



Université catholique de Louvain

Institute of Condensed Matter and Nanosciences

Molecular Chemistry, Materials and Catalysis

Design, Synthesis and Characterization of Ferrocene
Containing Aromatic Foldamers

&

Synthesis and Study of Novel Indole Based Chalcones for
Anti-cancer Activities

Dissertation submitted for the Degree of Doctor in Sciences

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LA VIANDE FAIT GROSSIR!
JE LE SAIS. JE SUIS UN
EXPERT.



István E. MARKO

This thesis is dedicated to the people who always support me but sadly passed away during my PhD period.

Boss, Grandfather and Mother-in-law

Acknowledgements

Undertaking a PhD abroad is a great challenge. This situation is especially particular for me and it would never be possible to finish without the support and guidance from lots of people.

First and foremost, I would like to express my appreciation to and memory of my former advisor professor István E. Markó (calling him “boss” is more familiar to me) who taught and influenced me through his extensive knowledge and views both on science and life. Under his guidance I successfully overcame the initial tough time and finished my first two years of PhD study as well as my 18-month defense. I really feel grateful to have had these experiences with him.

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Thanks to all my family members (my wife, parents, grandparents, brothers, sisters and also the new coming nephew) for their support. I should express my thank especially to Xiao, the love of my life. For me, she is the meaning of our family.

At last, I would like to thank my friends both in here and China. I am sorry I can't list your names as one page is not long enough.

Authors' Contributions

The Author, as well as supervisor Prof. Istvan Marko have designed the indole chalcone project.

The Author, as well as supervisor Prof. Michael Singleton have designed the ferrocene containing aromatic oligoamides foldamer work.

The Author carried out the experiments, recorded the analytical data and interpreted the results that are presented in this thesis with following exceptions:

The mass spectroscopic experiments were recorded by Mr. Raoul Rozenberg (Université Catholique de Louvain).

Crystallographic data was collected by Dr. Koen Robeyns (Université Catholique de Louvain).

Biological evaluations were carried out by Dr. Bouazza Es Saadi (Université Catholique de Louvain) and Miss Yang Jin (Key Laboratory of Chemistry for Natural Products, Chinese Academy of Sciences)

List of abbreviations

°	Degree
°C	Celsius degree
α	Alpha
β	Beta
Δ	Delta
ΔE_p	Peak to peak separation
δ	Delta
Å	Ångström
A	Ampere
Ac	Acetyl
Bn	Benzyl
Boc	<i>tert</i> -Butyloxycarbonyl
Bu	Butyl
CDCl ₃	Chloroform- <i>d</i>
COSY	Correlation spectroscopy
Cp	Cyclopentadienyl
CV	Cyclic voltammetry
DAN	1,5-dioxynaphthalenes
DCM	Dichloromethane
DFT	Density functional theory
DIPEA	Di-iso-propylethylamine
DMAD	Dimethylacetylene dicarboxylate
DMAP	4-Dimethyl aminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
E_a	Anodic peak potential
E_c	Cathodic peak potential
ESP	Electro-static potential

Et	Ethyl
Et ₃ N	Triethylamine
Eq.	Equivalent
Fc	Ferrocene
GBM	Glioblastoma multiforme
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HRMS	High resolution mass spectrometry
hDM2	Human oncogene product double minture 2
Hz	Hertz
<i>i</i> PrOH	<i>iso</i> -Propanol
i_a	Anodic peak current
i_a/i_c	Current ratio
i_c	Cathodic peak current
IR	Infrared spectroscopy
kJ	Kilo Joule
M	Molar
MS	Mass spectrometry
MS 4 Å	Molecular sieves 4 Ångström
mPE	<i>m</i> -Phenylene ethynylene
NIS	<i>N</i> -iodosuccinimide
NMR	Nuclear magnetic resonance spectroscopy
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
Nu	Nucleophile
Pd/C	Palladium on carbon
PDI	Pyromellitic di-imides
Ph	Phenyl
Pt	Platinum

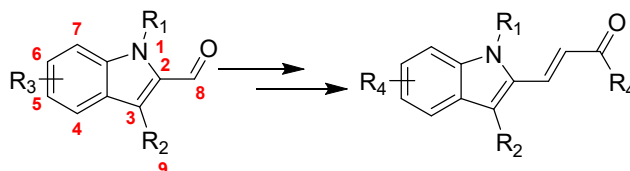
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
ROESY	Rotating frame Overhauser Effect Spectroscopy
RT	Room temperature
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TMEDA	Tetramethyl ethylenediamine
TMS	Trimethylsilyl
V	Voltage
WHO	World health organization

During my PhD research, I worked on two distinct and separate projects. The first involved the synthesis and study of novel indole based chalcones for anti-cancer activities. This project was developed and supervised initially by Prof. István Markó and then continued with Prof. Michael Singleton and Prof. Raphaël Frédérick. Upon joining the new group, I also worked on a second topic, the design, synthesis and characterization of ferrocene containing aromatic foldamers. Due to the different topics, my thesis will be divided into two independent parts as described in the abstracts below.

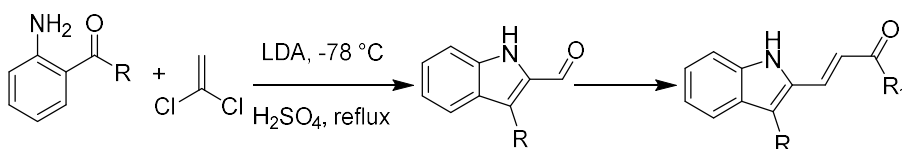
Development of new indole chalcones

Chalcones are a series of molecules containing an α,β -unsaturated ketone that connects two aromatic systems, and have been shown to have a variety of biological activities. Among these molecules, a new series of hybrid indole based chalcones have drawn great attention during the last decade for their novel biological activity such as inhibition of $A\beta_{1-42}$ aggregates and tumor cell growth. However, due to the convenience of their synthesis, biological evaluations were performed primarily on 3 or 5 position indole chalcones.

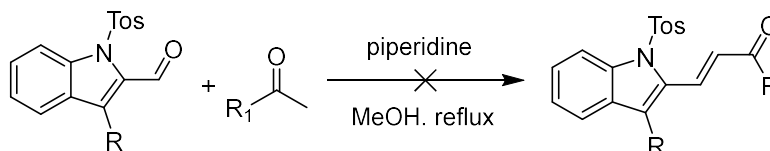
The Marko laboratory was not only interested in the development of new methodologies for the construction of complex natural products but also devoted to discovery of small molecules with novel biological applications. In the first part of this thesis, the work is focused on the construction of indole based chalcone scaffolds through a facile methodology and the verification of their biological activities.



Our group has developed a novel methodology which allowed facile construction of indole-2-aldehydes through 2-aminobenzylketones and dichloroethylene in two steps. To increase the yield of this transformation, a tosyl group was introduced for protecting the amine and to decrease the pK_a of the amino group. As a result, the yield of the indole compounds was increased from 20% to 83%.

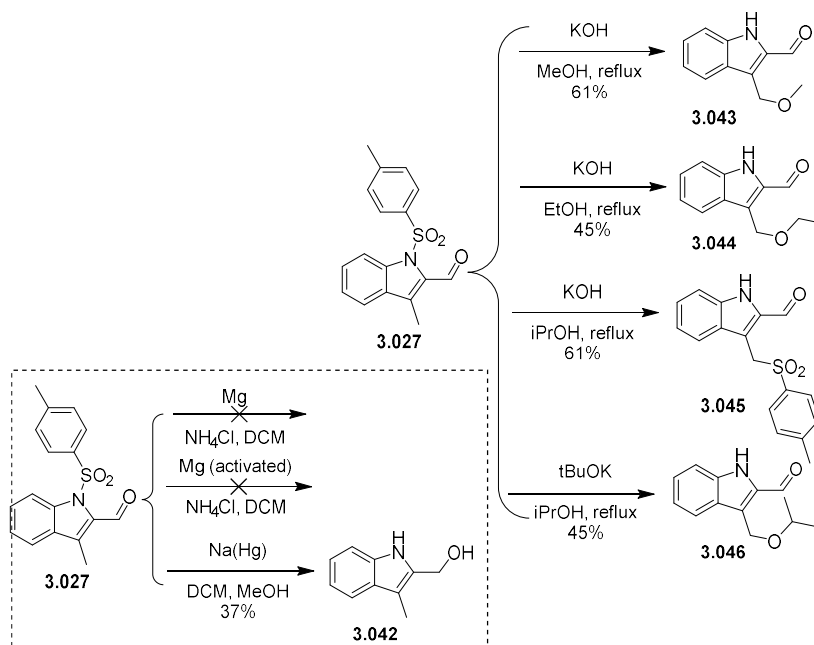


Direct condensation of unprotected indole aldehydes with ketones, except 2-acetylpyridine, worked well giving six new chalcones. However, when we attempted to use the tosyl protected aldehydes in the aldol condensation, no chalcone products were detected. Considering the success with the unprotected indole aldehydes, the tosyl group was considered to be the key cause for the failure of this reaction.

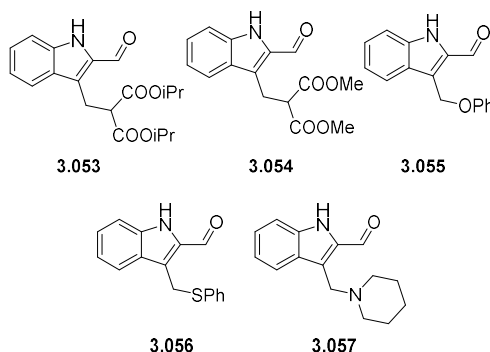


Different protecting groups were introduced and successfully used to obtain the corresponding indole aldehydes, but with low yields compared to tosyl protection. Among them, mesyl and trifluoroacetate were introduced with the idea of decreasing the size of the protecting group. However, neither mesyl nor trifluoroacetate protected indole aldehydes could react in the subsequent condensation reactions. Piv and Boc were also introduced with the idea of removing them in the acidic steps but the highest yield for the indole was 8%.

Another option is removing the tosyl after the formation of indole. Traditional methods using magnesium, sodium amalgam or bases were applied. Interestingly, basic conditions resulted in modification of the indole C9 position.



Inspired by this, a new approach for derivatizing the indole was developed, and C-O, C-C, C-S and C-N bonds were formed on indole C9 position during the deprotection reactions.

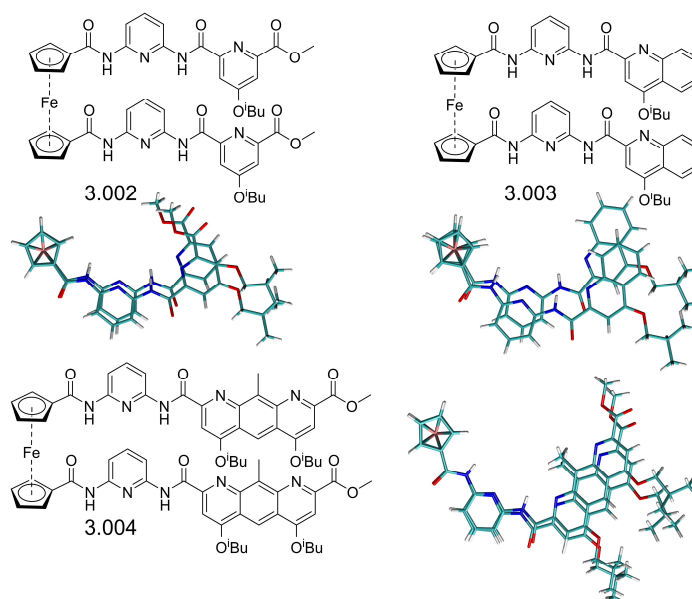


Based on these aldehydes, a series of indole based chalcones with different modification at indole C5 and C9 positions and the chalcone parts were obtained through aldol condensation. Biological evaluation of those series of chalcones on human leukemia HEL cells and breast cancer MDA-MB-231 cells indicated a phenyl group at the indole C9 position and a *p*-pyridine on the chalcone showed the best inhibition. Mechanistic studies indicated these chalcone may induce HEL cell death through apoptosis.

Abstract for ferrocene containing foldamers

α -Helix and β -sheets are the two main secondary structures found in proteins. Among them, bent β -sheets play key roles in the active sites of many proteins and are involved in molecular recognition, metabolic pathways and cell signaling. Aromatic oligoamides foldamers (AOFs) mimicking helical structures have been well established since the end of 20th century. Compared to the large amounts of research aimed at simulating helical structures, examples of AOFs mimicking sheet-like structures are rare. Most reported structures have relied on rather rigid turn-units to orient the aromatic strands in the same direction in order for them to interact. Still, the number of reported turn-units for this purpose is small. Moreover, in some cases the distance initially enforced between the strands is not ideal for good π -stacking. To this end, we were curious if ferrocene dicarboxylic acid could be a suitable turn-unit for the development of AOF β -sheet mimics. To study this, we focused on the design, synthesis and characterization of novel sheet-like aromatic oligoamides containing ferrocene as a central turn-unit and the factors that stabilize their structures.

A first generation of ferrocene AOF β -sheet mimics were developed through the combination of a pyridine functionalized ferrocene di-carboxylic acid moiety with pyridine, quinoline and diazaanthracene units. The different size of the monomers was expected to allow for stronger stacking interactions. Solid state studies illustrated that all three oligomers folded into stacked conformations with parallel dipoles. The distance between strands are 3.2 Å to 3.4 Å which suggests strong π stacking. Additionally, the curved structure resulting from the folding could be a potential molecular cleft for interacting with small molecules.



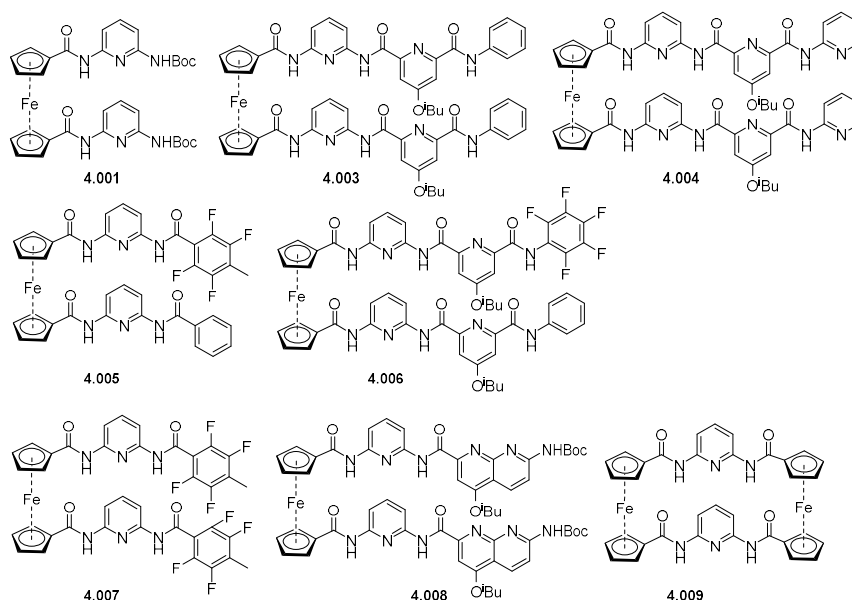
Solution studies of those oligomers, including solvent, concentration and variable temperature studies, indicated a high mobility in all three oligomers. Differences in stability resulting from the different aromatic sizes were difficult to interpret. Still, several pieces of evidence including comparison of shielding with single strands, NMR studies of asymmetric oligomers and theoretical calculations support the idea that the stacked conformation of these oligomers is also a stable state in solutions.

Electrostatic potential maps (ESP) of these oligomers indicated several potential H-bonding donor and acceptor sites in the cleft-like binding sites. To study if the ferrocene sheet structures could be functional, the host-guest properties of the oligomers were studied with small molecules including benzoic acid, catechol and resorcinol. Initial tests showed that the host-guest systems were all in fast exchange on the NMR timescale. Additionally, these tests also indicated catechol and resorcinol can be good guest molecules for **3.002** as well as benzoic acid, catechol and resorcinol for **3.003**.

Further studies of those five host-guest systems were carried out by NMR titrations. Results showed all of these pairs fitted well to 1:1 binding isotherm. Association constants were 238 M^{-1} (catechol $\subset$ **3.002**), 40 M^{-1} (catechol $\subset$ **3.003**), 108 M^{-1} (benzoic acid $\subset$ **3.003**), 80 M^{-1} (resorcinol $\subset$ **3.002**). Notably, **3.003** with resorcinol failed to fit with 1:1, 2:1, or 1:2 binding models. Different affinities illustrated a certain selectivity of host molecules to the guests.

NOESY studies on catechol $\subset$ **3.002** system showed correlations between 3-/6-position protons of the catechol with the protons of the Cp rings and with the methyl esters. Additionally, correlations are also observed between the 4-/5-position of catechol protons and the methyl esters of **3.002**. Combined with the chemical shifts observed during titrations, it strongly supports that the catechol is bound in the cleft of **3.002** in the sheet-like conformation. Computational studies of these five host-guest systems supported this type of binding as the lowest energy state. Energy differences for guests in and out of host molecules varied from 2.6 kJ/mol to 47 kJ/mol in favor of the guest molecules inside of hosts.

While the first generation highlighted the potential for ferrocene as a turn-unit in AOF β -sheet mimics, the main challenge to overcome was the mobility of the structures. With this in mind, a variety of second generation ferrocene containing AOF were designed with different ideas to further restrict mobility of oligomers and control their folded structures. Nine new ferrocene containing oligomers were synthesized.



Among these, solid-state, solution and DFT studies on the macrocyclic structure **4.009** indicated that strand flipping, rather than ferrocene rotation is likely responsible for the high-mobility. Although crystals of **4.009** gave a stacked conformation with a parallel dipole, only two sets of ferrocene signals were found in proton NMR. DFT calculation showed the energy difference of head to head and head to tail conformations was only 9 kJ/mol in favor of head to head structure. Variable temperature studies showed that the strand flipping remains fast even at low temperature. Methods to control this should be key for controlling the molecular mobility of the oligomers in solution.

One possibility for this came from oligomer **4.004**. NMR studies showed a strong dependence of the chemical shifts for the oligomer on the presence of water. Importantly, variable temperature studies showed that, in water containing deuterated DCM, the ferrocene signals split into four separate peaks at low temperature. In anhydrous deuterated DCM, no splitting of ferrocene signals was found. Thus, the presence of water has an important stabilizing effect on the structure. Solid state study of **4.009** showed a similar stacked conformation as observed for the first-generation oligomers. However, two water molecule hydrogen bonding network was found inside of the cavity of the oligomer. This network connects the two strands and likely limits the strand flipping motion. Methods to mimic this type of interaction can be an important design aspect for future ferrocene containing foldamers.

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Part A

Design, Synthesis and Characterization of Ferrocene Containing Aromatic Foldamers

Diverse of Natural Molecules and Their Mimics

1. Overview of natural molecules

The diversity of natural molecules and their specific function play an essential role in daily process. Proteins, nucleic acids and polysaccharides, the three main biopolymer backbones, sustain the ecological cycle through molecular recognition, transport, electron transfer, catalysis, etc.

This reactivity is a result of the hierarchical conformations of proteins and nucleic acids. For proteins, four distinct level of conformations (primary structure, secondary structure, tertiary structure and quaternary structure) have been defined. Primary structure refers to the sequence of amino acids in the peptide chain. Locally ordered conformations in this sequence, formed through weak interaction such as H-bonds in the backbone of peptide chains, give the secondary structural elements. α -Helices and β -sheets were predicted by Linus Pauling and Robert Corey in 1951 on the basis of H-bonding and cooperativity criteria, and are two of the most common conformations among protein secondary structure.^[1] The tertiary structure is then formed from the ordered arrangement of the secondary elements and the rest of the peptide chain. Finally, the association of independent tertiary structures gives the quaternary structure.^[2] Nucleic acids have three levels of conformations; primary structure denotes the nucleic acid sequence linked by phosphodiester bonds, secondary structure is formation of base pairs and construction of ordered structures such as single or double helical structures.^[3]

Notably for proteins, their diverse functions have led to significant studies of the different higher ordered structures. Among these, protein secondary structures (figure 1.1.1) in particular have drawn great attention due to the relative simplicity of their ordered structures allowing for relatively easy use in potential applications.^[4]

Chapter I

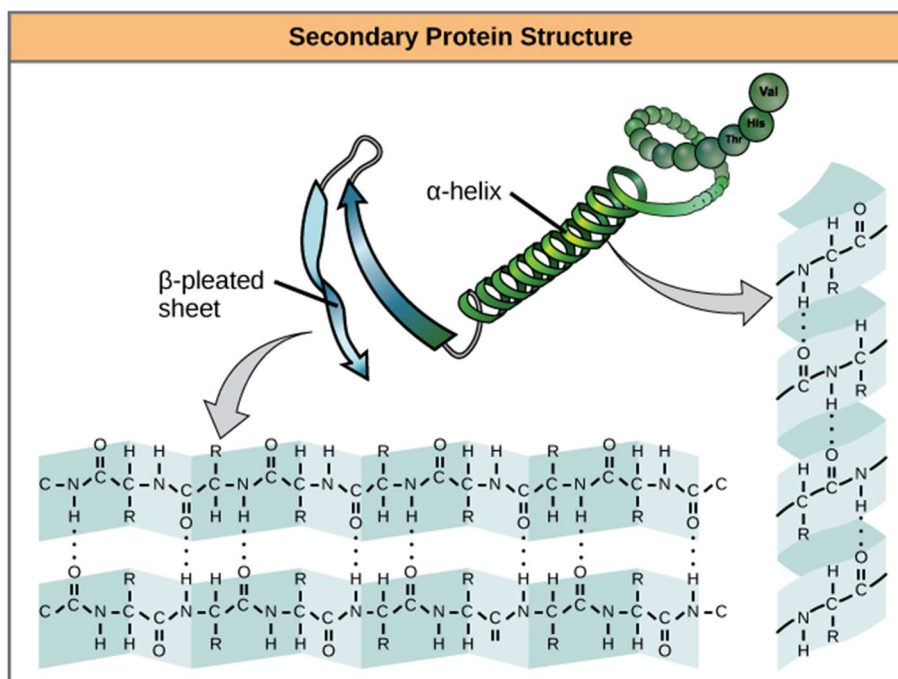


Figure 1.1.1 Structure of α -helices and β -sheets and their H-bonding forming. Image credit: OpenStax Biology

In helices, the hydrogen bonding is formed between the carbonyl (C=O) of one amino acid and amino H (N-H) of another amino acid located in the same chain. This type of H-bonding results in the formation of helical structure from polypeptide chain. Depending on the specific residues involved, different helices can be defined. For example, hydrogen bonding in α -helices occurs between the N-H group with C=O group located next three or four residues along the protein backbone. In β -sheets, two or more segments of polypeptide chains form a sheet-like structure by lining up next to each other. H-bonds are formed from the carbonyl (C=O) from one chain to amino H (N-H) of the other chain. Additionally, β -sheets can be described as parallel if the strands are arrayed in same direction (matching up of N- and C- termini). In the opposite sense, it is described as anti-parallel. [2b, 5]

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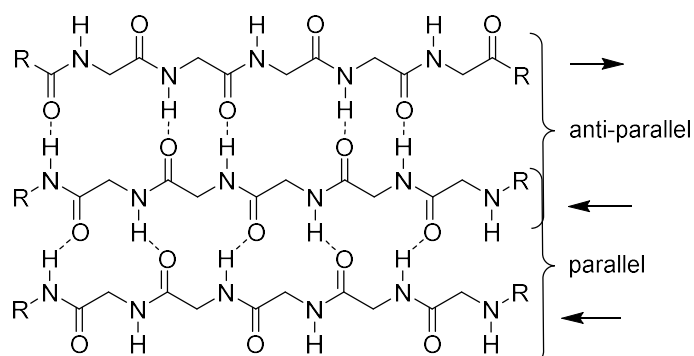


Figure 1.1.2 Schematic representation of parallel and antiparallel β -sheets.

In nature, parallel β -sheets structures are less common than anti-parallel structures although parallel β -sheets distribute better H-bonds when peptide chains are less extended. Based on a study of 144 proteins, twice as many anti-parallel β -strand turns were observed relative to parallel conformations.^[6] This may be due to the complexity of parallel conformations. Parallel β -sheets are generally composed of five or more strands and usually buried within the hydrophobic interior of proteins while anti-parallel are often smaller and can be composed of as few as 2 strands.^[7] Likely due to this difference and different requirements for interstrand H-bonding, it is also rare for mixed sheets (combination of parallel and anti-parallel segments) to occur.

Chapter I

2. Biomimicry

2.1 Biomimetic chemistry

Compared with the limited diversity of monomers that comprise the backbones of biopolymer molecules, they have a remarkably wide range of functions. The large number of different enzymatic reactions and molecular signalling pathways highlight this aspect. As such, being able to mimic these molecules could give important and powerful tools for meeting a number of complicated chemical challenges. To this end, the design and synthesis of molecules to imitate those occurring in nature, particularly enzymes, has been an area of significant interest. Ronald Breslow first used 'biomimetic chemistry' to describe those chemical processes that imitate aspects of biochemical reactions.^[8] The development of 'artificial enzymes' is one of the major topics in biomimetic chemistry, with the goal being to obtain the enormous rate accelerations and specificities of enzyme of catalysed reactions.^[8a-d, 9] Based on the knowledge of enzyme reactions at that time, early biomimetic designs typically involved attaching a catalytic site to a substrate binding site.

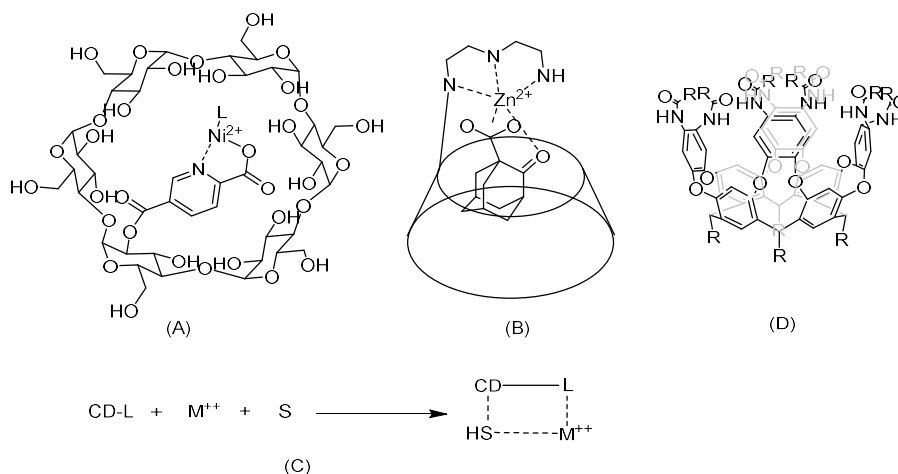


Figure 1.2.1 Examples of two artificial enzymes (A) First example of artificial enzyme designed by Ronald Breslow (B) artificial enzyme designed by Iwao Tabushi with multi-binding site. (C) scheme of bindings between guest molecule with artificial enzyme. (D) general structure of Rebek's cavitand

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As an early example, Breslow and co-workers, developed cyclodextrins functionalized with ligands that could bind a metal such as Ni (figure 1.2.1 A). A series of tests on the hydrolysis of *p*-nitrophenyl acetate (shown to be a good guest for cyclodextrin in a previous study^[10]) was performed. Taking into account different reaction parameters, the cyclodextrin connected with a nickel complex was shown to specifically accelerate the hydrolysis of *p*-nitrophenyl acetate versus the free nickel complex.^[9] Similar work has been done by Tabushi^[11] where cyclodextrin connected with a polyamine for binding to various metals was tested with different hydrophobic anions as guests. The result showed remarkable enhancement of binding of hydrophobic anions by the metal-complex hosts (especially for the zinc complex) illustrating double recognition between both the metal complex and cyclodextrin with the guest (figure 1.2.1 B & C). Rebek is another pioneer in this field and developed a variety of supramolecular catalysts which contain allosteric properties including very early examples of self-replicators or synthetic cavitands (figure 1.2.1 D).^[12] Those novel examples were some of the earliest applications of biomimetic chemistry which laid the foundation of it and also, pointed out a possible direction for overcoming chemical challenges. These series of work still have a profound influence in chemistry with numerous researches reported every year.^[13]

Beyond the increased reactivity, the above examples of biomimetic reactivity also highlight an important aspect of biomimetic chemistry. While taking inspiration from biological systems, they do not attempt to directly copy the active sites of enzymes. A similar approach has also been used with structural biomimetics of natural molecules.

Chapter I

2.2 Foldamer

Inspired by the complex structures of biomolecules, significant work in the last decades has focused on the development of synthetic oligomers capable of folding to mimic the structures and functions of biopolymers. This has given rise to a large class of molecules, foldamers.

2.2.1 Definition

The term “foldamer” was first coined by Gellman in 1996 followed by a general description “any polymer with a strong tendency to adopt a specific compact conformation” in 1998.^[14] Three years later, Moore and co-workers gave a more specific definition, stating that a foldamer was “any oligomer that folds into a conformationally ordered state in solution, the structure of which is stabilized by a collection of non-covalent interactions between nonadjacent monomer units.” He also classified two major groups of foldamers: “single-stranded foldamers that only fold and multiple-stranded foldamers that both associate and fold.”^[15]

Both Moore and Gellman’s definitions, agreed that the molecule must adopt a conformationally ordered structure. However, a different emphasis was placed on the level of order in the adopted conformation. Moore believed that foldamer should describe mimics of “secondary structures” and introduced “tyligomer” to describe more complex ordered structures, including tertiary or quaternary conformations, this notably involves polymers. Gellman, however, combined all of these under the same term.

Based on Moore’s definition, foldamers can be more clearly distinguished from other structures and should meet several requirements. A foldamer should first be a chain molecule with certain size. Second, the chain should be potentially dynamic in solution, but still adopt a specific preferred folded conformation. Last but not the least, this folded chain should be controlled by noncovalent interactions between non-adjacent monomers. Polymers as well as some rigid oligomers, based on this definition, could not be a foldamer.

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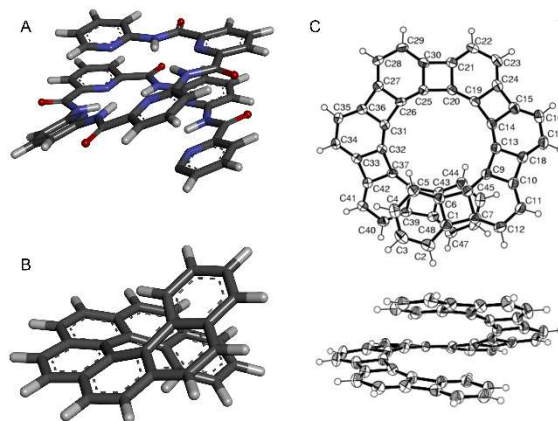


Figure 1.2.2 (A) Model of aromatic foldamer in helix conformation; (B) model of helicene; (C) crystal structure of flexible extended helicene developed by Vollhardt, copyright from Wiley 2002; (D) DFT calculation of possible mechanism for helicene and helicene analogs racemization, copyright from Royal Society of Chemistry 2011.

A clear and distinguishable definition is essential but sometimes, it will cause confusion. One example is helicenes (figure 1.2.2 B) and their derivatives. Due to the rigidity of classical helicenes, these series of molecules could adopt compact conformations in solutions, and no dynamic state of folding and unfolding can happen in solutions. Thus, it is not compatible with Moore's definition of foldamer. However, Vollhardt and co-workers uncovered a flexible case of helicenes (figure 1.2.2 C) which adopt dynamic state of racemization and preference of folded state in toluene which could be considered as foldamers.^[16] This series of molecules are at the edge of "foldamer" based on Moore's definition which led to difficulties of distinguish exactly what constitutes a foldamer.

More recently the definition has been greatly expanded to "artificial folded molecular architectures", which includes synthetic (non-natural) α -peptide sequences, artificial proteins, nucleic acids and helical polymers, some of which were studied extensively long before the term foldamer was coined.^[17] Thus, a wide range of polymers and oligomers including helicenes can be

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considered as foldamers.

2.2.2 Foldamer design

The concept of foldamer design is directly linked to the building blocks used for their synthesis and the specific interactions they contain that force them into well-defined conformers in solution. The driving forces to accomplish this are myriad and sophisticated. Generally, those could be attributed to both internal and external factors. Internal factors are predominantly based on the nature of the molecules (i.e. molecule's rigidity, steric hindrance of groups, and non-covalent interactions such as dipole-dipole interactions, intramolecular H-bonding and aromatic stackings). External factors, in general, include solvophobic effects and inter-molecular non-covalent interactions. In most cases, both features are involved in the stability of folded structures.

Helicene as well as other rigid molecules with folded conformations (molecular ribbons, molecular rods, i.e.) could be divided into internal factor dominated foldamers, named "rigid locked molecules" in the account of Hecht and Huc, since the rigidity of those molecules, in most cases, only allow compact conformations in solution.^[17-18]

Other than those extreme examples, most foldamer molecules contain more flexible backbones. These architectures are more controlled of H-bond, π -stacking, local steric hindrance or other interactions which restrict rotation of bonds in the structure. Examples of building blocks providing these interactions and the foldamers that make use of them are given below.

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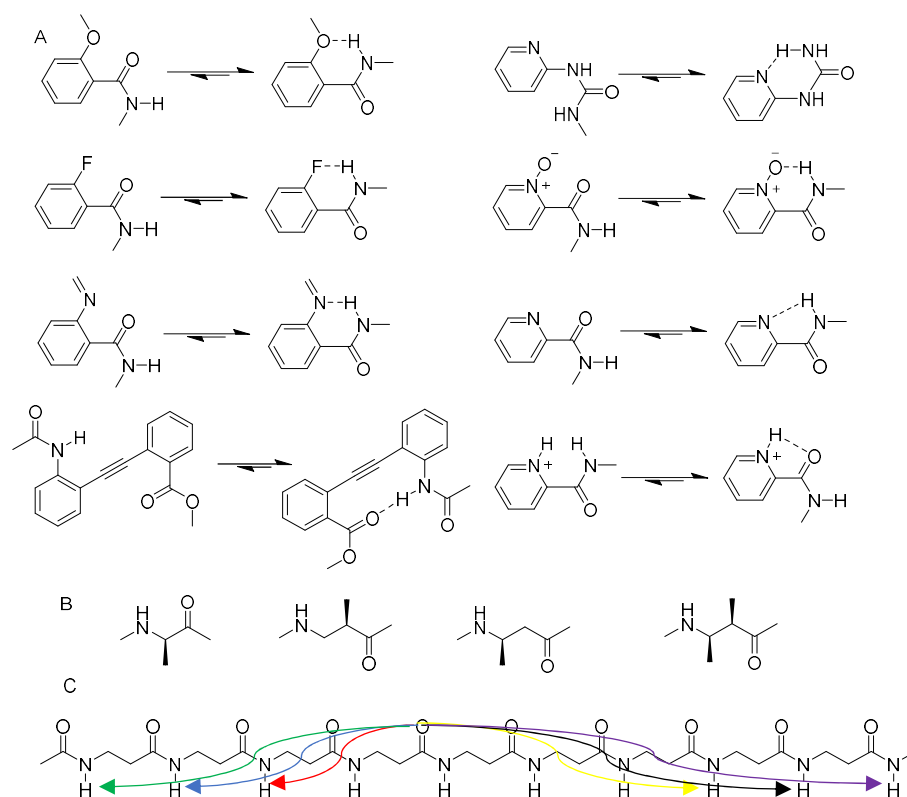


Figure 1.2.3 Some representative H-bond patterns for the construction of dominate conformation. **(A)** adjacent H-bond formation of aromatic units; **(B)** structure of amino acid unit, from left to right: α -amino acid, β^2 -amino acid, β^3 -amino acid, β^2 β^2 -amino acid; **(C)** possible remote H-bonds formed on β -peptide backbone, green arrow represent 14-helix, blue arrow represent 10-helix, red arrow represent 6-helix, yellow arrow represent 12-helix, black arrow represent 16-helix, purple arrow represent 20-helix

H-bonding is the most widely used interaction for the development of foldamers. Figure 1.2.3 lists some representative examples of monomers where H-bonds formed in foldamers give rise to one dominate conformation. Monomer types include both aromatic and aliphatic units. Many magnificent structures, aromatic crescents, helices, linear strands and macrocycles, were generated with these units.^[19] A representative work is an octamer comprised of quinoline and L-leucine monomers connected by amide bonds. H-bonds

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formed between amide protons and the quinoline nitrogens as well as between L-leucine amide proton with carbonyl oxygen give a right-handed helical conformation (figure 1.2.4 A & B).^[20]

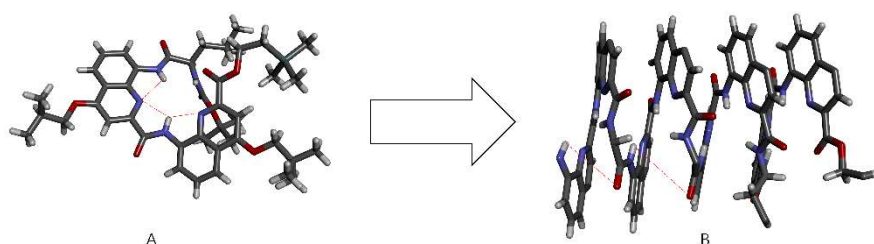


Figure 1.2.4 (A) Crystal structure of quinoline amide dimer; (B) crystal structure of octamer of quinoline amide showed single helix conformation, red dash lines represent H-bond

H-bonding has also been widely used with peptidomimetics.^[14b, 21] Peptidomimetics are molecules that aim to mimic the structures of natural peptides or proteins to further imitating their biological functions.^[15, 22] Both Gellman and Seebach found that exploiting remote H-bonds with β -amino acids building blocks could be used to stabilize the artificial peptide structures. This is still used as an efficient strategy for developing peptidomimetics.^[14b, 21a, 23] β -amino acids are unnatural amino acid segments similar to natural α -amino acids, but with an additional carbon. They can have side chains at the β^2 , β^3 or both positions. β -peptides were the first series of artificial peptides which were shown to adopt well-defined helical structures with different β -amino acid sequences giving structures such as 12-helix, 14-helix, 10/12-helix (figure 1.2.3 C).^[17]

Locally steric hindrance is also prevalently employed in the build blocks of foldamer structures. Unlike fully rigid oligomers, not all dihedral angles for the rotation of bonds are restricted, rather the steric bulk in the monomers limits backbone rotation to a certain range of angles. This kind of strategy is popular in the design of tertiary amides (figure 1.2.5 A, B and E), imides (figure 1.2.5 D), ureas (figure 1.2.5 C) and guanidine (figure 1.2.5 C). By introduction of

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groups on the amide/imide/urea/guanidine amide nitrogens, the *cis-trans* conversion in these groups can be partially locked. Itai revealed through both solid state and NMR studies that a number of tertiary amide bonds showed strong preference for *cis* conformations in several benzoic amides.^[24] Inspired by this character, longer sequence tertiary aromatic amides, imides, ureas and guanidines were developed that gave ordered secondary conformations.^[25]

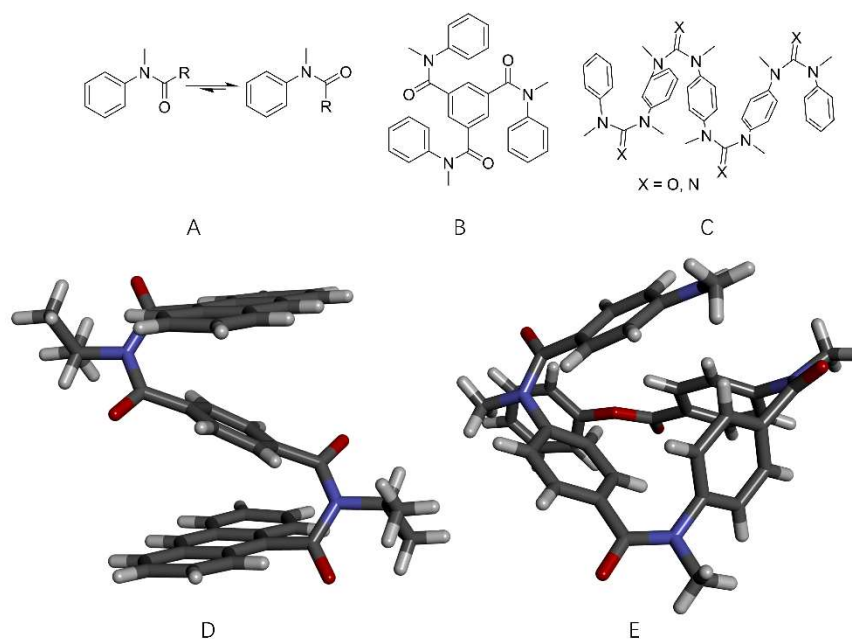


Figure 1.2.5 (A) Tertiary benzoic amide adopts *cis* conformation; (B) Trimesanilide adopt ordered planar conformation (C) Schematic representation of crystal structure of aromatic urea/guanidine adopts helix conformation; (D) crystal structure of tertiary aromatic imides adopts helix conformation; (E) longer sequence of tertiary aromatic amide adopts helix conformation

Peptoids, oligomeric *N*-substituted glycines, are another key example in peptidomimetics which mainly use locally steric hinderance to stabilize higher ordered structures.^[26] It belongs to the family of α -amino acids, but rather than side chain substitution on the C_α position, the side chains are attached on the amide nitrogens. This kind of design precludes the possibility of intramolecular H-bonds between carbonyl oxygens with amide protons and therefore should

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theoretically weaken the structural rigidity of these molecules. However, computational studies revealed the inclusion of bulky and even chiral chains on the amide nitrogen position could allow peptoids to adopt defined secondary structures, since the steric interactions limited the set of energetically accessible conformations of the molecules. This was subsequently proven by spectroscopic studies.^[26-27] In addition to linear peptoid sequences, cyclic peptoids have also raised attention for their variable size and diverse functions since they were first developed in 2007.^[28]

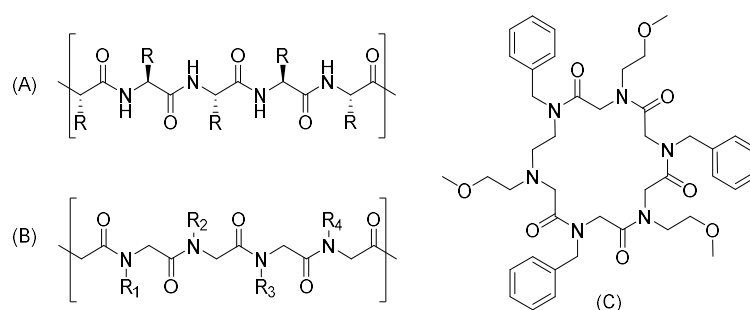


Figure 1.2.6 Schematic representation of peptide (A) and peptoid (B); (C) first example of cyclic peptide developed by Kirshenbaum using head-to-tail macrocyclization

Another weak interaction that cannot be ignored for the stabilization of foldamer structures is π -stacking. Indeed, compared to H-bonding and locally steric hinderance, aromatic stackings are relatively weaker, and typically regarded as only assisting in the stabilization of foldamers containing aromatic groups. In many aromatic helices, aromatic stacking occurs only when other noncovalent interactions have facilitated conformational preferences that place the aromatic groups in proximity. Because of this, it can be dominated by other factors (figure 1.2.4 B, 1.2.5 C and 1.2.7 A). In an early case of aromatic helices, Hamilton designed several aromatic amide scaffolds (figure 1.2.7 B) which formed helices or clefts conformations by using different monomers to control the formation of H-bonds.^[29] Still, π -stacking interactions have been shown in a number of cases to promote the formation of well-

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defined structures. For example, in an aromatic amide scaffold designed to mimic β -sheet structures (figure 1.2.7 C), Huc et al. introduced rigid turn units to orient two aromatic amide strands in the same direction. The interaction between the strands was found to be dependent on the size of the aromatic surface, with only long strands giving well-stacked structures.^[30] Through a variety of analytical techniques, especially NMR, evidence for aromatic stacking (close contact between aromatic groups) could be obtained from nuclear Overhauser effect (NOE) data and changes in shielding due to aromatic ring current effects.^[31] Additionally, π stacking has been important in the development of aedamers (figure 1.2.7 E and F), flexible aromatic oligomers that alternate electron rich and electron poor aromatics. In this case, the aromatic electron donor-accepter interactions and large aromatic groups allow for stronger π stacking that is able to stabilize stacked conformations in oligomers of several lengths.^[32]

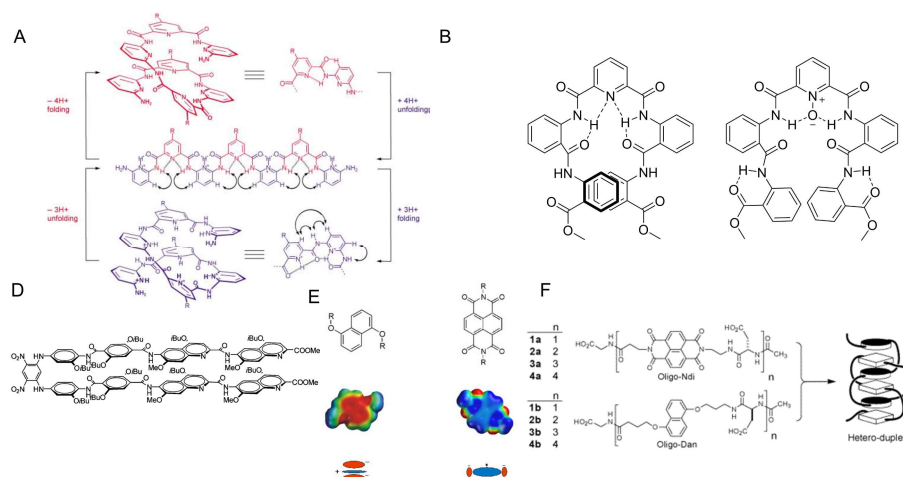


Figure 1.2.7 (A) Protonation-introduced transition on aromatic foldamers adopt other helix or linear conformation,^[33] copyright Wiley 2003; (B) Hamilton's design of aromatic scaffolds; (C) aromatic foldamers mimic β -sheets (D) classical electron-rich and electron-deficient aromatics used in aedamers (E) Iverson's aedamers adopted hetero-duplex in aqueous solution

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Finally, solvent effects are also an indispensable factor which play a major role in stabilization of foldamer conformations. When describing solvophobic effects, one should first mention the hydrophobic effect. Hydrophobic effects are the observed tendency of nonpolar substances to aggregate in an aqueous solution.^[34] In nature, this has a major effect on a variety of processes especially in protein formation and protein-protein interactions.^[35] Solvophobic effects are an extension of hydrophobic effects but occur with nonaqueous polar solutions.^[36]

Learning from the definition and significant research done on this topic, solvophobic effects are non-specific solvent-solute interactions. This feature makes it becomes a double-edged sword for forming ordered conformations.^[37] On the one hand, strong solvophobic effects could promote solutes forming a compact structure; on the other hand, strong solvophobic effects sometimes come along with poor solubility. Li developed a system which alternated DNA and large aromatic sequences which folded into a specific stacked conformation of the aromatic units at high temperature (figure 1.2.8 A).^[37b] Logically incorporating aedamers (aromatic electron donor-acceptor oligomers^[38]) when designing aromatic foldamer can also be a good method to direct folding. Chen reported a kind of zipper-like δ -peptide (figure 1.2.8 C) foldamer which is driven by aromatic electron donor-acceptor interactions between the 1,5-dioxynaphthalenes (DAN, electron-rich, figure 1.2.8 B) and pyromellitic di-imides (PDI, electron-deficient, figure 1.2.8 B). Solvent studies showed these molecules adopt folded conformations in both chloroform and DMF solvents even at 150 °C and have more compact folded forms than in other peptide skeletons.^[39] Solvophobic effects also work in some rigid systems. Inspired by corresponding macrocycles with similar monomers, Moore successfully developed *m*-phenylacetylene oligomers (figure 1.2.8 D) with semi-rigid aromatic backbones and with sidechains that allowed solubilization in various solvents, even acetonitrile-water mixtures. These foldamers adopted helical conformations in polar solvents due to local steric hinderance and solvophobic effects.^[40] In non-polar solvents, the

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oligomers adopt a more disordered unfolded conformation.

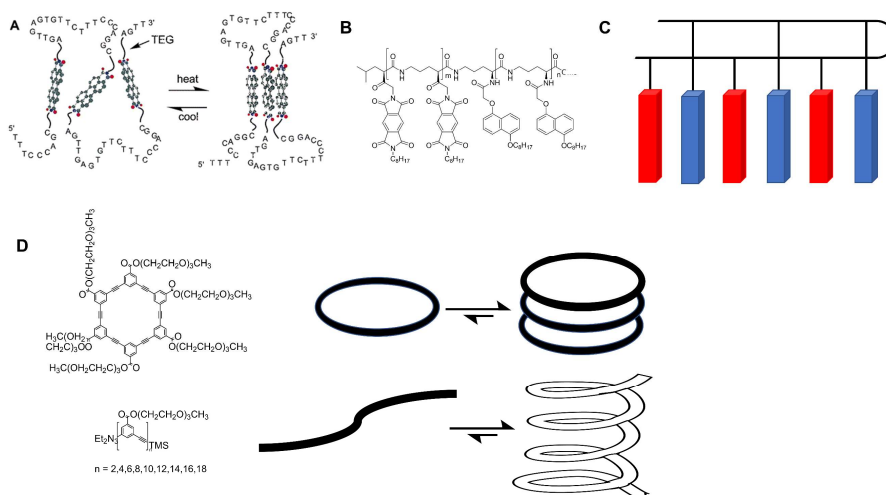


Figure 1.2.8 (A) Thermophilic property of chromophoric trimer linked by a mutated NF-κB site with no hybridization in the DNA loops, copyright from ACS 2003; (B) structure of δ -peptide foldamers developed by Chen; (C) a schematic diagram of aromatic electron donor-acceptor interactions on Chen's foldamer; (D) a schematic diagram of macrocycles and Moore's helical structure

The above interactions remain the most widely used for foldamer design. However, it should also be mentioned that a number of other interactions have been used in the design of foldamers including, metal-ligand interactions, salt bridges, or sigma-hole interactions, and have also been shown to have a strong influence on establishing structures with well-defined conformations. Still, ultimately a combination of multiple interactions is important for obtaining highly stable structures. This can be specifically seen in one class of foldamer, aromatic oligoamides.

2.3 Aromatic oligoamide foldamers

Aromatic oligoamide foldamers (AOF) are a series of oligomers with aromatic moieties connected with amide bonds.^[41] The conformation of these kind of oligomers can be highly predictable based on the nature of the building blocks.

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Compared to alkyl-amide bonds, rotation of aryl-amide bonds is restricted and energy minima are found to be *syn*- and *anti*-conformations due to the conjugation between the amide and aromatic groups. Specific attractive and repulsive interactions between the amide and aromatic groups (e.g. H-bonding, figure 1.2.3 A in section 2.2.2) gives strong preferences for one of these conformations and further forces the structures into well-defined conformations. Based on this idea, a number of monomers with specific electrostatic attractions/repulsions were designed. Molecules with the combination of those monomers were forced into folded structures (some early oligoamides are shown in figure 1.2.8). While most work in this area has been focused on helical structures^[17, 19, 42], a number of beta-sheet mimics have been developed recently. Section 4 gives an overview of systems that mimic beta sheets both with natural and non-biological monomers.

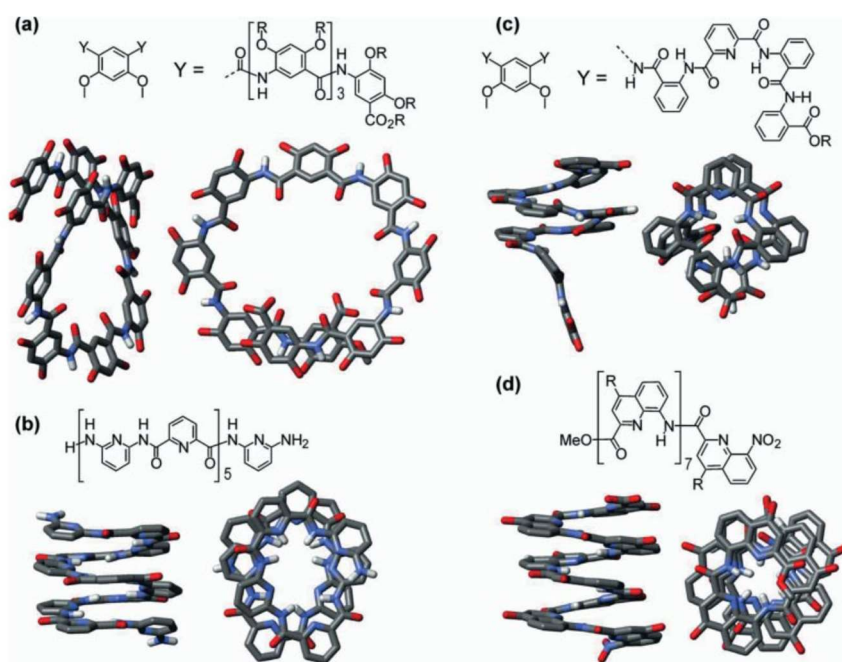


Figure 1.2.9 Some early representative examples of aromatic oligoamides

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3. β -Sheet mimics

α -helix and β -sheets are the most salient local well-defined structures in protein secondary structures. Their fundamental effect in stabilization of protein conformation and function have been recognized as early as middle 20th century although pure α -helix and β -sheets were isolated in 1980s and 1990s, respectively.^[43] Rather than α -helix which normally only need intra-strand non-covalent interactions to stabilize conformations, β -sheets typically require more complicated weak inter-strands interactions.^[44]

As mentioned in section 1, β -sheets are one of the main types of protein secondary structures and account for 30% of all protein structures. Based on the directions of the individual β -strands in the sheet, β -sheets can be distinguished into parallel, anti-parallel or mixed alignments structures (figure 1.1.2 in section 1). In natural β -sheet structures, the anti-parallel conformation is the simplest conformation, as compared to the other two, stable anti-parallel β -sheet can be formed from as little as two strands comprised of five amino acids. By contrast, parallel β -sheets generally contain at least 5 strands for which average distances of adjacent two strands are 6.5 Å (for anti-parallel this distance is usually 5 Å). The complexity of parallel β -sheet conformations has led to few mimics for this structural motif, with most efforts focused on anti-parallel structures. One of the main topics in foldamer research is using artificial monomers to construct molecules which could simulate sheet-like conformation. Based on this, Gellman and Seebech using the unnatural β -amino acid formed oligomer did the groundbreaking work which will show in section 3.2 and 3.3.

3.1 β -Strand

The major component of β -sheets are β -strands (figure 1.4.1 A&B). These are linear or saw-toothed arrangements of amino acids with amide bonds being almost coplanar. This is the simplest peptide structural element. Normally there is no intramolecular H-bonds between amino acid segments. Single β -strands are not common, and this motif typically exists only when stabilized

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by a larger protein structure, such as when two or more β -strands paired by H-bonds form β -sheets.

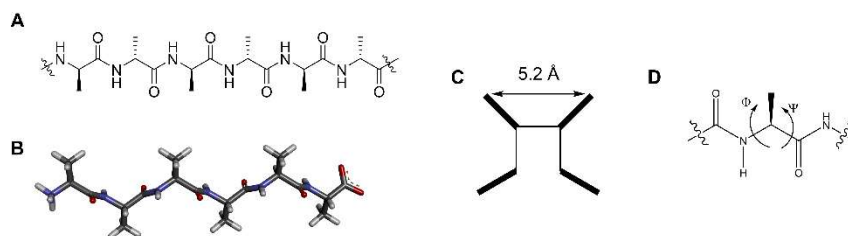


Figure 1.4.1 (A) & (B) Schematic representation and model of idealized β -strand composed of Ala residues; (C) Schematic representation typical β -turn and average distance (βC_{i+1} – βC_{i+2}); (D) ϕ and ψ torsion angles in peptide

Still, β -strands are a crucial structural element for recognition in metabolic cycles or in immune defenses. For example, proteolytic enzymes selectively bind their inhibitors through extended β -strand conformations.^[45] The aggregation of β -strands has also been implicated with some neurodegenerative diseases.^[46] Given these important functions, small molecules mimicking β -strand and β -sheet structures can be interesting for better understanding protein interactions and functions.^[47]

Single β -strand mimics are a great challenge since most of single strands in proteins are stabilized by their tertiary/quaternary structures, which remain challenging to mimic. Cyclization of the strands for restricting conformations is one of the simplest approaches to stabilize single peptide strands. This approach is also notably used in nature. Natural cyclic peptides have been observed and exhibit extensive biological activities compared to linear peptides. The lower conformational flexibility of cyclic peptides has been shown to enhance their interaction with target molecules.^[48] Encouraged by this, many cyclic peptides with bioactivities have been developed as lead compounds and some of them have been used in clinical studies (figure 1.4.2 show some representative examples). Based on this idea, other approaches for obtaining molecules that mimic β -strand conformations by constraining

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their flexibility have been developed. These include the use of disulfides, metal ions, double bonds, sterically hindered amino acids, the addition of aromatics and heterocyclic rings.

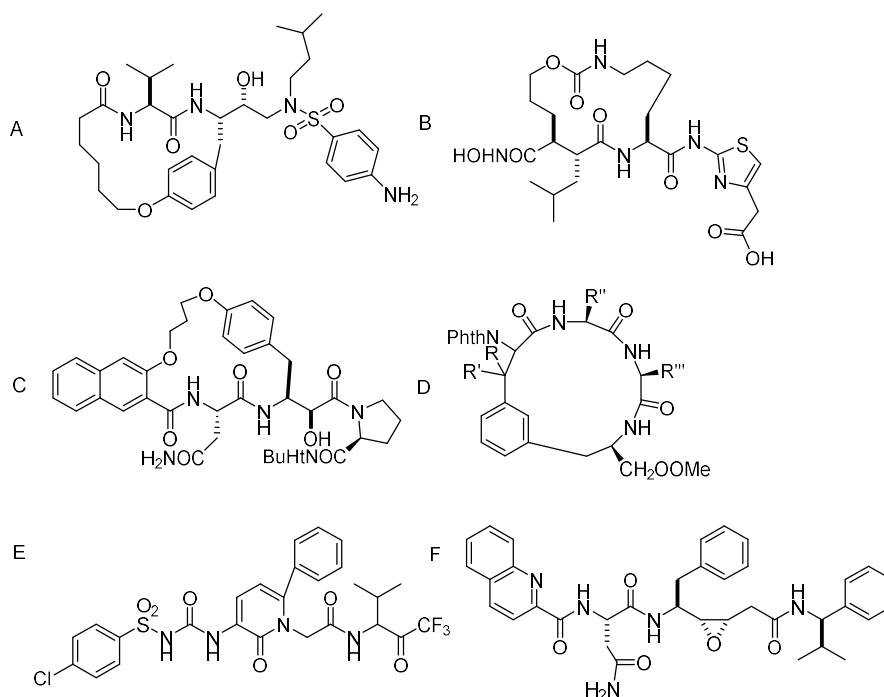


Figure 1.4.2 Some representative examples of β -strand mimics (A) & (B) & (C) & (D) β -strand mimic with inducing cyclic rings which have anti-HIV, anti-cancer activities, for A IC_{50} (HIV-1 infected MT2 cells) = 90 nM, for B IC_{50} (tumor necrosis factor α -whole blood assay) = 80 nM, for C IC_{50} (HIV-1 protease) = 19 nM; (E) β -strand mimics with inducing heteroatoms which have anti-liver cancer activity, IC_{50} (HLE) = 10 - 50 nM; (F) β -strand mimics with inducing rigid group which have anti-HIV activity, IC_{50} (HIV-1) = 10 - 50 nM

3.2 β -Turn

Another important secondary structural element of β -sheets are the turn units involved in orienting or changing the direction of the peptide strands.

β -turns, also known as tight turns, reverse turns, etc., are a major class of protein secondary structure which are the most prevalent type of non-

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repetitive structures.^[49] Geddes first discovered β -turn structures in silk proteins and then Venkatachlam characterized the conformation by a hydrogen bond between amide proton of an i residue and carbonyl oxygen of $i+3$ residue. A β -turn usually consists of 4 amino acid residues with the average distance between C_{i+1} and C_{i+2} being 5.2 Å (figure 1.4.1 C). Based on the difference of ϕ and ψ torsion angles (figure 1.4.1 D) in $i+1$ and $i+2$ residue, β -turn could be classified into nine types (figure 1.4.3).^[50]

Figure 1.4.3 Table of dihedral angles in peptide and difference of ϕ and ψ torsion angles in β -turns

Turn	Dihedral angles (°)			
	Φ_{i+1}	Φ_{i+2}	Ψ_{i+1}	Ψ_{i+2}
I	-60	-90	-30	0
I'	60	90	30	0
II	-60	80	120	0
II'	60	-80	-120	0
IV	-61	-53	10	17
Vla1	-60	-90	120	0
Vla2	-120	-60	120	0
Vlb	-135	-75	135	160
VIII	-60	-120	-30	120

It should be noted that in addition to β -turns, another kind of recognized turn is the γ -turn, which consists of three consecutive residues and with intramolecular H-bond formed between carbonyl oxygen of the i residue and amide proton of $i+2$ residue.^[51]

With the ability of connecting strands and changing strand directions, turn

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units especially β -turn mimics, have been developed extensively. A templating strategy was first introduced, which used rigid synthetic turns to enforce strand folding into an anti-parallel conformation. The first example was reported by Fiegel, who used phenoxathiine derivatives combined with cyclic peptides designed to adopt parallel or anti-parallel β -sheet like structures (figure 1.4.4 A). A series of analogues were designed and all were shown to force the cyclic peptides into defined sheet-like conformations.^[52]

Kelly developed dibenzofuran-based amino acid backbones (figure 1.4.4 B) to replace the $i+1$ and $i+2$ residue of β -turn. The rigid dibenzofuran backbone was designed to reverse the chain direction of the strands by facilitating H-bonds between amino acids. The flexibility of the initial ethylene spacers for the carboxy and amine functionalities was designed to let dibenzofuran skeleton be oriented perpendicular to the plane of the β -sheet.^[53]

Gellman developed ^DPro-DADME diamine and Gly-CHDA diacid turns in 2001 and 2006, respectively, for which, compared to natural amino acids (a natural α -amino acids strand could not adopt tight parallel sheet structures), could force to peptide strands into a parallel conformation, giving a simple artificial model system. Following work on these turns also illustrated that an increasing number of strands could enhance conformational stabilities.

In 1951, a new “complex” of iron with two cyclopentadienide ligands was reported, although the correct structure and name, ferrocene, were given only one year later. In ferrocene the iron is bound in an η_5 fashion to each of the two cyclopentadienyl (Cp) rings (figure 1.4.4 C). This results in these rings being parallel to each other and separated by a distance of 3.4 Å. Its stability and reversible redox functions have attracted much attention for different applications.^[54] As a result of the short distance, ferrocene has been of interest as a turn unit in peptide chemistry. Notably, the history of ferrocene as a turn, with each cyclopentadienyl ring connected to a single amino acid, started with Schlögl in 1957, the same decade of ferrocene invention.^[55] The advantage of ferrocene as a turn unit is mainly attributed to the fact that the distance of the

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two Cp rings are suitable for H-bonds formation in the attached strands, though its good redox properties also allow it to act as a redox-switching center. Hirao also reported that by controlling intra/inter-strand H-bonds, ferrocene dipeptide chains could adopt γ -turn like structures.^[56] Many of the initial studies on ferrocene-peptide conjugates forming well-defined conformations used ferrocene dicarboxylic acid coupled to two valine units. Two identical H-bonds were detected between the pairs of amide protons and carbonyl oxygens in CDCl_3 , and further supported by the X-ray structure. The replacement of the terminal esters for amides gave rise to better H-bonding, even allowing the two amino acids to form a sheet like structure in water.^[57]

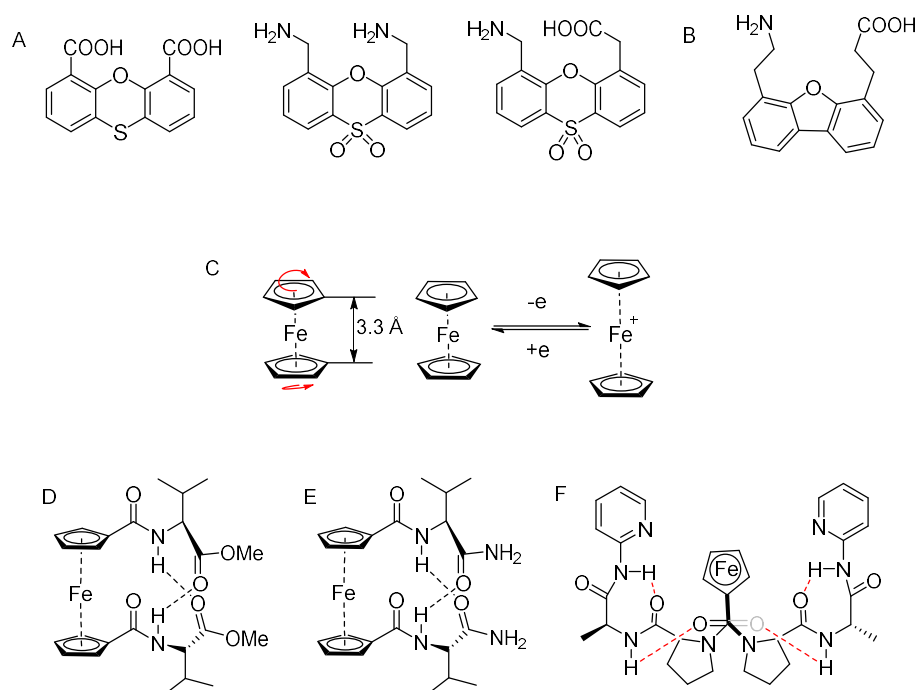


Figure 1.4.4 (A) First series of rigid turns developed by Fiegl; (B) dibenzofuran-based amine acid mimic turn; (C) representation of ferrocene and its characteristics; (D) & (E) & (F) some representative example of ferrocene dipeptides

Many other successful attempts at mimicking β -turns, as well as strands, have been employed for the formation β -hairpins or other types of β -sheet mimics. These will be introduced in next section.

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3.3 Hairpin

β -hairpin is a kind of supersecondary structure which contains two adjacent peptide strands linked by a short turn or loop. The number of residues of the turn or loop vary between 2 to 6.^[58] Compared with the broad definition and various types of β -sheets, the β -hairpin is simpler, consisting of any two β -strands with at least a 2 residue turn. This makes β -hairpins minimal models for mimicking protein β -sheet structures. The initial study of β -hairpins was reported in 1993 by Blanco with a nonameric peptide chain which equilibrated between folded and unfolded states in aqueous solution.^[59] This work opened an era of research aimed at stabilizing these peptide folds and a series of further studies based on this sequence was continued.^[60] Those early works revealed for the first time the possibility of short sequence β -sheet structures to exist independent of the stabilizing features of whole proteins.

