PART I: ASYMMETRIC SYNTHESIS OF SIXMEMBERED N-HETEROCYCLIC RINGS MEDIATED BY BIFUNCTIONAL THIOUREA CATALYSTS

PART II: EXPLORATION OF DNA CLEAVAGE ACTIVITIES BY CERTAIN SMALL ORGANIC MOLECULES

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Table of Contents

Summary

This thesis is divided into two parts. Part I is focused on the asymmetric synthesis of six-membered N-heterocyclic rings mediated by bifunctional thiourea catalysts. In addition, Part II describes the exploration of DNA cleavage activities by certain small organic molecules.

In chapter 1, a brief historic overview of asymmetric organocatalysis was provided and different types of catalysts were introduced. In particular, bifunctional thiourea catalysts based on cinchona alkaloids were discussed in detail. A selection of examples and the reaction mode of hydrogen bonding interaction were shown in this chapter to highlight the recent development in this field and emphasize the advantages of using this type of catalysts.

In Chapter 2, chiral 3-nitro-1,2-dihydroquinolines were synthesized catalyzed by cinchona alkaloids based bifunctional thiourea catalysts. A cascade reaction was successfully introduced to react between N-(2-formylphenyl)benzenesulfonamide and nitrooelfin. Installation of suitable electron withdrawing groups on the amino moiety could enhance the reactivity and improve the enantioselectivity of the reaction. The desired compounds were achieved in high yields and with up to 90% enantiomeric excesses. These six-membered N-heterocyclic rings were proved to be quite useful in the pharmaceutical industry and medicinal chemistry.

In Chapter 3, Chiral 2-Aryl-2,3-dihydroquinolin-4(1H)-ones, as an another class of six-memebered N-heterocyclic rings, were synthesized through a intramolecular

cyclization reaction catalyzed by quinidine-derived tertiary amine-thiourea catalyst. The resulting compounds were obtained followed by decarboxylation and desulfonylation reaction. The yields were high and the enantioselectivities could research to 98%. These 2-substituted dihydroquinolinones were proved to be valuable precursors for diversified medicinal compounds.

Chapter 4 described the synthesis of two classes of sufur-containing cyclic organic compounds. For disulfide-derived Beaucage's reagent analogue, aminomethylated Beaucage's reagent and its sulfoxide analogue were successfully synthesized. And for trithiol-derived cyclic organic compounds, varacin B were successfully synthesized. All the compounds synthesized were used to explore the DNA-cleaving activities and all of them showed the good effectiveness in converting circular supercoiled DNA to the corresponding circular nicked form.

In chapter 5, a new selected oligonuleotide was discovered to perform a site specific cleavage reaction based on guanine quadruplex structure catalyzed by the combination of Mg^{2+} and histidine. Different methods, such as: CD spectrum, PAGE, middle phosphorus (^{32}P) labeling, were used to prove this cleavage reaction occurred in a hydrolysis way at the specific site of this oligonucleotide in the presence of Mg^{2+} and histidine.

List of Tables

List of Schemes

List of Figures

List of Abbreviations

Ar	aryl
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BINOL	1,1'-bi-2-naphthol
Bu	butyl
Boc	*tert*-butoxycarbonyl
Cbz	benzyloxycarbonyl
DABCO	1, 4-diazabicyclo-[2.2.2]octane
DCC	dicyclohexyl carbodiimide
DCM	dichloromethane
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EA	ethyl acetate
ee	enantiomeric excesses
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectra
IPA	isopropanol
LRMS	low resolution mass spectra
LDA	lithium diisopropylamide
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
t-Bu	*tert*-butyl
TEA	triethylamine

Tf	trifluoromethyl sulfonyl
TFA	trifluoroacetic acid
Ts	toluenesulfonyl

List of Publications

Papers:

1. Xiaoqian Liu, Yixin Lu*. Asymmetric Synthesis of 2-Aryl-2, 3-dihydro-4-quinolones via Bifunctional Thiourea-mediated Intramolecular Cyclization. *Org. Lett.* **2010**, *23*, 5592.

2. Xiaoqian Liu, Yixin Lu*. Bifunctional thiourea-promoted cascade aza-Michael-Henry-dehydration reactions: asymmetric preparation of 3-nitro-1, 2-dihydroquinolines *Org. Bio. Chem.* **2010**, *8*, 4063.

3. Jiahui Zheng, Xiaoqian Liu (co-first author), Qing Yuan, Yoon-Joo Shin, Daekyu Sun, Yixin Lu*. Thiol-dependent DNA cleavage by aminomethylated Beaucage's reagent. *Org. Bio. Chem.* **2010**, *8*, 1293.

4. Xiaoqian Liu, Xinming Li, Magdeline Tao Tao Ng, Yifan Wang ,Wei Xu, Tianhu Li*. Site specific self-cleavage of certain assemblies of G-quadruplex. *Chem. Commun*, **2008**, *43*, 380, highlighted by chemical biology.

5. Yifan Wang, Xinming Li, Xiaoqian Liu, Tianhu Li*. An i-motif-containing DNA device that breaks certain forms of Watson–Crick interactions. *Chem. Commun.*, **2007**, *42*, 4369.

6. Xinming Li, Magdeline Tao Tao Ng, Yifan Wang, Xiaoqian Liu, Tianhu Li*. Dumbbell-shaped circular oligonucleotides as inhibitors of human topoisomerase I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4967.

Conference Posters:

7. Xiaoqian Liu, Yixin Lu *. "Thiol-dependent DNA cleavage by aminomethylated Beaucage's reagent", *Inaugural (1st) International conference on Molecular & Functional catalysis (ICMFC-1)*, Singapore, July 11-15, 2010.

8. Xiaoqian Liu, Yixin Lu * "Asymmetric synthesis of 3-Nitro-1, 2-dihydroquinolinescatalyzed by bifunctional organocatalyst", *6th Singapore International Chemical Conference*, National University of Singapore (NUS), December 15-18, 2009.

9. Xiaoqian Liu, TianhuLi *. "Constructions of DNA Rotaxane with G-quadruplex Dumbbell", *First International Meeting on Quadruplex DNA*, Louisville, KY, USA, April 21-24, 2007.

Chapter 1 Introduction

1.1 Asymmetric Organocatalysis

As an important element in nature, the chiralty of the molecular has been attracted much more attention in the pharmaceutical industry as well as in the other industries, such as: semiconductor industry and food industry.[1] Compounds with one or more chiral centers but determined as one single enantiomer usually show unique physical properties and biological activities. For example, one third of all sale drugs around the world are represented as the chiral molecules and the research conducted by FDA has shown that that the enantiomeric pure drug candidates could provide better therapeutic effect and less side effect over the racemic mixtures of the drug candidates.[2-3] So the asymmetric synthesis of optical pure molecules is on demand these days.

The development of efficient methods to obtain the chiral compounds is becoming the great challenge for organic chemists. Historically, these chiral compounds could obtained either by chemical transformation of chiral precursors or by kinetic resolution of the racemic mixtures. However, both methods have their own drawbacks. The former one requires suitable precursors and the latter one can only get 50% of the desired products theoretically.

Compared to these two methods, asymmetric catalysis shows great significance because according to this method, many new chiral products could generated only by introducing chiral catalysts and many versatile chiral sources could be act as chiral catalysts, such as: amino acids, nucleic acids as well as sugars.[4]

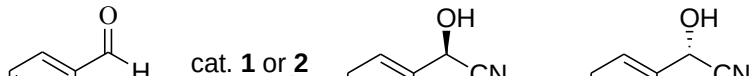
The types of asymmetric catalysis could be mainly divided into three categories.[5] For a long time, the field of asymmetric catalysis was dominated by metal [6-28] and biocatalysis.[29-44] However, the metal mediated catalysis usually requires the combination of the chiral ligand and the transition metal. The transition metals are usually toxic, expensive, easily contaminated and the reaction conditions are quite harsh and restricted with moisture and oxygen.[45] For the biocatalysis, because of the highly selectivity of the enzyme, the reaction type and substrates are quite narrow.

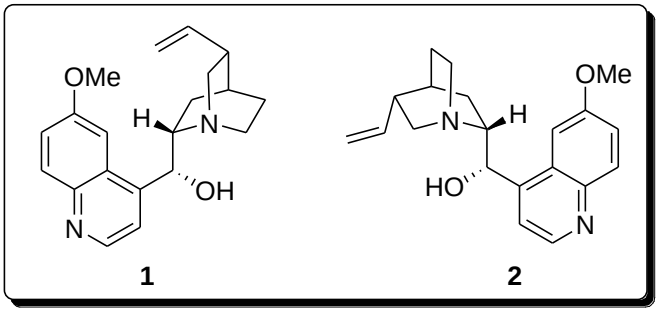
Until recently, the third discipline of asymmetric catalysis: asymmetric organocatalysis, has becoming new and hot area. In asymmetric organocatalysis, the chiral organic molecules are acted as the catalytically activated species. The advantages of organocatalysts are significant: they are metal free, nontoxic, readily available and different organocatalysts can promote various types of reactions through different activation modes.

The concept of "organic catalyst" was first introduced by Otswald in 1900, which was used to differentiate the organic molecules as catalyst from that of enzymes and other molecules involving the metals.[46] After that, the first example of an asymmetric reaction was reported by Bredig and the reaction was as following: The hydrocyanation reaction of benzaldehyde was catalyzed by natural cinchona alkaloids quinine **1** and quinidine **2** to give the product **3a** and **3b**.[47] Although the enantioselectivities of these reactions were very poor, this was still memorialized as the pioneer work in the field of organocatalysis. Another example of an asymmtric reaction in the early stage was that in the late 1950s, Pracejus reported the addition

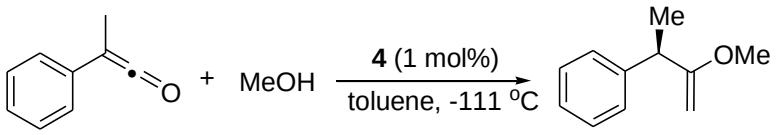
reaction of methanol to methyl phenyl ketene by using *O*-acetyl-quinine **4** as catalyst to obtain the product **5** in 74% ee.[48] However, the field of organocatalysis did not enormous developed until the last decade of the twentieth century, only few examples were reported such as proline-catalyzed asymmetric annulation [49-50] and phase-transfer catalyzed asymmetric alkylation reactions.[51-53] Around the turn of the century, MacMillan coined the term "organocatalysis" and over the last few years there has been enormous advancement in this field of research.[54-59]

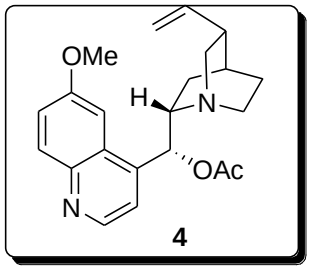
Bredig





Pracejus





Scheme 1-1 Examples of asymmetric reactions using organic catalysts in the early age

1.2 Hydrogen Bonding Mediated Asymmetric Catalysis

1.2.1 Introduction

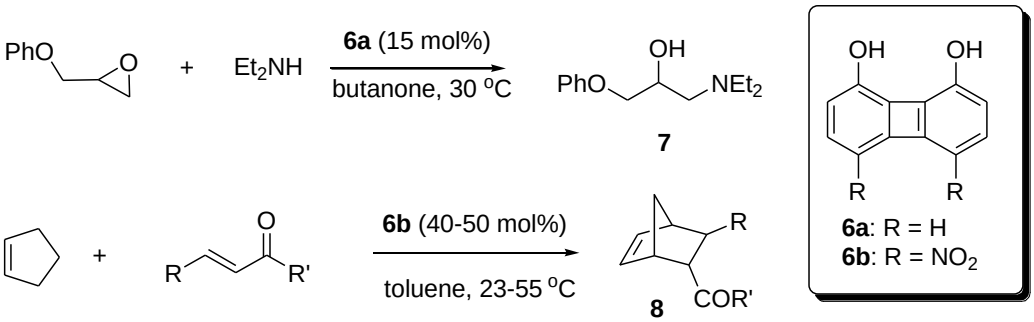
Hydrogen bonding is one of the most powerful forces in molecular interaction and recognition in the biological system.[60] It is responsible for forming various structures, like the ability of proteins to fold the three dimensional structures; the match of DNA base paring; the binding of ligands to acceptors. This unique noncovalent interaction is not only important to the structure determination, but also crucial in catalysis. For a very long time, scientists have tried much effort to design the small molecules that mimic the activities of enzymes. However, because lacking of the rigid and three dimensional structures as well as the functional groups in the molecules, the design was always facing the huge challenge. Over the four decades, the main strategy of enantioselective catalysis has involved the electrophile activation by metal-cenrtered chiral Lewis acids.[61] The supramolecular complex matches the sophistication of enzyme thus catalyzing enantioselective transformation.[62] However, the development of chiral Brønsted acids as activation forces is highly challenging because the hydrogen bonding interactions between promoter and substrates are quite weak. Recently, organic chemists have begun to appreciate the great potential by hydrogen bonding as a mechanism for electrophile activation in the small, synthetic catalyst system.[63-64] This type of catalysis is complementary to the existing Lewis acid system and the new applications and development appeared a rapid increasing pace in the last decade.[65-69]

According to the different activating modes of hydrogen bonding and various

scaffolds of the chiral sources, these catalysts will be classified into three catagories: Chiral diol-based organocatalysts, Chiral Phosphoric acids and Urea and thiourea catalysts. They will be introduced individually in this chapter.

1.2.2 Chiral Diol-Based Organocatalysts

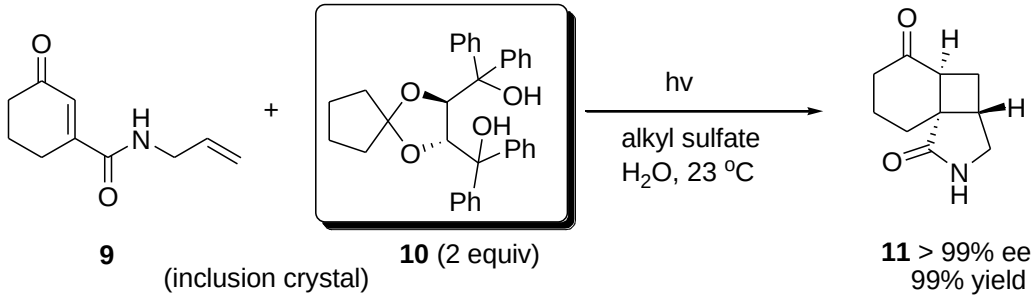
As early as in the 1980s, the first examples of biphenylenediol-accelerated epoxide aminolysis and Diels-Alder reaction were reported to demonstrate that chiral diols could not only act as good ligands in the enantioselective Lewis acid mediated reaction, but also perform well as general acid catalyzed asymmetric reactions (Scheme 1-2).[70-72] This is consistent with the computational study by Jørgensen who proposed that the water could coordinate the carbonyl group in the substrate to stabilize the transition state by hydrogen bonding.[73-74]



Scheme 1-2 Biphenylenediol-accelerated epoxide opening and cycloaddition reactions

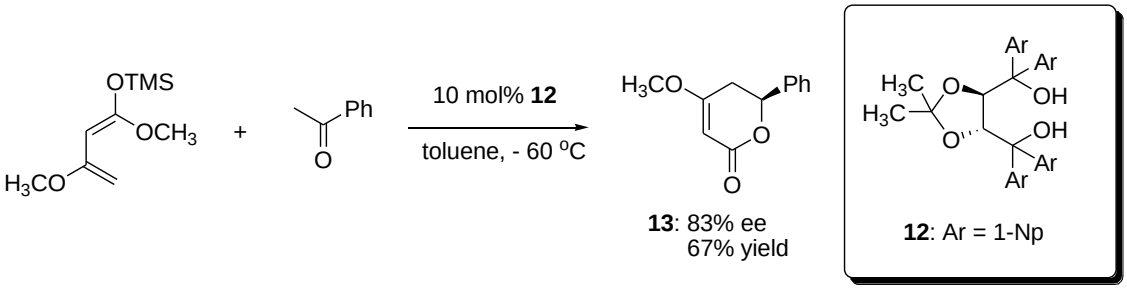
From initial study, α,α,α',α'- tetraaryl-1,3-dioxolane-4,5-dimethanol (TADDOL) derivatives and 1,1'-bi-2-naphthol (BINOL) became effective catalysts. The pioneer work of TADDOL derivatives was done by Toda and co-workers. They discovered that these derivatives are efficient resolving agents for numbers of the basic species

through the formation of H-bonded inclusion compounds.[75-77] For example, TADDOL **10** and diene **9** could react in the UV condition to result [2+2] cycloaddition product **11** with high enantioselecity.



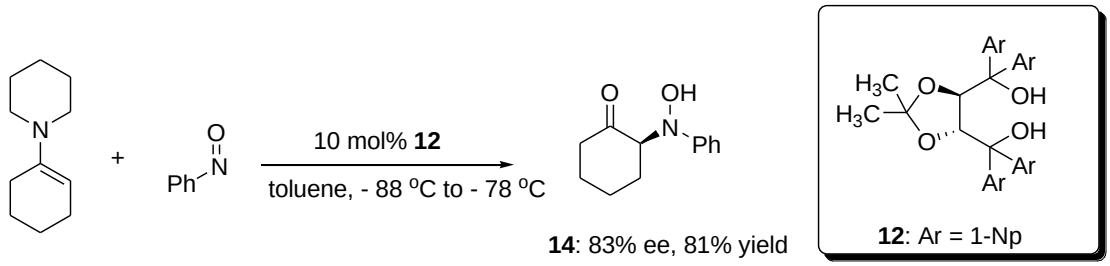
Scheme 1-3 Enantioselective solid-state photochemical [2 + 2] cycloaddition

Another example catalyzed by TADDOL was asymmetric hetero-Diels-Alder reaction of aminodienes with aldehydes in the presence of the 10 mol% **12**. This was the first successful application of chiral diol as hydrogen bonding catalyst. This reaction could be accelerated in the hydrogen bond donating solvent such as butanol and chloroform.[78]



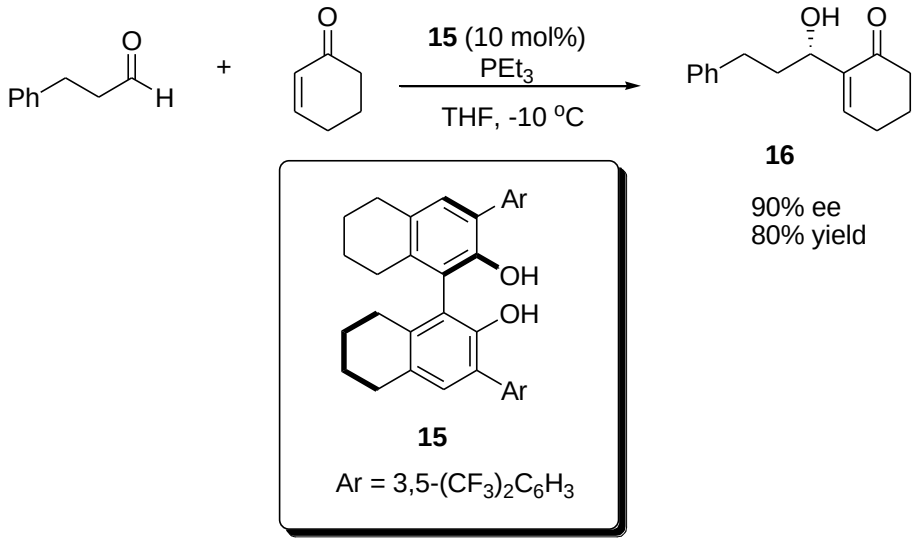
Scheme 1-4 Enantioselective hetero-Diels-Alder reaction by catalyst **12**

Subsequent research revealed that electrophiles rather than aldehydes may also be actived by catalyst **12.** The asymmetric aldo reaction of enamines could occur by the Hydrogen bond activation of nitrosobenzene.[79]

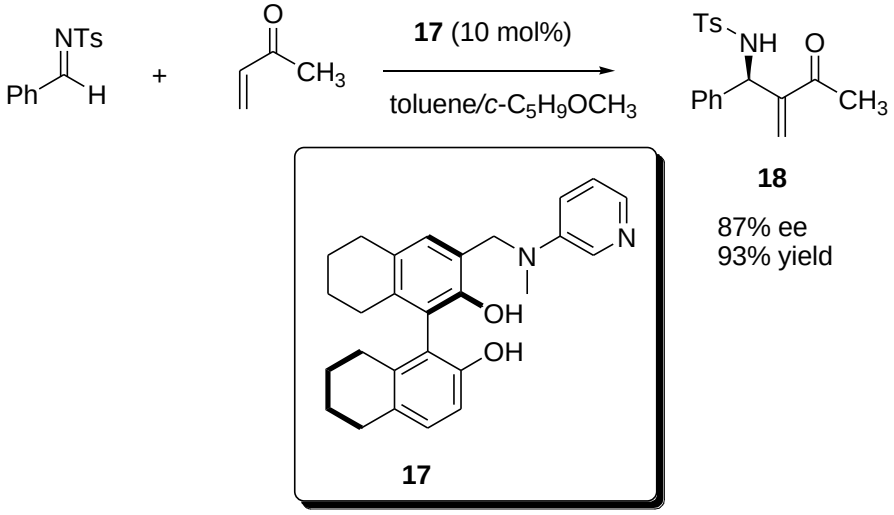


Scheme 1-5 Enantioselective aldo reaction of enamines

For BINOL structure, numbers of applications were found by optimizing the binaphthol scaffold. The ability of this structure to act as chiral hydrogen bond donor was found by McDougal and Schaus in the enantioselective Morita-Baylis-Hillman reactions. Arene-substitued octahydro (BINOL) derivatives such as catalyst **15** could afford the product **16** with high enantioselectivities. And another (BINOL) derivative **17** was found to catalyze the aza-Morita-Baylis-Hillman reaction. This catalyst played as bifunctional catalyst, in which phenol hydroxyl groups activated the electrophile and the pyridyl group act as the nucleophile to generate the required enolate.



Scheme 1-6 Enantioselective Morita-Baylis-Hillman reaction by catalyst **15**



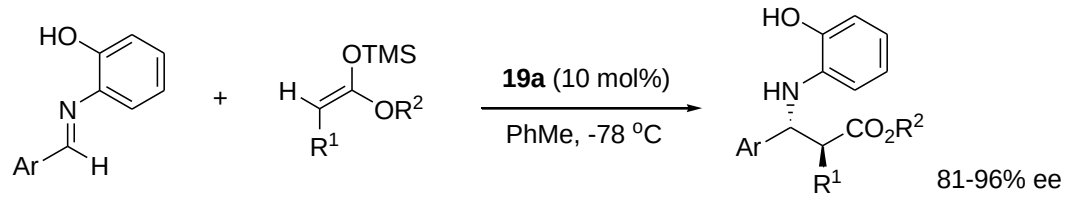
Scheme 1-7 Enantioselective aza-Morita-Baylis-Hillman reaction

1.2.3 Chiral Phosphoric Acids

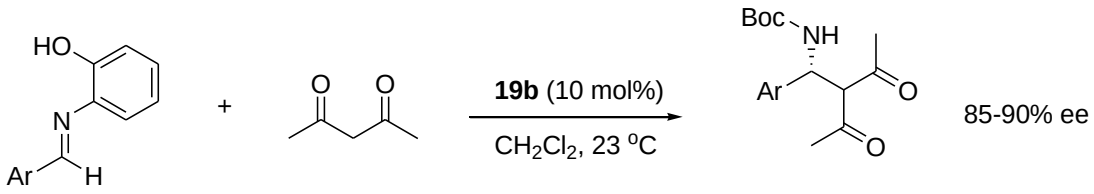
Chiral phosphoric acids derived from binaphthols have been proved to be useful in molecular recognition applications. In 2004, Akiyama [80] and Terada [81] groups independently reported the ennatioselective Mannich reaction catalyzed by phosphoric acids. Their researches showed that these catalysts had strong acidic

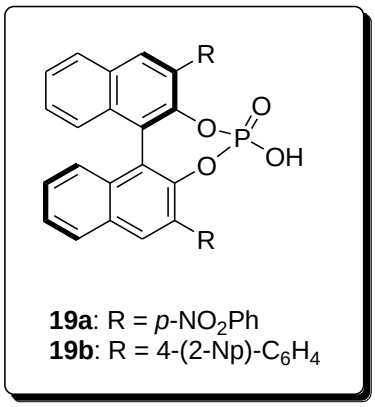
proton and could form highly ionic hydrogen bonds. The enantioselectivities were controlled by the variation of bulky groups in the 3,3'-position.

Akiyama et al.



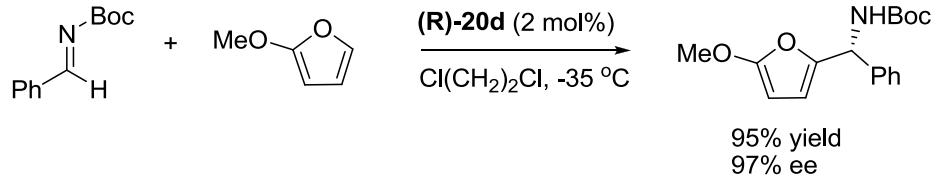
Terada et al.





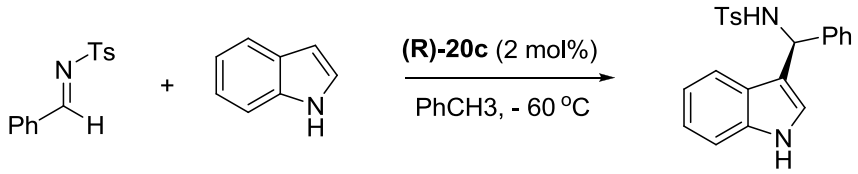
Scheme 1-8 Ennatioselective mannich reaction catalyzed by phosphoric acids

Chiral Phosphoric acids could also catalyze the aza-Friedel-Crafts reaction. The reactions could afford a range of functionalized, active amines and aromatic furans, [82] indoles [83-87] and pyrroles.[88] These reactions were catalyzed by chiral phosphoric acids through activation of imines.



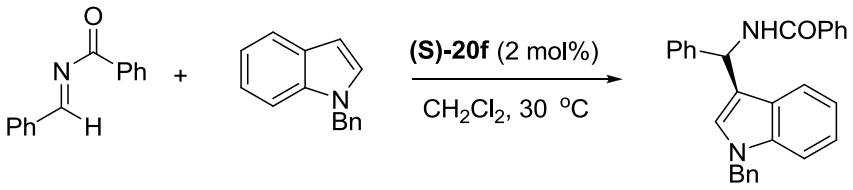
95% yield
97% ee

(a)



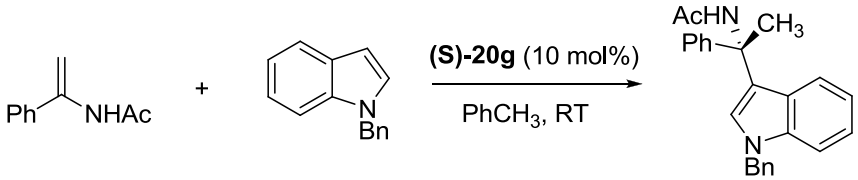
83% yield
98% ee

(b)



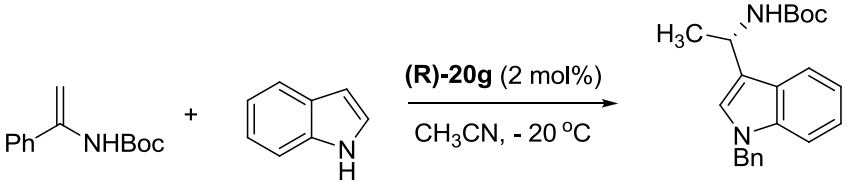
99% yield
92% ee

(c)



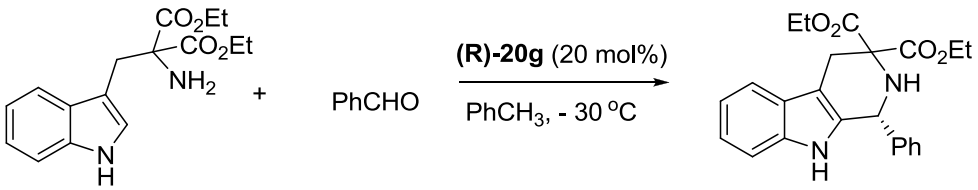
99% yield
92% ee

(d)

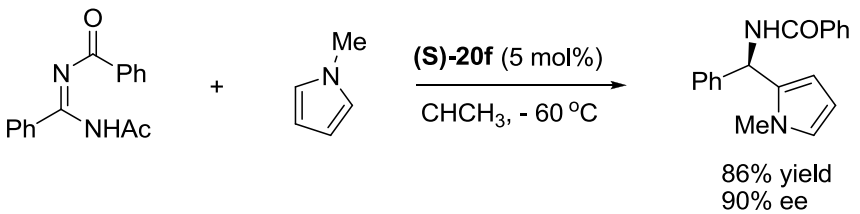


95% yield
93% ee

(e)

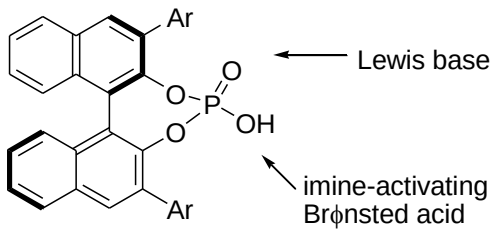


(f)



86% yield
90% ee

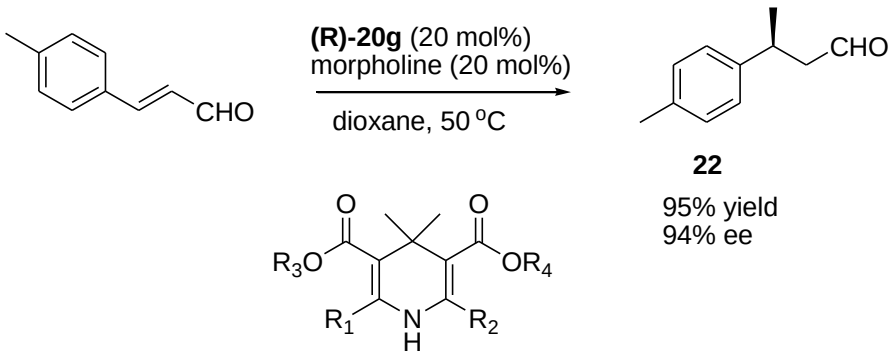
(g)



(**R) 20a:** Ar = 4-NO_2CH_4
(R) 20b: Ar = 4-β-naphthyl-C_5H_4
(R) 20c: Ar = 4-ClC_6H_4
(R) 20d: Ar = 3,5-dimesitylphenyl
(R) 20e: Ar = 9-anthryl
(R) 20f: Ar = $SiPh_3$
(R) 20g: Ar = 2,4,6-(iPr)$_3$C6H$_2$
(R) 20h: Ar = 3,5-$(CF_3)_2C_6H_3$
(R) 20i: Ar = 9-phenanthryl
(R) 20j: Ar = 4-PhC_6H_4

Scheme 1-9 Enantionselective aza-Friedel-Crafts reactions catalyzed by chiral phosphoric acids

The List group [89] and MacMillan [90] group first independently developed the asymmetric hydrofenation reaction by employing Hantzsch esters as hydride source for the reduction of imines and chiral phosphoric acid as chiral inducer. The yields and enantioselectivities were quite good.

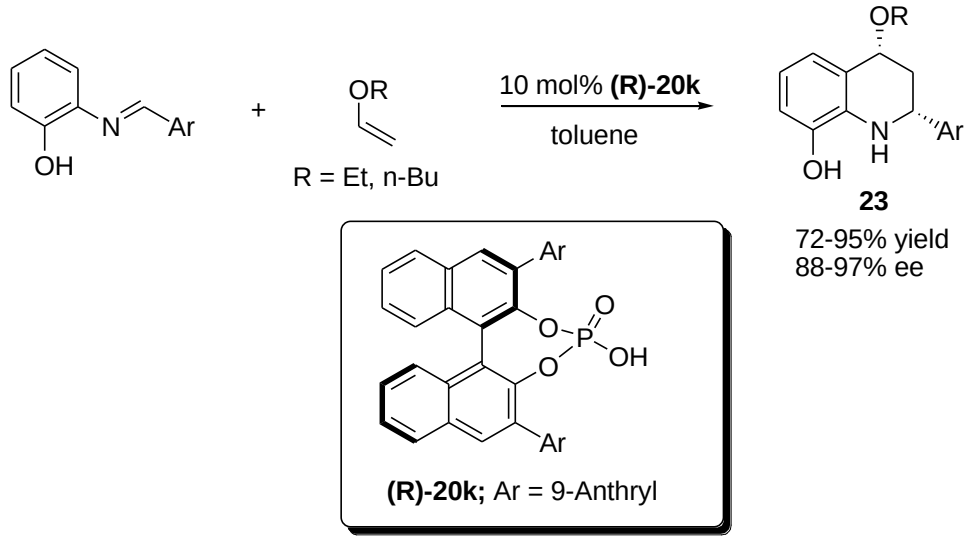


21a: R_1 = Me, R_2 = iPr, R_3 = R_4 = Me
21b: R_1 = R_2 = Me, R_3 = R_4 = Et

Scheme 1-10 Enantionselective hydrofenation reaction

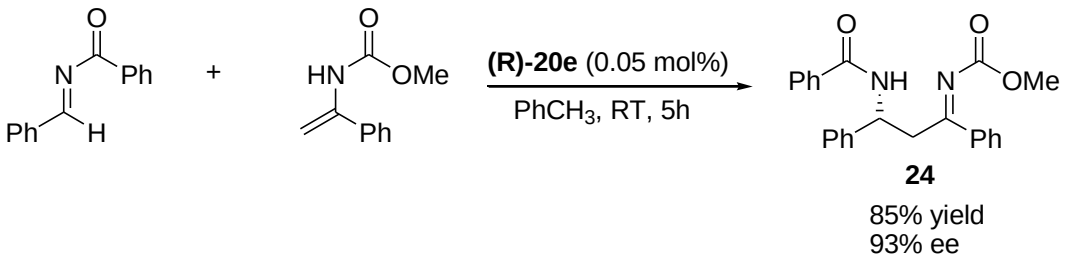
With the realization of strong activation ability of Brønsted acids with imines, the resulting imines could naturally participate in the aza-Diels-Alder reactions with electron-rich dienes. Akiyama et al. developed the catalyst **20k** for the asymmetric

aza-Diels-Alder reaction of aldimines with vinyl ethers.[91] The OH motif of the aldimine was found to be crucial for attaining the enantioselectivities and yields in the reaction which involved the hydrogen bonding interaction.



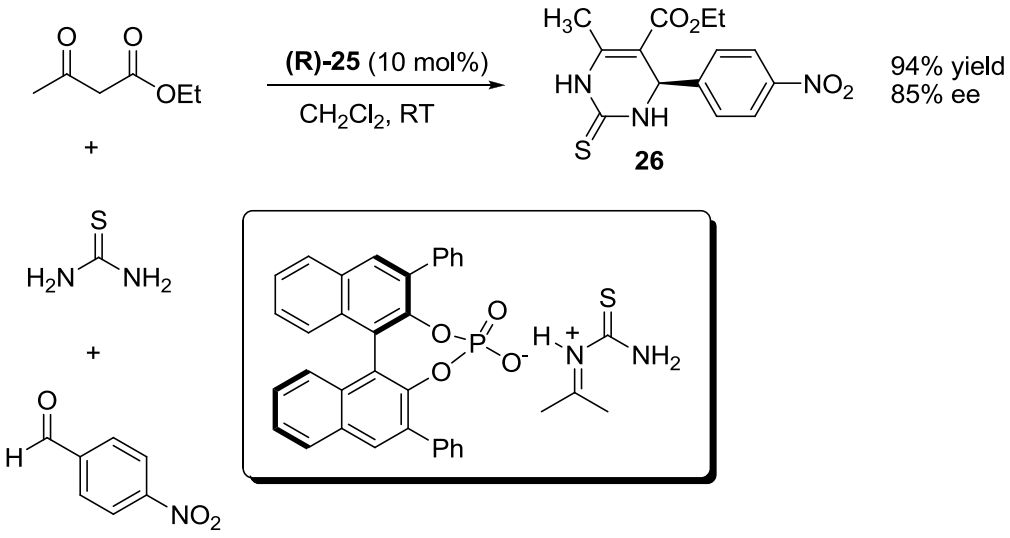
Scheme 1-11 Enantionselective aza-Diels-Alder reaction of aldimines with vinyl ethers

Terada et al. demonstrated an enantioselective azaene-type reaction between N-acylimines and enamides catalyzed by chiral phosphoric acid to obtain the β-aminoimines, which could further hydrolyzed to give the β-amino ketones with high yields and enantioselectivities.[92]



Scheme 1-12 Enatioselective aza-ene-type reaction catalyzed by **(R)-20e**

Gong's group reported the three component Biginelli reaction of ketoesters, thioureas and aromatic aldehydes catalyzed by chiral phosphoric acid **24** to give the desired products with high yields and enantioselectivities.[93]

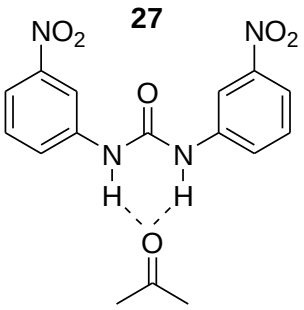


Scheme 1-13 Enatioselective three-component Biginelli reaction

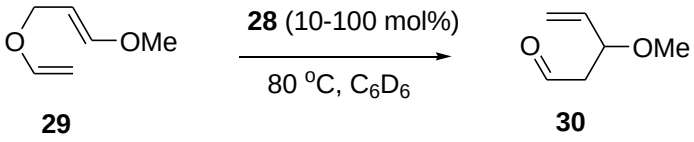
1.2.4 Urea and Thiourea Catalysts

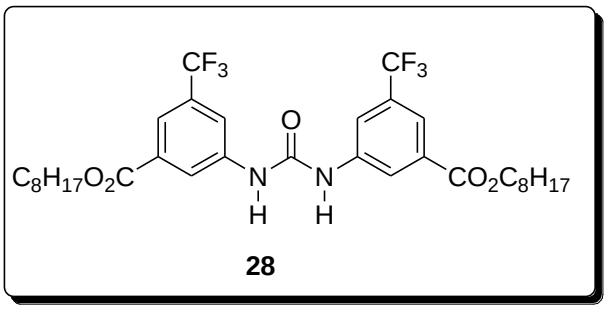
1.2.4.1 Monofunctional Urea and Thiourea Catalysts

The concept that urea and thioureas can function as catalysts by activation of electrophiles through double hydrogen bonding interaction has been explored by many research groups. The pioneer work was done by Etter's group and Curan's group. Etter and co-workers demonstrated that electro-poor diaryl ureas **27** could be cocrystallized with numbers of Lewis basic molecules by the formation of double hydrogen bonds.[94-95]



And Curran found that the diaryl urea **28** could promote the Claisen rearrangement of **29**.[96-97]

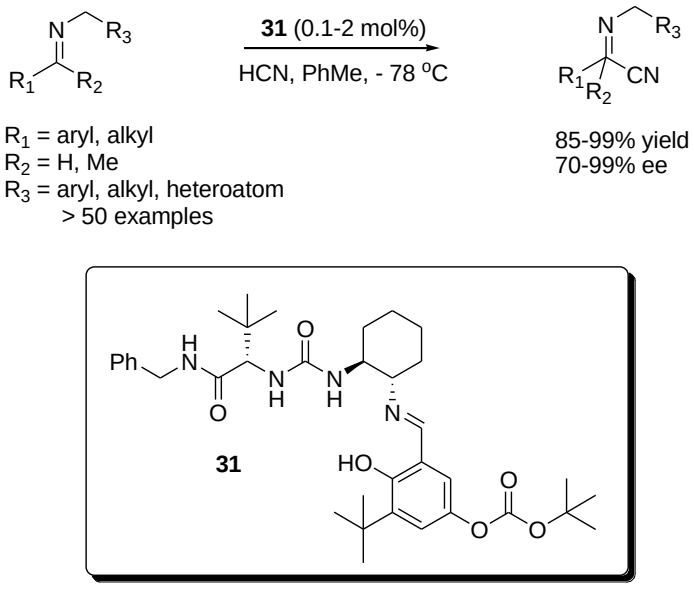




Scheme 1-14 Claisen rearrangement catalyzed by diaryl urea **28**

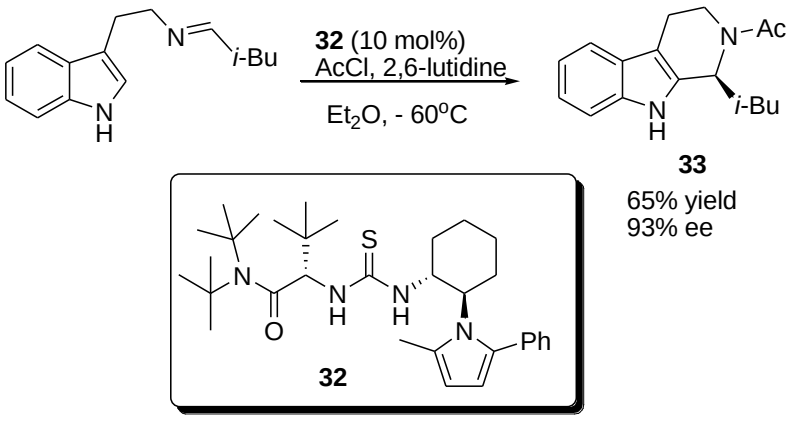
However, the utilization of different urea and thiourea as viable catalysts came from the work of Jacobsen. A series of Schiff bases derived from urea and thioureas were synthesized, which were originally designed as potential ligands for organometallic catalysis. They surprisingly found that thiourea effectively catalyzed the Strecker reaction without involving the metal. As a result of the library synthesis, catalyst **31** was identified as the best catalyst in the reaction of aldimines and

methylketoimines with HCN.[98-99]



Scheme 1-15 Enatioselective reaction of aldimines and methylketoimines with HCN

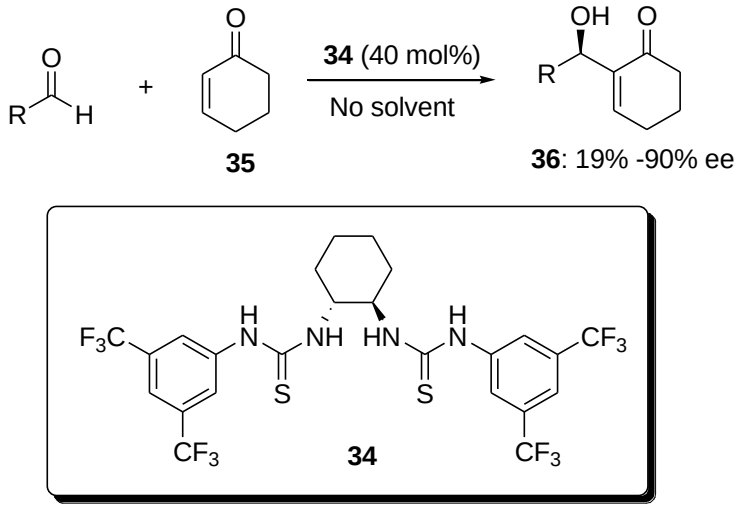
An enantioselective and catalytic acyl-Pictect-Spenger reaction using thiourea catalyst **32** was also developed by Jacobson group. The catalyst here not only represented the first example of asymmetric catalysis of this heterocycle forming reaction, but also demonstrated that N-acyliminium ions could be activated by chiral hydrogen bond donors. [100]



Scheme 1-16 Enatioselective acyl-Pictect-Spenger reaction

Nagasawa reported the use of chiral diaryl thiourea **34** to catalyze the

asymmetric Morita-Baylis-Hillman addition of cyclohexenone **35** to a range of activated aldehydes.[101] The high enantioselectivities were assumed to be the hydrogen bounding rising from both thiourea moieties and the substrates.



