DEVELOPMENT OF ENANTIOSELECTIVE AGENTS AND HEME-TARGETED ANTIMALARIALS

A Dissertation
Submitted to the Faculty of the
Graduate School of Arts and Sciences
of Georgetown University
in partial fulfillment of the requirements for the
degree of
Doctor of Philosophy
in Chemistry

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Date: May, 26 2011
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ABSTRACT

Several chiral 3,5-dinitrobenzoyl-derived amides were prepared and applied in enantioselective NMR analysis of protected amines. It was found that \((R)-N-(1-(\text{naphthalen-1-yl})\text{ethyl})-3,5\text{-dinitrobenzamide}\) is a very effective chiral solvating agent (CSA) that gives diastereomeric complexes exhibiting sharp and well resolved NMR signals with a wide range of substrates. Crystallographic analysis showed that this CSA forms a chiral cleft that can selectively bind one enantiomer of a substrate through simultaneous hydrogen bonding, \(\pi-\pi\) stacking, and \(\text{CH}/\pi\) interactions. The enantioselective complex formation results in strong upfield shifts in the proton NMR spectrum even in the presence of only 5 mol % of the CSA.

Five stereodynamic ICD probes based on a “frictionless” arylacetylene framework and terminal aldehyde units, such as 1,4-bis(2(2-formylphenylethynyl)phenylethynyl)benzene, were prepared through a series of Sonogashira cross-coupling reactions. The CD silent sensors generate a strong chiroptical response to substrate-controlled induction of axial chirality upon selective \([1+1]-, [2+2]-,\) and \([1+2]-\)condensation with amines. The chiral amplification results in intense Cotton effects that can be exploited for in situ ICD analysis of the absolute configuration and ee of a wide range of amines.
The continuous need for antimalarials that overcome resistance of *Plasmodium falciparum* to current antimalarial drugs, in particular chloroquine, has been addressed. Forty-two novel heme-targeted antimalarial drug candidates were prepared by systematic variations of the side chain length, basicity, branching and heteroatom substitution at the 4-position in the quinoline backbone. The basic chloroquine side chain and quinolyl *N* are known to facilitate the accumulation of the drug in the acidic digestive vacuole of the malaria parasite. Many of the compounds tested showed excellent potency against chloroquine sensitive and resistant strains which established several important structure activity relationships.
Acknowledgements

I would like to thank my mentor Dr. Christian Wolf for his never ending support and guidance throughout my Ph.D. studies. The passion Christian has for his group, his students and the chemistry is unparalleled. Before coming to Georgetown I thought I was an efficient individual… but then I met Christian and he truly is a “machine of efficiency.” From my first day, Christian has led by example and has taught me to work diligently, to work efficiently and always ask “why” (even if you think you know the answer). I know, without a doubt, that I made the right choice in coming to Georgetown and joining his group. Christian, thank you very much for your support as a mentor and as a friend over these past years.

I would like to sincerely thank the committee members who I have had the privileged of discussing chemistry with throughout the years: Dr. Timothy Warren, Dr. Bahram Moasser, Dr. Paul Roepe, Dr. Faye Rubinson and Dr. David Yang. My committee members have helped me in becoming a well-rounded researcher and in developing my scientific prowess by asking me to always think critically about scientific problems and theories. Thank you all very much for your time, support, guidance and belief in me.

I have had the pleasure of working with two very talented undergraduate students, Eric Whetmore and Nicholas Rosa. I would especially like to thank Eric for his help in the development of the antimalarial compounds, for returning a second summer to work on the enantioselective sensing project and for going beyond the chemistry and becoming a very good friend.
Over the years the Wolf group has become known as the ‘Wolf Pack.’ I have always felt this name was appropriate because the word “pack” promotes the idea of a family. When people spend so many hours together they share the good times and bad times, they help to support each other and when you become part of someone’s life you go beyond being an associate, colleague or coworker… you become a type of family, a “pack.” To Dr. Xuefeng Mei, Dr. Shuanglong Liu, Dr. Kekeli Ekoue-Kovi, Dr. Rachel Lerebours, Dr. Jayakumar K. Natarajan, Dr. Kimberly Yearick Spangler, Dr. Marwan Ghosn, Hanhui Xu, Mikki Boswell, Max Moskowitz, Peng Zhang, Andrea Griffith and Keith Bentley, I thank you all for sharing in my Ph.D. studies, for helping to shape me as a researcher, for experiencing everyday graduate school life and for being part of the “pack” with me. I would especially like to thank Dr. Kimberly Spangler for her constant support, scientific discussion and for her friendship which I cherish greatly.

I would like to thank the members of the Georgetown Malaria Collaboration: the de Dios group, the Roepe group and the Wolf group. I would like to thank Dr. John Alumasa for his antimalarial activity measurements and Dr. Kimberly Yearick Spangler, Dr. Kekeli Ekoue-Kovi and Dr. Jayakumar K. Natarajan for synthesizing the complimentary compounds in the various series.

A person’s first educators are their parents. I would like to thank my parents, Linda and Steve Iwaniuk, for instilling in me the ideas of hard work, determination and the importance of education. Without them, I would not be able to constantly push myself to the “next level” trying to go beyond what I have already accomplished.
I would like to thank a group of people affectionately known as the ‘Hume Ave” crowd, Stephen Drake, Stefan Wiese, Chris Martin and Satjana Pattanasak, for being great friends and such an important part in my life. I would especially like to thank Satjana for always being there for me, for her constant support, for her belief in me and for her knowing me better than I know myself… thank you “Babes” for everything… I love you!

Lastly, I would like to thank the Chemistry Department, the Graduate School of Arts and Sciences, and Georgetown University for the opportunity to pursue my Ph.D. Thank you to Mrs. Kay Bayne, Ms. Inez Traylor, Dr. Mo Itani, Dr. Steve Hannum, Mr. Travis Hall, Mr. Bill Craig and Mrs. Yen Miller for all of your help over the years.

I will always have fond memories of the time I have spent at Georgetown and those memories are shaped by many more people than possible to mention here, but to everyone who has influenced me and played a role in my life… thank you.
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<td>CD</td>
<td>circular dichroism</td>
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<td>high-performance liquid chromatography</td>
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<tr>
<td>HTA</td>
<td>heme-targeted antimalarials</td>
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<td>HTS</td>
<td>high-throughput screening</td>
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<td>IC50</td>
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<td>ICD</td>
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<td>IDA</td>
<td>Indicator-displacement assays</td>
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<tr>
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<td>ligand displacement assay</td>
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<td>α-methoxy-α-trifluoromethylphenylacetic acid</td>
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<td>nonsteroidal anti-inflammatory drugs</td>
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<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
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<td>WHO</td>
<td>World Health Organization</td>
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I. Introduction

“Unless you try to do something beyond what you have already mastered, you will never grow.”

Ralph Waldo Emerson

1.1 The Importance of Chirality

The preference for one enantiomer over the other in nature is expressed in the omnipresence of (S)-amino acids and D-sugars. Since Louis Pasteur realized that the universe is chiral (l’universe est dissymétrique) in 1848,1 the origin of homochirality has intrigued scientists and non-scientists alike.2,3 Because enantiomers are energetically equivalent in an achiral environment (such as the “prebiotic broth” of the Earth 3.85 billions years ago) there should not be a preference in the production or utilization of one enantiomer.4,5 Several theories have been presented in the past 60 years regarding the origin and timeline of biomolecular homochirality. These include autocatalytic reactions leading to an enantiomeric amplification,6-8 electrical current (lightening) passing through the CH₄, NH₃, H₂O, H₂ atmosphere,9,10 simple organic molecules forming on iron-sulfide surfaces present from the Earth’s iron core,11-13 circular polarized light inducing asymmetry in interstellar organic molecules14 and the Earth’s right-handed helical rotation about its axis and the Sun.4 While the scientific community may never agree on the origin of homochirality, the implications of chirality on life are undeniable.

Many biologically active compounds found in everyday life, such as flavors, fragrances, nutrients, agrochemicals and pharmaceuticals, are optically active and the enantiomers can have different biochemical and pharmacological activities. For a given
pharmaceutical, one enantiomer may possess the desired activity, while the other enantiomer may be inactive, severely toxic or antagonistic. In the late 1950s, thalidomide was prescribed to pregnant women as a non-barbiturate sedative to alleviate the symptoms associated with morning sickness. The drug was primarily distributed in Europe and was found to be very effective in combating morning sickness and nausea; however, in 1961 it was pulled from the market after an increasing number of reports describing children born with congenital malformations had appeared. Thalidomide was used in racemic form, and an investigation into the therapeutic profile of each enantiomer revealed that the \((R)\)-enantiomer possesses the desired activity, whereas \((S)\)-thalidomide is a potent teratogen responsible for the severe birth defects. Further studies have shown that thalidomide racemizes under physiological conditions, therefore, administration of enantiopure \((R)\)-thalidomide would still cause birth defects (Figure 1.1).

![Figure 1.1. Racemization of thalidomide under physiological conditions.](image)

More than 50% of today’s top-selling pharmaceuticals are sold as single enantiomer formulations. For example, the cholesterol reducing agent Lipitor (2008 annual sales of $12.4 billion), the anitplatelet agent Plavix (2009 annual sales of $6.1
billion)\textsuperscript{20} and the over-the-counter anti-inflammatory Ibuprofen (World Health Organization’s “Essential Medicines List” member)\textsuperscript{21} are all sold as single enantiomers (Figure 1.2).\textsuperscript{16} The increasing demand for enantiopure compounds has been met by remarkable growth in asymmetric synthesis,\textsuperscript{22-25} catalysis,\textsuperscript{26-30} combinatorial chemistry,\textsuperscript{31-33} and analytical techniques for the determination of stereochemical purity.\textsuperscript{16}

![Chemical Structures]

\textbf{Figure 1.2.} Examples of top-selling enantiopure drugs.

\textbf{1.2 Enantioselective Differentiation of Chiral Molecules}

The advancement in asymmetric synthesis and combinatorial techniques has made it possible to produce a large number of chiral compounds in a short time. This has directed attention toward the development of fast and accurate analytical techniques for the determination of enantiomeric purity.\textsuperscript{31,28} Stereoselective analysis is important in determining the enantiomeric purity and stereochemical stability of chiral compounds; in addition, it is essential for the development of new asymmetric reactions. Chiral HPLC and GC are often considered the “bottleneck” in such screening efforts because these techniques are usually laborious and time-consuming. The need for faster enantiomeric
excess determination has propelled the introduction of efficient assays based on chromatography, mass spectrometry, UV and fluorescence spectroscopy, IR thermography, circular dichroism, capillary electrophoresis, and biochemical methods. The ultimate goal is the development of methods capable of accurate real-time analysis and high-throughput screening (HTS) of a large number of samples.

Enantioselective sensing based on optical methods, such as fluorescence, UV-vis and circular dichroism spectroscopy, offers a variety of advantages over conventional techniques including different detection modes, high sensitivity, low instrument cost, waste reduction and time efficiency. Typically, a molecular sensor contains a chromophore and a binding site in close proximity. The binding of the substrate affects the chromophore and results in a measurable spectroscopic response such as a change in fluorescent emission, UV-vis absorbance (shift of the maximum wavelength or an increase/decrease in intensity) or a change in the interaction with circularly polarized light. The specific response of the chromophore may be associated with a particular electronic process (π-π* transitions, intra- or intermolecular energy transfer, photoinduced electron transfer, etc) and be used to determine absolute configuration and enantiomeric purity of the substrate.

Pu et al. developed BINOL-derived macrocycle 1 for the enantioselective recognition of mandelic acid, 2, and other α-hydroxy carboxylic acids (Figure 1.3). When (-)-1 was titrated with (S)-2, a fluorescence enhancement was observed at 424 nm; however, there was very little enhancement observed when (R)-2 was used under the same conditions. It was generally believed that the lone electron pairs at the amino
groups of 1 participate in photoinduced electron transfer (PET) and quench fluorescence emission. Hydrogen bonding between (-)-1 and (S)-2 would suppress the PET process and thus lead to fluorescence enhancement. However, a recent study by Pu et al. revealed that the amino groups do not affect the fluorescence response of the BINOL-derived sensor.\textsuperscript{43} Investigation of the fluorescence intensity change of (-)-1 with respect to the enantiomeric composition of 2 revealed a linear relationship between the change in fluorescence emission and the enantiopurity of 2 which provides a means for quantitative enantiomeric excess (ee) analysis.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Chiral BINOL-derived macrocycles developed for the enantioselective recognition of mandelic acid.}
\end{figure}

An indicator-displacement assay (IDA) with a chiral host is another tool for ee determinations. An IDA experiment is based on competitive binding between the host and either the indicator or the substrate. Upon addition of the substrate to the host, the indicator which is non-covalently associated with the host is displaced. This process can be monitored by UV-vis or other spectroscopic techniques.\textsuperscript{44,45} Anslyn \textit{et al.} introduced a
trans-diaminocyclohexane-derived Cu(II) complex, 3, for the enantioselective recognition of amino acids.\textsuperscript{36d} In order to enhance the optical response of the assay, a chromophoric ligand (pyrocatechol violet, PV) was used. PV competes with the amino acid substrate for the Cu(II) coordination sites without displacing the trans-diaminocyclohexane (Figure 1.4). Upon addition of 4 to a solution of (S,S)-3, PV is displaced as the diastereomeric complexes 6 are formed. Due to the difference in the thermodynamic stabilities of the diastereomeric complexes, the enantiomers of 4 have different abilities to displace PV which can ultimately be measured and used for enantioselective sensing. Monitoring the change in UV-vis absorbance of the indicator 5 allows quantification of the enantiomeric purity of valine and several other amino acids.

**Figure 1.4.** Anslyn's trans-diaminocyclohexane-Cu(II) complex developed for the enantioselective recognition of chiral amino acids.

Wolf *et al.* have developed 1,8-dianaphthalene-derived N,N-dioxides, 7 and 8, for the enantioselective detection of chiral amines, amino alcohols, unprotected amino
acids, and carboxylic acids (Figure 1.5). Job plot analysis revealed that both 7 and 8 form a 2:1 complex with Sc(III). The Sc[N,N'-dioxide 7]2 complex proved to have a strong fluorescence signal at 588 nm and Sc[N,N'-dioxide 8]2 shows a measurable metal charge transfer band between 360-480 nm. In analogy to an IDA experiment, Wolf et al. developed a ligand displacement assay (LDA) in which titration of the substrate to the Sc[N,N'-dioxide]2 complex results in the dissociation of the N,N'-dioxide ligands. This process coincides with a characteristic signal variation in either fluorescence emission or UV-vis absorbance. During titration with a scalemic substrate, displacement of the first N,N'-dioxide ligand from the Sc(III) center by either the (R)- or the (S)-enantiomer of the chiral substrate gives diastereomeric complexes. The difference in the thermodynamic stabilities of these diastereomeric complexes governs which enantiomer is more effective in replacing the second N,N'-dioxide ligand from the metal center (Figure 1.5). Displacement of the second N,N'-dioxide ligand results in the reduction of fluorescence emission or complete disappearance of the charge transfer band, respectively. The enantioselective recognition with Sc(III) complexes of 7 and 8 has allowed the quantification and determination of enantiomeric composition for several classes of compounds.33,46
Figure 1.5. 1,8-Diheteoarylnapthalene-derived \(N,N\)-dioxide sensors developed by Wolf for the enantioselective recognition of a variety of chiral substrates (top). Illustration of the formation of Sc(III)-derived diastereomeric complexes with an amino alcohol substrate.

1.3 Development of Heme-Targeted Antimalarials (HTA) Derived From a 7-Chloroquine Motif

The World Health Organization (WHO) reports that malaria is the 5\textsuperscript{th} leading cause of death from infectious diseases worldwide and the 2\textsuperscript{nd} leading cause of death from infectious diseases in Africa.\textsuperscript{47} Clearly, malaria is one of the world's most
widespread diseases responsible for 200-300 million clinical cases and over 1 million
deaths in 2008. This debilitating disease is found primarily in tropical and subtropical
regions of the world, an area which encompasses nearly 41% of the world’s population.
It is estimated that malaria is responsible for a child’s death every 30 seconds throughout
the world and roughly 3000 children die every day in Africa. Malaria is caused by a
protozoan parasite of the genus Plasmodium, of which five species can infect humans
with Plasmodium falciparum (P. falciparum) being the most lethal. The onset of malaria
is accompanied by very characteristic symptoms such as periodic chills, rigors, high
fevers and profuse sweating in cycles lasting between 48 and 72 hours.

The life cycle of the malaria parasite is dependent upon both human and mosquito
hosts and can be subdivided into five stages (Figure 1.6): (I) an infected female
Anopheles mosquito feeds on a human blood meal during which the mosquito passes
sporozoites into the human’s bloodstream through its saliva; (II) The sporozoites initially
infect the human liver cells where a single sporozoite produces 30,000-40,000 daughter
cells which eventually mature into merozoites infecting the red blood cells; (III) The
merozoites reproduce asexually eventually causing the red blood cell to rupture releasing
merozoites to infect more red blood cells; (IV) Some of the merozoites differentiate into
male and female gametocytes which are then ingested by a mosquito during a second
blood meal; (V) Once inside the mosquito host, the gametocytes mature into zygotes
which then multiple and form sporozoites in the mosquito saliva, thus restarting the
cycle.
One of the first reported treatments to combat malaria was to chew on the inner bark of the Cinchona tree of South America. In 1817, the antimalarial compound quinine was isolated from this tree bark and spawned the investigation into other cinchona alkaloids along with the development of a variety of compounds exhibiting a 4-substituted quinoline pharmacophore. Compounds such as chloroquine (CQ), mefloquine, sontoquine and amodiaquine have proven to be among the most effective antimalarial drugs (Figure 1.7). Chloroquine has been the most successful antimalarial agent since quinine and also the most cost-effective.
Figure 1.7. Structures of 4-substituted quinoline derivatives used as antimalarials.

Ferriprotoporphyrin IX (FPIX) is generated during proteolysis of host hemoglobin (Hb) which serves as a major source of amino acids during the protozoan life stages within the infected red blood cell. Since the soluble FPIX is toxic to Plasmodium, yet an unavoidable byproduct, it has developed an ingenious strategy for detoxification by converting FPIX into insoluble crystalline hemozoin. The 4-substituted quinolines shown in Figure 1.7 are known to form a complex with free FPIX and this interaction inhibits conversion to hemozoin and hence its detoxification via crystallization. The accumulation of significant concentrations of toxic FPIX-drug adducts is believed to be ultimately responsible for killing the parasite.

With the overwhelming success of chloroquine, worldwide malaria eradication was thought to be possible, ultimately leading to a decline in antimalarial research throughout the 1950s. Unfortunately, the first reports of chloroquine resistance (CQR) emerged in South America and Asia in the early 1960s. While the mechanism of CQR is not fully understood, it is known that resistant strains accumulate reduced amounts of CQ in the digestive food vacuole (DV) relative to their CQ sensitive (CQS) counterparts. An effective malaria vaccine is the ultimate goal in combating the disease. Because the
development of a vaccine is still underway, research on the resistance mechanism and the
development of new drugs that overcome CQR are the best current alternatives.

1.4 References


II. Objectives

To date, more than 50% of the world’s top selling pharmaceuticals are sold as single enantiomers. With this demand spawning the rapid advancement of asymmetric synthesis, catalysis, and combinatorial techniques the need for fast and accurate enantiomeric excess (ee) determination has increased dramatically. Enantioselective analysis remains the “bottleneck” in the drug development process because it often entails laborious and time-consuming chromatographic techniques. The limitations associated with current ee determination methods need to be addressed by the development of new techniques which are based on molecular recognition probes capable of real-time analysis and/or high-throughput screening (HTS) of asymmetric reactions.

Malaria remains one of the top five most devastating diseases affecting the world’s population and is responsible for nearly 1 million deaths per year. With the increased incidence of multi-drug resistant parasites, malaria threatens to become an even greater problem. Current research needs to place emphasis on developing drugs capable of overcoming the resistance while offering insight into the structure activity relationship (SAR) of the resistance mechanism.

The main objectives of this thesis were:

(a) to develop a chiral solvating agent (CSA) for the enantiodifferentiation of chiral amines and amides

Few CSA’s for the enantiodifferentiation of chiral amines and amides are known and proton NMR shift differences between the formed diastereomeric adducts
are often less than 0.1 ppm. Based on the success of the Whelk-O 1 chiral stationary
phase in HPLC, a CSA capable of forming diastereomeric adducts using a three-point
interaction motif was envisioned. The CSA would form a chiral cleft and have (1) a
large π-system for face-to-face π-π stacking, (2) a central amide group serving as
hydrogen bond donor, and (3) a second π-system for face-to-edge CH/π interactions.

![Figure 2.1. Whelk-O 1 structure.](image)

(b) to develop fluxional, CD silent probes exhibiting a stereodynamic
arylacetylene framework and two terminal aldehyde units for
enantioselective CD analysis of chiral amines

The rational design of a series of sensors exhibiting a diarylacetylene-based
framework capable of “frictionless” central-to-axial chirality induction upon
cyclocondensation was expected to provide a new tool for chiral in situ analysis.
Upon reaction with a diamine the sensor would be locked into a macrocyclic structure
in which the central chirality of the substrate dictates the axial chirality of the
diarylacetylene-based framework. The restriction in conformational freedom would
result in distinct chiral amplification and generate a highly CD active compound
providing quantifiable information on the absolute configuration and the enantiomeric composition of the diamine.

![Diagram of sensor and schematic illustration of a conformationally locked diimine analogue.](image)

**Figure 2.2.** Structure of sensor and schematic illustration of a conformationally locked diimine analogue.

(c) to develop novel heme-targeted antimalarials (HTA) derived from the chloroquine motif to probe the structure-function relationships of chloroquine (CQ) resistance

The increased reports of multi-drug resistant parasites have inspired the development of new antimalarials such as mefloquine, tafenoquine, and amodiaquine to combat this worldwide epidemic. It was assumed that systematic variation of the side chain length, basicity, branching and heteroatom substitution at the 4-position in the quinoline backbone will provide promising entries towards affordable heme-targeted antimalarials. In addition, these methodical variations of the side chain would allow identification of SAR principles which would lead to a better understanding of the resistance mechanism.
Structural modification of the side chain

Modification of side chain basicity

$X = \text{NH, O, S}$

Variation of the nucleophilicity/basicity of the quinolyl nitrogen

**Figure 2.3.** Modification sites of the CQ pharmacophore.
III: A Versatile and Practical Solvating Agent for Enantioselective Recognition and NMR Analysis of Protected Amines

3.1 Introduction

The rapid advancement of asymmetric synthesis,\textsuperscript{1-4} catalysis,\textsuperscript{5-9} and the general availability of combinatorial techniques\textsuperscript{10-12} that can produce large numbers of chiral compounds overnight have directed increasing attention to the development of fast, accurate and convenient methods for the determination of the enantiomeric composition of scalemic mixtures. The need for fast enantiomeric excess (ee) analysis has propelled the introduction of efficient assays based on chromatography,\textsuperscript{13} mass spectrometry,\textsuperscript{14} UV\textsuperscript{15} and fluorescence spectroscopy,\textsuperscript{16} IR thermography,\textsuperscript{17} circular dichroism,\textsuperscript{18} capillary electrophoresis,\textsuperscript{19} and biochemical methods.\textsuperscript{20}

Prior to the mid 1960s, the determination of the enantiomeric purity of chiral compounds was generally accomplished with chiroptical methods. In most cases, the optical rotation of a sample at well defined temperature, solvent, concentration and wavelength was compared to the known rotation of the enantiopure reference measured under identical conditions using Equation 5.1:

\[
\text{% optical purity} = 100 \left( \frac{[\alpha]_{\text{mixture}}}{[\alpha]_{\text{pure}}} \right) \quad \text{where} \quad [\alpha]_l = \frac{\alpha}{l \times c} \quad \text{(Equation 5.1)}
\]

\textsuperscript{1}Reproduced in part with permission from the American Chemical Society, Washington, DC, USA. Iwaniuk, D. P.; Wolf, C. J. Org. Chem. 2010, 75, 6724-6727. Copyright 2010 American Chemical Society.
where \( l \) is the path length in decimeters, \( c \) is the concentration in g/mL, \( T \) is the temperature in Celsius and \( \lambda \) is the wavelength.\(^{21,22}\) However, enantiomeric excess and optical rotation do not always follow a linear relationship due to possible aggregation in solution.\(^{28}\) There are examples in the literature in which incorrect optical rotations of enantiopure references were reported. For example, the specific rotation of enantiopure (+)-3-methylcyclopentene was believed to be \([\alpha]^{20}_{D} = +78^\circ\) until the exact value was determined as \([\alpha]^{20}_{D} = +174.5^\circ.\)^{21}\) Lastly, the accuracy of optical rotation measurements for enantioselective analysis can be compromised by contamination with an optically active impurity. This is particularly important when the impurity has a high rotation value compared to the analyte.\(^{21}\) For these reasons, the use of polarimetry for ee analysis has diminished significantly in the last decades.

NMR spectroscopy has become an invaluable tool for stereochemical analysis and quantitative ee determination.\(^{23}\) Enantiomers cannot be distinguished in an achiral environment because they have isochronous NMR signals. However, diastereoisomers have different chemical properties and often show anisochronous NMR shifts.\(^{24}\) Accordingly, conversion of enantiomers into diastereoisomers can afford distinguishable NMR spectra and integration then provides the diastereomeric ratio which is directly related to the enantiomeric composition of the original mixture.\(^{21,24}\)

Raban and Mislow were first to report \(^1\)H NMR chemical shift nonequivalences and thus demonstrated the potential of this technique for stereochemical analysis.\(^{25}\) They studied the NMR spectrum of a mixture of four stereoisomers (SS, RR, SR, RS) obtained from the reaction of racemic 1-(2-fluorophenyl)ethanol and racemic 2-phenylpropanoyl
chloride (Scheme 3.1).\textsuperscript{25} Integration of the diastereomeric methyl signals revealed a diastereomeric ratio of 68/32 which was in excellent agreement with orthogonal GC analysis.\textsuperscript{25}

\[
\begin{array}{c}
\text{HO} \quad \text{Cl} \\
\text{F} \quad \text{aryl} \\
\end{array}
\begin{array}{c}
\rightarrow \\
\text{O} \quad \text{aryl} \\
\end{array}
\text{F} \quad \text{aryl}
\]

\textit{Scheme 3.1.} Reaction studied by Raban and Mislow.\textsuperscript{25}

Transformation of enantiomers into diastereoisomers can be accomplished through covalent bond formation with a suitable enantiopure reagent. Additionally, non-covalent interactions such as metal coordination, hydrogen bonding, π-π interactions, etc. can produce diastereomeric adducts.\textsuperscript{24} A wide range of chiral derivatizing agents (CDAs) have been used to produce covalent diastereoisomers prior to NMR analysis (Figure 3.1).\textsuperscript{24,25} This approach is of limited practicality because it requires a synthetic step and work-up prior to ee determination. Alternatively, chiral lanthanide shift reagents (CLSRs) can be used to generate diastereomeric metal complexes via substrate coordination for direct NMR analysis (Figure 3.1).\textsuperscript{21} Similarly, chiral solvating agents (CSAs) form diastereomeric adducts based on hydrogen bonding, π-π interactions, CH/π interactions or ion-pairing which allows in situ ee quantification without cumbersome product purification (Figure 3.1).\textsuperscript{26}
3.1.1 Enantioselective NMR Analysis

There are many CDAs available for the stereochemical analysis of carboxylic acids, alcohols, and amines. A historically important, and still widely used, CDA is α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) developed by Mosher in the late 1960s for the enantiodifferentiation of chiral alcohols and amines (Figure 3.1). Mosher’s introduction of MTPA inspired the development of other CDAs with an α-substituted phenylacetic acid backbone. A key structural feature to these CDAs is the presence of an aromatic ring attached to the chiral center. Upon bond formation, a...
portion of the substrate is shielded by the aromatic ring thus producing different chemical shift nonequivalences for each diastereoisomer (Figure 3.2).\textsuperscript{27} For instance, the reaction of \((R)\text{-MTPA chloride and a chiral alcohol generates two diastereomeric esters that provide information about the absolute configuration and the relative amounts of the substrate. As shown in Figure 3.2, the R\textsubscript{1} group of the \((R,R)\text{-ester is intruding into the } \pi\text{-cloud of the phenyl group of MTPA resulting in an upfield shift. However, in the } (R,S)\text{-isomer the R}\textsubscript{2} group experiences an upfield shift for the same reason.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Possible conformations of the MTPA-derived diastereoisomers illustrating the origin of chemical shift nonequivalences.\textsuperscript{27}}
\end{figure}

Despite the general usefulness of CDAs, there are some potential problems that are worth mentioning. First, the CDA needs to be 100\% enantiopure, otherwise the measured % de will not reflect the original % ee. Second, the covalent bond formation reaction must occur without racemization of the CDA and the substrate.\textsuperscript{28} Third, kinetic resolution of the substrate can lead to false conclusions. Kinetic resolution can occur
when the enantiomers of a substrate react at different rates with the CDA. If the reaction is incomplete the ratio of the two diastereoisomers formed will not be equivalent to the ratio of the two enantiomers in the original mixture. Finally, the purification of the diastereoisomers formed must be conducted carefully to avoid selective enrichment of one diastereoisomer.

