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博士論文

Studies toward the Synthesis of Five, Six, Seven Member Heterocyclic Small Molecules as Possible Inhibitors of Vascular Endothelial Growth Factor Receptor-3 on Soluble Support

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PhD DISSERTATION

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DECLARATION

I, Kaushik chanda, declare that the thesis entitled Studies toward the Synthesis of Five, Six, Seven Member Heterocyclic Small Molecules as Possible Inhibitors of Vascular Endothelial Growth Factor Receptor-3 on Soluble Support and the work presented in it are my own.
I confirm that:

- This work was done wholly or mainly while in candidature for a research degree

at this University;

- Where I have consulted the published work of others, this is always clearly

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- I have acknowledged all main sources of help;

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Date:_____

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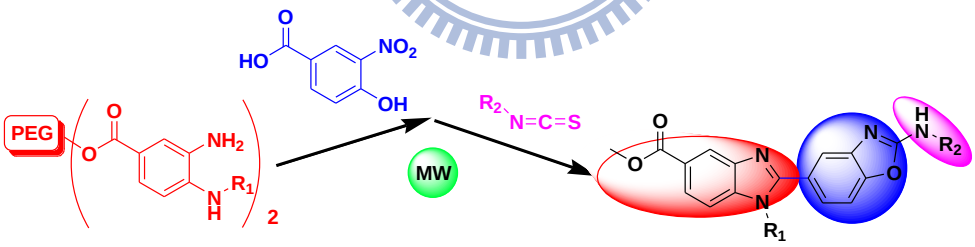
ABSTRACT

This dissertation comprises of three chapters:

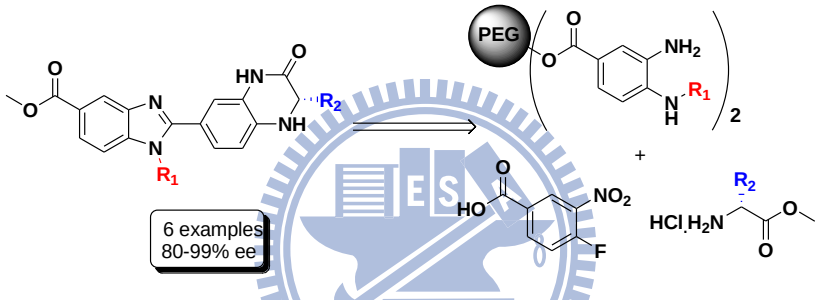
The work described in this dissertation involves the design, synthesis, and biological evaluation of novel bi-heterocyclic molecules on soluble support using focused microwave irradiation.

In Chapter 1, section A, we report the design of an efficient liquid-phase method for the parallel synthesis of substituted benzimidazolylbenzoxazols using focused microwave irradiation and subsequent bioactivity. A key step in this approach involves the attachment of 4-hydroxy-3-nitrobenzoic acid to polymer immobilized *o*-phenylenediamine. Mild acidic conditions then promote a ring closure and subsequent reduction to form substituted benzimidazole derivative. The so formed benzimidazole derivatives underwent efficient ring closure with various alkyl, aralkyl, aryl, heteroaryl and unsaturated isothiocyanates to generate the benzimidazolylbenzoxazols.

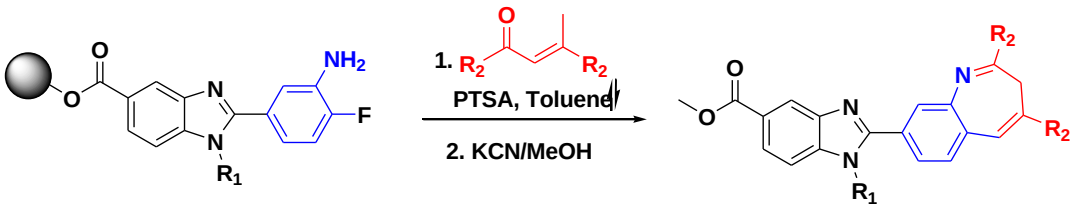


The derived polymer-bound compounds were finally cleaved from the support with KCN in methanol at room temperature resulted in the generation of molecular library with two points of structural diversity (Section A). Interestingly, all the members of the molecular library exhibited moderate to high inhibition against VEGFR-3. This novel synthetic methodology offers an easy access to benzimidazolylbenzoxazols on soluble polymeric support, from which a new class of anti-cancer drugs may be developed.

Section B involves the focused microwave irradiation to a multistep synthetic sequence of reactions designed to generate benzimidazolyl quinoxalinones using a soluble polymer support. They were obtained by the *ipso*-fluoro (S_NAr) displacement of the immobilized *ortho*-nitro fluoro benzimidazoles with chiral alpha amino esters under microwave irradiation. When subjected to neutral reduction, intermediate chiral organic-polymer conjugates underwent a spontaneous intramolecular ring closure. Cleavage of the polymer support, at room temperature did not cause any significant racemization resulting in the generation of a chiral molecular library with two points of structural diversity (Section B).

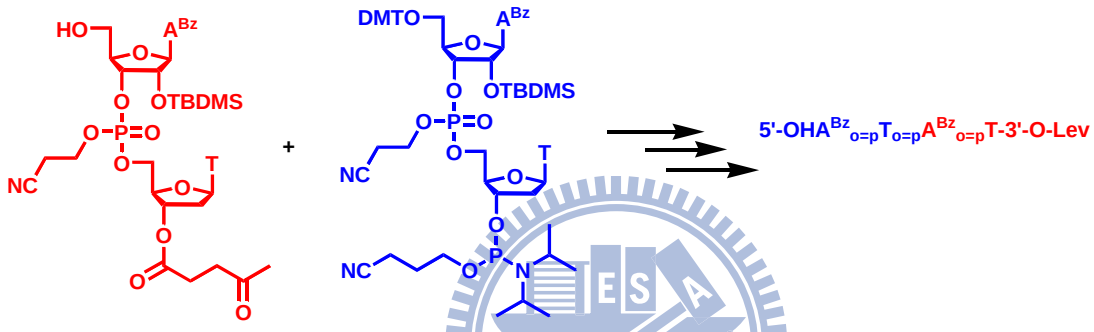


Section C involves the synthesis of Benzazepines, the subject of our interest having seven-membered aza-heterocyclic ring fusing aromatic unit, are of considerable interest owing to their varied biological activities. Polymer anchored 3-amino-4-fluorophenyl benzimidazole derivatives underwent intramolecular cyclisation with 1,3-disubstituted 2-butenone to generate the polymer anchored benzazepine derivatives.

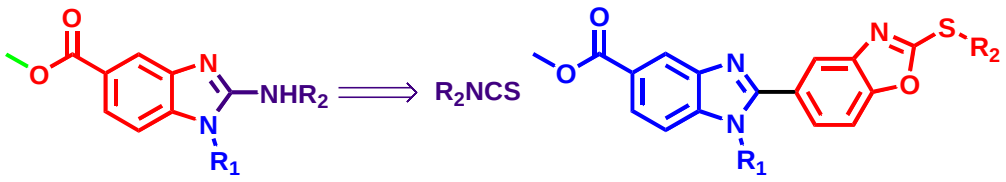


Subsequent cleavage from polymer support results the formation of architecturally diverse seven member heterocyclic molecules (Section C).

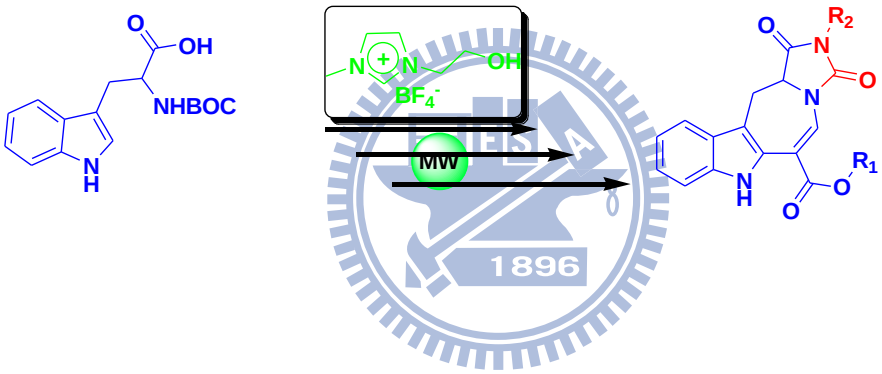
In chapter 2, we have developed a general procedure for the solution-phase synthesis of chimeric oligonucleotides (oNA) analogues using readily available phosphoramidite reagents with 2+2 and 3+3 protocol. The key feature of this method is using the solution-phase phosphoramidite procedure to assemble linear oligonucleotide sequences and sequential removal of 3′ levulinyl group as well as 5′ dimethoxytrityl group without the contamination of n−1 or shorter failures. This methodology offers an easy access to the scale up synthesis of oligonucleotides for clinical as well as commercial purposes.



In Chapter 3, of section A, approaches towards the synthesis of 2-(substituted amino) benzimidazoles derivatives under focused microwave irradiation are described. Ionic liquid were employed as soluble support. Ionic liquid bound 4-fluoro-3-nitro benzoic acid underwent nucleophillic substitution reaction with various primary amines which after reduction and subsequent cyclisation with various isothiocyanates generates the desired compounds in high purity and yields. In the same way an alternative strategy on ionic liquid support for the parallel synthesis of thioanlogues of benzoxazol derivatives under focused microwave irradiation. Herein, we have replaced the NH group of R_2 in chapter 1 of section A with sulphur analogues. A series of 16 member library were successfully synthesized with two structural variability present on benzoxazol moiety.



Section B develops the novel ionic-liquid supported synthetic protocol for hydantoin analogs tethered with azepino[4,5-*b*]indoles by the use of focused microwave irradiation. Ionic liquid bound tryptophan underwent Pictet-Spengler reaction and subsequent basification with various keto esters to generate the ionic liquid immobilized azepino[4,5-*b*]indoles. The so formed azepino[4,5-*b*]indole derivatives underwent cyclisation with various isocyanates to generate the three dimensional molecular architecture in traceless fashion (Section B).



Chapter One

Soluble Polymer Supported Synthesis of Bis Heterocyclic Compounds

1. Introduction

Cancer is a universal disease which has been known and written about for thousands of years. The word cancer is a generic term used to describe as many as 200 malignant diseases which arise in different tissues and cause different illnesses.[1] The disease cancer is characterized by abnormal cell growth and division that produces an expanding mass of disorganized tissues know as a tumor. As such, it is a disease of the cell and can develop in any tissue of the body. Cancerous cells multiply rapidly due to uncontrolled cellular growth and division. By the very nature of the disease, cancerous cells can infiltrate into other tissue types and leading to the spread of cancers.[2]

Sixty percent of patients with newly diagonised solid tumors have clinically evident or microscopic metastasis on diagnosis of primary tumors.[3] It is this statistic, and the fact that the metastases are the major cause of treatment failure and death in cancer patients, this makes the cancers is one of the deadly diseases that exists today. Once tumor have metastasized and spread to different tissues, treatment is very difficult, as local therapy can no longer be directed at the disease, making metastasis the most life threatening aspects of cancer.

A way of identifying the various causes of cancers is by studying populations and behaviours.This approach compares peoples of various groups of people exposed to

different factors or exhibiting different behaviors. A striking finding to emerge from population studies is that cancers arise with different frequencies in different areas of the world. For example, stomach cancer is prominent in Japan, colon cancer is prominent in USA, and skin cancer is common in Australia.[4] The reason for the high rates of the 2specific kinds of cancer in certain countries is explained by the several factors specific for each country. An example of this is Australia, where the rate of skin cancer is highest in the world, due to the amount of sunlight to which people are exposed.

Regions of Highest Incidence

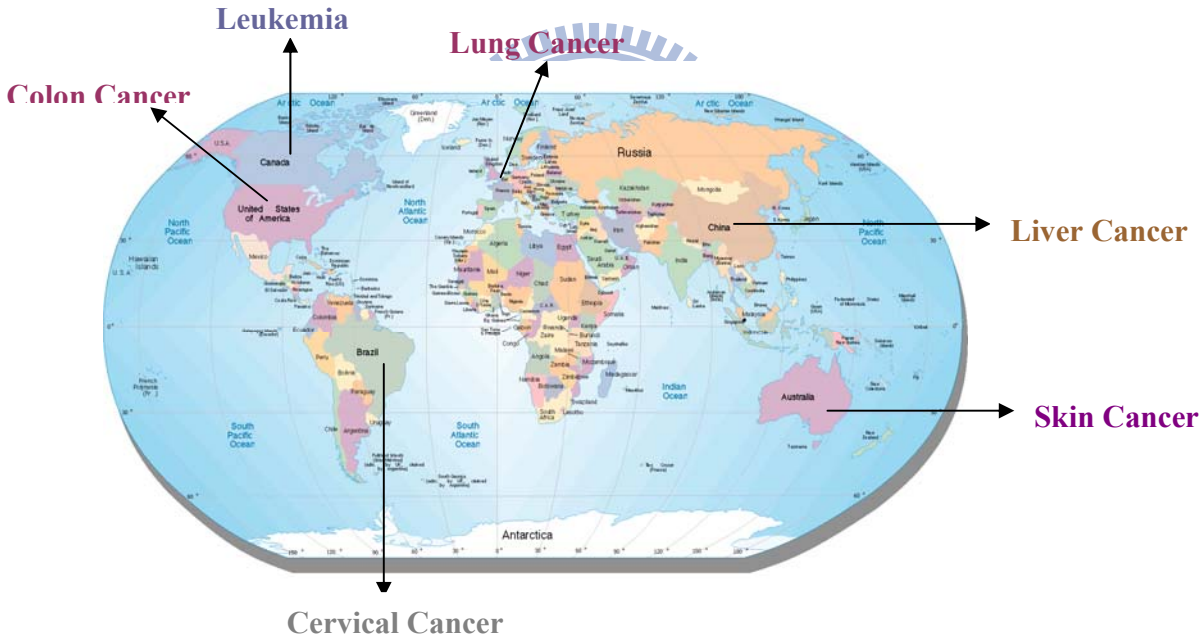


Figure 1.1. Regions of higher incidence of different forms of Cancers

As metastasis often proves fatal, there is an important need for improved understanding of this complicated process. The mechanism of invasion and metastasis, and patterns of spread of cancers need to be better understood in order to develop new therapies. Potential treatment interfering with this process could lead to better efficacy and

selectivity against cancers. The ultimate outcome would be to develop the treatment strategies that inhibit the metastasis and prevent the spread of cancers allowing the eradication of primary tumor and along with improved techniques capable of irradiating the secondary tumor metastasis.

1.1. Classification of Cancer

Cancer cells are classified into two main categories depending on whether or not they can spread by invasion and metastasis, as being either benign or malignant. Benign tumors are tumors that cannot spread by invasion or metastasis; hence, they only grow locally. They are not life threatening and are often treated by surgery. In some instances, particularly in benign brain tumors, the size of the tumors can lead to severe problems and ultimately death if untreated.[5]

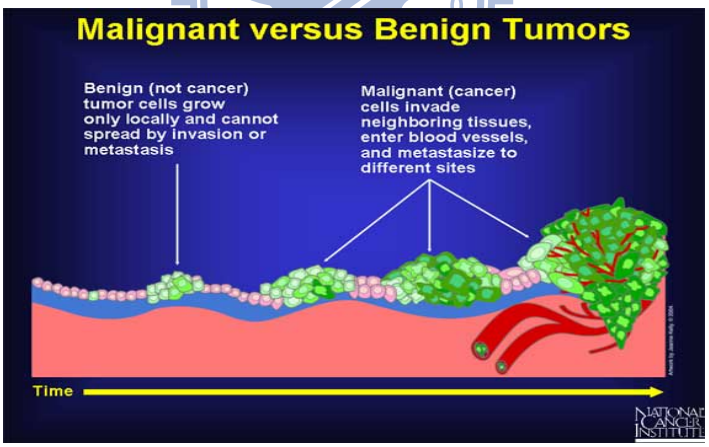
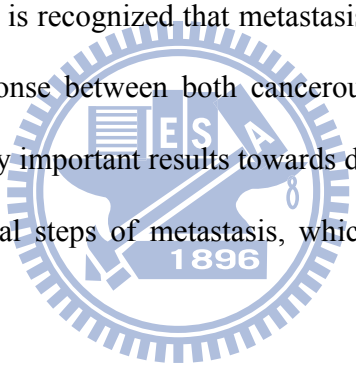


Figure 1.2. Malignant Vs Benign tumors

1.2. Metastatic Cancer

Joseph Claude Recamier, a French physician, introduced the term "metastasis in 1898.[6] Before that, physicians believed that secondary tumors were separate from the primary

tumor. Recamier showed that the secondary tumors (metastases) are caused by the spread of cancer cells from a primary tumor, and he described the mechanism of the invasion into the blood circulation and the growth of the secondary tumor (metastasis). The metastatic mechanism has been explained by two theories. The first is the "seed-and-soil" theory by Stephan Paget, first described in 1889 (for a review of this theory see ref 3). James Ewing proposed a second explanation in 1929, which described the process of metastasis on purely anatomical grounds. This theory suggests that most secondary growths occur at the first organ that tumor cells encounter. Researchers world wide have found that both theories are partially correct and both are used these days to explain the spread of cancer. Nowadays it is recognized that metastasis is a complicated process that involves interaction and response between both cancerous and normal cells. Recently researchers have produced very important results towards developing an understanding of the mechanisms and individual steps of metastasis, which is referred to as metastatic cascade.



1.3. The Metastatic Cascade

Metastasis is a complex process, which consists of several sequential steps beginning from the growth of the primary tumor at a localized site, invading the local host tissue, invading blood or lymphatic vessels travel in the circulatory system, settling at a different site in the body, and finally, growing a new colony.[7] Each step in the metastatic cascade is rate-limiting, and failure to complete any of these-steps prevents the tumor cell from producing a metastasis. Each of the processes leading to metastasis, as shown in Figure 1.3 will be described in turn.

1.3.1. Growth of primary tumor (Angiogenesis)

The first step shown in Figure 1.3 represents the growth of the primary tumor ata a localized site.[7] The primary tumor canot grow beyond the size of 1-2 mm without acquiring a blood supply. The acquisition of a new blood supply via the growth of new blood vessels is called angiogenesis (neovascularization), and allows tumor growth, invasion and metastasis to occur.[8] Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into the cancerous cells growth, supplying nutrients and oxygen and removing waste products.[9-13] Angiogenesis starts with cancer cells sending signals to surrounding normal cells. These signals activate some genes in the host tissue that, in turn, makes proteins to encourage the growth of new blood vessels. As a tumor becomes vascularized, the number of cells released into circulation correlates with the density of blood vessels in the primary tumor. The rate of proliferation of vascular endothelial cells is 20-2000 times faster in host-induced tumor endothelium than in normal endothelial cells.[14]

1.3.2. Detachment

The primary growth of cancerous cells is accompanied by several changes, both on the surface of the cells and with aberrant secretion of some materials. The underlying processes of invasion and metastasis are dependent on the modified behavior of tumor cells with regard to cell-cell and cell-matrix interaction.[5] Malignant cells have a reduced ability to adhere to each other, which helps them detach from the primary tumor and invade local tissue. These changes cause drastic alteration in aspects of the biological behaviors of the cells.

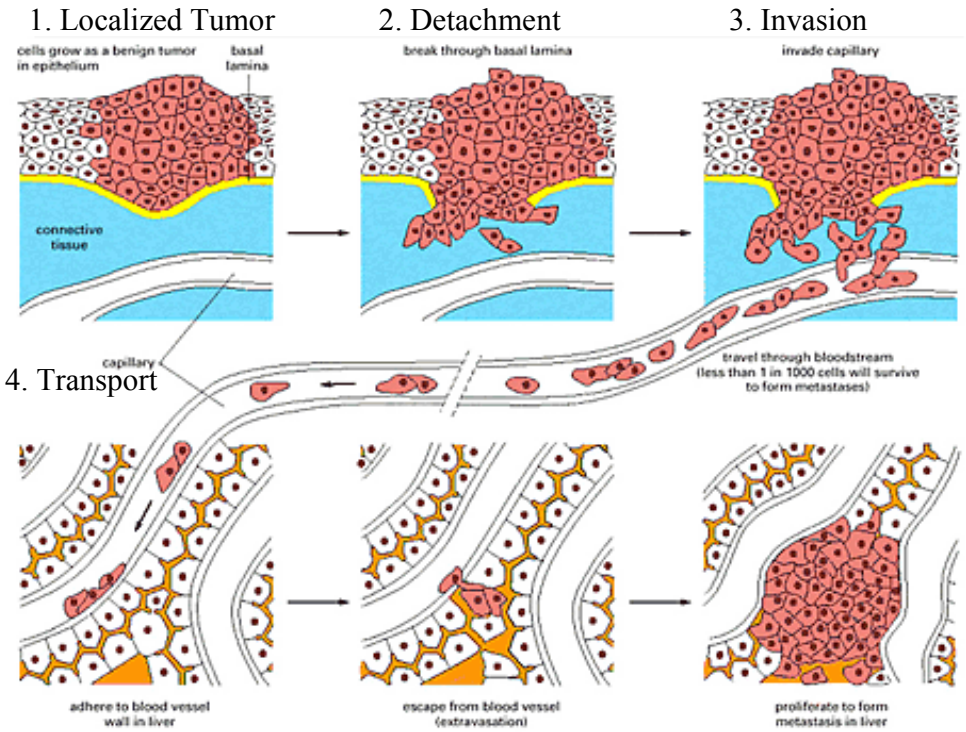


Figure 1.3. The Metastatic cascade

The most important change is the decrease of E-cadherin (a cell surface glycoprotein) that mediates the adhesion between cells. Loss of E-cadherin expression relates to more invasive and metastatic phenotype.[8,15] This occurs due to the over branching of the cell surface oligosaccharide, results in the change of negative charge of the cell surface. With the increase of negative charge on the cell surface, there is repulsion between cells which results to detachment of metastasizing tumor cells.

1.3.3. Invasion

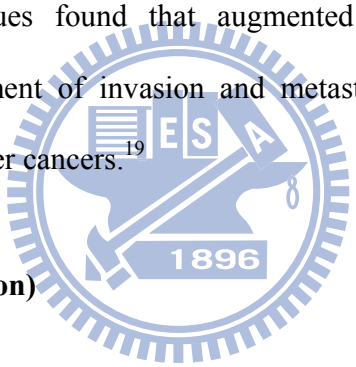
Historically, invasion was thought of as a passive force, where tumor cells were believed to be pushed by the pressure of growth into the circulation. Much research and experiments tested this growth-pressure theory, and showed that blocking the growth of metastatic tumor cells had no effect on their invasive and metastatic potential.[6] Since the pressure of a tumors growth does not effect the invasion of local tissue, tumor invasion is clearly an active process. Once malignant cells have detached from the primary tumor they will penetrate the extra cellular matrix (ECM) and enter the blood or lymph system. The invasion involves three steps: attachment, proteolysis and movement.

1.2.3.1. Attachment

The first step in invasion is the attachment of the tumor cells to the ECM or basement membrane. The ECM provides cell attachment ligands for extracellular receptors such as fibronectins, laminas and integrins. Some of these adhesion molecules including all ECM molecules and their cell surface receptors have been characterized.[16-17] All of these are important surface receptors that mediate the adhesion of cells to the ECM or basement membrane, and over expression of these receptors during tumor metastasis causes an increase in the progression of the disease. Fibronectins and lamins are cell-surface glycoprotein receptors that have the responsibility for activation of the host cells to produce proteolytic enzymes.[6] Inhibition of fibronectin or laminin has experimentally inhibited the metastasis.

1.3.3.2. Proteolysis

The second step in invasion is the degradation of the basement membrane. The breakdown of proteins including collagen, fibronectin, proteoglycans and other components in the ECM is mediated by degradative enzymes that are produced by the stimulative action of laminin or fibronectin to the host cells. There are three types of proteases produced from the tumor cells: serine proteases, collagenases (metalloproteinase), and cysteine proteases. The most important serine proteases are the highly regulated plasminogen activators, which catalyze the conversion of the extracellular plasminogen to plasmin, leading to the degradation of the ECM.[18] The metalloproteinase are the most important proteinases associated with the malignant process. Liotta and colleagues found that augmented levels of metalloproteinase correlated with the development of invasion and metastasis in human breast, colon, stomach, thyroid, lung and liver cancers.[19]



1.3.3.3. Movement (locomotion)

After completion of the degradation process the cells begin moving and this movement is the defining characteristic of the invasion stage. The cells are then free to circulate via the blood stream and invade other tissues in the body. Direction of locomotion is influenced by tumor cell derived motility factors, host derived chemotactic factors, components of ECM and their proteolytic digestion products and growth factors. The stimulation of the tumor cells movement is known as chemotactic activity.

1.3.4. Intravasion

Intravasion is the process by which tumor cells enter the blood or lymph system. The tumor cells attack the membrane of the venules or capillaries in the same way that occurred during the invasion process by degradation of the ECM. The tumor vessels formed in angiogenesis are often defective and leaky, allowing malignant cells to cross their walls fairly easily and enter the circulation.[6]

1.3.5. Transport

Most of the tumor cells that reach the blood circulation die, due to the presence of several resistance forces, such as mechanical trauma, attack by natural killer cells and anoikis(loss of th anchorage dependence). Only the most aggressive of tumor cells can successfully survive all the stages of the metastatic cascade, and it is estimated that less than0.01% of the tumor cells that reach the circulatory system survive. This vulnerability in the blood mean that anchorage independence of malignant cells is not complete, and many may die through apoptosis.[8]

1.3.6. Lodgement

The process of lodgement is not a random process. Different tumors exhibit different attachment factors and adhesion mediating molecules, specific cell recognition and affinity between adhesion molecules.[15] The patterns of spread of metastasis are explained to some extent on the basis of circulatory anatomy and the organ of first encounter. Since gastrointestinal tract tumors penetrate the portal venous system they lead to liver

metastasis, where as other tumors penetrate the systemic veins, eventually draining into the Vena Cava, leading to lung metastasis.[20] However, some tumor cells do not lodge in the first capillary bed they reach, but are transported to more distant organs and have a more selective pattern of metastatic colony formation.

1.3.7. Growth of secondary tumor (Matastasis)

After progressing through all the stages outlined above and shown in Figure 1.3 most tumors thereafter remain dominant and fail to complete the final stage of metastasis in the metastatic cascade. The failure may be due to lack of appropriate stimulatory growth factors. As with primary tumor cells, in order for the secondary tumor to grow larger than 0.5 mm in diameter a new blood supply is needed.[17] This process of angiogenesis is therefore crucial for the growth of the secondary tumor cells, and for this reason there is intensive research aimed at designing anti-angiogenic drugs to prevent the growth of both primary and secondary tumor cells.

1.4. Factors Influencing Malignancy in Cancer

There are two important factors that effect the development of a malignancy. These two factors are cell-to-cell adhesion and cell to extracellular matrix interaction. The interaction between malignant cells is particularly important, since they have a low ability to adhere to each other. This facilitates their detachment from the primary tumor site and subsequent entry into the circulatory system.

1.5. Current treatments of Cancer

The current treatment regimes for cancer are dependent upon the type of cancer being treated. Surgery is useful for large tumors that have not begun to metastasize. For small tumors or metastatic tumors, radiotherapy and/or chemotherapy are usually required. Both of these treatments cause serious side effects, primarily because they are targeting all cells that are rapidly dividing. Whilst cancerous cells are targeted, cells such as hair follicles or those cells lining gastrointestinal tract and bone marrow are also rapidly dividing cells and are therefore also affected during radiotherapy or chemotherapy regimes. The need for effective chemotherapeutic agent that can destroy the cancer cells without affecting the normal cells is paramount.

1.6. Cancer and VEGF

It is a well established fact in cancer biology that the tumor growth depends on the advancement of neovasculature. While there are more than 100 distinct types of cancer (and considerable heterogeneity within each tumor), the mechanisms that fuel tumor growth and survival are relatively similar. Across all cancers, sustained angiogenesis is reported to be the hall mark of cancer. Angiogenesis, a physiological process involves the formation of new blood vessel from the pre-existing ones is important for the supply of nutrients, oxygen, hormones and growth factors. For a tumor to grow beyond 1 to 2 mm diameter needs continuous supply of blood, which is acquired by expressing growth factor that recruit new vasculature from existing vessels as show in figure 1.4.[21-24]

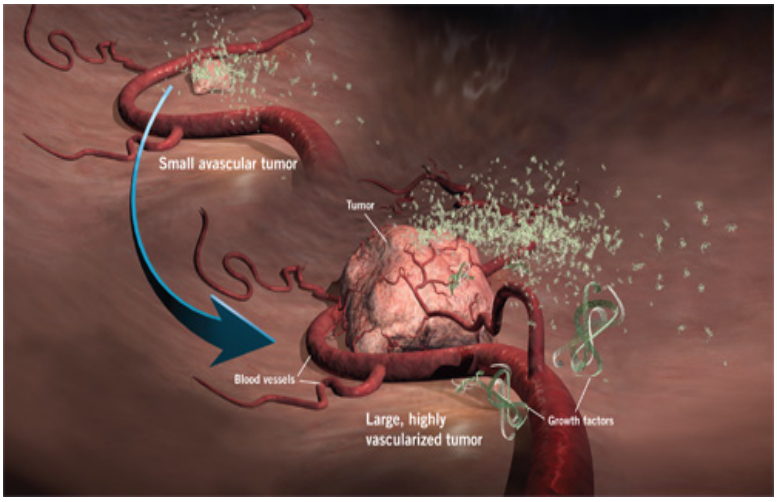


Figure 1.4. The Angiogeneic Process

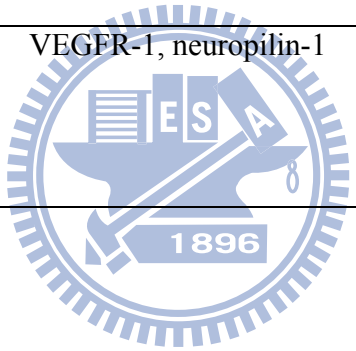
Among the many factors implicated in angiogenesis, VEGF has been identified as the most important one. The scope of scientific research involving VEGF continues to grow dramatically.

1.6.1. VEGF

VEGF (also known as VEGF-A, but commonly referred to simply as VEGF) stands for "vascular endothelial growth factor." Vascular endothelial growth factor (**VEGF**) is a chemical signal produced by cells that stimulates the growth of new blood vessels. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. This protein plays an important role in angiogenesis. As the name suggests, VEGF stimulates vascular endothelial cell growth, survival, and proliferation. The VEGF is a member of a family of 6 structurally related proteins that regulate the growth and differentiation of multiple components of the vascular system, especially blood and

lymph vessels. The six members are VEGF-A, placenta growth factor, VEGF-B, VEGF-C, VEGF-D and VEGF-E. [25]

VEGF Family Members	Receptors	Functions
VEGF (VEGF-A)	VEGFR-1, VEGFR-2, neuropilin-1	Angiogenesis, vascular maintenance
VEGF-B	VEGFR-1	Not established
VEGF-C	VEGFR-2, VEGFR-3	Lymphagiogenesis
VEGF-D	VEGFR-2, VEGFR-3	Lymphagiogenesis
VEGF-E (viral factor)	VEGFR-2	Angiogenesis
Placental growth factor (PIGF)	VEGFR-1, neuropilin-1	Angiogenesis Inflammation



1.6.2. VEGF receptors

Vascular endothelial growth factor (VEGF) ligands mediate their angiogenic effects by binding to specific VEGF receptors, leading to receptor dimerization and subsequent signal transduction. VEGF ligands bind to 3 primary receptors and 2-co-receptors. These six members can bind and activate the tyrosine kinase receptors, VEGF receptors 1, 2, and 3, which promotes the propagation, endurance, and migration of endothelial cells.[26] VEGFR-1 is able to bind VEGF-A, VEGF-B, and PlGF. VEGFR-2 is activated basically by VEGF-A, but of VEGF-C, VEGF-D, and VEGF-E may also activate this receptor. So, basically the angiogenesis is regulated by VEGFR-1 and VEGFR-2 (Figure 1).

Endothelial expression of VEGF receptors varies among the 3 primary recetors; VEGFR-2 is expressed on almost all endothelial cells, where as VEGFR-1 and -3 are selectively expressed in distinct vascular beds. The neuropilin-1 (NP-1 or NRP-1) and NP-2 (or NRP-2) receptors are thought to increase the binding affinity of the various VEGF ligands to these primary receptors, although the site specific roles of NP-1 and NP-2 in angiogenesis are not known.

1.6.3. The strategies for inhibiting the VEGF pathway

There are two primary pathways for inhibiting the VEGF signaling pathway which includes inhibiting either the VEGF ligand or the VEGF receptor. These are explained as below.

Extra cellular targeting of the VEGF ligand

Anti-VEGF strategies that directly target the VEGF ligand include ligand-binding antibodies and soluble receptors. These agents work extracellularly to provide specific inhibition of the VEGF pathway without disrupting other non-VEGF related targets. Therefore, they may inhibit angiogenesis without affecting other secondary or "off-target"pathways.[27] VEGF also promotes angiogenesis by signaling through neuropilin, a co-receptor to VEGFR-1 and -2 on endothelial cells. The presence of neuropilin is associated with tumor aggressiveness and poor prognosis. Neuropilin receptors lack a targetable intracellular kinase domain. Therefore, anti-VEGF strategies that target the ligand extracellularly may be capable of attenuating VEGF signaling that is mediated through neuropilin in Fig. 1.5.[27] Neuropilin receptors lack an intracellular kinase domain

but are able to induce signaling by recruiting ligands to the cell membrane. Emerging evidence also now suggests that neuropilin may be able to transduce VEGF signals in the absence of tyrosine kinase VEGF receptors. Accordingly, prevention of neuropilin-mediated signaling may require an extracellular strategy of VEGF inhibition.

Intracellular targeting of the VEGF receptor

Anti-VEGF strategies that target the VEGF receptor include tyrosine kinase inhibitors (TKIs) and receptor antibodies. Agents that target the VEGF receptor intracellularly, such as TKIs, have a wider range of inhibitory effects and may disrupt other secondary pathways that are also mediated through receptor kinases.[28]

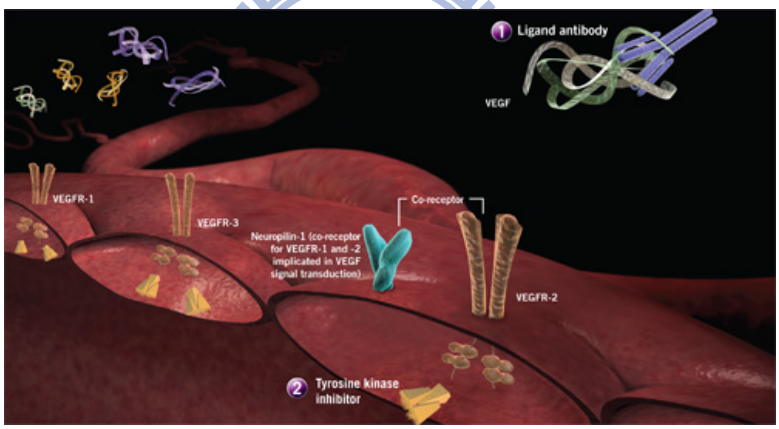


Figure 1.5. Stategies for inhibiting the VEGF pathways

1.6.4. Factors leading to the production and expression of angiogenesis by VEGF

Vascular endothelial growth factor (VEGF) production and subsequent angiogenesis can be triggered by a number of factors, including both genes and gene products, in the cellular microenvironment.

Entry	VEGF triggers	Description/subsequent effect	Factors involved
1	Hypoxia	Shortage of oxygen in the tumor environment	HIF-1α, HIF-1β
2	Oncogenes/Tumor suppressor genes	Genes that stimulate or suppress tumor formation	c-Src oncogene Bcr-Abl oncogene Ras oncogene *p*53 tumor suppressor gene
3	Cellular receptors	Proteins on the cell surface that are part of signaling pathways	EGFR HER-2 IGF-IR
4	Other growth factors and cytokines	Proteins secreted by cells that stimulate cellular signaling	COX-2 PDGF

Hypoxia triggers VEGF expression

Without an independent blood supply, tumors must rely on diffusion to obtain oxygen and other nutrients, and typically cannot grow more than 2 mm^3 in size. Thus, a growing tumor without sufficient vasculature will have hypoxic or lacking in oxygen areas. In

response to hypoxic conditions, tumors secrete vascular endothelial growth factor (VEGF) in order to recruit new vasculature, which then provides a supply of oxygen.[29] Hypoxia remains an important trigger of VEGF expression even once a tumor becomes vascularized. As the tumor grows, it continually outgrows its existing blood supply, leaving a rim of necrotic and hypoxic tissue. The tumor responds by up regulating VEGF gene expression, primarily through the activity of hypoxia inducible factor-1 (HIF-1), a protein consisting of 2 subunits (HIF-1α and HIF-1β).[30]

Oncogenes and tumor suppressor genes trigger VEGF expression

Oncogenes (genes that contribute to the production of a cancer) and tumor suppressor genes (genes encoding a protein that normally suppress tumor formation) are associated with increased vascular endothelial growth factor (VEGF) production. Oncogenes are generally mutated forms of proto-oncogenes (normal cellular genes capable of transforming a cell when activated). Some examples of oncogenes and tumor suppressor genes include

- **c-Src** is a proto-oncogene that appears to directly stimulate VEGF expression.
- **Bcr-Abl** is an oncogene formed from fusion of two proto-oncogenes[31] A preclinical study in tumor cell lines showed that transfection of **Bcr-Abl** caused an increase in VEGF expression, whereas blocking the function of **Bcr-Abl** reduced VEGF expression
- **Ras** oncogene: Ras proteins are part of the signaling cascade of growth factor—induced angiogenesis. The genes that encode for Ras proteins have been

associated with induction of VEGF expression in many solid tumors, including pancreatic, colorectal, and non-small cell lung cancers[32]

- **p53** tumor suppressor gene: Dysregulation of **p53**, normally a regulator of the cell cycle and trigger of apoptosis in damaged cells, has been implicated in the pathology of solid malignancies, including colorectal, breast, and endometrial carcinomas. Genetic alteration of tumor suppressor genes, including **p53**, has been shown to induce VEGF production[33]

1.6.4.1. The Role of VEGFR-1

Vascular endothelial growth factor (VEGF) receptor-1 (VEGFR-1) is a receptor for VEGF-A and can also bind VEGF-B and placental growth factor (PlGF). VEGFR-1 is a key receptor in developmental angiogenesis, but does not appear to be critical to pathogenic angiogenesis. Its role appears to vary with stages of development, physiologic and pathophysiologic conditions, and cell type.[34]

1.6.4.2. The Role of VEGFR-2

Vascular endothelial growth factor (VEGF) receptor-2 (VEGFR-2) mediates the majority of the downstream angiogenic effects of VEGF, including:

- Permeability
- Endothelial cell proliferation
- Invasion
- Migration
- Survival

Recent work suggests that VEGFR-2 can stimulate angiogenesis on its own. The activation and signaling of VEGFR-2 may be positively or negatively influenced by co-expression and activation of VEGFR-1

1.6.4.3. The Role of VEGFR-3

Vascular endothelial growth factor (VEGF) receptor-3 (VEGFR-3) promotes lymphangiogenesis. The term lymphangiogenesis involves the formation of new lymphatics, includes the spreading and invasion from tumors into surrounding stromal tissues, incursion into lymphatic walls and followed by lymph nodes implantation, and proliferation in the parenchyma of target organs. Recent studies have identified that because of single-layered endothelial cells having incomplete basal membranes, the lymphatic vessel composed, it lacks the tight junctions between endothelial cells. That's why, the tumor cells can easily enter the lymphatic vessels and metastasize to lymph nodes.[35] Unlike blood vascular system, the lymphatic system does not receive much attention owing to the lack of knowledge about the molecular mechanism of its development and function. Expression of VEGFR-3 is mostly restricted to lymphatic endothelium in normal tissues. However, recent studies have suggested that increased expression of VEGF-C and VEGF-D promotes tumor lymphangiogenesis and one can only curb this by inhibiting the VEGFR-3 signaling pathways. VEGFR-3 activation has been observed in several solid tumor types, including melanoma and breast cancer. In these tumors, elevated levels of VEGFR-3 ligands VEGF-C and VEGF-D are associated with lymph node metastases.[36] In recent years a promising approach to the therapeutic intervention of cancer has focused on antiangiogenesis therapies. This approach to

intervening in cancer progression takes advantage of the idea that inhibiting the blood supply to tumors will deplete the oxygen and nutrients. Resultantly it arrest tumor cell growth and proliferation. This approach has been found to be effective and there are presently twenty antiangiogenic drugs undergoing various stages of evaluation in clinical trials and numerous others in preclinical development. Recently two small molecule inhibitors sunitinib (**A**) and sorafenib (**B**) have been approved, in addition to AMG 706 (**C**), as antiangiogenic drugs (Figure 1).[37]

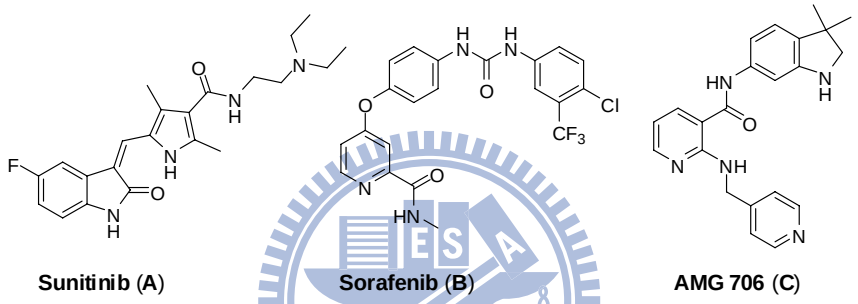


Figure 1.6. Examples of small molecule kinase inhibitors in clinical trials

In spite of these achievements, it remains an important challenge to develop new drugs in order to overcome the drug resistance and maintain the steady progress in the cancer research. Hence the synthesis of large number of molecules for screening and 'lead' generation plays an important role in cancer drug research. Concomitantly, coupled with high throughput screening, development of effective methodologies for rapid synthesis of diversified libraries of small heterocyclic molecules is of great importance.

1.7. Solid-Phase Methods in Organic Synthesis

Since the genesis of modern organic chemistry, classical organic reactions have been carried out in solution phase, implying the separation of the desired product from reagents and by-products after the reaction. This purification step can however turn out to be incredibly time consuming and often rigorous. In 1963 Merrifield first introduced the solid-phase synthesis of peptide and oligosaccharide.[38] This methodology, which was limited to the synthesis of peptides[39] and oligosaccharides, however remained predominantly limited to this field until the introduction of combinatorial techniques. There are two different ways of combining reagents to achieve chemical diversity. One is "Split-and-mix" synthesis (Figure 1.7 (a)), which was first developed by Furka, Lam and Houghten has now been used extensively in the pharmaceutical industry for the generation of thousands of compounds.[40-42] At every step, all beads are split equally into the number of reactions carried out (3 in the example). After reaction and washing, the beads are mixed together and then separated again into the next number of vessels required (3 again). The process continues until the last step with each bead containing a different compound (one bead / one compound). The advantage of this technique is the ability to generate exponentially growing numbers of compounds (3n after n steps) while keeping the number of reactions quite low (3 × n in the case of n steps). With split and mix, however, all compounds end up in the same mixture.

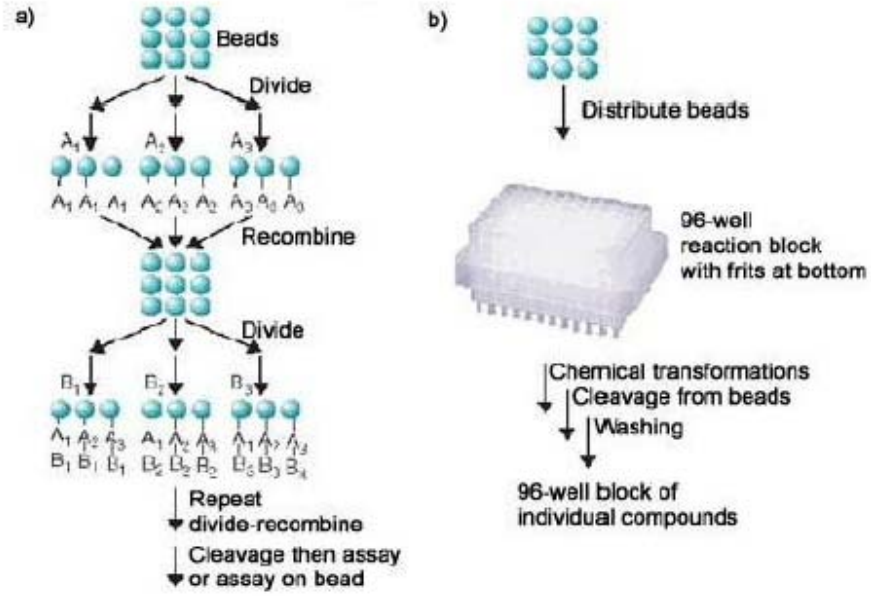


Figure 1.7. Differences between (a) split and mix and (b) parallel synthesis

The second approach is "parallel synthesis", where discrete compounds end up distributed in 96 or 384-well plates (figure 1.7 (b)). The technique is based on the distribution of diverse reagents having the same reactive functionality (i.e. carboxylic acids, amines, etc.) at each step of the synthesis. At the end of the sequence, each well will have followed a specific order, different from all other wells.

Since then, worldwide organic chemists have been widely adopted solid-phase organic synthesis (SPOS) to achieve combinatorial synthesis of structurally diverse heterocyclic molecular libraries. The key perception for SPOS methodology is to tether a simple molecule onto an insoluble polymer-support and then build-up density by planned chemical modifications and then finally cleave the final product off the resin (Merrifield's protocol). There are several advantages of SPOS over existing solution phase approaches. One major advantage is the facile separation and purification of

intermediates by simple filtration of the reaction mixture. Moreover, due to this easiness of purification, all transformations applied during SPOS can be achieved using a large excess of reagents to drive reactions to completion. With the introduction of ′pseudo-dilution effect′[43] facilitates macrocyclisation in SPOS relative to in solution phase synthesis. For example in 1995, Hauske introduced an acid-labile carbamate linker to deliver, high purity products with high purity following macrocyclisation of ′drug-like′ libraries mediated by Pd(0) under mild conditions.[44]

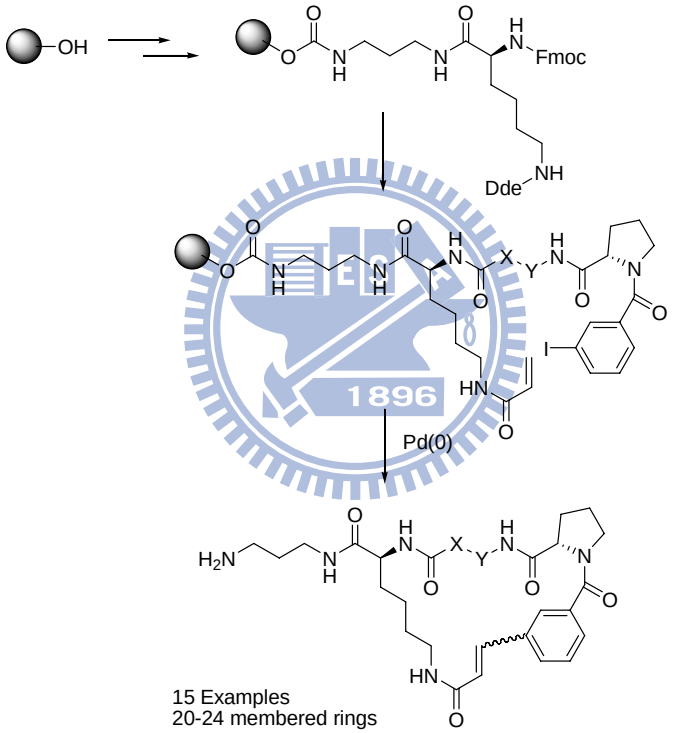


Figure 1.8. Hauske's macrocyclisation on solid-support resins.

All of the macrocycles (20- to 24-membered rings) were liberated from the resins under traceless cleavage conditions and obtained in good overall yields (Figure 1.8).

Although Merrifield's protocol for SPOS has many advantages over conventional solution phase techniques, it has some drawbacks: functionalized resins are difficult to

characterize and reactions are difficult to monitor using normal spectroscopic techniques in comparison with solution phase chemistry; reaction rates are slow; extra steps are required to attach and to release substrates from the resin; convergent synthetic strategies are not applicable; and certain reactions can only be carried out on particular types of resin. However, some of these disadvantages can be overcome by introducing solid-supported reagents. These are particularly reactive chemicals immobilized on a resin for carrying out desired chemical transformations in solution (Ley's protocol).[45] This concept also encompasses the development of solid-supported scavengers to remove excess organic or metallic reagents from solution phase reactions. Solid-supported reagents and scavengers facilitate *in situ* purification of reaction mixtures, leaving the desired products in solution for convenient identification. Conventional multi-step transformations in solution can be performed using sequential solid-supported reagents and scavengers to telescope workup and purification procedures. In addition, this approach can also be applied to convergent syntheses to achieve higher yields than in the corresponding linear counterparts. Prof Steven Ley demonstrated the former facet by employing solid-supported reagents to effect the clean and efficient total synthesis of several natural products (*e.g.* oxomartidine and epimartidine) (Figure 1.9).[46]

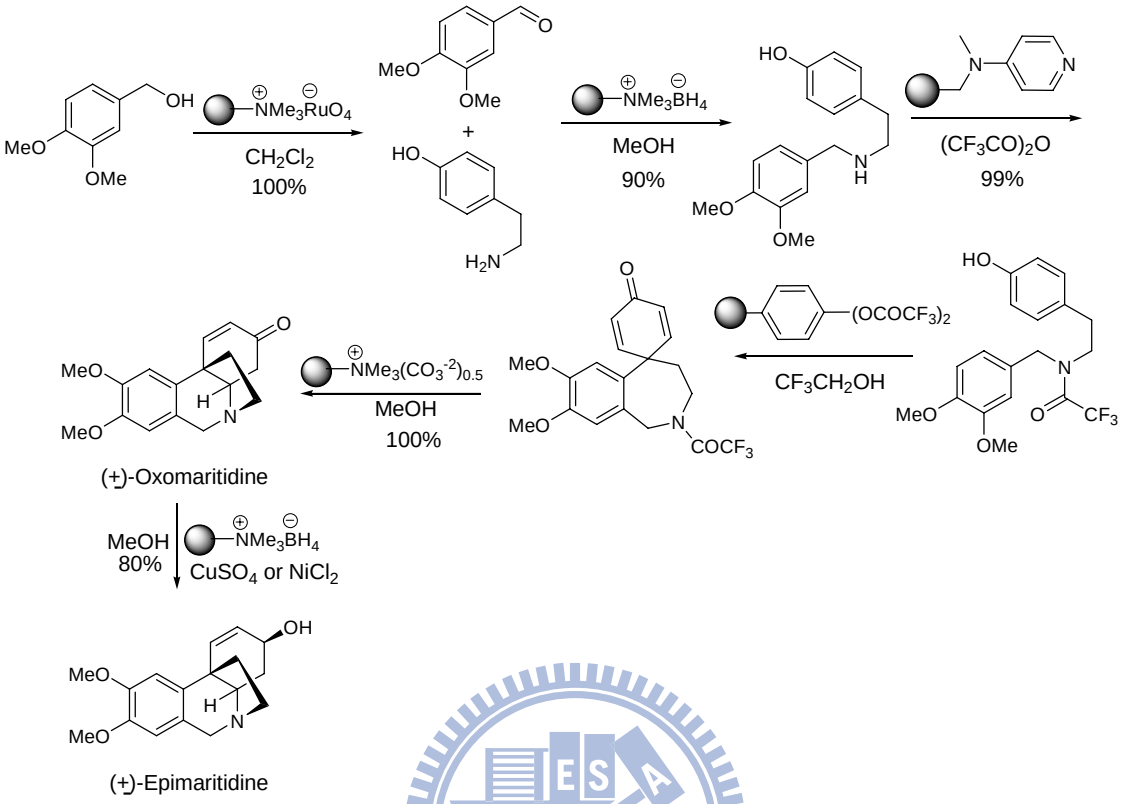


Figure 1.9. Ley's total synthesis of (±)-oxomaritidine and (±)-epimaritidine.[46]

Both the Merrifield and the Ley approaches to SPOS have been widely applied in automated combinatorial and parallel synthesis in industry for rapidly preparing drug-like small molecules for property screening (*e.g.* as new pharmaceuticals). However, slower conversion rates than for the corresponding transformations in solution remains a weakness due to the biphasic interactions between the polymer and solution.

1.8. Soluble Polymer Supported Technology in Organic Synthesis

To circumvent the drawbacks inherent to solid-supported technologies such as, nonlinear kinetics, unequal distribution to the chemical reaction, solvation problems, alternative

approaches using homogeneous 'beadless' phase-tagged chemistry have been introduced to facilitate separation whilst retaining solution phase kinetics. Among all of these beadless approaches, soluble polymer phase anchoring of substrates to enable easy separation, monitoring, analysis and characterisation has become the method of choice.[47] Normally the polymers employed as soluble supports in liquid phase organic synthesis should possess some basic qualities such as easy availability, good mechanical and chemical stabilities, having appropriate functional groups for easy anchoring to organic moiety and more importantly showing high solubility to dissolve molecular entities etc. Moreover, it has been observed that polymer supports normally used in organic synthesis should have macromolecules of varying sizes. These supports should withstand the reaction condition used in solution phase chemistry and consequently most polymer supports used in liquid phase synthesis possess alkyl ether backbone structures. By variation of functional groups of backbone structures, polymer properties are determined and may provide sites for attachment of organic moieties. There are number of polymers which are normally used for small molecule organic synthesis. These includes polyethylene glycol (PEG), polystyrene, poly(propylene oxide), poly(vinyl alcohol), polyethylene imine, polyacrylic acid, polyacryl amide, PEG with 3,5-diisocyanatobenzyl chloride, and cellulose (Figure 1.10) etc.

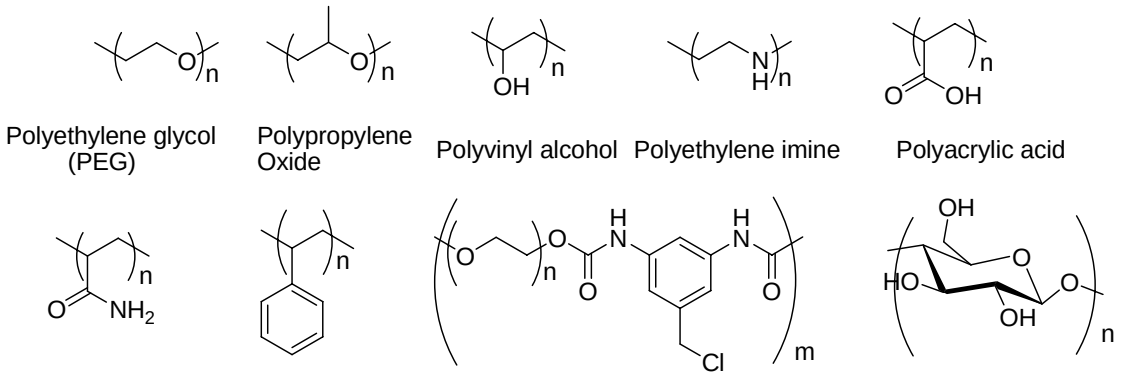


Figure 1.10. Different Soluble Supports used in Small Molecule Organic Synthesis

1.8.1. Application and recent development of polyethylene glycol as soluble support in organic synthesis

A number of polymeric reagents have been used for the simplification of organic synthesis. The liquid phase method of peptide synthesis on polyethylene glycol (PEG) was first introduced in 1971 and the PEG method has emerged as one of the most effective supports for the synthesis of oligopeptide, oligonucleotide, oligosaccharide as well as small molecules for the construction of combinatorial libraries.[48] Polyethylene glycol is cheap as compared to polyethylene oxide and polyoxyethylene. Normally for the supported synthesis PEG_{5000} and PEG_{4000}, PEG_{6000} are used as soluble supports based on the loading capacity and hydroxyl functionalities contained. PEG_{5000} contains one hydroxyl group and its loading capacity is 0.2 mmol/g, whereas PEG_{4000} and PEG_{6000} consist of two hydroxyl groups and their loading capacities are 0.5 mmol/g and 0.33 mmol/g, respectively. Employed as a protecting group, this linear homopolymer exhibits solubility in a wide range of organic solvents and water. PEG is insoluble in hexane, diethyl ether and *tert*-butyl methyl ether, and these solvents have been used to induce PEG precipitation. Careful precipitation conditions or cooling of polymer solutions in ethanol or methanol yields crystalline PEG due to the helical structure of the polymer that produces a strong propensity to crystallize. Thus, as long as the polymer backbone remains unaltered during liquid-phase synthesis, then purification by crystallization can be utilized at each reaction step. Furthermore, the solubilizing power of PEG not only allows homogeneous reactions under numerous reaction

conditions, but these solubility properties permit individual reaction steps to be monitored without requiring cleavage of product from the polymer support. The characterization of PEG-bound organic moieties is often straight forward as the polymer does not interfere with spectroscopic or chemical methods of analysis. In addition, MeOPEG (PEGME: polyetylene glycol monomethyl ether) contains a single methoxy group (δ = 3.38 ppm) and ethyl protons of PEG backbone (δ = 3.64 ppm) that provide internal standards for easy monitoring of reactions by 'H NMR spectroscopy.

Depending on polymerization conditions, PEG terminal may consist of a hydroxyl group or may be selectively functionalized. Commercially available PEG is produced through anionic polymerization of ethylene oxide to yield a polyether structure possessing either hydroxyl groups at both ends or a methoxy group at one end and a hydroxyl group at the other (MeO-PEG). The polymer MeO-PEG is considered mono functional, as typically the methoxy group of MeO-PEG remains unchanged throughout chemical manipulations. For identical chain lengths, the loading capacity of PEG is twice that of MeO-PEG as two hydroxyl groups serve as anchoring sites on PEG. Recently, varieties of PEG derivatives have been developed and are commercially available.

1.8.2. Applications of PEG in biological studies

A key property of PEG is that attachment to other molecules and surfaces provide a biocompatible, protective coating. This protective coating slows rejection of materials in biological systems (such as the human body), greatly reduces protein, cell and bacterial adsorption, and reduces the rate of kidney clearance (because of larger size). PEG also is

nontoxic and has been approved by the FDA for topical and interna1 use in humans. PEG is soluble in water and many organic solvents, and it forms aqueous two-phase systems when paired with certain other polymers (such as dextran). It is insoluble in ethyl ether and hydrocarbons such as hexane. The water solubility, lack of toxicity, high flexibility and well-defined chemistry of bifùnctional PEG makes it ideally suited for many cross linking or tethering applications. Seven technologies that have resulted from use of these properties are: (1) PEG-proteins for pharmaceutical use; (2) PEG-surfaces for electricdy-controlled, nonfouling materials; **(3)** PEG-liposomes for drug delivery; (4) molecule-molecule or molecule-surface coupling for drug and materials applications; (5) PEG-molecules for biological purifications; (6) biopolymer synthesis on PEG supports; and **(7)** PEG attachment for control of solubility (e.g., enzymes into organic solvents or water solubilization of enzyme substrates, dyes, flavors and chemotherapeutic agents).[49]

1.8.2.1 PEG-proteins for pharmaceutical use

It has been demonstrated that proteins with PEG attached remain active and have a greatIy diminished or negligible immune response. The result is that these PEG-proteins have greatly increased serum lifetimes. Examples include PEG-SOD, PEG-asparaginase, PEG-IL- 2 and PEG-hemoglobin. In addition, PEG attachment makes proteins much larger and thus reduces their rate of clearance through the kidney. PEG has also been attached to many small molecules (such as vitamin E, cholesterol, fluorouracil, etc.). The goal here is to reduce rate of kidney clearance and impart water solubility.

1.8.2.2 PEG-surfaces

In addition to the molecular modifications, PEG can also be attached to surfaces to form protective, bio-compatible coatings. A variety of applications result, including PEG coatings for arterial replacements, diagnostic apparatus and blood contacting devices. Similarly, capillary zone electrophoresis has emerged as an important new analytical technique in biochemistry, and PEG coatings on the capillaries prevent protein adsorption **and** provide critical control of electro osmosis.

1.8.2.3. PEG-liposomes

There has been intense interest in use of liposomes for controlled-release and selective delivery of drugs. A problem with this application is that liposomes, especially larger ones, are quickly attacked and cleared from the body. Recent research has shown that incorporation of PEG into the outer coating of liposomes can greatly increase serum lifetime, thus solving a critical problem blocking application of this promising drug delivery technique. The hydrophilic, biocompatible nature of PEGs and their mild, well-defined chemistry makes them ideal for coupling or tethering molecules to molecules or molecules to surfaces. This technology is critical for the next generation of drugs and biomaterials. Research has shown that use of PEG as a coupler to bind molecules to other molecules and surfaces provides highly active materials.

1.8.2.4 Biological Purification

The genetic engineering revolution has led to methods for production of a variety of physiologically active proteins. There is, however, a critical need in this industry for improved methods for isolation of the proteins produced. An approach to this problem that has recently received much interest is purification by partitioning in aqueous two-phase systems (analogous to oil and water) made by solution of PEG, other polymers and salts in water. In this approach, a PEG-ligand is made (such as a PEG-antibody), which binds specifically with the desired protein and pulls the protein into the PEG-rich phase.

1.8.2.5. Biopolymer syntheses

The three bio-oligomers (peptides, oligonucleotides, and oligosaccharides) can all be grown on PEG as a soluble carrier. The PEG-oligomer is precipitated after each step to isolate the product, which can then be cleaved or taken to the next addition step. Advantages of this method are that fewer errors result, chemistry is faster, and large quantities of materials can be handled. A variation on this theory is to build the bio-oligomer on a PEG chain that is bound to a solid polystyrene particle. This approach apparently provides advantages of both solid-phase and liquid-phase synthesis.

1.8.2.6. Solubilization of insoluble molecules

PEG is soluble both in water and in many organic solvents. This property has been utilized to solubilize other molecules by PEG attachment. An interesthg biotechnical application is solubilization of enzymes in organic solvents such as chlorinated hydrocarbons. Additionally, water-insoluble materials may become water-soluble after PEG attachment. Examples here include dyes, flavors, substrates for enzymes, cofactors, pharmaceuticals etc.

1.9. Small molecule Syntheses

Polyethylene glycol has long been applied as soluble supports for the synthesis of oligopeptide, oligosaccharide as well as small molecules. Till date numerous research efforts has been published using PEG as soluble supports. Janda *et* al. first reported the synthesis of pentapeptide and sulfonamide libraries using the PEG as a support. This was the first report of the use of PEG in small organic molecule synthesis, and the pentapeptide library preparation was the first application of this polymer in a combinatorial sense. Synthesized libraries were act as ligands for a monoclonal antibody against â-endorphin (Figure 1.11).[48]

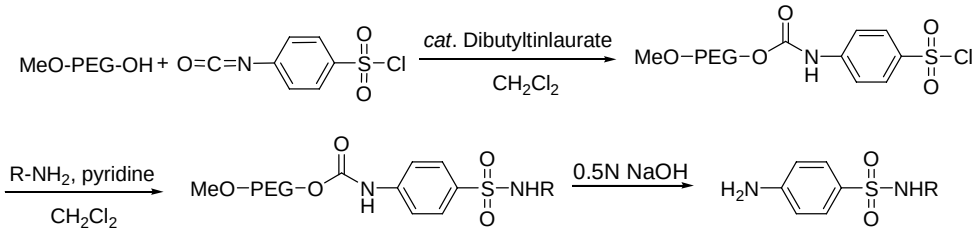


Figure 1.11. Synthesis of aryl sulfonamide libraries on soluble support.

Similar methodology was applied for the synthesis of a new class of peptidomimetics called azetides, alkylated malonates and 3,5-pyrazolidinediones which could be used for rheumatoid arthritis.[50] (Figure 1.12.)

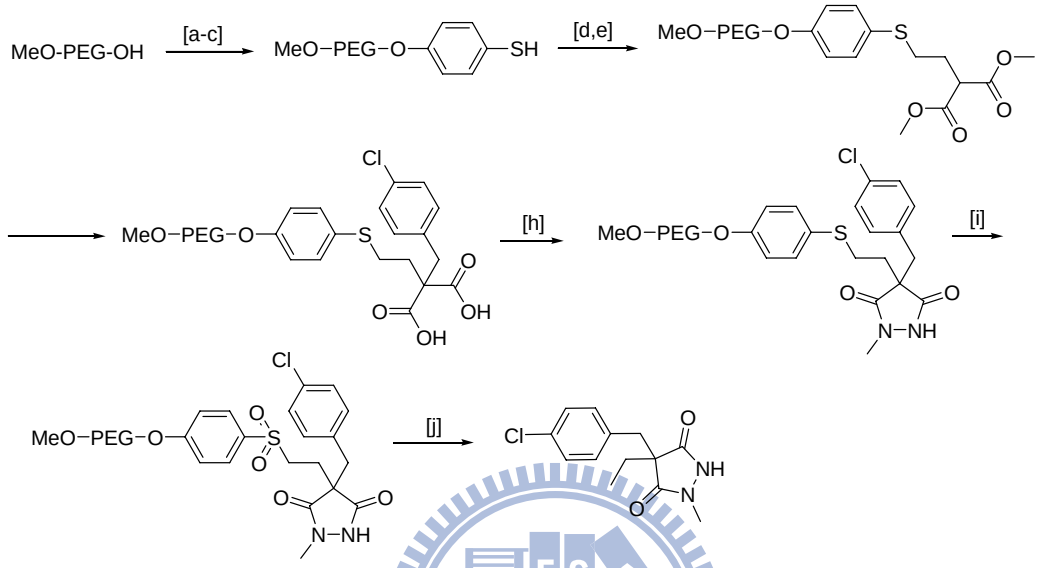


Figure 1. 12. Soluble Polymer-supported synthesis of 3,5-pyrazolidinediones. Conditions: (a) MsCl, Et_3N, CH_2Cl_2, room temperature, 12 h; (b) 4-HO-C_6H_4-S)$_2$, Cs_2CO_3, DMF, 65 °C, 6 h; (c) DTT, H2O, reflux, 3 h; (d) $BrCH_2CH_2Br$, Cs_2CO_3, DMF, room temperature, 14 h; (e) $CH_2(CO_2Me)_2$, Cs_2CO_3, DMF, room temperature, 17 h; (f) 4-Cl-$C_6H_4CH_2Cl$, Cs_2CO_3, DMF, room temperature, 17 h; (g) (i) NaOH, H_2O, room temperature, 5 h, (ii) Amberlite IR-120 (H+), 1 h; (h) CH_3NHNH_2, PyBOP, iPr_2EtN, DMF, room temperature, 43 h; (i) $KHSO_5$, H_2O, room temperature; (j) 5% Na/Hg, Na_2HPO_4, MeOH/DMF (1/8), room temperature, 18 h.

Furthermore, it has been observed that the intramolecular cyclitive cleavage to generate 3-aminoimidazoline-2,4-diones and intramolecular stille coupling have also occurred on soluble polymer supported synthesis.[51] Recently, it has been observed that the problem of low loading capacity was overcome by combining the basic principles of dendrimer chemistry with that of PEG polymers to generate new, soluble PEG supports of expanded functional group capacity as developed by Cozzi.[52]

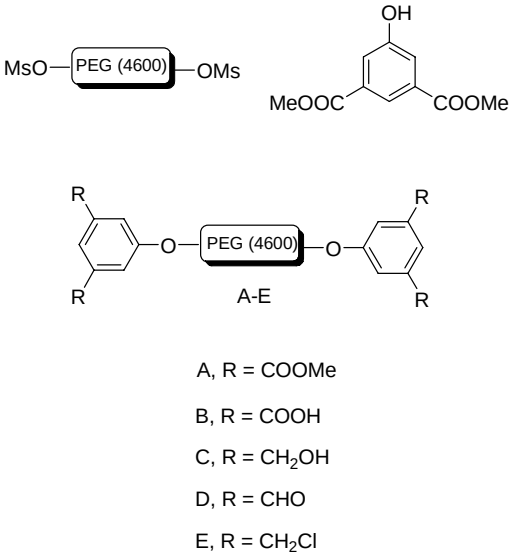


Figure 1.13: Synthesis of high molecular weight PEG- polymers.

The bis-mesylate obtained from PEG4600 was reacted (Figure 1.13) with dimethyl 5-hydroxyisophthalate and Cs_2CO_3 in DMF (50 °C, 15 h) to afford the tetra ester A in 95% yield. Hydrolysis of A with aqueous KOH at room temperature for 15 h and followed by acidification gave the tetra acid B in 70% yield. Reduction of the ester A with 2 N DIBAL in CH_2Cl_2 at -78 °C to room temperature for 15 h afforded the tetraol C in 83% yield. From this compound C, which features four easily removable benzylic hydroxyl groups, the tetra aldehyde D was synthesized by oxidation with MnO_2 in CH_2Cl_2 room temperature for 72 h in 71% yield, and the tetrachloride E obtained by reaction with $SOCl_2$ in the presence of pyridine in refluxing toluene for 15 h, in 60% yield. Thus five different functionalities amenable to a variety of synthetic manipulations could be easily attached to the polymer backbone. To further confirm the possibility of performing organic synthesis on this polyfunctionalized PEG, tetraol C was esterified with *N*-Boc

glycine using DCC as coupling reagent and catalytic amount of DMAP in refluxing CH_2Cl_2 for 15 h obtained tetra-*N*-Boc glycinate in 94% yield, which after BOC

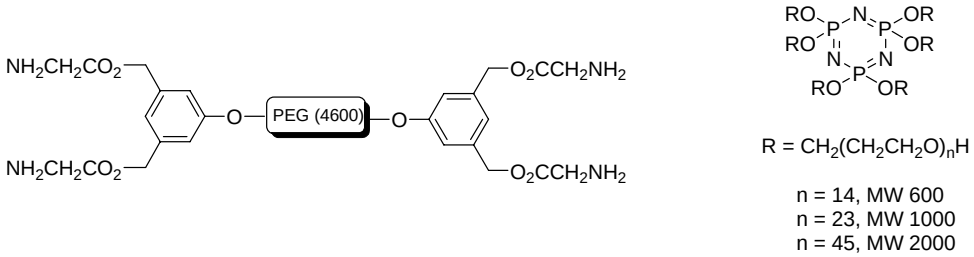


Figure 1.14. Reactions anchoring on PEG 4600

deprotection in 2:1 TFA: CH_2Cl_2 mixture gave tetra amine in 83% yield as shown in figure 1.14. Here we have observed that loading capacity of polyfunctionalized PEG increases to 0.8 mmol/g as compared to MeO-PEG_{5000} where loading capacity is 0.2 mol/g. Recently, Janda *et. al*. have synthesized a new high-loading (1 mmol/g) soluble-polymer based on a cyclotriphosphazene core with PEG arms known as stealth star polymer that exhibit advanced precipitation properties compared with those of linear PEG.[53] While it was extremely necessary to increase the loading capacity for soluble supports, Fan *et. al*. [54] developed the Janus dendrimer via a liquid phase approach which could easily be purified by simple precipitation technique without column chromatography. Their utility as soluble supports was carefully decorated in the Pd catalyzed Suzuki cross coupling reactions giving biaryl products in good yield in figure 1.15.

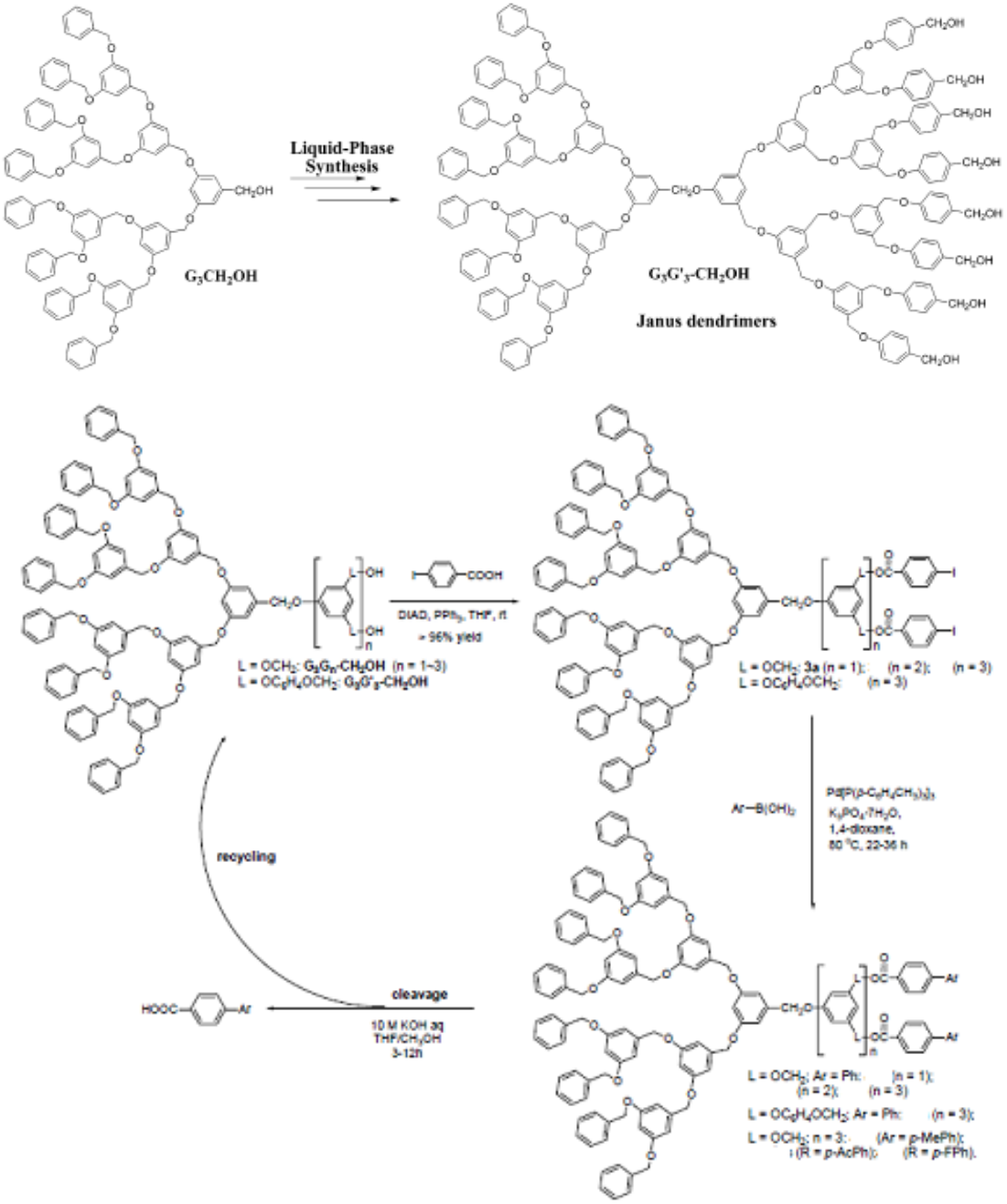


Figure 1. 15. Janus Dendrimers as Soluble Supports for the Pd-Catalyzed Suzuki Coupling Reaction.

Section A

1.10. Benzoxazols – Importance and synthesis.

Benzoxazoles are privileged *N-O* heterocycles known for a wide range of biological activities. Benzoxazoles are categorized as pervasive structural motif, because of their ability to interact with a wide range of different enzymes and receptors. Recently they are found in a number of natural products, agrochemical products, as well as medicinal utility (Figure 1.16). For example, the benzoxazole moiety is established in natural products like antimycobacterial pseudopteroxazole,[55] UK-1,[56] AJI9561 **1**[57] and salvianen.[58] Besides this activity, this moiety has been found as cathepsin S inhibitor **2**,[59] 5-HT_3 receptor agonist **3**,[60] HIV reverse transcriptase inhibitor L-697,661 **4**,[61] anticancer agent NSC-693638 **5**,[62] estrogen receptor-β agonist ERB-041 **6**,[63] orexin-1 receptor antagonist SB-334867 **7**[64], selective peroxisome proliferators-activated receptor γ antagonist JTP-426467 **8**,[65] Recently, it had been found that the benzoxazol ring **9**, is an intriguing heterocycle acts as a novel class of potent KDR inhibitors.[66] In spite of this activity it also act as herbicides, such as Fenoxaprop **10** and as fluorescent whitening dyes **11**.[67] 2-Phenylbenzoxazols are known as photo stable, highly efficient UV dyes, and are used as organic brightening agents, laser dyes, and organic plastic scientillators.

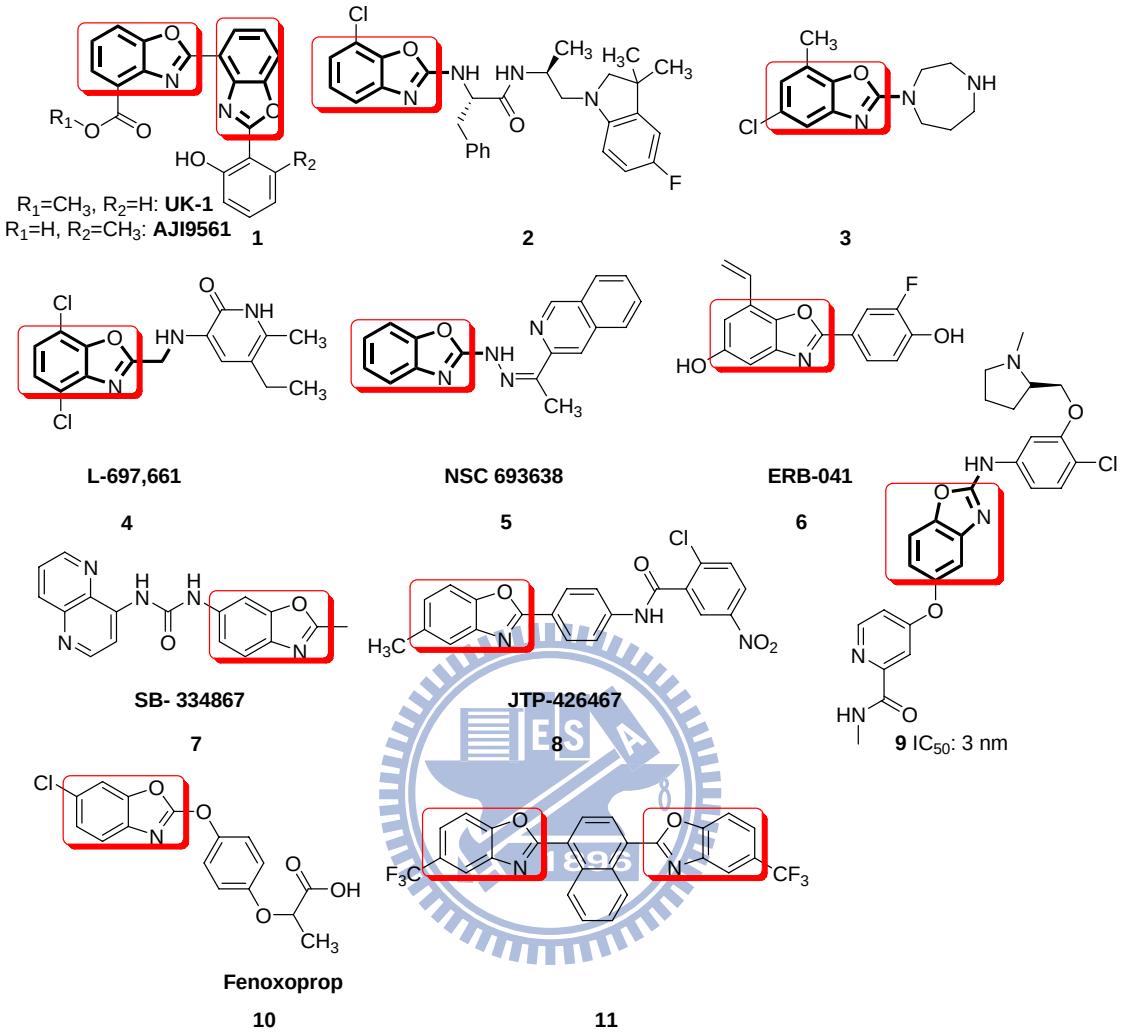
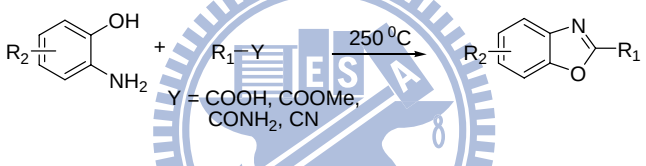


Figure 1.16. Biologically active benzoxazol derivatives.

