Synthesis and Evaluation of Densely-Functionalized Troponoids

Danielle R. Hirsch

The Graduate Center, City University of New York

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SYNTHESIS AND EVALUATION OF DENSELY-FUNCTIONALIZED TROPONOID S

by

DANIELLE R. HIRSCH

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree in Doctor of Philosophy, The City University of New York

2018
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Danielle R. Hirsch

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Synthesis and Evaluation of Densely-Functionalized Troponoids

by

Danielle R. Hirsch

Advisor: Ryan P. Murelli

Herein, we document our efforts to expand the scope of troponoid synthetic methodology towards densely-substituted scaffolds. This body of work has focused primarily on an oxidopyrylium cycloaddition/ring-opening strategy for the synthesis and biochemical evaluation of troponoids for assistance in a variety of medicinal chemistry studies. In Chapter 1, we outline the use of this synthetic strategy in the profiling of a library of synthetic αHTs against an aminoglycoside antibiotic resistance enzyme known as aminoglycoside-2”-O-nucleotidyltransferase [ANT(2’’)-Ia]. Enzymatic mechanistic insights have been gleaned from an assessment of troponoid/antibiotic synergistic potential. In particular, two synthetic constructs were identified as promising antibiotic adjuvant candidates, demonstrating a capacity to rescue gentamicin activity while in the presence of ANT(2’’)-Ia-expressing bacteria. These results validate the oxidopyrylium cycloaddition/ring-opening method as a viable approach to generating new ANT(2’’)-Ia inhibitors, and provide some preliminary insight into the structural changes required for effective inhibition.

In Chapter 2, we report the expansion of this synthetic strategy to a class of molecules called 3,7-dihydroxytropolones, which have been indicated as promising leads in the development of antiviral, antimelanoma and antimalarial agents. These synthetic developments were applied in
the synthesis of a prospective biosynthetic precursor to the natural products puberulic and puberulonic acid. Additionally, several new synthetic 3,7-dihydroxytropolones were identified as promising scaffolds for anti-HSV drug development. This synthetic work was further expanded towards additional investigations on the synthesis and physical evaluation of atropisomeric troponoids, as discussed in Chapters 3 and 4. The research described within these chapters aims to broaden our understanding of the physical properties of troponoids by using DFT and experimental techniques to assess the rotational barriers of a series of benzenoid and troponoid scaffolds, as well as by studying the atropselective bromination of a methoxytropolone. During the course of these studies, $^1$H- and $^{13}$C-NMR experiments unveiled several mechanistic findings pertinent to future development of the reaction.

Collectively, the studies described in this thesis aim to broaden our understanding of the physical and biological properties of troponoids.
In loving memory of my grandmothers, Phyllis Friedman Hirsch and Jana Tevet; and my grandfathers, Norman Hirsch and Yitzhak Barzilai. This is for you.

&

For Marley, Haley, and Turtle – with all the love in my heart.
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I consider myself extremely lucky to have been surrounded by many wonderful teachers throughout my educational experience who have nurtured in me a passion for learning. In particular, I would like to thank Mr. Jack Lehmann, Mr. Martin Shields, Ms. Natalie Macke, and Mr. Bernard Argeski at Pascack Hills High School for sparking my interest in science. I would also like to thank Profs. Stephen Anderson, Anita Brandolini, and Robert Mentore for providing me with an incredible learning environment at Ramapo College. Within the Ramapo College chemistry faculty, I would like to extend a special thank you to Prof. Jay Carreon, whose passion for organic chemistry was contagious enough to inspire me to ultimately pursue a Ph.D. He gave me the opportunity to work in his lab and taught me the foundations of everything I know.

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example she has set for me as an educator and as a person, and for all the time she took to help me better myself as an instructor.

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computational and physical organic chemistry. Their contributions to the troponoid bromination work (see Chapters 3 and 4) were vital to the success of these projects. Through working with them I have had the opportunity to learn techniques and gain perspectives that have proven truly invaluable to my growth as a scientist. I thank each of them for the opportunity to collaborate.

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The members of the Murelli group have contributed immensely to my personal and professional growth over the last six years. I consider myself extremely lucky to have been surrounded by such incredible people throughout my time here, and I would not be where I am today without their constant encouragement. I am exceptionally grateful to Lauren Bejcek, who became like a sister to me both in research and in scone baking. She has been a truly empowering presence in my life and I am so grateful I had the chance to overlap (and share an awesome apartment) with her. I would also like to thank Alex Berkowitz for all his patience and support over the years, and especially for all his invaluable help with this dissertation. His knowledge of both chemistry and Taylor Swift facts (in equal measure) never cease to amaze and inspire me. Another seminal figure to me throughout this process is Michael D’Erasmo, who from the very
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Å</td>
<td>angstrom</td>
</tr>
<tr>
<td>AAC</td>
<td>aminoglycoside acetyltransferase</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>Ac7c</td>
<td>1-aminocycloheptane-1-carboxylic acid</td>
</tr>
<tr>
<td>Acbc</td>
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<tr>
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<tr>
<td>CC50</td>
<td>half maximal cytotoxicity concentration</td>
</tr>
<tr>
<td>CCl4</td>
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</tr>
<tr>
<td>CF3</td>
<td>trifluoromethyl</td>
</tr>
<tr>
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</tr>
<tr>
<td>CHCl3</td>
<td>chloroform</td>
</tr>
<tr>
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<td>Substance/Description</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>CDCl₃</td>
<td>deuterated chloroform</td>
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<tr>
<td>CDV</td>
<td>cidofovir</td>
</tr>
<tr>
<td>CLSI</td>
<td>clinical and laboratory standards institute</td>
</tr>
<tr>
<td>Cle</td>
<td>cycloleucine (1-aminocyclopentane carboxylic acid)</td>
</tr>
<tr>
<td>CSP</td>
<td>chiral stationary phase</td>
</tr>
<tr>
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<td>dibromodimethylhydantoin</td>
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<td>density functional theory</td>
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<td>dichloromethane</td>
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<td>3.7-dihydroxytropolone</td>
</tr>
<tr>
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<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>Dmaa</td>
<td>β-dimethylaminoalanine</td>
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<tr>
<td>DMAD</td>
<td>dimethyl acetylenedicarboxylate</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
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<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>half maximal effective concentration</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalent(s)</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>Et₃N</td>
<td>triethylamine</td>
</tr>
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<td>ethyl acetate</td>
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<td>ethanol</td>
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<td>FICI</td>
<td>fractional inhibitory concentration index</td>
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<tr>
<td>Fmoc</td>
<td>fluorenylmethoxycarbonyl</td>
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<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
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<td>Gly</td>
<td>glycine</td>
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</table>
h hour(s)
HCMV human cytomegalovirus
HIV human immunodeficiency virus
HBV hepatitis B virus
HOBut hydroxybenzotriazole
HPLC high-performance liquid chromatography
HSV herpes simplex virus
αHT α-hydroxytropolone
Hyp(but) O-butyl-D-hydroxyproline
Hz hertz
IC50 half maximal inhibitory concentration
Ile isoleucine
IMPase inositol monophosphatase
IR infrared
KSHV Kaposi’s sarcoma-associated herpesvirus
μL microliter(s)
Leu leucine
Lys lysine
M molar
μM micromolar
2-Nap β-naphthylalanine
NBA N-bromoacetamide
NBP N-bromophthalimide
NBS N-bromosuccinimide
NBSac N-bromosacharin
NCS N-chlorosuccinimide
Me methyl
MeCN acetonitrile
Me2NPh N,N-dimethylaniline
mg milligram(s)
MHz megahertz
min  minute(s)
mL   milliliter(s)
mmol millimole(s)
MeOH methanol
MP   melting point
MPLC medium-pressure liquid chromatography
MS   mass spectrometry
MsOH methanesulfonic acid
nM   nanomolar
NMP  N-methylpyrrolidone
NMR  nuclear magnetic resonance
Npht naphthyl
NTS  nucleotidyl transferase superfamily
PAA  phosphonoacetic acid
3Pal D-β-3-pyridylamine
PBS  phosphate-buffered saline
PDB  protein data bank
PES  potential energy scan
Ph   phenyl
Phe  phenylalanine
Phe-F₅ pentafluorophenylalanine
Phg  phenylglycine
Piv  pivaloyl
Pmh  π-methyl histidine
PPh₃ triphenylphosphine
i-Pr  i-propyl
i-Pr₂NPh N,N-diisopropylaniline
Pro  Proline
PyBOP (benzotriazol-1-yloxy)tritylphosphonium hexafluorophosphate
Pyrr  pyrrolidinyl
Rₜ  retention factor
RNA  ribonucleic acid
RNase H  ribonuclease H
RT  reverse transcriptase
rt  room temperature
SAR  structure-activity relationship
TBS  \( t \)-butyldimethylsilyl
TFA  trifluoroacetic acid
TfOH  trifluoromethanesulfonic acid
THF  tetrahydrofuran
Thr  threonine
TLC  thin-layer chromatography
Tle  \( t \)-butyl-leucine
\( \Delta T_m \)  compound-induced changes in thermal stability
Tmga  tetramethylguanidine
TMS  trimethylsilyl
TMSI  trimethylsilyl iodide
TMSOTf  trimethylsilyl trifluoromethanesulfonate
TOF  time-of-flight
\( \mu \)wave  microwave
Chapter I

Synthesis and Biological Activity of α-Hydroxytropolones

1.1 Introduction to α-Hydroxytropolones

α-Hydroxytropolones (αHTs) are a subset of the troponoid family of natural products\(^1\) that possess therapeutic potential against a wide variety of disease targets.\(^2\) Structurally, the molecules represent non-benzenoid aromatic ring scaffolds with a contiguous array of three oxygen atoms (1.1a, αHTs) or four oxygens (1.1b, 3,7-dihydroxytropones [3,7-dHTs], vide infra). Under certain basic conditions, αHTs can adopt a dianionic form, which is capable of delocalizing charge throughout the molecule resulting in a trident-like assemblage of negatively charged oxygen atoms (Figure 1.1).\(^3\) This makes them exceptional inhibitors of dinuclear metalloenzymes.\(^2,4\) Studies on natural products β-thujaplicinol 1.2 and manicol 1.3 have demonstrated that αHTs can exhibit potent activity against hepatitis B virus (HBV),\(^5\) human immunodeficiency virus (HIV),\(^6\) herpes simplex virus 1 and 2 (HSV-1 and -2),\(^7\) and an aminoglycoside resistance enzyme, ANT(2′′)-Ia.\(^8\)

![Figure 1.1](image)

**Figure 1.1.** (A) General structure of 1.1a αHTs and 1.1b 3,7-dHTs. (B) Structural properties of αHTs responsible for therapeutic activity, and (C) αHT natural products.

1.2. Synthetic Routes Towards α-Hydroxytropolones

Synthetic routes towards αHTs have historically focused on a variety of strategies summarized in Scheme 1.1. One of the earliest reports of troponoid total synthesis was
published in 1960. Nozoe and coworkers were able to synthesize 3,7-dHT natural product puberulonic acid 1.6 via a series of brominations and hydolyzations on purpurogallin 1.4 (Scheme 1.1A). While this is an effective method of generating hydroxylated tropones, it suffers from a low yielding first step. Additionally, this oxidation-driven approach starts with the full structural framework on purpurogallin and no new carbon-carbon bonds are formed – as such, it does not lend itself well to generating diverse substitution patterns.