Chiral lanthanide shift reagents (CLSRs) generally consist of an enantiopure ligand coordinated to a paramagnetic metal center. Upon addition of a scalemic mixture of a chiral substrate, diastereomeric complexes are formed and the original enantiomeric composition can be determined by integration of the corresponding diastereotopic signals. In 1974, Whitesides was first to report the effectiveness of a chiral tris[d,d-dicampholylmethanato]europium(III) complex for analysis of the enantiomeric purity of chiral amines, alcohols and nitroalkanes (Figure 3.1). Since then, CLSRs have been applied successfully in ee analysis of amines, alcohols, carbonyl compounds, epoxides, sulfoxides, ethers, sulfides, nitroalkanes, olefins, alkyl halides, and other substrates. While CLSRs have found extensive use for ee analysis, determination of the absolute configuration is somewhat limited. The elucidation of the exact geometry of these diastereomeric complexes is severely hampered due to fast exchange of bound and free ligands which often compromises resolution and hinders the investigation of the chiral recognition mechanism. Additionally, the shortened NMR relaxation time of the substrate due to the proximity of a paramagnetic metal center frequently results in severe peak broadening. Line broadening increases with the strength of the NMR magnet,
therefore, a 500 MHz instrument would produce a spectrum with line broadening 25 times more severe than a spectrum collected with a 100 MHz instrument.\textsuperscript{21}

Pirkle introduced chiral solvating agents (CSA) such as α-phenylethylamine to convert enantiomers to diastereomeric adducts for quantitative ee determination.\textsuperscript{32} These early experiments established a fundamental principle of NMR analysis with CSAs: only the resolution of the diastereotopic signals, but not the integration result, is affected by the enantiopurity of the CSA. Therefore, a CSA does not need to be 100\% enantiopure, which is a great advantage over CDAs.\textsuperscript{24} Similar to CLSRs, a CSA and a substrate form noncovalent complexes that can vary in both thermodynamic and kinetic stability.\textsuperscript{33} The observed nonequivalences are either a result of quantitative formation of diastereomeric adducts with different chemical shifts, or the two enantiomers of a substrate have different affinities to the CSA and one adduct with a different chemical shift compared to the free substrate is formed predominantly. In some cases, substoichiometric amounts of the CSA can be used to generate NMR spectroscopic nonequivalences.\textsuperscript{26} Because noncovalent diastereoisomers are formed the effectiveness of a CSA for enantiodifferentiation is typically dependent on solvent, concentration and temperature.\textsuperscript{26} If the chiral recognition mechanism is well understood it is possible to assign the absolute configuration in addition to the ee.\textsuperscript{33}

Some CSAs have been developed during investigations aimed at elucidating the stereoselective interactions between a chiral substrate and a chiral stationary phase (CSP) used in high-performance liquid chromatography (HPLC).\textsuperscript{24} These studies are often
performed by NMR analysis with a soluble version of the CSP. In many cases, it was found that the soluble CSP analogue proved to be an efficient CSA.\textsuperscript{24}

\textbf{3.1.2 The Whelk-O 1 Chiral Stationary Phase}

The Whelk-O 1 chiral stationary phase was developed by Pirkle and coworkers for the HPLC separation of naproxen enantiomers and other arylpropionic acids commonly found in nonsteroidal anti-inflammatory drugs (NSAIDs).\textsuperscript{34} The concept of reciprocity in chiral recognition, which had played a major role in previously invented chiral stationary phases, was also utilized for the design of the Whelk-O 1 CSP.\textsuperscript{34-38} This concept requires that a CSP derived from the ultimate target molecule, e.g. \((S)\)-naproxen, is first prepared and used to chromatographically screen racemic mixtures of compounds that are expected to undergo highly stereoselective interactions with the immobilized target. One enantiomer of the analyte which affords the highest enantioselectivity is then immobilized on silica gel to afford a new CSP.\textsuperscript{39} Assuming that the stereoselective interactions are reciprocal and not altered by the immobilization, one would expect that this new CSP would be effective in the enantioseparation of the target and structurally similar compounds.\textsuperscript{34,39} Pirkle prepared a CSP derived from \((S)\)-naproxen to chromatographically screen several potential selectors containing a \(\pi\)-acidic 3,5-dinitrobenzoyl group (DNB) which was proposed to interact with the \(\pi\)-basic naphthalene ring of the NSAID.\textsuperscript{34} The outcome of this study was the development of the Whelk-O 1 CSP.
The Whelk-O selector consists of an amide group connecting an electron-deficient 3,5-dinitrobenzoyl group and an electron-rich naphthalene ring (Figure 3.3).\textsuperscript{10} Numerous chromatographic studies, crystallography, and NMR analysis have shown that the amide unit prefers the pseudoaxial position and thus affords a cleft-like structure which is essential for the chiral recognition mechanism.\textsuperscript{40-46} The cleft is formed by the π-acidic 3,5-dinitrobenzamide moiety forming the “wall” which is nearly perpendicular to the π-basic naphthyl “floor.”

![Diagram of Whelk-O selector](image)

\textbf{R = alkyl tether to silica support}

\textbf{Figure 3.3.} Whelk-O 1 CSP structure (left) and the main conformation showing the 3,5-dinitrobenzoyl group in the pseudoaxial position (right).\textsuperscript{47} Reproduced with permission from \textit{Tetrahedron} 2002, 58, 3597-3603.

Cyclohexene rings with an electronegative group placed in the allylic position prefer a pseudoaxial orientation over the pseudoequatorial conformation. This is due to the anomeric effect, e.g. resonance stabilization based on the overlap between the non-bonding double bond π-orbital and the antibonding orbital of the allylic σ-bond.\textsuperscript{39,48}
Because the benzylic position in the tetrahydrophenanthrene ring is structurally similar to an allylic position it is expected that the most stable conformation of the Whelk-O selector has the 3,5-dinitrobenzoyl group in the pseudoaxial position providing a cleft-like arrangement. It is generally assumed that only one enantiomer of a racemate can diffuse into the cleft to simultaneously undergo hydrogen bonding with the amide proton, \( \pi-\pi \) interactions with the 3,5-dinitrobenzoyl group, and CH/\( \pi \) interactions with the naphthyl moiety without adopting an energetically unfavored conformation (Figure 3.4). Immobilization of this selector on silica gel has led to an impressive range of HPLC enantioseparations. However, the synthesis of the Whelk-O selector requires several steps including preparative enantioseparation, which has been a remaining drawback limiting its use in NMR analysis and other applications.

**Figure 3.4.** Enantioselective recognition model of the Whelk-O 1 CSP. Reproduced with permission from *Tetrahedron* 2002, 58, 3597-3603.
3.2 Results and Discussion

We envisioned developing a CSA capable of forming diastereomeric adducts with aromatic substrates based on a three-point interaction motif similar to that of the Whelk-O selector. The following structural features were assumed to be desirable: (1) a large π-system for face-to-face π-π stacking, (2) a central amide group serving as hydrogen bond donor, (3) a second π-system for face-to-edge CH/π interactions, and (4) a benzylic/allylic heteroatom to favor a pseudoaxial conformation and a cleft-like structure. In contrast to the elaborate synthesis of the Whelk-O selector which requires several steps and preparative enantioseparation of a racemic mixture, a CSA that could be prepared from inexpensive, enantiopure starting materials was considered to be more practical.

3.2.1 Synthesis and Evaluation of (S)-N-(2,3-Dihydro-H-inden-1-yl)anthracene-9-carboxamide

Initially, (S)-N-(2,3-dihydro-H-inden-1-yl)anthracene-9-carboxamide, (S)-1, consisting of an amide group bridging an electron-rich 9-anthracene group and an aminooindane ring, was selected as a readily available probe for structural analysis and chiral recognition mechanism studies (Scheme 3.2). It was envisioned that the amide residue would prefer the pseudoaxial position, thus forming the desired chiral cleft with the anthracene unit representing the “wall” and the aminooindane unit being the “floor.” Based upon the above rationale, it was assumed that one enantiomer of a chiral aromatic substrate would diffuse selectively into the cleft to undergo hydrogen bonding, π-stacking and CH/π-interactions with the host. In contrast, the other enantiomer would either
participate in only two of these interactions inside the cleft thus having a different orientation or it would preferably reside outside the cleft. The corresponding diastereomeric complexes would then give rise to distinct chemical shifts (ΔΔδ) due to different shielding effects which could easily be measured by 1H NMR spectroscopy. CSA (S)-1 was prepared in a single step from 9-anthracene carbonyl chloride and (S)-aminoindane in the presence of triethylamine with a yield of 75% (Scheme 3.2).10

\[
\begin{align*}
\text{[9809518511835504947985427464192x-25458854217250768067256936875591410636554240]} & \quad \quad \text{Scheme 3.2. Synthesis of (S)-1.} \\
\end{align*}
\]

Upon slow evaporation of a concentrated solution of (S)-1 in dichloromethane, single crystals suitable for X-ray diffraction were obtained (Figure 3.5). The aliphatic ring of aminoindane adopts a half-puckered conformation which places the amide and anthracene group into a pseudoaxial conformation. This conformation permits the CSA to adopt the desired cleft which is considered critical for chiral recognition.59 The X-ray analysis suggests that (S)-1 adopts the desired three-dimensional geometry similar to the Whelk-O selector.
Stoichiometric amounts of (S)-1 and the enantiomers of analytes 2-6 were then dissolved in deuterated chloroform for NMR analysis (Figure 3.6). Unfortunately, chemical shift nonequivalences were observed only for the enantiomers of 3.

Selected excerpts of the $^1$H NMR spectra obtained with equimolar amounts of (S)-1 and amide 3 are shown in Figure 3.7. The protons in the 1- and 8-positions of the anthracene ring of (S)-1, H$_a$, appear as a broad singlet, probably due to the fast rotation of the anthracene ring about the C-N bond. As expected, the proton in the 10-position, H$_b$, appears as a singlet (Figure 3.7, spectrum A). Addition of (S)-3 produces a homochiral
diastereomeric adduct in which the H\textsubscript{b} proton is upfield shifted (\(\Delta\Delta\delta = 0.025\text{ppm}\)) indicating the H\textsubscript{b} proton is being shielded by the 3,5-dinitrobenzene ring of the guest. Resolution of the H\textsubscript{a} protons into a broad doublet accompanied by an upfield shift (\(\Delta\Delta\delta = 0.071\text{ppm}\)) indicates that the presence of the guest impedes the rotation of the anthracene ring and shields the H\textsubscript{a} protons (Figure 3.7, spectrum C). Addition of (\(R\))-3 produces the heterochiral diastereomeric adduct (Figure 3.7, spectrum D). The H\textsubscript{b} proton is slightly downfield shifted (\(\Delta\Delta\delta = 0.007\text{ppm}\)). Additionally, the H\textsubscript{a} protons are slightly upfield shifted (\(\Delta\Delta\delta = 0.012\text{ppm}\)) but to a lesser extent than in the homochiral adduct. Interestingly, the H\textsubscript{a} protons are not resolved which may be attributed to either weak association with the CSA or formation of an adduct that allows free rotation of the anthracene unit.
Figure 3.7. $^1$H NMR Spectra of (S)-1 and amide 3. A: Free (S)-1, B: Free 3, C: (S)-1 and (S)-3, D: (S)-1 and (R)-3. The spectra were collected in CDCl$_3$ at $1.1 \times 10^{-2}$ M with a CSA to substrate ratio of 1:1.
Slow evaporation of a concentrated equimolar mixture of (S)-1 and (S)-3 in dichloromethane yielded single crystals suitable for X-ray diffraction (Figure 3.8). Crystallographic analysis proves that the homochiral complex is based on a three-point interaction. The 3,5-DNB group of (S)-3 and the anthracene group of (S)-1 are cofacial and have a separation of 3.45 Å indicating π-π interactions. The amide proton of (S)-1 and the carbonyl oxygen of (S)-3 participate in hydrogen bonding, and the N-O distance was determined as 1.953 Å. Finally, the cyclohexane ring of the guest and the aminoindane ring of (S)-1 are cofacial with a distance of 3.54 Å suggesting CH/π interactions between the axial C-H bonds in the cyclohexane ring and the π-system of aminoindane. Contrary to the single crystal structure of (S)-1, which shows the anthracene moiety in a pseudoaxial conformation providing a chiral cleft (Figure 3.5), the analysis of the cocrystal with (S)-3 reveals that the introduction of a guest produces a conformational change in the CSA. Apparently, the interaction with the substrate causes a ring flip and the amide group is placed in the pseudoequatorial position. The lack of conformational rigidity of (S)-1 explains its poor performance as chiral solvating agent.

![Figure 3.8. Cocrystal of the homochiral complex (S)-1–(S)-3.](image)
3.2.2 Synthesis and Evaluation of Second Generation CSAs

As previously mentioned, the development of a CSA readily prepared from commercially available, enantiopure starting materials would be more attractive than a multiple step route involving enantioseparation. Examination of commercially available chiral amines, other than aminoindane, for the construction of the essential benzylic amide moiety favoring a cleft-like conformation was the starting point for the design of a CSA that would overcome the limitation of \((S)\)-1. Accordingly, 1-naphthylethylamine and 1,2,3,4-tetrahydro-1-naphthylamine were selected to (a) evaluate the importance of a cyclic structure versus a less rigid acyclic amine moiety and (b) compare the effect of a \(\pi\)-basic versus a \(\pi\)-acidic aryloyl group (Figure 3.9).

![Figure 3.9. Structures of the Whelk-O selector and CSAs 7-9.](image_url)

The synthesis of 7-9 employed inexpensive, enantiopure starting materials and proceeded with high yields. Chiral solvating agent 7 was synthesized in one step from 9-anthracene carbonyl chloride and \((S)\)-1-naphthylethylamine in the presence of triethylamine in 67% yield. The dinitrobenzoyl amides 8 and 9 were prepared by
condensation of 1-amino-1,2,3,4-tetrahydronaphthalene or 1-(1-naphthyl)ethylamine with 3,5-dinitrobenzoyl chloride in 85-90% yield (Scheme 3.3).¹⁰


Upon slow evaporation of a concentrated solution of (R)-9 in dichloromethane, single crystals were obtained (Figure 3.10). Crystallographic analysis showed that the benzylic amide group adopts the pseudoaxial conformation generating the chiral cleft formed between the naphthalene ring and the 3,5-DNB group, similar to the Whelk-O selector.³⁹ The amide proton resides in the interior of the cleft which should facilitate hydrogen bonding with a guest molecule.
Figure 3.10. Single crystal structure of CSA 9 showing the desired chiral cleft.

To test the usefulness of 7-9, pivaloyl derivatives of chiral aromatic amines and anthranyl amides of aminooindan and several aliphatic amines were prepared (Figure 3.11). The pivamides 10-16 were obtained in a single step by condensation of the corresponding enantiopure amine with pivaloyl chloride in 52-98% yield. Amides 17-20 were obtained by condensation with 9-anthracene carbonyl chloride in 43-75% yield (see Experimental Section).

Figure 3.11. Structures of analytes tested.
NMR analysis of equimolar mixtures of 7-9 and racemic 1-(1-naphthyl)ethylamide 10 in chloroform revealed that 9 affords diastereomeric adducts with quite different chemical shifts, whereas, the presence of 7 and 8 did not generate any nonequivalences of the proton signals of 10. Selected excerpts of the $^1$H NMR spectra of (R)-9 and amide 10 in chloroform are shown in Figure 3.12 (spectrum A and B, respectively). In the homochiral adduct (R)-9–(R)-10, the methyl signal of (R)-10, H$_a$, is significantly upfield shifted ($\Delta \Delta \delta = 0.282$ ppm) (Figure 3.12, spectrum C). The tert-butyl protons of 10, H$_b$, show a downfield shift of 0.059 ppm (Figure 3.12, spectrum C). As expected, identical chemical shifted were obtained for the homochiral adduct (S)-9–(S)-10. Interestingly, comparison of the NMR spectra of the heterochiral complex and the free analyte shows a very small change in the chemical shifts of 10 (Figure 3.12, spectrum D). This could be a result of a weak association between (R)-9 and (S)-10.
Figure 3.12. A: Free CSA 9, B: Free 10, C: (R)-CSA 9 and (R)-10, D: (R)-CSA 9 and (S)-10, E: (R)-CSA 9 with scalemic-10. The spectra were collected in CDCl3 at 6.85 mM with a CSA to substrate ratio of 1:1.

A co-crystal of the homochiral adduct (R)-9–(R)-10 was produced through slow diffusion of diethyl ether vapor into an equimolar mixture of (R)-9 and (R)-10 in
dichloromethane. The crystal structure shows that the CSA populates a cleft-like conformation having the 3,5-dinitrobenzoyl group almost perfectly orthogonal to its naphthalene plane (Figure 3.13). The proton of the amide group in (R)-9 points inside the cleft and undergoes hydrogen bonding with the carbonyl unit of (R)-10. The N-O distance was determined as 2.79 Å. This interaction is complemented by π−π stacking and CH/π forces. The distance between the cofacial 3,5-dinitrobenzoyl group and the aromatic ring of 10 is 3.66 Å. The CH/π attraction arises from the T-shaped orientation of the aromatic C-H bonds in 10 and the naphthalene plane of the CSA having a separation of 2.95 Å. Overall, this three-point interaction places (R)-10 into a highly ordered arrangement inside the cleft of (R)-9. As a result, the methyl group of (R)-10 intrudes into the π-cloud and diamagnetic ring current of the naphthalene unit of the CSA, which explains the strong upfield shift shown in Figure 3.12.

Figure 3.13. Crystal structure of the homochiral complex (R)-9-(R)-10. View along the cleft (left), from the top (middle) and into the cleft (right).
Next, CSA 9 was employed in NMR studies with equimolar amounts of amides 11-20. In all cases, nonequivalences that are sufficient for quantitative enantiodifferentiation were observed (Table 3.1). As seen in the previous example, the NMR signals in the homochiral adduct \((R)-9-(R)-15\) undergo more pronounced upfield shifts than the corresponding protons in the heterochiral complex while the tert-butyl groups show small downfield shifts (Figure 3.14). The nonequivalences of the aromatic signals \(H_a\) and \(H_b\) were determined as 0.103 and 0.063 ppm, respectively. The doublets of the methyl protons \(H_c\) are baseline resolved and even the protons \(H_c\) in the peripheral methoxy group have clearly different chemical shifts. The corresponding \(\Delta\Delta\delta\) values are 0.076 and 0.024 ppm, respectively. As expected, the diastereomeric adducts show the strongest NMR nonequivalences at the methine proton \(H_d\) which are separated by 0.210 ppm. The NMR spectra obtained with analytes 10-20 clearly demonstrate (a) the high resolving ability of CSA 9 and (b) the conserved signal sharpness which both contribute to high overall resolution (Figure 3.14 and Experimental Section).

Selected \(^1\text{H}\) NMR excerpts obtained with the anthracene analogue of aminoisindane, 17 are shown in Figure 3.15. In agreement with the previously discussed pivaloyl derivatives, the homochiral adduct \((R)-9-(R)-17\) shows greater nonequivalences than the heterochiral adduct. The benzylic proton of 17, \(H_b\), undergoes an upfield shift (\(\Delta\Delta\delta = 0.039 \text{ ppm}\)) due to shielding interactions with the naphthyl ring of \((R)-9\). In addition, the proton in the 10-position of the anthracene group of 17, \(H_a\), is upfield shifted by 0.006 ppm. The NMR signals of the diastereomeric adducts are not perfectly
resolved. However, ee values can still be determined by integration of the nonoverlapping regions of the diastereotopic $H_b$ signals.
Figure 3.14: A: Free CSA 9, B: Free 15, C: (R)-CSA 9 and (R)-15, D: (R)-CSA 9 and (S)-15, E: (R)-CSA 9 with scalemic-15. The spectra were collected in CDCl₃ at 6.85 mM with a CSA to substrate ratio of 1:1.
Figure 3.15: A: Free CSA 9, B: Free 17, C: (R)-CSA 9 and (R)-17, D: (R)-CSA 9 and (S)-17, E: (R)-CSA 9 with scalemic-17. The spectra were collected in CDCl₃ at 6.85 mM with a CSA to substrate ratio of 1:1.
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</tr>
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<tr>
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<td></td>
<td>Ar-OCH$_3$</td>
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<tr>
<td>8</td>
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The remarkable differences in the chemical shifts of the methyl protons in the homochiral adduct (R)-9–(R)-10 prompted further investigation of the feasibility of NMR enantiodifferentiation with substoichiometric amounts of the CSA. Indeed, the methyl groups adjacent to the chiral center in 10 are still baseline resolved in the presence of 20 and 5 mol% of CSA 9 (Figure 3.16). This experiment underscores the efficiency of CSA 9 in addition to the wide application spectrum summarized in Figure 3.16.
Figure 3.16: Variation of the amounts of CSA 9 used for the enantiodifferentiation of 10. A: 100% (R)-CSA 9, B: 20% (R)-CSA 9, C: 5% (R)-CSA 9. The substrate concentration was kept constant at 6.85 mM in CDCl₃.

In order to evaluate the practical use of CSA 9 for quantitative ee determination, 9 samples of scalemic mixtures of 10 with known enantiomeric composition were prepared to confirm a linear correlation between the actual ee’s and the values determined from
NMR integration results. The calculated ee’s were generally within 3% of the actual enantiopurity of the samples (Figure 17 and 18).

**Figure 3.17.** Linear correlation between the calculated and the theoretical ee values.
Figure 3.18: Excerpt of the $^1$H NMR spectrum of 10 with various enantiomeric compositions in the presence of CSA 9 in CDCl$_3$. The ee’s calculated by integration are shown in parentheses. The substrate concentration was 6.85 mM and equimolar amounts of 9 were used.
3.3 Conclusions

The principles of the design and chiral recognition mechanism of the Whelk-O selector were exploited for the development of 3,5-dinitrobenzoyl-derived 1-naphthylethyl amide 9. This readily prepared compound was found to be an attractive CSA for NMR analysis of amine derivatives. CSA 9 combines a wide application scope with practical resolution due to signal sharpness and effective signal separation. Crystallographic analysis showed that 9 forms a chiral cleft that can accommodate the homochiral enantiomer of amide 10 through hydrogen bonding, \( \pi-\pi \) stacking and CH/\( \pi \) interactions. The enantioselective complex formation causes strong upfield shifts in the \( ^1H \) NMR spectrum even in the presence of only 5 mol\% of 9. This NMR study suggests that immobilization of 9 on silica gel could afford a powerful chiral stationary phase that would be readily prepared from inexpensive enantiopure starting materials and be economically more attractive than the Whelk-O 1.

3.4 Experimental Section

3.4.1 Synthesis of CSAs and Analyte Derivatives

\( N-(2,3\text{-Dihydro-}H\text{-inden-1-yl})\text{anthracene-9-carboxamide, 1.} \)

To a solution of enantiopure aminoindan (0.05 mL, 0.39 mmol) and triethyl amine (0.16 mL, 1.14 mmol) in 3 mL of anhydrous CH\(_2\)Cl\(_2\) was added 9-anthracene carbonyl chloride (0.100 g, 0.41 mmol) dissolved in 3 mL of anhydrous CH\(_2\)Cl\(_2\). The reaction mixture was stirred at room temperature for 2 hours and extracted with NaHCO\(_3\), water and brine.
The combined organic layers were dried over MgSO\textsubscript{4} and concentrated in vacuo to afford 1 (0.098 g, 0.29 mmol) in 75% yield as an off-white solid. \textsuperscript{1}H NMR: \(\delta = 2.11\ (m, 1H)\), 2.87-3.06 (m, 3H), 6.01 (ddd, \(J = 7.5, 7.5, 7.5\ Hz, 1H\)), 6.23 (d, \(J = 7.9\ Hz, 1H\)), 7.45-7.56 (m, 2H), 7.99 (d, \(J = 8.4\ Hz, 2H\)), 8.46 (s, 1H). \textsuperscript{13}C NMR: \(\delta = 30.4, 34.2, 55.4, 124.0, 124.9, 125.4, 126.7, 126.8, 127.9, 128.2, 128.5, 131.0, 131.6, 142.6, 143.5, 169.1\).

\textbf{(S)-N-(1-(Naphthalen-1-yl)ethyl)anthracene-9-carboxamide, 7.}\textsuperscript{53}

To a solution of (S)-1-naphthylethyl amine (0.12 mL, 0.75 mmol) and triethyl amine (0.22 mL, 1.58 mmol) in 2 mL of anhydrous CH\textsubscript{2}Cl\textsubscript{2} was added 9-anthracene carbonyl chloride (0.150 g, 0.62 mmol) dissolved in 2 mL of anhydrous THF. The reaction mixture was stirred at room temperature for 5 hours and concentrated in vacuo. Purification by flash chromatography on silica gel (CH\textsubscript{2}Cl\textsubscript{2}:NEt\textsubscript{3} = 1:0.05, v/v) followed by extraction with water afforded 7 (0.155 g, 0.41 mmol) in 67% yield as a light brown solid. \textsuperscript{1}H NMR: \(\delta = 1.97\ (d, J = 7.3\ Hz, 3H)\), 6.23 (d, \(J = 7.3\ Hz, 1H\)), 6.50 (dt, \(J = 7.3, 7.3\ Hz, 1H\)), 7.40-7.58 (m, 8H), 7.71 (dd, \(J = 7.5, 7.9\ Hz, 1H\)), 7.80 (d, \(J = 8.2\ Hz, 1H\)) 7.89 (d, \(J = 8.2\ Hz, 1H\)), 7.95 (bs, 2H), 8.42 (s, 1H), 8.53 (d, \(J = 8.5\ Hz, 1H\)). \textsuperscript{13}C NMR: \(\delta = 20.8, 45.2, 122.7, 123.6, 125.2, 125.4, 126.1, 126.6, 128.1, 128.2, 128.5, 128.9, 131.0, 131.1, 131.6, 134.0, 137.7, 168.4\).

\textbf{(R)-N-1-[(1,2,3,4-Tetrahydronaphthyl)]-3,5-dinitrobenzamide, 8.}\textsuperscript{54}

To a solution of enantiopure (R)-1,2,3,4-tetrahydro-1-naphthylamine (0.10 mL, 0.69 mmol) and triethyl amine (0.23 mL, 1.65 mmol) in 2 mL of anhydrous CH\textsubscript{2}Cl\textsubscript{2} was added
3,5-dinitrobenzoyl chloride (0.150 g, 0.0.065 mmol) dissolved in 2 mL of anhydrous CH₂Cl₂. The reaction mixture was stirred at room temperature for 1 hour and then extracted with NaHCO₃, water and brine. The combined organic layers were dried over MgSO₄ and concentrated in vacuo to afford 8 (0.387 g, 0.58 mmol) in 90% yield as a light purple solid. ¹H NMR: δ = 1.88-2.06 (m, 3H), 2.19 (m, 1H), 2.79-2.95 (m, 2H), 5.43 (ddd, J = 5.5, 5.7, 7.9 Hz, 1H), 6.48 (d, J = 7.9 Hz, 1H), 7.16-7.22 (m, 3H), 7.29 (d, J = 7.6 Hz, 1H), 8.92 (s, 2H), 9.15 (s, 1H). ¹³C NMR: δ = 19.8, 29.1, 29.8, 48.9, 121.0, 126.6, 127.1, 127.9, 128.7, 129.6, 135.3, 137.9, 138.0, 148.7, 161.9.

(R)-N-(1-(Naphthalen-1-yl)ethyl)-3,5-dinitrobenzamide, 9.

