New DNA Based hybrid catalysts

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Abstract

DNA owing to its unique chiral structure offers the possibility to be utilized as catalyst. Combined with other elements it forms the basis for the formation of hybrid catalysts. In present study, group of DNA based hybrid catalysts incorporating Copper and Platinum have been developed and presented for various reactions. Reactions are found to have high enantioselectivities. NMR studies conducted showed the effectivity of catalysts developed.

Keywords: DNA, hybrid, NMR
Dedication

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Chapter 1
Literature Survey
Enzyme catalysis is known since long to have high activities and selectivities, achieved under mild conditions (such as in water) owing to their chirality. However, enzyme catalysts are limited by their synthesis and technology. The emerging field of “hybrid catalysts” addresses this problem by combining the catalytic power of transition metal catalysis with the chiral architectures and characteristics of biopolymers such as proteins and RNA. Only recently (eight years ago), the double helix of DNA has been shown to have activity as chiral environment. Considerable progress has been made in the development and processing of these catalysts since then. In this chapter, different concepts of DNA chemistry, how it reacts with other substances and progress in DNA based hybrid catalysts will be reviewed and discussed. Furthermore, an outline of the current work as described in this thesis is provided.
1. Introduction

**Deoxyribonucleic acid (DNA)** is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms. DNA is a nucleic acid, which comprises one of three major macromolecules essential for all known forms of life. DNA molecules are double-stranded helices, consisting of two long biopolymers made of simpler units called nucleotides—each nucleotide is composed of a nucleobase (guanine, adenine, thymine, and cytosine), recorded using the letters G, A, T, and C, and a backbone made of alternating sugars (deoxyribose) and phosphate groups. Nucleobases (G, A, T, C) are attached to the sugars. [1].

![Fig – 1: DNA double strand helix (used with permission)](image)

DNA is a promising candidate as a source of chirality in asymmetric analysis in many respects.

It is the fundamental hereditary material of living organisms and is widely distributed in the natural world. Studies of DNA have already greatly contributed to the progress of life science, in which the discovery of the DNA double helical structure more than 50 years ago had established the foundation of molecular biology, making scientists able to understand life on molecular level in the genome. Based on the knowledge about genetic code, DNA has been used in genetic engineering, forensics, phylogenies, history and anthropology.
More recently, the use of DNA as a bio macromolecule has been attracting additional attention of researchers in diverse areas of science. As a result, DNA is regarded as an ideal macromolecule for creating new functional materials. Utilization of DNA has become a lively field in science and its attracting more and more attention from scientists and engineers. Most of these investigations seem to rely on a few fundamental properties of DNA that relate to the famous double helical structure. The most important applications of DNA based upon double helical structure by focusing on three basic features of natural DNA molecule being polyelectrolyte, having selective affinity for small molecules and its biocompatibility as shown in Fig – 2.

![Fig – 2: Basic feature of natural DNA](image)

Among these characteristics, the **electrostatic property** of DNA as highly charged polyelectrolyte is most important, because it has been widely used for exploring various DNA based applications. A DNA molecule consists of two polynucleotide strands coiled around each other in a helical fashion, with a diameter of approximately 2 nm. Moreover, the double helix chains of DNA are negatively charged by the phosphate groups that are regularly arranged in the two back bones. Therefore, DNA is an ideal template to fabricate highly ordered nanostructures by binding cationic agents such as metal ions, cationic surfactants and polycationic agents. The **second feature** of DNA is its selective affinity for
small molecules. The most common DNA structure is the B–DNA type in which the stacked bases are regularly spaced 0.34 nm along the helix axis, and the helical structure possesses a wide major groove and a narrow minor groove of approximately same depth. Some small molecules can intercalate into the spaces between the stacked bases, or bind in the grooves between the two backbones. Both of the interaction patterns are highly selective towards the structure of small molecules. By this special affinity, DNA can be used as an environmental material to selectively remove toxic pollutants or as a template to arrange functional molecules. Thirdly, DNA is perfectly biocompatible, as it can be found in almost all living organisms. This offers DNA excellent prospects for serving as biomaterials.

The development of DNA utilization depends not only on the excellent properties but also on its availability. Currently, various DNAs with a designed sequence can be synthesized by automated synthesis systems and amplifies by the PCR method. However, such synthetic DNA is still expensive and not easy to produce in large quantities, therefore laboratories usually use it at micrograms or milligrams level. On the other hand, other type of DNA that is extracted from natural products, referred to natural DNA has a large potential for mass production. In fact, it has had commercial products on sale by a few companies, and can be supplied by the ton. As a substance widely existing in organisms, natural DNA is plentiful in the natural world. A typical case is salmon milt in fishery, which is often used for livestock feedstuff. The salmon milt contains over 10% of dry weight DNA and its manufacture is very facile. Because salmon fishery is an increasing industry with a worldwide supply exceeding 2.4 million tons/year. It is estimated that about 3000 tons of salmon DNA is available/year. Other resources include berring milt, scallop testis, and even artificial microorganisms produced by biological methods. Moreover, DNA can be efficiently extracted from animal tissues that have a high nucleas to cytoplasmic mass ratio, such as thymus gland and spleen, Undoubtedly, such facile and renewable resources will effectively promote further research of DNA.