Around the same time, a cyclopropanation/ring-opening approach was developed by Johnson and coworkers that addressed several of these limitations (Scheme 1.1B). 1,2,3-Trimethoxybenzene 1.7 was treated with ethyldiazoacetate and subsequently underwent a 6π-electrocyclic ring-opening followed by hydrolysis and oxidation to generate 3,7-dHT natural product puberulic acid. Along the same lines, Balci and coworkers more recently were able to generate αHT 1.12 via a cyclopropanation/6π-electrocyclic ring-opening on benzenoid 1.10 followed by a singlet oxygen-mediated rearrangement of 1.11 (Scheme 1.1C).

![Scheme 1.1: An overview of synthetic approaches to αHTs, derivatives, and natural products. Highlighted approaches feature key steps involving (A) direct oxidation, (B-E) cyclopropanation, and (F) cycloaddition.](image-url)
In contrast to these cyclopropanation approaches which are followed by $6\pi$-electrocyclic ring-openings, Banwell and coworkers developed a strategy leveraging cyclopropanated intermediates in Grob fragmentations for the generation of troponoid natural products $\beta$-thujaplicinol 1.2 (Scheme 1.1D) and puberulic acid 1.9 (vide infra). A notable advantage provided by this approach is the accessibility of bromotroponoid intermediates stemming from 1.14, which can be functionalized through cross-coupling\textsuperscript{11} and demethylated to generate $\alpha$HTs.\textsuperscript{13} An additional cyclopropanation approach was utilized several years later by Davies and coworkers towards dimethoxytropolones 1.17.\textsuperscript{10e} While not applied directly to $\alpha$HTs, this method granted quick access (only four steps from vinyl diazo carbonyl 1.15) to intermediates that are a simple demethylation away from $\alpha$HTs (Scheme 1.1E).

More recently, a cycloaddition/ring-opening approach was reported by Föhlisch and coworkers (Scheme 1.1F).\textsuperscript{14} Starting with substituted furans 1.18 and pentachloroacetone, 8-oxabicyclo[3.2.1]octenes 1.19 were generated via a cycloaddition. These modifiable intermediates were subsequently treated to a base-mediated ring-opening followed by demethylation to provide $\alpha$HTs 1.20, including $\beta$-thujaplicinol 1.2.

1.3. Previous Synthetic Chemistry-Driven Biological Studies on $\alpha$-Hydroxytropolones

Despite the scope of studied synthetic approaches to $\alpha$HTs as well as their promising therapeutic potential, there has existed a surprising lack of SAR-amenable general synthetic routes to access them.\textsuperscript{2} Fortunately, bioactive natural products can provide a suitable starting point for synthetic derivatization, as was demonstrated by Le Grice and coworkers.\textsuperscript{6c} In this work, the alkene moiety of natural product manicol (1.3, Figure 1.2) was functionalized via epoxidations, aminations, and dihydroxylations for an SAR study on HIV RT RNase H.
While none of the 13 new αHT constructs exhibited significant advantages over the natural product lead in enzymatic assays, all were active. More importantly, unlike manicol, some of the analogs were capable of inhibiting viral replication in cell-based assays. Furthermore, many of the analogs were also significantly less cytotoxic than manicol. This study demonstrates the importance of synthesis in medicinal chemistry studies.

In a similar vein, Piettre and coworkers were able to synthesize a series of arylated 3,7-dihydroxytropolones by modifying an existing troponoid. This synthetic work was utilized in SAR studies on inhibition of inositol monophosphatase (IMPase), an enzyme implicated in bipolar disorder and manic depression. They were able to generate compounds 1.24 and 1.25 in a 4.7:1 ratio (Scheme 1.2). These compounds were then protected with methyl groups using diazomethane, subjected to Suzuki cross-couplings using a variety of arylboronic acids, and subsequently deprotected with TMSI/MeCN. While they were able to synthesize 37 new dihydroxytropolones and subsequently screen them for their inhibitory activity against IMPase, their best molecule tested had only a slight increase in potency over the parent compound.

Marquet and coworkers followed a similar oxidation/cross-coupling method to synthesize arylated dihydroxytropolone analogs in an effort to develop broad activity HIV retroviral hydroxytropolones. They were able to generate compounds 1.24 and 1.25 and carry them...
through the subsequent Suzuki cross-coupling to generate a series of dihydroxytropolones 1.26a-c, which lead to some improved analogs against specific antiretroviral enzymes (Scheme 1.2). However, none of them were reported to have improved HIV replication activity over the parent compound, and all proved to be inactive in an HIV-1 cellular assay. Synthetically, the method is limited in that the initial bromination only goes to 57% conversion and gives a mixture of products.

\[
\text{HO} \quad \text{O} \quad \text{OH} \quad \text{NBS (slow addition)} \quad 57\% \text{ conversion} \quad \text{HO} \quad \text{O} \quad \text{OH} \\
\text{1.26a, Ar = Ph-(4-CHO)} \quad \frac{1.24/1.25 = 4.7:1 \text{ ratio}}{} \quad \text{HO} \quad \text{O} \quad \text{OH} \\
\text{1.26b, Ar = Ph-(3-NO}_2\text{)} \\
\text{1.26c, Ar = Ph-(4-NAC)} \\
\text{(Integrate 3' processing IC}_{50} = 0.15 \mu M) \\
\text{1.26a-c: No improved replication activity}
\]

\[
\text{HO} \quad \text{O} \quad \text{OH} \\
\text{1.26} \quad \text{Ar} \\
\text{Library of biaryl 3,7-dHTs} \\
\text{1.26, X = H} \\
\text{1.25, X = Br}
\]

**Scheme 1.2.** Direct bromination/cross-coupling method for functionalizing and modifying 3,7-dihydroxytropolone.

*De novo* synthetic methods offer opportunity to gain access to a more structurally diverse group of molecules. One additional approach was developed by Banwell and coworkers, who were able to accomplish late-stage introduction of substitution on αHTs. This method was used to synthesize natural products β-thujaplicinol (vide supra) and puberulic acid (Scheme 1.3).

\[
\text{OH} \quad \text{8 steps} \quad \text{OH} \quad \text{OH} \quad \text{OMe} \quad \text{OMe} \quad \text{OH} \quad \text{OH} \\
\text{1.27} \quad \text{1.28} \quad \text{1.29} \quad \text{1.29} \quad \text{1.29} \quad \text{1.29}
\]

**Scheme 1.3.** Multi-step synthesis of puberulic acid showing late-stage introduction of substitution.

While this method provides a route to modifiable late-stage synthetic intermediates and is viable
from a structure-function standpoint, it is problematic in practice. The synthesis is long (10 steps) and only allows access to one position on the αHT ring.

1.4. Murelli Synthesis and Antiviral Assessment of αHTs

As a method of overcoming these limitations, earlier work done in our lab based on established [5C + 2C] cycloadditions\textsuperscript{17} showed that 8-oxabicyclo[3.2.1]octenes 1.32 can be accessed through an intermolecular dipolar cycloaddition of kojic acid-derived salts 1.30 and diversely-substituted alkynes 1.31 (Scheme 1.4).\textsuperscript{18}

![Scheme 1.4. Formation of αHTs via an oxidopyrillum cycloaddition/ring-opening approach.](image)

A key advantage of this approach is the introduction of functionality via readily available, structurally diverse alkynes. Notably, salt 1.30 and related ylide sources have also found utility in catalytic, asymmetric [5 + 2] dipolar cycloadditions,\textsuperscript{19} most recently with an array of α,β-unsaturated aldehyde dipolarphiles,\textsuperscript{20} demonstrating the widespread utility of this starting material. Moreover, 1.30a can be synthesized on a large scale from kojic acid, which is a very inexpensive byproduct of sake production.\textsuperscript{21} Owing to the ease with which large quantities of this salt can be synthesized, triflate salt 1.30a becomes a \textit{de facto} starting material. Thus, diversely substituted 8-oxabicyclo[3.2.1] octenes 1.32 are readily synthesized in one step.
These bicyclic intermediates can undergo an acid-mediated ring-opening\(^{18,22}\) and demethylation to generate multi-substituted \(\alpha\)HTs in few steps from readily available 3-hydroxy-4-pyrone (kojic acid). This route has proven to be useful in a broad range of optimization-driven medicinal chemistry studies. Chiefly, it has been used to assess the antiviral properties of these molecules.

Much of these medicinal chemistry efforts have been geared towards the development of anti-HSV compounds. HSV-1 and -2 are highly related and destructive pathogens which currently infect almost 4 billion people worldwide.\(^{23,24}\) They chronically infect superficially damaged skin and mucosal surfaces, where they gain access to the nervous system and establish lifelong latency. In addition to ulcerative diseases, they are also associated with a rare form of encephalitis as well as corneal blindness.\(^{25}\) The current first line of treatment includes nucleoside analog drugs such as acyclovir (ACV) and cidofovir (CDV).\(^{26-28}\) However, these clinical agents are not completely effective, and the emergence of drug-resistant strains impedes efforts to control HSV, particularly in immunocompromised adults\(^{29}\) and children.\(^{30}\) This drug resistance therefore necessitates the development of new therapeutics.

Towards this end, Tavis, Morrison, and coworkers demonstrated that \(\alpha\)HT natural products manicol and \(\beta\)-thujaplicinol could inhibit wild-type and ACV-resistant HSV-1 and -2 replication in cellular assays.\(^ {31}\) Our lab subsequently synthesized and tested a library of \(\alpha\)HTs against HSV to assess their viability as drug candidates.\(^ {32}\) Among the top molecules to emerge from this study was biphenyl 1.35, which exhibited nanomolar inhibition in HSV replication assays that was improved over the natural product lead and first-line therapeutic ACV (Figure 1.3).
Subsequent studies were conducted in an attempt to determine the binding target of biphenyl αHT \textbf{1.35}. Due to structural similarities with HIV RT RNase H,\textsuperscript{33} dinuclear metalloenzyme terminase pUL15C emerged as a likely candidate, and thus an αHT-based SAR study was conducted on this target in collaboration with Le Grice and coworkers.\textsuperscript{34} The best molecule to emerge from the series was bromotropolone \textbf{1.36}, which displayed improved binding over the natural product lead in both inhibition and thermal stabilization experiments (Figure 1.4). However, antiviral lead \textbf{1.35} was found to bind weakly to pUL15C, suggesting that this enzyme might not be the antiviral target. Studies are currently ongoing to identify the primary target.\textsuperscript{35}
Nevertheless, the promising anti-HSV activity of αHTs has spurred SAR studies on other members of the Herpesviridae family, most recently focused on Kaposi’s sarcoma herpesvirus (KSHV).\textsuperscript{36} KSHV is the causative agent of Kaposi’s sarcoma,\textsuperscript{37} which remains one of the most common cancers in people living with HIV.\textsuperscript{38} The virus is also responsible for certain types of lymphomas and multicentric Castleman’s disease. These fatal diseases are currently largely untreatable due to a lack of effective therapies.\textsuperscript{39} Therefore, new molecules are needed in order to combat KSHV.

