by soil microorganisms, water-microorganisms, Pseudomonas putida and Escherichia coli.[21] For example, 1-methyl-3-butylimidazolium tetrafluoroborate can be biodegraded in the waste water streams by E. coli in about 15 days. Several researcher groups focused their attention on oxidative and thermal degradation of ILs in aqueous media. Ionic liquids have potential to affect the aquatic system through a variety of mechanisms including acetyl cholinesterase enzyme inhibition which leads to deleterious effects on aquatic systems.[22]

1.5. TOXICITY STUDIES OF RTILS

RTILs with low melting range are useful as solvents in industrial scale production of fine chemicals and they are potentially useful as low temperature electrolytes in lithium ion batteries, as they possess relatively high conductivities, wide electrochemical windows, and high thermal and chemical stabilities. There is also interest in fuel cell applications of proton conducting imidazolium based ionic liquids.[23]

The major objective of our current study of RTILs is to synthesize and evaluate the toxicity of the novel RTILs with oxygenated and fluoroalkyl side chains (compounds 6, 7 and 8). The side chains of alkoxyalkyl groups and fluoroalkyl groups on the imidazolium and related ionic liquids may render these compounds as thermally and electrochemically stable. For example, the electron withdrawing effects of these side chains will increase their oxidation potentials at the cathodes. These compounds also provide an opportunity to explore their toxicity effects as a function of their hydrophilic or hydrophobic effects. Introduction of fluorine into the side chains would increase their hydrophobicity whereas alkoxy groups increase their hydrophilicity. The toxicity properties of such alkoxymethyl and fluoroalkyl derived ionic liquids have not been investigated in the literature to date, although there are several studies on the toxicity of imidazolium based ionic liquids. The relatively low toxicity of imidazolium salts has been made use of them as gene deliver vectors. For example, a polymeric imidazolium ion liquid, poly[3-butyl-1-vinylimidazolium L-proline salt, can be used as a gene vector and as shown by propidium iodide assay, it intercalates with DNA protecting the genes from enzymatic degradation.[24] The toxicity of the imidazolium based ionic liquids is

dependent on the side chains, as shown by the earlier observations which show that the lower the toxicity the lower the length of the alkyl side chain.[25] The later authors also showed that the toxicity effect of the anions (chloride, hexafluorphosphate and triflate) is negligible. The concentration of the RTILs also plays a role on the exerted toxicities on bacterial cultures. At moderate concentration of the imidazolium salts, the bacteria adapt to them so that there is no effect on the bacterial growth, while at much higher concentrations, the bacterial growth is inhibited.[6] The RTILs with alkoxyalkyl and fluoroalkyl side chains are expected to have potential applications in industrial organic synthesis, as well as electrolyte materials in lithium ion batteries. This thesis addresses the synthesis of ionic liquids, 1-methyl-3-ethoxymethylimidazolium bis(trifluoromethanesulfonyl)imide (6), 1-methyl-3-ethoxymethylimidazolium tetrafluoroborate (7) and 1-methyl-3-(2-fluoroethyl)imidazolium bis(trifluoromethanesulfonyl)imide (8), their structural characterization using multinuclear NMR, and their toxicity studies using human bronchoalveolar cell lines. These thermal stabilities can also be determined by using thermogravimetric analysis.

1.6. RESULTS AND DISCUSSION

The newly synthesized imidazolium and related heterocyclic based ionic liquids (not discussed in this dissertation) containing ethoxymethyl, and fluoroalkyl substituents, and selected compounds 6, 7, and 8 for their toxicity assays in Figure 1.4**.** 1-Methylimidazole was reacted with the various alkyl halides and the resulting halide salts were reacted with lithium bis(trifluoromethylsulfonyl)imide (LiTFSI) in aqueous solution to give their corresponding TFSI salts, as liquids at room temperature. These ionic liquids

were characterized by ^{1}H, ^{13}C and ^{19}F NMR spectroscopy, and thermogravimetric analysis (TGA). In the study of these counteranions, the TFSI salts that have lower melting points, and wider electrochemical stabilities as compared to other counteranions.[26] Toxicity studies for several imidazolium based ionic liquids have been reported in the literature.[25-31] Even though most of the literature studies on RTILs show negligible in vitro or in vivo, some of the studies have found moderate to low toxicity.[25, 28, 30] These toxicity studies would help in designing related ionic liquids with lower toxicities.

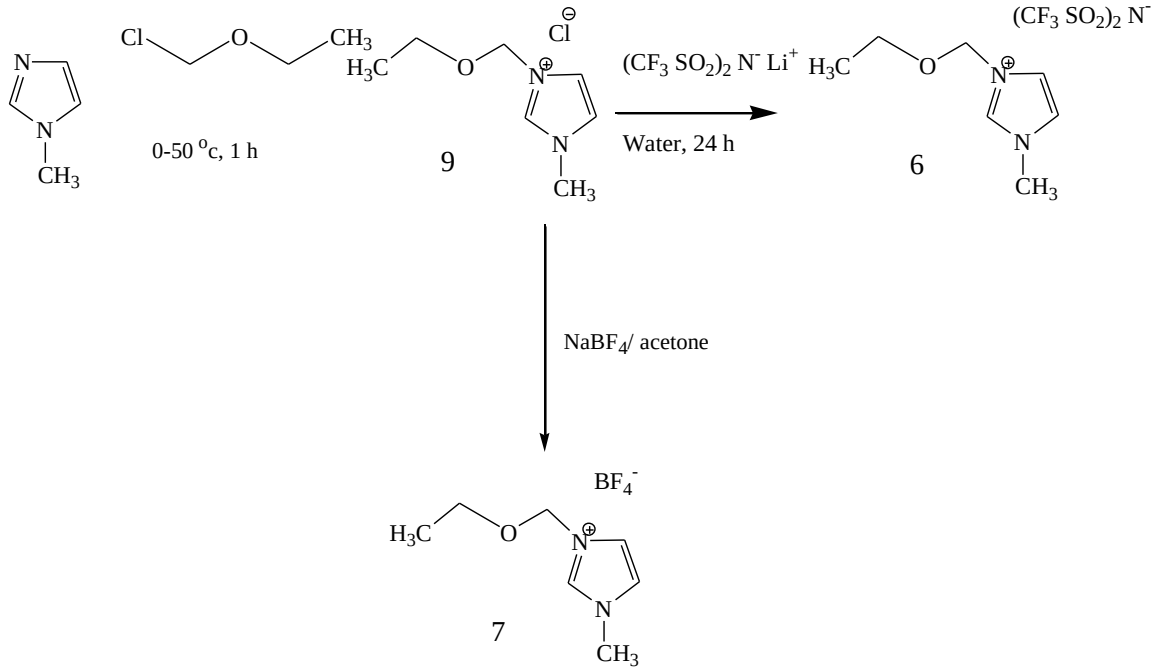
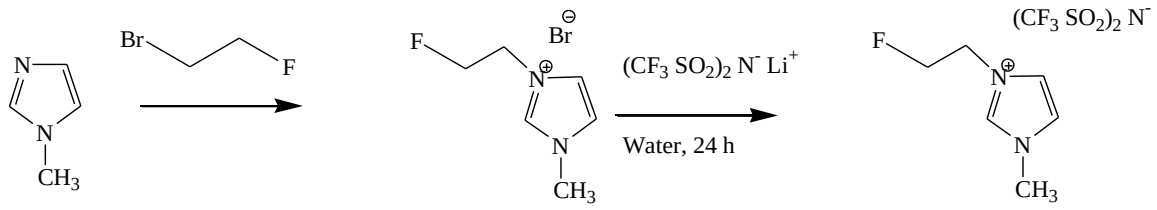


Figure1.4. Synthetic scheme for the preparation of ionic liquids

The reaction of 1-methylimidazole with 1-chloromethoxy ethane at 0 ^{o}C for 1 hour gave 1-methyl-3-(ethoxymethyl)imidazolium chloride (1) as a pale yellow hygroscopic solid which was filtered and washed with ether to remove any unreacted starting materials. Anion metathesis of compound 9 with LiTFSI in aqueous solution

gave compound 6 as a pale yellow viscous liquid. The compounds were characterized by ^{1}H, ^{13}C and ^{19}F NMR spectral analysis.

Similarly, reaction of 1-methylimidazole with 2-fluoroethyl bromide give 1-methyl-3-(2-fluoroethyl)imidazolium bromide (10) which upon anion metathesis using LiTFSI gave the ionic liquid, 1-methyl-3-(2-fluoroethyl)imidazolium TFSI (8) in Figure1.5.



10 8

Figure 1.5. Preparation of 1-methyl-3-(-2-fluoroethyl)imidazolium TFSI ionic liquid

Interestingly, reaction of 1-methylimidazole with 3,3,3-trifluoropropyl iodide was unsuccessful and the 1-methylimidazolium iodide salt was obtained as the major product. The following mechanism accounts for the unexpected dehydrohalogenation of the alkyl halide in Figure1.6. The electron withdrawing inductive effect of the trifluoromethyl group ensures the high acidity of the neighboring C-H bonds resulting in rapid E2-type of dehydrohalogenation. The 3, 3, 3-trifluoropropene byproduct could not be isolated due to its high volatility.

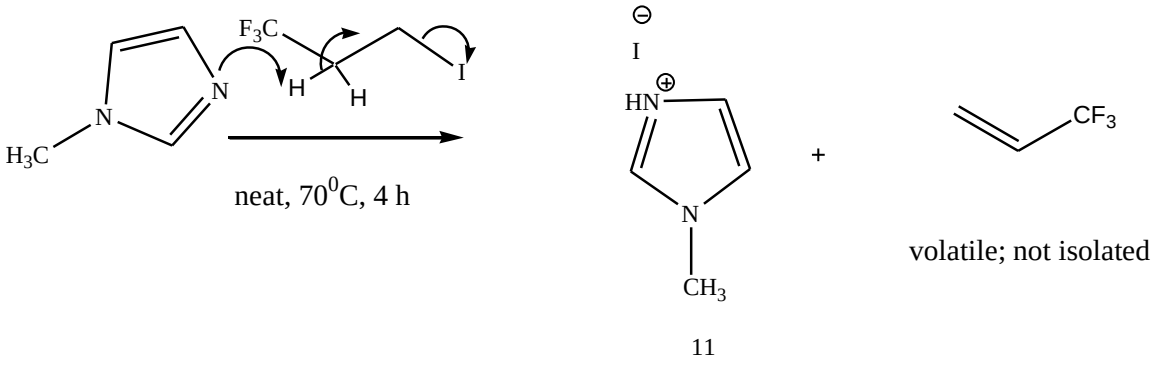


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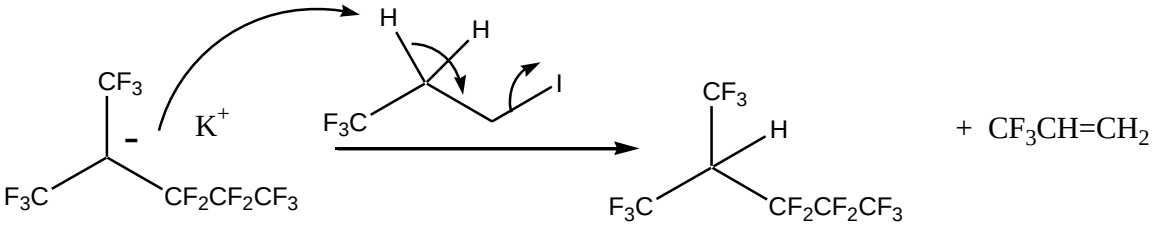
Figure1.6. Dehydrohalogenation of 1-methyl-3-(3,3,3-trifluoropropyl)imidazolium iodide

There is one precedent in the literature for a similar E2 reaction in Figure1.7 of the 3,3,3-trifluoropropyl iodide with a perfluoroalkyl-carbanion, 12, to give the corresponding conjugate acid, 13, and trifluoropropene, as the byproduct.[32] Therefore, we have selected the corresponding monofluorinated substrate, 3-fluoropropyl iodide for the synthesis of the monofluoroalkyl substituted imidazolium ionic liquid. S_N2 reaction of this compound with 1-methylimidazole proceeded in near quantitative yields without any attendant E2 competition.