Research on natural DNA, including marine DNA mentioned above, the calf thymus DNA and various microbial DNA such as bacteriophage λ – DNA, circular plasmid pBR322 DNA and others is the subject of most investigations. These studies prove that parameters of molecular weight, purity, molecular shape (linear of circular) content of Guanine (G) and cytosine © bases even the content
of single strand DNA (a byproduct in manufacture) are more important than the base sequence of DNA.

2. Helical anionic polynucleotide backbone

Compared to other anionic polymers, DNA is distinguished by the highly charged double helical structure, which shows local stiffness in a range of about 50 nm but long range flexibility in water. These characteristics make DNA feasible to create precisely ordered materials. Many novel materials consisting of natural DNA and various cationic substances, including metal ions, nanoparticles, proteins, surfactants and polycationic macromolecules have been reported recently. These are classified into four categories as shown in fig 3 below.

**Fig – 3:** Schematic representation of the structures of DNA/cation complexes. (a) The complex of linear DNA molecules with cationic ions or nanoparticles. (b) DNA condensation by multivalent cations resulting in a toroidal structure. (c) DNA–cationic surfactant complex. (d) DNA–cationic polymer complex formed by electrostatic layer-by-layer (LbL) deposition [used with permission of Royal Society for Chemistry, 2014]

In dilute solutions (< 1 mg ml⁻¹), DNA forms wormlike coils. However, the DNA molecules in dilute solution can be easily stretched to linear templates that can lead to ordered nanostructures Fig. 3(a). As the DNA is concentrated (> 1 mg ml⁻¹), the molecules spontaneously undergo unidirectional ordering and transform into liquid crystals of the ‘cholesteric’ type. In the presence of multivalent cations
such as polyamines, DNA undergoes a dramatic condensation to a compact, usually highly ordered toroidal structure Fig. 3(b). When DNA reacts with cationic surfactants such as hexadecyltrimethylammonium chloride, a precipitate is formed, producing a complex that is soluble in common organic solvents, and thus can be easily cast to thin films. The conformation of the DNA–surfactant complex is controllable and often locally ordered Fig. 3(c). Like other anionic polymers, DNA reacts with polycationic macromolecules, thus by some common protocols such as the process of electrostatic layer by layer (LbL) deposition, DNA can be made into many useful materials. Fig. 3(d).

3. Intercalation and groove binding

Besides the electrostatic interaction with anionic DNA, small molecules can interact with DNA in two other ways: groove binding and intercalation, both excellently selective. As implied by their names, some molecules shaped like the DNA groove can adhere to the edges of base pairs in both the major and minor grooves, and molecules with a planar structure are able to intercalate into the spaces between stacked base pairs. Such selective affinity for small molecules has been exploited for various applications.

3.1 Removing harmful substances with DNA

Generally, compounds that can interact with DNA are often toxic or carcinogenic. For example, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and some endocrine disruptors, are significant pollutants generated from incomplete combustion, often found in coal tar, automobile exhaust, or cigarette smoke. They are known or suspected to exhibit strong carcinogenicity. For removing such harmful chemicals before they interact with intracellular DNA, natural DNA has been considered as a favorable candidate for an adsorbent.
3.2 Applying DNA in stereochemistry
DNA is chiral as a result of the asymmetric centers in the ribose units and due to its helix structure. Therefore, DNA is an attractive material for use in stereochemistry. Natural DNA has been investigated for enantioselection because of the crucial importance of enantioselection in various fields such as drug and food analysis, biochemistry or clinical pharmacology [2]. Owing to this structure, it has been promoted as catalyst in many reactions. This novel concept of DNA – based asymmetric catalysis was introduced only eight years ago by Feringa and Roelfes in the form of copper (II) – catalyzed Diels – Alder reaction. They produced novel DNA based asymmetric catalysis on the basis of supramolecular assembly by using a copper complex of a non-chiral ligand that can bind to DNA achieving very high enantioselectivities of upto 99%. Since then, DNA hybrid catalysts have been successfully applied to various asymmetric carbon – carbon or carbon – heteroatom bond forming reactions. Such high enantioselectivity is attributed to a catalytically active copper complex anchored on the DNA molecule through a 2,2-bipyridine unit intercalating into the DNA double helix. It was reported that the DNA is the source of chirality in the Cu(II) catalyzed Diels–Alder reactions. When the Diels–Alder reaction was catalyzed by the Cu complex alone without DNA, both endo and exo cycloaddition products were formed. Although there is a preponderance of the endo product is of, both essentially were generated as racemic mixtures. As the active Cu(II) center is brought into proximity of the chiral environment of the DNA double helix, it allows for a transfer of chirality from DNA to the reaction product. The reactions performed in water allowed virtually complete regioselectivity (up to 99% endo) and excellent enantioselectivity (up to 99% ee). In following studies, a high enantioselectivity was also found in the Diels–Alder reaction of a,b-unsaturated-2-acyl imidazoles and a electrophilic fluorination of beta-keto esters. [2] Three years ago, same group reported first non – enzymatic catalytic enantioselective hydration of enones using a DNA hybrid catalyst and demonstrated remarkable potential of this field beyond traditional metal ligand based catalysts. It has also been shown recently that enantiomeric preference could be controlled by changing the binding ligands even if archetypal B – DNA is used only. That’s how opposite enantiomer of product can be obtained without replacing right hand B – DNA with left – hand DNA. [3]