Fortunately, KSHV features a pUL15C-like terminase known as pORF29C, which we considered a possible target for αHT inhibition. We thus examined inhibition of this enzyme by a library of synthetic αHTs\textsuperscript{36} and discovered that dimethylester 1.37 demonstrated significant thermal stabilization (Figure 1.5). As an extension of this finding, we sought to identify an inhibitor of KSHV replication. Based on prior HSV studies,\textsuperscript{34} biphenyl 1.35 was analyzed. As KSHV is primarily latent in the infected host, it must be reactivated in order to be susceptible to an antiviral agent, and so these assays were run in a tetracycline repressed (TREx) latently infected cell line in the presence of a known activator doxycycline. As expected, viral replication was significantly reduced in the presence of DNA polymerase inhibitor phosphonoacetic acid (PAA, 1.38). However, synthetic αHT 1.35 emerged as a more potent inhibitor, further reducing viral growth at a significantly lower concentration than that of 1.38. At this same concentration of 5 μM, 1.35 induced no cell death and so the antiviral effect is not due to cytotoxicity. These results represent a promising lead for the development of anti-KSHV drugs.
Synthetic αHTs have also demonstrated promising inhibitory activity towards dinuclear metalloenzymes in other viral targets. Based on previous SAR studies focused on αHT inhibition of HIV RT RNase H (vide supra), we tested our library of αHTs against this enzyme. Bromotropolone 1.36 was again identified as a potent substrate in both inhibition and thermal stabilization assays. The molecules were additionally screened against HBV RT RNase H, and while none of the synthetic constructs demonstrated improved enzymatic activity over the natural product lead β-thujaplicinol, methyl ketone 1.39 was found to inhibit HBV viral replication with an EC$_{50}$ of 340 nM (Figure 1.6). This infers a therapeutic index of ~100 – a 4-fold increase over the natural product lead. These studies all highlight the importance of synthesis in antiviral development.
Although the majority of our ongoing research has been aimed at antiviral developments, αHTs have demonstrated value towards other disease targets. The remainder of this chapter will focus on αHT activity towards the aminoglycoside antibiotic resistance enzyme aminoglycoside-2’-O-nucleotidyltransferase [ANT(2’’)-Ia]. This enzyme is prevalent among Gram-negative bacteria and is one of the most common determinants of enzyme-dependent aminoglycoside resistance.\textsuperscript{42} Our oxidopyrylium cycloaddition/ring-opening strategy was used in the synthesis and profiling of a library of synthetic αHTs against ANT(2’’)-Ia.\textsuperscript{43}

1.5. Introduction to ANT(2’’)-Ia

Antibiotic resistance is a global health threat that jeopardizes not only our ability to treat bacterial infections, but healthcare as we know it.\textsuperscript{44} Besides lifestyle changes,\textsuperscript{44f} one strategy to overcome resistance is to inhibit or disrupt resistance pathways in the form of antibiotic adjuvants.\textsuperscript{45} For example, clavulanic acid is a β-lactamase inhibitor commonly used in tandem with β-lactam antibiotics (such as amoxicillin, \textbf{1.40}) to prolong their efficacy (\textbf{Scheme 1.5}).\textsuperscript{46}
Aminoglycoside antibiotics are also prone to this type of enzymatic deactivation. One of these enzymes is ANT(2’’)-Ia, which catalyzes the adenylation of several clinically relevant antibiotics including gentamicin and tobramycin, rendering them inactive (Scheme 1.6). This target is prevalent among pathogenic Gram-negative bacteria, and has been ranked along with N-acetyltransferase-6’ [AAC-(6’)] as the most common determinant of enzyme-dependent aminoglycoside resistance in *Pseudomonas aeruginosa*. This is of particularly high clinical relevance, as *P. aeruginosa* is the most common hospital-acquired Gram-negative infection. Thus, inhibitors of ANT(2’’)-Ia could potentially find therapeutic utility when used in combination with ANT(2’’)-Ia-susceptible aminoglycoside antibiotics.
αHT, along with αHT natural product β-thujaplicinol, were identified as inhibitors of ANT(2’’)-Ia in a 1982 study by researchers at Eli Lilly.\textsuperscript{50} Tropolone, meanwhile, showed no activity against the enzyme, illustrating the importance of the 3 contiguous oxygens. While several compounds have emerged for targeting aminoglycoside-modifying enzymes,\textsuperscript{51} in particular aminoglycoside mimics,\textsuperscript{52} ANT(2’’)-Ia inhibitors have been elusive,\textsuperscript{53} and αHTs remain one of the only leads.

1.6. Preliminary Substrate Scope and SAR

Our oxidopyrylium cycloaddition/ring-opening synthetic strategy was used in a structure-function study of ANT(2’’)-Ia.\textsuperscript{43} The enzyme was overexpressed in \textit{Escherichia coli} BL21 (\lambdaDE3), and activity was monitored in 96 well format through the detection of pyrophosphate (EnzCheck pyrophosphate assay), a by-product of the adenylation of gentamicin (Scheme 1.6). All biological work was carried out by Prof. Gerard Wright and Dr. Georgina Cox. Previously described synthetic α-hydroxytropolones\textsuperscript{18,22a} and natural product β-thujaplicinol were tested for their inhibitory activity through an in vitro screen to obtain IC\textsubscript{50}
values (Table 1.1). $K_i$ experiments were obtained on active compounds (IC$_{50}$ < 200 μM) through dose curves with ATP and kanamycin B substrates.

Among the compounds tested, $\beta$-thujaplicinol was found to be capable of inhibiting the enzyme with the greatest potency, with a $K_i$ value of 6.4 μM. While the majority of the synthesized molecules showed no inhibition of the enzyme, methyl ketone 1.39 and nitroaryl 1.54 demonstrated some inhibitory potency. Notably, these compounds were among the least sterically demanding of the substrates tested and were approximately 5-10 fold less potent than $\beta$-thujaplicinol, which is the least substituted. This trend suggests a sterically congested enzymatic pocket.

<table>
<thead>
<tr>
<th>Table 1.1. Inhibition of ANT(2&quot;)-Ia by known, previously-synthesized αHTs as well as methylated variants.</th>
</tr>
</thead>
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<td><img src="image" alt="Chemical structures" /></td>
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<tr>
<td>1.37  IC$_{50}$ &gt; 200 μM</td>
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<td>1.50  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.51  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.52  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.39  IC$_{50}$ = 50 ± 8 μM  $K_i$ = 29 ± 6 μM</td>
</tr>
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<td>1.53  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.54  IC$_{50}$ = 20 ± 6 μM  $K_i$ = 21.4 ± 0.4 μM</td>
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<td>1.55  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>β-Thujaplicinol  IC$_{50}$ = 6.6 ± 0.8 μM  $K_i$ = 6.4 ± 0.9 μM</td>
</tr>
<tr>
<td>αHT (1.1a)  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.39a  IC$_{50}$ &gt; 200 μM</td>
</tr>
<tr>
<td>1.54a  IC$_{50}$ &gt; 200 μM</td>
</tr>
</tbody>
</table>
1.7. Speculation on Binding Mode

Previous studies suggest that the adenylation of ANT(2’’)-Ia may proceed via a mechanism involving the two magnesium ions in the catalytic pocket of the enzyme. Additionally, prior studies on αHT inhibition of ANT(2’’)-Ia demonstrated that activity is influenced more substantially by ATP than by the aminoglycoside antibiotic. This trend was also observed in our own studies. Furthermore, while direct binding of αHTs to ATP cannot be entirely discounted, this mechanism of action appears unlikely due to the discrepancy between the concentration of ATP (35 μM) and the observed IC$_{50}$ values (as low as 6 μM) in the inhibition assays.

More likely, the αHTs inhibit via competitive action with respect to ATP. In the instances where $K_i$ values were determined, all compounds demonstrated competitive inhibition with respect to ATP and mixed inhibition with respect to the antibiotic. This suggests that αHTs bind at or close to the ATP binding site, which is further supported by crystallographic data of αHT-bound HIV RT RNase H (Figure 1.7A). These cocrystal structures exhibit a metal-metal bond distance comparable to that found in an ATP-dinuclear enzyme complex (3.76 Å vs 3.91 Å, respectively) (Figure 1.7B). Furthermore, recent crystallographic data of ANT(2’’)-Ia in ternary complex with a nonhydrolyzable ATP analog (α,β-methyleneadenosine 5’-triphosphate lithium salt, AMPCPP) and gentamicin shows coordination of both divalent metal ions by AMPCPP phosphate groups as well as several Asp residues (Figure 1.7C). This suggests that the magnesium ions function as mediators of ATP transfer to the aminoglycoside substrate. αHT inhibition therefore likely occurs via inhibition of this mechanism.
Furthermore, tropolone is generally inactive against ANT(2’’)-Ia, and additional methylated congeners of active compounds 1.39 and 1.54 (1.39a and 1.54a respectively, Table 1.1) accessible through our synthetic method were also found to be inactive. While this could be due to other effects such as steric impacts of the added methyl group, this data is consistent with a dimetallic chelation binding mode.

1.8. Follow-Up Substrate Scope and SAR

We subsequently began to synthesize derivatives of the two lead synthetic αHTs, 1.39 and 1.54. Using the same oxidopyrylium cycloaddition/ring-opening route, a series of analogs was generated and assayed (Table 1.2). Among the ketones tested, only isopropyl ketone 1.62 showed any significant activity, which was comparable to 1.39. Cyclohexyl derivatives 1.63 and 1.64 were inactive, potentially due to the aforementioned steric demands of the binding pocket.

Additional support for the hypothesis of a sterically demanding enzymatic pocket can be found among the aryl series. Phenyl derivative 1.56 demonstrated comparable activity to parent molecule 1.54, while naphthyl derivatives 1.57 and 1.58 showed no activity. Comparisons among electron-withdrawing-substituted aryls further support this hypothesis. Halogenated analogs 1.59 and 1.60 both showed an almost 4-fold increase in activity over the other synthetic.
constructs; meanwhile, trifluoromethyl aryl 1.61 demonstrated no inhibition of the enzyme, presumably due to the steric effects of the CF₃ group even while electronic considerations remained consistent.

