CERTIFICATE OF ORIGINALITY

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Synthesis of Fluorinated Heterocyclic Compounds and Study of their Interaction with DNA

By

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Dedication

This research work is dedicated to my Father, Mother, and my children Matin, Mahta, and to all my family and friends.

Acknowledgement

I would like to express deepest gratitude to my supervisors Dr. George Weaver, Dr. Paul Lucas and Dr. Mark Elsegood for their full support, expert guidance, encouragement, enthusiastic supervision, and invaluable advice throughout the duration of the research project and allowing me to grow as a research scientist. Without their incredible patience and timely wisdom and counsel, my thesis work would have been a frustrating and overwhelming pursuit.

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Abstract

Over fifty, structurally diverse, novel fluorinated heteroarenes, have been successfully synthesised by S_NAr reaction of a range of fluorinated arenes including pentafluoropyridine, hexafluorobenzene, and methyl pentafluorobenzoate by introduction of a range of groups such as imidazole, triazole, benzimidazole, benzotriazole, and carbazole. Different water solubilising side chains were introduced to some of the successfully synthesised fluorinated heteroarenes to improve water solubility and potential biological activity. X-ray crystal structures of over 10 compounds were obtained including those of two macrocyclic compounds containing 21- and 24-membered rings. The synthesised compounds have been characterized by elemental analysis, IR, ^{1}H and ^{19}F spectroscopy and high resolution mass spectrometry. These compounds have been screened for their biological activities and possible interaction with DNA by methods including UV-visible spectroscopy, fluorescence spectroscopy, co-crystallization for X-ray diffraction analysis, and antimicrobial activity. A number of the fluoroaryl benzimidazole derivatives have been tested against K-562 and MCF-7 cell lines and G361 and HOS cell lines. From the all tested compounds three tethered fluoroaryl benzimidazole derivatives demonstrated micromolar inhibition against K-562 and MCF-7 cell lines. These compounds, in addition to 1-tetrafluoropyrid-4-yl-2-tetrafluoropyrid-4-ylsulfanyl-1H-benzimidazole, also demonstrated micromolar inhibition against G361 and HOS cell lines. Two of the compounds were found to activate caspases leading to apoptosis.

Abbreviations

- **ACTD** Actinomycin D
- **A** Adenine
- **AFM** Atomic-force microscopy
- **ChI** Chlorambucil
- **C** Cytosine
- **COX-2** Cyclooxygenase-2
- **CDKs** Cyclin-dependent kinases
- **$Cu(OTf)_2$** Copper(II) triflate
- **CT-DNA** Calf thymus-DNA
- **DNA** Deoxyribonucleic acid
- **DMF** Dimethylformamide
- **DCM** Dichloromethane
- **DMSO** Dimethylsulfoxide
- **Et_3N** Triethylamine
- **EB** Ethidium boromide
- **Eq.** Equation
- **EGFR** Epidermal growth factor receptor
- **EN** Electronegativity
- **ESI-MS** Electrospray ionisation mass spectrometry
- **ERK** Extracellular Regulated Kinase
- **GC-MS** Gas chromatography mass spectrometry
- **FT-IR** Fourier transform infrared
- **G** Guanine
- **ΔG** Gibbs free energy
- **g** Grams
- **h** Hours
- **5HTID** 5-hydroxytryptamine receptor 1D
- **HOS** Human Osteosarcoma cell
- **IR** Infra-red
- **IC50** Half maximal inhibitory concentration
- **K** Binding constant

- **K_{sv}** Stern-Volmer quenching constant
- **mL** Millilitres
- **m.p.** Melting point
- **MCF-7** Michigan cancer foundation-7
- **MAPK** Mitogen-activated protein kinase
- **NMR** Nuclear magnetic mesonance
- **NaH** Sodium hydride
- **$NaHCO_3$** Sodium bicarbonate
- **ppm** Parts per million
- **PPA** Polyphosphoric acid
- **PTKs** Protein tyrosine kinases
- **RNA** Ribonucleic acid
- **RT** Room temperature
- **S_NAr** Aromatic nucleophilic substitution
- **SS-DNA** Salmon sperm-DNA
- **T** Thymine
- **TS** Thymidylate synthase
- **tRNA** Transfer ribonucleic acid
- **TKI** Tyrosine-kinase inhibitor
- **THF** Tetrahydrofuran
- **TLC** Thin layer chromatography
- **UV** Ultraviolet

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1. Introduction

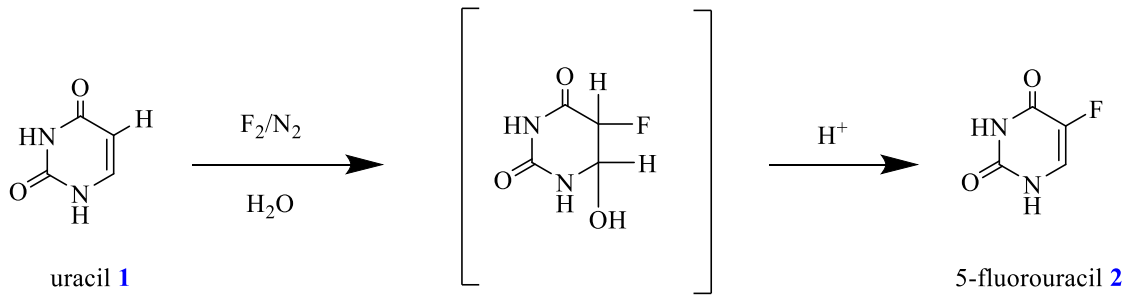
Heterocyclic compounds are the largest of the traditional divisions of organic chemistry and are of huge importance biologically and industrially. For more than one century heterocyclic chemistry has been the largest area for research in organic chemistry. Heterocycles have been used in the development of biologically active compounds, and also in the understanding of living processes, and in improving the quality of life. Large numbers of drugs such as morphine, atropine, procaine, codeine, and reserpine are heterocycles.[1,2] Also many synthetic drugs such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine are heterocycles due to their useful solubility profile and their rigid structures which can bind to a broad range of receptors for valuable biological activity.[3,4] Therefore heterocyclic chemistry is very important in the pharmaceutical industry for the synthesis of new drugs including antibacterial, antifungal, antimycobacterial, anti-inflammatory and especially anti-cancer agents because cancer is a leading cause of premature deaths in the world. In cancer, abnormal cells divide rapidly without control and are able to invade and destroy healthy tissues.[1,5] These uncontrolled, unregulated cells which divide rapidly and grow abnormally produce malignant tumours which affect and invade nearby parts of the body leading to human death. It has been estimated that in the United States over 1 million cases of cancer are reported per year and it is expected that by the year 2017 cancer-related deaths will reach 12 million world-wide.[1] Therefore in recent years, a wide range of research has been carried out in the field of anti-cancer drug development.[1] On the other, hand fluorine use in medicinal chemistry has exploded over recent decades and significant improvement in pharmaceutical and biological activity of drugs containing fluorine has been shown. Therefore fluorination of a heterocyclic compound or combination of heterocycles with fluorinated arenes is a major concern in drug design and discovery, and is the main focus of this thesis.

1.1. Fluorine in medicinal chemistry

Fluorinated compounds have shown important improvement of biological activity of pharmaceutical compounds and drugs in medicinal chemistry. The fundamental properties of fluorine impart significant improvement on the biological activity of the fluorinated molecules. Therefore many fluorinated analogues of natural compounds have been synthesised and investigated due to the increase in understanding of the impact of fluorination on the biological properties of a molecule. This has supported the design and synthesis of more active and selective pharmaceutical agents

during the last 15 years, and development of synthetic methodologies in organic fluorine chemistry. There are more than 150 fluorinated compounds among those marketed pharmaceutical drugs in the world which is a huge number compared with other halogen-containing pharmaceuticals. Organo-chlorine and -bromine compounds are far more abundant as natural compounds. [6]

As a good example of the effect of a fluorine atom modifying the activity of the parent compound is introduction of fluorine into quinolone derivatives which led to the first fluoroquinolone, (norfloxacin) that is a broad-spectrum antibiotic. Replacing a CH bond by a CF bond improves metabolic stability. Also replacement of hydrogen with fluorine in uracil **1** gives 5-fluorouracil **2** which shows potent antitumor activity (Scheme 1).[6,7] The fluorinated version of the molecule binds irreversibly to the active site of thymidylate synthetase preventing thymine biosynthesis in tumour cells.



Scheme 1: Commercial synthesis of 5-fluorouracil.

1.1.1. Physical characteristics of fluorine

Among all elements fluorine has the highest electronegativity value (EN) of 4 (on the Pauling scale) with very low polarizability and very low bond dissociation energy (155 kJ/mol).[8]

The low polarizability effect of fluorine and the three non-bonding electron pairs are important physical characteristics of biological activity of fluorinated compounds.[6, 9, 10]

The high elecronegativity of fluorine causes it to attract electrons more powerfully than any other element and it is never found as the free element in the natural environment. Moreover, the bonding between the ligand and the target in the active centre of a protein molecule possessing fluorophobic and fluorophilic areas can be improved due to the electronegativity of the fluorine atom which causes particular dipole-type interactions. It is necessary to mention that in organic compounds, the fluorine

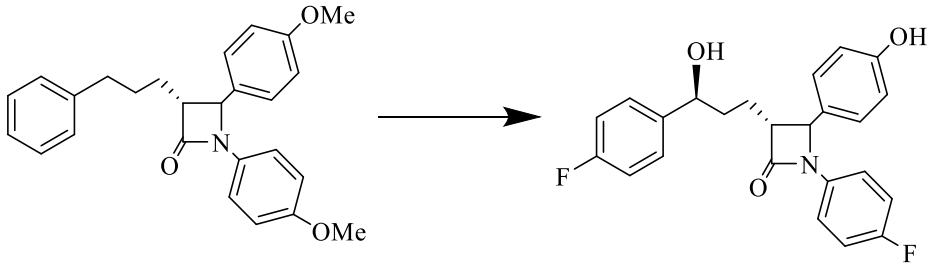
atoms can accept hydrogen bonds only in the absence of better acceptors, such as oxygen or nitrogen, as fluorine is more electronegative and not able to compete with stronger hydrogen-bond acceptors.[11] The fluorine atom has the second smallest van der Waals radius, (1.47 Å) which is sited between hydrogen (1.20 Å)12 and oxygen (1.52 Å)[112] and allows fluorine to behave like a hydroxyl group and contribute in hydrogen bonding interactions.[10] Also the C-F bond has greater strength than C-H bond which results in high chemical stability of fluorinated compounds.[10,13]

Furthermore, fluorine containing substituents (especially perfluorinated groups, like CF_3, C_6F_5, OCF_3) can provide significant steric volume distressing the arrangement of the parent molecule and its isomers, conformations or transition states. Therefore it affects chemical properties such as solubility, acidity, basicity and metabolic stability of molecules. In addition the incorporation of fluorine into organic molecules often leads to increasing lipophilicity thereby enhancing their affinity for natural receptors.[11]

In nuclear magnetic resonance spectroscopy, ^{19}F deserves special attention due to its desirable characteristics (nuclear spin ½, relatively narrow lines, high sensitivity, short longitudinal relaxation time, and 100% abundance). Therefore, ^{19}F NMR spectroscopy is ideally suited to following the fate of fluorinated drug molecules. The selective detection of molecules containing fluorine reduces the background and interfering signals. In addition, this method is very sensitive to changes in the environment due to larger chemical shifts than ^{1}H NMR. ^{19}F NMR can be used in laboratory research; for example in studying the mechanism of interaction with receptors in tumour and normal tissue localization of anti-cancer drugs. Moreover, ^{19}F NMR spectroscopy can be useful as a tool for therapeutic monitoring of fluoropyrimidine analogues and their prodrugs. ^{19}F NMR spectroscopy studies of the metabolism of the successful fluoropyrimidine anti-cancer drugs, such as fluorouracil, and prodrug capecitabine, as well as antifungal agents have been carried out. [9, 11]

1.1.2. Improving Metabolic Stability with Fluorine

One of the problems in many drug discovery projects is low metabolic stability because lipophilic compounds are likely to be oxidized by liver enzymes, especially cytochrome P450. There are different approaches to minimise this problem such as increasing the polarity of the molecule. Another strategy is to introduce a fluorine substituent to block the metabolically labile site and optimally the fluorine atom will not harm the bonding to the target protein. Certainly, this method is commonly employed and results in many successful compounds. The discovery of the cholesterol-absorption inhibitor Ezetimibe **4** (Scheme 2) is counted as one of the nicest examples of this strategy.[14,15,16]



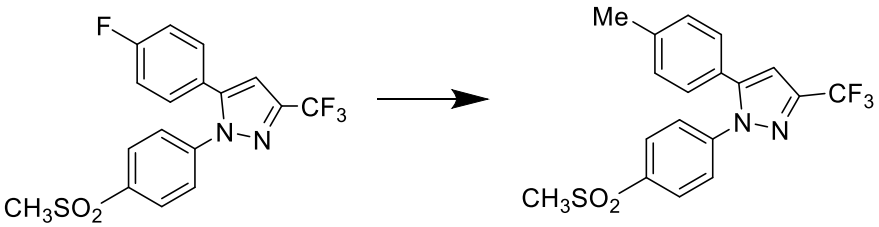
SCH 48461 **3**
ED50 (hamster)= 2.2 mg.kg^{-1}

Ezetimib **4** (oral cholesterol absorption inhabitor)
ED50 (hamster)=0.04 mg.kg^{-1}

Scheme 2. Development of Ezetimibe 4 by optimization of the lead.

As seen in (Scheme 2), the target compound SCH58235 (Ezetimibe) was afforded by introducing two fluorine substituents to starting compound SCH48461, which is a very effective compound that was recently approved by the FDA. By substitution of hydrogen by fluorine the phenyl ring does not oxidize to a phenol, and dealkylation of the methoxy group is prevented by replacement of OMe by F on the N-phenyl substituent.

Another significant example indicating the strong effect of fluorine on metabolic stability is the development of cyclo-oxygenase 2 (COX-2), inhibitor **4** (Scheme 3). In this case, fluorine was replaced by a metabolically labile methyl group to reduce the very long biological half-life to a more acceptable level by really decreasing the metabolic stability of the lead compound. Interestingly, there are also a few cases known for which the introduction of a fluorine substituent does not prevent oxidation at that site. This phenomenon is observed in particular for phenyl rings with a nitrogen substituent in the para position to the fluorine substituent.[17,18]



Early COX II inhibitor **5**
$t_{1/2}$ (rat) up to 220 h

Celecoxib **6**
$t_{1/2}$ (rat) = 3.5 h

Scheme 3. Discovery of the COX 2 inhibitor Celecoxib 6 by replacing the a fluorine group with methyl group which is reduces the very long half-life to an acceptable level.[14,17,18]

1.1.3. The effect of fluorine on the pKa and bioavailability

Fluorine has a very strong effect on the basicity or acidity (pKa) of neighbouring functional groups. pKa shift changing will be depend to the position of the fluorine substituent relative to the acidic or basic group in the molecule. For example, the pKa's of ethylamine and its β-fluorinated analogues decrease in almost linear fashion by introduction of fluorine, $CH_3CH_2NH_2$ = 10.7, $CH_2FCH_2NH_2$ = 8.97, $CHF_2CH_2NH_2$ = 7.52, and $CF_3CH_2NH_2$ = 5.7. Similarly, the acidities or pKa's of acetic acid and its α-fluorinated analogues increase with CH_3COOH = 4.76, CH_2FCOOH = 2.59, CHF_2COOH = 1.24 and CF_3COOH = 0.23 [14,19]

Also, the pKa value of the piperidine ring decreases about 2 log units by substitution of fluorine at the position 3 and 4. A change in the pKa has a result on both the pharmacokinetic properties of the molecule and its binding affinity. For example, for binding within a certain leads series a strongly basic group is essential, however simultaneously this basic group may cause low bioavailability of compounds as a result of the partial ability of a strong basic group to pass through membranes. Therefore a drug discovery project team should be trying to finding an optimum between these conflicting effects. This challenge is well emphasised by the work of van Niel et al.[20] on the development of novel fluorinated indole derivatives as selective 5HT1D receptor ligands.

As seen in figure 1, the pKa values of the compounds significantly decrease by amalgamation of fluorine. This reduction of basicity was shown potent effect on oral absorption or bioavailability but associated weakening of the affinity to the receptor.[20,21,22]

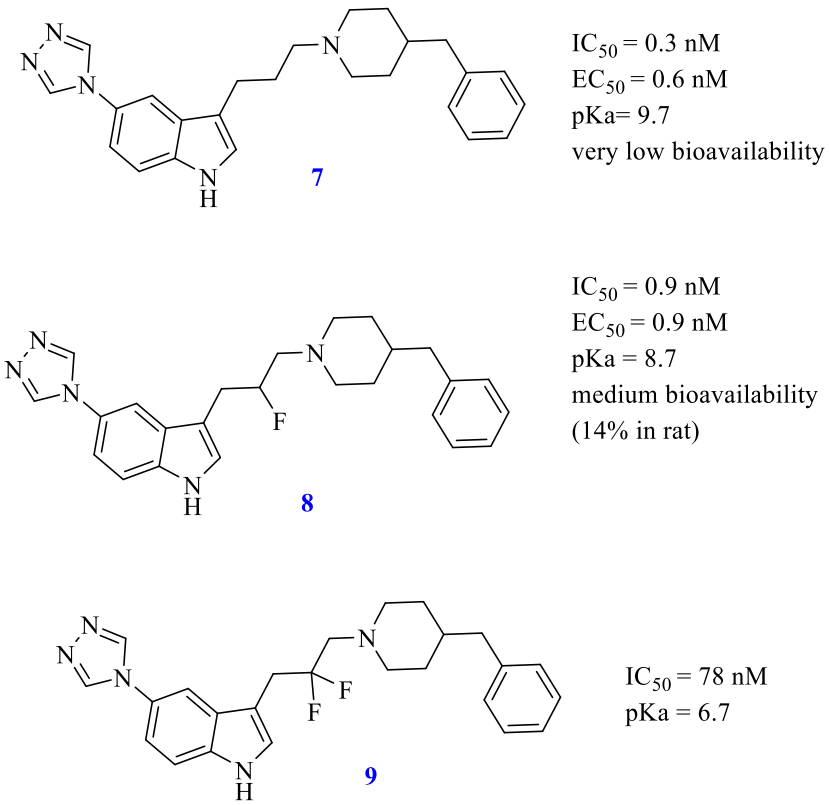


Figure 1. Effect of pKa value on the receptor binding and bioavailability for a set of 5HT1D agonists.[20]

The nonfluorinated parent compound **7** has very low bioavailability but it is a very strong receptor binding ligand. The monofluorinated compound **8** has a lower pKa which is still well-matched with the requirements for receptor binding, but significantly increased bioavailability of compound. The difluoro compound **9** is basic enough to achieve high binding affinity for the 5HT1D receptor due to low pKa value of 6.7.

1.1.4. The effect of fluorine on molecular lipophilicity

One of the important molecular parameters in medicinal chemistry is lipophilicity. Naturally the ligand needs appropriate lipophilicity for good binding affinity to the target protein but too high lipophilicity will reduce solubility and causes other undesirable properties for a compound.[14] Thus, the equilibrium between required lipophilicity and a certain minimal overall polarity of the molecule is another regular challenge for medicinal chemists. To improve the effect of fluorine on the lipophilicity of compound the hydrogen atom was replaced by fluorine atom in 293 pairs of molecules and log D values (logarithmic coefficient of the distribution of the compound between octanol and water at a given pH) was measured. The result indicated lipophilicity slightly increased

by substitution of hydrogen with fluorine.[23] Interestingly, in the compounds which are characterized by the presence of an oxygen atom close to the fluorine substitution show decreased lipophilicity. Possibility the fluorine polarizes the neighbouring oxygen atom causing stronger hydrogen bonds between the oxygen and neighbouring water molecules. Therefore the idea of H/F exchange should be used with care because it does not always increase lipophilicity, however in general an additional fluorine substituent will improve the binding affinity due to an increase in the lipophilicity of the molecule.[14,23]

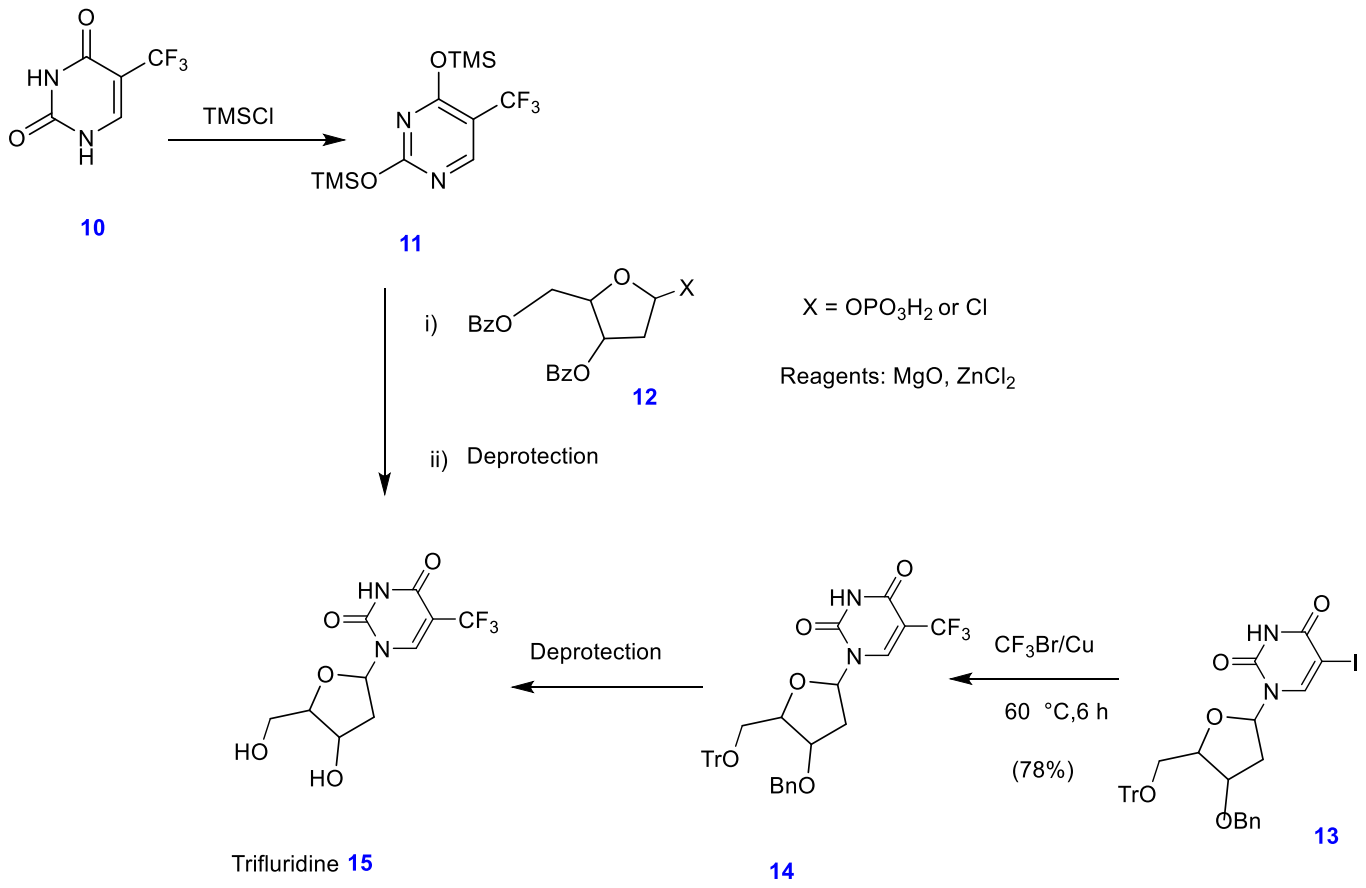
1.1.5. Fluorinated heterocyclic compounds based on natural products

In many therapeutic classes the presence of fluorinated drugs and drug candidates based on natural compounds, such as fluorocorticoid and fluorouracil derivatives are still used as major drugs for many clinical therapeutic classes, and recent research focuses on fluorinated nucleosides, alkaloids, macrolides, steroids, amino acids and prostaglandins. Most of the applications are found in anti-cancer, anti-viral and anti-infectious fields.[6,24]

1.1.5.1. Nucleosides

Fluorinated analogues of nucleosides, such as trifluridine **15**, have been developed as anti-cancer and anti-infection drugs, due to their interaction either with DNA and RNA, or their effect on an enzymatic reaction. The main targeted enzymes by fluoronucleosides and fluoronucleobases are thymidylate synthesis, ribonucleotide diphosphate reductase (RDPR), DNA polymerases and viral reverse transcriptases.

For example thymidylate synthesis (TS) is one of the main enzymes for DNA synthesis effecting transformation of 2-deoxyuridine monophosphate into thymidine monophosphate, which trifluridine as anti-viral drug can inactivate by blocking release of the substrate and co-factor from the active site.[6,24] Trifluridine **15** can be formed in two ways; it can be formed by trifluoromethylation of the protected iodonucleosides **13** or by radical trifluoromethylation of uracil followed by enzymatic or chemical coupling with the protected deoxyribose **12** as seen in Scheme 4.[6]

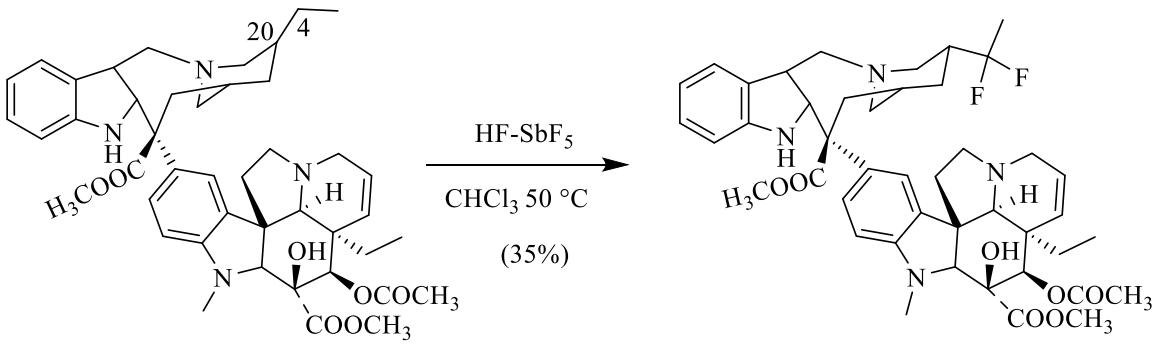


Scheme 4. Syntheses of trifluridine.

1.1.5.2. Alkaloids

Vinblastine and its analogue vinorelbine are anticancer drugs based Vinca indole dimer alkaloids which work by binding to tubulin and inhibiting polymerisation into microtubules. It is important to mention that second-generation vinca dimer alkaloids, (e.g. vinflumine) show a better therapeutic effect in cancer therapy and are more active than vinorelbine.[25,6]

Vinflunine **17** is formed by fluorination of vinorelbine **16** in presence of super-acid media (HF-SbF_5) and trichloromethane ($CHCl_3$) which generated a super-electrophilic agent such as difluoromethylation to abstract hydrogen from protonated alkaloid. Difluorination extraordinarily takes place selectively at C-4 of the clavamine part (Scheme 5).[6]



Vinorelbine (Navelbine) **16** Vinflunine **17**

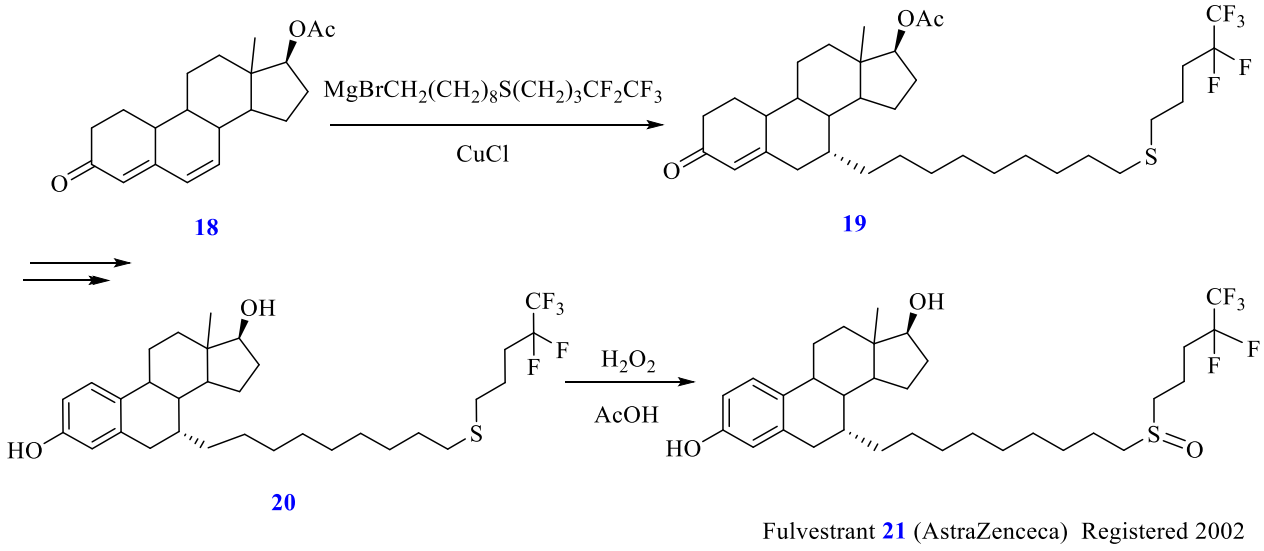
Scheme 5. Synthesis of vinflunine.[6]

1.1.5.3. Steroids

Pharmaceutical research in the fluorocorticoid field is now essentially devoted to the search for new formulation of registered drugs. Nevertheless, there has been recently a renewal of interest for steroids in medicinal chemistry. It mainly concerns selective ligands for steroid hormone receptors for the treatment of several hormonal disorders.

One of the most important drugs acting on steroid hormone receptors is Fulvestrant **21**, which works by binding to the oestrogen receptor in a competitive way with estradiol with similar affinity. Therefore fulvestrant can be used as a treatment for hormone-dependent breast cancer.[6,26]

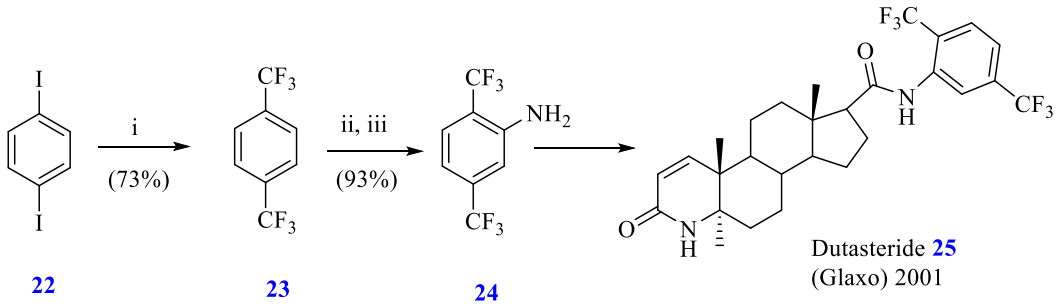
Fulvestrant **21** has been synthesised as shown in (Scheme 6), and contains a long-chain substituent with the terminal two carbons fluorinated.



Fulvestrant **21** (AstraZenceca) Registered 2002

Scheme 6. Synthesis of fulvestrant.[6]

Dutasteride is another fluorinated steroid drug which is a 4-azasteroid. It works by inhibition of type 1 and 2, 5α-reductases, enzymes which convert testosterone to dihydrotestosterone in the prostate. Therefore Dutasteride **25** can be used to treat the Benign Prostatic Hyperplasia (BPH). In the synthesis of Dutasteride **25** the fluorine containing starting compound 2,4-bis(trifluoromethyl)aniline **24** can be formed by trifluoromethylation of the corresponding di-iodobenzene **22**, followed by nitration and reduction (Scheme 7).[6]



i) CF_3COONa, CuI, NMP; ii) HNO_3, H_2SO_4; iii) H_2, Ni,iPrOH

Scheme 7. Synthesis of dustasteride.[6]

1.2. Heterocyclic chemistry

The cyclic compounds in which the ring contains carbon plus one or more atoms of other elements are called heterocyclic compounds, and the non-carbon atoms are named as hetero atoms. The most common hetero atoms are nitrogen, oxygen and sulphur. Among heterocyclic compounds, those containing five to six atoms in the ring are generally the most important. Heterocyclic compounds are widely distributed in nature and they are mostly important due to extensive variety of physiological activity attributed to them.

Some of the significant compounds containing heterocyclic rings are antibiotics, amino acids, dyes, drugs, enzymes, the genetic material DNA, and so on. A few of the fundamental ring systems of the heterocyclic compounds which were used in this study are listed below (Figure 2).[27]

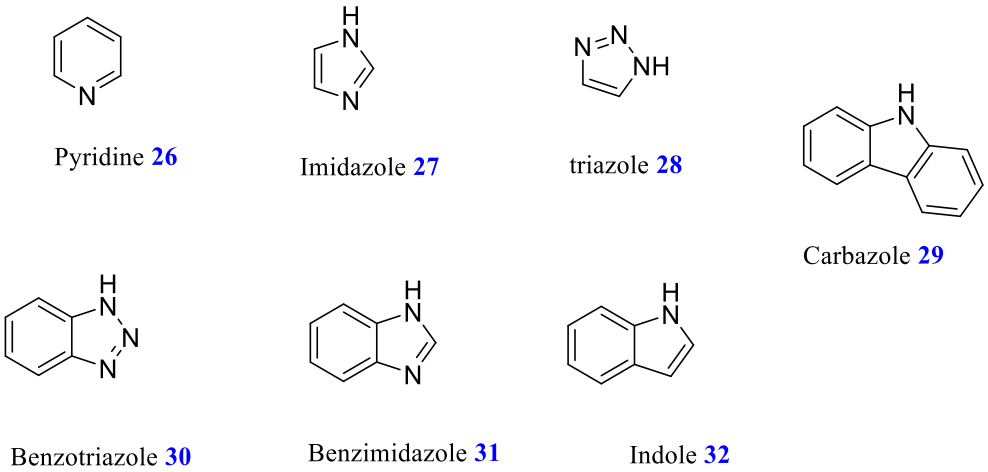


Figure 2. Fundamental heterocyclic rings.

1.2.1. Imidazole in medicinal chemistry

Imidazole is one of the heterocyclic compounds which is common and is very important in mechanical chemistry. Imidazole is a 5-membered planar ring containing two nitrogen atoms which is highly polar and therefore soluble in water and other polar solvents, due to its two nitrogen atoms easily leading to the formation of hydrogen bonds. It exists as two tautomers since the hydrogen can move and locate on either nitrogen atom. The imidazole contains a sextet of π-electrons, two electrons from the protonated nitrogen, and four from the remaining atoms in the ring therefore it is classified as an aromatic compound. Imidazole is amphoteric so it can act as an acid or a base.[5,28,29]

Also, because of these special structural features of imidazole ring, its derivatives can freely bind with a range of enzymes and receptors in biological systems through hydrogen bonds, ion–dipole, π–π stacking, and van der Waals forces, thus showing broad bioactivity.[5,30]

In fact, the imidazole ring is part of many natural compounds such as purine, histamine, histidine and nucleic acid.[31,32] Mainly, imidazole-based compounds are very important in medicinal chemistry and encouraged medicinal chemists to synthesize novel imidazole containing chemotherapeutic agents such as anticancer (dacarbazine), antifungal (clotrimazole), antiparasitic (metronidazole), antihistaminic (cimetidine) and antihypertensive (losartan) drugs which have been widely used to treat various types of diseases with high therapeutic potency.[33,34]

In terms of anti-cancer activity imidazole derivatives could delay DNA synthesis through weak interactions such as hydrogen bond or π-π stacking which stop cell growth or division.

Temporarily, imidazole could bind to protein molecules much easily compared with other heterocyclic rings. More importantly, incessant work has been done leading to synthesis newimidazole-based anticancer agents targeting various enzymes or receptors such as topoisomerases, microtubule, cytochrome P450 enzymes, rapidly accelerated fibrosarcoma (RAF) kinases and so on.[5]

So far, many imidazole derivatives as anticancer and antitumour drugs such as dacarbazine **33,** zoledronic acid **34** and azathioprine **35** have been widely used in the clinic (Figure 3).[5,35,36,37]

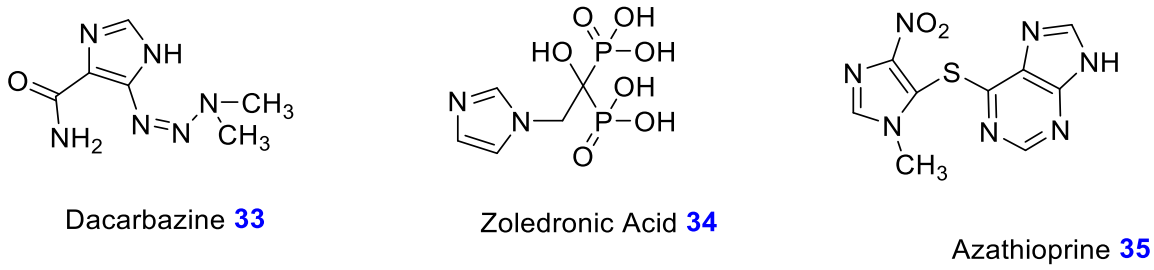


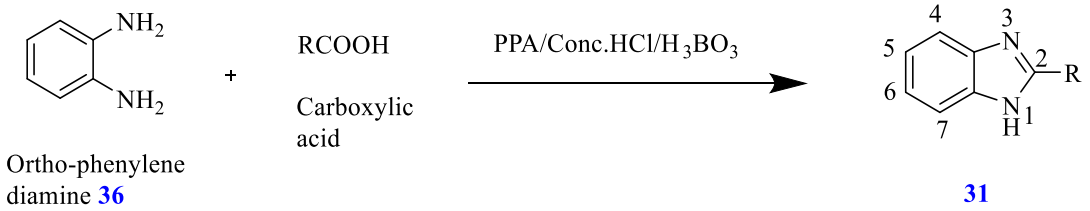
Figure 3. Some clinical imidazole-based anticancer drugs.[5]

1.2.2. Benzimidazole in medicinal chemistry

Benzimidazole is a heterocyclic aromatic organic compound which results from the formal fusion of benzene and imidazole. It is an essential pharmacophore and an advantaged or priviledged structure in medicinal chemistry.[38,39]

Benzimidazole is typically soluble in polar solvents and less soluble in non-polar ones due to the imide nitrogen. The solubility in non-polar solvents increases by introducing other non-polar substituents in various positions of the benzimidazole ring. On the other hand, by introducing a polar group into the molecule the solubility in polar solvents will be improved. Benzimidazoles are sufficiently acidic to be usually soluble in aqueous alkali. Also they are weakly basic. Benzimidazole is slightly less basic than imidazole and is usually soluble in dilute acids. Benzimidazole with unsubstituted NH groups, show fast prototropic tautomerism which leads to equilibrium mixtures of asymmetrically substituted compounds. benzimidazole ring is very stable and unaffected by strong acid such as concentrated sulfuric acid or hot hydrochloric acid in addition to alkalis. Is it fairly resistant to oxidation and reduction. A further characteristic of benzimidazoles is that they have the ability to form salts.[39-41]

Benzimidazoles **31** are generally synthesised from the reaction of 1, 2-diaminobenzenes **36** with carbonyl-containing compounds (carboxylic acids) under tough dehydrating reaction conditions, using strong acids such as polyphosphoric acid (PPA), hydrochloric acid, or boric acid (Scheme 8).[27]



Scheme 8. Synthesis of benzimidazole from ortho-phenylene diamine. [27]

The benzimidazole nucleus is a vital core in many compounds acting at different targets to cause a range of pharmacological effects. However all seven positions in the benzimidazle nucleus can be substituted with different chemical units, but in the most of the biologically benzimidazole based compounds functional groups are located at the 1, 2, 5 or 6 positions. Therefore, the compounds might be mono-, di- or tri-substituted derivatives of the nucleus. Suitably substituted benzimidazole derivatives have established different therapeutic applications such as in antimicrobial [39] antiviral, antifungal, anticancer, antiulcer, antihypertensive and antihistaminics1.[29,42,43]

The optimization of benzimidazole-based structures led to several drugs which are presently on the market.(Figure 4).[28]

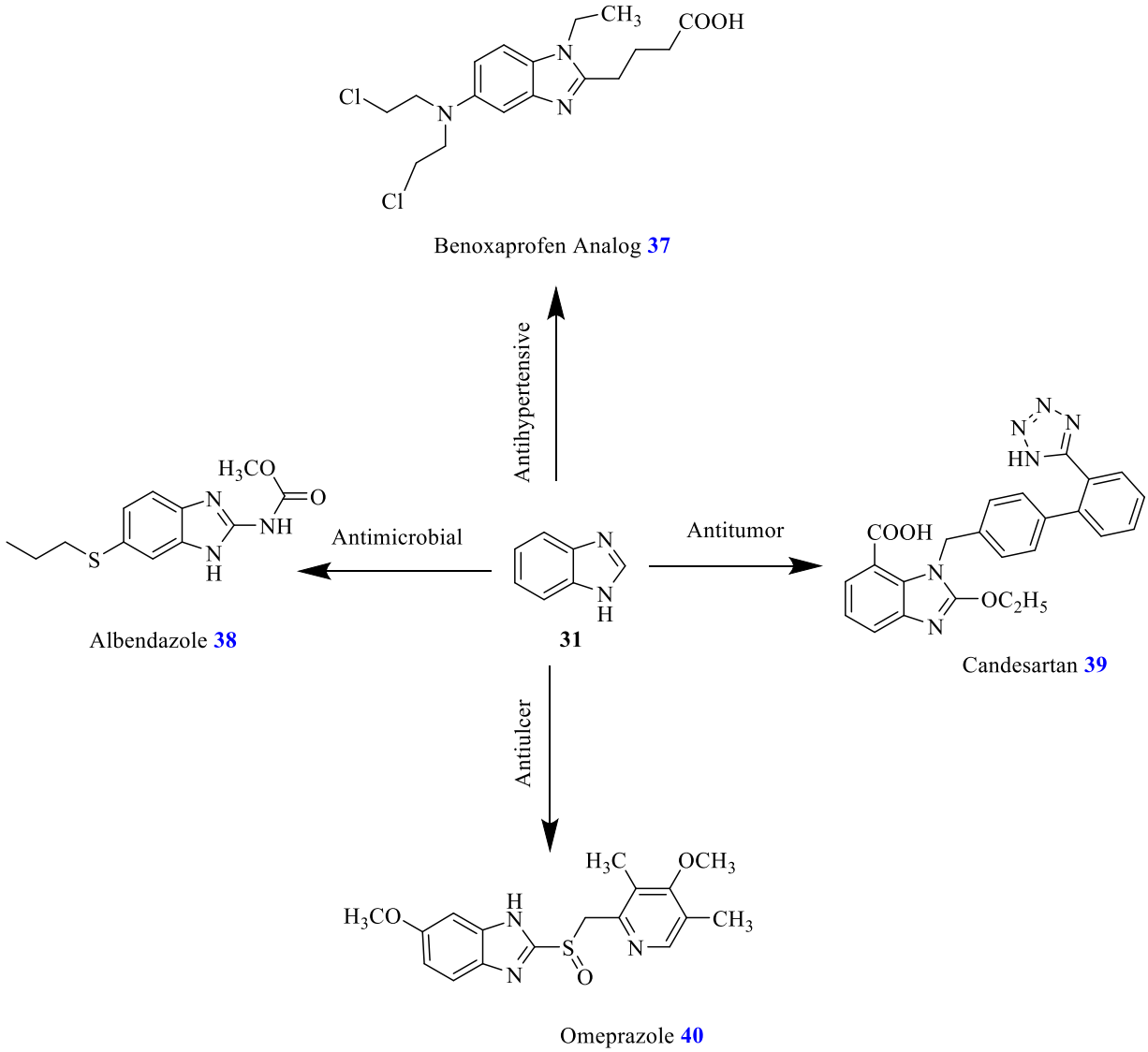
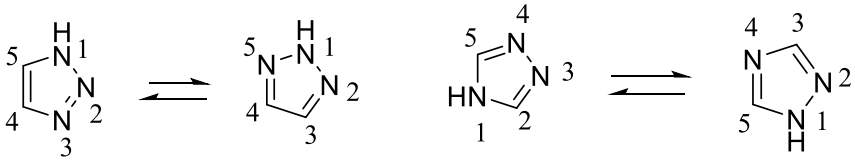


Figure 4. Benzimidazole-based structures drugs.[28]

1.2.3. Triazole in medicinal chemistry

Triazole is one of the organic heterocyclic compounds consisting of a five-membered ring structure. It contains three nitrogen atoms and two carbon atoms. Triazole is pale yellow crystalline solid which is soluble in water and alcohol. It exists as a pair of isomeric compounds, 1,2,3-triazole **28** and 1,2,4-triazole **41** (Figure 5) depending on whether the carbon atoms are adjacent or not.[43,44,45]

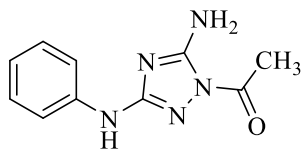


1,2,3-Triazole **28** 1,2,4-Triazole **41**

Figure 5. Pair of triazole isomers.

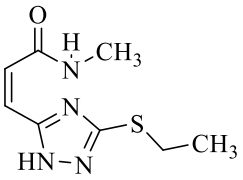
This unique structure of triazole allows its derivatives to freely bind with a range of receptors and enzymes in biological systems and to show an extensive spectrum of biological activitiy.[46-48] Moreover, the triazole ring can be combined with different pharmacophore groups to create novel drug molecules as an attractive linker. Therefore it provides a useful and effective pathway to develop different bioactive molecules.[49-51]

Triazole based compounds with pharmacological activity indicated some advantages such as low toxicity, high bioavailability, less multi-drug resistance, broad spectrum activity, better therapeutic effect, and fewer adverse effects. Therefore they have been regularly applied in the treatment of different types of disease including cancer.[52-54] In general, triazole derivatives have various pharmacological activities such as antifungal, antihistaminic, antimicrobial, anti-inflammatory, and antineoplastic. in addition to anticancer activities which is a major target for medicinal chemists (Figure 6).[43,55]



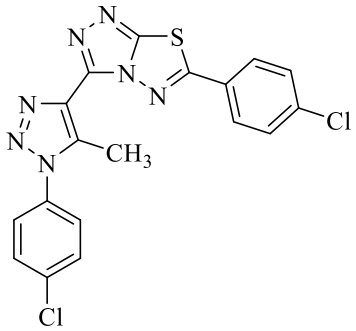
This compound indicated powerful and selective cyclin-dependent kinase CDK inhibitory activities and inhibited in vitro cellular propagation in different tumour cells.[78]

1-(5-amino-3-(phenylamino)-1H-1,2,4-triazol-1-yl)ethan-1-one **42**



This compound showed clearly anti-proliferative effect in breast carcinoma cells in vitro with the less cytotoxicity against of normal cell [79]

(*Z*)-3-(3-(ethylthio)-1*H*-1,2,4-triazol-5-yl) -*N*-methylacrylamide **43**



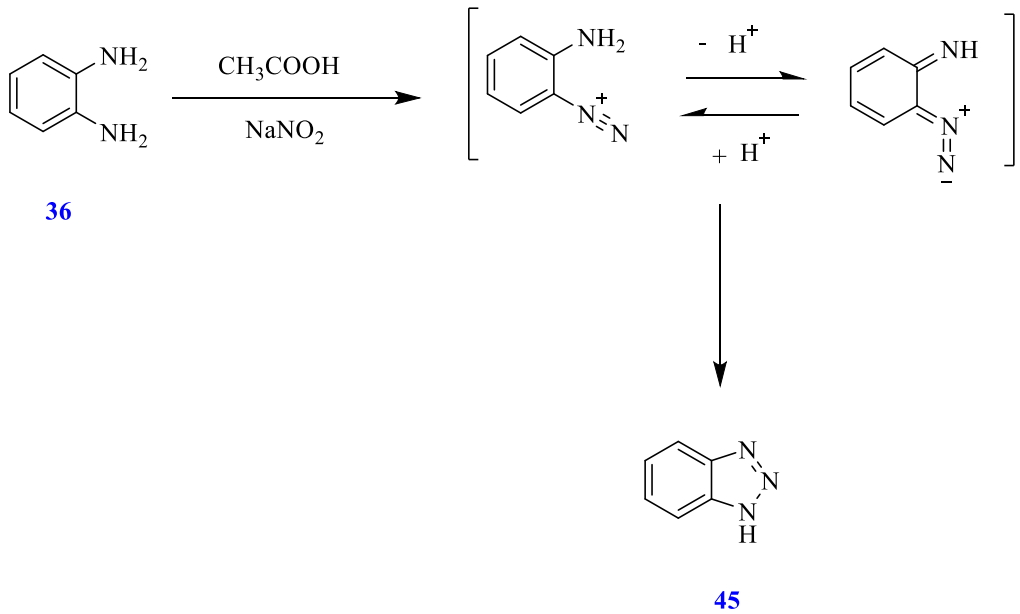
TDZ showed antitumor activity against of two different human cell line in vitro and in vivo it indicated inhibition of DNA replication [65]

6-(4-chlorophenyl)-3-(1-(4-chlorophenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (TDZ) **44**

Figure 6. Examples of antitumor triazole-base compounds in vitro.[43]

1.2.4. Benzotriazole in medicinal chemistry

Benzotriazole **45** is another of the important scaffolds found in many biologically active compounds and drugs. It is an inexpensive, non-toxic, highly stable, aromatic nitrogen heterocycle consisting of a benzene ring fused to a triazole. Benzotriazole can be produced by diazotization of one the amine groups in benzene-1,2-diamine **36** with sodium nitrite and an acid (often a carboxylic acid) (Scheme 9). The reaction is usually best conducted at lower temperature (5-10 °C) to avoid loss of nitrous acid or decomposition of the unstable diazonium intermediate.[56-59]



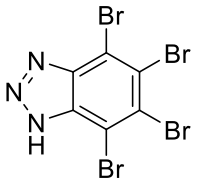
Scheme 9. Synthesis of benzotriazole.[59]

Benzotriazole, because of its fused, more highly conjugated structure, can form stronger π-π stacking interactions compared to triazole. In addition, the three nitrogen atoms allow it to form coordination and hydrogen bonds easily. Therefore the benzotriazole-based compounds can bind to enzymes and receptors in biological systems more easily through different non-covalent binding modes which results in an extensive spectrum of biological activity. Moreover, benzotriazole can form BTA-containing metal complexes by binding the benzotriazole nucleus to different metal ions which could exert a double action mechanism to overcome drug resistance, due to both benzotriazole derivatives themselves and their supramolecular agent activity.[60] For the above reasons, the benzotriazole moiety has been regularly employed to design novel drug molecules.[61-63]

Recently, medicinal chemists working on benzotriazole derivatives have reached great improvement. They have discovered a number of BTA-base compounds with effective pharmacological properties, low toxicity, few side effects, little multi-drug resistance, good water solubility, promising bioavailability, diversity of drug administration, as well as a broad bioactivity spectrum.[62-64]

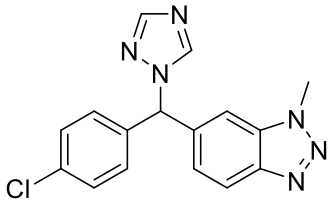
Generally, bioactive BTA-based compounds are being extremely investigated all over the world to treat different types of disease including cancers.

Some anticancer benzotriazole derivatives such as Vorozole and TBB have been in clinical use (Figure 7). The successful examination of these drug encourages continuation of work to make the new BTA-based anticancer compounds targeting different kinases or receptors.[65-68]



TBB has pro-apoptotic effect on a number of different tumor cell by inhibition of protein kinase CK2 [91,92]

4,5,6,7-Tetrabromobenzotriazole (TBB) **46**



Vorozole is potent and selective antineoplastic agent which causes reversible inhibition of cytochrome P450 aromatase[93,91]

6-[(4-Chlorophenyl)(1,2,4-triazol-1-yl)methyl]-1-methylbenzotriazole (Vorozole) **47**

Figure 7. Examples of clinical antitumor BTA-based compounds.

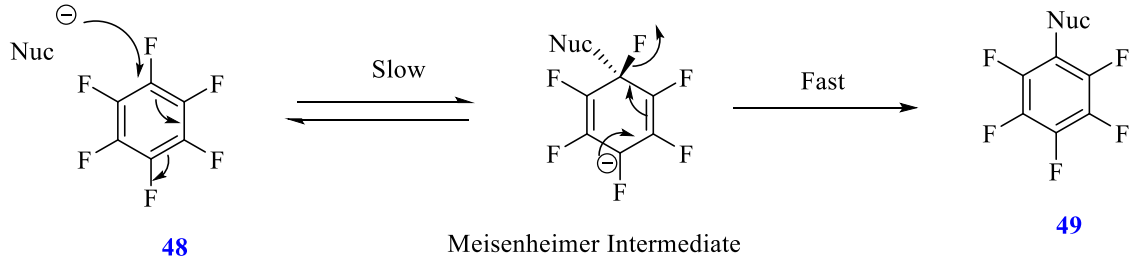
1.2.5. Reactions of Perfluorinated Arenes and Heteroarenes

Examining fluorinated arene and heteroarene systems for the chemical synthesis of a wide range of polyfunctional carbo- and hetero-cyclic derivatives, has been one of the early research programs that has been pursued in organofluorine chemistry since the 1960s. For synthesis of a wide range of highly functionalised heteroaromatic and ring fused polycyclic systems, perfluoroarenes such as pentafluoropyridine, and hexafluorobenzene can be used as core scaffolds.[69]

Reaction of a perfluoroaromatic as an acceptor moiety with various nucleophiles as the donor moiety involves nucleophilic aromatic substitution (S_NAr) and occurs readily under usually mild conditions. The reaction can involve displacement of fluorine from highly fluorinated aromatic systems by carbon, nitrogen, oxygen and sulphur centred nucleophiles, due to the strong electron withdrawing effect of the fluorine substituents, rendering the compounds extremely sensitive towards nucleophilic attack.

The majority of reactions of perfluoroaromatic systems proceed by the two-step addition-elimination nucleophilic aromatic substitution (S_NAr) mechanism. The first step of the reaction involves the flouting of the aromaticity of the fluoroaromatic ring and creation of a tetrahedral intermediate (the

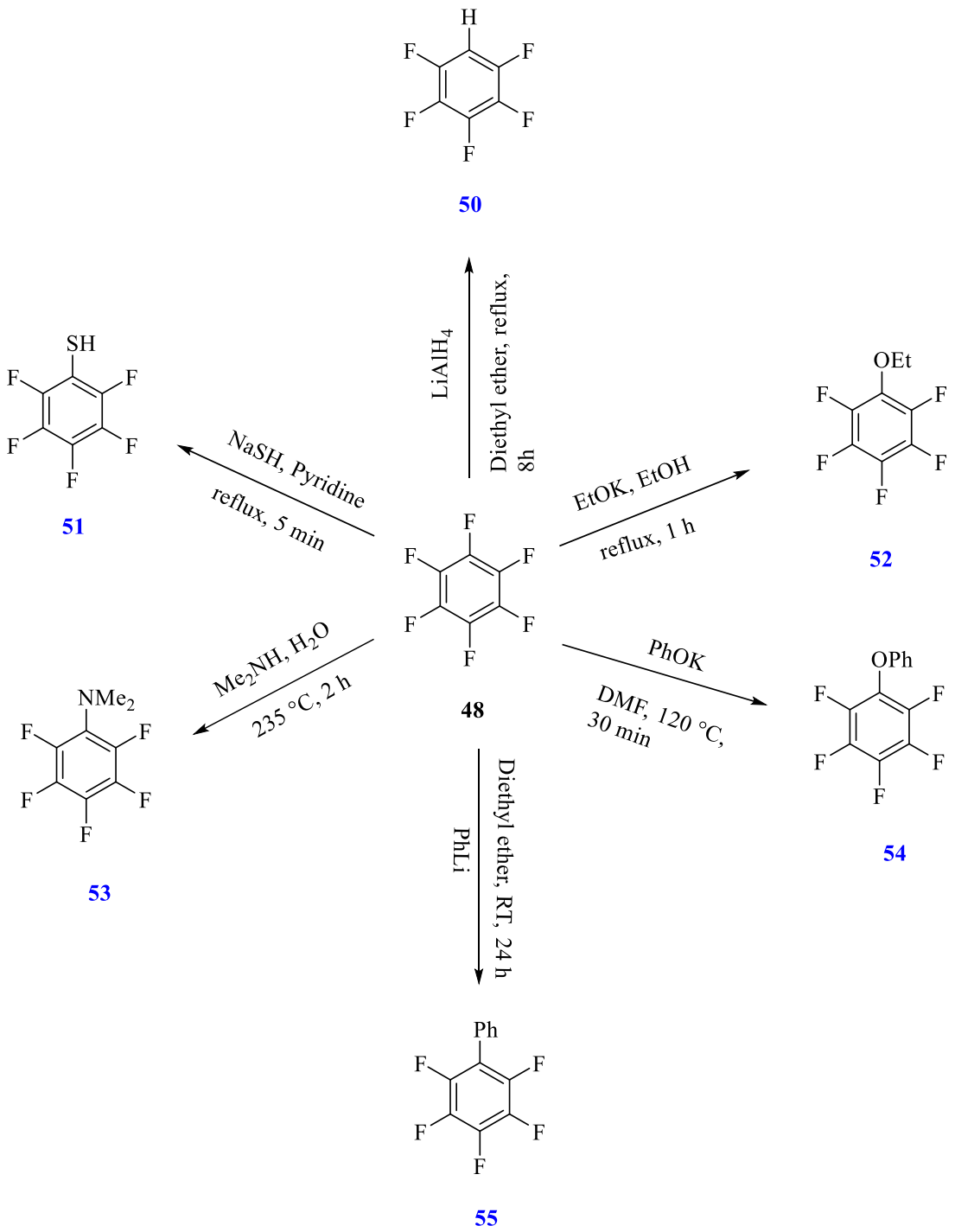
so-called Meisenheimer intermediate). The Meisenheimer intermediate usually breaks down quickly through the ejection of a fluoride ipso to the site of initial nucleophilic attack therefore aromaticity is recouped (Scheme 10). [70]



Scheme 10. Mechanism of nucleophilic substitution reaction.[71]

Highly fluorinated systems are more receptive towards S_NAr reaction compared to chlorinated or brominated complements, because the rate of reaction depends on the electrophilicity of the aromatic ring and the electronic stabilisation of the Meisenheimer intermediate.

S_NAr reactions of hexafluorobenzene **48** are well documented and interesting. Relevant examples have been selected for more detailed discussion.[70]

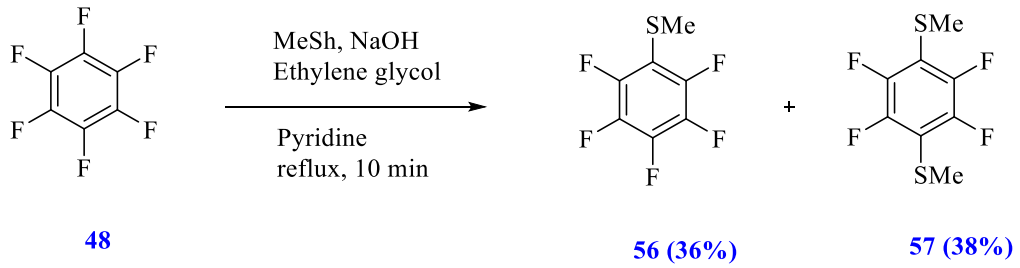


Scheme 11. Examples of S_NAr reactions of hexafluorobenzene.

As the examples shown in Scheme 11 these S_NAr reactions show how an extensive variety of substituted pentafluorobenzene derivatives may be formed in a single synthetic step.[70]

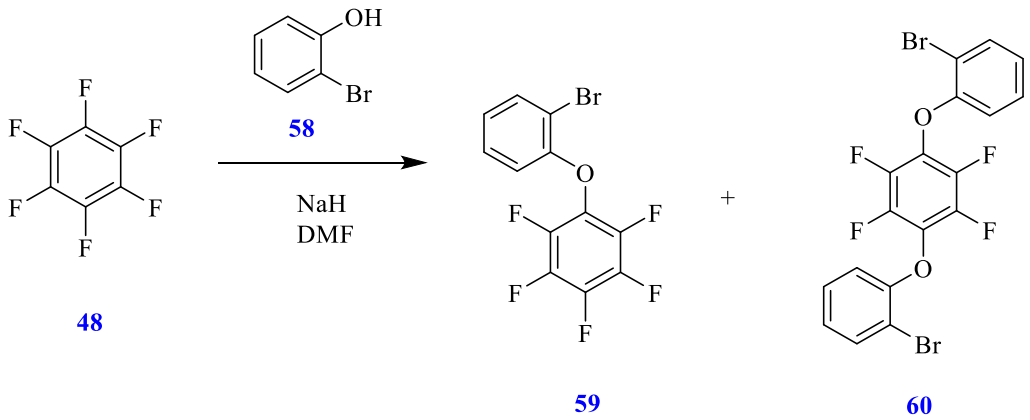
There is no selectivity matter regarding the first nucleophilic substitution due to the symmetrical structure of hexafluorobenzene **48**. Occasionally, some di-substituted product is isolated as well as the mono-substituted compound in S_NAr reactions of hexafluorobenzene **48** especially when the

added functional group is more activating than fluorine itself. For example, the reaction of sodium methanethiolate with an excess of hexafluorobenzene **48** which resulted in formation of mono- and di-substituted compounds **56** and **57** in approximately equal amounts from a single pot reaction (Scheme 12).[72]



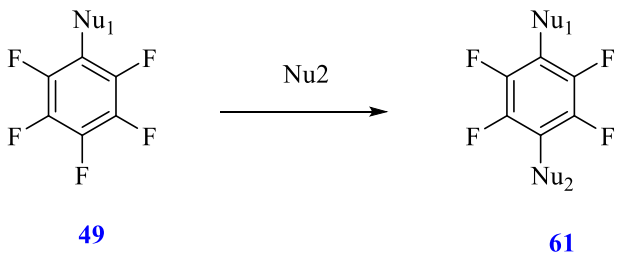
Scheme 12. Reaction of hexafluorobenzene 48 with methanethiolate.[72]

Also, the reaction of hexafluorobenzene **48** with 2-bromophenol **58** is another example of mono and di-substituted formation in single step of S_NAr reactions (Scheme 13).[73]



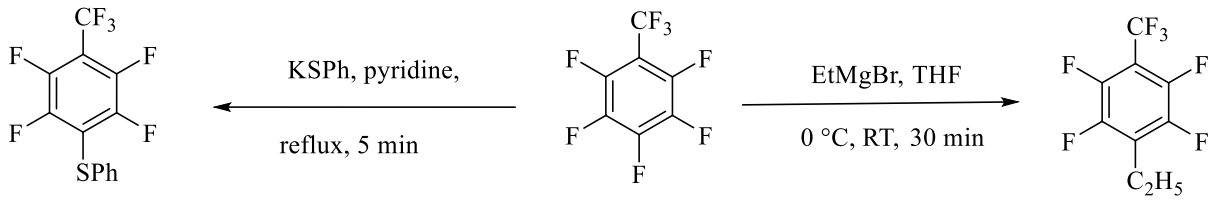
Scheme 13. Reaction of hexafluorobenzene 48 with 2-bromophenol 58.[73]

As seen in scheme 14, addition of a further nucleophile to mono-substituted perfluorobenzene is possible. The same, or a different, nucleophile substituent can add to pentafluorobenzene **49** derivatives.[74]



Scheme 14. Addition of second nucleophilic substituent to petafluorobenzene derivative.[74]

In the vast majority of cases, the 1,4-disubstituted product is obtained and nucleophilic substitution occurs preferentially at para position due to the destabilizing effect of a para-fluorine (Scheme 15). The lone pair on the fluorine atom is believed to repel the build up of charge at the centre of the pentadienyl anion system of the incipient Meisenheimer complex.

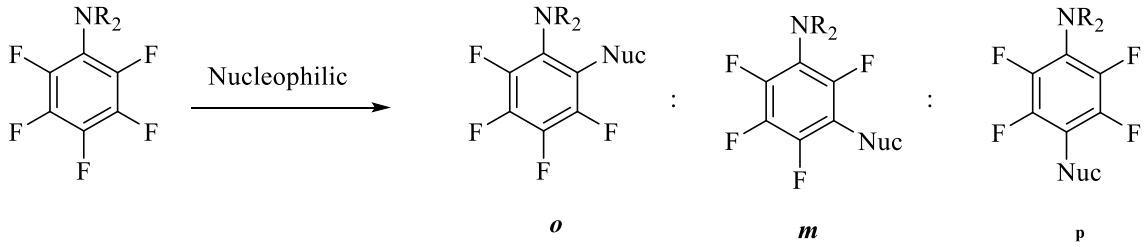


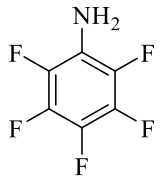
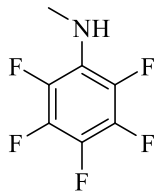
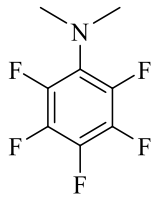
63 **62** **64**

Scheme 15. Examples where asecond nucleophilic substituent attaches to 1,2,3,4,5-pentafluoro-6-(trifluoromethyl)benzene.[70]

However, significant variation from total selectivity of *para* substituent is observed in several cases. (Table 1). Initially, S_NAr reactions of pentafluoroaniline **65** with ammonia, primary and secondary amines and alkoxide nucleophiles indicated nucleophilic substitution to occur mainly at the *meta* position. Corresponding reactions of pentafluoro-N-methylaniline **66** give about equal amounts of *meta* and *para*– substituted products, however practically exclusive para substitution is observed for reactions of pentafluoro-N,N-dimethylaniline **67**. The observed results indicate the amino group powerfully deactivated the *ortho* and *para* positions due to the resonance donation of electron density into the aromatic ring by the amino group lone pair. However as the dimethylamino group is much larger, and twists out of the plane, the lone pair can no longer conjugate with the π-system, and therefore cannot successfully deactivate the *ortho* and *para* sites.[71]

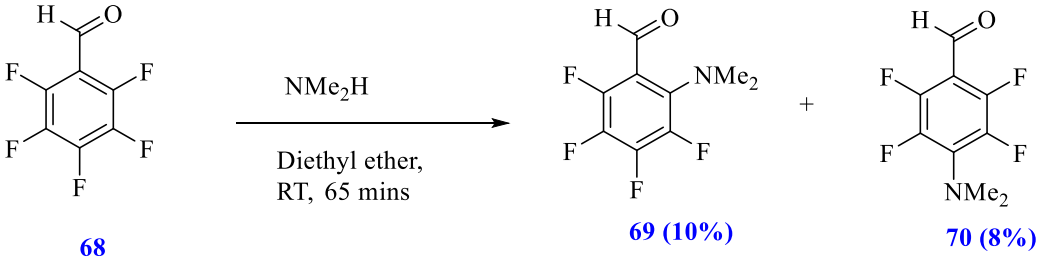
Table 1. Typical SNAr reactions of pentafluoroaniline, pentafluoro-N-methylaniline and pentafluoro-N,N-dimethyl aniline.[71]



Substrate	Nucleophile	Isomer substitution %		
		Ortho	***Meta***	***Para***
 **65**	NH_3	0	87	13
	$MeNH_2$	0	88	12
	Me_2NH	0	90	10
	NaOMe	5	79	16
 **66**	NH_3	0	40	60
	$MeNH_2$	0	60	40
	Me_2NH	0	52	48
	NaOMe	5	43	52
 **67**	NH_3	0	7	93
	$MeNH_2$	0	6	94
	Me_2NH	3	5	92
	NaOMe	1	2	97

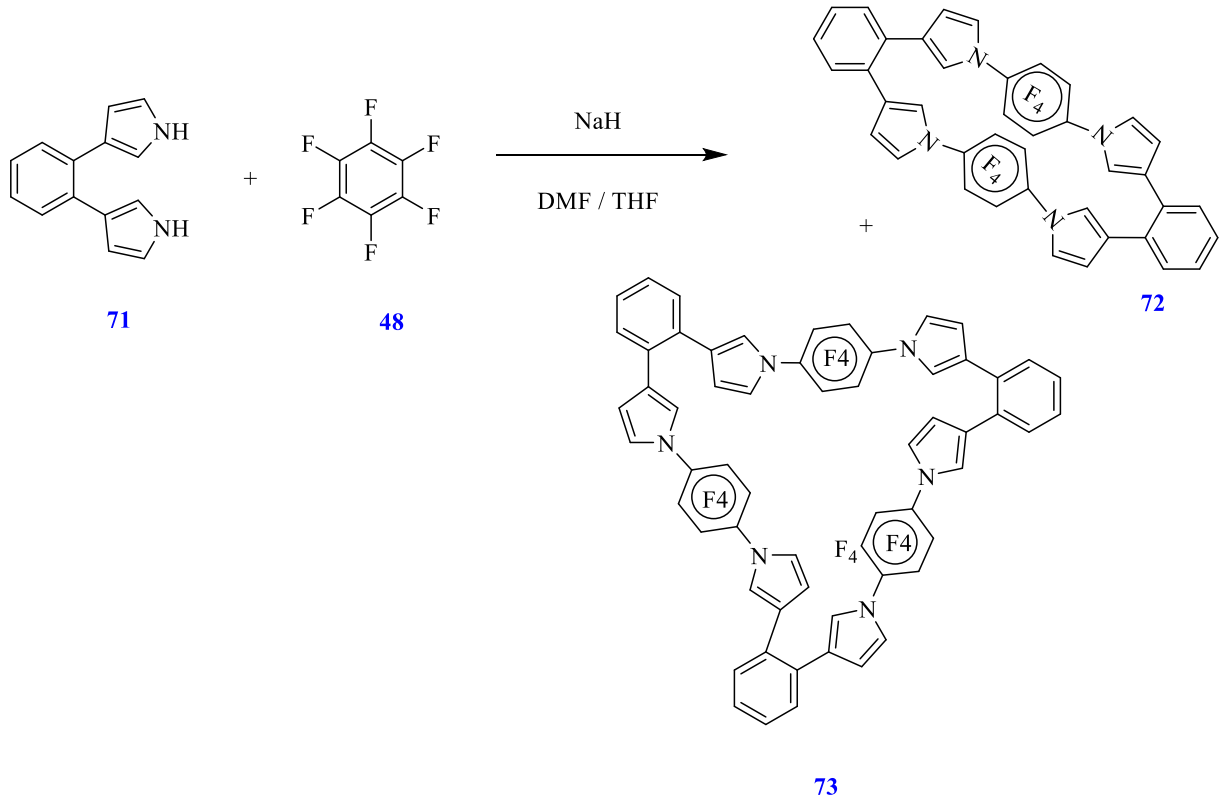
Similarly, reaction of pentafluorobenzaldehyde **68** with dimethylamine (Me_2NH) results in the formation of *ortho* and *para* substituted products in one step S_NAr reaction in diethyl ether as solvent (Scheme 16).[75] Hydrogen bonding of the amine to the carbonyl oxygen may be involved in directing

attack at the ortho position which would involve addition para to a fluorine with the associated repulsion effect of the fluorine lone pair.



Scheme 16. S_NAr reaction of pentafluorobenzaldehyde 68 with dimethyl amine.[75]

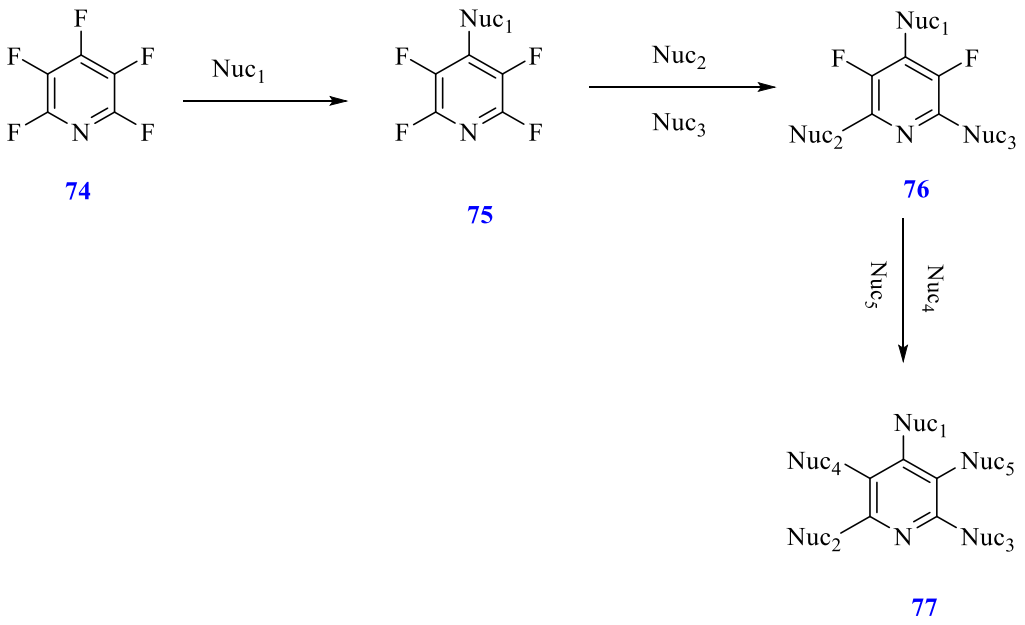
Another recent reaction of a perfluoroaromatic as acceptor moiety with various difunctional nucleophiles with nitrogen as the donor atom involved nucleophilic aromatic substitution (S_NAr) of ortho-dipyrrol-3-ylbenzene **71** with hexafluorobenzene **48** in the presence of NaH as base in DMF and THF to form the macrocyclic stacked fluoroarenes **72** and **73**, which can used in the field of molecular electronics as conductors and field effect transistors (FET) due to the π-stacked structure (Scheme 17).



Scheme 17. Reaction of ortho-dipyrrol-3-ylbenzene and hexafluorobenzene

1.2.5.1. Perfluorinated pyridine

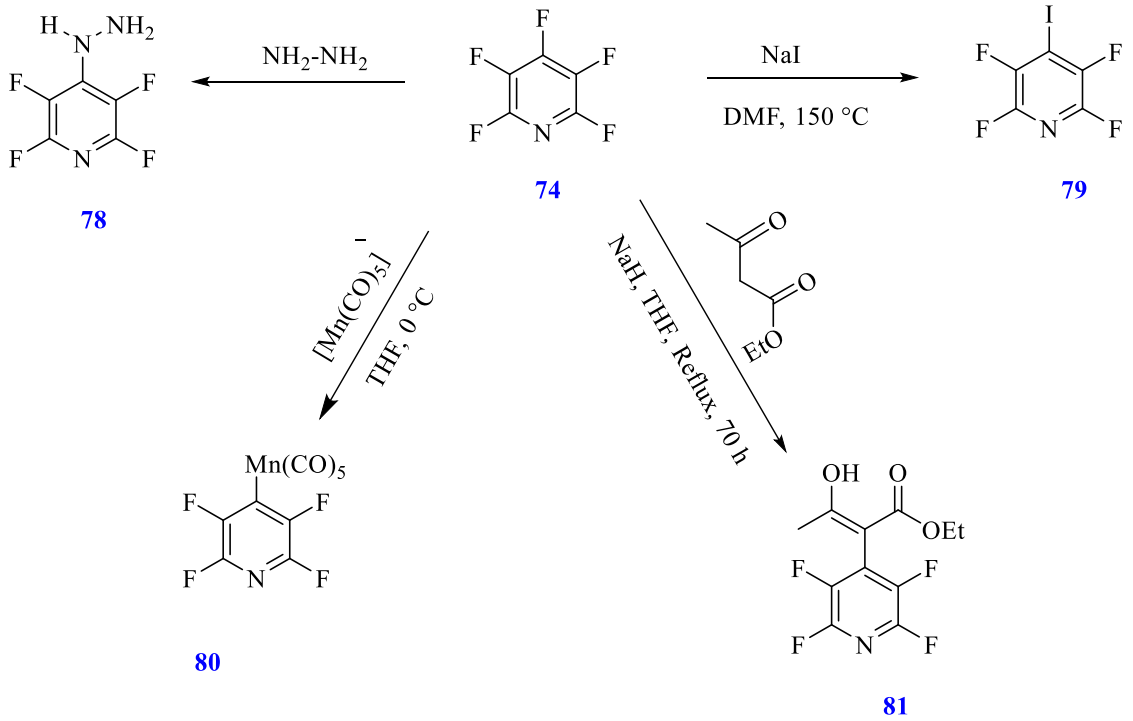
The perfluoroheteroaromatic systems such as pentafluoropyridine **74** are exceptional core scaffolds for the synthesis of different functionalized heteroaromatic and ring fused polycyclic compounds. Pentafluoropyridine, because of presence of five highly electronegative fluorine atoms and the ring nitrogen atom, is very reactive toward nucleophilic attack and all the five fluorine atoms can be replaced by nucleophiles in S_NAr reactions.[76]



Scheme 18. General reaction of pentafluoropyridine75 with difunctional nucleophiles.[77-79]

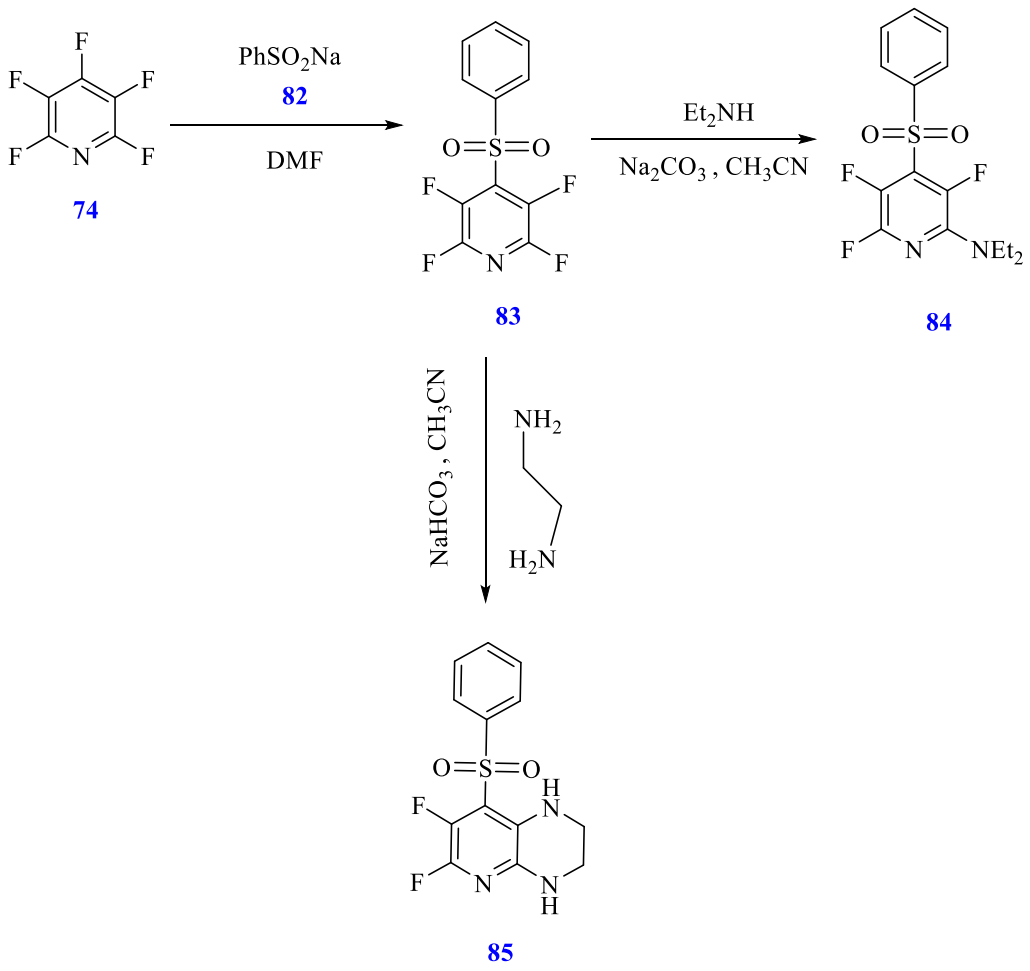
As seen in the above (Scheme 18), the first nucleophile adds at *para* position (4-position) of pentafluoropyridine **74** which is most active site, to the stabilizing nitrogen, and avoiding the repulsion of a *para*-fluorine atom. Following nucleophiles then attack at C-2 and C-6 of compound **75** which are adjacent to the ring nitrogen. The final nucleophiles tend to add to C-3 and C-5 of compound **76** to give final compound **77**.

Scheme 19 shows some examples of the one-step nucleophilic substitution of pentafluoropyridine in which all the nucleophilic substituents added at the para position as most active site.[80-82]



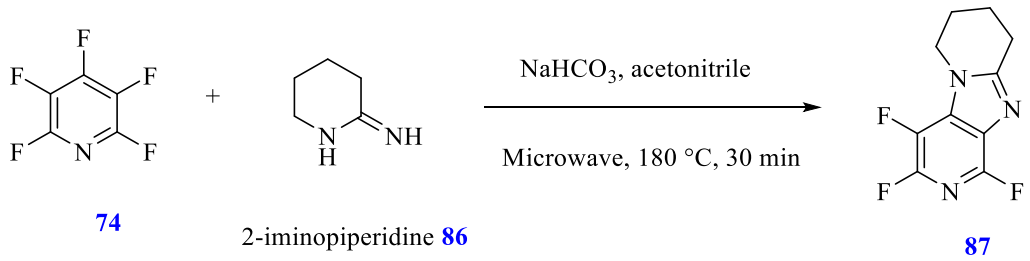
Scheme 19. S_NAr reactions of pentafluoropyridine.