Scheme 1-17 Enatioselective Morita-Baylis-Hillman addition of cyclohexenone to aldehydes

The urea and thiourea are considered as a useful class of catalysts because of their effect to enantioselective reactions of numbers of nucleophilic and electrophilic partners. In addition, thay are both easily prepared and tunable. They are readily available to prepare bifunctional catalysts by using amine coupling to introduce another acidic or basic group.

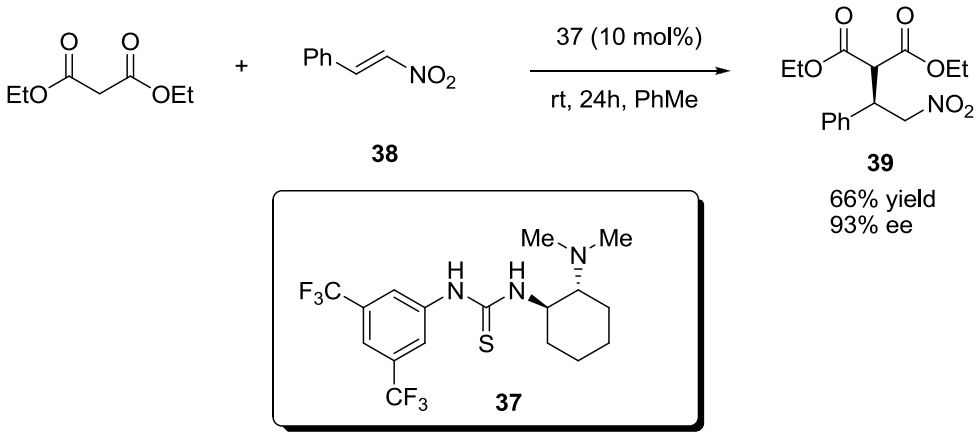
1.2.4.2 Bifunctional Urea and Thiourea Catalysts

The development of chiral catalysts which could simultaneously activate both electrophile and nucleophile is always under investigation. Much pioneer work in this field had involved Lewis acid together with additional Bronsted or Lewis basic functionality. A hydrogen bond donor (Brønsted acid) is used to activate the

electrophile, while a Lewis base is used to activate the nucleophile. Such bifunctional catalysts imitate the natural enzymetic system and potentially improve the catalytic activity and enantioselectivity.[102-104] In this section, the early development of chiral urea and thiourea based bifunctional catalysts will be discussed and they are classified into three categories based on the chiral frameworks where they are derived.

1.2.5 Chiral Cyclohexane Diamine Based Bifunctional Thiourea catalysts

A novel bifunctional tertiary amine thiourea catalyst was designed by Takemoto and co-workers.[105-106] Catalyst **37** was assembled on the same chiral framework of 1,2-trans diamine which Jacobsen used. It could successfully catalyze the Michael addition of malonate esters to nitro olefins **38**. In this dual activation mode shown in Figure 1-1, the nitro olefin interact with the thiourea moiety via double hydrogen bonding, thus enhance the electophilicity of the reacting carbon. At the same time, the tertiary amine part could fix the malonate to the activated enol form.



38 **39** 66% yield 93% ee **37**

Scheme 1-18 Enatioselective Michael addition of malonate esters to nitro olefins

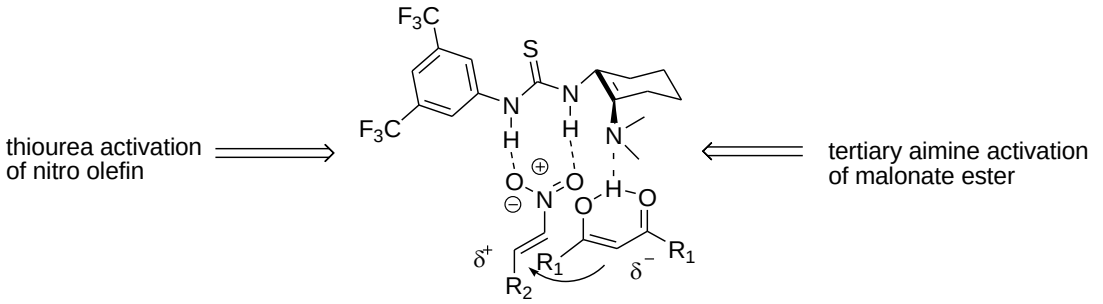
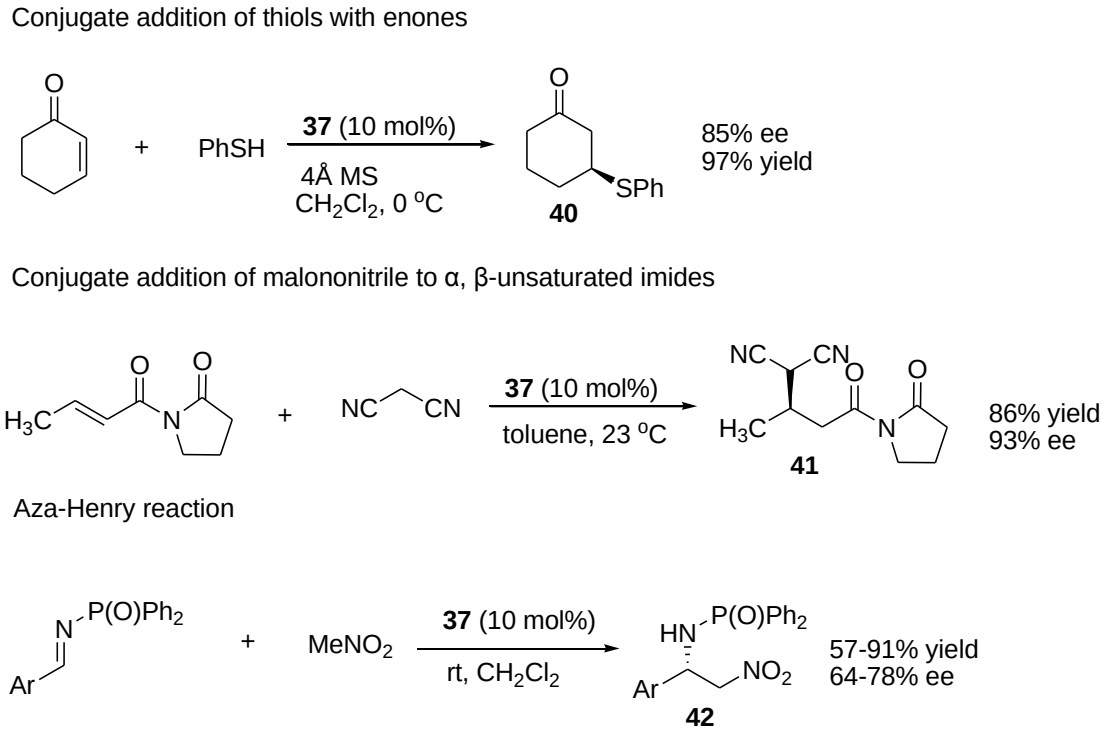


Figure 1-1 Dual activation mode of hydrogen bonding interaction of catalyst to malonate ester and nitro olefin

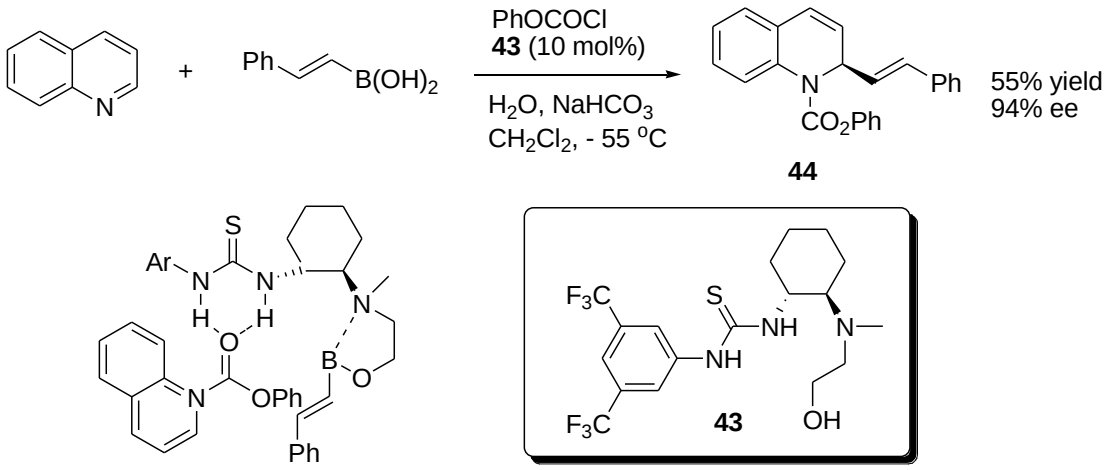
Further more, this bifunctional catalyst could extend to catalyze diverse asymmetric conjugate addition of thiols with enones,[107] malononitrile to α,β-unsaturated imides [108] and aza-Henry reaction.[109]



Scheme 1-19 Selective examples of asymmetric conjugate addition reactions

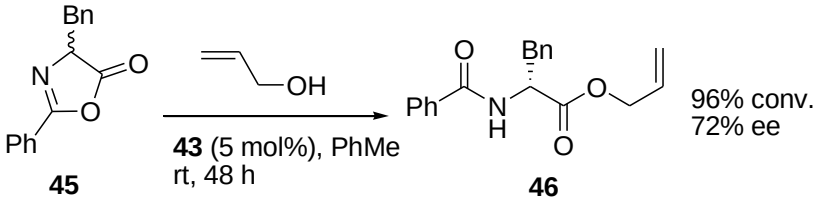
Later, Takemoto and co-workers developed new bifunctional aminol thiourea **43**

for the Petasis reaction of quinolines with vinyl boronic acid.[110] The 1,2-amino alcohol moiety in catalyst **43** was proved to be quite important in the reaction process to increase the reactivity and improve the enantioselectivity. It was proposed that the OH group involved in the formation of the five member ring with the vinyl boronic acid.



Scheme 1-20 Enatioselective Petasis reaction of quinolines with vinyl boronic acid

Berkessel et al. reported that catalyst **43** could efficiently undergo dynamic kinetic resolution of azalactones to give chiral α-amino acids.[111] In this reaction, it was proposed that the substrate was binding to the catalyst followed by general catalysis of ring opening reaction. And they also successfully extend this strategy for the kinetic resolution of oxazinones for preparing β-amino acids.[112]



Scheme 1-21 Dynamic kinetic resolution of azalactones

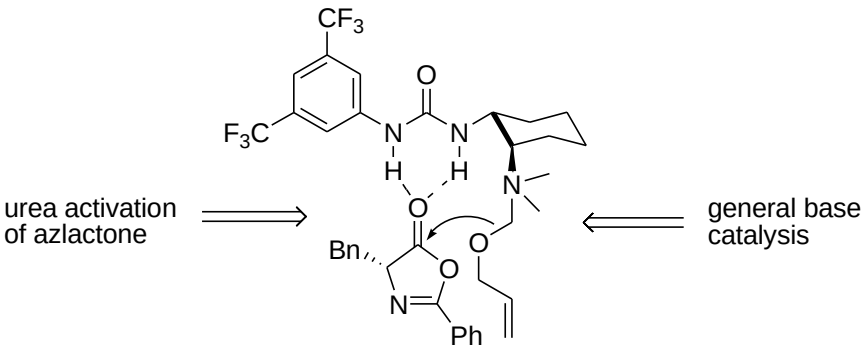
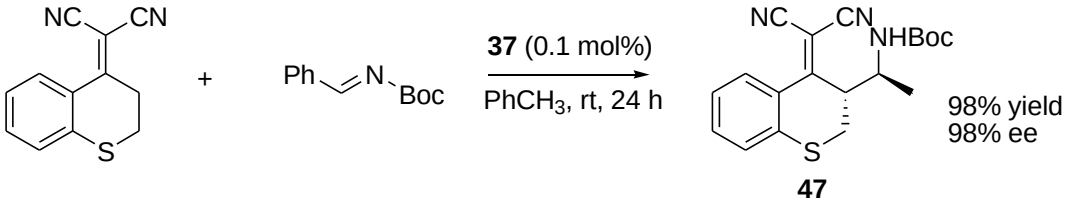


Figure 1-2 Activation mode of catalyst to azalactones

Chen and co-workers demonstrated the Mannich reaction could occur between dicyanoolefins and N-Boc benzaldimine catalyzed by catalyst **37** with high yield and ee values.[113] And the catalysts loading could decrease to 0.1 mol%.



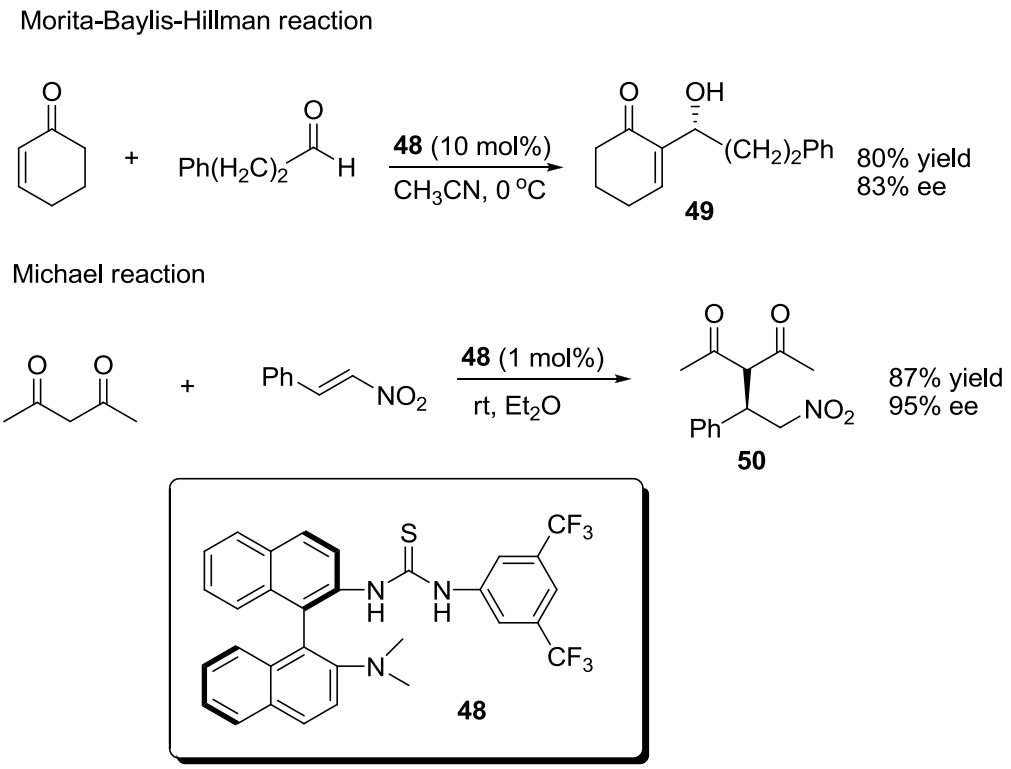
Scheme 1-22 Enatioselective mannich reaction of dicyanoolefins and N-Boc benzaldimine

1.2.6 Chiral Napthyl-Derived Bifunctional Thiourea catalysts

Chiral axial binaphthyl is another class of important scaffold and it is usually used in the organometallic reaction as ligands.[114] Wang and co-workers designed a new chiral binapthyl-derived bifunctional thiourea catalyst **48** incorporation with thiourea moiety into the chiral binapthyl framework. It worked at the Morita-Baylis-Hillman (MBH) reaction of cyclohexenone with a range of aldehyde

without additives.[115] Although the aromatic amine in catalyst **48** seemed weaker as nucleophile than aliphatic amines, it showed better result in this reaction compared to the Takemoto catalyst **37**.

This organocatalyst could also use in the Michael reaction of 1,3-diketone with nitro olefins. The catalyst loading could decrease to 1 mol% and the Michael adduct could be readily converted into α-substituted β-amino acids.[116]



Scheme 1-23 Enantioselective Morita-Baylis-Hillman and Michael addition reaction catalyzed by catalyst **48**

1.2.7 Cinchona Alkaloid -Derived Bifunctional Thiourea Catalysts

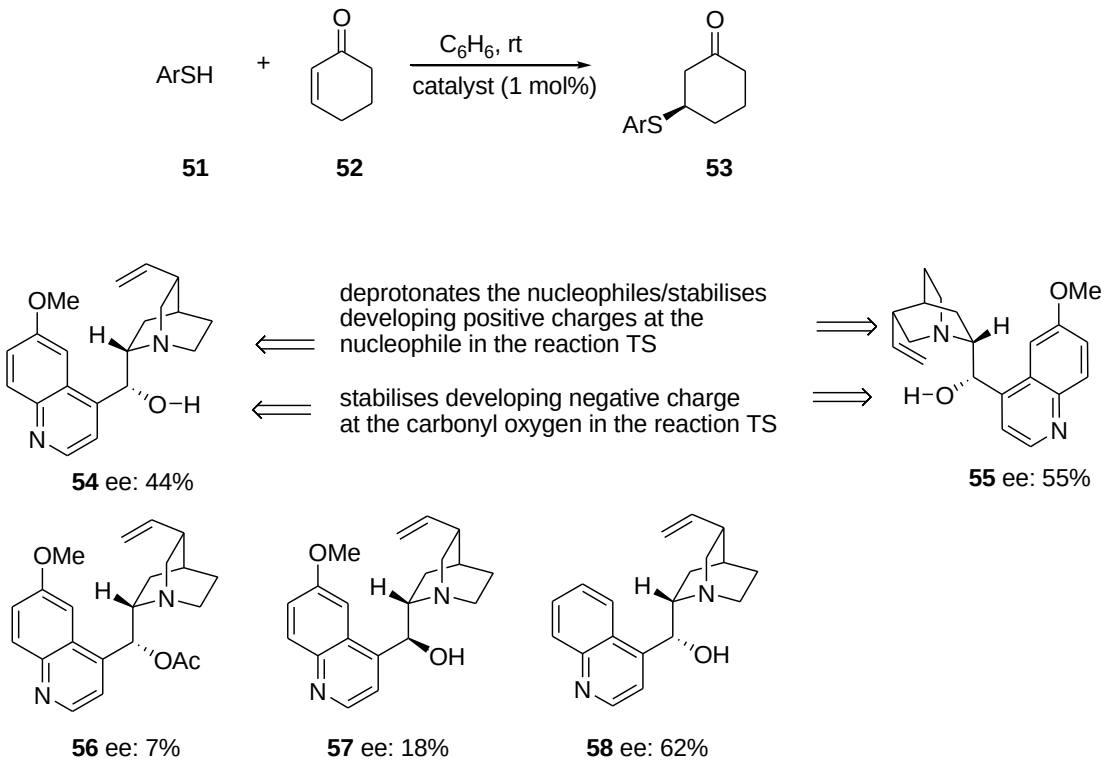
Cinchona alkaloids and their derivatives have shown great utilities in asymmetric organocatalysis. In the following sections, more information about cinchona alkaloids and their derivatives will be introduced and the representative reactions catalyzed by

cinchona alkaloid - derived bifunctional thiourea will also be listed accordingly.

Cinchona alkaloids are natural organic molecules isolated form the bark of several species of cinchona trees.[117] They have played important medicinal role for over 300 years. They could be used as an important antimalarial drug and muscle relaxant compounds. Cinchona alkaloids were first discovered as resolving agents by Pasteur in 1853.[118] Besides the resolution process, they can also be used as enantioselective analytical tools. The stereoselective syntheis of quinine 150 years ago laid the foundation of modern organic chemistry. However, the most interesting application of Cinchona alkaloids relied on their ability to promote enantioselecitve transformation in asymmetric catalysis.

The first asymmetric reaction carried out by cinchona base was reported by Bredig and Fiske in 1912.[119] They reported the addition of HCN to benzaldehyde in the presence of quinine and quinidine. However, the enantioselectivities were in the range of 10% ee. Four decades later, Pracejus obtained the useful enantioselectivities (74% ee) by using O-acetylquinine in the reaction of methanol to phenylmethylketene.[120] In 1981, a landmark research was done by Wynberg and co-workers to demonstrate that cinchona alkaloids catalyzed the addition of thiophenols to cyclic enones.[121]

This reaction made researchers to realize that the catalysts could act bifunctionally which was dependent on the presence of both tertiary amine part and hydrogen bond donating hydroxyl group in the catalysts.

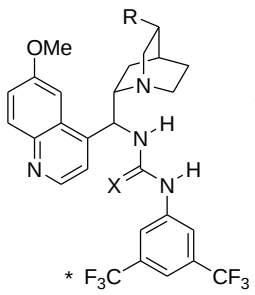


Scheme 1-24 Bifunctional alkaloid-mediated catalysis reported by Wynberg

The readily available and inexpensive cinchona alkaloids can be classified into two sets of pseudoenantiometic pairs: quinine and quinidine or cinchonine and cinchonidine. They are now privileged chiral catalysts in the area of asymmetric organocatalysis. The key feature which is responsible for the successful catalysts is that the chiral skeletons are versatile and tunable for different types of reactions. The quinuclidine nitrogen is acted as the chiral base to activate the nucleophilic reaction. The secondary 9-OH group can serve as an acid site or hydrogen bond donor. It can also be derivatized in to ureas or amides to provide a more powerful acidic site or hydrogen bond donors. The 6'-methoxy group of quinine and quinidine can also be derivated to the free OH group or thiourea moiety, which can serve as an effective H-bond donor as well.

C-9 sterocentre
synthetic route makes both diastereomers available
can tune the orietation of the bifuntioanl components

(Thio)urea N-aryl group
relavely unhindered substitution variable

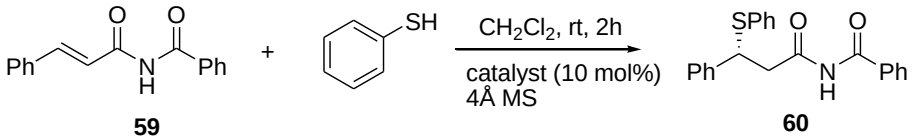


Quinuclidine ring
general base, activates the nuelophilic reaction component by deprotonation/nuleophilic catalysis
located in close to the (thio)urea moiety
varaible absolute configuration at C-8

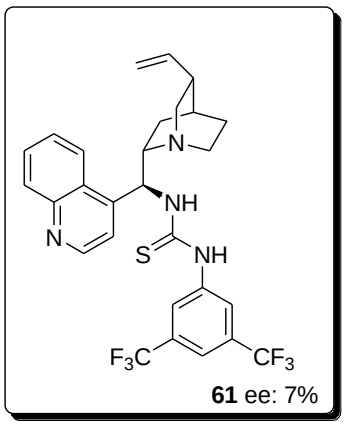
(Thio)urea moiety
can stabilise developing negative charge in the TS of addition reactions
known to bind to a variety of Lewis-basic functional groups and relative rigid

Figure 1-3 Chemical information and variation of cinchona alkaloids

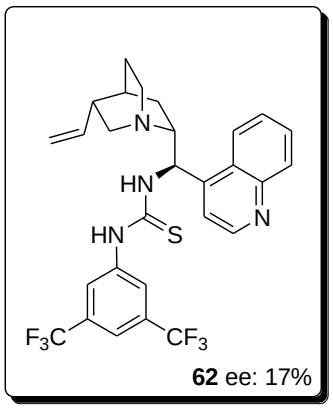
The first example came from Chen and co-workers who found catalyst **61** and **62** could catalyze the Michael addition of thiophenol to α, β-unsaturated imide **59**, but the enantioselectivity was quite low. [122]



59 **60**



61 ee: 7%

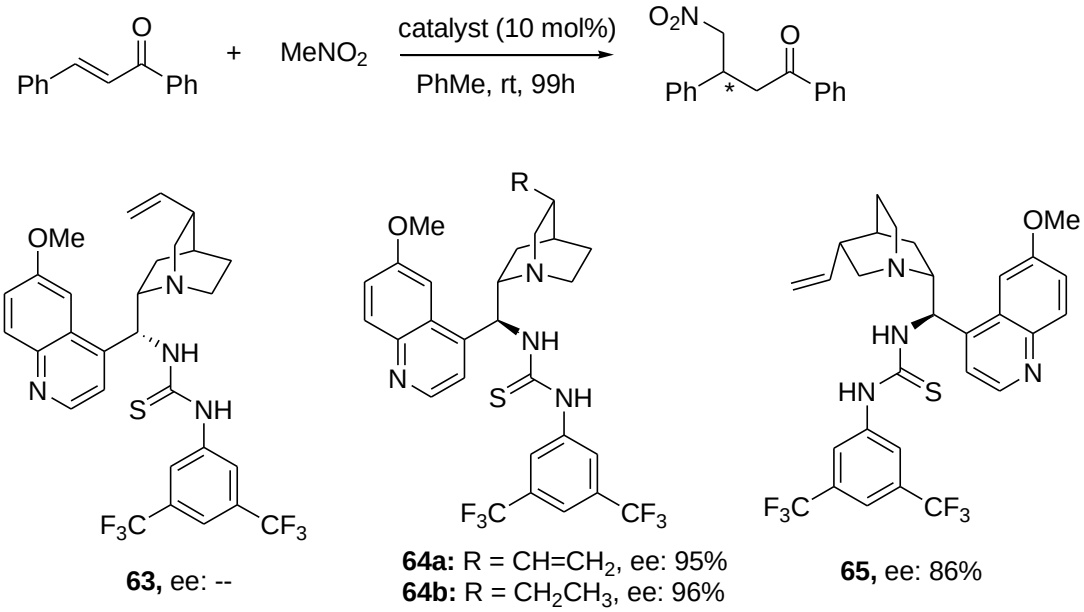


62 ee: 17%

Scheme 1-25 The first example of thiourea-substituted cinchona alkaloid mediated catalysis

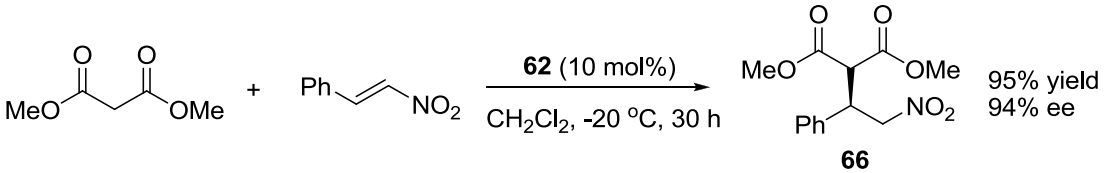
After that, the Soós and co-workers developed quinine and quinidine derived thiourea catalyst **63-65** for the enantioselective addition of nitromethane to chalcones.

Long time was necessary to achieve the satisfied yields and the lower catalyst loading in the higher temperature would not decrease the product yield or enantioselectivity.[123]



Scheme 1-26 Asymmetric catalysis of the addition of nitromethane to chalcone

The Dixon's group reported the cinchonine derived catalyst **62** to catalyze the reaction of malonate ester to nitro olefin at -20 ^{o}C to obtain the adduct **66** in excellent yield and enantioselectivity.[124]

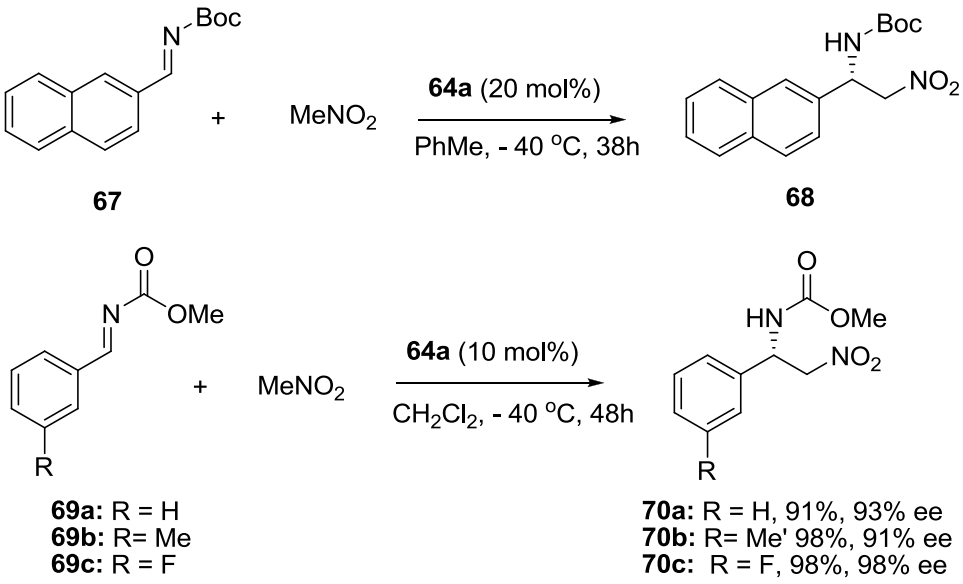


Scheme 1-27 Enantioselective reaction of malonate ester to nitro olefin

Inspired by the pioneer work by successfully using cinchona alkaloid derived organocatalyst in the asymmetric catalysis, remarkable numbers of the reactions were explored by several other researchers.

Ricci and co-workers first proved the catalyst **64a** could successfully catalyze the aza-Henry reaction between nitromethane and N-Boc imnine which was derived from benzaldehyde. Other carbamate protecting groups gave the similar results.[125]

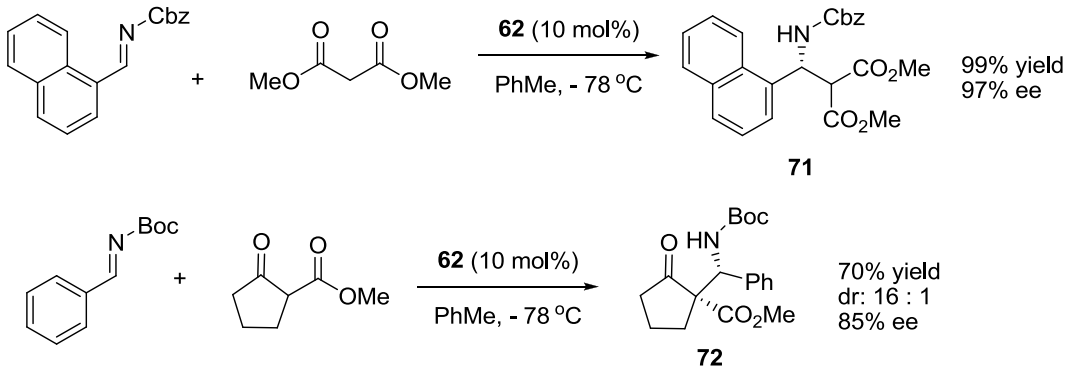
In a similar study, Schaus and co-workers used the catalyst **64b** to catalyze the same reaction class with methylcarbamate derivatives and large excess of nitromethane in CH_2Cl_2. The enantioselectivity were > 90%.[126]



Scheme 1-28 Modified cinchona alkaloid catalyzed aza-Henry reaction

An asymmetric Mannich reaction was also explored by researchers. Dixon's group demonstrated that acetyl actone, malonate esters and β-ketoesters could take part in the reaction with N-carbamoyl protected imines in the presence of the catalyst

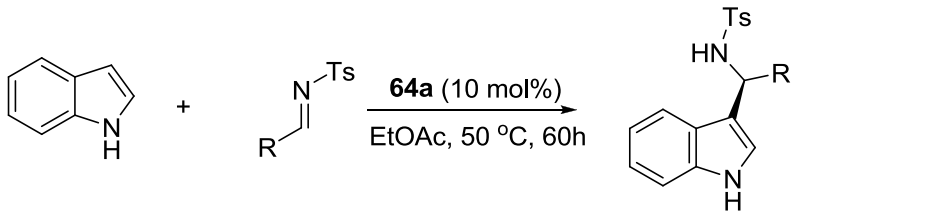
14.[127]



Scheme 1-29 Enantioselective Mannich reaction by catalyst **64**

Shortly after that Deng's group [128] and Schaus's group reported the similar results by using quinine and quinidine derived catalyst analogues. For Friedel-Crafts-type reaction, Deng's group firstly reported the asymmetric synthesis of 3-indolyl methanamine subunit from indoles and imines by using catalyst **64a**.[129]

The high enantioselectivity were observed no matter of the electronic character of the substrates. This study was the first example to participate the alkyl imines in the selective Friedel-Crafts-type reaction.



Scheme 1-30 Enantioselective Friedel-Crafts-Type reactions with imine substrates

Other reactions, such as: Diels-Alder reaction,[130] Decarboxylation reaction[131]

and desymmetrisation of meso anhydrides,[132] because of the constraint of the text, they will not listed in details in this chapter.

1.3 Project Objective

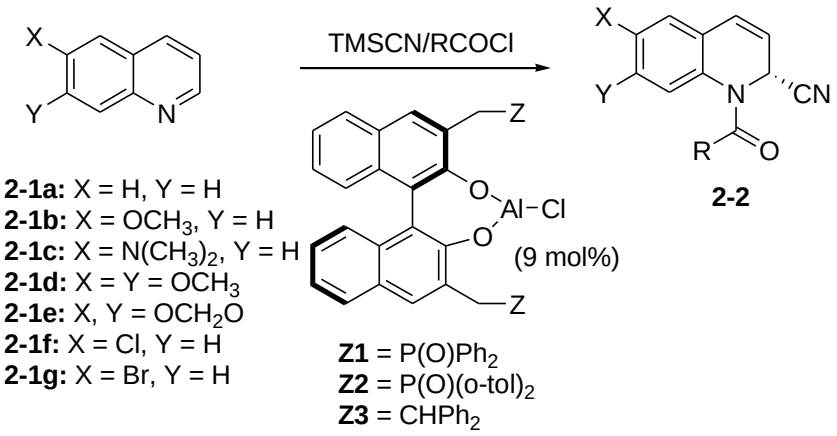
Since the cinchona alkaloids derived bifunctional thiourea catalysts have been proved to quite useful in the field of asymmetric organocatalysis, the purpose of our study is to design and synthesize the novel bifunctional thiourea catalyst in the asymmetric synthesis of six-membered N-heterocycles which have been found in biologically and medicinally important natural products. The next two chapters will focus on the asymmetric synthesis of two different types of six-membered N-heterocycles: Hydroquinolines and Dihydro-4-quinolones through two different methodologies. Chapter 2 will introduce how to synthesis of hydroquinolines by a cascade aza-Michael-Henry-dehydration reaction. In addition, chapter 3 is going to provide an efficient method to symmetric synthesis of chiral dihydro-4-quinolones through bifunctional thiourea-mediated intramolecular cyclization reaction.

Chapter 2 Bifunctional Thiourea Catalyzed Cascade Reaction: Asymmetric Synthesis of 3-nitro-1,2-dihydroquinolines

2.1 Introduction

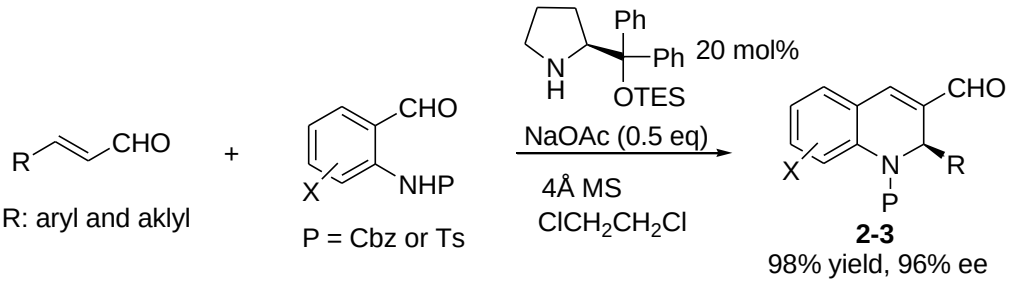
The functionalized six-membered heterocycles containing one nitrogen atom have been found quite important in biology and medicinal chemistry. Among these nitrogen heterocycles, hydroquinolines can be considered as a class of substrates which represent the most frequently encountered ones in natural or synthetic products.[133-135] It possess a broad spectrum of biological activities, are of great importance in pharmaceutical industry and medicinal chemistry. Some scaffolds containing the hydroquinolines cores have showed antimicrobial activity against fungal and bacterial species.[136] In particular, functionalized 1,2-dihydroquinolines are useful structural motifs and synthetic intermediates in natural product synthesis.[137-138]

Methods for the catalytic asymmetric synthesis of chiral 1,2-dihydroquinolines are very limited. Most literatures reported the synthesis of non-catalytic asymmetric synthesis of chiral 1,2-dihydroquinolines.[139-143] Shibasaki and co-workers developed an asymmetric Reissert-type reaction promoted by a Lewis acid-Lewis base bifunctional catalyst to access chiral 2-cyano-1,2-dihydroquinolines with good yield and enentioselectivity.[144]



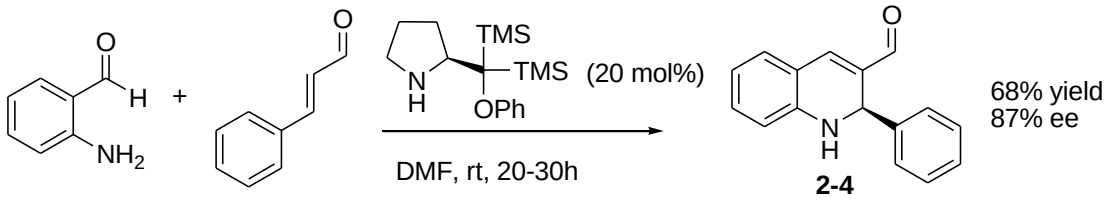
Scheme 2-1 Asymmetric Reissert-type reaction

Wang et al. disclosed an asymmetric synthesis of 3-formyl-1,2-dihydroquinolines employing a domino conjugate addition-aldol-dehydration reaction promoted by prolinol silyl ether via an iminium-enamine activation.[145]



Scheme 2-2 Enatioselective domino conjugate addition-aldol-dehydration reaction

A similar approach for the dihydroquinoline synthesis was independently reported by Córdova and co-workers.[146] The highly enantioselective organocatalytic cascade reaction is presented between 2-aminobenzaldehydes and a,b-unsaturated aldehydes proceed with excellent chemo- and enantioselectivity to give 1,2-dihydroquinolines derivatives in high yields with high ee.

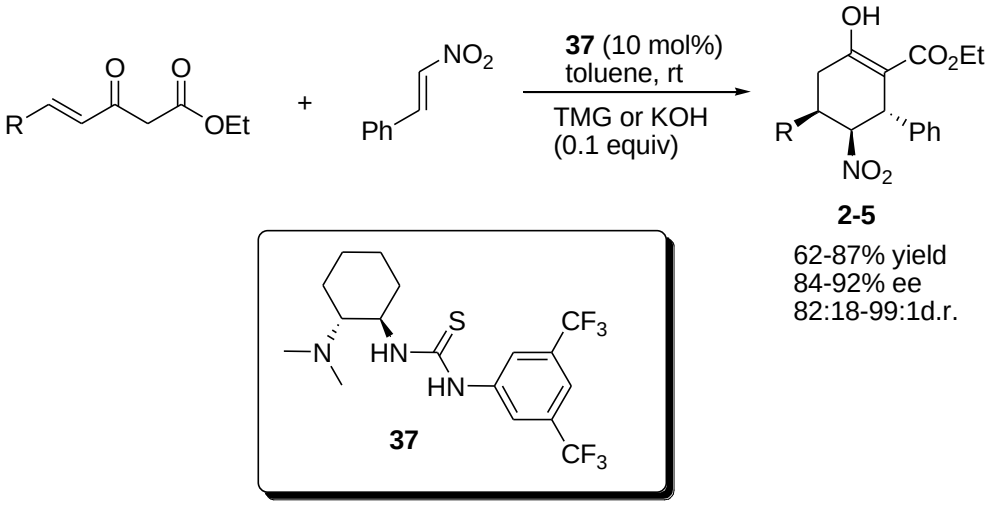


Scheme 2-3 Asymmetric domino reaction reported by Córdova

Given the importance of functionalized 1,2-dihydroquinoline compounds, and lack of efficient method for the preparation of chiral 3-nitro-1,2-dinhydroquinolines, developing an organocatalytic approach to tackle this synthetic problem became a challenge. From the first chapter, bifunctional organic molecules containing a tertiary amino group and a thiourea moiety have been established as remarkably useful organic catalysts. And inspired by other researchers' work, the cascade reaction has been chosen as a suitable method to synthesis of chiral 3-nitro-1,2-dinhydroquinolines. It is characterized by the efficiency and its biomimetic way, because the same principles are found in the biosynthesis of nature products. [147-149] Cascade reactions avoid the time-consuming and protection/deprotection processes and they usually come out the products with multiple stereocenters and excellent steroselectivities. In addition, they are environmentally friendly. Because of these advantages, cascade reactions become flourishing in organic chemistry over classical synthesis.

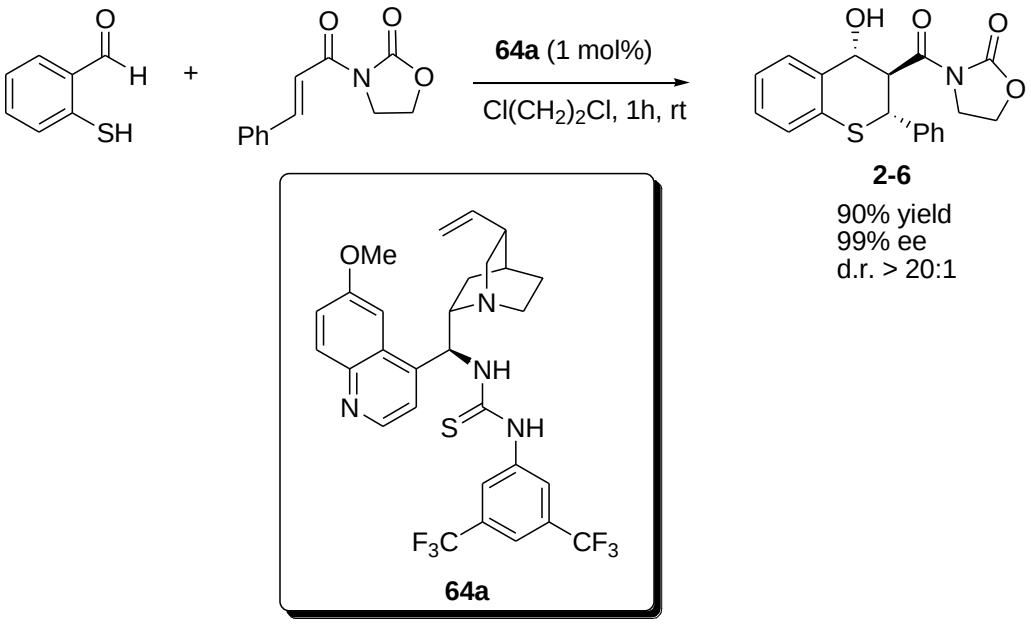
In fact, some examples of cascade reactions by bifunctional thiourea catalyst have been successfully explored. For example, Takemoto and co-workers reported the cascade Michael–Michael reaction of γ,δ-unsaturated β-ketoesters with nitroolefin catalyzed by amine thiourea **37** (Scheme 2-4).[150] The process gave products in high

yields (62 – 87%) and with high enantio- (84 – 92% ee) and diastereoselectivities (d.r. 82:18 – >99:1). However, the addition of a base (TMG or KOH) was essential for the second Michael reaction to occur.



