Synthesis of novel dimeric and oligomeric benzimidazoles targeted to nucleic acid minor groove binding

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Abstract

Amido-oligopyrroles/imidazoles and dimeric benzimidazoles are heterocyclic compounds which are nucleic acid minor groove binding agents. The targets for this project were symmetrical and unsymmetrical amide-linked di- and tri- and tetrameric benzimidazoles to evaluate as potential DNA binding agents. Syntheses involved linkage between C2 and C5 positions of 2-carboxy, 5-carboxy or 2,5-dicarboxy and 2-amino, 5-amino or 2,5-diamino-bearing benzimidazoles. This matrix of substrates could be coupled directly C2-C5 by amide linkage, or these same precursors could also be dimerized symmetrically using small bifunctional linkers (diamine or diacid), affording symmetrical C2-C2 or C5-C5 dimer types of orientation, such as head-to-head, and tail-to tail systems.

Monomeric building block benzimidazoles were prepared by condensation of *o*-phenylene diamines with either aldehydes or carboxylic acids affording the corresponding C2 or C5 amino and C5 carboxybenzimidazoles. 2-Carboxybenzimidazoles were however prepared by condensation of *o*-phenylene diamines with trichloroacetimidate followed by hydrolysis. New different novel symmetrical and unsymmetrical-bis(benzimidazole) libraries (C2-C2, C5-C5 and C5-C2 orientation) were prepared *via* coupling of different aminobenzimidazoles with different carboxybenzimidazoles from the matrix of available monomers.

Novel tris(benzimidazoles) with differently linked orientations were prepared either by coupling one equivalent of 2,5-dicarboxybenzimidazole with two equivalents of aminobenzimidazole or coupling one equivalent of 2,5-diaminobenzimidazole with two equivalents of carboxybenzimidazole. A second set of novel tris(benzimidazoles) was prepared *via* reduction of a nitro- to amino bearing dimer followed by coupling with another carboxybenzimidazole monomer.

Novel symmetrical tetra(benzimidazoles) (C2-C2-C2-C2 and C5-C2-C2-C5 orientation) were synthesized *via* either coupling one equivalent 2-dicarboxy dimeric benzimidazole with two equivalents of 2 or 5-aminobenzimidazole or *via* coupling one equivalent of 2-diaminobis(benzimidazole) with two equivalents of 2 or 5-carboxybenzimidazoles.

Novel symmetrical bis(benzimidazoles) piperidine derivatives were synthesized *via* coupling one equivalent of 2-dicarboxy dimericbenzimidazole with two equivalents of piperidine derivatives.

Forty oligomeric benzimidazoles were evaluated using Surface Plasmon Resonance (SPR) for binding to a series of three oligonucleotides containing A2T2, or A3T3 or A4T4 sequences.
Data are presented showing the identification of four optimum ligands from a screen of 40, primarily 2_L2552_L2 tetramers but also several 2_L5 dimers and dimeric 5,5-units *without* terminal additional benzimidazoles.

Declaration

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Shatha I. Alaqeel

Copyright

Dedication

This thesis is dedicated to my beloved Mother and Father

" the love and the hope"

Acknowledgements

I would like to thank my supervisor Dr John Gardiner, for his continuous support and encouragement and for the time and energy he gave to me and the constructive discussions we had. His integrity and remarkable dedication to quality research and teaching is a strong inspiration.

I also extend my sincere thanks to my colleague Kanan M. Wahedy, for his patience and guidance through out the project.

Special thanks to my family, including my father, mother, sisters and brothers, especially my sister Dr Aida, and my brothers Mohammed and Abdullah, for their continuous support, help and encouragement and to the Saudi Government, including the Saudi Cultural Bureau in the United Kingdom and King Saud University in Riyadh for their help and support.

In my years at the University of Manchester, I have enjoyed working alongside many enthusiastic people, current and previous postdocs and students. They have contributed to a stimulating environment, and I have benefited from their friendship, technical help and discussion especially Miss Val Boot, Dr Reynard Spiess and Dr Sanjay Nilapwar.

Finally I thank every one from Dr Gardiner and Dr Webb's group for their help.

Abbreviations

AcOH	Acetic acid
Bi	Benzimidazole
br	Broad
bs	Broad singlet
$CDCl_3$	Chloroform-d
CDI	*N,N*-Carbonyldiimidazole
COSY	Correlated spectroscopy
d	Doublet
dd	Boublet of doublets
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarisation transfer
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylslfoxide
DNA	Deoxyribonucleic acid
dq	Doublet of quartets
EDC	1-Ethyl-3-[3-(dimethylamino)propyl] carbodiimide
HMQC	Heteronuclear Multiple Quantum Coherence
HOBt	1-Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
IR	Infrared
LRMS	Low resolution mass spectrometry
m	multiplet
mp	Melting point
ms	Mass spectroscopy
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
ppm	Parts per million
q	Quartet
qd	Quartet of doublets
RNA	Ribonucleic acid
rt	Room temperature

s	Singlet
SPR	Surface plasmon resonance
t	triplet
TFO	Triplex forming oligonucleotide
THF	Tetrahydrofuran
TLC	Thin layer chromatography
tt	Triplet of triplets
UV	Ultraviolet

Chapter 1
Introduction

1.1 Heterocycles, medicinal chemistry and synthetic diversity

Heterocyclic aromatic chemistry is an important branch of organic chemistry because of the physiological and industrial significance of heterocyclic compounds.

We are now in the post-genome era, with great advances in molecular biology and high-throughput techniques, which enable us to better understand the biological processes and suggest new targets and techniques for the diagnosis and treatment of many diseases. These advances are due in large part to increasing investment in biological and pharmaceutical research and development.

The majority of medicines are small synthetic organic molecules and a high proportion of them contain a heterocyclic ring.[1] It is interesting to consider why most bioactive molecules have heterocyclic rings. One possibility is that heterocyclic rings have hydrogen bond donors and/or acceptors in a semi-rigid framework and they can therefore present a diverse array of pharmacophores.

Heterocyclic compounds are pervasive amongst anticancer, and anti-microbial and other categories of bioactive agents.[2] Some examples of bioactive heterocycles are shown in **Table 1**.

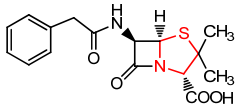
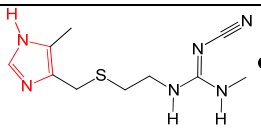
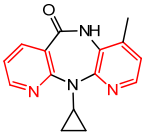
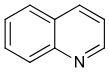
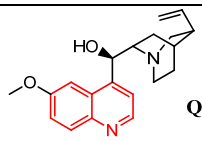
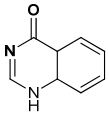
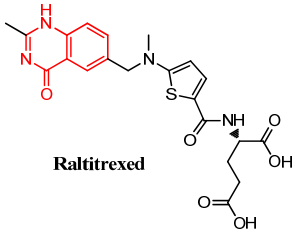
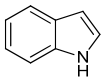
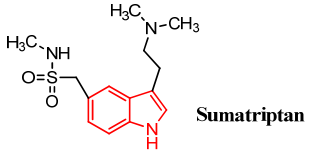
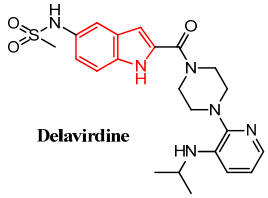
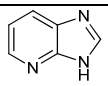
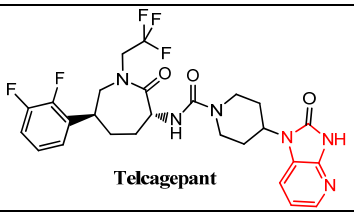
Heterocyclic pharmacophore	Pharmacological activities	Structure of drug
 Thiazole	Anti-bacterial [3]	 Pencillin G
 Imidazole	Treatment for analgesic,[4] anti ulcer,[5] anti cancer [6-7]	 Cimetidine
 pyridine	Anti-HIV [8]	 Nevirapine
 Quinoline	Anti-inflammatory, anti-malarial [9]	 Quinine
 Quinazoline	Anti-cancer [10]	 Raltitrexed
 Indole	Treatment for migraine headache [11]	 Sumatriptan
	Anti-HIV [12]	 Delavirdine
 Imidazo[4,5-b]pyridine	Treatment for migraine headache.[13]	 Telcagepant

Table 1 Some heterocyclic compounds with pharmacological activities.

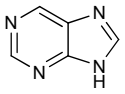
A variety of heterocyclic ring types are pharmacophoric groups in many drugs with various medicinal activities.

Examples of common heterocyclic pharmacophores include thiazoles, imidazoles, pyridines, quinolines, quinazolines, imidazo[4,5-b]pyridines, indoles, benzimidazoles, and related systems as shown in **Table 1**.[2]

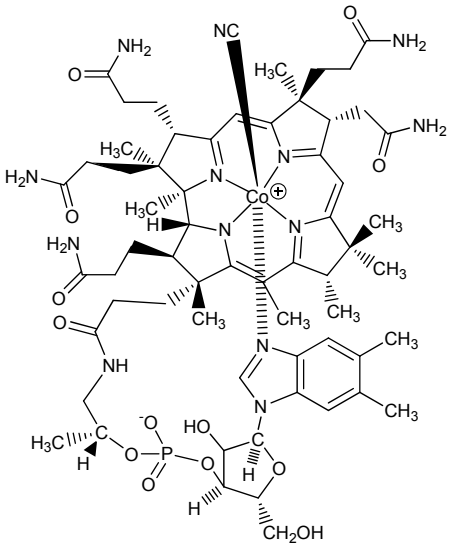
1.2 Benzimidazoles: biological occurrence and medicinal chemistry

Amongst heterocyclic pharmacophores, the benzimidazole ring system is quite common. These substructures are often called 'privileged' due to their wide recurrence in bioactive compounds. Although there is great interest in benzimidazole ligands and structural chemistry, the main interest is in their biological activities.

The early 1950s was an important period regarding discovery of the biological significance of benzimidazole-containing structures and the closely-related purines (**Figure 1**). The 5,6-dimethyl-1-(α-D-ribofuranosyl)benzimidazole ring system was discovered in 1948 as the an integral part of the structure of vitamin B12 [14] (**Figure 1**).



Purine



vitamin B 12

Figure 1 Some imidazole containing bioactive compounds

Subsequently pharmaceutical, veterinary and agrochemical products were discovered including thiabendazole, cimetidine, azomycin, metronidazole, misonidazole, and

chlotrimazole, antihistamines, astemizole and the anti-ulcerative omeprazole.[15] Benzimidazole-based drugs exhibit a wide range of different biological activities as a result of changing the groups on the core structure, as shown in **Figure 2**. These biological activities include anti-cancer (**1**),[16] bactericidal (**2**),[17] fungicidal (**3**)[18-19] analgesic (**4**) [20] and anti-viral properties (**5**).[21] Some have cardiovascular applications (**6**) [22] while some derivatives have been synthesized and evaluated for inhibition of HIV-1 infectivity.[23] Some benzimidazoles (dimers or oligomers) have been shown to have cytotoxic activity in several human tumor cell lines.

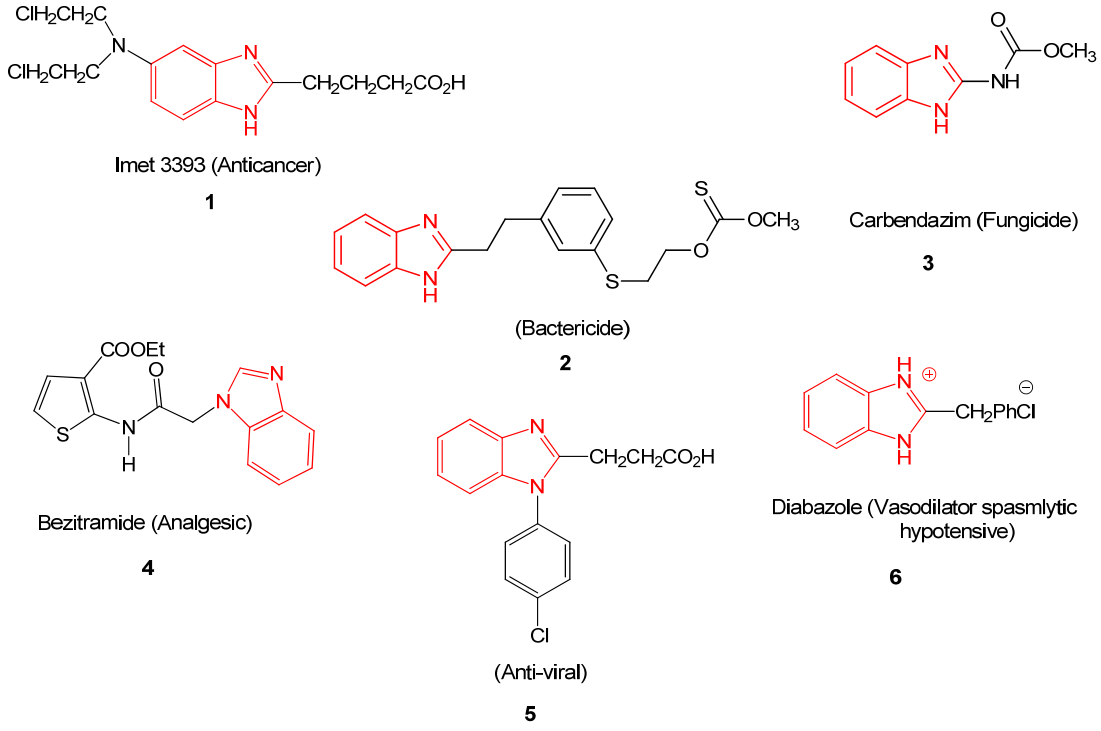


Figure 2 Some benzimidazole containing drugs

1.3 Tautomerism and amphoteric character [24] of N-H benzimidazoles

Benzimidazole (**7**), as the name implies, is a bicyclic ring system in which benzene has been fused to the 4-and 5-positions to imidazole ring (**8**) (**Figure 3**).

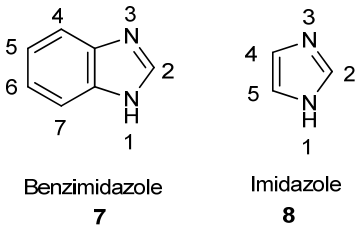


Figure 3 Imidazole and benzimidazole structures

Tautomerization occurs through intermolecular processes either involving two or more benzimidazole molecules or through interactions with a protic solvent such as water. This renders the 5- and 6- positions, and any group at that position in the ring system, chemically equivalent.

Structure **7** shows the systematic numbering of benzimidazole ring system, although benzimidazole is depicted in **7** as possessing the proton at N1, there actually exists a rapid exchange between the –NH- and =NH-nitrogen atoms, and two tautomers **7** and **7'** may be drawn for benzimidazole molecules (**Figure 4**).

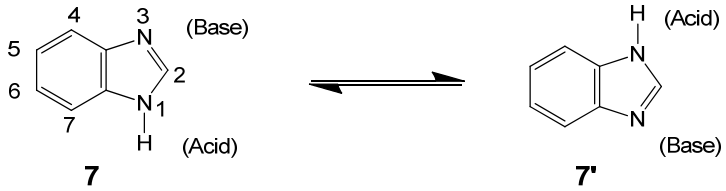
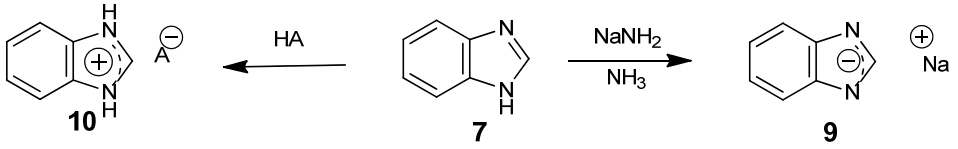


Figure 4 Tautomerism in N-H benzimidazole.

The pseudo-acidic character of benzimidazole (**7**) and its derivatives is reflected in the ability of such compounds to form metallic salts, such as sodium salt (**9**), which forms when sodium amide is added to a solution of benzimidazole in ammonia (**Scheme 1**).



Scheme 1 Acidic and basic behaviour of benzimidazole

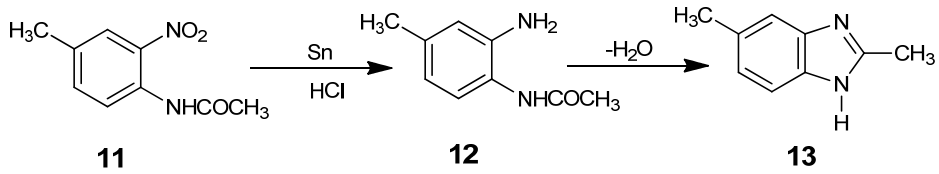
Electronegative groups on the benzimidazole ring increase its acidic nature, allowing a number of bases to react with it. Nitrobenzimidazoles, for example, are strong enough acids to dissolve in sodium carbonate or aqueous ammonia.

Benzimidazoles are also basic compounds having the ability to form salts (**10**) with acids (**Scheme 1**). The basic properties result from the ability of nitrogen to accept a proton.

As a result of the conjugation between the imidazole and benzene ring, benzimidazole (pK_b of 5.4 [25]) is a weaker base than imidazole pk_b of (6.95 [25]). This conjugation increases the number of contributing resonance structures, thus increasing the chemical stability of the molecule. At the same time, by removing electrons from the nitrogen, it decreases its proton affinity.

1.4 Synthesis of N-H benzimidazoles

The first benzimidazole was prepared in 1872 by Hoebrecker,[26] who obtained 2,5-dimethylbenzimidazole (**13**) by the reduction and dehydration of 2-nitro-4-methylacetanilide (**11**) (**Scheme 2**).



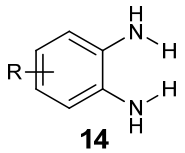
11 **12** **13**

Scheme 2 Synthesis of 2,5-dimethylbenzimidazole

A number of different synthetic approaches have been reported; some of the methods used for their preparation are summarized below.

1.4.1 Condensation reactions of *o*-phenylene diamines

Almost all syntheses of benzimidazoles start with benzene derivatives possessing nitrogen-containing functions ortho to each other (**14**); of the type in **Figure 5**.

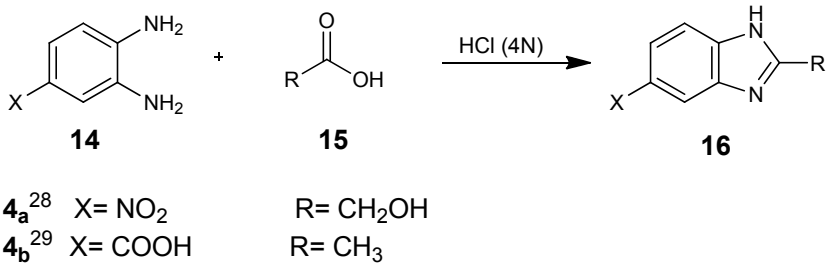


14

Figure 5 ortho di nitrogen compounds

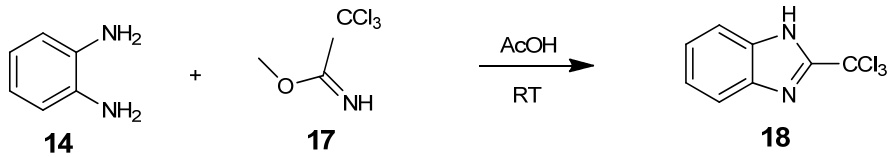
The most commonly used (Phillip's method),[27] involves the condensation of *o*-diaminobenzenes (**14**) with carboxylic acids (**15**) or its derivatives, including heating the reagents together in the presence of concentrated hydrochloric acid (**Scheme 3**).

This is the most common synthetic method for preparation of a wide range of benzimidazoles.



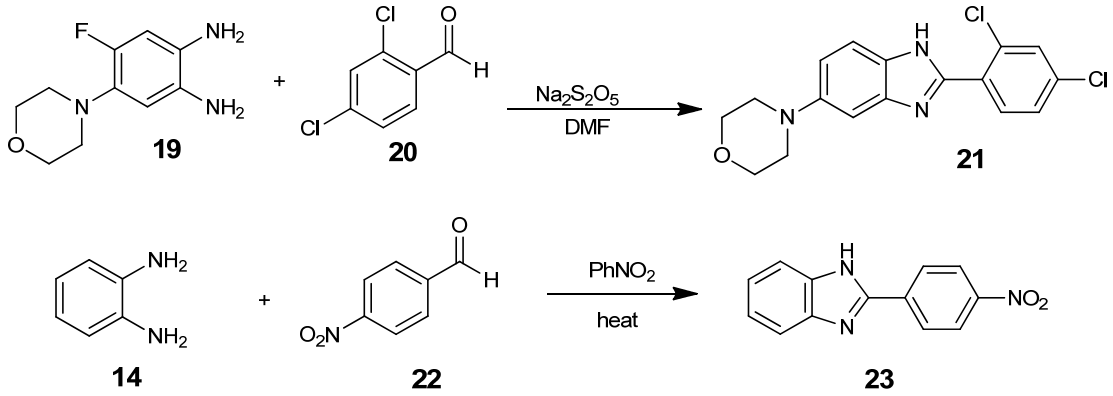
Scheme 3 Condensation of *o*-phenylenediamine and carboxylic acid

The reaction of the appropriate imidate ester (trichloroacetimidate) (**17**) with *o*-phenylenediamine (**14**) or its salt gives the 2-trichloromethyl benzimidazole (**18**) (**Scheme 4**) only at room temperature, and this is an important precursor for 2-carboxylic benzimidazoles.[30]



Scheme 4 Methylacetimidate condensation with phenylenediamine

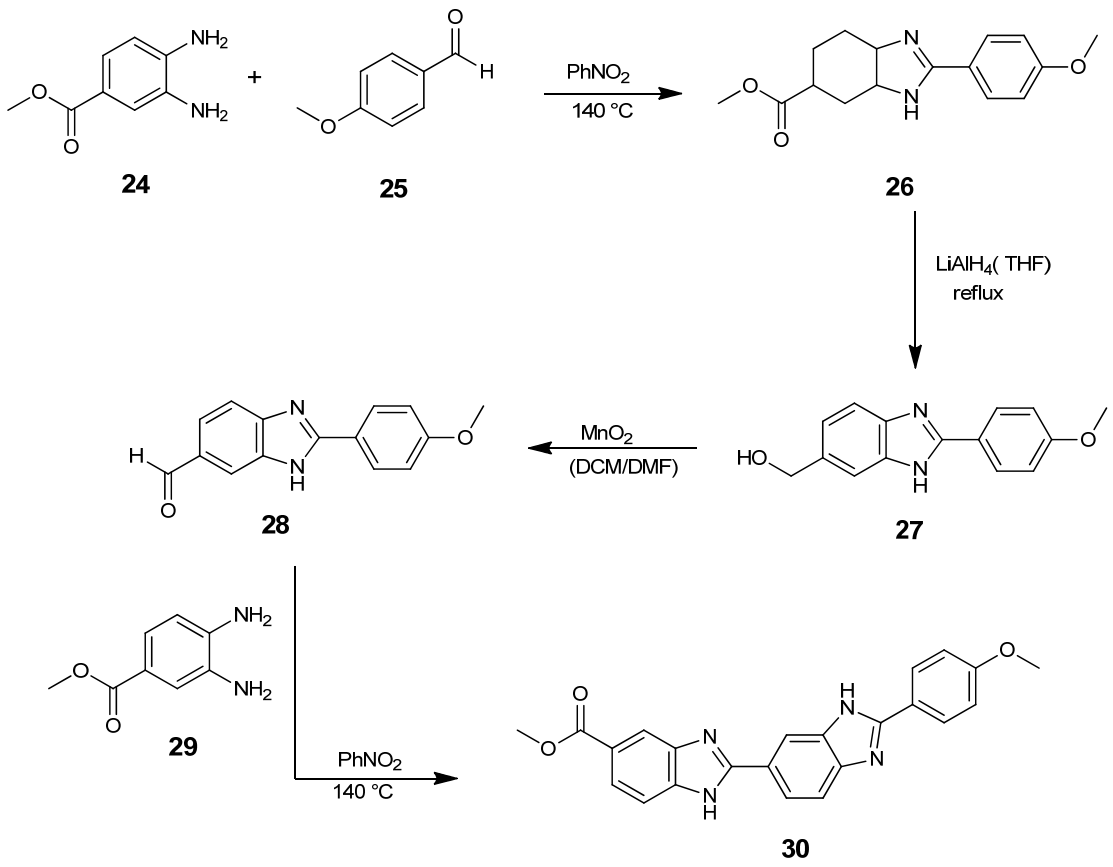
The condensation of phenylenediamine (**19**) and (**14**) with aldehydes (**20**) and (**22**) is achieved by various reported conditions. As shown in **Scheme 5**, this can be achieved in the presence of sodium metabisulphite,[31] or heating in the presence of nitro benzene.[32]



Scheme 5 Synthesis of benzimidazole *via* condensation of aldehydes and phenylenediamine in different oxidizing reagents, $Na_2S_2O_5$, and $PhNO_2$

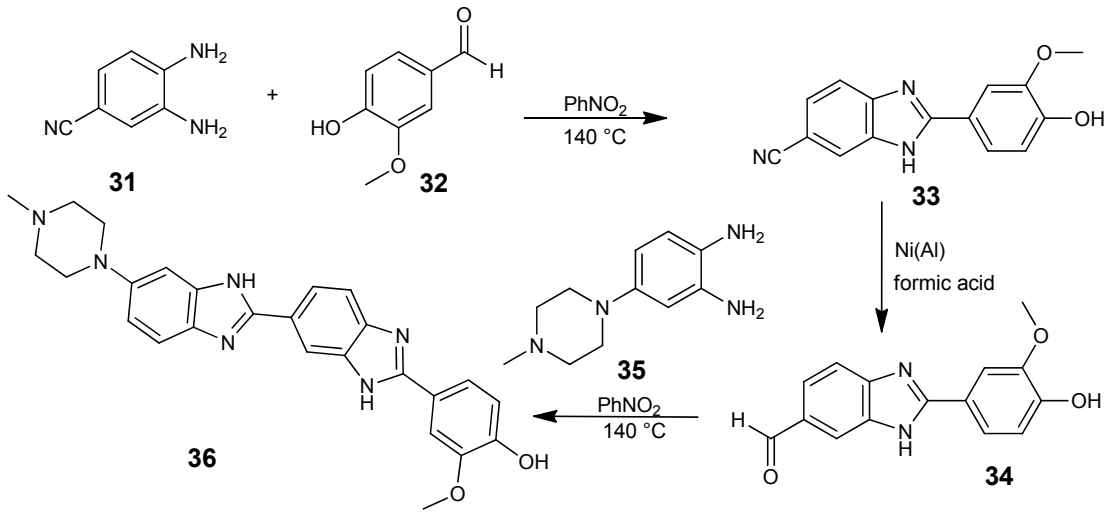
A number of bis(benzimidazoles) have synthesized by using different methods. Lown *et al* [33] have reported a synthesis of novel bisbenzimidazole (**30**) by condensation of 4-ester-*o*-arylenediamine (**24**) and benzaldehyde (**25**) derivatives,

leading to monomeric benzimidazole bearing ester (**26**) that was then converted to aldehyde by reduction and oxidation. The later was coupled with 4-substituted-*o*-arylenediamine (**29**) to form bisbenzimidazole (**30**) (**Scheme 6**).



Scheme 6 Lown's route for bisbenzimidazole synthesis [33]

Kelly, *et al* [34] have reported the synthesis of bisbenzimidazole (**36**) by condensation of monomeric benzimidazole (**33**) bearing a cyano group which was converted to aldehyde (**34**) by using Ni(Al) in formic acid followed by coupling with substituted-*o*-arylenediamine (**35**) to form bisbenzimidazole (**36**) (**Scheme 7**).

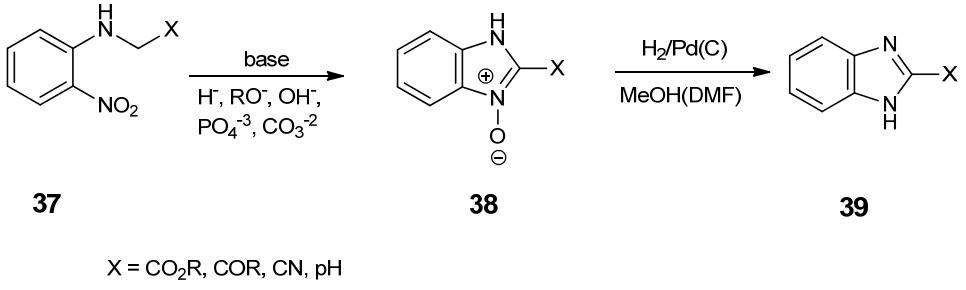


Scheme 7 Kelly's route for bisbenzimidazole synthesis [34]

1.4.2 Base-induced condensation [35]

This approach has utilized substrate groups possessing a relatively acidic proton adjacent to the nitrogen (**37**) *i.e*, X=CO_2R^1, COR^1, CN, or Ph. A variety of R groups have been reported, most typically H, or CO_2R^2, while $R^1$and R^2 are aliphatic or aromatic. The common used bases are hydride, alkoxide, hydroxide, phosphate or carbonate (**Scheme 8**).

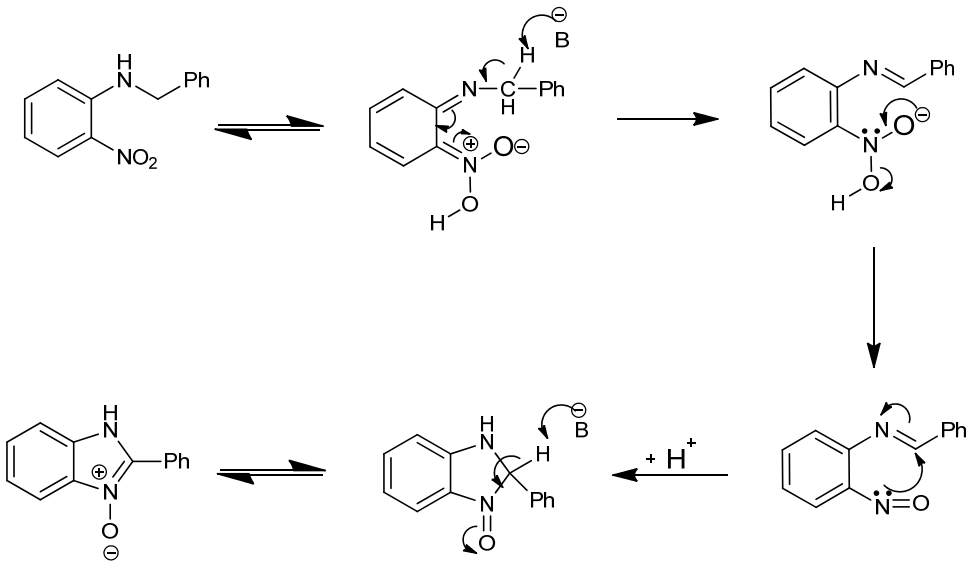
Reduction of of *N*-oxybenzimidazole (**38**) by using H_2/Pd (C) affording the desired benzimidazole (**39**) (**Scheme 8**).



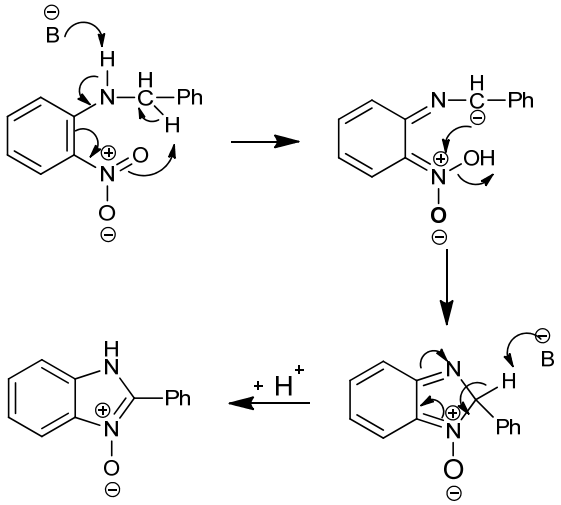
Scheme 8 Base induced cyclization

According to Machin [35] this reaction can occur *via* two different mechanistic pathways, pathway a or pathway b (**Scheme 9**).

Pathway a: -



Pathway b: -



Scheme 9 Two suggested mechanisms for based induced cyclization towards the synthesis of *N*-oxybenzimidazole

1.5 Nucleic acids and benzimidazoles

1.5.1 Background: general structure of nucleic acids

Deoxyribonucleic acid (DNA) forms our genes, and contains all the genetic information for life to develop and evolve from simple bacteria to complex multi-cellular organisms such as humans. DNA is a double stranded polymer, which consists of four monomers, with a backbone comprising phosphodiester chain, with a deoxyribose sugar attached to it. The base attached to the sugar.

There are four different heterocyclic bases in DNA; Adenine (**40**), Guanine (**41**), cytosine (**42**) and Thymine (**43**) (**Figure 6**). RNA (Ribonucleic acid) is a single stranded transcript from DNA. In RNA Thymine is replaced by Uracil. On the other hand, the deoxyribose sugar of DNA is replaced by a ribose sugar. The building block of sugar and base is termed a nucleoside, and when a 5'-phosphate is attached, it is a nucleotide (**Figure 7**).

The double helical structure of DNA was first discovered by James Watson and Francis Crick in 1953.[36] When the two strands come together, they only bond in a certain way with Adenine bonding to Thymine through two hydrogen bonds, and Cytosine to Guanine through three hydrogen bonds (**Figure 8**). The B-DNA form is the physiological form of the DNA double helix. The specific conformational constraints made it clear that the sequence of the bases in the polymer encodes the genetic information for the synthesis of proteins. The conformation of 'real' DNA, however, deviates slightly from the B-form in a sequence-dependent manner as well as depending on the interaction with DNA-binding proteins.

The two strands in DNA run parallel to each other through inter-strand hydrogen bonds, 5'-end on one side and the 3' on the other. During transcription (coding of DNA to RNA) nucleic acids can only be read by the RNA polymerase enzyme in the downstream direction 5' to the 3' (**Figure 9**).

Chemical instructions for protein synthesis in our body are encoded by the linear arrangement of the bases in our genes. Many diseases are the result of aberrant gene expression. Designing compounds to regulate and study the process of gene expression and organization is an area of vast interest, especially in the area of small molecules as gene regulators.

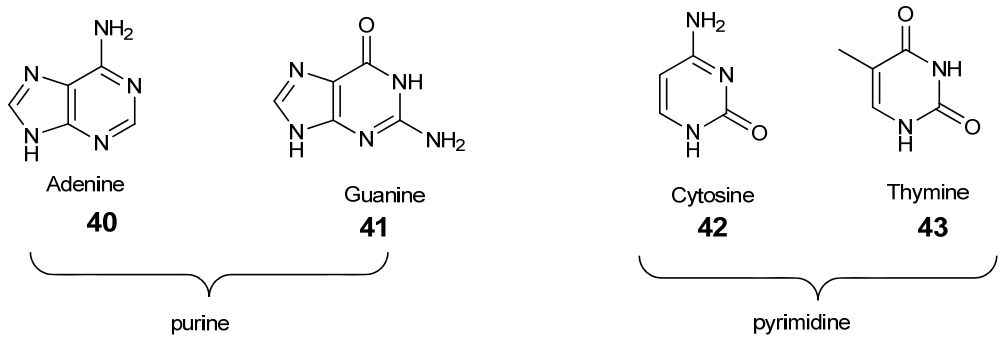
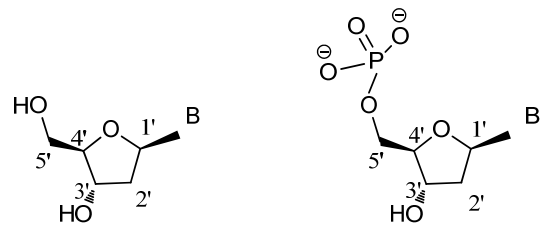


Figure 6 The four DNA bases



Deoxyribo-nucleoside **44**

Deoxyribo-nucleotide **45**

Figure 7 General structures for nucleosides and nucleotides

1.5.2 Major and minor grooves of DNA

In the double helix of DNA, there are minor and major grooves winding along the helix surface. The phosphate backbone in the double helix is closer together on one side of the helix than the other. As shown in figure 9 and 10, the smaller gap is called the minor groove and the major groove is the bigger gap, with the major groove being approximately 50% bigger than the minor groove. The grooves are defined structurally by the orientation of the base pairs, such as the N7 atom of the purine ring and C5 atom of the pyrimidine ring facing out into the major groove. Both grooves are lined with hydrogen donor (arrows out) and hydrogen acceptor sites (arrows in) (**Figure** 8).[37]

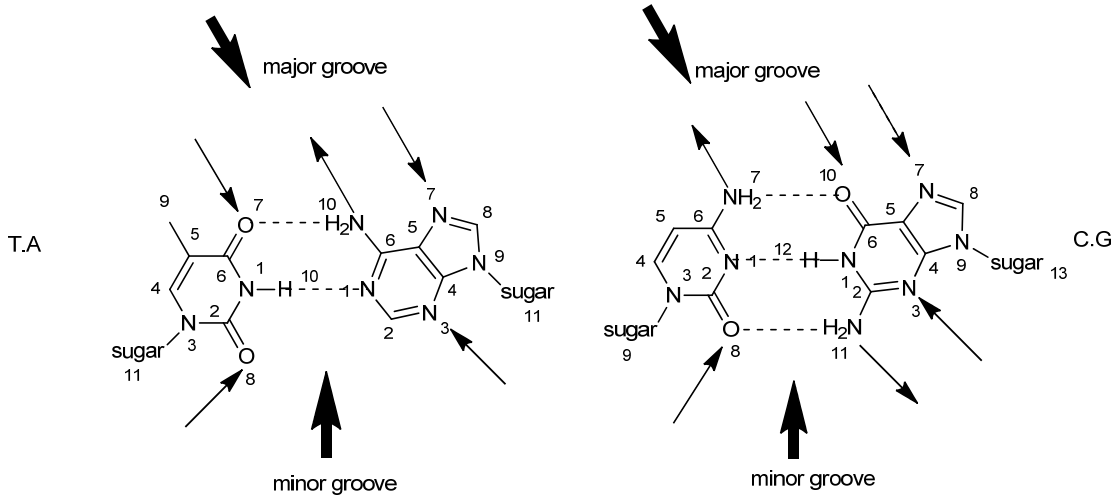


Figure 8 major and minor groove among the nucleic acid bases and hydrogen bonding sites

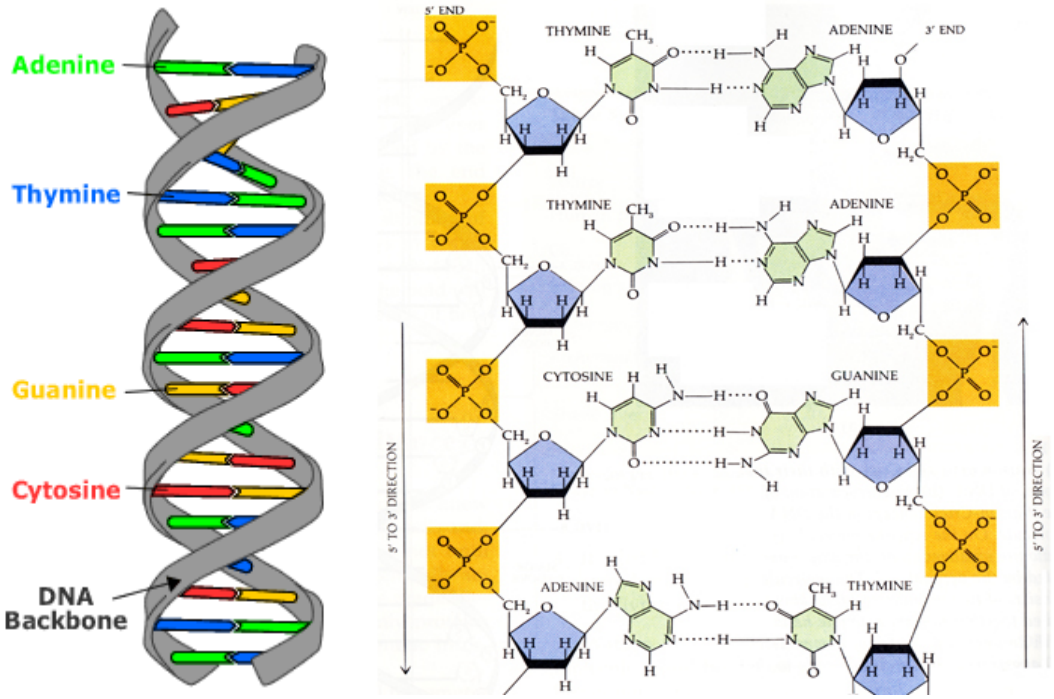


Figure 9 DNA Watson-Crick base-pairing and free H-bond sites [#]

1.5.3 DNA-drugs interactions

A number of drugs with various biological effects, exert their actions on either DNA or RNA *via* various mechanisms. These include many anti-neoplastic (anti-tumor), antifungal drugs, numerous anti-parasitic agents, antiviral compounds, and some antibiotics.

DNA is the basis for life. It is one of the three main classes of biopolymer (protein, polysaccharide and nucleic acid). DNA, as the carrier of genetic information is a major target for some drugs which interfere with DNA replication, a major step in cell division and growth, which is central for tumourigenesis and pathogenesis of diseases. Some other drugs also interfere with transcription (gene expression and protein synthesis).[38] Examples of DNA-drugs interactions are as follows:-

1.5.3.1 Intercalation:

Intercalation into DNA (insertion between a pair of base pairs) is a very important process, especially with regards to the function of many anticancer drugs.[39]

[#] (a) Taken from http ://evolution.berkeley.edu/…/ lllC2ReviewDNA.shtml ; (b) taken from www.mun.ca/.../BlOL2060/ BlOL2060-18/CB18.html

Intercalation results from insertion of a planar aromatic substituent between DNA base pairs, with concomitant unwinding and lengthening of the DNA helix.[39]

DNA intercalation of heterocyclic aromatic compounds has been an interesting subject due to their potential as cytostatic reagents and as probes in molecular biology.[40] Intercalation of DNA was originally explained by Lerman,[41] as the intercalator is sandwiched between two adjacent base pairs and lengthens the helix by 3.4 Å corresponding to van der Waals thickness of an aromatic intercalator, and simultaneously resulting in an unwinding of the helix (**Figure 10**).

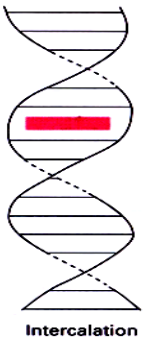


Figure 10 Drugs intercalation into DNA

These intercalating drugs are capable of slipping between the layers of nucleic acid base pairs and disrupting the shape of the double helix therefore preventing replication and transcription.

These drugs must be flat in order to fit between the base pairs, and must therefore be aromatic or heteroaromatic in nature. Some drugs prefer to bind to the helix *via* the major groove, whereas others prefer access *via* the minor groove (**Figure 11**).

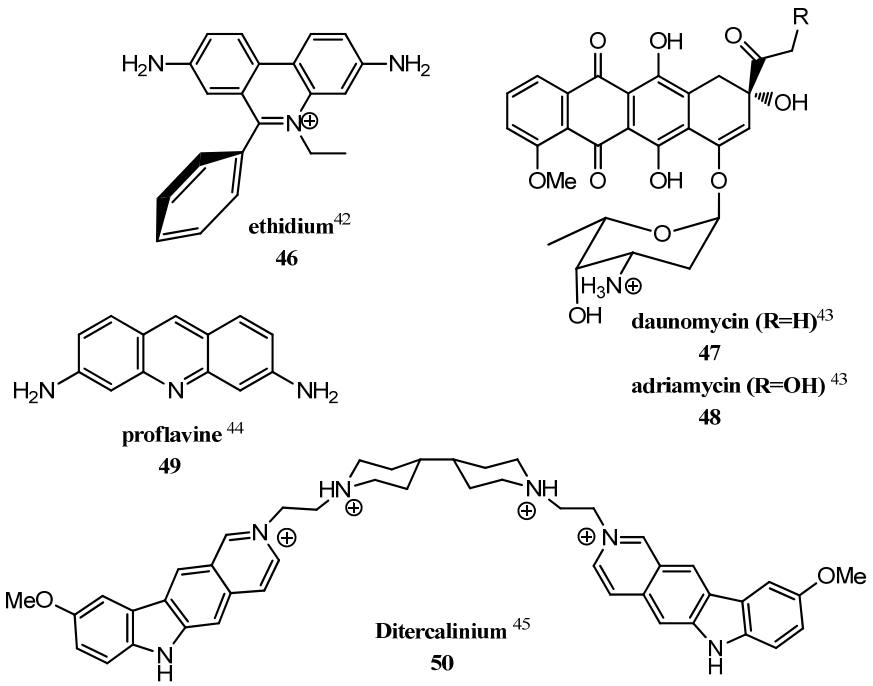
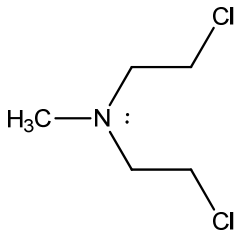


Figure11 Some of intercalating drugs

The static interaction between DNA and several interacting molecules has been studied and confirmed by X-ray diffraction.[46] This interaction is not random, as some compounds intercalate from the major groove of the helix whereas others (Hoechst benzimidazoles) do so only from the minor groove of the helix.[36]

1.5.3.2 Alkylating drugs

These drugs include chemically reactive species which act as alkylating agents, which have a highly electrophilic character and the ability to react with nucleophilic base pair, often A or G amino groups, to form strong covalent bonds (**Figure 12**).

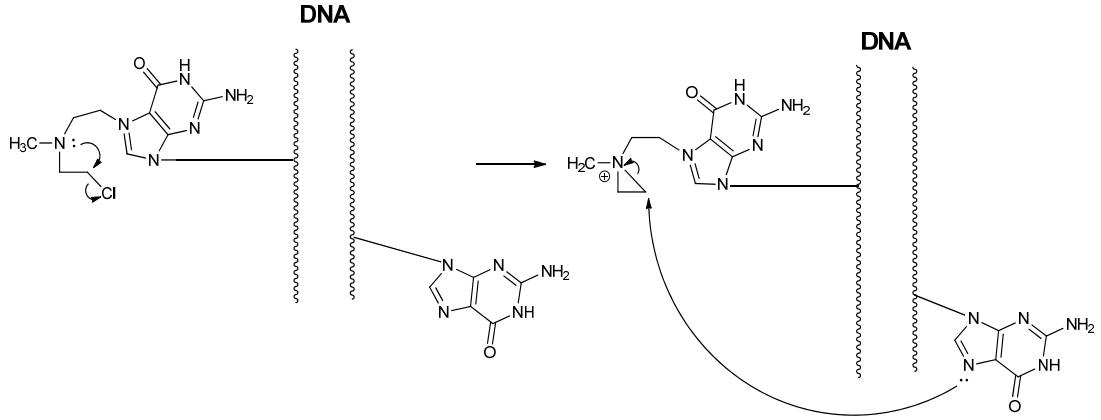


Mechlorethamine [47]

51

Figure 12 Alkylating drug

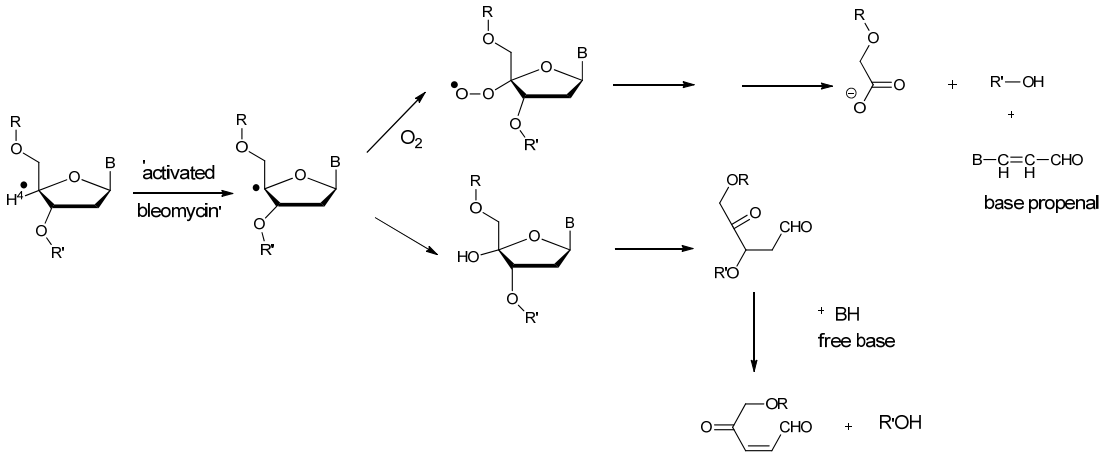
Drugs with two such alkylating groups react with nucleophilic groups in DNA and in particular the 7-nitrogen of guanine on each chain and cross-link the strands so that they cannot unravel during replication or transcription (**Scheme 10**).



Scheme 10 Nitrogen mustard as an example of cross linking alkylating agent

1.5.3.3 Chain cleavage drugs

Another type of chemical interaction includes the chain cleavage drugs. These drugs are able to cut the strands of DNA by radical abstraction of hydrogen atoms from DNA, then preventing the enzyme DNA ligase from repairing the damage. The resultant radicals react with oxygen to form peroxy species, which then fragment. Bleomycin-Fe(II) [48] is a metal-binding drug which acts as radical H abstractor . Strand cleavage occur with or without additional oxygen single strand cleavage [49] (**Scheme 11**).



Scheme 11 Example of chain cleavage drug [49]

1.5.4 DNA groove binders

They modify DNA action by either binding to the major groove; or by binding to the minor groove. Binding to one of the grooves usually alters the conformation of the double helix therefore the other groove is affected. They can be more sequence selective than simple intercalators, with possible multi-base pairs recognition. They typically have torsional twist (**Figure 13**).

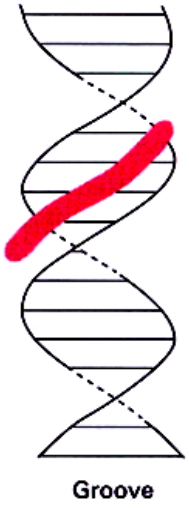


Figure 13 Drugs that are groove binders

1.5.4.1 Major groove binders-oligonucleotides

Oligonucleotides are among the compounds designed for artificial regulation of gene expression. A deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequence composed of several covalently linked nucleotides. Oligonucleotides are classified as deoxyribooligonucleotides or ribooligonucleotides. Fragments containing up to 50 nucleotides are generally termed oligonucleotides, and longer fragments are called polynucleotides. Deoxyribooligonucleotide consists of a 5-carbon sugar called deoxyribose joined covalently to phosphate at the 5′ and 3′ carbons of this sugar to form an alternating, unbranched polymer. A ribooligonucleotide consists of a similar repeating structure where the 5-carbon sugar is ribose (**Figure 14**).

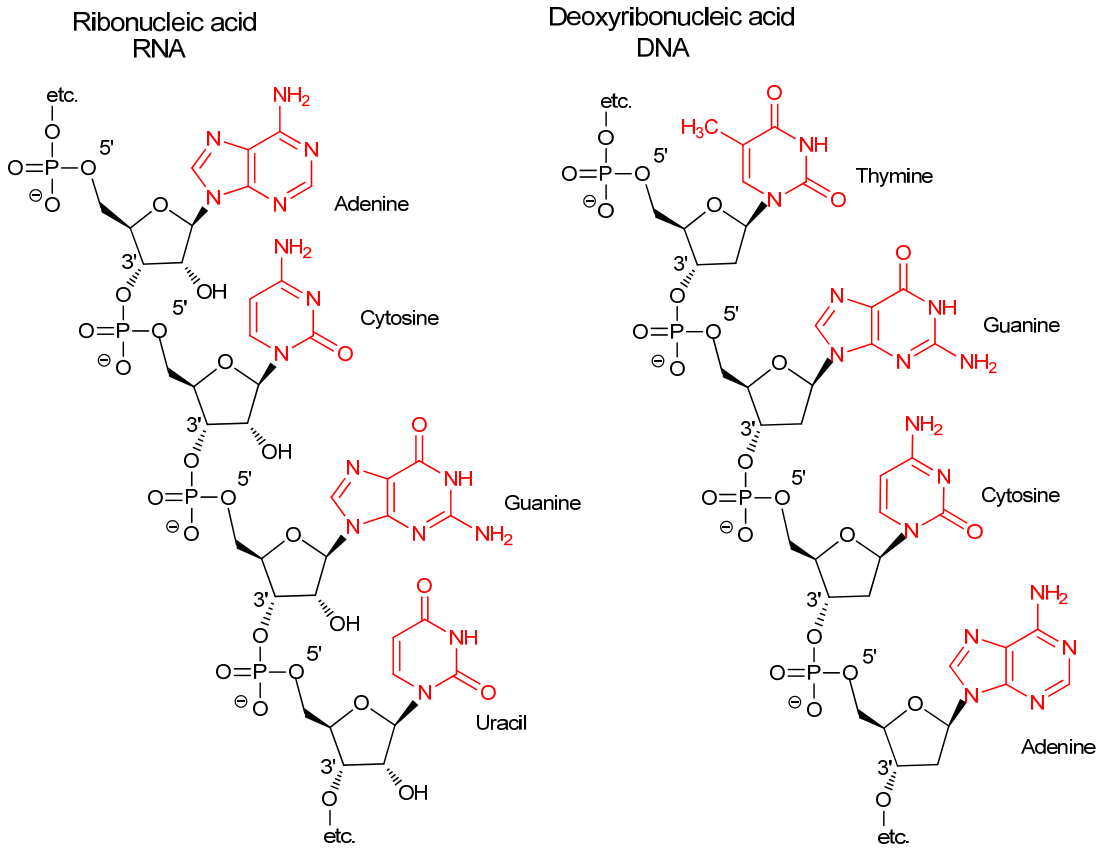


Figure 14 An oligonucleotide fragment containing the four nitrogen bases Adenine, Guanine, Cytosine and Thymine in DNA and Uracil in RNA

oligonucleotides can bind with a high specificity of recognition to the major groove of double helical DNA by forming Hoogsteen type bonds (as shown in the **Figure 15**) with purine bases of the Watson-Crick base pairs, resulting in triple helix formation [50] (**Figure 16**).

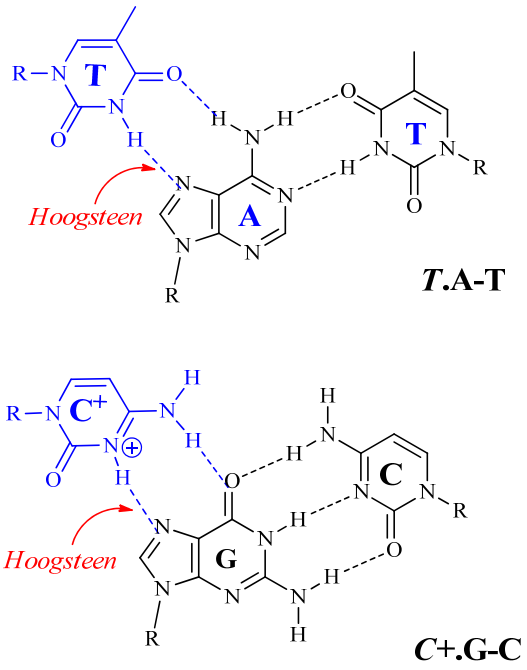


Figure 15 Formation of Hoogsteen type bond for triple helix

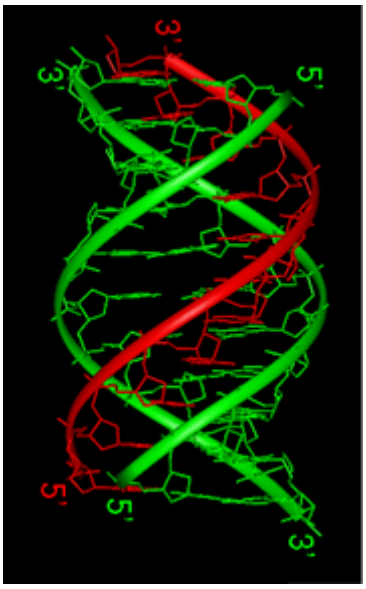


Figure 16 The formation of triple helix [#]

Gene expression can be artificially controlled by synthetic oligonucleotides that bind either to the gene itself or to its messenger RNA. Binding of an antigene oligonucleotide to the major groove of DNA involves the formation of a triple helix. Covalent attachment of an intercalating agent to the third strand oligonucleotide strongly stabilizes the triple-helical complex.

A triple helix can be formed on a single-stranded nucleic acid by an oligonucleotide

[#] Taken from http ://monod.biomath.nyu.edu/~yanglj/pre-research.html

made of two portions: one binds to the target sequence by forming Watson-Crick base pairs **(**see **Figure 8)**, the second one binds to this double-helical region to form a triple-helical complex **(Figure 15)**. These clamp oligonucleotides are able to arrest replication of a single-stranded DNA or reverse transcription of a viral RNA. The antigene and clamp strategies can be adapted to a gene therapy protocol.[51]

Triple helix formation involves the recognition of Watson-Crick base pairs by hydrogen bonding interactions within the major groove of the double helix Oligonucleotides and oligonucleotide analogues can wind around the double helix ; their orientation is dependent on base sequence.[52]

The triplex forming oligonucleotides (TFO) have found use as a major gene targeting tool.[53] They bind to the major groove of duplex DNA and inhibit gene expression. Their use includes to position DNA reactive agents to specific locations in the genome. TFOs are shown to inhibit gene expression providing a possible role for these compounds in cancer therapy. The formation of intermolecular DNA triple helices created the possibility of designing compounds with extensive sequence recognition properties. They may be useful as anti-gene agents or tools in molecular biology. In these structures a third strand oligonucleotide binds in the DNA major groove, making specific contacts with substituents on the exposed faces of the base pairs.[54] They bind in the major groove of duplex DNA at polypurine/ polypyrimidine stretches in a sequence-specific manner.[54-55]

Antisense oligonucleotides

Antisense oligonucleotides are single strands of DNA or RNA that are complementary to "sense" strands of nucleotides. They bind to and prevent protein translation of certain messenger RNA strands. They have been used in research, and may become useful for therapy of certain diseases. [55- 56] Messenger RNA (mRNA) is a single-stranded molecule used for protein production at the ribosome. Because its sequence is used for translation, mRNA is called a "sense" strand or sense sequence. A complementary sequence to that mRNA is an "antisense" sequence.

Antisense RNA is currently being investigated as a human therapy for certain forms of cancer. The goal is to use gene therapy techniques to insert an antisense gene into tumor cells.[57]

Antisense DNA strands can also be made (not in the double helix, but in the side of the DNA that is transcribed) (**Figure 17**). Short antisense strands of DNA can be introduced into cells, which then bind with target mRNA and prevent translation to protein.

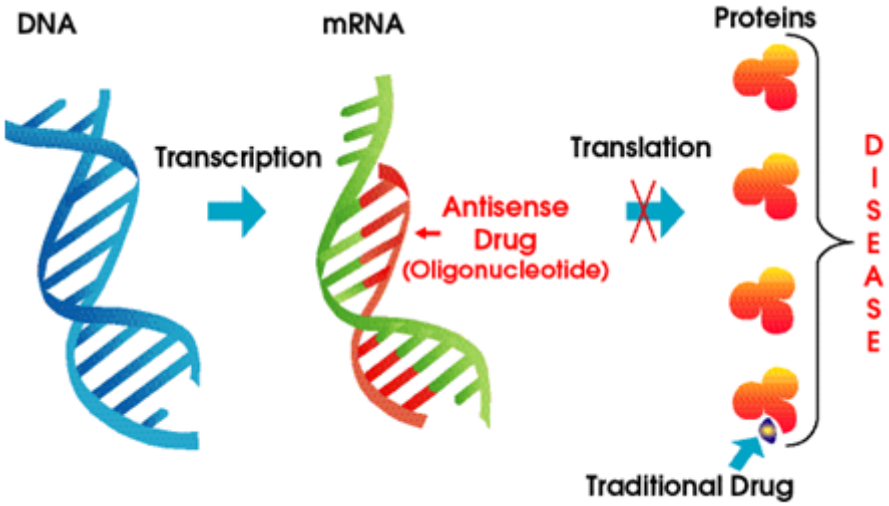


Figure 17 Antisense drug to DNA [#]

Antisense DNA is currently an approved therapy for cytomegalovirus infections of the eye, under the trade name Vitravene. Vitravene targets two different viral proteins. Antisense DNA is also being explored for therapy of HIV, some cancers, and other diseases. One difficulty in applying this therapy is successfully delivering the antisense DNA or RNA to all target tissues (for instance, making sure the antisense strands reach infected blood cells for HIV).[56, 59]

Modification of oligonucleotides

During the last decade there has been an upsurge of interest in the chemistry and biology of nucleic acid analogues. This has mainly been due to the realization that modified oligunucleotides, with improved properties, can be used in the sequence specific control of gene expression.

Chemical modification of oligonucleotide came to prominence in the early 1990s with the advent of the antisense approach to control gene expression.[60] The antisense field has suffered to some extent from unrealistically high expectations for clinical applications. Oligonucleotides carrying other chemical moieties besides the normal

[#] Taken from http ://employees.csbsju.edu/…/bind/oldrug devel.html

bases are called “modified oligonucleotides”. Many synthesis strategies for modifications have been developed to aid delivery, localization and imaging of modified oligonucleotides. The main development is in the chemistry and biology of modified oligonucleotides, including recent examples of chemical modifications.

These modifications can be in the backbone, sugar and/or nucleobase as well as derivatisation or conjugation of the modified oligonucleotide. This can improve several longstanding problems associated with using native nucleic acid, such as nuclease resistance, specificity, affinity, bioavailability, biodistribution, cell targeting immune response [61] (**Figure 18**).

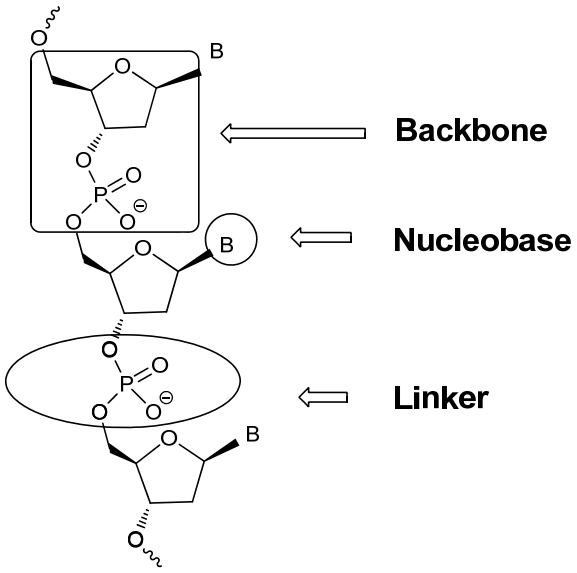


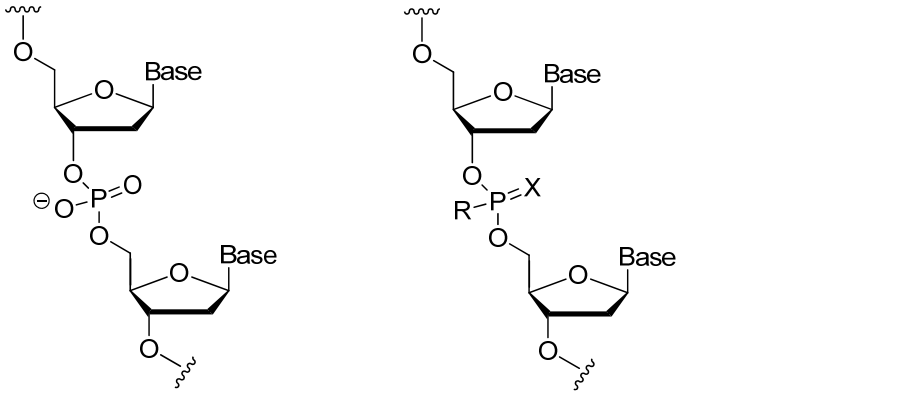
Figure 18 Chemical modification of oligonucleotides including The backbone, sugar and/or nucleobase of the nucleotide

Nucleic acids have been extensively modified by replacing the phosphodiester group, with alternative anionic, neutral and cationic structures. Several of these modified oligonucleotides exhibit improved properties including enhanced recognition and binding to RNA, duplex DNA and proteins. This has resulted in the development of new and more potent antisense and antigene agents. Furthermore, backbone modified oligonucleotides have also been used in the development of several alternative strategies.[60]

Modification of the backbone

Among the very first modifications made to oligonucleotides were alterations of the phosphodiester back bone to stabilize the oligonucleotides towards enzymatic

degradation within cells. Backbone modifications are still being explored (**Figure 19**).[61]



52 Phosphodiester

53 Phosphonoformate X = O , R = CO_2H
54 Phosphonoacetate X = O , R = CH_2CO_2H
55 Thiophosphonoacetate X = S , R = CH_2CO_2H

Figure 19 The natural Structure of phosphodiester and backbone modification by either phosphonoacetate, phosphonoformate and thiophosphonoacetate [61]

A number of studies have focused on modification of the native phosphodiester linkage in the backbone of oligonucleotides. For example, phosphonoacetate, phosphonoformate and thiophosphonoacetate are phosphodiester modifications (**Figure 19**).[61]

One of the most extensive contributions in this area has come from De Mesmaeker and co-workers who have synthesized neutral dinucleotide analogues with many different linker groups including complete replacement of phosphodiester linkage of oligonucleotide. Many moieties that are not chemically similar to phosphodiester, such as amide has been used to replace the phosphodiester linkage. In addition to amide other neutral modifications that are well accommodated with nucleic acid duplexes include acetal group. Only a few modified oligos have been reported with positively charged backbones replacing the native backbone where the phosphodiester group is replaced by a positively charged guanidinium group (**Figure 20**).[60]

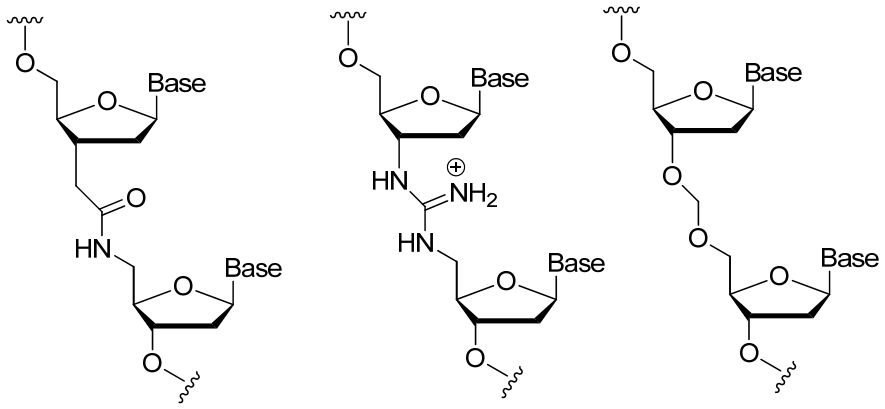


Figure 20 Replacement of phosphodiester linkage of oligonucleotides by either amide, guanidine or acetal linkers [60]

Modification of the ribose unit

Modification of the ribose unit in DNA and RNA profoundly influence biological properties of the nucleic acid .

Conformational restriction of the ribose units, as in LNA (Locked Nucleic Acids) (**56**) and tricyclo-DNA (**57**), has been identified as a powerful tool to increase DNA and RNA affinity as well as biological stability and antisense properties. Apart from that sugar modified DNA analogues, as homo DNA (**58**), have shown to be orthogonal base-pairing systems. This property can be used to produce DNA or RNA-diagnostic tools with higher selectivity (**Figure 21**).[62-63]

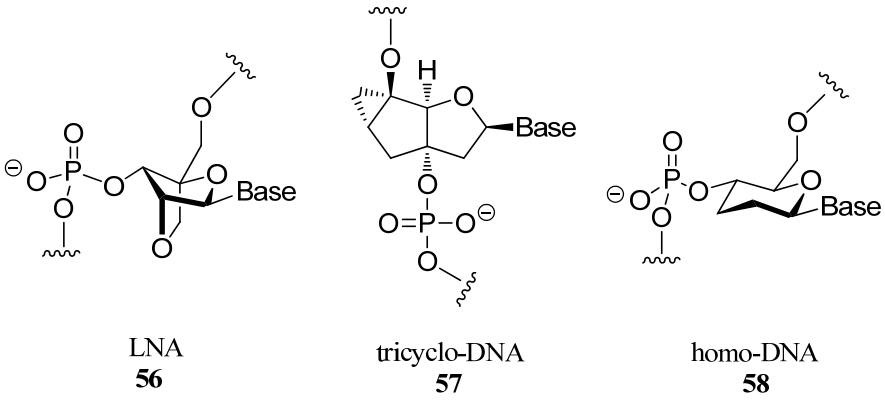
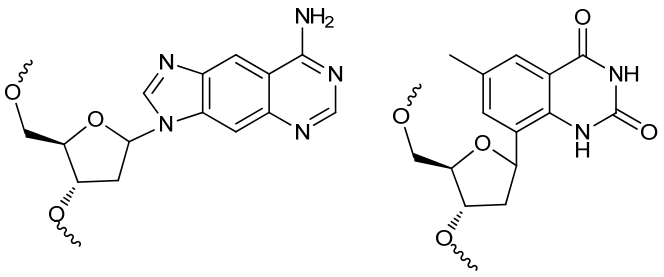


Figure 21 Chemical structures of selected oligonucleotide analogues with sugar modification [62-63]

Modification of the nucleobases

Modifications to the nucleobases have been explored to increase the stability of duplexes and also to increase the scope for targeting duplex DNA through the formation of triple helices with so-called TFO.[64] However , more recently the focus

has been on modification of nucleobases to create artificial DNA systems in which the majority of the native nucleobases have been altered. For example, Kool *et al* developed extended dxA (**59**) and dxT (**60**) nucleobases (**Figure 22**) and incorporated these into oligonucleotides, which formed a double helix with expanded internucleotide distances.[65]



dxA
59

dxT
60

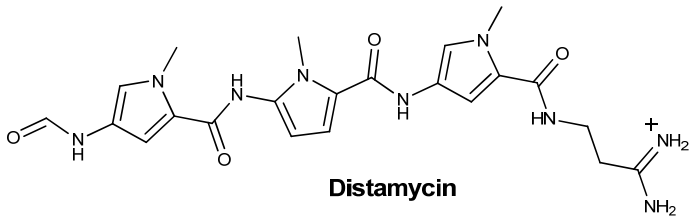
Figure 22 Chemical structures of base modifications by extended dxA and dxT

1.5.4.2 Minor groove binders

The minor groove of the double helix is the site of non-covalent sequence selective binding by a number of small molecules; therefore it is of great interest for developing new drugs.