Di- and tri-substitution is possible as shown by the examples in Scheme 20.[78, 83] The sodium salt of benzenesulfinic acid **82** reacted with pentafluoropyridine **74** to form compound **83** which was in turn treated with a second nucleophile (diethylamine) which added to the ortho position to form di-substituted compound **84** in moderate yield, On the other hand reaction of compound **83** with ethylenediamine led to the tri-substitution product. In this case addition is most likely to have occurred firstly at ortho (C-2) position and the other amino group then attacking the less reactive, but geometrically accessible, meta position (C-3) to form the piperazine ring. This reaction shows the possibility of forming an additional fused ring.[78,84]



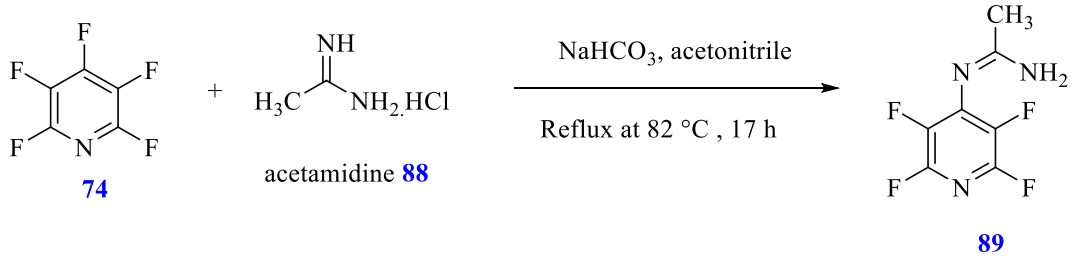
Scheme 20. Di-and tri-substitution reactions of pentafluoropyridine.[78]

An additional example of ring formation involved the reaction of pentafluoropyridine **74** with 2-iminopiperidine **86** to form the tricyclic compound **87** after heating with $NaHCO_3$ at 180 °C for 30 minutes under microwave irradiation. Initial attack at the para position is most likely to have occurred, followed by a second S_NAr reaction at the meta position to close the imidazole ring (Scheme 21).[78]



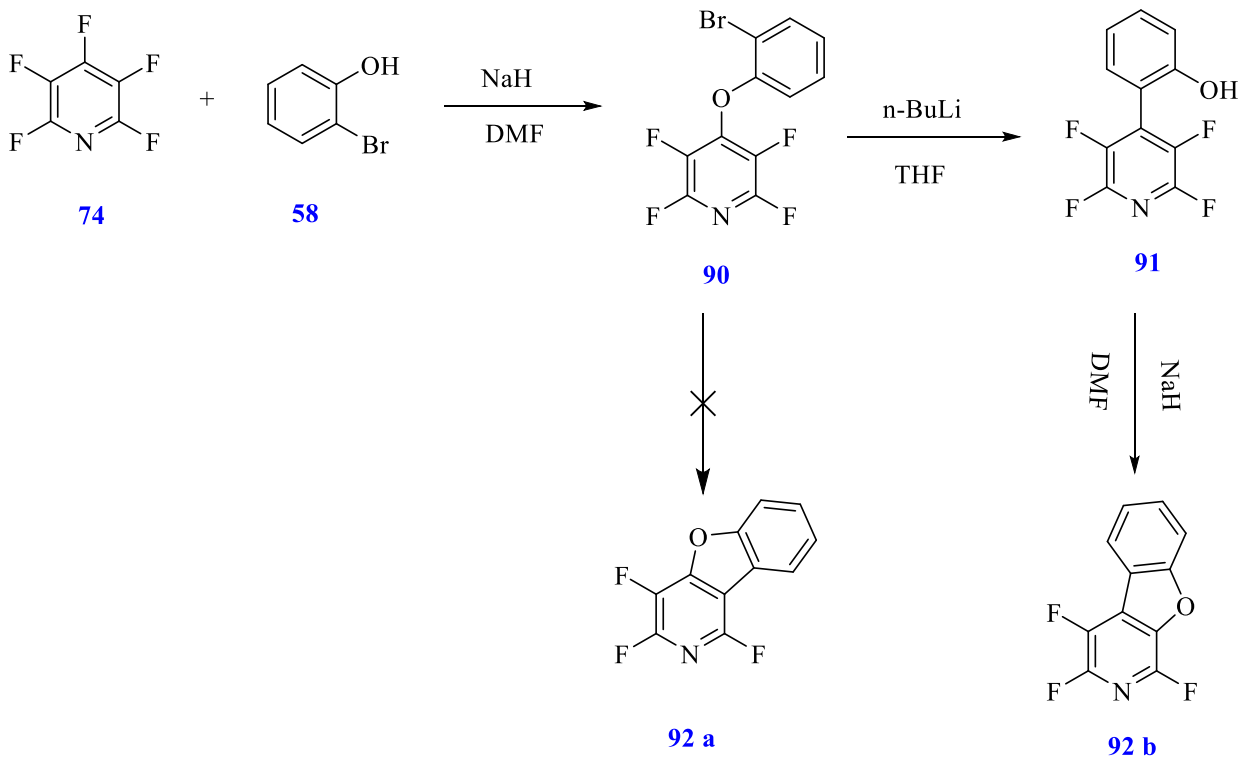
Scheme 21. Reaction of pentafluoropyridine with 2-iminopiperidine.[78]

However, in some case the cyclization failed and a ring did not form (Scheme 22). As seen in the reaction of pentafluoropyridine **74** with acetamidine **88** in the presence of $NaHCO_3$ under reflux in acetonitrile for 17 h which gave the uncyclised amidine derivative **89**.[78]



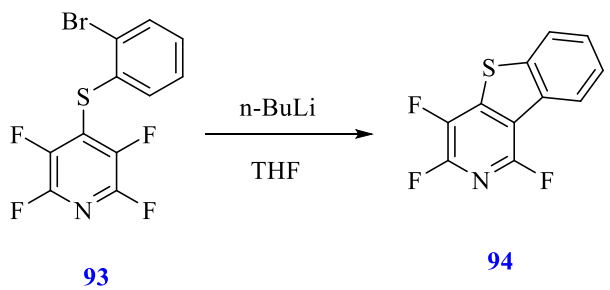
Scheme 22. Reaction of pentafluoropyridine with acetamidine.

Ring formation of fluorinated aryl ethers is another aim of the Weaver research group. As seen in Scheme 23, lithium-bromine exchange in ether was predicted to generate an aryllithium which would lead to intramolecular substitution of the fluorine at C-3 of the pyridine ring to close the furan ring forming **92a**. But treatment of the compound 34 with n-BuLi in THF at −78 °C followed by warming to room temperature gave a compound **91** which was not the expected tricyclic furan **92a**. The compound (2-(tetrafluoropyrid-4-yl)phenol) **91** was isolated indicating that the reaction had proceeded by a Smiles-type rearrangement. It seems that the aryllithium formed prefers to attack at C-4 rather than at C-3 to avoid the destabilizing effect of a para-fluorine. Treatment of the unexpected compound **91** with sodium hydride in DMF did then give the fused benzofuran **92b** quantitatively with the phenoxide oxygen effecting nucleophilic substitution of the fluorine atom at 3-position of the pyridine ring.[73]



Scheme 23. Smiles-type reaction in lithiation of 4-(2-bromophenoxy)tetrafluoropyridine.[73]

On the other hand, when the corresponding thioether **93** was treated with n-BuLi, direct cyclization occurred by S_NAr reaction at C-3 and no rearrangement was observed. The anion stabilizing effect of the sulfur is thought to lower the activation energy for attack at C-3 overcoming the repulsive effect of the para-fluorine (Scheme 24).[73]



Scheme 24. Cyclization of thioether 93 with n-BuLi

1.3. Fluorinated heterocyclic compounds as potential DNA binding ligands

1.3.1. DNA double helix structure

Deoxyribonucleic acid (DNA) is a double-stranded molecule where each strand is composed of a mixture of four different types of bases which are named as adenine (A), thymine (T), cytosine (C) and guanine (G). In the strand these bases are connected to deoxyribose sugars to form nucleosides which are joined by phosphodiester links at both the 3'-hydroxyl and 5'-hydroxyl groups (Figure 8). The two strands are held together by Watson Crick hydrogen bonds where A form two hydrogen bonds with T and C forms three hydrogen bonds with G.[85-86]

DNA plays a significant role in the life process as it carries genetic information which instructs the biological synthesis of enzymes and proteins through the process of transciption and translation of genetic information in living cells. It is a major target for drug interaction as it is the starting point of significant cellular processes of replication, transcription and translation which are vital for cell growth and division. Small ligand molecules bind to DNA and unnaturally change and/or inhibit DNA function. These small ligand molecules are used as drugs when inhibition of DNA function which is essential to treat or control a disease.[86]

The interaction mechanisms of drugs with DNA have an important role in biological studies in drug discovery, and pharmaceutical development procedures, which can be used for the determination of new drugs targeting DNA.[87]

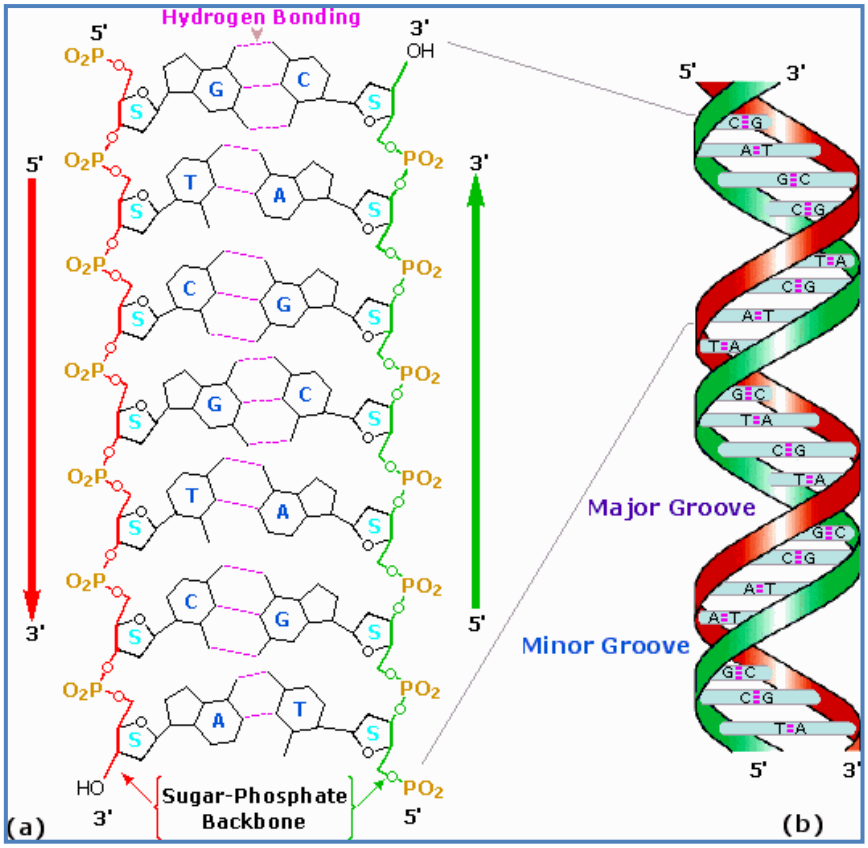


Figure 8. DNA double Helix structure.[88]

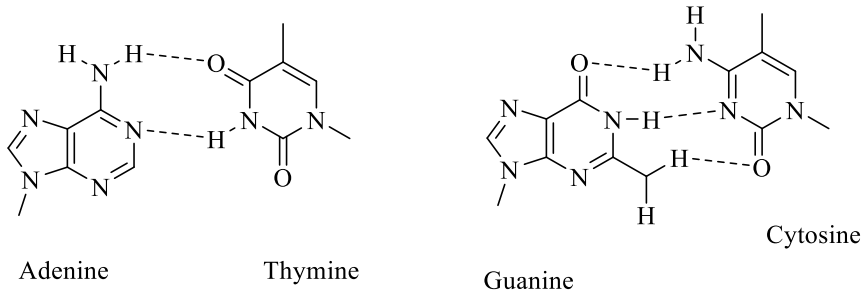


Figure 8a. DNA base pair structure which bind together by hydrogen binding.

1.3.2. Type of drug-DNA interactions

Drugs can interact with DNA in three different ways. i) by interaction of the drug with proteins that bind to the DNA, which control transcription factors and polymerases. ii) through the RNA binding to double helical DNA to form a triple helix structure, to open DNA single strand regions forming DNA-RNA hybrids that may affect transcriptional action and iii) by interaction of small aromatic drugs with DNA double strand structures. Interaction of drugs with DNA can involve binding of a planar aromatic ring compound between the base pairs of DNA which is called intercalation (for

example actinomycin D and daunomycin), or electrostatic interaction and DNA groove binding interactions of flexible chains which can wrap themselves around either the minor or major grooves.[86,89]

1.3.3. Modes of drug-DNA binding

There are two modes of drug–DNA binding, covalent and noncovalent.

1.3.3.1. Covalent mode of binding

Some anticancer drugs bind to DNA through covalent interaction such as via alkylation or inter- and intra-strand cross linking.[90] In covalent interactions the binding is irreversible and invariably proceeds to complete inhibition of DNA and as a result cell death (apoptosis) will take place. The covalent binders have very high binding strength. In addition, they can cause DNA backbone distortion, which in turn can affect both transcription and replication. The covalent binders are usually alkylating agents, and as a result of adduct formation, they are used in cancer treatment to cross link DNA.[9] DNA alkylating drugs are the oldest class of anticancer drug, but are still commonly used today. They show significant anti-cancer activity by damaging DNA and can cause cancer cells to undergo apoptosis.[6] Alkylation usually occurs on the N-7 of guanine in DNA, although alkylation of other positions and other types of cross-links can occur.

Another well-known covalent binder used as an anticancer drug is cis-platin, cis-diamminedichloroplatinum(II) which makes intra- and inter-strand cross-links through replacement of chloride ligands by the nitrogens on the purine bases(Figure 9).[91]

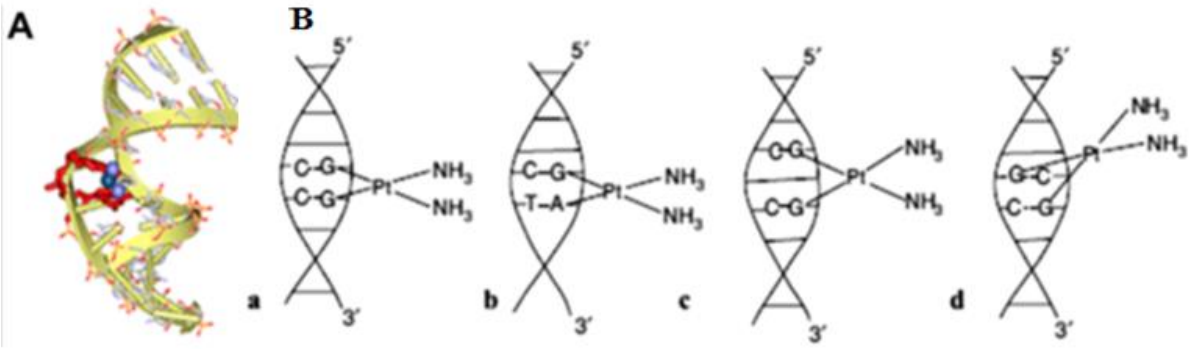


Figure 9. (A) Cisplatin covalently bonded to DNA. B. (a) Modes of binding of cisplatin to guanine (G) and adenine (A); (b) 1,2-intrastrand GpG (structure a), 1,2-intrastrand.

1.3.3.2. Non-covalent mode of binding

Non-covalent binding agents are normally considered less cytotoxic than DNA covalent binding agents. The non-covalent binding mode is reversible and is preferred over a covalent interaction which leads to irreversible DNA damage and severe toxic side effects.

Non-covalent binders cause DNA conformation change, interrupt protein–DNA interactions and potentially initiate DNA strand breaks which substantial effects on gene expression.[90]

The non-covalent mode of drug–DNA binding can be classified into intercalation and groove binding.[86]

1.3.3.3. Groove binding drug-DNA interaction

Minor groove binding drugs typically have several aromatic rings, such as pyrrole, furan or benzene connected by bonds possessing torsional freedom. Groove binder drugs can bind with DNA by van der Waals interaction and hydrogen bonding. In addition, these drugs can form hydrogen bonds to bases, typically to N-3 of adenine and O-2 of thymine.

Nucleotide asymmetry generates two grooves with different geometrical characteristics. The major groove is wide and relatively small and shallow, and approximately 22 Å in width. However the minor groove is deeper and narrower, and only about 12 Å in width (Figure 10).[87,92]

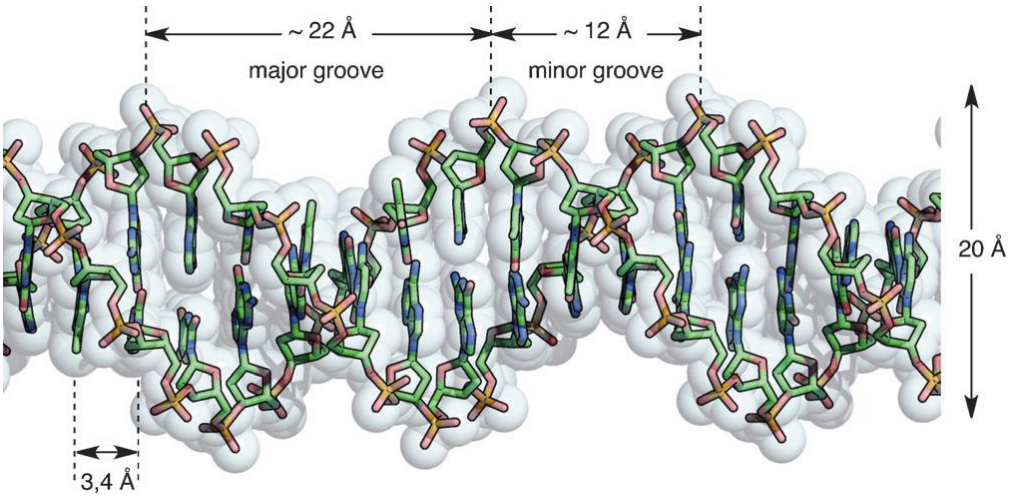


Figure 10. B-DNA conformation, with the main structural dimensions.[87]

Furthermore, studies of the functional groups on the base pairs shows that in the major groove the donor-acceptor pattern is more variable. Actually, in the minor groove A/T and T/A are degenerate while in the major groove all four possible base pairs can be distinguished by particular donor–

acceptor combinations.[87] In nature, proteins can bind to the major groove, some to the minor groove, and some require binding to both. Most DNA interactive proteins do bind in the major groove which is richer in the number and specificity of the hydrogen bond donor and acceptor sites compared to the minor groove. However, most small molecules of less than 1000 Da bind in the minor groove.[92]

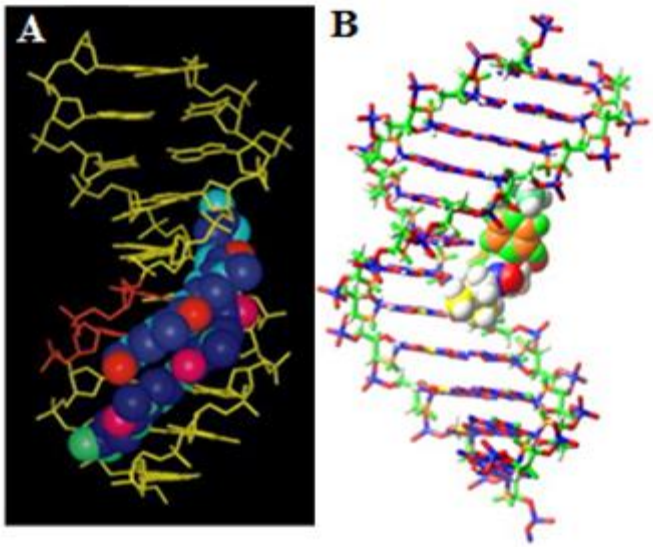
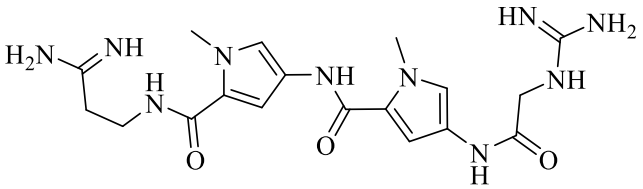


Figure 11. A) Minor groove filled with two drug molecules of diimidazole lexitropsin. B) major groove binding of DMAADD molecule.[85]

The minor groove binders are usually curved in shape, which matches well with the topology of double-stranded DNA, and increasingly are of interest as potential anti-cancer drugs [87,90] which can bind either non-covalently and covalently to DNA.[92]

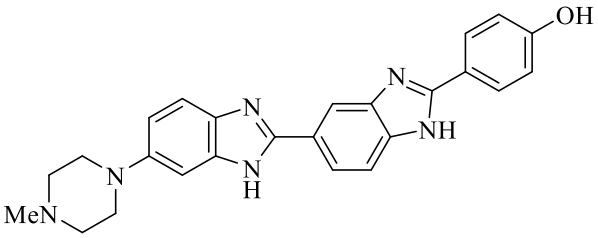
It is important to recognise that due to the sterically obstructive amine groups, G-C base pairs are not good binding sites in most minor groove DNA sections. However the anti-microbial agents that bind to A/T base pairs in the minor groove frequently have anti-tumour activity and are more selective than other typical DNA-reactive anti-tumour drugs which regularly cause wide damage to areas that are also vital for the operating of non-replicative cells. Moreover, such agents can cause damage to normal tissues by producing mutagenic lesions.[93]

Non-covalent minor groove binding drugs as potential anticancer compounds are interesting to work on. The polypyrrole, Bis (benzimidazole) and the bis-quaternary ammonium heterocycles are some examples of A/T minor groove binding drugs.[94]



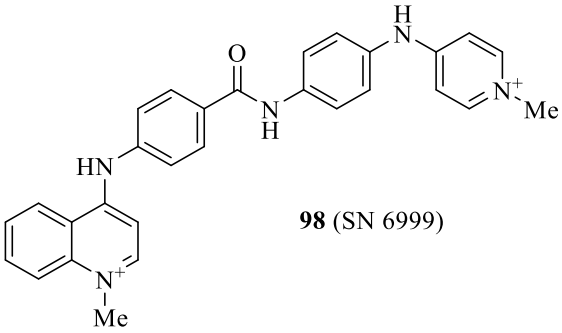
Netropsin **95**

Figure 12. Netropsin is an example of a polypyrrole which binds to A/T-rich sites, and acts as potent inhibitor of Werner and Bloom helicases.[94]



Hoechst 33258 (pibenzimol) **96**

Figure 13. Hoechst 33258 (pibenzimol) is example of a bis-(benzimidazole) which binds to AT-sites, and acts as potent cytotoxic drug by inhibition of topoisomerase and DNA helicase.[94]



98 (SN 6999)

Figure 14. SN 6999 is example of bis-quaternary ammonium heterocycles which bind to AT tracts with at least 4 base pairs, related to polyamidines, and causes significant distortion of the DNA structure as well as inhibition of DNA and RNA polymerases.[91, 94]

In addition the pyrrole-imidazole (Py-Im) polyamides show high binding affinity and specificity to the specific DNA sequences. They are effective inhibitors of RNA transcription, and modulate gene expression by modifying chromatin structure. This compound has been linked with the DNA-

alkylating agent chlorambucil (Chl) which then reacts covalently with specific sites of DNA. For example. polyamide 1R-Chl (Figure 16).[91]

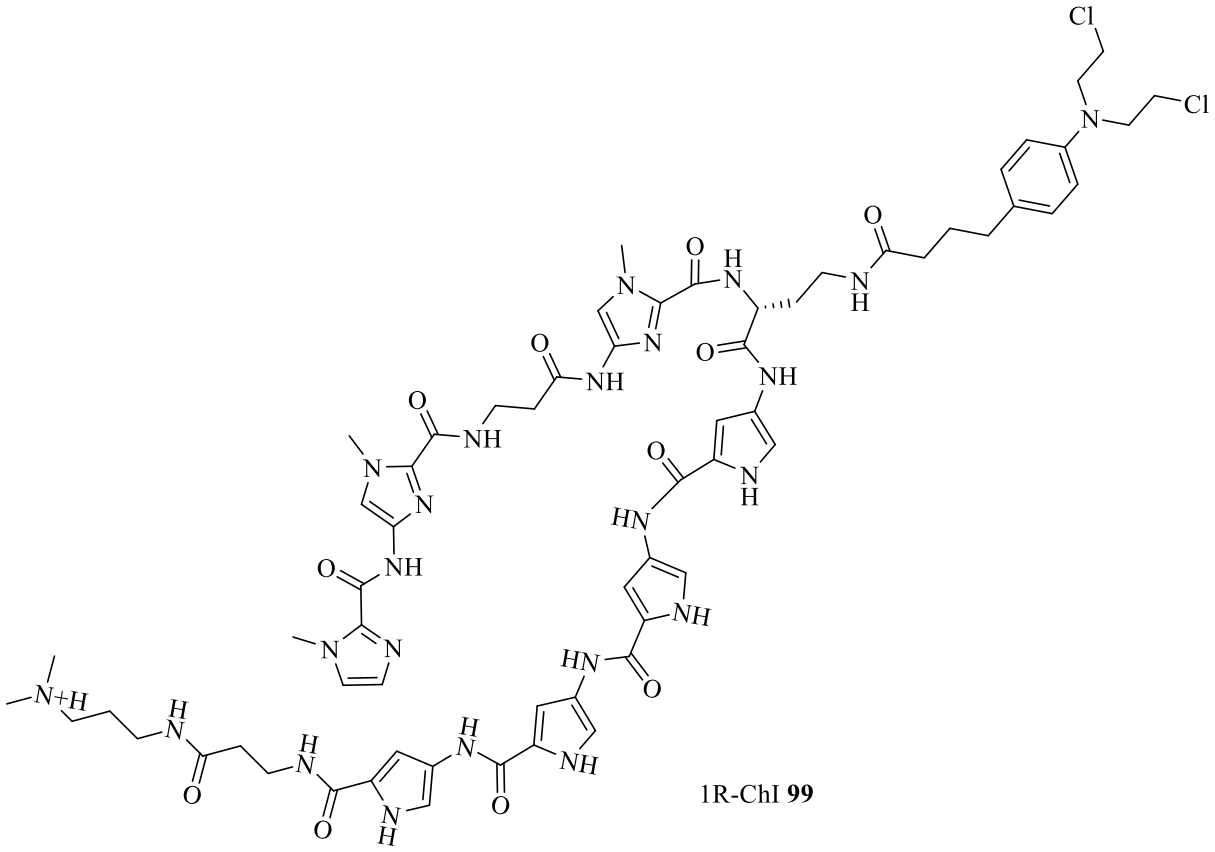


Figure 15. Structure of 1R-Chl which binds to a specific sequence of DNA and acts as inhibitor of cell proliferation in different cancer cell lines with no apparent cytotoxicity.[91]

1.3.3.4. Intercalating binding agents

The interaction between DNA base pairs and the acridine proflavione agent was described by Leman as intercalation. Intercalation is the selected binding manner of flat polyaromatic ligands of adequately large surface area and suitable steric properties (Figure 16). Intercalators stack between adjacent DNA base pairs leading to significant π-electron overlap, aligning perpendicular to the DNA backbone without breaking up the hydrogen bonds between the DNA bases. Van der Waals, hydrogen bonding, hydrophobic, and/or charge transfer are the known forces that maintain the stability of the DNA–intercalator complex, even more than DNA alone.[83,96] In addition the DNA intercalators complex is less sensitive to the ionic strength compared to DNA groove binding complexes due to the stabilization by π-π interaction.

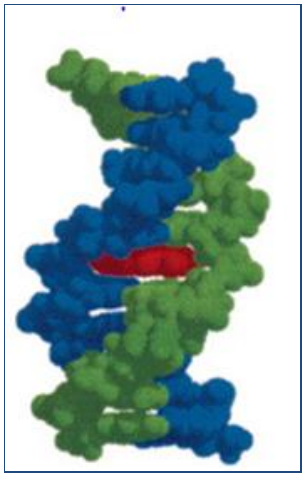
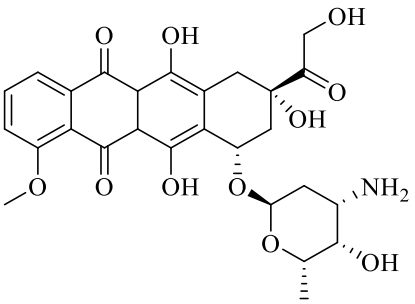


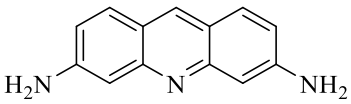
Figure 16. Intercalation of a planar aromatic molecule in the DNA base pairs by hydrophobic interaction and van der Waals forces. [97]

When an intercalating agent is inserted between DNA base pairs, a decrease in the DNA helical twist and lengthening of the DNA occurs that causes delay in cell replication. Therefore such compounds used in chemotherapeutic treatment to inhibit DNA replication in rapidly growing cancer cells.

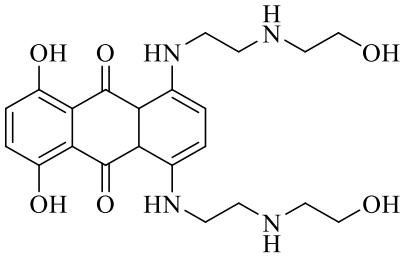
The binding mode can be determined by the size and nature of intercalating chromophore. For effective intercalation, at least a two-ring fused system is necessary, for example naphthalene- type, but these intercalate only if they have pendant cationic side chain, having similar affinities irrespective of the presence of charged or uncharged nitrogen atoms in the aromatic system. In comparison fused three-ring systems such as acridines have adequate stacking interactions to intercalate without the need for appended side chains.[93] Figure 17 shows some examples of intercalating clinical drugs.



Doxorubicin **100**



proflavine **101**



Mitoxantrone **102**

Figure 17. Example of DNA-intercalating agents [93]

1.3.4. Techniques used to study drug–DNA interactions

There are a number of different techniques which can be used to study the interaction of DNA with ligand molecules such as infrared (IR), UV–Visible, and nuclear magnetic resonance (NMR) and fluorescence spectroscopies, as well as macromolecular X-ray crystallography. Atomic force microscopy (AFM), electrophoresis, mass spectrometry, viscosity measurements, thermal denaturation studies, differential pulse voltammetry, Raman and circular dichroism, have also all been employed. All these techniques can be used as methods to characterise drug–DNA complexes, and effect of binding on DNA structure. In this study UV–Visible and fluorescence spectroscopies were used as these are of common use. Macromolecular x-ray crystallography was also attempted to see if co-crystals of DNA-drug addicts could be grown. In addition, some antibacterial activity also studied against one Gram negative strain (*E. coli*) and one gram positive strain (*Staphylococcus aureus*).

1.3.4.1. UV–Visible absorption spectroscopy

UV–Visible absorption spectroscopy is one the simplest and commonly employed instrumental techniques for studying both the stability of DNA and its interactions with small ligand molecules. In this technique, UV–Visible absorption spectra of the free drug are compared with drug–DNA complexes, which are usually different. If a small molecule has intercalated between DNA base pairs hypochromism (reduced absorbance) and bathochromism (red shift) should occur which is due to the stacking interaction between an aromatic chromophore of intercalator and a base pair of DNA. In terms of bathochromism (red shift) the π-orbitals of the DNA base pairs couple with π^*-orbital of the small molecules which causes an energy decrease and a decreasing of $\pi \rightarrow \pi^*$ transition energy. Therefore the absorption of the small molecule shows a red shift. Simultaneously, the empty π^*-orbital is partly filled by electrons, reducing the transition probability, which leads to hypochromism. In case of electrostatic attraction between the DNA and small molecules, a hyperchromic effect should occur, which is due to the corresponding changes of DNA in its conformation and structure after the complex–DNA interaction has occurred. The hyperchromic effect is the exceptional increase in absorbance of DNA upon denaturation.[86]

1.3.4.2. Fluorescence spectroscopy

Fluorescence spectroscopy is another common technique which can indicate interactions between small ligand molecules and DNA. The advantages of this technique compared to other techniques are high sensitivity, selectivity and large linear concentration range. Compounds containing aromatic

functional groups with low-energy π-π transition levels have large number of possible transitions compared to compounds containing aliphatic and alicyclic carbonyl structures or conjugated double-bond structures, and absorp strongly. Such rigid structures can often re-emit the excess energy as light in the form of fluorescence. Therefore aromatic systems usually give the most intense and the most useful fluorescence signals. Also using this technique based on fluorescence emission the mode of drug-DNA binding can often be determined. In the case of intercalation interactions, drugs bound to DNA enhance deactivation through fluorescence emission so a significant increase in the fluorescence emission will usually be observed.

But in other interactions like groove binding, electrostatic, hydrogen bonding or hydrophobic interactions drugs are close to the sugar-phosphate backbone; therefore it is possible to observe a decrease in the fluorescence intensity due to non-radiative energy loss. The quenching fluorescence assay is one method which provides further information about the binding mode of drugs to DNA.

Ethidium bromide (EB) is one of the common fluorophore probes. It can insert to the DNA base pair and bind to DNA strongly as an intercalator, which causes an increase in the fluorescence intensity. The improved fluorescence in presence of EB can be quenched by the addition of a second molecule. Therefore by intercalating a second molecule into DNA, the fluorescence intensity of the EB-DNA will decrease, because it will compete with EB in binding with DNA. In addition the extent of interaction between the drug and DNA can be determinate by the extent of fluorescence quenching of EB bound to DNA.[86] Figure 18, describes the interaction of Ni complex $[Ni_2(L)_2(NO_3)_2]$ with EB-DNA. The K_{SV} values of compound is $(7.0 \pm 0.097) \times 10^3\ M^{-1}$

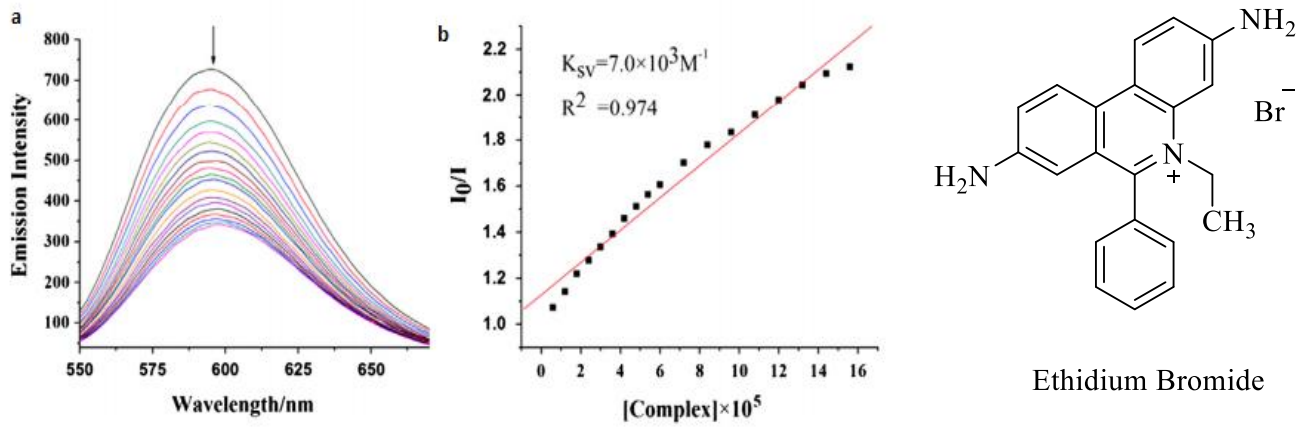


Figure 18. Emission spectra of EB bound to CT-DNA in the presence of free Ni complex [Ni2 (L)2(NO3)2] (a) Fluorescence quenching curves of EB bound to CT-DNA Ni complex (b). (Plots of I_0/I vs. [Compound]). [86]

1.3.4.3. Macromolecular X-ray crystallography

One of the useful and important methods which is used to determine the three dimensional structure of DNA-drug complex is macromolecular X-ray crystallography. This method involves growth of a co-crystal of DNA and the drug of interest and X-ray diffraction data collection. There are different methods to grow suitable crystals which all have the aim of bringing the biological macromolecules solution to a supersaturated state. The crystallization process can be affected by different factors which include biophysical and physico-chemical parameters. Biophysical parameters include stabilization, purification, storage and handling of macromolecules, as bacterial contamination are very significant in crystallization of the DNA. Fundamental physico-chemical parameters such as supersaturation (concentration of macromolecules and precipitants), temperature, pH, purity, ionic strength, pressure, magnetic and electric field, viscosity effects and the difference in concentration between DNA/drug solutions crystallizing solution also are significant factor in DNA crystallization. In addition, nucleation and crystal growth can be affected by the method used. Therefore it better to attempt different methods to achieve optimum crystallization. The most common method is the vapour diffusion technique which was investigated in this study.[98,99]

1.3.4.4. Vapour diffusion crystallization

This technique is suitable for small volumes less than 2 µl and was used for the first time to crystallize transfer ribonucleic acid (tRNA). In this technique the vapour pressure of a droplet containing biological macromolecule, buffer, crystallizing agent, additives, and any drug expected to bind, and the vapour pressure of a reservoir (containing a solution of crystallizing agent which is more concentrated than the droplet) are allowed to reach to equilibrium by diffusion of the volatile substances such as organic solvent or water molecules from the droplet. Vapour diffusion crystallization can involve different methods which include, hanging drop, sitting drop and sandwich drop as seen in (Figure 19).[99,37] In the present study the hanging drop method was employed.

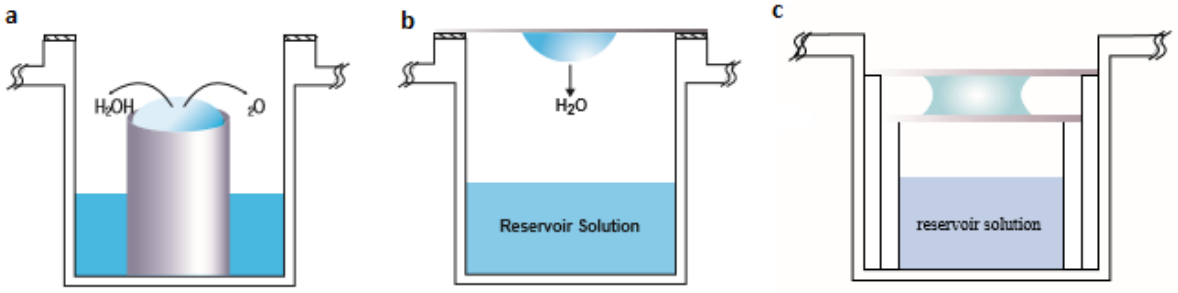


Figure 19. Vapour diffusion crystallization methods. a) Sitting drop, b) hanging drop, c) sandwich drop.[37]

1.4. Fluorinated heterocyclic compounds as protein kinases inhibitors

Due to toxicity of many anticancer drugs to normal tissues and various side effects of them the medicinal chemist is constantly trying to design and develop new therapeutic agents for the treatment of cancers with high selectively. An important area where selectivity is hoped for is inhibition against protein kinases and receptors.[100-102]. Inhibition of receptors or kinases is one of the greatest pathways to treat cancers due to their significant role of them in cell proliferation.[103]

Cancer cells vary from normal cells in their way of communicating with their neighbours because of abnormality in their signalling network. Therefore the understanding of abnormal signal transduction as the source of the altered phenotype of cancer cells has led to more attention to emerging therapies targeting this abnormality,[104] and identification of these signalling pathway is very important in signal transduction therapy.[105] One of the most important signalling elements are protein tyrosine kinases (PTKs) which were recognized as key drivers of cancer cells in the late 1980s. Protein tyrosine kinases (PTKs) show a crucial role in the regulation of cell proliferation, differentiation, metabolism, migration, and survival. They are classified as receptor PTKs and non-receptor PTKs. Selective receptor and non-receptor PTK inhibitors are known to function as anti-tumor agents. These agents are indicated to prevent various features of cancer cell development, including propagation, survival, incursion, and angiogenesis.[106]

Also the groups of serine /threonine kinases, cyclin dependent kinase (CDKs), Erks, Raf,and PKB/Akt, are very important in cell proliferation, cell division, and anti-apoptotic signalling. In addition EGFR (epidermal growth factor receptor), PDGFR (platelet derived growth factor receptor), and VEGFR (vascular endothelial growth factor receptor), were targeted having established their involvement in various malignancies.[107]

For example, the Ras-Raf-Mek-Erk pathway is one significant pathway in the hallmark of many cancers which can be activated by receptor-linked tyrosine kinases such as the epidermal growth factor receptor (EGFR). Therefore the inhibition of Raf, Mek, and Erk are very significant and useful for treatment of many cancers as their activities are highly improved because of the extensive oncogenic mutations in ras (Figure 18).[108-110] For example sorafenib (a Raf kinase inhibitor) can be useful in preventing activating mutations in B-Raf which occur in 66% of human melanomas.[111] Selumetinib (AZD6244) (a Mek kinase inhibitor) is used to treat various types of cancer, such as non-small cell lung (Figure 20). [112]

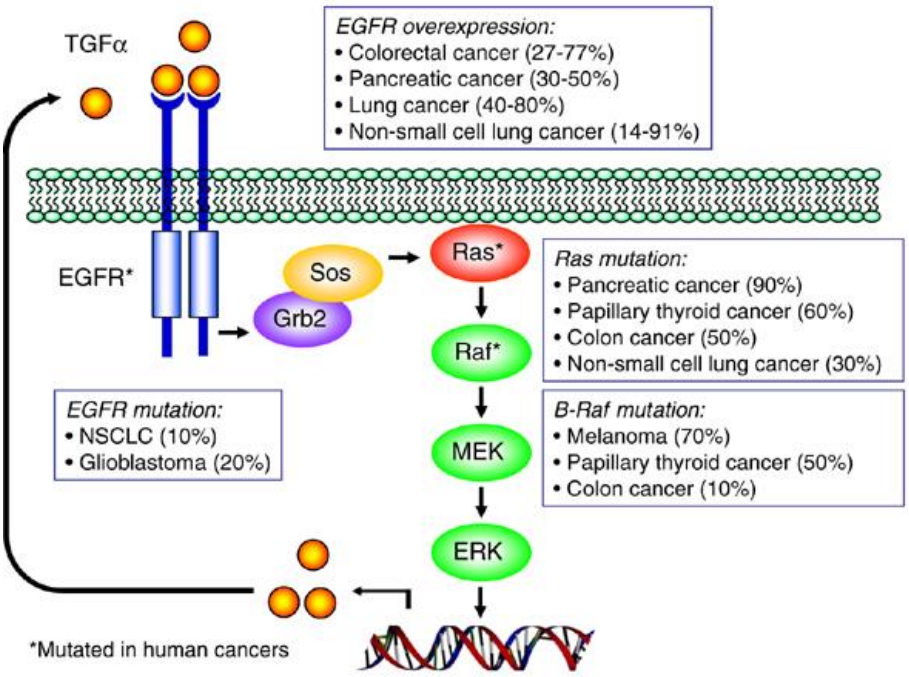
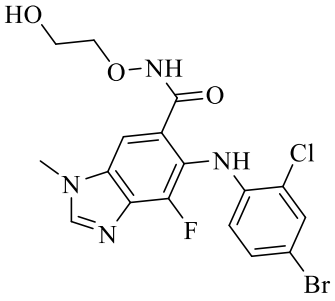


Figure 20. On cogene activation of the ERK MAPK cascade.

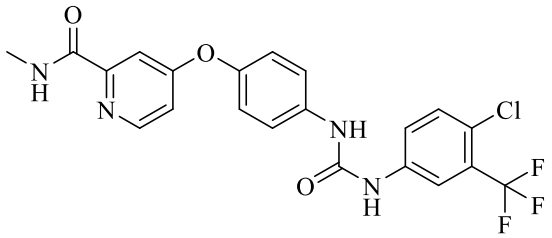
Mutationally activated B-Raf, Ras and mutationally activated and/or overexpressed EGFR causes persistent activation of the ERK MAPK cascade in human cancers. Activated ERKs translocate to the nucleus, where they phosphorylate and control various transcription factors causing changes in gene expression. Especially, ERK-mediated transcription can result in the upregulation of EGFR ligands, such as transforming growth factor alpha (TGF-α), therefore generating an autocrine feedback loop that is critical for Ras-mediated transformation and Raf-mediated gene expression changes.[108]

Also epidermal growth factor receptor (EGFR) is a tyrosine kinase cell-surface receptor for members of EGF-Family which contains an extracellular ligand-binding domain, a transmembrane lipophilic

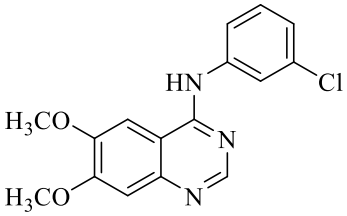
region and an intracellular protein tyrosine kinase domain. EGFR triggered by binding of a specific substrate which leads to cell propagation, invasion and metastasis by activation of signal transduction pathways. By binding EGFR inhibitors to epidermal growth factor receptor the EGFR pathway will be blocked, resulting in inhibition of the proliferation of malignant cells.[113-116] Therefore the role of EGFR in treatment of many cancers is found to be very significant. For example, Iressa and Tarceva are active epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) which prevent the growth and spread of non-small cell lung cancer and pancreatic cancer by blocking the EGFR pathway.[117-119] Although they show some side effects. Quinazoline AG 147829 is another example of EGFR inhibitor which is in clinical development for the treatment of glioblastoma multiforme (GBM) (Figure 20). [115]



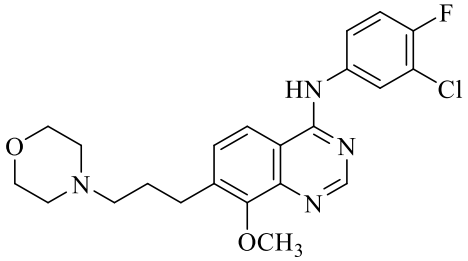
Selumetinib: Mek kinase inhibitor **103**



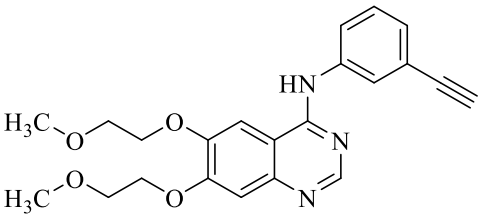
Sorafenib: Raf kinase inhibitor **104**



AG 1498 : EGFR inhibitor **105**



Iressa : EGFR inhibitor **106**



Tarceva: EGFR inhibitor **107**

Figure 21. Protein kinase inhibitors in the clinic and in development

In general this study was aimed at the synthesis of new heteroarene and fluorinated heteroarene compounds with potential bioactivity. In particular for the synthesis of new heterocycles, we planned to exploit the easy S_NAr reaction of fluorinated arenes such as hexafluorobenzene and pentafluropyridine to introduce a range of groups, specially bisimidazole and bisbenzoimidazole derivatives which will allow subsequent ring fusion processes to be carried to generate polycyclic structures, or allow biaryl type links to be set up to generate conformationally flexible polyaryl systems. A further aim was to introduce different water solubilising group into the successfully synthesised polycyclic structures to increase water solubility of the compounds for further

consideration and investigation of the biological activity. The binding properties of the synthesised compounds will be carried out using UV absorption, and ethidium bromide (EB) fluorescence displacement experiments to evaluate interactions between the synthesised compounds as groove or intercalating binding agents with DNA by different binding modes including hydrogen bonding and electrostatic interactions. Biological properties will be assessed by antimicrobial activity assay, and anticancer activity of the synthesised compounds will be investigated against different cancer cell lines. Continuing work should be undertaken to improve biological and anticancer activity of synthesised compounds and to develop the new drugs needed.

2. Results and discussion

As mentioned before fluorinated compounds have shown important improvement in the biological activity of pharmaceutical compounds and drugs in medicinal chemistry. The introduction of a fluorine atom into organic compounds can change the biological properties of the compound and make significant improvement on the biological activity of them by influencing its metabolism. On the other hand N-heterocycles are very important scaffolds found in many biologically active compounds, and drugs containing nitrogen atom play a significant role in interaction of small molecules and drugs target. These compounds have application and their utilization in organic synthesis is important. Therefore this research involves the use of perfluoroarenes, as convenient starting materials to different heterocycles compound especially N-heterocycles for synthesis of novel fluorinated heterocyclic compounds by S_NAr reaction.

2.1. Outcome of organic synthesis

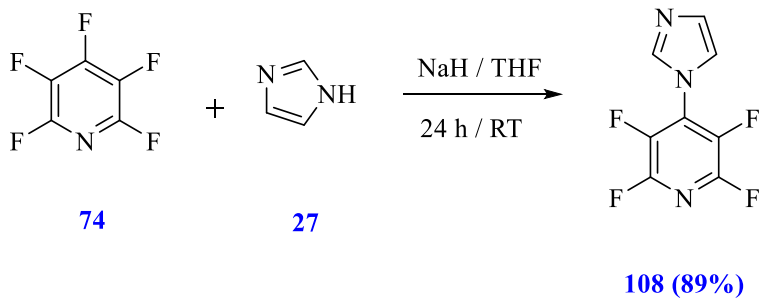
2.1.1. Synthesis of perfluoro heterocyclic compounds

The aim of this study was the synthesis of a range of perfluorobiheteroaryl compounds containing dissimilar heterocyclic rings in order to have possible interaction with DNA and potential biological activity. It was hoped that hetero atoms such as N or S could function as effective nucleophiles to promote S_NAr of the fluorine atoms in a perfluoroarene, and that this would provide an easy and flexible method to rapidly assemble linked multi-ring heterocycles as possible DNA binding agents.

2.1.1.1. Reaction of pentafluoropyridine with imidazole

The aim of this reaction (Scheme 25) was the synthesis of a biheteroaryl compound containing imidazole and a perfluoropyridine ring in order to develop scaffolds with potential to interact with DNA. It was hoped that nitrogen heterocycles such as imidazole could function as effective nucleophiles to promote S_NAr of the fluorine atoms in a perfluoropyridine. The reaction of pentafluoropyridine with imidazole in presence of NaH as base and THF as solvent did not successfully afford the desired product. TLC and NMR revealed that some starting materials were still present with several products formed. Optimization of reaction conditions showed that a large excess of pentafluoropyridine (3 equiv.) was essential to complete consumption of starting material and formation of 2,3,5,6-tetrafluoro-4-(1*H*-imidazol-1-yl)pyridine **108**. The structure of compound

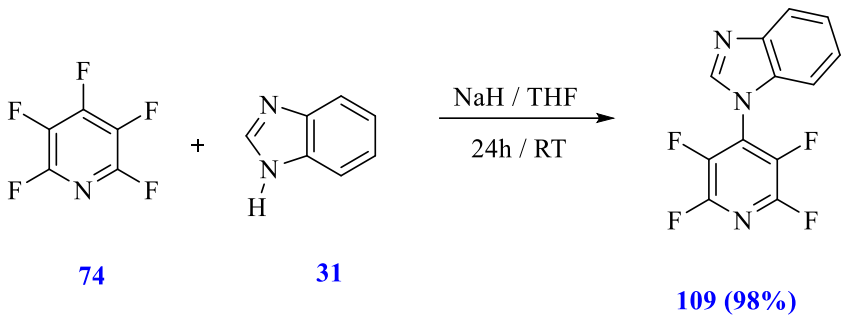
108, formed in 89% yield, was established by its ^{1}H NMR spectrum, with accurate mass measurement of m/z MS (ESI) (MH^{+}), 218.0335 matching $C_8H_3F_4N_3$. In addition the ^{19}F NMR spectrum showed signals for two pairs of fluorine atoms indicating that the tetrafluoropyridine ring was intact. Several crystallization methods were tried to crystallize the product but the compound did was not crystallize, and remained as a yellow oily liquid.



Scheme 25. Reaction of pentafluoropyridine with imidazole.

2.1.1.2. Reaction of pentafluoropyridine with benzimidazole

Initial attempts to make compound **109** using benzimidazole instead of imidazole (Scheme 26) led to its formation in a moderate 55% yield. To try to improve the yield slow addition was investigated. The reaction of pentafluoropyridine with benzimidazole in presence of NaH as base and THF as solvent was completed in 24 h at room temperature using a syringe pump to add the benzimidazole slowly. TLC and ^{1}H NMR spectroscopy indicated the compound **109** was made successfully as a shiny solid in a much improved 98% yield with accurate mass measurement m/z of 268.0491 (MH^{+}) for $C_{12}H_5F_4N_3$. The ^{19}F NMR spectrum showed signals for two pairs of fluorine atoms. The m.p. of the crystals was found to be 138-143 °C and the structure confirmed by x-ray crystallography (Figure 22). Therefore the yield was then improved to 98% by use of a syringe pump allowing a more controlled addition.



Scheme 26. Reaction of pentafluoropyridine with benzimidazole.

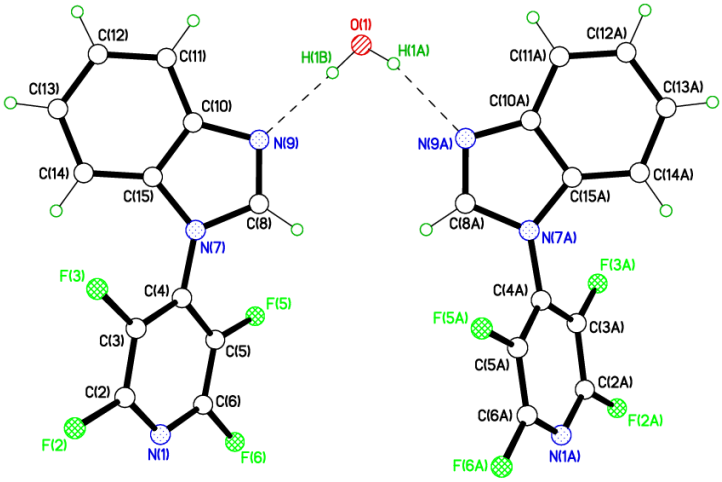
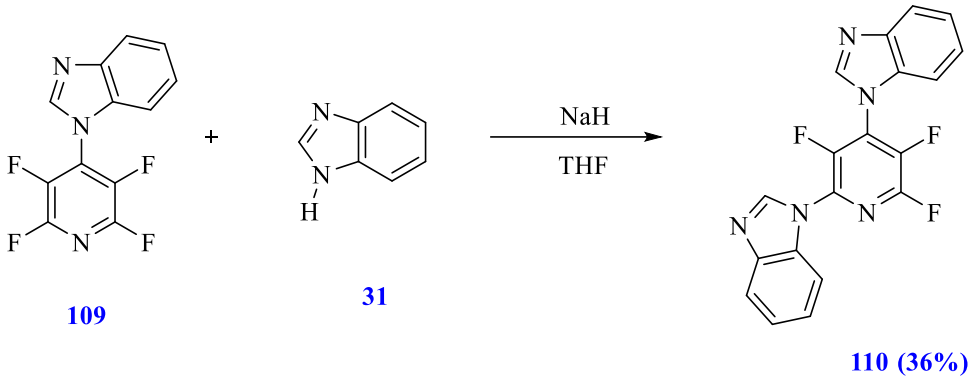


Figure 22. X-ray crystal structure of compound 109 which crystallises with one molecule of water with hydrogen bonds to the N-9 atoms of two benzimidazole rings.

2.1.1.3. Reaction of benzimidazolyltetrafluoropyridine derivative 109 with Benzimidazole

The aim of this reaction was formation of di-product **110** in order to form a linked three ring molecule with better potential as a groove binder for DNA (Scheme 27).



Scheme 27. Reaction of compound 109 with benzimidazole.

Therefore reaction of compound **109** (1 equiv.) with benzimidazole **31** (1 equiv.) was carried out under different conditions (Table 2)

Table 2. Different reaction conditions of compound 109 with benzimidazole

Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 24 h. N_2	Starting material, unknown compound and product 36%
2	NaH	DMF, RT, 24 h. N_2	Starting material and product 30%
3	NaH	THF, reflux, 24 h. N_2	Starting material, unknown compound and product 16%
3	NaH	Dioxane, reflux, 22 h. N_2	Starting material, product 13%

As seen in table 2 entry 1, reaction in THF at room temperature indicated the best yield of target compound **110** which afforded by column chromatography. The structure was confirmed by ^{1}H NMR spectroscopy and accurate mass measurement (ESI) (MH^+), m/z 366.0953 for $C_{12}H_5F_4N_3$ as expected.

The m.p. of the crystals was 186-190 °C. In addition the exact structure of compound **110** was confirmed by X-ray crystallography (Figure 23).

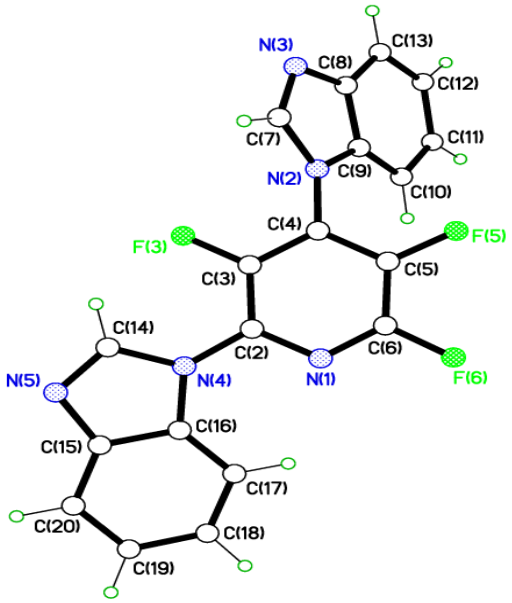
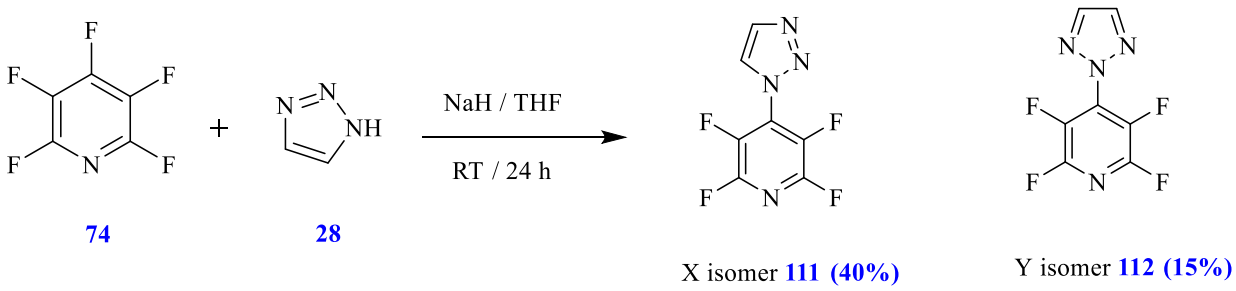


Figure 23. Crystal structure of compound. 110.

2.1.1.4. Reaction of pentafluoropyridine with triazole

The aim of this reaction (Scheme 28) was to complete the synthesis of another biheteroaryl compound containing 1,2,3-triazole and a perfluoropyridine ring as a scaffold with possible interaction with DNA. The reaction of pentafluoropyridine with triazole (ratio 2:1) in presence of NaH as base and THF as solvent for 24 h at RT was carried out successfully. ^{19}F and ^{1}H NMR spectra indicated the presence of two different compounds in the product. The product mixture was purified by column chromatography using 6:4 light petrol:ethyl acetate as eluting solvent to give X isomer **111** as white shiny solid (40%) and 100% ethyl acetate to give Y isomer **112** as shiny light yellow solid (15%). The structures of both isomers were confirmed by ^{1}H NMR and ^{19}F NMR spectroscopy. The ^{1}H NMR perfectly proved the difference between each isomer as the ^{1}H NMR spectrum of isomer X **111** indicated two signals each integrating for 1H, whereas the spectrum of isomer Y **112** showed only 1 signal which integrated for 2H. Also accurate mass measurement of each isomer confirmed the correct structure. Isomer X **111** indicated MS (ESI) (MH^+), m/z 219.0287 for $C_7H_3F_4N_4$ and isomer Y **112** showed MS (ESI) (MH^+), m/z 219.0289 for $C_7H_3F_4N_4$ as expected. In addition the exact structure of compound **111** (X isomer) was confirmed by X-ray crystallography (Figure 24).



Scheme 28. Reaction of pentafluoropyridine with 1,2,3-triazole.

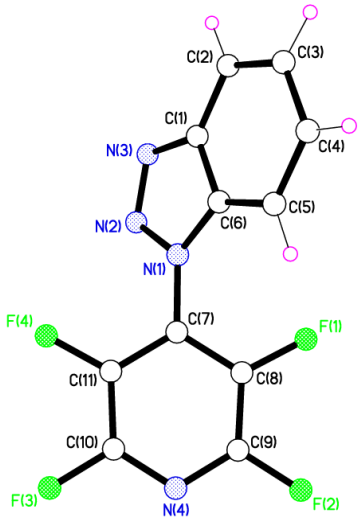
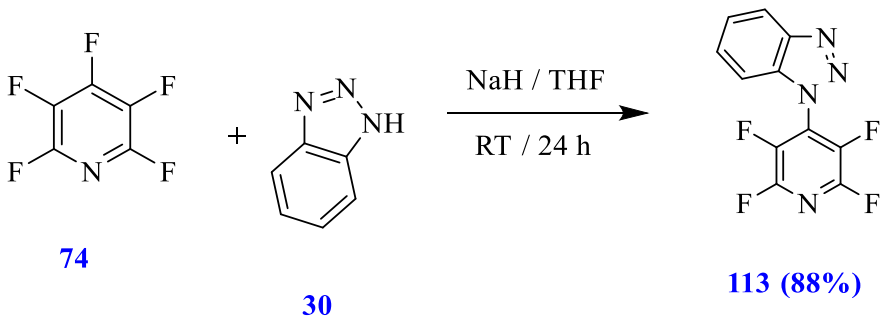


Figure 24. Crystal structure of compound 110.

2.1.1.5. Reaction of pentafluoropyridine with benzotrizole

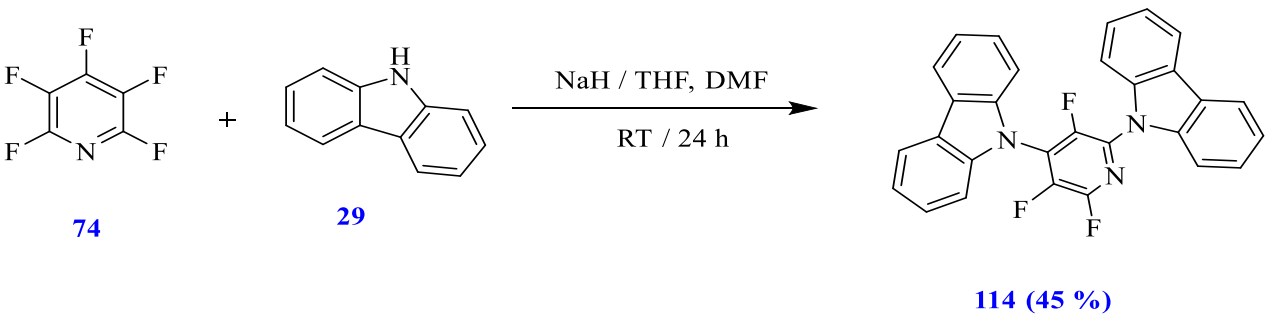
The aim of this reaction (Scheme 29) was to achieve the synthesis of another heteroaryl compound containing benzotriazole and a perfluoropyridine ring in order to have possible interaction with DNA and potential biological activity, as benzotrizole derivatives indicated usefull activity in medicinal chemistry. The reaction of pentafluoropyridine with benzotriazole (ratio 2:1) in presence of NaH as base and THF as solvent for 24h at RT was carried out successfully. The target compound **113** was precipitated by adding the water to give a white solid in 88% yield. The structure was confirmed by the ^{19}F and ^{1}H NMR spectra with the ^{19}F NMR spectrum displaying two signals, each corresponding to 2 F as expected. Also accurate mass measurement (ESI) (MH^+), *m/z* 269.0443 confirmed the expected formula, $C_{12}H_5F_4N_4$.



Scheme 29. Reaction of pentafluoropyridine with benzotriazole.

2.1.1.6. Reaction of pentafluoropyridine with carbozle

Reaction of pentafluoropyridine **74** with carbazole **29** was carried out at RT in the presence of NaH as base and a mixture of THF and DMF as solvents (Scheme 30). TLC analysis showed the presence of the starting carbazole **29**, and column chromatography purification allowed us to get the target product **114** as white crystals in 45 % yield. ^{1}H NMR and ^{19}F NMR spectroscopy proved the presence of the compound **114** with the ^{19}F NMR spectrum displaying 3 signals, each corresponding to 1 F as expected. Also mass spectrometry found the expected mass of the compound MS (ESI) (MH^{+}), $C_{29}H_{17}F_{3}N_{3}$ requires m/z 464.1369 found m/z 464.1370. In addition the exact structure of compound **114** was confirmed by X-ray crystallography (Figure 25).



Scheme 30. Reaction of pentafluoropyridine with carbazole.

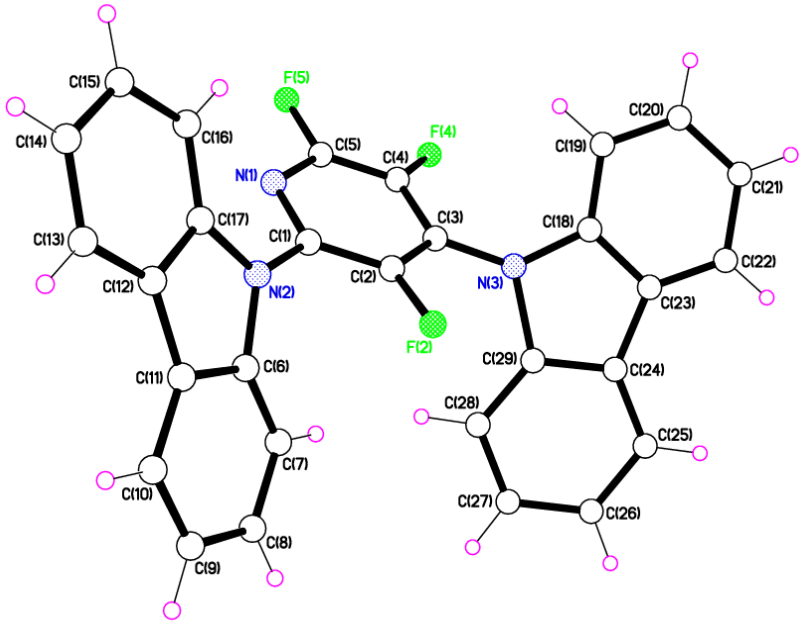
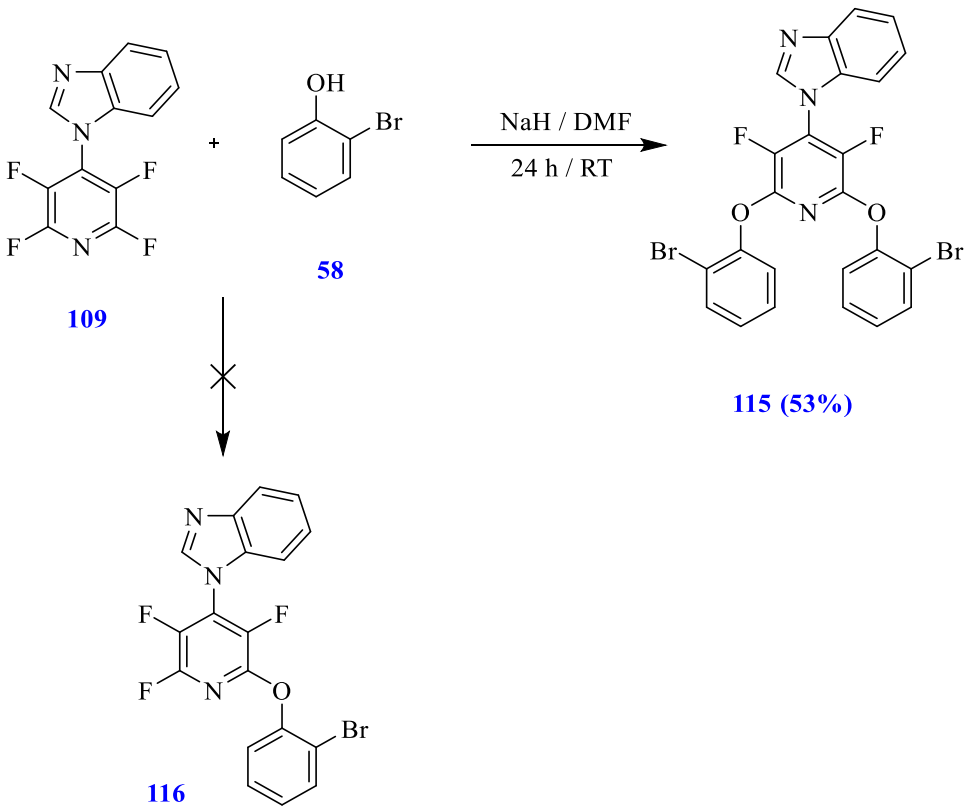


Figure 25. Crystal structure of compound 114.

2.1.1.7. Reaction of 2-bromophenol with compound 109

The aim of this reaction was the synthesis of polycyclic compounds with more than one heteroatom in their chemical structure in order to have potential interaction with DNA and useful biological activity. The reaction of compound **109** with 2-bromophenol in the presence of NaH as base and THF as solvent at room temperature for 24 h did not successfully afford the expected compound **116** (Scheme 31). The ^{1}H NMR and GC mass spectra did not show the product formed. Optimization of reaction condition showed that changing the solvent to DMF was essential to complete reaction and formation of product, although the TLC and NMR analysis showed presence of small quantity of impurity. After recrystalliztion, the product **115**, in which two molecules of bromophenol had added, was formed in 53% yield and confirmed by ^{1}H NMR spectroscopy and accurate mass measurement with *m/z* of 573.9383 for $C_{24}H_{15}Br_2F_2N_3O_2$. Moreover, the ^{19}F NMR spectrum showed one signal for one pair of fluorine atoms indicating that 2,6-disubstitution had occurred. Also product showed a m.p of 178-181 °C.

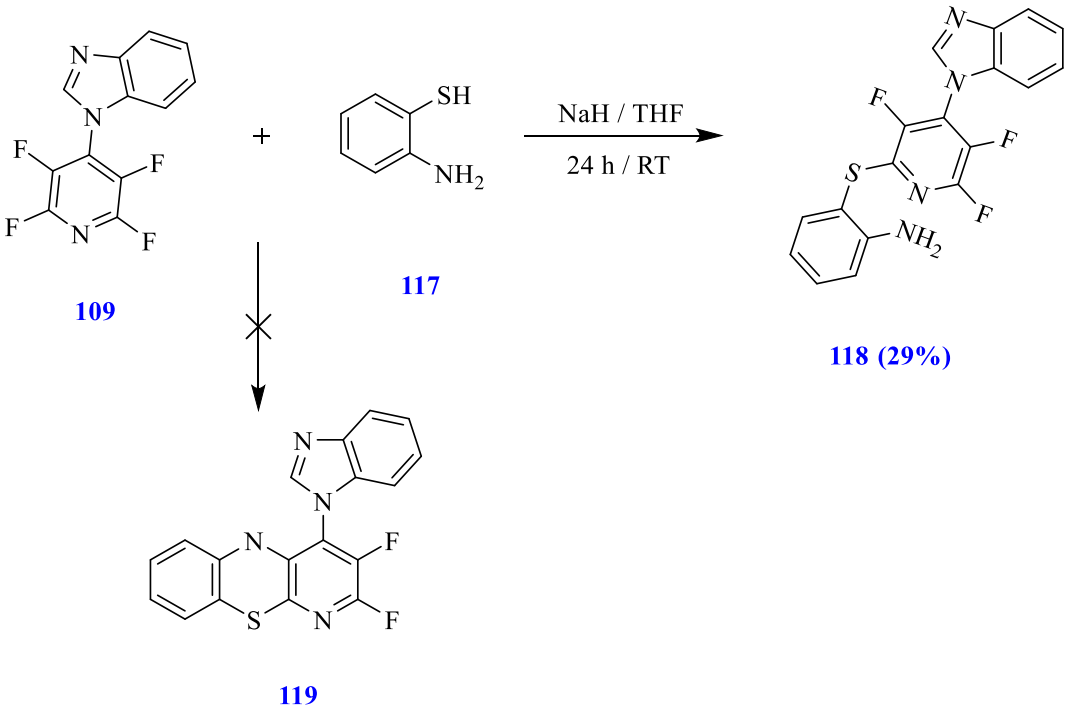


Scheme 31. Reaction of 2-bromophenol with compound 109.

2.1.1.8. Reaction of 2-aminothiophenol with compound 109

The aim of this reaction was synthesis of higher polycyclic compounds with more than one heteroatom in their chemical structure in order to have good interaction with DNA and enhance biological activity. The reaction of compound **109** with 2-aminothiophenol **117** in the presence of NaH as base and THF as solvent at room temperature for 24 h did not successfully afford the expected tricyclic compound **119** (Scheme 32). TLC revealed that some starting material was still present with two products being formed.

After column chromotographic purification the compound **118** was obtained in 29% yield as dark red solid which was confirmed by its ^{1}H NMR spectrum and high resolution mass spectrum (scheme 32). The accurate mass of 374.0728 matched the expected formula of $C_{18}H_{12}F_3N_4S$. While the ^{19}F NMR spectrum showed three signals for three fluorine atoms as expected. Also IR spectroscopy indicated the presence of a NH group by the signal at 3355 cm^{-1}.



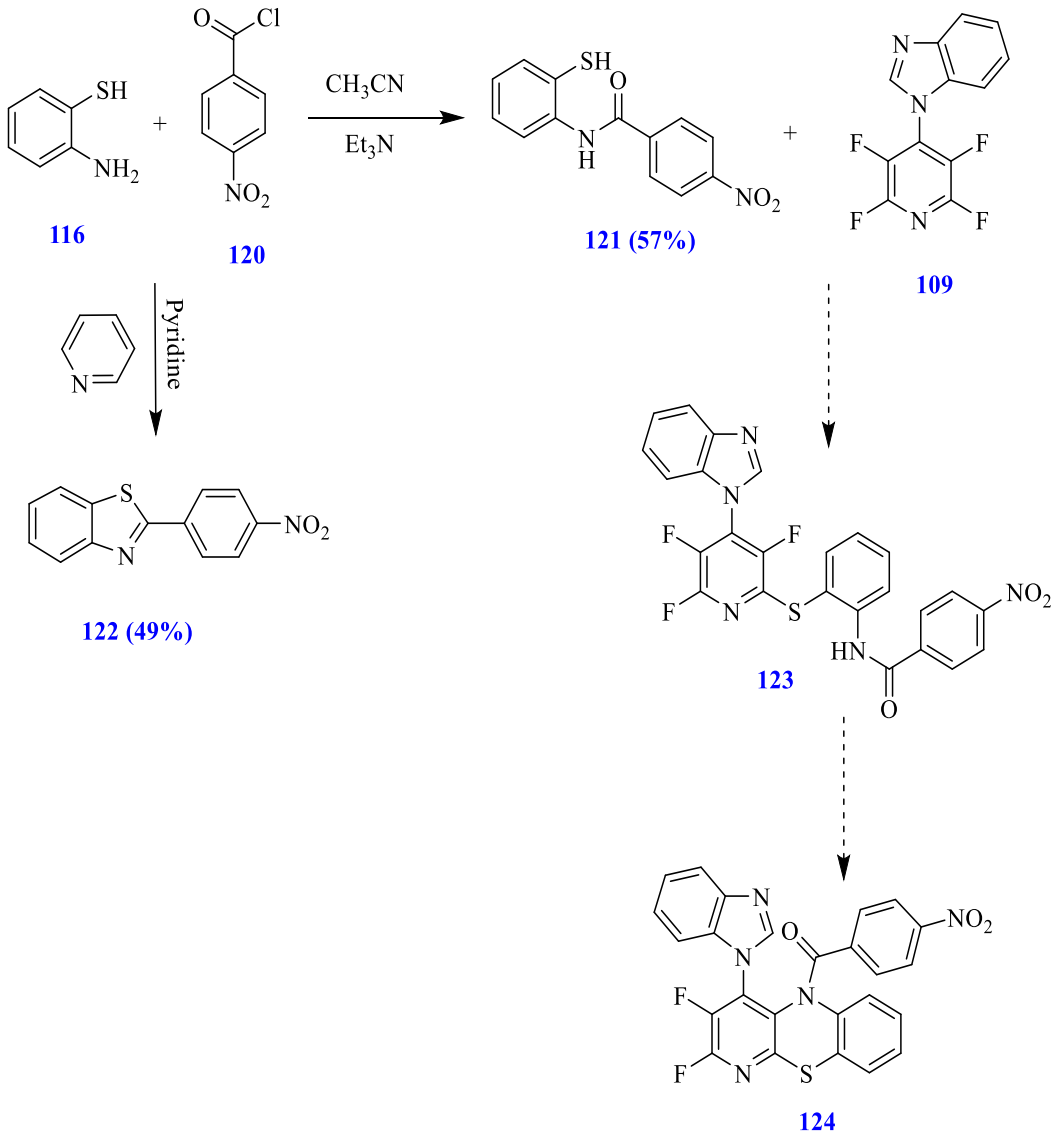
Scheme 32. Reaction of the 2-aminothiophenol with compound 109.

2.1.1.9. Reaction of 2-aminobenzenethiol with 4-nitrobenzoyl chloride

The aim of this reaction was the synthesis of target compound **121** as a starting compound for further reaction with pentafluoropyridine. It was hoped acylating the amino group would render the NH group more acidic and allow deprotonation to activate the nitrogen to nucleophilic attack leading to formation of the desired tricyclic thiazine derivative **124** (Scheme 33).

To synthesise target, *N*-(2-mercaptophenyl)benzamide **121**, 2-aminobenzenethiol was reacted with 4-nitrobenzoyl chloride **120** in the presence of Et_3N (base) and THF (solvent) at room temperature for 24 h. The ^{1}H NMR and mass analysis indicated some impurity and product was formed only in very low yield. Optimization of reaction conditions showed that changing the solvent to acetonitrile (CH_3CN) was essential to give complete reaction and formation of target product in 57% yield, which was confirmed by ^{1}H NMR spectroscopy and accurate mass measurement of 273.0339 for $C_{13}H_{10}N_2O_3S$. In addition the IR data proved the presence of NH and carbonyl groups with peaks at 3348.54 and 1681.98 cm^{-1}. Also the m.p. of the product was 178-181 °C. The thiol **121** was found to undergo easy oxidation in air to the disulfide. This then meant that an additional step to reduce the disulfide bond would be required for the proposed reactions with fluoroarenes.

2-(4-Nitrophenyl)benzothiazole **122** was formed when the reaction of 2-aminobenzenethiol with 4-nitrobenzoyl chloride was carried out in pyridine (as base and solvent) at room temperature for 24 h, showing it was unsuitable as a solvent to form **121** leading to dehydration and ring closure. The structure was confirmed by ^{1}H NMR spectroscopy and accurate mass measurement with *m/z* of 257.0377 for $C_{13}H_8N_2O_2S$. In addition in the IR spectrum, disappearance of the NH and carbonyl peaks at 3348.54 and 1681.98 cm^{-1} proved that cyclization of compound **121** had occurred, and compound **122** had formed. The m.p of the product was 224-229 °C. Due to the difficulty in preparing **121** and its easy aerial oxidation to the corresponding disulfide, it was not possible to study its reaction with **109** to make fused products such as **124**.



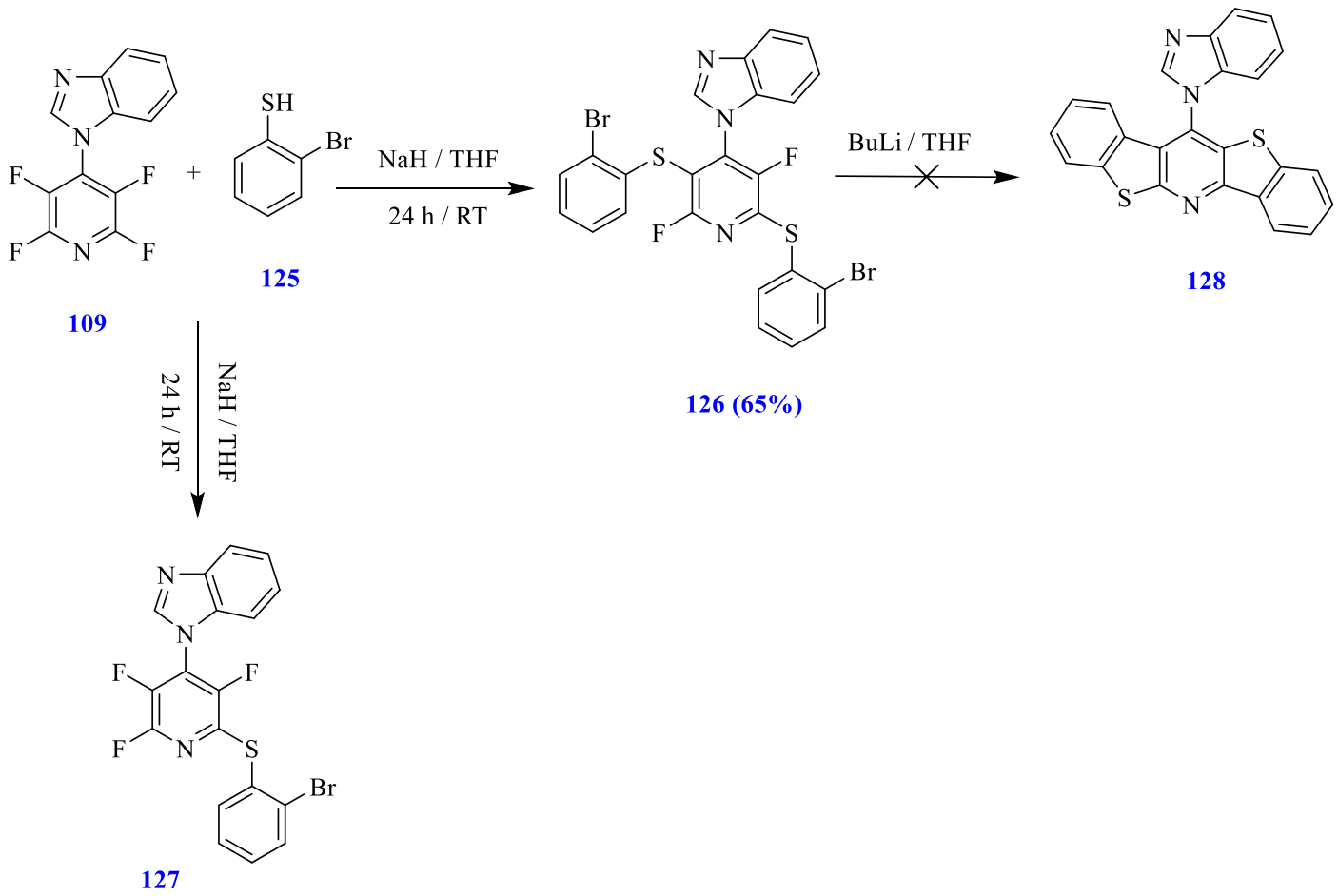
Scheme 33. Reaction of 2-aminobenzenethiol with 4-nitrobenzoyl chloride.