The below section briefly reviews methods to the construction of biologically active benzoxazols rings which involves the condensation of 2-aminophenol with either carboxylic acids or aldehydes by acid or base catalyzed reaction under high temperature conditions or metal induced cyclisation reaction.

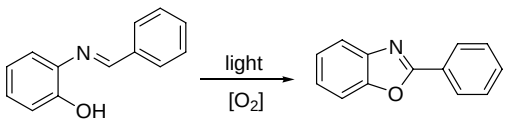
1.11. Chemical Methods for Synthesizing Benzoxazols derivatives.

In 1957, Leavit *et. al.*[68] first synthesized the benzoxazols derivatives using polyphosphoric acid as the condensing agent. The utility of polyphosphoric acid as a remarkably effective condensing agent, particularly for intra- and intermolecular condensations, has been extensively demonstrated in recent years. These condensations generally are carried out by thermal fusion, heating in solvents or in various concentrations of hydrochloric or sulfuric acid, or by heating under pressure in the presence of dilute hydrochloric acid. The catalyst polyphosphoric acid, which also serves as a suitable solvent for the reaction, is equally effective for the condensation of carboxylic acids, amides, esters or nitriles with *o*-aminophenols to generate the benzoxazol derivatives in good yields in scheme 1.



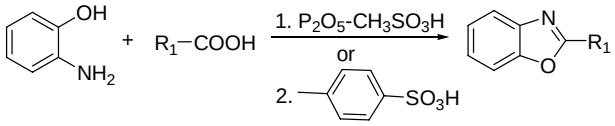
Scheme 1. Levait's method of benzoxazol synthesis.

In 1973, Grellmann *et. al* synthesized the benzoxazols derivatives in photochemical way which involves the reaction of benzylideneanilines substituted in the ortho position of the aniline ring by hydroxyl group in degassed as well as air saturated solution with high quantum yields as shown in scheme 2.[69]



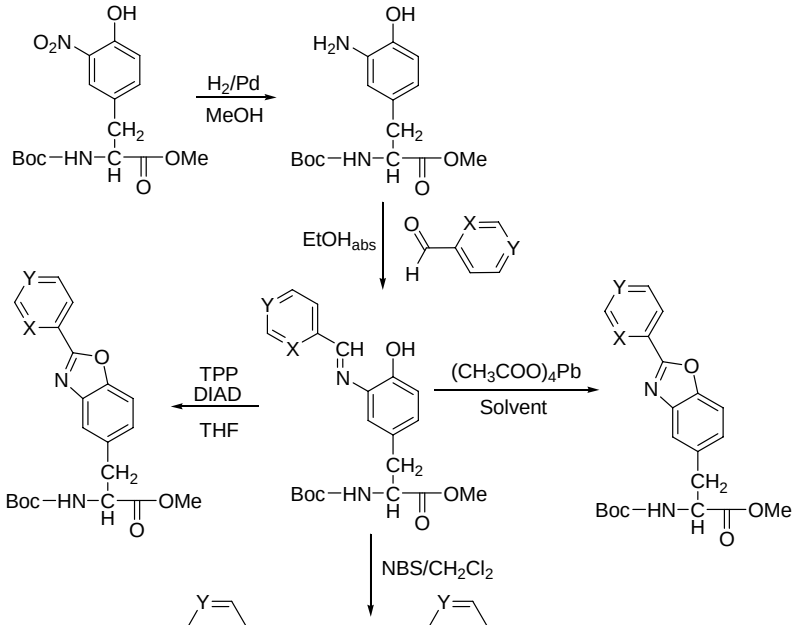
Scheme 2. Grellmann method of benzoxazol synthesis using light

In 1997, So *et. al*. and Kerwin *et. al*. syntheised the benzoxazol derivatives by the reaction of ortho aminophenols with benzoic acid in presence of phosphorous pentoxide-methane sulfonic acid (P_2O_5-CH_3SO_3H), which is a convenient alternative to PPA reagents under heating conditions and *para*-toluene sulfonic acid in refluxing benzene conditions in high yields respectively as hown in scheme 3.[70]



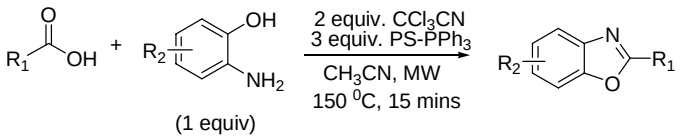
Scheme 3. So *et. al*. and Kerwin *et. al*. methods of benzoxazol synthesis.

In 2002, Guzow *et.al.*have synthesized the same scaffolds using oxidative cyclisation of azomethines (Schiff's base) obtained from N-boc-3-nitro-l-tyrosine methyl ester with appropriate aldehyde. They used the three oxidizing agent such as lead tetraacetate, N-bromosuccinimide, and Mitsunobu reagents (triphenylphosphine (TPP) and diisopropylazidodicarboxylate (DIAD) as shown in scheme 4.[71]



Scheme 4. Guzow *et. al*. methods of benzoxazol synthesis.

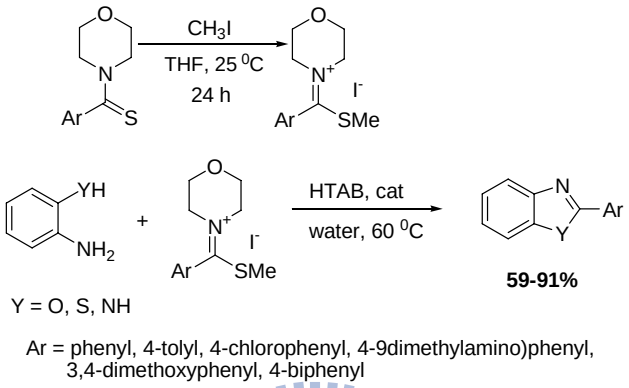
In 2006, Wang *et.al.* have synthesized the same scaffold using commercially available PS-PPh_3/CCl_3CN resin combined with microwave irradiation in high yields and purity.[72]



Scheme 5. Wang *et. al*. methods of benzoxazol synthesis.

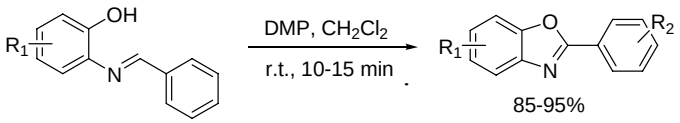
In 2009, Boeini *et.al.* have synthesized benzoxazoles by the aquatic reaction of the corresponding thioamidinium salts and 2-aminophenol in scheme 6. They have showed

that the thioamidinium salt was successfully applied as an alternative to a carboxylic acid derivative to react smoothly with an amino precursor and in the presence of catalytic amounts of hexadecyltrimethylammonium bromide salt to produce benzoxazoles in good to excellent yields.[73]



Scheme 6. Boeini *et. al*. methods of benzoxazol synthesis.

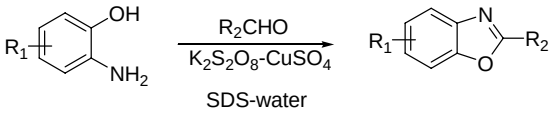
Similarly, Bose *et. al.* in 2010 have synthesized the benzoxazol derivatives by the intramolecular cyclization of phenolic azomethines/Schiff bases at ambient temp using a highly versatile hypervalent I(V) reagent, Dess-Martin periodinane (DMP) in scheme 7. Similar methodology has been applied by treating the reaction mixtures sequentially with Amberlyst A-26 thiosulfate resin and (diisopropylamino) methyl resin (PS-DIEA), which remove excess reagent and byproducts, to give pure products.[74]



Scheme 7. Bose *et. al*. methods of benzoxazol synthesis.

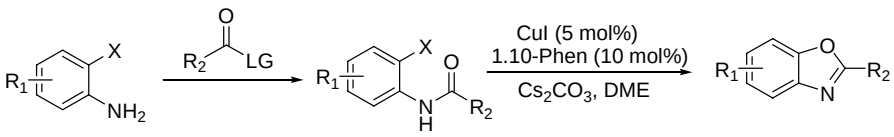
Recently, Atul *et. al.* have synthesized libraries of 2-substituted- benzoxazoles via potassium persulfate-$CuSO_4$-mediated oxidative coupling of aldehydes with o-

aminophenols in aq. Micelles as drawn in scheme 8. The reagent is com. available, cheap, and highly chemoselective. The yields were superior in aq. micelles to those in org. solvents. However the main advantages of this diversity-oriented synthesis involves the short reaction times, large-scale synthesis, excellent chemoselectivity, excellent yields, as well as environmental friendliness as shown in scheme 8.[75]



Scheme 8. Atul *et. al*. methods of benzoxazol synthesis.

In 2006, Batey *et. al* have introduced the general method for the formation of benzoxazoles via a copper-catalyzed cyclization of *ortho*-haloanilides which requires *o*-aminophenol as substrate. The reaction involves an intramolecular C-O cross-coupling of the ortho-haloanilides and is believed to proceed via an oxidative insertion/reductive elimination pathway through a Cu(I)/Cu(III) manifold. Ligands such as 1,10-phenanthroline and *N,N* -dimethylethylenediamine were shown to provide ligand acceleration/stabilization in the reaction. However, the optimal conditions for cyclization used a combination of CuI and 1,10-phenanthroline (10 mol %) as shown in the scheme 9. The rate of reaction follows the order I > Br > Cl, consistent with oxidative addition being the rate-determining step.[76]



Scheme 9. Batey *et. al*. methods of benzoxazol synthesis.

However the mechanism of the reaction involves the coordination of the amide group of **A** with CuI to give **B**, followed by an oxidative insertion to **C**, and then a reductive elimination to release product **D** with concomitant regeneration of CuI catalyst as shown in figure 1.20.

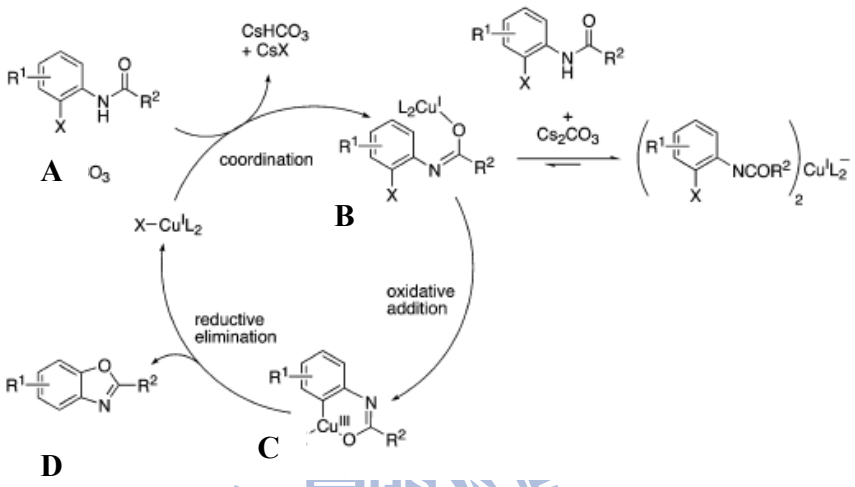
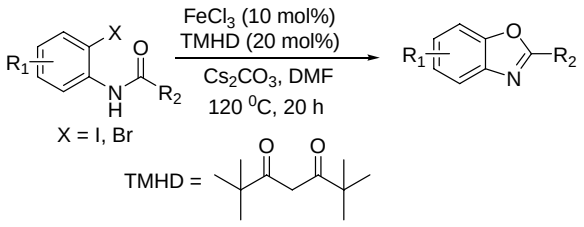


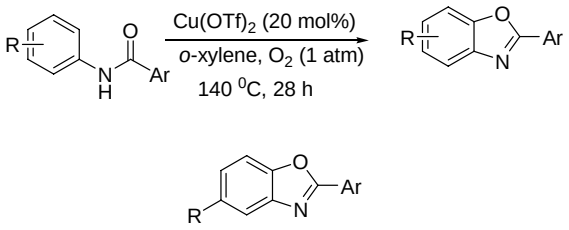
Figure 1.20. Mechanistic Proposal for the Copper-Catalyzed Formation of benzoxazol derivatives.

In 2008, Bolm *et. al.* synthesized the same scaffolds using the intramolecular iron-catalysed *o*-arylation of teadily available 2-haloanilines. The key step involves the use of FeCl3 and 2,2,6,6-tetramethyl-3,5 heptanedione (TMHD) as the catalyst system as shown in scheme 10.[77]



Scheme 10. Bolm *et. al*. methods of benzoxazol synthesis.

In 2008, Nagasawa's group developed a $Cu(OTf)_2$ catalyzed intramolecular cyclization via an oxidative C-H functionalization and C-O bond formation sequence to synthesize benzoxazoles from N-phenylbenzamide and its derivatives in *o*-xylene under an oxygen atmospherein scheme 11.[78]



Scheme 11. Nagasawa *et. al*. methods of benzoxazol synthesis.

Several functional groups including halogens (F, Cl, Br) and electron-donating or -withdrawing substituents are well tolerated. The regioselectivity of the cyclizations is highly impressive. They have already reported that these cyclization reactions in o-dichlorobenzene (*o*-DCB) also using $Cu(OTf)_2$ (20 mol%) as catalyst at 160 ^{0} C under an atmosphere of air gave superior results in most cases. The reaction of a substrate with the strongly electron-withdrawing nitro group did not proceed at all. The mechanism of the reaction involves the initial coordination of benzanilide to $Cu(OTf)_2$ would lead to direct ortho-metallation by electrophilic aromatic substitution at the Cu center. The presence of a directing group at the meta position of the anilide would promote this process by the formation of a doubly coordinated intermediate.

The formation of a six-membered metallacycle and subsequent reductive elimination affords benzoxazole and a reduced copper species that is then reoxidized by molecular oxygen to complete the catalytic cycle figure 1.21.

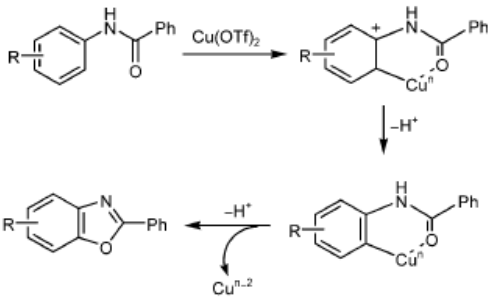
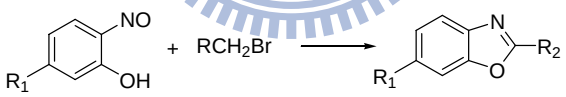


Figure 1.21. The mechanism for the synthesis of benzoxazoles.

In 2010, Huang's group discovered the synthesis of benzoxazols ring from 5-diethylamino-2-nitrosophenol with arylbromide with good functional group tolerance and high yield as shown in scheme 12.[79]



Scheme 12. Huang *et. al*. methods of benzoxazol synthesis.

When alkyne functionality was introduced in arylbromide moiety such as CH_3CCCH_2Br or $PhCCCH_2Cl$ with two equivalents 5-diethylamino-2-nitrosophenol, then the benzoxazine ring was also obtained along with benzoxazole ring. However, the mechanism for the formation of benzoxazine ring is shown in figure 1.22. The first *o*-nitrosophenol reacts with R-CH_2X to afford the benzoxazole. The alkynyl moiety in the

benzoxazole intermediate reacts with a second *o*-nitrosophenol through Diels-Alder reaction to form a benzoxazine intermediate with OH on the nitrogen. Rearrangement of the HO group occurred under the basic reaction conditions to give the bi-heterocyclic product.

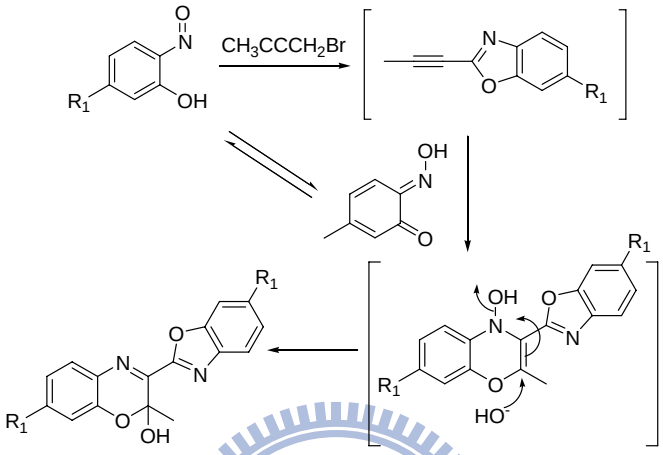
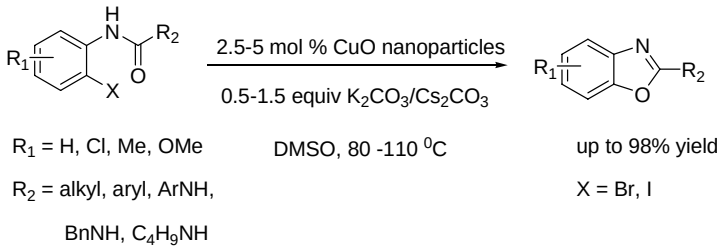


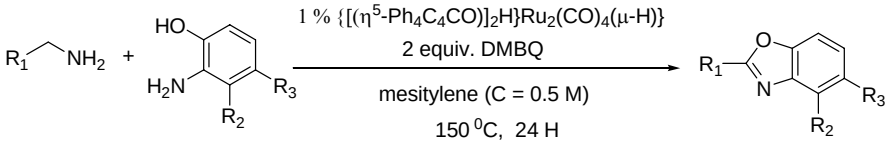
Figure 1.22. The mechanism for the synthesis of benzoxazoles.

In 2009, Punniyamurthy *et. al.* synthesized the substituted benzoxazoles derivatives via intramolecular cyclization of *o*-bromoaryl derivatives using copper (II) oxide nanoparticles in DMSO under air as shown in scheme 13.[80]



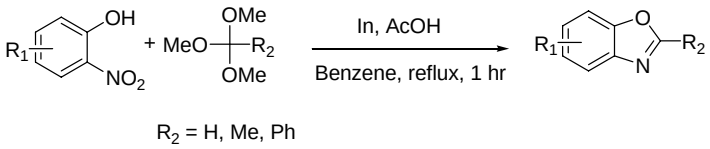
Scheme 13. Punniyamurthy *et. al*. methods of benzoxazol synthesis.

In 2009, Marsden *et. al.* are synthesized benzoxazoles directly by oxidative condensation of primary and secondary amines with *o*-aminophenols under hydrogen transfer catalysis. The optimal system utilizes 1 mol % of the Shvo catalyst, with dimethoxybenzoquinone as the hydrogen-accepting terminal oxidant as shown in scheme 13.[81]



Scheme 14. Marsden *et al*. methods of benzoxazol synthesis.

In 2009, Kim *et. al.* synthesized the benzoxazoles derivatives from the reductive intermolecular reaction between orthoesters such as trimethyl orthoformate, trimethyl orthoacetate, and trimethyl orthobenzoates with 2-nitrophenols in the presence of indium and acetic acid (10 equiv) in refluxing benzene in scheme 14.[82]



Scheme 15. Kim *et. al*. methods of benzoxazol synthesis.

However the mechanism of the reaction involves the consequent electron transfers and proton transfers, the nitro group could first transform into a nitroso intermediate. The nitroso intermediate may be transformed into the aniline species via electron transfer and

proton transfer processes, if the proton transfer reactions are highly favorable (path A in figure 1.23), and then it can react further to the imidate intermediate. Another possibility is the coupling reaction of the nitroso radical anion with the orthoester to form the imidate intermediate, as shown in path B in figure 1.23. After the imidate intermediate is formed by either path A or B, an acid-assisted attack by the hydroxyl group toward the neighboring imidate group followed by the loss of MeOH due to the aromatization driving forces would produce the benzoxazole ring.

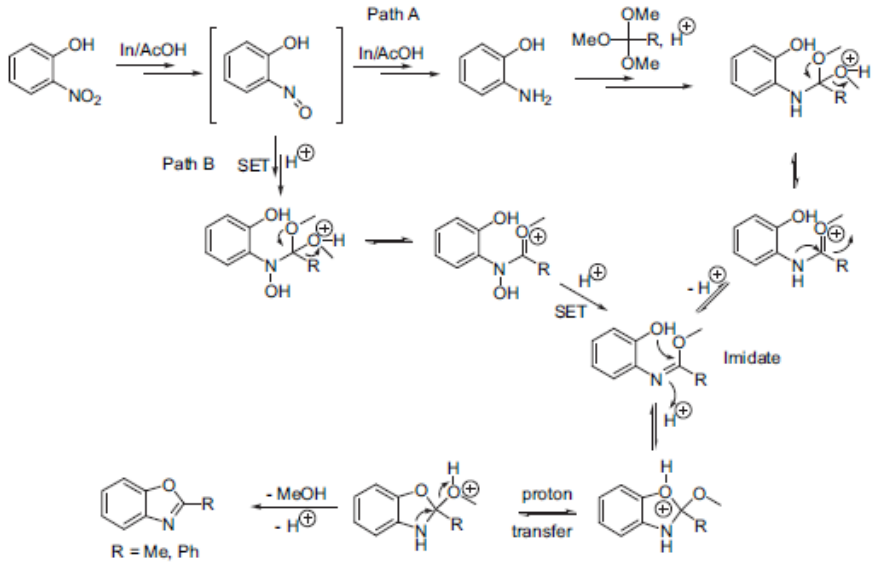


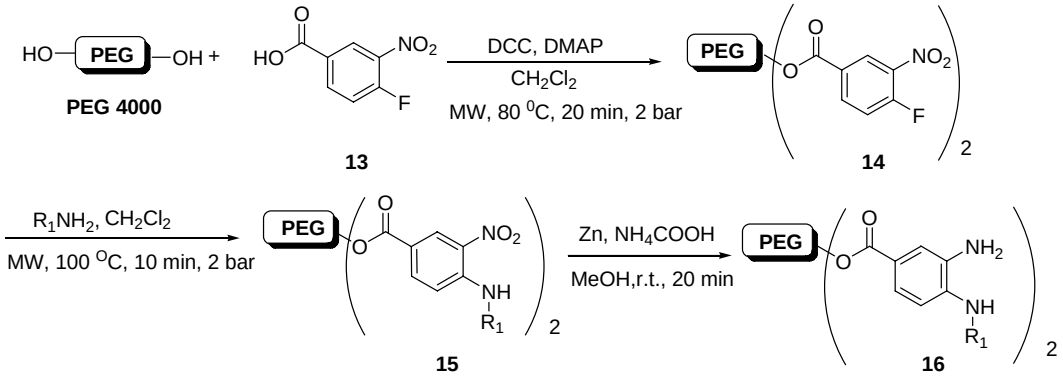
Figure 1.23. The mechanism for the synthesis of benzoxazoles.

However all these methods are suffer from drawbacks such as harsh reaction condition, incapability to generate diversified library, longer reaction time and metal induced reactions. Recently it has been observed that the significance of compounds consists of two or more heterocyclic rings are well acknowledged by the occurrence with which *bis*-heterocyclic compounds were branded as the most potent ones.[83] Although the medicinal

chemistry fraternity has put up a brave front to cover a wide range of structurally and functionally derivatized heterocyclic compounds, yet it lacks the effort to explore providential *bis*-heterocyclic compounds. Hence libraries of such type of compounds have not been extensively studied so far. Compared to mono heterocycles; fused or linked bis-heterocyclic scaffolds with two heteroatom-containing rings might be expected to afford additional binding opportunities as well as comprehensive options for diversification. Prompted by these above observation and in the interest of broadening the scope of starting materials that can be elaborated into benzoxazoles under milder conditions, we undertook the present investigation and the results of our study have been reported herein.

1.12. Results and Discussion

The present strategy commenced with the synthesis of polymer immobilized *o*-phenylene diamine **16** from 4-fluoro-3-nitrobenzoic acid **13** with built-in structural diversity (R_1) through three step protocol as developed by our group earlier (Scheme 16).[84]



Scheme 16. PEG supported synthesis of *o*-phenylenediamine **16**.

The synthetic route described in Scheme 15 was utilized HO-PEG-OH (MW: 4000) as a soluble support was reacted with the commercially available 4-fluoro-3-nitrobenzoic acid **13** through the *N,N′*-dicyclohexylcarbodiimide (DCC) and catalytic amount of 4-dimethylaminopyridine (DMAP) activation to afford the polymer immobilized *o*-fluoronitrobenzene **14** as yellow compounds in quantitative yields. However, we have observed that completion of the reaction was achieved in 1 day at room temperature condition. But with the application of sealed vessel microwave irradiation (80 °C, 2 bar), the same reaction completed in 20 minutes. After completion of the reaction time, the insoluble DCU was filtered off and purified by precipitating out the reaction mixtures with cold ether. The mechanism of the formation of compound 14 was explained in figure 1.24.

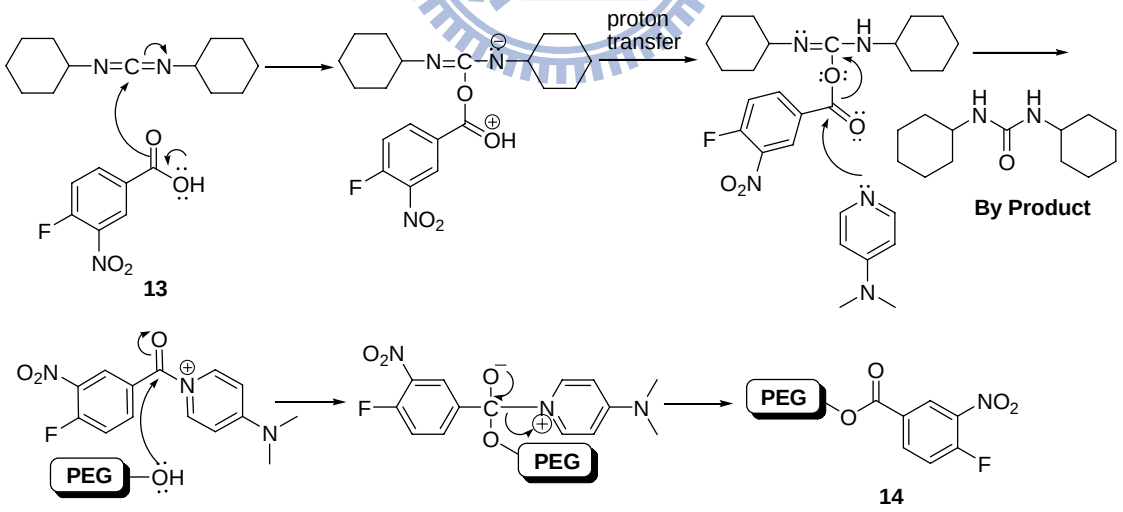


Figure 1.24. Mechanism of formation of compound **14** on soluble support

The first point of structural diversity was introduced by nucleophilic aromatic substitution (SnAr) of readily available primary amines with **14** via an *ipso*-fluoro displacement to give polymer bound nitroanilines **15**. NMR analysis of **15** showed complete conversion to **15** after a reaction time of 12 h at room temperature. The reaction proceeded efficiently with various amines without cleavage of the ester bond at the polymer attached site. With the application of microwave irradiation (100 °C, 2 bar) reduced the reaction time to 10 minutes. Purification was achieved by the precipitation with cold ether. Reduction of the aryl nitro group in the resulting polymer immobilized nitro derivative **15** was successfully accomplished with a suspension of Zn/$HCOONH_4$ in methanol to afford immobilized diamine **16** at room temperature for 20 minutes. Formation of the amine conjugates **16** was confirmed from change of yellow to blue color upon spotting on the TLC plate. Upon completion of the reaction, reaction mixtures were filtered through fritted funnel to get rid of the Zn. The reaction mixtures were evaporated and dichloromethane was added to salt out the ammonium formate to obtain the compound **16.** The exact mechanism reharding the formation of compound 16 was observed in figure 1.25.

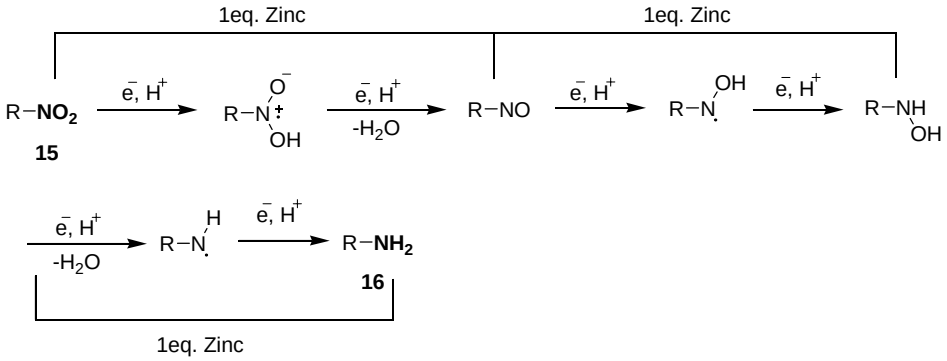


Figure 1.25. Mechanism of formation of compound **16** on soluble support.

For reaction purposes we have chooses the following amines as shown in figure 1.26.

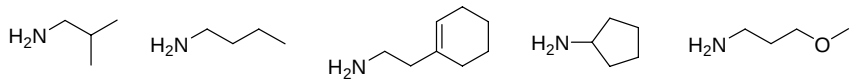


Figure 1.26. Set of amines required to introduce the first diversity (R_1) in the library.

In an effort to attain the target molecule, compound **16** was *N*-acylated at the primary amine functionality with 4-hydroxy-3-nitrobenzoic acid **17** via DCC activation as in scheme 17. But surprisingly DCC activation of amine functionality leads to complex reaction mixtures. Accordingly, we turned our attention to obtaine the anilide conjugates **18** by the condensation of acid **17** with PEG conjugates **16** through *in-situ* generated DCC/HOBT activated ester in *N,N'*-dimethylformamide in 12 hours reflux. However, the application of microwave irradiation under sealed vessel condition (90 °C, 5 bar) reduced the reaction time to 25 minutes. The mechanism of the reaction has been depicted in figure 1.27.

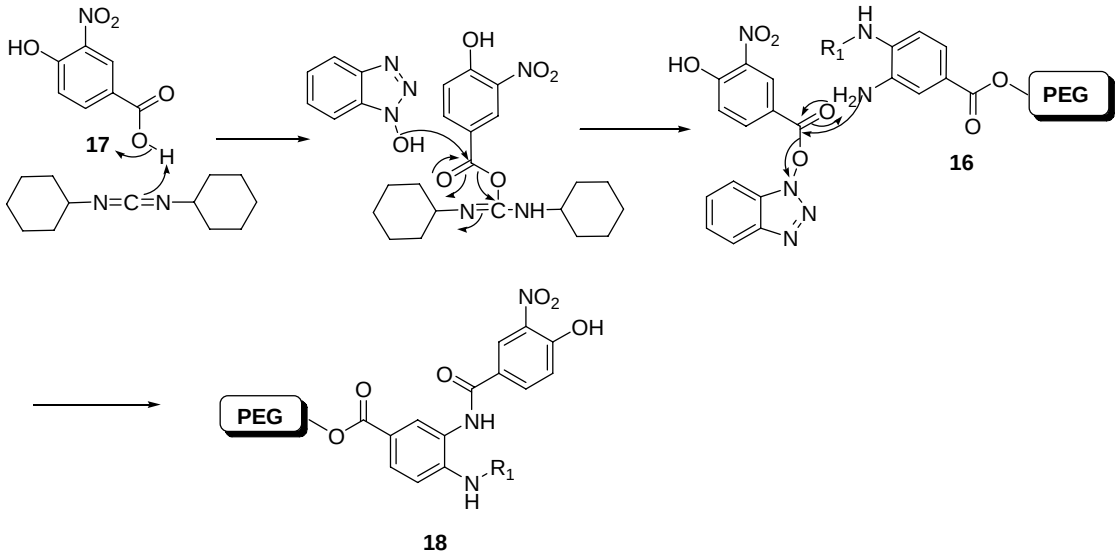
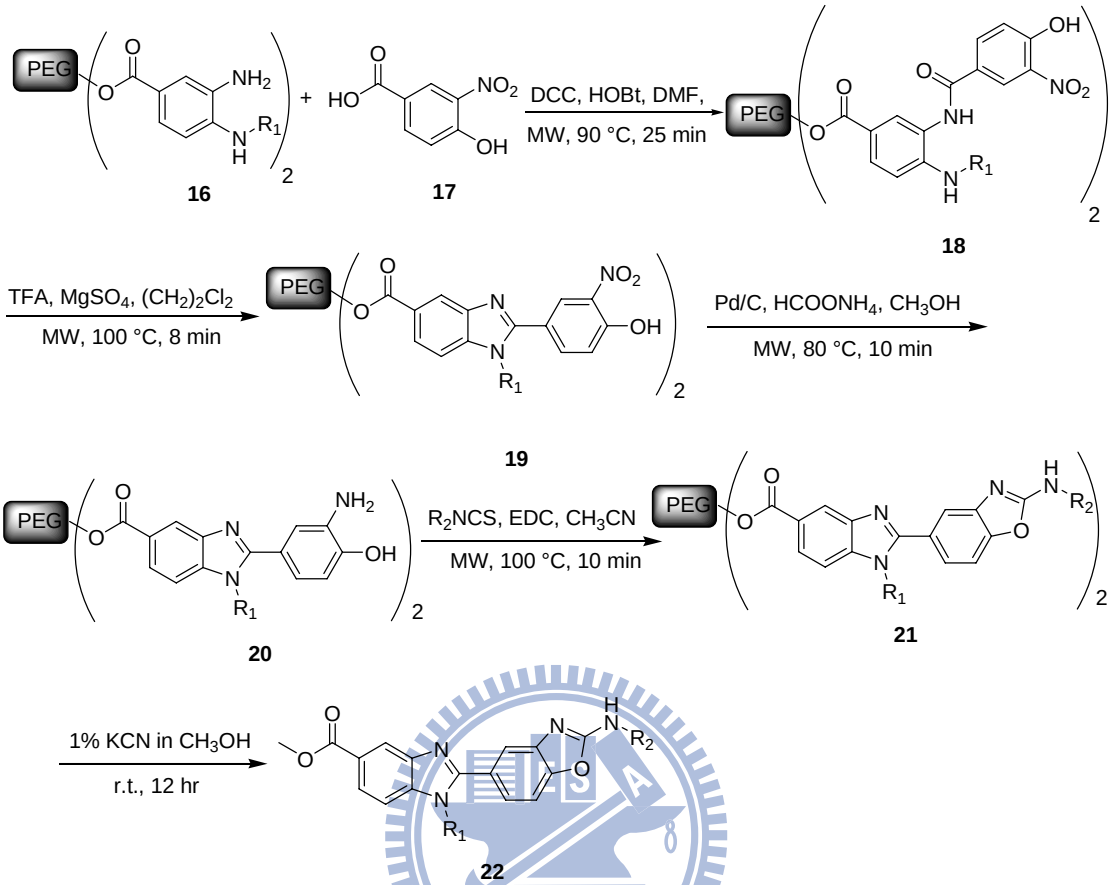


Figure 1.27. Mechanism for the formation of polymer conjugates **18.**

After completion of the reaction time, the reaction mixture was filtered through fritted funnel to separate the insoluble DCU and HOBt and the solvent was removed under reduced pressure. The reaction mixture was further purified and filtered by precipitation with cold ether which was subsequently dried. For the construction of benzimidazole ring, anilide conjugates **18** were subjected to acid catalyzed cyclisation in presence of 10 % trifluoroacetic acid in 1,2-dichloroethane under refluxing condition for 18 hours. The formation of the conjugate **19** was achieved by the intramolecular cyclization facilitated by the protonation of amide carbonyl by trifluoroacetic acid. However, it has been observed that addition of $MgSO_4$ facilitates cyclization by accelerating the removal of water and shortens the reaction time to 10 hours. In order to achieve the target compound quickly, we applied the microwave irradiation under sealed vessel condition (100 ^{o}C, 8 bar) at this stage, which further reduced the reaction time to 8 minutes.



Scheme 17. General strategy of microwave assisted synthesis of benzimidazolylbenzoxazoles.

After completion of the reaction, $MgSO_4$ was filtered off and the polymer conjugate was purified by precipitating out the reaction mixtures with excess of cold ether. For the reduction of nitro group of conjugate **19**, initially we attempt the strategy involving zinc dust in methanol buffered with ammonium formate. But this condition failed to reduce the nitro-group because of strong intramolecular hydrogen bonding between the nitro-group and hydroxyl group at *ortho* position. Hence we switch to another reducing condition involving 10% palladium on activated charcoal in methanol solution in presence of ammonium formate. Formation of the amine conjugates **20** was achieved

under refluxing condition for 2 hours. However, by the application of microwave irradiation at 80 °C, the desired conjugates **20** were obtained within 10 minutes. After completion, palladium and excess ammonium formate were removed by filtration. Amine conjugates **20** were obtained in pure form by further precipitation in cold ether.

Our main goal was the construction of benzoxazole ring in conjunction with introduction of additional sets of diversity. It has been realized that the elaboration of intermediate **20** to the desired core structure required one carbon electrophile, which could be fix through reaction with isothiocyantes as shown in figure 1.28. In an effort to mimic the bioactive compound as mentioned earlier in Figure 1.16, we decided to explore the building of benzoxazole ring with various isothiocyanates. Hence the amine conjugates **20** were condensed with selective isothiocyanates using *N*-3-(dimethylaminopropyl)-3-ethyl-carbodiimide as an activating agent in anhydrous CH_3CN under microwave irradiation at 100 °C (5 bar) to furnish the benzoxazole conjugate **21** in 10 minutes as shown in figure.

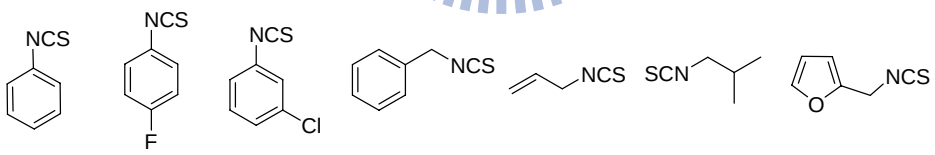


Figure 1.28. Set of isothiocyanates required to introduce the second diversities (R_2) in the library.

However, it has been observed that the same reaction required 10 hours under conventional refluxing condition, which in turn reflects the superiority of microwave irradiation. After completion of the reaction, the reaction mixture precipitated with cold ether and filtered through fritted funnel to obtain benzoxazole conjugate **21** in good

yields. The formation of the product involves the nucleophillic attack of amine group on the isothiocyanates moiety to form intermediate **a**. For mechanistic investigation, intermediate **a** was isolated before adding the coupling reagents and characterized (see Supporting Information). The involvement of *N*-3-(dimethylaminopropyl) -3-ethyl-carbodiimide as coupling agent promotes the further activation of thiocarbonyl (C=S) moiety of intermediate **a**; which after cyclization and electron reorganization generate the target compounds **21** as depicted in Figure 1.29.

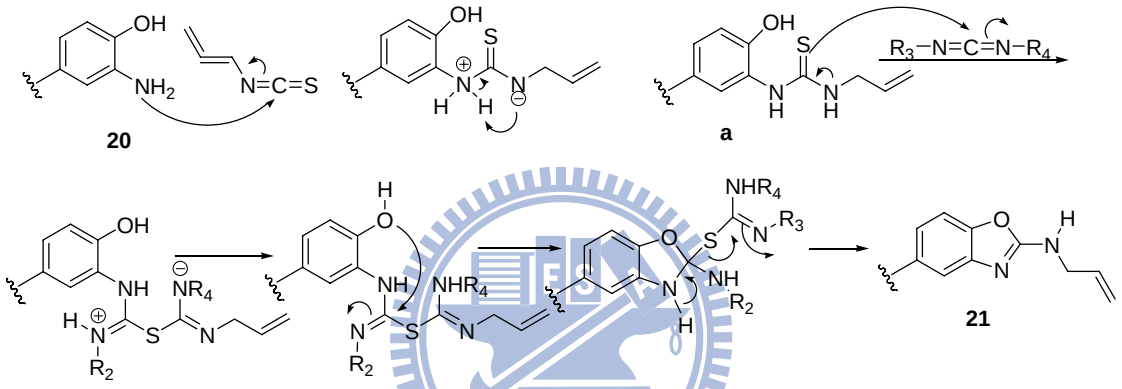
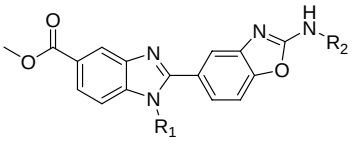


Figure 1.29. Plausible mechanism towards the formation of benzimidazolylbenzoxazoles.

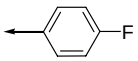
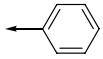
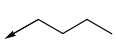
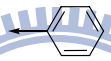
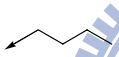
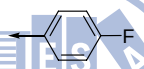
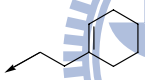
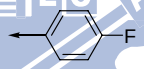
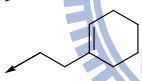
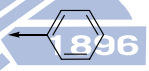
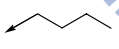
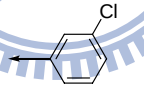
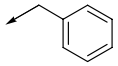
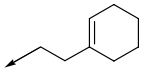
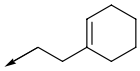
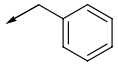
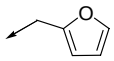
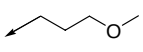
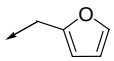
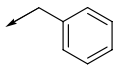
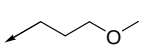
The benzimidazolylbenzoxazoles were finally cleaved from polymer support using 1 % solution of KCN in MeOH at room temperature within 12 hours. The reaction mixture was concentrated and polymer was precipitated by ether and removed by filtration. The filtrates were evaporated and subjected to HPLC analysis which indicated the 76-98 % crude purity of title compounds. Finally column chromatography purification afforded the benzimidazolylbenzoxazole derivatives **22** in good overall yields (Table 1). By utilizing the desired reaction sequence, we have synthesized various benzimidazolylbenzoxazole derivatives **22** with two diverse substitutions as shown in

Table 1. The data exhibit that the after five-step syntheses sequence benzimidazolylbenzoxazole derivatives were rapidly synthesized in excellent overall yields.

Table 1. Synthesized benzo[*d*]oxazol-5-yl-1*H*-benzo[*d*]imidazole-5-carboxylates **22**.



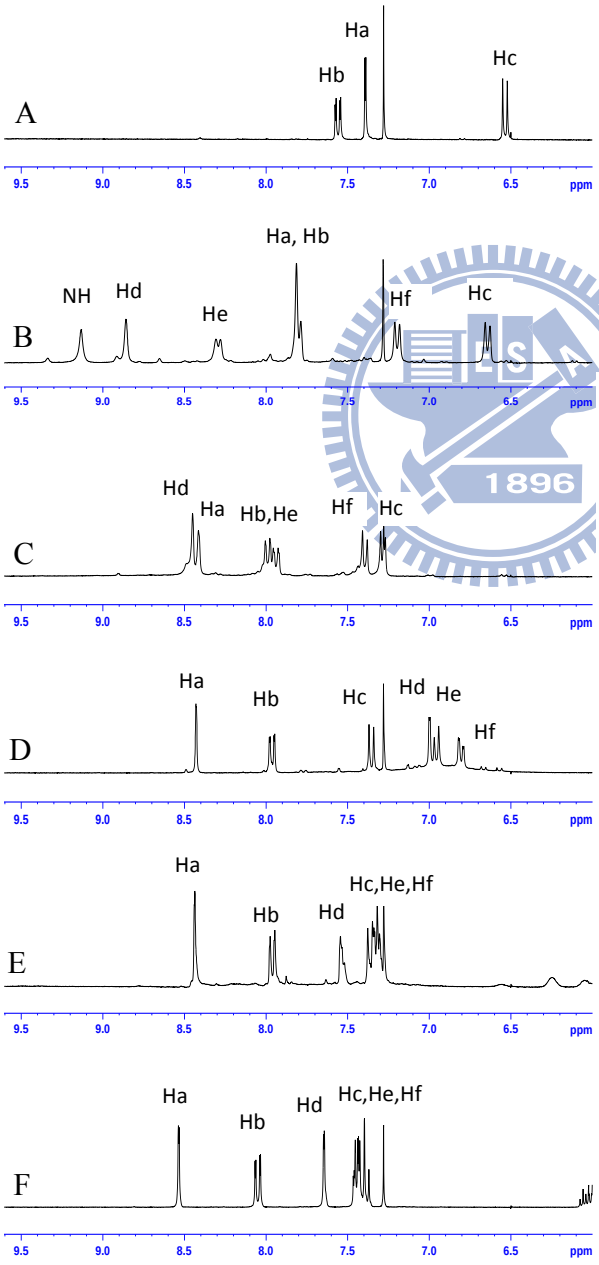
22

Compounds	R_1	R_2	Yield[a](%)	Purity[b](%)	LRMS[c]
22a			90	88	459
22b			85	89	441
22c			93	95	441
22d			91	97	459
22e			82	89	511
22f			92	96	493
22g			95	94	474
22h			82	80	405
22i			81	82	455
22j			89	94	457
22k			85	80	507
22l			95	98	457
22m			77	76	461
22n			79	77	467
22o			87	86	437
22p			96	98	417

[a]Determined based on the weight of crude samples (%). [b]Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%). [c]LRMS were detected with ESI ionization source.

Monitoring the progress of reaction on polymer support throughout a multistep synthetic sequence is a relatively difficult task. Typical methods such as thin-layer chromatography (TLC), GC and mass spectrometry (MS) are not providing substantial information for polymer-supported methods. However, nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopy is particularly useful strategies.[85] A significant advantage of NMR protocol in soluble polymer support is that it is nondestructive as compared to the customary *"cleave and characterize*" techniques used for solid supported synthesis. The soluble polymer support like polyethylene glycol is serves as a true alternative concerning its solubility in various organic solvents. Hence the monitoring of the present synthetic scheme was accomplished by ^{1}H NMR spectroscopy (Figure 1.30). In the spectra A, the most downfield three peaks belong to the three aromatic protons (Ha, Hb and Hc). Formation of the anilide conjugates were confirmed from the appearance of NH signals at 9.18 ppm, whereas the additional signals in aromatic region at 8.80, 8.31 and 7.24 ppm corresponds to Hd, He and Hf respectively were emerged from 4-hydroxy-3-nitrobenzoic acid in spectra B. Establishment of benzimidazole ring was evident from the disappearance of NH proton in spectra C and Ha, Hb and Hc protons were shifted to downfield due to electron withdrawing nature of benzimidazole derivatives. Reduction of nitro group was noticed from substantial shifting of Hd and He protons to upfield region and appeared at 7.00 and 6.81 ppm in spectra D. Final cyclization with allylisothicyanate to **21j** generated the electron withdrawing benzoxazole derivatives which was observed in spectra E. Due to the

electron withdrawing effect, the previously appeared upfield protons Hd and He at 7.0 and 6.81 ppm become deshielded and moved to 7.45 and 7.32 ppm. Finally, the cleavage of product from PEG support was confirmed by observing the absence of set of signals around 4.4 ppm due to PEG moiety along with slight shifting of all aromatic protons to downfield region. Other characteristic signals of different protons are in agreement with structure **22** in spectra F.



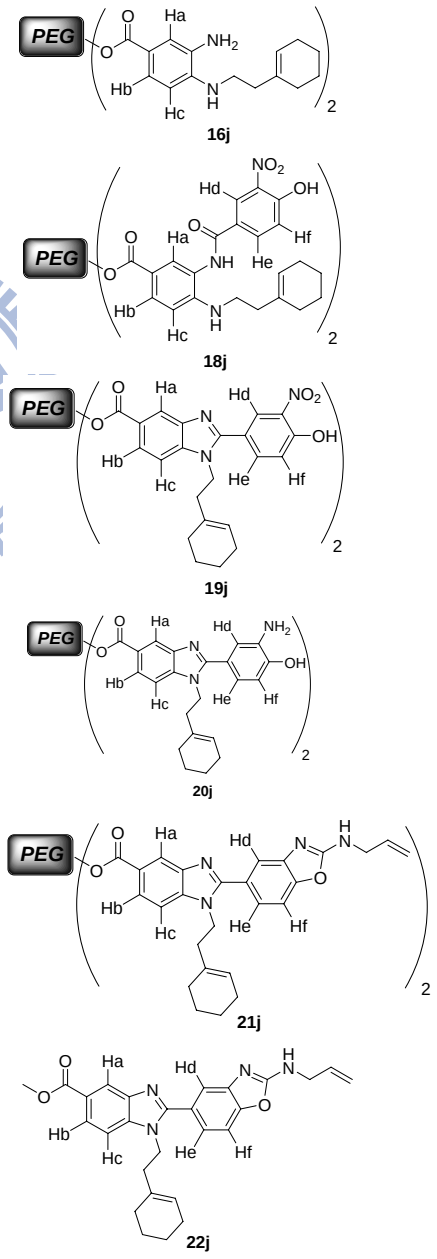


Figure 1.30. Stepwise monitoring of benzimidazolylbenzoxazoles by ^{1}H NMR spectroscopy.

Furthermore, additionally the structure of final compounds was unambiguously confirmed by the X-ray crystallographic study. Figure 1.31 depicts the ORTEP diagram of compound **22n** (see supporting information for the crystallographic data). The single crystal X-ray analysis of compound **22n** indicates that two rings of benzimidazole and benzoxazole moieties are in anti-periplanar orientation.

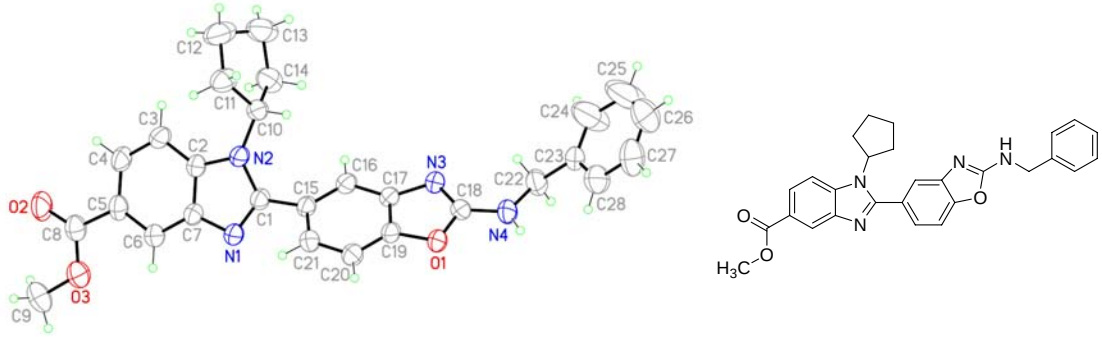


Figure 1.31. ORTEP diagram of compound **22n**.

Section B

1.2. Quinoxalinones- Importance and Synthesis

Quinoxalines[86] are privileged nitrogen heterocycles known for a wide range of biological activities, which have been found in clinically accepted drugs as well. Several derivatives of quinoxaline (e.g., HBY-097 and S-2720) display interesting activity against HIV as nonnucleosidic inhibitors of the reverse transcriptase (Figure 1.31, A).[87] Quinoxalinones have been reported as diazepine receptor antagonists[88] (Figure 1.31, B) and antithrombotic agents.[89] Their potential to act as inhibitors of blood coagulation factor Xa[90] and anti-diabetic glycogen phosphorylase (Figure 1.31, C)[91], have also been realized recently. Indeed, acting as glutamate receptors[92] or serine protease inhibitors,[93] quinoxalin-2-ones were proven active for treating diseases of the central nervous system like Huntington, or Parkinson and Alzheimer diseases. Indole-substituted quinoxalinone was also proven active as vascular endothelial growth factor (VEGF) inhibitor.[94] Receptor binding studies on human adenosine has revealed the antagonist property of the 2-2 isomers at the nanomolar level (Figure 1.31, D).[95,96] During the study of bi- and terbenzimidazoles, it was found that 6-2 linkage of the quinoxaline and the corresponding 2,3-dione moiety greatly enhanced the DNA topoisomerase I activity (Figure 1.31, E).[97]

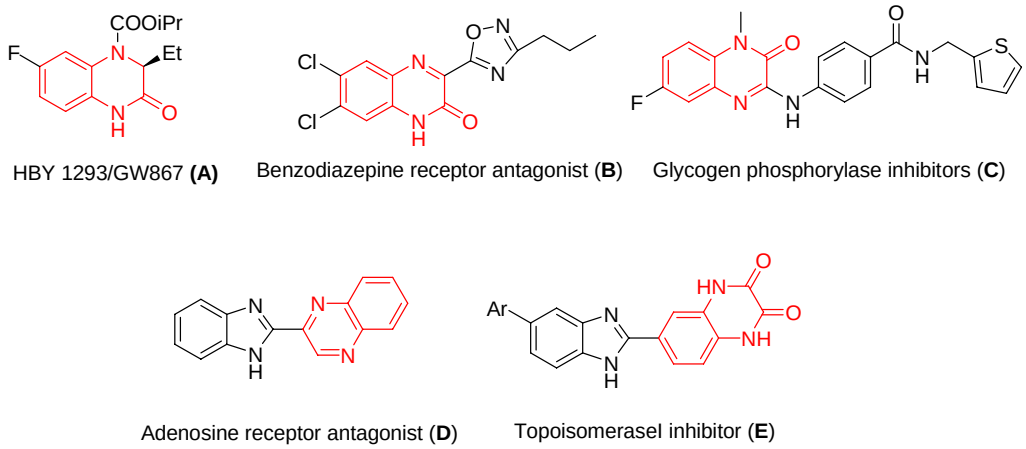


Figure 1.31. Biologically active quinoxalinone derivatives

1.21. Chemical Synthesis of quinoxalinone derivatives

In view of their wide range of biological activities, quinoxalinones have been synthetic targets for the solid phase,[98] liquid phase[99] and microwave assisted Ugi coupling methods.[100] Here I am discussing some of the relevant methods for the synthesis of quinoxalinones derivatives.

In 1975, Suschitzky *et. al*. have first synthesized the quinoxalinone derivatives by reacting with *o*-phenylene diamine derivative with dimethyl acetylenedicarboxylate in choloroform solution at 0 ^{O}C temperature which has been depicted in figure 1.32.[101]

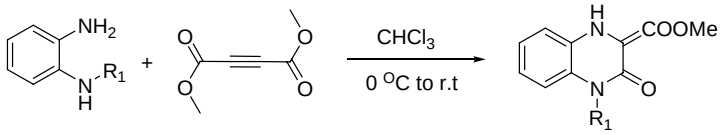


Figure 1.32. Suschitzky's method of quinoxalinone synthesis

In 2000, Krachňak *et. al.* have used the solid phase methodology for the synthesis of quinoxalinone deravatives. A polystyrene resin functionalized with aldehyde was underwent alkylation by amino acid methyl esters, and then the resin bound secondary amines were reacted with *o*-fluoronitrobenzenes. The resulting *o*-nitroanilines were reduced by tin chloride to the *o*-phenylenediamines, which spontaneously cyclized. The amide nitrogen of the dihydroquinoxalinones obtained was alkylated by alkyl halides. Furthermore it has been observed that after cleavage from the polymeric support, the dihydroquinoxalinones were air oxidized to generate the quinoxalinones as depicted in figure 1.33.[102]

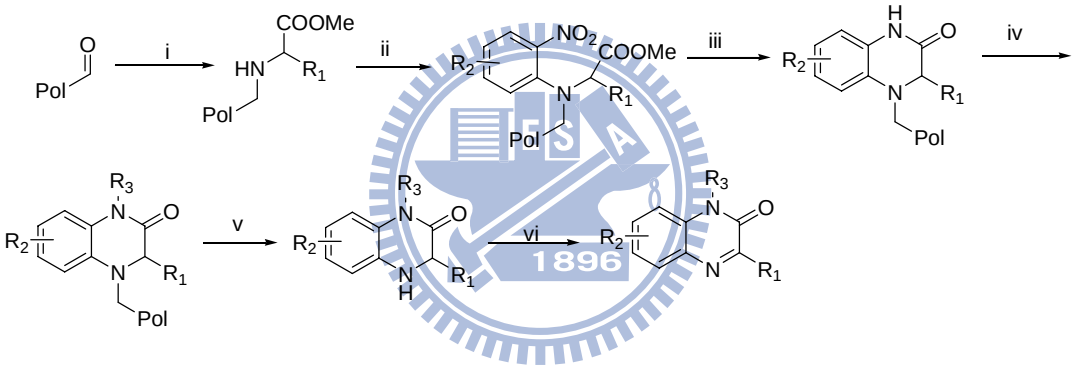


Figure 1.33. Traceless synthesis of quinoxalinones. Reagents: (i) amino acid ester, $NaB(AcO)_3H$, DMF/AcOH; (ii) *o*-fluoronitrobenzene, DMSO, 75 °C, 1–3 days, (iii) $SnCl_2.2H_2O$, NMP, r.t., 2 h. (iv) 2-*t*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP), alkyl halide, DMF, r.t., 2 h; (v) TFA or gaseous HCl or gaseous HF, r.t., 2 h; (vi) air oxidation, MeOH, r.t., overnight.

In 2005, Dudash, Jr *et. al.* have used the commercially available N-methyl-1,2-diaminobenzene was treated with ethyl chlorooxoacetate to give the quinoxalinone derivatives as drawn in figure 1.34.[103]

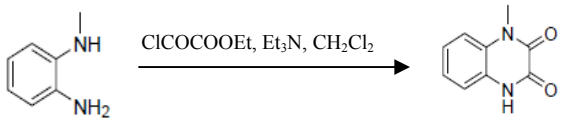


Figure 1.34. Synthetic metods for quinoxalinone scaffolds

In 2006, Mahaney *et. al.* have synthesized the 3,4-dihydroquinoxalin-2(1H)-one as a pure enantiomer in two steps starting with an appropriately substituted ortho-fluoronitrobenzene, compound and either (R)- or (S)-2-aminobutyric acid. Nucleophilic aromatic substitution under basic conditions afforded the 2-[(2-nitrophenyl) amnio]-butanoic acid, reduction of which via hydrogenation facilitated spontaneous ring cyclization to form the optically pure 3,4-dihydroquinoxalin-2(1H)-one as shown in figure 1.35.[104]

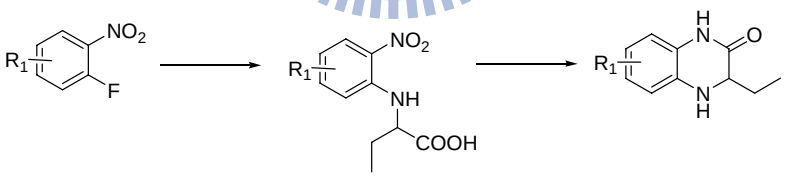


Figure 1.35. Synthesis of 3,4-dihydroquinoxalin- 2(1H)-ones. Reagents and conditions: (a) (R)- or (S)-2-aminobutyric acid, K_2CO_3, DMF, 100 ^{O} C; (b) 10 % Pd/C, H_2 (50 psi), EtOH; 49–53 % yield for two steps;

In 2006, Liu et. al have synthesized simultaneously quinoxalinone and benzimidazole scaffold library by using the solid-phase "split and-pool" approach as drawn in figure 1.36.[105]

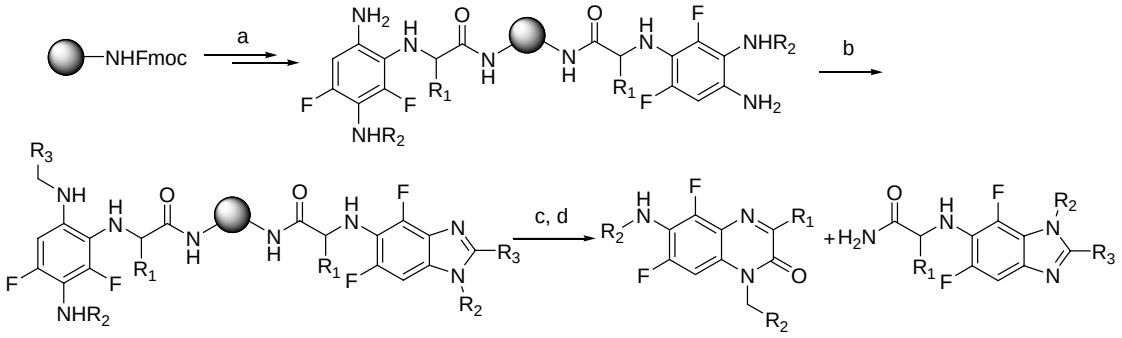
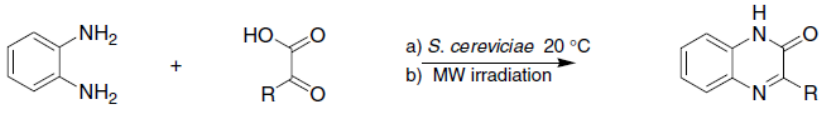


Figure 1.36. Reagents: (a) usual methods (b) R_3CHO (10 equiv), 5 % AcOH/DMF, r.t., 20 h; (c) $NaBH_3CN$ (20 equiv), 5 % AcOH/DMF, r.t., 14 h; (d) 95 % TFA/CH_2Cl_2, r.t., 1h.

In 2007, Moglioni *et. al.* have synthesized the quinoxalinone derivatives by heating *o*-phenylenediamines with a-ketoacids in 50–65% over all yields. Furthermore, it was necessary to vary the conditions of the reactions (e.g., temperature, reaction solvent, catalysis) for each particular synthesis as shown in the Figure 1.37.[106] Most importantly they have used the biocalysis as well as microwave irradiation for the reaction.



R= $-CH_3$ (**a**); $-C_2H_5$ (**b**); $-(CH_2)_2-CO_2H$ (**c**); $-CH_2-CO_2H$ (**d**); $-CH_2-C_6H_5$ (**e**); -OH (**f**)

Figure 1.37. Moglioni's methods of quinoxalinones synthesis

In 2008, Bi *et. al*. have discovered the synthetic routes to quinoxalinone derivatives via microwave-mediated smiles rearrangement as shown in the figure 1.38.[107]

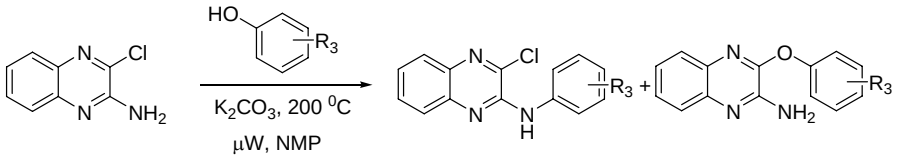


Figure 1.38. Bi's methods for quinoxalinone synthesis.

In 2008, Mamedov et. al. have found the new versatile one-step method for the synthesis of benzimidazoles from quinoxalinones and arylenediamines via a novel rearrangement in presence of acetic acid reagent as drawn in figure 1.39.[108]

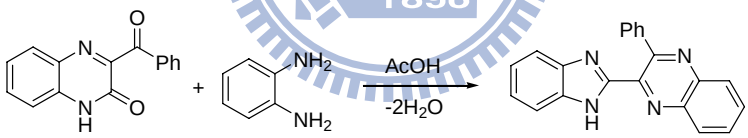


Figure 1.39. Mamedov methods of quinoxaline synthesis

In 2010, Ajani et. al. have synthesized the same scaffolds by the reaction between *o* phenylenediamines with oxalic acid which underwent further rearrangement to generate the scaffolds as predicted in figure 1.40.[109]

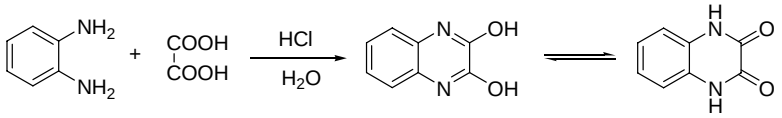


Figure 1.40. Ajani's way of quinoxalinone synthesis

In 2010, it has been observed that the lewis acid has also been used for the construction of quinoxalinone derivatives. Venable *et. al.* have used the ytterbium triflate $Yb(OTf)_3$ as lewis acid for the construction of the said skeleton. They have reacted the *o*-phenylenediamines with trimethoxy acetate using toluene as solvent under refluxing conditions as proposed in figure 1.41.[110]

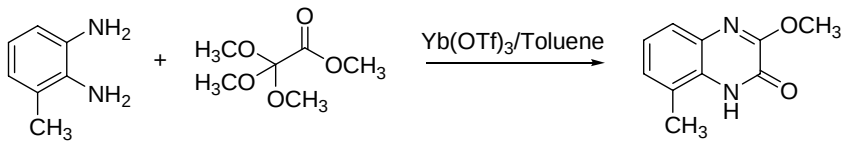


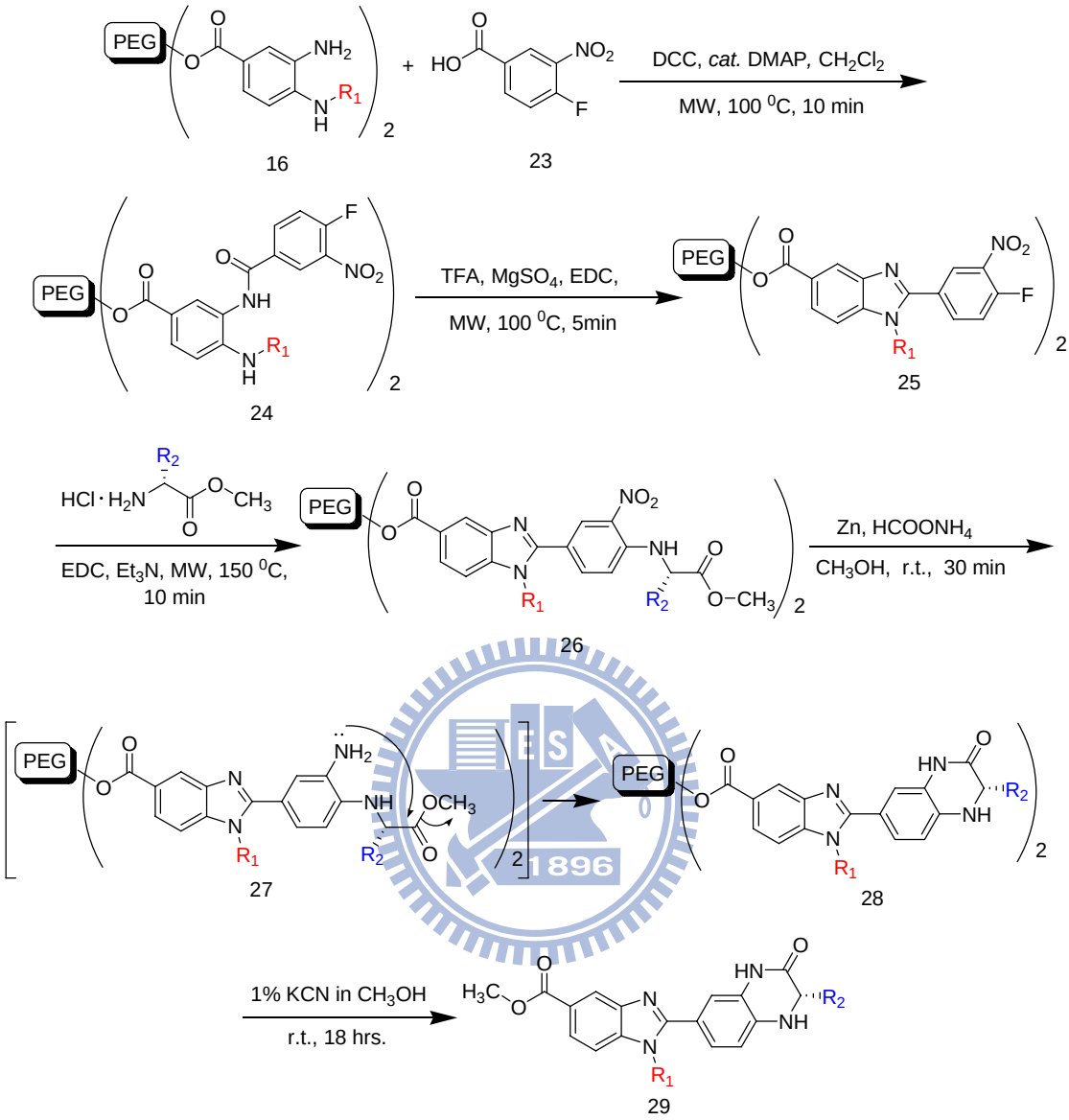
Figure 1.41. Lewis acid catalysed quinoxalinone ring formation

1.22. Results and Discussion

Ortho-diamino ester conjugates **16** (Scheme 16) which are the initiators for the present strategy with a built-in structural diversityhas been used for the present synthetic scheme. In order to introduce the second diversity, compound **16** was continuously reacted with 4-fluoro-3-nitro-benzoic acid **23** as shown in scheme 18. Anilide conjugates **24** were obtained by the condensation of acid **23** with the PEG conjugates **16** via the *in-situ* generated DCC activated ester in refluxing dichloromethane. Required refluxing time of 24 hours for this step was reduced to 40 minutes using domestic MW irradiation. When microwave irradiation under sealed vessel condition at 100 ^{0}C (8 bar) was applied to deliver anilide conjugates **24**, it took only 10 minutes. The obtained anilide conjugates **24** were converted into benzimidazoles **25** by an intramolecular ring closure through the nucleophilic attack of the secondary amine on to the amide carbonyl which was induced

by a mild acid (10% TFA). Addition of anhydrous magnesium sulphate in this transformation brought down the reaction time, by facilitating the removal of water during this step, which needed 15 h under refluxing conditions in dichloroethane. The time for the formation of benzimidazole was reduced to 15 minutes by domestic MW reactor. However, the reaction time was reduced to 5 minutes in sealed vessel MW conditions (5 bar, 100 ^{0}C). Magnesium sulphate was filtered off and the polymer conjugate **25** was purified by precipitating out the reaction mixtures with excess of cold ether.

SCHEME 18. Microwave assisted synthesis of benzoimidazolylquinoxalinones.



Polymer bound benzimidazoles **25** were found to contaminate no previous intermediates, and was used as such for the further steps in the present sequence. In further steps, reactivity of *ortho*-nitro fluoro groups in **25** was used in a cascaded manner. Introduction of the amino functional group and the chirality in the quinoxaline moiety was achieved by the use of optically active alpha-amino esters. The *ipso*-fluoro displacement with various chiral amino esters on the conjugates **25** was complete in 20 hrs in refluxing dichloroethane. The domestic MW irradiation brought down the reaction time to 40

minutes. We also have found that application of focused MW reactor at 150 ^{0}C (7 bar) drove the reaction to completion in 10 minutes (Table 2). The resulting *ortho*-nitro aniline conjugates 2**6** were yellow in colour and were purified by precipitation with cold ether, washing the solid with excess ether for purification and vacuum dried prior to the next step. Only step that did not warrant the application of MW was the neutral reduction of nitro group, which was effected by using zinc dust and ammonium formate in methanol at room temperature in 30 minutes. The intended reduction did result in the amine conjugate **27**. However, neutral conditions during the reduction in methanol did not result in the protonation of the amines. It was followed by nucleophilic attack of the *in-situ* generated amines on the ester carbonyl leading to the formation of quinoxalinone conjugates **28**. The aminolysis of nonactivated ester usually occurs under harsh conditions. In some cases, through the assistance of microwave irradiation or directly refluxing reaction mixtures in solvent-free conditions, aminolysis of nonactivated esters are much easily to proceed.[111] Spontaneous in situ cyclization from conjugates **27** to **28** involving the facile aminolysis of methyl esters at ambient temperature is successful to generate lactam ring of quinoxalinone.[112] The presently observed on-support cyclization is in accordance with previously reports on the synthesis of biologically active compound on solid phase support.[113] Finally cleavage of the soluble polymer support was achieved using 1% KCN in methanol at room temperature to obtain polymer free benzimidazolyl quinoxalinones **29** in good to excellent yields.

TABLE 2. Comparison of microwave and conventional heating for the coupling step, cyclization and S_NAr reactions

Entry	Substrates	Products	Optimized reaction conditions		
			time[a] (h)	time[b] (min)	time[c] (min)
1	16	24	24	40	10
2	24	25	15	20	5
3	25	26	20	40	10

[a] Reflux conditions. [b] Domestic microwave oven. [c] Biotage initiator

Normally for monitoring the progress of organic reaction on solid phase support by regular proton NMR is very difficult because of the insolubility of conjugated material in solvent but it is possible for the compounds attached with polyethylene glycol which itself act as soluble polymer support. Amenability of the present synthetic sequence to NMR monitoring reaction progress has been demonstrated in Figure 1.42. Formation of the anilides **24i** from conjugates **16i** is supported by the appearance of the low field NH proton around 9.2 ppm, which is also characterized by the presence of signals at 8.8 and 8.5 ppm (Spectrum B) due to the aromatic protons in the *ortho*-nitro fluoro moiety. Benzimidazole conjugates **25i** indicates the absence of the low field NH proton at 9.2 ppm, whereas the aromatic protons (Spectrum C) were shifted to the downfield due to the amide NH and secondary amine were converted into more electron withdrawing benzimidazole derivative. Reaction with amino acid esters results in the substitution of the electron withdrawing fluorine, which is indicated by the appearance of an upfield signal at 6.8 ppm (Spectrum D) of the proton attached to carbon atom near to the fluorine atom. Cyclization to the quinoxalinone conjugates **28** was only observed after detachment of the product from polymeric support which showed the absence of the low field triplet

around 4.4 ppm due to PEG. Observed signals characteristic of different protons are in agreement with structures **29i** (Figure 1.42. Spectrum E).

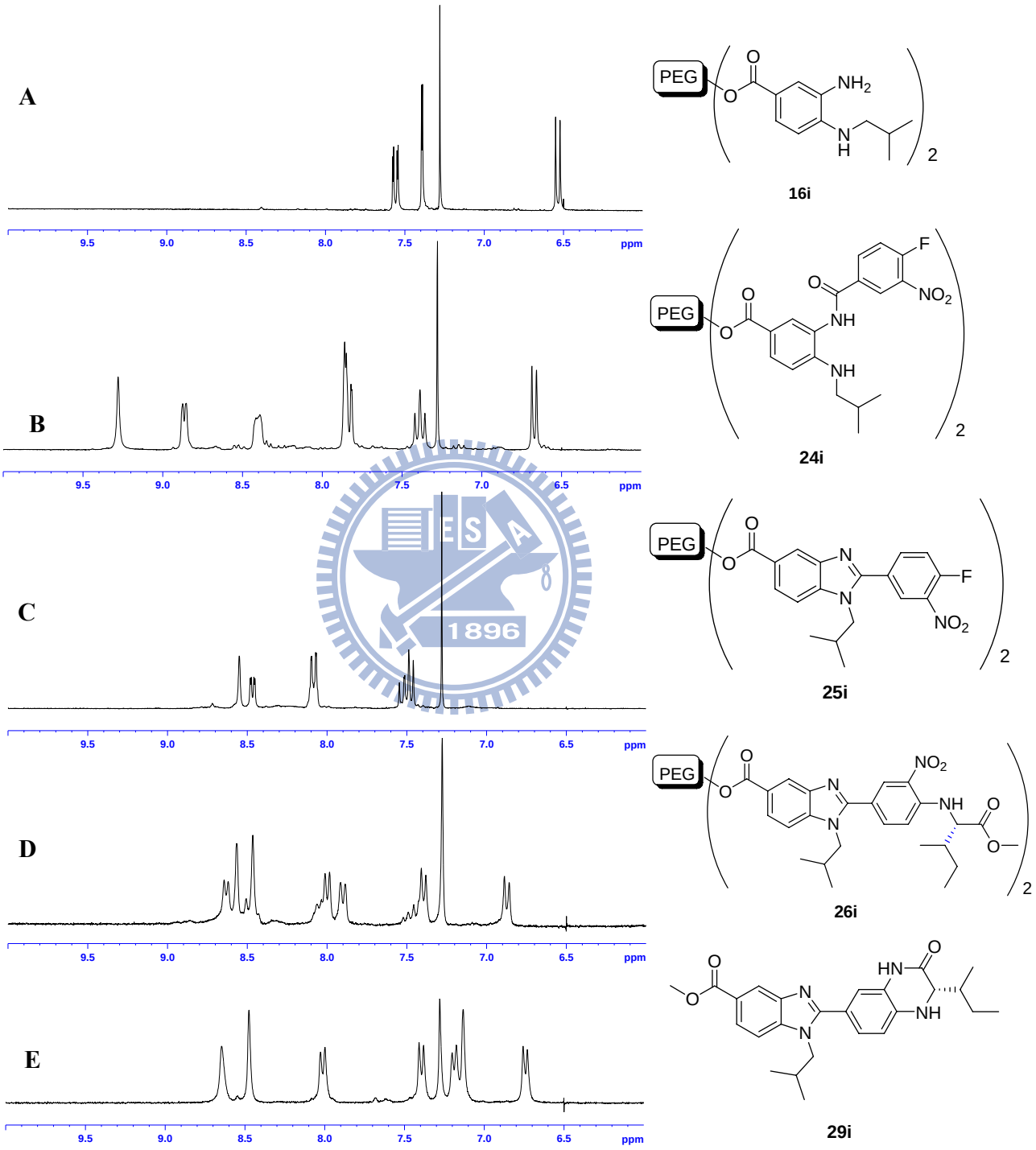
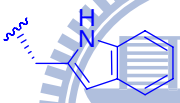
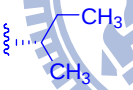


FIGURE 1.42. [1]H-NMR monitoring reactions of benzimidazolyl quinoxalinone

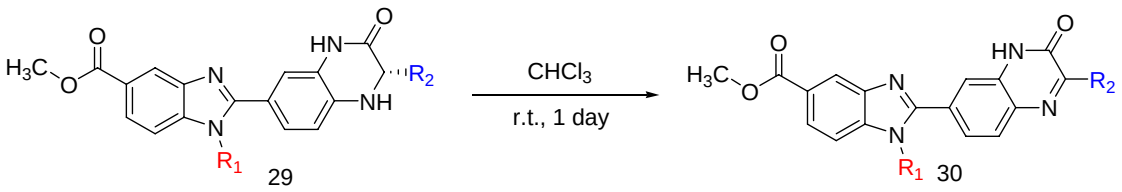
Chiral integrity of the conjugates **26** and **28** during reductive cyclization could not be monitored due to the presence of the macromolecular support. Chiral HPLC analysis of **29b** obtained after the final cleavage showed about 10 % racemization (80 % e.e.) High enantiomeric excess of final cleaved compounds were observed in majority of the cases, showing practically a very insignificant loss of chirality, during the three steps reaction on the polymer support in MW harsh conditions (Table 3).

TABLE 3. Microwave Assisted Liquid Phase Synthesis of Optically Active Benzimidazolylquinoxalinone Libraries 29(h-m)

Entry	R_1	R_2	m/z	Purity[a]	Yield[b]	ee[c]
29h			507	86	91	82
29i			434	95	70	97
29j			392	84	86	99
29k			420	89	76	95
29l			404	87	76	98
29m			432	93	81	80

[a] Determined by HPLC analysis (UV detection at 254 nm of the crude product (%); [b] Determined based on the weight of crude samples (%); [c] Determined by HPLC on chiral DIACEL CHIRACEL OD using h-hexane/2-prpoanol as solvents.