Gellman developed artificial peptide sequences which will folded into β -hairpin conformations through a 12-membered H-bonded (carbonyl oxygen of i residue and amide proton of $i+3$ residue) turn comprised of a heterochiral dinipicotic acid (figure 1.4.5 A).^[61] At nearly the same time, Seebach introduced a 10-membered H-bonded (amide proton of i residue and carbonyl oxygen of $i+3$ residue) turn with mixed β^2/β^3 -dipeptides (figure 1.4.5 B).^[62] Both of the designs gave well-defined β -hairpin populations characterized by X-ray (for Gellman's), NMR (for Seebach's) and computational studies (for Seebach's).^[61-62]

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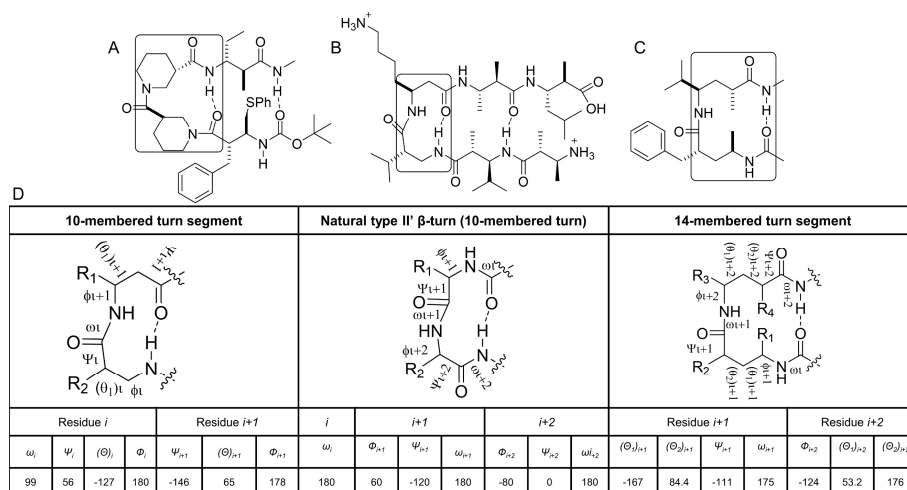


Figure 1.4.5 (A) Gellman's 12-membered H-bonding turn; (B) Seebach's 10-membered H-bonding chain; (C) 14-membered H-bonding turn; (D) comparison of 10- and 12-membered H-bonding turn and natural type II'

Other than β -hairpins, new types of artificial hairpins were also developed. γ -peptide type backbones formed turns with 14-membered H-bonded (amide proton of i residue and carbonyl oxygen of $i+1$ residue) rings (figure 1.4.5 C).^[63] Notably, both 10- and 12-membered H-bonding turns gave similar dihedral angles with natural type II' β -turns (figure 1.4.5 D), indicating that these could have a better impact in β -sheet mimetics as well as peptide drug design.

Except the turn sequence, the strands also play an important role in the stabilization of β -hairpin. This can be attributed to the intrinsic β -sheet propensities of the amino acids in the strands and the interaction between sidechains.^[64] It is obvious the "intrinsic propensities" of amino acids for forming β -sheets mainly refers to their ability to adopt conformations that can participate in inter-strand H-bonding and the influence of hydrophobic effects on their structures. However, the importance of sidechain-sidechain interactions for specific conformations also been confirmed and studied. Santiveri compared a variety of β -hairpin conformational changes through the mutation of individual residues, and found out that cross-strand sidechain-

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sidechain interactions may determine the hairpin conformation.^[65] Balaram successfully obtained a peptide hairpin by screening a number of strands connected to a three residue loop known as not a typical turn structures. This work gave an insight into strand influence on turn (figure 1.4.6 A & B).^[66] Waters developed a β -hairpin through the methylation of a lysine sidechain to form a cation. This modification was shown to enhance the interaction between two strands via a cation- π interaction (figure 1.4.6 C).^[67]

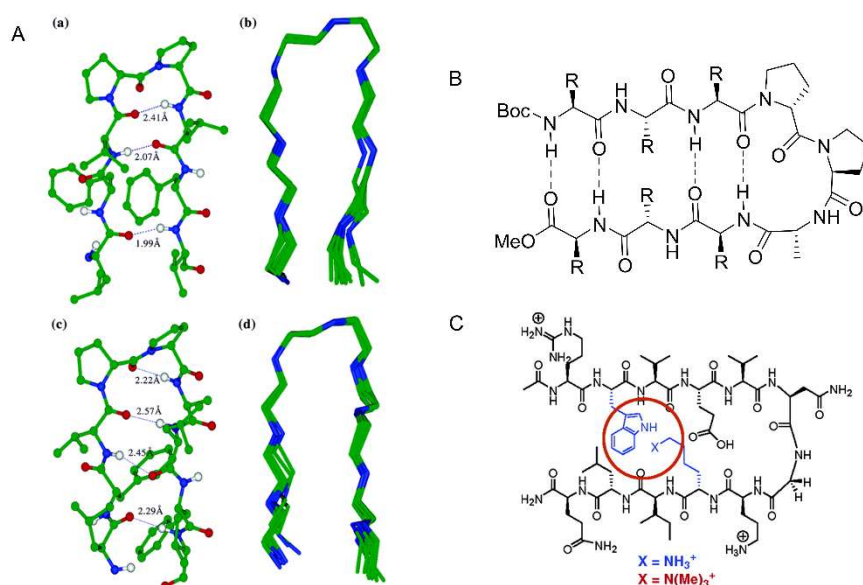


Figure 1.4.6 (A) a & c best position of sidechain for two artificial β -hairpins, c & d 10 of lowest energy conformation from calculation for two artificial β -hairpins; (B) general structure of designed β -hairpins; (C) designed artificial β -hairpins with sidechain forming cation- π interactions

3.4 Aromatic oligomers that mimic β -strands/sheets

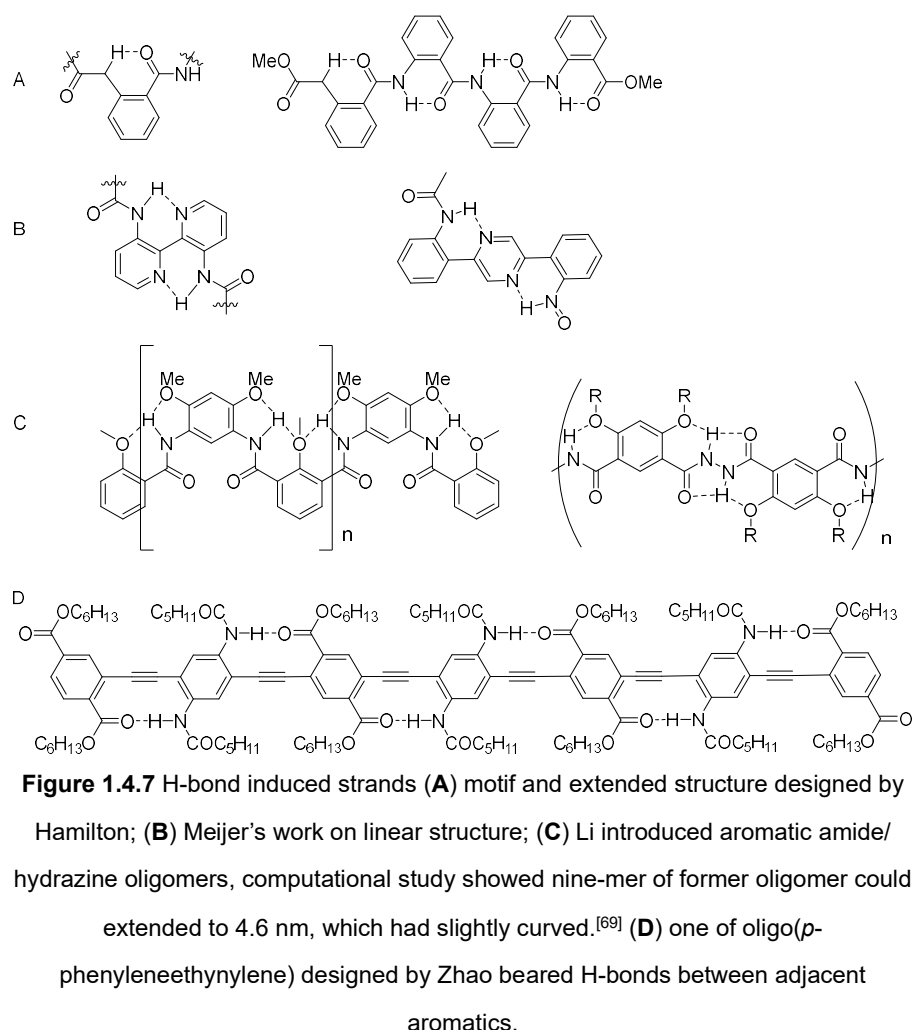
Aromatic oligomers with linear conformations were some of the earliest approaches used for β -sheet design since their rigidity combined with functionalizing could mimic the pleated structure of linear single β -strands. These were often based on building blocks that formed non-covalent interactions with adjacent monomers and limited remote intrastrand

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interactions.

For example, Hamilton using anthranilic acid as a motif designed the first aromatic extended strand. The reason for choosing this motif is it could form a stable six-membered H-bonding rings between adjacent amides, as well as, its intrinsic rigidity and *trans* preference of the amide bond (figure 1.4.7 A).^[68] Later, Meijer used bipyridyl derivatives that could also form six-membered H-bonding rings for obtaining similar extended structures (figure 1.4.7 B). For such systems with basic nitrogen sites, protonation can lead to distinct conformational changes. For example, disrupting the hydrogen bonding motifs in helical foldamers such as in figure 1.2.6 A (in section 2), through protonation results in the formation of linear conformations.^[33] Three centered H-bonding motifs have also been introduced for construction of linear (zig-zag) strands. Interestingly, subtle changes to the structures can decrease the magnitude (width) of the zig-zag structure to give more linear sequences. Notably, Li developed a series of aromatic amides or hydrazides which formed different extended conformation (figure 1.4.7 C).^[69] He showed that the hydrazide foldamers placed the aromatic rings in a more linear arrangement than in the amide derivative.^[70] Hydrogen bonding can also be used to enforce coplanar arrangements in more rigid systems. For example, the structure in figure 1.4.7 D has the aromatic groups directly connected through ethyne spacers. The flat structure is preferred as a result of an interesting 10-membered hydrogen bonding ring. Thus, in all of these structures H-bonding is important for controlling the linear structures. However, it should be mentioned that for optimal linear conformations H-bonds should be included on both sides of the strands to avoid curved structures in longer oligomers.

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Still, it was observed that aromatic strands that use adjacent H-bonds on only one side of the strand could potentially be functionalized on the other side to allow intermolecular H-bonds to give structures that mimic β -sheets-like dimers. Systems relying on multiple hydrogen bonding units could potentially give very stable dimers, similar to the base pairing that occurs in DNA/RNA. Gong studied “mismatching” in the hydrogen bonding patterns by replacing one of the terminal D groups for A group. This led to a dramatic decrease in the dimerization affinity of the two strands (40 times lower than the association constant of the “matched” dimer.) Differences in association constants for

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triply hydrogen bonded (ADA·DAD, DDA·AAD, DDD·AAA, D representative hydrogen bond donor; A representative hydrogen bond acceptor, figure 1.4.8 A&B) have also been observed due to secondary hydrogen bonding interactions.^[71] Gong also gave an evaluation of hybrid aromatic-aliphatic amide oligomers. The flexibility of the aliphatic subunits was minimized by intramolecular H-bonds between adjacent aromatic units giving oligomers with preferred strand-like conformations. At the same time, the free hydrogen bond donors / acceptors on the other side of two strands could be used to intermolecular H-bonds with a second strand (figure 1.4.8 C).^[72] Inspired by Gong's work, Chen developed ADDA·DAAD system with two oligomers associated by intermolecular H-bonds. Similar systems with DDAADDAA interactions have also been developed by Zimmerman. In both cases, stable dimers were formed between two strands. The above examples can be considered as aromatic mimics of sheet-like structures. Indeed, the interstrand H-bonding is similar to peptide β -sheets.

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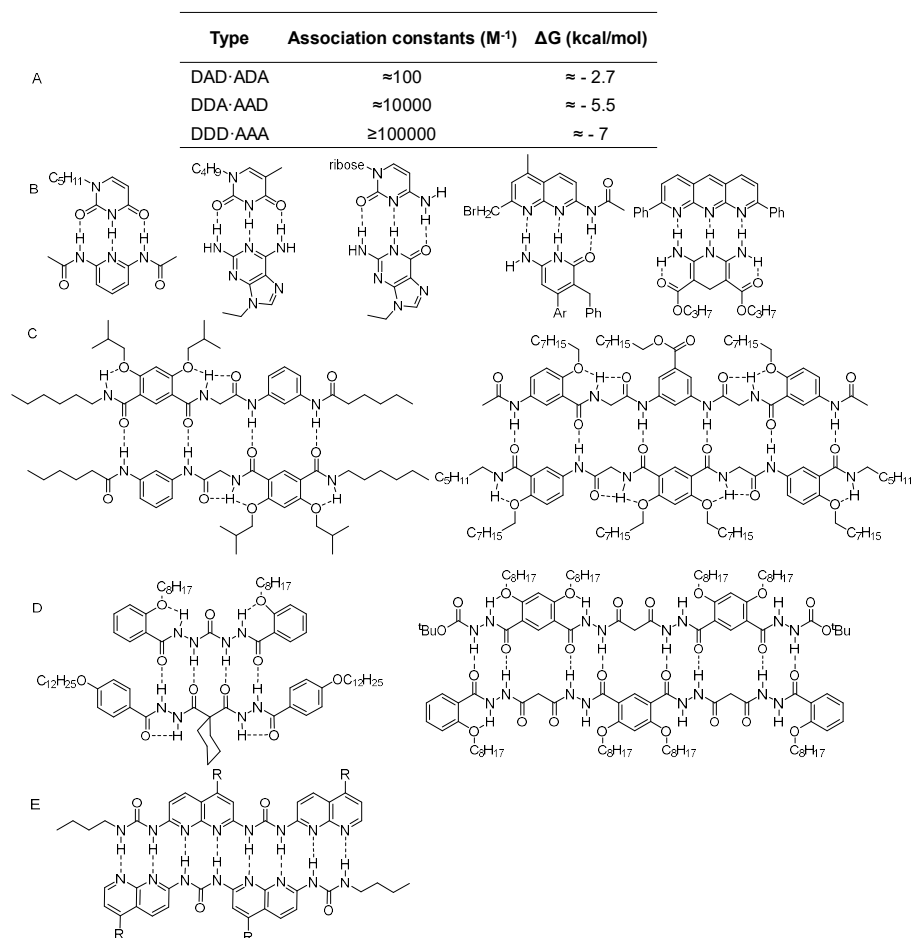


Figure 1.4.8 (A) Association constant and Gibbs free energy of different types of triply hydrogen-bonded systems; **(B)** representative examples of triply hydrogen bonded systems, from left to right: ADA·DAD, ADA·DAD, DDA·ADD, DDA·ADD, DDD·AAA; **(C)** representative cases of dimeric systems by design of Gong, right complex was also used for “mismatching” study by replacing one of the terminal D to A; **(D)** Chen’s strategy of DAAD·ADDA dimer and DA·AD - DA·AD - DA·AD - DA·AD dimer system; **(E)** DDAADDAA·AADDAAADD complex

Other than systems relying on interstrand hydrogen bonding, a handful of other aromatic β -sheet structures based on inter-strand aromatic interactions. Maurizot reported that an aromatic heptamer connected with amines adopted cylindrical β -sheet conformations (figure 1.4.9 A & B). In the solid state, this

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heptamer adopted a compact structure with the central 1,3-diamino-4,6-dinitrobenzene unit serving as a turn for connecting two curved parallel strands. The distance of those two strands is approximately 3.5 Å suggesting strong aromatic stacking interactions. ^1H NMR spectrum also showed significant upfield shifts of the aromatic proton resonances in the oligomer versus the monomers consistent with shielding from aromatic ring current effects. Additionally, 2D-ROESY NMR showed several correlations between remote protons, indicating the folded state also occurs in solution.^[73]

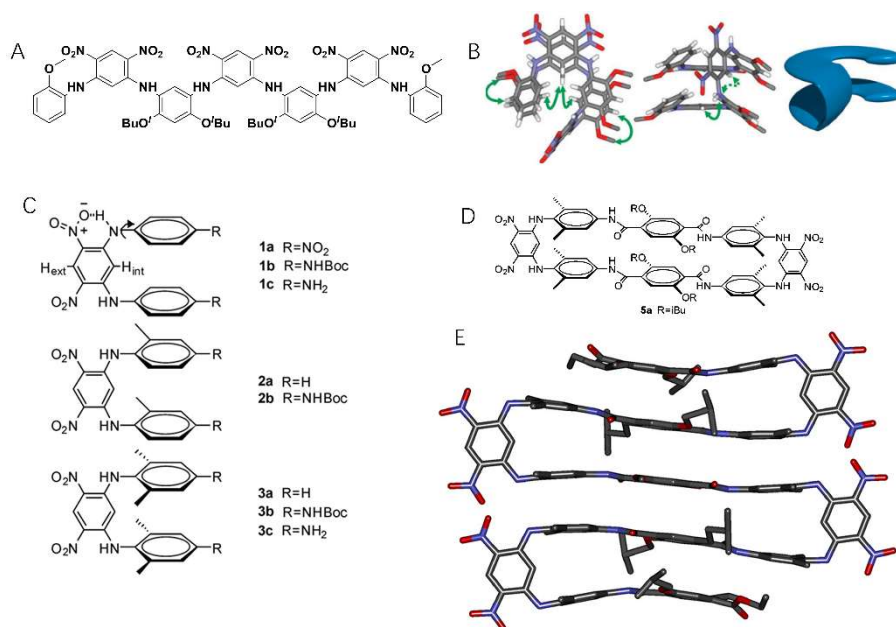


Figure 1.4.9 Examples of β -sheet mimic with aromatic stacking (**A**) chemical structure of aromatic heptamer; (**B**) top and side view and cartoon model of aromatic heptamer, the distance showed aromatic stacking; (**C**) structures of turn units; (**D**) structure of macrocycles; (**E**) crystal of penta-strands showed little bit twist of strands but the distance between strands showed aromatic stacking

Larger structures based on this idea were also developed that showed important contributions from the turn units (figure 1.4.9 C). Specifically, limiting the rotational freedom in the initial 4,6-dinitro-1,3-phenylenediamine units allowed for better-defined structures in both the solid state and in solution.

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Additionally, hindered aromatic units with *para*-isobutoxy groups (also for solubility) were chosen for extending the strands. Based on this, a series of aromatic oligomers and macrocycles were synthesized with different strand lengths and numbers of strands, including 2-strand, 3-strand, 5-strand structures (figure 1.4.9 D&E, figure 1.4.10 A) for mimicking β -sheet. Solution ^1H and 2D-ROESY NMR spectra and the solid-state structures showed positive evidence for the proximity of the aromatic strands in some cases. Still, in some cases limited stacking was observed. For example, a system using squaramides as the turn units was recently reported. While the distance in this case is ideal for stacking interactions, one of the strands in the sequence was found to be flipped in the solid state structure, giving minimal overlap between the two strands (figure 1.4.10 B & C).^[74] This may be due to the electrostatic repulsion between the strands and limited aromatic stacking due to the relatively short aromatic strands. Based on all of the above work, several design considerations could be seen for the development of aromatic β -sheet structures. First is choice of a suitable turn units. Typically, a suitable turn unit should be rigid in order to provide a good tendency for forcing the strands into a parallel orientation at a good distance for non-covalent interactions (mainly aromatic stacking). Second large aromatic surfaces in the strands should be needed for a good stacking interaction. Additionally, modifications on the aromatics to reduce strand flipping are also important.

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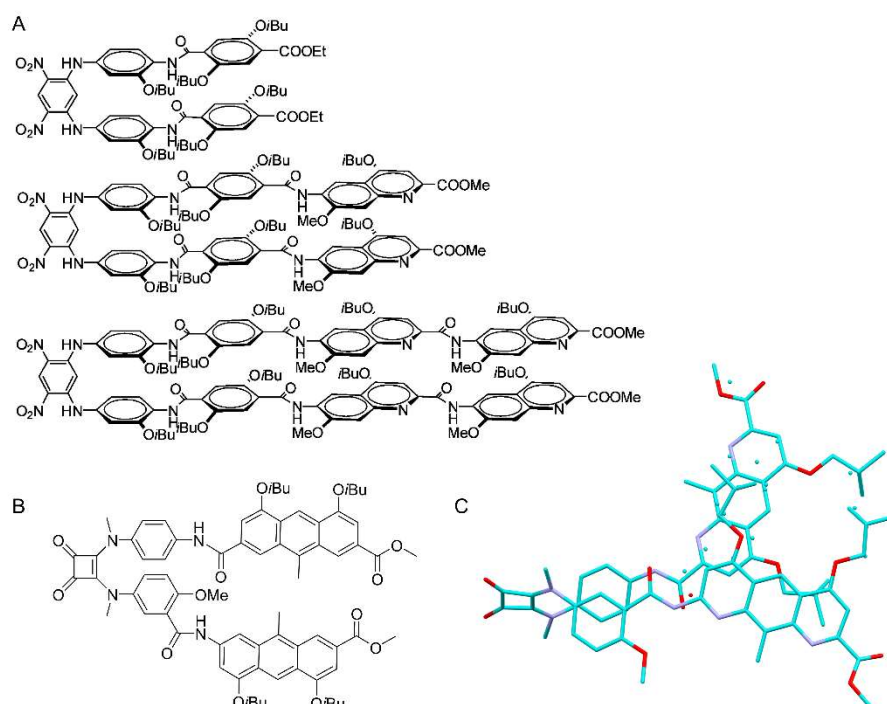


Figure 1.4.10 (A) Increasing numbers of aromatics of mimicking β -sheets; (B) & (C) structure and top view of crystal structure of mimicking β -sheets with squaramide with short sequence of aromatic strands

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4. Applications of Foldamers : Molecular recognition

Since the Nobel prize was awarded to Pederson, Lehn and Cram in 1987 for their work on cation recognition^[75], figure 1.3.1, Molecular recognition has become one of the major areas in supramolecular chemistry.^[76]

Molecular recognition is a fundamental process needed for life. It is widely seen, for example, in metabolism pathways which need specific interactions such as between substrates and enzyme catalysis, membrane transportation, and cellular signaling. Those recognitions in biological systems are based on the precise and regular structure of proteins or ribonucleases. In nature, molecular recognition is usually based on noncovalent interactions such as hydrogen-bonding, electrostatic interactions, steric hinderance, and the hydrophobic effect. In fact, in most cases specific recognition involves a combination of all of these. Because of the importance of this process, specific molecular recognition is also a major goal for mimics of natural structures. This includes foldamers, where these structures can act as either the host or the guest.

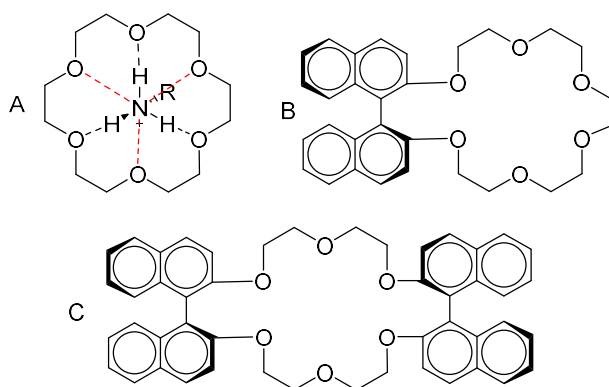


Figure 1.3.1 Some of representative initial work on cation host design (A) first 18-crown ether have binding ability with ammonia salt; (B) & (C) inducing of enantiomer mimic enzyme's system distinguishing racemic molecules

A brief of applications of foldamers for recognition is given in the following sections.

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4.1 Foldamer design as host

Host-guest chemistry “began” with the recognition of small molecules especially ions by cyclic molecules and highlighted the “lock and key” theory. With the development of foldamer chemistry, artificial defined arrangements such as helical, linear and turn structures occurred which corresponded to natural structures. Compared to cyclic structures, those molecules contain “open-chain” system which required complex noncovalent interactions to stabilize their conformations. And also, those noncovalent interactions endow the possibility of “catching” other molecules by, for example, H-bonds, or electrostatic effects.

The compact secondary structures of naturally occurring (or analogous aliphatic) amino acid foldamers limits their ability to act as hosts for internal recognition of small molecules. Indeed, the formation cavities are not feasible at the secondary structural level with these units. Still, some rare examples of curved β -sheet clefts have been reported that could bind guests and catalyze reactions.^[77] By contrast, aromatic foldamers have offered a substantially greater variety of different structures. Their variably size and modularity have allowed cavities or receptors to be developed that are capable of binding to a large range of small molecules as well as ions. This makes this class of foldamer in particular interesting for the design of host-guest systems.

A 2, 2' – bipyridine (figure 1.3.2 A) motif is one of the earliest monomers used for the design aromatic helical structures. Computational studies have shown that the *trans* conformation of 2, 2' – bipyridine motifs are preferred (energy difference between the *trans/cis* isomers is around 6 kJ/mol) due to electrostatic repulsion between the endocyclic nitrogens.^[78] Based on this, a series of pyridine/pyrimidine/naphthyridine-based helices and macrocycles were designed and studied by Lehn (figure 1.3.2 B). Two crystal structures of such aromatic helices showed variable size cavities with internal diameters of 2.6 Å - 3.8 Å. When the larger structure was treated with alkali cationic salts (potassium, cesium) or organic ions (guanidinium of C_3 - symmetry), they

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observed evidence for the formation of the ion complexes. This was described to occur through a process of “unfolding-binding to ion-folding”. A “close-chain” macrocycle was also synthesized and shown to form ion complexes, blocking the bond rotation in the cycle (figure 1.3.2 C). Additionally, cation-promoted self-assembly was also investigated with oligoamines, showing the potential for the formation of monomeric, dimeric, trimeric and tetrameric stacks of helical structures that mimic ions channels (figure 1.3.2 D).^[79]

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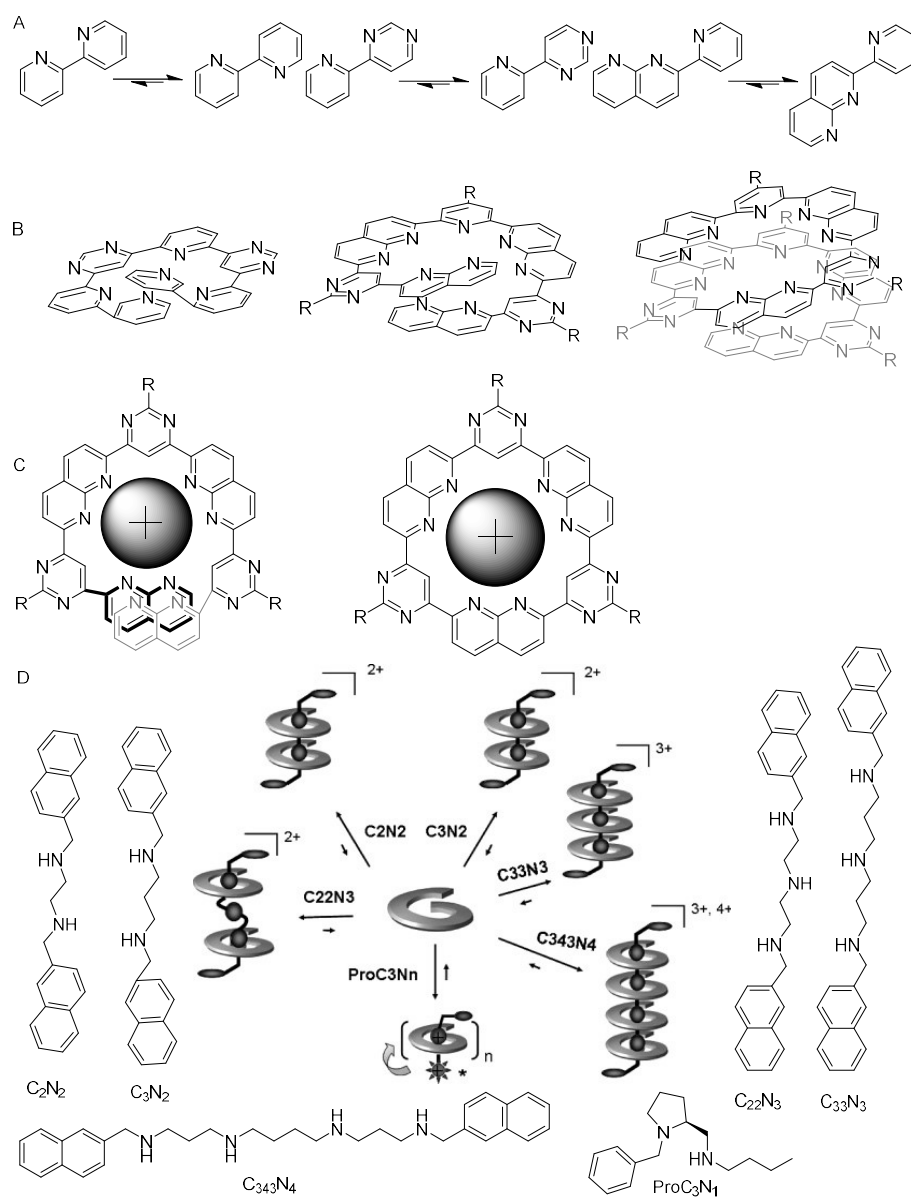


Figure 1.3.2 (A) Aromatic dimers exhibited preferred conformations; (B) heptamers and eleven-mer showed helix conformation; (C) model of heptamer and macrocycle binding with ions, black balls represent cation; (D) models of templates induced helical structures aggregation, C₂N₂, C₃N₂, C₂₂N₃, C₃₃N₃, C₃₄₃N₄ and ProC₃N₁ represent structure of templates

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Similarly, in the solvophobically folded *m*-phenylene ethynylene (mPE) oligomers developed by Moore, the hydrophobic cavities formed by folding exhibited diastereoselectivity for chiral monoterpenes recognition. In another series, their helical mPE oligomers functionalized with hexaethylene glycol became water soluble and could still bind the guests with acceptable diastereoselectivity.^[80]

Aromatic oligoamides also showed comparable cavities with good hydrogen bond donors and acceptors. In 2002, Huc reported a series of pyridine amide heptamers with crystal structures showing several guest water or DMSO molecules (figure 1.3.3 A).^[81] Three years later, the same group reported a new heptamer, described as a “molecular apple peel”. In the design of this helical structure, the diameter (curvature) has decreased from middle to end of the sequence giving closed cavities. This structure was described to encapsulate water molecules, both in solution and solid state, through a process of folding and unfolding (figure 1.3.3 B).^[82] Using this same strategy, they also developed larger aromatic capsules with bigger cavity sizes by increasing the number and types of monomers used in the sequence. This also had the effect of adding in new interactions for binding different polar molecules. Through this work, they also found that bigger helical capsules generally preferred one large guest (shape fitting) rather than several small guest which may contain different equilibria process. This indicated the possibility of rational design of molecular capsules for selective and tight molecular recognition and controllable release.^[42, 83]

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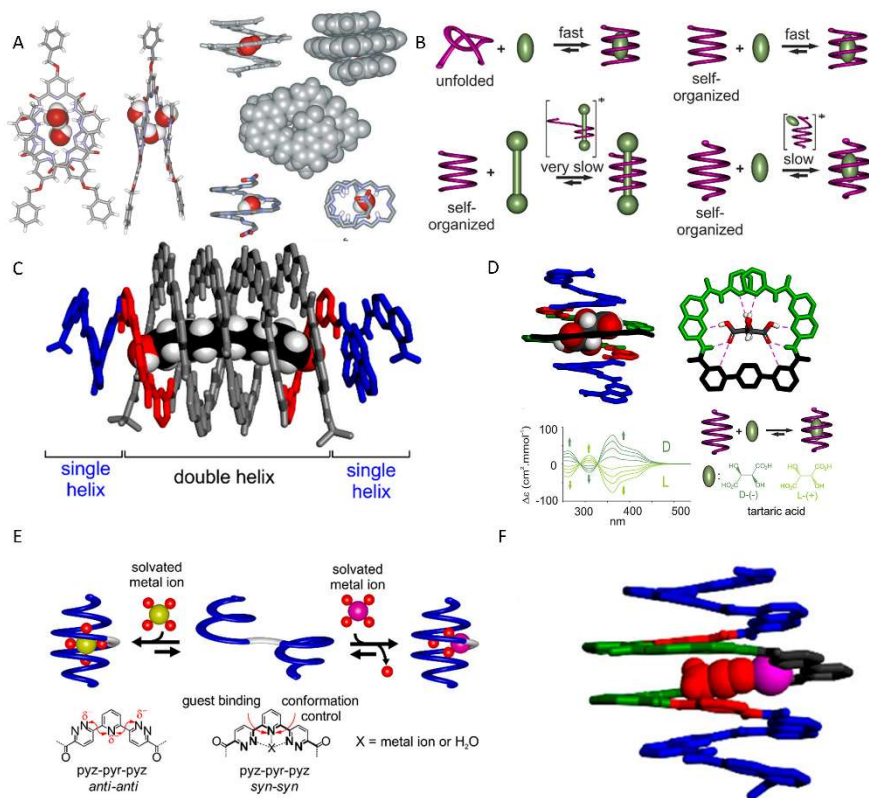


Figure 1.3.3 (A) Top and side view of aromatic heptamer amide crystal structure which contained water molecules; (B) different equilibrium processes of helical receptors and guest molecules; (C) guest induced double helices; (D) *P*-helix selectively binding L-tartaric acid and crystal structure showed H-bonds forming; (E) & (F) schematic representation of metal ion assisted helices and guest recognition

Other than single helices, double helical capsules were also discovered which encapsulated guest molecules (figure 1.3.3 C).^[84] By using the chirality of helical molecules, Huc designed and synthesized an aromatic oligomer which adopted a helical conformation and could diastereoselectively binding tartaric acid (L-tartaric acid fitted *M* helix and D-tartaric fitted *P* helix, figure 1.3.3 D). After, a series of helices which could specifically recognize chiral compounds such as monosaccharides were designed, synthesis and studied.^[42, 85] Additional designs were also developed which could specifically coordinate metal ions and recognize guest molecules (figure 1.3.3 E & F).^[42]

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4.2 Foldamer design as guest

Due to their nature as structural biomimetics, a significant amount of work has also gone into the development of foldamers with ordered structure that act as guests (ligands) for selectively binding to biomacromolecules. This is typically aimed at important applications in disease diagnosis (probe and sensor) and therapy (drug design and delivery).

For example, the p53 gene usually plays an important role in the occurrence and development of cancers because its tumor-suppressor activity can be stimulated to eliminate cancer cells. Certain evidence proved hDM2 (human oncogene product double minture 2, also known as MDM2) could regulate the expression of the p53 gene by specifically binding to a short helical motif of the p53 activation domain (p53AD).^[86] Therefore, foldamers mimicking the functional part of p53AD either by activation of p53 gene or selectively binding to hDM2 theoretically could activate p53 gene expression through foldamer-protein or foldamer-DNA interactions.

4.2.1 Protein-foldamer interaction

A number of protein-protein interactions are mediated by α -helical secondary structures. As a result, the design of many foldamer helices, both aliphatic and aromatic, has been aimed to disrupt these interactions by competing for binding in the protein-protein interaction.

Take the above example of p53-hDM2 interaction, a functional epitope comprised of three residues (F19, W23 and L26) on one side of p53AD helix contributes a lot to this interaction. Many synthetic foldamers were designed to mimic this functional part of p53AD in order to recognize the binding segment of hDM2 and so block the p53-hMD2 interactions. Schepartz developed a series of β^3 -peptides (figure 1.3.4 A) that adopted a 14-helix that successfully mimics the functional part of p53AD with 3 residues on one side and showed strong affinity to hDM2 with the most potent peptide showing 1.6-fold higher affinity than p53AD for hDM2.^[87] Except β -peptides, peptoids could

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also mimic this functional part. Appella used oligomeric peptoids as the scaffold for rational design of foldamers inhibiting the p53-hDM2 interaction (figure 1.3.4 B). They surprisingly found that the best affinity to hDM2 were the peptoids that lack any helix-promoting groups. Aromatic foldamers have been used as well. By studying the conformation and distance of three residues on the functional part on p53AD, Wilson and Edwards developed a series of oligobenzamide mimics, stabilized by H-bonds, that adopted slightly twisted linear conformations. Compared to the original p53AD, the conformation of oligobenzamide let the three residues be placed at suitable positions as well as distances which exhibited strong affinity to P53 (figure 1.3.4 C).^[88] To better control the geometry and increase the recognition selectivity, they introduced hybrid oligoamides which mimicked better the conformation that selectively inhibited the p53-hDM2 interaction (figure 1.3.4 D).^[89]

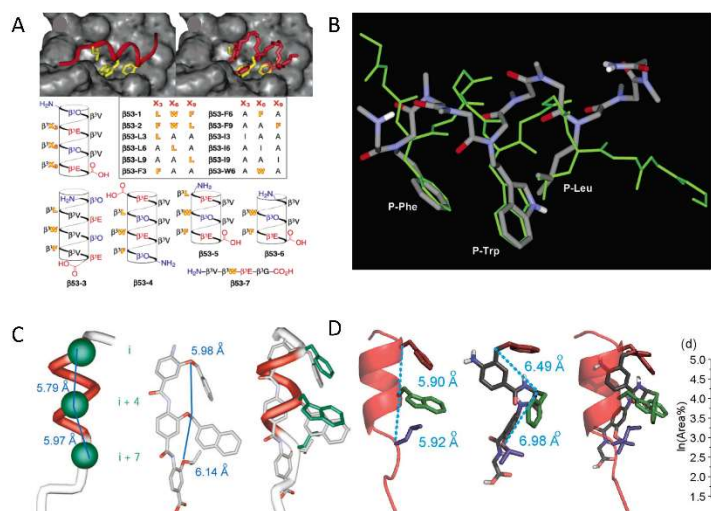


Figure 1.3.4 (A) Crystal structure of p53AD₁₅₋₂₉ (backbone in red, side-chain of F19, W23 and L26 in yellow) with hDM2₁₇₋₁₂₄ (gray) and model of an idealized β -peptide 14-helix bound in hDM2₁₇₋₁₂₄, bottom is helical net diagrams of β^3 -peptides; (B) structure comparison of the three hydrophobic sidechains of p53AD₁₅₋₂₉ peptide (green) and peptoid designed by Appella (stick model); (C) & (D) design of Edwards and Wilson oligoamides with the comparison of p53AD₁₅₋₂₉

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As one of the key secondary structures, around 15,000 high-affinity β -strand interaction pairs are active in protein-protein interactions. Compared to mimics of α -helices, β -sheet-like models have a challenge of solubility in water.^[90] The Noramy strategy was pointed out by Gellman with application of “heterogeneous backbones”. Early works showed that combinations of α/β amino acids could successfully be used as building blocks for forming β -hairpin conformations in aqueous solution. However, these works also found greater disorder of sidechains in synthetic foldamer relative to natural sequences. Based on this strategy, Horne systemically screened β -residues replacing $\alpha\alpha$ dipeptide segments and developed a series of α/β -peptides. $\alpha\alpha \rightarrow \beta^3/\beta^2$ and $\alpha\alpha \rightarrow \beta^{2,3}$ that exhibited stable β -hairpin conformations with sidechains corresponded to nature β -sheet structures.^[91]

Foldamers mimicking DNA, not only on conformation but also specific surface, may increase the interaction with DNA binding enzymes. The surface of DNA especially the charge distribution on the surface are important for recognition of enzymes that interact with DNA. Huc reported a series of quinoline foldamers that adopted a single helix conformation with phosphate sidechains in an arrangement that mimicked the surface charge distribution of B-DNA. Those foldamers are composed by Q^4 , mQ and Q^5 quinoline monomers (figure 1.3.5 A) which showed different sizes for the helical backbones. By adjusting these, they could match the phosphate positions of B-DNA (figure 1.3.5 B). Those foldamers showed inhibition on enzymes such as topoisomerase 1 and HIV-1 integrase.^[92] Recently, modification on side chains by using carboxylate instead of phosphate motifs on those quinoline foldamers could adopt better charge distribution and improve inhibition on enzymes.^[93]

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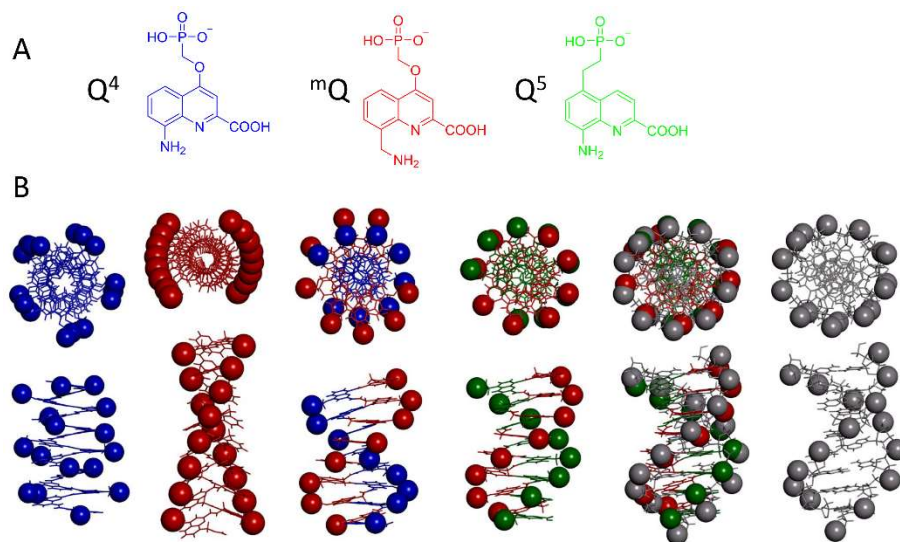


Figure 1.3.5 (A) Structure of quinoline monomer with different color. (B) side and top view of helical foldamers blue, red and green represent Q^4 , mQ and Q^5 monomers, grey represent B-DNA, comparison of hybrid helix of mQ and Q^5 monomers with B-DNA showed matching conformation

On the aspect of study protein-foldamer interactions, those examples were only the tip of the iceberg but cover the idea of developing these molecules for this kind of interactions though rationally designed foldamers that selectively replace desired protein structures in protein-protein interactions.

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5. Conformational exchange in foldamer

Although the emphasis in foldamer studies is usually placed on the specific folded conformation, it should be noted that folding is an equilibrium process. In solution exchange occurs between folded and unfolded states or even between multiple stable conformations. Control over this conformational exchange has been reported to have potential important applications for information storage or transfer, catalysis, and sensing.^[94]

A good example of this exchange process is the interconversion between P and M helicities in some foldamers.^[19] While many helical foldamers based on biologically related amino acids strongly favor only one helical handedness due to the chiral nature of the monomers used, for foldamers that are derived from non-chiral building blocks the two different helicities should be equally stable minima. Thus, with no directing forces, equal amounts of the P and M helices will exist in equilibrium in solution (figure 1.5.1 A). This can be detected in solution by, for example, using chiral shift reagents under NMR spectroscopy.^[95] The exchange between these minima should then pass through some number of higher energy unfolded or partially unfolded states. A number of factors including solvent, nature of the interactions, helix length, and temperature relate to how easily these intermediate folded states are accessed and so affect the rate of exchange between the P and M conformations. In some cases the rate of exchange is slow enough to allow separation by chiral chromatography and isolation of the two helicities.

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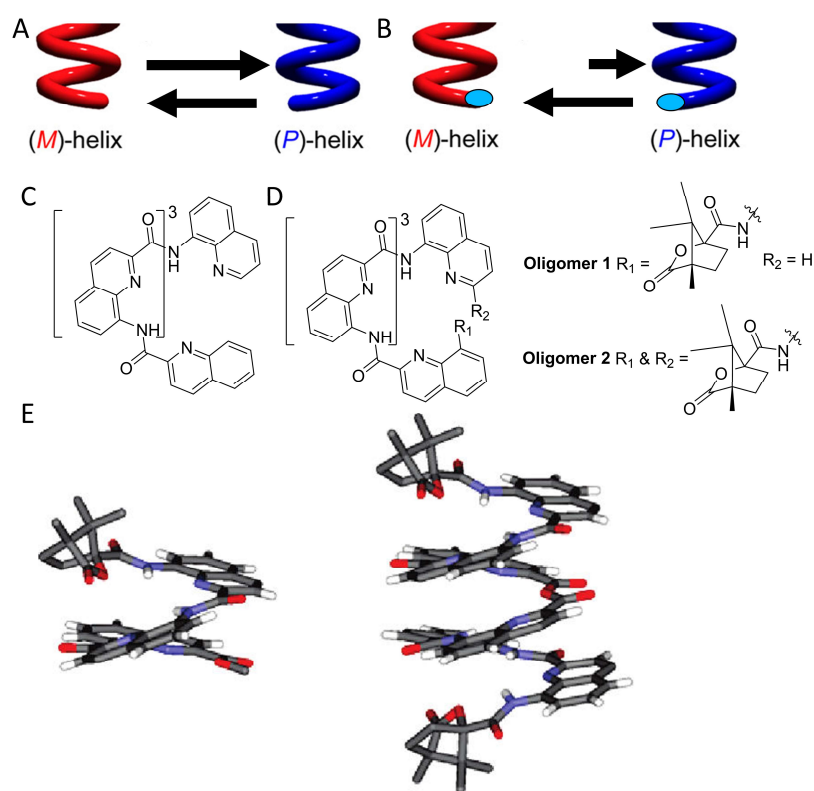


Figure 1.5.1, Representative examples of foldamer conformational exchange, (A) helix inversion between P and M conformations, (B) Use of a chiral group to direct helix handedness. (C) One of the chemical structures of oligoamides of 8-amino-2-quinolinecarboxylic acid (D) & (E) Chemical and Crystal structures of oligoamides of 8-amino-2-quinolinecarboxylic acid bearing (1R)-(+)-camphanyl group

The equilibrium between the P and M forms can be shifted towards one of the conformations by interaction with chiral molecules or through modification of the foldamer with a chiral end group (Sergeant-Soldier). For example, Huc and coworkers reported an aromatic foldamer capsule that adopts equally P and M helical conformations (figure 1.3.3 D in section 3 of chapter I). However, when a single enantiomer of a guest (D/L- tartaric acid) is introduced, the formed complex show a strong preference into one direction of P (for D tartaric acid) or M (for L tartaric acid) helicity.^[85] Additionally, oligoamides of 8-amino-2-quinolinecarboxylic acid have been extensively demonstrated to adopt

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stable helical conformations in a wide range of solvents (figure 1.5.1 C). Huc and coworkers reported that when these oligoamides are functionalized with chiral group at their terminus, there can also be a preference for one handedness over the other (figure 1.5.1 D & E).^[96]

Besides helix inversion, in double helical aromatic oligoamide foldamers other types of conformational exchange can be present related to the relative orientations of each strand. For example, Huc and coworkers reported a screw motion in the anti-parallel double helical aromatic oligoamide foldamer binding with different sized rodlike guests' systems. Both solid state and solution studies revealed the length induced screw motions of duplex systems (figure 1.5.2 A & B).^[97]

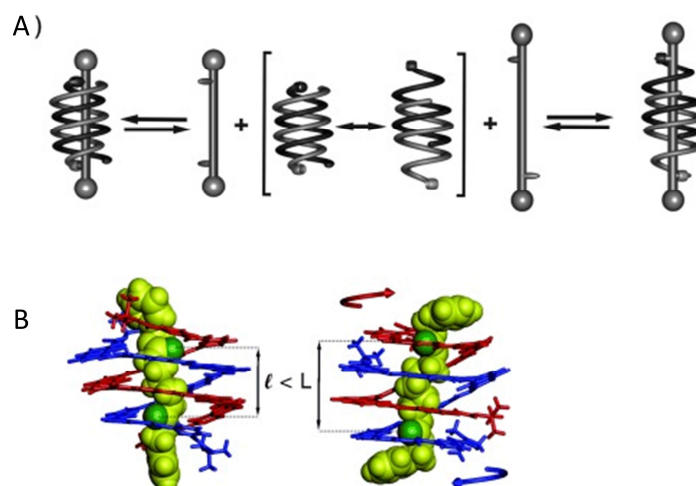


Figure 1.5.2 (A) Schematic representation of screw motion. (B) Solid state of double helix with strands. L and l represent the distance between the two pyridine clefts in each complex, and are equal to 6.8 Å and 9.0 Å, respectively.

Examples of exchange between distinctly different conformations such as helices and sheets are observed in nature but rare in artificial molecules.^[98] As this process requires major structural changes strong stimuli can be necessary to induce the change. For instance, Huc reported by inducing protons, oligoamides prepared from 2,6-pyridinedicarboxylic acid and 2,6-diaminopyridine adopt conformation exchanges between helical and linear

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(figure 1.2.7 A in section 2, chapter I).^[33] Additionally, some examples using light with styrene/azobenzene monomers or metal coordination have been reported.^[99] Despite this, systems containing more flexible units could allow for an easier exchange between large conformations potentially allowing it to be promoted just through simple interactions with small molecules and could be important for sensing applications. However, these have been much less studied.

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Research basis, Objective and Design

1. Research Basis

As described in Chapter I, the folded structures of biomolecules, notably proteins, are important for the biological processes necessary for life. The development of molecules able to mimic these folded structures, foldamers, is thus not only important for developing a better understanding of folding in biomolecules, but also, as foldamers can potentially mimic the functions of biomolecules they can be of interest for addressing challenges in chemistry related to catalysis, recognition, and drug development. As a result a large number of foldamer structures have been designed using a wide range of building blocks. The vast majority of these structures have been focused on mimicking the helical structures found in nature, and far fewer foldamers for mimicking β -sheet structures have been described. Still, given the importance of β -sheet motifs in the active sites of proteins or in protein-protein interactions, mimics of these structures can be highly valuable.

Unlike their aliphatic analogs, aromatic oligoamide foldamers are particularly interesting for the development of sheet-like structures as the individual strand conformations can be easily controlled through monomer choice, allowing the formation of either linear or curved strands with a variety of functional groups appended to them. The main requirement for these systems is then to have sufficient interaction between the strands in order to promote a well-stacked sheet-like conformation. In previous examples of AOF β -sheet mimics this has relied on the use of largely rigid turn-units for orienting the strands in the same direction.^[1] Still, large aromatic sequences are needed to observe well-stacked conformation of the strands. Only a few such structures with sheet-like motifs (figure 1.2.6 D & figure 1.2.16) have been reported. Developing new turn units which could provide suitable distances between two strands are essential.

To this end, we were interested if 1,1'-ferrocene dicarboxylic acid could be a suitable turn-unit. The distance between the Cp rings is ideal for promoting aromatic stacking between the strands. This has been applied for aliphatic β -sheets, but has not been reported with aromatic oligoamides.^[2] Still, ferrocene directly appended with aromatic groups has been shown to favor a *syn* stacked conformation, indicating that it could be an interesting turn-unit for AOF as well. Additionally, ferrocene is a fluxional molecule with low rotation barrier. This can potentially give access to multiple conformations making its

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use as a turn-unit of interest for controlling exchange between different structures.

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2. Objectives of this PhD thesis

The main objective of this research is to design and synthesize ferrocene containing aromatic oligoamide foldamers with an initial goal of obtaining stable sheet-like conformations. Through this, we aim to address several questions. The first is whether ferrocene can be a viable turn-unit for AOF sheet mimics. Second, what factors are important for controlling the balance between the fluxional behavior of the ferrocene versus the interaction between the strands and, thus, the structural stability of the foldamers.

In order to accomplish this, series of ferrocene containing AOF will be developed with the general design shown in figure 2.1 and 2.2. The ferrocene will act as a central unit that connects two aromatic oligoamide strands. In order to look at factors that influence the structures of these systems, several different modifications to this design were envisaged.

1. In an initial series, the size of the aromatic surfaces will be modified using aromatic monomers of different sizes such as pyridine, quinoline, and diazaanthracene. The hypothesis is that larger surfaces should increase the interaction between the strands. This will also be extended to the strand length. In order to potentially change preferences between head-to-head or head-to-tail conformations of the strands, it was envisaged to use curved strands.

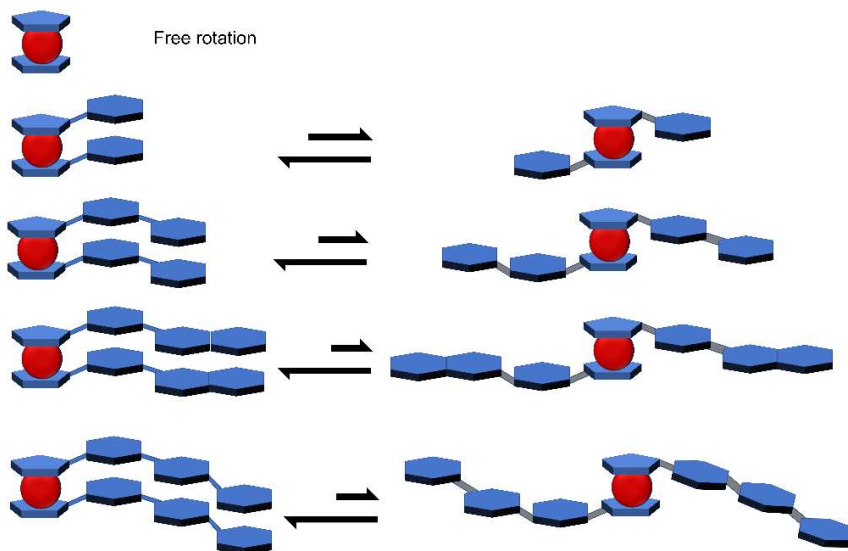


Figure 2.1 Schematic representation of possible conformation change of ferrocene aromatic oligoamides. Red ball represent iron, blue hemihedrons represent Cp rings, blue octahedrons represent aromatic rings, grey sticks represent possible connection

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- Next, based on the idea of aedamers, a series of oligomers will be synthesized that include electron rich (donor) /electron poor (acceptor) aromatic moieties, figure 2.2. This is expected to increase the π - π interactions and stabilize the structure. Depending on the match between donor and acceptor it can be interesting to see if these molecules further self-assemble into dimeric or larger structures, figure 2.2.

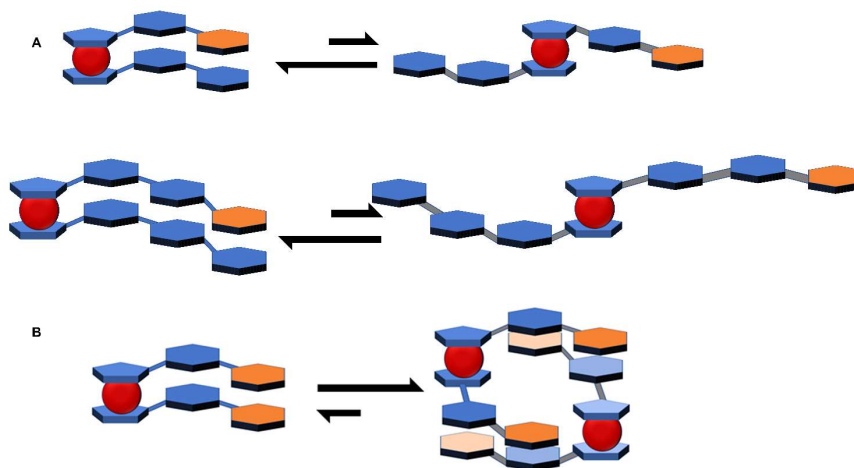


Figure 2.2 (A) Schematic representation of possible conformation change of ferrocene aromatic oligoamides with aedamer. Orange octahedrons represent opposite electrostatic potentials; (B) Enhancing electrostatic repulsion may decrease folding state while enhancing inter-molecular interactions. blue and orange color of aromatic rings represent opposite electrostatic potentials.

- Finally, with the use of curved strands, the resulting sheet-like structures should give novel molecular cleft-like structures. Studying the ability of these structures to interact with small molecules can provide an interesting functional aspect to these systems that can potentially mimic the functions of curved β -sheets in nature, figure 2.4.

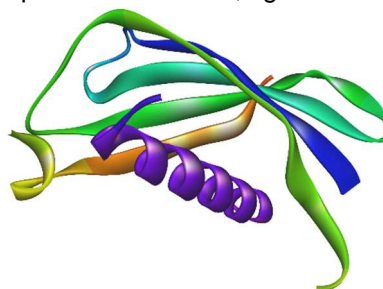


Figure 2.4 Schematic representation structure of hot dog fold interacting with a helix inside of its curvature.

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Chapter III

Stabilizing Attempts by Increasing Aromatic Size

This chapter focuses on the design, synthesis and characterization of our first series of aromatic oligoamide ferrocene derivatives (**3.002-3.004** as follows). These initial oligomers differ by the size of the strand aromatic surface in order to study if increased π -stacking can enhance the stability of the oligomers. Additionally, the ability of the series to bind small molecules was also studied.

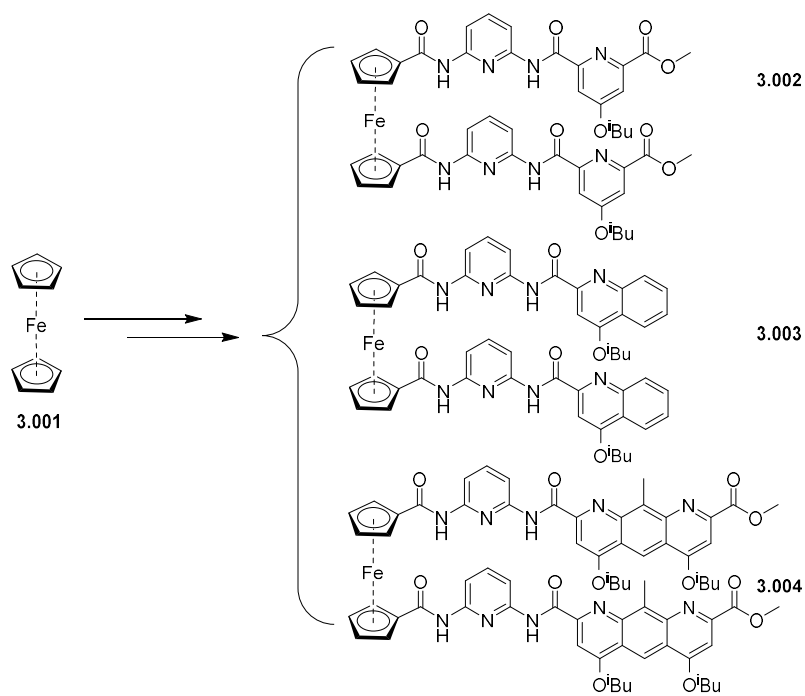
Part of the work of this chapter was published in *Liu et al. Chem. Euro. J.* **2020**, 26, 181-185. ^[1]

1. Foldamer Design

Our initial design uses aminopyridine functionalized ferrocene-1,1'-dicarboxylic acid with either pyridine, quinoline or diazaanthracene carboxylic acids coupled at the end of the strand to give oligomers **3.002**, **3.003**, or **3.004**. This was performed to study how the increasing aromatic surfaces, and so increasing aromatic stacking effects, could restrict the fluxionality of the oligomers.

3.002, **3.003** and **3.004** were designed for enhancing the potential π - π interactions between the two strands. Comparing these molecules can improve our understanding of structural elements needed for stabilizing sheet-like conformations ferrocene containing aromatic β -sheet mimics.

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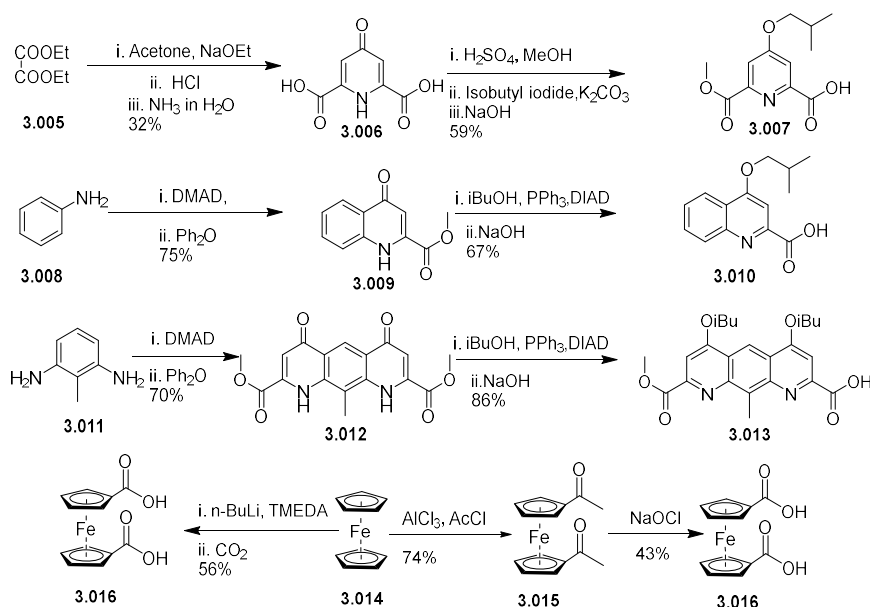
Scheme 3.1.1 Design of oligomer **3.002** to **3.004**

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2. First Generation of β -sheet Mimics

2.1 Synthesis

Syntheses of ferrocene-1,1'-dicarboxylic acid **3.017** and monomers **3.007**, **3.010**, **3.013** are shown and follow reported procedures. Chelidamic acid **3.006** was synthesized in three steps. Sodium ethoxide was used to generate the acetone enolate which was reacted with diethyl oxalate and then cyclized under acid conditions to form chelidonic acid. This was followed by reaction with an ammonia solution to form chelidamic acid. The chelidamic acid was then esterified with methanol and a catalytic amount of concentrated sulfuric acid. Finally, alkylation with isobutyl iodide followed by mono-saponification afforded pyridine monomer **3.007**.



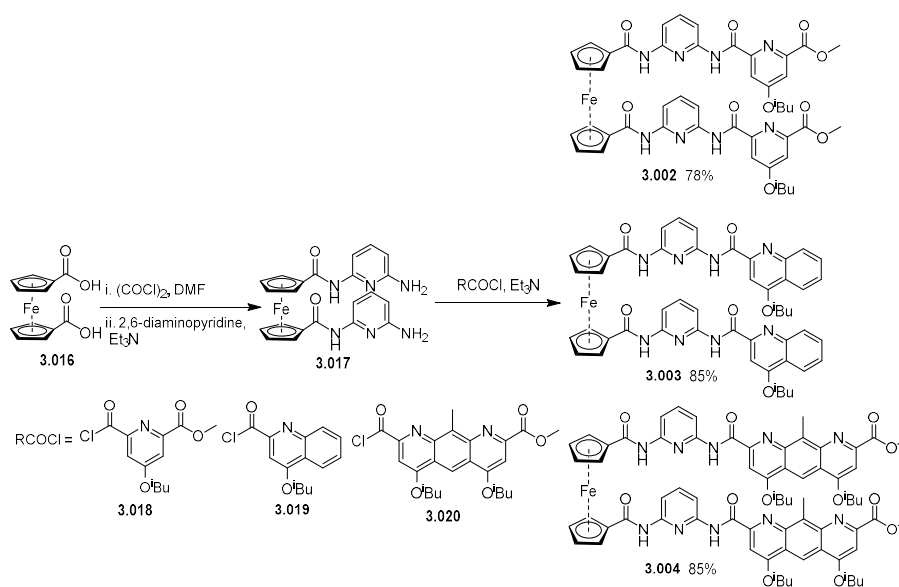
Scheme 3.2.1 Synthetic route of monomers

The quinoline monomer was synthesized starting from aniline **3.008** by reaction with DMAD in polyphosphoric acid to give quinolinone **3.009** in good yield. A Mitsunobu reaction was applied for adding the isobutyl side chain. Hydrolysis of the ester then gave monomer **3.010**. The diazaanthracene monomer was obtained in a similar fashion starting from 2,6-diaminotoluene **3.011**.

Ferrocene-1,1'-dicarboxylic acid **3.016** could be gained from either direct addition of carbon dioxide to the ferrocene dianion or through acetylation by Friedel-Crafts reaction followed by formation of the acid with sodium hypochlorite. Both of the above two methods gave good yields.

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Ferrocene-1,1'-dicarboxylic acid chloride was obtained through classical methods. Oxalyl chloride with catalytic DMF worked well. After, the acid chloride was directly reacted with excess 2,6-diaminopyridine with triethylamine in anhydrous THF. After overnight reaction and subsequent purification by column, ferrocene oligomer **3.017** could be obtained in good yield. This unit represents the turn unit used for all oligomers in the series. Acid chlorides **3.018**, **3.019**, or **3.020**, were obtained by reaction of the corresponding monomer carboxylic acids with refluxing thionyl chloride for 15 to 60 min. Those three acid chlorides were then reacted with the **3.017** in the presence of triethylamine in anhydrous THF to afford the oligomers **3.002**, **3.003** and **3.004** as orange to yellow solids in moderate yield.



Scheme 3.2.2 Synthetic route of oligomer **3.002** to **3.004**

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2.2 Solid State Study

Single crystals of **3.002** and **3.003** were obtained from the slow diffusion of methanol into dichloromethane solutions of each compound. While **3.004** could not be crystallized with the same method, slow evaporation of an acetone solution of the compound gave good results.

The top and side view of the three crystal structures are showed in figure 3.2.1, although there is a little twist for the amide between the ferrocene and pyridine groups, the three structures showed the expected parallel arrangement of the stacked aromatic strands. The ferrocene moiety was found in a largely eclipsed conformation with torsion angles of 4.3°, 7.3° and 3.4° for **3.002**, **3.003**, **3.004** respectively. Distances between the overlapped aromatics of each strand, ranging from 3.2 Å to 3.4 Å, suggested strong π - π interactions between the strands. From the top view, all of those three showed curved conformations resulting from H-bonding and dipole-dipole interactions between the amides and nitrogens of the aromatic rings. Those cleft-like structures are lined by H-bonds donors and acceptors and could thus be potential receptors for small molecules. The top view also illustrates that the dipole moments of two strands are parallel to each other. While unfavorable this should give better overlap of the aromatic parts. This arrangement was likely further stabilized by the anti-parallel packing between molecules in the crystal lattice.

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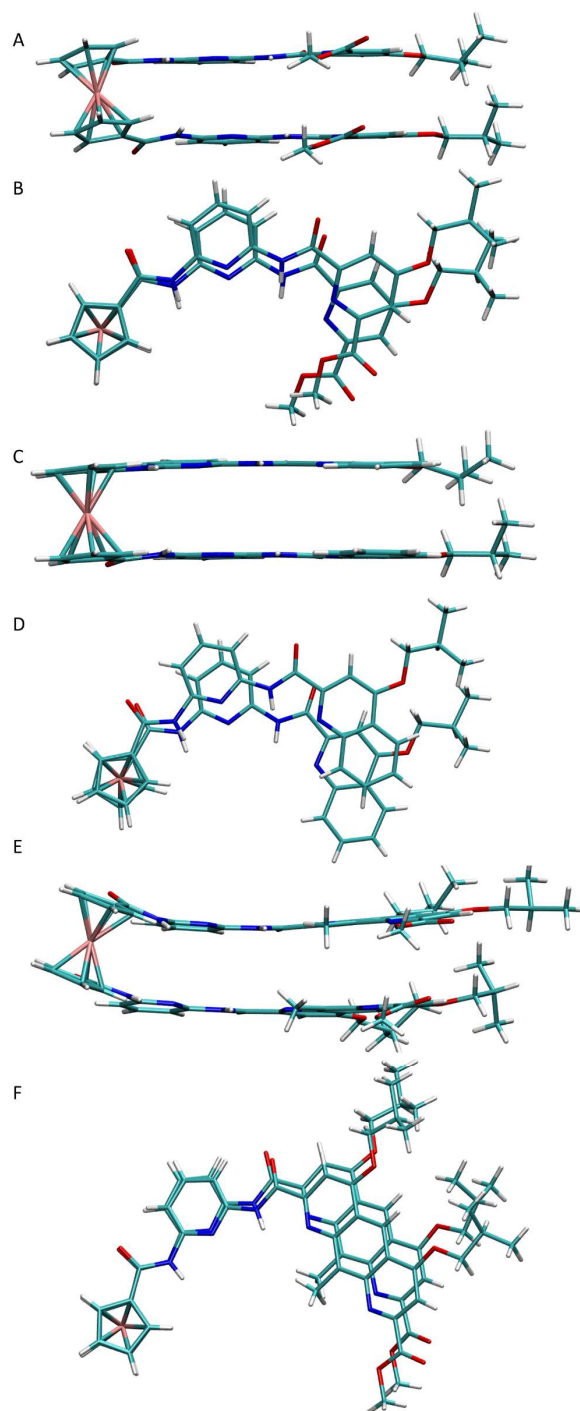


Figure 3.2.1 Side (A, C, E) and top (B, D, F) view of the three oligomers' crystal structures

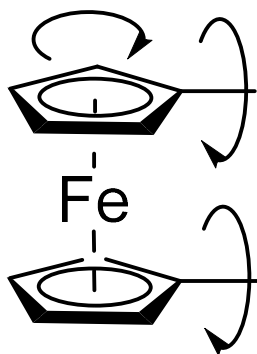
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2.3 Solution Studies

3.002 and **3.003** showed good solubility in chloroform and dichloromethane, with concentrations more than 10 mM at room temperature possible, as well as modest solubility in acetone, acetonitrile, or DMSO. **3.004** does not show the same solubility. Even in chloroform, its limited solubility required the ^{13}C NMR to be obtained at 313 K. Because of this, the remaining solution studies were focused primarily on **3.002** and **3.003**.