![Chemical Structures](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀</th>
<th>Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.56</td>
<td>117 ± 68 µM</td>
<td>25 ± 3 µM</td>
</tr>
<tr>
<td>1.57</td>
<td>IC₅₀ &gt; 200 µM</td>
<td></td>
</tr>
<tr>
<td>1.58</td>
<td>IC₅₀ &gt; 200 µM</td>
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</tr>
<tr>
<td>1.59</td>
<td>IC₅₀ = 8 ± 1 µM</td>
<td>Ki = 5.7 ± 0.8 µM</td>
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<tr>
<td>1.60</td>
<td>IC₅₀ = 9.9 ± 0.6 µM</td>
<td>Ki = 6.9 ± 0.2 µM</td>
</tr>
<tr>
<td>1.61</td>
<td>IC₅₀ &gt; 200 µM</td>
<td></td>
</tr>
<tr>
<td>1.62</td>
<td>IC₅₀ = 73 ± 31 µM</td>
<td>Ki = 19 ± 0.3 µM</td>
</tr>
<tr>
<td>1.63</td>
<td>IC₅₀ &gt; 200 µM</td>
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</tr>
<tr>
<td>1.64</td>
<td>IC₅₀ &gt; 200 µM</td>
<td></td>
</tr>
</tbody>
</table>

1.9. Assessment of Synergistic Activity

With a series of ANT(2'')-Ia-inhibiting troponoids in hand, we subsequently sought to assess whether these molecules could rescue the activity of aminoglycoside antibiotics against ANT(2'')-Ia-expressing bacteria. Occasionally, molecules that demonstrate potent enzymatic activity will show a lack of antibacterial activity in cells.56 This is typically attributed to issues with cell permeability and/or the molecule being removed from the cell via efflux pumps. Therefore, a hyper-permeable, ANT(2'')-Ia-expressing strain of *E. coli* BW25113 was constructed,57 leading to gentamicin resistance (MIC of 64 µg/mL vs. 0.25 µg/mL for wild type).
These bacteria were treated with gentamicin and the αHTs in a checkerboard fashion in order to assess their ability to potentiate the activity of the antibiotic (Figure 1.8).

Quantitatively, synergy can be determined by calculating each molecule’s FIC index (FICI)\textsuperscript{58} according to CLSI guidelines (Eq. 1.1).\textsuperscript{59}

\[
FICI = \frac{MIC \text{ (gentamicin with } \alphaHT \text{)}}{MIC \text{ (gentamicin)}} + \frac{MIC \text{ (} \alphaHT \text{ with gentamicin)}}{MIC \text{ (tropolone)}}
\]

Eq. 1.1

FICI values of less than 0.5 are considered indicative of synergistic activity, while values of 0.5 – 4 are classified as indicative of no interaction, and FICI values > 4 demonstrate antagonistic activity.\textsuperscript{45} Qualitatively, the shaded portions of the 2D graphs of synergistic molecules (FICI < 0.5) are triangular in shape as a result of the concerted effects of the αHT and gentamicin.

![Figure 1.8](image.png)

**Figure 1.8.** Checkerboard analysis and FICI values of αHT inhibitors with gentamicin in the presence of ANT(2')-la-expressing *E. coli*. As a negative control, β-thujaplicinol was also tested with APH(2')-I\text{d}-expressing bacteria.
Consistent with the previous reports from Eli Lilly, β-thujaplicinol demonstrated synergy with gentamicin in our experiments (FICI = 0.375). As a negative control, an alternative enzyme conferring resistance to gentamicin (aminoglycoside phosphotransferase [APH(2’’)-Id]) that is uninhibited by αHTs was expressed by E. coli. β-thujaplicinol showed no synergy with this enzyme, which indicates that this activity is likely due to inhibition specifically of ANT(2’’)-Ia and not simply of ATP-binding proteins.

Synergistic activity was also observed with ketones 1.39 and 1.62. ANT(2’’)-Ia-inhibiting biaryls 1.54, 1.55, 1.59 and 1.60 were all synergistically inactive. While the reason for this activity is currently unclear, a few possibilities exist such as low cell permeability, active efflux from the cell, and off-target interactions of the αHTs.

Interestingly, the checkerboard analysis revealed a difference in relative activity between β-thujaplicinol and synthetic construct 1.39. At 12 μM of β-thujaplicinol and 1.39, gentamicin had an MIC of 8 μg/mL and 32 μg/mL, respectively (see Figure 1.8). This trend reflects the difference in activity determined in the ANT(2’’)-Ia inhibition assay. However, at a 50 μM concentration of β-thujaplicinol all bacterial cells had died, while a considerable amount of cells (~80%) remained at the same concentration of 1.39. At these higher concentrations of 1.39, no growth was seen at the lowest concentration of gentamicin tested (2 μg/mL). Thus, the highest non-toxic concentrations of 1.39 were more effective at restoring antibiotic activity than the highest non-toxic concentrations of β-thujaplicinol. Though not directly therapeutically useful, it does imply that 1.39 could have advantages over β-thujaplicinol in studying ANT(2’’)-Ia inhibition in cell-based assays where background antibacterial activity is a hindrance.
1.10. Conclusions

A library of αHTs were synthesized and tested for their inhibition of ANT(2′′)-Ia. Several were identified as potent inhibitors, and two were found to have additional specific synergistic activity with gentamicin against gentamicin-resistant, ANT(2′′)-Ia-expressing *E. coli*. One of these compounds has significantly less background antibacterial activity with respect to *E. coli* than the natural product lead, β-thujaplicinol, and could have advantages in the study of the enzyme in cell-based assays. These results validate the oxidopyrylium cycloaddition/ring-opening method as a viable approach to generating new ANT(2′′)-Ia inhibitors, and provide some preliminary insight into the structural changes required for inhibitory activity and cellular efficacy.

1.11. References


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The only non-troponoid ANT(2'')-Ia inhibitor that we are aware of are 1,3-diamines reported by Serpersu and coworkers, of which the most potent showed a K\textsubscript{i} value of 125 ± 24 μM. See: Welch, K. T.; Virga, K. G.; Whittemore, N. A.; Ozen, C.; Wright, E.; Brown, C. L.; Lee, R. E.; Serpersu, E. H. Bioorg Med. Chem., 2005, 13, 6252-6263.

(a) Wright, E.; Serpersu, E. J. Biochemistry 2005, 44, 11581. For further studies on the mechanism of ANT(2'')-Ia, see: (b) Cox, G.; Stogios, P. J.; Savchenko, A.; Wright, G. D. mBio.,


1.12. Supplementary Experimental Details

Drs. Michael P. D’Erasmo and Christine Meck performed some of the synthetic work and Dr. Georgina Cox performed the biological assays for this study. All of the experimental details can be found in the Supporting Information for the publications on which this chapter is based.\textsuperscript{S1,S2} This material is available free of charge at the ACS (http://pubs.acs.org) and at ScienceDirect (https://www.sciencedirect.com).

1.12.1. General Information

All starting materials and reagents were purchased from commercially available sources and used without further purification, with exception of CH\textsubscript{2}Cl\textsubscript{2}, which was purified on a solvent purification system prior to the reaction. \textsuperscript{1}H NMR shifts are measured using the solvent residual peak as the internal standard (CHCl\textsubscript{3} δ 7.26), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet), coupling constant (Hz), and integration. \textsuperscript{13}C NMR shifts are measured using the solvent residual peak as the internal standard (CDCl\textsubscript{3} δ 77.20), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Microwave reactions were performed via the Biotage Initiator 2.5. Purification via column chromatography was performed on the Biotage Isolera Prime, with Biotage SNAP 10g or 25g cartridges, in a solvent system of ethyl acetate and hexanes.


1.12.2. Synthesis and Characterization of 8-oxabicyclo[3.2.1]octenes (S1.1a-d)

![Chemical Structure](image)

**General Procedure.** To a solution of salt 1.30a and alkyne (5-10 equiv) in CHCl₃ (0.5 M) was added N,N-diisopropylaniline (1.2 equiv). The reaction mixture was subjected to microwave irradiation at 100 °C for one hour. Reaction mixture was then loaded directly onto column for chromatography and purified.

3-methoxy-5-methyl-6-(naphthalen-1-yl)-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (S1.1a). To a solution of salt 1.30a (108.2 mg, 0.373 mmol) and 1-ethynylnapthalene (527 μL, 3.73 mmol) in CDCl₃ (746 μL) was added N,N-diisopropylaniline (87 μL, 0.447 mmol). After microwave irradiation at 100 °C for 60 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 2-5% EtOAc in hexanes (8 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield S1.1a as an orange oil (85.7 mg, 79% yield). Rf= 0.30 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 3057 (w), 2978 (w), 2930 (w), 2836 (w), 1710 (s), 1605 (m) 1345 (w), 1268 (w), 1132 (m), 1117 (w), 989 (w), 778 (w) cm⁻¹. **¹H NMR (200 MHz, CDCl₃)** δ 8.05 – 7.94 (m, 1H), 7.91 – 7.67 (m, 2H), 7.58 – 7.35 (m, 3H), 7.22 (d, J = 1.2 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.16 (s, 1H), 5.18 (d, J = 2.5 Hz, 1H), 3.67 (s, 3H), 1.51 (s, 3H). **¹³C NMR (100 MHz, CDCl₃)** δ 190.2 (s), 156.5 (s), 145.9 (s), 134.0 (s), 131.6 (s), 131.6 (s), 128.8 (s), 128.7 (s), 126.8
(s), 126.7 (s), 126.5 (s), 125.7 (s), 125.1 (s), 123.7 (s), 120.2 (s), 88.2 (s), 86.8 (s), 55.0 (s), 21.6 (s).

3-methoxy-5-methyl-6-(naphthalen-2-yi)-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (S1.1b). To a solution of salt 1.30a (17.0 mg, 0.058 mmol) and 2-ethynlnaphthalene (88.9 mg, 0.584 mmol) in CDCl₃ (117 μL) was added N,N-diisopropylaniline (13.7 μL, 0.070 mmol). After microwave irradiation at 100 °C for 60 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 2-5% EtOAc in hexanes (8 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield S1.1b as a yellow oil (14.4 mg, 84% yield). Rf= 0.28 in 25% EtOAc in hexanes. IR (thin film, KBr) 3056 (w), 2963 (w), 2933 (w), 2837 (w), 1708 (m), 1605 (w), 1174 (w), 1344 (w), 1131 (m), 1101 (w), 867 (w), 694 (w) cm⁻¹. ¹H NMR (200 MHz, CDCl₃) δ 7.89 – 7.80 (m, 3H), 7.71 (d, J = 1.5 Hz, 1H), 7.55 – 7.48 (s, 1H), 7.44 (d, J = 1.7 Hz, 1H), 7.40 (d, J = 1.8 Hz, 1H), 6.41 (d, J = 2.5 Hz, 1H), 6.30 (s, 1H), 5.05 (d, J = 2.5 Hz, 1H), 3.64 (s, 3H), 1.76 (s, 3H). ¹³C NMR (50 MHz, CDCl₃) δ 190.2 (s), 159.0 (s), 146.4 (s), 133.5 (s), 133.4 (s), 130.7 (s), 128.9 (s), 128.5 (s), 128.1 (s), 127.1 (s), 127.0 (s), 125.0 (s), 124.4 (s), 123.7 (s), 119.5 (s), 86.8 (s), 86.2 (s), 55.1 (s), 22.7 (s).