To a solution of (R)-1-naphthylethyl amine (0.20 mL, 1.25 mmol) and triethyl amine (0.52 mL, 3.73 mmol) in 5 mL of anhydrous CH₂Cl₂ was added 3,5-dinitrobenzoyl chloride (0.302 g, 0.1.31 mmol) dissolved in 5 mL of anhydrous CH₂Cl₂ at 0 °C. The reaction mixture was stirred at room temperature for 1 hour and then extracted with NaHCO₃, water and brine. The combined organic layers were dried over MgSO₄ and concentrated in vacuo to afford 9 (0.387 g, 1.06 mmol) in 85% yield as a yellow solid. ¹H NMR: δ = 1.18 (d, J = 7.1 Hz, 3H), 6.10 (dt, J = 6.9, 7.1 Hz, 1H), 6.59 (d, J = 7.1 Hz, 1H), 7.46-7.56 (m, 3H), 7.59 (d, J = 7.1 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 8.86 (s, 2H), 9.08 (s, 1H). ¹³C NMR: δ = 20.6, 45.9, 120.9, 122.7, 128.8, 125.2, 126.1, 126.9, 127.1, 128.8, 128.9, 130.8, 133.8, 137.0, 137.4, 148.4, 161.6.
Representative procedure for the synthesis of compounds 10-16.

To a solution of enantiopure 1-naphthylethyl amine and triethyl amine (0.23 mL, 1.43 mmol) in 4 mL of anhydrous CH$_2$Cl$_2$ was added pivaloyl chloride (0.22 mL, 1.78 mmol) at 0 °C. After stirring for 1 hour, the mixture was extracted with NaHCO$_3$, water and brine. The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo to afford 10 (0.300 g, 1.18 mmol) in 82% yield as a white solid.

$N$-(1-(Naphthalen-1-yl)ethyl)pivalamide, 10.

$^1$H NMR: δ = 1.16 (s, 9H), 1.65 (d, $J$ = 6.7 Hz, 3H), 5.81 (s, 1H), 5.88 (dt, $J$ = 6.7, 7.1 Hz, 1H), 7.43-7.52 (m, 4H), 7.78 (d, $J$ = 7.9 Hz, 1H), 7.84 (d, $J$ = 8.2 Hz, 1H), 8.03 (d, $J$ = 8.0 Hz, 1H). $^{13}$C NMR: δ = 20.5, 27.5, 38.6, 44.5, 122.4, 123.5, 125.1, 125.8, 126.4, 128.3, 128.6, 131.2, 133.9, 138.5, 177.1.

$N$-(1-(Naphthalen-2-yl)ethyl)pivalamide, 11.

Employing 0.100 g of 2-naphthylethyl amine (0.58 mmol) in the procedure described above afforded 11 (0.146 g, 0.57 mmol) in 98% yield as a white solid. $^1$H NMR: δ = 1.21 (s, 9H), 1.57 (d, $J$ = 6.9 Hz, 3H), 5.27 (dt, $J$ = 7.0, 7.2 Hz, 1H), 5.86 (d, $J$ = 5.5 Hz, 1H), 7.39-7.48 (m, 3H), 7.72 (s, 1H), 7.72-7.81 (m, 3H). $^{13}$C NMR: δ = 21.5, 27.5, 38.6, 48.5, 124.3, 124.6, 125.8, 126.1, 127.5, 127.8, 128.4, 132.6, 133.3, 140.8, 177.4.
N,N’-(1,2-Diphenylethane-1,2-diyl)bis(2,2-dimethylpropanamide), 12.

Employing 0.200 g of enantiopure 1,2-diphenylethylenediamine (0.094 mmol) in the procedure described above afforded 12 (0.208 g, 0.71 mmol) in 75% yield as a white solid. \(^1^H\) NMR: \(\delta = 1.17\) (s, 18H), 5.14 (dd, \(J = 2.3, 4.5\) Hz, 2H), 6.94 (s, 2H), 7.04 (dd, \(J = 1.5, 7.7\) Hz, 4H), 7.10-7.17 (m, 6H). \(^1^C\) NMR: \(\delta = 27.4, 38.6, 59.8, 127.2, 127.5, 128.4, 139.2, 179.3.\)

N-(1-Phenylethyl)pivalamide, 13.

Employing 0.20 mL of enantiopure methylbenylamine (1.57 mmol) in the procedure described above afforded 13 (0.308 g, 1.5 mmol) in 96% yield as a white solid. \(^1^H\) NMR: \(\delta = 1.20\) (s, 9H), 1.48 (d, \(J = 7.0\) Hz, 3H), 5.10 (dt, \(J = 7.0, 7.1\) Hz, 1H), 5.78 (s, 1H), 7.22-7.34 (m, 5H). \(^1^C\) NMR: \(\delta = 21.6, 27.5, 38.5, 48.4, 125.9, 127.2, 128.6, 143.4, 177.4.\)

N-(1,1-Diphenylpropan-2-yl)pivalamide, 14.

Employing 0.100 g of enantiopure 1,1-diphenyl-2-aminopropane (0.47 mmol) in the procedure described above afforded 14 (0.073 g, 0.25 mmol) in 52% as a white solid. \(^1^H\) NMR: \(\delta = 0.94\) (s, 9H), 1.12 (d, \(J = 6.4\) Hz, 3H), 3.84 (d, \(J = 9.9\) Hz, 1H), 5.30-5.32 (m, 1H), 7.13-7.19 (m, 2H), 7.22-7.30 (m, 8H). \(^1^C\) NMR: \(\delta = 20.3, 27.2, 38.4, 47.2, 58.5, 126.6, 126.7, 128.0, 128.4, 128.6, 141.9, 142.2, 177.3.\)
\textbf{N-(1-(4-Methoxyphenyl)ethyl)pivalamide, 15.}

Employing 0.10 mL of enantiopure 4-methoxy-methylbenzyl amine (0.68 mmol) in the procedure described above afforded 15 (0.140 g, 0.59 mmol) in 88\% as a white solid. $^1$H NMR: $\delta = 1.19$ (s, 9H), 1.46 (d, $J = 6.9$ Hz, 3H), 3.79 (s, 3H), 5.05 (dt, $J = 7.0$, 7.1 Hz, 1H), 5.71 (bs, 1H), 6.86 (d, $J = 8.7$ Hz, 2H), 7.21 (d, $J = 8.7$ Hz, 2H). $^{13}$C NMR: $\delta = 21.5, 27.5, 38.5, 47.8, 55.2, 114.0, 127.2, 135.6, 158.7, 177.3$.

\textbf{N-(1-(4-Methylphenyl)ethyl)pivalamide, 16.}

Employing 0.10 mL of enantiopure 1-(4-methylphenyl)ethylamine (0.68 mmol) in the procedure described above afforded 16 (0.125 g, 0.57 mmol) in 84 \% yield as a white solid. $^1$H NMR: $\delta = 1.19$ (s, 9H), 1.46 (d, $J = 6.9$ Hz, 3H), 2.33 (s, 3H), 5.06 (dt, $J = 7.0$, 7.1 Hz, 1H), 5.75 (bs, 1H), 7.13 (d, $J = 8.1$ Hz, 2H), 7.17 (d, $J = 8.1$ Hz, 2H). $^{13}$C NMR: $\delta = 20.9, 21.5, 27.4, 38.4, 48.0, 125.8, 129.2, 136.7, 140.4, 177.2$.

\textbf{Representative procedure for the synthesis of compounds 17-20.}

To a solution of enantiopure aminoindan (0.05 mL, 0.39 mmol) and triethyl amine (0.16 mL, 1.14 mmol) in 3 mL of anhydrous CH$_2$Cl$_2$ was added 9-anthracene carbonyl chloride (0.100 g, 0.41 mmol) dissolved in 3 mL of anhydrous CH$_2$Cl$_2$. The reaction mixture was stirred at room temperature for 2 hours and extracted with NaHCO$_3$, water and brine. The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo to afford 17 (0.098 g, 0.29 mmol) in 75\% yield as an off-white solid.
**N-(2,3-Dihydro-\(H\)-inden-1-yl)anthracene-9-carboxamide, 17.**

\(^1\)H NMR: \(\delta = 2.11\) (m, 1H), 2.87-3.06 (m, 3H), 6.01 (ddd, \(J = 7.5, 7.5, 7.5\) Hz, 1H), 6.23 (d, \(J = 7.9\) Hz, 1H), 7.45-7.56 (m, 2H), 7.99 (d, \(J = 8.4\) Hz, 2H), 8.46 (s, 1H). \(^{13}\)C NMR: \(\delta = 30.4, 34.2, 55.4, 124.0, 124.9, 125.4, 126.7, 126.8, 127.9, 128.2, 128.5, 131.0, 131.6, 142.6, 143.5, 169.1.\)

**N-(1-Cyclohexylethyl)anthracene-9-carboxamide, 18.**

Employing 0.08 mL of enantiopure cyclohexylethylamine (0.0.54 mmol) in the procedure described above afforded 18 (0.078 g, 0.24 mmol) in 43% yield as a white solid. \(^1\)H NMR: \(\delta = 1.05-1.30\) (m, 5H), 1.35 (d, \(J = 6.7\) Hz, 3H), 1.52 (m, 1H), 1.68-1.92 (m, 5H), 4.35-4.43 (m, 1H), 5.86 (d, \(J = 8.9\) Hz, 1H), 7.44-7.52 (m, 4H), 7.99 (d, \(J = 8.0\) Hz, 2H), 8.08 (d, \(J = 6.8\), 2H), 8.45 (s, 1H). \(^{13}\)C NMR: \(\delta = 18.2, 26.1, 26.3, 29.2, 42.9, 50.3, 125.4, 126.6, 127.9, 128.5, 131.1, 132.3, 168.7.\)

**N-(Heptan-2-yl)anthracene-9-carboxamide, 19.**

Employing 0.06 mL of enantiopure 2-aminoheptane (0.0.40 mmol) in the procedure described above afforded 19 (0.072 g, 0.23 mmol) in 57% yield as a yellow solid. \(^1\)H NMR: \(\delta = 0.91-0.95\) (m, 3H), 1.31-1.64 (m, 13H), 4.51 (m, 1H), 5.81 (d, \(J = 8.3\) Hz, 1H), 7.44-7.52 (m, 4H), 7.99 (d, \(J = 7.7\) Hz, 2H), 8.08 (d, \(J = 8.4\), 2H), 8.45 (s, 1H). \(^{13}\)C NMR: \(\delta = 13.0, 20.2, 21.6, 24.8, 30.6, 35.8, 45.1, 124.4, 125.5, 126.9, 127.0, 130.1, 131.2, 167.7.\)
**N-(2,6,6-Trimethylbicyclo[3.1.1]heptan-3-yl)anthracene-9-carboxamide. 20.**

Employing 0.07 mL of enantiopure isopinocampheylamine (0.0.41 mmol) in the procedure described above afforded 20 (0.082 g, 0.23 mmol) in 56% yield as a yellow solid. $^1$H NMR: $\delta = 0.79$ (d, $J = 10.0$ Hz, 1H), 1.19 (s, 3H), 1.27 (s, 3H), 1.35 (d, $J = 7.1$ Hz, 3H), 1.80 (m, 1H), 1.84 (t, $J = 6.4$ Hz, 2H), 2.03 (m, 1H), 2.41 (m, 1H), 2.93 (m, 1H), 4.81 (m, 1H), 5.97 (d, $J = 8.6$ Hz, 1H), 7.44-7.52 (m, 4H), 7.98 (d, $J = 7.9$ Hz, 2H), 8.08 (d, $J = 8.6$ Hz, 2H), 8.45 (s, 1H). $^{13}$C NMR: $\delta = 21.0$, 23.4, 28.0, 35.4, 37.3, 38.5, 41.6, 46.2, 47.7, 48.5, 125.0, 125.4, 126.6, 127.9, 128.0, 128.4, 131.1, 132.2, 168.7

### 3.4.2 Crystallographic Data

**Crystallographic data of N-(2,3-Dihydro-\textit{H}-inden-1-yl)anthracene-9-carboxamide, (S)-1**

Crystal data: C$_{12.80}$H$_{10.13}$N$_{0.53}$O$_{0.53}$, $M = 179.95$, 0.40 x 0.40 x 0.30 mm$^3$, orthorhombic, space group $P2_1_2_1_2_1$, $a = 11.5969(6)$, $b = 16.8534(9)$, $c = 18.2589(10)$ Å, $V = 3568.7(3)$ Å$^3$, $Z = 15$, $D_c = 1.256$ g/cm$^3$, $F_{000} = 1424$, MoKα radiation, $\lambda = 0.71073$ Å, $T = 173(2)$K, $2\theta_{\text{max}} = 50.0^\circ$, 26849 reflections collected, 6291 unique ($R_{\text{int}} = 0.0558$). Final $\text{GooF} = 1.012$, $R1 = 0.0362$, $wR2 = 0.0715$, $R$ indices based on 4646 reflections with I >2sigma(I) (refinement on $F^2$), 469 parameters, 0 restraints. Lp and absorption corrections applied, $\mu = 0.076$ mm$^{-1}$. Absolute structure parameter = 1.1(12) (Flack, H. D. Acta Cryst. 1983, A39, 876-881).
Crystallographic data of (S)-1-(S)-3

Crystal data: C_{39}H_{38}N_{4}O_{6}, M = 658.73, 0.30 x 0.30 x 0.10 mm³, orthorhombic, space group P2_12_12_1, a = 7.5905(8), b = 17.2998(19), c = 25.727(3) Å, V = 3378.3(6) Å³, Z = 8, D_c = 2.590 g/cm³, F_{000} = 2784, MoKα radiation, λ = 0.71073 Å, T = 173(2)K, 2θ_{max} = 50.0°, 25025 reflections collected, 5926 unique (R_{int} = 0.0413). Final Goof = 1.035, R1 = 0.0358, wR2 = 0.0872, R indices based on 4826 reflections with I >2σ(I) (refinement on F²), 462 parameters, 0 restraints. Lp and absorption corrections applied, μ = 0.177 mm⁻¹. Absolute structure parameter = -0.2(10) (Flack, H. D. Acta Cryst. 1983, A39, 876-881).

Crystallographic data of (R)-N-(1-(Naphthalen-1-yl)ethyl)-3,5-dinitrobenzamide, (R)-9

Crystal data: C_{76}H_{42}N_{2}O_{2}, M = 750.32, 0.40 x 0.30 x 0.10 mm³, monoclinic, space group P2_1, a = 9.063(2), b = 12.618(3), c = 15.351(3) Å, β = 96.704(3)°, V = 1743.5(7) Å³, Z = 2, D_c = 1.334 g/cm³, F_{000} = 700, MoKα radiation, λ = 0.71073 Å, T = 296(2)K, 2θ_{max} = 50.0°, 12677 reflections collected, 5967 unique (R_{int} = 0.0416). Final Goof = 1.068, R1 = 0.0908, wR2 = 0.2430, R indices based on 5365 reflections with I >2σ(I) (refinement on F2), 217 parameters, 1 restraint. Lp and absorption corrections applied, μ = 0.101 mm⁻¹. Absolute structure parameter = 1.5(19) (Flack, H. D. Acta Cryst. 1983, A39, 876-881).
Crystallographic Data of (R)-9-(R)-10

Crystal data: C\textsubscript{36}H\textsubscript{36}N\textsubscript{4}O\textsubscript{6}, \( M = 620.69 \), colorless plate, 0.56 × 0.46 × 0.03 mm\textsuperscript{3}, monoclinic, space group \( P2_1 \), \( a = 9.592(3) \), \( b = 17.603(5) \), \( c = 19.168(5) \) Å, \( \beta = 93.087(4) \)°, \( V = 3231.6(15) \) Å\textsuperscript{3}, \( Z = 4 \), \( D_c = 1.276 \) g/cm\textsuperscript{3}, \( F\textsubscript{000} = 1312 \), Siemens SMART, Bruker APEXII CCD, MoK\( \alpha \) radiation, \( \lambda = 0.71073 \) Å, \( T = 296(2) \)K, \( 2\theta_{\text{max}} = 54.0^\circ \), 25303 reflections collected, 13156 unique (\( R_{\text{int}} = 0.0479 \)). Final \( GooF = 0.977, R1 = 0.0482, wR2 = 0.1068 \), \( R \) indices based on 9726 reflections with I >2\( \sigma \)(I) (refinement on \( F^2 \)), 855 parameters, 1 restraint. Lp and absorption corrections applied, \( \mu = 0.088 \) mm\textsuperscript{−1}. Absolute structure parameter = 1.6(7) (Flack, H. D. Acta Cryst. 1983, A39, 876-881).
3.4.3 NMR Enantiodifferentiation of Amides 10-20 by CSA 9

The chiral solvating agent 9 and amides 10-20 were dissolved separately in CDCl$_3$ at a concentration of 13.69 mM. Then, 400 µL of each solution were combined in the NMR tube to produce an equimolar concentration of 6.85 mM.

$^1$H NMR Spectra of (R)-CSA 9 and amide 11

Figure 3.19. A: Free CSA 9, B: Free 11, C (R)-CSA 9 and (R)-11, D: (R)-CSA 9 and (S)-11, E: (R)-CSA 9 with scalemic-11. The spectra were collected in CDCl$_3$ at 6.85 mM with a CSA to substrate ratio of 1:1.
\(^1\)H NMR Spectra of \((R)\)-CSA 9 and amide 12

**Figure 3.20:** A: Free CSA 9, B: Free 12, C: \((R)\)-CSA 9 and \((R)\)-12, D: \((R)\)-CSA 9 and \((S)\)-12, E: \((R)\)-CSA 9 with scalemic-12. The spectra were collected in CDCl\(_3\) at 6.85 mM with a CSA to substrate ratio of 1:1.
Figure 3.21: A: Free CSA 9, B: Free 13, C: (R)-CSA 9 and (R)-13, D: (R)-CSA 9 and (S)-13, E: (R)-CSA 9 with scalemic-13. The spectra were collected in CDCl₃ at 6.85 mM with a CSA to substrate ratio of 1:1.
\(^1\)H NMR Spectra of (S)-CSA 9 and amide 14

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{spectrum_diagram.png}
\caption{A: Free CSA 9, B: Free 14, C: (S)-CSA 9 and (R)-14, D: (S)-CSA 9 and (S)-14, E: (S)-CSA 9 with scalemic-14. The spectra were collected in CDCl\textsubscript{3} at 6.85 mM with a CSA to substrate ratio of 1:1.}
\end{figure}
$^1$H NMR Spectra of (R)-CSA 9 and amide 16

**Figure 3.23:** A: Free CSA 9, B: Free 16, C: (R)-CSA 9 and (R)-16, D: (R)-CSA 9 and (S)-16, E: (R)-CSA 9 with scalemic-16. The spectra were collected in CDCl$_3$ at 6.85 mM with a CSA to substrate ratio of 1:1.
$^1$H NMR Spectra of (S)-CSA 9 and amide 18

Figure 3.24: A: Free CSA 9, B: Free 18, C: (S)-CSA 9 and (R)-18, D: (S)-CSA 9 and (S)-18, E: (S)-CSA 9 with scalemic-18. The spectra were collected in CDCl$_3$ at 6.85 mM with a CSA to substrate ratio of 1:1.
$^1$H NMR Spectra of (R)-CSA 9 and amide 19

**Figure 3.25**: A: Free CSA 9, B: Free 19, C: (R)-CSA 9 and (R)-19, D: (R)-CSA 9 and (S)-19, E: (R)-CSA 9 with scalemic-19. The spectra were collected in CDCl$_3$ at 6.85 mM with a CSA to substrate ratio of 1:1.
$^1$H NMR Spectra of (R)-CSA 9 and amide 20

Figure 3.26: A: Free CSA 9, B: Free 20, C: (R)-CSA 9 and (R)-20, D: (R)-CSA 9 and (S)-20, E: (R)-CSA 9 with scalemic-20. The spectra were collected in CDCl$_3$ at 6.85 mM with a CSA to substrate ratio of 1:1.
3.5 References


47 Reprinted from *Tetrahedron*, 58, Wolf, C.; Pirkle, W. H., Conformational effects on the enantioselective recognition of 4-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene derivatives by a Naproxen-derived chiral stationary phase, 3597-3603, Copyright (2002), with permission from Elsevier.


52 During this study we found that CSA 9 can also be used for the resolution of chiral sulfoxides. Deshmukh, M.; Dunach, E.; Juge, S.; Kagan, H. B. *Tetrahedron Lett.* **1984**, *25*, 3467-3470.


IV: Enantioselective Recognition of Amines Based on [1+1]-, [2+2]- and [1+2]-Condensation with Stereodynamic Arylacetylene-Derived Dialdehydes

4.1 Introduction

The unique structure and stereodynamic properties of axially chiral biaryls have been exploited in asymmetric synthesis, chiral amplification and for the development of fascinating technomimetic devices, including molecular propellers and switches.\(^1\) The use of internal or external means to control the energy barrier to rotation, such as incorporation of steric bulk, metal complexation or photochemical activation, often plays a crucial role in these applications. Elongation of the biaryl axis with an acetylene unit increases the aryl-aryl distance to approximately 4.0 Å. Because of the extended separation of the two aryl rings, the steric hindrance to rotation about the pivotal axis in diarylacetylenes is generally low and conformational isomers cannot be isolated at room temperature.\(^2\) This intrinsic rotational freedom is a key feature of diarylacetylene-derived molecular turnstiles\(^3\) and gyroscopes (Figure 4.1).\(^4\)

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The design of chromophoric stereodynamic receptors that report a molecular recognition event through substrate-controlled induction of axial or helical chirality has received increasing attention in recent years. The amplification of central chirality in supramolecular assemblies, molecular bevel gears, propellers and other well-defined arrangements is often expressed by characteristic induced circular dichroism (ICD) signals which can be used for configurational and conformational analysis. Berova, Nakanishi, Canary and others have introduced practical ICD probes that are based on chiral amplification via formation of hydrogen bond adducts and metal complexes exhibiting fluxional porphyrin or other aromatic ligands. Rosini and Toniolo have demonstrated that the covalent attachment of a conformationally flexible biphenyl unit to chiral amino acids, carboxylic acids and alcohols followed by isolation of the adduct and CD analysis provides a reliable means for the assignment of the absolute configuration of these important substrates (Figure 4.2).
Suzuki developed a dynamic molecular recognition process for the detection of diammonium substrates via hydrogen bonding with an \(N,N,N',N'\)-tetrasubstituted terephthalamide host (Figure 4.3). The two tertiary amide groups of the host have proven essential to generate a chiroptical readout. The tertiary amides cannot adopt a planar geometry due to steric hindrance, therefore, the host exists in an interconverting mixture of twisted \(\text{syn}\)- and \(\text{anti}\)-conformation. While the \(\text{anti}\)-conformer is more stable due to dipole offset, the \(\text{syn}\)-conformer has the appropriate geometry for dual hydrogen bonding between the guest and host. Suzuki’s host is equipped with long arylethynylbenzene “arms” as chromophores. Upon guest binding, the host is “locked” into the \(\text{syn}\)-conformation resulting in a strong ICD signal due to exciton coupling of the chromophores. The Cotton effect of the ICD signal is dependent upon the helical “arm-crossing” geometry resulting in either \((P)\)- or \((M)\)-configuration.
Figure 4.3. Structure of Suzuki's \(N,N,N',N'-\)tetrasubstituted terephthalamide host and conformational change upon substrate binding.\(^{5c}\)

Despite the advance of stereodynamic biphenyl probes, a rationally designed enantioselective sensor exhibiting a diarylacetylene-based framework capable of “frictionless” central-to-axial chirality induction has not been reported.\(^{14}\) Based on the relatively free rotation about the alkyne rods one can assume that such a sensor would exist as a mixture of rapidly interconverting conformers in solution. The molecular geometry, i.e. the relative orientation and the length of the central axis and the branches, would need to be carefully chosen to provide a platform for two subsequent condensation reactions between a diamine and the terminal aldehyde functions. Upon diimine formation the sensor would be locked into a macrocyclic structure in which the central chirality of the substrate dictates the axial chirality of the diarylacetylene-based framework. This substrate-controlled stereoselective cyclization would result in distinct chiral amplification and generate a highly CD active compound providing quantifiable
information on the absolute configuration and the enantiomeric composition of the diamine used.

![Chemical structure](image)

**Figure 4.4.** Schematic illustration of the conversion of the conformationally flexible sensor to the locked diimine analogue.

### 4.2 Results and Discussion

Based on the potential of 1,3-diarylacetylenes as versatile stereodynamic units in molecular devices, probes and other applications, a series of arylacetylene-based frameworks having a well-defined intramolecular separation and relative orientation of terminal formyl groups was prepared and evaluated (Figure 4.5).
The design of the CD silent arylacetylene moiety of stereodynamic dialdehydes 1-5 directs the reaction with diamines towards a [1+1]- or [2+2]-assembly which was expected to provide a distinct chiroptical response to a concomitant substrate-controlled chiral amplification process. It was assumed that bridging of 1,4-di(2-formylphenyl)buta-1,3-diyn, 4, and 1,4-bis(2-formylphenylethynyl)benzene, 5, through reaction with a diamine would favor formation of a large macrocyclic tetraimine via [2+2]-cyclocondensation. By contrast, 1,4-bis(2-formylphenylethynyl)phenylethynyl)benzene, 1, 1,4-bis(2-formyl-1-naphthylethynyl)phenylethynyl)benzene, 2, and 1,4-bis(2-formylphenylethynyl)phenylethynyl)anthracene, 3, were expected to produce a [1+1]-assembly.
Figure 4.6. Schematic illustration of the reaction with diamines towards a [1+1]- or [2+2]-diimine macrocycle (top). Structures of amine and diamine substrates (bottom).

4.2.1 Synthesis, Diimine Formation and Evaluation of ICD Sensors Capable of Forming a [1+1]- and [1+2]-Condensation Product

Sensor 1 was prepared in 4 steps and 62% overall yield (Scheme 1). Sonogashira coupling of 2-bromoiodobenzene and 1,4-diethynylbenzene gave 1,4-bis((2-bromophenyl)ethynyl)benzene, 17, in almost quantitative yields. Alkynylation with trimethylsilylacetylene followed by deprotection of 18 using TBAF then afforded
precursor 19. Finally, 1,4-bis((2-ethylphenyl)ethynyl)benzene, 19, was converted to 1 in 88% yield by palladium-catalyzed Sonogashira coupling with 2-bromobenzaldehyde.\textsuperscript{15}

\begin{center}
\includegraphics[width=\textwidth]{scheme41.png}
\end{center}

**Scheme 4.1.** Synthesis of sensor 1.

Palladium catalyzed Sonogashira coupling of 2-bromoiodobenzene and 1,4-diethynylbenzene gave dibromide 20 in quantitative amounts (Scheme 4.2). Cross-coupling with ethynyltrimethylsilane then furnished 21 in 93% yield which was followed by TBAF promoted deprotection towards the terminal dialkyne 22. Finally, coupling of 22 and two equivalents of 1-bromo-2-naphthaldehyde in the presence of 10 mol% of tetrakis(triphenylphosphine)palladium(0) and copper(I)iodide produced 2 in 54% yield.
A somewhat similar strategy was used to prepare the branches of compound 3 prior to the construction of the central rod. First, 2-(2-bromophenyl)ethynyl)benzaldehyde, 23, was obtained in almost quantitative yields by Sonogashira coupling of 2-iodobromobenzene and 2-ethynylbenzaldehyde. Alkynylation with ethynyltrimethylsilane and subsequent deprotection then gave 24 and 25 in 83% and 62% yield, respectively. 2-(2-Ethynylphenylethynyl)benzaldehyde, 25, was then attached to the anthracene core by palladium catalyzed cross-coupling with 9,10-dibromoanthracene to give 3 in 60% yield.
Scheme 4.3. Synthesis of sensor 3.

As expected, the condensation between sensors 1-3 and the amines 6-16 gave either a [1+1]-diimine macrocycle or an acyclic [1+2]-diimine (Figure 4.6). For example, the condensation of 1 with a stoichiometric amount of diamine 9 towards the expected macrocycle was monitored by NMR spectroscopy showing quantitative disappearance of the formyl protons of 1 (Figure 4.7). ESI-MS analysis of the resulting diimine condensation product showed formation of the [1+1]-macrocycle (Figure 4.8).

Figure 4.7. NMR spectrum of the diimine macrocycle obtained from sensor 1 and (R)-9.
Figure 4.8. MS spectrum of the diimine macrocycle obtained from sensor 1 and (R)-9.