2.1.1.10. Reaction of 2-bromothiophenol with compound 109

The aim of this reaction was the synthesis of condensed polycyclic compounds which contain more than one electronegative atom such as nitrogen, fluorine, or sulphur in their chemical structure. Introduction of a 2-bromophenylthio- or 2-bromophenoxy-groups has been shown to be a viable method to effect fusion of a benzothiophene or benzofuran ring by bromine-lithium exchange, and intermolecular S_NAr reaction. The reaction of 2-bromothiophenol **125** with compound **109** in presence of NaH as base and THF as solvent was thus carried out over 24 h at room temperature. TLC and NMR revealed that some starting material (compound **109**) was still present. However after recrystallization from hot ethanol the formation of difluoropyridine derivative **126** in 65% yields was confirmed by ^{1}H NMR spectroscopy and accurate mass measurement at 603.8952 for $C_{24}H_{13}Br_2F_2N_3S_2$. The ^{19}F NMR spectrum showed two signals for two fluorine atoms as expected for the 2,4,5 trisubstituted pyridine. Also the m.p. of the crystals was 166-170 °C. In addition the exact structure of **126** was confirmed by X-ray crystallography (Figure 26).

Cyclisation of dipyridine derivative **126** (Scheme 34) was attempted with the hope of forming the bis-benzothiophenopyridine **128** but this could not be achieved by using BuLi to effect bromin-lithium exchange.

To minimize the formation of the difluoropyridine derivative **126** and encourage formation of target compound **127**, the reaction was repeated under different conditions. Increasing the amount of tetrafluoropyridine derivative **109** (2 equiv.), and decreasing the time of reaction at 0 ºC was studied. The NMR spectra and accurate mass indicated that even under these conditions still compound **126** was still the main product and compound **127** did not form.



Scheme 34. Reaction of 2-bromothiophenol with compound 109.

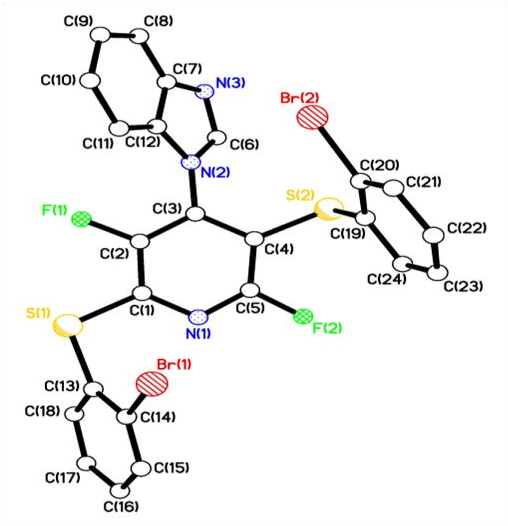
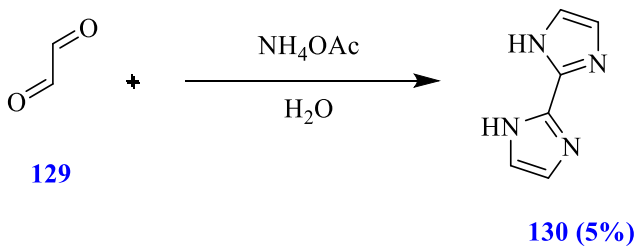


Figure 26. crystal structure of compound 126.

2.1.2. *Synthesis of Bis N-heterocyclic compounds as starting materials for reaction with fluoroarenes*

2.1.2.1. *Reaction of glyoxal with ammonium acetate* [120]

The aim of this reaction was synthesis of a bis-imidazole derivative by the condensation of glyoxal **129** (an α-dicarbonyl aldehyde compound) with two equivalents of ammonium acetate in order to use as starting material for further reaction with perfluorinated arenes.[(2)] The known 1*H*,1'*H*-2,2'-biimidazole **130** was formed in a low 5% yield. The structure was confirmed by ^{1}H NMR spectroscopy and by its mass spectrum with *m/z* 135.0668 for $C_6H_7N_4$. Also IR spectroscopy indicated the present of a NH group by showing a signal in the region 3000-3500 cm^{-1}. Although the yield is low, the product was easy to isolate by simple filtration and the starting materials were cheap.



Scheme 35. Reaction of glyoxal with ammonium acetate

2.1.3. *Synthesis of 2,2-(1,4-butanediyl)-bis-1H-imidazole and 2,2-(1,4-butanediyl)-bis- 1H-benzimidazole*

As the 1*H*,1*H*-2,2'-biimidazole **130** did not dissolve in most organic solvents and showed low reactivity towards perfluorinated compounds, it was decided to prepare a linked bis-imidazole with a flexible spacer as such compounds were of interest as possible cross-linking agents. *2,2-(1,4-butanediyl)-bis-1H-imidazole,* **132** with long chain hydrocarbon linker to increase their solubility were thus prepared.

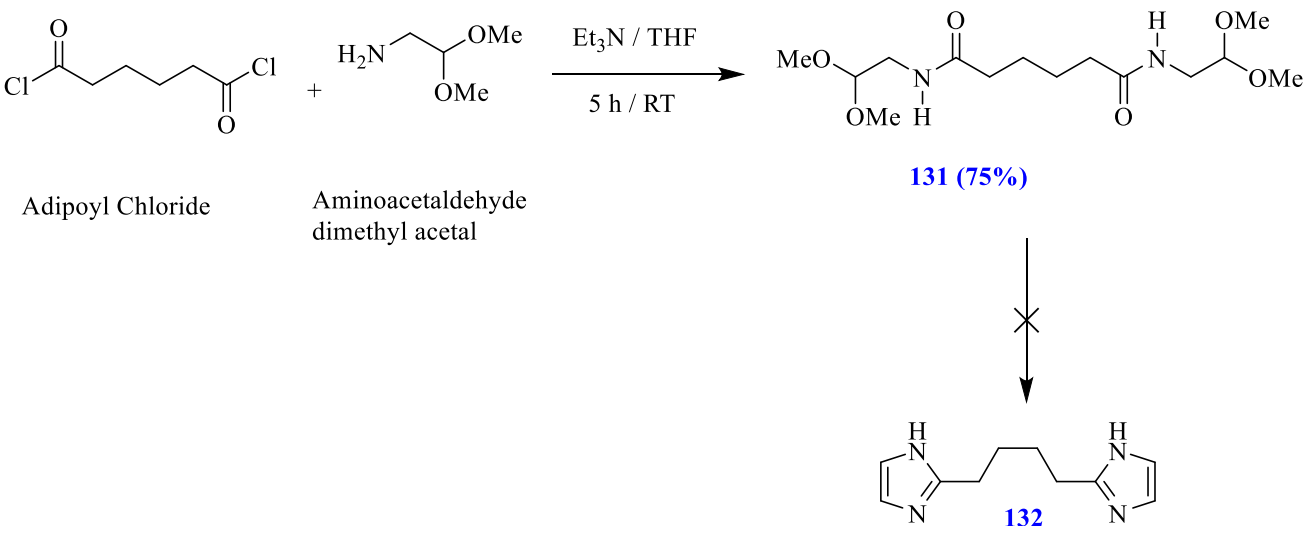
2.1.3.1. *Reaction of adipoyl chloride with aminoacetaldehyde dimethyl acetal*

The aim of this reaction was to synthesise intermediate compound **131** and to investigate cyclisation with acid to prepare target compound **132**. The reaction of adipoyl chloride with aminoacetaldehyde dimethyl acetal in the presence of Et_3N as base and THF as solvent for 5 h at room temperature

successfully afforded the desired product. The compound **131** was formed in 75% yield as white solid and its structure confirmed by its ^{1}H NMR spectrum and by its mass spectrum with *m/z* 321.2019 (MH^{+}) for $C_{14}H_{29}O_6N_2$. Also the IR spectrum indicated the present of NH group by showing the signal in area 3000-3500 cm^{-1} (broad peak). The compound **131** had an m.p of 93-95 °C. The reaction was followed by treating the successfully made compound **132** with different acid conditions (Table 3) with the aim of converting the acetyl groups to aldehydes and promoting cyclisation to the imidazole target compound **132.** Unfortunately the reactions were unsuccessful, and none of the conditions tried led to formation of target compound**132** as seen in (Scheme 36).

Table 3. different reaction conditions for cyclization of compound 132

Entry	Reagents	Conditions	Results
1	AcOH / NH_4OAc	80 °C, 24 h. N_2	Unsuccessful
2	PPA / NH_4OAc	80 °C, 24 h. N_2	Unsuccessful
3	H_2SO_4/AcOH / NH_4OAc	80 °C, 24 h. N_2	Unsuccessful



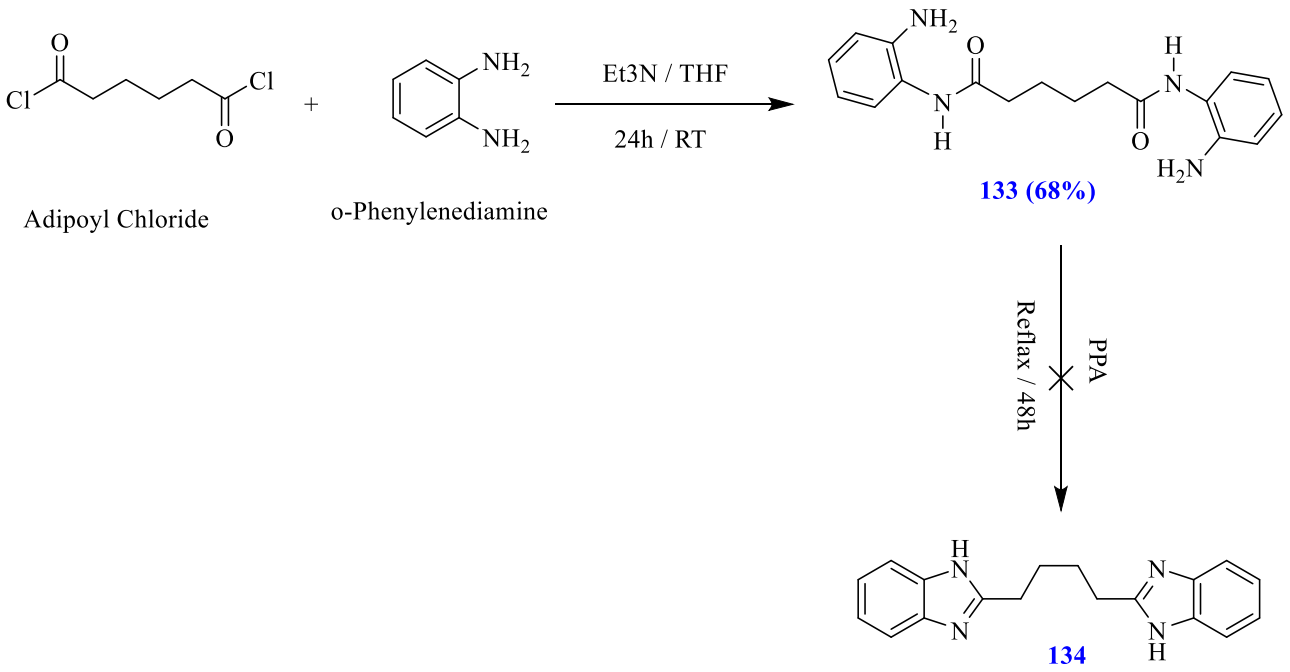
Scheme 36. Reaction of adipoyl chloride with aminoacetaldehyde dimethyl acetal

2.1.3.2. Reaction of adipoyl chloride with o-phenylenediamine

The aim this reaction was the synthesis of the linked bis-amide compound **133** and its cyclisation with acid to prepare the target linked benzimidazole **134**. The reaction of adipoyl chloride with *o*-phenylenediamine *in* the presence of Et_3N as base and THF as solvent for 24 h at room temperature

successfully afforded compound **133** which was formed in 68% yield as a white solid. The structure was confirmed by ^{1}H NMR spectroscopy and by its mass spectrum with *m/z* 327.1814 (MH^{+}) for $C_{18}H_{23}O_2N_4$. Also the IR spectrum indicated the presence of an NH group by signals in the area 3000-3500 cm^{-1} (broad peak). The compound **133** had a m.p. of 155-165 °C.

The reaction was followed by treating the compound **134** with polyphosphoric acid (PPA) at 85 °C for 48 h. ^{1}H NMR spectroscopy indicated presence of both cyclised and non-cyclised compounds which unfortunately could not be separated to isolate the target compound due to the high polarity of both compounds.



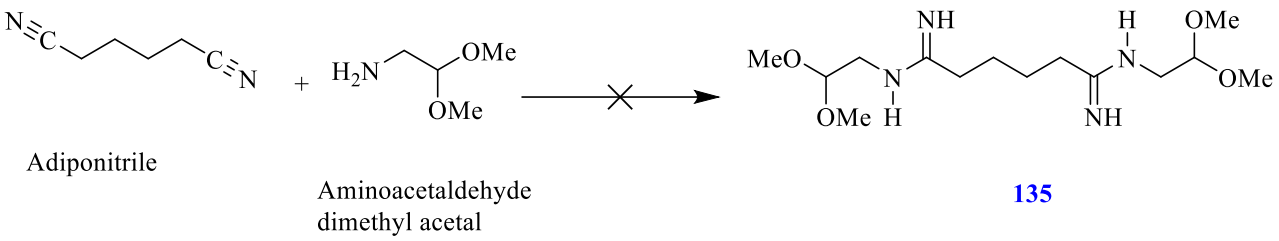
Scheme 37. Reaction of adipoyl chloride with aminoacetal aldehyde methyl ester.

2.1.3.3. Reaction of adiponitrile with aminoacetaldehyde dimethyl acetal

As synthesis of compound **132** by using the adipoyl chloride was not successful as shown in scheme 36, it was decided to study the reaction of adiponitrile, to change the carbonyl group in compound **131** to an imine which might help cyclisation and allow preparation of target compound **132**. Therefore the reaction of adiponitrile with aminoacetaldehyde dimethyl acetal was carried out under different conditions (Table 4). However, the reactions did not work at all and ^{1}H NMR spectroscopy indicated only the presence of starting material (adiponitrile) and the target compound **135** did not formed.(Scheme 38).

Table 4. different reaction conditions attempted for the formation of compound 135.

Entry	Reagents	Conditions	Results
1	$Cu(OTf)_2$	THF /reflux, 24 h, N_2	no reaction
2	-	THF/reflax, 24 h, N_2	no reaction
3	-	reflux, 24 h, N_2	no reaction



Scheme 38. Reaction of adiponitrile with aminoacetaldehyde dimethyl acetal.

2.1.3.4. Synthesis of imidate and formation of the target compound 132 and 134

The aim of these reactions was formation of imidate **136** as starting material to react with aminoacetaldehyde dimethyl acetal and *o*-phenylenediamine to allow synthesis of target compounds **132** and **134** which could not be formed using the conditions above**.**

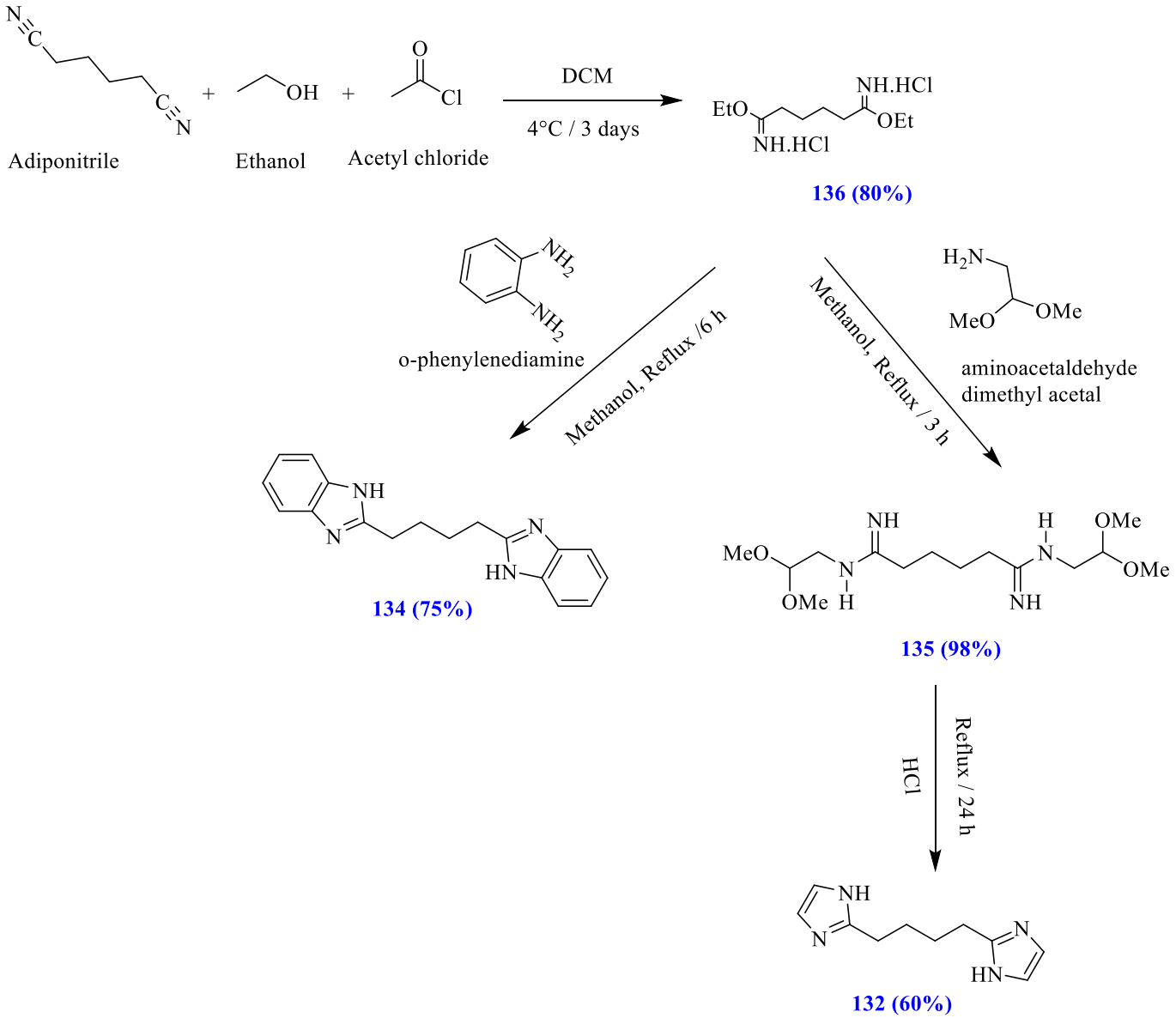
The reaction of adiponitrile with ethanol and acetyl chloride in DCM was completed in 3 days at 4 °C. The imidate salt **136** formed as white solid in 80% yield and was confirmed by ^{1}H NMR spectroscopy. Also the IR spectrum indicated the present of a NH group by showing a signal at 3000-3500 cm^{-1}. Compound **136** had a m.p. of 135-136 °C.

The intermediate compound **136** then reacted successfully with aminoacetaldehyde dimethyl acetal in methanol as solvent at 65 °C for 3 h. The compound **135** was formed as a yellow syrup in 98% yield, and was confirmed by its ^{1}H NMR spectrum and accurate mass measurement with *m/z* of 319.2315 (MH^+) for $C_{14}H_{31}O_4N_4$. Also the IR spectrum indicated the present of the NH group with a signal at 3000-3500 cm $^{-1}$.

Compound **135** was treated successfully with 37% HCl at 65 °C for 24 h to synthesise target bis-imidazole **132** as a brown solid in 60% yield (Scheme 39). The structure was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy. However, the ^{1}H NMR spectrum recorded in DMSO showed two singlet

signals at 6-7 ppm for 4H atoms and one singlet signal for NH at 12 ppm (Figure 27) whereas the ^{1}H NMR in methanol only indicated one singlet signal at 6.8 ppm for 4H atom and did not show any signal for NH (Figure 28), which could be caused by hydrogen exchange between two nitrogens in methanol. Accurate mass measurement confirmed the correct molecular formula with *m/z* of 191.1296 (MH^{+}) for $C_{10}H_{13}N_4$. The compound **132** failed to melt, but decomposed at 168 °C.

Reaction of the imidate salt with o-phenylenediamine was carried out successfully in the presence of methanol for 6 h at 80 °C (Scheme 38). The linked benzimidazole **134** formed as a light yellow solid in 75% yield and was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy and accurate mass measurement with *m/z* of 291.1581 (MH^{+}) for $C_{18}H_{18}N_4$. Also IR spectrum indicated the presence of NH group with a signal in area 3000-3500 cm $^{-1}$. The compound **134** m.p. was 258-260 °C in agreement with the literature value of 258 °C. [31] In addition the exact structure of **134** was confirmed by X-ray crystallography (Figure 29).



Scheme 39. Synthesis of bis-imidate to form linked imidazole and benzimidazoles 132 and 134.

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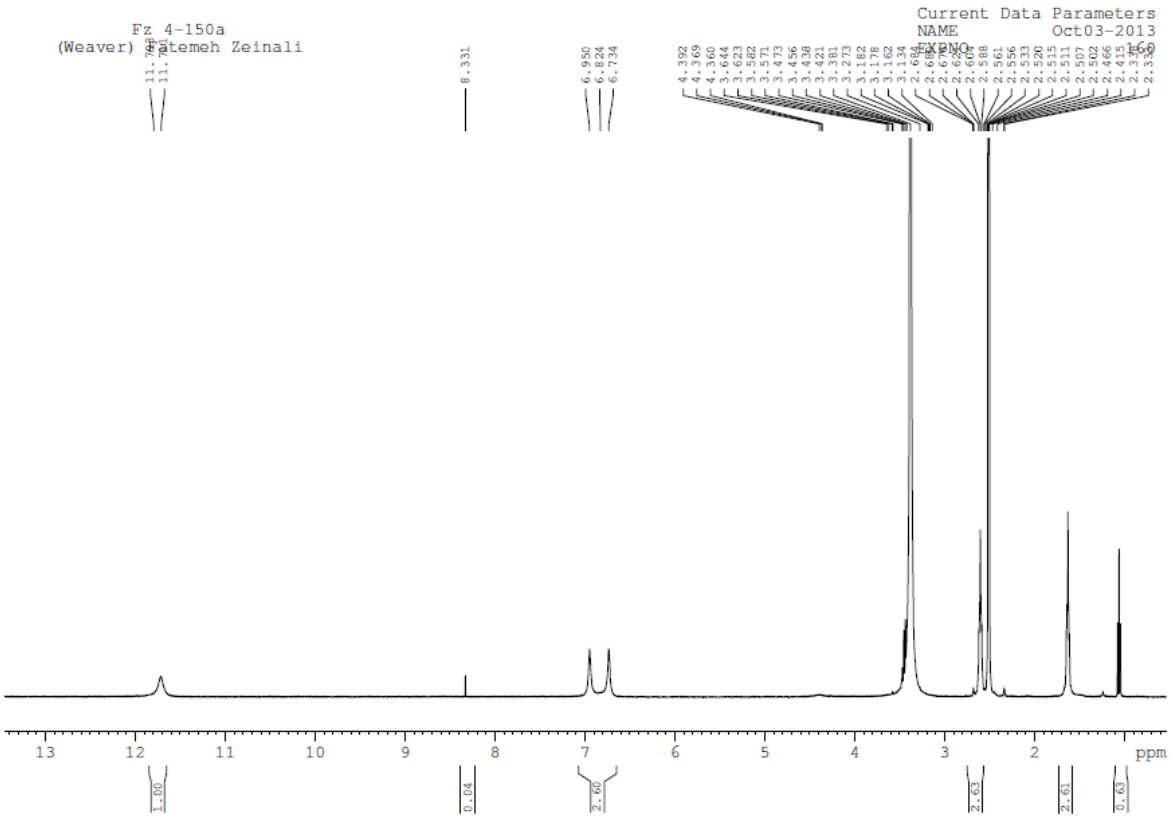


Figure 27. ^{1}H NMR (400 MHz, DMSO-d6) spectrum of compound 132.

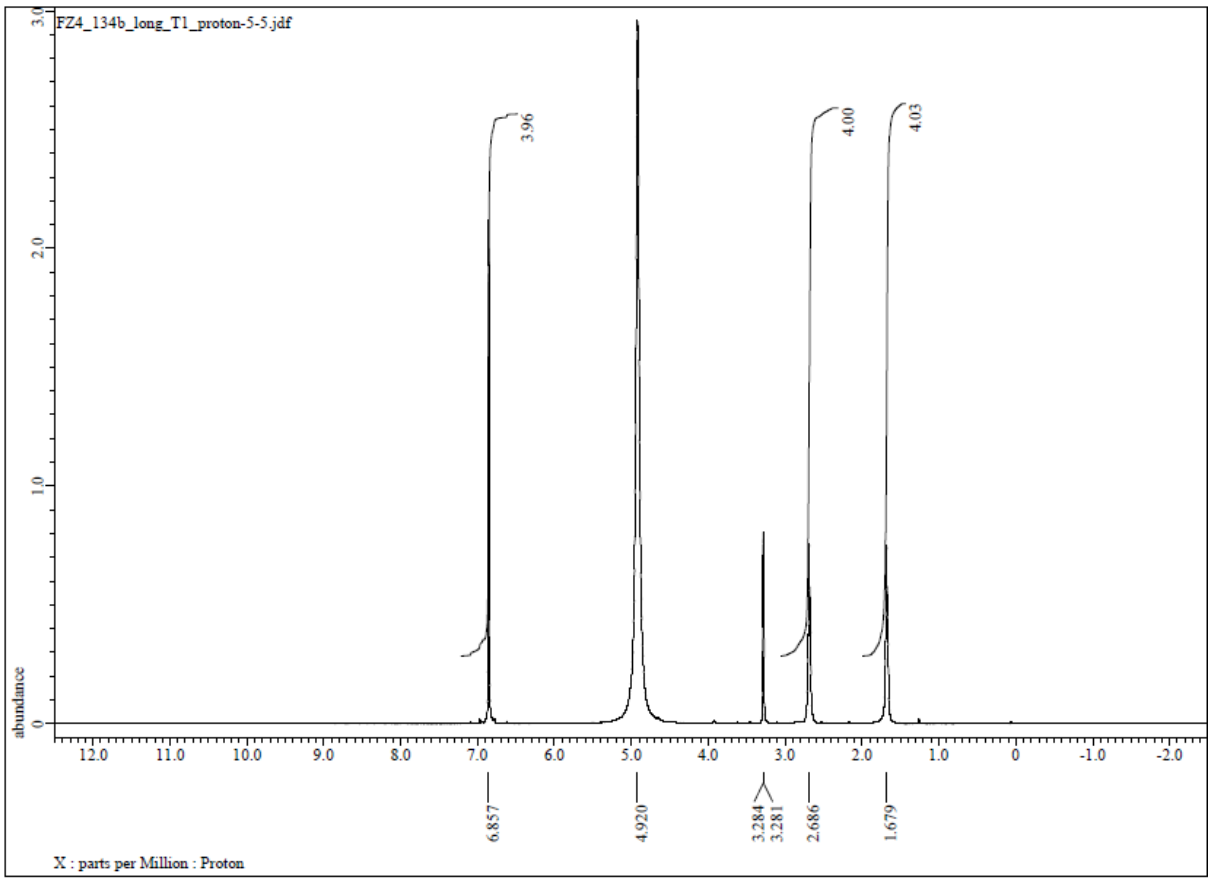


Figure 28. ^{1}H NMR (400 MHz, methanol-d$_4$) spectrum of compound 132.

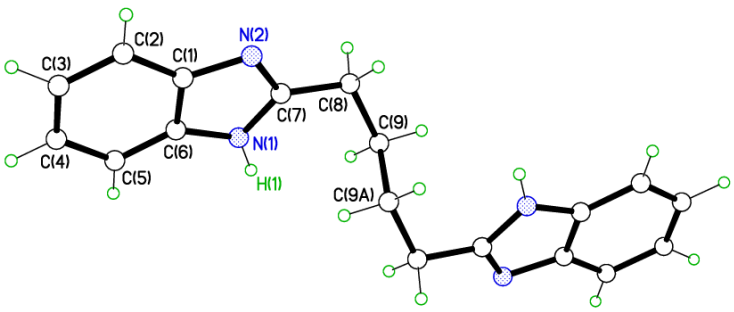


Figure 29. Crystal structure of compound 134.

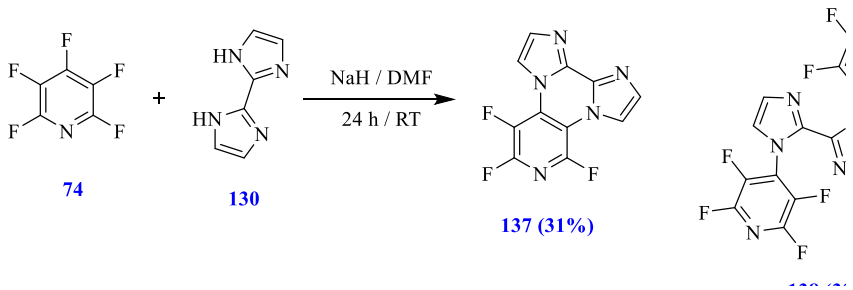
2.1.4. Synthesis of bis-imidazole and bis-benzimidazole containing perfluoro heterocyclic derivatives

Nitrogen containing heterocyclic aromatic compounds such as bis-imidazole and bis-benzimidazole are an important type of nitrogen containing heterocyclic aromatic substances in drug design and synthesis. Also they are of importance in medicinal chemistry due to their biological activity. Moreover, several functional groups are easily introduced into the structurally rigid of bis-imidazole and Bis-benzimidazole ring and alter the biological activities allowing using as cross linking agents for double-stranded DNA.[37]

We planned to synthesize compounds which contain more than one bio labile constituent, namely bis-imidazole or bis-benzimidazole and fluorinated containing heterocyclic substances to provide a biologically active DNA binding structure as lead scaffolds.

2.1.4.1. Reaction of the pentafluoropyridine with 1H,1H-2,2'-biimidazole

The reaction of pentafluoropyridine **74** with 1*H*,1*H*-2,2'-biimidazole **130** in the presence of NaH as base and dry DMF as solvent was conducted over 24 h at room temperature. TLC revealed that two compounds formed and some starting material was still present. By adding water compound **138** (0.14 g, 33%) was preciptated and collected as white solid. After an extraction process compound **137** (0.08 g, 31%) was isolated. Their structures confirmed by ^{1}H NMR spectroscopy and accurate mass measurement with *m/z* of 264.0413 (MH^+) for $C_{11}H_5F_3N_5$ for **137**, and 433.0441 (MH^+) for $C_{16}H_5F_8N_6$ matching **138**. The ^{19}F NMR spectra showed three signals (1 F for each signal) for compound **137** and 2 signals (4 F atom for each signal) for compound **138** as expected. Compound **137** had m.p. of 288-295 °C which is much higher than compound **138** (158-164 °C). The structure of **138** was confirmed by X-ray diffraction analysis (Figure 30).



Scheme 40. Reaction of the pentafluoropyridine with 1H,1H-2,2'-biimidazole.

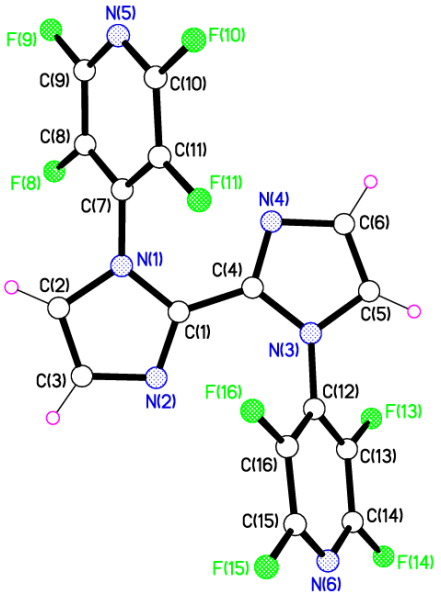
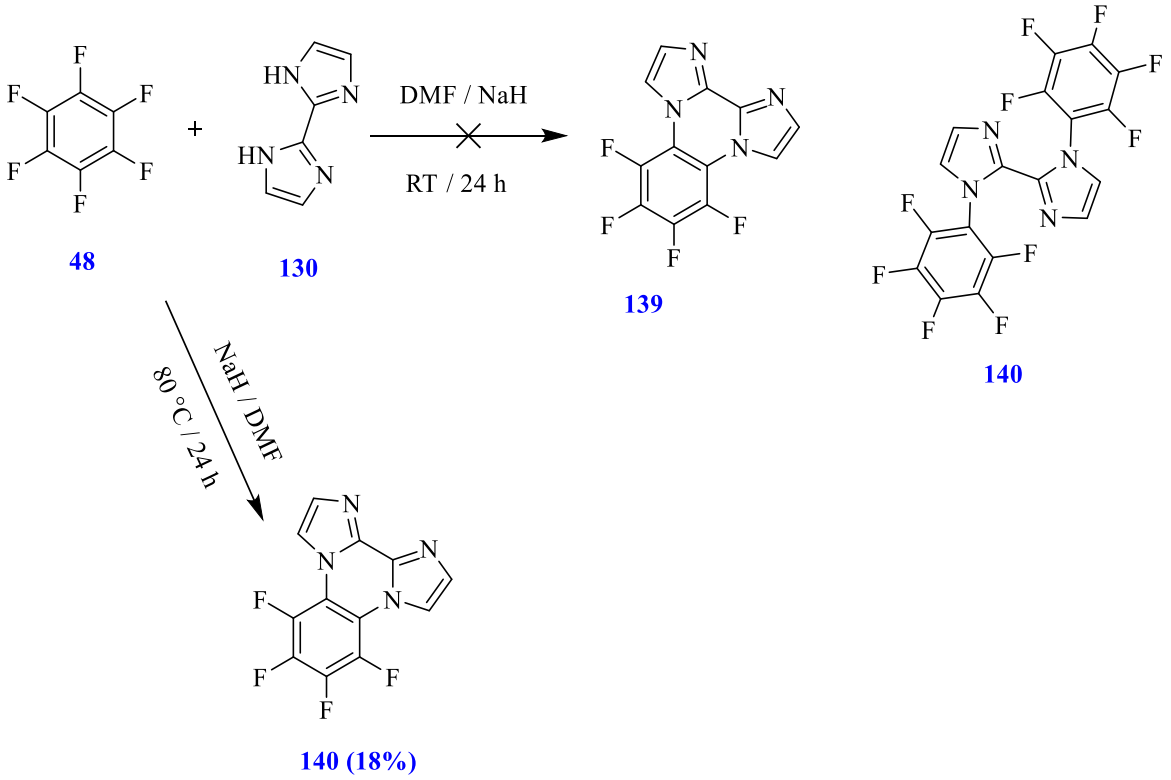


Figure 30. Crystal structure of compound 134.

2.1.4.2. Reaction of the hexafluorobenzene with 1H,1H-2,2'-biimidazole

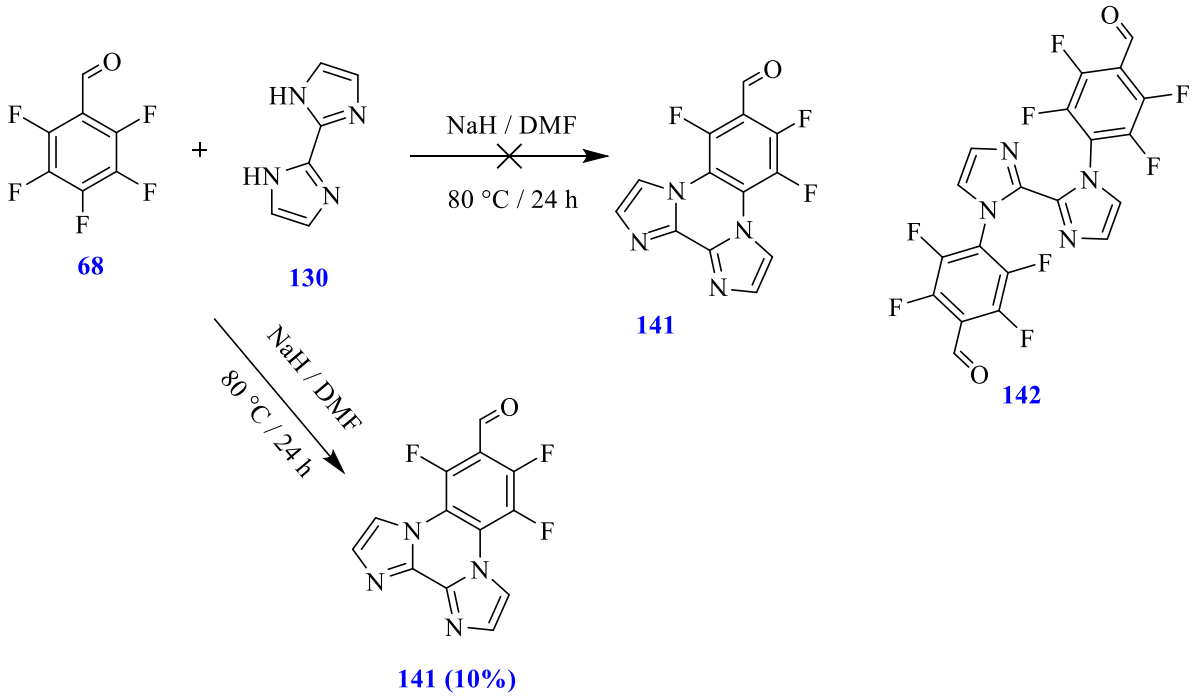
The reaction of hexafluorobenzene **48** (2 equiv.) with 1*H*,1*H*-2,2'-biimidazole **130** (1 equiv.) in the presence of NaH as base and dry DMF as solvent was conducted over 24 h at room temperature. According to the ^{1}H NMR spectrum and TLC the target compound did not form and starting material was present as the main compound. However, the reaction worked at 80 °C in the presence of NaH as base and dry DMF in 1:3 ratio of starting biimidazole and hexafluorobenzene. After recrystallization from hot ethanol the product **139** was obtained as a brown solid (18% yield) and the structure confirmed by ^{1}H NMR spectrum and accurate mass measurement with *m/z* of 281.0433 (MH^{+}) for $C_{12}H_5F_4N_4$. The ^{19}F NMR spectrum showed two signals (2F for each environment) as expected. No evidence for the formation of **140** was obtained.



Scheme 41. Reaction of the pentafluoropyridine with 1H,1H-2,2'-biimidazole.

2.1.4.3. Reaction of the pentafluorobenzaldehyde with 1H,1H-2,2'-biimidazole.

The reactions of pentafluorobenzaldehyde **68** with 1*H*,1*H*-2,2'-biimidazole **130** in 1:1 and 2:1 ratios were attempted with NaH as base and dry DMF as solvent and were conducted over 24 h at room temperature. TLC and NMR of both reactions revealed mixtures of different compound formed. Attempts to separate and purify the products by repeated recrystallization methods were unsuccessful. Attempts to isolate pure product by column chromatography afforded five fractions, but the ^{1}H NMR spectra of all fractions indicated they were still not pure. However, the reaction worked after heating the reactants in DMF at 80 °C for 24 h in the presence of NaH as base and in a 1:1 ratio. After recrystallization from DCM and light petrol the product **141** was obtained as a yellow solid in 10% yield, and the structure confirmed by ^{1}H NMR spectroscopy and accurate mass measurement with *m/z* of 291.476 (MH^{+}) for $C_{13}H_6F_3N_4$. The ^{19}F NMR spectrum showed three signals (1F for each signal) indicating cyclisation had occurred to form **141** rather than **142**.

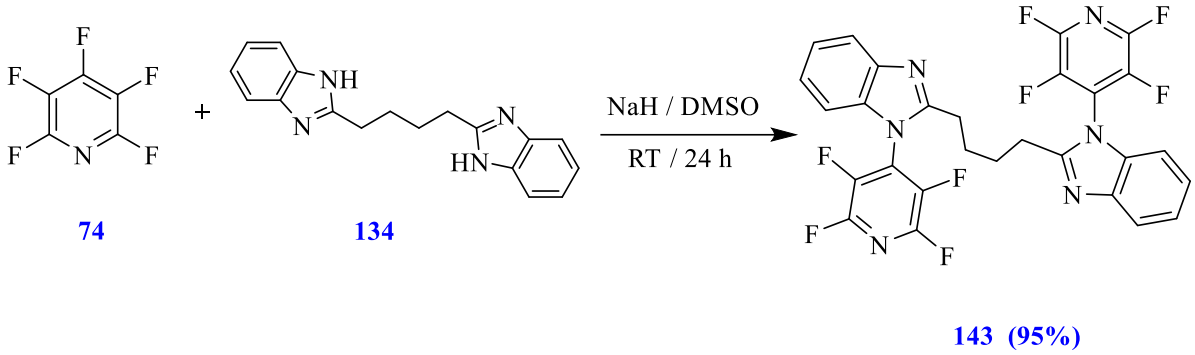


Scheme 42. Reaction of pentafluorobenzaldehyde with 1H,1H-2,2'-biimidazole.

2.1.4.4. Reaction of pentafluoropyridine with Bis-benzoimidazole 134

The reaction of pentafluoropyridine **74** (3 equiv) with bis-benzimidazole **134** (1 equiv.) was studied under two conditions; the first was with NaH as base and dry DMF as solvent over 24 h at room temperature. TLC and ^{1}H NMR spectroscopy indicated a mixture of different compounds. Therefore different crystallization methods were tried to isolate pure product, but they were unsuccessful and the ^{1}H NMR spectrum of the product mixture was messy and complicated.

The second condition employed DMSO as solvent instead of DMF and bis-benzimidazole was added dropwise by syringe pump. TLC revealed one main compound formed with only a trace impurity. After recrystallization from hot toluene, the product **143** (95%) was obtained as a white solid, and the structure assigned by ^{1}H NMR spectroscopy and accurate mass measurement with *m/z* of 589.1376 (MH^{+}) for $C_{28}H_{17}F_{8}N_{6}$. The ^{19}F NMR spectrum showed 2 signals (4 F atom for each signal) as expected. Compound **143**, had a m.p of 197-198 °C.

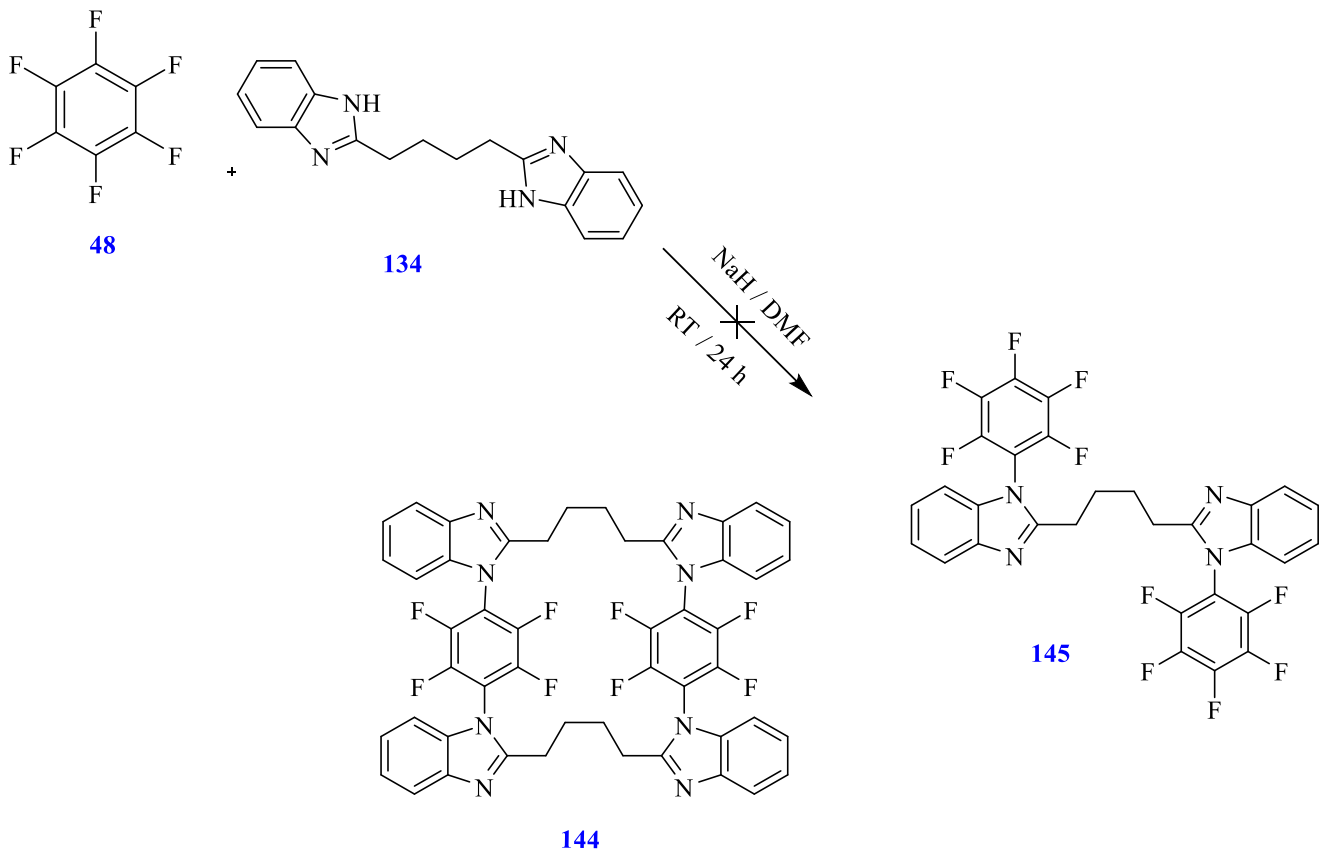


Scheme 43. Reaction of the pentafluoropyridine with bis-benzimidazole 134.

2.1.4.5. Reaction of the hexafluorobenzene with bis-benzimidazole 134

The reaction of hexafluorobenzene **48** with bis-benzimidazole **134** was tried under two conditions; firstly with NaH as base and dry DMF as solvent over 24 h at room temperature using a 10:1 ratio of hexafluorobenzene and bis-benzoimidazole. TLC indicated very polar products were formed and the ^{1}H NMR spectrum showed broad complex signals in the aromatic region. A different recrystallization method were tried but was unsuccessful and neither **144** nor **145** could be obtained.

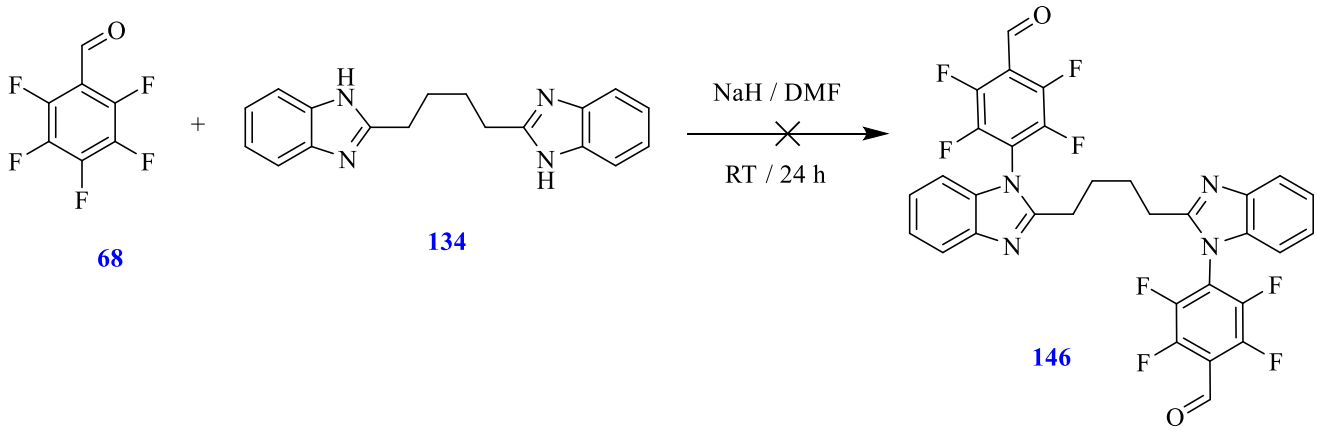
The second experiment used syringe pump addition of the bis-benzimidazole. This time TLC showed many spots which indicated many compounds formed. Recrystallization was attempted but was unsuccessful. Preparative TLC was then employed but only one component of the mixture was isolated pure, but proved to be in a negligible amount to characterise, while other spots were very close together and could not be separated. Also at the base line, preparative TLC showed a fluorescent compound formed, which was isolated but has not yet proved possible to identify.



Scheme 44. Reaction of the pentafluoropyridine with bis-benzimidazole 134.

2.1.4.6. Reaction of pentafluorobenzaldehyde with bis-benzimidazole 134

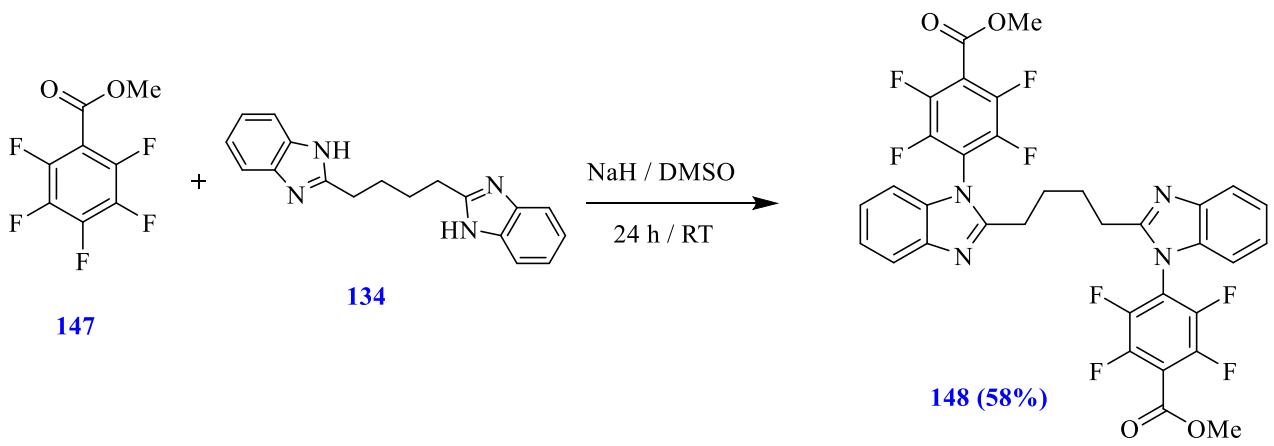
The reaction of pentafluorobenzaldehyde (2 equiv.) with bis-benzimidazole **134** (1 equiv.) was carried out in the presence of NaH as base and dry DMF as solvent over 24 h at room temperature using a syringe pump to ensure slow addition. The compound formed was a red solid which tailed on the TLC plate, and ^{1}H NMR spectroscopy showed only broad, messy peaks in the aromatic reaction. Attempts to isolate a pure product by recrystallization or chromatography were unsuccessful and no evidence for the formation of **146** could be obtained. (Scheme 45)



Scheme 45. Reaction of pentafluorobenzaldehyde with bis-benzimidazole 134.

2.1.4.7. Reaction of compound 134 with methyl pentafluorobenzoate

The reaction of methyl pentafluorobenzoate **147** (2 equiv.) with bis-benzimidazole **133** (1 equiv.) was carried out in present of NaH as base and DMSO as solvent over 24 h at room temperature. TLC revealed one main compound formed with only a trace impurity. After recrystallization from hot ethanol, the product **148** (58%) was obtained as a white solid, and the structure assigned by 1H NMR spectroscopy and accurate mass measurement with m/z of 703.1569 (MH^+) for $C_{34}H_{23}F_8O_4N_6$. The ^{19}F NMR spectrum showed 2 signals (4 F atoms for each signal) as expected. Compound **148**, had a m.p of 195-198 °C



Scheme 46. Reaction of compound 134 with methyl pentafluorobenzoate.

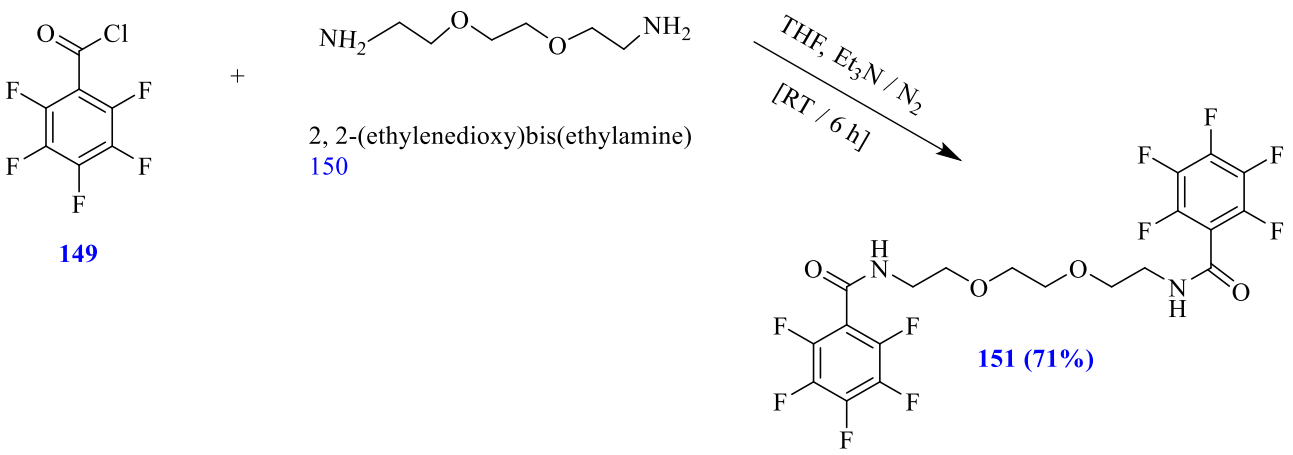
2.1.5. Synthesis of bis-linked binding agents via formation of an amide bond

Organic bis-linked binding agents are compounds that interact with double stranded DNA, usually reversibly. Bis-linked binding compounds contain two possible intercalating ring systems connected together by a linker. Bis-linked binding agents have higher DNA binding constants due to more than one intercalating unit and can exhibit sequence selectivity and show slower dissociation than mono compounds.

Therefore it was planned to exploit the reactivity of the acyl halide group in derivatives of pentafluorobenzoyl chloride towards di-amide formation by reaction with long chain diamines. It was hoped that diamide compounds would act as bis-nucleophiles and allow two potential binding compounds to be linked together to increase their DNA binding potency. The pentafluorophenyl benzamides could also be used as starting compounds for further functionalization to improve activity.

2.1.5.1. Reaction of pentafluorobenzoyl chloride with 2,2-(ethylenedioxy)bis(ethylamine)

In order to synthesise fluorinated bis-intercalating compounds, reaction of pentafluorobenzoylchloride **149** with the aliphatic amine 2, 2-(ethylenedioxy)bis(ethylamine) **150** was investigated in a 2:1 ratio at RT in THF as solvent and with Et_3N as base. The target compound **151** was afforded in 75% yield (Scheme 47). ^{19}F NMR spectroscopy displayed the expected fluorine signals for the target compound, δ_F (376 MHz, $CDCl_3$) 21.14-21.22 (4F, m), 11.04-11.20 (2F, m), 1.79-1.81(4F, m) and mass spectrometry gave the mass of the expected compound **151**, MS (ESI) (MH^+), $C_{20}H_{14}O_4F_{10}N_2$ requires m/z 537.0867, found m/z 537.0867. The structure of **151** was confirmed by X-ray diffraction analysis (Figure 31), m.p. 128 - 129 °C.



Scheme 47. Reaction of pentafluorobenzoyl chloride and 2,2-(ethylenedioxy)bis(ethylamine).

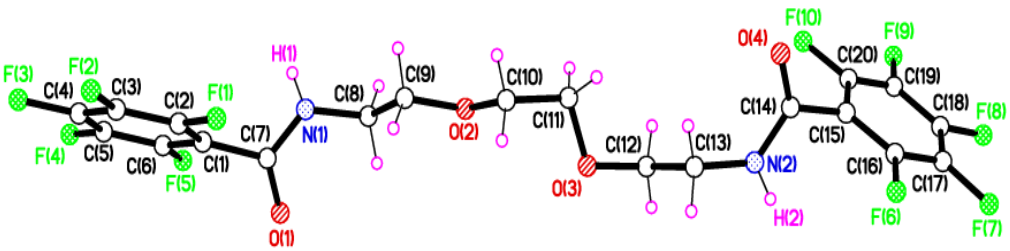


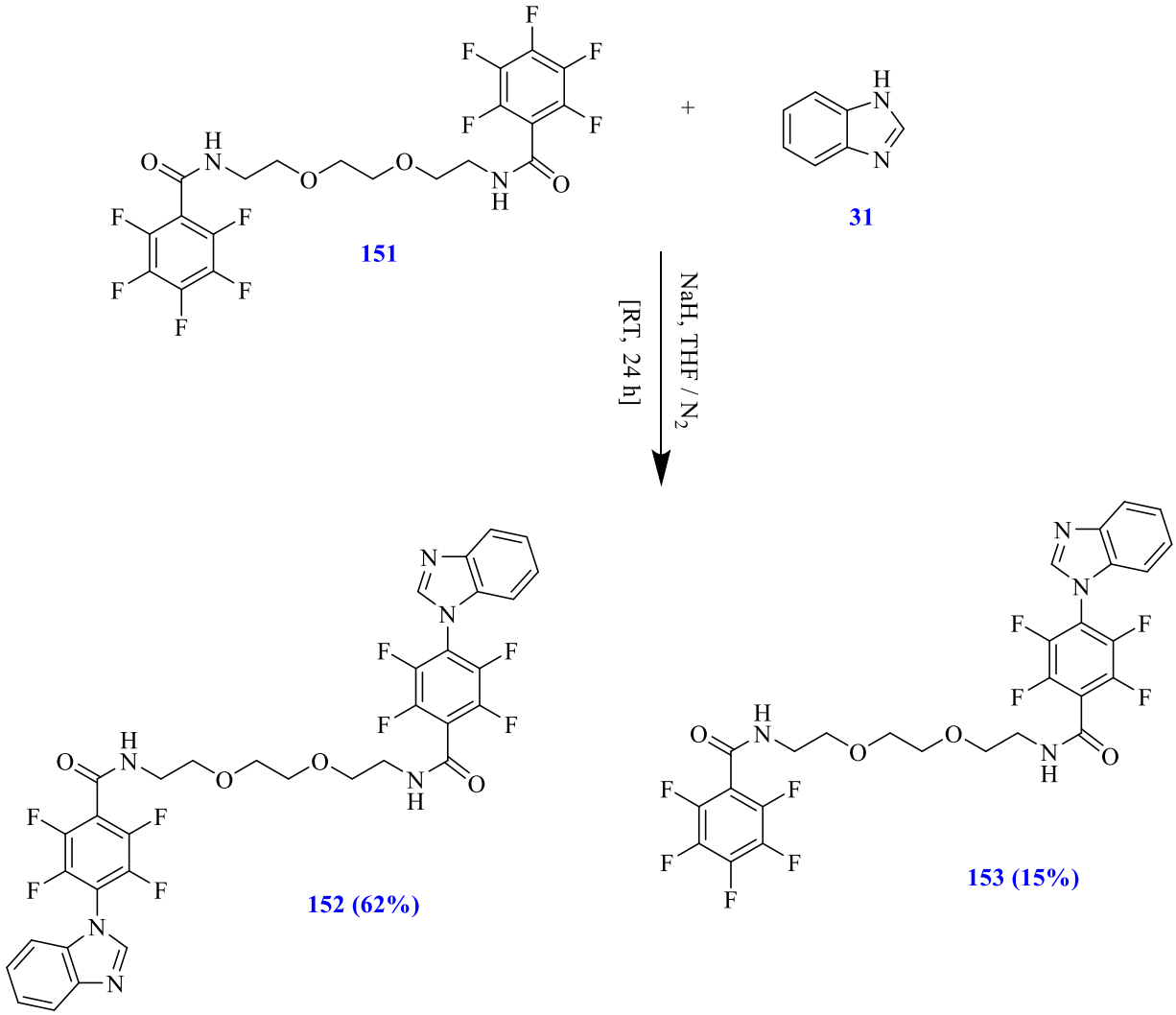
Figure 31. crystal structure of compound 151.

2.1.5.2. Substitution reaction of benzimdazole 31 with fluorinated bis-linked scaffold 151

After successful synthesis of the fluorinated bis-link binder **151** it was planned to investigate the possibility introducing different heterocyclic groups into the compound **151** by S_NAr reaction to make larger agents with increased surface area to interact with DNA as potential bis-intercalators. Therefore the reaction of benzimidazole **31** with compound **151** was carried out in a 2:1 ratio at RT in the THF as solvent and NaH as base. The workup procedure gave a white solid which TLC indicated to be a mixture of two compounds. Therefore column chromatography was used to separate the mixture to give target compound **152** (62%) and compound **153** (15%) (Scheme 44). ^{19}F NMR spectroscopy of the compound proved the di-substituted derivative **152** had formed by exhibiting two signals for eight fluorine atom each signal cantaining 4 F atoms.

and mass spectrometry was in accord with the expected formula, MS (ESI) (MH-), $C_{34}H_{23}O_4F_8N_6$ requires m/z 731.1659, found m/z 731.1657. Similarly ^{19}F NMR spectroscopy of the compound **153** proved mono substitution had occurred in one ring only, by exhibiting five signals for nine fluorine atoms, δ_F (376 MHz, $CDCl_3$) 22.48 (2F, dd, *J*, 22.93, 11.65), 22.07-21.01 (2F, m), 16.59 (2F, dd, *J*, 23.31, 11.65), 11.1 (1F, t, *J* 20.3), 1.83-1.70 (2F, m).

The mass spectrum confirmed the required formula, MS (ESI) (MH^-), $C_{27}H_{18}O_4F_9N_4$ requires m/z 633.1190 found m/z 633.1192. Also the structure of **152** was confirmed by X-ray diffraction analysis (Figure 32).



Scheme 48. Reaction of benzimdazole with fluorinated bis-linked scaffold 151.

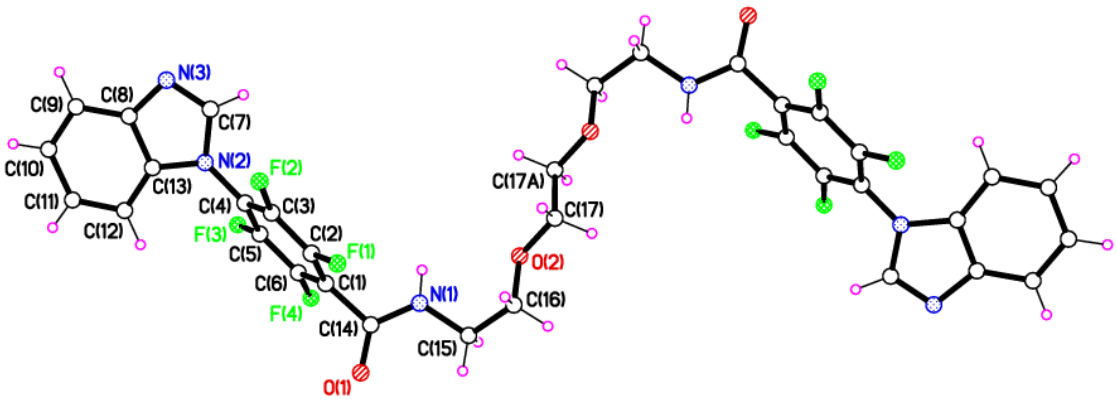


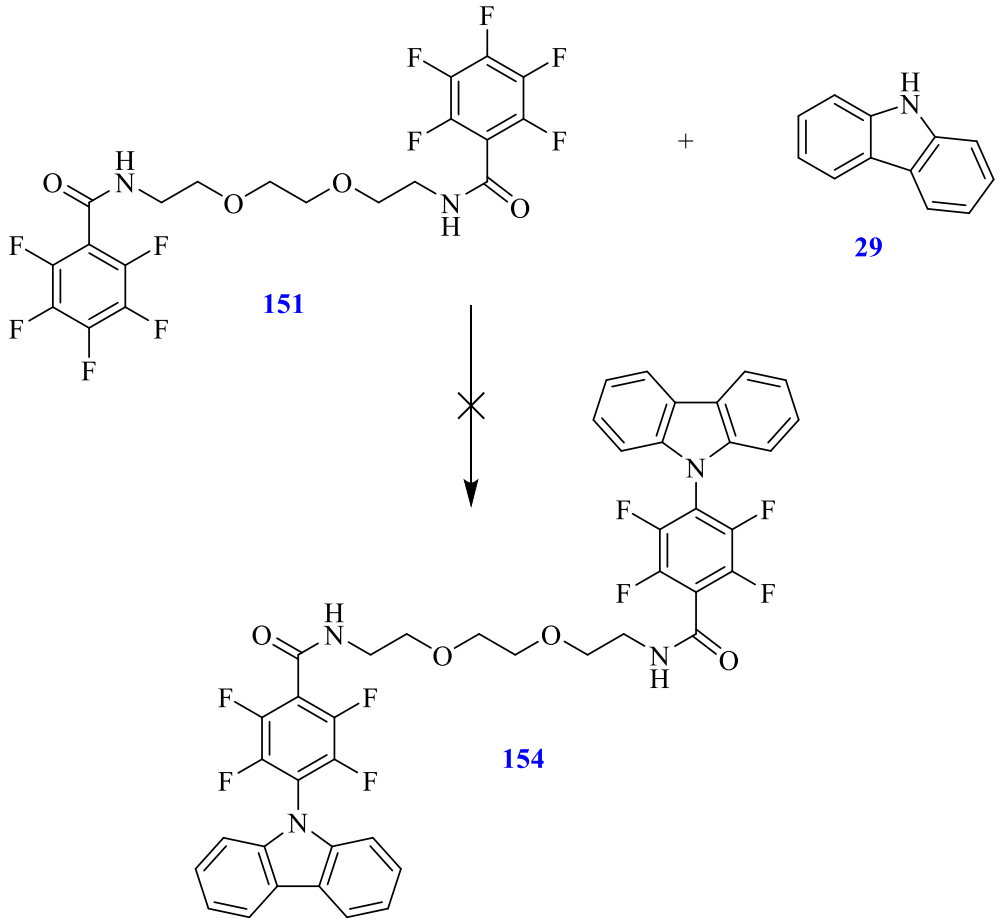
Figure 32. Crystal structure of compound 152.

2.1.5.3. Substitution reaction of carbazole with fluorinated bis-linked scaffold 151

The reaction of carbazole **29** with compound **151** was carried under different conditions as shown in table 5, hoping to displace the 4-position fluorine atom in compound **151** to form the target compound **154** by nucleophilic substitution reaction (Scheme 49). Unfortunately the reactions did not work and only the starting material was recover according to TLC and ^{1}H NMR spectroscopy, which showed only signal s in the aromatic region.

Table 5. Different reaction conditions attempted for the of carbazole with Fluorinated bis-linked scaffold 151.

Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 22 h. N_2	Starting material
2	NaH	THF, 65 °C, 22 h. N_2	Starting material
3	NaH	DMF, RT, 22 h. N_2	Starting material
4	NaH	DMF, 85 °C, 22 h. N_2	Starting material



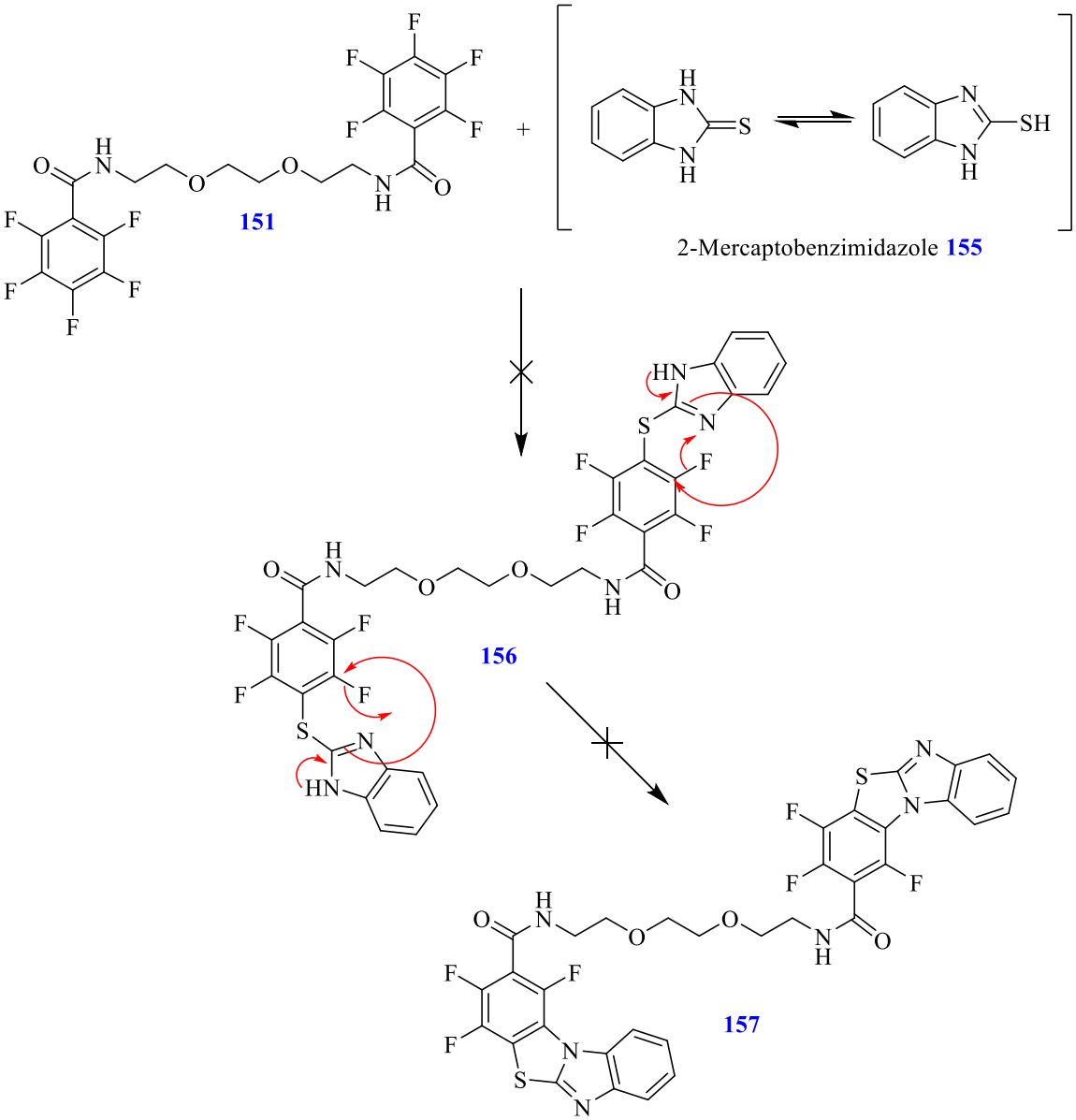
Scheme 49. Attempted reaction of carbazole with fluorinated bis-linked scaffold 151.

2.1.5.4. Substitution reaction of 2-mercaptobenzimidazole with fluorinated bis-linked scaffold 151

After the unsuccessful reaction of carbazole **29** with compound **151** the reaction of 2-mercaptobenzimidazole **155** with compound **151** was investigated using different conditions as shown in table 6, hoping to displace the 4-fluorine atom in compound **151** with formation of intermediate compound **156** by nucleophilic substitution reaction which may then cyclize to form the linked tetracyclic compound **157** (Scheme 50). Unfortunately the reactions did not work and only starting material was recovered according to TLC and ^{1}H and ^{19}F NMR spectroscopy. The ^{1}H spectroscopy did not show any signal at aromatic region and ^{19}F NMR spectroscopy indicated the same signals as compound **151.**

Table 6. Different reaction conditions of 2-mercaptobenzimidazole with fluorinated bis-linked scaffold 151

Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 22 h. N_2	Starting material
2	NaH	THF, Reflux (65 °C), 22 h, N_2	Starting material
3	NaH	DMF, RT, 22 h, N_2	Starting material
4	NaH	DMF, 85 °C, 22 h, N_2	Starting material

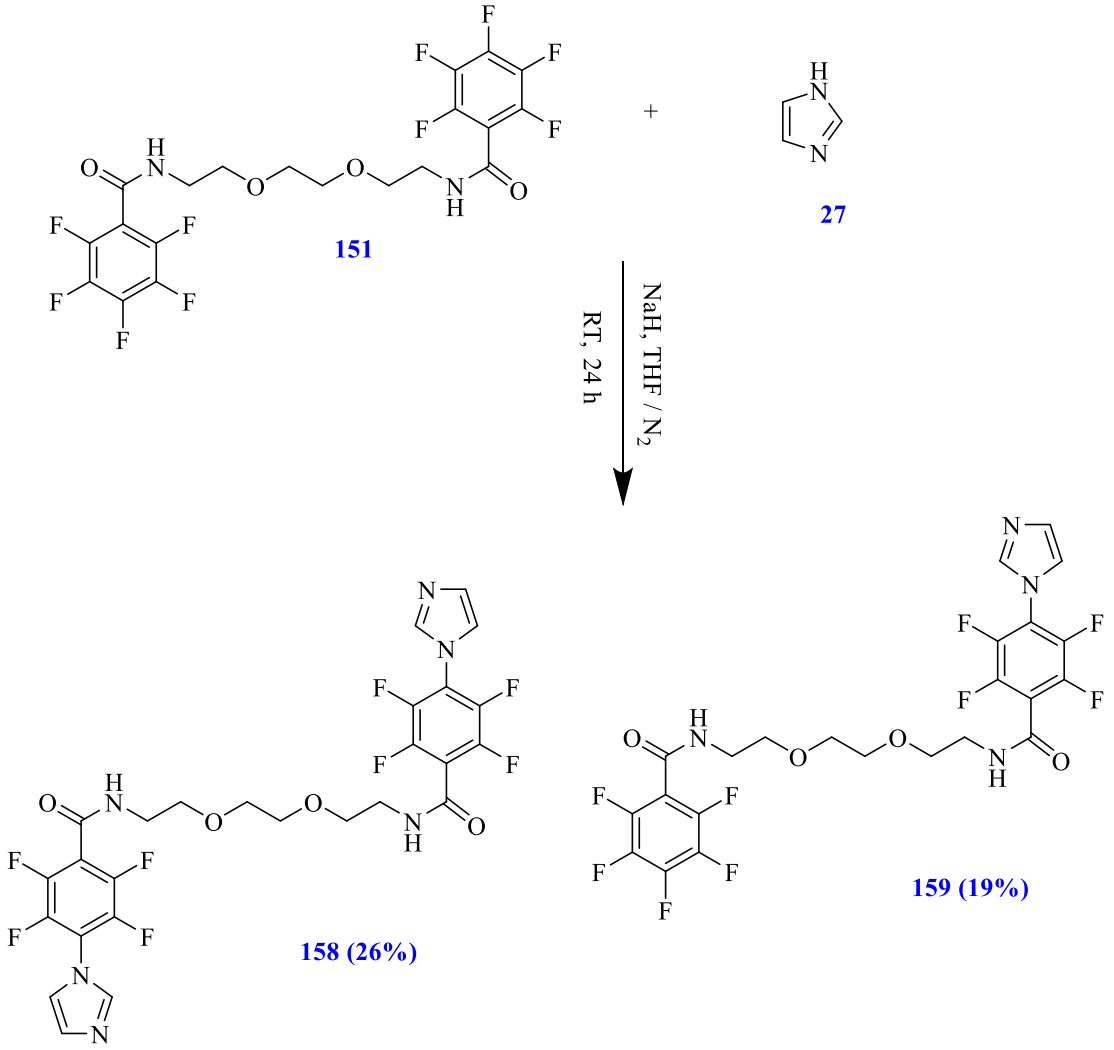


Scheme 50. Reaction of 2-mercaptobenzimidazole with fluorinated bis-linked scaffold 151.

2.1.5.5. Substitution reaction of imidazole with fluorinated bis-linked scaffold 151

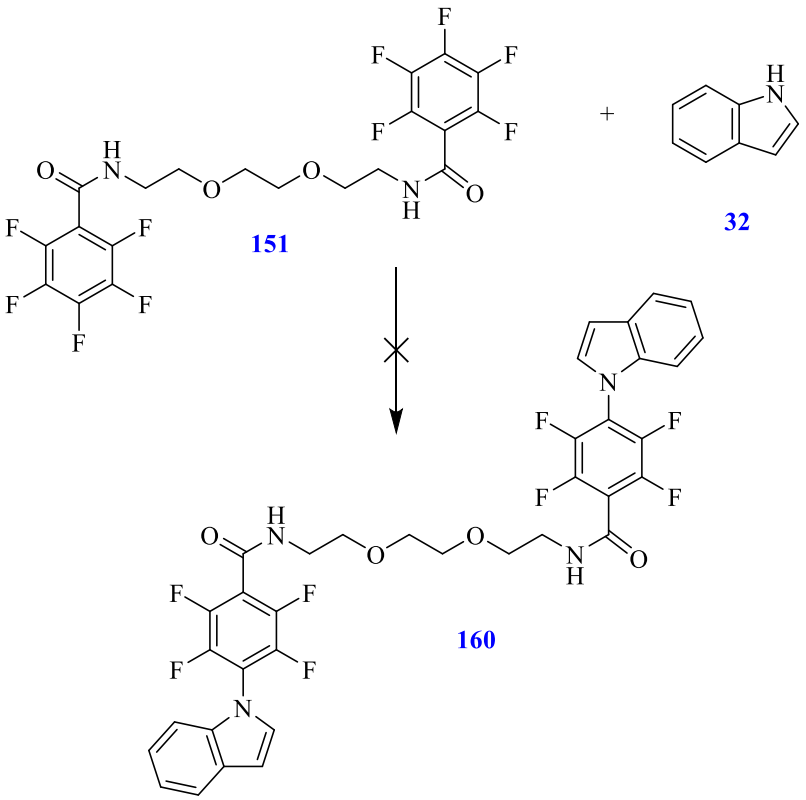
After the failed efforts to synthesis potential bis linked binding agents using carbazole or 2-mercaptobenzimidazole it was decided to try imidazole derivatives as nucleophiles as the benzimdazole had worked well before (section 2.1.5.2). Thus reaction of imidazole with synthesis compound **151** was carried out in 2:1 ratio at RT in THF as solvent and with NaH as base. The workup procedure gave an oily product and TLC indicated the presence of a mixture of two compounds plus some starting material. Therefore column chromatography purification was carried to give target compound **158** (26%) and compound **159** (19%) (Scheme 51). The ^{19}F NMR spectrum

of the compound proved di-substituent **158** had occurred by exhibiting two signals for eight fluorine atoms each signal containing 4F atoms. Also mass spectrometry confirmed the expected formula with (ESI)(MH^-), $C_{26}H_{21}F_8N_6O_4$ requires m/z 633.1491, found m/z 633.1473. Also ^{19}F NMR spectroscopy proved mono substitution **159** had occurred in by exhibiting five signals for nine fluorine atoms, δ_F (376 MHz, $CDCl_3$) 22.39-22.48 (2F, q, *J*, 12.03), 21.07-21.01 (2F, m), 16.59-16.67 (2F, q, *J*, 11.65), 11.1 (1F, t, *J* 20.3), 1.70-1.83 (2F, m). and mass spectrometry confirmed the formula, (ESI)(MH^-), $C_{23}H_{16}F_9N_4O_4$ requires m/z 583.1033, found m/z 583.1040. it is worth mentioning that the benzimdazole show better reactivity than imidazole and gave much higher yields of mono and di-substituted products as seen in Scheme 44 with the same reaction conditions.



Scheme 51. Reaction of imdazole with fluorinated bis-linked scaffold 151.

2.1.5.6. Substitution reaction of indole with fluorinated bis-linked binding scaffold 151



Scheme 52. Reaction of indole with fluorinated bis-linked binding scaffold 151

The reaction of indole **32** with compound **151**.(Scheme 52) was next carried out under the conditions shown in table 7 in a 2:1 ratio of reactants. Disappointingly again the reactions did not work and only the starting materials were identified as proved by TLC and NMR spectroscopy.