12 13

Figure1.7. Elimination reaction of 3,3,3,trifluoropropyliodide

The thermal stability of the ionic liquids synthesized in this study was studied using TGA instrument. The decomposition temperatures (T_d) for these ionic liquids were found to be over 300 ^oC showing their high thermal stabilities.

1.7 EXPERIMENTAL SECTION

1.7.1. Materials and Methods. Chloromethoxyethane, (>96%) and LiTFSI (>98.0%) were purchased from TCI America was purchased from Fisher Scientific. 1-methylimidazole, (>99%), diethyl ether (anhydrous, ACS certified) Acetone (ACS certified, >99.5 %), magnesium sulfate (anhydrous, 99.5%), silica gel (99%, 63-200 A^o mesh size), acetone-d_6 were purchased from Sigma Aldrich and were used as received.

^{1}HNMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a INOVA-Varian 400 MHz spectrometer at 400 MHz , 100 MHz and 376 MHz, respectively in acetone-d_6 solvent. The chemical shifts for ^{1}H and ^{13}C NMR were referenced with respect to residual solvent signals or internal tetramethylsilane and the ^{19}F spectra were referenced to internal trichlorofluoromethane ($\delta CFCl_3 = 0$). All the ^{13}C chemical shifts were assigned using proton coupled ^{13}C spectra. The ionic liquids with chloride or bromide counteranions are moisture sensitive and these reactions were done under inert nitrogen atmosphere. The anion metathesis using LiTFSI was done in aqueous solution, as the final products are not hygroscopic.

Thermogravimetric analysis, using TGA, model: TGA-Q50 V20 instrument was conducted at a heating rate of 10 ^oC/min (reported T_d was the onset temperature where the rapid decomposition started).

1.7.2. Synthesis of 1-ethoxymethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide (6). To a stirred solution of Chloromethoxyethane (11.2 g, 118 mmol) at 0 ^{o}C in a 50 mL RB flask , 1-methylimidazole (7.5 g, 91 mmol), was added dropwise in an inert atmosphere. The reaction mixture was stirred at 0 ^{o}C for 30 min, and at 50 ^{o}C in a water bath for 1 h. The reaction mixture was cooled to room temperature and washed with diethyl ether (28 mL). A light yellow solid was obtained after filtration, purification and drying in high vacuum (92% yield). The yellow solid (15 g, 85 mmol) was dissolved in water and stirred with LiTFSI (23.30 g, 81 mmol) for 24 h. The product was extracted from the reaction mixture using dichloromethane. The organic layer was dried ($MgSO_4$), and the solvent removed in high vacuum to give the compound 6 as an off-white viscous liquid (24.99 g, 85%); mp: ~ -35^{0}C.

^{1}H NMR in Figure1.8, F (400 MHz, Acetone-d_6): δ 9.13 (s, 1 H), 7.81 (bs, 1 H), 7.73 (bs, 1H), 5.69 (s, 2H, N- CH_2), 4.07(s, 3H, -N-methyl), 3.64(q, J_{CH} = 7 Hz, 2H, O-CH_2), 1.16 (t, J_{CH} = 7Hz, 3H, methyl) ; ^{13}C-NMR in Figure1.9, ^{19}F NMR in Figure1.10 and ^{13}C-NMR in Figure1.11 (^{1}H coupled) (400 MHz, Acetone-d_6), δ 137.6 (d, J_{CH} = 201 Hz), 123.9 (d, J_{CH} = 205 Hz), 122.0 (dm, J_{CH} = 203 Hz), 119.2 (s, J_{CF} = 320Hz), 116.0 (s), 79.6 (t, J_{CH}= 163Hz), 66 .2 (t, J_{CH} = 136 Hz), 36.7 (q, J_{CH} = 143 Hz), 14.2 (t, J_{CH} = 126Hz) ; ^{19}F NMR (376 MHz, Acetone-d_6) δ -78.91(s).

1.7.3. Thermogravimetric Analysis. The thermal stability of the ionic liquids synthesized in this study was studied using TGA. Thermogravimetric analysis, using TGA, model: TGA-Q50 V20 instrument was conducted at a heating rate of 10 ^{o}C/min (reported T_d was the onset temperature where the rapid decomposition started). The TGA

curves recorded for the compounds 6 is shown in Figures1.12. The thermal decomposition temperatures (T_d) estimated for compound 6 from TGA is 405 ^oC.

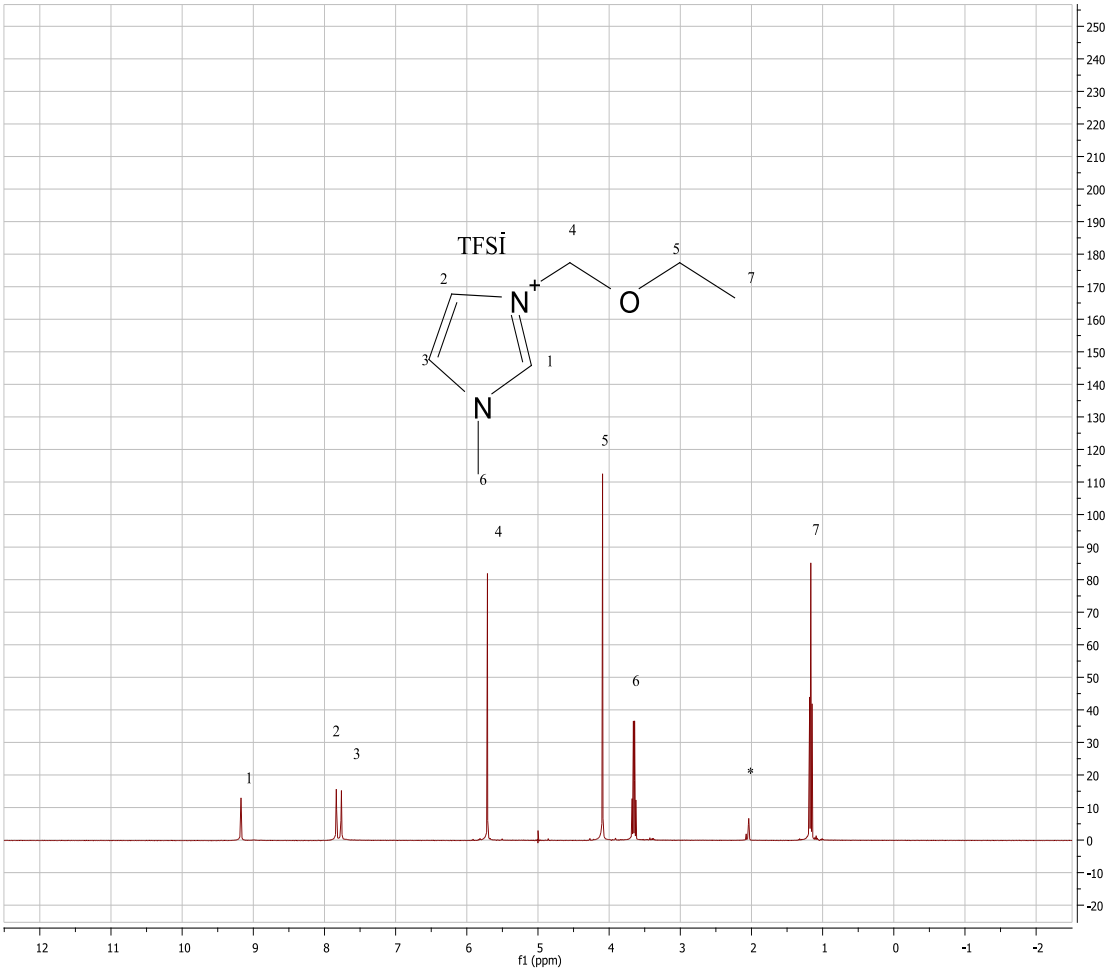


Figure1.8. ^1H NMR spectrum (400 MHz) of 1-ethoxymethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide

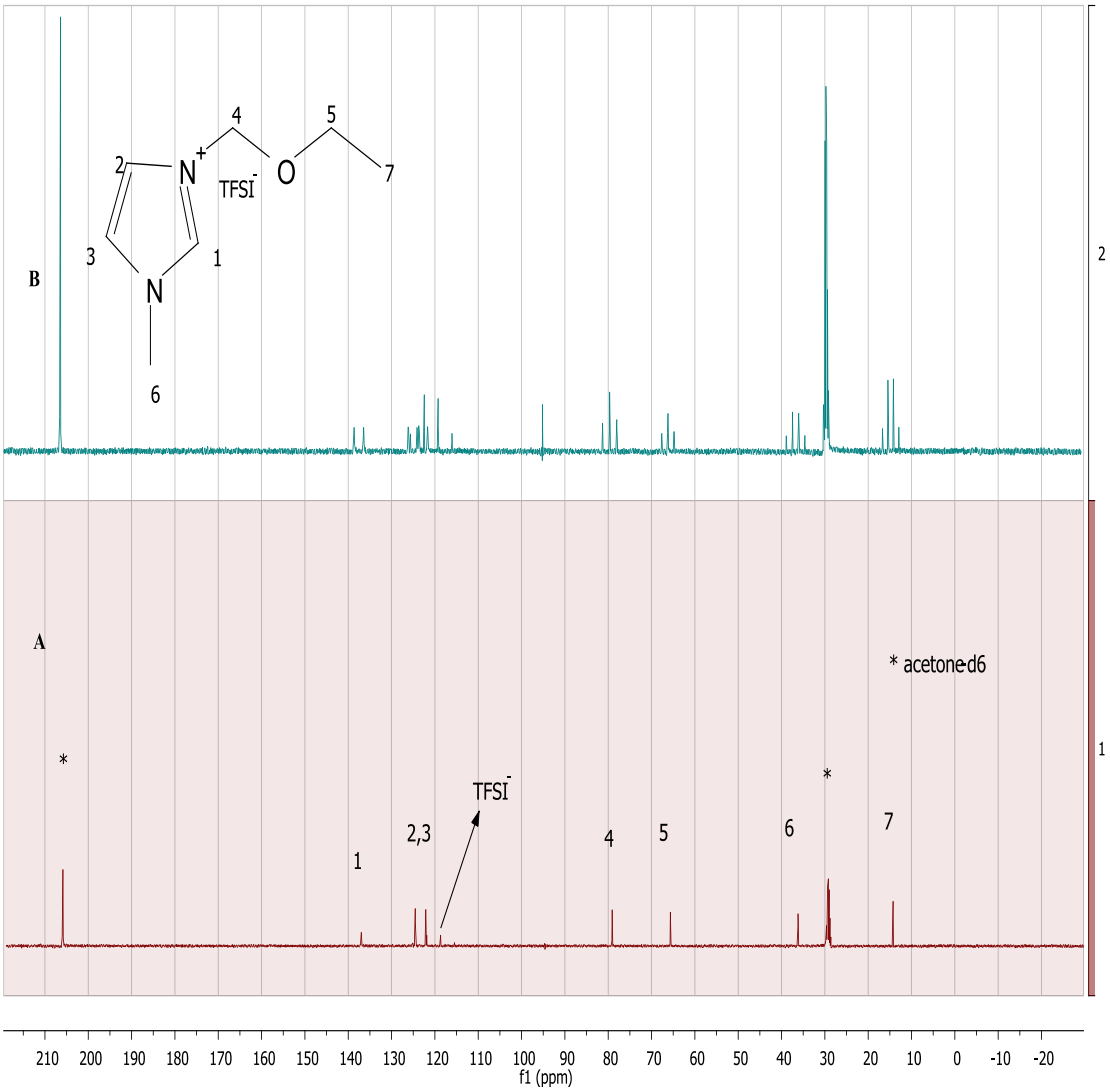


Figure1.9. ^{13}C NMR spectra (100 MHz, A, proton decoupled; B, proton coupled) of 1-ethoxymethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide

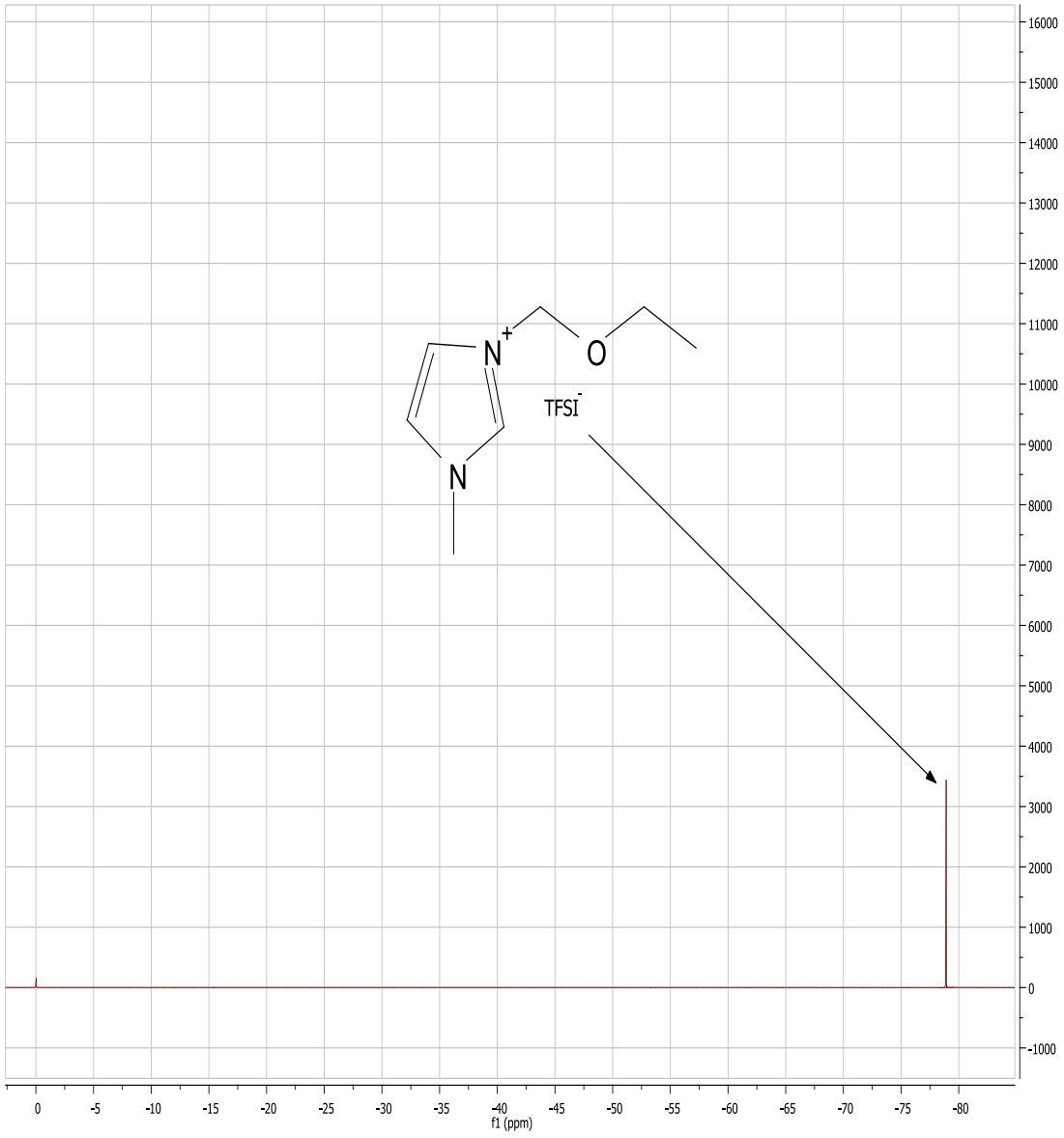


Figure1.10. ^{19}F NMR spectrum (376 MHz) of 1-ethoxymethyl-3-methylimidazolium bis (trifluoromethanesulfonyl)imide

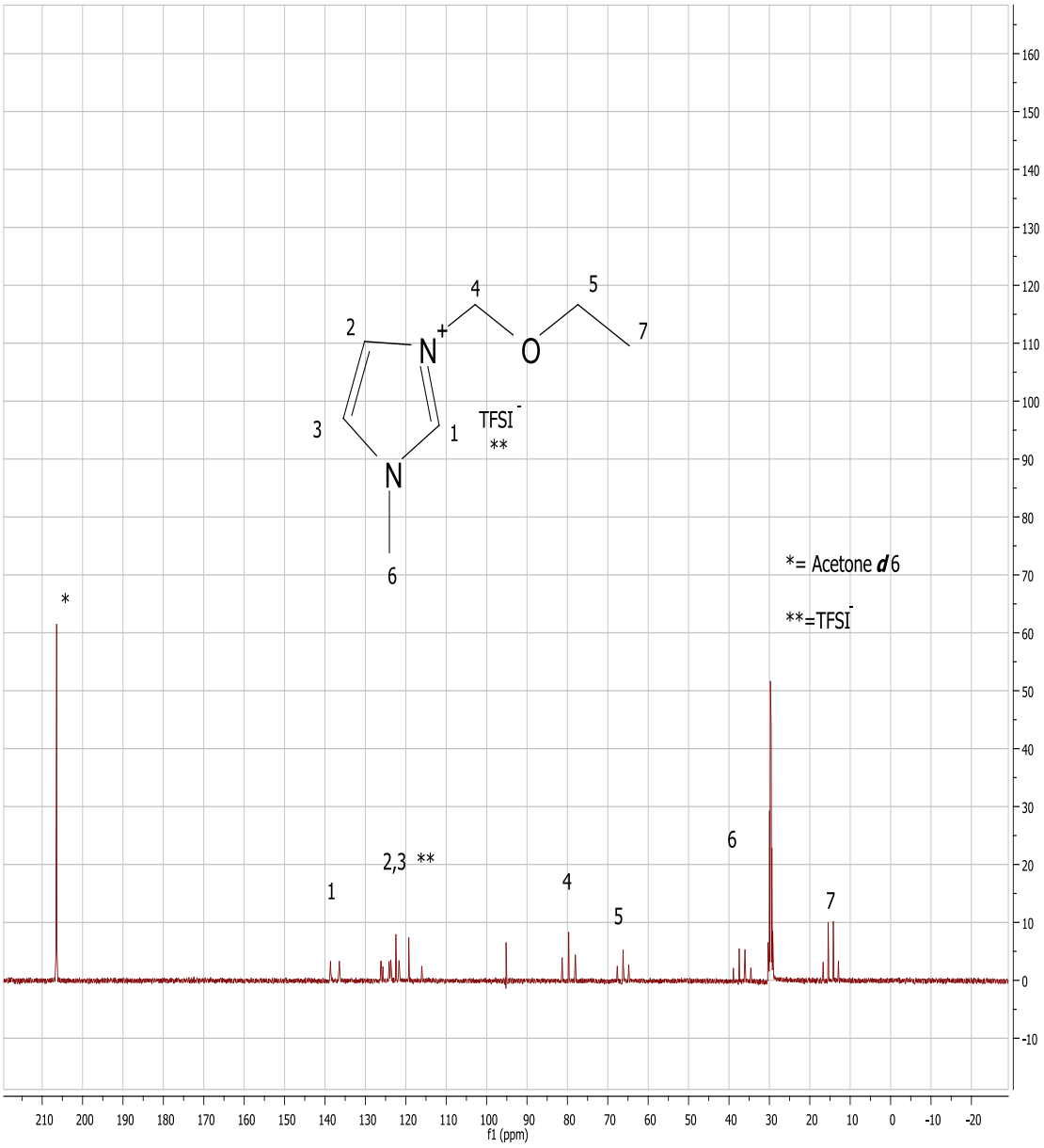


Figure1.11. ^{13}C NMR coupled spectrum (400 MHz) of 1-ethoxymethyl-3-methyl imidazolium bis(trifluoromethanesulfonyl)imide