Scheme 19. Oxidation of Compounds 29 to 30 after Cleavage



To this end, no any side products arising from the oxidation of the 3,4 carbon/nitrogen bond were found when polymer free compounds **29** were released, as predicted from an acid-free, concurrent self-cyclization step (**26** to **27**). Ito *et al* [28] have reported that acid treatment of quinoxalinones causes in oxidation of the 3,4 carbon/nitrogen bond. We did observe the compounds **29** slowly lost their chirality to the compound **30** when they were stirred in acidic chloroform solution in 24 h at room temperature (Scheme 19). However when the compounds **29** containing substituents as flexible aliphatic chain 29i-m stirred in chloroform solution underwent loss of chirality to form compound 30 where as it was difficult for aromatic substituents (Scheme 19).

Section C

1.3. Benzazepine, its importance and synthesis

The 1-benzazepine moiety is very important heterocycles and more frequently found in molecules of pharmaceutical interest.[114] It has observed that the vasopressin is an antidiuretic hormone, released from the posterior pituitary either in response to increased plasma osmolarity detected by brain osmoreceptors or decreased blood volume and blood pressure sensed by low pressure volume receptors and arterial baroreceptors.[115] The hormone exerts its action through well defined receptor subtypes: vascular Via and renal epithelial V2 receptors. One of the key roles of arginine vasopressin (AVP) is the control of salt (NaCl) balances. The blockade of V2 receptors may be useful in treating diseases characterized by excess renal absorption of free water. Thus V2 antagonists may correct the fluid retention in congestive heart failure, liver cirrhosis, nephrotic syndrome, CNS injuries, lung disease and hyponatremia. Thus it has been observed that structure 5-fluoro-2-methyl-N-[4-(5H-pyrrolo[2,1-e][1,4] benzodiazepin-10(llH)-ylcarbonyl)-3-chlorophenyl] benzamide **1** (VPA-985), which is currently undergoing clinical trials as a AVP antagonists. The benzazepine derivative, 5-dimethylamino-1-[4-(2 methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-1-benzazepine (OPC-31260) **2**,[116] is an orally effective, nonpeptide vasopressin V2 receptor antagonist and is now undergoing clinical trials as a promising aquaretic agent. However the most important activity concerns with the antiarrhythmic activity which has been demonstrated in tetrahydro compounds such as **3**[117] as observed in figure 1.43.

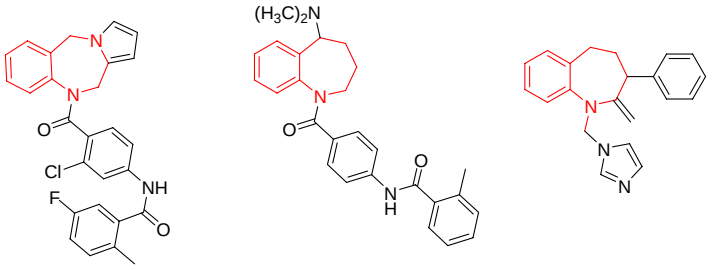


Figure 1.43. Biologically relevant heterocycles containing 1-benzazepine moiety

Although there are scattered accounts of 3*H*-1-benzazepines their syntheses are laborious and their chemistry has not been well studied.

1.31. Chemical methods of Benzazepine synthesis

In 1988, Brook *et. al*. used the pentafluoroaniline reacted with acetophenone in tetralin at reflux temperature in the presence of anhydrous zinc chloride to give the 3-azepine derivatives as 6,7,8,9-tetrafluoro-2,4,-diphenyl-3-1*H*-benzazepine derivatives. The self-condensation product of acetophenone, β-benzoyl-α-methylstyrene ("dypnone"), is an intermediate in the ring-forming reactions as shown in the figure 1.44.[118]

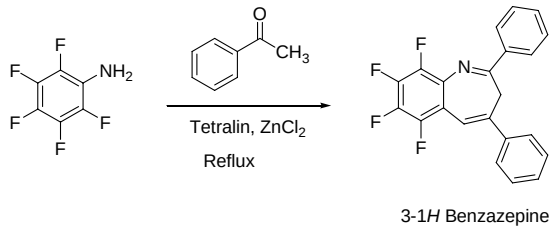


Figure 1.44. Brooker way to 3*H*-1-benzazepine derivatives synthesis.

However in 1996, Toupet *et. al.*[119a] had reported the synthesis of 5H-l-benzazepines **c** by electrochemical oxidation of 2,5-dihydro-IH-l-benzazepines **a**. In this report they also describe the preparation of 3H-l-benzazepines **d**, isomers of **c** , by two synthetic pathways The first is the chemical oxidation of the 2,5- and 2,3-dihydro-l*H*-l-benzazepines **a** and **b**, the second the rearrangement of 5H-l-benzazepines **c** as shown in figure 1.45.

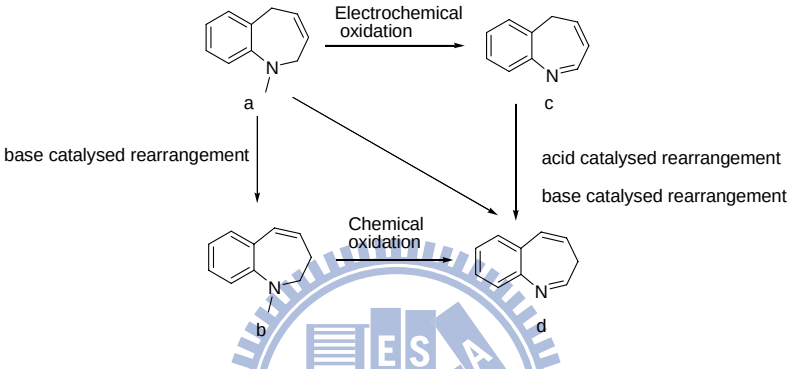


Figure 1.45. Toupet methods of 3*H*-1-benzazepine derivatives synthesis

In 2007, Ramig *et. al.*[119b] described a new synthesis of 3*H*-1-benzazepines using acetophenone heated with one equivalent of 2-fluoroaniline, with azeotropic removal of water, forming the imine **e**. Further heating yielded 2,4-diphenyl-3*H*-1-benzazepine **f** as drawn in figure 1.46.

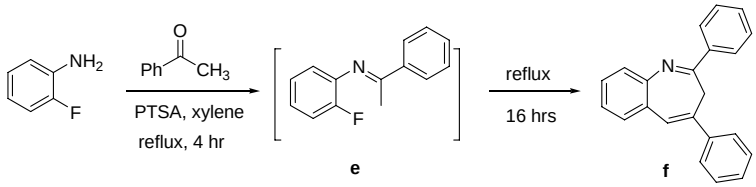


Figure 1.46. Ramig's methods of 3*H*-1-benzazepine synthesis

Similarly in 2007, Batra *et. al.*[119c] have used the $SnCl_2$-mediated reduction of nitro groups in 2-nitro-4-(2-nitrobenzylidene)alkanoates and 4-nitro-2-(2-nitroalkylidene)alkanoates allows the facile synthesis of substituted 1*H*-1-benzazepines and 3*H*-1-benzazepines. This reaction proceeds via S_N2 reaction of ethyl nitroacetate and nitroethane with the acetyl derivatives of Baylis–Hillman adducts deriving from 2-nitro-substituted benzaldehydes. However, while during the reaction an unprecedented rearrangement of an alkyl 1*H*benzazepine-2-carboxylate to a substituted isoquinoline has been observed as shown in figure 1.47.

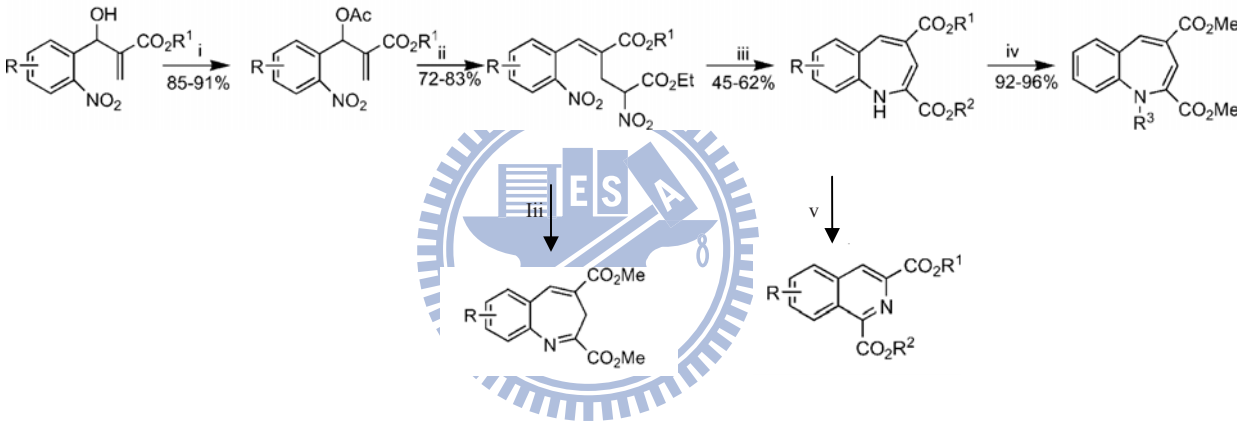
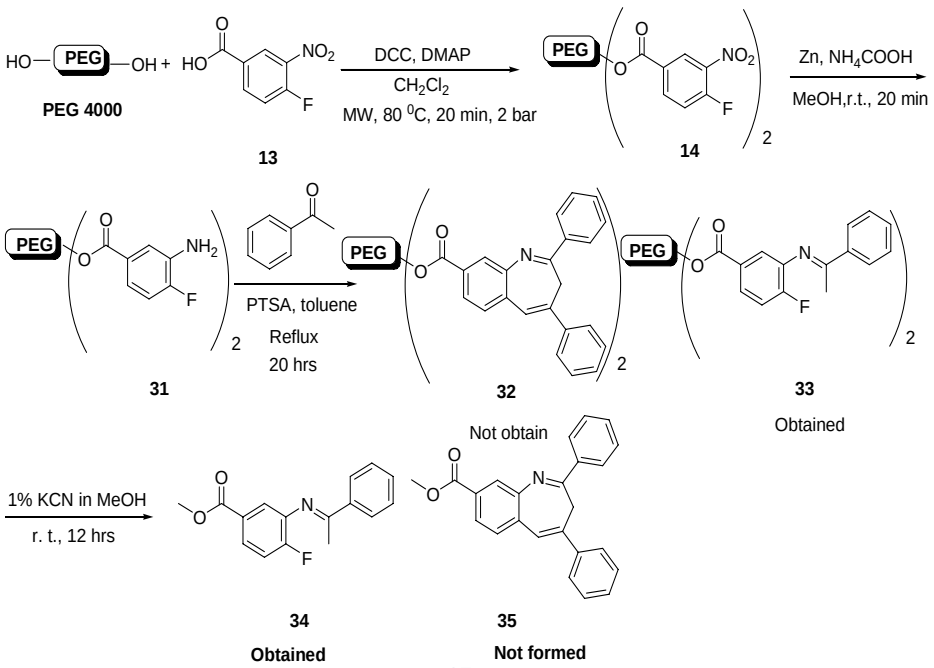


Figure 1.47. Reagents and conditions: i) AcCl, pyridine, CH_2Cl_2, room temp., 3 h; ii) $NO_2CH_2CO_2Et$, K_2CO_3, DMF, 3 h; iii) $SnCl_2{\cdot}2H_2O$, MeOH or EtOH, N_2, reflux, 2 h; iv) R_3COCl (R_3 = Me, Ph), Et_3N, CH_2Cl_2, room temp., 3 h; v) silica gel (60–120 mesh), room temp., 24 h or silica gel (60–120 mesh), hν, room temp., 6 h.

1.32. Results & Discussions

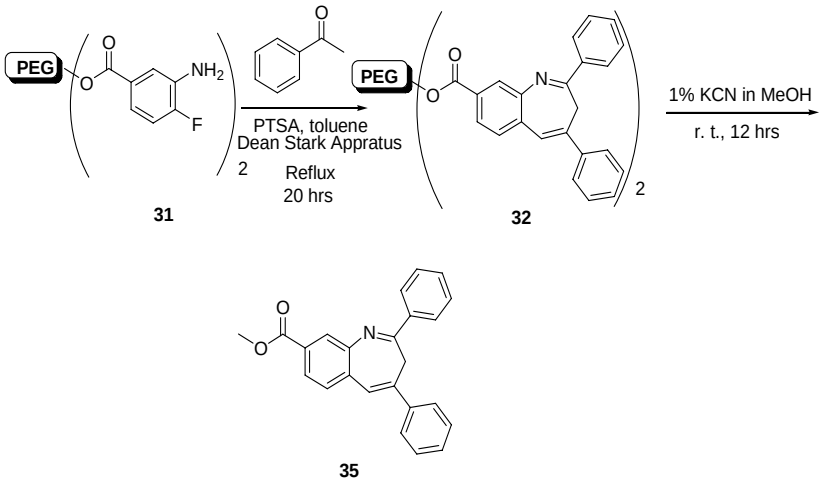
Initially, using our own synthetic pathway we have developed the polymer immobilized 4-Fluoro-3-nitro benzoic acid as the compound **14** by our group earlier. Reduction of the

aryl nitro group in the resulting polymer immobilized nitro derivative **14** was successfully accomplished with a suspension of Zn/$HCOONH_4$ in methanol to afford immobilized amine **31** at room temperature for 20 minutes. Formation of the amine conjugates **31** was confirmed from change of yellow to blue color upon spotting on the TLC plate. Upon completion of the reaction, reaction mixtures were filtered through fritted funnel to get rid of the Zn. The reaction mixtures were evaporated and dichloromethane was added to salt out the ammonium formate to obtain the compound **31.** NMR analysis of **31** showed complete conversion to **31** after a reaction time of 20 minutes at room temperature. In an effort to attain the target molecule, compound **31** was reacted with acetephenone in refluxing toluene using catalytic amount of *p*-toluenesulfonic acid (PTSA) for 20 hrs. Upon completion of the reaction time, solvent was evaporated under reduced pressure The reaction mixtures were further filtered and purified by precipitation with cold ether which was subsequently dried as shown in scheme 20. The formation of the compound 32 was not confirmed from the NMR studied. So it was decided to cleave the compound from the polymer support which was achieved using 1 % KCN in MeOH for 12 hrs. The reaction mixture was concentrated and polymer was precipitated by ether and removed by filtration. Subsequent NMR and MS analysis has confirmed the formation of imine derivatives **34.** Subsequently the compound **34** was further reacted with acetophenone in refluxing toluene solution yielded no cyclised product.



Scheme 20. Attempted synthesis of polymer immobilized 2,4-diphenyl-3*H*-1-benzazepine

Then we shifted our attention to synthesize the compound using dean stark apparatus, as we predicted that the imine **34** was the intermediate formed during the reaction which eventually leads to the release of water. So the water needed to be trapped during the reaction. So we did the same reaction using dean stark apparatus for 20 hours of refluxing in toluene solution. After completion of the reaction time as well as subsequent purification with ether, we cleaved the polymer conjugates to generate the desired compound as predicted in the scheme 21.



Scheme 21. Successful synthesis of 2,4-diphenyl-3*H*-1-benzazepine

The formation of the cyclised compound **35** and imine intermediate **34**was confirmed from ^{1}H NMR and MS spectroscopy as drawn in figure 1.48. In compound **34** we have observed the peak at δ = 2.4 ppm which indicates the methyl signal, where as in compound **35** there are two peaks at δ = 3.49 and 7.17 ppm respectively indicates the formation of the compound.

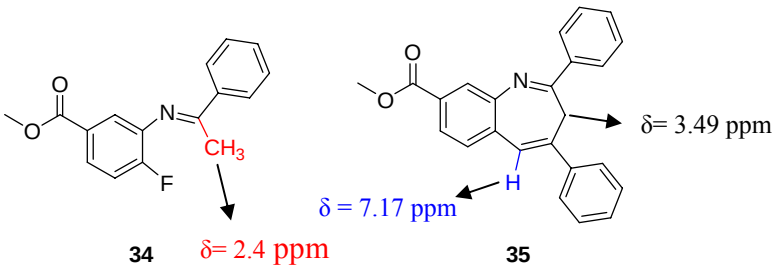
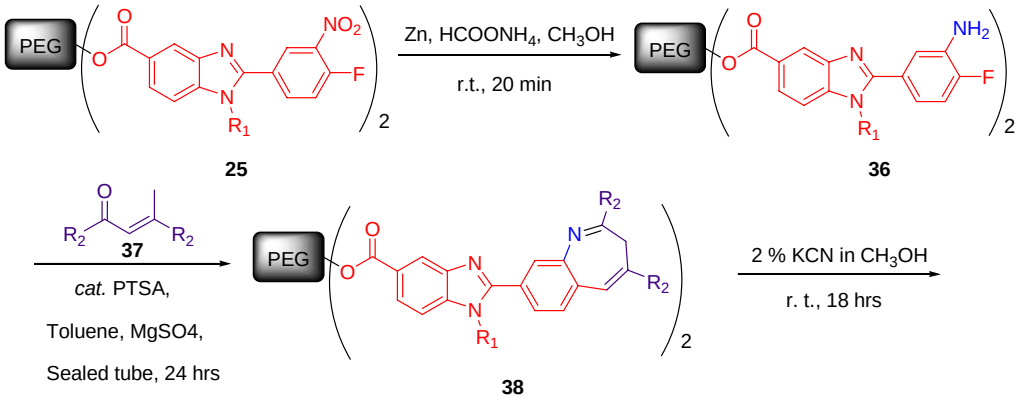


Figure 1.48. ^{1}HNMR interpretation of compound **34** and **35** in $CDCl_3$

Next we turned our attention to the synthesis of bis aryl ring system using the same procedure which required to take the compound **25** as the key starting material synthesized from the previous section B as shown in scheme 22. Reduction of the aryl

nitro group in the resulting polymer immobilized nitro derivative **25** was successfully accomplished with a suspension of Zn/$HCOONH_4$ in methanol to afford immobilized amine **36** at room temperature for 20 minutes. Formation of the amine conjugates **36** was confirmed from the NMR. Upon completion of the reaction, reaction mixtures were filtered through fritted funnel to remove the Zn. The reaction mixtures were evaporated and dichloromethane was added to salt out the ammonium formate to obtain the compound **36.** In an effort to attain the target molecule, compound **36** was undergoing cyclisation reaction with 3 equiv of acetophenone moiety. Initial studies for this reaction were carried out at refluxing temperature using toluene as solvent and PTSA as catalytic amount in dean stark apparatus yielded only imine intermediate **37.** Subsequently, addition of 5 equiv of acetophenone to the reaction mixture under pressured microwave irradiation failed to generate the polymer immobilized compound **38**. Subsequently, we have observed that the use of high boiling solvent like mesitylene or xylene did not yield the cyclised product. We, then next focused our attention to carried out the reaction using NaH as base and toluene as solvent under sealed tube condition failed to obtain the desired cyclised product. The failure of the reaction can be attributed by the fact that electron withdrawing nature of the benzimidazole moiety prevents the cyclisation of the intermediate imine **37** to the desired cyclised product **38**. In order to obtain the cyclised product **38**, we decided to carry out the aldol reaction of the acetophenone moiety. According to the literature method[121], we added the 1 equiv of acetophenone solution in dichloromethane to the 1 equiv of titanium chloride ($TiCl_4$) in hexane solution at 0 ^{O}C for over 10 minutes. To the same was added 2 equiv of Et_3N in dichloromethane solution and the reaction mixture was allowed to stir for overnight at room temperature. Upon

completion of the reaction as checked by tlc the reaction mixture was poured over cold water and extracted with ethyl acetate and dried as shown in scheme 21.



Scheme 22. General strategy towords the synthesis of benzimidazolylbenzaepines on soluble polymer support

The aldol product of acetophenone **37** was reacted with polymer conjugates of **36** under sealed tube condition using toluene as solvent and PTSA as catalytic amount obtained the desired product **38** in 26 hours of heating. Upon completion of the reaction time, the reaction mixture was evaporated under reduced pressure. Subsequently the reaction mixture was purified by precipitating with cold ether and filtered and dried. The formation of the product was confirmed from ^{1}H NMR analysis. However upon cleavage of the polymer conjugates **38**, we observed two spots in 50:50 ratios which correspond to the product as well as starting material. However, in an attempt to compete the reaction,

we then carried out the reaction under same set of condition using over dried $MgSO_4$ as dehydrating agent. The use of $MgSO_4$ implies to the fact that water formed during imine formation required dehydrating agent to absorb which further facilitates to the cyclisation of polymer conjugates **36** to the desired compound **38**. Moreover the use of $MgSO_4$ reduced the reaction time to 18 hrs of intense refluxing. After cessation of the reaction time, the polymer conjugates **38** were purified by precipitating out the reaction mixtures with excess of cold ether to remove the excess aldol product **37** and dried. The formation of the polymer conjugates **38** were confirmed from the study of the proton NMR spectra. However as anticipated, the mechanism for benzazepine formation **38** is shown in Figure 1.49. The results suggest that the PTSA catalyzed the formation of unsaturated fluoroimine **39a.** The conversion of fluoroimine **39** to enamine **40** could occur by a thermal [1,5] sigmatropic shift. Then, addition-elimination processe develops. Further rearrangement produces intermediate **41**. This could undergo acid-mediated cyclization accompanied by fluoride loss, giving cation **42**, which again rearomatizes to 5*H*-benzazepine **43** by the subsequent proton loss. Finally, benzazepine **43** isomerizes to benzazepine **38**.

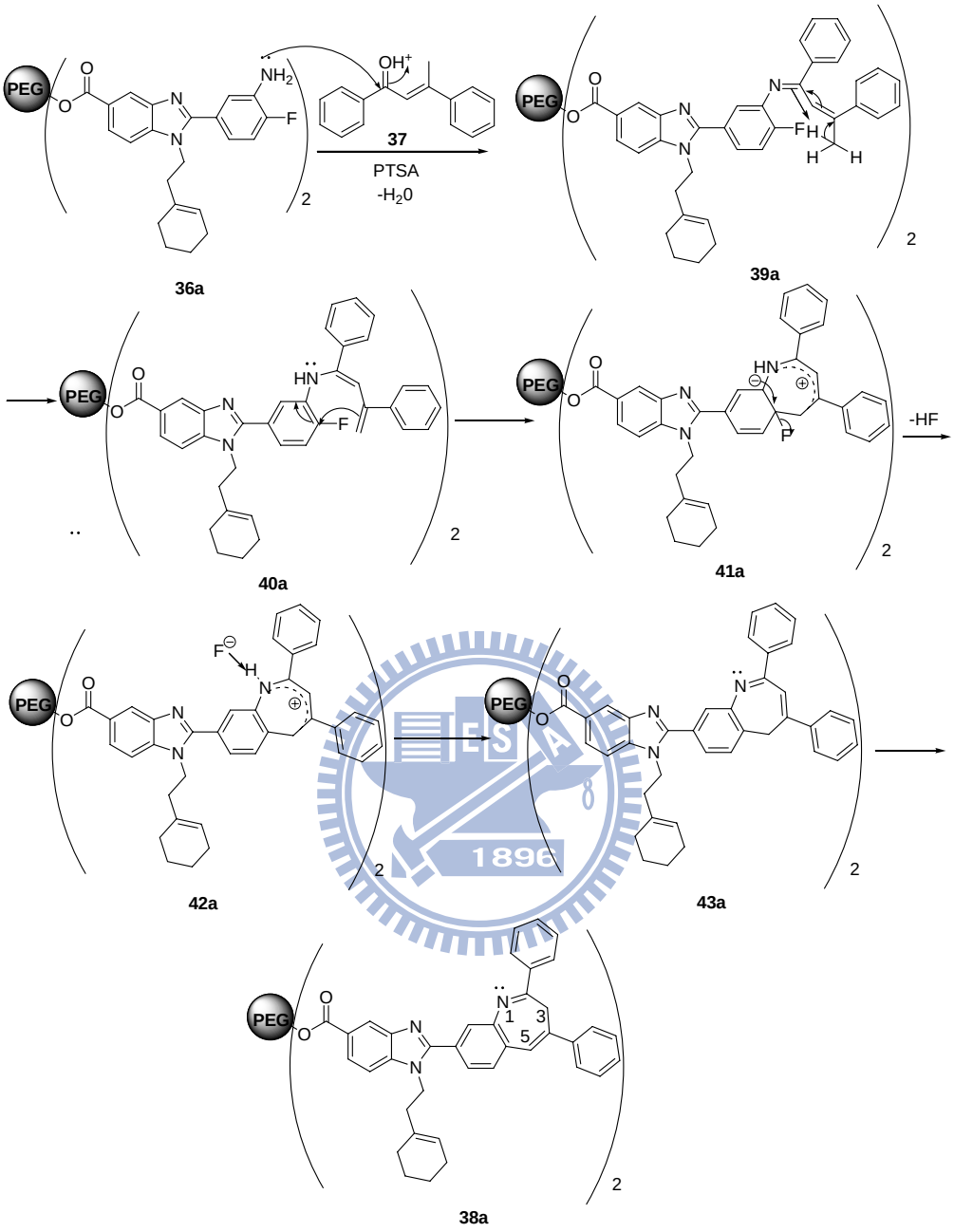
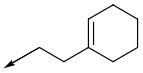
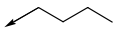
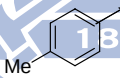
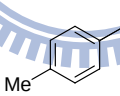
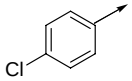
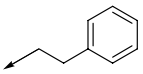
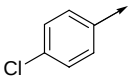
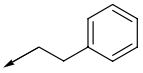
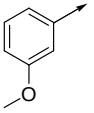
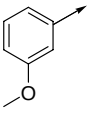
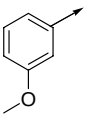


Figure 1.49. Mechanism of the formation of 2,4-diphenyl-3*H*-1-benzazepine.

Highly substituted 3*H*-1-benzazepine were finally cleaved from polymer support using 2 % KCN in MeOH solution at room temperature for 12 hours. After polymer filtration and washing, the filtrate and washes were evaporated to provide a residue that contained

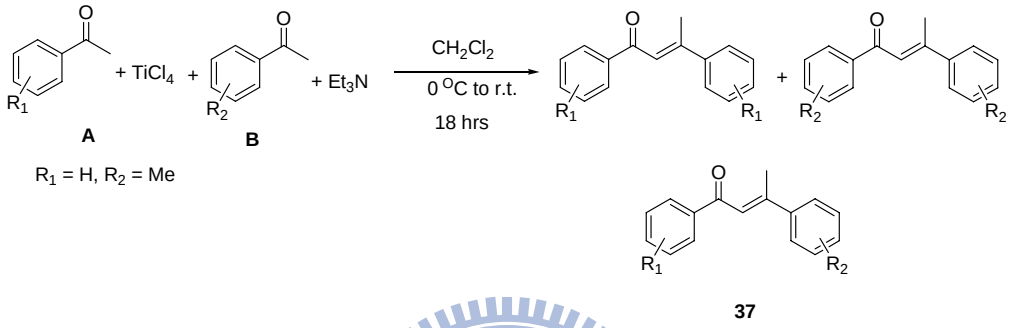
a major component of 72-92 % purity of title compounds as assessed by HPLC analysis. Crude products were typically purified by column chromatography to afford the compounds **44** in good overall yields (Table 4).

Table 4. Synthesized 2, 4-diaryl-3*H*-(benzo[*b*]azepin-8-yl)-1H-benzo[*d*]imidazole-5-carboxylates **44**.

Entry	R_1	R_2	m/z[a]	Purity[b]	Yield[c]
44a			578	n.d.[d]	79
44b			526	90	85
44c			538	72	77
44d			512	84	89
44e			526	81	85
44f			554	89	91
44g			540	92	82
44h			580	91	94
44i			642	81	72
44j			634	78	76
44k			572	83	80
44l			586	77	79

[a]LRMS were detected with ESI ionization source. [b]Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%). [c] Determined based on the weight of crude samples (%). [d] Performed under solution phase synthesis.

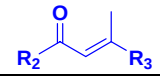
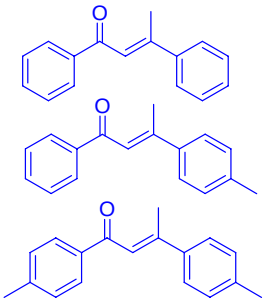
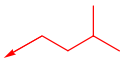
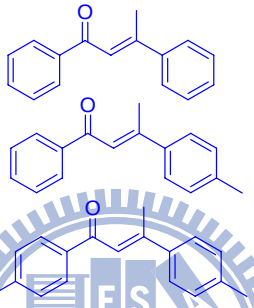
Here in order to establish the diversity in the methodology, we introduced the cross aldol product obtained from different substituted acetophenone as shown in scheme 23.



Scheme 23. Synthesis of mixed aldol condensation product from unsymmetrical ketones

Intersetingly we obtained three products in the cross aldol condensation without column purification which were further reacted with polymer conjugates **36**. The reaction went on smoothly in 18 hrs of intense refluxing under sealed tube. After cessation of the reaction time, the polymer conjugates **38** were purified by precipitating out the reaction mixtures with cold ether and dried. The polymer conjugates **38** were finally cleaved using 2 % KCN in MeOH solution at room temperature for 12 hours. After polymer filtration, and washing, the filtrate and washes were evaporated to provide a residue that contained a three component of varied purity of title compounds as assessed by HPLC analysis as in table 5.

Table 5. Synthesized 2, 4-diaryl-3*H*-(benzo[*b*]azepin-8-yl)-1H-benzo[*d*]imidazole-5-carboxylates **44**.

Entry	R_1		m/z[a]	Purity[b]	Yield[c]
44m			538 552 566	49+25+22	81
44n			540 554 568	66+14+07	79

[a] *LRMS were detected with ESI ionization source.* [b] *Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%).* [c] *Determined based on the weight of crude samples (%).*

It is interesting to note that the polarity of the product is same and was difficult to purify. The formation of the three compounds in one pot was verified by HPLC and mass spectroscopy. Because of the well-understood chemistry, the availability of a wide diversity of aliphatic amines as well as differently substituted ketones, the excellent synthetic purity and yields obtained during the liquid-phase synthesis of 3*H*-1-benzazepine on soluble polymeric support.

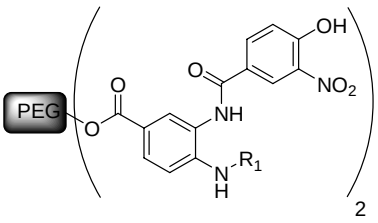
1.4. Experimental section

1.4.1. General directions

All reactions were performed under an inert atmosphere with unpurified reagents and dry solvents. Analytical thin-layer chromatography (TLC) was performed using 0.25mm silica gel coated Kiselgel 60 F_{254} plates which were developed with UV fluorescence (254 nm and 365 nm) and CAM (aq). Flash chromatography was performed using the indicated solvent and silica gel 60 (Merck, 230-400 mesh). All the microwave experiments were performed in a Biotage initiator under optimized reaction conditions of power and pressure. ^{1}H NMR (300 MHz): These were recorded on a Bruker DX-300 spectrometer. Chemical shifts are reported in parts per million (ppm) as referenced to the appropriate residual solvent peak on the scale from an internal standard. Broad signals are assigned as br. ^{13}C NMR spectra: These were recorded at 75 MHz on Bruker DX-300 instrument or at 125.1 MHz on a Bruker AV-500 instrument. Chemical shifts are given in parts per million (ppm) as referenced to $CHCl_3$. ^{19}F NMR spectra: There were recorded at 287.5 MHz on Bruker-AV 500 instruments. Mass Spectra: Low resolution and were recorded on a VG Prospec spectrometer, with molecular ions and major peaks being reported. High-resolution mass spectra (HRMS) were recorded on a JEOL TMS-HX 110 mass spectrometer. Normal phase HPLC was performed on a Shimadzu LC-10AT series machine with a Hypersil (250 x 4.6 mm) analytical column. PEG was purchased from SHOWA.

1.5. General Procedure for the synthesis of Intermediates

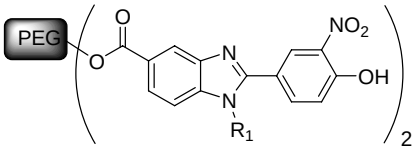
General procedure for the preparation of Polymer bound 3-(4-Hydroxy-3-nitrobenzamido)-4-(substituted amino) carboxylates 18.



18

To a solution of *N, N'*- dicyclohexylcarbodiimide (DCC) (139 mg, 0.67 mmol, 3.0 equiv) in *N, N'*-dimethylformamide (DMF) was added 4-hydroxy-3-nitrobenzoic acid **17** (122 g, 0.67 mmol, 3 equiv) and 1-hydroxybenzotriazole (HOBt) (91 mg, 0.67 mmol, 3.0 equiv) in a sequential order. The resulting slurry was stirred for 5 minutes at room temperature and then added polymer (PEG 4000) anchored *o*-phenylene diammine **16** (1.0 g, 0.22 mmol, 1.0 equiv) in DMF (5 mL). The reaction mixtures were subsequently heated with stirring in a 10 mL microwave process vial for 25 minutes in the appropriate mode of pressure and temperature to obtain the polymer conjugate **18**. After completion of the reaction, the suspensible byproducts were filtered through filter paper. The reaction mixtures were precipitated by slow addition of cold ether and precipitated amide conjugates **18** were filtered through fritted funnel. The crude product was washed in succession with ether (100 mL×3) to remove the undesired impurity and dried for further steps.

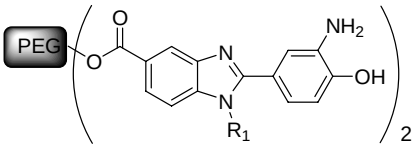
General procedure for the preparation of Polymer bound 2-(4-Hydroxy-3-nitrophenyl)- 1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 19.



19

To a solution of Polymer bound 3-(4-Hydroxy-3-nitrobenzamido)-4-(substituted amino) carboxylates **18** in 1,2-dichloroethane, trifluoroacetic acid (0.5 mL) and $MgSO_4$ (500 mg) was added and the mixture was subsequently heated with stirring in a 10 mL microwave process vial for 8 minutes in the appropriate mode of pressure and temperature. After completion of the reaction, $MgSO_4$ was removed through celite. The reaction mixtures were precipitated by slow addition of excess of cold ether (100 mL) and filtered through a fritted funnel to obtain the polymer bound 2-(4-Hydroxy-3-nitrophenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **19** in high purity.

General procedure for the preparation of Polymer bound 2-(3-Amino-4-hydroxyphenyl) -1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 20.

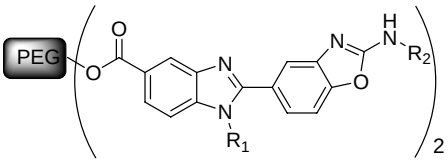


20

To a solution of **19** in methanol, Pd on charcoal (48 mg, 0.45 mmol, 1.0 equiv.) and ammonium formate (132 mg, 2.10 mmol, 10.0 equiv) were added. The reaction mixtures was subsequently heated with stirring in a 10 mL microwave process vial for 10 minutes in the appropriate mode of pressure and temperature to complete reduction of nitro group which was evident from color change (yellow to greenish blue) upon spotting on a TLC plate. After completion, the reaction mixtures were then subjected to centrifugation for

removal of Pd on charcoal and the supernatant liquid was concentrated by rotary evaporation to remove methanol. Dichloromethane (10 mL) was then added to salt out ammonium formate. The reaction mixtures were filtered through fritted funnel to remove ammonium formate to obtain the polymer bound 2-(3-Amino-4-hydroxyphenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **20.**

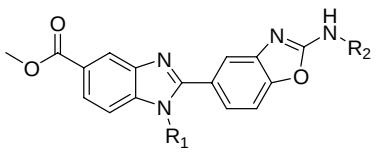
General procedure for the preparation of Polymer bound 2-(2-(Substituted amino) benzo[*d*]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 21.



21

To a stirred solution of polymer bound 2-(3-Amino-4-hydroxyphenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates conjugates **20** in CH_3CN (5 mL), various isothiocyanates (1.05 mmol, 5.0 equiv) and *N*-3(dimethylaminopropyl)-3-ethyl carbodiimide (EDC) (163 mg, 1.05 mmol, 5.0 equiv) as an activating agent were added. The reaction mixtures were exposed under pressured microwave irradiation for 10 minutes. Upon completion of cyclization by checking the NMR, the crude product mixtures were purified by precipitation with cold ether (100 mL×3) and dried to obtain the conjugate **21** in high purity.

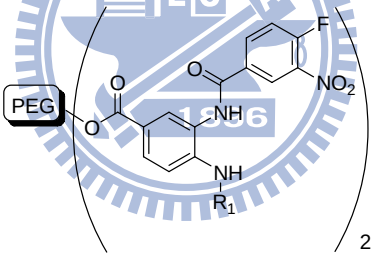
General procedure for the cleavage of Polymer bound substituted benzimidazolylbenzoxazoles 22.



22

To a solution of polymer conjugates **21** in methanol (20 mL), KCN (100 mg) was added and stirred for 12 hours at room temperature. After completion of the reaction, the crude product was precipitated with excess of cold ether (100 mL), the polymer was filtered off and subjected to evaporation. The residue was dried under vacuum, and subjected to crude HPLC analysis with UV detection at λ = 254 nm (column: Sphereclone 5μ Si (250 x 4.6 mm); gradient: 35 % ethyl acetate in hexane; flow rate: 1 mL/min.). The slurry obtained was loaded on silica gel column and eluted with a mixture of ethyl acetate and hexane (1:4) to get the title compounds **22** in 77-96 % overall yields.

General Procedure for the Preparation of Polymer Bound 3-(4-Fluoro-3-nitrobenzamido)-4-(substituted amino) carboxylates 24.

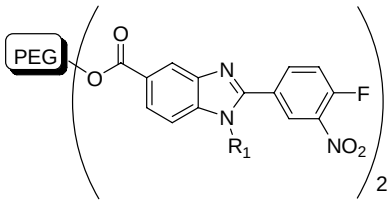


24

Polymer bound *o*-phenylene diamine **16** (PEG 4000) (1.0 g, 0.25 mmol, 1.0 equiv) dissolved in (5 mL) of dichloromethane was added to a solution of 4-Fluoro-3-nitrobenzoic acid **23** (0.11 g, 0.60 mmol, 2.4 equiv) in dichloromethane (5 mL) in the presence of *N,N'*–dicyclohexylcarbodiimide (DCC) (0.144 g, 0.70 mmol, 2.4 equiv.) and *N,N'*- dimethylamino pyridine (DMAP) (3 mg). The reaction mixture was stirred at room temperature and subsequently irradiated microwave for 10 minutes to obtain the polymer bound amide conjugate **24**. After completion of the reaction, the suspensible dicyclohexyl urea (DCU) was filtered through filter paper. The reaction mixtures were

precipitated by slow addition of cold ether and precipitated amide conjugate **3** was filtered through fritted funnel. The crude product was washed successively with ether to remove the undesired impurity and dried for further steps.

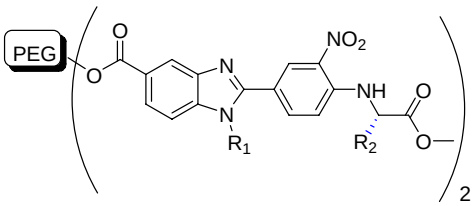
General Procedure for the Preparation of Polymer Bound Benzimidazole Derivatives 25.



25

To a solution of **24** in 1,2-dichloroethane, trifluoroacetic acid (0.5 mL) and of $MgSO_4$ (0.5 g) was added and irradiated under microwave condition for 5 minutes. After completion of the reaction, $MgSO_4$ was removed through celite. The reaction mixtures were precipitated by slow addition of excess of cold ether (100 mL) and filtered through fritted funnel to obtain the compound **25** in high purity.

General Procedure for the Preparation of Polymer Bound Substituted Benzimidazole Derivatives 26.

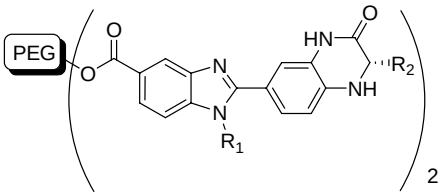


26

The polymer bound benzimidazole derivative **25** was treated with various chiral amino esters (5 equiv.) and Et_3N (3 equiv.) in 1,2-dichloroethane (5 mL). The reaction mixtures were irradiated under microwave condition for 10 minutes to complete S_NAr reaction and

the reaction mixtures were washed with cold ether (100 mL), dried to obtain the conjugate **26** in quantitative yields.

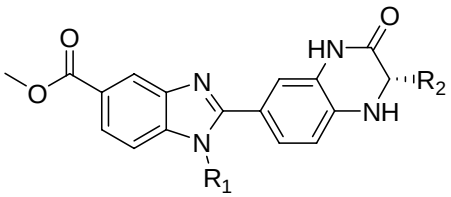
General Procedure for the Preparation of Polymer Bound Substituted Benzimidazolylquinoxalinone Derivatives 28.



28

To a solution of **26** in methanol, Zn (0.5 g, 7.5 mmol, 30 equiv.) and ammonium formate (0.24 g, 3.75 mmol, 15.0 equiv) were added. The crude mixtures were stirred for 30 minutes for complete reduction of nitro group which was evident from color change from yellow to colorless. The reaction mixtures were then subjected to centrifugation for removal of Zn and the supernatant liquid was concentrated by rotary evaporation to remove methanol. Dichloromethane (10 mL) was then added to salt out ammonium formate. The reaction mixtures were filtered through filter paper to remove ammonium formate to obtain the conjugate **28.**

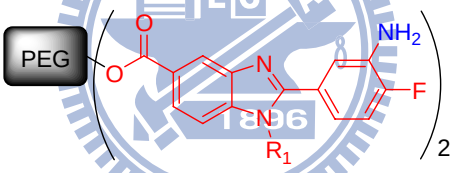
General Procedure for the Cleavage of Polymer Bound Substituted Benzimidazolylquinoxalinone Derivatives 29.



29

To a solution of conjugates **28** in methanol (30 mL), KCN (0.1 g) was added and stirred for 18 hours. After completion of the reaction, the crude product was precipitated with excess of cold ether (100 mL), the polymer was filtered off and subjected to rotavapor. The residue was dried under vacuum, and subjected to crude HPLC analysis (HPLC 254 nm). The residue was dissolved in dichloromethane (5 mL) and again subjected to evaporation. The slurry obtained was loaded on neutral silica gel column and eluted with a mixture of ethyl acetate and hexane (2:1) to get the title compounds **29** in good yields and subjected to chiral HPLC analysis using chiral column (Daicel Chiralcel OD) employing 2-propanol: *n*-hexane (1:9) as the eluent ratio.

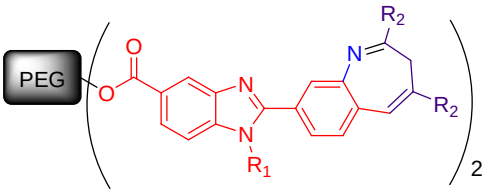
General Procedure for the Preparation of Polymer Bound Substituted Benzimidazole Derivatives 36.



36

To a solution of **25** in methanol, Zn (0.5 g, 7.5 mmol, 30 equiv.) and ammonium formate (0.24 g, 3.75 mmol, 15.0 equiv) were added. The crude mixtures were stirred for 30 minutes for complete reduction of nitro group which was evident from color change from yellow to colorless. The reaction mixtures were then subjected to centrifugation for removal of Zn and the supernatant liquid was concentrated by rotary evaporation to remove methanol. Dichloromethane (10 mL) was then added to salt out ammonium formate. The reaction mixtures were filtered through filter paper to remove ammonium formate to obtain the conjugate **36.**

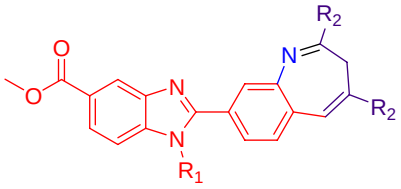
General Procedure for the Preparation of Polymer Bound Substituted 2, 4-Diaryl-3*H*-(benzo[*b*]azepin-8-yl)-1H-benzo[*d*]imidazole-5- carboxylates Derivatives 44.



38

A solution of enone **37** (5.0 equiv), polymer bound substituted benzimidazole derivatives **36** (1 equiv) and *p*-TsOH monohydrate (20 mg) in toluene (25 mL) was refluxed under N_2 for 18 h using sealed tube apparatus. Upon completion of the reaction time, solution was cooled to room temp. The solvent was evaporated under reduced pressure, and the residue was dissolved in dichloromethane (50 mL). The reaction mixtures were precipitated by slow addition of excess of cold ether (100 mL) and filtered through fritted funnel to obtain the compound **38** in high purity.

General Procedure for the Cleavage of Polymer Bound Substituted 2, 4-diaryl-3*H*-(benzo[*b*]azepin-8-yl)-1H-benzo[*d*]imidazole-5-carboxylates Derivatives 44.

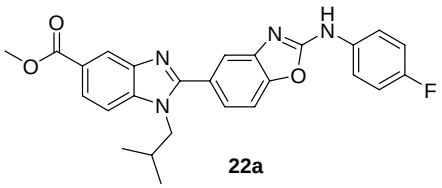


44

To a solution of conjugates **38** in methanol (30 mL), KCN (0.1 g) was added and stirred for 12 hours. After completion of the reaction, the crude product was precipitated with excess of cold ether (100 mL), the polymer was filtered off and subjected to rotavapor. The residue was dried under vacuum, and subjected to crude HPLC analysis with UV detection at λ = 254 nm (column: Sphereclone 5μ Si (250 x 4.6 mm); gradient: 35 %

ethyl acetate in hexane; flow rate: 1 mL/min.). The slurry obtained was loaded on silica gel column and eluted with a mixture of ethyl acetate and hexane (1:4) to get the title compounds **44** in 72-91 % overall yields.

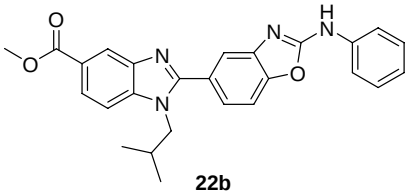
2-(2-(4-Fluorophenylamino)benzo[*d*]oxazol-5-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22a.



22a

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 1.5 Hz, 1H), 8.07 (dd, J = 8.5, 1.5 Hz, 1H), 7.98 (brs, 1H), 7.71 (d, J = 1.5 Hz, 1H), 7.64 (dd, J = 9.0 Hz, J_{HF} = 4.6 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.45 (dd, J = 8.2, 1.5 Hz, 1H), 7.36 (d, J = 8.2 Hz, 1H), 7.08 (dd, J_{HF} = 8.8 Hz, J = 7.4 Hz, 2H), 4.13 (d, J = 7.5 Hz, 2H), 3.98 (s, 3H), 2.11 (sept, J = 6.6 Hz, 1H), 0.74 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 159.8, 159.4 (d, $^1J_{CF}$ = 252.9 Hz), 156.5, 149.2, 143.2, 142.7, 139.3, 134.3, 126.7, 125.0, 124.7, 124.1, 122.4, 120.8 (d, $^3J_{CF}$ = 7.8 Hz), 118.1, 116.4 (d, $^2J_{CF}$ = 26.1 Hz), 110.7, 109.8, 52.5, 52.4, 29.4, 20.5, 20.4; IR (cm^{-1}, KBr): 3303, 1708; MS (ESI) m/z 459 (MH^+); HRMS (ESI, m/z) calcd for $C_{26}H_{23}FN_4O_3$: m/z 459.1832; Found 459.1834.

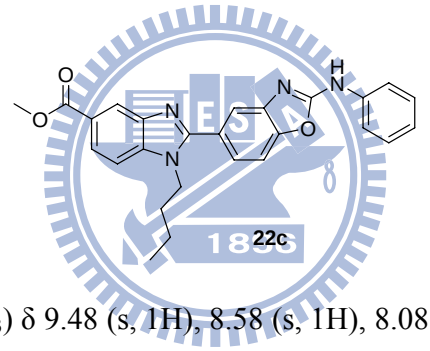
1-Isobutyl-2-(2-(phenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22b.



22b

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.5 Hz, 1H), 8.07 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.97 (brs, 1H), 7.74 (d, *J* = 1.4 Hz, 1H), 7.67 (dd, *J* = 8.7, 1.2 Hz, 2H), 7.48 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.47-7.39 (m, 4H), 7.14 (t, *J* = 7.4 Hz, 1H), 4.13 (d, *J* = 6.6 Hz, 2H), 3.98 (s, 3H), 2.13 (sext, *J* = 6.6 Hz, 1H), 0.73 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 159.4, 156.5, 149.2, 145.4, 143.4, 142.9, 139.4, 138.0, 129.8, 126.9, 124.8, 124.3, 123.9, 122.6, 118.9, 118.3, 110.6, 109.9, 53.2, 52.5, 29.2, 20.5, 20.4; IR (cm^{-1}, KBr): 3434, 1712; MS (ESI) m/z 441 (MH^+); HRMS (ESI, m/z) calcd for $C_{26}H_{24}N_4O_3$: m/z 441.1927; Found 441.1925.

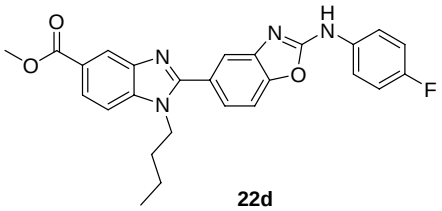
1-Butyl-2-(2-(phenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22c.



22c

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (s, 1H), 8.58 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.71 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.37-7.29 (m, 3H), 7.07 (t, *J* = 7.2 Hz, 1H), 4.27 (t, *J* = 7.1 Hz, 2H), 3.97 (s, 3H), 1.74 (quint, *J* = 7.1 Hz, 2H), 1.27-1.22 (m, 2H), 0.83 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 159.9, 156.3, 149.3, 143.4, 142.8, 139.1, 138.4, 129.7, 126.2, 124.7, 123.9, 123.7, 123.2, 122.4, 119.1, 117.8, 110.4, 109.8, 52.6, 45.1, 32.1, 20.3, 13.9; IR (cm^{-1}, KBr): 3436, 1714; MS (ESI) m/z 441 (MH^+); HRMS (ESI, m/z) calcd for $C_{26}H_{24}N_4O_3$: m/z 441.1927; Found 441.1924

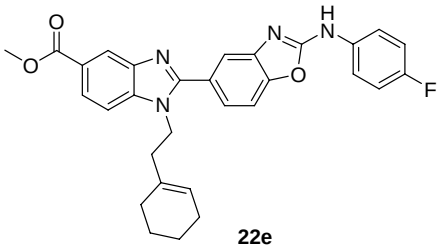
1-Butyl-2-(2-(4-fluorophenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22d.



22d

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.57 (s, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.68 (s, 1H), 7.63 (dd, J = 8.5 Hz, J_{HF} = 4.6 Hz, 2H), 7.47 (d, J = 8.5 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H), 7.05 (dd, J = 8.5 Hz, J_{HF} = 8.4 Hz, 2H), 4.27 (t, J = 7.2 Hz, 2H), 3.98 (s, 3H), 1.78 (quint, J = 7.2 Hz, 1H), 1.26-1.19 (m, 2H), 0.84 (t, J = 7.2 Hz, 3H); ^{19}F NMR (282.4 MHz, $CDCl_3$) δ -119.0; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 160.9, 159.9 (d, $^{1}J_{CF}$ = 260.6 Hz), 156.2, 149.3, 143.3, 142.7, 139.1, 134.5, 126.1, 125.1, 125.0, 124.6, 122.3, 120.9 (d, $^{3}J_{CF}$ = 4.0 Hz), 118.1, 116.5 (d, $^{2}J_{CF}$ = 23.1 Hz), 111.5, 110.4, 52.6, 45.1, 32.1, 20.3, 13.9; IR (cm^{-1}, KBr): 3303, 1709; MS (ESI) m/z 459 (MH^{+}); HRMS (ESI, m/z) calcd for $C_{26}H_{23}FN_4O_3$: m/z 459.1832; Found 459.1831.

1-(2-Cyclohexenylethyl)-2-(2-(4-fluorophenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22e.

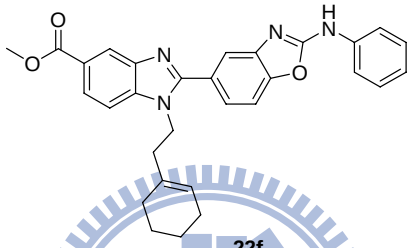


22e

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (brs, 2H), 8.08 (d, J = 8.5 Hz, 1H), 7.72 (s, 1H), 7.62 (dd, J = 8.6 Hz, J_{HF} = 4.5 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.09 (dd, J = 8.6 Hz, J_{HF} = 8.5 Hz, 2H), 5.19 (m, 1H), 4.39 (t, J = 7.2 Hz, 2H), 3.99 (s, 3H), 2.35 (t, J = 7.2 Hz, 2H), 1.83-1.75 (m, 4H), 1.44-1.34 (m, 4H); ^{19}F NMR (282.4

MHz, $CDCl_3$) δ -118.9; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 161.1, 159.7, 159.4 (d, $^1J_{CF}$ = 262.2 Hz), 156.2, 149.3, 143.3, 142.8, 139.2, 134.2, 133.4, 126.5, 125.1, 124.9, 124.7, 124.2, 122.5, 120.9 (d, $^3J_{CF}$ = 7.5 Hz), 118.0, 116.4 (d, $^2J_{CF}$ = 30.0 Hz), 110.4, 109.8, 52.6, 44.2, 38.1, 28.6, 25.5, 22.9, 22.3; IR (cm^{-1}, KBr): 3446, 1716; MS (ESI) m/z 511 (MH^+); HRMS (ESI, m/z) calcd for $C_{30}H_{27}FN_4O_3$: m/z 511.2145; Found 511.2142.

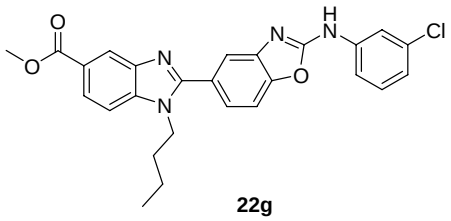
1-(2-Cyclohexenylethyl)-2-(2-(phenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22f.



22f

^1H NMR (300 MHz, $CDCl_3$) δ 9.00 (brs, 1H), 8.57 (d, *J* = 1.5 Hz, 1H), 8.08 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.73 (s, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.44-7.30 (m, 3H), 7.10 (t, *J* = 8.2 Hz, 1H), 5.18 (m, 1H), 4.37 (t, *J* = 7.2 Hz, 2H), 3.98 (s, 3H), 2.34 (t, *J* = 7.2 Hz, 2H), 1.81-1.73 (m, 4H), 1.44-1.41 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 159.8, 156.3, 149.2, 143.3, 142.8, 139.2, 138.3, 133.4, 129.7, 126.4, 125.1, 124.9, 124.7, 123.8, 123.6, 122.4, 119.0, 117.9, 110.5, 109.8, 52.6, 43.1, 38.1, 28.6, 25.5, 22.9, 21.8; IR (cm^{-1}, KBr): 3421, 1712; MS (ESI) m/z 493 (MH^+); HRMS (ESI, m/z) calcd for $C_{30}H_{28}N_4O_3$: m/z 493.2240; Found 493.2242.

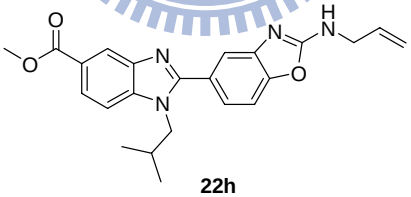
1-Butyl-2-(2-(3-chlorophenylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22g.



22g

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (brs, NH), 8.56 (s, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.80 (s, 1H), 7.72 (s, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 8.2, 1H), 7.30-7.25 (m, 2H), 7.05 (d, *J* = 7.8 Hz, 1H), 4.29 (t, *J* = 7.4 Hz, 2H), 3.99 (s, 3H), 1.80 (quint, *J* = 7.4 Hz, 2H), 1.30-1.22 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 159.1, 156.2, 149.2, 143.2, 142.7, 139.6, 139.1, 135.3, 130.7, 126.2, 125.1, 124.8, 124.3, 123.6, 122.3, 118.8, 118.2, 116.8, 110.4, 109.9, 52.6, 45.1, 32.1, 20.3, 13.9; IR (cm^{-1}, KBr): 3399, 1747; MS (EI) m/z 474 (M^+); HRMS (ESI, m/z) calcd for $C_{26}H_{23}ClN_4O_3$: m/z 475.1537; Found 475.1533.

2-(2-(Allylamino)benzo[*d*]oxazol-5-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22h.

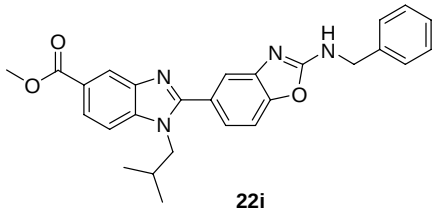


22h

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.4 Hz, 1H), 8.04 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.60 (s, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.38-7.34 (m, 2H), 6.11 (brs, 1H), 5.99 (m, 1H), 5.33 (dd, *J* = 17.0, 1.1 Hz, 1H), 5.22 (dd, *J* = 10.1, 1.1 Hz, 1H), 4.12 (m, 4H), 3.96 (s, 3H), 2.10 (sept, *J* = 6.6 Hz, 1H), 0.71 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 163.3, 156.6, 149.9, 143.8, 142.9, 139.4, 133.9, 126.6, 124.8, 124.5, 123.3, 122.6, 117.6, 117.4, 110.6, 109.6, 52.5, 52.4, 45.8, 29.1, 20.3; IR (cm^{-1}, KBr): 3278, 1710; MS

(ESI) m/z 405 (MH^+); HRMS (ESI, m/z) calcd for $C_{23}H_{24}N_4O_3$: m/z 405.1927; Found 405.1929.

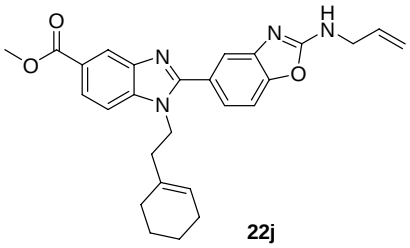
2-(2-(Benzylamino)benzo[*d*]oxazol-5-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22i.



22i

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, *J* = 1.6 Hz, 1H), 8.04 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.56 (d, *J* = 1.1 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.42-7.30 (m, 7H), 5.97 (t, *J* = 6.0 Hz, 1H), 4.72 (d, *J* = 6.0 Hz, 2H), 4.13 (d, *J* = 7.5 Hz, 2H), 3.97 (s, 3H), 2.10 (sept, *J* = 6.6 Hz, 1H), 0.73 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 163.2, 156.5, 149.9, 143.8, 142.9, 139.4, 137.8, 129.3, 128.4, 128.1, 126.8, 124.8, 124.5, 123.5, 122.6, 117.6, 110.6, 109.0, 52.5, 52.4, 47.6, 29.2, 20.3; IR (cm^{-1}, KBr): 3210, 1705; MS (ESI) m/z 455 (MH^+); HRMS (ESI, m/z) calcd for $C_{27}H_{26}N_4O_3$: m/z 455.2083; Found 455.2085.

2-(2-(Allylamino)benzo[*d*]oxazol-5-yl)-1-(2-Cyclohexenylethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22j.

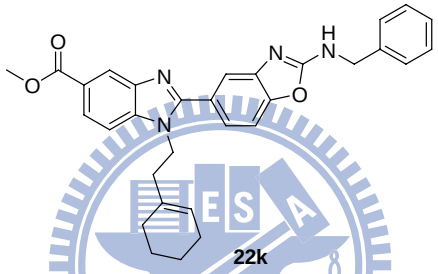


22j

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, *J* = 1.5 Hz, 1H), 8.05 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.64 (d, *J* = 1.5 Hz, 1H), 7.46 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 6.00 (m, 1H), 5.86 (m, 1H), 5.35 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.24 (dd, *J* =

10.3, 1.3 Hz, 1H), 5.18 (m, 1H), 4.36 (t, *J* = 7.2 Hz, 2H), 4.17-4.14 (m, 2H), 3.97 (s, 3H), 2.33 (t, *J* = 7.2 Hz, 2H), 1.81-1.74 (m, 4H), 1.45-1.43 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 163.2, 156.3, 150.1, 143.9, 142.9, 139.3, 133.9, 133.4, 126.4, 124.9, 124.8, 124.5, 123.3, 122.6, 117.4, 117.3, 110.3, 109.6, 52.5, 45.8, 44.1, 38.1, 28.6, 25.5, 22.9, 22.3; IR (cm^{-1}, KBr): 3424, 1714; MS (ESI) m/z 457 (MH^+); HRMS (ESI, m/z) calcd for $C_{27}H_{28}N_4O_3$: m/z 457.2240; Found 457.2239.

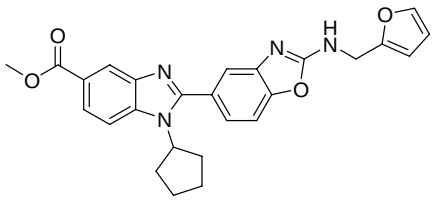
2-(2-(Benzylamino)benzo[*d*]oxazol-5-yl)-1-(2-Cyclohexenylethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22k.



22k

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, *J* = 1.4 Hz, 1H), 8.05 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.54 (d, *J* = 1.3 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.40-7.23 (m, 7H), 6.65 (m, 1H), 5.18 (m, 1H), 4.68 (d, *J* = 4.9 Hz, 2H), 4.34 (t, *J* = 7.2 Hz, 2H), 3.96 (s, 3H), 2.32 (t, *J* = 7.2 Hz, 2H), 1.81-1.73 (m, 4H), 1.45-1.42 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 163.4, 156.3, 150.1, 143.9, 142.9, 139.3, 137.9, 133.4, 129.2, 128.2, 128.0, 126.3, 125.0, 124.8, 124.5, 123.2, 122.5, 117.2, 110.3, 109.6, 52.5, 47.5, 44.1, 38.1, 28.6, 25.5, 23.0, 22.3; IR (cm^{-1}, KBr): 3209, 1714; MS (ESI) m/z 507 (MH^+); HRMS (ESI, m/z) calcd for $C_{31}H_{30}N_4O_3$: m/z 507.2396; Found 507.2395.

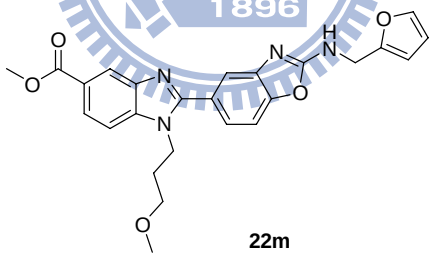
1-Cyclopentyl-2-(2-(furan-2-ylmethylamino)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22l.



22l

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.61 (s, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.45-7.39 (m, 3H), 6.42-6.32 (m, 2H), 5.62 (t, *J* = 5.5 Hz, 1H), 4.99 (quint, *J* = 8.9 Hz, 1H), 4.71 (d, *J* = 5.5 Hz, 2H), 3.98 (s, 3H), 2.36-2.29 (m, 2H), 2.14-2.06 (m, 4H), 1.75-1.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 163.0, 156.7, 150.9, 150.1, 143.6, 143.0, 142.9, 136.9, 126.6, 124.6, 124.0, 123.6, 122.9, 117.5, 111.9, 110.9, 109.7, 108.4, 58.1, 52.5, 40.4, 30.8, 25.6; IR (cm^{-1}, KBr): 3423, 1714; MS (ESI) m/z 457 (MH^+); HRMS (ESI, m/z) calcd for $C_{26}H_{24}N_4O_4$: m/z 457.1876; Found 457.1878.

2-(2-(furan-2-ylmethylamino)benzo[*d*]oxazol-5-yl)-1-(3-methoxypropyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 15m.

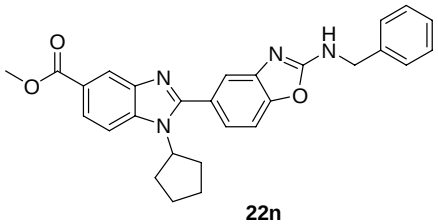


22m

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.5 Hz, 1H), 8.05 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.67 (d, *J* = 1.4 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.46-7.36 (m, 3H), 6.35 (dd, *J* = 5.6, 4.9 Hz, 2H), 6.13 (brs, 1H), 4.70 (s, 2H), 4.42 (t, *J* = 7.1 Hz, 2H), 3.97 (s, 3H), 3.24 (t, *J* = 6.0 Hz, 2H), 3.21 (s, 3H), 1.99 (quint, *J* = 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 162.9, 156.1, 150.9, 150.1, 143.7, 143.0, 142.8, 139.4, 125.3, 124.7, 124.6, 123.4, 122.5, 117.4, 110.9, 110.3, 109.7, 108.5, 69.1, 59.0, 52.5, 42.2, 40.4, 30.4; IR (cm^{-1}, KBr):

3211, 1708; MS (ESI) m/z 461 (MH^+); HRMS (ESI, m/z) calcd for $C_{25}H_{24}N_4O_5$: m/z 461.1825; Found 461.1822.

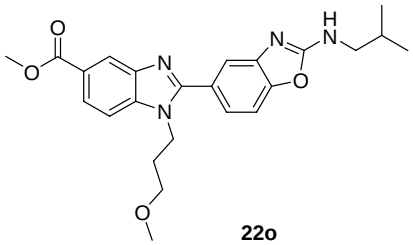
2-(2-(Benzylamino)benzo[*d*]oxazol-5-yl)-1-Cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22n.



22n

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (d, *J* = 1.5 Hz, 1H), 8.00 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.54 (d, *J* = 1.0 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.42-7.34 (m, 7H), 6.16 (t, *J* = 5.4 Hz, 1H), 4.98 (quint, *J* = 8.5 Hz, 1H), 4.71 (d, *J* = 5.4 Hz, 2H), 3.97 (s, 3H), 2.35-2.26 (m, 2H), 2.13-2.03 (m, 4H), 1.77-1.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 163.3, 156.7, 150.1, 143.7, 143.6, 137.8, 136.9, 129.3, 128.3, 128.0, 126.6, 124.5, 124.0, 123.5, 122.9, 117.4, 111.9, 109.6, 58.1, 52.5, 47.5, 30.8, 25.6; IR (cm^{-1}, KBr): 3266, 1712; MS (ESI) m/z 467 (MH^+); HRMS (ESI, m/z) calcd for $C_{28}H_{26}N_4O_3$: m/z 467.2083; Found 467.2080.

2-(2-(Isobutylamino)benzo[*d*]oxazol-5-yl)-1-(3-methoxypropyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 22o.

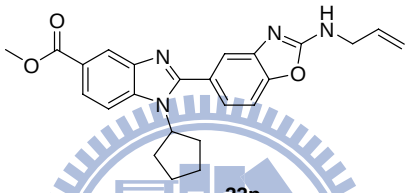


22o

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.5 Hz, 1H), 8.05 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.64 (d, *J* = 1.5 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.47 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.38 (d,

J = 8.2 Hz, 1H), 5.50 (m, 1H), 4.42 (t, *J* = 6.9 Hz, 2H), 3.95 (s, 3H), 3.34 (m, 2H), 3.27 (t, *J* = 6.2 Hz, 2H), 3.21 (s, 3H), 2.18 (m, 1H), 2.02-1.97 (m, 2H), 1.03 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 163.5, 156.2, 150.0, 145.4, 144.0, 142.9, 139.5, 124.9, 124.6, 123.1, 122.5, 117.1, 110.2, 109.5, 69.2, 59.0, 52.5, 51.0, 42.2, 30.3, 28.9, 20.4; IR (cm^{-1}, KBr): 3426, 1712; MS (ESI) m/z 437 (MH^+); HRMS (ESI, m/z) calcd for $C_{24}H_{28}N_4O_4$: m/z 437.2189; Found 437.2190.

2-(2-(Allylamino)benzo[*d*]oxazol-5-yl)-1-Cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic Acid Methyl Ester 15p.



22p

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.5 Hz, 1H), 8.00 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.58 (d, *J* = 1.0 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.42-7.37 (m, 2H), 6.01 (m, 1H), 5.64 (m, 1H), 5.36 (dd, *J* = 17.1, 1.2 Hz, 1H), 5.26 (dd, *J* = 10.3, 1.2 Hz, 1H), 4.98 (quint, *J* = 8.5 Hz, 1H), 4.16 (t, *J* = 5.5 Hz, 2H), 3.97 (s, 3H), 2.39-2.26 (m, 2H), 2.14-2.06 (m, 4H), 1.77-1.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 163.1, 156.7, 150.0, 143.8, 143.7, 136.9, 133.9, 126.7, 125.0, 124.0, 123.6, 122.9, 117.5, 117.4, 111.9, 109.7, 58.1, 52.5, 45.8, 30.8, 25.6; IR (cm^{-1}, KBr): 3413, 1714; MS (ESI) m/z 417 (MH^+); HRMS (ESI, m/z) calcd for $C_{24}H_{24}N_4O_3$: m/z 417.1927; Found 417.1926.

1-Butyl-2-[2-(1H-indol-2-ylmethyl)-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl]-1H benzoimidazole-5-carboxylic acid methyl ester (29h):

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.60 (s, 1H), 8.49 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.38 (t, J = 8.1, 2H), 7.23 - 7.08 (m, 5H), 6.60 (d, J = 8.1 Hz, 1H), 4.30-4.19 (m, 4H), 3.95 (s, 3H), 3.47 (dd, J = 14.1, 3.0 Hz, 1H), 3.08 (dd, J = 14.1, 10.5 Hz, 1H), 1.81 (m, 2H), 1.28 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 168.1, 155.7, 142.4, 139.2, 136.8, 135.1, 127.6, 125.9, 125.0, 124.9, 124.6, 123.9, 122.8, 122.5, 120.1, 119.1, 118.7, 116.8, 114.4, 111.9, 110.6, 110.5, 110.2, 57.0, 52.6, 45.3, 32.2, 28.8, 13.9; MS (EI) m/z: 507 (M+); HRMS (EI, m/z) calcd for $C_{30}H_{29}N_5O_3$: m/z 507.2270; Found 507.2272; $[\alpha]_D^{20}$ = -79.8 (*c* 0.8 , CH_2Cl_2) for 82 % ee ; IR (cm^{-1}, neat): 1720, 1683.

1-Isobutyl-2-(2-sec-butyl-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl)-1H-Benzoimida - zole-5-carboxylic acid methyl ester (29i):

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.42 (s, 1H), 8.04 (dd, J = 8.6, 1.6 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.19 - 7.13 (m, 2H), 6.75 (d, J = 8.1 Hz, 1H), 4.30 (s, 1H), 4.15-4.10 (m, 2H), 3.93 (s, 3H), 2.13 (m, 1H), 1.64 - 1.55 (m, 2H), 1.05 (d, J = 6.9 Hz, 2H), 0.80 (m, 6H), 0.79 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 167.5, 155.4, 138.9, 135.2, 125.4, 124.7, 124.5, 124.0, 121.7, 118.9, 116.2, 112.9, 109.9, 109.7, 61.1, 52.1, 44.8, 38.6, 31.9, 24.5, 19.9, 15.3, 13.2, 12.1; MS (EI) m/z: 434 (M+); HRMS (EI, m/z) calcd for $C_{25}H_{30}N_4O_3$: m/z 434.2318; Found 434.2331; $[\alpha]_D^{20}$ = -45.0 (*c* 0.1 , CH_2Cl_2) for 97% ee; IR (cm^{-1}, neat): 1715, 1661.

1-Butyl-2-(2-methyl-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl)-1H-benzoimidazole-5-carboxylic acid methyl ester (29j):

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.21 (s, 1H), 8.04 (dd, J = 8.5, 1.4 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.24 - 7.20 (m, 2H), 6.81 (d, J = 7.9 Hz, 1H), 4.23 (t, J= 7.9 Hz, 2H), 4.11-4.15 (m, 2H), 3.36 (s, 3H), 1.78-1.88 (m, 2H), 1.53 (d, J = 6.6 Hz, 3H), 1.37-1.28 (m, 2H), 0.92 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.8, 168.1, 155.5, 142.9, 139.3, 135.5, 126.2, 124.9, 124.5, 122.3, 116.8, 114.2, 110.0, 96.5, 52.5, 52.3, 45.2, 32.3, 21.2, 20.4, 18.7, 14.0; MS (EI) m/z: 392 (M+); HRMS (EI, m/z) calcd for $C_{22}H_{24}N_4O_3$:m/z 392.1848; Found 392.1843; $[\alpha]_D^{20}$ = -44.0 (*c* 0.1 , CH_2Cl_2) for 99 % ee; IR (cm^{-1}, neat): 1714, 1666.

1-Butyl-2-(2-isopropyl-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl)-1H-benzoimidazole -5-carboxylic acid methyl ester (29k)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.04 (dd, J = 8.5, 1.5 Hz, 1H), 7.80 (s, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.26-7.13 (m, 2H), 6.76 (d, J = 8.0 Hz, 1H), 4.24-4.29 (m, 3H), 3.97 (s, 3H), 3.92 (m, 1H), 2.27 (m, 1H), 1.78-1.89 (m, 2H), 1.37-1.25 (m, 2H), 1.09 (d, J = 6.8 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 167.3, 155.6, 143.0, 139.4, 135.5, 125.3, 125.0, 124.5, 122.3, 120.5, 116.5, 113.4, 109.9, 62.1, 52.5, 45.2, 37.0, 32.2, 25.1, 20.4, 19.3, 17.7, 13.9; MS (EI) m/z: 420 (M+); HRMS (EI, m/z) calcd for $C_{24}H_{28}N_4O_3$: m/z 420.2161; Found 420.2155; $[\alpha]_D^{20}$ = -67.0 (*c* 1.1 , CH_2Cl_2) for 95 % ee ; IR (cm^{-1}, neat): 1716, 1633.

1-Cyclopentyl-2-(2-methyl-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl)-1H benzoimida -zole-5-carboxylic acid methyl ester (29l):

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.38 (s, 1H), 7.98 (dd, J = 8.6, 1.5 Hz, 1H), 7.54 (d, J = 7.4 Hz, 1H), 7.17-7.15 (m, 2H), 6.80 (d, J = 8.5 Hz, 1H), 4.98 (m, 1H), 4.16-4.12 (m, 2H), 3.97 (s, 3H), 2.31 - 2.23 (m, 2H), 2.10-2.06 (m, 6H), 1.53 (d, J = 6.6 Hz,

3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 167.6, 155.8, 136.3, 135.3, 128.7, 125.8, 124.8, 124.3, 123.6, 121.9, 116.6, 113.6, 111.5, 57.8, 52.0, 51.7, 30.5, 28.9, 25.2, 23.7, 22.9, 18.4; MS (EI) m/z: 404 (M+); HRMS (EI, m/z) calcd for $C_{23}H_{24}N_4O_3$: m/z 404.1848; Found 404.1856; $[\alpha]_D^{20}$ = -69.0 (*c* 1.0, CH_2Cl_2) for 98 % ee; IR (cm^{-1}, neat): 1716, 1674.

1-Cyclopentyl-2-(2-isopropyl-3-oxo-1,2,3,4-tetrahydro-quinoxalin-6-yl)-1H-benzoimi -dazole-5-carboxylic acid methyl ester (29m):

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.15 (s, 1H), 8.46 (s, 1H), 7.95 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 7.15-7.08 (m, 2H), 6.70 (d, *J* = 8.1 Hz, 1H), 4.97 (m, 1H), 4.61 (m, 1H), 3.96 (s, 3H), 3.88 (m, 1H), 2.30-2.23 (m, 3H), 2.08-2.05 (m, 5H), 1.04 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 168.1, 156.6, 143.6, 136.9, 135.6, 125.6, 124.9, 124.1, 122.9, 119.9, 116.8, 113.3, 111.8, 61.8, 58.3, 52.5, 32.0, 30.8, 25.7, 23.0, 20.6, 19.3, 17.6; MS (EI) m/z: 432 (M+); HRMS (EI, m/z) calcd for $C_{25}H_{28}N_4O_3$: m/z 432.2161; Found 432.2163; $[\alpha]_D^{20}$ = -74.0 (*c* 1.1, CH_2Cl_2) for 80 % ee; IR (cm^{-1}, neat): 1715, 1667.

1-Isobutyl-2-(3-sec-butyl-2-oxo-1,2-dihydro-quinoxalin-6-yl)-1H-Benzoimidazole-5-carboxylic acid methyl ester (30i):

^{1}H NMR (300 MHz, CDCl3) δ 8.57 (s, 1H), 8.11 (d, *J* = 7.5 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.80 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 4.21 (d, *J* = 7.4 Hz, 2H), 3.98 (s, 3H), 3.50 (m, 1H), 2.15 (m, 1H), 1.68-1.62 (m, 2H), 1.33 (d, *J* = 6.8 Hz, 3H), 1.00-0.97 (m, 3H), 0.80 (d, *J* = 6.5 Hz, 6H); MS (ESI) m/z: 433 (MH+).

1-Butyl-2-(3-methyl-2-oxo-1,2-dihydro-quinoxalin-6-yl)-1H-benzoimidazole-5 carboxylic acid methyl ester (30j):

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.3 (s, 1H), 8.55 (s, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.79 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 4.32 (t, *J* = 7.5 Hz, 2H), 3.97 (s, 3H), 2.65 (s, 3H), 1.90-1.82 (m, 2H), 1.36-1.29 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H); MS (ESI) m/z: 391 (MH+).

1-Butyl-2-(3-isopropyl-2-oxo-1,2-dihydro-quinoxalin-6-yl)-1H-benzoimidazole-5-carboxylic acid methyl ester (30k):

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 2H), 7.63-7.54 (m, 2H), 4.48-4.43 (m, 2H), 3.99 (s, 3H), 3.60 (m, 1H), 1.78-1.70 (m, 2H), 1.17-1.10 (m, 8H), 0.89 (t, *J* = 7.1 Hz, 3H); MS (ESI) m/z: 419 (MH+).

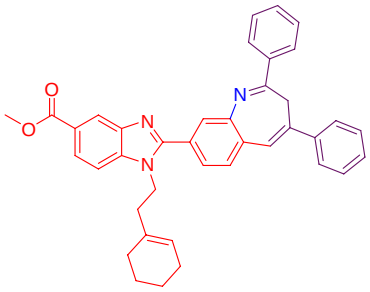
1-Cyclopentyl-2-(3-methyl-2-oxo-1,2-dihydro-quinoxalin-6-yl)-1H-benzoimidazole-5-carboxylic acid methyl ester (30l):

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.04 (s, 1H), 8.55 (s, 1H), 8.04 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 1H), 7.82 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 5.00 (m, 1H), 3.97 (s, 3H), 2.65 (s, 3H), 2.35-2.33 (m, 2H), 2.19-2.06 (m, 3H), 1.80-1.71(m, 3H); MS (ESI) m/z: 403 (MH+).

1-Cyclopentyl-2-(3-isopropyl-2-oxo-1,2-dihydro-quinoxalin-6-yl)-1H-benzoimidazole-5-carboxylic acid methyl ester (30m):

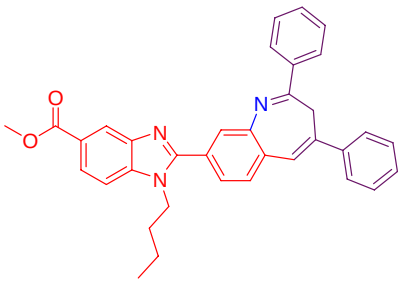
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 8.00-7.97 (m, 2H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 5.03 (m, 1H), 3.99 (s, 3H), 3.53 (m, 1H), 2.32-2.30 (m, 3H), 2.19-1.83 (m, 5H), 1.33 (d, *J* = 6.2 Hz, 6H); MS (ESI) m/z: 431 (MH+).