Table 7. Different reaction conditions for the attempted reaction of indole with fluorinated bis-linked scaffold 151

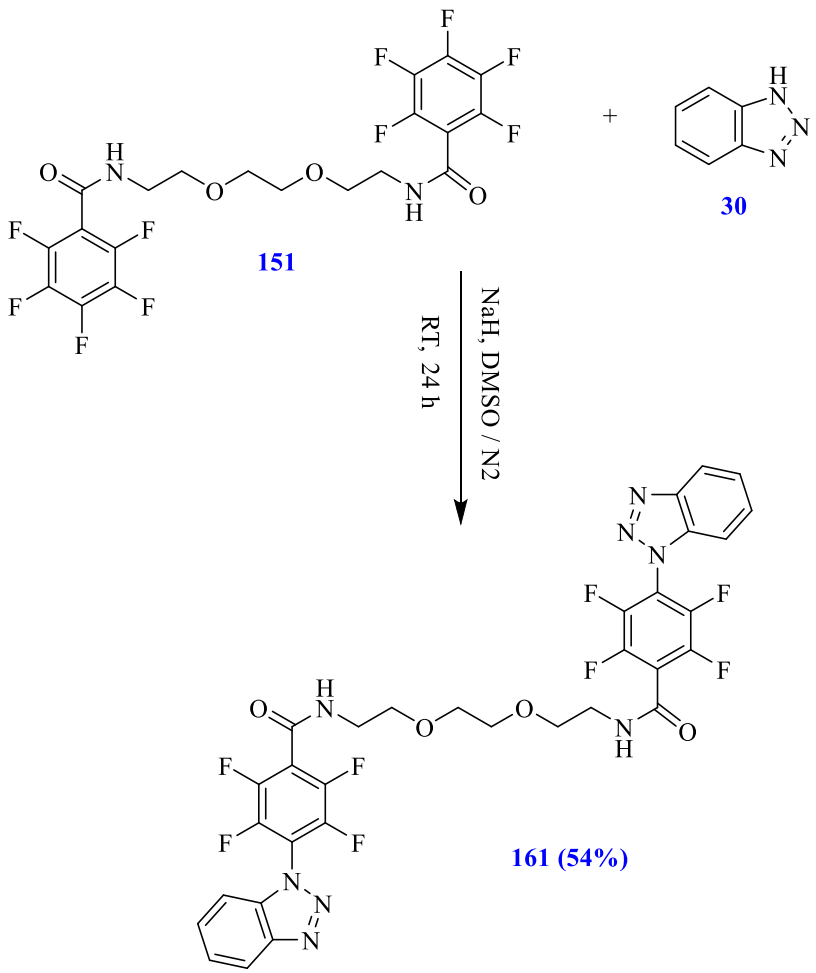
Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 22 h, N_2	Starting material
2	NaH	THF, Reflux (65 °C), 22 h, N_2	Starting material
3	NaH	DMF, RT, 22 h, N_2	Starting material

2.1.5.7. Substitution reaction of benzotrizole with fluorinated bis-intercalator scaffold 151

After the failed effort to synthesis a potential bis-linked binding using indole and successful reaction of benzimidazole it was planned to attempt reaction with benzotrizole as it is known to be a good nucleophile. Thus reaction of benzotriazole **30** with compound **151** (Scheme 53) was carried out under different conditions as shown in table 8, of these the reaction which was carried in a 2:1 ratio at RT in DMSO as solvent and NaH as base indicated the highest yield of target compound (entry 3). The workup procedure gave a colourless oily product. The ^{1}H NMR spectrum indicated the presence of some impurities.Column chromatography purification was therefore carried to give target compound **161** (54 %) as a white solid.^{19}F NMR spectroscopy showed two signals (4 F for each signal) as expected for target compound **161** and mass spectrometry displayed the expected mass of the compound, MS (ESI) (MH+), $C_{32}H_{23}F_8N_8O_4$ requires m/z 735.1709, found m/z 735.1705.

Table 8. Different reaction conditions for the attempted reaction of benzotrizole with fluorinated bis-linked scaffold 151.

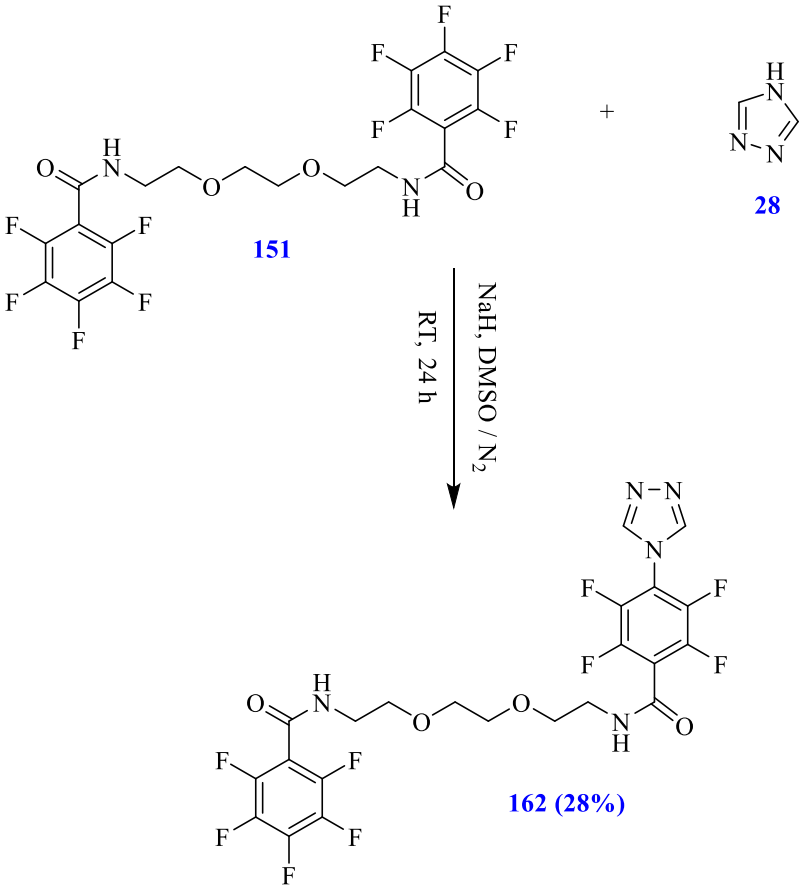
.Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 22 h, N_2	Starting material
2	NaH	DMF, 65 °C, 22 h, N_2	Starting material, product **161** (29%)
3	NaH	DMSO, RT, 22 h, N_2	Starting material, product **161** (54%)



Scheme 53. Reaction of benzotriazole with fluorinated bis-intercalator scaffold 159.

2.1.5.8. Substitution reaction of 1,2,4-trizole with fluorinated bis-intercalator scaffold 151

After the successful effort to synthesis a potential bis intercalator using benzotrizole it was planned to attempt to make the 1,2,4-triazole derivative. Thus reaction of 1,2,4-triazole **28** with synthesised compound **151** was carried out in a 2:1 ratio at RT in DMSO as solvent and NaH as base. The workup procedure gave colourless oily product. The ^{1}H NMR spectrum indicated the presence of some starting material. Therefore column chromatographic purification was carried to give target compound **162** (28 %) as white solid (Scheme 54). ^{19}F NMR spectroscopy showed the expected fluorine signals for the target compound, δ_F (376 MHz, $CDCl_3$) 22.74-22.64 (2F, q, *J*, 11.65), 21.15-21.05 (2F, m), 14.49-14.40 (2F, q, *J*, 11.28), 11.4 (1F, t, *J* 20.68), 1.95-1.81 (2F, m). Mass spectrometry displayed the expected mass of the compound, MS (ESI) (MH^-), $C_{22}H_{15}F_9N_5O_4$ requires m/z 584.0986, found m/z 584.0994



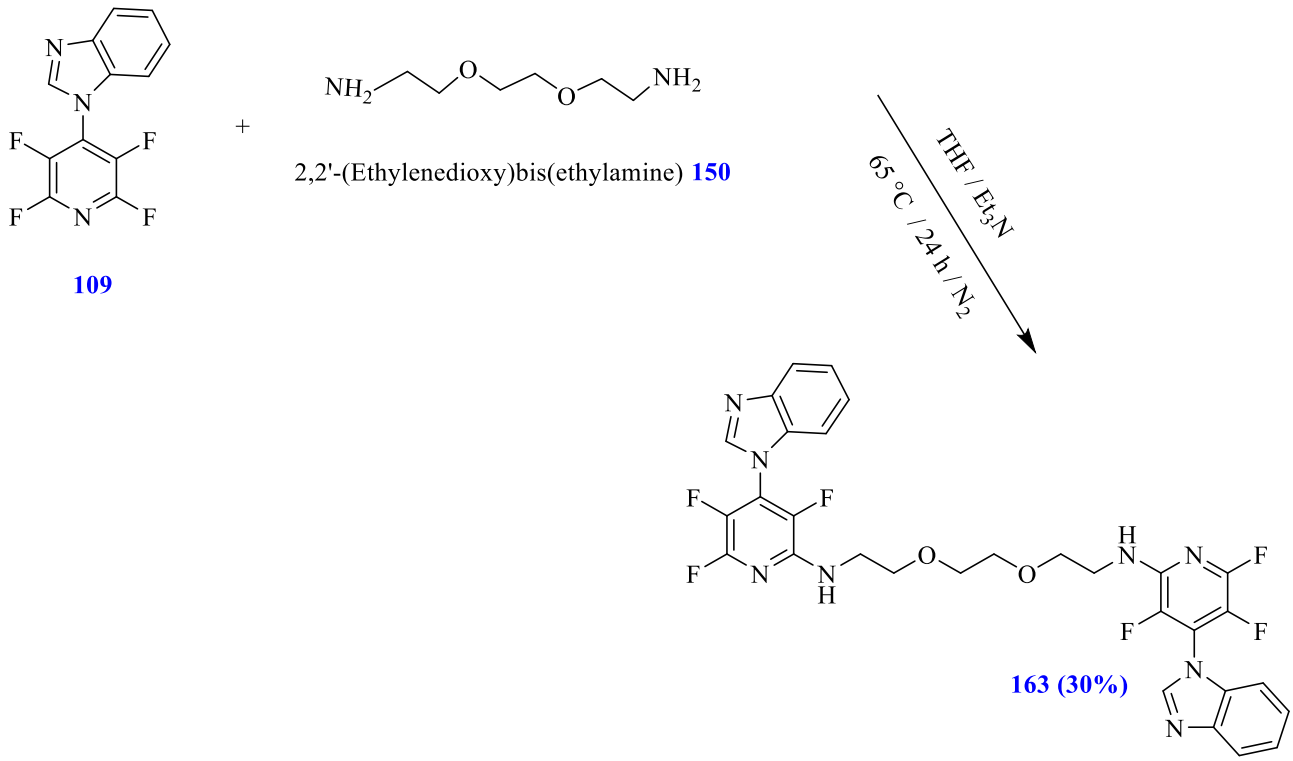
Scheme 54. reaction of 1,2,4-triazole with fluorinated bis-intercalator scaffold 151.

2.1.6. Synthesis of bis-linked binding agents by introducing of aliphatic hydrophilic link to synthesised fluorinated heterocyclic compounds.

In this part of the investigation we planned to make another series of novel bis-linked binding agent by introducing an aliphatic hydrophilic link to synthesised fluorinated heterocyclic compounds with better water solubility, and to investigate their DNA binding properties.

2.1.6.1. Reaction of compound 109 with 2,2-(ethylenedioxy)bis(ethylamine).

The aim of this reaction was formation of compound **163** by replacement of the 2-fluorine atom in compound **109** with 2,2′-(ethylenedioxy)bis(ethylamine) to make another bis-linked binding agent by formation of an aryl amine bond by S_NAr reaction (Scheme 55).



Scheme 55. Reaction of compound 109 with 2,2′-(ethylenedioxy)bis(ethylamine).

Therefore reactions of compound **109** (2 equiv.) with 2,2′-(ethylenedioxy)bis(ethylamine) **150** (1 equiv.) were carried with the different conditions shown in table 9. However the reaction which was carried in the present of THF as solvent and Et_3N as base under reflax (65 °C) for 24 h indicated highest yield of target compound (entry 3).

Table 9. Different methods for synthesising target compound 163.

Entry	Reagents	Conditions	Results
1	None	THF, RT, 24 h, N_2	Starting material
2	None	THF, Reflux (65 °C), 24 h, N_2	Starting material, product **163** (31%)
3	Et_3N	THF, Reflux (65 °C), 22 h, N_2	Starting material, product **163** (55%)

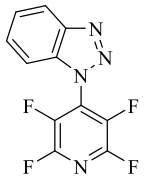
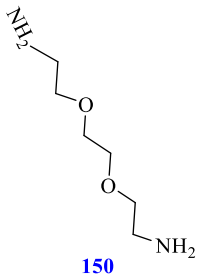
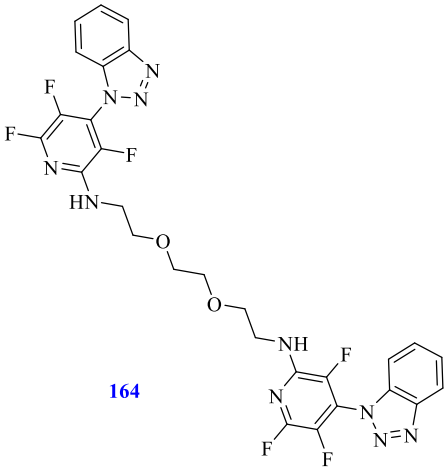
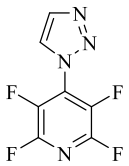
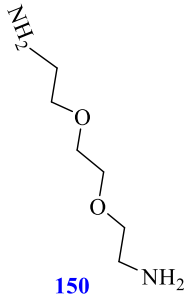
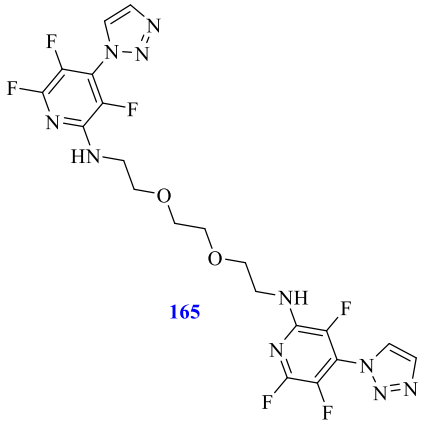
TLC revealed a mixture of two polar compounds one of which only just moved with ethyl acetate as eluent, while the other remained on the base line. Also some starting material was still present. After column chromatography purification processes the target product **163** was obtained in 55% yield and the structure was confirmed by its ^{1}H NMR spectrum and accurate mass measurement with *m/z* of 641.1845 (MH^-) for $C_{30}H_{23}F_6N_8O_2$. The ^{19}F NMR spectrum showed three signals (2F for each

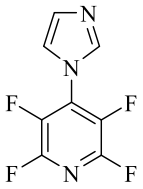
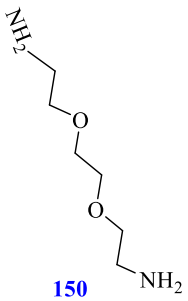
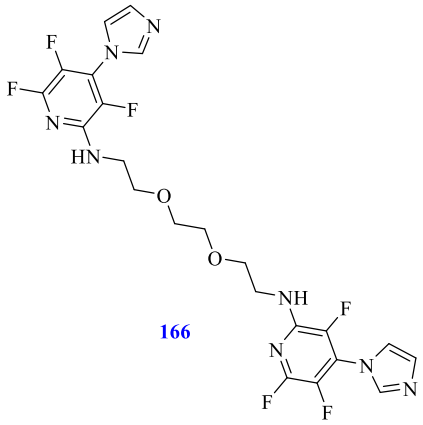
signal) as expected. Also IR spectroscopy indicated the presence of an NH group by a signal in the region 3000-3500 cm^{-1}.

2.1.6.2. Reaction of further perfluoropyridine derivatives with 2,2′(ethylenedioxy)bis(ethylamine)

After optimizing the conditions for the reaction of 1-(perfluoropyridin-4-yl)-1*H*-benzo[*d*]imidazole **109** with 2,2′-(ethylenedioxy)bis(ethylamine), we were decided to try different perfluoropyridine derivatives to synthesise a series of more polar fluorinated heterocyclic compounds using 2,2′-(ethylenedioxy)bis(ethylamine) as aliphatic hydrophilic linker as shown in Table 10.

Table 10. Reaction of different perfluoropyridine derivatives with 2,2′-(ethylenedioxy)bis(ethylamine).

Reactant	Reactant	Conditions	Expected product	Yield%
 **113**	 **150**	THF as solvent, Et_3N as base, Reflux (65 °C), 22 h, N_2	 **164**	70%
 **111**	 **150**	THF as solvent, Et_3N as base, Reflux (65 °C), 22 h, N_2	 **165**	30%

 **108**	 **150**	THF as solvent, Et_3N as base, Reflux (65 °C), 22 h, N_2	 **166**	65%

As seen in table 10 reaction of compound **113** with 2,2′-(ethylenedioxy)bis(ethylamine) in THF as solvent, and Et_3N as base under reflux (65 °C) for 22 h worked well and the target compound **164** was afforded in 70% yield. The ^{19}F NMR spectrum showed three signals (2F for each signal) as expected. Also mass spectrometry confirmed the expected mass for the target product **164**, MS (ESI) (MH^+), $C_{28}H_{23}F_6N_{10}O_2$ requires m/z 645.1904, found m/z 645.1908.

Compound **111** was reacted with 2,2′-(ethylenedioxy)bis(ethylamine) under the same conditions as for the preparation of compound **164** (table 10) but the desired compound **165** was afforded in only 30% yield which is much less then compound **164** therefore the benzotriazolyl perfluoropyridine derivative appears more reactive than the triazolyl perfluoropyridine. ^{19}F spectroscopy proved the presence of six fluorine atoms in the product by detecting three signals which each signal contain 2 F as expected. In addition mass spectrometry found the expected mass for target product **165,** MS (ESI)(MH^+), $C_{20}H_{29}F_6N_{10}O_2$ requires m/z 545.1591, found m/z 545.1576.

Also target compound **166** was afforded (65% yield) in a similar way (Table 10). The ^{19}F spectrum proved the product was formed and displayed three signals (2F atoms for each signal as expected. Moreover the mass spectrum displayed a signal for the expected molecular formula for the target compound **166**, MS (ESI) (MH^+), $C_{22}H_{21}F_6N_8O_2$ requires m/z 543.1686, found m/z 543.1686

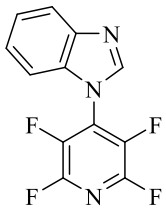
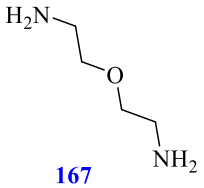
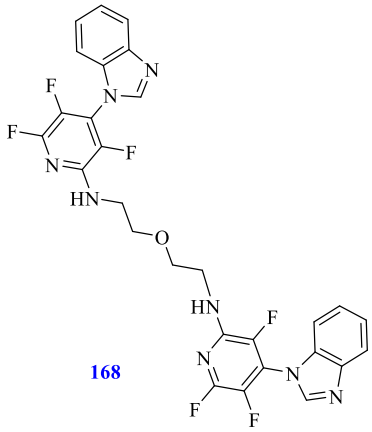
2.1.6.3. Reaction of different perfluoropyridine derivatives with 2-(2-aminoethoxy) ethylamine

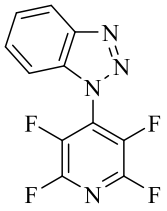
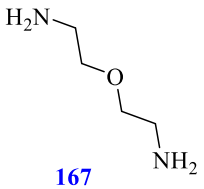
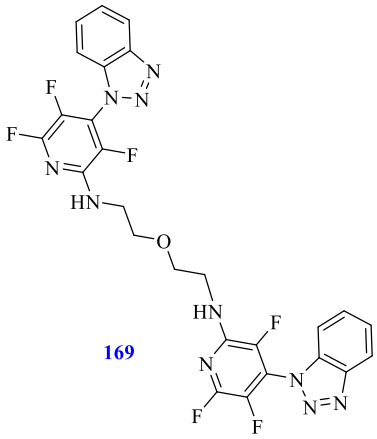
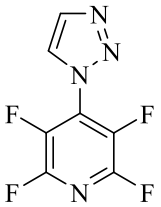
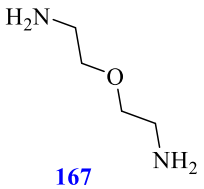
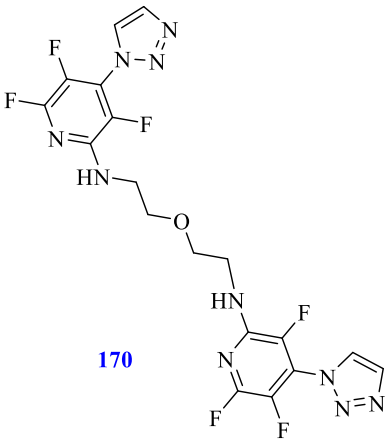
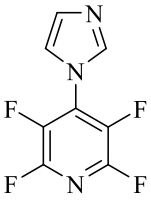
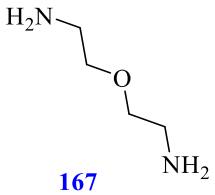
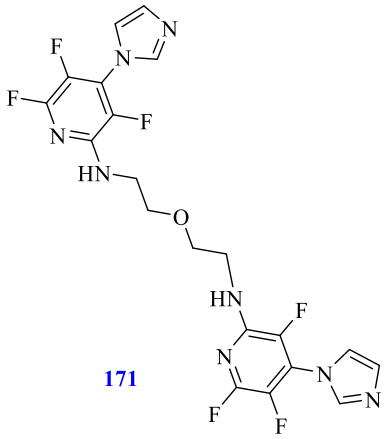
The aim of these reactions was formation of an alternative series of polar fluorinated heterocyclic novel compounds with a shorter linker. Varying the length of the linker could allow different binding modes with duplex DNA to be probed. Replace most of a fluorine atom in perfluoropyridine derivatives with 2-(2-aminoethoxy) ethylamine was expected again to proceed readily by S_NAr reaction.

After optimization of the conditions for reaction with 2,2′-(ethylenedioxy)bis(ethylamine) **148** (section 2.1.6.2), we were decided to test the same conditions for reactions of perfluoropyridine derivatives with 2-(2-aminoethoxy)ethylamine **165** as an alternative aliphatic hydrophilic linker (Table 11).

But as can be seen in table 11 reaction of compound **109** and **113** with 2-(2-aminoethoxy)ethylamine **165** resulted in lower yields compared to reactions with 2,2′-(ethylenedioxy)bis(ethylamine) **148** in table 10 Therefore was decided to try reaction of compound **111** with 2-(2-aminoethoxy)ethylamine in the more polar DMF instead of THF as solvent. The result indicated the reaction worked much better in DMF by showing higher % yield of product. Thus, reaction of compound **108** with 2-(2-aminoethoxy) ethylamine was also carried out in DMF.

Table 11. Reaction of different perfluoropyridine derivatives with 2-(2-aminoethoxy) ethylamine.

Reactant	Reactant	Conditions	Expected product	Yield
 **109**	 **167**	THF as solvent, Et_3N as base, Reflux (65 °C), 22 h, N_2	 **168**	23%

 **113**	 **167**	THF as solvent, Et_3N as base, Reflux (65 °C), 22h. N_2	 **169**	15%
 111	 **167**	DMF as solvent, Et_3N as base, 65 °C, 22 h, N_2	 **170**	55%
 **108**	 **167**	DMF as solvent, Et_3N as base, 65 °C, 22 h, N_2	 **171**	50%

As seen in table 11, reaction of compound **109** with 2-(2-aminoethoxy) ethylamine in THF as solvent, and Et_3N as base under reflux (65 °C) for 22 h worked and the target compound **168** was afforded in 23% yield. The ^{19}F NMR spectrum showed three signals (2F for each signal) as expected.

Also mass spectrometry showed the expected mass for target product **168**, MS (ESI) (MH^+), $C_{28}H_{21}F_6N_8O_1$ requires *m/z* 599.1737, found *m/z* 599.1720

Compound 113 was reacted with 2-(2-aminoethoxy)ethylamine under the same conditions as for compound **109** (Table 11) and target compound **169** was afforded in 15% yield. ^{19}F spectroscopy proved the product formed by detecting three signals (2F atoms for each signal as expected).. Also mass spectrometry found the expected mass for target product **169**, MS (ESI) (MH^+), $C_{26}H_{19}F_6N_{10}O_1$ requires *m/z* 601.1642, found *m/z* 601.1637

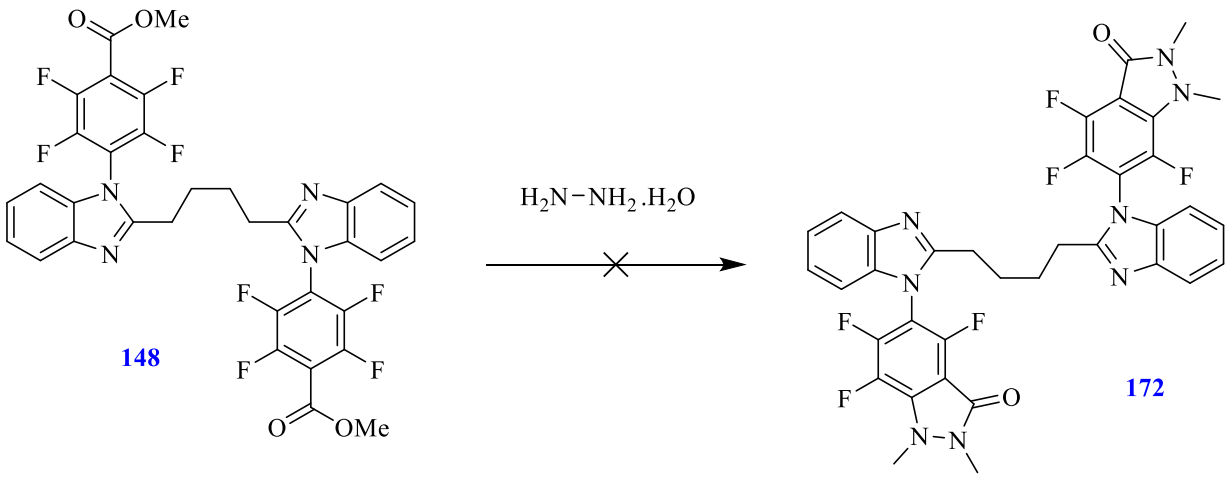
Due to the low yield of compounds **168** and **169**, reaction of compound **111** with 2-(2-aminoethoxy)ethylamine was carried out in DMF as solvent, using Et_3N as base but at the same temperature (65 °C) for 22 h. The reaction was successful affording the target compound **170** in 55% yields. ^{19}F spectroscopy proved the product formed by detecting three signals (2F atoms for each signal as expected). Also mass spectrometry found the expected mass for the target compound **170**, MS (ESI) (MH^+), $C_{18}H_{15}F_6N_{10}O_1$ requires *m/z* 501.1329, found *m/z* 501.1320.

In addition target compound **171**was afforded (50% yield) in similar conditions as for compound **170** (Table 11), and its structure proved by ^{19}F spectroscopy which found three signals (2F atoms for each signal as expected). Mass spectrometry showed the expected mass for target compound **171**, MS (ESI) (MH^+), $C_{20}H_{17}F_6N_8O_1$ requires *m/z* 499.1424, found *m/z* 499.1409.

2.1.7. *Further functionalization to make bis-linked binding agent*

Another approach taken was the design and synthesis of a series of potentially fluorinated bis-intercalating compounds involving potential cyclization of ester derivatives to make bi- or tri-cyclic bis-intercalators.

2.1.7.1. *Reaction of compound 148 with hydrazine*



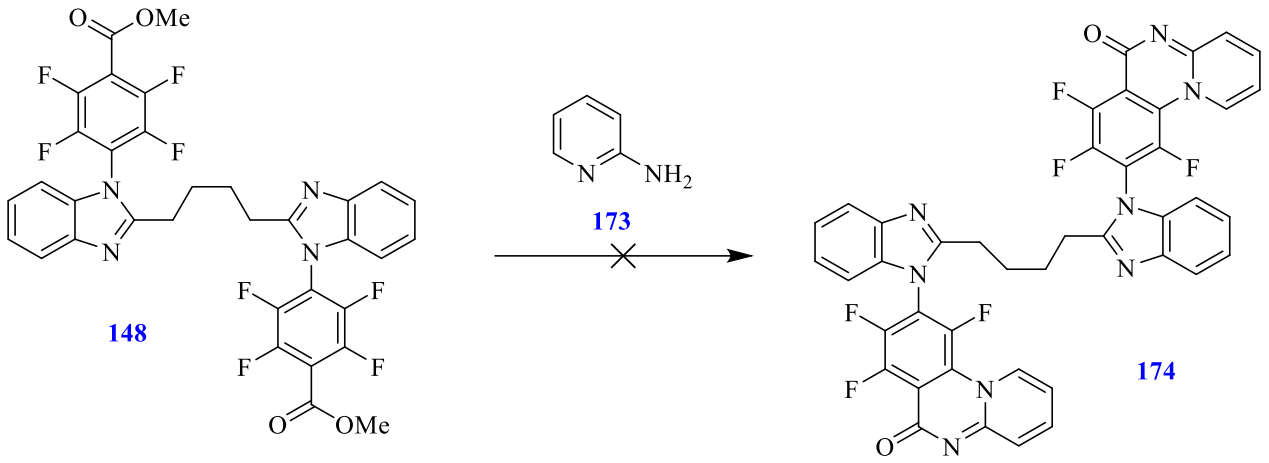
Scheme 56. Reaction of compound 148 with hydrazine.

The reaction of hydrazine with compound **148** (Scheme 56) was carried out under different conditions as shown in table 12 in a 4:1 ratio of reactants. As in entry 1 the hydrazine hydrate did not dissolve very well in THF and only starting material was recovered. Therefore in entry 2, methanol was added to dissolve the hydrazine and form a homogeneous reaction mixture. Unfortunately the reactions did not work and only the starting materials were recovered. Only in entry 3 did the ^{19}F NMR spectrum indicate reaction had occurred but it was such a complex spectrum and nothing was recovered, even after several different purification methods.

Table 12. Different condition attempted for synthesising target compound 172.

Entry	Reagents	Conditions	Results
1	None	THF, RT, 22 h, N_2	Starting material
2	None	THF and 2 ml of methanol, RT, 22 h, N_2	Starting material
3	None	methanol, 1 h, 65 °C and RT, 22 h, N_2	Starting material, unknown compounds formed

2.1.7.2. Reaction of compound 148 with 2-aminopyridine



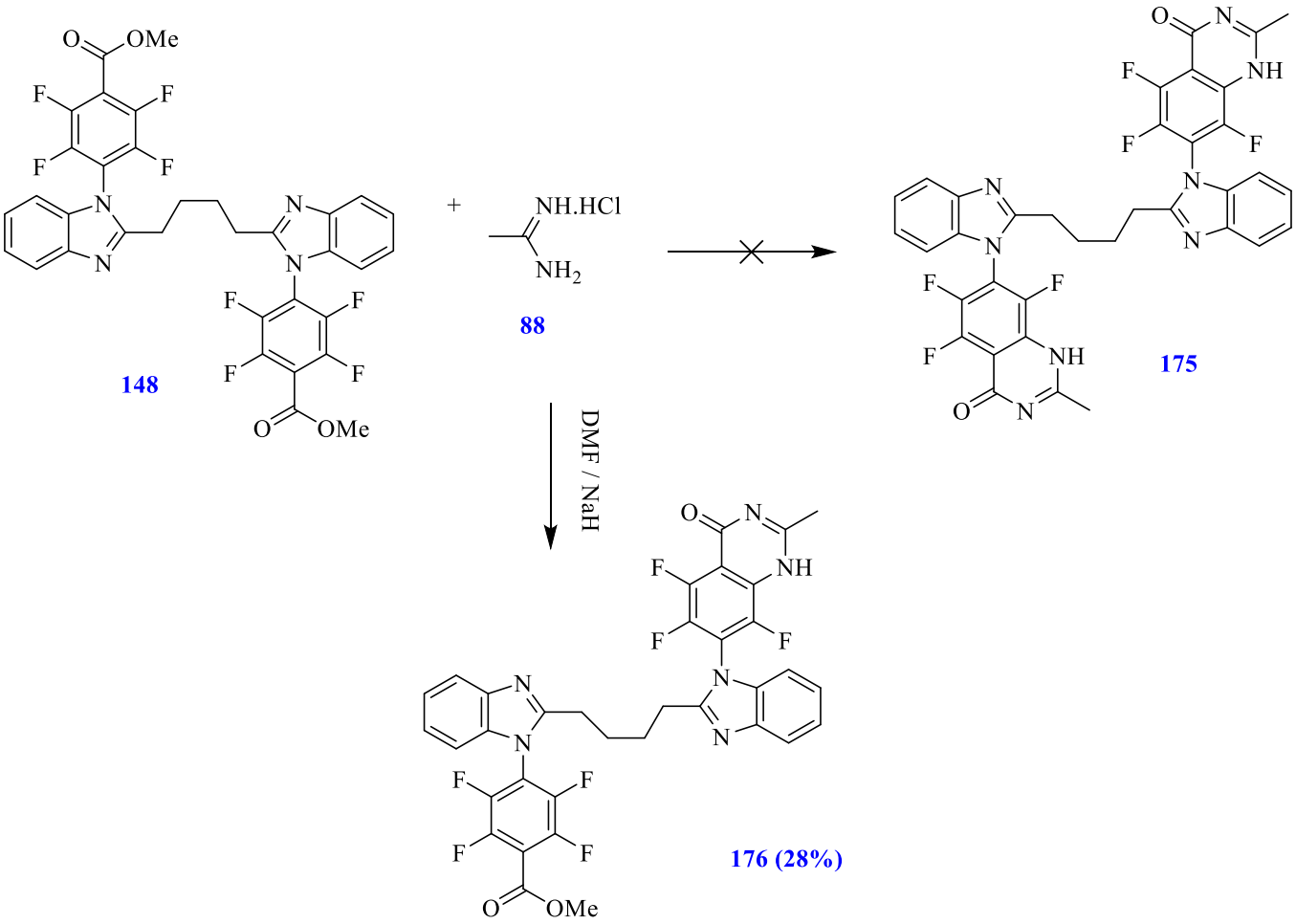
Scheme 57. Reaction of compound 148 with 2-aminopyridine.

After the unsuccessful reaction of hydrazine with ester compound 148 and the poor solubility of hydrazine in nonpolar solvents, it was decided to try 2-aminopyridine as a nucleophilic reagent which might form both an amide bond to the ester in **148** and cyclise by effecting S_NAr reaction of the adjacent fluorine to form tricyclic compound **174** (Scheme 57). Reactions of 2-aminopyridine with the successfully synthesised ester compound **148** were conducted with a higher ratio of 2-aminopyridine (4:1) under different conditions as shown in table 12 Unfortunately all the reactions did not work and only starting materials was recovered.

Table 13. Different condition attempted for synthesising target compound 174.

Entry	Reagents	Conditions	Results
1	NaH	THF, RT, 22 h, N_2	Starting material
2	None	Dioxane, 10 h, RT and 10 h 80 °C, 22 h, N_2	Starting material,
3	None	Acetic acid, 24 h, 100 °C and RT, 22 h. N_2	Starting material

2.1.7.3. Reaction of ester compound 148 with acetamidine hydrochloride



Scheme 58. Reaction of ester compound 148 with acetamidine hydrochloride.

After unsuccessful reaction of 2-aminopyridine with compound **148** it was decided to try another more reactive nucleophile like acetamidine (Scheme 58). The reactions of ester compound **148** and acetamidine hydrochloride were carried in a 1:2 ratio as shown in table 14, As seen in entry 4 the reaction which was carried at 85°C, in DMF as solvent and NaH as base indicated highest yield of target compound **176** (entry 3). The workup procedure gave yellow solid product. The ^{1}H NMR indicated the presence of some impurities. Therefore column chromatography purification was carried out to give compound **176** (28%) as a white solid. ^{19}F and ^{1}H NMR spectroscopy indicated that the acetamidine had added and cyclised on only one of the fluoroarene rings, leaving the ester intact on the other as seen in (Scheme 58). Also mass spectrometry displayed the expected mass of the compound **176**, MS (ESI) (MH^{+}), $C_{35}H_{24}F_{7}N_{6}O_{3}$ requires m/z 709.1793 found m/z 709.1775. The

formation of **176** showed the reaction to be viable, and further investigation to effect reaction on both rings should be continued.

Table 14. Different condition for synthesising target compound 176.

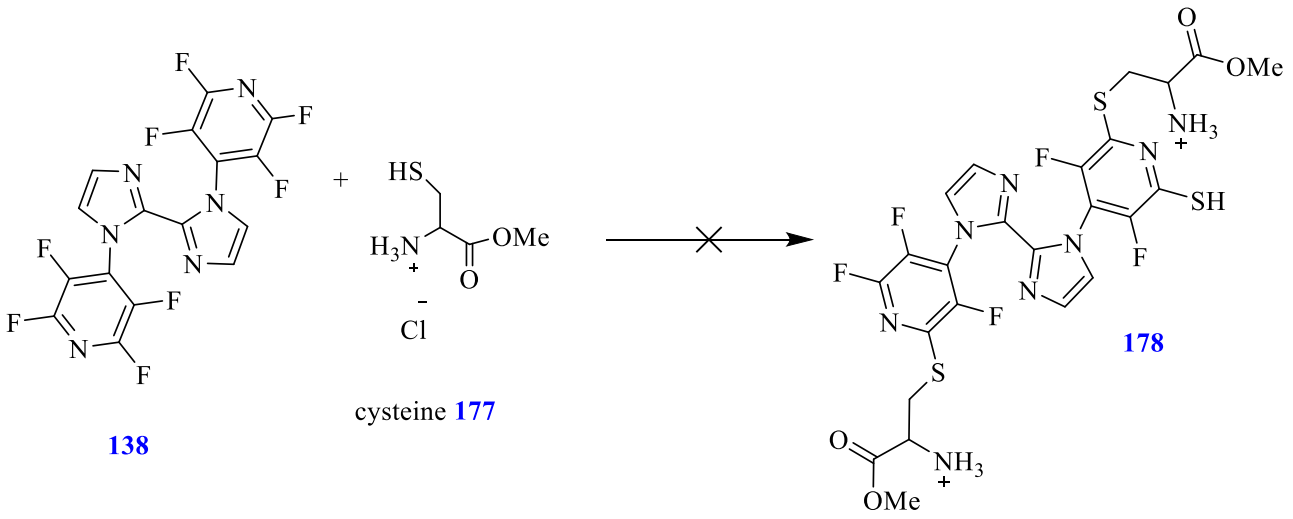
Entry	Reagents	Conditions	Results
1	None	Pyridine and 1 ml of methanol, 80 °C, 22 h, N_2	Starting material
2	Sodium acetate	Methanol, reflux, 24 h, N_2	Starting material
3	NaH	THF, RT, 22 h, N_2	Starting material,
4	NaH	DMF, RT, 22 h, N_2	Starting material, product **176** (10%)
5	NaH	DMF, 85°C, 22 h, N_2	Starting material, product **176** (28%)
6	NaH	DMSO, RT, 22 h, N_2	Starting material, complex NMR spectrum of unknown compound.

2.1.8. Addition of hydrophilic side chains to the successfully synthesised fluorinated heterocyclic compounds.

Throughout the biological assays it had been found that some of synthesised compounds had poor solubility in the buffers employed. For the purpose of improving the water solubility of the novel synthesised compounds it was planned to introduce hydrophilic side chains to the compounds. Therefore the aim of these reactions is to replace a fluorine atom in the successfully prepared fluorinated heterocycls with different side chains by an aromatic nucleophilic substitution reaction. Hopefully the aliphatic side chain bearing polar groups should make the compound more water-soluble and could improve DNA binding activity.

2.1.8.1. Reaction of compound 138 with cysteine

The aim of this reaction was to increase the water solubility and improve DNA binding activity of compound **138** by adding the amino acid cysteine **177** (protected as its methyl ester) as a side chain through reaction of its thiol side group. (Scheme 59)



Scheme 59.Reaction of the compound 138 with cysteine.

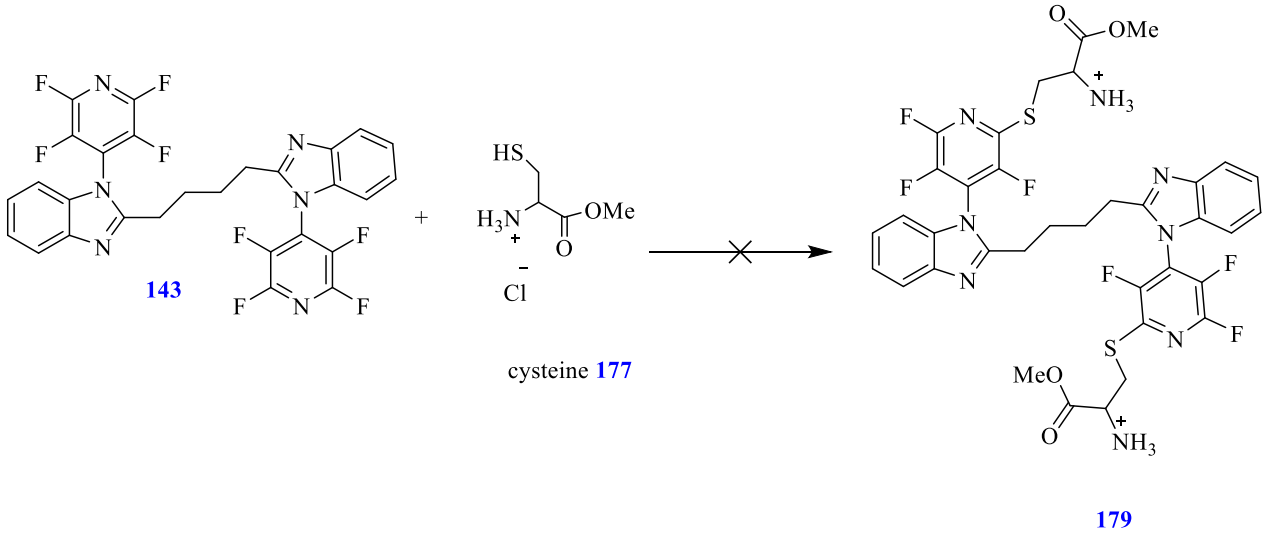
Therefore reaction of compound **138** (1 equiv) with methyl cysteine **177** (2 equiv) was carried under different conditions as shown in table15. TLC and mp point indicated only the presence of compound **138** as starting material. The reaction had not worked and the target compound **178** was not formed.

Table 15. different reaction conditions for compound 138 with cysteine.

Entry	Reagents	Conditions	Results
1	Et_3N	THF, RT, 22 h, N_2	Starting material
2	Et_3N	DMF, RT, 22 h, N_2	Starting material
3	Et_3N	DMF and THF, RT, 22 h, N_2	Starting material

2.1.8.2. Reaction of the compound 143 with cysteine

As the successfully synthesised compound **143** did show positive activity against some cancer cell lines, but had low water solubility, we decided to use this compound as a core biological agent and introduce different hydrophilic side chains by replacing the F atoms with further nucleophiles. It was hoped to synthesise a set of more water soluble fluorinated heterocycles with potential biological activity using this strategy. Therefore reaction of compound **143** with cysteine **177** was carried out in ratio 1:2 in the presence of Et_3N as base and DMF as solvent was carried out for 24 h at room temperature under N_2 in an attempt to form target compound **179**. Unfortunately NMR spectroscopy indicated only the presence of starting material and the target compound was not afforded.

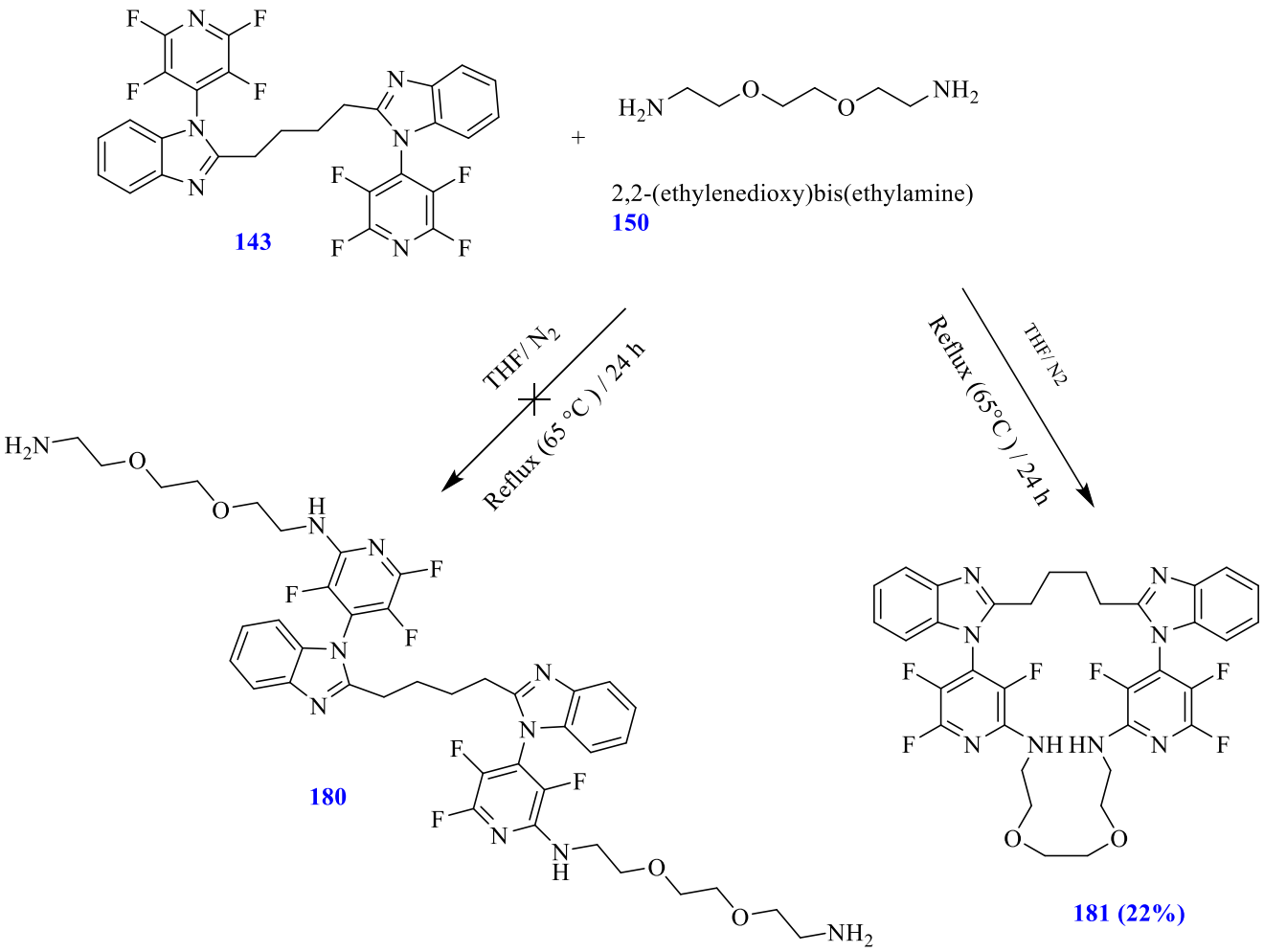


Scheme 60. Reaction of the compound 143 with cysteine.

2.1.8.3. Study of the reaction of compound 143 with 2,2-(ethylenedioxy)bis(ethylamine)

The aim of this reaction is improve the biological activity of compound **143** by replacement of two fluorine atoms of compound **143** with two amine chains and hopefully synthesising target compound **180** as a possible DNA groove binding agent. (Scheme 61)

The reaction of compound **143** (1 equiv.) with 2,2-(ethylenedioxy)bis(ethylamine) **150** (2 equiv.) in THF as solvent was conducted over 24 h at 65 °C under N_2. TLC revealed a mixture of starting material and a new compound. Therefore column chromatographic purification was carried out affording the interesting and unexpected macro-cyclized compound **181** in 22% yields, instead of the target compound **180**. The structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy and accurate mass measurement with *m/z* of 697.2469 (MH^+) for $C_{34}H_{31}F_6N_8O_2$. The ^{19}F NMR spectrum showed three signals (2F for each signal) as expected. Also the structure of **181** was confirmed by X-ray diffraction analysis (Figure 33).



Scheme 61. Reaction of compound 143 with 2,2-(ethylenedioxy)bis(ethylamine).

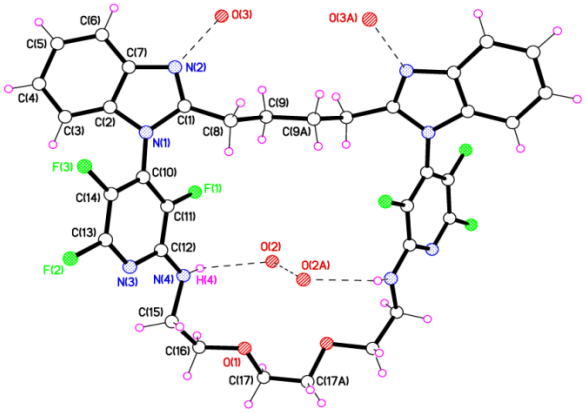


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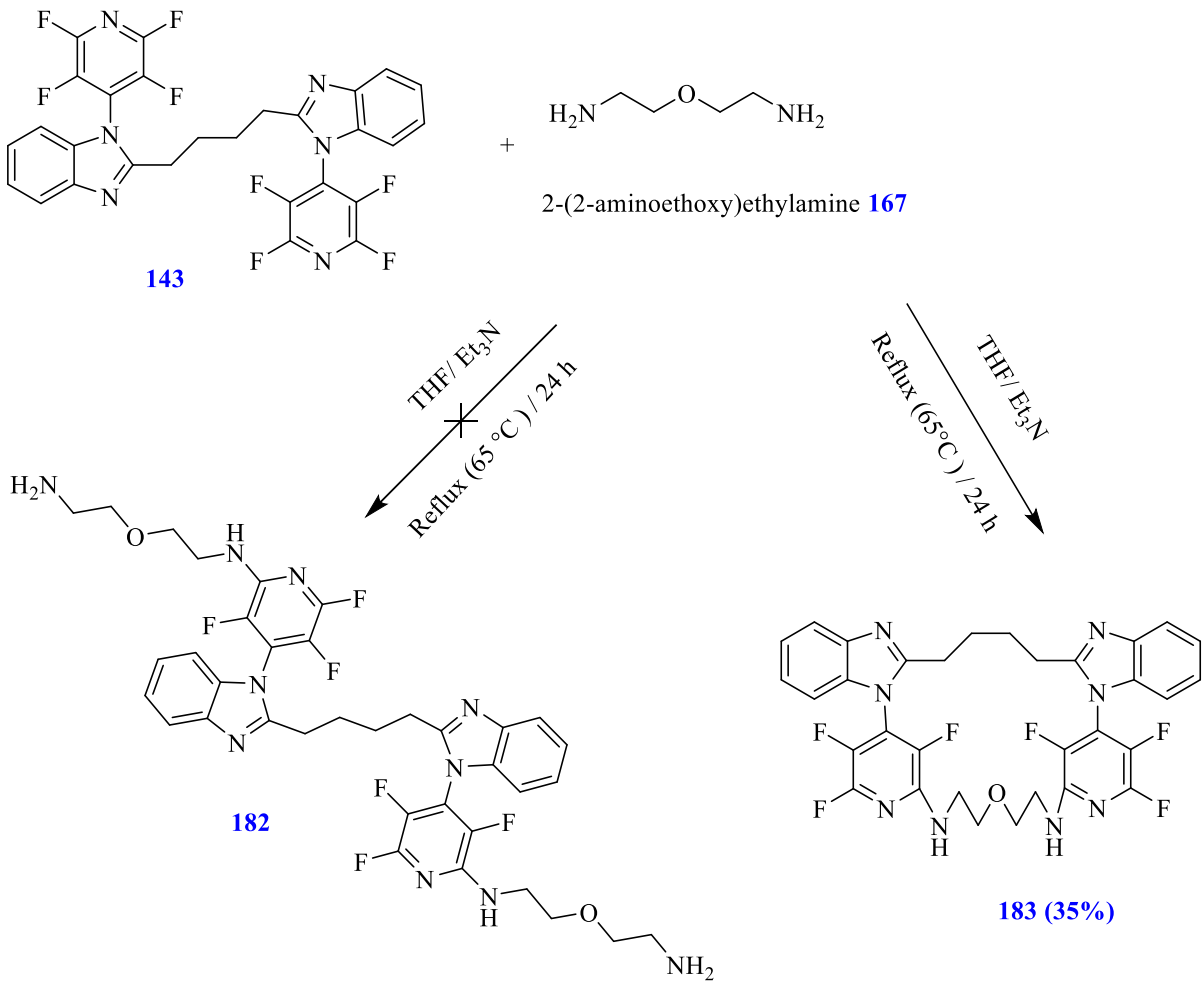
Figure 33. X-ray crystal structure of 181·2½H2O showing the 24-membered macrocycle with 2½ hydrogen bonded water molecules. The molecule lies on a 2-fold axis. O(3) and O(3A) are each ¼ occupied.

2.1.8.4. Investigation of the Reaction of compound 143 with 2-(2-Aminoethoxy)ethylamine

After synthesis of the unexpected macro cyclized compound **181** it was planned to try the shorter linker 2-(2-aminoethoxy)ethylamine to find out if this would still lead to a cyclized compound. Therefore reaction of compound **143** (1 equiv) with 2-(2-aminoethoxy)ethylamine **167** (2 equiv) in THF as solvent and with Et_3N as base was conducted over 24 h at 65°C under N_2 (scheme 62) TLC revealed a mixture of starting material and a new compound. After column chromatographic purification the macro cyclized compound **183** was obtained in 35% yield. As seen, even with a shorter linker macrocyclisation occurred, and target compound **182** was not formed. Compound **183** was confirmed by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy and accurate mass measurement with *m/z* of 653.2207 (MH^+) for $C_{32}H_{26}F_6N_8O$. The ^{19}F NMR spectrum showed three signals (2 F for each signal) as expected. Also the structure of **183** was confirmed by X-ray diffraction analysis (Figure 34).



Scheme 62. Reaction of compound 143 with 2-(2-aminoethoxy)ethylamine.

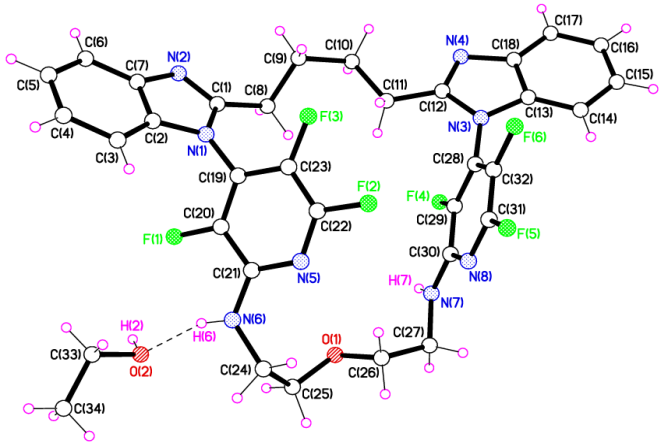
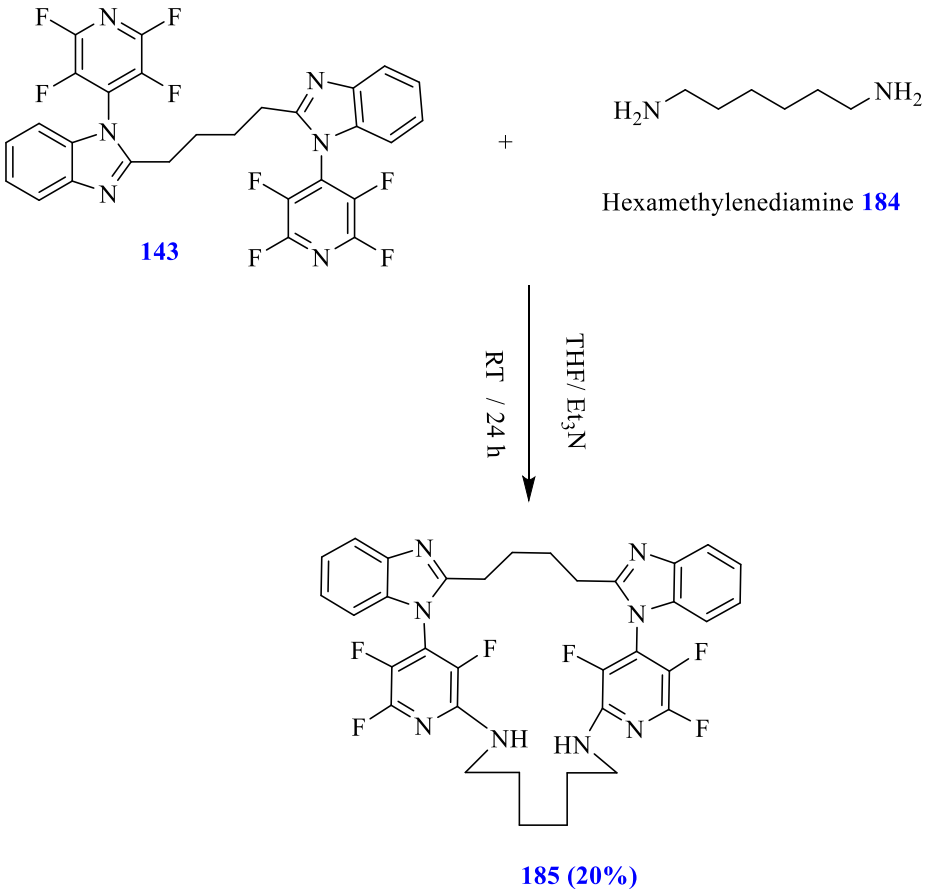


Figure 34. X-ray crystal structure of 183 showing the 21-membered macrocycle with a hydrogen-bonded ethanol solvate molecule.

2.1.8.5. Study of the reaction of perfluoro bis-benzimidazole derivative 143 with hexamethylenediamine

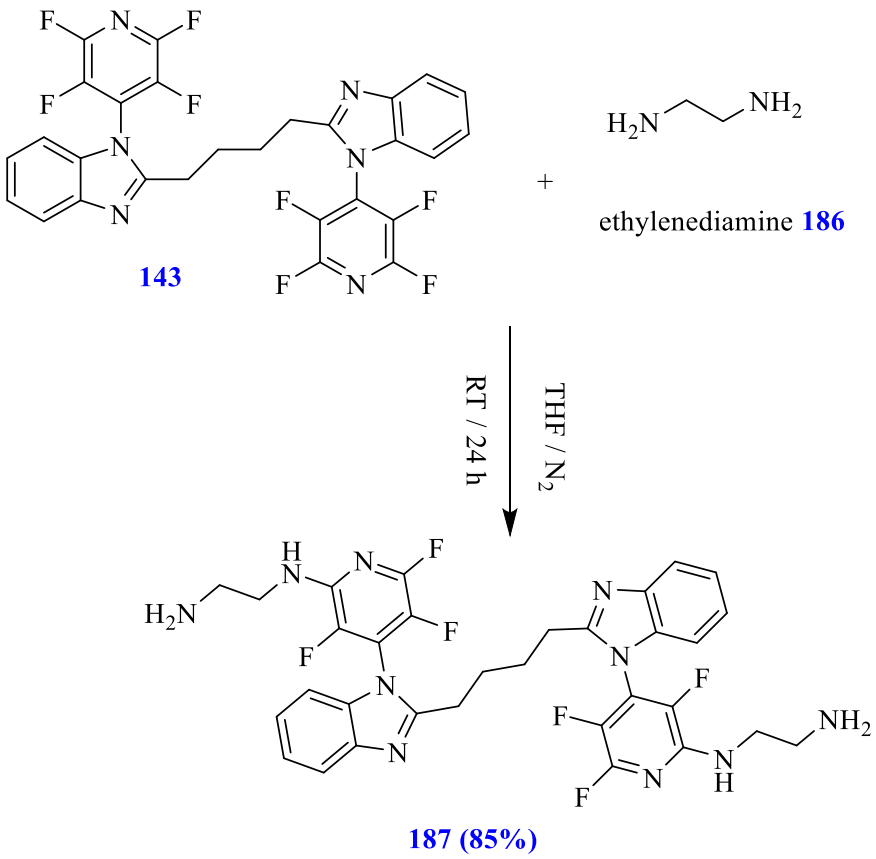
After successfully synthesising macro cyclized compounds **181** and **183** it was decided to try hexamethylenediamine in order to make further examples of such macro cycles**.** Thus reaction of perfluoro bis-benzimidazole derivative **143** with hexamethylenediamine **184** in 1:1 ratios was carried out in THF as solvent and in the presence of Et_3N as base at RT for 24 h (scheme 63). After purification the target macro cyclized compound **185** was again afforded in 20% yield. The structure was confirmed by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy and accurate mass measurement with *m/z* of 655.2570 (MH^+) for $C_{34}H_{31}F_6N_8$. The ^{19}F NMR spectrum showed three signals (2 F for each signal) as expected. Formation of a 22-membered ring in this case, confirms the relative ease of macrocycle formation in these systems, which is likely due to the hindered rotation of the aryl benzimidazole groups increasing the probablitiy of the final amino group encountering the other fluoropyridine ring, rather than reacting with a second molecule to form oligomers or polymers.



Scheme 63. reaction of perfluoro bis-benzimidazole derivative 143 with hexamethylenediamine.

2.1.8.6. Study of the reaction of compound 143 with ethylenediamine

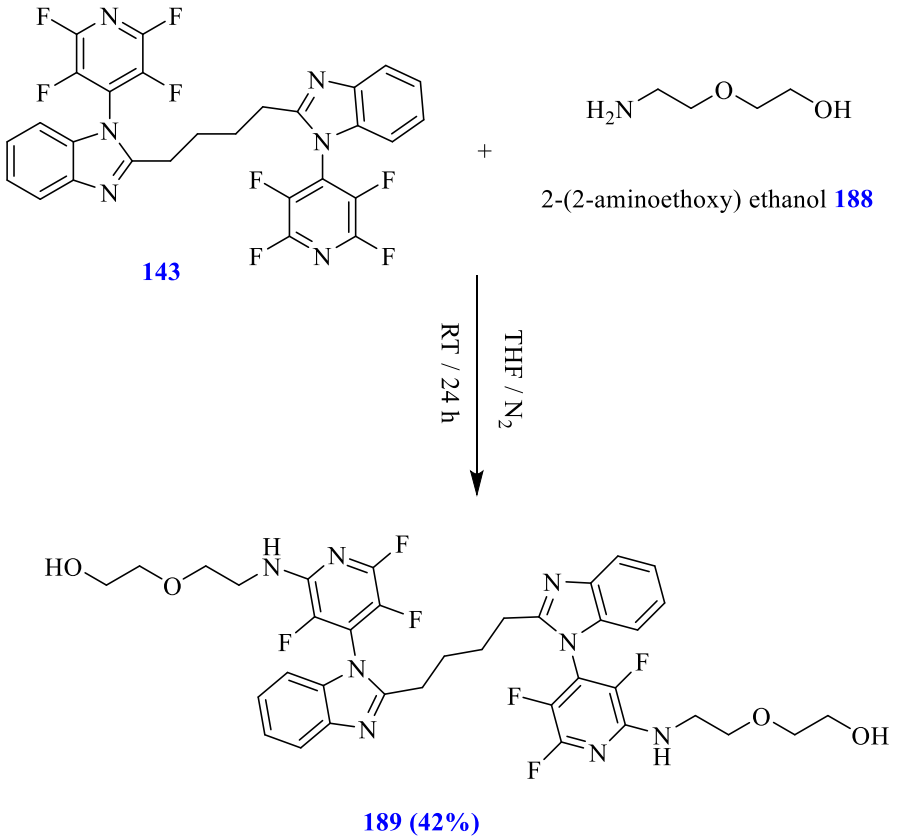
Next it was planned to study the possibility of adding two molecules of ethylenediamine **186** perfluoro bis-benzimidazole derivative **143** by treating it with two equivalents of ethylenediamine **186** in THF at RT for 24 h (Scheme 64). The target compound **187** was afforded easily by addition of water to the reaction mixture in 85% yield. The structure was confirmed by its ^{1}H NMR spectrum and accurate mass measurement with *m/z* of 669.2632 (MH^-) for $C_{32}H_{31}F_6N_{10}$. The ^{19}F NMR spectrum showed three signals (2F for each signal) as expected.



Scheme 64. Reaction of compound 143 with ethylenediamine.

2.1.8.7. Investigation of the reaction of compound 143 with 2-(2-aminoethoxy) ethanol

Two equivalents of 2-(2-aminoethoxy) ethanol **188** were then reacted with one equivalent of compound **143** in order to form a further target compound **189** that might possess potential biological activity or DNA binding activity. The reaction was carried out in THF at RT for 24 h. The target compound **189** was afforded in 42% by recrystallization from DCM and light petroleum (scheme 65). The structure was confirmed by its ^{1}H NMR spectrum and accurate mass measurement with m/z of 759.2836 (MH^{+}) for $C_{36}H_{37}F_{6}N_{8}O_{2}$. The ^{19}F NMR spectrum showed three signals (2 F for each signal) as expected. Elemental analysis fitted with the expected composition for a molecular formula of $C_{36}H_{36}F_{6}N_{8}O_{2}.3H_{2}O$ (785).



Scheme 65. Reaction of compound 188 with 2-(2-aminoethoxy)ethanol.

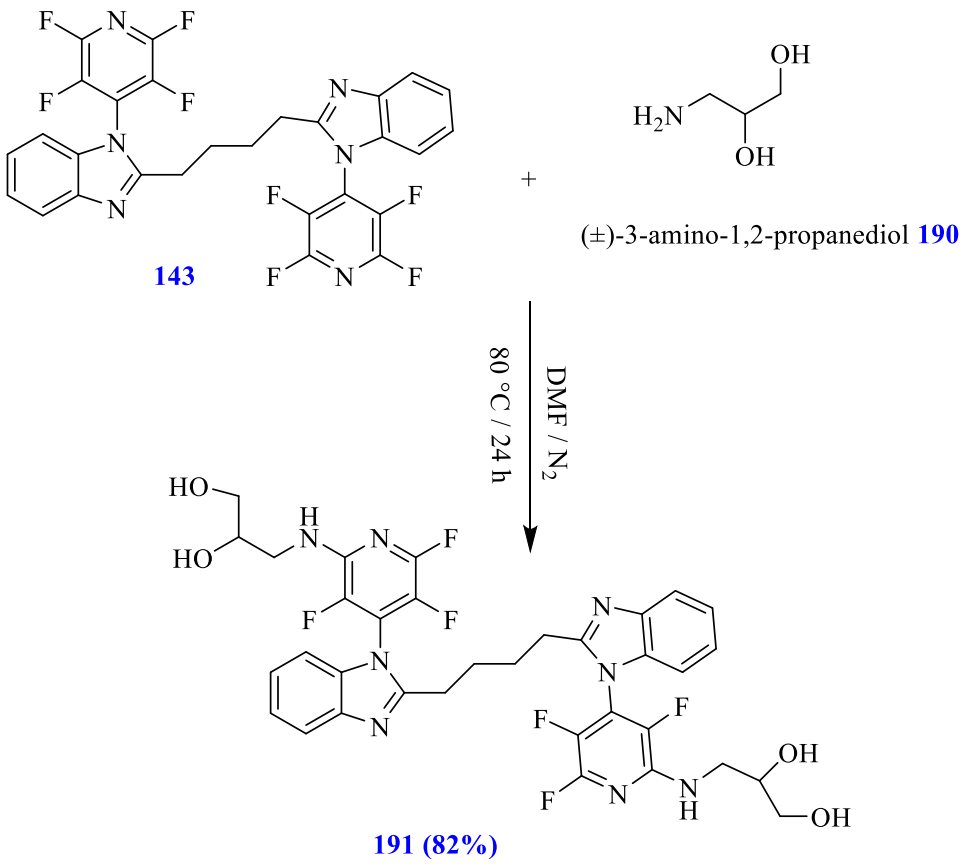
2.1.8.8. Investigation of the reaction of compound 143 with (±)-3-amino-1,2-propanediol

Reaction of two equivalents (±)-3-amino-1,2-propanediol **190** with one equivalent of compound **143** was tried in THF at RT similar to previous reactions. Unfortunately this time the reaction did not work well and different conditions were therefore studied (Table 16).

Table 16. different reaction conditions for compound 143 with (±)-3-amino-1,2-propanediol.

Entry	Reagents	Conditions	Results
1	None	THF, RT, 24 h. N_2	Starting material
2	None	DMF, RT, 24 h. N_2	Starting material and product (30%)
3	None	DMF, 80 °C, 24 h. N_2	Product (82%)

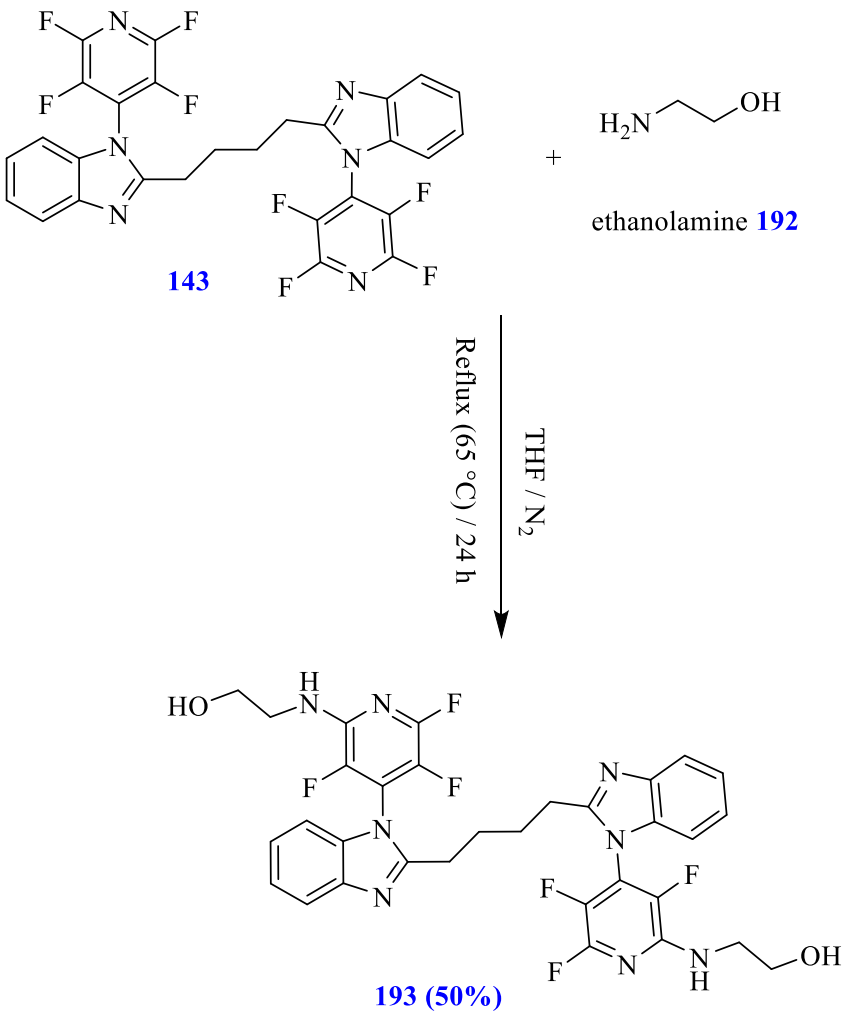
As seen in table 16, the reaction worked very well in DMF at 80 °C and target compound **191** was afforded in 82% (Scheme 66). The structure was confirmed by NMR spectroscopy. The ^{19}F NMR spectrum showed three signals (2 F for each signal) as expected. In addition accurate mass measurement gave m/z of 731.2523 (MH^+) for $C_{34}H_{33}F_6N_8O_8$



Scheme 66. Reaction of compound 143 with (±)-3-amino-1,2-propanediol.

2.1.8.9. Reaction of compound 143 with ethanolamine

In order to extend the number of novel hydrophilic fluorinated heterocyclic compounds as potential biological agents, two equivalents of ethanolamine **192** were reacted with one equivalent of compound **143** in order to form the target **193** (Scheme 67). The reaction was carried out in boiling THF at RT for 24 h and compound **193** was afforded in 50% after recrystallization from hot ethanol The structure was confirmed by its ^{1}H NMR spectrum and accurate mass measurement with m/z of 669.2161 (MH^{-}) for $C_{34}H_{27}F_{6}O_{2}N_{8}$. The ^{19}F NMR spectrum showed three signals (2F for each signal) as expected.

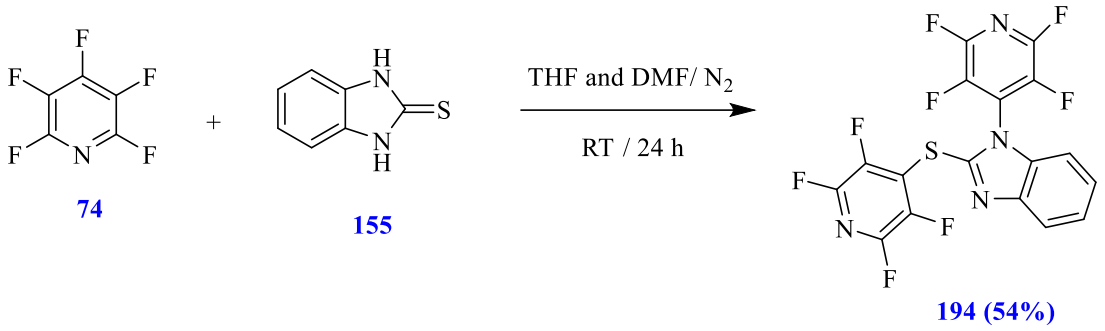


Scheme 67. Reaction of compound 143 with ethanolamine.

2.1.8.10. Reaction of pentafluoroprydine 74 with 2-mercaptobenzimidazole

As scaffold **194** showed positive activity against Trypanosoma parasites [127] and as this scaffold would have potential for further modification to allow synthesis of diverse libraries of molecules that can be screened for biological activity, we decided to synthesise this scaffold again, and also attempt to improve the yield by modifying the existing method as the reported yield [128] was very low. Therefore reaction of pentafluoropyridine **74** with 1,3-dihydro-2*H*-benzimidazole-2-thione **155** was carried out in a mixture of DMF and THF as solvent, and with NaH as base, but in this case the solution of 2-mercaptobenzimidazole in DMF : THF (3:10) was added by slow addition to a stirred

mixture of NaH and pentafluoropyridine in THF using a syringe pump to control the rate of addition. This helped to improve the reaction by increasing the yield from 27% to 54%.

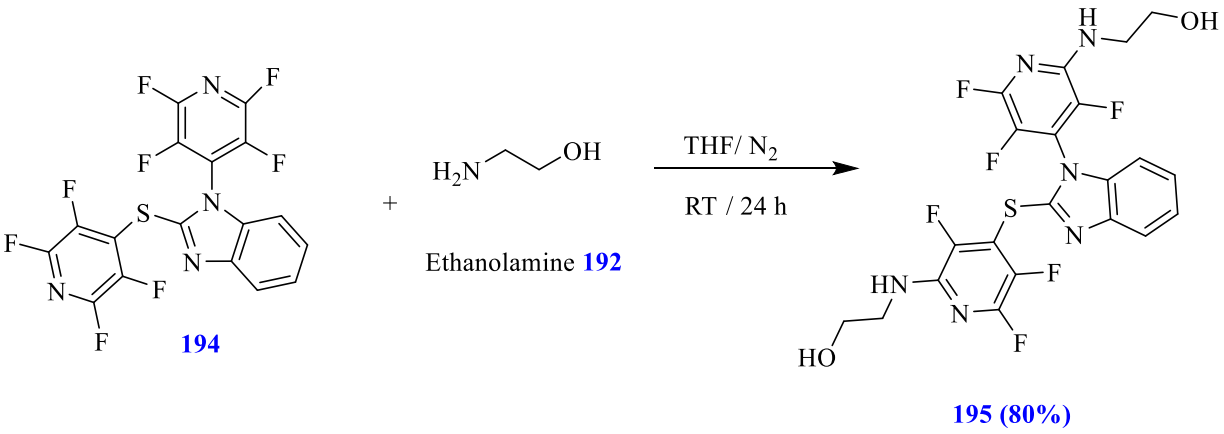


Scheme 68. Reaction of pentafluoropyridine with 2-mercaptobenzimidazole.

2.1.8.11. Investigation of further substitution of fluorine in scaffold 194 by ethanolamine

As mentioned above the bis-tetrafluorypyridyl benzimidazole derivative **194** had shown positive activity against Trypanosoma parasites [121] it was therefore decided take this compound on for further modification, and to add hydrophilic side chains to improve water solubility and biological activity, by further S_NAr reaction.

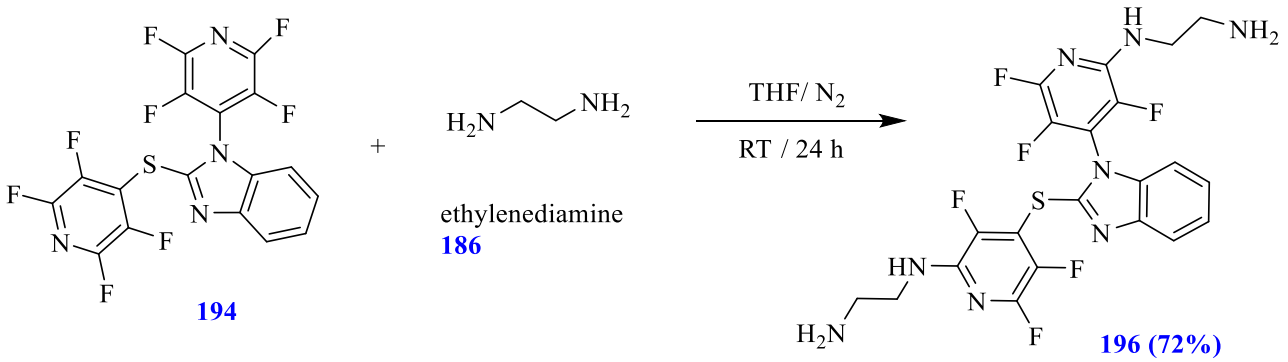
Thus, ethanolamine **192** was chosen as the first nucleophile to be investigated, and two equivalents were reacted with one equivalent of compound **194** in an approach to form the target compound **195** (Scheme 69). The reaction was carried out in THF as solvent and Et_3N as base for 24 h, at RT and the desired compound **195** was afforded in 80% after extraction as a shiny white sugary solid. The structure was confirmed by the ^1H NMR spectrum and accurate mass measurement with m/z of 531.1028 (MH^-) for $C_{21}H_{17}F_6N_6O_2S$. The ^{19}F NMR spectrum showed six signals (1F for each signal) as expected.



Scheme 69. Reaction of scaffold 194 with ethanolamine.

2.1.8.12. Subsituation reaction of scaffold 194 with ethylenediamine

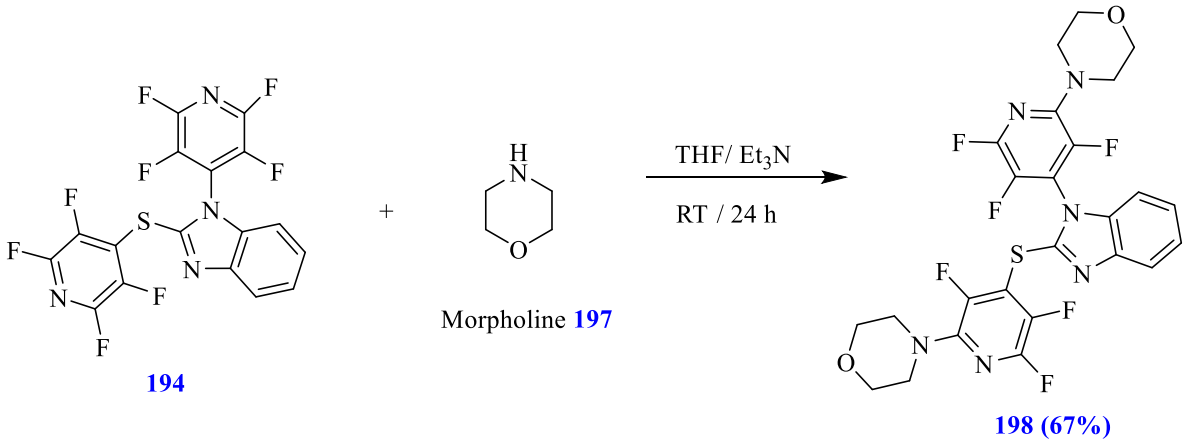
Reaction of two equivalents of ethylenediamine **186** with one equivalent of scaffold **194** was next tried with the same conditions (Scheme 70). The target compound **196** was afforded in 72% as a yellow sugary solid. The structure was confirmed by ^{1}H NMR spectroscopy and accurate mass measurement with m/z of 529.1350 (MH^-) for $C_{21}H_{19}F_6N_8S$. The ^{19}F NMR spectrum showed six signals (1F for each signal) as expected.



Scheme 70. Reaction of scaffold 194 with ethylenediamine.