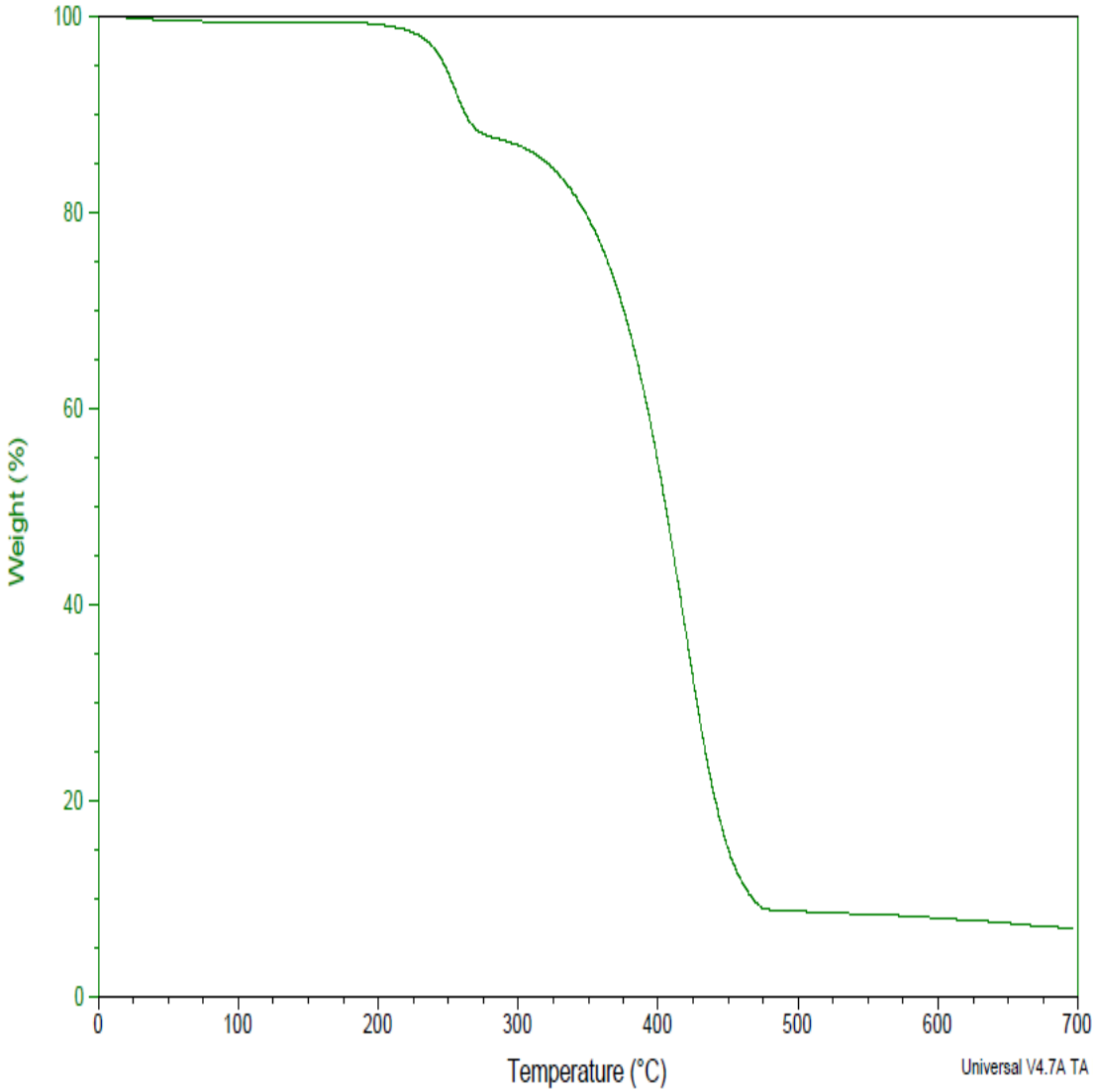


Figure 1.12. Thermogravimetric analysis of 1-ethoxymethyl-3-methyl imidazolium bis(trifluoromethanesulfonyl)imide ($T_d = 405^0C$)

1.7.4. Synthesis of 1-ethoxymethyl-3-methylimidazolium tetrafluoroborate (7). To a stirred solution of chloromethoxyethane (7.7 g, 81 mmol) at 0 ^oC in a 50 mL RB flask,1-methylimidazole (5g, 61mmol), was added drop wise in a nitrogen atmosphere. The reaction mixture was continued stirring, at 0 ^oC for 30 min. The solid precipitated was stirred at 50 ^oC in a water bath for 1 h. The mixture was cooled to room temperature and washed with diethyl ether (28 mL). A light yellowish solid was obtained

after filtration, purification and drying in high vacuum. The yellowish solid (10 g, 56 mmol, 92% yield) was dissolved in acetone and stirred with $NaBF_4$ (6.9 g, 63 mmol) for 54 h. The reaction mixture was filtered through column of silica gel, and dried ($MgSO_4$). The solvents were removed on a rotary evaporator under high vacuum. The compound **7** was obtained as a yellowish viscous liquid (32.2g**,** 14.12 mol, 95% yield); mp: ~ -20^0C.

^{1}H NMR in Figure1.13 (400 MHz, Acetone-d6): δ 8.9 (s, 1H-imidazole), 7.7(bs, 1H-imidazole), 7.8 (bs, 1H-imidazole), 5.6 (s, N- CH_2), 4.0 (s, -N-methyl), 3.6 (q, J = 7.0, O-CH_2), 1.1 (t, J = 7.0, methyl);^{13}C-NMR in Figure1.14 and Figure1.15 (Coupled) (400 MHz, Acetone-d6) δ 14.9 ((qt, J=126Hz,3Hz, CH_3), 36.6 (q, J =143 Hz, -O-CH_2), 66 .1 (tq, J = 147Hz, 6Hz, N-methyl), 79.4 (t, J= 165Hz, N-CH_2), 122.5 (dm, J = 209 Hz, imidazole CH), 124.9 (d, J=216Hz. Imidazole CH), 137.6 (d, J = 216 Hz, imidazole CH); ^{19}F NMR in Figure1.16 (376 MHz, Acetone-d6) δ 149.7.All the ^{1}H, ^{13}C chemical shifts were assigned using proton coupled ^{13}C spectra, ^{19}F NMR spectra that were recorded on a INOVA-Varian 400 MHz spectrometer at 400 MHz, 100 MHz and 376MHz, respectively, in acetone-d6 solvent.

1.7.5. Thermogravimetric Analysis. The thermal decomposition temperatures (Td) estimated for compound **7** from TGA is 305 ^{o}C in Figure1.17.

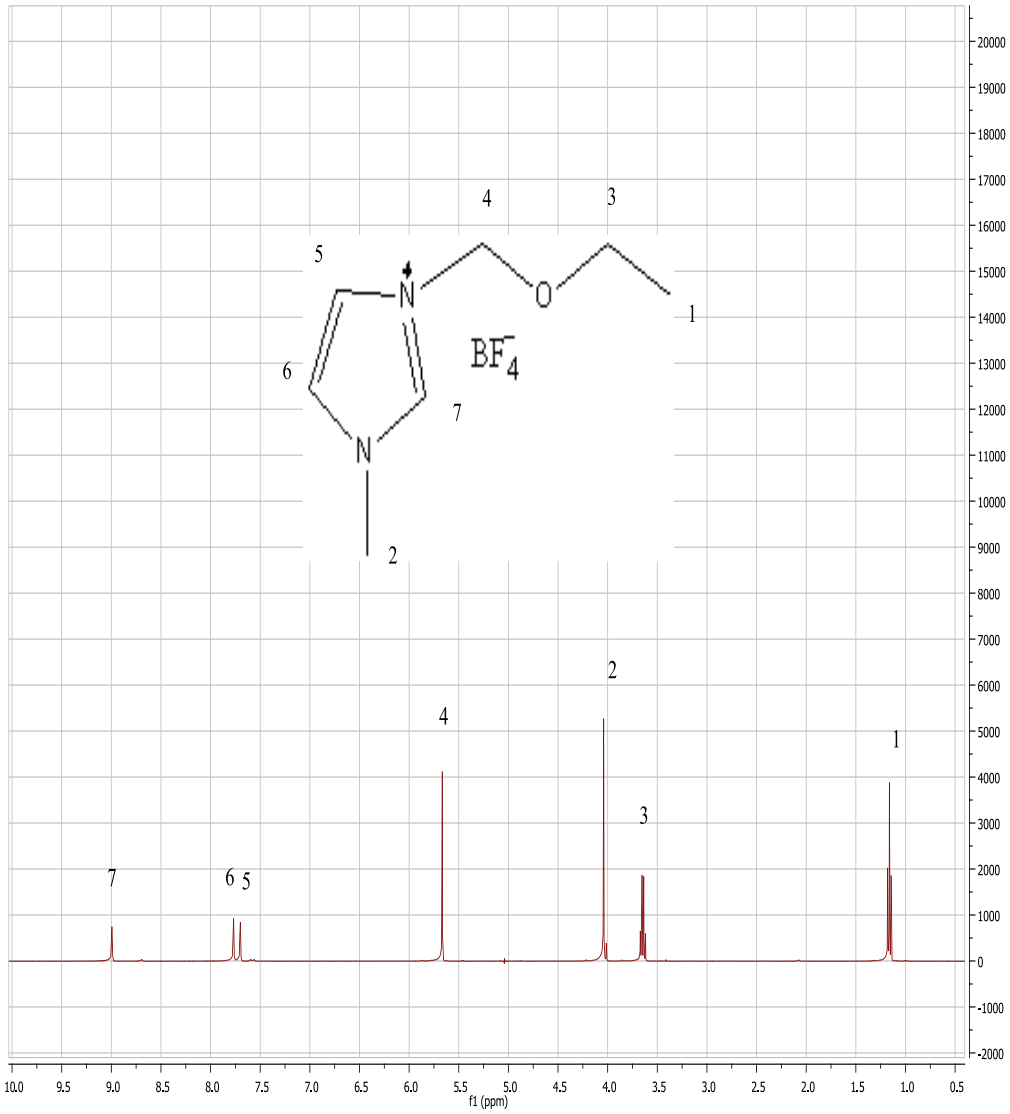


Figure1.13. ^{1}H NMR spectrum of 1-ethoxymethyl-3- methylimidazolium tetrafluoroborate