1-(2-Cyclohexenylethyl)-2-((1*E*,4*E*)-2,4-diphenyl-3H-benzo[*b*]azepin-8-yl)-1H-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44a.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.4 Hz, 1H), 8.09 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.98 (d, *J* = 1.4 Hz, 1H), 7.90 (dd, *J* = 7.6, 1.4 Hz, 2H), 7.66-7.63 (m, 4H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.52-7.35 (m, 6H), 7.17 (s, 1H), 5.28 (m, 1H), 4.49 (t, *J* = 7.3 Hz, 2H), 3.98 (s, 3H), 3.48 (brs, 2H), 2.47 (t, *J* = 7.3 Hz, 2H), 1.86-1.81 (m, 4H), 1.48-1.44 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 158.6, 155.4, 147.1, 142.8, 139.9, 139.3, 138.2, 136.8, 133.4, 131.7, 131.0, 130.9, 129.3, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 127.4, 125.4, 124.9, 124.8, 124.5, 122.6, 110.4, 52.5, 44.5, 38.4, 34.5, 28.7, 25.5, 25.9, 22.3; IR (cm^{-1}, KBr): 1714, 1612; MS (ESI) m/z 578 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{39}H_{36}N_3O_2$: m/z 578.2807; Found 578.2808.

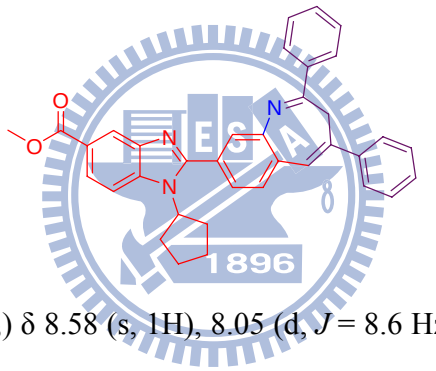
1-Butyl-2-((1*E*, 4*E*)-2, 4-diphenyl-3H-benzo[*b*]azepin-8-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44b.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, *J* = 1.4 Hz, 1H), 8.09 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.96 (d, *J* = 1.1 Hz, 1H), 7.91 (dd, *J* = 7.6, 1.4 Hz, 2H), 7.68-7.60 (m, 4H), 7.52 (d, *J* =

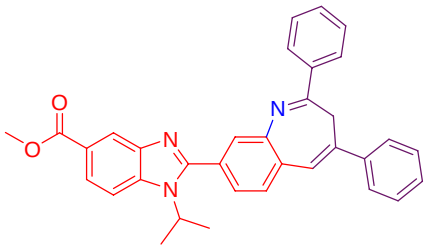
8.5 Hz, 1H), 7.50-7.33 (m, 6H), 7.17 (s, 1H), 4.41 (t, *J* = 7.5 Hz, 2H), 3.98 (s, 3H), 3.49 (brs, 2H), 1.90 (quint, *J* = 7.5 Hz, 2H), 1.32 (sext, *J* = 7.5 Hz, 2H), 0.91 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 158.7, 155.4, 147.2, 142.9, 139.9, 139.3, 138.3, 136.9, 131.7, 131.2, 130.9, 129.3, 129.2, 129.0, 128.6, 128.5, 128.4, 127.4, 125.1, 124.9, 124.8, 124.7, 122.6, 110.4, 52.5, 45.3, 34.6, 32.2, 20.4, 13.9; IR (cm^{-1}, KBr): 1707, 1612; MS (ESI) m/z 526 (MH^+); HRMS (ESI, m/z) calcd for $C_{35}H_{31}N_3O_2$: m/z 526.2494; Found 526.2491.

1-Cyclopentyl-2-((1*E*, 4*E*)-2, 4-diphenyl-3H-benzo[*b*]azepin-8-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44c.



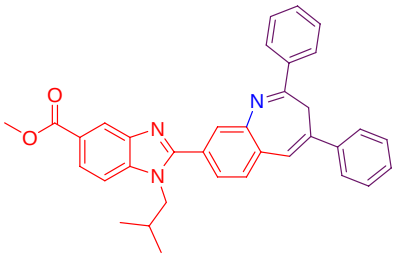
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 7.92 (d, *J* = 1.3 Hz, 1H), 7.91-7.90 (m, 2H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.62-7.57 (m, 4H), 7.49-7.34 (m, 6H), 7.17 (s, 1H), 5.28 (quint, *J* = 8.9 Hz, 1H), 3.98 (s, 3H), 3.49 (brs, 2H), 2.37-2.34 (m, 2H), 2.23-2.07 (m, 4H), 1.84-1.80 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 158.7, 156.0, 147.1, 143.5, 139.5, 138.2, 136.9, 136.8, 136.0, 131.6, 131.4, 130.9, 129.5, 129.3, 129.0, 128.7, 128.5, 127.3, 126.6, 125.0, 124.8, 124.3, 122.9, 112.0, 58.3, 52.5, 34.6, 30.9, 25.8; IR (cm^{-1}, KBr): 1714, 1614; MS (ESI) m/z 537 (MH^+); HRMS (ESI, m/z) calcd for $C_{36}H_{32}N_3O_2$: m/z 538.2494; Found 526.2492.

2-((1*E*,4*E*)-2,4-diphenyl-3H-benzo[*b*]azepin-8-yl)-1-isopropyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44d.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 1.4 Hz, 1H), 7.88 (dd, *J* = 7.0, 1.7 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.61 (dd, *J* = 7.0, 1.1 Hz, 2H), 7.55 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.48-7.34 (m, 6H), 7.17 (s, 1H), 5.07 (sept, *J* = 7.0 Hz, 1H), 3.98 (s, 3H), 3.49 (brs, 2H), 1.74 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 159.0, 154.6, 147.1, 143.1, 139.9, 138.1, 137.3, 136.8, 136.0, 132.6, 131.9, 131.0, 129.5, 129.3, 129.2, 128.7, 128.5, 127.4, 126.6, 125.1, 124.7, 124.3, 122.1, 112.6, 52.6, 50.0, 34.6, 21.9; IR (cm^{-1}, KBr): 1714, 1614; MS (ESI) m/z 512 (MH^+); HRMS (ESI, m/z) calcd for $C_{34}H_{30}N_3O_2$: m/z 512.2338; Found 512.2336.

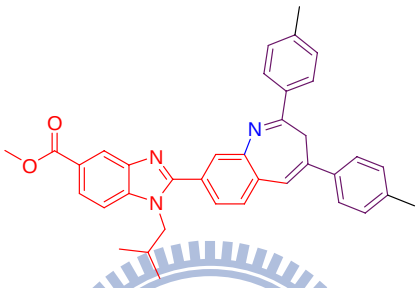
2-((1*E*, 4*E*)-2, 4-diphenyl-3H-benzo[*b*]azepin-8-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44e.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.94 (s, 1H), 7.90 (dd, *J* = 7.1, 1.0 Hz, 2H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.62-7.58 (m, 3H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.46-7.35 (m, 6H), 7.17 (s, 1H), 4.25 (d, *J* = 7.2 Hz, 2H), 3.98 (s, 3H), 3.48 (brs, 2H), 2.20 (sext, *J* = 6.6 Hz, 1H), 0.80 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ

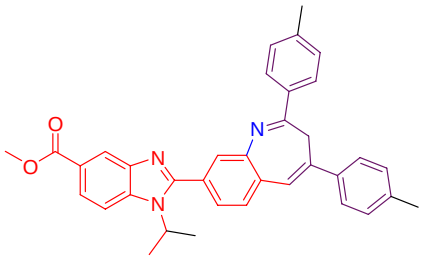
168.0, 158.8, 155.9, 147.2, 143.1, 139.9, 139.6, 138.3, 136.9, 131.6, 131.3, 130.9, 129.3, 129.2, 129.1, 129.0, 128.7, 128.4, 127.3, 126.7, 124.9, 124.7, 124.6, 122.7, 110.6, 52.6, 52.5, 34.6, 29.4, 20.5; IR (cm^{-1}, KBr): 1712, 1612; MS (ESI) m/z 526 (MH^+); HRMS (ESI, m/z) calcd for $C_{35}H_{32}N_3O_2$: m/z 526.2494; Found 526.2495.

2-((1*E*, 4*E*)-2, 4-di*p*-tolyl-3*H*-benzo[*b*]azepin-8-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44f.



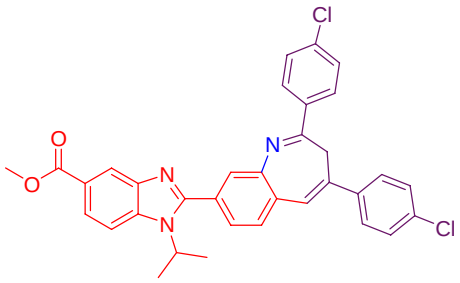
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.3 Hz, 1H), 8.07 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.92 (d, *J* = 1.4 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.57 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.52-7.46 (m, 4H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.14 (s, 1H), 4.26 (d, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 3.44 (brs, 2H), 2.41 (s, 3H), 2.36 (s, 3H), 2.20 (sext, *J* = 6.6 Hz, 1H), 0.80 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 158.6, 155.9, 147.2, 142.9, 141.2, 139.5, 138.6, 137.1, 136.8, 135.5, 131.6, 131.5, 129.7, 129.6, 128.8, 128.6, 128.5, 127.3, 125.8, 124.9, 124.7, 124.6, 122.7, 110.6, 52.6, 52.5, 34.6, 29.4, 21.7, 21.6, 20.5; IR (cm^{-1}, KBr): 1712, 1610; MS (ESI) m/z 554 (MH^+); HRMS (ESI, m/z) calcd for $C_{37}H_{36}N_3O_2$: m/z 554.2807; Found 554.2805.

2-((1*E*,4*E*)-2,4-di*p*-tolyl-3*H*-benzo[*b*]azepin-8-yl)-1-isopropyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44g.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.86 (s, 1H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 1H), 7.55-7.50 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.14 (s, 1H), 5.07 (sept, *J* = 7.0 Hz, 1H), 3.98 (s, 3H), 3.46 (brs, 2H), 2.41 (s, 3H), 2.36 (s, 3H), 1.74 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 158.6, 155.3, 147.1, 141.3, 138.7, 137.1, 136.9, 135.4, 131.7, 131.6, 129.9, 129.7, 129.4, 129.3, 129.0, 128.7, 128.5, 127.8, 127.2, 125.8, 124.8, 124.4, 122.7, 112.4, 52.5, 49.7, 34.4, 21.9, 21.8, 21.6; IR (cm^{-1}, KBr): 1716, 1612; MS (ESI) m/z 540 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{36}H_{34}N_3O_2$: m/z 540.2651; Found 540.2648.

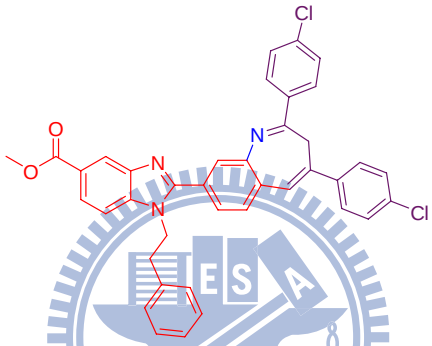
2-((1*E*, 4*E*)-2,4-bis(4-chlorophenyl)-3*H*-benzo[*b*]azepin-8-yl)-1-isopropyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44h.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 1.5 Hz, 1H), 8.04 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.86 (d, *J* = 1.6 Hz, 1H), 7.82 (dd, *J* = 7.0, 1.6 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.37 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.15 (s, 1H), 5.04 (sept, *J* = 7.0 Hz, 1H), 3.98 (s, 3H), 3.42 (brs, 2H), 1.67 (d, *J*

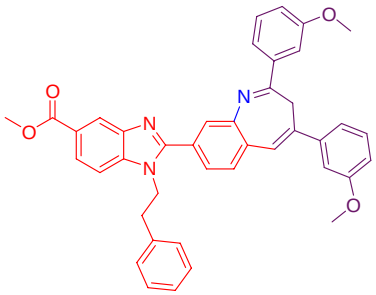
= 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 156.9, 155.0, 146.9, 138.2, 137.3, 137.2, 136.4, 135.3, 134.8, 131.7, 131.5, 129.7, 129.6, 129.4, 129.3, 129.1, 128.6, 127.3, 127.0, 125.2, 125.1, 124.4, 122.8, 112.4, 52.5, 49.7, 34.3, 21.9; IR (cm^{-1}, KBr): 1714, 1612; MS (ESI) m/z 580 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{34}H_{28}Cl_2N_3O_2$: m/z 580.1558; Found 580.1550.

2-((1*E*, 4*E*)-2,4-bis(4-chlorophenyl)-3*H*-benzo[*b*]azepin-8-yl)-1-phenethyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44i.



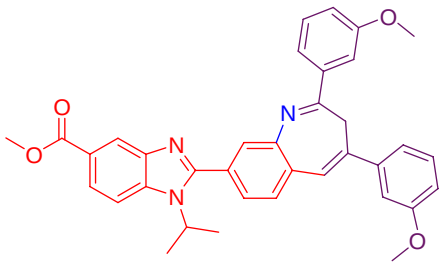
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.4 Hz, 1H), 8.08 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.85 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 1.4 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.51-7.49 (m, 2H), 7.46 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.19-7.16 (m, 4H), 7.13 (s, 1H), 6.99-6.97 (m, 2H), 4.63 (t, *J* = 7.3 Hz, 2H), 3.99 (s, 3H), 3.40 (brs, 2H), 3.16 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 156.8, 155.3, 146.8, 142.9, 139.0, 138.2, 137.4, 137.3, 136.4, 135.1, 135.0, 131.6, 130.9, 129.9, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.6, 127.5, 127.3, 125.1, 125.0, 124.9, 122.7, 110.3, 52.6, 47.0, 36.4, 34.1; IR (cm^{-1}, KBr): 1712, 1612; MS (ESI) m/z 642 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{39}H_{30}Cl_2N_3O_2$: m/z 642.1715; Found 642.1709.

2-((1*E*, 4*E*)-2,4-bis(3-methoxyphenyl)-3*H*-benzo[*b*]azepin-8-yl)-1-phenethyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44j.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.4 Hz, 1H), 8.08 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.52-7.40 (m, 3H), 7.38 (dd, *J* = 8.2, 1.7 Hz, 2H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.26-7.19 (m, 4H), 7.16 (s, 1H), 7.11 (t, *J* = 2.2 Hz, 1H), 6.94-6.92 (m, 4H), 4.63 (t, *J* = 7.3 Hz, 2H), 3.99 (s, 3H), 3.84 (s, 3H), 3.74 (s, 3H), 3.44 (brs, 2H), 3.16 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 160.3, 160.2, 158.4, 155.6, 147.0, 143.0, 141.4, 139.7, 139.1, 137.4, 136.7, 131.6, 131.3, 130.3, 130.0, 129.2, 129.1, 128.9, 128.5, 127.5, 126.6, 125.0, 124.9, 124.9, 122.7, 120.8, 119.9, 117.8, 114.4, 112.9, 112.8, 110.2, 55.7, 55.6, 52.6, 47.0, 36.4, 34.9; IR (cm^{-1}, KBr): 1712, 1608; MS (ESI) m/z 634 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{41}H_{36}N_3O_4$: m/z 634.2706; Found 634.2711.

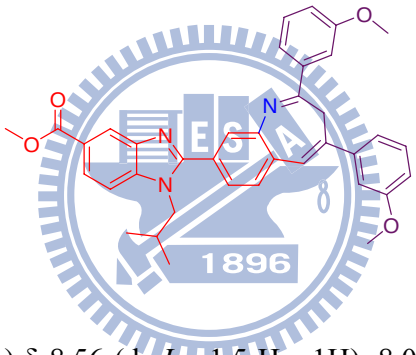
2-((1*E*, 4*E*)-2,4-bis(3-methoxyphenyl)-3*H*-benzo[*b*]azepin-8-yl)-1-isopropyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44k.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 1.4 Hz, 1H), 8.03 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.88 (d, *J* = 1.6 Hz, 1H), 7.68 (t, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 1.6 Hz, 1H), 7.50 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.45 (t, *J* = 2.4 Hz, 1H), 7.39-7.25 (m, 3H), 7.17 (s, 1H), 7.10 (t, *J* = 2.4

Hz, 1H), 6.94 (ddd, *J* = 6.2, 1.6, 0.6 Hz, 2H), 5.05 (sept, *J* = 7.0 Hz, 1H), 3.98 (s, 3H), 3.87 (s, 3H), 3.77 (s, 3H), 3.46 (brs, 2H), 1.68 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 160.3, 160.1, 158.5, 155.4, 147.1, 143.7, 141.4, 139.7, 137.3, 136.8, 131.6, 131.3, 130.3, 130.0, 129.4, 129.1, 126.6, 124.9, 124.7, 124.3, 122.9, 120.9, 119.9, 117.8, 114.3, 112.9, 112.7, 112.4, 55.7, 55.6, 52.5, 49.6, 35.0, 21.9; IR (cm^{-1}, KBr): 1712, 1606; MS (EI) m/z 572 (MH^+); HRMS (ESI, m/z) calcd for $C_{36}H_{34}N_3O_4$: m/z 572.2549; Found 572.2546.

2-((1*E*, 4*E*)-2,4-bis(3-methoxyphenyl)-3*H*-benzo[*b*]azepin-8-yl)-1-isopropyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 44l.



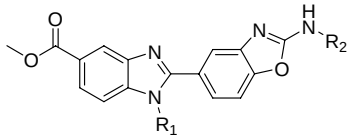
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 1.5 Hz, 1H), 8.07 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.95 (d, *J* = 1.5 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.59 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.50-7.46 (m, 3H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.25-7.22 (m, 1H), 7.17 (s, 1H), 7.13 (t, *J* = 2.4 Hz, 1H), 6.95 (ddd, *J* = 6.2, 1.7, 0.6 Hz, 2H), 4.26 (d, *J* = 7.5 Hz, 2H), 3.98 (s, 3H), 3.92 (s, 3H), 3.87 (s, 3H), 3.45 (brs, 2H), 2.19 (sext, *J* = 6.7 Hz, 1H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 160.3, 160.2, 158.6, 155.8, 147.2, 143.1, 141.5, 139.8, 139.6, 136.7, 131.6, 131.2, 130.3, 130.0, 129.3, 129.2, 126.7, 125.0, 124.9, 124.7, 122.7, 120.8, 119.9, 117.8, 114.3, 112.9, 112.7, 110.7, 55.7, 55.6, 52.7, 52.5, 35.1, 29.4, 20.4; IR (cm^{-1}, KBr): 1712, 1606; MS (EI) m/z 585 (M^+); HRMS (EI, m/z) calcd for $C_{37}H_{35}N_3O_4$: m/z 585.2628; Found 585.2623.

1.6. VEGFR-3 inhibitory activity

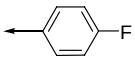
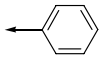
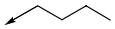
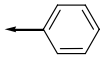
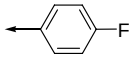
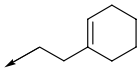
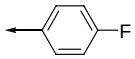
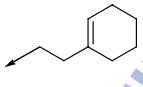
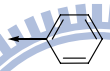
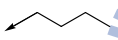
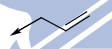
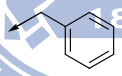
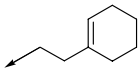
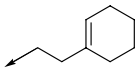
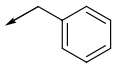
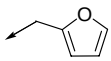
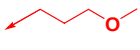
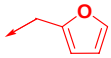
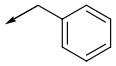
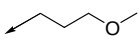
After the successful synthesis of benzoxazole *bis*-heterocyclic library, our attention for biological evaluation of these compounds was deviated towards the VEGFR inhibitors due to their structural resemblance with the potent KDR inhibitor and potential VEGFR-3 inhibitor. It is a well established fact in cancer biology that the tumor growth depends on the expression of various growth factors associated with angiogenesis and lymphangiogenesis. Recently, more advanced studies on the identification of roles of various VEGFR investigated that the VEGFR-3 has been implicated as key mediator of lymphangiogenesis in both normal biology and tumors. Consequently as an novel VEGFR target, we are intriguing to examine the new *bis*-heterocyclic scaffold for the inhibition of VEGFR-3.

Accordingly, the new benzimidazolyl linked benzoxazole derivatives **22a-p** were examined with *in vitro* inhibition of VEGFR-3 kinase cell based assay in the concentration of 3 μM on H928 cell lines. Interestingly, after preliminary screening it has been found that all the compounds have shown moderate to high inhibition from 15 % to 87 % (Table 6). Gratifyingly, compounds namely **22c**, **22f**, **22h-k**, **22m** and **22n** shows higher inhibitory activity against VEGFR-3. These selective compounds were further studied for the IC_{50} values and respective data was specified in Table 6. The comparative biological studies points to compound **22c**, **22m** and **22n** as the prominent compounds that inhibits receptor tyrosine kinase VEGFR-3. A generalization of this study suggests that the new benzimidazole linked benzoxazole *bis*-heterocyclic scaffold possess potential to inhibit receptor tyrosine kinase VEGFR-3 and further extension of this study can defiantly aid the development of novel cancer therapeutics.

Table 6. Percent inhibition of VEGFR-3 and IC_{50} (μM) value for selected compounds.



22

Compounds	R_1	R_2	Inhibition(%)[a]	IC_{50}[b]	LRMS[c]
22a			52		459
22b			40		441
22c			60	0.75	441
22d			29		459
22e			46		511
22f			69	1.19	493
22g			53		474
22h			76	1.03	405
22i			86	1.25	455
22j			63	1.18	457
22k			73	1.42	507
22l			15		457
22m			**81**	**0.56**	**461**
22n			87	0.70	467
22o			81		437
22p			22		417

[a] Results are expressed as the mean percent inhibition at 3μM level. [b] For the selective compounds having inhibition is more than 60%; values are an average of three individual determinations.

KIRA ELISA Assay (In vitro method for detecting VEGF R3 activity):

H928 cells ($2X10^5$) in 100 μl medium were added to each well in a flat bottom 24-well culture plate and cultured overnight at 37°C in 5% CO_2. After the supernatants were removed, the cells were serum-starved for 24h. A medium containing a test compound was added into each well and the cell culture was incubated for 30 minutes before it was stimulated by recombinant VEGF-C for 15 minutes. After the supernatants were removed, 100 μl of a lysis buffer were added into each well to lyse the cells and solubilize the VEGFR3. The lysis buffer included 150 mM NaCl containing 50 mM Hepes (Genentech media prep), 0.5% Triton-X100 (Genentech media prep), 0.01% thimerosol, 30 kIU/ml aprotinin (ICN Biochemicals, Aurora, Ohio), 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF; ICN Biochemicals), and 2 mM sodium orthovanadate. The plate was then put on a plate shaker (Bellco Instruments Vineland, N.J.) and the substance in each well of the plate underwent mixing for 6o minutes at room temperature. While the cells were being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the affinity-purified polyclonal anti-VEGFR3 (2.5 μg/well block buffer (PBS containing 0.5% BSA and 0.01% thimerosol) for 60 minutes at room temperature with gentle agitation. The anti-VEGFR3 coated plate was subsequently washed twice with a wash buffer (PBS containing 0.05% Tween 20 and 0.01% thimerosol). The lysate containing

solubilized VEGFR3 from the cell culture microtiter well were transferred (85 µl/well) to the anti-VEGFR3 coated ELISA plate and incubated for 2 h at room temperature with gentle agitation. The unbound receptors were removed by washing with a wash buffer. 100 µl of biotinylated 4G10 (antiphosphotyrosine) diluted to 0.2 µg/ml in dilution buffer (PBS containing 0.5% BSA, 0.05% Tween 20, 5 mM EDTA, and 0.01% thimerosol) were added into each well. After incubation for 2 h at room temperature, the plate were washed and 100 µl HRP-conjugated streptavidin (Zymed Laboratories, S.San Francisco, Calif.) diluted 1:2000 in dilution buffer will be further added. After the free avidin conjugate were washed away, 100 µl freshly prepared substrate solution (tetramethyl benzidine, TMB) was added to each well. The reaction was allowed to proceed for 10 minutes and the color development was stopped by the addition of 100 µl/well 1.0 M H_3PO_4. The absorbance at 450 nm and the absorbance at a reference wavelength of 650 nm ($A_{450/650}$) were measured using an ELISA reader and the data were repeated 3 times. The inhibition efficacy of each test compound is expressed as an inhibition percentage calculated according to following formula: 1-[(C-A)/(B-A)] X 100. In this formula, A is the basal amount of phosphotyrosine detected in a blank control, B is the amount of phosphotyrosine detected with VEGFR-C only, and C is the amount of phosphotyrosine detected with a test compound and VEGF-C. For IC_{50} generation, the final compound concentrations ranged from 0.1 µM to 10 µM.

1.7. References

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Chapter Two

Convergent Solution Phase Synthesis of Chimeric Oligonucleotides by 2+2 Phosphoramidite Strategy.

2.1. Introduction

1869 was a landmark year in genetic research, because it was the year in which Swiss physiological chemist Friedrich Miescher first identified a substance what he called "nuclein" inside the nuclei of human white blood cells. (The term "nuclein" was later changed to "nucleic acid" and eventually to "deoxyribonucleic acid or “DNA"). After 50 years of Miescher discovery, Russian biochemist Phoebus Levene discovered the the order of the three major components of a single nucleotide (phosphate-sugar-base); the first to discover the carbohydrate component of RNA (ribose), DNA (deoxyribose), and the first to correctly identify the way RNA and DNA molecules are put together. After thorough investigation, Levene's proposed the polynucleotide structure which was considered in many regards. For example, we now know that DNA is in fact composed of a series of nucleotides and that each nucleotide has three components: a phosphate group; either a ribose (in the case of RNA) or a deoxyribose (in the case of DNA) sugar; and a single nitrogen-containing base. We also know that there are two basic categories of nitrogenous bases: the purines (adenine [A] and guanine [G]), each with two fused rings, and the pyrimidines (cytosine [C], thymine [T], and uracil [U]), and each with a single ring. Furthermore, it is now widely accepted that RNA contains only A, G, C, and U (no T), whereas DNA contains only A, G, C, and T (no U) (Figure 1.1).

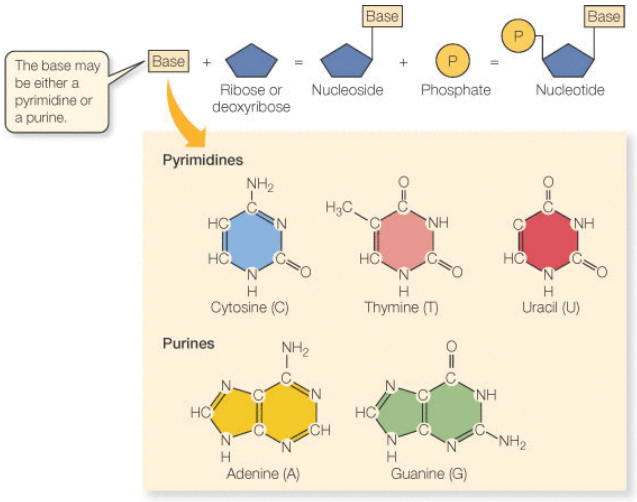


Figure 1.1. Nucleotides have three components

Based on these scientists discovery and putting all the experimental evidence together, Watson and Crick proposed the double helical structure of DNA and its model which has four major features remain the same today.[1]

Figure 1.2. James Watson and Francis Crick understanding the double helical model of ribonucleic acid

These four main features are

- DNA is a double-stranded helix, with the two strands connected by hydrogen bonds. Bases are always paired with Ts, and Cs is always paired with Gs, which is consistent with and accounts for Chargaff's rule.
- Most DNA double helices are right-handed; that is, if you were to hold your right hand out, with your thumb pointed up and your fingers curled around your thumb, your thumb would represent the axis of the helix and your fingers would represent the sugar-phosphate backbone. Only one type of DNA, called Z-DNA, is left-handed.
- The DNA double helix is anti-parallel, which means that the 5' end of one strand is paired with the 3' end of its complementary strand (and vice versa). Nucleotides are linked to each other by their phosphate groups, which bind the 3' end of one sugar to the 5' end of the next sugar.
- Not only are the DNA base pairs connected via hydrogen bonding, but the outer edges of the nitrogen-containing bases are exposed and available for potential hydrogen bonding as well. These hydrogen bonds provide easy access to the DNA for other molecules, including the proteins that play vital roles in the replication and expression of DNA.

For this remarkable discovery they have got the Nobel Prize in 1962 in Physiology or Medicine. With this discovery the door for the human life science research has opened. With the increasing demand human genome project, organic chemists are now actively engaged in the synthesis of DNA and RNA. Synthetic deoxyoligonucleotides of defined sequence have been used to solve important biochemical and biophysical problems.

Organic chemists interested in the synthesis of natural products are actively involves in the DNA and RNA synthesis to achieve the purpose of its application.

2.2. Introduction and Mechanism of RNA interference.

RNA interference (**RNAi**) is a system within living cells that helps to control the genes which are active and how active they are. Two types of small RNA molecules are there, one is microRNA (miRNA) and other is small interfering RNA (siRNA) are important to RNA interference. RNAs are the direct products of genes, and these small RNAs can bind to specific other RNAs and either increase or decrease their activity, for example by preventing a messanger RNA (miRNA) from producing a protein. RNA interference has an important role in defending cells against parasitic genes such as viruses and transposons. The RNAi pathway is found in many eularyotes including animals and is initiated by the enzyme dicer, which cleaves long double stranded RNA (dsRNA) molecules into short fragments of ~20 nucleotides. One of the two strands of each fragment, known as the *guide strand*, is then incorporated into the RNA-induced silencing complex (RSIC). The well-studied outcome is post-transcriptional gene silencing, which occurs when the guide strand base pairs with a complementary sequence of a messenger RNA molecule and induces cleavage by the catalytic component of the RISC complex. This process is known to spread systemically throughout the organism despite initially limited molar concentrations of siRNA as shown in Figure 1.3.

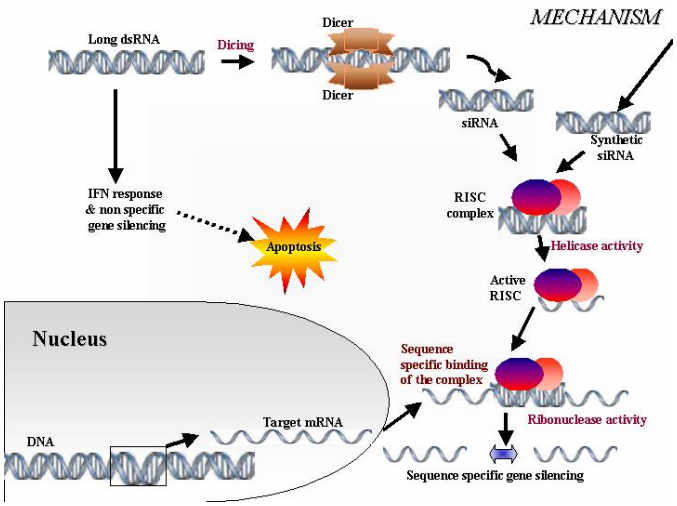


Figure 1.3. Mechanism of RNA interference.

The selective and robust effect of RNAi on gene expression makes it a valuable research tool, both in cell culture and in living organisms because synthetic dsRNA introduced into cells can induce suppression of specific genes of interest. RNAi may also be used for large-scale screens that systematically shut down each gene in the cell, which can help identify the components necessary for a particular cellular process or an event builds as a promising tool in biotechnology and medicine. Previously RNA interference was known as post transcriptional gene silencing. In 2006, Andrew Fire and Craig. C. mello shared the Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm C. elegans, which they published in 1998.[2]

2.2.1. Commonly used RNA Interference Drugs.

Fomivirsen (brand name **Vitravene**) is an antisence drug. It is used in the treatment of cytomegalovirus retinitis (CMV) in immunocompromised patients, including those with suffereing from AIDS. It is a synthetic 21 member oligonucleotide sequence with

phosphorthioate linkages (which are resistant to degradation by nucleases) and has the sequence 5'-GCG TTT GCT CTT CTT CTT GCG-3' which has show in figure 1.4. The mechanism of action involves the blocking of the translation of viral mRNA by binding to a coding segment of a key CMV gene. It was the first antisence antiviral approved by the FDA.[3]

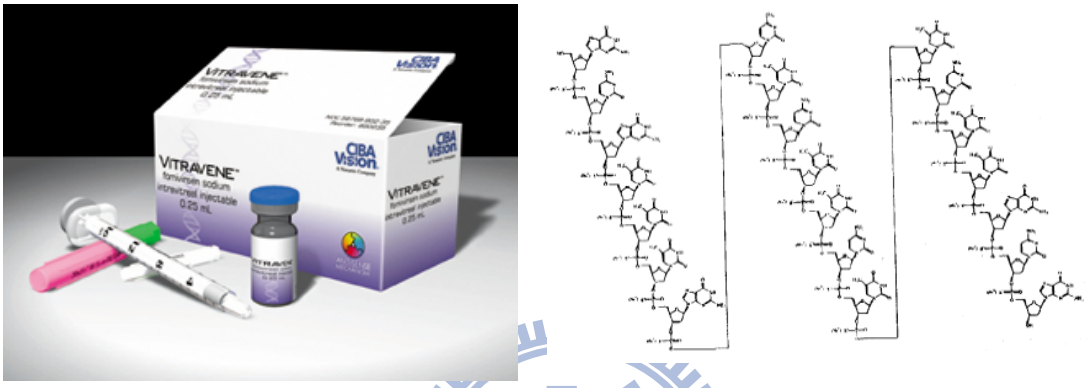


Figure 1.4. First antisence drug Vitravene.

2.3. Chemical Methods for Synthesizing Oligomeric Nucleotide

2.3.1. Solution Phase Synthetic Methods

In 1955, Michelson and Todd[4] first underwent the chemical synthesis of dinucleotide. They have achieved the target by reacting the 3′-O-acetylthymidine with *o*-benzylphosphorous-*oo*-diphenylphosphoric anhydride in dry acetonitrile solution using 2, 6-lutidine as base at room temperature to get the 5′-0-acetylthymidine-3′-Benzyl phosphate **2**. Subsequent reaction of **2** with N-chloro succinimide in dry benzene and acetonitrile generates the 3′-benzyl phosphorochloridate-5′-dibenzyl phosphate **3** which after condensation with 3′-*O*-acetylthymidine **4** and subsequent deprotection to get the dithymidine dinucleotide **6** in good yields as in figure 1.5.

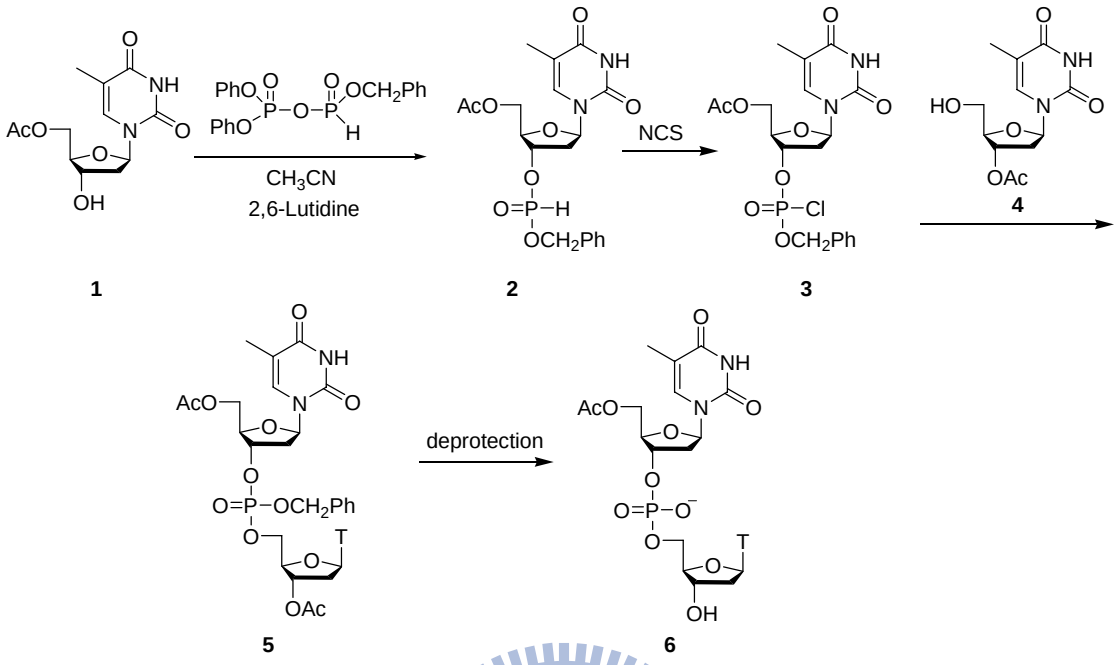


Figure 1.5. Michelson and Todd synthesis of 3′→5′ dithymidine dinucleotide.

In 1957, Hall and Todd[5] used the mixed anhydrides as Intermediates in the synthesis of dinucleoside phosphates. They used the 2′ and 3′ protected uracil hydrogen phosponate **7** which reacted with diphenylphosphorchloridate as an activator and 5′-OH-2′ and 3′ protected adenine **8** to generate the compound 9 which after deprotection with N-chlorosuccinimide to get the dinucleoside phosphate derivatives **9** as shown in scheme 1.6.

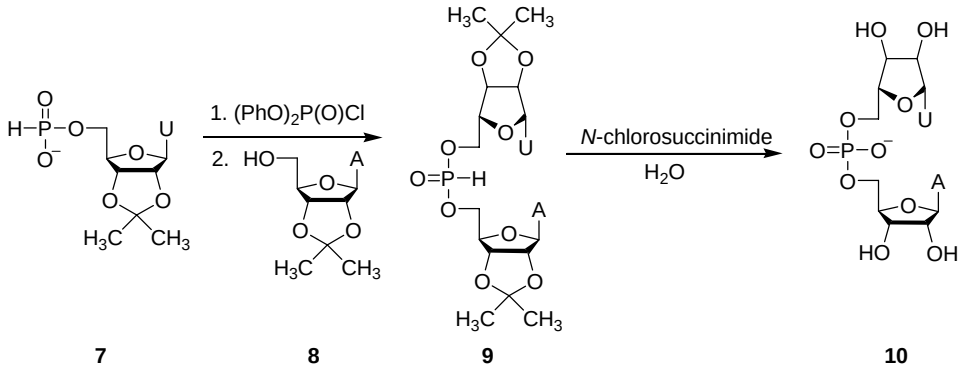


Figure 1.6. Hall and Todd synthesis of dinucleoside phosphates.

In 1968, H.G. Khorana[6] proposed the 'phosphotriester' strategy for the log chain oligonucleotide synthesis. In this methodology, while doing the synthesis four points had taken care of. First one, the 5'-hydroxyl group of the nucleoside component **11** is blocked by the classical bulky trityl group which can be removed by acid treatment when desired. Second one, the 3'-hydroxyl of the nucleoside component is free. Third one, the second component involved in the reaction is a 5'-mononucleotide **12** whose 3'-hydroxyl group is blocked by an acetyl group. This group is removed by very mild alkaline treatment when required which after condensation using DCC or aryl sulfonyl chloride generates **13** and further deprotection provides **14**. Fourth one, the phosphate group has been used as the monoesterified component directly for the condensation step as shown in figure 1.7.

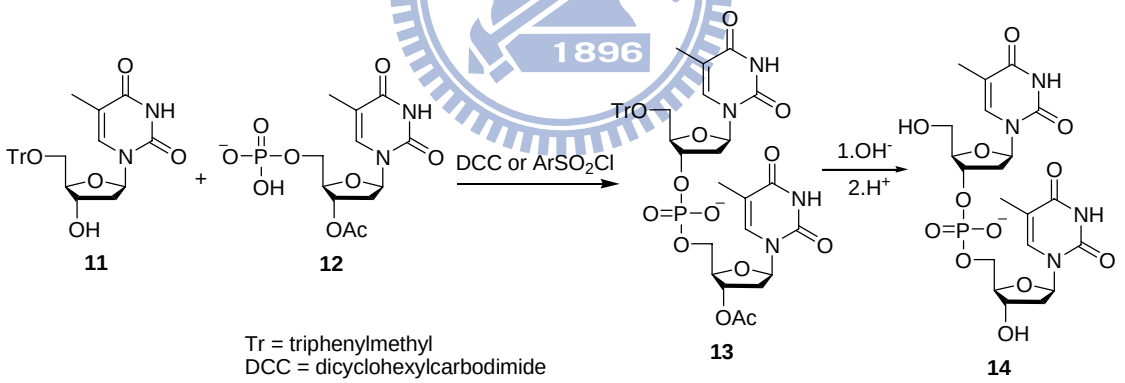


Figure 1.7. Khorana's phosphotriester strategy for the long chain oligo nucleotide synthesis.

However, for the last two decades this strategy proved useful for long chain oligonucleotide synthesis in solution phase. The only drawback of this methodology is

that both the starting material and product mixtures are ionic in nature which needed tedious ion-exchange chromatography for purification.

In an attempt to solve the purification problem, Letsinger and Ogilvile[7] used the methods which involves the protection of 5' position of thymidine nucleotide **15** with 4-methoxy trityl group and subsequent condensation with **16** in presence of MS-Cl (2,4,6-trimethylbenzenesulfonyl chloride) provided phosphate monoester 16 which after subsequent condensation with thymidine **17** in presence of TPS-Cl (2,4,6-triisopropylbenzenesulfonyl chloride) to get the phosphate diester **18**, which can be purified easily as shown in figure 1.8

In 1965, Letsinger and Mahadevan[8] used the solid phase synthetic methodology derived from the Merrifield idea of solid phase synthesis for the synthesis of nucleotide oligomers. The main advantages of this methodology involves the easy removal of excess reagent or by product by simple filtration and no column chromatography needed for the purification saved a lot of time.

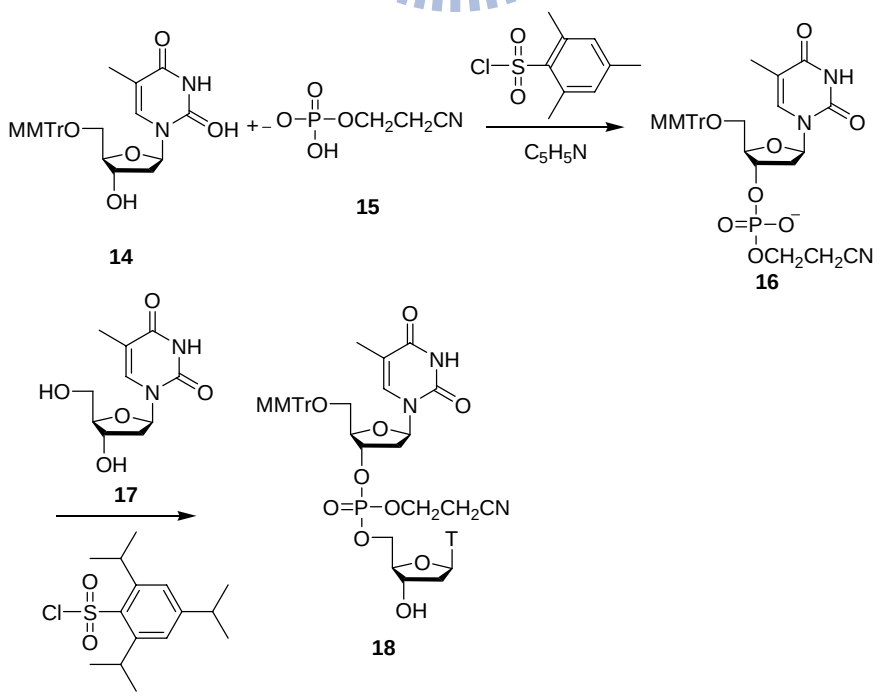


Figure 1.8. Letsinger and Ogilvile 'phosphotriester' strategy for the oligo nucleotide synthesis.

In 1975, Letsinger[9] discovered new strategy for the oligonucleotide synthesis. Letsinger identified that 5′ protected thymidine **19** reacted with 2,2,2-trichloroethylphosphodichloridite act as activator in 2,6-lutidine generated **20**, which further underwent condensation with 3′ protected thymidine derivative **21** to get the phosphate trimester **22**, which after oxidation with I_2 in aqueous solution to get the phosphate trimester **23** in good yields as shown in figure 1.9.

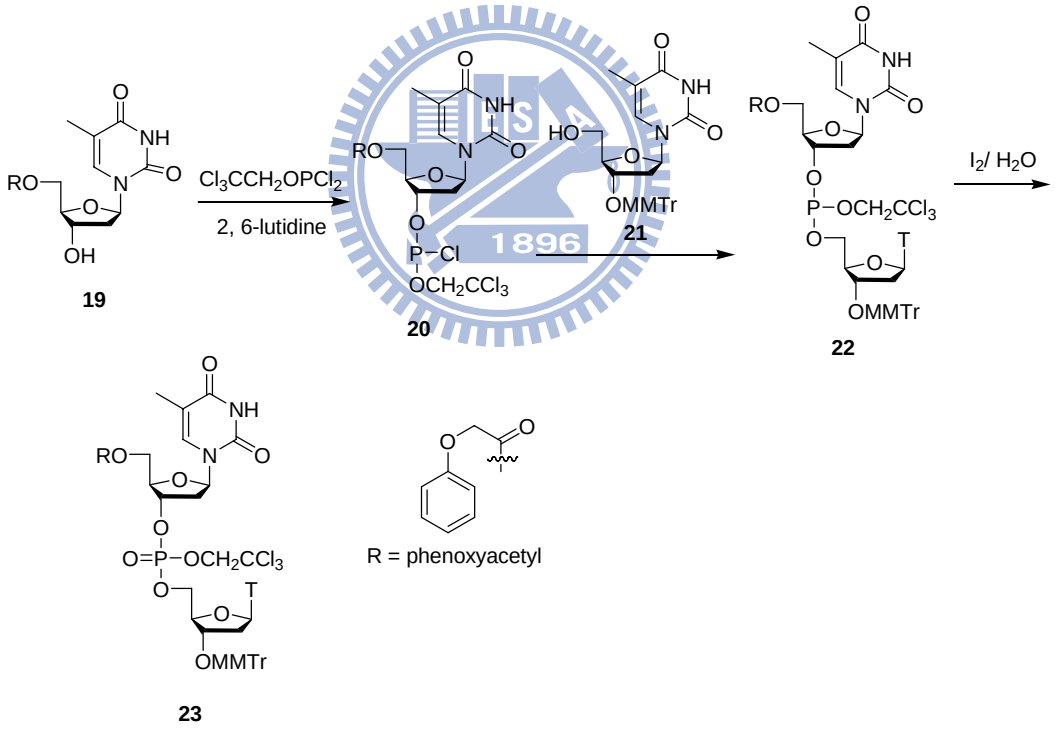


Figure 1.9. Letsinger's methodology for oligonucleotide synthesis

However this methodology offered drawbacks as the intermediate **20** is very air sensitive and decomposes easily on exposure to air.

In an attempt to solve the stability problem of intermediates, in 1981, Beaucage and Caruthers[11] developed new synthetic strategy involving 'phosphoramidite' approach. The synthetic strategy involves the protection of the 5′ position of thymidine by two methoxy trityl group to get **24**, which further reacted with chloro-(N, N-dimethylamino) methoxyphosphine **25** to achieve the phosphoramidite intermediate **2**6, the phosphoramidite **25** was then underwent condensation with thymidine **27** protected in the 3' position with levulinyl moiety using 1*H*-tetrazole as an activator to generate the phosphite trimester **28**. The final product was obtained by the oxidation of the product **28** to get phosphate triester **29** as shown in figure 1.10. However it has been observed that the replacing the *N*, *N*-dimethyl amino group with *N*,*N*-disopropyl amino group[12] involves the high stability and easy purification of the product mixture to get the automated synthesis of 12-mer oligonucleotide sequence.[13]

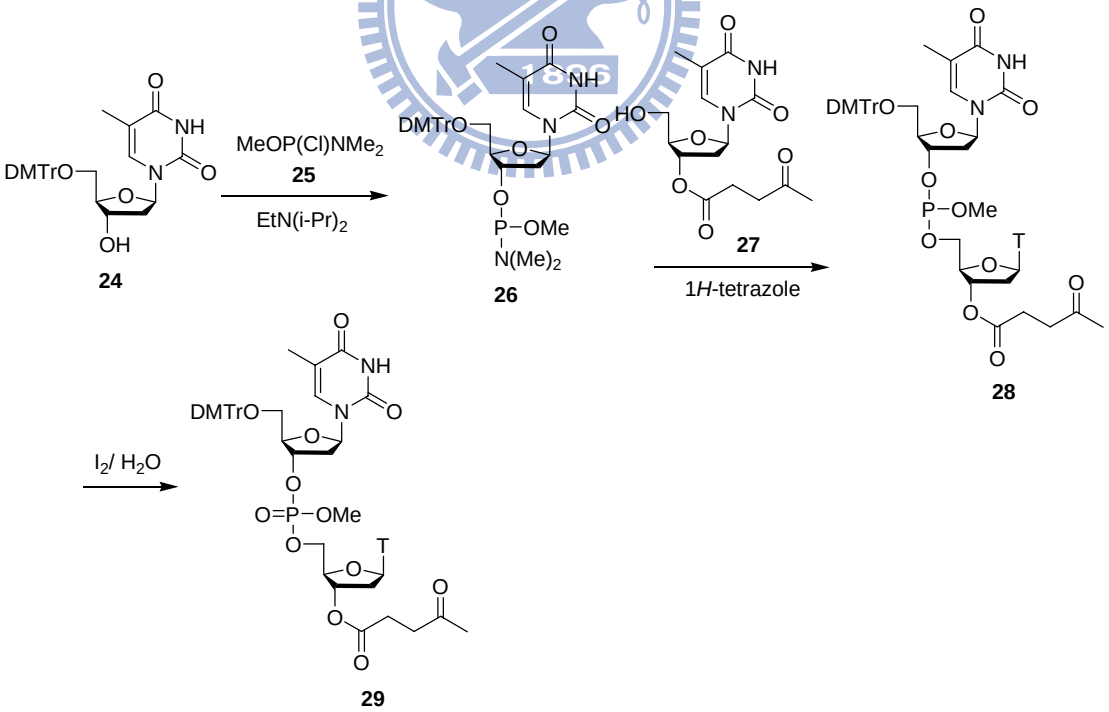


Figure 1.10. Beaucage and Caruthers developed new synthetic strategy involving 'phosphoramidite' approach.

In 1985, Gregg[14] invented the "1*H*-phosphonate" strategy for the oligonucleotide synthesis. This strategy involves the protected 5′ position of thymidine moiety by two methoxy trityl group **30** reacted with 3'protected thymidine derivative **31** in presence of **32** as an activator to generate the 33 which after aqueous oxidation with I_2 solution to get the dimeric nucleotide sequence **34** as in figure 1.11**.**

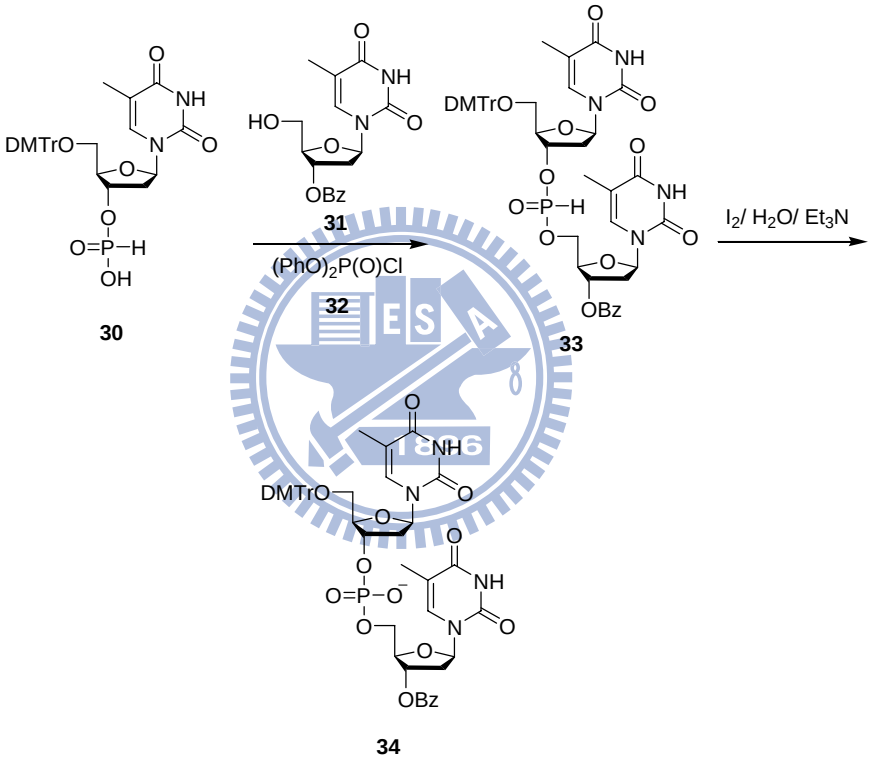


Figure 1.11. Gregg's methods of oligonucletide synthesis

Furthermore it has been observed that the "phosphoramidite" synthetic strategy is efficient and time saving and "H-phosphonate" synthetic strategy is only applicable to the short chain oligomeric nucleotide synthesis.

2.3.2 Polymer Supported Approach to Oligomeric Nucleotides

Because of excessive difficulty in purification process, it is not at all easy for oligomer synthesis, Scientists have changed their attention to the synthesis of on solid phase supports for which commonly used supports are controlled pore glass (CPG), polystyrene, silica gel, and cellulose paper. But by using solid-phase synthesis amount received for nucleotide oligomers relatively small, about 1 ~ 10 μmol. This amount may able to meet the demand of research needs, but not on the future clinical tests.

In 1993, Bonora[15] used the polyethylene glycol (PEG) as soluble support and the "phosphoramidite" synthetic strategy to synthesize oligonucleotides, The advantage of this method is that it can increase the capacity of nucleotide oligomers to 100 μmol. The use of soluble polymer support overcome several problems faced by solid phase synthesis such as polyethylene glycol is soluble in many organic solvents such as dichloromethane, acetonitrile, and methanol but remain insoluble in ether which facilitates the easy purification by adding the large amount of ether to precipitate out the reaction mixture and then simply filter the product. This process of purification reduces the excess reagent and shortens the reaction time. These two advantages reduce the costs of synthesis of nucleotide oligomers and improve synthetic efficiency. But only problem is that repeated precipitation will lead to the same amount of product loss to the synthetic sequence.

In this methodology the time required for the synthesis of nucleotide oligomers is about four hours, that is, also considering the time required in the cycle containing (detritylation), condensation (condensation), capping and oxidation (oxidation) time as shown in Table 1.

Table 1. Soluble polymer supported synthesis of oligonecleotide

Detritylation	3% TCA in CH_2Cl_2	20 ml	15 min
Condensation	Amidite 0.1 M Tetrazole 0.5 M in CH_3CN	2.5 equiv. 10.0 equiv. 10 ml	5 min
Capping	Acetic anhydride 2,6-lutidine NMI	1 ml 1 ml 1 ml	3 min
Oxidation	TBHP in CH_3CN	1 ml 20 ml	15 min

[a]Quantities are for 1.0 g of PEG_{5000}-oligonucleotide.

To overcome these limitations, Vasseur[16] designed a new approach where polymer-bound reagents promote chemical transformations of the oligonucleotide which is in solution. Indeed, solid-supported reagents reduce or even eliminate purification steps since they are simply removed from the reaction mixtures by filtration. This approach was applied to the synthesis of several di- and trinucleotides with oxo and thiono phosphotriester internucleosidic linkages.

Vasseur's examination focused on the phosphoramidite method using tetrazole which is the is the most extensively employed activator of the reaction between the 5′- hydroxyl function of nucleosides and nucleoside 3′-*O*-phosphoramidites leading to a phosphite triester internucleosidic linkage. Because of high purity owing to dangerous sublimation and easy to explode tetrazole is not used as activator. Polystyrene sulfonic acid resins (DOWEX 50W X8 or Amberlist 15) employed as polyvinylpyridinium tosylate **37** were tested as phosphoramidite activators. In this case, the coupling between phosphoramidites **35** and 5′-hydroxyl **36** performed with resins **37** gave the phosphite triesters **38** were contaminated with the excess of starting phosphoramidites **35** and with cyanoethyl-

phosphonate diesters **39** resulting from partial hydrolysis of **35** due to the water content of the resin which can be purified by addition of water. The crude mixtures were directly used for the next step which involves the conversion of phosphite triesters **38** to phosphate trimester **42** which was achieved by using the reagent **40** and **41** respectively to generate the oxo and thiono phosphotriester linkages **42a-b**, **43a-b** respectively. In this approach, the crude mixtures obtained from the oxidation or sulfurization were treated with 2 % *p*-toluenesulfonic acid in CH_2Cl_2-MeOH, 7:3 v/v, at 0 °C to deprotect the 5′-*O*-DMTr **44a-b** and **45a-b** respectively. To allow elongation from the 3′-side of building blocks, the delevulinylation reaction was performed from fully protected crudes containing oxo- or thiono-phosphotriesters **43a-b** respectively as in figure 1.12.

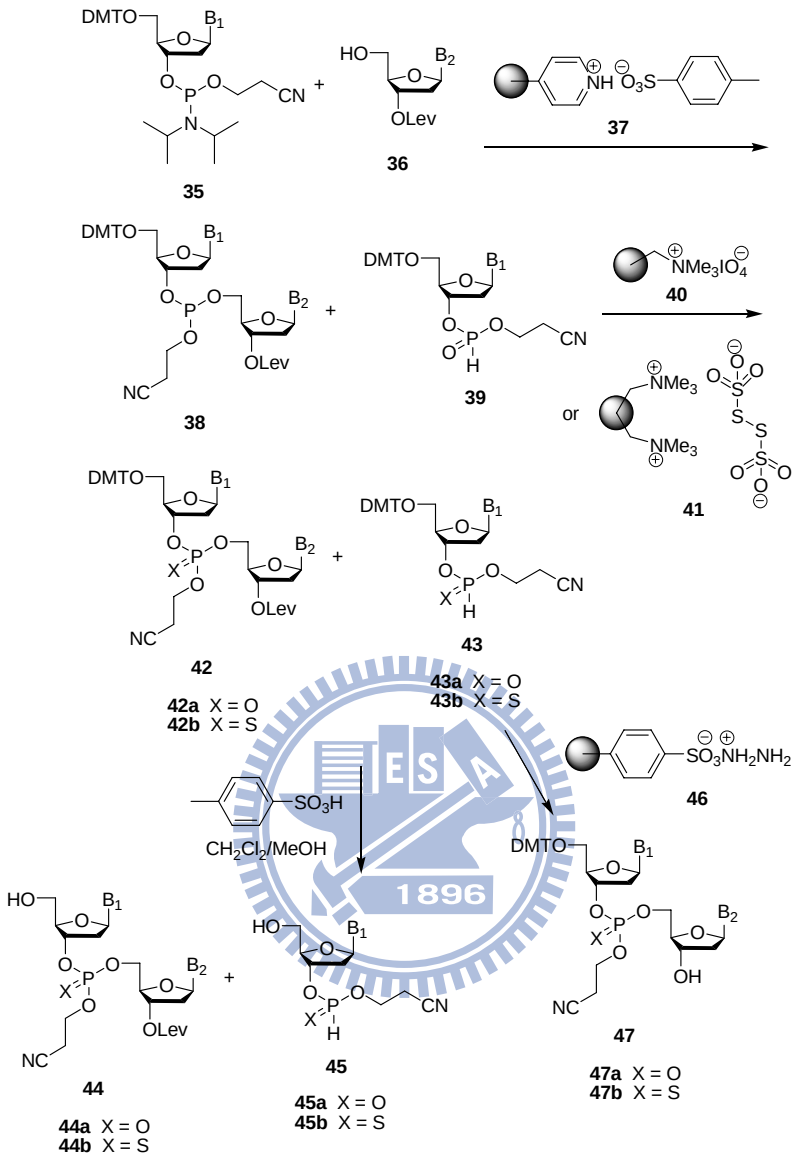
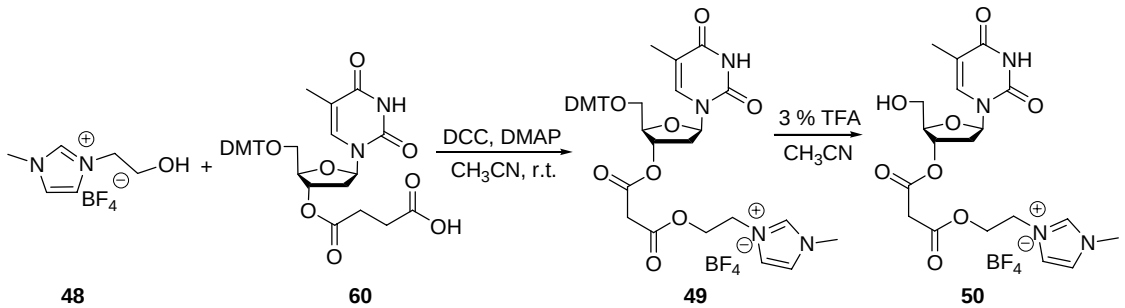


Figure 1.12. A new solution-phase phosphoramidite approach for oligonucleotide synthesis employing recyclable solid-supported reagents

The 3′-*O*-levulinyl protecting group is readily removed by the polymer supported reagent **46** in 3 hours to generate the compound **47a-b** as shown in figure 1.12.

Damha *et.al.*[17] have described the synthesis of oligonucleotides in solution using a soluble ionic liquid as support. Short oligomers of varying base composition were synthesized using this method in high yields and high purity, requiring no chromatography for purification prior to cleavage from the support. The synthesis of the ionic liquid supported oligonucleotide began with the ionic liquid **48**. The succinylated 5′-DMT-thymidine derivative **60** was then coupled to the ionic liquid **48** using dicyclohexylcarbodiimide (DCC) and catalytic amounts of 4-(dimethylamino) pyridine (DMAP) in acetonitrile to give the ionic liquid supported nucleoside **49**. Exposure of **49** to acidic conditions yielded compound **50**. The dinucleoside phosphotriesters TpT, ApT, CpT, and GpT **51** were prepared at the 250 µmol scale by reacting the ionic liquid supported nucleoside **50** with a 1.5-fold excess of the appropriate phosphoramidite derivatives **a-d** using 4,5- dicyanoimidazole (DCI) as the activating agent in CH_3CN and stirring for 1-2 h. To carry out the oxidation of the phosphite triester intermediates, compounds from previous steps was again dissolved in a small amount of CH_3CN, pyridine, or 2,4,6-collidine, and a large excess (2-5 equiv) of a 0.1 M solution of iodine in 2:1 THF: water was added. Similarly 5′detritylation of **51** was achieved using 3 % trifluoroacetic acid in 20 minutes. In addition to the dimmers, a thymidine trimer, and a tetramer were also synthesized at the 50-100 µmol scale as shown in figure 1.13.



48 **60** **49** **50**

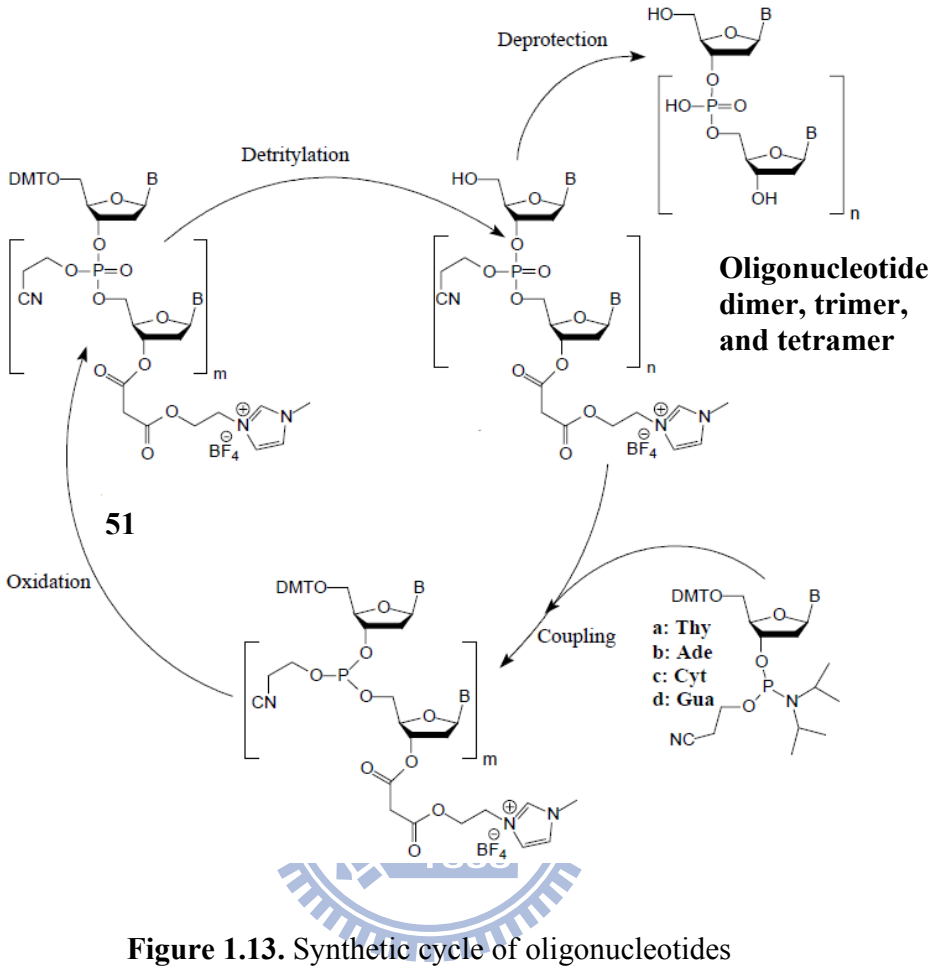


Figure 1.13. Synthetic cycle of oligonucleotides

The solution-phase-synthesized oligomers were compared to the same sequences prepared using standard gene machine techniques by LCMS. This methodology may provide a cheaper route for the large-scale synthesis of oligonucleotides.

2.4. Purification of oligomeric nucleotides.

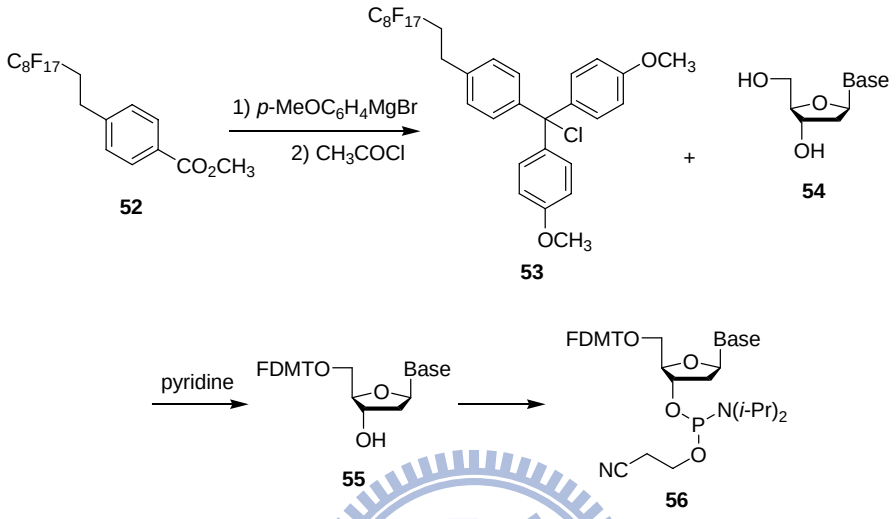
There are commonly used method for the purification of oligonucleotides such as anion exchange chromatography (AX), reverse phase chromatography and polyacrylamide gel electrophoresis. Most commonly used method is the affinity chromatography as proposed by Seliger in 1978.[18] Seliger observed that the solid phase approach to oligomeric nucleotide synthesis has the disadvantage that the support is a polyfunctional blocking group. This fact essentially necessitates a synchronous growth of the grafted chains. If this cannot be achieved the product released from the support contains the desired sequence together with homologous and truncated compounds. The difficulties encountered in separating this mixture may limit the applicability of the support synthesis. For this reason, Seliger had used affinity blocking groups in the separation of products from the nucleotide condensation. With this introduction of terminal affinity blocking groups permits the removal rapidly and selectively, of the desired sequence from a solid phase oligonucleotide product, clearly improves the efficiency of the support method and extend its range to longer chains oligonucleotide synthesis.

In 2005, Pearson *et. al.*[19] described the fluorous affinity purification of oligonucleotides, a rapid and convenient method that, when compared to existing methods that rely on 5′ affinity tagging, results in unusually high recoveries of materials that are free of failure sequences, even with long oligonucleotides. It has been found that the automated synthesis is the best available strategy for the production of oligonucleotides but due to the production of heterogeneous mixtures complicates the product purification as well as limiting scale-up. These problems are exacerbated as the length of the strand increases. A popular purification strategy uses reversed-phase HPLC is often performed on oligonucleotides with a 4,4′-dimethoxytrityl (DMT) group on the 5′-terminus, so-called

"DMT-on" or "trityl-on" purification, wherein the DMT group of the final nucleotide is left on during synthesis, The DMT group functions as a lipophilic handle, allowing the separation of DMT-bearing oligonucleotides from those without DMT groups (e.g., failure sequences), since the DMT group imparts added hydrophobicity to the molecule and thus causes it to be more strongly retained. The DMT group is subsequently removed with acid to afford the desired oligonucleotide. If that level of purification is not required, RP-HPLC may be used to purify target oligonucleotides of up to about 50 nt by the DMT-on technique (depending on the particular case), with varying degrees of success in removing failure sequences. When the oligonucleotide becomes lengthy, the failure sequences and other undesired by products are more strongly retained, often running close to (or merging with) the desired product peak, making separation difficult or impossible using this technique. An additional limitation of trityl-on purification by RP-HPLC is low recovery. Researchers have explored more lipophilic trityl groups in an attempt to remove these limitations by developing stronger hydrophobic affinity interactions with the adsorbent. Trityl groups bearing long alkyl chains or extended aromatic systems have been described as removable lipophilic tags for RP-HPLC.

Ideally, the fluorous moiety would be incorporated into a standard DMT group in such a way that the resultant FDMT group behaves identically, so that the phosphoramidite coupling and detritylation steps will also be similar. So for this reason, Pearson reacted the methyl 4-(1*H*,1*H*,2*H*,2*H*-perfluorodecyl) benzoate **52** with 4-methoxyphenylmagnesium bromide in dry THF to generate the 3^{o}-alcohol which further reacted with acetyl chloride to get the compound **53** containing fully fluorinated long chain alkyl (C_8F_{17}) group. Compound **53** then reacted with DNA base pair **54** under

optimized condition of coupling generated **55**. 5′–FDMT protected compound **55** underwent phosphotidylation reaction with two different reagent gave phosphoramidites **56** in good yields as shown in figure 1.14.



Series	Base	yield of **55**	yield of **56**	phosphitidylation condition*
a	thymidine	73 %	79 %	A
b	N^6-benzoyladenine	91 %	71 %	B
c	N^4-benzoylcytosine	96%	62 %	B
d	N^2-isobutyrylguanine	96 %	84 %	B

*phosphitidylation condition:
A : $(i\text{-Pr})_2NP(Cl)O(CH_2)_2CN$, $(i\text{-Pr})_2NEt$
B : $[(i\text{-Pr})_2]_2NPO(CH_2)_2CN$, tetrazole

Figure 1.14. Synthesis of 5′–*O*–FDMT protected phosphoramidites using fluorous tagged reagent.

From a synthetic standpoint, the FDMT group was found to behave identically to a DMT group throughout this sequence. Examples of oligodeoxyribonucleotides (ODNs) **57-60** were prepared as in figure 1.15, where phosphoramidite **56** was used in the last coupling step to install a fluorous-tagged thymidine at the 5′ terminus followed by oxidation.

After HPLC analysis of the product mixture, solid phase extraction (SPE) method using fluorous phase column was used. It has based upon the principle that fluorous-tagged materials are highly retained in preference to non fluorous materials such as failure sequences and DMT-bearing impurities.

57 (30 mer): 5' **FDMT**-TTTCTCTAGACAATTGTGCAATACGTCTTT

58 (50-mer): 5'-**FDMT**-TTTCTGTTGACAATTTATCATCGGTCGTAT-AATGTGTGGAATTGGTCTTT

59 (75-mer): 5'-**FDMT**-TTTCTGGTTAAGGTGTGTATATGCTCGGC-TACTAATTAGTGAGTATTATCTCGCTAC-ATTAACAGTTGTCTTT

60 (00-mer): 5'-**FDMT**-TTTCTGGTTAAGGTGTGTAATATGCTCAGC-TACTAATTAACAGTTGTCTAAGCTGGTTAA-CGTGAGTAATATGATCAGCTACTATTTAAC-AGTTGTCTTT

Figure 1.15. . Examples of oligodeoxyribonucleotides (ODNs) **57-60.**

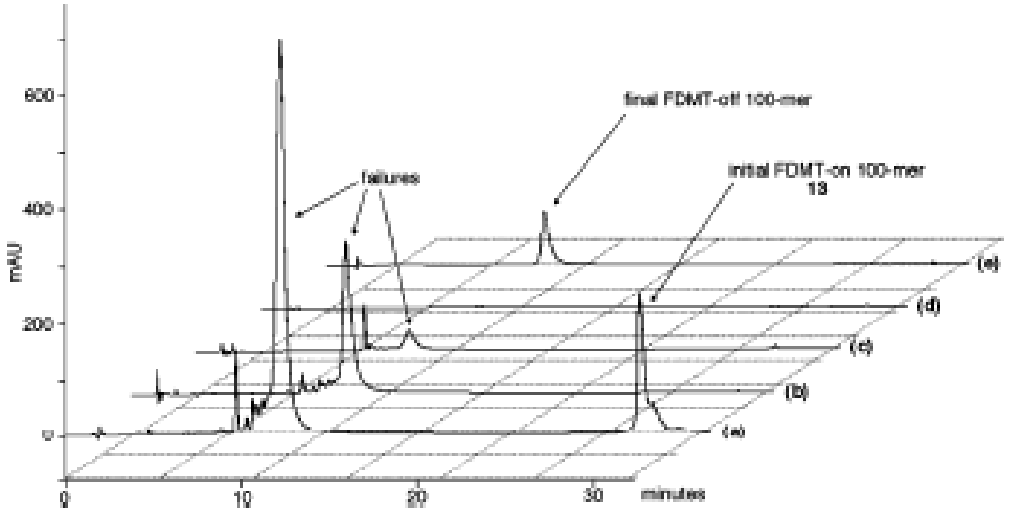


Figure 1.16. High Performance Liquid Chromatography (HPLC) analysis of fluorine-phase solid phase extraction (SPE) purification of 100 base pairs of nucleotide oligomers. Trace (a): Crude synthesis products before purification. Trace (b): Eluate from loading of FDMT-100-mer **60** onto a Fluoro-Pak column, showing that was fully loaded in one pass. Trace (c): Eluate from washing the column with 10 % acetonitrile in 0.1M TEAA followed by water, showing that the remainders of the failure sequences are eluted

without eluting the fluorous-tagged oligonucleotide. Trace (d): Eluate from a second washing of the column with 10 % acetonitrile in 0.1 M TEAA followed by water, showing that all of the failure sequences had been removed by the first failure wash. Trace (e): Elution of the final FDMT-off 100-mer after on-column detritylation with trifluoroacetic acid aas in figure 1.16.

In 2005, Bannwarth[20] also made the same oligonucleotide purification method. The only difference is the use of the protecting group with two fully fluorinated long alky chain. Compound **61** was reacted with n-butyl lithium then twice with **62** methyl tertiary alcohol to get the compound **63**, continued to react with acid chlorides generated compound **64** which further reacted with 5 'position of thymidine **65** to get the 5' protected base **66**. Compound **66** can undergo phosphitidylation reaction with phosphoramidite to get the compound **67** as in figure 1.17.

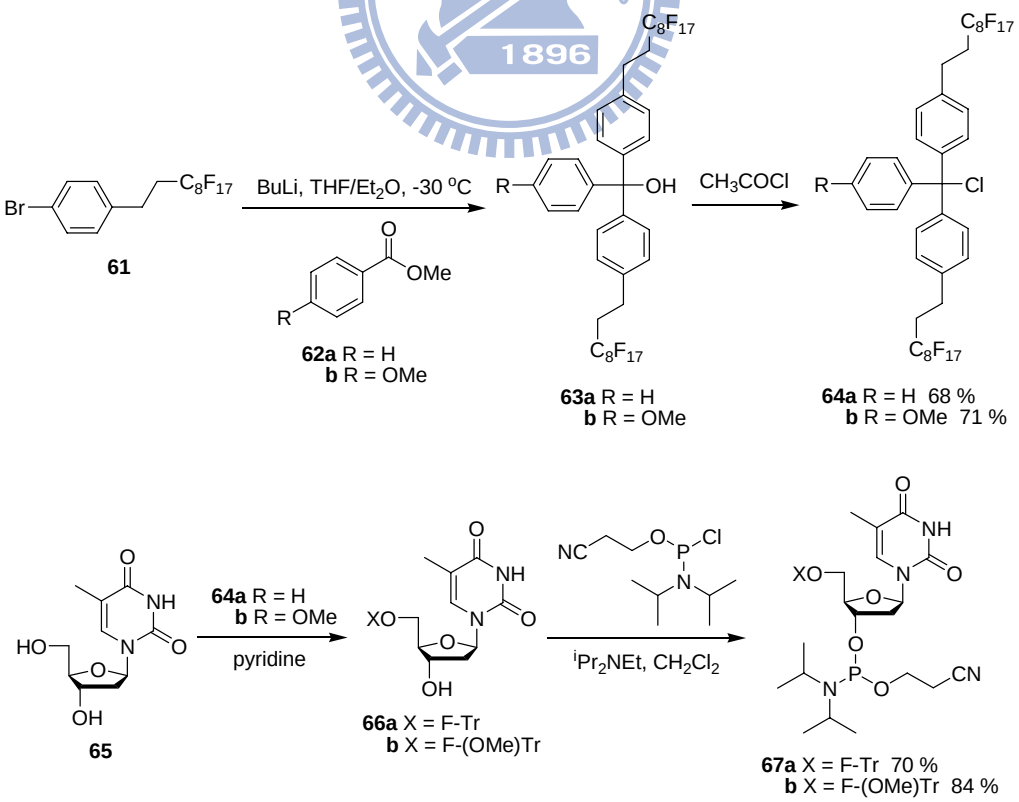


Figure 1.17. Bannworth oligonucleotide purification

2.5. Results and Discussions.

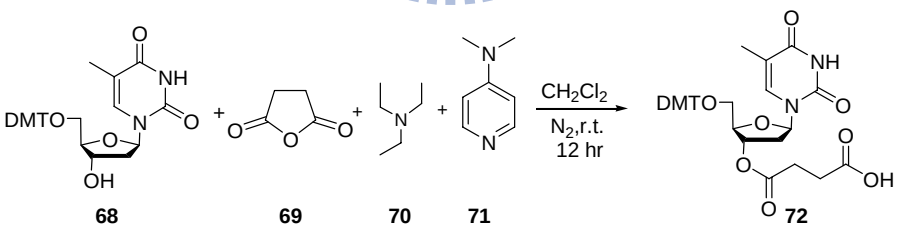
In this study, we tried to present a novel convergent soluble polymer supported approach to synthesize oligonucleotides using the phosphoramidite method. Convergent 2+2 and 3+3 synthetic routes can minimize the number of synthesis steps and produce a high quality product that does not suffer from the pollution of $n-1$ failures. Polyethylene glycol-2000 (PEG-2000) as soluble support and phosphoramidite as synthon. The stepwise formation of the oligonucleotide up to 4th stage was confirmed by ^{31}P, ^{1}H NMR as well as MALDI-TOF-MS. Herein, we can observe that the analytical techniques currently used for characterizing of synthetic biopolymer, mass spectrometry is of increasing importance. Among the modern soft ionization techniques such as electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) has an enormous impact on the analysis of biopolymers such as oligonucleotides, protein and peptides[1]. It has been recognized that ESI and MALDI MS are amenable to polymer analysis as well, although the synthetic polymer situation is more complex than the protein situation due to the coexistence of several molecular weight distribution[2-4]

- Instead of a single molecular weight, we have to deal with a molecular weight distribution (MWD) as a result of polymer synthesis,
- The polymer chain might have different end group chemistries due to different initiation and termination processes, thereby creating a functionality type distribution (FTD)

- In case of random copolymers, the polymer chains show a chemical composition distribution (CCD)
- In case of block copolymer, additional sequence and block length distribution are present.
- Copolymers show an architecture distribution (linear, cyclic, branched, dendritic).