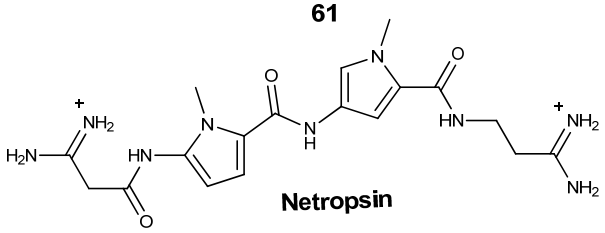
Molecules that bind in the minor groove are usually flat and crescent shaped, so that they fit tightly into the groove.

The natural products distamycin (**61**) and netropsin (**62**) bind to the minor groove *via* H-bonding of the benzimidazole functionality between the nitrogens of A, and the oxygens of T. The dye Hoechst 33258 (**63**) is an example of a synthetic heterocycle which binds in the DNA minor groove. It is a bis(benzimidazole) which is characterized by cationic charge, narrow molecular cross section, and its concave shape [66] (**Figure 23**). The binding preference for the A-T base pairs is due to the closeness of the aromatic H atoms of the Hoechst molecule and the floor of the minor groove. These examples have been the primary leads (leading compounds) in searching for new synthetic DNA minor groove binding heterocycles.



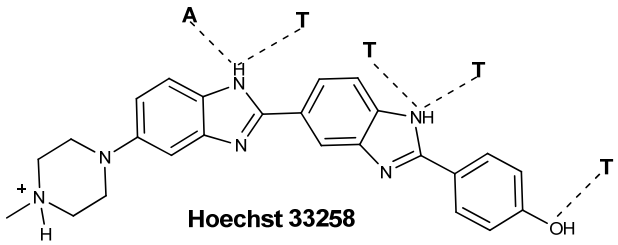
Distamycin

61



Netropsin

62



Hoechst 33258

63

Figure 23 Representatives of minor groove binding drugs [67a] **and interaction of Hoechst 33258 with DNA douplex** [67b, 67c]

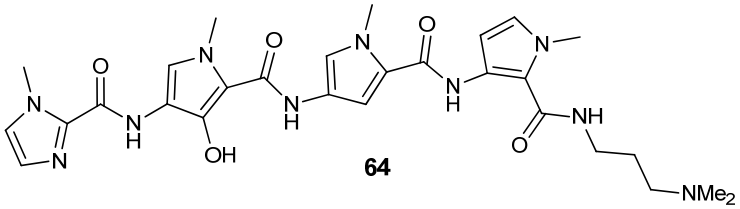
Sequence specific DNA-binding are small molecules that can permeate cells could potentially regulate transcription of specific genes. The leads in **Figure 23** are sequence selective but not specific for particular sequences This has spurred efforts in the development of new minor groove binding agents that could control expression of specific genes, It is possible to specifically inhibit a specific gene's expression and potentially thereby, for example, control the growth of tumour cells. The ideal is designing a drug capable of recognizing a specific sequence in DNA. A series of bis(benzimidazole) compounds which have a head-to-head orientation have been designed as selective DNA minor groove binders. The analysis confirms that the predicted optimal site for the core bis(benzimidazole) motifing of Hoechst 33258 is the four base sequence 5-AATT. This sequence selectivity results in inhibition of transcription at A/T rich sites and may be responsible for cytotoxic and antitumor effects shown by this head-to-head bis(benzimidazole).[36,68]

1.5.4.3 Amide-linked benzimidazoles

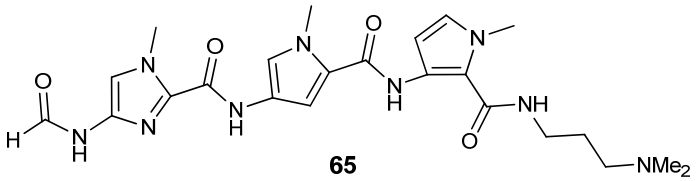
Amides play a key role for medicinal chemists, as the amide functionality is a common feature in many small or complex synthetic and natural molecules. This

amide is of course the core linkage in proteins, and nearly all known enzymes are proteins, all antibodies are proteins, proteins provide mechanical support e.g (collagen). The carboxamide group appears in a significant percentage of known drugs, as carboxamides are neutral, stable and have both hydrogen-bond accepting and donating properties.

Synthesis of Hoechst 33258 and its analogues as non-amide systems in the last three decades was followed by synthesis of polyheterocyclic compounds, such as Dervan polyamides,[40] which have seen considerable interest as sequence selective DNA minor groove binding agents in recent years **(Figure 24)**.



64



65

Figure 24 Two of Dervan's oligopyrrole molecules

Dervan *et al* have succeeded in synthesizing alternative heterocycles for DNA recognition that include a benzimidazole/imidazole pair. These compounds incorporated into DNA- binding polyamides comprised of pyrrole/imidazole pairs in which they not only increase the binding site size but significantly increase the binding affinity of the ligands as well. This benzimidazole-imidazole polyamide represents a novel class of DNA binding ligands.

The experimental results indicated that the benzimidazole–heterocycle building blocks can replace pyrrole-pyrrole, pyrrole-imidazole, and pyrrole-hydroxypyrrole constructs.[40]

The benzimidazole-containing hairpin polyamides represent a novel class of DNA binding ligands.

The hairpin consisted of a methylene group across the backbone of the DNA linking the two anti-parallel dimers together (**Figure 25**).

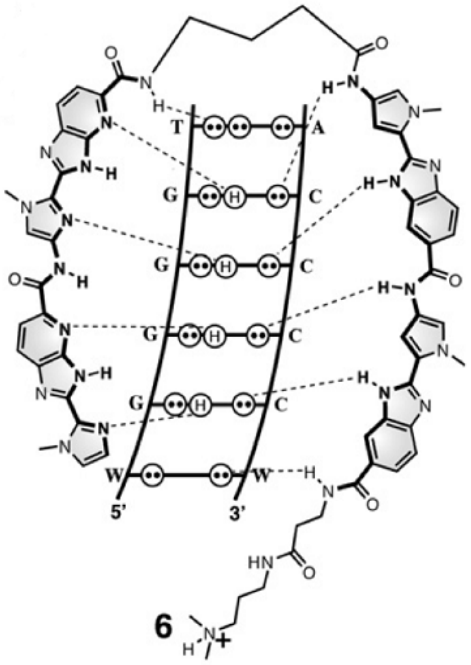


Figure 25 Hairpin structure binding two anti parallel dimers to base pairs in the minor groove. [40]

1.6 Chemistry of dimeric and oligomeric benzimidazoles

The general synthesis of monomeric benzimidazoles can be achieved by either condensation of arylenediamine and aryl carboxylic acids and their derivatives in the presence of strong acid such as mineral acids, or condensation of *o*-arylenediamine and aldehyde in the presence of oxidant such as metabisulfite or nitrobenzene (see **Section 1.4**). The lead compound for all bis(benzimidazoles) is the head-to-tail dimer Hoechst 33258, which has been used as the basis for the preparation of many potential DNA binders (**Figure 26**) shows a variety of benzimidazoles which have been reported to contain bioactive features. A number of studies indicates that benzimidazoles show very good binding activity in the minor groove of DNA and there are examples of such ligands which possess various biological activities.

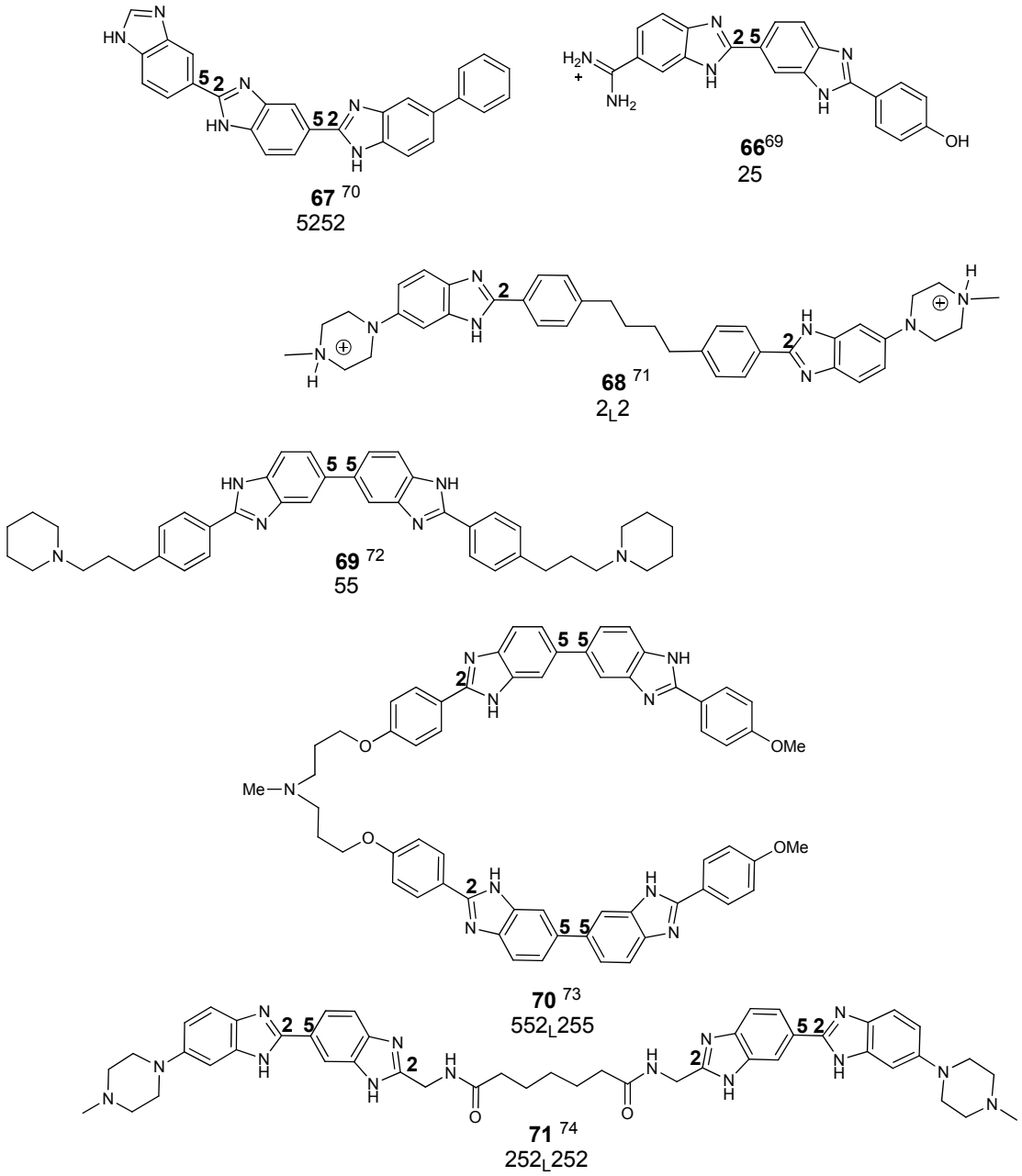


Figure 26 Some selective benzimidazole containing bioactive features

The bis benzimidazole (**66**) is related to the Well-known minor groove agent, Hoechst 33258. Biosensor-surface Plasmon Resonance (SPR) results clearly show that the (**66**) bind to the TTAA sequence, the dimer-binding mode of (**66**) is a new DNA recognition motif and offers a novel design for selective targeting of DNA sequences with minor groove.

The tribenzimidazole (**67**) is a class of anticancer agent that binds in the DNA minor groove of duplex DNA.

An alkyl-linked bis (benzimidazole) (**68**) with the base sequence of two self-complementary DNA duplexes, d(CGCGAATTCGCG)$_2$ and d(CGCAAATTTGC G)$_2$ (A2T2 and A3T3) interact with DNA by intercalation and groove-binding and that binds preferentially in the minor groove of AT regions of DNA oligomers.

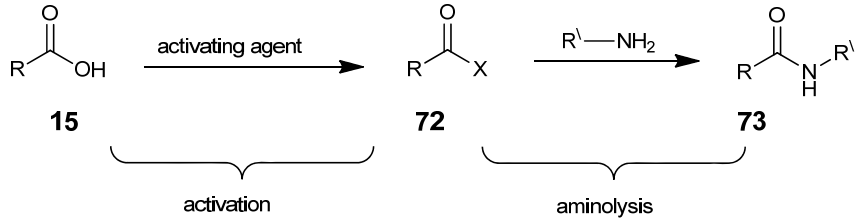
The binding of symmetric bis-benzimidazole (**69**) to the minor groove of DNA has been studied by SPR methods and found that compound (**69**) has enhanced affinity and selectivity for AT sequence in DNA, this compound which easily penetrate into cells to accumulate in the nucleus, represent a novel family of DNA-targeted potential anticancer agents.

Tetramer (**70**) exhibited high sequence-selectivity for four consecutive A/T base pairs in the dodecanucleotide sequence d[CGCGAATTCGCG],which is a model for longer oligonucleotide sequences in the A/T region of the minor groove, it also exhibited sub-micromolar anticancer activity.

Tetramer (**71**) bond to double-strand DNA is an inhibitor of HIV-1 integrase. The design of this compound is an important pharmacological task since chemotherapeutic efficacy of most of the currently used anticancer drugs depends on selectivity and efficiency of their interaction with DNA. These features vary according to monomers (benzimidazole unit), these can be coupled to each other, this is important in the synthesis of dimeric (**66, 68, 69**) and oligomeric benzimidazoles such as trimer (**67**) and tetramer (**70-71**). However these monomers can combine together through linker, such as extended inter-benzimidazole linkers (**68, 70, 71**). Other monomers can bind directly without any linker (**66, 67, 69**).

1.7 Amide formation

Carboxy components (**15**) can be activated as acyl halides, then coupled with an amine to produce an amide (**Scheme 12**).



Scheme 12 General equation for amide synthesis using activating agent

There are different ways of coupling reactive carboxy derivatives with an amine.

- An intermediate acylating agent is formed and isolated then subjected to aminolysis.
- A reactive acylating agent is formed from the acid in a separate step(s), followed by immediate treatment with the amine.
- The acylating agent is generating *in situ* from the acid in the presence of the amine, by the addition of an activating or coupling agent.

1.8 Aims and objectives overall strategy

The aim of this project is synthesis of novel benzimidazole oligomers linked through positions 2 and 5 and the use of such oligomers to evaluate their potential for nucleic acid groove binding (**Figure 27**).

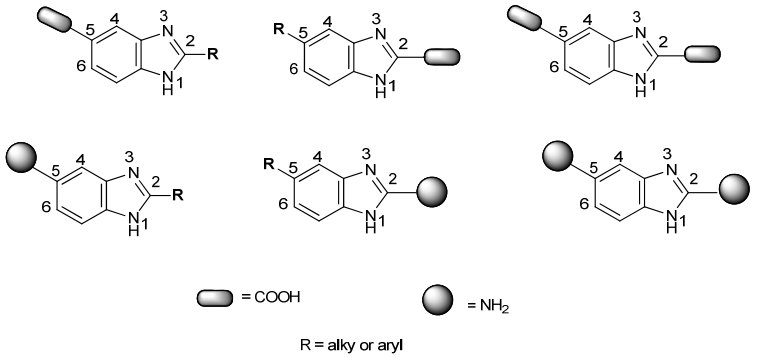


Figure 27 Different novel benzimidazoles with carboxy or amino functionality

So the main aim of this project was to develop and optimize methodolofy to prepare diverse structures, and then determine DNA-binding efficacy to design new ligand synthesis. A range of methods are available to assess DNA binding, such as, footprinting and affinity cleavage, coupled with high resolution definition of structural detail of binding within such sequences by X-ray crystallography, NMR and mass spectrometry. In this project we have initially targeted use of SPR.

1.9 Specific target structures

1.9.1 Amide-linked bis(benzimidazole)

Target structures are illustrated in **Figure 28**. Monomers (carboxybenzimidazole and aminobenzimidazole) can be coupled to each other to form unsymmetrical (**Sections 2.4**) and symmetrical amide-linked bis(benzimidazoles) (**Sections 2.5**).

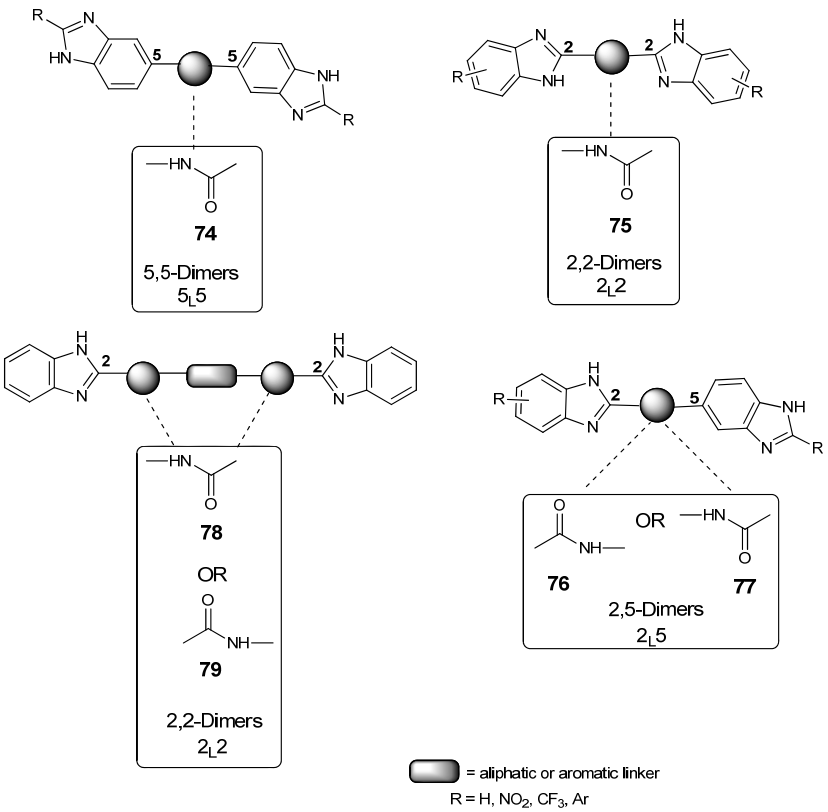


Figure 28 Generic structures of unsymmetrical and symmetrical amide-linked bis(benzimidazoles)

1.9.2 Amide-linked tris(benzimidazoles)

Target structures are illustrated in **Figure 29**. Trimers with various functionality were proposed. In principle, Utilization of the bifunctionality of 2,5-dicarboxybenzimidazole **106** allowed coupling with two equivalents of 2- or 5-aminobenzimidazole affording tris(benzimidazole) with different linkage and orientation (**Section 3.1.1**). Combination of 2,5-diaminobenzimidazole **108** coupled with two equivalents of 2- or 5-carboxybenzimidazole should afford different orientations of tris(benzimidazole) (**Section 3.1.2**). A third set of tris (benzimidazoles) was envisaged starting from bis(benzimidazoles) bearing a nitro group, reduction to the corresponding amines and coupling with 2-carboxy benzimidazole (**Sections 3.1.3**).

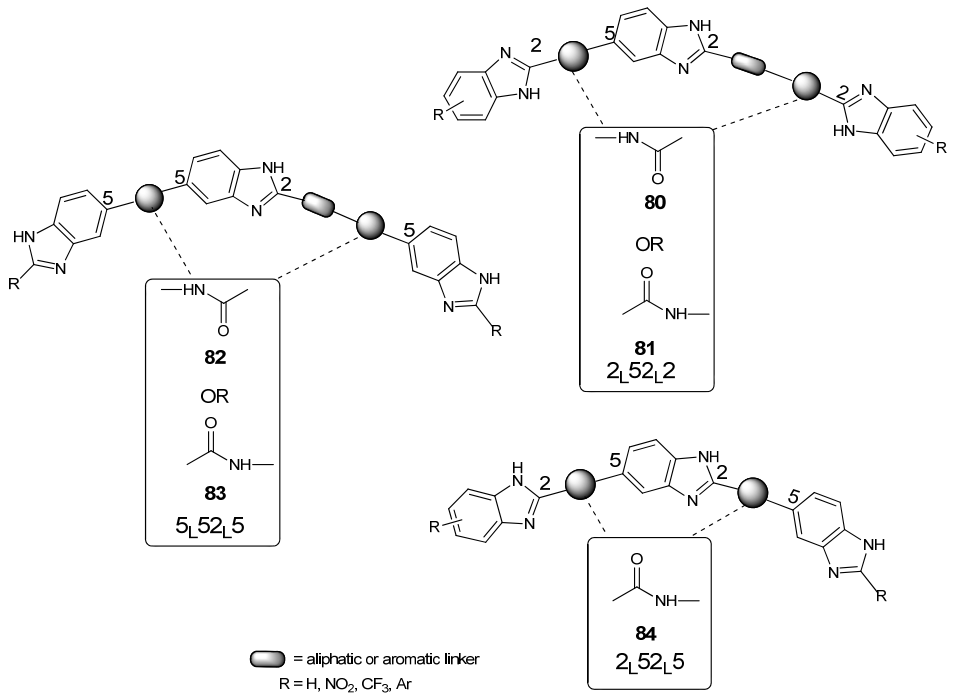


Figure 29 Generic structures of amide-linked tris(benzimidazoles)

1.9.3 Symmetrical amide-linked tetra(benzimidazoles)

Target structures are illustrated in **Figure 30**. A group of symmetrical tetrameric benzimidazoles with different functionalites were proposed. Such tetramers can be envisaged *via* coupling of bis(2-carboxybenzimidazole) **167** with different 2- and 5-aminobenzimidazole **112**, **132**, **101** and **110** (**Sections 3.2.1**, and **3.2.2**), another type of bis(2-carboxybenzimidazole) **166** with 2-aminobenzimidazole **112** (**Section 3.2.3**). Other tetramers (**177** and **178**) were prepared by coupling bis(2-aminobenzimidazole) **169** with 2- and 5-carboxybenzimidazole **125, 116** (**Sections 3.2.4**, and **3.2.5**)**,** another type of bis(2-aminobenzimidazole) **168** was coupled with 2- and 5-carboxybenzimidazole **123** and **115** (**Section 3.2.6**).

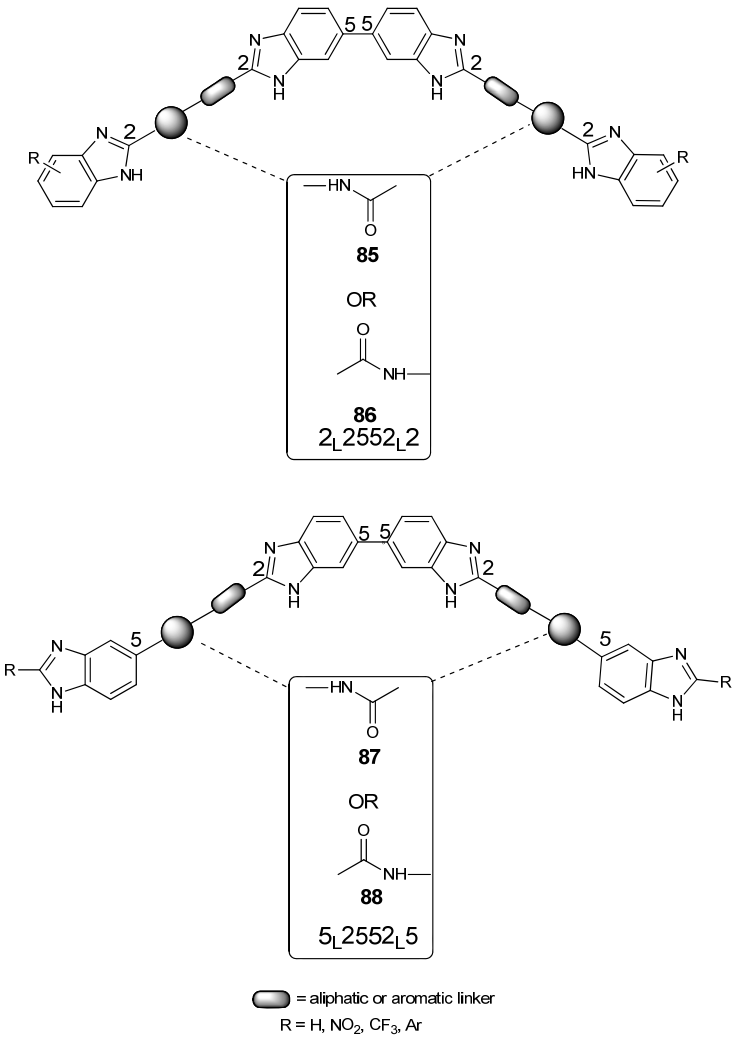


Figure 30 Generic structures of amide-linked tetra(benzimidazoles)

1.9.4 Symmetrical bis(benzimidazole) piperidine derivatives

Target structures are illustrated in **Figure 31**. piperidine derivatives can also be coupled to bis(carboxybenzimidazole) **167** to form symmetrical amide-linked bis(benzimidazoles) (**Sections 3.3**).

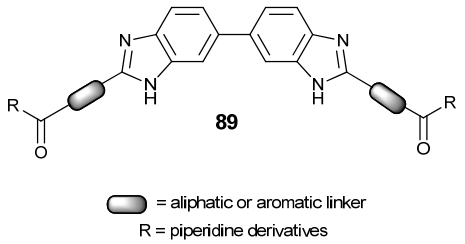


Figure 31 Generic structures of symmetrical amide-linked bis(benzimidazoles) with piperidine derivatives

The targets for this project were symmetrical and unsymmetrical amides and directing (C2-C5) linked, di-, tri- and tetrameric benzimidazoles.

Chapter 2

Synthesis of monomeric and dimeric benzimidazoles

2.1 Synthesis of amino and carboxy1-*H* benzimidazoles

This section discusses the synthesis of benzimidazole building blocks bearing a 5-NH_2 (*via* the nitro), 5-CO_2H, or a group at C2 bearing an CO_2H or NH_2 as the precursors for dimerization and oligomerization (**Figure 32**). Preparation of these derivatives involved condensation of *o*-phenylenediamines with either aldehydes or carboxylic acids to afford the corresponding amino or carboxybenzimidazoles (except 2-carboxy benzimidazole, which was prepared by condensation of *o*-phenylenediamines with trichloroacetimidate followed by hydrolysis).

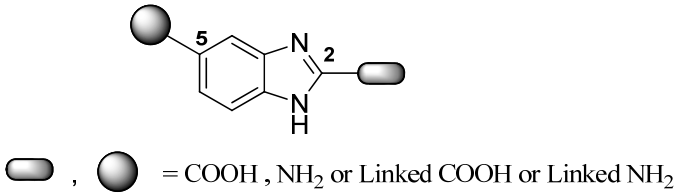


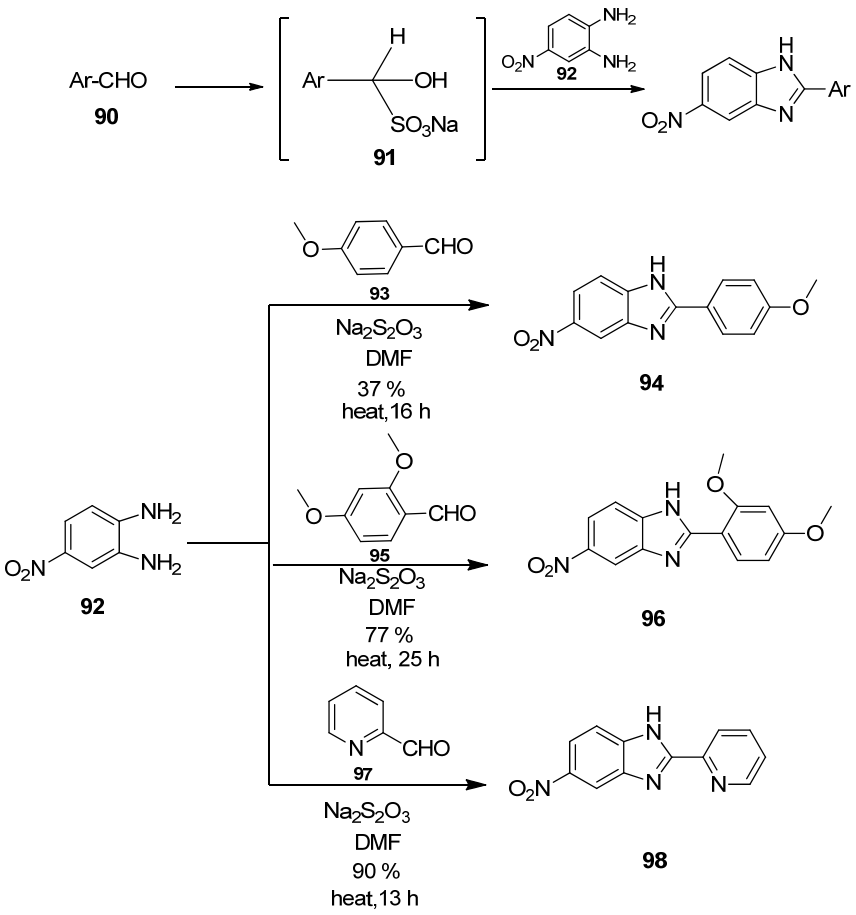
Figure 32 Generic structures of 2- or 5- amino or carboxybenzimidazole

2.1.1 Condensation of *o*-phenylenediamine with aldehyde

Aldehydes can be condensed with *o*-phenylenediamines to afford a benzimidazole product. In this project, two different methods were used employing (i) a sodium metabisulfite [31] promoted reaction and (ii) heating in nitrobenzene. [32]

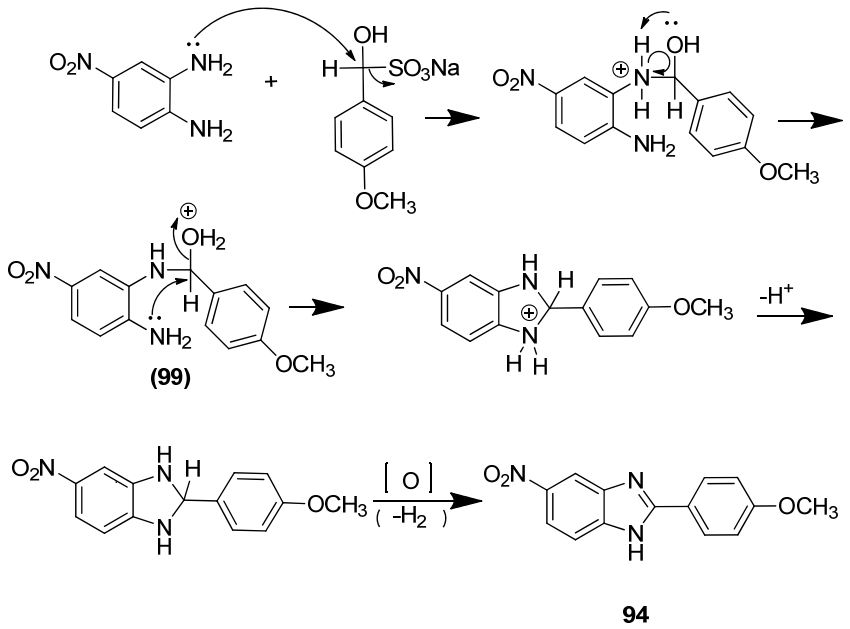
The synthesis of N-H benzimidazoles bearing a 5-amino group as precursors to dimerization and oligomerization. For that purpose a phenylenediamine (in this case 5-nitro) was condensed with aldehyde or affording the corresponding nitrobenzimidazole, which was then reduced to the corresponding aminobenzimidazole.

Specifically, aldehydes **90** can be condensed with *o*-phenylenediamines to afford the corresponding benzimidazole, and the method employed here used sodium metabisulfite promoted reaction. These reactions rely on pre-forming the bisulfite adduct of the aryl aldehyde **91** (see **Scheme 13**).



Scheme 13 5-Nitrobenzimidazole synthesis using sodium metabisulfite

A proposed mechanism of the reaction is shown in **Scheme 14**. The bisulfite adduct facilitates forming initial aminal intermediate **99**, and the key ring closure step is a dehydration.



Scheme 14 Reaction mechanism for the synthesis of 5-nitro benzimidazole using sodium metabisulfite.

This type of reaction was used to prepare benzimidazoles **94** and **96**, in which an ethanolic solution of 4-methoxybenzaldehyde (**93**) or 2,4-dimethoxybenzaldehyde (**95**) were added seperately to an aqueous solution of sodium metabisulphite; the adduct formed precipitated from the reaction was filtered and dried. The *o*-phenylenediamine (**92)** in DMF was then added to each adduct and the mixture was heated at 130^{o}C for (16-48 h) affording 2-(4'-methoxyphenyl)-5-nitrobenzimidazole (**94**) and 2(2',4'-di methoxyphenyl)-5-nitrobenzimidazole (**96**).

The structures of **94** and **96** were confirmed by spectral data. The ^{1}H NMR for **94** showed the methoxy protons as a singlet at 3.92 ppm and the NH proton appeared as a broad peak in the range of 13.4-13.6 ppm. The MH^{+} peak appeared as expected at *m/z* 270.

In the ^{1}H NMR for compound **96** the six protons of the two methoxy groups appeared as singlets at 3.99 and 4.18 ppm, respectively, (**Figure 33**)**,** whilst MS showed the MH^{+} peak at *m/z* 301.

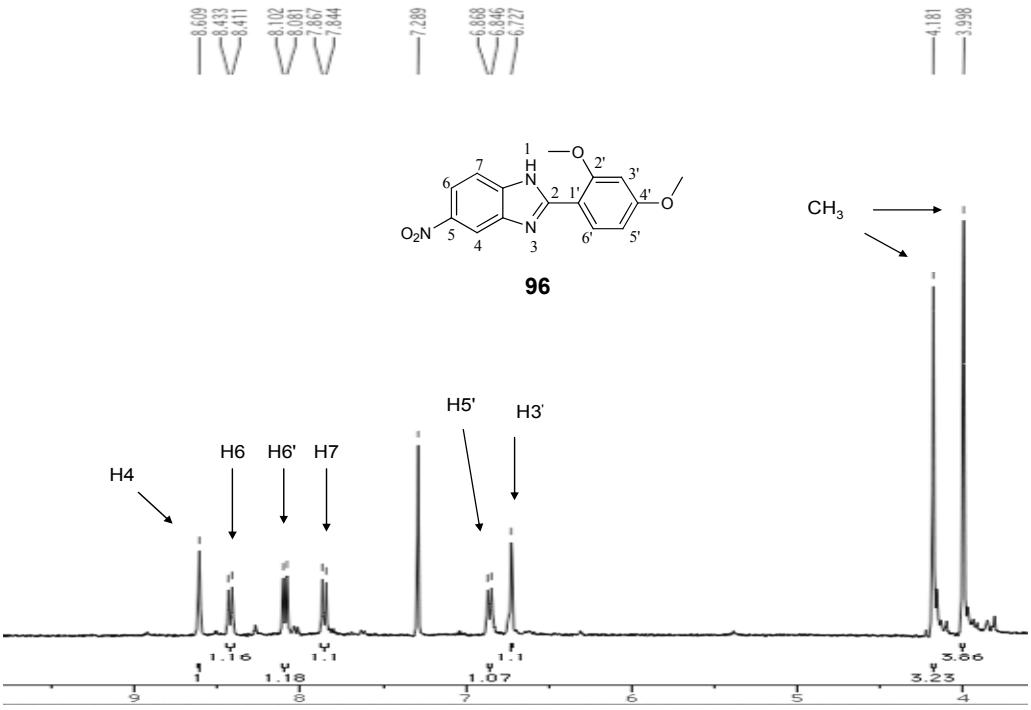


Figure 33 ^{1}H NMR (400 MHz) of 96 in $CDCl_3$ [#].

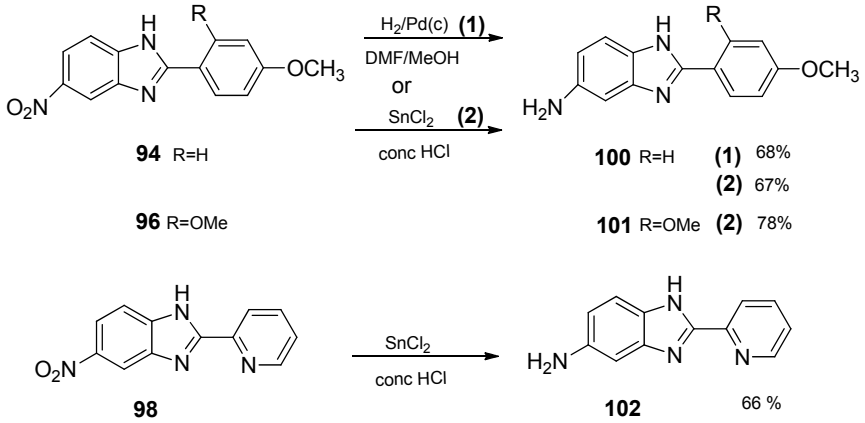
With successful reactions demonstrated using aryl aldehydes, similar reactions were evaluated with a pyridine system.

Thus, an ethanolic solution of 2-formylpyridine (**97**) was added to an aqueous solution of sodium metabisulphite, the adduct formed was filtered and dried. The *o*-phenylenediamine (**92**) in DMF was then added and the mixture was heated at 130 °C for 13 hours affording 5-nitropyridylbenzimidazole (**98**).

2.1.2 Reduction of 5-nitrobenzimidazoles

This step was crucial in the project, because preparing the amines is a key requirement for using amide coupling to link the benzimidazole with different compounds having carboxylic acids (e.g. at position 2). Reduction of 2-(*p*-methoxyphenyl-) or 2-(2,4-di-methoxyphenyl-) or 5-nitropyridyl-) benzimidazoles was carried out using either (H_2/ Pd) or $SnCl_2$ /HCl. TLC showed complete disappearance of the starting materials and gave high yields of the amine products (**Scheme 15**).

[#] This assignment has been confirmed by using 2D NMR (COSY)



Scheme 15 Reduction of 5-nitrobenzimidazoles to the corresponding amines

Thus, reduction of 2-(4'-methoxyphenyl)-5-nitrobenzimidazole (**94**) by $SnCl_2$/HCl and H_2(Pd/C) afforded **100** with 67 % or 68 % yield respectively. The ^{1}H NMR for **100** showed a singlet peak at 3.91 ppm arising from the methoxy group. The MS showed MH^+ peak at *m/z* 240, consistent with the structure shown.

Another attempt to reduce compound **94** to the corresponding amine **100** using H_2(Pd/C) was successful. The structure of amine **100** was confirmed by ^{1}H NMR, which showed a singlet peak at 3.86 ppm, from the three protons of the methoxy group (**Figure 34**). The ^{13}C NMR showed a methoxy peak at 55.7 ppm, consistent with the structure.

2-(2',4'-dimethoxyphenyl)-5-nitrobenzimidazole (**96**) was reduced using $SnCl_2$ / HCl affording 5-amino-2-(2',4'-dimethoxyphenyl) benzimidazole (**101**) in 78 % yield. The ^{1}H NMR for **101** showed singlet peaks for the two methoxy protons at 3.91 and 4.08 ppm. The NH_2 proton appeared as a broad peak in the range of 3.43-3.47 ppm, and NH proton appeared as a broad peak at 10.18-10.70 ppm, whilst the ^{13}C NMR showed the two methoxy peaks at 56.5 and 57.1 ppm. The MS was consistent with the appearance of the MH^+ peak at *m/z* 270.

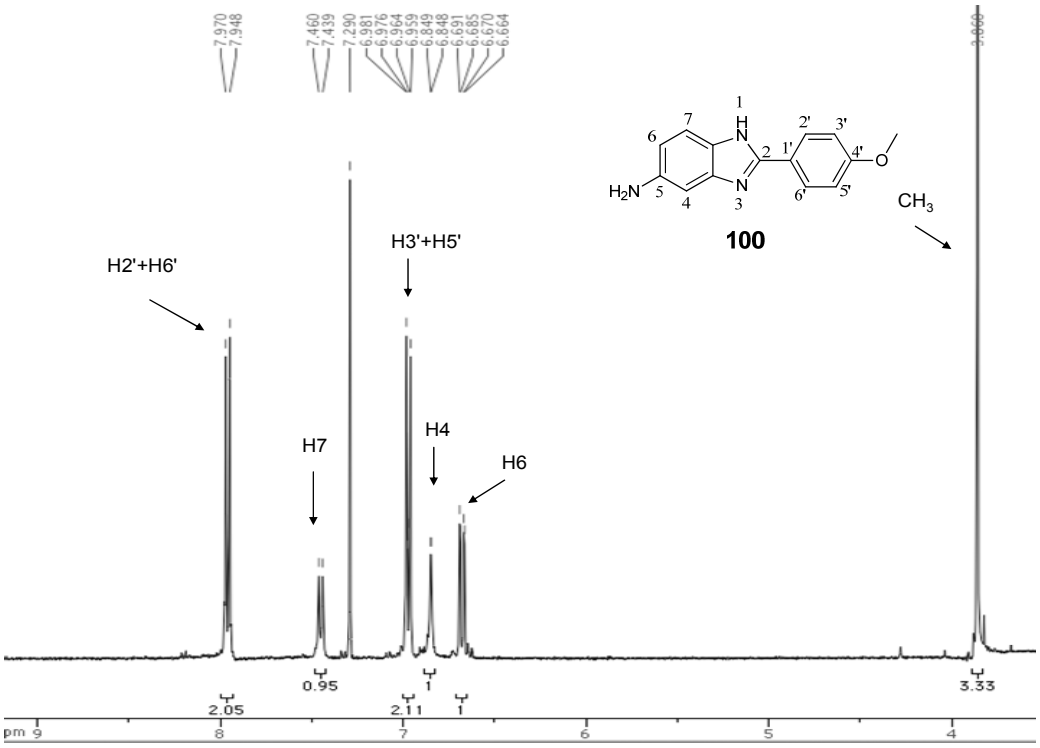


Figure 34 ^{1}H NMR (400 MHz) of 100 in $CDCl_3$ [#]

Reduction of 5-nitropyridylbenzimidazole (**98**) using $SnCl_2$/HCl afforded the corresponding amine **102** (**Scheme 15**) whose structure was confirmed by all spectral data. The ^{1}H NMR for **102**, showed benzimidazole protons H6, H4 and H7 at 7.50, 7.84 and 7.93 ppm respectively, while H5', H4', H3' and H6' in the pyridine ring appeared at 7.74, 8.19, 8.44 and 8.89 ppm respectively (**Figure 35**). The MS showed MH^+ ion at *m/z* 211.

[#] This assignment has been confirmed by using 2D NMR (COSY)

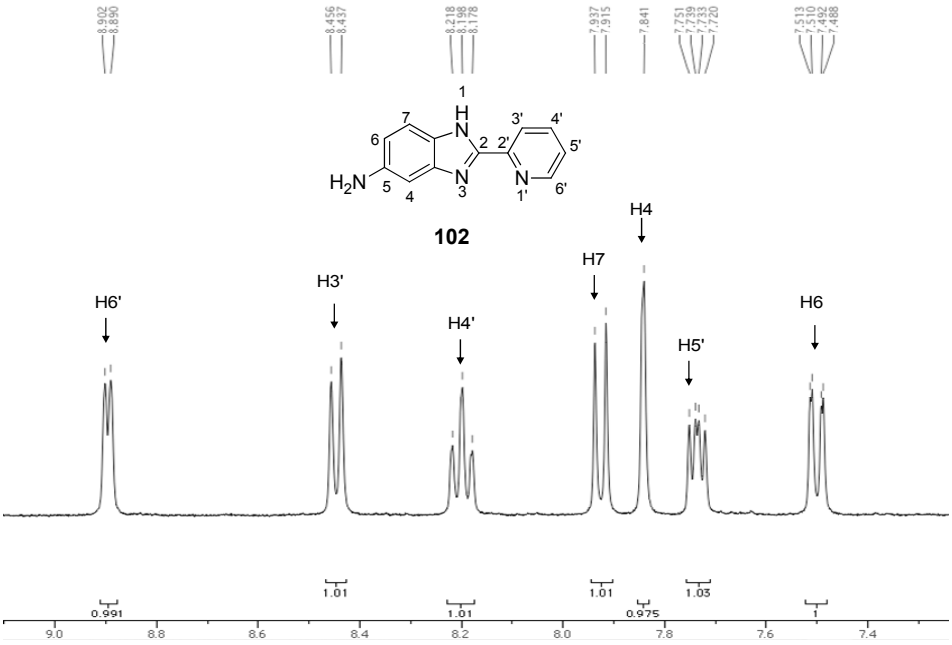
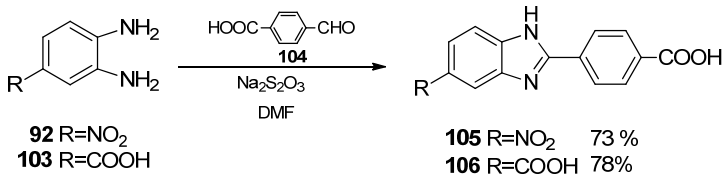


Figure 35 H NMR (400 MHz) of 102 in d_6-DMSO [#]

2.1.3 Synthesis of 2-(*p*-carboxyphenyl)-1*H*-benzimidazole [31]



Scheme 16 Synthesis of 2-(4'-carboxyphenyl benzimidazole

The 2-(4'-carboxyphenyl)-5-nitrobenzimidazoles **105** and **106** were prepared by the reaction between 4-carboxy benzaldehyde (**104**) and 4-nitro-1,2-phenylenediamine (**92**) or 3,4-diaminobenzoic acid (**103**), respectively. In the presence of sodium metabisulfite under conditions analogous to those used for preparation of the 2-aryl alkoxy derivatives (using DMF as a solvent and heating at 140 °C for 3 days) (**Scheme 16**).

NMR and MS confirmed the formation of benzimidazoles **105** and **106** using this method.

The ^{1}H NMR for **105** showed a broad singlet of 13.22-13.23 ppm, related to NH or COOH groups, the ^{13}C NMR showed a C=O at 166.9 ppm and the MH^{+} peak was

[#] This assignment has been confirmed by using 2D NMR (COSY)

observed at *m/z* 284. Whilst for **106**, the IR showed C=O peaks at 1685, 1700 cm^{-1} for the two carboyxlates and an NH stretch at 3305cm^{-1}, fully consistent with the structure shown. The ^{1}H NMR showed a broad peak in the range of 13.07-13.62 ppm related to the two COOHs (**Figure 36**). The structure was further confirmed by ^{13}C NMR which showed a C=O at 167.2, 168.1 ppm, respectively, whilst MS showed the MH^{+} peak at *m/z* 283.

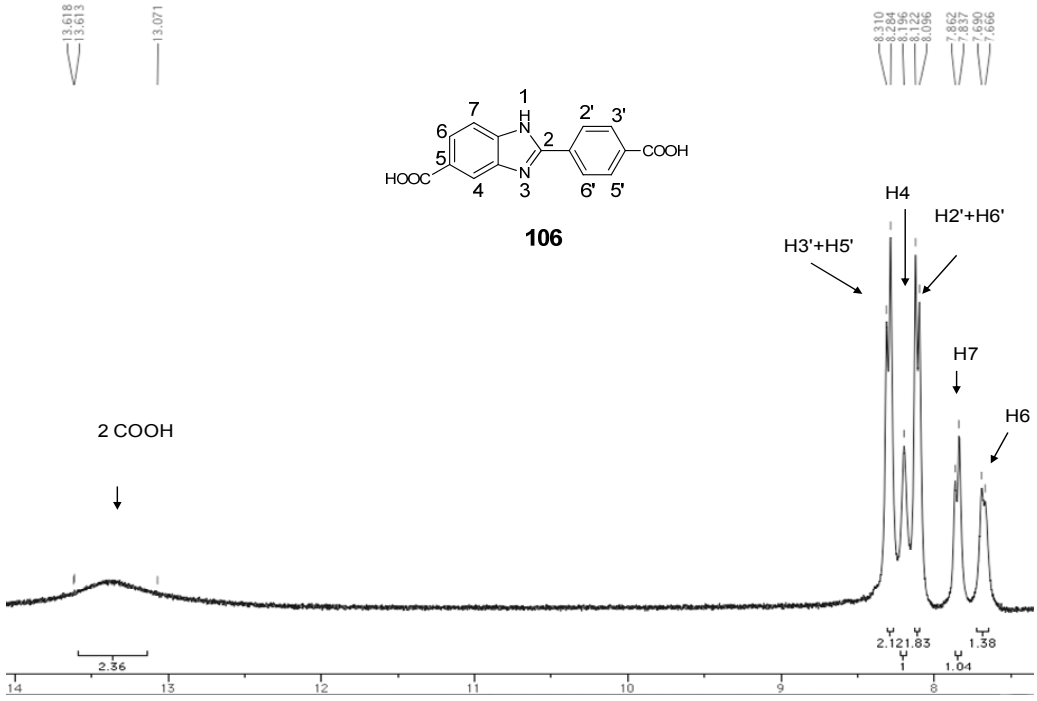
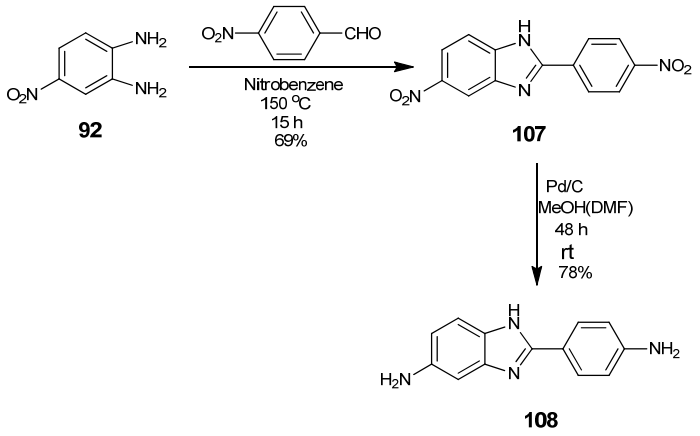


Figure 36 ^{1}H NMR (400 MHz) of 106 in d_6-DMSO #

2.1.4 Phenylenediamine condensation with aldehydes in nitrobenzene [32]

This method is used to synthesize benzimidazoles *via* condensation of aromatic aldehydes and phenylenediamines by high temperature reaction in nitrobenzene. In this case, the nitrobenzene acts as a solvent and oxidant at the same time. On the other hand some aromatic aldehydes (e.g. *p*-nitrobenzaldehyde) failed to condense with phenylene diamine in sodium metabisulphite reaction. However, 5-nitro-2-(4-nitrophenyl) benzimidazole (**107**) was successfully prepared using the nitrobenzene method.

This assignment has been confirmed by using 2D NMR (COSY)



Scheme 17 Synthesis of 2,5-diaminobenzimidazole by using $PhNO_2$

^{1}H NMR, ^{13}C-NMR and MS confirmed the structure of the above products.

The ^{1}H NMR for **107** showed a singlet peak at 13.93 ppm related to NH. The MS showed MH^+ peak at *m/z* 285, consistent with the structure shown.

Reduction of **107** using Pd/C in MeOH and DMF afforded the corresponding diamino benzimidazole **108** in 78 % yield (**Scheme 17**) whose structure was confirmed by all spectral data. The ^{1}H NMR for **108**, showed a broad peak in the range of 6.06-6.38 ppm for the NH_2 proton (**Figure 37**). Mass spectroscopy showed the *m/z* at 225 as the base peak.

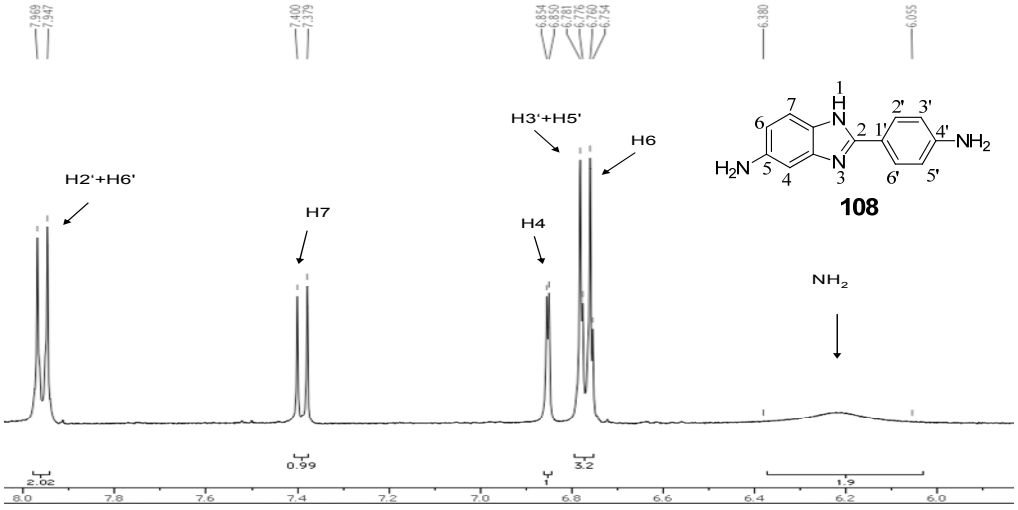


Figure 37 ^{1}H NMR (400 MHz) of 108 in d_6-DMSO [#]

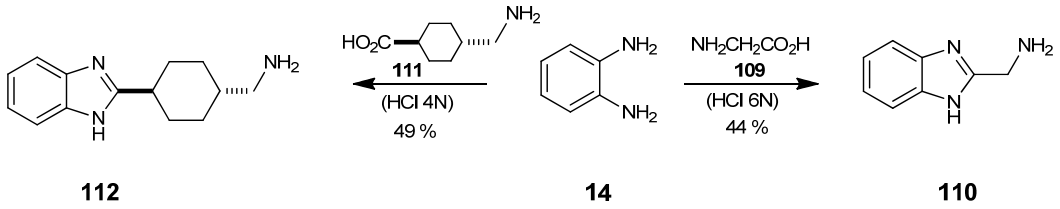
[#] This assignment has been confirmed by using 2D NMR (COSY)

2.2 Condensation of *o*-phenylenediamine with carboxylic acids

2.2.1 Condensation with aliphatic carboxylic acid (Phillip's method) [27]

Aliphatic carboxylic acids can condense with *o*-phenylenediamines affording benzimidazoles. This method involves condensation of phenylenediamine with carboxylic acid in refluxing HCl, affording the benzimidazole.

A series of 2-aminobenzimidazoles **110** and **112** (**Scheme 18**) and 5-carboxy benzimidazoles **115** and **116** (**Scheme 19**) were prepared using this type of chemistry. All spectroscopic data (NMR and MS) were consistent with the formation of target benzimidazoles.



112 **14** **110**

Scheme 18 Condensation of phenylene diamine with carboxylic acid according to Phillip's method

2-Aminomethyl benzimidazole **110** was formed by condensation of *o*-phenylene diamine **14** with glycine **109** in refluxing HCl (6N) for 24 hours**.**

^{1}H NMR for **110** showed the two methylene protons as a singlet peak at 4.56 ppm, the two doublet of doublets for aromatic protons at 7.36 and 7.67 ppm respectively, and a broad peak in the range of 4.74-6.78 ppm related to NH_2 protons. The imidazole NH proton appears clearly as a broad singlet peak at 9.15-9.18 ppm (**Figure 38**). ^{13}C NMR showed the methylene carbon at 35 ppm (**Figure 39**) and the MS showed the expected MH^+ peak at *m/z* 148.

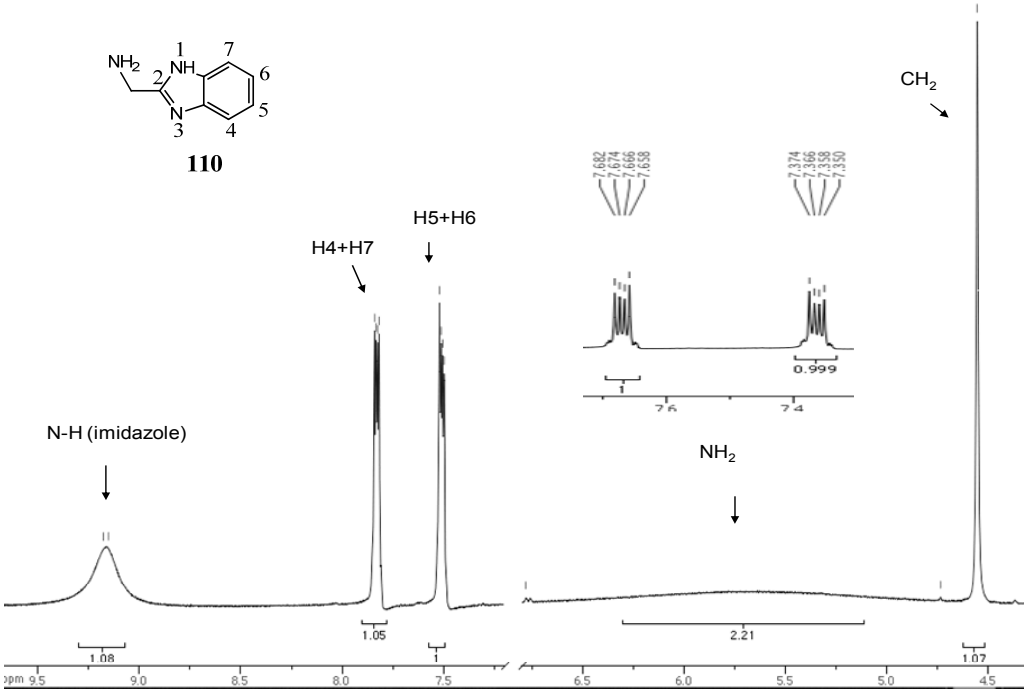


Figure 38 1H NMR (400 MHz) of 110 in d_6-DMSO [#], the inset shows expansion for the aromatic part

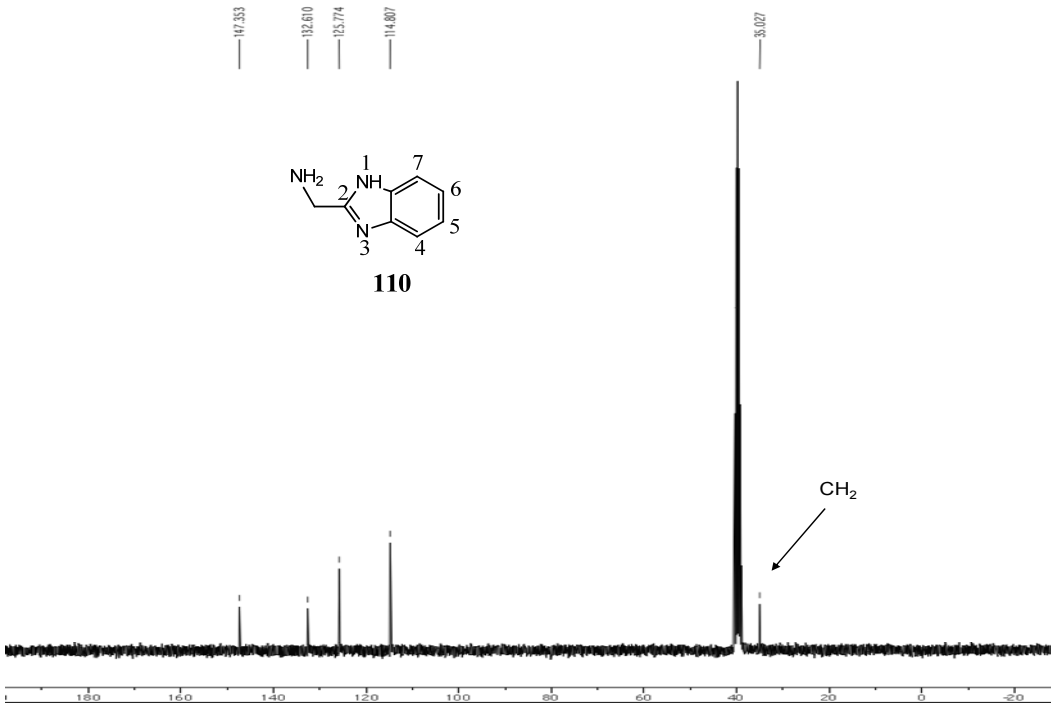


Figure 39 ^{13}C NMR (300 MHz) of 110 in d_6-DMSO

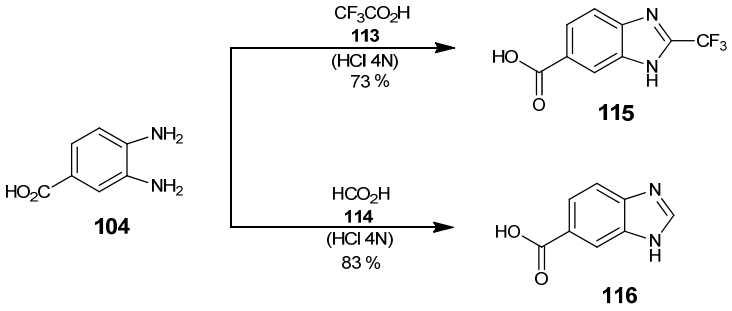
[#] This assignment has been confirmed by using 2D NMR (COSY)

4-(1-*H*-Benzimidazol-2-yl)-cyclohexyl) methanamine (**112**) has been synthesized from condensation of 1,2-phenylenediamine **14** with *trans*-4-aminomethyl cyclohexane carboxylic acid **111** in refluxing HCl (4N) for 6 hours.

The ^{1}H NMR for **112**, showed a broad singlet peak for the NH_2 in the range of 3.36-3.64 ppm, two doublet of doublets at 6.87 and 7.23 ppm for the four aromatic protons, whilst the two methylene protons appear clearly as a doublet at 2.29 ppm. The ^{13}C NMR showed the signal for the methylene carbon at 44.4 ppm, and cyclohexyl carbons in the range of 29-35.9 ppm. The expected MH^+ peak was observed at *m/z* 230.

2.2.2 Synthesis of 5-carboxybenzimidazoles

5-Carboxybenzimidazoles **115** and **116** were prepared using this type of chemistry (**Scheme 19**). All spectroscopic data (MS and NMR) were consistent with the formation of benzimidazoles **115** and **116**.



Scheme 19 Some 5-carboxybenzimidazole synthesized *via* Phillip's method [27]

Condensation of 3,4-diaminobenzoic acid **104** with trifluoro acetic acid **113** in refluxing HCl (4N) for 4 hours, gave 5-carboxy-2-trifluoromethyl benzimidazole (**115**) (**Scheme 19**).

The ^{13}C NMR for **115** showed the C=O peak at 168.2 ppm, and CF_3 carbon at 143ppm, which appears as quarter with coupling constant J_{CF} = 40.0 Hz. (**Figure 40**) and MS showed the expected MH^+ peak at *m/z* 231, and the ^{1}H NMR showed a broad peak for the COOH group in the range of 14.25-14.62 ppm, while the imidazole NH appears as a broad peak in the range of 12.86-13.27 ppm (**Figure 41**) and all other spectral data was consistent with the structural assignment.

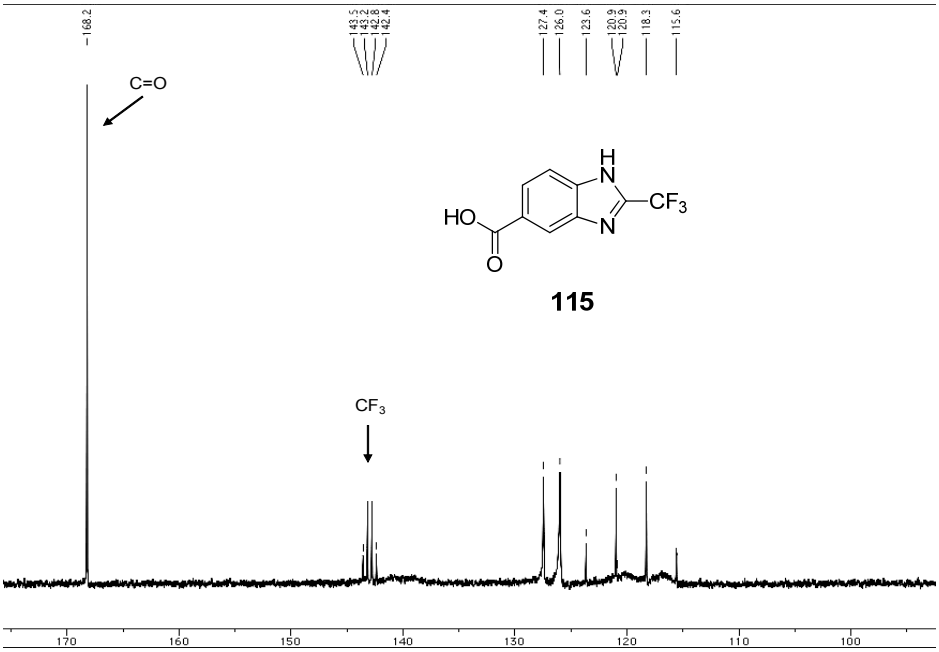


Figure 40 ^{13}C NMR (100 MHz) of 115 d_6-DMSO

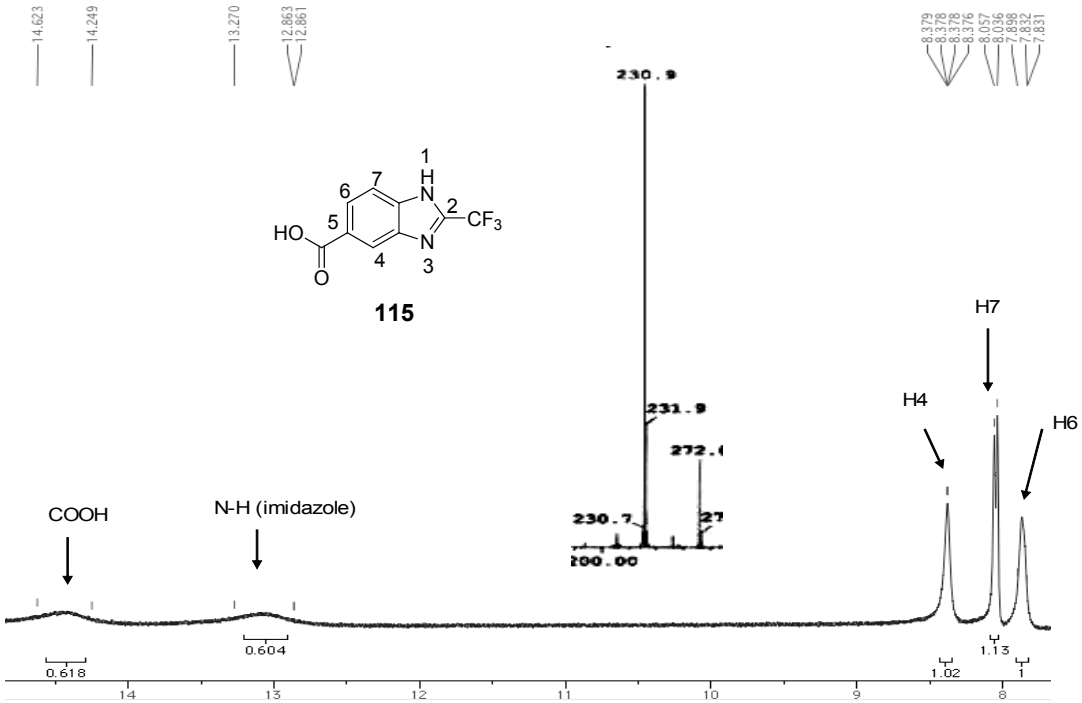


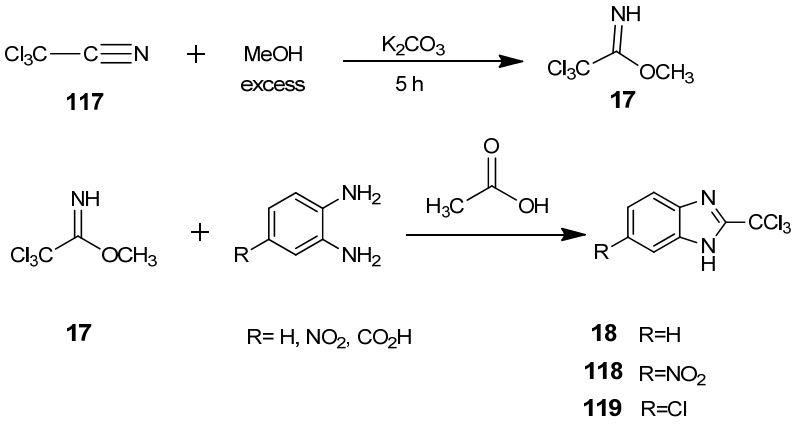
Figure 41 ^{1}H NMR (400 MHz) of 115 in d_6-DMSO [#], the inset shows LR mass spectrum

[#] This assignment has been confirmed by using 2D NMR (COSY)

5-carboxybenzimidazole (**116**) was synthesized from condensation of 3,4-diamino benzoic acid **104** with formic acid **114** in refluxing HCl (4N) for 4 hours. The ^{1}H NMR for **116**, showed a broad singlet at 12.53-13.22 ppm for the COOH group, while the imidazole NH appears as a broad singlet peak in the range of 7.21-7.49 ppm. The ^{13}C NMR showed a C=O at 166.7 ppm and the MH^{+} peak was observed at *m/z* 163.

