SYNTHESIS AND ANTICANCER STUDIES OF METHYLENE BRIDGED *N*-HETEROCYCLIC CARBENE SILVER(I) AND PALLADIUM(II) COMPLEXES DERIVED FROM IMIDAZOL-2-YLIDENES

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by

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LIST OF ABBREVIATIONS AND SYMBOLS

Å	Angstrom, 1×10^{-10} m
°C	Degrees Celsius
FT-NMR	Fourier transform nuclear magnetic resonance
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
CHN	Carbon, hydrogen and nitrogen
δ	Chemical shift in ppm
d	Doublet
t	Triplet
m	Multiplet
DMSO	Dimethylsulfoxide
h	Hours
Hz	Hertz
J	Coupling constant
MHz	Megahertz
mmol	Millimoles
mol	Moles
RT	Room temperature
M	Molar
NHC	*N*-heterocyclic carbene
μM	Micromolar
MCF-7	Human breast cancer
Et	Ethyl

LIST OF PUBLICATIONS

Haque, R.A., Budagumpi, S., Zetty Zulikha, H., **Hasanudin, N**., Khadeer Ahamed, M.B. and Abdul Majid, A.M.S. (2014). Silver(I)-N-heterocyclic carbene complexes of nitrile-functionalized imidazol-2-ylidene ligands as anticancer agents. *Inorganic Chemistry Communications*, 44: 128-133.

Haque, R.A., **Hasanudin, N**., Iqbal, M.A., Ahmed, A.,Hashim, S., Abdul Majid, A. and Ahamed, M.B.K. (2013). Synthesis, crystal structures, in vitro anticancer, and in vivo acute oral toxicity studies of bis-imidazolium/benzimidazolium salts and respective dinuclear Ag(I)-N-heterocyclic carbene complexes. *Journal of Coordination Chemistry*, 66(18): 3211-3228.

Haque, R. A., Iqbal, M. A., Budagumpi, S., Khadeer Ahamed, M.B., Abdul Majid, A.M.S. and **Hasanudin, N.** (2013). Binuclear meta-xylyl-linked Ag(I)-N-heterocyclic carbene complexes of N-alkyl/aryl-alkyl-substituted bis-benzimidazolium salts: Synthesis, crystal structures and in vitro anticancer studies. *Applied Organometallic Chemistry*. 27(4): 214-223.

Haque, R. A., Iqbal, M. A., **Hasanudin, N**. and Nasri, S. F. (2010). Synthesis and characterization of methylated and benzylated imidazole linked new carboxamides. *Science International-Lahore*, 22: 275-279.

SINTESIS DAN KAJIAN ANTIKANSER KOMPLEKS PERAK(I) DAN PALLADIUM(II) TITIAN METIL *N*-HETEROSIKLIK KARBENA TERBITAN IMIDAZOL-2-ILIDENA

ABSTRAK

Tesis ini menerangkan sintesis, pencirian (^{1}H, ^{13}C NMR, CHN, takat lebur) dan aktiviti kanser bagi titian metil garam *bis*-imidazolium (**1**-**4**) dan kompleks perak(I)-*N*-heterosiklik karbena (NHC). Kesemua garam *bis*-imidazolium ditindak balas dengan punca logam bes, perak oksida, melalui tindak balas pengkompleksan ringkas untuk menghasilkan empat kompleks perak baru (**5**-**8**) dengan hasil yang sangat memuaskan (61-71%). Penggunaan kompleks perak(I)-NHC sebagai reagen pemindahan karbena kepada kompleks palladium(II)-NHC (**9**) telah berjaya disintesis. Kesemua sebatian yang terhasil telah dicirikan melalui teknik spektal dan analitikal seperti NMR spektroskopi (^{1}H dan ^{13}C) dan analisis unsur. Struktur molekul untuk tiga garam *bis*-imidazolium, dua kompleks perak dan satu kompleks palladium telah dikenalpasti melalui teknik pembelauan sinaran-X kristal tunggal. Kajian struktur sinar-X untuk kompleks perak mengesahkan bahawa struktur kompleks perak terdiri daripada dua kation perak di apit oleh dua unit ligan. Kesemua garam, kompleks perak dan palladium adalah bukan bersifat hidroskopik dan menunjukkan kestabilan terhadap udara, lembapan dan haba. Aktiviti antikanser untuk semua garam *bis*-imidazolium, kompleks perak dan kompleks palladium telah diuji melawan sel kanser payudara manusia (MCF-7), dengan menggunakan tamoxifen sebagai ubat piawai (IC_{50} = 2.4 μM). Garam-garam *bis*-imidazolium menunjukkan tiada aktiviti untuk garisan MCF-7, kecuali garam **4**; menunjukkan aktiviti sederhana baik (IC_{50} = 30 μM). Sementara itu, kesemua kompleks perak dan

palladium memaparkan aktiviti antikanser yang signifikan dengan nilai IC_{50} diantara 0.5-6.2 μM terhadap garisan sel yang diuji.

SYNTHESIS AND ANTICANCER STUDIES OF METHYLENE BRIDGED *N*-HETEROCYCLIC CARBENE SILVER(I) AND PALLADIUM(II) COMPLEXES DERIVED FROM IMIDAZOL-2-YLIDENES

ABSTRACT

This thesis presents the synthesis, characterization (^{1}H, ^{13}C NMR, CHN, melting point) and anticancer activity of new methylene-bridged *bis*-imidazolium salts (**1**-**4**) and their respective silver(I)-*N*-heterocylic carbene (NHC) complexes. All *bis*-imidazolium salts were treated with a basic metal source, silver oxide, *via* a simple complexation reaction to afford four silver complexes (**5**-**8**) in exceptionally good yields (61-71%). The use of silver(I)-NHC complex as NHC transfer reagents was shown to be successful to synthesize palladium(II)-NHC complex (**9**). All isolated compounds were characterized by spectral and analytical techniques viz., NMR (^{1}H and ^{13}C) spectroscopy and elemental analysis. Molecular structure of three imidazolium salts, two silver complexes and one palladium complex were established through the single crystal X-ray diffraction technique. X-ray structural studies for silver complexes established that the structures of each silver complex are comprised of two silver cations sandwiched by two NHC units. The structural analysis of palladium complex **9** show that it is a mononuclear Pd(II) complex, chelated by two units of ligands. All the prepared salts, silver complexes and palladium complexes were non-hygroscopic and showed stability towards air, moisture and heat. The *in vitro* anticancer activity of all *bis*-imidazolium salts, silver complexes and palladium complexes were investigated against the human breast cancer (MCF-7) cell line, using tamoxifen as standard drug (IC_{50} = 2.4 µM). The *bis*-imidazolium salts demonstrated no activity for MCF-7 cell line, except salt **4**; showing moderate activity (IC_{50} = 30 µM). Whereas, all the silver and palladium

complexes displayed significant activity with IC_{50} values in the range 0.5-6.2 μM against tested cell line.

CHAPTER ONE

INTRODUCTION

1.1 General background (carbene and *N*-heterocylic carbene)

A carbene is defined as an organic molecule containing a divalent carbon atom with two non-bonding electrons and has general formula :CR_2. Most of the carbenes are very reactive, unstable and easier said than done to isolate. Carbenes can be classified as either in singlet state or triplet state depending on their electronic configurations. Singlet carbenes have two electrons (antiparallel spin-paired) that are not involved in bonding, whereas in the triplet carbenes, two electrons reside in two different orbitals with parallel spins as illustrated in Figure 1.1 (Bourissou *et al.*, 2000).

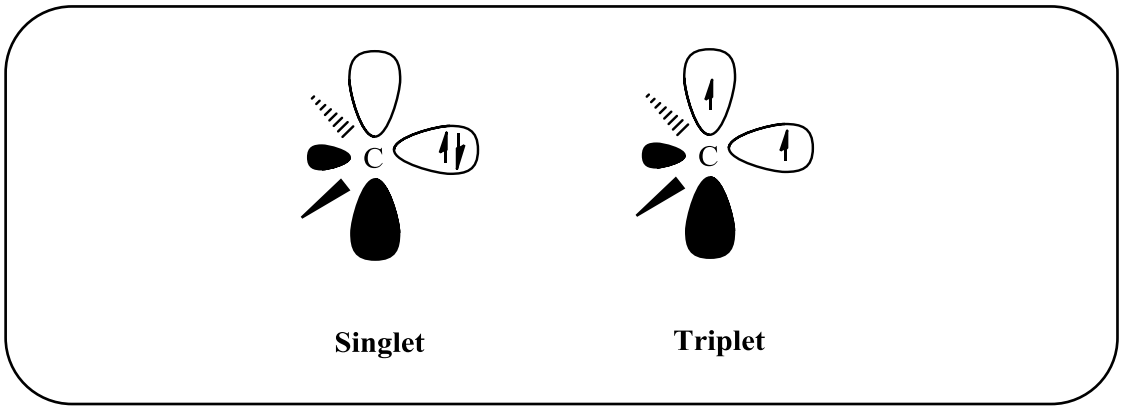
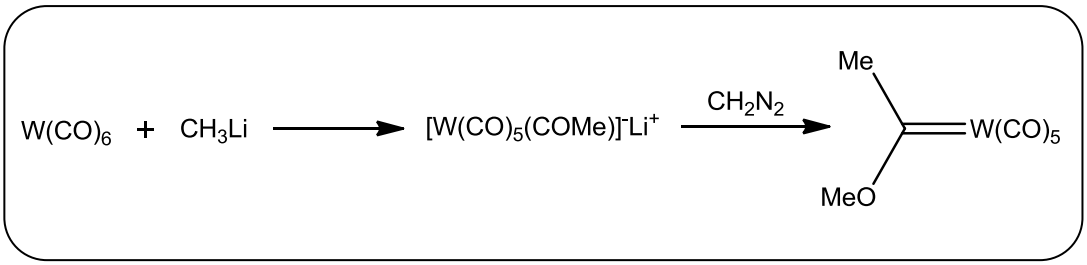


Figure 1.1: Ground state multiplicity of the carbene.