2.3.1 Concentration and Solvent Studies

Concentration studies by NMR were used to see if the oligomers aggregated substantially in solution.^[2] ^1H NMR of **3.002** and **3.003** in Chloroform-*d* were launched at 0.1, 0.5, 1, 5 and 10 mM. These spectra are shown in figure 3.2.2 and 3.2.3. For the most part, the resonances for the oligomer protons did not show substantial shifts with increased concentration, indicating limited aggregation. Importantly, in the proton NMR of **3.002** and **3.003** in chloroform-*d* the ferrocene protons appeared as only two signals corresponding to the 2/2',5/5' and 3/3',4/4' pairs of protons on both rings. The same behavior was detected in the proton NMR of **3.004**. This phenomenon indicated that despite the well-folded crystal structure, those molecules in solution are highly dynamic. This likely comes from rotations of Cp rings and C-C bonds between ferrocene and first amide groups (the arrows showed in follow).



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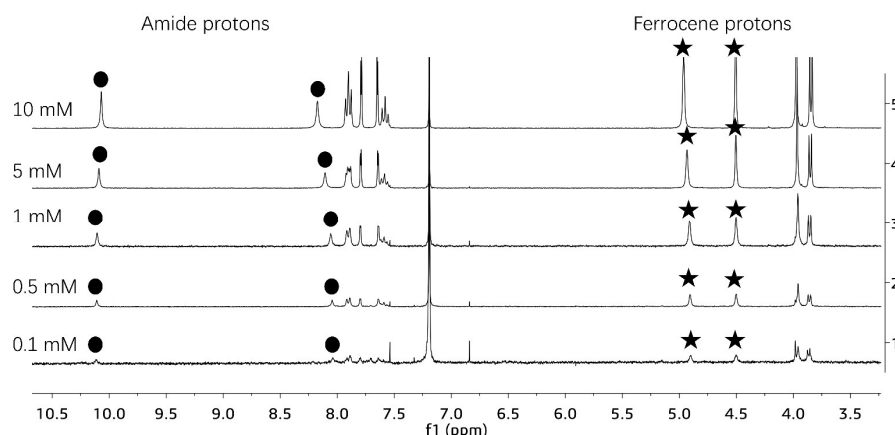


Figure 3.2.2 Partial ^1H NMR spectrum of **3.002** in Chloroform-*d* at different concentration. Black balls represent amide protons and pentagrams represent ferrocene protons.

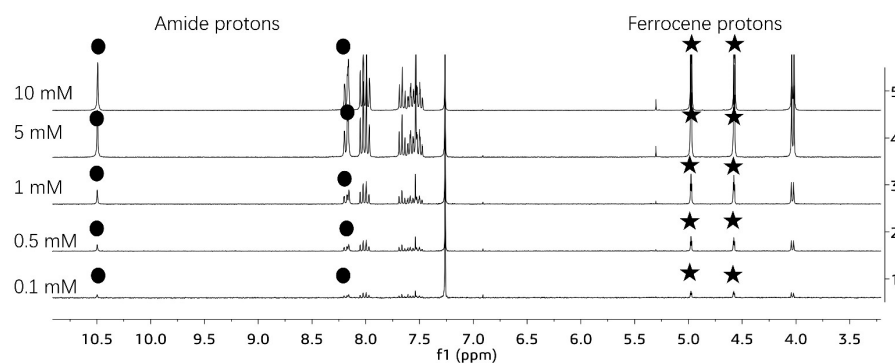


Figure 3.2.3 Partial ^1H NMR spectrum of **3.003** in Chloroform-*d* at different concentration. Black balls represent amide protons and pentagrams represent ferrocene protons.

As solvent can play an important role in stabilizing the stacked conformation, the NMR spectra of the oligomers were also taken in more polar solvents. This can potentially have two opposing effects. First, in more polar solvents a solvophobic effect could potentially increase the stability of the stacked conformation and lead to a slowing of the fluxional process. However, a second possibility is that the more polar solvent weakens the electrostatic component of the π -stacking leading to a less favorable stacked conformation. Based on this, proton NMRs for **3.002** were further carried out in chloroform-*D*, acetone-*D*₆, acetonitrile-*D*₃ at 1 mM and DMSO-*D*₆ as well as for **3.003** in chloroform-*D* and DMSO-*D*₆ at 1 mM, respectively. These are shown in Figure 3.2.4 and 3.2.5.

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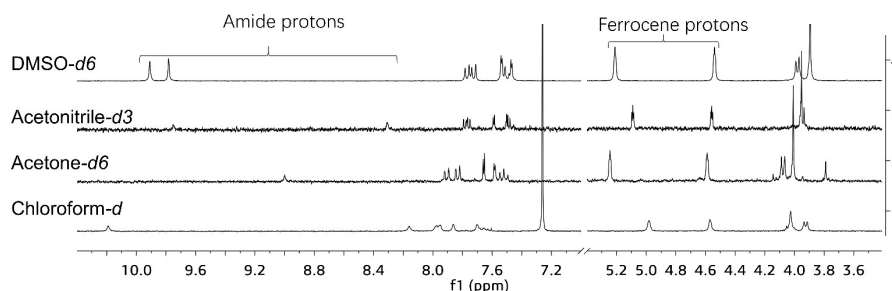


Figure 3.2.4 Partial NMR spectrum of **3.002** in solvents of chloroform-D, acetone-D6, acetonitrile-D3 and DMSO-*d*6 at 1mM.

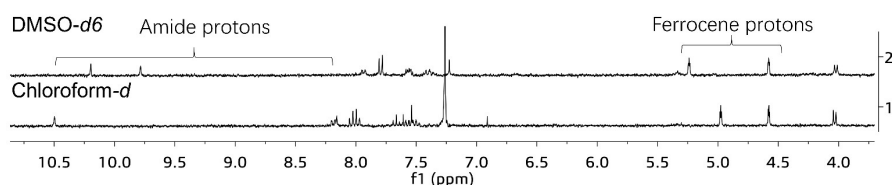


Figure 3.2.5 Partial NMR spectrum of **3.003** in solvents of chloroform-D and DMSO-*d*6 at 1mM.

3.002 showed only partial solubility in acetone and acetonitrile resulting in less intense signals. In all cases, the ferrocene protons did not show any splitting and the only major changes, *i.e.* shifting of signals, were attributed to solvent effects. Thus, the molecules remain reasonably fluxional even in more polar solvents. Nevertheless, several observations for **3.002** and **3.003**, indicate that the folded stacked conformations are important in solution as well.

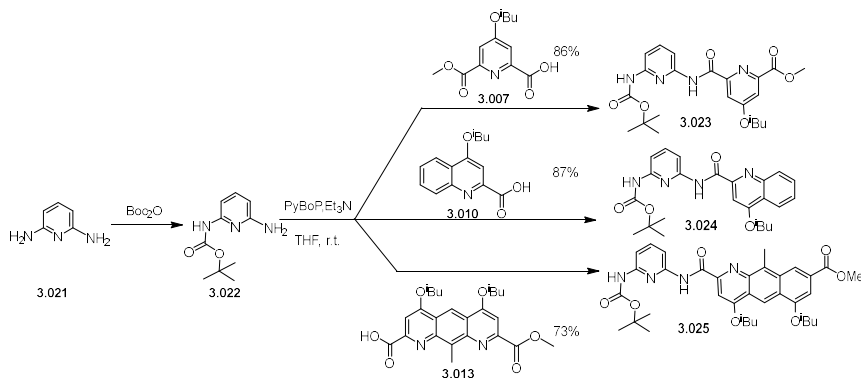
2.3.2 Comparison with Aromatic Dimers

A protons chemical shift will be influenced by its environment and in dynamic systems the extent of this influence from a specific conformation will be related to the prevalence of this conformation.^[3] Notably, aromatic ring current effects, which should lead to a shielding of the proton signals, should be quite noticeable for stacked aromatic systems.^[4] For oligomers **3.002**, **3.003** and **3.004**, stacked conformations in solution should cause a shielding effect compared to their single strands. For comparison of their chemical shifts, pyridine-pyridine dimer **3.023**, pyridine-quinoline dimer **3.024** and pyridine-diazaanthracene dimer **3.025** were synthesized as single strands.

Aromatic dimer **3.023** was obtained followed the reported literature method.^[5] Aromatic dimers **3.024** and **3.025** were obtained by reacting **3.022** with **3.010** and **3.013** respectively in the presence of PyBoP and Et₃N. All of those three

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dimers showed good solubility in chloroform and dichloromethane.



Scheme 3.2.3 synthetic route of dimer **3.023** to **3.025**

Proton NMR of the three dimers were carried out in chloroform-*d* at 1 mM. The comparison of dimers with their corresponding ferrocene oligomers are given in figure 3.2.6 to figure 3.2.8. Compared to their free dimers, chemical shifts for the oligomers' aromatic protons are obviously more upfield, suggesting more shielding. Considering the results of the NMR concentration study of **3.002** and **3.003** which showed limited intermolecular aggregation at 1mM, the increased shielding observed in **3.002**, **3.003** and **3.004** should come from the intramolecular stacking of the strands. Additionally, the magnitude of the upfield shift range of the aromatic protons (figure 3.2.8 > figure 3.2.7 > figure 3.2.6) may suggest a larger preference for strand stacking in the ferrocene oligomers may be correlated with the size of the aromatic parts in each strand.

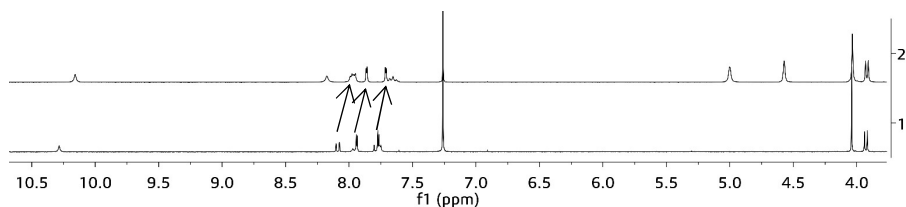


Figure 3.2.6 Partial NMR spectrum of **3.002** (up, 1 mM) with **3.023** (down, 1 mM) in chloroform-*d*.

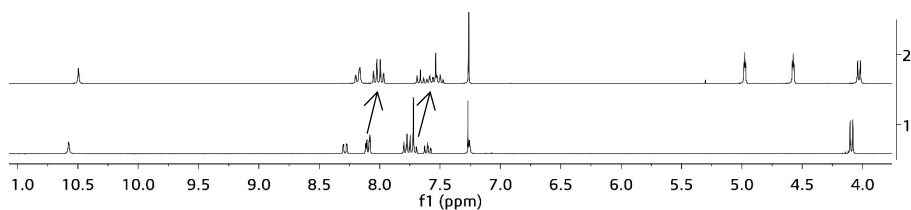


Figure 3.2.7 Partial NMR spectrum of **3.003** (up, 1 mM) with **3.024** (down, 1 mM) in chloroform-*d*.

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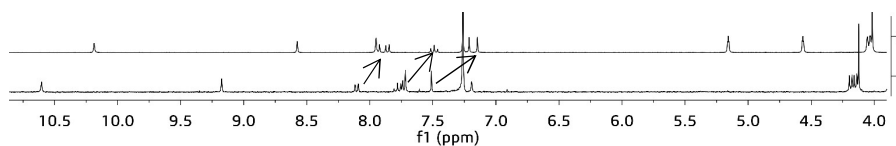
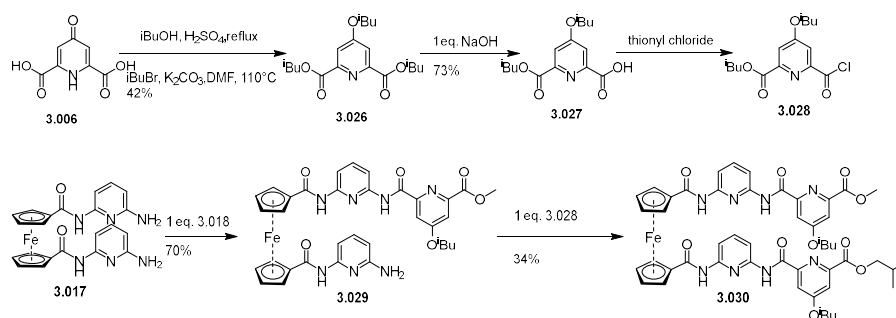


Figure 3.2.8 Partial NMR spectrum of **3.004** (up, 1 mM) with **3.025** (down, 1 mM) in chloroform-*d* .

2.3.3 ROESY Study

To further look at the conformation in solution, an asymmetric ferrocene aromatic oligoamide, **3.030**, was synthesized for 2D NMR studies. Pyridine monomer **3.028** was synthesized through the same procedure as **3.007**, using *iso*-butanol for the esterification. **3.017** was first reacted with 1 equivalent of **3.018** in anhydrous THF to give the mono-coupled product **3.029**. Then **3.029** was further reacted with **3.028** to obtain the final asymmetric oligomer **3.030**. The yield of getting **3.030** from **3.017** is 21% which is acceptable considering the difficulties we met in the synthesis of **4.005** and **4.006** which are also asymmetric oligomers showed in chapter IV.



Scheme 3.2.4 Synthetic route of asymmetric oligomer **3.030**

^1H - ^1H ROESY NMR experiments were conducted on **3.030**. Weak NOE correlations detected between H_a and H_b supporting the stacked conformation. The weakness of the correlations could be attributed to two possible reasons, first the motions of oligomer **3.030** in chloroform is rapid which limits the interaction between these protons. This could result from the movement of ferrocene part and the movement of ester part. Second, the size of our molecule is 968 g/mol for which the NOE_{max} is expected to be relatively weak. Still, the ^1H - ^1H ROESY spectrum of asymmetric oligomer **3.030** supports that the oligomer adopts a conformation in solution that places the two esters in proximity of each other in chloroform. Combined with the evidence from the shielding effects from the proton NMR comparison between the ferrocene oligomers with their corresponding single strands, the stacked conformation in the ferrocene oligomers should exist in solution as well.

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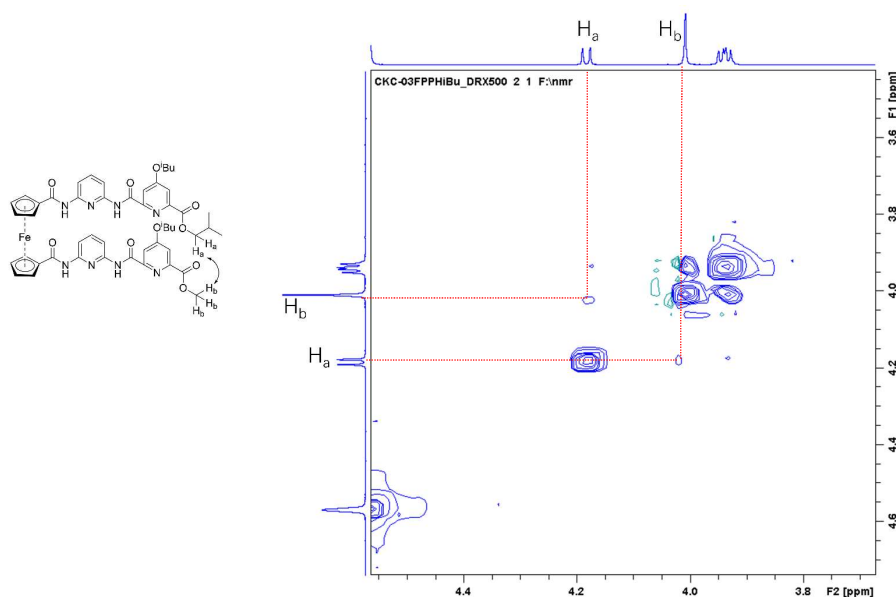


Figure 3.2.10 ^1H - ^1H ROESY spectrum of asymmetric ferrocene conjugate **3.030** at 1 mM in chloroform- d , chemical shift of methylene proton of isobutyl ester is located at 4.2 ppm (H_a), chemical shift of methyl proton of methyl ester is located at 4.0 ppm (H_b)

2.3.4 Temperature-dependent Proton NMR Study

Proton NMR spectra of **3.002**, **3.003** and **3.004** at room temperature showed only two signals for the Cp protons whereas a total of four peaks would be expected given their crystal structures. As mentioned before, this is attributed to the rapid movements of the molecules. Lower temperature may slow down those movements and lead to the splitting of the Cp signals.^[6] Variable temperature NMR spectra from 183 K to 253 K of oligoamide **3.002** and **3.003** are shown below. Unfortunately, no splitting of the Cp peaks occurred within the temperature range studied. Because of solubility of **3.004** decreasing the temperature only led to its precipitation, making it not possible to study by low-temperature proton NMR. For **3.002** and **3.003**, the most obvious change with decreasing temperature is that the aromatic signals of both spectra are upfield shifted. While we could attribute this to the tendency of the two strands to overlap more at lower temperature, the general dependence of chemical shift on temperature makes this at best speculative. Still, it is interesting to note that the pyridine oligomer shows substantially more broadening of the signals at low temperature than the quinoline containing oligomer.

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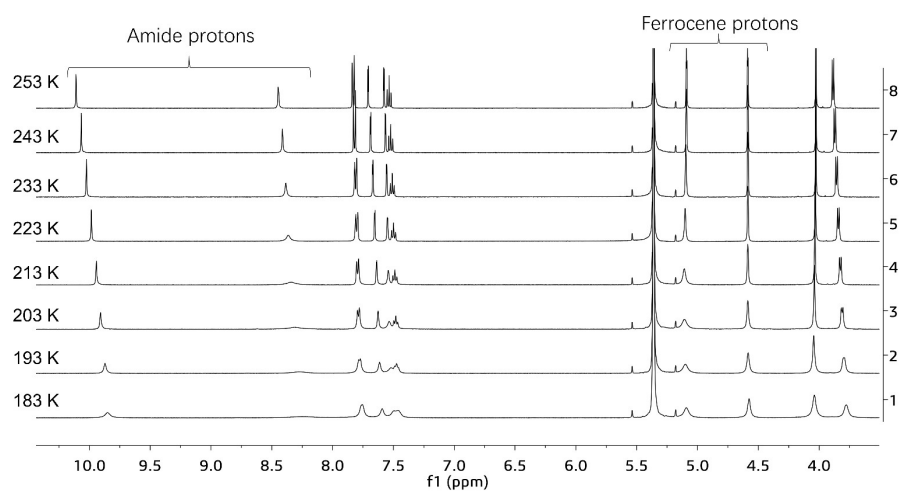


Figure 3.2.11 Temperature dependent ^1H NMR of **3.002** (2 mM) in CDCl_3 , from top to bottom: 253K, 243K, 233K, 223K, 213K, 203K, 193K, 183K.

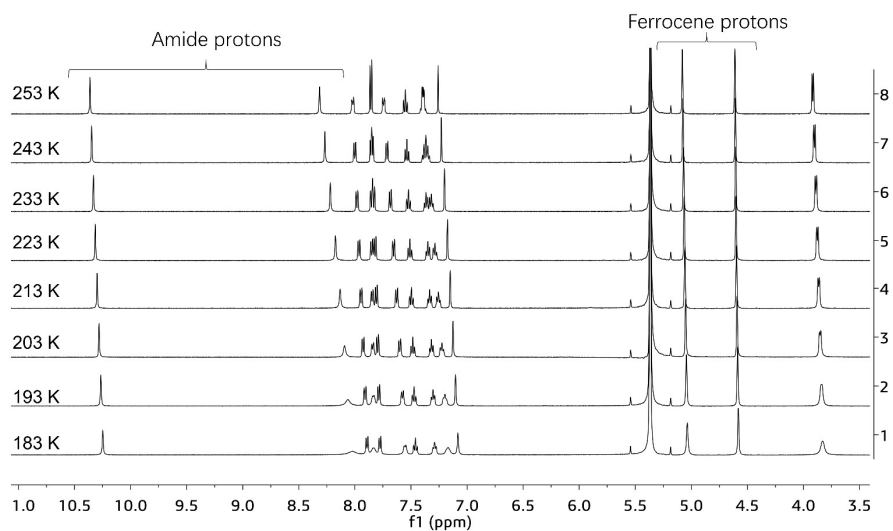


Figure 3.2.12 Temperature depended ^1H NMR of **3.003** (2 mM) in CDCl_3 , from top to bottom: 253K, 243K, 233K, 223K, 213K, 203K, 193K, 183K.

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3. Host-guest evaluations

As one the most important moieties of protein secondary structures, β -sheets contain locally well-defined conformations. One of the key features for this kind of conformations is that they can uniquely binding specific molecules. Crystal structures of our ferrocene oligomers clearly show compact conformations, with curves containing potential H-bond donors and acceptors. Solution studies also support a preference for this folded conformation. Structural analyses indicate these could act as molecular clefts, able to interact with small molecules with suitable H-bonds donors or acceptors. This led us to study their host-guest properties in order to show that the cleft-like structures can be potentially functional. Additionally, interaction with a guest could potentially further stabilize the folded structure in solution.

Nitrobenzene, benzoic acid, catechol and resorcinol, due to their differently spaced H-bond acceptor/donor groups, were chosen for testing the guest binding abilities of our ferrocene β -sheet structures. Because of the solubility of **3.004** and as the methyl group on the diazaanthracene could be an obstacle for the guest interacting inside of the curvature, host-guest evaluations of ferrocene oligomers were only carried out with **3.002** and **3.003**.

3.1 NMR titrations

Initial tests using **3.002/3.003** with Nitrobenzene/benzoic acid/catechol/resorcinol were conducted first. Chemical shifts of amide protons on **3.002/3.003** were chosen for evaluation. The results showed that the host-guest systems were all in fast exchange on the NMR timescale. Neither **3.002/3.003** with nitrobenzene, nor **3.002** with benzoic acid showed obvious chemical shift changes of their amide protons. However, for **3.002** with catechol/resorcinol and **3.003** with benzoic acid/catechol/resorcinol significant changes were observed suggesting host-guest interactions for each pair. Further full titrations were performed on these host-guest systems.

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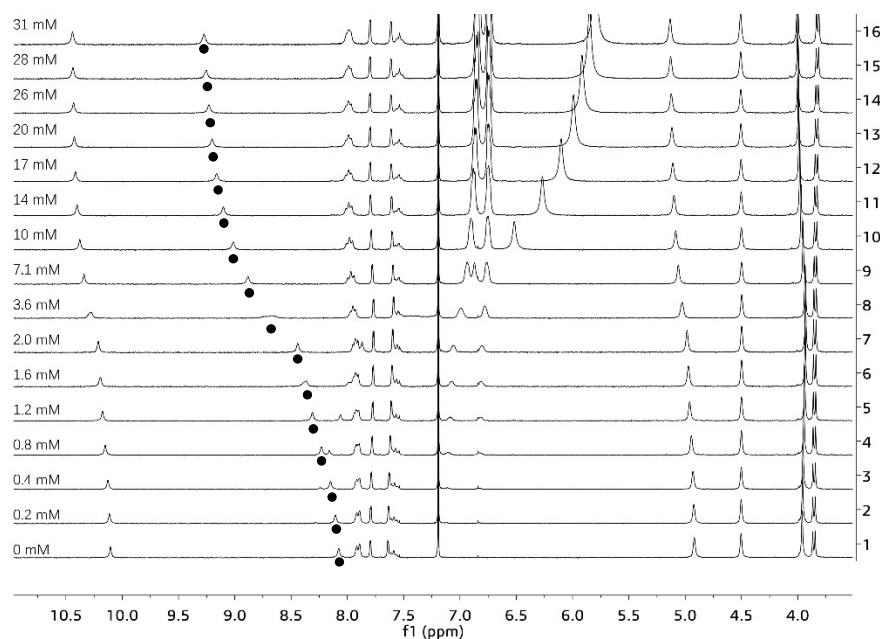


Figure 3.3.1 ^1H NMR spectra recorded during the titration of **3.002** (2 mM) with catechol in $\text{Chloroform-}d$ at 300 MHz $K_a = 238 \text{ M}^{-1}$. Chemical shifts of amide protons pointed out by black balls were chosen for calculated.

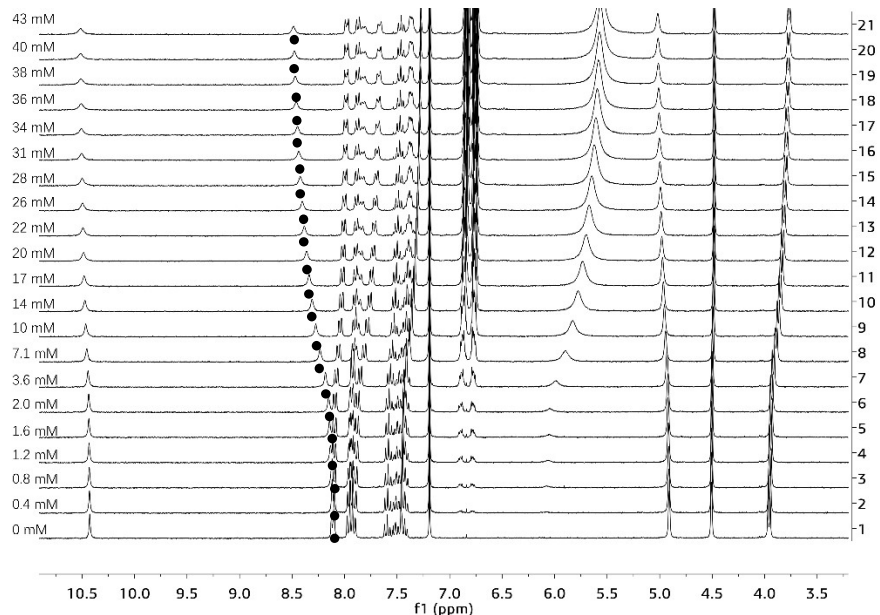


Figure 3.3.2 ^1H NMR spectra recorded during the titration of **3.003** (2 mM) with catechol in $\text{Chloroform-}d$ at 300 MHz $K_a = 40 \text{ M}^{-1}$. Chemical shifts of amide protons pointed out by black balls were chosen for calculated.

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NMR titrations of **3.002** and **3.003** with catechol are shown in figure 3.3.1 and 3.3.2, respectively. A number of protons exhibited chemical shift changes with increasing amount of the guest molecules. For **3.002** with catechol, one of the most obvious changes is that the amide protons shift downfield by 1.19 and 0.32 ppm for the amide proton next to ferrocene (8.09 ppm) and between pyridines (10.12 ppm) respectively. Catechol with **3.003** system does not produce as strong of a shift. For the same amide protons, shifts of 0.39 ppm and 0.07 ppm were detected. The downfield shift for the amides in both cases is consistent with their participation in hydrogen bonding. Additionally, small upfield shifts are observed for the aromatic protons on both oligomers during the titration. Plots of the chemical shift changes in **3.002** and **3.003** versus equivalents of catechol could be fit well to a 1:1 host-guest systems with association constant values of 238 and 41 M⁻¹, respectively. Although not high, to our best knowledge, the association constant for **3.002** with catechol still ranks as one of the highest reported for cleft molecules with this guest.^[7]

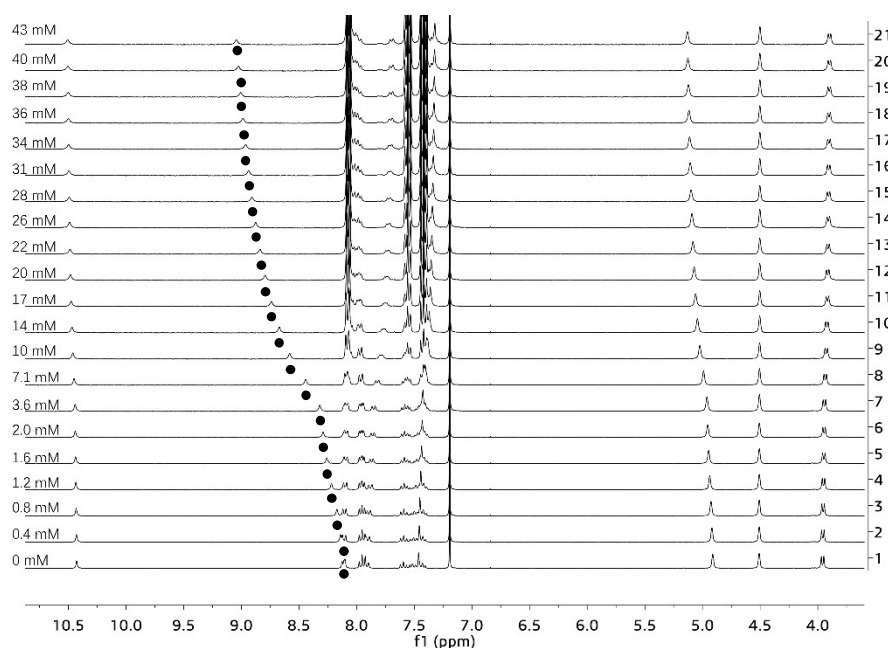


Figure 3.3.3 ¹H NMR spectra recorded during the titration of **3.003** (2 mM) with benzoic acid in Chloroform-*d* at 300 MHz $K_a = 108 \text{ M}^{-1}$. Chemical shifts of amide protons pointed out by black balls were chosen for calculated.

Titration of **3.003** with benzoic acid was carried out and the spectra are shown in figure 3.3.3. Increasing equivalents of benzoic acid results in significant downfield shift of amide protons with the values of 0.95 and 0.08 ppm for ferrocene amide and quinoline amide, respectively. Obvious upfield chemical shift changes were also observed for the aromatic protons on the oligomer,

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suggesting similar behavior of this host-guest system as for catechol. Similarly, the data with benzoic acid also fit with 1:1 binding with an association constant of 108 M^{-1} .

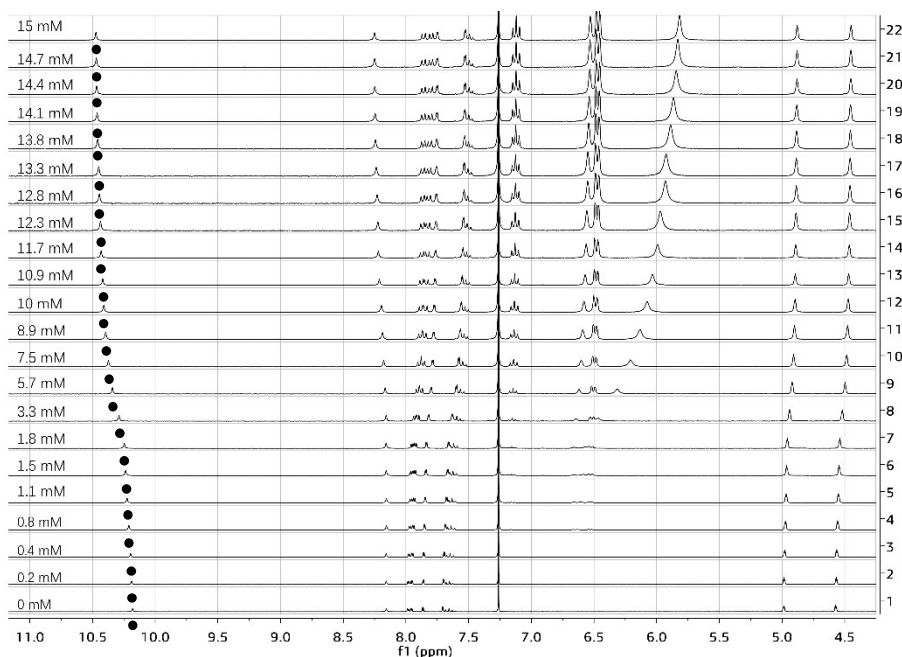


Figure 3.3.4 ^1H NMR spectra recorded during the titration of **3.002** (2 mM) with resorcinol in $\text{Chloroform-}d$ at 300 MHz $K_a = 80 \text{ M}^{-1}$. Chemical shifts of amide protons pointed out by black balls were chosen for calculated.

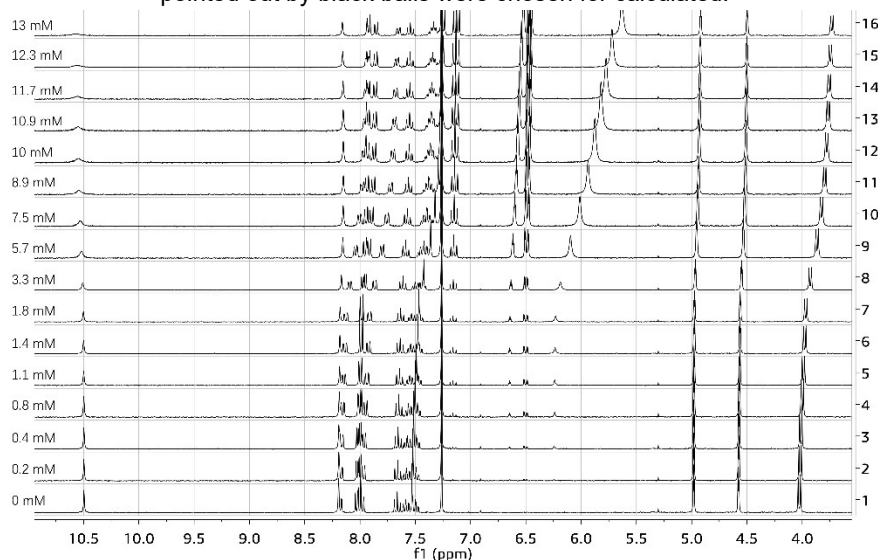


Figure 3.3.5 ^1H NMR spectra recorded during the titration of **3.003** (2 mM) with resorcinol in $\text{Chloroform-}d$ at 300 MHz.

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NMR titration spectra of **3.002/3.003** with resorcinol host-guest pairs are shown in figure 3.3.4 and 3.3.5. Several proton peaks shifted obviously with increasing concentrations of resorcinol. Notably, with resorcinol as the guest molecule, chemical shift changes for the amide protons are different than with the other guests. The ferrocene amide protons ($\Delta\delta = 0.09$ ppm in **3.002** and 0.03 ppm in **3.003**) shifted less than the other amide protons ($\Delta\delta = 0.29$ ppm in **3.002** and ppm 0.06 in **3.003**). This difference suggests that resorcinol may bind in a different fashion to the hosts. Fitting of the titration data showed **3.002** with resorcinol was also consistent with a 1:1 binding model with an association constant of 80 M^{-1} . However, the data for **3.003** with resorcinol failed to fit to 1:1, 2:1, or 1:2 binding models and gave large errors for the association constants. This may be due to competition between multiple binding sites with **3.003** resorcinol as described below.

In addition to the above host-guest systems, NMR titration of pyridine-pyridine dimer **3.023** with catechol/resorcinol were also performed. Certain interactions were detected during the addition of catechol to the pyridine dimer and the result is consistent with 1:1 binding model with a binding constant of 115 M^{-1} (figure 3.3.6), while no interaction was observed with resorcinol (figure 3.3.7). These results further suggest the important role of two strands in the oligomer.

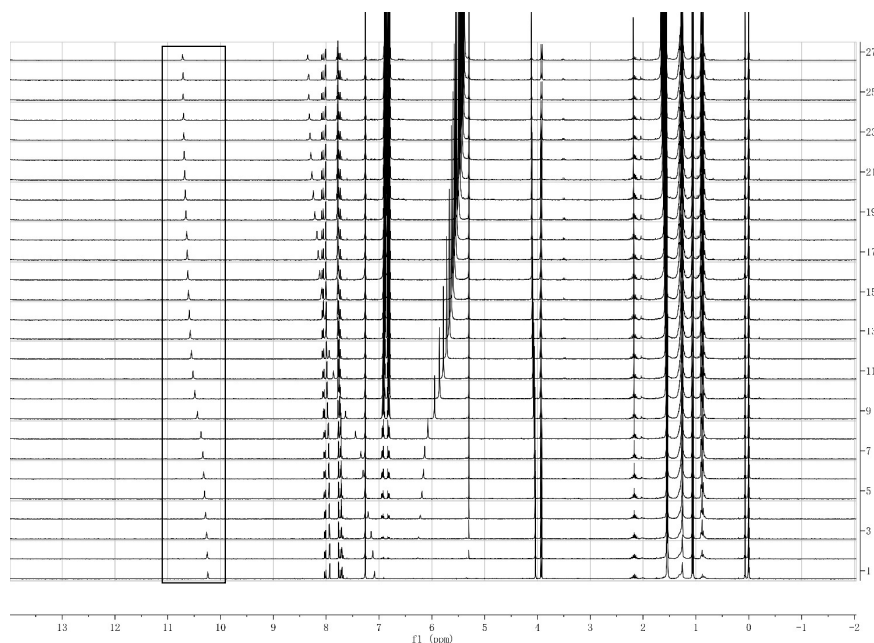


Figure 3.3.6 ^1H NMR spectra recorded during the titration of pyridine dimer **3.023** (2 mM) with catechol in Chloroform-*d* at 300 MHz. Chemical shifts of amide protons pointed out by black box were chosen for calculated

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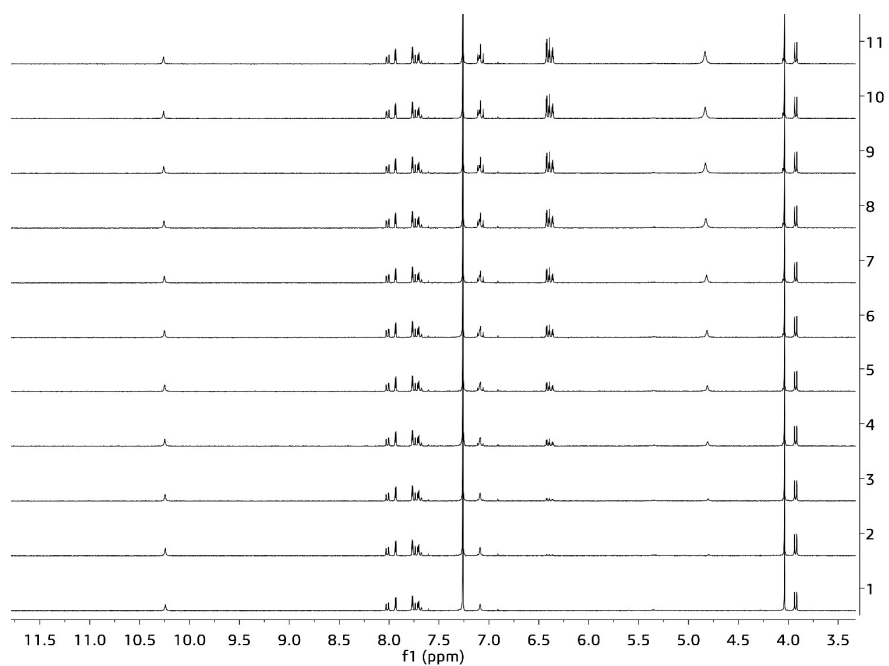


Figure 3.3.7 Part of ^1H NMR spectra recorded during the titration of pyridine dimer **3.023** (2 mM) with resorcinol in Chloroform- d at 300 MHz. No interaction was observed.

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3.2 NOESY studies

From the titrations, the observation that both amides of the hosts are potentially participating in H-bonding interactions with the guest molecules suggests an in cleft binding mode. To further look at how the hosts and guests interact with each other, they were further studied by ^1H - ^1H NOESY NMR.

The ^1H - ^1H NOESY spectrum of **3.002** with catechol is shown below. Correlations between the 3-/6-position protons of the catechol with the protons of the Cp rings and with methyl esters were observed. Additionally, correlations are also observed between the 4-/5-position protons and the methyl esters. Combined with the chemical shifts observed during titrations, it strongly supports that the catechol is bound in the cleft of **3.002** in the sheet-like conformation.

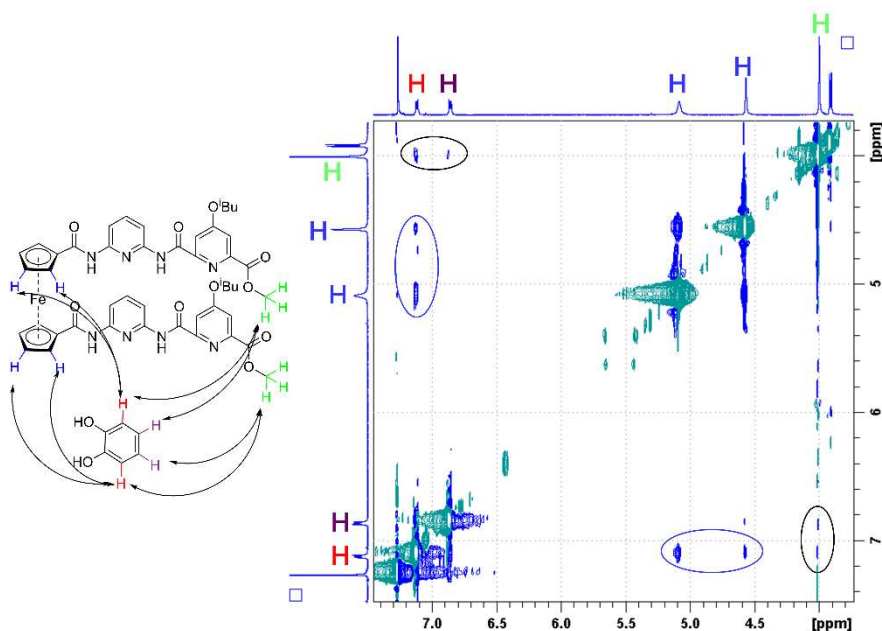


Figure 3.3.8 Part of ^1H - ^1H NOESY spectrum of ferrocene conjugate **3.002** (1mM) with catechol (1mM). Key protons were pointed out by different color.

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Likely due to the weak binding interaction, the ^1H - ^1H NOESY spectrum for **3.003** with catechol does not show any correlations between the host and guest molecules. Based on HMBC, HMQC, COSY as well as the proton NMR spectra, signals of **3.003** with benzoic acid were attributed to the specific protons. However, too much overlap for the peaks of host and guest molecules makes it difficult to draw definite conclusions as for **3.002** with catechol.

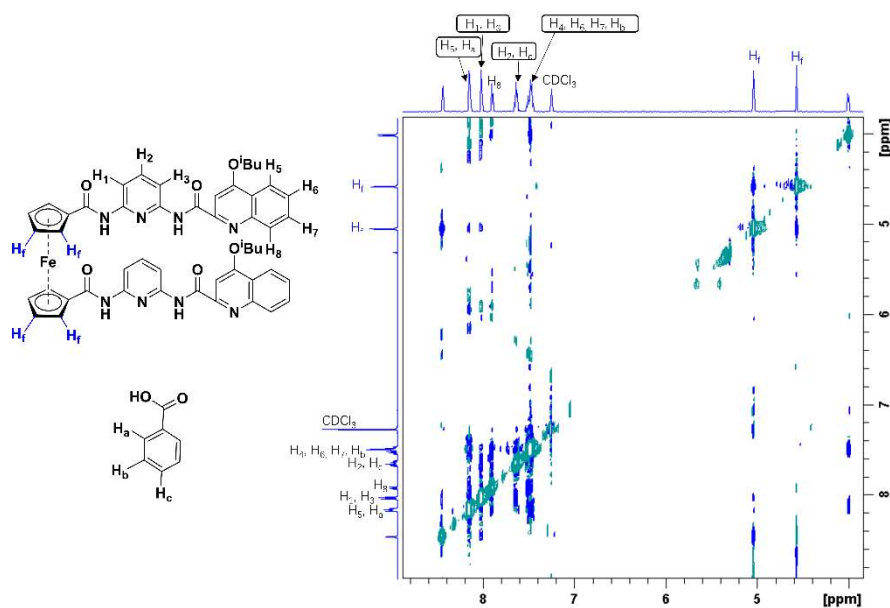


Figure 3.3.9 Part of ^1H - ^1H NOESY spectrum of ferrocene conjugate **3.003** (1mM) with benzoic acid (1mM). Key protons were pointed out by different number.

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4. Cyclic Voltammetry

One of the key features for ferrocene is its excellent reversible one-electron Fc/Fc^+ redox couple. This feature coupled with the host-guest chemistry can be of interest for electrochemical sensing of small molecules. Additionally, the redox reactivity of ferrocene is ubiquitous in many fields including development of organometallic medicine, catalysis and molecular machines.

To study how our foldamers influence the redox properties of the ferrocene moiety, the redox properties of **3.002**, **3.003** and **3.004** were investigated by cyclic voltammetry (CV). The measurements were performed in dry and degassed dichloromethane with 0.1 M NBu_4PF_6 as the supporting electrolyte. Measurements used a glassy carbon working electrode, Pt wire as counter electrode and a silver/silver nitrate reference electrode. For all the electrochemical experiments, foldamer concentrations were 1 mM in DCM solution and scan rates of 10 mV/s, 50 mV/s, 100 mV/s, 250 mV/s and 500 mV/s were used. Ferrocene was added as internal reference.

Four parameters can be observed from the voltammograms which are anodic peak potential (E_a), current (i_a), cathodic peak potential (E_c) and current (i_c). From this, the half-wave potential ($E_{1/2}$), peak to peak separation (ΔE_p) and current ratio (i_a/i_c) can be calculated.

The CV data, voltammograms and redox parameters, for the foldamers with different scan rates are shown in the figures and tables below.

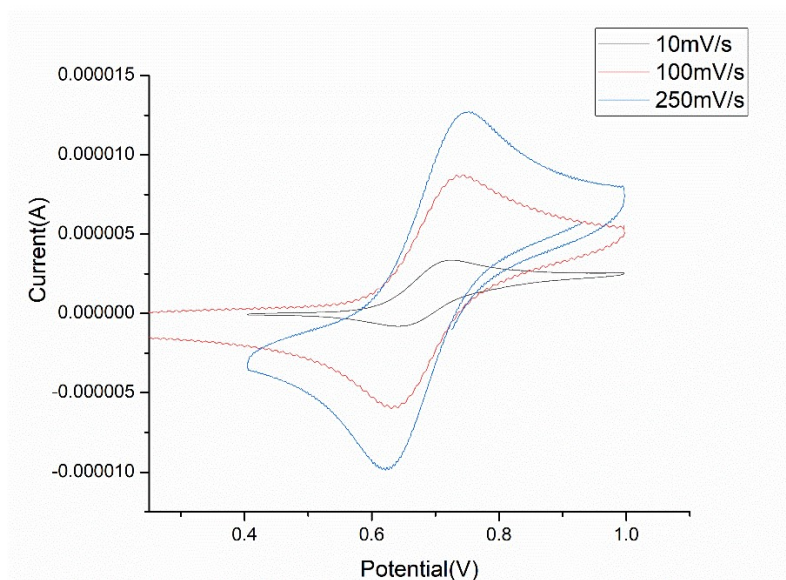


Figure 3.4.1 Cyclic Voltammograms showing the reversible oxidation for foldamer **3.002** at different scan rates

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Table 3.4.1 Redox parameters for foldamer **3.002** ^a

Foldamer	E_a	E_c	i_a	i_c	i_a/i_c	$E_{1/2}$	ΔE_p
10 mV/s	0.72	0.64	2.30	1.73	1.33	0.69	0.08
100 mV/s	0.74	0.63	8.51	6.17	1.38	0.69	0.09
250 mV/s	0.76	0.62	12.76	10.52	1.21	0.69	0.08
Ferrocene ^b	0.27	0.18	6.53	6.06	1.08	0.23	0.09

a: The unit for potential is V; the unit for current is μA ; the parameter for ferrocene as internal stranded was collected at 100 mV/s

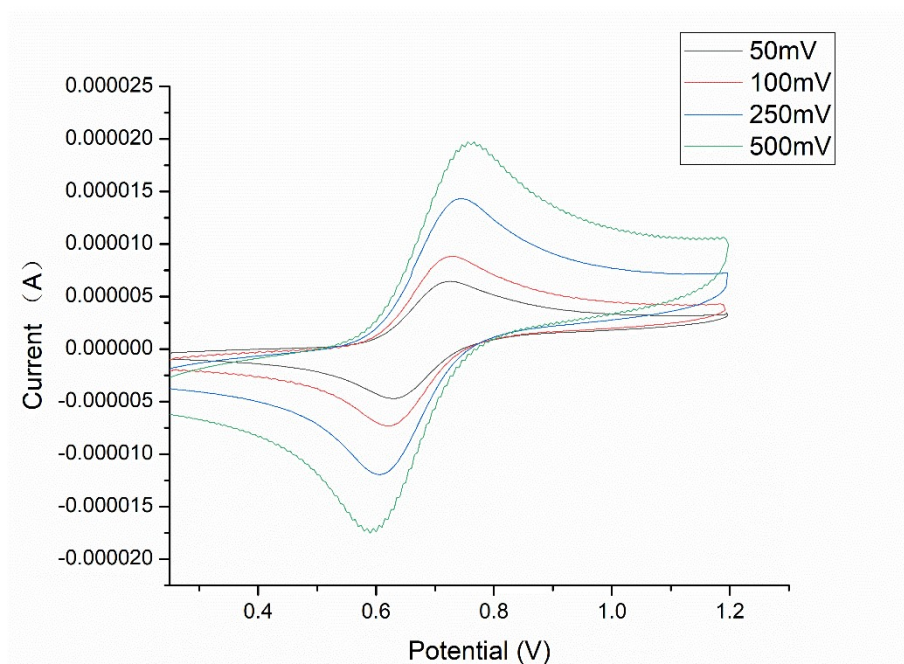


Figure 3.4.2 Cyclic Voltammograms showing the reversible oxidation for foldamer **3.003** at different scan rates

Table 3.4.2 Redox parameters for foldamer **3.003** ^a

Foldamer	E_a	E_c	i_a	i_c	i_a/i_c	$E_{1/2}$	ΔE_p
50 mV/s	0.73	0.63	6.21	4.88	1.27	0.68	0.10
100 mV/s	0.72	0.62	9.56	7.64	1.25	0.67	0.10
250 mV/s	0.74	0.61	14.68	12.03	1.22	0.68	0.13
500 mV/s	0.77	0.59	21.79	17.51	1.3	0.68	0.18
Ferrocene ^b	0.28	0.17	10.5	9.27	1.13	0.23	0.09

a: The unit for potential is mV; the unit for current is μA ; the parameter for ferrocene as internal stranded was collected at 100 mV/s

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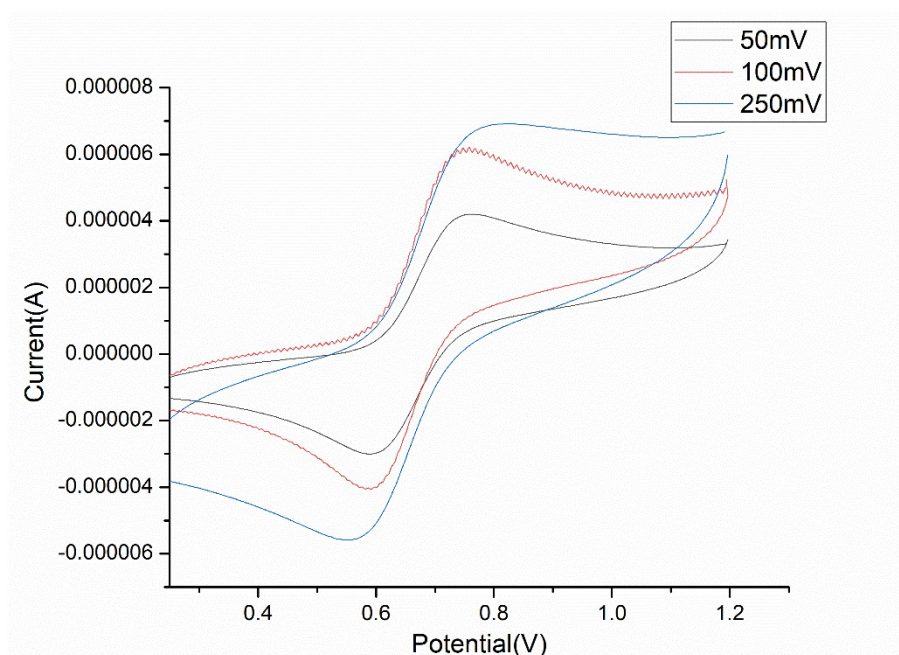


Figure 3.4.3 Cyclic Voltammograms showing the reversible oxidation for foldamer **3.004** at different scan rates

Table 3.4.3 Redox parameters for foldamer **3.004** ^a

Foldamer	E_a	E_c	i_a	i_c	i_a/i_c	$E_{1/2}$	ΔE_p
50 mV/s	0.75	0.59	4.21	3.05	1.38	0.67	0.16
100 mV/s	0.75	0.60	5.92	4.15	1.41	0.68	0.15
250 mV/s	0.80	0.55	6.95	5.58	1.25	0.67	0.33
Ferrocene ^b	0.27	0.18	8.65	8.19	1.09	0.23	0.09

a: The unit for potential is mV; the unit for current is μA ; the parameter for ferrocene as internal standard was collected at 100 mV/s

For all three oligomers, a single reversible oxidation event is observed. This was evidenced by the different electrochemical parameters. The reversibility was evidenced from the electrochemical parameters. The current ratio (i_a/i_c) for the three foldamers is slightly over 1 but not exceptionally higher. The peak separations (ΔE_p) are above the ideal 57 mV for a reversible wave, however, larger values are common, especially in non-polar solvents. Peak to peak separation values of ferrocene and **3.002** and **3.003** are similar, suggesting the stable redox reversibility of these oligomers. The only exception is oligomer **3.004**, for which the values deviate significantly from the ferrocene standard.

Compared to ferrocene, the half-wave potential for all three oligomers is

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obviously higher, consistent with the electron withdrawing nature of the carboxyl groups on the Cp rings and indicating that the oxidation of the oligomers is more difficult. Within the series almost no change in the oxidation potential is observed. This shows that the nature of the aromatic groups does not influence the electronic properties of the ferrocene moiety. Thus, changes in the fluxional properties in the series should not be a result of electronic changes at the ferrocene, but rather due to interactions between the strands.

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5. Computational Studies

Density functional theory (DFT) is one of the most widely used computational quantum mechanical modeling methods to investigate the electronic structure of molecules. For our designed foldamer, DFT could reveal their relationship between energy and conformation, of which theoretically explain and predict preference structure.

5.1 Geometry and Energy Optimization

DFT calculation of **3.002**, **3.003** and **3.004** using Gaussian09^[8] were first applied to obtain the minimized geometries with functional of B3LYP-D3BJ^[9] and basis sets of 6-311G**^[10] for C, H, O, N and Lanl2dz^[11] for Fe, respectively. Single point energies were then obtained for the optimized geometries with the functional and cc-PVTZ^[12] basis set, which has been shown to give better minimal in the literature.^[13] Figure 3.5.1 shows the calculated structures for the molecules compared with their crystal structures. Calculated structures matched well with the crystal structures and exhibited the same stacked conformation of the strands with only minor differences observed in the isobutyl side-chains and methyl ester groups.

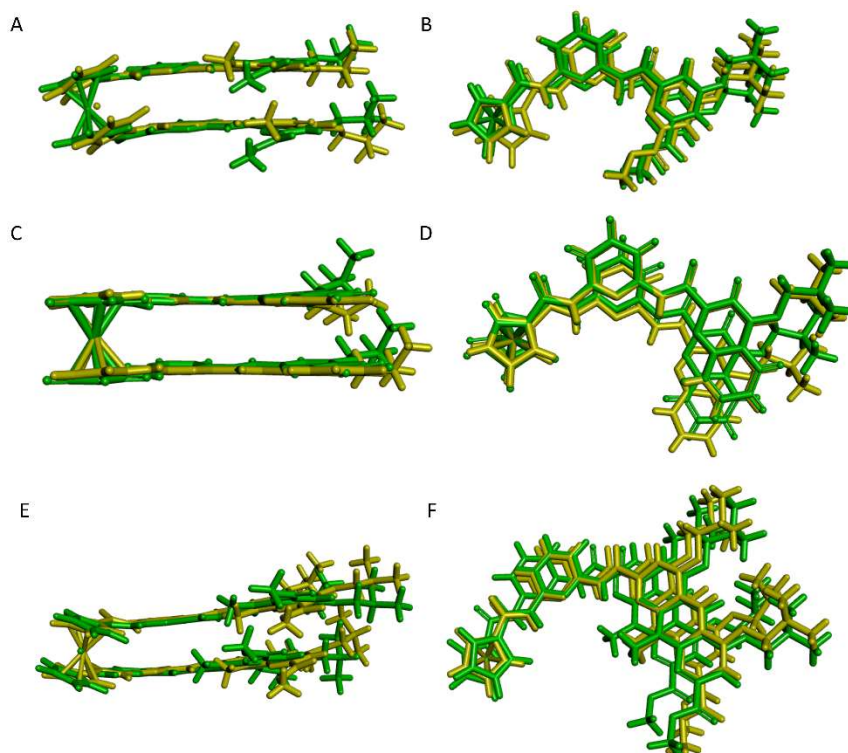


Figure 3.5.2 Side (A, C, E) and top (B, D, F) view of overlap of the crystal structure (yellow) and optimized structure (green) of ferrocene oligomers

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In the series, the electrostatic potential maps support the idea that the clefts can function as potential guest binding sites. Figure 3.5.2 shows the electrostatic potential maps of **3.002** and **3.003**, which were calculated with Gaussian and created multiwfn^[14]. Those maps show the surface of clefts in both **3.002** and **3.003** contains several regions of positive and negative electrostatic potential which correspond to the expected binding site for guests. Regions outside of the cleft, notably the amide carbonyl oxygens, also show negative electrostatic potentials and so could also be potential binding sites. Moreover, for **3.002**, negative regions were also detected of the terminal ester carbonyl oxygens and may be another potential binding site for stabilizing guest molecules. Additionally, from the electrostatic potential maps it is observed that the cavity of **3.002** is expected to be smaller than **3.003**.

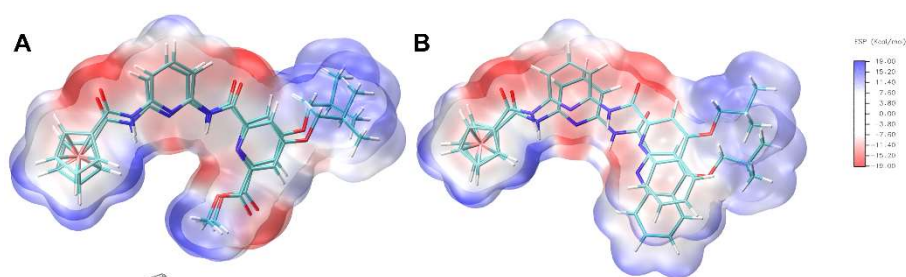


Figure 3.5.2 Electrostatic potential maps of **3.002** and **3.003**. ESP were calculated by Gaussian and draw by multiwfn. Positive potential is shown in blue and negative showed in red.

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5.2 DFT Study on Ferrocene Rotation and Strand flipping

Although geometry optimization of three foldamers show the stacked conformation, having started from the crystal structure coordinates this does not imply that this is a global minimum. Importantly, open structures resulting from rotation of the Cp rings, or head-to-tail conformations resulting from amide rotation could give lower energies. This was studied using **3.002** by manually changing the dihedral angle of the ferrocene by 10° over 360° and then performing a full geometry optimization with the same functional and basis sets above mentioned. After optimization, 29 structures are successfully obtained.

Table 3.5.1 Energies of **3.002** in different rotation states

Original dihedral angles	Dihedral angles after optimization	Energy (kJ/mol)	Energy difference with crystal structure (kJ/mol)
10	3.66	-11121971.00	1.59
20	15.38	-11121972.24	0.35
30	15.37	-11121972.24	0.34
40	15.37	-11121972.24	0.34
50	15.39	-11121972.24	0.34
60	15.41	-11121972.23	0.35
70	55.08	-11121920.96	51.62
80	55.11	-11121920.96	51.63
100	71.62	-11121916.24	56.34
110	71.61	-11121916.24	56.34
120	71.62	-11121916.24	56.34
140	127.55	-11121898.62	73.97
150	127.58	-11121898.94	73.64
170	108.31	-11121899.67	72.92
180	127.57	-11121898.94	73.64
190	232.77	-11121899.00	73.58
200	232.78	-11121899.00	73.58
210	205.95	-11121890.33	82.25
240	232.8	-11121899.00	73.58
250	248.55	-11121888.94	83.64
260	288.59	-11121916.14	56.44
270	253.3	-11121896.83	75.76
290	304.88	-11121921.07	51.52
300	304.87	-11121921.07	51.52

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310	333.76	-11121964.61	7.97
320	333.76	-11121964.60	7.97
330	333.78	-11121964.61	7.97
340	339.41	-11121963.52	9.06
350	358.02	-11121972.92	-0.34
Head to tail conformation	-	-11121950.35	-22.23
optimization structure from crystal	356.53	-11121972.58	0.00

Calculation results are shown in Table 3.5.1. Both the starting dihedral angle and the final (optimized) angles are shown. It should be noted that in some cases these values varied substantially. The most obvious differences being between 6° and 60°. As shown in figure 3.5.3, even starting from different conformations, the dihedral angles of ferrocene moiety in optimized structures are more concentrated to some specific angles including 15°, 55°, 71° and 127°. For all of the structures, the calculated energies are positively correlated with the dihedral angle, indicating that the stacked conformations are more stable. A trend line, figure 3.5.3, for a plot of the dihedral angle versus that energy of each minima shows an increase in energy from 0° to 180°. The highest energy structure is calculated to be more than 80 kJ/mol. As the energy barrier for ferrocene rotation is less than 4 kJ/mol this should not come from the ferrocene^[15]. Thus, these calculations suggest that there is an important stabilizing interaction between the aromatic strands. It should be noted however that these values were obtained from the gas phase calculations and solvation should be expected to greatly influence the energies, likely decreasing the difference in energy between the ground state and open forms. Still, these results fit with the experimental data suggesting that the stacked conformation is a stable structure.

At the same time, strand flipping was also investigated through comparison of head to tail and head to head conformations. These two conformations were optimized though DFT with same functional and basis set mentioned above and the energy difference between them is approximately 22 kJ/mol with head to head conformation favored. This result indicated stacked conformation with parallel dipoles is still the most stable conformation, likely due to it allowing the best overlap between the aromatic rings. Indeed, optimized structures of the head to tail conformation displayed a favorable overlap of the middle pyridines but a large separation between the last pyridines.

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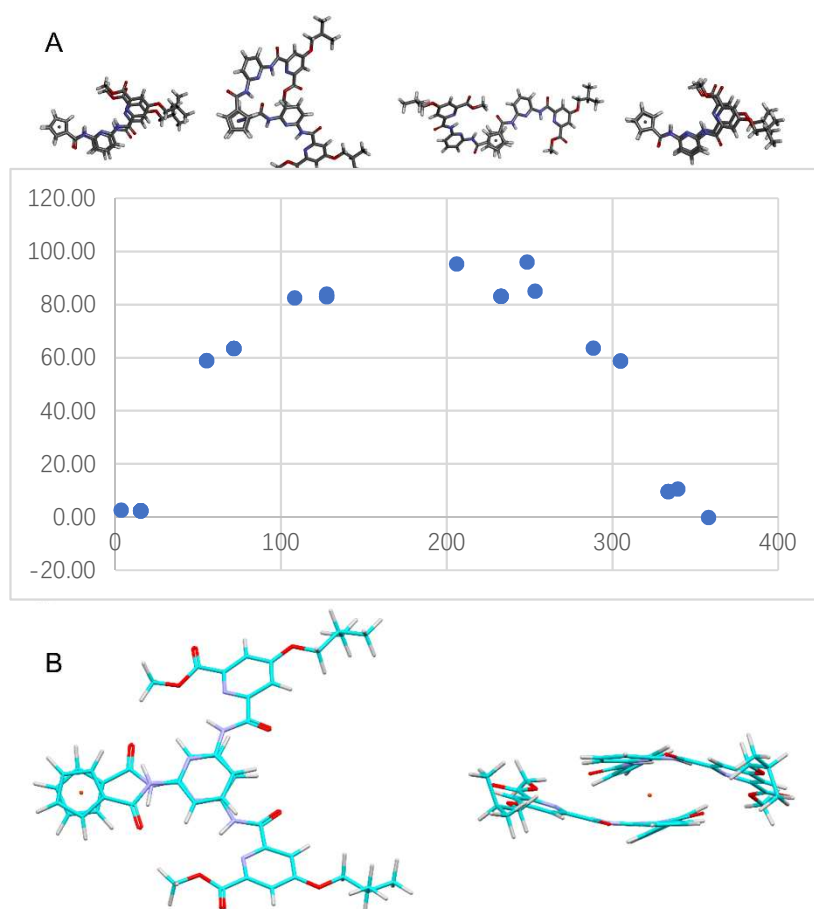


Figure 3.5.3 (A) Energies difference of 1 in different rotation states compared with optimization structure from crystal data, calculated by DFT, 4 representative structures of dihedral angle values of 3.66°, 71.62°, 232.78° and 358.02° were shown at top; (B) minimized structure of head to tail conformation

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5.3 DFT Study on Host-guest Chemistry

Computational studies were also performed to look at the Host-Guest chemistry of the oligomers. To achieve this, we developed a method of progressive optimization of the host-guest pairs observed with the oligomers. The procedure is as shown in figure 3.5.4, Molclus^[16] is used for generating initial configurations. The idea for molclus is the guest molecule will move from the geometry center of host molecule to all directions, when all the atoms of two molecules are placed so that no-unfavorable distances (based on VDW contacts) are experienced, it is recorded as one configuration. This continues until a pre-set number of configurations is obtained. Then those series of configurations were optimized by MOPAC^[17] to remove the highest energy combinations or combine highly similar configurations. The resulting structures were then optimized by DFT using Gaussian in order to compare their energies.