Mass spectrometry, in particular MALDI-MS, has often been used as a powerful method to characterize the large molecular weight oligonucleotide also it is a valuable tool for analyzing potential defects in their structure which results from incomplete synthetic strategy.

We need to first synthesized the 5'-O-DMT-2-deoxynucleoside-3-O-succinate **72** started from the 5'-*O*-DMT-2-deoxynucleoside-3'-OH **68**, succinic anhydride **69**, triethyl amine **70**, and *N,N'*-dimethyl amino pyridine (DMAP) **71** in dichloromethane under N_2 atmosphere. The scheme **11** has been depicted below.



Scheme 1. Synthesis of starting material 72.

The formation of the compound can be better understood from studying the mechanistic pathway. Here we have found that the lone pair present on the N-atom of pyridine moiety of compound **71** attacks the ester linkage of succinic anhydride **69**, to form **a** which was further attacked by lone pair of oxygen atom of 5'-*O*-DMT-2-deoxynucleoside-3'-OH **68**

to generate the compound **72** as shown in figure 1.18. After completion of the reaction as checked by TLC, the crude product was purified by column chromatography using ethyl acetate as eluant to isolate the compound **72**.

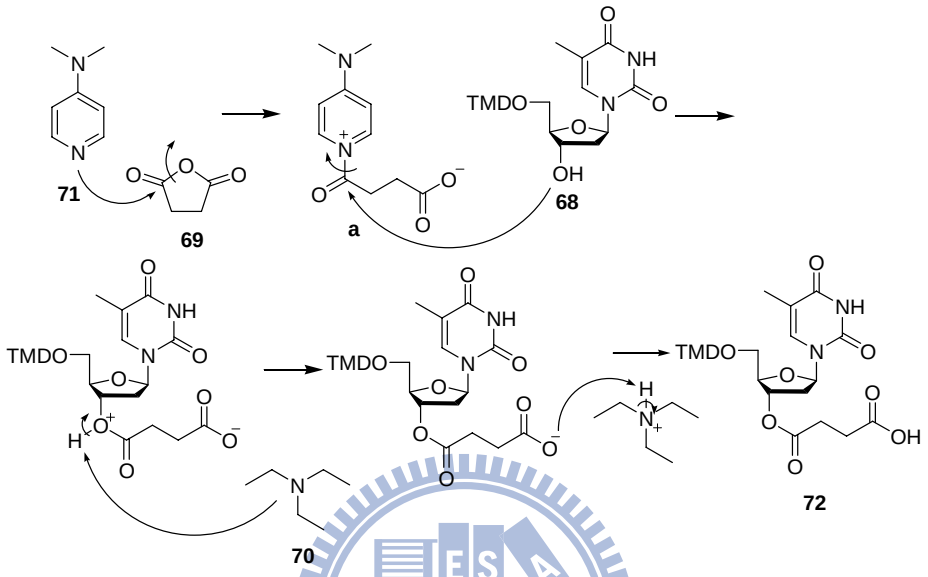
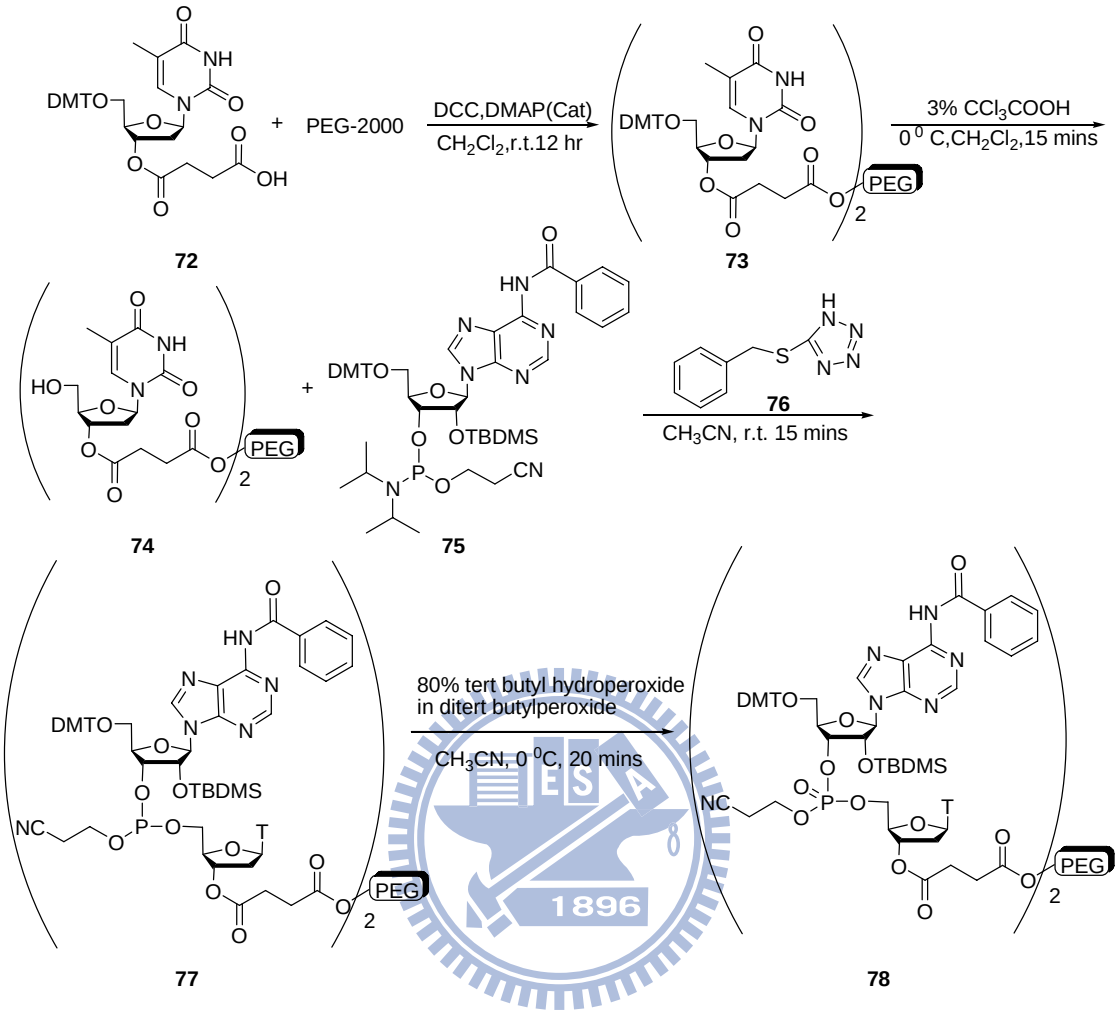


Figure 1.18. Mechanism of formation of compound 72.

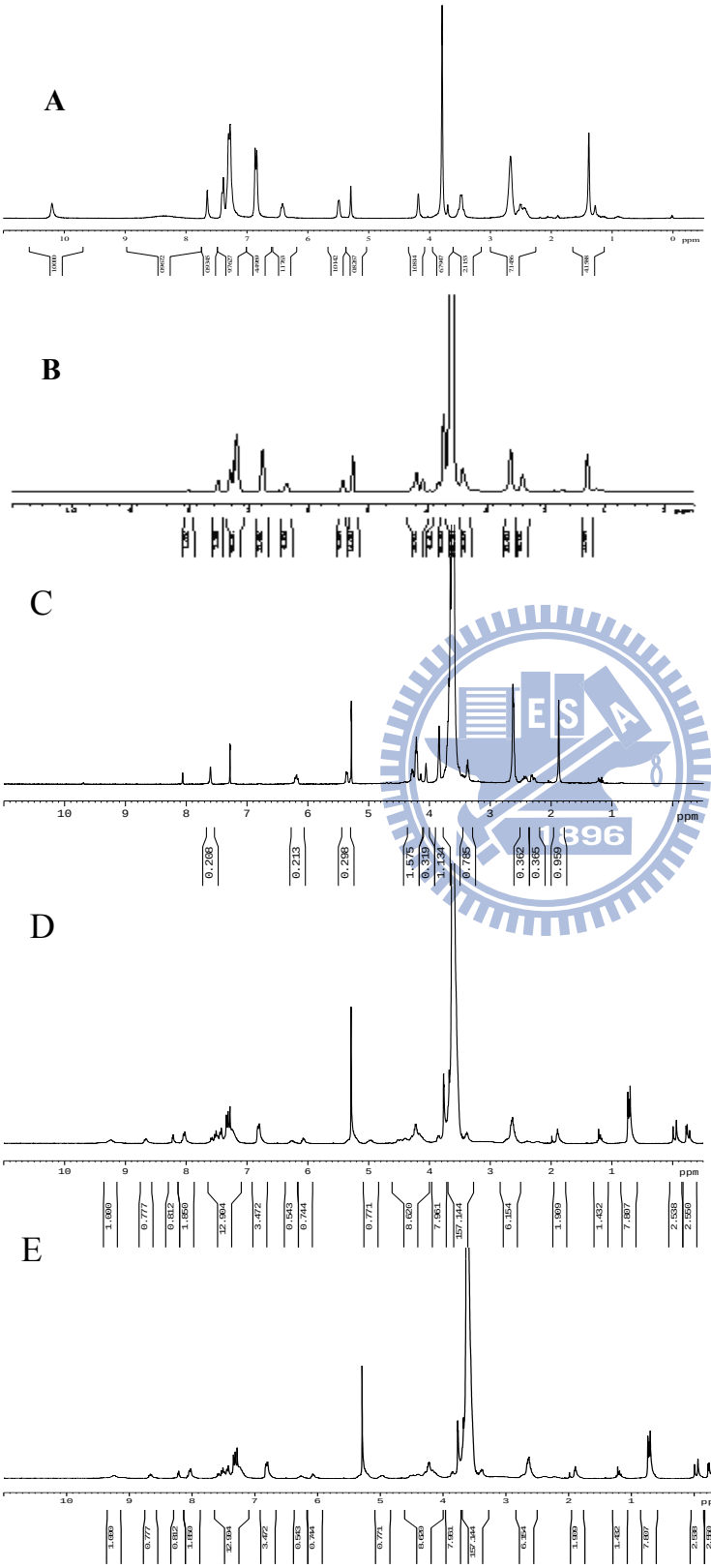
The monitoring of the present synthetic scheme was accomplished by ^{1}H NMR spectroscopy (Figure 1.19). In spectra A, the formation of the product **72** was confirmed by the change of the chemical shift of the proton attached to the 3'-OH of deoxynucleoside ring in the starting material came at 4.59 ppm but after reaction it moved to downfield at 5.48 ppm in the product. Coupling of **72** was achieved using PEG-2000 as soluble support in presence of *N,N'*-dicyclohexylcarbodiimide (DCC) as coupling reagent in CH_2Cl_2 at room temperature under N_2 atmosphere to obtained compound **73** as shown in scheme 2.

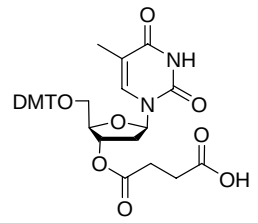


Scheme 2. Stepwise formation of 5'-*O*-DMT $A^{Bz}T$ 3'-*O*-Succinate on soluble polymer support.

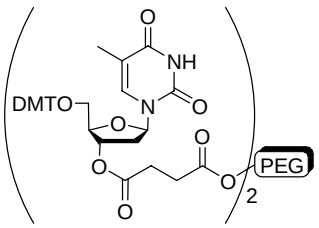
The formation of **73** was confirmed by ^{1}H NMR spectrum. The peak at 4.26 ppm indicates the terminal ester -OCH_2 group of PEG moiety as shown in spectrum B. Subsequent step involves the removal of 5'-O-DMT group of compound **73** which was achieved in 3 % tricholoroacetic acid in CH_2Cl_2 at 0 ^{0}C in 15 minutes to obtain the compound **74** .The formation of compound **74** which was confirmed by ^{1}H NMR spectrum where all the aromatic protons belong to the dimethoxy trityl group has

disappeared except at 7.6 and 8.1 ppm which indicates the CH and NH proton of the pyrimidione ring in spectra C. The compound **74** was then undergone condensation reaction with 4 equivs of amidite **75** and 10 equivalents of tetrazol **76** using acetonitrile as solvent under N_2 atmosphere at room temperature for 15 minutes to obtain the compound **77** *i.e* .PEG-phosphite derivative which has been confirmed from spectrum D. Compound **77** was further oxidized to obtain the phosphate derivative **78** using 80 % tert butyl hydro peroxide in di-tertiary butyl hydro peroxide for 15 minutes at 0 ^{0}C to get the compound **78** as shown in Scheme 2. However the ^{1}HNMR was not clear.

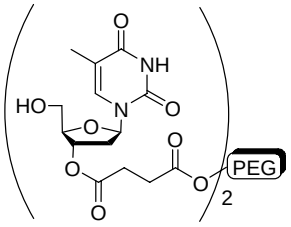

A
B
C
D
E
ppm


DMTO
NH
O
N
OH

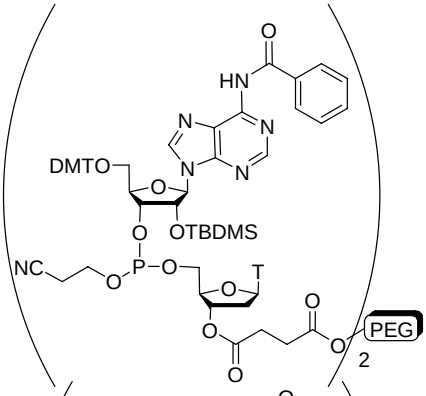
72


DMTO
NH
PEG
2

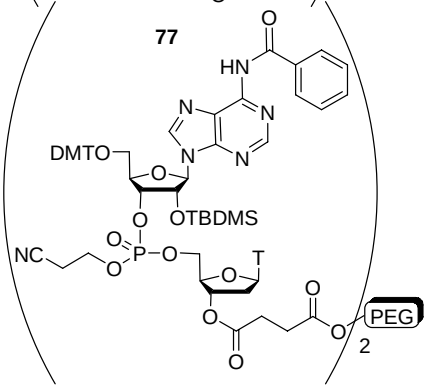
73


HO
NH
PEG
2

74


HN
DMTO
OTBDMS
NC
T
PEG
2

77

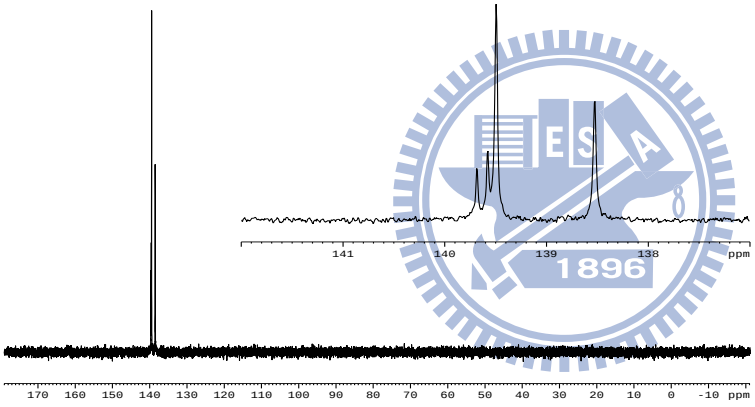

HN
DMTO
OTBDMS
NC
T
PEG
2

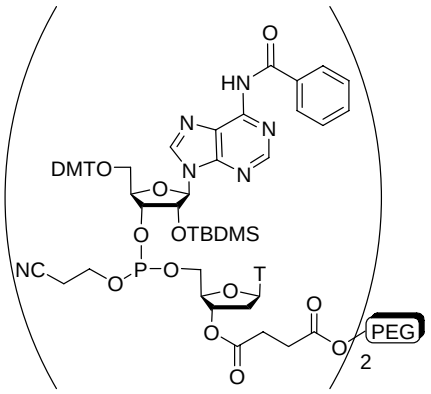
78

Figure 1.19. Stepwise formation of compound **78** from compound **72**.

However, the formation of the compound was exclusively confirmed from ^{31}P spectrum where the peak at 139.5 ppm indicates the presence of P(III) oxidation state in the compound and also the peak at 138.5 ppm indicates the excess phosphoramidite present in the reaction mixture. Subsequently the formation of the compound 66 was confirmed from ^{31}P spectrum where the peak at -2.2 ppm indicates the presence of P(V) oxidation state of the PEG-phosphate derivative **78** as shown in figure 1.20.

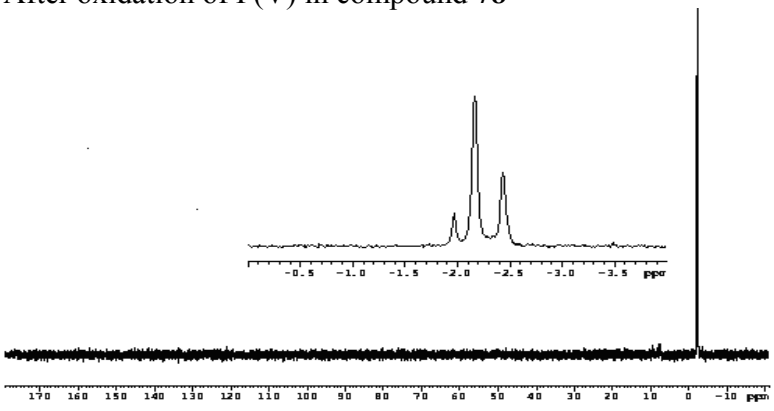
Before oxidation of P(III) in compound **65**

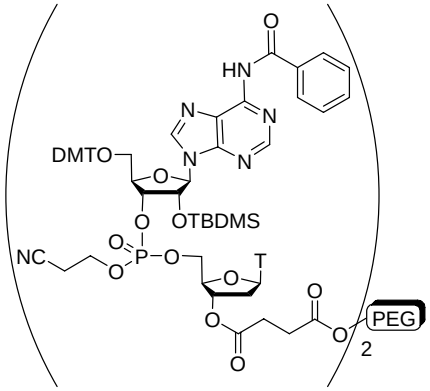




77

After oxidation of P(V) in compound **78**





78

Figure 1.20. Conversion of P(III) to P(V).

Our main goal is to study the formation of each product by MALDI MS in subsequent steps which do not require to cleavage products from the support

We have used the α-Cyano-hydroxycinnamic acid as matrix to dissolve polymer conjugate compound in CH_3CN as 30 mg per ml. The target compound was dissolved in 2:1 ratio of CH_3CN and water. Then it was degassed and then injected in to the MALDI spectrometer. Before going to record the MALDI MS of the target compound, first we had done the study on PEG-2000 as our starting material.

2nd Step MW 3252

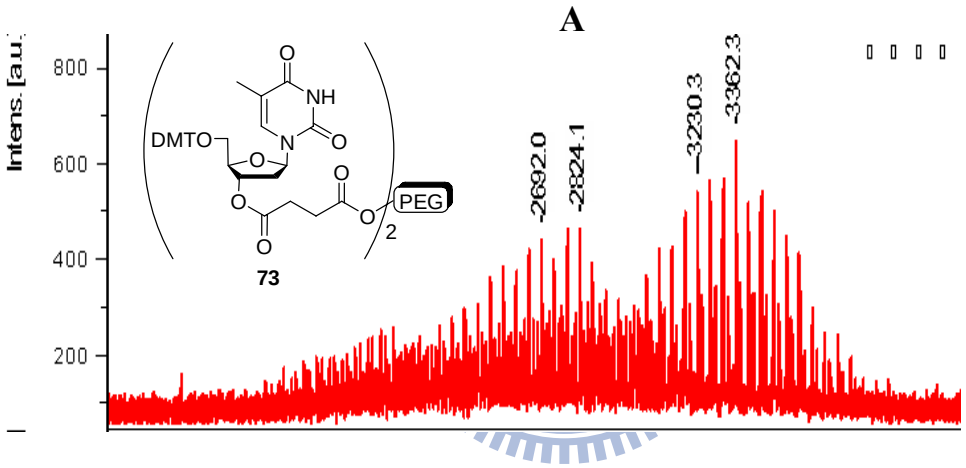


Figure 1.21. MALDI-MS spectra of compound 73

In MALDI spectrum of **A** of Compound **73** indicates two peak one is at 3362 and 3230 which indicates the MW of the compound 3252 is present in between them and other peak at 2692 which probably indicates the detachment of dimethoxy trityl group from the molecule .Because the MW of each of the dimethoxy trityl group is 303 and the compound **73** contains two DMT group which indicates that nearly 606 MW is missing from the MW of compound **73** as in figure 1.21.

3rd step MW 2648

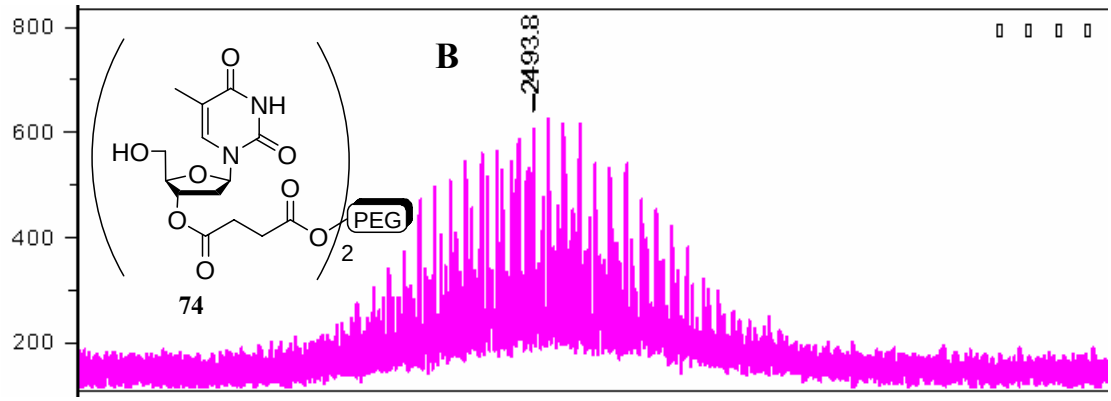


Figure 1.22. MALDI-MS spctra of compound 74

In the 2nd spectrum **B** there is a highest peak at 2649. The MW of the compound is 2648. We can attribute the peak at 2649 because of detachment of dimethoxy trityl group from the polymer conjugates **74** as in figure 1.22.

4th step MW 4422.38

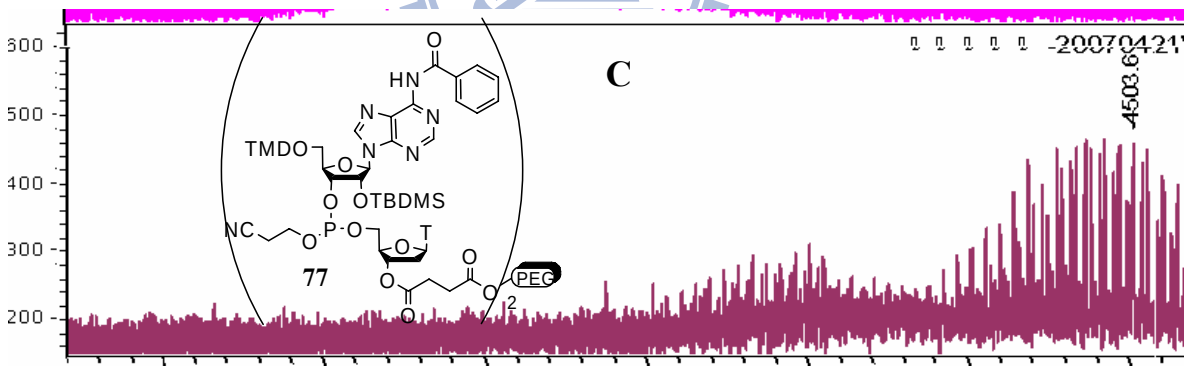


Figure 1.23. MALDI-MS spctra of compound 77

In the 3rd spectrum **C** we have seen the peak at 4500 which indicates that the desired compound **77** has formed whose MW is 4422.2 as in figure 1.23.

5th step MW 4454.38

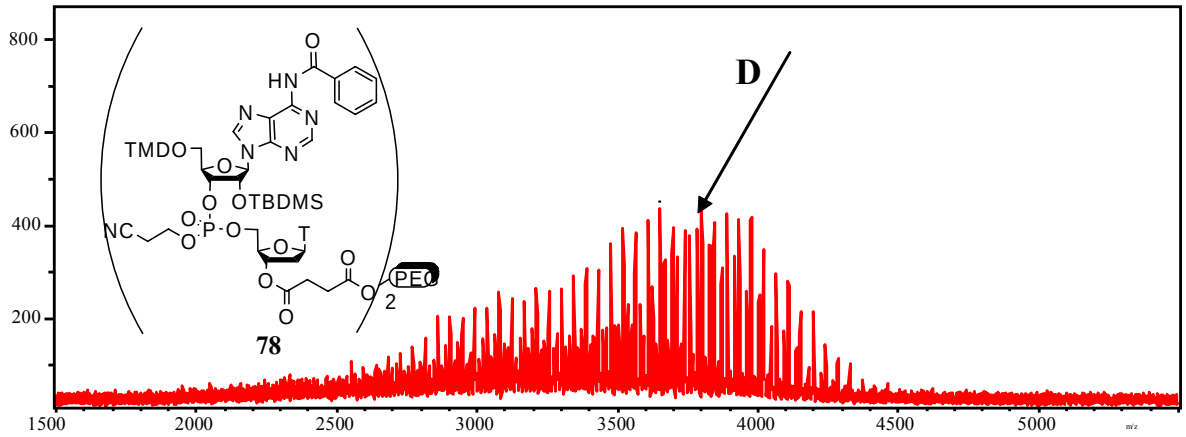
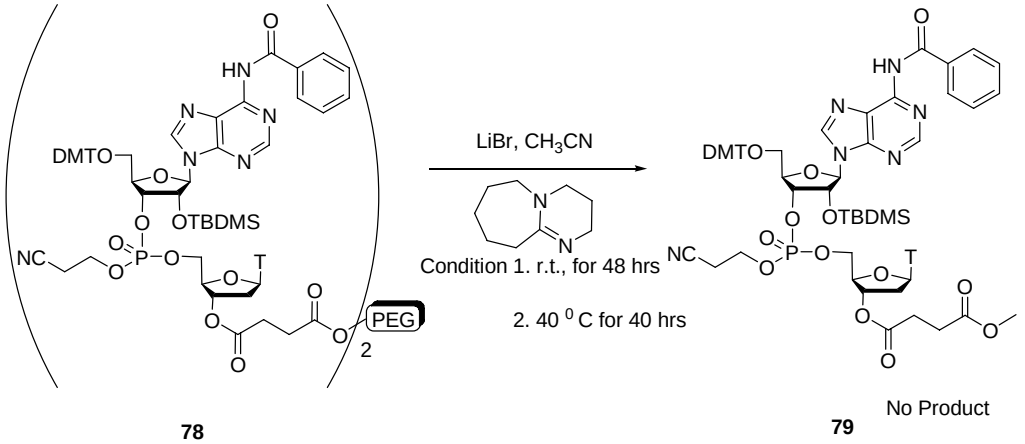


Figure 1.24. MALDI-MS spctra of compound 78

In the spectra **D**, we have seen that the highest peak observed at 3800 where as the MW of the compound is 4454.38 which indicates that the 5'-*O*-DMT group has been removed from the polymer conjugates **78** molecule so that is the reason highest molecular peak appeared around 3800 as in figure 1.24.

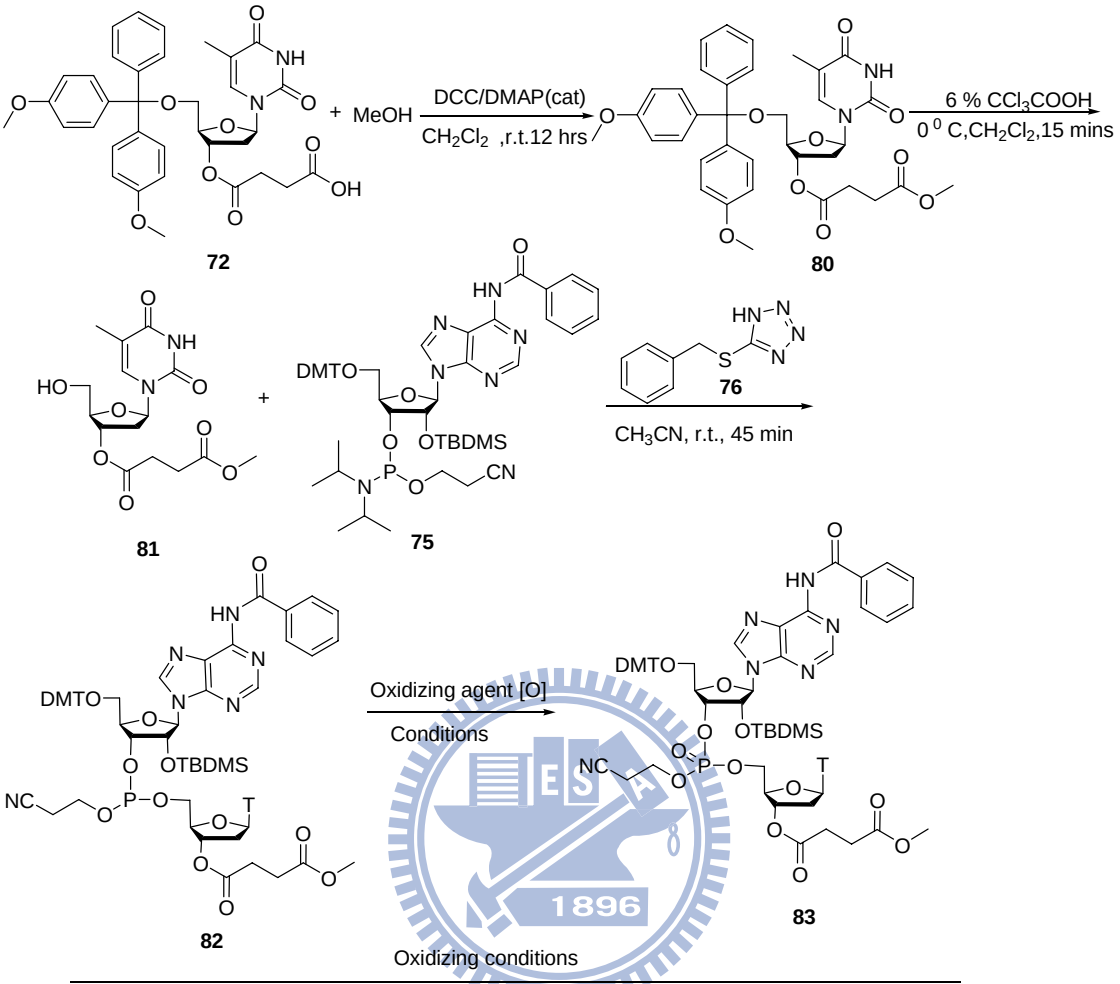
Since the MALDI MS spectrum of the compound **78** was not producing any convincing results, therefore we tried to detach the molecule from the soluble support PEG. For this reason we employed the mild cleaving agent such as LiBr and DBU in acetonitrile as solvent as shown in scheme 3.



Scheme 3. Cleavage of compound **78** from polymeric support.

First we tried the cleavage reagent at room temperature. But after two days room temperature stirring polymer did not cleave from the conjugates **78**. Then we tried the same reaction at elevated temperature *i.e*. by raising the reaction temperature around 40 ^{0}C which resulted the polymer cleavage upon spotting on the TLC plate. After precipitating with ether and subsequently filtering the polymer, we isolated the two spots by column chromatography. But from NMR it was confirmed that the phosphorus bond had been cleaved during the reaction.

However, in view of the drawbacks achieved during the solid phase synthesis of oligonucleotide we turned our attention towards the solution phase synthesis. In this case we tried to protect the succinic acid group of compound 60 to methyl ester derivative 68. After that followed the same pattern of reaction which has been depicted in scheme 4.



Entry	Reagent	Condition	Result
1	NBS/ DMSO/CH_3CN (W/V/V) (100/1.4/20)	0 ^{0}C, 15 min	Decomposed
2	TBHP in 80% di-tert-butyl peroxide/water3:2		Not commercially available
3.	TBHP in 5.5 M Deceane soln	0 ^{0}C, 45 min	85 % yield

Scheme 4. Stepwise formation of 5'-*O*-DMT $A^{Bz}T$ 3'-*O*-Succinate on solution phase.

The first step involves the coupling of the 5'-O- DMT-2-deoxynucleoside-3-succinate **72** with excess of MeOH in the presence of coupling reagent *N,N'*-dicyclohexylcarbodiimide (DCC) and at room temperature under N_2 atmosphere to obtained compound **80.** The

formation of **80** was confirmed by ^{1}H NMR spectrum which showed the at 3.72 ppm there is peak corresponds to OMe group attached to succinyl moiety as in spectrum A in Figure 1.25. After deprotection of dimethoxytrityl group from compound **80** using 6 % tricholoroacetic acid in dichloromethane at 0 ^{0}C for 15 minutes we obtained compound **81**. The formation of the compound **81** was confirmed from ^{1}H NMR study which showed the disappearance of aromatic proton as well as DMT group from the spectrum B in figure 1.25. Compound **81** was purified by column chromatography using 50 % ethyl acetate in Hexane. Coupling of compound **81** was achieved using 1.3 equivalent of phosphoramidate rA **75** and 4.5 equivalent of tetrazol **76** under nitrogen atmosphere for 45 minutes at room temperature. The completion of the reaction was observed in TLC which showed the two spots being formed along with the top impurity. The purification of the product was achieved using neutral silica gel packed with 1% triethylamine in Hexane solution. The reaction mixture was evaporated to remove the acetonitrile under reduced pressure and diluted with dichloromethane and packed in column. First few fractions were eluted with 50 % EA-HX containing 1 % Et_3N to remove some non polar impurity. While analyzing the impurity, we observed that phosphoramide rA **75** underwent oxidation to generate the compound **84** which was characterized by both 1H NMR and MALDI MS spectra. Then the column was eluted with 70% EA-HX which contains only the pure product mixture. Formation of the compound **82** was confirmed from ^{1}H NMR (spectra c, Figure 1.25) as well as ^{31}P NMR spectra (Spctra A, Figure 1.20). In order to oxidize the compound **82**, we use the N-bromosuccinimide in presence of DMSO and acetonitrile as solvent under 0^{0} C for 15 minutes. After checking the TLC, which was unclear, then the solvent were evaporated under reduced pressure and added a

drop of triethyl amine and tried to purify through neutral silica gel packed in hexane containing 1 % triethyl amine, but after addition of triethyl amine the reaction mixture was changed drastically. Some new spots appeared on TLC plate while purifying the compound which points to the decomposition of the product mixture. Next we turned our attention to oxidize the phosphite trimester bond of compound **82** to phosphate bond. For that we tried different oxidizing agent including NBS/DMSO/ACN and TBHP in di-tert-butylhydroperxide under 0 ^{0}C. But NBS/DMSO/ACN method suffers drawbacks during column purification where as TBHP in ditert-butyl hydroperxide are not commercially available. So then, we turned our attention to TBHP in 5.5 M Decane solution which is commercially available. Compound **82** was reacted with 6 equivalent of TBHP in 5.5 M decane solution in anhydrous acetonitrile solution under 0 ^{0}C for 45 minutes to get the compound **83** under vigorous stirring. The completion of the reaction was monitored by tlc. The purification was achieved by column chromatography using neutral silica gel.

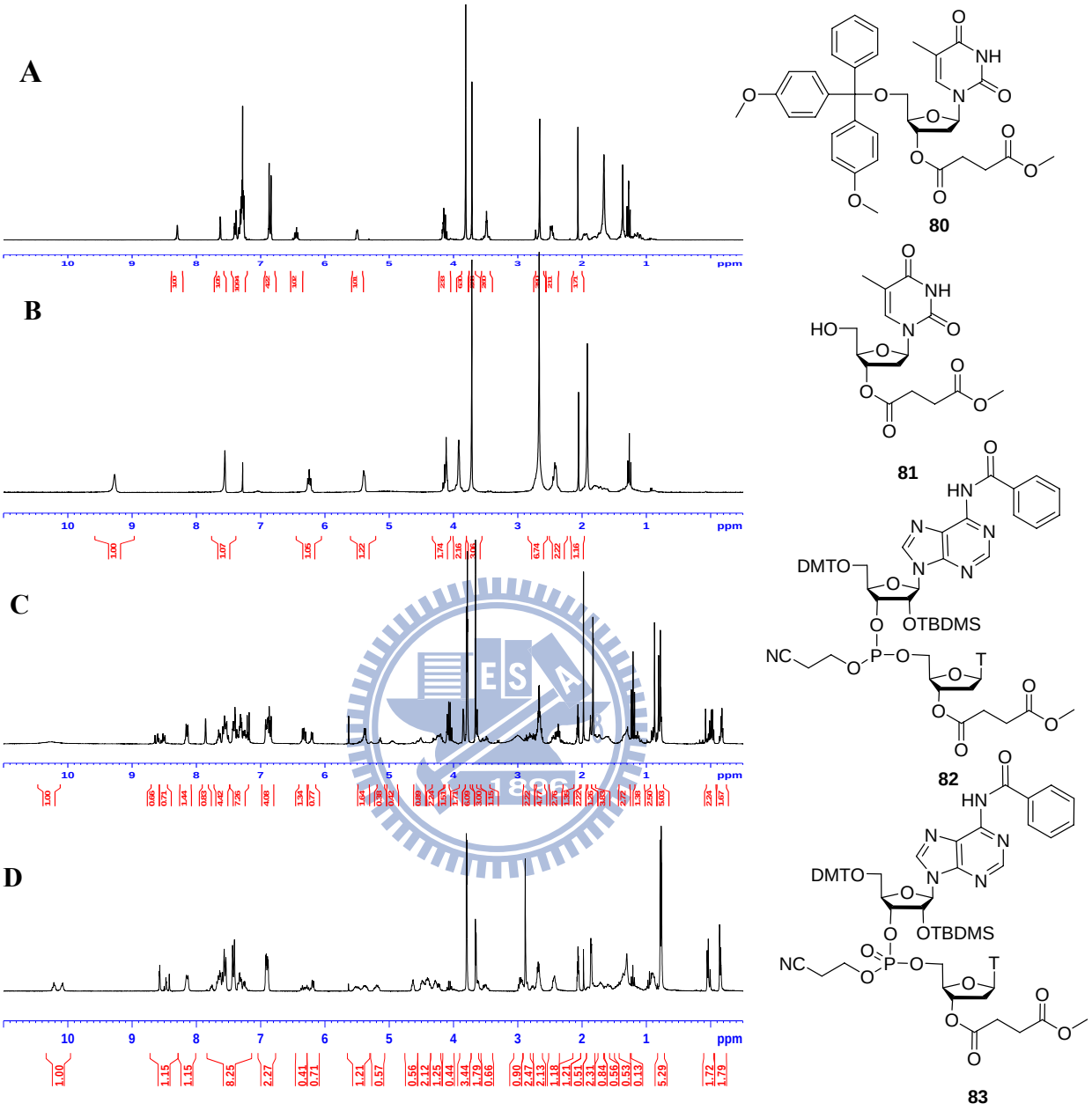
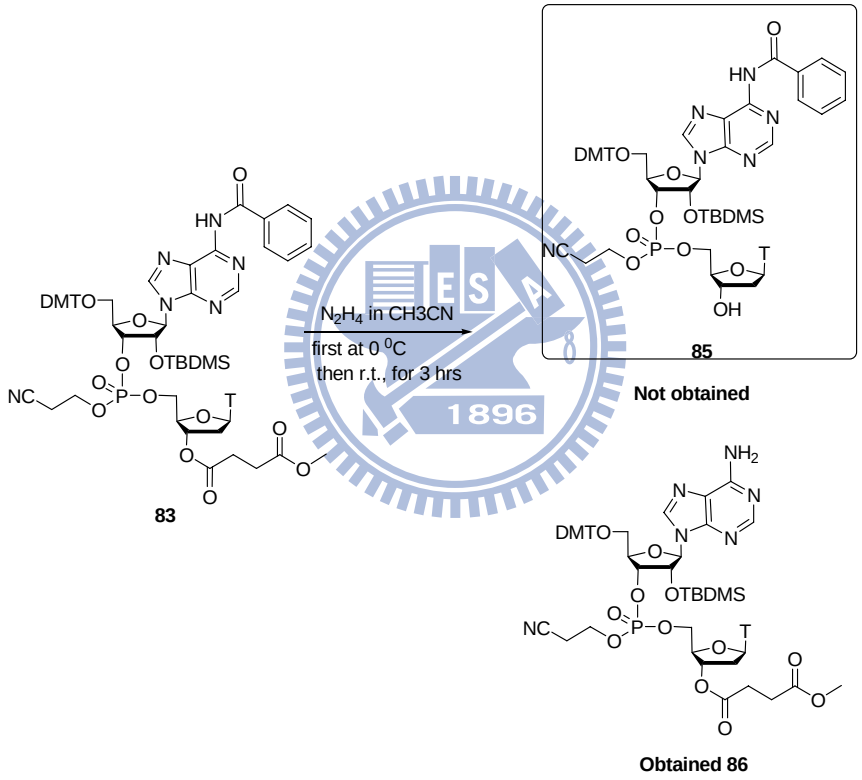


Figure 1.25. Stepwise monitoring for the formation of 5'-*O*-DMT $A^{Bz}T$ 3'-*O*-Succinate on solution phase.

First eluted with 100 ml of 50% Ea- Hx to get out the undesired impurity, the pure product obtained in 70% EA-Hx used as an eluant. Yield was obtained in 85 %. The

formation of the product was confirmed by ^{1}HNMR (Spectra D, Figure 1.25), ^{31}PNMR (Spectra B, Figure 1.20).

Our next goal was to cleave the succinyl linker attached to 3' position of the compound **71** using hydrazine hydrate in anhydrous acetonitrile as solvent first at 0 ^{o}C then at room temperature. But the reaction did not proceed at all at 0 ^{o} C, and then it was continued at room temperature for 3 hours which showed the complete conversion of the product as shown in the scheme 5.



Scheme 5. Detachment of 3' - succinyl linker from compound **83**.

We purified the product using neutral silica gel column chromatography to get the pure compound **85**. But after careful ^{1}H NMR and MALDI MS analysis, we came to conclusion that the amide bonds in compound **83** underwent cleavage in basic reaction condition involving hydrazine hydrate solution to get the compound **86** as shown in Figure 1.26.

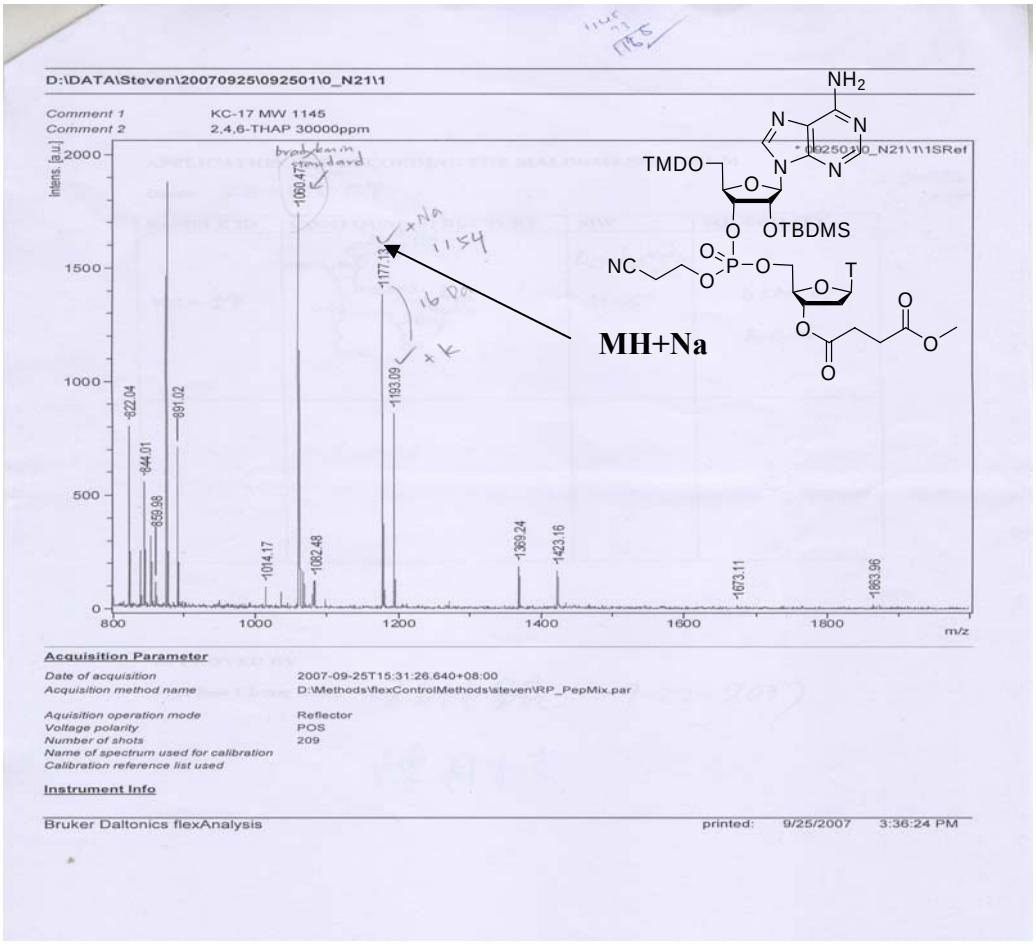
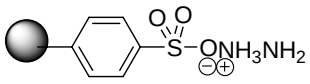
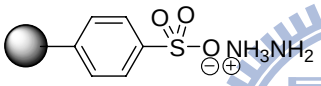
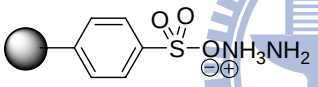
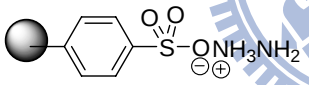


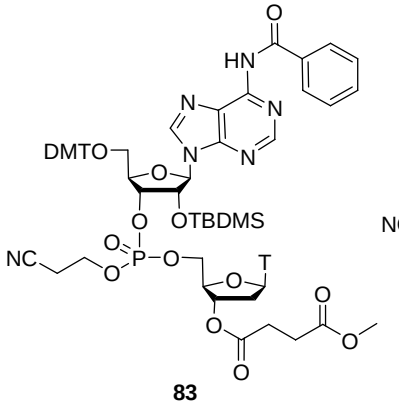
Figure 1.26. MALDI mass spectrum of compound **86.**

Till now we have successfully synthesized oligonucleotide dimer $T_{o=p}$A-5'-ODMT by synthetic methods as described above in pursuit for 2+2 strategy.

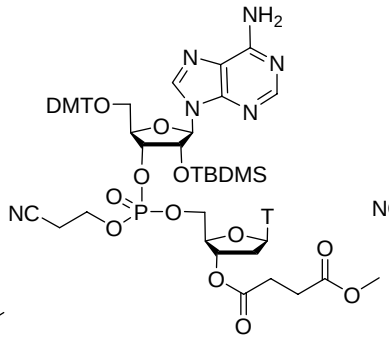
In order to achieve the cleavage of the 3'-succinyl linker from compound **83**, we turned our attention to polymer supported reagent. For this reason we have used polymer-supported sulfonic acid hydrazinium reagent. We tried different conditions to effectively remove the 3' position methyl succinate protecting groups as shown in table 2. But no desired product **85** was obtained.

Table 2. Summarizes the conditions applied for 3'-deprotection of compound **70.**

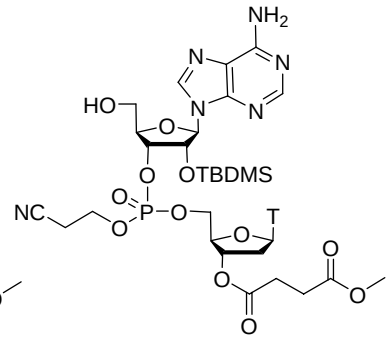
Condition	Reagent	Solvent	Base	Product
1	NH_2NH_2-H_2O	CH_3CN		**86**
2		CH_3CN/ r.t.	X	**No reaction**
3		CH_3CN/ 55 ^{0} C	X	**87**
4		Pyridine	X	**No reaction**
5		CH_3CN	Et_3N	**No reaction**



83



86

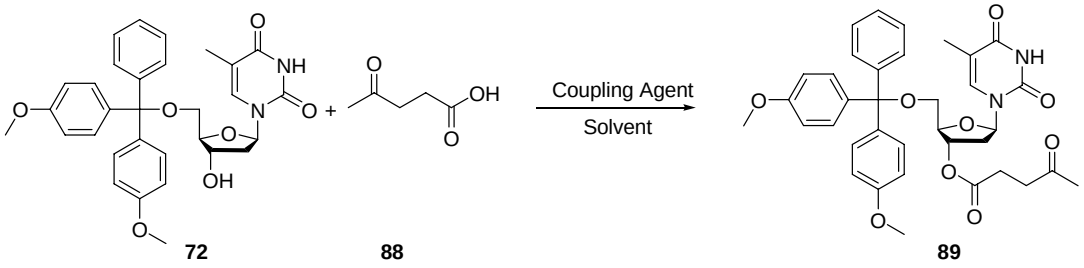


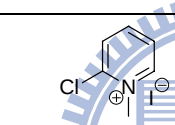
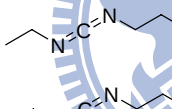
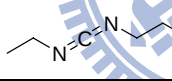
87

Till now we tried to deprotect the 3' OH which was protected with succinyl ester linker using different cleaving agent both in solid phase as well as solution phase. Since it was

not successful then, we have turned our focus to protect 3' OH group of compound **60** with leuvulinyl moiety. The scheme 6 has been depicted here. The 3'-hydroxy group of thymidine **60** was first protected with levulinic acid **88**.

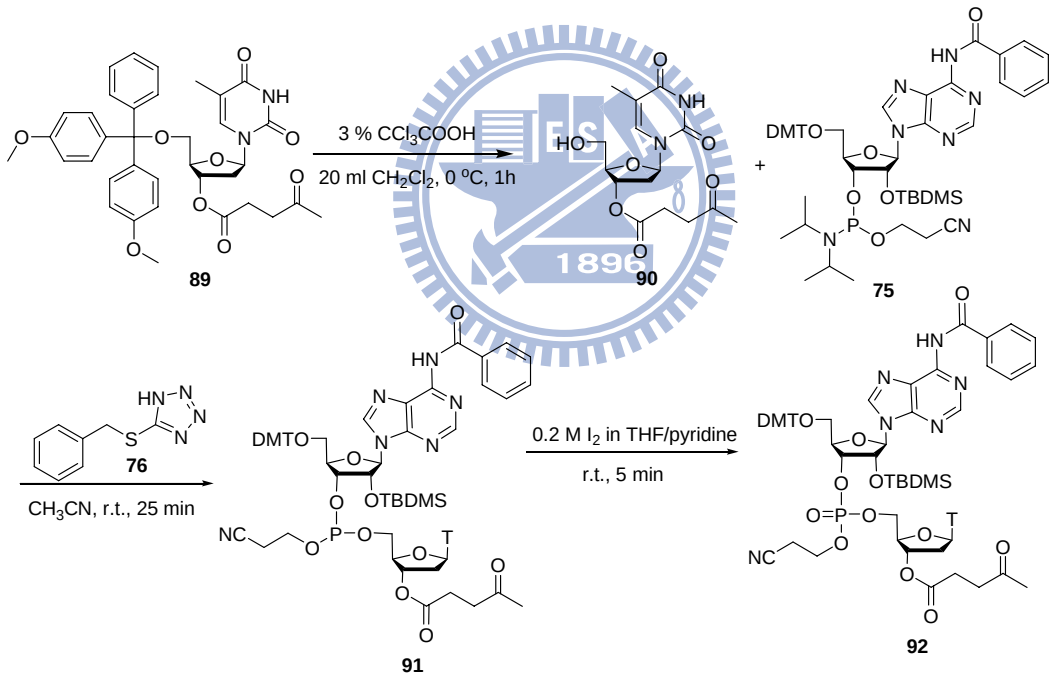
Scheme 6. Different coupling reagents were tasted for levulinylation.



Condition	Coupling reagent	Solvent	Yield (%)
1		CH_3CN/dioxane	0
2		Dioxane	93
3		Dioxane	90

For this reaction, I tried with three different coupling reagents such as Mukaiyama reagent in condition 1, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in condition 2, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in condition 3 in scheme 6. However, the best results were obtained when EDC in condition 2. After levulinylation, of compound **72** at room temperature for 1.5 hours, we obtained compound **89**. The formation of compound **89** was confirmed by ^{1}H NMR spectra, which indicates the three signals belonging to the levulinyl moiety of compound **77** were appeared around the region of δ = 2.75, 2.50, 2.15 ppm. Detritylation of **89** with

3 % trichloroacetic acid in dichloromethane afforded 5'-OH thymidine **90**. Similarly the formation of compound **90** has been confirmed from the disappearance of three sets of signals in the region of δ = 2.75, 2.50, 2.15 ppm in NMR spectrum. Condensation of compound **90** was achieved using 1.3 equivalent of phosphoramidate rA **75** and 5.0 equivalent of 5-(benzylthio)-1*H*-tetrazol **76** acts as an activator under nitrogen atmosphere for 30 minutes at room temperature resulted the phosphite trimester **91**, which was then further oxidized to phosphate trimester **92** by using 0.2 M I_2 in 1:4 pyridine: THF ratio for 10 minutes as shown in scheme 7.

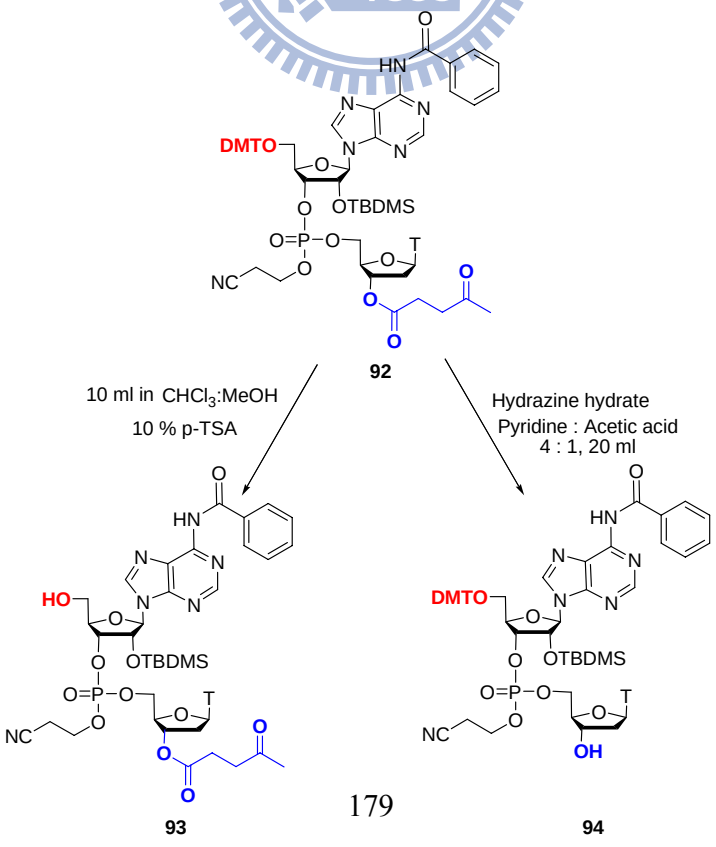


Scheme 7. Synthesis of compound 92

Here, we have observed that after condensation, the crude product was purified through column chromatography with neutral silica gel deactivated with 3 % pyridine in hexane.

The formation of compound **91** and **92** was confirmed from ^{31}P NMR spectra. However, it has been observed that while during condensation with phosphoramidate rA **75** compound **91** was decomposed to small extent which eventually lowers the yield of the product. The signal of phosphate trimester appeared at 140 ppm in ^{31}P NMR spectrum after condensation, and it was shifted to 0 ppm after oxidation. More importantly it has been observed that product of oxidation was purified by simple extraction.

In an attempt to obtain the 5' deprotection in compound **92**, was treated with 5 % *p*-toluenesulfonic acid in $CHCl_3$ and MeOH used as solvent in the ratio of 7:3 at 0 ^{O}C temperatures to obtain the compound **93**. However, in an attempt to synthesis oligonucleotide tetramer, we then moved to deprotect the 3' position of levulinyl moiety of compound **92** with hydrazine hydrate solution in 4:1 pyridine and acetic acid for 20 minutes and the reaction mixture was quenched by the addition 2,4-pentanedione as shown in scheme 8.



Scheme 8. 5' and 3' deprotection of compound 92

The formation of the product **94** was accompanied by the attack of the hydrazine moiety on the ketone of the levulinyl group; whereas the other amine of the hydrazine cleaved the ester bond of **92** to get the 3' deprotected dimer oligonucleotide as drawn in figure 1.27.

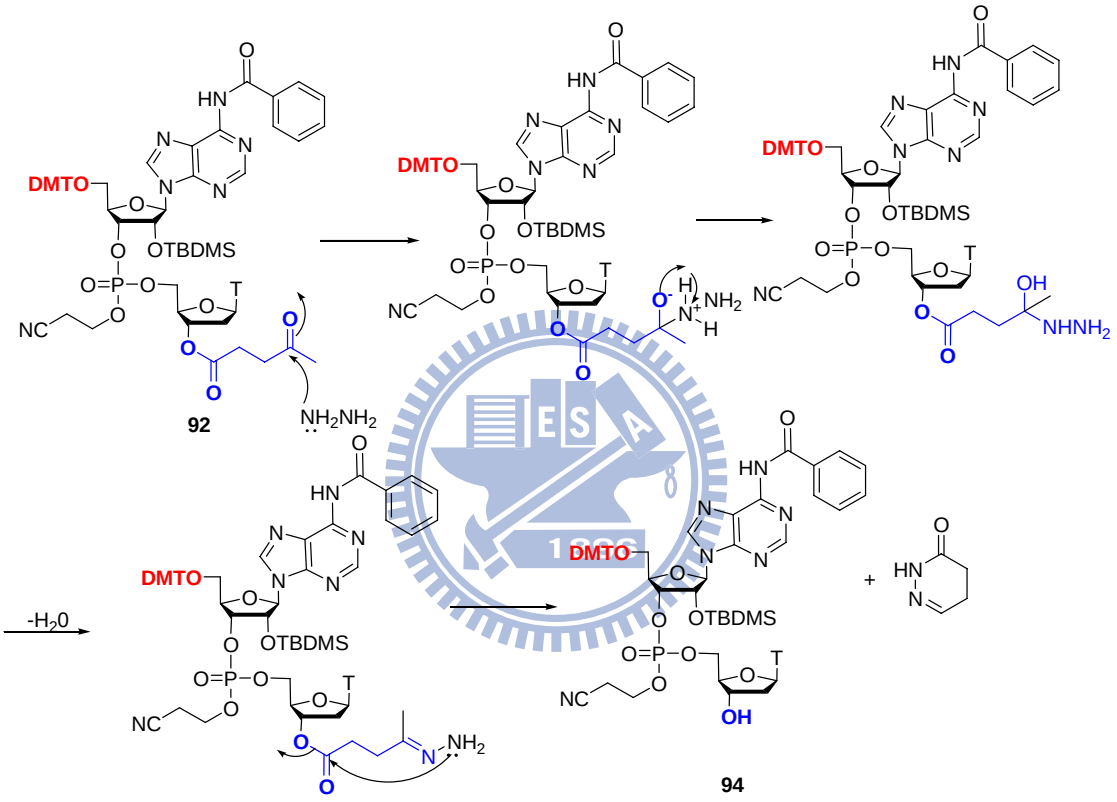
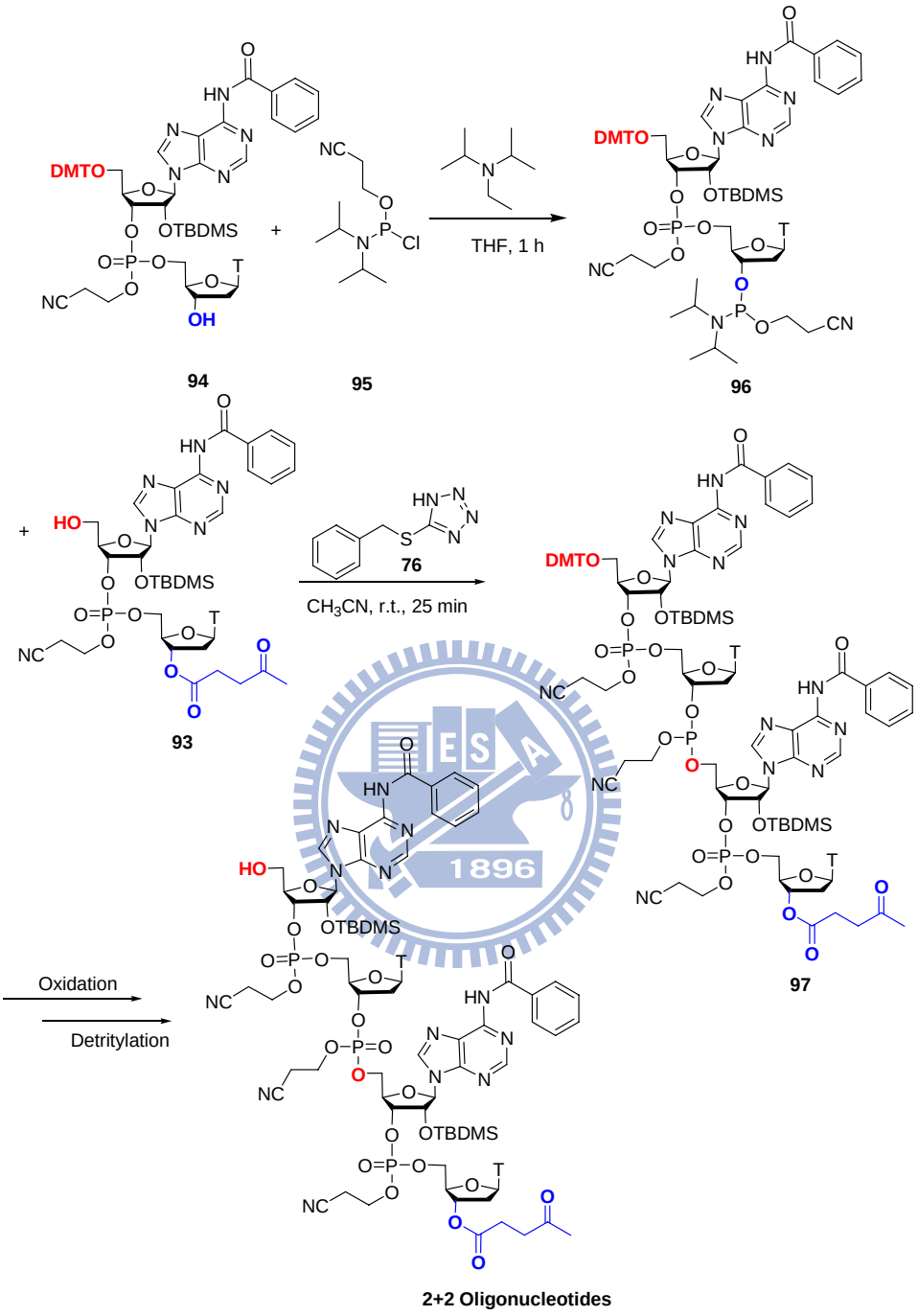


Figure 1.27. Mechanism of formation of compound **94** from **92.**

3' -OH deprotected dimer oligonuclotide was then converted to dimer phosphoramidite **96** under acidic as well as basic conditions as shown in scheme 9. Basic condition of deprotection was performed with chloro(2-cyanoethoxy)-(diisopropylamino) phosphine **95** and diisopropyl ethyl amine. However, the low yield obtained for the product **96** in this reaction due to the sensitivity of phosphoramidite to the weakly acidic silica gel during column purification.



Scheme 9. 2+2 Synthetic strategy for oligonucleotide synthesis

The signal of phosphoramidite was appeared at 150 ppm in ^{31}P NMR spectrum after phosphitylation. The compound dimer 5'-OH **93** was condensed with dimer

phosphoramidite **96** to generate the tetramer phosphate trimester **97** using CH_3CN as solvent and 5-(benzylthio)-1*H*-tetrazol **76** as an activator.

The compound 97 was further underwent oxidation and detritylation to generate the target compound (which is beyond the scope of this dissertation).

In summary, the synthesis of oligonucleotide tetramer through the solution phase 2+2 strategy has been achieved. This convergent synthesis established a practical method for the large-scale synthesis of oligonucleotides. It reduced the synthetic steps for constructing longer oligonucleotides and reduced the time needed to reach targeted molecules. Moreover, this methodology avoided the products from the pollution of $n-1$ or shorter failures and it is applicable for scale-up production to fit clinical trials and commercialization.

2.6. Experimental Section

^{1}H NMR spectra were recorded at 300MHz (Bruker DRX-300) and the chemical shifts were measured from the solvent peak as an internal standard (CD_2HCN in CD_3CN) as an external standard. 13C and DEPT NMR spectra were recorded at 75MHz (Bruker DRX-300) and the chemical shifts were measured from the solvent peak (in CD_3CN) as an internal standard. 31P NMR spectra were recorded at 65MHz and the chemical shifts were measured from 85% H3PO4 as an external standard. Acetonitrile was distilled from CaH2 and stored under nitrogen. TLC was performed on Merck Kieseigel 60 F254 precoated glass plates. Column chromatography was performed with silica gel SiliaFlash, G60 (Silicycle Co. Ltd). The MALDI time-of-flight (TOF) mass spectrometry was carried out by use of a Bruker Daltonics Biflex III (Leipzig, Germany).

Synthesis of 5'-O-Dimethoxytrityl-3'-O-levulinoyl thymidine 89.

To a solution of 5'-*O*-dimethoxytritylthymidine (10.0 g, 18.4 mmol) in dioxane (132 mL), levulinic acid (4.2 g, 36.0 mmol), EDC (5.8 g, 36.0 mmol), and dimethylaminopyridine (DMAP, 0.22 g, 1.8 mmol) were added. After stirring for 2.5 h, TLC analysis (5% MeOH/DCM) indicated complete conversion of the starting material. The solvent was evaporated by vacuum, and the residue was dissolved in dichloromethane (200 mL). After washing of the organic phase with water, 10 % $KHSO_4$, and 10% $NaHCO_3$ (three times), the organic phase was dried ($MgSO_4$), filtered, and concentrated to give a foam **89**, which was used directly in the next reaction.

^{1}H NMR (300 MHz, CD_3CN) δ 10.02 (br, 1H), 7.58 (s, 1H), 7.49 (d, J = 7.5 Hz, 2H), 7.43–7.21 (m, 7H), 6.9 (d, J = 8.7 Hz, 4H), 6.35 (t, J = 6.4 Hz, 1H), 5.46 (s, 1H), 4.13 (s, 1H), 3.77 (s, 6H), 3.63 (s, 2H), 3.42 (dd, J = 19.0, 7.7 Hz, 2H), 2.76 (t, J = 6.2 Hz, 2H), 2.54 (t, J = 6.4 Hz, 2H), 2.54–2.36 (m, 2H), 2.14 (s, 3H), 1.50 (s, 3H). ^{13}C NMR (75 MHz, CD_3CN) δ 207.3, 178.2, 164.6, 159.2, 151.2, 145.3,136.2, 136.1, 135.9, 130.5, 128.5,128.4, 127.5, 117.8, 113.7, 111.2, 87.1, 84.8, 84.1, 75.4, 67.1, 55.4, 54.8, 37.9, 37.5, 29.4, 28.3, 11.8.

Synthesis of 5'-Hydroxyl-3'-O-levulinoyl-thymidine 90.

To a solution of 5'-O-dimethoxytrityl-3'-O-levulinoyl-thymidine **89** (11.6 g, 18.0 mmol) in dichloromethane (200 mL), 6 % trichloroacetic acid in dichloromethane (200 mL) was added at 0◦C for 1 h. After reaction completion, solvent was removed and the crude product was subjected to column purification. First, 33% hexane in ethyl acetate was used as eluent to remove the byproduct and then 100% ethyl acetate was used to elute product **90**. The product was obtained as white foam in 94% yield.

^{1}H NMR (300 MHz, CD_3CN) δ 7.64 (s, 1H), 6.22 (t, *J* = 7.0 Hz, 1H), 5.26 (d, *J* = 1.6 Hz, 1H), 4.03 (d, *J* = 2.2 Hz, 1H), 3.75 (d, *J* = 2.1 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.53 (t, *J* = 6.5 Hz, 2H), 2.36–2.25 (m, 2H), 2.14 (s, 3H), 1.85 (s, 3H). ^{13}C NMR (75 MHz, CD_3CN) δ 208.0, 173.2, 165.1, 151.7, 137.2, 118.3, 111.3, 87.7, 85.6, 75.7, 62.7, 38.3, 37.7, 29.8, 28.7, 12.5.

General Procedures for the Condensation, Oxidation, Detritylation, Delevulinylation, and Phosphitylation Reaction.

Condensation

5'-OH oligonucleotide **90** (16.9 mmol, 1.0 equiv), phosphoramidite (22.0 mmol, 1.3 equiv), and activator, 5-(benzylthio)-1*H*-tetrazol (84.5 mmol, 5.0 equiv) was added in a 500 mL flask under nitrogen. Acetonitrile was added and the reaction mixture was stirred for 30 min until reaction completion. Acetonitrile was removed by rotary evaporation and the crude product was purified by column chromatography (column was packed with 5% pyridine in hexane) to obtain 5'- O-DMTr-rABzpCNEdT-3'-O-Lev **91.**

^{31}P NMR (65 MHz, CD_3CN) δ 139.3, 139.3.

Oxidation

A solution of 0.2M I_2 in 4/1 THF/pyridine was added to the phosphite trimester and the reaction mixture was stirred for 10 min. The reaction was quenched by extraction with 1M $Na_2S_2O_3$, 10% $KHSO_4$, 10% $NaHCO_3$, and a mixture of brine/water (1/1), the organic layer was collected and dried by $MgSO_4$. After filtration and solvent removal, product was obtained as brown foam 5'-O-DMTr-rABzpoCNEdT-3'-O-Lev **92**.

^{31}P NMR (65 MHz, CD_3CN) δ −1.9, −2.1.

Detritylation for Dimer Oligoribonucleotide

To the solution of phosphate triester in 7/3 dichloromethane/methanol at 0◦C, 10% *p*-toluenesulfonic acid in dichloromethane/methanol (7/3) was added and the resulting mixture was stirred for 30 min at 0 ^{0}C. After completion, water was added and the resulting mixture was vigorously stirred for 10 min at 0 ^{0}C.Asaturated $NaHCO_3$ aqueous solutionwas added and again, the reaction mixture was stirred strongly for 10 min at 0 ^{0}C. The reaction mixture was then washed with saturated $NaHCO_3$ solution, 0.2M $Na_2S_2O_3$, and 0.5M NaCl.The organic layer was separated and dried over anhydrous $MgSO_4$. After filtration the solvent was evaporated under rotary evaporation. The crude product was purified by column choromatography or ether precipitation. The product was obtained as a white solid **93**.

Delevulinylation

To the solution of phosphate triester in 4/1 pyridine/acetic acid, hydrazine monohydrate (1.5 equiv) was added and reacted for 2 h. After reaction completion, acetylacetone (2.0 equiv) was added to quench the excess hydrazine, and the mixture was stirred for 10 min. The solvents were removed under vacuum and the residue was dissolved in dichloromethane and extracted with water, 10% $KHSO_4$ (two times), 10% $NaHCO_3$ (two times), and brine. After drying by $MgSO_4$ and filtration, the solvents were removed by rotary evaporation. The crude product was purified by column chromatography (column was packed with 3% pyridine in hexane).

Basic Conditions for Phosphitylation

To a solution of 3'-OH oligonucleotides (1.0 equiv) in THF under nitrogen at 0◦C, *N*,*N*-diisopropylethylamine (3.0 equiv) and chloro(2-cyanoethoxy)(diisopropylamino) phosphine (1.5 equiv) were added and the reaction mixture was stirred for 1 h. After

completion, the mixture was washed with 5 % aqueous $NaHCO_3$ and dried over $MgSO_4$. The crude product was purified by column chromatography (column was packed with 3 % pyridine in hexane) and product was obtained as yellow form.

^{31}P NMR (65 MHz, CD_3CN) δ 148.9, 148.9, 148.8, 148.7, −1.7, −1.8, −1.9, −2.0.

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Chapter Three

Ionic Liquid supported Synthesis of Novel Small Molecules

3.1. Introduction

A new kind of chemical revolution, ‘‘green chemistry’’, is brewing—150 years after the first chemical revolution transformed modern life with a host of conveniences, which protects the environment, not by cleaning it up, but by inventing new chemistry and new chemical processes that prevent pollution. In essence, it prompts the chemical and pharmaceutical manufacturer to consider how human life is impacted after these chemicals are generated and introduced into their society. By rethinking chemical design from the ground up, chemists are developing new ways to manufacture products that fuel the economy and lifestyles, without the damages to the environment that have become all too evident in recent years.[1-2] The use of volatile organic compounds (VOCs) poses a risk to those people working in or living close to such processing facilities. In addition VOCs have been heavily implicated in causing changes to the global climate, the formulation of smog as well as being identified as a source of ozone depletion.[3] In order to prevent the source of ozone depletion, the Montreal Protocol (a protocol to the Vienna convention for the protection of the ozone layer) has been introduced worldwide. It is an international treaty designed to protect the ozone layer by phasing out the production of a number of substances believed to be responsible for ozone depletion. The treaty was opened for signature on September 16, 1987, and entered into force on January 1, 1989, followed by a first meeting in Helsinki, May 1989. Since then, it has undergone seven revisions, in 1990 (London), 1991 (Nairobi), 1992 (Copenhagen), 1993 (Bangkok), 1995 (Vienna),

1997 (Montreal), and 1999 (Beijing). It is believed that if the international agreement is adhered to, the ozone layer is expected to recover by 2050.[4] Due to its widespread adoption and implementation it has been hailed as an example of exceptional international co-operation with Kofi Annan, the former secretary general of UNO quoted as saying that "perhaps the single most successful international agreement to date has been the Montreal Protocol". It has been ratified by 196 states. Exploration of non-toxic, environment friendly synthetic methodologies for organic reactions is one of the prime challenges to the organic chemists. The need to have alternative solvents that are environmentally friendly, and can serve as effective substitutes for conventional organic solvents, has driven this rapid growth.

There appears to be four main alternative strategies:

(1) solvent-free synthesis

(2) The use of water as a solvent

(3) The use of supercritical fluids as solvents

(4) The use of ionic liquids as solvents.

Ionic liquid has emerged as a true alternative for overcoming the long existed problem. Research in the field of ionic liquids (ILs) has grown exponentially in recent years. Ionic liquid is no more a new word to a scientific community today. The use of ionic liquids has recently received more and more attention as eco-friendly reaction media in organic synthesis.

3.2. What is an Ionic Liquid?

• Any salt above its melting point

• Technically, all molten salts, such as NaCl

(mp = 800 ^{o}C), are ionic liquids

• Obviously not practical for organic synthesis

However, the definitions from literature states that

According to Sheldon "The term ionic liquid implies a material that is fluid at (or close to) ambient temperature, is colorless, has a low viscosity and is easily handled. Room temperature ionic liquids are generally salts of organic cations, *e.g.* tetraalkylammonium, tetraalkylphosphonium, *N*-alkylpyridinium, 1,3-dialkylimidazolium and trialkylsulfonium cations as shown in figure 1."

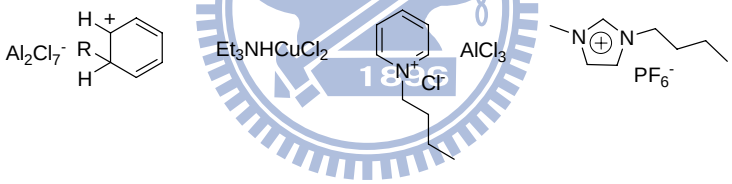


Figure 1. Room temperature ionic liquids

According to Handy "Most basic definition of a room temperature ionic liquid is a salt that has a melting point at or near room temperature."

According to Maio "Organic salts with melting points below ambient or reaction temperature."

According to Wilkes "Ionic liquid is a salt with a melting temperature below the boiling point of water." Salt of organic cation which has a melting point near ambient temperature (up to ~100 ^{o}C).

3.2.1. History of Ionic Liquid:

In 1800s while doing Friedel-Crafts reactions “red oil” formed during reaction which was later identified as long presumed intermediate called sigma complex.[5] The date of discovery of the "first" ionic liquid is disputed, along with the identity of the discoverer. Ethanolammonium nitrate (m.p. 52–55 °C) was reported in 1888 by S. Gabriel and J. Weiner.[6] The first report of a room-temperature ionic liquid was ethyl ammonium nitrate (m.p. 12 °C), synthesized in 1914 by Paul Walden.[7] In the 1970s and 1980s there was interest in ionic liquids based on alkyl-substituted imidazolium and pyridinium cations, with halide or trihalogenoaluminate anions, initially developed for use as electrolytes in battery applications.[8-9] Start of modern era of ionic liquids began with discovery of 1-butylpyridinium chloride-aluminum chloride mixture whose melting point was 40 ^{o} C, and butylpyridinium cation easily reduced. It was noticed that large anions with many degrees of freedom inhibited crystallization until lower temperature. In early 1990s ethylmethylimidazolium halides prepared, then anion metathesis with various silver salts provided a small library of room temperature ionic liquids. By the mid 1990s, the basic understanding of the ionic liquids concept was well known in a narrow scientific community, mostly electrochemists, but this area of esoteric curiosity was of little interest, or too specialized, for synthetic industrial applications.

3.2.2. Characteristics of ionic liquids

Ionic liquids are often characterized by different names. There are several synonyms of ionic liquids.

• Room temperature ionic liquid (RTIL)

• Molten salt

• Room temperature molten salt

• Ambient temperature molten salt/ionic liquid

• Task specific ionic liquid (TSIL)

• Liquid organic salt

• Fused salt

• Neoteric solvent

The most important characteristics of ionic liquids are non-flammability, negligible vapor pressure, high thermal/chemical/electrochemical stability, good solvating ability, large liquidus range (span of temperatures between melting and boiling point of a liquid) and easy recyclability

They are highly polar, non-coordinating, can be easily tunable between water and organic solvents and generally do not coordinate with metals, enzymes. They have the ability to be stored for long time without decomposition. Some chiral ionic liquids may control stereoselectivity.