In 1964, Fischer and Maasböl synthesized the first stable Fischer carbene complex with tungsten (Scheme 1.1). Fischer carbenes are considered as singlet carbenes which donate their electron pair as σ mode from sp^2 hybrid orbital and use the *p*-orbital to accept back donation from the metal in a π mode (Figure 1.2) (Fischer and Maasböl, 1964).

One decade after Fischer discovery, the second type of carbene was discovered by Schröck in 1974. The treatment of $Ta(CH_2CMe_3)_3Cl_2$ with two equivalents of $Li(CH_2CMe_3)$ resulted in the first stable Schröck carbene complex $(CH_2CMe_3)_3Ta(CHCMe_3)$ (Scheme 1.2). Schröck carbenes are considered as triplet and nucleophilic carbenes. This type of carbene is found in early transition metal complexes with higher oxidation states, such as Ta(V), Ti(IV) and W(V) (Schröck, 1974). These carbenes tend to form two covalent bonds with the metal. The bonding between metal and carbene takes place by interaction between two unpaired electrons on the carbene and the metal as depicted in Figure 1.3.

In contrast to Fisher and Schröck carbenes, *N*-heterocyclic carbenes (NHCs) are singlet neutral carbenes and are generally prepared by deprotonation of azolium salts, using a strong base such as potassium hydroxide or sodium hydroxide. Deprotonation leaves electron pair on the carbon thus producing carbene. This electron pair occupies a σ orbital, leaving an empty *p*-orbital that remains available to share the electron density of the lone pairs of the nitrogen atoms on both sides of it. The lone pair of the carbene is donated to the metal in order to form Metal−Carbon bond. The bonding in NHC complex is depicted in Figure 1.4.



Scheme 1.1: Synthesis of first Fischer carbene.

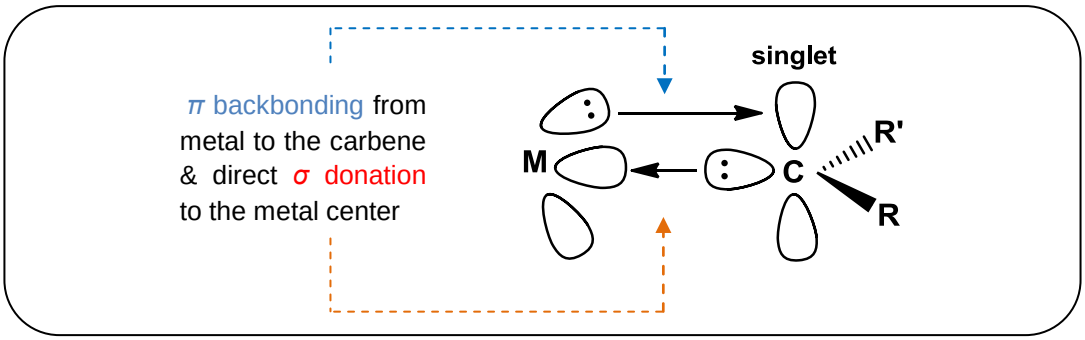


Figure 1.2: Bonding in Fischer carbene complexes.

Scheme 1.2: Synthesis of Schröck carbene.

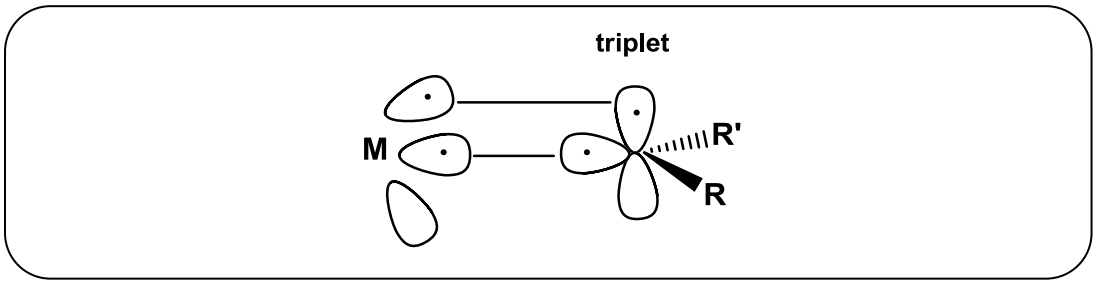


Figure 1.3: Bonding in Schröck carbene complexes.

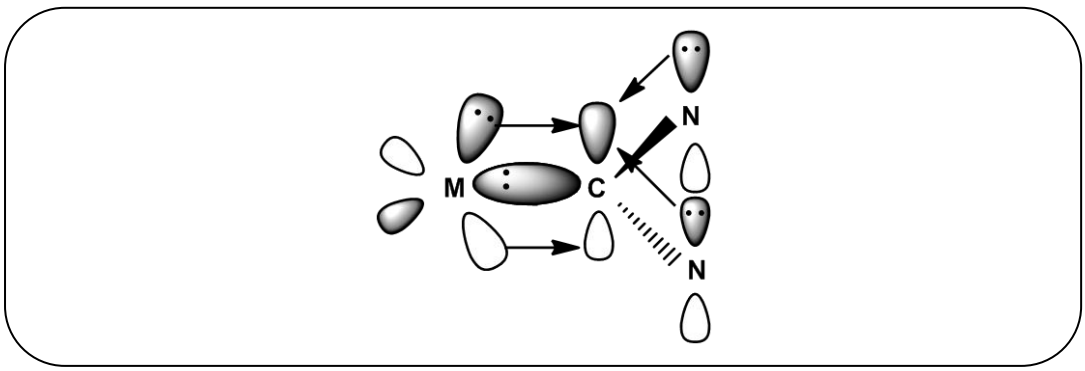


Figure 1.4: Bonding in *N*-heterocylic carbene complexes.

1.2 Stability of NHCs

Diaminocarbenes, or NHCs, are unique class of carbene family which has attracted much attention of organometallic chemists. As ligands, NHCs are very good σ donors and can coordinate with metal center in a wide variety of oxidation states (low and high oxidation states) (Herrmann, 2002). Conversely to Fisher and Schröck type carbene, NHCs are extremely air-stable and inert ligands when coordinated to a metal center. The stability of NHCs are mainly resulted by electronic effects (Arduengo *et al.*, 1993). This stabilization is due to the ′push-pull′ mechanism. In brief, the electronegative nitrogens inductively stabilize by pulling the electron density away from the carbene center (σ stabilization), while the energy of the vacant p_π orbitals increases by interaction with the symmetric combination of the two π electrons from both nitrogen atoms (π stabilization) (Figure 1.5) (Bourissou *et al.*, 2000).

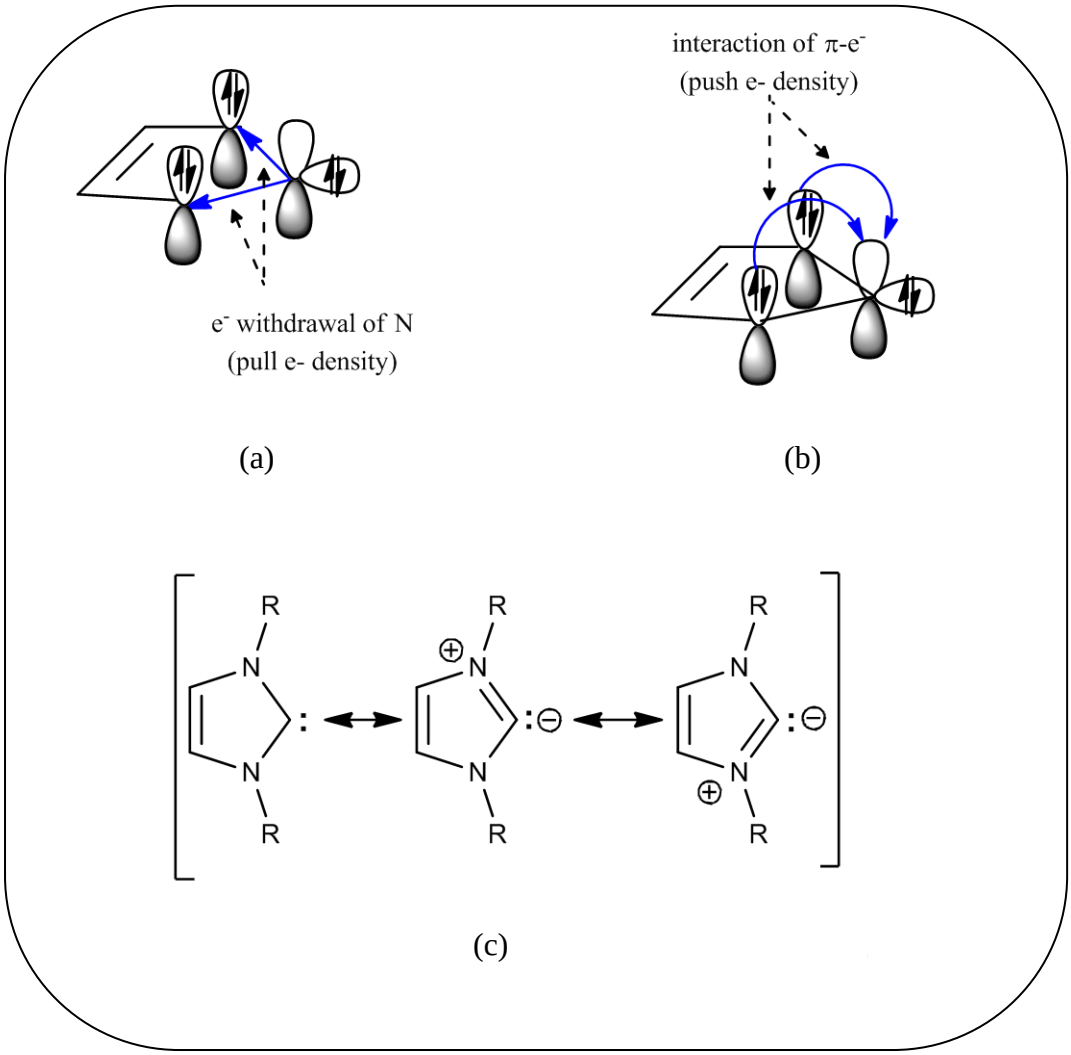


Figure 1.5: Electronic stabilization of NHC (a) Inductive effect, (b) Mesomeric effect, (c) Representations of resonance in imidazole-2-ylidenes.