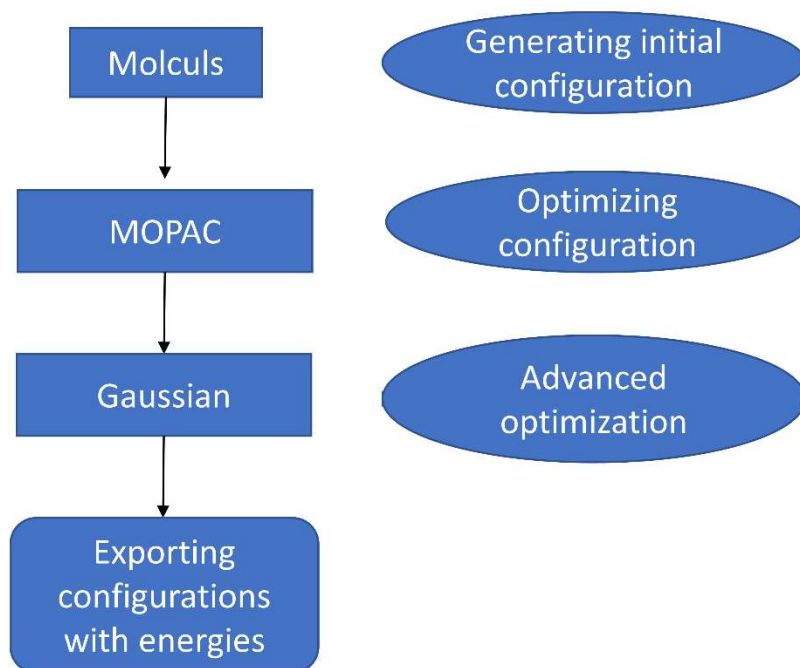


Figure 3.5.4 General procedure for host-guest optimization.

Using the above approach, the five pairs of host-guest molecules were put into molclus to give 100 initial configurations. Then those 100 configurations are optimized by MOPAC to obtain around 45 configurations with the first 20 lowest energy conformations subsequently optimized with Gaussian with the same functional and basis set as above. Finally, 15 to 20 configurations for each system were obtained.

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For the pair of **3.002/3.003** with catechol, the results are listed in table 3.5.2. It shows all of models with catechol located inside of clefts are lower in energy than the ones where the guest is bound outside of the cleft. Energy differences between the guest inside and outside are as larger as 47 kJ/mol for **3.002** and 18 kJ/mol for **3.003**. Additionally, there are two models (gx18 and gc18) where catechol was found inside of clefts but that still show higher energy due to the hydroxyl groups of catechol being oriented away from the cleft and not interacting with the amide protons or endocyclic nitrogens of the host molecules.

Table 3.5.2 Calculated models for ferrocene conjugates **3.002/3.003** with catechol, sorting by energy (Geometry optimization: B3LYP-D3BJ/6-311G**/LANL2DZ; Single point energy calculation: B3LYP-D3BJ/ CC-PVTZ)

3.002 with catechol	Energy Difference with lowest energy geometry (kJ/mol)	status [†]	3.003 with catechol	Energy Difference with lowest energy geometry (kJ/mol)	status [†]
gx11	0	in	gc8	0	in
gx1	7.41	in	gc4	-0.02	in
gx2	23.9	in	gc1	4.48	in
gx7	34.17	in	gc2	7.89	out
gx8	40.96	in	gc17	17.93	out
gx4	41.2	in	gc6	18.7	out
gx3	46.77	in	gc12	20.11	out
gx14	47.41	out	gc10	23.88	in
gx5	47.42	out	gc11	26.09	out
gx6	47.42	out	gc16	27.42	out
gx10	49.03	out	gc7	28.2	out
gx17	53.05	out	gc14	30.34	out
gx18	58.3	in (‡)	gc3	35.99	out
gx16	58.3	out	gc20	37.19	out
gx12	69.78	out	gc9	44.01	out
gx20	71.29	out	gc13	44.02	out
gx19	89.2	out	gc18	52.18	in (‡)
gx9	108.64	out	gc19	54.8	out
gx13	-	-	gc5	58.64	out
gx15	-	-	gc15	-	-

[†] guest molecules location state; [‡] Polar parts of guest compounds are located opposite to **3.002** or **3.003**

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Table 3.5.3 5 lowest energy of configurations for open form of ferrocene conjugates **3.002** with catechol (Geometry optimization: B3LYP-D3BJ//6-311G**/LANL2DZ; Single point energy calculation: B3LYP-D3BJ/CC-PVTZ)

Open-form of 3.002 with catechol	Energy Difference with lowest energy geometry (kJ/mol)
gx1open	0
gx2open	7.1
gx3open	25.21
gx4open	34.88
gx5open	34.88

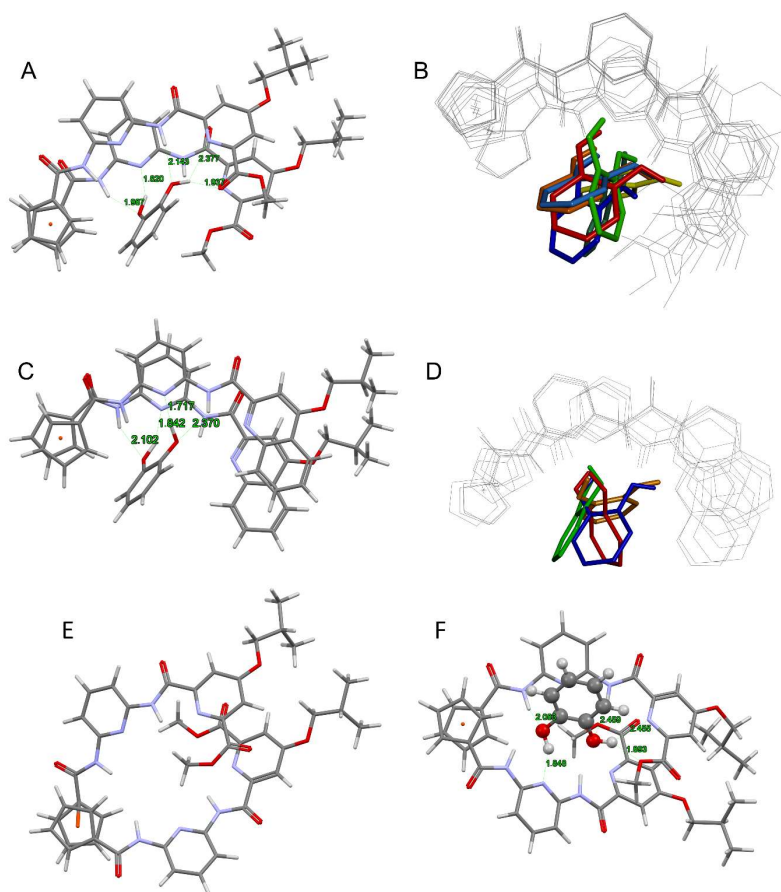


Figure 3.5.5 Lowest energy model of **3.002** (A and B)/**3.003** (C and D) with catechol and overlay of the catechol inside of clefts models. E and F show lowest energy conformation of open-form **3.002** and its interaction with catechol. Potential H-bonds and their distances are shown

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Figure 3.5.5 (A) shows the lowest energy model from calculation and B shows overlay of models where catechol located inside of **3.002** clefts. The first 7 low energy models show catechol located inside of cleft, with positions that are consistent with the data obtained from the ^1H - ^1H NOESY experiments. Interestingly, there appears to be significant room for the guest to move or rotate inside of the cavity. The lowest energy structure showed several potential H-bonds with the distances from 1.8 to 2.4 Å between the donor protons and the acceptor. In almost all of the structures, the ferrocene amides are closer to the guest than the strand amide. This is consistent with the observations from the titration studies. The models of **3.003** with catechol, showed similar behavior but only the first 3 had catechol inside of **3.003** cleft. The lowest energy model of this series showed only the pyridine nitrogen and amide proton of **3.003** participated in forming H-bond with catechol while the nitrogen of quinoline was not involved. Compared with those two calculated results and their association constant, H-bond between catechol with terminal ester of **3.002** as well as the size of cavity may be the main reasons for increased stability of this host-guest system.

Additionally, literature reports have shown that ferrocene substituted pyridine amide molecules may act as chelator-like hydrogen bonding receptors.^[18] While the open forms of the oligomers were not calculated to be stable, it is possible that with the guest a different conformation could be important. To look at this, a chelator like (open-form, head-to-tail) structure of **3.002** with a small dihedral angle and its interaction with catechol were studied computationally. After the optimization (B3LYP-D3BJ//6-311G**/cc-PVTZ), the chelator like structure of **3.002** shown in figure 3.5.5 (E), was calculated to have an energy about 90 kJ/mol higher than in the stacked conformation indicating that this sort of conformation is unfavorable. Five models of its host-guest system with catechol were carried out with the above method. Even the lowest model is about 70 kJ/mol higher than the stacked conformation of **3.002** with catechol.

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Table 3.5.4 Calculated models for ferrocene conjugates **3.003** with benzoic acid, sorting by energy (Geometry optimization: B3LYP-D3BJ/6-311G**/LANL2DZ; Single point energy calculation: B3LYP-D3BJ/ CC-PVTZ)

3.003 with benzoic acid	Energy Difference with lowest energy geometry (kJ/mol)	status [†]
gb10	0	in
gb7	0	in
gb9	0.01	in
gb3	13.66	in
gb1	13.78	in
gb2	20.43	in
gb11	25	in
gb8	30.14	out
gb13	33.16	out
gb18	34.1	out
gb20	40.96	in (‡)
gb15	42.59	out
gb19	43.09	out
gb17	44.49	out
gb12	46.62	in (‡)
gb14	50.7	out
gb6	58.05	out
gb4	-	-
gb5	-	-
gb16	-	-

[†] guest molecules location state; [‡] Polar parts of guest compounds are located opposite to **3.003**

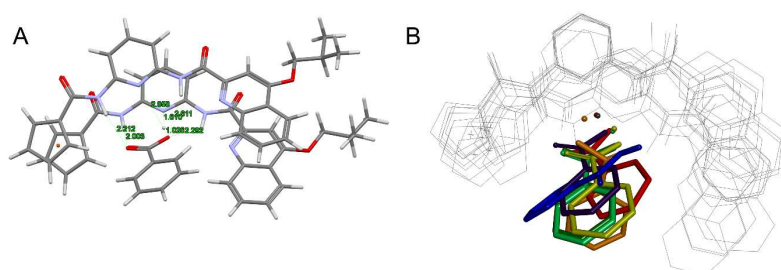


Figure 3.5.5 Lowest energy model of **3.003** with benzoic acid and overlay of the benzoic acid inside of clefts models. Potential H-bonds and their distances are shown

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Seven models out of 17 with benzoic acid were found with the guest inside of the **3.003** cleft. The inside model is 30 kJ/mol lower in energy than the outside ones. The lowest configuration showed similar behavior as for **3.003** with catechol where no H-bond formed between the quinoline nitrogen with the guest molecule. Again, the guest acts primarily with the ferrocene amides and pyridine nitrogens. This place the aromatic portion of the guest close to the quinoline ring. In accordance with the experimental results, the larger cleft size of **3.003** can potentially allow better interaction with benzoic acid than **3.002**, where the esters occupy this region.

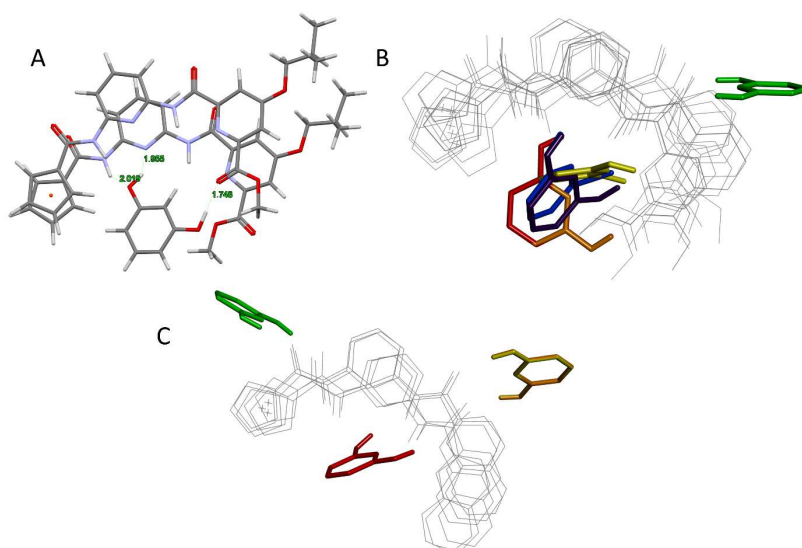


Figure 3.5.6 A. Lowest energy model of **3.002** with resorcinol, potential H-bonds and their distances are shown; B. Overlay of the resorcinol inside of **3.002** clefts models; C. Overlay of the resorcinol inside of **3.003** clefts models;

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Table 3.5.5 Calculated models for ferrocene conjugates **3.002/3.003** with resorcinol, sorting by energy (Geometry optimization: B3LYP-D3BJ/6-311G**/LANL2DZ; Single point energy calculation: B3LYP-D3BJ/ CC-PVTZ)

3.002 with resorcinol	Energy Difference with lowest energy geometry (kJ/mol)	status [†]	3.003 with resorcinol	Energy Difference with lowest energy geometry (kJ/mol)	status [†]
gd9	0	in	ge3	0	in
gd5	0.03	in	ge1	1.75	out
gd10	5.56	in	ge8	1.75	out
gd1	7.2	out	ge13	26.62	out
gd7	9.02	in	ge14	30.56	-
gd4	13.22	in	ge5	39.13	in
gd11	22.02	in	ge17	43.35	-
gd3	24.08	in	ge16	54.68	out
gd2	24.16	in	ge20	70.13	-
gd6	24.85	in	ge18	600.69	in
gd17	30.18	out	ge11	612.98	in
gd15	39.47	out	ge15	614.83	out
gd12	50.05	out	ge9	639.31	in
gd16	57.19	out	ge19	651.76	-
gd19	61.03	out	ge10	682.05	-
gd8	66.15	out	ge2	-	-
gd13	69.06	out	ge4	-	-
gd20	73.23	out	ge6	-	-
gd18	85.66	out	ge7	-	-
gd14	86.96	out	ge12	-	-

[†] guest molecules location state;

Table 3.5.5 lists the energy for the resorcinol with **3.002/3.003** host-guest systems. The lowest energy models are shown in figure 3.5.6 (A), (B) and (C). For resorcinol with **3.002**, calculated results showed resorcinol located inside of clefts for most of lowest energy models except one. H-bonds are shown in figure 3.5.6 (A), except the H-bonds between amide proton with resorcinol oxygen and pyridine nitrogen with resorcinol hydroxyl, the ester oxygen of **3.002** was also found to participate in forming a H-bond with another hydroxyl of resorcinol. In calculated result of **3.003** with resorcinol system, resorcinol was located in a variety of positions around the host. The first three lowest energy models are shown in figure 3.5.6 C. For these the energy difference between the in and out conformations are less than 2 kJ/mol. These results indicate that multiple potential binding sites can exist for this host-guest system and may explain the difficulties obtained in fitting the titration data.

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6. Conclusions

In this chapter, we have discussed the design, synthesis and characterization of an initial series of aromatic oligoamide foldamers with ferrocene as a turn unit. Crystal structures of **3.002**, **3.003** and **3.004** showed compact conformations which corresponded with our initial ideas. NMR studies in various deuterated solvents (from non-polar to polar solvents) as well as temperature-dependent spectra indicated those molecules were fluxional. With a process equating the protons of Cp rings. Still, solution studies including comparison studies and 2D ROESY spectrum support that these oligomers adopt preferentially folded stacked conformations in solution.

The curvature of these molecules generated by the interaction in the strands makes them interesting molecular clefts for host-guest chemistry. Titrations of the oligomers with several small molecules with different potential H-bond donors and acceptors showed that these oligomers can be potentially functional. Among them, benzoic acid, catechol and resorcinol were shown to form 1:1 host-guest systems with **3.002/3.003**. The exception to this was **3.003** with resorcinol which could not be fit to any combination of host guest ratio. Different chemical shifts of the amide protons in host molecules when treated with different guests as well as different association constant indicated showed that the oligomers has some selectivity for the guests. Moreover, **3.002** with catechol ranks among the strongest host-guest interactions for cleft molecules with catechol. As one the function group of series of bioactive molecules including dopamine and epinephrine, this host-guest studies may be of interest for developing electrochemical detectors. ^1H - ^1H NOESY NMR were applied to further study their interactions and indicated that catechol fitted into the cleft of **3.002**. Even though no direct evidence was observed from the ^1H - ^1H NOESY spectrum of the other host-guest systems, given the similar observations during titration of those three pairs, we hypothesize that catechol and benzoic acid bind in similar fashion with **3.003** as catechol with **3.002**.

DFT calculations of **3.002** in different conformations showed that the parallel stacked conformations are the lowest energy structures and fit well with the experimental results. Computational studies on those host-guest systems also showed that lowest energy states of those geometries were consistent with the experimental observations from the titration and ^1H - ^1H NOESY results.

All in all, both experimental and computational results suggest that ferrocene can be an interesting candidate for turn units in the development of aromatic β -sheet like structures. Still, the systems should be further optimized to obtain greater stability. Approaches for this are discussed in the next section.

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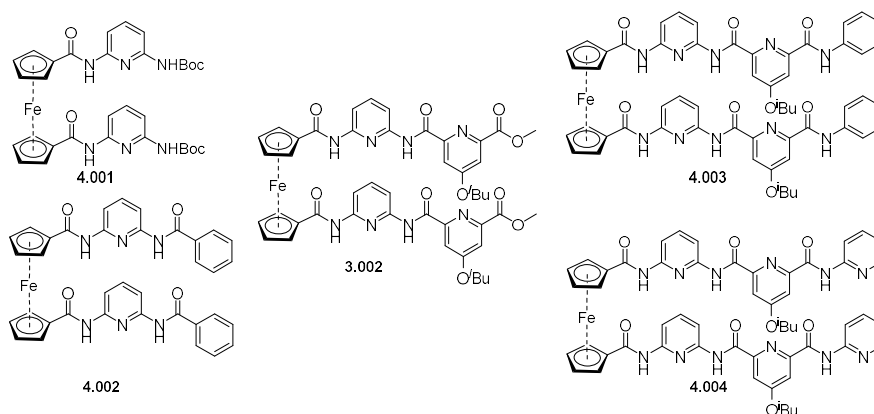
Second Generation of Controlling Molecular Mobility

This chapter focuses on the design, synthesis and characterization of a second generation of aromatic oligoamide ferrocene derivatives **4.001-4.009**. With the initial series described in chapter III, **3.002**, **3.003** and **3.004** exhibited well-defined structures in solid state and both solution and computational results supported that the stacked structure should be favored. These exciting preliminary results showed the potential of ferrocene for the design of sheet like foldamers. Still, while some observations, such as differences in aromatic shielding with larger aromatic groups, suggest some minor effect of aromatic surface on the conformational stability of the stacked structures, these were not very conclusive. Within the experimental limits, the variable temperature results could not give definitive difference between **3.002** and **3.003** and the limited solubility of **3.004** prevented its comparison. Thus, the systems developed in this chapter were aimed at studying other designs or factors that could potentially increase the stacked structure stability by rational replacing moieties to realize further control of the structures adopted by the ferrocene oligomers.

1. Foldamer design

For the second generation of ferrocene aromatic foldamers, a number of designs were envisaged that could influence the stability of the stacked structures. These include:

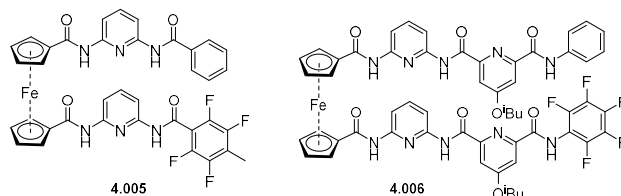
- a. Increasing the number of aromatics on each strand to increase the aromatic surface;



Scheme 4.1.1 Design of increasing numbers of aromatic rings

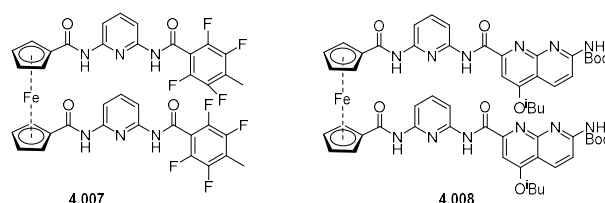
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- b. Introduction of electron rich – electron poor aromatics into the strands, which should give stronger π -stacking interactions.



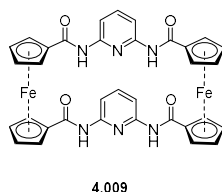
Scheme 4.1.2 Design of asymmetric oligomers by inducing aedamers

- c. Using the same idea, **4.007** and **4.008** for could potentially have less favorable intra-molecular interactions between the strands due to the similar nature of the aromatics. However, by adopting larger dimeric structures the π stacking interactions could be potentially better.



Scheme 4.1.3 Design of oligomers by reducing intra-molecular interactions

- d. Besides of those “open” form molecules, “close” form (macrocyclic) systems could be used to limit ferrocene rotation and see to what extent the rotation of the aromatic groups is responsible for the equating of the ferrocene signals (**4.009**).



Scheme 4.1.4 Design of “closed” form of foldamer

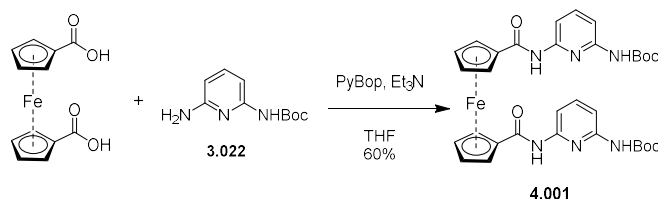
The study of the stability and conformational preferences in these series should improve the understanding of the factors needed for controlling the molecular mobility oligomers and for directing exchange between multiple well-defined conformations such as sheets or helices.

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2. Synthesis and characterization

2.1 Synthesis

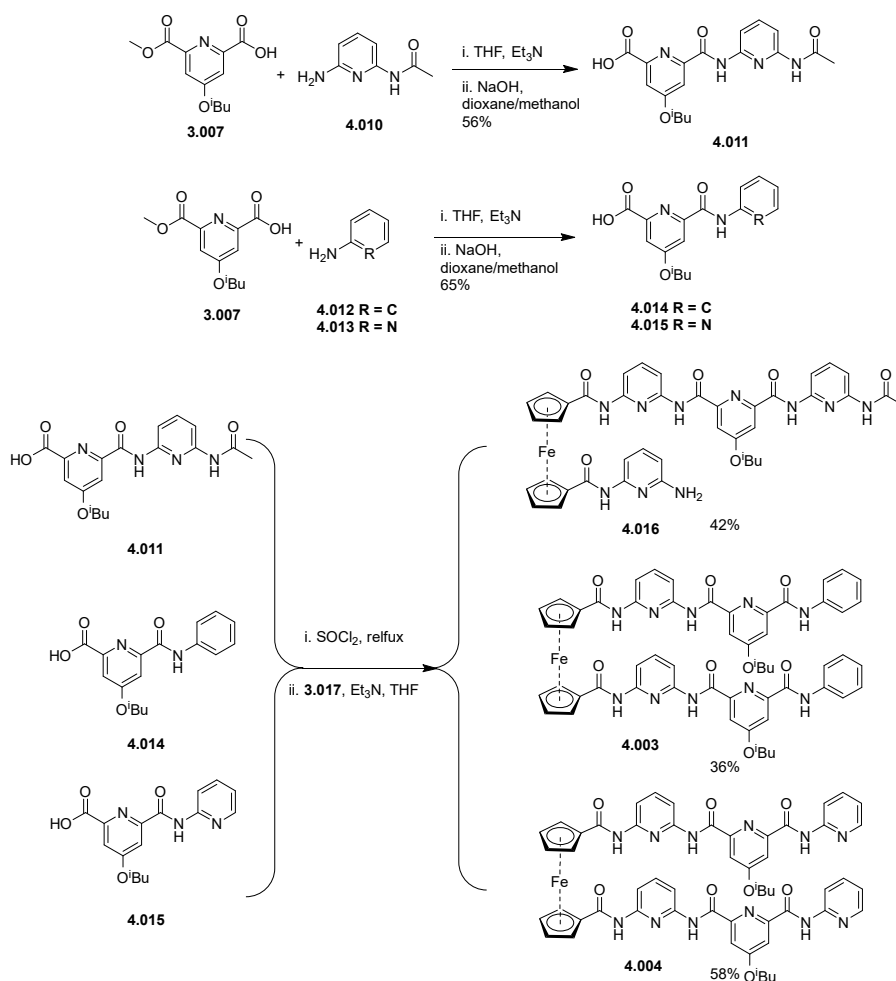
Synthesis of **4.001** was performed by coupling of ferrocene dicarboxylic acid with two equivalents of a monoprotected pyridine using PyBOP and gave the desired product in good yield.



Scheme 4.2.1 Synthesis of short sequence oligomer **4.001**

Longer oligomers, **4.003**, **4.004**, and **4.016** were synthesized in a similar fashion as the initial series of ferrocene oligomers by coupling of the corresponding dimers to the ferrocene derivative **3.017**. Initially, dimer **4.011** was synthesized from the corresponding acid chloride of **3.007** and mono-acetyl protected 2,6-diaminopyridine **4.010** in freshly distilled THF followed by hydrolysis using sodium hydroxide. **4.011** was then refluxed with thionyl chloride to afford the corresponding acid chloride which was subsequently reacted with **3.017** in dry THF or DCM. However, the desired product could not be obtained and no evidence for its formation was observed by NMR. **3.017** was then reacted with **4.011** in the presence of various peptide coupling reagents including PyBOP and HATU as well as triethyl amine. A pure compound could be isolated from these reactions, but characterization by NMR and MS indicated that the product was the half-connected ferrocene derivative **4.016**. Interestingly, even upon reaction of **4.016** with excess **4.011**, the desired symmetric bis-coupled ferrocene conjugates could not be obtained.

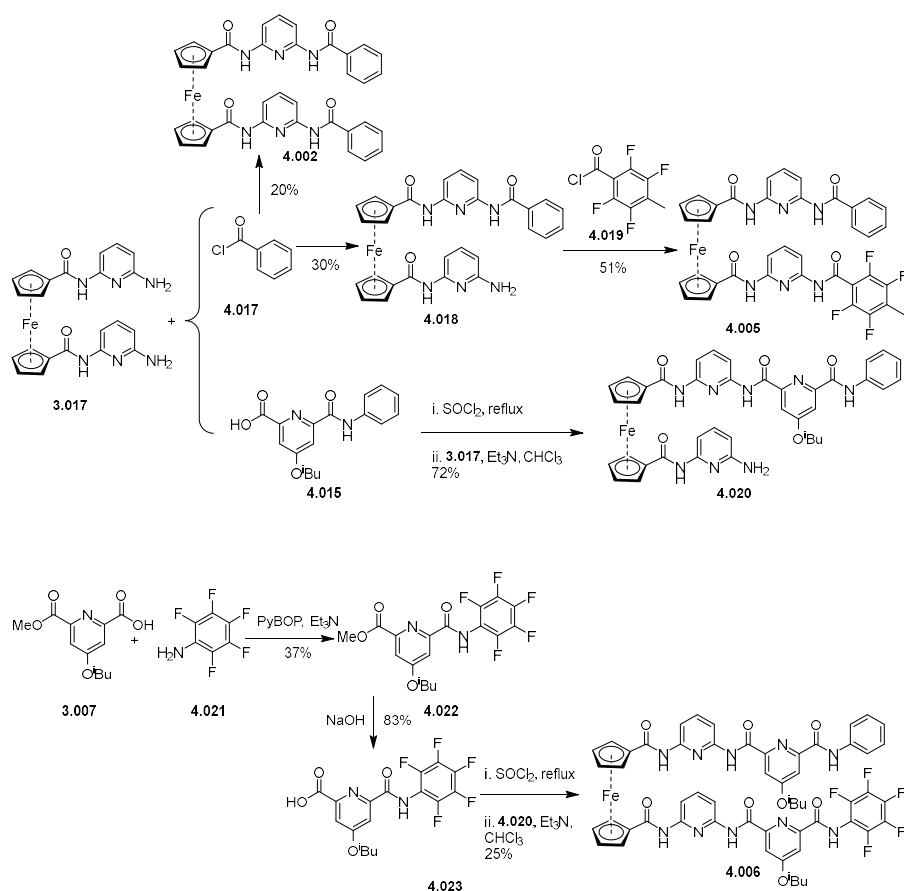
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Scheme 4.2.2 Attempts of synthesizing foldamer with increased number of aromatic rings

The failure with dimer **4.011** to afford ferrocene heptamer may indicate that the ferrocene becomes too sterically encumbered with highly curved oligomers preventing further reaction. Smaller dimer **4.014** was obtained with the same method starting from **3.007** and aniline. The acid chloride of **4.014** was subsequently obtained in refluxing thionyl chloride. After, the acid chloride was reacted with **3.017** in freshly distilled chloroform in the presence of triethylamine successfully affording **4.003** with moderate yield. **4.004** was obtained under the same procedure with replacement of aniline by 2-aminopyridine.

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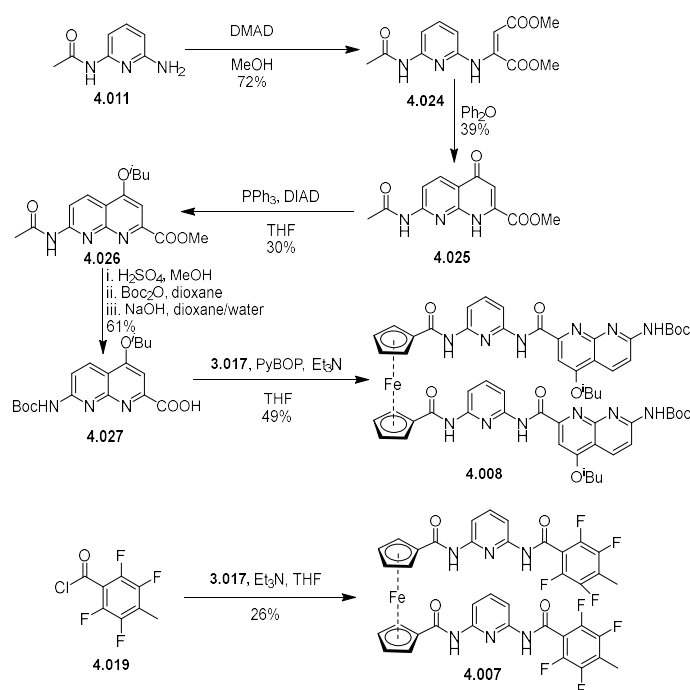
Scheme 4.2.2 Attempts of synthesizing asymmetric foldamers with inducing aedamers

For the synthesis of asymmetric foldamer **4.005** and **4.006**, a similar step by step coupling procedure was used as for asymmetric foldamer **3.030**. **3.017** was first reacted with 1 equivalent of the more electron-rich **4.017** or **4.015** to form asymmetric oligomers **4.018** (oligomer **4.002** was obtained as a by-product with a yield of 20%) or **4.020**. After, the coupling of these oligomers to the either the electron deficient aromatic unit **4.019** or **4.023** afforded the final asymmetric foldamers, **4.005** and **4.006**. The yield for the asymmetric foldamers is not as high as the symmetric ones, which is due to the low yields of the step-by-step coupling method. All of the acid chlorides were coming from their corresponding acids by refluxing them in thionyl chloride for 10 to 30 minutes. Notably, the corresponding ester of dimer **4.022** from the coupling of pentafluoroaniline **4.021** with mono-ester pyridine **3.007** gave a relatively low yield (37% isolated by column), but hydrolysis of this dimer ester using potassium hydroxide worked surprisingly well as its acid could be obtained

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with no decomposition products detected. Both compounds showed moderate solubility in chloroform, dichloromethane, and methanol.

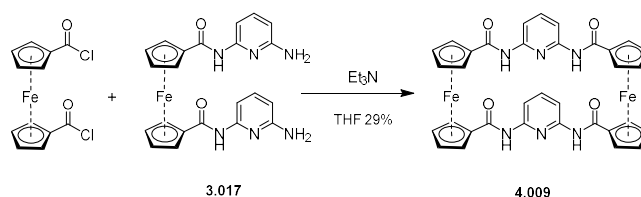
For oligomers **4.007** and **4.008**, the synthetic route relied on direct coupling of **3.017** with the corresponding monomers. The synthesis and purification of naphthyridine monomer **4.027** followed the reported procedure.^[1] Interestingly, unlike the other ferrocene oligomers, **4.007** showed good solubility in methanol but poor solubility in chloroform or dichloromethane at room temperature. However, when heated to 40 °C, **4.007** showed good solubility in dichloromethane.



Scheme 4.2.3 Attempts of synthesizing foldamers with reduced intramolecular interactions

Finally, for the synthesis of the macro-cycle ferrocene derivative **4.009**, ferrocene-1,1'-dicarboxylic acid chloride was stirred with **3.017** in the presence of triethylamine to give the macrocyclic product with yield of 30%. **4.009** shows moderate solubility in chloroform, dichloromethane and methanol.

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Scheme 4.2.3 Attempts of synthesizing “close” foldamer

2.2 Characterizations

2.2.1 “Close” form structure study

DFT calculation on strand rotation and flipping of **3.002** gave an energy map of different potential conformations. Energy differences between different conformations of optimized structures versus the optimized structure obtained from the X-ray data were lower for flipping of strands (Head-to-head versus head-to-tail was about 22kJ/mol difference) than for rotation of the strands about the ferrocene. The macrocyclic structure **4.009**, could be further used to study the conformation change by the “strand” flipping and the idea that the splitting of the ferrocene protons can be a good indication of strand stability.

Single crystals of **4.009** were obtained by slow diffusion of dichloromethane into a methanol solution of the compound. Top and side views of crystal structure **4.009** are shown below, the two strands are directly parallel stacked to each other. The distance between them ranged from 3.4 to 3.6 Å which are suitable for π - π interaction. The two ferrocenes are both in an “eclipsed” conformation.

To our surprise, the NMR of **4.009** again shows only two sets of signals for the ferrocene protons. As rotation about the ferrocene should not be possible with this compound, the strand rotation in this constrained system must still be occurring rapidly.

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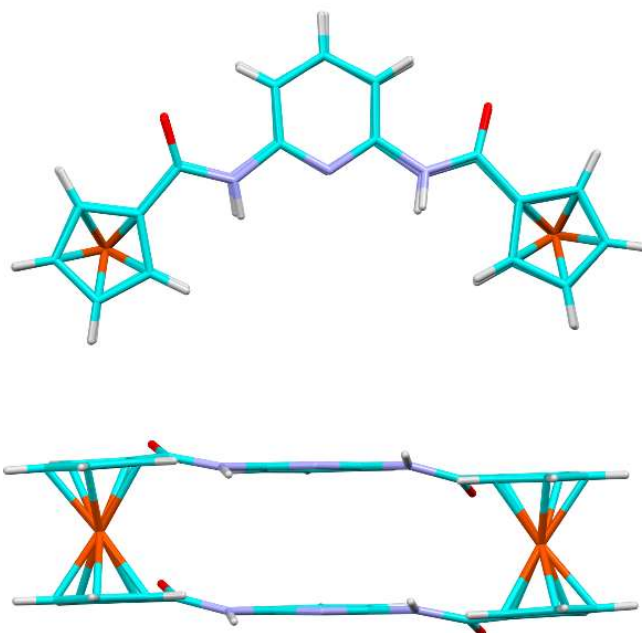


Figure 4.2.1 Top and side view of crystal structure of “close” form molecule **4.009**

Computational optimization from the X-ray coordinates of **4.009** was carried out by DFT with functional of B3LYP-D3 and basis set of 6-311G** for C, H, O, N and LANL2DZ for Fe.

The optimized structure matched well with the crystal structure which both showed parallel stacking of the two strands. Still, a slight twisting of the oligomer was observed in the calculated structure.

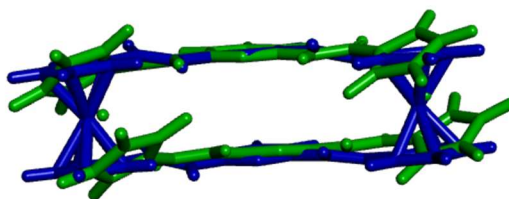


Figure 4.2.2 Overlay of crystal structure (blue) and calculated (green) of “close” form of molecule **4.009**

Using the same functions and methods, different conformation of **4.009** (flip of strands) were calculated. The energy difference between the head to head conformation (left in figure 4.2.3) and head to tail conformation (right in figure 4.2.4) is approximately 9 kJ/mol in favor of the head to head structure.

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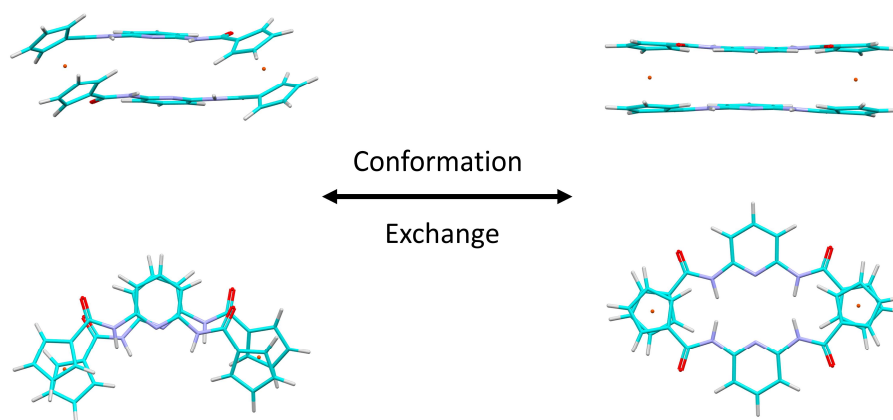


Figure 4.2.3 Top and side view of head-to-head and head-to-tail conformation optimized by Gaussian 09 from molecule **4.009**

To further study the strand flipping of **4.009**, variable temperature NMR was conducted from 183 K to 293 K, figure 4.2.4. Unfortunately, at some point it appears that the compound underwent some decomposition, resulting in the presence of impurities in the spectra. Still, similar to the previous variable temperature NMR results of **3.002** to **3.004**, protons in the ferrocene region do not show splitting within the temperature range studied. However, the signals become broad and flatten out below 213 K. This may be attributed to slow exchange between the head to head and head to tail conformations or reduced solubility of **4.009** at low temperature. Considering the moderate solubility of **4.009** in various solvents (especially methanol), additional variable temperature NMR experiments need to be performed in different solvents. Nevertheless, these results highlight that the mobility of these systems comes from the strand flipping rather than rotation about the ferrocene, and that ways to slow this could give more stable sheet like structures.

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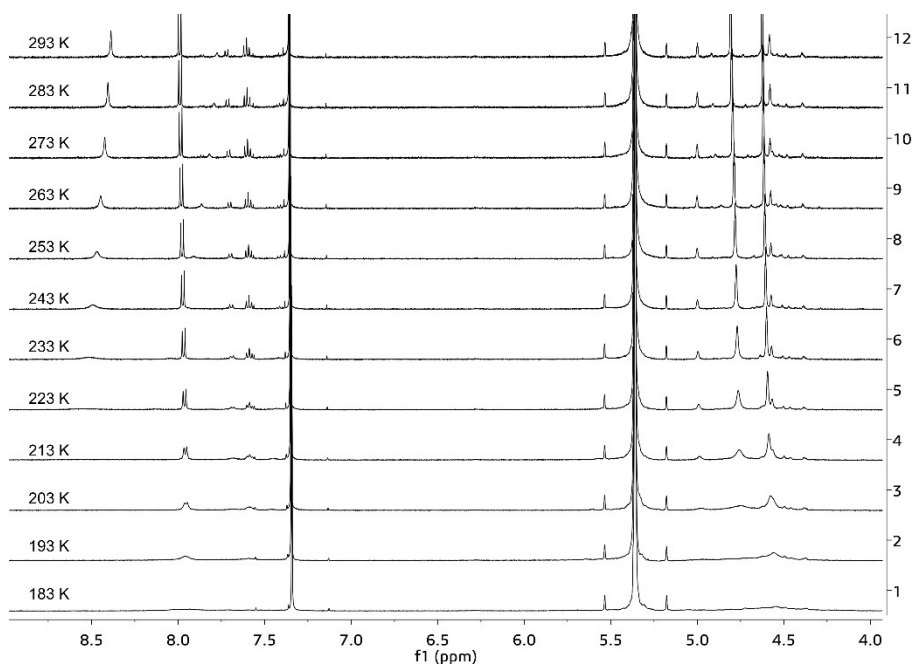


Figure 4.2.4 Temperature dependent ^1H NMR of **4.009** (1 mM) in dichloromethane- D_2 .

2.2.2 Water as a stabilization factor

After several attempts, single crystals of **4.004** were obtained through slow diffusion of hexane into a THF solution of the compound at 4°C in the refrigerator. Top and side view of crystal structure are shown in figure 4.2.5. Compared to the crystal structure of **3.002**, the increased number of aromatic rings in **4.004**, give highly curved strands that form a central cavity. This curve also results in the distance of 2.8 Å between the Cp ring of the ferrocene moiety and last pyridine in the strand. This narrow distance may explain the difficulty for coupling of **3.017** with **4.012**. Although the structure of **4.004** becomes twisted because of the small distance, the two strands are overall parallel to each other.

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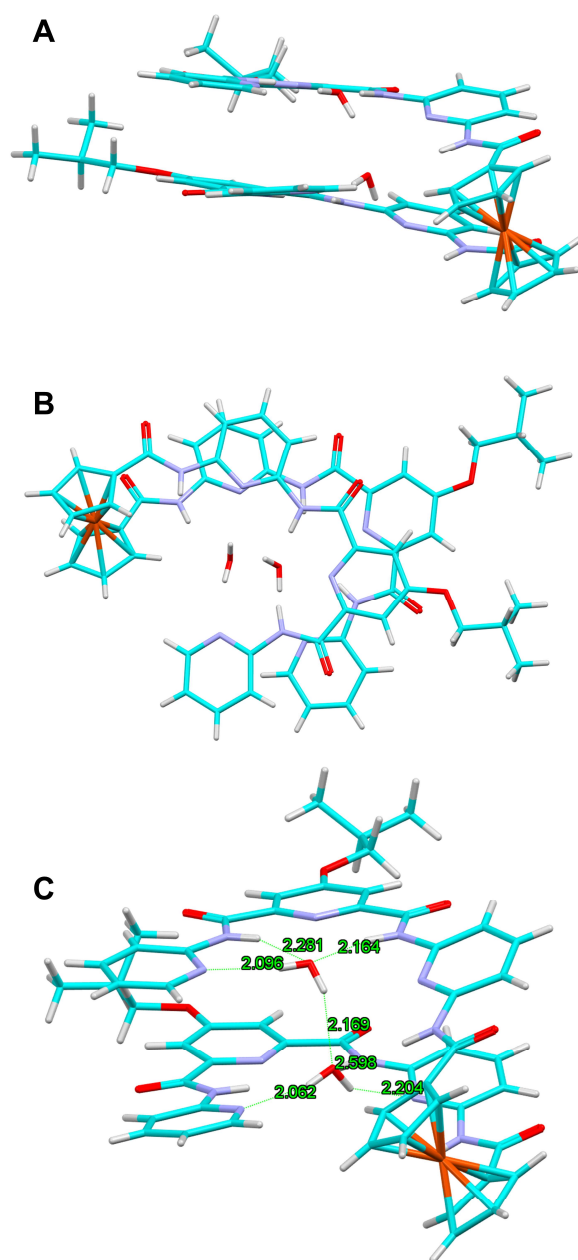


Figure 4.2.5 Top (A) and side (B) view of co-crystal structure of oligomer **4.004** with two water molecules located inside. (C) show distance between oligomer and water molecules.

Additionally, two water molecules are found inside of the structure. Distances between endocyclic nitrogens of **4.004** and hydrogens of water molecules as well as between amide protons of oligomer and oxygens water molecules are

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2.096 to 2.204 Å and 2.164 to 2.598 Å suggesting multi-hydrogen bonds are formed. The distance between two water molecules is also short (2.169 Å) suggesting a hydrogen bonding network that connects the two strands.

Evidence for the overall structure of the oligomer was also obtained from solution studies. The ROESY spectrum of **4.004** showed correlations between the ferrocene protons and the protons of terminal pyridines (H_A) with medium NOE correlation strength. This is expected given the curvature for the strands.

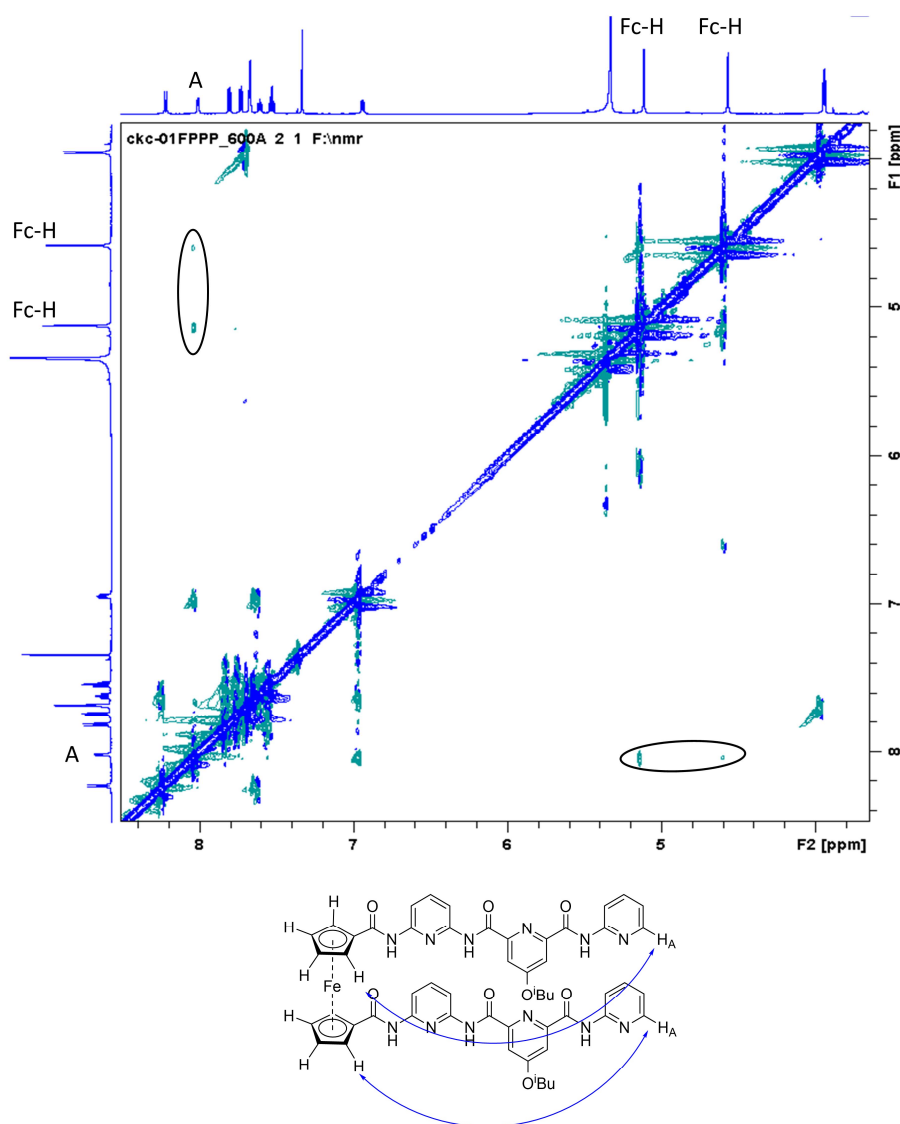


Figure 4.2.6 Part of ROESY spectrum and structure of **4.004**; blue arrows show correlations.

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From the solid-state structure of **4.004**, interactions between the oligomer and water also could be an important potential stabilizing interaction for the structure. The presence of water also had marked effects on the proton NMR in deuterated dichloromethane. Compared to in anhydrous deuterated DCM, when **4.004** was dissolved in deuterated DCM with water, the amide protons are significantly downfield shifted. Additionally, the water signal is also down field shift from the typical 1.52 ppm in “pure” deuterated DCM to 1.8 ppm. Similar behavior of water signals were also reported in aromatic oligoamide helical structure.^[2] Shift of these signals suggest H-bonding may form between oligomer and waters.

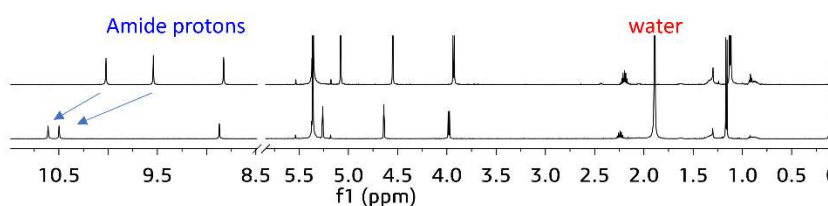


Figure 4.2.7 Part of the proton NMR spectrum for **4.004** in deuterated dichloromethane with (up)/ without (down) water. Blue arrows show the shifts of amide proton which may due to hydrogen bond forming.

To see if the water has an influence on the structural mobility of **4.004**, variable temperature proton NMR from 298 K to 193 K were recorded in deuterated dichloromethane both with or without water. When dissolved in with high quality deuterated DCM (water less than 0.01%), the ferrocene singlets became broad at low temperature, but no splitting was detected within the temperature limits of the instrument. However, when treated with deuterated chloroform containing water, the water signal was obviously downfield shifted from 1.8 ppm to 5.6 ppm with decreasing temperature. Additionally, each of the ferrocene signals split into two different sets of signals. No splitting was observed for the other signals of the oligomer. These results indicate that the presence of water has an important stabilizing effect on the structure. Indeed, a hydrogen bonding network between the strands, as observed in the crystal structure, should limit the ability of the strands to flip. This can be seen as one way of controlling the strand flipping. Finding other ways to incorporate similar interactions could be a useful design component for future oligomers.

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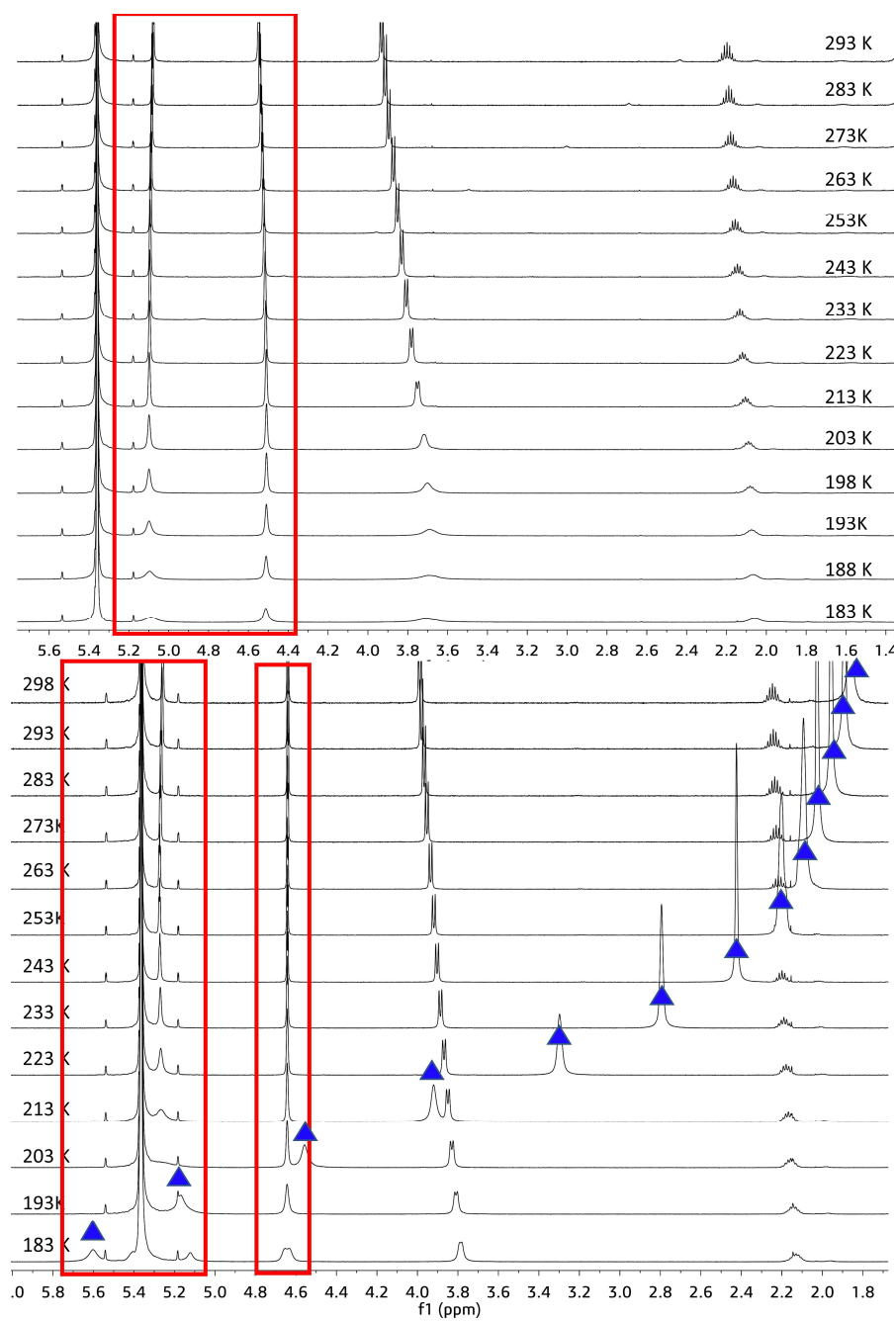


Figure 4.2.8 Part of temperature-depended proton NMR spectrums of **4.004** in deuterated dichloromethane(top: without saturated water, down: with saturated water). Temperatures were listed in the spectrums; water single (blue triangle in spectrum) were shifted from 1.8 ppm to 5.6 ppm in the solvent with water. ferrocene singles (red box) are located round 4.6 and 5.1 ppm

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2.2.3. Characterization of Other Oligomers

Due to lack of time, characterization of asymmetric oligomers **4.005** and **4.006** as well as symmetric oligomers **4.007** and **4.008** were not fully performed. However, some interesting results were obtained.

For asymmetric oligomer **4.005**, which was designed for enhancing the intramolecular interactions, the proton NMR peaks became broad and a small amount of solid precipitated out over time in deuterated chloroform. When the deuterated chloroform was dried and replaced with deuterated methanol, the solution became clear and sharp signals were observed in the proton NMR. This may be due to self-assembly through intermolecular π stacking. More experiments need to be performed including high temperature NMR, concentration dependent kinetic, and solid-state studies to draw confirm this.

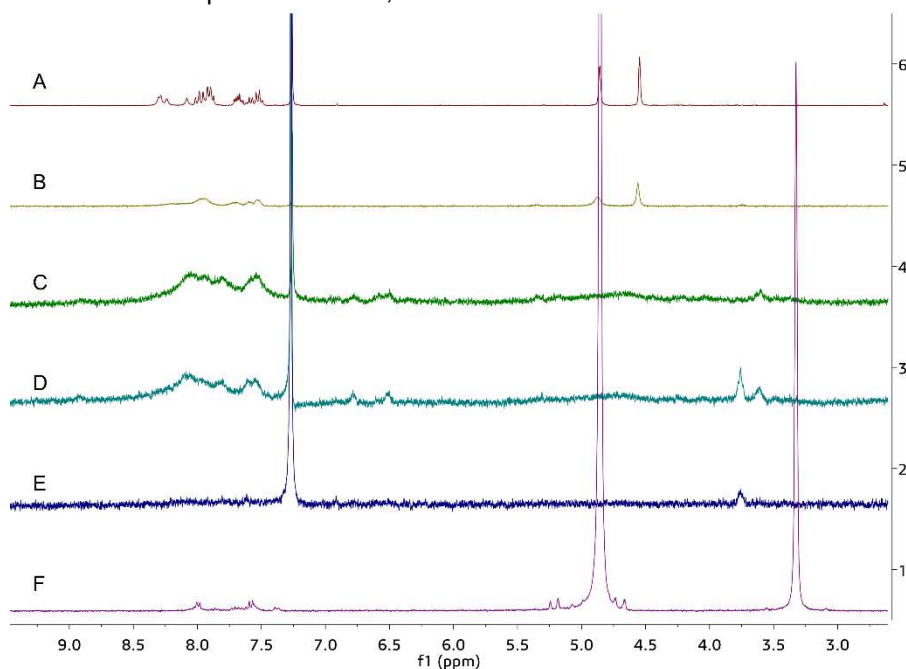


Figure 4.2.9 Time dependent NMR of **4.005** (A) standard proton NMR of **4.009** in deuterated chloroform; (B) proton NMR of **4.009** in deuterated chloroform at 0 h; (C) proton NMR of **4.009** at in deuterated chloroform 12 h; (D) proton NMR of **4.009** in deuterated chloroform at 24 h; (D) proton NMR of **4.009** in deuterated chloroform at 36 h; (E) proton NMR of **4.009** in deuterated chloroform at 48 h; (F) proton NMR of **4.009** in deuterated methanol at 48 h

Characterization of other oligomers will be performed to further study the stabilization of ferrocene aromatic oligoamides.

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3. Conclusions

In this chapter, nine second generation of foldamers using ferrocene as a turn moiety were designed and synthesized. Among those, the crystal structure of **4.009** showed an ideal stacked conformation with parallel dipoles. Distances between two pyridine moieties are around 3.5 Å. Both the conformation of the crystal structure and the distance between two strands suggest the importance of the π - π interactions. Still, DFT optimization of two possible conformation of **4.009** suggested that the strand flipped (head to tail) conformation was potentially similar in energy to the head-to-head conformation.

4.004 was initially designed to study how increasing strand length affected the interactions between two strands. Proton NMR of **4.004** in dry dichloromethane and water containing dichloromethane showed obvious shifts of the amide proton and water protons. This indicated the oligomer has important interactions with the water molecules. Additionally, variable temperature proton NMR studies showed that the ferrocene protons split into 4 signals in water containing deuterated dichloromethane with decreasing temperature from 298 K to 193 K. No such changes were observed in dry deuterated dichloromethane, which further indicated water has an important stabilizing effect in **4.004**. The crystal structure of **4.004** and water showed a highly curved structure with 2 water molecules incorporated, forming a hydrogen bond network between the strands. ROESY spectrum of **4.004** also give the support of this curved conformation. All of those results suggest that **4.004** can adopt well-defined conformations stabilized by water molecules both in solid and solution state.

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References

- [1] Y. Ferrand, A. M. Kendhale, J. Garric, B. Kauffmann, I. Huc, *Angew. Chem. Int. Ed.* **2010**, *49*, 1778-1781.
- [2] J. Garric, J.-M. Léger, I. Huc, *Angew. Chem. Int. Ed.* **2005**, *44*, 1954-1958.

Chapter V

Conclusions & Perspectives

1. Conclusions

The prevalence and role of β -sheets in proteins makes them interesting targets for the development of synthetic models that can mimic their structure and potentially function. Although various aliphatic amino acids have been used for generating β -sheet mimics, aromatic foldamers that imitate sheet-like structures are rare. Still, a handful of examples have been reported. In this work, we have aimed to develop a series of aromatic oligoamide foldamers that adopt sheet-like structures. The main novelty in our system is the use of ferrocene dicarboxylic acid as a turn-unit for connecting the aromatic oligoamide strands. Unlike, previously reported AOF β -sheets, this turn-unit is significantly more flexible, as the Cp rings of ferrocene are known to rotate rapidly. Still, because it can allow for distances between the strands that are ideal for aromatic stacking, we envisaged that it could be an interesting new turn-unit that could expand the design possibilities for sheet-like mimics. As such, we outlined two key questions that we wanted to address through this work. First, can ferrocene be a viable turn-unit for generating aromatic oligoamide foldamers that adopt sheet-like conformations? Second, what factors are important for controlling the structures of these systems? To this end, twelve new ferrocene containing oligomers were synthesized, Figure 5.1.1.

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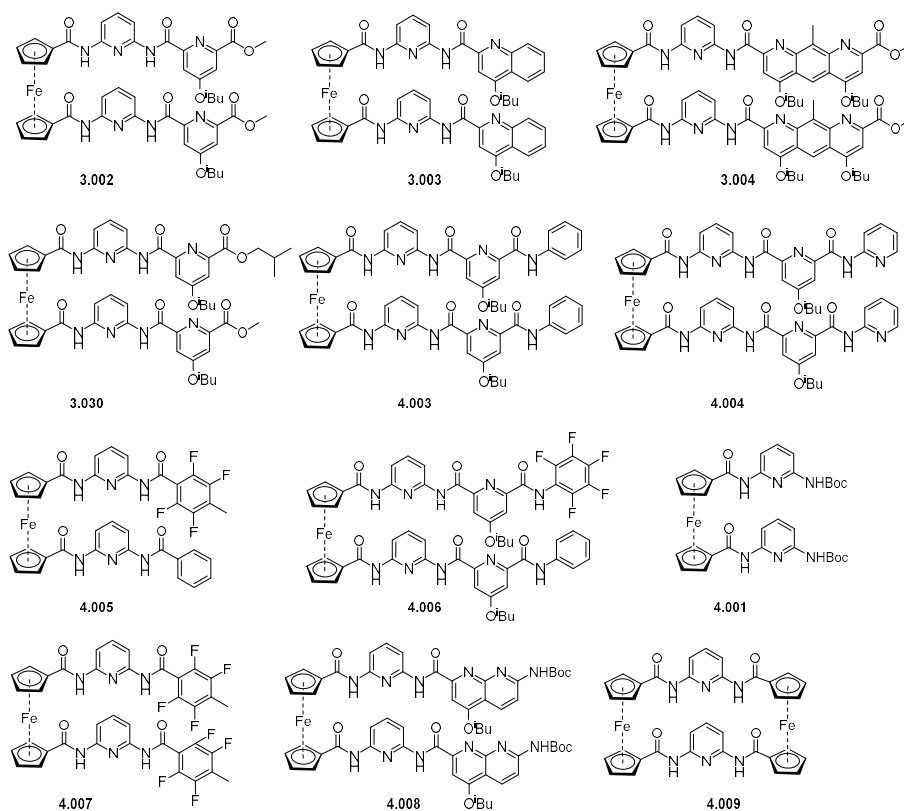


Figure 5.1.1 Structure of obtained ferrocene containing aromatic oligoamide oligomers

In the initial generation of these compounds (**3.002-3.004**), the structures differed by the size of the last aromatic unit. This was based on the idea that larger aromatic surfaces could better stabilize the stacked structures. However, the variable temperature NMR analysis of these compounds did not give conclusive results about this point. Still, the solid state structures showed that all three of the oligomers adopt parallel stacked conformations. Moderate evidence from NMR studies indicates that this conformation is also important in solution. Additionally, computational studies looking at rotation of the ferrocene moiety as well as strand flipping were performed. These show that the observed stacked head-to-head conformation is more stable than open (unstacked) or head-to-tail conformations. The collection of these results shows that indeed ferrocene can be a viable turn-unit for the design of AOF sheet-like structures.

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Importantly, due to their folding, these molecules adopt cleft like structures that can function as hosts for the recognition of small guest molecules. The guest binding ability on **3.002/3.003** was studied. The chosen guests, nitrobenzene, benzoic acid, catechol and resorcinol, differ in the position of the potential hydrogen bond donor/acceptor sites. Titration results showed **3.002** could better bind to catechol and resorcinol than **3.003**, which itself was a better host for benzoic acid. NOESY studies for **3.002** with catechol support that the guest molecules bind inside of cleft structure. This type of interaction was also supported by the computational results. 100 initial structures were randomly produced of each host-guest system. These structures were further optimized by MOPAC and then Gaussian, the final lowest energy models corresponded well with the experimental results. Moreover, those models also potentially offer an explanation for the different selectivity of **3.002/3.003** for the guest molecules based on the clefts sizes and functional group interactions. While one example of a helix-sheet-helix aromatic oligoamide has been reported to bind guests, the ability of purely sheet-like aromatic oligoamides has not been reported. Thus, this work shows that these types of structures can have interesting applications that mimic aspects of the reactivity of bent β -sheets found in nature.

Still, the initial series of compounds was found to be highly fluxional and methods to control the molecular motion of the compounds needed to be developed. Encouraged with the novel properties of **3.002**, **3.003** and **3.004**, a second generation of ferrocene containing AOF were synthesized for this purpose. These structures incorporated different approaches (longer strand lengths, donor/acceptor interactions, etc..) for potentially controlling the molecular motions.

Among these, the crystal structure of macrocycle **4.009** gave a good support of π - π interactions between the two pyridine units with its parallel stacked conformations. Still, NMR studies showed that the ferrocene protons still appear as only two signals indicating that a fluxional process was still present. As this cannot be due to rotation around the ferrocene, the strand flipping appears to be the primary cause. Notably, DFT calculations revealed the

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energy difference between head to head and head to tail conformations of the macrocycle to be only 9 kJ/mol. Limiting this conformational exchange is thus one of the main factors needed for controlling the stability of ferrocene AOF.

A potential option for this was found with **4.004**, which also showed a well-defined conformation in the solid state and in solution. Importantly, it was observed in the crystal structure that the two strands in the oligomers were connected by a hydrogen bonding network formed from two water molecules. Proton NMR studies also showed marked changes in the chemical shifts of the oligomers in dry versus wet solvent. Importantly, while variable temperature proton NMR studies of **4.004** in dry DCM showed no splitting of the ferrocene proton resonances at low temperature, when water was present in the sample, the ferrocene signals split into four different peaks indicating that the fluxional process is restricted.

Unfortunately, due to limited time, full structural characterizations of other oligomers were not performed. Still, the new compounds, especially the donor/acceptor systems can have potentially interesting properties and should be explored more.

In all, this research has been able to partially address the initial questions set for these molecules. Namely, ferrocene can be a suitable turn unit in aromatic oligoamide foldamers β -sheet mimics. However, more work needs to be done towards stabilizing different conformations and controlling exchange between them.

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2. Perspectives

Ferrocene as turn moiety to design foldamers has its own advantages and challenges. Ferrocene containing aromatic oligoamide foldamers were shown in this thesis to both adopt well-defined conformations in the solid state and have a preference for these compact conformations in solution. However, there are still some aspects that need to be investigated.

One is achieving the better control over the conformation of ferrocene aromatic oligoamide foldamers. While the initial studies did not give conclusive evidence of a strong dependence of aromatic surface size on structural stability, this point should be important and studied more in the future. Using larger aromatic surfaces with increasing aromatic stacking effects could be the first option. One limitation for the curved design, is that after three oligomers the ferrocene moiety prevents the addition of additional monomers. This could be overcome by using larger monomers or monomers that give linear structures (figure 5.2.1 A). Additionally, given the importance of strand flipping, approaches like “stapling” the two strands together could be interesting for controlling the structures (figure 5.2.1 B). Furthermore, based on the above results, ferrocene oligoamide foldamers with multiple strands (3 or more) need to be designed by choosing suitable aromatic moieties or through potentially using a ferrocene amino acid (figure 5.2.1 C).

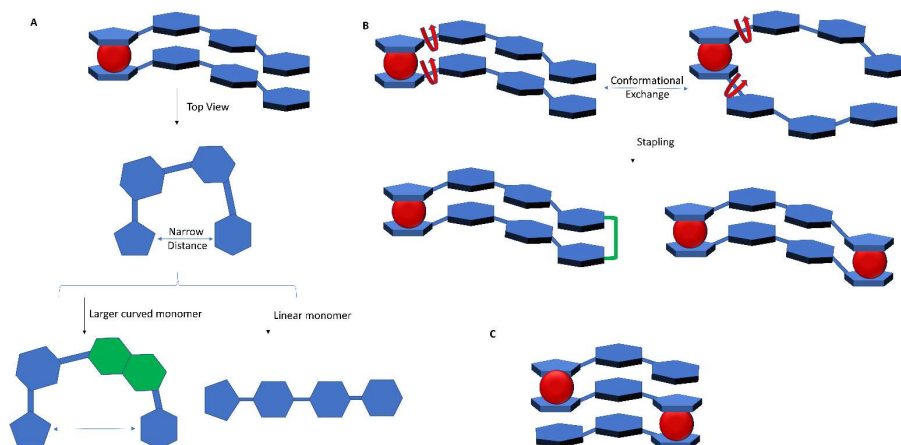


Figure 5.2.1 Schematic representation of achieving better control over the conformation of ferrocene aromatic oligoamide foldamers. (A) using longer strands with stronger aromatic stackings; (B) forming macrocycles for restricting strand flipping. (C) enhancing aromatic stackings by using multistrands

Additional DFT calculations on some of the molecules including **4.004** and **4.009** will be interesting. Particularly looking transition state energies for the strand flipping could give us an idea of potentially how low the energy barrier for this process is and ways to limit it.