Scheme 2-4 Asymmetric Michael-Michael reactions catalyzed by catalyst **37**

Wang and co-workers recently reported a powerful **64a** catalyzed cascade thiol-Michael – aldol reaction of 2-mercaptobenzaldehyde with α,β-unsaturated oxazolidinone.[151] The products **2-6** had three stereogenic centers in excellent stereoselectivity which may be attributed to the noncovalent hydrogen-bonding interactions in the bifunctional amine thiourea unit in **9b** and the substrates.



Scheme 2-5 Asymmetric cascade Michael-aldol reactions catalyzed by catalyst **64a**

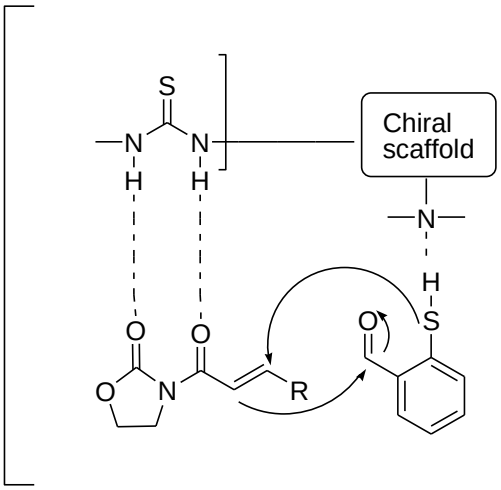
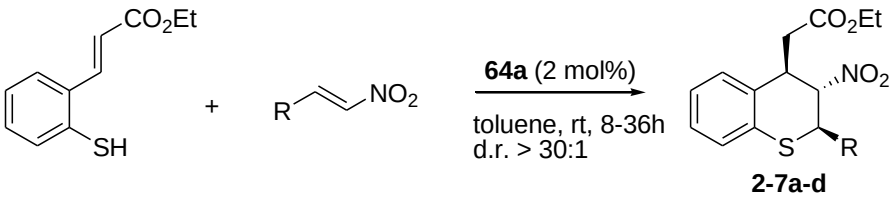
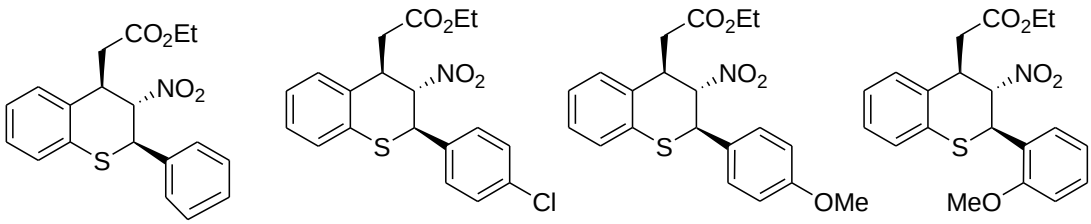


Figure 2-1 Activation mode of cascade thiol-Michael – aldol reaction

Later on, the same group developed a new cascade Michael-Michael reaction catalyzed by **64a** between the ethyl with a range of nitroalkenes to afford the thiochromans with new stereocenters and high stereoselectivity.[152]



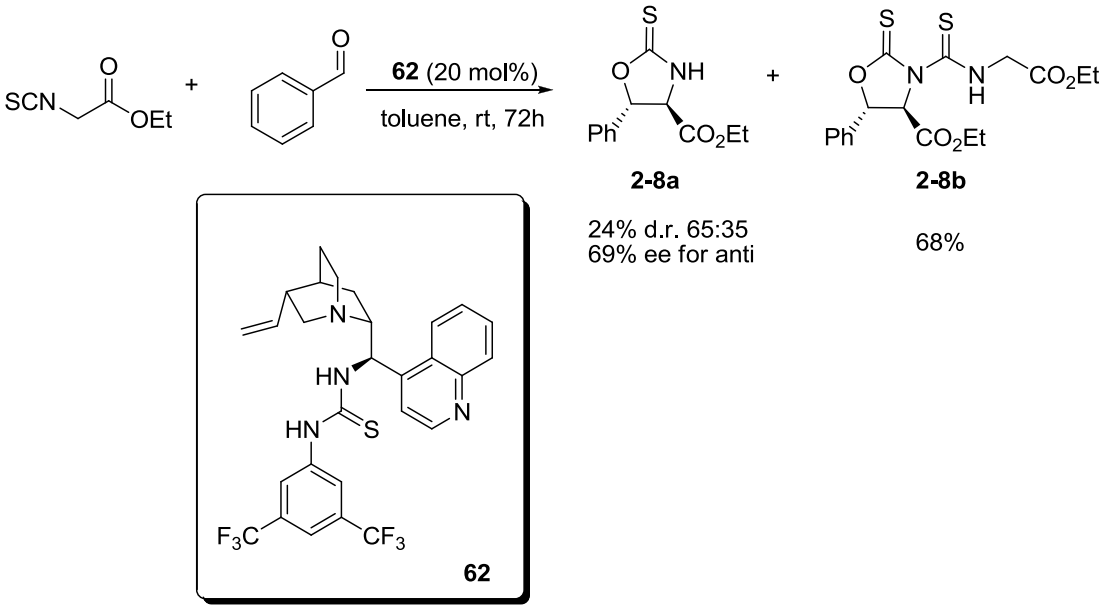
Selective examples:



90%, 97% ee | 99%, 97%ee | 88%, 97% ee | 90%, 99% ee

Scheme 2-6 Asymmetric cascade Michael-Michael reactions catalyzed by catalyst **64a**

The quinidine-derived thiourea catalyst **62** was also applied to the aldol addition of the α-isothiocyanato imide to benzyladehyde to afford the mixture of thiooxazolidinones **2-8a** and **2-8b**.[153]



Scheme 2-7 Asymmetric aldol addition of the α-isothiocyanato imide to

benzyladehyde

Inspired by these pioneer work of cascade reactions and the successfully reported of racemic synthesis of 3-nitro-1,2-dihydroquinoline,[154] a chiral version of synthesis of 3-nitro-1,2-dihydroquinoline catalyzed by cinchona alkaloids derived bifunctional thiourea catalyst through a cascade reaction seems reasonable and practical.

We envisage that a cascade process mediated by tertiray amine-thiourea catalyst can lead to a one-pot generation of chiral 3-nitro-1,2-dihydroquinoline (Figure 2-2). Installation of an electron-withdrawing group on the amino moiety of 2-aminobenzaldehyde is anticipated to render aniline N-H increased acidity, the abstraction of which by the tertiary amine leads to an aza-Michael reaction. The thiourea group in the chiral catalyst is anticipated to have hydrogen bonding interactions with the nitro group. The subsequent Henry reaction with the aldehyde, followed by dehydration is expected to generate 3-nitro-1,2-dihydroquinoline.

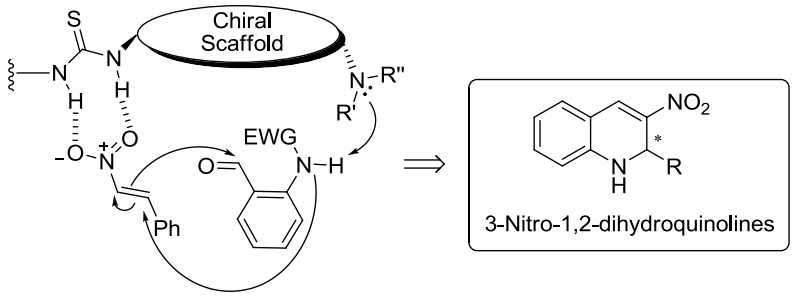


Figure 2-2 Synthesis of 3-Nitro-1,2-dihydroquinoline via a Cascade aza-Michael-Henry-Dehydration Reaction

2.2 Result and Discussion

2.2.1 Catalyst and Solvent Screening

In this reaction, sulfone was used to activate the amino group, and the reaction between N-(2-formylphenyl)benzenesulfonamide and nitrooelfin was chosen for the initial exploration.

As cinchona alkaloids are well-established powerful organic catalysts, a number of cinchona alkaloid-based organocatalysts were examined in our study.

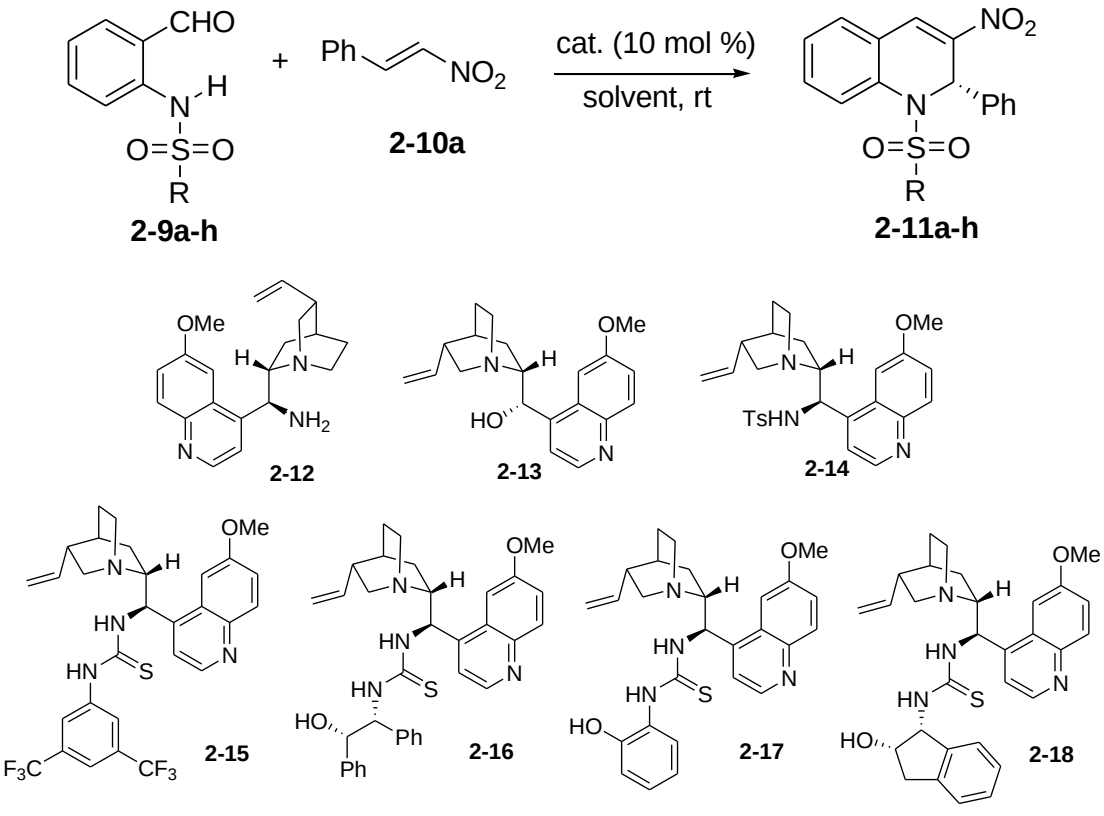
From the result of catalysts screening, quinine-derived **2-12** and quinidine **2-13** effectively promoted the projected cascade reactions, yielding the desired 3-nitro-1,2-quinolines in excellent yields, albeit in poor enantioselectivites (entries 1 and 2). Quinidine-derived sulfonamide **2-14** was completely ineffective (entry 3). While quinidine-derived thiourea catalysts **2-15**, **2-16** and **2-17** offered low enantioselectivities (entries 4-6). However, thiourea **2-18** was found to be a good catalyst, affording the desired quinoline in moderate enantioselectivity (entry 7).

Enantioselectivity was substantially improved when toluene was used as the solvent (entry 8). Electron-withdrawing sulfone group in the sulfonamide substrate is essential for the observed reactivity, as no reaction was observed when 2-aminobenzaldehyde or its N-Cbz-protected derivative was used. It suggested that sulfonamide moiety in the substrate might play an important role in asymmetric induction, thus various sulfonamides were prepared and their influences were investigated.

Mesylate afforded the product in high yield, but the enantioselectivity was

significantly decreased, suggesting that the steric hindrance of the sulfonamide may be important in the asymmetridc induction (entry 9). Sulfonamides containing various electron-withdrawing groups on the phenyl rings or naphathylene gave results comparable to those obtained with tosylsulfonamide (entries 10 to 13). By employing sterically hindered 2,4,6-trimethylbenzenesulfonamide substrate, very good enantioselectivity was attainable (entry 14). Using more hindered 2,4,6-triisopropylbenzenesulfonamide led to further improvement in the enantioselectivity of the reaction. Under the optimized reaction conditions, the desired 3-nitro-1,2-dihydroquinoline was obtained in 81% yield and with 90% ee (entry 16).

Table 2-1 Screening of Catalysts for the Organocatalytic Cascade aza-Michael-Henry-Dehydration reaction [a]



entry	cat.	R	solvent	*t* (h)	yield[b] (%)	ee[c] (%)
1	**2-12**	Ts	CH_2Cl_2	12	95	13
2	**2-13**	Ts	CH_2Cl_2	48	95	12
3	**2-14**	Ts	CH_2Cl_2	73	-	-
4	**2-15**	Ts	CH_2Cl_2	24	95	23
5	**2-16**	Ts	CH_2Cl_2	48	70	15
6	**2-17**	Ts	CH_2Cl_2	50	80	9
7	**2-18**	Ts	CH_2Cl_2	50	95	48
8	**2-18**	Ts	Toluene	72	95	70
9	**2-18**	Ms	Toluene	48	95	40
10	**2-18**	*p*CF_3-Ph	Toluene	72	43	63
11	**2-18**	*p*NO_2-Ph	Toluene	72	79	58
12	**2-18**	3,5-CF_3-Ph	Toluene	18	> 95	55
13	**2-18**	1-naphthyl	Toluene	72	> 95	72
14	**2-18**	2,4,6-Me-Ph	Toluene	60	80	86
15	**2-18**	2,4,6-*i*Pr-Ph	Toluene	60	57	90
16[d]	**2-18**	2,4,6-*i*Pr-Ph	Toluene	**72**	**81**	**90**
17	**2-18**	2,4,6-*i*Pr-Ph	CH_2Cl_2	72	60	65
18	**2-18**	2,4,6-*i*Pr-Ph	Xylene	72	70	86
19	**2-18**	2,4,6-*i*Pr-Ph	DMF	48	95	0
20	**2-18**	H	Toluene	72	--	--

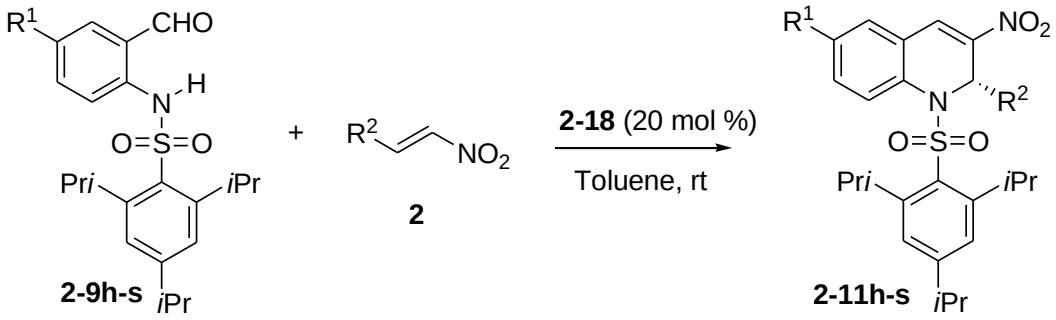
[a] The reactions were performed with **2-9** (0.025 mmol), **2-10a** (0.075 mmol) and catalyst (0.0025 mmol) in anhydrous solvent (0.2 mL) at room temperature, unless otherwise specified; [b] Isolated yield. [c] The ee value was determined by chiral HPLC analysis. [d] 20 mol% catalyst was used

2.2.2 Reaction Scope

This cascade aza-Michael-Henry-dehydration reaction is applicable to different nitroolefins (Table 2-2). Various meta- or para-substituted aryl nitroolefines were suitable substrates, and the electronic nature of the aromatic rings can also be varied, the ee vary from 81% to 90%. In addition, aliphatic nitroolefin could be used (entry 10) in the condition of increasing the catalyst loading to 50 mol%. Moreover, employment of different ortho-formyl anilines easily led to the formation of different quinolines (entry 12). However, ortho-substituted aryl nitroolefins were poor substrates, the ee could surprisingly drops a lot (entry 13) likely due to the steric

repulsion resulted from the nearby bulky sulfonamide group.

Table 2-2 Asymmetric Synthesis of Various 3-Nitro-1,2-dihydroquinolines via **2-18**-promoted Organocatalytic Cascade aza-Michael-Henry-Dehydration Reaction [a]



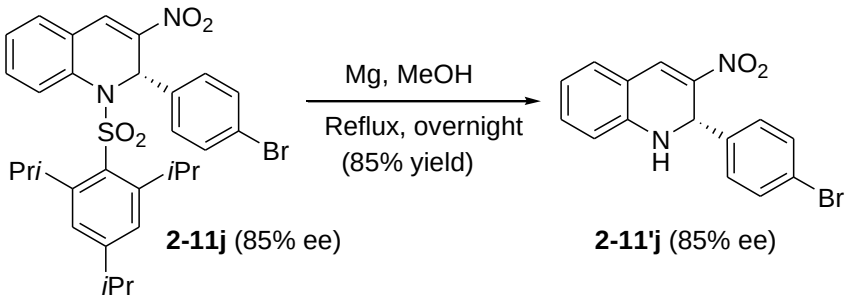
entry	product	R_1/R_2	*t* (h)	yield[b] (%)	ee[c] (%)
1	**2-11h**	H/Ph	72	81	90
2	**2-11i**	H/4-F-Ph	36	77	82
3	**2-11j**	H/4-Br-Ph	36	91	85
4	**2-11k**	H/4-CN-Ph	36	92	87
5	**2-11l**	H/4-CH_3-Ph	48	83	81
6	**2-11m**	H/4-CH_3O-Ph	48	75	84
7	**2-11n**	H/3-Cl-Ph	72	90	87
8	**2-11o**	H/3-Br-Ph	72	86	89
9	**2-11p**	H/3-CH_3-Ph	72	83	81
10[d]	**2-11q**	H/$(CH_3)_2$C-Ph	72	86	70
11	**2-11r**	H/2-Naphthyl	72	75	82
12	**2-11s**	Cl/Ph	72	78	88
13	**2-11t**	H/2-CH_3-Ph	72	60	17

[a] The reactions were performed with **2-9** (0.025 mmol), **2-10** (0.075 mmol) and **2-18** (0.0025 mmol) in anhydrous toluene (0.5 mL) at room temperature; [b] Isolated yield. [c] The ee value was determined by chiral HPLC analysis. [d] 50 mol % catalyst was used

2.2.3 Synthesis of 3-nitro-1,2-dihydroquinolines

The sulfonamide group could be easily removed by reductive cleavage. When **2-11j** was treated with magnesium in methanol under reflux, 2-aryl-substituted 3-nitro-1,2-dihydroquinoline **2-11'j** was obtained in good yield. It should be noted

that the enantioselectivity was maintained and the bromine atom on the benzene ring was not affected. (Scheme 2-8)



Scheme 2-8 Cleavage of sulfonamide group

2.3 Conclusion

The examples described above have successfully demonstrated that the power of cascade reaction catalyzed by cinchona alkaloids derived bifunctional thiourea catalyst. These cascade reactions have provided new and efficient approaches to construct the complex chiral molecules from simple achiral substrates. For the cascade aza-Michael-Henry-dehydration reaction developed by our group, it was demonstrated the efficient preparation of chiral 3-nitro-1,2-quinolines which were quite important and useful in the medicinal chemistry. By installing an electron-withdrawing sulfone group on anilines, the substrates could be activated by well-established tertiary amine-thiourea catalysts, and this strategy may find wide applications in the preparation of nitrogen-containing heterocycles.

Mechanistic understanding of this cascade reaction, development of other organocatalytic cascade processes and their applications to the asymmetric synthesis of flavonoids and their structural analogues are currently in progress.

2.4 Experimental Section

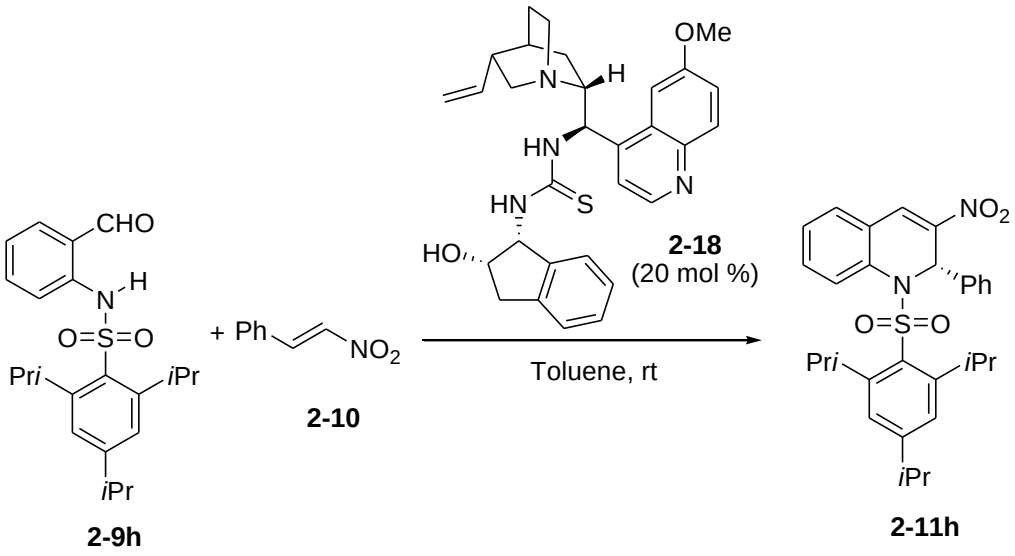
2.4.1 General Information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 or DPX300 (300 MHz) or AMX500 (500 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.0). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants were reported in Hertz (Hz). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode, and a Finnigan/MAT 95XL-T mass spectrometer in FAB mode and mass spectrometer in EI mode. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. For thin-layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F_{254}) were used, and compounds were visualized with a UV light at 254 nm. Flash chromatography separations were performed on Merck 60 (0.040 - 0.063 mm) mesh silica gel.

Different sulfonamide-containing 2-aminobenzaldehydes were synthesized according to the literature procedure,[155] and the thiourea catalysts were prepared following the literature procedures.[156]

2.4.2 Representative Procedures

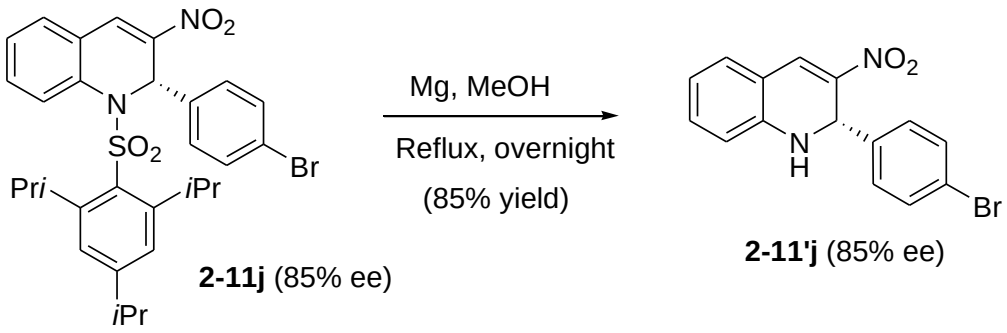
2.4.2.1 Representative Procedure for Cascade aza-Michael-Henry-Dehydration Reaction



Scheme 2-9 Cascade aza-Michael-Henry-Dehydration reaction by catalyst **2-18**

A solution of *N*-(2-formylphenyl)-2,4,6-triisopropylbenzenesulfonamide **2-9h** (9.7 mg, 0.025 mmol), (*E*)-(2-nitrovinyl)benzene **2-10** (11.2 mg, 0.075 mmol) and thiourea **2-18** (2.6 mg, 0.005 mmol) in toluene (200 μL) was stirred at room temperature for 72 hrs. The mixture was then diluted with water, and extracted with CH_2Cl_2 several times. The combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash chromatographic column (EtOAc: Hexanes = 1: 10) to afford **2-11h** as a light yellow solid (10.5 mg, 81% yield).

2.4.2.2 Representative Procedure for the Reductive Desulfonylation



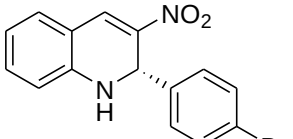
Scheme 2-10 Reductive desulfonylation by Mg and MeOH

To a mixture of **2-11j** (44.7 mg, 0.075 mmol) and activated Mg (18 mg, 0.75 mmol) in a 25 mL round bottom flask was added anhydrous methanol (10 mL). The reaction solution was brought to reflux overnight. After cooling down to room temperature, the reaction mixture was quenched by adding 1 M aqueous HCl, and extracted by CH_2Cl_2 several times. The combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash chromatographic column (EtOAc: hexanes = 1: 8) to afford **2-11'j** as a dark red solid (21 mg, 85% yield).

2.4.3 Determination of the Absolute Configurations of Products

The absolute configuration of **2-11'j** was determined by comparing the HPLC traces of **2-11'j** with those reported in the literature,[157] and the absolute configuration of **2-11j** was deduced accordingly. The configurations of other quinolines were assigned by analogy.

(*S*)-2-(4-Bromophenyl)-3-nitro-1,2-dihydroquinoline **2-11'j**

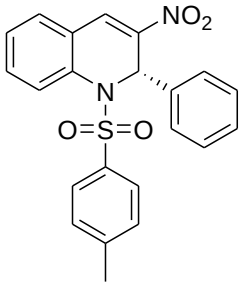


2-11'j

HRMS (EI) m/z calcd for $C_{15}H_{11}O_2N_2{}^{79}Br_1$ $[M]^+$ = 330.0004, found = 329.9994; $C_{15}H_{11}O_2N_2{}^{81}Br_1$ $[M]^+$ = 331.9983, found = 331.9989. The NMR spectra of **2-11'j** were consistent with the literature data. The enantiomers of **2-11'j** were analyzed by chiral phase HPLC using OD-H column at 254 nm (2-propanol/hexane = 15/85), flow rate = 1.0 mL/min; major enantiomer: t_R = 19.8 min, minor enantiomer: t_R = 15.9 min (literature:[3] major enantiomer: t_R = 21.0 min, minor enantiomer: t_R = 15.6 min).

2.4.4 Analytical data of substrates and products

(*S*)-3-Nitro-2-phenyl-1-tosyl-1,2-dihydroquinoline **2-11a**

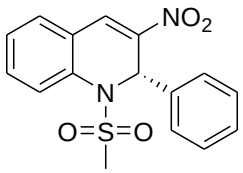


2-11a

A yellow solid; Isolated in 95% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5: 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H), 6.90 (s, 1H), 7.11-7.14 (d, *J* = 8.4 Hz, 2H), 7.28-7.32 (m, 4H), 7.48 (m, 1H), 7.57 (s, 1H), 7.77-.7.79 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 55.5, 125.1, 126.6, 126.8, 127.6,128.5, 128.6, 128.7, 128.8, 129.6, 129.8, 132.5, 134.2,

134.8, 135.1, 144.0, 144.6. The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 10: 90), 1.0 mL/min; Major enantiomer: t_R = 15.8 min, minor enantiomer: t_R = 13.0 min. HRMS (ESI) m/z calcd for $C_{22}H_{18}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 429.0880, found = 429.0894.

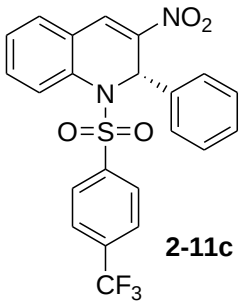
(*S*)-1-(Methylsulfonyl)-3-nitro-2-phenyl-1,2-dihydroquinoline **2-11b**



2-11b

A yellow oil; Isolated in 95% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5: 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.78 (s, 3H), 6.88 (s, 3H), 7.25-7.34 (m, 6H), 7.44-7.52 (m, 2H), 7.65-7.66 (d, *J* = 8.2 Hz, 1H), 8.15(s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.6, 55.7, 124.0, 126.3, 126.6, 127.1, 128.8, 129.0, 129.2, 130.5, 133.0, 134.4, 135.4, 144.7; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 10: 90), 1.0 mL/min; Major enantiomer: t_R = 26.9 min, minor enantiomer: t_R = 20.0 min. HRMS (EI) m/z calcd for $C_{16}H_{14}O_4N_2$ $^{32}S_1$ $[M]^+$ = 330.0674, found = 330.0672.

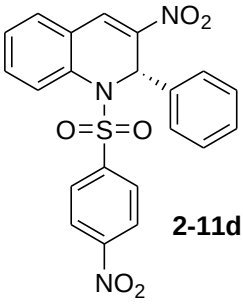
(*S*)-3-Nitro-2-phenyl-1-(4-(trifluoromethyl)phenylsulfonyl)-1,2-dihydroquinoline **2-11c**



2-11c

A yellow solid; Isolated in 43% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92-6.95 (m, 1H), 7.27-7.31 (m, 5H), 7.35-7.38 (m, 2H), 7.50-7.62 (m, 6H), 7.79-7.83 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 121.0, 124.6, 124,9, 125.9, 126.0, 126.1, 126.2, 126,7, 127.1, 128.0, 128.3, 128.4, 128.8, 129.0, 130.1, 132.8, 133.4, 134.5, 134.9, 135.4, 141.0, 144.0; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 10: 90), 1.0 mL/min; Major enantiomer: t_R = 16.0 min, minor enantiomer: t_R = 9.0 min. HRMS (EI) m/z calcd for $C_{22}H_{15}O_4N_2F_3\,^{32}S_1$ $[M]^+$ = 460.0705, found = 460.0711.

(*S*)-3-Nitro-1-(4-nitrophenylsulfonyl)-2-phenyl-1,2-dihydroquinoline **2-11d**

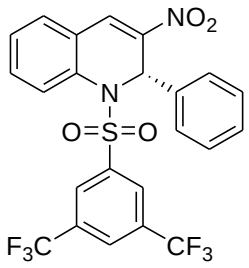


2-11d

A yellow oil; Isolated in 79% yield after flash column chromatographic purification (hexane: ethyl acetate = 5: 1 to 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91

(s, 1H), 7.21-7.27 (m, 5H), 7.34-7.37 (m, 2H), 7.51-7.60 (m, 4H), 7.76-7.79 (d, *J* = 7.9 Hz, 1H), 8.15-8.18 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8, 124.1, 124.9, 126.7, 127.9, 128.2, 128.4, 128.9, 129.1, 130.2, 132.9, 133.1, 134.2, 143.0, 144.0, 150.4. The enantiomers were analyzed by HPLC using an IA -H column at 254 nm (2-propanol: hexane = 10: 90), 1.0 mL/min; Major enantiomer: t_R = 54.8 min, minor enantiomer: t_R = 26.5 min. HRMS (EI) m/z calcd for $C_{21}H_{15}O_6N_3{}^{32}S_1$ $[M]^+$ = 437.0682, found = 429.0680.

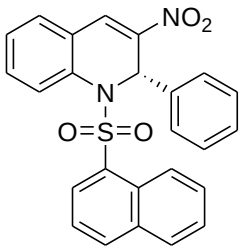
(*S*)-1-(3,5-bis(trifluoromethyl)phenylsulfonyl)-3-nitro-2-phenyl-1,2-dihydroquinoline **2-11e**



2-11e

A yellow solid, isolated in 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (s, 1H), 7.22-7.46 (m, 7H), 7.53-7.61 (m, 2H), 7.80-7.82 (d, *J* = 1.3 Hz, 2H), 8.05 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 123.7, 124.9, 126.6, 126.7, 126.8, 127.9, 128.4, 128.5, 128.9, 129.2, 130.2, 132.6, 132.9, 133.0, 133.9, 139.8, 144.0; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 2: 98), 0.5 mL/min; Major enantiomer: t_R = 13.3 min, minor enantiomer: t_R = 11.6 min. HRMS (EI) m/z calcd for $C_{23}H_{14}O_4N_2F_6$ $^{32}S_1$ $[M]^+$ = 528.0578, found = 528.0572.

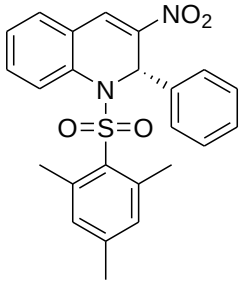
(*S*)-1-(Naphthalen-1-ylsulfonyl)-3-nitro-2-phenyl-1,2-dihydroquinoline **2-11f**



2-11f

A yellow solid; Isolated in 95% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (s, 1H), 7.23-7.31 (m, 7H), 7.33-7.42 (m, 2H), 7.50-7.67 (m, 3H), 7.78-7.80 (d, *J* = 8.5 Hz, 2H), 7.85-7.89 (m, 2H), 8.00-8.01 (d, *J* = 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 121.5, 125.1, 126.7, 127.6, 127.8, 127.8, 128.2, 128.3, 128.4, 128.7, 128.8, 129.1, 129.2, 129.3, 129.8, 131.7, 132.5, 134.0, 134.6, 134.9, 135.0, 143.9; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 10: 90), 1.0 mL/min; Major enantiomer: t_R = 26.7 min, minor enantiomer: t_R = 21.3 min. HRMS (EI) m/z calcd for $C_{25}H_{18}O_4N_2{}^{32}S_1$ $[M]^+$ = 442.0987, found = 442.0986.

(*S*)-1-(Mesitylsulfonyl)-3-nitro-2-phenyl-1,2-dihydroquinoline **2-11g**

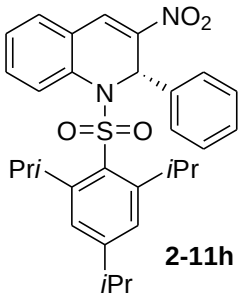


2-11g

A yellow solid; Isolated in 80% yield after flash column chromatographic

purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H), 2.46 (s, 6H), 6.68 (s, 1H), 6.94 (s, 2H), 7.19-7.30 (m, 7H), 7.40-7.43 (m, 2H), 7.53 (m, 1H), 7.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9, 22.3, 54,4, 125.1, 126.7, 126.9, 128.6, 128.7, 129.0, 130.2, 132.1, 132.3, 132.5, 135.0, 135.3, 140.3, 143.6, 144.6; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 5: 95), 1.0 mL/min; Major enantiomer: t_R = 13.5 min, minor enantiomer: t_R = 15.4 min. HRMS (ESI) m/z calcd for $C_{24}H_{22}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 457.1193, found = 457.1173.

(*S*)-3-Nitro-2-phenyl-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11h**

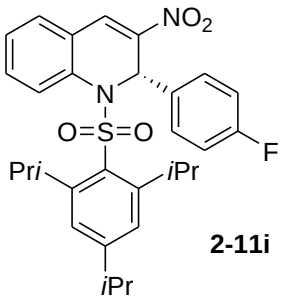


2-11h

A yellow oil; Isolated in 81% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.17 (t, *J* = 6.8 Hz, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.90-2.95 (m, 1H), 3.88-3.92 (m, 2H), 6.78 (s, 1H), 7.17-7.40 (m, 12H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.5, 24.8, 54.2, 124.1, 125.2, 126.5, 126.6, 126.8, 128.6, 128.7, 129.3, 130.4, 132.2, 135.1, 135.7, 145.2, 151.8, 154.2; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 5: 95), 1.0 mL/min; Major enantiomer: t_R = 17.1 min, minor enantiomer: t_R = 20.3 min.

HRMS (ESI) m/z calcd for $C_{30}H_{34}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 541.2132, found = 541.2157.

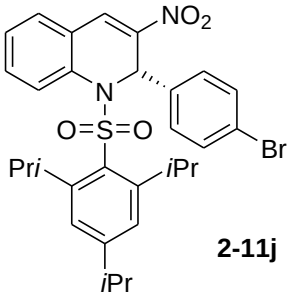
(*S*)-2-(4-Fluorophenyl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11i**



2-11i

A yellow oil; Isolated in 77% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.14 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.90-2.95 (m, 1H), 3.88-3.92 (m, 2H), 6.75 (s, 1H), 6.90-6.93 (m, 2H), 7.15-7.17 (m, 2H), 7.30 (s, 2H), 7.39-7.41 (m, 3H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 30.0, 34.1, 53.6, 115.5, 115.8, 124.2, 124.9, 126.4, 126.7, 128.6, 128.7, 129.4, 130.4, 130.8, 131.6, 132.4, 135.0, 145.0, 151.8, 154.3; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 16.2 min, minor enantiomer: t_R = 21.9 min. HRMS (ESI) m/z calcd for $C_{30}H_{33}O_4N_2{}^{35}F_1{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 559.2037, found = 559.2018.

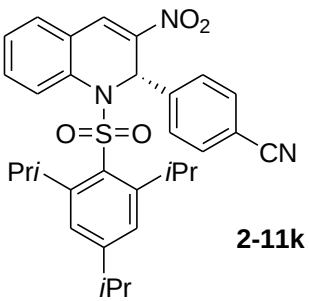
(*S*)-2-(4-Bromophenyl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11j**



2-11j

A yellow solid; Isolated in 91% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300MHz, $CDCl_3$) δ 1.12-1.14 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.90-2.95 (m, 1H), 3.85 (m, 2H), 6.74 (s, 1H), 7.08-7.10 (d, *J* = 8.5 Hz, 2H), 7.17 (s, 2H), 7.30-7.42 (m, 6H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.6, 24.8, 30.1, 34.1, 53.7, 122.9, 124.2, 124.8, 126.4, 126.7, 128.5, 129.6, 130.5, 130.7, 131.9, 132.5, 134.9, 135.0, 144.6, 151.8, 154.4; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 17.8 min, minor enantiomer: t_R = 22.2 min; HRMS (ESI) m/z calcd for $C_{30}H_{33}O_4N_2{}^{79}Br_1{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 619.1237, found = 619.1254; calcd for $C_{30}H_{33}O_4N_2{}^{81}Br_1{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 621.1216, found = 621.1240.

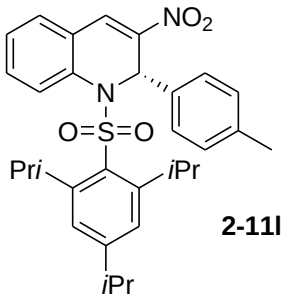
(*S*)-4-(3-Nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinolin-2-yl)benzonitrile **2-11k**



2-11k

A yellow oil; Isolated in 92% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11-1.17 (m, 12H), 1.26-1.28 (d, *J* = 6.9 Hz, 6H), 2.91 (m, 1H), 3.84 (m, 2H), 6.81 (s, 1H), 7.17 (s, 2H), 7.17-7.45 (m, 7H), 7.55-7.58 (d, *J* = 8.2 Hz, 2H), 8.08 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.6, 24.8, 30.2, 34.1, 53.8, 112.8, 118.0, 124.8, 126.2, 126.9, 127.6, 130.0, 130.5, 130.7, 132.5, 132.7, 134.9, 141.1, 143.9, 151.8, 154.6; The enantiomers were analyzed by HPLC using an IA -H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 36.6 min, minor enantiomer: t_R = 73.0 min. HRMS (ESI) m/z calcd for $C_{31}H_{33}O_4N_3{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 566.2084, found = 566.2085.

(*S*)-3-Nitro-2-p-tolyl-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11l**

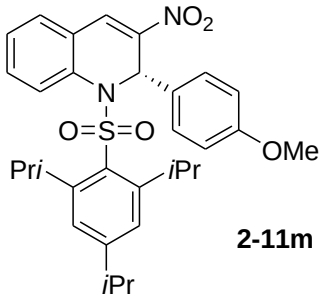


2-11l

A yellow oil; Isolated in 83% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.17 (t, *J* = 7.2 Hz, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.28 (s, 3H), 2.90 (m, 2H), 3.90 (m, 2H), 6.74 (s, 1H), 7.05-7.06 (m, 4H), 7.17 (s, 2H), 7.17-7.41 (m, 5H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 21.0, 23.3, 23.4, 24.6, 24.8, 30.0, 34.1, 124.1, 125.2, 126.4, 126.5, 126.7, 129.2, 129.3, 130.3, 131.0, 132.2, 132.7, 135.1,

138.6, 145.3, 151.8, 154.1; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 22.4 min, minor enantiomer: t_R = 18.5 min. HRMS (ESI) m/z calcd for $C_{31}H_{36}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 555.2288, found = 555.2305.

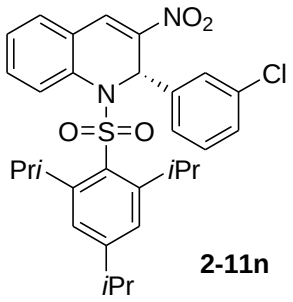
(*S*)-2-(4-Methoxyphenyl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11m**



2-11m

A yellow oil; Isolated in 75% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.17 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.93 (m, 1H), 3.75 (s, 1H), 3.90 (m, 2H), 6.72-6.77 (m, 3H), 7.07-7.08 (d, *J* = 3.1 Hz, 2H), 7.12 (s, 2H), 7.30-7..49 (m, 3H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.6, 24.8, 29.6, 30.0, 53.8, 55.1, 114.0, 124.1, 125.1, 126.4, 126.5, 127.8, 128.2, 129.1, 130.3, 130.9, 132.2, 135.1, 145.4, 151.8, 154.1, 159.9; The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 24.5 min, minor enantiomer: t_R = 33.0 min. HRMS (ESI) m/z calcd for $C_{31}H_{36}O_5N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 571.2237, found = 571.2249.

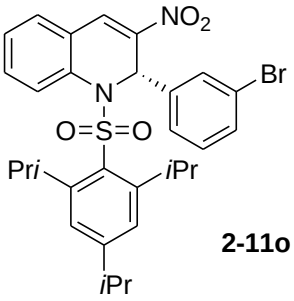
(*S*)-2-(3-Chlorophenyl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11n**



2-11n

A yellow oil; Isolated in 90% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.17 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.90-2.95 (m, 1H), 3.88-3.92 (m, 2H), 6.76 (s, 1H), 7.14-7.29 (m, 10H), 7.40-7.41 (d, *J* = 3.6 Hz, 2H), 8.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 23.4, 24.5, 24.8, 30.1, 34.1, 53.7, 124.2, 124.9, 125.0, 126.6, 126.9, 127.1, 129.0, 129.7, 129.9, 130.6, 132.5, 134.6, 134.8, 137.7, 144.5, 151.9, 154.4. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 14.1 min, minor enantiomer: t_R = 20.7 min. HRMS (ESI) m/z calcd for $C_{30}H_{33}O_4N_2{}^{35}Cl_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 575.1742, found = 575.1730.

(*S*)-2-(3-Bromophenyl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11o**

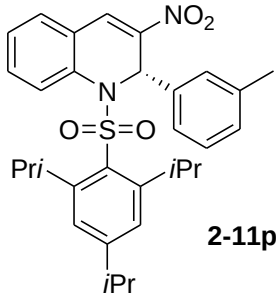


2-11o

A yellow solid; Isolated in 86% yield after flash column chromatographic

purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.15 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.91-2.96 (m, 1H), 3.85 (m, 2H), 6.74 (s, 1H), 7.11-7.13 (m, 2H), 7.19 (s, 2H), 7.33-7.42 (m, 3H), 7.48-7.51 (d, *J* = 7.2 Hz, 1H), 8.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.5, 25.8, 53.6, 122.7, 124.2, 125.1, 125.3, 126.6, 126.9, 129.8, 130.0, 130.2, 131.9, 132.5, 134.8, 138.0, 144.5, 151.9, 154.5; The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3 : 97), 0.5 mL/min; Major enantiomer: t_R = 14.0 min, minor enantiomer: t_R = 20.5 min. HRMS (ESI) m/z calcd for $C_{30}H_{33}O_4N_2{}^{79}Br_1{}^{23}Na_1{}^{32}S_1$ and $C_{30}H_{33}O_4N_2{}^{81}Br_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 619.1237 and 621.1216, found = 619.1260 and 621.1248.