1.3 NHC metal complexes

In 1968, the use of NHCs as ligands for transition metal-NHC complexes was first time explored by Öfele and Wanzlick who prepared Cr(III)- (**I**) and Hg-(II)-NHC (**II**), respectively (Figure 1.6) (Wanzlick & Schönherr, 1968). After that, NHCs have been extensively explored and used as catalysts in a number of reactions, such as transfer hydrogenation, Heck and Suzuki coupling, aryl amination, and olefin polymerization. Furthermore, metal-NHC complexes have received much attention for their catalytic ability due to their ability to stabilize metal centers in variety of

oxidation states, which play important role in homogeneous catalysis (Herrmann *et al.*, 1998; Herrmann *et al.*, 2002, Scholl *et al.*, 1999, Sanford *et al.*, 2001). Although many metal complexes of carbenes were synthesized much earlier but no free carbenes were reported as isolable in any of these cases. Later, in 1991 Arduengo and co-workers made the breakthrough of isolating the first crystalline carbenes, 1,3-di-1-adamantyl-imidazol-2-ylidene (**III**), which subsist as a solid but it is air and moisture-sensitive (Arduengo *et al.*, 1991). Few years after, the same group was able to synthesize the first air-stable carbene, 1,3-dimesityl-4,5-dichloroimidazol-2-ylidene, (**IV**) (Figure 1.6) (Arduengo *et al.*, 1997). The popularity and interest in metal-complexes of NHCs increased among researchers after various reports showing their tendency in catalytic activity (Scholl *et al.*, 1999).

In the early 1990s, striking similarities between NHCs and phosphine ligands, in terms of ligand properties and metal coordination chemistry, were recognized by Herrmann and co-workers (Herrmann *et al.*, 1993). Like phosphines, NHCs ligands are excellent σ donors and having both steric and electronic tunability which make them capable to promote a wide variety of catalytic reactions. Initially, Grubb and co-workers synthesized the ruthenium-based catalyst, $(PCy_3)_2(Cl)_2$-Ru=CHPh (**a**) and $(PPh_3)_2(Cl)_2$-Ru=CHPh (**b**), that swiftly gained popularity due to their stability and catalytical activity in olefin metathesis reactions. Subsequently, the same group replaced a phosphine by an NHC ligand to develop "second generation of Grubbs catalyst". This NHC-Ru complex (**c**) was synthesized and used to improve the performance of Grubb metathesis reaction (Figure 1.7) (Sanford *et al.*, 2001; Trnka *et al.*, 2003).

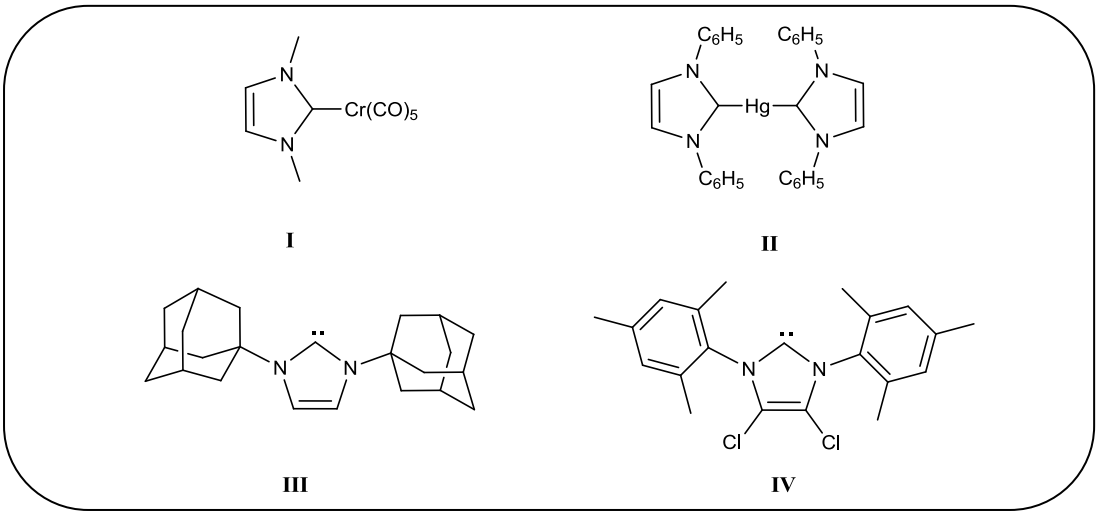


Figure 1.6: Structures of Cr(III)-NHC (**I**), Hg(II)-NHC (**II**), the early isolated free carbenes (1,3-di-1-adamantyl-imidazol-2-ylidene) (**III**) and the (1,3-dimesityl-4,5-dichloroimidazol-2-ylidene) (**IV**).

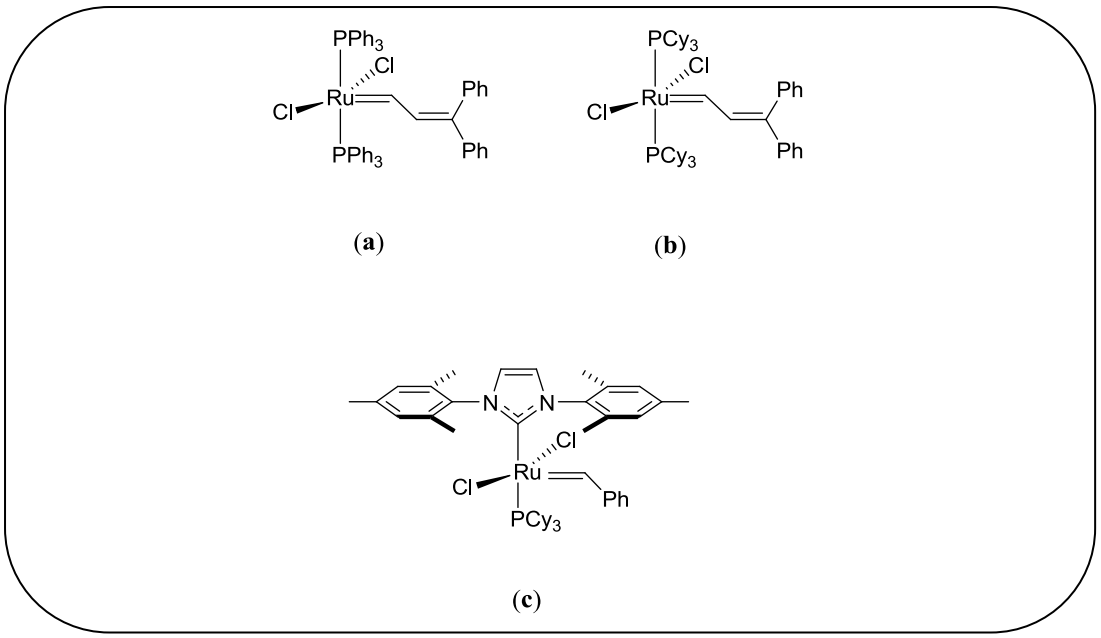


Figure 1.7: (**a**) and (**b**), First-generation ruthenium-based metathesis and (**c**) Second-generation ruthenium-based metathesis.

Miscellaneous of NHC structures has been explored as their applications have grown. One of which is biscarbenes system which have been further explored in the current research. BisNHCs offer entropic stability to their complexes and this type of

ligands, in general, has established to be very useful in organometallic catalysis. The first strategy to synthesize biscarbene ligands are normally by direct reaction between *N*-alkyl or –arylimidazole with one of the desired alkyl dihalide (Albrecht *et al.*, 2002; Köcher & Herrmann, 1997). Figure 1.8 illustrates general structure of bidentate chelating alkane-bridged diNHC complex and this type of complex can be simply modified by either varying the *N*-subsituents or the length of the alkyl chain between the two imidazoylidene units.