"Pure imidazolium ionic liquids can be described as polymeric hydrogen bonded supramolecules and in some cases when mixed with other molecules, they should better be regarded as nonstructural materials with polar and non polar regions rather than homogeneous solvent." (Dupont). The solubility of different species in imidazolium ionic liquids depends mainly on polarity and hydrogen bonding ability. Saturated aliphatic compounds are generally only sparingly soluble in ionic liquids, whereas olefins show somewhat greater solubility, and aldehydes can be completely miscible. This can be

exploited in biphasic catalysis, such as hydrogenation and hydrocarbonylation processes, allowing for relatively easy separation of products and/or unreacted substrates. Gas solubility follows the same trend, with carbon dioxide gas showing exceptional solubility in many ionic liquids, carbon monoxide being less soluble in ionic liquids than in many popular organic solvents, and hydrogen being only slightly soluble (similar to the solubility in water) and may vary relatively little between the more commonly used ionic liquids. Different analytical techniques have yielded somewhat different absolute solubility values.[10-11]

3.2.3. Application of Ionic Liquids

Nowadays ionic liquids find a number of industrial applications which vary greatly in character. A few of their industrial applications are briefly described below; more detailed information can be found in a recent review article.[12]

3.2.3.1. Chemical industry

The first major industrial application of ionic liquids was the BASIL (*B*iphasic *A*cid *S*cavenging utilizing *I*onic *L*iquids) process by BASF, in which a 1-alkylimidazole was used to scavenge the acid from an existing process. This then results in the formation of an ionic liquid which can easily be removed from the reaction mixture. This increased the space/time yield of the reaction by a factor of 80,000.[13] Eastman operated an ionic liquid-based plant for the synthesis of 2,5-dihydrofuran from 1996 to 2004. The dimersol process is a traditional way to dimerize short chain alkenes into branched alkenes of higher molecular weight. Y. Chauvin and H. Olivier-Bourbigou have developed an ionic

liquid-based add-on to this process called the difasol process. Ionikylation is an ionic liquid-based process developed by Petrochina for the alkylation of four-carbon olefins with iso-butane. Their 65,000 tonne per year plant is claimed to be the biggest industrial application of ionic liquids to date.

3.2.3.2. Cellulose processing

Occurring at a volume of some 700 billion tons, cellulose is the earth's most widespread natural organic chemical and, thus, highly important as a bio-renewable resource. But even out of the 40 billion tons nature renews every year, only approx. 0.2 billion tons are used as feedstock for further processing. A more intensive exploitation of cellulose as a bio-renewable feedstock has to date been prevented by the lack of a suitable solvent that can be used in chemical processes. Robin Rogers and co-workers at the University of Alabama have found that by means of ionic liquids, real solutions of cellulose can now be produced for the first time at technically useful concentrations.[14] This technology therefore opens up great potential for cellulose processing. Although it has been presented as a new idea, the use of ionic liquids in cellulose processing originally dates back to 1934 in a patent by Graenacher where mixtures of 1-ethylpyridinium chloride with free nitrogen containing bases were used.[15] Making cellulosic fibers from so-called dissolving pulp currently involves the use, and subsequent disposal, of great volumes of various chemical auxiliaries, esp. carbon disulfide (CS_2). Major volumes of waste water are also produced for process reasons and need to be disposed of. These processes can be greatly simplified by the use of ionic liquids, which serve as solvents and are nearly entirely recycled. The "Institut für Textilchemie und Chemiefasern" (ITCF) in

Denkendorf and BASF are jointly investigating the properties of fibers spun from an ionic liquid solution of cellulose in a pilot plant setup.[16] The dissolution of cellulose based materials like tissue paper waste, generated at chemical industries and at research laboratories, in room temperature ionic liquid, 1-butyl-3-methylimidazolium chloride, bmimCl, was studied and the recovery of valuable compounds by electrodeposition was studied from this cellulose matrix.[17-18]

3.2.3.3. Dispersants

Ionic liquids can be used as dispersing agents in paints to enhance the finish, appearance and drying properties. Examples are the TEGO brand dispersers by Evonik, used in their Pliolite brand paints. Ionic liquds are used for the dispersion of nano-materials at IOLITEC.

3.2.3.4. Gas handling

Ionic liquids have several properties that make them useful in gas storage and handling applications. Their vapor pressure is typically very low, they are stable at high temperatures, and are able to act as solvents for a wide variety of compounds and gases. They also have weakly coordinating anions and cations which are able to stabilize polar transition states. Many ionic liquids can be reused with minimal loss of activity. The ionic liquid 1-butyl-3-methylimidazolium chloride has been used for separating hydrogen from ammonia borane.[19 -20]

3.2.3.5. Gas treatment

As profiled in the July 13, 2009 issue of C&E News, ION Engineering is commercializing technology using ionic liquids and amines for CO_2 capture and natural gas sweetening.[21]

3.2.3.6. Nuclear industry

Ionic liquids have been proposed for use in the nuclear industry. The liquid 1-butyl-3-methylimidazolium chloride has been investigated as non-aqueous electrolyte media for the recovery of uranium and other metals from spent nuclear fuel and other sources.[22-24] A task-specific ionic liquid, protonated betaine bis(trifluoromethanesulfonyl) imide has been investigated as a solvent for uranium oxides.[25]

3.2.3.7. Solar energy

Ionic liquids show great potential for use as a heat transfer and storage medium in solar thermal energy systems. Concentrating solar thermal facilities such as parabolic troughs and solar power towers utilize the energy of the sun by focusing it onto a receiver which can generate temperatures of around 600 °C. This heat can then be used to generate electricity in a steam or other cycle. For buffering during cloudy periods or to enable generation overnight, some of this energy can be stored by heating an intermediate fluid. Although nitrate salts have been the medium of choice since the early 1980s, those used freeze at 220 °C and thus require heat tracing overnight to prevent solidification. Ionic liquids such as $[C_4mim][BF_4]$ have been identified with more favorable liquid-phase temperature ranges (-75 to 459 °C) and could therefore be excellent liquid thermal storage media and heat transfer fluids in solar thermal power plants.[26]

3.2.3.8. Food and bio products

The ionic liquid 1-butyl-3-methylimidazolium chloride is able to completely dissolve freeze dried banana pulp and the solution with an additional 15 % DMSO lends itself to Carbon-13 NMR analysis. In this way the entire banana compositional makeup of starch, sucrose, glucose, and fructose can be monitored as a function of banana ripening.[27] Ionic liquids have been used for the extraction of specific natural compounds from plants for pharmaceutical, nutritional and cosmetic applications, such as the antimalarial drug artemisinin from the plant *Artemisia annua*.[28]

3.2.3.9. Waste recycling

Ionic liquids can be developed for the recycling of synthetic goods, plastics and metals. They offer the specificity required to separate similar compounds from each other, such as in the separation of polymers from plastic waste streams. This has been achieved using lower temperature extraction processes than current approaches and could be the answer to avoiding tones of plastics being incinerated or consigned to landfill each year.

3.2.3.10. Batteries

Researchers have identified IL's that can replace water as the electrolyte in metal-air batteries. IL's have great appeal because they evaporate at much lower rates than water, increasing battery life by drying slower. Further, IL's have an electrochemical window of up to six volts [29](versus 1.23 for water) supporting more energy-dense metals.

3.2.3.11. High Purity Organometallics

Following Green Chemistry principles, ionic liquids are used along with micro reactors to synthesize and purify extremely reactive organometallic compounds for ALD and CVD applications, with improved safety in operations and higher purity products. [30-31]

3.2.3.12. Safety

Due to their non-volatility, effectively eliminating a major pathway for environmental release and contamination, ionic liquids have been considered as having a low impact on the environment and human health, and thus recognized as solvents for green chemistry. However, this is distinct from toxicity, and it remains to be seen how 'environmentally-friendly' ILs will be regarded once widely used by industry. Research into IL aquatic toxicity has shown them to be toxic as or more so than many current solvents already in use. Review papers on this aspect have been published in 2007.[32-33] Available research also shows that mortality isn't necessarily the most important metric for measuring their impacts in aquatic environments, as sub-lethal concentrations have been shown to change organisms' life histories in meaningful ways. According to these researchers balancing between zero VOC emissions, and avoiding spills into waterways (via waste ponds/streams, etc.) should become a top priority. However, with the enormous diversity of substituents available to make useful ILs, it should be possible to design them with useful physical properties and less toxic chemical properties. With regard to the safe disposal of ionic liquids, a 2007 paper has reported the use of ultrasound to degrade solutions of imidazolium-based ionic liquids with hydrogen peroxide and acetic acid to relatively innocuous compounds.[34] Despite their low vapor pressure many ionic liquids have also found to be combustible and therefore require careful handling.[35] Brief

exposure (5 to 7 seconds) to a flame torch will ignite these IL's and some of them are even completely consumed by combustion.

3.3. Structural Manifestation of Ionic Liquids

Ionic liquids consist of cations and anions. Cations should be infinite in number. The most common cations are dialkylimidazolium, alkylpyridinium, tetraalkylammonium, tetraalkylphosphonium. The cations can be tuned according to the properties needed. For liquid at room temperature, should be unsymmetrical. Functionalizing side chain on cation can change melting point of straight-chain. However, the presence of C_2-methyl group generally increases melting point of unsubstituted. Other common cations are pyridinium which is possibly unstable in presence of nucleophiles and alkyl chain length effect on melting point and tetraalkylammonium ions which are known for much longer than imidazolium and highly viscous/difficult to handle. Long alkyl chain/decreased symmetry and lower melting point. Other than this triazolium, pyrazolium, thiazolium, benzimidazolium, guanidinium, phosphonium, sulfonium are also forms the cationic parts of ionic liquids. The anion part should be more diverse. Some of the common anionic parts are tetrafluoroborate, hexafluorophosphate, bis(trifluoromethanesulfonyl)imide, halogen, mesylate/tosylate/triflate. Anions should be larger and more weakly coordinating and having lower melting temperatures as shown in figure 2.

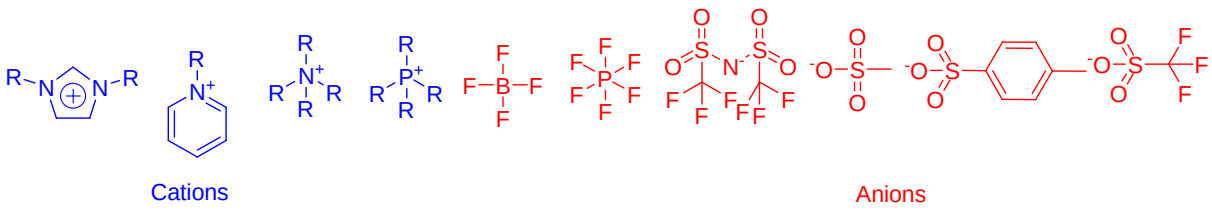


Figure 2. Cations and Anions used to make Ionic Liquids

3.4. IL Functionalization

The chemophysical properties of ILs depend on the combination of cation and anion; in addition, the length of the alkyl chain and functionality has a striking influence on their properties. Thus, the preparation of "task-specific" or functionalized ILs is normally realized through the incorporation of functional groups on the cations, to date, mostly the imidazolium cation, although a few examples involving functionalized anions are known.[36]

3.4.1. IL Functionalization via Imidazolium Cation Modification

Starting from 1-alkylimidazoles and using functionalized alkyl halides, the quaternization method usually gives the desired functionalized ILs as imidazolium halides in good yield (Figure 3). Both components are often commercially available and the reaction is usually facile. In many cases solvent is not required unless the reaction is highly exothermic. However, in most cases, solvent is required to purify the imidazolium halide from unreacted starting materials. If a long chain alkyl halide is used, longer reaction times are needed and heating is also necessary. Electron-withdrawing groups attached to the alkyl halide enhance electrophilicity, and consequently shorten the reaction time.

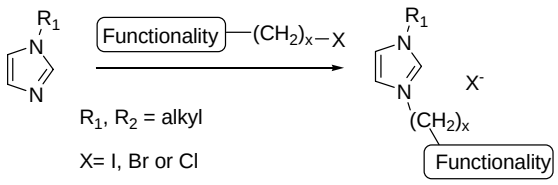


Figure 3. Synthetic route used to prepare functionalized ILs precursors

This method is suitable for the synthesis of nearly all the ILs which are stable towards base, however, because of the relatively strong basicity of imidazole, elimination of hydrogen halide or Hoffmann elimination can occur in some cases.[37] So far most functional groups have been introduced directly to the imidazolium moiety using this direct quartinazation route. For example, imidazolium cations with hydroxyl groups,[38] carboxylic groups,[39] thiol groups[8], alkyne groups,[40] alkene groups,[41] diene groups,[42] and fluorous chains were successfully prepared.[43-44] Certain functionalities requires alternative synthetic routes to be attached to the imidazole backbone, for example, amine[14] and amide groups,[45] phosphine,[46] urea and thiourea groups,[47] and chiral centres.[48] The imidazolium centre is relatively inert and functional groups can after be further reacted, eg. carboxylic ester groups can be converted to carboxylic acid groups,[49] and sulphides can be converted into thiol groups.[50]

3.4.2. ILs containing alkene and alkyne functionality

Alkene and alkynes can be easily introduced into imidazole ring systems using the quaternization method.[51] Figure 4 illustrates some examples of such ILs. Alkene ILs are often less viscous than their saturated counterparts.[52] Due to the highly developed chemistry of C=C and C≡C bonds, they are very good precursors for the preparation of further functionalized ILs. For example, they can be brominated to give dense halogenated ILs.[53] The resulting brominated ILs with $[PF_6]^-$ or $[Tf_2N]^-$ anions are immiscible with water and miscible with dichloromethane, however, they are hardly miscible with chloroform. They are more thermally stable than the non-halogenated imidazolium ILs and therefore have potential applications in separation processes.

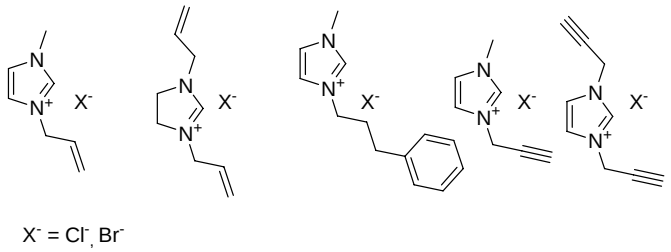


Figure 4. Examples of ILs containing alkene and alkyne functionalities.

3.4.3. ILs containing alcohol, ether and carboxyl functionalities

Direct quaternisation of imidazole with chloroalkylalcohol afford OH or OMe functionalized ILs. Similarly, ester groups can be introduced into imidazolium systems, which can be converted into carboxylic acid groups by reaction with HCl. They dissolve a wide range of transition metal salts and react to form coordination polymers.[54] Figure 5 presents some examples of these ILs.

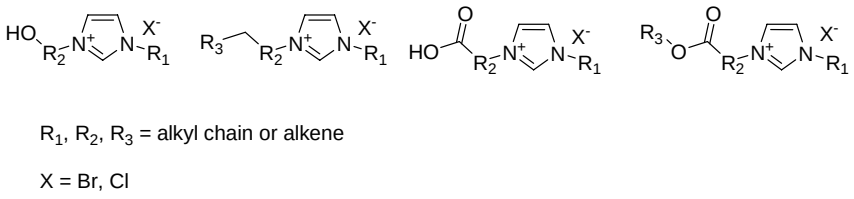


Figure 5. Examples of ILs of containing alcohol, ether and carboxyl groups

3.4.4. ILs containing silicon, nitrogen, phosphous and transition metal substituents

Many other functional groups have also been introduced onto the imidazolium backbone using

standard quaternization reactions. For example, the imidazolium salts with $SiOMe_3$ groups can be prepared in high yield. By anion exchange the hydrophillicity of these salts can be controlled on Si/SiO_2 surface (Figure 6).[55-56]

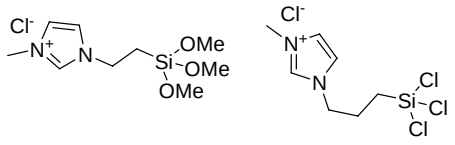


Figure 6. Examples of imidazolium salts of functionality containing $SiOMe_3$ group

The thiol group was also incorporated into the imidazolium moiety. Reduction of disulfide ILs gives thiol-functionalized ILs that can be used to stabilize gold nanoparticles, which can be ransferred from water to ILs through anion exchange (Figure 7).

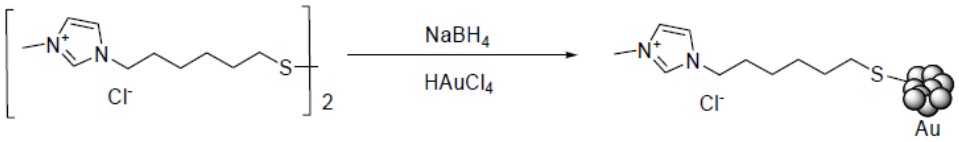


Figure 7. Synthesis of gold nanoparticles using a thiol IL as stabilizer

Imidazolium salts with the SO_3H group are strong Brønsted acids, they can serve as catalyst for Fischer esterification, dehydrodimerization and the pinacol rearrangement.[57] Amino, amido[58], and have also been introduced into the imidazolium backbone. The biphenyl phosphine fragment was introduced into the imidazolium unit, [59] and used in hydroformylation reactions.[60] (Figure 8) Phosphonate can also be introduced to the imidazole system, which is stable in air and can be used as mechanic lubricant.[61]

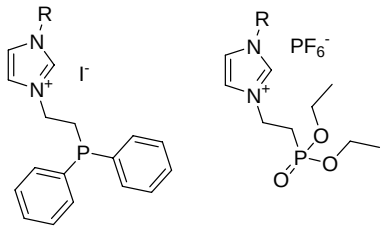


Figure 8. Imidazolium salts with phosphine functionality and ILs with phosphonate functionality

Transition metals can also be incorporated into ILs. For example, the cobalt carbonyl moiety was introduced into imidazolium to form a compound with a melting point of 100°C, which is designated as the criteria to distinguish ILs from other salts (Figure 9).

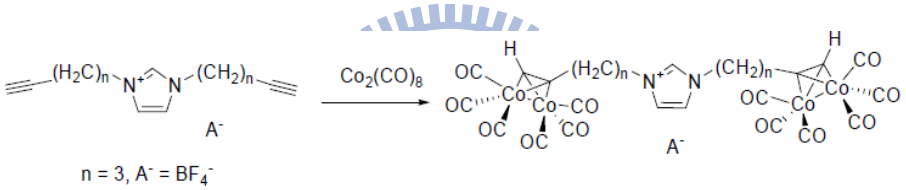


Figure 9. ILs containing cobalt carbonyl fragments

ILs containing the ferrocene moiety, i.e. (ferrocenylmethyl)imidazolium and (ferrocenylmethyl)triazolium, have been prepared. [62] Through adjustment of anion, an IL with melting point of 86°C can be obtained (Figure 10).

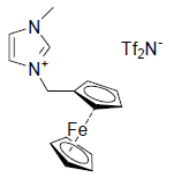


Figure 10. An IL containing ferrocene

3.4.5. N,N-bis(functionalized)-imidazolium ILs (double armed)

Many of the above mentioned functionalized ILs were mono-functionalized, however, *N*,*N* bisfunctionalized-imidazolium ILs can also be obtained using two general methods. Firstly, the deprotonation of imidazole by NaH followed by addition of two equivalents of the functionalized alkyl halide precursor usually gives high yield of *N*,*N*-bisfunctionalized-imidazolium ILs. [63] However this method requires stronger inorganic base, thus it cannot be applied to functional groups which are sensitive to base (Figure 11, left).

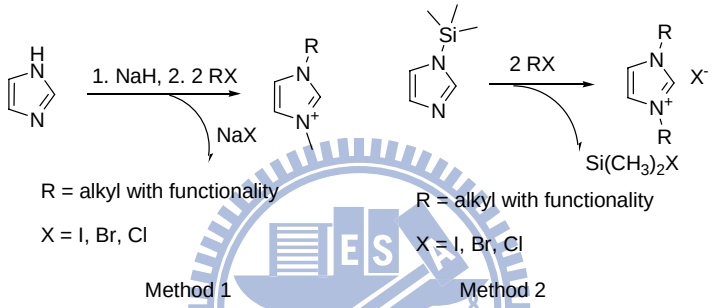


Figure 11. Synthetic routes N, N-bisfunctionalized-imidazolium salts

The second method employs 1-trimethylsilyimidazole as a precursor. Heating a mixture of 1- trimethylsilyimidazole and two equivalent of functionalized alkyl halide gives the desired 1,3- bisfunctionalized-imidazolium in high yield (Figure 11, right).[64] The $SiMe_3Cl$ by-product can be easily removed by distillation. Because no strong base agent is need for the process, this method can be applied to a wide range of precursors.

3.4.6. Alternative methods

It is well know that the imidazolium halides usually have high melting points and are solid at room temperature. To obtain low melting point ILs anion-exchange with

imidazolium halides is required. The drawback of such process is the difficulty to prevent halide contamination, which requires repeated extraction/washing water is often used. Since it has been demonstrated recently that the presence of halide in ILs can drastically change the physical properties of ILs, [65] and may result in catalyst poisoning and deactivation if used as solvents for catalytic reactions,[66] a halide-free route has been developed for synthesizing ILs with the $[BF_4]^-$ anion, in which trimethyloxonium tetrafluoroborate is used to react directly with *N*alkylimidazole.[67] Another method to prepare ILs is by neutralization of a methanol solution of 1-ethyl-3 methylimidazolium methylcarbonate ($MeOCO_2^-$) with acids containing the corresponding anions as shown in figure 12, where MeOH and CO_2 are the only by-products.[68]

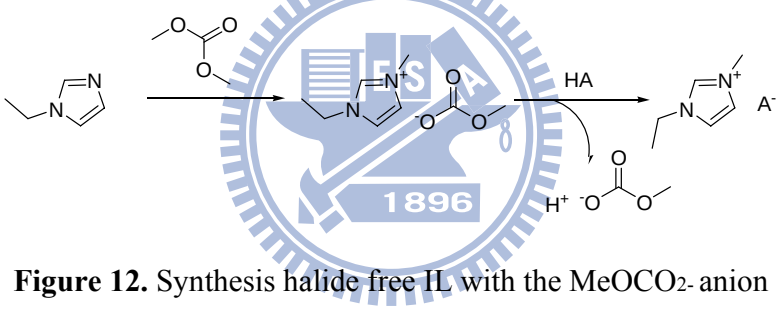


Figure 12. Synthesis halide free IL with the $MeOCO_2^-$ anion

Transformation of imidazolium based zwitterions can also give halide free ILs. For example, imidazolium zwitterions with carboxylate groups can be converted into IL bearing the carboxylic acid functionality (Figure 13. up). A similar process can be used to convert the sulfonate group into an alkanesulfonic acid.[69] (Figure 13. down).

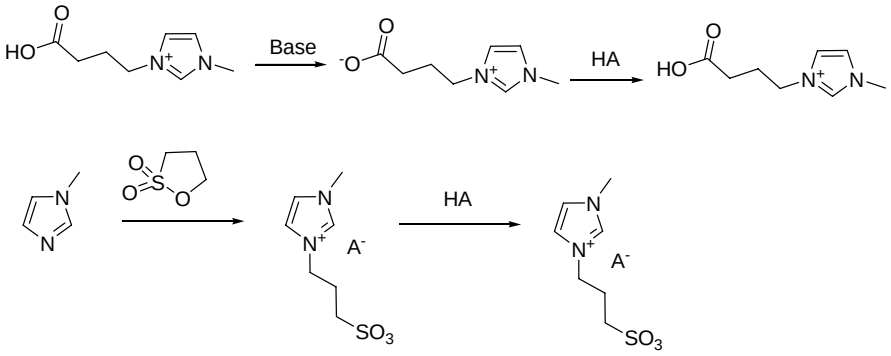


Figure 13. Preparation of ILs containing carboxyl acid group (up) and sulfonic acid (down)

3.4.7. Non-imidazolium based functionalized ILs

Pyridinium functionalized ILs have received less attention than imidazolium systems, but they have been successfully functionalized with the nitrile[70] and $(CH_2)_m(CF_2)_nCF_3$ functionalities (Figure 14).[71]

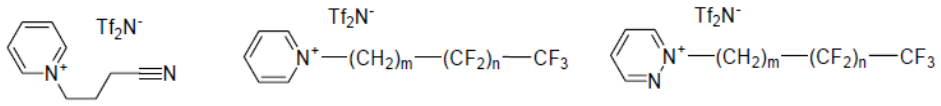


Figure 14. Functionalized ILs based on pyridinium and pyridazinum cations

It has also been reported that a series of 1,2,4-substitute-triazolium salts form ILs, prepared via a multi-step route (Figure 15).[72]

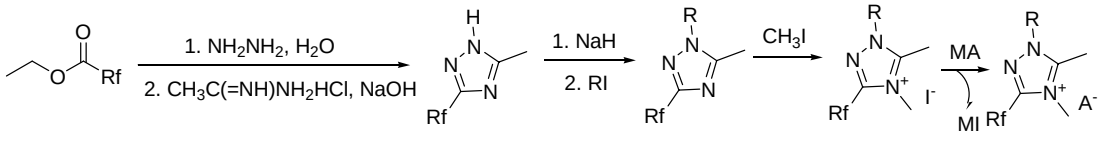


Figure 15. Preparation of triazolium based functionalized ILs

Low melting N-4-functionalized-1-alkyl or polyfluoroalkyl-1,2,4-triazolium salts have also been prepared from zwiterions [73-74] Low melting salts with alkyl, fluoroalkyl, alkyl ether, and fluoroalkyl ether oxazolidine and morpholine cations have been reported.[75] Triazine-based polyfluorinated triquaternary liquid salts were also prepared and tested in hydrophormylation reactions.[76] Phosphazene-based ILs were also prepared and show application potentials as lubricant additives (Figure 16).[77]

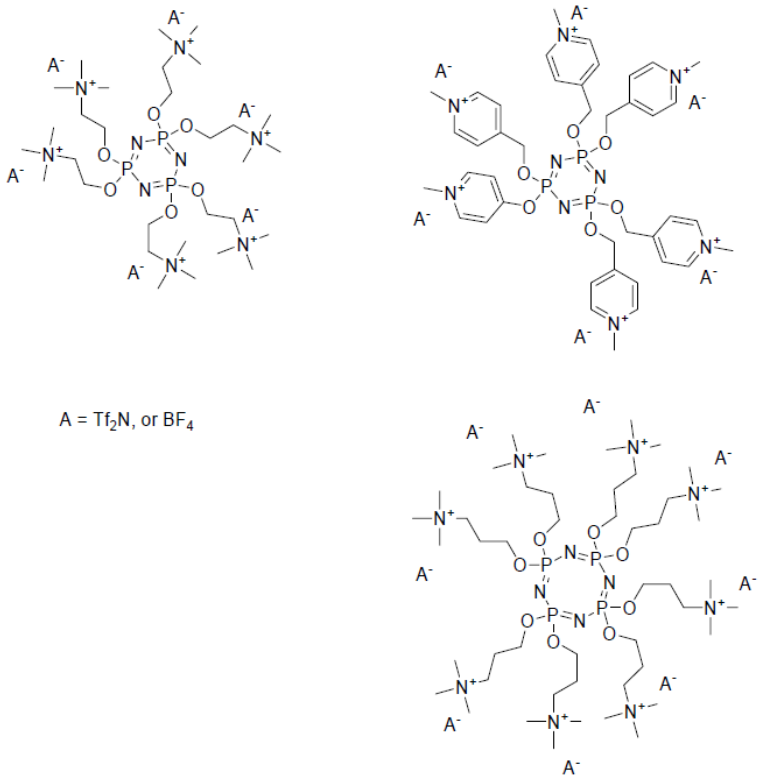


Figure 16. Phosphazene-based ILs

Quaternization of pyrazine, pyridazine, and pyrimidine with alkyl and polyfluoroalkyl halides to form low melting salts has also been reported.[78] Functionalized ammonium ILs, for example, ammonium salts with ether groups have been synthesized. All these quaternary ammonium salts exhibit improved cathodic and anodic stability, such as larger electrochemical window than that of the corresponding 1,3-dialkyimidazolium salts.[79] Quaternary trialkyl(polyfluoroalkyl)ammonium salts have been prepared and their physical properties were found to be mainly determined by the type of anion, regardless of substituents on the cation.[80] Low melting and low viscosity ILs prepared via protonation of trialkylamines by perfluoroalkyl *β*-diketones have been reported (figure 17).[81] The viscosities of this kind of IL, claimed by the authors, were as low as 3.4 cp.

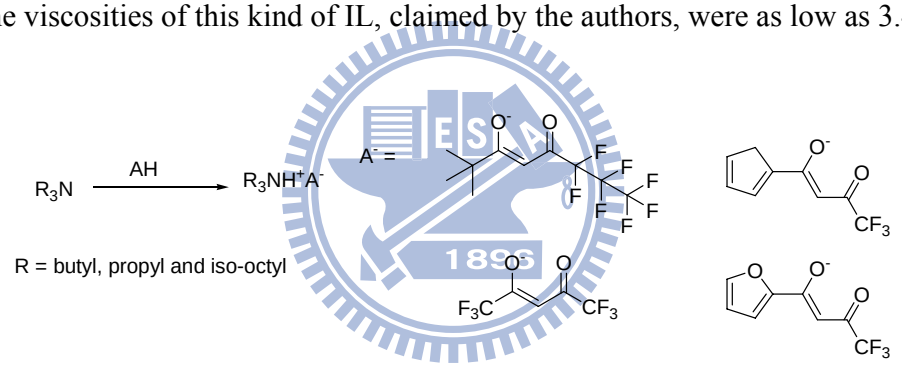


Figure 17. Protonated ammonium ILs with perfluoroalkyl β-diketonate anions

Pyrrolidinonium ILs, with the vinyl substituent, have been synthesized; their intended use is as liquid electrolytes.[82] Phosphonium ILs represent another important member in the IL family. These ILs have been used as media for degradation of phenol,[83] electrolytes for super capacitors,[84] and solvents for use in highly basic environments.[85-86]

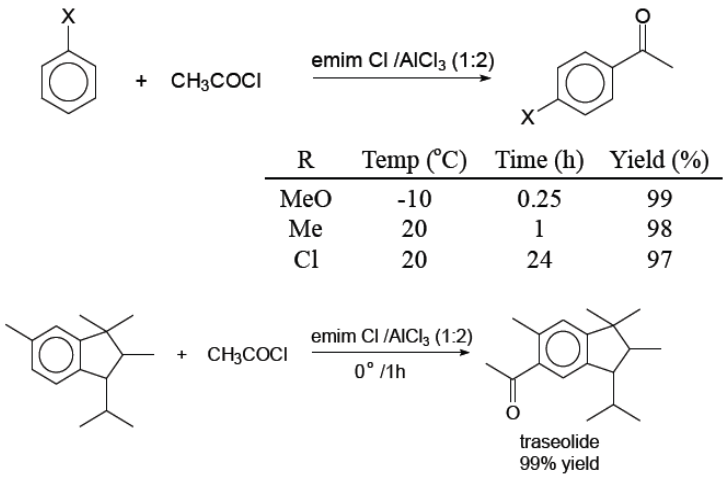
3.5. Application of ionic liquid in organic synthesis

The medicinal chemistry community has been under increased pressure to produce, in an environmentally benign fashion, the myriad of drugs required by society in short periods of time. Because of high molecular complexity in drug discovery processes accompanied by time constraints, the primary driver of pharmaceutical green chemistry has become the development of efficient and environmentally benign synthetic protocols. This can be achieved through the proper choice of starting materials, atom economic methodologies with a minimum number of chemical steps, the appropriate use of greener solvents and reagents, and efficient strategies for product isolation and purification. Thus, green chemistry has emerged as a discipline that permeates all aspects of synthetic chemistry. A major goal of this endeavor must thus be to simultaneously maximize the efficient use of safer raw materials and to reduce the waste produced in the process. The Global need for Ionic Liquids Conventional organic solvents are used in a variety of industrial applications that include the production of pharmaceuticals, the manufacturing of electronic components, the processing of polymers, refrigeration systems and the synthesis of chemicals. Reactions such as hydrogenation, hydroformylation, Heck reactions, dimerization/oligomerization of olefins, Friedel-Crafts reactions, enzyme catalyzed reactions, hydrogenations, benzoylation, Heck reaction, Fischer indole synthesis, etc being carried out using RTILs as solvents.

3.5.1. Friedel-Crafts Acylation in Ionic Liquid

In 1998, K. R. Seddon *et. al.* demonstrated the Friedel-Crafts acylation reaction in ionic liquid and $AlCl_3$ in the ratio of 1:2. They used the differently substituted benzene

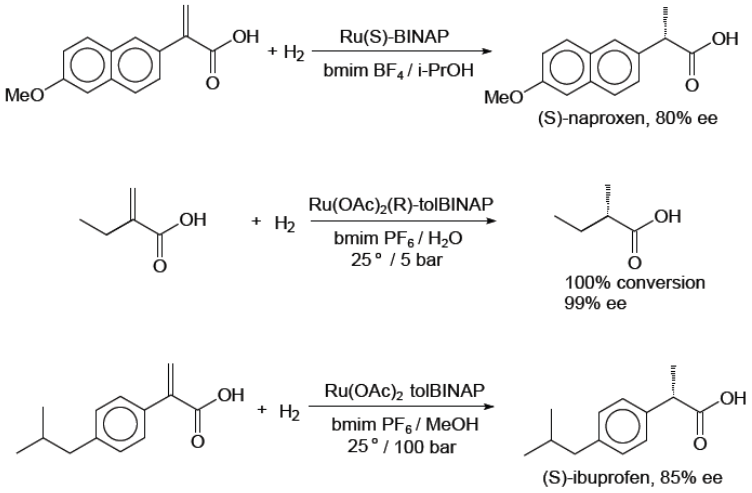
derivatives reacted with acyl choloride to generate the Friedel-Crafts acylated product as shown in scheme1.[87]



Scheme 1. Friedel-Crafts Acylation in Ionic Liquid

3.5.2. Hydrogenation

In 1995, Chauvin demonstrated the first example of hydrogenation using ionic liquid as reaction medium. Dupont *et. al.* showed the asymmetric hydrogenation using $RuCl_2(Ph_3P)_3$ as catalyst in [bmim][BF_4]/organic solvent as solvent. Interestingly the metal catalyst was also recyclable and reusable as shown in Scheme 2.[88]



Scheme 2. Asymmetric hydrogenation in Ionic Liquid

3.5.3. Cross coupling Reaction

In 1996, Kaufmann showed the first example in ionic liquid by use of ammonium and phosphonium salts. Under basic conditions, deprotonation and formation of palladium complexes of imidazolium carbenes become facile. He observed that the competition of cationic and neutral pathways for enol ethers nonexistent in ionic liquids and α-arylation (cationic) regiospecific as shown in figure 19.[89]

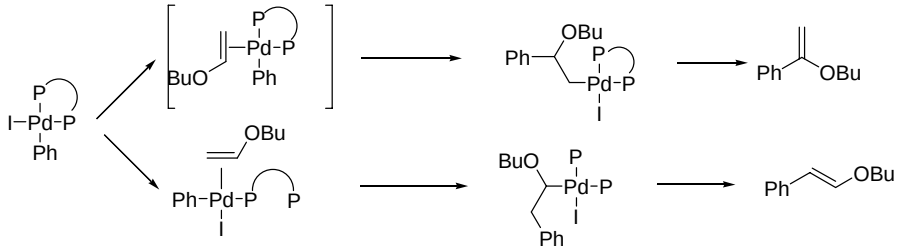
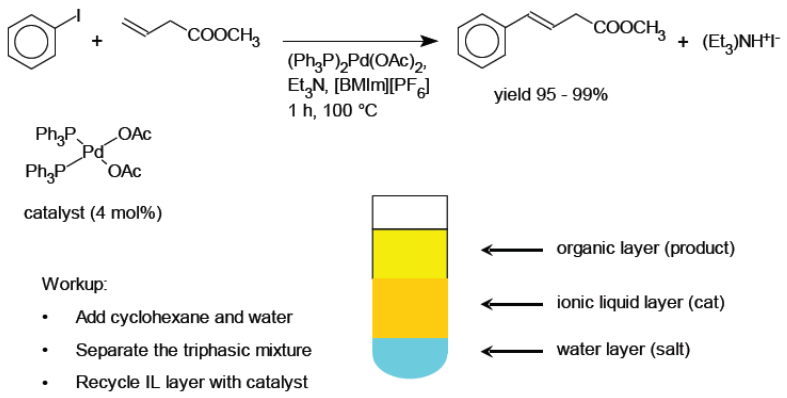


Figure 19. Mechanistic pathway in Cross coupling reaction.

In 1999, Seddon found tri-phasic system – organic: product, ionic liquid: catalyst, aqueous: salt which allows catalyst to be recovered and reused. Similar results for Suzuki, Stille, and Negishi (although yield decreases on recycle experiments for Negishi).

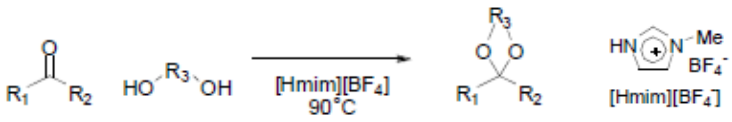
In 1999, A. J. Carmichael *et. al.,* showed the palladium catalysed Heck arylation in ionic liquid medium as shown in scheme 3.[90]



Scheme 3. Palladium catalysed Heck arylation in ionic liquid medium.

3.5.4. Diol/Carbonyl Protection

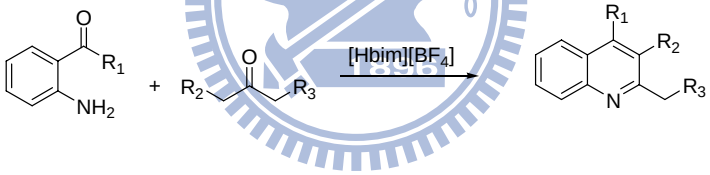
In 2004, He *et. al.* found that carbonyl functionality in organic molecules can be protected using diol in ionic liquid medium as shown in Scheme 4. It has been observed that in molecular solvents, large excess of reagents are required. Monosubstituted imidazolium ionic liquids serve as brønsted acids, moreover acidic ionic liquids afford protected product with no added catalyst, 1:1 ratio of carbonyl to diol, no refluxing/Dean-Stark trap, and no molecular solvent. The product acetals immiscible with ionic liquid and no need to remove water.[91]



Scheme 4. Carbonyl group protection in ionic liquid medium.

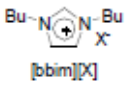
3.5.5. Friedlander Synthesis

In 2003, Palimkar *et.al.* showed the ionic liquids can be used as reaction medium for the Friedlander quinoline synthesis. Normally this reaction requires common additives such as HCl, H_2SO_4, PTSA, microwave, $ZnCl_2/NEt_3$, and ruthenium or palladium complexes. But using ionic liquids as reaction medium does not require any additives as shown in scheme 5.[92]

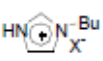


Scheme 5. Friedlander Synthesis in ionic liquid medium.

The efficiency of this reaction related to the basicity of anions of ionic liquid in figure 20. Moreover, ionic liquid can be recovered almost completely and recycled at least twice.



[bbim][X]



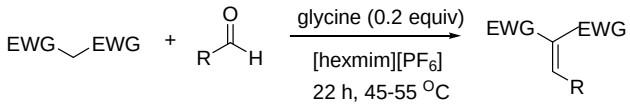
[Hbim][X]

Ionic Liquid	pK_a of Acid of Anion, HX	Yield (%)
[bbim][ClO4]	-11	37
[bbim][Br]	-9	50
[bbim][Cl]	-7	50
[bbim][PF6]		70
[bbim][BF4]	0.5	75
[Hbim][ClO4]	-11	50
[Hbim][Br]	-9	75
[Hbim][Cl]	-7	73.8
[Hbim][PF6]		90
[Hbim][BF4]	0.5	96

Figure 20. Effect of basicity of anions in Friedlander synthesis.

3.5.6. Knoevenagel Condensation/Robinson Annulation

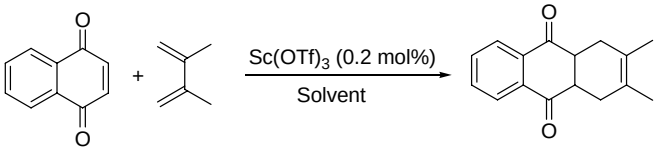
In 2001, Morrison et. al. have used the Knoevenagel condensation reactions performed in air without rigorous drying of ionic liquid and the product was extracted with toluene. The Ionic liquid washed with toluene and reused without further purification as shown in scheme 6.[93]



Scheme 6. Knoevenagel Condensation in ionic liquid medium.

3.5.7. Diels-Alder Reaction

In 2001, Song *et.al.* have identified that the Diels-Alder reaction can be performed in ionic liquid medium. The main advantage was that ionic liquid allows for catalyst recovery, rate acceleration, selectivity enhancement as observed in scheme 7.[94]

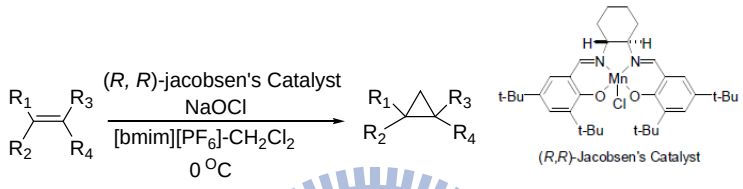


Entry	Solvent	Yield (%)
1	CD_2Cl_2	22
2	[bmim][PF_6] (1 eq)+ CD_2Cl_2	46
3	[bmim][PF_6]	>99

Scheme 7. Diels alder reaction in ionic liquid medium

3.5.8. Olefin Epoxidation

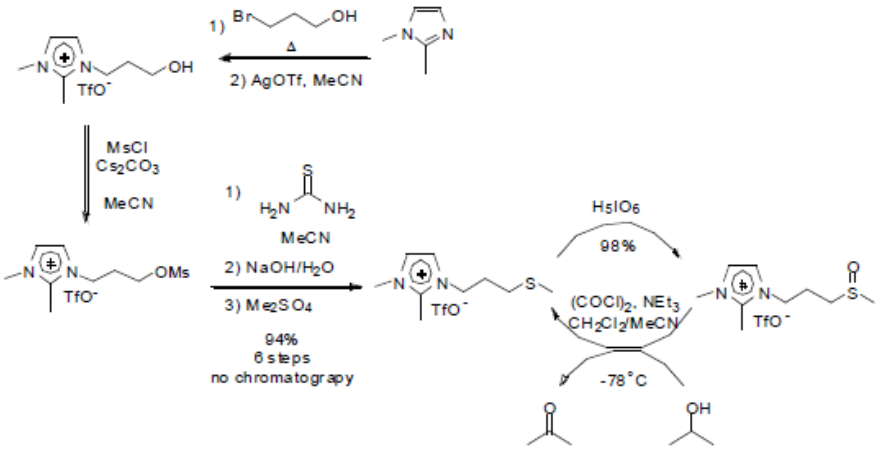
In 2001, Song *et. al.* carried out the olefin epoxidation using (*R*,*R*)-Jacobsen's catalyst immobilized in ionic liquid as shown in scheme 8 and observed the rate enhancement over molecular solvents. But enantioselectivity and activity decrease upon reuse of catalyst because of possible degradation over time. The co-solvent used for reactions below room temperature, because ionic liquid solid at reaction temperature.[95]



Scheme 8. Olefin Epoxidation reaction in ionic liquid medium.

3.5.9. Swern Oxidation

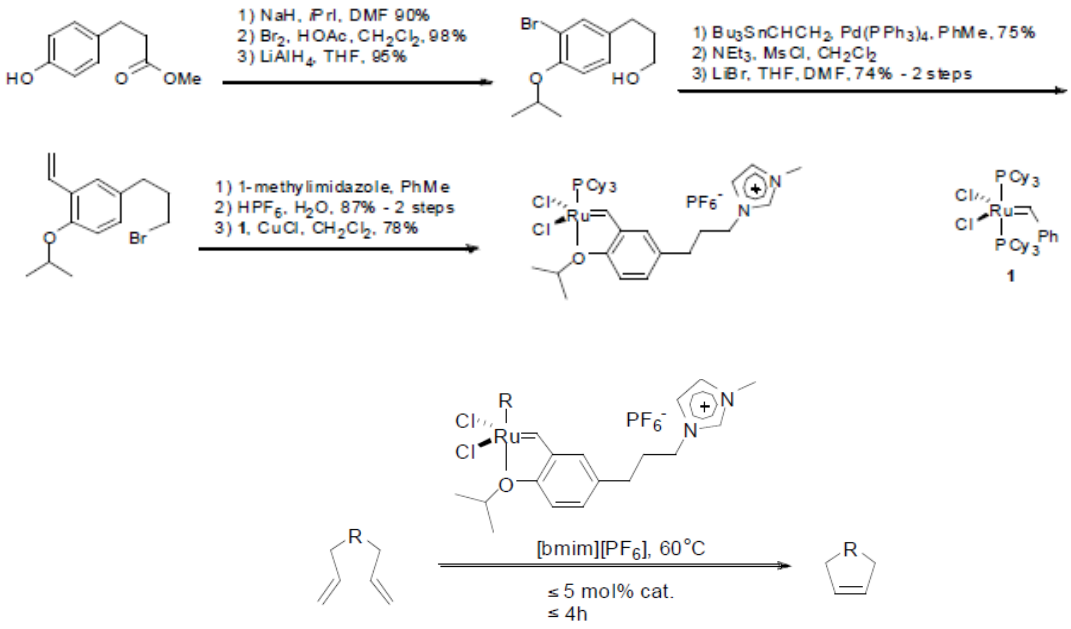
In 2006, Chan *et. al*. have used the ionic liquid as reaction medium for Swern oxidation as shown in scheme 9. The reaction involves the ionic liquid tethered "dimethyl sulfoxide" which can be prepared with no chromatography and no use of volatile (smelly) organosulfur reagents. The Products separated from ionic liquid by phase extraction with ether. The reduced sulfide may be reoxidized and reused for at least 4 recycles with small loss of activity and also able to tether TEMPO catalyst.[96]



Scheme 9. Swern oxidation using ionic liquid tethered dimethyl sulfoxide.

3.5.10. Ring Closing Metathesis

In 2003, Audic *et.al.* have used the ionic liquid in ring ring closing metathesis as shown in scheme 10.[97]



Scheme 10. Ring closing metathesis in ionic liquid medium.

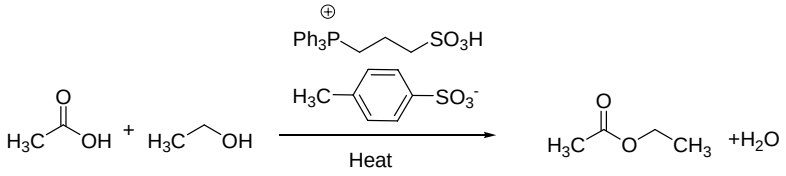
3.6. Ionic liquid used as support for organic synthesis

Despite all those application now a day's ionic liquid are used for the synthesis of new heterocyclic compound. Till now we have mentioned the use of ionic liquid as a designer green solvent, there are few literature report where ionic liquid was used as support. Research in the field where ionic liquid was used as a support is the challenging and one of the hot topics for the medicinal and organic chemist. An attractive feature of ionic liquids is that their solubilities, depending on the choice of cations and anions, can be tuned readily so that they can phase separate from organic as well as aqueous media. Substrate solubility can also be tuned. This suggests the possibility of using these low molecular weight ionic liquids as soluble supports for organic synthesis. This novel liquid-phase strategy can embrace several possibilities: (a) supported catalysts, (b) supported reagents, and (c) supported substrates. In each case, the IL-supported species can be dissolved in a solvent (usually more polar), and the reaction can be conducted in a homogeneous solution. After the reaction, a less polar organic solvent is added in which the IL supported species is not soluble, and the IL phase separates from the organic phase. The recovered IL supported species can be regenerated (for reagent) or reused (for catalyst) or further reacted to give the final product, which would then be detached from the ionic liquid support.

3.6.1. The use of Ionic-Liquid as Supported Catalysis.

Davis was the first to recognize that functionalized ionic liquids can serve not just as reaction media but as catalyst as well.[98] Scheme 11 shows the example is the

phosphonium salt catalyzes the formation of esters from alcohols and acids, dehydration of alcohols to ethers, and pinacol rearrangement of vicinal diols.[99] Phosphonium salt behaves as a strong bronsted acid.



Scheme 11. Phosphonium salts catalyses the organic reaction

The product can be easily removed from the IL-supported catalyst by simple distillation which can be reuesd. Ionic liquid supported sulfonic acid can catalyse the esterification of aliphatic acids with olefin and hetero-Michael additions. [100] Gao and Bao [101] reported the IL supported 2,2,6,6-tetramethyl-piperidinyloxy (Figure 21, a, b, c), free radical TEMPO catalysts in the oxidation of alcohols.

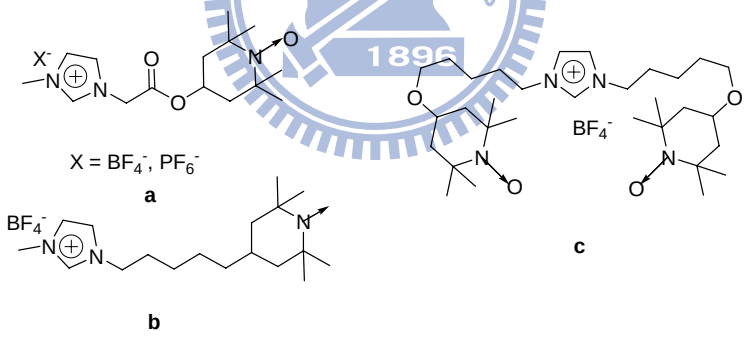
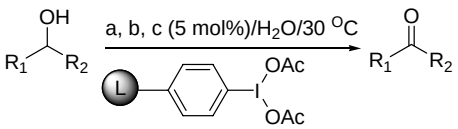


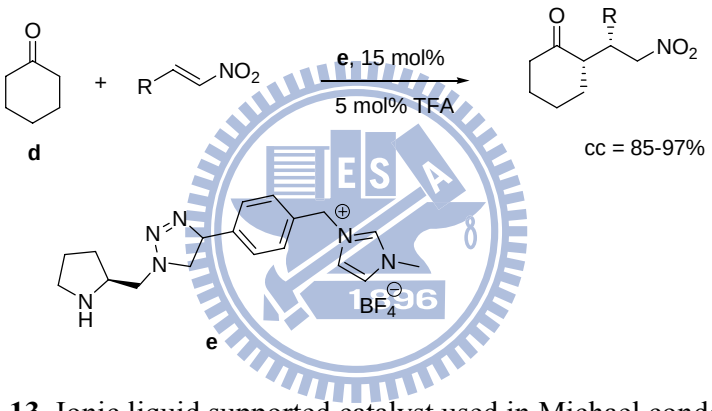
Figure 21. Ionic liquid supported catalyst

All the catalysts showed similar activity to that of free TEMPO and could be reused up to many times without loss of activity in scheme 12.



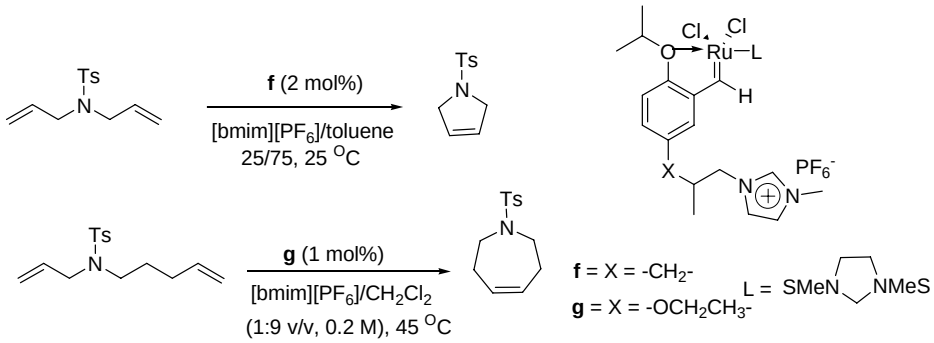
Scheme 12. Catalytic activity of Ionic liquid supported catalyst

The main advantage of using an IL-supported catalyst is that the catalyst can be recovered simply by solubility difference. This is especially important in a case where the catalyst has to be used in quite substantial amounts. Ionic-liquid-supported triazole-pyrrolidine **e**, for the direct asymmetric Michael reaction was successfully synthesized. The supported catalyst demonstrated good activity and high enantioselectivity in the addition of cyclohexanone to nitroolefins. Furthermore, the supported catalyst can be readily recovered and reused four times without significant loss of catalytic activity.[102]



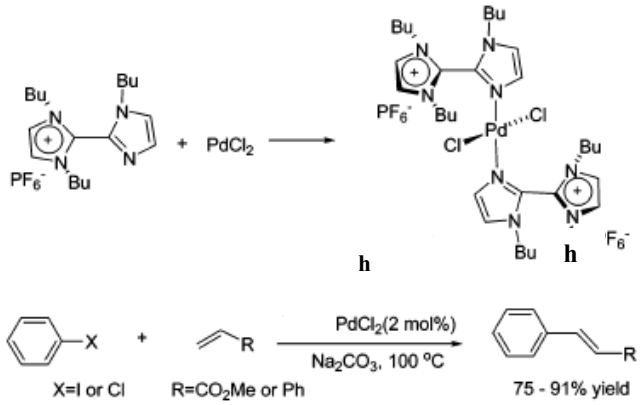
Scheme 13. Ionic liquid supported catalyst used in Michael condensation

The efficient recycling of IL-supported catalysts suggests that the approach can be useful in metal-catalyzed reactions where the reuse of expensive ligands, metal, or both is critical. In 2003, Guillemine[103] and Yao[104] reported independently the synthesis of IL-supported catalysts for the ring-closing metathesis (RCM) of olefins (Scheme 14).



Scheme 14. IL-supported catalysts for the ring-closing metathesis (RCM) of olefins

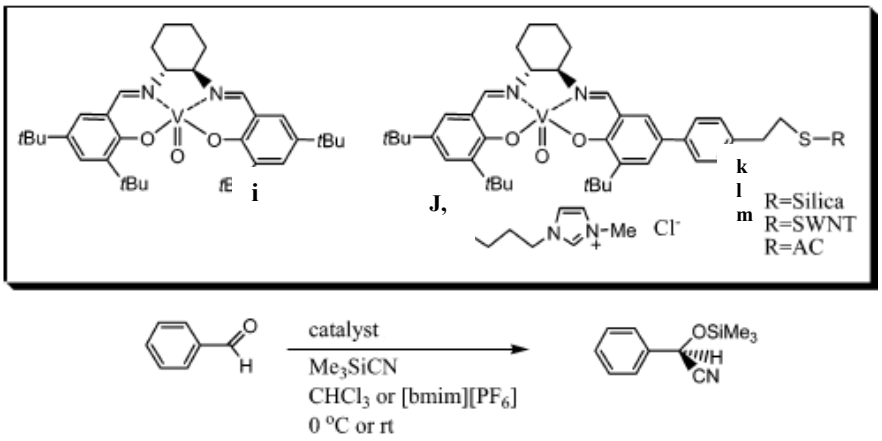
The IL-supported palladium complex **h** was found to catalyze the Heck reactions (Scheme 15) with good recyclability of up to 10 cycles.[105]



Scheme 15. Ionic liquid supported Heck reactions

In an interesting study, Garcıa compared silica, single wall carbon nanotube (SWNT), activated carbon (AC), and imidazolium ion as support for chiral vanadyl salen complex **i** in the enantioselective cyanosilylation of benzaldehyde (Scheme 16).[106] The IL-supported complex **j** was compared with the unsupported complex **i**, as well as similar complexes

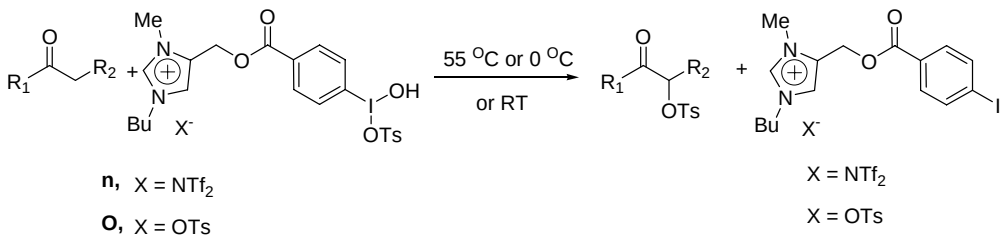
k-l anchored on solid insoluble matrix such as silica, SWNT, and AC. Among the four recoverable catalysts **j-m**, **j** showed the highest activity.



Scheme 16. Enantioselective cyanosilylation of benzaldehyde

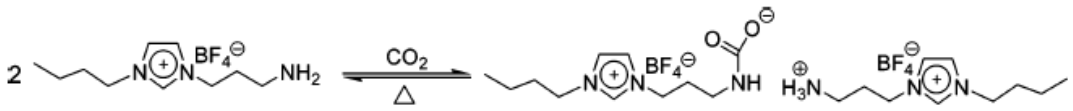
3.6.2. The use of Ionic-Liquid as Supported Reagents.

Synthetic reagents anchored onto ionic liquids can be separated readily from the reaction mixture by simple phase separation after the desired chemical transformation and then be regenerated and reused. Recently it has been observed that the IL-supported hypervalent iodine(III) reagents **n** and **o** have been prepared by Handy for the R-tosylation of ketones (Scheme 17).[107] The reaction demonstrated the advantage of the tunable separation properties of the ionic liquid support.



Scheme 17. Tosylation of ketones using ionic-liquid as supported reagents

A significant application of IL-supported reagents is their use as recyclable scavengers. Commercially, large scale CO_2 capture in the removal of carbon dioxide from natural gas uses aqueous amines to chemically trap the CO_2 by the formation of ammonium carbamate **q**. Davis showed that when the IL supported amine **p** was exposed to a stream of carbon dioxide for 3 h at 1 atm at room temperature, nearly 0.5 mol of CO_2 was captured per mole of **p**. (Scheme 18).[108]



p **q**

Scheme 18. The use of IL-supported reagents as recyclable scavengers

The free amine **p** can be recovered and reused with no observed loss of efficiency for five cycles. Recently, the IL-supported amine has been proposed as scavenger for benzoyl chloride, *p*-toluenesulfonyl chloride, phenyl isothiocycanate, and chlorophenyl isocyanate compounds in combinatorial synthesis.[109]

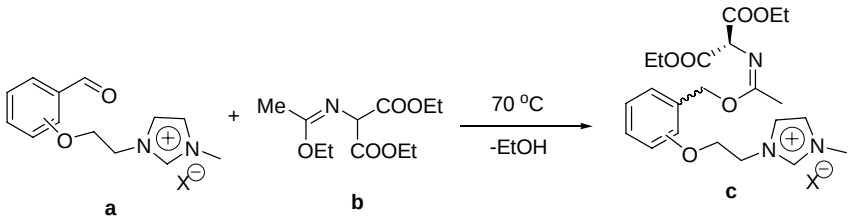
3. 7. Ionic-Liquid-Supported Synthesis of Small Molecules and Combinatorial Synthesis

As we all aware of the fact that the supported synthesis is a widely employed technique that has greatly facilitated the synthesis of many compounds and is the critical element behind the explosion in combinatorial synthesis. Traditionally, the supported synthesis has employed a heterogeneous material such as cross linked polystyrene to support one of the reactants. The primary advantage of such a choice is that the supported material,

being heterogeneous, can be readily separated by simple filtration from the reaction medium and by-products. At the same time, this heterogeneity limits the types of reactions and reaction conditions that can be employed. Further, using simple polystyrene supports, which are typically functionalized to <10%, the maximum loading is <2 mmol/g. These limitations have led more recently to the development of a variety of 'soluble' supports (e.g. the polyethylene glycol (PEG) supports popularized by Janda). Since the supports are homogeneous in a variety of conventional organic solvents, reactions can be performed under conventional solution-phase conditions. At the same time, by changing the polarity of the solvent (most frequently by the addition of methanol), the support and supported molecule will precipitate, resulting in facile separation by filtration. While this is a major step forward, there are still limitations to the current supports. In order to circumvent the drawback, most recently ionic liquid has emerged as alternative soluble support for carrying out the organic synthesis of biologically relevant compounds. An attractive feature of ionic liquids is that their solubility can be tuned readily. Therefore, phase separation from organic solvent or aqueous phase is allowed depending on the choice of cations and anions. This suggests the possibility of using these small molecular ionic liquids as soluble supports for organic synthesis. Ionic liquid attached substrates are expected to retain their reactivity, as in solution reactions, and allowed the use of conventional spectroscopic analysis such as NMR during the synthetic process.

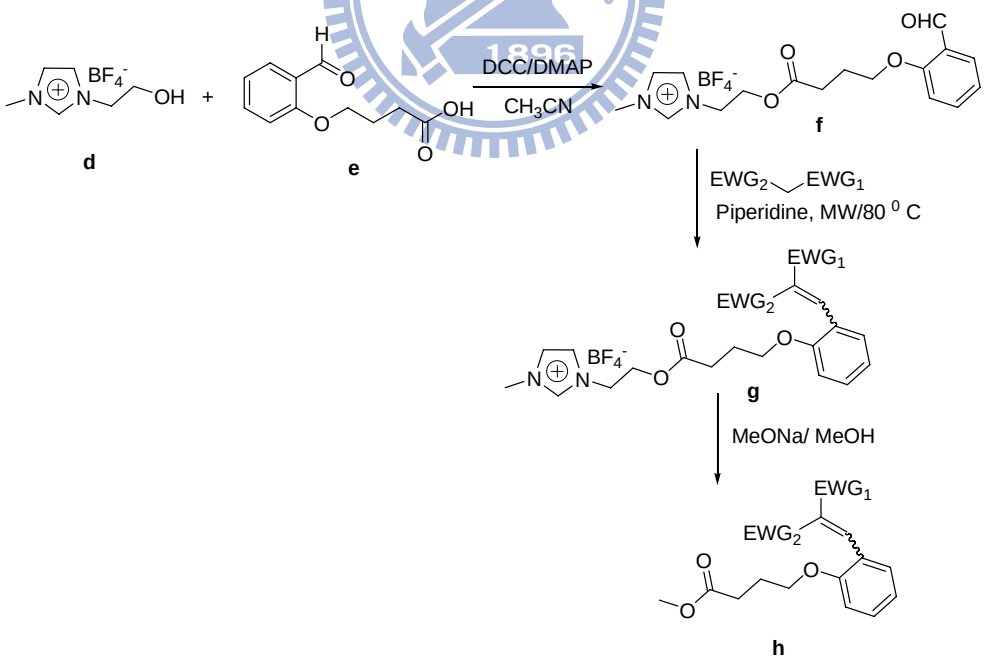
Bazureau was the first to propose the use of ionic liquid as soluble support for the synthesis of small organic molecules.[110] They observed that the reaction of the IL anchored dipolarophile **a** (*ortho*) with the imidate **b** to give the adduct **c** (Scheme 19) was

faster than that of the reaction of free 2-ethoxybenzaldehyde with the ionic liquid [emim][NfO](emim)1-ethyl-3-methylimidazolium).[111]



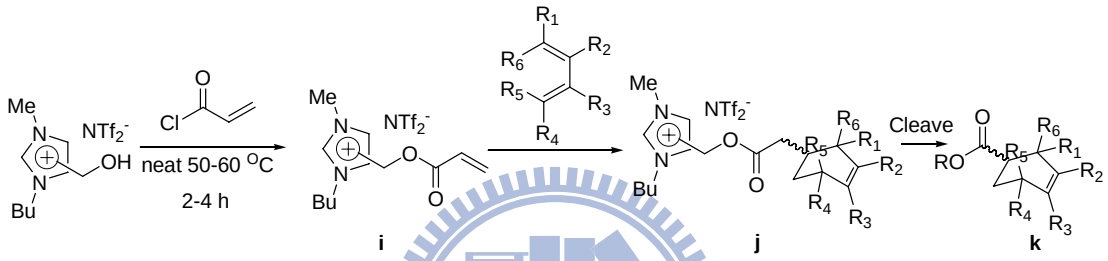
Scheme 19. IL supported synthesis of small molecules

They also examined the Knoevenagel and 1,3-dipolar cycloadition reactions with the IL-supported benzaldehyde **f** (Scheme 20). Thus, the substituted benzaldehyde **e** was anchored onto the IL support **d** to give **f**. Knoevenagel reaction of **f** under homogeneous conditions gave the products **g** in high yields. Cleavage of the ionic liquid support from **g** by basic methanolysis gave the small molecule **h** in good isolated yield.



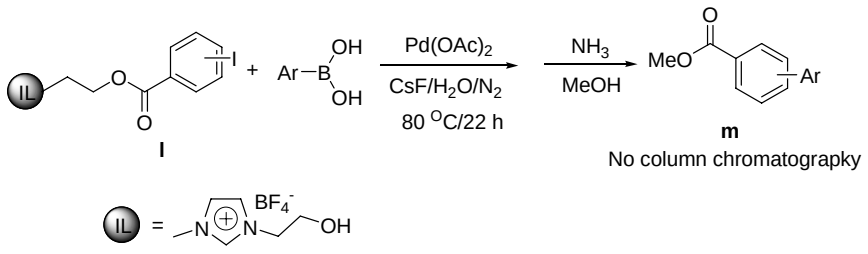
Scheme 20. Ionic Liquid supported synthesis of **h**

In an attempt to show the 4+2 cycloaddition reaction on ionic liquid support, Handy and Okello have reacted the acrolyl chloride with ionic liquid to get the IL anchored compound **i**.[112] The Diels-Alder reactions between **i** and various substituted butadienes gave the Diels-Alder adducts **j** (Scheme 21), which could be readily isolated by removal of the volatiles under reduced pressure. Cleavage of **j** led to the esters **k** in good overall yields as shown in scheme 21. The ionic liquid support could be recovered in greater than 90% yield and reused.



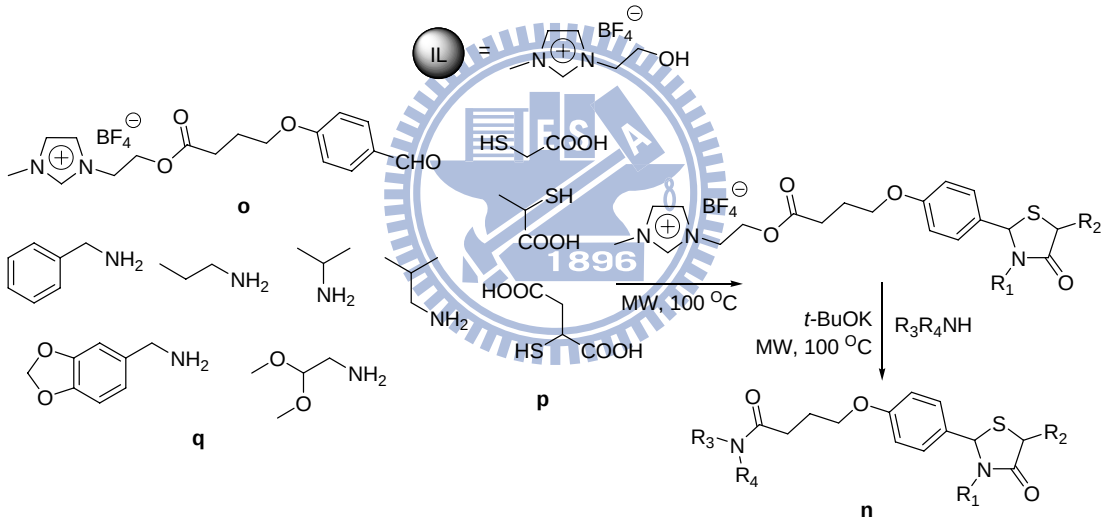
Scheme 21. Ionic liquid supported Diels-Alder reaction

In order to demonstrate the advantages of IL-supported synthesis over the conventional solution-phase synthesis, Chen *et. al.* examined a series of Suzuki coupling reactions between boronic acids and the IL-supported iodobenzoates **l** as shown in Scheme 22.[113] IL-supported biaryl products, which were subjected to cleavage with ammonia/methanol to give **m** in pure form, which was easily separated from the ionic liquid phase by ether extraction without chromatographic purification.



Scheme 22. Ionic Liquid Supported Suzuki coupling reactions.

Furthermore it has been observed that the IL-supported strategy for combinatorial synthesis was demonstrated by the preparation of a small library of 4-thiazolidinones **n** (Scheme 23).[114] The synthesis of the IL-bound 4-thiazolidinones was accomplished by a one-pot condensation among **o**, mercapto acids **p**, and amines **q** under microwave dielectric heating. The cleavage of **n** from the IL support was realized by amide formation also under microwave irradiation. Diversity was introduced at the phenoxy component **o**, the thiol **p**, the amine **q**, and the cleavage step.

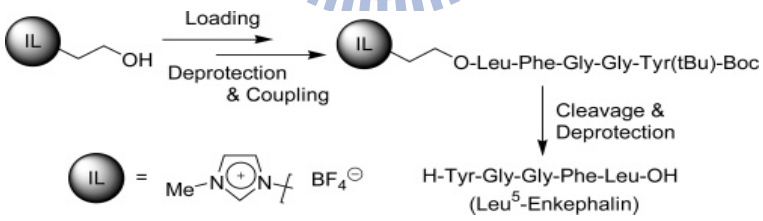


Scheme 23. Ionic Liquid Supported synthesis of small library of 4-thiazolidinones.

The advantages of using ionic liquid support are as follows: (1) it provides the ease of product isolation because the side products are removed by simple washing with appropriate solvent; (2) in each step, the reaction can be followed by standard analytical technique; (3) due to the high polarity of the ionic liquid support, microwave dielectric heating can be easily applied to enhance the reaction; (4) the final product was usually

obtained in high purity after flash chromatography. A small library of 4-aminophenyl ethers was also prepared using an ionic liquid as support.[115]

With the success of IL-supported synthesis for small molecules, the same strategy has been tried to apply to the syntheses of important oligomers of biomolecules. Chen *et. al.* have used the synthesis of the bioactive pentapeptide Leu5-enkephalin **r** using ionic liquid support (Scheme 24).[116] The approach is similar to the solid-phase and most liquid-phase peptide syntheses in that the IL support is linked to the C-terminus of the growing peptide and serves as a carboxylic acid protecting group. Most importantly, no racemization or epimerization was observed in the deprotection/coupling sequence. This generic protocol of deprotection along with coupling was applicable IL-supported pentapeptide without any difficulties. Their structural assaignment and purities could be confirmed easily by conventional analytical methods such as NMR and MS. Finally, the product Leu5-enkephalin was cleaved from the ionic liquid support by basic hydrolysis followed by removal of the protecting groups.

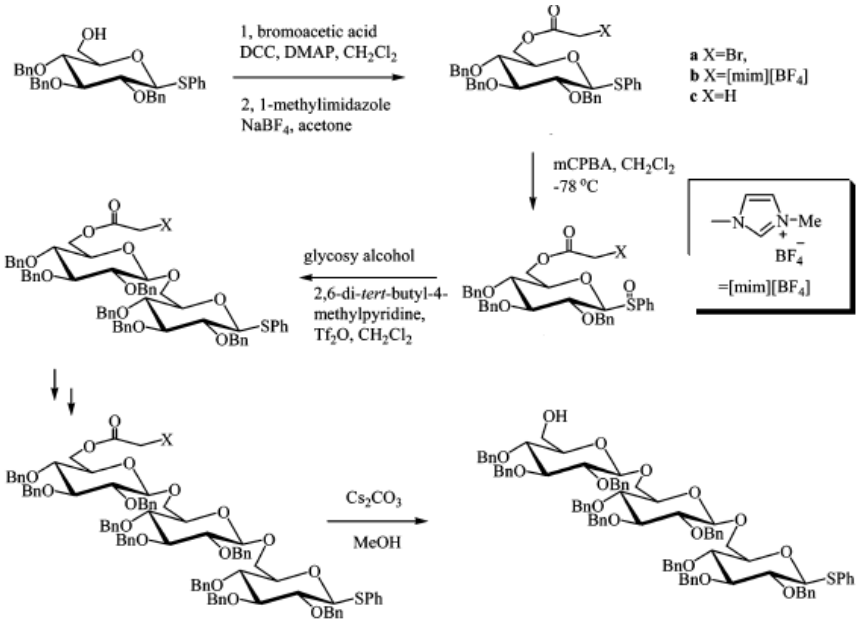


r

Scheme 24. Ionic liquid supported synthesis of Leu5-enkephalin **r**

Buoyed by the success synthesis of oligopeptides in 2006, Chen *et. al*. initiated the, adopted the approach for the synthesis of oligosaccharides as shown in (Scheme 25).[117] The efficient synthesis of oligosaccharides is a challenging task because the traditional

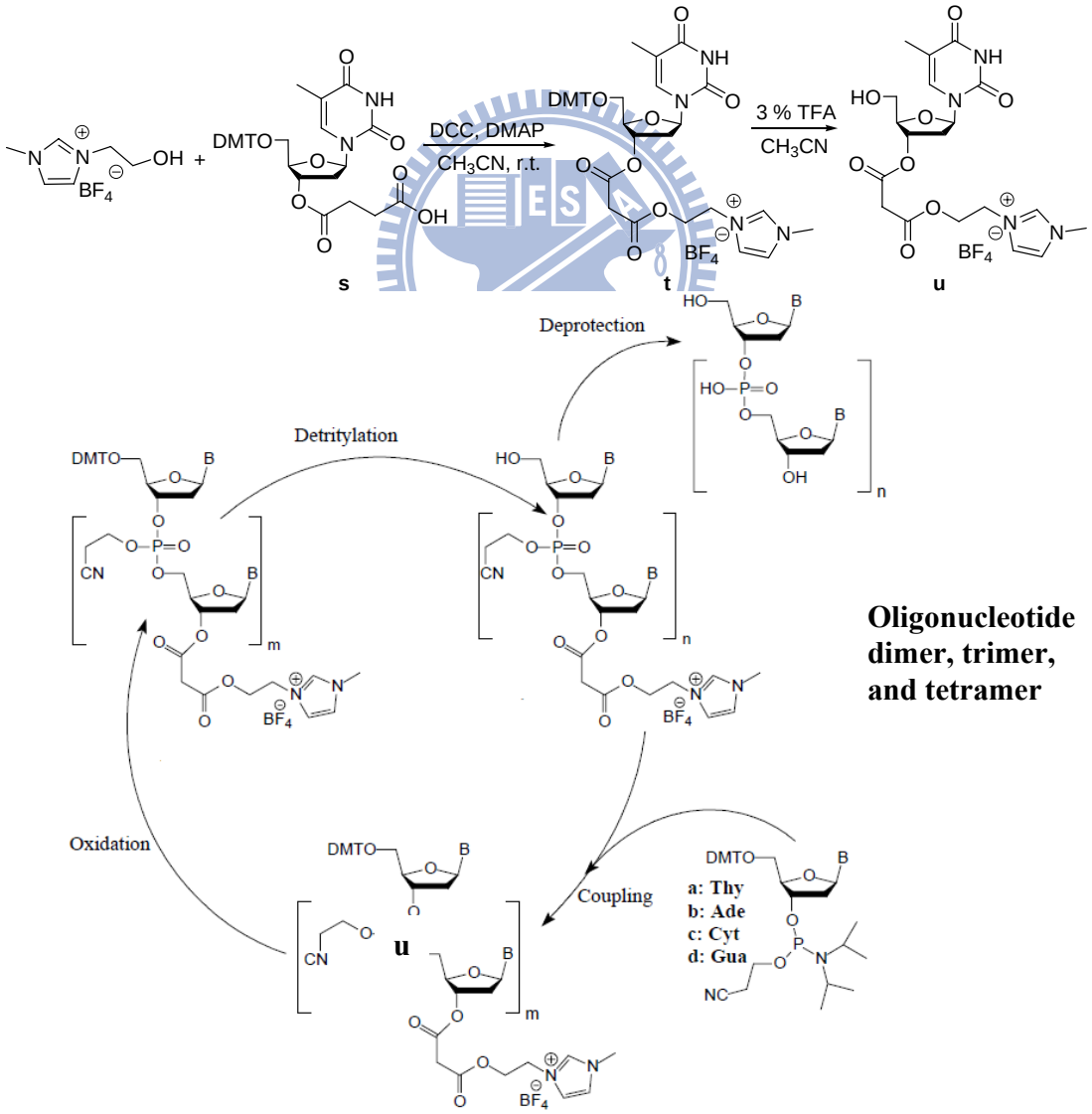
solution-phase synthesis is laborious and requires purification by chromatography after each step.



Scheme 25. Ionic liquid supported synthesis of oligosaccharides.

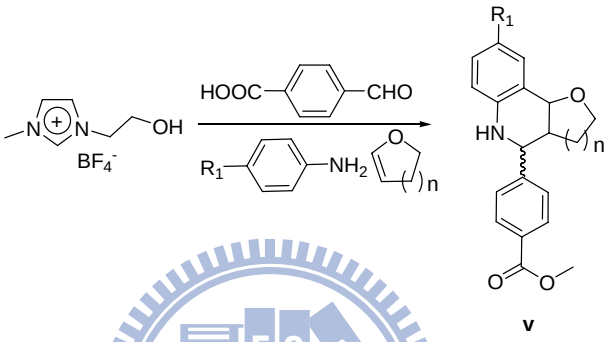
Damha *et.al.*[118] have described the synthesis of oligonucleotides in solution using a soluble ionic liquid as support. Short oligomers of varying base composition were synthesized using this method in high yields and high purity, requiring no chromatography for purification prior to cleavage from the support. The synthesis of the ionic liquid supported oligonucleotide began with the ionic liquid. The succinylated 5′-DMT-thymidine derivative **s** was then coupled to the ionic liquid using dicyclohexylcarbodiimide (DCC) and catalytic amounts of 4-(dimethylamino) pyridine (DMAP) in acetonitrile to give the ionic liquid supported nucleoside **t**. Exposure of **t** to acidic conditions yielded compound **u**. The dinucleoside phosphotriesters TpT, ApT, CpT, and GpT were prepared at the 250 μmol scale by reacting the ionic liquid supported

nucleoside **t** with a 1.5-fold excess of the appropriate phosphoramidite derivatives using 4,5- dicyanoimidazole (DCI) as the activating agent in CH_3CN and stirring for 1-2 h. To carry out the oxidation of the phosphite triester intermediates, compounds from previous steps was again dissolved in a small amount of CH_3CN, pyridine, or 2,4,6-collidine, and a large excess (2-5 equiv) of a 0.1 M solution of iodine in 2:1 THF: water was added. Similarly 5′detritylation of **u** was achieved using 3 % trifluoroacetic acid in 20 minutes. In addition to the dimmers, a thymidine trimer, and a tetramer were also synthesized at the 50-100 µmol scale as shown in scheme 26 and 27.