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For the other oligomers that were synthesized during this work, the designs were aimed at studying how π - π stacking influences the oligomer mobility and structure. Structural characterization of these molecules with both NMR and X-ray crystallography still need to be performed. Of particular interest for stabilizing the structures are the oligomers containing strands with complimentary donor/acceptor interactions.

However, oligomers containing symmetric strands with donor/acceptor groups could also give interesting results. For these, when the strands are the same, the interaction between them should be less favorable. However, if the system dimerizes to give a double helical structure, there could be potentially better interactions (figure 5.2.2). By controlling this type of self-assembly, ferrocene aromatic oligoamide foldamers that switch between sheet and helix forms could be developed. To achieve this kind of conformations, more specific control of interactions between strands need to be incorporated. Characterization of **4.007** and **4.008** will be interesting for this purpose. This should include both solution and solid state studies. Concentration dependent studies will be particularly interesting here.

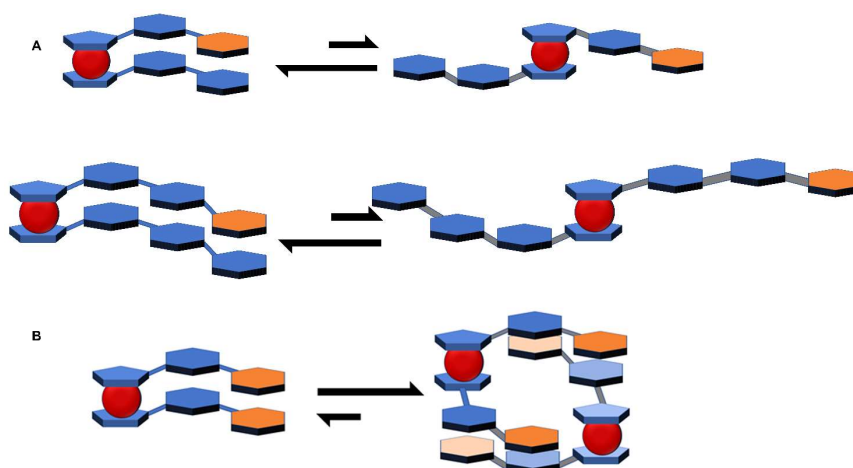


Figure 5.2.2 (A) Schematic representation of possible conformation change of ferrocene aromatic oligoamides with aedamer. Orange octahedrons represent opposite electrostatic potentials; (B) Enhancing electrostatic repulsion may decrease folding state while enhancing inter-molecular interactions. blue and orange color of aromatic rings represent opposite electrostatic potentials.

In addition, as bent β -sheet structures in nature are important for guest recognition and catalysis, looking at the function of this class of molecules will be interesting. **3.002**, **3.003** and **4.004** showed incorporation of guest molecules by the cleft formed by the strand curvature. This could be divided into two aspects. One is modifying the existing ferrocene aromatic foldamers to have modified properties like water-solubility which could allow them to bind

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with more bioactive molecules such as dopamine. Given the stable reversible electrochemical properties of the ferrocene group, this could allow for interesting electrochemical sensors to be developed. The other aspect is replacing certain aromatic moieties to generate different sized cavities which could be potential interesting for binding to larger guests such as peptide based α -helices.

Experimental Section

1. Instrumentation

^1H and ^{13}C nuclear magnetic resonance spectra were recorded in Louvain-la-Neuve on Bruker Avance II 300 MHz spectrometer (^1H 300 MHz and ^{13}C 75 MHz) and Bruker Avance 500 MHz spectrometer. ^1H chemical shifts are reported in ppm downfield from internal tetramethylsilane ($\delta = 0$ ppm), Chloroform-*d* ($\delta = 7.26$ ppm), DMSO-*d*₆ ($\delta = 2.50$ ppm), Acetone-*d*₆ ($\delta = 2.05$ ppm) or Acetonitrile- *d*₃ ($\delta = 1.94$ ppm). ^1H chemical shifts are reported in ppm using Chloroform-*d* ($\delta = 77.16$ ppm). If necessary, the structures were determined by two-dimensional analysis (COSY, HMQC and HMBC). NOESY and ROESY spectra were recorded in Louvain-la-Neuve on Bruker Avance 500 MHz spectrometer and in Taipei on Bruker Avance 500 MHz spectrometer and Bruker Avance 600 MHz spectrometer. ^1H spectra described as follow: chemical shift (multiplicity, coupling constant (Hz), integration). ^{13}C spectra are described as follow: chemical shift. Multiplicity is designed as : s = singlet; d = doublet; t = triplet; q = quartet; qui = quintet; sex= sextet; sep = septet; b = broad; m = multiplet.

High-resolution mass spectra were recorded by Mr. Raoul Rozenberg from Mass Spectrometry Service of UCLouvain.

X-ray diffraction were recorded by Dr. Koen Robeyns form Institute of Condensed Matter and Nanosciences of UCLouvain.

Calculation and conformation analyses were performed on UCLouvain cluster (manneback) with Gaussian 09.

Cyclic voltammetry was carried out on Autolab PGSTAT12/30/302 with the following conditions: Platinum (working electrode), Carbon (counter electrode) and Ag/AgNO₃ DCM TBABF 0.1 M (reference electrode).

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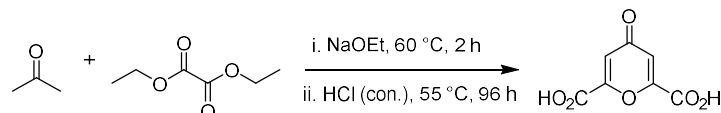
2. Procedure

Unless otherwise noticed, all manipulations were performed under an argon atmosphere using standard glassware. Diethyl ether and tetrahydrofuran were distilled on sodium/benzophenone under argon atmosphere. Dichloromethane, triethylamine and toluene were distilled on calcium hydride. Solvents used for work-up were of technical grade. Commercial reagents were purchased from Acros, Sigma-Aldrich, ABCR, TCI, Merck or Fluorochem and used as received unless stated otherwise.

Thin layer chromatography was performed on Merck Millipore F₂₅₄ silica gel 60 plates (200 µm thickness, aluminium supported). The plates were visualized using ultra-violet light (254 nm) and stained using an alkaline potassium permanganate, vanillin or ninhydrin solution. Chromatography was performed under pressure with the stated solvents using Sigma-Aldrich silica gel 60 (70 – 230 mesh).

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4-oxo-4H-pyran-2,6-dicarboxylic acid

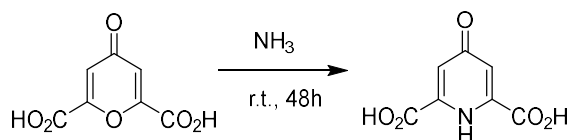


To a solution of sodium ethoxide (17.02 g, 250.3 mmol, 2.0 eq.) in ethanol (100 mL), was added acetone (9.0 mL, 122.8 mmol, 1.0eq.), diethyl oxalate (38.0 g, 260.2 mmol, 2.12 eq.) and ethanol (30 mL). After the addition was complete, the reaction mixture was allowed to stir for 2 h at 60 °C. Subsequently, 36% HCl (50 mL) and water (25 mL) were slowly added and the reaction mixture was further allowed to proceed for 24 h at 55 °C. Approximately 100 mL of solvent was removed by rotary evaporation. Subsequently, 36% HCl (23 mL) and water (70 mL) were slowly added and the reaction was further allowed to proceed for 72 h at 55 °C. The precipitated crystals were filtered and washed sequentially with water (200 mL) and acetone (100 mL) and the residue was recrystallized from boiling water in the presence of charcoal (4 g) to afford 8 g (35%) of product as a white solid.

^1H NMR (300 MHz, DMSO- d_6) δ 6.86 (s, 2H).

The characteristic data was corresponding with reported literature.^[1]

4-oxo-1,4-dihydropyridine-2,6-dicarboxylic acid



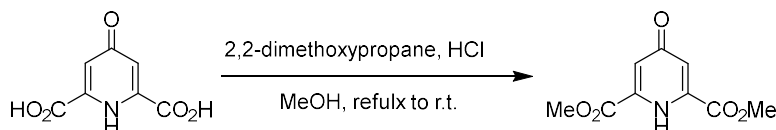
4-oxo-4H-pyran-2,6-dicarboxylic acid (8 g, 43.5 mmol) was suspended into 250 mL NH_3 (25% aqueous solution) at 0 °C. The resulting suspension was allowed to warm to room temperature and stirred for 48 h. Solvent was removed by rotary evaporation and the solid was redissolved in boiling water with 4g charcoal for 15 min and filtered. The filtrate was cooled down to 0 °C and acidified to pH 1 with HCl (36% aqueous solution) resulting in the precipitation of a white powder. The solid was obtained by filtration, washed with ice cold water and then dried in vacuum to give 7.1 g (90%) of product.

^1H NMR (300 MHz, DMSO- d_6) δ 7.54 (s, 2H).

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The characteristic data was corresponding with reported literature.^[1]

Dimethyl 4-oxo-1,4-dihydropyridine-2,6-dicarboxylate

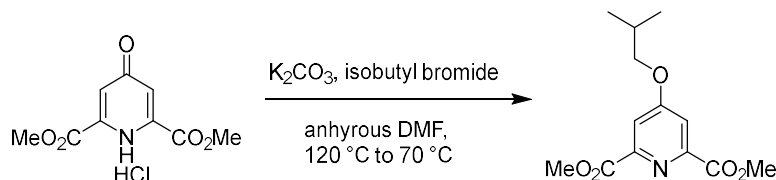


A suspension of chelidamic acid (5.3 g, 28.9 mmol) in MeOH (150 mL) was placed in a 500 mL round-bottomed flask. To this, 2,2-dimethoxypropane (35 mL, 289 mmol) and concentrated HCl (4.8 mL, 57.8 mmol) were added. The reaction mixture was refluxed for 4 hours under a CaCl₂ tube, and then at room temperature for 13 hours. After removing the solvent, the solid was triturated with Et₂O (50 mL x3), and the solution was separated from the solid by decantation. After drying the solid under vacuum, the product was obtained as a white solid (6.1 g, 99%).

¹H NMR (300MHz, DMSO-*d*₆) δ 11.59 (s, 1H), 7.56 (s, 2 H), 3.86 (s, 6H)

The characteristic data was corresponding with reported literature.^[1]

Dimethyl 4-isobutoxypyridine-2,6-dicarboxylate



Dimethyl 4-oxo-1,4-dihydropyridine-2,6-dicarboxylate (6.1 g, 25.0 mmol) and potassium carbonate (6.9 g, 50 mmol) were dissolved in 50 mL of anhydrous DMF and heated and stirred at 120 °C for 1.5 h. After, the system was cooled down to 70 °C followed by addition of isobutyl bromide (4.1 mL, 37.5 mmol) and stirred for another 3 h. The solution was cooled down to room temperature and the solid filtered off. 50 mL of water were added, and the mixture was extracted for 3 times with 50 mL of toluene. The combined organic phases were dried with sodium sulfate and evaporated under vacuum to obtain the product as a white solid (5.3 g, 80%).

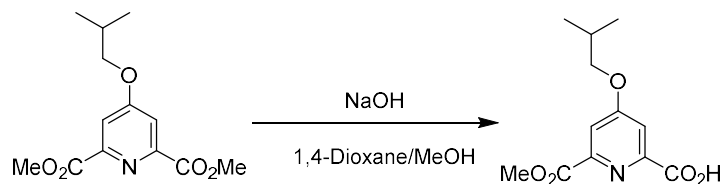
¹H NMR (300 MHz, Chloroform-*d*) δ 7.83 (s, 2H), 4.03 (s, 6H), 3.92 (d, *J* = 6.5

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Hz, 2H), 2.18 (dt, $J = 13.3, 6.6$ Hz, 1H), 1.08 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d): δ =166.4, 164.5, 149.3, 114.0, 74.5, 51.9, 29.0, 17.85.

The characteristic data was corresponding with reported literature.^[2]

4-isobutoxy-6-(methoxycarbonyl)picolinic acid

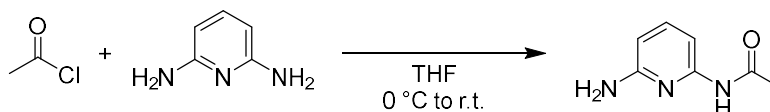


Dimethyl 4-oxo-1,4-dihydropyridine-2,6-dicarboxylate (1.34g, 5 mmol) was dissolved in a mixture of 1,4-dioxane (24 mL) and methanol (7 mL) with an ice bath. Sodium Hydroxide (0.2 g, 5 mmol) was added in one portion and the mixture was stirred at room temperature for 2.5 h while monitored by TLC. After the hydrolysis was completed, 0.5 mL of acetic acid was added, and the mixture was poured into 100 mL of water. Then extracted 3 times with 50 mL of DCM. The combined organic phases were dried over sodium sulfate and evaporated by rotary evaporation to afford the product (0.95 g, 74%) as a white solid without further purification.

^1H NMR (300 MHz, Chloroform- d) δ 7.78 (d, $J = 2.4$ Hz, 1H), 7.75 (d, $J = 2.4$ Hz, 1H), 3.94 (s, 3H), 3.85 (d, $J = 6.5$ Hz, 2H), 2.10 (dt, $J = 13.3, 6.7$ Hz, 1H), 0.99 (d, $J = 6.7$ Hz, 6H).

The characteristic data was corresponding with reported literature.^[2]

N-(6-aminopyridin-2-yl) acetamide



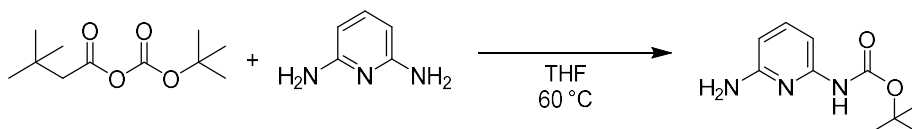
To 50 mL of a THF solution of 2,6-diaminopyridine (5.5 g, 50 mmol) was added acetyl chloride (1.96 g, 25 mmol) in 20 mL of THF dropwise with an ice bath. The reaction was allowed to warm to room temperature and stirred overnight. Then the solid was filtered off and the remaining solvent was removed by rotary evaporation. The residue was recrystallized in a mixture of ethanol and toluene to afford the pure product as a white solid (1.8 g, 47%).

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^1H NMR (300 MHz, Chloroform-*d*) δ 7.52 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 6.35 – 6.20 (m, 1H), 4.33 (s, 3H), 2.18 (s, 4H).

The characteristic data was corresponding with reported literature.^[3]

Tert-butyl (6-aminopyridin-2-yl) carbamate

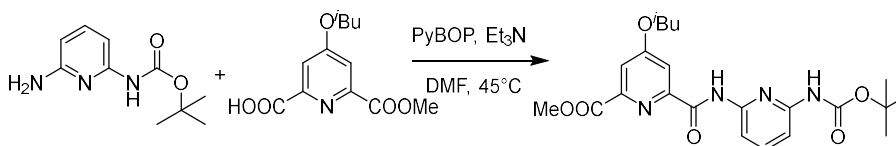


Di-tert-butylcarbonate (10.0 g, 45.8 mmol) was added to a solution of 2,6-diaminopyridine (5.0 g, 45.8 mmol) in 150 mL THF. The resulting solution was stirred at 60 °C overnight. After, the solvent was removed under vacuum to give the product as white solid (9.8 g, 70%) without further purification.

^1H NMR (300 MHz, CDCl₃): δ 7.45-7.40 (m, 1H), 7.24-7.23 (d, J = 8.1 Hz, 1H), 7.17 (s, 1H), 6.18-6.16 (d, J = 8.4 Hz, 1H), 4.35 (s, 2H), 1.51 (s, 9H).

The characteristic data was corresponding with reported literature.^[4]

Methyl 6-((6-((tert-butoxycarbonyl) amino) pyridin-2-yl) carbamoyl)-4-isobutoxypicolinate



PyBOP (1.0g, 2 mmol) was added to a mixture of tert-butyl (6-aminopyridin-2-yl) carbamate (209 mg, 1 mmol) and 4-isobutoxy-6-(methoxycarbonyl) picolinic acid (253 mg, 1 mmol) in 30 mL of DMF. Triethyl amine (385 mg, 3.8 mmol) was added and the mixed solution was stirred at 45 °C overnight. After, 50 mL of saturated sodium bicarbonate solution was added and the solution extracted 3 times with 30 mL of DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by column with the ratio of solvent (PE/EA = 4/1) to afford the pure product as a white solid (400 mg, 86%).

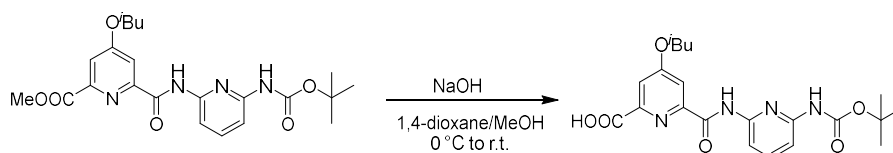
^1H NMR (300 MHz, Chloroform-*d*) δ 10.40 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.95 (d, J = 2.5 Hz, 1H), 7.80 (d, J = 2.3 Hz, 2H), 7.77 (s, 1H), 4.07 (s, 3H),

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3.95 (d, $J = 6.5$ Hz, 2H), 2.28 – 2.08 (m, 1H), 1.56 (s, 9H), 1.09 (d, $J = 6.7$ Hz, 6H).

The characteristic data was corresponding with reported literature.^[2]

6-((6-((tert-butoxycarbonyl) amino) pyridin-2-yl) carbamoyl)-4-isobutoxypicolinic acid

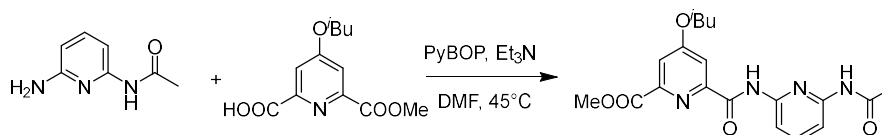


Methyl 6-((6-((tert-butoxycarbonyl) amino) pyridin-2-yl) carbamoyl)-4-isobutoxypicolinate (200 mg, 0.45 mmol) was dissolved in 1,4-dioxane (10 mL) and methanol (3 mL) and cooled with an ice bath. Sodium hydroxide (18 mg, 0.45 mmol) in 2 mL of methanol was added dropwise. After, the mixture was allowed to stir at room temperature for 4 h while monitored by TLC. 0.1 mL of acetic acid was added when TLC showed that the reaction was completed followed by 20 mL of water. The mixture was extracted 3 times with 15 mL of DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation to afford the product (170 mg, 87%) as a white solid without further purification.

¹H NMR (300 MHz, Chloroform-*d*) δ 10.40 (s, 1H), 8.04 (d, $J = 7.6$ Hz, 1H), 7.95 (d, $J = 2.5$ Hz, 1H), 7.80 (d, $J = 2.3$ Hz, 2H), 7.69 (s, 1H), 3.95 (d, $J = 6.5$ Hz, 2H), 2.28 – 2.08 (m, 1H), 1.56 (s, 9H), 1.09 (d, $J = 6.7$ Hz, 6H).

The characteristic data was corresponding with reported literature.^[2]

Methyl 6-((6-acetamidopyridin-2-yl) carbamoyl)-4-isobutoxypicolinate



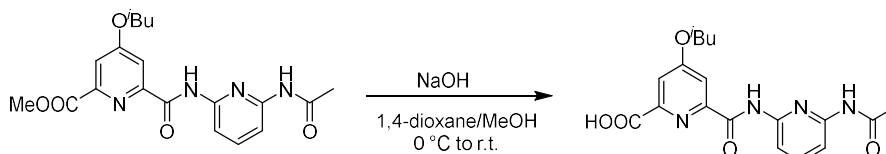
PyBOP (1.0g, 2 mmol) was added to a mixture of N-(6-aminopyridin-2-yl)acetamide (269 mg, 1.8 mmol) and 4-isobutoxy-6-(methoxycarbonyl) picolinic acid (450 mg, 1.8 mmol) in 30 mL of DMF. Triethyl amine (385 mg, 3.8 mmol) was added and the mixed solution was stirred at 45 °C overnight. After, 50 mL

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of saturated sodium bicarbonate solution was added and the solution was extracted 3 times with 30 mL of DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by column with the ratio of solvent (PE/EA = 4/1) to afford the pure product as a white solid (502 mg, 72%).

^1H NMR (300 MHz, Chloroform-*d*) δ 10.26 (s, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 2.5 Hz, 2H), 7.74 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 2.5 Hz, 1H), 3.97 (s, 3H), 3.86 (d, J = 6.5 Hz, 2H), 2.18 (s, 3H), 2.15 – 1.99 (m, 1H), 0.99 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.70, 165.06, 161.55, 157.66, 151.49, 149.45, 148.25, 140.00, 114.91, 110.97, 104.78, 103.33, 75.25, 52.95, 28.02, 19.04.

6-((6-acetamidopyridin-2-yl) carbamoyl)-4-isobutoxypicolinic acid

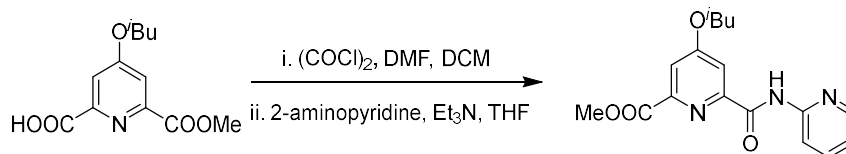


Methyl 6-((6-acetamidopyridin-2-yl) carbamoyl)-4-isobutoxypicolinate (386 mg, 1 mmol) was dissolved in 1,4-dioxane (15 mL) and methanol (5 mL) and cooled with an ice bath. Sodium hydroxide (40 mg, 1 mmol) in 3 mL of methanol was added dropwise. After, the mixture was allowed to stir at room temperature for 4 h and monitored by TLC. 0.1 mL of acetic acid was added when TLC showed complete reaction followed by 20 mL of water. The mixture was extracted 3 times with 15 mL of DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation to afford the product (297 mg, 80%) as a white solid without further purification.

^1H NMR (300 MHz, Chloroform-*d*) δ 10.30 (s, 1H), 7.97 (s, 1H), 7.87 (d, J = 2.5 Hz, 1H), 7.79 – 7.63 (m, 2H), 3.86 (d, J = 6.5 Hz, 2H), 2.18 (s, 2H), 2.14 – 1.97 (m, 1H), 0.99 (d, J = 6.7 Hz, 5H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.70, 166.84, 161.55, 157.66, 140.00, 114.91, 110.97, 104.78, 103.33, 75.25, 28.02, 19.04.

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Methyl 4-isobutoxy-6-(pyridin-2-ylcarbamoyl) picolinate

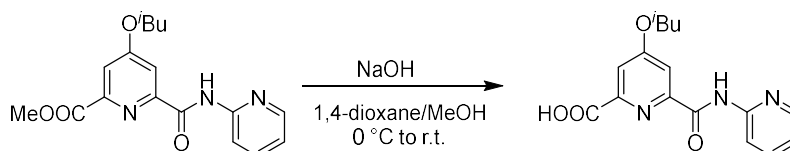


To a solution of 4-isobutoxy-6-(methoxycarbonyl) picolinic acid (176 mg, 0.5 mmol) in 10 mL DCM, catalytic DMF followed by oxalyl chloride (0.43 mL, 5mmol) were added dropwise. The mixture was allowed to stir for 2 h. Solvent was removed by rotary evaporation. The solid was dried under vacuum for another 2 h and then used for next step without further purification. The residue was suspended in 10 mL THF with 2-aminopyridine (47 mg, 0.5 mmol), triethyl amine (152 mg, 1.5 mmol) was added dropwise. The mixture was stirred at room temperature overnight. 30 mL of saturated sodium bicarbonate solution was added and extracted 3 times with 20 mL of DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation. The product was purified by column (PE/ EA = 5/1) to give the product as a white solid (130 mg, 78%).

¹H NMR (300 MHz, Chloroform-*d*) δ 11.22 (s, 1H), 8.56 (dt, *J* = 8.5, 0.9 Hz, 1H), 8.51 – 8.36 (m, 1H), 8.08 – 7.95 (m, 2H), 7.88 (ddd, *J* = 8.7, 7.4, 1.8 Hz, 1H), 7.19 (ddd, *J* = 7.4, 5.1, 1.0 Hz, 1H), 4.08 (s, 3H), 3.97 (d, *J* = 6.5 Hz, 2H), 2.19 (dt, *J* = 13.3, 6.7 Hz, 1H), 1.08 (d, *J* = 6.7 Hz, 6H). ¹³C NMR (75 MHz, Chloroform-*d*) δ 165.02, 162.51, 160.47, 150.90, 148.76, 146.59, 139.55, 120.18, 115.52, 114.19, 112.76, 75.42, 52.15, 28.01, 19.05.

HRMS (ESI): *m/z* calcd for C₁₇H₂₀O₄N₃, [M+H]⁺ 330.13461, found 330.13466

4-isobutoxy-6-(pyridin-2-ylcarbamoyl) picolinic acid



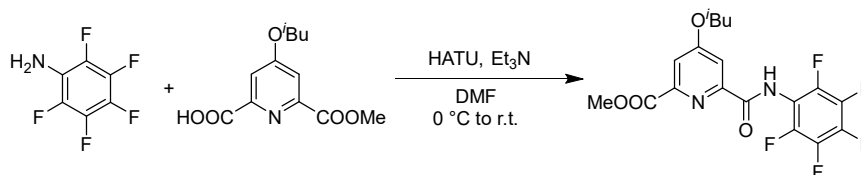
Methyl 4-isobutoxy-6-(pyridin-2-ylcarbamoyl) picolinate (100 mg, 0.3 mmol) was dissolved in 1,4-dioxane (6 mL) and methanol (2 mL) and cooled with an ice bath. Sodium hydroxide (12 mg, 0.3 mmol) dissolved in 1 mL of methanol

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was added to the mixture dropwise. After the addition was complete, the system was allowed to stir at room temperature for 4 h and monitored by TLC. 0.1 mL of acetic acid was added to the mixture followed by 10 mL of water and the solution was extracted 3 times with 10 mL of DCM. The combined organic solution was dried over sodium sulfate and concentrated by rotary evaporation to afford the product as a white solid (79 mg, 80%). ^1H NMR (300 MHz, Chloroform- d) δ 11.29 (s, 1H), 8.56 (dt, J = 8.5, 0.9 Hz, 1H), 8.51 – 8.36 (m, 1H), 8.08 – 7.95 (m, 2H), 7.88 (ddd, J = 8.7, 7.4, 1.8 Hz, 1H), 7.19 (ddd, J = 7.4, 5.1, 1.0 Hz, 1H), 3.97 (d, J = 6.5 Hz, 2H), 2.19 (dt, J = 13.3, 6.7 Hz, 1H), 1.08 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 168.02, 167.40, 162.51, 150.90, 148.76, 146.59, 139.55, 120.18, 115.52, 114.19, 112.76, 75.42, 28.01, 19.05.

HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{18}\text{O}_4\text{N}_3$, $[\text{M}+\text{H}]^+$ 316.12957, found 316.12954

Methyl 4-isobutoxy-6-((perfluorophenyl)carbamoyl) picolinate



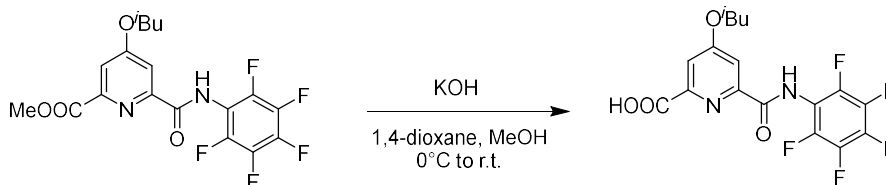
HATU (1140 mg, 3 mmol) with triethylamine (303 mg, 3 mmol) was added to 4-isobutoxy-6-(methoxycarbonyl) picolinic acid (506 mg, 2 mmol) in 15 mL DMF with an ice bath and stirred for 15 min. 2,3,4,5,6-pentafluoroaniline (366 mg, 2 mmol) in 5 mL DMF was added to the solution and the mixture was allowed to stir at room temperature for 60 h. After, the solution was poured into 80 mL of ice water and the precipitate filtered to give the product (312 mg, 37%) as an off-white solid without further purification.

^1H NMR (300 MHz, Chloroform- d) δ 9.63 (s, 1H), 7.92 (d, J = 2.5 Hz, 1H), 7.80 (d, J = 2.5 Hz, 1H), 4.02 (s, 3H), 3.93 (d, J = 6.5 Hz, 2H), 2.24 – 2.09 (m, 1H), 1.06 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 167.80, 164.76, 162.02, 150.19, 148.31, 115.43, 111.52, 75.41, 53.43, 53.04, 31.94, 29.71, 29.37, 28.02, 22.71, 19.02, 14.13.

HRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{16}\text{O}_4\text{N}_2\text{F}_5$, $[\text{M}+\text{H}]^+$ 419.10247, found 419.10242

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4-isobutoxy-6-((perfluorophenyl)carbamoyl) picolinic acid

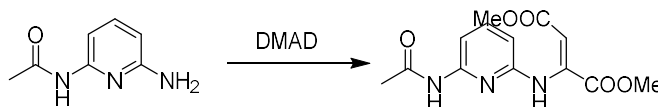


Methyl 4-isobutoxy-6-((perfluorophenyl)carbamoyl) picolinate (300 mg, 0.75 mmol) was dissolved in 1,4-dioxane (12 mL) and methanol (4 mL) and cooled with an ice bath. Potassium hydroxide (67 mg, 1.2 mmol, >85% purity) dissolved in 1 mL of methanol was added to the mixture dropwise. After the addition was complete, the system was allowed to stir at room temperature for 3 h. After, 0.6 mL of acetic acid was added to the mixture followed by 30 mL of water and the solution was extracted 3 times with 10 mL of DCM. The combined organic solution was dried over sodium sulfate, concentrated by rotary evaporation and dried under vacuum (252 mg, 83%). The residue was used directly without further purification.

^1H NMR (300 MHz, Chloroform-*d*) δ 9.63 (s, 1H), 7.92 (d, J = 2.5 Hz, 1H), 7.80 (d, J = 2.5 Hz, 1H), 4.02 (s, 3H), 3.93 (d, J = 6.5 Hz, 2H), 2.24 – 2.09 (m, 1H), 1.06 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.80, 164.76, 162.02, 150.19, 148.31, 115.43, 111.52, 75.41, 53.43, 53.04, 31.94, 29.71, 29.37, 28.02, 22.71, 19.02, 14.13.

HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{13}\text{O}_4\text{N}_2\text{F}_5$, $[\text{M}+\text{H}]^+$ 405.08682, found 405.08688

Dimethyl 2-((6-acetamidopyridin-2-yl)amino)maleate



To a solution of N-(6-aminopyridin-2-yl)acetamide (8.6 g, 28.4 mmol) in 50 mL of methanol was added DMAD (3.5 mL, 28.4 mmol). The resulting mixture was heated at reflux overnight. The solution was then cooled, and the solvent was evaporated to ca. 20 mL. The flask was then placed at -20 °C for 4 hours leading to precipitation of a solid. The resulting yellow prisms were collected by filtration and washed several times with cold methanol and dried under

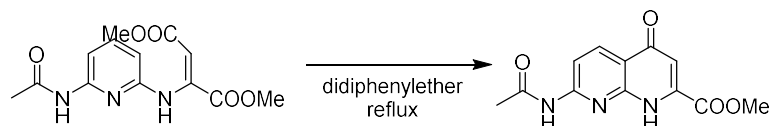
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reduced pressure to afford the pure product (12.0 g, 72%).

^1H NMR (300 MHz, Chloroform-*d*) δ 9.91 (br s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.53 (br s, 1H), 6.55 (d, J = 7.8 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 2.17 (s, 3H).

The characteristic data was corresponding with reported literature.^[5]

Methyl 7-acetamido-4-oxo-1,4-dihydro-1,8-naphthyridine-2-carboxylate

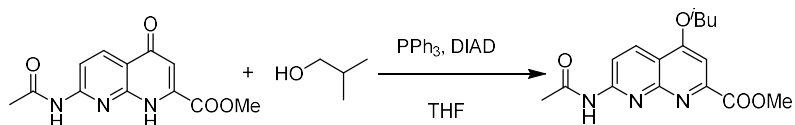


The fumarate derivative (12.0 g, 40 mmol) was added to refluxing diphenyl ether (250 mL at 260 °C) and the mixture heated at reflux temperature for 40 min. The reaction mixture was cooled down to room temperature and petroleum ether (800 mL) was added. The precipitate was triturated and filtered with another 500 mL of petroleum ether to afford the product (4.1 g, 39%) which was used without further purification for next step.

^1H NMR (300 MHz, DMSO-*d*₆) δ 11.73 (br s, 1H); 10.67 (s, 1H); 8.40 (d, 3 J = 8.7 Hz, 1H); 8.07 (d, 3 J = 8.7 Hz, 1H); 6.66 (s, 1H); 3.94 (s, 3H); 2.21 (s, 3H).

The characteristic data was corresponding with reported literature.^[5]

7-Acetamido-4-isobutoxy-1,8-naphthyridine-2-carboxylic acid



In a flame dried flask placed under the protection of argon, naphthyridone (4.1 g, 15.6 mmol), 2-methyl-1-propanol (1.9 mL, 20 mmol) and triphenylphosphine (5.2 g, 20 mmol) were suspended in 50 mL of freshly distilled THF and cooled with ice bath. Then DIAD was added to the mixture dropwise and stirred at this temperature for another 30 min and subsequently at room temperature for 24 hours. Solvent was removed by rotary evaporation and the residue was purified by chromatography (PE/EA = 1/1) and recrystallized from methanol at -20 °C to afford pure product (1.5 g, 30%).

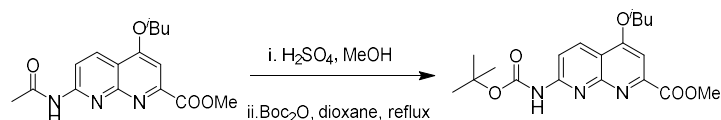
^1H NMR (300 MHz, Chloroform-*d*) δ 8.90 (br s, 1H); 8.58 (d, 3 J = 8.8 Hz, 1H);

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8.53 (d, 3 J = 8.8 Hz, 1H); 7.55 (s, 1H); 4.06 (d, 3 J = 6.2 Hz, 2H); 4.04 (s, 3H), 2.28-2.24 (1H, m); 1.12 (d, 3 J = 6.8 Hz, 6H).

The characteristic data was corresponding with reported literature.^[5]

7-((tert-butoxycarbonyl)amino)-4-isobutoxy-1,8-naphthyridine-2-carboxylic acid

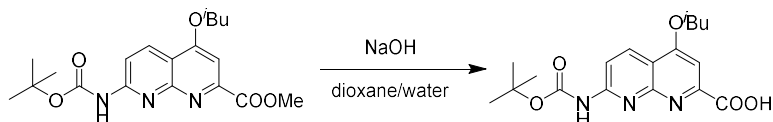


Naphthyridine (1.5 g, 4.7 mmol) was dissolved in 50 mL of methanol and cooled by ice bath. Then concentrated H₂SO₄ (1.3 mL, 25 mmol) was added dropwise. The reaction was stirred while cooled with an ice bath for another 1 hour and then allowed to stir at room temperature overnight. The mixture was then neutralized with a saturated sodium bicarbonate solution and extracted with DCM. The organic layers were then washed with distilled water, brine and dried over sodium sulfate and concentrated to afford a crude solid that was used without further purification. The solid was dissolved in 50 mL of dioxane with Boc₂O (2.2 g, 10 mmol). The reaction mixture was heated to reflux for 4 hours and solvent was removed by rotary evaporation. The residue was purified by chromatography (PE/EA = 4/1 to 2/1) (1.16 g, 68%).

¹H NMR (300 MHz, CDCl₃) δ 8.52 (d, 3 J = 8.8 Hz, 1H); 8.32 (d, 3 J = 8.8 Hz, 1H); 7.57 (br s, 1H); 7.52 (s, 1H); 4.04 (d, 3 J = 6.6 Hz, 1H); 4.03 (s, 3H); 2.27 (m, 1H); 1.56 (s, 9H); 1.12 (d, 3 J = 6.8 Hz, 1H).

The characteristic data was corresponding with reported literature.^[5]

7-((tert-butoxycarbonyl)amino)-4-isobutoxy-1,8-naphthyridine-2-carboxylic acid



Naphthyridine (1.16 g, 3.0 mmol) was dissolved in 20 mL of dioxane/water (8/2). Sodium hydroxide (0.24 g, 6 mmol) was added and the mixture was stirred for 5 hours. The reaction was then quenched with 0.5 mL of acetic acid

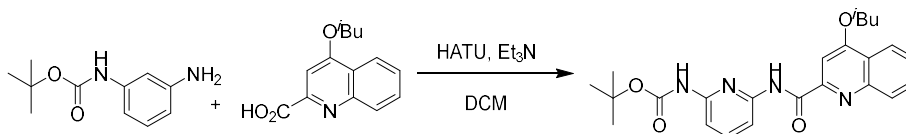
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and the solvent was removed under reduced pressure. The residue was dissolved in 50 mL of DCM and washed with water, brine, dried over sodium sulfate and concentrated by rotary evaporation to afford the pure product (0.97 g, 90%).

^1H NMR (300 MHz, DMSO- d_6) δ 10.38 (s, 1H), 8.54 (d, 3 J = 9 Hz, 1H); 8.18 (d, 3 J = 9 Hz, 1H); 7.48 (s, 1H); 4.10 (d, 3 J = 6.2 Hz, 2H); 2.19 (m, 1H); 1.51 (s, 9H); 1.07 (d, 3 J = 6.4 Hz, 6H).

The characteristic data was corresponding with reported literature.^[5]

Tert-butyl (6-(4-isobutoxyquinoline-2-carboxamido) pyridin-2-yl) carbamate



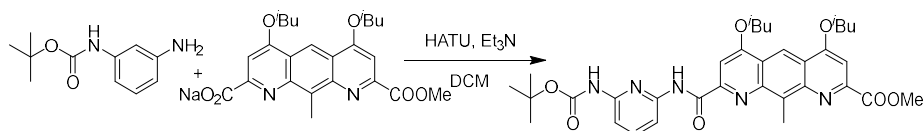
HATU (760 mg, 2 mmol) and triethylamine (202 mg, 2 mmol) were added to the solution of 4-isobutoxyquinoline-2-carboxylic acid (274 mg, 1 mmol) in 8 mL anhydrous DMF and the mixture was stirred for 15 min with an ice bath. Tert-butyl (3-aminophenyl) carbamate (208 mg, 1 mmol) in 2 mL DMF was added and the reaction was allowed to stir overnight. The reaction was poured in 20 mL of ice-water. The solid was filtered and purified by column (PE/EA = 4:1) to afford the product as a light green solid (376 mg, 87%).

^1H NMR (300 MHz, Chloroform- d) δ 10.59 (s, 1H), 8.26 (dd, J = 8.4, 1.4 Hz, 1H), 8.07 (ddd, J = 13.6, 7.9, 1.2 Hz, 2H), 7.82 – 7.70 (m, 3H), 7.68 (s, 1H), 7.63 – 7.54 (m, 2H), 4.05 (d, J = 6.5 Hz, 2H), 2.48 – 2.19 (m, 1H), 1.47 (s, 9H), 1.14 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 163.36, 163.02, 152.44, 150.71, 150.57, 149.56, 147.55, 140.71, 130.56, 129.51, 127.25, 122.51, 122.14, 108.38, 108.08, 98.26, 81.20, 75.26, 28.39, 28.30, 19.59, 19.38.

HRMS (ESI): m/z calcd for $\text{C}_{24}\text{H}_{29}\text{O}_4\text{N}_4$, $[\text{M}+\text{H}]^+$ 437.21833, found 437.21815

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Methyl 8-((6-((tert-butoxycarbonyl)amino)pyridin-2-yl)carbamoyl)-4,6-diisobutoxy-10-methylpyrido[3,2-g]quinoline-2-carboxylate

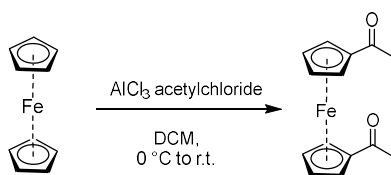


HATU (304 mg, 0.8 mmol) and triethylamine (81 mg, 0.8 mmol) were added to the solution of 4-isobutoxyquinoline-2-carboxylic acid (183 mg, 0.4 mmol) in 8 mL anhydrous DMF and the mixture was stirred for 15 min while cooled with an ice bath. Tert-butyl (3-aminophenyl) carbamate (83 mg, 0.4 mmol) in 2 mL DMF was added and stirred overnight. The reaction was poured into 20 mL of ice-water. The solid was filtered and recrystallized in MeOH to afford the product as a light green solid (184 mg, 73%).

^1H NMR (300 MHz, Chloroform-*d*) δ 10.48 (s, 1H), 9.17 – 8.92 (m, 1H), 8.08 (dd, J = 7.3, 1.5 Hz, 1H), 7.83 – 7.69 (m, 2H), 7.62 (s, 1H), 7.44 (s, 1H), 7.37 (s, 1H), 4.35 – 3.98 (m, 7H), 3.45 (d, J = 0.9 Hz, 3H), 2.36 (td, J = 6.6, 1.7 Hz, 1H), 1.54 (s, 9H), 1.20 (d, J = 6.7 Hz, 12H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 166.48, 164.05, 163.50, 162.79, 152.37, 151.09, 150.66, 149.93, 149.29, 145.87, 144.79, 140.66, 137.80, 121.59, 121.45, 113.77, 108.23, 108.06, 98.76, 96.42, 81.19, 75.33, 75.17, 53.36, 28.42, 19.29, 13.06.

HRMS (ESI): m/z calcd for $\text{C}_{34}\text{H}_{42}\text{O}_7\text{N}_5$, $[\text{M}+\text{H}]^+$ 632.30788, found 632.30895.

1,1'-diacetylferrocene



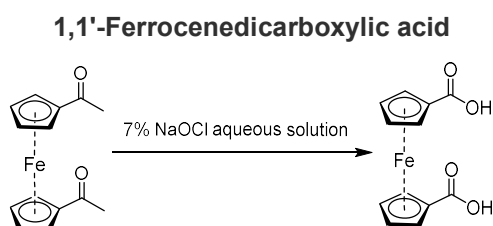
To a solution of anhydrous aluminium chloride (6.7 g, 50 mmol) in 25 mL freshly distilled DCM, acetyl chloride was added dropwise, during which time the aluminium chloride dissolved. A solution of ferrocene (3.7 g, 20 mmol) in 25 mL DCM was added dropwise at room temperature over a period of 45 min and the mixture was allowed to stir at room temperature for 3 h. The solution was poured into 100 mL of water-ice and the organic phase extracted. The

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organic phase was washed with water until neutral and dried over sodium sulfate, and concentrated by rotary evaporation. The residue was purified by column (PE/EA = 10/1) to give an orange solid (10 g, 74%).

^1H NMR (300 MHz, Chloroform- d) δ , 4.51 (4H, s), 4.77 (4H, s), 2.35 (6H, s)

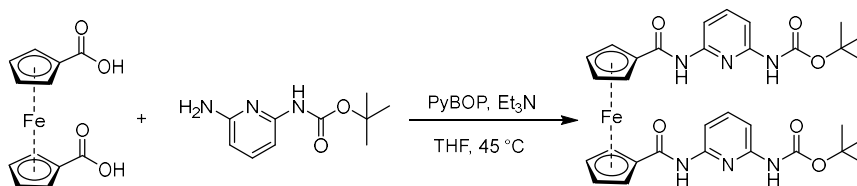
The characteristic data was corresponding with reported literature.^[6]



1,1'-diacetylferrocene (9.5 g, 35 mmol) was added to 250 mL of 7% sodium hypochlorite solution at 60 °C and stirred for 2 h while protected from light. A further 250 mL of sodium hypochlorite solution was added and the reaction was stirred for another 6 h at 60 °C. After, the reaction was filtered while it was hot and acidified to pH 1-2 with concentrated hydrochloric acid solution to give an orange precipitate. The crude was re-dissolved in a sodium hydroxide solution and the solid filtered off, the aqueous solution was re-acidified with concentrated hydrochloride acid and filtered to give the product as a pure orange solid (4.1 g, 43%).

^1H NMR (300 MHz, Chloroform- d) δ 12.25 (s, 2H), 4.70 (d, J = 6.4 Hz, 4H), 4.47 (d, J = 6.4 Hz, 4H).

The characteristic data was corresponding with reported literature.^[6b]



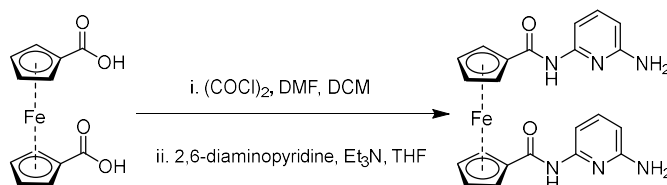
PyBOP (1.56 g, 3 mmol) and triethylamine (0.30 g, 3 mmol) were added to a solution of 1,1'-ferrocenedicarboxylic acid (0.27 g, 1 mmol) and tert-butyl (6-aminopyridin-2-yl) carbamate (0.21 g, 1 mmol) in 20 mL THF and the reaction was stirred at 45 °C for 30 h. The reaction was quenched by 50 mL saturated

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sodium bicarbonate solution and extracted 3 times with 30 mL DCM. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by column (MeOH/DCM = 1/100) to afford the pure product (0.39 g, 60%).

^1H NMR (300 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 7.85 – 7.74 (m, 1H), 7.71 – 7.52 (m, 3H), 4.81 (t, J = 2.0 Hz, 2H), 4.53 (t, J = 2.0 Hz, 2H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.93, 152.24, 150.30, 149.41, 140.45, 108.98, 108.13, 81.38, 72.54, 70.53, 28.30.

HRMS (ESI): m/z calcd for $\text{C}_{32}\text{H}_{37}\text{O}_6\text{N}_6^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 655.21662, found 655.21664.



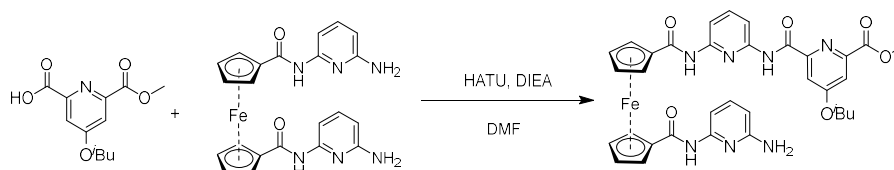
To a solution of ferrocene dicarboxylic acid (0.19 g, 1 eq.) in 15 mL DCM, a catalytic amount of DMF was added. Then oxalyl chloride (0.60 mL, 10 eq.) was added dropwise and the reaction mixture was allowed to stir at room temperature for 2 hours. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the crude acid chloride without further purification. The acid chloride was re-dissolved in freshly distilled THF with a excess of 2,6-diaminopyridine (0.30 g, 4 eq.). Triethyl amine (0.40 mL, 4 eq.) was added slowly and the mixture was stirred at room temperature overnight. Then the solvent was removed under reduced pressure. The solid was redissolved in ethyl acetate and the mixture was washed with water. The aqueous phase was extracted with ethyl acetate one time. The organic phases were combined, dried over sodium sulfate and concentrated by rotary evaporation. The mixture was purified by column (methanol/dichloromethane = 1/20) to afford the product (82%, 0.26 g) as an orange solid.

^1H NMR (300 MHz, Chloroform-*d*) δ 8.07 (s, 2H), 7.62 (dd, J = 8.0, 0.7 Hz, 2H), 7.47 (t, J = 7.9 Hz, 2H), 6.28 (dd, J = 7.9, 0.8 Hz, 2H), 4.86 (t, J = 1.9 Hz,

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4H), 4.53 (t, $J = 2.0$ Hz, 4H), 4.37 (s, 4H). ^{13}C NMR (75 MHz, Chloroform- d) δ 167.82, 158.65, 149.44, 140.64, 109.58, 109.26, 103.66, 72.71, 72.55, 70.53, 70.28, 30.94, 29.71, 24.77.

The characteristic data was corresponding with reported literature.^[7]

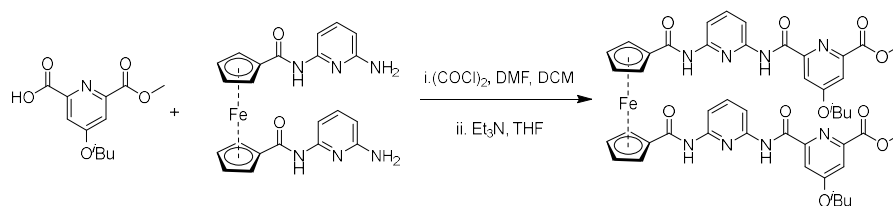


The pyridine monoacid (152 mg, 0.6 mmol), HATU (500 mg, 1.32 mmol) and DIEA (200 μL) in 10 mL DMF were stirred at 0 $^{\circ}\text{C}$ for 15 min. Ferrocene conjugate **b** (300 mg, 0.66 mmol) was added to the reaction mixture and stirred at 0 $^{\circ}\text{C}$ for another 1 hour then stirred overnight at room temperature. 50 mL of saturated sodium bicarbonate solution was added to the solution and extracted with 30 mL of DCM two times. The combined organic phases were dried over sodium sulfate and concentrated by rotary evaporation. The mixture was purified by column (methanol/dichloromethane = 1/50) to afford product as orange solid (70%, 0.28 g).

^1H NMR (300 MHz, Chloroform- d) δ 10.35 (s, 1H), 8.70 (s, 1H), 8.16 – 8.03 (m, 2H), 7.97 (s, 1H), 7.96 (d, $J = 2.5$ Hz, 1H), 7.81 – 7.73 (m, 2H), 7.43 – 7.36 (m, 2H), 6.27 (dd, $J = 7.0, 1.8$ Hz, 1H), 5.16 (s, 2H), 4.89 (dt, $J = 18.1, 2.0$ Hz, 4H), 4.55 (dt, $J = 4.5, 2.0$ Hz, 4H), 4.07 (s, 3H), 3.95 (d, $J = 6.5$ Hz, 2H), 2.17 (s, 1H), 1.07 (d, $J = 6.7$ Hz, 7H). ^{13}C NMR (75 MHz, Chloroform- d) δ 167.95, 167.88, 161.71, 161.59, 157.73, 151.56, 150.03, 149.09, 148.09, 140.52, 139.91, 133.83, 115.26, 111.09, 110.67, 109.69, 104.43, 103.73, 77.94, 75.26, 72.46, 70.33, 67.98, 53.06, 28.06, 25.59, 19.09.

HRMS (ESI): m/z calcd for $\text{C}_{34}\text{H}_{34}\text{O}_6\text{N}_7^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 690.19630, found 690.19612.

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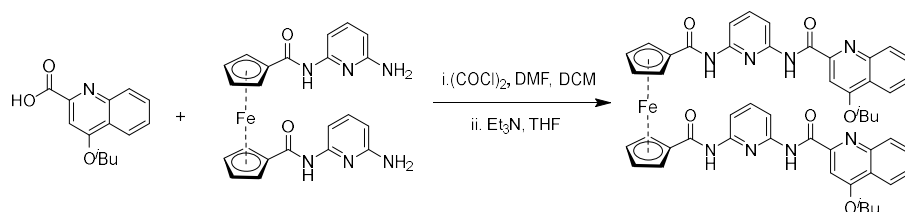


To a solution of mono acid (121 mg, 0.5 mmol) in distilled DCM, a catalytic amount of DMF was added followed by oxalyl chloride (610 mg, 5 mmol). The reaction was stirred at room temperature for 3 hours. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the mono acyl chloride which was used without further purification. In a pre-dried round bottom flask under the protection of argon, the acyl chloride was redissolved in dry THF with the ferrocene diamine (100 mg, 0.2 mmol). Triethylamine (89 mg, 0.9 mmol) was added dropwise at room temperature and the reaction was allowed to stir overnight. Then the solvent was removed by rotary evaporation and the mixture was redissolved into a mixture of dichloromethane and methanol (1:2). After removing the dichloromethane by rotary evaporation, a solid precipitated which was filtered and dried to afford the product (159 mg, 78%) as orange solid.

^1H NMR (300 MHz, Chloroform-*d*) δ 10.12 (s, 2H), 8.09 (s, 2H), 7.89 (d, J = 8.1 Hz, 4H), 7.79 (s, 2H), 7.67 – 7.49 (m, 4H), 4.91 (s, 4H), 4.50 (s, 4H), 3.97 (d, J = 7.9 Hz, 6H), 3.86 (d, J = 6.5 Hz, 4H), 1.01 (d, J = 6.6 Hz, 12H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 167.63, 164.93, 161.55, 151.42, 149.80, 148.83, 148.17, 140.63, 115.03, 111.03, 109.46, 75.35, 72.76, 70.33, 28.05, 19.10.

HRMS (ESI): m/z calcd for $\text{C}_{46}\text{H}_{46}\text{O}_{10}\text{N}_8^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 925.28058, found 925.28168.

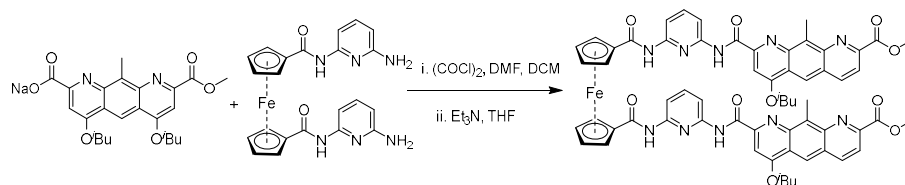
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To a solution of mono acid (118 mg, 0.5 mmol) in distilled DCM, a catalytic amount of DMF was added followed by oxalyl chloride (610 mg, 5 mmol). The reaction was stirred at room temperature for 3 hours. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the mono acyl chloride which was used without further purification. In a pre-dried round bottom flask under the protection of argon, the acyl chloride and ferrocene diamine (100 mg, 0.2 mmol) were redissolved in THF. Triethylamine (89 mg, 0.9 mmol) was added dropwise at room temperature. The reaction was allowed to stir overnight. Then the solvent was removed by rotary evaporation and the mixture was re-dissolved into a mixture of dichloromethane and methanol (1:2). After removing the dichloromethane by rotary evaporation, a solid precipitated which was filtered and dried to afford the product (170 mg, 85%) as an orange solid.

^1H NMR (300 MHz, Chloroform- d) δ 10.43 (s, 2H), 8.11 (d, J = 8.7 Hz, 4H), 8.07 – 7.82 (m, 6H), 7.69 – 7.34 (m, 8H), 4.91 (s, 4H), 4.51 (s, 4H), 3.95 (d, J = 6.5 Hz, 4H), 2.23 (dt, J = 13.3, 6.6 Hz, 2H), 1.09 (d, J = 6.7 Hz, 12H). ^{13}C NMR (75 MHz, Chloroform- d) δ 206.97, 167.22, 162.99, 162.59, 150.28, 149.78, 149.20, 147.22, 140.60, 130.12, 129.25, 126.92, 122.20, 121.83, 109.54, 109.29, 97.97, 77.83, 75.03, 72.49, 70.22, 30.95, 28.19, 19.28.

HRMS (ESI): m/z calcd for $\text{C}_{50}\text{H}_{46}\text{O}_6\text{N}_8^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 911.30092, found 911.30169.



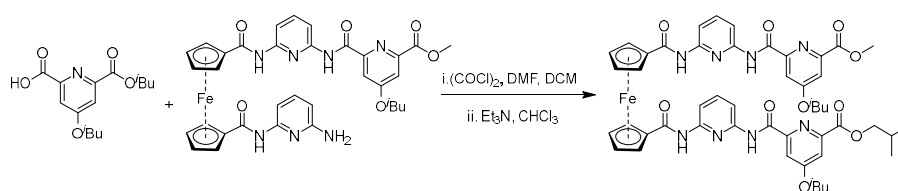
To a solution of the mono acid salt (222 mg, 0.5 mmol) in distilled DCM, a catalytic amount of DMF was added followed by oxalyl chloride (610 mg, 5

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mmol). The reaction was stirred at room temperature for 3 hours. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the mono acyl chloride which was used without further purification. In a pre-dried round bottom flask under the protection of argon, the acyl chloride and ferrocene diamine (100 mg, 0.2 mmol). Triethylamine (89 mg, 0.9 mmol) was then added dropwise at room temperature. The reaction was allowed to stir overnight. Then the solvent was removed by rotary evaporation and the mixture was re-dissolved into a mixture of dichloromethane and methanol (1:2). After removing the dichloromethane by rotary evaporation, a solid precipitated which was filtered and dried to afford the product (158 mg, 85%) as an orange solid.

NMR data was collect at 318 K. ^1H NMR (500 MHz, Chloroform-*d*) δ 10.22 (s, 1H), 8.61 (s, 1H), 7.99 (s, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.24 (s, 1H), 7.15 (s, 1H), 5.13 (t, J = 2.0 Hz, 2H), 4.55 (t, J = 1.9 Hz, 2H), 4.08 – 4.00 (m, 7H), 3.39 (s, 3H), 2.42 – 2.28 (m, 2H), 1.22 (dd, J = 8.6, 6.7 Hz, 12H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 206.97, 167.22, 162.99, 162.59, 150.28, 149.78, 149.20, 147.22, 140.60, 130.12, 129.25, 126.92, 122.20, 121.83, 109.54, 109.29, 97.97, 77.83, 75.03, 72.49, 70.22, 30.95, 28.19, 19.28.

HRMS (ESI): m/z calcd for $\text{C}_{62}\text{H}_{56}\text{O}_{10}\text{N}_{10}^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 1299.48001, found 1299.48063.



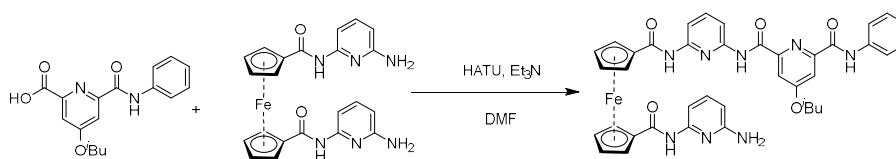
To a solution of mono pyridine acid (71 mg, 0.2 mmol) in distilled DCM, a catalytic amount of DMF was added followed by oxalyl chloride (305 mg, 2.4 mmol). The reaction was stirred at room temperature for 3 hours. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the mono acyl chloride which was used without further purification. In a pre-dried round bottom flask under the protection of argon, the acyl chloride and ferrocene diamine (152 mg, 0.2 mmol). Triethylamine

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(45 mg, 0.5 mmol) was then added dropwise at room temperature. The reaction was allowed to stir overnight. Then the solvent was removed by rotary evaporation and the mixture was purified by column (methanol/dichloromethane = 1/100) to afford asymmetric ferrocene conjugate as an orange solid (66 mg, 34%).

^1H NMR (300 MHz, Chloroform- d) δ 10.23 (d, J = 23.7 Hz, 2H), 8.15 (d, J = 16.2 Hz, 2H), 8.02 – 7.93 (m, 4H), 7.87 (dd, J = 2.6, 1.1 Hz, 2H), 7.76 – 7.60 (m, 4H), 4.98 (dt, J = 3.4, 1.9 Hz, 4H), 4.57 (t, J = 1.9 Hz, 4H), 4.20 (d, J = 6.7 Hz, 2H), 4.02 (s, 3H), 3.93 (dd, J = 6.5, 1.9 Hz, 4H), 2.32 – 2.10 (m, 3H), 1.14 – 1.05 (m, 18H). ^{13}C NMR (75 MHz, Chloroform- d) δ 167.63, 167.48, 164.90, 164.14, 161.52, 151.42, 149.81, 148.86, 148.30, 148.13, 140.58, 115.06, 114.94, 110.98, 110.48, 109.89, 109.39, 75.29, 72.72, 71.95, 70.29, 53.00, 30.93, 28.04, 27.91, 19.26, 19.07.

HRMS (ESI): m/z calcd for $\text{C}_{49}\text{H}_{52}\text{O}_{10}\text{N}_8^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 969.32289, found 969.32294.



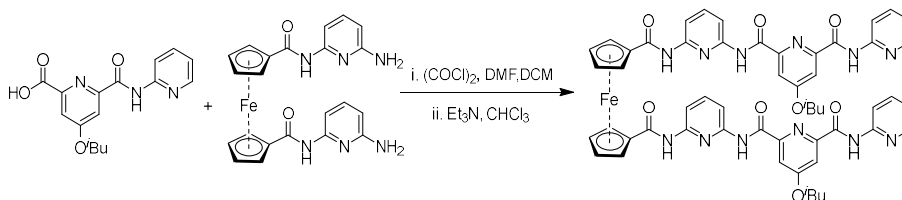
HATU (380 mg, 1 mmol) and triethylamine (101 mg, 1 mmol) were added to the dimer acid (157 mg, 0.5 mmol) in 15 mL anhydrous DMF and stirred for 15 min with an ice bath. Ferrocene diamine (228 mg, 0.5 mmol) in 5 mL anhydrous DMF was added in the reaction mixture. The reaction was stirred at room temperature overnight. After, the reaction solution was poured into 50 mL of a saturated sodium bicarbonate solution while cooled with an ice bath. The aqueous solution was extracted 3 times with 30 mL DCM. The combined organic solution was dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by column (DCM/MeOH = 100/1 to 50/1) to afford the product as an orange solid (271 mg, 72%).

^1H NMR (300 MHz, Chloroform- d) δ 10.30 (s, 1H), 9.99 (s, 1H), 8.50 (s, 1H), 8.10 (d, J = 8.4 Hz, 2H), 8.04 – 7.93 (m, 2H), 7.89 (d, J = 8.0 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.74 (t, J = 8.2 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.36 – 7.28 (m,

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2H), 7.22 (t, $J = 7.9$ Hz, 1H), 7.12 (t, $J = 7.4$ Hz, 1H), 6.09 (d, $J = 7.9$ Hz, 1H), 4.83 (dt, $J = 23.0, 1.9$ Hz, 4H), 4.62 – 4.45 (m, 4H), 4.29 (s, 2H), 3.96 (d, $J = 6.6$ Hz, 2H), 2.30 – 2.06 (m, 1H), 1.07 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 168.48, 168.23, 167.94, 161.91, 161.59, 157.13, 151.40, 150.47, 149.99, 149.61, 149.21, 140.82, 140.04, 137.45, 129.08, 124.92, 120.92, 112.14, 110.69, 110.03, 104.50, 103.60, 75.51, 72.68, 72.59, 70.80, 70.61, 38.76, 28.16, 19.21.

HRMS (ESI): m/z calcd for $\text{C}_{39}\text{H}_{37}\text{O}_5\text{N}_8^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 751.22776, found 751.22784.



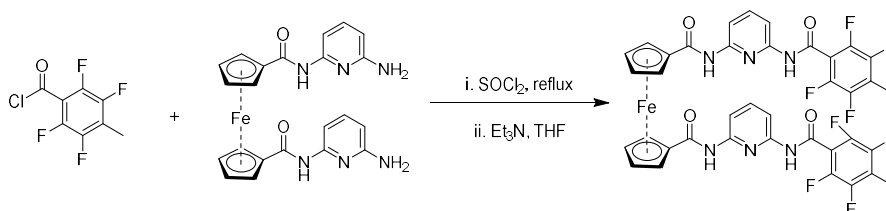
To a solution of Pyridine dimer acid (200 mg, 0.6 mmol) in freshly distilled DCM, a catalytic amount of anhydrous DMF was added followed by oxalyl chloride (813 mg, 6.4 mmol) and the reaction was stirred for 4 h at room temperature. Then the solvent was removed by rotary evaporation and the residue was dried under vacuum to give the mono acyl chloride without further purification. In a pre-dried round bottom flask under the protection of argon, the acyl chloride and ferrocene diamine (137 mg, 0.3 mmol) were dissolved in chloroform. Triethylamine (71 mg, 0.7 mmol) was added dropwise at room temperature. The reaction was allowed to stir overnight. Then the solvent was removed by rotary evaporation and the mixture was purified by column (methanol/dichloromethane = 1/100) to afford the ferrocene conjugate as an orange solid (183 mg, 58%).

^1H NMR (300 MHz, Chloroform- d) δ 10.45 (s, 2H), 10.36 (s, 2H), 8.63 (ddd, $J = 4.7, 1.7, 0.9$ Hz, 2H), 8.24 (dt, $J = 7.8, 1.1$ Hz, 2H), 8.19 – 8.06 (m, 4H), 7.92 – 7.83 (m, 4H), 7.79 (t, $J = 8.1$ Hz, 2H), 7.71 (d, $J = 2.5$ Hz, 2H), 7.45 (ddd, $J = 7.6, 4.7, 1.2$ Hz, 2H), 4.01 (s, 6H), 3.87 (d, $J = 6.5$ Hz, 4H), 2.22 – 2.01 (m, 2H), 1.00 (d, $J = 6.7$ Hz, 12H). ^{13}C NMR (75 MHz, Chloroform- d) δ 167.75,

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165.01, 162.57, 161.89, 151.32, 149.54, 149.26, 148.30, 140.91, 137.64, 126.83, 122.54, 115.02, 111.14, 109.88, 75.30, 53.02, 28.03, 19.05.

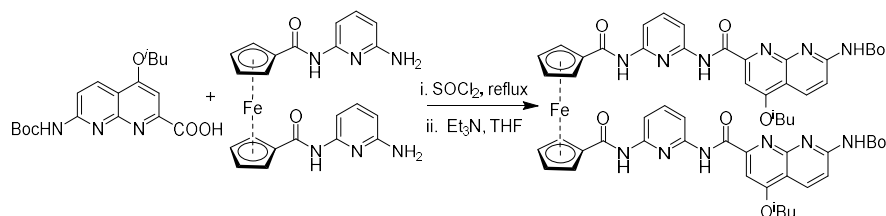
HRMS (ESI): m/z calcd for $C_{54}H_{51}O_8N_{12}^{54}Fe$, $[M+H]^+$ 1049.33435, found 1049.33466.



2,3,5,6-Tetrafluoro-4-methylbenzoic acid (211 mg, 1 mmol) was dissolved in 5 mL thionyl chloride and stirred at reflux for 30 min. The reaction solution was concentrated by rotary evaporation and dried under vacuum for 2 h. The residue was used as directly without further purification. The acid chloride and ferrocene diamine (205 mg, 0.5 mmol) were then dissolved in freshly distilled THF. The reaction was stirred for 60 h at room temperature. After, the reaction mixture was poured in 50 mL of a saturated sodium bicarbonate solution and washed 2 times with 30 mL DCM. After, the aqueous solution was filtered and an orange solid was obtained (107 mg, 26%).

1H NMR (300 MHz, $DMSO-d_6$) δ 11.26 (s, 2H), 9.75 (s, 2H), 8.02 – 7.63 (m, 6H), 5.12 (s, 4H), 4.67 – 4.37 (m, 4H), 2.34 (s, 3H). ^{13}C NMR (75 MHz, $DMSO-d_6$) δ 168.12, 150.92, 149.58, 140.56, 110.25, 77.59, 72.94, 70.73, 8.11.