The second strategy to obtain biscarbene precursors was shown in Scheme 1.3. The nucleophilic substitution reaction of the dibromomethyl compound with 2 imidazole units was followed by quaternization process with methyl iodide, give the desired *bis*-imidazolium salt. This *bis*-imidazolium salt was further used for metallation with palladium and nickel (Clyne *et al.*, 2000). In 1995, Fehlhammer *et al.* reported the first example of chelating diNHC-palladium complex (Scheme 1.4) (Fehlhammer *et al.*, 1995). Subsequently, Herrmann and coworkers synthesized the bidentate free NHC in high yield and this free carbene was further used to synthesize the *bis*-NHC complexes of Ru(II), Os(II), Rh(I), Ir(I) and Pd(II) (Herrmann *et al.*, 1996). Further work on catalytic study has shown that diNHC complexes are catalytically active for a wide range of reactions such as Kumada-Corriu and Suzuki Coupling reactions. Because of their common applications in catalytic field, the chemistry of poly-NHC complexes is in continuous development. Several palladium-NHC complexes with *cis*-chelating diNHC ligands have been shown to efficiently catalyze cross coupling reactions (Chiu *et al.*, 2005; Schaub & Radius, 2005; Xi *et al.*, 2007).

Another interesting application of chelating biscarbene complexes was reported by Quezada and co-workers. They found that Rh(II)-complexes obtained *via*

transmetallation of Ag(I)-carbene may have applications in radiation therapy (Scheme 1.5) (Quezada *et al.*, 2004). Later, in 2005, Garrison and co-workers believe that chelating-NHC ligands may have potential to be used as metal chelators in the synthesis of radiopharmaceuticals (Garrison *et al.*, 2005).

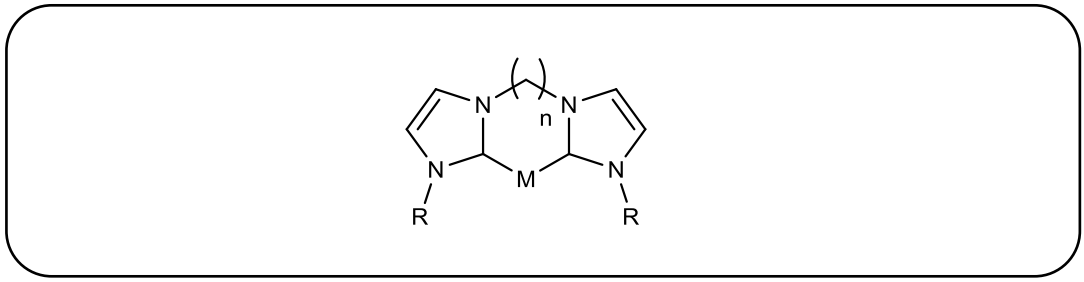
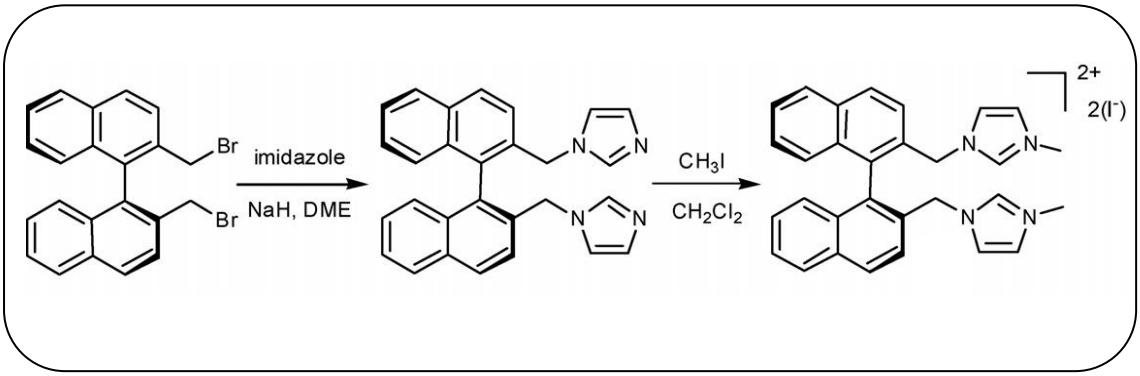
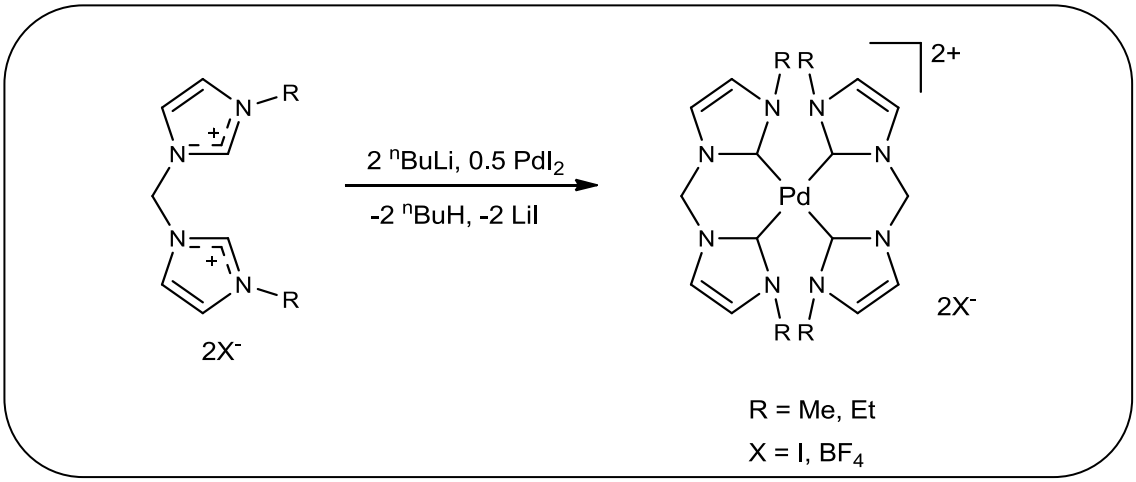


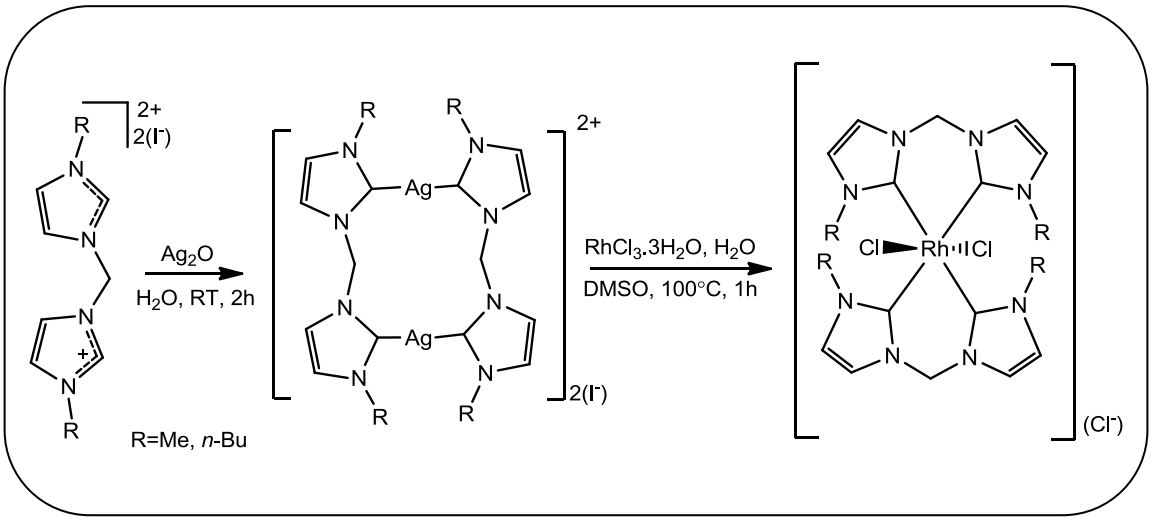
Figure 1.8: General structure of alkane-bridged diNHC complex.



Scheme 1.3: Synthesis of biscarbene precursor.



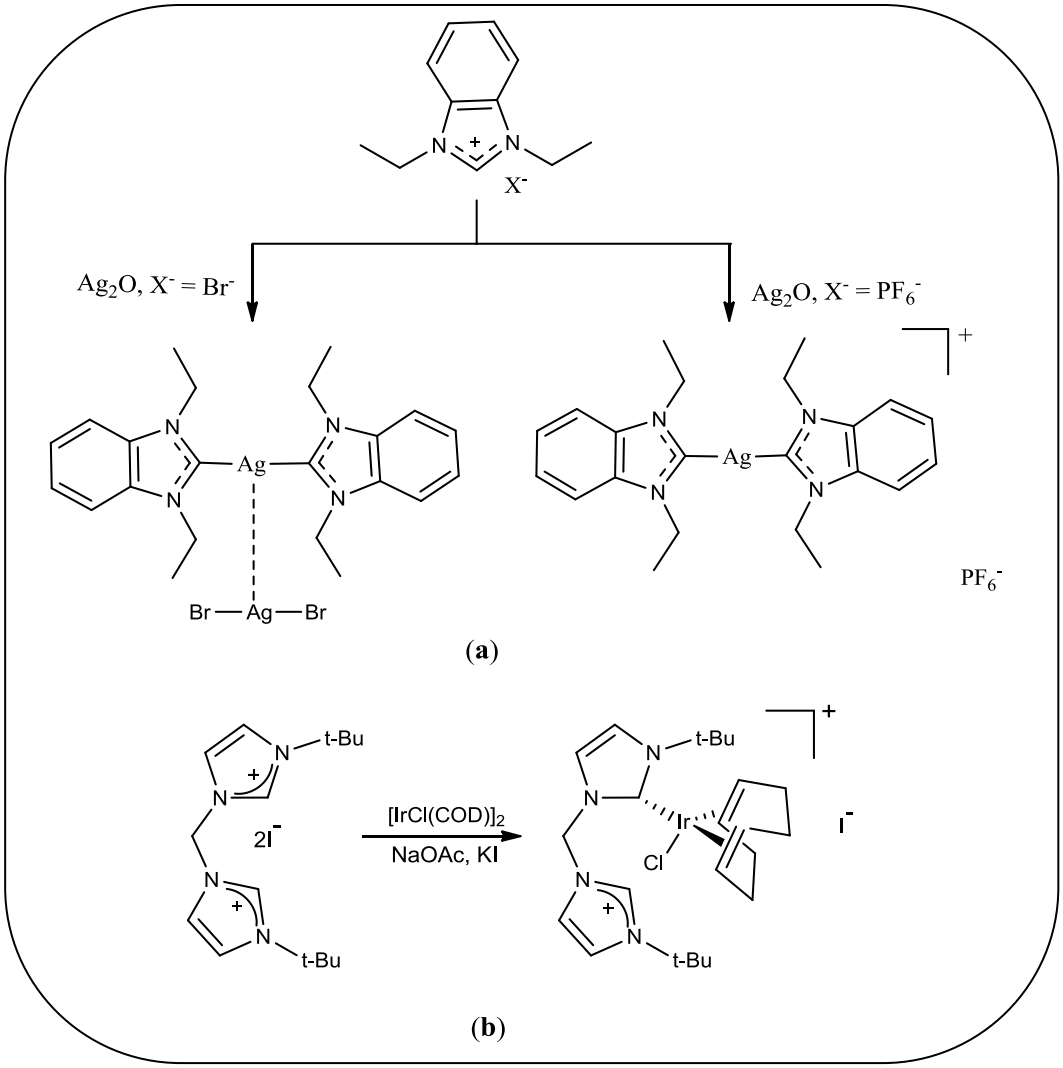
Scheme 1.4: Synthesis of Pd-diNHC complexes.