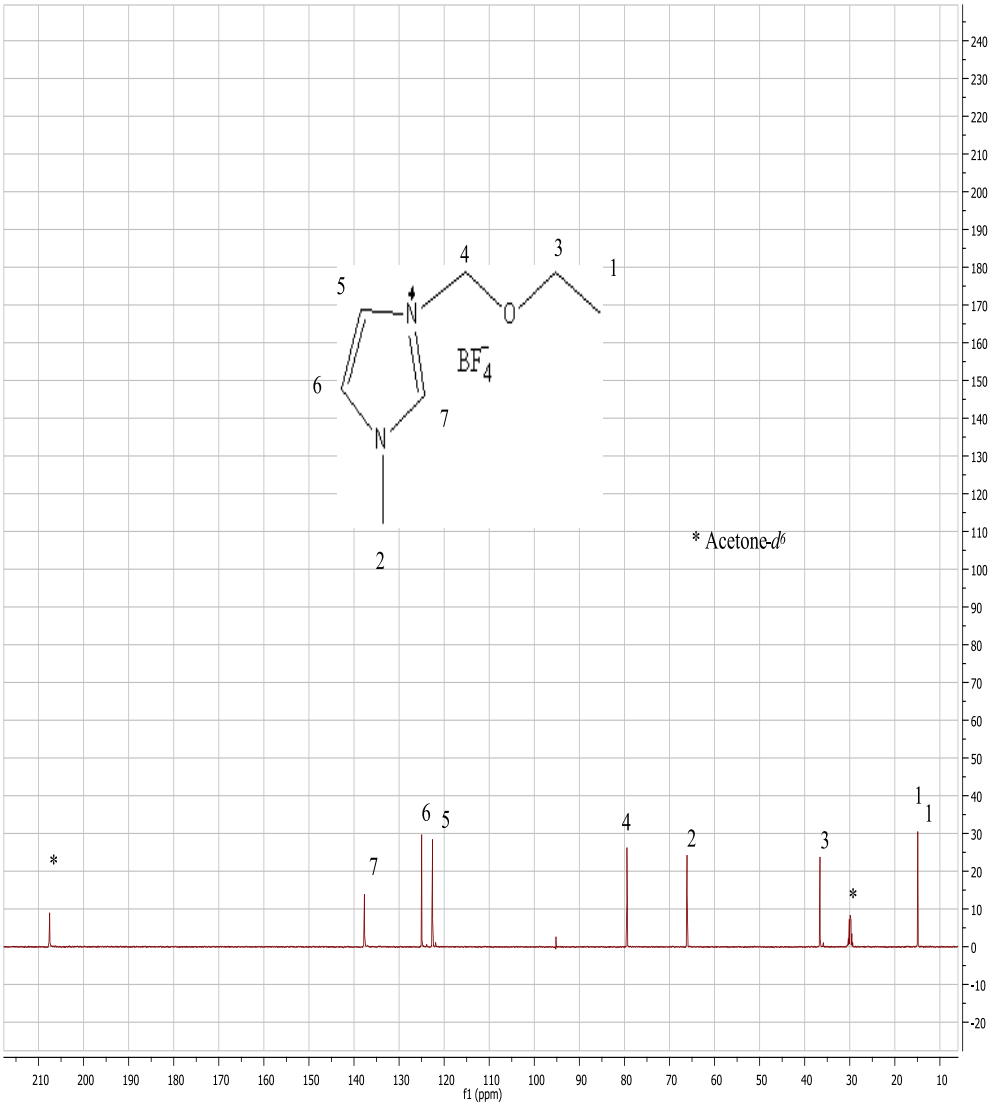


Figure 1.14. ^{13}C NMR spectrum of 1-ethoxymethyl-3-methylimidazolium tetrafluoroborate

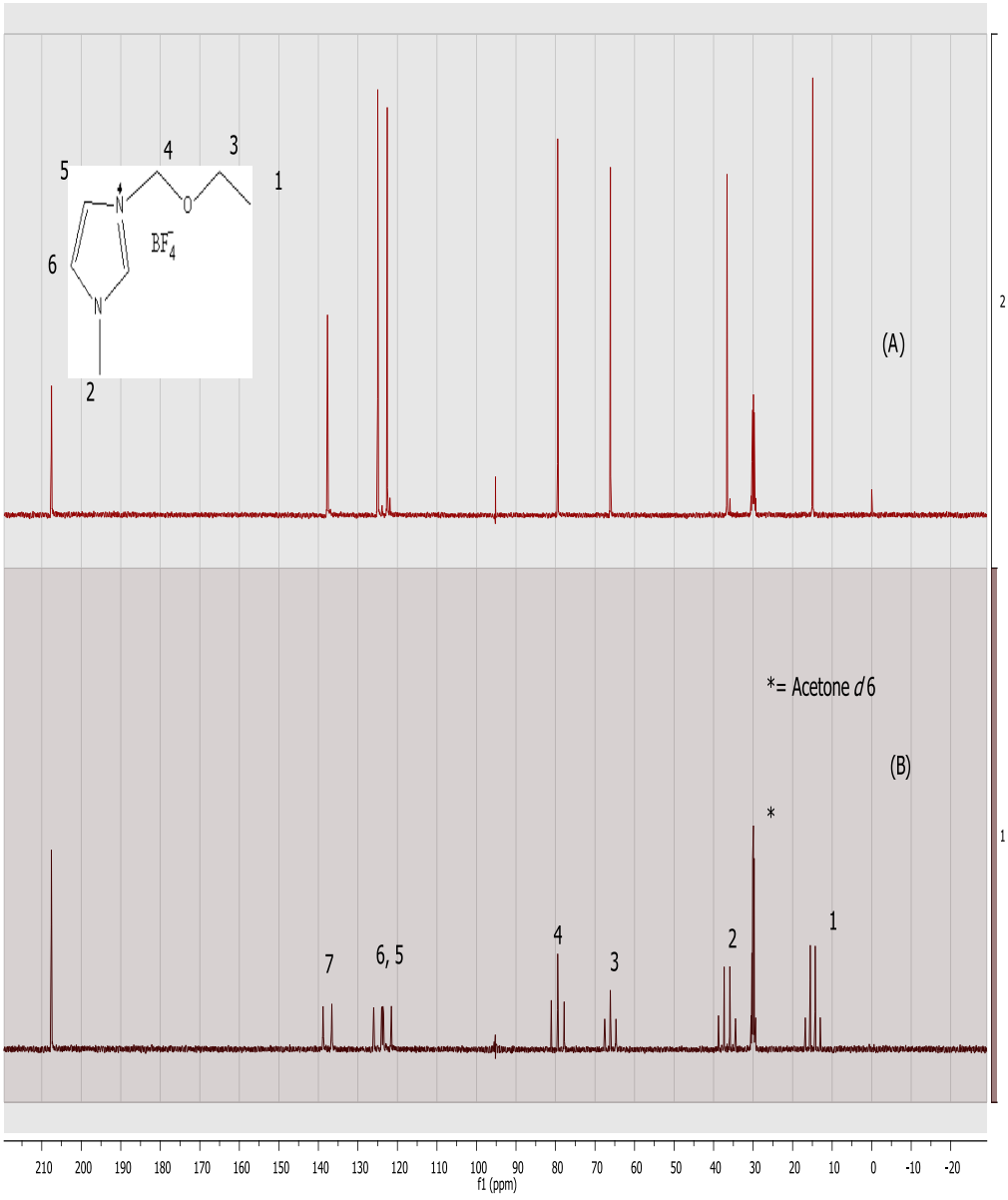


Figure1.15. ^{13}C NMR spectra (A, proton decoupled; B, proton coupled) of 1-ethoxymethyl-3-methylimidazolium tetrafluoroborate

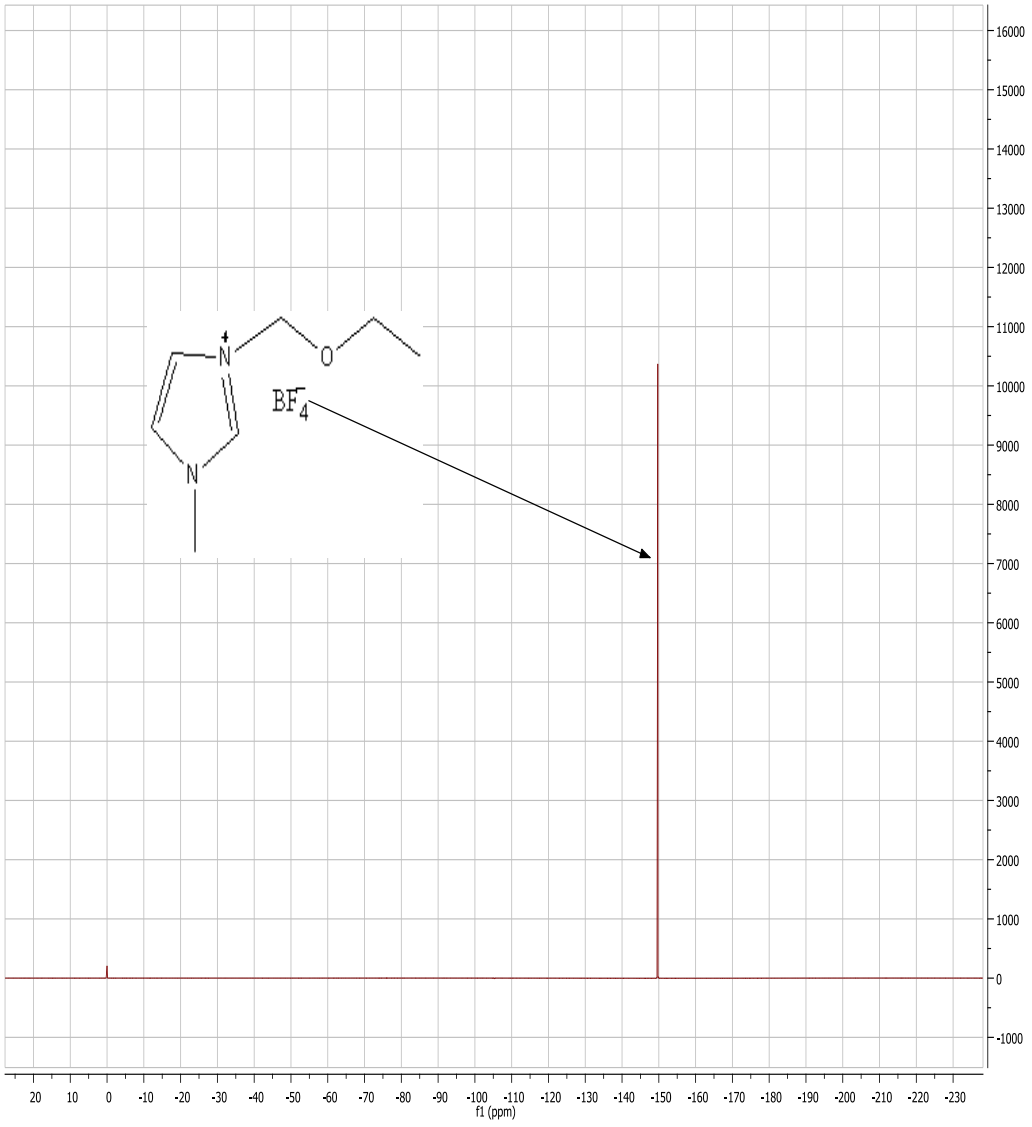


Figure1.16. ^{19}F NMR spectrum of 1-ethoxymethyl-3-methylimidazolium tetrafluoroborate