The corresponding diimines have very strong Cotton effects even at micromolar concentrations. As shown in Figure 4.9, the CD spectra of the cyclic diimines obtained from 1 and the enantiomers of trans-1,2-diaminocyclohexane, 6, have an amplitude greater than 100 mdeg at 3.76 x 10^{-5} M. Interestingly, this probe is also suitable for the CD analysis of monoamines 10-16. A representative example obtained with two equivalents of 16 proves that this diimine affords distinctive Cotton effects (Figure 4.10). The general CD activity of the acyclic condensation products derived from 10-16 is quite remarkable since these acyclic diimines can populate a complex mixture of discrete conformations.
Figure 4.9. ICD spectra of the diimines formed from 1 and (1R,2R)-6 (blue) and (1S,2S)-6 (red) at room temperature (3.75 mM in chloroform). For CD analysis the samples were diluted to 3.76 $10^{-5}$ M.

Figure 4.10. ICD spectra of the diimines formed from 1 and (1R,2R,3R,5S)-16 (blue) and (1S,2S,3S,5R)-16 (red) at room temperature (3.75 mM in chloroform). For CD analysis the samples were diluted to 7.52 $10^{-5}$ M.
Apparently, cyclocondensation of the CD silent probe generates a rigid ring topology that is controlled by the structure of the diamine. Computational analysis suggests that 6 adopts a diaxial chair conformation upon cyclization (Figure 4.11). The chirality of the substrate is thus imprinted into the previously fluxional diarylacetylene-based probe and the high CD activity can be attributed to the induction of three chiral axes represented by the central 1,4-di(phenylethynyl)benzene rod and the two 2-iminophenylethynyl branches. According to the MM2 calculation of the diimine derived from 1 and (1S,2S)-6, the central rod of the sensor accepts a (P)-conformation whereas the two branches are locked into (M)-axes.

**Figure 4.11.** MM2 Computation of the structure of the diimine derived from 1 and (1S,2S)-6. For better clarity, the hydrogens are omitted in the space filling model.

In order to evaluate the practical use of sensor 1 for quantitative ee determination of an amine substrate, a calibration curve was constructed using 6 in varying ee. The diimine mixtures were obtained at 3.75 mM and the samples were diluted to 1.88 $10^{-5}$ M for CD analysis. The CD amplitudes (mdeg) at 288 nm were plotted versus %ee showing a perfectly linear relationship (Figure 4.12). Five scalemic samples of 6 were then
prepared and treated with sensor 1 as described above. Using the linear regression equation calculated from the calibration curve and the measured CD amplitudes at 288 nm, the enantiomeric excess of these samples was determined. Experimentally obtained data were within 3.9% of the actual values (Table 1).\(^6\)

**Figure 4.12.** Linear correlation between the CD amplitudes (mdeg) at 288 nm and the theoretical %ee values.

**Table 4.1.** Experimentally determined ee’s of five scalemic samples of 6 using sensor 1.

<table>
<thead>
<tr>
<th>Actual % ee ((R))</th>
<th>Calculated % ee ((\bar{R}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>-90.0</td>
<td>-86.1</td>
</tr>
<tr>
<td>30.0</td>
<td>30.8</td>
</tr>
<tr>
<td>-10.0</td>
<td>-13.9</td>
</tr>
<tr>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>-44.0</td>
<td>-41.6</td>
</tr>
</tbody>
</table>
While the stereochemical outcome of the cyclocondensation between 1 and diamines such as 6 is well-defined and predictable, the reaction with two equivalents of a scalemic monoamine can potentially result in a mixture of homochiral and heterochiral adducts. The formation of a diastereomeric mixture would be likely to complicate quantitative ee analysis due to smaller Cotton effects and a nonlinear relationship between the observed CD amplitude and the enantiomeric composition of the amine used. However, a linear calibration curve and experimentally obtained ee’s within 5.5% of the actual values were obtained when the sensor was applied in the enantioselective analysis of monoamine 16 (Table 4.2).

**Table 4.2.** Experimentally determined ee’s of five scalemic samples of 16 using sensor 1.

<table>
<thead>
<tr>
<th>Actual % ee ($R$)</th>
<th>Calculated % ee ($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86.0</td>
<td>84.6</td>
</tr>
<tr>
<td>30.0</td>
<td>26.2</td>
</tr>
<tr>
<td>-10.0</td>
<td>-13.7</td>
</tr>
<tr>
<td>50.0</td>
<td>55.5</td>
</tr>
<tr>
<td>-44.0</td>
<td>-46.4</td>
</tr>
</tbody>
</table>

ESIS-MS examination of a mixture containing an acyclic diimine of 1 in the presence of stoichiometric amounts of another monoamine showed that the condensation is reversible under the conditions used in our assay (Scheme 4.4). Accordingly, the linear
relationship between the CD amplitude of the diimines obtained and the enantiomeric composition of 16 may be attributed to highly stereoselective formation of thermodynamically favored homochiral products.

Scheme 4.4. Reversibility of the diimine formation: ESI-MS analysis of a reaction mixture containing an acyclic diimine of 1 and 16 (3.75 mM in chloroform) in the presence of stoichiometric amounts of 15. After the mixture was allowed to react for 90 minutes, an ESI-MS spectrum was collected showing considerable formation of diimines derived from 15.
The suitability of dialdehydes 2 and 3 for enantioselective sensing of the amines shown in Figure 4.6 was then evaluated. As expected, mass spectrometric analysis of the reaction mixtures obtained with diamines 6-9 proved formation of [1+1]-macrocycles while the monoamines generate [1+2]-cyclocondensation products and NMR spectroscopy showed quantitative disappearance of the formyl protons. In situ CD analysis of the diimines showed strong Cotton effects that can be attributed to population of a highly CD active axially chiral conformer (Figure 4.13-4.17).

![ICD Spectra of the [1+1]-macrocycles obtained using 2 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at 9.75 x 10^{-5} M in CHCl3 at room temperature.](image-url)
Figure 4.14. ICD spectra of the acyclic [1+2]-cyclocondensation products produced from 2 and (R)-14 (blue) or (S)-14 (red) at 1.13 x 10^{-4} M in CHCl₃ at room temperature.

Figure 4.15. ICD Spectra of the [1+1]-macrocycles obtained using 3 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at 5.64 x 10^{-5} M in CHCl₃ at room temperature.
Figure 4.16. ICD spectra of the acyclic [1+2]-cyclocondensation products produced from 3 and (R)-13 (blue) or (S)-13 (red) at $3.39 \times 10^{-4}$ M in CHCl$_3$ at room temperature.

In analogy to the study with dialdehyde 1, MM2 calculations of the dimines obtained by cyclocondensation of sensors 2 and 3 with (1S,2S)-6 showed that this distinctive chiral amplification is controlled by the central chirality of the amine substrate (Figure 4.17). The chirality of the substrate is thus imprinted into the previously fluxional diarylacetylene-based probe and the high CD activity can be attributed to the induction of three chiral axes represented by the central rod and the two arylacetylene branches. The induced chiroptical properties of the diimines formed was expected to provide quantifiable information on the absolute configuration and the enantiomeric composition of the substrate.
Figure 4.17. MM2 Computation of the structures of the diimines derived from (1S,2S)-6 and 2 (top) or 3 (bottom). For better clarity, the hydrogens are omitted in the space filling model.

Linear calibration curves using 2 and 3 with either diamine 8 or monoamine 13, respectively, were obtained as described with sensor 1. The linear regression equation calculated from these calibration curve and the measured CD amplitudes of the diimines generated from scalemic mixtures of amines 8 and 13 gave experimentally obtained data that were within 7.4% of the actual values (Tables 4.3 and 4.4).
Table 4.3. Experimentally determined ee’s of five scalemic samples of \textbf{8} using sensor \textbf{2}.

<table>
<thead>
<tr>
<th>Actual % ee ($R$)</th>
<th>Calculated % ee ($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-90.0</td>
<td>-92.6</td>
</tr>
<tr>
<td>30.0</td>
<td>22.6</td>
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<tr>
<td>-22.0</td>
<td>-21.4</td>
</tr>
<tr>
<td>-46.0</td>
<td>-45.9</td>
</tr>
<tr>
<td>86.0</td>
<td>92.7</td>
</tr>
</tbody>
</table>

Table 4.4. Experimentally determined ee’s of five scalemic samples of \textbf{13} using sensor \textbf{3}.

<table>
<thead>
<tr>
<th>Actual % ee ($R$)</th>
<th>Calculated % ee ($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-90.0</td>
<td>-82.6</td>
</tr>
<tr>
<td>30.0</td>
<td>30.8</td>
</tr>
<tr>
<td>86.0</td>
<td>83.5</td>
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<tr>
<td>54.0</td>
<td>48.5</td>
</tr>
<tr>
<td>-46.0</td>
<td>-39.9</td>
</tr>
</tbody>
</table>
4.2.2 Synthesis, Diimine Formation and Evaluation of ICD Sensors Capable of Forming a [2+2]-macrocycle and [2+2]-Condensation Product

Sensor 4 was readily available through oxidative homocoupling of 2-ethynylbenzaldehyde as described in the literature (Scheme 4.5).\textsuperscript{17} The elongated analogue 5 was prepared by Sonogashira coupling of 2-bromobenzaldehyde and 1,4-diethynylbenzene in 80\% yield. Slow evaporation of a solution of 3 in dichloromethane gave single crystals suitable for X-ray diffraction. The crystallographic analysis of 5 showed the expected linear geometry and the lack of steric hindrance to rotation about the aryl-alkyne bonds (Figure 4.18).

Scheme 4.5. Synthesis of sensors 4 and 5.

Figure 4.18. Crystal structure of 5.
As previously discussed, the expected geometry of 4 and 5 should afford tetrainine macrocycles upon [2+2]-cyclocondensation with diamines.\textsuperscript{18} The reaction between stoichiometric amounts of either enantiomer of \textit{trans}-diaminocyclohexane and 4 was monitored by NMR spectroscopy showing quantitative disappearance of the formyl protons. Formation of the expected [2+2]-macrocycle was evident from ESI-MS analysis but the condensation with 1,2-diamino-1,2-diphenylethane, 8, yielded an acyclic product. Importantly, the 4-derived imines obtained with the enantiomers of \textit{trans}-diaminocyclohexane and other amines gave measurable Cotton effects (Figure 4.19 and Experimental Section).

Figure 4.19. ICD Spectra of the [2+2]-macrocycles obtained using 4 and (1\textit{R},2\textit{R})-6 (blue) or (1\textit{S},2\textit{S})-6 (red) at 2.82 x 10^{-4} M in CHCl\textsubscript{3} at room temperature.
Encouraged by the results obtained with 4, the condensation between 5 and several amines were screened (Figure 4.6). ICD sensor 5 undergoes [2+2]-cyclocondensation with all diamines tested and generates acyclic diimines with amines 10-16 (Figure 4.20 and 4.21, respectively). Both the enantiomeric tetraimines and diimines formed show remarkable Cotton effects that can be used for quantitative ee analysis of the amine substrates.

Figure 4.20. ICD Spectra of the [2+2]-macrocycles obtained using 5 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at 1.32 \(10^{-4}\) M in CHCl\(_3\) at room temperature.
Figure 4.21. ICD Spectra of the [1+2]-cyclocondensation products obtained using 5 and (R)-12 (blue) or (S)-12 (red) at 1.51 x 10^{-4} M in CHCl₃ at room temperature.

To demonstrate the use of 5 in enantioselective sensing applications, a calibration curve was constructed using scalemic mixtures of diaminocyclohexane, 6. The corresponding 5-derived macrocyclic tetraimines were prepared in chloroform at 3.75 mM and the samples were diluted to 7.48 x 10^{-4} M for CD analysis. Plotting of the CD amplitudes (mdeg) measured at 300 nm versus %ee revealed a perfectly linear relationship. Four scalemic samples of 6 having -90.0%, -46.0%, 30.0% and 86.0% ee were then prepared and treated with sensor 5 as described above. Using the linear regression equation calculated from the calibration curve and the measured CD amplitudes of these samples, the enantiomeric excess was determined. Experimentally obtained data were within 3.9% of the actual values (Table 4.5).
Table 4.5. Experimentally determined ee’s of four scalemic samples of 12 using sensor 5.

<table>
<thead>
<tr>
<th>Actual % ee (R)</th>
<th>Calculated % ee (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-90.0</td>
<td>-92.7</td>
</tr>
<tr>
<td>30.0</td>
<td>28.4</td>
</tr>
<tr>
<td>-46.0</td>
<td>-49.9</td>
</tr>
<tr>
<td>86.0</td>
<td>87.4</td>
</tr>
</tbody>
</table>

4.3 Conclusions

Five fluxional, CD silent probes, 1-5, exhibiting a stereodynamic arylacetylene framework and two terminal aldehyde units have been developed. The well-defined geometry of these compounds either favors [1+1]- or [2+2]-cyclocondensation with diamines while monoamines generate acyclic [1+2]-diimines. Computational analysis suggests that the strong Cotton effects observed upon di- and tetraamine formation are due to substrate controlled induction of axial chirality. The observed Cotton effects occur at high wavelengths which excludes misinterpretation due to overlapping CD signals of the substrate and interference with chiral impurities. These sensors provide means for metal-free enantioselective CD analysis of a wide range of chiral amines and the distinct CD output allows determination of the absolute configuration and the enantiomeric composition. The use of sensitive ICD sensors 1-5 for enantioselective analysis of amines avoids the cumbersome isolation of reaction products and it eliminates the need for an elaborate synthesis of an enantiomerically pure chiral probe.
4.4 Experimental Section

4.4.1 Synthesis of ICD probes 1-5

1,4-Bis((2-bromophenyl)ethynyl)benzene (17)\(^{19}\)

A solution of 2-iodobromobenzene (3 mL, 23.4 mmol), 1,4-diethynylbenzene (0.999 g, 7.8 mmol), Pd(PPh\(_3\))\(_4\) (0.901 g, 0.78 mmol) and CuI (0.148 g, 0.78 mmol) in 6 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 12 hours in a closed vessel. The resulting mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH\(_2\)Cl\(_2\)) followed by recrystallization from CH\(_2\)Cl\(_2\) and hexanes afforded 17 (3.36 g, 7.73 mmol) in 99% yield as a yellow solid.

\(^1\)H NMR: δ = 7.18 (ddd, \(J = 1.7, 7.6, 7.9\) Hz, 2H), 7.29 (ddd, \(J = 1.1, 7.6, 7.9\) Hz, 2H), 7.55 (dd, \(J = 1.7, 7.6\) Hz, 2H), 7.56 (s, 4H), 7.62 (dd, \(J = 1.1, 7.9\) Hz, 2H). \(^{13}\)C NMR: δ = 89.9, 93.5, 123.1, 125.1, 125.6, 127.0, 129.6, 131.6, 132.5, 133.2.

1,4-Bis(2-(trimethylsilylethynyl)phenylethynyl)benzene (18)\(^{20}\)

A solution of 17 (3.40 g, 7.80 mmol), ethynyltrimethylsilane (3.5 mL, 24.7 mmol), Pd(PPh\(_3\))\(_4\) (0.900 g, 0.78 mmol) and CuI (0.148 g, 0.78 mmol) in 6 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 18 hours in a closed vessel. The reaction mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH\(_2\)Cl\(_2\): hexanes, 2:1, v/v) afforded 18 (4.41 g, 7.26 mmol) in 93% yield as a red solid.
$^1$H NMR: δ = 0.28 (s, 18H), 7.22-7.31 (m, 4H), 7.49-7.51 (m, 4H), 7.54 (s, 4H). $^{13}$C NMR: δ = 0.0 90.1, 93.1, 103.3, 123.2, 125.7, 125.8, 128.0, 128.2, 131.5, 131.6, 132.2.

1,4-Bis(2-ethynylphenylethynyl)benzene (19)$^{20}$

A solution of 18 (3.24 g, 6.89 mmol) and tetrabutylammonium fluoride (1.0 M in THF, 10.34 mL, 35.80 mmol) in 8 mL of anhydrous THF was stirred at room temperature for 90 minutes. The reaction mixture was concentrated in vacuo and purification by flash chromatography on silica gel (hexanes:CH$_2$Cl$_2$, 2:1, v/v gradually changed to pure CH$_2$Cl$_2$) afforded compound 19 (1.91 g, 3.28 mmol) in 76% yield as a red solid.

$^1$H NMR: δ = 3.40 (s, 2H), 7.23-7.33 (m, 4H), 7.53-7.56 (m, 8H). $^{13}$C NMR: δ = 81.4, 82.2, 89.9, 93.3, 123.2, 124.7, 126.0, 128.2, 128.6, 131.7, 131.8, 132.6.

1,4-Bis(2-(2-formylphenylethynyl)phenylethynyl)benzene (1)

A solution of 19 (0.083 g, 0.22 mmol), 1-bromobenzaldehyde (0.14 mL, 1.20 mmol), Pd(PPh$_3$)$_4$ (0.029 g, 0.025 mmol) and CuI (0.005 g, 0.026 mmol) in 2 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 20 hours in a closed vessel. The mixture was cooled to room temperature, diluted with 4 mL of diethyl ether and then stored at -20 °C for 12 hours. The precipitate was collected via vacuum filtration, dissolved in CH$_2$Cl$_2$ and washed with H$_2$O. The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo to afford 1 (0.107 g, 0.20 mmol) in 88% yield as a yellow solid. NMR analysis showed that 1 cocrystallizes with 1 equivalent of water.
\[ ^1\text{H NMR: } \delta = 7.35\text{-}7.38 (m, 4H), 7.46 (dd, J = 7.6 \text{ Hz}, 2H), 7.53 (s, 4H), 7.56\text{-}7.61 (m, 6H), 7.68 (d, J = 7.7 \text{ Hz}, 2H), 7.95 (d, J = 7.7 \text{ Hz}, 2H), 10.78 (s, 2H).\]

\[ ^{13}\text{C NMR: } \delta = 88.9, 89.9, 93.6, 95.0, 110.0, 123.0, 124.7, 125.8, 126.8, 127.1, 128.3, 128.7, 128.8, 131.7, 132.1, 132.3, 133.3, 133.8, 135.9, 191.7.\]

\text{Anal. Calcd. } C_{40}H_{22}O_2 \cdot H_2O: C, 86.94; H, 4.38; \text{ Found: } C, 87.01; H, 3.96.

\text{1,4-Bis(2-bromophenylethynyl)benzene (20)\textsuperscript{19}}

A solution of 2-iodobromobenzene (3 mL, 23.4 mmol), 1,4-diethynylbenzene (0.999 g, 7.8 mmol), \( \text{Pd(PPh}_3\text{)}_4 \) (0.901 g, 0.78 mmol) and \( \text{CuI} \) (0.148 g, 0.78 mmol) in 6 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 12 hours in a closed vessel. The resulting mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH\textsubscript{2}Cl\textsubscript{2}) followed by recrystallization from CH\textsubscript{2}Cl\textsubscript{2} and hexanes afforded 20 (3.36 g, 7.73 mmol) in 99% yield as a yellow solid.

\[ ^1\text{H NMR: } \delta = 7.18 (ddd, J = 1.7, 7.6, 7.9 \text{ Hz}, 2H), 7.29 (ddd, J = 1.1, 7.6, 7.9 \text{ Hz}, 2H), 7.55 (dd, J = 1.7, 7.6 \text{ Hz}, 2H), 7.56 (s, 4H), 7.62 (dd, J = 1.1, 7.9, 2H).\]

\[ ^{13}\text{C NMR: } \delta = 89.9, 93.5, 123.1, 125.1, 125.6, 127.0, 129.6, 131.6, 132.5, 133.2.\]

\text{1,4-Bis(2-(trimethylsilylethynyl)phenylethynyl)benzene (21)\textsuperscript{20}}

A solution of 20 (3.40 g, 7.80 mmol), ethynyltrimethylsilane (3.5 mL, 24.7 mmol), \( \text{Pd(PPh}_3\text{)}_4 \) (0.900 g, 0.78 mmol) and \( \text{CuI} \) (0.148 g, 0.78 mmol) in 6 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 18 hours in a closed vessel.
The reaction mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH$_2$Cl$_2$: hexanes, 2:1, v/v) afforded 21 (4.41 g, 7.26 mmol) in 93% yield as a red solid.

$^1$H NMR: $\delta = 0.28$ (s, 18H), 7.22-7.31 (m, 4H), 7.49-7.51 (m, 4H), 7.54 (s, 4H). $^{13}$C NMR: $\delta = 0.09, 90.1, 93.1, 103.3, 123.2, 125.7, 125.8, 128.0, 128.2, 131.5, 131.6, 132.2.$

1,4-Bis(2-ethynylphenylethynyl)benzene (22)$^{20}$

A solution of 21 (3.24 g, 6.89 mmol) and tetrabutylammonium fluoride (1.0 M in THF, 10.34 mL, 35.80 mmol) in 8 mL of anhydrous THF was stirred at room temperature for 90 minutes. The reaction mixture was concentrated in vacuo and purification by flash chromatography on silica gel (hexanes:CH$_2$Cl$_2$, 2:1, v/v gradually changed to pure CH$_2$Cl$_2$) afforded compound 22 (1.91 g, 3.28 mmol) in 76% yield as a red solid.

$^1$H NMR: $\delta = 3.40$ (s, 2H), 7.23-7.33 (m, 4H), 7.53-7.56 (m, 8H). $^{13}$C NMR: $\delta = 81.4, 82.2, 89.9, 93.3, 123.2, 124.7, 126.0, 128.2, 128.6, 131.7, 131.8, 132.6.$

1,4-Bis(2(2-formyl-1-naphthylethynyl)phenylethynyl)benzene (2)

A solution of 22 (0.500 g, 1.38 mmol), 1-bromo-2-naphthaldehyde (1.29 g, 5.49 mmol), Pd(PPh$_3$)$_4$ (0.159 g, 0.14 mmol) and CuI (0.026 g, 0.14 mmol) in 6 mL of triethylamine:ACN:THF (3:2:1, v/v) was stirred at 80 ºC for 20 hours in a closed vessel. The mixture was cooled to room temperature, diluted with 20 mL of diethyl ether and then stored at -20 ºC for 6 hours. The precipitate was collected via vacuum filtration, dissolved in CH$_2$Cl$_2$ and washed with H$_2$O. The combined organic layers were dried over
MgSO₄ and concentrated in vacuo to afford 2 (0.465 g, 0.75 mmol) in 54% yield as a yellow solid. NMR analysis showed that 2 cocrystallizes with 1 equivalent of water.

1H NMR: δ = 7.32 (ddd, J = 1.1, 1.3, 8.2 Hz, 2H), 7.41-7.44 (m, 4H), 7.51 (s, 4H), 7.56 (ddd, J = 1.1, 1.1, 8.1 Hz, 2H), 7.65-7.68 (m, 2H), 7.72-7.75 (m, 2H), 7-84-7-89 (m, 4H), 7.99 (d, J = 8.6 Hz, 2H), 8.75 (d, J = 8.0 Hz, 2H), 11.00 (s, 2H). 13C NMR: δ = 86.9, 90.2, 93.7, 100.9, 122.1, 123.0, 124.7, 125.6, 127.5, 127.9, 128.4, 129.1, 129.2, 129.5, 131.9, 132.5, 132.6, 133.0, 134.3, 135.7, 192.1. Anal. Calcd. C₄₈H₂₈O₃: C, 88.32; H, 4.32; Found: C, 88.08; H, 4.21.

2-((2-Bromophenyl)ethynyl)benzaldehyde (23)

A solution of 2-iodobromobenzene (0.73 mL, 5.70 mmol), 2-ethynylbenzaldehyde (0.500 g, 3.84 mmol), Pd(PPh₃)₄ (0.443 g, 0.38 mmol) and CuI (0.073 g, 0.38 mmol) in 3.5 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 24 hours in a closed vessel. The resulting mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH₂Cl₂:hexanes, 2:1, v/v) afforded 23 (1.05 g, 3.70 mmol) in 97% yield as a light yellow solid.

1H NMR: δ = 7.21 (ddd, J = 1.7, 7.6, 7.9 Hz, 1H), 7.31 (ddd, J = 1.1, 7.6, 7.7 Hz, 1H), 7.45 (m, 1H), 7.55-7.62 (m, 3H), 7.67 (d, J = 7.8 Hz, 2H), 7.95 (d, J = 7.8 Hz, 1H), 10.74 (s, 1H). 13C NMR: δ = 89.3, 94.6, 124.5, 125.7, 126.4, 127.1, 127.2, 128.9, 130.1, 132.6, 133.3, 133.4, 133.7, 136.0, 191.8. Anal. Calcd. C₁₅H₉BrO: C, 63.18; H, 3.18; Found: C, 62.93; H, 3.08.
2-(2-(Trimethylsilylethynyl)phenylethynyl)benzaldehyde (24)

A solution of 23 (0.509 g, 1.79 mmol), ethynyltrimethylsilane (0.30 mL, 2.66 mmol), Pd(PPh₃)₄ (0.210 g, 0.18 mmol) and CuI (0.0.33 g, 0.17 mmol) in 4 mL of acetonitrile:triethylamine (1:1, v/v) was stirred at 80 °C for 4 hours in a closed vessel. The reaction mixture was cooled to room temperature and concentrated in vacuo. Purification by flash chromatography on silica gel (CH₂Cl₂: hexanes, 7:1, v/v) afforded 24 (0.449 g, 1.49 mmol) in 83% yield as a red oil. NMR analysis showed that 24 cocrystallizes with 0.5 equivalents of water.

1H NMR: δ = 0.26 (s, 9H), 7.31 (ddd, J = 1.8, 2.0, 2.5 Hz, 4H), 7.45 (m, 1H), 7.50-7.56 (m, 2H), 7.59 (dd, J = 1.3, 7.5 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 10.75 (s, 1H).

13C NMR: δ = 0.0, 88.7, 95.0, 99.4, 110.1, 124.9, 125.8, 127.0, 128.4, 128.7, 128.9, 132.0, 132.9, 133.4, 133.8, 135.9, 192.0. Anal. Calcd. C₄₀H₃₈O₃Si₂: C, 77.13; H, 6.15; Found: C, 77.35; H, 5.88.

2-(2-Ethynylphenylethynyl)benzaldehyde (25)

A solution of 24 (0.10 g, 0.33 mmol) and tetrabutylammonium fluoride (1.0 M in THF, 0.37 mL, 0.37 mmol) in 2 mL of anhydrous THF was stirred at room temperature for 5 minutes. The reaction mixture was concentrated in vacuo and purification by flash chromatography on silica gel (CH₃Cl) afforded compound 25 (0.04 g, 0.17 mmol) in 62% yield as a red solid. NMR analysis showed that 25 cocrystallizes with 1 equivalent of water.
1H NMR: δ = 3.42 (s, 1H), 7.29-7.36 (m, 2H), 7.43 (m, 1H), 7.52-7.58 (m, 3H), 7.65 (d, J = 7.7 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 10.78 (s, 1H). 13C NMR: δ = 81.7, 88.8, 94.6, 124.8, 125.4, 126.7, 126.9, 128.6, 128.7, 128.8, 131.9, 132.7, 133.3, 133.6, 136.0, 192.3. Anal. Calcd. C34H22O3: C, 85.34; H, 4.63; Found: C, 85.33; H, 4.35.

1,4-Bis(2-formylphenylethynyl)phenylethynyl)anthracene (3)
A solution of 25 (0.075 g, 0.33 mmol), 9,10-dibromoanthracene (0.027 g, 0.08 mmol), Pd(PPh3)4 (0.025 g, 0.02 mmol) and CuI (0.004 g, 0.02 mmol) in 3 mL of acetonitrile:triethylamine:tetrahydrofuran (1:1:1, v/v) was stirred at 80 °C for 20 hours in a closed vessel. The mixture was cooled to room temperature, dissolved in dichloromethane and washed with water. Purification by flash chromatography on silica gel (CH2Cl2:hexanes, 1:1, v/v) afforded 3 (0.030 g, 0.047 mmol) in 60% yield as a red solid. NMR analysis showed that 3 cocrystallizes with 2 equivalents of water.

1H NMR: δ = 7.35-7.38 (m, 4H), 7.46 (dd, J = 7.6 Hz, 2H), 7.53 (s, 4H), 7.56-7.61 (m, 6H), 7.68 (d, J = 7.7 Hz, 2H), 7.95 (d, J = 7.7 Hz, 2H), 10.78 (s, 2H). 13C NMR: δ = 88.9, 89.9, 93.6, 95.0, 110.0, 123.0, 124.7, 125.8, 126.8, 127.1, 128.3, 128.7, 128.8, 131.7, 132.1, 132.3, 133.3, 133.8, 135.9, 191.7. Anal. Calcd. C48H30O4: C, 85.95; H, 4.51; Found: C, 86.05; H, 4.79.