Scheme 1.5: Synthesis of biscarbene Rh-NHC complexes that shown potential to be used in radiation therapy (Quezada *et al.*, 2004).

1.4 Syntheses of NHC metal complexes

Nowadays, there are numerous ways of generating NHC metal complexes. The most widely used method is *in situ* deprotonation of azolium salts in the presence of suitable metal ion. The basic ligands (OR^- or OAc^-) present in the metal precursor can act as an internal base (Baker *et al.*, 2001). One of the most commonly used bases, for deprotonation which also acts as a metal source, is Ag_2O (Catalano & Etogo, 2007; Paulose *et al.*, 2008; Simons *et al.*, 2003). The use of external bases (lithium or potassium tert-butoxide or sodium acetate) can be added for *in situ* deprotonation (Scheme 1.6) (Baker *et al.*, 2006; Han *et al.*, 2008). By using this strategy, the isolation of the free NHC is not necessary. This strategy is also suitable to perform one-pot synthesis, thus making this method popular in preparing NHC complexes.



Scheme 1.6: (**a**) Syntheses of Ag(I)-NHC complexes using Ag_2O act as internal base,(**b**) Synthesis of Ir(II)-NHC complex using NaOAc as an external base and $IrCl(COD)_2$ as a metal source.

Apart from *in situ* deprotonation method, the isolation of the free carbene is also commonly used. This method necessitates deprotonation of azolium salt using strong base such as NaH and *t*-BuOk or dimsyl anions to produce the free carbene, (Arduengo *et al.*, 1991) and this carbene can be isolated or directly used without isolation. The main advantage by using this method is the large diversity of metal precursors that can be used without special requirements regarding the ligand sphere and the oxidation state of metal center (Weskamp *et al.*, 2000). However, this

method can pose problems such as ligand decomposition and dimerisation of the free carbene by attaining Wanzlick equilibrium (Figure 1.9) (Böhm & Herrmann, 2000). Another method is the carbene transfer method (transmetallation) by using Ag-NHC complexes. This method was introduced by Lin and co-workers in 1998. The lability of Ag-carbene bond allowed silver to be replaced by another metal center to form new metal-NHC complexes. As of today, many metal-carbene complexes such as Pd-, Rh- (Scheme 1.7) (Simons *et al.*, 2003), Ru-NHC complexes are prepared by using this route (Trnka *et al.*, 2003).

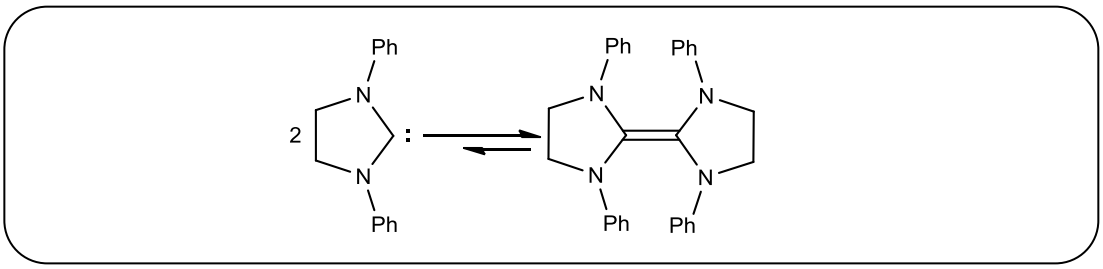
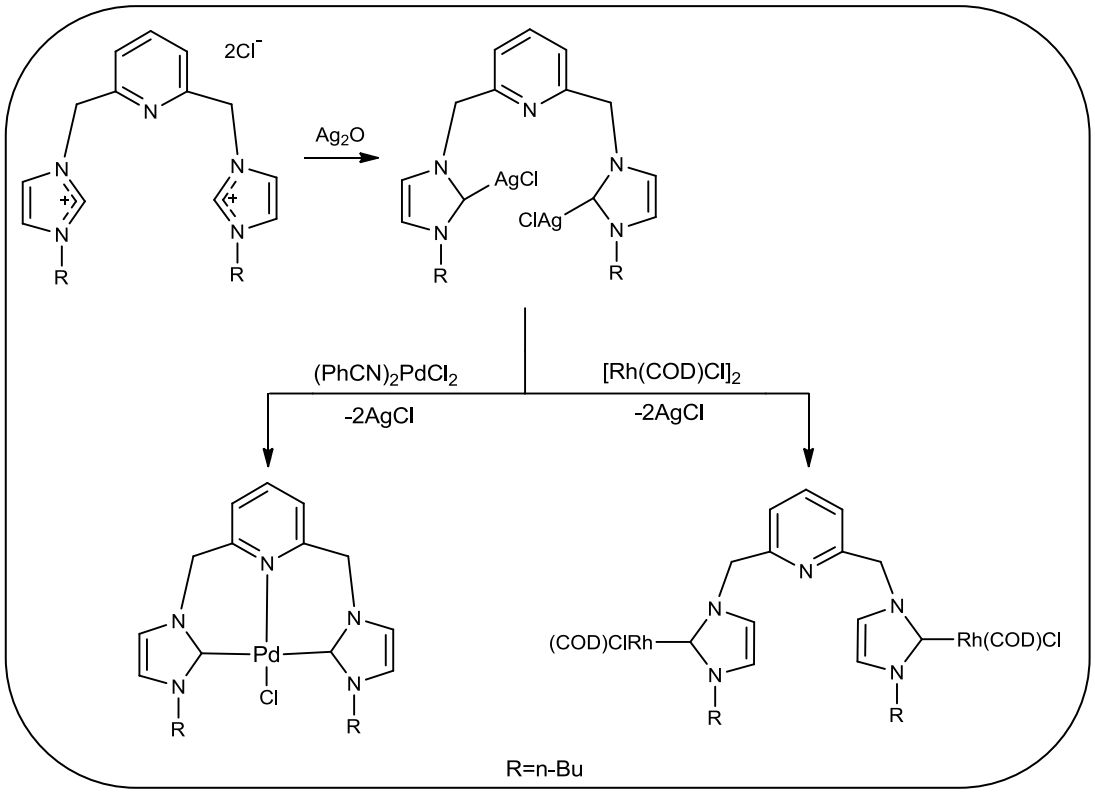


Figure 1.9: Wanzlick′s equilibrium.

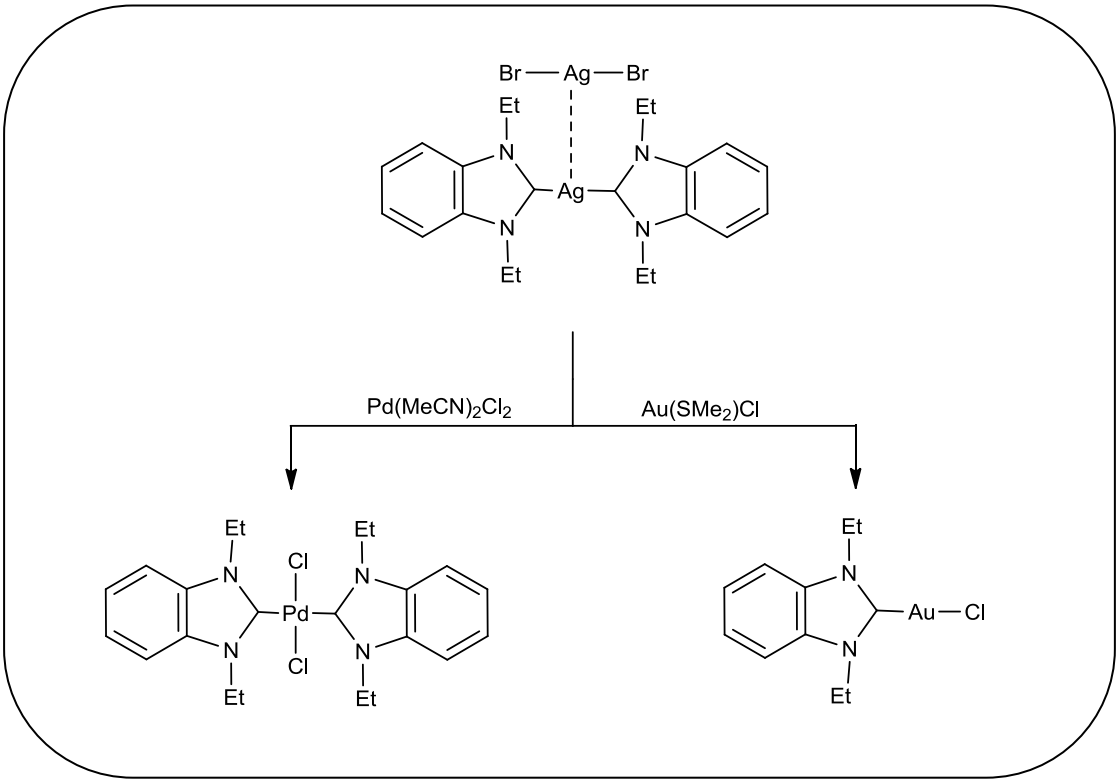


Scheme 1.7: Transmetallation reactions leading to Pd- and Rh-NHC complexes.