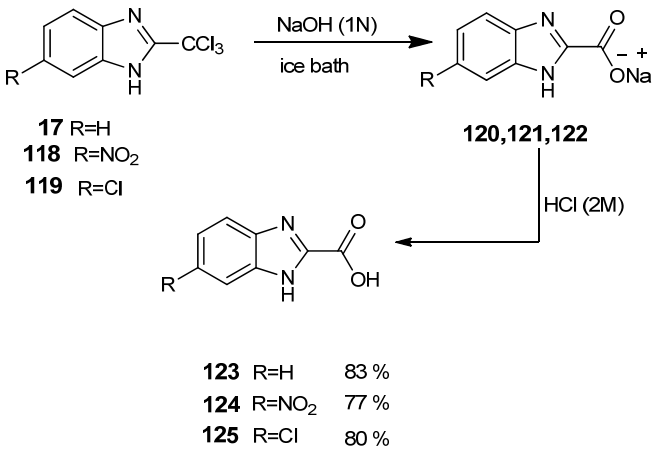
2.3 Synthesis of 2-carboxy benzimidazoles (123,124,125) by using trichloroacetonitrile

To introduce a 2-carboxyl group, the use of a precursor 2-trichloroacetimidate was employed. This route uses the condensation of *o*-phenylenediamine and trichloro acetimidate **17** [75] prepared from the reaction of trichloroacetonitrile **117** and methanol in the presence of potassium carbonate affording 2-trichloromethyl benzimidazoles **18**, **118** and **119** (**Scheme 20**).



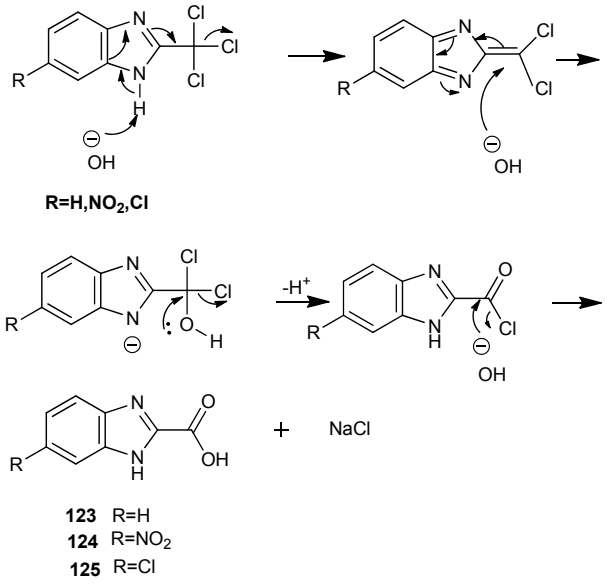
Scheme 20 Synthesis of 2-trichloromethyl benzimidazole

The 2-trichloro methyl benzimidazoles **18, 118** and **119** were stirred with NaOH (1M) in an ice bath giving the carboxylate salts [76] **120, 121** and **122**. Once TLC indicated no starting material remained (after 2-6 hours) the reaction was neutralized with HCl (2M), yielding **123-125** in 77-83 % yields (**Scheme 21**).



Scheme 21 Synthesis of 2-carboxybenzimidazole *via* alkaline hydrolysis of 2-trichloromethyl benzimidazole

A proposed mechanism of the reaction is shown in **Scheme 22**.



Scheme 22 Reaction mechanism for the synthesis of 2-carboxy benzimidazole *via* alkaline hydrolysis of 2-trichloromethyl benzimidazole

The structure of the products **123**, **124** and **125** were confirmed by ^{1}H NMR, ^{13}C NMR. The ^{1}H NMR for **123** showed two doublet of doublets at 7.16 and 7.45 ppm respectively, related to the four aromatic protons. The MS showed the expected MH^{+} peak at *m/z* 163 and the ^{13}C NMR showed a C=O resonance at 159.3 ppm.

The MS for **124** showed the MH^+ peak at *m/z* 206, and the ^1H NMR showed a broad peak for the NH in the range of 3.98-4.83 ppm (**Figure 42**). The ^{13}C NMR showed the peak C=O at 160.4 ppm.

Whilst the ^{13}C NMR for **125** showed C=O at 160.4 ppm. The ^1H NMR showed a singlet peak at 8.30 ppm, related to the imidazole NH or COOH groups. The expected MH^+ peak was observed at *m/z* 196. The structure was further confirmed by IR which showed C=O at 1638 cm^{-1} and NH stretch at 3378 cm^{-1}.

Thus, in all cases, all spectroscopic data were consistent with isolation of the 2-carboxylate.

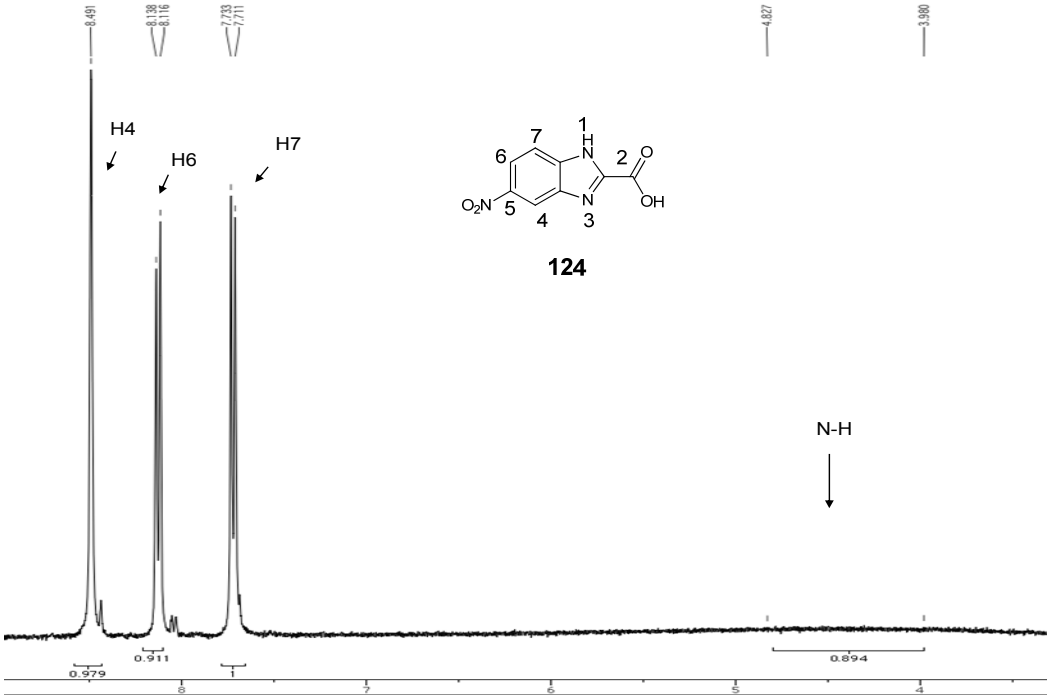


Figure 42 ^1H NMR (400 MHz) of 124 in d_6-DMSO [#]

[#] This assignment has been confirmed by using 2D NMR (COSY)

2.4 Dimerization

The synthesis of monomeric benzimidazoles is a clearly pre-requisite towards dimerization and oligomerization.

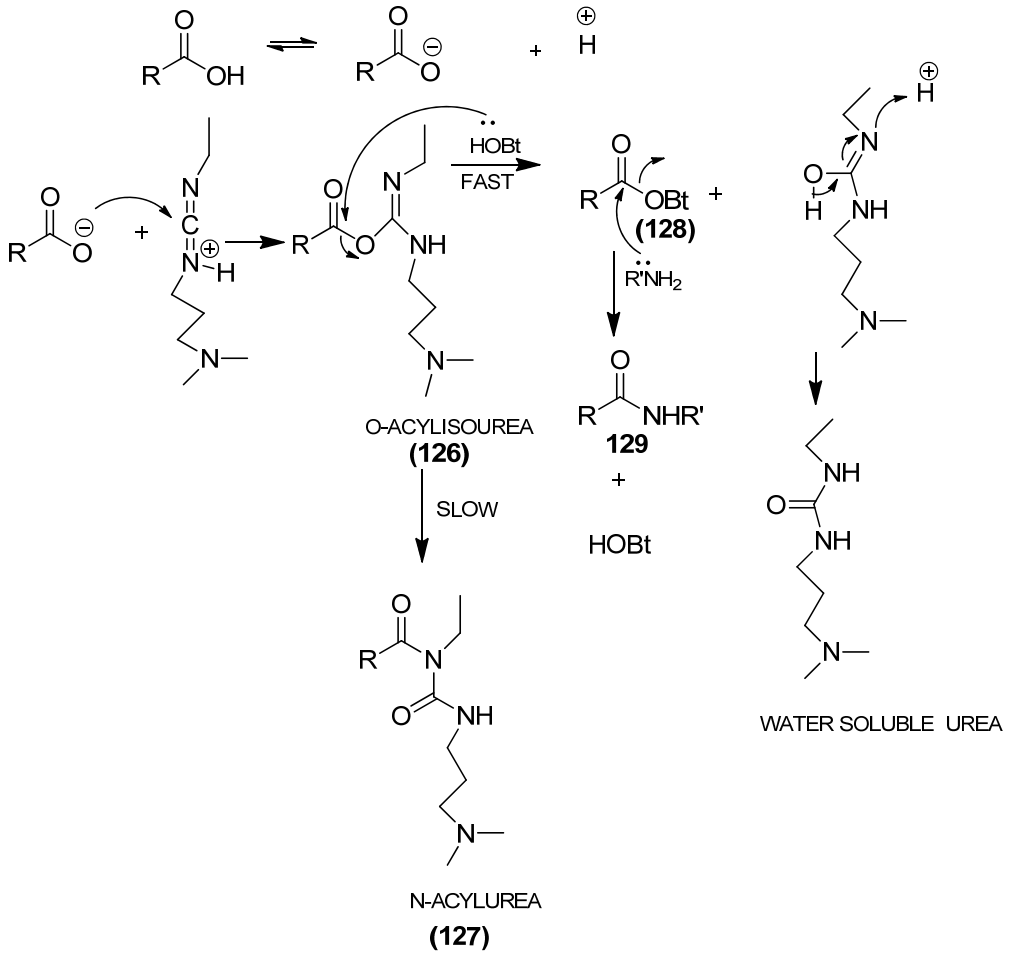
Different directions of linkages were suggested (**Section 1.9.1**) to target either symmetrical or unsymmetrical bis(benzimidazoles) with amide C2-C2, C2-C5 and C5-C5 linkages.

2.4.1 Synthesis of unsymmetrical bis(benzimidazoles)

This sort of dimeric system can be obtained *via* coupling of aminobenzimidazoles and 2-carboxy or 5-carboxy monomeric benzimidazoles. Examples include the syntheses using 2-carboxy benzimidazoles and 5-carboxy-2-trifluoro methyl benzimidazole and 2-(4'-carboxyphenyl)-5-nitrobenzimidazole that afford two different orientations of head-to-head and head-to-tail bis(benzimidazoles).

2.4.2 Amide synthesis *via* coupling of carboxybenzimidazole and aminobenzimidazole by using EDC/HOBt system [77]

Conversion of carboxylic acid to an amide requires the transformation of the acid into a more reactive intermediate. For that purpose, the coupling reagent used was ethyl-3-dimethylaminopropyl carbodiimide (**EDC**). The acid and the coupling reagent EDC/HOBt were dissolved in DMF on ice bath for 30 minutes followed by adding amino benzimidazole. The reaction was left stirring, and once the TLC showed completion of the reaction, the key step for purification was the addition of water to the reaction mixture. The product precipitated, while the urea side product is water-soluble. Finally, compounds were purified either by column chromatography or recrystallization. The amide coupling reaction mechanism using EDC/HOBt is as below (**Scheme 23**).

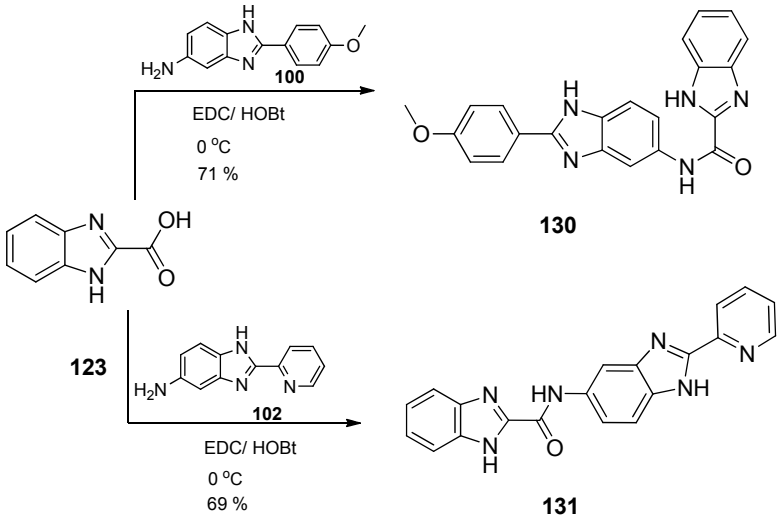


Scheme 23 Reaction mechanism for amide synthesis *via* coupling of carboxy benzimidazole and amino benzimidazole by using EDC/HOBt reagents

The use of HOBt minimizes the formation of unreactive *N*-acylurea **127**, which is formed from *O*-acylisourea **126**. This compound **126** reacts with HOBt which is a fast step and gives a carbonylbenzotriazole intermediate **128**, which reacts with amine to give the amide **129** (**Scheme 23**).

2.4.3 Library of carboxybenzimidazoles

Synthesis of 2- and 5-carboxybenzimidazoles is the precursor step for introducing amide linkage for dimeric or oligomeric systems. Coupling of **123** to **100** or **102** was achieved with EDC/HOBt (**Scheme 24**).



Scheme 24 Unsymmetrical bis(benzimidazoles) *via* reaction of 5-aminobenzimidazole and 2-carboxybenzimidazole

The ^{1}H NMR for **130** showed a signal for the methoxy group as a singlet at 3.92 ppm,, while the characteristic signal for the amide proton appeared at 11.0 ppm, and the imidazole NH proton appears clearly as a broad singlet peak in the range of 13.45-13.63 ppm (**Figure 43**). The ^{13}C NMR showed the characteristic signal for carbonyl at 161.6 ppm, and the methoxy group at 55.8 ppm, whilst the MS showed MH^{+} ion at *m/z* 384. The IR showed a stretching at 3387 cm^{-1}, for the NH bond. Thus, all data were consistent with the structure assignment.

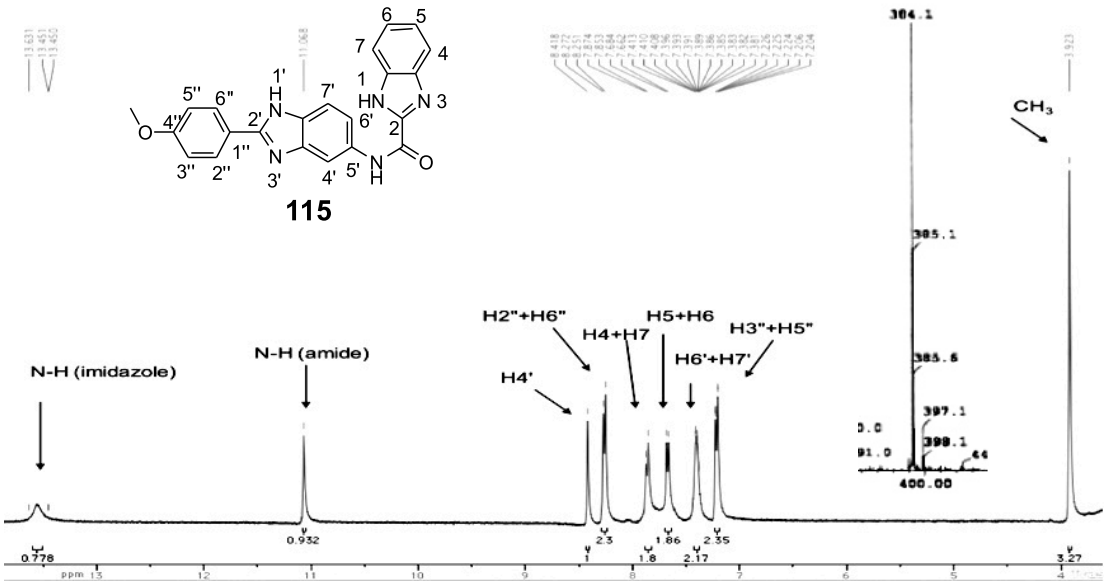


Figure 43 ^{1}H NMR (400 MHz) of 130 in d_6-DMSO [#], the inset shows LR mass spectrum

The dimer **131** is the expected product of the coupling between 1-*H*-benzimidazole-2-carboxylic acid **123** and 5-amino pyridylbenzimidazole **102** using EDC/HOBt (**Scheme 24**). After 12 days TLC showed no starting material remaining and the desired amide **131** was isolated in 69% yield.

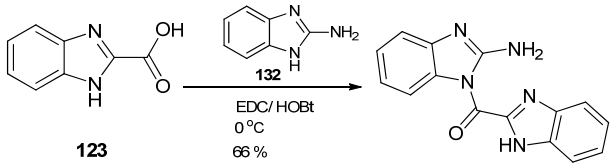
The ^{1}H NMR for **131** showed an amide proton at 10.94 ppm, and the iminol OH proton as a singlet peak at 10.99 ppm. The two imidazole NHs, appear as singlets at 13.15 and 13.49 ppm, respectively, and the iminol NH appears as a singlet at 13.46 ppm. The ^{13}C NMR showed the characteristic signal for the carbonyl at 157.6 ppm. The expected MH^+ peak at *m/z* 355, was also observed, moreover, the IR showed the NH stretch at 3355 cm^{-1}.

2.4.3.1 Synthesis of (2-amino-benzimidazol-1-yl)-(1*H*-benzimidazol-2-yl)methanone (133)

In couplings between 2-amino benzimidazole and 2-carboxylate benzimidazoles the amidine-like reactivity affects the site of amide formation. Consequently, coupling 1-*H*-benzimidazole-2-carboxylic acid **123** and 2-amino-1*H*-benzimidazole **132** with EDC/

[#] This assignment has been confirmed by using 2D NMR (COSY)

HOBt leads to (2-amino-benzoimidazol-1-yl)-(1*H*-benzoimidazol-2-yl) methanone (**133**) being isolated in 66 % yield (**Scheme 25**)



Scheme 25 dimeric benzimidazole from coupling of 2-aminobenzimidazole and 2-carboxybenzimidazole

The ^{1}H NMR for **133** showed four doublet of doublets at 7.08, 7.24, 7.43 and 7.63 ppm, respectively, which arise from the eight aromatic protons. A broad singlet at 3.65-4.68 ppm is observed for the NH_2 (**Figure 44**). ^{13}C NMR showed C=O at 163.1 ppm, the IR showed a stretching at 3350 cm^{-1} for the amine NH bond and MS showed MH^{+} peak at *m/z* 278.

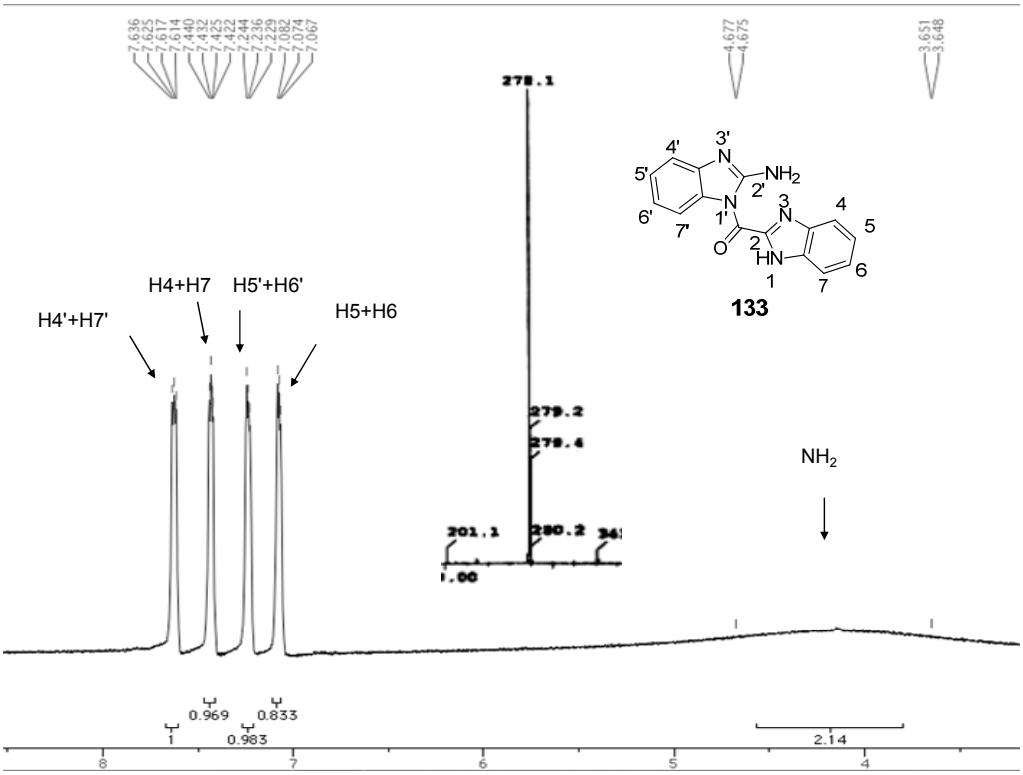


Figure 44 ^{1}H NMR (400 MHz) of 133 in d_6-DMSO [#], the inset shows LR mass spectrum

[#] This assignment has been confirmed by using 2D NMR (COSY)

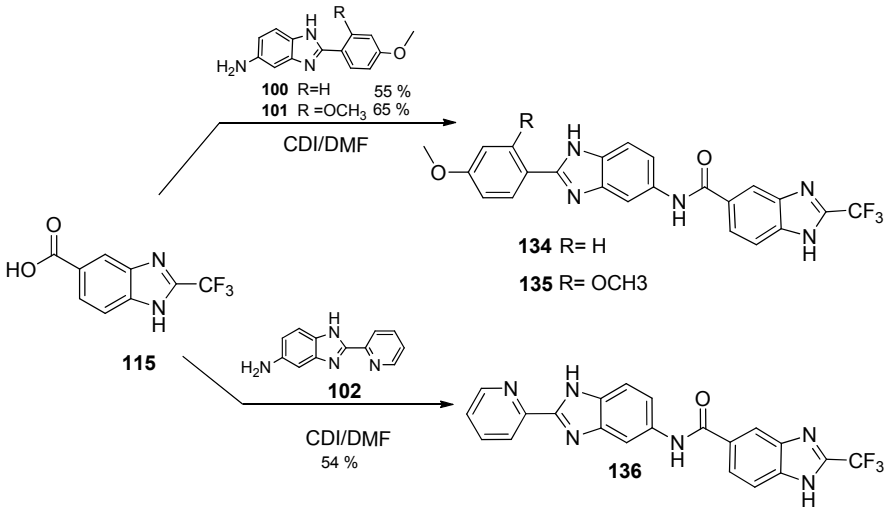
2.4.4 Amide synthesis *via* coupling of carboxybenzimidazole and aminobenzimidazole by using CDI system [78]

Whilst EDC/HOBt was used for 2-carboxy benzimidazoles (**Section 2.4.2**), the coupling reagent *N*,*N*-carbonyl diimidazole (**CDI**) was used for 5-carboxy-2-trifluoromethyl benzimidazoles. The acid was dissolved in DMF then the solution was treated with CDI at room temperature and after 1 h the amino benzimidazole was added.

The coupling reagent CDI is a useful coupling reagent that allows one-pot amide formation. Acyl carboxyl imidazole and imidazole are initially formed but readily react together to yield the activated species as the acylimidazole. Practically, the acyl imidazole is normally preformed then the amine is added.

2.4.4.1 Coupling of 5-carboxybenzimidazoles to generate 5,5-dimers.

A small range of unsymmetrical head-to-tail and tail to tail amide bis (benzimidazoles) were prepared by coupling 5-carboxy-2-trifluoromethyl benzimidazole with 2- and 5-aminobenzimidazoles using CDI as the coupling agent.



Scheme 26 Unsymmetrical bis(benzimidazole) *via* aminolysis of 5-carboxy-2-trifluoromethyl benzimidazole using 5-aminobenzimidazole

Synthesis of dimer **134** was initially attempted by coupling 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and 5-amino-2-(4'-methoxyphenyl) benzimidazole (**100**) with EDC/HOBt. However, no product could be isolated. Coupling

with CDI instead, the reaction went to completion after 10 days affording the desired amide in 55% isolated yield (**Scheme 26**).

The IR for **134**, showed an NH stretch at 3458 cm^{-1}, and the ^{1}H NMR showed the methoxy proton as a singlet at 3.98 ppm, and the amide proton as a singlet peak at 10.65 ppm (**Figure 45**). The ^{13}C NMR showed the C=O peak at 163.6 ppm, the methoxy group at 54.9 ppm and CF_3 carbon at 138.2 ppm which appears as a quartet with coupling constant J_{CF} = 39.3 Hz. The MH^+ ion appeared in MS as the base peak at *m/z* 452.

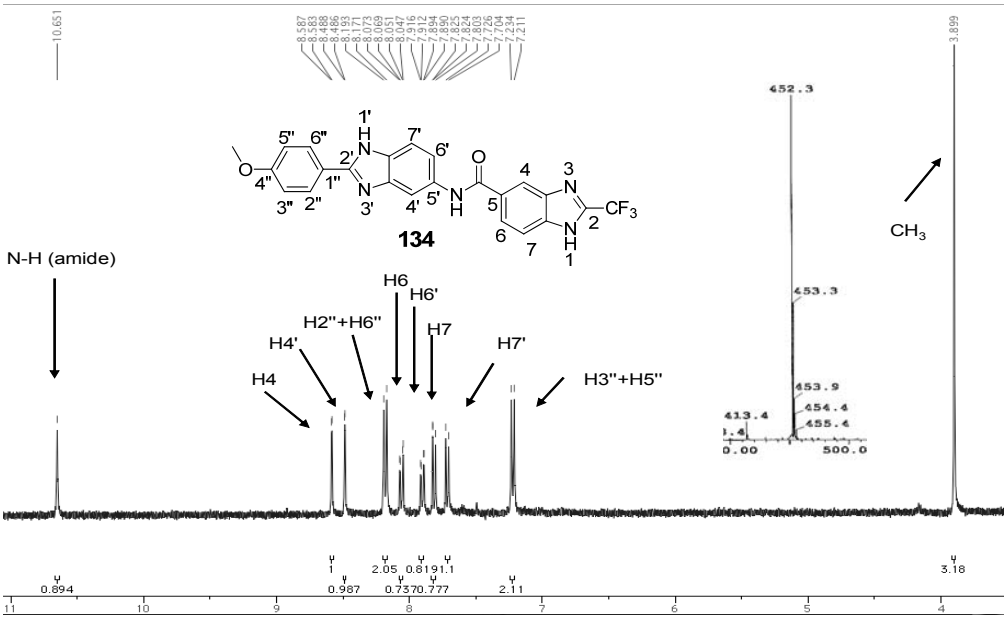


Figure 45 ^{1}H NMR (400 MHz) of 134 in d_6-DMSO [#], the inset shows LR mass spectrum

An attempt to prepare dimer **135** involved coupling 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and 5-amino-2-(2',4'-dimethoxyphenyl) benzimidazole (**101**) with EDC/ HOBt, but this was unsuccessful. Using CDI, however, **135** was isolated in 65% yield after 6 days (**Scheme 26**).

The MS for **135** showed the MH^+ peak at *m/z* 482. The IR showed NH stretch at 3416 cm^{-1}, and the ^{1}H NMR showed the six protons of the two methoxy groups as singlets at

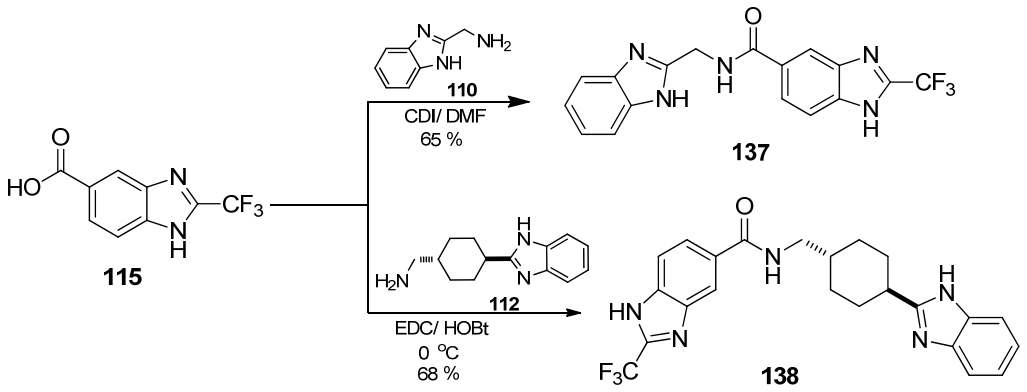
[#] This assignment has been confirmed by using 2D NMR (COSY)

3.94 and 4.11 ppm. A singlet peak at 10.75 ppm, was seen for NH amide, while the imidazole NH proton appears as a broad peak in the range of 13.99-14.46 ppm. The ^{13}C NMR showed two methoxy groups at 55.9 and 56.4 ppm, C=O peak at 166.1 ppm and CF_3 carbon at 142.4 ppm appears as a quartet with coupling constant J_{CF} = 39.4 Hz.

Dimer **136** was also prepared in 54% isolated yield by coupling 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and 2-pyridin-2-yl-1*H*-benzimidazol-5-ylamine (**102**) using CDI over 8 days (no product could be isolated using EDC/HOBt) (**Scheme 26**).

The ^{13}C NMR for **136**, showed the C=O, peak at 166.1 ppm, while CF_3 carbon at 142.1 ppm appears as a quartet with coupling constant J_{CF} = 39.5 Hz. The ^{1}H NMR showed the amide NH proton as a singlet peak at 10.72 ppm. The structure was confirmed by IR which showed NH stretch at 3420 cm^{-1}, while the MS showed MH^+ peak at *m/z* 423, which is consistent with the structure shown.

2.4.4.2 Systems with extended amine-bearing linkers at carbon-2



Scheme 27 Unsymmetrical bis(benzimidazole) *via* aminolysis of 5-carboxy-2-trifluoromethyl benzimidazole using 2-aminobenzimidazole

Synthesis of dimer **137** involved coupling 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and 2-aminomethyl benzimidazole (**110**) using EDC/HOBt. However, no product could be isolated from these conditions. Switching to use of CDI/DMF for 6 days, led to the desired amide in 65% yield (**Scheme 27**).

The IR for **137** showed NH stretch at 3470 cm^{-1}, and the ^{1}H NMR showed the two methylene protons as a broad singlet at 4.76 ppm, and a singlet peak at 9.36 ppm was

seen for NH amide, while the imidazole NH proton appears as a broad peak in the range of 11.79-13.0 ppm (**Figure 46**). The ^{13}C NMR showed the methylene carbon at 38.3 ppm, and the C=O was observed at 166.9 ppm whilst the CF_3 carbon resonates at 142.2 ppm as a quartet with coupling constant J_{CF} = 39.1 Hz. The MS showed the MH^+ ion at *m/z* 359 as the base peak.

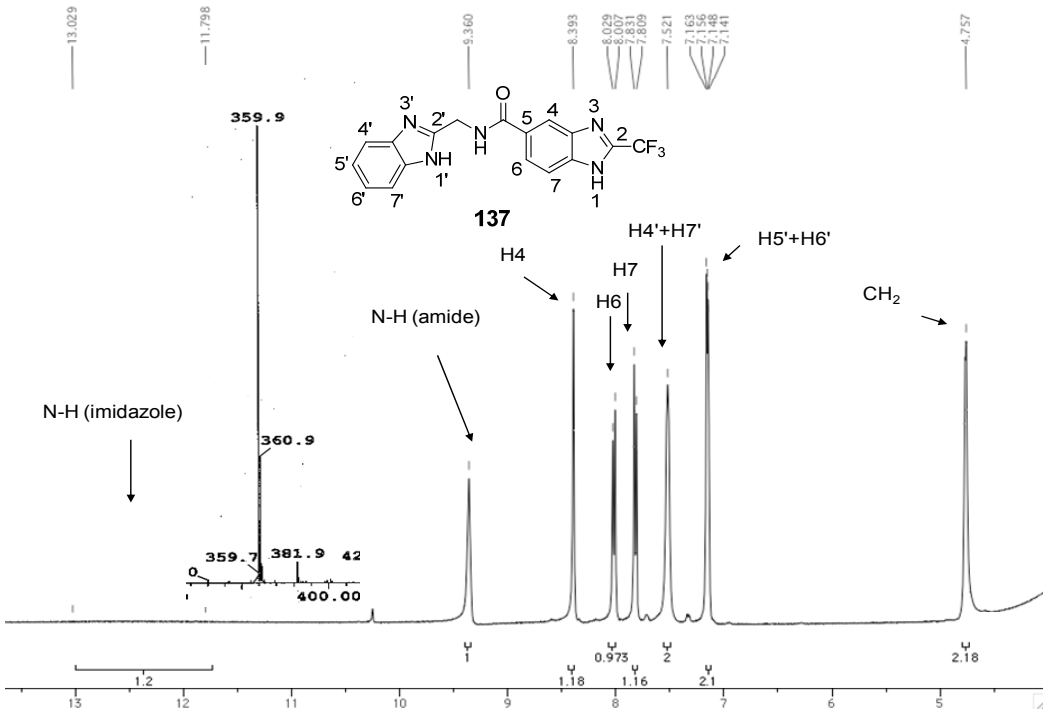


Figure 46 ^{1}H NMR (400 MHz) of 137 d$_6$-DMSO [#], the inset shows LR mass spectrum

A second type of extended C2-linked dimer **138** was also prepared by coupling 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and 4-(1-*H*-benzimidazol-2-cyclohexyl)methanamine (**112**) using EDC/HOBt (**Scheme 27**). After 2 days TLC showed no starting material remaining and the desired amide **138** was isolated in 68% yield. Spectroscopic data (NMR and M.S) confirmed the formation of this dimer.

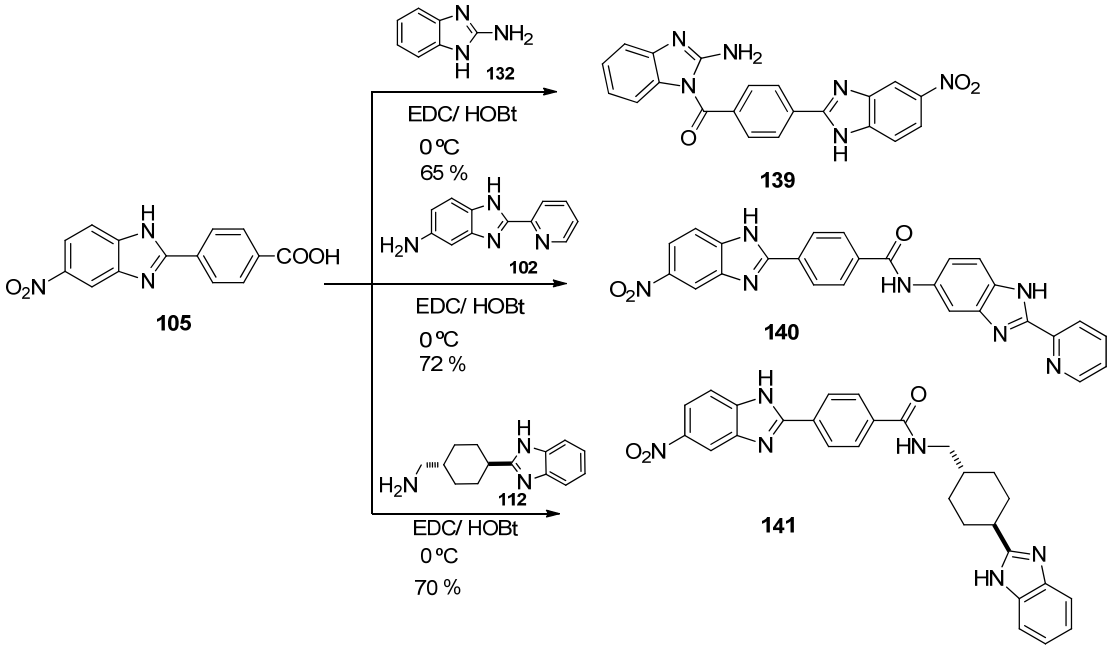
The ^{1}H NMR for **138** showed the amide proton as a singlet proton at 8.65 ppm, while the two imidazole NH protons appeared as a broad peak in the range of 11.85-12.55 and 13.90-14.60 ppm respectively, whilst two methylene protons appear clearly as triplet at 3.23 ppm. The ^{13}C NMR showed methyelene carbon at 45.9 ppm, and cyclohexyl

[#] This assignment has been confirmed by using 2D NMR (COSY)

carbons in the range of 30.5-38.2 ppm, while CF_3 carbon at 142.3 ppm appears as a quartet with coupling constant J_{CF} = 39.2 Hz. The IR showed NH stretch at 3401 cm^{-1}. The MH^+ ion appeared in the MS as a base peak at *m/z* 442.

2.4.5 Dimers from *2*-(4'-Carboxy) phenyl Systems

Several of unsymmetrical head-to-head and head to tail amide bis(benzimidazoles) were prepared by coupling of 2-(4'-carboxyphenyl)-5-nitrobenzimidazole (**105**) with 2- and 5-aminobenzimidazoles. This sort of coupling was achieved by dissolving the acid and EDC/HOBt in DMF at 0 ºC for 30 minutes followed by adding the amine, and stirring for several days, affording the dimers **139-141** in good yields 65-72 % (**Scheme 28**).



Scheme 28 Unsymmetrical bis(benzimidazole) *via* reaction of 2- or 5-aminobenzimidazole and carboxybenzimidazole

Dimer **139** is the expected product of the coupling between 2-(4'-carboxyphenyl)-5-nitrobenzimidazole (**105**) and 2-amino-1*H*-benzimidazole (**132**) using EDC/HOBt. After 8 days TLC showed no starting material remaining and the desired amide **126** was isolated in 65% yield (**Scheme 28**). The ^1H NMR for **139** showed the imidazole NH as a broad peak in the range of 12.28-12.51 ppm. A broad peak in the range 8.30-8.47 ppm was observed for the NH_2. The ^{13}C NMR showed a C=O peak at 169.4 ppm. The structure was confirmed by IR which showed NH stretch at 3366 cm^{-1}, while mass

spectroscopy showed MH^+ peak at *m/z* 399, which is consistent with the structure shown.

Dimer **140** was also prepared in 68% isolated yield by coupling 2-(4'-carboxyphenyl)-5-nitrobenzimidazole (**105**) and 2-pyridin-2-yl-1*H*-benzimidazol-5-ylamine (**102**) using EDC/HOBt. After 8 days no starting material remaining (**Scheme 28**). Spectroscopic data (NMR and MS) confirmed the formation of this dimer.

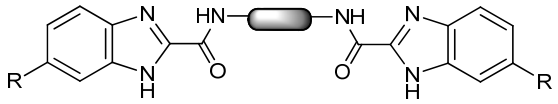
The IR for **140** showed an NH stretch at 3400 cm^{-1}, and the ^1H NMR showed NH amide as a singlet peak at 10.29 ppm and the imidazole NH as a broad singlet peak in the range of 8.49-8.51 ppm. Whilst the MH^+ ion appeared in MS as the base peak at *m/z* 476.

The extended dimer **141** was also prepared by coupling 2-(4'-carboxyphenyl)-5-nitrobenzimidazole (**105**) and 4-(1-*H*-benzimidazol-2-yl)cyclohexyl)methanamine (**112**), however **141** was isolated after 6 days reaction in 70% yield (**Scheme 28**).

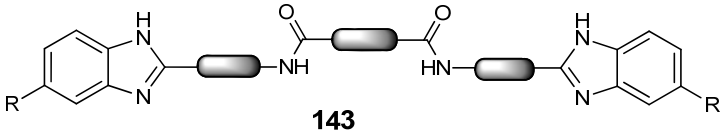
This was confirmed by ^1H NMR of **141** which showed a triplet peak at 8.62 ppm for the NH amide, while the imidazole NH appears as a broad peak in the range of 11.96-12.39 ppm, and the two methylene protons appear as triplets at 3.23 ppm. The ^{13}C NMR showed methylene carbon at 45.8 ppm, and cyclohexyl carbons in the range of 30.5-38.3 ppm, whilst The MS showed MH^+ peak at *m/z* 495 and the IR showed an NH stretch at 3410 cm^{-1}.

2.5 Synthesis of C2-C2 and C5-C5 symmetrical bis(benzimidazoles)

Synthesis of dimeric systems can be achieved by two different methods. Firstly, coupling one equivalent of a diamine with two equivalents of 2- or 5-carboxy benzimidazoles or secondly, coupling one equivalent of a dicarboxylic acid with two equivalents of 2-aminobenzimidazoles. These methods afford two different types of orientation, head-to-head (C2-C2) and tail-to-tail (C5-C5) bis(benzimidazoles) (**Figure 47**).

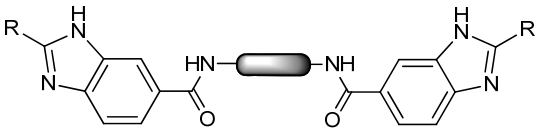


142



143

C2-C2 head-to-head bis(benzimidazoles)



144

C5-C5 tail-to-tail bis(benzimidazoles)

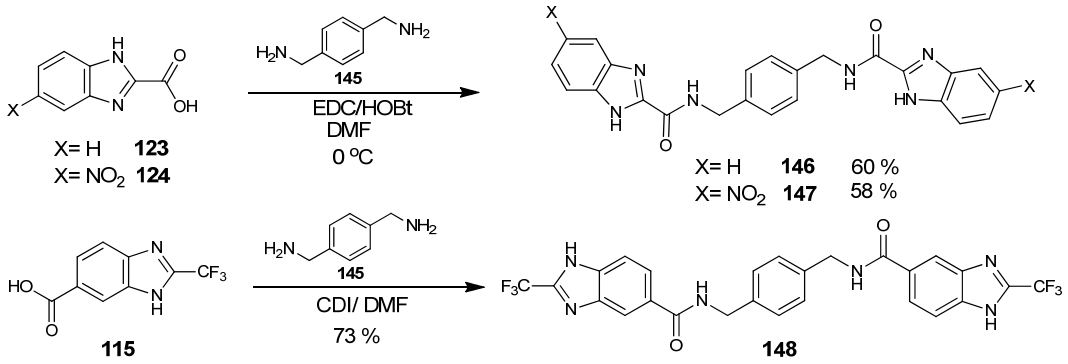
Figure 47 Generic structures for head-to-head and tail-to-tail symmetrical bis(benzimidazoles)

2.5.1 Coupling of bisamine with monocarboxybenzimidazole

For the synthesis of such bis(benzimidazoles), *p*-xylenediamine (**Scheme 29**) and ethylenediamine (**Scheme 30**) were selected as the bis(amines). These were coupled with three 2- and 5-carboxybenzimidazoles **123, 124** and **115** affording novel symmetrical bis(benzimidazoles) **146-148**.

2.5.1.1 Coupling of *p*-xylenediamine with 2- or 5-carboxybenzimidazole.

In these syntheses, coupling of 2-carboxybenzimidazole **123** or 2-carboxy-5-nitro benzimidazole **124** with *p*-xylenediamine **145** was achieved using 1:1 EDC/HOBt at 0 °C. The reactions went to completion after 8-14 days affording the desired amides **146** and **147** in 60 and 58% yield, respectively. However, the reagent system did not work using 5-carboxy-2-trifluromethylbenzimidazole (**115**) and no product could be isolated from these conditions. Employing CDI facilitated this coupling affording dimer **148** in 73 % yield after 9 days (**Scheme 29**).



Scheme 29 Symmetrical bis(benzimidazoles) from *p*-xylenediamine

Spectroscopic data supported the structures of these dimers in all cases. The ^{1}H NMR of compounds **146, 147** and **148** showed benzylic methylene groups at 4.48 and 4.49 ppm while the characteristic signals of amide protons were seen at 9.53, 9.77 and 9.16 ppm respectively (see **Figure 48** for ^{1}H NMR of **146**). The mass spectra showed the MH^{+} ion for all dimers as the base peak at *m/z* 425, 515 and 561 respectively. The ^{13}C NMR showed the characteristic signals for the amide carbonyl at 159.2 and 166.5 ppm respectively for **146** and **148** (see **Figure 49** for ^{13}C NMR of **146**).

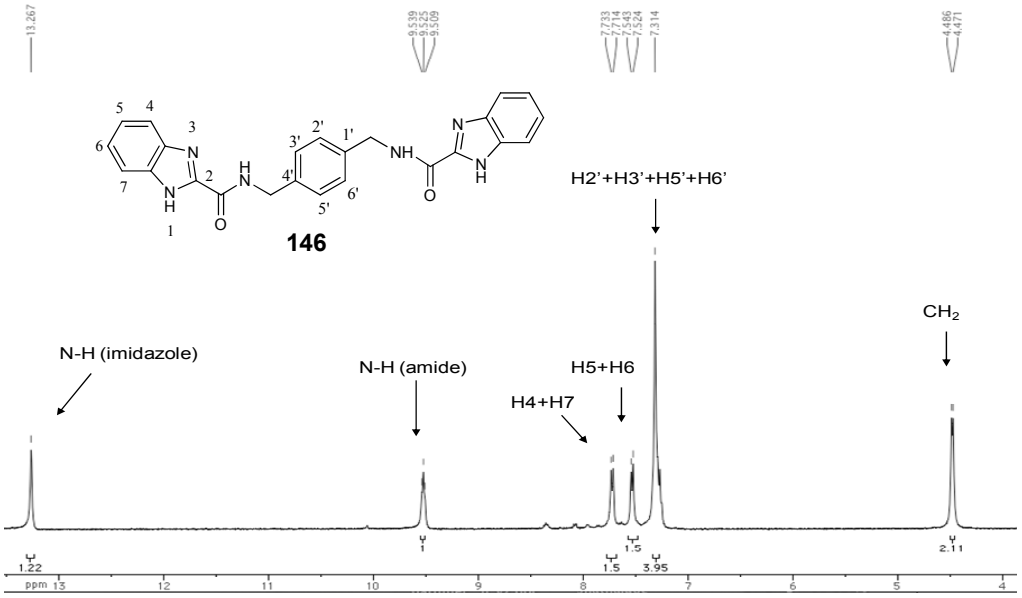


Figure 48 ^{1}H NMR (400 MHz) of 146 d_6-DMSO #

This assignment has been confirmed by using 2D NMR (COSY)

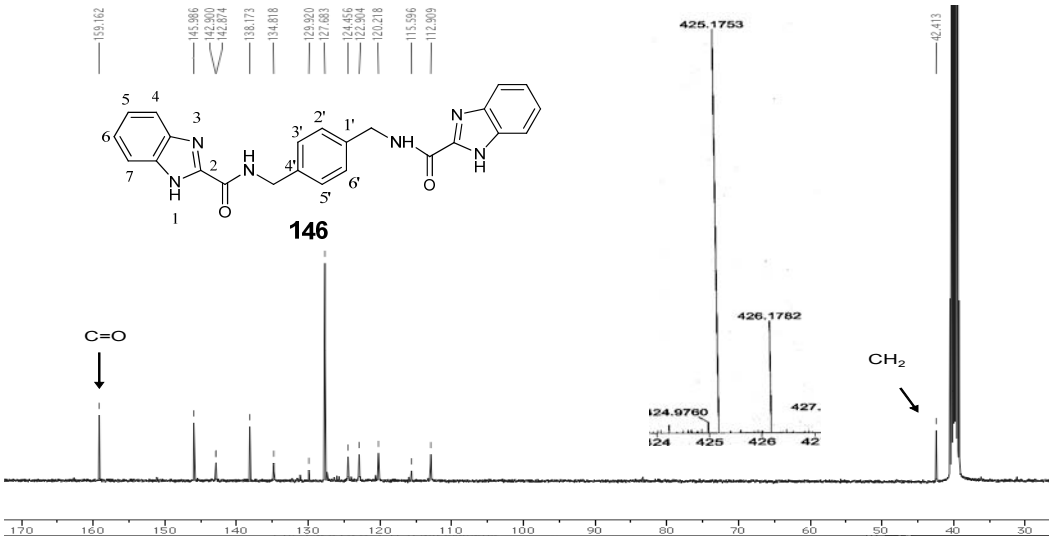
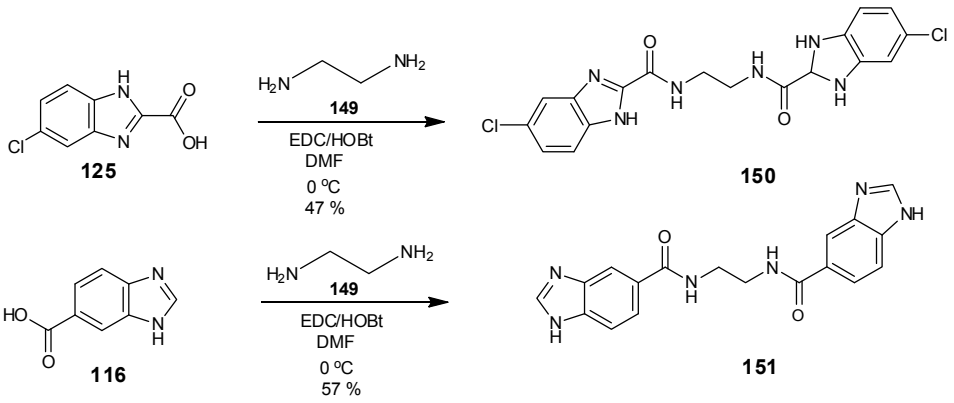


Figure 49 ^{13}C NMR (100 MHz) of 146 d_6-DMSO, the inset shows LR mass spectrum

2.5.1.2 Coupling of ethylene diamine with of 2- or 5-carboxybenzimidazole.

In these syntheses coupling of 2-carboxy-5-chlorobenzimidazole (**125**) or 5-carboxybenzimidazole (**116**) with ethylenediamine **149** was achieved using 1:1 EDC/HOBt at 0 °C. The reactions went to completion after 5-6 days affording the desired amides **150** and **151** in 47 and 57 % yield, respectively.



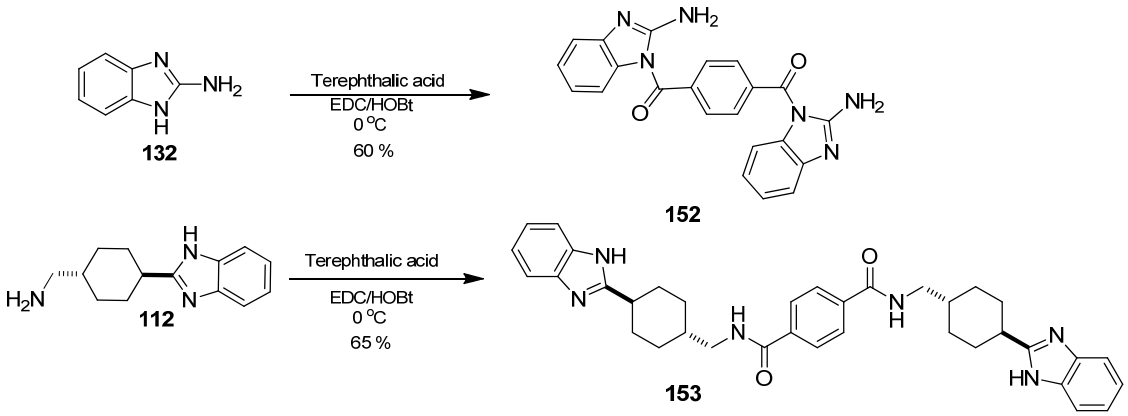
Scheme 30 Symmetrical bis(benzimidazoles) synthesis from coupling ethylene diamine with 2- or 5-carboxybenzimidazole

Similarly, the ^{1}H NMR of the compounds **150** and **151** showed methylene groups at 3.55 and 3.50 ppm respectively, while the characteristic signals of amide protons were seen at 9.22 , 8.61 and 8.65 ppm respectively. The dimer **150** showed the base peaks *m/z* at 417

and dimer **151** showed MH^+ peak at *m/z* 349. The ^{13}C NMR showed the characteristic signals for the amide carbonyl at 159.0 and 167.7 ppm respectively for **150-151** .

2.5.2 Coupling of benzimidazole amine with dicarboxylic acid

For the synthesis of such symmetrical bis(benzimidazoles), terephthalic acid was coupled with two different aminobenzimidazoles **132** and **112** affording the novel symmetrical bis(benzimidazoles) **152** and **153** in 60 and 65 % yields respectively (**Scheme 31**).



Scheme 31 Symmetrical bis(benzimidazoles) from coupling 2-aminobenzimidazole with terephthalic acid

Dimer **152** was formed in 60% yield using 2-aminobenzimidazole (**132**) and terephthalic acid coupled by using EDC/HOBt in DMF for 14 days. This product was confirmed by MS which showed *m/z* as the base peak at 397. The ^1H NMR showed the two NH_2 protons as a broad peak in the range of 3.25-3.69 ppm and the eight aromatic protons as two doublet of doublets at 7.32 and 7.62 ppm (**Figure 50**). ^{13}C NMR showed C=O at 165.6 ppm.

The dimeric amide **153** was formed in 65 % yield using the same method, with 4-(1-*H*-benzimidazol-2-yl)cyclohexyl)methan amine (**112**) and terephthalic acid. This product was confirmed by ^1H NMR through the appearance of amide protons at 8.66 ppm as a triplet peak and the eight aromatic protons as two doublet of doublets at 7.53 and 7.78 ppm. ^{13}C NMR showed C=O at 165.7 ppm (**Figure 51**), the MS showed *m/z* for doubly charged peak at 295/589 (see **Figure 52** for **153**).

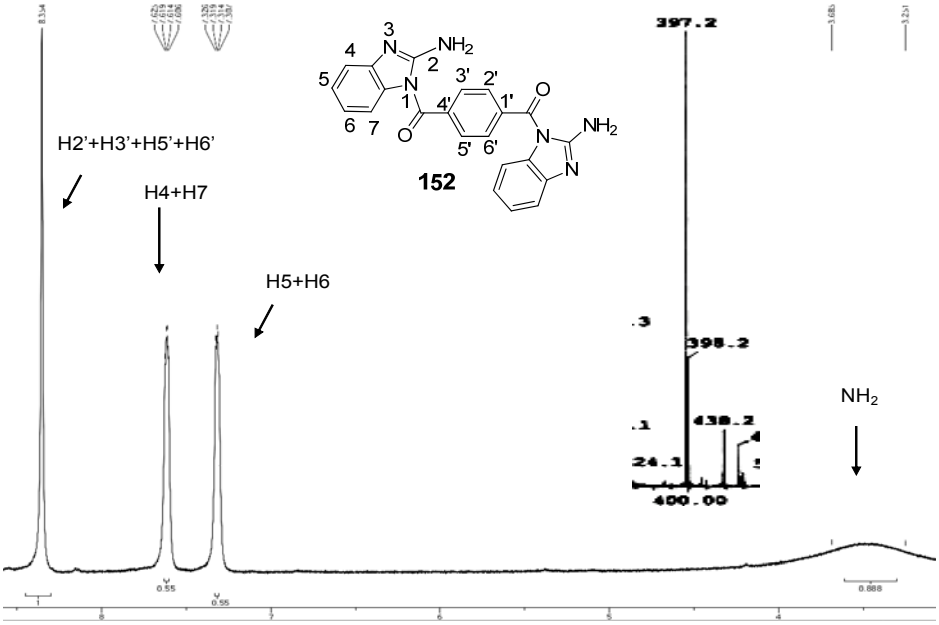


Figure 50 ^{1}H NMR (400 MHz) of 152 d_6-DMSO [#], the inset shows LR mass spectrum

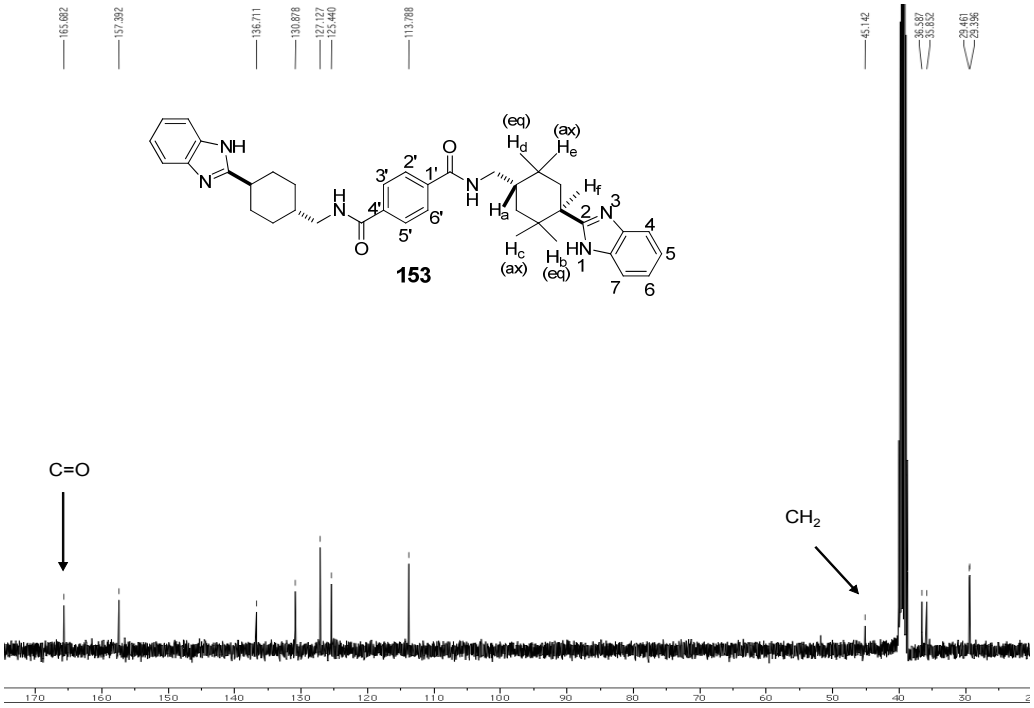


Figure 51 ^{13}C NMR (100 MHz) of 153 d_6-DMSO

[#] This assignment has been confirmed by using 2D NMR (COSY)

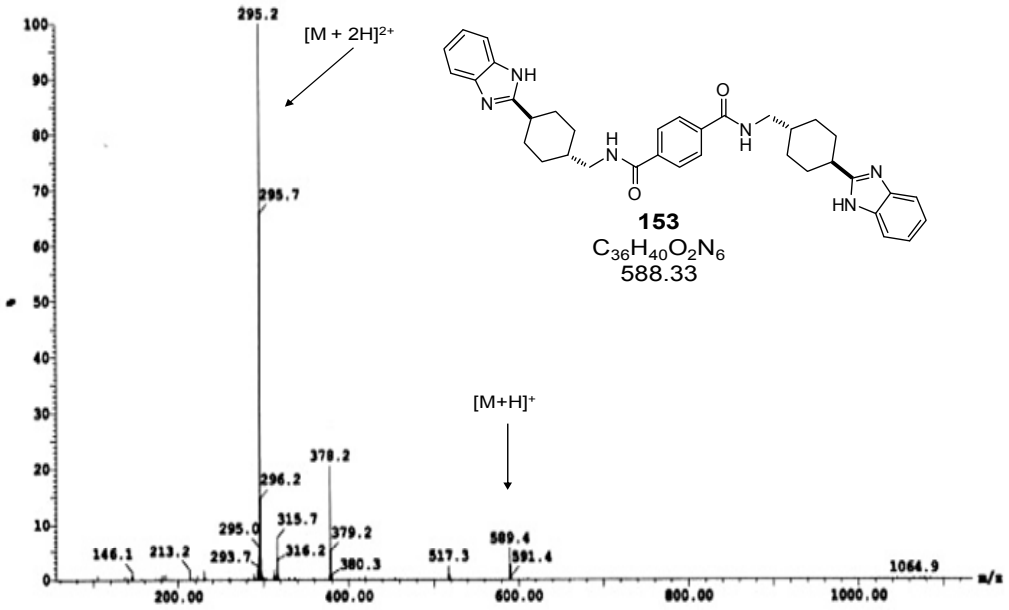


Figure 52 LRMS of 153

A number of unsymmetrical and symmetrical bis(benzimidazoles) were prepared with different directions of amide linked C2-C2 (head-to-head), C2-C5 (head-to-tail) and C5-C5 (tail-to-tail) linkages, these dimers differ in having a 2-CO_2H or 5-CO_2H directly on the benzimidazole ring, or a aryl spacer at C2. These were coupled with amines by using two different coupling reagents (EDC/HOBt) for the 2-carboxybenzimidazole and (CDI) with the 5-carboxybenzimidazoles.

In parallel, a series of dimers were also prepared from amines (2-NH_2 or 5-NH_2), thus in effect using the reverse direction of amide bond.

Chapter 3

Synthesis of trimeric and tetrameric benzimidazoles and bis(benzimidazoles) piperidine derivatives

3.1 Synthesis of amide linkage trimeric benzimidazole

Three different routes were used in order to synthesize tris(benzimidazoles) as follows.

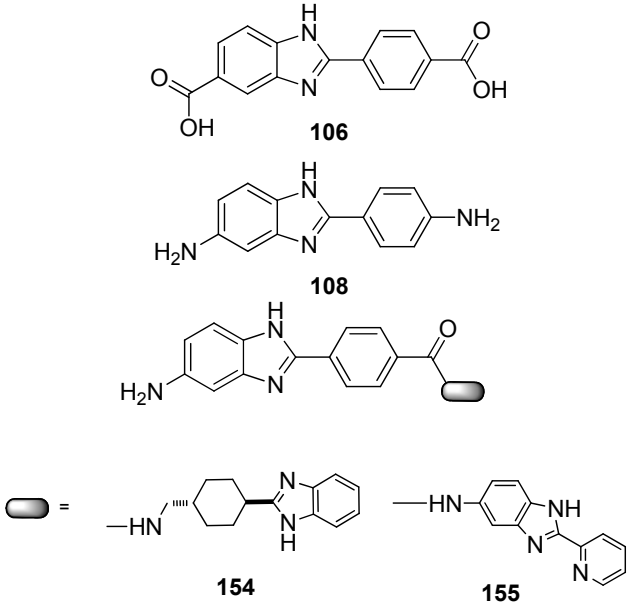
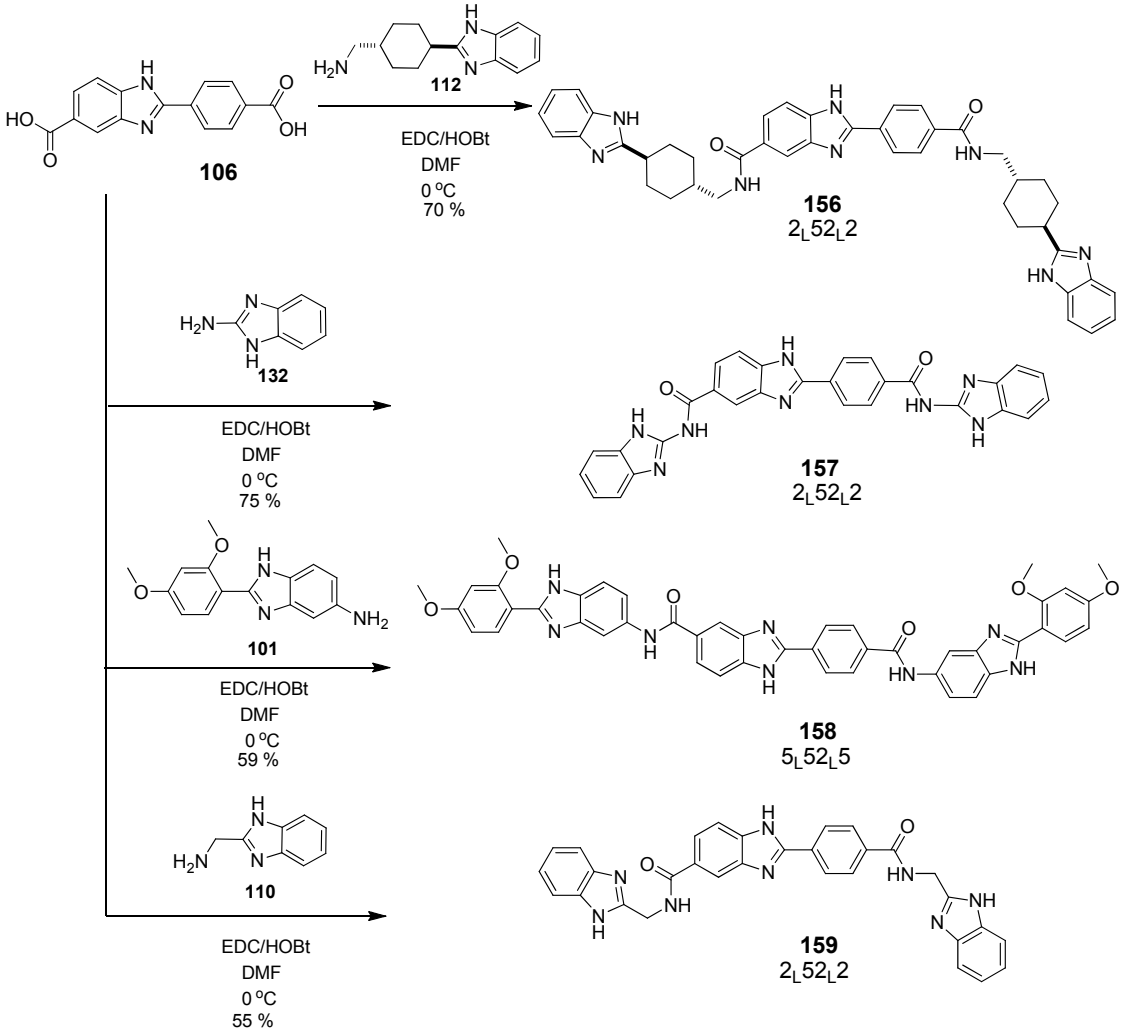


Figure 53 Starting materials used in synthesis of trimeric benzimidazole

3.1.1 Bifunctionality of 2,5-dicarboxybenzimidazole: -

Several novel tris(benzimidazoles) were synthesized *via* the utilization of the bifunctionality of 2,5-dicarboxy benzimidazole **106** in which one equivalent of the diacid was coupled with two equivalents of different 2- or 5-aminobenzimidazoles.

Using this method the trimers **156-159** were synthesized in yields of 55-75% (**Scheme 32**).



Scheme 32 Tris(benzimidazole) synthesis from coupling of 2,5-dicarboxybenzimidazole and 2- or 5-aminobenzimidazoles

The four trimers were prepared by using EDC/ HOBt (**Scheme 32**). Trimers **158** and **159** were obtained after 14 days, whilst **156** was a 12 days reaction and **157** was obtained after 13 days when TLC showed no starting material remaining and the desired trimer **157** was isolated in 75 % yield (**Scheme 32**).

The ^{1}H NMR for trimer **156** showed N-H amide protons as triplet at 8.47 and 8.64 ppm, whilst four methylene protons appear clearly as triplet at 3.23 ppm. The ^{13}C NMR showed the characteristic signals for two amide carbonyls at 164.7 and 165.8 ppm, and methyelene carbons at 44.4 ppm, the MS showed MH^{+} ion at *m/z* 705. The IR showed the C=O at 1622 and 1636 cm^{-1} and the NH stretch in the range 3245-3395 cm^{-1}.

The IR for **157** showed an NH stretch at 3364-3534 cm^{-1} and C=O at 1653 and 1664 cm^{-1}, and the ^{1}H NMR showed NH amide as a singlet peaks at 8.41 and 8.58 ppm respectively, while the ^{13}C NMR showed two amide C=O peaks at 152.7 and 153.6 ppm respectively. The mass spectra showed MH^{+} ion as a base peak at *m/z* 513.

The ^{1}H NMR for trimer **158** showed N-H amide protons as singlet peaks at 10.69 and 10.79 ppm. The protons of the methoxy groups as singlets at 3.94 and 4.11 ppm (**Figure 54**). The ^{13}C NMR showed the characteristic signals for two amide carbonyls at 163.9 and 164.9, whilst the MS showed MH^{+} peak at *m/z* 785. The IR showed the C=O at 1640 and 1653 cm^{-1} and the NH stretch at 3282-3507 cm^{-1}.

The mass spectra for **159** showed MH^{+} ion as a base peak at *m/z* 541, whilst ^{1}H NMR showed N-H amide protons as triplet at 9.19 and 9.34 ppm respectively, while the four methylene protons appear clearly as doublet at 4.73 ppm. The structure was confirmed by IR which showed NH stretch at 3204-3555 cm^{-1} and the two C=O at 1640 and 1647 cm^{-1}. The ^{13}C NMR showed methylene carbons at 37.9 ppm, and two amide C=O peaks at 165.9 and 166.9 ppm respectively.