6-(4-chlorophenyl)-3-methoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (S1.1c). To a solution of salt 1.30a (100.0 mg, 0.344 mmol) and 1-chloro-4-ethynylbenzene (469.83 mg, 3.44 mmol) in CHCl₃ (689 μL) was added N,N-diisopropylaniline (80 μL, 0.413 mmol). After microwave irradiation at 100 °C for 60 min, the reaction mixture was
purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 2-5% EtOAc in hexanes (8 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield S1.1c as a yellow solid (37.8 mg, 40% yield). Melting Point (MP) = 138-141 °C. Rf= 0.27 in 25% EtOAc in hexanes. IR (thin film, KBr) 3066 (w), 2974 (w), 2935 (w), 2839 (w), 1711 (s), 1604 (m), 1490 (w), 1174 (w), 1131 (w), 1092 (w), 864 (w), 827 (w) cm\(^{-1}\). \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 7.36 (d, \(J = 8.7\) Hz, 2H), 7.21 (d, \(J = 8.7\) Hz, 2H), 6.29 (d, \(J = 2.3\) Hz, 1H), 6.16 (s, 1H), 4.98 (d, \(J = 2.5\) Hz, 1H), 3.60 (s, 3H), 1.66 (s, 3H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 189.9 (s), 157.9 (s), 146.2 (s), 134.8 (s), 131.7 (s), 129.2 (s), 127.5 (s), 123.9 (s), 119.2 (s), 86.5 (s), 85.9 (s), 55.0 (s), 22.3 (s).

3-methoxy-5-methyl-6-(4-(trifluoromethyl)phenyl)-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (S1.1d). To a solution of salt 1.30a (100.2 mg, 0.345 mmol) and 1-ethynyl-4-(trifluoromethyl)benzene (563 \(\mu\)L, 3.45 mmol) in CDCl\(_3\) (690 \(\mu\)L) was added N,N-diisopropylaniline (80 \(\mu\)L, 0.414 mmol). After microwave irradiation at 100 °C for 60 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 2-5% EtOAc in hexanes (8 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield S1.1d as a yellow oil (77.6 mg, 72% yield). Rf= 0.30 in 25% EtOAc in hexanes. IR (thin film, KBr) 3063 (w), 2981 (w), 2938 (w), 2840 (w), 1713 (s), 1606 (m), 1410 (w), 1327 (s), 1129 (s), 1016 (m), 865 (w), 832 (w) cm\(^{-1}\). \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 7.63 (d, \(J = 8.1\) Hz, 2H), 7.38 (d, \(J = 8.1\) Hz, 2H), 6.40 (d, \(J = 2.5\) Hz, 1H), 6.16 (s, 1H), 5.01 (d, \(J = 2.5\) Hz, 1H), 3.60 (s, 1H), 1.66 (s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 189.7
(s), 157.8 (s), 146.3 (s), 136.9 (s), 130.9 (q, $J = 32.8$ Hz), 126.6 (s), 126.0 (q, $J = 3.8$ Hz), 125.9 (s), 124.2 (q, $J = 272.1$ Hz), 118.9 (s), 86.6 (s), 86.2 (s), 55.1 (s), 22.3 (s).

### 1.12.3. Synthesis and Characterization of aHTs via One-Pot TfOH/HBr/AcOH

#### General Procedure.

In a 15 mL round-bottom flask was placed a solution of 8-oxabicyclo[3.2.1]octene S1.1 in CHCl$_3$ (0.1 M). To the solution at rt was added 4 equivalents of triflic acid. The mixture was stirred for 30 minutes before quenching with an equivalent volume of pH 7 phosphate buffer and extracting with CH$_2$Cl$_2$ to isolate the methoxytropolone. To this compound was added HBr in AcOH (33%) at reflux for 4 hours. Reaction mixture was then quenched to pH 5 using a pH 7 phosphate buffer and extracted with CH$_2$Cl$_2$.

#### 2,7-dihydroxy-4-methyl-5-(naphthalen-1-yl)cyclohepta-2,4,6-trienone (1.57).

To a solution of bicycle S1.1a (35.6 mg, 0.1218 mmol) in CDCl$_3$ (1.22 mL) was added triflic acid (43.1 μL, 0.487 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching to pH 7 with pH 7 phosphate buffer and extracting with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure.

To the isolated methoxytropolone (18.2 mg) was added 800 μL 33% HBr/AcOH solution. The reaction was heated to reflux (120 °C) for 7 hours before being quenched to pH 6 with 10 mL of pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under
reduced pressure to yield **1.57** as a brown oil (13.4 mg, 77% yield). **IR (thin film, KBr)** 3248 (br), 1526 (m), 1447 (w), 1383 (m), 1278 (w), 1239 (w), 1220 (w), 1086 (w), 783 (w), 730 (w) cm⁻¹.

**¹H NMR (200 MHz, CDCl₃)** δ 7.92 (dd, J = 7.8, 4.3 Hz, 2H), 7.63 (s, 1H), 7.52 (s, 1H), 7.61 – 7.27 (m, 5H), 2.08 (s, 3H). **¹³C NMR (100 MHz, CDCl₃)** δ 167.9 (s), 158.3 (s), 156.8 (s), 147.4 (s), 142.1 (s), 138.9 (s), 130.9 (s), 130.6 (s), 130.3 (s), 129.2 (s), 129.1 (s), 126.0 (s), 125.7 (s), 124.5 (s), 123.7 (s), 123.0 (s), 30.0 (s), 26.5 (s).

**2,7-dihydroxy-4-methyl-5-(naphthalen-2-yl)cyclohepta-2,4,6-trienone (1.58).** To a solution of bicycle **S1.1b** (42.4 mg, 0.145 mmol) in CDCl₃ (1.45 mL) was added triflic acid (51.3 μL, 0.580 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching to pH 6 with pH 7 phosphate buffer and extracting with CH₂Cl₂ (5 x 10 mL). Combined organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. To the isolated methoxytropolone (15.6 mg) was added 686 μL 33% HBr/AcOH solution. The reaction was heated to reflux (120 °C) for 8 hours before being quenched to pH 6 with 10 mL of pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH₂Cl₂ (5 x 10 mL). Combined organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield **1.58** as a brown oil (12.5 mg, 85% yield). **IR (thin film, KBr)** 3248 (br), 1526 (w), 1446 (w), 1392 (w), 1284 (w), 1231 (w), 1205 (w), 1122 (w), 1092 (w), 907 (w), 858 (w) 795 (w), 730 (w) cm⁻¹. **¹H NMR (200 MHz, CDCl₃)** δ 7.89 (m, 3H), 7.71 (s, 1H), 7.61 (s, 1H), 7.59 (s, 1H), 7.55 (dd, J = 2.9 Hz, 1H), 7.55 (d, J = 9.5 Hz, 1H), 7.36 (dd, J = 8.4, 1.6 Hz, 1H), 2.29 (s, 3H). **¹³C NMR (100 MHz, CDCl₃)** δ 167.62 (s), 158.13 (s), 156.79 (s), 143.95 (s), 141.39 (s), 139.43 (s), 133.58 (s), 132.87 (s), 128.64 (s), 128.40 (s), 128.14 (s), 127.52 (s), 127.06 (s), 126.87 (s), 126.76 (s), 124.59 (s), 53.75 (s), 26.82 (s).
4-(4-bromophenyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (1.59). To a solution of previously-made 8-oxabicyclo[3.2.1]octene intermediate\textsuperscript{3} (17.6 mg, 0.0548 mmol) in CDCl\textsubscript{3} (548 μL) was added triflic acid (19.4 μL, 0.219 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching to pH 6 with pH 7 phosphate buffer and extracting with CH\textsubscript{2}Cl\textsubscript{2} (5 x 10 mL). Combined organics were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated under reduced pressure. To the isolated methoxytropolone (12.5 mg) was added 550 μL 33% HBr/AcOH solution. The reaction was heated to reflux (120 °C) for 4 hours before being quenched to pH 5 with 10 mL of pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (5 x 10 mL). Combined organics were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated under reduced pressure to yield 1.59 as an orange/brown oil (10.3 mg, 86% yield). IR (thin film, KBr) 3248 (br), 1528 (m), 1487 (w), 1447 (w), 1388 (w), 1279 (w), 1208 (w), 1128 (w), 1092 (w), 1072 (w), 1012 (w), 814 (w), 669 (w) cm\textsuperscript{-1}. \textsuperscript{1}H NMR (200 MHz, CDCl\textsubscript{3}) δ 7.58 (d, J = 6.8 Hz, 2H), 7.43 (s, 1H), 7.26 (s, 1H), 7.12 (d, J = 8.4 Hz, 2H), 2.23 (s, 3H). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) δ 167.61 (s), 158.35 (s), 156.55 (s), 142.76 (s), 142.52 (s), 139.09 (s), 132.21 (s), 130.36 (s), 124.54 (s), 124.00 (s), 122.43 (s), 26.79 (s).

4-(4-chlorophenyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (1.60). To a solution of bicycle S1.1c (19.2 mg, 0.0694 mmol) in CHCl\textsubscript{3} (694 μL) was added triflic acid (24.5 μL, 0.278 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching with sodium acetate (56.9 mg, 0.693 mmol). After stirring for an additional 15 minutes, the solution was concentrated under reduced pressure. To the mixture was added 696 μL AcOH and 151 μL 33% HBr/AcOH solution. The reaction was heated to 90 °C for 4 hours before being quenched to pH 5 with 10 mL of pH 7 phosphate buffer. The organic layer
was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Upon finding clean conversion to the methoxytropolone, the product was resubjected to the HBr/AcOH reflux conditions (847 μL 33% HBr/AcOH solution, 120 °C) for two hours to yield 1.60 as a black oil (16.5 mg, 90% yield). IR (thin film, KBr) 3066 (w), 2974 (w), 2935 (w), 2839 (w), 1711 (s), 1604 (m), 1490 (w), 1174 (w), 1131 (w), 1092 (w), 864 (w), 827 (w) cm$^{-1}$. $^1$H NMR (200 MHz, CDCl$_3$) δ 7.56 (s, 1H), 7.43 (s, 1H), 7.42 (d, $J = 8.3$ Hz, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 2.24 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 167.62 (s), 158.11 (s), 156.78 (s), 142.55 (s), 139.09 (s), 134.04 (s), 130.14 (s), 129.31 (s), 124.55 (s), 124.04 (s), 29.99 (s), 26.83 (s).

2,7-dihydroxy-4-methyl-5-(4-(trifluoromethyl)phenyl)cyclohepta-2,4,6-trienone (1.61). To a solution of bicycle S1.1d (27.3 mg, 0.0880 mmol) in CDCl$_3$ (880 μL) was added triflic acid (31.1 μL, 0.352 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching to pH 7 with pH 7 phosphate buffer and extracting with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. To the isolated methoxytropolone (10.5 mg) was added 461 μL 33% HBr/AcOH solution. The reaction was heated to reflux (120 °C) for 7 hours before being quenched to pH 5 with 10 mL of pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 1.61 as a brown oil (6.3 mg, 63% yield). IR (thin film, KBr) 3247 (br), 1617 (w), 1530 (m), 1392 (w), 1324 (s), 1281 (w), 1166 (w), 1126 (m), 1068 (m), 1017 (w), 822 (w) cm$^{-1}$. $^1$H NMR (200 MHz, CDCl$_3$) δ 7.72 (d, $J = 8.5$ Hz, 2H), 7.58 (s, 1H), 7.41 (s, 1H), 7.38 (d, $J = 8.5$ Hz, 2H), 2.24 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 167.62 (s), 158.11 (s), 156.78 (s), 142.55 (s), 139.09 (s), 134.04 (s), 130.14 (s), 129.31 (s), 124.55 (s), 124.04 (s), 29.99 (s), 26.83 (s).
MHz, CDCl$_3$) δ 167.8 (s), 158.3 (s), 156.8 (s), 147.4 (s), 142.1 (s), 138.9 (s), 130.4 (q, $J = 32.7$ Hz), 129.1 (s), 126.0 (q, $J = 3.7$ Hz), 124.5 (s), 124.3 (q, $J = 270.0$ Hz) 123.7 (s), 26.7 (s).