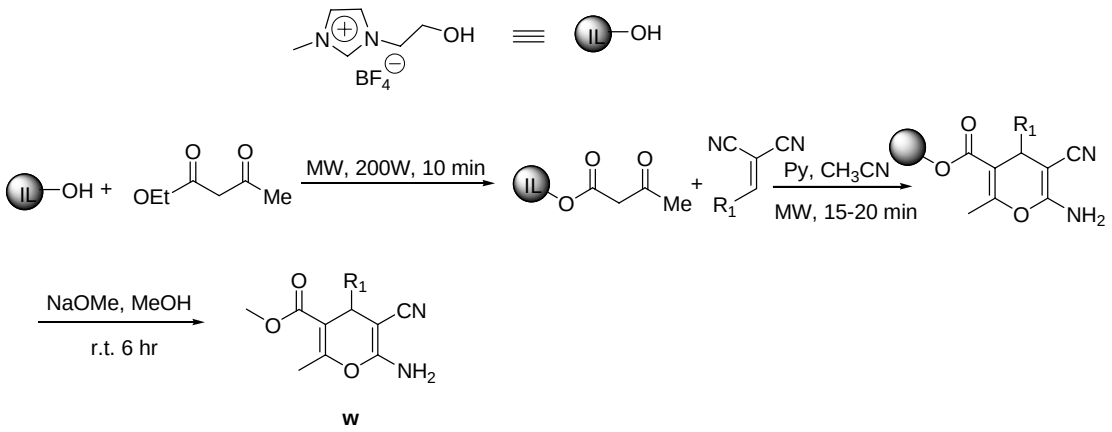
Scheme 26 and 27. Synthetic cycle of oligonucleotides

In 2007, Li *et.al*. have used the ionic liquid as soluble support for the synthesis of tetrahydropyrano and tetrahydrofuranoquinolines **v**, an important heterocyclic compounds under microwave irradiation as elaborated in scheme 28.[119]



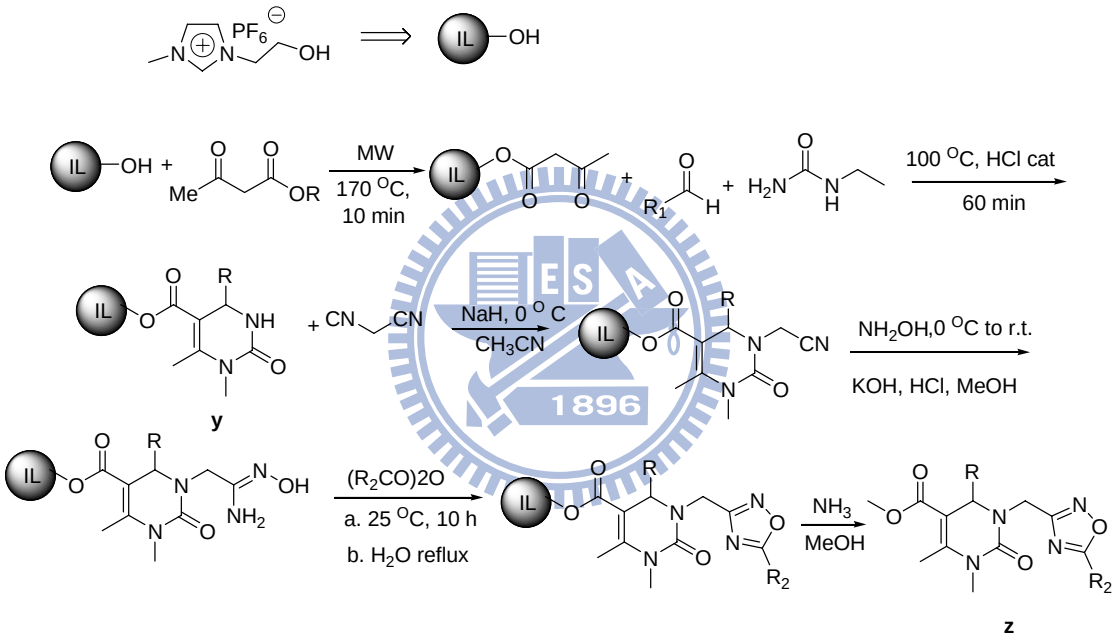
Scheme 28. Synthetic cycle of tetrahydropyrano and tetrahydrofuranoquinolines

Song et al.[120] reported the synthesis of 2-amino-4*H*-pyrans **w** from malononitrile, aryl aldehydes, and 1,3-dicarbonyl compounds using ionic liquids as soluble support as elaborated in scheme 29.



Scheme 29. Synthesis of pyran derivatives.

Bazureau *et. al.* performed multicomponent reaction in ionic liquid as support to obtain 2 thioxotetrahydropyrimidin4-(1*H*)-ones.[121] The methodology employs microwave irradiation and a matrix of PEG ILPs used for an ionic liquid phase organic synthesis. Continuing their work in this area, Bazureau *et al*.[122] have developed the synthesis of N-3 functionalized 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) **z** (Scheme 30) with a 1,2,4-oxadiazole **y** group via the three component Biginelli condensation without solvent, according to the "ionic liquid-phase organic synthesis" (IoLiPOS) methodology.



Scheme 30. 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) **z.**

Section A

3.8. Benzimidazoles and its amino derivatives, Importance& Synthesis

Benzimidazole and its amino derivatives are important class of biologically active heterocyclic molecules in the field of drugs and pharmaceuticals.[123] First in 1954, Tamm, Folkers and co-workers reported the synthesis and antiviral activity of halogenated benzimidazole nucleosides.[124] They observed that 5,6-dichloro-1-(*β*-D ribofuranosyl)benzimidazole (DRB) has multiple biological activities including activity against RNA and DNA viruses.[125] Moreover, the benzimidazole scaffolds possess significant activity against several viruses such as HIV,[126] herpes (HSV-1),[127] RNA,[128] influenza,[129] and human cytomegalovirus (HCMV). In spite of these, some benzimidazole derivatives have been demonstrated to be potent topoisomerase I inhibitors,[130] selective neuropeptide YY1 receptor antagonists[131] (Figure 22, **1**), angiotensin II (AII) inhibitors[132] (Figure 22, **2**), potential antitumour agents[133], antimicrobial agents[134], and inhibitors of the hepatitis C virus RNA polymerase[135], inhibitors of HCMV replication[136]. However it has been found that the 2-aminobenzimidazoles are important organic scaffolds due to their extensive applications in biological and therapeutic activities. It has been observed that the gastrointestinal infections are among the major public health problems in developing countries. Especially amoebiasis (Entamoeba histolytica) and giardiasis (Giardia lamblia) have high mortality indexes due to the effects of severe diarrhea and invasive infections. Although current drug therapy for the treatment of amoebiasis and giardiasis is effective, most

available drugs have established that benzimidazole carbamates (BZC) such as Albendazole, Mebendazole, Flubendazole and Fenbendazole (Figure 22, **3, 4, 5, 6**) inhibit the in vitro growth of Trichomonas vaginalis[137] and G. lamblia.[138] Clinical reports have shown that Albendazole is as effective as Metronidazole, the choice drug for the treatment of giardiasis. Astemizole (Figure 19, **7**), containing 2-amino benzimidazole moiety was a second generation antihistamine drug which has a long duration of action.

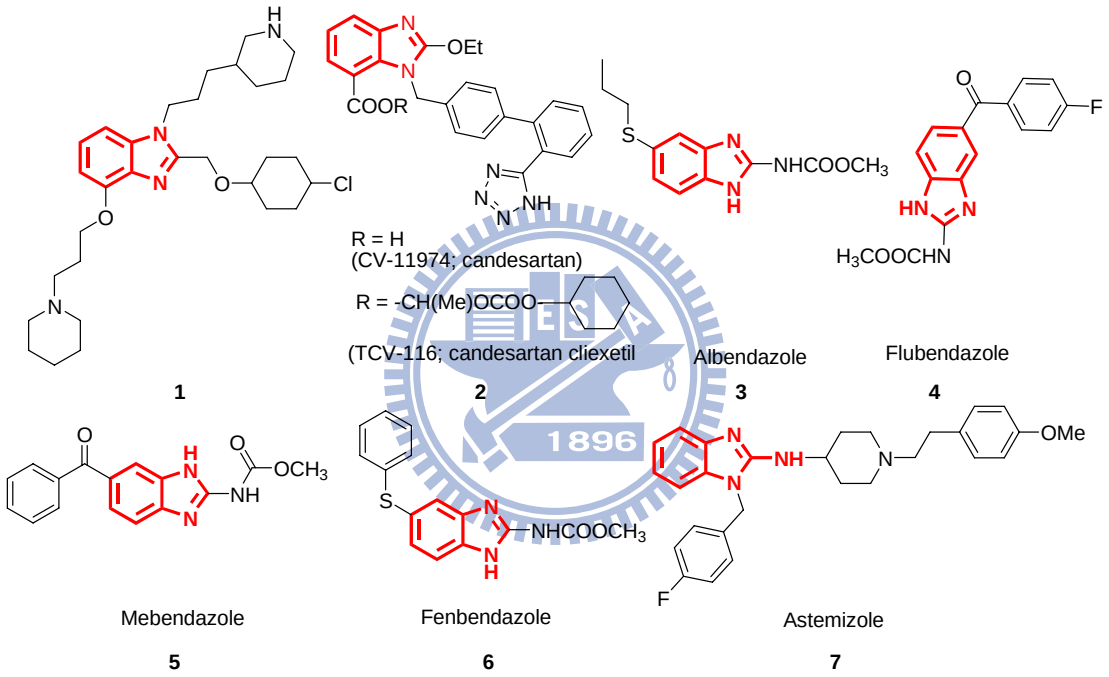
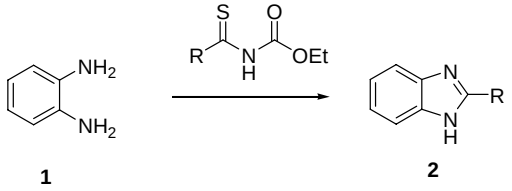


Figure 22. Biologically active benzimidazole derivatives

The below section briefly reviews methods to the construction of biologically active benzimidazoles as well as 2-aminobenzimidazole rings which involves the condensation of 1,2-phenylenediamine with either carboxylic acids or aldehydes by acid or base catalyzed reaction under high temperature conditions or metal induced cyclisation reaction.

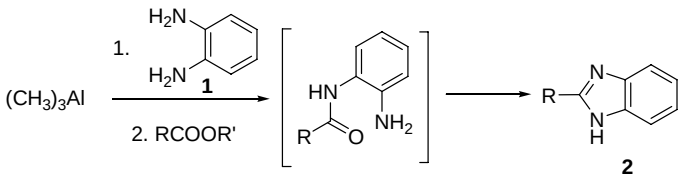
3.9. Chemical methods for synthesizing benzimidazole derivatives.

In 1977, Papadopulous *et. al.* have synthesized the benzimidazole derivatives using 1,2-phenylenediamine derivatives **1** with *N*-ethoxycarbonylthioamides with the evolution of H_2S gas as shown in scheme 31.[139]



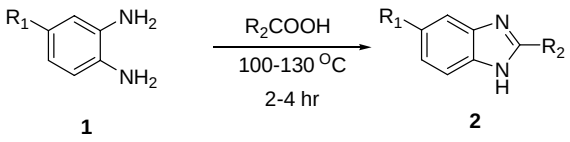
Scheme 31. Papadopulous method of benzimidazole synthesis

In 1981, Neef *et. al*. have synthesized the benzimidazole derivatives using organo aluminium reagents. They have reacted the *o*-phenylenediamine derivatives **1** with alkyl esters in presence of trimethyl aluminium as activating reagent as shown in the scheme 32. The mechanism of the reaction involves the coupling of o-amino group of 1, 2-phenylenediamine with esters and subsequent cyclisation to the benzimidazole derivatives **2**.[140]



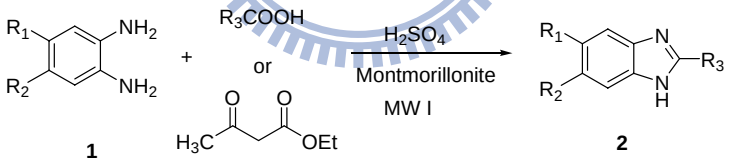
Scheme 32. Neef method of benzimidazole synthesis

n 1985, Raban *et. al*. used the reaction of an appropriate 1,2-phenylenediamine **1** with carboxylic acid and its derivative to produce the benzimidazole derivatives **2** as shown in the scheme 33.[141]



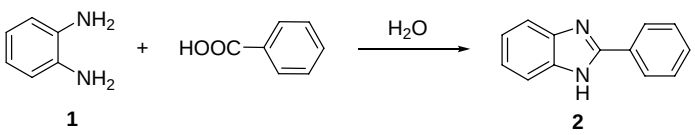
Scheme 33. Raban method of benzimidazole synthesis

In 1995, Soufiaoui *et. al*. have identified the methods for the synthesis of benzimidazole derivatives involving an appropriate 1,2-phenylenediamine **1** with carboxylic acid or with ethyl acetoacetate using H_2SO_4 or montmorillonite or K-10 as acidic catalyst using microwave irradiation as shown in scheme 34.[142]



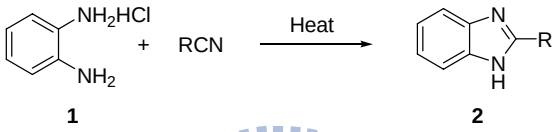
Scheme 34. Soufiaoui method of benzimidazole synthesis

In 2003, Poliakoff *et. al*. have used the high temperature water for the synthesis of benzimidazole derivatives **2** in green synthetic pathway as shown in scheme 35.[143]



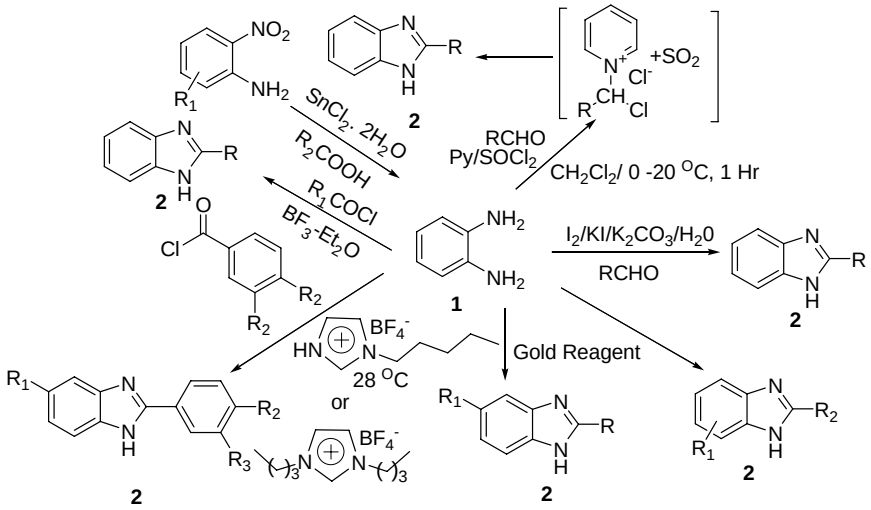
Scheme 35. Poliakoff method of benzimidazole synthesis

Structurally the nitriles are regarded as nitrogen system "anhydrides" of the amidines which was also used for the synthesis of benzimidazole derivatives. In 1944, Wagner et .al have used this synthons by reacting with hydrochloride salts of 1,2-phenylenediamine under refluxing conditions as appeared in scheme 36.[144]



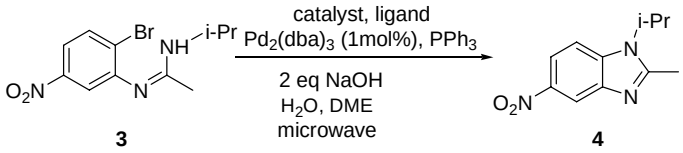
Scheme 36. Wagner method of benzimidazole synthesis

Recently, several methods have been introduced, where aldehydes, acid chloride, o-dinitrobenzene, Gold's reagent, and 2-nitroanilines] are used as starting materials for this synthesis as shown in scheme 37.[145]



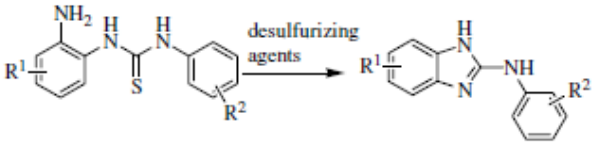
Scheme 37. Different routes to benzimidazoles

Brain and co-worker have also developed a new procedure for the preparation of benzimidazole compounds by palladium-catalyzed aryl-amination chemistry as drawn in scheme 38.[146]



Scheme 38. Brains routes to benzimidazoles

Compounds containing a 2-aminobenzimidazole group have been shown to exhibit a broad spectrum of pharmacological activities.[147] Therefore, an efficient practical method for the synthesis of a diverse collection of aminobenzimidazoles would be of great value for drug discovery. Several synthetic methodologies have been reported in the literature for the synthesis of 2-aminobenzimidazoles. Most involve formation of thioureas using isothiocyanates followed by cyclodesulfurization using desulfurizing agents such as mercury(II) oxide, mercury(II) chloride, copper(I) chloride, methyl iodide, tosyl chloride, dicyclohexylcarbodiimide (DCC) and PS-carbodiimide as shown in Scheme 39.[148]

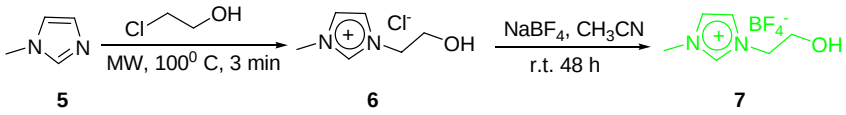


Scheme 39. Methods to synthesis of 2-(N-substitutedamino)benzimidazoles

Although these methods are suitable for certain synthetic conditions, sometimes, there exist some drawbacks such as long reaction time, high temperature, low yields of products in some cases, use of an additional microwave oven, corrosive reagents and large amounts of solid supports which would eventually result in the generation of a large amount of toxic waste. Moreover more than one step is involved in the synthesis of these compounds in some procedures. Therefore, the pursuance of more convenient and practical synthetic methods for these compounds still remains an active research area. Prompted by these above observation and in the interest of broadening the scope of starting materials that can be elaborated into 2-aminobenzimidazoles under milder conditions, we undertook the present investigation and the results of our study have been reported herein.

3.10. Results and Discussions

The present strategy commenced with the synthesis of ion support 7 equipped with hydroxyl group linker, 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([hydemim][BF_4]) was easily prepared in two steps using microwave irradiation. The synthetic sequence is depicted in scheme 41 which involves the reaction of 1-methylimidazole with chloroethanol under microwave irradiation for 3 minutes at 100 ^{O} C temperature. The progress of the reaction was monitored by repeated cooling of the reaction mixture after every one minute interval. On completion of the reaction time, the reaction mixture was repeatedly washed with cold ether and dried under vacumm for overnight to get the compound **6**. Compound **6** was then stirred with sodium tetrafluoroborate in CH_3CN as solvent under N_2 atmosphere for 48 hours for anion exchange reaction. Upon completion of the reaction time, the insoluble sodium chloride was filtered off and washed the precipitate with successive addition of acetonitrile. The supernatant liquid was evaporated and dried under vacuum to obtain the ion support **7**.

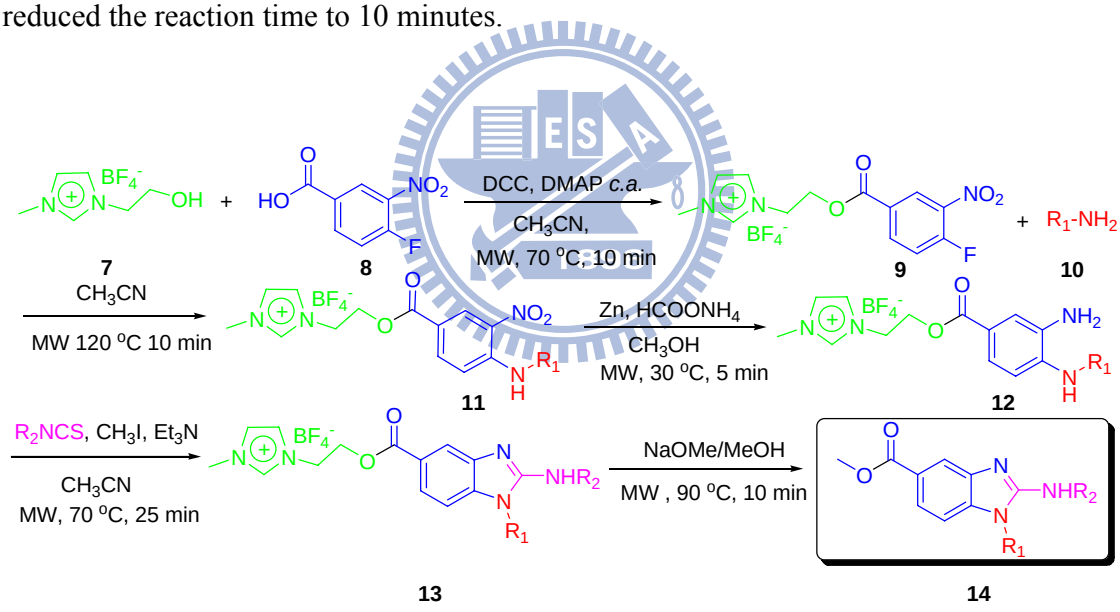


5 **6** **7**

Scheme 40. Preparation of ionic liquid support 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([hydemim][BF_4]).

To attain the target compound on ionic liquid support, which was synthesized in (Scheme 40), the most essential reaction involves the coupling of 4-fluoro-3-nitrobenzoic acid **8** to

1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([hydemim][BF_4]) **7** in scheme 41. The ionic liquid 7 was reacted with 4-fluoro-3-nitrobenzoic acid **8** in the presence of catalytic amount of 4-dimethylaminopyridine and *N, N'*-dicyclohexylcarbodiimide (DCC) in anhydrous CH_3CN at room temperature for 72 hours to complete coupling of carboxylic acid to ion tag. In order to compare the reaction conditions, this coupling reaction was carried out under a set of system, which involves the room temperature coupling conditions for 72 hours getting reduced to refluxing temperature for 12 hours under thermal conditions. Significantly, upon application of microwave irradiation in a closed vessel system under pressure (70 °C, 2 bar) further reduced the reaction time to 10 minutes.



Scheme 41. General strategy of microwave assisted synthesis of 2-(Substituted amino) benzimidazoles on Imidazolium Ion Tag as Support.

After completion of the reaction, the suspended dicyclohexyl urea (DCU) was filtered off and ionic liquid conjugates **9** were precipitated with slow addition of cold ether which

was then filtered to remove the excess un-reacted reagents to obtain the IL-conjugates **9**. In the first-generation diversification step to IL-conjugates **9** which was achieved by nucleophilic aromatic substitution (SnAr) of readily available primary amines as the first building block **10** via an *ipso*-fluoro displacement to give IL-bound *o*-nitroanilines **11**. In this protocol, components **8** and **10** were added to a vigorously stirred CH_3CN solution for 10 hrs under room temperature condition. However, we have observed that with the application of microwave irradiation in a closed vessel system under pressure (120 °C, 4 bar) further reduced the reaction time to 5 minutes as observed in refluxing condition for 3 hrs. The reaction proceeded smoothly with various amines without cleavage of the ester bond at the ionic liquid attached site. Subsequently, upon completion of reaction time, the resulting mixtures were precipitated with ice-cold ether and filtered to remove the excess un-reacted amines to obtain ionic liquid conjugates **11**. To reduce quantitatively the *o*-nitrogroups in **11** and produce ionic liquid conjugates **12,** we systematically investigated the reduction with neutral reducing agent such as $Zn/HCOONH_4$ in methanol. The reaction was successfully accomplished in 20 minutes. The reaction time reduced to 5 minutes upon application of microwave irradiation in a closed vessel system (30 ^{0}C, 1 bar) for 5 minutes. Formation of the amine conjugates **12** was confirmed from change of yellow to blue color upon spotting on the TLC plate. Upon completion of the reaction, reaction mixtures were filtered through fritted funnel to get rid of the Zn. The reaction mixtures were evaporated and acetonitrile was added to salt out the ammonium formate to obtain the compound **12.** In an effort to attain the target molecule, compound **12** underwent key synthetic sequence which involves the efficient ring closure of ionic liquid bound *o*-phenylenediamine **12**. It has been realized that the elaboration of intermediate **12**

to the desired core structure required one carbon electrophile, which could be attach through reaction with isothiocyantes. Hence the amine conjugates **12** were condensed with various isothiocyantes using methyl iodide as an activating agent and triethylamine (Et_3N) as base in one pot manner which provided a more efficient route to the targeted compounds. The reaction was successfully finished in conventional thermalcondition for 5 hrs whereas the application of microwave irradiation condition (70 ^{0}C, 2 bar) reduced the reaction time to 25 minutes. After completion of the reaction, the reaction mixtures were precipitated with cold ether and filtered through fritted funnel to obtain 2-(substituted amino) benzimidazoles on ion tag **13** in good yields.

The multistep mechanism of the one pot formation of benzimdazole makes it an intriguing and challenging subject for study. Of particular interest is the nucleophillic attack on isothiocyantes moiety by the secondary amine functionality of conjugates **12** followed by the activation of the C=S bond by methyliodide (CH_3I) is believed to facilitate the formation of intermediate A. Furthermore the intermediate A after cyclization and electron reorganization by triethy amine (Et_3N) generated the target compound **13** as shown in figure 23.

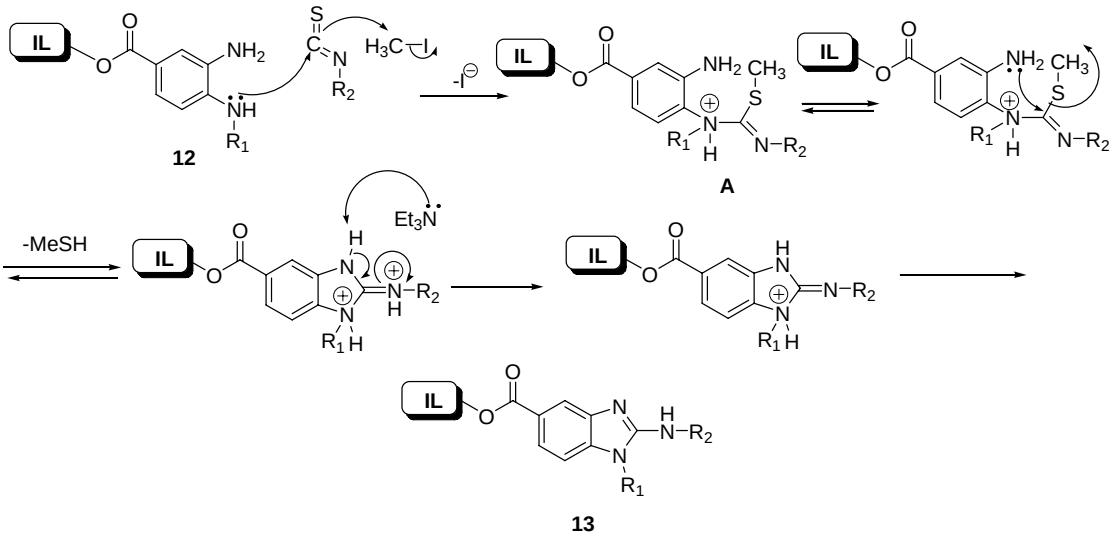
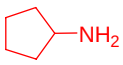
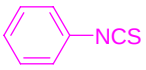
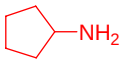
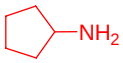
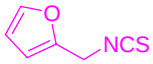
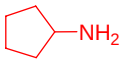
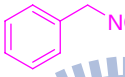
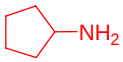
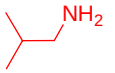
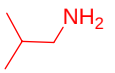
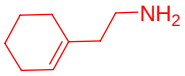
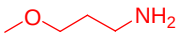
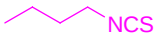
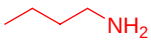
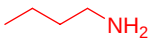
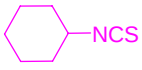
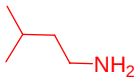
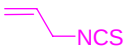
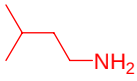
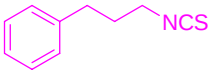
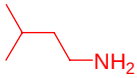
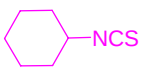


Figure 23. Proposed Mechanism for the formation of 2-(substituted amino) benzimidazoles **13** on ion tag.

The 2-(substituted amino) benzimidazoles **13** were finally cleaved from ion support using 0.1 M NaOMe solution in MeOH at room temperature within 12 hours. However, in a bid to attain the cleavage from the ion support in a shortest possible way, we applied the microwave irradiation in a closed vessel condition (90 ^{O}C, 4 bar) which eventually finished in 12 minutes. Upon completion of the reaction as judged by tlc, the reaction mixture was concentrated and ion support was precipitated by ether and removed by filtration. The filtrates were evaporated and subjected to HPLC analysis which indicated the 71-97 % crude purity of title compounds. Finally column chromatography purification afforded the 2-(substituted amino) benzimidazoles derivatives **14** in good overall yields (Table 1). By utilizing the desired reaction sequence, we have synthesized various 2-(substituted amino) benzimidazoles derivatives **14** with two points of structural diversity as shown in Table 1. The data exhibit that the after five-step syntheses sequence

2-(substituted amino) benzimidazoles derivatives were rapidly synthesized in excellent overall yields.

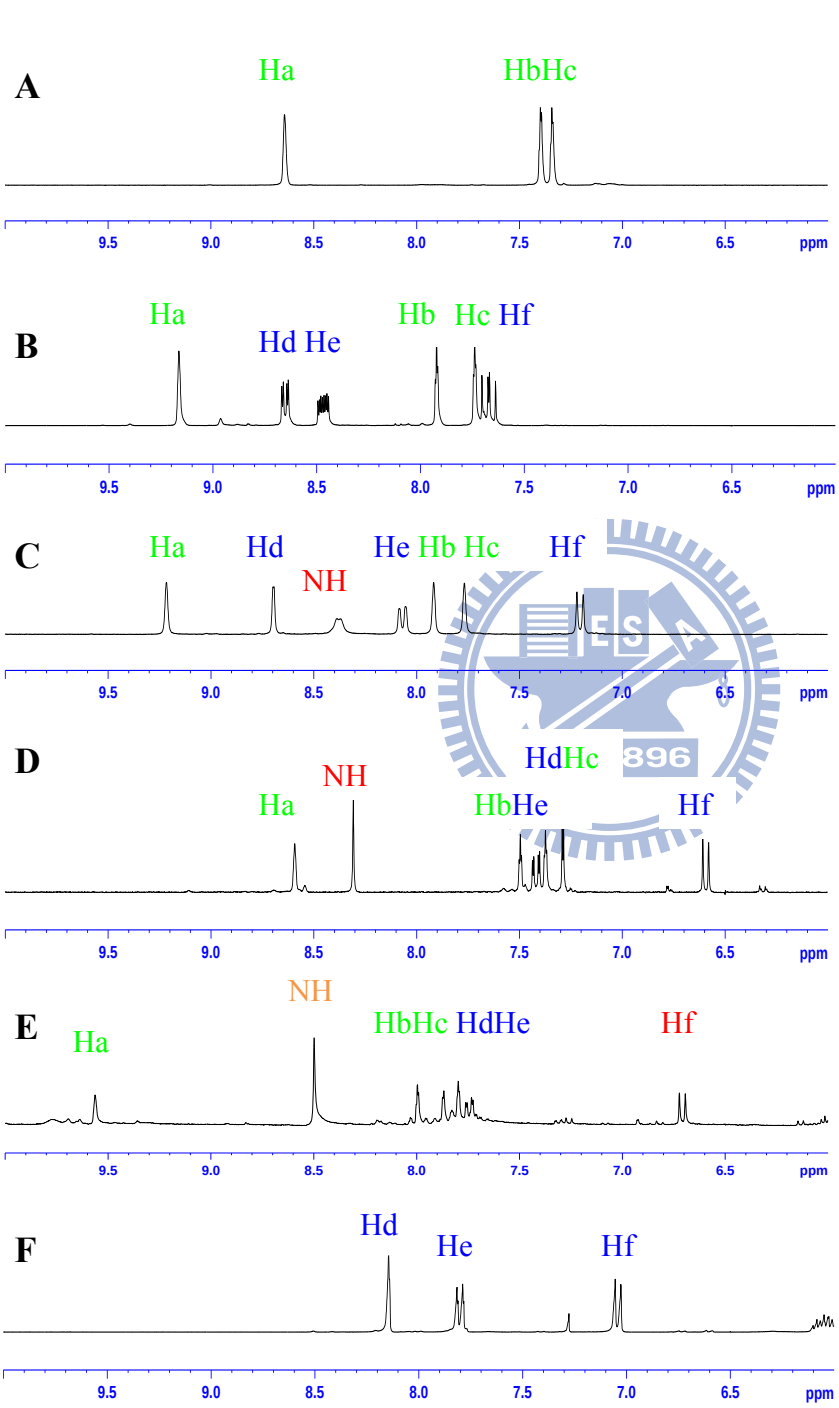
Table 1. Synthesis of 2-(Substitutedamino)benzimidazol Derivatives **14a-14n** using Microwave Irradiation on Ionic Liquid Support

Entry	R_1NH_2	R_2NCS	Purity[a](%)	Yield[b](%)	LRMS[c]
14a			92	94	335
14b			97	91	299
14c			71	84	339
14d			78	88	349
14e			86	91	315
14f			75	79	351
14g			96	89	329
14h			88	78	381
14i			91	93	319
14j			93	85	289
14k			82	91	329
14l			86	90	301
14m			81	87	379
14n			77	91	343

[a] Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%.).[b] Determined based on the weight of crude samples (%). [c] LRMS were detected with ESI ionization source.

However, the main advantage of using the ionic liquid as support was its direct monitoring capacity by standard analytical techniques such as ^{1}H and ^{13}C NMR and mass spectroscopy in comparison to different solid supports where the conjugated material almost remain insoluble in organic solvents. Here in, we have demonstrated the quantitative product conversion by regular proton NMR spectroscopy in each intermediate step with an attached IL-tag. It has been found that the three protons Ha, Hb, Hc of free *IL*-tag appeared at 8.64, 7.45, and 7.34 ppm respectively in proton NMR spectra of A in figure 24. However, the chemical shift of these three protons were shifted to more down field alongwith the appearance of three more peaks at 8.67, 8.57, and 7.65 ppm respectively due to the attachment of 4-fluoro-3-nitrobenzoic acid **8** to the IL-tag **7.** This observation could be attributed to the electron-withdrawing nature of the ester linkage to the *IL*-tag in spectra B. Formation of *o*-nitroaniline conjugates **11** were confirmed from the appearance of NH peak at 8.44 ppm in spectra C which further established by the shifting of Hf proton to more upfield position. Subsequent reduction of *o*-nitroanilines **11** to the *o*-phenylenediamines **12** was established by the shifting of Hd protons at 7.35 ppm. Establishment of benzimidazole ring was evident from the appearance of Hd, He and Hf protons to downfield region due to electron withdrawing nature of benzimidazole derivatives. The previously appeared upfield protons Hd, He, and Hf at 7.35, 7.40, and 6.60 ppm become deshielded and moved to 7.81, 7.70, and 6.72 ppm. Finally, the cleavage of product from ionic liquid support was confirmed by observing the absence of set of three signals at 9.50, 8.00, and 7.95 ppm due to ionic

liquid moiety along with slight shifting of all aromatic protons to downfield region. Other characteristic signals of different protons are in agreement with structure **14** in spectra F.



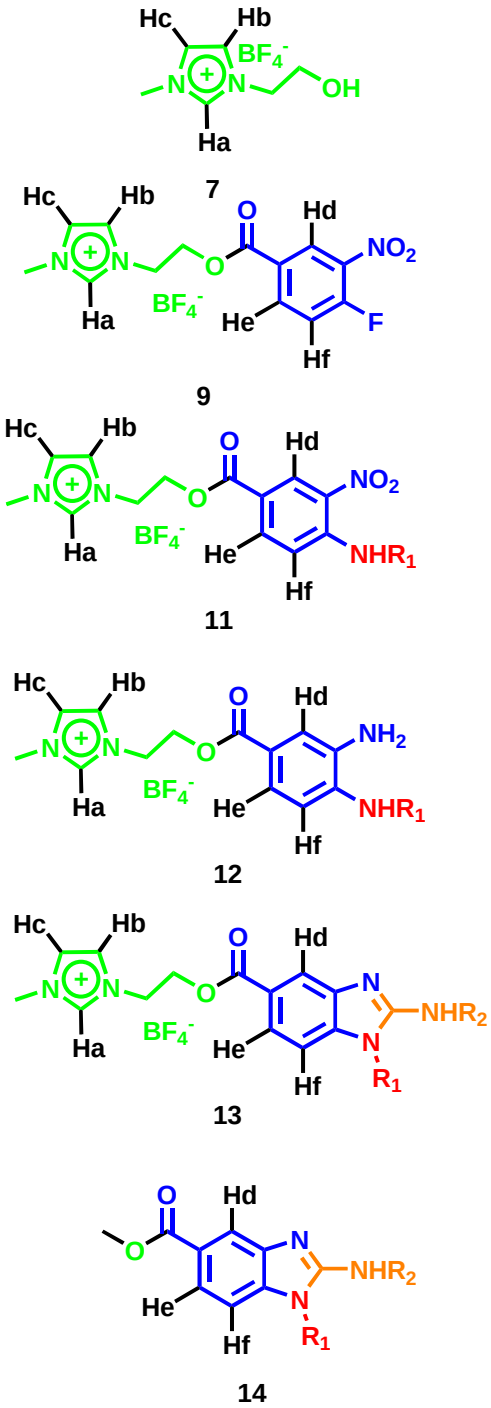
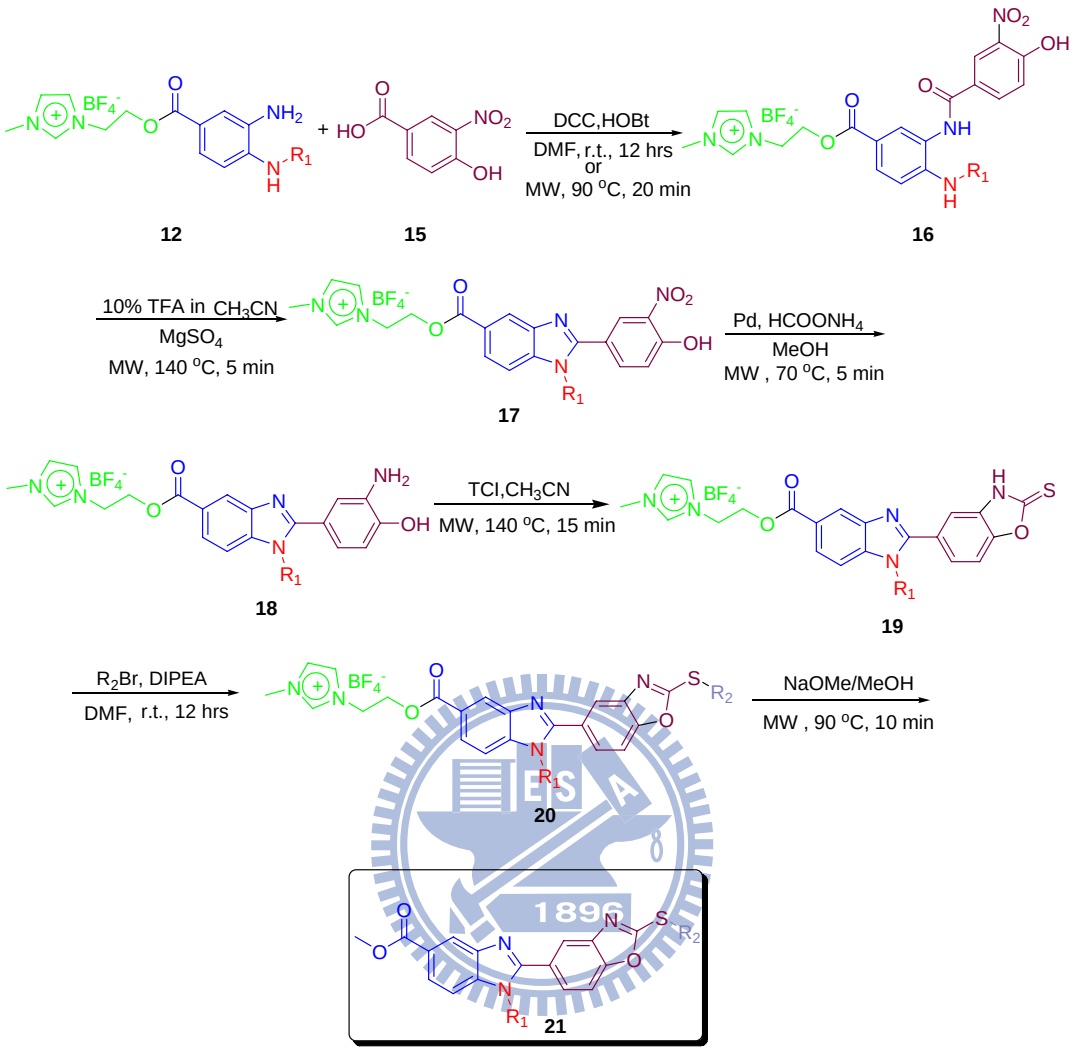


Figure 24. Stepwise monitoring of benzimidazolylbenzoxazoles by ^{1}H NMR spectroscopy.

In an effort to attain the target molecule, ionic liquid bound compound **12** was *N*-acylated at the primary amine functionality with 4-hydroxy-3-nitrobenzoic acid **15** via DCC activation as in scheme 42. The anilide conjugates **16** was obtained by the condensation of acid **15** with ionic liquid conjugates **12** through *in-situ* generated DCC/HOBT activated ester in *N,N*'-dimethylformamide in 12 hours reflux. However, the application of microwave irradiation under sealed vessel condition (90 °C, 5 bar) reduced the reaction time to 20 minutes. After completion of the reaction time, the reaction mixture was filtered through fritted funnel to separate the insoluble DCU and HOBt and the solvent was removed under reduced pressure. The reaction mixture was further purified and filtered by precipitation with cold ether which was subsequently dried. For the construction of benzimidazole ring, anilide conjugates **16** were subjected to acid catalyzed cyclisation in presence of 10 % trifluoroacetic acid in CH_3CN under refluxing condition for 5 hours. The formation of the conjugate **17** was achieved by the intramolecular cyclization facilitated by the protonation of amide carbonyl by trifluoroacetic acid. In order to achieve the target compound quickly, we applied the microwave irradiation under sealed vessel condition (140 °C, 5 bar) at this stage, which further reduced the reaction time to 5 minutes.



Scheme 42. General strategy of microwave assisted synthesis of benzimidazolylbenzoxazoles.

After completion of the reaction, $MgSO_4$ was filtered off and the ionic liquid conjugates were purified by precipitating out the reaction mixtures with excess of cold ether. For the reduction of nitro group of conjugate **17**, we tried with a reducing condition involving 10% palladium on activated charcoal in methanol solution in presence of ammonium formate. Formation of the amine conjugates **18** was achieved under refluxing condition for 2 hours. However, by the application of microwave irradiation at 70 °C, the desired

conjugates **18** were obtained within 5 minutes. After completion, palladium and excess ammonium formate were removed by filtration. Amine conjugates **18** were obtained in pure form by further precipitation in cold ether.

Our main goal was the construction of benzoxazole ring in conjunction with introduction of additional sets of diversity. It has been realized that the elaboration of intermediate **18** to the desired core structure required one carbon electrophile, which could be fix through reaction with thiocarbonyl diimidazole (TCDI). In an effort to mimic the bioactive compound as mentioned earlier in Figure 1.16, we decided to explore the building of benzoxazole ring with thiocarbonyl diimidazole. Hence the amine conjugates **18** were condensed with thiocarbonyl diimidazole in anhydrous CH_3CN under microwave irradiation at 140 $^{\circ}$C (5 bar) to furnish the benzoxazole conjugate **19** in 15 minutes as shown in scheme 43. However, it has been observed that the same reaction required 6 hours under conventional refluxing condition, which in turn reflects the superiority of microwave irradiation. After completion of the reaction, the reaction mixture precipitated with cold ether and filtered through fritted funnel to obtain benzoxazole conjugate **19** in good yields. The most plausible mechanism for the formation of the product involves the nucleophillic attack of amine group on the C=S fragment of the thiocarbonyl diimidazole moiety to form intermediate **a** with the leaving of one imidazole moety. The intermediate **a** then isomerizes to intermediate **b** which was further attacked by the lone pair of electron present on the oxygen atom of hydroxyl group to form the intermediated **c** with the simultaneous removal of the remaining imidazole moiety. The intermediate **c** after cyclization and electron reorganization to generate the target compounds **19** as depicted in Figure 25.

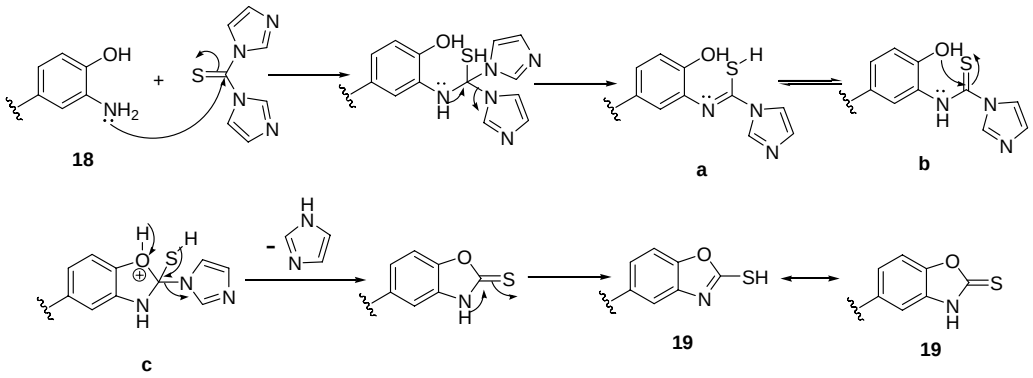
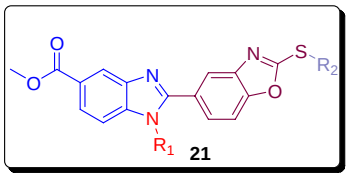


Figure 25. Plausible mechanism towards the formation of benzimidazolylbenzoxazoles.

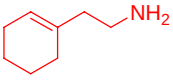
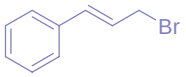
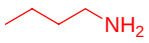
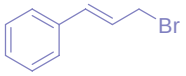
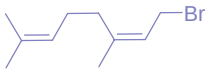
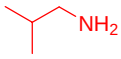
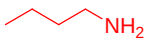
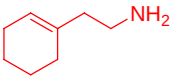
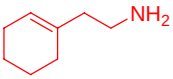
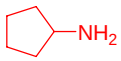
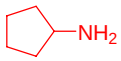
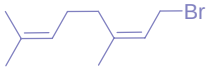
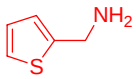
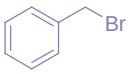
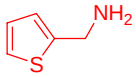
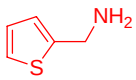
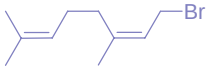
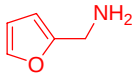
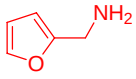
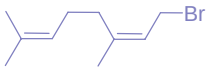
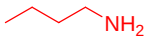
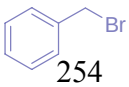
The second point of chemical diversity was introduced by the N-alkylation of ionic liquid conjugates benzoxazolin-2-thione **19**. Under mild alkaline conditions, S-alkylation has been reported to occur and was employed to synthesize a number of biologically active sulfides, as well as to be kinetically investigated. However, we have observed that the use of 1,3-dialkyl halides leads to tricyclic thiazinobenzimidazoles via S-N-bis-alkylation. In accordance with these reports, alkylation of **19** in principle can result either N- or S-alkylation. Under the room-temperature conditions employed, the reactions with various alkyl bromides in presence of diisopropylethylamine led to S-alkylated conjugates **20**. After completion of the reaction, the reaction mixture precipitated with cold ether and filtered through fritted funnel to obtain benzimidazolylbenzoxazoles conjugate **20** in good yields. The benzimidazolylbenzoxazoles were finally cleaved from ionic liquid support using 0.1 M solution of NaOMe in MeOH at room temperature within 12 hours. But in order to obtain the title compound in quick fashion, we applied the microwave irradiation at 90 $^{\circ}$C (2 bar) for 10-15 minutes depending on the substrate. The reaction mixture was concentrated and ionic liquid support was precipitated by ether and removed by filtration.

The filtrates were evaporated and subjected to HPLC analysis which indicated the 76-97 % crude purity of title compounds. Finally column chromatography purification afforded the thio analogs of bis benzimidazolylbenzoxazole derivatives **21** in good overall yields (Table 2). By utilizing the desired reaction sequence, we have synthesized various thio analogs of bis benzimidazolylbenzoxazole derivatives **21** with two diverse substitutions as shown in Table 2. The data exhibit that the after six-step syntheses sequence benzimidazolylbenzoxazole derivatives were rapidly synthesized in excellent overall yields.

Table 2. Synthesis of Thio anlogues of Benzimidazolylbenzoxazol Derivatives **21a-21q** using Microwave Irradiation on Ionic Liquid Support



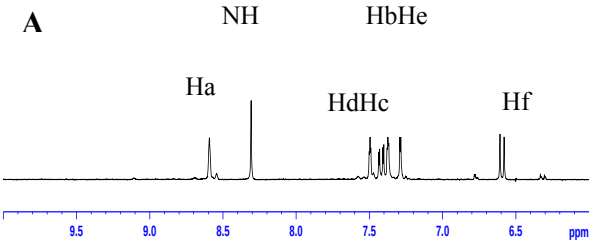
21

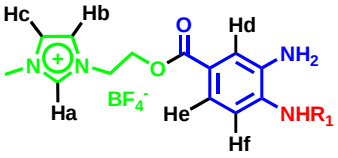
Entry	R_1NH_2	R_2Br	Yield[a]	Purity[b]	LRMS[c]
1			79	77	550
2			91	95	498
3			90	98	518
4			92	91	450
5			89	99	518
6			91	95	570
7			95	97	502
8			88	95	434
9			84	97	530
10			89	87	511
11			88	86	461
12			90	93	557
13			89	92	446
14			80	76	542
15			94	97	422
16			90	92	472

[a]Determined based on the weight of crude samples (%). [b]Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%). [c]LRMS were detected with ESI ionization source.

Monitoring the progress of reaction on polymer support throughout a multistep synthetic sequence is a relatively difficult task. Typical methods such as thin-layer chromatography (TLC), GC and mass spectrometry (MS) are not providing substantial information for polymer-supported methods. The soluble support like ionic liquid is serves as a true alternative concerning its solubility in various organic solvents. Hence the monitoring of the present synthetic scheme was accomplished by ^{1}H NMR spectroscopy (Figure 26). In the spectra **A**, the most downfield three peaks belong to the three aromatic protons (Hd, He and Hf) along with Ha, Hb, Hc for ionic liquid moiety. Formation of the anilide conjugates were confirmed from the appearance of NH signals at 9.40 ppm, whereas the additional signals in aromatic region at 9.15, 8.25 and 7.30 ppm corresponds to Hg, Hh and Hi respectively were emerged from 4-hydroxy-3-nitrobenzoic acid in spectra **B**. Establishment of benzimidazole ring was evident from the disappearance of NH proton in spectra **C** and Hd, He and Hf protons were shifted to downfield due to electron withdrawing nature of benzimidazole derivatives. Reduction of nitro group was noticed from substantial shifting of Hg and Hh protons to upfield region and appeared at 7.10 and 6.99 ppm in spectra **D**. Final cyclization with thiocarbonyl diimidazole to **19** generated the electron withdrawing benzoxazole derivatives which was observed in spectra **E**. Due to the electron withdrawing effect, the previously appeared upfield protons Hg and Hh at 7.10 and 6.99 ppm become deshielded and moved to downfield region. Subsequently the S-alkylation with cinammyl bromide generated the compound 20 which is evident from spectra **F** Finally, the cleavage of

product from ionic liquid support was confirmed by observing the absence of set of signals around 4.5 ppm due to ionic liquid moiety along with slight shifting of all aromatic protons to downfield region. Other characteristic signals of different protons are in agreement with structure **21** in spectra **G**.





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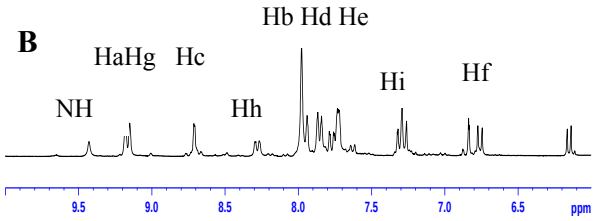

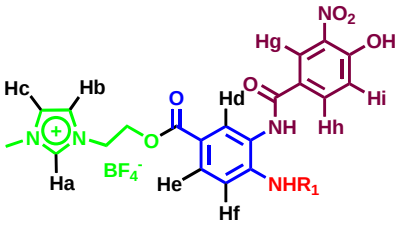

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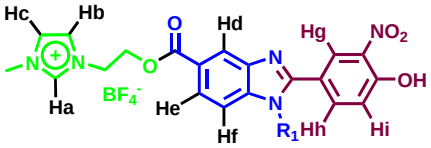

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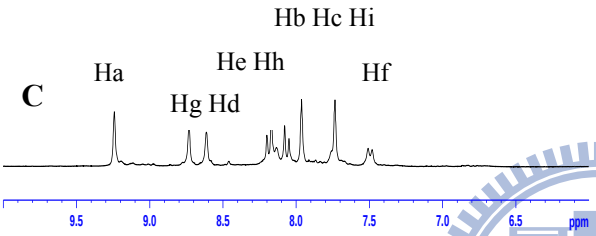

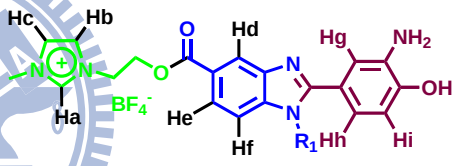

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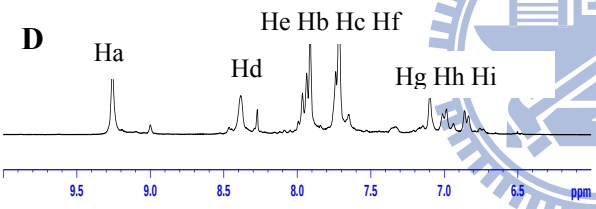

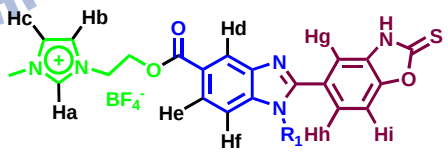

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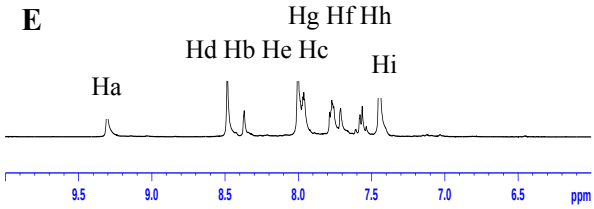

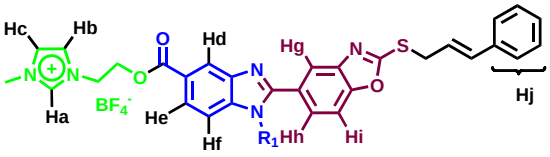

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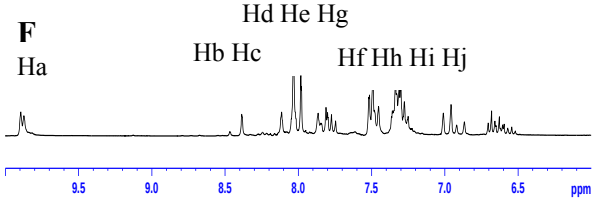
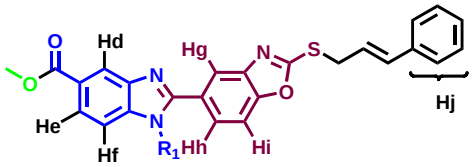




21

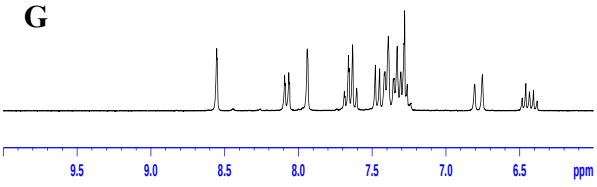
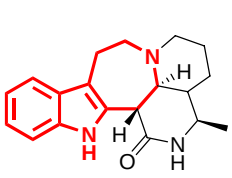


Figure 26. Stepwise monitoring of benzimidazolylbenzoxazoles by ^{1}H NMR spectroscopy.

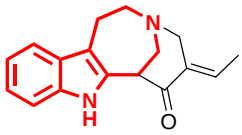
Section C

3.11. Hydantoin fused tetrahydroazepino[4,5-*b*]indoles

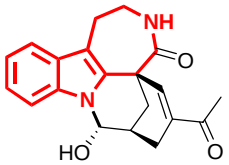
The biological activity and structural complexity associated with alkaloids containing seven membered rings from several natural sources form a significant role in the discovery of novel medicinal agents for curing diseases. The complex molecular architecture seven-membered heterocyclic ring system such as azepino[4,5-*b*]indoles, have recently been observed in several important natural products such as arboflorine (**A**) and subincanadine (B) in Figure 22. [149]. Moreover, It has been witnessed that the core of important natural product such as tronocarpine (C) and malassezindoles (D) contains azepino[4,5-*b*]indolone carcass in figure 27 which is the presursor of all important azepino[4,5-*b*]indoles, the reduced derivatives have been found to display significant activity against central nervous system diseases for new class of antipsychotics selective for the hD1-receptor family (E).[150] Recently, it has been found that hydantoin derivatives also possess several pharmacological properties including dual action for anticonvulsant and antimuscarinic activity, insulinotropic properties, and antifungal activity.[151]



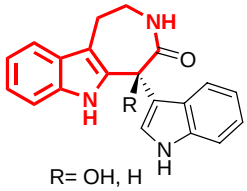
Arboflorine (A)



Subinacanadine (B)

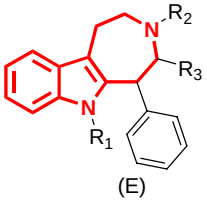


Tronocarpine (C)



R= OH, H

Malassezeindole (D)



(E)

Figure 27. Structures of interesting azepino[4,5-*b*]indoles containing natural products.

The preparation of both of these classes of compounds has not been studied extensively in the literature except few methods described in the next section.

3.12. Chemical synthesis of azepino[4,5-*b*]indoles and its derivatives.

In 1982, Ward *et. al*. have observed the formation of azepino[4,5-*b*]indoles by the thermal decomposition of the azidoacrylates in xylene solution as shown in figure 28.[152]

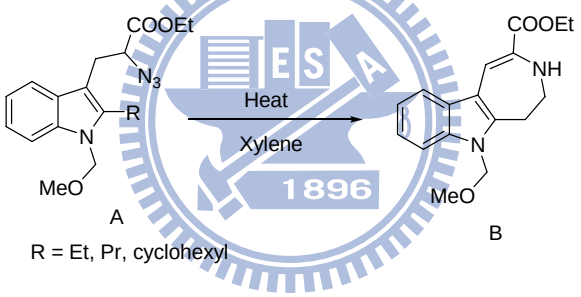


Figure 28. Ward's methods of azepino[4,5-*b*]indole synthesis.

In 2000, Griemer *et. al*. have synthesized the azepino[4,5-*b*]indole moiety using sequence of key synthetic steps such as Pictet-Spengler cyclizations, Michael addition reactions, lactamization, directed metallation, and reductive amination from readily available building blocks as shown in figure 29.[153]

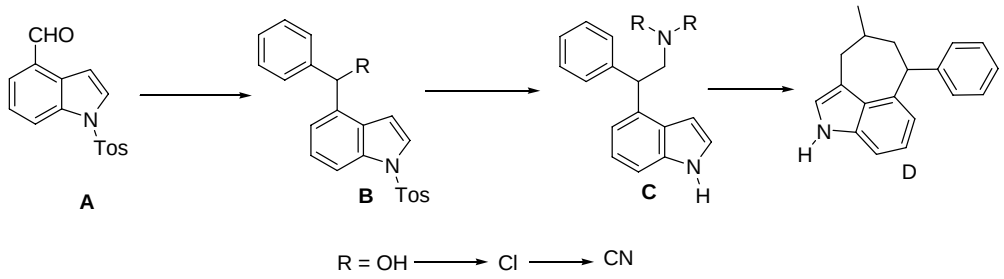


Figure 29. Griemer's methods of azepino[4,5-*b*]indole synthesis.

In 2009, Stewert *et. al*. have discovered the domino single-step Tsuji–Trost/Heck reactions in the synthesis of azepino[4,5-*b*]indole ring systems as shown in the figure 30.[154]

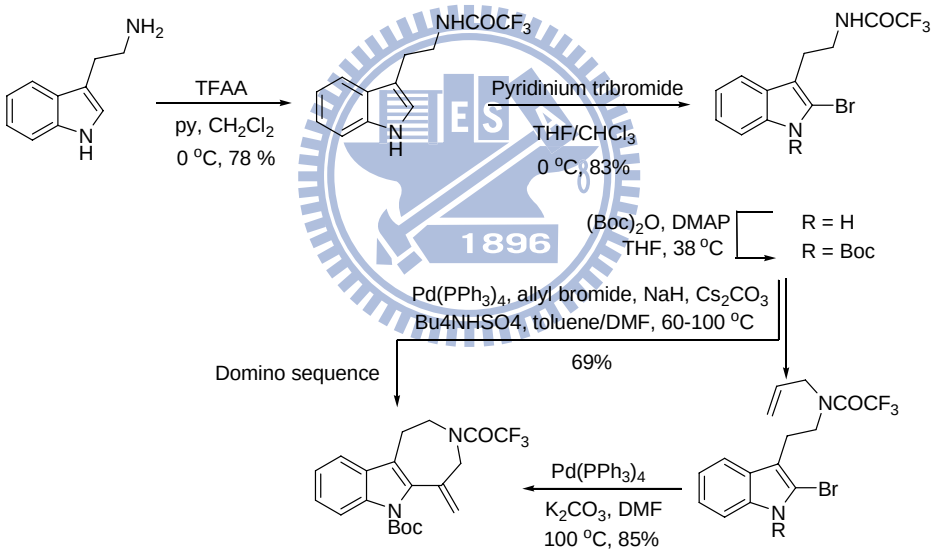
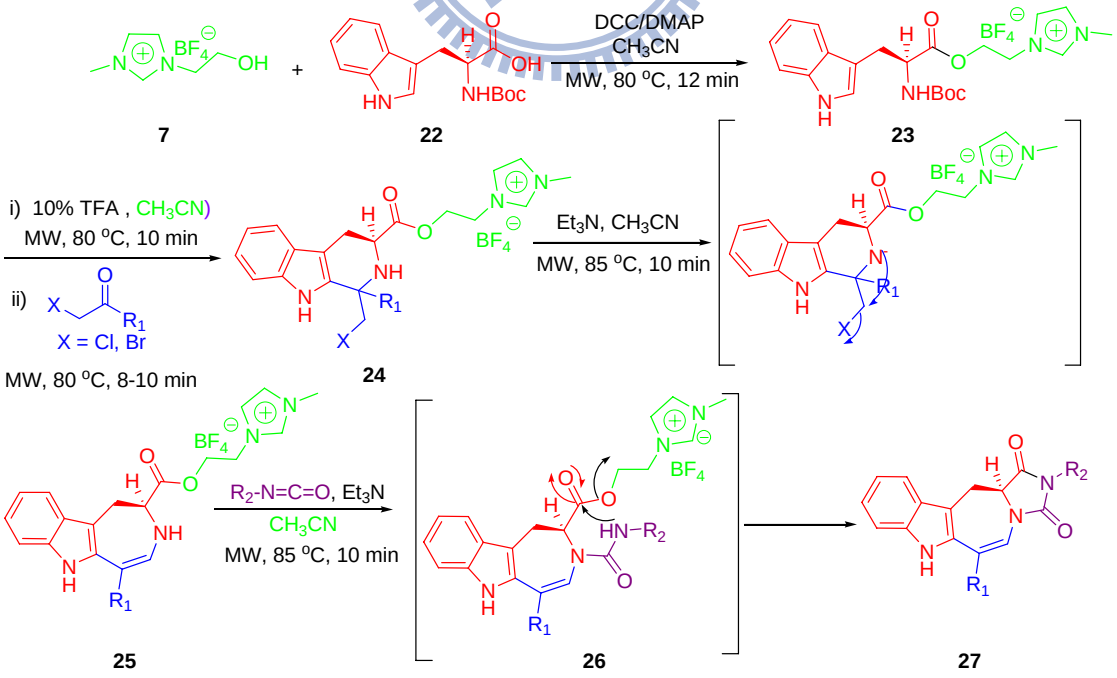


Figure 30. Construction of the azepino[4,5-b]indole ring system through single-step or domino Tsuji–Trost/Heck reactions.

3.13. Results & Discussions

To attain the target compound on ionic liquid support, the most essential reaction involves the coupling of Boc protected L-tryptophan **22** to hydroxyl ethyl methyl imidazolium tetrafluoroborate **7** (Scheme 43). For comparison purposes, this coupling reaction was carried out under a set of different conditions, involving (i) room temperature for 48 h; (ii) thermal heating at refluxing temperature for 12 h; and (iii) microwave irradiation in a closed vessel system under pressure (80 °C, 2 bar) which reduced the time to 12 min. After completion of the reaction, the dicyclohexyl urea (DCU) was filtered off and IL-conjugates **23** were precipitated with addition of cold ether, which was then filtered to obtain the. Unlike other solid supports, the main advantage of using ionic liquid soluble support was its direct monitoring capacity by standard analytical technique such as ^{1}H and ^{13}C NMR and mass spectroscopy. Having prepared the IL-conjugates **23,** our next challenges were the NHBoc deprotection and subsequent Pictet-Spengler cyclization with differently substituted α-halo keto esters to afford the corresponding β-carbolines derivatives. In the first approach, the intermediate free primary amine was isolated in high yield after NHBoc deprotection of **23** using 10 % trifluoroacetic acid in CH_3CN at room temperature for 4 hours. However, we have observed that the application of microwave irradiation could facilitate the NHBOC deprotection in 10 minutes. From this free amine, the six-membered β-carbolines ring system was constructed under Pictet-Spengler condition using [5 + 1] approach affording IL conjugates **24** in good overall yield. It was also observed that the IL conjugates **23** was directly transformed into the β-carbolines ring system **24** in a suspension of 10 % trifluoroacetic acid in CH_3CN, followed by the addition of α-halo keto esters in one pot manner in excellent yield and in a shorter time under microwave irradiation (80 °C, 2 bar)

for 18-20 min as compared to 12 hrs under refluxing conditions. Using this protocol, the deprotection/cyclization sequence could be carried out in a one-step, one purification process to afford the β-carbolines **24** in excellent yield. After the completion of the reaction, CH_3CN was removed from the reaction mixtures under reduced pressure, and the residue was further precipitated with ether, and filtered through a fritted funnel to remove unreacted α-halo keto esters and other side products to obtain the IL conjugates **24**. This substrate **24** was subsequently set up for the convertion of the six membered β-carbolines moiety to the seven membered olefinic azepines. For this reason we reacted the IL conjugates **24** with 3 equivalent of triethyl amine in CH_3CN solution under micrwave irradiation (85 ^oC, 2 bar) for 10 minutes as compared to 12 hrs under refluxing conditions. After the completion of the reaction, the reaction mixtures was purified by precipitation with ether, and filtered through a fritted funnel to remove side products to obtain the IL conjugates azepines **25**.

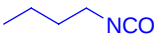
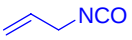
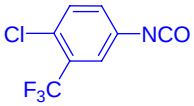
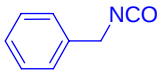
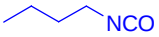
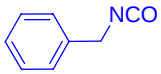
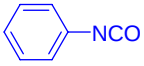
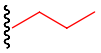
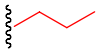
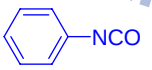
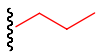
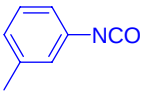
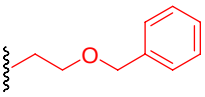
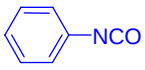
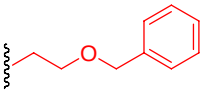
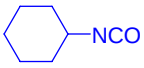
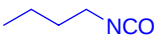


Scheme 43. Ionic-Liquid Supported Synthesis of Architecturally Diverse Hydantoin Fused Tetrahydroazepino[4,5-b]Indoles

The formation of the IL conjugates **25** can be explained by the fact that the reactions under basic conditions presumably proceeded via intramolecular N-alkylation and formation of an aziridine intermediate. Subsequent proton abstraction in basic condition gives the olefin **25.** To generate the second diversity in the proposed structure, we explored the construction of terminal hydantoin moiety across IL immobilized olefinic carbolines **25** by the reaction with various isocyanates and triethyl amine as a base under microwave irradiation to form urea intermediate **26.** Subsequent nucleophilic attack by the amide nitrogen to the ester carbony moiety leads to the intramolecular cyclisation with concomitant traceless cleavage of the ionic liquid support. This leds to the traceless synthesis of architecturally diverse tetracyclic scaffolds in high purity and good oveall yields. It has to be noticed that the cyclization of the hydantoin ring and subsequent traceless cleavage of the ionic liquid support was carried out in one step way under mild basic conditions using triethylamine. The reaction was finally completed in 10 min (85 °C, 4 bar) as compared to 14 hrs under refluxing conditions. The progress of the final cyclisation was carefully monitored by TLC which signifies the complete discharge of the tetracyclic scaffolds 20 from the ionic liquid supportwith the confirmation of the traceless nature of the final cyclisation. Upon completion of the reaction time, residue was purified by precipitation in ether to obtain the crude compounds **27**. Further HPLC analysis of the crude compounds indicated the 73-99 % crude purity of title compounds.

Finally column chromatography purification afforded the tetracyclic architectures **27** in good overall yields (Table 3).

Table 3. Microwave-Assisted, IL-Supported Synthesis of Hydantoin Fused Tetrahydroazepino[4,5-b]Indoles **(27a-27q)**

Entry	R_1	R_2NCO	Yield[a](%)	Purity[b](%)	LRMS[c]
27a			81	n.d.	382
27b			92	89	366
27c			86	77	526
27d			91	97	415
27e			89	99	395
27f			80	85	429
27g			90	95	415
27h			80	73	435
27i			87	81	409
27j			84	88	437
27k			91	91	395
27l			97	97	415
27m			79	78	451
27n			80	91	429
27o			83	94	507
27p			93	96	513
27q			85	80	367

[a] Determined based on the weight of crude samples (%.) [b] Determined by HPLC analysis (UV detection at 254 nm) of the crude product (%.) [c] LRMS were detected with ESI or EI ionization sources.

The formation of the product hydantoin fused tetrahydroazepino[4,5-b]indoles is confirmed on spectral data as well as the 1D NOE analysis of compound **27l**, which showed that there is no correlation between Hd and Ha protons, which demonstrates clearly from the (Figure 31 and 32). Moreover, the irradiation of He caused the enhancement of NH, Hc and H protons by 0.55 %, 1.65 % , 1.12 % and 1.13 % respectively where as the irradiation of Hf caused the enhancement of signal of the Hd by 3.97 % and vice versa. Similarly, the irradiation of Ha caused the enhancement of Hb by 20.02%. In the same way, the irradiation of Hb caused the enhancement of Ha and Hc signals by 23.09 and 3.85 % respectively. We also obtained the signal enhancement of Ha and Hb by 0.91 % and 2.64 % followed by irradiation of Hc.

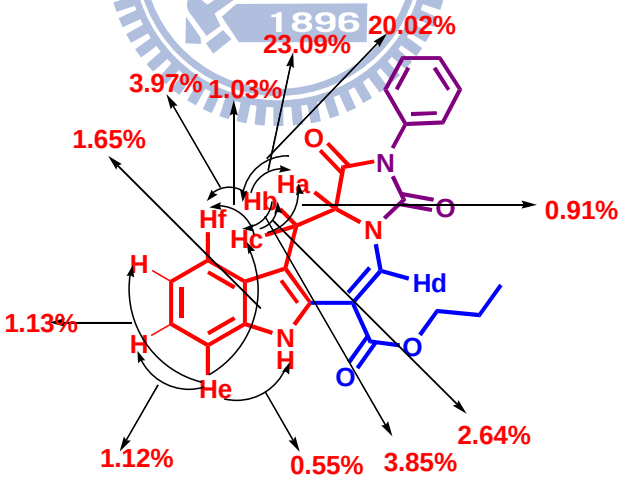


Figure 31. Some important NOE interactions in the compound 27l.

Furthermore, additionally the structure of final compounds was unambiguously confirmed by the X-ray crystallographic study. Figure 1.31 depicts the ORTEP diagram of compound **27l**.

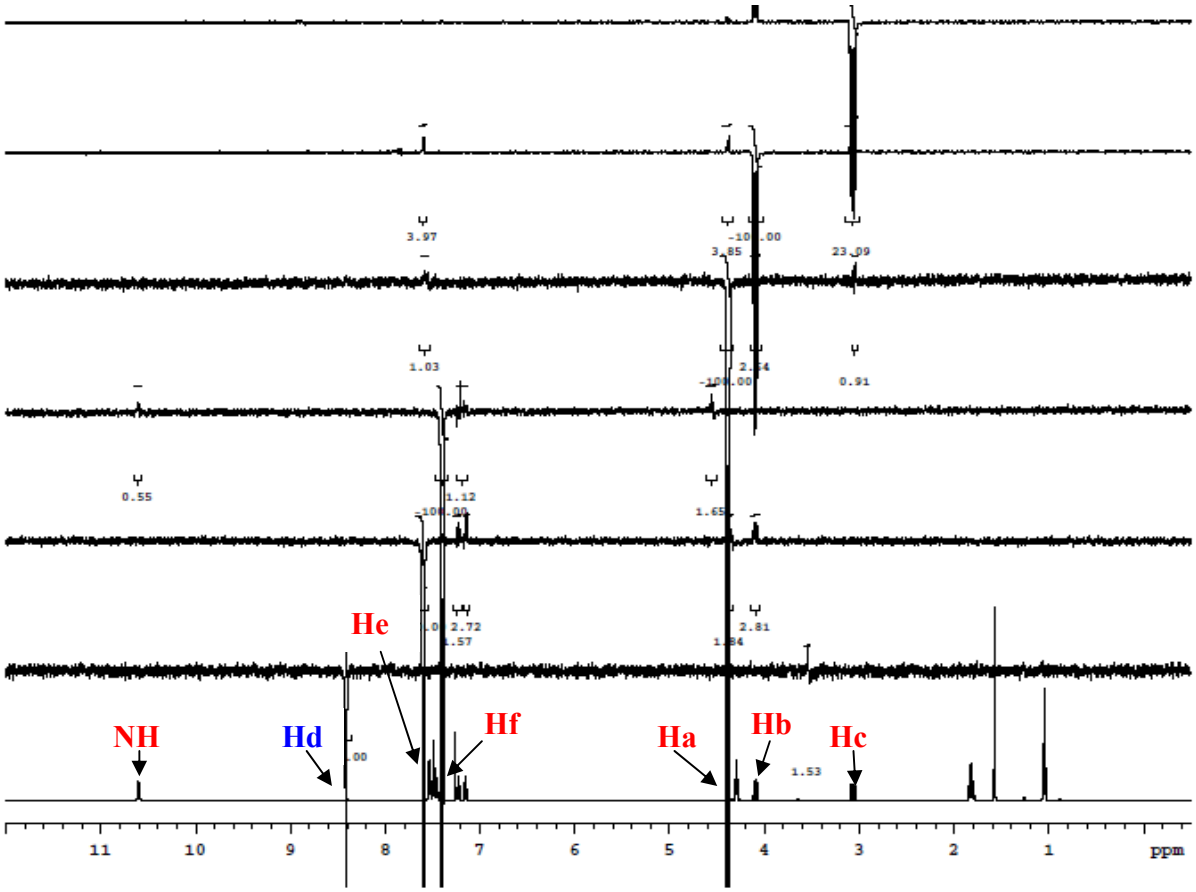


Figure 32. Structural correlation established by 1D nOe measurement

The single crystal X-ray analysis of compound **27l** indicates that two rings of tetrahydroazepino[4,5-b]indoles and hydantoin moieties are in anti-periplanar orientation.

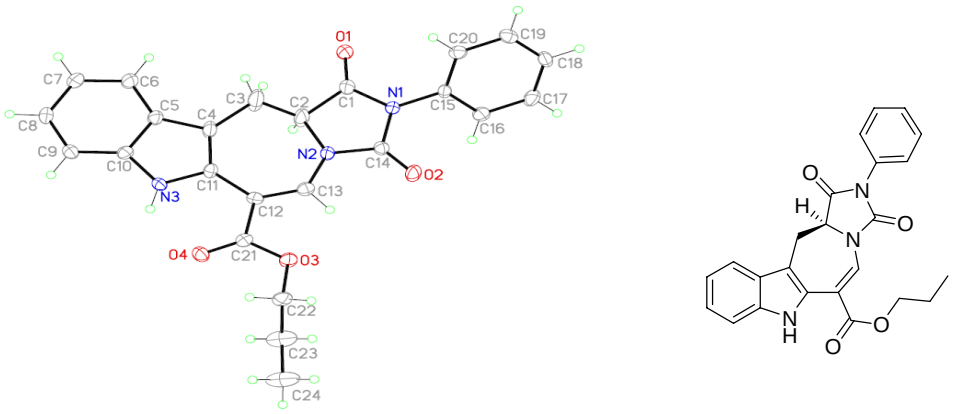


Figure 33. ORTEP diagram of Compound **27l**

3.14. Experimental Section

General Procedure for the Synthesis of 1-(2-Hydroxyethyl)-3-methylimidazolium Chloride (6).

A mixture of 1-Methylimidazole **5** (1.0 g, 12.2 mmol) and 2-Chloroethanol (0.975 g, 12.2 mmol) was placed in a 10 mL microwave vial, and the vial was then irradiated for 3 min at 100^{o} C. After the reaction was cooled to ambient temperature, the formed viscous solid was successively washed with ether (20 mL×3), and then dried under vacuum for 6 h. The hydroxyl-functionalized ionic liquid **6** was obtained as white crystals (1.85 g, 94%). ^{1}H NMR (300 MHz, D_2O): δ3.79 (s, 3H), 3.82 (t, J = 3.7 Hz, 2H), 4.20 (t, J = 4.0 Hz, 2H), 7.34 (s, 1H), 7.39 (s, 1H), 8.64 (s, 1H).

General Procedure for the Synthesis of 1-(2-Hydroxyethyl)-3-methylimidazolium Tetrafluoroborate (7). A mixture of **1**-(2-Hydroxyethyl)-3-methylimidazolium Chloride **6** (1.85 g, 11.4 mmol), $NaBF_4$ (2.5 g, 22.8 mmol) and dry acetonitrile (50 mL) was stirred at room temperature for 48 h under nitrogen. The so formed white precipitate was filtered off and washed with acetonitrile (30 mL×3). Concentration of combined filtrates gave product **7** as light yellow oil (2.39 g, 98%). ^{1}H NMR (300 MHz, acetone-d_6): δ 3.94 (t, J = 5.1 Hz, 2H), 4.04 (s, 3H), 4.42 (t, J = 5.1 Hz, 2H), 7.66 (s, 1H), 7.70 (s, 1H), 8.91 (s, 1H); MS (FAB): *m/z* [M^+] (100%): 127.

General Procedure for the Synthesis of Ionic Liquid (IL) bound 4-Fluoro-3-nitro benzoic acid (9).

4-Fluoro-3-nitro benzoic acid **8** (0.573 g, 3.14 mmol, 1.34 Equiv), 1-Methyl-3-ethyl imidazolium tetrafluoroborate **7** (0.50 g, 2.34 mmol, 1.0 Equiv) and *N, N'*-dimethylamino pyridine (DMAP) (0.005 g) are placed in a dry, nitrogen-purged 100 mL round-bottom flask containing dry CH_3CN (15 mL). To the mixtures were added dropwise *N, N'*-dicyclohexylcarbodiimide (DCC) (0.675 g, 3.28 mmol, 1.4 Equiv) dissolved in dry CH_2Cl_2 (5 mL) for a period of 5 minutes. The reaction mixtures were stirred for another 15 minutes at room temperature. Then this O-acylation reaction was carried out in a tube using microwave radiation at 40 w (75 °C, 1 bar) for 15 minutes. After the completion of the reaction, the insoluble DCU byproduct was allowed to settle, and the reaction mixtures were filtered and washed with CH_3CN (50 mLx3). The solvent was evaporated, and the residue was again precipitated with cold ether which was filtered through fritted funnel to remove any unreacted acid and DCC, finally collected and dried under vacuum.

General Procedure for the Substitution of the Aryl fluoride in Ionic Liquid (IL) bound 4-Fluoro-3-nitro benzoic acid (9) with amines.