(*S*)-3-Nitro-2-m-tolyl-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11p**

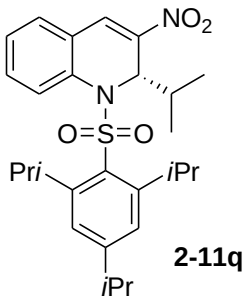


2-11p

A yellow oil; Isolated in 83% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.17 (m, 12H), 1.27-1.29 (d, *J* = 6.9 Hz, 6H), 2.27 (s, 3H), 2.91-2.95 (m, 1H), 3.88-3.90 (m, 2H), 6.75 (s, 1H), 6.98 (m, 2H), 7.07-7.11 (m, 2H), 7.18 (s, 2H), 7.30 (m, 1H), 7.37-7.40 (m, 2H), 7.46 (m, 1H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 23.3, 23.4, 24.5, 24.9, 29.6, 30.0, 34.1, 54.2, 123.7, 124.1, 125.3, 126.6, 127.6,

128.5, 129.3, 129.5, 130.4, 130.9, 132.2, 135.1, 135.5, 138.4, 145.3, 151.8, 154.2; The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 6.4 min, minor enantiomer: t_R = 8.8 min. HRMS (ESI) m/z calcd for $C_{31}H_{36}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 555.2288, found = 555.2304.

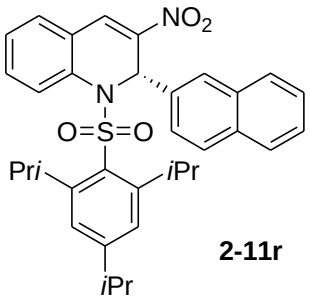
(*S*)-2-Isopropyl-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11q**



2-11q

A yellow oil; Isolated in 86% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87-0.89 (d, *J* = 6.7 Hz, 3H), 1.03-1.05 (d, *J* = 6.6 Hz, 3H), 1.11-1.17 (m, 12H), 1.24-1.26 (d, *J* = 6.9 Hz, 6H), 2.84-2.94 (m, 1H), 3.72-3.81 (m ,2H), 5.63-5.67 (dd, *J* = 10.0 Hz, 4.11 Hz, 1H), 7.12 (s, 2H), 7.30-7.35 (m, 1H), 7.40-7.48 (m, 2H), 7.53-7.56 (m, 1H), 7.68 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 23.1, 23.4, 24,6, 25.0, 30.2, 34.1, 40.5, 50.6, 123.9, 125.6, 126.7, 127.3, 127.8, 130.2, 131.3, 131.8, 134.6, 147.3, 151.6, 153.9; The enantiomers were analyzed by HPLC using two combined IC-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 12.2 min, minor enantiomer: t_R = 10.6 min. HRMS (ESI) m/z calcd for $C_{27}H_{36}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 507.2408, found = 507.2428.

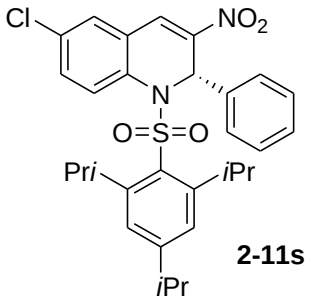
(*S*)-2-(Naphthalen-2-yl)-3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11r**



2-11r

A yellow oil; Isolated in 75% yield after flash column chromatographic purification (hexane: ethyl acetate = 8:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.18 (m, 12H), 1.28-1.47 (d, *J* = 6.9 Hz, 6H), 2.91-2.96 (m, 1H), 3.92 (m, 2H), 6.97 (s, 1H), 7.19 (s, 2H), 7.18-7.52 (m, 8H), 7.68-7.78 (m, 3H), 8.09 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 23.5, 24.7, 25.0, 30.2, 34.2, 54.4, 124.3, 124.7, 125.3, 126.0, 126.3, 126.5, 126.7, 126.8, 127.6, 128.1, 128.9, 129.7, 130.5, 131.1, 132.3, 132.9, 133.0, 133.3, 135.1, 145.1, 151.9, 154.3; The enantiomers were analyzed by HPLC using two combined IC-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 13.3 min, minor enantiomer: t_R = 20.6 min. HRMS (ESI) m/z calcd for $C_{34}H_{36}O_4N_2{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 591.2288, found = 591.2310.

(*S*)-6-Chloro-3-nitro-2-phenyl-1-(2,4,6-triisopropylphenylsulfonyl)-1,2-dihydroquinoline **2-11s**



2-11s

A yellow oil; Isolated in 78% yield after flash column chromatographic purification (hexane: ethyl acetate = 10: 1 to 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12-1.13 (m, 12H), 1.24-1.25 (m, 6H), 2.87-2.92 (m, 1H), 3.81-3.87 (m, 2H), 6.70 (s, 1H), 7.11-7.15 (m, 2H), 7.22-7.26 (m, 3H), 7.31-7.33 (m, 2H), 7.42 (s, 1H), 7.88 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 23.4, 24.5, 24.8, 30.1, 34.1, 124.2, 126.5, 126.7, 127.6, 128.0, 128.76, 128.8, 128.9, 129.0, 129.6, 130.4, 131.8, 132.1, 133.5, 135.3, 146.1, 151.9, 154.5; The enantiomers were analyzed by HPLC using two combined IC-H columns at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 8.8 min, minor enantiomer: t_R = 12.2 min; HRMS (ESI) m/z calcd for $C_{30}H_{33}O_4N_2{}^{35}Cl_1{}^{23}Na_1{}^{32}S_1$ $[M+Na]^+$ = 575.1742, found = 575.1760.

Chapter 3 Asymmetric Synthesis of 2-Aryl-2,3-dihydro-4-quinolones via Bifunctional Thiourea-mediated Intramolecular Cyclization

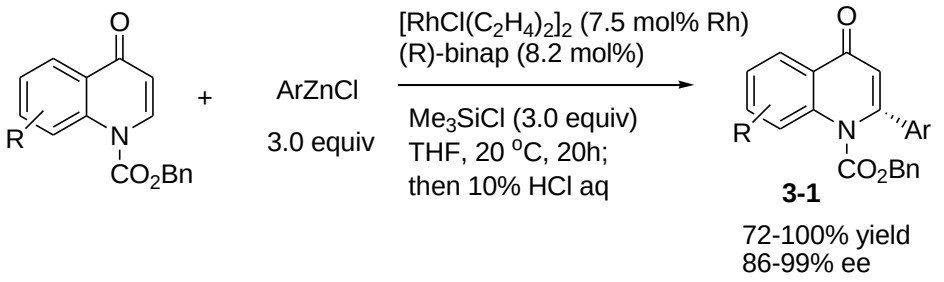
3.1 Introduction

As another important class of six-membered N-heterocycles, the quinolones can be considered as a family of compounds with broad-spectrum antibiotic properties.[158] In particular, 2-aryl-2,3-dihydro-4-quinolones represent a new class of antitumor agents.[159-160]

They have shown potent cytotoxic and antitubulin effects. Strong cytotoxic effects with ED_{50} values were demonstrated by these compounds in the nanomolar or subnanomolar range in almost all tumor cell lines. Since individual stereoisomers of quinolones displayed different biological acitivities, it is highly desirable to develop enantioselective routes to access this class of important antibiotics.

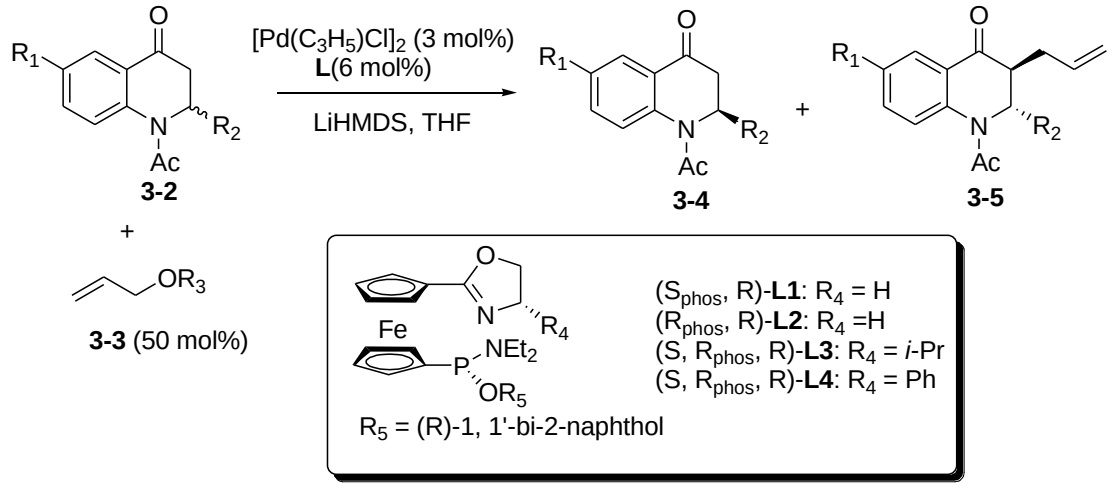
However, the methods of asymmetric synthesis of chiral 2-aryl-2,3-dihydro-4-quinolones were very limited. Generally, the synthesis of these racemic compounds are carried out by acid or base catalyzed isomerization of 2'-aminochalcones via the aza Michael reaction in the alumina or silica gel supported conditions.[161-166] So far to the best of our knowledge, only two examples have been reported in the literature to synthesize the chiral compounds, both making use of transition metal-mediated catalytic processes. Hayashi and coworker reported an asymmetric synthesis of 2-aryl-2,3-dihydro-4-quinolones via a rhodium-catalyzed 1,4-addition of arylzinc reagent to 4-quinolones.[167] These 1,4-adducts can be obtained

with high enantioselectivity by using (R)-binap as a ligand, and high yields are realized by conducting the reactions in the presence of chlorotrimethylsilane.



Scheme 3-1 Asymmetric synthesis of **3-1** via a rhodium-catalyzed 1,4-addition

Recently, Hou et al. disclosed a kinetic resolution of 2,3-dihydro-2-substituted 4-quinolones by a palladium-catalyzed asymmetric allylic alkylation, affording chiral 2,3-disubstituted 2,3-dihydro-4-quinolones in excellent enantioselectivity. [168]

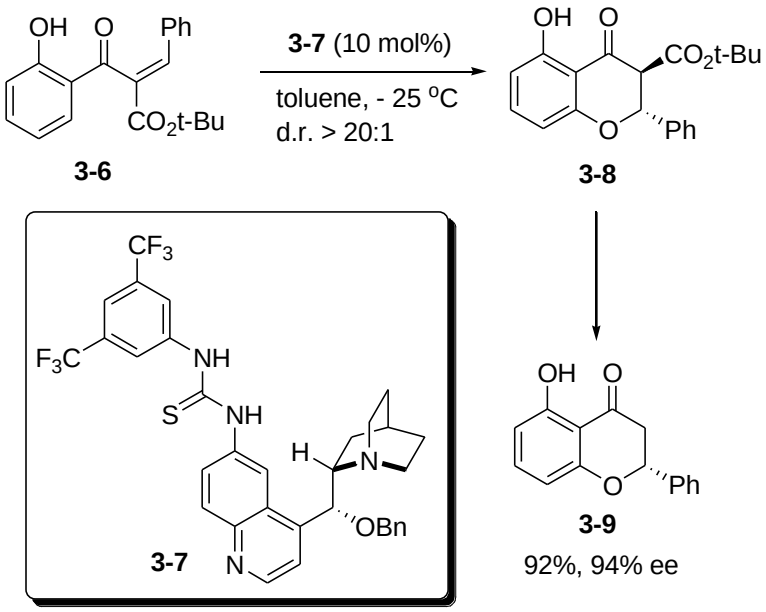


Scheme 3-2 Kinetic resolution of palladium-catalyzed asymmetric allylic alkylation

Given the biological importance of 2-aryl-2,3-dihydro-4-quinolones, developing organocatalytic synthetic methods for the practical preparation of these valuable

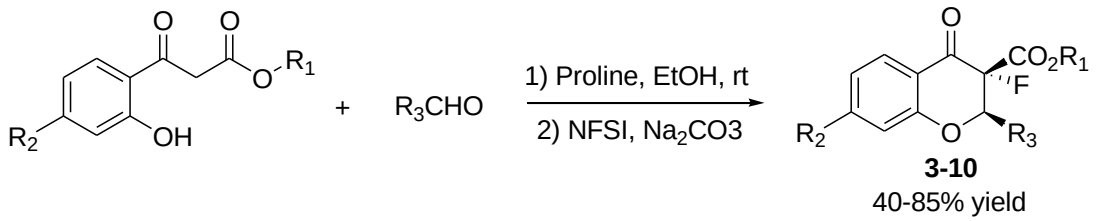
molecules is on demand.

Recently, a great method was induced by Scheidt group in the asymmetric synthesis of flavanones and chromanones.[169] Cinchona alkaloids derived bifunctional thiouea catalysts were used to efficiently catalyze the cyclization reaction. After decarboxylation reaction, the chiral flavanones and chromanones were obtained with high yields and enantioselectivities.



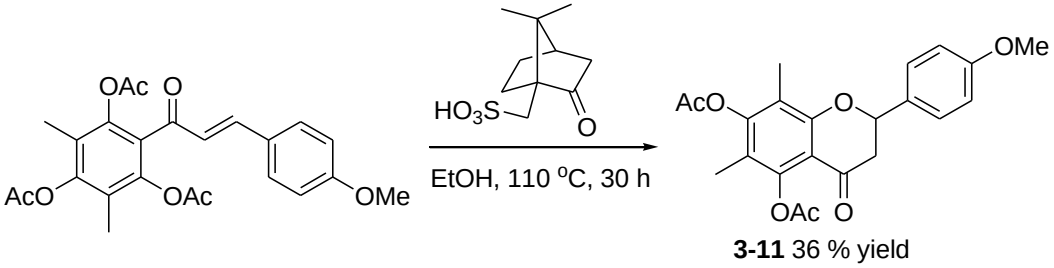
Scheme 3-3 Asymmetric synthesis of flavanones and chromanones

Zhao group [170-171] later extended this work by introducing fluorine to the chiral center formed by Oxa-Michael Addition to get the fluorinated flavanone derivatives in this one-pot tandem reaction.



Scheme 3-4 Asymmetric synthesis of fluorinated flavanone derivatives

These flavanones could also be prepared by Asymmetric Cyclization of 2-Hydroxychalcones catalyzed by Chiral CSA. [172]



Scheme 3-5 Asymmetric Cyclization of 2-Hydroxychalcones catalyzed by Chiral CSA

Inspired by these excellent work and considered about the advantages of cyclization reaction and its great utility in the natural product synthesis,[173-174] an attractive strategy was designed to stereoselective synthesis of 2-aryl-2,3-dihydro-4-quinolones through an intramolecular conjugate addition of aniline to a properly activated alkene moiety by cinchona alkaloids derived bifunctional thiourea catalysts (Figure 3-1). The key issue here is how to affect the cyclization in an efficient and stereocontrolled manner.

In the previous chapter, the asymmetric preparation of 3-nitro-1,2-dihydroquinolines was reported by a bifunctional thioued-promoted cascade aza-Michael-Henry-dehydration reaction,[175] in which the nitrogen

nucleophiles were activatived by installing a sulfonyl group. Thus, we envisioned that the sufonyl substituted aniline could be used as the nucleophilic component; in the presence of bifunctional tertiary amine thiourea catalysts, [176-181] the acidic proton of the sulfonamide can be readily removed, initiating the subsequent aza-Michael type[182-188] intramolecular cyclization reaction.

To better activate the electrophiles, an ester group could be installed at the α-position of the carbonyl function. Such an ester group is expected to facilitate hydrogen bonding interactions between the substrates and the thiourea catalysts, leading to a better stereochemical control. With the established protocols for its decarboxylative cleavage, the ester group can be readily removed at the end of the synthesis, without affecting the integrity of the newly created stereogenic center at the 2-position of chiral quinolones.

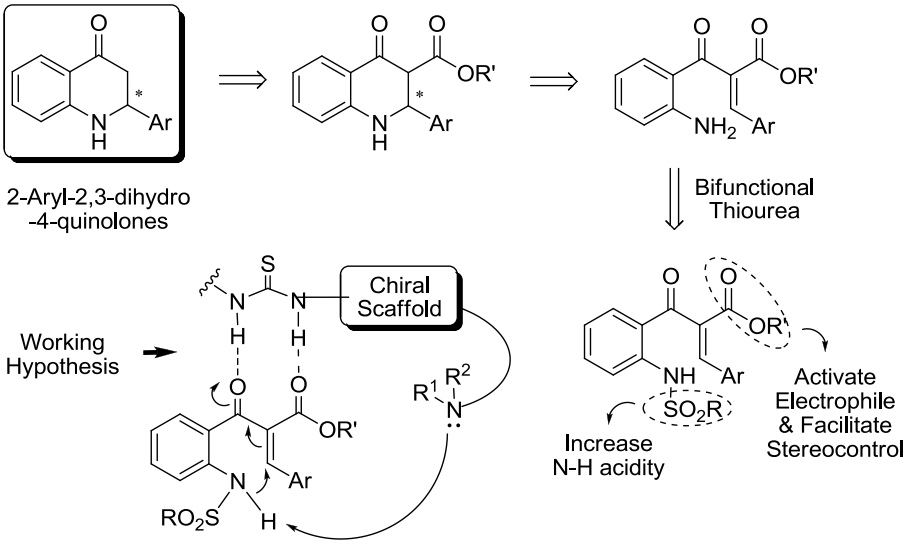


Figure 3-1 Preparation of 2-Aryl-2,3-dihydro-4-quinolones via an intramolecular cycliztion Reaction

3.2 Result and Discussion

3.2.1 Catalysts and Solvent Screening

To prove the validity of our proposed synthetic strategy, alkylidene β-ketoester **3-15a** with a properly installed neighboring sulfonamide group was treated with catalyst **3-12** (Figure 3-2)**.**

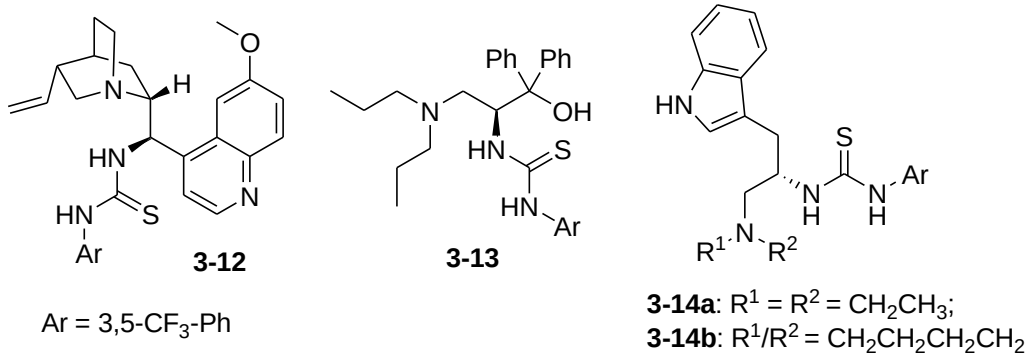
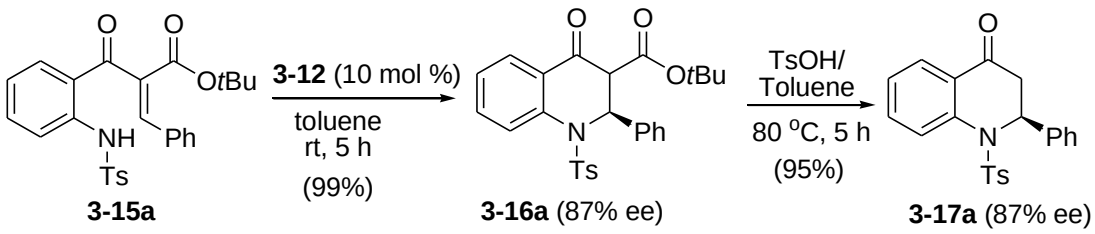


Figure 3-2 Bifunctional tertiary amine thiourea catalysts employed in the study

To our delight, the intramolecular cyclization occurred smoothly to yield product **3-16a** in quantitative yield and with 87% ee. The next step is the cleavage of the ester group. Upon further treatment with TsOH at the elevated temperature, the decarboxylation [189-193] took place readily, and 2-phenyl-2,3-dihydro-4-quinolone **3-17a** was obtained in excellent yield and with 87% ee (Scheme 3-6).



Scheme 3-6 Synthesis of 2-Phenyl-2,3-dihydro-4-quinolone **3-17a** via Conjugate

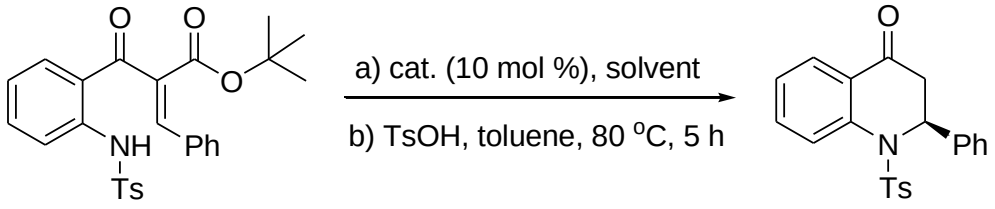
addition- Decarboxylation Sequence

The enantiomeric purity of the final quinolone products is determined by the stereochemical outcome of the first intramolecular cyclization step, as the second decarboxylation step will not compromise the stereogenic integrity of the quinolone intermediates created. The absoluted configuration of final product **6a** was determined by comparing the exact HPLC condition referring to the literature.[194]

The catalytic effects of various bifunctional catalysts (Table 3-1) and different reaction conditions in the intramolecular conjugate addition step were investigated, aiming to improve the enantioselectivity of the process. L-Threonine-derived **3-13**, and L-tryptophan-based bifunctional catalysts **3-14a** [195] and **3-14b** all could promote the reactions which have shown great reactivities and enantioselectivities in the Michael reaction with other donors and accepters. However, the results in this reaction were disappointing. Only quinidine-derived bifunctional thiourea catalyst **3-12** could give the satisfied result. The desired products were obtained with low enantioselectivity (Table 3-1, entries 2-4). A solvent screening revealed that toluene was the best reaction medium for the reaction. Other polar solvent, such as EtOAc and CH_3CN did not provide the better results (Table 3-1, entries 8 and □10), which indicate that polar reaction medium may disturb the hydrogen bonding interaction between the substrates and the catalysts. By lowering the reaction temperature to 0 ^oC, the desired quinolone **3-17a** could be obtained in high yield and with 94% ee (entry 12). The sulfonyl group on the nitrogen was crucial, employment of alkylidene β-ketoester with the free amino group resulted in formation of the product with only

78% ee, comparing to a 94% ee under otherwise same reaction conditions.

Table 3-1 Catalyst Screening for the Synthesis of Quinolones via an Intramolecular Cyclization-Decarboxylation Sequence [a]



3-15a **3-17a**

entry	cat.	solvent	t[b] (h)	T (°C)	Yield[c] (%)	ee[d] (%)
1	**3-12**	Toluene	5	rt	99/95	87
2	**3-13**	Toluene	72	rt	79/89	11
3	**3-14a**	Toluene	24	rt	99/95	57
4	**3-14b**	Toluene	12	rt	99/95	20
5	**3-12**	MeOH	9	rt	99/95	35
6	**3-12**	CH2Cl2	51	rt	99/95	79
7	**3-12**	Hexane	72	rt	<30	--
8	**3-12**	EtOAc	3	rt	99/95	42
9	**3-12**	THF	51	rt	99/95	36
10	**3-12**	CH3CN	5	rt	99/91	73
11	**3-12**	Acetone	5	rt	99/95	42
12	**3-12**	Toluene	28	0	92/91	94

[a] The reactions were performed with **3-15a** (0.05 mmol) and catalyst (0.005 mmol) in anhydrous toluene (0.2 mL) at the temperature specified. [b] Time for step a. [c] Isolated yields for both steps. [d] The ee value was determined by HPLC analysis on a chiral stationary phase. [e] Not determined.

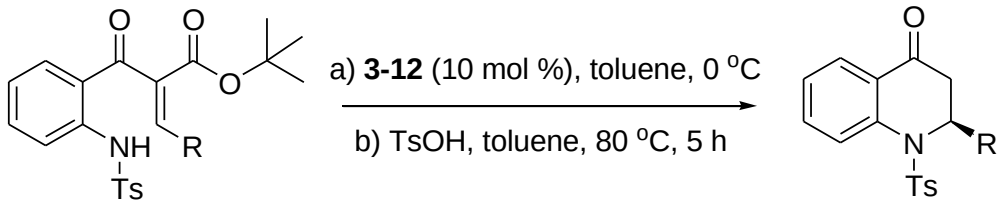
3.2.2 Reaction Scope

The reaction scope was next studied, various alkylidene β-ketoesters with different substituents were examined, and the results are summarized in Table 3-2.

It was found that para- or meta- substituted aromatic rings, regardless their

electronic nature, were well-tolerated for the reaction. In all the examples examined, good yields and excellent enantioselectivities were obtained (entries 1-6). For the alkene bearing an ortho-methyl-phenyl substituent, the desired product could still be obtained in good yield and with excellent enantioselectivity, although much longer reaction was required (entry 7). 2-Naphthyl-substituted alkylidene could also be employed, and high ee was attainable (entry 8). When the alkyl substituent was used, the final 2-*i*Pr-2,3-dihydro-4-quinolone was prepared with 78% ee (entry 9).

Table 3-2 Preparation of Various 2-Substituted-2,3-dihydro-4-quinolones [a]



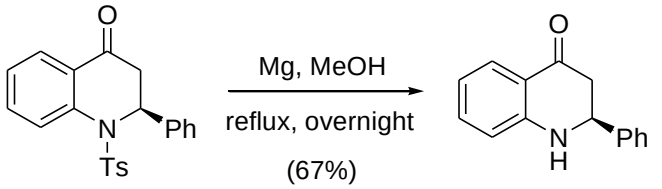
3-15 **3-17**

entry	R	product	t (h)	yield [b] (%)	ee [c] (%)
1	Ph	3-17a	28	92/91	94
2	4-ClPh	3-17b	24	98/88	91
3	4-CH_3-Ph	3-17c	24	98/87	89
4	3-CH_3-Ph	3-17d	28	95/70	92
5[d]	2-CH_3-Ph	3-17e	96	93/86	83
6	4-BrPh	3-17f	24	96/76	87
7	3-F-Ph	3-17g	42	95/90	83
8	2-Naphthyl	3-17h	24	92/88	84
9	4-CH_3CH_2Ph	3-17i	24	98/91	94
10	$(CH_3)_2CH$	3-17j	48	97/85	78

[a] The reactions were performed with **3-15** (0.05 mmol), **3-12** (0.005 mmol) and 4Å molecular sieves in anhydrous toluene (0.2 mL) at 0 °C for the first step; 0.05 mmol TsOH in toluene (0.2 mL) was used for the second step. [b] Isolated yields for both steps. [c] The ee value was determined by HPLC analysis on a chiral stationary phase. [d] The reaction was performed at -30 °C.

3.2.3 Synthesis of 2-Aryl-2,3-dihydro-4-quinolones

Chiral 2-aryl-2,3-dihydro-4-quinolones could be synthesized by deprotection of the sulfonamide group to get the free amino group.[196-197] The reductive cleavage of the sulfonyl protection was demonstrated in Scheme 3-7. When **3-17a** (94% ee) was treated with magnesium in methanol at the refluxing condition, the desulfonated 2,3-dihydro-2-aryl-quinolin-4(1H)-one **3-18** with 93% ee was obtained. The integrity of the newly created stereogenic center at the 2-position of chiral quinolones was not effected and the enantioselectivity could maintained after desufonylation.



3-17a (94 % ee) **3-18** (93 % ee)

Scheme 3-7 Cleavage of the N-Sulfonyl Group

3.3 Conclusion

In conclusion, a new approach for the asymmetric preparation of biologically important 2-aryl-2,3-dihydro-quinolin-4(1H)-ones was developed. By installing a sulfonyl group on the nitrogen of anilines and an ester function on the alkylidene β-ketoesters, the intramolecular cyclization reactions proceeded in a highly enantioselective manner in the presence of tertiary amine-thiourea organic catalysts, and the subsequent removal the ester group was realized via an acidic decarboxylative process without affecting the newly generated 2-stereogenic center of the quinolones.

The reported procedure represents a practical asymmetric route to 2-substituted-2,3-dihydro-4-quinolones, and this method may find wide applications in the synthesis of other bioactive nitrogen-containing cyclic structures.

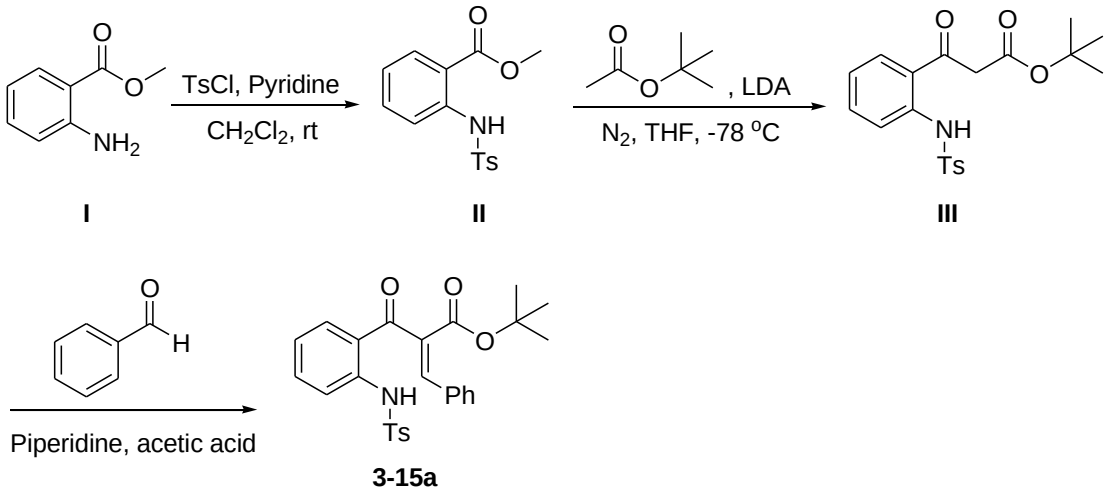
3.4 Experimental Section

3.4.1 General Information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 or DPX300 (300 MHz) or AMX500 (500 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.0). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants were reported in Hertz (Hz). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. For thin-layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F_{254}) were used, and compounds were visualized with a UV light at 254 nm. Flash chromatography separations were performed on Merck 60 (0.040 - 0.063 mm) mesh silica gel.

3.4.2 Representative Procedures

3.4.2.1 Preparation of Starting Material 3-15a



Methyl 2-(tosylamino)benzoate **II**

To a solution of methyl 2-aminobenzoate **I** (1.51g, 10 mmol) in CH_2Cl_2 (15 mL) was added pyridine (0.97 mL, 12 mmol) dropwise over 2 min. The reaction was stirred for 25 min, and a solution of 4-methylbenzene-1-sulfonyl chloride (2.28g, 12 mmol) in CH_2Cl_2 was added dropwise. After stirring overnight, the reaction was quenched with sat. aq.NH_4Cl (30 mL). The layers were separated and the aqueous layer extracted with CH_2Cl_2 several times. The combined organic layers were washed with brine (30 mL), and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure and purified via column chromatography (EtOAc: Hexanes = 1: 8) to give 2.20 g of pale yellow solid **II** (72% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H), 3.89 (s, 3H), 7.03-7.06 (m, 1H), 7.23-7.24 (m, 2H), 7.29 (m, 1H), 7.45-7.47 (m, 1H), 7.70-7.77 (m, 2H), 7.92-7.94 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 52.4, 115.9,

119.0, 122.8, 127.3, 129.6, 131.2, 134.5, 136.5, 140.6, 143.9, 168.3; HRMS (ESI) m/z calcd for $C_{15}H_{15}N_1O_5S_1{}^{32}Na_1$, $[M+Na]^+$ = 328.0713, found = 328,0722.

tert-Butyl 3-oxo-3-(2-(tosylamino)phenyl)propanoate **III**

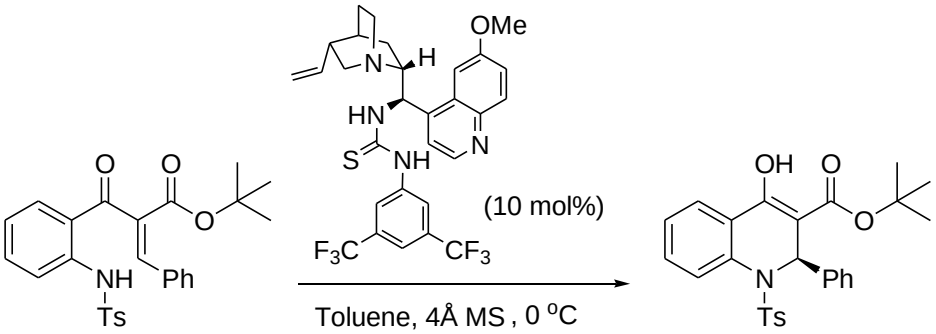
To a N_2 purged 100 mL round bottom flask was added 20 mL of dry THF and diisopropylamine (5 mL, 36 mmol). The solution was cooled to -78 °C and *n*-BuLi (15 mL, 2.5 M) was added and the solution is warmed to 0 °C for 45 min. The solution is cooled to -78 °C and *t*-butyl acetate (3 mL, 24 mmol) in 20 mL THF was added dropwise over 10 minutes. After 90 min., methyl 2-(tosylamino)benzoate **II** (1.83 g, 6 mmol) in 15 mL of THF was added. The solution was allowed to warm to RT overnight and quenched with aq. NH_4Cl (sat.), extracted with EtOAc (2 x 25 mL), washed brine (40 mL), dried Na_2SO_4, filtered, concentrated in vacuo. Purified via column chromatography (EtOAc: Hexanes = 1: 8) to give pale yellow solid III (1.45 g, 62% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.37 (s, 3H), 3.86 (s, 2H), 7.08-7.10 (t, *J* = 1.1 Hz, 1H), 7.23-7.26 (d, *J* = 8.1 Hz, 2H), 7.48 (t, *J* = 1.3 Hz, 3H), 7.70-7.78 (m, 4H), 11.29 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 27.8, 48.3, 76.5, 76.9, 77.4, 82.4, 118.7, 121.2, 122.4, 127.2, 130.0, 131.5, 135.2, 136.5, 140.5, 143.9, 166.0, 196.8; HRMS (ESI) m/z calcd for $C_{20}H_{23}N_1O_5S_1{}^{32}Na_1$, $[M+Na]^+$ = 412.13, found = 412.38.

(*Z*)-tert-butyl 2-(2-(4-methylphenylsulfonamido)benzoyl)-3-phenylacrylate **3-15a**

To a solution of 5 mL toluene in 25 mL round bottom flask was added benzaldehyde (41 μL, 0.4 mmol), tert-butyl

3-oxo-3-(2-(tosylamino)phenyl)propanoate **III** (156mg, 0.4 mmol) with piperidine (2 μL, 0.02 mmol) and glacial acetic acid (1μL, 16.5 mmol) as well as 4Å molecular sieve (30 mg, 0.1 mmol). The heterogenous mixture was stirred at RT for 5 h. The reaction was taken up in EtOAc (10 mL) and washed with brine (25 mL). The organic layer was dried with Na_2SO4. After concentration, the residue was purified by flash column (EtOAc: Hexanes = 1: 8) to afford **3-15a** as a white solid (227 mg, 68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.43 (s, 3H), 6.94-7.00 (m, 1H), 7.22-7.34 (m, 7H), 7.45-7.50 (m, 1H), 7.68-7.91 (m, 5H), 11.51 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 27.7, 82.4, 117.6, 121.3, 122.2, 127.3, 128.8, 129.7, 129.9, 130.3, 132.3, 132.6, 133.1, 135.5, 136.8, 140.7, 141.8, 143.9, 163.3, 199.4. HRMS (ESI) m/z calcd for $C_{27}H_{27}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 500.1502, found = 500.1520.

3.4.2.2 Representative Procedure for Intramolecular Cyclization Reaction

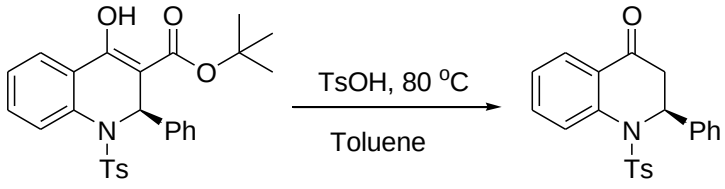


3-15a **3-16a**

(Z)-Tert-buty-2-(2-(4-methylphenylsulfonamido)benzoyl)-3-phenylacrylate **3-15a** (23.4 mg, 0.05 mmol), thiourea **3-12** (2.97 mg, 0.005 mmol) and 4Å molecular sieve (20 mg) in toluene (200 μL) was stirred in toluene (200 μL) at 0 °C for 28 h.

The mixture was then diluted with water, and extracted with CH_2Cl_2 several times. The combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash chromatographic column (EtOAc: Hexanes = 1: 10) to afford **3-16a** as a light yellow solid (21.06 mg, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 9H), 2.39 (s, 3H), 6.33 (s, 1H), 7.13-7.44 (m, 11H), 7.65-7.71 (m, 2H), 12.04 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.1, 55.7, 82.4, 124.1, 125.1, 126.7, 126.8, 127.0, 127.6, 128.0, 128.1, 129.2, 131.5, 135.5, 135.9, 138.8, 143.8, 162.3, 169.8. HRMS (ESI) m/z calcd for $C_{27}H_{27}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 500.1502, found = 500.1520.

3.4.2.3 Representative Procedure for Decarboxylation Reaction

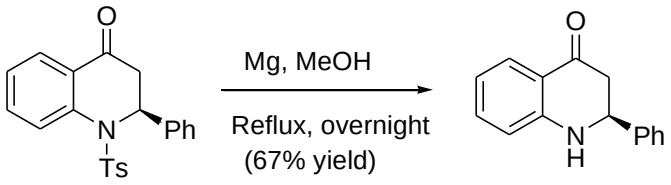


3-16a **3-17a**

p-Toluenesulfonic acid (8.6 mg, 0.05 mmol) was added to the solution of tert-butyl 4-hydroxy-2-phenyl-1-tosyl-1,2-dihydroquinoline-3-carboxylate **3-16a** (23.4 mg, 0.05 mmol) in toluene and the solution was heated to 80 °C, and the reaction was completed in 5h. The solution was allowed to cool down, diluted with EtOAc (5 mL) and then washed with brine. The combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash chromatographic column (EtOAc: Hexanes = 1: 10) to afford **3-17a** as a light yellow

solid (19.60 mg, 91 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.63-2.67 (m, 1H), 3.01-3.05 (m, 1H), 5.96 (m, 1H), 7.19-7.32 (m, 8H), 7.54-7.62 (m, 3H), 7.86-7.92 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 39.5, 57.8, 125.7, 126.0, 126.2, 126.9, 127.0, 127.1, 127.9, 128.6, 130.2, 135.0, 136.7, 137.7, 140.2, 144.7, 192.0. HRMS (ESI) m/z calcd for $C_{22}H_{19}O_3N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 400.1168, found = 400.1185.

3.4.2.4 Representative Procedure for the Reductive Desulfonylation

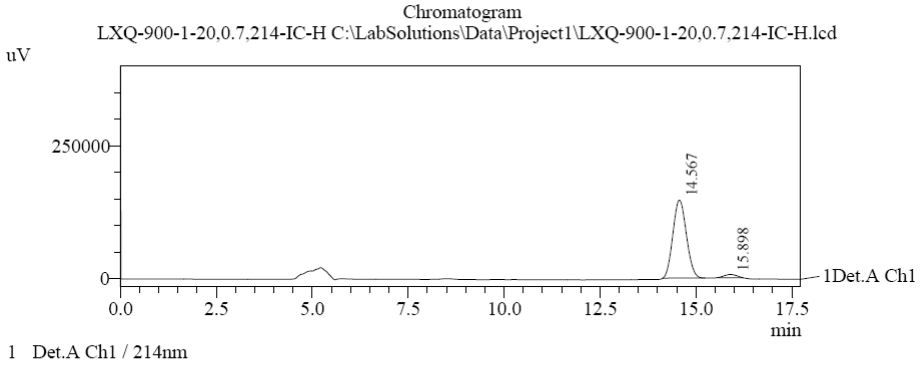


3-17a (ee: 94 %) **3-18** (ee: 93 %)

To a mixture of **3-17a** (23.4 mg, 0.05 mmol) and activated Mg (12 mg, 0.05 mmol) in a 25 mL round bottom flask was added anhydrous methanol (5 mL). The reaction solution was brought to reflux overnight. After cooling down to room temperature, the reaction mixture was quenched by adding 1 M aqueous HCl, and extracted by CH_2Cl_2 several times. The combined organic layers were dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash chromatographic column (EtOAc: hexanes = 1: 8) to afford **3-18** as a yellow solid (7.47 mg, 67 % yield).

3.4.3 Determination of the Absolute Configurations of Products

The NMR spectra of **3-18** were consistent with the literature data.[1] The enantiomers of **3-18** were analyzed by chiral phase HPLC using IC-H column at 214 nm (2-propanol/hexane = 20/80), flow rate = 0.7 mL/min; major enantiomer: t_R = 14.6 min, minor enantiomer: t_R = 15.9 min (literature[1]: major enantiomer: t_R = 14.9 min, minor enantiomer: t_R = 16.2 min).



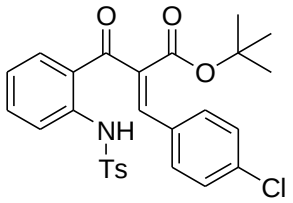
PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	14.567	3582692	146630	96.409	96.064
2	15.898	133463	6008	3.591	3.936
Total		3716155	152639	100.000	100.000

3.4.4 Analytical Data and HPLC Chromatogram of Products

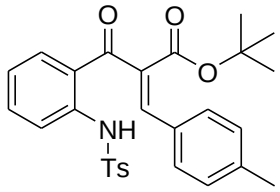
(*Z*)-tert-butyl 3-(4-chlorophenyl)-2-(2-(4-methylphenylsulfonamido)benzoyl)acrylate **3-15b**



A white solid; Isolated in 60% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 9H),

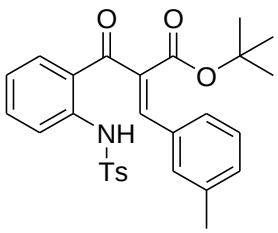
2.45 (s, 3H), 6.95-7.01 (m, 1H), 7.18 (s, 4H), 7.30-7.32 (m, 2H), 7.46-7.47 (m, 1H), 7.49-7.52 (m, 1H), 7.64-7.67 (m, 1H), 7.76-7.90 (m, 3H), 11.44 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 27.7, 82.7, 117.8, 121.2, 122.3, 127.3, 129.1, 129.7, 131.0, 131.1, 132.9, 135.7, 136.4, 136.8, 140.2, 140.7, 144.0, 163.1, 199.0. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1{}^{35}Cl_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 534.1112, found = 534.1122.