1,4-Di(2-formylphenyl)buta-1,3-diyn (4)
A solution of 2-ethylbenzaldehyde (0.199 mg, 1.53 mmol), N,N,N’,N’-tetramethyl ethylenediamine (0.09 mL, 0.72 mmol) and CuI (0.058 g, 0.31 mmol) in 3 mL of acetone
was stirred under air at room temperature for 1 hour. The resulting mixture was concentrated in vacuo, dissolved in dichloromethane and washed with water. Purification by flash chromatography on silica gel (CH$_2$Cl$_2$) afforded 4 (0.139 g, 0.54 mmol) in 35% yield as a light yellow solid.

$^1$H NMR: $\delta = 7.50$-$7.54$ (m, 2H), 7.60 (ddd, $J = 1.3$, 7.5, 7.6 Hz, 2H), 7.68 (dd, $J = 1.3$, 7.5 Hz, 2H), 7.93 (ddd, $J = 1.4$, 7.8 Hz, 2H), 10.50 (s, 2H). $^{13}$C NMR: $\delta = 79.2$, 79.7, 124.5, 127.9, 129.8, 133.8, 134.5, 137.5, 190.7.

1,4-Bis(2-formylphenylethynyl)benzene (5)

A solution of 2-bromobenzenaldehyde (0.37 mL, 3.17 mmol), 1,4-diethynylbenzene (0.102 g, 0.81 mmol), Pd(PPh$_3$)$_4$ (0.092 g, 0.08 mmol), CuI (0.015 g, 0.08 mmol) and triethylamine (0.22 mL, 1.58 mmol) in 5 mL of anhydrous acetonitrile was stirred at 80 °C for 18 hours in a closed vessel. The resulting mixture was cooled to room temperature, concentrated in vacuo then washed with water and brine. Purification by flash chromatography on silica gel (CH$_2$Cl$_2$:hexanes, 2:1, v/v gradually changed to pure CH$_2$Cl$_2$) afforded 5 (0.211 g, 0.63 mmol) in 80% yield as a yellow solid. NMR analysis showed that 5 cocrystallizes with 1 equivalent of water.

$^1$H NMR: $\delta = 7.43$-$7.47$ (m, 2H), 7.55 (s, 4H), 7.57 (ddd, $J = 1.3$, 7.3, 7.7 Hz, 2H), 7.63 (dd, $J = 1.3$, 7.7 Hz, 2H), 7.93 (ddd, $J = 1.3$, 7.4 Hz, 2H), 10.60 (s, 2H). $^{13}$C NMR: $\delta = 87.2$, 95.6, 122.9, 126.3, 127.5, 128.9, 131.7, 133.3, 133.7, 135.9, 191.2. Anal. Calcd. C$_{48}$H$_{36}$O$_5$: C, 83.95; H, 4.40; Found: C, 83.60; H, 4.09.
4.4.2. Diimine Formation with ICD Sensor 1

To monitor the conversion of sensor 1 to its diimine derivative, the reaction between 1 and a stoichiometric amount of amine 9 was analyzed by $^1$H-NMR spectroscopy in CDCl$_3$. The disappearance of the formyl signals at 10.8 ppm and the appearance of the imine signal at 9.0 ppm showed quantitative conversion after 90 minutes. The condensation is also evident from ESI-MS analysis. All other diimine formations were monitored by ESI-MS as shown below.

$^1$H-NMR spectrum of the diimine macrocycle obtained from sensor 1 and (R)-9.
MS spectrum of the diimine macrocycle obtained from sensor 1 and (R)-9.

ESI-MS: m/z 573.3 (M+1)^+

MS spectrum of the diimine macrocycle obtained from sensor 1 and (1R,2R)-8.

ESI-MS: m/z 711.2 (M+1)^+
MS spectrum of the diimine macrocycle obtained from sensor 1 and (1R,2R)-6.

ESI-MS: m/z 613.3 (M+1)^+

MS spectrum of the diimine obtained from sensor 1 and (R)-10.

ESI-MS: m/z 741.3 (M+1)^+
MS spectrum of the diimine obtained from sensor 1 and (R)-11.

ESI-MS: m/z 841.3 (M+1)^+

MS spectrum of the diimine obtained from sensor 1 and (R)-12.

ESI-MS: m/z 765.3 (M+1)^+
MS spectrum of the diimine obtained from sensor 1 and (R)-13.

![Graph showing MS spectrum of (R)-13 with m/z 921.4 (M+1)⁺]

ESI-MS: m/z 921.4 (M+1)⁺

MS spectrum of the diimine obtained from sensor 1 and (R)-14.

![Graph showing MS spectrum of (R)-14 with m/z 729.4 (M+1)⁺]

ESI-MS: m/z 729.4 (M+1)⁺
MS spectrum of the diimine obtained from sensor 1 and (R)-15.

ESI-MS: m/z 753.4 (M+1)^+

MS spectrum of the diimine obtained from sensor 1 and (R)-16.

ESI-MS: m/z 805.4 (M+1)^+
4.4.3. Diimine Formation with ICD Sensor 2

To monitor the conversion of sensor 2 to its diimine derivative, the reaction between 2 and a stoichiometric amount of amine 6 and 7 was analyzed by $^1$H-NMR spectroscopy in CDCl$_3$. The disappearance of the formyl signals at 10.8 ppm and the appearance of the imine signal at 9.0 ppm showed quantitative conversion. The condensation is also evident from ESI-MS analysis. All other diimine formations were monitored by ESI-MS as shown below.

$^1$H-NMR spectrum of the diimine macrocycle obtained from sensor 2 and (R)-7
$^1$H-NMR spectrum of the diimine macrocycle obtained from sensor 2 and (S)-7

MS spectrum of the diimine macrocycle obtained from sensor 2 and (1S,2S)-6

ESI-MS: m/z 713.2 (M+1)$^+$
MS spectrum of the diimine macrocycle obtained from sensor 2 and (S)-7

ESI-MS: m/z 883.4 (M+1); (macrocycle + H₂O) m/z 901.2 (M+1)

MS spectrum of the diimine macrocycle obtained from sensor 2 and (1R,2R)-8

ESI-MS: m/z 811.2 (M+1)
MS spectrum of the diimine macrocycle obtained from sensor 2 and (S)-9

ESI-MS: m/z 673.6 (M+1)

MS spectrum of the diimine obtained from sensor 2 and (R)-10

ESI-MS: m/z 841.2 (M+1)
MS spectrum of the diimine obtained from sensor 2 and (S)-11

ESI-MS: m/z 941.2 (M+1)

MS spectrum of the diimine obtained from sensor 2 and (S)-12

ESI-MS: m/z 865.1 (M+1)
MS spectrum of the diimine obtained from sensor 2 and (S)-13

ESI-MS: m/z 1021.3 (M+1)^+

MS spectrum of the diimine obtained from sensor 2 and (S)-14

ESI-MS: m/z 829.3 (M+1)^+
MS spectrum of the diimine obtained from sensor 2 and (S)-15

![MS spectrum of the diimine obtained from sensor 2 and (S)-15](image)

ESI-MS: m/z 853.4 (M+1)+

MS spectrum of the diimine obtained from sensor 2 and (S)-16

![MS spectrum of the diimine obtained from sensor 2 and (S)-16](image)

ESI-MS: m/z 905.3 (M+1)+
4.4.4. Diimine Formation with ICD Sensor 3

To monitor the conversion of sensor 3 to its diimine derivative, the reaction between 3 and a stoichiometric amount of amine 6 was analyzed by $^1$H-NMR spectroscopy in CDCl$_3$. The disappearance of the formyl signals at 10.8 ppm and the appearance of the imine signal at 9.0 ppm showed quantitative conversion. The condensation is also evident from ESI-MS analysis. All other diimine formations were monitored by ESI-MS as shown below.

$^1$H-NMR spectrum of the diimine macrocycle obtained from sensor 3 and (1S,2S)-6
MS spectrum of the diimine macrocycle obtained from sensor 3 and (1S,2S)-6.

![MS spectrum](image)

**ESI-MS:** m/z 713.3 (M+1)^+ 

MS spectrum of the diimine macrocycle obtained from sensor 3 and (S)-7.

![MS spectrum](image)

**ESI-MS:** m/z 883.3 (M+1); (macrocycle + H₂O) m/z 901.3
MS spectrum of the diimine macrocycle obtained from sensor 3 and (1R,2R)-8

ESI-MS: m/z 811.2 (M+1)^+

MS spectrum of the diimine macrocycle obtained from sensor 3 and (S)-9.

ESI-MS: m/z 673.3 (M+1)
MS spectrum of the diimine obtained from sensor 3 and (R)-10.

ESI-MS: m/z 841.2 (M+1)

MS spectrum of the diimine obtained from sensor 3 and (S)-11.

ESI-MS: m/z 941.2 (M+1)
MS spectrum of the diimine obtained from sensor 3 and \((S)-12\).

![MS spectrum of \((S)-12\)](image)

ESI-MS: \(m/z\ 941.2\ (M+1)\)

MS spectrum of the diimine obtained from sensor 3 and \((R)-13\).

![MS spectrum of \((R)-13\)](image)

ESI-MS: \(m/z\ 1021.4\ (M+1)\)
MS spectrum of the diimine obtained from sensor 3 and (1S,2S,3S,5R)-16.

ESI-MS: m/z 905.4 (M+1)

4.4.5. Diimine and Tetraimine Formation with ICD Sensor 4

The conversion of sensor 4 to its diimine derivative was evident by ESI-MS. The condensation reactions between 4 and a stoichiometric amount of amines 6, 8 and 15 were monitored by ESI-MS as shown below.
MS spectrum of the diimine macrocycle obtained from sensor 4 and (1S,2S)-6.

ESI-MS: m/z 709.3 (M+1)$^+$

MS spectrum of the diimine obtained from sensor 4 and (1R,2R)-8.

ESI-MS: m/z 647.3 (M+1)$^+$
MS spectrum of the diimine obtained from sensor 4 and (S)-15.

ESI-MS: m/z 495.3 (M+1)^+

4.4.6. Diimine and Tetraimine Formation with ICD Sensor 5

To monitor the conversion of sensor 5 to its diimine derivative, the reaction between 5 and a stoichiometric amount of amine 6 and 7 were analyzed by ^1H-NMR spectroscopy in CDCl₃. The disappearance of the formyl signals at 10.8 ppm and the appearance of the imine signal at 9.0 ppm showed quantitative conversion. The condensation is also evident from ESI-MS analysis. All other diimine formations were monitored by ESI-MS as shown below.
$^1$H-NMR spectrum of the tetraimine macrocycle obtained from sensor 5 and (1S,2S)-6

$^1$H-NMR spectrum of the tetraimine macrocycle obtained from sensor 5 and (S)-7
MS spectrum of the tetraimine macrocycle obtained from sensor 5 and (1S,2S)-6

ESI-MS: m/z 825.3 (M+1)^+

MS spectrum of the tetraimine macrocycle obtained from sensor 5 and (S)-7

ESI-MS: m/z 1165.5 (M+1)^+
MS spectrum of the tetraimine macrocycle obtained from sensor 5 and (1R,2R)-8

![MS Spectrum of Tetraimine Macrocycle](image)

ESI-MS: m/z 1021.1 (M+1)

MS spectrum of the diimine obtained from sensor 5 and (S)-10

![MS Spectrum of Diimine](image)

ESI-MS: m/z 541.2 (M+1)
MS spectrum of the diimine obtained from sensor 5 and (S)-11

ESI-MS: m/z 641.2 (M+1)^+

MS spectrum of the diimine obtained from sensor 5 and (S)-12

ESI-MS: m/z 565.2 (M+1)^+
MS spectrum of the diimine obtained from sensor 5 and (R)-14

Exact Mass: 528.35

ESI-MS: m/z 529.3 (M+1)^+

MS spectrum of the diimine obtained from sensor 5 and (R)-15

Exact Mass: 552.35

ESI-MS: m/z 553.3 (M+1)^+
MS spectrum of the diimine obtained from sensor 5 and \((1S,2S,3S,5R)-16\)

**ESI-MS:** \(m/z\) 605.4 (M+1)^+

MS spectrum of the diimine obtained from sensor 5 and \((R)-13\)

**ESI-MS:** \(m/z\) 721.3 (M+1)^+
4.4.7. UV/Vis Spectra of ICD Sensor 1-5 and the Diimine Obtained with 6

UV-Vis spectra of 1 (blue) and the diimines obtained with (S,S)-6 (red) at $2.50 \times 10^{-5}$ M in CDCl$_3$.

UV-Vis spectra of 2 (blue) and the diimines obtained using 2 and (S,S)-6 (red) at $2.50 \times 10^{-5}$ M in CDCl$_3$. 
UV-Vis spectra of 3 (blue) and the diimines obtained using 3 and (S,S)-6 (red) at 2.50 x 10^{-5} M in CDCl_{3}.

UV-Vis spectra of 4 (blue) and the diimines obtained with (S,S)-6 (red) at 4.56 x 10^{-5} M in CDCl_{3}.
UV-Vis spectra of 5 (blue) and the tetraimines obtained using 5 and (S,S)-6 (red) at 3.75 x 10^{-5} M in CDCl₃.

4.4.8. Enantioselective Sensing Experiments using ICD Sensor 1

A stock solution of 1 (0.00375 M) in anhydrous CDCl₃ was prepared and 350 µL of this solution were placed in 4 mL vials. Then, solutions of the substrates (0.1316 M for diimines and 0.2626 M for monoamines) in CDCl₃ were prepared. For each diimine formation, 10 µL of a substrate stock solution were placed in a vial containing the sensor solution (350 µL) and molecular sieves (4Å, 8-12 mesh) were added. The reactions were stirred at room temperature for 90 minutes. Prior to each use, the CD instrument was purged with nitrogen for 20 minutes at room temperature. CD spectra were collected with a standard sensitivity of 100 mdeg, a data pitch of 0.5 nm, a band width of 1 nm, a scanning speed of 500 nm s⁻¹ and a response of 0.5 s using a quartz cuvette (1 cm path length). The data were baseline corrected and smoothed using a binomial equation. The
CD analysis was conducted with sample concentrations of $3.76 \times 10^{-5}$ M. Control experiments with 6-16 at concentrations between $1.87 \times 10^{-4}$ M and $1.32 \times 10^{-3}$ M showed that the free substrates are CD silent in the region of interest.

CD Spectra of the macrocycles obtained using 1 and (1$R$,2$R$)-8 (blue) or (1$S$,2$S$)-8 (red) at $3.76 \times 10^{-5}$ M in CHCl$_3$.

CD Spectra of the macrocycles obtained using 1 and (1$R$,2$R$)-6 (blue) or (1$S$,2$S$)-6 (red) at $3.76 \times 10^{-5}$ M in CHCl$_3$.
CD Spectra of the macrocycles obtained using 1 and (R)-9 (blue) or (S)-9 (red) at 3.76 x 10^{-5} M in CHCl₃.

CD Spectra of the diimines obtained using 1 and (R)-10 (blue) or (S)-10 (red) at 7.52 x 10^{-5} M in CHCl₃.
CD Spectra of the diimines obtained using 1 and (R)-11 (blue) or (S)-11 (red) at 7.52 x 10^{-5} M in CHCl₃.

CD Spectra of the diimines obtained using 1 and (R)-12 (blue) or (S)-12 (red) at 7.52 x 10^{-5} M in CHCl₃.
CD Spectra of the diimines obtained using 1 and (R)-13 (blue) or (S)-13 (red) at 7.52 x $10^{-5}$ M in CHCl₃.

CD Spectra of the diimines obtained using 1 and (R)-14 (blue) or (S)-14 (red) at 7.52 x $10^{-5}$ M in CHCl₃.
CD Spectra of the diimines obtained using 1 and (R)-15 (blue) or (S)-15 (red) at 7.52 x \(10^{-5}\) M in CHCl₃.

CD Spectra of the diimines obtained using 1 and (1R,2R,3R,5S)-16 (blue) or (1S,2S,3S,5R)-16 (red) at 7.52 x \(10^{-5}\) M in CHCl₃.
4.4.9. Enantioselective Sensing Experiments with ICD Sensor 2

The diimines were prepared as described above and the CD spectra were collected using the previous described parameters. Upon completion, the CD analysis was conducted with sample concentrations of $5.64 \times 10^{-5}$ M to $9.41 \times 10^{-5}$ M. Control experiments with 6-16 at concentrations between $9.35 \times 10^{-5}$ M and $1.32 \times 10^{-3}$ M showed that the free substrates are CD silent in the region of interest.

CD Spectra of the macrocycles obtained using 2 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at $9.76 \times 10^{-5}$ M in CHCl₃.
CD Spectra of the macrocycles obtained using 2 and (R)-7 (blue) or (S)-7 (red) at $5.65 \times 10^{-5}$ M in CHCl$_3$.

CD Spectra of the macrocycles obtained using 2 and (1R,2R)-8 (blue) or (1S,2S)-8 (red) at $9.76 \times 10^{-5}$ M in CHCl$_3$. 
CD Spectra of the macrocycles obtained using 2 and (R)-9 (blue) or (S)-9 (red) at 2.26 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 2 and (R)-10 (blue) or (S)-10 (red) at 1.13 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 2 and (R)-11 (blue) or (S)-11 (red) at 1.13 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 2 and (R)-12 (blue) or (S)-12 (red) at 1.13 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 2 and (R)-13 (blue) or (S)-13 (red) at 1.13 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 2 and (R)-14 (blue) or (S)-14 (red) at 1.13 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 2 and (R)-15 (blue) or (S)-15 (red) at 1.13 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 2 and (1R,2R,3R,5S)-16 (blue) or (1S,2S,3S,5R)-16 (red) at 3.39 x 10^{-4} M in CHCl₃.
4.4.10 Enantioselective Sensing Experiments with ICD Sensor 3

The diimines were prepared as described above and the CD spectra were collected using the previous described parameters. Upon completion, the CD analysis was conducted with sample concentrations of $9.41 \times 10^{-5}$ M to $3.39 \times 10^{-4}$ M. Control experiments with 6-16 at concentrations between $1.87 \times 10^{-4}$ M and $1.32 \times 10^{-3}$ M showed that the free substrates are CD silent in the region of interest.

CD Spectra of the macrocycles obtained using 3 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at $5.64 \times 10^{-5}$ M in CHCl$_3$. 

![CD Spectra](image-url)
CD Spectra of the macrocycles obtained using 3 and (R)-7 (blue) or (S)-7 (red) at 9.41 x 10^{-5} M in CHCl₃.

CD Spectra of the macrocycles obtained using 3 and (1R,2R)-8 (blue) or (1S,2S)-8 (red) at 9.41 x 10^{-5} M in CHCl₃.
CD Spectra of the macrocycles obtained using 3 and (R)-9 (blue) or (S)-9 (red) at 9.40 x 10^{-5} M in CHCl₃.

CD Spectra of the macrocycles obtained using 3 and (R)-10 (blue) or (S)-10 (red) at 1.07 x 10^{-4} M in CHCl₃.
CD Spectra of the macrocycles obtained using 3 and (R)-11 (blue) or (S)-11 (red) at 1.13 x 10^{-4} M in CHCl3.

CD Spectra of the macrocycles obtained using 3 and (R)-12 (blue) or (S)-12 (red) at 1.13 x 10^{-4} M in CHCl3.
CD Spectra of the macrocycles obtained using 3 and (R)-13 (blue) or (S)-13 (red) at 3.39 $\times$ 10$^{-4}$ M in CHCl$_3$.

CD Spectra of the macrocycles obtained using 3 and (1R,2R,3R,5S)-16 (blue) or (1S,2S,3S,5R)-16 (red) at 1.07 $\times$ 10$^{-4}$ M in CHCl$_3$. 

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4.4.11. Enantioselective Sensing Experiments with ICD Sensor 4

The diimines were prepared as described above and the CD spectra were collected using the previous described parameters. Upon completion, the CD analysis was conducted with sample concentrations of $2.82 \times 10^{-4}$ M. Control experiments with 6, 8 and 15 at concentrations between $6.59 \times 10^{-4}$ M and $1.32 \times 10^{-3}$ M showed that the free substrates are CD silent in the region of interest.

CD Spectra of the macrocycles obtained using 4 and (1\(R,2R\))-6 (blue) or (1\(S,2S\))-6 (red) at $2.82 \times 10^{-4}$ M in CHCl\(_3\).
CD Spectra of the macrocycles obtained using 4 and (1\textit{R},2\textit{R})\textbf{-8} (blue) or (1\textit{S},2\textit{S})\textbf{-8} (red) at 2.82 x 10^{-4} M in CHCl₃.

![CD Spectra of macrocycles](image)

CD Spectra of the diimines obtained using 4 and (\textit{R})\textbf{-15} (blue) or (\textit{S})\textbf{-15} (red) at 2.82 x 10^{-4} M in CHCl₃.

![CD Spectra of diimines](image)
4.4.12. Enantioselective Sensing Experiments with ICD Sensor 5

The diimines were prepared as described above and the CD spectra were collected using the previous described parameters. Upon completion, the CD analysis was conducted with sample concentrations of $6.59 \times 10^{-5}$ M to $2.63 \times 10^{-4}$ M. Control experiments with 6-16 at concentrations between $9.35 \times 10^{-4}$ M and $1.32 \times 10^{-3}$ M showed that the free substrates are CD silent in the region of interest.

CD Spectra of the macrocycles obtained using 5 and (1R,2R)-6 (blue) or (1S,2S)-6 (red) at $1.32 \times 10^{-4}$ M in CHCl₃.
CD Spectra of the macrocycles obtained using 5 and (R)-7 (blue) or (S)-7 (red) at 6.59 x 10^{-5} M in CHCl₃.

![Image](image1.png)

CD Spectra of the macrocycles obtained using 5 and (1R,2R)-8 (blue) or (1S,2S)-8 at 2.63 x 10^{-4} M in CHCl₃.

![Image](image2.png)
CD Spectra of the diimines obtained using 5 and (R)-10 (blue) or (S)-10 (red) at 1.50 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 5 and (R)-11 (blue) or (S)-11 (red) at 1.51 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 5 and (R)-12 (blue) or (S)-12 (red) at 1.51 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 5 and (R)-13 (blue) or (S)-13 (red) at 1.50 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 5 and (R)-14 (blue) or (S)-14 (red) at 1.50 x 10^{-4} M in CHCl₃.

CD Spectra of the diimines obtained using 5 and (R)-15 (blue) or (S)-15 (red) at 1.50 x 10^{-4} M in CHCl₃.
CD Spectra of the diimines obtained using 5 and (1R,2R,3R,5S)-16 (blue) or (1S,2S,3S,5R)-16 (red) at 1.50 x 10^{-4} M in CHCl₃.

4.4.13 Calibration Curve and ee Determination Using the 1-Derived Diimine Obtained with Substrate 16

In order to evaluate the practical use of sensor 1 for ee determination, a calibration curve was constructed using samples of 16 in varying ee. A stock solution of 1 (0.00375 M) in anhydrous CDCl₃ was prepared and 350 µL of this solution were placed in 4 mL vials. Stock solutions of 16 (0.1316 M) with varying ee composition (+100.0, +80.0, +60.0, +40.0, +20.0, 0.0, -20.0, -40.0, -60.0, -80.0, -100.0) were prepared in anhydrous CDCl₃. For each diimine formation, 10 µL of a substrate stock solution were placed in a vial containing the 350 µL the sensor solution and molecular sieves (4Å, 8-12 mesh) were added. The reactions were stirred at room temperature for 90 minutes. Upon completion
of the condensation reaction, the solutions (0.00375 M) were diluted to 9.38 x 10^{-5} M for CD analysis. The data were baseline corrected and smoothed using a binomial equation. The CD amplitudes (mdeg) at 350 nm were plotted versus % ee. The calibration curve shows a linear relationship (mdeg = 0.2234(% ee) - 1.6766) with R^2 = 0.9906.

4.4.14 Enantiomeric Excess: Calibration curve and ee determination using the 2-derived diimine macrocycle obtained from substrate 8

The diimines were prepared as described above. Upon completion, the reaction solutions (0.00375 M) were diluted to 7.48 x 10^{-5} M for CD analysis. The data were baseline corrected and smoothed using a binomial equation. The CD amplitudes (mdeg) at 288
nm were plotted versus % ee. The calibration curve shows a linear relationship ($mdeg = -0.4998(\% ee) + 0.5056$) with $R^2 = 0.9939$.

4.4.15 Enantiomeric Excess: Calibration curve and ee determination using the 3-derived diimine obtained from substrate 13

The diimines were prepared as described above. Upon completion, the reaction solutions (0.00315 M) were diluted to $1.58 \times 10^{-4}$ M for CD analysis. The data were baseline corrected and smoothed using a binomial equation. The CD amplitudes (mdeg) at 344 nm were plotted versus % ee. The calibration curve shows a linear relationship ($mdeg = 0.3826(\% ee) - 0.319$) with $R^2 = 0.9949$. 
4.4.16 Enantiomeric Excess: Calibration curve and ee determination using the 5-derived diimine obtained from substrate 12

The diimines were prepared as described above. Upon completion, the reaction solution (0.00375 M) was diluted to $7.48 \times 10^{-4}$ M for CD analysis. The data were baseline corrected and smoothed using a binomial equation. The CD amplitudes (mdeg) at 300 nm were plotted versus % ee. The calibration curve shows a linear relationship (mdeg = $0.3649(\% \text{ ee}) - 4.605$) with $R^2 = 0.9711$. 

![Calibration curve graph]

\[ y = 0.3826x - 0.319 \]
\[ R^2 = 0.9949 \]
4.4.17 Crystallographic Data of ICD Sensor 5

Slow evaporation of a solution of sensor 5 (50 mg, 0.150 mmol) in dichloromethane (2.0 mL) gave single crystals suitable for X-ray diffraction.
Crystal data: C$_{24}$H$_{14}$O$_2$, $M = 334.35$, $0.34 \times 0.21 \times 0.10$ mm$^3$, monoclinic, space group $P2_1/c$, $a = 8.9286(5)$, $b = 8.2593(4)$, $c = 11.8381(6)$ Å, $\beta = 107.0110(10)^\circ$, $V = 834.79(7)$ Å$^3$, $Z = 2$, $D_c = 1.330$ g/cm$^3$, $F_{000} = 348$, MoK$\alpha$ radiation, $\lambda = 0.71073$ Å, $T = 100(2)$K, $2\theta_{\text{max}} = 56.0^\circ$, 7294 reflections collected, 1989 unique ($R_{\text{int}} = 0.0175$). Final $\text{Goof} = 1.045$, $R_I = 0.0363$, $wR^2 = 0.1038$, $R$ indices based on 1786 reflections with $I > 2\sigma(I)$ (refinement on $F^2$), 118 parameters, 0 restraints. $Lp$ and absorption corrections applied, $\mu = 0.084$ mm$^{-1}$.