4. Biocompatibility

Natural DNA has several suitable characteristics for biomaterials. Biocompatibility with humans is the primary advantage of natural DNA in
biomedical applications. Because the molecular structure of DNA in vertebrate species is homogenous, unlike other biopolymers such as proteins and sugars, it has no or low immunogenic properties, so that may limit both innate and acquired immune responses in the human body. Second, DNA has the advantage in its capability of binding small molecules, which means that some pharmacological molecules can be attached to DNA via electrostatic interaction, groove binding and intercalation. This characteristic has made DNA-based materials beneficial for loading drugs. Third, DNA is degradable in the human body, thus greatly increasing its serviceability for medical devices and controlled drug-release systems. Actually, natural DNA combined with other materials has been tried for several medical applications, including improved compatibility of medical devices, dental and medical implantology, and cell cultures [2].

For example, DNA has been used to modify PSf membranes by blending or immobilizing DNA on its surface. Blood compatibility is a common problem of PSf when it is used as a hollow fiber in hemodialysis. However, by combining with salmon DNA, it was reported that the surface of the hybrid membrane became more hydrophilic. DNA coatings to improve the biocompatibility of biomaterial surfaces has also been explored by fabricating multilayered DNA-coatings by LbL self-assembly, and using poly-D-lysine or poly(allylamine hydrochloride) as the cationic counterparts. In order to evaluate the biocompatibility of the DNA coatings in implantology, both in vitro and in vivo experiments were carried out. The in vitro experiments of rat primary dermal fibroblasts (RDF) revealed that the presence of multilayered DNA-coatings do not affect RDF cell viability and morphology but increase proliferation while in vivo rat model studies showed that the presence of the multilayered DNA-coating did not induce any adverse effects in terms of inflammation and healing of wounds. Similarly, DNA–chitosan bilayer membranes were investigated for treatment of wounds, and the results showed that the membranes will adhere to rabbit peritoneum tissue. [2]

5. DNA based hybrid catalysts: the new generation?

In order to answer the question “What can DNA based catalysts add to the field of catalysts?” It is important to understand what is ideal catalyst? From a catalytic point of view an ideal catalyst is highly active and selective under mild conditions, very stable and can be separated from the product using a relatively
simple process. In this respect, DNA has attracted attention of many chemists owing to its chiral double helix structure. Compared with RNA and proteins,

1. DNA is more stable and is easy to handle for many chemical reactions.
2. It has well defined chiral structure. The ionic right handed double helix of B – DNA is the predominant conformation found in organisms, but depending on the laboratory conditions (such as hydration of the grooves, the ionic strength of solvent, and the presence of DNA – binding molecules) other structures such as left handed Z – DNA and A – DNA are also accessible.

**Fig – 6:** Three polymorphs of DNA [5] [used with permission of John Wiley and Sons. 2014]

3. Using Watson – Crick base pairing rules, DNA not only can form ubiquitous double helix but also other three dimensional structures such as triplex and quadruplex on the basis of four letter alphabet.
Fig – 7: Chemical structure of DNA showing the connectivity of four bases to the phosphate – sugar backbone [5] [used with permission of John Wiley and Sons. 2014]

4. DNA is starting point for the development of a water compatible catalyst because of high solubility in water – An important attribute with respect to Green Chemistry.

5. Finally, DNA is readily commercially available from both natural and synthetic sources and is cheap. Both bulk and st – DNA can be bought at relatively cheaper prices. [4, 5].