(*Z*)-tert-butyl 2-(2-(4-methylphenylsulfonamido)benzoyl)-3-p-tolylacrylate **3-15c**



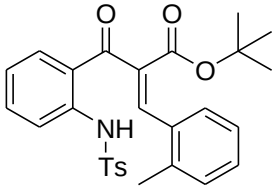
A white solid; Isolated in 68% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 9H), 2.33 (s, 3H), 2.43 (s, 3H), 6.94-6.99 (m, 3H), 7.03-7.05 (d, *J* = 8.0 Hz, 2H), 7.15-7.18 (d, *J* = 8.2 Hz, 2H), 7.29-7.32 (m, 1H), 7.68-7.69 (d, *J* = 1.5 Hz, 1H), 7.71-7.81 (m, 2H), 7.89-7.91 (m, 2H), 11.53 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 21.5, 27.7, 82.2, 117.6, 121.4, 122.2, 127.3, 129.6, 129.7, 129.8, 130.1, 131.1, 133.1, 135.4, 136.8, 140.7, 140.9, 141.8, 143.8, 163.5, 199.7. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found = 514.1678.

(*Z*)-tert-butyl 2-(2-(4-methylphenylsulfonamido)benzoyl)-3-m-tolylacrylate **3-15d**



A white solid; Isolated in 66% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.27 (s, 3H), 2.42 (s, 3H), 6.94-6.99 (m, 1H), 7.04-7.14 (m, 4H), 7.28-7.30 (m, 2H), 7.46-7.48 (t, *J* = 1.3 Hz, 3H), 7.67-7.68 (d, *J* = 1.5 Hz, 2H), 7.70-7.80 (m, 2H), 7.88-7.91 (d, *J* = 8.4 Hz, 2H), 11.52 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 21.4, 27.7, 82.3, 117.5, 121.4, 122.2, 126.7, 127.3, 128.6, 129.7, 131.0, 131.2, 132.0, 132.5, 133.1, 135.4, 136.8, 138.5, 140.6, 142.0, 143.9, 163.4, 199.5. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found = 514.1678.

(*Z*)-tert-butyl 2-(2-(4-methylphenylsulfonamido)benzoyl)-3-o-tolylacrylate **3-15e**

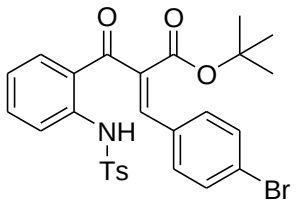


A white solid; Isolated in 59% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.41 (s, 3H), 2.45 (s, 3H), 6.93-7.06 (m, 3H), 7.19-7.30 (m, 5H), 7.41-7.47 (m, 1H), 7.66-7.74 (m, 2H), 7.82-7.85 (m, 2H), 8.09 (s, 1H), 11.42 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.9, 21.4, 27.7, 82.4, 117.6, 121.6, 122.0, 126.0, 127.2, 128.3, 129.5, 129.7, 129.9, 130.5, 132.0, 133.5, 135.2, 136.7, 137.76, 140.50, 140.96, 143.84, 198.9.. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found =

514.1678.

(*Z*)-tert-butyl 3-(4-bromophenyl)-2-(2-(4-methylphenylsulfonamido)benzoyl)acrylate

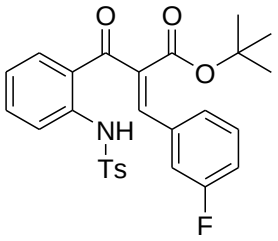
3-15f



A light yellow solid; Isolated in 48% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 9H), 2.43 (s, 3H), 6.95-6.97 (m, 1H), 7.08-7.10 (d, *J* = 5.1 Hz, 2H), 7.29-7.32 (m, 4H), 7.48 (m, 1H), 7.63-7.65 (m, 1H), 7.72 (s, 1H), 7.80-7.82 (d, *J* = 5.3 Hz, 1H), 7.86-7.88 (m, 2H), 11.43 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.60, 27.76, 82.79, 117.9, 121.3, 122.5, 124.9, 127.3, 129.8, 131.3, 131.6, 132.1, 133.0, 133.1, 135.8, 136.9, 140.8, 144.1, 163.2, 199.2. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1{}^{79}Br_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 578.0607, found = 578.0629 and for $C_{27}H_{26}O_5N_1{}^{81}Br_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 580.0587, found = 580.0595.

(*Z*)-tert-butyl 3-(3-fluorophenyl)-2-(2-(4-methylphenylsulfonamido)benzoyl)acrylate

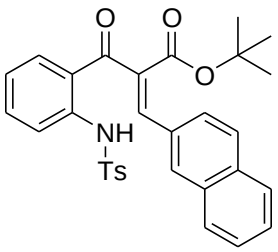
3-15g



A yellow oil; Isolated in 36% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 2.41 (s, 3H), 6.95-7.08 (m, 4H), 7.19-7.31 (m, 3H), 7.45-7.47 (m, 1H), 7.65-7.68 (m, 1H), 7.74-7.77 (d, *J* = 8.0 Hz, 2H), 7.87-7.90 (d, *J* = 8.2 Hz, 2H), 11.44 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 27.6, 82.8, 116.1, 116.4, 117.1, 11.4, 117.6, 121.1, 122.2, 125.6, 127.2, 129.7, 130.4, 130.5, 132.9, 133.7, 135.7, 136.6, 140.1, 140.2, 140.7, 144.0, 163.0, 198.8. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1F_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 518.1408, found = 518.1408.

(*Z*)-tert-butyl 2-(2-(4-methylphenylsulfonamido)benzoyl)-3-(naphthalen-2-yl)acrylate

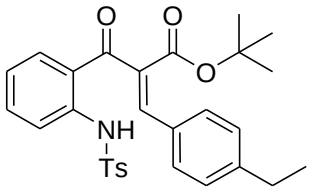
3-15h



A white solid; Isolated in 62% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 2.45 (s, 3H), 6.95 (m, 1H), 7.25-7.28 (m, 3H), 7.30-7.54 (m, 3H), 7.61-7.64 (d, *J* = 8.6 Hz, 1H), 7.73-7.80 (m, 4H), 7.88-7.93 (m, 3H), 8.00 (s, 1H), 11.58 (s, 1H); ^{13}C

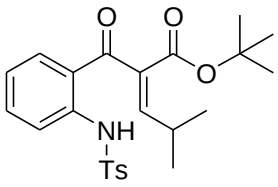
NMR (75 MHz, $CDCl_3$) δ 21.5, 27.7, 82.4, 11.6, 121.4, 122.3, 125.5, 126.7, 127.3, 127.5, 127.7, 128.6, 128.7, 129.7, 130.1, 131.7, 132.2, 133.0, 135.5, 136.8, 140.7, 141.8, 143.9, 163.4, 199.6. HRMS (ESI) m/z calcd for $C_{31}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 550.1659, found = 550.1665.

(*Z*)-tert-butyl 3-(4-ethylphenyl)-2-(2-(4-methylphenylsulfonamido)benzoyl)acrylate **3-15i**



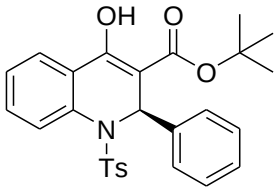
A white solid; Isolated in 68% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (m, 3H), 1.33 (s, 9H), 2.42 (s, 3H), 2.61-2.63 (m, 2H), 6.96-6.99 (t, *J* = 0.8 Hz, 1H), 7.05-7.08 (d, *J* = 8.2 Hz, 2H), 7.18-7.21 (d, *J* = 8.2 Hz, 2H), 7.28-7.30 (m, 2H), 7.47-7.48 (m, 1H), 7.69 (d, 1H), 7.78-7.80 (m, 2H), 7.88-7.91 (m, 2H), 11.56 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0, 21.5, 27.7, 28.6, 82.2, 117.6, 121.4, 122.3, 127.3, 128.4, 128.5, 129.7, 130.0, 130.2, 131.1, 133.1, 135.4, 136.8, 140.6, 141.8, 143.9, 147.1, 163.5, 200.0. HRMS (ESI) m/z calcd for $C_{29}H_{31}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 528.1815, found = 528.1830.

(*Z*)-tert-butyl 4-methyl-2-(2-(4-methylphenylsulfonamido)benzoyl)pent-2-enoate **3-15j**



A white solid; Isolated in 56% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95-0.97 (d, *J* = 6.6 Hz, 6H), 1.27 (s, 9H), 2.04-2.27 (m, 1H), 2.37 (s, 3H), 6.78-6.81 (m, 1H), 7.01-7.07 (m, 1H), 7.22-7.24 (d, *J* = 8.0 Hz, 2H), 7.46-7.51 (m, 1H), 7.59-7.62 (m, 1H), 7.76-7.80 (m, 3H), 11.34 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 21.6, 27.6, 29.0, 81.9, 118.4, 122.1, 122.3, 127.1, 129.6, 132.6, 133.3, 135.2, 136.7, 140.3, 143.8, 152.8, 163.1, 198.5. HRMS (ESI) m/z calcd for $C_{24}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_{1,}$ $[M+Na]^+$ = 466.1659, found = 466.1675.

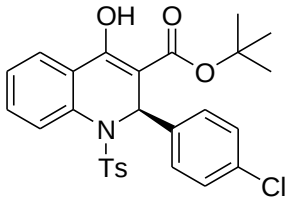
<u>*tert*-Butyl 4-hydroxy-2-phenyl-1-tosyl-1,2-dihydroquinoline-3-carboxylate **3-16a**</u>



A light yellow solid; Isolated in 92 % yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 9H), 2.39 (s, 3H), 6.33 (s, 1H), 7.13-7.44 (m, 11H), 7.65-7.71 (m, 2H), 12.04 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.1, 55.7, 82.4, 124.1, 125.1, 126.7, 126.8, 127.0, 127.6, 128.0, 128.1, 129.2, 131.5, 135.5, 135.9, 138.8, 143.8, 162.3, 169.8.The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 5: 95), 1.0 mL/min; Major enantiomer: t_R = 7.6 min, minor enantiomer: t_R =

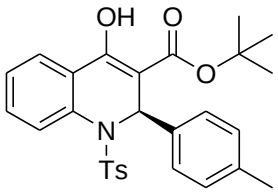
9.1 min. HRMS (ESI) m/z calcd for $C_{27}H_{27}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 500.1502, found = 500.1520.

tert-Butyl 2-(4-chlorophenyl)-4-hydroxy-1-tosyl-1,2-dihydroquinoline-3-carboxylate **3-16b**



A light yellow solid; Isolated in 98% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.39 (s, 3H), 6.27 (s, 1H), 7.12-7.15 (m, 2H), 7.19-7.34 (m, 7H), 7.41-7.45 (m, 1H), 7.65-7.69 (m, 2H), 12.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.1, 55.2, 82.6, 97.5, 124.1, 124.9, 126.8, 128.1, 128.3, 128.4, 129.2, 131.6, 133.5, 135.2, 135.6, 137.4, 144.0, 162.5, 169.6. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 9.2 min, minor enantiomer: t_R = 12.3 min. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1{}^{35}Cl_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 534.1112, found = 534.1122.

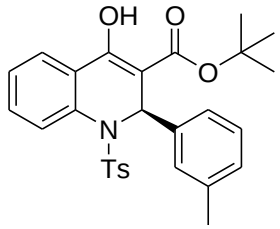
tert-Butyl 4-oxo-2-p-tolyl-1-tosyl-1,2,3,4-tetrahydroquinoline-3-carboxylate **3-16c**



A white solid; Isolated in 98% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 9H),

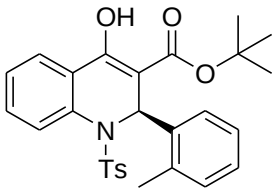
2.28 (s, 3H), 2.39 (s, 3H), 6.29 (s, 1H), 7.02-7.43 (m, 11H), 7.64-7.70 (m, 2H), 12.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9, 21.4, 28.1, 55.5, 82.3, 98.1, 124.1, 125.1, 126.6, 126.8, 126.9, 128.1, 128.8, 129.2, 131.4, 135.5, 135.8, 135.9, 137.2, 143.8, 162.2, 169.9. The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 5 : 95), 1.0 mL/min; Major enantiomer: t_R = 7.9 min, minor enantiomer: t_R = 13.4 min. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found = 514.1678.

tert-Butyl 4-hydroxy-2-m-tolyl-1-tosyl-1,2-dihydroquinoline-3-carboxylate **3-16d**



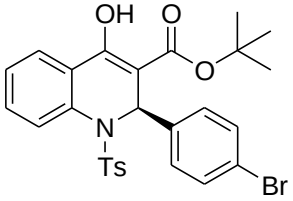
A white solid; Isolated in 95% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.30 (s, 3H), 2.39 (s, 3H), 6.29 (s, 1H), 7.00-7.16 (m, 6H), 7.26-7.35 (m, 3H), 7.41-7.46 (m, 1H), 7.65-7.72 (m, 2H), 12.04 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 21.4, 28.1, 55.6, 82.4, 98.1, 123.9, 124.1, 125.1, 126.6, 126.8, 127.8, 127.9, 128.2, 128.4, 129.2, 131.4, 135.5, 135.9, 137.7, 138.7, 143.8, 162.3, 169.8.The enantiomers were analyzed by HPLC using an AS-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 27.3 min, minor enantiomer: t_R = 20.8 min. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found = 514.1678.

tert-butyl 4-hydroxy-2-o-tolyl-1-tosyl-1,2-dihydroquinoline-3-carboxylate **3-16e**



A white solid; Isolated in 93% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.39 (s, 3H), 2.80 (s, 3H), 6.57 (s, 1H), 6.65-6.67 (m, 1H), 6.83-6.86 (m, 1H), 7.09-7.14 (m, 3H), 7.20-7.34 (m, 3H), 7.36-7.43 (m, 2H), 7.51-7.54 (m, 2H), 7.67-7.70 (m, 1H), 11.9 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.9, 21.4, 53.5, 82.3, 123.8, 125.3, 126.5, 127.0, 127.2, 127.3, 127.9, 128.9, 129.1, 130.9, 131.3, 134.4, 135.1, 135.8, 137.5, 143.8, 162.4, 169.5. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 10.2 min, minor enantiomer: t_R = 8.7 min. HRMS (ESI) m/z calcd for $C_{28}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 514.1659, found = 514.1678.

tert-Butyl
2-(4-bromophenyl)-4-oxo-1-tosyl-1,2,3,4-tetrahydroquinoline-3-carboxylate **3-16f**

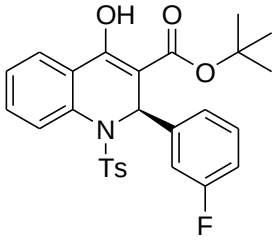


A light yellow solid; Isolated in 96% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 9H), 2.40 (s, 3H), 6.25 (s, 1H), 7.12-7.20 (m, 4H), 7.30-7.45 (m, 6H), 7.64-7.69 (m, 2H), 12.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.1, 55.23, 82.6, 97.4, 121.7,

124.2, 124.9, 126.8, 126.9, 128.1, 128.8, 129.2, 130.9, 131.3, 131.6, 132.4, 135.2, 135.6, 138.0, 144.0, 162.5. The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3 : 97), 0.5 mL/min; Major enantiomer: t_R = 18.3 min, minor enantiomer: t_R = 32.2 min. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1{}^{79}Br_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 578.0607, found = 578.0629 and for $C_{27}H_{26}O_5N_1{}^{81}Br_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 580.0587, found = 580.0595.

tert-Butyl 2-(3-fluorophenyl)-4-oxo-1-tosyl-1,2,3,4-tetrahydroquinoline-3-carboxylate

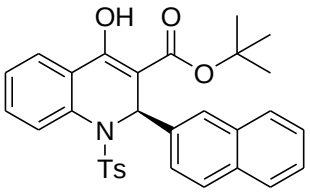
3-16g



A light yellow solid; Isolated in 95% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 1H), 2.39 (s, 3H), 6.30 (s, 3H), 6.90-7.00 (m, 1H), 7.03-7.10 (m, 1H), 7.13-7.22 (m, 4H), 7.28-7.35 (m, 3H), 7.43-7.46 (m, 1H), 7.65-7.72 (m, 2H), 12.04 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 28.1, 55.3, 82.6, 97.5, 113.9, 114.2, 114.4, 114.7, 122.6, 124.2, 124.9, 126.8, 128.1, 129.2, 129.5, 129.6, 131.6, 135.2, 135.7, 141.6, 141.7, 144.0, 162.5, 164.3. The enantiomers were analyzed by HPLC using an AS-H column at 254 nm (2-propanol: hexane = 3: 97), 0.2 mL/min; Major enantiomer: t_R = 60.6 min, minor enantiomer: t_R = 47.7 min. HRMS (ESI) m/z calcd for $C_{27}H_{26}O_5N_1F_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 518.1408, found = 518.1408.

tert-Butyl 4-hydroxy-2-(naphthalen-2-yl)-1-tosyl-1,2-dihydroquinoline-3-carboxylate

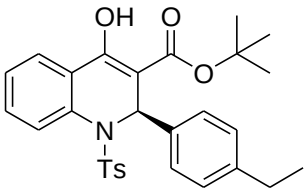
3-16h



A white solid; Isolated in 92% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.41 (m, 3H), 6.49 (s, 1H), 7.15-.18 (d, *J* = 8.1 Hz, 2H), 7.27-7.28 (d, *J* = 1.1 Hz, 2H), 7.30-7.46 (m, 5H), 7.57 (s, 1H), 7.63-7.80 (m, 6H), 12.15 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 28.1, 55.9, 82.5, 97.7, 124.1, 125.1, 125.6, 125.7, 125.9, 126.7, 126.9, 127.4, 128.0, 128.2, 129.2, 131.5, 132.9, 135.4, 135.8, 136.2, 143.9, 162.6, 169.9. The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 43.9 min, minor enantiomer: t_R = 27.3 min. HRMS (ESI) m/z calcd for $C_{31}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 550.1659, found = 550.1665.

tert-Butyl 2-(4-ethylphenyl)-1,2,3,4-tetrahydro-4-oxo-1-tosylquinoline-3-carboxylate

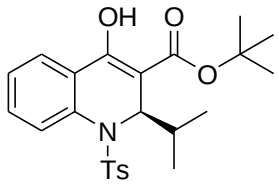
3-16i



A light yellow solid; Isolated in 98% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (m, 3H), 1.52 (s, 9H), 2.39 (s, 3H), 2.58-2.60 (m, 4H), 6.30 (s, 1H), 7.04-7.07 (d, *J* = 8.2 Hz,

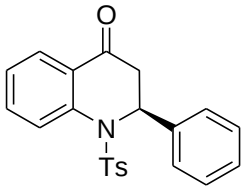
2H), 7.12-.7.15 (d, *J* = 8.1 Hz, 2H), 7.20-7.22 (d, *J* = 8.1 Hz, 2H), 7.28-7.36 (m, 3H), 7.41-7.43 (m, 1H), 7.65-7.72 (m, 2H), 12.03 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 21.4, 28.1, 28.3, 55.5, 76.5, 77.0, 77.4, 82.3, 98.2, 124.1, 125.1, 126.6, 126.8, 126.9, 127.5, 128.1, 129.2, 131.4, 135.5, 136.0, 136.1, 143.4, 143.8, 162.2, 169.8. The enantiomers were analyzed by HPLC using an AD-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 21.0 min, minor enantiomer: t_R = 37.5 min. HRMS (ESI) m/z calcd for $C_{29}H_{31}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 528.1815, found = 528.1830.

tert-Butyl 2-isopropyl-4-oxo-1-tosyl-1,2,3,4-tetrahydroquinoline-3-carboxylate **3-16j**



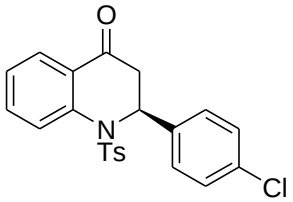
A light yellow solid; Isolated in 97% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88-1.02 (m, 6H), 1.60 (s, 9H), 2.35 (s, 3H), 4.75-4.78 (d, *J* = 9.4 Hz, 1H), 7.06-7.08 (d, *J* = 8.1 Hz, 1H), 7.24-7.52 (m ,3H), 7.64-7.67 (m, 1H), 7.79 (d, *J* = 0.8 Hz, 1H), 7.81-7.82 (d, *J* = 0.84 Hz, 1H), 11.94 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0, 19.4, 21.4, 28.2, 32.6, 59.0, 82.0, 98.7, 124.2, 125.2, 126.4, 126.6, 127.8, 129.0, 131.2, 135.8, 136.2, 143.4, 161.2. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 2: 98), 0.5 mL/min; Major enantiomer: t_R = 18.7 min, minor enantiomer: t_R = 15.3 min. HRMS (ESI) m/z calcd for $C_{24}H_{29}O_5N_1{}^{23}Na_1{}^{32}S_1$, $[M+Na]^+$ = 466.1659, found = 466.1675.

2-Phenyl-1-tosyl-2,3-dihydroquinolin-4(1H)-one **3-17a**



A light yellow solid; Isolated in 91% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H), 2.63-2.67 (m, 1H), 3.01-3.05 (m, 1H), 5.96 (m, 1H), 7.19-7.32 (m, 8H), 7.54-7.62 (m, 3H), 7.86-7.92 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 39.5, 57.8, 125.7, 126.0, 126.2, 126.9, 127.0, 127.1, 127.9, 128.6, 130.2, 135.0, 136.7, 137.7, 140.2, 144.7, 192.0. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 5: 95), 1.0 mL/min; Major enantiomer: t_R = 24.6 min, minor enantiomer: t_R = 27.2 min. HRMS (EI) m/z calcd for $C_{22}H_{19}O_3N_1{}^{32}S_1$, $[M]^+$ = 377.1086, found = 377.1082. $[\alpha]^{25}_D$ -1.9 (c 0.47, $CHCl_3$).

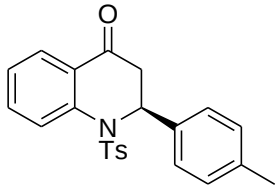
(S)-2-(4-chlorophenyl)-2,3-dihydro-1-tosylquinolin-4(1H)-one **3-17b**



A light yellow solid; Isolated in 88% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.61-2.65 (dd, *J* = 18.0 MHz, 1H), 2.96-3.00 (dd, *J* = 18.0 MHz, 1H), 5.91-5.92 (d, *J* = 5.7 MHz, 1H), 7.21-7.29 (m, 8H), 7.56-7.61 (m, 3H), 7.87-7.92 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 39.4, 57.3, 125.8, 125.9, 126.1, 127.0, 127.2, 128.3, 128.8,

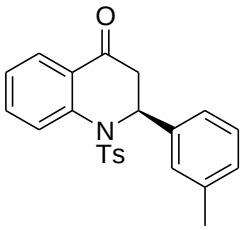
130.2, 133.9, 135.2, 136.3, 136.5, 139.9, 144.8, 191.7. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1 mL/min; Major enantiomer: t_R = 37.0 min, minor enantiomer: t_R = 40.0 min. HRMS (ESI) m/z calcd for $C_{22}H_{17}ClO_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 410.0623, found = 410.0609. $[\alpha]^{25}_D$ -0.4 (c 0.78, $CHCl_3$).

(*S*)-2,3-dihydro-2-p-tolyl-1-tosylquinolin-4(1H)-one **3-17c**



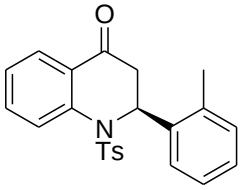
A light yellow solid; Isolated in 87% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, CDCl3) δ 2.23 (s, 3H), 2.40 (s, 3H), 2.58-2.62 (dd, *J* = 17.7 MHz, 1H), 2.96-3.00 (dd, *J* = 18.0 MHz, 1H), 5.89-5.90 (d, *J* = 5.7 MHz, 1H), 7.01-7.03 (m, 2H), (m, 2H), 7.16-7.19 (m, 3H), 7.23-7.26 (m, 2H), 7.51-7.53 (m, 1H), 7.57-7.59 (m, 2H), 7.84-7.88 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9, 21.6, 39.5, 57.6, 125.7, 126.0, 126.2, 126.8, 127.0, 127.1, 129.3, 130.1, 134.7, 134.9, 136.7, 137.6, 140.1, 144.6, 192.2. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1 mL/min; Major enantiomer: t_R = 34.7 min, minor enantiomer: t_R = 37.8 min. HRMS (ESI) m/z calcd for $C_{23}H_{20}O_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 390.1169, found = 390.1173. $[\alpha]^{25}_D$ -0.8 (c 1.31, $CHCl_3$).

(*S*)-2,3-dihydro-2-m-tolyl-1-tosylquinolin-4(1H)-one **3-17d**



A white solid; Isolated in 70% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.24 (s, 3H), 2.40 (s, 3H), 2.57-2.62 (dd, *J* = 18.0 MHz, 1H), 2.97-3.00 (d, *J* = 17.0 MHz, 1H), 5.88-5.89 (d, *J* = 5.6 MHz, 1H), 6.97-7.10 (m, 2H), 7.17-7.26 (m, 3H), 7.50-7.59 (m, 6H), 7.84-7.90 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.4, 21.6, 39.5, 57.7, 123.9, 125.7, 126.0, 126.2, 127.0, 127.1, 127.8, 128.4, 128.6, 130.1, 135.0, 136.7, 137.7, 138.3, 140.2, 144.6, 192.1. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 28.4 min, minor enantiomer: t_R = 39.0 min. HRMS (ESI) m/z calcd for $C_{23}H_{20}O_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 390.1169, found = 390.1173. $[\alpha]^{25}_D$ -1.7 (c 0.43, $CHCl_3$).

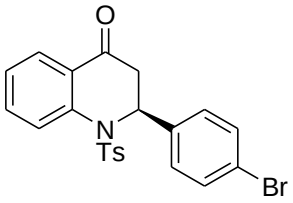
(*S*)-2,3-dihydro-2-o-tolyl-1-tosylquinolin-4(1H)-one **3-17e**



A light yellow solid; Isolated in 86% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (s, 3H), 2.65 (s, 3H), 2.87 (s, 2H), 6.13-6.15 (m, 1H), 6.75-6.76 (d, *J* = 8.2 MHz, 1H), 6.91 (m, 1H), 7.11-7.21 (m, 3H), 7.26-7.29 (m, 2H), 7.44-7.46 (d, *J* = 8.2 MHz, 1H), 7.54-7.56 (m, 1H), 7.75-7.73 (d, *J* = 8.2 MHz, 1H), 7.91-7.92 (d, *J* = 8.2 MHz, 1H); ^{13}C NMR

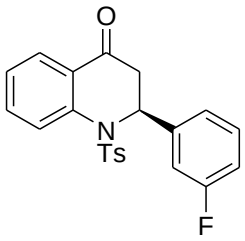
(125 MHz, $CDCl_3$) δ 20.2, 21.6, 40.2, 56.3, 125.8, 126.4, 126.6, 126.9, 127.0, 127.3, 127.4, 128.2, 129.9, 131.5, 135.0, 136.0, 136.1, 137.3, 140.5, 144.6, 192.7. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 26.5 min, minor enantiomer: t_R = 22.3 min. HRMS (ESI) m/z calcd for $C_{23}H_{20}O_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 390.1169, found = 390.1173. $[\alpha]^{25}_D$ +2.4 (c 0.99, $CHCl_3$).

(*S*)-2-(4-bromophenyl)-2,3-dihydro-1-tosylquinolin-4(1H)-one **3-17f**



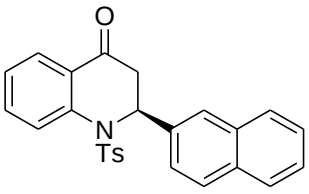
A light yellow solid; Isolated in 76% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.40 (s, 3H), 2.58-2.63 (dd, *J* = 18.0 MHz, 1H), 2.93-2.97 (dd, *J* = 18.0 MHz, 1H), 5.86-5.87 (d, *J* = 6.2 MHz, 1H), 7.16-7.19 (m, 3H), 7.20-7.26 (m, 2H), 7.34-7.36 (d, *J* = 8.2 MHz, 2H), 7.54-7.58 (m, 3H), 7.84-7.89 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6, 39.3, 57.3, 122.1, 125.8, 125.9, 126.1, 127.0, 127.2, 128.7, 130.2, 131.8, 135.2, 136.5, 136.8, 139.9, 144.8, 191.6. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 0.5 mL/min; Major enantiomer: t_R = 75.2 min, minor enantiomer: t_R = 80.6 min. HRMS (ESI) m/z calcd for $C_{22}H_{17}{}^{79}BrO_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 454.0118, found = 454.0114; calcd for $C_{22}H_{17}{}^{81}BrO_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 456.0084, found = 456.0100. $[\alpha]^{25}_D$ -0.5 (c 2.09, $CHCl_3$).

(*S*)-2-(3-fluorophenyl)-2,3-dihydro-1-tosylquinolin-4(1H)-one **3-17g**



A light yellow solid; Isolated in 90% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.41 (s, 3H), 2.59-2.63 (dd, *J* = 18.0 MHz, 1H), 2.94-2.98 (dd, *J* = 17.7 MHz, 1H), 5.90-5.91 (d, *J* = 5.7 MHz, 1H), 6.87-6.88 (m, 1H), 7.02-7.05 (m, 2H), 7.19-7.25 (m, 3H), 7.55-7.59 (m, 3H), 7.84-7.92 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 39.3, 57.3, 114.1, 114.4, 114.8, 115.1, 122.4, 125.9, 126.1, 126.9, 127.1, 130.1, 130.2, 135.2, 136.3, 139.9, 144.8, 191.5. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 36.1 min, minor enantiomer: t_R = 41.4 min. HRMS (ESI) m/z calcd for $C_{22}H_{17}FO_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 394.0919, found = 394.0919. $[\alpha]^{25}_D$ +6.3 (c 0.50, $CHCl_3$).

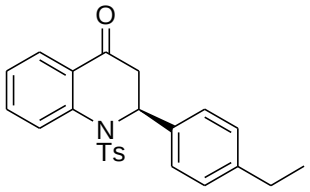
(*S*)-2,3-dihydro-2-(naphthalen-3-yl)-1-tosylquinolin-4(1H)-one **3-17h**



A white solid; Isolated in 88% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.67-2.75 (dd, *J* = 18.0 MHz, 1H), 3.16-3.22 (d, *J* = 18.0 MHz, 1H), 6.10-6.12 (d, *J* = 5.9 MHz, 1H), 7.17-7.20 (t, *J* = 7.2 MHz, 1H), 7.21-7.22 (s, 3H), 7.29-7.72 (m, 7H), 7.74-7.95 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 39.1, 57.8, 125.2, 125.7, 125.8,

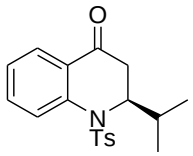
125.9, 126.2, 126.3, 126.9, 127.0, 127.4, 128.0, 128.6, 130.1, 132.6, 132.7, 135.1, 136.6, 139.9, 144.7, 192.1. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1.0 mL/min; Major enantiomer: t_R = 50.3 min, minor enantiomer: t_R = 58.8 min. HRMS (ESI) m/z calcd for $C_{26}H_{20}O_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 426.1169, found = 426.1176. $[\alpha]^{25}_D$ -0.4 (c 0.59, $CHCl_3$).

(*S*)-2-(4-ethylphenyl)-2,3-dihydro-1-tosylquinolin-4(1H)-one **3-17i**



A light yellow solid; Isolated in 91% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.17 (m, 3H), 2.42 (s, 3H), 2.55-2.66 (m, 3H), 2.99-3.03 (m, 1H), 5.92-5.93 (d, *J* = 5.6 MHz, 1H), 7.06-7.08 (d, *J* = 8.9 MHz, 1H), 7.19-7.22 (m, 3H), 7.26-7.29 (m, 2H), 7.52-7.56 (m, 1H), 7.60-7.62 (m, 2H), 7.87-7.92 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.2, 21.6, 28.3, 39.6, 57.6, 125.6, 125.9, 126.1, 126.9, 127.0, 128.1, 130.1, 134.9, 135.0, 136.7, 140.2, 143.8, 144.6, 192.2. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 3: 97), 1 mL/min; Major enantiomer: t_R = 32.8 min, minor enantiomer: t_R = 35.8 min. HRMS (ESI) m/z calcd for $C_{24}H_{22}O_3N_1{}^{32}S_1$, $[M\text{-}H^+]^-$ = 404.1326, found = 404.1319. $[\alpha]^{25}_D$ -1.1 (c 1.82, $CHCl_3$).

(*S*)-2,3-dihydro-2-isopropyl-1-tosylquinolin-4(1H)-one **3-17j**



A light yellow solid; Isolated in 85% yield after flash column chromatographic purification (EtOAc: Hexanes = 1: 8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97-1.03 (m, 6H), 1.29 (m, 2H), 1.56-1.66 (m, 3H), 4,89-4.92 (d, *J* = 9.1 MHz, 1H), 7.09-7.11 (d, *J* = 8.1 MHz, 1H), 7.31-7.38 (m, 3H), 7.59 (m, 1H), 7.74-7.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.8, 19.2, 21.4, 29.6, 32.5, 50.8, 96.5, 124.5, 124.8, 126.6, 126.7, 127.8, 129.1, 132.4, 135.5, 136.9, 143.8, 164.3, 174.6. The enantiomers were analyzed by HPLC using an IA-H column at 254 nm (2-propanol: hexane = 10: 90), 1 mL/min; Major enantiomer: t_R = 9.3 min, minor enantiomer: t_R = 8.4 min. HRMS (EI) m/z calcd for $C_{19}H_{21}O_3N_1{}^{32}S_1$, $[M]^+$ = 343.1242, found = 343.1238. $[\alpha]^{25}_D$ -1.4 (c 0.49, $CHCl_3$).

Chapter 4 Thiol-dependent DNA cleavage by sulfur-containing cyclic molecules

4.1 Introduction

Sulfur-containing cyclic organic compounds, with their unique chemical properties and potential synthetic applications,[198-201] as well as interesting biological and physiological activities,[202-204] have attracted much attention in recent years. These sulfur-containing cyclic organic molecules can be classified on the basis of the functional groups and chemical reactions involved with DNA.[205] The antitumor antibiotic leinamycin can be considered as a structural type of DNA-damaging agent. (Figure 4-1)

This natural product was isolated by researchers at Kyowa Hakko Kogyo Ltd. from a strain of *Streptomyces* found in soil samples collected near Miyagi, Japan.[206] The structure was analyzed by NMR and IR spectroscopy and X-ray crystallography and also confirmed by total synthesis.[207] Interestingly, in vitro experiments revealed that leinamycin was a thiol-dependent DNA cleavage agent.[208] The strand cleavage was caused by involving the conversion of molecular oxygen to DNA-cleaving oxygen radicals as shown in the Scheme 4-1.[209] By inspection of its structure, it was suggested that 1,2-dithiolan-3-one 1-oxide heterocycle was the most reactive portion of antibiotic and it probably played a crucial role in thiol dependent DNA cleavage reaction.

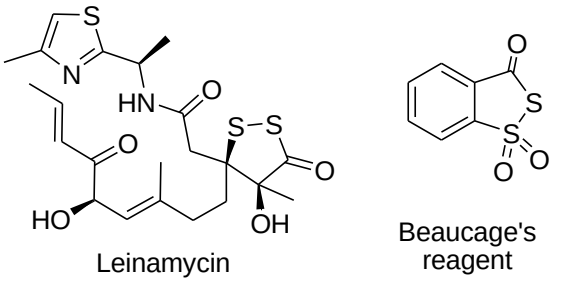
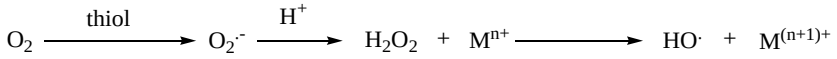


Figure 4-1 Selective examples shown DNA-cleaving acvtivities



Scheme 4-1 Propose mechanism of DNA cleavage

Based on these discoveries, numbers of leinamycin derivatives with 1,2-dithiolan-3-one 1-oxide heterocycle cores were synthesized and a model substrate 3H-1,2-benzodithiol-3-one 1,1-dioxide was applied to investigate the DNA-cleavage reaction. This molecule was later known as Beaucage's sulfurizing agent, in which micromolar concentration could efficiently lead to thiol-dependent DNA cleavage.[210]

Besides disulfide-derived functionalities, the trithiol functional groups have also be proven to be sufficient in causing DNA cleavage reaction. Varacin C, as a family member of varacin, which possesses an unique benzotrithiol 1-oxide functionality, has been found to exhibit potent antifungal activity against Candida albicans and possess potent antitumor activity against human colon tumor cell line HCT 116.[211-214] It was discovered in the Far Eastern ascidian Polycitor sp. by Makarieva and coworkers in 1995.[215] The total synthesis of varacin C was first reported in 2002,[216] and it was demonstrated that this antibiotic displayed potent thiol-dependent DNA-cleaving activity [217] that far exceeded that of varacin. It was further demonstrated that an acidic

environment promoted the DNA-cleaving activities of varacin C.

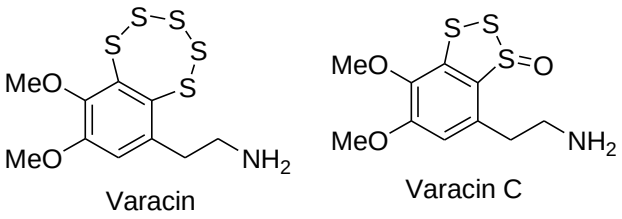


Figure 4-2 Structure of varacin and varacin C

4.2 Project Objectives

Although numbers of examples have shown that sulfur-containing cyclic organic compounds possess great biological activities especially in thiol dependent DNA cleavage reaction, the whole picture has not been described in details for this magnificent reaction and many points could be thought over further. For example, although 1,2-dithiolan-3-one 1-oxide heterocycle in leinamycin seemed to be proved to be crucial in thiol dependent DNA cleavage reaction, the effect of side chain in this core has not been investigated yet. It may play important role as well in the reaction. In addition, other family members of varacin C, like varacin B, which is quite similar structure, has not been applied in the DNA cleavage reaction. It may be a good sulfurizing agent as well. So all these provide us enough room to expand our understanding and go further inside to see more clearly of this reaction.

In this project, new sulfur-containing organic molecules were synthesized and the DNA-cleaving abilities were evaluated as well. More details about the synthesis of the new designed sulfur-containing organic molecules and experimental results of

their thiol dependent DNA-cleaving reactions will be described in the following sections.

4.3 Result and Disscusion

4.3.1 Synthesis of Aminomethylated Beaucage's Reagent

Beaucage's reagent, which contains 3H-1,2-benzodithiol-3-one 1,1-dioxide, has been proved to be efficient sulfurizing agent in catalyzing the thiol dependent DNA cleavage reaction. The concentration of the Beaucage's reagent could be low into micromolar scale. However, besides 3H-1,2-benzodithiol-3-one 1,1-dioxide core, the other functionalities in the Beaucage's reagent have never been investigated and the effect of these side chains are unknown so far. Introduction of amino moiety in Beaucage's reagent is supposed to result in better DNA cleaving ability compared to the parent compounds without amino side chains, due to the favourable electrostatic interactions between the protonated amino group and the negatively charged phosphate backbone in DNA. So two aminomethylated Beaucage's reagents were synthesized to evaluate the effect of the amino side chain, and thiol-dependent DNA cleavage reaction were also done to investigate the efficiency of DNA cleaving reaction by these two products.

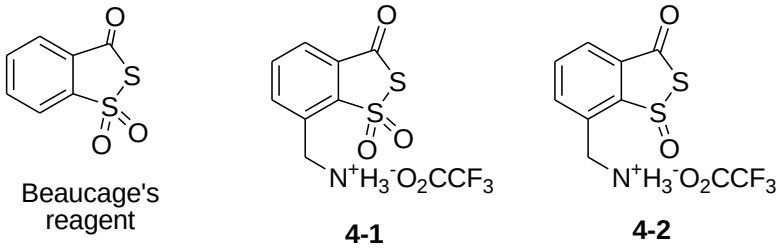
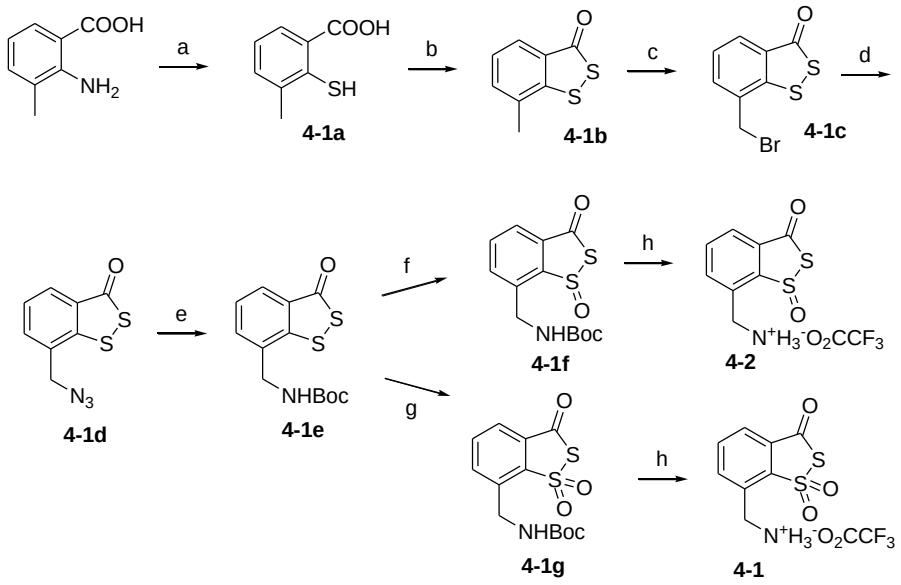


Figure 4-3 Selected sulfur-containing organic molecules

The synthesis of aminomethylated Beaucage's reagent **4-1** and its sulfoxide analogue **4-2** is outlined in Scheme 4-2. The synthesis of **4-1** began with the diazonium reaction on 2-amino-3-methyl benzoic acid following the procedure described by Allen and coworkers.[218] As the corresponding diazonium salt was unstable at elevated temperatures, the reaction temperature was strictly maintained below 5 °C. Upon its formation, the salt was treated immediately with sulfide to generate disulfide under strongly alkaline conditions. Using activated zinc in refluxing glacial acetic acid, the disulfide bond was cleaved off to yield thiol **4-1a.** The reaction of thioacetic acid with **4-1a** in concentrated sulfuric acid gave rise to the cyclic compound **4-1b**.[219] Radical bromination at the benzylic position was performed by treating **4-1b** with N-bromosuccinimide (NBS) and AIBN, yielding bromide **4-1c.** The subsequent reaction of **4-1c** with sodium azide in DMF then afforded the corresponding azide **4-1d** in good yield. Attempts to synthesize the corresponding amine from **4-1d** using triphenylphosphine following a typical Staudinger reduction [220] were unsuccessful. The Boc-protected product, **4-1e**, was then obtained with an in situ procedure, [221] albeit in low yield. m-Chloroperoxybenzoic acid (m-CPBA) was used to accomplish the S-oxidation of **4-1e** to produce the sulfoxide **4-1f.**[222] However, using a large excess of m-CPBA did not yield the corresponding sulfone.[223] So an alternative approach was explored by employing dimethyldioxirane as potential oxidizing agent. A one-pot procedure in which Oxone™ was added to a solution of **4-1e** in acetone led to the successful preparation of the sulfone **4-1g**. Finally, removal of the Boc protection in **4-1g** and **4-1f** afforded sulfone **4-1** and sulfoxide **4-2**,

respectively.