1.12.4. NMR Spectra of Synthesized Compounds

$^1$H NMR of S1.1a

$^{13}$C NMR of 5e
$^{1}H$ NMR of S1.1b

$^{13}C$ NMR of S1.1b
$^1$H NMR of S1.1c

$^{13}$C NMR of S1.1c
$^1$H NMR of S1.1d

$^{13}$C NMR of S1.1d
$^{1}H$ NMR of 1.57

$^{13}C$ NMR of 1.57
$^1$H NMR of 1.58

$^{13}$C NMR of 1.58
$^1$H NMR of 1.59

$^{13}$C NMR of 1.59
$^1$H NMR of 1.60

$^{13}$C NMR of 1.60
$^1$H NMR of 1.61

$^{13}$C NMR of 1.61
1.12.5. Supplementary References


Chapter II

Synthesis and Biological Assessment of 3,7-Dihydroxytropolones

2.1. Introduction

7-Hydroxytropolones (α-hydroxytropolones, or αHTs; 2.1a) and 3,7-dihydroxytropolones (3,7-dHTs, 2.1b) (Scheme 2.1A) are troponoids with three and four contiguous oxygen atoms, respectively, and display an extraordinarily broad range of biological activity. While this activity is most often attributed in both cases to their ability to serve as metal binding fragments for many physiologically relevant dinuclear metalloenzymes (2.2, Scheme 2.1A), stark differences in bioactivity have been observed between them. For example, αHTs appear to be substantially more potent than 3,7-dHTs as inhibitors of HIV ribonuclease H, a promising target for HIV antivirals that remains untargeted clinically, as well as aminoglycoside-2″-O-nucleotidyltransferase, one of the most common determinants of enzyme-dependent aminoglycoside resistance in Pseudomonas aeruginosa. On the other hand, the 3,7-dHT natural product puberulic acid has demonstrated potent

![Figure 2.1. (A) Contiguously hydroxylated tropolones and common mode of metalloenzyme binding. (B) A divergent, unifying galactose approach towards both tropolone classes. (C) Overview of kojic acid route developed by our lab and extended in this study to 3,7-dHTs.](image)
antimalarial activity, with selectivity on par with clinical agent artesunate, whereas in similar assays αHTs have substantially lower potency and selectivity.\textsuperscript{5} 3,7-dHT also possesses activity against B16 melanoma cells an order of magnitude greater than αHTs, and increases the lifespan of mice bearing B16 melanoma comparably to mice administered the chemotherapeutic agent mitomycin C.\textsuperscript{6}

In order to better understand the nuances of structure-bioactivity differences between αHTs and 3,7-dHTs, efficient synthetic methods are needed to access both classes, and of particular value would be those that also allow comparable substitution.\textsuperscript{7} Recently, a galactose-based method previously used by Sunazuka and Omura to synthesize puberulic acid\textsuperscript{8} was further adapted to develop a small library of both 3,7-dHTs and αHTs (\textbf{Scheme 2.1B}), as well as other related troponoids.\textsuperscript{9} Testing of these molecules for their antimalarial activity revealed that a carboxylic acid was key to providing selectivity against \textit{Plasmodium falciparum K1} versus MRC-5 cells, and it did so whether on an αHT or a 3,7-dHT, but provided more selectivity with 3,7-dHT. These studies highlight the value of a divergent, unifying strategy in SAR determination of oxygenated troponoids, and their importance in assessing the inhibitory potential of these oxygenated troponoids.

Our lab has previously reported an extremely efficient oxidopyrylium cycloaddition/ring opening strategy for αHT synthesis that has to date generated over 50 published αHTs with varying substitution patterns,\textsuperscript{10,11} and has been used to provide SAR and pursue synthetic chemistry-based optimization studies related to various human diseases.\textsuperscript{12-18} We herein report the adaptation of this route to access a series of 3,7-dHTs. We highlight this strategy through the synthesis of a potential biosynthetic precursor to the 3,7-dHT natural products puberulonic and puberulic acid. The molecules were assessed as part of biochemical and biological studies related to HIV, hepatitis B
virus (HBV), and herpes simplex virus (HSV)-1 and -2. These studies help provide an understanding of the advantages and disadvantages of 3,7-dHTs and αHTs as chemotypes for drug discovery and development.

2.2. Synthesis of 3,7-Dihydroxytropolones

2.2.1. Preliminary Library of 3,7-dHTs

Our synthesis started with a cycloaddition between oxidopyrylium trflate salt 2.8a, which can be made on gram scale in 3 steps from commercially-available kojic acid, and iodopropiolates (Scheme 2.2A, 2.12a/b), which are prepared from the corresponding propiolates using silver nitrate and N-iodosuccinimide. Bromopropiolates are also capable of efficient oxidopyrylium cycloaddition and have been used in the synthesis of bromohydroxytropolones, but their volatility and strong lachrymator properties pose technical challenges.
The iodobicycles can then be converted into methoxybicycles through a DMAP-catalyzed methanolysis (2.13a/b → 2.14a/b). These conditions were deemed necessary when we found that Brønsted base-mediated methanolysis led to dimethyl acetals (e.g. 2.13d), while DMAP incorporation was observed as the major product when chloroform was employed as the solvent in an attempt at hydrolysis (2.13c, Scheme 2B). Interestingly, subsequent ring-opening attempts on these bicycles (2.14a/b → 2.15a/b) using sulfonic acids led to formation of oxidopyrylium dimers as the major product, as observed by crude ¹H-NMR (e.g. 2.17a, Scheme 2.2C). We hypothesize that this may proceed via protonation and elimination of the β-methoxyenoate 2.20, which would lead to a rapidly dimerizing oxidopyrylium ylide (2.8* → 2.17a). Fortunately, an alternative procedure using the Lewis acid boron trichloride promoted clean conversion to 3,7-dimethoxytropolones 2.15a and 2.15b, which were subsequently converted to the 3,7-dHTs 2.16a and 2.16b using hydrobromic acid in acetic acid at elevated temperatures.

Additionally, in the presence of excess water along with extended reaction times, methyl ester 2.15a was readily decarboxylated (2.16c, Scheme 2.2D), which can be attributed to the participation of a β-keto acid tautomer. Thus, while the ester provides a convenient synthetic handle for efficient oxidopyrylium cycloaddition and subsequent iodide for methoxide exchange, it can be readily removed if desired.

2.2.2. Synthetic Efforts Towards Total Synthesis of a Potential Biosynthetic Intermediate

With an efficient method for 3,7-dHT synthesis in hand, we sought to test the new strategy in total synthesis. The two most widely studied 3,7-dHTs are puberulonic and puberulic acid, which have been isolated from the fungus Penicillum puberulum. These natural products are also close structural homologs of the 6-hydroxytropolones stipitatonic and stipitatic acid, the focus of
recent biosynthetic studies by Cox and coworkers.\textsuperscript{25} These studies demonstrated that stipitatic acid is formed from stipitatonic acid, which is, in turn, formed from the lactone stipitalide (2.21, Scheme 2.3). While a similar biosynthetic pathway seems plausible for puberulonic and puberulic acid, the 3,7-dHT stipitalide homolog 2.16d is unknown. Access to this molecule could be useful in elucidating the biosynthesis of puberulic and puberulonic acid, and our synthetic strategy appeared uniquely well suited for this task. Thus, we set out to conduct a total synthesis of 2.16d.

We began our synthesis with the chloromethylene-containing oxidopyrylium salt 2.8b (Scheme 2.4). Initial attempts towards bicycle 2.13e using our prior conditions (‘a’, Scheme 2.4) lead to a significant amount of compound 2.13b, which created purification challenges given the similar polarities of 2.13b and 2.13e. We hypothesized that this product was formed through a hydride transfer from the \( N,N \)-diisopropylaniline,\textsuperscript{26} and thus, leveraging the readily reversible nature of this class of oxidopyrylium ylides (formed following deprotonation of 2.8b),\textsuperscript{10} we synthesized and isolated dimer 2.17b as the source of the ylide.\textsuperscript{27} Indeed, this alternative procedure led to the dihalogen 2.13e without any noticeable 2.13b. While this intermediate offered two electrophilic handles, namely a \( \beta \)-iodoacrylate for methanolysis and a primary alkyl chloride for eventual lactonization, we reasoned that the alkyl chloride would be stable to the methanolysis conditions because of the adjacent tertiary center. Indeed, methanolysis of 2.13e proceeded efficiently while maintaining the chloride’s integrity, even though higher temperatures were
needed for this transformation than for 2.13a/b. Subsequent ring-opening afforded methoxytropolone 2.15c, which was then advanced to 2.16d through a three-step acetylation/lactonization/demethylation sequence. During the course of these studies, we also discovered that reverse-phase chromatography of 2.15c facilitated on-column lactonization to afford 2.15e, providing a more direct synthetic method.

2.3. Biological Studies of αHTs versus 3,7-dHTs

2.3.1. HIV-Associated Activity

One of the more active areas of research in our group has been αHT antiviral development, as several viral nucleases have been identified as targets for these molecules. The most widely studied in these contexts is the ribonuclease H function of HIV reverse transcriptase (HIV RT RNaseH), a promising candidate for therapeutic development that remains untargeted
clinically. αHTs are potent inhibitors of the enzyme with 50% inhibition concentrations (IC_{50}) less than 200 nM, and several synthetic αHTs have been developed with modest antiviral activity in cell-based assays. While both 3,7-dHTs and αHTs are known inhibitors of the enzyme, and the collection of literature sources indicate that αHTs are superior inhibitors, head-to-head comparisons of both classes of molecules have not to the best of our knowledge been conducted. We thus tested 3,7-dHTs **2.16a–2.16d**, αHT congener **2.26**, and methoxytropolone congeners **2.15a** and **2.27** for their ability to inhibit HIV-1 RT RNaseH.