At first, DNA based hybrid catalysts have been proposed to be very efficient systems for conducting catalytic reactions in water. The question is that are they really promising? Although, DNA based hybrid catalysts have many advantages listed above, they have several disadvantages

1. As many organic reagents are not soluble in water and some are unstable in water, application of DNA hybrid catalytic system in organic media is questionable. Till now, 33% v/v of water – miscible organic solvents such as MeCN, DMF, and alcohols have been reported to be applied without decrease in ee compared to DNA hybrid catalysis in water.
2. It has also been reported that DNA duplexes performed in water are sufficiently soluble and stable in organic solvents.

3. Large scale production of catalysts is limited by cost.

4. Mechanism of stereoinduction is still not fully understood. Research is underway to apply molecular dynamics and density functional theory with the help of computer simulations to understand the relation of helical chirality of DNA and enantioselectivity of chemical reaction and design of new catalysts [5].

DNA is found to exhibit different character with different organic/polymer materials depending on type and nature of polymer and type and nature of DNA base pair interacting. This is the aim of the present research and forms the basis of new DNA based hybrid catalysts as outlines below.

5.1 Diels – Alder reaction

The potential of DNA-based hybrid catalysts for asymmetric catalysis was demonstrated for the first time in a Diels–Alder reaction combined with a supramolecular approach. Feringa and Roelfes chose a copper(II)-catalyzed Diels–Alder reaction between cyclopentadiene and an azachalcone in water as a model reaction (Fig – 8). The Cu$^{II}$ center acted as a Lewis acid and activated the dienophile by coordination to the ketone oxygen atom and the pyridyl nitrogen atom. A catalytically active complex was formed in situ between Cu$^{II}$ and a ligand that consisted of three functional components: a DNA-binding domain, such as 9-aminoacridine, a spacer moiety, and a metal-binding group. In the presence of st-DNA or calf thymus DNA (ct-DNA), the copper complex was anchored to the DNA double helix through the acridine intercalator. The reactions proceeded smoothly in water and were monitored to above 80% completion. Product 3 was obtained as a mixture of endo (major) and exo (minor) isomers, both with significant enantioselectivity. Without DNA, the products were obtained as racemates. Therefore, the enantioselectivity of the reaction originated from the DNA. The enantioselectivity of the Diels–Alder reaction proved to be dependent on the substituent, R, of the ligand, and on the spacer length, n. When R was 1-naphthylmethyl, an endo/exo ratio of 98:2 was observed, and the endo isomer was obtained with 49\% ee. In contrast, the endo isomer was formed with the opposite enantioselectivity with 37\% ee when R was a 3, 5-dimethoxybenzyl group. Furthermore, the absolute configuration of the product could be changed simply by changing the spacer. These stereochemical outcomes might be attributed to π – π stacking interactions between the ligand and the substrate and
imply that each enantiomer can be obtained selectively through the appropriate design of the ligand, although the chirality of the DNA is identical. Elongation of the spacer (n=5) caused the ee value of the product to decrease dramatically. Irrespective of the source of the DNA used (st-DNA or ct-DNA), similar enantioselectivities were observed. An ee value of 53% was observed for the endo isomer when a ligand was used with a spacer length n=2, a 3, 5-dimethoxybenzyl group as substituent R, and a methoxy group as substituent X.

![Figure 8: Diels–Alder reaction catalyzed by a supramolecular hybrid of Cu$^{II}$–ligand complex and DNA [2] [used with permission of John Wiley and Sons. 2014]](image)

5.2 Michael reaction

A DNA-based hybrid catalyst was used to promote a highly enantioselective Michael reaction in water (Fig 9; the Michael adduct was obtained in some cases with > 99% ee). The maximum ee value observed previously for the product of a
Michael reaction in water was 86% ee with a Pd–binap complex. The DNA hybrid catalyst was self-assembled from st-DNA and a copper complex with L6; this catalyst also promoted Diels–Alder reactions with high enantioselectivity. The Michael adducts were obtained with up to 99% ee when dimethyl malonate was used as the nucleophile, and an α, β-unsaturated 2-acyl imidazole was used as the Michael acceptor. Nitromethane was also a good nucleophile in this reaction: the corresponding products were formed with up to 94% ee. Like the stereochemical outcome of the Diels–Alder reaction, the observed high stereoselectivity can be explained as a consequence of the nucleophile attacking the Si face of the Michael acceptor because of the chiral environment provided by the DNA hybrid catalyst system. Because the Cu$^{II}$–L6/st-DNA complex remains in the aqueous phase during extraction of the products, the DNA hybrid catalyst could be recovered. In the case of the DNA-mediated Michael reaction, the recovered catalyst solution was reused for another reaction on a 1 mmol scale without a significant decrease in the enantioselectivity or yield. These results demonstrate the potential of the DNA based hybrid catalyst for practical applications in synthesis.