1.5 Applications of silver(I)-NHC complexes

1.5.1 Ligand transfer chemistry

The metal-NHC chemistry, especially Ag(I)-NHCs, has received a great deal of attention from many researchers. The first use of Ag(I)-NHCs in transmetallation was introduced by Wang and Lin (Wang and Lin, 1998) (Scheme 1.8). The growing interest of Ag(I)-NHCs due to their straightforward method, stability of NHCs, fascinating structural diversity and also most prominently excellent as transfer agents (Barnard *et al.*, 2004; Lin & Vasam, 2004, 2007). In transmetallation route, the NHC salt is reacted first with Ag_2O to give the Ag(I)-NHC complex. Further reaction of Ag(I)-NHC complexes with the targeted metal source affords the metal-NHC complex by transferring NHC ligand from silver center to the other metal centers (Garrison *et al.*, 2005). Recently, Ag(I)-NHC complexes also have been reported as convenient precursors to prepare a variety of metal-NHC complexes such as Cu(I) (Liu *et al.*, 2013), Pt(II) and Ni(II), (Chen *et al.*, 2011), Pd(II), Ru(II) and Rh(I) (Lang *et al.*, 2013).



Scheme 1.8: The first NHC transfer reaction from Ag(I)-NHC complex to Pd(II) and Au(I).

1.5.2 Medicinal chemistry

Aside from their place in ligand transfer chemistry, silver compounds also have prominent place in bioorganometallic chemistry. In the history of medicinal chemistry, silver has been extensively used throughout for a variety of medical purposes such as in cancer treatment. Today, cancer treatment has been a major focus on research and development in academia and pharmaceutical fields. Cancer is often referred to as "the silent killer." At the onset of cancer, cancer cells are difficult to identify; by the time they have been detected, not much can be done to cure the patient. Cancer is a broad term for a class of diseases in which abnormal cells divide without control and invade other normal cells in the body.

The first historical example of metal-based drugs that used in cancer treatment is cisplatin. Cisplatin is commonly used for the treatment of a variety of

cancers. The metal-based drug show 70-90% cure rate for testicular cancer and is effective against brain, ovarian, bladder and breast cancer when combined with other drugs (Marzano *et al.*, 2002). Later on, various cisplatin analogous compounds such as carboplatin, oxaliplatin, nedaplatin and lobaplatin have been approved for current tumor therapy (Figure 1.10) (Wheate *et al.*, 2010). However, the use of platinum-based anticancer drugs in clinical applications is restricted as most of the tumors have resistance against the mentioned drugs and their side effects can be severe. Due to this situation, the quest for selective anti-invasive agents with low toxicity for cancer treatment is urgently required. Where silver and gold becomes the suitable canditates due to their lower toxicity compared to platinum.

In 2008, Youngs and co-workers have reported anticancer properties of Ag(I)-NHC complexes (Figure 1.11) against the human derived cancer cell lines, OVCAR-3 (ovarian), MB157 (breast), and Hela (cervical) (Medvetz *et al.*, 2008). Later, Tacke group have tested some of symmetrical and unsymmetrical Ag(I)-NHC complexes against renal cancer (Caki-1) cell line and the results are consistent with those reported by Youngs group (Patil *et al.*, 2010, 2011a, 2011b). Recently, Gautiers and co-workers have investigated the anticancer potential of Ag(I)-NHC complexes against several cancerous cell lines (KB: oral carninoma, HL60: promyelocytic leukaemia, HL60R: resistant HL60, MCF-7: breast cancer, MCF-7R: resistant MCF-7, T47D: breast cancer (Gautier & Cisnetti, 2012).

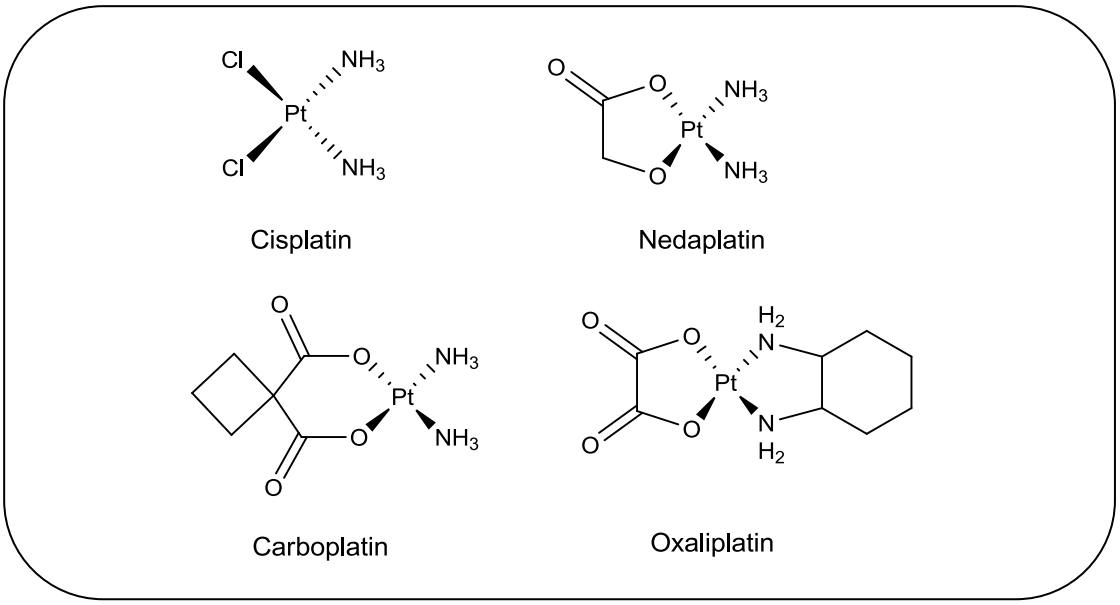


Figure 1.10: Platinum-based anticancer drugs.

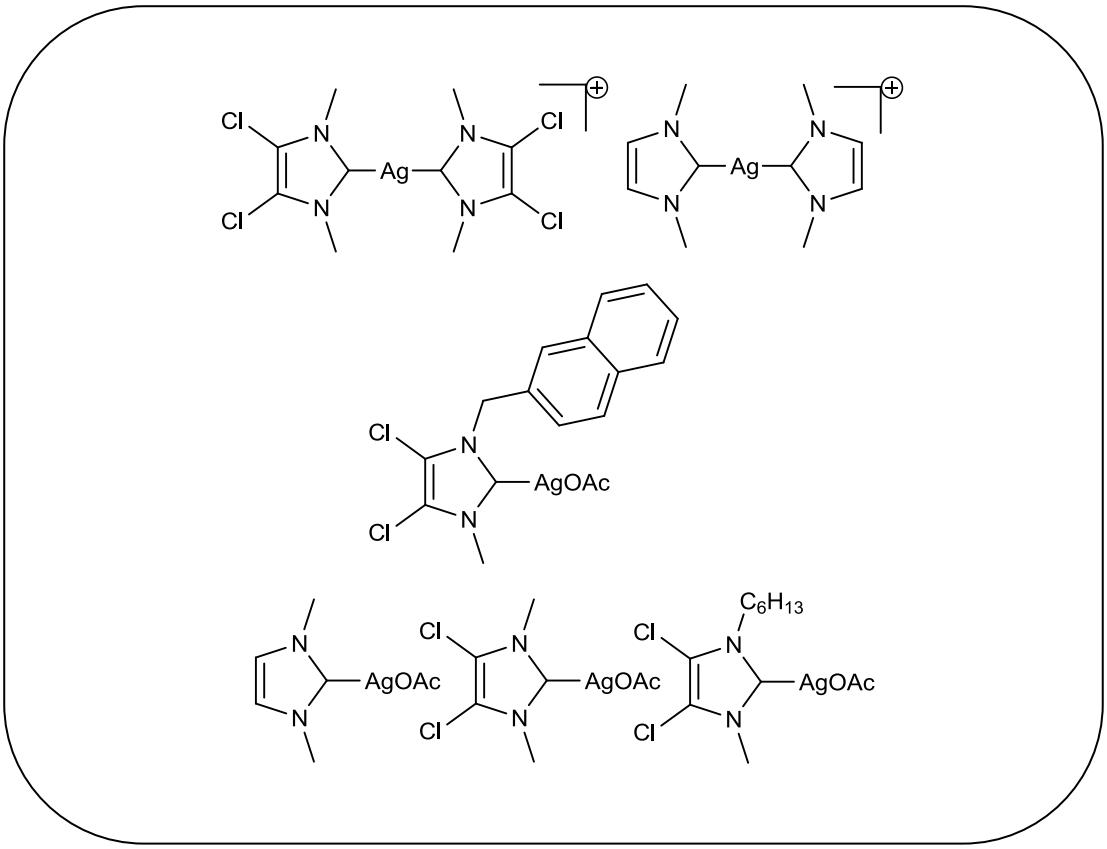


Figure 1.11: Ag(I)-NHC complexes for anticancer studies by Youngs *et al.*

Silver commonly used as antimicrobial for more than hundred years and it was targeted for water purification, wound care antiseptics and infections (Klasen,