2.1.8.13. Substitution Reaction of scaffold 194 with morpholine

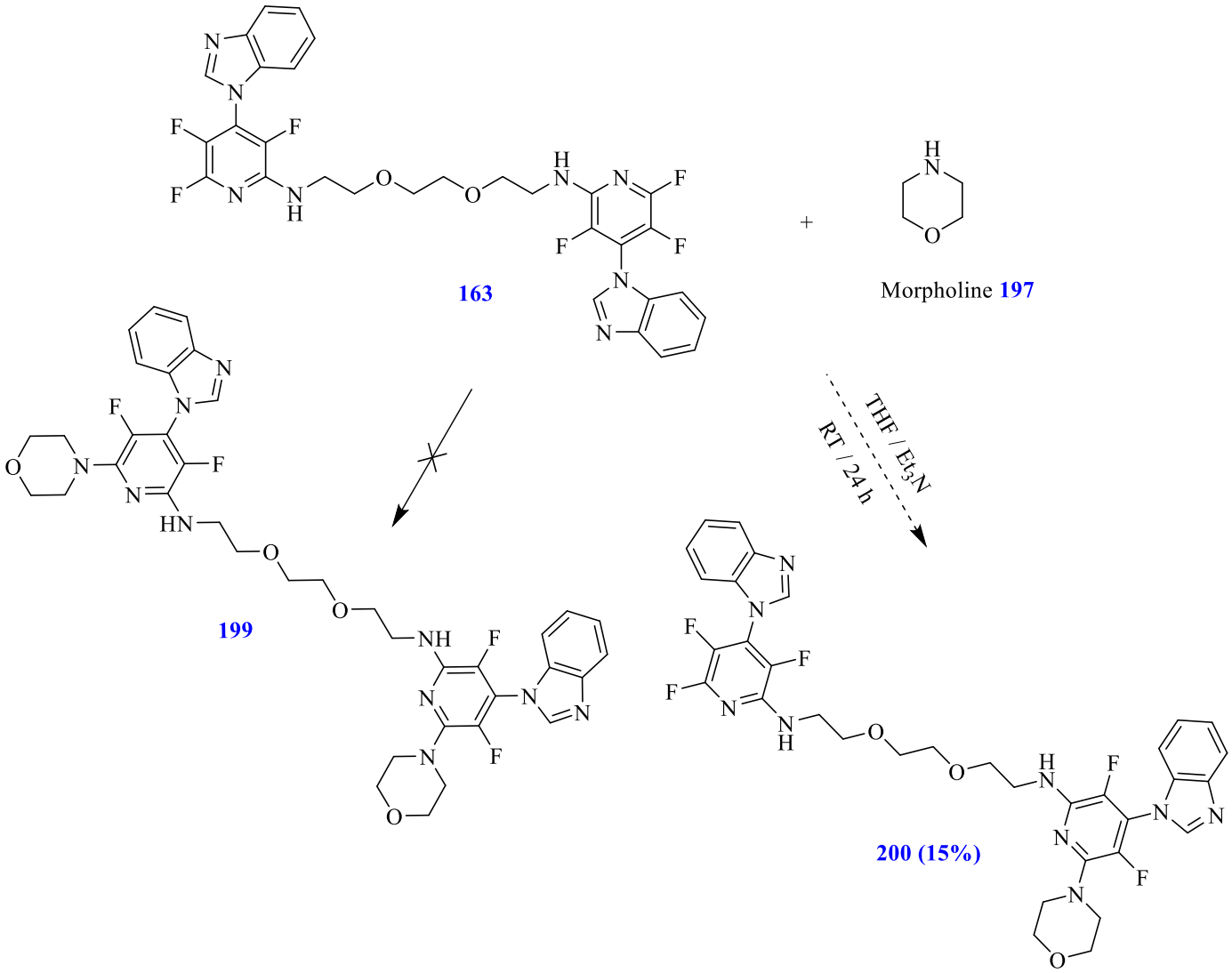
Similarly reaction of morpholine **197** with compound **194** in ratio 2:1 was investigated in THF as solvent and Et_3N as base at RT for 24 h. pleasingly the target compound **198** was again afforded in good yield (67%) after chromatographic purification. The structure was confirmed by NMR spectroscopy and accurate mass measurement with m/z of 583.1345 (MH^-) for $C_{25}H_{21}F_6N_6O_2S_1$. The ^{19}F NMR spectrum showed six signals (1F for each signal) as expected for the two differently substituted trifluoropyridine rings.



Scheme 71. Reaction of scaffold 194 with Morpholine.

2.1.8.14. Investigation of reaction of morpholine with the tethered bis-benzimidazolyl pyridine 163

After successful substitution of fluorine by morpholine **197** in compound **194** and synthesis of tethered compound **198** it was decided to try reaction of compound **163** with morpholine **197** in the hope of replacing a third fluorine in the pyridyl rings to form compound **199** Therefore reaction of morpholine with compound **163** in ratio 2:1 was undertaken in THF as solvent and Et_3N as base at RT for 24 h. After separation by column chromatography the compound **200** was afforded in 15% yield. However compound **199** was not afforded as shown in (Scheme 72), and in this reaction morpholine had added to only one of the pyridine rings and mono-substitution occurred. The structure of compound **200** was confirmed by ^{1}H NMR and ^{19}F NMR spectroscopy with tiny impurity which could not be more purify due to lack of the time and polarity of the compound. The ^{19}F NMR spectrum showed five signals (1F for each signal) as expected. Reaction at only one pyridine ring illustrates the decreasing reactivity of the remaining fluorines on increasing substitution, particularly with electron donating groups such as amines. Reaction at higher temperature may lead to morpholine adding to both pyridine end groups to form the symmetrical compound **199**.



Scheme 72. Reaction of morpholine with tethered compound 163.

2.1.9. Investigation of reaction of perfluoroarenes with aliphatic side chains which contain thiol

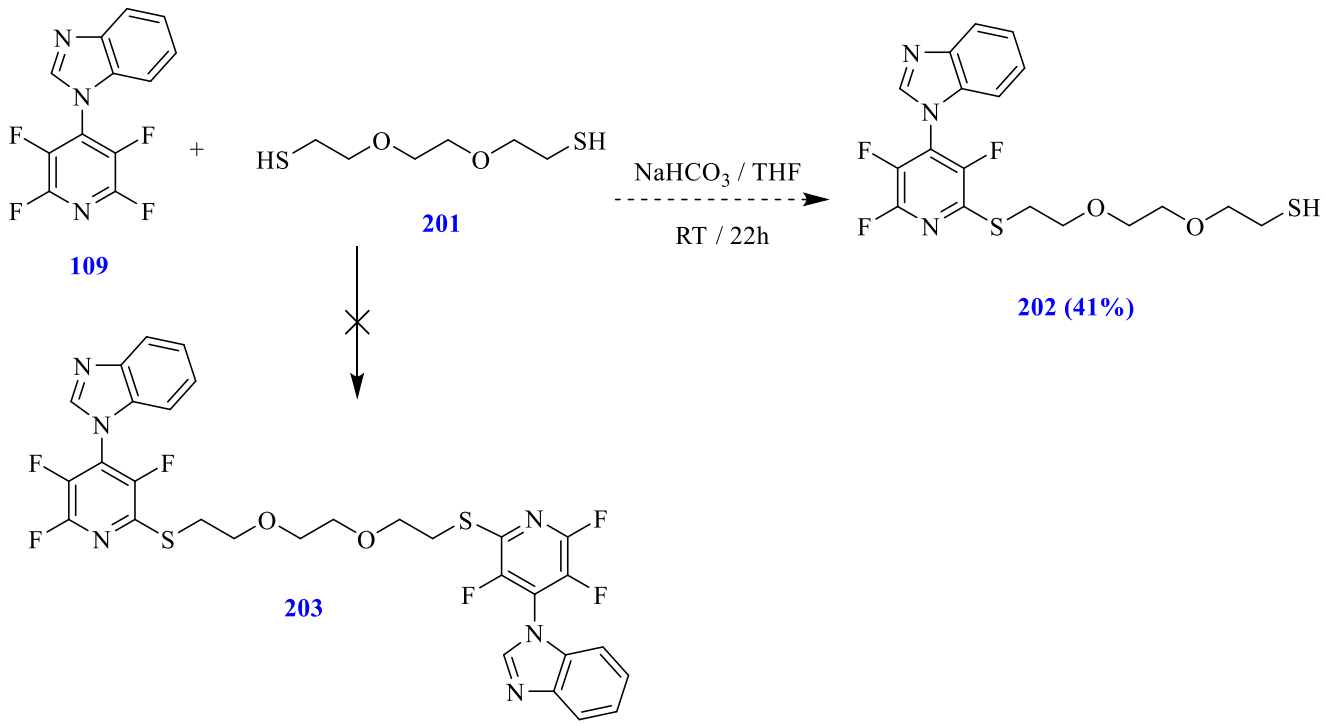
After successfully reaction of perfluroarenes with aliphatic containing hydrophilic amino and make series of novel linked and fused heterocyclic scaffolds containing amine group. We was decided to try another aliphatic side chain which contain thiol to make series of novel linked and fused heterocyclic scaffolds containing thiol group and compare the biological activity of each series.

2.1.9.1. Reaction of compound 109 with 3,6-dioxa-1,8-octanedithiol

To start we decided try to react compound **109** with **201** to make bis-linked binding agent containing sulphur group therefore reaction of compound **109** (2 equiv.) with 3,6-dioxa-1,8-octanedithiol **201** (1equiv.) was carried under different conditions. As seen in table 17, the reactions did not work well and only the reaction which was carried at 65 °C, in THF as solvent and $NaHCO_3$ as base indicated presence of compound **202** which was afforded by column chromatography as white solid in 41 % yield (entry 5). However the ^1H NMR spectrum was not completely clean and still showed some impurity, and the compound could therefore not be fully characterised. Although ^{19}F NMR spectroscopy showed 3 signals as expected, each signal contained 1 F atom. Also mass spectrometry displayed the expected mass of the compound **202**. MS (ESI) (MH^+), $C_{18}H_{19}F_3N_3O_2S_2$ requires m/z 430.0871 found m/z 430.0869. Although the target bis linked intercalator compound **203** not formed, the formation of compound **202** showed the reaction to be viable and further investigation to effect reaction on both rings should be continued.

Table 17. different reaction conditions for compound 109 with 3,6-dioxa-1,8-octanedithiol.

Entry	Reagents	Conditions	Results
1	Et_3N	THF, reflux (65 °C), 22 h. N_2	Complex NMR spectrum of unknown compound.
2	Et_3N	THF, RT, 22 h. N_2	Still complex NMR spectrum
3	No base	THF, RT. 22 h. N_2	Starting material
4	$NaHCO_3$	THF, RT, 22 h, N_2	Starting material
5	$NaHCO_3$	THF, 65 °C, 22 h, N_2	Starting material and compound **202**



Scheme 72. Reaction of compound 109 with 3,6-dioxa-1,8-octanedithiol.

2.2. DNA binding studies

2.2.1. UV absorption spectroscopy experiment

The interaction of the synthetic compounds prepared with DNA was determined by a UV absorption spectroscopy which is a simple and useful method for analysing binding. In the UV absorption assay the change in the absorbance of the test compound with and without SS-DNA gives an idea about the interaction of the substance with DNA. Known compounds, Actinomycin D (an intercalator) and naproxen (a groove binder) were used as controls. Compounds binding with DNA through intercalation usually result in hypochromism and bathochromism (red shift). As the intercalative mode involving a stacking interaction between an aromatic chromophore and the base pair of DNA, the extent of the hypochromism is usually consistent with the strength of intercalative interaction.[44] In the case of electrostatic attraction between the compound and DNA, a hyperchromic effect is observed that reflects the corresponding changes of DNA in its conformation and structure after the complex–DNA interaction has occurred. The hyperchromic effect is the noticable increase in absorbance of DNA upon denaturation.

All spectral characteristics indicated some change which were causedby change in conformation and structure of DNA after the compounds binds to it. Also the association/ binding constants of all DNA-complexes were determinate by Benesi-Hildebrand equation, Eq. (1)

$$\frac{Ao}{A-A0} = \frac{\in G}{\in H-G-\in G} + \frac{\in G}{\in H-G-\in G} \times \frac{1}{K[DNA]} \qquad (1)$$

Where *K* is the binding constant, Ao is absorbance of drug without any DNA, and A is absorbance of the DNA-Drug complex. ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of the drug and DNA-Drug complex, respectively. The *K* value was determined from the intercept-to- slope ratio of Ao/A-Ao vs. 1/ [DNA] plot.

In addition the Gibbs free energy (ΔG) was used to calculate for each complex using Eq. (2)

$$\Delta G = -RT \ln K \qquad (2)$$

Where R is general gas constant (8.314 $JK^{-1}mol^{-1}$) and T is room temperature (298 K).

2.2.1.1. UV-visible spectroscopy of ACTD as known intercalate agent and naproxen as known groove binder

ACTD is a known DNA-interacting transcription blocker with anti-cancer activity.it interferes with both DNA replication and transcription by intercalation between bases. Also it acts as a cytotoxic inducer of apoptosis in tumour cells. Therefore it was used as reference in this study (Figure 35)

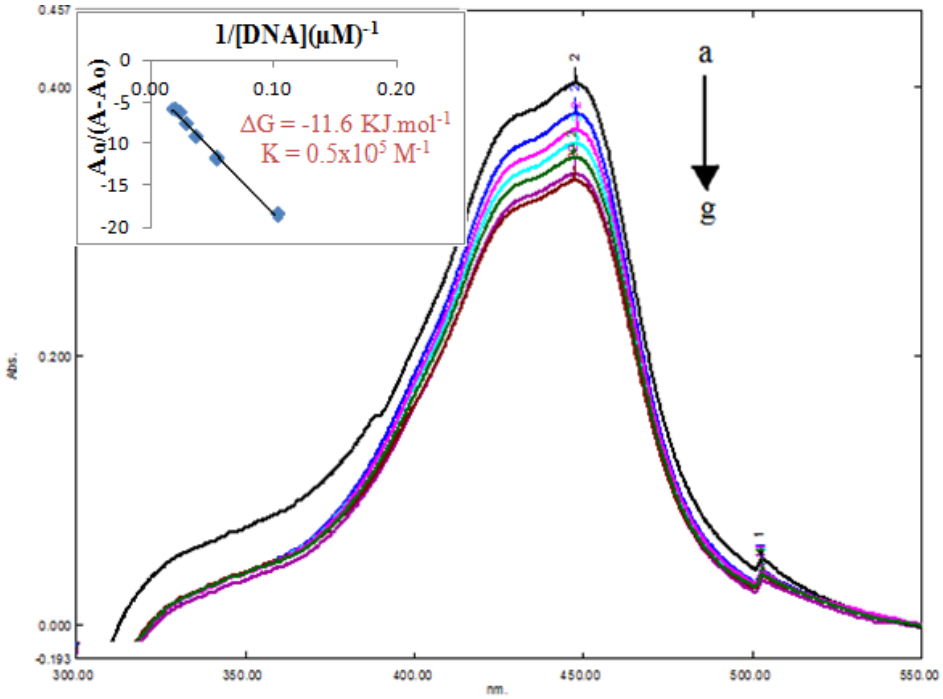


Figure 35. Absorption spectra of 1 x 10^{-5} M ACTD in absence (a) and presence of 9.6 µM (b), 18 µM (c), 27 µM (d), 35 µM (e), 43 µM (f) and 50 µM (g) of DNA.

The arrow shows increasing DNA concentration. Inside graph is plot of Ao/ (A-Ao) vs. 1/ [DNA] for determination of binding constant (K) and Gibbs free energy (ΔG) of ACTD. (R^2 = 0.9926 for six point)

According to Figure 35, the ACTD as known intercalator control showed strong absorption in the region (440-460 nm) which is accredited to the long-living triplet excited state of aromatic system. Also the spectra indicated that by increasing concentration of DNA, the absorption bonds of the complex were decreased which resulting in the tendency of hypochromism as expected due to the intercalation of ACTD with DNA. Hypochromism is caused by contraction of DNA helix, as well as from conformational change of DNA. The binding constant value for ACTD is 0.5x10^5 M^{-1} which

indicated strong affinity of the drug to SS-DNA. The Gibbs free energy is -11.6 $kJmol^{-1}$. The negative value of ΔG shows a spontaneous process in this interaction.

Naproxen is another known DNA binding agent which was used as possible groove binder control. As seen in (Figure 36) it showed strong absorption at300 nm.The spectra indicated that by increasing concentration of DNA, the absorption decreased therefore in the case of groove binding still we have the hypochromism as well as the intercalation interaction. The *K* value (1×10^5 M^{-1}) and the Gibb's free energy (-12.3 $kJmol^{-1}$) of naproxen was higher than the *K* value and the Gibbs free energy of ACTD (figure 14) which indicated that naproxen has higher affinities to SS-DNA than AD. The negative value of ΔG shows a spontaneous process.

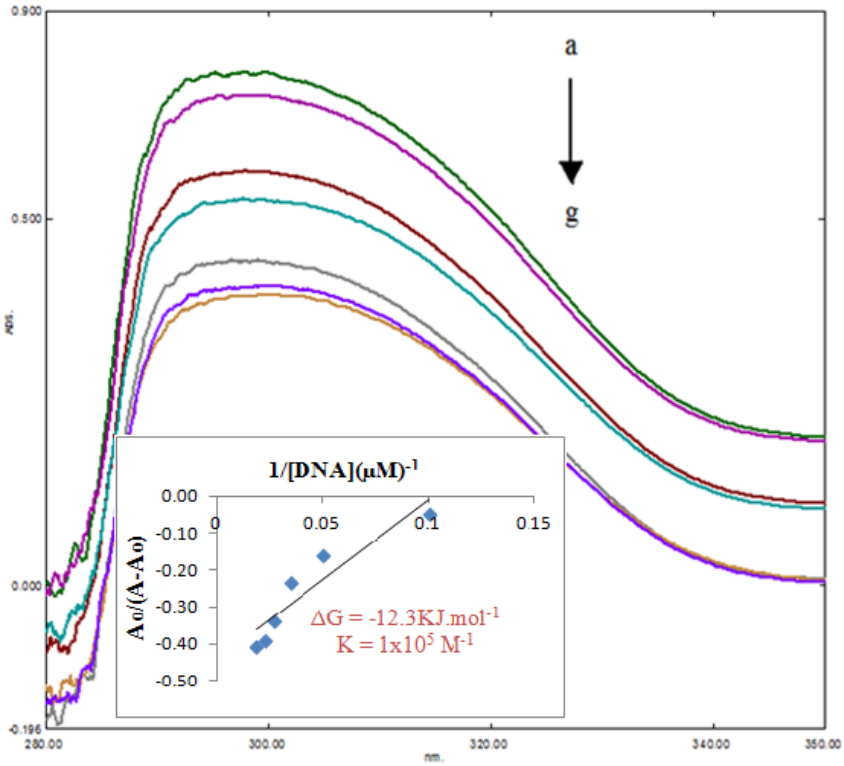


Figure 36. Absorption spectra of 1×10^{-5} M of naproxen in absence (a) and presence of 9.6 µM (b), 18 µM (c), 27 µM (d), 35 µM (e), 43 µM (f) and 50 µM (g) DNA.

The arrow shows increasing of DNA concentration. Inside graph is plot of Ao/ (A-Ao) vs. 1/ [DNA] for determination of binding constant (*K*) and Gibbs free energy (ΔG) of.naproxen (R^2 = 0.8614 for six points)

2.2.1.2. Studies of UV absorption results and cooperation of binding constant data

Table 18. Absorption changes caused by synthesised compounds intraction with DNA.

Compounds	Absorption	*K* value (M^{-1})
108	Decrease (hypochromism)	0.5×10^4
111	Decrease (hypochromism)	2.9×10^4
113	Decrease (hypochromism)	1.1×10^5
114	Decrease (hypochromism)	1.5×10^5
137	Decrease (hypochromism)	0.1×10^5
138	Decrease (hypochromism)	0.3×10^4
143	Decrease (hypochromism)	0.6×10^5
151	Decrease (hypochromism)	4.7×10^4
152	Decrease (hypochromism)	1.4×10^5
161	Decrease (hypochromism)	6.9×10^4
162	Decrease (hypochromism)	2.5×10^4
163	Decrease (hypochromism)	0.8×10^4
164	Decrease (hypochromism)	1.5×10^3
165	Increase (hyperchromic effect)	1.5×10^5
166	Decrease (hypochromism)	8.9×10^4
169	Decrease (hypochromism)	4.4×10^4
170	Decrease (hypochromism)	9.8×10^4
171	Decrease (hypochromism)	8.8×10^4
181	Decrease (hypochromism)	1.1×10^5
183	Decrease (hypochromism)	7.6×10^4
187	Decrease (hypochromism)	4.4×10^4
189	Decrease (hypochromism)	1×10^5
191	Decrease (hypochromism)	3×10^4
195	Decrease (hypochromism)	2.4×10^5
196	Decrease (hypochromism)	1.8×10^5
198	Increase (hyperchromic effect)	2.1×10^5

According to the above table the K values (bonding constant) more than $1x10^{-5}$ indicates good binding affinity, the K values between $1x10^{-4}$ – $1x10^{-5}$ indicate moderate binding affinity and the K values below $1x10^{-4}$ indicate poor binding affinity. Also most of the compounds on adding the DNA in different concentrations showed a decrease in the absorption which indicated a hypochromic effect due to the intercalative interaction. Only compounds **165** and **198** showed an increase in the absorption due to a hypochromic effect which can be result of electrostatic attraction between the compounds and DNA.

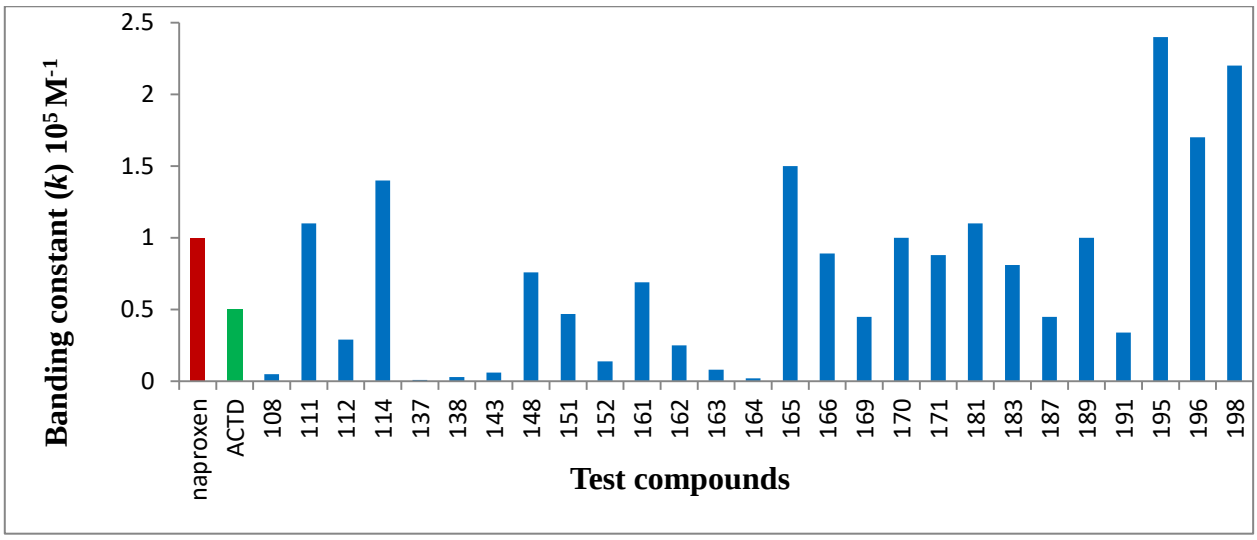
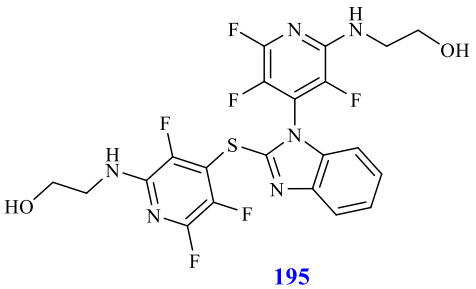


Figure 37. UV absorption of synthesised compounds with different concentrations of DNA. Each bar in this graph represents the binding constant of different synthesised compounds.

According to figure 37, all of the synthesised compounds showed some affinity to SS-DNA in the UV absorption assay, although in comparison to the reference compounds and each other, binding is very different. Interestingly compounds **165**, **196** and **198** showed the greatest *k* values compared to the other compounds and even references which indicated highest bonding affinity to SS-DNA than other synthesised compounds. In addition compound **195** and **198** showed closest and higher binding constants in comparison to **196** which might be because of presence of oxygen in both structure, as seen in below.



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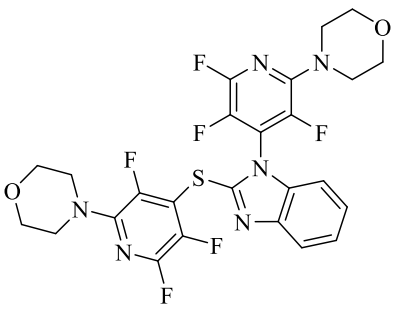

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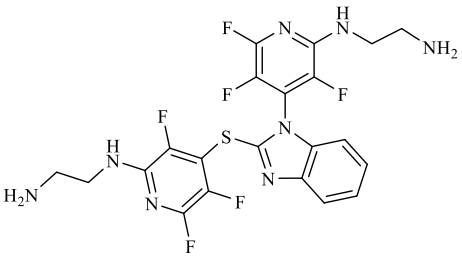


196

Compounds **114** and **165** have the second largest binding constant values which are still higher then reference's *K* value. Compounds **113**, **170, 181** and **189** indicated very similar *K* values to naproxen. Along with the *K* value of compounds **169** and **187,** which was very close to ACTD, compounds **108**, **112**, **137**, **143**, **162**, **163**, **164**, and **191**, showed very weak DNA binding affinity compared to the referece and the other compounds.

2.2.2. Ethidium bromide (EB) fluorescence displacement experiment

Ethidium bromide (EB) fluorescence displacement assay was another useful method for studying the interaction of the synthetic compounds prepared with DNA. In this method the EB fluorescence intensity will increase in the presence of DNA by intercalation of EB into the double helix (Figure 38). It is quenched by addition of another compound as the quencher then displaces EB from DNA. Therefore if the fluorescence intensity decreased on adding the compound it means that the test compound binds to the DNA and displaces the EB. If no change in intensity occurs it does not mean the compound does not bind to DNA as it may bind in another non-intercalating way.

The linear Stern-Volmer quenching constant K_{sv} was calculated from the linear Stern-Volmer equation (3), by considering the quenching of EB bound to DNA by different concentration of quencher or compound.

$$I_o/I = 1 + K_{sv}[Q] \qquad (3)$$

Where I_o and I represent the fluorescence intensities in absence and presence of the compound and Q is the concentration of the compound. K_{vs} values were calculated from the slope of the regression line in the derived plot of I_o/I vs compound concentration.

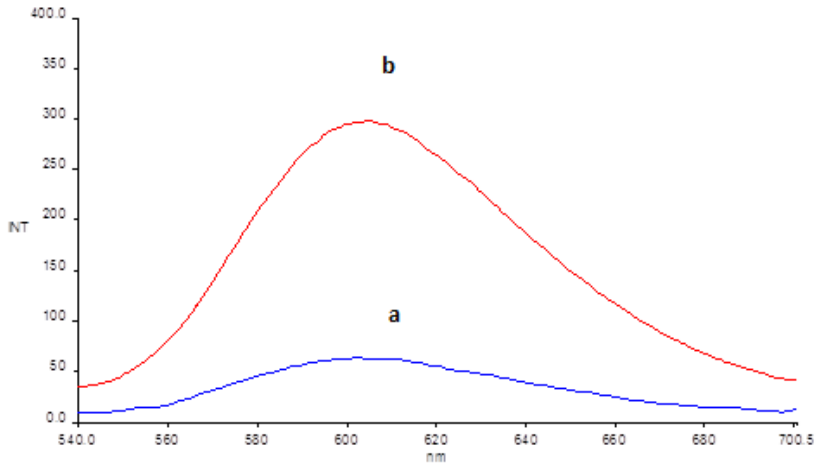


Figure 38.) Emission spectra of EB 1 x 10-6 M in the absence of DNA and b) Emission spectra in presence of DNA 3.17 x 10-5 M

2.2.2.1. EB fluorescence displacement assay of Actinomycin D as intercalating agent

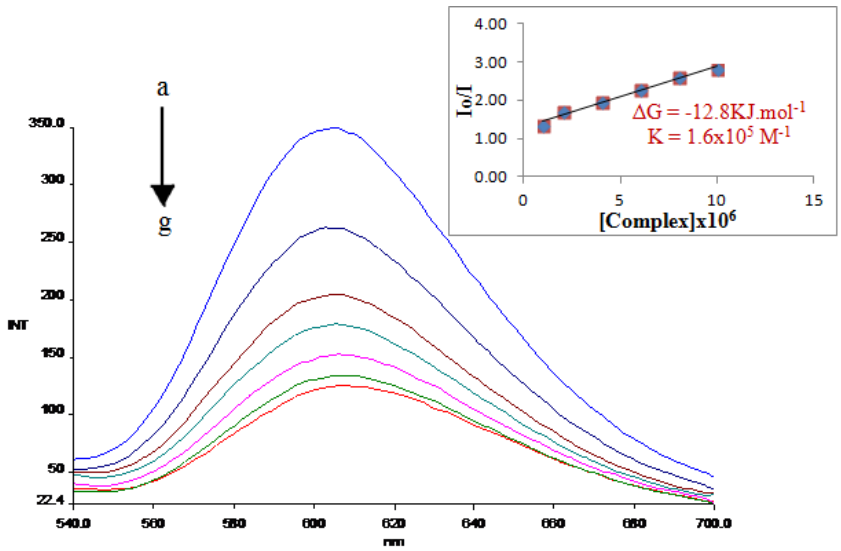


Figure 39. Emission spectra of SS-DNA-EB in tris-HCl buffer on titration of ACTD. Kex = 480 nm; [EB] = 1 x 10^{-6} M; [DNA] = 3.17 x 10^{-5} M;[ACTD] (a) 0.0, (b) 1 x 10^{-6}, (c) 2 x 10^{-6}, (d) 4 x 10^{-6}, (e) 6 x 10^{-6}, (f) 8 x 10^{-6}, (g) 10 x 10^{-6} M. The arrow shows the increase of the complex concentration. Inside graph is plot of Io/ I vs. [DNA] for determination of *K* and ΔG values. (R^2= 0.9782 for six points)

According to figure 39, by increasing of the ACTD concentration, the fluorescence intensity at 605 nm decreased significantly, which indicated the ACTD had displaced the EB completely and bound to DNA by intercalation interaction as expected. Also the K_{vs} value for ACTD complex was found to be 1.6 x 10^5 which indicated a very strong affinity of ACTD complex to SS-DNA. The Gibbs free energy is -12.8 kJ.mol^{-1}. The negative value of ΔG shows a spontaneous process in this interaction.

2.2.2.2. EB fluorescence displacement assay of naproxen as groove binding agent reference.

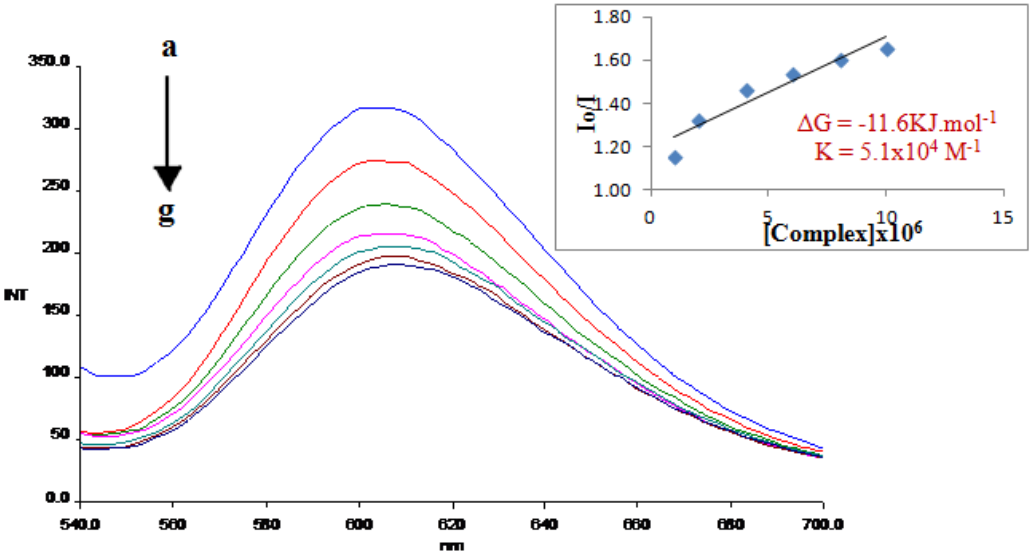


Figure 40. Emission spectra of SS-DNA-EB in tris-HCl buffer on titration of naproxen. Kex = 480 nm; [EB]= 1 x 10^{-6} M; [DNA] = 3.17 x 10^{-5} M; [AD] (a) 0.0, (b) 1 x 10^{-6}, (c) 2 x 10^{-6},(d) 4 x 10^{-6}, (e) 6 x 10^{-6}, (f) 8 x 10^{-6}, (g) 10 x 10^{-6} M. The arrow shows the increase of the complex concentration. Inside graph is plot of Io / I vs. [DNA] for determination *K* and ΔG values. (R^2= 0.9782 for six points)

According to Figure 40, by increasing of the naproxen concentration the fluorescence intensity decreased which indicated naproxen complex interact with DNA as groove binder and releasing some EB from EB-DNA system. Another possibility is naproxen binds to DNA in different binding sites and changes the conformation of DNA which causes a decrease in the fluorescence intensity. Also the K_{vs} value for naproxen complex was found to be 5.1 x 10^4 M^{-1} which indicated affinity of naproxen complex to compete at binding site of SS-DNA which EB binds as an intercalating agent is much less then ACTD (Figure 39) as expected. The Gibbs free energy is -12.8 kJ. mol^{-1}. The negative value of ΔG again shows a spontaneous process.

2.2.2.3. *Binding constant comparison of synthesis compound using EB quenching assay*

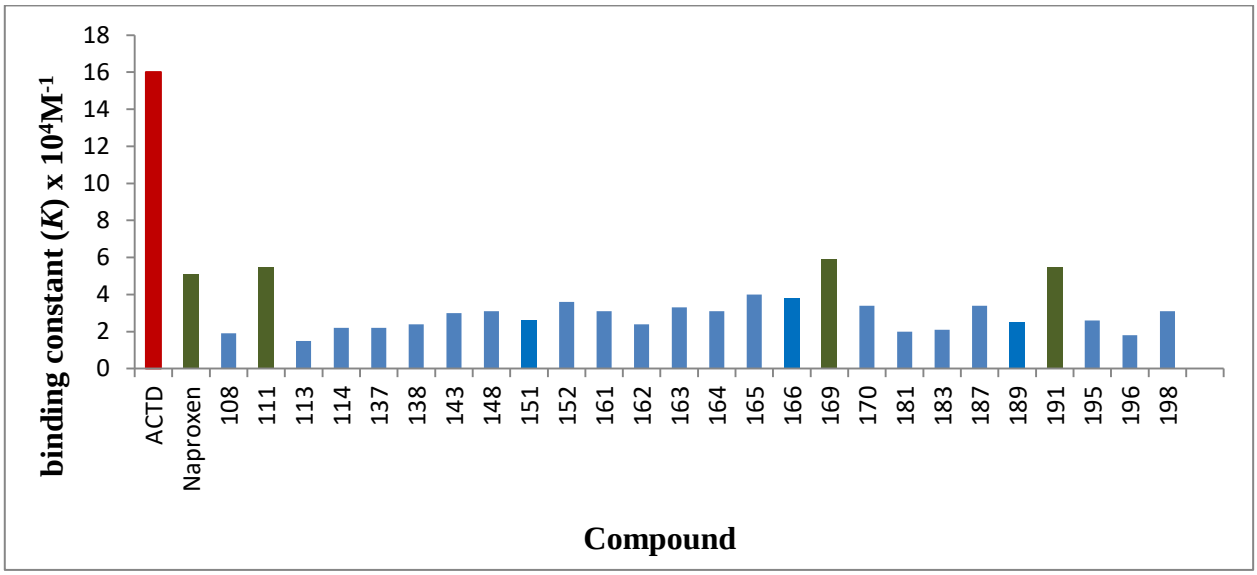
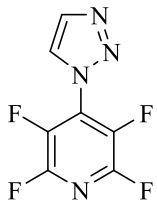
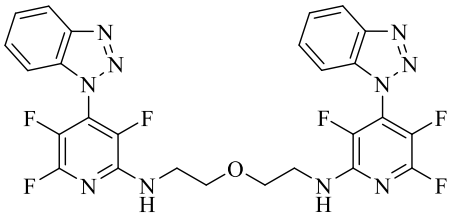


Figure 41. EB displacement assay of synthesis compound. Each bar in this graph represents binding constant of different synthesised compounds. ACTD and naproxen were used as references.

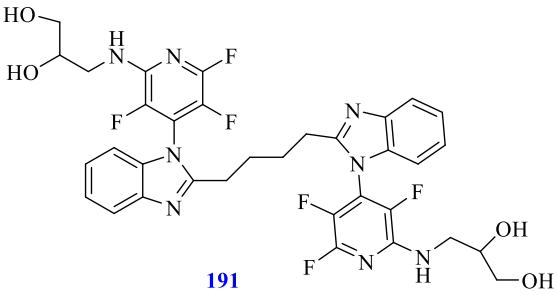
According to Figure 41, all the synthesised compounds showed some affinity to SS-DNA in this assay, although in comparison to the intercalating reference compound ACTD binding was very weak. Compounds **111**, **169**,and **191**, indicated the greater *K* values compared to others. Therefore theses compound showed stronger affinity to SS-DNA than other synthesised compound and their K_{vs} values are closest to the K_{vs} value of naproxen**.**



X isomer **111**



169



191

2.3. Anticancer activity in vitro

The anticancer activities of synthesis compounds (**143**, **148**, **151**, **152**, **181**, **183**, **187**, **189**, **191**, **193** and **194**) were determined against breast carcinoma MCF-7 and leukemia K562 cell lines after 72 h of incubation using the Calcein assay, and the CDK inhibitor, roscovitine, as a control compound. The results obtained (Table 19), show that compounds **143**, **187**, **183** and **194** display cytotoxicity in which IC_{50} values reached low micromolar ranges.

Table 19. Cytotoxicity of fluoroarylbenzimidazole derivatives

.Compound	Cell line IC_{50} (μM)	
	K-562	MCF-7
143	2.8 ± 0.4	7.8 ± 2.0
148	>12.5	>12.5
152	>12.5	>12.5
183	7.2 ± 1.5	4.8 ± 0.4
187	3.4 ± 0.1	8.0 ± 2.6
189	>12.5	>12.5
191	>12.5	>12.5
193	>12.5	>12.5
194	13.2 ± 1.3	>25
Roscovitine	42 ± 3	11 ± 1

These four compounds were further screened by collaborating partners against melanoma G361 and osteosarcoma HOS cell lines to expand information about selectivity towards various types of cancers (Table 20). Concentration-dependent activity it was observed in all cases with these four compounds. Three of the active compounds contained the tetramethylene bis-benzimidazole linker group **134** with tri- or tetrafluorinated pyridine rings, whilst the smaller molecule **194** also contains two fluorinated pyridine rings, separated by the benzimidazole ring. This suggests that two fluorinated pyridine rings separated by 4-6 Å is desirable for activity. However the mechanism of the activity is not yet clear.

Table 20. Cytotoxicity of lead derivatives.

Compound	IC50 (μM)	
	G361	HOS
143	2.0 ± 0.1	1.8 ± 0.1
183	6.0 ± 0.1	8.3 ± 2.0
187	19.9 ± 1.2	16.3 ± 3.0
194	4.4 ± 0.6	2.5 ± 0.9
Roscovitine	22.4 ± 0.2	24.3 ± 0.2

Further experiments were conducted to gain information about the mechanism underlying the observed cytotoxicity. Firstly, the effect of compounds **183**, **187** and **194** on the cell cycle of K562 and MCF-7 cells was analysed. Asynchronously growing cells were treated for 24 h with compounds in two doses, corresponding to 1× and 3× IC_{50} values, followed by analysis by flow cytometry. As shown in Figure 42, the compounds markedly influenced cell cycle profiles in both cell lines although each compound demonstrated this effect in a different way. Treatment of MCF-7 cells with a low dose of **143** led to a decrease in the S phase, whilst a higher dose significantly decreased G1-phase population and increased sub-G1 population in both K562 and MCF-7 cell lines. Compound **187** had no effect at the lower dose used, but the higher dose significantly increased G2/M population in MCF-7 cells. Interestingly, treated K562 cells completely altered the profile, with apparent block of S phase. Macrocyclic compound **183** markedly reduced S and G2/M phases in MCF7 cells, while the cell cycle profile in K562 was not changed at all.

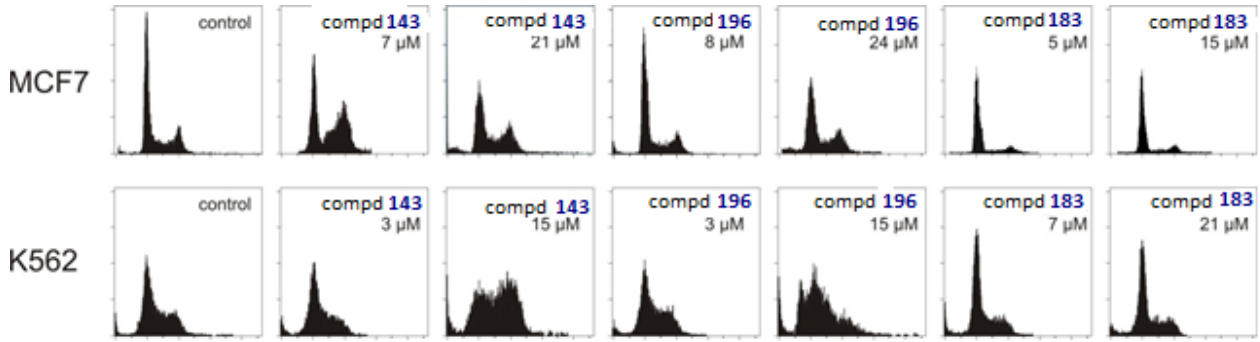


Figure 42. Effect of studied compounds on the cell cycle of MCF7 and K562 cells treated for 24 h.

Flow cytometric analysis revealed that some compounds increase sub-G1 population, which is a well accepted indicator of ongoing apoptotic cell death. We therefore analyzed treated K562 cells for activity of caspases 3 and 7, proteases that are activated during apoptotic cell death. (Figure 43) shows the results of biochemical assay of caspase 3/7 activity in cells treated for 24 h with the studied compounds at doses corresponding to 1× and 3× IC_{50} values. While **187** was inactive in the assay, compounds **183** and **194** significantly stimulated activation of the caspases, with the increase in activity more than twenty-fold, and five-fold, over untreated control cells respectively. Based on flow cytometric results and biochemical assay of caspases we conclude that **183** and **194** induce apoptosis in treated cells. However, the flow cytometric experiments suggest that the active compounds exhibit their toxicity through different cellular targets. Further work is ongoing to identify the mode of action of these fluorinated compounds.

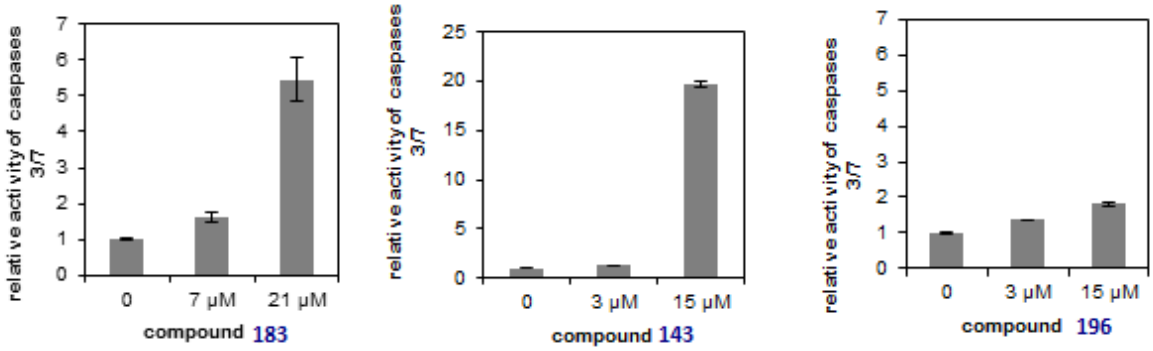


Figure 43. Fluorimetric caspase activity assay. K562 cells treated with studied compounds for 24 h were lysed and the activities of caspases were measured using the fluorogenic substrate Ac-DEVD-AMC and normalised to untreated control.

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2.4. Antibacterial activity of synthesised compounds

The synthesised compounds were examined against a gram positive (*Staphylococcus aureus*) and a gram negative (*Escherichia coli*) bacterium *in vitro* by using the disc diffusion method with each experiment performed in triplicate. Amoxicillin was used as reference antibiotic in this assay. Criteria for activity is based on the diameter of the inhibition zone (mm); an inhibition zone more than 20 mm indicates significant activity, for 18-20 mm inhibition is good, 15-17 mm is low, and below 11-14 mm is non-significant activity.[35, 66] The following compounds at 10^{-3} M were tested, **108**, **114**, **137**, **138**, **143**, **148**, **161**, **162**, **163**, **164**, **165**, **171**, **181**, **183**, **198** , but showed no zone

inhibition at all. Compounds **111**, **113**, **251**, **252**, **166**, **169**, **170**, **187**, **189**, **191**, **195** and **196** showed an inhibition zone and reading of the zone inhibition represents the mean value of these readings, which are shown in Table 21.

Unfortunately the antibacterial activity assay confirmed that the tested compounds showed non-significant activity against the bacteria, because the inhibition zones were below 11-14 mm. As the high concentration 10^{-3} M did not show a significant inhibition we did not study any lower concentrations of the compounds. Although the compound **191** showed highest activity against *E. coli* and compound **187** showed the highest activity against *S. aureus*. Therefore the fluorinated synthesized compounds in the present study did not show good antibacterial activity against either *Escherichia coli* or *Staphylococcus aureus* strains, despite the evidence for DNA binding. Further studies would be required to understand the exact interaction of the compounds with bacterial cells.

Table 21.Antimicrobial activity of some fluorinated synthesized compounds

Compound	Average zone of inhibition (mm)	
	Escherichia coli	*Staphylococcus aureus*
Amoxicillin	19	40
111	9	0
113	9	0
151	11	0
152	10	7
166	10	8
169	7	9
170	9	0
187	0	14
189	10	0
191	12	0
195	0	7
196	0	9

2.5. Hanging drop DNA crystallization

In DNA crystallization assay a piece of melting point tube was used to hold a drop of DNA-compound mixture in a cacodylate buffer solution. This was attached to wall of sample vial above

cotton wool wetted with the more concentrated buffer solution to help to absorb solvent from the drop to concentrate it, leading to crystallisation. The sample tube containing the drop was left at RT for a prolonged time to form crystals. Cacodylate buffer was used to dissolve the short synthetic oligonucleotide CGCGAATTCGCG by adding 600 µl of the buffer to DNA to prepare conc. 0.5 mM of DNA. Synthesised compounds were dissolved in DMSO (0.01 mol, 10 ml) in separate vials and the drop was making by mixing 2 µl of each compound stock solution with 20 µl of CGCGAATTCGCG DNA. Unfortunately to date no crystals have formed and this method has given no information about the DNA-compound complexes formed.

3. Conclusions and future work

Over fifty new composite, linked and fused heterocyclic scaffolds have been successfully synthesised using S_NAr substitution reactions of perfluorinated arene building blocks including pentafluoropyridine, hexafluorobenzene, and methyl pentafluorobenzoate with different nucleophile hetarenes such as imidazole, triazole, benzimidazole, benzotriazole, and carbazole. Furthermore, these scaffolds would have potential for further modification by introducing different water solublising side chains to allow synthesis of diverse libraries of molecules which are more water souluble and have improved potential for biological activity. X-ray crystal structures of ten compounds were obtained including those of two macrocyclic compounds **181** and **183** containing 24- and 21-membered rings.

Cyclization of ester derivative **148** to make bi- or tri-cyclic bis-intercalators by using different nucleophiles reagent such as hydrazine, 2-aminopyridineas and acetamidine did not prove successful. Only compound **176** cyclised on just one of the fluoroarene rings, leaving the ester intact on the other, by using acetamidine which was a more reactive nucleophile than the other reagents tested.

A number of compounds synthesised have been shown to bind to DNA by a combination of UV absorption and fluorescence measurement, although the exact nature of binding has not been determined.

From UV absorption most of the test compounds showed decrease in the absorption which indicated a hypochromic effect due to the intercalative interaction. Only compounds **195** and **168** showed a hypochromic effect by an increase in the UV absorption, which can be result of electrostatic attraction between the compound and DNA.

In addition, interestingly compounds **195**, **196** and **198** showed the greatest *K* values in binding constant compared to the other compounds and known references which indicated higher bonding

affinity to SS-DNA than other synthesised compounds. Compounds **113**, **170**, **181** and **189** indicated very similar *K* values to naproxen. Along with *K* value of compounds **169** and **187** was very close to ACTD.

In terms of fluorescence assay, only compounds **191**, **169** and **112** indicated greater *K* values compared to other scaffolds, which showed stronger affinity to SS-DNA than the other synthesised compounds, although in comparison to the *K* value of intercalating reference compound ACTD are still much lower.

For anticancer activity of the test compounds, only three compounds **143**, **183** and **187** demonstrated micromolar inhibition against K-562 and MCF-7 cell lines. These compounds, in addition to **194**, also demonstrated micromolar inhibition against G361 and HOS cell lines. Compounds **143** and **183** were found to activate caspases leading to apoptosis.

Attempts to co-crystallize the compounds with a synthetic oligonucleotide to allow X-ray diffraction studies to determine binding were unsuccessful. Also studies on the antimicrobial activity of a range of the new compounds indicated non-significant activity against the bacteria because the inhibition zones were below 11-14 mm. However, compound **187** showed the highest activity against of *S. aureus* and compound **191** showed highest activity against *E. coli*.

The synthetic work has generated several areas where additional study would be desirable. The formation of **176** showed the cyclization of ester derivatives to make bi- or tri-cyclic bis-intercalators to be viable, and further investigation to effect reaction on both rings should be continued. Also cyclization of ester derivative using more reactive nucleophiles reagents could be explored. Further development of methods to foresee and control the stepwise addition of nucleophiles to perfluoroarenes should be undertaken to study the orientation effects of the added groups, and to improve reactivity as the number of available fluorine atoms falls with progressive substitution. To improve biological activity of the compounds further work could be carried out to replace the remaining fluorine atoms in the ring systems synthesised with alternative aromatic or aliphatic containing hydrophilic amino, hydroxyl and thiol groups. Also synthesis of positively charged derivatives should be undertaken to improve binding to DNA.

Additional attempts to co-crystallize the products synthesised with the DNA strands of known composition should be carried out, to allow a more detailed understanding of the molecular interaction involved in binding activity. A number of the successfully synthesised compounds in this work are being taken forward in a further project to test for anticancer and anti-parasitic activity.

4. Experimental

4.1. General

All starting materials and solvents were obtained commercially. THF was distilled under a nitrogen atmosphere from sodium/benzophenone ketyl radical. Anhydrous conditions were obtained by using oven/flame-dried glassware purged with nitrogen prior to the addition of chemical reagents. A nitrogen atmosphere was maintained throughout reactions where necessary through the use of a nitrogen balloon. NMR spectra were recorded on a Bruker DPX 400 operating instrument or a Jeol ECS-400 at 400 MHz for ^{1}H or 376 MHz for ^{19}F NMR spectra or 100 MHz for ^{13}C spectra were recorded in $CDCl_3$, DMSO-d_6 or methanol-d_4. Chemical shifts are given in parts per million (ppm) and coupling constants are recorded in hertz. Tetramethylsilane was used as internal standard for ^{1}H and ^{13}C spectra. ^{19}F NMR spectra re referenced to hexafluorobenzene. DEPT was used to assign environment (CH, CH_2, and CH_3) to each carbon atom in the ^{13}C spectra. Mass spectra were recorded on a Thermo Scientific Exactive (Orbi) ESI (Ethanol) via nanomate (Advion) or were recorded at the EPSRC National mass Spectrometry Facility. The solvent used for all samples was methanol. GC-MS was performed on a Fisons 8060 with a DB5MS column of 30 m length and splitless injection.

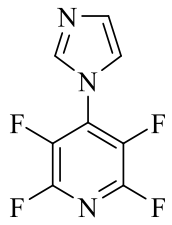
IR spectra were obtained using a Perkin-Elmer spectrum 65 FT-IR spectrophotometer. Sodium chloride plates were used to acquire thin film spectra, or samples were recorded as KBr discs. Melting points were recorded using an Electrothermal-IA 9100 melting point instrument and are uncorrected. Elemental analysis was determined using a Perkin-Elmer 2400 analyser.

The reactions were checked using thin layer chromatography (TLC) on Merck TLC silica gel 60 F254 aluminum backed plates. UV radiation at a wavelength of 254 nm using a U.V.P chromate-vue cabinet model CC-60 used to visualise TLC plates. Column chromatography was carried out on Merck Kiesel 60 silica gel.

For biological activity assays salmon sperm (SS) DNA, naproxen, ACTD and EB were purchased from Sigma. A Spectra UV-2550 instrument was used for UV absorption assay and a Perkin Elmer Luminescence Spectrometer S50B was used for fluorescence assay. For DNA crystallization experiments the DNA sequence CGCGAATTCGCG was purchased from Sigma. Cacodylate buffer was pH 6.5; 50 mM-isopropanol 15% solution. Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* strains were used for antibacterial studies. Muller Hinton agar, petri dish 90 × 14 mm (diameter and height respectively) and Townson + Mercer incubator was used to incubate the bacteria in anti-microbial activity studies.

4.2. Organic synthesis

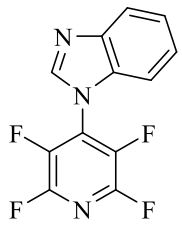
4.2.1. 2,3,5,6-Tetrafluoro-4-(1H-imidazol-1-yl)pyridine 108[129]



108 (89%)

A solution of imidazole **27** (2 mmol, 0.136 g) in THF (4 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2.5 mmol, 0.1 g) in THF (5 mL). The mixture was reacted at room temperature for 30 min. A solution of pentafluoropyridine **74** (6 mmol, 0.7 mL) in THF (3 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (25 mL × 3) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give compound **108** (0.39g, 89%) as yellow oil.
ν_{max} /cm^{-1} (film) 2986, 1645, 1479, 1265, 1189, 1078, 968, 832, 738 ; MS (ESI) (MH^+), $C_8H_3F_4N_3$ requires *m/z* 218.0328; found *m/z* 218.0335; δ_H (400 MHz, $CDCl_3$) 8.01 (1H, s), 7.43 (1H, t, *J 1.6* Hz), 7.32 (1H, s); δF (376 MHz, $CDCl_3$) 175.54-75.70 (2F, BB'), 13.13-13.23 (2F, AA')

4.2.2. 1-(Perfluoropyridin-4-yl)-1H-benzo[d]imidazole 109[130]



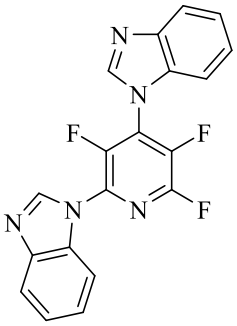
109 (98%)

A solution of pentafluoropyridine **74** (10 mmol, 1.1 mL) in THF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (15 mmol, 0.6g) in THF (10 mL). A solution of benzimidazole **31** (10 mmol, 1.2 g) in THF (15 mL) was added dropwise to the mixture by using a syringe pump and the reaction left to stir at room temperature for 24 h under

N_2. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (25 mL × 3) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give compound **109** (2.62g, 98%) as a shiny, creamy solid.

m.p. 136-140°C; ν_{max} /cm^{-1} (film) 2988, 1712, 1478, 1265, 1222, 969, 896, 748; MS (ESI) (MH^+), $C_{12}H_5F_4N_3$ requires *m/z* 268.0469 found *m/z* 268.0491; δ_H (400 MHz, $CDCl_3$,) 8.11 (1H, s), 7.94-7.90 (1H, m), 7.47-7.44 (2H, q, *J*, 7.2, 4.4) 7.34-7.33 (1H, m); δ_F (376 MHz, $CDCl_3$) 75.98-76.14 (2F, BB'), 16.34-16.15 (2F, AA'); δ_C (100 MHz, $CDCl_3$) 144.3 (td, *J*, 240, 11, C-F), 143.2 (Cq), 141.1 (N-CH=N), 136.9 (dd, *J*, 270, 37, C-F), 132.3 (Cq), 127.0-127.2 (m, Cq-N), 125.2 (CH), 124.4 (CH), 121.3 (CH), 110.9 (CH).

4.2.3. 1,1'-(3,5,6-Trifluoropyridine-2,4-diyl)bis(1H-benzo[d]imidazole) 110

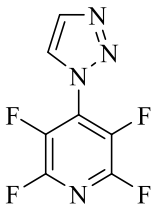


110 (36%)

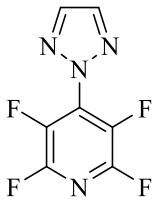
A solution of tetrafluoropyridine derivative **109** (2 mmol, 0.53 g) in THF (5 mL) was added dropwise to the stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2.5 mmol, 0.1 g) in THF (5 mL). A solution of benzimidazole **31** (2 mmol, 0.24 g) in THF (5 mL) was added dropwise to the mixture using pressure compensating dropping funnel and the reaction left to stir at room temperature overnight. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (25 mL × 3) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give brown sticky solid (0.6 g).Purification was carried out by column chromatography and 1-(2-(benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-4-yl)-1H-benzo[d]imidazole **110** was obtained as a brown solid (0.08 g, 36%).

m.p. 186-190 °C ; MS (ESI) (MH^+), $C_{12}H_5F_4N_3$ requires *m/z* 366.0953; found *m/z* 366.0960; δ_H (400 MHz, $CDCl_3$,) 8.43 (1H, d, *J* 2.8), 8.11 (1H, t, *J* 2), 8.01 (2H, d, *J* 7.6), 7.90-7.88 (1H, m), 7.85-7.83 (1H, m), 7.41-7.38 (4H, m), 7.33 -7.31 (1H, m); δ_F (376 MHz, $CDCl_3$) 79.3 (1F, t, *J* 23.3), 27.8 (1F, d, *J* 28.9), 18.4 (1F, d, *J* 23.3).

4.2.4. 2,3,5,6-Tetrafluoro-4-(1H-1,2,3-triazol-1-yl)pyridine 111 and 2,3,5,6-tetrafluoro-4-(2H-1,2,3-triazol-2-yl)pyridine 112



X isomer **111 (40%)**



Y isomer **112 (15%)**

A solution of pentafluoropyridine **74** (40 mmol, 4.4 mL) in THF (4 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (30 mmol, 1.2 g) in THF (10 mL). A solution of 1,2,3-triazole **28** (20 mmol, 1.38 g) in THF (15 mL) was added dropwise to the mixture and the reaction left to stir at room temperature for 24 h under N_2. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (25 mL × 3) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give oily semi solid compound (3.5 g). ^{19}F and ^{1}H NMR spectra indicated the presence of two different compounds in the product. The mixture was separated by column chromatography using 6:4 light petrol: ethyl acetate as eluting solvent to give isomer X **111** as a shiny white solid (1.72 g, 40%) and 100% ethyl acetate to give isomer Y **112** as shiny light yellow solid (0.66 g, 15%).

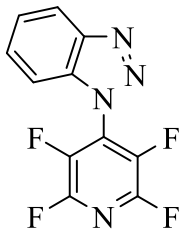
X isomer **111**:

m.p. 63-65 °C; ν_{max} /cm^{-1} (film) 1643, 1465, 1257, 1180, 956, 848, 671. MS (ESI) (MH^+), $C_7H_3F_4N_4$ requires *m/z* 219.0288 found *m/z* 219.0287; δ_H (400 MHz, $CDCl_3$,) 8.61 (1H, s), 8.28 (1H, s); δ_F (376 MHz, $CDCl_3$) 77.48-77.32 (2F, BB'), 14.41-14.25 (2F, AA'); δ_C (100 MHz, $CDCl_3$) 154.5 (CH), 145.2 (CH), 144.1 (td , *J* 270, 23, C-F), 136.7 (dd, *J* 210, 30 C-F), 127.15-127.98 (m, Cq-N); Analysis (%) for $C_7H_2F_4N_4$ (218) required: C, 38.55; H, 0.92; N, 25.69 Found: C, 38.34; H, 0.83; N, 25.08.

Y isomer **112**:

m.p. 150-152°C; ν_{max} /cm^{-1} (film) 1651, 1427, 1265, 1057, 956, 833; MS (ESI) (MH^+), $C_7H_3F_4N_4$ requires *m/z* 219.0288 found *m/z* 219.0289; δ_H (400 MHz, $CDCl_3$) 8.64 (2H, t, *J* 2); δ_F (376 MHz, $CDCl_3$) 76.14-75.68 (2F, BB'), 15.00-18.84 (2F, AA'); δ_C (100 MHz, $CDCl_3$) 144.5 (td, *J* 190, 11 C-F), 141.0 (t, *J*, 4 CH) , 135.3 (dd, *J* 200, 10, C-F), 124.5-124.7 (m, Cq-N); Analysis (%) for $C_7H_2F_4N_4$ (218) required: C, 38.55; H, 0.92; N, 25.69 Found: C, 38.37; H, 0.85; N, 25.04

4.2.5. 1-(Perfluoropyridin-4-yl)-1H-benzo[d][1,2,3]triazole 113[131]

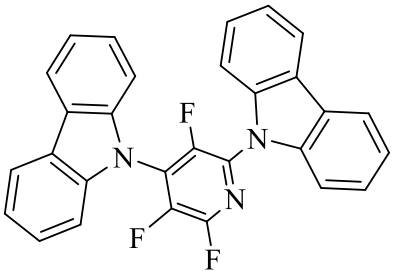


113 (88%)

A solution of pentafluoropyridine **74** (40 mmol, 4.4 mL) in THF (5 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (30 mmol, 1.2 g) in THF (10 mL). A solution of benzotriazole **30** (20 mmol, 2.4 g) in THF (15 mL) was added dropwise to the mixture by syringe pump and the reaction left to stir at room temperature for 24 h under N_2. The solvent was evaporated and distilled water (10 mL) was added to the residue. A white solid was precipitated and was collected by suction filtration to give compound **113** (4.72 g, 88%) as white solid.

m.p. 135-138°C; ν_{max} /cm^{-1} (film) 2924, 2854, 1695, 1473, 1249, 1041, 956, 840, 624; MS (ESI) (MH^+); $C_{12}H_5F_4N_4$ requires *m/z* 269.0445 found *m/z* 269.0443; δ_H (400 MHz, $CDCl_3$,) 8.22 (1H, d, *J* 8.4), 7.67 (1H, t, *J* 7.2), 7.56-7.48 (2H, m); δ_F (376 MHz, $CDCl_3$) 75.99-75.76 (2F, BB'), 17.24-17.04 (2F, AA'); δ_C (100 MHz, $CDCl_3$) 145.6 (Cq), 144.2 (dt, *J* 230, 14, C-F), 136.7 (dd, *J* 270, 37, C-F), 132.6 (Cq), 127.0-127.1 (m, Cq-N), 125.9 (CH), 130.0 (CH), 109.8 (CH), 121.0 (CH).

4.2.6. 9,9'-(3,4,6-Trifluoropyridine-2,5-diyl)bis(9H -carbazole) 114



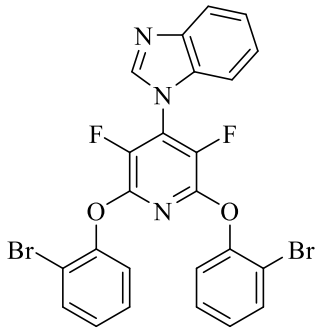
114 (45 %)

A solution of pentafluoropyridine **74** (50 mmol, 8.85 g) in THF (3 mL) was added dropwise to to a stirred suspension of NaH (60% dispersion in mineral oil) (20 mmol, 0.8 g) in THF (5 mL). A solution of carbazole **29** (10 mmol, 1.67 g) in THF (10 mL) and DMF (2 mL) was added dropwise to

reaction mixture under N_2 and left to stir at RT for 24 h. The solvent was evaporated and water (20 mL) was added to the residue. a yellow solid precipitated and was filtered (3.47 g). The product was purified by column chromatography using 8:2 light petrol:diethyl ether eluting solvent to give compound **114** (4.1g, 45%) as white crystals.

m.p. 203-205 °C; ν_{max} /cm^{-1} (film) 1597, 1419, 1118, 972 , 856; MS (ESI) (MH^+), $C_{29}H_{17}F_3N_3$ requires m/z 464.1369 found m/z 464.1370; δ_H (400 MHz, $CDCl_3$) 8.15 (1H, d, *J* 8), 8.11 (1H, d, *J* 7.6), 7.54-7.45 (3H, m), 7.41-7.31 (3H, m); δ_F (376 MHz, $CDCl_3$) 77.89 (1F, t, 28.95), 38.30 (1F, d, *J* 30.01), 22.84 (1F, d, *J* 26.32); δ_C (100 MHz, $CDCl_3$),147.28 (dd, *J* 250, 20, C-F), 146.0 (dd, *J* 260, 10, C-F),139.2 (Cq), 139.0 (Cq), 139.8 (dd, *J* 220, 31, C-F), 132.01-132.85 (m, Cq-N), 127.45-128.05 (m, Cq-N), 126.8 (CH), 126.5 (CH), 124.7 (Cq), 124.6 (Cq), 122.1 (CH), 121.8 (CH), 120.8 (CH), 120.5 (CH), 110.9 (d, *J* 2.8, C-H), 110.3 (C-H); Analysis (%) for $C_{29}H_{16}F_3N_3$. (445) required: C, 75.16; H, 3.48; N, 9.07 Found: C, 74.93; H, 3.35; N, 9.02.

4.2.7. 1-(2,6-bis(2-Bromophenoxy)-3,5-difluoropyridin-4-yl)-1H-benzo[d]imidazole 115



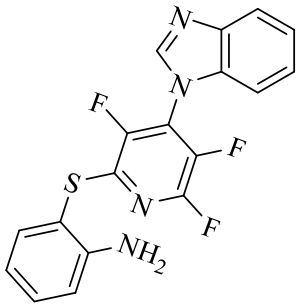
115 (53%)

A solution of 2-bromophenol **58** (1 mmol, 0.11 mL) in THF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (1.5 mmol, 0.06 g) in THF (5 mL) and left 30 min at room temperature. A solution of tetrafluoropyridine derivative **109** (1 mmol, 0.267 g) in THF (5 mL) and was added dropwise to the reaction mixture and the reaction left to stir at room temperature overnight. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (3 × 25 mL) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give cream colour solid (0.53 g). Recrystallization from DCM and hexane gave shiny white crystals of 1-(2,6-bis-(2-bromophenoxy)-3,5-difluoropyridine-4-yl)-1*H*-benzo[*d*]imidazole **115** as shiny white crystals (0.31 g, 53%).

m.p. 178-181 °C; ν_{max} /cm^{-1} (film): 1712, 1635, 1519, 1435, 1350, 1287, 1080, 979, 871, 732;

MS (ESI) (MH^+), $C_{24}H_{16}{}^{79}Br_2F_2N_3O_2$ requires *m/z* 573.9387; found *m/z* 573.9383; δ_H (400 MHz, $CDCl_3$,) 8.23 (1H, t, *J* 2), 7.85 (1H, dt, *J* 6, 1.6), 7.48-7.46 (5H, m), 7.17 (2H, dt, *J* 8, 1.2), 7.06-7.02 (4H, m); δ_F (376 MHz, $CDCl_3$) 14.9 (2F, s).

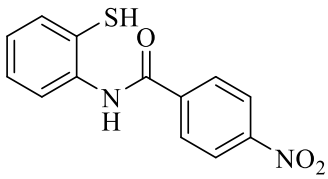
4.2.8. 2-((4-(1H-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-2-yl)thio)aniline 118



118 (29%)

A solution of tetrafluoropyridine derivative **109** (1 mmol, 0.267 g) in THF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (1.3 mmol, 0.05 g) in THF (5 mL). 2-aminothiophenol **117** (1 mmol, 0.11 mL) was mixed with sodium borohydride (1 mmol, 0.038 g) in THF (5 mL) and was added dropwise to the mixture using a pressure compensating dropping funnel, and the reaction left to stir at room temperature overnight. The reaction was quenched with a few drops of methanol. Distilled water (10 mL) was added, and the mixture was extracted with DCM (3×20 mL). The organic extract was washed with aqueous sodium chloride and dried over $MgSO_4$, filtrated and evaporated to give dark yellow sticky solid (0.41 g). Purification was carried out by column chromatography giving 2-((4-(1*H*-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridine-2 yl)thiol)aniline **118** as dark red solid (0.11 g, 29%).
m.p. 60-64 °C; ν_{max} /cm^{-1} (film) 3333 (broad, N-H), 1612, 1512, 1435, 1303, 1257, 802, 740 ;
MS (ESI) (MH^+), $C_{18}H_{11}F_3N_4S$ requires *m/z* 374.0729, found *m/z* 374.0728; δ_H (400 MHz, $CDCl_3$,) 8.01 (1H, s), 7.86-7.82 (1H, m), 7.41-7.39 (1H, dd, *J* 7.6, 1.2), 7.36-7.32 (2H, m), 7.25-7.21 (2H, m), 6.78 (2H, dd *J* 8, 1.2), 4.27 (2H, s, N-H); δ_F (376 MHz, $CDCl_3$) 78.5 (1F, t, *J* 24), 35.1 (1F, d, *J* 29), 14.9 (1F, d, *J* 24).

4.2.9. N-(2-Mercaptophenyl)-4-nitrobenzamide 121

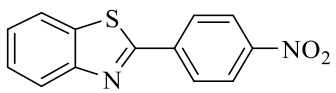


121 (57%)

2-Aminothiophenol **116** (2 mmol, 0.22 mL) was mixed with sodium borohydride (2 mmol, 0.08 g) in CH_3CN (5 mL) and was added dropwise to a stirred solution of Et_3N (2 mmol, 0.28 mL) in CH_3CN (4 mL). A solution of 4-nitrobenzoyl chloride **120** (2 mmol, 0.371 g) in CH_3CN (5 mL) and was added dropwise to the reaction mixture and the reaction left to stir at room temperature overnight. After distilled water (10 mL) was added solid precipitated and was collected by suction filtration to give yellow solid (0.095 g). Recrystallization from hot ethanol gave shiny yellow crystals of N-(2-mercaptophenyl)-4-nitrobenzamide **121** (0.31 g, 57%).

m.p. 178-181 °C ; v_{max} /cm^{-1} (film) 3348 (N-H), 1681 (C=O), 1581 and 1519 (NO_2), 1311, 1249, 1103, 1010, 833, 756, 632, 540; MS (ESI) (MH^-), $C_{13}H_{10}N_2O_3S$ requires m/z 273.0348; found m/z 273.0339; δ_H (400 MHz, $CDCl_3$,) 8.91 (1H, s, N-H), 8.43 (1H, d, *J* 8.0), 8.34-8.31 (2H, m), 7.93 (1H, d, *J*, 8.8), 7.81 (1H, dd, *J* 2.4, 6.8), 7.56 (1H, dd *J* 7.6, 1.2), 7.33-7.30(1H, m), 7.05 (1H, t *J* 6), 4.25 (1H, s, SH).

4.2.10. 2-(4-Nitrophenyl)benzo[d]thiazole 122



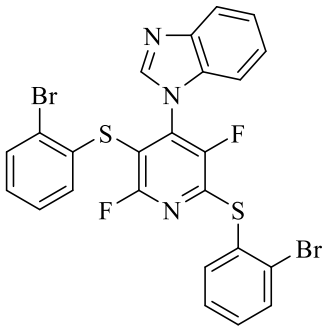
122 (49%)

2-Aminothiophenol **116** (2 mmol, 0.22 mL) was mixed with sodium borohydride (2 mmol, 0.076 g) in pyridine (5 mL) and left to stir at room temperature for 30 min. A solution of 4-nitrobenzoyl chloride **120** (2 mmol, 0.371 g) in pyridine (5 mL) and was added dropwise to the reaction mixture and the reaction left to stir at room temperature overnight. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (3 × 25 mL) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give yellow sticky solid

(0.54 g). The compound was washed with cold ethanol and collected by suction filtration to give compound **122** (0.25 g, 49%) as a shiny yellow solid.

m.p. 224-229 °C; ν_{max} /cm^{-1} (film) : 1597 and 1519 (NO_2), 1342, 1219, 1111, 972, 848, 763, 732, 686; MS (ESI) (MH^+), $C_{13}H_9N_2O_2S$ requires m/z 257.0371; found m/z 257.0377; δ_H (400 MHz, $CDCl_3$,) 8.4 (2H, d, *J* 2), 8.29 (2H, d, *J* 8.8), 8.16 (1H, d, *J* 8.4), 7.99 (1H, d, *J* 8), 7.58(1H, t, *J* 7.6), 7.49 (1H, d, *J* 7.2).

4.2.11. 1-(2,5-bis((2-Bromophenyl)thio)-3,6-difluoropyridin-4-yl)-1H-benzo[d]imidazole 126

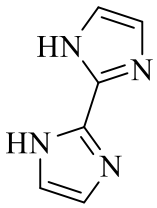


126 (65%)

A solution of 2-bromothiophenol **125** (1 mmol, 0.12 mL) in THF (3 mL) was added dropwise to stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (1.5 mmol, 0.05 g) in THF (5 mL). A solution of tetrafluoropyridine derivative **109** (1 mmol, 0.267 g) in THF (5 mL) added dropwise to the reaction mixture and left to stir at room temperature overnight. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (3 × 25 mL) and the combined organic layers dried over $MgSO_4$, filtered and evaporated to give creamy solid (0.4 g). Recrystallization from hot ethanol gave shiny white crystals of 1-[2,6-bis-(2-bromo-phenylsulfanyl)-3,5-difluoro-pyridin-4-yl]-1*H*-benzoimidazole **126** (0.39 g, 65%).

m.p. 162-165 °C; ν_{max}/cm^{-1} (film): 1581, 1450, 1333, 1149, 1018, 1080, 884, 740; MS (ESI) (MH^+), $C_{24}H_{14}Br_2F_2N_3S_2$ requires m/z 603.8973; found m/z 603.8952; δ_H (400 MHz, $CDCl_3$,) 7.79 (1H, s), 7.72-7.67 (3H, m), 7.35 (1H, t, *J* 1.2), 7.28 (1H, dd, *J 6*, 2), 7.26-7.22 (3H, m), 7.03 (1H, d, *J* 4.8), 6.83-6.81 (2H, m), 6.73-6.71 (1H, m); δ_F (376 MHz, $CDCl_3$) 101.9 (1F, d, *J* 27), 33.30 (1F, d, *J* 27).

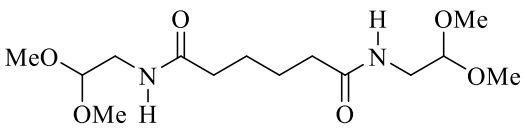
4.2.12. 1H,1'H-2,2'-Biimidazole 130 [120]



130 (5%)

Aqueous glyoxal **129** (50%, 0.1 mol, 11.5 mL) was added dropwise to a stirred solution of ammonium acetate (0.26 mol, 20 g) in water (10 mL) and the reaction left to stir at room temperature overnight. The dark brown solid formed (0.71 g) was collected by suction filtration and washed with water (3 × 10 mL) and acetone (3 × 10 mL) to give 1*H*,1'*H*-2,2'-biimidazole **130** (0.7 g, 5%)
m.p. 347-350 °C (literature value : > 300 °C) [27]; MS (ESI) (MH^+), $C_6H_6N_4$ requires *m/z* 135.0668; found *m/z* 135.0662; ν_{max}/cm^{-1} (film) 3368 (broad peak , H_2O), 2922 and 2852 (2 N-H), 1651, 1402, 1329, 1102, 937, 745; δ_H (400 MHz, DMSO-d_6) 12.85 (2H, s, N-H), 7.18 (2H, s), 7.06 (2H, s).

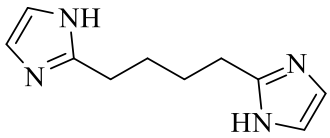
4.2.13. N1,N6-bis(2,2-Dimethoxyethyl)adipamide 131



131 (75%)

A solution aminoacetaldehyde dimethyl acetal (4 mmol, 0.46 g) in THF (2 mL) was added dropwise to a stirred solution of triethylamine (4 mmol, 0.61 mL) in THF (3 mL). The mixture was stirred at room temperature for 30 min. A solution of adipoyl chloride (2 mmol, 0.29 mL) in THF (2 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The reaction was quenched with distilled water (10 mL), and the mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give compound **131** (0.48 g, 75%) as a white solid.
m.p. 93-95°C; ν_{max}/cm^{-1} (film) 3315-3284 (broad peak) NH group, 1649 (C=O), 1423, 1384, 1138, 1199, 966, 832, 738, 607; MS (ESI) (MH^+), $C_{14}H_{29}N_2O_6$ requires *m/z* 321.2020; found *m/z* 321.2019; δ_H (400 MHz, $CDCl_3$) 5.88 (2H, s), 4.38 (2H, t, *J 5.2*), 3.39-3.41(16H, m), 2.21-2.26 (4H, m), 1.64-1.68 (4H, m).

4.2.14. 1,4-di(1H-imidazol-2-yl)butane 132



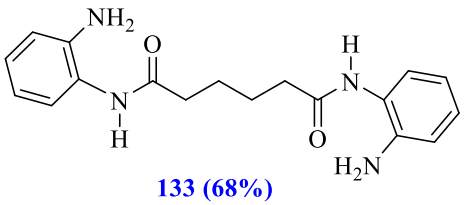
132 (60%)

The intermediate compound **135** (8.3 mmol, 1.58 g) was treated with HCl (40 mmol, 6 mL) and the mixture heated at 65 °C for 24 h under N_2. After 24 h the reaction mixture was cooled and neutralized with dry sodium carbonate (Na_2CO_3). Addition of water precipitated the target compound **132** (0.79 g, 50%) which was filtered as a light brown solid.

mp: decomposed at 168 °C; ν_{max} /cm^{-1} (film) 3000-3450 (broad peak, NH), 1639, 1577, 1423, 1342, 1153, 1103, 991, 887, 763, 675; MS (ESI) (MH^+), $C_{10}H_{15}N_4$ requires m/z 191.1291 found m/z 191.1296; MS (ESI) (MH^-), $C_{10}H_{13}N_4$ requires m/z 189.1146, found m/z 189.1147

δ_H (400 MHz, DMSO-d_6) 11.79 (2H, s), 6.95 (2H, s), 6.73 (2H, s), 2.57-2.54 (4H, m), 1.61-1.56 (4H, m); δ_H (400 MHz, Methanol-d_4) 6.85 (4H, s), 2.66-2.70 (4H, m), 1.69-1.67 (4H, m); δ_C (100 MHz, Methanol-d_4) 149.9 (Ar Cq), 120.1 (Ar CH), 27.6 (CH_2), 27.3 (CH_2).

4.2.15. N1,N6-bis(2-Aminophenyl)adipamide 133

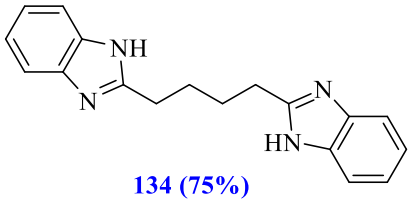


133 (68%)

A solution of *o-phenylenediamine* (4 mmol, 0.47 g) in THF (4 mL) was added dropwise to a stirred solution of triethylamine (4 mmol, 0.6 mL) in THF (3 mL). The mixture was stirred at room temperature for 30 min. A solution of adipoyl chloride (2 mmol, 0.29 mL) in THF (2 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and water (20 mL) was added to the residue. A yellow solid precipitated and was filtered to give compound **133** (0.49 g, 75%) as white solid.

m.p. 155-165°C; ν_{max}/cm^{-1} (film) 3250 (broad peak) NH group, 1658 (C=O), 1643, 1512, 1481, 1290, 1136, 1068, 966, 832, 752, 607; MS (ESI) (MH^+), $C_{18}H_{22}N_4O_{24}$ requires *m/z* 327.1815 found *m/z* 327.1814; δ_H (400 MHz, DMSO-d_6) 9.34 (1H, s), 9.15 (1H, s), 7.15 (2H, d, *J* 8), 6.89 (2H, t, *J* 7.2) 6.70 (2 H, t, *J* 7.6), 6.54 (2 H, t, *J* 7.6), 4.32 (2H, s), 2.34-2.33- (4H, m), 1.64-1.63 (4H, m).

4.2.16. 1,4-bis(1H-benzo[d]imidazol-2-yl)butane 134

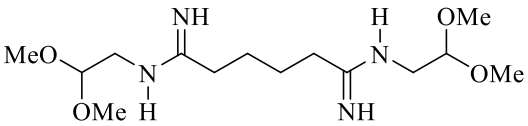


134 (75%)

A solution of *o-phenylenediamine (*4 mmol, 0.43 g) in methanol (6 mL) was added to a suspension of compound **18** (2 mmol, 0.55 g) in methanol (3 mL). The mixture was refluxed for 3 h under N_2. After 3 h the reaction was stopped and treated with water (20 mL). Compound **134** precipitated and was collected by suction filtration as shiny light yellow solid (0.44 g, 75%). m.p. 258-260°C; v_{max}/cm^{-1} (film) 3441-3163 (broad, NH), 1620, 1535, 1415, 1141, 1006, 840, 744; MS (ESI) (MH$^+$), $C_{18}H_{19}N_4$ requires *m/z* 291.1592, found *m/z* 291.1581

MS (ESI) (MH$^-$), $C_{18}H_{17}N_4$ requires *m/z* 289.1549 found *m/z* 289.1559; δ_H (400 MHz, DMSO-d_6), 12.2 (2H, s), 7.44 (4H, dd, *J* 6, 2.8), 7.16 (4H, dd, *J* 6, 3.2), 2.96-2.89 (4H, m), 1.85-1.77 (4H, m). δ_C (100 MHz, DMSO-d_6) 155.5 (Ar Cq), 138.8 (broad Ar Cq), 127.14-127.7 (m, N-Cq=N), 114.5 (Ar CH), 120.4 (Ar CH), 28.7 (CH2), 27.5 (CH_2).

4.2.17. N1,N6-bis(2,2-Dimethoxyethyl)adipimidamide 135

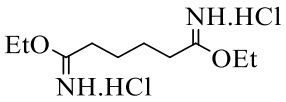


135 (98%)

A solution of aminoacetaldehyde dimethyl acetal (0.0045 mol, 0.47 g) in methanol (3 mL) was added to stirred suspension of compound **136** (0.002 mol, 0.48 g) in methanol (3 mL). The reaction mixture was heated under reflux for 3 h. The solvent was evaporated to give intermediate compound **135** (0.6 g, 95%) as a yellow syrup.