Scheme 4-2 Synthesis of **4-1** and **4-2** Reagents and conditions: (a) i) 1 eq. $NaNO_2$, conc. HCl, 0-5 ^oC; ii) 1.1 eq. Na_2S, 1.1 eq. S8, NaOH, rt, 2 h; iii) 4 eq. Zn, CH_3COOH, reflux, 16 h, 59 %; (b) 1 eq. CH_3COSH, conc. H_2SO_4, 50 ^oC, 2 h, 94 %; (c) 1.5 eq. NBS, 0.05 eq. AIBN, CCl_4, Reflux, 50 %; (d) 5 eq. NaN_3, DMF, 60 ^oC, 16 h, 85 %; (e) 2.1 eq. Boc_2O, 3 eq. K_2CO_3, 1.2 PPh_3, CH_2Cl_2, rt, 19 h, 35 %; (f) 1.5 eq. m-CPBA, CH_2Cl_2, -20 ^oC, 24 h, 60 %; (g) 4 eq. Oxone , 12 eq. $NaHCO_3$, acetone, 0 ^oC, 24 h, 80 %; (h) 10 eq. CF_3COOH, CH_2Cl_2, rt, 12 h, 100 %.

It was shown in the previous reports that Beaucage's reagent and other benzotrithiole 2-oxides could efficiently cause single-stranded breaks in duplex DNA in the presence of excess thiol, as measured by conversion of circular supercoiled DNA (form I) to circular relaxed (form II) DNA. The binding of substrates to DNA is believed to be strengthened by electrostatic interactions between the protonated amino side chain and the negatively charged phosphate backbone.

4.3.2 Testing of DNA Cleavage Reaction

To examine the potential DNA cleaving activities of compound **4-1**, plasmid

pBR322 with 1 were incubated and its concentrations were varied from 5 μM to 100 μM, at pH 7.0 and in the presence of 2-mercaptoethanol, and the results are shown in Figure 4-4.

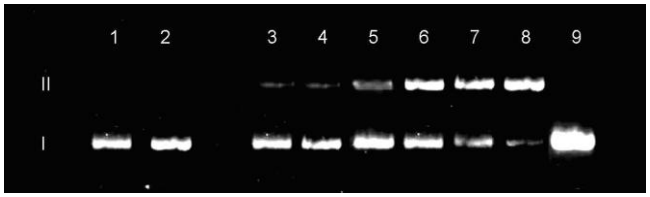


Figure 4-4 Thiol-dependent DNA cleavage by **4-1** with various concentrations at pH 7.0. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-1** (5 μM); Lane 3, **4-1** (5 μM) + thiol (25 μM); Lane 4, **4-1** (10 μM) + thiol (50 μM); Lane 5, pBR322 DNA with **4-1** (20 μM) + thiol (100 μM); Lane 6, pBR322 DNA with **4-1** (30 μM) + thiol (150 μM); Lane 7, pBR322 DNA with **4-1** (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with **4-1** (80 μM) + thiol (400 μM); Lane 9, pBR322 DNA with **4-1** (100 μM).

Similar to Beaucage's reagent, the DNA cleavage caused by **4-1** was also thiol-dependent. In the absence of thiol, compound **4-1** alone did not cause any DNA cleavage (Lanes 2 and 9). In the presence of thiol, sulfone 1 triggered DNA cleavage (Lanes 3 to 7). At a concentration of 80 μM (Lane 8), 1 led to very efficient cleavage of single-stranded DNA; the form I plasmid was almost completely converted to form II.

The pH dependence of compound 1-triggered DNA cleavage was next examined, and plasmid pBR322 was incubated with **4-1** in different buffer solutions (pH ranging from 6 to 8). As shown in Figure 4-5, there was no significant enhancement on DNA-cleaving activities when the pH values were varied. These results are certainly very different from the varacin C-caused cleavage which could be effectively

promoted under acidic conditions.

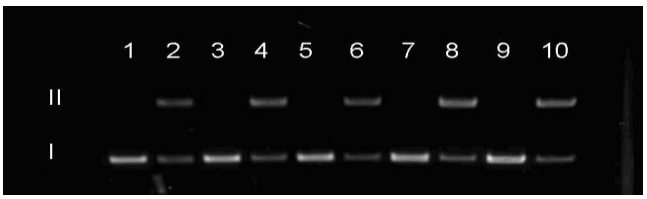


Figure 4-5 Thiol-dependent DNA cleavage by compound **4-1**. Assays were performed in 50 mM sodium phosphate buffer with different pH containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** (50 μM) and 2-mercaptoethanol (250 μM). The total volume of reaction was 20 μL. The reaction time was 12 h. Lane 1/2, pH 6.0 without/with thiol; Lane 3/4, pH 6.5 without/with thiol; Lane 5/6, pH 7.0 without/with thiol; Lane 7/8, pH 7.5 without/with thiol; Lane 9/10, pH 8.0 without/with thiol.

In order to achieve effective cleavage, reactions needed to be carried out for 12 hours. Longer reaction time could lead to better yield of the cleaving products, but not very significantly (Figure 4-6). Reaction could be almost completed in 24 hours.

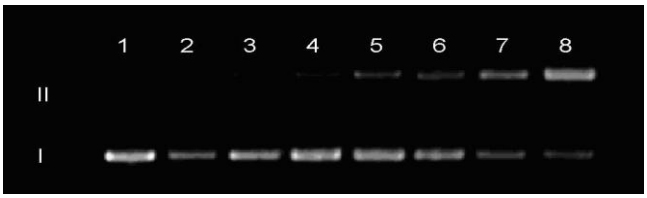


Figure 4-6 Time dependence of DNA cleavage by compound **4-1** (30 μM). Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction times corresponding to lanes 2 to 8 were 2, 4, 6, 8, 10, 12, 24 hours, respectively.

The DNA-cleaving activities of sulfoxide 2 was also investigated (Figure 4-7). It was found that **4-2** was not an effective DNA-cleaving agent, even with concentration of **4-2** at 1 mM, no DNA cleavage was observed. This result suggested the presence of sulfone functional group, as well as disulfide bond, are important for the observed DNA-cleaving activities.

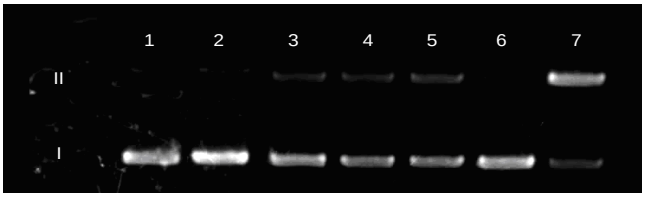


Figure 4-7 Thiol-dependent DNA cleavage by various concentrations of compound **4-2** compared to compound **4-1**. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-2** (5 μM) + thiol (25 μM); Lane 3, **4-2** (100 μM) + thiol (500 μM); Lane 4, **4-2** (300 μM) + thiol (1.5 mM); Lane 5, pBR322 DNA with **4-2** (1 mM) + thiol (5 mM); Lane 6, pBR322 DNA with **4-1** (5 μM) + thiol (25 μM); Lane 7, pBR322 DNA with **4-1** (100 μM) + thiol (500 μM).

Since compound **4-1** had shown great reactivity in catalyzing the thiol-dependent DNA cleavage reaction, next comparing effectiveness of compound **4-1** and Beaucage's reagent in causing DNA cleavage were carried out for experiment and the results are shown in Fig. 4-8, also quantifications of DNA cleavages are summarized in Table 4-1. The extents of DNA cleavage caused by aminomethylated Beaucage's reagent (**4-1**) with concentrations of 5 μM, 30 μM and 80 μM are shown in lanes 2 to 5, respectively. Cleavages of DNA by Beaucage's reagent are illustrated in lanes 7 to 9, with concentrations of Beaucage's reagent at 50 μM, 100 μM and 250 μM, respectively. Apparently, compound **4-1** is more efficient in causing DNA cleavage. In a quantitative comparison, compound **4-1** caused 76.6% cleavage of form I DNA at 80 μM (entry 5, Table 4-1), however, to reach similar level of cleavage (75.8%, entry 9, Table 4-1), a 250 μM of Beaucage's reagent needed to be used. These results clearly demonstrate that the presence of amino group effectively promotes DNA-cleaving activities.

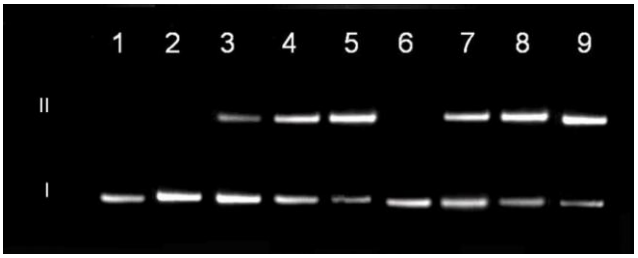


Figure 4-8 Thiol dependent cleavage by various concentrations of **4-1** at pH 7.0 compared to Beaucage's reagent (BR) at different concentrations. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-1** (80 μM) without thiol; Lane 3, **4-1** (5 μM) + thiol (25 μM); Lane 4, **4-1** (30 μM) + thiol (150 μM); Lane 5, pBR322 DNA with **4-1** (80 μM) + thiol (400 μM); Lane 6, pBR322 DNA with BR (250 μM) but without thiol; Lane 7, pBR322 DNA with BR (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with BR (100 μM) + thiol (500 μM); Lane 9, pBR322 DNA with BR (250 μM) + thiol (1.25 mM).

Table 4-1 Intensity of form I and II DNA after treatments with **4-1** and Beaucage's reagent [a]

Entry	I	II	% of I	% of II
1	95813	0	100	0
2	149121	0	100	0
3	150320	55233	73.1	26.9
4	94991	109390	46.5	53.5
5	45617	149348	**23.4**	**76.6**
6	106179	0	100	0
7	118073	109762	51.8	48.2
8	68102	149350	31.3	68.7
9	48113	150828	**24.2**	**75.8**

[a] Quantification of extent of DNA cleavage was done by performing densitometric scanning to measure the volume of each band. The same experiment was repeated twice, seeing Supporting Information for the full details.

4.3.3 Synthesis of Varacin B

Another class of sulfur-containing cyclic organic compounds, varacin B was designed to synthesis. As a family member of varacin, varacin B was not fully studied as well as varacin C although the differences of two seemed to be very tiny. The total synthesis of varacin C was reported in 2002 [216] and the examination of varacin C

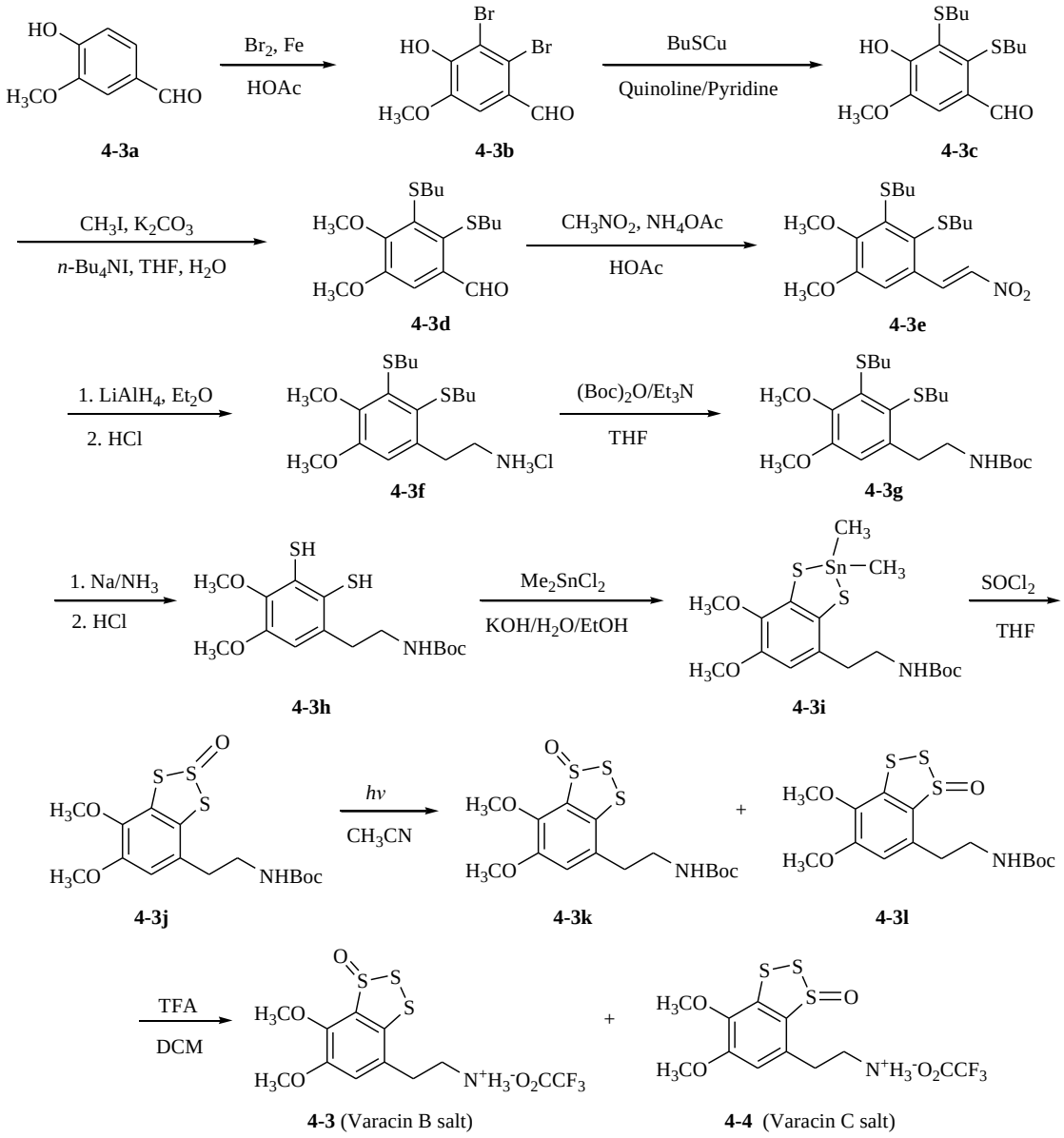
revealed that it exhibited potent antifungal and antimicrobial activities against *Staphylococcus aureus, Candida albicans* and *Bacillus subtilus*. [224] In thiol dependent DNA cleavage reaction, varacin C has shown great reactivity, and the catalytic concentration could be decreased to micromolar scale. However, surprisingly, the synthesis of varacin B was not reported so far and the bioactivity of varacin B was not investigtated as well. In order to compare the difference of varacin B and varacin C based on their structures of 1-oxide and 3-oxide respectively, the total of varacin B was done and the exploration of DNA cleavage reaction was done as well.

On the other hand, most tumor cells possess lower extracellular pH than that in normal tissue, an intrinsic feature of the tumor phenotype and is caused by alterations either in acid export or in clearance of extracellular acid.[313] Antibiotics that possess acid-triggered functionalities are very rare in nature,[308]

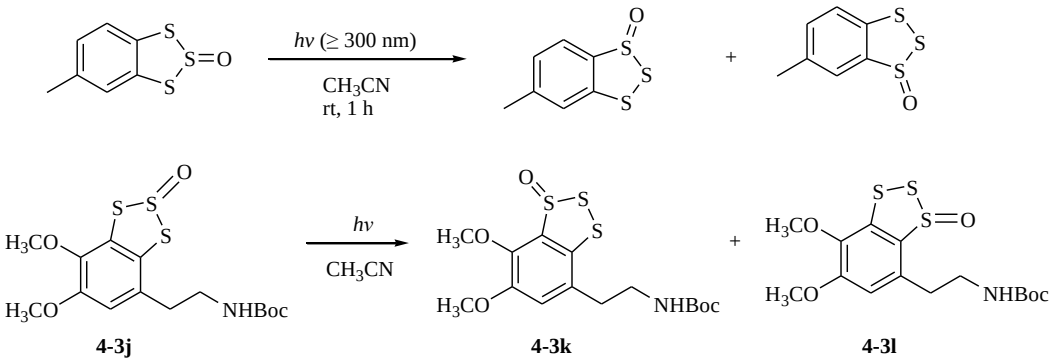
The synthetic routes leading to the synthesis of varacin B is illustrated in Scheme 4-3, which was modified from the earlier reported procedures by Davidson,[225] Li and their co-workers. [216]

5,6-Dibromovanillin **4-3b** was prepared from vanillin **4-3a** in the presence of bromine and ion power by refluxing condition. The yield of this single step was 70%. Compound **4-3c** was obtained in 61% chemical yield by treatment of **4-3b** with cuprous *n*-butylmercaptide in quinoline/pyridine at 160 ^{o}C. Next, **4-3c** was further derived by methylation to give the intermediate **4-3d** (65% yield) and followed by reaction in the condition of nitromethane/NH_4OAc to gave nitrostyrene **4-3e** in 78 % yield. After reduction of **4-3e** with $LiAlH_4$, the phenethylamine side chain was

obtained, then protected with *tert*-butoxycarbonyl (Boc) group, to give **4-3g** in 65% chemical yield from **4-3e**. The *n*-butyl groups could be deprotected in the Na/NH_3 condition to get the free dithiol groups. After treatment with dimethyltin dichloride which was considered to activate two free dithiol groups, dithiolate anion **4-3i** was formed. Upon addition of thionyl chloride, **4-3j** was obtained in 28 % chemical yield for three steps. A novel UV-induced isomerization was occurred to give a mixture of the corresponding **4-3k** and **4-3l**. The UV condition was followed by that in Sato's report (Scheme 4-4).[226] After removal of the Boc group with TFA in dichloromethane solution, the desired product **4-3** was obtained finally.

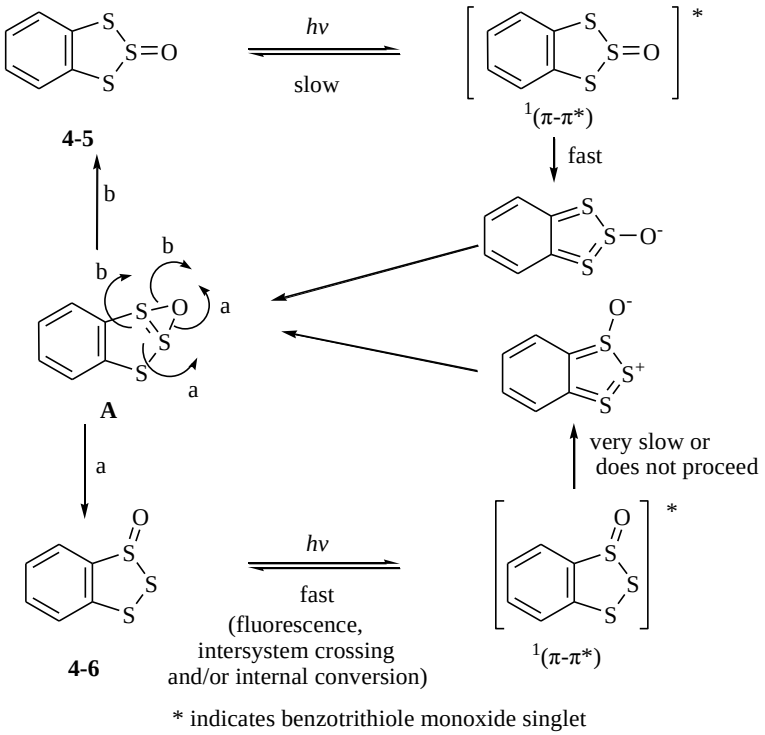


Scheme 4-3 Synthesis of varacin B and varacin C



Scheme 4-4 UV-induced isomerization

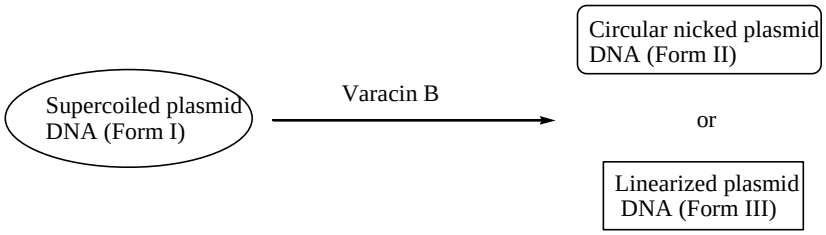
In the UV-induced isomerization reaction, the mechanism of oxygen shifting in trithiole 2-oxide ring could be followd by photochemical rearrangements without ring opening (Scheme 4-5). Benzotrithiole 2-oxide is first photolysed to form singlet excited **4-5**, which is rapidly transformed into the strained intermediate A. According to path *a* and path *b*, intermediate A could convert into benzotrithiole 1-oxide **4-6** and back to the 2-oxide **4-5**, respectively. The rate of quenching of singlet excited **4-6** is faster than that of conversion of singlet excited **4-6** into intermediate A.



Scheme 4-5 Plausible mechanism for the rearrangement from **4-5** to**4-6**

4.3.4 DNA-Cleaving Activities of Varacin B

The DNA-cleaving activity of varacin B was examined by monitoring its effectiveness in converting circular supercoiled DNA (form I) to the corresponding circular nicked form (form II). Plasmid pBR322 was used the same as that in the DNA-cleaving reaction of aminomethylated Beaucage's reagent (**4-1**).



Scheme 4-6 The conversion of circular supercoiled DNA

The discovery of the DNA-cleaving activities of varacin C revealed that thiol-dependent DNA cleavage reaction could efficiently occur in the acid condition, which could be considered as a good sign for the further exploration of other biological activities. Because usually, most tumor cells possess lower extracellular pH than that in normal tissue, an intrinsic feature of the tumor phenotype and is caused by alterations either in acid export or in clearance of extracellular acid.[227]

As shown in Figure 4-9, different pH values of phosphate buffer were used in the the thiol dependent DNA cleavage reaction. It was shown that by decreasing pH value of phosphate buffer, the amount of DNA cleavage by varacin B increases. At pH 5.5, the cleavage reaction could perform better, thus obtaining more amounts of form II (85%, lane 2). It was proven that the reaction could be accelerated in the acidic

condition so the pH value 5.5 was chose as the necessary reaction condition for further study.

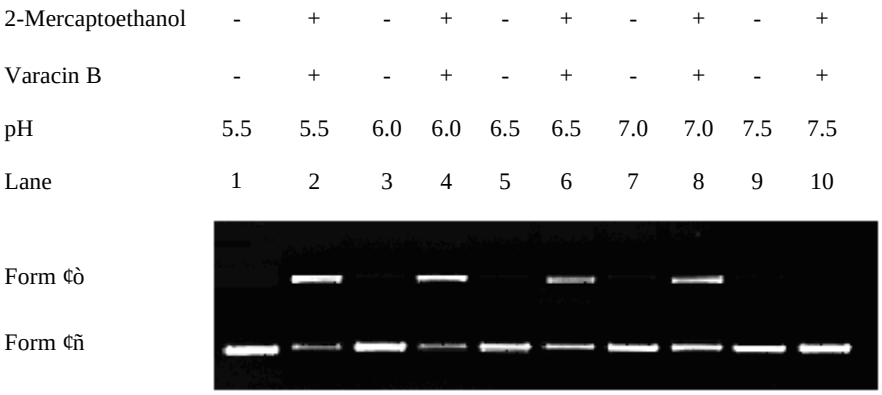


Figure 4-9 Acid-promoted DNA cleavage by varacin B in sodium phosphate buffer solutions at various pH values. Reactions were incubated at 37 °C for 12 h in 50 mM sodium phosphate containing 10% (v/v) acetonitrile, 500 ng of supercoiled pBR322 DNA and in the presence or absence of varacin B and 1 mM 2-mercaptoethanol activating reagents; lane 1, pH 5.5, no added thiol and varacin B; lane 2, pH 5.5, 150 nM varacin B and 1 mM 2-mercaptoethanol; lane 3, pH 6.0, no added thiol and varacin B; lane 4, pH 6.0, 150 nM varacin B and 1 mM 2-mercaptoethanol; lane 5, pH 6.5, no added thiol and varacin B; lane 6, pH 6.5, 50 nM varacin B and 1 mM 2-mercaptoethanol; lane 7, pH 7.0, no added thiol and varacin B; lane 8, pH 7.0, 150 nM varacin B and 1 mM 2-mercaptoethanol; lane 9, pH 7.5, no added thiol and varacin B; lane 10, pH 7.5, 150 nM varacin B and 1 mM 2-mercaptoethanol.

Various concentrations of varacin B under pH 5.5 conditions were tested. As agarose gel shown in Figure 4-10, with the increasing the concentrations of varacin B, the amount of DNA cleavage could also increase. It was found that the concentration of varacin B could be as low as 10 nM at pH 5.5 (lane 2) to produce the relaxed form of DNA (form II).

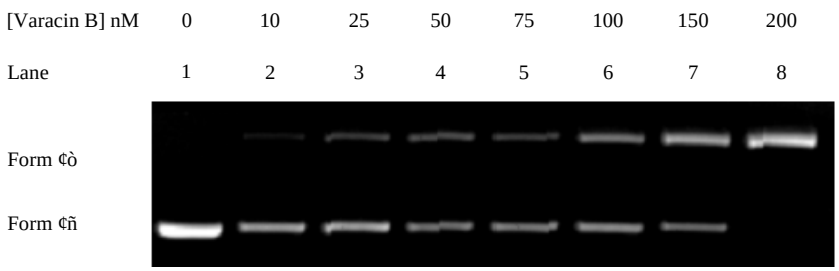


Figure 4-10 Acid-promoted DNA cleavage by various concentrations of varacin B. Reactions were incubated at 37 °C for 12 h in 50 mM sodium phosphate (pH 5.5) containing 10% (v/v) acetonitrile, 500 ng supercoiled pBR322 DNA, varacin B and 1 mM 2-mercaptoethanol; Lane 1, pBR322 alone; lane 2, 10 nM varacin B; lane 3, 25 nM varacin B; lane 4, 50 nM varacin B; lane 5, 75 nM varacin B; lane 6, 100 nM varacin B; lane 7, 150 nM varacin B; lane 8, 200 nM varacin B.

Last, different types of nucleophiles were used to further explore the reaction mode of DNA cleavage. The results are shown in Figure 4-11**.** The DNA-cleavage by varacin B did not occur when thiol was abscent in the reaction (lane 1). In addition, glutathione and cysteine were both capable of triggering varacin B-mediated DNA cleavage as effectively as 2-mercaptoethanol (lanes 4 and 6). However, phenol or aniline did not promote the DNA cleavage reaction as shown in lane 8 and 10 below.

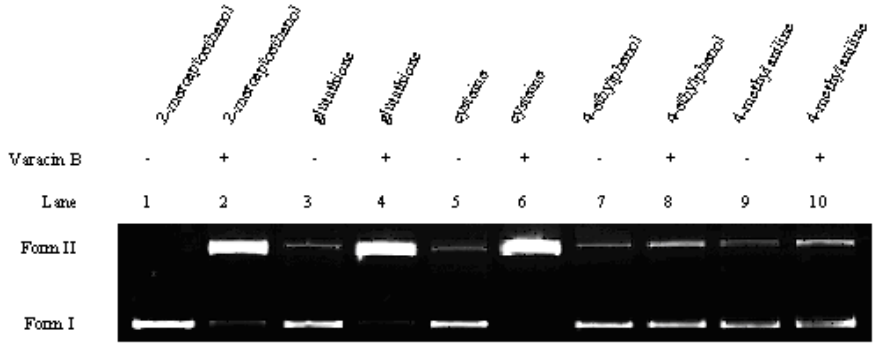


Figure 4-11 Activation of varacin B-promoted DNA cleavage by various nucleophiles. Reactions were incubated at 37 °C for 6 h in 50 mM sodium phosphate (pH 5.5) containing 10% v/v acetonitrile, 500 ng of supercoiled pBR322 DNA, 200 nM varacin B and in the presence or absence of nucleophile; lane 1, no added 2-mercaptoethanol; lane 2, 1 mM 2-mercaptoethanol; lane 3, no added glutathione; lane 4, 1 mM glutathione; lane 5, no added cysteine; lane 6, 1 mM cysteine; lane 7, no added 4-ethylphenol; lane 8, 1 mM 4-ethylphenol; lane 9, no added 4-methylaniline; lane 10, 1 mM 4-methylaniline.

4.4 Conclusion

In conclusion, two classes of sufur-containing cyclic organic compounds were synthesized. For disulfide-derived Beaucage's reagent analogue, aminomethylated Beaucage's reagent **4-1**, and its sulfoxide analogue **4-2** were successfully synthesized . The DNA-cleaving activities of **4-1** and **4-2** have been investigated by examining their effectiveness in converting circular supercoiled DNA (form I) to the corresponding circular nicked form (form II). The presence of amino side chain in **4-1** improved its DNA cleaving activity, leading to substantial potency improvement comparing to its non-amino analogue (3H-1,2-benzodithiol-3-one 1-oxide). The sulfoxide **4-2**, however, was found to be ineffective in cleaving DNA. The results suggested that combination of the amino group and sulfur-containing DNA-cleaving functionality could lead to the generation of novel effective DNA-cleaving agents, which may find potential applications in anti-cancer research.

For trithiol-derived cyclic organic compounds, varacin B were successfully synthesized and biological test methods of varacin B for the DNA cleavage reaction were developed compared with that of varacin C. The test results of acid-promoted DNA-cleaving activities of natural varacin B strongly support that it possess novel, important DNA cleavage abilities, and it also sheds the light on the development of anti-cancer agents by further modification and optimization of these sulfur-containing cyclic organic compounds

Further investigation to understand the origin of observed DNA-cleavage and to search for more potent sulfur-containing DNA-cleaving functionalities are in progress.

4.5 Experimental Section

4.5.1 General Information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 or DPX300 (300 MHz) or AMX500 (500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.0). Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants are reported in Hertz (Hz). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode, and a Finnigan/MAT 95XL-T mass spectrometer in FAB mode. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. For thin-layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F_{254}) was used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine, or ceric ammonium molybdate followed by heating on a hot plate. Flash chromatography separations were performed on Merck 60 (0.040 - 0.063 mm) mesh silica gel. Microwave irradiation was performed using a CEM Discover Synthesis Unit with a 0.5-2 microwave reaction vial.

4.5.2 Materials

All reagents and chemicals were purchased from Sigma-Aldrich Chemical Co. and used as received, unless otherwise stated. Handheld UV Lamp (Brand: Uvitec, UK; Model: LF 106L) used in our studies was purchased from its Science and Medical Pte. Ltd. Bromine was purchased from VWR Singapore Pte Ltd. THF was distilled over sodium wire under nitrogen atmosphere before use. Dichloromethane (DCM) and triethylamine (Et_3N) were distilled over calcium hydride prior to use.

The pBR322 DNA was purchased from New England Biolabs (Cat. Number: N3033L, 1,000 μg/mL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 at 25 ^{o}C). This DNA is isolated from *E. coli* ER2272 by a standard plasmid purification procedure, and should be stored at -20 ^{o}C. It is a commonly used plasmid cloning vector in *E. coli*.[228] It contains a double-stranded circle 4,361 base pairs in length.[229] pBR322 contains the genes for resistance to ampicillin and tetracycline, and can be amplified with chloramphenicol. The molecular weight is 2.83 x 10^6 daltons.[230]

4.5.3 General Methods

Analytical thin-layer chromatography (TLC) was carried out on Whatman TLC plates precoated with silica gel 60 (Merck 60 F_{254}). Visualization was performed using a UV lamp (λ= 254 nm). Column chromatography was performed on Merck silica gel (230-400 mesh) eluting with ethyl acetate and hexane, using compressed air.

All DNA-cleaving reactions by aminomethylated Beaucage's reagent (**4-1**) and varacin B were analyzed by electrophoresis on 1% agarose gels in 1 M Tris-acetaed

buffer (100 v, 2 h). The resulting gels were subsequently photographed on a UV trans-illuminator after attaining with ethidium bromide. The amount of DNA in each band of ethidium-stained gels was quantified using Lumi-Imager (LumiAnalyst 3.1).

General procedures for preparation of 1% agarose gel electrophorosis

1) Prepare 1x TBE (Prepared from the 10x stock).

2) 1g of agarose was added into the conical flask and 100 mL 1x TBE was added subsequently.

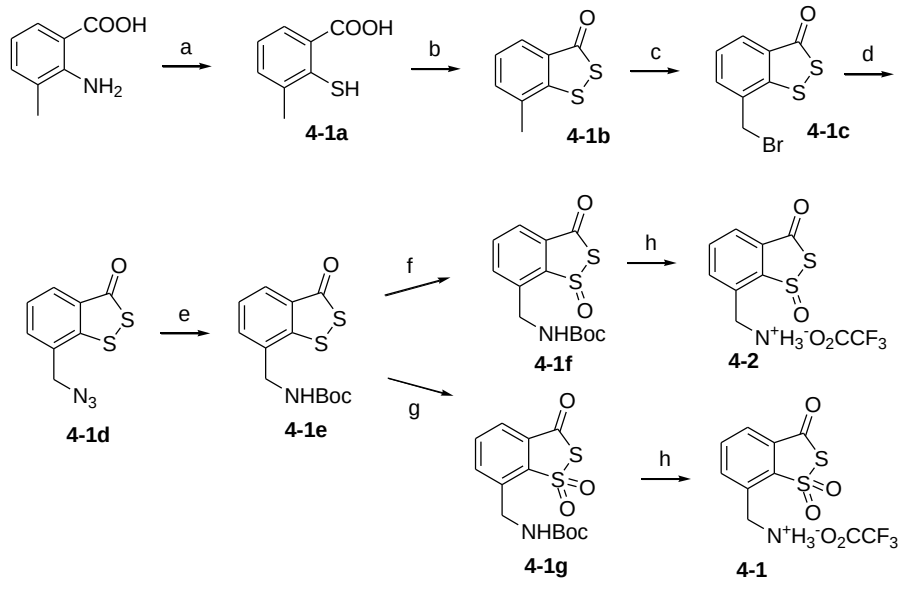
3) Heat the solution to boiling to dissolve the agarose.

4) Add 5 µl of ethidium bromide to the dissolved agarose and mix.

5) Get a gel plate and a comb. Put the two dams into the slots on each side of the gel plate. Make sure that they fit tight. Pour the melted agarose onto the gel plate in the Agarose gel electrophoresis box. Next, place the comb (well-maker) with teeth in place.

6) Let the gel cool to room temperature.

4.5.4 Experimental Procedures and Characterization of 4-1 and Intermediates



Scheme 4-2 Synthesis of **4-1** Reagents and conditions: (a) i) 1 eq. $NaNO_2$, conc. HCl, 0-5 ^oC; ii) 1.1 eq. Na_2S, 1.1 eq. S8, NaOH, rt, 2 h; iii) 4 eq. Zn, CH_3COOH, reflux, 16 h, 59 %; (b) 1 eq. CH_3COSH, conc. H_2SO_4, 50 ^oC, 2 h, 94 %; (c) 1.5 eq. NBS, 0.05 eq. AIBN, CCl_4, Reflux, 50 %; (d) 5 eq. NaN_3, DMF, 60 ^oC, 16 h, 85 %; (e) 2.1 eq. Boc_2O, 3 eq. K_2CO_3, 1.2 PPh_3, CH_2Cl_2, rt, 19 h, 35 %; (f) 1.5 eq. m-CPBA, CH_2Cl_2, -20 ^oC, 24 h, 60 %; (g) 4 eq. Oxone , 12 eq. $NaHCO_3$, acetone, 0 ^oC, 24 h, 80 %; (h) 10 eq. CF_3COOH, CH_2Cl_2, rt, 12 h, 100 %.

2-Mercapto-3-benzoic acid (**4-1a**)

To a mixture of 2-amino-3-methyl-benzoic acid (0.30 g, 1.98 mmol), water (1 mL) and concentrated HCl (0.4 mL) at 0 °C, was added a pre-cooled solution of $NaNO_2$ (0.14 g, 1.98 mmol) in water (0.5 mL). The diazonium salt solution was tested with starch-iodine paper and a blue coloration was observed. $Na_2S{\cdot}9\ H_2O$ (0.52 g, 2.18 mmol) and powdered sulfur (0.067 g, 2.18 mmol) were dissolved in boiling water (2 mL), a solution of 10 M NaOH (0.2 mL) was then added and the resulting yellow solution was cooled in an ice bath. Along with crushed ice, the diazonium solution was added to the stirred alkaline sulfide solution over 30 minutes. The temperature of the reaction mixture as kept below 5 °C by directly adding ice. The mixture was allowed to warm up to room temperature and stirred for another 2 hours. Concentrated HCl solution (0.24 ml) was added to adjust the pH of solution to 3. The precipitate was filtered, and washed with water. To further remove excess sulfur, the precipitate was boiled in a solution of Na_2CO_3 (0.12 g) in water (4 mL), and the mixture was filtered off while hot. The pH of the filtrate was adjusted to 3, and the precipitate was filtered, and washed with water. The crude disulfide was then mixed with zinc dust (0.5 g, 7.69 mmol) and glacial acetic acid (1 mL), and the mixture was brought to reflux for 16 h. After cooling down to room temperature, the mixture was

filtered, and the precipitate was washed with water, and then suspended in water (5 mL). An aqueous solution of 33 % NaOH (0.08 mL) was added, and the mixture was boiled for 20 min to ensure complete extraction of product from the precipitate. After filtration, concentrated HCl was added to the filtrate, and the precipitate was collected and washed with water to afford desired **4-1a** as a pale pink powder (0.20 g, 59%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.43 (s, 3H), 6.60 (s, 1H), 7.13 (t, 1H, *J* = 7.7 Hz), 7.40 (d, 1H, *J* = 7.4 Hz), 8.08 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 21.4, 123.6, 124.4, 130.8, 134.7, 137.0, 138.9, 172.6; HRMS (IT-TOF) calcd for $[C_8H_7O_2{}^{32}S]^-$ 167.0172, found 167.0141; m.p. 195.0 °C.

7-Methyl-3H-benzo[c][1,2]dithiol-3-one (**4-1b**)

To a suspension of **4-1a** (0.14 g, 0.82 mmol) in concentrated H_2SO_4 (2 mL) at ambient temperature was added thioacetic acid (0.12 ml, 1.64 mmol) dropwise over a period of 40 minutes. The mixture was heated at 50 °C for 2 h, and then quenched by pouring the mixture onto crushed ice (300 g). The precipitate was filtered through a Büchner funnel, thoroughly washed with water and suspended in chloroform (10 mL). The mixture was shaken with saturated aqueous sodium bicarbonate (5 mL), and solid was filtered and triturated in boiling chloroform several times, and the organic layers were combined. The organic phase was washed with saturated sodium bicarbonate (10 mL) and water (2 x 10 mL), and dried over anhydrous magnesium sulfate. After filtration and concentration, the crude was purified by column chromatography (EtOAc/hexane 1:3) to afford **4-1b** as a yellow needle (0.140 g, 94%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.49 (s, 3H), 7.35 (t, 1H, *J* = 7.6 Hz), 7.47 (d, 1H, *J* = 7.1 Hz), 7.80 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 18.4, 124.9, 125.9, 129.0, 133.6, 133.9, 147.9, 194.0; HRMS (EI) calcd for $C_8H_6O_1{}^{32}S_2$ 181.9860, found 181.9865; m.p. 96.0 – 97.0 °C.

7-(Bromomethyl)-3H-benzo[c][1,2]dithiol-3-one (**4-1c**)

To a solution of **4-1b** (0.14 g, 0.746 mmol) in CCl_4 (5 mL) were added *N*-bromosuccinimide (0.20 g, 1.59 mmol) and AIBN (6 mg, 0.036 mmol). The reaction mixture was then brought to reflux for 24 h, and quenched by adding cold water. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by column chromatography (EtOAc/hexane 1:20) to give **4-1c** as a pale yellow needle (0.097 g, 50%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 4.63 (s, 2H), 7.40 (t, 1H, *J* = 7.9 Hz), 7.67 (d, 1H, *J* = 7.9 Hz), 7.89 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.6, 126.3, 127.7, 130.2, 133.6, 134.1, 147.7, 193.1; HRMS (EI) calcd for $C_8H_5O_1{}^{79}Br_1{}^{32}S_2$ 259.8965, found 259.8962; $C_8H_5O_1{}^{81}Br_1{}^{32}S_2$ 261.8945, found 261.8942; m.p .119.0 – 120.0 °C.

7-(Azidomethyl)-3H-benzo[c][1,2]dithiol-3-one (**4-1d**)

To a solution of **4-1c** (0.25 g, 0.960 mmol) in DMF (3 mL) was added NaN_3 (0.310 g, 4.79 mmol), and the resulting mixture was stirred at 60 °C for 24 h. The

solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (20 mL). The organic phase was washed with water (3 x 15 mL), and dried over anhydrous $MgSO_4$. After filtration and concentration *in vacuo*, the crude was purified by column chromatography (EtOAc/hexane 1:4) to give **4-1d** as a white solid (0.182 g, 85%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 4.63 (s, 2H), 7.48 (t, 1H, *J* = 7.7 Hz), 7.67 (d, 1H, *J* = 7.2 Hz), 7.97 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 52.0, 126.1, 127.5, 130.2, 131.8, 133.0, 147.2, 193.2; HRMS (EI) calcd for $C_8H_5O_1N_3{}^{32}S_2$ 222.9874, found 222.9872; m.p. 62.0 – 63.0 °C.

tert-Butyl (3-oxo-3H-benzo[c][1,2]dithiol-7-yl)methylcarbamate (**4-1e**)

To a solution of **4-1d** (0.11 g, 0.490 mmol) in CH_2Cl_2 (6 mL) were added PPh_3 (0.15 g, 0.588 mmol), Boc_2O (0.23 g, 1.03 mmol), K_2CO_3 (0.20 g, 1.76 mmol) and water (2 mL), the reaction mixture was stirred at room temperature for 24 hours. The organic and aqueous phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic extracts were washed with brine (1 x 15 mL), and dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/hexane 1:6) to give **4-1e** as a white solid (0.050 g, 35%)

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.49 (s, 9H), 4.53 (d, 2H, *J* = 5.9 Hz), 5.15 (s, 1H), 7.41 (t, 1H, *J* = 7.6 Hz), 7.62 (d, 1H, *J* = 7.2 Hz), 7.87 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.3, 42.2, 80.2, 126.0, 126.4, 129.8, 132.1, 134.9, 146.5, 155.6,

193.6; HRMS (EI) calcd for $C_{13}H_{15}O_3N_1{}^{32}S_2$ 297.0493, found 297.0494; m.p. 96.0 – 97.0 °C.

7-(*N*-Boc-aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1-oxide (**4-1f**)

To a stirred solution of **4-1e** (0.020 g, 0.067 mmol) in CH_2Cl_2 (2 mL) at -20 °C was added slowly a solution of *m*-chloroperoxybenzoic acid (0.040 g, 0.202 mmol) in CH_2Cl_2 (2 mL). The mixture was kept stirring at below -30 °C for 45 minutes, and then at room temperature for 24 hours. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous $Na_2S_2O_3$ (2 x 5 mL) and $NaHCO_3$ (1 x 5 mL). The organic phase was concentrated *in vacuo* and purified by column chromatography (EtOAc/hexane 1:5) to give **4-1f** as a white solid (0.013 g, 60%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.47 (s, 9H), 4.52 (d, 2H, *J* = 5.9 Hz), 4.99 (s, 1H), 7.41 (t, 1H, *J* = 7.9 Hz), 7.61 (d, 1H, *J* = 6.8 Hz), 7.88 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 42.3, 80.4, 126.1, 126.6, 129.9, 132.2, 135.0, 146.7, 155.6, 193.6; HRMS (ESI) calcd for $C_{13}H_{15}O_4N_1{}^{32}S_2{}^{23}Na_1$ 336.0335, found 336.0323; m.p. 94.0 – 95.0 °C.