2000; Russel, 1994; Silver *et al.,* 2006). Although the use of silver as antibiotic agent had proven effective for many years, its popularity declined with the discovery of penicillin and other antibiotics (Lansdown, 2004). In few years back, Ag(I)-NHC complexes have gained a significant amount of interest in medicinal research. The first use of Ag(I)-NHC complexes as antimicrobial agents was reported by Youngs and co-workers in 2004 (Figure 1.12). These Ag(I)-NHC complexes showed better antimicrobial activity than $AgNO_3$ against the microorganisms; *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* (Youngs *et al.*, 2004). Today, many of articles dealing with Ag(I)-NHC complexes shown a great potential as anticancer and antimicrobial agents (Budagumpi *et al.*, 2012; Patil *et al.*, 2011a).

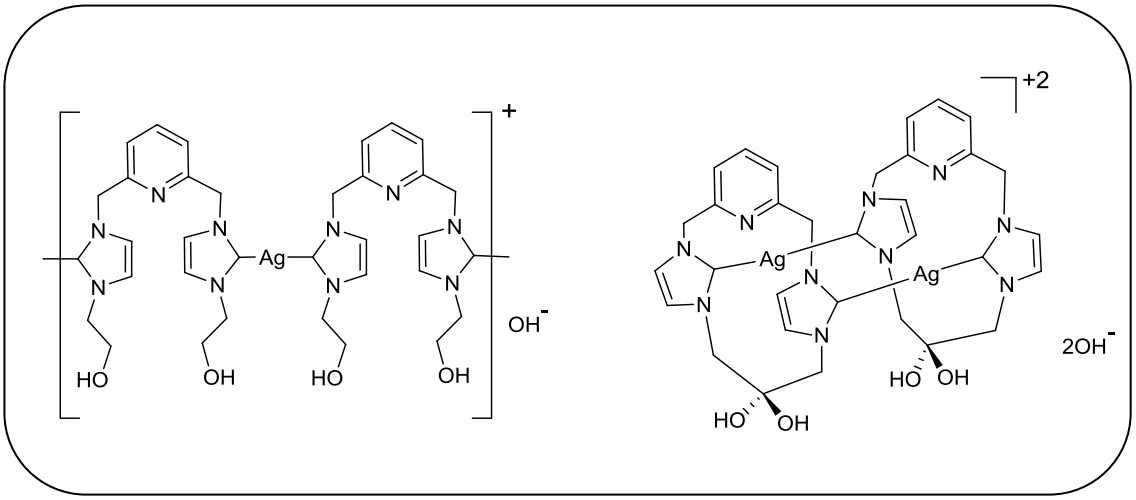


Figure 1.12: The first Ag(I)-NHC complexes as potential antimicrobial agents.

1.5.3 Catalysis

Since the last two decades, Ag(I)-NHC complexes have been explored for their catalytic potential. Ramírez and co-workers reported the use of Ag(I)-NHC complexes as catalyst for diboration reactions with terminal and internal alkenes (Ramírez *et al.*, 2005). Futhermore, Ag(I)-NHC complexes were reported to be used

as catalysts for the ring-opening polymerisation of lactide (Sentman *et al.*, 2005). Nevertheless, compared to the other metal-NHC complexes such as palladium, ruthenium, nickel, rhodium and iridium very few studies have been reported for Ag(I)-NHC complexes and this indicates Ag(I)-NHC complexes are not very stunning in this field (Herrmann, 2002).

1.6 Applications of palladium(II)-NHC complexes

1.6.1 Medicinal chemistry

Pd(II)-NHC complexes are not very prominent in medicinal field but still have been explored as a potential alternative to treat several cancers unresponsive to current cancer treatments. In 2007, Ray and co-workers synthesize and studied the efficiency of Pd(II)-NHC complexes against HCT 116 (colon adenocarcinoma), HeLa (cervical cancer) and MCF-7 (breast cancer) and the results were found to be considerably stronger than cisplatin (Ray *et al.*, 2007). Recently, Pd(II)-NHC complexes were tested against *Escherichia coli* and *Staphylococcus aureus* bacteria and displayed comparable antimicrobial activities at the MIC level. These Pd(II)-NHC complexes were also investigated on their anticancer activities against HCT 116 cancer cell lines and one of Pd(II)-NHC complexes showed significant anticancer activity (Haque *et al.*, 2013). Figure 1.13 shows a few of Pd(II)-NHC complexes tested for their biological properties.

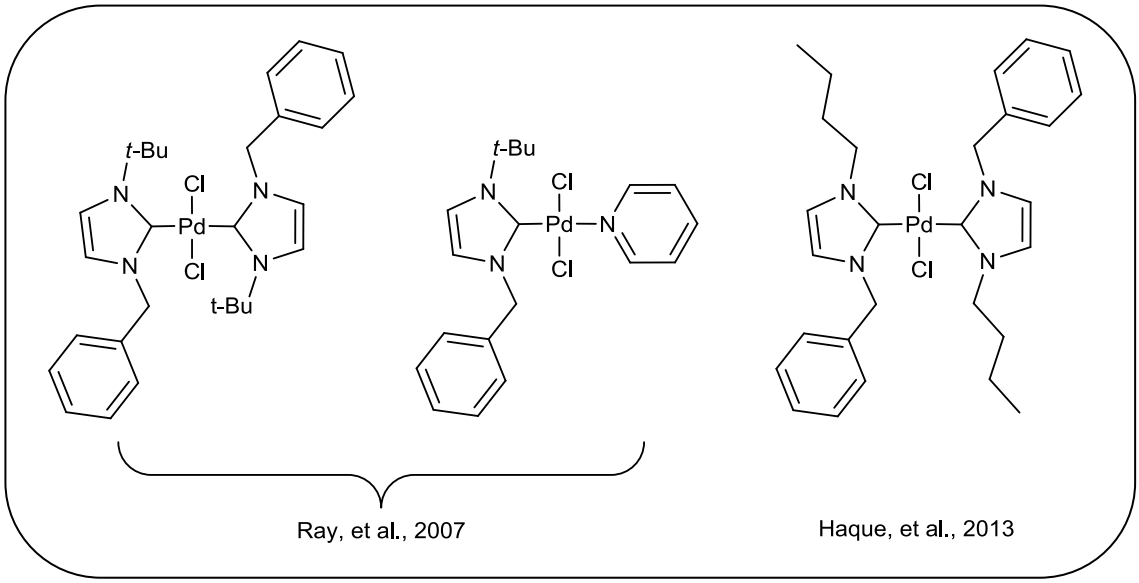


Figure 1.13: Pd(II)-NHC complexes tested for their biological properties.

1.6.2 Catalysis

Over the past two decades, Pd(II)-NHC complexes were first reported by Hermann and co-workers as catalyst in the Heck coupling of aryl halides with n-butyl acrylate to afford high yield product. Figure 1.14 shows the Pd(II)-NHC complex that was used in Heck reaction and this complex exhibited high thermal stability of the Pd-$C_{carbene}$ bonds of the catalyst in solution (Herrmann *et al.*, 1998; Herrmann *et al.*, 1995). Later on, the interest of Pd(II)-NHC complexes in catalysis fields have grown especially in cross-coupling chemistry. Numerous of Pd(II)-NHC complexes have been applied as catalysts in various organic syntheses due to their stability towards air and moisture, low-to-moderate cost and the availability of palladium in the stable and variable oxidation states. Specifically, diNHC of Pd(II)-complexes have shown extreme stability in the presence of heat and moisture leading to notable catalytic properties (Budagumpi *et al.*, 2012).

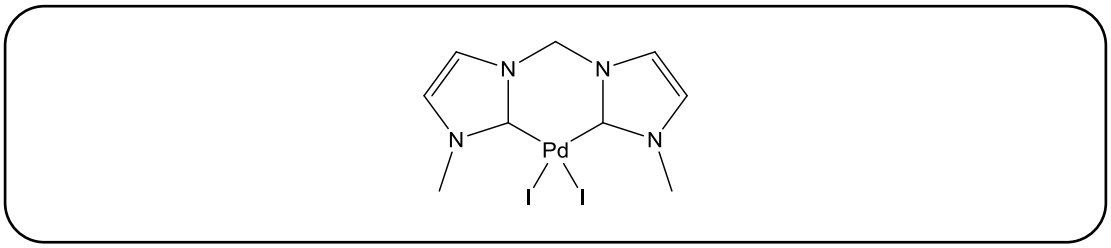


Figure 1.14: Pd(II)-NHC complex used as catalyst in Heck reaction.