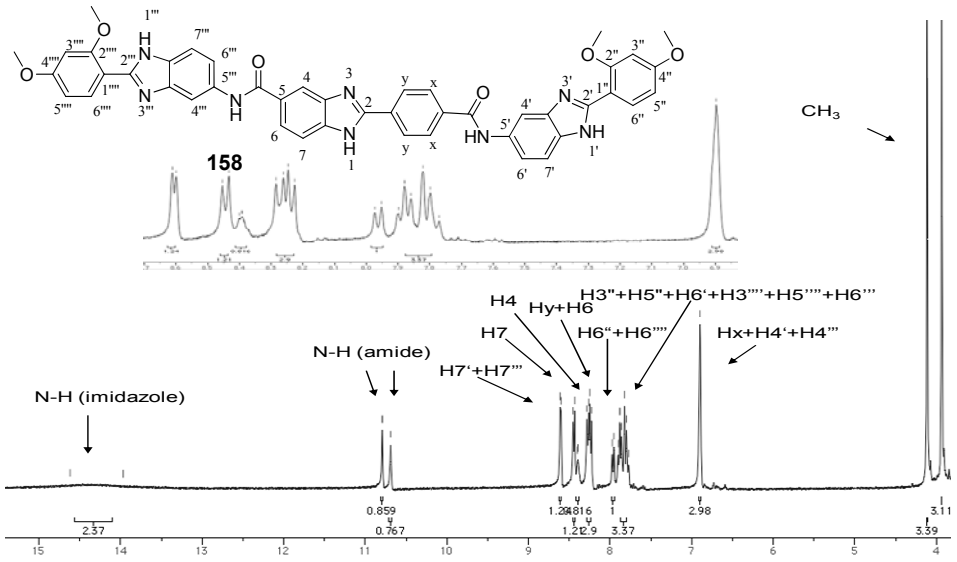


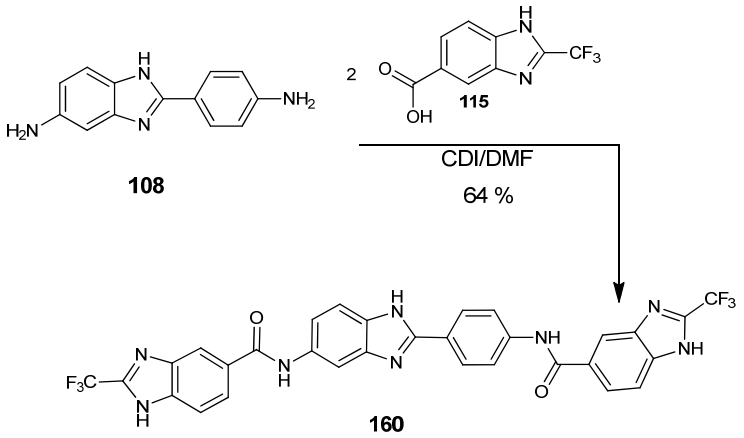
Figure 54 ^{1}H NMR (400 MHz) of 158 in d_6-DMSO [#], the inset shows expansion for the aromatic part

[#] This assignment has been confirmed by using 2D NMR (COSY)

3.1.2 Bifunctionality of 2,5-diaminobenzimidazole.

With 2,5-diaminobenzimidazole **108** in hand, novel tris(benzimidazoles) were synthesized *via* utilization of the bifunctionality of this diaminobenzimidazole in which one equivalent of the diamine was coupled with two equivalents of 2- or 5-carboxybenzimidazoles **124** and **115**.

Attempts to prepare trimer **160**, using EDC/HOBt with 5-carboxy-2-trifluro methylbenzimidazole (**115**) failed and the starting material was recovered in near-quantitative amount. However, pleasingly, employing CDI facilitated this coupling affording trimer **160** in 64 % yield after 12 days (**Scheme 33**).

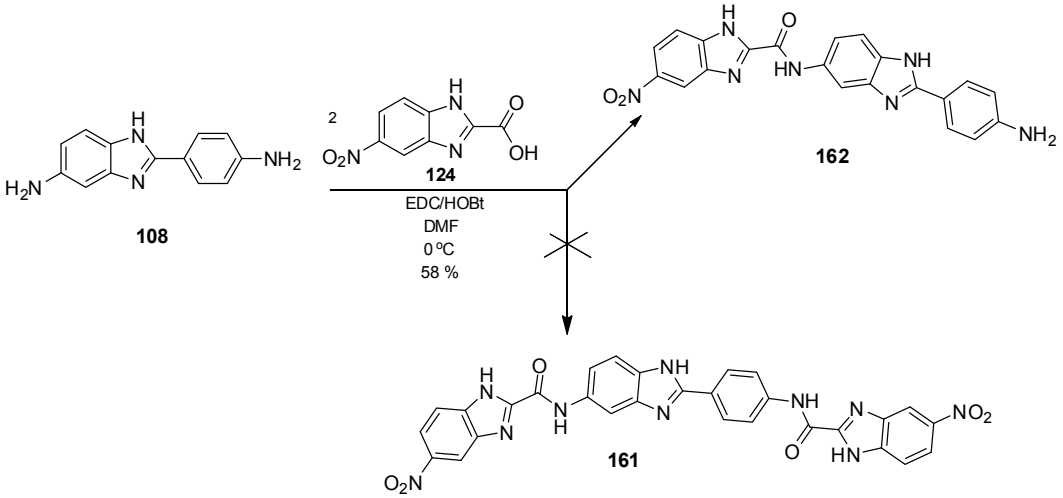


Scheme 33 Synthesis of tris(benzimidazole) *via* coupling of diaminobenzimidazole 108 and carboxybenzimidazole 115

The ^{1}H NMR of this trimer showed two different amide protons appearing at 10.65 and 10.79 ppm. The MS showed the MH^{+} peak at *m/z* 649. ^{13}C NMR showed two amide carbonyls at 165.9 and 166.2 ppm and two CF_3 carbons which are coincident at 142.9 ppm appearing as a quarter with coupling constant (J_{CF} = 39.6 Hz). The IR showed the C=O at 1633 and 1653 cm^{-1} and the NH stretch in the range 3114-3420 cm^{-1}

For attempted coupling with 2-carboxy-5-nitrobenzimidazole (**124**) and 5-amino-2-(4-aminophenyl) benzimidazole (**108**), using EDC/HOBt in DMF or CDI in DMF, but this was unsuccessful, No trimer **161** could be isolated from these conditions (**Scheme 34**). The dimeric amide **162** was formed in 58 % yield after 17 days.

All data was consistent with this assigned structure.



Scheme 34 Synthesis of dimeric benzimidazole *via* coupling of diaminobenzimidazole 108 and carboxybenzimidazole 124

The structure was confirmed by ^{1}H NMR which showed the amide proton as a singlet proton at 11.43 ppm, while the NH_2 protons appear as a broad peak in the range of 6.04-6.56 ppm. The MH^+ ion appeared in the MS as a base peak at *m/z* 414. The ^{13}C NMR showed a C=O peak at 156.6 ppm (**Figure 55**).

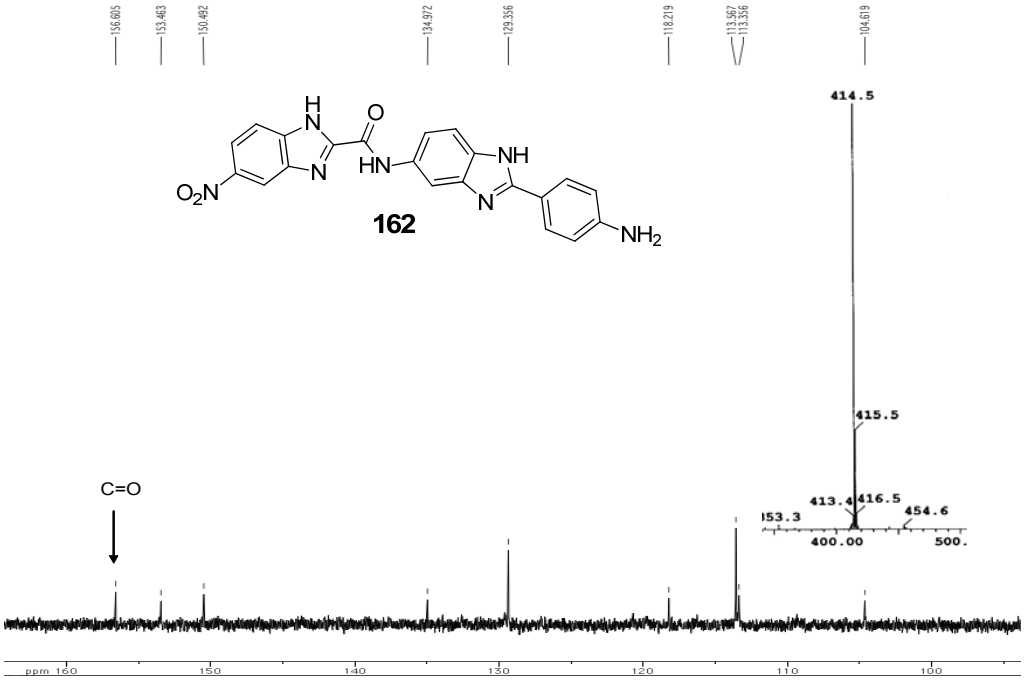
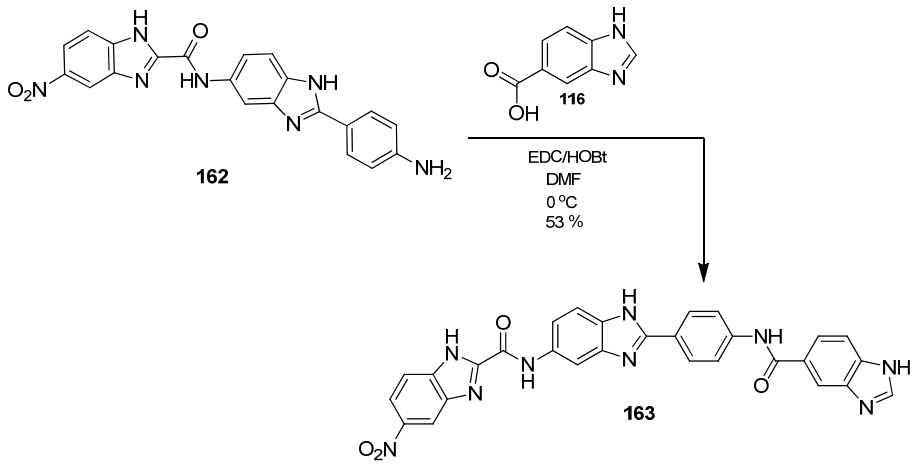


Figure 55 ^{13}C NMR (100 MHz) of 162 d_6-DMSO, the inset shows LR mass spectrum

The dimeric amide **162** was converted to the trimer **163** in 53 % yield *via* its coupling with 5-carboxybenzimidazole (**116**) over 12 days (**Scheme 35**). The ^{1}H NMR of the trimer **163** showed two amide protons at 10.66 and 11.43 ppm. The MS showed the MH^{+} peak at *m/z* 558. The IR showed the C=O at 1622 and 1659 cm^{-1}.



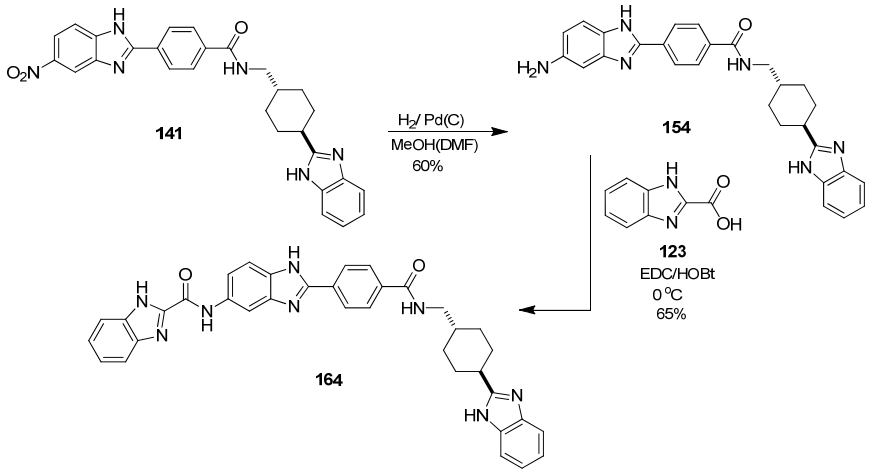
Scheme 35 Tris(benzimidazole) synthesis from coupling of dimeric amide 162 and 5-carboxybenzimidazole

3.1.3 Extendability feature of nitro bis(benzimidazole)

Bis(benzimidazoles) bearing a nitro group can be utilized for further coupling, in which the nitro group can be reduced to the corresponding amine which can be used as an amine for coupling with other carboxybenzimidazoles. Two examples of this type of trimer **164** and **165** were synthesized in this way.

3.1.3.1 Synthesis of trimer 164

The nitrobis(benzimidazole) **141** was reduced by using H_2/Pd(C) for 5 days affording the desired amine **154** in 60 % yield.



Scheme 36 Tris(benzimidazole) synthesis from coupling of aminobis(benzimidazole) and carboxybenzimidazole

The ^{1}H NMR of **154** showed a change in chemical shift of the amide proton from 8.62 ppm in the nitrobis(benzimidazole) **141** to 8.63 ppm. Moreover, the ^{13}C NMR appeared C=O at 166.7 ppm compared with that in the nitro compound at 169.7 ppm. The amine **154** showed *m/z* for doubly charged peak at 233/465 (**Figure 56**).

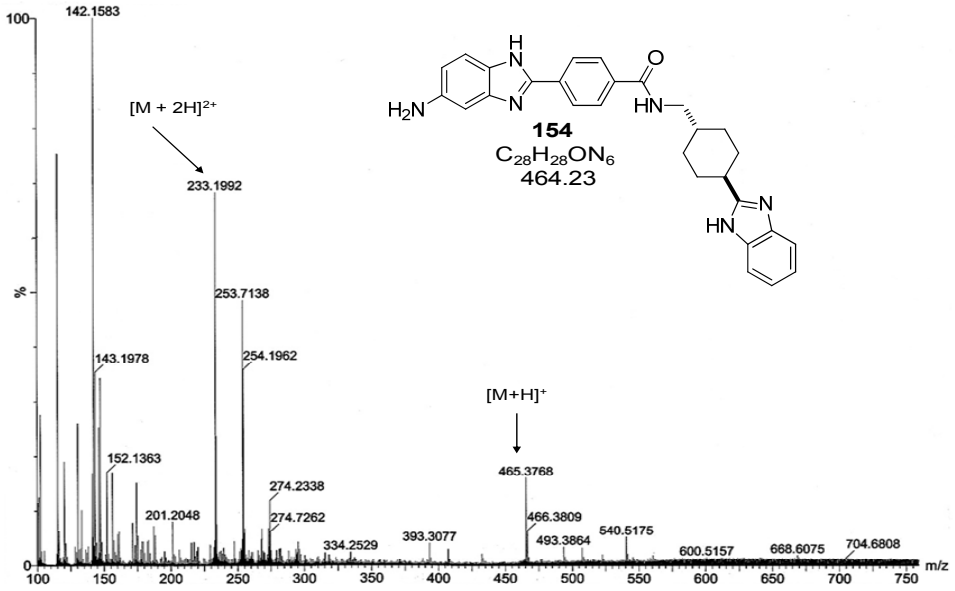


Figure 56 LRMS of 154

This aminobis(benzimidazole) **154** was then converted to the trimer **164** in 65 % *via* coupling with 2-carboxybenzimidazole (**123**) (**Scheme 36**). The ^{1}H NMR of the trimer

164 showed the appearance of two amide protons as triplet at 8.73 and a single peak 11.03 ppm. Mass spectral data was also consistent with a base peak at *m/z* 609 (**Figure 57**), whilst ^{13}C NMR showed C=O resonsances at 165.1 and 165.7 ppm.

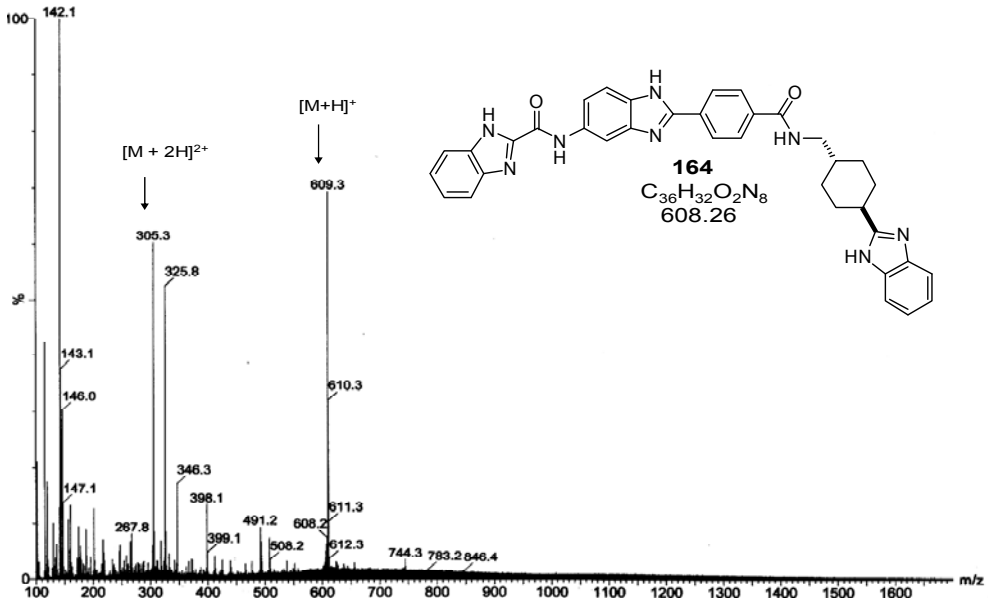
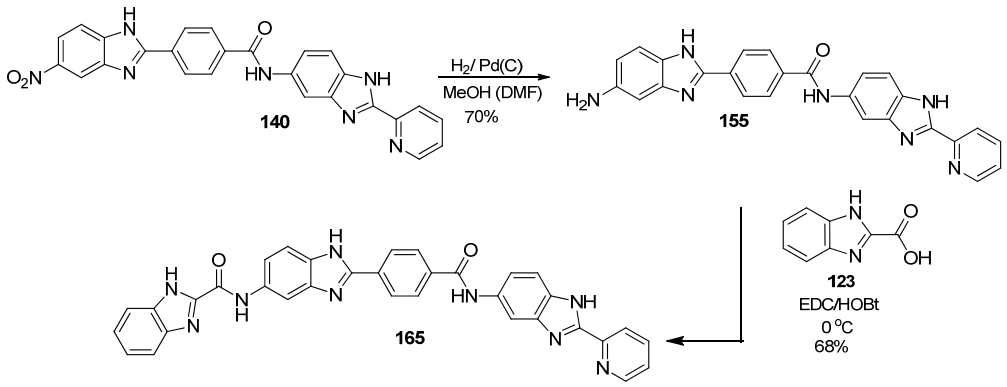


Figure 57 LRMS of 164

3.1.3.2 Synthesis of trimer 165

The nitrobis(benzimidazole) **140** was reduced by using H_2/Pd(C) for 6 days affording the desired amine **155** in 70 % yield. ^1H NMR spectrum of this amino dimer showed a change in chemical shift for the amide NH from 10.29 ppm in nitro dimer **140** to 10.42 ppm, and IR which showed the C=O at 1638 cm^{-1} (compared with the nitro compound **140** at 1654 cm^{-1}). The compound **155** showed the base peak at *m/z* 446.

Aminobis(benzimidazole) **155** was converted to the trimer **165** in 68 % yield *via* its coupling with 2-carboxybenzimidazole (**123**) over 7 days (**Scheme 37**). The ^1H NMR of the trimer **165** showed two amide protons at 8.43 and 10.48 ppm. The ^{13}C NMR showed C=O signals at 164.5 and 166.6 for the two different carbonyls whilst mass spectral data showed the molecular ion at *m/z* 590.



Scheme 37 Coupling of bis(benzimidazole) amine with 2-carboxybenzimidazole towards tris(benzimidazole) synthesis

3.2 Synthesis of symmetrical tetrabenzimidazole

Symmetrical tetramers were prepared from two different bisbenzimidazole diacids **166** and **167** (**Figure 58**). These differ in having a 2-CO_2H directly on the benzimidazole ring, or a aryl spacer at C2. These were coupled with amines.

In parallel, a series of tetramers were also prepared from diamine **168** and **169** (**Figure 58**), thus in effect using the reverse direction of amide bond.

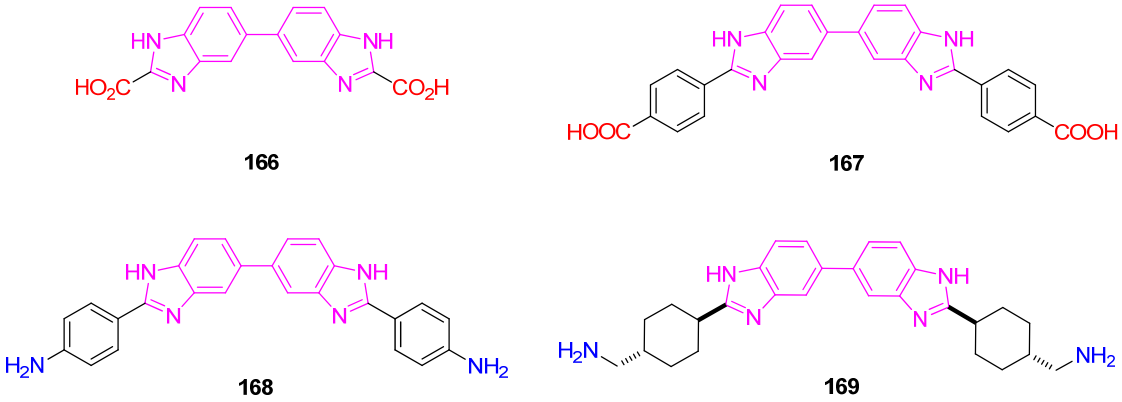
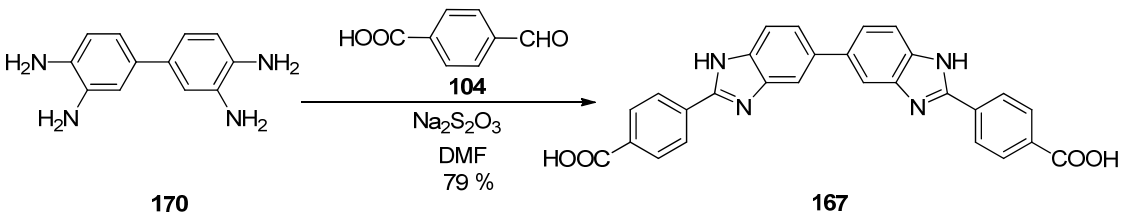


Figure 58 Structures of bisbenzimidazole diacids and diamines used in synthesis of symmetrical tetrabenzimidazoles

3.2.1 Amidation of aminobenzimidazoles *via* 2,2 bis-carboxybenzimidazole

Appaorach to symmetrical tetrabenzimidazoles employed the bis (carboxy benzimidazole) **167** as a precursor for coupling with two equivalents of 2- or 5-aminobenzimidazole (**112**, **132**, **101** and **110**).



Scheme 38 Synthesis of bis(carboxybenzimidazole) 167

This diacid **167** was synthesized in 79 % yield by condensation of 3,3'-diamino benzidine (**170**) with 4-carboxy benzaldehyde (**104**), in the presence of sodium meta bisulfite,[31] under conditions analogous to those used for preparation of the 2-aryl alkoxy derivatives described in **Section 2.1.1** (using DMF as a solvent and heating at 140 °C for 2 days)(**Scheme 38**).

All spectral data confirmed the structure. Mass spectral data showed a base peak with *m/z* 475, and IR showed NH stretching at 3450 cm^{-1} and C=O at 1682 cm^{-1}. The ^{13}C NMR showed two C=O peaks at 167.7 ppm and the ^{1}H NMR showed two doublets at 8.14 and 8.34 ppm related to H2'+H6' and H3'+H5' respectively. Two COOHs appear clearly as a broad peaks at 12.48-13.83 ppm in the ^{1}H NMR spectrum (**Figure 59**).

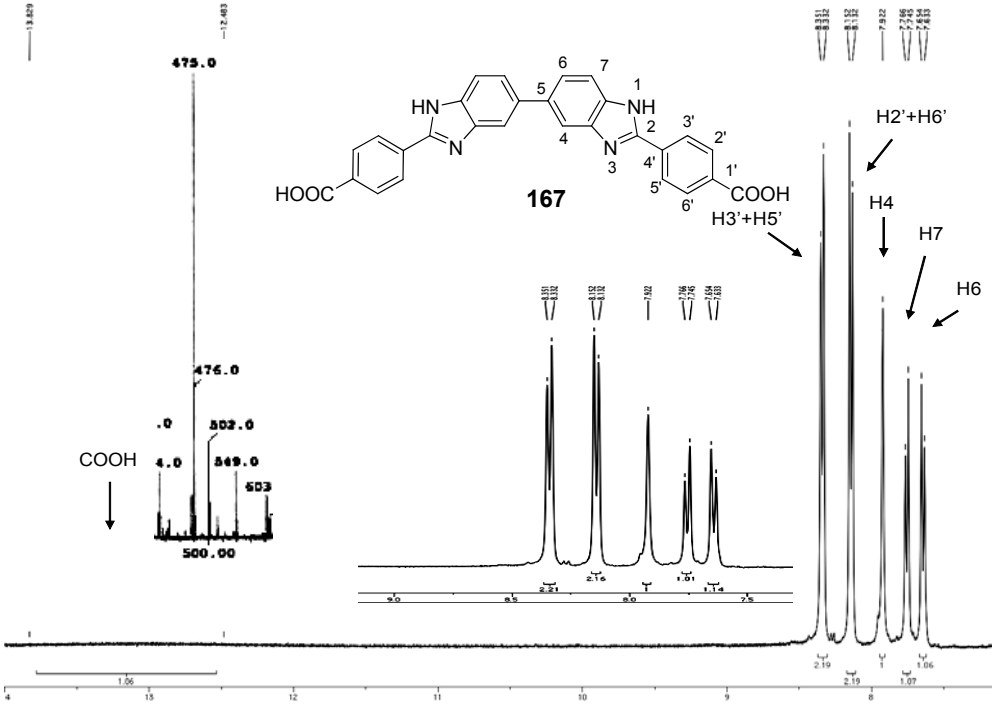


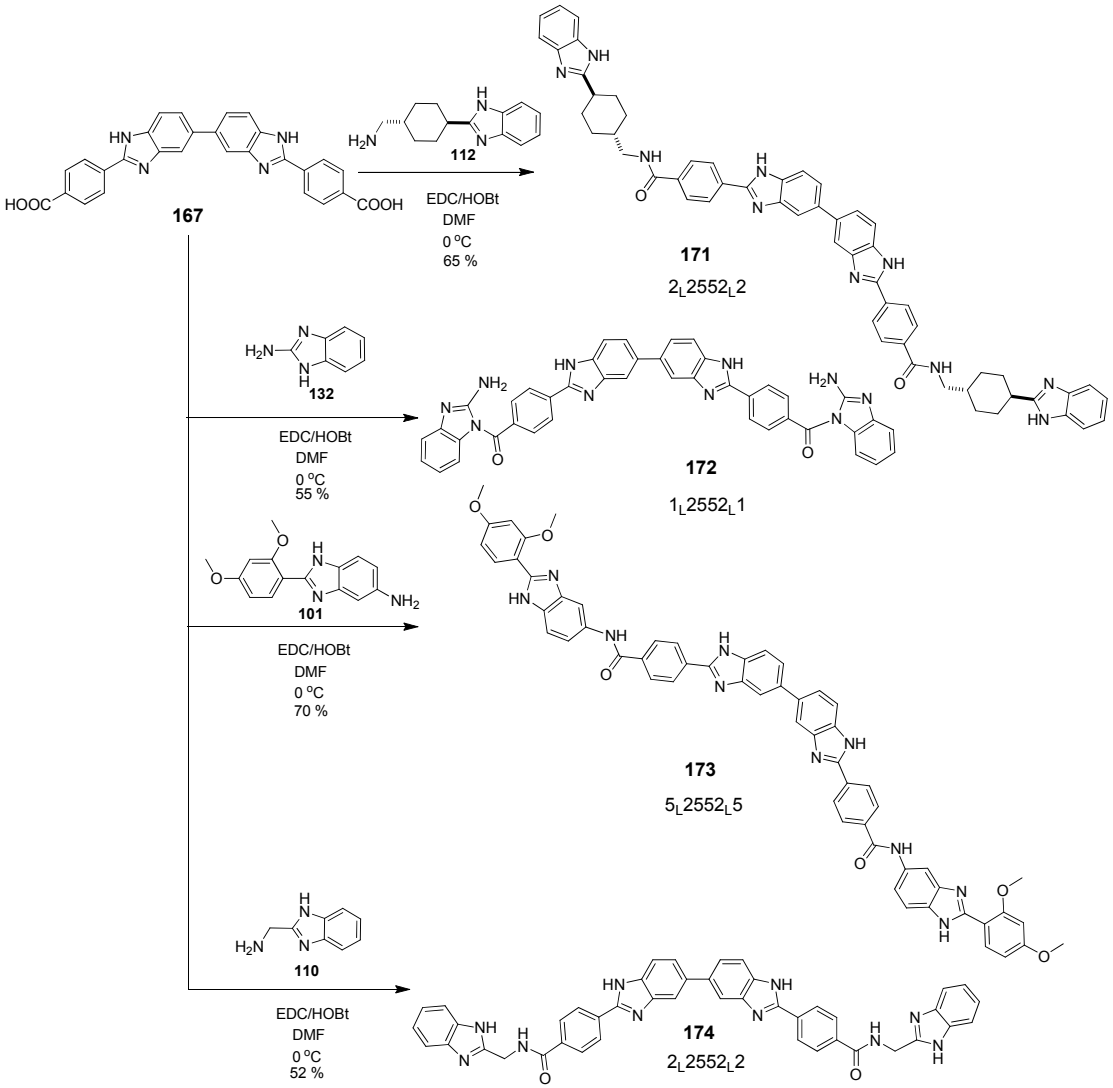
Figure 59 ^{1}H NMR (400 MHz) of 167 d_6-DMSO [#], the inset shows LR mass spectrum and expansion for the aromatic part

[#] This assignment has been confirmed by using 2D NMR (COSY)

3.2.2 Synthesis of symmetrical tetrameric benzimidazole from coupling of 167 and aminobenzimidazoles.

In this route symmetrical tetrabenzimidazoles were synthesized by reaction of one equivalent of bis(benzimidazole) **167** with two equivalents of the amines **112**, **132**, **101**and **110** using EDC/HOBt in DMF as follow.

Yields of tetrabenzimidazoles **171-174** were 52-70 % yield (**Scheme 39**).



Scheme 39 Synthesis of symmetrical tetra(benzimidazoles) 171-174

The four tetramers were prepared by using EDC/HOBt (**Scheme 39**). Tetramers **172** and **173** were obtained after 11 days, whilst **171** was a 14 days reaction and **174** was

obtained after 20 days when TLC showed no starting material remaining and the desired tetramer **174** was isolated in 52 % yield (**Scheme 39**).

The ^{1}H NMR of tetramer **171** showed N-H amide protons as triplet at 8.68 ppm, whilst four methylene protons appear clearly as triplet at 3.24 ppm. The ^{13}C NMR showed the characteristic signals for two amide carbonyls at 168.2 ppm, and methyelene carbons at 44.4 ppm, the MS showed MH^{+} peak at *m/z* 897. The IR showed the C=O at 1635 cm^{-1} and the NH stretch in the range 4040-3405 cm^{-1} .

The IR for **172** showed an NH stretch at 3378 cm^{-1} and C=O at 1630 cm^{-1} , and the ^{1}H NMR showed the two NH_2 protons as abroad singlet peaks in the range of 7.71-7.79 ppm and the eight aromatic protons as two doublet of doublets at 8.34 and 8.39 ppm respectively, while the ^{13}C NMR showed two amide C=O peaks at 168.1 . The MH^{+} ion appeared in the MS as a base peak at *m/z* 705.

Spectroscopic data (NMR and M.S) confirmed the formation of tetramer **173**, the ^{1}H NMR for tetramer **173** showed N-H amide protons as singlet peaks at 10.39 ppm. The protons of the methoxy groups as singlets at 3.88 and 4.05 ppm. The ^{13}C NMR showed the characteristic signals for two amide carbonyls at 165.0, whilst the MS showed MH^{+} ion at *m/z* 977. The IR showed the C=O at 1636 cm^{-1} and the NH stretch at 3416-3462 cm^{-1}.

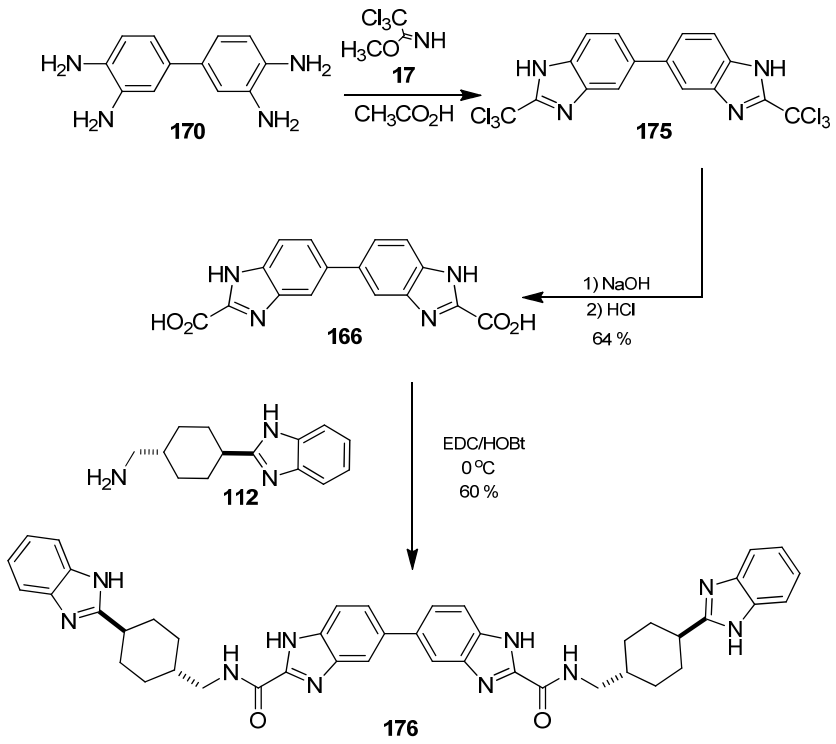
The MH^{+} ion of tetramer **174** appeared as a base peak at *m/z* 733, whilst ^{1}H NMR showed N-H amide protons as triplet at 9.53 ppm, while the four methylene protons appear clearly as triplet at 4.77 ppm . The structure was confirmed by IR which showed NH stretch at 3187-3400 cm^{-1} and the C=O at 1615 cm^{-1}. The ^{13}C NMR showed methylene carbons at 37.8 ppm, and two amide C=O peaks at 165.9 ppm.

3.2.3 Synthesis of tetramer 176

Alternatively to the 2-*p*-carboxy phenyl group of **167**, systems using the 2-CO_2H bisbenzimidazole (in effect thus lacking the phenyl ring of **167**).

Condensation of 3,3'-diaminobenzidine (**170**) in which condensation of bisdiamine with trichloroacetimidate (**17**) afforded bis(2-trichloromethyl)benzimidazole **175**.

Conversion of **175** to the corresponding dicarboxylic acid **166** under conditions analogous to those used for preparation of the 2-carboxy benzimidazoles described in **Section 2.3** in 64 % yield (**Scheme 40**).



Scheme 40 Synthesis of symmetrical tetra(benzimidazole)

The symmetrical bis(benzimidazole) **166** was identified by mass spectral data (*m/z* at 323 as the base peak), and the ^{1}H NMR which showed H6 as a doublet of doublets arising from coupling with H7 and H4 with two coupling constants ($J_{4,6}$ = 1.6 Hz and $J_{6,7}$ = 8.4 Hz), and H4 appeared as a doublet with J = 1.6 Hz, H7 appeared as a doublet with J = 8.4 Hz. A broad peak in the range of 9.98-10.14 ppm related to the two COOHs, and the two imidazole NH protons appear as singlet peak at 9.07 ppm. The ^{13}C NMR showed the carbonyl at 166.9 ppm (see **Figure 60** for ^{1}H NMR and ^{13}C NMR of **166**).

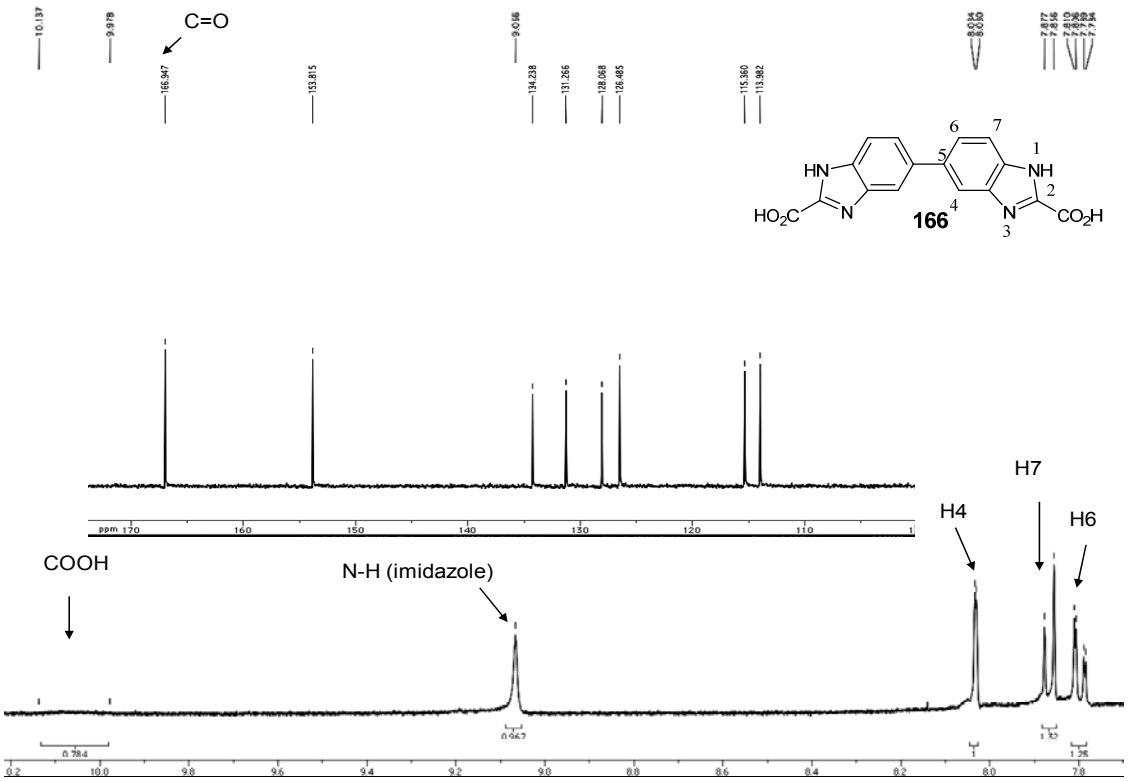


Figure 60 ^{1}H NMR (400 MHz) of 166 in d_6-DMSO [#],· the inset shows ^{13}C NMR (100 MHz) of 166 d_6-DMSO

Coupling of **166** with 4-(1-*H*-benzimidazol-2-yl)cyclohexyl) methanamine (**112**) using EDC/HOBt in DMF afforded the tetramer **176** in 60 % yield.

The ^{1}H NMR of the compound **176** showed good NMR evidence since the two equivalent amide protons appeared as triplet peak at 9.01 ppm, while the four methylene protons appeared as a triplet peak at 3.25 ppm. Spectral data confirmed the structure with IR showing two distinct peaks at the range 3215-3355 and 1661 cm^{-1} related to N-H and C=O stretching respectively, and the mass spectral data showed *m/z* for doubly charged peak at 373/745 (see **Figure 61** for LR mass spectrum of **176**).

[#] This assignment has been confirmed by using 2D NMR (COSY)

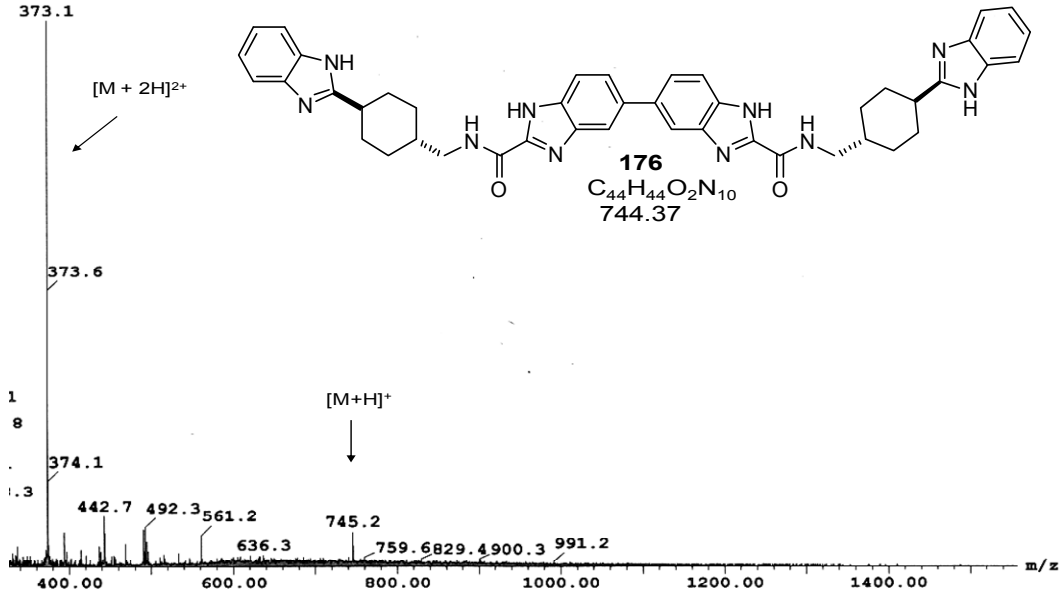
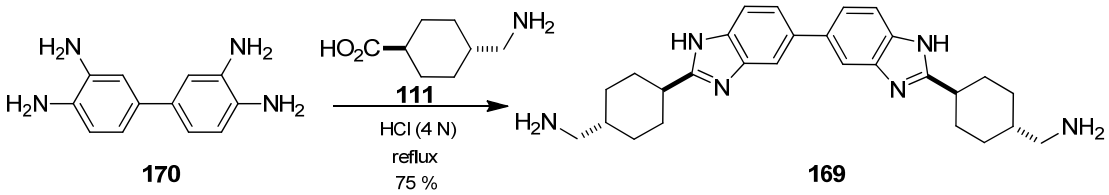


Figure 61 LRMS of 176

3.2.4 Amidation of carboxybenzimidazoles *via* diamino bis(benzimidazole)

Appaorach to a tetra(benzimidazole) employed the bis(aminobenzimidazole) **169** as a precursor for coupling with two equivalents of 5-aminobenzimidazole **112** and **123**.



Scheme 41 Synthesis of diaminobis(benzimidazole) 169

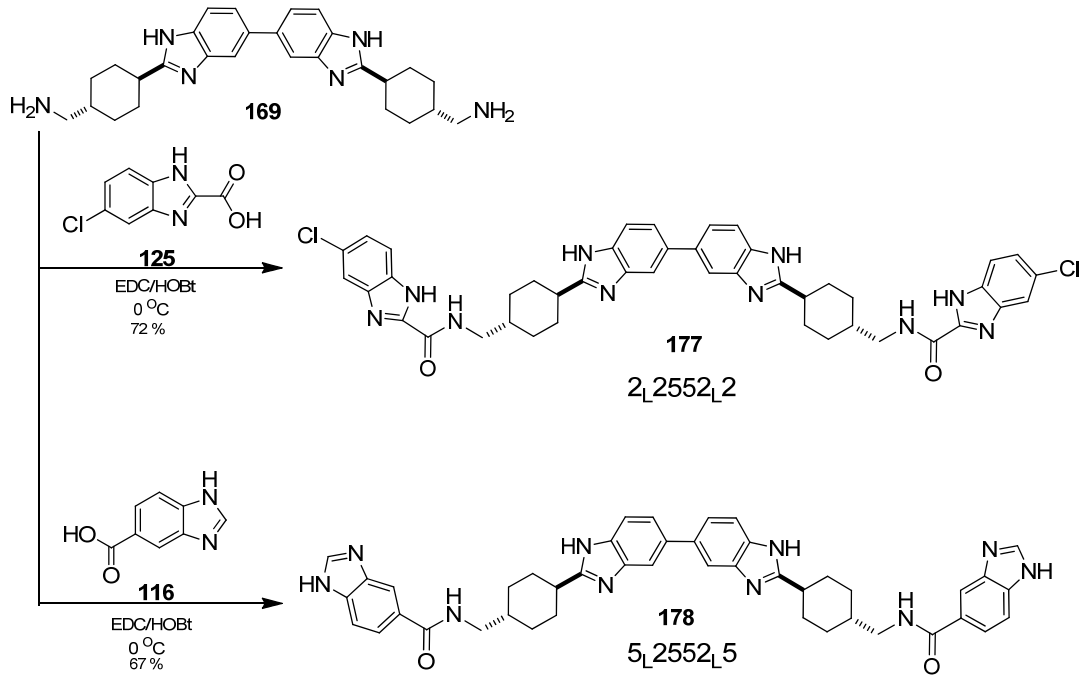
This diaminobis(benzimidazole) **169** was synthesized in 75 % yield by condensation of bis-*o*-diamine **170** with *trans*-4-aminomethycyclohexane carboxylic acid (**111**) using 4 N HCl for 72 h (**Scheme 41**).

Spectroscopic data supported the structure of this diamine. Mass spectral data showed the base peak with *m/z* 457, and ^{1}H NMR showed the two methylene protons as a doublet at 2.45 ppm. The ^{13}C NMR showed two methylene carbons at 48.6 ppm, and cyclohexyl carbons in the range of 30.5-40.6 ppm.

3.2.5 Synthesis of symmetrical tetrameric benzimidazole from coupling of 2,2 bis-aminobenzimidazole and 5-carboxybenzimidazoles

The tetra(benzimidazoles) were synthesized as symmetrical type in which one equivalent of diamine **169** was coupled with two equivalents of the acids **125** and **116** using EDC/HOBt in DMF as follows.

Tetra(benzimidazoles) **177** and **178** were synthesized in 67 and 72 % yield (**Scheme 42**).



Scheme 42 Synthesis of symmetrical tetra(benzimidazoles) from 2,2 bis-aminobenzimidazole

Similarly, ^{1}H NMR of the tetramers **177** and **178** showed the characteristic N-H amide proton as a triplet at 9.08 and 8.51 ppm for **177** and **178** respectively (see **Figure 62** for ^{1}H NMR of **178**), the tetramer **177** showed the base peaks *m/z* at 813 and tetramer **178** showed MH^{+} peak at *m/z* 745. The ^{13}C NMR showed the characteristic signals for the amide carbonyl at 157.3 and 166.8 ppm respectively for **177** and **178**. Spectral data confirmed the structure with IR showing two distinct peaks at 3400 and 3100-3364 related to N-H stretching and C=O at 1663 and 1621 cm^{-1} respectively.

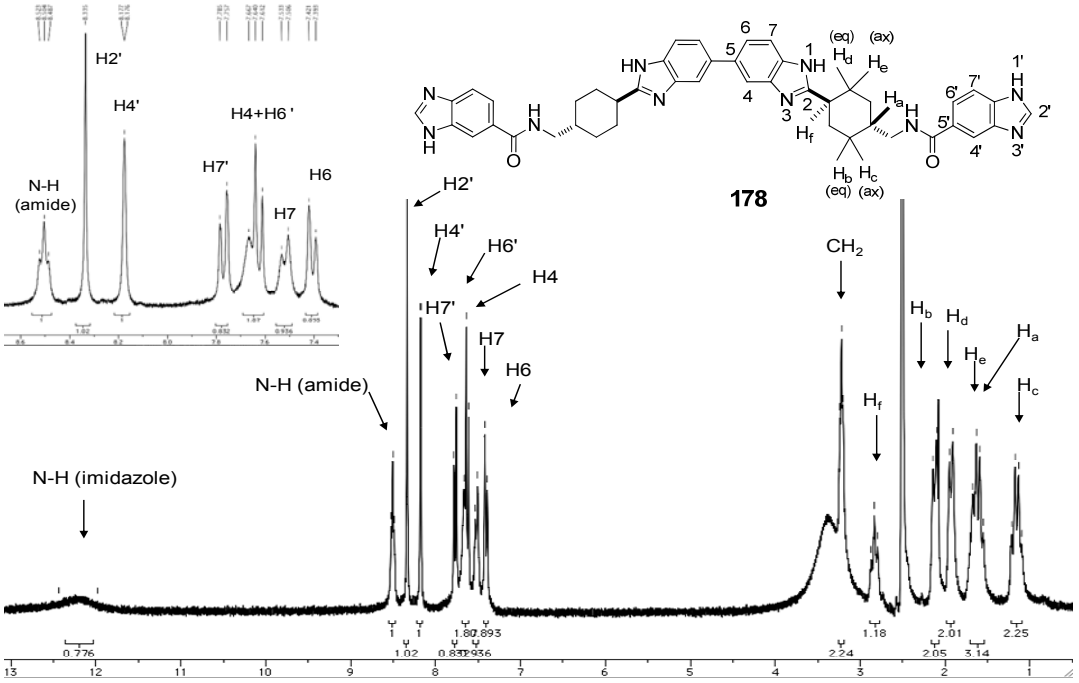


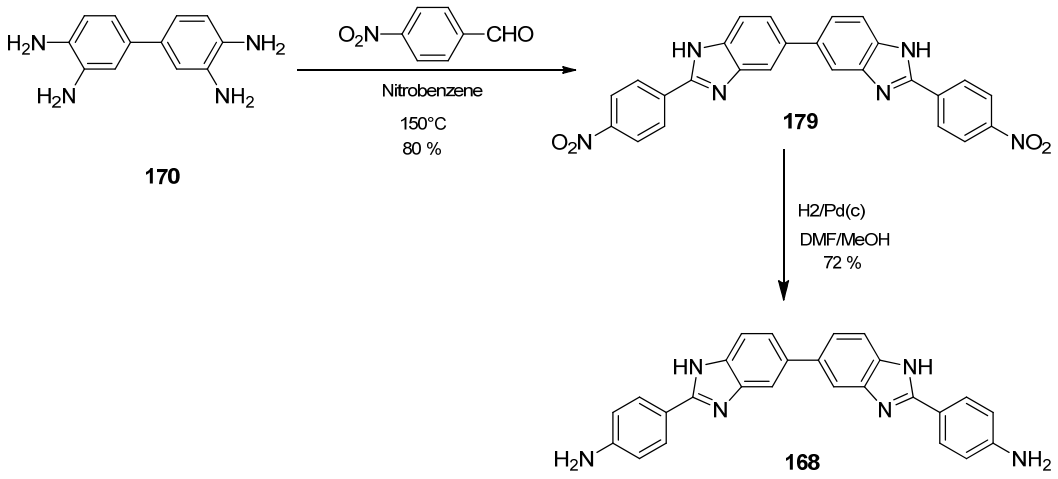
Figure 62 ^{1}H NMR (400 MHz) of 178 in d_6-DMSO [#], the inset shows expansion for the aromatic part, N-H amide

3.2.6 Amidation of bis(aminobenzimidazole) *via* aromatic carboxy benzimidazole

Synthesis of symmetrical tetra(benzimidazoles) requires dinitrobis(benzimidazole) **179** was synthesized in 80 % yield by condensation of 4-nitro benzaldehyde and bis-*o*-diamine **170**. In the presence of nitrobenzene under conditions analogous to those used for preparation of the 5-nitro-2-(4-nitrophenyl) benzimidazole described in **Section 2.1.4** for 5 days (**Scheme 43**).

Reduction of **179** by using H_2/Pd(C) for 24 h affording the desired diamine bis (benzimidazole) **168** in 72 % yield (**Scheme 43**).

[#] This assignment has been confirmed by using 2D NMR (COSY)



Scheme 43 Synthesis of 2,2-diaminobis(benzimidazole) by using $PhNO_2$

Spectroscopic data supported the structure of this diamine.

The ^1H NMR for **168** showed a singlet peak at 5.63 ppm related to NH_2 protons, two doublet at 6.69 and 7.87 ppm for the eight aromatic protons, while the imidazole NH protons appeared as a singlet peak at 7.96 ppm. The MS showed *m/z* for doubly charged peak at 209/417 (**Figure 63**), consistent with the structure shown.

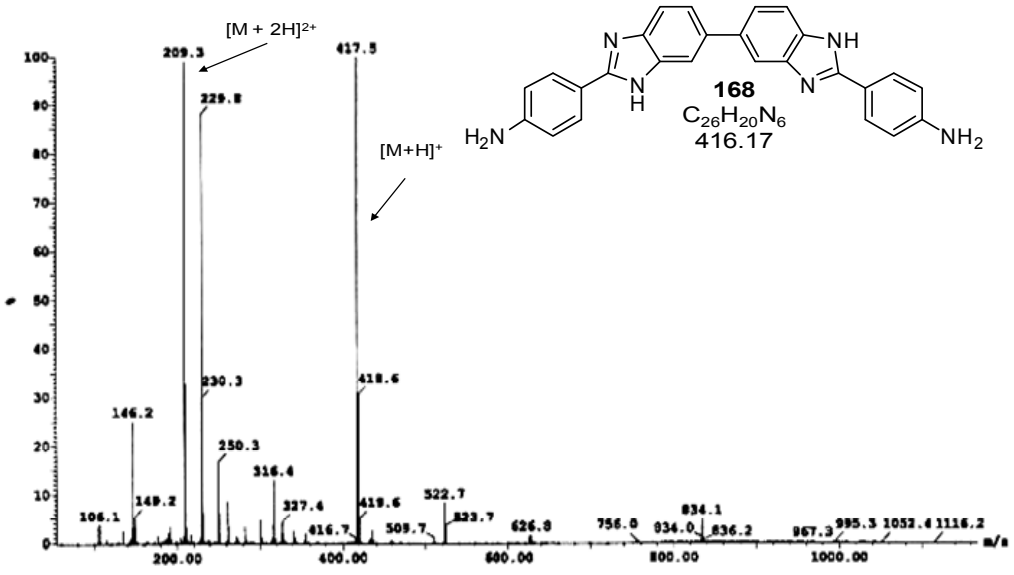
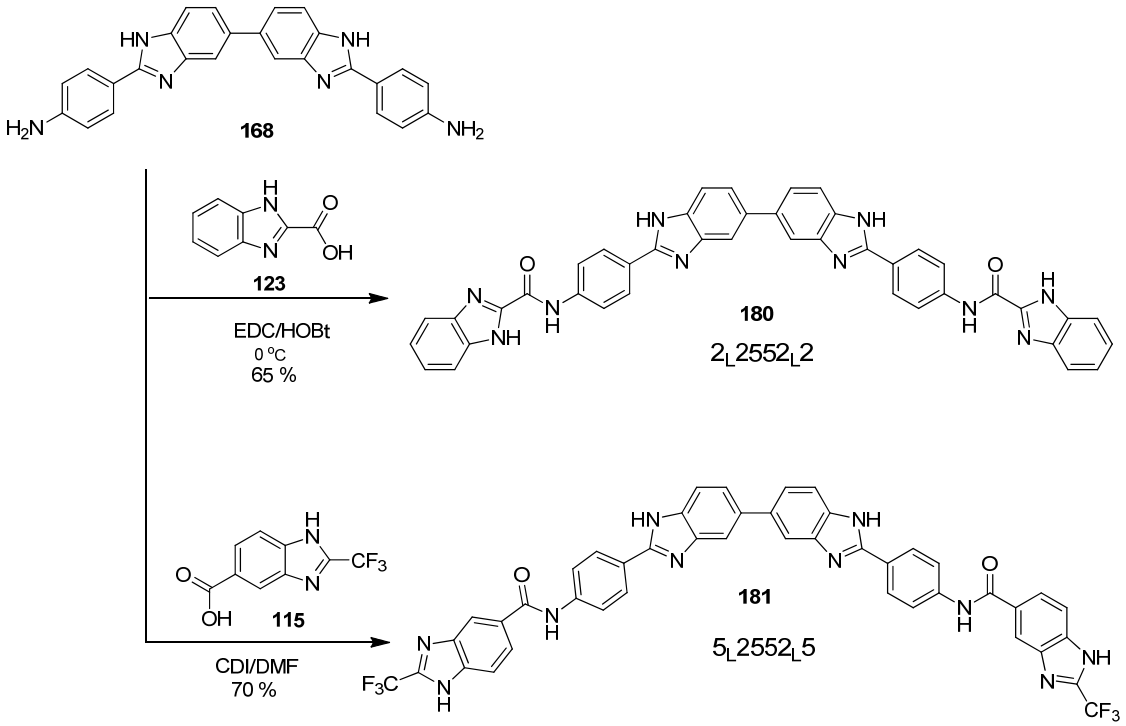


Figure 63 LRMS of 168

Diaminobis(benzimidazole) **168** was converted to the tetramers *via* the utilization of this benzimidazole in which one equivalent of the diamine will couple with two equivalents

of 2- or 5-carboxybenzimidazoles **123** and **115** (**Scheme 44**), affording the novel symmetrical tetra(benzimidazoles) **180** and **181** in 65 and 70 % yield respectively.



Scheme 44 tetra(benzimidazoles) synthesis from coupling of 2,2-diaminobis(benzimidazole) and 2- or 5-carboxybenzimidazoles

Tetramer **180** was formed in 65% yield using 1*H*,1′*H*-[5,5′]bisbenzimidazolyl-2,2′-diphenyl-4,4'-diamine (**168**) and benzimidazole-2-carboxylic acid (**123**) coupled by using EDC/HOBt in DMF for 15 days. This product was confirmed by MS which showed *m/z* as the base peak at 705. The ^{1}H NMR showed the N-H amide proton as a singlet peak at 11.17 ppm and The imidazole protons appeared as a broad peak in the range 12.85-13.09 and a singlet peak at 13.52 ppm, (see **Figure 64** for ^{1}H NMR of **180**). ^{13}C NMR showed C=O at 157.5 ppm, the structure was confirmed by IR showing two distinct peaks at the 3344 and 1662 cm^{-1} related to N-H and C=O stretching respectively.

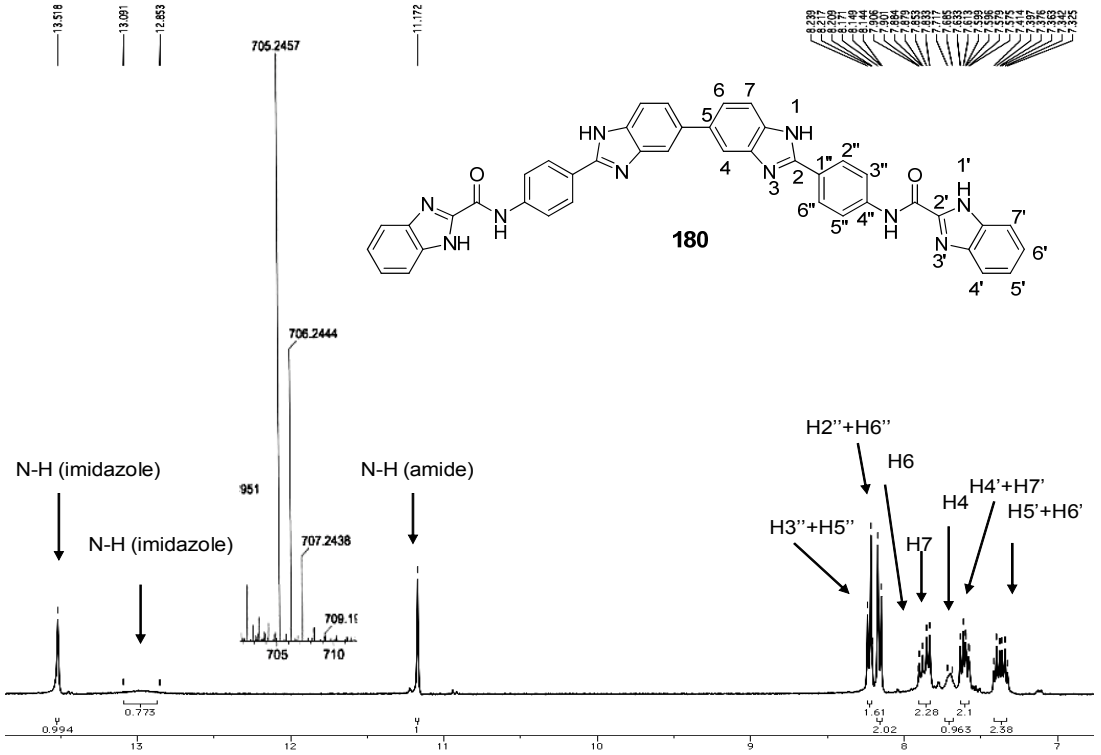


Figure 64 ^{1}H NMR (400 MHz) of 180 d$_6$-DMSO [#],· the inset shows LR mass spectrum

However, the reagent system did not work using 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (**115**) and no product could be isolated from these conditions. Employing CDI facilitated this coupling affording tetramer **181** in 70 % yield after 18 days (**Scheme 44**).

Spectroscopic data supported the structure of this tetramer. This product was confirmed by ^{1}H NMR through the appearance of the characteristic signals of amide protons at 8.22 ppm. ^{13}C NMR showed the characteristic signals for the amide carbonyl at 162.2 ppm and two CF_3 carbons which are coincident at 142.3 ppm appearing as a quarter with coupling constant (J_{CF} = 39.8 Hz). The mass spectra showed MH^+ peak at *m/z* 841. The IR showed the C=O at 1634 cm^{-1} and the NH stretch at 3221-3546 cm^{-1} .

[#] This assignment has been confirmed by using 2D NMR (COSY)

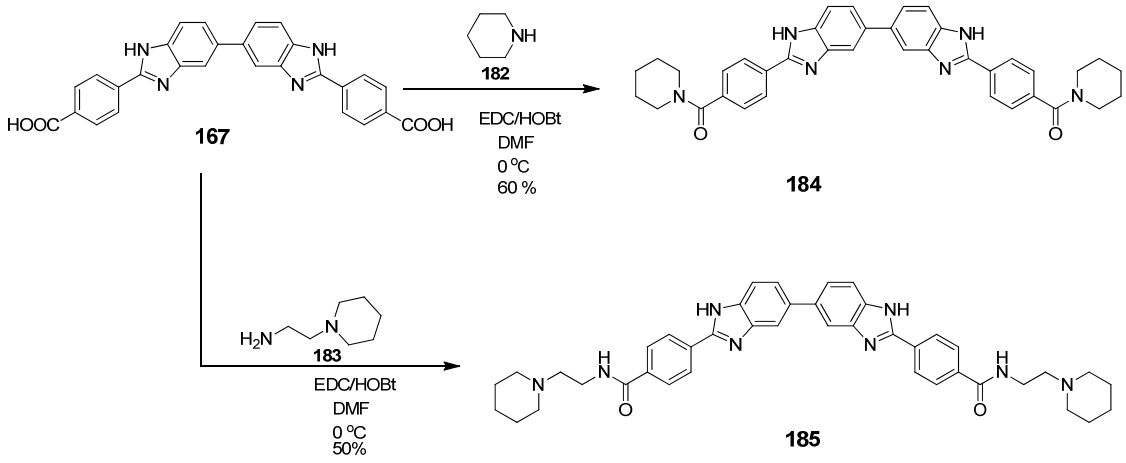
3.3 Synthesis, structural characterization of symmetrical bis(benzimidazoles) piperidine derivatives

We describe new methodology for the synthesis of symmetric bis-benzimidazoles carrying piperidine derivatives, may lead to novel compounds that may possess significantly higher affinity for DNA-binding and show bioactive behaviour.

The synthetic procedure was employed to prepare benzimidazole derivatives involving of the bis(carboxybenzimidazole) **167** as a precursor for coupling with two equivalents of piperidine derivatives.

3.3.1 Coupling of 2,2 bis-carboxybenzimidazole with piperidine derivatives

For the synthesis of such benzimidazoles, one equivalent of bis(carboxybenzimidazole) **167** was coupled with two equivalents of the piperidine derivatives **182** and **183** affording the novel symmetrical bis(benzimidazoles) **184** and **185** in 60 and 50 % yields respectively (**Scheme 45**). Two products were confirmed by ^{1}H NMR, ^{13}C NMR and MS



Scheme 45 Synthesis of symmetrical bis(benzimidazoles) 184-185

Dimer **184** was formed in 60% yield using 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid (**167**) and piperidine **182** coupled by using EDC/HOBt in DMF for 6 days. This product was confirmed by MS which showed the MH^{+} ion at *m/z* 609 as

the base peak, ^{1}H NMR showed five of methylene protons in the piperidine ring as abroad singlet peaks in the range of 1.48-3.66 ppm. A singlet peak at 7.82 ppm for the NH imidazole. The ^{13}C NMR showed C=O at 168.4 ppm and methylene carbons of the piperidine ring at 24.0, 29.6 and 35.3 ppm respectively. The IR showed C=O at 1593 cm^{-1}.

Synthesis of dimer **185** involved coupling between 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid (**167**) and 1-(2-amino ethyl)-piperidine (**183**) using EDC/HOBt, however **185** was isolated after 9 days reaction.

whose structure was confirmed by all spectral data. ^{1}H NMR showed five of methylene protons in the piperidine ring as multi peaks in the range of 1.19-2.46 ppm. A triplet peak at 8.58 ppm for the NH amide, ^{13}C NMR showed the characteristic signal for the amide carbonyls at 165.3 ppm, whilst MS showed *m/z* for doubly charged peak at 348/695 (**Figure 65**), consistent with the structure shown. The IR showed the C=O at 1639 cm^{-1}.

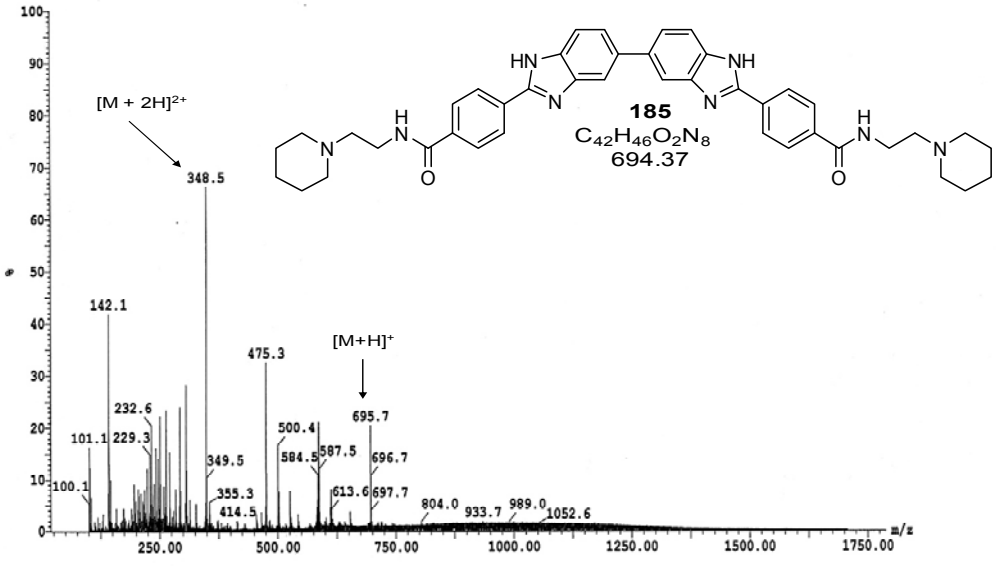


Figure 65 LRMS of 185

3.4 Conclusion

Our main aim of this research thesis was to prepare and characterize novel dimeric, trimeric and tetrameric benzimidazoles.

This was successfully achieved *via* condensation of *o*-phenylenediamine derivatives with the relevant aldehydes or carboxylic acids for the preparation of different monomeric benzimidazoles. However in the case of 2-carboxybenzimidazole systems, the condensation of *o*-phenylenediamine derivatives with methyltrichloroacetimidate was developed affording 2-trichloromethylbenzimidazole derivatives that were carefully hydrolyzed to the corresponding 2-carboxybenzimidazoles. This was a critical development as various alternative routes to 2-carboxy systems are poor and this method opens up the capability to generate a wide range of 2-carboxyl-bearing oligomers. These monomers were characterized by amino and carboxy substitutions at 2 or 5 positions. The main type of linkage between these monomers was developed, namely amidation (for dimers, trimers and tetramers), enabling oligomerization.

3.4.1 Synthesis of unsymmetrical bis(benzimidazoles)

Different categories of linked bisbenzimidazoles were synthesized differing in the C2 and/or C5 link sites employed. These categories comprise benzimidazoles linked by amide linkages.

Two different coupling reagents were used, (EDC/HOBt) for the 2-carboxy benzimidazoles and (CDI) with the 5-carboxybenzimidazoles for the synthesis of a number of unsymmetrical and symmetrical amide linked C2-C2, C2-C5 and C5-C5 bis(benzimidazoles), *via* coupling of the aminobenzimidazole monomers with carboxy benzimidazole monomers.

A library of unsymmetrical tail-to-tail (C5-C5) (**Figure 66**) head-to-head (C2-C2) and head-to-tail (C2-C5) (**Figure 67**) bis(benzimidazoles) were prepared in which 5-carboxy-2-trifluoromethylbenzimidazole was coupled with 2- and 5-amino benzimidazoles using CDI coupling. Another library of unsymmetrical head-to-head (C2-C2) and head-to-tail (C2-C5) bis(benzimidazoles) were generated in which 2- and 5-aminobenzimidazole were coupled with 2- carboxybenzimidazoles using EDC/HOBt coupling.