HRMS (ESI): m/z calcd for $C_{36}H_{24}O_4N_6F_8^{54}Fe^{23}Na$, $[M+H]^+$ 857.10196, found 857.10229.

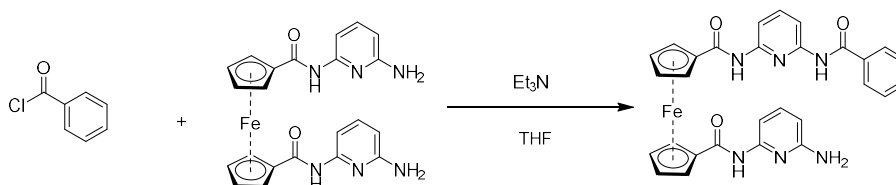


The naphthyridine acid (0.1 g, 0.27 mmol) was dissolved in 5 mL of $SOCl_2$ and then stirred at reflux for 0.5 hours. The reaction was then concentrated by rotary evaporation and dried under vacuum for 2 hours. The residue was used

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directly without further purification. The acid chloride and ferrocene diamine (60 mg, 0.13 mmol) were then dissolved in freshly distilled THF. The reaction was added triethylamine (0.1 mL, 0.6 mmol) stirred for 24 h at room temperature. After, the reaction mixture was poured in 50 mL saturated sodium bicarbonate solution and washed 2 times with 30 mL DCM. The organic layers were combined and concentrated to 10 mL. After, 10 mL of methanol were added to the solution. The product was precipitated by slow removal of DCM by rotary evaporation. After filtration the product was obtained as an orange solid (151 mg, 49%).

^1H NMR (300 MHz, Chloroform- d) δ 10.44 (s, 1H), 8.56 (d, J = 9.1 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 7.80 – 7.71 (m, 2H), 7.66 (s, 1H), 7.52 (t, J = 7.9 Hz, 1H), 6.30 (dd, J = 7.9, 0.7 Hz, 1H), 4.33 (s, 2H), 4.10 (d, J = 6.5 Hz, 2H), 3.49 (s, 2H), 2.29 (dp, J = 13.2, 6.6 Hz, 1H), 1.13 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 172.44, 163.90, 162.38, 157.39, 154.82, 153.84, 151.95, 149.60, 139.84, 134.17, 114.05, 113.52, 104.52, 103.47, 98.70, 81.83, 75.48, 28.21, 19.13.



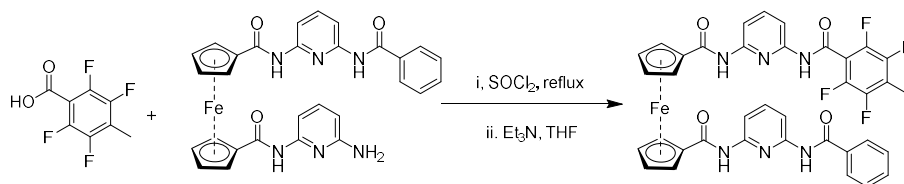
Benzoyl chloride (70 mg, 0.5 mmol) was added to a solution of ferrocene pyridine diamine (228 mg, 0.5 mmol) in 20 mL freshly distilled DCM. The reaction mixture was stirred overnight. After, 30 mL of saturated sodium bicarbonate solution was added and the solution was extracted 3 times with 30 mL DCM. The combined organic solution was dried over sodium sulfate and concentrated by rotary evaporation. The residue was purified by column (DCM/MeOH = 50/1) to afford the product as an orange solid (90mg, 30%).

^1H NMR (300 MHz, Chloroform- d) δ 8.53 (s, 2H), 8.36 (s, 1H), 8.02 (dd, J = 8.0, 0.8 Hz, 1H), 7.94 (ddd, J = 8.2, 1.9, 1.0 Hz, 3H), 7.68 (t, J = 8.1 Hz, 1H), 7.64 – 7.43 (m, 4H), 7.35 (t, J = 8.0 Hz, 1H), 6.12 (dd, J = 8.0, 0.7 Hz, 1H), 4.94 (dt, J = 17.4, 2.0 Hz, 4H), 4.54 (q, J = 2.0 Hz, 4H), 4.42 (s, 2H). ^{13}C NMR

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(75 MHz, Chloroform-*d*) δ 167.88, 140.78, 140.58, 132.43, 128.99, 127.39, 110.05, 109.63, 104.47, 103.63, 72.64, 70.57.

HRMS (ESI): m/z calcd for $C_{29}H_{25}O_3N_6^{54}Fe$, $[M+H]^+$ 559.13788, found 559.13820.

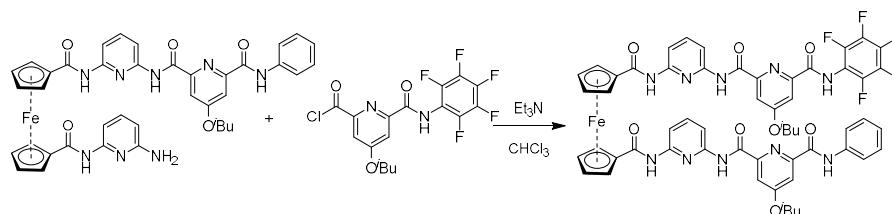


2,3,5,6-Tetrafluoro-4-methylbenzoic acid (42 mg, 0.2 mmol) was dissolved in 5 mL thionyl chloride and stirred at reflux for 30 min. The solution was then concentrated by rotary evaporation and dried under vacuum for 2 h. The residue was used directly without further purification. The acid chloride and asymmetric ferrocene conjugate (90 mg, 0.2 mmol) were dissolved in freshly distilled THF. The reaction was added triethylamine (0.06 mL, 0.4 mmol) and stirred for 48 h at room temperature. After, the reaction mixture was poured into 20 mL of a saturated sodium bicarbonate solution and washed 2 times with 20 mL DCM. The organic layers were combined and concentrated to 10 mL. After, 10 mL of methanol were added to the solution. The product was precipitated by slow removal of DCM by rotary evaporation. After filtration the product was obtained as an orange solid (62 mg, 51%).

1H NMR (300 MHz, Chloroform-*d*) δ 8.27 (d, J = 15.3 Hz, 3H), 8.08 (s, 1H), 8.03 – 7.94 (m, 2H), 7.94 – 7.86 (m, 3H), 7.74 – 7.46 (m, 4H), 4.86 (d, J = 2.7 Hz, 4H), 4.55 (s, 4H), 2.33 (t, J = 2.1 Hz, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 168.24, 161.64, 161.05, 150.25, 149.63, 149.12, 140.78, 128.88, 124.62, 120.31, 112.32, 111.46, 109.68, 72.45, 72.33, 70.73, 29.70, 28.04, 19.04.

HRMS (ESI): m/z calcd for $C_{37}H_{27}O_4N_6F_4^{54}Fe$, $[M+H]^+$ 749.14206, found 749.14219.

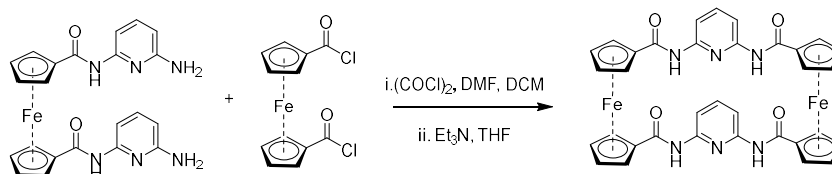
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The pyridine dimer acid (100 mg, 0.17 mmol) was dissolved in 10 mL thionyl chloride and stirred at reflux for 30 min. The reaction solution was concentrated by rotary evaporation and dried under vacuum for 2 h. The residue was used directly without further purification. The acid chloride and asymmetric ferrocene conjugate (132 mg, 0.17 mmol) were dissolved in freshly distilled chloroform. The reaction was stirred for 72 h at room temperature. After, the reaction mixture was poured in 50 mL saturated sodium bicarbonate solution and washed 2 times with 50 mL DCM. The organic layers were combined and concentrated to 10 mL. After, 10 mL of methanol were added to the solution. The product was precipitated by slow removal of DCM by rotary evaporation. After filtration the product was obtained as an orange solid (48 mg, 25%).

^1H NMR (300 MHz, Chloroform-*d*) δ 10.48 (s, 1H), 10.22 (s, 1H), 10.11 – 9.96 (m, 1H), 9.80 (s, 1H), 8.52 (s, 1H), 8.41 (s, 1H), 7.98 – 7.90 (m, 3H), 7.87 – 7.74 (m, 6H), 7.66 (t, J = 8.1 Hz, 1H), 7.61 – 7.48 (m, 2H), 7.28 (t, J = 7.8 Hz, 1H), 7.08 (t, J = 7.4 Hz, 1H), 4.84 (dt, J = 21.4, 1.9 Hz, 4H), 4.56 (dt, J = 4.1, 1.9 Hz, 4H), 3.94 (dd, J = 15.0, 6.5 Hz, 4H), 2.27 – 2.08 (m, 2H), 1.11 (dd, J = 6.7, 3.2 Hz, 11H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 168.24, 161.82, 161.65, 161.05, 150.92, 150.25, 149.62, 149.13, 140.78, 137.27, 128.87, 124.61, 120.31, 112.31, 111.97, 111.79, 111.45, 110.00, 109.68, 72.45, 72.33, 70.74, 29.71, 28.04, 19.04.

HRMS (ESI): m/z calcd for $\text{C}_{56}\text{H}_{48}\text{O}_8\text{N}_{10}\text{F}_5^{54}\text{Fe}$, $[\text{M}+\text{H}]^+$ 1137.29674, found 1137.29720.



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1,1'-ferrocene dicarboxylic acid (311 mg, 1 mmol) was dissolved in a catalytic amount of anhydrous DMF and 10 mL of freshly distilled DCM and stirred for 4 h. Solvent was then removed by rotary evaporation and the resulting solid dried under vacuum for another 2 h. The residue was used as without further purification. The acid chloride and ferrocene diamine (456 mg, 1 mmol) were dissolved in freshly distilled THF. The reaction was stirred for 45 h at room temperature. After, the reaction mixture was poured into 50 mL of saturated sodium bicarbonate solution and washed 2 times with 30 mL DCM. The organic layers were combined and concentrated to 10 mL. After, 10 mL of methanol were added to the solution. The product was precipitated by slow removal of DCM by rotary evaporation. After filtration the product was obtained as an orange solid (203 mg, 29%).

^1H NMR (300 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 8.39 (s, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.57 (q, J = 8.4 Hz, 1H), 4.95 (s, 2H), 4.80 (t, J = 2.0 Hz, 2H), 4.56 (dd, J = 4.1, 2.1 Hz, 4H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 168.37, 149.55, 139.63, 109.61, 72.05, 71.50.

HRMS (ESI): m/z calcd for $\text{C}_{34}\text{H}_{26}\text{O}_4\text{N}_6\text{F}_4^{54}\text{Fe}_2$, $[\text{M}+\text{H}]^+$ 691.07831, found 691.07874.

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3. X-ray crystallography

All diffraction data were recorded on a MAR345 image plate using Mo-K α radiation generated by a Rigaku UltraX 18S rotating anode (Xenocs Fox3d mirrors). Prior to data collection the crystals were flash frozen to 150 K. Data integration and reduction was performed by CrysAlisPRO,^[8] and the implemented absorption correction was applied. The structures were solved by SHELXT^[9] and refined against F² by SHELXL-2014/7.^[10] All non-hydrogen atoms were re-refined anisotropically, hydrogen atoms were added in calculated positions and refined in riding mode, with temperature factors 1.2 times higher than their parent atoms.

Molecular No.	3.002	3.003	3.004
CCDC code	UCL957_ms_Fe_diaza-antr 1	UCL966_ms_ferr_3 2	UCL973_ms_Ferr_antr 3
Empirical formula	C ₄₇ H ₄₈ Cl ₂ Fe N ₈ O ₁₀	C ₅₀ H ₄₆ ClO Fe N ₈ O ₆	C ₁₆₁ H ₁₈₆ Fe ₂ N ₂₀ O ₃₁
Formula weight	1011.68	910.80	3008.99
Temperature	150(2) K	150(2) K	150(2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	<i>P</i> -1	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> -1
Unit cell dimensions	a = 9.0626(3) Å	a = 14.2837(2) Å	a = 13.7442(5) Å
	b = 12.8066(5) Å	b = 12.30642(17) Å	b = 17.8552(6) Å
	c = 20.1239(8) Å	c = 28.6685(4)	c = 17.8594(4) Å

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		Å	
	= 92.222(3)°	= 90°	= 66.392(3)°
	= 100.717(3)°	= 94.3849(13)°	= 89.139(3)°
	= 95.145(3)°	= 90°	= 71.044(3)°
Volume	2281.94(15) Å ³	5024.61(13) Å ³	3764.5(2) Å ³
Z	2	4	1
Density (calculated)	1.472 Mg/m ³	1.204 Mg/m ³	1.327 Mg/m ³
Absorption coefficient	0.517 mm ⁻¹	0.354 mm ⁻¹	0.275 mm ⁻¹
F(000)	1052	1904	1592
Crystal size	0.20 x 0.14 x 0.04 mm ³	0.35 x 0.30 x 0.07 mm ³	0.35 x 0.30 x 0.26 mm ³
range	3.154 to 26.305°.	2.981 to 26.383°.	2.973 to 26.183°.
Reflections collected	29469	43092	54886
Independent reflections	9045 [R _(int) = 0.0499]	10216 [R _(int) = 0.0484]	14858 [R _(int) = 0.0522]
Compl. to = 25.242°	99.3 %	99.7 %	99.4 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min.	1.00000 and 0.83860	1.00000 and	1.00000 and 0.93848

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transm.		0.67136	
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restr. / param.	9045 / 0 / 619	10216 / 0 / 594	14858 / 16 / 1014
Goodness-of-fit on F^2	1.037	1.037	1.031
Final R indices [$I > 2 \sigma(I)$]	$R_1 = 0.0443$, $wR_2 = 0.1090$	$R_1 = 0.0493$, $wR_2 = 0.1304$	$R_1 = 0.0515$, $wR_2 = 0.1435$
R indices (all data)	$R_1 = 0.0534$, $wR_2 = 0.1145$	$R_1 = 0.0649$, $wR_2 = 0.1383$	$R_1 = 0.0574$, $wR_2 = 0.1480$
min and max	0.539 and -0.563 e.Å ⁻³	0.404 and -0.353 e.Å ⁻³	0.670 and -0.544 e.Å ⁻³

Molecular No.	4.004	4.009
CCDC code	-	-
Empirical formula	C120 H134 Fe2 N24 O24	C18 H15 Fe N3 O3
Formula weight	2408.20	377.18
Temperature	150(2) K	150(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Tetragonal
Space group	$P-1$	$P2_1/n$
Unit cell dimensions	a 14.0152(3) Å	a = 10.18350(19) Å

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	b = 32.0600(7) Å	b = 10.18350(19) Å
	c = 27.4484(5) Å	c = 29.9259(9) Å
	$\alpha = 90$	$\alpha = 90^\circ$
	$\beta = 101.8778(18)$	$\beta = 90^\circ$
	$\gamma = 90$	$\gamma = 90^\circ$
Volume	12069.2(4) Å ³	3103.43 (15) Å ³
Z	4	8
Density (calculated)	1.325 Mg/m ³	1.615 Mg/m ³
Absorption coefficient	0.321 mm ⁻¹	0.996 mm ⁻¹
F(000)	5064	1552
Crystal size	0.50 x 0.35 x 0.10 mm ³	0.12 x 0.11 x 0.09 mm ³
θ range	2.944 to 23.817°.	2.829 to 25.306°.
Reflections collected	48565	7731
Independent reflections	9045 [$R_{\text{int}} = 0.0499$]	10216 [$R_{\text{int}} = 0.0484$]
Compl. to $= 25.242^\circ$	99.3 %	99.7 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transm.	1.00000 and 0.56765	1.00000 and 0.07319
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restr. / param.	9045 / 0 / 619	10216 / 0 / 594
Goodness-of-fit on F^2	1.073	1.005

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Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.3470$, $wR_2 = 0.6593$	$R_1 = 0.0459$, $wR_2 = 0.0914$
R indices (all data)	$R_1 = 0.1062$, $wR_2 = 0.1741$	$R_1 = 0.0622$, $wR_2 = 0.0948$
min and max	0.567 and -0.462 e.Å ⁻³	0.460 and -0.231 e.Å ⁻³

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4. Computational Details

Computational modeling was performed with the Gaussian 09 software package^[11] using the B3LYP-D3BJ^[12] functional. All carbon, oxygen, nitrogen and hydrogen atoms were represented using the 6-311G(d,p)^[13] basis set and the LANL2DZ^[14] basis set was used for iron.

For comparing the energy of the oligomers as a function of dihedral angles around ferrocene, the dihedral angle was manually changed by 10° through a range of 0° to 360°. The structures were then allowed to optimize with no frozen coordinates. Energies given (Figure S10) are relative to the energy of the structure optimized from the X-ray coordinates.

For studying the Host-Guest chemistry of the oligomers with catechol, benzoic acid, and resorcinol:

- i. 100 configurations of each host-guest systems were randomly generated using molclus^[15].
- ii. These 100 configurations were then optimized at the PM6-DH+ level using MOPAC.^[16]
- iii. From these structures, the 20 lowest energy conformations with different geometries were chosen and optimized at the DFT level with Gaussian 09 using the same functional and basis sets as above (B3LYP-D3BJ/6-311G(d,p)&LANL2DZ).

Coordinates of all the geometries are available via the link <https://onlinelibrary.wiley.com/doi/full/10.1002/chem.201904719>

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Part B

Synthesis and Study of Novel Indole based Chalcones for Anti-cancer Activities

Chapter VII

Introduction

1. Methuosis

Cell death is the event where a biological cell stops to perform its function and it is a fundamental cellular process which prevent pathologies relating to autoimmunity, cancer and neurodegeneration. Cell death could be divided into Programmed cell deaths (referring type I: apoptosis and type II: autophagy) and other cell deaths (necroptosis, methuosis, etc.). Those different cell death pathways could be distinguished by obvious morphological criteria (Table 7.1.1).

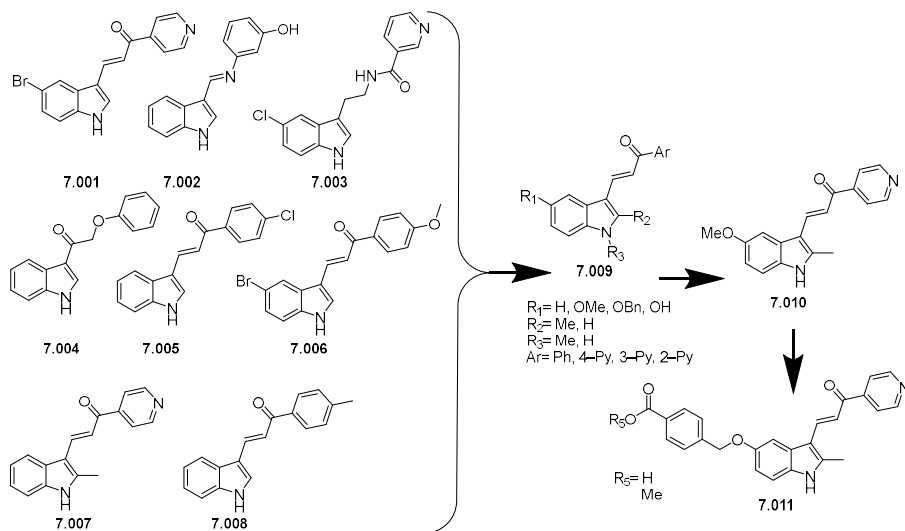
Table 7.1.1 Different features and significance of four cell death ways				
Feature	Apoptosis	Autophagic	Necroptosis	Methuosis
Rupture of plasma membrane	No	Varies	Yes	Yes
Cell swelling	No	No	Yes	Yes
Membrane blebbing	Yes	No	No	No
Chromatin condensation	Yes	Yes (late)	No	No
DNA fragmentation	Yes	Yes (late)	No	Varies
Accumulation of autophagosomes	No	Yes	Yes	Yes
ATP depletion	Yes	Varies	Yes	Yes

Methuosis is a unique form of non-apoptotic cell death triggered by alterations in the trafficking of clathrin-independent endosomes, leading to extreme vacuolization and rupture of the cell.^[1] This kind of cell death pathway was first observed in glioblastoma cells by Chi and co-workers. When they induced the ectopic expression of constitutively active oncoprotein, H-Ras (G12V), the cell experienced accumulated multiple large, phase-lucent cytoplasmic vacuoles and eventually underwent cell death.^[2] Later the Maltese group systemically studied the mechanism of methuosis and proposed a model wherein activation of the Ras oncoprotein causes extensive endosomal vacuolization, not only because of an increase in micropinocytosis, but also because of the disruption in normal endocytic trafficking and lysosomal fusion of macropinosomes.^[3] This process was further mediated by downstream

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mediator, Rho guanosine triphosphatase (GTPase) family, Rac 1 GTPase expression of which promotes the accumulation of Ras-induced vacuoles. Further study revealed that Arf6, a GTPase involved in macropinosome recycling, is also affected by constitutive Ras expression. Thus, the Ras-induced extreme vacuolization is a result of an increase in macropinosome formation (activation of Rac 1) and decrease in macropinosome recycling (deactivation of Arf6).^[4]

Maltese and co-workers screened a series of indole-based molecules (scheme 7.1.1) and found indole chalcones **7.007** could induce obvious vacuolization of glioblastoma multiforme (GBM) cells detected by phase-contrast microscopy.^[5] This kind of phenomenon corresponded with a novel type of non-apoptotic cell death mechanism, methuosis.^[3, 5] Further modification of this indole chalcone gave more than twenty analogs that were evaluated for their inhibitory activity on U251 GBM cell lines. 6 of them showed obvious vacuoles during treating the U251 cell line and among them, MOMIPP (**7.010**) showed potent antiproliferations on the U251 cell line with IC₅₀ values of 12 μ M, also causing vacuoles during the inhibitory of cell growth. Further modification (**7.011**) based on **7.010** did not show better inhibition. This was not only with U251 GBM cell lines. Photographs of phase-contrast microscopy of **7.007** and **7.010** on breast cancer MCF-7 cell lines and endothelial HMEC cell lines also showed large vacuoles (figure 7.1.1).^[5]



Scheme 7.1.1 Structure of indole based chalcones during the discovery of anti-GBM cell lines through methuosis cell death way.

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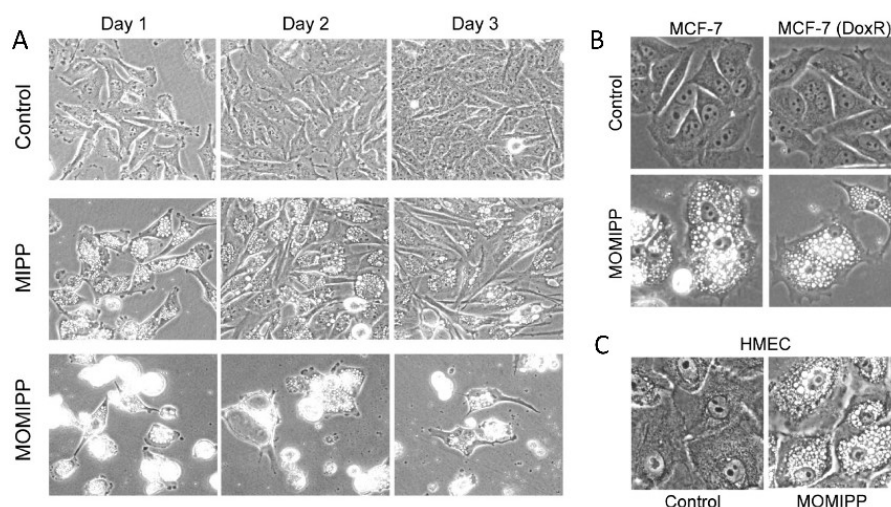


Figure 7.1.1 Photographs observed from the phase-contrast microscopy during **7.007** and **7.010** treat with different cell lines. Among which, (A) was **7.007** (MIPP) and **7.010** (MOMIPP) treated with U251 cell line, (B) was **7.010** (MOMIPP) treated with MCF-7 cell line and (C) was **7.010** treated with HMEC cell line. Control was only treated with DMSO.

Structure activity relationships of **7.010** indicated the importance of the pyridinyl group. Further studies of these series of indole chalcone molecules inducing methuosis was carried out by modification of indolyl-2 position (figure 7.1.2). Ethyl, propyl, isopropyl and isobutyl groups were introduced instead of methyl at the indolyl-2 position. However, modification of the methyl group only results in reducing the IC_{50} as well as the ability of causing vacuoles.^[6]

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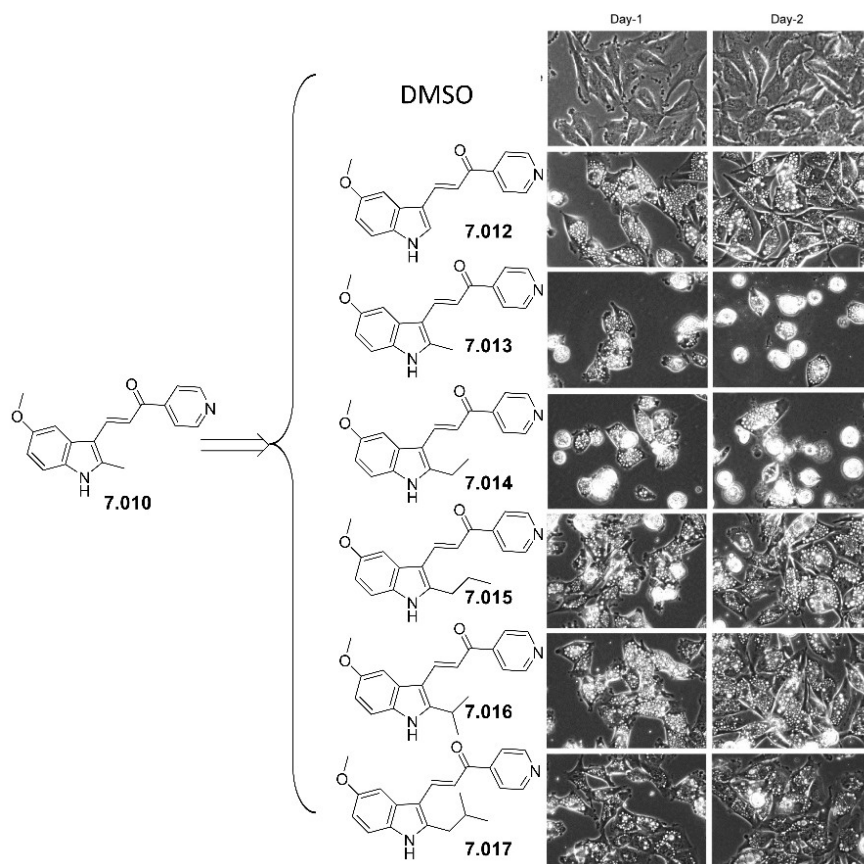


Figure 7.1.2 Deviations of indolyl-2 position and the photographs recorded during treated with U251 cell line in 24h and 48h.

Recently, more modified structures were reported through modification of the methoxy and methyl to various groups including esters, amides and acids.^[7] Some of those molecules in cell growth and morphology studies showed better performance. Further study on cell cycles indicated some of them induced mitotic catastrophe and those were subsequently tested on microtubule polymerization. Compared to colchicine which was caused microtubule disruption, some of those derivatives showed stronger effects of tubulin polymerization in U251 cells.^[8] For now, all of those indole chalcones were divided into three classes as shown figure 7.1.3.^[9]

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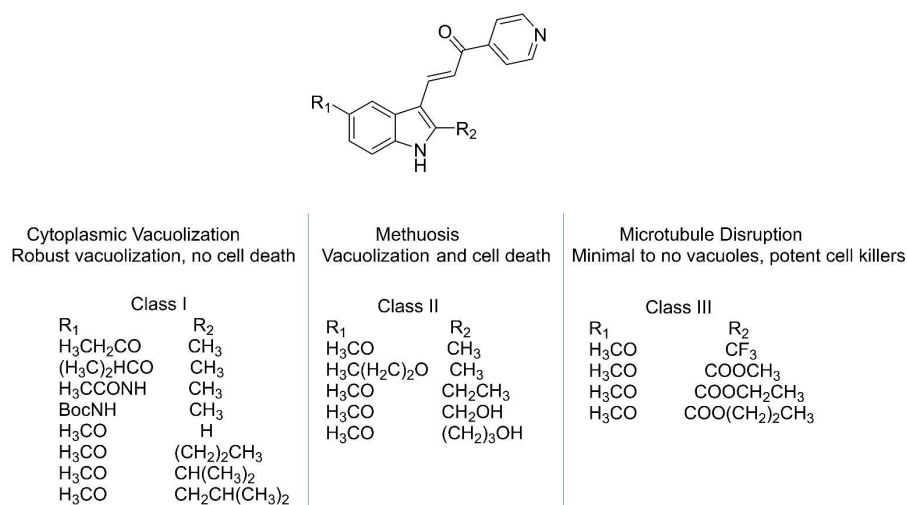


Figure 7.1.3 Classification of indolyl-3-chalcones by different mechanisms.

Except for the indole chalcone derivatives, other small molecules including a quinoline derivative (Vacquinol-1), ursolic acid derivative and azaindole based derivative (figure 7.1.4) were reported to display cytostatic properties via methuosis against several cancer cell lines.^[10]

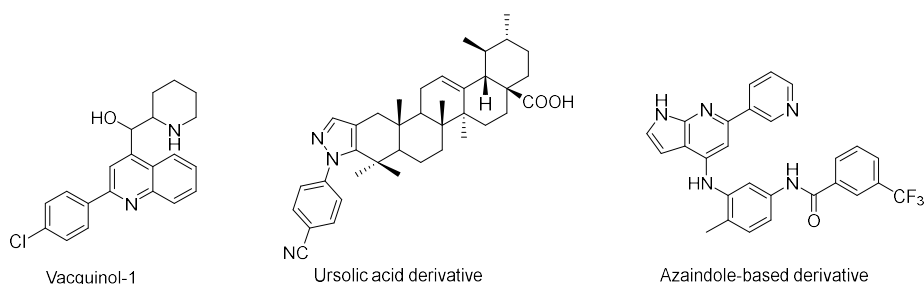


Figure 7.1.4 Recently reported methuosis inducer.

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2. Indole and Chalcone

2.1 indole

Indole is a planar heteroaromatic molecule with a ten-electron pi system formed by a pair of electrons from the nitrogen atom and eight electrons from the carbon atoms.^[11] Indole and its derivatives represent one series of the most abundant and important natural products in nature. A huge diversity of biologically significant compounds are indole-containing molecules, for instance, serotonin (neurotransmitter, figure 7.2.1 A),^[12] vinblastine (anticancer, figure 7.2.1 B),^[13] mitomycin C (anticancer, figure 7.2.1 C)^[14] and reserpine (antihypertensive, figure 7.2.1 D)^[15]. Additionally, non-natural indole-containing molecules also common in drugs including sumatriptan, tadalafil, rizatriptan and fluvastatin (figure 7.2.1 E-H).

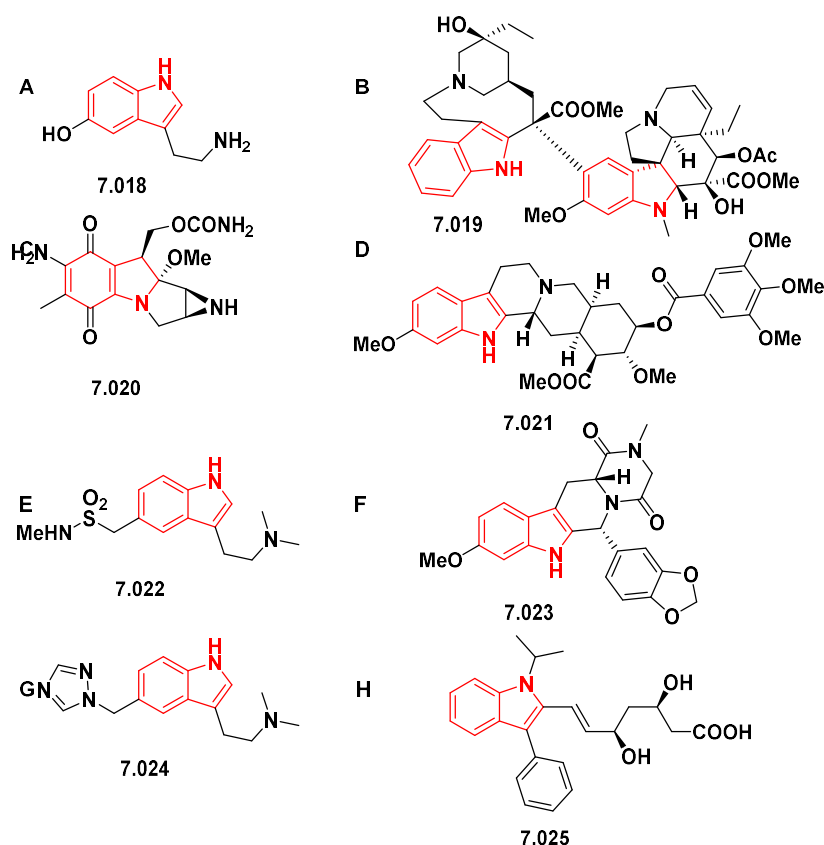


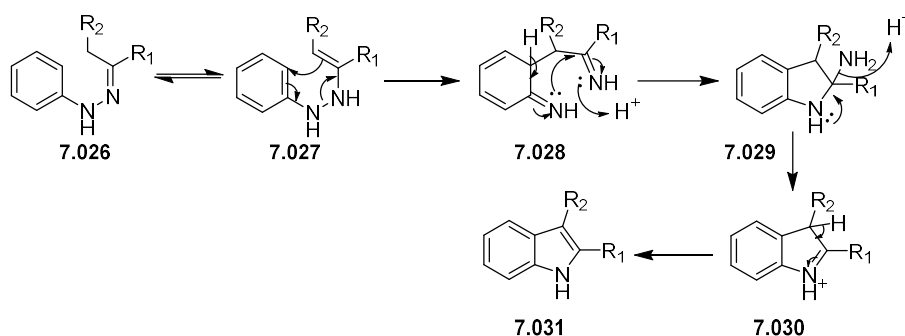
Figure 7.2.1 Structures of some representative bioactive natural and synthetic molecules containing an indole core (in red) A serotonin; B. mitomycin C; C. vinblastine; D. reserpine. E. sumatriptan; F. tadalafil; G. rizatriptan; H. fluvastatin

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The diverse bio-activities of indole and its derivatives make these molecules popular for finding bio-active molecules. The central theme for indole chemistry is how to construct as well as derivatize indole containing molecules.

Lots of researches have been carried out toward a variety of synthetic methodologies since the second half of 19th century^[16]. Lots of books and reviews summarized indole synthetic methodologies from the point of cyclization types or positions.^[17]

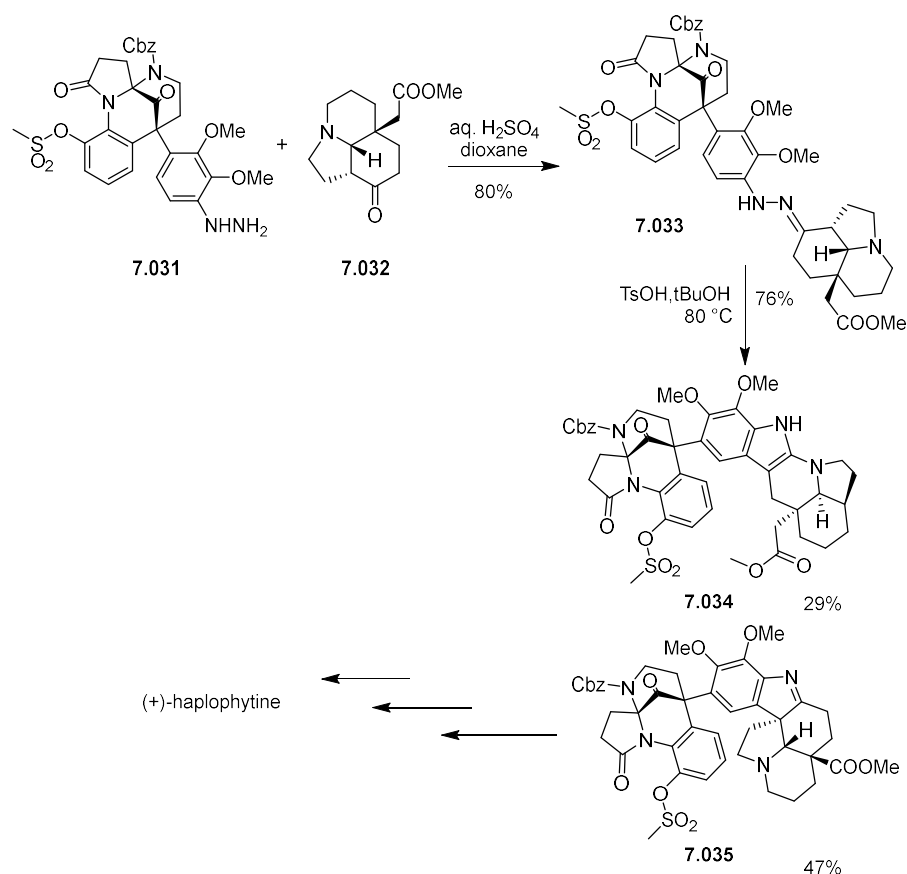
Among them, sigma-tropic rearrangement represented by Fischer indole synthesis remains the most versatile and widely applied one after it was first reported in 1883.^[16a] The classical Fischer indole synthesis reaction is cyclization of arylhydrazones to indoles with acid at elevated temperature. Although the mechanism of classical Fischer indole synthesis is still not fully clear, the possible mechanism involving a sigma-tropic rearrangement is widely accepted (scheme 7.2.1). The Gassman reaction and Bartoli reaction also go through sigma-tropic rearrangements during indole synthesis.



Scheme 7.2.1 Possible mechanism of Fischer indole synthesis

One of the outstanding among the wide applications of the Fischer indole synthesis is synthesis of haplophytine^[18] (scheme 7.2.2). In this total synthesis, the key step was using the Fischer indole synthesis for building the central indole fragment. After the formation of **7.033**, they first obtained a 20% total yield of **7.034** and **7.035** by refluxing in acetic acid. This was successfully used in the total synthesis of aspidospermine.^[19] Then after a series of optimizations finally obtained **7.035** with a moderate yield.

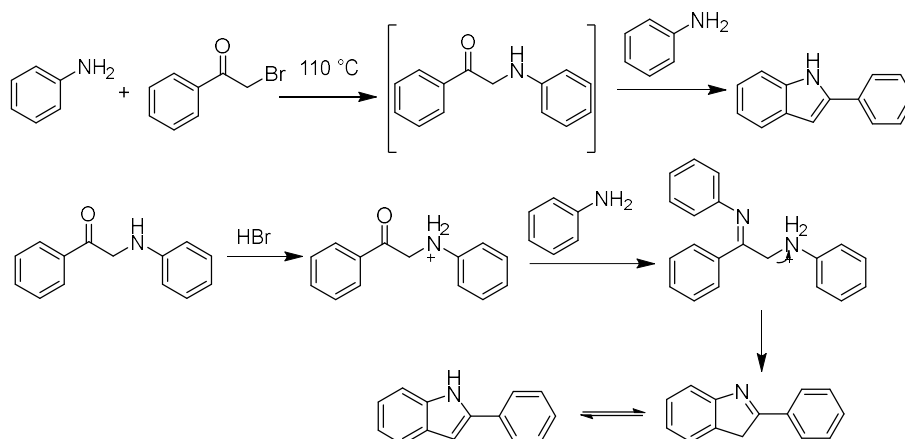
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Scheme 7.2.2 Application of Fischer indole synthesis in total synthesis of haplophytine.

Bischler indole synthesis, also known as Bischler-Mohlau indole synthesis is another pioneering reaction discovered a decade after the Fischer indole synthesis.^[20] The basic Bischler reaction is starting from aniline and α -bromoacetophenone reacted at high temperature followed by addition of another equivalent of aniline and cyclization (Scheme 7.2.3). The mechanism is still disputed, but Lewis acid activation and electrophilic cyclization steps are widely accepted.^[20] This key cyclization step also exists in many other synthetic methodologies such as Sundberg, Hemetsberger and Taber indole syntheses which are categorized as electrophilic cyclization indole synthesis methodologies.

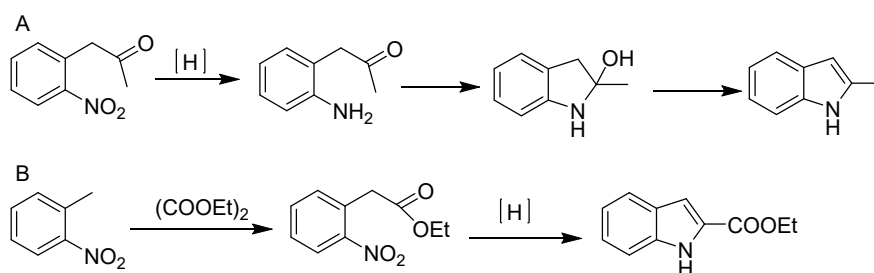
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Scheme 7.2.3 Bischler indole synthesis and its possible mechanism

Opposite to electrophilic cyclization, some indole synthesis methods use strong bases to form nucleophiles which give the indole through nucleophilic cyclization. Representative case such as Madelung reaction and its modifications.^[21]

Reduction of the *o*-nitrobenzoyl ketone, cyanide or nitrostyrene can generate *o*-aminobenzoyl groups followed by cyclization by the *o*-amine to the β -position of the chain and aromatization by dehydration to form the indole. One of the most useful reductive cyclization reactions for indole synthesis was developed by Reissert which including base catalyzed condensation of *o*-nitrotoluene with an oxalate ester followed by reductive cyclization to 2-carboxylic acid indole (scheme 7.2.4).^[22]



Scheme 7.2.4 A. Reductive cyclization indole synthesis. B. Reissert indole synthesis

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2.2 Chalcone

A chalcone is a series of molecules containing α,β -unsaturated ketones connecting two aromatic systems (figure 7.2.2 A). In nature, these compounds are widely spread in plants and mainly involved in the biosynthesis of isoflavonoids/flavonoids. Herbs containing chalcone based bioactive molecules have attracted much attention, and have been used for thousands of years for treating injuries and diseases.^[23] More recently, chalcones such as metochalcone marketed for choleric drug, sofalcone used for antiulcer, are still being approved for clinical use (figure 7.2.2 B & C).

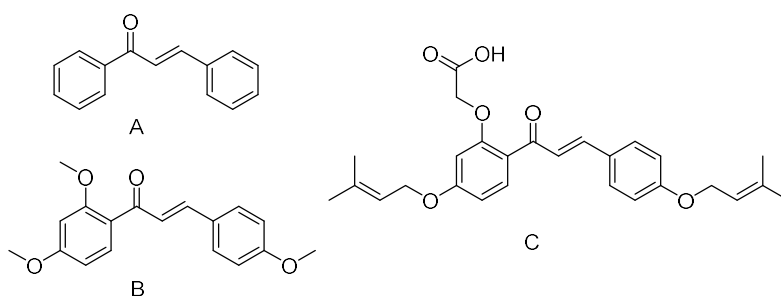


Figure 7.2.2 A. General structure of chalcone. B. metochalcone. C. Sofalcone

Condensation reactions under basic or acid conditions is the general method for the synthesis of chalcones. The Claisen-Schmidt condensation is one the most classical reactions used and is catalyzed by either strong bases from enolate mechanism or acids from enol mechanism.^[24] Other strategies like Friedel-Crafts acylation starting from cinnamoyl chloride, Wittig reaction starting from aromatic ylide ketone, Julia-Kicienski Olefination starting from aromatic α -tosyl-ketone and Suzuki cross coupling reaction starting from α,β -unsaturated acid chlorides with some boron compounds are also quite efficient.^[25] Most of those methodologies will give *trans*-chalcones as the *trans* isomer is thermodynamically more stable. However, some examples reported the synthesis of *cis*-isomers of chalcone. Yoshizawa used siloxypropynes and *t*BuOK at -78°C to obtain siloxyallenes, which were treated with a strong acid (H_2SO_4) and 1,2-dimethoxyethane (DME) to produce *cis*-chalcones. This procedure will give up to 99:1 *cis/trans* ratio.^[26]

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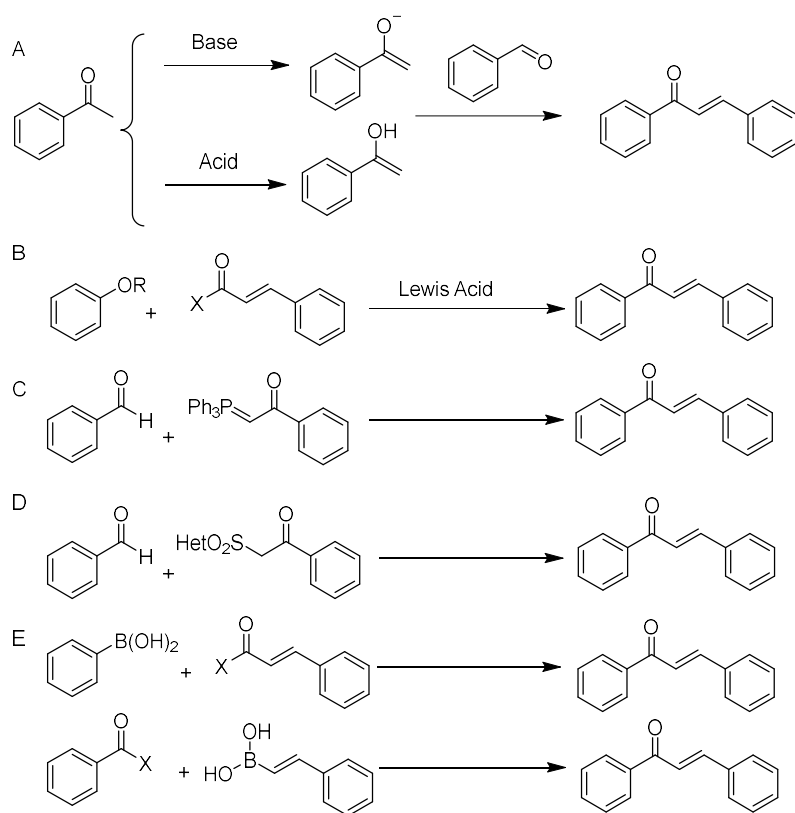
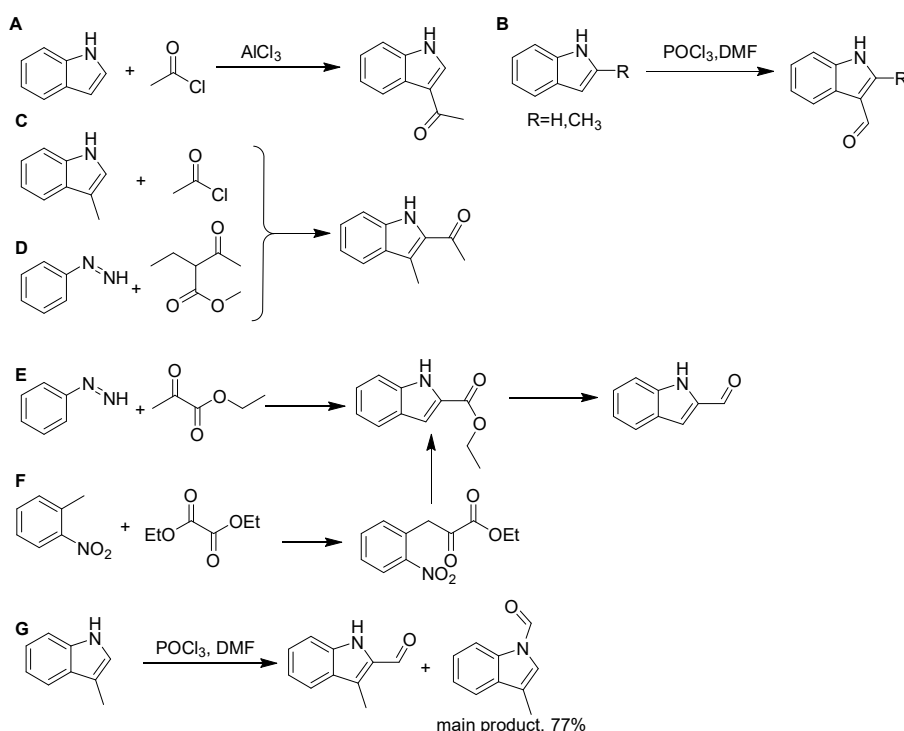


Figure 7.2.3 General representations of chalcone synthesis methodologies. A. Claisen-Schmidt condensation B. Friedel-Crafts acylation. C. Wittig reaction D. Julia-Kicienski Olefination. E. Suzuki coupling reaction

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2.3 indole chalcone

For the synthesis of indole chalcones, the most convenient method is condensation from aldehyde with ketone. Indole-2/3-ketone as well as indole-3-aldehyde are usually obtained through Friedel-Craft acetylation (scheme 7.2.5 A & C) and Vilsmeier-Haack reaction (scheme 7.2.5 B), respectively.^[44] 3-methylindole-2-aldehyde could also be obtained by Fischer indole synthesis (scheme 7.2.5 D).^[45] 3-hydroindole-2-aldehyde could be achieved by Fischer indole synthesis or Reissert indole synthesis followed by reduction of the acid to the aldehyde (scheme 7.2.5 E & F).^[29, 46]

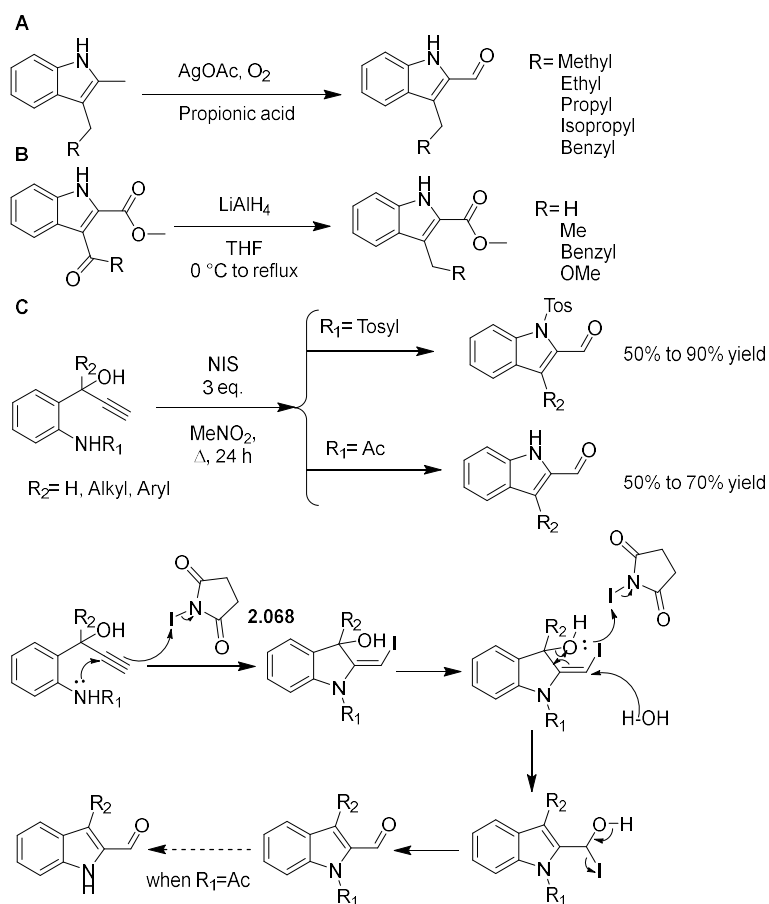


Scheme 7.2.5 Indole-2/3-aldehyde/ketone synthesis method.

However, due to poor nucleophilic property of the indole-2-position, direct formylation such as with the Vilsmeier-Haack reaction is challenging for functionalization at this position even for 3-methylindole (scheme 7.2.5 G). For now, three popular approaches were developed to obtain 3-substituted indole-2-aldehydes. First is an oxidation procedure. Itahara reported selective oxidation of 3-substituted 2-dimethylindole under acid conditions with silver acetate to give 3-substituted 2-indolealdehydes (scheme 7.2.6 A).^[47] Additionally, reductive deoxygenation is also useful (scheme 7.2.6 B).^[48] Other than modification on the indole ring, Chan reported a *N*-iodosuccinimide (NIS) mediated method for preparing indole-2-carbaldehydes from cyclo-

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isomerization of 1-(2-aminophenyl)prop-2yn-1-ols with moderate yield (Scheme 7.2.6 C).^[49]



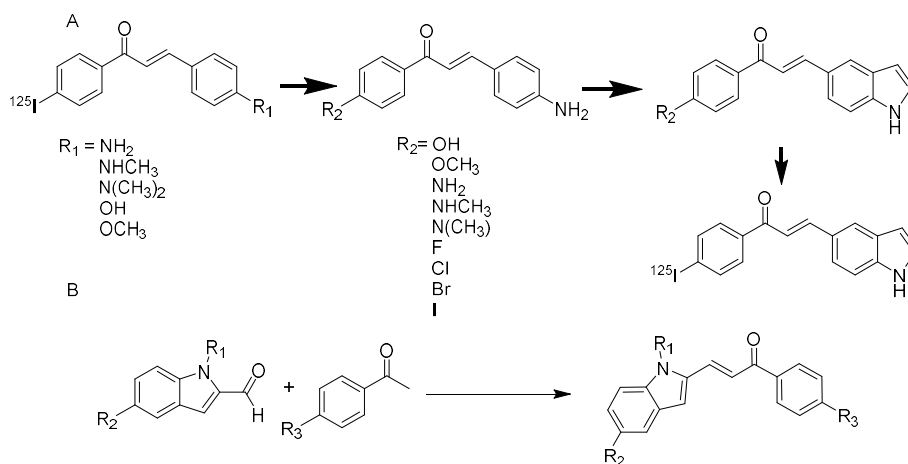
Scheme 7.2.6 Indole-2-aldehyde synthesis through oxidation reaction (A) and reduction reaction (B); (C) 2-aldehydeindole synthesis method with the proposed methodology.

Even though those existing methodologies could give 2-aldehydeindoles, it is undeniable that harsh conditions are necessary and also usually result in relatively low yields.

Except for the hybrid indole chalcones as methuosis inducers mentioned in section 1, many other chalcones also exhibited novel bio-activities in various fields, due not only to the Michael acceptor of the α,β -unsaturated ketone but also from the modified aromatics. In recent years, indole chalcone scaffolds have been reported to act against various cancer cells through diverse mechanisms, and to be useful β -amyloid imaging probes. Saji and coworkers reported that a series of 4-pehnylsubstituted indolyl-5-chalcone structures (figure 7.2.7 A) derived from chalcones showed competitive affinity property

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with A β ₁₋₄₂ aggregates versus [¹²⁵I]IMPY. The affinities of those compounds ranged from 4 to over 1000 nM depending on the substitutions. Among those, iodo-chalcone exhibited selectivity on labeling A β plaques when tested in brain tissue from an animal model but further experiments on normal mice indicated its poor passivity through the blood brain barrier.^[27] Ozdemir and co-workers reported nine indolyl-3-chalcone compounds (figure 7.2.7 B) and evaluated their activity in vitro COX-1 and COX-2 inhibitions. Four of them exhibited COX-2 inhibitory activity with IC₅₀ values less than 10.0 μ g/mL and in vivo studies indicated their anti-inflammatory and antioxidant activities.^[28]



Scheme 7.2.7 (A) Discovery of indole chalcone derivatives as β -amyloid imaging probes. (B) Chalcones of anti-inflammatory activity

Hybrid chalcones have attracted more attention for their anticancer properties. Kumar reported indolyl-1,3,4-oxadiazoles exhibited potent inhibitory effects as anticancer agents.^[29] Inspired by these skeletons, a series of indolyl/*N*-methylindolyl-3-chalcones were designed and synthesized and further research evaluated their anticancer properties. Results revealed the skeleton containing indolyl-chalcone with a methoxyphenyl group showed the potent anticancer activities on A549, PaCa-2 with IC₅₀ values less than 5 μ M while *N*-methyl groups do not show obvious influence on activities.^[30] Gurkan-Alp reported indole retinoid derivatives showed potent inhibitory effects on various cancer cells especially breast cell lines including Cama1, T47D, MDA and MCF7 cell lines. Further studies such as flow cytometry analysis by one of the most potent molecules (IC₅₀= 1.83 μ M) on MDA-MB-231 cell lines showed it could induce apoptosis in such cell line. Additionally, some docking works of this molecule with RXR α and RXR γ to gain more insight into the binding mechanism were carried out by molecular dynamics and it showed fitted well to those two proteins (figure 7.2.4).^[31]

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Breast cancer	Positive Control (CPT)	2.014
Cama1	0.07	<0.01
T47D	<0.01	1.16
MCF7	<0.01	1.71
BT474	12.75	1.91
MDA-MB-453	<0.01	<0.01
BT20	<0.01	1.31
SK-BR-3	<0.01	1.29
MDA-MB-361	0.17	0.06
MDA-MB-157	0.02	1.46
MDA-MB-231	<0.01	1.83

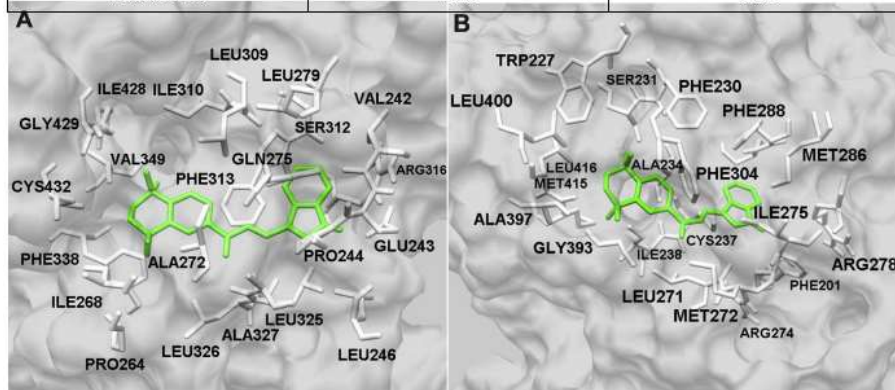
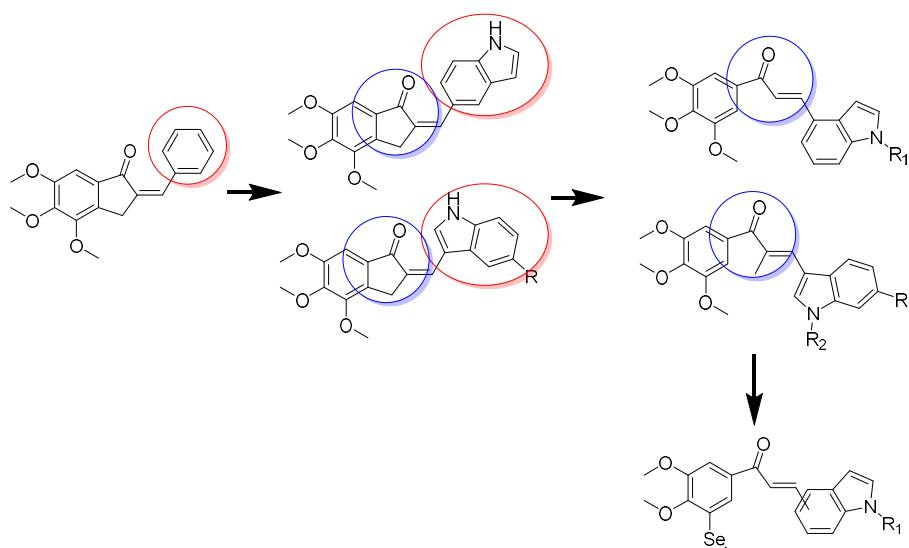


Figure 7.2.4 Anti-proliferative capacity of to 10 breast cancer cell lines. IC₅₀ levels were shown in form and CPT as the positive control. MD computation result showed binding site of (A) RXR α and (B) RXR γ at 10 ns.

Li and co-workers found a class of benylideneindanone derivatives showed inhibition on tubulin polymerization (scheme 7.2.8).^[32] During the optimization, a series of indole chalcone derivatives were discovered with better performance on inhibition of cell growth by tubulin polymerization both in *vitro* and *vivo*.^[33] Additionally, another series of selenium-containing indole chalcones was also developed which could also inhibit tubulin polymerization.^[34]

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Scheme 7.2.8 Discovery of indole chalcones as novel microtubule disruption agents

Except those above representative indole-based structures, other combinations with the moieties of indole and chalcone were discovered during the optimization of bio-active molecules. Abid reported a series of 1,2,3-triaolyl-appended indole chalcone hybrids had potent antioxidant activity and good affinity with DNA sequences through the mechanism of antiproliferation of human embryonic kidney cells (HEK293, figure 7.2.5 A).^[35] Notably, Miao and Zhao reported a series of indole-2-chalcones which usually inhibited human lung cancer A549 cell lines by inducing apoptosis both in *vitro* and *vivo* (figure 7.2.5 B).^[36]

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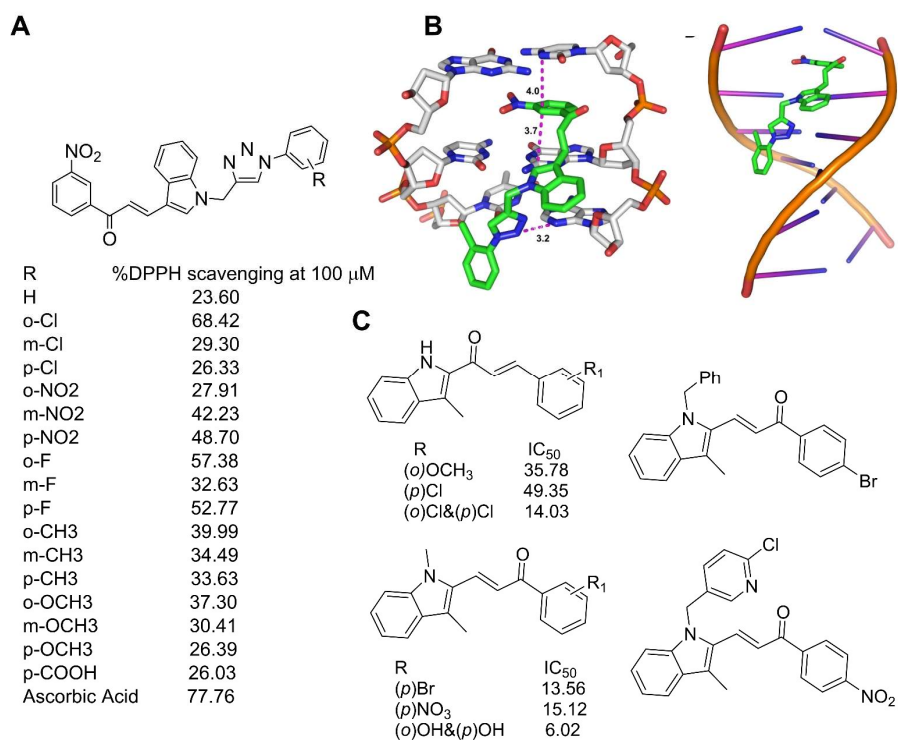


Figure 7.2.5 (A) Abid designing of indole chalcone molecules with their antioxidant activity. (B) Molecular docking with DNA base pairs. (C) structure and their IC₅₀ on indole-2-chalcone reported by Miao and Zhao.

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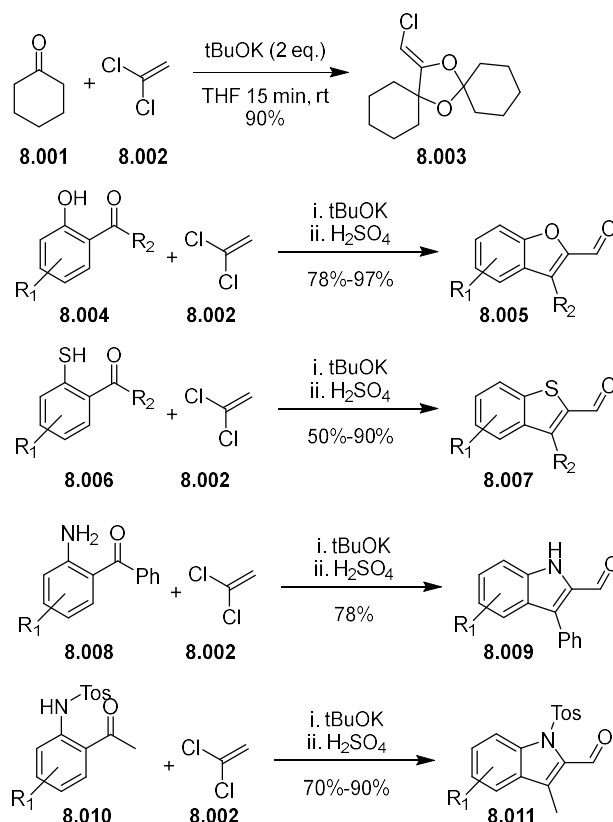
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Previous Work and Objective

1. Group researches

The Marko group has developed a new methodology based on chloromethylene-ketals by Florian Schevenels. After some modifications, this methodology was extended to the synthesis of heterocycles including benzofurans, and benzothiophenes, as well as, indoles with moderate to excellent yields in mild conditions (figure 8.1.1).^[1]



Scheme 8.1.1 New methodology developed in group and its wide applications.

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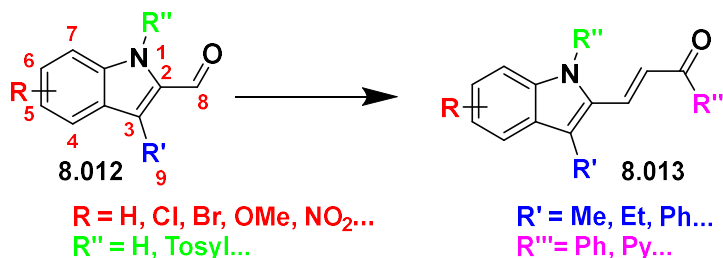
2. Objectives of this project

Methuosis is a kind of novel non-apoptotic cell death mechanism. Finding molecules inducing methuosis would be meaningful not only on the study and understanding of methuosis but also for the development of new anti-cancer chemotherapeutics.

For now, only a few examples of molecules were reported to induce methuosis. Of these, indole-3-chalcones have been one of the major classes.^[2] More recently, a report by found one example of an indole-2-chalcone which could potentially induce apoptosis in A549 lung cancer cells.^[3] Still, few examples of this family of molecule have been studied for this purpose. This is likely due primarily to the difficulty in obtaining indole-2-chalcone.

The main objective of this part of my research is to investigate synthesize and study the bioactivity of indole-2- chalcones as potential methuosis inducers.

The synthetic pathways for these series of indole-2-chalcones will be started from the indole-2-aldehyde synthesized via the methodology developed in the Marko group, followed by condensation with various ketones. With this strategy, we expect to be able to obtain multiple modifications on both the indole rings (red, blue and green positions in scheme 8.2.1) and ketone part (pink position in scheme 8.2.1).



Scheme 8.2.1 Synthetic representation on construction of indole-2-chalcones

The bioactivity evaluation of the synthesized indole-2-chalcones will then be used for verifying the inhibition of various cancer cells and their cell death mechanisms. In doing so we aim to address the questions: If these series of hybrid chalcones could induce methuosis in cancer cells, does it have the same targets as indole-3-chalcones? If not, which kind of mechanism will it induce?