Fig – 9: Asymmetric Michael addition catalyzed by complexes formed between Cu$^{II}$ ions and L6 in the presence of DNA. [used with permission of John Wiley and Sons. 2014]
5.3 Friedel Crafts Alkylation

A DNA-based hybrid catalyst also found application in the Friedel–Crafts alkylation, one of the most important Lewis acid mediated reactions; no other catalytic asymmetric Friedel–Crafts alkylation of olefins has been described with water as the solvent. The DNA-based hybrid catalyst was self-assembled by combining a Cu\textsuperscript{II} complex with st-DNA or oligonucleotides. The DNA-mediated catalytic enantioselective Friedel–Crafts alkylation reaction of 5-methoxyindole (8) in water was established by using a, b-unsaturated 2-acyl imidazoles as electrophiles under aqueous conditions (Fig 10).

The DNA sequence proved to be an important variable in the optimization of the reaction. A series of synthetic double- and single-stranded DNA molecules were evaluated, and a similar pattern was observed to that found for the Diels–Alder reaction. The best results were obtained when Cu–dmbpy was used in combination with the self-complementary oligonucleotide d(TCAGGGCCCTGA)\textsubscript{2} (93%ee). This sequence also gave the best results in the Diels–Alder reaction. The enantioselectivity decreased significantly with AT-rich duplexes and single-stranded DNA.
5.4 Flourination

Although less studied than the above-mentioned carbon–carbon bond-forming reactions, the creation of carbon–heteroatom bonds with a DNA-based hybrid catalyst has also been demonstrated. First DNA-based asymmetric C–F bond-forming reaction was developed by combining a chemical fluorination procedure and the DNA hybrid system established for the asymmetric Diels–Alder reaction by Roelfes and Feringa. The fluorination of indanone b-ketoesters with the Cu$^{II}$–dmbpy–DNA catalyst was carried out in an aqueous buffer with Selectfluor as the fluorine-transfer reagent. The effect of the ligand structure on the enantioselectivity is consistent with the previous observations for asymmetric Diels–Alder reactions. In the presence of dmbpy, fluorinated products were obtained with good DNA-induced enantioselectivity (up to 74%ee).

5.4.1 Allylic Amination with an Iridium(I) – Diene – DNA hybrid catalysts

It has been demonstrated that the application of DNA hybrid catalysis could be extended to organometallic chemistry beyond Lewis acid catalysis. However, the enantioselectivities or yields are very very low.

A DNA-based system was utilized for an allylic substitution on the basis of iridium(I) diene chemistry in an aqueous medium. Bicyclo[2.2.2]octadiene ligands,[34] which had shown good activity in iridium(I)-catalyzed allylic substitution reactions, were selected and modified for use as anchoring ligands for DNA hybrid catalysis (Fig 11). The DNA–diene hybrid ligand was examined in the iridium-catalyzed allylic substitution of 1-phenylallyl acetate (12) with morpholine (13) in an aqueous medium. In the presence of the DNA-based diene ODN3, a slightly higher activity was observed than with the iridium(I) complex of the free diene.
**Fig – 11:** Allylic Amination with an Iridium (I) – Diene – DNA hybrid catalysts [used with permission of John Wiley and Sons. 2014]
Chapter 2
Experimentation
Chapter 2

Synthesis and processing of DNA based hybrid catalysts and their incorporation in 3 + 2 cycloaddition reaction

Various reactions were carried out in the presence of water to check and validate the effectiveness of DNA to act as catalyst for 3 + 2 cycloaddition reactions. The scheme of reactions is simplified on the basis that presence of DNA ensures the possibility of reaction while DNA itself does not losses its integrity – which is the basis of catalysis. Reaction(s) are divided into groups involving preparation of precursors and then their incorporation into main reaction in the presence of prepared DNA solution. Investigations revealed that DNA act as effective catalyst for carrying out reaction in the presence of water at lower temperature(s). The enantioselectivities or yields of reactions are also found to be good.
DNA based asymmetric catalysis are prepared by two step approach. Effect of Pt. is checked by introducing Pt. in the form of complexes. These complexes are prepared in following ways