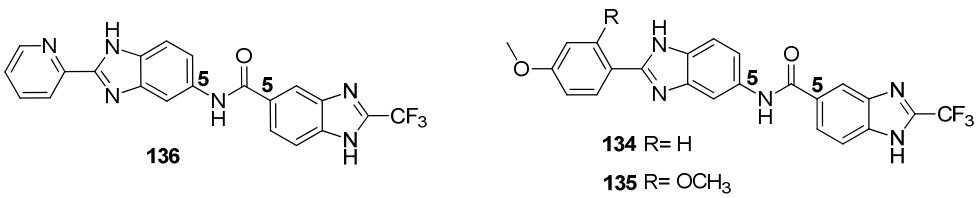


Figure 66 C5-C5 unsymmetrical bis(benzimidazoles)

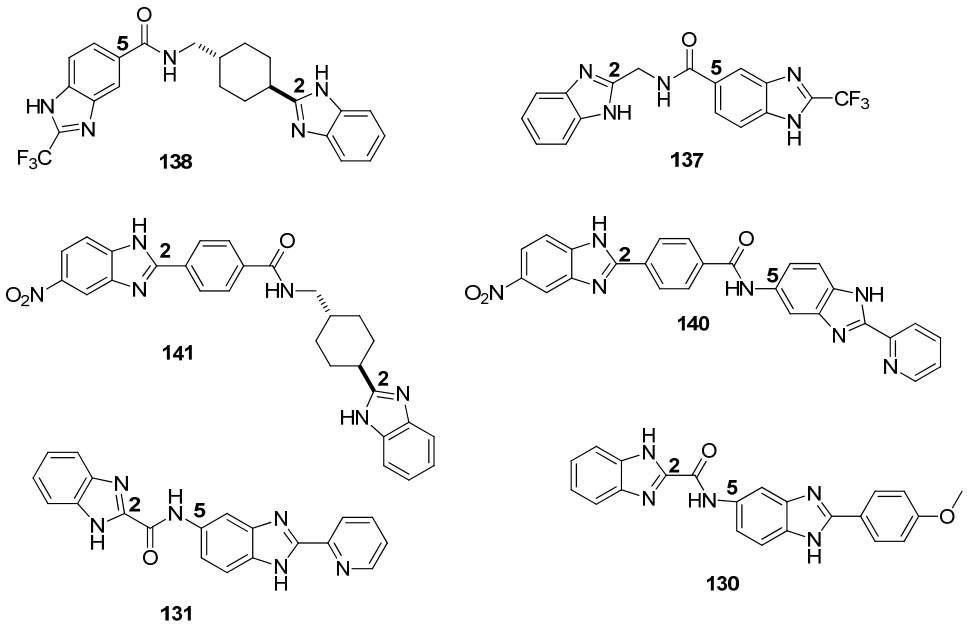
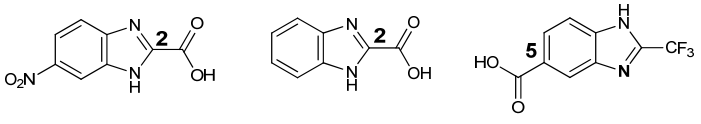


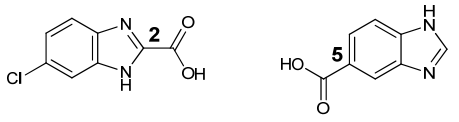
Figure 67 C2-C2 and C2-C5 unsymmetrical bis(benzimidazoles)

3.4.2 Synthesis of symmetrical bis(benzimidazoles)

Bis(benzimidazole) dimer syntheses involved utilization of 2-carboxybenzimidazole (**123**), 2-carboxy-5-nitrobenzimidazole (**124**), 5-carboxy-2-trifluoromethyl benzimidazole (**115**), 2-carboxy-5-chlorobenzimidazole (**125**) and 5-carboxy benzimidazole (**116**) (**Figure 68**). These acids were coupled with two different diamines (*p*-xylene diamine and ethylene diamine) affording two small libraries for C2-C2 (head-to-head) symmetrical bis(benzimidazoles) (**Figure 69**) and C5-C5 (tail-to-tail) symmetrical bis(benzimidazoles) (**Figure 70**).

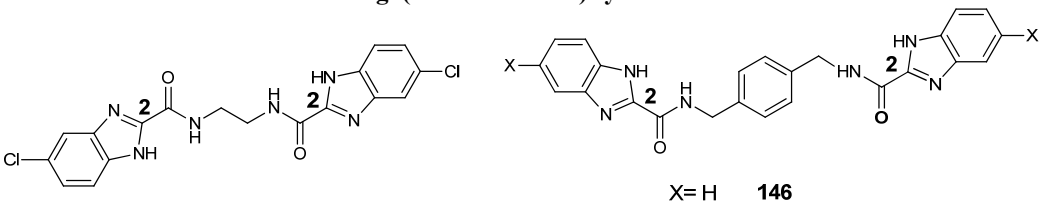


124 123 115



125 116

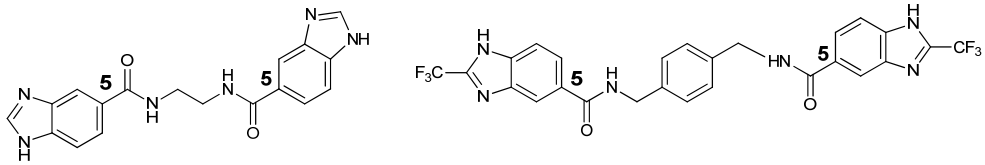
Figure 68 2- and 5-Carboxybenzimidazoles used in bis- and oligo(benzimidazoles) synthesis



150

X= H **146**
X= NO_2 **147**

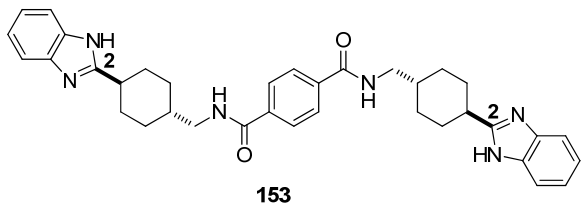
Figure 69 C2-C2 (head-to-head) symmetrical bis(benzimidazoles)



151 148

Figure 70 C5-C5 (tail-to-tail) symmetrical bis(benzimidazoles) *via* coupling diamine with carboxybenzimidazole

Other symmetrical bis(benzimidazoles) (C2-C2 and C5-C5) were synthesized *via* coupling one equivalent of terephthalic acid with two equivalents of selected 2-aminobenzimidazoles (**Figure 71**).



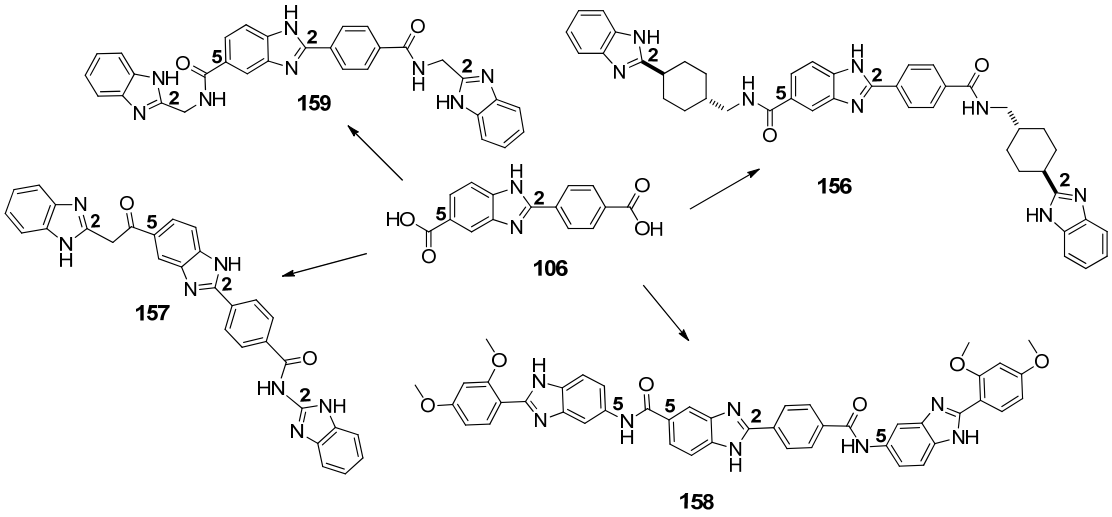
153

Figure 71 C2-C2 (head-to-head) symmetrical bis(benzimidazoles) *via* coupling dicarboxylic acid with aminobenzimidazole

3.4.3 Synthesis of tris(benzimidazoles)

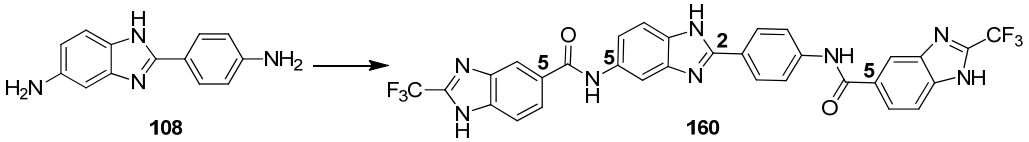
a) Bi-functionality feature of benzimidazoles

Three different routes were followed in this project towards trimerization. Firstly, six novel tris(benzimidazoles) **156-160** and **163** were synthesized *via* utilization of the bifunctionality of benzimidazolediacid **106** or the analogous diamine **108**. Coupling of diacid **106** with 2 equivalents of 2- or 5-aminobenzimidazole to generat trimers **156-159** (**Scheme 46**).

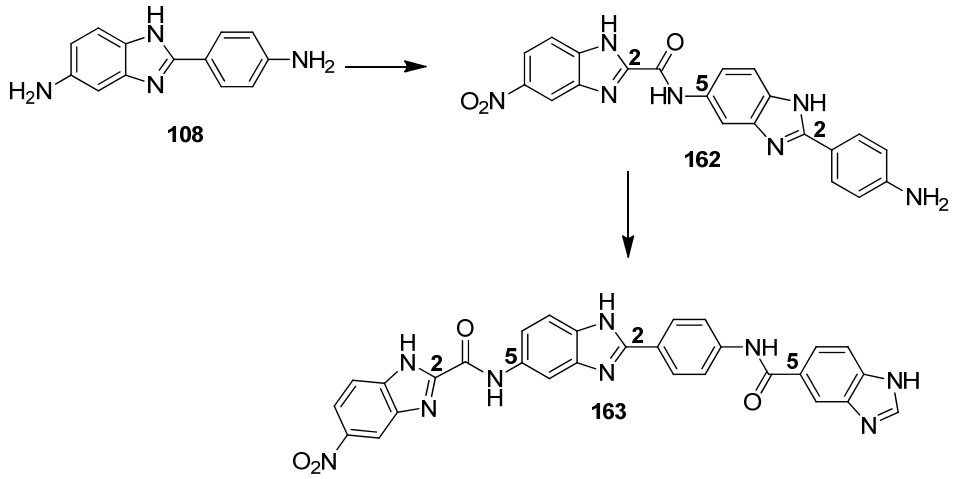


Scheme 46 Tris(benzimidazoles) synthesis *via* coupling of dicarboxybenzimidazole with different aminobenzimidazoles

Secondly two novel trisbenzimidazoles were synthesized *via* utilization of the bifunctionality of benzimidazole diamine **108**. Coupling of diamine **108** with 2 equivalent of 2-trifluoromethyl-5-carboxybenzimidazole **115** provided trimer **160** (**Scheme 47**). The dimer **162** was synthesized from coupling of diamine **108** with 2-carboxy-5-nitrobenzimidazole (**124**), which was coupled with 5-carboxy benzimidazole (**116**) affording the trimer **163** (**Scheme 48**).



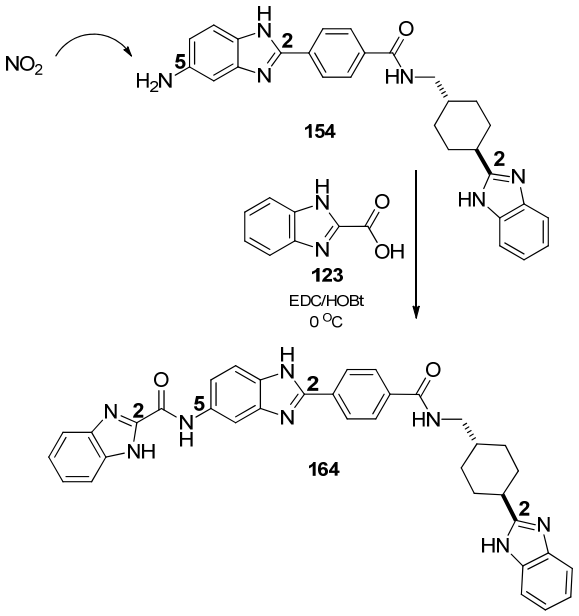
Scheme 47 Coupling of diaminobenzimidazole with two equivalents of carboxybenzimidazole towards trimerization



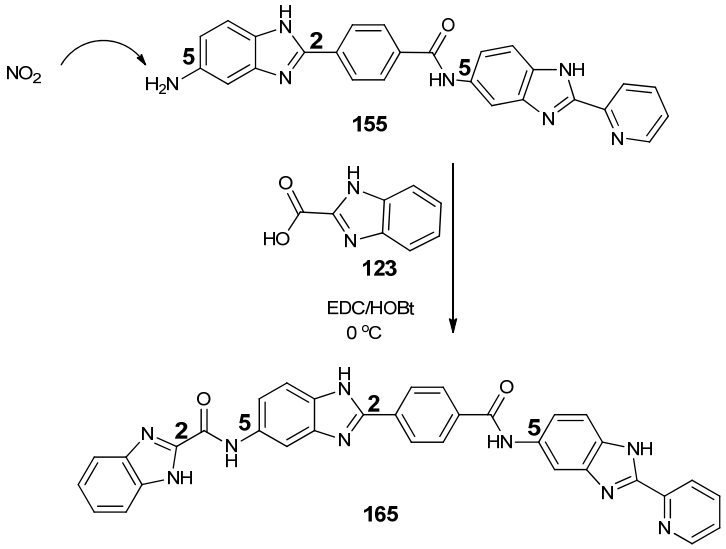
Scheme 48 Tris(benzimidazole) synthesis from coupling of dimer and 5-carboxybenzimidazole

b) Extendability feature of nitro bis(benzimidazoles)

A third route was reduction of bis(benzimidazoles) bearing a nitro group to the corresponding amine (*e.*g. **141** and **140**) then utilized for further coupling to trimers. Thus, **154** and **155** were coupled with 2-carboxybenzimidazole (**123**) (**Schemes 49 and 50**) to give tris(benzimidazoles), **164** and **165** respectively.



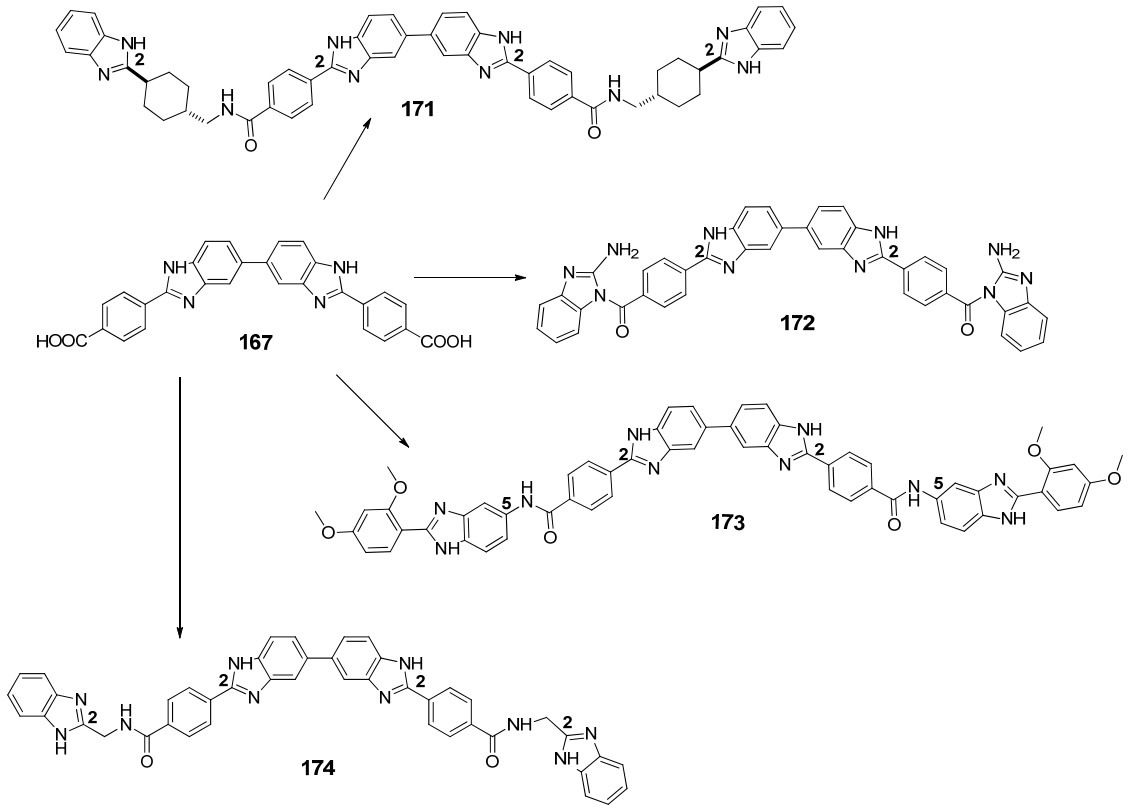
Scheme 49 Tris(benzimidazole) synthesis *via* coupling of bis(benzimidazole) amine with 2-carboxybenzimidazole



Scheme 50 Tris(benzimidazole) synthesis from coupling of aminobis(benzimidazole) and carboxybenzimidazole

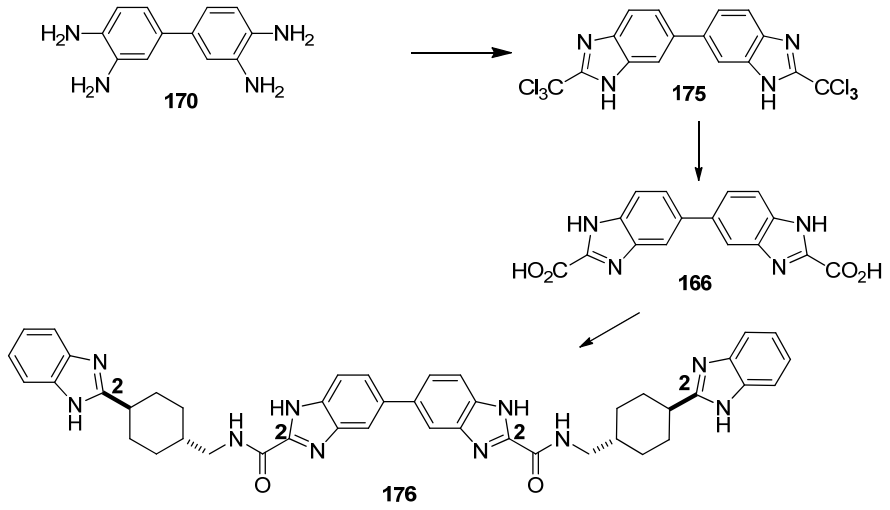
3.4.4 Synthesis of symmetrical tetra(benzimidazoles)

The other type of oligomer synthesis investigated in this research was the synthesis of four different sets of tetra (benzimidazoles). 3,3'-diaminobenzidine **170** was the precursor for the synthesis of the four different sets of bis (benzimidazoles) **167, 166, 169** and **168**. One set of symmetrical tetra(benzimidazoles) were synthesized (**Scheme 51**) *via* coupling of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylicacid **167** (which was synthesized by condensation of 3,3'-diaminobenzidine **170** with 4-carboxy benzaldehyde **104**, in the presence of sodium metabisulfite) with different 2- and 5-aminobenzimidazoles.



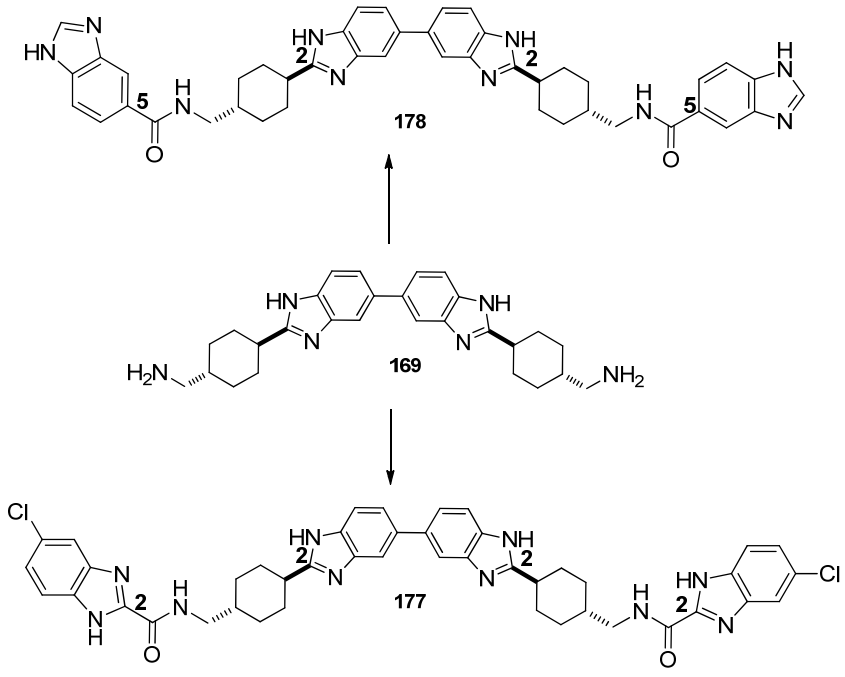
Scheme 51 Synthesis of symmetrical tetra(benzimidazoles) *via* coupling diacarboxy bis(benzimidazole) with 2- and 5- aminobenzimidazoles

Another set of symmetrical tetra(benzimidazoles) were synthesized (**Scheme 52**) by bis(benzimidazole) **175** could be hydrolysed to the dimer **166** which was then coupled with **112** affording tetramer **176**.



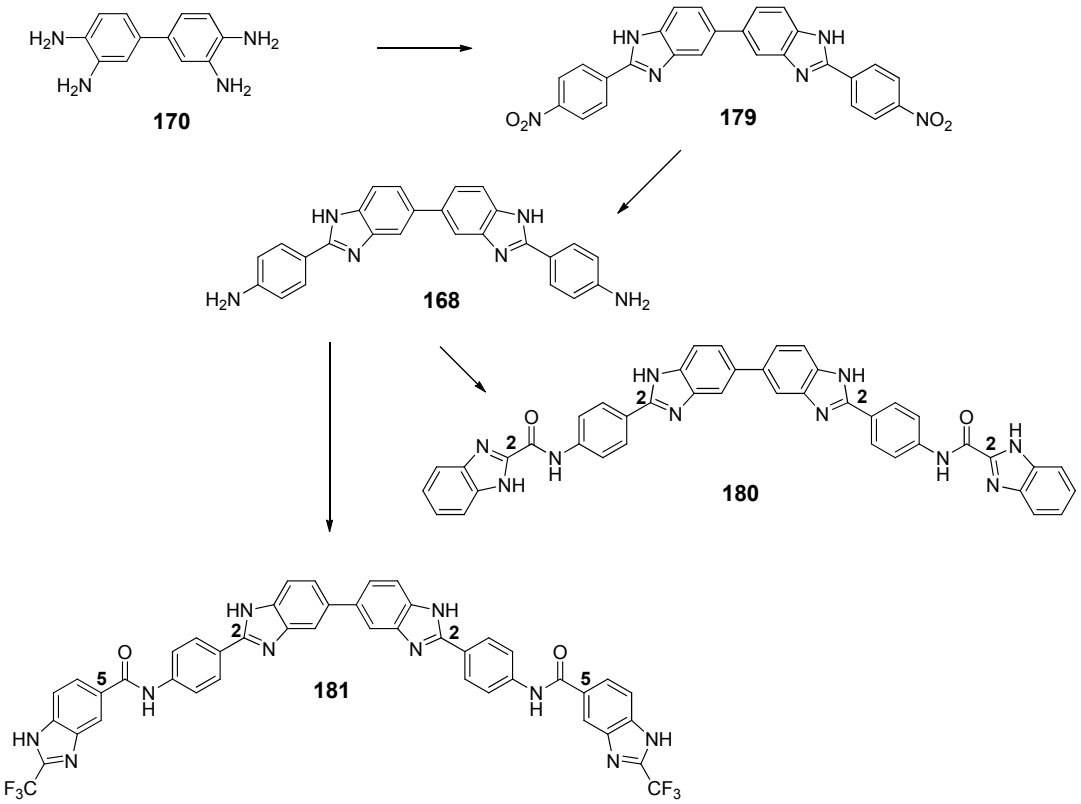
Scheme 52 Synthesis of symmetrical tetra(benzimidazole) *via* coupling dicarboxybis(benzimidazole) with 2-aminobenzimidazole

A third set of symmetrical tetra(benzimidazoles) were synthesized *via* coupling of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diamine **169** (which was synthesized by condensation of 3,3'-diaminobenzidine **170** with *trans*-4-aminomethyl cyclohexane carboxylic acid (**111**) with different 2- and 5-carboxybenzimidazole (**Scheme 53**).



Scheme 53 Synthesis of symmetrical tetra(benzimidazoles) *via* coupling diaminobis(benzimidazole) with 2- and 5- carboxybenzimidazoles

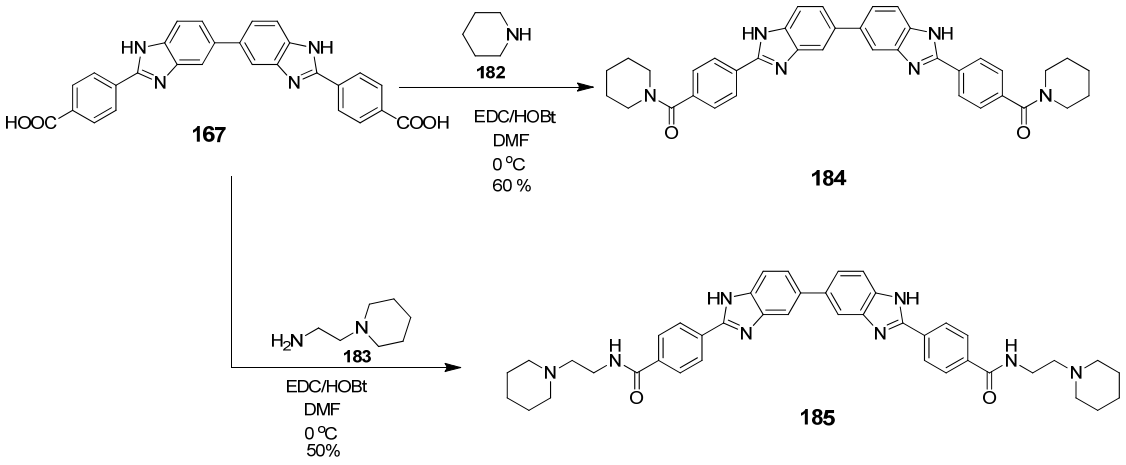
The last set of symmetrical tetra(benzimidazoles) were synthesized *via* coupling of 2,2'-Bis-(4-amino-phenyl)-3*H*,3'*H*-[5,5']bibenzimidazolyl **168** (which was synthesized by condensation of 3,3'-diaminobenzidine **170** to give 2,2'-Bis-(4-nitro-phenyl)-3*H*,3'*H*-[5,5']bibenzimidazolyl **179**, then reduction of bis(benzimidazoles) **179** bearing a nitro group to the corresponding amine) with 2-carboxybenzimidazole **123** and 2-trifluoromethyl-5-carboxybenzimidazole **115** (**Scheme 54**) to give tetra(benzimidazoles) **180** and **181** respectively.



Scheme 54 Synthesis of symmetrical tetra(benzimidazoles) *via* coupling diamino bis(benzimidazole) with different carboxybenzimidazoles

3.4.5 Synthesis of symmetrical bis(benzimidazoles) piperidine Derivatives

The last benzimidazole synthesized in this research was the synthesis of bis (benzimidazole) dimer **184,** and **185** (**Scheme 55**) *via* coupling of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** with two different piperidine derivatives.



Scheme 55 Symmetrical bis(benzimidazoles) synthesis *via* coupling of dicarboxybenzimidazole with piperidine derivatives

In this project categories of mono-, di-, tri- and tetrameric benzimidazoles with differently linked orientation have been prepared.

Chapter 4

Evaluation of DNA binding of oligobenzimidazoles by SPR technique

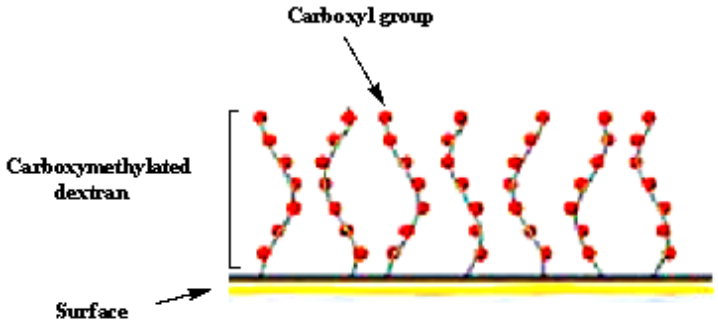
4.1 Sequence-specific minor groove binding of novel oligo(benzimidazoles)

4.1.1 Surface plasmon resonance technique

The interaction of nucleic acids with small molecules has been a primary area of interest for therapeutic development for over half a century. Surface plasmon resonance (SPR)-biosensor techniques directly provide essential information for the study and characterization of small molecule –nucleic acid interactions, and the use of these methods is steadily increasing.[79] The method is label-free and monitors the interactions in real time. Both dynamic and steady-state information can be obtained for a wide range of reaction rates and binding affinities.

Biosensor-SPR methods, particularly as implemented in the Biacore technology, offer significant advantages in many measurements of nucleic acid interactions. For small molecules, which frequently have large binding constants with weak spectroscopic signals and low interaction enthalpies, SPR approaches may provide the only practical method for accurate determination of the binding and rate constants.

The Biacore sensor chip is the most versatile chip available and the first choice for immobilization is *via* covalent attachment to -NH_2, -SH, -CHO, -OH or -COOH groups (eg **Figure 72**). A common non-covalent attachment involves streptavidin /biotin, with these attached to chip surface and ligand respectively. It supports a wide range of immobilization levels and permits attachment of proteins, nucleic acids, carbohydrates and small molecules.[79]



Matrix: carboxymethylated dextran covalently attached to a chip surface, molecules are covalently coupled to the sensor surface *via* carboxyl group

Figure 72 Immobilization of chip by carboxyl group [#]

[#] Taken from www.biacore.com/…/guide/cm5/index.html

Covalent attachment by amino or thiol coupling requires that the immobilized molecule has a free amino or thiol group that is not involved in the interaction. Although other immobilization techniques are available, the biotin-streptavidin coupling is popular in Biacore SPR experiments and it is particularly useful for nucleic acids immobilization.

The large affinity constant for the biotin-streptavidin complex results in a stable surface for binding studies. An important step in sensor chip immobilization is to decide how much biomolecule to immobilize. It is usually best to immobilize the smallest amount of materials.

The response represented by the sensorgarm is the difference between the response in the cell with immobilized DNA minus the response of blank flow cell without DNA, due to modification of the refractive index/mass at the surface and a resulting change in the SPR angle. The angle change is reported in Biacore technology as response units (RU) where a 1000 RU response is equivalent to a change in surface concentration of about 1ng/mm^2 of DNA (the relationship between RU and ng of material bound will vary with the refractive index of the bound molecule). The experiments thus measure changes in refractive index when the mass of material on the surface changes due to ligand binding to DNA.

Additionally, the ‘‘off’’ response is determined as related to the drop in mass as ligand dissociates from DNA when the solvent system consists of pure buffer, and the ligand is washed from the DNA.

The true responses obtained were used to get K_d by plotting it against the drug concentration and fitting it with a steady- state affinity fit .

4.1.2 Buffers, DNA and oligonucleotides

For our studies we used three different DNA oligonucleotides with a 5'- biotin attached to, then immobilize on a streptavidin-coated chip (**Figure 73** and **74**). All experiments were conducted in PBS buffer, filtered and degassed, was purchased from Sigma-Aldrich (dissolve 1 tablet in 200 mL of water). The three different 5'-labeled hairpins (Eurofins MWG Operon.;HPLC purified) used in SPR studies were :d(biotin-CGAAATTTCGTCTCCGAAATTTCG);d(biotin-CGAATTCGTCTCCGAATTCG);d(biotin-CGAAAATTTTCGTCTCCGAAAATTTTCG) (HPSF purified). All other chemicals were analytical grade reagents.

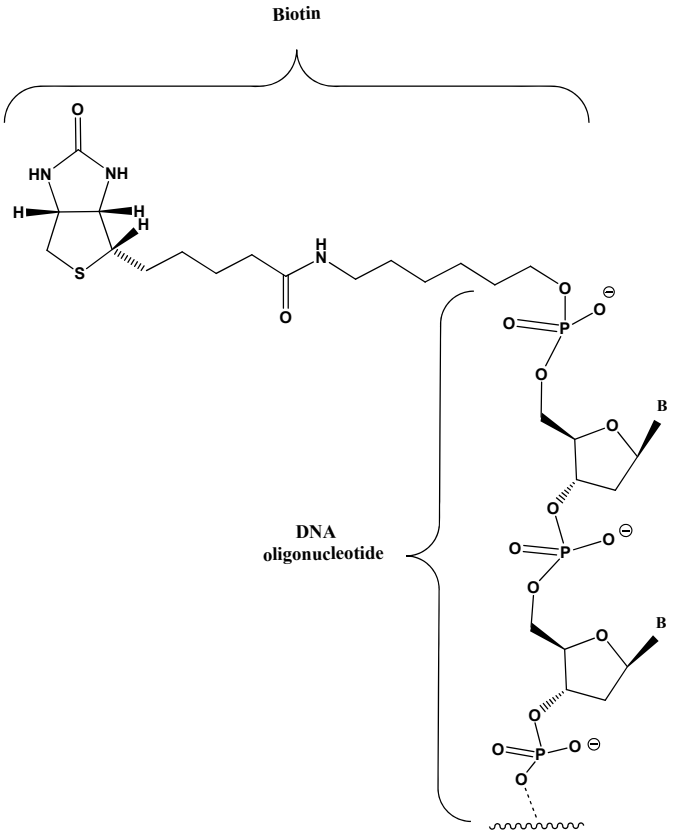


Figure 73 Biotin -conjugated DNA oligonucleotides

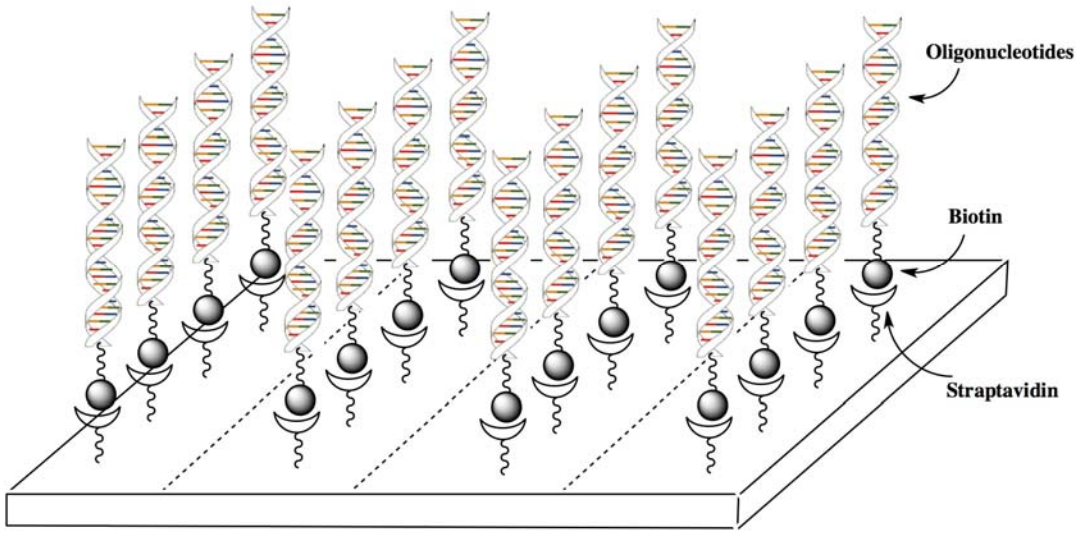


Figure 74 Attachment of streptavidin/biotin with chip surface and oligonucleotides respectively

4.1.3 Determination of binding constant by SPR [79]

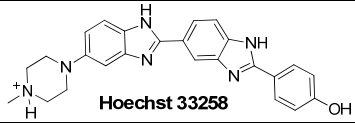
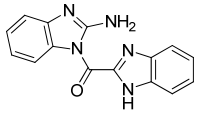
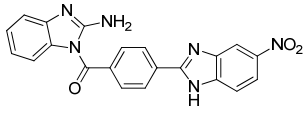
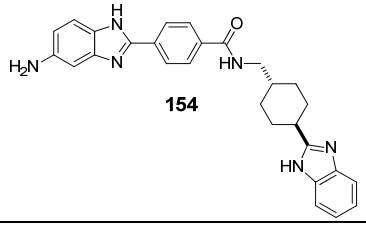
SPR measurements were performed with a four-channel BIAcore 3000 optical biosensor system and streptavidin-coated sensor chips (SA). Three consecutive 1 min injections of 1M NaCl in 50 mM NaOH followed by extensive washing with buffer were used to prepare the sensor chips. Nearly the same amounts of 5'-biotinylated

oligomers (25 nM) in PBS buffer were immobilized on the surface by non-covalent capture, leaving one of the flow cells blank as a control. Manual injection was used with a flow rate of 2 μL/min to achieve long contact times with the surface and to control the amount of the DNA bound to the surface. Solutions of compound with known concentrations were prepared in filtered and degassed buffer by serial dilutions from stock solution [#] and passed over the immobilized DNA surfaces for a predetermined time period (typically 5 min) at a flow rate of 20-30 μL/min and 25 °C. Buffer flow alone during 10 min was generally sufficient to dissociate the drug from DNA for surface regeneration.

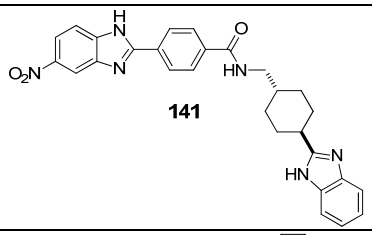
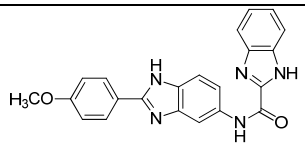
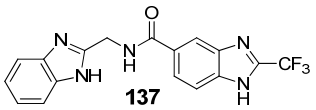
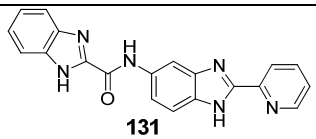
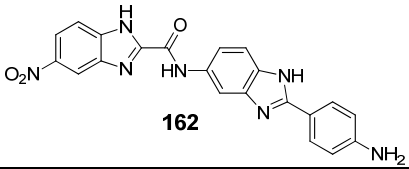
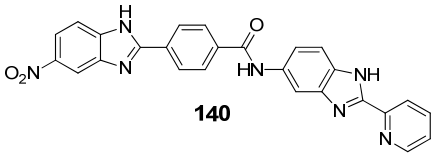
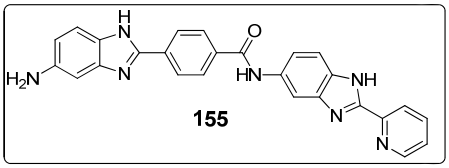
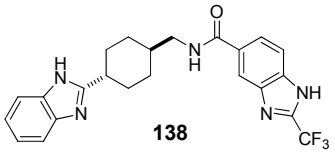
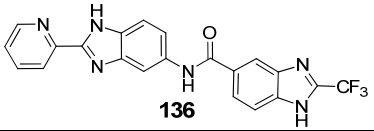
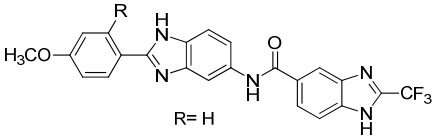
Average fitting of the sensorgrams at the steady-state level was performed with the BIAevaluation 4.1 and Origin 7 programs.

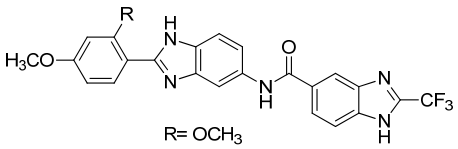
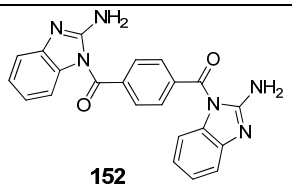
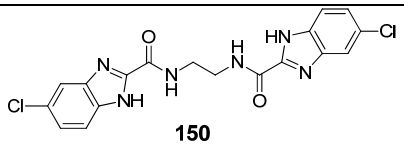
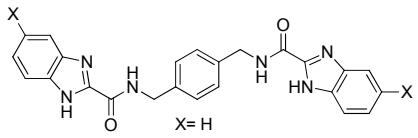
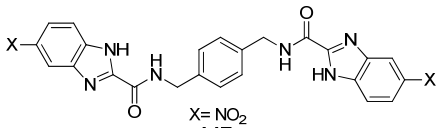
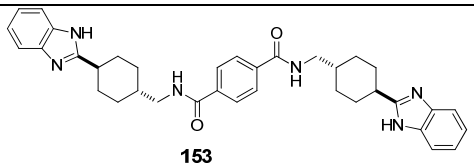
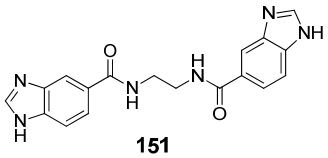
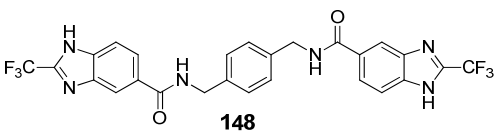
4.1.4 DNA binding assays

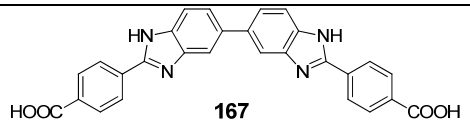
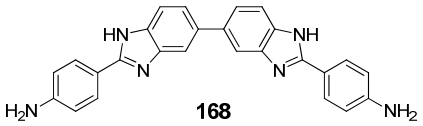
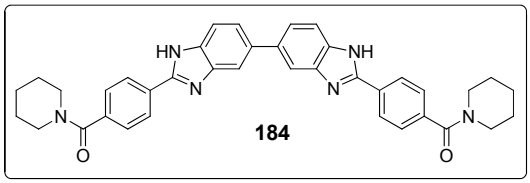
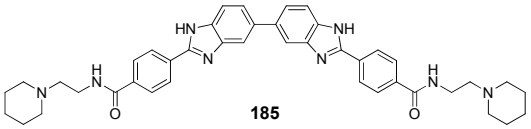
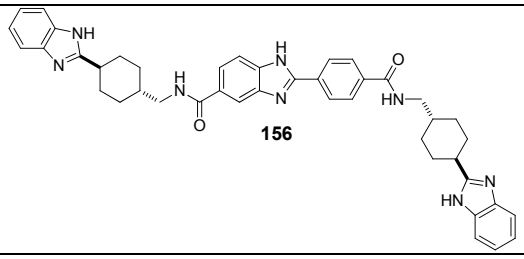
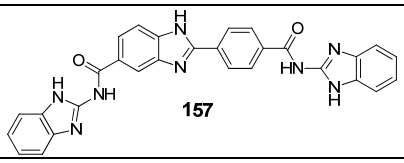
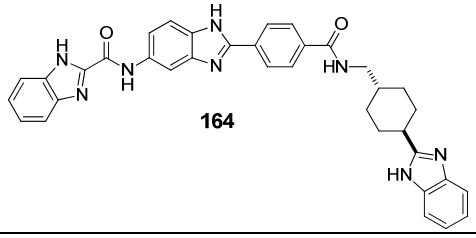
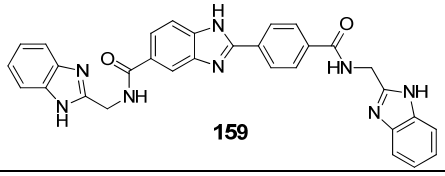
DNA oligonucleotides containing A2T2, or A3T3 or A4T4 sequences were immobilized and SPR used to evaluate the binding affinities of a library of different oligomeric ligands (**Table 2**).

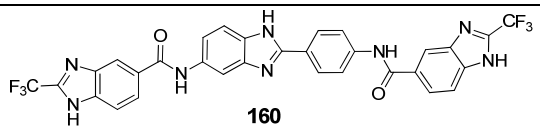
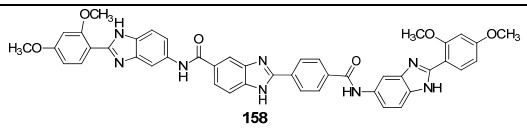
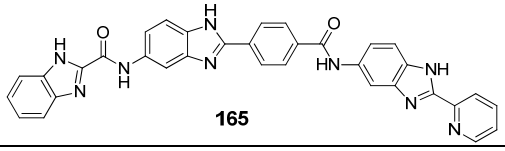
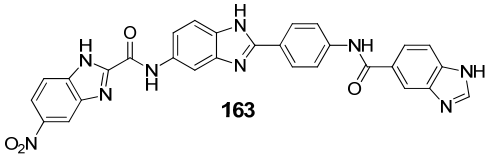
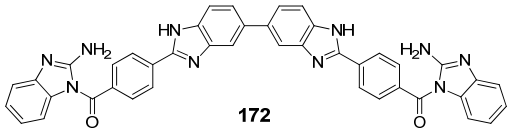
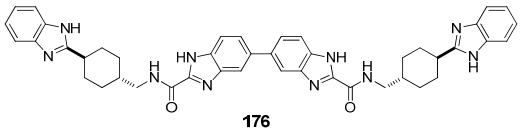
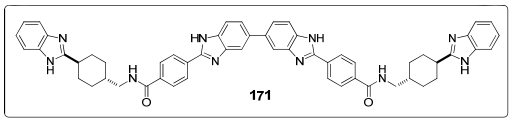
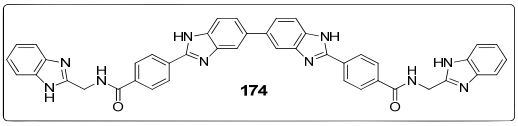
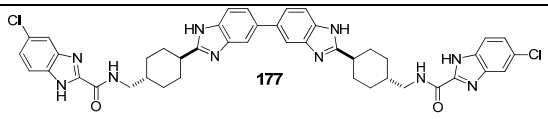
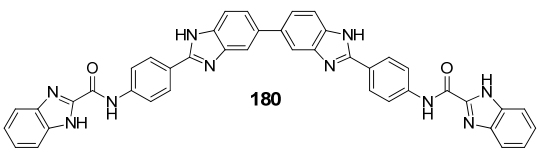
Linkage Type	Structure	A2T2 K_d(μM)	A3T3 K_d(μM)	A4T4 K_d(μM)
25	 Hoechst 33258	0.129	Less than 0.01	Less than 0.01
1_L2	 **133**	X	X	X
1_L2	 **139**	X	X	X
2_L2	 **154**	X	X	X

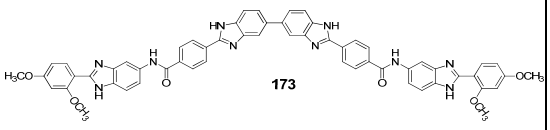
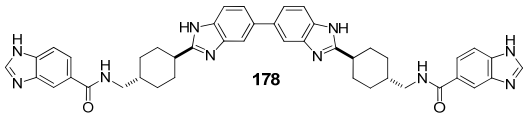
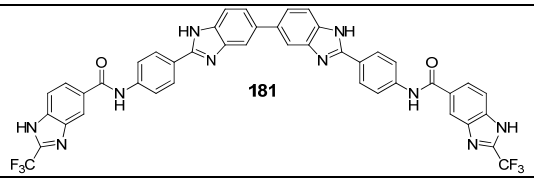
[#] See Section 4.1.5 for details.

2_L2	 **141**	X	X	X
2_L5	 **130**	X	X	X
2_L5	 **137**	173 very weak binding	X	X
2_L5	 **131**	X	X	X
2_L5	 **162**	X	X	X
2_L5	 **140**	X	X	X
2_L5	 **155**	131 Very weak binding	37.3 Strong binding	23.56 Strong binding
2_L5	 **138**	X	X	X
5_L5	 **136**	X	X	X
5_L5	 R= H **134**	X	X	X

5_L5	 R= OCH_3 **135**	X	X	X
	Symmetrical with central linker			
1_L1	 **152**	less than 200 very weak binding	less than 200 very weak binding	less than 200 very weak binding
2_L2	 **150**	X	X	X
2_L2	 X= H **146**	X	X	X
2_L2	 X= NO_2 **147**	X	X	X
2_L2	 **153**	X	X	X
5_L5	 **151**	X	X	X
5_L5	 **148**	X	X	X

	Symmetrical biphenyl core dimers			
55	 **167**	X	X	X
55	 **168**	X	X	X
55	 **184**	1.40 Strong binding	0.8 Strong binding	X
55	 **185**	X	X	X
	Trimers			
2_L52_L2	 **156**	X	X	X
2_L52_L2	 **157**	X	X	X
2_L52_L2	 **164**	X	X	X
2_L52_L2	 **159**	X	X	X

5_L52_L5	 **160**	in millimolar range very weak binding	~ 210 very weak binding	~ 211 very weak binding
5_L52_L5	 **158**	X	X	X
2_L52_L5	 **165**	X	X	X
2_L52_L5	 **163**	X	X	X
	Symmetrical biphenyl core tetramers			
1_L2552_L1	 **172**	X	X	X
2_L2552_L2	 **176**	X	X	X
2_L2552_L2	 **171**	46.9 Strong binding	0.16 Strong binding	0.06 Strong binding
2_L2552_L2	 **174**	8.5 Strong binding	14.2 Strong binding	1.3 Strong binding
2_L2552_L2	 **177**	X	X	X
2_L2552_L2	 **180**	X	X	X

5_L2552_L5	 173	X	X	X
5_L2552_L5	 178	X	X	X
5_L2552_L5	 181	X	X	X

K_d = Dissociation constant (micro molar) X = no binding

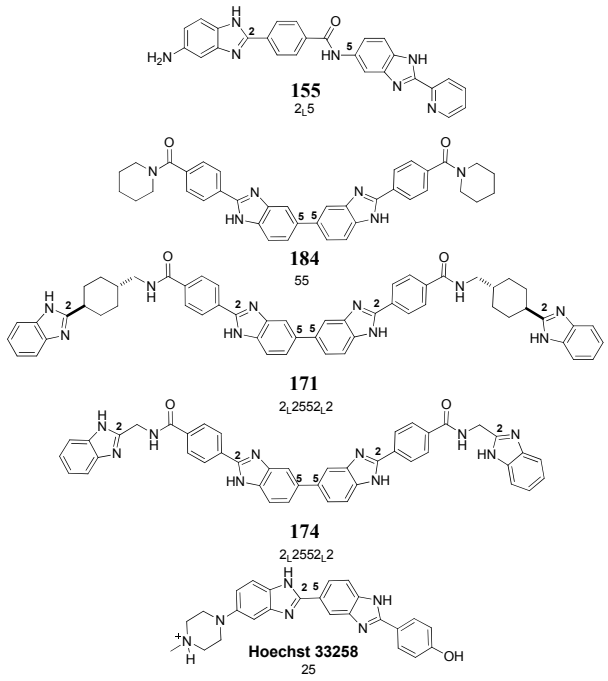
Table 2 Affinities for $(AT)_n$ targets

Data presented in **Table 2** showing our ligands binding to DNA are related to the well-known minor groove agent Hoechst 33258.

Hoechst binds very strongly to the three DNA sequences (A2T2, A3T3 and A4T4), as can be seen from comparing the binding of our ligands to DNA to Hoechst binding.

Some ligands **155**, **171**, **174** and **184** bind strongly to the three DNA sequences (A2T2, A3T3 and A4T4) others **137**, **152** and **160** are very weak binding to the three DNA sequences (A2T2, A3T3 and A4T4), other ligands show no binding to DNA sequence.

The best four (from a screen of 40) are 2_L2552_L2 tetramers **171** and **174**, and 2_L5 dimer **155** and dimeric 5,5-system **184** *without* terminal additional benzimidazoles (**Figure 75**).



A2T2 (uM)	A3T3 (uM)	A4T4 (uM)
131	37.3	23.56
1.4	0.8	X
46.9	0.16	0.06
8.5	14.2	1.3
0.129	< 0.01	< 0.01

Figure 75 Four highest affinity ligands identified from SPR screens

This screen subset identifies three new ligands with micromolar or lower affinities for one or more of the three DNA sequences (A2T2, A3T3 and A4T4). The two tetrameric benzimidazole-amides **171** and **174**, evidence higher affinities for the longer target sequences, with **171** showing the best affinity of 60 nM for the longer A4T4 oligonucleotide target.

The compound **184** binds more strongly to the A2T2 DNA duplex (**Figure 76**) than compound **155**, **171**, and **174** which have slower binding affinities. The sensorgram results were fit in the steady-state region as described in the Materials and Methods (**Section 4.1.2** and **4.1.3**) and binding affinities are collected in **Table 2.**

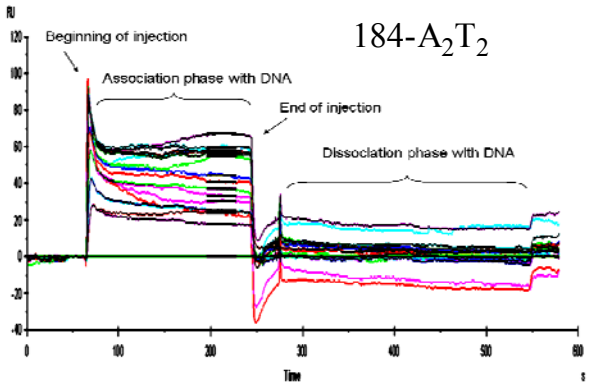


Figure 76 BIAcore SPR sensorgrams for interaction of compound 184 with the A2T2 sequence DNA hairpins in PBS buffer at 25 °C

Colour in Fig.76	Conc	Response (RU)
●	137	23.6
●	137	30.3
●	68.5	54.7
●	68.5	59.8
●	34.3	57.5
●	34.3	57.4
●	17.1	67
●	17.1	56.3
●	8.56	59.5
●	8.56	57.1
●	4.28	44.5
●	4.28	40.9
●	2.14	33.6
●	2.14	37.1
●	1.07	25.3
●	1.07	25.2
●	0.535	24.2
●	0.535	17.8
●	0	0

Table 3 The concentrations of compound 184 from 0 (reference line, green) to 137 µM at each RU value

Visual observation of the binding sensorgrams for the **171** compound interacting with the A3T3 DNA sequence indicated that it binds strongly (**Figure 77**). The sensorgrams for compound **184** decrease slightly with time at the highest concentrations used in the experiments. The binding of compounds **155** and **174** to the A3T3 sequence are, however, weaker than its interaction with A4T4 DNA.

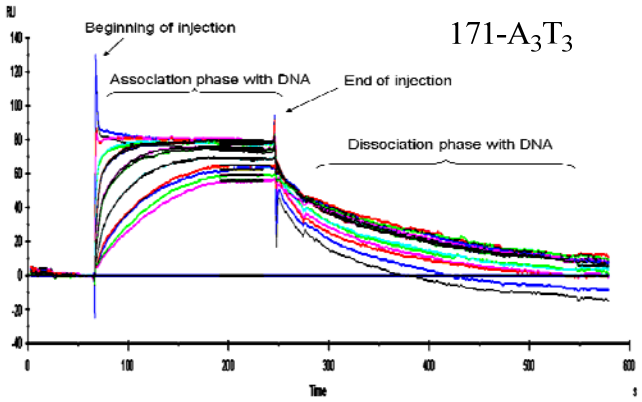


Figure 77 BIAcore SPR sensorgrams for compound 171 with the A3T3 DNA minor groove in PBS buffer at 25 °C, compound 171 bind strongly to A3T3 sequence

Colour in Fig.77	Conc	Response (RU)
	67.5	73.1
	67.5	78.6
	33.8	79.3
	33.8	80
	16.9	77.5
	16.9	77.9
	8.44	79.2
	8.44	79.1
	4.22	74.9
	4.22	74.3
	2.11	68.7
	2.11	68.8
	1.05	62.9
	1.05	64.8
	0.527	55.5
	0.527	58.8
	0	0

Table 4 The RU values at the concentration range of compound 171, 0-67.5 µM.

With the A4T4 sequence, quite significant binding of compound **171** is observed, (**Figure 78**) due to the very strong binding of compound **171** to the A4T4 DNA as can be seen from the results in **Table 1**, while the binding of compound **155** to the A4T4 sequence (**Figure 79**) is weaker than compound **171,** however, no binding of compound **184** to the A4T4 sequence is observed.

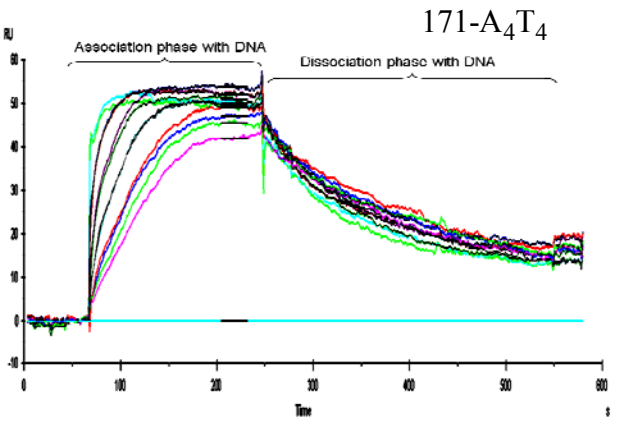


Figure 78 SPR sensorgrams for binding of compound 171 to A4T4 sequence DNA hairpins at 25 °C in PBS buffer

Colour in Fig.78	Conc	Response (RU)
●	16.9	49
●	16.9	50
●	8.44	53.7
●	8.44	52.2
●	4.22	52
●	4.22	50.9
●	2.11	49.4
●	2.11	49.2
●	1.05	46.9
●	1.05	49.1
●	0.527	42
●	0.527	45.4
●	0	0

Table 5 The concentration ranges of compound 171 are 0 (reference line, blue) to 16.9 µM in the presence of RU value.

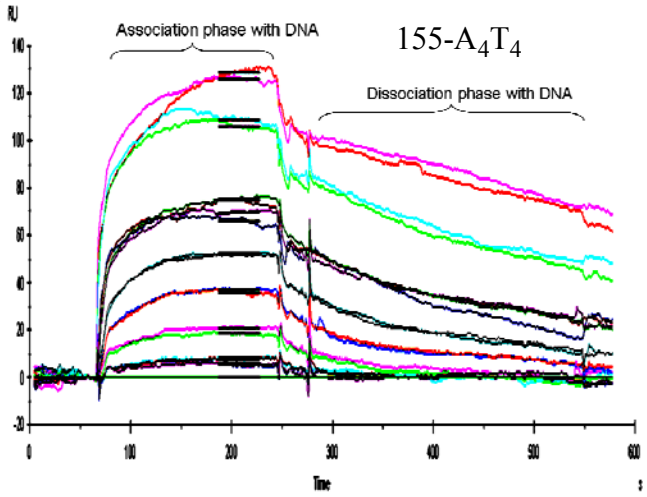


Figure 79 BIAcore SPR sensorgrams for interaction of compound 155 with the A4T4 sequence DNA hairpins in PBS buffer at 25 °C, the compound 155 bind weekly to A4T4 sequence.

colour in Fig.79	Conc	Response (RU)
●	187	129
●	187	126
●	94	106
●	94	109
●	47	66.2
●	47	74.4
●	23.5	69.8
●	23.5	75.6
●	11.8	52.6
●	11.8	51.7
●	5.88	37.3
●	5.88	35.9
●	2.93	21.2
●	2.93	18.3
●	1.47	8
●	1.47	5.31
●	0.734	7.83
●	0	0

Table 6 The concentrations of compound 155 from 0 (reference line, green) to 187 µM at each RU value.

4.1.5 Dilution of benzimidazoles

Benzimidazoles were dissolved first in DMSO: solution A of a compound was prepared using the following as an example calculation:

Mw = 896

n = 5.58 µmole

V= 1 mL

$$no.of\ moles \quad n = \frac{mass\,(g)}{Mwt\,(g.mol^{-1})}$$

$$= \frac{5\times10^{-3}}{896} = 5.58\times10^{-6}\ mole = 5.58\ \mu mole$$

$$C_A = \frac{n}{V} = \frac{5.58\mu\,mole}{1\,mL} = \frac{5.58\times10^{-6}}{1\times10^{-3}} = 0.00558\ M = 5.58\ mM$$

A 20 µL aliquot of this solution **A** was mixed with 380 µL of 1.05x PBS buffer to prepare solution **B** with total volume of 400 µL calculated as follows:

$$C_A \times V_A = C_B \times V_B$$

$$5.58\times 20 = C_B \times 400$$

$$C_B = \frac{5.58\times 20}{400}$$

$$C_B = 0.279\ mM$$

This dilution of 20 µL into 400 µL results in a twenty-fold dilution to give a final concentration of 0.279 mM.

Further dilution provided solution **C** with

$\mathbf{C_C}$ = 0.139 M, $\boldsymbol{V_C}$ =200 µL

This solution was generated by taking 100 µL of solution **B** and adding 100 µL of 1.05x PBS buffer, generating solution **C** with total volume of 200 µL. The final molarity of sample C was calculated as follows:

$$C_B \times V_B = C_C \times V_C$$

$$0.279\times100 = C_C \times 200$$

$$C_C = \frac{0.279\times100}{200}$$

$$C_C = 0.139\,mM = 139\,\mu M$$

Compound	V (mL)	n (μmole)	C_A (mM)	V_A (μL)	V_A* (μL)	V_1 (μL)	V_B (μL)	V_B* (μL)	V_C (μL)	C_C (μM)
171	1	5.58	5.58	20	380	400	100	100	200	139
174	1	6.83	6.83	20	380	400	100	100	200	170
155	1	11.23	11.23	20	380	400	25	50	75	187
184	1	8.22	8.22	20	380	400	25	50	75	137

Table 7 Dilution of benzimidazoles soluble in DMSO

V = Volume of DMSO used to dissolve sample

n = Number of moles of sample

C_A = Molarity of solution A

V_A = Volume that was taken from V

V_A^* = Volume of 1.05x PBS buffer that was added to V_A

$V_1 = V_A + V_A^*$

V_B = Volume that was taken from V_1

V_B^* = Volume of 1.05x PBS buffer that was added to V_B

$V_C = V_B + V_B^*$

C_C = Molarity of solution C

4.2 Analysis and conclusion

The SPR studies reveal two types of binding motif. The symmetrical biaryl core of **171**, **174** and **184** and the unsymmetrical dimeric backbone of **155**. The efficacy of binding is higher for the symmetrical (and larger) systems. Interestingly, the dimer **155** binds about 4-5 fold better to the longer A3T3 and A4T4 sequences. This is a good new compound which leads for future work. However, the symmetrical systems show significantly tighter binding, with examples in the low micromolar to nanomolar ranges. There is some evidence of a trend to binding better to the longer sequence for the tetrameric systems, where binding to the A4T4 sequences is an order of magnitude better than to A3T3.

Recently, a series of symmetric head-to-head bis-benzimidazoles have been reported in the literature [72] (see **Section 1.6**) (**186** and **187**) and been evaluated for their binding affinities to four consecutive AT base pairs in the minor groove of the duplex d(CGCGAATTCGCG)$_2$ (**Figure 80**).

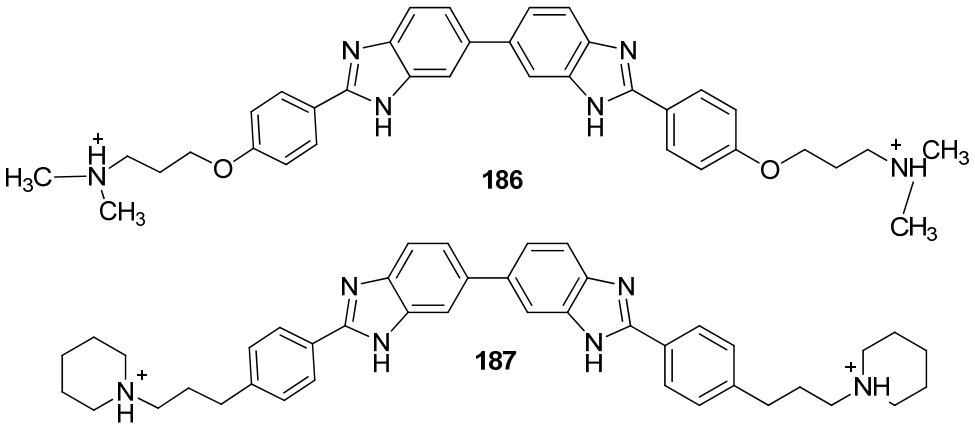


Figure 80 Structures of symmetric head-to-head bis-benzimidazoles have been reported in the literature [72]

The structural studies of the two reported symmetric head-to-head bis-benzimidazoles (**186** and **187**) provide us with the basis to identify factors controlling AATT DNA sequence recognition. The majority of reported compounds are symmetrical and possess a 2,2'-diphenyl-5,5'-bis benzimidazole core (**Figure 81**).

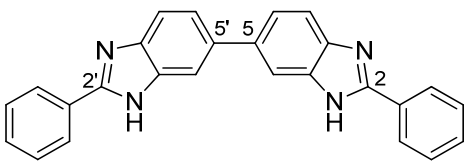


Figure 81 Common structure of the core of comparable ligands

This thesis reports that benzimidazole systems **155**, **171**, **174** and **184** provide a very favourable and flexible DNA recognition.

This information can be of use in guiding the design of effective DNA-binding molecules and binding affinity for AATT sequences. A key binding feature is due to the presence of NH atom, which acts as hydrogen donors (**Figure 82**).

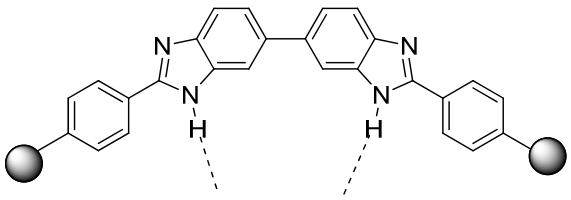


Figure 82 Generic structure showing hydrogen bonding formation

Binding of Hoechst 33258 to AT-rich regions is well known to involve NH protons of 33258 interacting with adenine N3 and/ or thymine O2, which are capable of acting as hydrogen acceptor through specific hydrogen bonding (**Figure 83**).

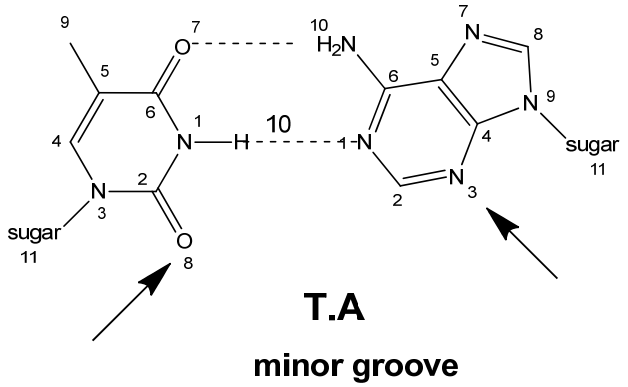


Figure 83 The structure of adenine and thymine acting as hydrogen acceptors.

Our novel compounds **155**, **171**, **174** and **184** (**Figure 84**) bind to the minor groove of a 2-6 bp long AT-tract oligonucleotide and from precedents, it is likely that this also involves the NH atom in the benzimidazole rings of these compounds and the linker amide nitrogen atoms binding to DNA bases *via* hydrogen bonding interactions.

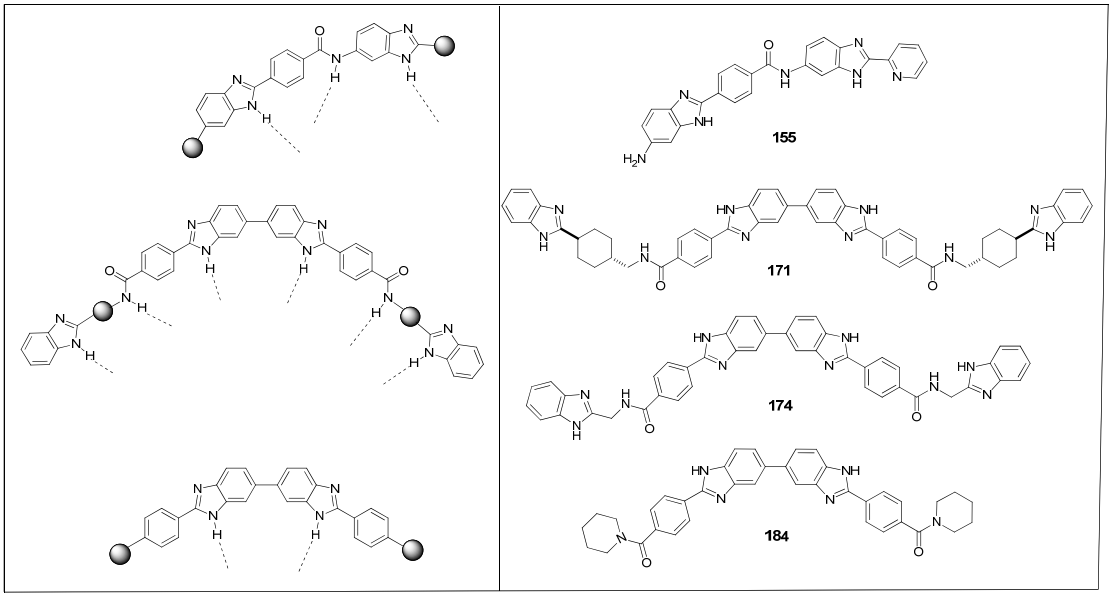


Figure 84 Generic structures showing the ligand-DNA interface on left side compared to our binding examples on right side

For the novel ligands in this thesis. The affinity of binding is higher for the symmetrical systems, and encourages the future design of analogues better ablity to target specific sites in DNA.