1.7 Research objectives

The available literature shows that the Ag(I)-NHC complexes reported as anticancer agents mostly are mono-nuclear type. However, there is very less published work related to the anticancer properties of metal-NHC complexes to date. With this background, the current research embarked on the following objectives:

(i) To synthesize novel *bis*-imidazolium salts and their respective Ag(I)-NHC complexes.

(ii) To use selected Ag(I)-NHC complex as transfer agent in order to synthesize Pd(II)-NHC complex.

(iii) To characterize all the synthesized compounds using different spectral and analytical methods.

(iv) To evaluate the anticancer activity of *bis*-imidazolium salts, Ag(I)- and Pd(II)-NHC complex against breast cancer cell line MCF-7.

CHAPTER 2

EXPERIMENTAL

2.1 Reagents and instruments

Imidazole (Merck, 99.0%), 2-bromomethylbenzonitrile (Merck, for synthesis), 3-bromomethylbenzonitrile (Merck, for synthesis), 2-methylbenzylbromide (Sigma-Aldrich, 96.0%), 3-methylbenzylbromide (Sigma-Aldrich, 96.0%), potassium hexafluorophosphate (Across Organics, 99.0%), potassium hydroxide pallets (R&M chemicals, 85%), dichloromethane (QReC, 99.8%), silver(I) oxide (Merck, 99.0%), dichloro(1,5-cyclooctadiene)palladium(II) (Sigma-Aldrich), and celite (Merck, size: 0.02-0.1 nm) were obtained from commercial sources and used as received. Di(*1H*-imidazol-1-yl)methane was prepared according to the literature method with slight modifications (Panzner, 2006).

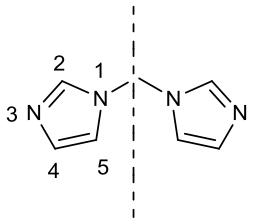
All the reactions were carried out under aerobic conditions. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at ambient temperature from solutions in d_6-DMSO or d_3-CD_3CN using TMS as an internal reference. Data were represented as follow: chemical shift (integration, spin multiplicity (s = singlet, d = doublet, t = triplet, q= quartet, m = multiplet), coupling constant in Hertz (Hz), assignment). Elemental analyses (CHN) were performed on Perkin Elmer series II, 2400 microanalyzer. X-ray diffraction data were recorded using a Bruker SMART APEX2 CCD area-detector diffractometer and were performed by X-ray Crystallography Unit, The School of Physics, USM. The melting points were assessed by using a Stuart Scientific SMP-1 (UK) instrument.

2.2 Cell line and culture condition

Breast cancer (MCF-7) cell line was purchased from American type culture collection (Rockvill, MD, USA). MCF-7 cell line is derived from pleural metastasis of ductal human breast carcinoma and was grown in DMEM medium. The growth media was supplemented with 10% HIFBS and 1% PS. Cells were cultured in 5% CO_2- humidified atmosphere at 37 ^oC.

2.3 Synthesis of reagent

Synthesis of di(*1H*-imidazol-1-yl)methane was prepared according to the literature method (Panzner, 2006) with slight modifications (As has been described in page 34).

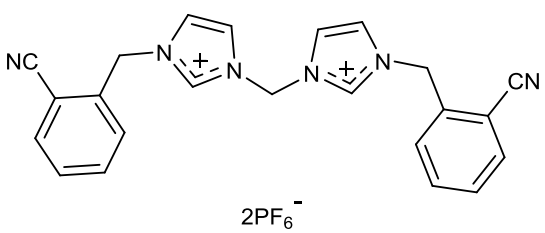


di(*1H*-imidazol-1-yl)methane

2.4 Synthesis of methylene-bridged *bis*-imidazolium salts

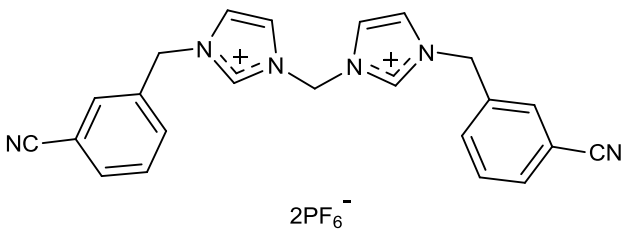
For salt **1-4**, in metathesis reaction, the ratios of KPF_6 were used at least more than 0.5 moles excess from bromide salts.

2.4.1 di(1,1′-(2-cyano)benzylimidazolium)-3,3′methylene dihexafluorophosphate (**1**)



2-(Bromomethyl)-benzonitrile (2.65 g, 0.0136 mole) was added to a stirred solution of di(*1H*-imidazol-1-yl)methane) (1.0 g, 0.0068 mole) in 1,4-dioxane (100 mL). The mixture was refluxed at 100 °C for 24 h. The solution was evaporated in vacuo to yield brownish mother-liquor (92.6%). For characterization purpose the hexafluorophosphate salt of the compound was used whereas for further synthesis halide salt was used. So obtained bromide salt (2.5g, 0.0046 mole) was directly converted to its hexafluorophosphate salt by metathesis reaction using KPF_6 (1.27 g, 0.0069 mole) in 50 mL of methanol and stirred for 3 h. The white precipitates obtained were collected and recrystallized using acetonitrile to yield colorless crystals. Yield: 2.0 g (66.7%), m.p. = 200-201 °C, ^{1}H NMR (500 MHz, d_6-DMSO): δ 5.74 (4H, s, 2 × N_{imid}-CH_2-Ar), 6.67 (2H, s, N_{imid}-CH_2-N_{imid}), 7.52 (2H, d, J = 7.5 Hz, Ar-H), 7.65 (2H, m, Ar-H), 7.81 (2H, m, Ar-H), 7.89 (2H, t, 2 × imidazolium H5′), 7.98 (2H, d, J = 7.5 Hz, Ar-H), 8.05 (2H, t, 2 × imidazolium H4′), 9.52 (2H, s, 2 × imidazolium H2′); ^{13}C{1H} NMR (125 MHz, d_6-DMSO); 50.7 (2 × N_{imid}-C-Ar, benzylic), 58.5 (2 × N_{imid}-C-N_{imid}, methylene), 122.8, 129.6 (imidazolium C5′ and C4′), 111.1 (C-CN), 116.7 (CN), 129.6, 129. 9, 133.7, 134.0, 136.8 (Ar-C), 138.4 (NCN). Anal. Calc for $C_{23}H_{22}N_6F_{12}P_2.H_2O$: C, 41.12; H, 3.22; N, 12.21%. Found: C, 40.74, H, 2.50, N, 12.19%.

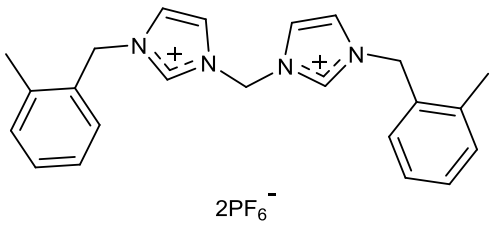
2.4.2 di(1,1′-(3-cyano)benzylimidazolium)-3,3′methylene dihexafluorophosphate (**2**)



3-(Bromomethyl)-benzonitrile (2.37 g, 0.012 mole) was added to a stirred solution of di(*1H*-imidazol-1-yl)methane (0.9 g, 0.0061 mole) in dioxane (100 mL).

The mixture was refluxed at 100 °C for 24 h. The solution was evaporated in vacuo to yield white precipitate (77.6%). So obtained bromide salt (1.56 g, 0.0029 mole) was directly converted to its hexafluorophosphate salt by metathesis reaction using KPF_6 (1.06 g, 0.0058 mole) in 50 mL of methanol and stirred for 3 h. The white precipitates obtained were collected and then recrystallized using acetonitrile to yield colorless crystals. Yield: 1.30 g (67.3%), m.p. = 192-193 °C, ^{1}H NMR (500 MHz, d_6-DMSO): δ 5.55 (4H, s, 2 × N_{imid}-CH_2-Ar), 6.59 (2H, s, N_{imid}-CH_2-N_{imid}), 7.66 (2H, t, 2 × imidazolium H5′), 7.80 (2H, t, 2 × imidazolium H4′), 7.89, 7.90, 7.92 (Ar-H), 7.99 (2H, s, Ar-H), 9.51 (2H, s, 2 × imidazolium H2′); ^{13}C{1H} NMR (125 MHz, d_6-DMSO); 51.4 (2 × N_{imid}-C-Ar, benzylic), 58.5 (2 × N_{imid}-C-N_{imid}, methylene), 122.7, 123.2 (imidazolium C5′ and C4′), 111.9 (C-CN), 118.3(C-N), 130.2, 132.1, 1.32.6, 133.5, 135.6 (Ar-C), 138.1 (NCN). Anal. Calc for $C_{23}H_{22}N_6F_{12}P_2$: C, 41.21; H, 3.01; N, 12.54%. Found: C, 40.96, H, 2.36, N, 12.20%.

2.4.3 di(1,1′-(2-methyl)benzylimidazolium)-3,3′methylene dihexafluorophosphate (**3**)



2-Methylbenzylbromide (2.50 g, 0.0135 mole) was added to a stirred solution of di(*1H*-imidazol-1-yl)methane (1.0 g, 0.0068 mole) in dioxane (60 mL). The mixture was refluxed at 100 °C for 24 h. The solution was evaporated in vacuo to yield white precipitates (77.7%). So obtained bromide salt (1.0 g, 0.0019 mole) was directly converted to its hexafluorophosphate salt by metathesis reaction using KPF_6 (0.8 g, 0.0044 mole) in 50 mL of methanol and stirred for 3 h. The white precipitates