**Scheme 1**

1. **Preparation of neutral Pt(DMSO)\(_2\)Cl\(_2\)**

The neutral platinum Sulfoxide complexes were prepared by adding calculated amount of dimethyl sulfoxide (DMSO) in aqueous solution of K\(_2\)[PtCl\(_2\)]. For this, 100 mg (0.241 m mol) of K\(_2\)[PtCl\(_2\)] (Mol. Wt. 415.09 g/mol) was measured on weighing balance. Measured amount is placed in 50 ml round bottom flask (RBF). 100 ml of distilled water is poured in the flask and solution is gently stirred to form suspension. DMSO (Mol. Wt. 78.19 g/mol, Density 0.907 gcm\(^{-3}\)) in 1:3 molar ratio to K\(_2\)[PtCl\(_2\)] is used. For this 0.722 m mol (56.40 mg/0.062 ml/62.18 µl) of DMSO is taken with the help of micro pipette. Care was taken each time to use clean syringe and apparatus. Measured DMSO is added in above solution and waited for 5 min to 1 hr until yellow crystal appear. The crystals appear after 48 hrs as light yellow needles at the bottom of transparent solution. The crystals are filtered in vacuum, washed with water, ethanol and ether. These are dried in vacuum for 4 hrs. 83.6 mg weight was obtained.

**Reaction**

\[
1 \text{K}_2[\text{PtCl}_4] + 3 \text{DMSO} \rightarrow \text{Pt(DMSO)}_2\text{Cl}_2
\]
2. Preparation of Pt(bipym)Cl₂

A solution of 2,2’-bipyrimidine (Mol. Wt. 158.16) was prepared by adding 32.5 mg (0.205 m mol) of solid in 5 ml Methyl alcohol (CH₃OH). The solid dissolves in CH₃OH instantly without stirring. Similarly a suspension of Pt(DMSO)₂Cl₂ was prepared by dissolving 75 mg of crystals in 10 ml CH₃OH. The suspension was used as base solution in which 2,2’-bipyrimidine solution is added drop wise with gentle stirring. The final solution is kept under gentle stirring for 12 hrs at room temperature. Red precipitates were treated in ultrasonic bath for 3 hrs, washed with C₂H₅OH (150 ml) and methyl ether (150 ml) under vacuum (vacuum filtration). The solid precipitates were subjected to vacuum drying for 12 hrs. The resulting solid is weighed on weighing balance. 60 mg of solid precipitates were obtained. The enantioselectivity or yield was 68.8% [1]

Reaction

![Scheme 1](image)

Scheme 1

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1. Preparation of (E) – methyl 2 – (benzylideneamino)acetate

2.8132 g (22.4052 m mol) of Methyl ester (Mol. Wt. 125.56 g/mol) was placed in 100 ml round bottom flask after weighing it on weighing balance. 3.43 ml (24.6457 m mol) of Triethylamine (Mol. Wt. 101.19 g/mol, density 0.727 gcm⁻³) was poured in it after carefully taking it with the help of syringe. 22 ml of CH₂Cl₂ was taken with syringe and placed in above solution. Excess amount (~12 g) of MgSO₄ was taken and poured into
above solution with the help of spatula. Mixture is stirred for 1 hr on magnetic stirrer at 280 rpm. Measured quantity (2.27 ml, (22.4052 m mol)) of Benzylaldehyde is poured in above solution. Mixture is stirred at 280 rpm for 11 hr on magnetic stirrer. Reaction was stopped after 11 hrs and product is filtered on 12 No. filter paper to separate out MgSO₄. Undissolved MgSO₄ filtered out on filter paper is discarded as waste. Decant (organic phase) is mixed with excess CH₂Cl₂ (~100ml) to dissolve all organic phase (from equipment, walls etc.)

**Washing**

After filtration, washing with water is performed. Washing is done three times in following sequence

**Procedure:** Organic phase (decant) is mixed with water in 500 ml, 1.5 ml ø separating funnel. The solution is shaken well and allowed to settle. Organic phase settles to the bottom and water floats [organic phase ~ 250 ml, water/aqueous phase ~150 ml] based upon differential specific gravity and immiscibility. Same procedure is repeated three times to ensure complete separation. Each time aqueous phase is thrown away and fresh water is used. After three washings, organic phase (~ 80 ml) is mixed with MgSO₄ (250 mg) to separate out water. Mixture/solution is filtered again to separate out MgSO₄ which is discarded.

**CH₂Cl₂ separation**

CH₂Cl₂ is separated from organic phase by evaporation under vacuum on rotavapor apparatus. For this solution is placed in RBF (100 ml) and attached to rotating end of rotavapor apparatus as shown.

**Fig – 12:** Schematic of attachments of flasks to rotavapor apparatus
Evaporation is performed in two steps. 490 mbar (step – 1) and 1.08 mbar (step – 2). This results in removal of all CH₂Cl₂ in collecting flask which is thrown away/discarded. The solution free from CH₂Cl₂ is taken from RBF and placed in Vile. A part of it (~ 20 ml) is mixed with chloroform for NMR. The rest of solution is again subjected to vacuum evaporation to remove last bit of/remaining of CH₂Cl₂. 3.008 g (E) – methyl 2 – (benzylideneamino)acetate was obtained with enantioselectivity or yield of 76%.