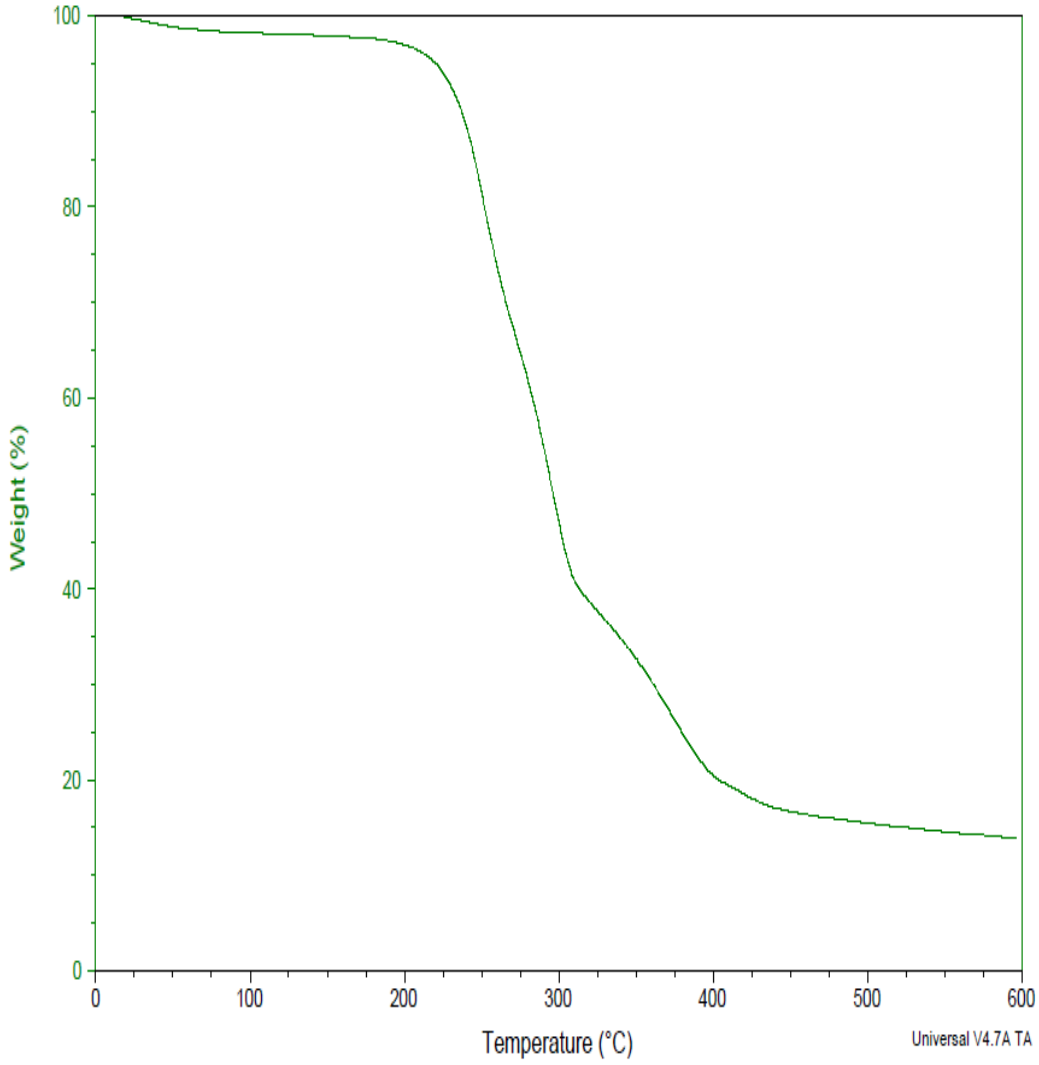


Figure1.17. Thermogravimetric analysis of 1-ethoxymethyl-3-methylimidazolium tetrafluoroborate (T_d = 305^{0}C)

1.7.6. Synthesis of 1- methyl-3-(2-fluoroethyl)imidazolium bis (trifluoromethanesulfonyl)imide (8). To a stirred solution of 1-bromo2-fluoroethane at room temperature in a 50 mL RB flask, 1-methyl imidazole was added and kept at 50 ^{0}C for 15 h. The reaction was continued stirring at 50 ^{0}C. Lithium bis (trifluoromethanesulfonyl) imide (1 mol equiv) was added to the reaction and stirred for

24 hours at room temperature. Compound 8 was obtained as a colorless viscous liquid; mp. ~ -30^0 C.

^{1}H NMR in Figure1.18 and ^{19}F NMR in Figure1.19 (400 MHz, Acetone-d_6): δ 9.07 (s, 1H-imidazole), 7.77 (apparent dd, 1H-imidazole), 4.97(dd, J = 80Hz, F-CH_2), 4.85(dd, J = 80Hz, F-CH_2), 4.79(dd, J = 80 Hz, N-CH_2), 4.72(dd, J = 80Hz, N-CH_2), 4.10 (s, N-methyl); ^{13}C-NMR in Figure1.20 and Figure1.21 (Coupled) (400 MHz, Acetone-*d6*) δ 36.66 (qt, J = 143 Hz, N-CH_3), 50.85 (dt, J =142 Hz, 31 Hz N-CH_2), 82.31(qd, J =152 Hz, 16 Hz, F-CH_2), 124.15(dd, J = 199 Hz, 7H-Imidazole CH), 121.25 ((q, J = 319 Hz, TFSI), 137.80 (d, J = 223 Hz, imidazole-CH); ^{19}F NMR(376 MHz, Acetone-*d6*) δ-223.29 (m, J = 45 Hz, F-CH_2), 78.91(s, TFSI).

1.7.7. Thermogravimetric Analysis. The thermal decomposition temperatures (T_d) estimated for compound 8 from TGA is 426 ^{o}C in Figure1.22.

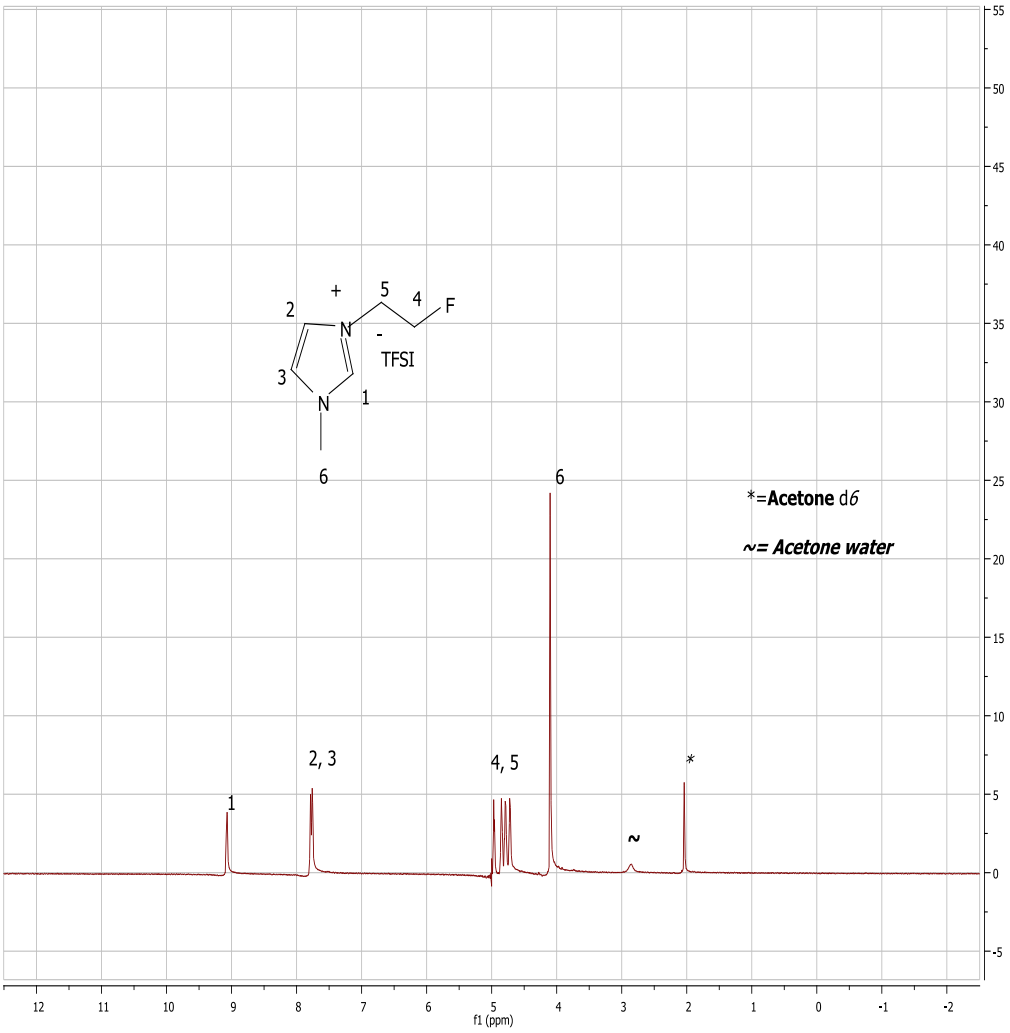


Figure 1.18: ^{1}H NMR spectrum of 1- methyl-3-(2-fluoroethyl)imidazolium bis(trifluoromethanesulfonyl)imide

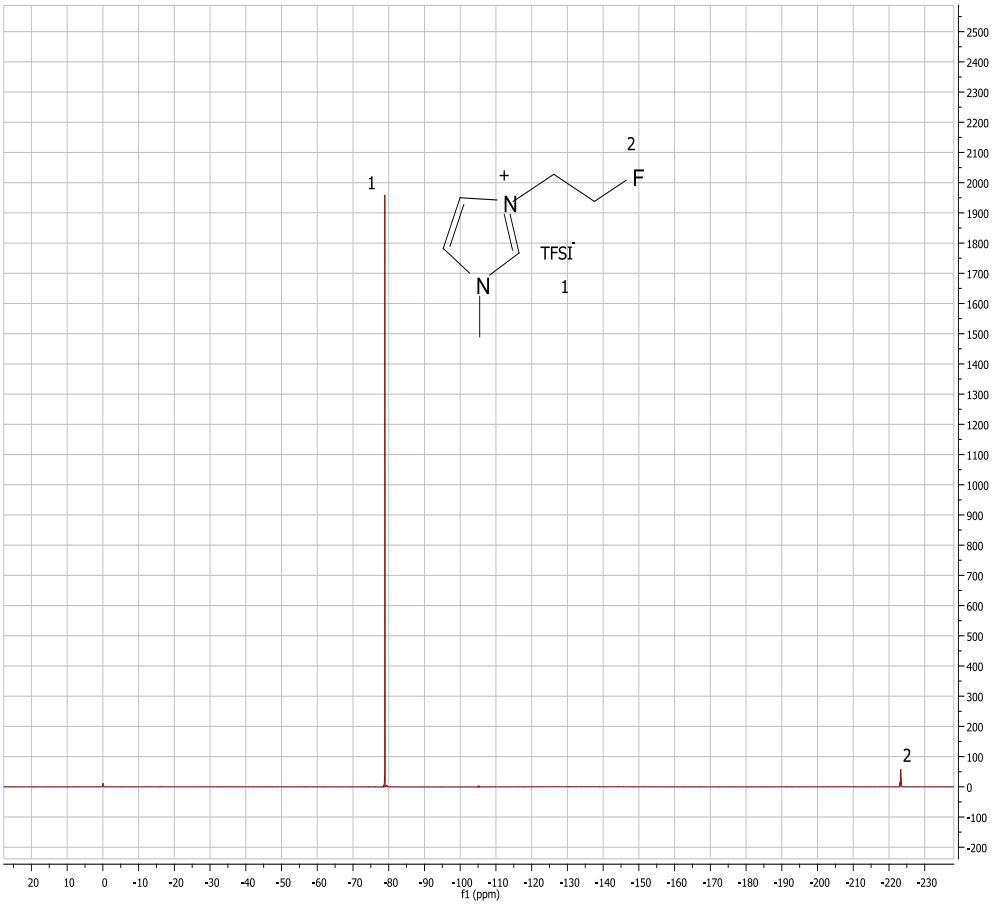


Figure1.19. ^{19}F NMR spectrum of 1- methyl-3-(2- fluoroethyl) imidazolium bis(trifluoromethanesulfonyl)imide