To the ionic liquid (IL) bound 4-Fluoro-3-nitro benzoic acid **9** (1.20 gm, 3.14 mmol, 1.0 Equiv) was added various aliphatic primary amines **10** (6.28 mmol, 2.0 Equiv) and 10 mL of dry CH_3CN at room temperature for 5 minutes. The mixtures were irradiated in a pressurized microwave reactor at 75 w (120 °C, 4 bar) for 5 minutes. After completion of the reaction time, the reaction mixtures were precipitated with slow addition of cold ether (100 mL) which was filtered through a fritted funnel to obtain the ionic liquid (IL) bound 4-(Substituted amines)-3-nitro benzoic acid **11** in high purity.

General Procedure for the Reduction of the Aryl nitro group in Ionic Liquid (IL) bound 4-(Substituted amino)-3-nitro benzoic acid (11).

To a solution of **11** in methanol, Zn (1.50 gm, 22.48 mmol, 7.0 equiv.) and ammonium formate (3.0 gm, 48.17 mmol, and 15.0 equiv) were added. The reaction mixtures was subsequently stirred at room temperature for 10 minutes to complete reduction of nitro group which was evident from color change (yellow to greenish blue) upon spotting on a TLC plate. After completion, the reaction mixtures were then subjected to centrifugation for removal of Zn and the supernatant liquid was concentrated by rotary evaporation to remove methanol. Acetonitrile (10 mL) was then added to salt out ammonium formate. The reaction mixtures were filtered through fritted funnel to remove ammonium formate to obtain the ionic liquid bound 3-Amino-4-(substituted amino) benzoic acid **12.**

General Procedure for the Preparation of Ionic Liquid Bound 2-(Substituted amino) benzoimidazole carboxylates 13.

To a stirred solution of ionic liquid bound 3-Amino-4-(substituted amino) benzoic acid **12** in CH_3CN (5 mL), various isothiocyanates (9.67 mmol, 3.0 equiv), CH_3I (1.30 gm, 9.67 mmol, 3.0 equiv) as activating agent and triethyl amine (Et_3N) (0.980 gm, 9.67 mmol, 3.0 equiv) were added in a sequential order. The reaction mixtures were exposed under pressured microwave irradiation at 40 w (70 ^{0} C, 1 bar) for 10 minutes. Upon cyclisation by checking NMR, the crude product mixtures were purified by precipitation with cold ether (100 mL×3) and dried to obtain the conjugate **13** in high purity.

General Procedure for the Cleavage of Ionic Liquid Bound 2-(substituted amino) benzimidazole Derivatives 14.

To a solution of conjugates **13** in methanol (20 mL), NaOMe (100 mg) was added and irradiated under pressured microwave irradiation at 60 w (90 ^{0}C, 2 bar) for 12 minutes. After completion of the reaction, the crude product was precipitated with excess of cold ether (100 mL), the ionic liquid was filtered off and subjected to evaporation. The residue was dried under vacuum, and subjected to crude HPLC analysis with UV detection at λ=254 nm (column: Sphereclone 5μ Si (250 x 4.6 mm); gradient: 35 % ethyl acetate in hexane; flow rate: 1 mL/min.). The slurry obtained was loaded on silica gel column and eluted with a mixture of ethyl acetate and hexane (2:3) to get the title compounds **14** in good yields.

General Procedure for the Preparation of Ionic Liquid Bound 3-(4-Hydroxy-3-nitrobenzamido)-4-(substituted amino) carboxylates 16.

To a solution of *N, N'*-dicyclohexylcarbodiimide (DCC) (710 mg, 3.45 mmol, 1.4 equiv) in *N, N'*-dimethylformamide (DMF) was added 4-hydroxy-3-nitrobenzoic acid **15** (585 mg, 3.20 mmol, 1.3 equiv) and 1-hydroxybenzotriazole (HOBt) (432 mg, 3.20 mmol,

3.20 equiv) in a sequential order. The resulting slurry was stirred for 5 minutes at room temperature and then added ionic liquid (IL) anchored *o*-phenylene diammine **12** (1.0 g, 2.46 mmol, 1.0 equiv) in *N, N'*-dimethylformamide (5 mL) (DMF). The reaction mixtures were subsequently heated with stirring in a 10 mL microwave process vial at 60 w (90 ^{o}C, 1 bar) for 20 minutes to obtain the ionic liquid conjugate **16**. After completion of the reaction, the suspensible byproducts were filtered through filter paper. The reaction mixtures were precipitated by slow addition of cold ether and precipitated amide conjugates **16** were filtered through fritted funnel. The crude product was washed in succession with ether (100 mL×3) to remove the undesired impurity and dried for further steps.

General Procedure for the Preparation of Ionic Liquid Bound 2-(4-Hydroxy-3-nitrophenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 17.

To a solution of ionic liquid bound 3-(4-Hydroxy-3-nitrobenzamido)-4-(substituted amino) carboxylates **16** in acetonitrile, trifluoroacetic acid (0.5 mL) and $MgSO_4$ (500 mg) was added and the mixture was subsequently heated with stirring in a 10 mL microwave process vial at 80 w (140 ^{o}C, 4 bar) for 5 minutes. After completion of the reaction, $MgSO_4$ was removed through celite. The reaction mixtures were precipitated by slow addition of excess of cold ether (100 mL) and filtered through a fritted funnel to obtain the ionic liquid 2-(4-Hydroxy-3-nitrophenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **17** in high purity.

General Procedure for the Preparation of Ionic liquid Polymer Bound 2-(3-Amino-4-hydroxyphenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 18.

To a solution of **17** in methanol, Pd on charcoal (1.30 g, 12.65 mmol, 5.0 equiv.) and ammonium formate (1.12 g, 17.72 mmol, and 7.0 equiv) were added. The reaction mixtures was subsequently heated with stirring in a 10 mL microwave process vial for 5 minutes in the appropriate mode of pressure and temperature to complete reduction of nitro group which was evident from color change (yellow to greenish blue) upon spotting on a TLC plate. After completion, the reaction mixtures were then subjected to centrifugation for removal of Pd on charcoal and the supernatant liquid was concentrated by rotary evaporation to remove methanol. Acetonitrile (10 mL) was then added to salt out ammonium formate. The reaction mixtures were filtered through fritted funnel to remove ammonium formate to obtain the ionic liquid bound 2-(3-Amino-4-hydroxyphenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **18.**

General Procedure for the Preparation of Ionic liquid Bound 2-(2-Mercaptobenzo[*d*]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 19.

To a stirred solution of ionic liquid bound 2-(3-Amino-4-hydroxyphenyl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates conjugates **18** in CH_3CN (5 mL), 1,1-thiocarbonyldiimidazole (663 mg, 3.73 mmol, 1.5 equiv) was added. The reaction mixtures were subsequently heated with stirring in a 10 mL microwave process vial for 5 minutes in the appropriate mode of pressure and temperature for complete cyclisation. After completion, the reaction mixture were precipitated by slow addition of excess of cold ether (100 mL) and filtered through a fritted funnel to obtain the ionic liquid 2-(2-Mercaptobenzo[*d*]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **19** in high purity.

General Procedure for the Preparation of Ionic liquid Bound 2-(2-(alkylthio)benzo[d]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates 20

To a stirred solution of ionic liquid bound 2-(2-(alkylthio)benzo[*d*]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates conjugates **19** in *N, N'*-dimethylformamide (5 mL) (DMF), *N,N*-diisopropylethyl amine (480 mg, 3.71 mmol, 1.5 equiv) and various alkyl bromides (3.71 mmol, 1.5 equiv) were added. The reaction mixtures were subsequently heated with stirring in a 10 mL microwave process vial for 5 minutes in the appropriate mode of pressure and temperature for complete alkylation. After completion, the reaction mixture were precipitated by slow addition of excess of cold ether (100 mL) and filtered through a fritted funnel to obtain the ionic liquid 2-(2-(alkylthio)benzo[d]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylates **12** in high purity.

General Procedure for the Cleavage of Ionic liquid Bound 2-(2-(alkylthio)benzo[d]oxazol-5-yl)-1-alkyl-1*H*-benzo[*d*]imidazole carboxylate 21.

To a solution of conjugates **20** in methanol (20 mL), NaOMe (100 mg) was added and irradiated under pressured microwave irradiation at 60 w (90 ^{0}C, 2 bar) for 12 minutes. After completion of the reaction, the crude product was precipitated with excess of cold ether (100 mL), the ionic liquid was filtered off and subjected to evaporation. The residue was dried under vacuum, and subjected to crude HPLC analysis with UV detection at λ=254 nm (column: Sphereclone 5μ Si (250 x 4.6 mm); gradient: 35 % ethyl acetate in hexane; flow rate: 1 mL/min.). The slurry obtained was loaded on silica gel column and eluted with a mixture of ethyl acetate and hexane (1:3) to get the title compounds **21** in good yields.

General Procedure for the Synthesis of Ionic Liquid (IL) bound Boc-*L*-tryptophan (23).

Boc-L-tryptophan **22** (0.76 g, 2.52 mmol), 1-Methyl-3-ethyl imidazolium tetrafluoroborate **7** (0.40 g, 1.87 mmol) and *N, N'*-dimethylamino pyridine (DMAP) (0.005 g) are placed in a dry, nitrogen-purged 100 mL round-bottom flask containing dry CH_3CN (15 mL). To the mixtures were added dropwise *N, N'*-dicyclohexylcarbodiimide (DCC) (0.54 g, 2.62 mmol) dissolved in dry CH_2Cl_2 (5 mL) for a period of 5 minutes. The reaction mixtures were stirred for another 15 minutes at room temperature. Then this O-acylation reaction was carried out in a tube using microwave radiation at 40 w (80 °C, 1 bar) for 12 minutes. After the completion of the reaction, the insoluble DCU byproduct was allowed to settle, and the reaction mixtures were filtered and washed with CH_3CN (50 mLx3). The solvent was evaporated, and the residue was again precipitated with cold ether which was filtered through fritted funnel to remove any unreacted acid and DCC, finally collected and dried under vacuum gave product **23** as pale white solid (1.18 g, 95%).

General Procedure for the Preparation Ionic Liquid (IL) bound Tetrahydro-β-carboline (24). Ionic Liquid bound Boc-L-tryptophan **23** (1.30 g, 2.60 mmol) was dissolved in 10 % TFA (4 mL) in CH_3CN (16 mL). The mixtures were irradiated in a pressurized microwave reactor at 40 w (80 °C, 2 bar) for 10 minutes. After Boc protective group was removed, various α-halo keto esters (5 mmol) were added to the reaction mixtures. Then the reaction mixtures were further irradiated in a microwave oven at 40 w (80 °C, 2 bars) for 10 minutes in one pot manner. After completion of the reaction time, the solvent was evaporated under reduced pressure. The residue was

precipitated with cold ether and filtered through a fritted funnel to remove any unreacted α-halo keto esters used in the Pictet-Spenger reaction. Finally the product obtained is the ionic liquid supported tetrahydro-β-carboline derivatives **24.**

General Procedure for the Generation of Ionic Liquid (IL) bound Tetrahydroazepino[4,5-*b*]Indole Derivatives (25).

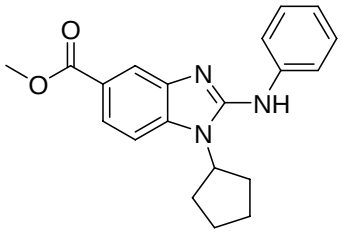
The tricyclic tetrahydro-β-carboline Derivatives **24** (1.0 mmol) was dissolved in CH_3CN (10 mL) and treated with triethylamine (0.30g, 3.0 mmol). The reaction mixtures was kept into microwave synthesis apparatus and irradiated at 45 w (85 °C, 2 bar) for 10 minutes. After the completion of the reaction time, the reaction mixtures were further precipitated with cold ether which was then filtered through fritted funnel and the residue was repeatedly washed with ether (50 mLx3). Finally the product obtained is the ionic liquid supported tetrahydroazepino [4,5-*b*]indoles **25**.

General Procedure for the Generation of Tetracyclic Hydantoin Fused Tetrahydroazepino[4,5-*b*]Indole Derivatives (27).

The tricyclic tetrahydroazepino [4,5-*b*]indoles derivatives **25** (1.0 mmol) was dissolved in CH_3CN solvent (10 mL) and treated with substituted isocyanates (1.8 mmol) in triethylamine (0.30g, 3.0 mmol). The reaction mixtures was kept into microwave synthesis apparatus and irradiated at 45 w (85 °C, 2 bar) for 10 minutes. After the completion of the reaction time, TLC was checked which indicates the complete release of the targeted compound from ionic liquid support. Then the reaction mixtures were precipitated with cold ether which was then filtered through fritted funnel and the residue was repeatedly washed with ether (50 mLx3). The combined filtrate were subjected to evaporation to obtain the crude compounds **27** and was checked by crude HPLC for crude

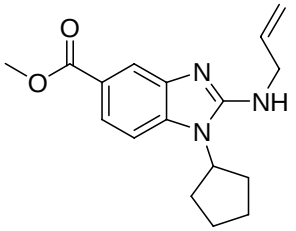
purity with UV detection at λ=254nm (column: Sphereclone 5μ Si (250 x 4.6 mm); gradient: 35 % ethyl acetate in hexane; flow rate: 1 (mL/min). The crude mixtures were further purified by column chromatography and eluted with ethyl acetate: hexane (1:9) to afford the final compounds in 79-97 % yields and 73-99 % purities.

1-Cyclopentyl-2-(phenylamino)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14a).



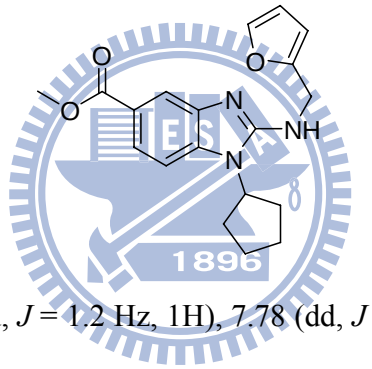
^{1}H NMR (300 MHz) δ 8.23 (d, *J* = 1.3 Hz, 1H), 7.87 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.29-7.24 (m, 2H), 7.20 (d, *J* = 8.2 Hz, 1H), 6.94 (t, *J* = 7.4 Hz, 1H), 4.81 (quin, *J* = 8.7 Hz, 1H), 3.93 (s, 3H), 2.19-2.13 (m, 4H), 2.02-1.99 (m, 2H), 1.81-1.77 (m, 2H); ^{13}C NMR (300 MHz) δ 168.16, 151.58, 141.17, 140.78, 135.87, 129.61, 123.99, 123.15, 123.00, 119.43, 119.30, 109.96, 56.26, 52.42, 30.15, 25.65; IR (cm^{-1}, KBr): 3270, 1707; MS (ESI) m/z 336 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{20}H_{21}N_3O_2$: m/z 335.1634; Found 335.1632.

2-Allylamino-1-Cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14b).



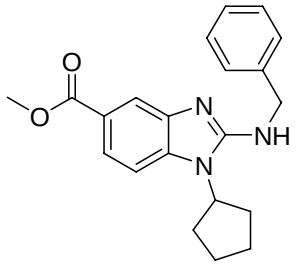
^{1}H NMR (300 MHz) δ 8.17 (d, *J* = 1.3 Hz, 1H), 7.79 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.03 (m, 1H), 5.32 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.21 (dd, *J* = 10.3, 1.3 Hz, 1H), 4.78 (brs, NH), 4.60 (quint, *J* = 8.3 Hz, 1H), 4.21 (d, *J* = 5.2 Hz, 2H), 3.96 (s, 3H), 2.16-2.11 (m, 4H), 2.04-1.98 (m, 2H), 1.82-1.78 (m, 2H); ^{13}C NMR (300 MHz) δ 168.3, 154.8, 141.8, 136.8, 134.8, 123.6, 122.2, 118.4, 117.3, 109.0, 55.6, 52.3, 43.4, 29.9, 25.6; IR (cm^{-1}, KBr): 3251, 1712; MS (ESI) m/z 300 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{17}H_{22}N_3O_2$: m/z 300.1712; Found 300.1711.

1-Cyclopentyl-2-(furan-3-yl-methylamino)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14c).



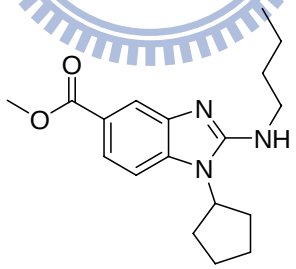
^{1}H NMR (300 MHz) δ 8.18 (d, *J* = 1.2 Hz, 1H), 7.78 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.37 (d, *J* = 1.0 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.33 (m, 2H), 5.01 (brs, NH), 4.73 (d, *J* = 7.1 Hz, 2H), 4.56 (quint, *J* = 8.2 Hz, 1H), 3.95 (s, 3H), 2.18-2.05 (m, 4H), 2.04-1.92 (m, 2H), 1.81-1.73 (m, 2H); ^{13}C NMR (300 MHz) δ 168.4, 154.8, 151.8, 142.7, 142.26, 136.9, 123.5, 122.2, 118.7, 110.9, 109.1, 108.4, 55.6, 52.3, 41.1, 30.0, 25.5; IR (cm^{-1}, KBr): 3295, 1710; MS (ESI) m/z 340 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{19}H_{22}N_3O_2$: m/z 340.1661; Found 340.1659.

2-(Benzylamino-1-cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14d).



^{1}H NMR (300 MHz) δ 8.19 (d, *J* = 1.3 Hz, 1H), 7.80 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 2H), 7.39-7.29 (m, 3H), 7.18 (d, *J* = 8.4 Hz, 1H), 4.86 (brs, NH), 4.75 (s, 2H), 4.54 (quin, *J* = 8.7 Hz, 1H), 3.93 (s, 3H), 2.19-2.13 (m, 4H), 2.02-1.99 (m, 2H), 1.81-1.77 (m, 2H); ^{13}C NMR (300 MHz) δ 168.3, 154.9, 141.9, 138.6, 136.8, 129.2, 128.5, 128.2, 123.6, 122.3, 118.6, 109.1, 55.7, 52.3, 48.3, 30.0, 25.5; IR (cm^{-1}, KBr): 3249, 1714; MS (ESI) m/z 350 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{21}H_{24}N_3O_2$: m/z 350.1868; Found 350.1866.

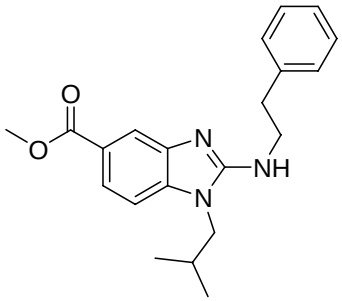
2-(Butylamino)-1-cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14e).



^{1}H NMR (300 MHz) δ 8.17 (d, *J* = 1.4 Hz, 1H), 7.77 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 4.68 (brs, NH), 4.64 (quint, *J* = 8.2 Hz, 1H), 3.96 (s, 3H), 3.57 (q, *J* = 7.5 Hz, 2H), 2.15-2.01 (m, 4H), 2.01-1.97 (m, 2H), 1.83-1.79 (m, 2H), 1.70 (quint, *J* = 7.5 Hz, 2H), 1.43 (sext, *J* = 7.5 Hz, 2H), 0.94 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (300 MHz) δ 168.2, 154.8, 141.1, 136.5, 123.6, 122.3, 117.9, 109.0, 55.6, 52.3, 44.0, 32.2, 29.2, 25.6,

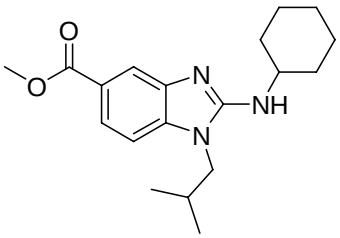
20.5, 14.2; IR (cm^{-1}, KBr): 3322, 1709; MS (ESI) m/z 316 (MH^+); HRMS (ESI, *m/z*) calcd for $C_{21}H_{24}N_3O_2$: m/z 316.2025; Found 316.2027.

1-Isobutyl-2-(phenylethylamino)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14f).



^{1}H NMR (300 MHz) δ 8.18 (d, *J* = 1.3 Hz, 1H), 7.78 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.33-7.31 (m, 2H), 7.25-7.16 (m, 3H), 7.01 (d, *J* = 8.3 Hz, 1H), 4.35 (t, *J* = 5.7 Hz, 1H), 3.92 (s, 3H), 3.82 (q, *J* = 6.6 Hz, 2H), 3.51 (d, *J* = 7.5 Hz, 2H), 3.03 (t, *J* = 6.6 Hz, 2H), 1.95 (sext, *J* = 6.0 Hz, 1H), 0.85 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (300 MHz) δ 168.5, 155.5, 142.4, 139.2, 139.1, 129.3, 129.1, 127.1, 123.5, 122.2, 118.4, 107.4, 52.3, 50.3, 44.5, 35.8, 28.9, 20.5; IR (cm^{-1}, KBr): 3326, 1691; MS (EI) m/z 351 (M^+); HRMS (EI, *m/z*) calcd for $C_{21}H_{25}N_3O_2$: m/z 351.1947; Found 351.1942.

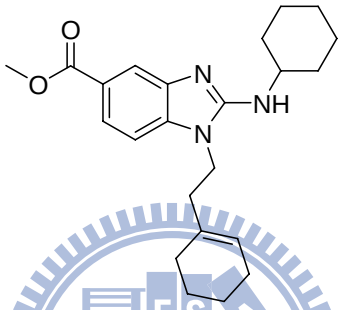
2-(Cyclohexylamino)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14g).



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, *J* = 1.4 Hz, 1H), 7.77 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.02 (d, *J* = 8.3 Hz, 1H), 4.08 (d, *J* = 7.5 Hz, 1H), 3.98 (m, 1H), 3.89 (s, 3H), 3.65 (d, *J* = 7.4 Hz, 2H), 2.17-2.13 (m, 2H), 1.93 (sext, *J* = 6.6 Hz, 1H), 1.77-1.49 (m, 4H), 1.31-1.22

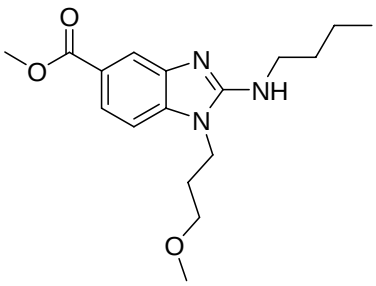
(m, 4H), 0.97 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 154.9, 142.5, 138.9, 123.4, 122.0, 118.2, 107.3, 52.2, 52.2, 50.3, 34.2, 29.1, 26.0, 25.1, 20.7; IR (cm^{-1}, KBr): 3326, 1706; MS (EI) m/z 329 (M^+); HRMS (EI, *m/z*) calcd for $C_{19}H_{27}N_3O_2$: m/z 329.2103; Found 329.2110.

1-(2-Cyclohexenylethyl)-2-cvyclohexylamino-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14h).



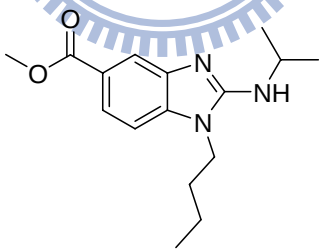
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J = 1.4 Hz, 1H), 7.79 (dd, J = 8.3, 1.4 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 5.41 (m, 1H), 4.12 (d, J = 7.5 Hz, 1H), 3.94 (t, J = 6.8 Hz, 2H), 3.90 (s, 3H), 3.84 (m, 1H), 2.34 (t, J = 6.8 Hz, 2H), 2.20-2.17 (m, 2H), 1.93-1.90 (m, 4H), 1.75-1.61 (m, 2H), 1.59-1.46 (m, 8H), 1.28-1.23 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 154.6, 142.3, 138.4, 134.2, 125.4, 123.5, 122.1, 118.3, 106.9, 52.2, 52.2, 42.2, 37.0, 34.3, 29.2, 26.1, 25.6, 25.3, 23.1, 22.3. IR (cm^{-1}, KBr): 3361, 1708; MS (EI) m/z 381 (M^+); HRMS (EI, *m/z*) calcd for $C_{23}H_{31}N_3O_2$: m/z 381.2416; Found 381.2410.

2-(Butylamino)-1-(3-methoxypropyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14i).



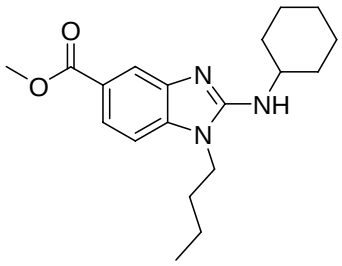
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, *J* = 1.4 Hz, 1H), 7.78 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.02 (d, *J* = 8.3 Hz, 1H), 5.41 (m, NH), 4.00 (t, *J* = 6.0 Hz, 2H), 3.89 (s, 3H), 3.54-3.48 (m, 2H), 3.38, (s, 3H), 3.33 (t, *J* = 6.0 Hz, 2H), 2.01 (t, *J* = 6.0 Hz, 2H), 1.66 (quint, *J* = 7.5 Hz, 2H), 1.41 (sext, *J* = 7.5 Hz, 2H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 154.4, 142.2, 138.4, 123.6, 122.3, 117.9, 106.8, 68.0, 58.9, 52.3, 43.6, 38.7, 32.3, 28.7, 20.5, 14.2; IR (cm^{-1}, KBr): 3320, 1708; MS (EI) m/z 319 (M^+); HRMS (EI, *m/z*) calcd for $C_{17}H_{25}N_3O_2$: m/z 319.1896; Found 319.1890

1-Butyl-2-isopropylamino-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14j).



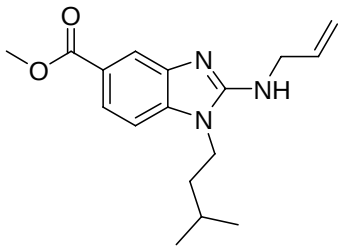
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 4.31 (m, 1H), 4.09 (d, *J* = 7.2 Hz, 1H), 3.95 (s, 3H), 3.86 (t, *J* = 7.2 Hz, 2H), 1.72 (quint, *J* = 7.2 Hz, 2H), 1.41-1.36 (m, 2H), 1.33 (d, *J* = 6.5 Hz, 6H), 0.94 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 154.7, 142.6, 138.5, 123.5, 122.1, 118.4, 106.9, 52.2, 45.6, 42.5, 31.4, 23.9, 20.6, 14.1; IR (cm^{-1}, KBr): 3241, 1714; MS (EI) m/z 289 (M^+); HRMS (EI, *m/z*) calcd for $C_{16}H_{23}N_3O_2$: m/z 289.1790; Found 289.1783.

1-Butyl-2-cyclohexylamino-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14k).



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, *J* = 1.4 Hz, 1H), 7.77 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 1H), 4.21 (d, *J* = 7.5 Hz, 1H), 3.98 (m, 1H), 3.88 (s, 3H), 3.83 (t, *J* = 7.1 Hz, 2H), 2.15- 2.13 (m, 2H), 1.77-1.61 (m, 5H), 1.52-1.21 (m, 7H), 0.92 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 154.8, 142.6, 138.6, 123.3, 121.9, 118.2, 106.9, 52.2, 52.2, 42.5, 34.2, 31.3, 26.0, 25.3, 20.6, 14.1; IR (cm^{-1}, KBr): 3215, 1708; MS (EI) m/z 329 (M^+); HRMS (EI, *m/z*) calcd for $C_{19}H_{27}N_3O_2$: m/z 329.2103; Found 329.2113.

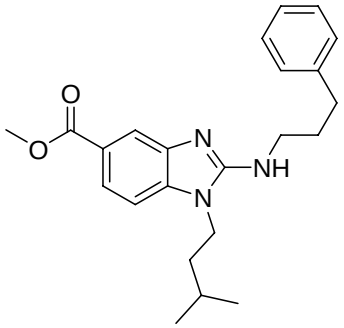
2-Allylamino-1-1sopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14l).



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, *J* = 1.5 Hz, 1H), 7.77 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 6.04 (m, 1H), 5.28 (dd, *J* = 17.1, 1.4 Hz, 1H), 5.17 (dd, *J* = 10.2, 1.4 Hz, 1H), 4.63 (m, NH), 4.19 (d, *J* = 6.7 Hz, 2H), 3.93-3.87 (m, 5H), 1.62-1.60 (m, 3H), 0.96 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 155.1, 142.3, 138.5, 135.0, 123.5, 122.3, 118.5, 116.9, 107.0, 52.3, 46.2, 41.2, 37.9, 26.3, 22.8; IR (cm^{-1}, KBr):

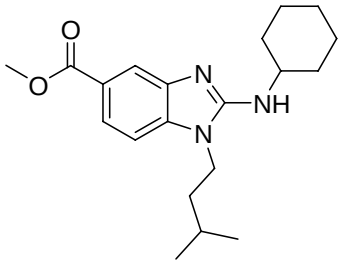
3220, 1714; MS (EI) m/z 301 (M^+); HRMS (EI, *m/z*) calcd for $C_{17}H_{23}N_3O_2$: m/z 301.1790; Found 301.1788.

1-Isopentyl-2-phenylpropylamino-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14m).



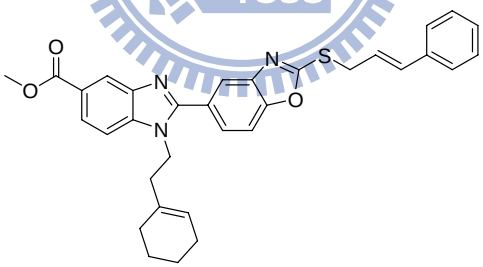
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 1.4 Hz, 1H), 7.81 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.33-7.19 (m, 5H), 7.02 (d, *J* = 8.3 Hz, 1H), 4.15 (m, 1H), 3.91 (s, 3H), 3.74 (t, *J* = 7.5 Hz, 2H), 3.67-3.66 (dt, *J* = 5.8, 6.7 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.11 (quint, *J* = 7.4 Hz, 2H), 1.63 (sext, *J* = 6.7 Hz, 1H), 1.56 (quint, *J* = 7.5 Hz, 2H), 0.97 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 155.2, 142.3, 141.9, 138.4, 128.9, 128.9, 126.5, 123.6, 122.3, 118.4, 106.9, 52.3, 43.7, 41.1, 38.9, 33.9, 31.6, 26.2, 22.8; IR (cm^{-1}, KBr): 3221, 1711; MS (EI) m/z 379 (M^+); HRMS (EI, *m/z*) calcd for $C_{23}H_{29}N_3O_2$: m/z 379.2260; Found 379.2264.

2-Cyclohexylamino-1-1sopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester (14n).



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J = 1.3 Hz, 1H), 7.80 (dd, J = 8.2, 1.3 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 4.06 (d, J = 7.5 Hz, 1H), 3.91 (m, 1H), 3.89 (s, 3H), 3.85 (t, J = 7.5 Hz, 2H), 2.18-2.15 (m, 2H), 1.79-1.59 (m, 6H), 1.58-1.48 (m, 2H), 1.28-1.25 (m, 3H), 1.00 (d, J = 6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 154.7, 142.6, 138.5, 123.5, 122.1, 118.4, 106.9, 52.3, 52.2, 45.6, 42.6, 31.4, 23.9, 23.8, 20.6, 14.1; IR (cm^{-1}, KBr): 3324, 1714; MS (EI) m/z 343 (M^+); HRMS (EI, m/z) calcd for $C_{23}H_{29}N_3O_2$: m/z 343.2260; Found 343.2262.

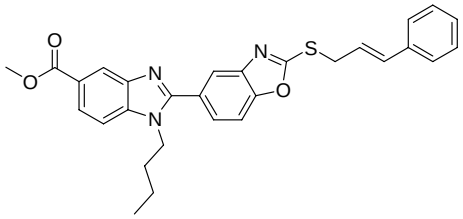
2-(2-(cinnamylthio)benzo[*d*]oxazol-5-yl)-1-(2-cyclohexenylethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21a.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 1.2 Hz, 1H), 8.07 (dd, J = 8.5, 1.2 Hz, 1H), 7.95 (d, J = 1.4 Hz, 1H), 7.68 (dd, J = 8.4, 1.4 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.48-7.30 (m, 6H), 6.77 (d, J = 15.6 Hz, 1H), 6.44 (m, 1H), 5.20 (m, 1H), 4.38 (t, J = 7.1 Hz, 2H), 4.20 (d, J = 7.2 Hz, 2H), 3.98 (s, 3H), 2.37 (t, J = 7.1 Hz, 2H), 1.85-1.83 (m, 2H), 1.74-1.70 (m, 4H), 1.48-1.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 166.6, 155.6, 153.2, 142.9, 142.8, 139.3, 136.5, 135.1, 133.3, 129.1, 128.5, 126.9, 126.8, 126.1, 125.2,

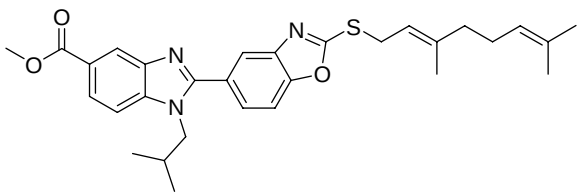
124.9, 124.7, 123.3, 122.7, 119.7, 110.9, 110.4, 52.6, 44.3, 38.2, 35.4, 28.6, 25.5, 22.9, 22.3; MS (ESI) *m/z*: 550 (MH^+); HRMS (ESI, m/z) calcd for $C_{33}H_{32}N_3O_3S$: *m/z* 550.2164; Found 550.2168; IR (cm^{-1}, neat): 2927, 1712, 1612.

1-Butyl-2-(2-(cinnamylthio)benzo[*d*]oxazol-5-yl)-1-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21b.



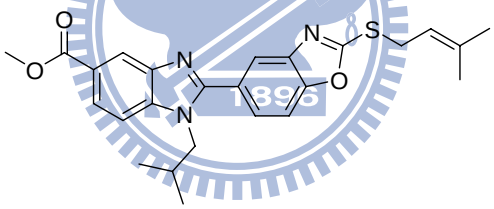
^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 1.4 Hz, 1H), 8.07 (dd, J = 8.5, 1.4 Hz, 1H), 7.94 (s, 1H), 7.65 (dd, J = 8.5, 1.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.41-7.39 (m, 2H), 7.35-7.30 (m, 2H), 6.77 (d, J = 15.6 Hz, 1H), 6.43 (m, 1H), 4.29 (t, J = 7.2 Hz, 2H), 4.20 (d, J = 7.3 Hz, 2H), 3.96 (s, 3H), 1.80 (quint, J = 7.2 Hz, 2H), 1.33-1.21 (sext, J = 7.2 Hz, 2H), 0.86 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 166.6, 155.5, 153.2, 143.1, 142.8, 139.3, 136.6, 135.1, 129.0, 128.5, 127.0, 126.9, 126.2, 125.1, 124.8, 123.3, 122.7, 119.7, 110.8, 110.2, 52.5, 45.2, 35.3, 32.2, 20.3, 13.9; MS (ESI) *m/z*: 498 (MH^+); HRMS (EI, m/z) calcd for $C_{29}H_{28}N_3O_3S$: *m/z* 498.1851; Found 498.1849; IR (cm^{-1}, neat): 2956, 1707, 1606.

2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1-isobutyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21c.



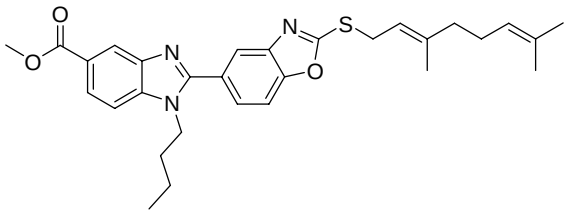
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 1.3 Hz, 1H), 8.05 (dd, J = 8.6, 1.3 Hz, 1H), 7.88 (s, 1H), 7.62 (dd, J = 8.3, 1.2 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 5.46 (t, J = 7.1 Hz, 1H), 5.06 (m, 1H), 4.12 (d, J = 7.5 Hz, 2H), 4.02 (d, J = 7.6 Hz, 2H), 3.96 (s, 3H), 2.13-2.04 (m, 5H), 1.79 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 0.72 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 167.4, 155.9, 153.1, 143.2, 142.9, 142.8, 139.4, 132.3, 127.2, 126.2, 124.9, 124.7, 123.9, 122.7, 119.6, 117.4, 110.8, 110.7, 52.5, 39.9, 31.0, 29.2, 26.6, 26.1, 20.4, 18.1, 16.8; MS (ESI) *m/z*: 518 (MH^+); HRMS (EI, m/z) calcd for $C_{30}H_{36}N_3O_3S$: *m/z* 518.2477; Found 518.2474; IR (cm^{-1}, neat): 2960, 1714, 1614.

1-Isobutyl-2-(2-(3-methylbut-2-enylthio)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21d.



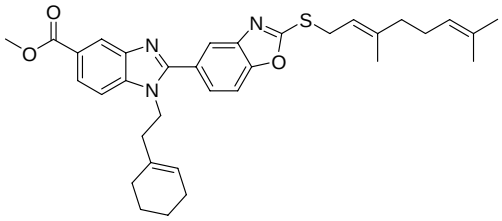
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 1.2 Hz, 1H), 8.06 (dd, J = 8.5, 1.2 Hz, 1H), 7.89 (s, 1H), 7.64 (dd, J = 8.5, 1.3 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 5.46 (t, J = 7.6 Hz, 1H), 4.14 (d, J = 7.5 Hz, 2H), 4.03 (d, J = 7.8 Hz, 2H), 3.98 (s, 3H), 2.10 (m, 1H), 1.79 (d, J = 6.0 Hz, 6H), 0.74 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 167.3, 155.9, 155.1, 142.9, 142.8, 139.7, 139.5, 127.2, 126.2, 124.9, 124. 122.7, 119.7, 117.6, 110.8, 110.6, 52.5, 31.1, 29.2, 26.2, 20.4, 18.4; MS (ESI) *m/z*: 450 (MH^+); HRMS (ESI, m/z) calcd for $C_{25}H_{28}N_3O_3S$: *m/z* 450.1851; Found 450.1853; IR (cm^{-1}, neat): 2960, 1706, 1614.

1-Butyl-2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21e.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 1.4 Hz, 1H), 8.07 (dd, J = 8.5, 1.4 Hz, 1H), 7.90 (d, J = 1.1 Hz, 1H), 7.66 (dd, J = 8.4, 1.5 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 5.47 (t, J = 6.9 Hz, 1H), 5.07 (m, 1H), 4.29 (t, J = 7.5 Hz, 2H), 4.04 (d, J = 7.5 Hz, 2H), 3.98 (s, 3H), 2.09-1.98 (m, 4H), 1.80 (s, 3H), 1.74-1.67 (m, 5H), 1.56 (s, 3H), 1.33-1.20 (sext, J = 7.2 Hz, 2H), 0.86 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 167.3, 155.5, 153.2, 143.2, 143.0, 139.3, 132.3, 126.9, 126.1, 125.0, 124.8, 123.9, 122.7, 119.5, 117.4, 110.8, 110.2, 52.5, 45.2, 39.9, 32.2, 30.1, 26.7, 26.1, 20.3, 18.1, 16.7, 13.9; MS (ESI) *m/z*: 518 (MH^+); HRMS (ESI, m/z) calcd for $C_{30}H_{36}N_3O_3S$: *m/z* 518.2477; Found 518.2480; IR (cm^{-1}, neat): 2931, 1716, 1616.

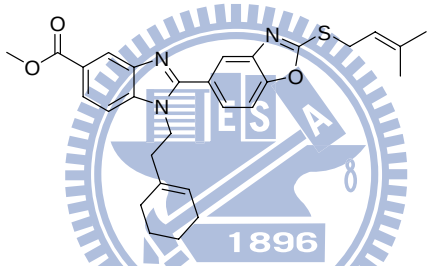
1-(2-Cyclohexenylethyl)-2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21f.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.93 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 5.46 (t, J = 7.8 Hz,

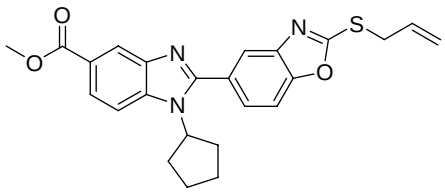
1H), 5.19 (m, 1H), 5.07 (m, 1H), 4.40 (t, J = 7.3 Hz, 2H), 4.04 (d, J = 7.7 Hz, 2H), 3.98 (s, 3H), 2.37 (t, J = 7.3 Hz, 2H), 2.09-1.96 (m, 4H), 1.83-1.80 (m, 2H), 1.79 (s, 3H), 1.73-1.71 (m, 4H), 1.68 (s, 3H), 1.60 (s, 3H), 1.49-1.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 167.5, 155.8, 153.3, 143.2, 143.0, 142.9, 138.9, 133.2, 132.4, 126.7, 126.2, 125.3, 125.0, 123.9, 122.3, 119.5, 117.4, 110.9, 110.6, 52.6, 44.4, 39.9, 36.1, 31.2, 28.6, 26.7, 26.1, 25.5, 22.9, 22.3, 18.1, 16.7; MS (ESI) *m/z*: 570 (MH^+); HRMS (EI, m/z) calcd for $C_{34}H_{40}N_3O_3S$: *m/z* 570.2790; Found 570.2793; IR (cm^{-1}, KBr): 1710, 1608.

1-(2-Cyclohexenylethyl)-2-(2-(3-methylbut-2-enylthio)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21g.



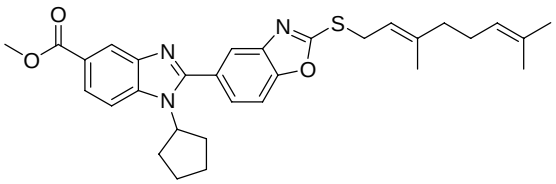
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 1.4 Hz, 1H), 8.08 (dd, J = 8.5, 1.4 Hz, 1H), 7.93 (d, J = 1.5 Hz, 1H), 7.69 (dd, J = 8.4, 1.5 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 5.47 (t, J = 7.6 Hz, 1H), 5.19 (m, 1H), 4.39 (t, J = 7.3 Hz, 2H), 4.03 (d, J = 7.8 Hz, 2H), 3.98 (s, 3H), 2.37 (t, J = 7.3 Hz, 2H), 1.83-1.82 (m, 2H), 1.79 (d, J = 6.0 Hz, 6H), 1.73-1.71 (m, 2H), 1.50-1.38 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 166.9, 154.9, 152.9, 142.5, 141.0, 139.2, 138.5, 132.7, 125.8, 125.7, 124.9, 124.5, 121.9, 119.1, 117.2, 110.4, 110.0, 52.1, 43.9, 37.7, 30.7, 28.2, 25.7, 25.0, 22.5, 21.8, 17.9; MS (ESI) *m/z*: 502 (MH^+); HRMS (ESI, m/z) calcd for $C_{29}H_{32}N_3O_3S$: *m/z* 502.2164; Found 502.2162; IR (cm^{-1}, neat): 2927, 1712, 1602.

2-(2-(Allylthio)benzo[*d*]oxazol-5-yl)-1-cyclopentyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21h.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 1.2 Hz, 1H), 8.01 (dd, J = 8.5, 1.2 Hz, 1H), 7.85 (s, 1H), 7.62 (m, 2H), 7.55 (d, J = 8.6 Hz, 1H), 6.06 (m, 1H), 5.43 (dd, J = 16.9, 1.2 Hz, 1H), 5.25 (dd, J = 10.0, 1.2 Hz, 1H), 4.94 (quint, J = 8.6 Hz, 1H), 4.00 (d, J = 6.5 Hz, 2H), 3.96 (s, 3H), 2.37-2.30 (m, 2H), 2.17-2.05 (m, 4H), 1.77-1.72 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 166.1, 155.5, 152.8, 143.1, 142.2, 136.4, 131.8, 126.6, 125.9, 124.4, 123.8, 122.5, 119.6, 119.4, 111.5, 110.4, 57.8, 52.1, 34.9, 30.4, 25.2; MS (ESI) *m/z*: 434 (MH^+); HRMS (ESI, m/z) calcd for $C_{24}H_{24}N_3O_3S$: *m/z* 434.1538; Found 434.1535; IR (cm^{-1}, KBr): 2952, 1714, 1614.

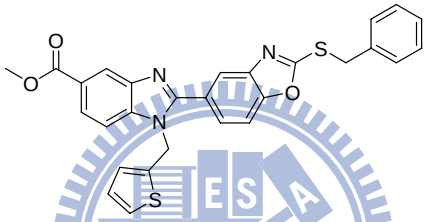
1-Cyclopentyl-2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21i.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 1.2 Hz, 1H), 7.99 (dd, J = 8.5, 1.2 Hz, 1H), 7.84 (s, 1H), 7.63-7.59 (m, 2H), 7.54 (d, J = 8.6 Hz, 1H), 5.44 (t, J = 7.6 Hz, 1H), 5.05 (m, 1H), 4.91 (q, J = 8.1 Hz, 1H), 4.02 (d, J = 7.5 Hz, 2H), 3.95 (s, 3H), 2.38-2.26 (m,

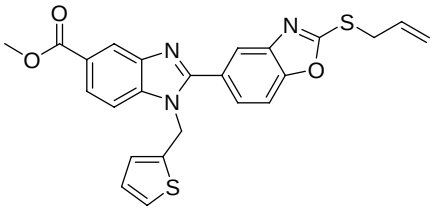
2H), 2.16-1.93 (m, 8H), 1.78 (s, 3H), 1.76-1.72 (m, 2H), 1.65 (s, 3H), 1.59 (s, 3H), 1.33-1.20 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 167.3, 156.0, 153.1, 143.6, 143.1, 142.8, 136.8, 132.3, 126.2, 125.5, 124.2, 123.7, 123.9, 122.9, 119.6, 117.4, 111.9, 110.8, 58.2, 52.5, 37.5, 31.0, 30.8, 26.6, 26.0, 25.6, 18.1, 16.7; MS (ESI) *m/z*: 530 (MH^+); HRMS (ESI, m/z) calcd for $C_{31}H_{36}N_3O_3S$: *m/z* 530.2477 Found 530.2480; IR (cm^{-1}, KBr): 2956, 1714, 1614.

2-(2-(Benzylthio)benzo[*d*]oxazol-5-yl)-1-(thiophen-2-ylmethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21j.



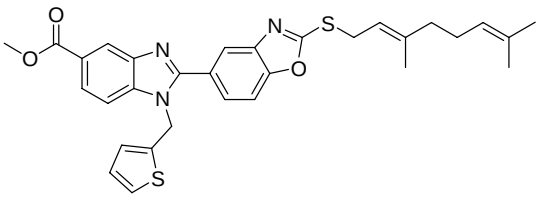
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.3 Hz, 1H), 8.05 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.99 (d, *J* = 1.5 Hz, 1H), 7.68 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.50-7.47 (m, 2H), 7.40 (d, *J* = 5.5 Hz, 2H), 7.37-7.31 (m, 2H), 7.24 (d, J = 5.5 Hz, 1H), 6.95 (dd, *J* = 5.0, 3.5 Hz, 1H), 6.85 (d, *J* = 3.5 Hz, 1H), 5.62 (s, 2H), 4.60 (s, 2H), 3.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 166.8, 155.4, 153.4, 142.9, 142.8, 139.2, 138.6, 135.9, 129.5, 129.2, 128.5, 127.7, 126.4, 126.3, 126.2, 126.1, 125.6, 125.2, 122.7, 119.9, 110.9, 110.6, 52.6, 44.7, 37.1; MS (ESI) *m/z*: 512 (MH^+); HRMS (ESI, m/z) calcd for $C_{28}H_{22}N_3O_3S_2$: *m/z* 512.1103 Found 512.1104; IR (cm^{-1}, KBr): 3052, 1714, 1616.

2-(2-(Allylthio)benzo[*d*]oxazol-5-yl)-1-(thiophen-2-ylmethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21k.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 1.3 Hz, 1H), 8.05 (dd, J = 8.4, 1.3 Hz, 1H), 7.96 (d, J = 1.5 Hz, 1H), 7.69 (dd, J = 8.4, 1.5 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 5.1 Hz, 1H), 6.95 (dd, J = 5.0, 3.4 Hz, 1H), 6.84 (d, J = 3.4 Hz, 1H), 6.05 (m, 1H), 5.62 (s, 2H), 5.44 (dd, J = 16.9, 1.1 Hz, 1H), 5.25 (dd, J = 10.1, 1.1 Hz, 1H), 4.00 (d, J = 6.5 Hz, 2H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 166.6, 155.4, 153.4, 142.9, 142.8, 139.2, 138.6, 132.2, 127.7, 126.3, 126.1, 125.5, 125.1, 124.5, 120.5, 120.4, 120.0, 119.9, 110.9, 110.6, 52.6, 44.7, 35.4; MS (ESI) *m/z*: 462 (MH^+); HRMS (ESI, m/z) calcd for $C_{24}H_{20}N_3O_3S_2$: *m/z* 462.0946; Found 462.0949; IR (cm^{-1}, KBr): 2929, 1714, 1614.

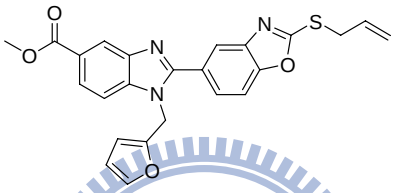
2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1-(thiophen-2-ylmethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21l.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 1.4 Hz, 1H), 8.05 (dd, J = 7.2, 1.4 Hz, 1H), 7.95 (d, J = 1.5 Hz, 1H), 7.69 (dd, J = 8.4, 1.5 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.24 (d, J = 5.0 Hz, 1H), 6.95 (dd, J = 5.0, 3.4 Hz, 1H), 6.84 (d, J = 3.4 Hz, 1H), 5.62 (s, 2H), 5.40 (d, J = 7.1 Hz, 2H), 5.08 (m, 1H), 4.03 (d, J = 7.6 Hz, 2H), 3.91 (s, 3H), 2.10-2.05 (m, 3H), 1.79 (s, 3H), 1.71 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (75

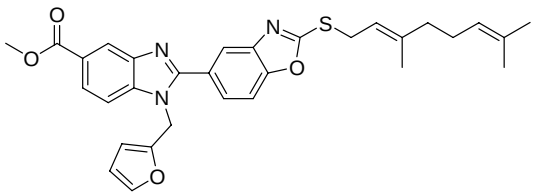
MHz, $CDCl_3$) δ 167.8, 167.5, 155.4, 153.5, 143.2, 142.9, 142.0, 139.1, 138.5, 132.3, 127.7, 126.3, 126.2, 126.1, 126.0, 125.9, 123.9, 122.8, 119.7, 117.4, 110.9, 110.6, 52.6, 44.7, 39.9, 31.0, 26.1, 25.9, 18.1, 16.8; MS (ESI) *m/z*: 558 (MH^+); HRMS (ESI, m/z) calcd for $C_{31}H_{32}N_3O_3S_2$: *m/z* 558.1885; Found 558.1887; IR (cm^{-1}, KBr): 2929, 1710, 1610.

2-(2-(Allylthio)benzo[*d*]oxazol-5-yl)-1-(furan-2-ylmethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21m.



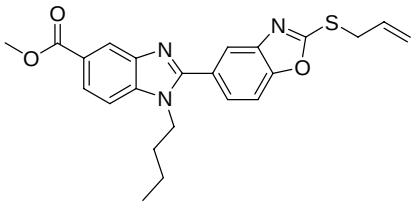
^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 1.3 Hz, 1H), 8.08 (d, J = 1.9 Hz, 1H), 8.06 (dd, J = 8.4, 1.3 Hz, 1H), 7.78 (dd, J = 8.4, 1.7 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.42 (dd, J = 2.5, 1.9 Hz, 1H), 6.38 (dd, J = 3.2, 1.9 Hz, 1H), 6.31 (d, J = 3.2 Hz, 1H), 6.09 (m, 1H), 5.44(dd, J = 16.9, 1.1 Hz, 1H), 5.40 (s, 2H), 5.26 (dd, J = 10.1, 1.1 Hz, 1H), 4.00 (d, J = 6.5 Hz, 2H), 3.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 166.7, 155.5, 153.5, 148.8, 145.5, 145.0, 143.7, 142.8, 139.2, 133.2, 132.2, 126.6, 125.7, 125.4, 122.4, 120.2, 120.0, 111.1, 110.9, 110.8, 52.6, 42.8, 33.6; MS (ESI) *m/z*: 446 (MH^+); HRMS (ESI, m/z) calcd for $C_{24}H_{20}N_3O_4S$: *m/z* 446.1174; Found 446.1175; IR (cm^{-1}, KBr): 2925, 1710, 1614.

2-(2-(3,7-dimethylocta-2,6-dienylthio)benzo[*d*]oxazol-5-yl)-1-(furan-2-ylmethyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21n.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.06 (t, J = 8.3 Hz, 2H), 7.77 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.42 (s, 1H), 6.38 (s, 1H), 6.31 (d, J = 3.1 Hz, 1H), 5.46 (t, J = 7.1 Hz, 2H), 5.39 (s, 2H), 5.07 (t, J =), 4.06 (d, J = 7.6 Hz, 2H), 3.93 (s, 3H), 2.09-2.06 (m, 3H), 1.80 (s, 3H), 1.66 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 167.4, 155.5, 153.5, 148.9, 143.7, 143.2, 142.9, 142.0, 139.2, 132.3, 126.5, 126.3, 123.9, 122.8, 119.7, 117.4, 111.1, 110.9, 110.6, 109.5, 52.6, 42.8, 39.9, 30.1, 26.7, 26.1, 25.9, 18.1, 16.7; MS (ESI) *m/z*: 542 (MH^+); HRMS (ESI, m/z) calcd for $C_{31}H_{32}N_3O_4S_2$: *m/z* 542.2113; Found 542.2110; IR (cm^{-1}, KBr): 2925, 1716, 1616.

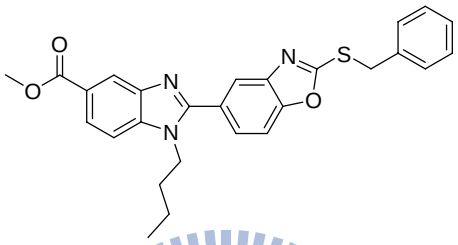
2-(2-(Allylthio)benzo[*d*]oxazol-5-yl)-1-butyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21o.



^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 1.4 Hz, 1H), 8.04 (dd, J = 8.5, 1.4 Hz, 1H), 7.88 (d, J = 1.5 Hz, 1H), 7.64 (dd, J = 8.3, 1.5 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 6.04 (m, 1H), 5.42 (dd, J = 16.8, 1.2 Hz, 1H), 5.24 (dd, J = 10.2, 1.2 Hz, 1H), 4.26 (t, J = 7.5 Hz, 2H), 3.98 (d, J = 6.9 Hz, 2H), 3.96 (s, 3H), 1.77 (quint, J = 7.5 Hz, 2H), 1.28-1.20 (sext, J = 7.5 Hz, 2H), 0.83 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz,

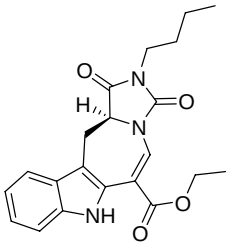
$CDCl_3$) δ 168.0, 166.6, 155.5, 153.2, 143.0, 142.7, 139.2, 132.2, 126.9, 126.2, 125.0, 124.8, 122.7, 120.0, 119.6, 110.8, 110.3, 52.5, 45.2, 35.4, 32.2, 20.3, 13.9; MS (ESI) *m/z*: 422 (MH^+); HRMS (EI, m/z) calcd for $C_{23}H_{24}N_3O_3S$: *m/z* 422.1538; Found 422.1539; IR (cm^{-1}, KBr): 2925, 1716, 1612.

2-(2-(Benzylthio)benzo[*d*]oxazol-5-yl)-1-butyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid methyl ester 21p.



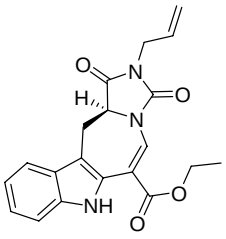
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, *J* = 1.4 Hz, 1H), 8.08 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.94 (d, *J* = 1.5 Hz, 1H), 7.66 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.51-7.45 (m, 3H), 7.40-7.32 (m, 3H), 4.65 (s, 2H), 4.29 (t, *J* = 7.3 Hz, 2H), 3.98 (s, 3H), 1.82 (quint, *J* = 7.3 Hz, 2H), 1.27 (sext, *J* = 7.3 Hz, 2H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 167.0, 154.8, 153.5, 142.8, 138.6, 135.9, 129.6, 129.5, 129.2, 128.5, 126.4, 125.8, 125.3, 122.1, 122.0, 119.7, 111.1, 110.6, 52.6, 45.4, 37.1, 32.1, 20.3, 13.9; MS (ESI) *m/z*: 472 (MH^+); HRMS (ESI, m/z) calcd for $C_{27}H_{26}N_3O_3S$: *m/z* 472.1695; Found 472.1696; IR (cm^{-1}, KBr): 2861, 1710, 1614.

Ethyl (12a*R*)-2-butyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27a).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.59 (s, NH), 8.34 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.17 (dd, *J* = 10.3, 1.7 Hz, 1H), 3.99 (dd, *J* = 15.8, 1.7 Hz, 1H), 3.68 (t, *J* = 7.2 Hz, 2H), 2.87 (dd, *J* = 15.8, 10.3 Hz, 1H), 1.73 (quint, *J* = 7.2 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.40-1.37 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.2, 154.2, 135.1, 130.5, 128.8, 127.4, 123.1, 120.2, 118.1, 111.6, 110.0, 104.1, 62.0, 59.4, 40.0, 30.3, 27.7, 20.3, 14.7, 14.0; MS (ESI) *m/z*: 404 (M+Na); HRMS (ESI, *m/z*) calcd for $C_{21}H_{24}N_3O_4$: *m/z* 382.1767; Found 382.1769; IR (cm^{-1}, neat): 3405, 2954, 1789, 1718, 1618.

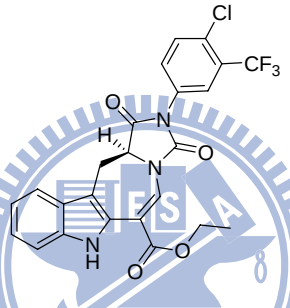
Ethyl (12a*R*)-2-allyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27b).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.57 (s, NH), 8.30 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 5.89 (m, 1H), 5.36-

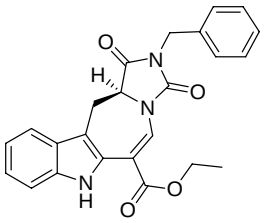
5.32 (m, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 4.26 (d, *J* = 5.9 Hz, 2H), 4.07 (d, *J* = 10.1, 1.1 Hz, 1H), 3.95 (dd, *J* = 15.8, 1.1 Hz, 1H), 2.84 (dd, *J* = 15.8, 10.1 Hz, 1H), 1.43 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 168.1, 153.7, 135.1, 130.6, 130.4, 128.8, 126.1, 123.2, 120.2, 119.7, 118.1, 111.7, 110.0, 104.2, 61.9, 59.4, 42.1, 27.6, 14.7 MS (ESI) *m/z*: 366 (MH+); HRMS (ESI, *m/z*) calcd for $C_{20}H_{20}N_3O_4$: *m/z* 366.1454; Found 366.1452; IR (cm^{-1}, neat): 3401, 2360, 1778, 1718, 1685.

Ethyl(12a*R*)-2-(4-chloro-3-trifluoromethylpheny)-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27c).



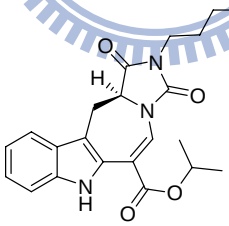
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, NH), 8.38 (s, 1H), 7.95 (s, 1H), 7.74 (d, *J* = 1.8 Hz, 1H), 7.71 (d, *J* = 6.0 Hz, 2H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.23-7.16 (m, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 4.28 (dd, *J* = 10.3, 1.7 Hz, 1H), 4.08 (dd, *J* = 15.8, 1.7 Hz, 1H), 3.03 (dd, *J* = 15.8, 10.3 Hz, 1H), 0.99 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 167.9, 152.4, 135.3, 132.9, 132.8, 130.2, 130.4, 129.6, 128.5, 127.3, 125.4, 125.3, 123.5, 120.5, 118.2, 111.8, 110.2, 105.7, 62.2, 59.3, 53.1, 27.8, 14.7; MS (ESI) *m/z*: 525 (M+Na); HRMS (ESI, *m/z*) calcd for $C_{24}H_{17}ClF_3N_3O_4Na$: *m/z* 526.0757; Found 526.0759; IR (cm^{-1}, neat): 3423, 2923, 1789, 1729, 1679.

Ethyl (12a*R*)-2-benzyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27d).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.56 (s, NH), 8.29 (s, 1H), 7.54-7.48 (m, 3H), 7.40-7.35 (m, 4H), 7.17 (t, J = 7.9 Hz, 1H), 7.09 (t, J = 7.9 Hz, 1H), 4.81 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 4.02 (dd, J = 10.3, 1.8 Hz, 1H), 3.93 (dd, J = 15.8, 1.8 Hz, 1H), 2.80 (dd, J = 15.8, 10.3 Hz, 1H), 1.42 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 168.1, 153.9, 135.4, 135.1, 130.3, 129.3, 128.9, 128.8, 127.7, 127.3, 123.1, 120.2, 118.0, 111.6, 109.9, 104.2, 61.9, 59.4, 43.7, 27.5, 14.7; MS (EI) *m/z*: 415 (M^+); HRMS (ESI, *m/z*) calcd for $C_{24}H_{21}N_3O_4$: *m/z* 415.1532; Found 415.1538; IR (cm^{-1}, neat): 3397, 2360, 1783, 1724, 1685.

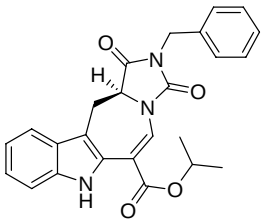
Isopropyl (12a*R*)-2-butyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27e).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.63 (s, NH), 8.30 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 5.26 (sept, J = 6.2 Hz, 1H), 4.12 (dd, J = 10.3, 1.6 Hz, 1H), 3.97 (dd, J = 15.8, 1.6 Hz, 1H), 3.68 (t, J = 7.3 Hz, 2H), 2.85 (dd, J = 15.8, 10.3 Hz, 1H), 1.71-1.66 (quint, J = 7.3 Hz, 2H), 1.43-1.34 (m, 8H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 167.7, 154.3, 135.1, 130.3, 128.9, 127.4, 123.1, 120.2, 118.1, 111.6, 109.9, 104.5, 69.7, 59.4, 39.9, 30.3, 27.7,

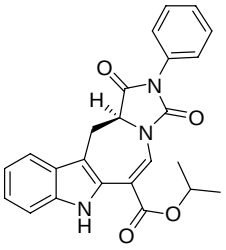
22.3, 20.4, 14.1; MS (EI) *m/z*: 395 (M^+); HRMS (ESI, *m/z*) calcd for $C_{22}H_{25}N_3O_4$: *m/z* 395.1845; Found 395.1852; IR (cm^{-1}, neat): 3407, 2865, 1782, 1716, 1691.

Isopropyl (12a*R*)-2-benzyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27f).



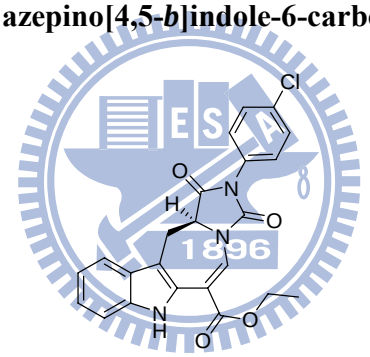
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.63 (s, NH), 8.30 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.2 Hz, 1H), 7.39-7.29 (m, 5H), 7.21 (t, J = 7.8 Hz, 1H), 7.13 (t, J = 7.8 Hz, 1H), 5.25 (sept, J = 6.2 Hz, 1H), 4.83 (s, 2H), 4.20 (dd, J = 10.3, 1.6 Hz, 1H), 3.99 (dd, J = 15.8, 1.6 Hz, 1H), 2.89 (dd, J = 15.8, 10.3 Hz, 1H), 1.39 (d, J = 6.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 167.7, 153.9, 135.4, 135.1, 130.2, 129.3, 129.2, 128.9, 127.9, 127.3, 123.2, 120.2, 118.1, 111.7, 109.9, 104.7, 69.7, 59.6, 43.7, 27.6, 22.3 ; MS (EI) *m/z*: 429 (M^+); HRMS (ESI, *m/z*) calcd for $C_{25}H_{23}N_3O_4$: *m/z* 429.1689; Found 429.1691; IR (cm^{-1}, neat): 3417, 2360, 1781, 1718, 1687.

Isopropyl (12a*R*)-2-phenyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27g).



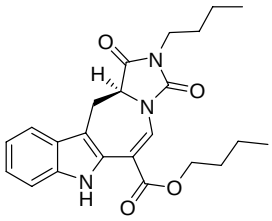
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.67 (s, NH), 8.39 (s, 1H), 7.60-7.50 (m, 6H), 7.48 (d, *J* = 7.0 Hz, 1H), 7.25 (t, *J* = 7.0 Hz, 1H), 7.17 (t, *J* = 7.0 Hz, 1H), 5.28 (sept, *J* = 6.2 Hz, 1H), 4.37 (dd, *J* = 10.3, 1.7 Hz, 1H), 4.10 (dd, *J* = 15.7, 1.7 Hz, 1H), 3.08 (dd, *J* = 15.7, 10.3 Hz, 1H), 1.42 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 167.2, 152.7, 134.8, 130.8, 129.7, 129.3, 128.9, 128.4, 126.9, 125.9, 122.8, 119.8, 117.7, 111.3, 109.7, 104.9, 69.4, 58.9, 21.5, 21.9; MS (EI) *m/z*: 415 (M^+); HRMS (EI, *m/z*) calcd for $C_{24}H_{21}N_3O_4$: *m/z* 415. 1532; Found 415.1544; IR (cm^{-1}, neat): 3386, 2360, 1785, 1726, 1687.

Ethyl(12a*R*)-2-(4-Chlorophenyl)-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27h).



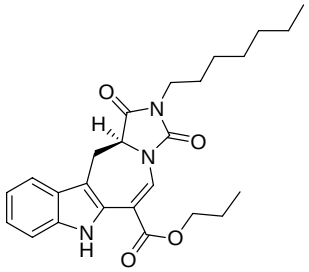
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, NH), 8.38 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.59-7.46 (m, 5H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.26-7.17 (m, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 4.36 (dd, *J* = 10.3, 1.7 Hz, 1H), 4.09 (dd, *J* = 15.8, 1.7 Hz, 1H), 3.06 (dd, *J* = 15.8, 10.3 Hz, 1H), 1.43 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 168.0, 152.8, 135.3, 135.1, 130.0, 129.9, 127.5, 127.3, 126.7, 126.1, 123.4, 120.3, 118.2, 111.7, 110.2, 105.2, 62.1, 59.3, 22.4, 14.7; MS (EI) *m/z*: 435 (M^+); HRMS (ESI, *m/z*) calcd for $C_{23}H_{18}ClN_3O_4$: *m/z* 435.0986.; Found 435.0981; IR (cm^{-1}, neat): 3386, 2360, 1780, 1722, 1687.

Butyl (12a*R*)-2-butyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27i).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.59 (s, NH), 8.34 (s, 1H), 7.57 (d, J = 7.4 Hz, 1H), 7.39 (d, J = 7.4 Hz, 1H), 7.22 (t, J = 7.4 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 4.34 (t, J = 6.5 Hz, 2H), 4.20 (dd, J = 10.3, 1.6 Hz, 1H), 4.01 (dd, J = 15.8, 1.6 Hz, 1H), 3.68 (t, J = 7.3 Hz, 2H), 2.91 (dd, J = 15.8, 10.3 Hz, 1H), 1.78 (quint, J = 7.3 Hz, 2H), 1.73 (quint, J = 6.5 Hz, 2H), 1.49 (sext, J = 7.3 Hz, 2H), 1.37 (sext, J = 6.5 Hz, 2H), 1.03 (t, J = 7.3 Hz, 3H), 0.97 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.3, 154.2, 135.1, 130.4, 128.8, 127.4, 123.1, 120.2, 118.1, 111.6, 110.0, 104.1, 65.8, 59.4, 39.9, 31.1, 30.3, 27.7, 20.3, 19.7, 14.2, 13.9; MS (EI) *m/z*: 409 (M^+); HRMS (ESI, *m/z*) calcd for $C_{23}H_{27}N_3O_4$: *m/z* 409.2002; Found 409.2007; IR (cm^{-1}, neat): 3413, 2360, 1782, 1718, 1685.

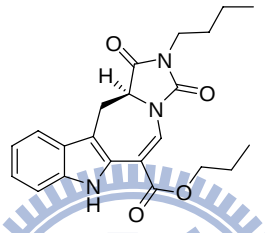
Propyl (12a*R*)-2-heptyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27j).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.60 (s, NH), 8.36 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 4.30 (t, J = 6.7 Hz, 2H), 4.22 (dd, J = 10.3, 1.7 Hz, 1H), 4.01 (dd, J = 15.8, 1.7 Hz, 1H), 3.67 (t, J = 7.0 Hz, 2H), 2.91 (dd, J = 15.8, 10.3 Hz, 1H), 1.84 (quint, J = 7.0 Hz, 2H), 1.73 (quint, J = 6.7

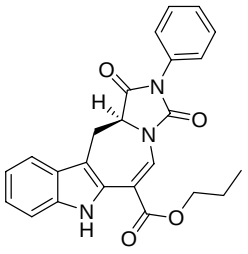
Hz, 2H), 1.36-1.31 (m, 8H), 1.06 (t, J = 6.7 Hz, 3H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.3, 154.2, 135.2, 130.4, 127.9, 127.4, 123.2, 120.2, 118.1, 111.7, 110.1, 104.2, 67.5, 59.4, 40.3, 32.0, 29.2, 28.3, 27.7, 27.0, 22.9, 22.5, 14.5, 10.9; MS (EI) *m/z*: 437 (M^+); HRMS (ESI, m/z) calcd for $C_{25}H_{31}N_3O_4$: *m/z* 437.2315; Found 437.2312; IR (cm^{-1}, neat): 3415, 2360, 1782, 1718, 1685.

Propyl (12a*R*)-2-butyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27k).



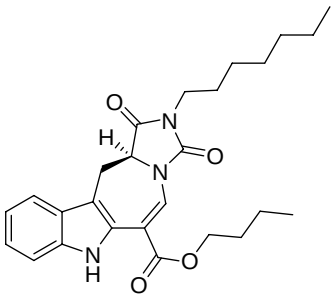
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.59 (s, NH), 8.36 (s, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.22 (t, J = 7.5 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 4.29 (t, J = 6.6 Hz, 2H), 4.21 (d, J = 10.3 Hz, 1H), 4.00 (d, J = 15.7 Hz, 1H), 3.69 (t, J = 6.9 Hz, 2H), 2.91 (dd, J = 15.7, 10.3 Hz, 1H), 1.84 (sext, J = 6.6 Hz, 2H), 1.73 (quint, J = 6.9 Hz, 2H), 1.40 (sext, J = 6.9 Hz, 2H), 1.06 (t, J = 6.6 Hz, 3H), 0.99 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.3, 154.2, 135.2, 130.4, 128.9, 127.4, 123.2, 120.2, 118.1, 111.6, 110.1, 104.2, 67.5, 59.4, 39.9, 30.3, 27.7, 22.5, 20.3, 14.1, 11.0; MS (EI) *m/z*: 395 (M^+); HRMS (EI, m/z) calcd for $C_{22}H_{25}N_3O_4$: *m/z* 395.1845; Found 395.1840; IR (cm^{-1}, neat): 3413, 2360, 1786, 1722, 1689.

Propyl (12a*R*)-2-phenyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27l).



^{1}H NMR (500 MHz, $CDCl_3$) δ 10.60 (s, NH), 8.41 (s, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.58-7.56 (m, 5H), 7.41 (d, J = 7.5 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H), 4.37 (dd, J = 10.5, 1.5 Hz, 1H), 4.31 (t, J = 6.5 Hz, 2H), 4.09 (dd, J = 16.0, 1.5 Hz, 1H), 3.06 (dd, J = 16.0, 10.5 Hz, 1H), 1.82 (quint, J = 7.5 Hz, 3H), 1.05 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 167.8, 152.7, 134.8, 130.8, 129.8, 129.3, 128.9, 128.3, 127.0, 125.9, 122.9, 119.9, 117.8, 111.3, 109.8, 104.5, 67.2, 58.9, 27.5, 22.0, 10.5; MS (EI) *m/z*: 415 (M^+); HRMS (EI, *m/z*) calcd for $C_{24}H_{21}N_3O_4$: *m/z* 415.1532; Found 415.1537; IR (cm^{-1}, neat): 3396, 2360, 1786, 1728, 1697.

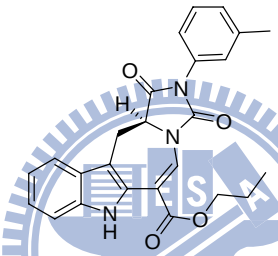
Butyl (12a*R*)-2-heptyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27m).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.59 (s, NH), 8.36 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 4.35 (t, J = 6.6 Hz, 2H), 4.24 (dd, J = 10.3, 1.6 Hz, 1H), 4.03 (dd, J = 15.8, 1.6 Hz, 1H), 3.67 (t, J = 7.7 Hz,

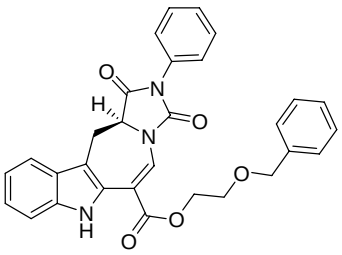
2H), 2.92 (dd, J = 15.8, 10.3 Hz, 1H), 1.81-1.70 (m, 4H), 1.50 (quint, J = 6.6 Hz, 2H), 1.36-1.32 (m, 8H), 1.02 (t, J = 7.7 Hz, 3H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.3, 154.2, 135.2, 130.4, 128.9, 127.4, 123.2, 120.2, 118.1, 111.6, 110.1, 104.2, 65.8, 59.4, 40.3, 32.0, 31.1, 29.2, 28.3, 27.7, 27.0, 22.9, 19.7, 14.5, 14.2; MS (EI) *m/z*: 451 (M^+); HRMS (EI, *m/z*) calcd for $C_{26}H_{33}N_3O_4$: *m/z* 451.2471; Found 451.2475; IR (cm^{-1}, neat): 3407, 2360, 1782, 1718, 1684.

Propyl (12a*R*)-2-(3-methylphenyl)-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27n).



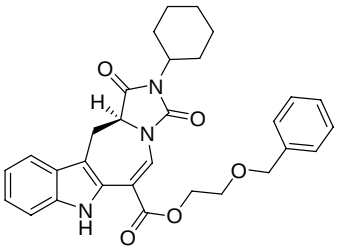
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, NH), 8.41 (s, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.8 Hz, 2H), 7.29 7.26 (m, 3H), 7.24 (t, J = 7.8 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 4.33-4.28 (m, 3H), 4.08 (dd, J = 15.8, 1.7 Hz, 1H), 3.04 (dd, J = 15.8, 10.3 Hz, 1H), 2.45 (s, 3H), 1.85 (sext, J = 7.3 Hz, 2H), 1.06 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 168.2, 153.3, 139.9, 135.2, 131.0, 130.3, 130.2, 129.6, 128.8, 127.4, 127.0, 123.6, 123.3, 120.3, 118.9, 111.7, 110.2, 104.8, 67.6, 59.2, 27.9, 22.5, 21.8, 10.9; MS (EI) *m/z*: 429 (M^+); HRMS (EI, *m/z*) calcd for $C_{25}H_{23}N_3O_4$: *m/z* 429.1689; Found 429.1696; IR (cm^{-1}, neat): 3399, 2360, 1790, 1732, 1691.

2-(Benzyloxy)ethyl (12a*R*)-1,3-dioxo-2-phenyl-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27o).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, NH), 8.46 (s, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.57-7.47 (m, 5H), 7.42-7.30 (m, 6H), 7.22 (t, J = 7.8 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 4.67 (s, 2H), 4.54 (t, J = 5.0 Hz, 2H), 4.41 (dd, J = 10.2, 1.7 Hz, 1H), 4.12 (dd, J = 15.8, 1.7 Hz, 1H), 3.84 (t, J = 5.0 Hz, 2H), 3.09 (dd, J = 15.8, 10.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 167.9, 153.1, 138.1, 135.3, 131.2, 130.6, 129.8, 129.3, 128.9, 128.5, 128.3, 128.2, 127.4, 126.4, 123.3, 120.3, 118.2, 111.7, 110.3, 104.9, 73.8, 68.1, 64.9, 59.4, 27.9; MS (EI) *m/z*: 507 (M^+); HRMS (EI, *m/z*) calcd for $C_{30}H_{25}N_3O_5$: *m/z* 507.1794; Found 507.1799; IR (cm^{-1}, neat): 3399, 2359, 1788, 1720, 1697.

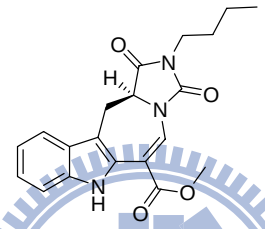
2-(Benzyloxy)ethyl (12a*R*)-2-cyclohexyl-1,3-dioxo-1,2,3,7,12,12a-hexahydropyrrolo[2',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27p).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, NH), 8.46 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.40-7.31 (m, 6H), 7.22 (t, J = 7.6 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 4.67 (s, 2H), 4.51 (t, J = 4.5 Hz, 2H), 4.14-4.06 (m, 2H), 3.96 (d, J = 15.8 Hz, 1H), 3.84 (t, J = 4.5 Hz, 2H), 2.85 (dd, J = 15.8, 10.8 Hz, 1H), 2.24-2.16 (m, 2H), 1.94-1.90 (m, 2H), 1.80-1.69 (m, 2H),

1.39-1.27 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 168.1, 153.8, 138.2, 135.2, 130.9, 128.9, 128.7, 128.2, 128.1, 127.5, 123.1, 120.2, 118.1, 111.6, 110.1, 103.9, 73.7, 68.1, 64.8, 58.9, 53.0, 29.9, 29.6, 29.5, 27.3, 26.2, 25.4; MS (EI) *m/z*: 513 (M^+); HRMS (EI, *m/z*) calcd for $C_{30}H_{31}N_3O_5$: *m/z* 513.2264; Found 513.2266; IR (cm^{-1}, neat): 3401, 2359, 1783, 1722, 1616.

Methyl (12a*R*)-2-butyl-1,3-dioxo-1,2,3,7,12,12a-hexahydroimidazo[5',1':7,1]azepino[4,5-*b*]indole-6-carboxylate (27q).



^{1}H NMR (300 MHz, $CDCl_3$) δ 10.56 (s, NH), 8.36 (s, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 4.22 (dd, J = 10.4, 1.8 Hz, 1H), 4.02 (dd, J = 15.7, 1.8 Hz, 1H), 3.93 (s, 3H), 3.69 (t, J = 7.2 Hz, 2H), 2.90 (dd, J = 15.7, 10.4 Hz, 1H), 1.70 (quint, J = 7.2 Hz, 2H), 1.42 (sext, J = 7.2 Hz, 2H), 0.99 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.7, 154.2, 135.2, 130.7, 128.7, 127.4, 123.2, 120.2, 118.1, 111.6, 110.1, 103.9, 59.4, 52.9, 39.9, 30.3, 27.7, 20.3, 13.9; MS (EI) *m/z*: 367 (M^+); HRMS (EI, m/z) calcd for $C_{20}H_{21}N_3O_3$: *m/z* 513.2264; Found 367.1530; IR (cm^{-1}, neat): 3415, 2359, 1781, 1717, 1685.

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