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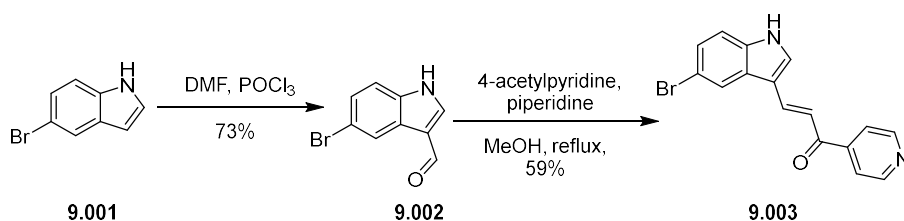
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Synthesis and Biological Evaluation

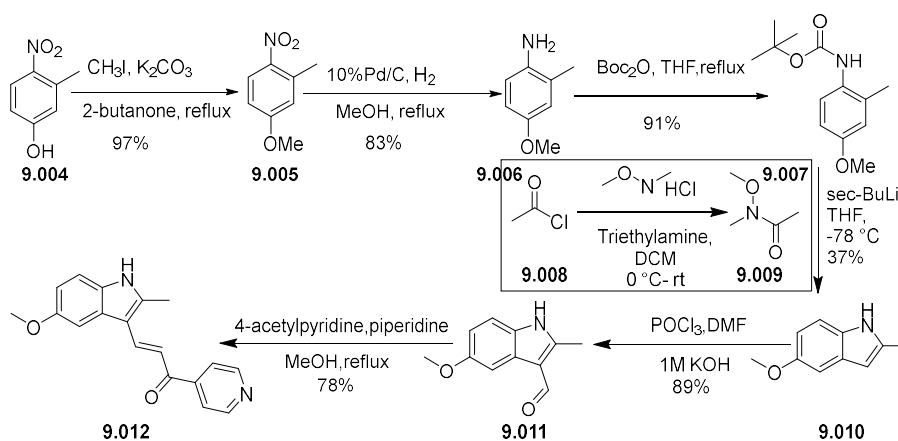
1. Re-synthesis of Reported Indole Chalcones as Positive Control

Two indole chalcone compounds were prepared which were claimed in the literature to exhibit potent anticancer activity by inducing methuosis.^[1] Compound **9.003** was easily obtained by a Vilsmeier-Haack reaction and aldol condensation.



Scheme 9.1.1 Synthesis of indole-3-chalcone **9.003**

For compound **9.011**, the synthesis followed the literature procedure. For this, the key step is the Weinreb amide synthesis followed by a condensation to get the desired indole.^[2] Then the same route including Vilsmeier-Haack reaction and aldol condensation, as shown in the following scheme, were used to give **9.012** in good overall yield.

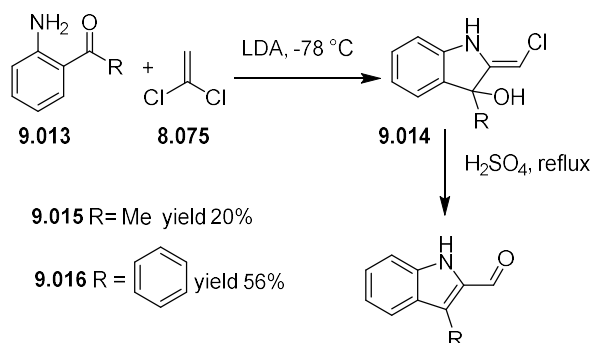


Scheme 9.1.2 Synthesis of indole-3-chalcone **9.012**

2. Discovery of a New Indole Chalcone Synthetic Strategy

2.1 Construction of Indole-2-aldehydes and Attempts of Directly Reach Indole-2-chalcones

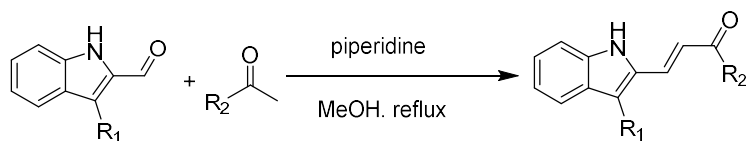
Based on our group's methodology, the 2-aldehyde indoles were prepared in two steps as shown in scheme 3.2.1. First step is reaction of 2-aminobenzylketone **9.013** with 1,1-dichloroethylene in the presence of LDA to obtain the cyclized indolone **9.014**. Next, the cycle is re-aromatization under acid conditions. Due to the similar pKa of the acetyl α -proton with the amine proton, attempts at deprotonation of the amine with LDA also deprotonated the acetyl proton. The yield for synthesizing 2-aldehyde-3-methylindole is only 20% while when the methyl was replaced by phenyl, the yield was obviously higher (56%).



Scheme 9.2.1 Synthesis of 2-aldehyde indoles

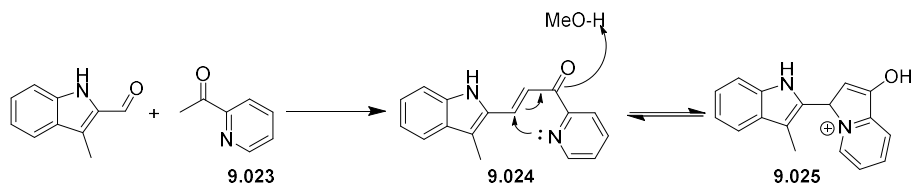
With the obtained indole-2-aldehyde, the condensation with a series of ketones using catalytic amounts of piperidine worked well, in moderate yield, to give a series of chalcone compounds. Notably, unlike the indole-3-chalcones which can precipitate from methanol, this series of indole-2-chalcones showed good solubility in methanol.

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	R ₁	R ₂	Yield
9.017	Me	3-pyridine	71%
9.018	Me	4-pyridine	63%
9.019	Me	Me	49%
9.020	Ph	3-pyridine	58%
9.021	Ph	4-pyridine	65%
9.022	Ph	Me	52%

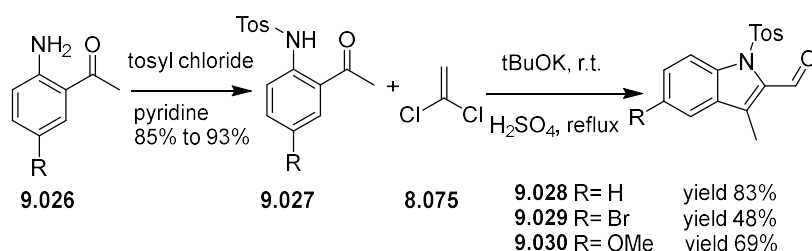
However, when this aldehyde was reacted with 2-acetylpyridine, we did not isolate the desired chalcone **9.024**. This was also reported in the construction of indole-3-chalcone when 2-acetylpyridine was used.^[1] This may be because after indole-2-chalcone **9.024** formed, it subsequently equilibrated to **9.025** by attack of the pyridine moiety lone pair on C4 position of Michael acceptor and cyclization.



Scheme 9.2.2 Possible transform of indole-2-aldehyde **9.023** to cyclized compound **9.025**

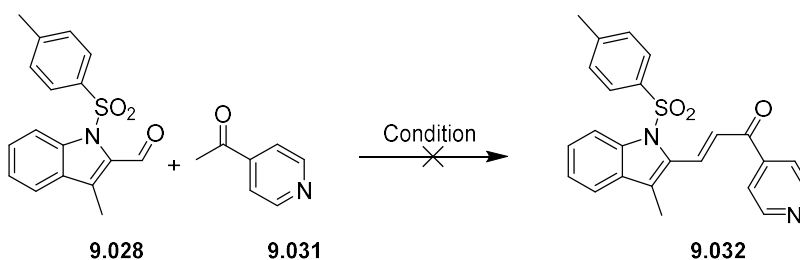
Although six chalcones were successfully obtained, this strategy of reaching indole-2-chalcones is limited by the low yield of indole-2-aldehyde. To increase the yield of the 2-aldehyde-3-alkylindole series compounds, one of the options is introducing an electrophilic group to lower the pK_a of the amine proton. By this idea, p-toluenesulfonyl (tosyl) chloride was used to form a series of tosyl protected anilines **9.027** and the yield of indole aldehyde **9.028** was dramatically increased (83%). Additionally, synthesis of 5-substituted indole aldehydes **9.029** and **9.030** were also obtained though tosyl protected method.

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Scheme 9.2.3 synthesis of tosyl protected indole-2-aldehydes

However, when this tosyl protected indole-2-aldehyde **9.028** was used for condensation with 3/4-acetylpyridine with catalytic piperidine, only starting materials were detected after 48 hours refluxing in methanol. Different bases as well as acids were applied but none of them gave the desired product. Notably, when the tosyl protected aldehyde was treated with sodium hydroxide, *n*-butyllithium (*n*-BuLi) or lithium di-isopropyl-amide (LDA), crude NMR showed the aldehyde proton shifted potentially indicating 9-methoxyindole-2-aldehyde from deprotection of tosyl group. Given that the aldehyde was still there, we did not isolate that compound.

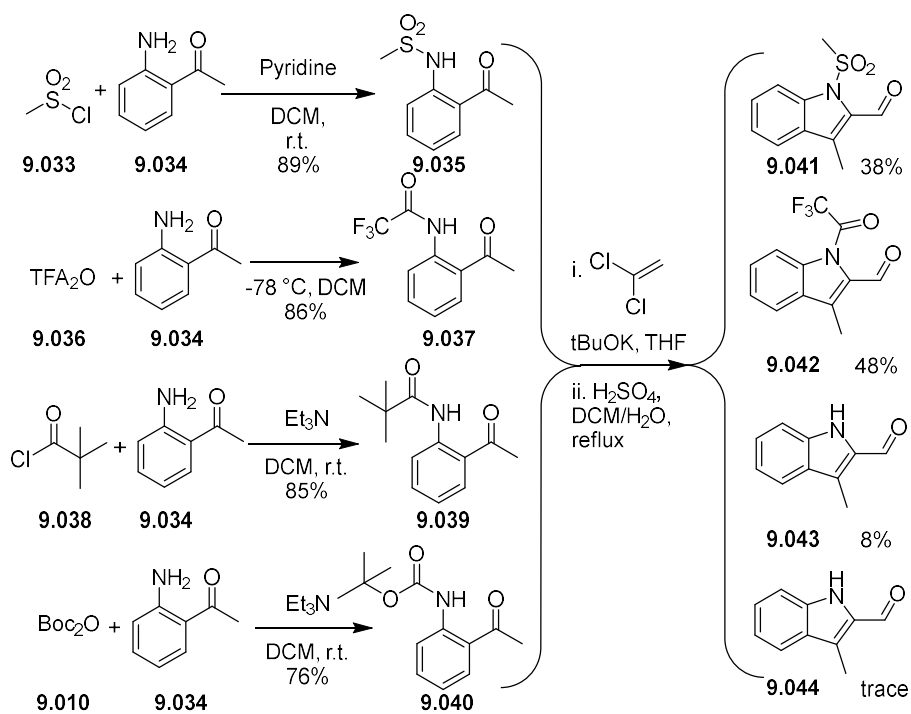


Base or Acid	Solvent	Condition	Result
Piperidine	MeOH	reflux	Starting materials
TEA	MeOH	reflux	Starting materials
DIPEA	MeOH	reflux	Starting materials
NaOH	MeOH	0°C-r.t.	New aldehyde*
<i>n</i> -BuLi	THF	-78°C	New aldehyde*
LDA	THF	-78°C	New aldehyde*
TiCl ₄	DCM	-78°C	Starting materials

* Crude NMR showed after 2 hour's reactions, aldehyde proton was not the same shift as the tosyl protected.

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The difficulties in obtaining the tosyl protected indole-2-chalcones while unprotected indole-2-aldehydes **9.015** and **9.016** worked well to obtain chalcones, indicated that the tosyl protecting group may be key factor for the failure of the chalcone synthesis. Replacing tosyl by the other electron withdrawing groups could be a possible option of solving this problem. Methanesulfonyl (mesyl) and trifluoroacetyl groups were chosen as alternatives for tosyl. In the meantime, acid sensitive groups including pivalic (Piv) and tert-butylcarbonate (Boc) were also introduced to lower the pKa of the amine proton in the cyclization step while still being easily removed by the following acid step. Their corresponded protected *o*-acetylanilines were obtained as shown in scheme 3.2.4 in moderate to good yield. These were then used for cyclization to the corresponding indoles. Unfortunately, none of the protecting groups gave yields comparable to the tosyl protected starting material. Moreover, **9.041** and **9.042** were tested for the condensation with acetylpyridine but only the starting material was detected after the reaction.

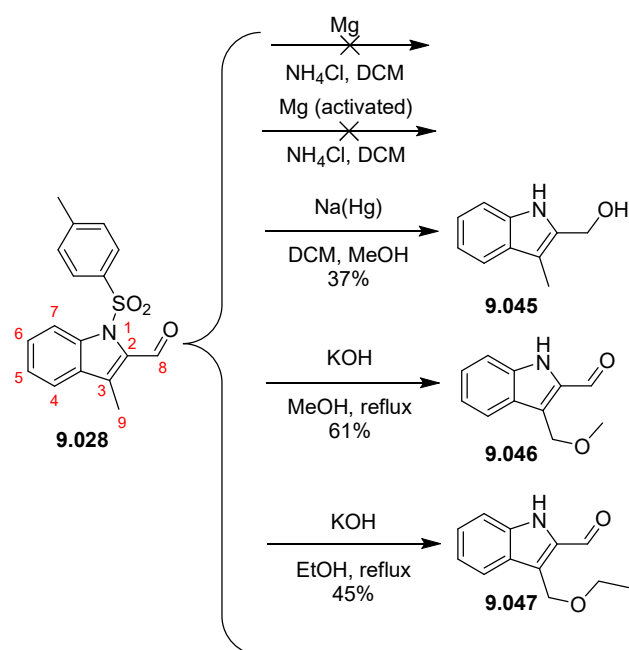


Scheme 9.2.4 Attempts at synthesis of indole aldehydes with different protecting groups

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2.2 Attempts of Removing Protecting Group

For now, tosyl is still the best protecting group for launching this indole synthesis reaction. In view of the successful condensation reaction with the unprotected indole-2-aldehyde, removing the tosyl before the condensation step offers another possible synthetic route. Hoping to directly remove the tosyl group via a radical reaction, magnesium was tried but did not give the desired result. Sodium amalgam in a mixed solvent system of dichloromethane/methanol worked to remove the tosyl group, however the aldehyde was reduced as well.^[3] Based on the literature description, potassium hydroxide was also utilized in methanol/ethanol and resulted in tosyl-removal, but also addition of a methoxy/ethoxy group onto the C9 of the indole and formed **9.046** and **9.047**.^[3]

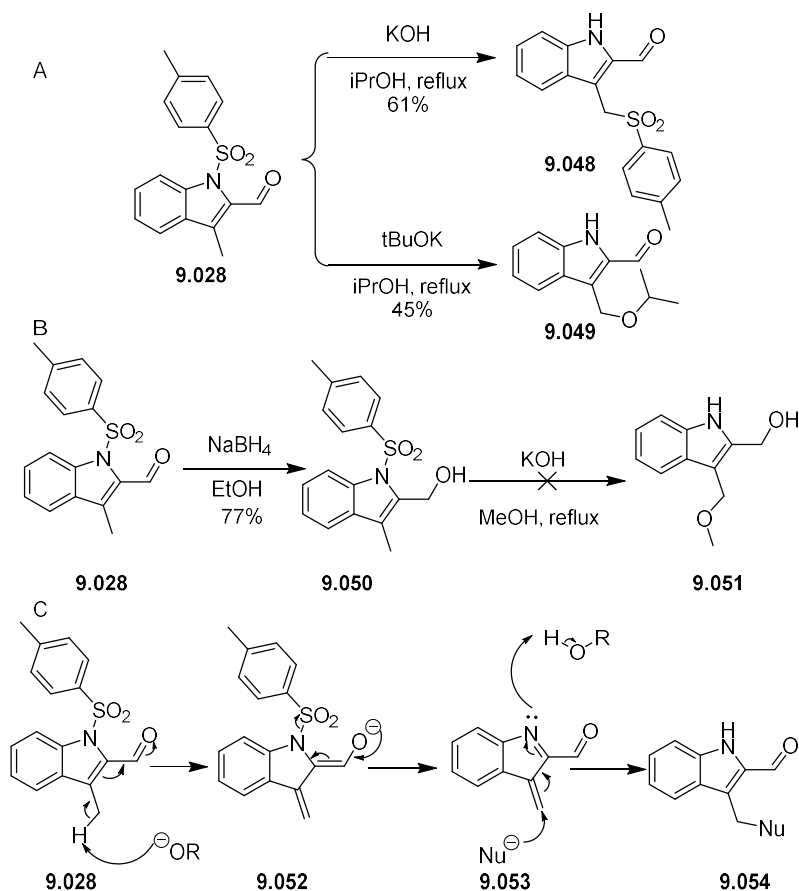


Scheme 3.2.5 Attempts of deprotection reaction

Interestingly, when we tried to extend this deprotection method from methanol/ethanol to isopropanol, in order to attempt to enrich the library of indole aldehydes, the tosyl group was detected “moving” from nitrogen to the C9 position of the indole giving **9.048**. When the base was changed to potassium tertbutoxide, the “normal” tosyl remove indole aldehyde **9.049** was obtained with the addition of the isopropyl group. The pK_a of H₂O is about 15.7, iPrOH is about 16.5 and tBuOH is about 17. We suspected that no isopropyl anions existed to act as the nucleophile when potassium hydroxide was used as the base. Reduction of the indole aldehyde to the alcohol also

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alters the deprotection reactivity. When indole alcohol **9.050** was refluxed in methanol with potassium hydroxide the desired tosyl deprotected compound **9.048** was not detected. This result further supported the mechanism of this deprotection reaction proposed in the literature.^[3] This involves the deprotonation of the C9 position to give enolate **9.052**. **9.052** then kicks out the tosyl group forming the aldehyde. Finally, attack of the nucleophile on the C9 position forms **9.054**. In the absence of the aldehyde group, this reaction does not occur.

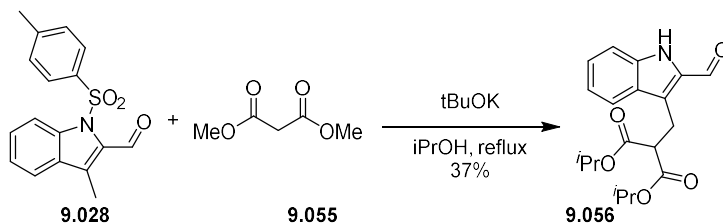


Scheme 3.2.6 (A) Tosyl deprotection with different bases result in different products; (B) attempts of tosyl deprotection reaction of using indole alcohol; (C) Proposed mechanism for this reaction

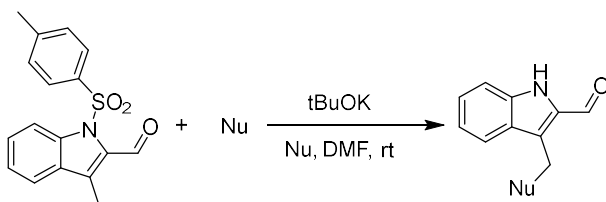
We postulated that by controlling the pKa/nucleophilicity of the base, a variety of nucleophiles could be added to give new functionalized indole aldehydes. Dimethyl malonate was first used to verify this idea. Refluxing **9.028** with dimethyl malonate and 2.5 eq. of potassium tertbutoxide in isopropanol, we successfully isolated product **9.056** with a yield of 37%. In the meantime, certain

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amount of **9.049** was also isolated. DMF as solvent was also tested, the reaction could occur at room temperature and gave the desired compound **9.057** with higher yield (62%). Phenol, Thiophenol and piperidine were subsequently used as the nucleophile and the desired compounds could be obtained with moderate yield.



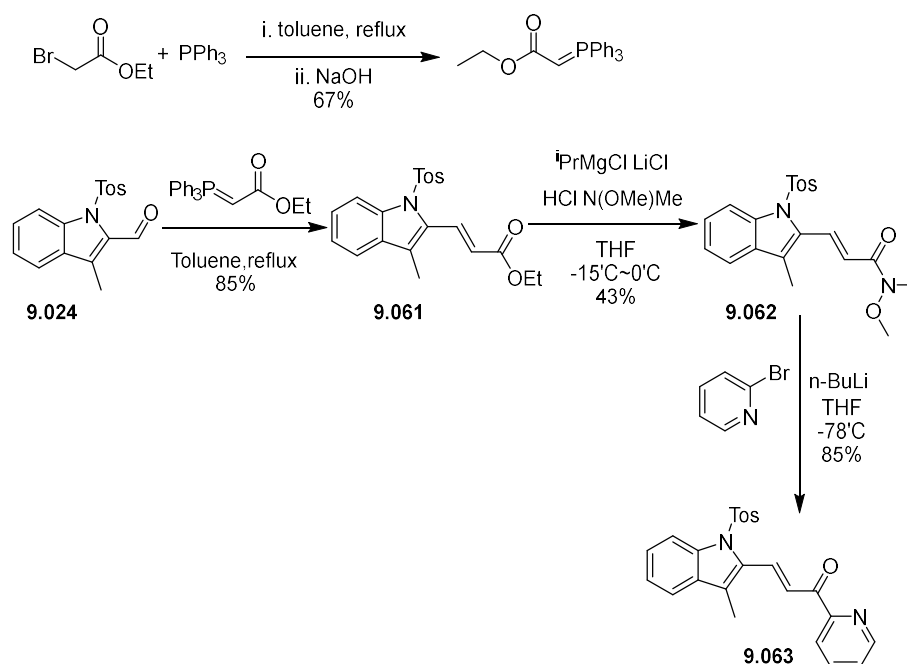
Scheme 3.2.7 Attempts of one-step deprotection and modification reaction



Nu	Product	Yield
dimethyl malonate	 9.057	62%
thiophenol	 9.058	53%
phenol	 9.059	62%
piperidine	 9.060	50%

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Considering high temperature during the formation of 2-pyridine indole chalcone such as **9.024** could cause cyclization. A new strategy based on Wittig reaction and Weinreb amide were designed to solve the issue of cyclization by avoiding high temperature. This synthetic strategy was first attempted with **9.024** reacted with ylide and formed **3.061**. **3.061** was subsequently transferred to its Weinreb amide type **3.062** followed by reacting with lithium substituted pyridine and produce the final chalcone **9.063**.



Scheme 3.2.8 Approach of indole based chalcone from 2-acetylpyridine

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With the successful deprotection reaction and the produced series of indole aldehydes, the condensation reaction to give the chalcones was performed successfully with yields range from 48% to 60%. A series of indole based chalcones were obtained. Those chalcones included the modification of C9 position of indole with hydrogen, methoxy, ethoxy and tosyl as well as bromination at C5 position; the other modifications came from different ketones.

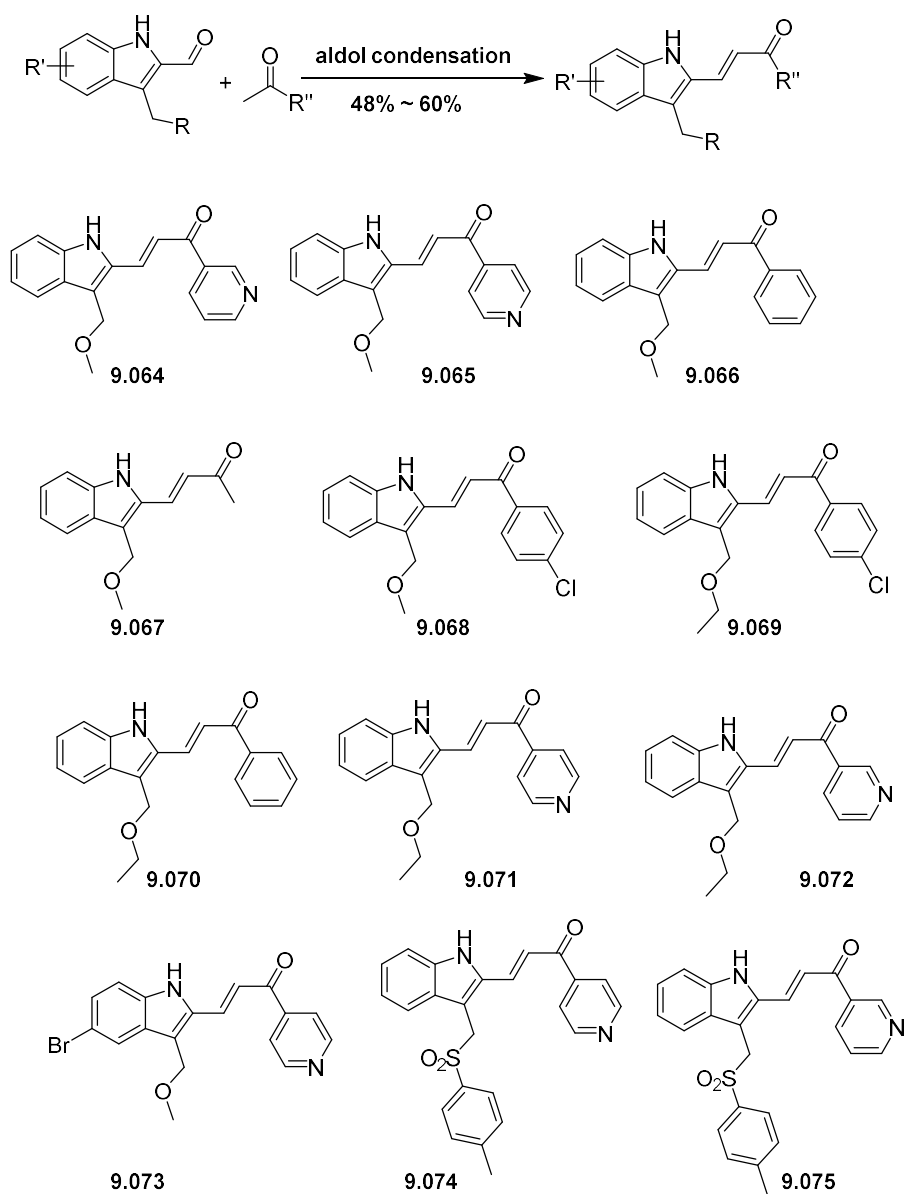


Figure 3.2.9 List of indole hybrid chalcone molecules

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Additionally, to enrich the library of indole-2-chalcones, indole-2-acids with 5-methoxy and chloro substitutions were reduced by lithium aluminum hydride and re-oxidized by manganese dioxide with yield around 60% of the corresponding aldehydes. Aldol condensation was successfully performed and 4 indole chalcones were obtained with the relatively low yield.

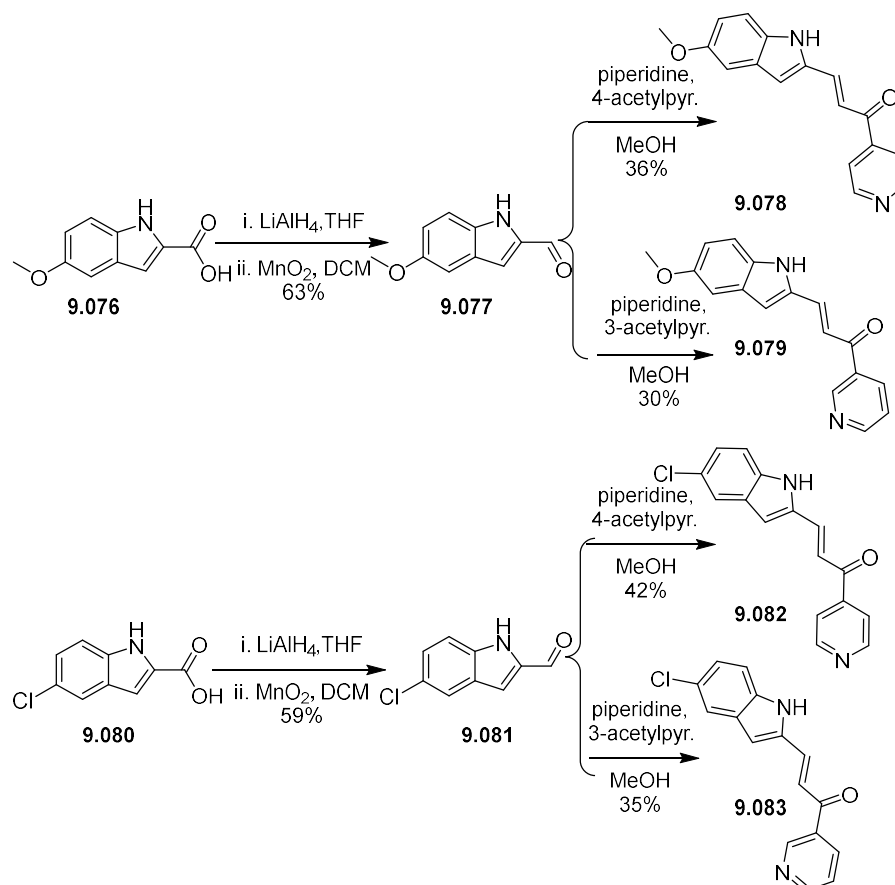


Figure 3.2.10 Indole chalcone molecules coming from commercially available indole acid.

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3. Biological Study

The biological study of this series of indole chalcones were initially performed in collaboration with a research group in Norway. However, the collaboration was terminated due to the sudden passing away of Prof. Marko without obtaining any biological result. A new cooperation was established with Prof. Frederick (tested on MDA-MB-231 cell line) and Prof. Pan (HEL and MDA-MB-231 cell lines).

Various literature studies indicated that indole-3/5-chalcones may induce cancer cell death via mechanisms other than programmed cell death or via novel cell death pathways including methuosis. These were summarized in Chapter II. As few studies were performed with indole-2-chalcones, verification of indole-2-chalcone cytotoxicity is the primary task. Twenty-two indole-2-chalcones, indole-2-aldehydes as well as the previously reported indole-3-chalcone **9.011**^[1, 4] were used to treat human leukemia HEL cell lines and human breast cancer MDA-231 cell lines with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.

Cytotoxicity results of indole-2-chalcone were listed in table 9.3.1. Nine of indole-2-chalcones showed inhibition on HEL cell line. Although more than half of the twenty-two chalcones did not show obvious inhibition on HEL cell line, some structure activity relations could be summarized from the result. **9.061**, the only tosyl protected compound, exhibited the strongest inhibition with IC₅₀ value 11.512 ± 1.061 μ M. Besides, all of the 3 chalcones with phenol group at R₂ position also displayed strong inhibition with IC₅₀ values around/below 20 μ M. Additionally, for R₂, the inhibition strength is phenyl > ethoxy > methoxy > methyl group. For MDA-MB-231 cell line, not enough inhibition results were obtained to have a good idea of what aspects are best, still, **9.021** with R₁ and R₂ as *para* pyridine and phenyl group, respectively, presented the strongest inhibitions.

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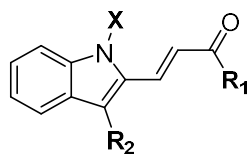
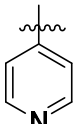
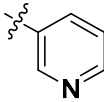
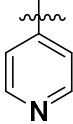
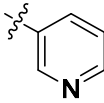
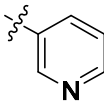


Table 9.3.1 Activity of indole chalcone molecules

Compound	R ₁	R ₂	X	IC ₅₀ (μM)	
				HEL	MDA-MB-231
9.011	-	-	-	17±1	26±1
9.018		CH ₃	H	108±2	>100
9.019	Me	CH ₃	H	20±1	>100
9.020			H	19±1	25±1
9.021			H	15±1	18±1
9.022	Me		H	16±1	115±1
9.061	OMe	CH ₃	Tosyl	11±1	65±2
9.064		CH ₂ OMe	H	108±2	>100
9.070		CH ₂ OEt	H	24±1	>100
9.072		CH ₂ OEt	H	100±1	>100
Fluorouracil	-	-	-	13±1	95±2

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Further biological studies were carried out on HEL cells treated with **9.061** and **9.021**, respectively (figure 9.3.2). Dose-response curves for those two chalcones on cell viability are shown in figure 9.3.1. For **9.021**, high concentration ($> 5 \mu\text{M}$) exhibited potent and continuous inhibition on HEL cells during 72 h. However, **9.061** showed potent inhibition on HEL cells even on the concentration level of $4 \mu\text{M}$.

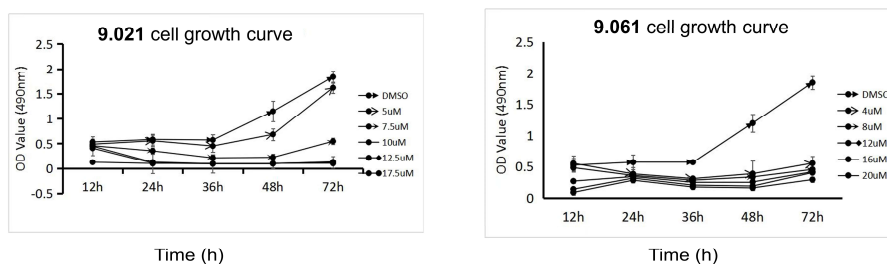


Figure 9.3.1 Does-response cell growth curve of HEL cells treated with **9.021** and **9.061**

Morphology studies were performed with the concentration of 0, 5, 10 and 15 μM for **9.021** (figure 9.3.2) and 0, 8, 12 and 16 μM for **9.061** (figure 9.3.3). Compared with the control set, cell density reduced with increasing compound concentration in both sets. Unfortunately, under phase contrast microscopy, none of those except indole-3-chalcone **9.011** showed phase-lucent macropinosome-derived vacuoles (figure 9.3.2 in right-up box). As the irregular vacuolization is the iconic sign in the early stage of methuosis, the morphology study of these indole-2-chalcones suggest a non-methuosis cell death mechanism. Additionally, HEL cells showed shrinking as well as bleb protrusions formed on the cell membrane indicated these two chalcones may induce HEL cell death though apoptosis.

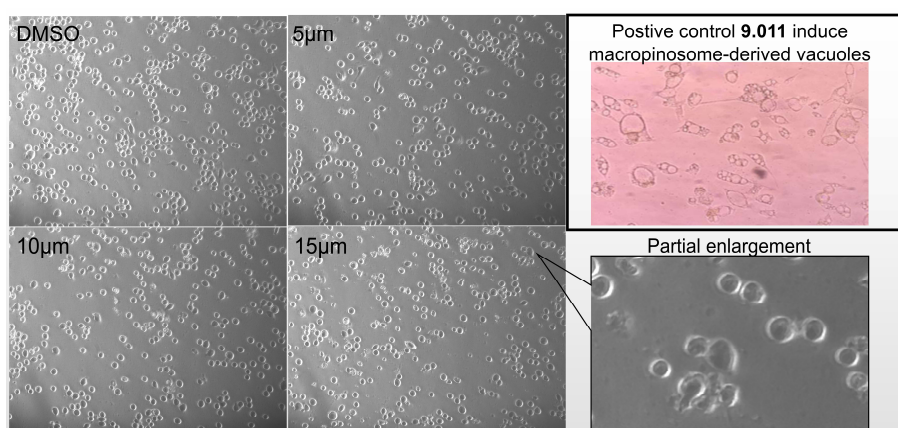


Figure 9.3.2 Morphology studies of chalcone **9.021** on HEL cell line at the concentration of 0, 5, 10, 15 μM ; Right up box showed postive control **9.011** induce macropinosome-derived vacuoles on HEL cell line.

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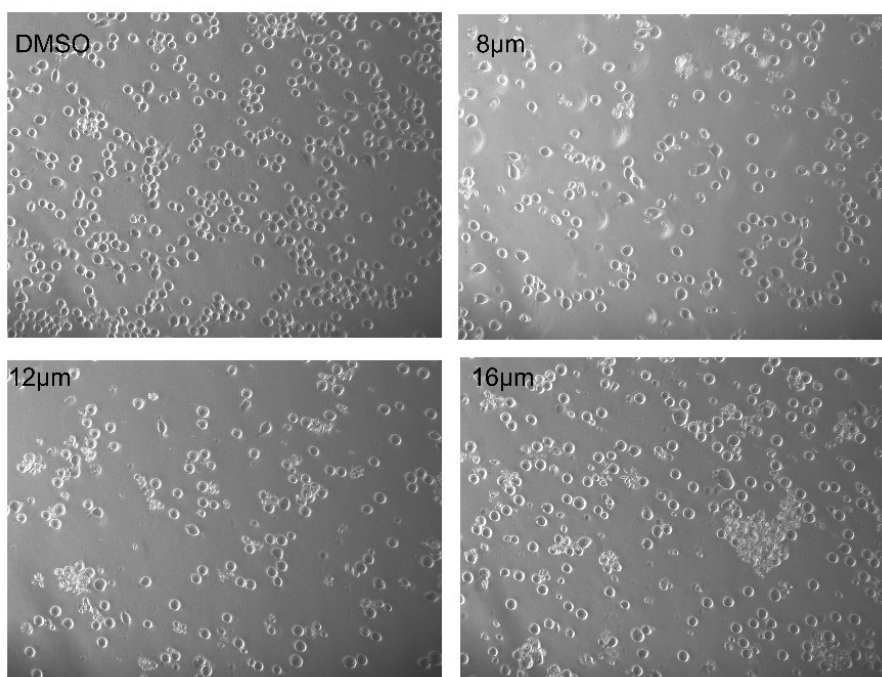


Figure 9.3.3 Morphology studies of chalcone **9.061** on HEL cell line at the concentration of 0, 8, 12, 16 μM

Biological studies indicated this series of indole chalcones exhibited certain inhibition on HEL cell line and MDA-MB-231 cell line. However, further morphological studies of HEL cells treated with **9.021** and **9.061** showed non-irregular vacuolization and some typical signs of apoptosis indicating these chalcones may induce cell death by apoptosis rather than methuosis. Flow cytometry and western blot experiments need to be performed for these cells to verify their cell death pathway.

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References

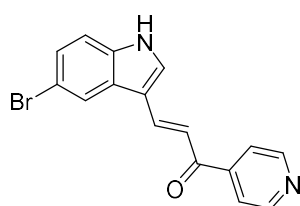
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Conclusions and Perspectives

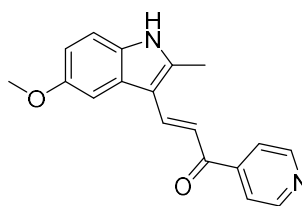
1. Conclusions

In recent decades, indole chalcones have represented a series of novel bioactive molecules especially for their anticancer and potent inhibition properties on cells both in vitro and in vivo, and have been of interest for mechanistic studies. Among these, few indole-2-chalcones were studied for their bioactivity. That is mainly due to the harsh conditions for obtaining the indole-2-aldehydes, especially the 3-substituted-indole-2-aldehydes.

In my work, two indole-3-chalcones which were reported previously in the literature as positive controls were synthesized.

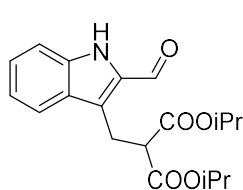


9.003

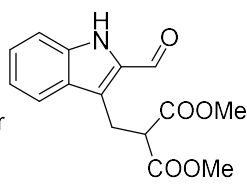


9.011

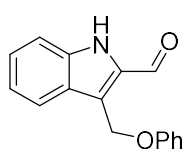
Through the development of a methodology for indole deprotection and modification in one step a large series of indol-2-aldehydes were obtained. A number of different nucleophiles were successfully substituted onto the C9 position of the indole.



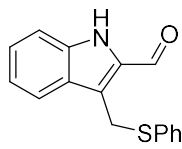
9.053



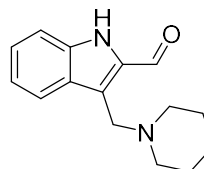
9.054



9.055



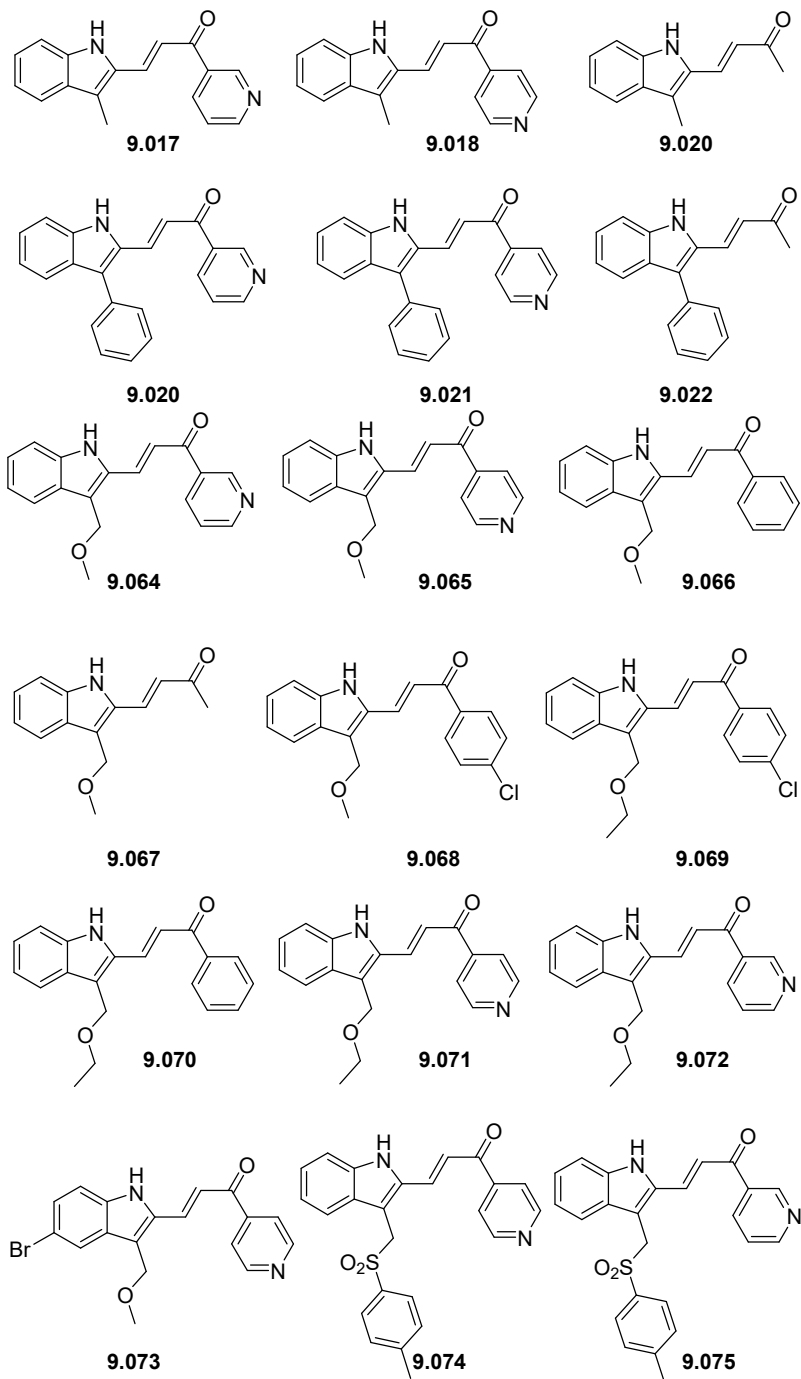
9.056



9.057

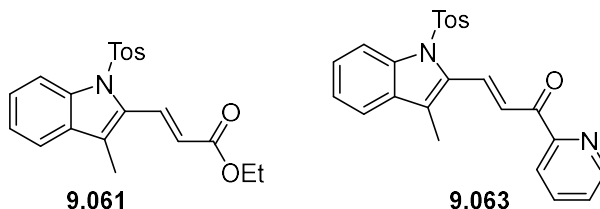
Chapter X

Using the unprotected indole aldehydes as the starting material, 18 indole chalcone analogues in total were obtained through aldol condensation with moderate yield.

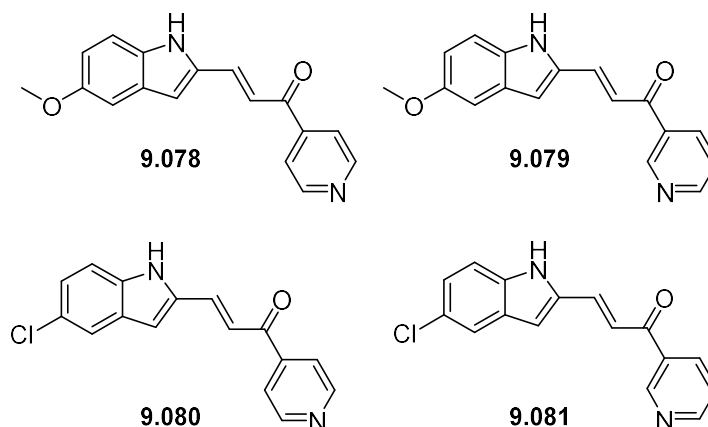


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Additional compounds could be obtained through other synthetic pathways. **9.061** was obtained via a Wittig reaction and **9.063** was obtained using a Weinreb amide at low temperature to avoid issues of cyclization.



Additionally, four indole-2-chalcones were obtained through commercially available indole-2-acids, via an initial reduction and oxidation to afford indole-2-aldehydes and subsequent aldol condensation.



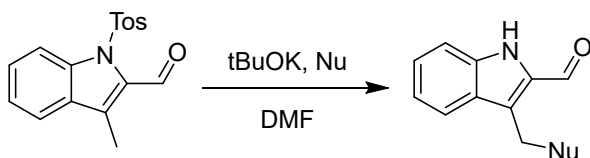
To verify those new indole chalcone's bioactivity, anti-proliferation studies were performed on human leukaemia HEL and human breast MDA-MB-231 cell lines. The result show nine of twenty-two chalcones exhibited inhibitions on HEL cell. Primary SARs demonstrated some useful insights. First, for R₁, *para* pyridine exhibited the most potent inhibition. Second, for R₂, inhibition strength is phenyl > OEt > OMe > H. Last, the protected indole chalcone displays better activity than the unprotected chalcones.

Cell growth curves and morphology studies of **9.021** and **9.060** were subsequently performed on HEL cell line. Both showed improved inhibitions on HEL cells with the increase of chalcone concentration. Morphology studies illustrated those two compounds may induce cell death through apoptosis.

Chapter X

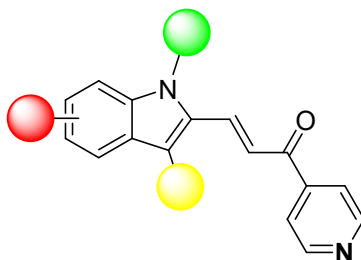
2. Perspectives

The deprotection reaction of tosyl protected indole-2-aldehyde should be further studied in order to enrich the library of unprotected indole-2-aldehydes.



Based on the initial biological studies of the indole-2-chalcones, it is worth continuing the modification of those molecules. The details are as follows,

First, based on the primary SARs, modifications on this molecule could be applied such as using different protecting groups (green part), modification on C9 position especially using aromatic group (yellow part).



Second is to further enrich the library of indole-2-chalcones. One possible way is using modified *o*-acetyl-aniline to form the different substituted indole-2-aldehyde (red part). Additionally, the pyridine moiety could also be replaced by other heterocycles.

Further biological experiments could also be performed to verify the cell death mechanism of **9.021/9.061** on HEL cells.

Experimental Section

1. Instrumentation

^1H and ^{13}C nuclear magnetic resonance spectra were recorded in Louvain-la-Neuve on Bruker Avance II 300 MHz spectrometer (^1H 300 MHz and ^{13}C 75 MHz) and Bruker Avance 500 MHz spectrometer. ^1H chemical shifts are reported in ppm downfield from internal tetramethylsilane ($\delta = 0$ ppm), Chloroform- d ($\delta = 7.26$ ppm), DMSO- d_6 ($\delta = 2.50$ ppm), Acetone- d_6 ($\delta = 2.05$ ppm) or Acetonitrile- d_3 ($\delta = 1.94$ ppm). ^1H chemical shifts are reported in ppm using Chloroform- d ($\delta = 77.16$ ppm). If necessary, the structures were determined by two-dimensional analysis (COSY, HMQC and HMBC). ^1H spectra described as follow: chemical shift (multiplicity, coupling constant (Hz), integration). ^{13}C spectra are described as follow: chemical shift. Multiplicity is designed as : s = singlet; d = doublet; t = triplet; q = quartet; qui = quintet; sex= sextet; sep = septet; b = broad; m = multiplet.

High-resolution mass spectra were recorded by Mr. Raoul Rozenberg from Mass Spectrometry Service of UClouvain.

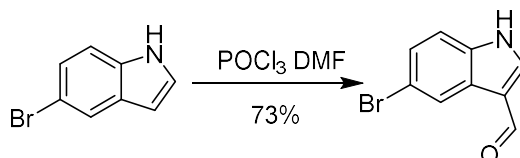
2. Procedure

Unless otherwise noticed, all manipulations were performed under an argon atmosphere using standard glassware. Diethyl ether and tetrahydrofuran were distilled on sodium/benzophenone under argon atmosphere. Dichloromethane, triethylamine and toluene were distilled on calcium hydride. Solvents used for work-up were of technical grade. Commercial reagents were purchased from Acros, Sigma-Aldrich, ABCR, TCI, Merck or Fluorochem and used as received unless stated otherwise.

Thin layer chromatography was performed on Merck Millipore F₂₅₄ silica gel 60 plates (200 μm thickness, aluminium supported). The plates were visualized using ultra-violet light (254 nm) and stained using an alkaline potassium permanganate, vanillin or ninhydrin solution. Chromatography was performed under pressure with the stated solvents using Sigma-Aldrich silica gel 60 (70 – 230 mesh).

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5-bromo-1H-indole-3-carbaldehyde (9.002)

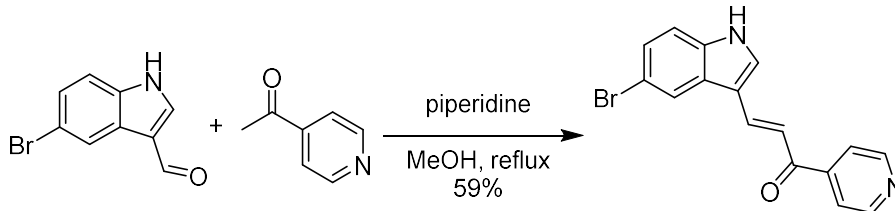


To a solution of POCl_3 (0.9 mL, 10 mmol) in 10 mL of DMF cooled with an ice bath, 5-bromo-indole (1.1 g, 5 mmol) in 5 mL of DMF was added dropwise. The mixture was stirred under the protection of argon overnight. Then the solution was poured into 40 mL of saturated sodium hydroxide solution and stirred for another 1 hour while cooled with an ice bath. The precipitate was collected and dried under vacuum to give the product without further purification.

Yield, 73%, dark-red solid. $^1\text{H-NMR}$ (300 MHz, DMSO) δ 7.66 (d, J = 8.2 Hz, 1H), 7.32 (d, J = 8.9 Hz, 1H), 7.08 (s, 1H), 7.03 (s, 1H).

The characteristic data was corresponding with reported literature.^[1]

3-(5-bromo-1H-indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.003)



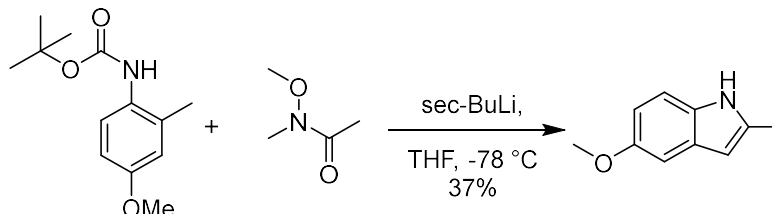
5-bromo-1H-indole-3-carbaldehyde **9.002** (112 mg, 0.5 mmol) was dissolved in anhydrous methanol. 4-acetylpyridine (121 mg, 1 mmol) and piperidine (112 mg, 1 mmol) were added, and the solution was heated at reflux overnight. After TLC showed no starting material, the precipitate was collected and washed with cold methanol, dried under vacuum and used without further purification.

Yield: 59%, light-yellow solid. $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ = 8.79 (d, J = 8.4 Hz, 2H), 7.71-7.83 (m, 4H), 7.20 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.2 Hz, 1H), 6.69 (d, J = 8.8 Hz, 1H).

The characteristic data was corresponding with reported literature.^[2]

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5-methoxy-2-methyl-1H-indole (9.010)

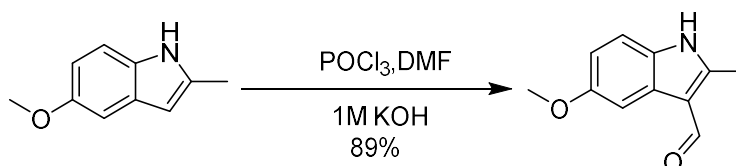


Tert-butyl (4-ethoxy-2-methylphenyl) carbamate **9.009** (0.40 g, 1.7 mmol) was dissolved in 10 mL freshly distilled THF under the protection of argon. The solution was cooled to -40 °C and sec-butyllithium (1.4 M, 0.27 mL) was added dropwise. After, the reaction mixture turned a bright yellow signifying deprotonation of the amide nitrogen. The reaction mixture was cooled to -50 °C with a solution of N-methoxy-N-methylacetamide **9.007** (210 mg, 2 mmol) in 3 mL THF was added dropwise. The reaction mixture was warmed to -10 °C and stirred for another one hour. Then the mixture was poured into 20 mL of 0.5 M HCl and extracted with diethyl ether. The organic phases were combined and concentrated by rotary evaporation to afford crude cyclized indoline. This crude material was dissolved into 10 mL of DCM and 2 mL of TFA and stirred for forty-eight hour. The reaction was poured into saturated sodium bicarbonate solution and extracted with DCM. The crude product was purified by chromatography (PE/EA = 9:1) to yield a brown solid.

Yield: 34%, brown solid. ¹H NMR (300 MHz, CDCl₃) δ 7.72 (s, 1H), 7.16–7.14 (d, 1H, J = 8.6 Hz), 6.99 (d, 1H, J = 2.4 Hz), 6.77–6.75 (dd, 1H, J = 2.4 Hz, 8.7 Hz), 6.13 (s, 1H), 4.04 (s, 3H), 2.41 (s, 3H).

The characteristic data was corresponding with reported literature. ^[2]

5-methoxy-2-methyl-1H-indole-3-carbaldehyde (9.011)



0.1 mL of POCl₃ was added to 1 mL of DMF while cooled with an ice bath and stirred for 10 min. A solution of 5-methoxy-2-methyl-1H-indole **9.010** (0.097 g, 0.60 mmol) was added dropwise and then stirred for an additional thirty minutes and warmed to rt. The reaction mixture was added to an ice-cold sodium hydroxide solution and stirred for another one hour at 0 °C. The

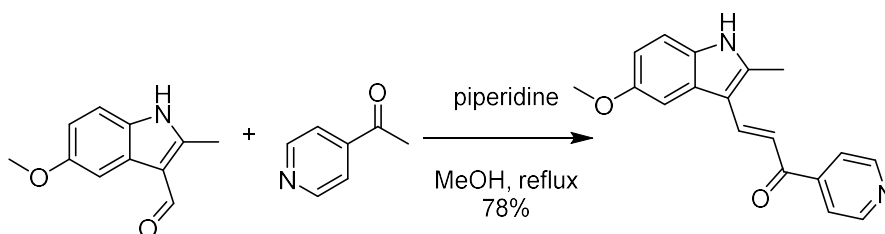
Chapter XI

resulting precipitate was collected, washed with ice-cold water and dried under vacuum to afford a yellow solid that was used without further purification.

^1H NMR (300 MHz, DMSO- d_6) δ 11.85 (s, 1H), 10.00 (s, 1H), 7.55 (d, 1H, J = 2.34 Hz), 7.27–7.25 (d, 1H, J = 8.7 Hz), 6.78–6.76 (dd, 1H, J = 2.4 Hz, 8.7 Hz), 4.02 (s, 3H), 2.64 (s, 3H).

The characteristic data was corresponding with reported literature. ^[2]

3-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.012)



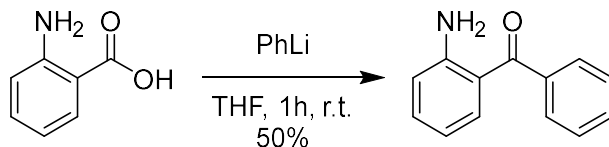
5-Methoxy-2-methylindole-3-carboxaldehyde **9.011** (0.10 g, 0.54 mmol) was dissolved in 10 mL of anhydrous methanol. 4-Acetylpyridine (0.08 g, 0.72 mmol) and piperidine (0.06 g, 0.72 mmol) were added and the reaction mixture was heated at reflux overnight. A precipitate slowly formed which was collected, washed with ice-cold methanol and dried under vacuum to yield a bright orange powder.

^1H NMR (300 MHz, DMSO- d_6) δ 11.88 (s, 1H), 8.81 (dd, J = 1.8 Hz, 4.2 Hz, 2H), 8.08 (d, J = 15.0, 1H), 7.94 (dd, J = 1.8 Hz, 4.2 Hz, 2H), 7.43 (d, J = 2.4 Hz, 1H), 7.36 (d, J = 15.0), 7.31 (d, J = 8.4, 1H), 6.84 (dd, J = 2.4 Hz, 8.4 Hz, 1H), 3.860 (s, 3H), 2.572 (s, 3H).

The characteristic data was corresponding with reported literature. ^[2]

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(2-aminophenyl)(phenyl)methanone (9.013)

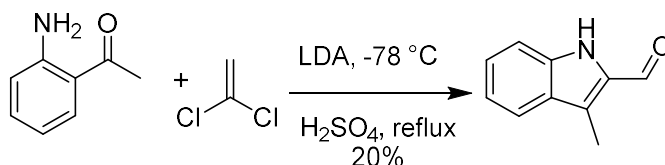


To a solution of 1.37 g of 2-aminobenzoic acid (10 mmol) in 20 mL of freshly distilled THF and cooled with an ice bath, phenyl lithium (2.0 M, 15 mL, 30 mmol) was added dropwise. After two hours, 30 mL of aqueous ammonium chloride solution was added. The solution was extracted with DCM and the combined organic phases washed with sodium sulfate solution and dried by rotary evaporation. The crude product was purified by chromatography (PE/EA= 15:1) to afford the desired compound **9.013**.

Yield: 50%, yellow solid. ^1H NMR (300 MHz, CDCl_3): δ = 7.62 (m, 2H), 7.56 (m, 2H), 7.46 (m, 2H), 7.35 (dd, J = 8.8 Hz, J = 2.3 Hz, 2 H), 6.64 (d, J = 8.8 Hz, 1H), 4.2 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3): 196.2, 152.1, 141.0, 133.2, 132.4, 129.2, 129.2, 128.4, 128.4, 120.0, 118.7, 106.9.

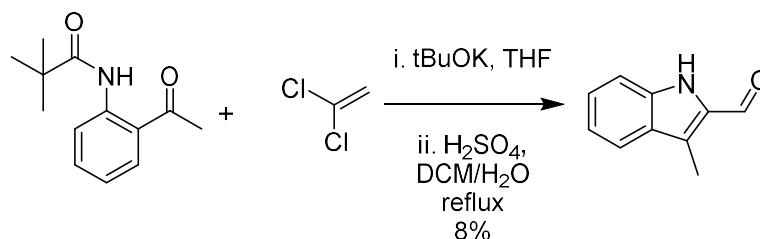
The characteristic data was corresponding with reported literature.^[3]

3-methyl-1H-indole-2-carbaldehyde (9.015)



Method 1: To a solution of 1.69 mL of diisopropylamine (12 mmol) in 10 mL of THF, cooled with ice bath, were added 4.8 mL of $n\text{BuLi}$ (12 mmol, 2.5 M). The mixture was cooled down to -78°C and 0.36 mL of dichloroethylene (4.5 mmol) were added. The reaction mixture was warmed to 0°C and 2-aminophenone (0.4 g, 3 mmol) in 2 mL of THF were added dropwise. The reaction mixture was stirred while cooled with an ice bath for one more hour and 20 mL of aqueous ammonia chloride solution was added. The aqueous solution was extracted with 10 mL of DCM for 3 times. The organic phase was combined and 50 mL 0.1 M sulfuric acid solution was added and the solution refluxed for eighteen hours. After, the aqueous layer was extracted with DCM and the organic layer was dried, filtered and concentrated. The crude compound was purified by chromatography (PE/EA = 10:1) to afford the pure compound.

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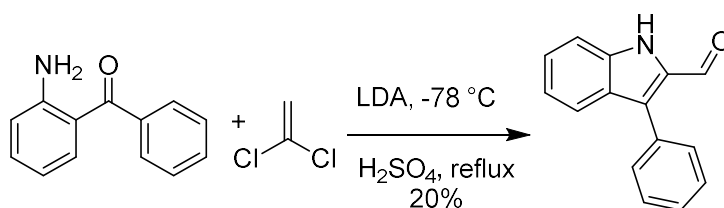


Method 2: To a solution of Piv protected 2-acetyl aniline (0.87 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and the solution was stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous sulfuric acid solution and refluxed for 24 h. After the aqueous layer was extracted with DCM and the organic phases combined, dried over sodium sulfate and concentrated by rotary evaporation. The crude product was recrystallized in acetone to afford the pure product.

Yield, 20% for method 1 and 8% for method 2, off-white solid. ^1H NMR (300 MHz, CDCl_3): δ = 10.12 (s, 1H), 8.64 (broad, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.38 (m, 2H), 7.16 (m, 1H), 2.46 (s, 3H). ^{13}C (75 MHz, CDCl_3): δ = 180.8, 137.6, 132.3, 128.5, 128.4, 127.9, 124.9, 121.8, 120.3, 112.3, 8.5.

The characteristic data was corresponding with reported literature. ^[4]

3-phenyl-1H-indole-2-carbaldehyde (9.016)



To a solution of 1.69 mL of diisopropylamine (12 mmol) in 10 mL of THF, cooled down with ice bath, were added 4.8 mL of *n*BuLi (12 mmol, 2.5 M). The mixture was cooled down to $-78\text{ }^{\circ}\text{C}$ and 0.36 mL of dichloroethylene (4.5 mmol) were added. The reaction mixture was warmed to $0\text{ }^{\circ}\text{C}$ and (2-aminophenyl)(phenyl)methanone (0.4 g, 3 mmol) in 2 mL of THF were added dropwise. The reaction mixture was stirred while cooled with an ice bath for one more hour and 20 mL of aqueous ammonia chloride solution was added.

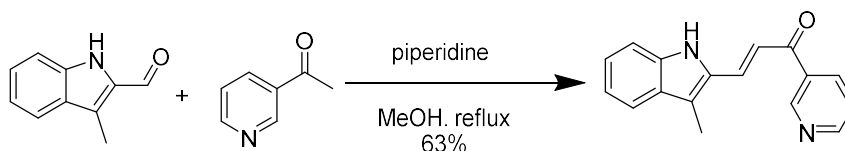
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The aqueous solution was extracted with 10 mL of DCM for 3 times. The organic phase was combined and 50 mL 0.1 M sulfuric acid solution was added and the solution refluxed for eighteen hours. After, the aqueous layer was extracted with DCM and organic layers dried, filtered and concentrated. The crude compound was purified by chromatography (PE/EA = 10:1) to afford the pure compound.

Yield, 20%, off-white solid. ^1H NMR (300 MHz, CDCl_3): δ = 10.03 (s, 1H), 9.28 (broad, 1H), 7.62 (m, 2H), 7.54 (m, 2H), 7.48 (m, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.21 (m, 1H). ^{13}C (75 MHz, CDCl_3): δ = 182.6, 137.3, 132.1, 131.9, 130.9, 129.7, 129.0, 129.0, 128.4, 127.9, 127.0, 122.4, 121.6, 112.3.

The characteristic data was corresponding with reported literature. ^[5]

3-(3-methyl-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.017)



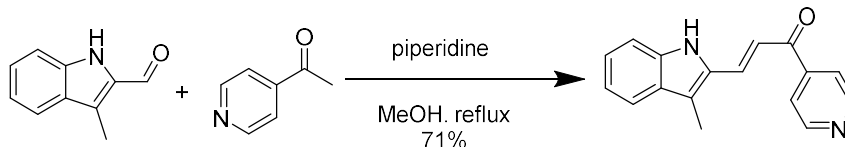
3-methyl-1H-indole-2-carbaldehyde **9.014** (0.10 g, 0.63 mmol) was dissolved in 10 mL of anhydrous methanol. 3-Acetylpyridine (0.09 g, 0.76 mmol) and piperidine (0.08 g, 0.76 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution was added and the aqueous phase extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 63%, bright yellow amorphous solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.75 (s, 1H), 9.35 – 9.26 (m, 1H), 8.86 (dd, J = 4.8, 1.7 Hz, 1H), 8.40 (dd, J = 8.0, 2.0 Hz, 1H), 7.93 (s, 2H), 7.73 – 7.63 (m, 2H), 7.45 (d, J = 8.2 Hz, 1H), 7.38 – 7.21 (m, 1H), 7.09 (d, J = 8.0 Hz, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 206.51, 187.49, 153.35, 149.37, 137.45, 135.60, 133.11, 132.52, 131.70, 127.95, 125.09, 124.12, 119.98, 119.38, 118.99, 111.50, 63.63, 57.12.

HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{15}\text{ON}_2$, $[\text{M}+\text{H}]^+$ 293.12845, found 293.12831.

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3-(3-methyl-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.018)

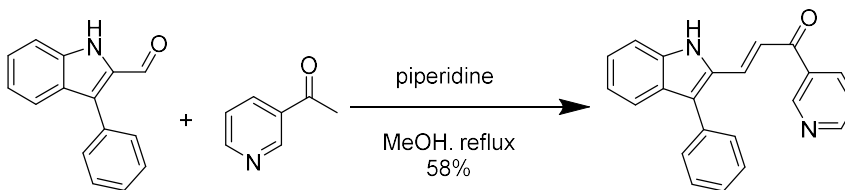


3-methyl-1H-indole-2-carbaldehyde **9.014** (0.10 g, 0.63 mmol) was dissolved in 10 mL of anhydrous methanol. 4-Acetylpyridine (0.09 g, 0.76 mmol) and piperidine (0.08 g, 0.76 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution was added and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 71%, bright yellow amorphous solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.81 (s, 1H), 8.95 – 8.86 (m, 2H), 8.01 – 7.89 (m, 3H), 7.86 (d, J = 15.5 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.46 (dt, J = 8.2, 1.0 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 4.78 (s, 2H), 3.29 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 202.12, 188.74, 151.49, 144.65, 138.25, 133.18, 128.74, 125.83, 121.87, 120.63, 120.57, 120.15, 119.57, 112.13, 64.23, 57.73.

HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{15}\text{ON}_2$, $[\text{M}+\text{H}]^+$ 293.12845, found 293.12831.

3-(3-phenyl-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.020)



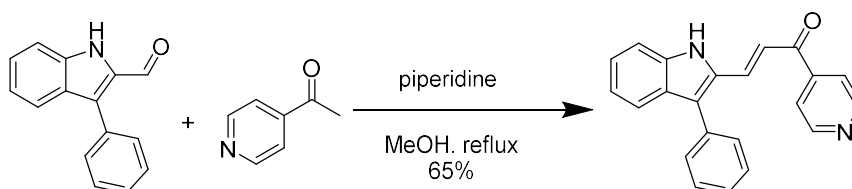
3-phenyl-1H-indole-2-carbaldehyde **9.015** (0.10 g, 0.45 mmol) was dissolved in 10 mL of anhydrous methanol. 3-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution was added and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

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^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.91 (s, 1H), 9.26 (s, 1H), 8.81 (s, 1H), 8.34 (d, $J = 7.8$ Hz, 1H), 7.97 (d, $J = 15.4$ Hz, 1H), 7.72 (d, $J = 15.2$ Hz, 1H), 7.66 – 7.36 (m, 8H), 7.32 (d, $J = 7.7$ Hz, 1H), 7.08 (t, $J = 7.7$ Hz, 1H). ^{13}C NMR (75 MHz, $\text{Chloroform}-d$) δ 193.57, 156.18, 149.26, 143.16, 139.03, 138.56, 135.99, 135.32, 134.30, 132.66, 132.03, 131.04, 130.10, 126.61, 125.94, 125.44, 124.61, 117.16.