These structural studies provide a basis to identify factors controlling DNA recognition and design DNA molecules that could ultimately be used as tools or agents to control gene expression in cells and that would guide the synthesis of possible targets and employ extensive simulation methods to produce a set of structures.

It is now well established that design of low molecular organic compounds such as benzimidazoles that can site-specifically recognize sequences in double stranded DNA is important for creating tools for studying molecular biology, on the other hand design of these compounds is an important pharmacological task, since chemotherapeutic efficacy of most of the currently used anti cancer drug depends on selectivity and efficiency of their interaction with DNA.

In the future, we will compare the sequence recognition properties of our compounds by means of NMR and other methods, to obtain more information for DNA properties and may be used in guiding the design of future effective DNA-binding molecules.

However the pharmacokinetics, bioavailability and toxicity of benzimidazoles remain to be established.

Considering the increasing contribution of cancer to the overall mortality rate, a more rational design and application of novel DNA binding compounds, with both higher efficiency and selectivity, constitutes an urgent task in medicinal chemistry.

4.3 Future work:-

It is now established that benzimidazole systems can provide favourable and flexible DNA recognition modules. Benzimidazoles, for example, form the core of Hoechst 33258 (H258), one of the most extensively studied minor groove binding agents, as well as of a variety of other related DNA-binding compounds (see **Section 1.6**).

To probe these interaction differences further, we have synthesized several new types of benzimidazole derivatives, which can be related to the well-known minor groove agent, Hoechst 33258.

The work has developed chemistry enabling synthesis of several different types of di-, tri- and tetra-benzimdiazoles with different sorts of linker units. In the future, this should enable the synthesis of a range of differently functionalized systems as the synthesis is designed to be modular. In this thesis a diversity of dimeric, trimeric and tetrameric ligands have been prepared.

These agents have to date been evaluated for their ability to bind to DNA using Surface Plasmon Resonance (SPR), which provides a practical method for determination of the binding affinity and rate constants. In this thesis the affinity of binding is higher for the our novel symmetrical ligands, and encourages the future design of analogues with better ability to target specific sites in DNA. Our new unsymmetrical dimeric structure **155** binds about 4-5 fold better to the longer A3T3 and A4T4 sequences. However, the symmetrical biaryl core of **171**, **174** and **184** show significantly tighter binding, with examples in the low micromolar to nanomolar ranges. There is some evidence of a trend of binding being tighter to the longer DNA sequence for the tetrameric systems, where binding to the A4T4 sequences is an order of magnitude better than to A3T3. These are good new compounds which can form the basis for future work.

The aims of future work are to identify features leading to DNA binding and to determine and prove the type of binding. They will require the use of other techniques including 2D-NMR, CD spectroscopy, T_m studies, gel chromatography, foot printing and fluorescence. Future collaborative work can aim to determine more details of sequence preferences, with the aim of guiding the design of future specific DNA-binding molecules.

The work in this thesis shows that with initial leads identified, future work is needed to further define structure-activity features, including the effects of physico-chemical

features to be addressed, such as polarity, lipophobicity or lipophilicity, and also solubility.

Chapter 5
Experimental

5.1 General experimental

^{1}H NMR spectra were recorded at 300 and 400 MHz on a Bruker DPX 300 and 400 spectrometer and ^{13}C NMR and DEPT spectra at 75 MHz and 100 MHz on a Bruker DPX300 and DPX 400 spectrometer. In addition to 1D NMR (^{1}H NMR, ^{13}C NMR and DEPT), 2D NMR such as (HMQC and COSY) were used in spectra interpretation. Chemical shifts are denoted in ppm (δ) relative to internal solvent standard (TMS in the case of ^{1}H). The splitting patterns for NMR-spectra are designated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), tt (triplet of triplets), dt (doublet of triplets), q quartet, dq (doublet of quartets), qd (quartet of doublets), and m (multiplet). Coupling constants (J) are designated in Hz. Mass spectra were recorded on a VG 70/70 Hybrid or a Kratos MS-50 mass spectrometer by ES. Melting points were determined with an electrothermal melting point apparatus. IR spectra were obtained using a Perkin Elmer 1605 FTIR spectrometer (KBr disc). Analytical TLC was performed on Merck 60 F254 aluminium backed plate and were developed using an UV lamp. Column chromatography employed Prolabo Silica Gel 60 (70-230 Mesh), using pre-distilled hexane and AnalaR (AR) ethyl acetate. THF was dried by distillation from sodium benzophenone ketyl. All reactions were conducted in dry glassware under an nitrogen atmosphere, unless otherwise stated. All chemicals were used directly from suppliers vessel without further purification, unless otherwise stated.

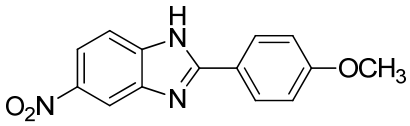
5.2 Condensation of aldehyde or carboxylic acid with phenylenediamine.

5.2.1 General method for condensation in the presence of $Na_2S_2O_5$.

An appropriate substituted benzaldehyde (15 mmol) was dissolved in EtOH (50 mL). Sodium bisulfite (1.6 g) in water (10 mL) was added to the cooled solution in portions and stirred vigorously. The precipitate which formed was filtered off and dried *in vacuo*. The mixture of this adduct (2 mmol) and substituted *o*-phenylene diamine (2 mmol) in DMF (5 mL) were heated at 140 ^{o}C for defined time after which the reaction mixture

was cooled, poured into water, and the solid was filtered off and purified by recrystallization from an appropriate solvent.

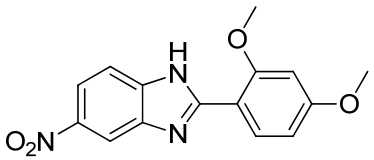
5.2.1.1 2-(4'-Methoxyphenyl)-5-nitrobenzimidazole (94) [80, 81]



The title compound was synthesized by condensation of 4-nitro-1,2-phenylenediamine and *p*-anisaldehyde bisulfite adduct for 16 h according to procedure **5.2.1** and purified by recrystallization from water: ethanol (1:1 v/v) affording **94** as a yellow solid (1.49 g, 37%); Mp 239 °C, (Lit. [80, 81] 238 °C).

^{1}H NMR (400 MHz, DMSO) 3.92 (s, 3H), 7.21 (d, *J* = 7.4 Hz, 2H), 7.77 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 2H), 8.40-8.59 (bs, 1H), 13.40-13.60 (br, NH). ^{13}C NMR (100 MHz, DMSO) 55.8 (CH_3), 114.9 (2CH), 115.7 (C), 118.1 (CH), 121.8 (C), 127.2 (CH), 129.0 (2CH), 129.1 (CH), 142.6 (C), 142.8 (C), 156.2 (C), 161.8 (C). LRMS: (ES) *m/z* $(MH)^+$ 270.8 for $C_{14}H_{12}N_3O_3$.

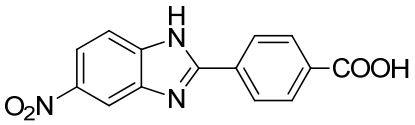
5.2.1.2 2-(2',4'-Dimethoxyphenyl)-5-nitrobenzimidazole (96) [81]



The title compound was synthesized by condensation of 4-nitro-1,2-phenylenediamine and 2,4-dimethoxybenzaldehyde bisulfite adduct for 48 h according to procedure **5.2.1** and purified by recrystallization from water: ethanol (1:3 v/v) affording **96** as a yellow solid (0.46 g, 77%); Mp 201 °C, (201 °C [81]).

^{I}H NMR (400 MHz, $CDCl_3$) 3.99 (s, 3H), 4.18 (s, 3H), 6.73 (s, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 8.8 Hz, 1H), 8.61 (s,1H). ^{13}C NMR (75 MHz, $CDCl_3$) 56.6 (CH_3), 57.1 (CH_3), 99.9 (CH), 101.6 (CH), 108.5 (CH), 109.2 (CH), 114.5 (CH), 122.3 (C), 130.1 (C), 131.9 (C), 132.0 (CH), 134.4 (C), 146.0 (C), 151.1(C), 153.3 (C). LRMS: (ES) *m/z* $(MH)^+$ 301.4 for $C_{15}H_{14}N_3O_4$.

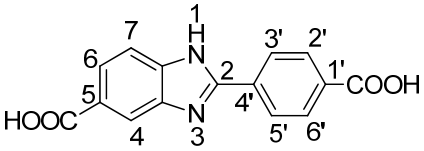
5.2.1.3 2-(4'-Carboxyphenyl)-5-nitro-1*H*-benzimidazole (105) [81]



The title compound was synthesized by condensation of 4-nitro-1,2-phenylenediamine and 4-carboxybenzaldehyde bisulfite adduct for 72 h according to procedure **5.2.1** and purified by recrystallization from acetic acid affording **105** as a brown solid (0.41 g, 73%); Mp > 300 °C, (> 300 °C [81]).

^{1}H NMR (400 MHz, DMSO) 7.96 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 2H), 8.11 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 2H), 8.95 (s, 1H), 13.22-13.23 (bs, 1H, NH or COOH). ^{13}C NMR (100 MHz, DMSO) 113.9 (2CH), 115.4 (2CH), 126.5 (2CH), 128.1 (CH), 131.3 (C), 134.2 (C), 153.8 (2C), 166.9 (C=O). LRMS: (ES) *m/z* $(MH)^+$ 284.0 for $C_{14}H_9N_3O_4$.

5.2.1.4 2-(4'-Carboxyphenyl)-5-carboxy-1*H*-benzimidazole (106)



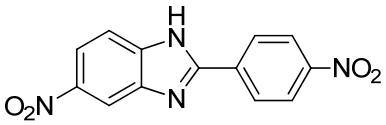
The title compound was synthesized by condensation of 3,4-diamino benzoic acid and 4-carboxybenzaldehyde salt for 24 h according to procedure **5.2.1** and purified by recrystallization from water: ethanol (1:1 v/v) affording **106** as a beige solid (044 g, 78%); Mp > 300 °C.

^{1}H NMR (300 MHz, DMSO) 7.68 (d, *J* = 7.5 Hz, 1H, H7), 7.85 (d, *J* = 7.5 Hz, 1H, H6), 8.11 (d, *J* = 7.8 Hz, 2H, H2'+H6'), 8.19 (s, 1H, H4), 8.29 (d, *J* =7.8 Hz, 2H, H3'+H5'), 13.07-13.62 (br, 2H, 2COOH). ^{13}C NMR (75 MHz, DMSO) 124.2 (CH), 124.4 (CH), 125.3 (CH), 127.1 (2CH), 130.3 (2CH), 132.5 (C), 132.7 (C), 133.7 (C), 134.0 (C), 152.8 (C), 152.9 (C), 167.2 (C=O), 168.1 (C=O). IR υ_{max} cm $^{-1}$ 1279 (C-N), 1060 (C-O), 1460 (C=C), 1654 (C=N), 1685 (C=O), 1700 (C=O), 3120 (C-H, aromatic), 3343 (N-H, amine) 3417 (O-H). HRMS: (ES) *m/z* $(MH)^+$ obsd. 283.0722, calcd. 283.0713 for $C_{15}H_{11}N_2O_4$.

5.2.2 General method for condensation in the presence of nitro benzene

A mixture of unsubstituted or substituted phenylenediamine (10 mmol) and appropriate aldehyde (10 mmol) in nitrobenzene (50 mL) was heated at 140 °C. Once tlc showed there was no starting material, the mixture was cooled and filtered after adding (50 mL) of water. The crude preciptate was purified by recrystallization or column chromatography.

5.2.2.1 5-Nitro-2(4-nitrophenyl)-1*H*-benzimidazole (107) [82,81]



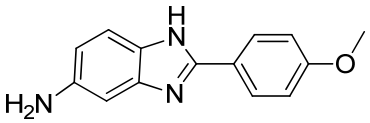
The title compound was synthesized by condensation of 5-nitro-1,2-phenylenediamine and *p*-nitrobenzaldehyde sulfite adduct for 48 h according to procedure **5.2.2** and purified by column chromatography using DCM:MeOH (10:1) affording **107** as a yellow solid (1.70 g, 69 %); Mp > 300 °C (Lit. [82] 356 °C, [81] > 300 °C).

^{1}H NMR (300 MHz, DMSO) δ 7.81 (d, *J* = 8.4 Hz, 1H,), 8.15 (d, *J* = 8.4 Hz, 1H,), 8.43 (s, 4H), 8.51 (s, 1H), 13.93 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO) 118.9 (CH), 124.3 (CH), 124.4 (2CH), 124.7 (CH), 127.9 (C) 128.4 (2CH), 131.0 (CH), 135.1 (C), 143.5 (C), 148.8 (CH), 153.8 (C). LRMS: (ES) *m/z* $(MH)^+$ 285.0 for $C_{13}H_9N_4O_4$.

5.3 General procedure for reducing nitrobenzimidazoles *via* $SnCl_2$/HCl.

A solution of nitrobenzimidazole (2 mmol) in conc. HCl (10 mL) was treated portionwise with $SnCl_2$ (12 mmol, 2.28 g) and the resulting solution was stirred at room temperature for several hours. The reaction mixture was poured into ice-water and neutralized with NaOH 10% solution, extracted with ethyl acetate (5 x 25 mL) washed with water (3 x 15 mL), dried over anhydrous Na_2SO_4, and then solvent removed *in vacuo* leaving an oily material. The residue was crystallized from acetone:hexane (1:10 v/v) and purified either by recrystallization or column chromatography.

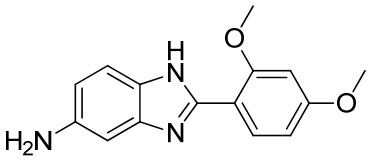
5.3.1 5-Amino-2-(4'-methoxyphenyl) benzimidazole (100) [83,81]



The title compound was synthesized according to method **5.3** by reaction over 24 hour and purified by recrystallization from ethanol affording **100** as a pale brown solid (0.32 g, 67 %); Mp 201-203 °C. *

^{1}H NMR (400 MHz, DMSO) 3.91 (s, 3H), 7.32 (d, J = 9.0 Hz, 2H), 7.48 (dd, J_1 = 8.5, J_2 = 1.5 Hz, 1H), 7.81 (d, J = 1.5 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 9.0 Hz, 2H) . LRMS: (ES) *m/z* $(MH)^+$ 240.9 for $C_{14}H_{14}N_3O_1$, (^{13}C NMR see **5.4.1**).

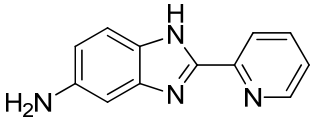
5.3.2 5-Amino-2-(2',4'-dimethoxyphenyl) benzimidazole (101) [81]



The title compound was synthesized according to method **5.3** by reaction over 24 h and purification by column chromatography DCM:MeOH (10:1 v/v) will afford **101** as a brown solid (0.42 g, 78 %); Mp 187 °C, (187 °C [81]).

^{1}H NMR (400 MHz, $CDCl_3$) 3.43-3.74 (br, 2H, NH_2), 3.91 (s, 3H), 4.08 (s, 3H), 6.62 (s, 1H), 6.70 (d, J = 8.4 Hz, 2H), 6.90-6.94 (bs, 1H), 7.44-7.50 (bs, 1H), 8.49 (d, J = 8.4 Hz, 1H), 10.18-10.70 (br, NH) ^{13}C NMR (75 MHz, $CDCl_3$) 56.5 (CH_3), 57.1 (CH_3), 100.1 (CH), 101.4 (CH), 108.5 (CH), 110.1 (CH), 115.6 (CH), 121.9 (C), 127.8 (C), 130.9 (CH), 131.2 (C), 131.6 (C), 149.9 (C), 160.3 (C), 167.8 (C). LRMS: (ES) *m/z* $(MH)^+$ 270.1 for $C_{15}H_{16}N_3O_2$.

5.3.3 2-Pyridin-2-yl-1*H*-benzimidazol-5-ylamine (102) [84, 81]



* This compound is known, but there is no data regarding its melting point.

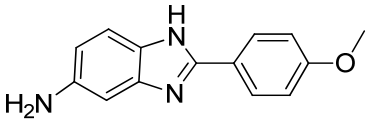
The title compound was synthesized according to method **5.3** by reaction over 7 h and purified by recrystallization from ethanol affording **102** as a brown solid (0.28 g, 66 %); Mp 215 °C (Lit. [84, 81] 217 °C)

^{1}H NMR (400 MHz, DMSO) 7.50 (dd, J_1 = 8.8, J_2 = 1.6 Hz, 1H), 7.74 (dd, J_1 = 8.0, J_2 = 4.8 Hz, 1H), 7.84 (d, J = 1.6 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 8.19 (t, J = 8.0 Hz, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.89 (d, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) 108.8 (CH), 116.2 (CH), 121.3 (CH), 123.7 (CH), 126.2 (CH), 127.8 (CH), 131.9 (C), 133.2(C), 138.8 (C), 142.6 (C), 149.4 (CH), 150.6 (C). LRMS: (ES) *m/z* $(M+2H)^+$ 212.1 for $C_{12}H_{11}N_4$.

5.4 General procedure for reducing nitrobenzimidazoles *via* H_2/Pd(C)

A solution of nitrobenzimidazole (3.5 mmol) in DMF/MeOH (10 mL:10 mL) was hydrogenated over 10% Pd/C (0.15 g), under an atmospheric pressure of H_2. TLC showed the reduction went to a completion after 24-48 h, the catalyst was removed by filtration through Celite®, and then water was added and the ppt was purified by either recrystallization or column chromatography.

5.4.1 5-Amino-2-(4'-methoxyphenyl) benzimidazole (100) [83,81]



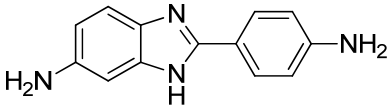
The title compound was synthesized from reduction of 2-(4'-methoxyphenyl)-5-nitrobenzimidazole (3.5 mmol, 0.941 g) according to method **5.4** with the reaction time extended for 24 h. The crude product was purified by recrystallization from ethanol affording **100** as a pale brown solid (0.57 g, 68 %); Mp 201-203 °C. *

^{1}H NMR (400 MHz, $CDCl_3$) 3.86 (s, 3H), 6.68 (d, J = 8.5 Hz, 1H), 6.85 (s, 1H), 6.97 (d, J = 9.1 Hz, 2H), 7.45 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75

* This compound is known, but there is no data regarding its melting point.

MHz, $CDCl_3$) 55.7 (CH_3), 99.0 (CH), 113.0 (2CH), 114.7 (CH), 116.8 (C), 122.6 (C), 128.1(2CH), 134.3 (CH), 138.8(C), 143.0 (C), 150.9 (C), 161.2(C). LRMS: (ES) *m/z* $(MH)^+$ 240.7 for $C_{14}H_{14}N_3O_1$.

5.4.2 2 (4-Amino-phenyl)-3*H*-benzimidazol-5-ylamine (108) [85, 81]



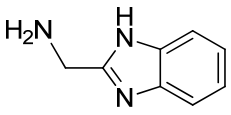
The title compound was synthesized by reduction of 5-nitro-2(4-nitro phenyl)benzimidazole (3.5 mmol, 0.994 g) according to method **5.4**, with the reaction extended to 48 h. The crude product was purified by recrystallization from ethanol affording **108** as a brown solid (0.61 g, 78 %); Mp 247 °C. *

^{1}H NMR (400 MHz, DMSO) 6.06-6.38 (br, 4H, NH amine), 6.76 (dd, J_1 = 8.4, J_2 = 2.0 Hz, 1H), 6.77 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 1.8 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) 96.9 (C), 111.2 (C), 114.4 (2CH), 114.6 (CH), 120.0 (C), 125.8 (CH), 129.6 (2CH), 134.9(C), 146.9 (CH), 148.8 (C), 153.6 (C). LRMS: (ES) *m/z* $(MH)^+$ 225.1 for $C_{13}H_{13}N_4$.

5.5 General method for condensation with aliphatic carboxylic acid (Phillip's method)

A solution of unsubstituted or substituted *o*-phenylene diamine (10 mmol) and 4N HCl (15 mL) was treated with appropriate aliphatic carboxylic acid (15 mmol) and heated under reflux for 3-24 h. The solution was cooled and allowed to stand overnight. The mixture was neutralized with 2 N NaOH, the solid was filtered, washed with water and purified by recrystalization from suitable solvent.

5.5.1 2-(Aminomethyl)-1*H*-benzimidazole (110) [86, 81]

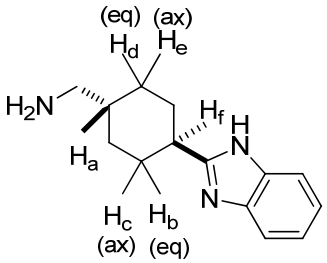


* This compound is known, but there is no data regarding its melting point.

The title compound was synthesized by condensation of *o*-phenylenediamine (1.08 g) with glycine (1.12 g) for 24 h according to procedure **5.5** and purified by recrystallization from diethyl ether affording **110** as a pale green crystals (0.65g, 44 %); Mp 252 °C (Lit. [86, 81] 252 °C)

^{1}H NMR (400 MHz, DMSO) 4.56 (s, 2H, CH_2), 4.74-6.78 (br, 2H, NH_2), 7.36 (dd, J_1 = 6.2, J_2 = 3.2 Hz, 2H), 7.67 (dd, J_1 = 6.1, J_2 = 3.2 Hz, 2H), 9.15-9.18 (bs, 1H NH). ^{13}C NMR (75 MHz, DMSO) 35.0 (CH_2), 114.8 (2CH), 125.8 (2CH), 132.6 (2C), 147.4 (C). LRMS: (ES) *m/z* $(MH)^+$148.1 for $C_8H_{10}N_3$.

5.5.2 4-(1-*H*-Benzimidazol-2-yl)cyclohexyl)methanamine (112) [87,81]

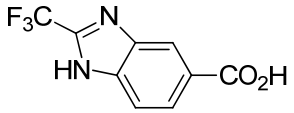


The title compound was synthesized by condensation of *o*-phenylenediamine (1.08 g) with *trans* 4-aminomethylcyclohexane carboxylic acid (2.32 g) for 9 h according to procedure **5.5** and purified by recrystalisation from ethanol afforded **112** as a beige powder (1.12g, 49%); Mp 238 °C.*

^{1}H NMR (300 MHz, DMSO) 0.83 (qd, J_1 = 12.5, J_2 = 2.8 Hz, 2H, H_c), 1.16-1.21 (m, 1H, H_a), 1.36 (qd, J_1 = 12.5, J_2 = 2.8 Hz, 2H, H_e), 1.68 (dq, J_1 = 12.0, J_2 = 0.8 Hz, 2H, H_b), 1.85 (dq, J_1 = 12.0, J_2 = 0.8 Hz, 2H, H_d), 2.29 (d, J = 7.5 Hz, 2H, CH_2), 2.55 (tt, J_1 = 12.0, J_2 = 0.8 Hz, 1H, H_f), 3.36-3.64 (br, 2H, NH_2), 6.87 (dd, J_1 = 6.0, J_2 = 3.0 Hz, 2H), 7.23 (dd, J_1 = 6.0, J_2 = 3.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) 29.0 ($2CH_2$), 29.5 ($2CH_2$), 34.9 (CH), 35.9 (CH), 44.4 (CH_2), 114.2 (2CH), 125.8 (2CH), 131.2 (2C), 157.4 (C). LRMS: (ES) *m/z* $(MH)^+$ 230.2 for $C_{14}H_{20}N_3$.

* This compound is known, but there is no data regarding its melting point.

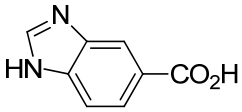
5.5.3 2-(Trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid (115) [88,81]



The title compound was synthesized by condensation of 3,4-diaminobenzoic acid (1.52 g) with trifluoroacetic acid (1.71 g) for 3 h according to procedure **5.5** and purified by recrystallization from ether affording **115** as a white powder (1.68 g, 73 %); Mp 286 °C (Lit. [88, 81] 285 °C) .

^{1}H NMR (400 MHz, DMSO) 7.83-7.89 (bs, 1H), 8.05 (d, J = 8.5 Hz, 1H), 8.37 (s, 1H), 12.86-13.27 (br, 1H, NH), 14.25-14.62 (br, 1H, COOH). ^{13}C NMR (100 MHz, DMSO) 115.6 (CH), 118.3 (CH), 120.9 (CH), 123.6 (C), 126.0 (C), 127.4 (C), 143.0 (C, CF_3, J_{CF} = 40.0 Hz), 168.2 (C=O). LRMS: (ES) *m/z* $(MH)^+$ 231.9 for $C_9H_5F_3N_2O_2$.

5.5.4 1*H*-Benzimidazole-5-carboxylic acid (116) [89,81]



The title compound was synthesized by condensation of 3,4-diaminobenzoic acid with formic acid for 4 h according to procedure **5.5** and purified by recrystallization from ethanol affording **116** as a white solid (1.34 g, 83 %); Mp 243 °C [*].

^{1}H NMR (400 MHz, DMSO) δ 7.21-7.49 (bs, 1H, N-H Imidazole), 7.62-7.69 (bs, 1H), 7.83 (d, J = 8.4 Hz, 1H), 8.20 (s, 1H), 8.39 (s, 1H), 12.53-13.22 (br, 1H, COOH). ^{13}C NMR (100 MHz, DMSO) 116.9 (CH), 121.9 (C), 122.5 (C), 122.8 (CH), 123.2 (C), 124.1 (CH), 143.2 (CH), 166.7 (C=O). LRMS: (ES) *m/z* $(MH)^+$163.0 for $C_8H_7N_2O_2$.

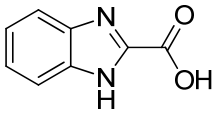
5.6 General procedure for the synthesis of unsubstituted and substituted 2-carboxybenzimidazole

Fresh methyltrichloroacetimidate (5 mmol, 0.88 g) was added dropwise to a cooled solution of unsubstituted or substituted *o*-phenylenediamine (4 mmol) in acetic acid (20 mL). At the end of the addition (30 minutes), the reaction was kept at room temperature

[*] This compound is known, but there is no data regarding its melting point.

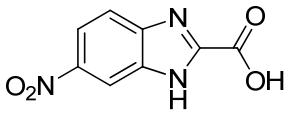
for 1 h and the resulting precipitate was filtered, washed with acetic acid (10 mL) and dried *in vacuo* to give unsubstituted or substituted 2-(trichloromethyl) benzimidazole as a white solid which was added to a cooled solution of sodium hydroxide (100 ml; 1N). The resulting solution was filtered and the filtrate was acidified with hydrochloric acid (1N) to pH 4. The precipitate was filtered off, washed twice with a solution of water and acetonitrile 3:1 v/v (30 mL) and ether (2 x 30 mL).

5.6.1 1-*H*-Benzimidazole-2-carboxylic acid (123) [90,81]



The title compound was synthesized according to method **5.6** in which *o*-phenylendiamine condensed with methyltrichloroacetimidate affording 2-trichloro methylbenzimidazole **17,** that was then hydrolysed affording the corresponding 2-carboxy benzimidazole **123** as an off-white solid (0.67 g, 83 %); Mp 65 °C * (65 °C [81]). ^{1}H NMR (400 MHz, DMSO) 7.16 (dd, J_1 = 8.0, J_2 = 3.8 Hz, 2H), 7.45 (dd, J_1 = 8.0, J_2 = 3.6 Hz, 2H), 8.18-8.96 (br, 2H, NH+COOH). ^{13}C NMR (100 MHz, DMSO) 116.3 (2CH), 124.8 (2CH), 136.6 (2C), 144.9 (C), 159.3 (C=O). LRMS: (ES) *m/z* $(MH)^+$ 163.0 for $C_8H_5N_2O_2$.

5.6.2 5-Nitro-1*H*-benzimidazole-2-carboxylic acid (124) [90,81]



The title compound was synthesized according to method **5.6** in which 5-nitro-*o*-phenylenediamine condensed with methyltrichloroacetimidate affording 5-nitro-2-trichloromethylbenzimidazole **118**, that was then hydrolysed affording the corresponding 5-nitro-2-carboxy-1*H*-benzimidazole **124** as a pale yellow solid (0.64 g, 77 %); Mp 46 C * (46 °C [81]).

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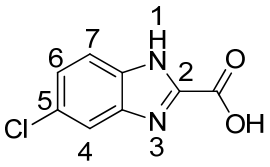
* Because of decarboxylation, this compound is known in references as decomposed

^{1}H NMR (400 MHz, DMSO) 3.98-4.83 (br, 1H, N-H), 7.72 (d, *J* = 6.9 Hz, 1H), 8.13 (d, *J* = 6.9 Hz, 1H), 8.49 (s, 1H), ^{13}C NMR (75 MHz, DMSO) 114.7 (CH), 116.2 (CH), 17.9 (C), 119.5 (C), 126.6 (CH), 143.9 (C), 147.9(C), 160.4 (C=O). LRMS: (ES) *m/z* $(MH)^+$ 206.0 for $C_8H_4N_3O_4$.

5.6.3 5-Chloro-1*H*-benzimidazole-2-carboxylic acid (125) [90]



The title compound was synthesized according to method **5.6** in which 5-chloro-*o*-phenylenediamine condensed with methyltrichloroacetimidate affording 5-chloro-2-trichloromethylbenzimidazole **119** that was then hydrolysed affording the corresponding 5-chloro-2-carboxy-1*H*-benzimidazole **124** as a pale yellow solid (0.79 g, 80 %); Mp 155 °C*.

^{1}H NMR (400 MHz, DMSO) 7.22 (dd, J_1 = 8.4, J_2 = 2.0 Hz, 1H, H6), 7.61 (d, *J* = 8.4 Hz, 1H, H7), 7.67 (d, *J* = 2.0 Hz, 1H, H4), 8.30 (s, 1H, NH or COOH), ^{13}C NMR (100 MHz, DMSO) 115.5 (CH), 116.3 (CH), 116.7 (C), 122.4 (C), 124.7 (CH), 126.5 (C), 143.8 (C), 160.4 (C=O). IR υ_{max} cm $^{-1}$ 809 (C-Cl), 1294 (C-N), 1063 (C-O), 1441 (C=C), 1609 (C=N), 1636 (C=O), 3100 (C-H, aromatic), 3378 (N-H, amine) 3503 (O-H). HRMS: (ES) *m/z* $(MH)^+$ obsd. 196.0028, calcd. 196.0034 for $C_8H_5ClO_2N_2$.

5.7 General procedure for synthesis of dimeric and trimeric benzimidazoles by using the coupling reagent EDC/HOBt.

The unsubstituted or substituted 2-carboxybenzimidazole (1.1 mmol) was dissolved in dry DMF (10 mL) with HOBt (1.1 mmol) and EDC (1.1 mmol). The reaction was then cooled to 0 °C and, after half an hour, the amine or the aminobenzimidazole (1 mmol)

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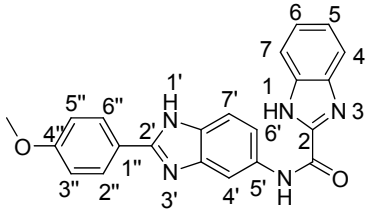
* This compound is known, but there is no data regarding its melting point.

was added and the reaction left stirring at room temperature. Once the TLC showed no starting material, the solvent was removed *in vacuo*, then (25 mL) water and ethyl acetate (50 mL) were added. The organic layer was washed with HCl 5% (5 mL) then with $NaHCO_3$ (10%) (5 mL) and finally with brine (5 mL). The organic layers were combined, dried ($MgSO_4$) concentrated and left over night *in vacuo*. The crude product was purified either by column chromatography or recrystallization.

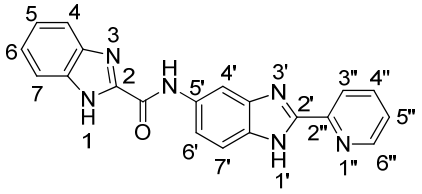
5.7.1 1*H*-Benzimidazole-2-carboxylic[2-(4'-methoxy-phenyl)-1*H*-benzimidazol-5-yl]-amide (130)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-carboxybenzimidazole with 5-amino-2-(4'-methoxyphenyl) benzimidazole by reaction over 20 h. The product was purified by column chromatography using DCM:MeOH (10:l v/v) affording **130** as a brown solid (0.30 g, 71 %); Mp 301-303 °C.

^{1}H NMR (400 MHz, DMSO) 3.92 (s, 3H, CH_3), 7.16 (d, *J* = 8.5 Hz, 2H, H3"+H5"), 7.35-7.45 (bs, 2H, H6'+H7'), 7.62 (d, *J* = 7.8 Hz, 2H, H5+H6), 7.81 (d, *J* = 7.8 Hz, 2H, H4+H7), 8.21 (d, *J* = 8.5 Hz, 2H, H2"+H6"), 8.36 (s, 1H, H4'), 11.07 (s, 1H, N-H Amide), 13.45-13.63 (bs, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 55.8 (CH_3), 105.9 (CH), 114.9 (2CH), 115.2 (CH), 117.1 (2CH), 120.0 (C), 121.1 (2CH), 125.2 (C), 128.9 (2CH), 134.2 (CH), 134.4 (C), 135.3 (C), 136.9 (C), 146.1 (2C), 151.3 (C), 157.5 (C), 161.6 (C=O). IR υ_{max} cm $^{-1}$ 1257 (C-N), 1078 (C-O), 1427 (C=C), 1644 (C=N), 1669 (C=O), 2928 (C-H, aliphatic), 3076 (C-H, aromatic), 3221 (N-H, amide), 3387 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 384.1453, calcd. 384.1455 for $C_{22}H_{17}N_5O_2$.

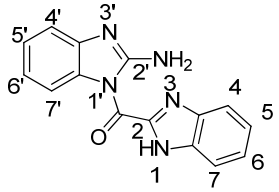
5.7.2 1*H*-Benzimidazole-2-carboxylica cid(2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-amide (131)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-carboxybenzimidazole with 2-pyridin-2-yl-1*H*-benzimidazol-5-ylamine by reaction over 12 days. The product was purified by recrystallization from ethanol affording **131** as a pale brown solid (0.27 g, 69%); Mp > 300 °C .

^{1}H NMR (400 MHz, DMSO) 7.36 (dq, J_1 = 7.6, J_2 = 7.2 Hz, 2H, H5+H6), 7.53 (dd, J_1 = 9.2, J_2 = 2.8 Hz, 1H, H5"), 7.61 (dd, J_1 = 7.6, J_2 = 3.2 Hz, 1H, H4), 7.69 (s, 1H, H4'), 7.83 (d, J = 8.0 Hz, 1H, H7'), 8.02 (dd, J_1 = 7.6, J_2 = 3.2 Hz, 1H, H7), 8.34 (dd, J_1 = 8.0, J_2 = 2.4 Hz, 1H, H6'), 8.31-8.36 (m, 2H, H6'+H4"), 8.39-8.41 (bs, 1H, H3"), 8.75 (d, J = 2.8, 1H, H6"), 10.94 (s, 0.6 H, N-H Amide), 10.99 (s, 1H, O-H Iminol), 13.15 (s, 1H, N-H Imidazole), 13.46 (s, 1H, N-H Iminol), 13.49 (s, 0.7 H, N-H Imidazole). ^{13}C NMR (75 MHz, DMSO) 105.2 (CH), 113.5 (C), 114.7 (CH), 116.6 (2CH), 121.1 (CH), 123.6 (2CH), 124.4 (CH), 130.7 (CH), 137.3 (CH), 138.3 (2C), 138.8 (C), 140.8 (C), 142.2 (C), 145.3 (C), 147.9 (CH), 150.8 (C), 157.6 (C=O). IR υ_{max} cm $^{-1}$ 1280 (C-N), 1097 (C-O), 1490 (C=C), 1636 (C=N), 1670 (C=O), 3058 (C-H, aromatic), 3221 (N-H, amide), 3355 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 355.1288, calcd. 355.1302 for $C_{20}H_{15}N_6O$.

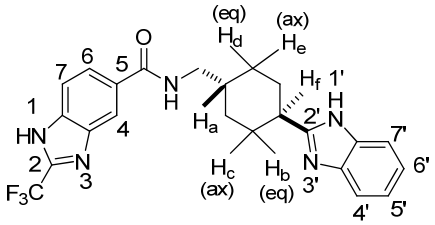
5.7.3 (2-Amino-benzimidazol-1-yl)-(1*H*-benzimidazol-2-yl)methanone (133)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-carboxybenzimidazole with 2-aminobenzimidazole by reaction over 11 days. The product was purified by recrystallization from ethanol affording **133** as a pale yellow solid (0.20 g, 66%); Mp > 300 °C .

^{1}H NMR (400 MHz, DMSO) 3.65-4.68 (br, 2H, NH_2), 7.08 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H5+H6), 7.24 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 2H, H5'+H6'), 7.43 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H4+H7), 7.63 (dd, J_1 = 8.8, J_2 = 4.4 Hz, 2H, H4'+H7'). ^{13}C NMR (75 MHz, DMSO) 113.3 (2CH), 116.6 (2CH), 120.8 (2CH), 122.9 (2CH), 135.9 (2C), 139.6 (C), 150.5 (C), 154.1 (C), 154.2 (C), 163.1 (C=O). IR υ $_{max}$ cm $^{-1}$ 1275 (C-N), 1060 (C-O), 1472 (C=C), 1628 (C=N), 1670 (C=O), 3056 (C-H, aromatic), 3350 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 278.1044, calcd. 278.1036 for $C_{15}H_{12}N_5O$.

5.7.4 2-Trifluoromethyl-1*H*-benzimidazole-5-carboxylic acid [4-(1*H*-benzimidazol-2-y1)-cyclohexylmethyl]-amide (138)

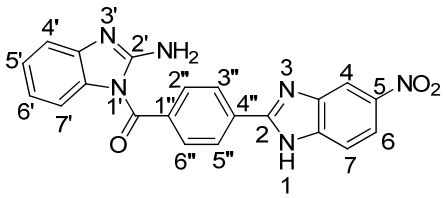


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 4-(1-*H*-benzimidazol-2-yl) cyclohexyl)methanamine by reaction over 2 days. The product was purified by recrystallization from ethanol: water (3:1 v/v) affording **138** as a pale yellow solid (0.33 g, 68 %); Mp 259-260 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 10.8, J_2 = 2.2 Hz, 2H, H_c), 1.59 (qd, J_1 = 10.8, J_2 = 2.2 Hz, 2H, H_e), 1.67-1.71 (m, 1H, H_a), 1.92 (dq, J_1 = 12.0, J_2 = 0.7 Hz, 2H, H_d), 2.11 (dq, J_1 = 12.0, J_2 = 0.7 Hz, 2H, H_b), 2.82 (tt, J_1 = 10.8, J_2 = 0.4 Hz, 1H, H_f), 3.23 (t, J = 6.4 Hz, 2H, CH_2), 7.11 (d, J = 6.0 Hz, 2H, H5'+H6'), 7.43-7.51 (bs, 2H, H4'+H7'), 7.78 (d, J = 8.4 Hz, 1H, H7), 7.94 (d, J = 8.4 Hz, 1H, H6), 8.28 (s, 1H, H4), 8.65 (s, 1H, N-H Amide), 11.85-12.55 (br, 1H, N-H Imidazole), 13.90-14.60 (br, 1H, N-H Imidazole). ^{13}C NMR (75 MHz, DMSO) 30.5 ($2CH_2$), 31.2 ($2CH_2$), 37.5 (CH), 38.2 (CH), 45.9 (CH_2), 114.7 (2CH), 117.4 (C), 120.6 (C), 121.0 (CH), 121.5 (2CH), 123.9 (CH), 125.7 (CH), 131.1 (2C), 141.7 (C), 142.3 (C,CF_3, J_{CF} = 39.2 Hz), 159.0 (C), 159.1 (C), 166.5 (C=O). IR υ $_{max}$ cm $^{-1}$ 612 (C-F), 1274 (C-N), 1046 (C-O), 1422 (C=C), 1620 (C=N), 1625 (C=O), 2876 (C-H, aliphatic), 3020 (C-H, aromatic), 3330 (N-H, amide),

3401 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$obsd. 442.1859, calcd. 442.1849 for $C_{23}H_{22}F_3N_5O$.

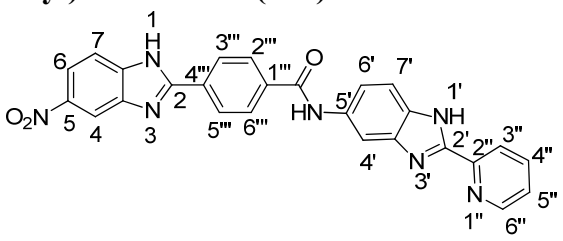
5.7.5 (2-Amino-benzimidazol-1-yl)-[4-(1*H*-benzimidazol-2-yl)-phenyl]-methanone (139)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-nitrobenzimidazole with 2-aminobenzimidazole by reaction over 8 days. The product was purified by column chromatography using DCM:MeOH (15:1 v/v) affording **139** as a pale yellow solid (0.28 g, 65 %); Mp > 300 °C .

^{1}H NMR (400 MHz, DMSO) 7.18 (dd, J_1 = 5.6, J_2 = 3.2 Hz, 2H, H5'+H6'), 7.48 (dd, J_1 = 5.4, J_2 = 2.7 Hz, 2H, H4'+H7'), 7.84 (d, J = 8.9 Hz, 1H, H7), 8.17 (d, J = 9.0 Hz, 1H, H6), 8.30-8.47 (br, 2H, NH_2), 8.30-8.48 (bs, 4H, H2"+H3"+H5"+H6"), 8.60 (s, 1H, H4), 12.28-12.51 (br, 1H, N-H Imidazole). ^{13}C NMR (75 MHz, DMSO) 112.4 (CH), 113.2 (2CH), 115.4 (C), 118.8 (CH), 122.2 (2CH), 122.3 (C), 127.2 (2CH), 129.4 (2CH), 132.0 (CH), 133.2 (2C), 137.7 (C), 143.2 (2C), 144.6 (C), 150.4 (C), 169.4 (C=O). IR υ_{max} cm $^{-1}$ 740 (N-O), 1298 (C-N), 1107 (C-O), 1421 (C=C), 1603 (C=N), 1630 (C=O), 3092 (C-H, aromatic), 3100 (N=O), 3366 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 399.1210, calcd. 399.1200 for $C_{21}H_{15}N_6O_3$.

5.7.6 4-(5-Nitro-1*H*-benzimidazol-2-yl)-*N*-(2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-benzamide (140)

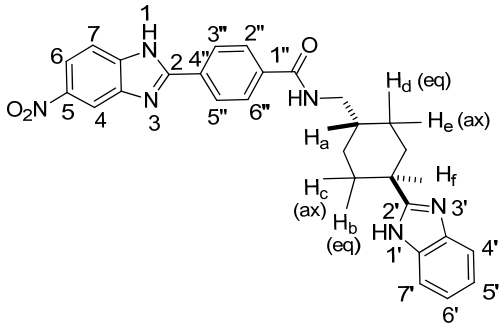


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-nitrobenzimidazole with 2-pyridin-2-yl-1*H*-benzimidazol-5-ylamine

by reaction over 8 days. The product was purified by recrystalization from ethanol affording **140** as a brown solid (0.38 g, 72%); Mp > 300 °C .

^{1}H NMR (400 MHz, DMSO) 7.46 (dd, J_1 = 8.0, J_2 = 5.2 Hz, 1H, H5"), 7.58 (d, J = 8.8 Hz, 1H, H7'), 7.71 (d, J = 7.8 Hz, 1H, H3"), 7.76 (d, J = 8.8 Hz, 1H, H6'), 7.90 (t, J = 7.8 Hz, 1H, H4"), 8.13 (d, J = 7.2 Hz, 1H, H7), 8.16 (d, J = 7.2 Hz, 1H, H6), 8.20 (d, J = 7.6 Hz, 2H, H3'"+H5'"), 8.24 (d, J = 7.6 Hz, 2H, H2'"+H6'"), 8.41 (s, 1H, H4'), 8.48 (s, 1H, H4), 8.49-8.51 (bs, 1H, N-H Imidazole), 8.66 (d, J = 5.2 Hz, 1H, H6"), 10.29 (s, 1H, N-H Amide). ^{13}C NMR : There were no peaks for ^{13}C on NMR, as the tested sample did not dissolve in DMSO. IR υ_{max} cm $^{-1}$ 795 (N-O), 1267 (C-N), 1065 (C-O), 1444 (C=C), 1647 (C=N), 1654 (C=O), 3065 (C-H, aromatic), 3266 (N=O), 3364 (N-H, amide), 3400 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 476.1471, calcd. 476.1466 for $C_{26}H_{18}N_7O_3$.

5.7.7 *N*-[4-(1*H*-Benzimidazol-2-yl)-cyclohexylmethyl]-4-(5-nitro-1*H*-benzimidazol-2-yl)-benzamide (141)

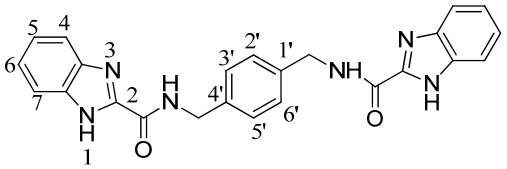


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-nitrobenzimidazol with 4-(1-*H*-benzimidazol-2-yl) cyclohexyl)methan amine by reaction over 6 days. The product was purified by recrystalization from acetone affording **141** as a pale yellow solid (0.38 g, 70%); Mp >300 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 11.6, J_2 = 1.2 Hz, 2H, H_c), 1.56-1.65 (m, 1H, H_a), 1.60 (qd, J_1 = 11.6, J_2 = 1.2 Hz, 2H, H_e), 1.93 (dq, J_1 = 10.8, J_2 = 0.3 Hz, 2H, H_d), 2.11 (dq, J_1 = 10.8, J_2 = 0.3 Hz, 2H, H_b), 2.82 (tt, J_1 = 12.0, J_2 = 1.0 Hz, 1H, H_f), 3.23 (t, J = 6.0 Hz, 2H, CH_2), 7.11 (d, J = 6.0 Hz, 2H, H5'+H6'), 7.46-7.47 (bs, 2H, H4'+H7'), 7.62 (d, J = 8.8 Hz, 1H, H7), 7.95-8.11 (m, 3H, H2"+H6"+H4), 8.25 (d, J = 8.8 Hz, 1H, H6), 8.41(d, J = 6.0 Hz, 2H, H3"+H5"), 8.5 (s, 1H, N-H Imidazole) 8.62 (t,

J = 5.6 Hz, 1H, N-H Amide), 11.96-12.39 (br, 1H, N-H Imidazole). ^{13}C NMR (75 MHz, DMSO) 30.5 ($2CH_2$), 31.2 ($2CH_2$), 37.5 (CH), 38.3 (CH), 45.8 (CH_2), 112.8 (CH), 115.4 (CH), 116.5 (2CH), 121.4 (2CH), 126.6 (CH), 127.1 (2CH), 127.9 (2CH), 129.9 (C), 135.4 (2C), 141.2 (C), 143.3 (C), 148.7 (C), 159.1 (C), 160.4 (C), 166.3 (C), 169.7 (C=O). IR υ $_{max}$ cm $^{-1}$ 740 (N-O), 1250 (C-N), 1064 (C-O), 1432 (C=C), 1634 (C=N), 1636 (C=O), 2925 (C-H, aliphatic), 3064 (C-H, aromatic), 3286 (N=O), 3310 (N-H, amide), 3410 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 495.2134, calcd. 495.2139 for $C_{28}H_{27}N_6O_3$.

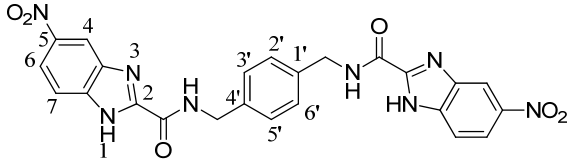
5.7.8 *N,N'*-Bis(1*H*-benzimidazole-2-carbonyl)-*p*-xylylenediamine (146)



The title compound was synthesized according to procedure **5.7** *via* amidation of benzimidazole-2-carboxylic acid by using *p*-xylenediamine over 8 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **146** as a white solid (1.27 g, 60 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 4.48 (d, *J* = 6.0 Hz, 4H, 2 CH_2), 7.31 (s, 4H, H2'+H3'+H5'+H6'), 7.53 (d, *J* = 7.6 Hz, 4H, H5+H6), 7.72 (d, *J* = 7.6 Hz, 4H, H4+H7), 9.53 (t, *J* = 6.0 Hz, 2H, N-H Amide), 13.27 (s, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 42.4 ($2CH_2$), 112.9 (2CH), 115.6 (2CH), 120.2 (2CH), 122.9 (2CH), 124.5 (2CH), 127.7 (2CH), 129.9 (2C), 134.8 (C), 138.2 (C), 142.9 (2C), 145.9 (2C), 159.2 (2C=O). IR υ $_{max}$ cm $^{-1}$ 1281 (C-N), 1114 (C-O), 1446 (C=C), 1622 (C=N), 1661 (C=O), 2931 (C-H, aliphatic), 3070 (C-H, aromatic), 3222 (N-H, amide), 3391 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 425.1723, calcd. 425.1726 for $C_{24}H_{21}N_6O_2$.

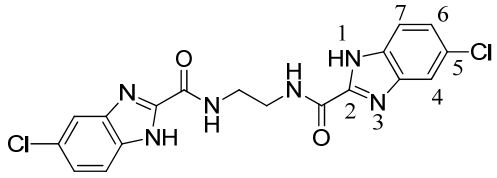
5.7.9 *N,N'*-Bis(5-nitro-1*H*-benzimidazole-2-carbonyl)-*p*-xylylenediamine (147)



The title compound was synthesized according to procedure **5.7** *via* amidation of 5-nitro-1*H*-benzimidazole-2-carboxylic acid by using *p*-xylenediamine over 14 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **147** as a pale brown solid (1.49 g, 58 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 4.49 (d, *J* = 6.4 Hz, 4H, 2 CH_2), 7.33 (s, 4H, H2'+H3'+H5'+H6'), 7.42 (s, 2H, H4), 7.77-7.85 (br, 2H, N-H Imidazole), 8.22 (d, *J* = 7.8 Hz, 2H, H7), 8.52-8.61 (m, 2H, H6), 9.77 (t, *J* = 6.4 Hz, 2H, N-H Amide). ^{13}C NMR : There were no peaks for ^{13}C on NMR, as the tested sample did not dissolve in DMSO. IR υ $_{max}$ cm $^{-1}$ 823 (N-O), 1261 (C-N), 1065 (C-O), 1441 (C=C), 1624 (C=N), 1664 (C=O), 2851 (C-H, aliphatic), 2918 (C-H, aromatic), 3090 (N=O), 3179 (N-H, amide), 3381 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 515.1439, calcd. 515.1427 for $C_{24}H_{19}N_8O_6$.

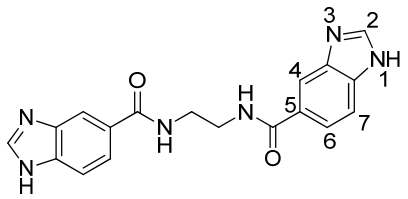
5.7.10 *N*,*N*'-Bis(5-chloro-1*H*-benzimidazole-2-carbonyl) ethylenediamine (150)



The title compound was synthesized according to procedure **5.7** *via* amidation of 5-chloro-1*H*-benzimidazole-2-carboxylic acid by using ethylenediamine over 6 days. The product was purified by recrystallization from ethanol affording **150** as an off brown solid (0.98 g, 47 % yield); Mp 298-300 °C.

^{1}H NMR (400 MHz, DMSO) δ 3.55 (s, 4H, 2 CH_2), 7.32 (dd, J_1 = 8.4, J_2 = 1.6 Hz, 2H, H6), 7.54 (d, *J* = 1.6, 2H, H4), 7.75 (d, *J* = 8.4 Hz, 2H, H7), 7.96 (s, 2H, N-H Imidazole), 9.22 (s, 2H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) 40.5 ($2CH_2$), 112.5 (2CH), 114.3 (2CH), 119.5 (2CH), 121.6 (2C), 123.4 (2C), 124.7 (2C), 147.0 (2C), 159.0 (2C=O). IR υ $_{max}$ cm $^{-1}$ 803 (C-Cl), 1246 (C-N), 1057 (C-O), 1425 (C=C), 1656 (C=N), 1659 (C=O), 2961 (C-H, aliphatic), 3183 (C-H, aromatic), 3333 (N-H, amide), 3400 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 417.0627, calcd. 417.0633 for $C_{18}H_{14}Cl_2N_6O_2$.

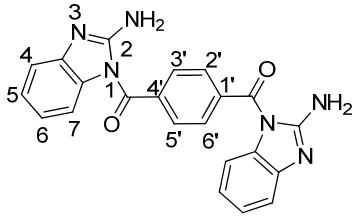
5.7.11 *N,N'*-Bis(1*H*-benzimidazole-5-carbonyl)ethylenediamine (151)



The title compound was synthesized according to procedure **5.7** *via* amidation of benzimidazole-5-carboxylic acid by using ethylenediamine over 5 days. The product was purified by recrystallization from ethanol affording **151** as a pale brown solid (0.05 g, 57 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 3.50 (s, 4H, 2 CH_2), 7.57 (d, *J* = 8.0 Hz, 2H, H6), 7.72 (d, *J* = 10.0 Hz, 1H, N-H Imidazole), 7.79 (d, *J* = 8.0 Hz, 2H, H7), 8.08 (s, 2H, N-H Imidazole), 8.25 (s, 2H, H4), 8.34 (d, *J* = 10.0 Hz, 2H, H2), 8.61 (s, 1H, N-H Amide), 8.65 (s, 1H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) δ 40.2 ($2CH_2$), 111.7 (2CH), 118.6 (2CH), 121.1 (2CH), 122.4 (2C), 125.9 (2C), 128.4 (2CH), 143.8 (2C), 167.7 (2C=O). IR υ $_{max}$ cm $^{-1}$ 1253 (C-N), 1055 (C-O), 1448 (C=C), 1608 (C=N), 1630 (C=O), 2945 (C-H, aliphatic), 3229 (C-H, aromatic), 3395 (N-H, amide), 3488 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 349.1402, calcd. 349.1413 for $C_{18}H_{17}N_6O_2$.

5.7.12 [4-(2-Amino-benzimidazole-1-carbonyl)-phenyl]-(2-amino-benzimidazol-1-yl)-methanone (152)

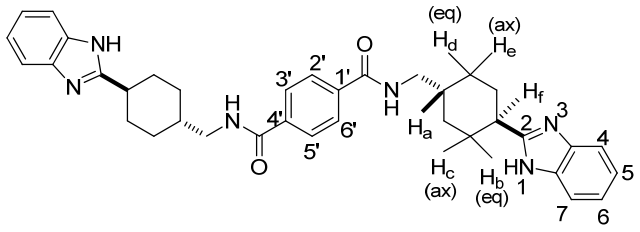


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-aminobenzimidazole by using terephthalic acid over 14 days. The product was purified by by column chromatography using DCM: MeOH (10:l) affording **152** as a pale yellow solid (0.26 g, 60 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 3.25-3.69 (br, 4H, 2 NH_2), 7.32 (dd, J_1 = 4.8, J_2 = 2.0 Hz, 4H, H5+H6), 7.62 (dd, J_1 = 5.2, J_2 = 3.2 Hz, 4H, H4+H7), 8.35 (s, 4H, H2'+H3' +H5'+H6'). ^{13}C NMR (100 MHz, DMSO) δ 113.4 (4CH), 124.7 (4C), 128.8 (4CH), 128.9 (4CH), 135.9 (2C), 144.1 (2C), 165.6 (2C=O). IR υ $_{max}$ cm $^{-1}$ 1271 (C-N), 1040

(C-O), 1428 (C=C), 1635 (C=N), 1664 (C=O), 3030 (C-H, aromatic), 3358 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 397.1422, calcd. 397.1413 for $C_{22}H_{17}N_6O_2$.

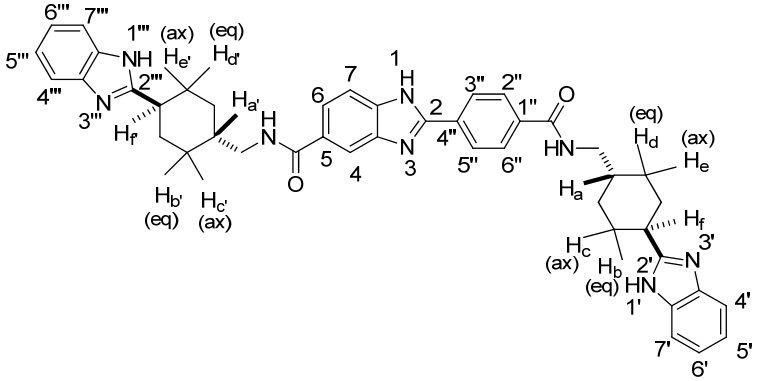
5.7.13 *N,N'*-Bis-[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-rephthalamide (153)



The title compound was synthesized according to procedure **5.7** *via* amidation of 4-(1-*H*-benzimidazol-2-yl)cyclohexyl)methanamine by using terephthalic acid over 8 days. The product was purified by column chromatography using DCM: MeOH (8:l) affording **153** as a beige solid (0.42 g, 65 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 1.12-1.22 (m, 2H, H_a), 1.17 (qd, J_1 = 12.4, J_2 = 3.2 Hz, 4H, H_c), 1.68 (qd, J_1 = 12.4, J_2 = 3.2 Hz, 4H, H_e), 1.94 (dq, J_1 = 11.2, J_2 = 1.2 Hz, 4H, H_d), 2.19 (dq, J_1 = 11.2, J_2 = 1.2 Hz, 4H, H_b), 3.14-3.22 (m, 6H, H_f+2CH_2), 7.53 (dd, J_1 = 6.4, J_2 = 3.2 Hz, 4H, H5+H6), 7.78 (dd, J_1 = 6.4, J_2 = 3.2 Hz, 4H, H4+H7), 7.94 (s, 4H, H2'+H3'+H5'+H6'), 8.66 (t, J = 6.0 Hz, 2H, N-H Amide),. ^{13}C NMR (100 MHz, DMSO) δ 29.4 ($4CH_2$), 29.5 ($4CH_2$), 35.9 (2CH), 36.6 (2CH), 45.2 ($2CH_2$), 113.8 (4CH), 125.4 (4CH), 127.1 (4CH), 130.9 (4C), 136.7 (2C), 157.4 (2C), 165.7 (2C=O). IR υ_{max} cm $^{-1}$ 1294 (C-N), 1076 (C-O), 1431 (C=C), 1620 (C=N), 1636 (C=O), 2922 (C-H, aliphatic), 3085 (C-H, aromatic), 3283 (N-H, amide), 3390 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 589.3301, calcd. 589.3291 for $C_{36}H_{41}N_6O_2$.

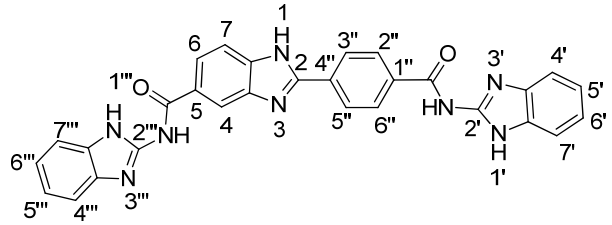
5.7.14 2-(4-{[4-(1*H*-Benzimidazol-2-yl)-cyclohexylmethyl]- carbamoyl}–phenyl)-1*H*-benzimidazole-5-carboxylic acid [4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-amide (156)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-Carboxyphenyl)-5-carboxybenzimidazole by using 4-(1-*H*-benzimidazol-2-yl) cyclo hexyl)methanamine over 12 days. The product was purified by recrystallization from ethanol affording **156** as a pale brown solid (0.54 g, 70 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 12.0, J_2 = 2.2 Hz, 4H, H_c+$H_{c'}$), 1.55-1.70 (m, 2H, H_a+$H_{a'}$), 1.61 (qd, J_1 =12.0, J_2 = 2.2 Hz, 4H, H_e+$H_{e'}$), 1.93 (dq, J_1 = 12.2, J_2 = 0.7 Hz, 4H, H_d+$H_{d'}$), 2.11 (dq, J_1 = 12.2, J_2 = 0.7 Hz, 4H, H_b+$H_{b'}$), 2.83 (tt, J_1 = 12.0, J_2 = 0.4 Hz, 2H, H_f+$H_{f'}$), 3.23 (t, J = 5.6 Hz, 4H, 2CH_2), 7.11 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 4H, H5'+H6'+H5'''+H6'''), 7.44-7.49 (m, 4H, H4'+H7'+H4'''+H7'''), 7.62 (d, J = 8.0 Hz, 1H, H6), 7.72 (d, J = 8.0 Hz, 1H, H7), 8.03 (d, J = 8.4 Hz, 2H, H2''+H6''), 8.15 (s, 1H, H4), 8.33 (d, J = 8.4 Hz, 2H, H3''+H5''), 8.47 (t, J = 5.4 Hz, 1H, N-H Amide), 8.64 (t, J = 5.4 Hz, 1H, N-H Amide), 12.08-12.33 (br, 3H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 29.1 (4CH_2), 29.7 (4CH_2), 36.1 (2CH), 36.8 (2CH), 44.4 (2CH_2), 109.7 (CH), 116.9 (CH), 119.9 (2CH), 120.3 (2CH), 124.6 (C), 125.1 (2C), 125.3 (2CH), 126.7 (2CH), 127.4 (2CH), 131.8 (CH), 134.3 (2CH), 138.8 (C), 140.9 (C), 147.2 (C), 149.8 (C), 152.0 (2C), 154.9 (C), 157.7 (2C) 164.7 (C=O), 165.8 (C=O). IR υ_{max} cm $^{-1}$ 1233 (C-N), 1044 (C-O), 1452 (C=C), 1615 (C=N), 1622 (C=O), 1636 (C=O), 2852 (C-H, aliphatic), 2923 (C-H, aromatic), 3330 (N-H, amide), 3395 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 705.3652, calcd. 705.3660 for $C_{43}H_{45}N_8O_2$.

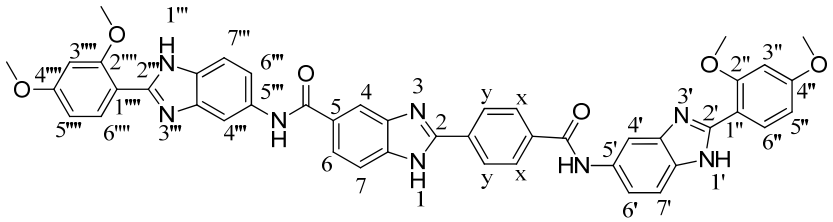
5.7.15 2-[4-(1*H*-Benzimidazol-2-ylcarbamoyl)-phenyl]-1*H*-benzimidazole-5-carboxylic acid (1*H*-benzimidazol-2-yl)-amide (157)



The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-carboxybenzimidazole by using 2-aminobenzimidazole over 13 days. The product was purified by column chromatography using DCM:MeOH (15:l v/v) affording **157** as a pale yellow solid (0.42 g, 75 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 7.14-7.19 (m, 4H, H5'+H6'+H5'''+H6'''), 7.49 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 4H, H4'+H7'+H4'''+H7'''), 7.70 (d, J = 8.8 Hz, 1H, H2''), 7.82 (d, J = 8.4 Hz, 1H, H3''), 8.08 (d, J = 8.4 Hz, 1H, H5''), 8.12 (d, J = 8.8 Hz, 1H, H6''), 8.36 (d, J = 7.2 Hz, 2H, H6+H7), 8.39 (s, 1H, H4), 8.41 (s, 1H, N-H Amide), 8.58 (s, 1H, N-H Amide), 12.29-12.39 (bs, 3H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 111.7 (CH), 112.7 (CH), 113.3 (CH), 118.5 (C), 118.9 (2CH), 120.1 (2C), 121.7 (2CH), 122.2 (2CH), 122.9 (C) 123.9 (C), 126.8 (2CH), 126.9 (2CH), 129.4 (2CH), 132.8 (C), 135.1 (C), 138.3 (C), 143.8 (2C), 146.9 (2C), 152.7 (C=O), 153.6 (C=O). IR υ_{max} cm $^{-1}$ 1227 (C-N), 1026 (C-O), 1420 (C=C), 1629 (C=N), 1653 (C=O), 1664 (C=O), 3069 (C-H, aromatic), 3390 (N-H, amide), 3534 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 513.1782, calcd. 513.1775 for $C_{29}H_{21}N_8O_2$.

5.7.16 2-{4-[2-(2,4-Dimethoxy-phenyl)-1*H*-benzimidazol-5-ylcarbamoyl]-phenyl}-1*H*-benzimidazole-5-carboxylic acid [2-(2,4-dimethoxy-phenyl}-1*H*-benzimidazol-5-yl]-amide (158)

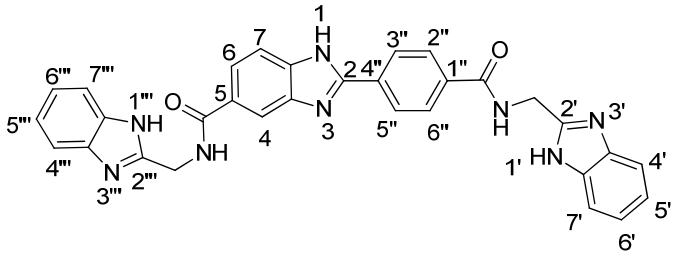


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-carboxybenzimidazole by using 5-amino-2-(2',4'-dimethoxyphenyl)

benzimidazole over 14 days. The product was purified by recrystallization from ethanol affording **158** as a brown solid (0.51 g, 59 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 3.94 (s, 6H, 2CH_3), 4.11 (s, 6H, 2CH_3), 6.89-6.91 (m, 4H, Hx+H4'+H4'''), 7.70-7.89 (m, 6H, H3''+H5''+H6'+H3''''+H5''''+H6'''), 7.98 (d, *J* = 8.4 Hz, 2H, H6''+H6''''), 8.25 (q, *J* = 8.4 Hz, 3H, Hy+H6), 8.39-8.40 (bs, 1H, H4), 8.44 (d, *J* = 8.4 Hz, 1H, H7), 8.61 (d, *J* = 8.2 Hz, 2H, H7'+H7'''), 10.69 (s, 1H, N-H Amide), 10.79 (s, 1H, N-H Amide), 13.97-14.61 (br, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 54.9 (2CH_3), 55.3 (2CH_3), 97.9 (2CH), 102.9 (C), 103.1 (CH), 103.3 (CH), 106.2 (2CH), 112.6 (2CH), 112.7 (2CH), 116.9 (2CH), 117.4 (C), 117.8 (CH) 121.5 (C), 125.5 (2CH), 127.4 (2CH), 130.2 (2CH), 130.7 (C), 131.2 (C), 134.7 (2C), 135.6 (2C), 136.1 (2C), 145.0 (2C), 145.2 (2C), 151.2 (C), 158.4 (2C), 163.6 (2C), 163.9 (C=O), 164.9 (C=O). IR υ_{max} cm $^{-1}$ 1266 (C-N), 1086 (C-O), 1437 (C=C), 1610 (C=N), 1640 (C=O), 1653 (C=O), 2851 (C-H, aliphatic), 3043 (C-H, aromatic), 3400 (N-H, amide), 3507 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 785.2836, calcd. 785.2831for $C_{45}H_{37}N_8O_6$.

5.7.17 2-{4-[(1*H*-Benzimidazol-2-ylmethyl)-carbamoyl]-phenyl}-1*H*-benzimidazole-5-carboxylic acid(1*H*-benzimidazol-2-ylmethyl)-amide (159)

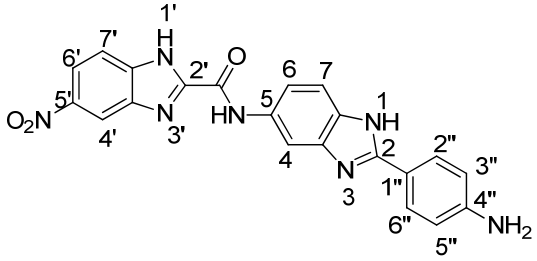


The title compound was synthesized according to procedure **5.7** *via* amidation of 2-(4'-carboxyphenyl)-5-carboxybenzimidazole by using 2-(aminomethyl)-1*H*-benzimidazole over 14 days. The product was purified by column chromatography using DCM:MeOH (15:l v/v) affording 1**59** as a pale brown solid (0.33 g, 55 %); Mp 290-292 °C.

^{1}H NMR (400 MHz, DMSO) 4.73 (d, *J* = 5.6 Hz, 4H, 2CH_2), 7.14-7.15 (m, 4H, H5'+H6'+H5'''+H6'''), 7.50 (dd, J_1 = 8.6, J_2 = 1.2 Hz, 4H, H4'+H7'+H4'''+H7'''), 7.63 (d, *J* = 1.2 Hz, 1H, H4), 7.77 (d, *J* = 8.8 Hz, 1H, H7), 7.88 (dd, J_1 = 8.8, J_2 = 1.2 Hz, 1H, H6), 8.14 (d, *J* = 8.0 Hz, 2H, H2''+H6''), 8.17 (s, 1H, N-H Imidazole), 8.31-8.36 (m, 4H,

H3"+H5"+N-H Imidazole), 9.19 (t, *J* = 5.6 Hz, 1H, N-H Amide), 9.34 (t, *J* = 5.6 Hz, 1H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) 37.9 ($2CH_2$), 99.5 (2C), 111.2 (2CH), 118.3 (2CH), 121.0 (2CH), 121.8 (2CH), 122.5 (C), 122.8 (C), 126.5 (2CH), 128.2 (2CH), 132.3 (CH), 134.3 (CH), 134.7 (C), 135.1 (C), 143.1 (CH), 143.3 (C), 146.7 (C), 152.2 (2C), 152.6 (2C), 165.9 (C=O), 166.9 (C=O). IR υ $_{max}$ cm $^{-1}$ 1271 (C-N), 1054 (C-O), 1443 (C=C), 1622 (C=N), 1640 (C=O), 1647 (C=O), 2852 (C-H, aliphatic), 3054 (C-H, aromatic), 3379 (N-H, amide), 3555 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 541.2090, calcd. 541.2095 for $C_{31}H_{25}N_8O_2$.