**Reaction**

![Chemical reaction diagram]

**Scheme 2**

**Scheme 3**

**Preparation of MOPs – DNA**

Different weights of DNA and Hemisodium salts (MOPs) are taken and mixed to prepare DNA for reaction. For this, 20.14 mg of DNA is weighed on weighing balance in a vile and then mixed with 143 mg (Mol. Wt. 220.25 g/mol) MOPs. The mixture is dissolved in 20 ml water and placed in orbital stirrer for 12 hrs. After orbital stirring solution was divided into two equal parts (10 ml each) and placed in two separate bottles.

**Addition of Pt(bipyrm)Cl₂**

Weighed amount (19.08 mg each) of red precipitates of Pt(bipyrm)Cl₂ prepared earlier is mixed with DNA – MOPS hemisodium salt solution. The mixture is stirred well to ensure and allow the homogeneous and thorough mixing of Pt(bipyrm)Cl₂ in solution.
Addition of Copper(II)trifluromethanesulfonate

16.275 mg of Cu(II)trifloromethanesulfonate is weighed on weighing balance and transferred to above solution separately to each bottle. The solution is transferred to orbital stirrer for 10 minutes to ensure complete and homogeneous mixing.

Scheme 3

Addition of N – Phenylmaleimide, N – Methylmaleimide and (E) – methyl 2 – (benzylideneamino)acetate

Addition of N – Phenylmaleimide and (E) – methyl 2 – (benzylideneamino)acetate (S – A)

Calculated amount (38.96 mg) of N – Phenylmaleimide (Mol. Wt. 173.17 g/mol) is weighed on weighing balance and transferred to one of prepared DNA solutions in the presence of Triethylamine (≈ 3 ml). The solution is stirred gently followed by addition of 119.6 mg (E) – methyl 2 – (benzylideneamino)acetate by syringe. The solution is stirred for mixing and transferred to orbital stirrer for 6 days at a speed of 70 rpm.

Addition of N – Methylmaleimide and (E) – methyl 2 – (benzylideneamino)acetate (S – B)

Calculated amount (24.77 mg) of N – Methylmaleimide (Mol. Wt. 110.10 g/mol) is weighed on weighing balance and transferred to the other prepared DNA solutions in the presence of Triethylamine (≈ 3 ml). The
solution is stirred gently followed by addition of 119.6 mg (E) – methyl 2 – (benzylideneamino)acetate by syringe. The solution is stirred for mixing and transferred to orbital stirrer for 6 days at a speed of 70 rpm.
Chapter 3

Results and discussion
Chapter 3

Results and Discussion

Nuclear Magnetic Resonance was carried out in order to determine the nature of compounds and to check and ensure the completeness, integrity and quality of reaction completion at every stage. Liquid State Proton ($^1$H) NMR (Bruker DMX400 spectrometer (Bruker BioSpin, Germany)) (Jose Mari Korta Zentroa, Avenida de Tolosa 72, Donostia – San Sebastian, Spain) was used for this purpose and presented.
1. Color Change

One of the evidence of successful completion of experiment and effectivity of catalyst is that the color of final solution (Cu(II)trifloromethanesulfonate plus Pt(bipyrm)Cl₂ after addition of N – Phenylmaleimide and N – Methylmaleimide respectively in DNA + MOPs) after remaining in orbital stirrer for 06 days, changes to blue to brownish green. This is unique with this reaction and is indication and evidence that reaction took place to completion.

Fig 13: Initial solution
Fig 14: Color change (blue) of final solution after 24 hours

(A)

Fig – 15: Color change (brown) of final solution after 06 days (A & B)

(B)
2. Nuclear Magnetic Resonance (NMR)

NMR spectra of Pt(bipyrm)Cl$_2$ and (E) – methyl 2 – (benzyldieneamino)acetate is shown in Fig 17 and 18 respectively.

2.1 Pt(bipyrm)Cl$_2$

Liquid-state NMR spectroscopy was performed on a Bruker DMX400 spectrometer (Bruker BioSpin, Germany) present at Jose Mari Korta Zentroa, Avenida de Tolosa 72, Donostia – San Sebastain, 20018, Spain operating at $^1$H Larmor frequency of 400 MHz.