MS (ESI) (MH$^+$), $C_{14}H_{31}N_4O_4$ requires *m/z* 319.2341 found *m/z* 319.2315; v_{max}/cm^{-1} (film) 3483, 3051 (NH), 1656, 1452, 1502, 1237, 1195, 1066, 798, 742; δ_H (400 MHz, Methanol-d_4) 4.54 (2H, t, *J* 4.8 Hz), 3.44 (16H, m), 2.53 (4H, m), 1.74 (4H, m).

4.2.18. Diethyl adipimidate dihydrochloride 136

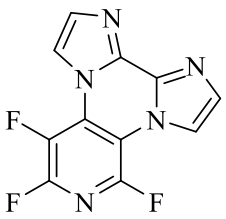


136 (80%)

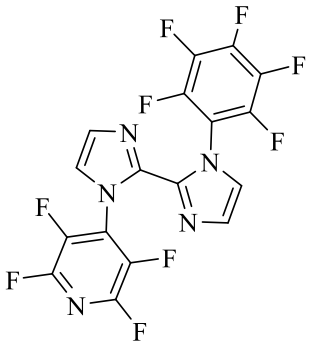
Ethanol (0.4 mol, 18.4 g) and acetyl chloride (0.25 mol, 17.8 mL) were added to a solution of adiponitrile (0.1 mol, 10.8 g) in DCM (10 mL). The mixture was stirred for 30 min then left at 4 ºC for 3 days. After this time, compound **136** had precipitated and was collected as white solid by suction filtration (23 g, 80%).

m.p. 135-136°C; ν_{max} /cm^{-1} (film) 3383 (broad peak) NH group, 1651, 1462, 1377, 1215, 1114, 879, 777; δ_H (400 MHz, D_2O) 4.66 (4H, q, *J* 6) 2.37-2.35 (4H, m), 1.58-1.59 (4H, m), 1.20 (6H, t, *J* 5.6 Hz). (N-H, peak not detected)

4.2.19. 5,7,8-Trifluorodiimidazo[1,2-a:2',1'-c]pyrido[3,4-e]pyrazine 137 and 1-(perfluorophenyl)-1'-(perfluoropyridin-4-yl)-1H,1'H-2,2'-biimidazole 138



137 (31%)



138 (33%)

A solution of pentafluoropyridine 74 (2 mmol, 0.22 g) in THF (5 mL) was added dropwise to stirred suspension of sodium hydride NaH (60 % dispersion in mineral oil) (2.5 mmol, 0.1 g) in THF (5 mL). A solution of 1*H*,1'*H*-2,2'-biimidazole 130 (1 mmol, 0.134 g) in THF (5 mL) and was added dropwise at room temperature and the mixture stirred overnight. The reaction was quenched with a few drops of methanol. After distilled water (10 mL) was added some solid formed and was collected by suction filtration to give compound **138** (0.14 g, 33%) as a white solid. The aqueous filtrate was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give dark yellow sticky solid which recrystallized from hot ethanol to give the pure compound **137** (0.08 g, 31%) as yellow solid.

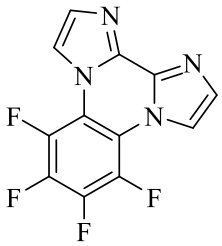
Compound **137**

m.p. 288-295 °C; ν_{max} /cm^{-1} (film), 1681, 1243, 1187, 957, 787; MS (ESI) (MH$^+$), $C_{11}H_5F_3N_5$ requires *m/z* 264.0483; found *m/z* 264.0413; δ_H (400 MHz, DMSO-d$_6$) 8.42-8.33 (2H, dd, J, 32, 1.2), 7.74-7.69 (2H, dd, *J* 1.6, 18.4); δ_F (376 MHz, $CDCl_3$) 8.31 (1F, dt, *J* 23.3, 2.6), 69.31 (1F, q, *J* 11.7), 86.90 (1F, dq, *J* 13.5, 3.0).

Compound **138**

m.p. 158-164 °C; ν_{max} /cm^{-1} (film), 1651, 1203, 1087, 927, 717; MS (ESI) (MH$^+$), $C_{16}H_5F_8N_6$ requires *m/z* 433.0434; found *m/z* 433.0441; δ_H (400 MHz, $CDCl_3$,) 8.13-8.08 (2H, m), 7.68-7.66 (4H, dd, *J* 10.4, 1.2); δ_F (376 MHz, $CDCl_3$) 74.70-74.53 (4F, BB'), 16.03-15.77 (4F, AA').

4.2.20. 5,6,7,8-Tetrafluorodiimidazo[1,2-a:2',1'-c]quinoxaline 140



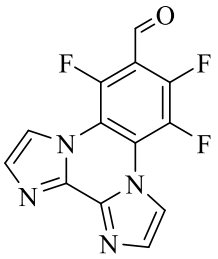
140 (18%)

A solution of hexafluorobenzene **48** (2 mmol, 0.37 g) in DMF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2.5 mmol, 0.1 g) in DMF (5 mL). 1*H*,1*H*-2,2'-biimidazole **130** (1 mmol, 0.134 g) in DMF (3 mL) and was added dropwise to the mixture. The mixture was heated at 80°C overnight under N_2. The solvent was evaporated and water (20 mL) was added to the residue. Some yellow solid precipitated and was filtered to give a brown solid which after recrystallization from ethanol afforded compound **140** (0.05 g, 18%) as a brown solid. m.p. decomposed at 240 °C

ν_{max}/cm^{-1} (film), 29.33, 1631, 1223, 1187, 957, 787; MS (ESI) (MH$^+$), $C_{12}H_5F_5N_4$ requires *m/z* 281.0445 found *m/z* 281.0433; δ_H (400 MHz, $CDCl_3$,) 8.16 (2H, d, *J* 1),7.67 (2H, d, *J* 1);

δ_F (376 MHz, $CDCl_3$) 14.04-13.94 (2F, BB'), 4.57-4.54 (2F, AA').

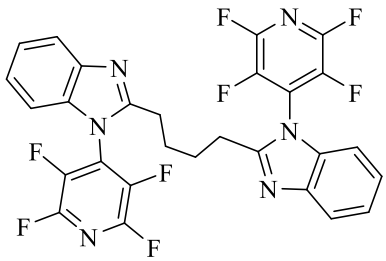
4.2.21. 5,7,8-Trifluorodiimidazo[1,2-a:2',1'-c]quinoxaline-6-carbaldehyde 141



141 (10%)

A solution of pentafluorobenzaldehyde **68** (1 mmol, 0.19 g) in DMF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (1.5 mmol, 0.06 g) in DMF (5 mL). 1*H*,1*H*-2,2'-biimidazole **130** (1 mmol, 0.134 g) in DMF (3 mL) and was added dropwise to the mixture. The mixture was heated at 80 °C overnight under N_2. The solvent was evaporated and distilled water (10 mL) was added. Solid formed which was collected by suction filtration to give impure brown solid after recrystelazaion by DCM and light petrol compound **141** (10%) was isolated as yellow solid. v_{max}/cm^{-1} (film), 2923, 1709, 1646, 1500, 1260, 10.91, 796, 682; MS (ESI) (MH^+), $C_{13}H_6F_3N_5O$ requires *m/z* 291.0488 found *m/z* 291.0476; δ_H (400 MHz, $CDCl_3$,) 10.46 (1H, s), 8.22 (2H, d, *J* 1.2), 7.69 (2H, d, *J* 1.6) ;δ_F (376 MHz, $CDCl_3$) 13.21-13.11 (1F, m), 20.16 (1F, d, *J* 21.8), 32.91 (1F, d, *J* 18.4).

4.2.22. 1,4-bis-1-Tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane 143



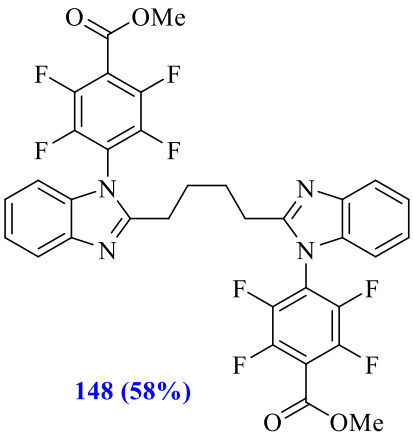
143 (95%)

A solution of pentafluoropyridine **74** (1.5 mmol, 0.25 g) in DMSO (2 mL) was added dropwise to stirred suspension of sodium hydride NaH (60 % dispersion in mineral oil) (1.5 mmol, 0.06 g) in DMSO (2 mL). Compound **133** (0.5 mmol, 0.15 g) in DMSO (4 mL) and was added dropwise by syringe pump and the reaction mixture left for 24 h at room temperature under N_2. The solvent was

evaporated and water (20 mL) was added to the residue. Solid precipitated and was collected by suction filtration to give compound **143** (0.28 g, 95%) as a white shiny solid.

m.p. 195-198 °C; v_{max}/cm^{-1} (film), 2924, 1642, 1471, 1255, 1136, 972, 743; MS (ESI) (MH$^+$), $C_{28}H_{17}F_8N_6$ requires *m/z* 589.1381 found *m/z* 589.1376; δ_H (400 MHz, $CDCl_3$,) 7.81 (2H, d, *J* 7.6), 7.36 (4H, m), 7.07 (2H, d, *J* 7.6), 2.87 (4H, m), 2.06 (4H, m); δ_F (376 MHz, $CDCl_3$) 76.4-76.1 (4F, m), 18.2-17.9 (4F, m); δ_C (100 MHz, $CDCl_3$) 153 (Ar N-Cq=N), 144.2 (dd, *J* 230, 26, C-F), 142.8 (Ar Cq), 136.8 (dd, *J* 267, 36, C-F), 134.2 (Ar Cq), 128.1-127.2 (m, Cq-N), 124.2 (Ar CH), 124.0 (Ar CH), 120.1 (Ar CH), 109.4 (Ar CH), 29.8 (CH2-C=N), 26.4 (CH_2-CH_2-CH_2); Analysis (%) for $C_{28}H_{16}F_8N_6$.½H_2O (597) requires: C, 56.28; H, 2.85; N, 14.07, found: C, 56.38; H, 3.01; N, 13.86.

4.2.23. 1,4-bis-1-(4-Methoxycarbonyltetrafluorophenyl)-1H-benzimidazol-2-ylbutane 148



148 (58%)

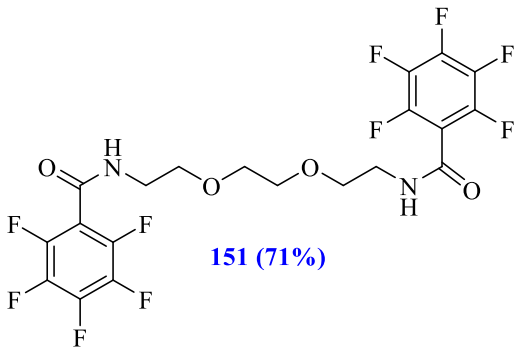
A solution of methyl pentafluorobenzoate **147** (2 mmol, 0.45 g) in DMSO (3 mL) was added dropwise to stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2 mmol, 0.08 g) in DMSO (3 mL). Compound **134** (1 mmol, 0.3 g) in DMSO (4 mL) was added dropwise to the reaction mixture after 30 min by syringe pump and it left 24 h at room temperature under a N_2 atmosphere. By adding distilled water (10 mL) to the mixture light yellow solid (0.8 g) precipitated and was collected by suction filtration which was recrystallized from hot ethanol to give compound **148** (0.4 g, 58%) as white shiny crystals.

m.p. 195-198 °C; v_{max}/cm^{-1} (film), 2924, 1642, 1471, 1255, 1136, 972, 743

MS (ESI) (MH$^+$), $C_{34}H_{23}F_8N_6O_4$ requires *m/z* 703.1586 found *m/z* 703.1569; δ_H (400 MHz, $CDCl_3$,) 7.81 (2H, d, *J* 7.6), 7.30-7.38 (m, 4H), 7.04 (2H, d, *J* 8.0), 4.09 (6H, 2), 2.87 (4H, m), 2.06 (4H, m); δ_F (376 MHz, $CDCl_3$) 25.3-25.2 (4F, m), 19.3-19.2 (4F, m); δ_C (100 MHz, $CDCl_3$) 159.3 (C=O), 154.0 (Ar N -Cq=N), 145.4 (dd, *J* 180, 21, C-F), 142.9 (Ar Cq), 142.8 (dd, *J* 170, 13, C-F), 135.0

(Ar Cq), 123.8 (Ar CH), 123.5 (Ar CH), 119.9 (Ar CH), 114.1 (t, *J* 17, Ar Cq-C=O), 109.3 (Ar CH), 53.8 (CH3), 27.1 (CH_2-C=N), 26.5 (CH_2-CH_2-CH_2), (1 ArF Cq not detected).

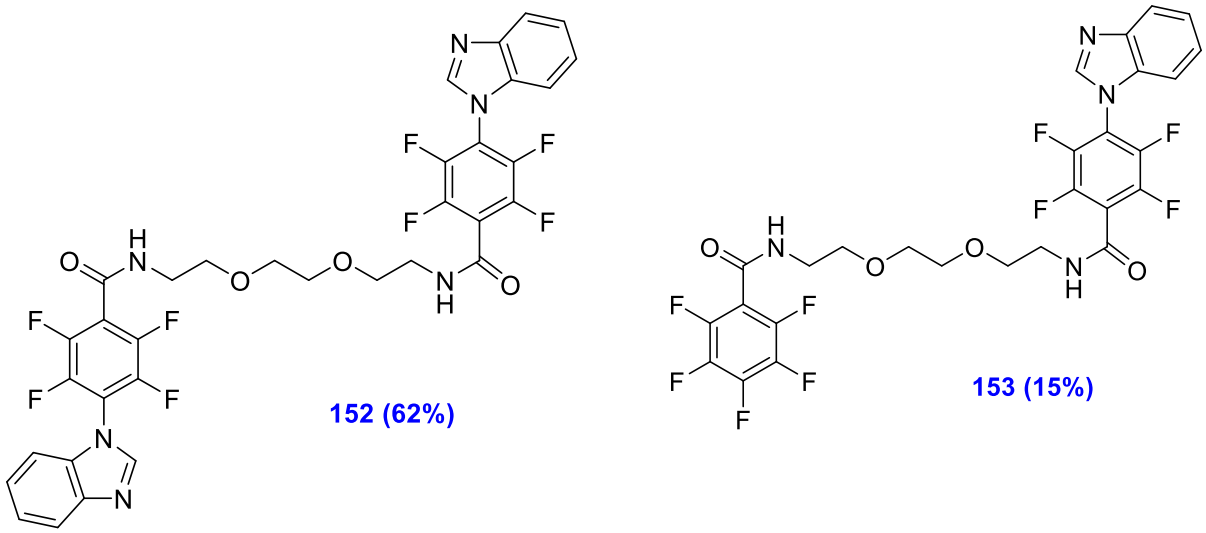
4.2.24. 2,2-(Ethylenedioxy)-bis-ethyl pentafluorobenzamide 151



A solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (1 mmol, 0.15 g) in THF (2 mL) was added dropwise to stirred solution of triethylamine (2 mmol, 0.20 g) in THF (2 mL). A solution of pentafluorobenzoyl chloride **149** (2 mmol, 0.46 g) in THF (2 mL) and was added dropwise to the reaction mixture and left for 24 h at room temperature under N_2. The solvent was evaporated and water (10 mL) was added to the residue. Yellow orange solid precipitated, and was filtered (0.5 g). Recrystallization using DCM and light petroleum gave compound **151** (0.38 g, 71%) as white shiny crystals.

m.p. 128-129 °C; ν_{max}/cm^{-1} (film), 3279 (N-H), 2872, 1658 (C=O), 1501, 1135, 1119, 992, 731; MS (ESI) (MH^+), $C_{20}H_{14}O_4F_{10}N_2$ requires *m/z* 537.0867, found *m/z* 537.0867; δ_H (400 MHz, $CDCl_3$) 6.55 (2H, s, NH), 3.70-3.66 (8H, m), 2.87 (4H, m); δ_F (376 MHz, $CDCl_3$) 21.2-21.1 (4F, m), 11.26 (2F, t, *J* 20.3), 1.74-1.90 (4F, m); δ_C (100 MHz, $CDCl_3$) 157.6 (C=O), 144. 2 (dd, *J* 255, 12, C-F), 142.3 (dd, *J* 260, 25, C-F), 137.5 (dd, *J* 230, 32. C-F), 111.5 (t, *J* 30, Ar Cq-C=O), 70.8 (CH_2-O), 69.8 (CH_2-O), 40.1 (CH_2-N). Analysis (%) for $C_{20}H_{14}F_{10}N_2O_4$ (536) required: C, 44.79; H, 2.63; N, 5.22 Found: C, 44.78; H, 2.44; N, 5.25.

4.2.25. 2,2'-(Ethylenedioxy)-bis-ethyl 4-benzimidazol-1-yltetrafluorobenzamide 152 and N-(2-(2-(2-(4-(1H-benzo[d]imidazol-1-yl)-2,3,5,6-tetrafluorobenzamido)ethoxy)ethoxy)ethyl)-2,3,4,5,6-pentafluorobenzamide 151



A solution of benzimidazole **31** (2 mmol, 0.24 g) in THF (3 mL) was added dropwise to stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2 mmol, 0.08 g) in THF (3 mL) after 30 min. A solution of compound **151** (1 mmol, 0.54 g) in THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was left to stir for 24 h at room temperature under N_2. Distilled water (10 mL) was added to the reaction mixture and it was extracted with DCM (3×25 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give white solid (0.65 g)**.** TLC indicated presence of mixture of two compounds and traces of starting material in the product. Thus further purification was carried out by column chromatography. Compound **153** (0.1 g, 15%) was afforded by using 99:1 ethyl acetate and methanol eluting as a colourless oil. Compound **152** (0.45 g, 62%) was eluted using 90:10 ethyl acetate and methanol to afford the compound **153** as a shiny white solid.

ν_{max} /cm^{-1} (film) 3279 (broad N-H), 2831, 1665 (C=O), 1496,1280, 1211, 1118, 987, 740

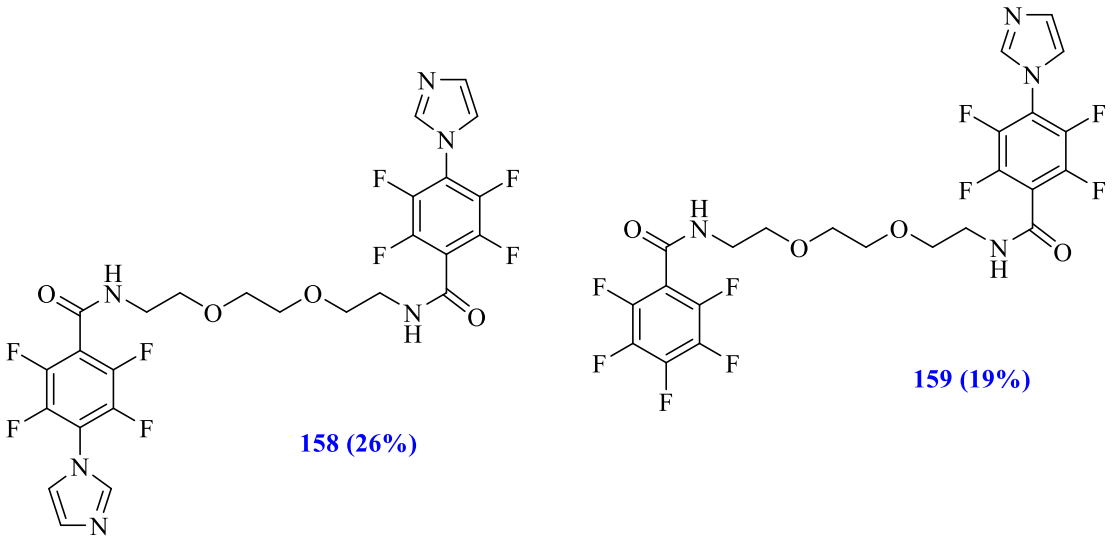
MS (ESI) (MH$^-$), $C_{27}H_{18}F_9N_4O_4$ requires *m/z* 633.1190 found *m/z* 633.1192; δ_H (400 MHz, $CDCl_3$,) 8.04 (1H, s), 7.88 (1H, d, *J* 3.6), 7.42-7.40 (2H, m), 7.23-7.22 (1H, m), 7.12 (1H, s, NH), 6.82 (1H, s, NH), 3.75-3.64 (12H, m); δ_F (376 MHz, $CDCl_3$) 22.48 (2F, dd, *J* 22.93, 11.65), 22.07-21.01 (2F, m), 16.59 (2F, dd, *J* 23.31, 11.65), 11.1 (1F, t, *J* 20.3), 1.83-1.70(2F, m).

Compound **152**

m.p. 115-117 °C; ν_{max} /cm^{-1} (film) 3194 (N-H), 2877, 1666 (C=O), 1550, 1418, 1280, 1134, 987, 710, 748; MS (ESI) (MH$^-$), $C_{34}H_{23}F_8N_6O_4$ requires *m/z* 731.1659 found *m/z* 731.1657;

δ_H (400 MHz, $CDCl_3$,) 8.03 (2H, s), 7.91-7.89 (2H, m), 7.43-7.38 (4H, m), 7.22 (2H, d, *J* 6), 7.00 (2H, s), 3.75-3.73 (12H, m); δ_F (376 MHz, $CDCl_3$) 22.6-22.5 (4F, m), 17.8-17.7 (4F, m);δ_C (100 MHz, $CDCl_3$) 157.5 (C=O), 142.9 (Ar CH=N), 141.1 (Ar Cq), 133.0 (Ar Cq), 124.9 (Ar CH), 123.9 (Ar CH), 120.9 (Ar CH), 110.4 (Ar CH), 70.5 (CH_2-O), 69.4 (CH_2-O), 40.2 (CH_2-N) (C-F and C-N could not be detected); Analysis (%) for $C_{34}H_{24}F_8N_6O_4$ (732) required: C, 55.74; H, 3.30; N, 11.47 Found: C, 55.59; H, 3.37; N, 11.16.

4.2.26. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(2,3,5,6-tetrafluoro-4-(1H-imidazol-1-yl)benzamide) 158 and 2,3,4,5,6-pentafluoro-N-(2-(2-(2-(2,3,5,6-tetrafluoro-4-(1H-imidazol-1-yl)benzamido)ethoxy)ethoxy)ethyl)benzamide 159



A solution of imidazole **27** (1 mmol, 0.07 g) in THF (3 mL) was added dropwise to stirred suspension of sodium hydride NaH (60 % dispersion in mineral oil) (2 mmol, 0.08 g) in THF (3 mL). The reaction mixture was left to stir for 30 min at RT then a solution of compound **151** (0.5 mmol, 0.26 g) in THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was left to stir for 24 h at room temperature under N_2. Distilled water (10 mL) was added to the reaction mixture and it was extracted with DCM (3×25 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give colourless oil (0.3 g)**.** TLC indicated the presence of mixture of two compounds and traces of starting material in the product. Thus further purification was carried out by column chromatography. Compound **158** (0.076 g, 26%) was afforded, after elution with 100% ethyl acetate, as a white oil. Compound **159** (0.05 g, 19%) was afforded using 99:1 ethyl acetate and methanol as eluent as a colourless oil.

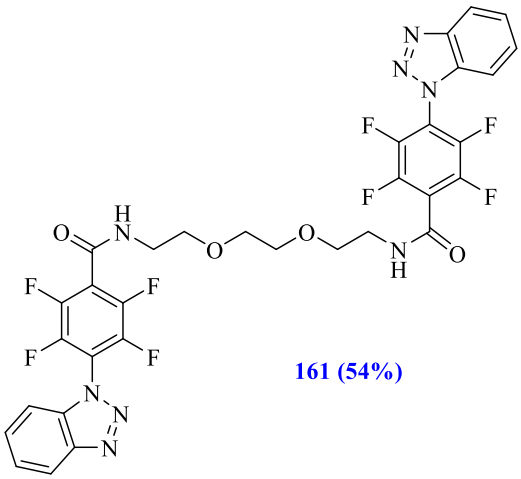
Compound **159**

ν_{max} /cm^{-1} (film) 3252 (NH), 2931, 1678 (C=O), 1498, 1349, 1294, 1118, 978, 746; MS (ESI) (MH$^-$), $C_{23}H_{16}F_9N_4O_4$ requires *m/z* 583.1033 found *m/z* 583.1040; δ_H (400 MHz, $CDCl_3$,) 7.80 (1H, s), 7.27 (2H, d, *J* 9.6), 6.99 (1H, s, NH), 6.82 (1H, s, NH), 3.75-3.64 (12H, m); δ_F (376 MHz, $CDCl_3$) 22.39-22.48 (2F, q, *J* 12.03), 21.07-21.01 (2F, m), 16.59-16.67 (2F, q, *J* 11.65), 11.1 (1F, t, *J* 20.3), 1.70-1.83 (2F, m).

Compound **158**

ν_{max} /cm^{-1} (film) 3192 (NH), 2881, 1668 (C=O), 1481, 1309, 1138, 978, 810; MS (ESI) (MH$^-$), $C_{26}H_{21}F_8N_6O_4$ requires *m/z* 633.1491 found *m/z* 633.1473; δ_H (400 MHz, $CDCl_3$,) 7.77 (2H, s), 7.26 (4H, d, *J* 11.6), 3.74-3.65 (12H, m), 1.90 (2H, s, N-H); δ_F (376 MHz, $CDCl_3$) 22.30-22.39 (4F, q, *J* 11.65), 14.52-14.60 (4F,q, *J* 9.0); δ_C (100 MHz, $CDCl_3$) 157.5 (C=O), 137.4 (Ar CH), 130.2 (Ar CH), 119.9 (Ar CH), 115.6 (Ar Cq-C=O), 70.4 (CH_2-O), 69.3 (CH_2-O), 40.1 (CH_2-N), (C-F and C-N could not be detected).

4.2.27. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(4-(1H-benzo[d][1,2,3]triazol-1-yl)-2,3,5,6-tetrafluorobenzamide) 161



161 (54%)

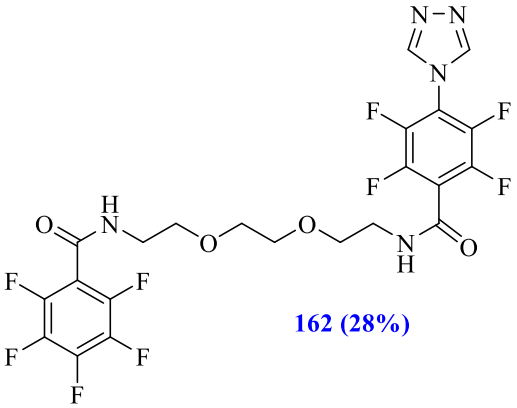
A solution of benzotriazole **30** (0.2 mmol, 0.02 g) in DMSO (2 mL) was added dropwise to stirred suspension of sodium hydride NaH (60 % dispersion in mineral oil) (0.2 mmol, 0.02 g) in DMSO (2 mL) and after 30 min a solution of compound **151**.(0.1 mmol, 0.05 g) in DMSO (2 mL) was added dropwise to the reaction mixture. The reaction mixture was left to stir for 24 h at room temperature under a N_2 atmosphere.

Distilled water (10 mL) was added to the reaction mixture and it was extracted with DCM (3×25 mL). The organic extract was washed with aqueous sodium chloride and dried over $MgSO_4$, filtered and evaporated to give white oil (0.06 g). TLC indicated the presence of some impurity in the

product. Thus further purification was carried out by column chromatography. Compound **161** was afforded after elution with 6:4 ethyl acetate and petrol followed by recrystallization from DCM and petrol to give a shiny white solid (0.041 g, 54%). m.p. 206-208 °C

ν_{max} /cm^{-1} (film) 3248 (N-H), 2877, 1658 (C=O), 1489, 1280,1041,979, 740; MS (ESI) (MH$^+$), $C_{32}H_{23}F_8N_8O_4$ requires *m/z* 735.1709 found *m/z* 735.1705; δ_H (400 MHz, $CDCl_3$,) 8.21 (2H, d, *J* 8.4), 7.64 (2H, td, *J* 6.8, 0.8), 7.52 (2H, td, *J* 6, 0.8), 7.42 (2H, d, *J* 8), 7.13 (2H, s, NH), 3.76-3.68 (12H, m); δ_F (376 MHz, $CDCl_3$) 22.70-22.61 (4F, q, *J* 11.28), 18.52-18.42 (4F, q, *J* 11.25,); δ_C (100 MHz, $CDCl_3$) 157.5 (C=O), 145 (Ar Cq), 133.4 (Ar Cq), 129.6 (Ar CH), 125.9 (Ar CH), 120.6 (Ar CH), 109.6 (Ar CH), 70.6 (CH_2-O), 69.4 (CH_2-O), 40.3 (CH_2-N) (C-F and C-N could not be detected); Analysis (%) for $C_{32}H_{22}F_8N_8O_4.1/2H_2O$ (743) required: C, 51.68; H, 3.09; N, 15.07 Found: C, 51.99; H, 3.08; N, 14.86.

4.2.28. 2,3,4,5,6-Pentafluoro-N-(2-(2-(2-(2,3,5,6-tetrafluoro-4-(4H-1,2,4-triazol-4-yl)benzamido)ethoxy)ethoxy)ethyl)benzamide 162

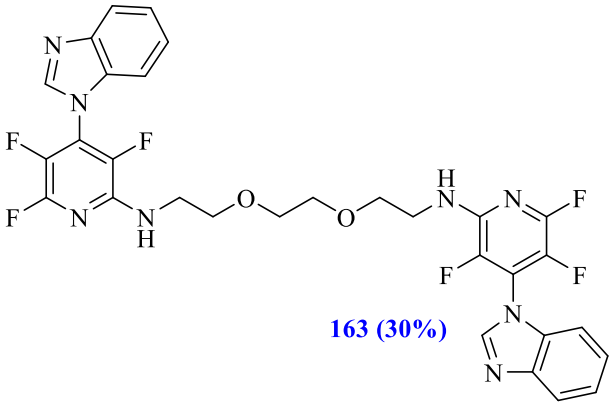


A solution of 1,2,4-triazole **28** (1 mmol, 0.07 g) in DMSO (3 mL) was added dropwise to stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (2 mmol, 0.08 g) in DMSO (3 mL). The reaction mixture was left to stir for 30 min at RT then a solution of compound **151** (0.5 mmol, 0.26 g) in DMSO (3 mL) was added dropwise. The reaction mixture was left to stir for 24 h at room temperature under a N_2 atmosphere.

Distilled water (10 mL) was added to the reaction mixture and it was extracted with ethyl acetate (3×25 mL). The organic extract was washed with aqueous sodium chloride and dried over $MgSO_4$, filtered and evaporated to give colourless oil (0.15 g). Purification was carried out by column chromatography, eluting with 9:1 ethyl acetate and petrol. Recrystallization from DCM and petrol then gave compound **162** (0.08 g, 28%) as a white solid.

m.p. 92-94 °C; v_{max} /cm^{-1} (film) 3282 (NH), 2921, 1662 (C=O), 1503, 1519, 1323, 1131, 990, 739; MS (ESI) (MH$^-$), $C_{22}H_{15}F_9N_5O_4$ requires *m/z* 584.0986 found *m/z* 584.0994; δ_H (400 MHz, $CDCl_3$,) 8.47 (1H, s), 8.26 (1H, s), 6.90 (1H, s, NH), 3.71-3.65 (12H, m); δ_F (376 MHz, $CDCl_3$) 22.69 (2F, q, *J* 11.65), 21.15-21.05 (2F, m), 14.45 (2F, q, *J* 11.28), 11.4 (1F, t, *J* 20.68), 1.95-1.81 (2F, m); Analysis (%) for $C_{22}H_{16}F_9N_5O_4$ (743) required: C, 45.14; H, 2.75; N, 11.96 Found: C, 45.05; H, 2.98; N, 11.37.

4.2.29. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(4-(1H-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-2-amine) 163



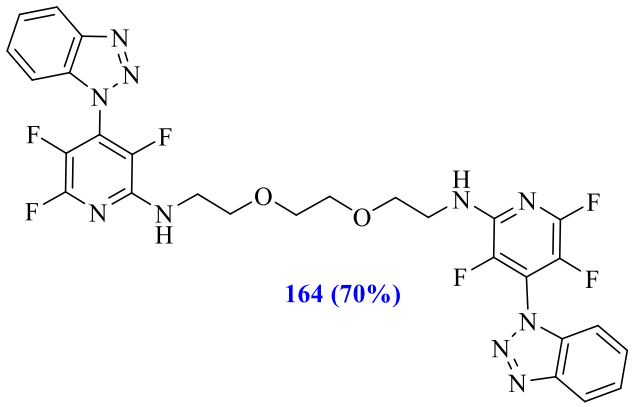
163 (30%)

A solution of compound **109** (2 mmol, 0.53 g) in THF (3 mL) was added dropwise to a stirred solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (1 mmol, 0.15 g) in THF (3 mL). The reaction mixture was refluxed at 65 °C for 24 h under N_2. The solvent was evaporated and distilled water (10 mL) was added to residue. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtrated and evaporated to give a sugary shiny solid (0.4 g). ^{19}F and ^{1}HNMR spectrum indicated the presence of some impurities in the product. Therefore further purification was carried out by column chromatography and compound **163** (0.19 g, 30%) was afforded as shiny solid using 100% ethyl acetate as eluting solvent.

m.p. 258-260 °C; v_{max}/cm^{-1} (film) 3302 (N-H), 2921, 1653, 1513, 1206, 744; MS (ESI) (MH$^+$), $C_{30}H_{25}F_6N_8O_2$ requires *m/z* 643.1999 found *m/z* 643.1953**;** MS (ESI) (MH$^-$), $C_{30}H_{23}F_6N_8O_2$ requires *m/z* 641.1843 found *m/z* 641.1845**;** δ_H (400 MHz, $CDCl_3$,) 8.02 (2H, s), 7.89 (2H, d, *J* 6.8), 7.38-7.32 (4H, m), 7.28-7.26 (2H, m), 5.25 (2H, s), 3.72-3.64 (12H, m); δ_F (376 MHz, $CDCl_3$) 72.11(2F, t, *J* 29), 11.2 (2F, dd, *J* 29, 9), 2.04 (2F, dd, *J* 23, 11); δ_C (100 MHz, $CDCl_3$) 146.4 (dd, *J* 230, 11, C-F), 143.1 (Ar CH=N) , 142.5 (t, *J*, 15, Ar Cq-NH), 142.1 (Ar Cq), 136.5 (dd, *J* 260, 7, C-F), 132.8 (Ar Cq), 129.6 (dd, *J* 260, 21, C-F) 124.5 (Ar CH), 123.7 (Ar CH), 123.1-122.9 (m, Cq-N), 120.8 (Ar

CH), 111.1 (Ar CH), 70.3 (CH2-O), 69.5 (CH2-O), 40.9 (CH2-N); Analysis (%) for $C_{30}H_{24}F_6N_8O_2$ (642) required: C, 56.08; H, 3.76; N, 17.44 Found: C, 56.09; H, 3.91; N, 17.35.

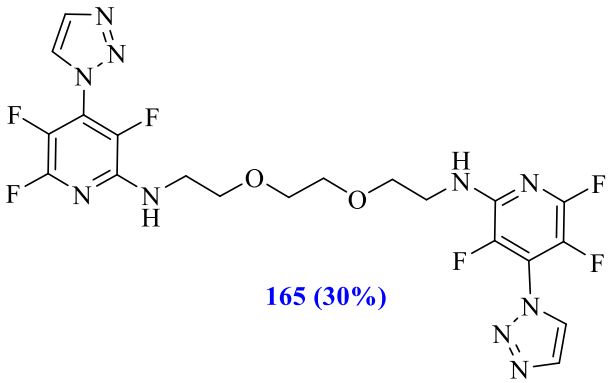
4.2.30. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(4-(1H-benzo[d][1,2,3]triazol-1-yl)-3,5,6-trifluoropyridin-2-amine) 164



A solution of compound **113** (6 mmol, 1.6 g) in THF (6 mL) and DMF (2 mL) was added dropwise to a stirred solution of triethylamine (6 mmol, 0.8 mL) in THF (3 mL). A solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (3 mmol, 0.437 g) in THF (6 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and water (10 mL) was added to the residue to give a sugary solid precipitate (1.56 g). TLC indicated the presence of some starting material in the product. Therefore further purification was carried out by column chromatography. Compound **164** (1.36 g, 70%) was afforded after elution with 1:1 ethyl acetate and petroleum ether as a white solid.

m.p.162-164 °C; ν_{max} /cm^{-1} (film) 3394 (NH), 2921, 1651, 1427, 1257, 1057, 979, 740; MS (ESI) (MH^+), $C_{28}H_{23}F_6N_{10}O_2$ requires *m/z* 645.1904 found *m/z* 645.1908; δ_H (400 MHz, $CDCl_3$,) 8.16 (2H, d, *J* 8), 7.58 (1H, t, *J* 7.2), 7.48-7.43 (4H, m), 5.25 (2H, s), 3.72-3.68 (12H, m); δ_F (376 MHz, $CDCl_3$) 72.11 (2F, td, *J* 23, 7), 12.08 (2F, dd, *J* 29, 10), 1.51 (2F, dd, *J* 22, 9); δ_C (100 MHz, $CDCl_3$) 145.5 (Ar Cq), 133.1 (Ar Cq), 129.3 (Ar CH), 125.0 (Ar CH), 120.5 (Ar CH), 110.1 (Ar CH), 70.3 (CH_2-O), 69.5 (CH_2-O), 40.9 (CH_2-N), (C-F and C-N could not be detected); Analysis (%) for $C_{28}H_{22}F_6N_{10}O_2$ (644) required: C, 52.18; H, 3.44; N, 21.73 Found: C, 52.21; H, 3.30; N, 21.94.

4.2.31. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(3,5,6-trifluoro-4-(1H-1,2,3-triazol-1-yl)pyridin-2-amine) 165

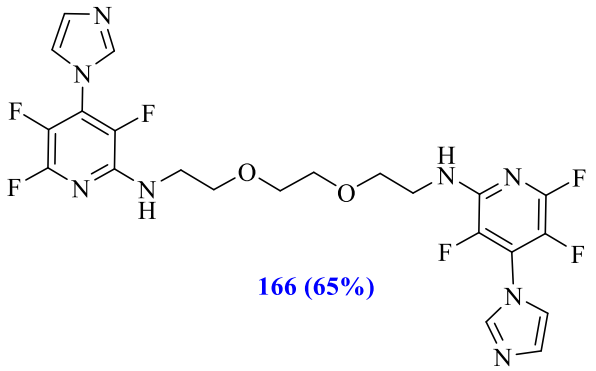


165 (30%)

A solution of compound **111** (1 mmol, 0.218 g) in THF (3 mL) was added dropwise to a stirred solution of triethylamine (1 mmol, 0.13 mL) in THF (3 mL). A solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (0.5 mmol, 0.07 g) in THF (3 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and distilled water (10 mL) was added. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtrated and evaporated to give sugary solid (0.3 g) which was purified by column chromatography. Compound **165** (0.08 g, 30%) was afforded after elution with 100% ethyl acetate as a white solid.

m.p. 111-113 °C; v_{max}/cm^{-1} (film) 3279 (NH), 2924, 1643, 1504, 1419, 1350, 1257, 1064, 927, 740 ; MS (ESI) (MH^+), $C_{20}H_{29}F_6N_{10}O_2$ requires *m/z* 545.1591 found *m/z* 545.1576; δ_H (400 MHz, $CDCl_3$,) 8.52 (2H, s), 8.24 (2H, s), 5.29 (2H, s), 3.69-3.62 (12H, m); δ_F (376 MHz, $CDCl_3$) 71.26 (2F, t, J 28.9), 10.13 (2F,dd, *J* 28.9, 11.6), 4.02 (2F, d, *J* 21.1); δ_C (100 MHz, $CDCl_3$) 154.2 (Ar CH), 146.2 (dd, *J* 240, 11 , C-F), 145.3 (Ar CH), 142 (t, *J* 13, Cq-NH), 135.5 (d, *J* 260, C-F), 128.3 (dd, *J*, 280, 33, C-F), 124.2-123.8 (m, Cq-N), 70.3 (CH_2-O), 69.6 (CH_2-O), 40.9 (CH_2-N).

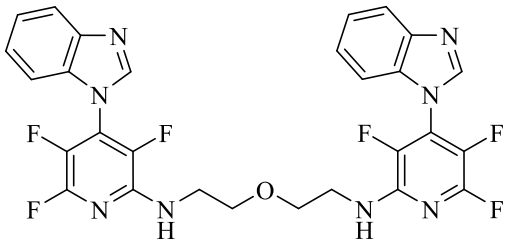
4.2.32. N,N'-((Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(3,5,6-trifluoro-4-(1H-imidazol-1-yl)pyridin-2-amine) 166



166 (65%)

A solution of compound **108** (6 mmol, 1.3 g) in THF (6 mL) was added dropwise to a stirred solution of triethylamine (6 mmol, 0.8 mL) in THF (3mL). A solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (3 mmol, 0.44 g) in THF (6 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under the N_2.The solvent was evaporated and distilled water (10 mL) was added to the residue. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtrated and evaporated to give sugary shiny solid (1.26 g). Purification was carried out by column chromatography. The compound **166** (1.05 g, 65%) was afforded by using 50 % Ethyl acetate and 50% Petroleum ether which washed with diethyl ether as creamy shiny solid.
m.p.117-120°C; v_{max} /cm^{-1} (film) 3217, 31.24 (N-H), 2931, 1655, 1419, 1350, 1273, 1087, 927, 740, 601; MS (ESI) (MH^+), $C_{22}H_{21}F_6N_8O_2$ requires *m/z* 543.1686 found *m/z* 543.1686; δ_H (400 MHz, $CDCl_3$,) 7.89 (2H, s), 7.38 (4H, d, *J* 10.8), 5.28 (2H, s), 3.61-3.72 (12H, m); δ_F (376 MHz, $CDCl_3$) 71.62 (2F, t, *J* 24.4), 8.11 (2F, dd, *J* 7.14, 27.44), 4.68 (2F, dd, *J* 8.64 , 24.8); δ_C (100 MHz, $CDCl_3$) 146.6 (dd, *J* 180, 8.7 C-F), 142.2 (t, *J* 11, Ar Cq-NH), 137.4 (Ar N-CH=N), 135.1 (ddd, *J* 200, 4.6, 1.7, C-F), 130.1 (Ar CH), 128.5 (dd, *J* 200, 26.3 C-F), 124.1-123.9 (M, Cq-N), 111.1 (Ar CH), 119.5 (Ar CH), 69.6 (CH_2-O), 70.2 (CH_2-O),40.8 (CH_2-N); Analysis (%) for $C_{22}H_{20}F_6N_8O_2$ (542) required: C, 48.71; H, 3.72; N, 20.66 Found: C, 48.80; H, 3.75; N, 20.05.

4.2.33. N,N'-(Oxybis(ethane-2,1-diyl))bis(4-(1H-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-2-amine) 168



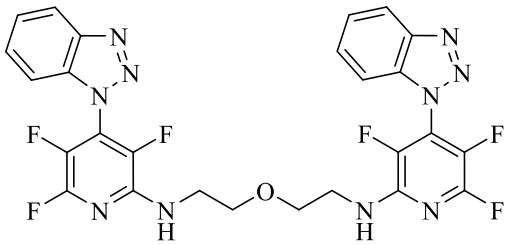
168 (23%)

A solution of compound **109** (2 mmol, 0.43 g) in THF (3 mL) was added dropwise to a stirred solution of triethylamine (4 mmol, 0.52 mL) in THF (3 mL). A solution of 2-(2-aminoethoxy)ethylamine) **167** (1 mmol, 0.178 g) in THF (3 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and distilled water (10 mL) added to residue. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtrated and evaporated to give a creamy solid (0.35 g) which was purified by column chromatography. Compound **168** (0.013 g, 23%) was afforded after elution with 100% ethyl acetate and recrystallization from DCM and petrol as a shiny white solid.

m.p. 140-141 °C; ν_{max}/cm^{-1} (film) 3299 (NH), 2941, 1681, 1532, 1459, 1226, 1157, 962, 695

MS (ESI) (MH^+), $C_{28}H_{21}F_6N_8O_1$ requires *m/z* 599.1737 found *m/z* 599.1720; δ_H (400 MHz, $CDCl_3$,) 8.01 (2H, s), 7.89 (2H, d, *J* 7.2), 7.38-7.33 (4H, m), 7.28-7.24 (2H, m), 5.14 (2H, s), 3.75-3.70 (8H, m); δ_F (376 MHz, $CDCl_3$) 72.11 (2F, t, *J* 23), 12.14 (2F, dd, *J* 30, 9), 1.16 (2F,dd, *J* 23, 9); δ_C (100 MHz, $CDCl_3$) 155.1 (Ar N-CH =N), 142.0 (Ar Cq),), 136.5 (Ar Cq), 124.6 (Ar CH), 123.8 (Ar CH), 120.5(Ar CH), 111.0 (Ar CH), 69.5 (CH_2-O), 41.9 (CH_2-N), (C-F, C-NH and C-N could not be detected).

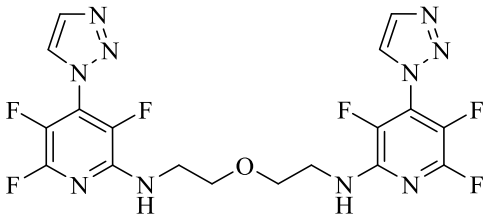
4.2.34. N,N'-(Oxybis(ethane-2,1-diyl))bis(4-(1H-benzo[d][1,2,3]triazol-1-yl)-3,5,6-trifluoropyridin-2-amine) 169



169 (15%)

A solution of compound **113** (2 mmol, 0.54 g) in THF (3 mL) was added dropwise to a stirred solution of triethylamine (4 mmol, 0.52 mL) in THF (3 mL). A solution of 2-(2-aminoethoxy)ethylamine) **167** (1 mmol, 0.18 g) in THF (3 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and distilled water (10 mL) was added precipitating a creamy solid (0.43 g). Purification was carried out by column chromatography. Compound **169** was afforded as a white solid (0.09g, 15%) using 1:1 ethyl acetate and petroleum ether as eluent followed by recrystallized from DCM and petrol. m.p. 157-159 °C;ν_{max} /cm^{-1} (film) 3279 (NH), 2931, 1651, 1512, 1419, 1265, 1057, 972, 617; MS (ESI) (MH$^+$), $C_{26}H_{19}F_6N_{10}O$ requires *m/z* 601.1642 found *m/z* 601.1637; δ_H (400 MHz, $CDCl_3$,) 8.14 (2H, d, *J*, 8), 7.56 (2H, t, *J* 8), 7.50-7.41 (4H, m), 5.15 (2H, s), 3.76-3.69 (8H, m); δ_F (376 MHz, $CDCl_3$) 72.11 (2F, t, *J* 23), 11.29 (2F,dd, *J* 28, 7), 1.62 (2F, dd, *J* 23, 8); δ_C (100 MHz, $CDCl_3$) 155.1 (Ar CH),146.3 (dd, *J* 240, 18, C-F), 145.5 (Ar Cq), 142.3 (t, *J* 13, Cq-NH), 136.5 (d, *J*, 260, C-F), 133.0 (Ar Cq), 129.8 (dd, *J* 230, 21, C-F), 129.3 (Ar CH), 125.0 (Ar CH), 123.7-122.7 (m, Cq-N), 120.5 (Ar CH), 111.0 (Ar CH), 69.5 (CH_2-O), 40.9 (CH_2-N); Analysis (%) for $C_{26}H_{18}F_6N_{10}O_1$. 1/2H_2O (609) required: C, 51.23; H, 3.11; N, 22.98 Found: C, 51.29; H, 2.81; N, 22.35.

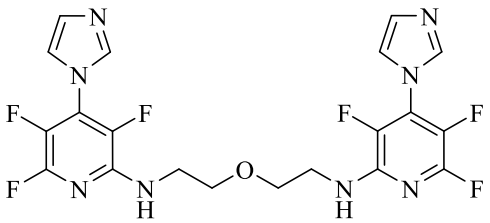
4.2.35. N,N'-(Oxybis(ethane-2,1-diyl))bis(3,5,6-trifluoro-4-(1H-1,2,3-triazol-1-yl)pyridin-2-amine) 170



170 (55%)

A solution of compound **111** (1 mmol, 0.22 g) in DMF (3 mL) was added dropwise to a stirred solution of triethylamine (2 mmol, 0.26 mL) in THF (3 mL). A solution of 2-(2-aminoethoxy) ethylamine) **167** (0.5 mmol, 0.18 g) in THF (3 mL) was added dropwise to the mixture and the reaction was refluxed at 65 °C for 24 h, under N_2. The solvent was evaporated and distilled water (10 mL) was added to give a cream solid (0.37 g) which was filtrated by suction filtration. The TLC plate indicated the presence of some starting material in the product. Thus the product was recrystallized by hot ethanol to cover compound **170** (0.138 g, 55%) as white solid. m.p. 127-129°C; ν_{max}/cm^{-1} (film) 3363, 3132 (N-H), 2947, 1658, 1519, 14350, 1265, 1126, 979, 67; MS (ESI) (MH$^+$), $C_{18}H_{15}F_6N_{10}O_1$ requires *m/z* 501.1329 found *m/z* 501.1320; δ_H (400 MHz, $CDCl_3$,) 8.50 (2H, s), 8.22 (2H, s), 5.12 (2H, s), 3.73-3.62 (8H, m); δ_F (376 MHz, $CDCl_3$) 72.11(2F, t, *J* 29), 10.10 (2F, dd, *J* 29, 11), 3.66 (2F, dd, *J* 23, 11); δ_C (100 MHz, $CDCl_3$) 153.2 (Ar CH), 146.2 (dd, *J* 230, 21, C-F), 145.3 (Ar CH), 142.3 (t, *J* 16, Cq-NH), 135.4 (d, *J* 250, C-F), 128.4 (dd, *J* 240, 33, C-F), 123.8-12.31 (m, Cq-N), 69.4 (CH_2-O), 40.9 (CH_2-N); Analysis (%) for $C_{18}H_{14}F_6N_{10}O_1$. (500) required: C, 43.21; H, 2.82; N, 27.99 Found: C, 43.15; H, 2.65; N, 26.93.

4.2.36. N,N'-(Oxybis(ethane-2,1-diyl))bis(3,5,6-trifluoro-4-(1H-imidazol-1-yl)pyridin-2-amine) 171



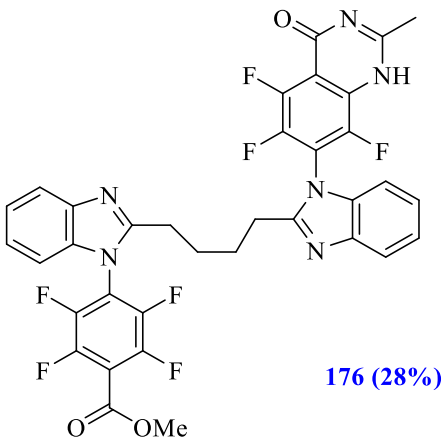
171 (50 %)

A solution of compound **108** (1 mmol, 0.22 g) in DMF (3 mL) was added dropwise to a stirred solution of triethylamine (2 mmol, 0.26 mL) in THF (3 mL). A solution of 2-(2-aminoethoxy) ethylamine) **167** (0.5 mmol, 0.12 g) in THF (3 mL) was added dropwise to the mixture and the reaction was heated at 65 °C for 24 h, under N_2. The solvent was evaporated and distilled water (10 mL) was added to the residue. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give yellow sticky oil (0.31 g). The product was triturated with diethyl ether to recover a white solid (0.16 g). TLC indicated the presence of some starting material in the product. Therefore the product was recrystallized from hot ethanol to give compound **171** (0.12 g, 50%) as a shiny white solid.

m.p. 144-145 °C; v_{max} /cm^{-1} (film) 3333, 3225 (N-H), 2931, 1651, 1519, 1350, 1280, 1080, 979, 748, 671; MS (ESI) (MH^+), $C_{20}H_{17}F_6N_8O_1$ requires *m/z* 499.1424 found *m/z* 499.1409

δ_H (400 MHz, $CDCl_3$,) 7.89 (2H, s), 8.29 (4H, d, *J* 20), 5.08 (2H, s), 3.73-3.64 (8H, m); δ_F (376 MHz, $CDCl_3$) 72.11(2F, t, 22.93), 8.14 (2F,d, *J* 28.95), 4.36 (2F,d, *J* 23.31); δ_C (100 MHz, $CDCl_3$) 144.5 (Ar N-CH=N), 153.2 (Ar C-H), 133.5 (Ar CH), 120.3 (Ar CH), 69.5 (CH_2-O), 40.9 (CH_2-N). (C-F, C-N and C-NH could not be detected); Analysis (%) for $C_{20}H_{16}F_6N_8O_1$. (498) required: C, 48.20; H, 3.24; N, 22.48 Found: C, 48.27; H, 3.20; N, 21.84.

4.2.37. Methyl2,3,5,6-tetrafluoro-4-(2-(4-(1-(5,6,8-trifluoro-2-methyl-4-oxo-1,4-dihydroquinazolin-7-yl)-1H-benzo[d]imidazol-2-yl)butyl)-1H-benzo[d]imidazol-1-yl)benzoate 176



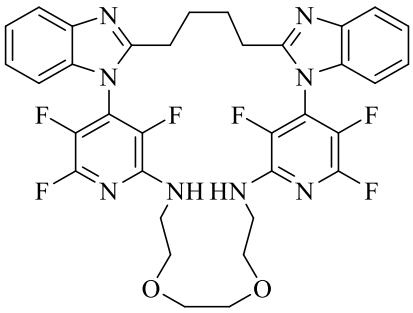
176 (28%)

A solution of acetamidine hydrochloride **88** (0.2 mmol, 0.02 g) in DMF (3 mL) was added dropwise to a stirred suspension of sodium hydride NaH (60% dispersion in mineral oil) (0.3 mmol, 0.012 g) in DMF (3 mL). A solution of compound **148** (0.1 mmol, 2.38 g) in DMF (3 mL) was added dropwise to the reaction mixture after 30 min by syringe pump and the reaction was refluxed at

85 °C for 24 h under a N_2 atmosphere. The solvent was evaporated and distilled water (10 mL) was added. The yellow solid partials precipitated and were collected by suction filtration (0.07 g). TLC plate indicated the presence of some starting material and impurity in the product therefore further purification was carried out by column chromatography. Compound **176** (0.02 g, 28%) as white crystal was afforded using 7:3 ethyl acetate: light petrol eluting solvent which was recrystallized from DCM and petrol.

m.p. 203-205 °C; vmax /cm^{-1} (film) 3394 (N-H), 2931, 1705 (C=O), 1620, 1489, 1280, 1157, 1003, 748, 624; MS (ESI) (MH$^+$), $C_{35}H_{24}F_7N_6O_3$ requires m/z 709.1793 found m/z 709.1775; δ_H (400 MHz, $CDCl_3$,) 11.24 (1H, s, NH), 7.78 (2H, dd, *J* 7.6, 16.4), 7.28-7.21 (4H, m), 7.02 (2H, t, *J* 8.4), 3.71 (3H, s, CH_3), 2.81-2.71 (4H, m), 2.61 (3H, s, CH_3), 1.85-2.03 (4H, m); δ_F (376 MHz, CDCl3) 28.25 (1F, d, *J* 15.04), 24.57-24.46 (2H, m), 20.60 (1F, t, *J* 22.56), 15.72-15.66 (2F, m), 14.97 (1F, d, *J* 18.8).

4.2.38. Rection of 1,4-bis-1-tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane 143 with 2,2-(ethylenedioxy)bis(ethylamine): macrocycle 181



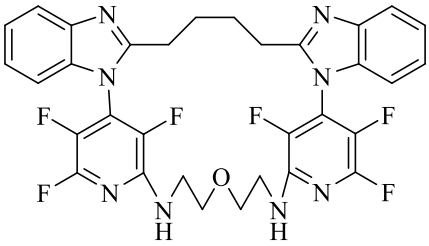
181 (22%)

A solution of 2,2-(ethylenedioxy)bis(ethylamine) **150** (0.4 mmol, 0.06 g) in THF (3 mL) was added dropwise to stirred solution of compound **143** (0.4 mmol, 0.24 g) in THF (5 mL). The reaction mixture was refluxed at 65°C for 24 h under N_2. The solvent was evaporated and distilled water (10 mL) added to residue. The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give a white sugary solid (0.23 g). Column chromatography purification was carried using 1:1 ethyl acetate and light petroleum as eluting solvent give compound **181** (0.06 g, 22%) as white crystals.

m.p. 133-135 °C; ν_{max} /cm^{-1} (film) 3311 (N-H), 2925, 2840, 1652, 1508,1454,1263,1097, 990, 744; MS (ESI) (MH$^+$), $C_{34}H_{31}F_6N_8O_2$ requires *m/z* 697.2469 found *m/z* 697.2462; δ_H (400 MHz, $CDCl_3$) 7.79 (2H, d, *J* 7.6), 7.37-7.33 (2H, td, 7.2, 0.8), 7.30-7.26 (2H, td, *J* 8.0, 1.2), 7.04 (2H, d, *J* 7.6), 5.37

(2H, t, *J* 4.8), 3.83-3.59 (12H, m), 2.82-2.63 (4H, m), 2.03-1.87 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.9 (2F, t, *J* 29.0), 11.95 (2F, d, *J* 35.0), -0.74 (2F, dd, *J* 19.9, 5.6); δ_C (100 MHz, $CDCl_3$) 153.9 (Ar N-Cq=N), 142.6 (Ar Cq), 134.7 (Ar Cq), 123.6 (Ar CH), 123.3 (Ar CH), 119.7(Ar CH), 109.4 (Ar CH), 69.9 (CH_2-O), 69.0 (CH_2-O), 40.7 (CH_2-N) 26.9 (CH_2-C=N), 26.2 (CH_2-CH_2-CH_2), (C-F and C-N could not be detected). Analysis (%) for $C_{30}H_{30}F_6N_8O_2{\cdot}2H_2O$ (732) requires: C, 55.74; H, 4.64; N, 15.30, found: C, 55.88; H, 4.37; N, 14.91.

4.2.39. Reaction of 1,4-bis-1-tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane 143 with 2-(2-aminoethoxy)ethylamine: macrocycle 183



183 (35%)

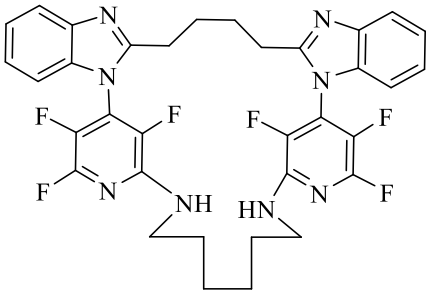
A solution of 2-(2-aminoethoxy)ethylamine **167** (0.4 mmol, 0.07 g) in THF (3 mL) was added to stirred solution of triethylamine (0.8 mmol, 0.08 g) in THF (2 mL). After 15 min a solution of compound **143** (0.2 mmol, 0.12 g) in THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was heated at 65 °C for 24 h under N_2.

The solvent was evaporated and water (10 mL) was added to the residue. A yellow orange solid precipitated, and was filtered (0.12 g). Column chromatography purification using 9:1 ethyl acetate and light petroleum as eluting solvent gave compound **183** (0.04 g, 35%) as white sugary crystals.

m.p. 203-205 °C; ν_{max} /cm^{-1} (film) 3434 (NH), 2099, 1648, 1550, 1453,1260,1126,1011, 744;

MS (ESI) (MH^+), $C_{32}H_{26}F_6N_8O$ requires *m/z* 653.2207 found *m/z* 653.2196; δ_H (400 MHz, $CDCl_3$,) 7.75 (2H, d, *J* 8.0), 7.29-7.18 (4H, m), 6.99 (2H, d, *J* 8.4), 5.28 (2H, s), 4.05-4.00 (2H, m), 3.81-3.73 (4H, m), 3.45-3.39 (2H, m), 2.93-2.82 (2H, m), 2.68-2.63 (2H, m), 2.04-1.87 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.6 (2F, t, *J* 29.0), 11.7 (2F, d, *J* 29.0), 0.55 (2F, d, *J* 17.3); δ_C (100 MHz, $CDCl_3$) 153.8 (Ar N-Cq=N), 142.8 (Ar Cq), 142.1(t, *J*, 14 Ar Cq-NH), 138.0 (dd, *J*, 290, 11, C-F), 134.8 (Ar Cq), 130.9 (dd, *J*, 250, 30, C-F), 123.5 (Ar CH), 123.3 (Ar CH), 123.5-123.0 (m Ar Cq-N), 119.9 (Ar CH), 109.4 (Ar CH), 68.6 (CH_2-O), 40.6 (CH_2-N), 27.0 (CH_2-C=N), 26.0 (CH_2-CH_2-CH_2), (1 ArFCq not detected); Analysis (%) for $C_{32}H_{26}F_6N_8O{\cdot}H_2O$ (670) requires: C, 57.31; H, 4.17; N, 16.72, found: C, 57.38 H, 4.18; N, 16.41.

4.2.40. Reaction of 1,4-bis-1-tetrafluoropyrid-4-yl-1H-benzimidazol-2-ylbutane 143 with 2-(2-aminoethoxy)ethylamine : macrocycle 185

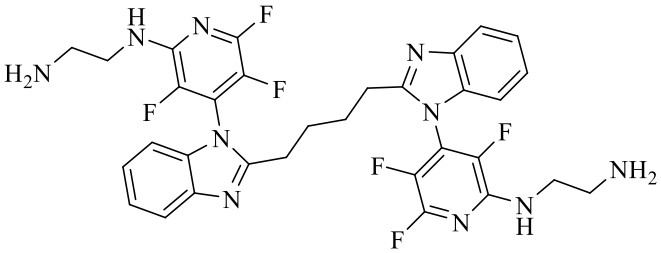


185 (20%)

A solution of hexamethylenediamine **184** (0.5 mmol, 0.06 g) in THF (3 mL) was added to a stirred solution of triethyleamine (1 mmol, 0.1 g) in THF (2 mL) after 15 min solution of compound**143** (0.2 mmol, 0.12 g) in THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was refluxed at 65 °C for 24 h under N_2.The solvent was evaporated and water (10 mL) was added to the residue. A yellow orange solid precipitated and was filtered (0.3 g). Column chromatography purification using 8:2 light petrol and ethyl acetate eluting solvent gave compound **185** as yellow sugary crystals (0.03 g, 20 %).

Decomposed at 180°C; ν_{max} /cm^{-1} (film) 3279 (broad peak, NH), 2924, 1651, 1512, 1427, 1257, 964,833; MS (ESI) (MH$^+$), $C3_4H_{31}F_6N_8$ requires m/z 655.2570 found m/z 655.2569; δH (400 MHz, CDCl3,) 7.72 (2H, d, *J* 8), 7.31-7.19 (4H, m), 6.98 (2H, d, *J* 8), 5.36 (2H, s), 3.82-3.72 (8H, m), 3.65-3.51 (4H, m), 2.84-2.66 (4H, m), 2.02-2.15 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.32 (2F, t, *J* 25.56), 12.71 (2F,dd, *J* 7.5, 30.08), 1.29 (2F, dd, *J* 8.6, 21.43) δ_C (100 MHz, $CDCl_3$) 153.8 (Ar N-Cq=N), 142.9 (Ar Cq), 134.8 (Ar Cq), 123.4 (Ar CH), 123.3 (Ar CH), 119.9 (Ar CH), 109.4 (Ar CH), 70.8 (CH_2-CH_2-CH_2), 69.6. (CH_2-CH_2- CH_2), 40.6 (CH_2-N), 27.0 (CH_2-C=N), 26.0 (CH_2-CH_2-CH_2), (C-F, C-NH and C-N could not be detected).

4.2.41. 1,4-bis-1-[2-(2-Aminoethylamino)-trifluoropyrid-4-yl-1H-benzimidazol-2-ylbutane 187

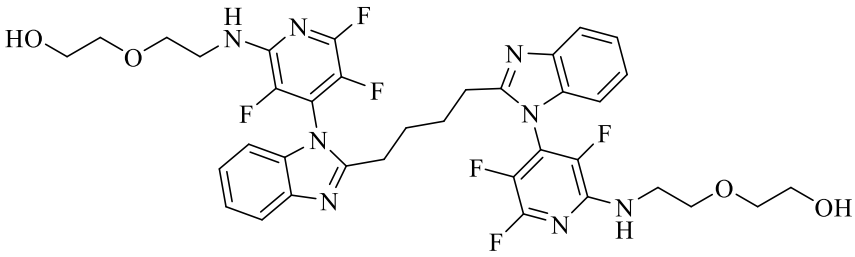


187 (85%)

A solution of ethylenediamine **186** (0.4 mmol, 0.025 g) in THF (2 mL) was added dropwise to a stirred solution of compound **143** (0.2 mmol, 0.12 g) in THF (3 mL) and the mixture stirred at room temperature for 24 h under N_2. Distilled water (10 mL) was added and the mixture extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give compound **187** (0.11 g, 85%) as a white shiny solid.

m.p. 195-198 °C; ν_{max}/cm^{-1} (film), 3400 (broad, NH), 2095, 1647, 1509, 1453, 1388, 1264; MS (ESI) (MH^-), $C_{32}H_{31}F_6N_{10}$ requires *m/z* 669.2632, found *m/z* 669.2621; δ_H (400 MHz, $CDCl_3$,) 7.80 (2H, d, *J* 7.6), 7.35-7.29 (4H, m), 7.11 (2H, d, *J*, 7.6), 5.50-5.38 (2H, td, *J* 8.4, 5.4), 3.57 (4H, dd, *J* 11.2, 5.2), 3.05 (4H, t, *J* 5.6), 2.81 (4H, s), 1.99-1.95 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.3 (2F, m), 13.09 (2F, dd, *J* 23.3, 8.6), 1.44 (2F, dd, *J* 23.3, 8.6).

4.2.42. 1,4-bis-1-{2-[2-(2-Hydroxyethoxy)ethylamino)-trifluoropyrid-4-yl]}-1H-benzimidazol-2-ylbutane 189

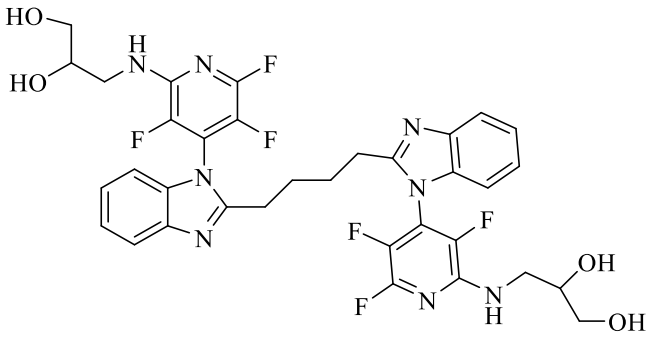


189 (42%)

A solution of 2-(2-aminoethoxy)ethanol **188** (0.2 mmol, 0.02 g) in THF (4 mL) was added dropwise to stirred solution of compound **143** (0.1 mmol, 0.06 g) in THF (4 mL) and the mixture stirred at room temperature for 24 h under N_2. Distilled water (10 mL) was added to reaction mixture and it was extracted with ethyl acetate (3×25 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give white oily product (0.07 g) which was recrystallized from DCM and light petroleum to give compound **189** (0.03 g, 42%) as colourless shiny crystals.

m.p. 168-170 °C; v_{max}/cm^{-1} (film), 3333 (N-H), 2870,1651,1512, 1427, 1388, 1273, 1118, 1072, 987, 740; MS (ESI) (MH$^+$), $C_{36}H_{37}F_6N_8O_2$ requires *m/z* 759.2836 found *m/z* 759.2829; δ_H (400 MHz, $(CD_3)_2CO$) 7.67 (2H, d, *J* 8.0), 7.46 (2H, s), 7.33-7.24 (6H, m), 4.68 (2H, t, *J* 4.8), 3.61 (8H, t, *J* 5.6), 3.54-3.47 (8H, m (overlap with water peak)), 2.78 (4H, m), 1.84 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.2 (2F, t, 11.7), 13.6-13.3 (2F, m), –1.93-(–1.46) (2F, m); Analysis (%) for $C_{36}H_{36}F_6N_8O_2.3H_2O$ (785) required: C, 55.03; H, 4.96; N, 14.26 Found: C, 55.37; H, 4.67; N, 13.87.

4.2.43. 1,4-bis-1-[2-(2,3-Dihydroxypropylamino)-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane 191



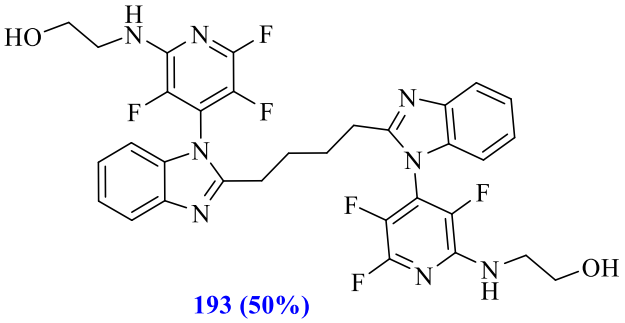
191 (82%)

A solution of (±)-3-amino-1,2-propanediol **190** (0.2 mmol, 0.018 g) in DMF (3 mL) was added dropwise to a stirred solution of compound **143** (0.1 mmol, 0.058 g) in DMF (mL) and the mixture heated at 80 °C for 24 h under N_2. Distilled water (10 mL) was added and some solid formed which collected by suction filtration to give compound **191** (0.08 g, 82%) as a white solid.

m.p. 123-126 °C; v_{max}/cm^{-1} (film), 3417 (broad N-H), 2931, 1651,1427, 1257, 1057, 979,748;

MS (ESI) (MH$^+$), $C_{34}H_{33}F_6N_8O_8$ requires *m/z* 731.2523 found *m/z* 731.2516; δ_H (400 MHz, $CDCl_3$) 1.92-1.86 (4H, m), 2.90-2.75 (4H, m), 3.47-3.42 (2H, m), 3.84 -3.56 (6H, m), 3.90 (4H, m), 4.25 (2H, s), 7.28-7.18 (6H, m), 7.64 (2H, d, *J* 7.6); δ_F (376 MHz, $CDCl_3$) 72.53 (2F, t, *J* 25.9), 14.02 (2F, dd, *J* 29.0, 8.6), 5.09 (2F, dd, *J* 25.9, 8.6); δ_C (100 MHz, $CDCl_3$) 154.6 (Ar N -Cq=N), 142.9 (Ar Cq) , 135.1 (Ar Cq), 123.8 (Ar CH), 123.7 (Ar CH), 123.4(Ar CH), 119.5 (Ar CH), 110.7 (Ar CH), 70.0 (CH-O), 64.5 (CH_2-O), 44.8 (CH_2-N), 26.6 (CH_2-C=N), 26.4 (CH_2-CH_2-CH_2), (Ar C-F and C-N could not be detected).

4.2.44. 1,4-bis-1-[2-(2-Hydroxyethylamino)-trifluoropyrid-4-yl]-1H-benzimidazol-2-ylbutane 193



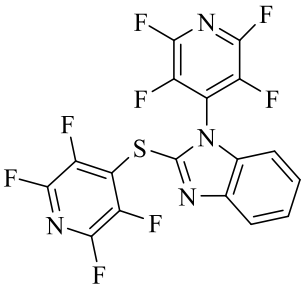
193 (50%)

A solution of ethanolamine **192** (0.4 mmol, 0.024 g) in THF (4 mL) was added dropwise to stirred solution of synthesise compound **143** (0.2 mmol, 0.108 g) in THF (4 mL) at 80 °C for 24 h under a N_2 atmosphere. Distilled water (10 mL) was added and the mixture extracted with DCM (3×20 ml). The organic extract was dried over $MgSO_4$, filtered and evaporated to give a white solid (0.14 g) which was recrystallized from hot ethanol to give the pure compound **193** (0.06, 50%) as white shiny crystals.

m.p. 175-178 °C; ν_{max}/cm^{-1} (film), 3425 (broad N-H), 2947, 1651, 1512, 1427, 1149, 1057, 987, 748. MS (ESI) (MH$^-$), $C_{34}H_{27}F_6N_8O_2$ requires *m/z* 669.2161 found *m/z* 669.2175;

δ_H (400 MHz, $(CD_3)_2CO$) 7.71-7.68 (2H, dd, *J*, 8.3, 1.6), 7.32-7.25 (4H, m), 6.60 (2H, s), 4.12 (2H, broad s), 3.78 (4H, t, *J* 11.2), 3.60-3.58 (4H, m), 2.88 (4H, m), 1.99 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.5 (2F, t, *J* 14.7), 13.9 (2F, dd, *J* 29.0, 11.7), 5.1 (2F, dd, *J* 22.9, 8.6).

4.2.45. 1-Tetrafluoropyrid-4-yl-2-tetrafluoropyrid-4-ylsulfanyl-1H-benzimidazole 194



194 (54%)

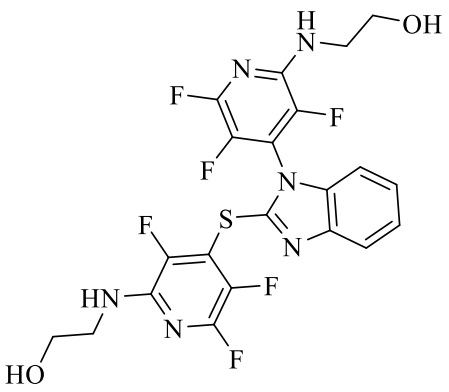
A solution of pentafluoropyridine **74** (100 mmol, 16.9 g) in THF (3 mL) and was added dropwise to a stirred suspension of NaH (60 % dispersion in mineral oil) (40 mmol, 1.6 g) in THF (4 mL). A solution of 2-mercaptobenzimidazole **155** (20 mmol, 3.0 g) in DMF (3 mL) and THF (10 mL) was added to reaction mixture dropwise using a syringe pump under N_2 atmosphere and left to stir at RT

for 24 h. The solvent was evaporated and water (20 mL) was added to the residue. The reaction mixture was extracted with DCM (3x25 mL) and organic extract was dried over $MgSO_4$, filtered and evaporated to give compound **194** (4.8 g, 54%) as creamy white crystals.

m.p. 113-115 °C

ν_{max} /cm^{-1}(film) 1630, 1474, 1246, 975, 952; MS (ESI) (MH-), $C_{17}H_3F_8N_4S$ found m/z 446.9962 requires m/z 446.9956; δ_H (400 MHz, $CDCl_3$) 7.85-7.80 (1H, m), 7.49-7.47 (2H, m), 7.23-7.19 (1H, m); δ_F (376 MHz, $CDCl_3$) 77.42-77.05 (2F, m), 74.37-73.97 (2F, m), 19.26-19.12 (2F, m), 27.03-26.87 (2F, m); δ_C (125 MHz, $CDCl_3$) 144.1 (dtd, *J* 248, 15, 4), 143.5 (dddd, *J* 247, 18, 13, 3), 143.4, 142.0, 141.2 (dm, *J* 260), 138.1 (dm, *J* 266), 135.0, 126.1, 126.0 (tt, *J* 13, 5), 125.0, 124.9 (tt, *J* 18, 3), 120.7, 110.1; Analysis (%) calculated for $C_{17}H_4F_8N_4S$ (448): C, 45.55; H, 0.90; N, 12.50. Found C, 45.41; H, 0.92; N, 12.00

4.2.46. 2-((3,5,6-Trifluoro-4-((1-(2,3,5-trifluoro-6-((2-hydroxyethyl)amino)pyridin-4-yl)-1H-benzo[d]imidazol-2-yl)thio)pyridin-2-yl)amino)ethan-1-ol 195



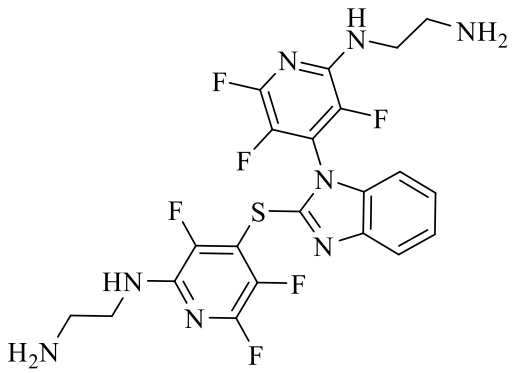
195 (80%)

A solution of ethanolamine **192** (1 mmol, 0.06 mL) in THF (4 ml) was added dropwise to stirred solution triehylamine (1 mmol, 0.14 ml) in THF (3 mL). A solution of scaffold **194** (0.5 mmol, 0.22 g) in THF (6 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under a N_2 atmosphere. Distilled water (10 mL) was added to the mixture and it was extracted with ethyl acetate (3×20 mL). The organic extract was washed with aqueous sodium chloride and dried over $MgSO_4$, filtrated and evaporated to give compound **195** (0.21 g, 80%) as white shiny solid.

m.p. 94-97 °C; ν_{max}/cm^{-1} (film), 3295 (NH), 2924, 2355, 2160,1651, 1519, 1427, 1265, 1111, 964; 671.MS (ESI) (MH$^-$), $C_{21}H_{17}F_6N_6O_2S_1$ requires *m/z* 531.1032 found *m/z* 531.1028

δ_H (400 MHz, $CDCl_3$) 7.80 (1H, dd, *J* 6, 2), 7.34-7.37 (2H, m), 7.11 (1H, d, *J* 7.6), 5.36 (2H broad s), 5.28 (2H, broad s) 3.91-3.75 (4H, m), 3.72-3.47 (4H, m); δ_F (376 MHz, $CDCl_3$) 72.54 (1F, t, *J* 23.3), 69.59 (1F, t, *J* 22.9), 23.99 (1F, dd, *J* 29.9, 7.1), 14.27 (1F, dd, *J* 30.8, 10.2), 8.33 (1F, dd, *J* 22.9, 7.1), 0.27 (1F, dd, *J* 23.3, 7.7); δ_C (100 MHz, $CDCl_3$) 143.2 (Ar Cq), 135.6 (Ar Cq), 123.2 (Ar CH), 120.4 (Ar CH) , 110.0 (Ar CH), 61.7 (CH_2-O), 61.5 (CH_2-O), 53.5 (CH_2-N), 43.4 (CH_2-N), (C-F, C-S and C-N could not be detected).

4.2.47. N1-(4-((1-(2-((2-Aminoethyl)amino)-3,5,6-trifluoropyridin-4-yl)-1H-benzo[d]imidazol-2-yl)thio)-3,5,6-trifluoropyridin-2-yl)ethane-1,2-diamine 196

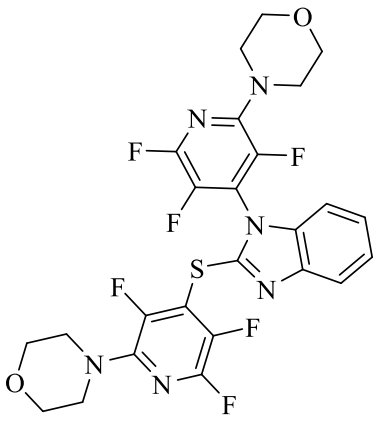


196 (72%)

A solution of ethylenediamine **186** (1 mmol, 0.06 g) in THF (5 mL) was added dropwise to a stirred solution triethylamine (1 mmol, 0.14 mL) in THF (3 mL). A solution of scaffold **194** (0.5 mmol, 0.22 g) in THF (8 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under a N_2 atmosphere. Distilled water (10 mL) was added to reaction mixture and it was extracted with ethyl acetate (3×20 mL). The organic extract was washed with aqueous sodium chloride and dried over $MgSO_4$, filtrated and evaporated to give yellow sugary solid **196** (0.19 g, 72%).

Decomposed at 119 °C; v_{max}/cm^{-1} (film), 3464 (NH), 3232 (N-H), 2893, 1651, 1427, 1257, 1072, 972, 810, 663; MS (ESI) (MH^-), $C_{21}H_{19}F_6N_8S_1$ requires m/z 529.1352 found m/z 529.1350; δH (400 MHz, $CDCl_3$) 7.78 (1H, dd, *J* 2.4, 5.2), 7.31-7.32 (2H, m), 7.14 (1H, d, *J* 6.8), 5.45 (1H, s, N-H), 5.08 (1H, s, N-H), 3.73-3.38 (4H, m), 3.01-2.87 (4H,m), 1.23 (4H, s, N-H); δF (376 MHz, $CDCl_3$) 75.38 (1F, t, *J* 23.3), 69.62 (1F, t, *J* 24.4), 23.60 (1F, dd, *J* 5.6, 28.5), 13.91 (1F, dd, *J* 8.6, 28.9), 7.76 (1F, dd, *J* 4.5, 23.3), 0.94 (1F, dd *J* 10.1, 23.3).