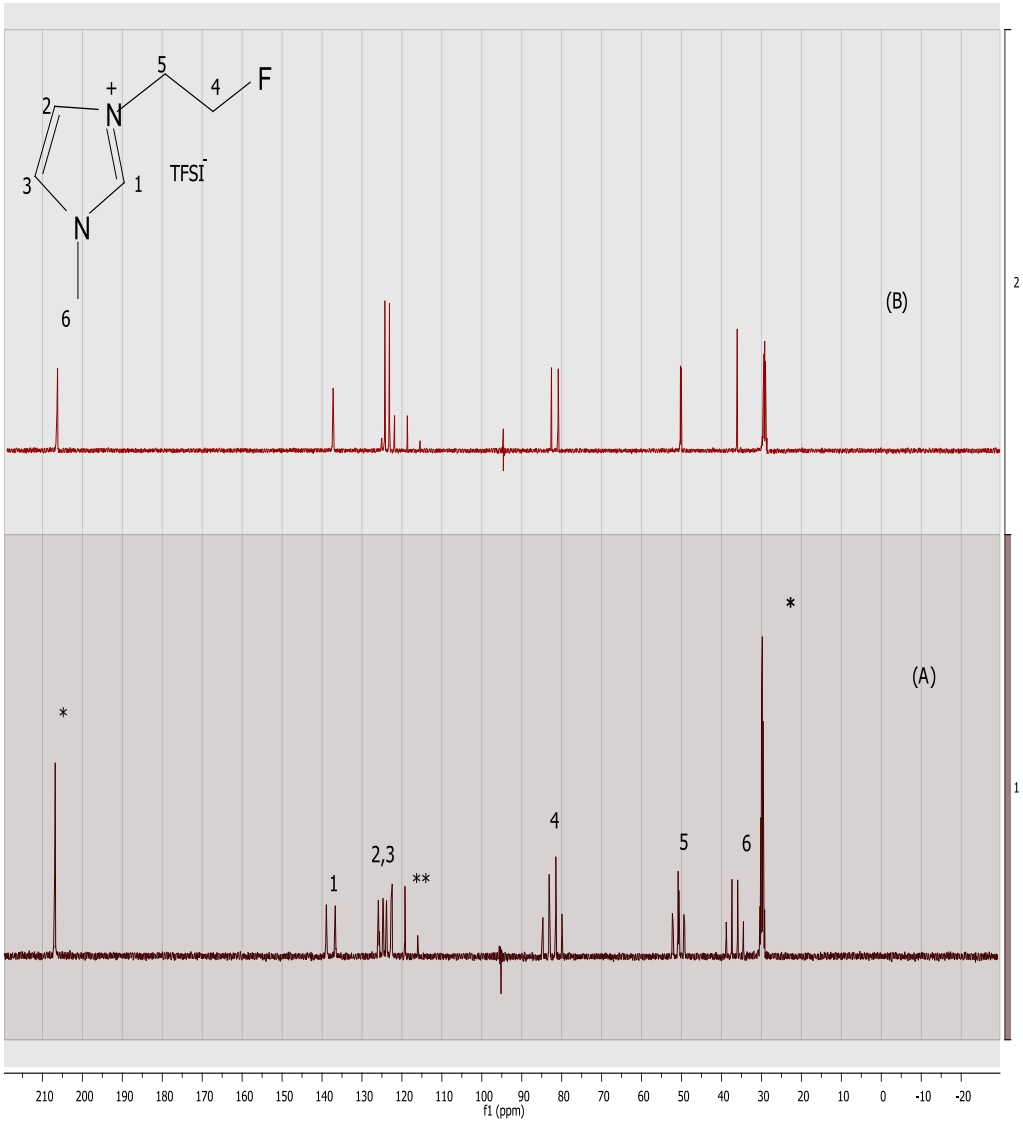


Figure1.20. ^{13}C NMR spectra (A, proton coupled; B, proton decoupled) of 1-methyl-3-(2- fluoroethyl)imidazolium bis(trifluoromethanesulfonyl)imide

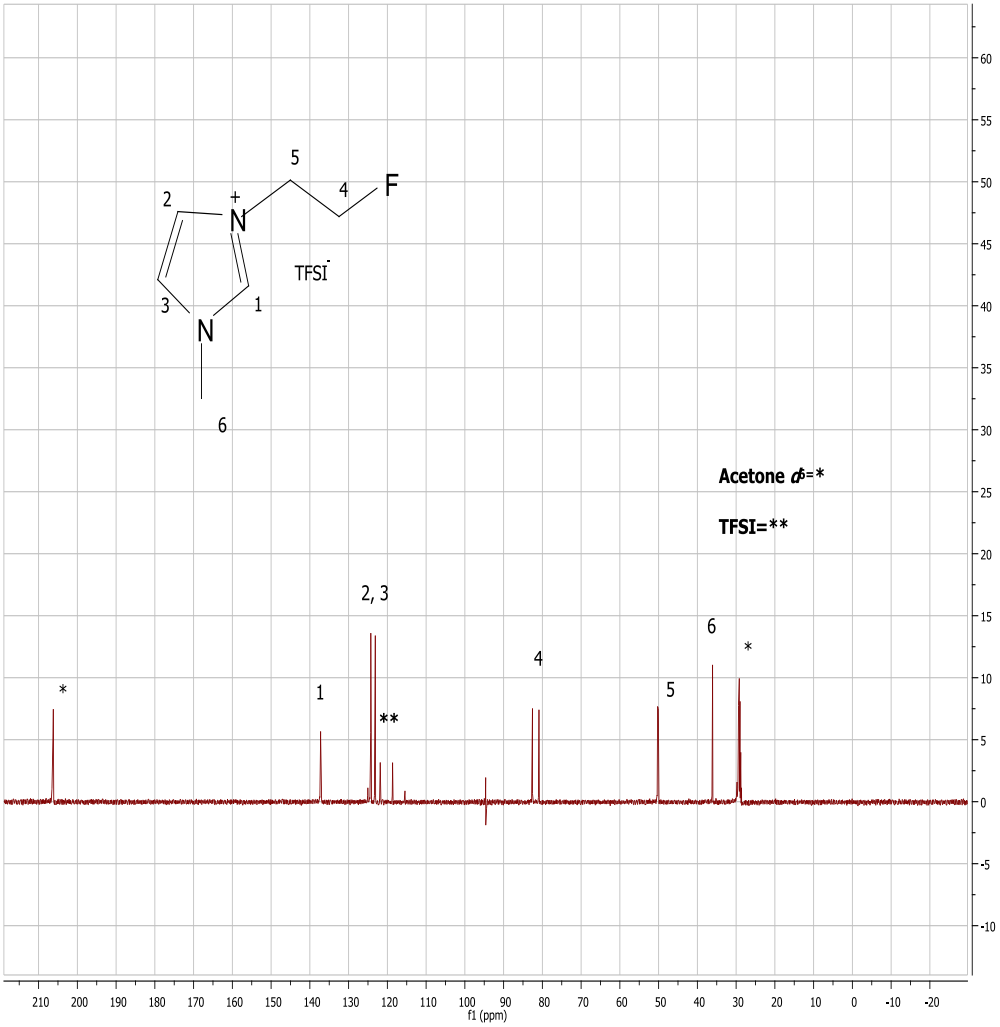


Figure1.21. ^{13}C NMR spectrum of 1- methyl-3-(2-fluoroethyl)imidazolium bis(trifluoromethanesulfonyl)imide

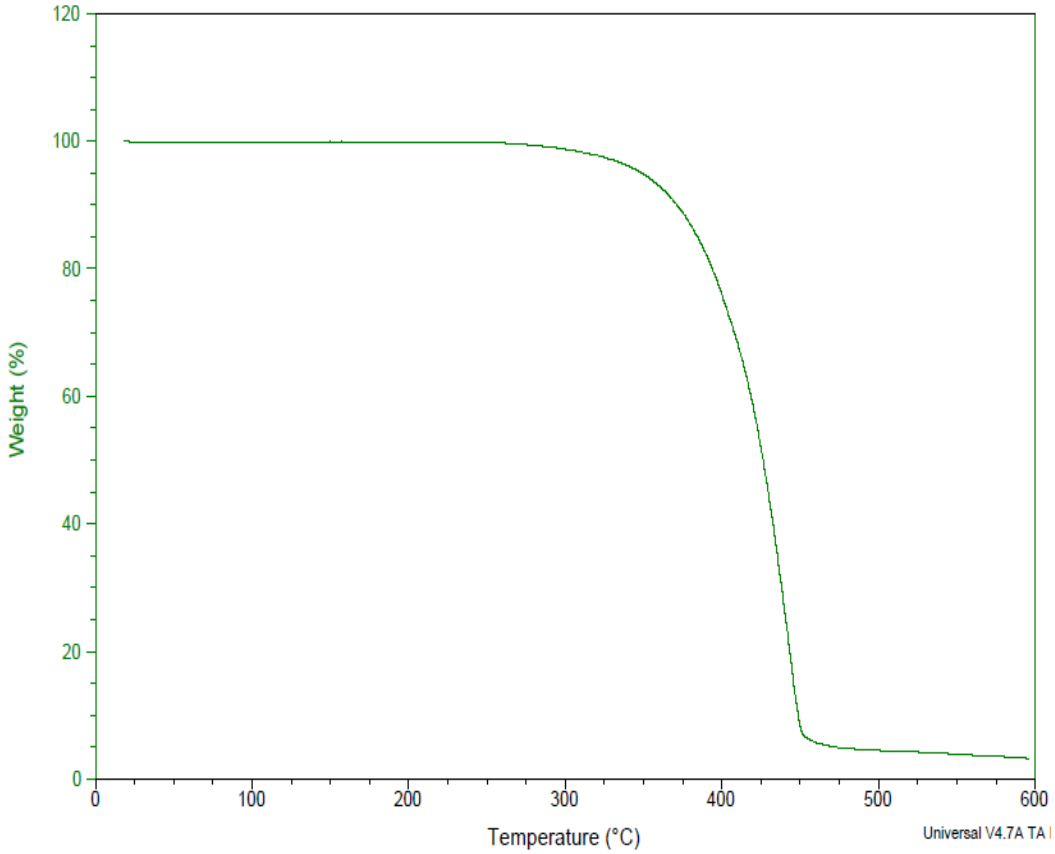


Figure1.22. Thermogravimetric analysis of 1-methyl-3-(2-fluoroethyl)imidazolium bis(trifluoromethanesulfonyl) imide (($T_d = 426^0C$)

2. DETERMINATION OF TOXICITY OF IONIC LIQUIDS

2.1. INTRODUCTION

Although there are several studies on the toxicity studies of imidazolium based ionic liquids.[6, 24-25], the toxicity properties of alkoxymethyl and fluoroalkyl derived ionic liquids have not been investigated. In this section we describe our results of toxicity studies of the ionic liquids 6-8 using a human bronchoalveolar cell line. These studies are more directly related to human health as compared to literature studies using bacterial cell cultures. In comparing the results with those of reported values of other compounds, The conducted experiments on LiTFSI as a test case.