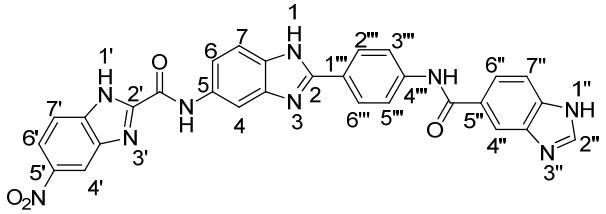
5.7.18 5-Nitro-1*H*-benzimidazole-2-carboxylic acid[2-(4-amino-phenyl)-1*H*-benzimidazol-5-yl]-amide (162)



The title compound was synthesized according to procedure **5.7** *via* amidation of 5-nitro-1*H*-benzimidazole-2-carboxylic acid by using 2(4'-aminophenyl)-1*H*-benzimidazol-5-ylamine over 17 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **162** as a brown solid (0.53 g, 58 % yield); Mp > 300 °C.

^{1}H NMR (300 MHz, DMSO) δ 6.04-6.56 (br, 2H, NH_2), 6.76 (d, *J* = 8.7 Hz, 2H, H2"+H6"), 7.70 (d, *J* = 8.7 Hz, 1H, H7), 7.79 (dd, J_1 = 8.8, J_2 = 0.9 Hz, 1H, H6'), 7.97 (d, *J* = 8.7 Hz, 2H, H3"+H5"), 8.25 (dd, J_1 = 8.7, J_2 = 0.9 Hz, 2H, H6+H7'), 8.43 (d, *J* = 0.9 Hz, 1H, H4), 8.63-8.69 (bs, 1H, H4'), 11.43 (s, 1H, N-H Amide), 14.12-14.28 (bs, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 99.5 (C), 104.6 (2CH), 113.4 (2CH), 113.6 (2CH), 118.2 (2CH), 120.7 (C), 129.4 (2CH), 129.6 (C), 134.0 (C), 134.9 (C), 150.5 (2C), 150.6 (2) 153.5 (2C), 156.6 (C=O). IR υ $_{max}$ cm $^{-1}$ 736 (N-O), 1232 (C-N), 1062 (C-O), 1473 (C=C), 1604 (C=N), 1630 (C=O), 3060 (C-H, aromatic), (N=O) 3281, 3338 (N-H, amide), 3447 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 414.1309, calcd. 414.1309 for $C_{21}H_{16}N_7O_3$.

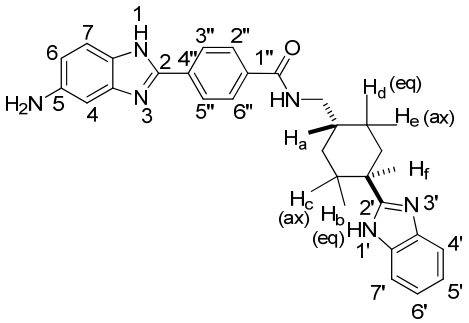
5.7.19 5-Nitro-1*H*-benzimidazole-2-carboxylic acid (2-{4-[(1*H*-benzimidazol-5-carbonyl)-amino]-phenyl}-1*H*-benzimidazol-5-yl)-amide (163)



The title compound was synthesized according to procedure **5.7** *via* amidation of 5-Nitro-1*H*-benzoimidazole-2-carboxylic acid[2-(4-amino- phenyl)-1*H*-benzoimidazol-5-yl]-amide **162** by using benzimidazole-5-carboxylic acid over 12 days. The product was purified by column chromatography using DCM: MeOH (12:l) affording 1**63** as a brown solid (0.59 g, 53 % yield); Mp > 300 °C.

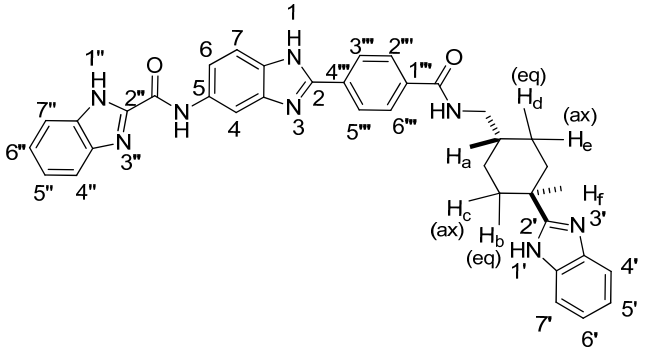
^{1}H NMR (400 MHz, DMSO) δ 6.77 (d, J = 8.8 Hz, 1H, H7"), 7.71 (dd, J_1 = 8.8, J_2 = 2.0 Hz, 1H, H6"), 7.78 (d, J = 8.4 Hz, 1H, H7), 7.81-8.03 (m, 3H, H4", H6, H4), 8.09 (d, J = 8.8 Hz, 1H, H7'), 8.17 (d, J = 9.2 Hz, 1H, H2"), 8.22 (d, J = 8.4 Hz, 2H, H2'"+H6'"), 8.42 (d, J = 8.4 Hz, 2H, H3'"+H5'"), 8.64 (dd, J_1 = 8.8, J_2 = 2.0 Hz, 2H, H4'+H6'), 10.66 (s, 1H, N-H Amide), 11.30 (d, J = 9.2 Hz, 1H, N-H Imidazole), 11.43 (s, 1H, N-H Amide), 14.11-14.25 (bs, 1H, N-H Imidazole). ^{13}C NMR : There were no peaks for ^{13}C on NMR, as the tested sample did not dissolve in DMSO. A large amount of precipitate was observed in the tube. IR υ $_{max}$ cm $^{-1}$ 734 (N-O), 1242 (C-N), 1064 (C-O), 1471 (C=C), 1599 (C=N), 1622 (C=O), 1659 (C=O), 3075 (C-H, aromatic), (N=O) 3185, 3333 (N-H, amide), 3389 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 558.1649, calcd. 558.1638 for $C_{29}H_{20}N_9O_4$.

5.8 4-(5-Amino-1*H*-benzimidazol-2-yl)-*N*-[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl-benzamide (154)



The title compound was synthesized from reduction of N-[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-4-(5-nitro-1*H*-benzimidazol-2-yl)-benzamide **141** according to method **5.4** over 5 days. The crude product was purified by column chromatography DCM: MeOH (10:1) affording **154** as a brown solid in (0.97 g, 60 %); Mp 298-300 °C. ^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 11.6, J_2 = 1.2 Hz, 2H, H_c), 1.24-1.29 (m, 1H, H_a), 1.62 (qd, J_1 = 11.6, J_2 = 1.2 Hz, 2H, H_e), 1.93 (dq, J_1 = 11.2, J_2 = 0.7 Hz, 2H, H_d), 2.13 (dq, J_1 = 11.2, J_2 = 0.7 Hz, 2H, H_b), 2.87-2.91 (bs, 1H, H_f), 3.22 (t, J = 6.0 Hz, 2H, CH_2), 6.63 (d, J = 8.8 Hz, 1H, H7), 6.77 (s, 1H, H4), 7.18 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H5'+H6'), 7.35 (d, J = 8.8 Hz, 1H, H6), 7.52 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H4'+H7'), 8.00 (d, J = 8.4 Hz, 2H, H3"+H5"), 8.17 (d, J = 8.4 Hz, 2H, H2"+H6"), 8.63 (t, J = 5.6 Hz, 1H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) δ 29.9 ($2CH_2$), 30.6 ($2CH_2$), 35.8 (CH), 37.5 (CH), 45.4 (CH_2), 114.3 (CH), 115.8 (CH), 121.8 (2CH), 125.5 (2CH), 127.3 (2CH), 127.8 (2CH), 129.8 (C), 130.6 (CH), 134.7 (C), 135.9 (C), 137.3 (C), 138.3 (C), 153.1 (C), 162.3 (C), 165.7 (2C), 166.7 (C=O). IR υ_{max} cm $^{-1}$ 1229 (C-N), 1434 (C=C), 1613 (C=N), 1643 (C=O), 2855 (C-H, aliphatic), 3064 (C-H, aromatic), 3208 (N-H, amide), 3338 (N-H, amine). HRMS: (ES) m/z $(M+2H)^{2+}$ obsd 233.1247 calcd. 233.1241 for $C_{28}H_{28}N_6O$.

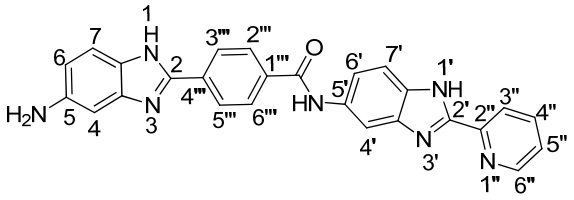
5.9 1*H*-Benzimidazole-2-carboxylic acid [2-(4-{[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-carbamoyl}-phenyl)-1*H*-benzimidazol-5-yl]-amide (164)



The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*-benzimidazole-2-carboxylic acid by using 4-(5-Amino-1*H*-benzimidazol-2-yl)-N-[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl-benzamide **154** over 6 days. The product was purified by column chromatography using DCM: MeOH (12:l) affording **164** as a brown solid (0.79 g, 65 % yield); Mp 284-286 °C.

^{1}H NMR (400 MHz, DMSO) 1.19 (qd, J_1 = 12.0, J_2 = 2.2 Hz, 2H, H_c), 1.71 (qd, J_1 = 12.0, J_2 = 2.2 Hz, 2H, H_e), 1.75-1.77 (m, 1H, H_a), 1.97 (dq, J_1 = 11.6, J_2 = 0.8 Hz, 2H, H_d), 2.21 (dq, J_1 = 11.6, J_2 = 0.8 Hz, 2H, H_b), 3.16 (tt, J_1 = 11.6, J_2 = 0.8 Hz, 1H, H_f), 3.25 (t, J = 6.0 Hz, 2H, CH_2), 7.35 (dd, J_1 = 5.6, J_2 = 2.4 Hz, 1H, H5'), 7.42 (dd, J_1 = 5.6, J_2 = 2.4 Hz, 1H, H4'), 7.49 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H5"+H6"), 7.55 (dd, J_1 = 8.4, J_2 = 2.0 Hz 1H, H6), 7.66 (d, J = 8.4 Hz, 1H, H7), 7.72 (s, 1H, H4), 7.76 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 2H, H4"+H7"), 7.82 (dd, J_1 = 5.6, J_2 = 2.4 Hz, 1H, H6'), 7.99 (d, J = 5.6 Hz, 1H, H7'), 8.07 (d, J = 8.0 Hz, 2H, H2'''+H6'''), 8.29 (d, J = 8.0 Hz, 2H, H3'''+H5'''), 8.39 (s, 1H, N-H imidazole), 8.73 (t, J = 6.0 Hz, 1H, N-H Amide), 11.03 (s, 1H, N-H Amide), 13.38-13.75 (br, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) δ 29.5 ($2CH_2$), 29.7 ($2CH_2$), 36.0 (CH), 36.7 (CH), 43.5 (CH_2), 109.6 (CH), 113.8 (2CH), 119.1 (CH), 119.9 (C), 124.5 (2CH), 125.1 (2CH), 126.2 (2CH), 127.3 (2CH), 127.8 (2CH), 127.9 (CH), 131.4 (C), 135.5 (2C), 140.7 (C), 143.1 (C), 145.7 (C), 145.9 (C), 154.5 (2C), 157.4 (C), 157.7 (C), 165.1 (C=O), 165.7 (C=O). IR υ_{max} cm $^{-1}$ 1230 (C-N), 1448 (C=C), 1555 (C=N), 1616 (C=O), 1637 (C=O), 2855 (C-H, aliphatic), 3058 (C-H, aromatic), 3214 (N-H, amide), 3361 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 609.2734, calcd. 609.2726 for $C_{36}H_{33}N_8O_2$.

5.10 4-(5-Amino-1*H*-benzimidazol-2-yl)-*N*-(2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-benzamide (155)

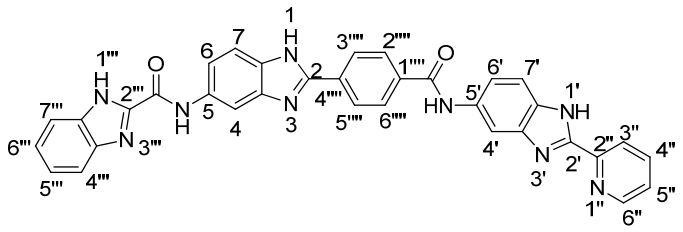


The title compound was synthesized from reduction of 4-(5-nitro-1*H*-benzimidazol-2-yl)-*N*-(2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-benzamide **140** according to method **5.4** over 6 days. The crude product was purified by column chromatography DCM: MeOH (10:1) affording **155** as a brown solid in (1.09 g, 70 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 6.56 (dd, J_1 = 8.8, J_2 = 1.6 Hz, 1H, H6), 6.68-6.71 (bs, 1H, H3"), 7.32 (d, J = 8.8 Hz, 1H, H7), 7.51-7.64 (m, 1H, H5"), 7.96 (s, 1H, H4), 8.04 (d, J = 8.4 Hz, 2H, H2'''+H6'''), 8.09 (d, J = 8.4 Hz, 1H, H7'), 8.14 (t, J = 8.0 Hz, 1H, H4"), 8.17 (d, J = 8.4 Hz, 2H, H3'''+H5'''), 8.22-8.35 (m, 2H, H4'+H6'), 8.74 (dd, J_1 =

4.8, J_2 = 1.2 Hz, 1H, H6''), 10.42 (s, 1H, N-H Amide), 12.42-12.63 (br, 1H, N-H Imidazole), 13.07-13.22 (br, 1H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) δ 105.9 (CH), 116.9 (CH), 119.0 (CH), 120.8 (CH), 122.4 (C), 124.4 (2CH), 127.8 (CH), 128.6 (2CH), 129.8 (CH), 130.1 (CH), 133.0 (CH), 135.9 (C), 138.4 (CH), 141.5 (C), 141.7 (CH), 143.4 (C), 143.5 (C), 147.6 (C), 149.8 (C), 150.4 (C), 155.9 (C), 156.0 (C), 158.7 (C), 164.4 (C=O). IR υ $_{max}$ cm $^{-1}$ 1232 (C-N), 1018 (C-O), 1475 (C=C), 1610 (C=N), 1638 (C=O), 3058 (C-H, aromatic), 3215 (N-H, amide), 3350 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 446.1734, calcd. 446.1729 for $C_{26}H_{20}N_7O$.

5.11 1*H*-Benzimidazole-2-carboxylic acid {2-[4-(2-pyridin-2-yl-1*H*-benzimidazol-5-ylcarbamoyl)-phenyl]-1*H*-benzimidazol-5-yl}-amide (165)

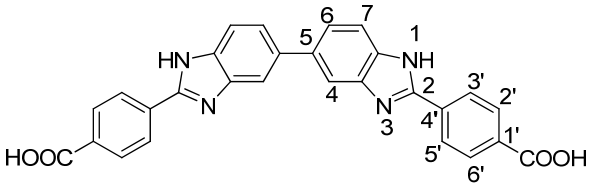


The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*-benzimidazole-2-carboxylic acid by using 4-(5-Amino-1*H*-benzimidazol-2-yl)-N-(2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-benzamide **155** over 7 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **165** as a brown solid (0.80 g, 68 % yield); Mp 296-298 °C.

^{1}H NMR (400 MHz, DMSO) 7.33-7.36 (bs, 2H, H5'''+H6'''), 7.53 (dd, J_1 = 7.6, J_2 = 4.8 Hz, 1H, H5''), 7.59 (d, J = 7.6 Hz, 1H, H3''), 7.62-7.68 (m, 2H, H4'''+H7'''), 7.79 (dd, J_1 = 4.8, J_2 = 1.6 Hz, 2H, H6''), 8.01 (t, J = 7.6 Hz, 1H, H4''), 8.09 (d, J = 8.4 Hz, 2H, H2''''+H6''''), 8.13 (dd, J_1 = 8.0, J_2 = 1.6 Hz, 1H, H6'), 8.17 (d, J = 8.0 Hz, 1H, H7'), 8.22 (d, J = 8.4 Hz, 2H, H3''''+H5''''), 8.25-8.29 (bs, 1H, N-H Imidazole), 8.33 (s, 1H, N-H Imidazole), 8.34 (d, J = 8.0 Hz, 1H, H7), 8.36 (d, J = 1.6 Hz, 1H, H4'), 8.39 (dd, J_1 = 8.0, J_2 = 2.8 Hz, 1H, H6), 8.43 (s, 1H, N-H Amide), 8.75 (d, J = 2.8 Hz, 1H, H4), 10.48 (s, 1H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) δ 106.8 (CH), 113.3 (2CH), 116.3 (CH), 119.8 (CH), 121.6 (C), 122.3 (C), 123.6 (CH), 123.9 (C), 125.9 (2CH), 126.2 (CH), 129.8 (2CH), 130.3 (2CH), 133.2 (CH), 133.5 (CH), 135.3 (C), 136.4 (CH), 136.6

(CH), 141.6 (C), 142.3 (CH), 145.8 (C), 147.1 (C), 152.0 (C), 154.2 (C), 156.5 (2C), 157.1 (C), 157.6 (C), 158.9 (C), 164.5 (C=O), 166.6 (C=O). IR υ $_{max}$ cm $^{-1}$ 1223 (C-N), 1071 (C-O), 1447 (C=C), 1611 (C=N), 1650 (C=O), 1664 (C=O), 3068 (C-H, aromatic), 3203 (N-H, amide), 3372 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 590.2065, calcd. 590.2053 for $C_{34}H_{24}N_9O_2$.

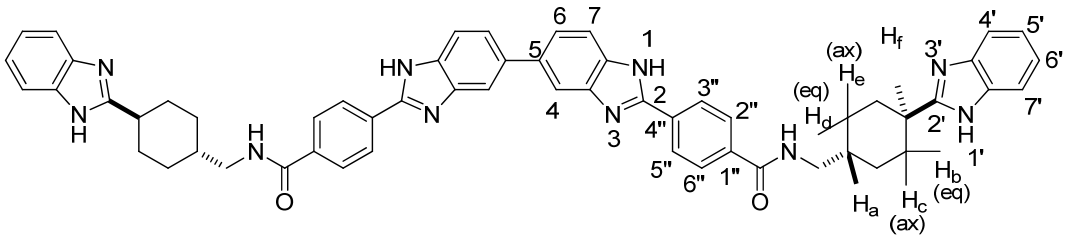
5.12 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid (167)



The title compound was synthesized by condensation of 3,3'-diaminobenzidine and 4-carboxybenzaldehyde salt for 48 h according to procedure **5.2.1** and purified by recrystallization from water:ethanol (1:1 v/v) affording **167** as a yellow solid (0.75 g, 79 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) δ 7.64 (d, *J* = 8.4 Hz, 2H, H6), 7.76 (d, *J* = 8.4 Hz, 2H, H7), 7.92 (s, 2H, H4), 8.14 (d, *J* = 8.0 Hz, 4H, H2'+H6'), 8.34 (d, *J* = 8.0 Hz, 4H, H3'+H5'), 12.48-13.83 (br, 2H, 2COOH). ^{13}C NMR (100 MHz, DMSO) 113.4 (2C), 116.2 (2CH), 118.9 (2CH), 122.9 (2CH), 126.9 (4CH), 130.4 (4CH), 132.1 (2C), 133.9 (4C), 136.5 (2C), 151.1 (2C), 167.3 (2 C=O). IR υ $_{max}$ cm $^{-1}$ 1292 (C-N), 1050 (C-O), 1460 (C=C), 1620 (C=N), 1682 (C=O), 3120 (C-H, aromatic), 3360 (N-H, amine) 3450 (O-H). HRMS: (ES) *m/z* $(MH)^+$ obsd. 475.1399, calcd. 475.1401 for $C_{28}H_{19}N_4O_4$.

5.13 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid bis-{[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-amide} (171)

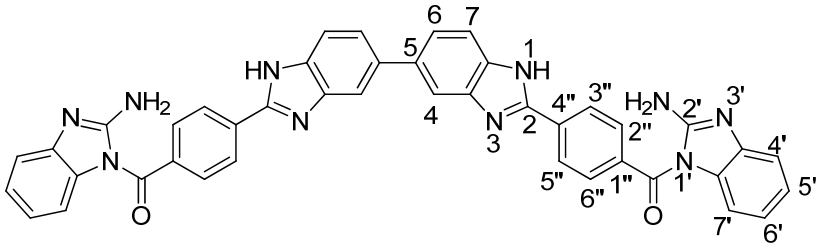


The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using 4-(1-

H-benzimidazol-2-yl)cyclohexyl)methanamine over 14 days. The product was purified by recrystallization from ethanol affording 1**71** as a pale brown solid (0.64 g, 65 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 12.0, J_2 = 1.2 Hz, 4H, H_c), 1.55-1.68 (m, 2H, H_a), 1.63 (qd, J_1 = 12.0, J_2 = 1.2 Hz, 4H, H_e), 1.93 (dq, J_1 = 11.6, J_2 = 0.8 Hz, 4H, H_d), 2.11 (dq, J_1 = 11.6, J_2 = 0.8 Hz, 4H, H_b), 2.83 (tt, J_1 = 11.6, J_2 = 0.8 Hz, 2H, H_f), 3.24 (t, J = 6.0 Hz, 4H, $2CH_2$), 7.10 (dd, J_1 = 6.3, J_2 = 2.7 Hz, 4H, H5'+H6'), 7.42-7.55 (m, 4H, H4'+H7'), 7.60 (d, J = 8.1 Hz, 2H, H6), 7.70-7.75 (bs, 2H, H4), 7.86-7.93 (br, 2H, N-H Imidazole), 8.07 (d, J = 8.4 Hz, 2H, H2"+H6"), 8.18 (d, J = 8.1 Hz, 2H, H7), 8.35 (d, J = 8.4 Hz, 2H, H3"+H5"), 8.68 (t, J = 6.0 Hz, 2H, N-H Amide), 12.22 (s, 2H, N-H imidazole). ^{13}C NMR (100 MHz, DMSO) 29.0 ($4CH_2$), 29.7 ($4CH_2$), 36.0 (2CH), 36.8 (2CH), 44.4 ($2CH_2$), 99.5 (2CH), 119.9 (2C), 124.5 (4CH), 125.1 (4CH), 126.8 (4CH), 128.4 (4CH), 129.3 (2CH), 131.3 (2CH), 134.3 (2C), 140.3 (2C), 149.9 (2C), 150.9 (2C), 157.7 (4C), 162.3 (2C) 164.6 (2C), 168.2 (2 C=O). IR υ_{max} cm $^{-1}$ 1271 (C-N), 1070 (C-O), 1460 (C=C), 1616 (C=N), 1635 (C=O), 2852 (C-H, aliphatic), 3033 (C-H, aromatic), 3193 (N-H, amide), 3405 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 897.4352, calcd. 897.4347 for $C_{56}H_{53}N_{10}O_2$.

5.14 (4-{2'-[4-(2-Amino-benzimidazole-1-carbonyl)-phenyl]-3*H*,3'*H*-[5,5']bibenzimidazolyl-2yl}-phenyl)-(2-amino-benzimidazol-1-yl)-methanone (172)

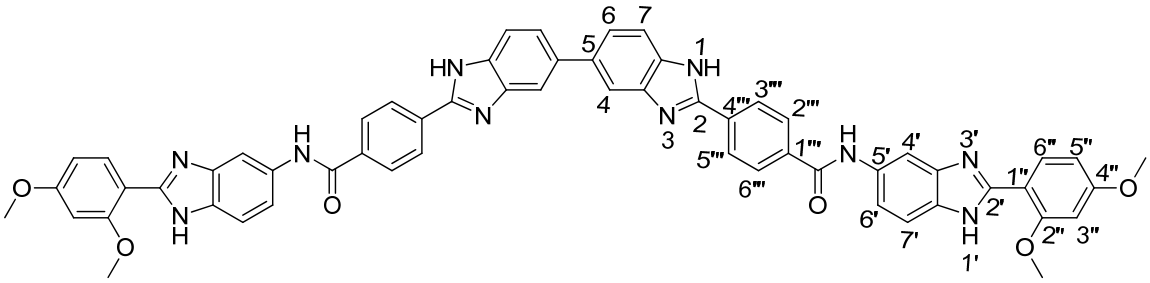


The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using 2-aminobenzimidazole over 11 days. The product was purified by column chromatography using DCM: MeOH (10:l) affording **172** as a brown solid (0.43 g, 55 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 7.17 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 4H, H5'+H6'), 7.49 (dd, J_1 = 5.2, J_2 = 2.0 Hz, 4H, H4'+H7'), 7.61 (q, J = 8.0 Hz, 2H, H4), 7.71-7.79 (bs, 4H, NH_2),

7.89-7.97 (bs, 2H, N-H Imidazole), 8.09 (d, *J* = 8.0 Hz, 2H, H6), 8.19 (d, *J* = 8.0 Hz, 2H, H7), 8.34 (d, *J* = 8.0 Hz, 2H, H2"+H6"), 8.39 (d, *J* = 8.0 Hz, 2H, H3"+H5"), 12.20-12.77 (br, 2H, N-H Imidazole) ^{13}C NMR (100 MHz, DMSO) 111.8 (2CH), 116.9 (2C), 120.5 (4CH), 124.5 (2CH), 125.1 (4CH), 127.9 (4CH), 128.4 (4CH), 129.2 (2CH), 131.8 (2C), 132.5 (2C), 134.9 (2C), 140.4 (2C), 140.5 (2C), 149.9 (4C), 150.9 (2C), 168.1 (2 C=O). IR υ_{max} cm $^{-1}$ 1270 (C-N), 1015 (C-O), 1470 (C=C), 1620 (C=N), 1630 (C=O), 3103 (C-H, aromatic), 3378 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 705.2546, calcd. 705.2542 for $C_{42}H_{29}N_{10}O_2$.

5.15 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid bis-{[2-(2',4'-dimethoxy-phenyl)-1*H*-benzimidazol-5yl]-amide} (173)

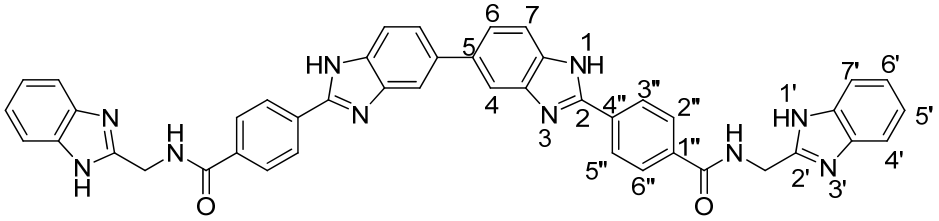


The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using 5-amino-2-(2',4'-dimethoxyphenyl) benzimidazole over 11 days. The product was purified by recrystallization from ethanol affording **173** as a brown solid (0.75 g, 70 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 3.88 (s, 6H, $2CH_3$), 4.05 (s, 6H, $2CH_3$), 6.73 (dd, J_1 = 8.4, J_2 = 2.0 Hz, 2H, H5"), 6.77 (d, *J* = 2.0 Hz, 2H, H3"), 7.55 (q, *J* = 8.4 Hz 6H, H4+H6+H7), 7.67 (d, *J* = 7.6 Hz, 4H, H4'+H7'), 7.87 (s, 2H, N-H Imidazole), 8.04 (d, *J* = 7.8 Hz, 4H, H2'"+H6'"), 8.18 (d, *J* = 7.8 Hz, 4H, H3'"+H5'"), 8.27(d, *J* = 8.4 Hz, 2H, H6"), 8.43 (dd, J_1 = 7.6, J_2 = 2.0 Hz, 2H, H6'), 10.39 (s, 2H, N-H Amide), 11.98-12.07 (br, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 55.9 ($2CH_3$), 56.2 ($2CH_3$), 98.9 (2CH), 106.6 (2CH), 112.0 (2C), 112.5 (2C), 124.6 (4CH), 126.3 (2CH), 126.7 (2CH), 128.7 (2CH), 130.1 (4CH), 131.0 (2C), 131.2 (2C), 132.3 (2C), 132.5 (2C), 132.9 (2CH), 133.8 (2CH), 135.0 (2CH), 136.2 (2C), 136.4 (2CH), 150.9 (2C), 151.4 (2C), 151.9 (4C), 158.4 (4C), 165.0 (2 C=O). IR υ_{max} cm $^{-1}$ 1240 (C-N), 1050 (C-O), 1458

(C=C), 1617 (C=N), 1636 (C=O), 2810 (C-H, aliphatic), 3040 (C-H, aromatic), 3235 (N-H, amide), 3441 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 977.3533, calcd. 977.3524 for $C_{58}H_{45}N_{10}O_6$.

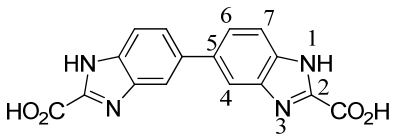
5.16 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid bis-{[(1*H*-benzimidazol-2-ylmethyl)]-amide} (174)



The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using 2-(aminomethyl)-1*H*-benzimidazole over 20 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **174** as a brown solid (0.42 g, 52 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 4.77 (t, J = 5.6 Hz, 4H, $2CH_2$), 7.16 (dd, J_1 = 6.0, J_2 = 3.2 Hz, 4H, H5'+H6'), 7.53-7.56 (m, 4H, H4'+H7'), 7.61 (dd, J_1 = 8.4, J_2 = 2.7 Hz, 2H, H6), 7.71-7.75 (bs, 2H, N-H Imidazole), 7.89-7.93 (bs, 2H, N-H Imidazole), 8.10 (d, J = 8.4 Hz, 2H, H7), 8.16 (d, J = 2.7 Hz, 2H, H4), 8.19 (d, J = 8.8 Hz, 4H, H2"+H6"), 8.36 (d, J = 8.8 Hz, 4H, H3"+H5"), 9.53 (t, J = 5.6 Hz, 2H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) 37.8 ($2CH_2$), 114.9 (2CH), 118.3 (2CH), 125.7 (2CH), 126.2 (4CH), 128.2 (4CH), 129.6 (4CH), 132.7 (2C), 133.2 (2C), 134.7 (4CH), 137.6 (2C), 138.2 (2C), 147.1 (2C), 147.4 (2C), 154.4 (2C), 154.7 (4C), 165.9 (2 C=O). IR υ_{max} cm $^{-1}$ 1273 (C-N), 1060 (C-O), 1443 (C=C), 1590 (C=N), 1615 (C=O), 2924 (C-H, aliphatic), 3058 (C-H, aromatic), 3310 (N-H, amide), 3400 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 733.2776, calcd. 733.2788 for $C_{44}H_{33}N_{10}O_2$.

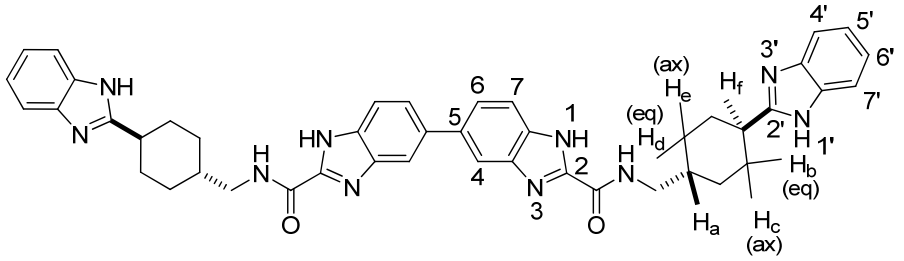
5.17 1*H*,1′*H*-[5,5′]Bibenzimidazolyl-2,2′-dicarboxylic acid (166) [81]



The title compound was synthesized according to method **5.6**. One equivalent of 3,3'-diaminobenzidine was condensed with two equivalents of methyl trichloroacetimidate affording 5,5'-bis(2-trichloromethyl-1*H*-benzimidazole) which was hydrolysed affording the corresponding bisbenzimidazolyl-2,2′-dicarboxylic acid **166** as a pale yellow solid (0.82 g, 64 % yield); Mp > 300 °C, (> 300 °C [81]).

^{1}H NMR (400 MHz, DMSO) 7.79 (dd, 2H, J_1 = 8.4, J_2 = 1.6 Hz, H6), 7.87 (d, 2H, J = 8.4 Hz, H7), 8.03 (d, 2H, J = 1.6 Hz, H4), 9.07 (s, 2H, N-H Imidazole), 9.98-10.14 (br, 2H, 2COOH). ^{13}C NMR (100 MHz, DMSO) δ 113.9 (2CH), 115.4 (2CH), 126.5 (2CH), 128.1 (2C), 131.3 (2C), 134.2 (2C), 153.8 (2C), 166.9 (2 C=O). LRMS: (ES) *m/z* $(MH)^+$ 323.3 for $C_{16}H_{21}N_4O_4$.

5.18 3*H*,3′*H*-[5,5′]Bibenzimidazolyl-2,2′-dicarboxylic acid bis-{[4-(1*H*-benzimidazol-2-yl)-cyclohexylmethyl]-amide} (176)

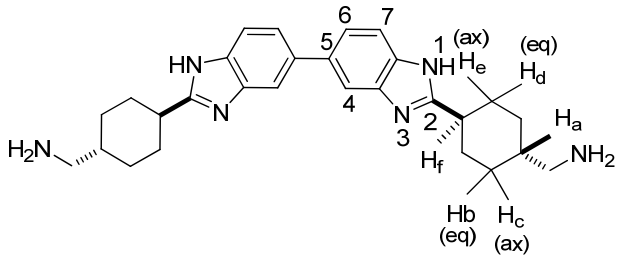


The title compound was synthesized according to procedure **5.7** *via* amidation of 3*H*,3′*H*-[5,5′]bibenzimidazolyl-2,2′-dicarboxylic acid **166** by using 4-(1-*H*-benzimidazol-2-yl)cyclohexyl)methanamin over 13 days. The product was purified by recrystallization from ethanol affording **176** as a brown solid (0.49 g, 60 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 1.18 (qd, J_1 = 12.4, J_2 = 2.8 Hz, 4H, H_c), 1.58 (qd, J_1 = 12.4, J_2 = 2.8 Hz, 4H, H_e), 1.69-1.75 (m, 2H, H_a), 1.89 (dq, J_1 = 11.6, J_2 = 1.2 Hz, 4H, H_d), 2.08 (dq, J_1 = 11.6, J_2 = 1.2 Hz, 4H, H_b), 2.80 (tt, J_1 = 11.6, J_2 = 1.2 Hz, 2H, H_f), 3.25 (t, J = 6.0 Hz, 4H, $2CH_2$), 7.59 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 4H, H5'+H6'), 7.76 (dd, J_1 = 8.4, J_1 = 0.8 Hz, 2H, H6), 7.84 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 4H, H4'+H7'), 7.85 (d, J = 8.4 Hz, 2H, H7), 7.96 (d, J = 0.8 Hz, 2H, H4), 9.01 (t, J = 6.0 Hz, 2H, N-H Amide), 12.11 (s, 4H, N-H imidazole). ^{13}C NMR : There were no peaks for ^{13}C on NMR, as the tested sample did not dissolve in DMSO. IR υ_{max} cm $^{-1}$ 1273 (C-N), 1037 (C-O), 1450 (C=C), 1537 (C=N), 1661 (C=O), 2854 (C-H, aliphatic), 3070 (C-H, aromatic), 3221

(N-H, amide), 3355 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 745.3734, calcd. 745.3727 for $C_{44}H_{45}N_{10}O_2$.

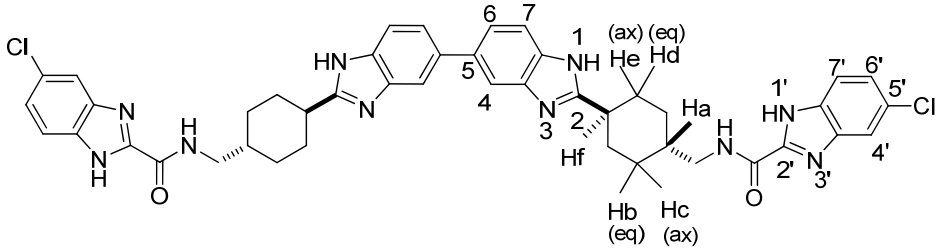
5.19 {4-[2'-(4''-Aminomethyl-cyclohexyl)-1*H*,1'*H*-[5,5']bibenzimidazolyl -2-yl]-cyclohexyl}-methylamine (169) [81]



The title compound was synthesized by condensation of 3,3'-diaminobenzidine with *trans* 4-aminomethyl cyclohexane carboxylic acid over 72 h according to procedure **5.5** and purified by recrystallization from (water:ethanol; 1:3, v/v) affording **169** as a brown powder (0.34 g, 75 % yield); Mp > 300 °C, (> 300 °C [81]).

^{1}H NMR (400 MHz, DMSO) 1.03 (qd, J_1 = 12.4, J_2 = 3.2 Hz, 4H, H_c), 1.27-1.32 (m, 2H, H_a), 1.61 (qd, J_1 = 12.4, J_2 = 3.2 Hz, 4H, H_e), 1.90 (dq, J_1 = 10.8, J_2 = 0.7 Hz, 4H, H_d), 2.09 (dq, J_1 = 10.8, J_2 = 0.7 Hz, 4H, H_b), 2.45 (d, J = 6.4 Hz, 4H, $2CH_2$), 2.79 (tt, J_1 = 10.8, J_2 = 0.7 Hz, 2H, H_f), 7.42 (dd, J_1 = 8.4, J_2 = 1.6 Hz, 2H, H6), 7.53 (d, J = 8.4 Hz, 2H, H7), 7.69 (s, 2H, H4). ^{13}C NMR (100 MHz, DMSO) δ 30.4 ($4CH_2$), 31.4 ($4CH_2$), 38.6 (2CH), 40.6 (2CH), 48.6 ($2CH_2$), 113.0 (2CH), 114.7 (2CH), 121.2 (2CH), 127.7 (2C), 135.5 (2C), 138.8 (2C), 159.9 (2C). LRMS: (ES) *m/z* $(MH)^+$457.3 for $C_{28}H_{37}N_6$.

5.20 6-Chloro-1*H*-benzimidazole-2-carboxylic acid {4-[2'-(4-{[(1*H*-benzimidazole-2-carbonyl)-amino]-methyl}-cyclohexyl)-1*H*,1'*H*-[5,5']bibenzimidazolyl-2-yl]-cyclohexylmethyl}-amide (177)

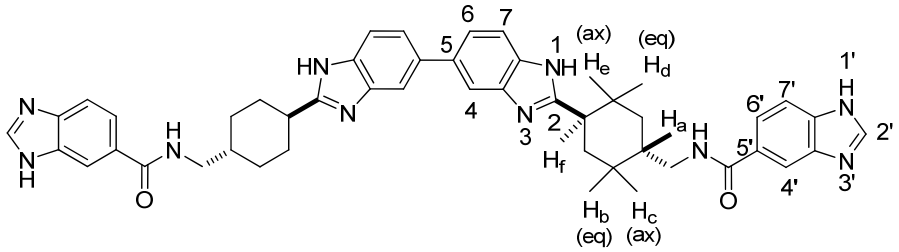


The title compound was synthesized according to procedure **5.7** *via* amidation of 5-chloro-1*H*-benzimidazole-2-carboxylic acid by using **169** over 5 days. The product was

purified by recrystallization from ethanol affording **177** as a beige solid (1.76 g, 72 % yield); Mp 238-240 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 11.6, J_2 = 2.2 Hz, 4H, H_c), 1.61 (qd, J_1 = 11.6, J_2 = 2.2 Hz, 4H, H_e), 1.71-1.74 (m, 2H, H_a), 1.98 (dq, J_1 = 11.2, J_2 =0.7 Hz, 4H, H_d), 2.12 (dq, J_1 = 11.2, J_2 = 0.7 Hz, 4H, H_b), 2.84 (tt, J_1 = 11.2, J_2 = 0.7 Hz, 2H, H_f), 3.25 (t, J = 6.4 Hz, 4H, $2CH_2$), 7.33 (dd, J_1 = 8.8, J_2 = 2.0 Hz, 2H, H6), 7.42 (d, J = 8.0 Hz, 2H, H7'), 7.52 (s, 2H, H4'), 7.54 (d, J = 8.0, J = 8.0 Hz, 2H, H6'), 7.68 (s, 2H, H4), 7.76 (d, J = 8.8 Hz, 2H, H7), 7.79 (s, 2H, N-H imidazole), 9.08 (t, J = 6.4 Hz, 2H, N-H Amide), 12.01-12.43 (br, 2H, N-H imidazole). ^{13}C NMR (100 MHz, DMSO) δ 28.8 ($4CH_2$), 29.7 ($4CH_2$), 35.9 (2CH), 36.7 (2CH), 43.8 ($2CH_2$), 110.9 (2CH), 112.8 (2CH), 116.9 (2C), 117.9 (2C), 120.2 (2CH), 121.9 (2CH), 123.2 (2CH), 125.7 (2CH), 127.2 (2C), 132.1 (2C), 133.9 (2C), 140.2 (2C), 142.2 (2C) 146.1 (2C), 157.3 (2 C=O). IR υ_{max} cm $^{-1}$ 808 (C-Cl), 1235 (C-N), 1058 (C-O), 1423 (C=C), 1560 (C=N), 1663 (C=O), 2857 (C-H, aliphatic), 3090 (C-H, aromatic), 3400 (N-H). HRMS: (ES) *m/z* $(MH)^+$ obsd. 813.2930, calcd. 813.2942 for $C_{44}H_{43}Cl_2N_{10}O_2$.

5.21 *N*-(4-{2'-[4-(2-Benzimidazolyl-5-carboxamido-methyl)-cyclohexyl]-1*H*,1'*H*-[5,5'] bibenzimidazolyl-2-yl}-cyclohexylmethyl)-2-benzimidazole-2-carboxamide (178)

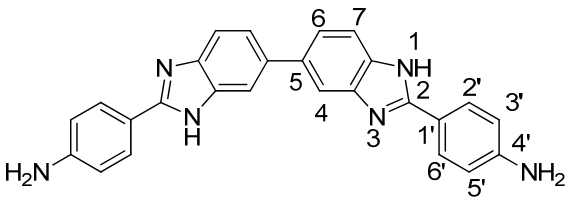


The title compound was synthesized according to procedure **5.7** *via* amidation of benzimidazole-5-carboxylic acid by using **169** over 8 days. The product was purified by recrystallization from ethanol affording **178** as a brown solid (1.49 g, 67 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 1.16 (qd, J_1 = 11.4, J_2 = 1.2 Hz, 4H, H_c), 1.55-1.71 (m, 2H, H_a), 1.61 (qd, J_1 = 11.4, J_2 = 1.2 Hz, 4H, H_e), 1.93 (dq, J_1 = 11.4, J_2 = 0.4 Hz, 4H, H_d), 2.12 (dq, J_1 = 11.4, J_2 = 0.4 Hz, 4H, H_b), 2.84 (tt, J_1 = 11.4, J_2 = 0.4 Hz, 2H, H_f), 3.22 (t, J = 5.7 Hz, 4H, $2CH_2$), 7.41 (d, J = 8.4 Hz, 2H, H6), 7.52 (d, J = 8.4 Hz, 2H,

H7), 7.61 (s, 2H, H4), 7.65 (d, *J* = 8.1 Hz, 2H, H6'), 7.77 (d, *J* = 8.1 Hz, 2H, H7'), 8.18 (s, 2H, H4'), 8.34 (s, 2H, H2'), 8.51 (t, *J* = 5.7 Hz, 2H, N-H Amide), 11.98-12.44 (br, 2H, N-H imidazole). ^{13}C NMR (100 MHz, DMSO) δ 30.2 ($4CH_2$), 30.9 ($4CH_2$), 37.2 (2CH), 38.0 (2CH), 45.5 ($2CH_2$), 114.4 (2CH), 115.1 (2CH), 115.3 (2CH), 120.8 (2CH), 121.4 (2CH), 122.7 (2CH), 128.5 (2C), 135.1 (2C), 138.1 (2C), 138.3 (2C), 139.7 (2C), 139.8 (2C), 143.9 (2CH), 159.4 (2C), 166.8 (2 C=O). IR υ_{max} cm^{-1} 1281 (C-N), 1042 (C-O), 1453 (C=C), 1574 (C=N), 1661 (C=O), 2850 (C-H, aliphatic), 3080 (C-H, aromatic), 3189 (N-H). HRMS: (ES) *m/z* $(MH)^+$ obsd. 745.3722, calcd. 745.3721 for $C_{44}H_{45}N_{10}O_2$.

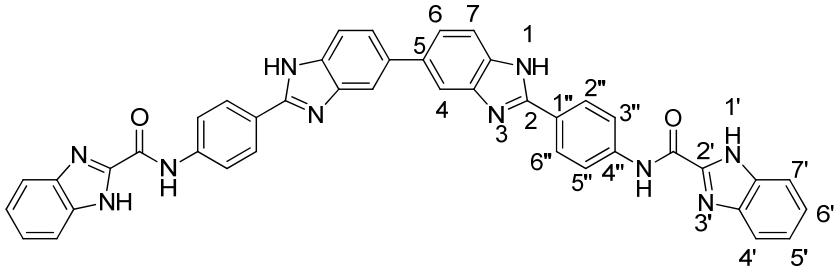
5.22 1*H*,1′*H*-[5,5′]Bisbenzimidazolyl-2,2′-diphenyl-4,4'-diamine (168) [73]



The title compound was synthesized from reduction of 1*H*,1′*H*-[5,5′]bisbenzimidazolyl-2,2′-diphenyl-4,4'-dinitro **179** according to method **5.4**, with the reaction extended to 48 h. The crude product was purified by recrystallization from ethanol affording **168** as a brown solid (1.05 g, 72 %); Mp 242-244 °C (Lit. [73] 242-244 °C).

^{1}H NMR (400 MHz, DMSO) 5.63 (s, 4H, NH amine), 6.69 (d, *J* = 8.8 Hz, 4H, H2'+H6'), 7.45(d, *J* = 8.4 Hz, 2H, H7), 7.60-7.64 (m, 2H, H6), 7.81 (s, 2H, H4), 7.87 (d, *J* = 8.8 Hz, 4H, H3'+H5'), 7.96 (s, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) δ 79.3 (2C), 110.8 (2C), 112.8 (2C), 113.5 (4CH), 114.5 (2C), 117.2 (2CH), 118.1 (2C), 127.7 (4CH), 131.8 (2CH), 150.6 (2CH), 153.1 (2C). IR υ_{max} cm^{-1} 1276 (C-N), 1443 (C=C), 1605 (C=N), 3099 (C-H, aromatic), 3336 (N-H amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 417.1837, calcd. 417.1827 for $C_{26}H_{21}N_6$.

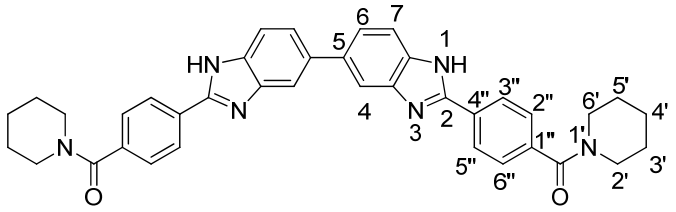
5.23 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-diamine bis-[benzimidazol-2-yl]-amide (180)



The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1′*H*-[5,5′]bisbenzimidazolyl-2,2′-diphenyl-4,4'-diamine **168** by using benzimidazole-2-carboxylic acid over 15 days. The product was purified by column chromatography using DCM: MeOH (10:l) affording **180** as a brown solid (1.37 g, 65 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 7.33-7.41 (dq, J_1 = 6.8, J_2 = 6.7 Hz, 4H, H5'+H6'), 7.61 (dd, J_1 = 6.2, J_2 = 1.6 Hz, 4H, H4'+H7'), 7.69-7.72 (bs, 2H, H4), 7.84 (d, J = 8.0 Hz, 2H, H7), 7.89 (dd, J_1 = 8.0, J_2 = 2.0 Hz, 2H, H6), 8.16 (d, J = 8.8 Hz, 4H, H2"+H6"), 8.23 (d, J = 8.8 Hz, 4H, H3"+H5"), 11.17 (s, 2H, N-H Amide), 12.85-13.09 (br, 2H, N-H Imidazole), 13.52 (s, 2H, N-H Imidazole) . ^{13}C NMR (100 MHz, DMSO) 112.7 (2CH), 115.2 (2C), 120.1 (4CH), 120.5 (4CH), 122.0 (2C), 122.9 (2CH), 125.7 (4CH), 126.9 (4CH), 132.7 (2C), 134.7 (2C), 134.8 (2C), 139.8 (2C), 142.5 (2CH), 145.4 (4C), 151.6 (2C), 157.5 (2 C=O). IR υ_{max} cm $^{-1}$ 1280 (C-N), 1118 (C-O), 1446 (C=C), 1596 (C=N), 1662 (C=O), 3053 (C-H, aromatic), 3232 (N-H, amide), 3344 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 705.2467, calcd. 705.2475 for $C_{42}H_{29}N_{10}O_2$.

5.24 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid-di(piperidin-1-yl)methanone (184)

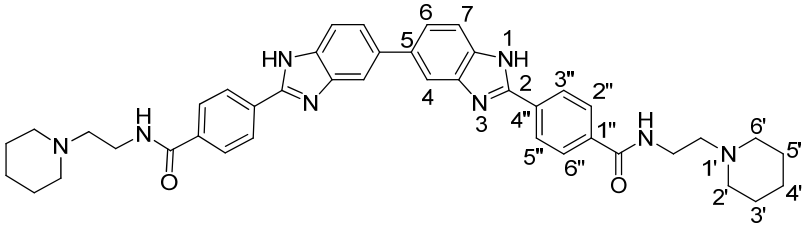


The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using

piperidine over 6 days. The product was purified by recrystallization from ether affording **184** as a pale brown solid (0.40 g, 60 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 1.48-1.59 (bs, 4H, 2CH_2(3')), 1.61-1.66 (bs, 4H, 2CH_2(4')), 3.30-3.39 (bs, 8H, 4CH_2(2'+5')), 3.58-3.66 (bs, 4H, 2CH_2(6')), 7.54 (dd, J_1 = 8.8, J_2 = 3.2 Hz, 4H, H2"+H6"), 7.65-7.70 (m, 4H, H3"+H5"), 7.82 (s, 2H, N-H imidazole), 8.08 (dd, J_1 = 8.0, J_2 = 2.0 Hz, 2H, H6), 8.21 (d, J = 8.0 Hz, 2H, H7), 8.33 (q, J = 8.0 Hz, 2H, H4). ^{13}C NMR (100 MHz, DMSO) 24.0 (2CH_2), 29.6 (4CH_2), 35.3 (4CH_2), 116.2 (2C), 120.6 (2C), 120.8 (2C), 121.8 (2CH), 125.7 (2CH), 126.5 (4CH), 127.3 (4CH), 129.5 (2CH), 141.9 (2C), 142.2 (2C), 151.9 (2C), 168.4 (2 C=O). IR υ_{max} cm $^{-1}$ 1279 (C-N), 1020 (C-O), 1445 (C=C), 1549 (C=N), 1593 (C=O), 2851 (C-H, aliphatic), 2935 (C-H, aromatic), 3288 (N-H, amide), 3417 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 609.2966, calcd. 609.2978 for $C_{38}H_{37}N_6O_2$.

5.25 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid bis-{[(piperidin-1-ylethyl)]-amide} (185)



The title compound was synthesized according to procedure **5.7** *via* amidation of 1*H*,1'*H*-[5,5']bibenzimidazolyl-2,2'-diphenyl-4,4'-dicarboxylic acid **167** by using 1-(2-amino ethyl)-piperidine over 9 days. The product was purified by recrystallization from methanol affording **185** as a pale yellow solid (0.38 g, 50 % yield); Mp > 300 °C.

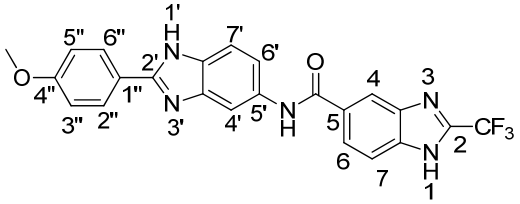
^{1}H NMR (300 MHz, DMSO) 1.19-1.24 (m, 4H, 2CH_2(4')), 1.33-1.41 (m, 4H, 2CH_2(3')), 1.47-1.54 (m, 4H, 2CH_2(5')), 2.37-2.46 (m, 8H, 4CH_2(2'+6')), 2.49-2.51 (bs, 4H, 2CH_2(amine)), 3.36-3.53 (bs, 4H, 2CH_2(amide)), 7.57 (dd, J_1 = 7.8, J_2 = 1.6 Hz, 2H, H6), 7.68-7.74 (bs, 2H, H4), 7.82-7.90 (bs, 2H, N-H imidazole), 8.01-8.09 (m, 4H, H2"+H6"), 8.19 (d, J = 7.8 Hz, 2H, H7), 8.34 (d, J = 8.1 Hz, 4H, H3"+H5"), 8.58 (t, J = 5.1 Hz, 2H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) 24.0 (2CH_2), 24.4 (2CH_2), 25.6 (2CH_2), 36.5 (2CH_2), 36.7 (2CH_2), 54.1 (2CH_2), 57.7 (2CH_2), 107.4 (2C), 107.9 (2C), 119.5 (2C), 120.0 (2CH), 125.6 (2CH), 126.3 (4CH), 127.8 (4CH), 129.6 (2CH),

146.3 (2C), 153.8 (2C), 156.4 (2C), 165.3 (2 C=O). IR υ_{max} cm $^{-1}$ 1233 (C-N), 1040 (C-O), 1419 (C=C), 1616 (C=N), 1639 (C=O), 2851 (C-H, aliphatic), 3058 (C-H, aromatic), 3288 (N-H, amide), 3490 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 695.3824, calcd. 695.3822 for $C_{42}H_{46}N_8O_2$.

5.26 General procedure for synthesis of amides that arised from coupling of amines with 2-trifluoromethyl 1*H*-benzimidazole-5-carboxylic acid by using the CDI.

A solution of 2-trifluoromethyl 1*H*-benzimidazole-5-carboxylic acid (2 mmol, 0.46 g) in DMF (10 mL) was treated with CDI (2 mmol, 0.325 g) at room temperature. The mixture was stirred at room temperature for 30 minutes, then treated with appropriate aminobenzimidazole (1 mmol) in DMF (5 mL), and the reaction stirred at room temperature. After 6-12 days the TLC showed no starting material left. After removing the solvent *in vacuo*, ethyl acetate (50 mL) was added to the crude, and washed with saturated $NaHCO_3$ (20 mL), brine (10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in *vacuo*. The residue was purified by either column chromatography or recrystalization.

5.26.1 2-Trifluoromethyl-1*H*-benzimidazole-5-carboxylicacid[2-(4'-methoxy-phenyl)-1*H*-benzimidazol-5-yl]-amide (134)

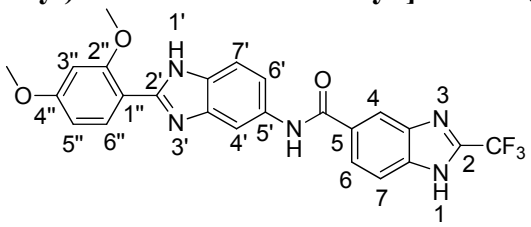


The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 5-amino-2-(4'-methoxy phenyl)benzimidazole, by reaction over 10 days. The product was purified by column chromatography using DCM: MeOH (15:1 v/v) affording **134** as a brown solid (0.49 g, 55 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 3.89 (s, 3H, CH_3), 7.22 (d, *J* = 9.2 Hz, 2H, H3"+H5"), 7.72 (d, *J* = 8.8 Hz, 1H, H7'), 7.81 (d, *J* = 8.6 Hz, 1H, H7), 7.90 (d, *J* = 8.8 Hz, 1H, H6'), 8.06 (d, *J* = 8.6 Hz, 1H, H6), 8.18 (d, *J* = 9.2 Hz, 2H, H2"+H6"), 8.49 (s, 1H, H4'), 8.59

(s, 1H, H4), 10.65 (s, 1H, N-H Amide). ^{13}C NMR (75 MHz, DMSO) 54.9 (CH_3), 104.2 (CH), 112.4 (CH), 113.7 (CH), 114.7 (CH), 114.9 (2CH), 115.5 (C), 116.2 (C), 117.4 (2CH), 119.5 (CH), 121.3 (C), 124.1 (CH), 127.7 (C), 129.6 (C), 130.8 (C), 131.8 (C), 137.9 (C), 138.2 (C, CF_3, J_{CF} = 39.3 Hz), 148.9 (C), 161.8 (C), 163.6 (C=O). IR υ $_{max}$ cm $^{-1}$ 595 (C-F), 1235 (C-N), 1073 (C-O), 1472 (C=C), 1644 (C=N), 1610 (C=O), 2865 (C-H, aliphatic), 3088 (C-H, aromatic), 3378 (N-H, amide), 3458 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 452.1336, calcd. 452.1329 for $C_{23}H_{16}F_3N_5O_2$.

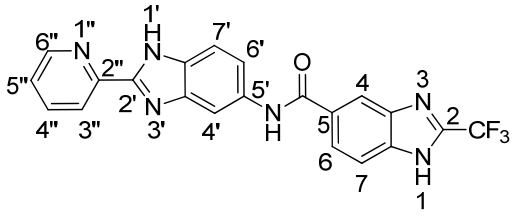
5.26.2 2-Trifluoromethyl-1*H*-benzimidazole-5-carboxylic acid [2-(2',4'-dimethoxy-phenyl)-1*H*-benzimidazol-5-y1]-amide (135)



The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 2-(2',4'-dimethoxyphenyl)-5-aminobenzimidazole, by reaction over 6 days. The product was purified by column chromatography using DCM: MeOH (12:1 v/v) affording **135** as a brown solid (0.63 g, 65 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 3.94 (s, 3H, CH_3), 4.11 (s, 3H, CH_3), 6.91 (d, *J* = 7.2 Hz, 1H, H5"), 6.93 (d, *J* = 1.1 Hz, 1H, H3"), 7.79 (d, *J* = 8.4 Hz, 1H, H7), 7.83 (d, *J* = 9.2 Hz, 1H, H6'), 7.87 (d, *J* = 7.2 Hz, 1H, H6"), 8.05 (d, *J* = 9.2 Hz, 1H, H7'), 8.09 (d, *J* = 8.4 Hz, 1H, H6), 8.46 (s, 1H, H4'), 8.61 (s, 1H, H4), 10.75 (s, 1H, N-H Amide), 13.99-14.46 (br, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 55.9 (CH_3), 56.4 (CH_3), 99.0 (CH), 103.5 (CH), 104.4 (CH), 107.5 (CH), 115.9 (CH), 117.7 (CH), 120.4 (CH), 124.3 (C), 127.3(C), 130.8 (CH), 131.2 (CH), 131.5 (C), 137.7 (C), 138.5 (C), 139.6 (C), 142.2 (C), 142.4 (C, CF_3, J_{CF} = 39.4 Hz), 143.0 (C), 146.4 (C), 159.9 (C), 165.4 (C), 166.1 (C=O). IR υ $_{max}$ cm $^{-1}$ 590 (C-F), 1230 (C-N), 1050 (C-O), 1463 (C=C), 1620 (C=N), 1614 (C=O), 2847 (C-H, aliphatic), 3090 (C-H, aromatic), 3360 (N-H, amide), 3416 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 482.1435, calcd. 482.1435 for $C_{24}H_{19}F_3N_5O_3$.

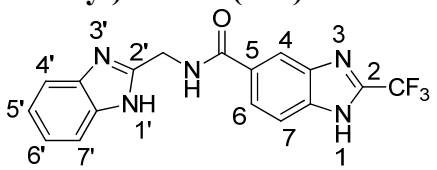
5.26.3 2-Trifluoromethyl-1*H*-benzimidazole-5-carboxylic acid (2-pyridin-2-yl-1*H*-benzimidazol-5-yl)-amide (136)



The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 2-pyridin-2-yl-1*H*-benzimidazol-5-ylamine by reaction over 6 days. The product was purified by recrystalization from ethanol affording **136** as a brown solid (0.46 g, 54 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 7.71 (dd, J_1 = 8.0, J_2 = 4.4 Hz, 1H, H5"), 7.81 (d, J = 8.4 Hz, 1H, H6'), 7.83 (d, J = 8.4 Hz, 1H, H7'), 7.96 (d, J = 8.8 Hz, 1H, H7), 8.03 (d, J = 8.8 Hz, 1H, H6), 8.18 (t, J = 8.0 Hz, 1H, H4"), 8.39 (d, J = 8.0 Hz, 1H, H3"), 8.40 (s, 1H, N-H Imidazole), 8.46 (s, 1H, H4'), 8.58 (s, 1H, H4), 8.70 (s, 1H, N-H Imidazole), 8.88 (d, J = 4.4 Hz, 1H, H6"), 10.72 (s, 1H, N-H Amide). ^{13}C NMR (75 MHz, DMSO) 104.6 (CH), 114.6 (CH), 115.9 (CH), 117.2 (CH), 117.8 (CH), 119.3 (C), 120.6 (CH), 120.8 (CH), 123.4 (CH), 124.3 (CH), 127.6 (C), 128.2(C), 130.6 (C), 132.3 (C), 138.5 (CH), 139.6 (C), 142.1 (C, CF_3, J_{CF} = 39.5 Hz), 142.7 (C), 147.4 (C), 150.6 (C), 166.1 (C=O). IR υ_{max} cm $^{-1}$ 610 (C-F), 1240 (C-N), 1050 (C-O), 1447 (C=C), 1630 (C=N), 1647 (C=O), 3040 (C-H, aromatic), 3360 (N-H, amide), 3420 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 423.1175, calcd. 423.1176 for $C_{21}H_{14}F_3N_6O$.

5.26.4 2-Trifluoromethyl-1*H*-benzimidazole-5-carboxylic acid(1*H*-benzimidazol-2-ylmethyl)-amide (137)

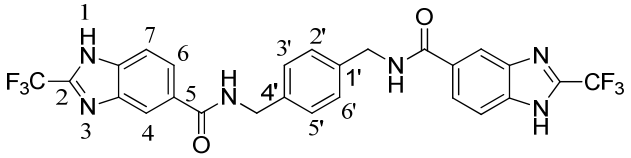


The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 2-(aminomethyl)-1*H*-

benzimidazole by reaction over 6 days. The product was purified by recrystalization from ethanol affording **137** as a brown solid (0.47 g, 65 %); Mp 184-186 °C.

^{1}H NMR (400 MHz, DMSO) 4.76 (s, 2H, CH_2), 7.15 (dd, J_1 = 6.0, J_2 = 2.8 Hz, 2H, H5'+H6'), 7.52 (d, J = 6.0 Hz, 2H, H4'+H7'), 7.82 (d, J = 8.8 Hz, 1H, H7), 8.02 (d, J = 8.8 Hz, 1H, H6), 8.39 (s, 1H, H4), 9.36 (s, 1H, N-H Amide), 11.79-13.03 (br, 1H, N-H Imidazole). ^{13}C NMR (75 MHz, DMSO) 38.3 (CH_2), 115.2 (C), 116.3 (CH), 117.4 (CH), 121.0 (CH), 121.8 (2CH), 124.1 (2CH), 124.6 (C), 130.3 (2C), 138.2 (C), 139.3 (C), 142.2 (C, CF_3, J_{CF} = 39.1 Hz), 152.8 (C) 166.9 (C=O). IR υ $_{max}$ cm $^{-1}$ 610 (C-F), 1273 (C-N), 1100 (C-O), 1443 (C=C), 1622 (C=N), 1646 (C=O), 2840 (C-H, aliphatic), 3065 (C-H, aromatic), 3320 (N-H, amide), 3470 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 360.1071, calcd. 360.1067 for $C_{17}H_{13}F_3N_5O$.

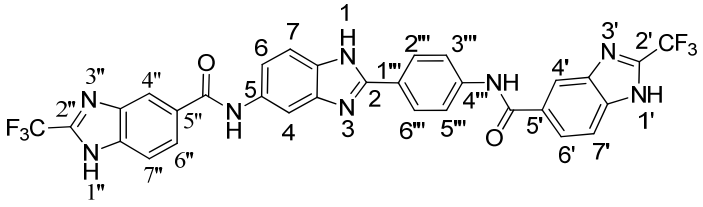
5.26.5 *N*,*N'*-Bis(2-Trifluoromethyl-1*H*-benzimidazole-5-carbonyl)-*p*-xylylenediamine (148)



The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid by using *p*-xylenediamine over 9 days. The product was purified by column chromatography using DCM: MeOH (15:l) affording **148** as a white solid (1.23 g, 73 % yield) ; Mp 290-292 °C.

^{1}H NMR (400 MHz, DMSO) δ 4.49 (d, J = 6.0 Hz, 4H, 2 CH_2), 7.32 (s, 4H, H2' +H3'+H5'+H6'), 7.77 (d, J = 8.8 Hz, 2H, H7), 7.93 (d, J = 8.8 Hz, 2H, H6), 8.29 (s, 2H, H4), 9.16 (t, J = 6.0 Hz, 2H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) δ 42.9 ($2CH_2$), 116.2 (2C), 116.9 (2C), 117.9 (2C), 120.7 (2CH), 123.4 (2C), 123.8 (2CH), 127.6 (4CH), 130.6 (2CH), 138.6 (2C), 142.4 (2C, $2CF_3$, J_{CF} = 39.2 Hz), 166.5 (2C=O). IR υ $_{max}$ cm $^{-1}$ 595 (C-F), 1232 (C-N), 1051 (C-O), 1440 (C=C), 1542 (C=N), 1640 (C=O), 2851 (C-H, aliphatic), 3064 (C-H, aromatic), 3325 (N-H, amide), 3434 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 561.1473, calcd. 561.1473 for $C_{26}H_{19}F_6N_6O_2$.

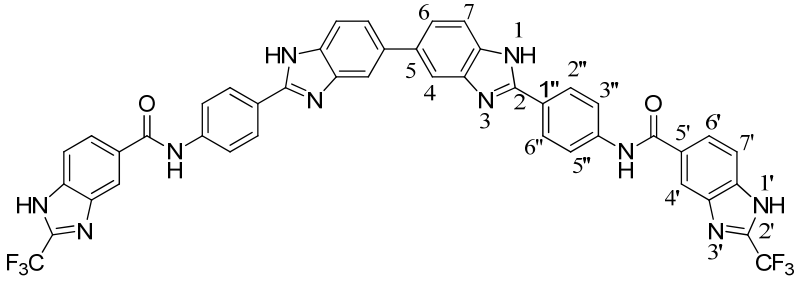
5.26.6 *N*-[4-(5(5-Benzimidazolyamino)-2-trifluoromethyl-1*H*-benzimidazol-5-yl)-phenyl]-benzimidazol-5-amide (160)



The title compound was synthesized according to procedure **5.26** *via* amidation of 2-(trifluoromethyl)-1*H*-benzimidazole-5-carboxylic acid with 2(4'-aminophenyl)-1*H*-benzimidazol-5-ylamine by reaction over 12 days. The product was purified by column chromatography using DCM: MeOH (12:1 v/v) affording **160** as a pale brown solid (1.66 g, 64 %); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 6.79 (d, J = 8.0 Hz, 1H, H7), 7.75 (d, J = 8.8 Hz, 2H, H7'+H7"), 7.83 (dd, J_1 = 8.8, J_2 = 1.6 Hz, 2H, H6'+H6"), 7.96 (d, J = 8.0 Hz, 1H, H6), 8.04-8.08 (bs, 1H, H4), 8.14 (d, J = 8.4 Hz, 2H, H2'"+H6'"), 8.26 (d, J = 8.4 Hz, 2H, H3'"+H5'"), 8.42 (d, J = 1.6 Hz, 2H, H4'+H4"), 8.52-8.59 (br, 1H, N-H Imidazole), 10.65 (s, 1H, N-H Amide), 10.79 (s, 1H, N-H Amide), 14.32-14.49 (br, 2H, N-H Imidazole). ^{13}C NMR (100 MHz, DMSO) 105.0 (CH), 113.5 (C), 113.7 (C), 113.9 (CH), 114.9 (C), 115.1 (C), 117.9 (2CH), 118.5 (2CH), 118.7 (C), 120.5 (CH), 120.6 (2CH), 120.8 (2CH), 123.2 (2C), 123.3 (C), 128.4 (2CH), 129.9 (2C), 136.6 (2C), 142.9 (2C, $2CF_3$, J_{CF} = 39.6 Hz), 149.9 (2C), 165.9 (C=O) 166.2 (C=O). IR υ_{max} cm $^{-1}$ 595 (C-F), 1258 (C-N), 1060 (C-O), 1471 (C=C), 1620 (C=N), 1633 (C=O), 1653 (C=O), 3050 (C-H, aromatic), 3391 (N-H, amide), 3420 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 649.1539, calcd. 649.1530 for $C_{31}H_{19}F_6N_8O_2$.