Sample preparation

All glassware and stir bars were treated with aqua regia, washed with copious water and acetone, and dried before each use. Few mille grams (4) of
Pt(bipym)Cl$_2$ is taken in a vile and 0.8 ml of DMSO is poured into it. The solution is well shaken and treated in ultrasonicator (10 min) to homogeneously dissolve the complex in DMSO solution. The resulting solution is transferred to NMR tube via pipette. The tube is marked with experiment number and transfer to NMR.

**Fig – 17: 1H - NMR spectra of Pt(bipym)Cl$_2$**

Peak(s) at 8, 9.4 and 9.7 ppm shows the presence of CH node resulting from 2 – pyrimidine and 1 benzene. The shift in the peaks is due to different atom index associated with each peak as shown in below table (Table – 1)
### Table – 1: Coupling Constants of 1H – NMR Spectra (Pt(bipym)Cl₂)

<table>
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<th>Sr. No.</th>
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<th>Atom Index</th>
<th>Coupling partner</th>
<th>Constant and Vector</th>
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This is an indication of existence of two equal-intensity resonances relative to uncoordinated DMSO. The narrow lower field resonance has Pt satellites consistent with s bonded DMSO. A somewhat broader higher field resonance is assigned, on which Pt coupling is not resolved, to o-bonded DOMO. Addition of excess ligand shifts this resonance up field but has no effect on the S-bonded methyl resonance at 40°. These NMR spectra are consistent with retention of two s bonded and two o bonded DMSO ligands in CH$_3$NO$_2$. Rapid o bonded ligand exchange leaves open the possibility of slight o bonded DMSO dissociation in nitro methane solution. The kinetic instability of the oxygen coordinated sulfoxide may be used to prepare mixed-ligand complexes of the form (Pt(DMSO)$_2$L)$_2$$^+$ [8]. The resonance of the azo-nitrogen in I was detectable via a small $^5J$(N,H) long-range coupling to the hydrogen atom in the para-position. Similarly, $^{195}$Pt satellites [$^2J$(Pt, H) $\approx$ 75 Hz] were clearly visible for the Pt–CH$_3$ resonances of all Pt(IV) complexes and permitted rapid measurement of $\delta^{195}$Pt. These long-range couplings were also efficiently identified by recording satellite-edited $^1$H NMR spectra that were difficult to resolve in normal $^1$H NMR spectra. [9]. However detection of Pt(II) remained unsuccessful via indirect measurements owing to the fact that both $^1$D satellite-edited and conventional $^1$H NMR spectra gave no evidence for the presence of resolved $^{195}$Pt satellites on any of the signals. [9 – 11]

2.2 (E) – methyl 2 – (benzylideneamino)acetate

Liquid-state NMR spectroscopy was performed on a Bruker DMX400 spectrometer (Bruker BioSpin, Germany) present at Jose Mari Korta Zentroa, Avenida de Tolosa 72, Donostia – San Sebastain, 20018, Spain operating at $^1$H Larmor frequency of 400 MHz.
**Fig – 18:** 1H – NMR Spectra of (E) – methyl 2 – (benzylideneamino)acetate

*Sample preparation*

All glassware and stir bars were treated with aqua regia, washed with copious water and acetone, and dried before each use. Few mille liters (≈ 20 ml) of (E) – methyl 2 – (benzylideneamino)acetate is taken in a vile and 0.8 ml of Chloroform is poured into it. The solution is well shaken and treated in ultrasonicator (10 min) to homogeneously dissolve the material in chloroform solution. The resulting solution is transferred to NMR tube via pipette. The tube is marked with experiment number and transfer to NMR.

Peaks at 7.52, 7.83 and 8.44 ppm show the presence of CH node resulting from benzylidenimin. Peaks at 3.68 ppm show the presence of CH₃ node resulting from methyl group while Peaks at 4.51 ppm show the presence of CH₂ node resulting from methylene group. The coupling constants of spectra are shown in below table (Table – 2)
**Table – 2: Coupling Constants of 1H – NMR Spectra (E) – methyl 2 – (benzylideneamino)acetate**

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<th>Sr. No.</th>
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<th>Atom Index</th>
<th>Coupling partner</th>
<th>Constant and Vector</th>
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3. **Future work**

a. Evaporate the water from product and conduct the FE – SEM, Dark field STEM – EDS, AFM, FTIR studies. To determine whether the proposed catalyst (DNA – complex) was formed or not?

b. FE – SEM will confirm its morphology (structure, shape and length) [12]

c. Dark field – Scanning TEM Energy dispersive spectroscopy will confirm which elements were present/left in DNA – complex [12]

d. FTIR will confirm the self-assembly of DNA – complex [13]

e. AFM will confirm topography and type of different phases [14]
References


[10] Scott JD, Puddephatt RJ. *Organometallics* (1986); 5: 1538


Appendix 1