4.2.48. 4-(3,5,6-Trifluoro-4-((1-(2,3,5-trifluoro-6-morpholinopyridin-4-yl)-1H-benzo[d]imidazol-2-yl)thio)pyridin-2-yl)morpholine 198

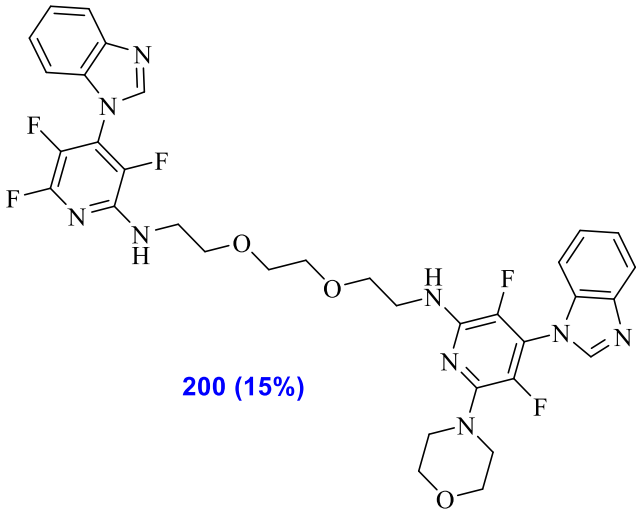


198 (67%)

A solution of morpholine **197** (1 mmol, 0.086 mL) in THF (4 mL) was added dropwise to a stirred solution triethylamine (1 mmol, 0.14 mL) in THF (3 mL). A solution of scaffold **194** (0.5 mmol, 0.22 g) in THF (6 mL) was added dropwise to the mixture and the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and water (10 mL) was added to the residue. A white solid precipitated, and was filtered (0.3 g). Column chromatography purification using eluting solvent 80% ethyl acetate and 20% light petroleum gave white sugary **198** (0.2 g, 67%).

m.p. 99-102 °C; ν_{max}/cm^{-1} (film) 2854, 1620, 1442, 1257, 1116, 972, 740; MS (ESI) (MH^-), $C_{25}H_{21}F_6N_6O_2S_1$ requires *m/z* 583.1345 found *m/z* 583.1345; δ_H (400 MHz, $CDCl_3$) 7.77 (1H, dd, *J* 2.4, 8), 7.37-7.33 (2H, m), 7.11 (1H, d, *J* 8), 3.83 (4H, t, *J* 4.4), 3.75 (4H, t, *J* 4.4), 3.54 (4H, t, *J* 4), *3*.39 (4H, t, *J* 4.4), δ_F (376 MHz, $CDCl_3$) 75.18 (1F, t, *J* 26.3), 72.28 (1F, t, *J* 24.4), 36.32 (1F, d, *J* 30.4), 25.26 (1F, d, *J* 31.5), 15.53 (1F, dd, *J* 5.6, 25.9), 5.96 (1F, d *J* 24.4); δ_C (100 MHz, $CDCl_3$) 143.6 (Ar Cq), 135.5 (Ar Cq),), 125.1 (Ar CH), 124.2 (Ar CH), 120.3 (Ar CH), 109.9 (Ar CH), 66.62 (d, *J* 4, CH_2-O), 47.8 (d, *J* 5.7, CH_2-N), 47.6 (d, *J* 5.7, CH_2-N), (C-F, C-S and C-N could not be detected); Analysis (%) for $C_{25}H_{21}F_6N_6O_2S_1$ (582) required: C, 51.55; H, 3.46; N, 14.43 Found: C, 51.63; H, 3.30; N, 13.94.

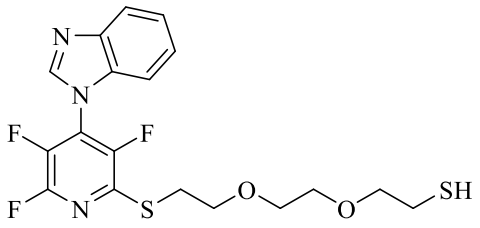
4.2.49. N-(2-(2-(2-((4-(1H-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-2-yl)amino)ethoxy)ethoxy)ethyl)-4-(1H-benzo[d]imidazol-1-yl)-3,5-difluoro-6-morpholinopyridin-2-amine 200



A solution of morpholine **197** (1 mmol, 0.086 mL) in dioxane (4 mL) was added dropwise to a stirred solution of scaffold **163** (0.5 mmol, 0.32 g) in dioxane (6 mL). the reaction left to stir at room temperature overnight under N_2. The solvent was evaporated and water (10 mL) was added to the residue. A white solid precipitated, and was filtered (0.35 g). Column chromatography purification using eluting solvent 95% ethyl acetate and 5% light petroleum gave **200** as white sugary solid (0.05 g, 15%). Only partial data available due to lack of time, and the polarity of compound which made purification difficult.

δ_H (400 MHz, $CDCl_3$) 8.06 (1H, s), 8.03 (1H, s), 7.92-7.88 (2H, m), 7.42-7.30 (6H, m), 5.28-5.27 (1H, m), 5.0-4.98 (1H, m), 3.83 (4H, t, *J* 3.6), 3.78-3.68 (12H, m), 3.46 (2H, t, J 2.8), 2.92 (2H, t, *J* 3.6); δF (376 MHz, $CDCl_3$) 75.16 (1F, dt, *J* 18.8, 3.8), 14.21 (1F, dd, *J* 22.5, 7.5), 11.49 (1F, dd, *J* 18.8, 8.2), 5.37 (1F, d, *J* 7.53), 1.13-1.01 (1F, m).

4.2.50. 2-(2-(2-((4-(1H-benzo[d]imidazol-1-yl)-3,5,6-trifluoropyridin-2-yl)thio)ethoxy)ethoxy)ethane-1-thiol 202



202 (41%)

A solution of 3,6-dioxa-1,8-octanedithiol **201** (1mmol, 0.18 g) in THF (3 mL) was added dropwise to stirred solution of $NaHCO_3$ (2 mmol, 0.17 g) in THF (3 mL). A solution of compound **109** (2 mmol, 0.56 g) in THF (6 mL) was added dropwise to the mixture and the reaction mixture was refluxed at 65 °C for 24 h under N_2. The solvent was evaporated and distilled water (10 mL) added to residue.The mixture was extracted with DCM (3×20 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated to give a white solid (0.67 g). Column chromatography purification using 45% ethyl acetate and 55% light petroleum as eluting solvent gave compound **202** (0.18 g, 41%) as white oil with some impurity which could not be purified further due to lack of time, therefore only partial data are available.

MS (ESI) (MH^-), $C_{18}H_{19}F_3N_3O_2S_2$ requires *m/z* 430.0871 found *m/z* 430.0869; δ_F (376 MHz, $CDCl_3$) 72.20 (1F, t, *J* 23.1), 33.24 (1F, d, *J* 28.9), 11.54 (1F, d, *J* 23.3).

4.3. Biological activity studies

4.3.1. Preparation of Trisma base buffer

Trisma base (0.30 g) was dissolved into distilled water (500 ml) to make 2.5 x 10^{-3} M trisma base buffer. The pH was adjusted to 7.55 by adding 0.1 M HCl.

4.3.2. Preparation of DNA stock solution

SS-DNA (50 mg) was purchased from Sigma and was dissolved by stirring overnight in double deionized water (pH 7.0) and kept at 4 °C. A solution of (SS-DNA) in the trisma buffer gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of ca. ~1.8, showing the DNA was free from any protein. SS-DNA concentration was found from absorption spectroscopy using molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ (260 nm).

4.3.3. Preparation of synthesis and references compounds solution

Each synthesised compound or reference compound (ACTD and naproxen) was weighed and dissolved in DMSO to make 10^{-2} M stock solutions of each substance. Further dilutions were performed with DMSO to prepare different concentrations of each compound (10^{-3}–10^{-6} $mol.dm^{-3}$).

4.3.4. Method of UV absorption assay

The UV absorption assay for each synthesis and references compound was carry out by keeping the fixed concentration of each compound while varying SS-DNA concentration. In this method, different amounts of SS-DNA solution were added to samples containing fixed concentrations of each compound. The mixtures were shaken well and were allowed to incubate for 30 min at room temperature to measure the absorption. Absorption spectra were recorded using cuvettes of 1 cm path length at room temperature (25 ± 1 °C).

4.3.5. Method of fluorescence displacement assay

In this method 0.3 mL of EB solution (10^{-4} M) in trisma was added to 0.3 mL of SS-DNA solution then each test compound solution (30, 60, 120, 180, 240, 300 µL of 10^{-6} M)) was titrated into the DNA/EB mixture. Each mixture were diluted up to 3 mL with trisma buffer, making the solutions

with varied concentration of (10^{-6}, 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, 8×10^{-6} and 10^{-5} M) each compound to SS-DNA. The mixtures were shaken and incubated at room temperature for 30 min. The fluorescence spectra of EB bound to SS-DNA were recorded at on emission wavelength of 605 nm with excitation of 480 nm.

4.3.6. Hanging drop DNA crystallization method

4.3.6.1. Preparation of DNA solution

Cacodylate buffer (pH 6.5); 50 mM isopropanol 15% was used to prepare a stock buffer solution containing $MgCl_2$ (10 mM) by adding $MgCl_2$ (9 mg) to cacodylate buffer (10 ml). DNA solution (0.5 mM) was prepared by adding (600 µl) of buffer stock solution to CGCGAATTCGCG DNA vial which contained (314 nmol).

4.3.6.2. Preparation of the DNA-compound complex sample

In hanging drop DNA crystallization method a sample vial and a piece of glass melting point tube to hold the drop were used. In order to make the drop, 20 µL of DNA solution was added to 2 µL of compound solution (0.01 M). The drop was held in the m.p. tube which was streaked to a sample vial which containing cotton wool wetted with buffer in the bottom. The sample vial was left at RT until the drops partially evaporated and crystals appeared.

4.3.7. Antimicrobial activity studies

The synthesized compounds at 10^{-2} and 10^{-3}M concentration were tested against two bacterial strains Gram-positive strain (*Escherichia coli*) and Gram-negative strain (*Staphylococcus aureus*). The disk diffusion method was used for the determination of antibacterial activity. The bacteria (100 µL) were added to Muller-Hinton agar media (25 mL), mixed well and poured into 90 × 14 mm petri plate (diameter and height respectively) and the media was allowed to solidify. Sterilized disks (6 mm in diameter) were saturated with the compound solution and placed gently on the inoculated agar plates. Each plate contained a blank disk, which was saturated with DMSO, the compound-containing disk and a control disk containing the antibiotic penicillin. The petri plates were incubated at 37 °C for 48 h.

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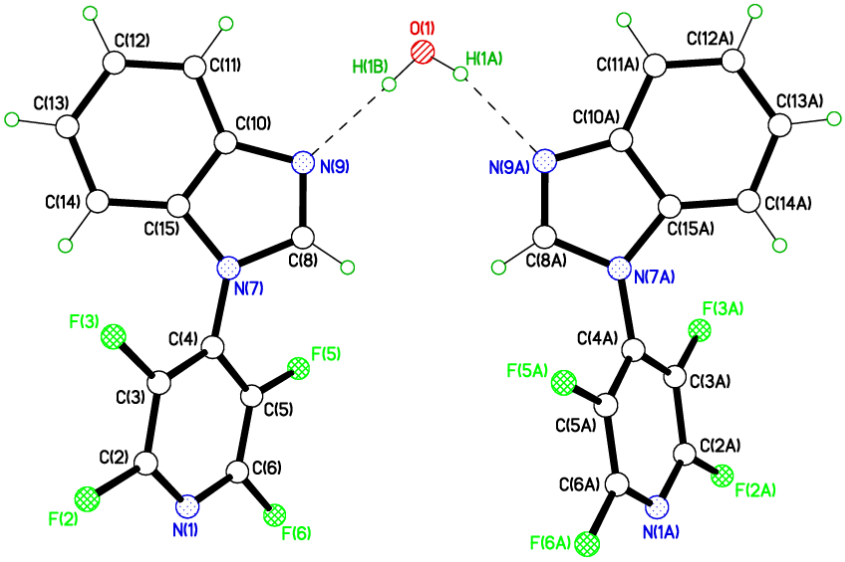
6. Appendix

6.1. X-ray crystallography data

6.1.1. Compound 109 X-ray crystal structure date

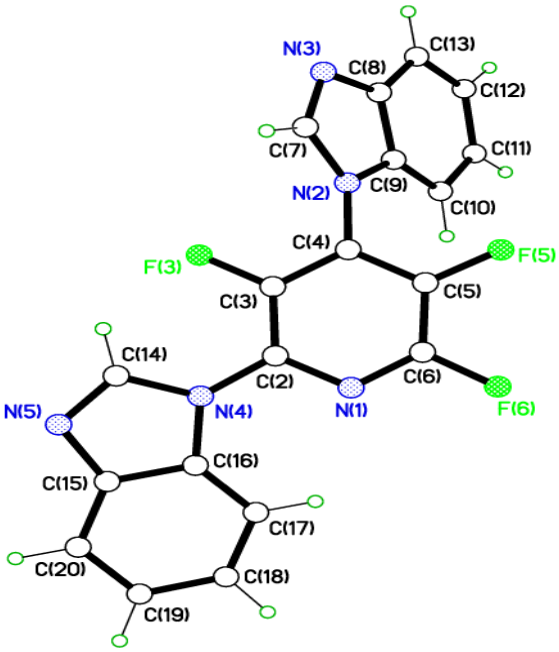


Crystal data and structure refinement

Identification code	gw85	
Chemical formula	$C_{12}H_6F_4N_3O_{0.50}$	
Formula weight	276.20	
Temperature	150(2) K	
Radiation, wavelength	MoKα, 0.71073 Å	
Crystal system, space group	monoclinic, Cc	
Unit cell parameters	a = 27.76(4) Å	α = 90°
	b = 7.483(10) Å	β = 99.29(2)°
	c = 11.133(15) Å	γ = 90°
Cell volume	2282(5) $Å^3$	
Z	8	
Calculated density	1.608 g/cm^3	
Absorption coefficient μ	0.148 mm^{-1}	
F(000)	1112	
Crystal colour and size	colourless, 0.76 × 0.46 × 0.05 mm^3	
Reflections for cell refinement	933 (θ range 3.31 to 21.56°)	
Data collection method	Bruker APEX 2 CCD diffractometer	

	ωrotation with narrow frames
θrange for data collection	2.82 to 28.49°
Index ranges	h –37 to 36, k 0 to 9, l 0 to 14
Completeness to θ = 26.00°	98.6 %
Intensity decay	0%
Reflections collected	2746
Independent reflections	2746 (R_{int} = 0.1161)
Reflections with $F^2>2\sigma$	1450
Absorption correction	semi-empirical from equivalents
Min. and max.transmission	0.896 and 0.993
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.1352, 0.0000
Data / restraints / parameters	2746 / 2 / 359
Final R indices [$F^2>2\sigma$]	R1 = 0.0780, wR2 = 0.1981
R indices (all data)	R1 = 0.1376, wR2 = 0.2206
Goodness-of-fit on F^2	0.937
Extinction coefficient	0.0060(14)
Largest and mean shift/su	0.000 and 0.000
Largest diff. peak and hole	0.456 and –0.386 e $Å^{-3}$

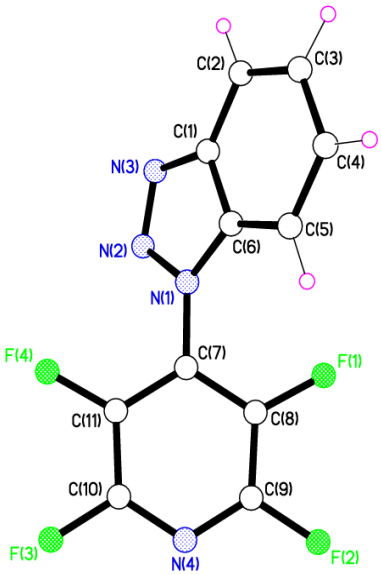
6.1.2. Compound 110 X-ray crystal structure data



Crystal data and structure refinement

Identification code	gw86
Chemical formula	$C_{19}H_{10}F_3N_5$
Formula weight	365.32
Temperature	150(2) K
Radiation, wavelength	MoKα, 0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell parameters	a = 12.0121(6) Å α = 90° b = 8.7895(5) Å β = 100.5037(8)° c = 14.8138(8) Å γ = 90°
Cell volume	1537.84(14) $Å^3$
Z	4
Calculated density	1.578 g/cm^3
Absorption coefficient μ	0.124 mm^{-1}
F(000)	744
Crystal colour and size	colourless, 0.67 × 0.50 × 0.20 mm^3
Reflections for cell refinement	7716 (θ range 2.71 to 30.56°)
Data collection method	Bruker APEX 2 CCD diffractometer
	ωrotation with narrow frames
θrange for data collection	2.71 to 30.59°
Index ranges	h –17 to 17, k –12 to 12, l –21 to 20
Completeness to θ = 30.59°	99.3 %
Intensity decay	0%
Reflections collected	17691
Independent reflections	4707 (R_{int} = 0.0258)
Reflections with $F^2>2\sigma$	3987
Absorption correction	semi-empirical from equivalents
Min. and max.transmission	0.921 and 0.976
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0741, 0.3637
Data / restraints / parameters	4707 / 0 / 284
Final R indices [$F^2>2\sigma$]	R1 = 0.0415, wR2 = 0.1189
R indices (all data)	R1 = 0.0483, wR2 = 0.1243
Goodness-of-fit on F^2	1.054
Largest and mean shift/su	0.001 and 0.000
Largest diff. peak and hole	0.546 and –0.256 e $Å^{-3}$

6.1.3. Compound 111 (X isomer) X-ray crystal structure data



Crystal data

$C_{11}H_4F_4N_4$	$F(000) = 536$
$M_r = 268.18$	$D_x = 1.729$ Mg m^{-3}
Monoclinic, $P2_1/n$	Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å
$a = 7.4413$ (7) Å	Cell parameters from 6886 reflections
$b = 13.0851$ (12) Å	$\theta = 2.5$–$30.6°$
$c = 10.9628$ (10) Å	$\mu = 0.16$ mm^{-1}
$\beta = 105.1556$ (13)°	$T = 150$ K
$V = 1030.32$ (16) Å^3	Block, colourless
$Z = 4$	0.89 × 0.43 × 0.15 mm

Data collection

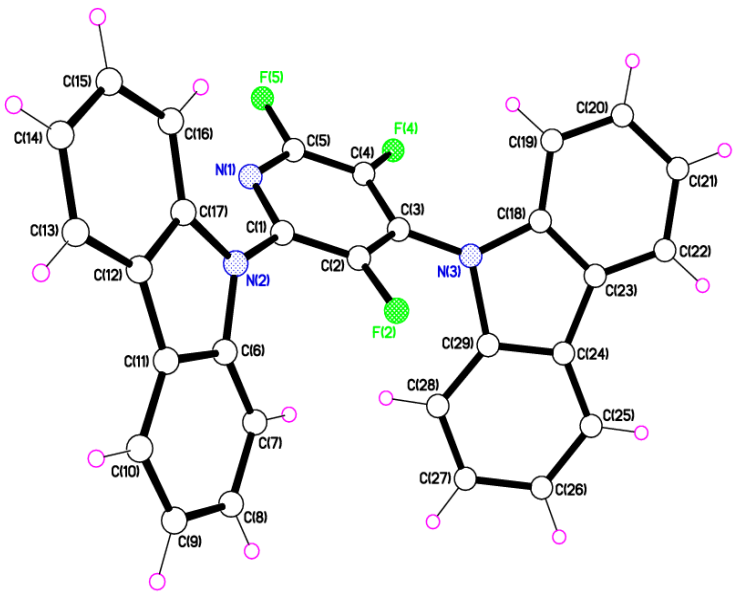
Bruker APEX 2 CCD area detector diffractometer	2817 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	$R_{int} = 0.027$
ω rotation with narrow frames scans	$\theta_{max} = 30.6°$, $\theta_{min} = 2.5°$
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	$h = -10 \rightarrow 10$

T_{min} = 0.870, T_{max} = 0.976	k = -18→18
12124 measured reflections	l = -15→15
3156 independent reflections	

Refinement

Refinement on F^2	Primary atom site location: structure-invariant direct methods
Least-squares matrix: full	Hydrogen site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)]$ = 0.038	All H-atom parameters refined
$wR(F^2)$ = 0.115	$w = 1/[\sigma^2(F_o^2) + (0.0686P)^2 + 0.2212P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.04	$(\Delta/\sigma)_{max}$ < 0.001
3156 reflections	$\Delta\rangle_{max}$ = 0.41 e Å^{-3}
188 parameters	$\Delta\rangle_{min}$ = -0.24 e Å^{-3}
0 restraints	

6.1.4. Compound 114 X-ray crystal structure data



Crystal data

$C_{29.02}H_{16.02}F_3N_3O_0$	D_x = 1.392 Mg m^{-3}
M_r = 463.77	Mo $K\alpha$ radiation, λ = 0.71073 Å
Orthorhombic, $P2_12_12_1$	Cell parameters from 4470 reflections
a = 8.1301 (5) Å	θ = 2.2–22.0°
b = 18.1470 (12) Å	μ = 0.10 mm^{-1}
c = 29.9929 (19) Å	T = 150 K
V = 4425.1 (5) Å^3	Plate, colourless
Z = 8	0.40 × 0.21 × 0.05 mm^3
F(000) = 1905	

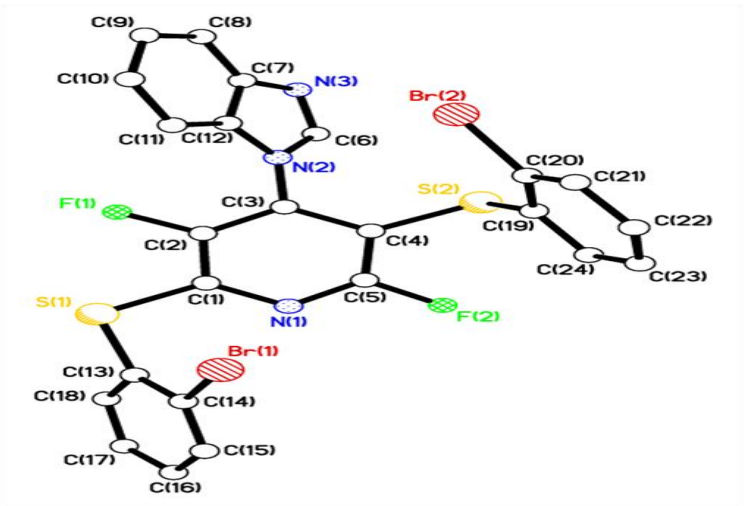
Data collection

Bruker APEX 2 CCD diffractometer	9768 independent reflections
Radiation source: fine-focus sealed tube	6291 reflections with $I > 2\sigma(I)$
Graphite monochromator	R_{int} = 0.057
ω rotation with narrow frames scans	θ_{max} = 27.1°, θ_{min} = 1.8°
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	h = -10→9
T_{min} = 0.961, T_{max} = 0.995	k = -23→23
30333 measured reflections	l = -38→36

Refinement

Refinement on F^2	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: inferred from neighbouring sites
$R[F^2 > 2\sigma(F^2)] = 0.045$	H-atom parameters constrained
$wR(F^2) = 0.089$	$w = 1/[\sigma^2(F_o^2) + (0.0303P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$S = 1.00$	$(\Delta/\sigma)_{max} = 0.001$
9768 reflections	$\Delta\rangle_{max} = 0.15$ e Å^{-3}
760 parameters	$\Delta\rangle_{min} = -0.15$ e Å^{-3}
546 restraints	Absolute structure: Flack x determined using 2058 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Primary atom site location: dual	Absolute structure parameter: 0.5 (4)

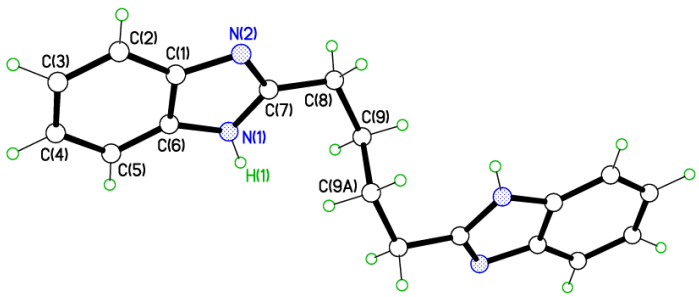
6.1.5. Compound 126 Xray crystal structure data



Crystal data and structure refinement

Identification code	gw83	
Chemical formula	$C_{24}H_{13}Br_2F_2N_3S_2$	
Formula weight	605.31	
Temperature	150(2) K	
Radiation, wavelength	MoKα, 0.71073 Å	
Crystal system, space group	triclinic, P $\bar{1}$	
Unit cell parameters	a = 8.2633(9) Å	α = 73.7151(15)°
	b = 10.5751(11) Å	β = 78.7097(16)°
	c = 13.8671(14) Å	γ = 70.2669(15)°
Cell volume	1088.0(2) $Å^3$	
Z	2	
Calculated density	1.848 g/cm^3	
Absorption coefficient μ	3.954 mm^{-1}	
F(000)	596	
Crystal colour and size	colourless, 0.37 × 0.29 × 0.06 mm^3	
Reflections for cell refinement	4741 (θ range 2.30 to 28.42°)	
Data collection method	Bruker APEX 2 CCD diffractometer	
	ωrotation with narrow frames	
θrange for data collection	1.54 to 30.55°	
Index ranges	h –11 to 11, k –15 to 14, l –19 to 19	
Completeness to θ = 29.00°	99.5 %	
Intensity decay	0%	
Reflections collected	17054	
Independent reflections	6544 (R_{int} = 0.0366)	
Reflections with $F^2>2\sigma$	4835	
Absorption correction	semi-empirical from equivalents	
Min. and max.transmission	0.3224 and 0.797	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	
Weighting parameters a, b	0.0520, 1.3591	
Data / restraints / parameters	6544 / 0 / 298	
Final R indices [$F^2>2\sigma$]	R1 = 0.0421, wR2 = 0.1024	
R indices (all data)	R1 = 0.0660, wR2 = 0.1129	
Goodness-of-fit on F^2	1.032	
Largest and mean shift/su	0.001 and 0.000	
Largest diff. peak and hole	1.985 and –0.886 e $Å^{-3}$	

6.1.6. Compound 134 X-ray crystal structure data



Crystal data

$C_{18}H_{18}N_4$	D_x = 1.324 Mg m^{-3}
M_r = 290.36	Mo $K\alpha$ radiation, λ = 0.71073 Å
Orthorhombic, *Pbca*	Cell parameters from 3582 reflections
a = 8.788 (3) Å	θ = 2.3–30.5°
b = 9.340 (3) Å	μ = 0.08 mm^{-1}
c = 17.743 (5) Å	T = 150 K
V = 1456.3 (8) Å^3	Lath, colourless
Z = 4	1.13 × 0.23 × 0.15 mm
F(000) = 616	

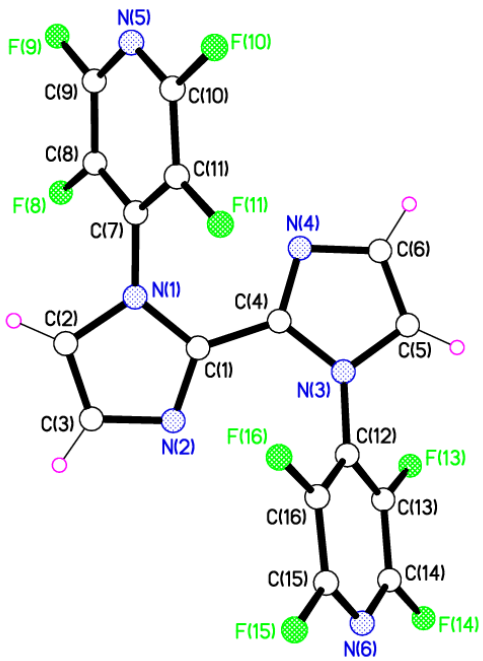
Data collection

Bruker APEX 2 CCD diffractometer	1773 reflections $\omega\iota\tau\eta$ $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	R_{int} = 0.047
ω rotation with narrow frames scans	θ_{max} = 30.6°, θ_{min} = 2.3°
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	h = -12→12
T_{min} = 0.914, T_{max} = 0.988	$\kappa = -13 \rightarrow 13$
15966 measured reflections	$\lambda = -25 \rightarrow 25$
2226 independent reflections	

Refinement data

Refinement on F^2	Primary atom site location: structure-invariant direct methods
Least-squares matrix: full	Hydrogen site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)] = 0.042$	All H-atom parameters refined
$wR(F^2) = 0.115$	$w = 1/[\sigma^2(F_o^2) + (0.0566P)^2 + 0.3564P]$ where $P = (F_o^2 + 2F_c^2)/3$
$S = 1.05$	$(\Delta/\sigma)_{max} < 0.001$
2226 reflections	$\Delta\rangle_{\mu\alpha\xi} = 0.34$ ε ⊕$^{-3}$
136 parameters	$\Delta\rangle_{\mu\iota\nu} = -0.21$ ε ⊕$^{-3}$
0 restraints	

6.1.7. Compound 137 X-ray crystal structure data



Crystal data

$C_{16}H_4F_8N_6$	$F(000) = 856$
$M_r = 432.25$	$D_x = 1.809$ Mg m^{-3}
Monoclinic, $P2_1/c$	Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å
$a = 7.4893$ (7) Å	Cell parameters from 7665 reflections
$b = 20.898$ (2) Å	$\theta = 2.2–30.5°$
$c = 10.2489$ (10) Å	$\mu = 0.18$ mm^{-1}
$\beta = 98.348$ (2)°	$T = 150$ K
$V = 1587.1$ (3) Å^3	Rod, colourless
$Z = 4$	0.80 × 0.23 × 0.21 mm

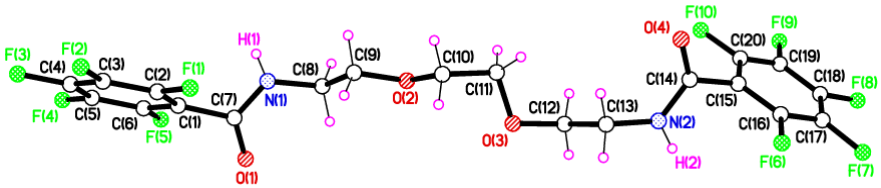
Data collection

Bruker APEX 2 CCD diffractometer	4039 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	$R_{int} = 0.025$
ω rotation with narrow frames scans	$\theta_{max} = 30.6°$, $\theta_{min} = 2.0°$
Absorption correction: multi-scan *SADABS* v2009/1, Sheldrick, G.M., (2009)	$h = -10 \rightarrow 10$
$T_{min} = 0.869$, $T_{max} = 0.963$	$k = -29 \rightarrow 29$
18321 measured reflections	$l = -14 \rightarrow 14$
4813 independent reflections	

Refinement

Refinement on F^2	Primary atom site location: structure-invariant direct methods
Least-squares matrix: full	Secondary atom site location: all non-H atoms found by direct methods
$R[F^2 > 2\sigma(F^2)] = 0.037$	Hydrogen site location: difference Fourier map
$wR(F^2) = 0.104$	All H-atom parameters refined
$S = 1.04$	$w = 1/[\sigma^2(F_o^2) + (0.051P)^2 + 0.488P]$ where $P = (F_o^2 + 2F_c^2)/3$
4813 reflections	$(\Delta/\sigma)_{max} = 0.001$
287 parameters	$\Delta\rangle_{max} = 0.37$ e Å^{-3}
0 restraints	$\Delta\rangle_{min} = -0.22$ e Å^{-3}

6.1.8. Compound 151 Xray crystal structure data



Crystal data

$C_{20}H_{14}F_{10}N_2O_4$	$F(000) = 3240$
$M_r = 536.33$	$D_x = 1.696$ Mg m^{-3}
Monoclinic, $P2_1/c$	Synchrotron radiation, $\lambda = 0.7749$ Å
$a = 17.4161$ (6) Å	Cell parameters from 9918 reflections
$b = 14.3221$ (5) Å	$\theta = 2.3–25.1°$
$c = 25.7502$ (9) Å	$\mu = 0.17$ mm^{-1}
$\beta = 101.207$ (2)°	$T = 150$ K
$V = 6300.5$ (4) Å^3	Plate, colourless
$Z = 12$	$0.05 \times 0.03 \times 0.01$ mm^3

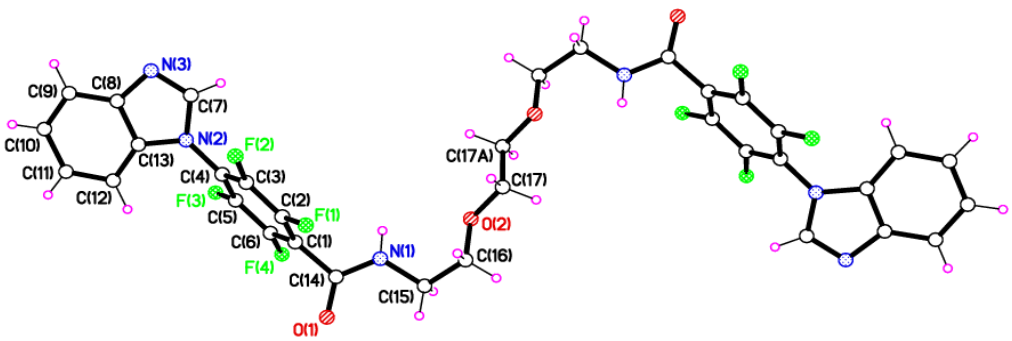
Data collection

Bruker D8 with PHOTON 100 detector diffractometer	9083 independent reflections
Radiation source: Advanced Light Station 11.3.1	6863 reflections with $I > 2\sigma(I)$
Silicon 111 monochromator	$R_{int} = 0.056$
Detector resolution: 10.42 pixels mm^{-1}	$\theta_{max} = 25.6°$, $\theta_{min} = 2.1°$
ω rotation with shutterless scans	$h = -19 \rightarrow 19$
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	$k = -15 \rightarrow 15$
$T_{min} = 0.992$, $T_{max} = 0.998$	$l = -28 \rightarrow 28$
44141 measured reflections	

Refinement

Refinement on F^2	Primary atom site location: iterative
Least-squares matrix: full	Secondary atom site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)] = 0.055$	Hydrogen site location: mixed
$wR(F^2) = 0.142$	H atoms treated by a mixture of independent and constrained refinement
$S = 1.09$	$w = 1/[\sigma^2(F_o^2) + (0.0421P)^2 + 15.2253P]$ where $P = (F_o^2 + 2F_c^2)/3$
9083 reflections	$(\Delta/\sigma)_{max} < 0.001$
991 parameters	$\Delta\rangle_{max} = 0.83$ e $Å^{-3}$
0 restraints	$\Delta\rangle_{min} = -0.32$ e $Å^{-3}$

6.1.9. Compound 152 X-ray crystal structure data



Crystal data

$C_{34}H_{24}F_8N_6O_4$	$F(000) = 748$
$M_r = 732.59$	$D_x = 1.517$ Mg m^{-3}
Monoclinic, $P2_1/n$	Synchrotron radiation, $\lambda = 0.7749$ Å
$a = 12.7854$ (5) Å	Cell parameters from 9938 reflections
$b = 8.9381$ (4) Å	$\theta = 2.5–33.6°$
$c = 14.1393$ (5) Å	$\mu = 0.12$ mm^{-1}
$\beta = 96.872$ (2)°	$T = 150$ K
$V = 1604.19$ (11) Å^3	Plate, colourless
$Z = 2$	0.35 × 0.18 × 0.02 mm^3

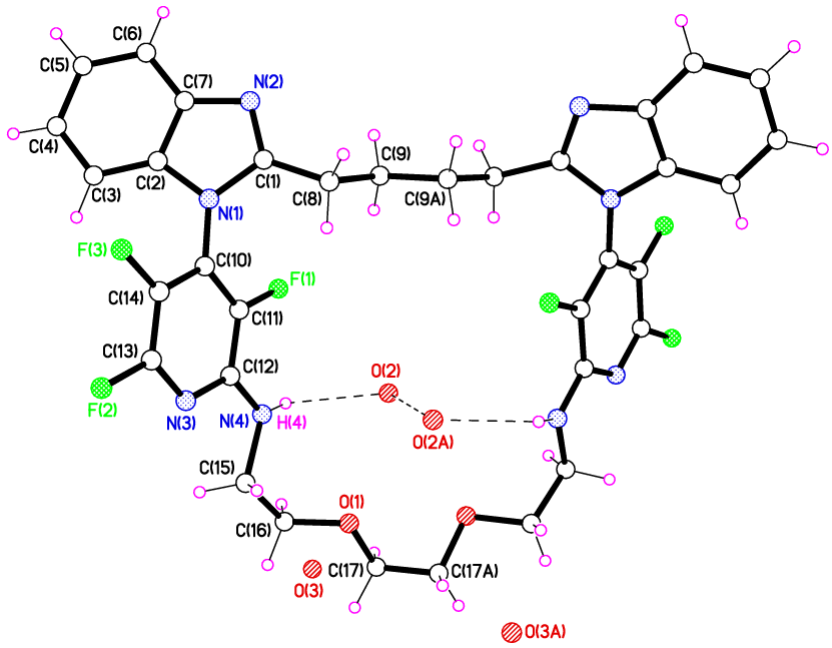
Data collection

Bruker D8 with PHOTON 100 detector diffractometer	4910 independent reflections
Radiation source: Advanced Light Station 11.3.1	3877 reflections with $I > 2\sigma(I)$
Silicon 111 monochromator	$R_{int} = 0.030$
ω rotation with shutterless scans	$\theta_{max} = 33.7°$, $\theta_{min} = 2.2°$
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	$h = -18 \rightarrow 18$
$T_{min} = 0.959$, $T_{max} = 0.998$	$k = -12 \rightarrow 12$
20949 measured reflections	$l = -20 \rightarrow 20$

Refinement

Refinement on F^2	Primary atom site location: interative
Least-squares matrix: full	Secondary atom site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)] = 0.043$	Hydrogen site location: mixed
$wR(F^2) = 0.121$	H atoms treated by a mixture of independent and constrained refinement
$S = 1.07$	$w = 1/[\sigma^2(F_o^2) + (0.058P)^2 + 0.4702P]$ where $P = (F_o^2 + 2F_c^2)/3$
4910 reflections	$(\Delta/\sigma)_{max} < 0.001$
239 parameters	$\Delta\rangle_{max} = 0.41$ e Å^{-3}
0 restraints	$\Delta\rangle_{min} = -0.20$ e Å^{-3}

6.1.10. Compound 181 X-ray crystal structure data



Crystal data

$C_{34}H_{30}F_6N_8O_2 \cdot 2.5(H_2O)$	D_x = 1.478 Mg m^{-3}
M_r = 741.70	Synchrotron radiation, λ = 0.7749 Å
Orthorhombic, $P2_12_12$	Cell parameters from 5058 reflections
a = 22.3299 (9) Å	θ = 2.7–29.5°
b = 6.0739 (3) Å	μ = 0.15 mm^{-1}
c = 12.2892 (5) Å	T = 100 K
V = 1666.78 (13) Å^3	Lath, colourless
Z = 2	0.10 × 0.03 × 0.02 mm^3
F(000) = 770	

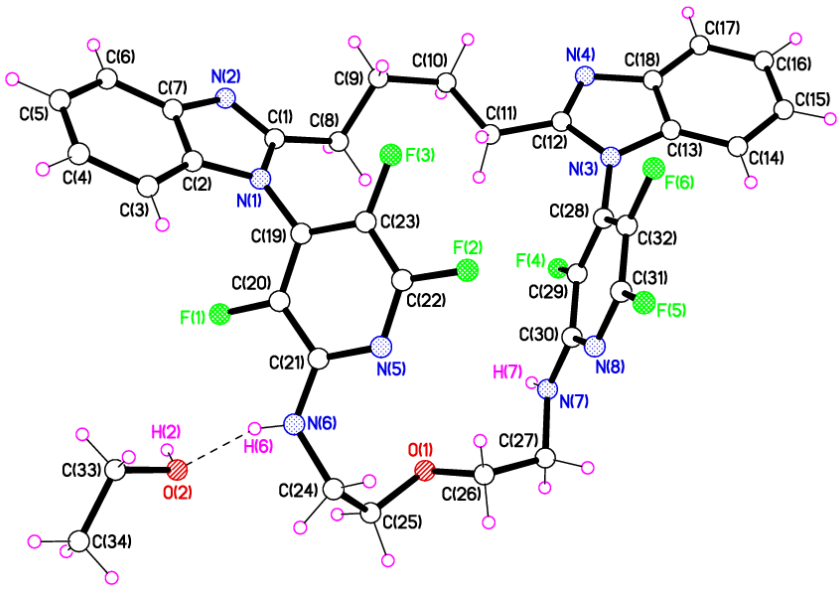
Data collection

Bruker APEX 2 CCD area detector diffractometer	3344 reflections with $I > 2\sigma(I)$
Radiation source: ALS Station 11.3.1	R_{int} = 0.068
ω & ϕ rotation with narrow frames scans	θ_{max} = 30.8°, θ_{min} = 2.7°
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	h = -29→29
T_{min} = 0.985, T_{max} = 0.997	k = -8→8
16401 measured reflections	l = -16→16
4015 independent reflections	

Refinement

Refinement on F^2	Hydrogen site location: inferred from neighbouring sites
Least-squares matrix: full	H-atom parameters constrained
$R[F^2 > 2\sigma(F^2)] = 0.053$	$w = 1/[\sigma^2(F_o^2) + (0.0652P)^2 + 0.4696P]$ where $P = (F_o^2 + 2F_c^2)/3$
$wR(F^2) = 0.140$	$(\Delta/\sigma)_{max} < 0.001$
$S = 1.05$	$\Delta\rangle_{max} = 0.29$ e Å^{-3}
4015 reflections	$\Delta\rangle_{min} = -0.38$ e Å^{-3}
278 parameters	Extinction correction: *SHELXL*, $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$
49 restraints	Extinction coefficient: 0.015 (3)
Primary atom site location: intrinsic phasing	Absolute structure: Flack x determined using 1238 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Secondary atom site location: difference Fourier map	Absolute structure parameter: 0.4 (5)

6.1.11. Compound 183 X-ray crystal structure data



Crystal data

$C_{32}H_{26}F_6N_8O{\cdot}C_2H_6O$	$F(000)$ = 2896
M_r = 698.67	D_x = 1.436 Mg m^{-3}
Monoclinic, $C2/c$	Mo $K\alpha$ radiation, λ = 0.71073 Å
a = 14.1373 (7) Å	Cell parameters from 7141 reflections
b = 17.2114 (8) Å	θ = 2.2–27.7°
c = 27.3518 (13) Å	μ = 0.12 mm^{-1}
β = 103.7199 (8)°	T = 150 K
V = 6465.4 (5) Å^3	Tablet, colourless
Z = 8	0.25 × 0.18 × 0.07 mm^3

Data collection

Bruker APEX 2 CCD area detector diffractometer	5498 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	R_{int} = 0.047
ω rotation with narrow frames scans	θ_{max} = 28.3°, θ_{min} = 1.9°
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	h = -18→18
T_{min} = 0.971, T_{max} = 0.992	k = -22→22
33520 measured reflections	l = -36→36
8064 independent reflections	

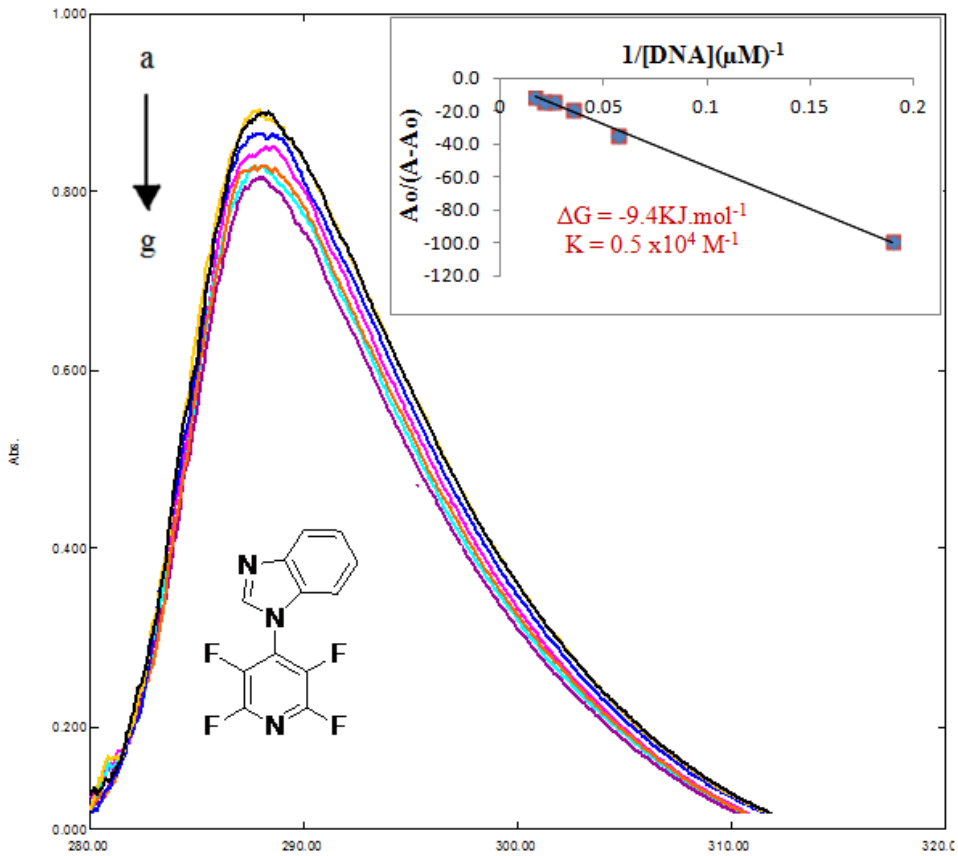
Refinement

Refinement on F^2	Primary atom site location: structure-invariant direct methods
Least-squares matrix: full	Hydrogen site location: mixed
$R[F^2 > 2\sigma(F^2)] = 0.042$	H atoms treated by a mixture of independent and constrained refinement
$wR(F^2) = 0.101$	$w = 1/[\sigma^2(F_o^2) + (0.0375P)^2 + 2.548P]$ where $P = (F_o^2 + 2F_c^2)/3$
$S = 1.03$	$(\Delta/\sigma)_{max} = 0.001$
8064 reflections	$\Delta\rangle_{max} = 0.24$ e Å^{-3}
477 parameters	$\Delta\rangle_{min} = -0.21$ e Å^{-3}
15 restraints	

6.2. UV-Visible spectroscopy data

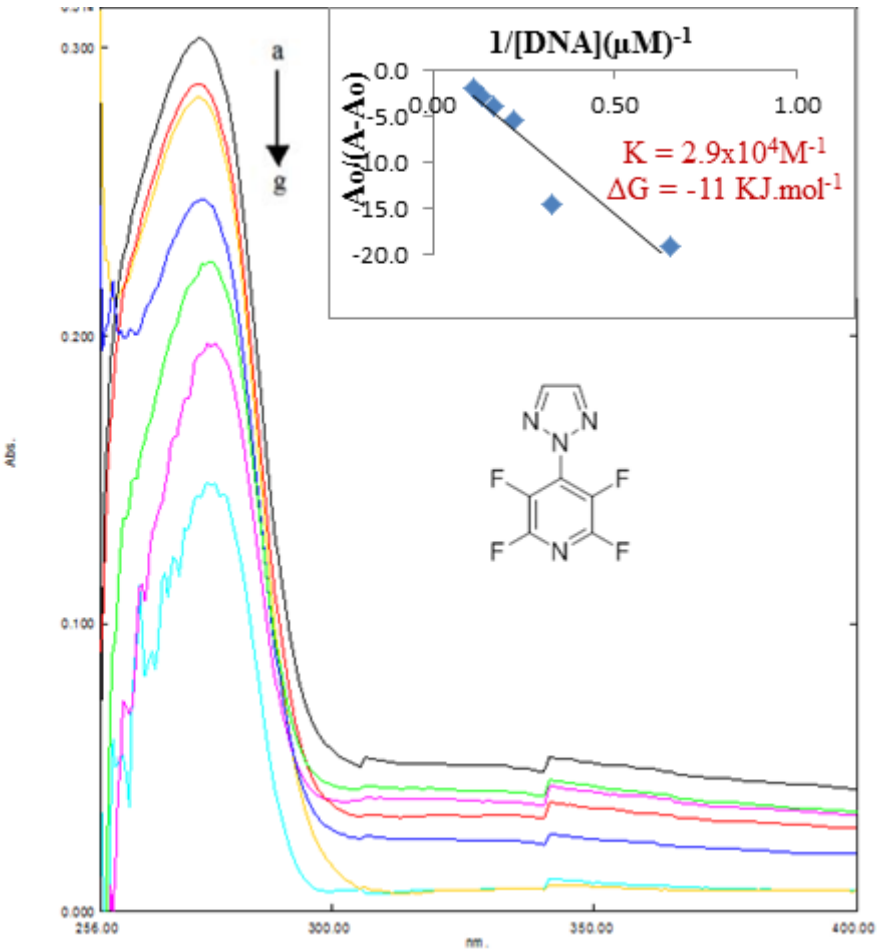
The spectra showed the absorbance of the of synthesis compound s in absence (a) and presence of 5.2 µM (b), 18 µM (c), 28 µM (d), 39 µM (e), 48 µM (f) and 57 µM (g) of DNA. The arrow shows increasing of DNA concentration. Inside graph is plot of Ao/ (A-Ao) vs. 1/ [DNA] for determination K and ΔG values.

6.2.1. UV-visible spectroscopy of compound 108



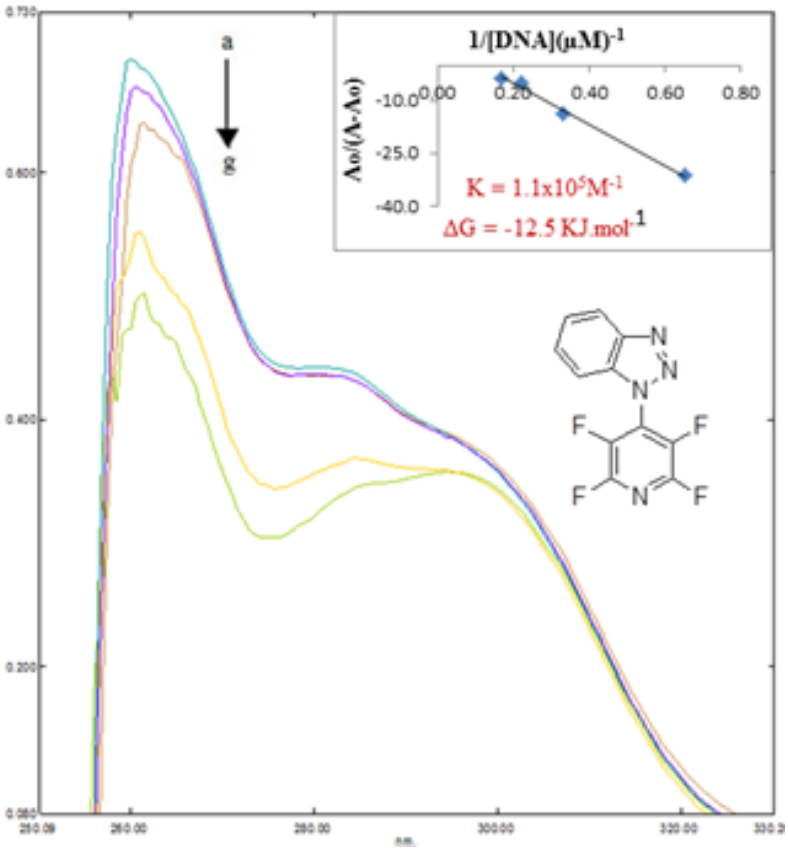
Absorption spectra of 1 x 10^{-5} M of compound 108 (R^2 = 0.9976 for six points)

6.2.2. UV-visible spectroscopy of compound 111



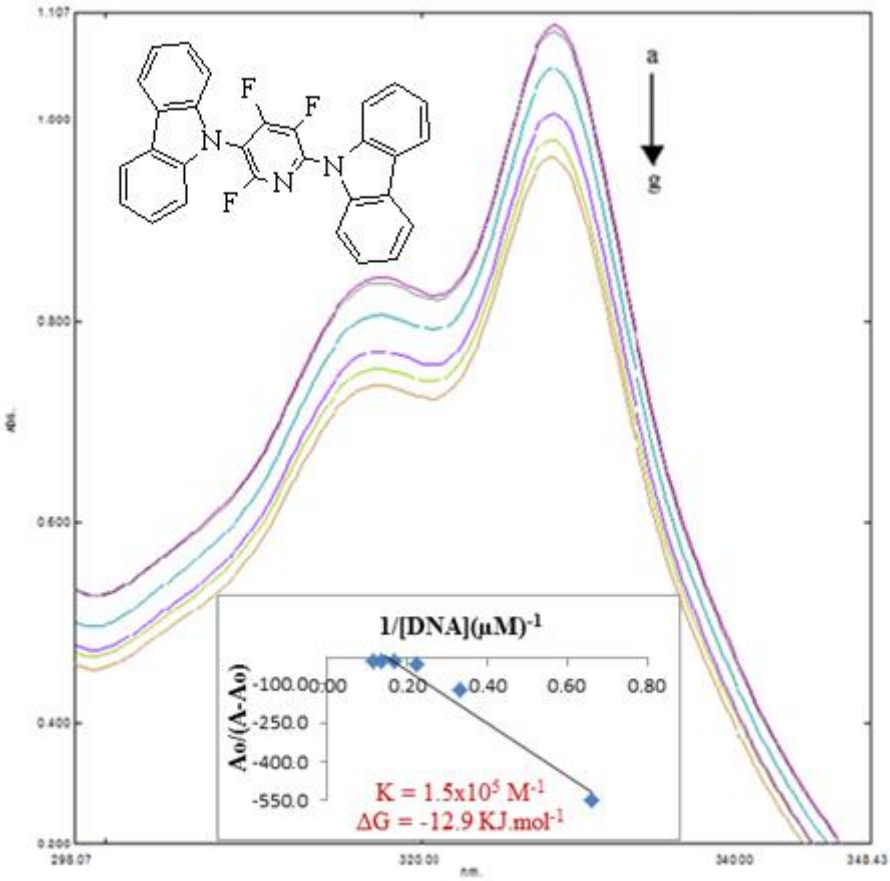
Absorption spectra of 9 x 10 $^{-5}$ M of compound 112. (R^2 =0.9529 for six points)

6.2.3. UV-visible spectroscopy of compound 113



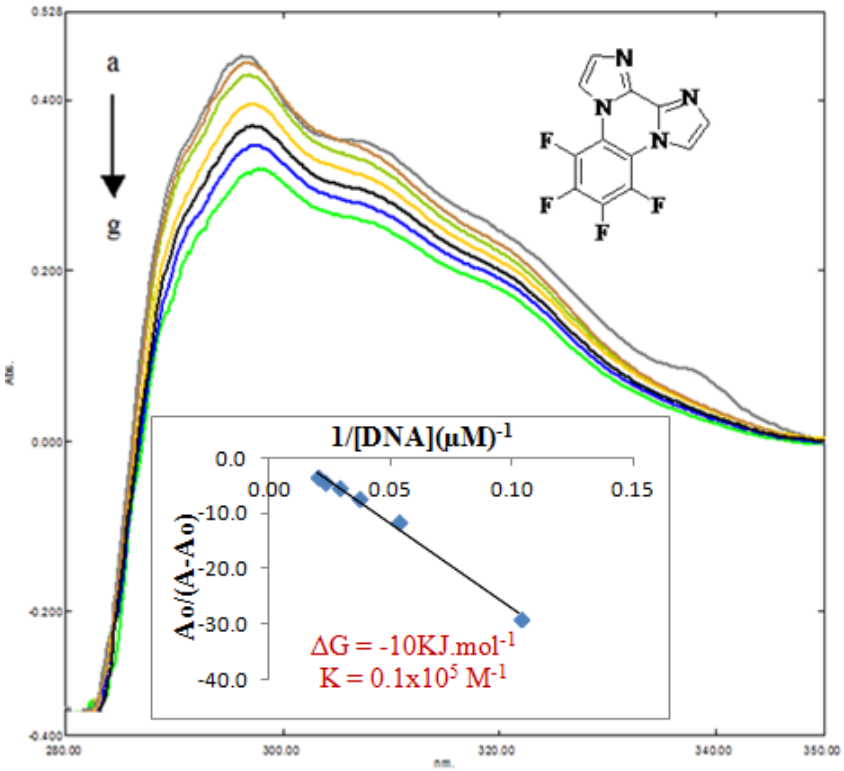
Absorption spectra of 2 x 10 $^{-5}$ M of compound 113. (R^2 =0.9940 for six points)

6.2.4. UV-visible spectroscopy of compound 114



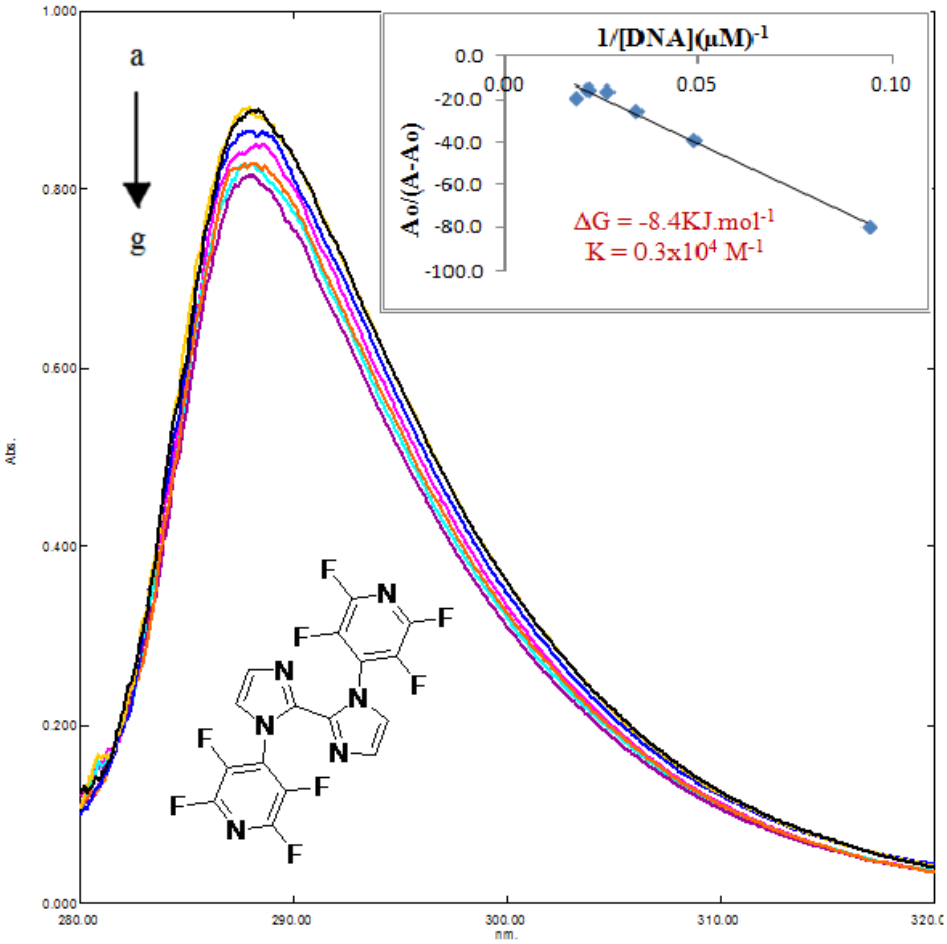
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 114. (R^2 =0.9563 for six points)

6.2.5. UV-visible spectroscopy of compound 137



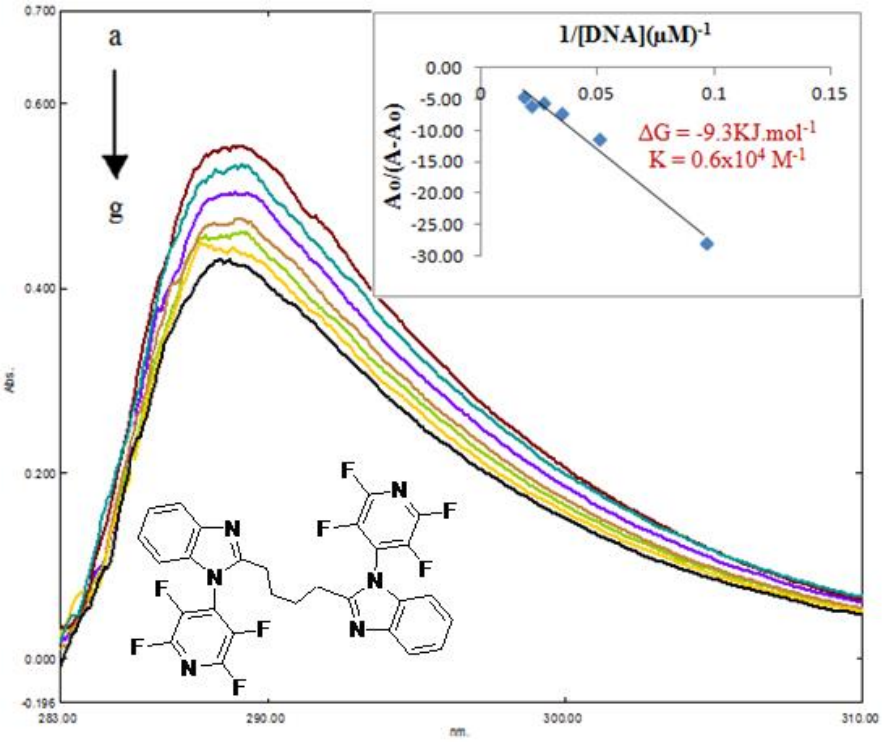
Absorption spectra of 1x 10^{-5} M of compound 137 (R^2 = 0.9912 for six points)

6.2.6. UV-visible spectroscopy of compound 138



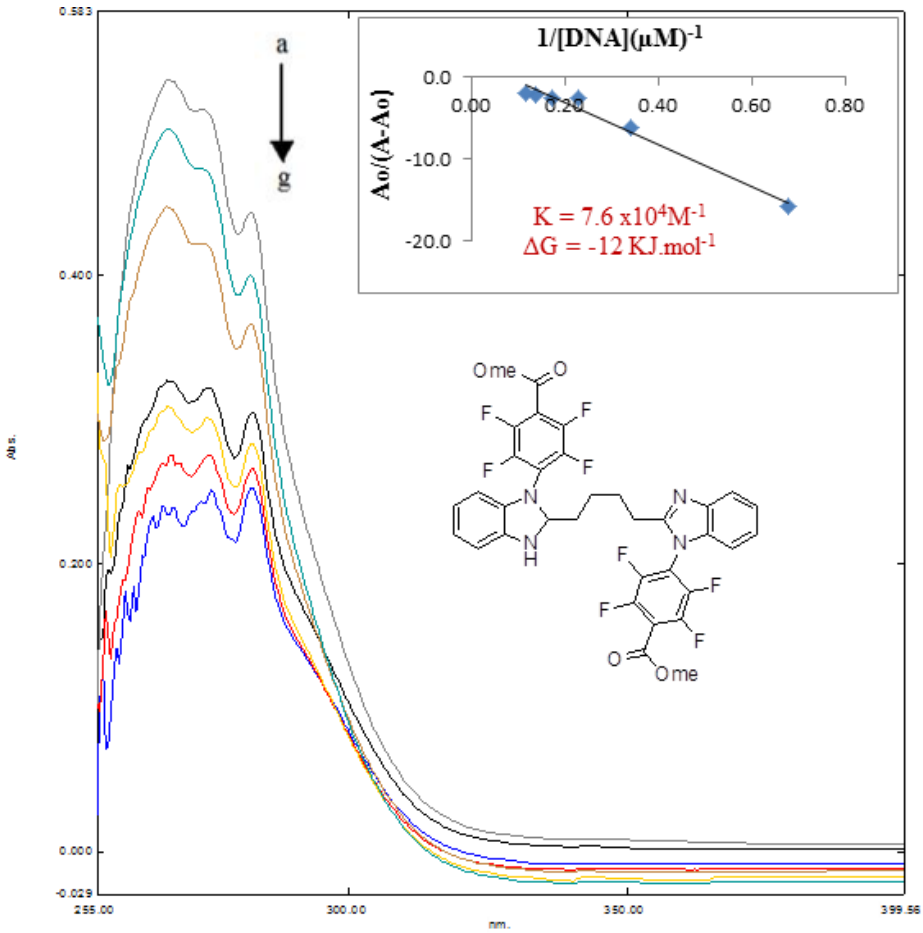
Absorption spectra of 1 x 10^{-5} M of compound 138 (R^2 = 0.9826 for six points)

6.2.7. UV-visible spectroscopy of compound 143



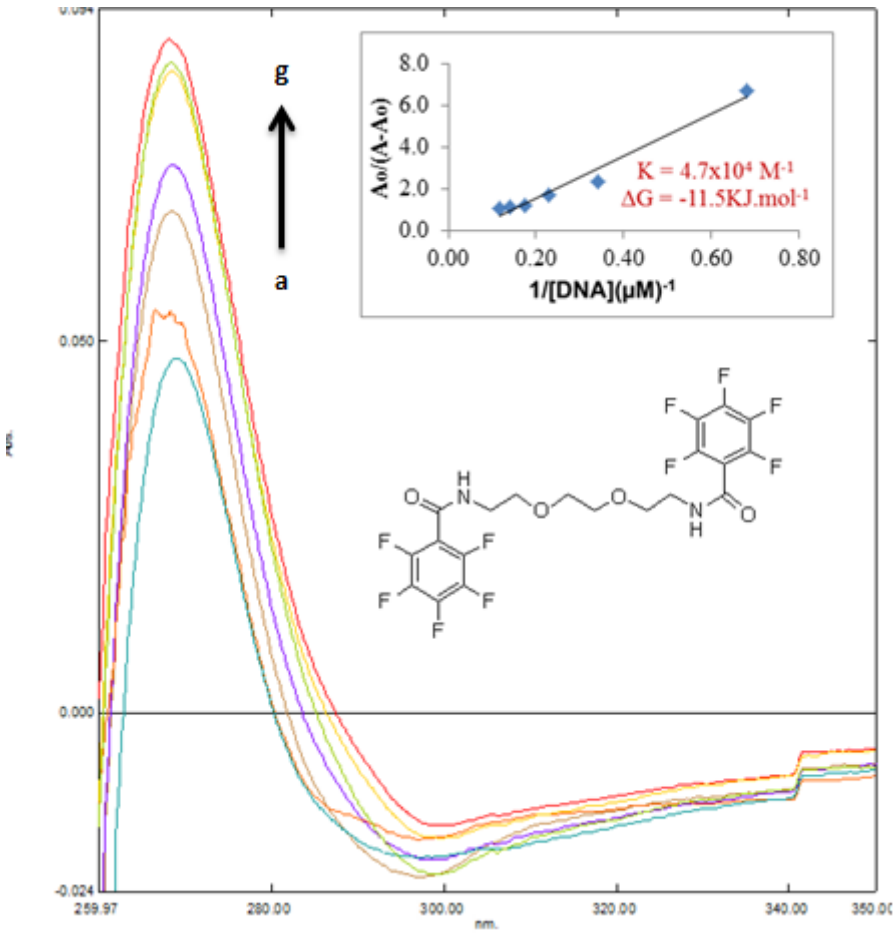
Absorption spectra of 1 x 10^{-5} M of compound 143. (R2 = 0.9744 for six points)

6.2.8. UV-visible spectroscopy of compound 148



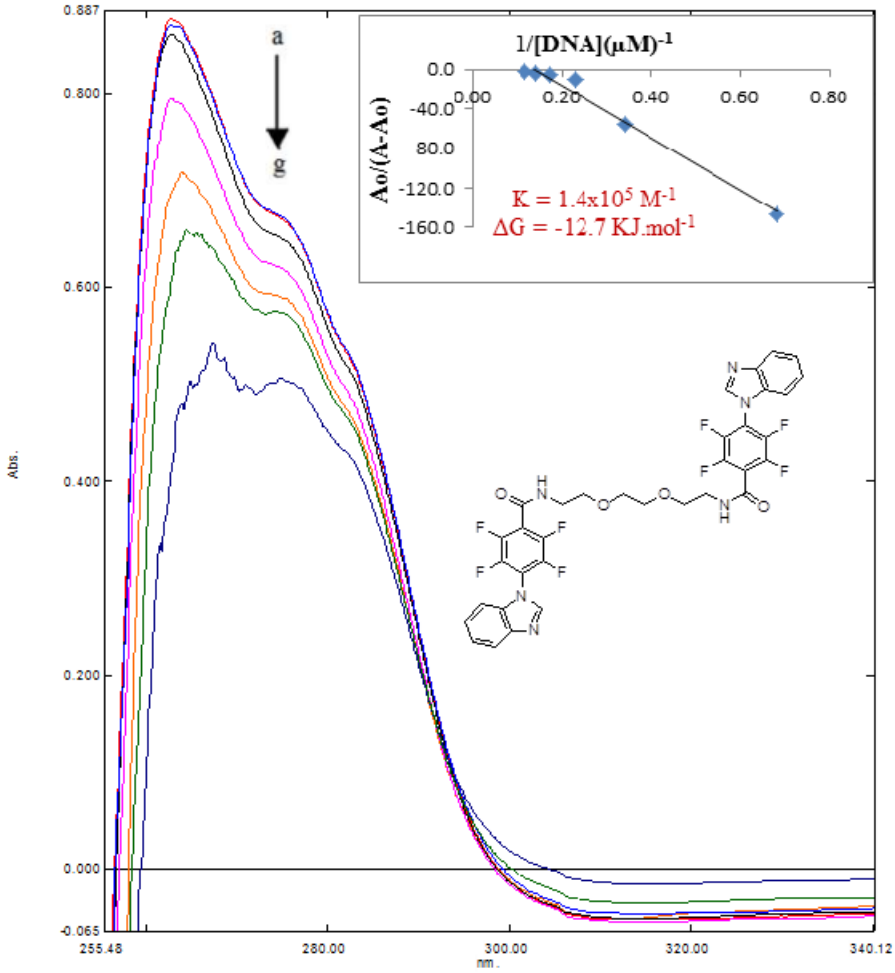
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 148. (R^2 =0.9785 for six points)

6.2.9. UV-visible spectroscopy of compound 151



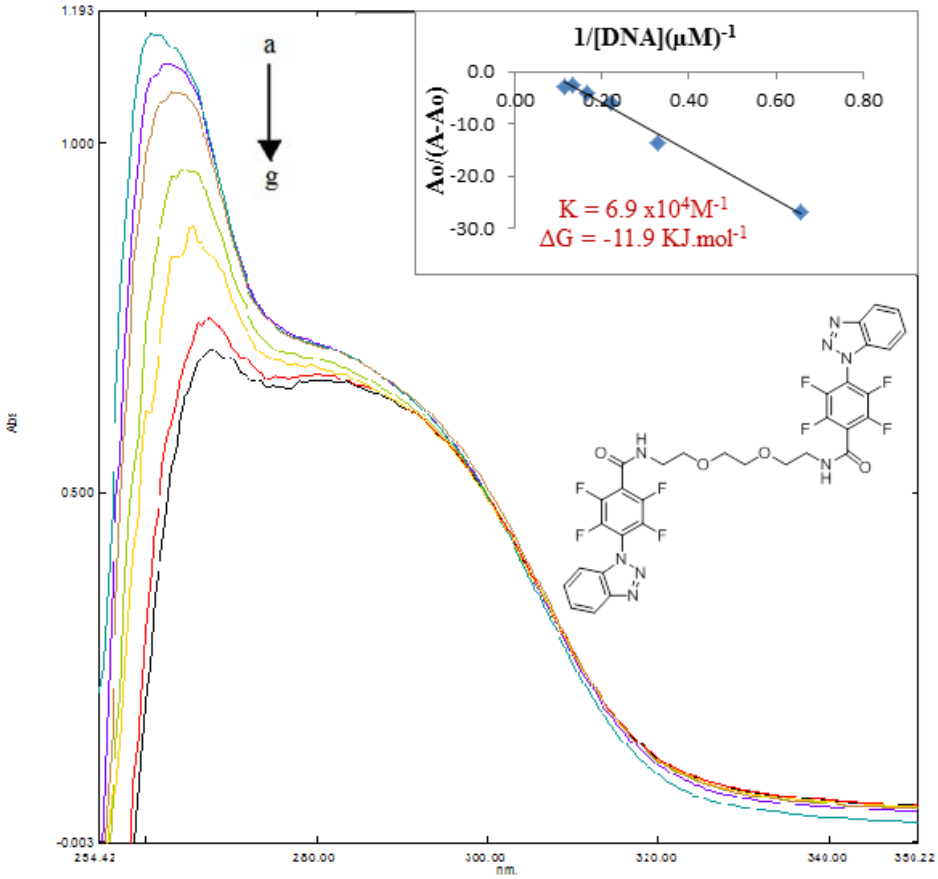
Absorption spectra of 6.5 x 10 $^{-5}$ M of compound 151. (R^2 =0.971 for six points)

6.2.10. UV-visible spectroscopy of compound 152



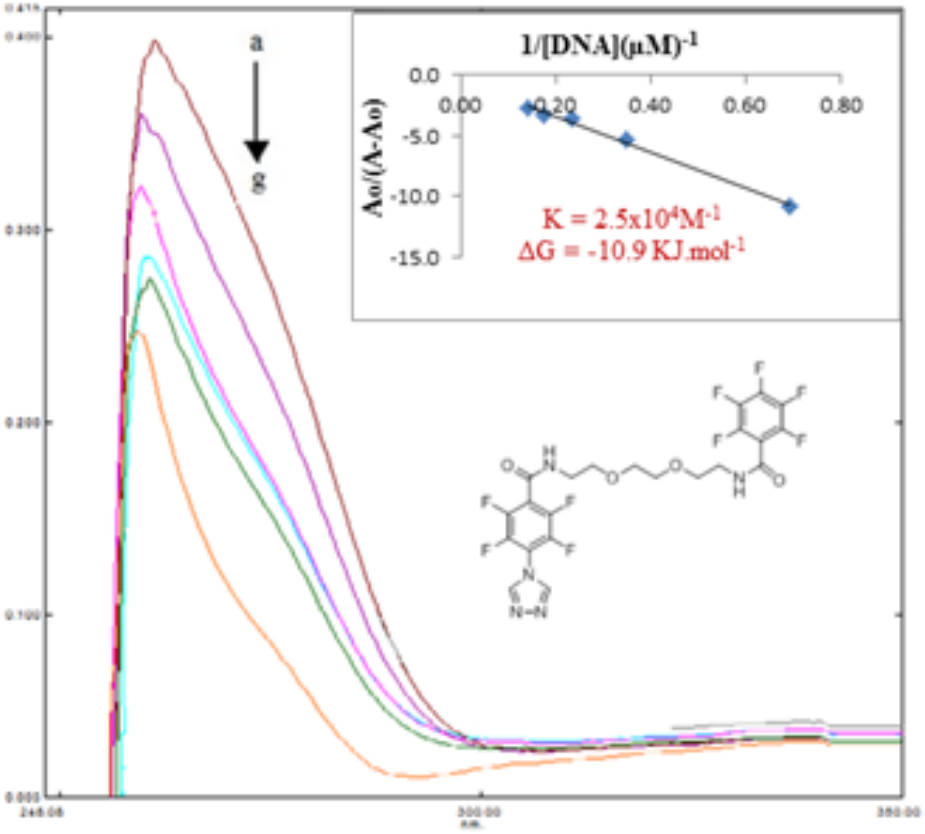
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 152 (R^2 =0.9824 for six points)

6.2.11. UV-visible spectroscopy of compound 161



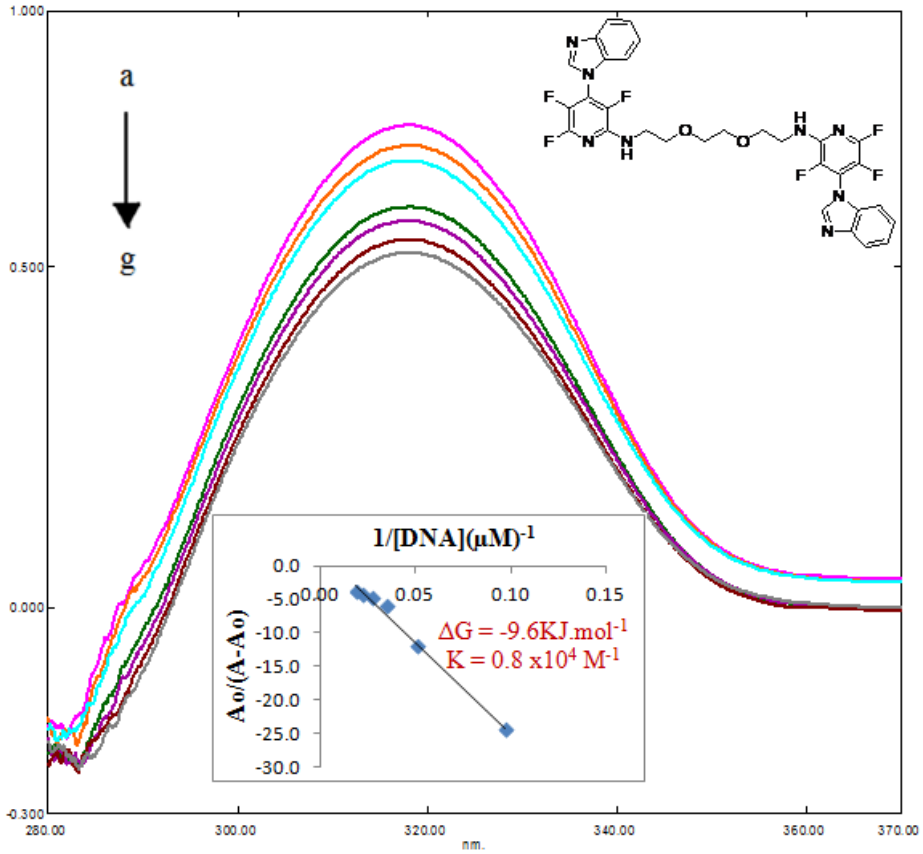
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 161. (R^2 =0.9886 for six points)

6.2.12. UV-visible spectroscopy of compound 162



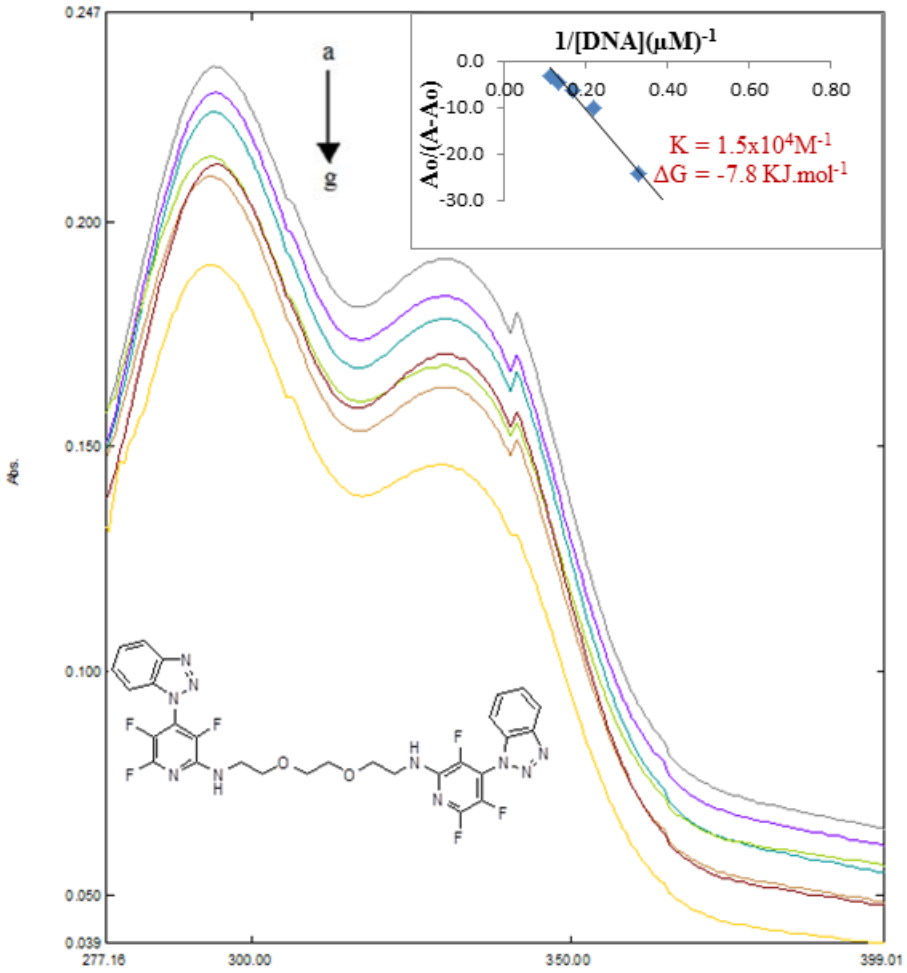
Absorption spectra of 9 x 10 $^{-5}$ M of compound 162 (R^2 =0.9951 for six points)

6.2.13. UV-visible spectroscopy of compound 163



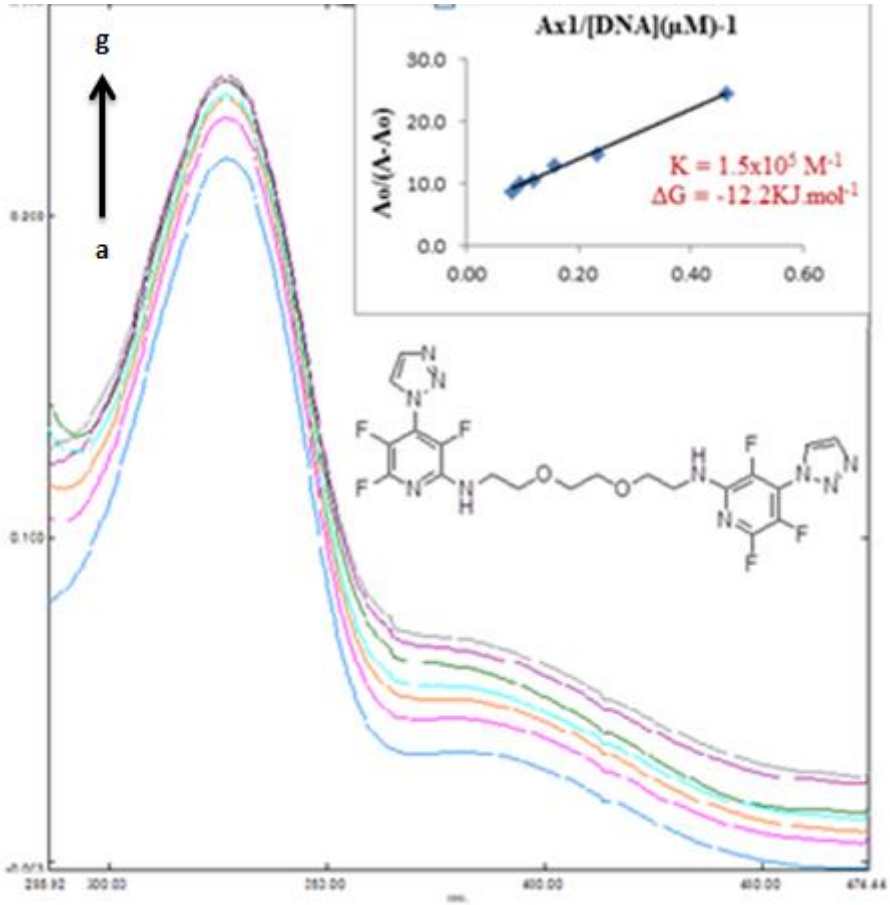
Absorption spectra of 1 x 10^{-5} M of compound 163. (R^2 = 0.9909 for six points)