4.5 References


5.1 Introduction

Malaria remains one of the world’s most widespread infectious diseases and is currently ranked as the 5th leading cause of death following respiratory infections, HIV/AIDS, diarrheal diseases and tuberculosis. The World Health Organization (WHO) estimated that malaria was responsible for 200-300 million clinical cases and over 1 million deaths in 2008. Malaria is caused by a protozoan parasite of the genus Plasmodium, of which five species can infect humans with Plasmodium falciparum (P. falciparum) being the most lethal. One of the first synthetic antimalarial drugs was the dye methylene blue, but due to its low activity and cell staining properties it was never seriously considered for therapeutic use. More importantly, methylene blue, as well as quinine, served as a structural platform eventually leading to the development of other aminoquinoline and aminoacridine antimalarials. The transition from methylene blue to more commonly known drugs began with the modification of the dimethylamino group to a more basic side chain (Figure 5.1). However, the potential for skin discoloration and other side effects remained. The incorporation of a tertiary amino group at the terminus


175
of the side chain and the screening of alternative aromatic ring systems propelled antimalarial drug design into a completely new direction that is still pursued to date. In particular, the modifications of quinoline or acridine derived drugs such as pamaquine and quinacrine has paved the way to some of the most successful antimalarial drugs (Figure 5.1).²,³

![Figure 5.1. Antimalarial drug development](image)

The toxic side effects observed with both pamaquine and quinacrine provided a driving force for the development of structural analogues exhibiting a superior therapeutic index. Pamaquine promotes the development of severe hemolytic reactions in patients.⁴ Quinacrine has several side effects including impotence, skin discoloration, eye discoloration and gastrointestinal problems.⁵ Around 1932, a drug that was remarkably more efficient than quinacrine was developed simultaneously (but independently) in Germany and Russia. This drug, later known as chloroquine (CQ), affords therapeutic
responses in a very short dosing course. For example, administration of 1.5 g over three days proved highly effective against acute attacks of *P. vivax* and the use of 0.3 g per week to maintain a plasma drug level of 14-42 µg/L was found sufficient for complete suppression of *P. vivax*\(^5\).\(^6\) Eventually, CQ was marketed as the drug of choice for antimalarial therapy and for prophylactic use. The high antimalarial potency of compounds containing a 4-substituted quinoline ring (CQ, quinine and other cinchona alkaloids) initiated the development of many other agents containing this pharmacophore. In particular, mefloquine, sontoquine, and amodiaquine have proved to be among the most effective antimalarial drugs developed to date (Figure 5.2).\(^7\)-\(^9\)

![Figure 5.2. Structures of 4-substituted quinoline derivatives used as antimalarials](image)

Aminoquinolines are known to form a complex with ferriprotoporphyrin IX (FPIX). FPIX is generated during proteolysis of host hemoglobin (Hb) which serves as a major source of amino acids during the protozoan life stages within the infected red blood cell. Free FPIX is toxic to *Plasmodium* which therefore has developed a strategy for detoxification, converting it into insoluble crystalline hemozoin.\(^10\) The drug-FPIX interactions inhibit conversion of hematin to hemozoin in the digestive food vacuole and
hence its detoxification via crystallization. The accumulation of significant concentrations of toxic FPIX-aminoquinoline adducts is believed to be ultimately responsible for killing of the parasite.\textsuperscript{11-15} It is widely accepted that the 4-aminoquinoline pharmacophore plays a crucial role in the complexation to FPIX resulting in inhibition of hemozoin formation and parasite growth.\textsuperscript{16} The presence of a basic amino group in the side chain is generally considered essential for trapping high concentrations of the drug in the acidic food vacuole of the parasite.\textsuperscript{17}

Worldwide malaria eradication was thought to be feasible based on the overwhelming success of chloroquine and this led to a decline in antimalarial drug research throughout the 1950s. However, first reports of CQ drug resistance surfaced in South America and Asia in the early 1960s.\textsuperscript{18,19} The occurrence of chloroquine resistance (CQR) was most likely the result of well-intentioned prophylactic attempts to eradicate the disease with the widespread introduction of CQ-containing insecticides, CQ-containing table salt, etc. The use of sub-lethal CQ concentrations facilitated the evolutionary selection of CQ resistant parasites.\textsuperscript{18,19} The mechanism of CQR is not fully understood but it is known that resistant strains accumulate reduced amounts of CQ in the digestive food vacuole (DV) relative to their CQ sensitive (CQS) counterparts. This has been attributed to mutations in the DV membrane protein Pfert (\textit{P. falciparum} chloroquine resistance transporter) which is suggested to participate in the active efflux of CQ (Figure 5.3).\textsuperscript{20-22}
Figure 5.3. Acidic parasitic DV containing FPIX and the Pfcrt membrane transport protein

Since the trapping of high concentrations of heme-targeted antimalarial drugs in the DV is essential, many efforts have been directed to overcome the resistance mechanism and the drug recognition by Pfcrt via modification of the CQ side chain. To address the ever-increasing threat of CQR, promising chloroquine resistant reversal agents\textsuperscript{23,24} and new therapeutics\textsuperscript{25} such as artemisinin and other endoperoxides have been introduced\textsuperscript{26-31}. A remaining drawback of endoperoxides is that they are generally less affordable in third world countries and resistance to some organoperoxides has already emerged\textsuperscript{11,32}. A second approach to combating resistance is to modify previously established antimalarial drugs that are effective against chloroquine sensitive (CQS) strains but have become ineffective towards CQR strains. 4-Substituted aminoquinolines, such as quinine, chloroquine and mefloquine, are among the most successful antimalarial drugs ever used, and additional lead compounds with improved CQR activity have been discovered via synthetic modifications of these prototypes\textsuperscript{33-35}. In general, 4-aminoquinolines carrying an aliphatic side chain are well tolerated and afford excellent
activity-toxicity profiles. The critical need for safe, effective and inexpensive antimalarials that are equally active against multiple strains of *P. falciparum* and *P. vivax* has provided a compelling driving force for the design of new CQ analogues.

Since modification of the 7-chloroquinoline ring, i.e. incorporation of other electron-withdrawing or electron-donating substituents into the various positions in the quinoline ring, have generally proved detrimental to the antimalarial activity, a systematic variation of the side chain structure and basicity seems to be more promising. Although few comprehensive and systematic modifications of the CQ side chain have been reported to date, it has been established that both shortening and lengthening of the separation of the two aliphatic amino groups to either 2-3 or 10-12 carbon atoms, as well as the incorporation of a phenol moiety, can lead to increased activity against CQR strains. Several studies revealed that introduction of a branched dialkylamino motif at the side chain terminus of CQ, e.g. replacement of the ethyl by isopropyl or tert-butyl groups, can furnish metabolically more stable antimalarials with enhanced life-time and retained activity against drug resistant strains of *P. falciparum*.

We envisioned that incorporation of an increasing number of basic amino groups, along with systematic structural variations (length and branching) of the aliphatic side chain attached to the potent 4-amino-7-chloroquinoline pharmacophore, would provide new candidates that overcome antimalarial drug resistance. The structural modifications planned were to systematically change the α-segment and the β-segment of an α,ω-triamine (Figure 5.4). Upon optimization of the length of the α-portion, the β-portion would undergo the same modifications to furnish a fully optimized side chain
establishing two basic amino groups. Because it is assumed that the pH of the DV differs
between CQS and CQR strains of malaria,\textsuperscript{45} fine-tuning of both the basicity of the
quinolyl nitrogen and of the side chain seemed promising (Figure 5.5).

\[
\begin{align*}
\text{α-segment} & \quad \beta\text{-segment} \\
\text{Cl} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\left(\text{NET}_2\right) & \quad \left(\text{NET}_2\right)
\end{align*}
\]

\textbf{Figure 5.4.} Side chain modification of 4-amino-7-chloroquinoline

In addition, attachment of other side chain motifs to the aminoquinoline ring was
expected to provide new entries to antimalarials effective against CQR strains. Sulphonamides including the protease inhibitor and antiretroviral agent fosamprenavir, the
nonsteroidal anti-inflammatory drug celecoxib, and sumatriptan, which has been used to
treat migraine headaches, have found widespread use as pharmaceuticals.\textsuperscript{46-49} Among the
few examples of antimalarial sulphonamides reported to date, some exhibit remarkable
potency.\textsuperscript{46-49} Additionally, ureas and thioureas have been used as potent blood glucose
reducing agents for the treatment of diabetes and other diseases.\textsuperscript{50} These compounds
could potentially serve as antimalarials because there is evidence supporting that some
antimalarials may affect glucose metabolism.\textsuperscript{50} It was therefore decided to synthesize
CQ analogues having a rigid sulphonamide, urea or thiourea moiety in the side chain. By
systematically modifying the CQ structure at specific sites we expected to discover new
structure-activity relationships (SARs), find new drug candidates with high potency against CQR strains, and better understand the mechanism of drug resistance.

**Figure 5.5.** Modification sites of the CQ pharmacophore

### 5.2 Results and Discussion

#### 5.2.1 Synthesis of 4-amino-7-chloroquinolines with linear dibasic side chains: α-portion modifications

Previous structural modifications of CQ have shown that variations in the side chain are less detrimental to the compound’s antimalarial activity than structural changes in the ring system. In a study reported by Krogstad et al., 4-amino-7-chloroquinolines having N,N-diethylaminoalkyl side chains with spacers consisting of two to twelve methylene units were found to be as effective as chloroquine against sensitive strains. Importantly, the homologues with either short or very long linkers between the two amino functions showed remarkable activity against CQR strains. The underlying idea was to extend this study to CQ analogues with an additional amino function incorporated in the linear side chain. The first series of new 4-amino-7-chloroquinolines carrying a triamino side chain is shown in Figure 5.6. In comparison to previously reported 4-
amino-7-chloroquinolines,\textsuperscript{39,43} compounds 4a-e an 5a-e afford an additional titratable amino group in the linear side chain while the length of the $\alpha$-segment is systematically varied.

![Chemical structures of 4- and 5-amino-7-chloroquinolines with $\alpha$-segment variation](image)

\textbf{Figure 5.6.} 4-Amino-7-chloroquinoline series with $\alpha$-segment variation
Retrosynthetic analysis of the desired drug candidates suggested that they could be prepared via reductive amination of the corresponding secondary amine with sodium borohydride and acetic acid (Figure 5.7). The secondary amines could be formed from the amide precursors 3a-e which should be available from the corresponding primary amine and N,N-diethylamino-3-propionic acid followed by reduction. The N-(7-chloro-4-quinolyl)diaminoalkanes would be obtained via nucleophilic aromatic substitution using 4,7-dichloroquinoline and the corresponding diamine.

![Diagram of retrosynthetic analysis](image)

**Figure 5.7.** Retrosynthetic analysis of drug candidates

The synthesis of the heme-targeted antimalarials involved inexpensive materials and high-yielding steps in most cases. For example, amination of 4,7-dichloroquinoline, 1, with commercially available 1,3-diaminopropane and 1,5-diaminopentane gave N-(7-chloro-4-quinolyl)-1,3-diaminopropane, 2b, and N-(7-chloro-4-quinolyl)-1,5-diaminopentane, 2d, in 88% and 87% yield, respectively (Scheme 5.1). Coupling of 2b and 2d with N,N-diethylamino-3-propionic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) furnished amides 3b and 3d in
54% and 62% yields. These amides were reduced with borane-dimethyl sulfide to the corresponding secondary amines $4b$ and $4d$ in 82% and 68% yield, respectively. Finally, tertiary amines $5b$ and $5d$ were prepared by treatment of precursors $4b$ and $4d$ with sodium borohydride in glacial acetic acid in yields of 81% and 31%. The other compounds shown in Figure 5.6 were prepared by Dr. Kimberly Yearick, Dr. Kekeli Ekoue-Kovi and Dr. Jayakumar K. Natarajan.

Scheme 5.1. Synthesis of selected 4-amino-7-chloroquinolines

5.2.2 Antiplasmodial activity of 4-amino-7-chloroquinolines with a linear dibasic side chain: α-portion modifications

The antimalarial activity of these new chloroquine analogues versus two CQS (HB3 and GCO3) and two CQR (Dd2 and FCB) strains were determined by Dr. Paul Roepe’s research group at Georgetown University using a standardized, inexpensive
assay based on SYBR Green I intercalation.\textsuperscript{53-55} The IC\textsubscript{50} values were calculated from experiments carried out in triplicate and compared to CQ. The resistance index (RI) is the ratio of the IC\textsubscript{50} for a resistant strain versus a sensitive strain (Dd2/HB3; FCB/GCO3). A high RI factor indicates that the drug is less effective against the CQR strain and low values point towards drugs overcoming CQR.

The RI of CQ was determined as approximately 10, whereas the compounds tested have RI values between 1.1 and 4.4 (Table 5.1). Compounds 4a-e and 5a-e show activity versus CQS strains similar to that of CQ and, more importantly, many retain their potency against CQR strains. Comparison of the antimalarial activity of 4a and 4b versus 5a and 5b (Table 5.1 entries 2, 3, 7 and 8) suggests that the presence of a central tertiary amino group in the series tested is crucial for the activity against Dd2, GC03, and FCB but not critically important for the CQS HB3 strain. Comparison with CQ reveals that 4b, 5a, 5b and 5d afford superior activity against the CQ resistant strains Dd2 and FCB. In particular, the tribasic 4-aminoquinolines, 5a and 5b, carrying a short linear side chain with two aliphatic tertiary amino functions are highly potent antimalarials and equally effective against both CQS and CQR strains. The impressive RI values of all compounds tested demonstrate that systematic variations of both the CQ side chain structure and basicity provide new venues to overcome antimalarial drug resistance. This could be combined with the introduction of a third basic amino function which should improve accumulation of the drug within the acidic food vacuole of the parasite. However, a further increase in titratable amino groups could limit the ability of the compounds to cross the DV membrane.
Table 5.1. Antiplasmodial activity of 4-aminoquinolines with a linear dibasic side chain: α-portion modifications

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Strain/IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HB3</td>
</tr>
<tr>
<td>1</td>
<td><img src="image" alt="CQ" /></td>
<td>13.5</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="4a" /></td>
<td>29.2</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="4b" /></td>
<td>27.3</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="4c" /></td>
<td>72.5</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="4d" /></td>
<td>46.0</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="4e" /></td>
<td>82.8</td>
</tr>
</tbody>
</table>
The Resistance Index (RI) is the ratio of the IC$_{50}$ for the resistant versus sensitive strain (Dd2/HB3 and GCO3/FCB).
5.2.3 Synthesis of 4-amino-7-chloroquinolines with a linear dibasic side chain: β-portion modifications

In the previous family of 4-amino-7-chloroquinolines the length of the α-segment of the linear side chain was varied. Promising antiplasmodial activities were obtained when the quinolyl nitrogen and the central amino group were kept within close proximity. Having optimized the α-segment, the next logical step to fully optimize the linear dibasic side chain was to prepare a series of compounds in which the β-segment is altered, while the α-segment remains constant (Figure 5.8).
Figure 5.8. 4-Amino-7-chloroquinoline series with β-segment variation
The synthesis of these 4-amino-7-chloroquinoline analogues began with the conversion of 4,7-dichloroquinoline, 1, with excess of 1,2-diaminoethane to almost quantitative amounts of \( N-(7\text{-chloro-4-quinolyl}) \) diaminoethane, 9, as described previously.\(^5\) Primary amine 9 then served as the common starting material for the synthesis of amides 6a-6e, secondary amines 7a-7e and tertiary amines 8a-8e (Scheme 5.2). For example, one-pot amidation of 9 with bromoacetyl chloride followed by peripheral substitution with excess of diethylamine gave 6a in 60% yield. Reduction with borane and subsequent reaction with sodium borohydride in glacial acetic acid then furnished the corresponding amines 7a and 8a in 78% and 37% yield, respectively. The aminoquinolines 6b, 7b, and 8b were available from previous studies.\(^5\) For comparison, we prepared branched aminoquinoline 11 using our established nucleophilic substitution and reductive alkylation protocols.

**Scheme 5.2.** Synthesis of CQ-derived analogues carrying a linear side chain and of a branched 4-aminoquinoline 11
5.2.4 Antiplasmodial activity of 4-amino-7-chloroquinolines with a linear dibasic side chain: β-portion modifications

Analysis of the antimalarial potency of 6a-8e showed that several of these novel aminoquinolines have similar activity against HB3 as CQ and many compounds are significantly more potent against the CQ resistant Dd2 strain (Table 5.2). In general, the antiplasmodial activity of the tertiary amines 8a-8e proved superior over that of their secondary analogues 7a-7e and amides 6a-6e. The same trend was observed with the series of 4-amino-7-quinolines having a varied α-segment (Table 5.1). Importantly, treatment of Dd2 with tertiary amines 8a-8e gave IC₅₀ values between 19.9 and 53.0 nM, which compares favorably with CQ (Table 5.2, entries 1 and 12-16). It is significant that compounds 8b-8e show high antimalarial activity against HB3 and still retain their potency against the Dd2 strain as evident by their low RI values ranging from 1.1 to 7.1. The stepwise variation of the β-segment of the CQ side chain reveals that small changes in the spacer length can have dramatic effects on the activity versus the two strains tested. For example, a shortening or lengthening of the β-segment in the side chain in compound 8b by only one methylene group increases the RI from 1.1 to 7.1 and 10.5, respectively (Table 5.2, entries 12-14). But excellent resistance indices are again obtained when the β-chain length is further increased (entries 15 and 16). To establish a comparison between a linear dibasic side chain and a highly branched side chain, the aminoquinoline 11 was tested. While compound 11 showed high potency against HB3 it showed significantly lower activity against Dd2 (Table 5.2, entry 17). This may suggest that for CQ analogues with multiple basic amino groups linearity is a better side chain motif than branching and
that a further increase in titratable amino groups may hinder the crossing of the DV membrane. Another possibility is that 11 is more readily recognized by the Pfcrt membrane protein and therefore removed from the DV at faster rate.

Table 5.2. Antiplasmodial activity of 4-aminoquinolines with a linear dibasic side chain: β-portion modifications

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Strain/IC\textsubscript{50} (nM)</th>
<th>HB3</th>
<th>Dd2</th>
<th>RI\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="CQ" /></td>
<td>8.4</td>
<td>122</td>
<td></td>
<td>14.6</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="6a" /></td>
<td>715</td>
<td>3362</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="6b" /></td>
<td>32</td>
<td>760</td>
<td></td>
<td>24.1</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="6c" /></td>
<td>225</td>
<td>1187</td>
<td></td>
<td>5.3</td>
</tr>
</tbody>
</table>
5.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]

6.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]

7.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]

8.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]

9.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]

10.  
\[
\begin{align*}
\text{HN} & \quad \text{H} \\
\text{N} & \quad \text{NEt}_2 \\
\text{O} & \quad \text{N}\text{Cl} \\
\text{N} & \quad \text{H}
\end{align*}
\]
11 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 7e \]
68.9 \hspace{1cm} 231 \hspace{1cm} 3.4

12 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 8a \]
5.0 \hspace{1cm} 53.0 \hspace{1cm} 10.5

13 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 8b \]
27.3 \hspace{1cm} 31.2 \hspace{1cm} 1.1

14 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 8c \]
5.1 \hspace{1cm} 36.2 \hspace{1cm} 7.1

15 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 8d \]
8.9 \hspace{1cm} 38.7 \hspace{1cm} 4.3

16 \[ \text{HN} \text{N} \text{Cl} \text{HN} \text{N} \text{NEt}_2 \] 
\[ 8e \]
5.0 \hspace{1cm} 19.9 \hspace{1cm} 4.0
5.2.5 Synthesis of 4-alkoxy-7-chloroquinolines with linear dibasic side chains

As discussed above, the basicity of the quinolyl nitrogen is another important parameter that has not been fully explored to date. A series of 4-alkoxy-7-chloroquinolines was therefore synthesized to study the significance of the basicity of the quinolyl nitrogen for the antiplasmodial activity (Figure 5.9). The replacement of the amino group at position 4 in the quinoline ring of these 7-chloroquinoline derivatives by an ether group reduces the pKa of the quinolyl nitrogen from 8.5 to 4.5. This change in the quinolyl basicity can influence the drug’s interaction with the Fe center in FPIX and therefore influence the parasite’s ability to convert free FPIX to hemozoin during the parasite’s detoxification process.
The synthesis of the 4-alkoxy-7-chloroquinolines is shown below (Scheme 5.3). We first prepared the amide derivatives in two steps from 4,7-dichloroquinoline and an amino alcohol in the presence of strong base followed by EDC-promoted coupling with 3-diethylaminopropionic acid. However, we found that 12a is not stable under typical amide reduction conditions and we therefore produced the corresponding amines via mesylation of alcohol 15 and subsequent substitution with either N,N-diethylldiaminopropane or N,N,N′-triethylldiaminopropane. Compounds 12b, 13b and 14b were prepared accordingly by Dr. Kekeli Ekoue-Kovi.
5.2.6 Antiplasmodial activity of 4-alkoxy-7-chloroquinolines with a linear dibasic side chain

Based on the relatively low basicity of the 4-alkoxyquinolines compared to the 4-aminoquinolines 6a-8e, ethers 12-14 can be expected to have a free, nonprotonated quinoline ring in the acidic DV which generally has a pH ranging from 5 to 6. Amides 12a and 12b are essentially monoprotic bases under physiological conditions and thus less prone to effective bioaccumulation in the acidic DV which explains their relatively low antiplasmodial activity (Table 5.3). The secondary and tertiary amines 13a, 13b, 14a, and 14b possess two basic groups in the side chain which favors migration into the DV. These compounds show enhanced antimalarial activity versus HB3 and Dd2.
Comparison with the screening results obtained for the 4-aminoquinolines 4b, 5c, 7b and 8b, reveals that ethers 13a, 14a, 13b, and 14b exhibit lower antimalarial potency but remarkable RI’s. In particular, tertiary amine 14b (RI 1.4) is a promising new antimalarial lead and slightly more effective against Dd2 than CQ.

Table 5.3. Antiplasmodial activity of 4-alkoxy-7-chloroquinolines

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Strain/IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>HB3</th>
<th>Dd2</th>
<th>RI&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="CQ" /></td>
<td><img src="image" alt="HB3 Dd2 RI" /></td>
<td>9.8</td>
<td>138</td>
<td><strong>14.1</strong></td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="12a" /></td>
<td><img src="image" alt="HB3 Dd2 RI" /></td>
<td>1706</td>
<td>1456</td>
<td><strong>0.9</strong></td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="12b" /></td>
<td><img src="image" alt="HB3 Dd2 RI" /></td>
<td>94.6</td>
<td>214</td>
<td><strong>2.3</strong></td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="13a" /></td>
<td><img src="image" alt="HB3 Dd2 RI" /></td>
<td>321</td>
<td>409</td>
<td><strong>1.3</strong></td>
</tr>
</tbody>
</table>
The Resistance Index (RI) is the ratio of the IC\textsubscript{50} for the resistant versus sensitive strain (Dd2/HB3).

\textit{5.2.7 Synthesis of 4-amino-7-chloroquinolines sulfonamide derivatives}

A series of CQ-derived sulfonamides containing titratable amines on the terminus of the CQ pharmacophore was prepared (Figure 5.10). A closer look reveals that the side chain termini contain residues with slightly basic groups having a pKa of 5-6. This structural feature was specifically chosen to capitalize on the pH gradient into the acidic DV and to further take advantage of the pH difference between the CQS DV (pH = 5.4) and the slightly more acidic CQR DV (pH = 5.2); therefore, trapping higher concentrations of the drug in the DV.
Following a previously reported procedure, \( N-(7\text{-chloro}-4\text{-quinolyl})\text{-1,3-diaminopropane} \) was prepared from 4,7-dichloroquinoline and 1,3-diaminopropane.\(^{56}\) The CQ-derived sulfonamides shown above were produced using commercially available arylsulfonyl chlorides. Due to competition with the nucleophilic quinolyl nitrogen, yields were often low. For example, 21 was synthesized in one step from \( N-(7\text{-chloro}-4\text{-quinolyl})\text{-1,3-diaminopropane} \) and the corresponding arylsulfonyl chloride in the presence of triethylamine (Scheme 5.4).\(^{58}\) The other compounds shown in Figure 5.10
were prepared by Dr. Kekeli Ekoue-Kovi, Dr. Kimberly Yearick and Dr. Jayakumar K. Natarajan.

![Scheme 5.4. Synthesis of sulfonamide CQ analogue 21](image)

**5.2.8 Antiplasmodial activity of 4-amino-7-chloroquinoline sulfonamides**

The CQ sulfonamide analogues can be further subdivided into two classes: (1) sulfonamide CQ derivatives possessing a central tertiary amino function and a peripheral 5-dimethylaminonaphthyl group (dansyl group) in the side chain (16a-16e and 17), and (2) CQ derivatives without a tertiary amino group (18-21). Treatment of Dd2 with sulfonamides 16-17 gave IC\(_{50}\) values comparable to CQ with 16a providing outstanding IC\(_{50}\)’s of 18 and 23 nM against HB3 and Dd2, respectively. Antimalarial testing with HB3 and Dd2 revealed that the RI of 16-17 ranged from 0.5 to 3.6. Most remarkable is the short chain sulfonamide 16a which has proven significantly more potent against the resistant strain Dd2 than CQ (Table 5.4, entries 1 and 2). An increase in the chain length (compounds 16a-16e) proved detrimental to the antimalarial activity (Table 5.4, entries 2-7). Comparison of compounds 16c and 17, which bears a methyl group at the \(\alpha\)-carbon in the side chain similar to CQ, shows that branching reduces the activity against both strains tested (Table 5.4, entries 4 and 5). The IC\(_{50}\)’s of sulfonamides 18-21 increased
into the micromolar range due to their lack of a basic tertiary amino function in the side chain which is believed to be crucial for the drug’s accumulation in the acid DV.

**Table 5.4.** Antiplasmodial activity of 4-amino-7-chloroquinolines sulfonamides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Strain/IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HB3</td>
</tr>
<tr>
<td>1</td>
<td><img src="image.png" alt="Compound 1" /> CQ</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td><img src="image.png" alt="Compound 2" /> 16a</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td><img src="image.png" alt="Compound 3" /> 16b</td>
<td>115</td>
</tr>
<tr>
<td>4</td>
<td><img src="image.png" alt="Compound 4" /> 16c</td>
<td>82</td>
</tr>
</tbody>
</table>
5.2.9 Synthesis of 4-amino-7-chloroquinoline-derived urea and thiourea compounds

A series of CQ analogues carrying a rigid urea or thiourea moiety at the terminus of the side chain was then investigated (Figure 5.11). Some of the compounds in this family contain a titratable amino functionality at the side chain periphery, and as discussed above, this amino function is expected to increase the accumulation of the drug in the acidic DV. However, ureas and thioureas may affect the malaria parasite through a different mechanism. Patients with severe falciparum malaria are susceptible to hypoglycemia and it is known that urea and thiourea residues are common in glucose controlling medicines. Therefore, CQ-derived urea and thiourea compounds may have a different target than heme and the presence of aliphatic amino groups could be less important.
Figure 5.11. 4-Amino-7-chloroquinoline-derived urea and thiourea analogues

The 4-amino-7-chloroquinolyl-derived ureas and thioureas 22-29 were prepared in good to high yields from \( N-(7\text{-chloro-4-quinolyl})-1,3\text{-diamine} \) and the corresponding isocyanate and isothiocyanate, respectively, as shown in Scheme 5.5.\(^{58}\)
5.2.10 Antiplasmodial activity of 4-amino-7-chloroquinolines urea and thiourea derivatives

Most of the compounds studied showed submicromolar antiplasmodial activity (Table 5.5). However, the urea/thiourea analogues have RI values ranging from 1.5 to 3.9 which compare favorably with that of CQ and with the majority of previously reported CQ-derived ureas and thioureas.\(^{50,60}\) In general, the thiourea derivatives are slightly more potent. For example, 22 has IC\(_{50}\) values of 316 nM and 596 nM against HB3 and Dd2, respectively and its counterpart 26 has corresponding IC\(_{50}\) values of 229 nM and 353 nM. In analogy to the sulfonamides discussed above, the low RI values of 22-29 suggest that
incorporation of a rigid urea or thiourea group into the side chain provides a new
direction that could overcome drug resistance to heme-targeted antimalarials.

**Table 5.5.** Antiplasmodial activity of 4-amino-7-chloroquinolines urea and thiourea
derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>HB3</th>
<th>Dd2</th>
<th>RI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Compound 1" /></td>
<td>10</td>
<td>127</td>
<td>12.7</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Compound 2" /></td>
<td>316</td>
<td>596</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Compound 3" /></td>
<td>281</td>
<td>&gt;1000</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Compound 4" /></td>
<td>436</td>
<td>674</td>
<td>1.6</td>
</tr>
</tbody>
</table>
a The Resistance Index (RI) is the ratio of the IC$_{50}$ for the resistant versus sensitive strain (Dd2/HB3).
5.3 Conclusions

In conclusion, 42 novel heme-targeted antimalarial drug candidates have been prepared by systematic variations of the side chain length, basicity, branching and heteroatom substitution at the 4-position in the quinoline backbone. From the antiplasmodial studies performed with the 7-chloroquinoline analogues discussed, several important SARs have been determined:

1. The incorporation of a second titratable amino group in the side chain is advantageous.
2. A linear dibasic side chain is more effective than branched analogues.
3. The optimal length for the α-segment of the side chain is 2-3 carbons.
4. The central amino group should be tertiary rather than secondary.
5. The optimal length for the β-segment of the side chain is 3 carbons.
6. A change in the heteroatom at position 4 in the quinoline ring from \(N\) to \(O\) generates less potent HTA drugs.
7. Incorporation of a sulfonamide, urea or thiourea residue results in reasonable RI values indicating that these compounds are potential leads that could overcome the problems with CQR strains.

In summary, these studies reveal that methodical variations of the side chain in chloroquine provides promising entries towards affordable heme-targeted antimalarials that overcome the ever-increasing problem of worldwide drug resistance. In the future,
the feasibility of the above needs to be tested by \textit{in vivo} studies to evaluate toxicity, bioavailability and metabolic stability of these leads.