![Chemical structures of compounds](image)

<table>
<thead>
<tr>
<th>Cmpd.</th>
<th>HIV-1 RT RNase H (IC_{50}, μM)^a</th>
<th>Antiviral (EC_{50}, μM)^b</th>
<th>Cytotoxicity (CC_{50}, μM)^c</th>
<th>Antiviral (EC_{50}, μM)^d</th>
<th>Cytotoxicity (CC_{50}, μM)^e</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.16a</td>
<td>1.5 ± 0.02</td>
<td>n.p.</td>
<td>1.4</td>
<td>(**) 6.0 ± 6.3</td>
<td>6.0 ± 3.6</td>
</tr>
<tr>
<td>2.16b</td>
<td>1.1 ± 0.04</td>
<td>n.p.</td>
<td>0.77</td>
<td>(**) 7.7 ± 1.0</td>
<td>12.2 ± 7.5</td>
</tr>
<tr>
<td>2.16c</td>
<td>1.3 ± 0.03</td>
<td>n.p.</td>
<td>2.1</td>
<td>-</td>
<td>10.2 ± 4.9</td>
</tr>
<tr>
<td>2.16d</td>
<td>1.2 ± 0.05</td>
<td>n.p.</td>
<td>19</td>
<td>(*) 42 ± 0.1</td>
<td>61</td>
</tr>
<tr>
<td>2.15a</td>
<td>13.5 ± 0.4</td>
<td>n.p.</td>
<td>32</td>
<td>(*) 5.5 ± 2.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2.26</td>
<td>0.28 ± 0.003</td>
<td>5.3</td>
<td>8.5</td>
<td>(**) 1.4 ± 0.7</td>
<td>92</td>
</tr>
<tr>
<td>2.27</td>
<td>14.7 ± 0.3</td>
<td>n.p.</td>
<td>&gt;50</td>
<td>-</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

**Figure 2.1.** Viral ribonuclease H-related studies. Compounds tested in antiviral assays with summary of data from RNase H-related biological studies for HIV and HBV. ^a^HIV-1 RT RNaseH IC_{50} values are reported as the average from triplicate assays ± standard deviation. ^b^Concentration of 50% viral replication suppression (EC_{50}) are reported from a single run. n.p. Reflects not protective. ^c^CC_{50} represents concentration at 50% cytotoxicity against the host CEM-SS cells, and are calculated from a single experiment. ^d^HBV replication inhibition at 20 μM, unless otherwise defined. ** Indicates that the amount of (+)-strand DNA is <25% and (-)-strand DNA is >60% relative to DMSO control. * Indicates that the amount of (+)-strand DNA is <50% of the amount of (-)-strand DNA relative to DMSO control. - refers to no evidence of selective inhibition of (+)-strand DNA. EC_{50} values are shown for active compounds, and are reported as the average of two or three dose response curves ± standard deviation. ^e^HepDES19 cytotoxicity values are likewise reported as an average of 2-4 assays ± standard deviation.
Consistent with literature trends, αHT 2.26 was the most potent of the compounds tested, with a 4–5 fold increase over the 3,7-dHTs (Figure 2.1). Methoxytropolones were even less potent, consistent with tropolone activity. Furthermore, while promising cell-based HIV antiviral activity of 3,7-dHT has been described in the literature, our synthetic 3,7-dHTs showed no protective effects, possibly due to a combination of weaker anti-RNaseH activity and higher cytotoxicity. Compound 2.26, which is known to have modest HIV protective effects, was tested simultaneously as a positive control. Thus, early indications suggest that the extra hydroxyl in 3,7-dHTs is unfavorable for HIV therapeutic development targeting RNaseH.

2.3.2. HBV-Associated Activity

Hepatitis B also has an RNase H function that has become a target for antiviral drug development, and αHTs have emerged as one of the most promising scaffolds. A 3,7-dHT has never been tested for this activity, and thus we assessed our new compounds’ HBV RNase H-specific antiviral activity by monitoring the amount of viral (+)-DNA strand versus (−)-DNA, since inhibiting RNase H activity suppresses production of the former. As expected, αHT 2.26 showed only minor cytotoxicity against the host cell line, HepDES19, while selectively inhibiting synthesis of the viral (+)-DNA. Selective inhibition of viral (+)-DNA was also observed with 3,7-dHTs 2.16a and 2.16b, although they were significantly more cytotoxic. It is worth noting the structural similarities of the two selective inhibitors, 2.16a and 2.16b, to compound 2.26 and the lack of activity of compound 2.16c, which could indicate an important role of the carbonyl appendage. Unfortunately, the EC50 values of the viral suppression of 2.16a/b coupled with their CC50 values revealed virtually no therapeutic window, as compared to the 60-fold window of αHT 2.26. Thus, while these experiments demonstrate antiviral potential for 3,7-dHTs that may warrant
further studies, early indications suggest they are not advantageous over αHTs. On the other hand, the 3,7-dimethoxytropolone 2.15a displayed moderate inhibition selectivity for (+)-DNA, had an antiviral EC\textsubscript{50} value of 5.5 μM, and was nontoxic at concentrations of up to 100 μM. Thus, 3,7-dimethoxytropolone appears to be a viable chemotype for further anti-HBV development.

2.3.3. HSV-associated activity

While 3,7-dHTs had never been tested for anti-HSV activity prior to our studies, αHTs were identified as anti-HSV agents, with the natural product manicol displaying antiviral EC\textsubscript{50} values against HSV-1 and -2 of 350 nM and 580 nM, respectively.\textsuperscript{32} Furthermore, tests against 20 synthetic αHTs synthesized through our oxidopyrylium cycloaddition/ring opening procedure identified a synthetic αHT with antiviral EC\textsubscript{50} values against HSV-1 and -2 of 120 nM and 80 nM, respectively (see 2.28, Figure 2.3).\textsuperscript{15} In all cases, cytotoxicity was not observed against Vero cells within the 24 hours experiment. We thus tested our library of tropolones and found that 3,7-dHTs 2.16a, 2.16b, and 2.16c all strongly inhibited replication of HSV-1 at a concentration of 5 μM, with suppression levels comparable to the therapeutic anti-HSV agent acyclovir (ACV) and exceeding that of cidofovir (CDV) (Figure 2.2A). αHT 2.26 only showed moderate replication inhibition at 5 μM, and no activity at 1 μM, whereas the activity for 2.16a–2.16c was maintained. Methoxytropolones 2.15a and 2.27 were the least active compounds among the series tested. 3,7-dHT 2.16d was inactive, illustrating how changes in the side-chains can impact activity. 3,7-dHTs 2.16a–c inhibited HSV-2 replication with comparable suppression levels and potencies, and this activity was maintained against three other HSV-2 clinical isolates at 5 μM (Figure 2.2B). The EC\textsubscript{50} values of compounds 2.16a and 2.16c were comparable to ACV for HSV-1, and showed no cytotoxicity versus the Vero cell line at up to 100 μM for 24 hours (Figure 2.2C). Furthermore,
while ACV was nearly an order of magnitude less potent against HSV-2 than HSV-1, 2.16a and 2.16c maintained significant potency.

Figure 2.2. HSV-related studies. (A) HSV-1 suppression by synthetic tropolones at concentrations of 5 and 1 μM. Also shown are clinical anti-HSV agents acyclovir (ACV) and cidofovir (CDV) at 5 μM. (B) Replication inhibition studies of 3,7-dHTs at 5 μM. L/R 2.16a-d against HSV-2, compound 2.16a against 3 HSV-2 clinical isolates, and comparisons of 2.16a and ACV against wild-type (WT) and thymidine kinase negative (TK) HSV strains resistant to ACV. (C) EC50 values obtained for 2.16a and 2.16c against HSV-1 and HSV-2, with data representing the average from 2 dose response curves +/- standard deviation. Also shown are values for ACV obtained previously by Tavis et al. under identical conditions. EC50 represents concentration at 50% cytotoxicity against the host Vero cells. n.d. = not determined.
2.3.4. Mechanistic Insights on Mode of Action

Although the mechanism of action is currently under investigation, experiments with 2.16a against the ACV-resistant TK-HSV-1 and HSV-2 strains confirmed a different mechanism of action than ACV (Figure 2.2B). One hypothesis is that the molecules could engage one or more viral nucleotidyltransferases. For example, the HSV-1 DNA packing terminase pUL15 encodes a C-terminal nuclease activity that can be inhibited by αHTs. Thus, we tested 3,7-dHTs against recombinant pUL15C and found them to be inhibitors of the enzyme, but with less potency than αHT 2.26 (Figure 2.2C).

Given the SAR suggesting benefits of the additional oxygen for HSV inhibition potency, a 3,7-dHT analog of the most potent anti-HSV αHT described to date, compound 2.28, was synthesized (2.16e, Scheme 2.5).

![Scheme 2.5. Synthesis of 3,7-dHT 2.16e. Reagents and conditions: (a) AgNO3, N-iodosuccinimide, acetone, 0 °C, 4 h, 57%, (b) 2.12d and 2.17a, CH2Cl2, 120 °C, 30 min (c) MeOH/DMAP, 70 °C, 15 min, 68% over two steps. (d) BCl3, CH2Cl2, 0 °C, 6 min, 84%. (e) HBr/AcOH, 120 °C, 70 min, 70%.](image)

Anti-HSV-1 activity was assessed in parallel with 3,7-dHT 2.16a and αHTs 2.28 and 2.26 (Figure 2.3B). As expected, compound 2.26 showed no antiviral activity, even at 5 μM, whereas some viral suppression was observed for 2.28, 2.16a and 2.16e. At 1 μM, 2.16e and 2.28 antiviral activity remained at a maximum, but activity of 2.16a began to drop. At 200 nM, 2.16a was inactive, and both 2.28 and 2.16e began to lose some activity. EC50 values of 2.16e were ~50 nM against both
HSV-1 and -2, which is the most potent anti-HSV activity of a tropolone we have found to date. Unfortunately, moderate cytotoxicity of 2.16e was also observed.

While increases in potency against HSV were anticipated through additive effects, surprising effects were observed with pUL15 inhibition. While both a change from an αHT to a 3,7-dHT (2.26 → 2.16a), and from a methyl ester to a biphenyl ketone (2.26 → 2.28) resulted in a decrease in pUL15 potency, changing both provided an increase in potency (2.26 → 2.16e). One hypothesis for this change is that the added hydroxyl permits the biphenyl side chain to adopt a new configuration that would promote new favorable contacts (Figure 2.4A vs. B). *In silico* modelling was thus carried out by superimposing the active site of the crystal structure of pUL15C (PDB id 4IOX)\(^{34}\) to the active site of the crystal structure of HIV RT RNase H bound to manganese cations and β-thujaplicinol (PDB id 3K2P).\(^{35}\) All computational work was performed by Prof. Emilio Gallicchio and Rajat Pal. The resulting structure of pUL15C was refined by energy minimization and simulated annealing using the Impact molecular modeling program.\(^{36}\) The receptor grid was generated using the default parameters available in the Schrodinger Suite 2016-
3 with one special adjustment where metal constraints were applied to allow metal–ligand interaction at the binding site. Glide docking\textsuperscript{37} were performed with the ligands αHT 2.28 and 3,7-dHT 2.16e. Several binding poses were obtained for the molecule 2.16e, out of which the best binding pose is shown in Figure 2.4.