6.2.14. UV-visible spectroscopy of compound 164



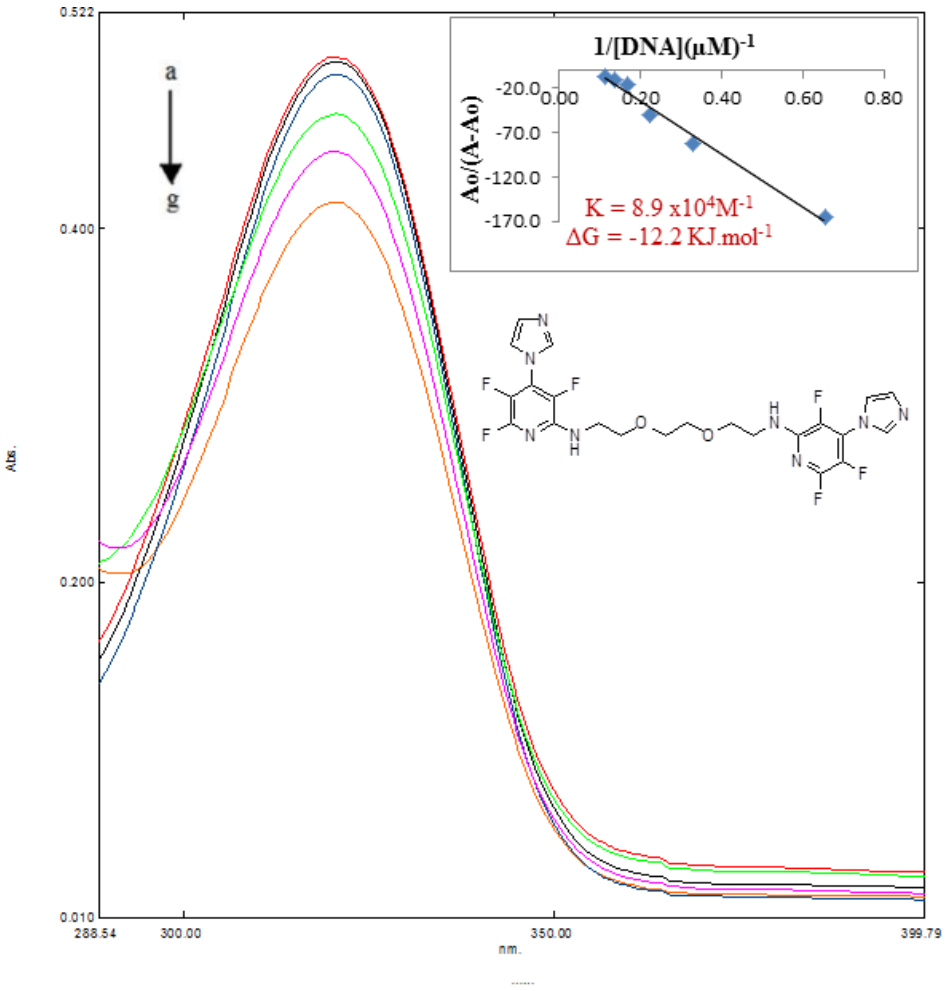
Absorption spectra of 9 x 10 $^{-5}$ M of compound 164. (R^2 =0.9949 for six points)

6.2.15. UV-visible spectroscopy of compound 165



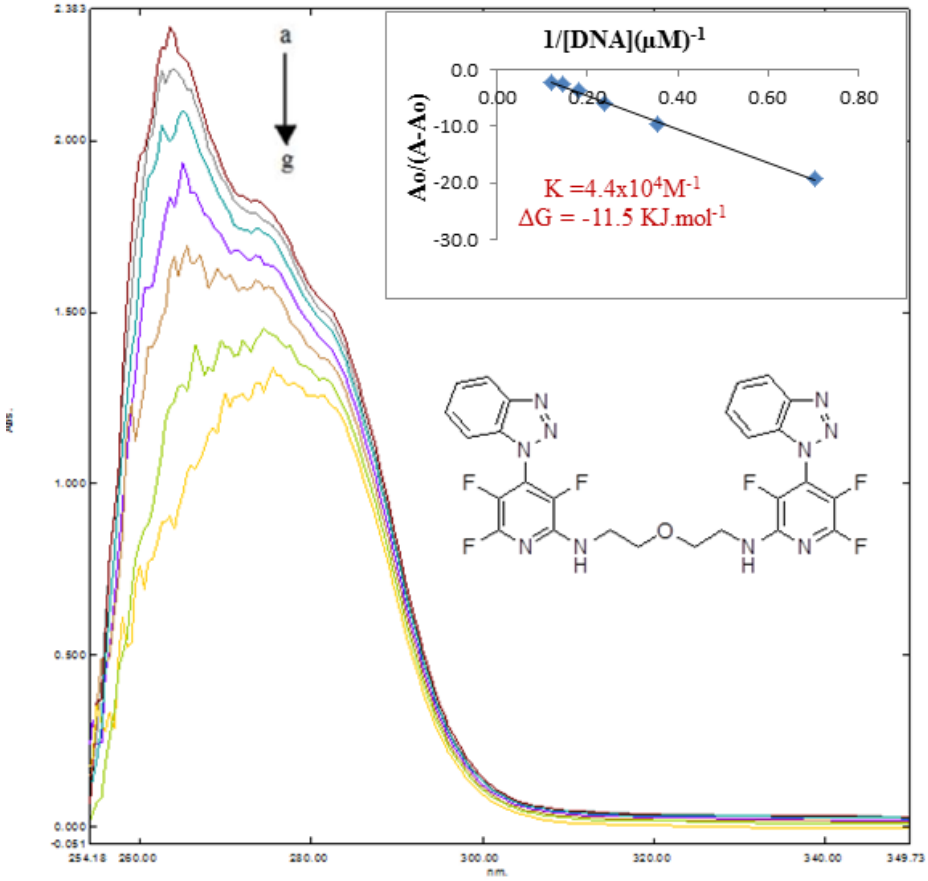
Absorption spectra of 7 x 10 $^{-5}$ M of compound 165. (R^2 =0.9933 for six points)

6.2.16. UV-visible spectroscopy of compound 166



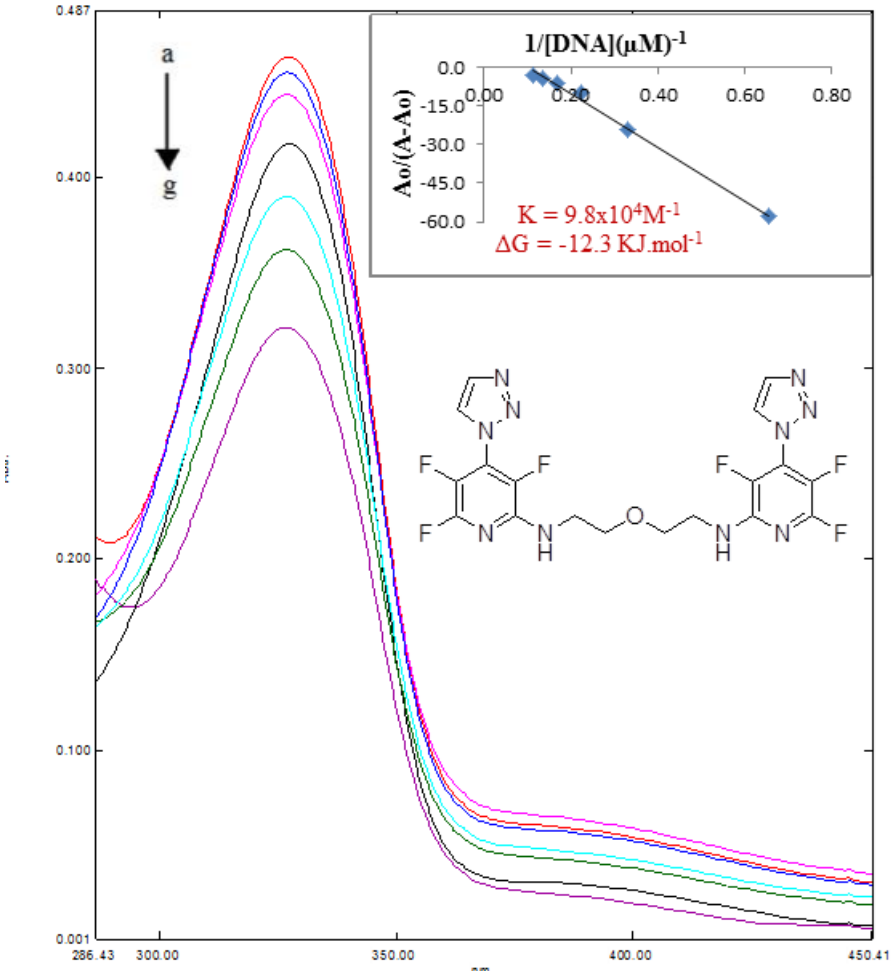
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 166. (R^2 =0.9837 for six points)

6.2.17. UV-visible spectroscopy of compound 169



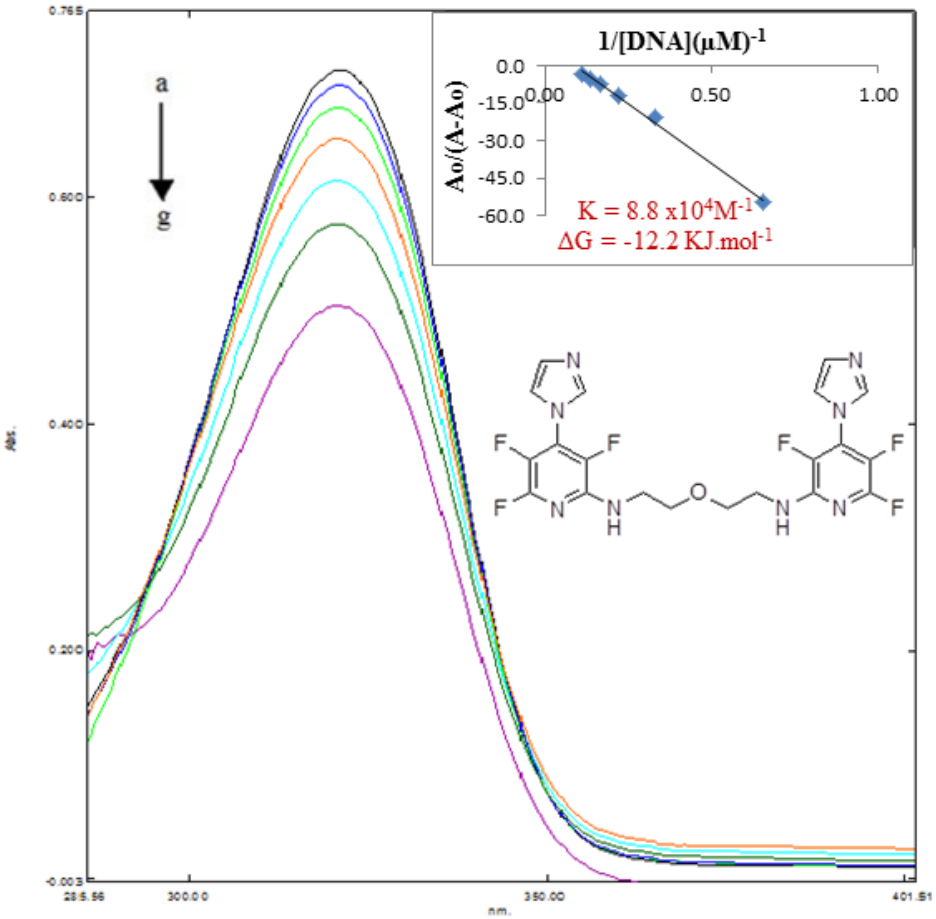
Absorption spectra of 9 x 10 $^{-5}$ M of compound 169 (R2 =0.9973 for six points)

6.2.18. UV-visible spectroscopy of compound 170



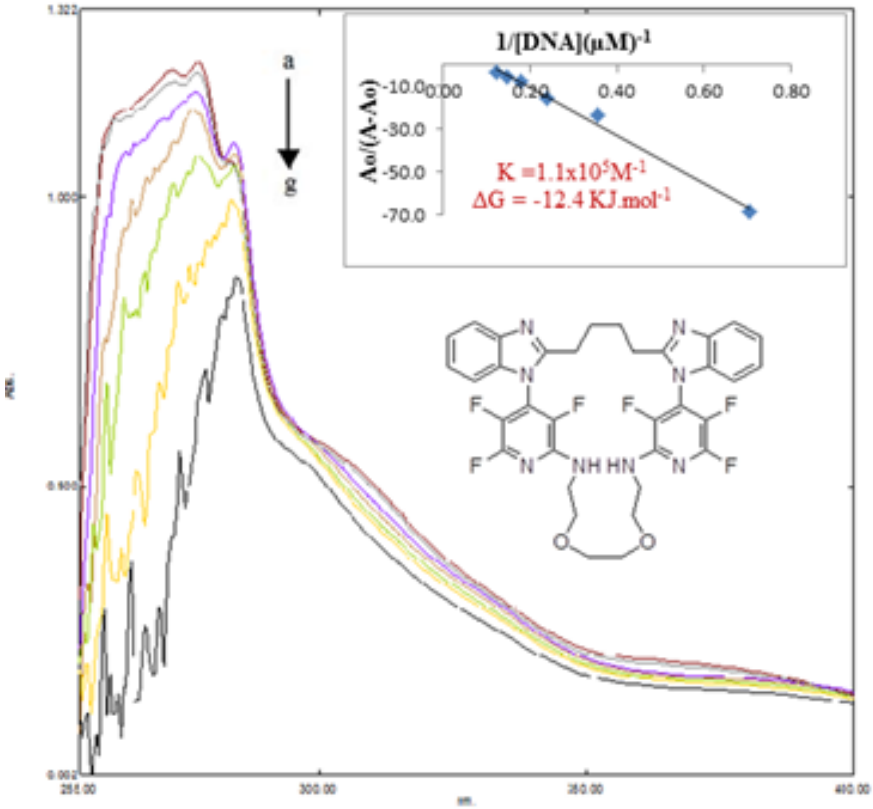
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 170 (R^2 =0.9949 for six points)

6.2.19. UV-visible spectroscopy of compound 171



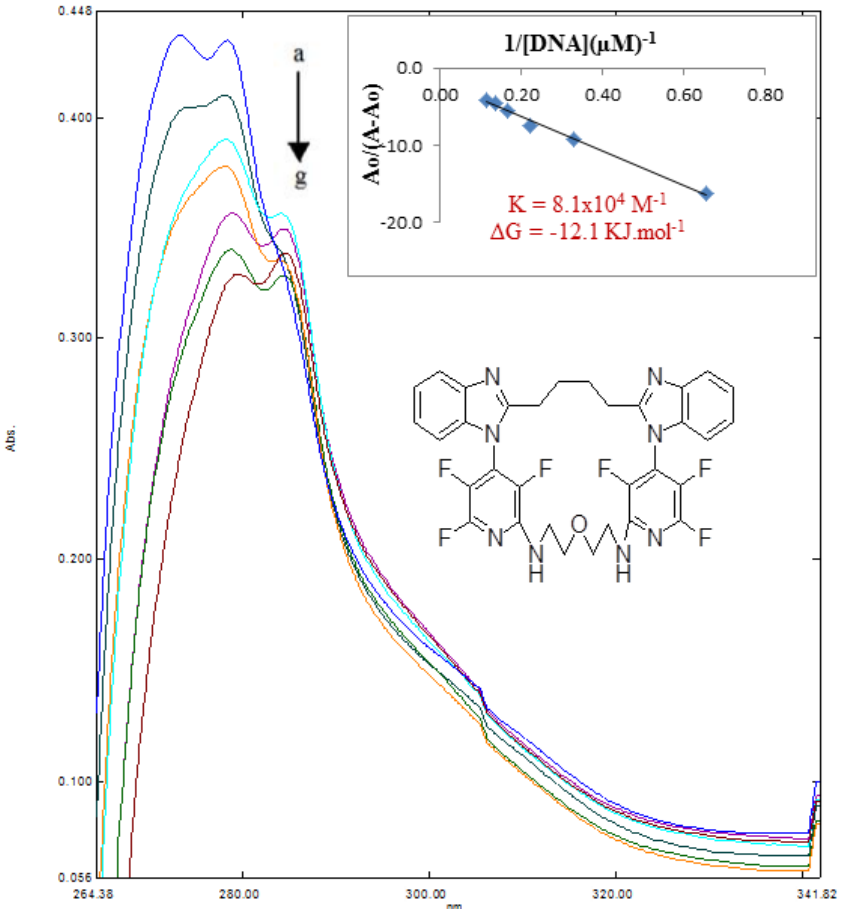
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 171. (R^2 =0.9961 for six points)

6.2.20. UV-visible spectroscopy of compound 181



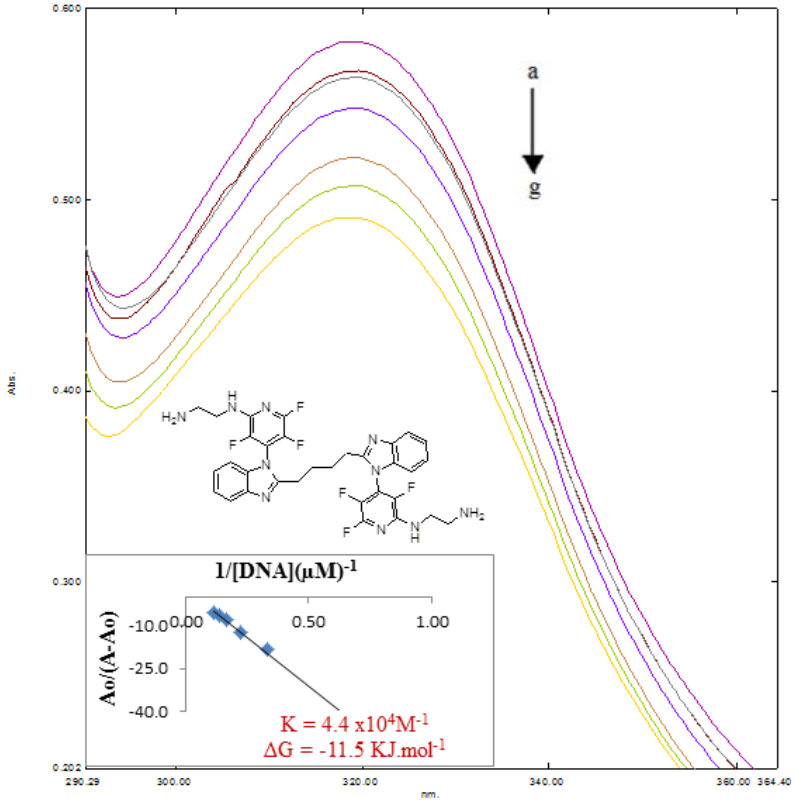
Absorption spectra of 9 x 10 $^{-5}$ M of compound 181 (R2 =0.9923 for six points)

6.2.21. UV-visible spectroscopy of compound 183



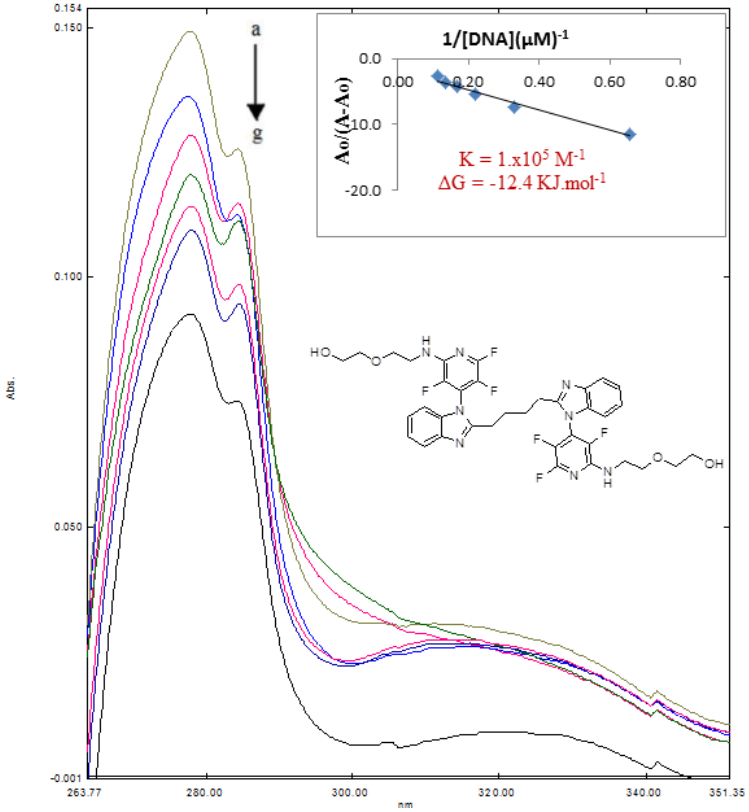
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 183. (R^2 =0.9934 for six points)

6.2.22. UV-visible spectroscopy of compound 187



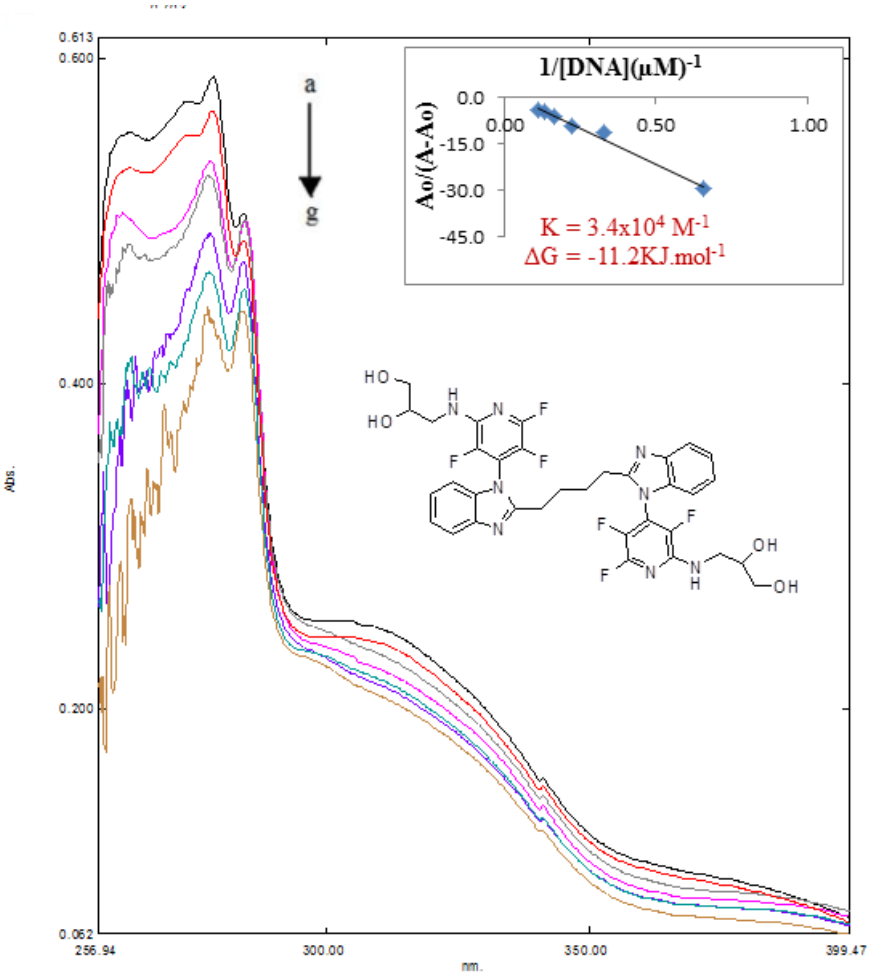
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 187 in (R^2 =0.9837 for six points)

6.2.23. UV-visible spectroscopy of compound 189



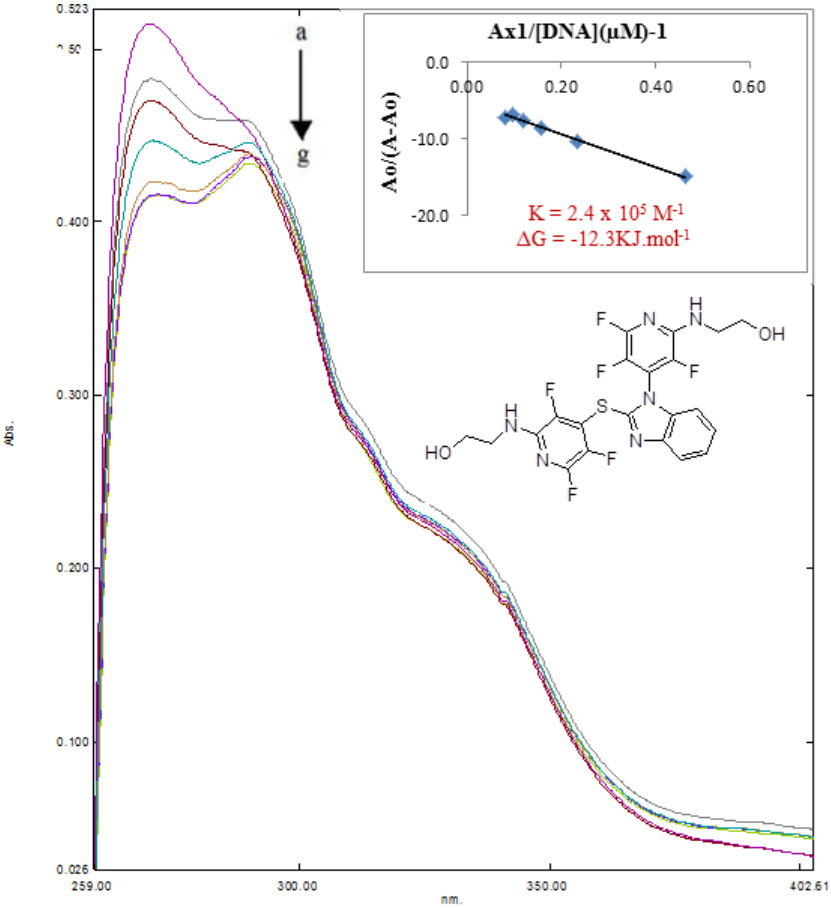
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 189. (R^2 =0.9769 for six points)

6.2.24. UV-visible spectroscopy of compound 191



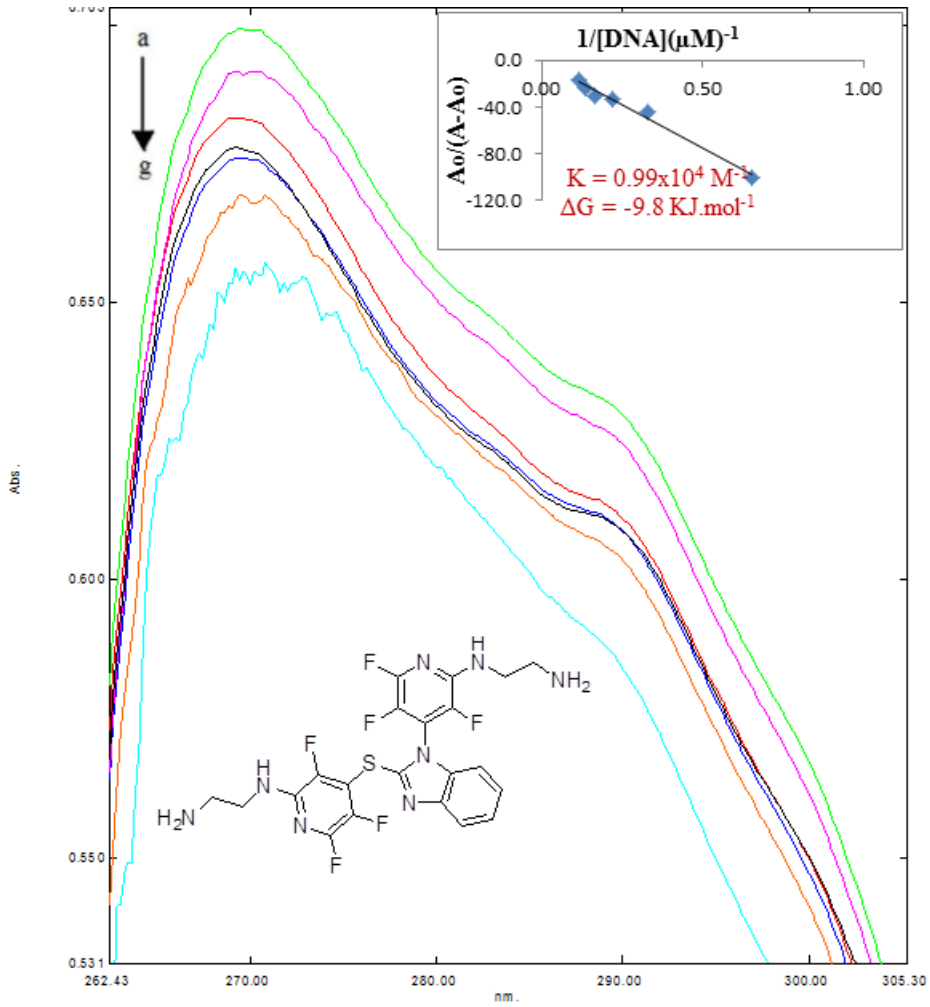
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 191. (R^2 =0.9866 for six points)

6.2.25. UV-visible spectroscopy of compound 195



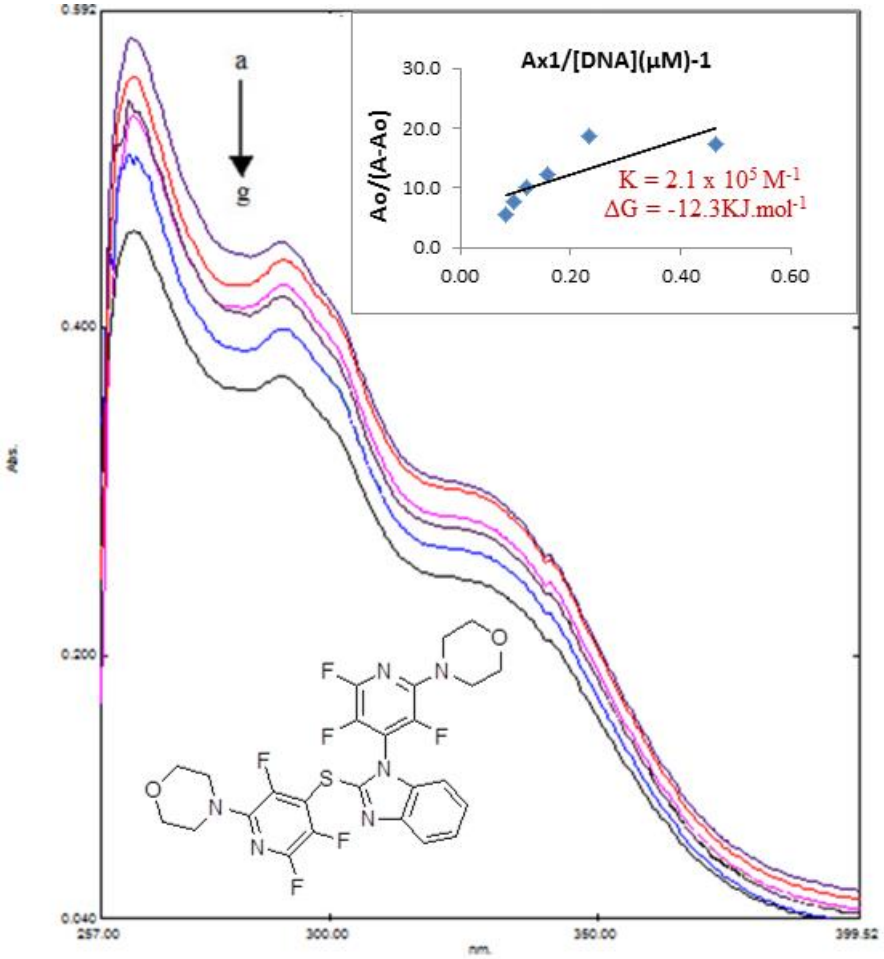
Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 195. (R^2 =0.9689 for six points)

6.2.26. UV-visible spectroscopy of compound 196



Absorption spectra of 3.8 x 10 $^{-5}$ M of compound 196. (R2 =0.9848 for six points)

6.2.27. UV-visible spectroscopy of compound 198

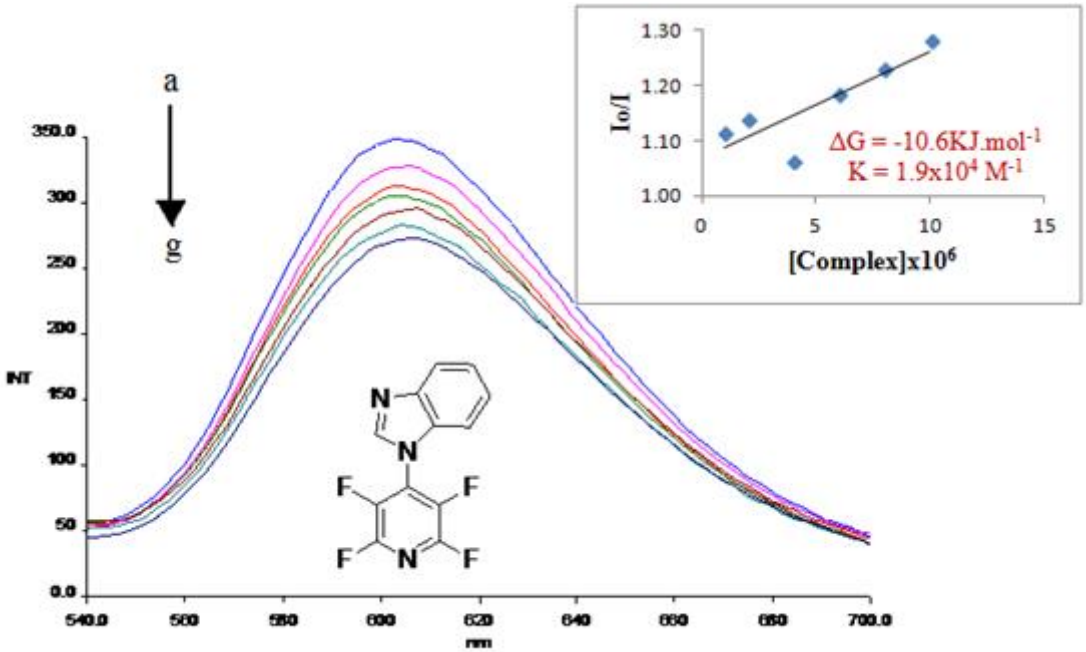


Absorption spectra of 9 x 10 $^{-5}$ M of compound 198. (R^2 =0.8887 for six points)

6.3. Fluorescence spectroscopy data for the ethidium boromide displacement assay

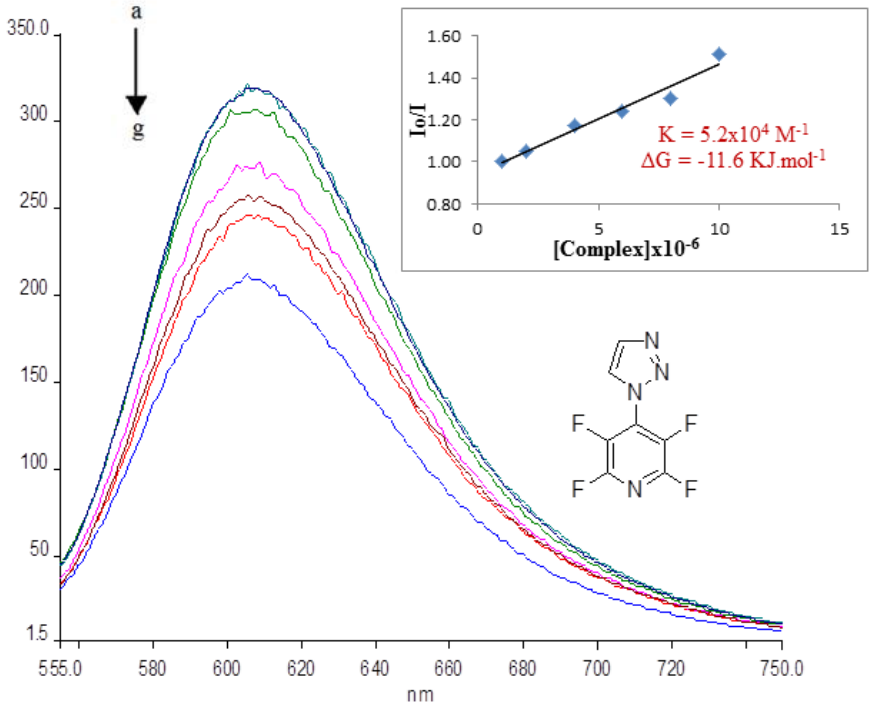
Emission spectra of SS-DNA in trisma base buffer on titration of the sucssessfuly synthesised compounds .K_{ex} = 480 nm; [EB] = 1x10^{-6} M; [DNA]=1.5 x10^{-4}M. The arrow shows the increase of the complex concentration (a) 0.0, (b) 1x10^{-6}, (c) 2 x 10^{-6}, (d) 4 x10^{-6}, (e) 6 x10^{-6}, (f) 8 x10^{-6}, (g) 1 x10^{-6} M .inside graph in plot of I_0 / I vs. complex concentration for determination of k and ΔG values.

6.3.1. Fluorescence spectra of compound 108



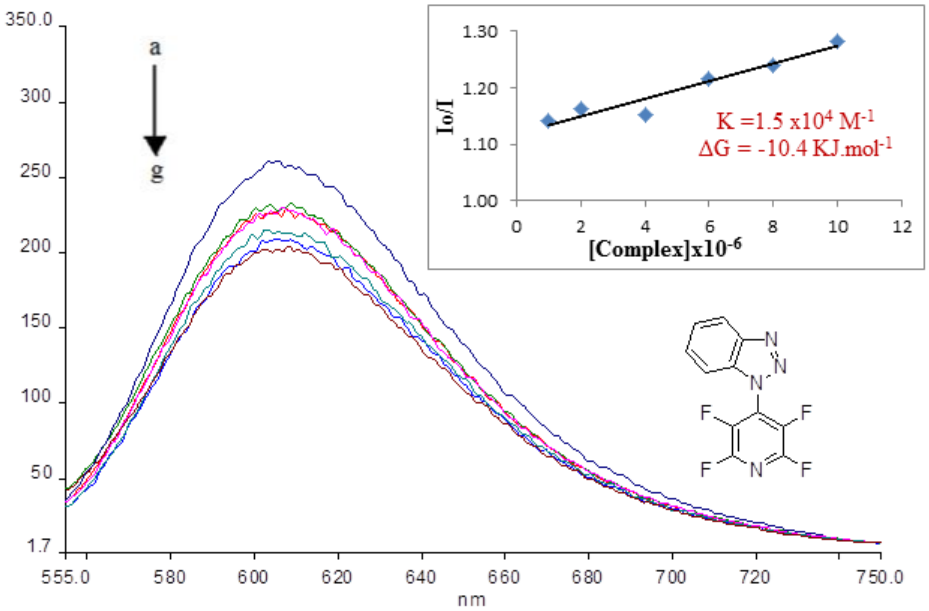
R^2 = 0.7217 for six points

6.3.2. Fluorescence spectra of compound 111



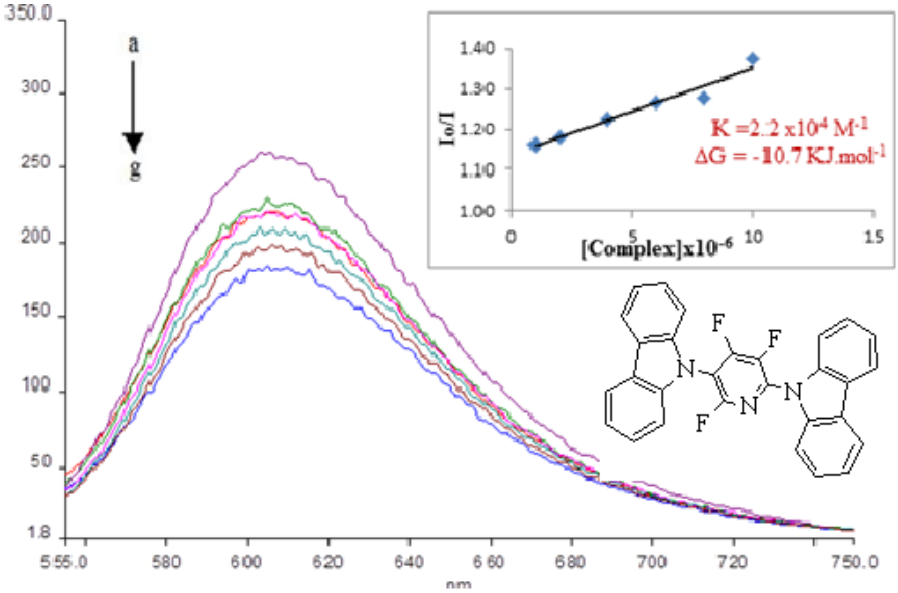
R^2 = 0.965 for six points.

6.3.3. Fluorescence spectra of compound 113



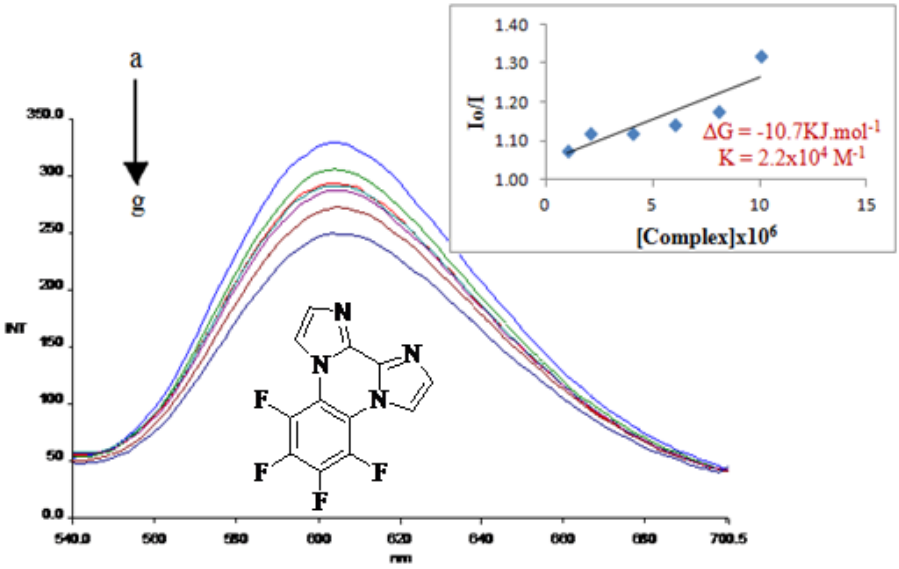
R^2 = 0.9296 for six points.

6.3.4. Fluorescence spectra of compound 114



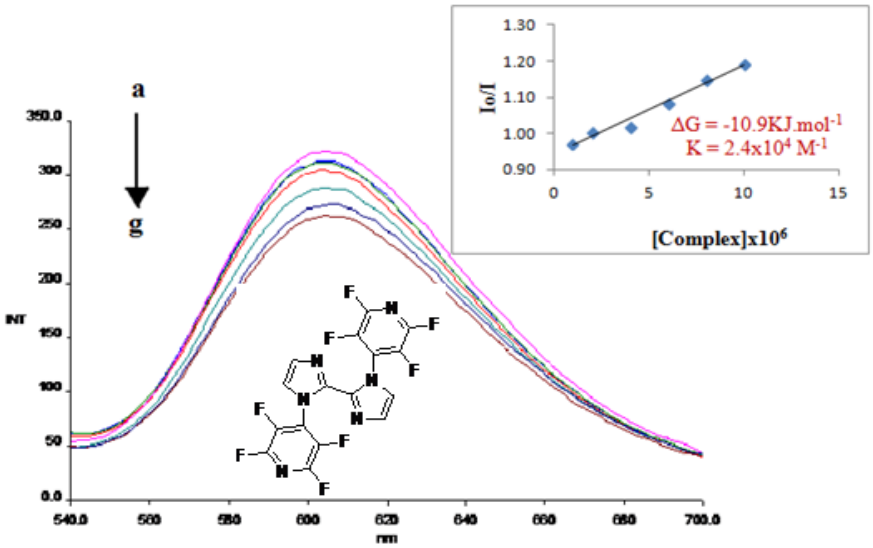
R^2 = 0.9472 for six point

6.3.5. Fluorescence spectra of compound 137



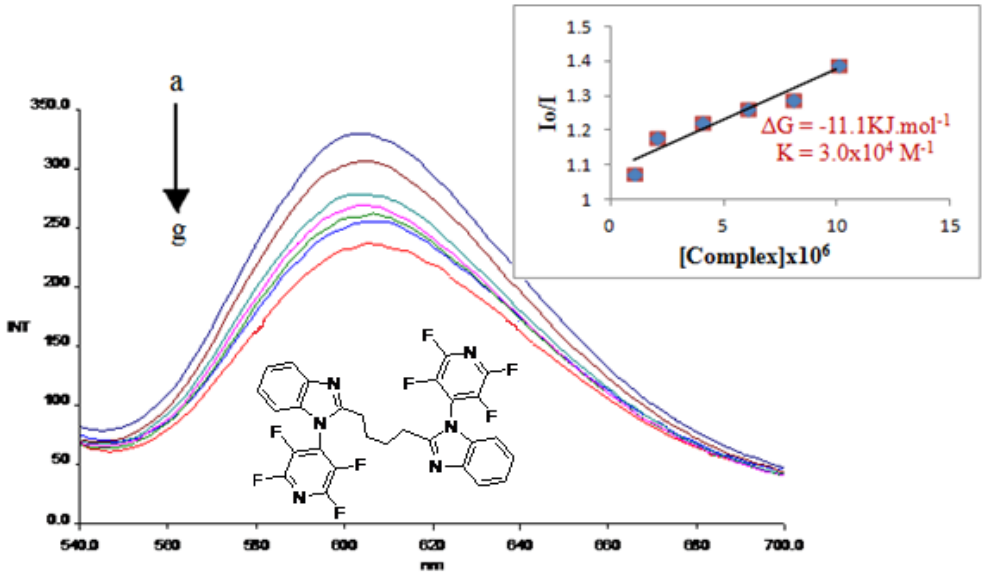
R^2 = 0.7375 for six points

6.3.6. Fluorescence spectra of compound 138



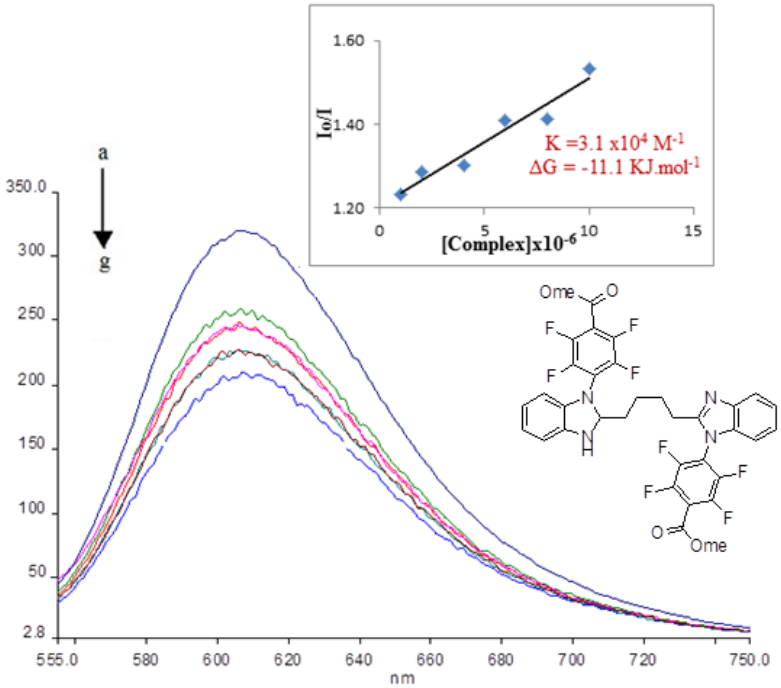
R^2 = 0.9786 for six points

6.3.7. Fluorescence spectra of compound 143



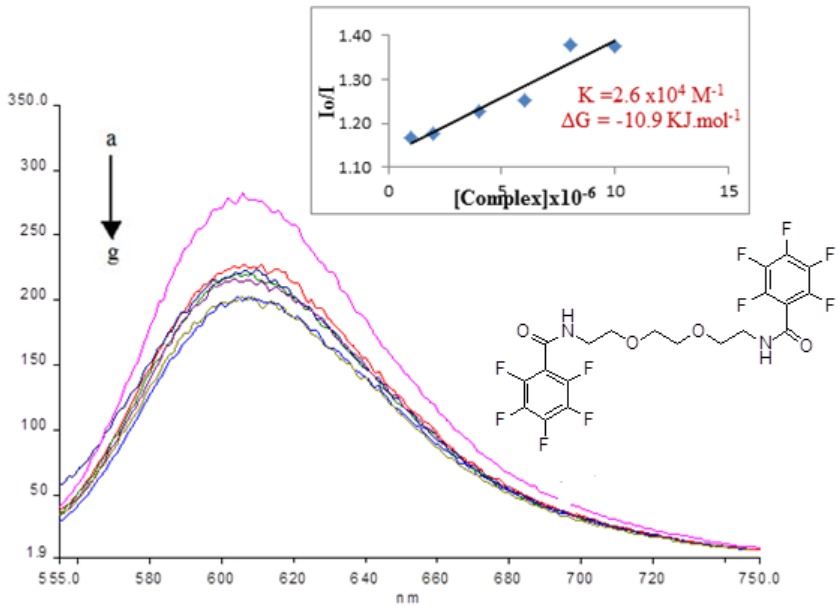
R^2 = 0.9921 for six points

6.3.8. Fluorescence spectra of compound 148



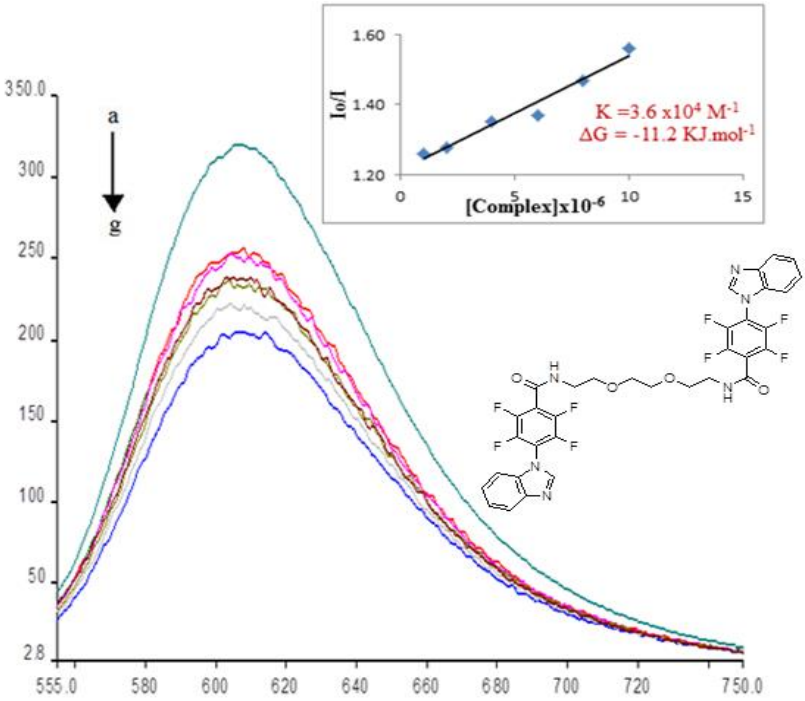
R^2 = 0.9414 for six points

6.3.9. Fluorescence spectra of compound 151



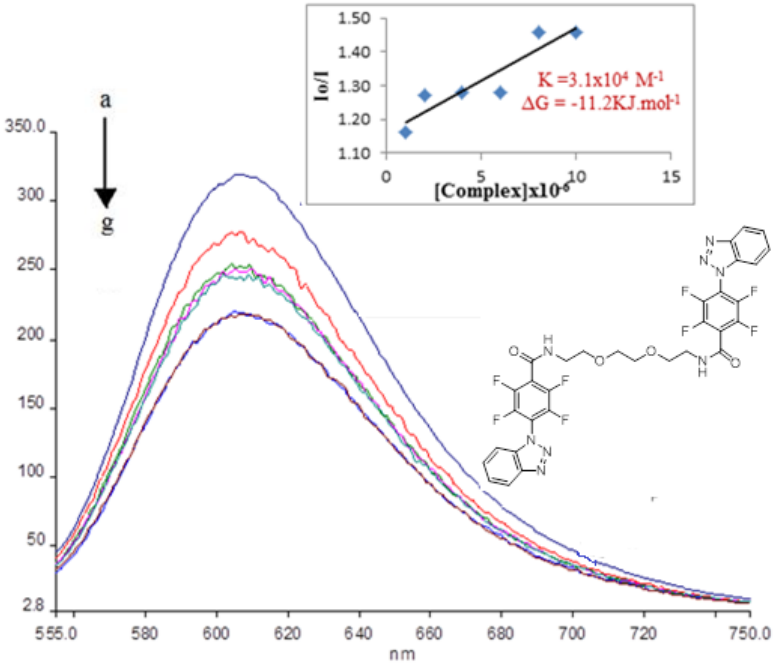
R^2 = 0.9318 for six point

6.3.10. Fluorescence spectra of compound 152



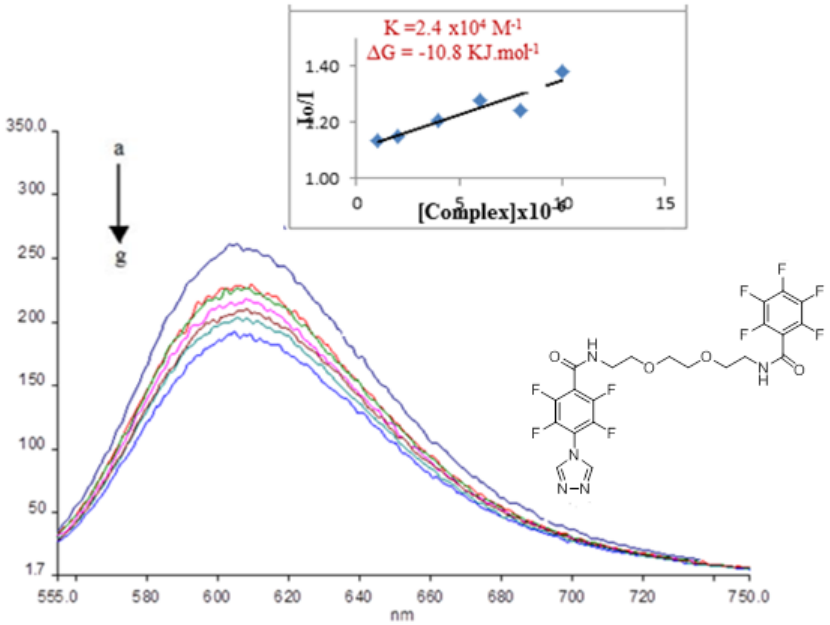
R^2 = 0.9622 for six points

6.3.11. Fluorescence spectra of compound 161



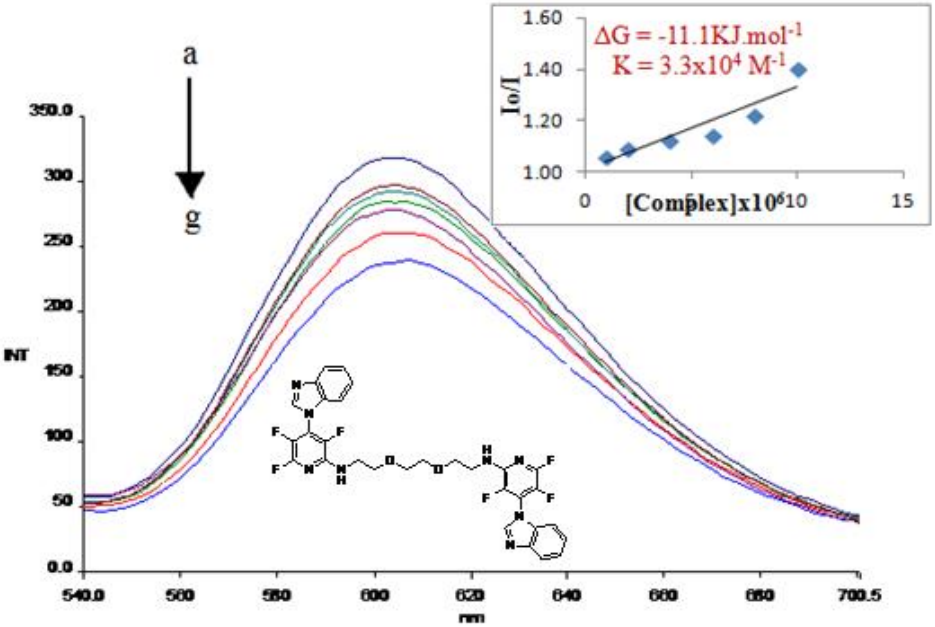
R^2 = 0.8476 for six points**.**

6.3.12. Fluorescence spectra of compound 162



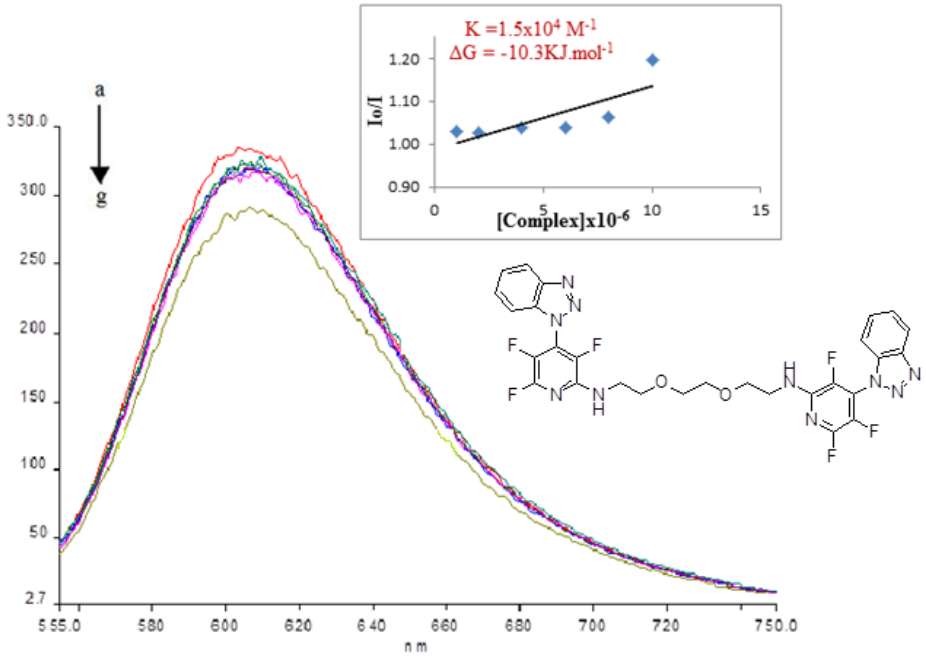
R^2 = 0.8765 for six points.

6.3.13. Fluorescence spectra of compound 163



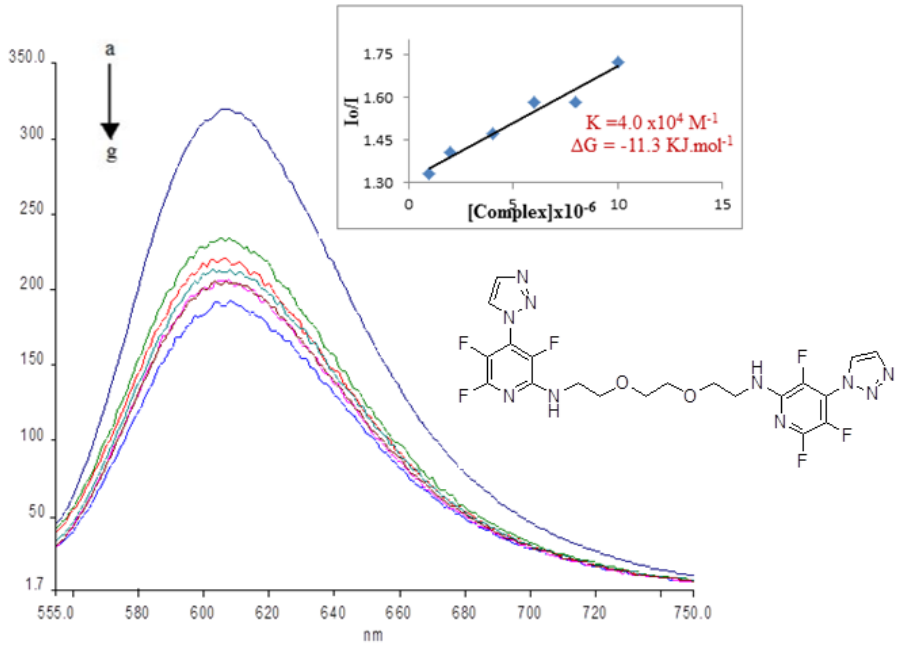
R^2 = 0.8559 for six points

6.3.14. Fluorescence spectra of compound 164



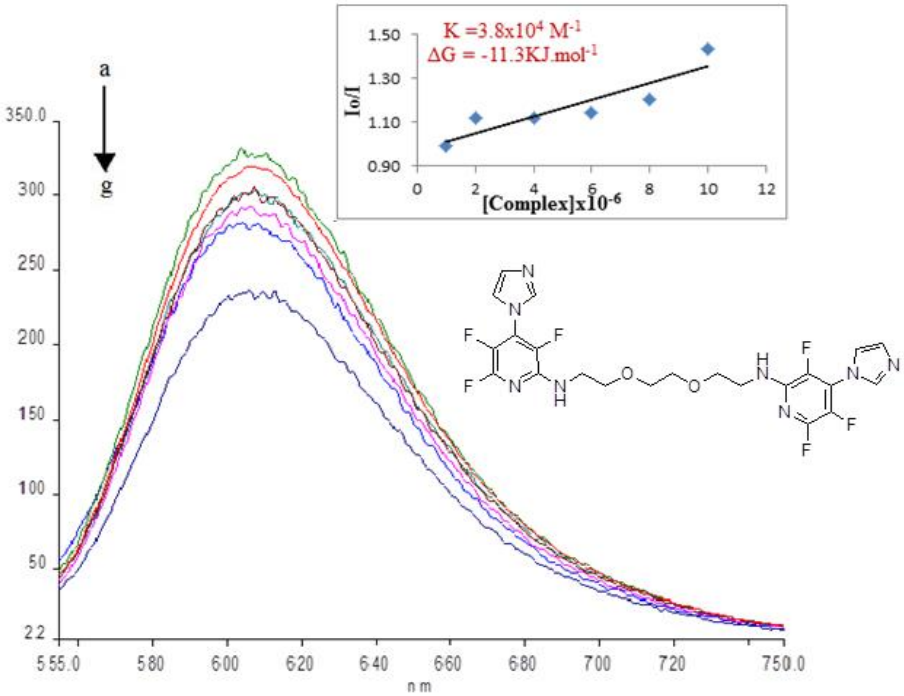
R^2 = 0.6235 for six point

6.3.15. Fluorescence spectra of compound 165



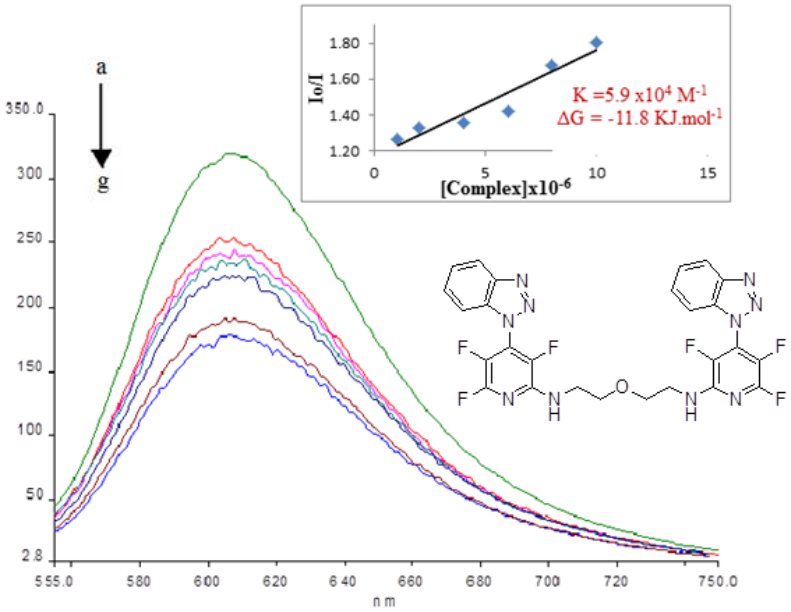
R^2 = 0.9601 for six points.

6.3.16. Fluorescence spectra of compound 166



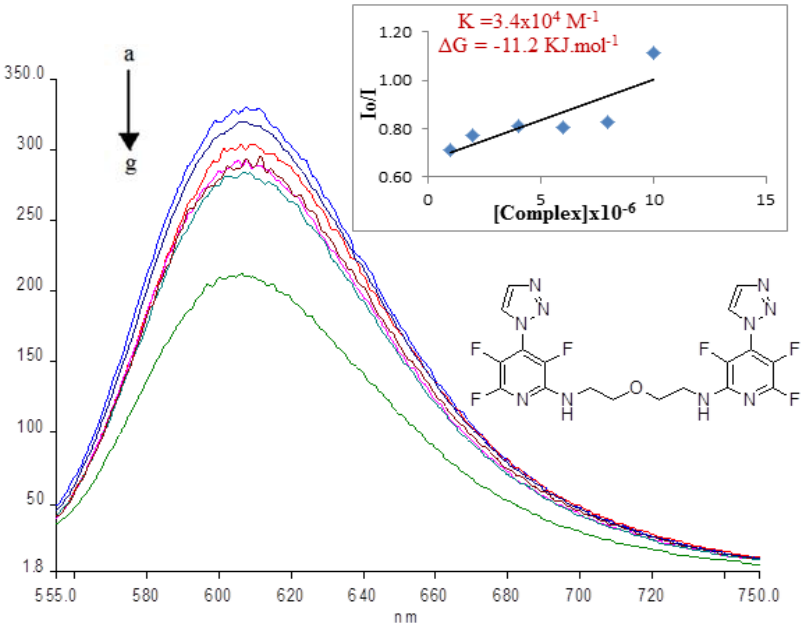
R^2 = 0.819 for six points.

6.3.17. Fluorescence spectra of compound 169



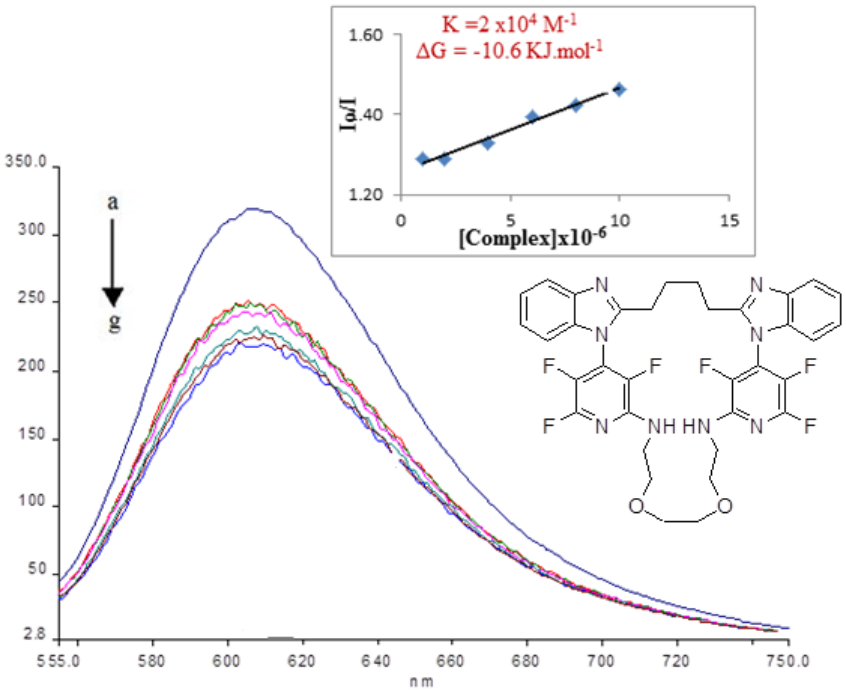
R^2 = 0.9221 for six points

6.3.18. Fluorescence spectra of compound 170



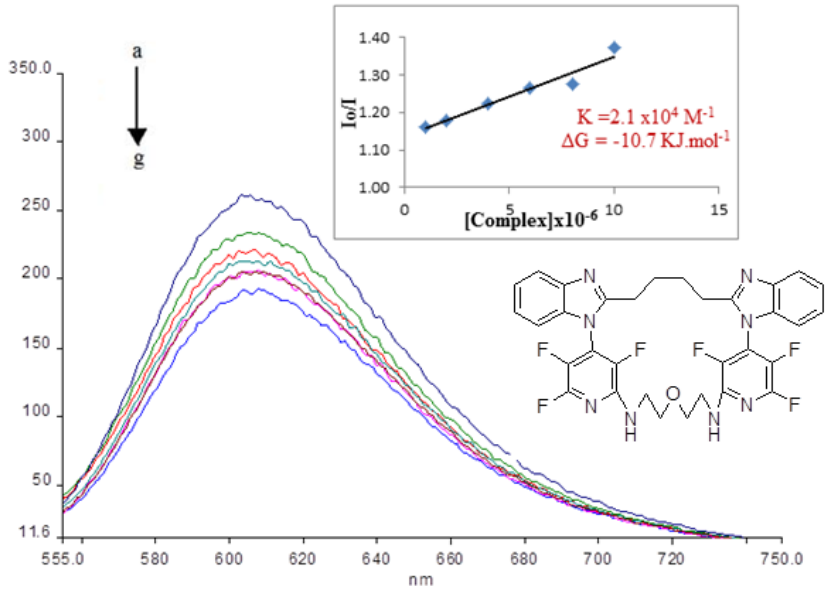
R^2 = 0.7004 for six points.

6.3.19. Fluorescence spectra of compound 181



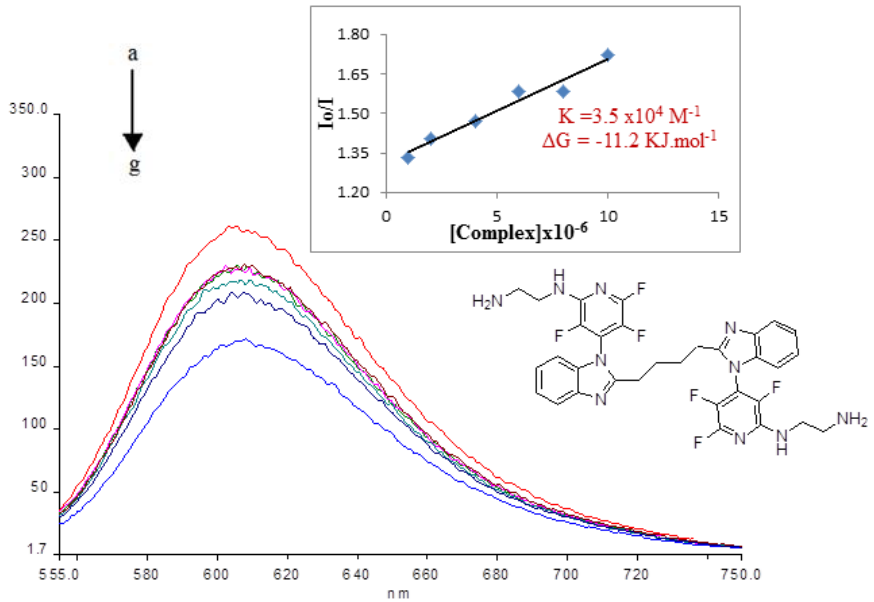
R^2 = 0.9827 for six point

6.3.20. Fluorescence spectra of compound 183



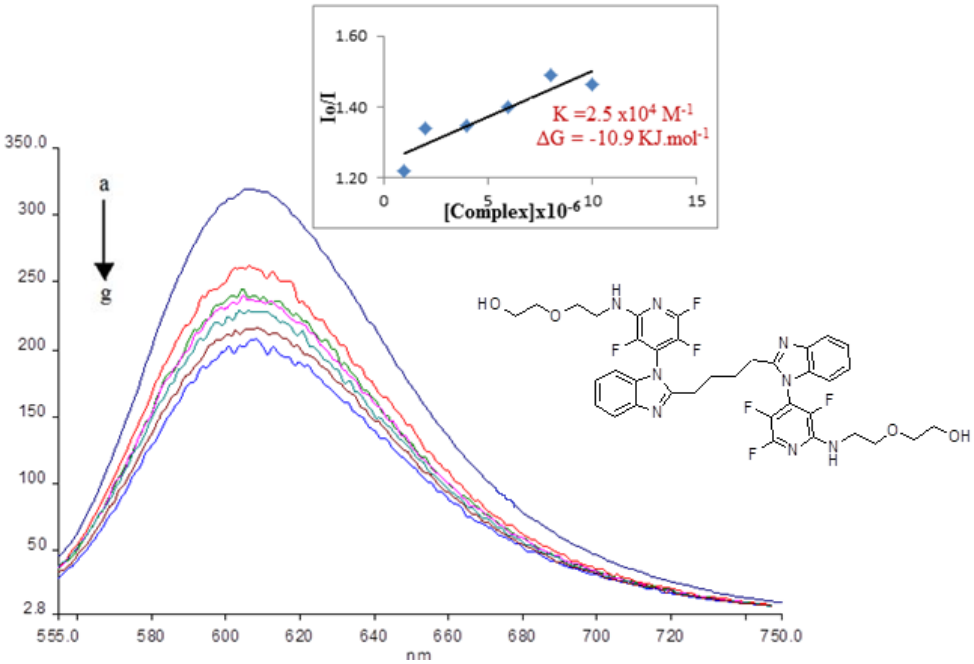
R^2 = 0.9456 for six point

6.3.21. Fluorescence spectra of compound 187



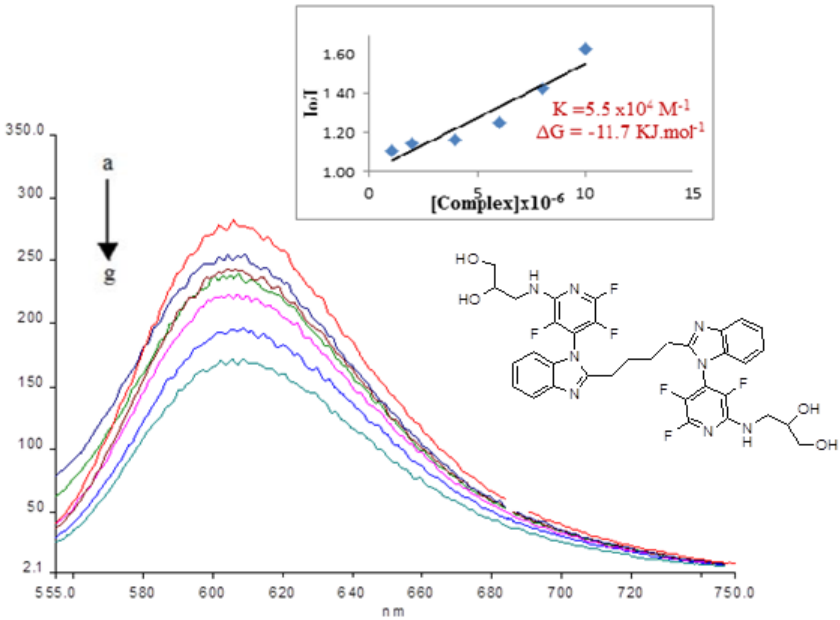
R^2 = 0.9601 for six points

6.3.22. Fluorescence spectra of compound 189



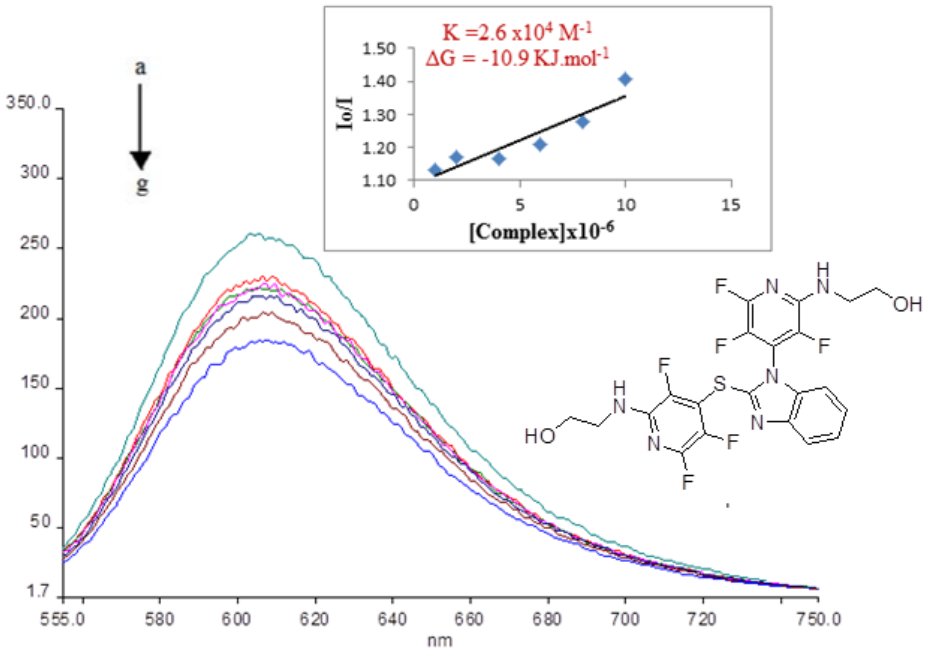
R^2 = 0.8443 for six point

6.3.23. Fluorescence spectra of compound 191



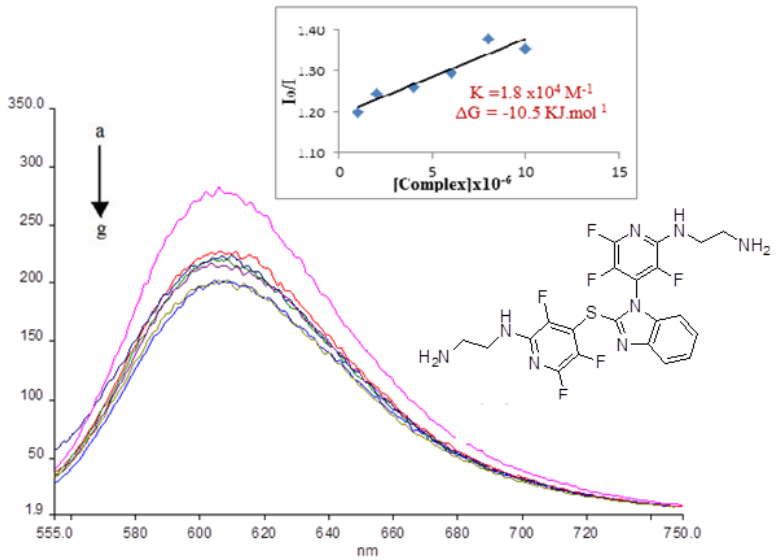
R^2 = 0.9071 for six point

6.3.24. Fluorescence spectra of compound 195



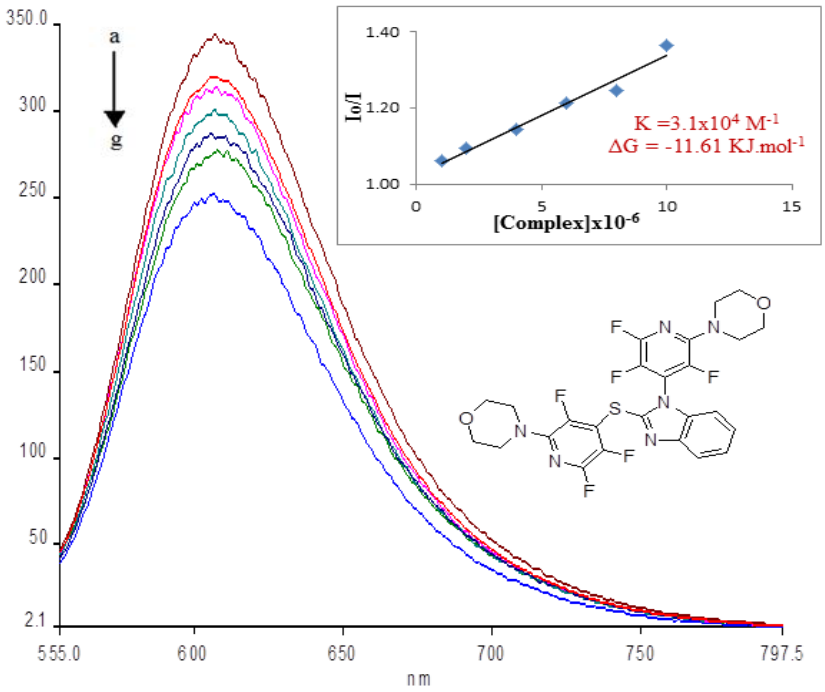
R^2 = 0.8712 for six point

6.3.25. Fluorescence spectra of compound 196



R^2 = 0.8935 for six point

6.3.26. Fluorescence spectra of compound 198



R^2 = 0.9744 for six points.