7-(*N*-Boc-aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (**4-1g**)

To a stirred solution of **4-1e** (10 mg, 0.034 mmol) in acetone (1.5 mL) at 0 °C was added Oxone™ (83 mg, 0.135 mmol) and $NaHCO_3$ (33.6 mg, 0.404 mmol) over 20 minutes. The reaction mixture was then allowed to warm up to room temperature. Upon completion of reaction as judged by TLC, solvent was removed *in vacuo*. The

residue was taken up in CH_2Cl_2, and the mixture was filtered. Concentration of the filtrate under reduced pressure yielded **4-1g** as a white solid (9 mg, 80%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.43 (s, 9H), 4.57 (dd, 1H, *J* = 4.6 Hz and 15.6 Hz), 4.90 (dd, 1H, *J* = 7.7 Hz and 15.5 Hz), 5.27 (s, 1H), 7.77 (t, 1H, *J* = 7.6 Hz), 7.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.4, 41.4, 80.6, 126.6, 129.9, 130.3, 131.9, 134.0, 136.6, 140.8, 192.7; HRMS (IT-TOF) calcd for $C_{13}H_{15}O_5N_1{}^{32}S_2Na_1$ 352.0289, found 352.0254; m.p. 192 .0 °C.

7-(Aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1-oxide (**4-2**)

To a solution of **4-1f** (15 mg, 0.051 mmol) in CH_2Cl_2 (10 mL) was added CF_3COOH, and the resulting solution was stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure and co-evaporated with CH_2Cl_2 several times to afford **4-2** as pale yellow syrup (10 mg, 100 %).

7-(Aminomethyl)-3H-benzo[c][1,2]dithiol-3-one 1,1-dioxide (**4-1**)

A light yellow powder; ^{1}H NMR (CD_3OD, 300 MHz) δ = 4.64 (dd, *J* = 14.5 Hz and 26.6 Hz), 8.00 (t, 1H, *J* = 7.6 Hz), 8.10 (m, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ = 70.5, 128.2, 130.1, 134.5, 134.8, 137.8, 148.8, 180.5; HRMS (IT-TOF) calcd for $[C_8H_8N_1O_3{}^{32}S_2]^+$ 229.9946, found 229.9912.

4.5.5 Figures of DNA Cleavage Experiments by 4-1 and 4-2

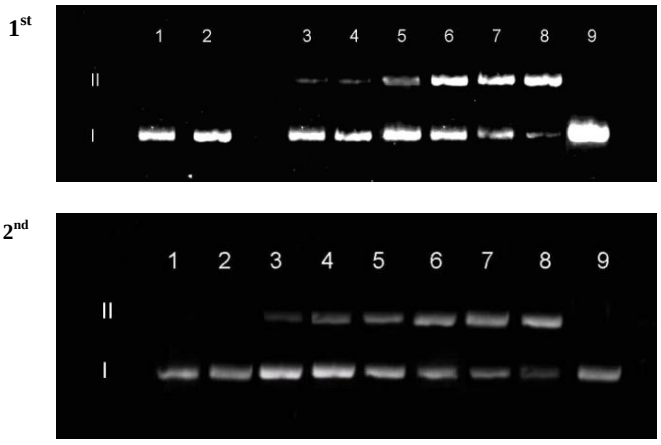


Figure 4-4 Thiol-dependent DNA cleavage by **4-1** with various concentrations at pH 7.0. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-1** (5 μM); Lane 3, **4-1** (5 μM) + thiol (25 μM); Lane 4, **4-1** (10 μM) + thiol (50 μM); Lane 5, pBR322 DNA with **4-1** (20 μM) + thiol (100 μM); Lane 6, pBR322 DNA with **4-1** (30 μM) + thiol (150 μM); Lane 7, pBR322 DNA with **4-1** (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with **4-1** (80 μM) + thiol (400 μM); Lane 9, pBR322 DNA with **4-1** (100 μM).

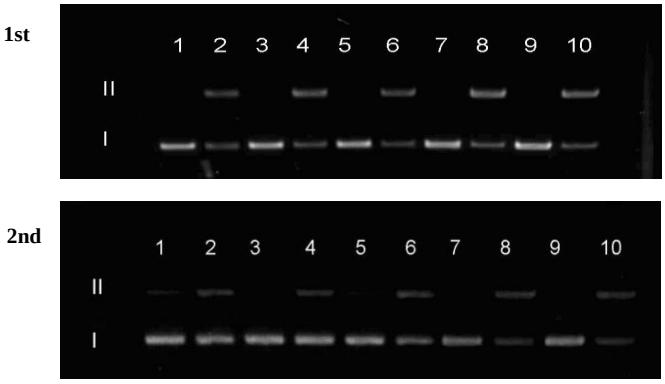


Figure 4-5 Thiol-dependent DNA cleavage by compound **4-1**. Assays were performed in 50 mM sodium phosphate buffer with different pH containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** (50 μM) and 2-mercaptoethanol (250 μM). The total volume of reaction was 20 μL. The reaction time was 12 h. Lane 1/2, pH 6.0 without/with thiol; Lane 3/4, pH 6.5 without/with thiol; Lane 5/6, pH 7.0 without/with thiol; Lane 7/8, pH 7.5 without/with thiol; Lane 9/10, pH 8.0 without/with thiol.

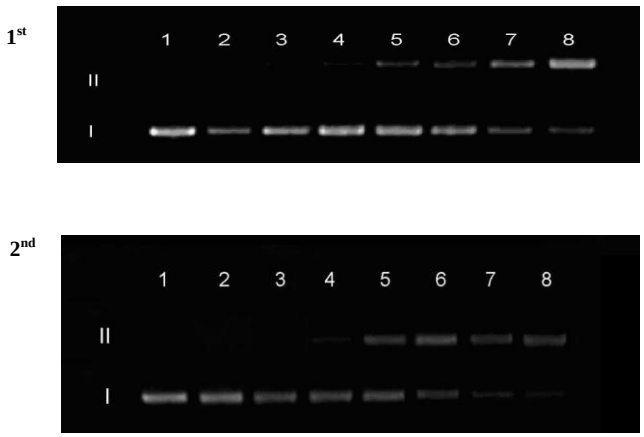


Figure 4-6 Time dependence of DNA cleavage by compound **4-1** (30 μM). Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction times corresponding to lanes 2 to 8 were 2, 4, 6, 8, 10, 12, 24 hours, respectively.

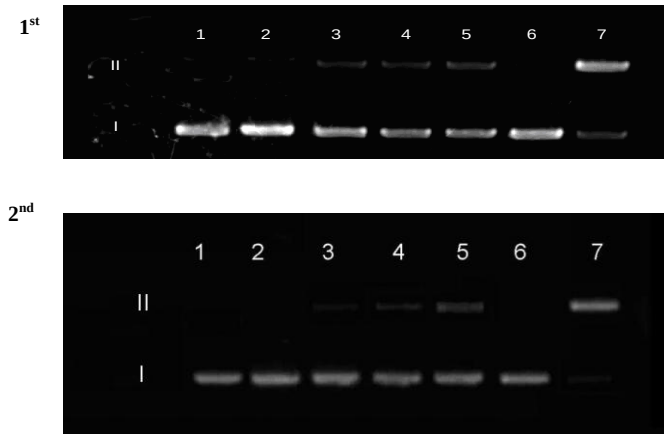


Figure 4-7 Thiol-dependent DNA cleavage by various concentrations of compound **4-2** compared to compound 4-1. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **4-1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-2** (5 μM) + thiol (25 μM); Lane 3, **4-2** (100 μM) + thiol (500 μM); Lane 4, **4-2** (300 μM) + thiol (1.5 mM); Lane 5, pBR322 DNA with **4-2** (1 mM) + thiol (5 mM); Lane 6, pBR322 DNA with **4-1** (5 μM) + thiol (25 μM); Lane 7, pBR322 DNA with **4-1** (100 μM) + thiol (500 μM).

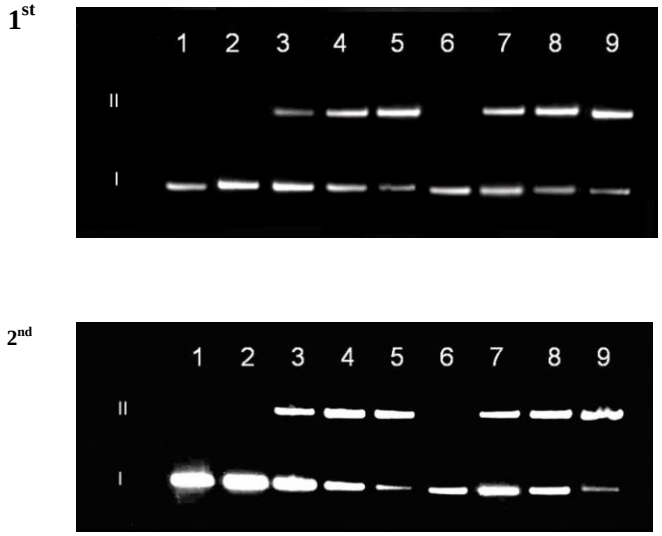


Figure 4-8 Thiol dependent cleavage by various concentrations of **4-1** at pH 7.0 compared to Beaucage's reagent (BR) at different concentrations. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **4-1** (80 μM) without thiol; Lane 3, 1 (5 μM) + thiol (25 μM); Lane 4, **4-1** (30 μM) + thiol (150 μM); Lane 5, pBR322 DNA with **4-1** (80 μM) + thiol (400 μM); Lane 6, pBR322 DNA with BR (250 μM) but without thiol; Lane 7, pBR322 DNA with BR (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with BR (100 μM) + thiol (500 μM); Lane 9, pBR322 DNA with BR (250 μM) + thiol (1.25 mM).

4.5.6 Quantifications of Extents of Cleavages by 4-1 and 4-2

Table 4-2 quantifications of cleavage with various concentrations at pH 7.0

	Sum intensity				Sum intensity							
Entry	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	116408	0	100	0	168814	0	100	0	100	0	0	0
2	119369	0	100	0	165865	0	100	0	100	0	0	0
3	192432	27148	87.6	12.4	166339	18114	90.2	9.8	88.9	11.1	1.8	1.8
4	194185	58639	76.8	23.2	133427	18908	87.6	12.4	82.2	17.8	7.6	7.6
5	132248	66976	66.4	33.6	211213	54324	79.5	20.5	73.0	27.0	9.3	9.3
6	109848	106571	50.8	49.2	166993	149468	52.8	47.2	51.8	48.2	1.4	1.4
7	65937	118751	35.7	64.3	97974	140493	41.1	58.9	38.4	61.6	3.8	3.8
8	40351	130613	23.6	76.4	19609	166285	10.5	89.5	17.1	82.9	9.2	9.2
9	118619	0	100.0	0.0	374249	0	100	0	100	0	0	0

Table 4-3 quantifications of thiol-dependent DNA cleavage by compound **4-1**

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	135986	10053	93.1	6.9	80858	0	100	0	96.6	3.4	4.87	4.87
2	113579	20520	84.7	15.3	35142	35424	49.8	50.2	67.2	32.8	24.68	24.68
3	129394	0	100.0	0.0	93217	0	100.0	0.0	100.0	0.0	0.00	0.00
4	125688	25608	83.1	16.9	33404	46735	41.7	58.3	62.4	37.6	29.27	29.27
5	113337	4332	96.3	3.7	88295	0	100.0	0.0	98.2	1.8	2.60	2.60
6	64548	33348	65.9	34.1	28518	37958	42.9	57.1	54.4	45.6	16.29	16.29
7	78943	0	100.0	0.0	97602	0	100.0	0.0	100.0	0.0	0.00	0.00
8	30514	30445	50.1	49.9	40834	63367	39.2	60.8	44.6	55.4	7.69	7.69
9	93515	0	100.0	0.0	103124	0	100.0	0.0	100.0	0.0	0.00	0.00
10	28355	29734	48.8	51.2	33233	53280	38.4	61.6	43.6	56.4	7.35	7.35

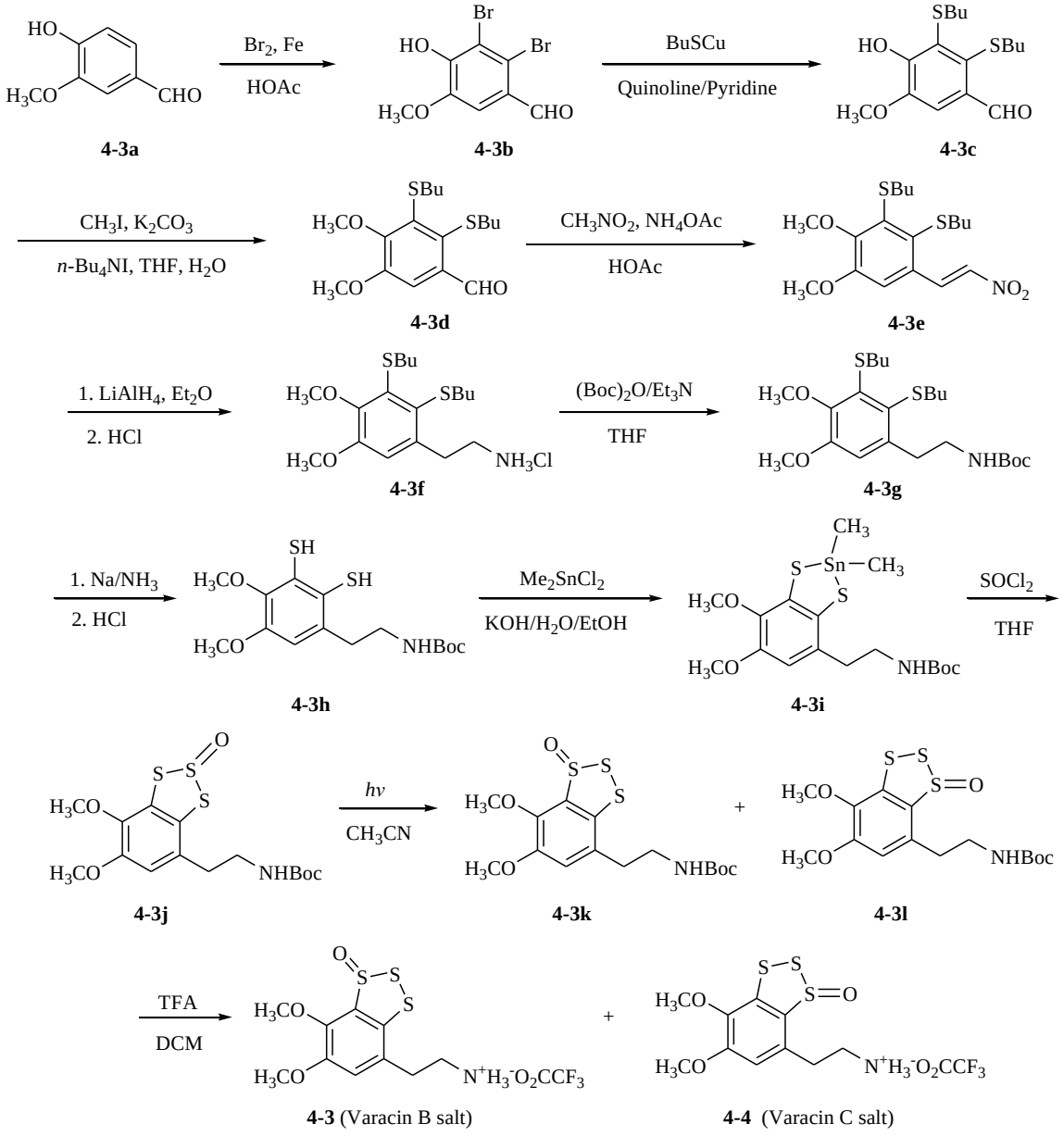
Table 4-4 quantifications of time dependence of DNA cleavage by compound **4-1**

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	163532	0	100	0	113394	0	100	0	100.0	0.0	0.00	0.00
2	62503	0	100.0	0.0	104938	0	100.0	0.0	100.0	0.0	0.00	0.00
3	108855	0	100.0	0.0	58072	0	100.0	0.0	100.0	0.0	0.00	0.00
4	162707	0	100.0	0.0	62278	8809	87.6	12.4	93.8	6.2	8.76	8.76
5	147879	23650	86.2	13.8	68718	46950	59.4	40.6	72.8	27.2	18.95	18.95
6	55494	25456	68.6	31.4	35986	69861	34.0	66.0	51.3	48.7	24.43	24.43
7	38632	60707	38.9	61.1	15421	37356	29.2	70.8	34.1	65.9	6.84	6.84
8	27417	150350	15.4	84.6	10833	45351	19.3	80.7	17.4	82.6	2.73	2.73

Table 4-5 quantifications of various concentrations of **4-1** at pH 7.0 compared to Beaucage's reagent (BR)

	Sum intensity				Sum intensity							
	I	II	% of I	% of II	I	II	% of I	% of II	AVG I	AVG II	STDEV I	STDEV II
1	391198	0	100	0	95813	0	100	0	100.0	0.0	0.00	0.00
2	352192	0	100	0	149121	0	100	0	100.0	0.0	0.00	0.00
3	269758	135568	66.6	33.4	150320	55233	73.1	26.9	69.8	30.2	4.65	4.65
4	158716	176404	47.4	52.6	94991	109390	46.5	53.5	46.9	53.1	0.62	0.62
5	57741	149861	27.8	72.2	45617	149348	23.4	76.6	25.6	74.4	3.12	3.12
6	123395	0	100	0	106179	0	100	0	100.0	0.0	0.00	0.00
7	196990	122179	61.7	38.3	118073	109762	51.8	48.2	56.8	43.2	7.00	7.00
8	142584	154397	48.0	52.0	68102	149350	31.3	68.7	39.7	60.3	11.80	11.80
9	44269	172340	20.4	79.6	48113	150828	24.2	75.8	22.3	77.7	2.65	2.65

4.5.7 Experimental Procedures and Characterization Varacin B and Intermediates



4-3a **4-3b** **4-3c**

4-3d **4-3e**

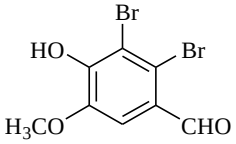
4-3f **4-3g**

4-3h **4-3i**

4-3j **4-3k** **4-3l**

4-3 (Varacin B salt) **4-4** (Varacin C salt)

2,3-Dibromo-4-hydroxy-5-methoxybenzaldehyde (**4-3b**):

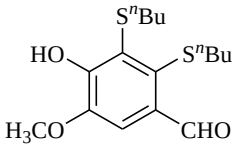


4-3b

Compound **4-3b**: a pink solid; 62% yield; ^{1}H NMR (300 MHz, DMSO-D$_6$) δ = 3.89 (s,

3H, OCH_3), 7.38 (s, 1H, ArH),10.06 (s, 1H, CHO); ^{13}C NMR (75 MHz, DMSO-D_6) δ = 191.0, 151.4, 147.6, 126.3, 122.3, 113.9, 110.5, 56.5; MS (ESI) calcd for $[C_8H_7Br_2O_3]^+$ M: 308.9; found *m/z* 309.1.

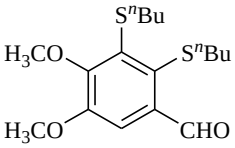
2,3-Bis(butylthio)-4-hydroxy-5-methoxybenzaldehyde (**4-3c**)



4-3c

Compound **4-3c**: a yellow solid; 65% yield; ^1H NMR (300 MHz, $CDCl_3$) δ = 0.86-0.88 (m, 6H, $2CH_3$), 1.39-1.54 (m, 8H, $2CH_2CH_2$), 2.84-2.87 (m, 4H, $2SCH_2$), 3.95 (s, 3H, OCH_3), 7.47 (s, 1H, ArH), 10.68 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 192.2, 152.8, 147.9, 129.4, 127.8, 126.5, 121.1, 110.7, 56.2, 38.7, 36.3, 31.6, 31.4, 21.9, 13.6; MS (ESI) calcd for $[C_{16}H_{23}S_2O_3]^-$ M: 327.1; found *m/z* 327.2.

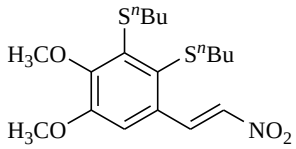
2,3-Bis(butylthio)-4,5-dimethoxybenzaldehyde (**4-3d**)



4-3d

Compound **4-3d**: an orange syrup; 71% yield; ^1H NMR (300 MHz, $CDCl_3$) δ = 0.86-0.88 (m, 6H, $2CH_3$), 1.39-1.52 (m, 8H, $2CH_2CH_2$), 2.82-2.99 (m, 4H, $2SCH_2$), 3.93 (s, 6H, $2OCH_3$), 7.43 (s, 1H, ArH), 10.74 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 192.9, 155.3, 153.5, 137.3, 135.5, 110.4, 60.4, 56.0, 38.3, 35.3, 31.9, 31.4, 22.0, 13.6; MS (ESI) calcd for $[C_{17}H_{27}S_2O_3]^+$ M: 343.1; found *m/z* 343.0.

2,3-Bis(butylthio)-4,5-dimethoxy-1-((*E*)-2-nitrovinyl)benzene (**4-3e**)

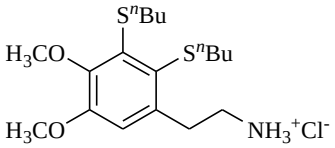


4-3e

A mixture of 2,3-bis(*n*-butylthio)-4,5-dimethoxybenzaldehyde **4-3d** (0.177 g, 0.5 mmol), nitromethane (5 mL), glacial acetic acid (0.074 mL, 1.25 mmol) and NH_4OAc (0.0794 g, 1.03 mmol) was heated and kept the temperature 60 ^oC for 8 hours followed by the addition of ice water (15 mL) to quench the reaction. After extraction of the aqueous mixture with diethyl ether, the combined organic phase was washed with water and brine and dried over Na_2SO_4. After removal of solvent under reduced pressure, the crude product was purified by flash chromatography on silica gel eluting with hexane:ethyl acetate (5:1, v/v) to afford **4-3e** (0.1657 g, 86% yield) as an yellow/brown oil.

Compound **4-3e**: an yellow oil; 86% yield; ^1H NMR (300 MHz, $CDCl_3$) δ = 0.86-0.88 (m, 6H, $2CH_3$), 1.39-1.49 (m, 8H, $2CH_2CH_2$), 2.78-3.00 (m, 4H, $2SCH_2$), 3.92 (s, 6H, $2OCH_3$), 6.99 (s, 1H, ArH), 7.45-7.49 (d, J = 13.8 Hz, 1H, CH), 8.89-8.93 (d, J = 13.7 Hz, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 153.4, 139.1, 137.6, 134.2, 131.2, 125.2, 114.5, 109.7, 60.4, 56.0, 37.8, 35.4, 31.9, 31.4, 22.0, 21.9, 13.7, 13.6.

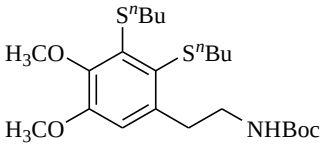
2-(2,3-Bis(butylthio)-4,5-dimethoxyphenyl)-*N*-chloroethanamine salt (**4-3f**)



4-3f

To a slurry of LAH (0.32 g, 8.43 mol) in distilled THF (10 mL) was added a solution of **4-3e** (0.9208 g, 2.39 mmol) in THF (10 mL). The mixture was heated at reflux for 1.5 h followed by cooling the reaction to 4 ^{0}C in an ice water bath. After the addition of 15% v/v aqueous HCl, the aqueous mixture was extracted with ethyl acetate. The combined organic extracts were washed with water and brine and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded the crude amine salt (0.8916 g) as a yellow syrup, which was used without further purification.

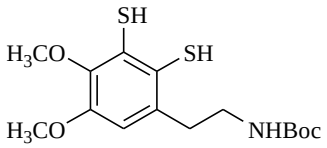
tert-Butyl 2,3-bis(butylthio)-4,5-dimethoxyphenethylcarbamate (**4-3g**)



4-3g

Compound **4-3g**: an orange syrup; 35% yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84-0.86 (m, 6H, $2CH_3$), 1.32-1.61 (m, 17H, $2CH_2CH_2$, $3CH_3$), 2.74-2.97 (m, 4H, $2SCH_2$), 2.98-3.32 (m, 4H, CH_2CH_2), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.72 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.8, 152.9, 148.9, 140.1, 136.5, 129.2, 112.8, 79.0, 59.9, 55.7, 41.7, 37.1, 35.3, 31.8, 31.4, 28.3, 22.1, 21.9, 13.7, 13.6.

tert-Butyl 2,3-dimercapto-4,5-dimethoxyphenethylcarbamate (**4-3h**):

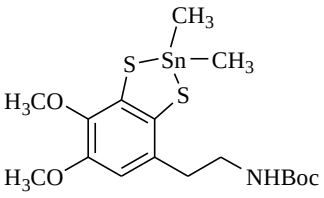


4-3h

Liquid ammonia was condensed by means of a dry-ice condenser into a 50 mL two-necked round bottom flask containing small pieces of sodium metal (about 0.8 g). A solution of **4-3g** (0.3805 g, 0.83 mmol) in distilled THF (10 mL) was injected in this round bottom flask at -78 ^{0}C to -60 ^{0}C when a deep blue solution persisted. After stirring for 1 h, the reaction was quenched by the addition of solid NH_4Cl. The excess ammonia was allowed to evaporate under a stream of N_2. The resultant white residue was dissolved in water and acidified with 15% (v/v) aqueous HCl. After extraction of the aqueous solution with DCM, the combined organic extracts were washed with water and brine and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded **4-3h** as an orange paste (0.2505 g crude product).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.41 (s, 9H, $3CH_3$), 2.94-3.34 (m, 4H, CH_2CH_2), 3.81-3.82 (s, 6H, $2OCH_3$), 6.56 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.8, 153.9, 151.6, 142.6, 142.5, 141.5, 137.0, 132.5, 117.1, 117.0, 110.7, 79.0, 67.9, 59.6, 55.8, 40.5, 35.9, 28.3, 25.5.

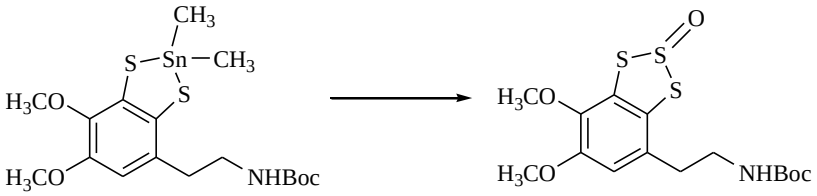
tert-Butyl2-(4,5-dimethoxy-2,2-dimethylbenzo[d][1,3,2]dithiastannol-7-yl) ethylcarbamate (**4-3i**)



4-3i

Compound **4-3i**: an white syrup; 86% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.80-0.96 (m, 6H, $2SnCH_3$), 1.42 (s, 9H, $3CH_3$), 2.96-3.41 (m, 4H, CH_2CH_2), 3.79-3.87 (m, 6H, $2OCH_3$), 6.50 (s, 1H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 155.9, 154.6, 149.8, 146.4, 130.1, 115.5, 111.3, 79.5, 56.2, 56.0, 39.9, 28.4, 28.0, 2.8.

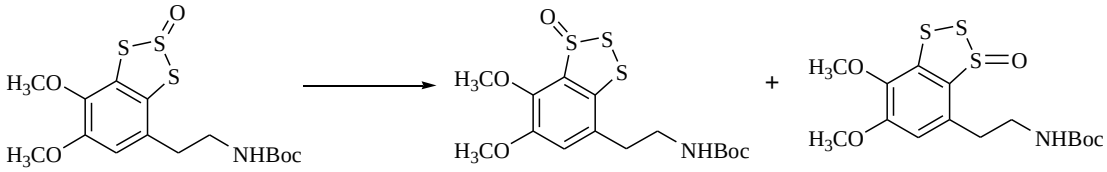
N-(Boc)-4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 2-oxide (**4-3j**)



5-9 **5-10a**

Compound **4-3j**: an yellow solid; 33% yield for two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.40 (s, 9H, $3CH_3$), 2.83-3.34 (m, 4H, CH_2CH_2), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.83 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.7, 152.2, 143.0, 131.7, 130.8, 127.2, 114.2, 79.3, 60.5, 56.5, 43.4, 28.5, 14.0.

N-(Boc)-4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 1-oxide (**4-3k**) and *N*-(Boc)-4-ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide (**4-3l**)



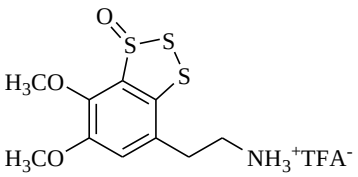
4-3j **4-3k** **4-3l**

A solution of **4-3j** (0.1133 g, 0.2893 mmol) in CH_3CN (5 mL) was irradiated with the wavelength of 300 nm for 4 hours. After removal of solvents by evaporation, the crude product was purified by flash chromatography on silica gel eluting initially with DCM:EA (50:1, v/v) and then with DCM:EA (10:1, v/v) to afford **4-3k** (0.0227 g, 20% yield) and **4-3l** (0.0272 g, 24% yield) as light yellow solid.

Compound **4-3k**: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.44 (s, 9H, $3CH_3$), 2.94-2.98 (m, 2H, CH_2), 3.44-3.45 (m, 2H, CH_2), 3.91 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.95 (s, 1H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 155.9, 154.7, 151.2, 143.2, 133.8, 125.0, 116.0, 112.9, 79.5, 60.7, 56.9, 43.4, 28.5; MS (ESI) calcd for $[C_{15}H_{20}S_3NO_5]^-$ M: 390.1; found *m/z* 390.1.

Compound **4-3l**: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.43 (s, 9H, $3CH_3$), 3.10-3.29 (m, 2H, CH_2), 3.46-3.48 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.78 (s, 1H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 154.7, 151.2, 142.4, 133.8, 125.0, 116.0, 113.0, 79.5, 60.5, 56.6, 41.4, 28.4; MS (ESI) calcd for $[C_{15}H_{21}S_3NO_5Na]^+$ M: 414.1; found *m/z* 413.9.

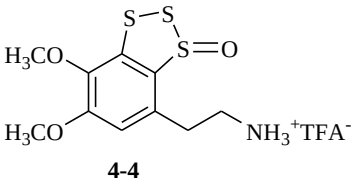
4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide trifluoroacetate salt (**4-3**)



4-3

Compound **4-3**: an yellow solid; 94% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.10-3.13 (m, 4H, CH_2CH_2), 3.93 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.23 (s, 1H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 153.1, 147.5, 137.3, 128.8, 119.9, 114.9, 62.8, 57.53, 40.0, 35.5; HRMS (ESI) calcd for $[C_{10}H_{14}S_3NO_3]^+$ M: 292.0130; found *m/z* 292.0134.

4-Ethylamino-6,7-dimethoxy-1,2,3-benzotrithiole 3-oxide trifluoroacetate salt (**4-4**)



4-4

Compound **4-4**: an yellow solid; 98% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.26-3.33 (m, 4H, CH_2CH_2), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.05 (s, 1H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 156.6, 144.3, 143.9, 142.4, 132.7, 114.9, 61.2, 57.4, 41.5, 34.2; MS (ESI) calcd for $[C_{10}H_{14}S_3NO_3]^+$ M: 292.0; found *m/z* 291.9; HRMS (ESI) calcd for $[C_{10}H_{14}S_3NO_3]^+$ M: 292.0130; found *m/z* 292.0

Chapter 5 DNA-cleavage of Certain Assemblies G-quadruplex Catalyzed by Natural Amino Acid

5.1 Introduction

In the previous chapter, two classes of sufur-containing cyclic organic compounds were reported to be synthesized and both of them have shown great bioactivities in the thiol-dependent DNA cleavage reactions. In those reactions, pBR 322 DNA were used. For the information of this DNA, it contains a double-stranded circle 4,361 base pairs in length with supercoiled topology. In fact, other oligonucleotides which are known as DNA fragments with genes information, can also show the cleavage activities when they are in certain topologies. In the following sections, new designed nucleotides which are stable as topology of G-quadruplex will be introduced to show DNA cleavage activities in the presence of natural amino acid and metal ions.

DNA (Deoxyribonucleic acid) is a nucleic acid which contains the genetic information used in the development and functioning of all known living organisms.[231] From chemistry perspective, it contains two long polymers which are called nucleotides, together with backbones made of sugars and phosphate groups joined by ester bonds. These two strands of nucleotides can twist and wrap together to form a ladder which is called double helix structure.

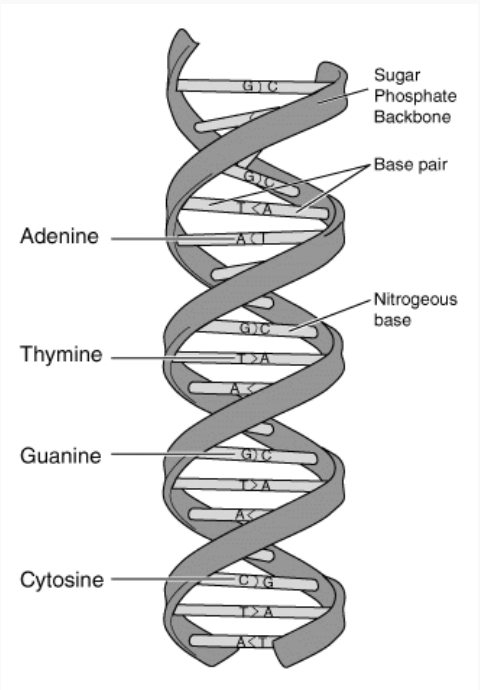


Figure 5-1 Double helix structure of DNA

There are four types of nucleobases in DNA, which are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). A GC base pair is stabilized with three hydrogen bonds while an AT base pair is stabilized with two hydrogen bonds. These different bases could fit perfectly like a lock and key, which is called "Watson-Crick base paring" named by discovers.

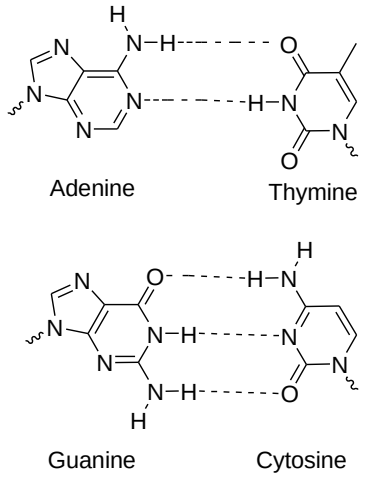


Figure 5-2 "Watson-Crick base paring"

Although DNA commonly exists in double helix structure by Watson-Crick base paring, certain guanine (G)-riched DNA four-stranded structures that are called G-quadruplexes.[232-235] The basic units of G-quadruplexes are guanine tetrads which are shown in the Figure 5-3. Guanine tetrad is a square co-planar array of four guanine bases, in which each base is both the donor and acceptor of two hydrogen bonds with its neighbors. Positively charged metal ions can be sandwiched between the tetrads to stabilize the structure.

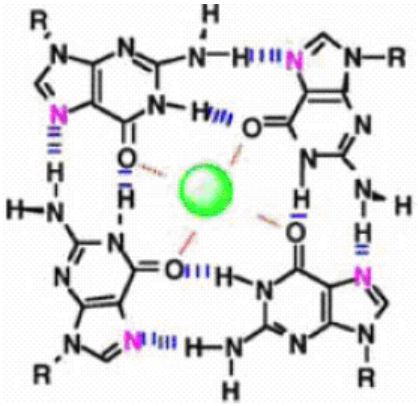


Figure 5-3 Structure of guanine tetrads

G-quadruplexes show unique dependence on the specific metal ions, usually K^+ and occasionally Na^+.[236] Their sizes are well suited to hold the guanine quadruplex structure because they can efficiently coordinate the two planes of quartets which are lined by eight carboxyl O_6 atoms from guanine. Other wide variety of cations are capable of occupying the central cavity of quadruplex structures,[236] including monovalent ions such as NH^{4+} and Tl^+ and divalent cations such as Sr^{2+}, Ba^{2+}, and Pb^{2+}.

The structure was known as early as 19th century that quanosine and its

derivatives could form viscous gels in water.[237] Until 1962, David R. Davis and co-workers [238] proposed the structure on the basis of X-ray diffraction data that four guanine bases form a planar structure through Hoogsteen hydrogen bonding interaction.[237]

Besides, these ions also have great effects on the topology of guanine quadruplex structures. The structure of the G-quadruplex can involve just one or two telomere strands looping back on themselves or four separate telomere strands. In each case, different ions can dominate the sequence to display as parallel or disparallel form, or they can assemble as unimolecular, biomolecular and tetramolecular forms.

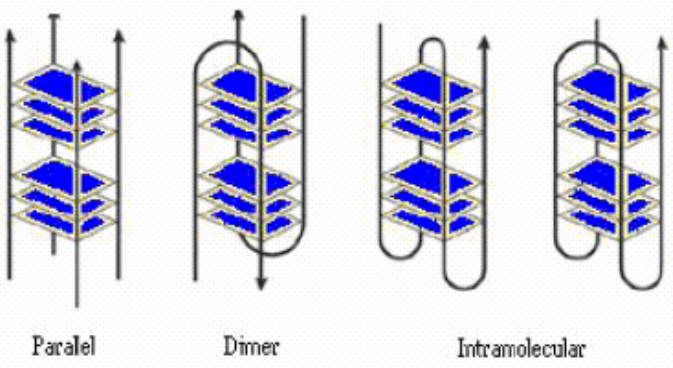


Figure 5-4 Topology of G-quadruplex structure

The possible biological roles for guanine quadruplex were paid little attention until it was elucidated in 1962 by David R. Davis. In the 1980s, the interest in guanine quadruplex structure was stimulated by several applications which were involved in the telomere protection and elongation also in the transcription regulation.[239-240]

In addition, self-assembly of guanine-rich oligonucleotides could form rod-shaped liquid crystals of cholesteric and act as the scaffold of artificial ion

channels and ion carriers.[241-243] Moreover, certain deoxyribozymes [244-254] and aptamers [255-258] are believed to rely on the formation of G-quadruplex for their biological actions.

Referring to the deoxyribozymes, which also known as catalytic DNA, are DNA molecules with catalytic reactivity. There are no DNA molecules in nature with catalytic activity involved, while RNA molecules with their catalytic reactivity do exist. This may be because that the double helical structure of DNA with genetic information inside is restricted to its catalytic potential. So far, all the deoxyribozymes (catalytic DNA) are single stranded like single stranded RNA. The single stranded form of DNA makes it potential to form more specific ordered structures with different topology.

Without a natural deoxyribozyme to study, much effort had been focused on exploring the manmade catalytic DNA, establishing its stability on some specific structure and discovering its catalytic reactivity like RNA or protein with enzyme behaviors. The first deoxyribozyme was discovered in 1994 by Ronald R. Breaker and Gerald Joyce at The Scripps Research Institute in La Jolla, CA.[246] In the presence of divalent ion lead, the short 38 nt DNA could form the specific structure which promoted the RNA cleavage reaction undertaken by the mechanisium of phosphoester transfer. (Figure 5-5) This catalytic DNA reveals that series of metal ions combined with amino acids as cofactors are essential for DNA functioned as a catalyst.[245] Catalytic amplification in this reaction is found to be 100-fold compared to the uncatalyzed reaction. Subsequently, some researches revealed that DNA could not

only act as the catalyst but also as the enzyme. It meant that deoxyribozyme could survive in the biological system which provided a promising environment using catalytic DNA as diagnostic and therapeutic tools.

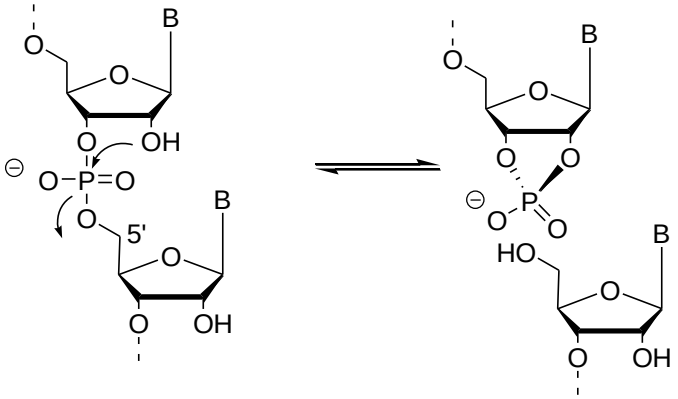


Figure 5-5 RNA cleavage reaction by phosphoester transfer

This important discovery in the field of deoxyribozyme points out a new direction by providing a new method to find the other deoxyribozymes. To broadening the reaction scope and finding more catalytic DNAs, PCR (polymerase chain reaction) technique was developed to create the catalytic DNA sequence by the process in vitro selection. This process is analogous to nature selection on an accelerated time scale. By processing in vitro selection, deoxyribozymes have been developed to catalyze many different types of reaction such as RNA cleavage, DNA cleavage, DNA modification and ligation, porphyrin rings metalation. Some of these deoxyribozyme can enhance the catalytic rate constant for their corresponding uncatalyzed reactions by 10 billion fold.

In the following paragraphs, DNA-cleaving deoxyribozyme will be mainly discussed based on the mechanism and its specific structure. DNA-cleaving deoxyribozyme is quite interesting because it involves some biological process such as RNA replication. Recently, two alternative mechanisms for DNA cleavage have been exploited by deoxyribozymes. One of the mechanisms is referred as the oxidation reaction.[259] The spontaneous oxidative cleavage of DNA by hydroxyl radicals can be induced using redox-active metals such as Cu^{2+} and reducing agents such as ascorbate.[259-260] Another mechanism of DNA cleavage is followed by a site-specific depurination process.[261] However by oxidative mechanism or depurination process, a nucleotide could be eliminated from the target DNA chain during the cleavage process, and in turn the elimination process will finally result in the loss of sequence-encoded information.

In order to overcome this shortcoming, hydrolysis of phosphate ester bond may be considered as an attractive method since in the DNA cleavage reaction, no genetic information is lost in this process. But this is very challenging because of the natural property of DNA. The absence of 2'-hydroxyl groups makes DNA approximately 105 times more resistant to hydrolysis compared to the rate constant of the related internal RNA transesterification reaction.[262] However, this functional limitation can be overcome by utilizing the metal ions combined natural amino acid, plus varying of DNA structures. The metal ions combined with natural amino acid could partially neutralize the negative charge of backbones of single stranded DNA and coordinate with water to carry out the acid/base catalysis for phosphodiester bond hydrolysis.

And the specific topology of single stranded DNA, especially G-quadruplex structures have been proved to be crucial in maintaining the reactivity of deoxyribozyme such as porphyrinmetalating deoxyribozyme, HD RNA-cleaving DNA enzyme and ATP-utilizing kinase deoxyribozyme.

So based on the previous cases reported and mechanisms involved inside, it becomes possible to explore of DNA cleavage reaction by direct hydrolysis of a phosphoester bond based on G-quadruplex structure catalyzed by natural amino acid combined with metal ions. This type of reaction has never been explored in previous work mainly because of the extraordinarily slow background rate of hydrolytic cleavage of DNA. Although the reaction is very challenging, if this reaction can be proved, this will be the first study to report this type of reaction. It may also provide the basis for further research in the vitro process to see whether this is suitable for using as an anticancer drug candidate.

The overall objective of this project is to search and confirm the deoxyribozymes based on the G-quadruplex catalyzed by natural amino acid combined with metal ions and the specific aims are:

(1) To develop the DNA sequences based on the different topology of G-quadruplex.

(2) To confirm the site-specific DNA cleavage by different methods such as PAGE, CD and Mass.