2.2. CELL VIABILITY ASSESSMENT

The cytotoxicity of the oxygenated and fluoroalkyl- derived imidazolium salts, 6-8 and LiTFSI using a human bronchoalveolar cell line, A549, using sulforhodamine-B (SRB) assay. The SRB assay has been used to estimate cell viability of various cancer cell lines. [33-34] 5% DMSO that killed 70% of population used as a positive control. 0.02% DMSO that did not cause cytotoxicity was used as the solvent of test chemical. The experiments were performed in triplicate and the data was expressed as mean ±SD.

2.3. MATERIALS AND METHODS

Ham's-F-12, (1x) modified medium, fetal bovine serum (FBS), penicillin-streptomycin, sulforhodamine-B in Figure 2.1, acetic acid and dimethylsulfoxide were purchased from Fisher Scientific. All the ionic liquids were synthesized as described in

Section 1 of this thesis.

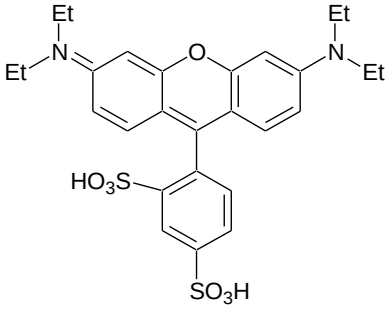


Figure2.1. Structure of sulforhodamine-B

2.4. CELL CULTURE

The human bronchoalveolar carcinoma derived cell line (A549) was purchased from ATCC (Manassas, VA, USA). Cells were treated in Ham's F-12 medium (1x) supplemented with 10% FBS, 1% penicillin-streptomycin and grown at 37 ^{0}C in an incubator.

2.5. CELL VIABILITY MEASUREMENT

Cell viability was measured using the SRB assay.[35-36] Stock solutions of ionic liquids and DMSO were 0.25 mM to 2.5 mM. Cell line was maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS), and 1% penicillin streptomycin, and grown at 37 ^{0}C in presence of 5% CO_2 humidified environment. First the cells were cultured with FBS and incubated at 37 ^{0}C for 48 h. A549 cells were seeded at a final concentration of 1 X 10^3 cells ml into 24-well plates. After the cells were treated with FBS for 48 hours, the old medium was discarded, a series of

concentrations ranging from 0.20 mM to 2.5 mM were added in incremental amounts. Stocks of ionic liquids were prepared in culture medium with 0.02% DMSO, a noncytotoxic level, to improve the solubility of the ionic liquids. 5% DMSO that kills 70% of cell populations is used as positive control. After cells being exposed to test chemical for 24 h at 37 ^{0}C, the experiment was terminated by discarding the culture media, adding 10% TCA (trichloroacetic acid) solution. The plates refrigerated for an hour at 4 ^{0}C. The TCA solution was removed and the cells were washed with water for at least three times. 500 µL 0.2% SRB in 1% acetic acid was added to each wells and kept it on Tilter for 20 min. The media was dispensed, and washed two more times with 1% acetic acid. The solution was discarded and 300 mL of 10 mM Tris-Base solution was added and kept on the Tilter for 20 min., and an aliquot of 100µl solution was transferred from each well into a new 96 well- plate.

Absorbance was measured using FLOUstar Optima (BMG Labtechnologies, Durham, NC, USA) microplate reader at 490 nm in Figure 2.2 and Figure 2.3.

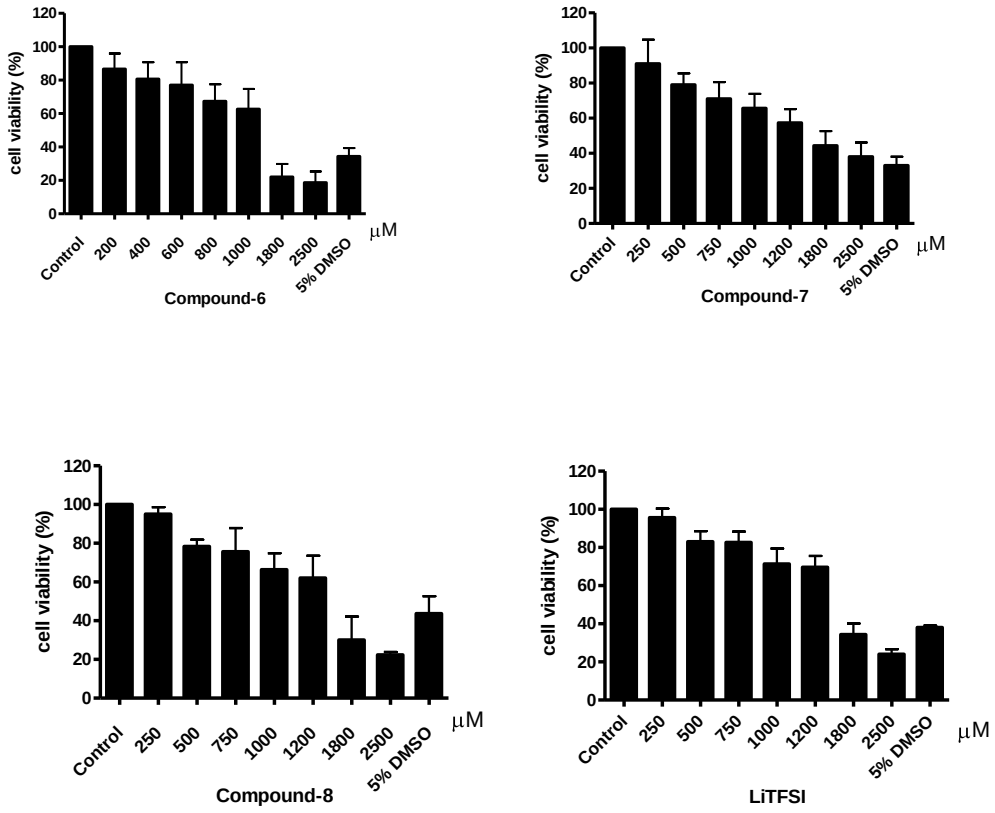


Figure2.2. Cell viability after being exposed to varying concentrations of ionic liquids (6-8 and LiTFSI) for 24 hours

Test chemicals were dissolved in cell culture medium containing 0.02% DMSO as this is not cytotoxic. 5% DMSO was used as a positive control. The mean ±SD represents values for four independent experiments.

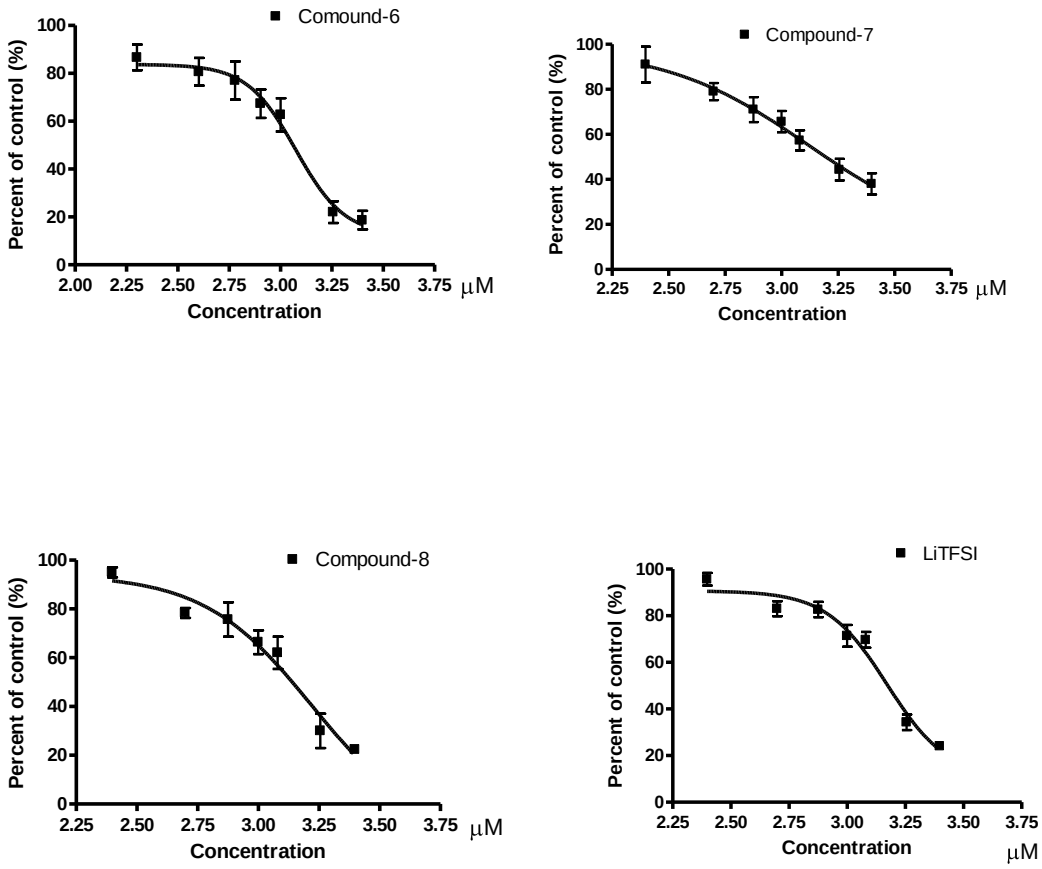


Figure2.3. Curve fitting of cell viability for ionic liquids (6-8 and LiTFSI)

Cell viability for ionic liquids (6-8 and LiTFSI) to obtain EC50 values in Table 2.1 three independent experiments of triplicates for each concentration was performed for each test chemical.

Table2. 1. Comparisons of EC50 value of the ionic liquids (6-8 and LiTFSI).

Compound	Solvent (100µl) used	Correlation Coefficient ($R^{2)}$	EC50 (mM)
6	DMSO	0.964	1.19
7	DMSO	0.925	1.32
8	DMSO	0.917	1.66
LiTFSI	Water	0.888	1.48

Cell viability after being exposed to varying concentrations of ionic liquids (6-8 and LiTFSI) for 24 hours. These values were obtained from the curve fitting of cell viability

3. CONCLUSION AND FUTURE WORK

The newly synthesized alkoxyalkyl and fluoroalkyl derived imidazolium ionic liquids show that these compounds are relatively nontoxic to human health and their toxicity is comparable with LiTFSI salt. Due to their wide electrochemical window, high thermal stabilities, wide liquid range, high anodic stability and relatively unharmed nature, these chemicals may be further developed for green chemistry.

There are only limited amount of studies on RTILs. There is a wide variety of basic structures (Imidazolium, Pyrrolidine, Pyridinium) and counter ions combination (tetrafluoroborate, hexafluorophosphate, bis(trifluoromethanesulfonyl)imide, tosylates) from which to choose, and various RTIL mixtures can synthesized and their toxicities and properties can measured.

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VITA

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