HRMS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{17}\text{ON}_2$, $[\text{M}+\text{H}]^+$ 325.13348, found 325.13354.

3-(3-phenyl-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.021)



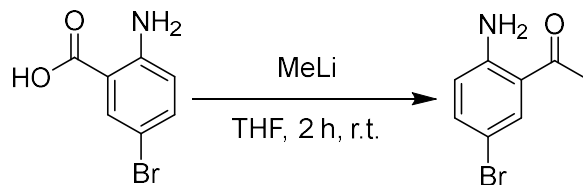
3-phenyl-1H-indole-2-carbaldehyde **9.015** (0.10 g, 0.45 mmol) was dissolved in 10 mL of anhydrous methanol. 4-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution was added and the solution extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.91 (s, 1H), 9.26 (s, 1H), 8.81 (s, 1H), 8.34 (d, $J = 7.7$ Hz, 1H), 7.97 (d, $J = 15.4$ Hz, 1H), 7.72 (d, $J = 15.2$ Hz, 1H), 7.64 – 7.46 (m, 7H), 7.42 (s, 1H), 7.32 (d, $J = 7.6$ Hz, 1H), 7.14 – 7.05 (m, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.03, 153.86, 149.90, 138.28, 136.12, 133.91, 133.67, 133.30, 131.34, 131.06, 130.56, 129.53, 127.83, 127.32, 126.10, 124.88, 124.64, 121.11, 120.61, 120.23, 112.35.

HRMS (ESI): m/z calcd for $\text{C}_{22}\text{H}_{17}\text{ON}_2$, $[\text{M}+\text{H}]^+$ 325.13348, found 325.13354.

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1-(2-amino-5-bromophenyl)ethan-1-one (9.026)

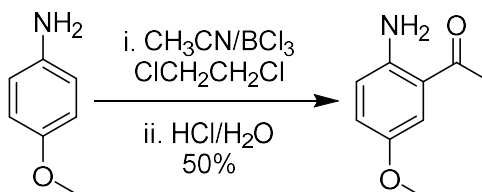


To a suspension of 5-bromoanthranilic acid (1.51 g, 7.0 mmol) in 30 mL of THF and cooled down to 0 °C using an ice bath. Methyllithium (15.3 mL, 1.6 M, 24.5 mmol) was added dropwise. After two hours, 40 mL of aqueous ammonium chloride were poured into the reaction mixture. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried, filtered and concentrated by rotary evaporation. The crude product was purified over chromatography to afford the pure compound.

Yield: 17%, brown solid. ^1H NMR (300 MHz, CDCl_3) δ 7.80 (d, J = 8.8 Hz, 1H), 6.29 (broad, 2H), 2.56 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 199.9, 149.2, 137.1, 134.5, 119.5, 119.1, 106.9, 28.0.

The characteristic data was corresponding with reported literature.^[6]

1-(2-amino-5-methoxyphenyl)ethan-1-one (9.026)



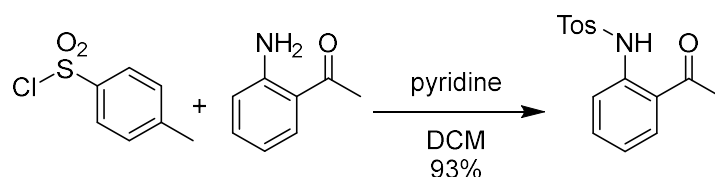
To a solution of boron trichloride in 10 mL of DCM at 0 °C was slowly added a solution of p-anisidine (1 g, 81.2 mmol) in 10 mL of 1,2-dichloroethane. The mixture was stirred for 20 min at 0 °C before the addition of anhydrous acetonitrile (4 mL, 80 mmol). The reaction was heated to 80 °C overnight. After it was cooled down to 0 °C, 80 mL of 2 M HCl aqueous solution was added and the resulting heterogeneous mixture was heated 80 °C until a homogeneous solution was obtained (about 30 min). The solution was allowed to cool down to room temperature and was neutralized with sodium bicarbonate. The mixture was extracted with DCM and the organic layer was washed with aqueous ammonium chloride. The organic layer was dried, the solid was filtered and the filtrate was concentrated to give the desired compound.

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Yield: 50%, brown oil. ^1H NMR (300 MHz, CDCl_3) δ 7.19 (d, J = 9.0 Hz, 1H), 6.99 (dd, J = 8.8 Hz, 2.8 Hz, 1H), 6.65 (d, J = 8.8 Hz, 1H), 6.24 (broad, 2H), 3.78 (s, 3H), 2.49 (s, 3H).

The characteristic data was corresponding with reported literature.^[7]

N-(2-acetylphenyl)-4-methylbenzenesulfonamide (9.027)

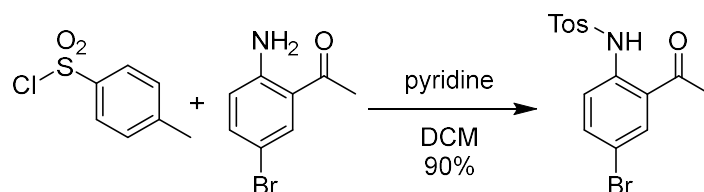


To a solution of 2-acetylaniline (0.41 g, 3 mmol) in 25 mL DCM was added 7.3 mL of pyridine followed by tosyl chloride (0.78 g, 4 mmol) in 5 mL of DCM dropwise at 0 °C. The reaction mixture was then stirred overnight. After the reaction was complete, the mixture was poured into ice-cold water and extracted with DCM. The combined organic layer was washed with 1 M HCl solution and dried over sodium sulfate and concentrated by rotary evaporation. The compound was purified by chromatography (EA/PE = 1/10) to afford the desired compound.

Yield: 93%, white needle solid. ^1H NMR (300 MHz, CDCl_3): δ 11.52 (s, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.74 (s, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.51-7.44 (m, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.12-7.03 (m, 1H), 2.58 (s, 3H).

The characteristic data was corresponding with reported literature.^[8]

N-(2-acetyl-4-bromophenyl)-4-methylbenzenesulfonamide (9.027)



To a solution of 4-bromo-2-acetylaniline (0.64 g, 3 mmol) in 25 mL DCM was added with 7.3 mL of pyridine followed by tosyl chloride (0.78 g, 4 mmol) in 5 mL of DCM dropwise at 0 °C. The reaction mixture was then stirred overnight. After the reaction was complete, the mixture was poured into ice-cold water and extracted with DCM. The combined organic layer was washed with 1 M

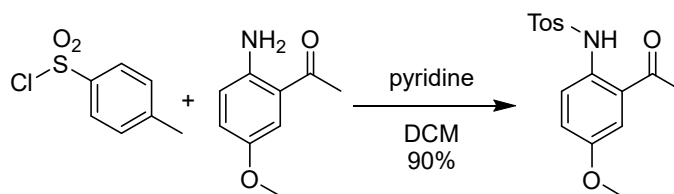
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HCl solution and dried over sodium sulfate and concentrated by rotary evaporation. The compound was purified by chromatography (EA/PE = 1/10) to afford the desired compound.

Yield: 90%, white needle solid. ^1H NMR (300 MHz, CDCl_3): δ 11.32 (s, 1H), 7.88 (s, 1H), 7.83 (s, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.57-7.51 (m, 2H), 7.46 (t, J = 7.6 Hz, 2H), 2.68 (s, 3H), 2.54 (s, 3H).

The characteristic data was corresponding with reported literature.^[9]

N-(2-acetyl-4-methoxyphenyl)-4-methylbenzenesulfonamide (9.027)



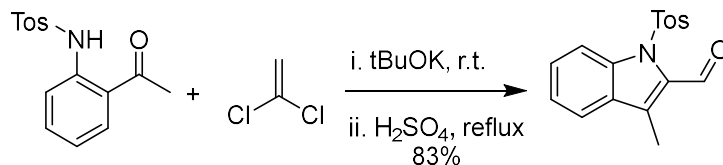
To a solution of 4-bromo-2-acetylaniline (0.5 g, 3.5 mmol) in 25 mL DCM was added with 7.3 mL of pyridine followed by tosyl chloride (0.78 g, 4 mmol) in 5 mL of DCM dropwise at 0 °C. The reaction mixture was then stirred overnight. After complete reaction, the mixture was poured into ice-cold water and extracted with DCM. The combined organic layer was washed with 1 M HCl solution and dried over sodium sulfate and concentrated by rotary evaporation. The compound was purified by chromatography (EA/PE = 1/10) to afford the desired compound.

Yield: 90%, yellow needle solid. ^1H NMR (300 MHz, CDCl_3): δ 10.66 (s, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 2.8 Hz, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 8.8 Hz, 1H), 3.85 (s, 3H), 2.64 (s, 3H), 2.48 (s, 3H).

The characteristic data was corresponding with reported literature.^[10]

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3-methyl-1-tosyl-1H-indole-2-carbaldehyde (9.028)

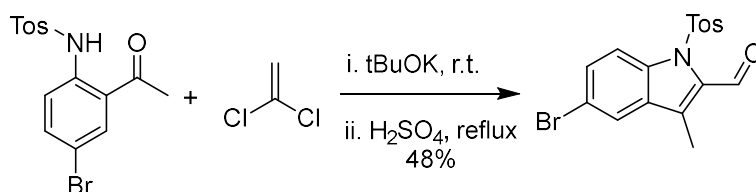


To a solution of tosyl protected 2-acetyl aniline (1.16 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and the reaction was stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous sulfuric acid solution and refluxed for 24 h. After, the aqueous layer was extracted with DCM and the organic phases combined, dried over sodium sulfate and concentrated. The crude product was recrystallized in acetone to afford the pure product.

Yield: 83%, off-yellow solid. ^1H NMR (300 MHz, Chloroform- d) δ 10.63 (s, 1H), 8.23 (dt, $J = 8.5, 0.9$ Hz, 1H), 7.64 – 7.50 (m, 4H), 7.34 (ddd, $J = 8.0, 7.2, 0.9$ Hz, 1H), 7.16 (d, $J = 8.1$ Hz, 2H), 2.53 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, Chloroform- d) δ 185.29, 145.28, 137.53, 134.16, 132.99, 132.18, 130.58, 129.77, 129.11, 126.69, 124.62, 121.58, 115.82, 21.59, 10.54.

The characteristic data was corresponding with reported literature.^[5]

5-bromo-3-methyl-1-tosyl-1H-indole-2-carbaldehyde (9.029)



To a solution of tosyl protected 4-bromo-2-acetyl aniline (1.47 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and the reaction was stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous

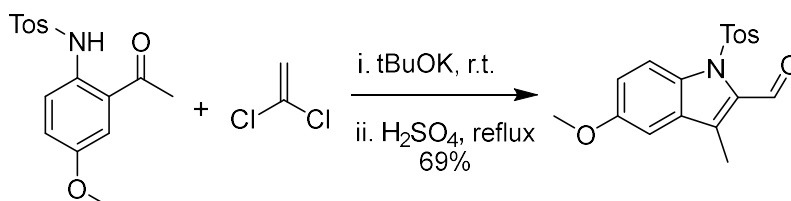
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sulfuric acid solution and refluxed for 24 h. After the aqueous layer was extracted with DCM and the organic phases combined, dried over sodium sulfate and concentrated. The crude product was recrystallized in acetone to afford the pure product.

Yield: 48%, light-brown solid. ^1H NMR (300 MHz, Chloroform- d) δ 10.51 (s, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.64 – 7.50 (m, 4H), 7.34 (ddd, J = 8.0, 7.2, 0.9 Hz, 1H), 2.49 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, Chloroform- d) δ 182.17, 144.76, 138.75, 135.11, 132.99, 132.18, 130.58, 129.77, 129.11, 126.69, 124.62, 121.58, 115.82, 21.59, 10.54.

The characteristic data was corresponding with reported literature.^[5]

5-methoxy-3-methyl-1-tosyl-1H-indole-2-carbaldehyde (9.030)

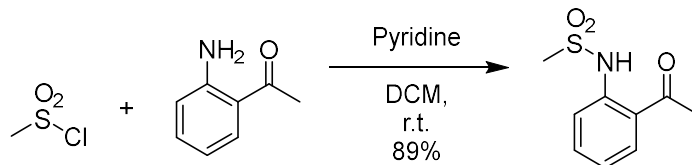


To a solution of tosyl protected 4-methoxy-2-acetyl aniline (1.27 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous sulfuric acid solution and refluxed for 24 h. After the aqueous layer was extracted with DCM and the organic phases combined, dried over sodium sulfate and concentrated. The crude product was recrystallized in acetone to afford the pure product.

Yield: 69%, yellow solid. ^1H NMR (300 MHz, Chloroform- d) δ 10.66 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.62 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 8.2 Hz, 1H), 6.9 (d, J = 8.8 Hz, 1H), 3.92 (s, 3H), 2.49 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, Chloroform- d) δ 180.23, 155.29, 145.19, 139.07, 135.42, 131.54, 131.14, 130.58, 128.67, 128.44, 112.47, 112.52, 100.79, 69.61, 21.59, 9.62.

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N-(2-acetylphenyl)methanesulfonamide (9.035)

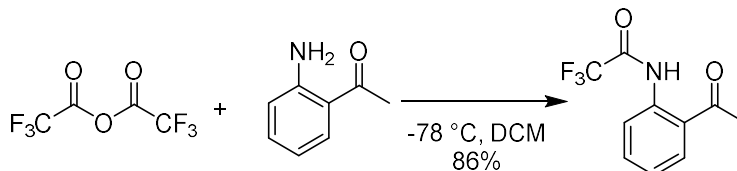


To a solution of 2-acetaniline (1.16 g, 7 mmol) in 20 mL DCM were added pyridine (1.7 mL, 21 mmol) and mesyl chloride (1.2 mL, 15.4 mmol) and stirred overnight. After TLC showed that the reaction had finished, the reaction was quenched with 20 mL of water. The organic phase was dried over sodium sulfate and concentrated. The crude compound was purified by chromatography (PE/EA = 10/1).

Yield solid, 89%, off-yellow solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 11.32 (s, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.73 (dd, J = 8.4, 1.0 Hz, 1H), 2.45 (ddd, J = 8.7, 7.6, 1.5 Hz, 1H), 3.05 (s, 3H), 2.65 (s, 3H).

The characteristic data was corresponding with reported literature.^[11]

N-(2-acetylphenyl)-2,2,2-trifluoroacetamide (9.037)



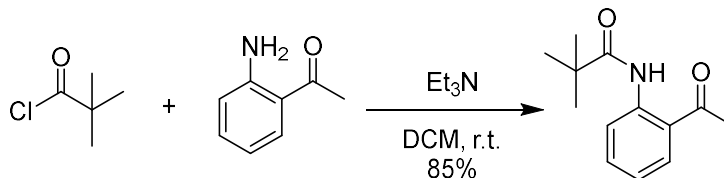
Trifluoroacetic anhydride (0.28 mL, 2 mmol) was added to the mixture of 2-acetaniline (0.27 g, 2 mmol) and triethylamine (0.28 mL, 2 mmol) in 15 mL of DCM under -78 °C. After 1.5 hour, the reaction was complete as indicated by TLC. The reaction was warmed to room temperature and diluted with water and extracted with DCM. The combined organic phase was dried over sodium and concentrated. The crude product was purified by chromatography (PE/EA = 15/1).

Yield: 86%, white solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 13.01 (s, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 2.38 (s, 3H).

The characteristic data was corresponding with reported literature.^[12]

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N-(2-acetylphenyl)pivalamide (9.039)

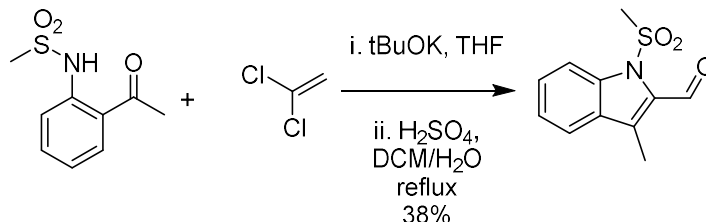


In 20 mL DCM solution of 2-acetylaniline (1.35 g, 10 mmol) and triethylamine (2.8 mL, 20 mmol) with ice bath. Add the Piv chloride and keep the temperature at 0 °C for 1 hour. Then warm to room temperature and keep stirring for 16 h. Then the organic phase was washed with aqueous sodium bicarbonate, dried over sodium sulfate and concentrated by rotary evaporation. The crude product was purified by chromatography (PE/EA = 15/1).

Yield: 85%, off-yellow oil. ^1H NMR (300 MHz, Chloroform- d) δ 8.79 (d, J = 8.4 Hz, 1H), 7.51 (dd, J = 8.4, 1.6 Hz, 1H), 7.08 (dd, J = 8.0, 1.6 Hz, 1H), 2.48 (s, 3H), 1.24 (d, J = 7.0 Hz, 6H).

The characteristic data was corresponding with reported literature.^[13]

3-methyl-1-(methylsulfonyl)-1H-indole-2-carbaldehyde (9.041)



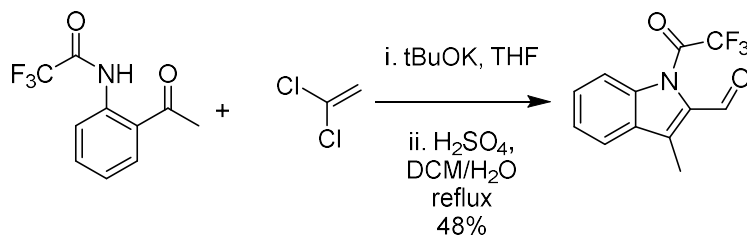
To a solution of mesyl protected 2-acetyl aniline (0.85 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and the reaction was stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous sulfuric acid solution and refluxed for 24 h. After the aqueous layer was extracted with DCM and combined, dried over sodium sulfate and concentrated. The crude product was recrystallized in acetone to afford the pure product.

Yield: 38%, off-yellow solid. ^1H NMR (300 MHz, Chloroform- d) δ 10.63 (s, 1H), 8.23 (dt, J = 8.5, 0.9 Hz, 1H), 7.64 – 7.50 (m, 4H), 7.34 (ddd, J = 8.0, 7.2, 0.9

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Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 2.53 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, Chloroform- d) δ 185.29, 145.28, 137.53, 134.16, 132.99, 132.18, 130.58, 129.77, 129.11, 126.69, 124.62, 121.58, 115.82, 21.59, 10.54.

3-methyl-1-(2,2,2-trifluoroacetyl)-1H-indole-2-carbaldehyde (9.042)



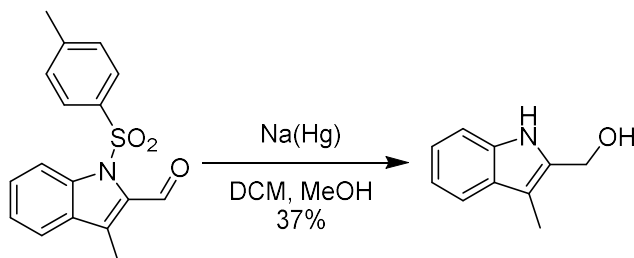
To a solution of mesyl protected 2-acetyl aniline (0.92 g, 4 mmol) in 30 mL of THF were added 1,1-dichloroethylene (0.46 mL, 5.6 mmol) and potassium *tert*-butoxide (1.72 g, 15 mmol) and the reaction was stirred for 90 min. Then, the reaction mixture was neutralized with sulfuric acid to pH equal 7. The aqueous layer was extracted with 20 mL of DCM for 3 times. The organic phase was dried over sodium sulfate and concentrated. The crude product was suspended into 50 mL of DCM and 50 mL of 0.5 M aqueous sulfuric acid solution and refluxed for 24 h. After the aqueous layer was extracted with DCM and the organic phases combined, dried over sodium sulfate and concentrated. The crude product was recrystallized in acetone to afford the pure product.

Yield: 48%, off-white solid. ^1H NMR (300 MHz, Chloroform- d) δ 10.63 (s, 1H), 8.23 (dt, J = 8.5, 0.9 Hz, 1H), 7.64 – 7.50 (m, 4H), 7.34 (ddd, J = 8.0, 7.2, 0.9 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 2.53 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, Chloroform- d) δ 185.29, 145.28, 137.53, 134.16, 132.99, 132.18, 130.58, 129.77, 129.11, 126.69, 124.62, 121.58, 115.82, 21.59, 10.54.

The characteristic data was corresponding with reported literature.^[14]

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(3-methyl-1H-indol-2-yl)methanol (9.045)

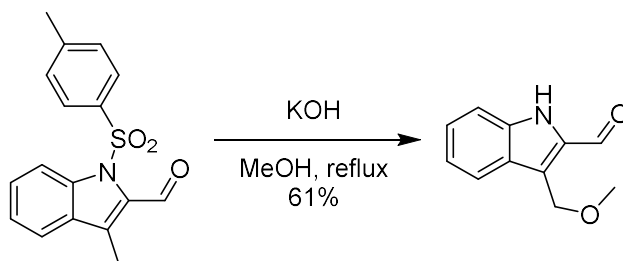


An excess of 2% sodium amalgam (4 g) was added with stirring over the period of 16 hours to 2-formyl-3-methyl-1-phenylsulphonylindole (0.1 g, 0.36 mmol) in the mixture of DCM and methanol. After, the mercury residues were removed by filtration and the filtrate was concentrated and the aqueous solution was extracted with 20 mL of DCM for 3 times. The combined extracts were dried over sodium sulfate and evaporated to give the crude product. Then the crude was purified by chromatography (pure DCM).

Yield: 37%, white solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 8.20 (s, 1H), 7.58 – 7.11 (m, 4H), 4.84 (s, 2H), 2.31 (s, 3H). ^{13}C (75 MHz, Chloroform-*d*) δ 135.9, 132.8, 129.5, 122.2, 119.3, 118.9, 110.4, 107.9, 56.6, 8.48.

The characteristic data was corresponding with reported literature.^[15]

3-(methoxymethyl)-1H-indole-2-carbaldehyde (9.046)



Tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added to powdered potassium hydroxide (0.38 g, 0.68 mmol) in 15 mL of methanol and the mixture was stirred under reflux for 1 hour and then concentrated under reduced pressure. 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

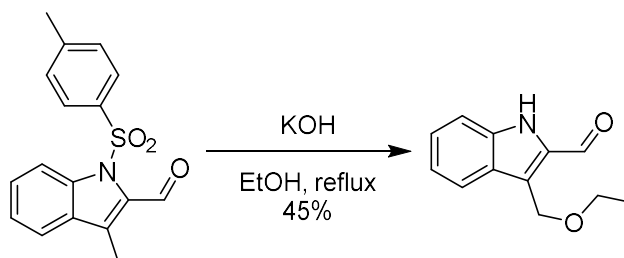
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Yield: 61%, off-white solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 10.08 (s, 1H), 8.93 (s, 1H), 7.73 (dq, J = 8.1, 0.9 Hz, 1H), 7.36 – 7.32 (m, 2H), 7.13 (ddd, J = 8.1, 5.4, 2.6 Hz, 1H), 4.91 (s, 2H), 3.41 (s, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 181.53, 137.02, 132.93, 127.53, 127.12, 123.59, 121.72, 121.19, 112.29, 64.75, 58.35.

HRMS (ESI): m/z calcd for $\text{C}_{11}\text{H}_{11}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 190.08626, found 190.08623.

The characteristic data was corresponding with reported literature.^[15]

3-(ethoxymethyl)-1H-indole-2-carbaldehyde (9.047)



Tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added to powdered potassium hydroxide (0.38 g, 0.68 mmol) in 15 mL of ethanol and the mixture was stirred under reflux for 1 hour and then concentrated under reduced pressure. 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

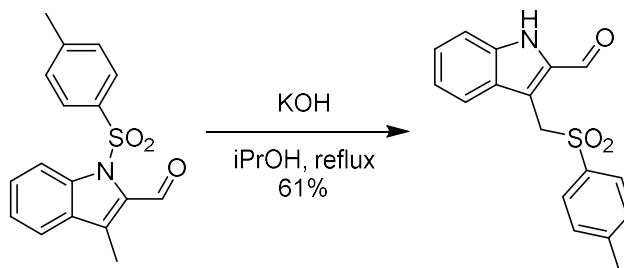
Yield: 73%, off-white amorphous solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 10.19 (s, 1H), 9.20 (s, 1H), 7.81 (dq, J = 8.2, 1.0 Hz, 1H), 7.49 – 7.34 (m, 2H), 7.19 (ddd, J = 8.0, 6.0, 1.9 Hz, 1H), 5.03 (s, 2H), 3.66 (q, J = 7.0 Hz, 2H), 1.29 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 181.91, 127.60, 121.87, 121.19, 112.46, 66.32, 63.12, 15.39.

HRMS (ESI): m/z calcd for $\text{C}_{12}\text{H}_{13}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 204.10191, found 204.10199.

The characteristic data was corresponding with reported literature.^[15]

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3-Tosylmethyl-1H-indole-2-carbaldehyde (9.048)

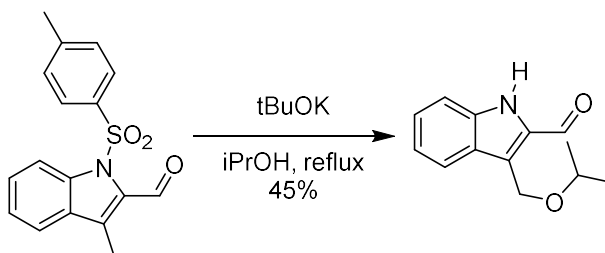


Tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added to powdered potassium hydroxide (0.38 g, 0.68 mmol) in 15 mL of isopropanol and the mixture was stirred under reflux for 1 hour and then concentrated under reduced pressure. 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

Yield: 61%, white amorphous solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 9.55, 8.96, 7.45 (d, J = 1.9 Hz), 7.43 (d, J = 1.8 Hz), 7.41 – 7.36 (m), 7.33 – 7.29 (m), 7.12 (d, J = 8.0 Hz), 7.03 (ddd, J = 8.1, 5.7, 2.2 Hz), 4.73, 2.31. ^{13}C NMR (75 MHz, Chloroform-*d*) δ 180.04, 145.29, 136.50, 134.16, 133.68, 129.74, 128.62, 127.68, 127.63, 121.76, 121.06, 112.86, 112.32, 52.73, 21.61.

HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{15}\text{O}_3\text{N}^{23}\text{Na}^{32}\text{S}$, $[\text{M}+\text{H}]^+$ 336.06649, found 336.06633.

3-isopropoxymethyl-1H-indole-2-carbaldehyde (9.049)



Tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 15 mL of isopropanol and the mixture was stirred under reflux for 1 hour and then concentrated under reduced pressure. 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was

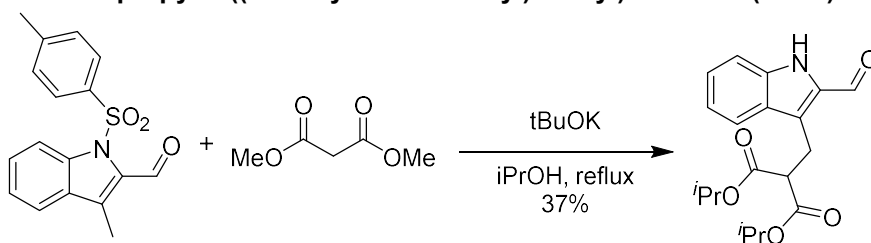
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combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

^1H NMR (300 MHz, Chloroform- d) δ 10.12 (s, 1H), 9.10 (s, 1H), 7.71 (dd, J = 8.2, 1.0 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.10 (ddd, J = 8.1, 6.0, 2.0 Hz, 1H), 4.94 (s, 2H), 3.75 (hept, J = 6.1 Hz, 1H), 1.20 (d, J = 6.1 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 182.00, 145.29, 136.50, 134.16, 133.68, 129.74, 128.62, 121.76, 120.98, 112.33, 71.52, 22.13.

HRMS (ESI): m/z calcd for $\text{C}_{13}\text{H}_{16}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 218.27639, found 218.27597.

Diisopropyl 2-((2-formyl-1H-indol-3-yl)methyl)malonate (9.056)



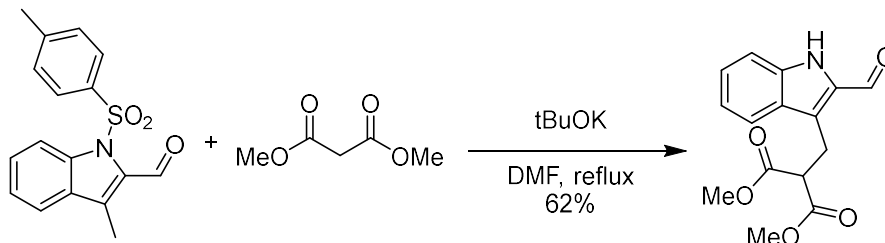
Dimethyl malonate ester (0.05 mL, 0.44 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 15 mL of isopropanol and the mixture was stirred under reflux for 15 min and then tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added and stirred under reflux for another 2 hours. After, 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

^1H NMR (300 MHz, Chloroform- d) δ 10.08 (s, 1H), 8.84 (s, 1H), 7.74 (dd, J = 8.2, 1.0 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.18 (dt, J = 8.1, 4.0 Hz, 1H), 4.99 (dq, J = 12.2, 6.1 Hz, 2H), 4.12 (q, J = 7.2 Hz, 1H), 3.79 – 3.63 (m, 3H), 1.21 (d, J = 6.3 Hz, 7H), 1.13 (d, J = 6.3 Hz, 6H). ^{13}C NMR (75 MHz, Chloroform- d) δ 181.24, 170.19, 143.82, 137.26, 130.42, 130.68, 129.48, 128.62, 121.76, 118.56, 112.33, 56.29, 56.52, 23.70, 22.89.

HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{24}\text{O}_5\text{N}$, $[\text{M}+\text{H}]^+$ 346.16502, found 346.16490.

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Dimethyl 2-((2-formyl-1H-indol-3-yl)methyl)malonate (9.057)

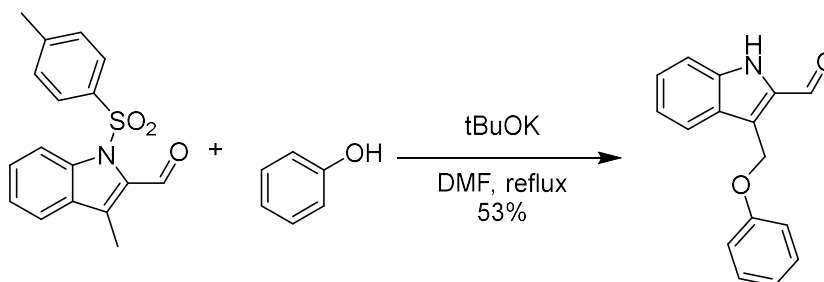


Dimethyl malonate ester (0.05 mL, 0.44 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 8 mL of DMF and the mixture was stirred at room temperature for 15 min and then tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added and stirred for another 2 hours. After, 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

¹H NMR (300 MHz, Chloroform-*d*) δ 10.00 (s, 1H), 8.90 (s, 1H), 7.65 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.11 (ddd, *J* = 8.0, 5.2, 2.7 Hz, 1H), 3.63 (s, 7H), 2.10 (M, 2H). ¹³C NMR (75 MHz, Chloroform-*d*) δ 181.24, 170.19, 143.82, 137.26, 130.42, 130.68, 129.48, 128.62, 121.76, 118.56, 112.33, 56.87, 23.67.

HRMS (ESI): *m/z* calcd for C₁₅H₁₆O₅N, [M+H]⁺ 290.10218, found 290.10230.

3-(phenoxyethyl)-1H-indole-2-carbaldehyde (9.058)



Phenol (0.04 g, 0.43 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 8 mL of DMF and the mixture was stirred at room temperature for 15 min and then tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added and stirred for another 2 hours. After, 20 mL of water was added, and the mixture was extracted with 15 mL of DCM

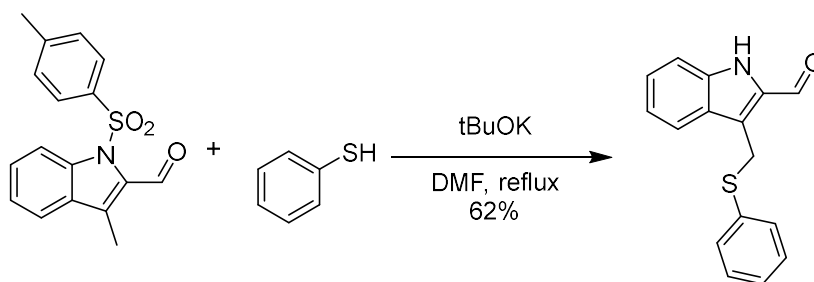
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for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.95 (s, 1H), 8.13 – 8.07 (m, 2H), 7.98 (d, J = 15.4 Hz, 1H), 7.78 – 7.68 (m, 3H), 7.65 – 7.41 (m, 7H), 7.34 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 7.12 (ddd, J = 8.1, 7.0, 1.0 Hz, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 180.24, 158.97, 143.22, 137.29, 130.58, 129.74, 126.48, 121.72, 120.99, 118.36, 117.98, 114.6, 111.8, 62.06.

HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 252.10232 found 252.10269.

3-((phenylthio)methyl)-1H-indole-2-carbaldehyde (9.059)



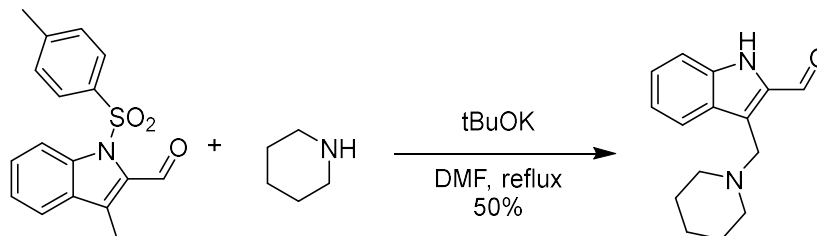
Phenol (0.04 g, 0.43 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 8 mL of DMF and the mixture was stirred at room temperature for 15 min and then tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added and stirred for another 2 hours. After, 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

Yield: 62%, off-yellow solid. ^1H NMR (300 MHz, $\text{Chloroform}-d$) δ 9.41 (s, 1H), 8.78 (s, 1H), 7.73 (dq, J = 8.2, 1.0 Hz, 1H), 7.33 (dq, J = 4.5, 1.4 Hz, 2H), 7.25 – 7.08 (m, 7H), 4.42 (s, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 180.24, 158.97, 143.22, 137.29, 130.58, 129.74, 126.48, 121.72, 120.99, 118.36, 117.98, 114.6, 111.8, 62.06.

HRMS (ESI): m/z calcd for $\text{C}_{15}\text{H}_{17}\text{O}_5\text{N}$, $[\text{M}+\text{H}]^+$ 268.07942, found 267.07988.

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3-(piperidin-1-ylmethyl)-1H-indole-2-carbaldehyde (9.060)

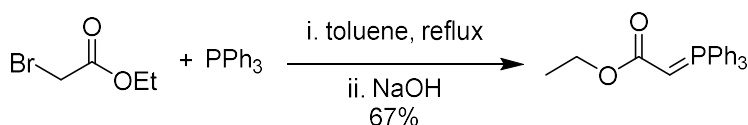


Piperidine (0.05 mL, 0.5 mmol) was added to powdered potassium tertbutoxide (0.76 g, 0.68 mmol) in 8 mL of DMF and the mixture was stirred at room temperature for 15 min and then tosyl protected 3-methylindole-2-aldehyde **9.024** (0.52 g, 0.17 mmol) was added and stirred for another 2 hours. After, 20 mL of water was added, and the mixture was extracted with 15 mL of DCM for 3 times. The organic phase was combined and washed with 10 mL 0.5 M HCl solution and then 20 mL water, dried over sodium sulfate and concentrated. The crude product was purified by chromatography (PE/EA = 8:1).

Yield: 50%, white solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 10.29 (s, 1H), 9.35 (s, 1H), 7.90 (dd, J = 8.2, 1.0 Hz, 1H), 7.49 – 7.33 (m, 2H), 7.18 (ddd, J = 8.1, 6.3, 1.6 Hz, 1H), 3.98 (s, 2H), 2.50 (t, J = 5.3 Hz, 4H), 1.83 – 0.71 (m, 9H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 182.38, 137.24, 133.29, 128.11, 127.25, 125.05, 122.30, 120.59, 112.24, 54.71, 52.67, 26.09, 24.31.

HRMS (ESI): m/z calcd for $\text{C}_{15}\text{H}_{18}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 243.14920, found 243.14919.

Ethyl 2-(triphenyl-15-phosphaneylidene)acetate



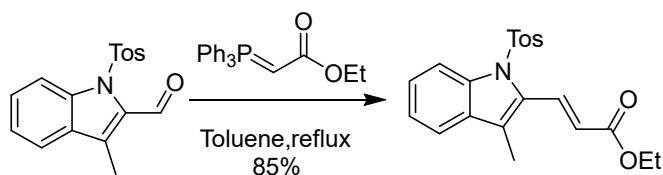
Ethyl bromoacetate (1.67 g, 10 mmol) is added dropwise to a suspension of triphenylphosphine (2.62 g, 10 mmol) in 50 mL toluene and the reaction mixture is stirred 48 hours. The reaction was cooled to room temperature and filtered through a sintered funnel. The precipitate was then washed with toluene and dried under vacuum to afford the corresponding phosphonium salt. The phosphonium salt was suspended into 10 mL of DCM with 10 mL of water. sodium hydroxide saturated solution was added till pH > 14 and stirred overnight. The aqueous layer was then extracted with 10 mL of DCM for 2 times. The organic phase was combined, dried over sodium sulfate and

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concentrated to afford the ylide without further purification.

The characteristic data was corresponding with reported literature.^[16]

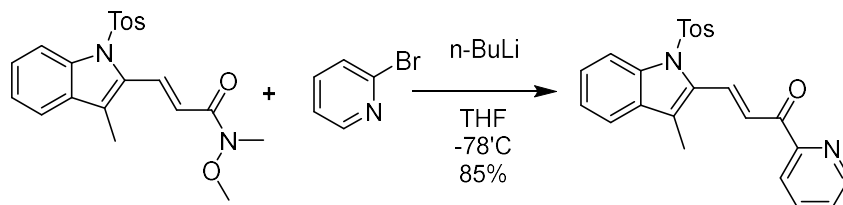
Ethyl -3-(3-methyl-1-tosyl-1H-indol-2-yl)acrylate (9.061)



To a solution of indole aldehyde **9.024** (470 mg, 1.5mmol) in 15mL of freshly distilled toluene was added (Carbethoxymethylene)triphenylphosphorane (522 mg, 1.5mmol) and stirred with refluxing overnight. Solvent was evaporated and the residue was purified by chromatography (PE/EA = 4/1).

Yield: 93%, light yellow amorphous solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.18 – 8.05 (m, 2H), 7.65 (ddt, *J* = 7.4, 6.1, 1.2 Hz, 4H), 7.65 – 7.40 (m, 3H), 7.34 (ddd, *J* = 8.1, 7.2, 1.0 Hz, 1H), 6.30 (d, *J* = 16.0 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 2.32 (d, *J* = 0.8 Hz, 3H), 1.31 (t, *J* = 7.1 Hz, 3H).

3-(3-methyl-1-tosyl-1H-indol-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one (9.063)



At -78 °C, to a solution of 2-bromopyridine (119 mg, 0.75 mmol, 1.5 eq.) in 10 mL of freshly distilled THF was slowly added *n*-BuLi (2.5 M in hexanes, 0.3 mL, 1.5eq.) and the reaction was stirred for 1 h. Indole weinreb amide **9.061** (199 mg, 0.5 mmol, 1 eq.) in 2 mL THF was added drop-wise to the reaction and it was stirred for another 1 hour. After, saturated NH₄Cl solution was added then diluted with 20mL of EtOAc. The organic phase was washed with brine dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 1/1).

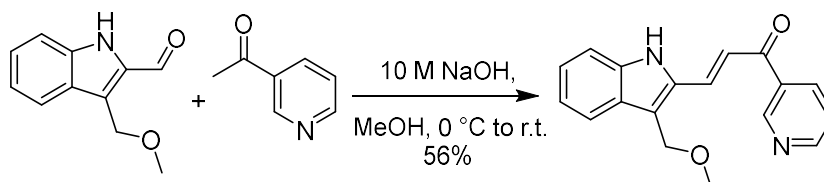
Yield: 80%, light yellow amorphous solid. ¹H NMR (300 MHz, DMSO) δ 8.84 (d, *J* = 4.7 Hz, 1H), 8.42 (s, 1H), 8.36 (s, 1H), 8.23 – 7.95 (m, 4H), 7.81 – 7.63 (m, 2H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.54 – 7.44 (m, 1H), 7.38 (d, *J* = 7.6 Hz, 1H),

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7.31 (d, $J = 8.2$ Hz, 2H), 2.45 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 188.65, 153.71, 149.83, 145.99, 138.35, 137.10, 134.29, 133.23, 132.72, 131.49, 130.62, 128.28, 127.62, 126.73, 125.23, 125.15, 124.96, 123.15, 121.13, 115.38, 21.44, 11.47.

HRMS (ESI): m/z calcd for $\text{C}_{24}\text{H}_{20}\text{O}_3\text{N}_2\text{S}$, $[\text{M}+\text{H}]^+$ 417.12674, found 417.12665.

(E)-3-(3-(methoxymethyl)-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.064)



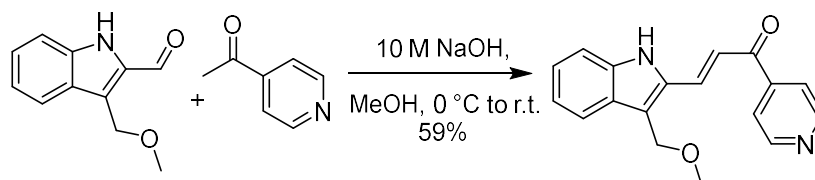
3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.1 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 3-acetylpyridine (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for two more hours. After, 30 mL of water were added, and the aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 2/1).

Yield: 63%, bright yellow amorphous solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.75 (s, 1H), 9.35 – 9.26 (m, 1H), 8.86 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.40 (dd, $J = 8.0, 2.0$ Hz, 1H), 7.93 (s, 2H), 7.73 – 7.63 (m, 2H), 7.45 (d, $J = 8.2$ Hz, 1H), 7.38 – 7.21 (m, 1H), 7.09 (ddd, $J = 8.0, 6.9, 1.0$ Hz, 1H). ^{13}C NMR (75 MHz, $\text{Chloroform}-d$) δ 206.51, 187.49, 153.35, 149.37, 137.45, 135.60, 133.11, 132.52, 131.70, 127.95, 125.09, 124.12, 119.98, 119.38, 118.99, 111.50, 63.63, 57.12.

HRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{16}\text{O}_2\text{N}_2$, $[\text{M}+\text{H}]^+$ 293.12845, found 293.12831.

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(E)-3-(3-(methoxymethyl)-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.065)

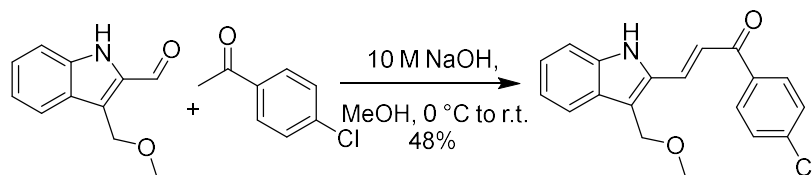


3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.1 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hours. After, 30 mL of water were added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 2/1).

bright yellow amorphous solid, yield 71%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.81 (s, 1H), 8.95 – 8.86 (m, 2H), 8.01 – 7.89 (m, 3H), 7.86 (d, *J* = 15.5 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.46 (dt, *J* = 8.2, 1.0 Hz, 1H), 7.30 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 7.10 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.78 (s, 2H), 3.29 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 202.12, 188.74, 151.49, 144.65, 138.25, 133.18, 128.74, 125.83, 121.87, 120.63, 120.57, 120.15, 119.57, 112.13, 64.23, 57.73.

HRMS (ESI): *m/z* calcd for C₁₈H₁₆O₂N₂, [M+H]⁺ 293.12845, found 293.12831.

(E)-3-(3-(methoxymethyl)-1H-indol-2-yl)-1-phenylprop-2-en-1-one (9.066)



3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.1 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. Acetophenone (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hours. After, 30 mL of water was added, and the aqueous layer was extracted with 15 mL of dichloromethane

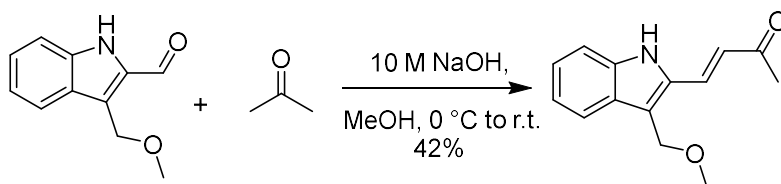
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for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 2/1).

Yield: 48%, bright yellow solid amorphous solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.75 (s, 1H), 8.15 – 8.05 (m, 2H), 7.89 (s, 2H), 7.76 – 7.63 (m, 3H), 7.43 (dt, J = 8.2, 1.0 Hz, 1H), 7.27 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.07 (ddd, J = 8.0, 6.9, 1.0 Hz, 1H), 4.75 (s, 2H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 187.63, 138.52, 137.83, 136.95, 133.06, 131.99, 130.48, 129.51, 128.40, 125.40, 120.36, 119.72, 119.10, 111.91, 64.08, 57.55.

HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{16}\text{O}_2\text{NCl}$, $[\text{M}+\text{H}]^+$ 325.79263, found 325.79271.

(E)-4-(3-(methoxymethyl)-1H-indol-2-yl)but-3-en-2-one (9.067)



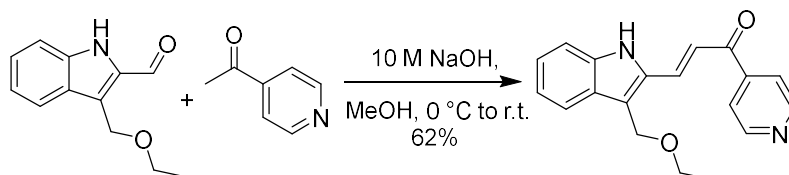
3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.1 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. Acetone (0.05 mL, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature and stirred for two more hour. After, 30 mL of water were added, and the aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 4/1).

Yield: 42%, light yellow solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.75 (s, 1H), 8.15 – 8.06 (m, 2H), 7.75 – 7.64 (m, 3H), 7.43 (dt, J = 8.2, 0.9 Hz, 1H), 7.27 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.07 (ddd, J = 8.0, 6.9, 1.0 Hz, 1H), 4.75 (s, 2H), 3.28 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 187.63, 138.52, 137.83, 136.95, 133.06, 131.99, 130.48, 129.51, 128.40, 125.40, 120.36, 119.72, 119.10, 111.91, 64.08, 57.55.

HRMS (ESI): m/z calcd for $\text{C}_{14}\text{H}_{15}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 229.97263, found 229.97268.

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(E)-3-(3-(ethoxymethyl)-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.071)

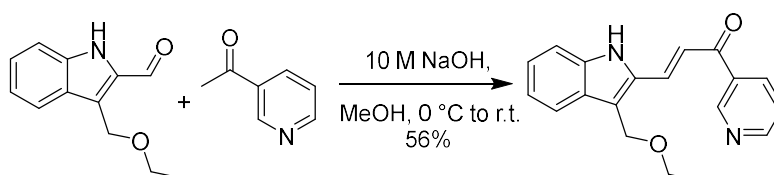


3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.10 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.06 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature for two more hour. After, 30 mL of water were added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 62%, bright orange solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.71 (s, 1H), 9.42 – 9.15 (m, 1H), 9.00 – 8.74 (m, 1H), 8.38 (ddd, J = 8.0, 2.3, 1.7 Hz, 1H), 7.92 (d, J = 2.2 Hz, 2H), 7.71 – 7.63 (m, 2H), 7.43 (dt, J = 8.2, 0.9 Hz, 1H), 7.27 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.07 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 4.80 (s, 2H), 3.50 (qd, J = 7.0, 1.8 Hz, 2H), 1.12 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.74, 151.33, 144.42, 138.03, 133.23, 132.63, 128.30, 125.68, 121.74, 120.92 – 119.94 (m), 119.43, 111.98, 65.11, 62.31, 15.62.

HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{19}\text{O}_2\text{N}_2$, $[\text{M}+\text{H}]^+$ 307.14410, found 307.14396.

(E)-3-(3-(ethoxymethyl)-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.072)



3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.10 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.06 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hour. After, 30 mL of water were

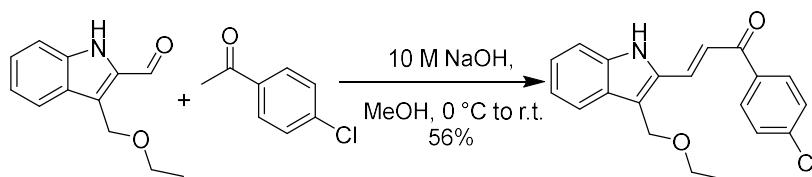
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added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 56%, bright orange solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.76 (s, 1H), 8.96 – 8.83 (m, 2H), 7.97 (s, 0H), 7.94 – 7.87 (m, 3H), 7.81 (d, J = 15.5 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.43 (dt, J = 8.2, 1.0 Hz, 1H), 7.28 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H), 7.07 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 4.79 (s, 2H), 3.50 (q, J = 7.0 Hz, 2H), 1.12 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.74, 151.33, 144.42, 138.03, 133.23, 132.63, 128.30, 125.68, 121.74, 120.92, 119.94, 119.43, 111.98, 65.11, 62.31, 15.62.

HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{19}\text{O}_2\text{N}_2$, $[\text{M}+\text{H}]^+$ 307.14410, found 307.14396.

(E)-1-(4-chlorophenyl)-3-(3-(ethoxymethyl)-1H-indol-2-yl)prop-2-en-1-one (9.069)



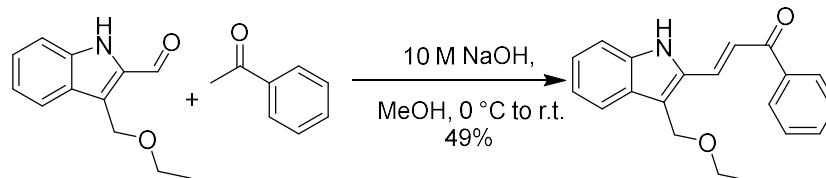
3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.10 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-chloro-1-acetylphenone (0.09 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hours. After, 30 mL of water were added, and the aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 56%, bright-yellow solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.74 (s, 1H), 8.19 – 8.01 (m, 2H), 7.91 (s, 2H), 7.71 (t, J = 8.2 Hz, 3H), 7.44 (d, J = 8.2 Hz, 1H), 7.28 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 4.80 (s, 2H), 3.51 (q, J = 7.0 Hz, 2H), 1.26 – 1.02 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 187.67, 138.50, 137.85, 132.86, 132.10, 130.48, 129.51, 128.35, 125.40, 120.41, 119.64, 119.54, 111.89, 65.05, 62.26, 15.63.

HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{NCl}$, $[\text{M}+\text{H}]^+$ 339.81927, found 339.81919.

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(E)-3-(3-(ethoxymethyl)-1H-indol-2-yl)-1-phenylprop-2-en-1-one (9.070)

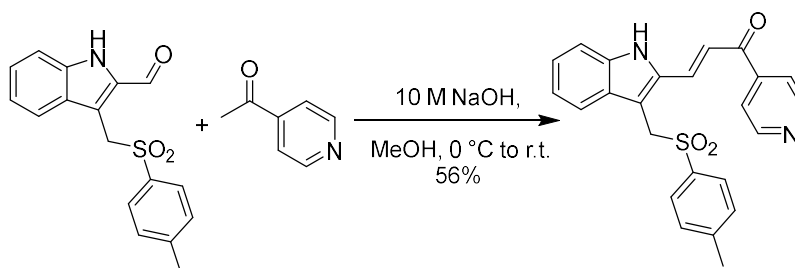


3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.10 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. Acetylphenone (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature for two more hour. After, 30 mL of water were added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 49%, yellow solid. ^1H NMR (300 MHz, Chloroform-*d*) δ 7.74 – 7.68 (m, 1H), 7.61 – 7.51 (m, 1H), 7.49 – 7.20 (m, 2H), 7.11 (ddd, J = 8.0, 6.8, 1.2 Hz, 1H), 4.80 (s, 2H), 3.59 (p, J = 7.0 Hz, 2H), 1.36 – 1.08 (m, 5H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 190.11, 138.18, 137.42, 132.85, 132.14, 129.32 – 127.86 (m), 125.40, 120.64, 120.34, 119.71, 119.59, 111.17, 65.70, 62.74, 15.32.

HRMS (ESI): m/z calcd for $\text{C}_{20}\text{H}_{19}\text{O}_2\text{N}$, $[\text{M}+\text{H}]^+$ 305.37770, found 305.37762.

(E)-1-(pyridin-4-yl)-3-(3-(tosylmethyl)-1H-indol-2-yl)prop-2-en-1-one (9.074)



3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.16 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature for two more hour. After, 30 mL of water were added, and

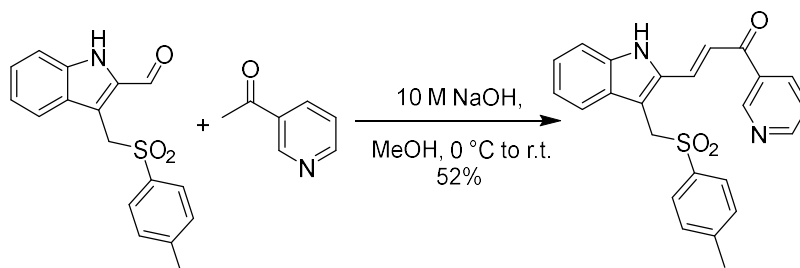
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the aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 56%, brown-yellow solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.83 (s, 1H), 9.05 – 8.78 (m, 2H), 8.03 – 7.78 (m, 2H), 7.73 – 7.36 (m, 6H), 7.28 (dd, J = 7.6, 5.5 Hz, 3H), 7.01 (t, J = 7.4 Hz, 1H), 5.05 (s, 2H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.34, 151.36, 144.27, 137.76, 133.16, 130.08, 128.60, 125.61, 121.76, 120.94, 120.37, 119.77, 111.86, 110.21, 52.47, 31.17, 21.39.

HRMS (ESI): m/z calcd for $\text{C}_{24}\text{H}_{21}\text{O}_3\text{N}_2\text{S}$, $[\text{M}+\text{H}]^+$ 417.12674, found 417.12665.

(E)-1-(pyridin-3-yl)-3-(3-(tosylmethyl)-1H-indol-2-yl)prop-2-en-1-one (9.075)



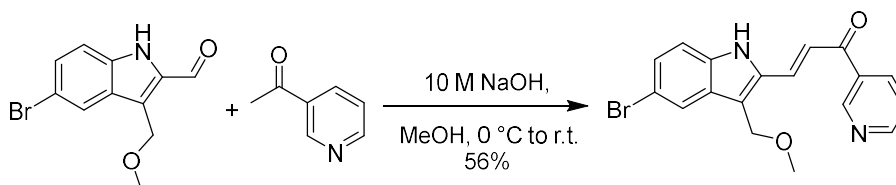
3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.16 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hour. After, 30 mL of water were added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

Yield: 52%, yellow solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.66 (s, 1H), 8.96 – 8.84 (m, 2H), 8.03 (d, J = 15.6 Hz, 1H), 7.95 – 7.87 (m, 2H), 7.81 (s, 1H), 7.78 – 7.67 (m, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.26 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 5.04 (s, 2H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.34, 151.36, 144.27, 137.76, 133.16, 130.08, 128.60, 125.61, 121.76, 120.94, 120.37, 119.77, 111.86, 110.21, 52.47, 31.17, 21.39.

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HRMS (ESI): m/z calcd for $C_{24}H_{21}O_3N_2S$, $[M+H]^+$ 417.12674, found 417.12665.

(E)-3-(5-bromo-3-ethoxy-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.076)

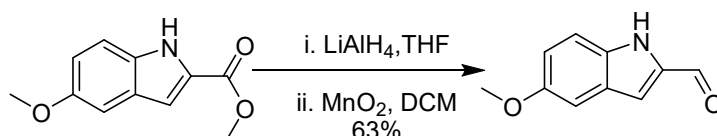


3 mL of 10 M aqueous sodium hydroxide solution was added to a solution of indole aldehyde (0.13 g, 0.5 mmol) in 10 mL of methanol and the mixture was cooled to 0 °C. 4-acetylpyridine (0.07 g, 0.6 mmol) in 3 mL of methanol was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and raised to room temperature, and stirred for two more hour. After, 30 mL of water were added, and aqueous layer was extracted with 15 mL of dichloromethane for 3 times. The organic layer was washed with brine and dried over sodium sulfate and concentrated by rotary evaporation. The crude compound was purified by column (PE/EA = 1/1).

1H NMR (300 MHz, $DMSO-d_6$) δ 11.99 (s, 1H), 8.90 (d, J = 8.2 Hz, 2H), 7.94 – 7.86 (m, 5H), 7.43 – 7.35 (m, 2H), 4.77 (s, 3H), 3.34 (s, 5H). ^{13}C NMR (75 MHz, $DMSO-d_6$) δ 188.71, 151.38, 144.19, 136.52, 134.01, 132.61, 130.05, 127.95, 122.71, 121.76, 120.56, 119.00, 114.08, 112.93, 63.89, 57.62.

HRMS (ESI): m/z calcd for $C_{18}H_{16}O_2N_2^{79}Br$, $[M+H]^+$ 371.03886, found 371.03897.

5-methoxy-1H-indole-2-carbaldehyde (9.077)



5-methoxy-indole-2-methylester (1.06 g, 4.83 mmol) in 30 mL of THF was stirred at 0 °C. $LiAlH_4$ (0.38 g, 10 mmol) was added in one portion and stirred 1 hour before the solvent was evaporated in vacuo, The residue was carefully quenched with ice-cold water and then 0.5 M HCl and then extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate and

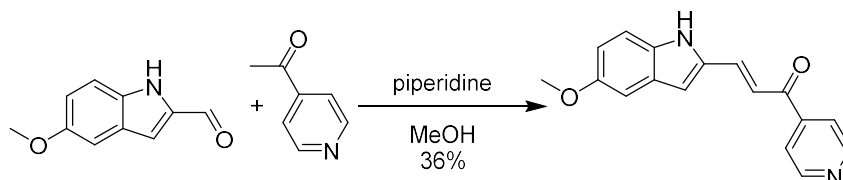
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concentrated. The crude product was re-dissolved into DCM with MnO₂ (2.10 g, 25 mmol) for 48 hours. The residue was filtered through a silica pad and the organic solvent was concentrated by rotary evaporation.

Yield: 63%, yellow solid. ¹H NMR (300 MHz, Chloroform-*d*) δ 10.17 (s, 1H), 7.60 (d, *J* = 2.6 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 6.81 (dd, *J* = 8.8, 2.6 Hz, 1H), 5.72 (t, *J* = 5.6 Hz, 1H), 4.96 (d, *J* = 5.6 Hz, 2H), 3.77 (s, 3H). ¹³C NMR (75 MHz, Chloroform-*d*) δ 184.2, 155.6, 151.2, 130.2, 126.4, 112.8, 112.5, 112.3, 102.5, 55.3, 55.2.

The characteristic data was corresponding with reported literature.^[17]

(E)-3-(5-methoxy-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.078)



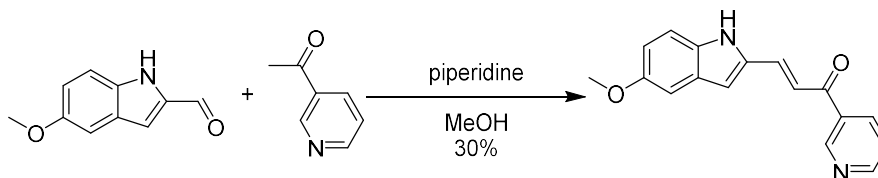
5-methoxy-indole-2-carbaldehyde **9.077** (0.09 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol. 4-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 36%, brown solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.65 (s, 1H), 8.93 – 8.86 (m, 2H), 7.95 – 7.89 (m, 2H), 7.77 (s, 2H), 7.35 (d, *J* = 8.9 Hz, 1H), 7.09 (d, *J* = 2.5 Hz, 1H), 7.04 (dd, *J* = 2.1, 0.9 Hz, 1H), 6.92 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.78 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 188.90, 154.49, 151.43, 148.16, 144.29, 136.31, 135.27, 134.41, 128.98, 121.90, 118.98, 116.61, 113.10, 110.92, 102.37, 55.85.

HRMS (ESI): *m/z* calcd for C₁₇H₁₅O₂N₂, [M+H]⁺ 279.11280, found 279.11268.

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(E)-3-(5-methoxy-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.079)

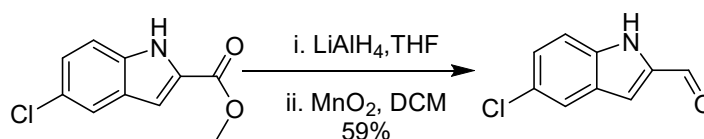


5-methoxy-indole-2-carbaldehyde **9.077** (0.09 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol. 3-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 30%, brown solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.59 (s, 1H), 9.27 (dd, $J = 2.3, 0.9$ Hz, 1H), 8.84 (dd, $J = 4.8, 1.7$ Hz, 1H), 8.38 (dt, $J = 8.0, 2.0$ Hz, 1H), 7.91 – 7.72 (m, 2H), 7.64 (ddd, $J = 8.0, 4.8, 0.9$ Hz, 1H), 7.35 (d, $J = 8.9$ Hz, 1H), 7.08 (d, $J = 2.4$ Hz, 1H), 7.01 (dd, $J = 2.0, 0.9$ Hz, 1H), 6.90 (dd, $J = 8.9, 2.5$ Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 188.06, 154.31, 153.63, 149.76, 136.03, 135.24, 134.15, 133.62, 128.87, 124.49, 119.20, 116.22, 112.88, 110.33, 102.28, 55.72.

HRMS (ESI): m/z calcd for $\text{C}_{17}\text{H}_{15}\text{O}_2\text{N}_2$, $[\text{M}+\text{H}]^+$ 279.11280, found 279.11268.

5-chloro-1H-indole-2-carbaldehyde (9.081)



5-methoxy-indole-2-methylester (1.06 g, 4.83 mmol) in 30 mL of THF was stirred at 0 °C. LiAlH_4 (0.38 g, 10 mmol) was added in one portion and stirred 1 hour before the solvent was evaporated in vacuo. The residue was carefully quenched with ice-cold water and then 0.5 M HCl and then extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate and concentrated. The crude product was re-dissolved into DCM with MnO_2 (2.10 g, 25 mmol) for 48 hours. The residue was filtered through a silica pad and the organic solvent was concentrated by rotary evaporation.

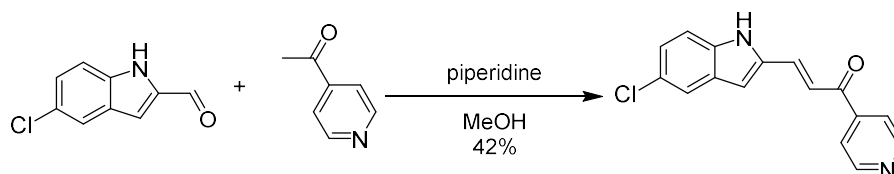
Yield: 59%, yellow solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.86 (s, 1H), 9.09 (s,

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1H), 7.73 (m, 1H), 7.39 (d, $J = 8.8$ Hz, 1H), 7.35 (dd, $J = 8.8, 1.8$ Hz, 1H), 7.22 (m, 1H).

The characteristic data was corresponding with reported literature.^[18]

(E)-3-(5-chloro-1H-indol-2-yl)-1-(pyridin-4-yl)prop-2-en-1-one (9.082)

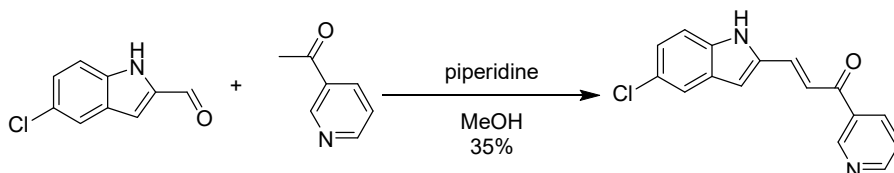


5-chloro-indole-2-carbaldehyde **9.081** (0.09 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol. 4-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium bicarbonate solution and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 35%, yellow-brown solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.95 (s, 1H), 8.95 – 8.86 (m, 2H), 7.95 – 7.89 (m, 2H), 7.82 (d, $J = 2.2$ Hz, 2H), 7.69 (d, $J = 2.1$ Hz, 1H), 7.52 – 7.44 (m, 1H), 7.25 (dd, $J = 8.7, 2.1$ Hz, 1H), 7.10 (dd, $J = 2.1, 0.9$ Hz, 1H). ¹³C NMR (75 MHz, Chloroform-*d*) δ 193.69, 156.08, 148.99, 141.89, 141.00, 140.37, 134.21, 129.71, 126.55, 125.60, 125.21, 118.43, 114.81.

HRMS (ESI): m/z calcd for C₁₆H₁₁ON₂Cl, [M+H]⁺ 282.05586, found 282.05579.

(E)-3-(5-chloro-1H-indol-2-yl)-1-(pyridin-3-yl)prop-2-en-1-one (9.083)



5-chloro-indole-2-carbaldehyde **9.081** (0.09 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol. 3-Acetylpyridine (0.07 g, 0.6 mmol) and piperidine (0.06 g, 0.6 mmol) were added and the reaction mixture was heated at reflux overnight. After TLC showed no starting material, 25 mL of saturated sodium

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bicarbonate solution was added and extracted with 20 mL of DCM for 3 times. The combined organic phase was washed with brine and dried over sodium sulfate, and concentrated by rotary evaporation. The crude compound was purified by chromatography (PE/EA = 4:1 to 2:1) to afford the pure compound.

Yield: 35%, yellow-brown solid. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.90 (s, 1H), 9.33 – 9.26 (m, 1H), 8.85 (dd, J = 4.8, 1.7 Hz, 1H), 8.40 (dt, J = 8.0, 2.0 Hz, 1H), 7.98 – 7.75 (m, 2H), 7.69 (d, J = 2.3 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.52 – 7.42 (m, 1H), 7.24 (dd, J = 8.7, 2.1 Hz, 1H), 7.13 – 7.05 (m, 1H). ^{13}C NMR (75 MHz, Chloroform- d) δ 192.93, 156.08, 155.23, 148.99, 141.37, 140.37, 134.21, 129.71, 126.55, 125.60, 125.21, 118.43, 114.81.

HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{11}\text{ON}_2\text{Cl}$, $[\text{M}+\text{H}]^+$ 282.05586, found 282.05579.

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