(3) To optimize the reaction conditions and consider the factors which will affect the reaction greatly. These factors could be: pH value, DNA

concentration, metal ions and etc.

So in the following sections, site specific cleavage reaction of a guanine-riched oligonucleotide is thoroughly studied during the investigation. Subsequent examinations on certain factors are described, such as variation of metal ions, pH values and concentration of DNA. In addition, kinetic analysis of self-cleavage of G-quadruplex is also carried out. Last methods and materials are also included.

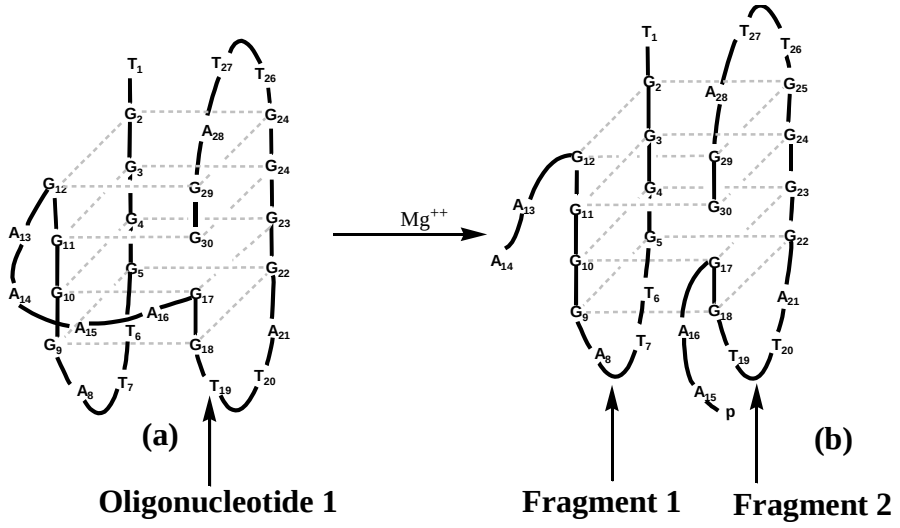
5.2 Results and Discussion

5.2.1 Emergence of Self-cleavage Activity

Besides the physical and chemical properties of G-quadruplex described previously, certain assemblies of G-quadruplex could also perform self-cleaving action in site specific fashions catalyzed by natural amino acid combined with metal ions.

A schematic diagram of a DNA self-cleavage processes uncovered during recent investigations is depicted in Figure 5-6. A guanine-rich 30-mer oligonucleotide (Oligonucleotide 1 in Figure 5-6) was designed previously in our laboratory with the expectation that this oligonucleotide would form an externally looped G-quadruplex assembly (a in Figure 5-6) under proper conditions. The initial intention in designing such a guanine-rich oligonucleotide was to examine whether a transesterification reaction could be feasible between the hydroxyl group at its 3' end and the phosphodiester bond between A_{16} and G_{17} since these functional groups are proximal to each other upon G-quadruplex formation. Instead of observing such a designed

transesterification reaction, a selfcleavage reaction of Oligonucleotide 1 at one of the two phosphodiester bonds between A_{14} and A_{15} was observed (Figure 5-6).



Oligonucleotide 1: 5' $T_1G_2GGGTTAGGGGAA_{14}A_{15}AGGTTAGGGGTTAG_{29}G_{30}$ 3' (30-mer)
Fragment 1: 5' $T_1G_2GGGTTAGGGGA_{13}A_{14}$ 3' (14-mer)
Fragment 2: 5' p-$A_{15}A_{16}GGTTAGGGGTTAG_{29}G_{30}$ 3' (16-mer)
p: phosphate group

Figure 5-6 Schematic representation of a self-cleavage process of Oligonucleotide 1 uncovered in these studies

Oligonucleotide 1 was accordingly phosphorylated at its 5' end with [γ-^{32}P] ATP in the presence of T_4 polynucleotide kinase and further purified by polyarylamide gel electrophoresis and gel filtration chromatography in the studies. In order to allow the formation of proper G-quadruplex assemblies, this guanine-rich oligonucleotide was next incubated at 20 ^{0}C in the presence of 5 mM NaCl for 12 hours followed by addition of KCl (final concentration 5 mM), which was then kept at the same temperature for additional 12 hours. The self-cleavage reactions of Oligonucleotide 1 were initiated next by adding a premixed solution of $MgCl_2$ and histidine to the

mixture, which was further kept at 34 ^{0}C for a different period of time. As shown in Figure 5-7, a new fast moving band was observed when such a reaction was allowed to proceed for 2 hours (Band 1 in Lane 3). The mobility shift of this new band is close to that of a molecular weight marker of 14-mer (5' *p-TGGGGTTAGGGGAA 3', Lane 5), which implied that a cleavage reaction took place between A_{14} and A_{15} of this guanine-rich sequence.

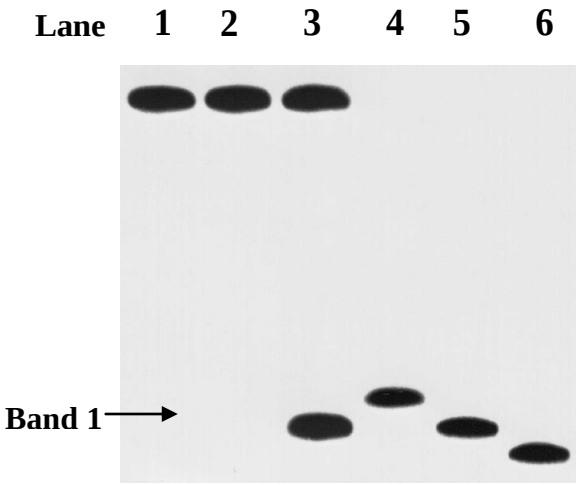


Figure 5-7 Polyacrylamide gel electrophoretic analysis of self-cleavage of DNA visualized by autoradiography. Lane 1: Oligonucleotide 1 alone; Lanes 2 to 3: self-cleavage reactions lasting for 0 and 2 h respectively; Lane 4: a 15-mer Oligonucleotide (*p-TGGGGTTAGGGGAAA) alone; Lane 5: a 14-mer (*p-TGGGGTTAGGGGAA) alone; Lane 6: a 13-mer (*p-TGGGGTTAGGGGA) alone.

5.2.2 Confirmation of Self-Cleaving Action of a Particular G-quadruplex

If a DNA cleavage reaction indeed occurred in the middle of the sequence of Oligonucleotide 1, a second fragment of 16-mer should in theory be generated at the same time. In order to visualize the two fragments of 14-mer and 16-mer (Fragment 1 and Fragment 2 shown in Figure 5-6) simultaneously, the experiment of methylene

blue staining was conducted next.

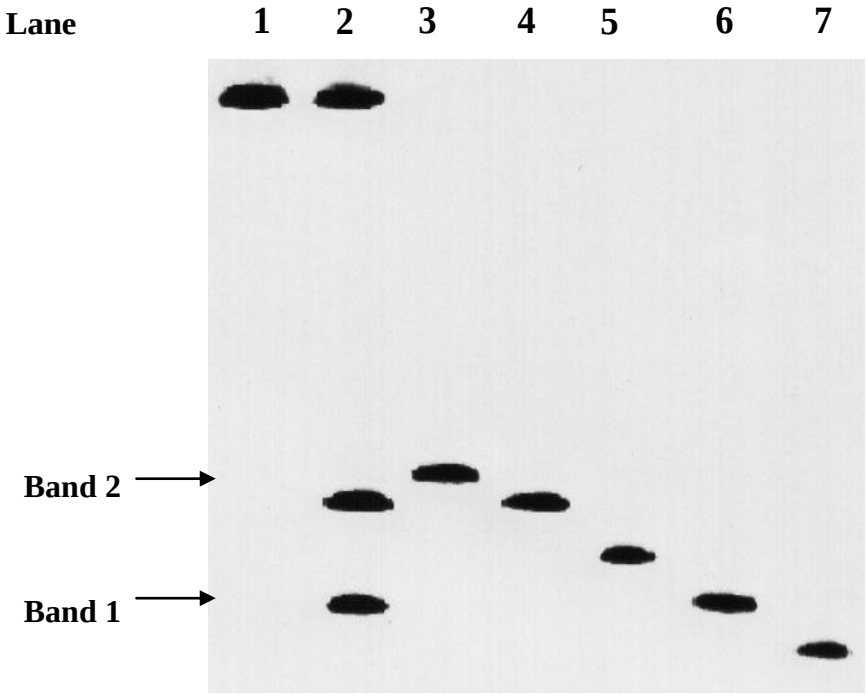


Figure 5-8 Polyacrylamide gel electrophoretic analysis of self-cleavage of DNA visualized by methylene blue staining. The same procedures as those for preparing samples loaded in Lane 3 was used except that 5' 32P-labeled Oligonucleotide 1 was replaced with 5' hydroxyl Oligonucleotide 1 and methylene blue staining protocol was adopted for visualizing the DNA bands. Lane 1: Oligonucleotide 1 alone; Lane 2: self-cleavage reaction lasting for 2 hr. Lane 3: a 17-mer (5' AAAGGTTAGGGGTTAGG 3') alone; Lane 4: a 16-mer (5' AAGGTTAGGGGTTAGG 3') alone; Lane 5: a 15-mer (5' TGGGGTTAGGGGAAA 3') alone; Lane 6: a 14-mer (5' TGGGGTTAGGGGAA 3') alone; Lane 7: a 13-mer (5' TGGGGTTAGGGGA 3') alone.

As shown in Figure 5-8, two fast moving bands (Band 1 and Band 2 in Lane 2) were visible from the stained polyacrylamide gel, which displayed the same mobility shifts as those of a 14-mer marker (Lane 6) and a 16-mer marker (Lane 4) respectively. These electrophoretic analysis data are indications that a cleavage reaction indeed took place between A_{14} and A_{15} in the middle of the sequence of Oligonucleotide 1 as shown in Figure 5-6.

5.2.3 Confirmation of Self-Cleaving Action at specific site

Oligonucleotide 1 that contains radiolabeled phosphorus (^{32}P) between A_{14} and A_{15} (5’ TGGGGTTAGGGGAA-^{32}p-AAGGTTAGGGGTTAGG 3’, internally ^{32}P-labeled Oligonucleotide 1) was next synthesized and examined during the investigations in order to determine which of the two fragments possesses the phosphate group.

As shown in Figure 5-9, the only observable self-cleavage product from the internally ^{32}P-labeled Oligonucleotide 1 is a 16-mer fragment (5’ *p-A_{15}AGGTTAGGGGTTAGG_{30} 3’) while not even a trace amount of 14-mer (5’ T_1GGGGTTAGGGGAA_{14}-*p 3’) is detectable, which is the sign that the phosphate group goes exclusively along with the 16-mer fragment rather than with the 14-mer as illustrated in Figure 5-6.

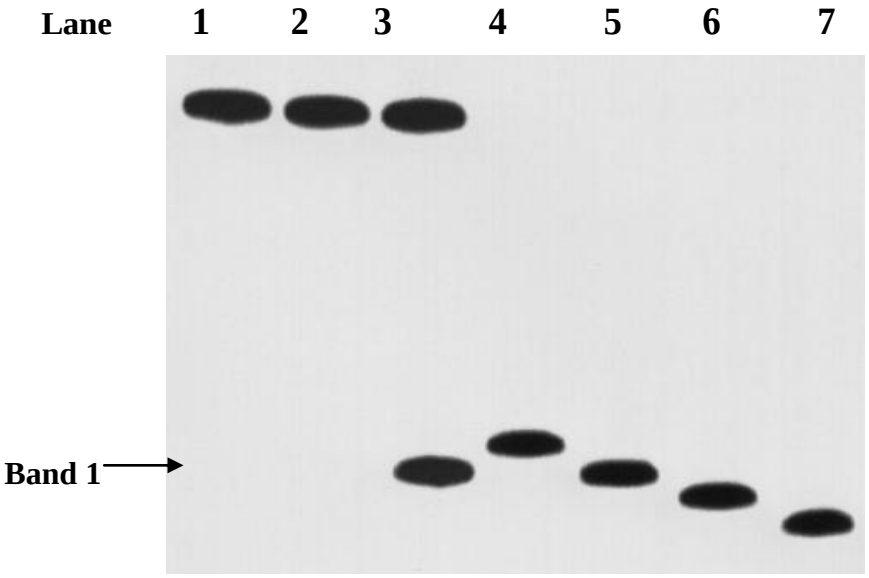


Figure 5-9 Polyacrylamide gel electrophoretic analysis of internally ^{32}P-labeled Oligonucleotide 1 (5' TGGGGTTAGGGGAA-32p-AAGGTTAGGGGTTAGG 3') in its self-cleavage reactions. Lane 1: internally 32P–labeled Oligonucleotide 1 alone; Lane 2 to 3: self-cleavage reactions lasting for 0 and 2 hr; Lane 4: a 17-mer (5' *p-AAAGGTTAGGGGTTAGG 3') alone; Lane 5: a 16-mer (5' *p-AAGGTTAGGGGTTAGG 3') alone; Lane 6: a 15-mer (5' *p-AAGGTTAGGGGTTAGG 3') alone; Lane 7: a 14-mer (5' *p-TGGGGTTAGGGGTT 3').

In addition, the oligonucleotide fragment in Band 1 in Lane 3 in Figure 5-9 was purified and further analyzed through hydrolysis by exonuclease I, an enzyme that digests single-stranded DNA in a 3' to 5' direction in a stepwise fashion. As shown in Figure 5-10, the purified ^{32}P-containing oligonucleotide fragment was completely degraded in the presence of the single strand-specific nuclease (Lane 3), which could be taken as an indication that this oligonucleotide fragment (Fragment 2 in Figure 5-6) holds a linear structure in its backbone.

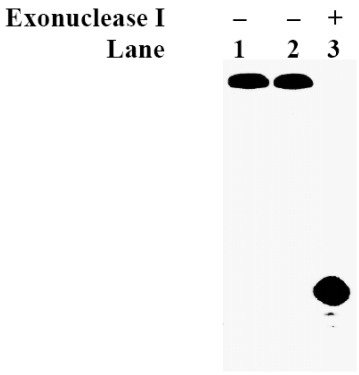


Figure 5-10 Hydrolysis of Fragment 2 (see **Figure 5-6** for its sequence information) generated in theself-cleavage reaction of Oligonucleotide 1 by exonuclease I. The oligonucleotide fragment in Band 1 in Lane 3 in Fig. 5 was cut out, eluted and further purified using gel filtration chromatography (NAP-25, GE Healthcare). A mixture (40 μL) containing 1 x exonuclease I buffer, the purified 32P-containing oligonucleotide fragment (Fragment 2) and 5 units of exonuclease I was incubated next at 37 ^{o}C for 30 min. Lane 1: Fragment 2 alone; Lane 2: a reaction mixture containing no exonuclease I and Lane 3: a reaction mixture containing 5 units of exonuclease I.

Based on the above observations, it can be suggested that the self-cleaving reaction of Oligonucleotide 1 took place at one of the phosphodiester bonds near the 3' end of A_{14} in the middle of its sequence as illustrated in Figure 5-11. Nevertheless, more direct evidence is evidently needed to further verify this suggested mechanism in the future.

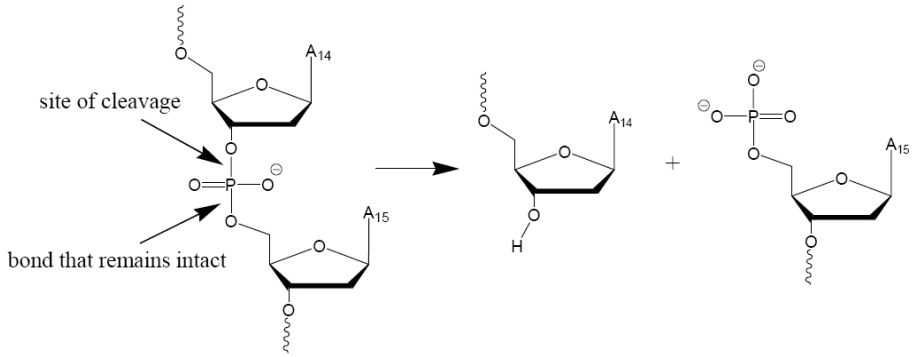


Figure 5-11 Diagrammatic illustration of a possible self-cleaving reaction at one of the two phosphodiester bonds between AB_{14B} and $AB_{15}B$ of Oligonucleotide 1

5.2.4 Reaction Parameters of the DNA Cleavage Reaction

5.2.4.1 Time Dependence

In addition, the time dependent self-cleavage reactions were examined in the studies. As shown in Figure 5-12, the yield of the self-cleavage reactions increased with the increasing reaction time and ~50% cleavage of Oligonucleotide 1 could be achieved within ~2 hours.

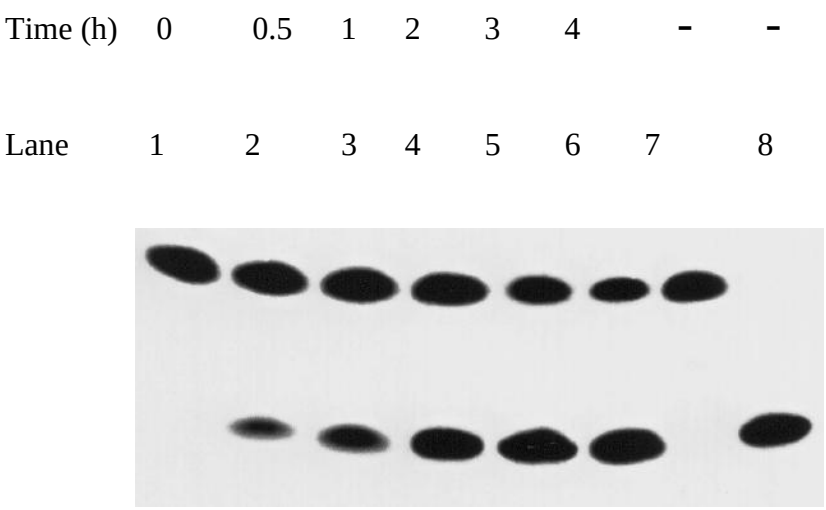


Figure 5-12 Time dependence of self-cleavage reaction of Oligonucleotide 1. The same procedures as those for preparing samples loaded in Lane 3 in Figure 2 were used except that the reactions were stopped at different time intervals. Lane 7: Oligonucleotide 1 alone; Lane 8: a 14-mer (*p-TGGGGTTAGGGGAA) alone. The time of reaction in Lanes 1, 2, 3, 4, 5 and 6 were 0, 0.5, 1, 2, 3 and 4 hours respectively.

5.2.4.2 Ion Dependence

With the aim of verifying that G-quadruplex structure could be formed by Oligonucleotide 1 as anticipated, CD spectroscopic analysis on a solution containing this guanine-rich sequence was also carried out. As shown in Figure 5-13, this Oligonucleotide 1-containing solution displayed a maximum absorption at 295 nm, which is a characteristic sign of the presence of anti-paralleled G-quadruplex structures in the mixture. [263-264] Potassium ion is, on the other hand, known to be one of the preferable monovalent cations for stabilizing G-quadruplex structures of DNA.

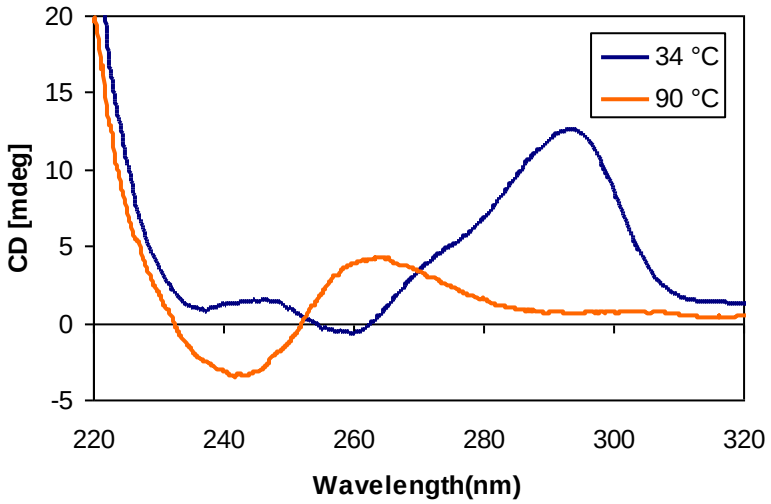


Figure 5-13 CD spectroscopic analysis of Oligonucleotide 1. A mixture (pH 7.0) containing 5 mM HEPS, 5 mM NaCl, 5 mM KCl and 10 μM Oligonucleotide 1 was examined with a CD Spectropolarimeter at 34 oC (black) and 90 oC (red) respectively over an range of wavelengths from 220 nm to 330 nm.

5.2.4.3 Potassium Ion Concentration Dependence

As a comparison, additional self-cleavage reactions of Oligonucleotide 1 were carried out in our studies in which the concentration of potassium ion varied. As shown in Fig. 5-14, there was no DNA cleavage detectable when potassium ion was

absent from the corresponding reaction mixture (Lane 2). This observation is consistent with the suggestion that formation of stable G-quadruplex is a prerequisite for the self-cleavage reaction of Oligonucleotide 1.

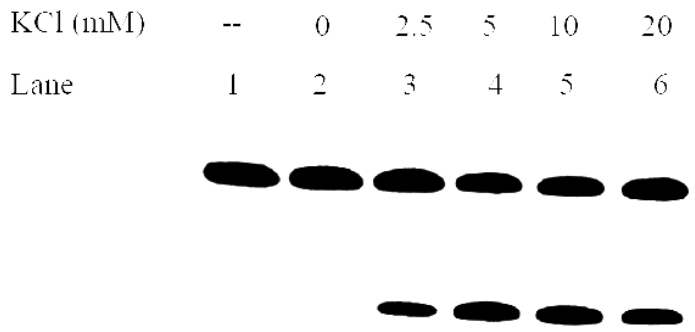


Figure 5-14 Effect of potassium ion concentration on the self-cleavage reaction of Oligonucleotide 1. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that concentration of potassium chloride in the new experiments varied. Lane 1: Oligonucleotide 1 alone; Lane 2: 0 mM KCl; Lane 3: 2.5 mM KCl; Lane 4: 5 mM KCl; Lane 5: 10 mM KCl and Lane 6: 20 mM KCl.

5.2.4.4 Temperature Dependence

Furthermore, the temperature dependence of the self-cleavage reactions of Oligonucleotide 1 was examined during our investigations. It appeared that the self-cleaving reactivity of this oligonucleotide was lost completely when the temperature of the corresponding reaction increased to 45 ^{o}C (Lane 7 in Figure 5-15), which could result from the dissociation of a G-quadruplex structure at relatively high temperatures.

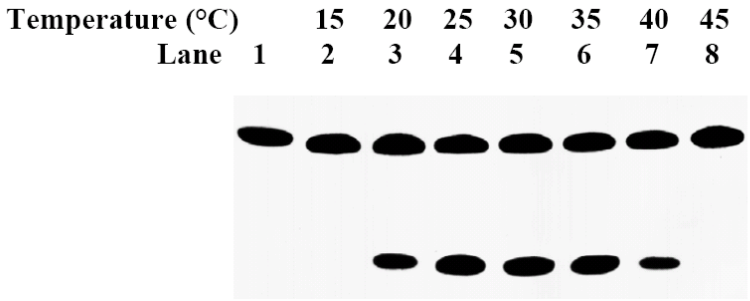


Figure 5-15 Temperature dependence of self-cleavage reactions of Oligonucleotide 1.The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that the new reaction mixtures were incubated at different temperatures. Lane 1: Oligonucleotide 1 alone. Reaction temperatures of the samples loaded in Lanes 2 to 8 were set at 15 ^{o}C, 20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C and 45 C respectively.

5.2.4.5 Sequence Dependence

Two new guanine-rich oligonucleotides were further designed during our investigations that contain the same sequences as Oligonucleotide 1 except that one or two guanines are replaced with non-guanine nucleotides (Oligonucleotide 2 and Oligonucleotide 3 in Table 5-1).

Table 5-1 Guanine-rich oligonuelotides that were examined I our studies

(a) Oligonucleotide 1:

5’ TGGGGTTAGGGGAAAAGGTTAGGGGTTAGG 3’

(b) Oligonuelotides that contain one and two “mismatched” guanines.

Oligonucleotide 2:

5’ TGGCGTTAGGGGAAAAGGTTAGGGGTTAGG 3’

Oligonucleotide 3:

5’ TGGCGTTAGAGGAAAAGGTTAGGGGTTAGG 3’

(c) Oligonuleotides that contain alternative nucleotides in the loops appearing at the ends of their columnar structures.

Oligonucleotide 4:

5’ TGGGGTTAGGGGAAAAGGTTTGGGGTTAGG 3’

Oligonucleotide 5:

5’ TGGGGTTAGGGGAAAAGGTTTTGGGGTTAGG 3’

(d) Oligonucleotides that contain five and three consecutive adenosines in their side loops.

Oligonucleotide 6:

5’ TGGGGTTAGGGGAAAAAGGTTAGGGGTTAGG 3’

Oligonucleotide 7:

5’ TGGGGTTAGGGGAAAGGTTAGGGGTTAGG 3'

These two new oligonucleotides are in theory unable to form ordinary G-quadruplex structures due to the presence of ‘mismatched’ guanine bases. It turned out that neither of these two mismatched sequences displayed a detectable self-cleaving activity under our standard reaction conditions (Lane 4 and Lane 6 in Figure 5-16).

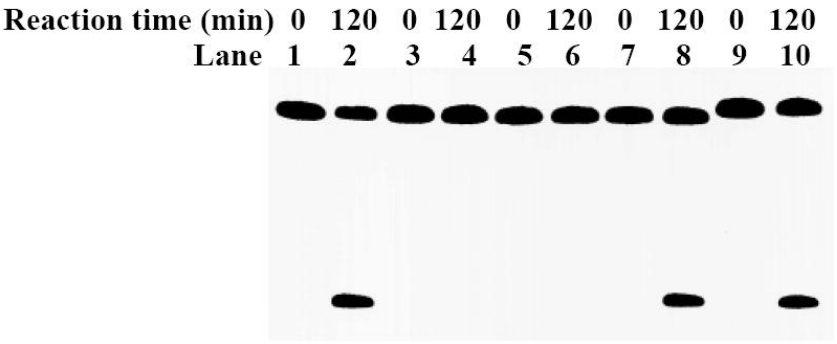


Figure 5-16 Sequence dependence of self-cleavage reaction of G-quadruplexes. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 2 were used except that Oligonucleotide 1 was replaced with Oligonucleotide 2, Oligonucleotide 3, Oligonucleotide 4, Oligonucleotide 5, Oligonucleotide 6 and Oligonucleotide 7 (see Table S1) respectively. Lane 1 and Lane 2: reactions of Oligonucleotide 1 lasting for 0 and 120 min respectively; Lane 3 and Lane 4: reactions of Oligonucleotide 2 (5' TGGCGTTAGGGGAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 5 and Lane 6: reactions of Oligonucleotide 3 (5' TGGCGTTAGAGGAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min, respectively; Lane 7 and Lane 8: reactions of Oligonucleotide 4 (5' TGGGGTTAGGGGAAAAGGTTTGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 9 and Lane 10: reactions of Oligonucleotide 5 (5'TGGGGTTAGGGGAAAAGGTTTTGGGGTTAGG 3') lasting for 0 and 120 min respectively.

However, when alterations of nucleotides in some loops located at the ends of the columnar structure of Oligonucleotide 1 were made, the resulting oligonucleotides (Oligonucleotide 4 and Oligonucleotide 5 in Table 5-1) still exhibited self-cleaving activity (Lanes 8 and 10 in Figure 5-17). These observations could be indications that Oligonucleotide 1 relies on the formation of a G-quadruplex structure for its self-cleaving activity.

As a comparison with Oligonucleotide 1 which possesses four consecutive adenosines in the middle of its sequence, new G-quadruplex structures containing five and three adenosines in a row (Oligonucleotides 6 and 7 in Table 5-1) in the loop located at the sides of their columnar structures were also designed and examined.

As shown in Figure 5-17, the self-cleavage rate of Oligonucleotide 6 (a five adenosine containing structure) was close to that of Oligonucleotide 1 (Lane 6) while there was no self cleavage product observable (Lane 2) when the size of its side loop was reduced to three consecutive adenosines (Oligonucleotide 7). These results suggest that besides the formation of a G-quadruplex assembly, the geometry of the side loop within the tetraplex columnar structure plays certain crucial roles in the site specific self DNA cleaving processes.

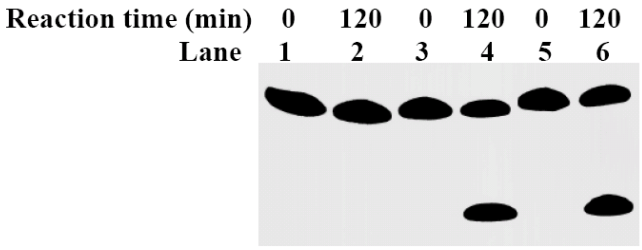


Figure 5-17 Effect of side loop variation on the self-cleaving reactivity of G-quaduplex structures. The same procedures as those for preparing samples loaded in Lane 3 in Fig. 5.2.1.2 were used except that Oligonucleotide 1 was replaced by Oligonucleotide 6 and Oligonucleotide 7 respectively. Lane 1 and Lane 2: reactions of Oligonucleotide 7 (5' TGGGGTTAGGGGAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively; Lane 3 and Lane 4: reactions of Oligonucleotide 1 lasting for 0 and 120 min respectively; Lane 5 and Lane 6: reactions of Oligonucleotide 6 (5' TGGGGTTAGGGGAAAAAGGTTAGGGGTTAGG 3') lasting for 0 and 120 min respectively.

5.3 Conclusion

This chapter described a new deoxyribozyme which could undergo the DNA self-cleavage reaction based on guanine quadruplex structure catalyzed by the combination of Mg^{2+} and histidine. It was proved that a new DNA sequence was found based on the topology of G-quadruplex. The CD spectrum shows that the G-quadruplex structure is definitely formed in the presence of the K^{+} ions. The curve indicates that this structure is most likely formed as a parallel topology because it has

a characterized peak at 298 nm which is an indication of parallel structure. Although such a guanine-rich oligonucleotide (Oligonucleotide 1) is designed for the transesterification reaction initially, it was unexpected to discover this self-cleavage reaction between the loop of the hydroxyl group at its 3' end and the phosphordiester bond between A16 and G17 in this sequence of Oligonucleotide 1.

In addition, other oligonucleotides analogues with various lengths of loops based on the G-quadruplex could also undergo the self-cleavage reactions. This fact suggests that as long as the G-quadruplex structures form at the specific topology, the reaction can occur at the proper condition. This finding has provided valuable insight that G-quadruplex is a crucial structure for some deoxyribozymes as a catalyst and this tetraplex assembly has its unique spatial arrangement as well as its functionality in the biological process.

This chapter also examined the self-cleavage reaction of DNA based on G-quadruplex by the process of hydrolysis of phosphordiester bond. Many different methods were developed to confirm the site-specific DNA cleavage by different methods such as PAGE, CD and Mass spectrum. Phosphorus (^{32}P) is radiolabeled in the Oligonucleotide 1 between A14 and A15 (5' TGGGGTTAGGGGAA-^{32}P-AAGGTTAGGGGTTAGG 3') and it was proved by comparing the marker that the phosphate group went exclusively along with the 16-mer fragment. This was also proved by the methylene blue staining which showed both 16-mer and 14-mer fragment in the gel. In addition, the hydrolysis of the process was suggested by the TOF- Mass spectrum. The spectrum showed the exact molecular

weight of the product after the self-cleavage reaction. It reveals that the product contains the phosphor group which indicates that the reaction can only occur in the way of hydrolysis. Compared to other mechanisms such as oxidative mechanism or depurination process, hydrolysis has obvious advantages over other mechanisms. The nucleotide is not eliminated from the target DNA chain during the cleavage process, and in turn the elimination process does not loss the sequence-encoded information which is very important in the biological process. Although DNA hydrolysis is much difficult because of the absence of 2'-hydroxyl groups in the DNA, this is the first study to report that the self-cleavage reaction can be carried out by the hydrolysis process. It has provided a new perspective that it is possible to have reaction occure in a hydrolysis way only if the G-quadruplex structure is formed with specific topology. It also shed light on searching of other new types of deoxyribozymes which could perform the cleavage reaction.

Thirdly, to optimize the reaction conditions, many reaction factors were also investigated one by one. These factors were: metal ions, DNA concentration and pH value. For the Metal ion dependence of the reaction, the result shows that potassium ion is required in this DNA-cleaving reaction. This has been well established by the references compared to the preference of monovalent ions by G-quadruplex. The following order is: $K^+ >> Na^+ > Rb^+ > Cs^+ > Li^+$. For the DNA concentration dependance of the reaction, the result indicates that it is necessary that the concentration of Oligonucleotide 1 is higher than 100 nM. This is most likely due to a different template of G-quadruplex generated before the cleavage course under high

concentration of Oligonucleotide 1. Also for the pH dependence of the reaction, the variation of pH values has little effect on the stability of G-quadruplex, the high yield product of the self-cleavage reaction of Oligonucleotide 1 could be obtained at pH range of 7.5 to 6.5 which is more favorable to the catalytic process. All the factors which are fully investigated help us know more about the reaction condition of the reaction. It provides much efficient data and these data could be very useful for other researchers to explore this type of reaction further.

In summary, Self-cleavage reaction of a guanine-riched oligonucleotide (Oligonucleotide 1) was thoroughly studied during the investigation. It appeared that the presence of potassium ions is a prerequisite for the self-cleavage reaction. The results of the studies are consistent with the suggestion that G-quadruplex is the core structure needed for the self-cleavage process by hydrolysis.

However, further applications of this reaction are discussed in less detail in this thesis because this is only the first example in self-cleavage reaction based on the G-quadruplex structure by hydrolysis mechanism. Although many researches have shown the importance of G-qudruplex in the biological process, there is still not enough knowledge about the role of the DNA self-cleavage reaction in biological system. So there is still long way to apply the reaction to the real biological system

To further extend the research, the terometic repeated G-quadruplex could be used to test the self-cleavage reaction because the structure is similar to the Oligonucleotide 1 and it is more close to the real sequence of DNA in the cell.

5.4 Materials and Methods

5.4.1 Oligonucleotides

All oligonucleotides used in our studies are purchased from Sigma-Proligo (Singapore) which provided custom oligonucleotides synthesis. Oligonucleotides are usually supplied as a lyophilized power that could be disolved in water and diluted to suitable concentration for reactions. All oligonucleotides samples should be stored at – 20 °C. Common synthetic oligonucleotides are provided with 5’ end hydroxyl group which is suitable for 5’ end labeling reactions.

5.4.2 Kinase

T_4 DNA ligase used in our studies is purchased from New England Biolabs (Cat. Number: M0202L, 100,000 units per package, 400 units/μL in 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% Glycerol, pH 7.4 @ 25 °C). The enzyme should be stored at -20 °C.

T_4 DNA ligases catalyze the formation of a phosphodiester bond between 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

One unit of T4 DNA ligase gives 50% ligation of HindIII fragments of λ DNA (5′ DNA termini concentration of 0.12 μM, 300 μg/ml) in a total reaction volume of 20 μl in 30 minutes at 16 °C in 1x T_4 DNA Ligase Reaction Buffer. The enzyme can also catalyze the ligation at room temperature. For cohesive ends, use 1 μl of T_4 DNA

Ligase in a 20 µl reaction for 10 minutes. For blunt ends, use 1 µl of T_4 DNA Ligase in a 20 µl reaction for 2 hours or 1 µl high concentration T_4 DNA Ligase for 10 minutes. The enzyme was supplied with 10 x reaction buffer (0.5 M Tris-HCl, 100 mM MgCl2, 10 mM ATP, 100 mM Dithiothreitol, pH 7.5 @ 25°C).

5.4.3 Buffer and Solution

L-Histidine, sodium chloride solution, potassium chloride solution, magnesium chloride solution, HEPES and HEPES buffer were purchased from Sigma while all oligonucleotides used in these studies were purchased from Sigma-Proligo.

a) TBE buffer

TBE buffer used in our studies are purchased from 1st Base Pte Ltd. (Cat.Number: 3010-10 x 1L, 1L per package). It is supplied in 10 times concentration solution which contains 1M Tris, Borate Acid and EDTA (pH 8.3).

b) TAE buffer

TAE buffer used in our studies are purchased from 1st Base Pte Ltd. (Cat.Number: 3000-10 x 1L, 1L per package). It is supplied in 10 times concentration solution which contains 1M Tris, Acetate Acid and EDTA (pH 8.0).

c) Tris-HCl

Tris-HCl buffer used in our studies are purchased from 1st Base Pte Ltd. (Cat.Number: 1415 -1L, 1L per package, contains 1M Tris (pH 7.0).

d) MES

MES used in our studies are purchased from Sigma-Aldrich (Cat. Number:

M3671-50G) in solid state. It was dissolved in deionized water at desired concentration and adjusted to appropriate pH value by using 1M NaOH or 1M HCl.

e) HEPES

HEPES used in our studies are purchased from Sigma-Aldrich (Cat. Number: H3375-25G) in solid state. It was dissolved in deionized water at desired concentration and adjusted to appropriate pH value by using 1M NaOH or 1M HCl.

5.5 Methodology

5.5.1 5' End Labeling of DNA

End labeling is a rapid and sensitive method in visualizing small amount of DNA. It provides 3' End labeling and 5' end labeling onto 3' or 5' end of target sequences via enzyme catalytic reaction respectively.

Compare to the rapidly developed nonradioactive method, radioactive labeling is still our first choice because 1) High sensitivity, only trace amount of molecules need to be detected; 2) Same chemical and physical properties were provided in end labeling. The material for the labeling reaction was conducted as following described:

5' hycroxyl oligonucleotide (100 μM) 2 μL

10 x reaction buffer 2 μL

T_4 PNK (10 units/ μL) 1 μL

[γ-^{32}P] ddATP (10 μCi/ μL) 2 μL

H_2O 2 μL

10 x reaction buffer including: 0.7 M Tris-HCl, pH 7.6, 100 mM MgCl, 50 mM

Dithiothreitol.

The reaction mixture was incubated at 37 °C for 1 hour and 10 μL denaturing loading buffer was added before loading on a 20% (19:1) denature PAGE for further purification.

5.5.2 Polyacrylamide Gel Electrophoresis (PAGE)

Two types of polyacrylamide gels are in common use: One is non-denaturing polyacrylamide gel for the separation and purification of oligonuleotides without destroying their tertiary structures while denaturing polyacrylamide gel does in the other way. Preparations of denaturing and non-denaturing gels are similar and the only difference is that denaturing gels needs urea while non-denaturing gels don not. The materials for preparing these gels are as following:

Acrylamide 190 g

N,N'-methylenebisacrylamide 10 g

10 x TBE buffer 100 mL

Urea 420 g

H_2O

To 1 L 20% acrylamide solution was prepared via dissolving these materials described in H_2O. The solution may be stored at 4 °C for several weeks. Place the required quantity of acrylamide solution in a clean beaker. Add 30 μL of TEMED (N,N,N',N'-tetramethylethylenediamine) to acrylamide solution, then 500 μL 10% (w/v) ammonium persulfate water solution. Mix the solution by stirring. Pour into the

gel plate. The gel will be solidified in 15 minnutes.

5.5.3 DNA Purification

Purifications of oligonucleotides are needed in all reactions. They can be eluted from gel and dissolve in buffer solutions. Various of methods have been developed for the purification of DNA, such as dialysis, ion-exchange and solid phase extraction.

a) Solid phase extraction

C_{18} cartridge (WAT023590) can be used in the solid phase extraction with all kinds of solvents. It was activated by washing 500 μL CH_3CN for two times, followed by 500 μL H_2O and lastly loaded with the desired buffer which was 500 μL 1M Tris-HCl pH 7.0 buffer in the reaction. The oligonucleotides solution was subsequently loaded to the cartridge. Then it was washed with 500 μL water for 3-5 times and eluted from column by 500 μL CH_3CN/H_2O (1:1 v/v). The purified oligonucleotides were collected drop by drop and dried in the sample vials.

b) Ion-exchange purification

To purify the radio-labeled oligonucleotides, NAP-25 columns from GE Healthcare can be used. The columns are pre-packed with Sephadex G-25 DNA Grade in distilled water (which contains 0.15% Kathon CG/ICP Biocide, used as a preservative).

They are designed for the rapid and efficient desalting, buffer exchange and purification of DNA and oligonucleotides (equal or greater than 10 mers) utilizing

gravity flow. The maximum sample volume for purification is 2.5 ml, and the volume of eluted sample is 3.5 ml. The columns should be equilibrated with 25 ml 10 mM sodium phosphate buffer (pH 6.8) first. After equilibrated, 2.5 ml and additional 3.5 ml of equilibration buffer is then added in twice to elute the sample. 1.5 ml of solution containing the oligonucleotides can be collected then and dried in the sample vials.

5.6 Presentative Procedures for DNA Cleavage Reaction

5.6.1 Detailed Procedures for Polyacrylamide Gel Electrophoretic Analysis of Self-cleavage of DNA Visualized by Autoradiography

Oligonucleotide 1 was labeled with [γ-^{32}P] ATP at its 5' end in the presence of T4 polynucleotide kinase followed by purification via polyacrylamide gel electrophoresis (20 %). The slice of band containing ^{32}P-labled Oligonucleotide 1 was cut out from the polyacrylamide gel and kept at an elution buffer (5 mM HEPES, pH 7.0, 5 mM NaCl and 5 mM histidine) for 3 hour followed by purification with gel filtration chromatography (NAP-25, GE Healthcare) eluted with the same elution buffer. The obtained Oligonucleotide 1 (~20 nM) in 5 mM HEPES (pH 7.0), 5 mM NaCl and 5 mM histidine was then kept at 20 ^{o}C for 12 hr. KCl was then added and the resultant solution was further adjusted to contain in 5 mM HEPES (pH 7.0), 5 mM NaCl, 5 mM KCl, 5 mM histidine and ~10 nM Oligonucleotide 1, which was further maintained at 20 ^{o}C for additional 12 hr. Self-cleavage reactions of Oligonucleotide 1 was initiated next by mixing a premixed solution of $MgCl_2$ and L-histidine with other reaction components and the resultant mixture [5 mM HEPES (pH 7.0), 5 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, 5 mM L-histidine and ~5 nM Oligonucleotide 1] was

further kept at 34 ^{0}C for different time periods. The self-cleavage reaction products were analyzed via 20% polyacrylamide gel electrophoresis after the reactions were stopped by addition of loading buffers followed by placing the reaction mixtures on ice.

5.6.2 Detailed Procedures for Polyacrylamide Gel Electrophoretic Analysis of Internally ^{32}P-labeled Oligonucleotide 1 in the Self-cleavage Reactions

A 16-mer oligonucleotide (5'AAGGTTAGGGGTTAGG 3') was labeled with [γ-^{32}P] ATP at its 5' end in the presence of T_4 polynucleotide kinase. The purified 5' phosphorylated 16-mer was further ligated with a 14-mer (5' TGGGGTTAGGGGAA 3') on the template of 5' CCTAACCTTTTCCCCTAA 3' in the presence of T_4 DNA ligase. The produced internally ^{32}P labeled Oligonucleotide 1 was further purified with polyacrylamide gel electrophoresis (20 %) and gel filtration chromatography (NAP-25, GE Healthcare). The same procedures as those for preparing samples loaded were further carried out except that 5' ^{32}P-labeled Oligonucleotide 1 was replaced with the internally ^{32}P-labeled Oligonucleotide 1.

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