5.26.7 1*H*,1'*H*-[5,5']Bibenzimidazolyl-2,2'-diphenyl-4,4'-diamine bis-[2-trifluoromethylbenzimidazol-5-yl]-amide (181)



The title compound was synthesized according to procedure **5.26** *via* amidation of 1*H*,1′*H*-[5,5′]bisbenzimidazolyl-2,2′-diphenyl-4,4'-diamine **168** by using 2-(trifluoro methyl)-1*H*-benzimidazole-5-carboxylic acid over 18 days. The product was purified by column chromatography using DCM: MeOH (10:l) affording **181** as a brown solid (2.35 g, 70 % yield); Mp > 300 °C.

^{1}H NMR (400 MHz, DMSO) 6.55 (dd, J_1 = 8.4, J_2 = 2.0 Hz, 4H, H2"+H6"), 6.98 (dd, J_1 = 8.0, J_2 = 1.6 Hz, 2H, H6), 7.02 (d, J = 1.6 Hz, 2H, H4), 7.24 (d, J = 8.0 Hz, 2H, H7), 7.51 (d, J = 8.4 Hz, 2H, H7'), 7.58 (dd, J_1 = 8.4, J_2 = 1.2 Hz, 2H, H6'), 7.95 (dd, J_1 = 8.4, J_2 = 2.0 Hz, 4H, H3"+H5"), 8.06 (d, J = 1.2 Hz, 2H, H4'), 8.22 (s, 2H, N-H Amide). ^{13}C NMR (100 MHz, DMSO) 113.4 (4CH), 114.5 (2CH), 117.1 (2CH), 118.7 (2C), 120.8 (2CH), 121.1 (2CH), 125.3 (2C), 126.5 (2CH), 127.2 (2C), 127.4 (4CH), 132.3 (2C), 132.9 (2C), 135.5 (2C), 142.3 (2C, $2CF_3$, J_{CF} = 39.8 Hz), 146.5 (2CH), 146.9 (2C), 151.2 (2C), 154.3 (2C), 157.8 (2C), 159.2 (2C), 162.2 (2 C=O). IR υ_{max} cm $^{-1}$ 610 (C-F), 1285 (C-N), 1080 (C-O), 1440 (C=C), 1611 (C=N), 1634 (C=O), 3040 (C-H, aromatic), 3310 (N-H, amide), 3411 (N-H, amine). HRMS: (ES) *m/z* $(MH)^+$ obsd. 841.2233, calcd. 841.2222 for $C_{44}H_{27}F_6N_{10}O_2$.

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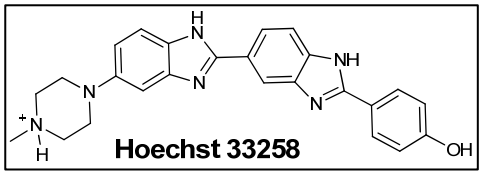
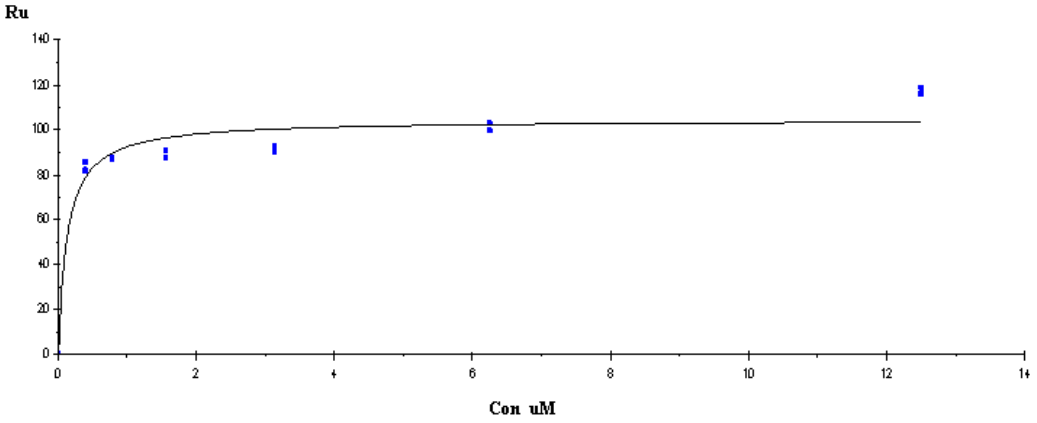
Appendix

Appendix I :SPR binding data for A2T2

I.1 The binding data of Hoechst 33258 to A2T2 DNA sequence (CGAATTCGTCTCCGAATTCG)

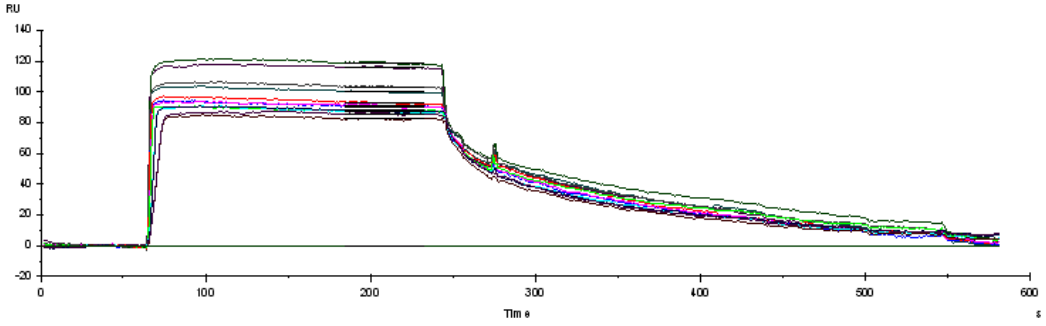




The Dissociation constant of Hoechst 33258 was evaluated by fitting the data with BIA evaluation 4.1 program

	KA(1/M)	KD(M)	R_{max}	n	Chi2
	7.76	0.129	104		67.9
Req vs. Conc				1	

Dissociation constant for binding of Hoechst 33258 to A2T2 DNA sequence

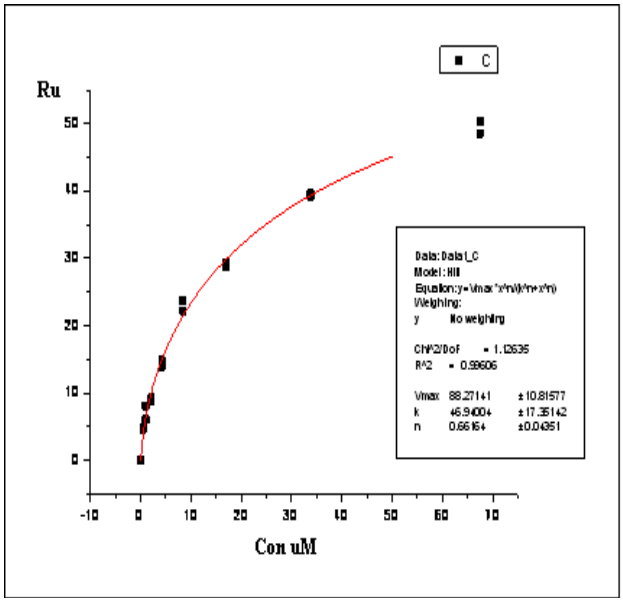


Binding curve for the interaction between Hoechst 33258 and A2T2 DNA sequence

	Req	SE(Req)	Conc
hoechst_s Fc=2-1 - 27	116	0.0695	12.5
hoechst_s Fc=2-1 - 28	119	0.0695	12.5
hoechst_s Fc=2-1 - 29	99.9	0.0695	6.25
hoechst_s Fc=2-1 - 30	103	0.0695	6.25
hoechst_s Fc=2-1 - 31	90.5	0.0695	3.13
hoechst_s Fc=2-1 - 32	92.7	0.0695	3.13
hoechst_s Fc=2-1 - 33	90.8	0.0695	1.56
hoechst_s Fc=2-1 - 34	87.8	0.0695	1.56
hoechst_s Fc=2-1 - 35	87.1	0.0695	0.781
hoechst_s Fc=2-1 - 36	87.6	0.0695	0.781
hoechst_s Fc=2-1 - 37	82.2	0.0695	0.391
hoechst_s Fc=2-1 - 38	85.7	0.0695	0.391
hoechst_s Fc=2-1 - 39	0	0.0695	0

The RU (Response unit) values at the concentration range of compound Hoechst 33258 , 0-12.5 μM

I.2 The binding data of compound 171 to A2T2 DNA sequence (CGAATTCGTCTCCGAATTCG)

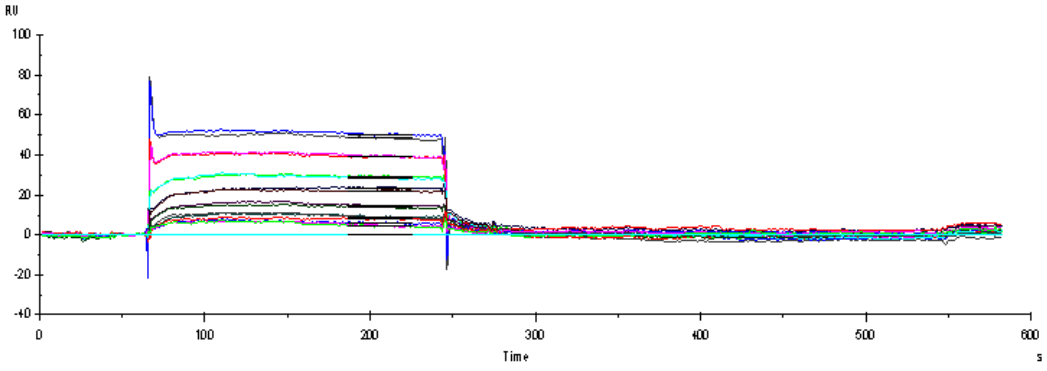


The Dissociation constant of compound 171 (Sh61) was evaluated by fitting the data with BIA evaluation Origin 7 program

Chi^2/DoF	R^2
1.12635	**0.99606**

Parameter	Value	Error
V_{max}	**88.2714**	**10.81577**
k	**46.94**	**17.35142**
n	**0.66164**	**0.04351**

Dissociation constant for binding of compound 171 (Sh61) to A2T2 DNA sequence

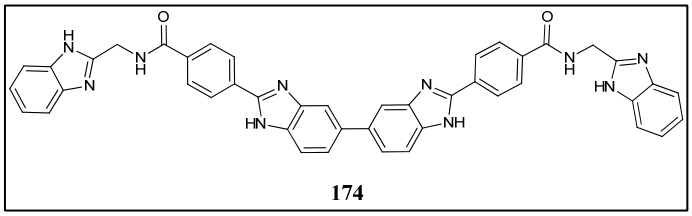


Binding curve for the interaction between compound 171 (Sh61) and A2T2 DNA sequence

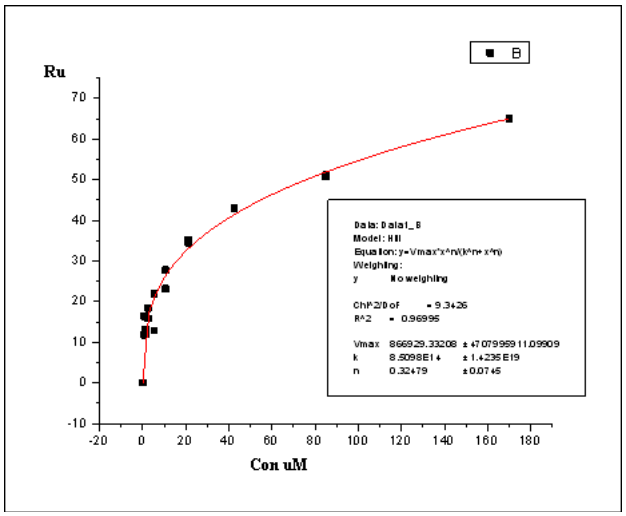
	Req	SE(Req)	Conc
Sh61_s Fc=2-1 - 43	48.6	0.0574	67.5
Sh61_s Fc=2-1 - 44	50.3	0.0574	67.5
Sh61_s Fc=2-1 - 45	39.2	0.0574	33.8
Sh61_s Fc=2-1 - 46	39.6	0.0574	33.8
Sh61_s Fc=2-1 - 47	29.3	0.0574	16.9
Sh61_s Fc=2-1 - 48	28.8	0.0574	16.9
Sh61_s Fc=2-1 - 49	23.7	0.0574	8.44
Sh61_s Fc=2-1 - 50	22.2	0.0574	8.44
Sh61_s Fc=2-1 - 51	14.8	0.0574	4.22
Sh61_s Fc=2-1 - 52	13.9	0.0574	4.22
Sh61_s Fc=2-1 - 53	9.24	0.0574	2.11
Sh61_s Fc=2-1 - 54	8.71	0.0574	2.11
Sh61_s Fc=2-1 - 55	6.04	0.0574	1.05
Sh61_s Fc=2-1 - 56	8.1	0.0574	1.05
Sh61_s Fc=2-1 - 57	4.89	0.0574	0.527
Sh61_s Fc=2-1 - 58	4.63	0.0574	0.527
Sh61_s Fc=2-1 - 59	0	0.0574	0

The RU (Response unit)values at the concentration range of compound 171 (Sh61) , 0-67.5 µM

I.3 The binding data of compound 174 to A2T2 DNA sequence (CGAATTCGTCTCCGAATTCG)



174

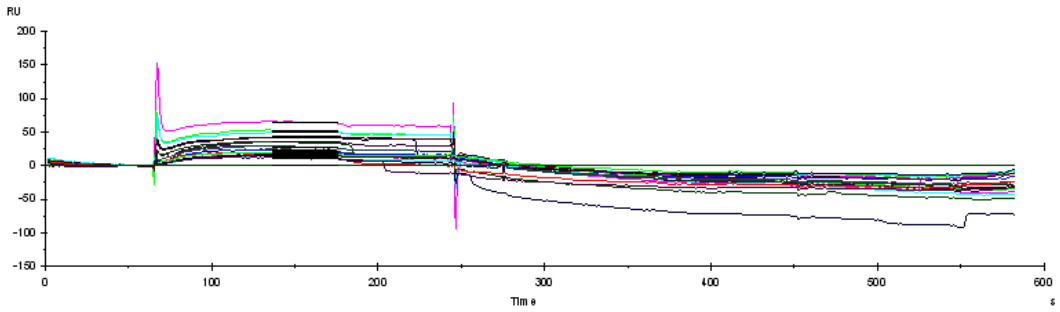


Chi^2/DoF	R^2
9.3426	0.96995

Parameter	Value	Error
V_{max}	866929.3321	4707995911
k	8.5098E+14	1.42E+19
n	0.32479	0.0745

The Dissociation constant of compound 174 (Sh72) was evaluated by fitting the data with BIA evaluation Origin 7 program

Dissociation constant for binding of compound 174 (Sh72) to A2T2 DNA sequence

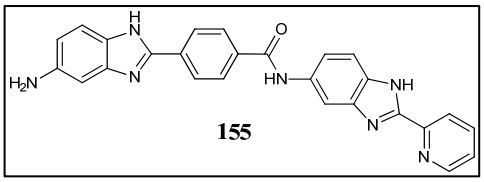


Binding curve for the interaction between compound 174 (Sh72) and A2T2 DNA sequence

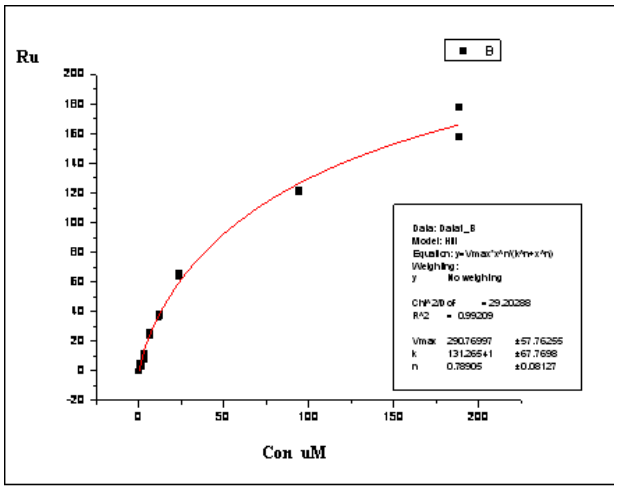
	Req	SE(Req)	Conc
Sh72_s Fc=2-1 - 62	65.1	0.154	170
Sh72_s Fc=2-1 - 63	51.2	0.154	85
Sh72_s Fc=2-1 - 64	50.9	0.154	85
Sh72_s Fc=2-1 - 65	42.8	0.154	42.5
Sh72_s Fc=2-1 - 66	43	0.154	42.5
Sh72_s Fc=2-1 - 67	35.3	0.154	21.3
Sh72_s Fc=2-1 - 68	34.5	0.154	21.3
Sh72_s Fc=2-1 - 69	27.8	0.154	10.6
Sh72_s Fc=2-1 - 70	23.2	0.154	10.6
Sh72_s Fc=2-1 - 71	21.9	0.154	5.31
Sh72_s Fc=2-1 - 72	12.9	0.154	5.31
Sh72_s Fc=2-1 - 73	15.9	0.154	2.65
Sh72_s Fc=2-1 - 74	18.4	0.154	2.65
Sh72_s Fc=2-1 - 75	13.1	0.154	1.32
Sh72_s Fc=2-1 - 76	12.1	0.154	1.32
Sh72_s Fc=2-1 - 77	16.4	0.154	0.664
Sh72_s Fc=2-1 - 78	11.8	0.154	0.664
Sh72_s Fc=2-1 - 79	0	0.154	0

The RU (Response unit) values at the concentration range of compound 174 (Sh72) , 0-170 μM

I.4 The binding data of compound 155 to A2T2 DNA sequence (CGAATTCGTCTCCGAATTCG)



155

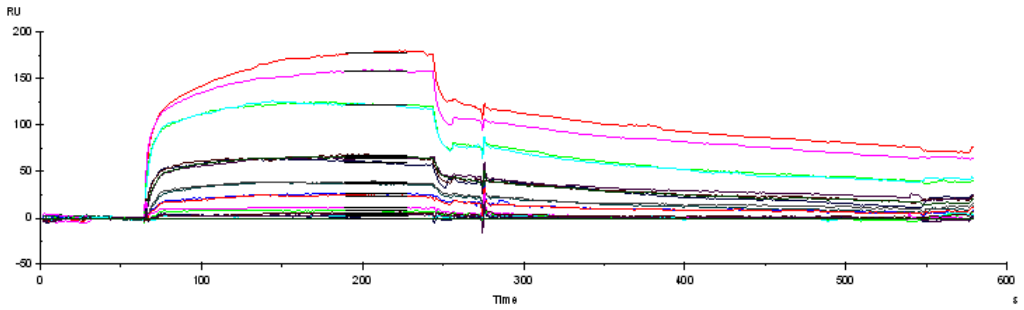


Chi^2/DoF	R^2
29.20288	0.99209

Parameter	Value	Error
V_{max}	290.77	57.76255
k	131.265	67.7698
n	0.78905	0.08127

The Dissociation constant of compound 155 (Sh74) was evaluated by fitting the data with BIA evaluation Origin 7 program

Dissociation constant for binding of compound 155 (Sh74) to A2T2 DNA sequence

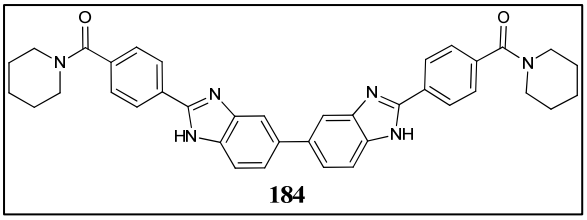


Binding curve for the interaction between compound 155 (Sh74) and A2T2 DNA sequence

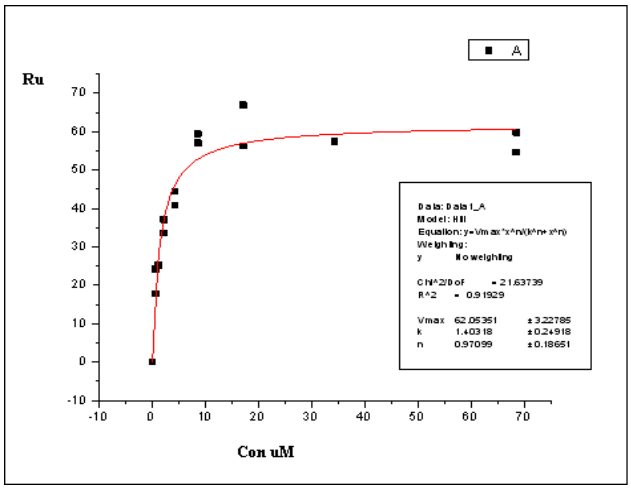
	Req	SE(Req)	Conc
SH74_ Fc=2-1 - 1	178	0.107	188u
SH74_1 Fc=2-1 - 1	158	0.107	188u
SH74_1 Fc=2-1 - 2	122	0.107	94u
SH74_1 Fc=2-1 - 3	121	0.107	94u
SH74_1 Fc=2-1 - 4	59.7	0.107	47u
SH74_1 Fc=2-1 - 5	66.7	0.107	47u
SH74_1 Fc=2-1 - 6	64	0.107	23.5u
SH74_1 Fc=2-1 - 7	65.4	0.107	23.5u
SH74_1 Fc=2-1 - 8	36.8	0.107	11.75u
SH74_1 Fc=2-1 - 9	38.4	0.107	11.75u
SH74_1 Fc=2-1 - 10	26.1	0.107	5.875u
SH74_1 Fc=2-1 - 11	24.1	0.107	5.875u
SH74_1 Fc=2-1 - 12	11.2	0.107	2.93u
SH74_1 Fc=2-1 - 13	7.96	0.107	2.93u
SH74_1 Fc=2-1 - 14	3.21	0.107	1.4687u
SH74_1 Fc=2-1 - 15	3.68E-04	0.0151	1.4687u
SH74_1 Fc=2-1 - 16	5.36	0.107	0.7343u
SH74_1 Fc=2-1 - 17	4.16	0.107	0.7343u
SH74_1 Fc=2-1 - 18	0	0.107	0

The RU (Response unit)values at the concentration range of compound 155 (Sh74) , 0-188 μM

I.5 The binding data of compound 184 to A2T2 DNA sequence (CGAATTCGTCTCCGAATTCG)



184

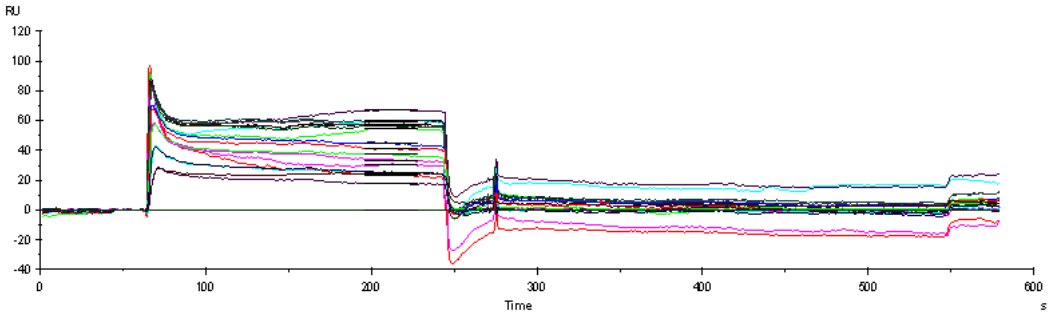


The Dissociation constant of compound 184 (Sh83) was evaluated by fitting the data with BIA evaluation program Origin 7

Chi^2/DoF	R^2
21.63739	0.91929

Parameter	Value	Error
V_{max}	62.0535	3.22785
k	1.40318	0.24918
n	0.97099	0.18651

Dissociation constant for binding of compound 184 (Sh83) to A2T2 DNA sequence



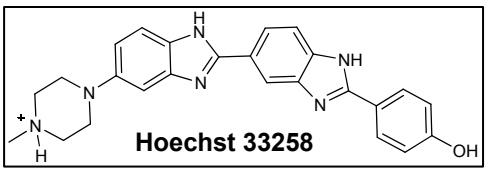
Binding curve for the interaction between compound 184 (Sh83) and A2T2 DNA sequence

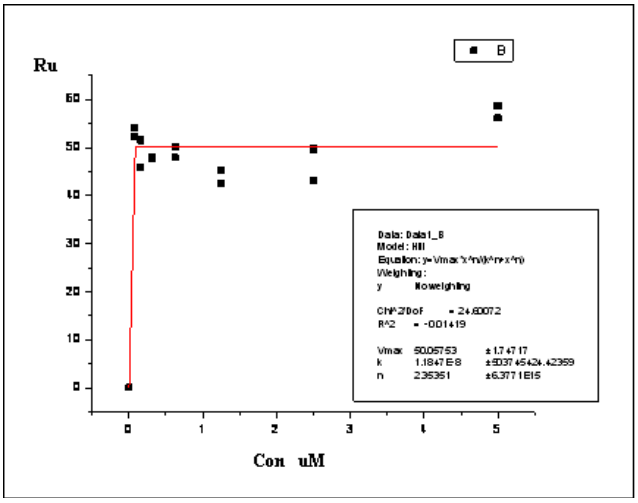
	Req	SE(Req)	Conc
SH83_1 Fc=2-1 - 20	23.6	0.0823	137
SH83_1 Fc=2-1 - 21	30.3	0.0823	137
SH83_1 Fc=2-1 - 22	54.7	0.0823	68.5
SH83_1 Fc=2-1 - 23	59.8	0.0823	68.5
SH83_1 Fc=2-1 - 24	57.5	0.0823	34.3
SH83_1 Fc=2-1 - 25	57.4	0.0823	34.3
SH83_1 Fc=2-1 - 26	67	0.0823	17.1
SH83_1 Fc=2-1 - 27	56.3	0.0823	17.1
SH83_1 Fc=2-1 - 28	59.5	0.0823	8.56
SH83_1 Fc=2-1 - 29	57.1	0.0823	8.56
SH83_1 Fc=2-1 - 30	44.5	0.0823	4.28
SH83_1 Fc=2-1 - 31	40.9	0.0823	4.28
SH83_1 Fc=2-1 - 32	33.6	0.0823	2.14
SH83_1 Fc=2-1 - 33	37.1	0.0823	2.14
SH83_1 Fc=2-1 - 34	25.3	0.0823	1.07
SH83_1 Fc=2-1 - 35	25.2	0.0823	1.07
SH83_1 Fc=2-1 - 36	24.2	0.0823	0.535
SH83_1 Fc=2-1 - 37	17.8	0.0823	0.535
SH83_1 Fc=2-1 - 38	0	0.0823	0

The RU (Response unit)values at the concentration range of compound 184 (Sh83), 0-137 uM

Appendix II :SPR binding data for A3T3

II.1 The binding data of Hoechst 33258 to A3T3 DNA sequence (CGAAATTTCGTCTCCGAAATTTCG)



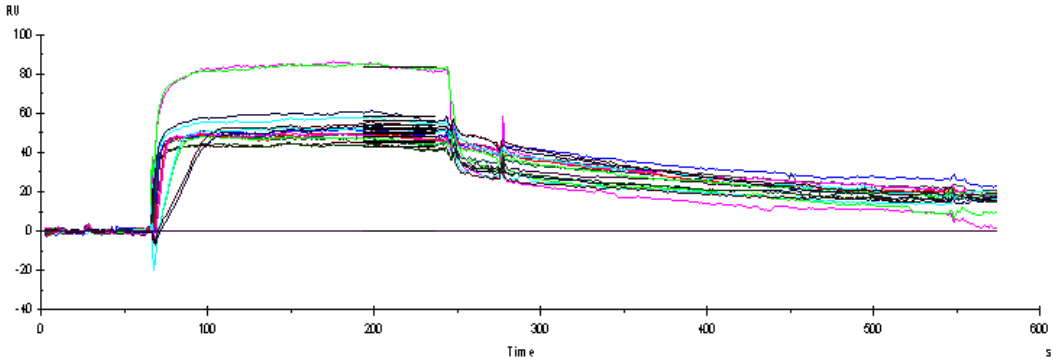


The Dissociation constant of Hoechst 33258 was evaluated by fitting the data with BIA evaluation 4.1 program

Chi^2/DoF	R^2
24.60072	-0.01419

Parameter	Value	Error
V_{max}	50.05753	1.74717
k	1.1847E-08	503745424
n	2.35351	6.38E+15

Dissociation constant for binding of Hoechst 33258 to A3T3 DNA sequence

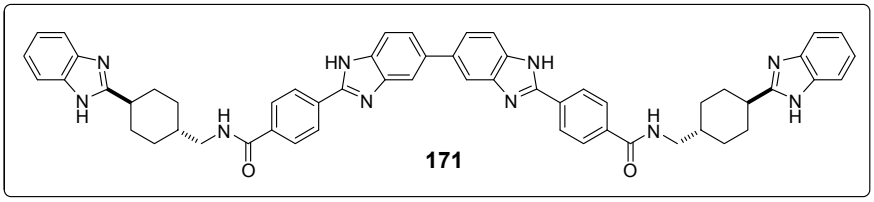


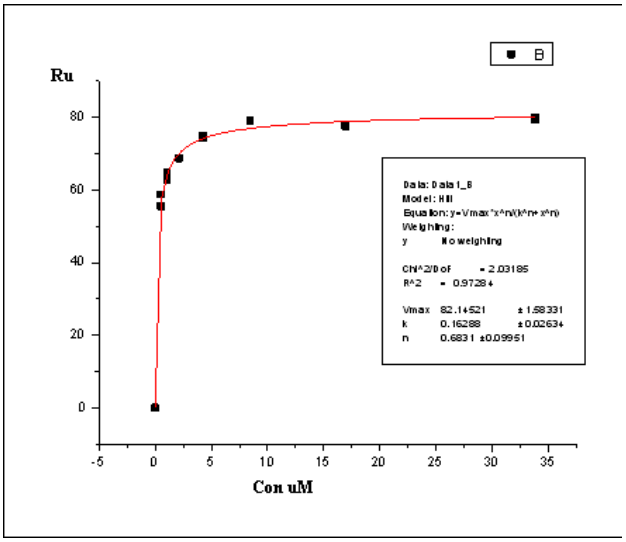
Binding curve for the interaction between Hoechst 33258 and A3T3 DNA sequence

	Req	SE(Req)	Conc
hoechst_1 Fc=3-1 - 42	83.3	0.172	10u
hoechst_1 Fc=3-1 - 43	83.7	0.172	10u
hoechst_1 Fc=3-1 - 44	56.2	0.172	5u
hoechst_1 Fc=3-1 - 45	58.7	0.172	5u
hoechst_1 Fc=3-1 - 46	43.1	0.172	2.5u
hoechst_1 Fc=3-1 - 47	49.7	0.172	2.5u
hoechst_1 Fc=3-1 - 48	42.6	0.172	1.25u
hoechst_1 Fc=3-1 - 49	45.3	0.172	1.25u
hoechst_1 Fc=3-1 - 50	47.9	0.172	0.625u
hoechst_1 Fc=3-1 - 51	50.1	0.172	0.625u
hoechst_1 Fc=3-1 - 52	48	0.172	0.3125u
hoechst_1 Fc=3-1 - 53	47.7	0.172	0.3125u
hoechst_1 Fc=3-1 - 54	45.9	0.172	0.156u
hoechst_1 Fc=3-1 - 55	51.5	0.172	0.156u
hoechst_1 Fc=3-1 - 56	52.3	0.172	0.07817u
hoechst_1 Fc=3-1 - 57	54.2	0.172	0.07817u
hoechst_1 Fc=3-1 - 58	0	0.172	0

The RU (Response unit) values at the concentration range of compound Hoechst 33258, 0-10 μM

II.2 The binding data of compound 171 to A3T3 DNA sequence (CGAAATTTCGTCTCCGAAATTTCG)



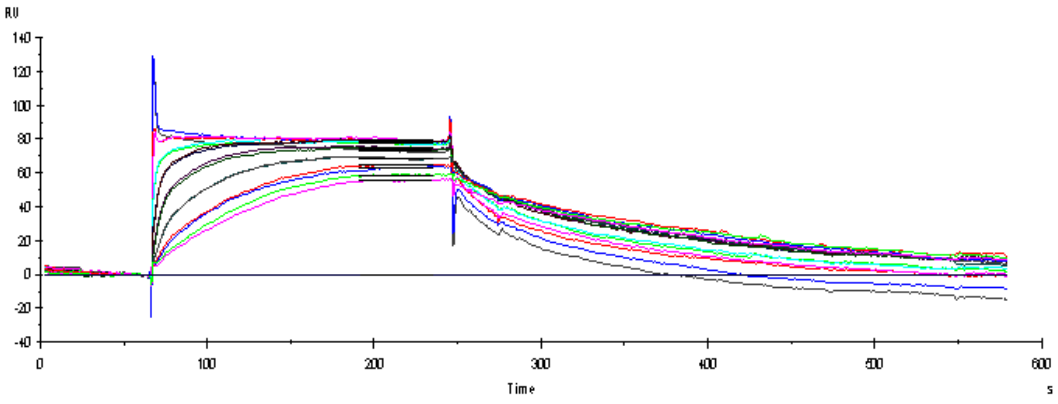


The Dissociation constant of compound 171 (Sh61) was evaluated by fitting the data with BIA evaluation Origin 7 program

Chi^2/DoF	R^2
2.03185	0.97284

Parameter	Value	Error
V_{max}	82.14521	1.58331
k	0.16288	0.02634
n	0.6831	0.09951

Dissociation constant for binding of compound 171 (Sh61) toA3T3 DNA sequence

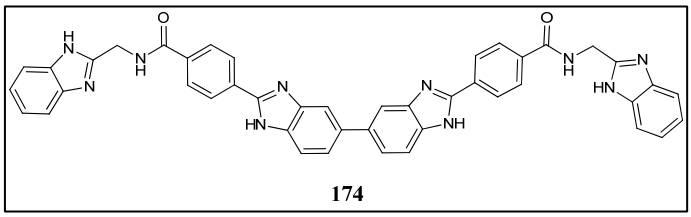


Binding curve for the interaction between compound 171 (Sh61) and A3T3 DNA sequence

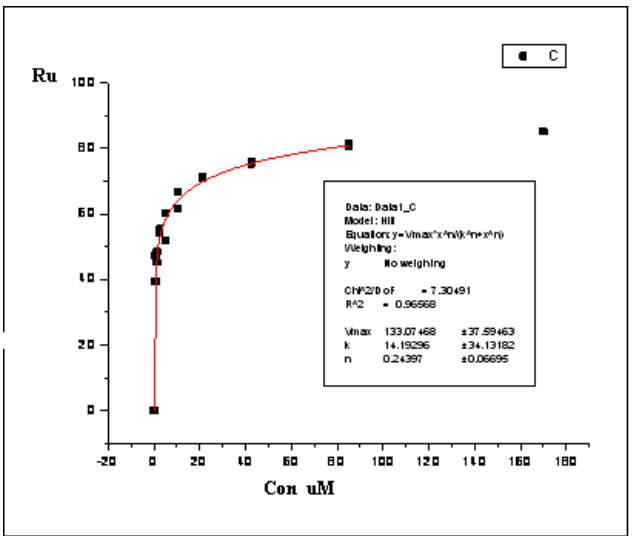
	Req	SE(Req)	Conc
Sh61_s Fc=3-1 - 43	73.1	0.0782	67.5
Sh61_s Fc=3-1 - 44	78.6	0.0782	67.5
Sh61_s Fc=3-1 - 45	79.3	0.0782	33.8
Sh61_s Fc=3-1 - 46	80	0.0782	33.8
Sh61_s Fc=3-1 - 47	77.5	0.0782	16.9
Sh61_s Fc=3-1 - 48	77.9	0.0782	16.9
Sh61_s Fc=3-1 - 49	79.2	0.0782	8.44
Sh61_s Fc=3-1 - 50	79.1	0.0782	8.44
Sh61_s Fc=3-1 - 51	74.9	0.0782	4.22
Sh61_s Fc=3-1 - 52	74.3	0.0782	4.22
Sh61_s Fc=3-1 - 53	68.7	0.0782	2.11
Sh61_s Fc=3-1 - 54	68.8	0.0782	2.11
Sh61_s Fc=3-1 - 55	62.9	0.0782	1.05
Sh61_s Fc=3-1 - 56	64.8	0.0782	1.05
Sh61_s Fc=3-1 - 57	55.5	0.0782	0.527
Sh61_s Fc=3-1 - 58	58.8	0.0782	0.527
Sh61_s Fc=3-1 - 60	0	0.0782	0

The RU (Response unit) values at the concentration range of compound 171 (Sh61), 0-67.5 μM

II.3 The binding data of compound 174 to A3T3 DNA sequence (CGAAATTTCGTCTCCGAAATTTCG)



174

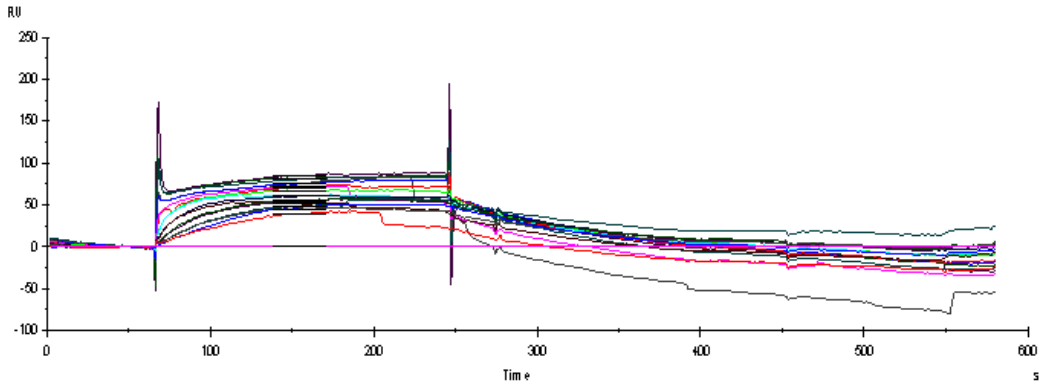


Chi^2/DoF	R^2
7.30491	0.96568

Parameter	Value	Error
Vmax	133.07468	37.59463
k	14.19296	34.13182
n	0.24397	0.06695

The Dissociation constant of compound 174 (Sh72) was evaluated by fitting the data with BIA evaluation Origin 7program

Dissociation constant for binding of compound 174 (Sh72) to A3T3 DNA sequence

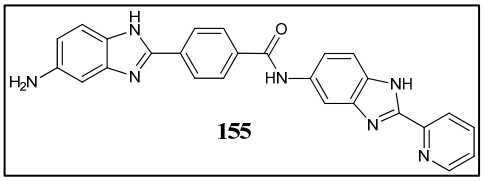


Binding curve for the interaction between compound 174 (Sh72) and A3T3 DNA sequence

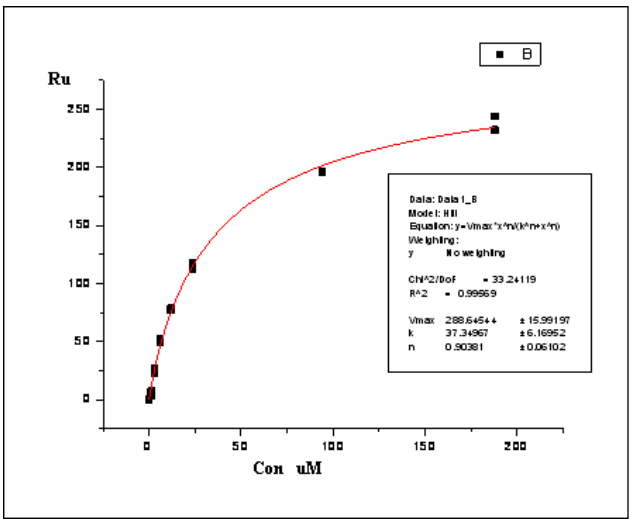
	Req	SE(Req)	Conc
Sh72_s Fc=3-1 - 62	85.1	0.172	170
Sh72_s Fc=3-1 - 63	81.6	0.172	85
Sh72_s Fc=3-1 - 64	80.6	0.172	85
Sh72_s Fc=3-1 - 65	75.9	0.172	42.5
Sh72_s Fc=3-1 - 66	75.3	0.172	42.5
Sh72_s Fc=3-1 - 67	70.8	0.172	21.3
Sh72_s Fc=3-1 - 68	71.2	0.172	21.3
Sh72_s Fc=3-1 - 69	66.7	0.172	10.6
Sh72_s Fc=3-1 - 70	61.5	0.172	10.6
Sh72_s Fc=3-1 - 71	60.3	0.172	5.31
Sh72_s Fc=3-1 - 72	52	0.172	5.31
Sh72_s Fc=3-1 - 73	54.1	0.172	2.65
Sh72_s Fc=3-1 - 74	55.4	0.172	2.65
Sh72_s Fc=3-1 - 75	48.4	0.172	1.32
Sh72_s Fc=3-1 - 76	45.3	0.172	1.32
Sh72_s Fc=3-1 - 77	47.4	0.172	0.664
Sh72_s Fc=3-1 - 78	39.5	0.172	0.664
Sh72_s Fc=3-1 - 79	0	0.172	0

The RU (Response unit) values at the concentration range of compound 174 (Sh72), 0-170 μM

II.4 The binding data of compound 155 to A3T3 DNA sequence (CGAAATTTCGTCTCCGAAATTTCG)



155

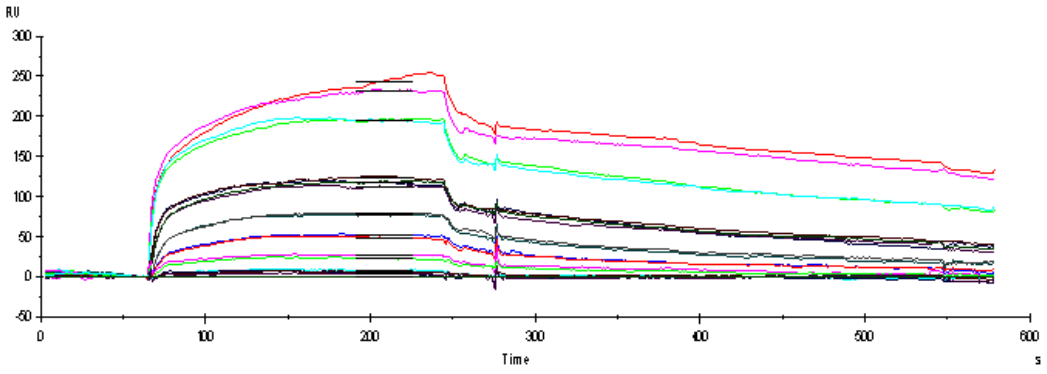


The Dissociation constant of compound 155 (Sh74) was evaluated by fitting the data with BIA evaluation Origin 7 program

Chi^2/DoF	R^2
33.24119	0.99569

Parameter	Value	Error
Vmax	288.64544	15.99197
k	37.34967	6.16952
n	0.90381	0.06102

Dissociation constant for binding of compound 155 (Sh74) to A3T3 DNA sequence

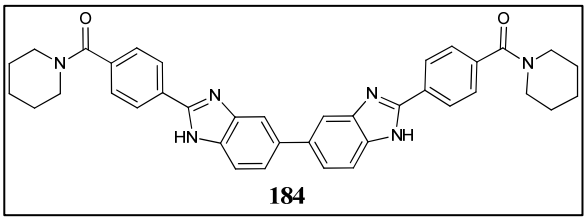


Binding curve for the interaction between compound 155 (Sh74) and A3T3 DNA sequence

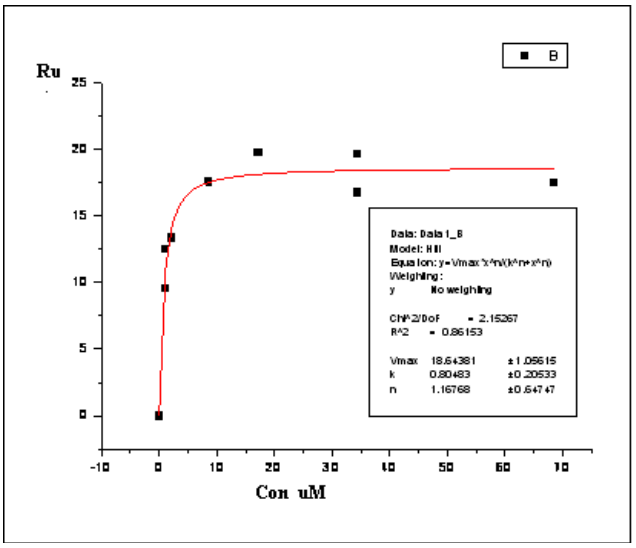
	Req	SE(Req)	Conc
SH74_ Fc=3-1 - 1	244	0.229	188u
SH74_1 Fc=3-1 - 1	232	0.229	188u
SH74_1 Fc=3-1 - 2	196	0.229	94u
SH74_1 Fc=3-1 - 3	195	0.229	94u
SH74_1 Fc=3-1 - 4	117	0.229	47u
SH74_1 Fc=3-1 - 5	125	0.229	47u
SH74_1 Fc=3-1 - 6	112	0.229	23.5u
SH74_1 Fc=3-1 - 7	118	0.229	23.5u
SH74_1 Fc=3-1 - 8	77	0.229	11.75u
SH74_1 Fc=3-1 - 9	78.7	0.229	11.75u
SH74_1 Fc=3-1 - 10	52.4	0.229	5.875u
SH74_1 Fc=3-1 - 11	48.8	0.229	5.875u
SH74_1 Fc=3-1 - 12	26.8	0.229	2.93u
SH74_1 Fc=3-1 - 13	22.6	0.229	2.93u
SH74_1 Fc=3-1 - 14	7.76	0.229	1.4687u
SH74_1 Fc=3-1 - 15	3.47	0.229	1.4687u
SH74_1 Fc=3-1 - 16	5.86	0.229	0.7343u
SH74_1 Fc=3-1 - 17	4.13	0.229	0.7343u
SH74_1 Fc=3-1 - 18	0	0.229	0.0u

The RU (Response unit) values at the concentration range of compound 155 (Sh74), 0-188 μM

II.5 The binding data of compound 184 to A3T3 DNA sequence (CGAAATTTCGTCTCCGAAATTTCG)



184

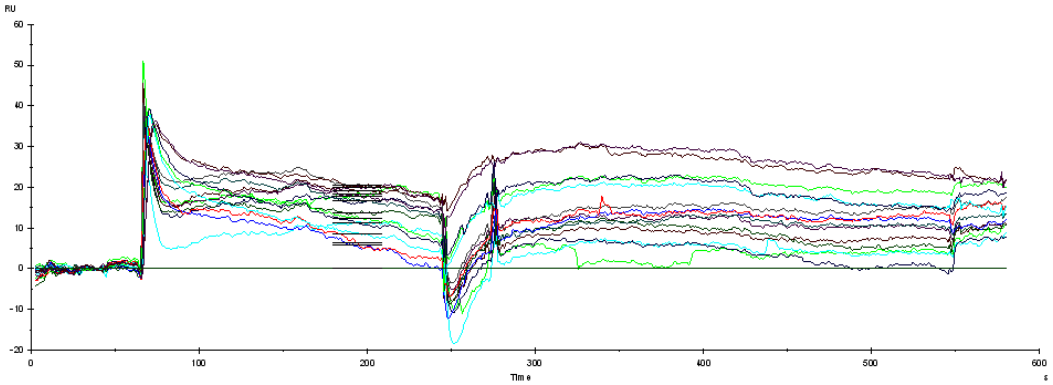


Chi^2/DoF	R^2
2.15267	0.86153

Parameter	Value	Error
Vmax	18.64381	1.05615
k	0.80483	0.20533
n	1.16768	0.64747

The Dissociation constant of compound 184 (Sh83) was evaluated by fitting the data with BIA evaluation Origin 7 program

Dissociation constant for binding of compound 184 (Sh83) to A3T3 DNA sequence



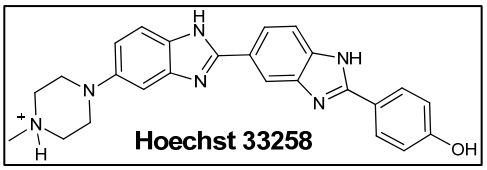
Binding curve for the interaction between compound 184 (Sh83) and A3T3 DNA sequence

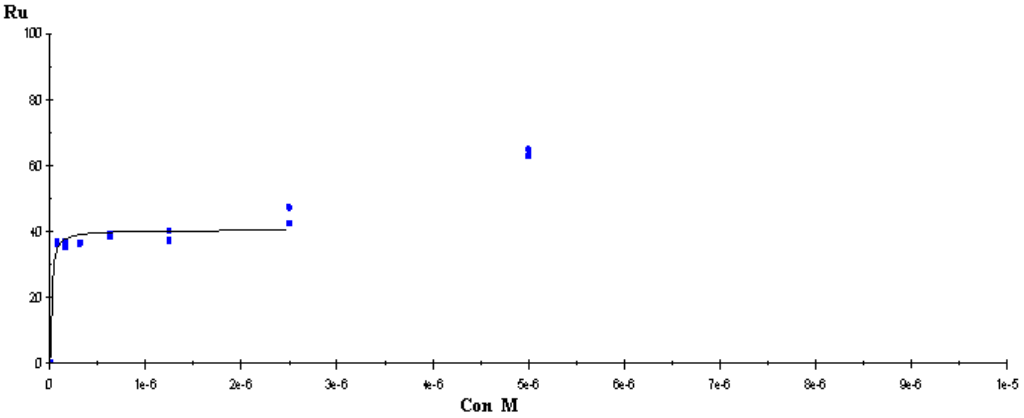
	Req	SE(Req)	Conc
SH83_1 Fc=3-1 - 22	18.2	0.121	68.5
SH83_1 Fc=3-1 - 23	11.1	0.121	68.5
SH83_1 Fc=3-1 - 24	16.6	0.121	34.3
SH83_1 Fc=3-1 - 25	19.2	0.121	34.3
SH83_1 Fc=3-1 - 26	19.9	0.121	17.1
SH83_1 Fc=3-1 - 27	13.8	0.121	17.1
SH83_1 Fc=3-1 - 28	16.8	0.121	8.56
SH83_1 Fc=3-1 - 29	20.5	0.121	8.56
SH83_1 Fc=3-1 - 30	5.85	0.121	4.28
SH83_1 Fc=3-1 - 31	6.48	0.121	4.28
SH83_1 Fc=3-1 - 33	12.5	0.121	2.14
SH83_1 Fc=3-1 - 34	8.65	0.121	1.07
SH83_1 Fc=3-1 - 35	11.3	0.121	1.07
SH83_1 Fc=3-1 - 36	20.6	0.121	0.535
SH83_1 Fc=3-1 - 37	17.9	1.21E-01	0.535
SH83_1 Fc=3-1 - 38	0	0.121	0

The RU (Response unit) values at the concentration range of compound 184 (Sh83), 0-68.5μM

Appendix III :SPR binding data for A4T4

III.1The binding data of Hoechst 33258 to A4T4 DNA sequence (CGAAAATTTTCGTCTCCGAAAATTTTCG)

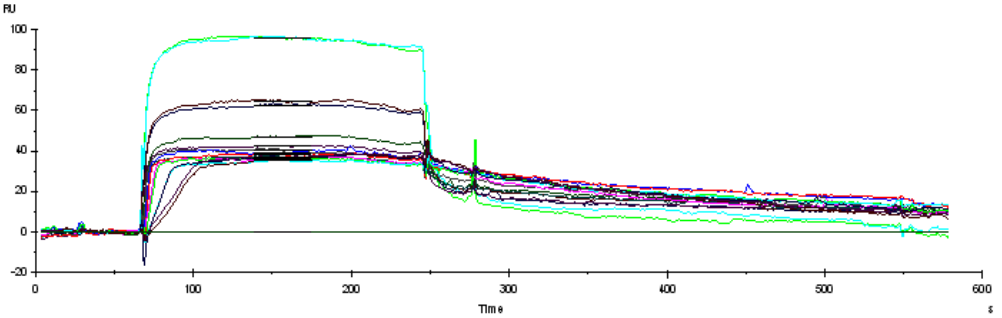




The Dissociation constant of Hoechst 33258 was evaluated by fitting the data with BIA evaluation 4.1 program

	KA(1/M)	KD(M)	R_{max}	n
	8.09E+07	1.24E-08	40.5	
Req vs. Conc				1

Dissociation constant for binding of Hoechst 33258 to A4T4 DNA sequence

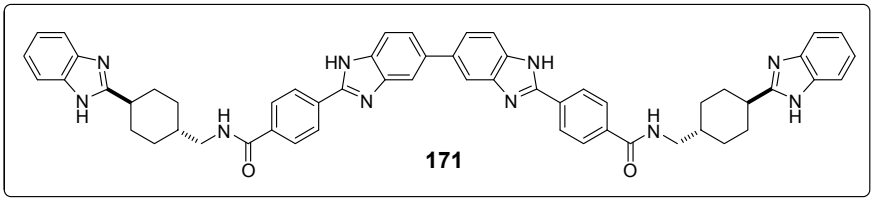


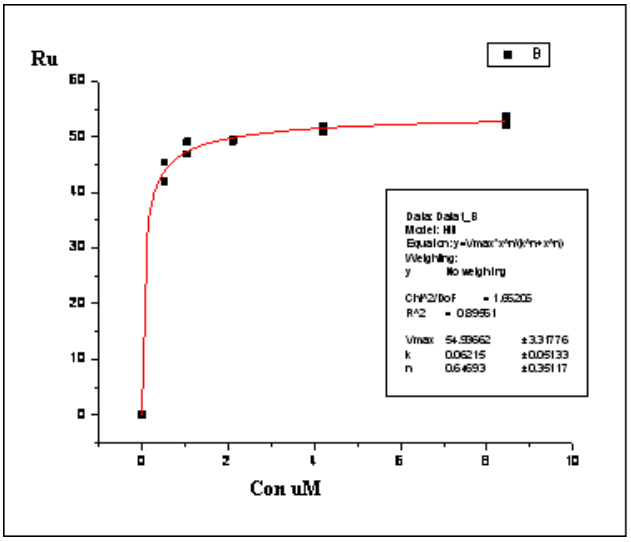
Binding curve for the interaction between Hoechst 33258 and A4T4 DNA sequence

	Req	SE(Req)	Conc
hoechst_1 Fc=4-1 - 42	96.1	0.0689	10u
hoechst_1 Fc=4-1 - 43	96.2	0.0689	10u
hoechst_1 Fc=4-1 - 44	62.9	0.0689	5u
hoechst_1 Fc=4-1 - 45	64.8	0.0689	5u
hoechst_1 Fc=4-1 - 46	42.5	0.0689	2.5u
hoechst_1 Fc=4-1 - 47	47.2	0.0689	2.5u
hoechst_1 Fc=4-1 - 48	37.1	0.0689	1.25u
hoechst_1 Fc=4-1 - 49	40.2	0.0689	1.25u
hoechst_1 Fc=4-1 - 50	39.2	0.0689	0.625u
hoechst_1 Fc=4-1 - 51	38.4	0.0689	0.625u
hoechst_1 Fc=4-1 - 52	36.6	0.0689	0.3125u
hoechst_1 Fc=4-1 - 53	36.2	0.0689	0.3125u
hoechst_1 Fc=4-1 - 54	35.2	0.0689	0.156u
hoechst_1 Fc=4-1 - 55	37	0.0689	0.156u
hoechst_1 Fc=4-1 - 56	36.2	0.0689	0.07817u
hoechst_1 Fc=4-1 - 57	37	0.0689	0.07817u
hoechst_1 Fc=4-1 - 58	0	0.0689	0u

The RU (Response unit) values at the concentration range of compound Hoechst 33258 , 0-10 μM

III.2 The binding data of compound 171 to A4T4 DNA sequence (CGAAAATTTTCGTCTCCGAAAATTTTCG)



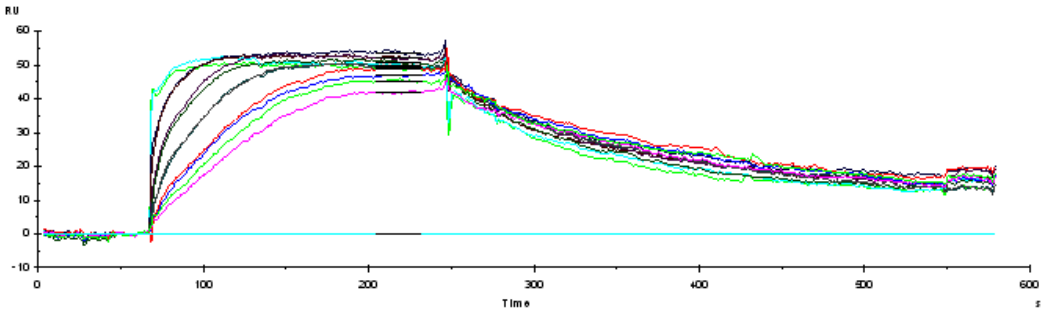


Chi^2/DoF	R^2
1.66206	0.89561

Parameter	Value	Error
V_{max}	54.93662	3.31776
k	0.06215	0.05133
n	0.64693	0.35117

The Dissociation constant of compound 171 (Sh61) was evaluated by fitting the data with BIA evaluation Origin 7 program

Dissociation constant for binding of compound 171 (Sh61) toA4T4 DNA sequence

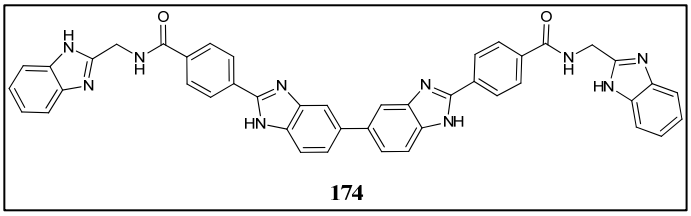


Binding curve for the interaction between compound 171 (Sh61) and A4T4 DNA sequence

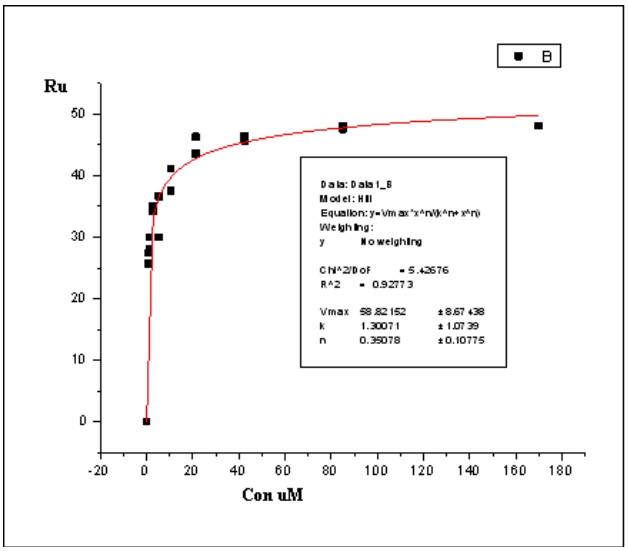
	Req	SE(Req)	Conc
Sh61_s Fc=4-1 - 47	49	0.0579	16.9
Sh61_s Fc=4-1 - 48	50	0.0579	16.9
Sh61_s Fc=4-1 - 49	53.7	0.0579	8.44
Sh61_s Fc=4-1 - 50	52.2	0.0579	8.44
Sh61_s Fc=4-1 - 51	52	0.0579	4.22
Sh61_s Fc=4-1 - 52	50.9	0.0579	4.22
Sh61_s Fc=4-1 - 53	49.4	0.0579	2.11
Sh61_s Fc=4-1 - 54	49.2	0.0579	2.11
Sh61_s Fc=4-1 - 55	46.9	0.0579	1.05
Sh61_s Fc=4-1 - 56	49.1	0.0579	1.05
Sh61_s Fc=4-1 - 57	42	0.0579	0.527
Sh61_s Fc=4-1 - 58	45.4	0.0579	0.527
Sh61_s Fc=4-1 - 59	0	0.0579	0

The RU (Response unit) values at the concentration range of compound 171 (Sh61), 0-16.9 μM

III.3 The binding data of compound 174 to A4T4 DNA sequence (CGAAAATTTTCGTCTCCGAAAATTTTCG)



174

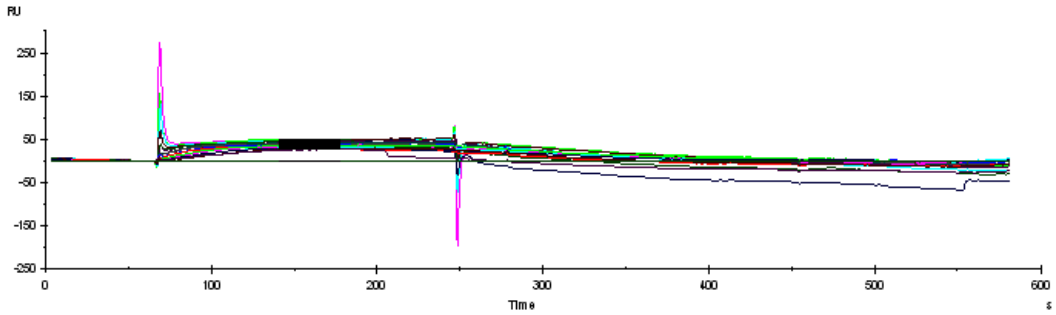


The Dissociation constant of compound 174 (Sh72) was evaluated by fitting the data with BIA evaluation Origin 7 program

Chi^2/DoF	R^2
5.42676	0.92773

Parameter	Value	Error
V_{max}	58.82152	8.67438
k	1.30071	1.0739
n	0.35078	0.10775

Dissociation constant for binding of compound 174 (Sh72) to A4T4 DNA sequence

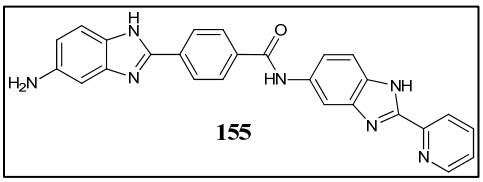


Binding curve for the interaction between compound 174 (Sh72) and A4T4 DNA sequence

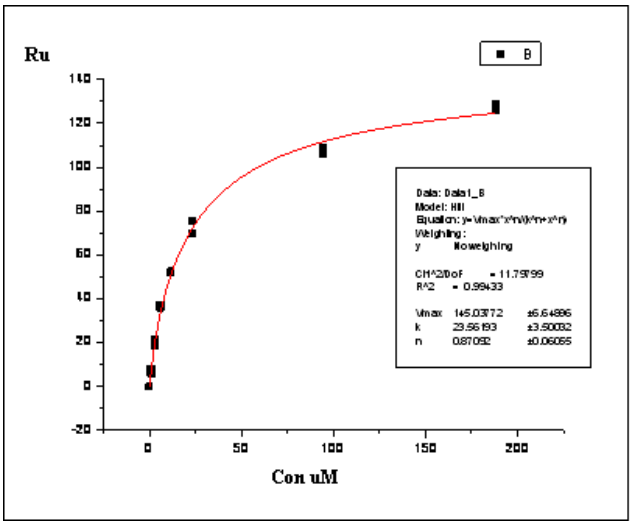
	Req	SE(Req)	Conc
Sh72_s Fc=4-1 - 62	48.1	0.12	170
Sh72_s Fc=4-1 - 63	48.1	0.12	85
Sh72_s Fc=4-1 - 64	47.6	0.12	85
Sh72_s Fc=4-1 - 65	45.7	0.12	42.5
Sh72_s Fc=4-1 - 66	46.4	0.12	42.5
Sh72_s Fc=4-1 - 67	43.6	0.12	21.3
Sh72_s Fc=4-1 - 68	46.3	0.12	21.3
Sh72_s Fc=4-1 - 69	41.1	0.12	10.6
Sh72_s Fc=4-1 - 70	37.6	0.12	10.6
Sh72_s Fc=4-1 - 71	36.6	0.12	5.31
Sh72_s Fc=4-1 - 72	30	0.12	5.31
Sh72_s Fc=4-1 - 73	34.2	0.12	2.65
Sh72_s Fc=4-1 - 74	35.1	0.12	2.65
Sh72_s Fc=4-1 - 75	30	0.12	1.32
Sh72_s Fc=4-1 - 76	28.1	0.12	1.32
Sh72_s Fc=4-1 - 77	27.4	0.12	0.664
Sh72_s Fc=4-1 - 78	25.6	0.12	0.664
Sh72_s Fc=4-1 - 79	0	0.12	0

The RU (Response unit) values at the concentration range of compound 174 (Sh72) , 0-170 μM

III.4 The binding data of compound 155 to A4T4 DNA sequence (CGAAAATTTTCGTCTCCGAAAATTTTCG)



155

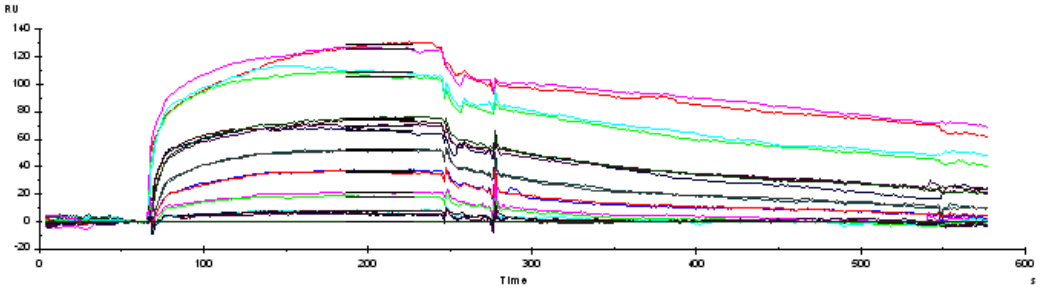


Chi^2/DoF	R^2
11.79799	0.99433

Parameter	Value	Error
V_{max}	145.03772	6.64896
k	23.56193	3.50032
n	0.87092	0.06055

The Dissociation constant of compound 155 (Sh74) was evaluated by fitting the data with BIA evaluation Origin 7 program

Dissociation constant for binding of compound 155 (Sh74) to A4T4 DNA sequence

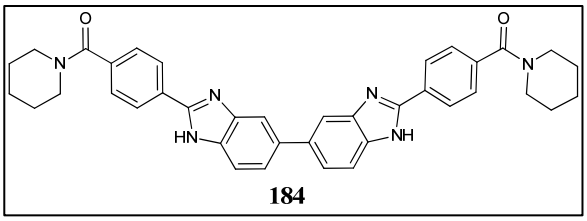


Binding curve for the interaction between compound 155 (Sh74) and A4T4 DNA sequence

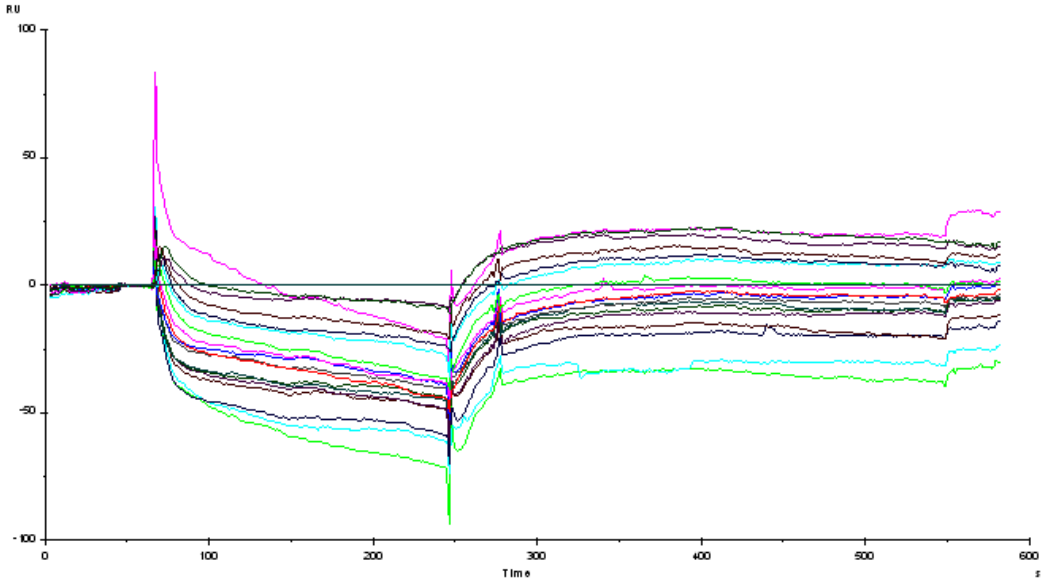
	Req	SE(Req)	Conc
SH74_ Fc=4-1 - 1	129	0.104	187
SH74_1 Fc=4-1 - 1	126	0.104	187
SH74_1 Fc=4-1 - 2	106	0.104	94
SH74_1 Fc=4-1 - 3	109	0.104	94
SH74_1 Fc=4-1 - 4	66.2	0.104	47
SH74_1 Fc=4-1 - 5	74.4	0.104	47
SH74_1 Fc=4-1 - 6	69.8	0.104	23.5
SH74_1 Fc=4-1 - 7	75.6	0.104	23.5
SH74_1 Fc=4-1 - 8	52.6	0.104	11.8
SH74_1 Fc=4-1 - 9	51.7	0.104	11.8
SH74_1 Fc=4-1 - 10	37.3	0.104	5.88
SH74_1 Fc=4-1 - 11	35.9	0.104	5.88
SH74_1 Fc=4-1 - 12	21.2	0.104	2.93
SH74_1 Fc=4-1 - 13	18.3	0.104	2.93
SH74_1 Fc=4-1 - 14	8	0.104	1.47
SH74_1 Fc=4-1 - 15	5.31	0.104	1.47
SH74_1 Fc=4-1 - 16	7.83	0.104	0.734
SH74_1 Fc=4-1 - 17	5.87	0.104	0.734
SH74_1 Fc=4-1 - 18	0	0.104	0

The RU (Response unit) values at the concentration range of compound 155 (Sh74), 0-187 μM

III.5 The binding data of compound 184 to A4T4 DNA sequence (CGAAAATTTTCGTCTCCGAAAATTTTCG)



184



No binding of compound 184 (Sh83) to the A4T4 DNA sequence