\subsection*{5.4 Experimental Details}

\textbf{Representative procedure for the synthesis of \(N\)-(7-chloro-4-quinolyl)-1,\(n\)-diaminoalkanes.} A mixture of 4,7-dichloroquinoline (1.0 g, 5.1 mmol) and excess 1,3-diaminopropane was heated to 110 °C for 6 h under inert atmosphere and then cooled to room temperature. Aqueous NaOH (1N, 10 mL) was then added and the mixture was extracted with CH\(_2\)Cl\(_2\). The organic layers were washed with water, brine, dried over anhydrous Na\(_2\)SO\(_4\) and evaporated under reduced pressure. \(N\)-(7-Chloro-4-quinolyl)-1,3-diaminopropane (1.05 g, 4.5 mmol, 88\% yield) was obtained as pale yellow crystals and used without further purification.

\(N\)-(7-Chloro-4-quinolyl)-1,3-diaminopropane, 2b. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta = 1.48\) (bs, 2H), 1.84-1.96 (m, 2H), 3.00-3.10 (m, 2H), 3.38-3.48 (m, 2H), 6.33 (d, \(J = 5.4\) Hz, 1H), 7.30 (dd, \(J = 2.1\) Hz, 9.0 Hz, 1H), 7.72 (d, \(J = 9.0\) Hz, 1H), 7.92 (d, \(J = 2.1\) Hz, 1H), 8.50 (d, \(J = 5.4\) Hz, 1H); \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta = 29.5, 40.8, 42.8, 97.8, 117.1, 122.0, 124.2, 127.6, 133.9, 148.6, 150.0, 151.5\).

\(N\)-(7-Chloro-4-quinolyl)-1,5-diaminopentane, 2d. Employing 1.0 g (5.1 mmol) of 4,7-dichloroquinoline in the procedure described above gave 1.16 g (4.4 mmol, 87\% yield) of
pale yellow crystals. $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ = 1.15 (bs, 2H), 1.40-1.60 (m, 4H), 1.66-1.86 (m, 2H), 2.71 (t, $J = 6.6$ Hz, 2H), 3.20-3.38 (m, 2H), 5.49 (t, $J = 4.8$ Hz, 1H), 6.36 (d, $J = 5.4$ Hz, 1H), 7.28 (dd, $J = 2.1$, 9.0 Hz, 1H), 7.72 (d, $J = 9.0$ Hz, 1H), 7.92 (d, $J = 2.1$ Hz, 1H), 8.50 (d, $J = 5.4$ Hz, 1H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$ = 24.3, 28.5, 33.1, 41.9, 43.0, 98.9, 117.0, 120.9, 125.0, 128.6, 134.6, 149.0, 149.6, 151.9.

$\text{N-(7-Chloro-4-quinolyl)} \cdot \text{N'-(3-diethylaminopropanoyl)-1,3-diaminopropane, 3b.}$ A mixture of $\text{N-(7-chloro-4-quinolyl)-1,3-diaminopropane (1.0 g, 4.24 mmol), N,N-diethylamino-3-propionic acid (0.78 g, 4.3 mmol)}$, EDC (0.98 g, 5.1 mmol) and triethylamine (1.8 mL, 12.9 mmol) in 30 mL of anhydrous DMF and chloroform (1:1 v/v) was stirred at room temperature for 2.5 days. The reaction mixture was concentrated in vacuo, then dissolved in dichloromethane and extracted with aqueous NaOH. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (1:1:0.05ethanol:hexanes:triethylamine, v/v) to give 0.83 g of (2.3 mmol, 54% yield) pale yellow crystals. $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ = 1.02 (t, $J = 7.1$ Hz, 6H), 1.74-1.83 (m, 2H), 2.41 (t, $J = 5.7$ Hz, 2H), 2.53 (q, $J = 7.1$ Hz, 4H), 2.67 (t, $J = 5.9$ Hz, 2H), 3.32-3.43 (m, 4H), 6.37 (d, $J = 5.6$ Hz, 1H), 6.76 (t, $J = 5.7$ Hz, 1H), 7.36 (dd, $J = 2.1$ Hz, $J = 9.0$ Hz, 1H), 7.90 (d, $J = 2.1$ Hz, 1H), 8.02 (d, $J = 9.0$ Hz, 1H), 8.45 (d, $J = 5.6$ Hz, 1H), 9.04 (t, $J = 5.7$ Hz, 1H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$ = 11.8, 28.6, 32.7, 35.7, 39.2, 46.5, 49.2, 98.6, 117.9, 122.5, 125.7, 128.5, 135.4, 149.4, 150.5, 151.9, 174.8; MS (ESI) m/z calcd for C$_{19}$H$_{27}$ClN$_4$O 362.2. Found (M + H)$^+$: 362.9.
**N-(7-Chloro-4-quinolyl)-N’-(3-diethylaminopropyl)-1,3-diaminopropane, 3d.** To *N*- (7-chloro-4-quinolyl)-N’-(3-diethylaminopropanoyl)-1,3-diaminopropane (0.2 g, 0.55 mmol) in 9 mL of anhydrous DMF, borane-dimethyl sulfide complex (0.35 mL, 3.69 mmol) was added dropwise at 0 °C. The reaction mixture was heated to reflux for 2.5 h and then quenched with 1.6 mL of water. Concentrated HCl (1.0 mL) was added and the reaction was refluxed for another 1.5 h. The reaction mixture was cooled to room temperature, basified (pH > 10) with NaOH and extracted with chloroform. The combined organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo to give a yellow oil (0.16 g, 0.46 mmol, 82% yield). ¹H-NMR (300 MHz, CDCl₃) δ = 1.01 (t, J = 7.1 Hz, 6H), 1.71-1.80 (m, 2H), 1.90-1.97(m, 2H), 2.48-2.55 (m, 6H), 2.74 (t, J = 6.9 Hz, 2H), 2.89-2.93 (m, 2H), 3.37-3.42 (m, 2H), 6.30 (d, J = 5.6 Hz, 1H), 7.29 (dd, J = 2.2 Hz, J = 9.0 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 2.2 Hz, 1H), 8.50 (d, J = 5.4 Hz, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ = 11.6, 25.1, 26.4, 42.2, 46.9, 49.6, 52.2, 53.7, 98.4, 117.9, 123.2, 125.2, 128.4, 135.0, 149.3, 150.7, 152.1; MS (ESI) m/z calcd for C₁₉H₂₉ClN₄ 348.2. Found (M + H)⁺: 349.1.

**N-(7-Chloro-4-quinolyl)-N’-ethyl-N’-(3-diethylaminopropyl)-1,3-diaminopropane, 4b.** To a solution of *N-(7-chloro-4-quinolyl)-N’-(3-diethylaminopropyl)-1,3-diaminopropane* (0.09 g, 0.24 mmol) in 4 mL of glacial acetic acid, sodium borohydride (0.24 g, 6.3 mmol) was added at 5 °C. The reaction was warmed to room temperature for 1 h and then heated to 60 °C for 30 h. After cooling to room temperature, the mixture was basified (pH > 10) with NaOH and extracted with dichloromethane. The combined
organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo. The crude product was purified by flash chromatography (1.0:0.05 ethanol:triethylamine, v/v) to give 0.075 g (0.12 mmol, 81% yield) of a yellow oil. $^1$H-NMR (300 MHz, CDCl₃) δ = 0.94 (t, $J = 7.2$ Hz, 6H), 1.11 (t, $J = 7.2$ Hz, 3H), 1.62-1.73 (m, 2H), 1.88-1.98 (m, 2H), 2.38-2.48 (m, 6H), 2.56 (t, $J = 7.7$ Hz, 2H), 2.62-2.71 (m, 4H), 3.32-3.42 (m, 2H), 6.31 (d, $J = 5.4$ Hz, 1H), 7.32 (dd, $J = 2.1$ Hz, 8.8 Hz, 1H), 7.71 (d, $J = 8.8$ Hz, 1H), 7.86 (bs, 1H), 7.93 (d, $J = 2.1$ Hz, 1H), 8.50 (d, $J = 5.4$ Hz, 1H); $^{13}$C-NMR (75 MHz, CDCl₃) δ = 11.8, 24.7, 24.9, 44.9, 47.1, 48.1, 51.4, 52.3, 54.2, 98.6, 117.9, 122.3, 124.9, 128.9, 134.8, 149.5, 150.8, 152.5; MS (ESI) m/z calcd for C₂₁H₃₃ClN₄ 376.2. Found (M + H)$^+$: 376.9.

$N$-(7-Chloro-4-quinolyl)$-N'$-(3-diethylaminopropanoyl)-1,5-diaminopentane, 4d. A mixture of $N$-(7-chloro-4-quinolyl)-1,5-diaminopentane (0.25 g, 0.95 mmol), $N,N$-diethylamino-3-propionic acid (0.17 g, 0.93 mmol), EDC (0.22 g, 1.14 mmol), and triethylamine (0.4 mL, 2.9 mmol) in 12 mL of anhydrous DMF and chloroform (1:1 v/v) was stirred at room temperature for 2.5 days. The reaction mixture was concentrated in vacuo, then dissolved in dichloromethane and extracted with aqueous NaOH. The combined organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo. The crude product was purified by flash chromatography (1.0:0.05 methanol:ammonium hydroxide, v/v) to afford 0.045 g (0.11 mmol, 12%) of colorless crystals. $^1$H-NMR (300 MHz, CDCl₃) δ = 1.01 (t, $J = 7.2$ Hz, 6H), 1.49-1.59 (m, 4H), 1.82-1.87 (m, 2H), 2.53 (t, $J = 6.0$ Hz, 2H), 2.53 (q, $J = 7.2$ Hz, 4H), 2.64 (t, $J = 6.0$ Hz, 2H), 3.26-3.33 (m, 4H), 5.46 (bs, 1H), 6.37 (d, $J = 5.4$ Hz, 1H), 7.35 (dd, $J = 2.2$ Hz, 8.8 Hz, 1H), 7.94 (d, $J = 2.2$ Hz, 1H).
Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H), 8.51 (d, J = 5.4 Hz, 1H), 8.80 (bs, 1H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ = 11.8, 24.3, 28.0, 30.0, 32.8, 37.9, 43.4, 46.3, 49.2, 100.6, 117.6, 122.0, 128.9, 134.9, 149.5, 150.3, 152.3, 173.7; MS (ESI) m/z calcd for C$_{21}$H$_{31}$ClN$_4$O 390.2.

Found (M + H)$^+$: 391.0.

$N$-(7-Chloro-4-quinolyl)-N’-(3-diethylaminopropyl)-1,5-diaminopentane, 5b. To $N$-(7-chloro-4-quinolyl)-N’-(3-diethylaminopropanoyl)-1,5-diaminopentane (0.14 g, 0.35 mmol) in 9 mL of anhydrous DMF, borane-dimethyl sulfide complex (0.23 mL, 2.42 mmol) was added dropwise at 0 °C. The reaction mixture was heated to reflux for 2.5 h and then quenched with 1.8 mL of water. Concentrated HCl (0.75 mL) was added and the mixture was refluxed for another 1.5 h. The product mixture was cooled to room temperature, basified (pH > 10) with NaOH and extracted with dichloromethane. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuo. The residue was purified by flash chromatography (1.0:1.0:0.10 ethanol:dichloromethane:triethylamine, v/v) to afford 0.09 g (0.24 mmol, 68% yield) as a light yellow oil. $^1$H-NMR (300 MHz, CDCl$_3$) δ = 0.99 (t, J = 7.1 Hz, 6H), 1.53-1.61 (m,2H), 1.67-1.83 (m, 6H), 2.50-2.58 (m, 6H), 2.74 (t, J = 6.7 Hz, 2H), 2.84 (t, J = 6.4 Hz, 2H), 3.36 (q, J = 6.1 Hz, 2H), 5.72 (bs, 1H), 6.28 (d, J = 5.4 Hz, 1H), 7.37 (dd, J = 2.2 Hz, 9.0 Hz, 1H), 7.93 (d, J = 2.2 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.51 (d, J = 5.4 Hz,1H). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ = 11.8, 24.8, 26.3, 28.5, 29.2, 43.1, 46.9, 49.5, 51.9, 99.2, 117.5, 121.8, 125.4, 128.9, 135.0, 149.4, 150.1, 152.2; MS (ESI) m/z calcd for C$_{21}$H$_{33}$ClN$_4$ 376.2. Found (M + H)$^+$: 377.1.
N-(7-Chloro-4-quinolyl)-N′-ethyl-N′-(3-diethylaminopropyl)-1,5-diaminopentane, 5d. To N-(7-chloro-4-quinolyl)-N′-(3-diethylaminopropyl)-1,5-diaminopentane (0.064 g, 0.17 mmol) in 4 mL of glacial acetic acid, sodium borohydride (0.16 g, 4.3 mmol) was added at 5 °C. The reaction was stirred at room temperature for 40 minutes and then heated to 55 °C for 36 h. After cooling to room temperature, the mixture was basified (pH > 10) with NaOH and extracted with dichloromethane. The combined organic layers were dried over anhydrous MgSO4 and concentrated in vacuo. The crude product was purified by flash chromatography (1.0:0.05 ethanol:triethylamine, v/v) to afford 0.021 g (0.05 mmol, 31% yield) as colorless crystals. \(^{1}\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta = 1.02 (t, J = 7.2 \text{ Hz}, 6\text{H}), 1.04 (t, J = 7.2 \text{ Hz}, 3\text{H}), 1.46-1.55 (m, 6\text{H}), 1.74-1.83 (m, 2\text{H}), 2.43-2.59 (m, 12\text{H}), 3.29-3.36 (m, 2\text{H}), 5.11 (bs, 1\text{H}), 6.41 (d, J = 5.5 \text{ Hz}, 1\text{H}), 7.37 (dd, J = 2.2 \text{ Hz}, 9.0 \text{ Hz}, 1\text{H}), 7.72 (d, J = 9.0 \text{ Hz}, 1\text{H}), 7.95 (d, J = 2.2 \text{ Hz}, 1\text{H}), 8.54 (d, J = 5.5 \text{ Hz}, 1\text{H}). \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta = 11.5, 11.8, 24.4, 25.3, 27.0, 28.9, 43.4, 46.3, 47.1, 47.8, 51.2, 51.8, 99.3, 117.4, 121.4, 125.4, 128.5, 135.0, 149.4, 150.0, 152.3; MS (ESI) m/z calcd for C\(_{23}\)H\(_{37}\)ClN\(_4\) 404.3. Found (M + H\(^+\)): 405.1.

Representative procedure for the synthesis of compounds 6a-6e.

A mixture of 9 (0.500 g, 2.26 mmol) and N,N-diisopropylethylamine (0.91 mL, 5.29 mmol) in anhydrous DMF was stirred vigorously at room temperature under nitrogen atmosphere. Bromoacetyl chloride (0.24 mL, 2.88 mmol) was added dropwise to the mixture and allowed to stir for 1 hour. Diethylamine (2.35 mL, 22.62 mmol) was then added and the reaction was stirred at room temperature for 72 hours. The mixture was concentrated in vacuo and purified by flash chromatography (1.1:1.0:0.1
hexanes:ethanol:triethylamine). Extraction of a solution in dichloromethane with aqueous NaHCO₃ and NaOH solution, dried over MgSO₄ and concentrated in vacuo to give 6a as a yellow solid (0.453 g, 1.36 mmol, 60%).

**N-(7-Chloro-4-quinolyl)-N-(2-diethylaminoethanoyl)-1,2-diaminoethane, 6a.** ¹H-NMR (400 MHz, CDCl₃) δ = 0.98 (t, J = 7.1 Hz, 6H), 2.54 (q, J = 7.1 Hz, 4H), 3.08 (s, 2H), 3.39 (m, 2H), 3.74 (m, 2H), 6.28 (d, J = 5.4 Hz, 1H), 6.92 (bs, 1H), 7.38 (dd, J = 2.1, 8.9 Hz, 1H), 7.81 (d, J = 8.9 Hz, 1H), 7.93 (d, J = 2.1 Hz, 2H), 8.48 (d, J = 5.4 Hz, 1H); ¹³C-NMR (100 MHz, CDCl₃) δ = 12.3, 38.5, 46.1, 48.2, 57.4, 98.0, 117.3, 122.1, 125.4, 128.4, 134.8, 149.1, 150.1, 151.9, 175.4; MS (ESI) m/z calcd for C₁₇H₂₃ClN₄O 334.2. Found (M + H)⁺: 335.2.

**N-(7-Chloro-4-quinolyl)-N-(4-diethylaminobutanoyl)-1,2-diaminoethane, 6c.**

Employing 0.700 g (3.17 mmol) of 9 and 0.48 mL 4-bromobutyryl chloride (4.13 mmol) in the procedure described above gave 0.624 g (1.72 mmol, 54%) of 6c as a yellow solid. ¹H-NMR (400 MHz, CDCl₃) δ = 1.00 (t, J = 7.3 Hz, 6H), 1.76-1.82 (m, 2H), 2.41 (t, J = 6.6 Hz, 2H), 2.47-2.56 (m, 6H), 3.35 (m, 2H), 3.64 (m, 2H), 6.24 (d, J = 5.4 Hz, 1H), 7.11 (bs, 1H), 7.34 (dd, J = 2.1, 8.9 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.89 (s, 1H), 8.44 (d, J = 5.4 Hz, 1H), 8.86 (t, J = 5.8 Hz, 1H). ¹³C-NMR (100 MHz, CDCl₃) δ = 10.9, 22.4, 35.4, 38.4, 46.5, 52.7, 97.8, 117.3, 122.6, 125.1, 125.5, 127.8, 134.8, 148.8, 150.3, 151.7, 176.2; MS (ESI) m/z calcd for C₁₉H₂₇ClN₄O 362.2. Found (M + H)⁺: 363.7.
N-(7-Chloro-4-quinolyl)-N-(5-diethylaminopentanoyl)-1,2-diaminoethane, \(6d\).

Employing 0.700 g (3.17 mmol) of 9 and 0.55 mL 5-bromovaleryl chloride (4.11 mmol) in the procedure described above gave 0.800 g (2.12 mmol, 67%) of 6d as a yellow solid.

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 0.92\) (t, \(J = 7.1\) Hz, 6H), 1.38-1.46 (m, 2H), 1.61-1.69 (m, 2H), 2.29 (t, \(J = 7.3\) Hz, 2H), 2.35 (t, \(J = 7.1\) Hz, 2H), 2.41 (q, \(J = 7.1\) Hz, 4H), 3.34 (m, 2H), 3.67 (m, 2H), 6.23 (d, \(J = 5.4\) Hz, 1H), 7.09 (bs, 1H), 7.32 (dd, \(J = 2.1, 8.7\) Hz, 1H), 7.83 (d, \(J = 5.3\) Hz, 2H), 7.85 (s, 1H), 8.40 (d, \(J = 5.4\) Hz, 1H). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 11.4, 23.8, 26.4, 36.2, 38.7, 46.2, 46.6, 52.4, 98.0, 117.2, 122.1, 125.4, 128.3, 134.8, 149.0, 150.1, 151.9, 176.3; MS (ESI) m/z calcd for C\(_{20}\)H\(_{29}\)ClN\(_4\)O 376.2. Found (M + H\(^+\)): 377.3.

N-(7-Chloro-4-quinolyl)-N-(6-diethylaminohexanoyl)-1,2-diaminoethane, \(6e\).

Employing 0.700 g (3.17 mmol) of 9 and 0.62 mL 6-bromohexanoyl chloride (4.14 mmol) in the procedure described above gave 0.642 g (1.65 mmol, 52%) of 6e as a yellow solid. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 0.95\) (t, \(J = 7.1\) Hz, 6H), 1.21-1.29 (m, 2H), 1.35-1.42 (m, 2H), 1.62-1.70 (m, 2H), 2.25-2.29 (m, 4H), 2.44 (q, \(J = 7.1\) Hz, 4H), 3.34 (m, 2H), 3.68 (m, 2H), 6.23 (d, \(J = 5.4\) Hz, 1H), 7.07 (bs, 1H), 7.32 (dd, \(J = 2.0, 9.0\) Hz, 1H), 7.76 (t, \(J = 6.0\) Hz, 1H), 7.82 (d, \(J = 2.0\) Hz, 1H), 7.84 (s, 1H), 8.39 (d, \(J = 5.4\) Hz, 1H). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 11.4, 25.5, 26.7, 27.0, 36.4, 38.7, 46.1, 46.7, 52.5, 98.0, 117.3, 122.1, 125.4, 128.3, 134.8, 149.0, 150.1, 151.8, 176.3; MS (ESI) m/z calcd for C\(_{21}\)H\(_{31}\)ClN\(_4\)O 390.2. Found (M + H\(^+\)): 391.3.
Representative procedure for the synthesis of compounds 7a-7e.

A solution of 6a (0.300 g, 0.90 mmol) in 10 mL of THF was heated to reflux and borane-dimethyl sulfide complex (0.45 mL, 5.4 mmol) was added. After 2.5 hours, 6 M HCl (1.45 mL, 9.0 mmol) and 2 mL of water were added and the mixture was heated to reflux for 1.5 hours. The clear solution was cooled to room temperature, basified with saturated NaOH and extracted with a 1:1 mixture of CH₂Cl₂ and CHCl₃. The combined organic layers were dried over anhydrous MgSO₄ and concentrated in vacuo. Purification by flash chromatography using EtOH:hexanes:Et₃N (1:1:0.1 v/v) as the mobile phase gave 7a (0.224 g, 0.70 mmol, 78% yield) as a brown oil.

\[ N-(7-C\text{hloro-4-quinolyl})-N-(2-diethylaminoethyl)-1,2-diaminoethane, \textit{7a}. \]

$^1$H-NMR (400 MHz, CDCl₃) $\delta$ = 1.01 (t, $J$ = 7.1 Hz, 6H), 2.50-2.58 (m, 6H), 2.70 (t, $J$ = 5.8 Hz, 2H), 3.02 (t, $J$ = 5.8 Hz, 2H), 3.33 (m, 2H), 5.97 (bs, 1H), 6.38 (d, $J$ = 5.4 Hz, 1H), 7.34 (dd, $J$ = 2.1, 8.9 Hz, 1H), 7.73 (d, $J$ = 8.9 Hz, 1H), 7.93 (d, $J$ = 2.1 Hz, 1H), 8.51 (d, $J$ = 5.4 Hz, 1H); $^{13}$C-NMR (100 MHz, CDCl₃) $\delta$ = 11.7, 42.0, 46.8, 47.0, 47.4, 52.5, 99.1, 117.4, 121.4, 125.1, 128.6, 134.7, 149.1, 149.9, 152.0; MS (ESI) m/z calcd for C₁₇H₂₅ClN₄ 320.2. Found (M+H)+: 321.1.

\[ N-(7-C\text{hloro-4-quinolyl})-N-(4-diethylaminobutyl)-1,2-diaminoethane, \textit{7e}. \]

Employing 0.300 g (0.83 mmol) of 6c in the procedure described above gave 0.101 g (0.29 mmol, 35%) of 7e as yellow oil. $^1$H-NMR (400 MHz, CDCl₃) $\delta$ = 1.01 (t, $J$ = 7.2 Hz, 6H), 1.53-1.56 (m, 4H), 2.45 (m, 2H), 2.53 (q, $J$ = 7.2 Hz, 4H), 2.68 (m, 2H), 3.03 (m, 2H), 3.34
(m, 2H), 6.10 (bs, 1H), 6.36 (d, \( J = 5.4 \) Hz, 1H), 7.34 (dd, \( J = 2.1, 8.9 \) Hz, 1H), 7.79 (d, \( J = 8.9 \) Hz, 1H), 7.92 (d, \( J = 2.1 \) Hz, 1H), 8.50 (d, \( J = 5.4 \) Hz, 1H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \( \delta = 11.4, 24.8, 28.1, 41.8, 46.7, 47.3, 49.2, 52.7, 99.0, 117.5, 121.6, 125.2, 128.6, 134.8, 149.2, 149.9, 152.0; MS (ESI) m/z calcd for \( \text{C}_{19}\text{H}_{29}\text{ClN}_4 \) 348.2. Found (M + H\(^+\)): 349.2.

\( \text{N-(7-Chloro-4-quinolyl)-N-(5-diethylaminopentyl)-1,2-diaminoethane,} \quad 7d \). Employing 0.300 g (0.80 mmol) of 6d in the procedure described above gave 0.170 g (0.47 mmol, 59%) of 7d as a yellow oil. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \( \delta = 1.07 \) (t, \( J = 7.2 \) Hz, 6H), 1.37 (m, 2H), 1.52-1.57 (m, 4H), 2.48 (m, 2H), 2.61 (q, \( J = 7.2 \) Hz, 4H), 2.68 (t, \( J = 7.0 \) Hz, 2H), 3.04 (m, 2H), 3.34 (m, 2H), 5.99 (bs, 1H), 6.38 (d, \( J = 5.4 \) Hz, 1H), 7.35 (dd, \( J = 2.1, 8.9 \) Hz, 1H), 7.76 (d, \( J = 8.9 \) Hz, 1H), 7.93 (d, \( J = 2.1 \) Hz, 1H), 8.51 (d, \( J = 5.4 \) Hz, 1H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \( \delta = 11.0, 25.2, 26.3, 29.8, 41.7, 46.8, 47.4, 48.9, 99.2, 121.5, 125.2, 128.7, 134.8, 149.2, 150.0, 152.1; MS (ESI) m/z calcd for \( \text{C}_{20}\text{H}_{31}\text{ClN}_4 \) 362.2. Found (M + H\(^+\)): 363.4.

\( \text{N-(7-Chloro-4-quinolyl)-N-(6-diethylaminohexyl)-1,2-diaminoethane,} \quad 7e \). Employing 0.300 g (0.77 mmol) of 6e in the procedure described above gave 0.147 g (0.39 mmol, 51%) of 7e as a yellow oil. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \( \delta = 1.01 \) (t, \( J = 7.2 \) Hz, 6H), 1.25-1.55 (m, 9H), 2.38 (m, 2H), 2.51 (q, \( J = 7.2 \) Hz, 4H), 2.64 (m, 2H), 3.02 (m, 2H), 3.30-3.34 (m, 2H), 5.94 (bs, 1H), 6.37 (d, \( J = 5.4 \) Hz, 1H), 7.34 (dd, \( J = 2.1, 8.9 \) Hz, 1H), 7.71 (d, \( J = 8.9 \) Hz, 1H), 7.93 (d, \( J = 2.1 \) Hz, 1H), 8.50 (d, \( J = 5.4 \) Hz, 1H); \(^{13}\)C-NMR
(100 MHz, CDCl$_3$) $\delta$ = 11.6, 27.0, 27.3, 27.6, 30.2, 41.9, 46.9, 47.2, 49.2, 52.9, 99.2, 117.4, 121.3, 125.2, 128.8, 134.8, 149.2, 149.9, 152.1; MS (ESI) m/z calcd for C$_{21}$H$_{33}$ClN$_4$ 376.2. Found (M + H)$^+$: 377.5.

**Representative procedure for the synthesis of compounds 8a-8e.**

To a solution of 7a (0.070 g, 0.22 mmol) in 5 mL of glacial acetic acid, NaBH$_4$ (0.293 g, 7.7 mmol) was added at 0 °C and the reaction temperature was increased to 60 °C. After 48 hours, the reaction mixture was cooled, basified with saturated NaOH, extracted with CH$_2$Cl$_2$ and washed with brine. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated in vacuo. Purification by flash chromatography using CH$_2$Cl$_2$:hexanes (1:1 v/v) as the mobile phase gave 8a (0.029 g, 0.080 mmol, 37% yield) as a yellow oil.

**N-(7-Chloro-4-quinolyl)-N'-ethyl-N'-(2-diethylaminoethyl)-1,2-diaminoethane, 8a.**

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ = 0.99 (t, $J = 7.1$ Hz, 6H), 1.06 (t, $J = 7.1$ Hz, 3H), 2.53-2.64 (m, 10H), 2.83 (t, $J = 5.5$ Hz, 2H), 3.24 (m, 2H), 6.34 (d, $J = 5.4$ Hz, 1H), 6.36 (bs, 1H), 7.32 (dd, $J = 2.1$, 8.9 Hz, 1H), 7.77 (d, $J = 8.9$ Hz, 1H), 7.92 (d, $J = 2.1$ Hz, 1H), 8.50 (d, $J = 5.4$ Hz, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ = 11.3, 11.9, 40.0, 47.3, 47.5, 50.6, 50.8, 99.1, 117.6, 121.8, 124.8, 134.6, 149.2, 150.0, 152.1; MS (ESI) m/z calcd for C$_{19}$H$_{29}$ClN$_4$ 348.2. Found (M + H)$^+$: 349.1.
**N-(7-Chloro-4-quinolyl)-N'-ethyl-N'-((4-diethylaminobutyl)-1,2-diaminoethane, 8c.**

Employing 0.100 g (0.29 mmol) of 7c in the procedure described above gave 0.086 g (0.23 mmol, 80%) of 8c as a light yellow oil. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta = 0.99$ (t, $J = 7.1$ Hz, 6H), 1.07 (t, $J = 7.1$ Hz, 3H), 1.46-1.48 (m, 4H), 2.39 (m, 2H), 2.43-2.48 (m, 4H), 2.52 (m, 2H), 2.59 (m, 2H), 2.81 (t, $J = 5.6$ Hz, 2H), 3.24 (m, 2H), 6.06 (bs, 1H), 6.35 (d, $J = 5.3$ Hz, 1H), 7.34 (dd, $J = 2.0$, 8.9 Hz, 1H), 7.64 (d, $J = 8.9$ Hz, 1H), 7.93 (d, $J = 2.0$ Hz, 1H), 8.51 (d, $J = 5.3$ Hz, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta = 11.6$, 11.9, 25.0, 25.4, 39.7, 46.8, 46.9, 51.1, 52.8, 52.9, 99.3, 117.4, 121.0, 125.2, 128.7, 134.7, 149.1, 149.8, 152.1; MS (ESI) m/z calcd for C$_{21}$H$_{33}$ClN$_4$ 376.2. Found (M + H)$^+$: 377.4.

**N-(7-Chloro-4-quinolyl)-N'-ethyl-N'-((5-diethylaminopentyl)-1,2-diaminoethane, 8d.**

Employing 0.100 g (0.28 mmol) of 7d in the procedure described above gave 0.075 g (0.19 mmol, 70%) of 8d as a light yellow oil. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta = 0.98$ (t, $J = 7.1$ Hz, 6H), 1.07 (t, $J = 7.1$ Hz, 3H), 1.29 (m, 2H), 1.42-1.54 (m, 4H), 2.33 (m, 2H), 2.45-2.51 (m, 6H), 2.60 (q, $J = 7.1$ Hz, 4H), 2.81 (t, $J = 5.6$ Hz, 2H), 3.24 (m, 2H), 6.07 (bs, 1H), 6.36 (d, $J = 5.2$ Hz, 1H), 7.34 (dd, $J = 2.1$, 8.9 Hz, 1H), 7.63 (d, $J = 8.9$ Hz, 1H), 7.93 (d, $J = 2.1$ Hz, 1H), 8.51 (d, $J = 5.4$ Hz, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta = 11.5$, 12.0, 25.5, 27.0, 27.3, 39.7, 46.8, 46.9, 51.2, 52.8, 52.9, 99.3, 117.5, 121.1, 125.2, 128.8, 134.7, 149.2, 149.8, 152.2; MS (ESI) m/z calcd for C$_{22}$H$_{33}$ClN$_4$ 390.2. Found (M + H)$^+$: 391.3.
**N-(7-Chloro-4-quinolyl)-N\(^\prime\)-ethyl-N\(^\prime\)-(6-diethylamino-hexyl)-1,2-diaminoethane, 8e.**

Employing 0.100 g (0.27 mmol) of 7e in the procedure described above gave 0.085 g (0.21 mmol, 79%) of 8e as a light yellow oil. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 1.04\) (t, \(J = 7.2\) Hz, 6H), 1.07 (t, \(J = 7.1\) Hz, 3H), 1.24-1.50 (m, 6H), 2.41 (m, 2H), 2.47-2.50 (m, 4H), 2.53-2.60 (m, 6H), 2.81 (t, \(J = 5.6\) Hz, 2H), 3.24 (m, 2H), 6.12 (bs, 1H), 6.35 (d, \(J = 5.3\) Hz, 1H), 7.34 (dd, \(J = 2.2, 8.9\) Hz, 1H), 7.65 (d, \(J = 8.9\) Hz, 1H), 7.93 (d, \(J = 2.2\) Hz, 1H), 8.51 (d, \(J = 5.3\) Hz, 1H); \(^1^3\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 11.0, 12.0, 26.3, 27.2, 27.4, 27.5, 29.7, 39.7, 46.7, 46.8, 47.0, 51.2, 52.6, 52.7, 99.3, 117.5, 121.2, 125.2, 128.7, 134.7, 149.1, 152.2; MS (ESI) m/z calcd for C\(_{23}\)H\(_{37}\)ClN\(_4\) 403.3. Found (M + H\(^+\))\(^+\): 404.2.

**N-(7-Chloro-4-quinolyl)-tris(2-aminoethyl)amine, 10.** A mixture of 4,7-dichloroquinoline (1.0 g, 5.0 mmol) and tris(2-aminoethyl)amine (8.0 mL, 53.5 mmol) was heated to 90 °C for 30 hours under nitrogen atmosphere in a closed vessel with good stirring. The reaction was quenched with 20 mL of concentrated NaOH and extracted between CH\(_2\)Cl\(_2\) and brine. The combined organic layers were dried over MgSO\(_4\) and concentrated in vacuo to give a yellow solid (1.47 g, 4.79 mmol, 95%). \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 1.44\) (bs, 4H), 2.61 (t, \(J = 6.0\) Hz, 4H), 2.80-2.83 (m, 4H), 2.85 (m, 2H), 3.28-3.32 (m, 2H), 6.33 (d, \(J = 5.4\) Hz, 1H), 6.62 (bs, 1H), 7.28 (dd, \(J = 2.1, 8.9\) Hz, 1H), 7.89 (dd, \(J = 2.1\) Hz, 1H), 7.93 (d, \(J = 8.9\) Hz, 1H), 8.48 (d, \(J = 5.4\) Hz, 1H); \(^1^3\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 39.8, 40.8, 52.0, 56.6, 99.1, 117.6, 122.0, 125.0, 128.5, 134.7, 149.2, 150.2, 152.0; MS (ESI) m/z calcd for C\(_{15}\)H\(_{22}\)ClN\(_5\) 307.2. Found (M + H\(^+\))\(^+\): 308.2.
**N-(7-Chloro-4-quinolyl)-N',N”-tetraethyl-tris(2-aminoethyl)amine, 11.** To a solution of 10 (0.462 g, 1.5 mmol) in 10 mL of glacial acetic acid, NaBH₄ (4.26 g, 112.7 mmol) was added at 5 °C and the reaction was stirred at room temperature for 24 hours and then heated to 60 °C for 8 hours. The cooled reaction mixture was basified with saturated NaOH, extracted with CH₂Cl₂ and washed with brine. The combined organic layers were dried over anhydrous MgSO₄ and concentrated *in vacuo* to give a yellow solid (0.315 g, 0.75 mmol, 50%). ¹H-NMR (400 MHz, CDCl₃) δ = 0.97 (t, J = 7.2 Hz, 12H), 2.50-2.56 (m, 12H), 2.64-2.68 (m, 4H), 2.86 (m, 2H), 3.25 (m, 2H), 6.33 (d, J = 5.4 Hz, 1H), 6.51 (bs, 1H), 7.31 (dd, J = 2.1, 8.9 Hz, 1H), 7.84 (d, J = 8.9 Hz, 1H), 7.91 (d, J = 2.1 Hz, 1H), 8.49 (d, J = 5.4 Hz, 1H); ¹³C-NMR (100 MHz, CDCl₃) δ = 11.1, 40.2, 47.2, 50.7, 51.5, 52.1, 99.1, 117.6, 122.2, 124.6, 128.5, 134.6, 149.2, 150.1, 152.1; MS (ESI) m/z calcd for C₂₃H₃₈ClN₅ 419.2. Found (M + H)⁺: 420.3.

**1-(7-Chloro-4-O-quinolyl)-N-(3-diethylaminopropanoyl)-2-aminoethane, 12a.** A mixture of O-(7-chloro-4-quinolyl)-2-aminoethanol, (0.300 g, 1.35 mmol), 3-diethylaminopropionic acid (0.270 g, 1.49 mmol), EDC (0.311 g, 1.62 mmol) and Et₃N (0.62 mL, 4.0 mmol) in 6 mL of anhydrous DMF and CHCl₃ (1:1 v/v) was stirred at room temperature for 20 hours. The reaction was concentrated *in vacuo*. Flash chromatography using CH₂Cl₂:Et₃N (1:0.2 v/v) as the mobile phase gave 0.045 g (0.13 mmol, 16% yield) of white crystals. ¹H-NMR (400 MHz, CDCl₃) δ = 0.97 (t, J = 7.1 Hz, 6H), 2.47-2.53 (m, 6H), 2.79 (t, J = 7.1 Hz, 2H), 3.57 (m, 2H), 4.49 (t, J = 5.1 Hz, 2H), 5.68 (bs, 1H), 6.39 (d, J = 5.3 Hz, 1H), 7.36 (dd, J = 2.1, 8.9 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.94 (d, J =
2.1 Hz, 1H), 8.53 (d, \(J = 5.3\) Hz, 1H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 11.7, 32.7, 43.1, 46.8, 48.2, 62.3, 98.9, 117.2, 125.5, 128.8, 135.0, 149.1, 149.5, 152.0, 173.8\); MS (ESI) m/z calcd for C\(_{18}\)H\(_{24}\)ClN\(_3\)O\(_2\) 349.2. Found (M + H\(^+\)): 350.1.

1-(7-Chloro-4-\(O\)-quinolyl)-\(N\)-(3-diethylaminopropyl)-2-aminoethane, 13a. To a solution of 15 (0.200 g, 0.90 mmol) and Et\(_3\)N (0.39 g, 2.7 mmol) in 4 mL of anhydrous THF at room temperature was added dropwise methanesulfonyl chloride (0.21 mL, 2.7 mmol). The reaction proceeded with good stirring for 15 minutes and was then quenched with saturated NaHCO\(_3\). The mixture was extracted with CH\(_2\)Cl\(_2\), dried over anhydrous MgSO\(_4\), and concentrated \textit{in vacuo}. The residue was dissolved in anhydrous CH\(_3\)CN (3.0 mL) under inert atmosphere and Et\(_3\)N (0.62 g, 2.7 mmol) and \(N,N\)-diethyl diaminopropane (2.9 mL, 18.0 mmol) were added. The reaction mixture was stirred at 40 °C for 21 hours and saturated NaHCO\(_3\) solution was added. The mixture was extracted with CH\(_2\)Cl\(_2\), dried over anhydrous MgSO\(_4\), and concentrated \textit{in vacuo}. The product was purified by flash column chromatography using CH\(_2\)Cl\(_2\):Et\(_3\)N (1:0.2 v/v) as the mobile phase to give a light yellow oil (0.265 g, 0.79 mmol, 88% yield) \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 1.01\) (t, \(J = 7.2\) Hz, 6H), 1.76 (m, 2H), 2.37 (t, \(J = 6.0\) Hz, 2H), 2.50 (q, \(J = 7.2\) Hz, 4H), 3.30 (t, \(J = 5.1\) Hz, 2H), 3.48 (t, \(J = 6.0\) Hz, 2H), 3.95 (t, \(J = 5.1\) Hz, 2H), 6.85 (d, \(J = 5.1\) Hz, 1H), 7.36 (dd, \(J = 2.2, 9.0\) Hz, 1H), 7.96 (d, \(J = 2.2\) Hz, 1H), 8.33 (d, \(J = 9.0\) Hz, 1H), 8.61 (d, \(J = 5.1\) Hz, 1H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta = 10.0, 22.7, 45.7, 49.5, 58.4, 60.0, 109.7, 123.0, 125.8, 125.9, 128.7, 134.8, 150.8, 151.1, 155.5\); MS (ESI) m/z calcd for C\(_{18}\)H\(_{26}\)ClN\(_3\)O 335.2. Found (M + H\(^+\)): 336.2.
1-(7-Chloro-4-O-quinolyl)-N'-ethyl-N'-(3-diethylaminopropyl)-2-aminoethane, 14a.

To a solution of 15 (0.200 g, 0.90 mmol) and Et$_3$N (0.39 g, 2.7 mmol) in 4 mL of anhydrous THF at room temperature was added dropwise methanesulfonyl chloride (0.21 mL, 2.7 mmol). The reaction proceeded with good stirring for 15 minutes and was then quenched with saturated NaHCO$_3$. The mixture was extracted with CH$_2$Cl$_2$, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The residue was dissolved in anhydrous CH$_3$CN (3.0 mL) under inert atmosphere and Et$_3$N (0.62 g, 2.7 mmol) and N,N,N'-triethyl diaminopropane (0.340 g, 6.75 mmol) were added. The reaction mixture was stirred at 45 °C for 36 hours and saturated NaHCO$_3$ solution was added. The mixture was extracted with CH$_2$Cl$_2$, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The product was purified by flash column chromatography using EtOH:hexanes:Et$_3$N (1:1.5:0.05 v/v) as the mobile phase to give a light yellow oil (0.035 g, 0.16 mmol, 18% yield) $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ = 1.01 (t, $J$ = 7.1 Hz, 6H), 1.10 (t, $J$ = 7.1 Hz, 3H), 1.68 (m, 2H), 2.46 (t, $J$ = 7.5 Hz, 2H), 2.52 (q, $J$ = 7.1 Hz, 4H), 2.59 (t, $J$ = 7.5 Hz, 2H), 2.68 (q, $J$ = 7.1 Hz, 2H), 3.02 (t, $J$ = 6.0 Hz, 2H), 4.25 (t, $J$ = 6.0 Hz, 2H), 6.72 (d, $J$ = 5.2 Hz, 1H), 7.42 (dd, $J$ = 2.0, 8.9 Hz, 1H), 8.00 (d, $J$ = 2.0 Hz, 1H), 8.12 (d, $J$ = 8.9 Hz, 1H), 8.72 (d, $J$ = 5.2 Hz, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$ = 11.5, 12.0, 25.0, 46.8, 48.5, 50.9, 51.9, 52.6, 67.6, 101.0, 119.9, 123.5, 126.4, 127.9, 135.6, 149.7, 152.5, 161.5; MS (ESI) m/z calcd for C$_{20}$H$_{30}$ClN$_3$O 363.2. Found (M + H)$^+$: 364.0.
N-\((N'-3-(7\text{-}chloro-4\text{-}quinolyl)\text{aminopropyl)}\text{-}4\text{-}methyl-3,4\text{-}dihydro-2H-benzo}[b][1,4]\text{oxazine-7-sulfonamide, 21}: To a mixture of \(N\text{-}(7\text{-}chloro-4\text{-}quinolyl)-1,3\text{-}diaminopropane (0.102 g, 0.43 mmol)\) in 4.5 mL of anhydrous THF under nitrogen at room temperature was added triethylamine (0.084 g, 0.83 mmol) and 1,4-benzoazinesulfonyl chloride (0.123 g, 0.49 mmol). After stirring for 36 hours at room temperature, the mixture was quenched with water and extracted with dichloromethane. The combined organic layers were dried over anhydrous MgSO\(_4\), concentrated \textit{in vacuo}, and purified by recrystallization from chloroform to give 35 as a off-white solid (0.02 g, 0.04 mmol, 10\% yield). \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta = 1.79\text{-}1.86 (m, 2H), 2.76 (s, 3H), 3.02 (t, J = 4.5 \text{ Hz}, 2H), 3.19 (t, J = 3.4 \text{ Hz}, 2H), 3.44 (t, J = 4.5 \text{ Hz}, 2H), 4.23 (t, J = 3.8 \text{ Hz}, 2H), 5.79 (bs, 1H), 6.25 (d, J = 4.1 \text{ Hz}, 1H), 6.72 (d, J = 6.0 \text{ Hz}, 1H), 6.99 (d, J = 1.5 \text{ Hz}, 1H), 7.06 (dd, J = 1.8 \text{ Hz}, J = 6.6 \text{ Hz}, 1H), 7.28 (dd, J = 1.8 \text{ Hz}, J = 6.6 \text{ Hz}, 1H), 7.72 (d, J = 6.6 \text{ Hz}, 1H), 7.83 (d, J = 1.2 \text{ Hz}, 1H), 8.37 (d, J = 4.1 \text{ Hz}, 1H); \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)) \(\delta = 26.8, 32.0, 37.5, 38.6, 39.5, 47.3, 63.9, 97.7, 109.3, 115.0, 116.3, 120.5, 124.5, 127.2, 134.2, 135.9, 146.8, 148.7, 150.4, 164.2.

\textbf{Representative procedure for the synthesis of urea (22-25) and thiourea (26-29) analogues:} A mixture of \(N\text{-}(7\text{-}chloro-4\text{-}quinolyl)-1,3\text{-}diaminopropane (0.15 g, 0.64 mmol)\) and the appropriate isothiocyanate or isocyanate (0.53 mmol) in anhydrous THF was stirred at room temperature until the reaction was complete. In all cases, the desired urea or thiourea product precipitated from solution. The precipitate was collected via vacuum filtration and dried \textit{in vacuo}. 

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**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(4-methoxyphenyl)urea, 22:** Employing 0.195 g (0.83 mmol) of N-(7-chloro-4-quinolyl)-1,3-diaminopropane and 4-methoxyphenyl isocyanate (0.09 mL, 0.69 mmol) in the procedure described above gave 0.244 g (0.64 mmol, 89 % yield) of white crystals. \(^1\)H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta = 1.81-1.89\) (m, 2H), 3.19-3.38 (m, 4H), 3.73 (s, 3H), 6.18 (t, \(J = 5.6\) Hz, 1H), 6.52 (d, \(J = 5.6\) Hz, 2H), 6.85 (dd, \(J = 2.1\) Hz, \(J = 6.7\) Hz, 2H), 7.31-7.37 (m, 3H), 7.49 (dd, \(J = 3.2\) Hz, \(J = 10.0\) Hz, 1H), 7.83 (d, \(J = 2.2\) Hz, 1H), 8.30 (d, \(J = 2.7\) Hz, 1H), 8.32 (s, 1H), 8.44 (d, \(J = 5.4\) Hz, 1H); \(^13\)C-NMR (75 MHz, DMSO-d\(_6\)) \(\delta = 25.8, 29.3, 37.8, 55.8, 114.9, 118.2, 120.2, 124.8, 128.2, 134.1, 134.3, 149.8, 150.8, 152.7, 154.7, 156.4.

**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(2-methoxy-4-nitrophenyl)urea, 23:** Employing 0.146 g (0.65 mmol) of N-(7-chloro-4-quinolyl)-1,3-diaminopropane and 2-methoxy-4-nitrophenyl isocyanate (0.1 g, 0.52 mmol) in the procedure described above gave 0.186 g (0.43 mmol, 83 % yield) of yellow crystals. \(^1\)H-NMR (300 MHz, DMSO-d\(_6\)) \(\delta = 1.85-1.90\) (m, 2H), 3.25-3.31 (m, 3H), 3.61 (t, \(J = 6.4\) Hz, 1H), 4.00 (s, 3H), 6.51 (d, \(J = 5.4\) Hz, 1H), 7.32 (t, \(J = 5.3\) Hz, 2H), 7.46 (dd, \(J = 2.2\) Hz, \(J = 8.8\) Hz, 1H), 7.78 (dd, \(J = 2.2\) Hz, \(J = 10.7\) Hz, 2H), 7.88 (dd, \(J = 2.4\) Hz, \(J = 9.0\) Hz, 1H), 8.29 (d, \(J = 9.3\) Hz, 1H), 8.40 (d, \(J = 3.4\) Hz, 1H), 8.43 (s, 1H), 8.57 (s, 1H); \(^13\)C-NMR (75 MHz, DMSO-d\(_6\)) \(\delta = 28.9, 57.1, 79.9, 106.1, 116.4, 118.2, 118.4, 124.8, 128.2, 134.1, 137.4, 140.9, 147.2, 149.8, 150.7, 152.6, 155.2.
**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(4-dimethylaminophenyl)urea, 24:**

Employing 0.178 g (0.75 mmol) of N-(7-chloro-4-quinolyl)-1,3-diaminopropane and 4-dimethylaminophenyl isocyanate (0.1 g, 0.62 mmol) in the procedure described above furnished 0.208 g (0.52 mmol, 84 % yield) of white crystals. $^1$H-NMR (300 MHz, DMSO-d$_6$) δ = 1.81-1.88 (m, 2H), 2.84 (s, 6H), 3.24 (q, $J$ = 6.4 Hz, 2H), 3.31-3.39 (m, 2H), 6.11 (t, $J$ = 5.7 Hz, 1H), 6.52 (d, $J$ = 5.4 Hz, 1H), 6.69 (d, $J$ = 9.0 Hz, 2H), 7.23 (d, $J$ = 9.0 Hz, 2H), 7.37 (t, $J$ = 5.0 Hz, 1H), 7.49 (dd, $J$ = 2.2 Hz, $J$ = 9.0 Hz, 1H), 7.83 (d, $J$ = 2.2 Hz, 1H), 8.12 (s, 1H), 8.30 (d, $J$ = 9.0 Hz, 1H), 8.44 (d, $J$ = 5.7 Hz, 1H); $^{13}$C-NMR (75 MHz, DMSO-d$_6$) δ = 2.8, 29.4, 37.8, 67.7, 95.2, 99.3, 113.9, 116.2, 118.2, 120.6, 124.8, 128.2, 131.2, 134.1, 135.5, 138.2, 143.7, 146.8, 149.3, 150.7, 152.6, 156.6.

**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(2-methoxyphenyl)urea, 25:**

Employing 0.191 g (0.81 mmol) of N-(7-chloro-4-quinolyl)-1,3-diaminopropane and 2-methoxyphenyl isocyanate (0.10 mL, 0.75 mmol) in the procedure described above gave 0.254 g (0.66 mmol, 82 % yield) of white crystals. $^1$H-NMR (300 MHz, DMSO-d$_6$) δ = 1.85-1.89 (m, 2H), 3.23-3.39 (m, 4H), 3.86 (s, 3H), 6.53 (d, $J$ = 5.9 Hz, 1H), 6.84-7.02 (m, 4H), 7.36 (t, $J$ = 5.1 Hz, 1H), 7.49 (dd, $J$ = 2.2 Hz, $J$ = 8.8 Hz, 1H), 7.83 (d, $J$ = 2.2 Hz, 1H), 7.94 (s, 1H), 8.14 (dd, $J$ = 2.0 Hz, $J$ = 7.1 Hz, 1H), 8.32 (d, $J$ = 9.0 Hz, 1H), 8.44 (d, $J$ = 5.6 Hz, 1H); $^{13}$C-NMR (75 MHz, DMSO-d$_6$) δ = 25.8, 29.2, 37.7, 67.7, 99.4, 118.2, 118.7, 121.7, 124.7, 128.2, 130.2, 134.1, 148.0, 149.8, 150.8, 152.6, 156.0.
\(N-(3-(7\text{-chloro-4-quinolyl})\text{aminopropyl})-N'-(4\text{-methoxyphenyl})\text{thiourea}, \text{ 26:}\)

Employing 0.162 g (0.69 mmol) of \(N-(7\text{-chloro-4-quinolyl})\text{-1,3-diaminopropane}\) and 4-methoxyphenyl isothiocyanate (0.08 mL, 0.58 mmol) in the procedure described above gave 0.116 g (0.29 mmol, 51 \% yield) of white crystals. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 1.95-1.99\) (m, 2H), 3.35 (q, \(J = 6.3\) Hz, 2H), 3.38 (bs, 2H), 3.77 (s, 3H), 6.52 (d, \(J = 5.4\) Hz, 1H), 6.93 (dd, \(J = 2.2\) Hz, \(J = 6.8\) Hz, 2H), 7.25 (d, \(J = 9.0\) Hz, 2H), 7.40 (t, \(J = 5.1\) Hz, 1H), 7.50 (dd, \(J = 2.2\) Hz, \(J = 9.0\) Hz, 1H), 7.64 (bs, 1H), 7.83 (d, \(J = 2.2\) Hz, 1H), 8.29 (d, \(J = 9.0\) Hz, 1H), 8.44 (d, \(J = 5.4\) Hz, 1H), 9.38 (bs, 1H); \(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 25.8, 28.2, 55.9, 67.7, 114.7, 118.2, 124.8, 126.8, 128.0, 132.3, 134.2, 149.6, 150.8, 152.4, 157.3, 181.4.\)

\(N-(3-(7\text{-chloro-4-quinolyl})\text{aminopropyl})-N'-(2\text{-methoxy-4-nitrophenyl})\text{thiourea, \text{ 27:}\)

Employing 0.136 g (0.58 mmol) of \(N-(7\text{-chloro-4-quinolyl})\text{-1,3-diaminopropane}\) and 2-methoxy-4-nitrophenyl isothiocyanate (0.101 g, 0.48 mmol) in the procedure described above afforded 0.144 g (0.33 mmol, 76 \% yield) of yellow crystals. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 1.98-2.03\) (m, 2H), 3.36-3.42 (m, 3H), 3.63-3.69 (m, 2H), 4.02 (s, 3H), 6.54 (d, \(J = 5.4\) Hz, 1H), 7.37 (t, \(J = 5.3\) Hz, 1H), 7.49 (dd, \(J = 2.4\) Hz, \(J = 9.0\) Hz, 1H), 7.83 (t, \(J = 2.4\) Hz, 2H), 7.90 (dd, \(J = 2.7\) Hz, \(J = 9.0\) Hz, 3H), 8.31 (d, \(J = 9.3\) Hz, 1H), 8.45 (d, \(J = 5.4\) Hz, 1H), 8.79 (d, \(J = 9.0\) Hz, 1H), 9.13 (bs, 1H); \(^{13}\)C-NMR (75 MHz, DMSO-\(d_6\)) \(\delta = 27.7, 42.5, 57.2, 79.9, 106.5, 116.8, 118.2, 124.8, 128.2, 134.1, 136.1, 142.9, 149.8, 150.7, 152.7, 180.5.\)
**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(4-dimethylaminophenyl)thiourea, 28:**

Employing 0.159 g (0.67 mmol) of N-(7-chloro-4-quinolyl)-1,3-diaminopropane and 4-dimethylaminophenyl isothiocyanate (0.101 g, 0.57 mmol) in the procedure described above gave 0.157 g (0.38 mmol, 67 % yield) of white crystals. $^1$H-NMR (300 MHz, DMSO-d$_6$) $\delta = 1.90$-$1.99$ (m, 2H), 2.90 (s, 6H), 3.30-3.38 (m, 2H), 3.61-3.64 (m, 2H), 6.50 (d, $J = 5.4$ Hz, 1H), 6.71 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 8.8$ Hz, 2H), 7.36 (t, $J = 5.3$ Hz, 1H), 7.49 (dd, $J = 2.2$ Hz, $J = 9.0$ Hz, 2H), 7.82 (d, $J = 2.2$ Hz, 1H), 8.27 (d, $J = 9.0$ Hz, 1H), 8.44 (d, $J = 5.4$, 1H), 9.28 (s, 1H); $^{13}$C-NMR (75 MHz, DMSO-d$_6$) $\delta = 28.2$, 79.9, 113.3, 118.2, 124.7, 126.7, 128.2, 134.0, 149.0, 149.8, 150.7, 152.6, 181.3.

**N-(3-(7-chloro-4-quinolyl)aminopropyl)-N’-(4-dimethylaminonaphthyl)thiourea, 29:**

N-(7-chloro-4-quinolyl)-1,3-diaminopropane (0.123 g, 0.52 mmol) and 4-dimethylamino-1-naphthyl isothiocyanate (0.10 g, 0.44 mmol) were employed in the procedure described above. The solution was then cooled to -45 °C and 0.175 g (0.38 mmol, 96 % yield) of white crystals were obtained. $^1$H-NMR (300 MHz, DMSO-d$_6$) $\delta = 1.92$ (bs, 2H), 2.87 (s, 6H), 3.28 (bs, 2H), 3.59-3.66 (m, 2H), 6.43 (s, 1H), 7.12 (d, $J = 8.1$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 2H), 7.48 (dd, $J = 2.2$ Hz, $J = 9.0$ Hz, 1H), 7.54-7.57 (m, 2H), 7.81-7.87 (m, 2H), 8.20-8.27 (m, 2H), 8.40 (d, $J = 5.6$ Hz, 1H), 9.57 (bs, 1H); $^{13}$C-NMR (75 MHz, DMSO-d$_6$) $\delta = 28.1$, 45.6, 79.9, 99.3, 118.2, 124.7, 126.1, 128.2, 129.5, 130.4, 132.1, 134.1, 149.8, 150.6, 151.6, 152.6, 167.4, 182.5.
5.5 References