This conformation reveals favorable π–cation interaction between the quaternary ammonium cation of Lys 640 and the phenyl ring π system of the biphenyl group of the ligand. The biphenyl group is thought to be further stabilized by accommodating within a hydrophobic groove formed between Lys 640 and Asn 583 with possible hydrophobic interaction with the Leu 636 present inside the groove. Further stabilization could also be achieved through interactions of the biaryl side chain with Asn 583 through NH–π interactions. These interactions are never observed in in silico modelling with 2.28. While further studies are needed to understand the specific mechanism of this increase in activity, these analyses highlight the consequences of the additional binding modes allowed by 3,7-dHTs, which might also result in greater likelihood of off-target effects and be a reason for the higher cytotoxicity observed with this class of tropolones.
2.4. Conclusions

We have developed an oxidopyrylium cycloaddition/ring opening approach for the synthesis of 3,7-dHTs. This strategy was applied in the synthesis of a prospective biosynthetic precursor to the natural products puberulonic and puberlic acid. Finally, the new 3,7-dHTs were tested for antiviral activity against HIV, HBV and HSV. Through these studies we found that 3,7-dHTs are weaker inhibitors than αHTs against HIV RNaseH, revealed 3,7-dimethoxytropolone as a promising chemotype for HBV-based antiviral development, and demonstrated that 3,7-dHTs can have greater potency against HSV than analogous αHTs. Furthermore, enzymatic inhibition studies coupled with preliminary in silico modelling highlight how additional configurations of 3,7-dHTs may manifest themselves in unexpected potency increases, and could also be responsible for observed cytotoxicity increases.
2.5. References


For a prior example where purified oxidopyrylium dimer was advantageous, see: D’Erasamo, M. P.; Meck, C.; Lewis, C. A.; Murelli, R. P. J. Org. Chem., 2016, 81, 3744–3751.


2.6. Supplementary Experimental Details

Daniel Schiavone performed some of the synthetic work described in this study. The biological assays were performed by the Morrison, Tavis, Beutler, and Le Grice labs. Homology docking studies were carried out by Rajat Pal and Prof. Emilio Gallicchio. All of these experimental details can be found in the Supporting Information for the publication on which this chapter is based. This material is provided free of charge by the RSC (http://pubs.rsc.org/).

2.6.1. General Information

All starting materials and reagents were purchased from commercially available sources and used without further purification, with exception of CH$_2$Cl$_2$, which was purified on a solvent purification system prior to the reaction. $^1$H NMR shifts are measured using the solvent residual peak as the internal standard (CHCl$_3$ $\delta$ 7.26, CD$_3$OD $\delta$ 3.31, (CD$_3$)$_2$SO $\delta$ 2.50), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet), coupling constant (Hz), and integration. $^{13}$C NMR shifts are measured using the solvent residual peak as the internal standard (CDCl$_3$ $\delta$ 77.2, CD$_3$OD $\delta$ 49.0, (CD$_3$)$_2$SO $\delta$ 39.5), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Microwave reactions were performed via the Biotage Initiator 2.5. Purification via normal phase column chromatography was performed on the Biotage Isolera Prime, with Biotage SNAP 10 g or 25 g cartridges, in a solvent system of ethyl acetate and hexanes. Reverse phase chromatography was performed on the Biotage Isolera Prime with Biotage SNAP C18 12 g cartridges, in a solvent system of water and acetonitrile with a 0.05% trifluoroacetic acid additive. Column gradients are measured in terms of column
volumes (CV). Mass spectra were recorded on a spectrometer by the electrospray ionization (ESI) technique with a time-of-flight (TOF) mass analyzer.

2.6.2. Synthesis and Characterization of Iodobicycles (2.13a, 2.13b, 2.13e)

General Procedure A. To a solution of salt $2.8a^{S2}$ and alkyne (10 equiv) in CH$_2$Cl$_2$ (0.5 M) was added N,N-diisopropylaniline (1.2 equiv). The reaction mixture was subjected to microwave irradiation at 120 °C for 20 minutes. The reaction mixture was then loaded directly onto column for chromatography and purified.

General Procedure B. To a solution of dimer $2.17b^{S2}$ in CH$_2$Cl$_2$ (0.5 M) was added alkyne (20 equiv). The reaction was subjected to microwave irradiation at 100 °C for one hour. The reaction mixture was then loaded directly onto column for chromatography and purified.

Methyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (2.13a).

Procedure A: To a solution of salt $2.8a$ (185.0 mg, 0.6373 mmol) and methyl 3-iodopropiolate$^3$ (1.3365 g, 6.3734 mmol) in CH$_2$Cl$_2$ (2 mL) was added N,N-diisopropylaniline (148.8 μL, 0.7649 mmol). After microwave irradiation at 120 °C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (8 CV); 10-15% EtOAc in hexanes (10 CV); 15-20% EtOAc in hexanes (10 CV); 20-25% EtOAc in hexanes (10 CV); 25-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield $2.13a$ as a yellow solid (126.2 mg, 57% yield). Melting point (mp) = 132-135 °C. Rf = 0.25 in 25% EtOAc in hexanes. IR (thin film, KBr) 2953 (w), 2840 (w), 1711 (s), 1610 (m), 1436 (w), 1379 (w), 1311
(m), 1205 (m), 1121 (w), 860 (w), 693 (w) cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 6.06 (s, 1H), 5.03 (s, 1H), 3.84 (s, 3H), 3.55 (s, 3H), 1.75 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 186.9 (s), 163.0 (s), 151.0 (s), 145.1 (s), 119.2 (s), 103.4 (s), 93.8 (s), 88.0 (s), 54.9 (s), 52.3 (s), 21.8 (s). HRMS (ESI+) m/z calc’d for C₁₁H₁₂IO₅⁺: 350.9724. Found: 350.9752.

Ethyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (2.13b).

Procedure A: To a solution of salt 2.8a (12.47 mg, 0.0430 mmol) and ethyl 3-iodopropiolate⁵³ (0.0963 g, 0.430 mmol) in CH₂Cl₂ (0.2 mL) was added N,N-diisopropylaniline (10.03 μL, 0.0516 mmol). After microwave irradiation at 120 °C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (8 CV); 10-15% EtOAc in hexanes (10 CV); 15-20% EtOAc in hexanes (10 CV); 20-25% EtOAc in hexanes (10 CV); 25-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield 2.13b as a yellow oil (15.3 mg, 98% yield). Rf= 0.29 in 25% EtOAc in hexanes. IR (thin film, KBr) 2981 (w), 2936 (w), 1710 (s), 1609 (m), 1448 (w), 1324 (w), 1307 (w), 1259 (m), 1122 (w), 869 (w), 693 (m) cm⁻¹. ¹H NMR (200 MHz, CDCl₃) δ 6.02 (s, 1H), 4.96 (s, 1H), 4.24 (q, J = 11.1 Hz, 2H), 3.50 (s, 3H), 1.70 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 187.0 (s), 162.6 (s), 150.9 (s), 145.1 (s), 119.3 (s), 103.0 (s), 93.7 (s), 88.0 (s), 61.7 (s), 55.0 (s), 21.7 (s), 14.3 (s). HRMS (ESI+) m/z calc’d for C₁₂H₁₄IO₅⁺: 364.9880. Found: 364.9886.
**Ethyl 5-(chloromethyl)-7-iodo-3-methoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (2.13c). Procedure B:** To a solution of dimer 2.17b (369.4 mg, 1.06 mmol) in CH₂Cl₂ (2.12 mL) was added ethyl 3-iodopropiolate³ (4.86 g, 21.7 mmol). After microwave irradiation at 100 °C for one hour, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 50 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield 2.13c as a yellow oil (596.0 mg, 71% yield). Rf = 0.32 in 25% EtOAc in hexanes. IR (thin film, KBr) 2980 (w), 2936 (w), 2839 (w), 1713 (s), 1613 (s), 1453 (w), 1369 (w), 1311 (m), 1270 (m), 1130 (w), 834 (w), 639 (m) cm⁻¹. ¹H NMR (200 MHz, CDCl₃) δ 5.99 (s, 1H), 5.13 (s, 1H), 4.44 – 4.22 (m, 2H), 4.26 – 4.00 (dd, 2H), 3.59 (s, 3H), 1.38 (t, J = 7.1 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 186.1 (s), 162.1 (s), 148.0 (s), 146.1 (s), 114.6 (s), 103.6 (s), 93.8 (s), 89.9 (s), 62.1 (s), 55.2 (s), 44.7 (s), 14.3 (s). HRMS (ESI⁺) m/z calc’d for C₁₂H₁₂ClINaO₅⁺: 420.9310. Found: 420.9314.

2.6.3. **Synthesis and Characterization of Methoxy-8-oxabicyclo[3.2.1]octenes (2.13a, 2.13b, 2.13e)**

Methyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (2.14a).

**Procedure:** To a solution of bicycle 2.13a (8.6 mg, 0.025 mmol) in methanol (1.23 mL) was added 4-dimethylaminopyridine (3.0 mg, 0.025 mmol). After microwave irradiation at 120°C for 20 min, the reaction mixture was concentrated under reduced pressure, taken up in CH₂Cl₂ and purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (40
CV)). Product fractions were concentrated to yield 2.14a as a clear oil (5.8 mg, 94% yield). Melting
point (mp) = 138-141 °C. Rf = 0.19 in 25% EtOAc in hexanes. IR (thin film, KBr) 2953 (w), 1696
(s), 1635 (s), 1606 (s), 1449 (w), 1371 (m), 1209 (m), 1112 (s), 834 (w), 723 (m) cm⁻¹. ¹H NMR
(200 MHz, CDCl₃) δ 6.20 (s, 1H), 5.05 (s, 1H), 3.96 (s, 3H), 3.76 (s, 3H), 3.56 (s, 3H), 1.75 (s,
3H). ¹³C NMR (100 MHz, CDCl₃) δ 188.8 (s), 168.7 (s), 163.8 (s), 145.1 (s), 121.8 (s), 114.2 (s),
86.0 (s), 83.4 (s), 60.7 (s), 54.9 (s), 51.7 (s), 23.0 (s). HRMS (ESI+) m/z calc’d for C₁₂H₁₅O₆⁺:
255.0863. Found: 255.0873.

Ethyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate
(2.14b).

Procedure: To a solution of bicycle 2.13b (155.1 mg, 0.4259 mmol) in methanol (12 mL) was
added 4-dimethylaminopyridine (139.5 mg, 1.142 mmol). After microwave
irradiation at 120 °C for 20 min, the reaction mixture was purified by
chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient:
5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (35 CV)). Product fractions were
concentrated to yield 2.14b as a clear oil (73.85 mg, 64% yield). Melting point (mp) = 84-86 °C.
Rf = 0.48 in 50% EtOAc in hexanes. IR (thin film, KBr) 2937 (w), 1709 (s), 1692 (s), 1635 (m),
1604 (m), 1451 (w), 1352 (w), 1241 (w), 1132 (w), 1112 (w), 986 (m), 723 (w) cm⁻¹. ¹H NMR
(200 MHz, CDCl₃) δ 6.20 (s, 1H), 5.04 (s, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.97 (s, 1H), 3.58 (s,
1H), 1.75 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 188.7 (s), 168.2 (s),
163.2 (s), 144.9 (s), 121.7 (s), 114.2 (s), 85.8 (s), 83.3 (s), 60.5 (s), 60.4 (s), 54.7 (s), 22.9 (s), 14.3
(s). HRMS (ESI+) m/z calc’d for C₁₃H₁₇O₆⁺: 269.1020. Found: 269.1019.
Ethyl 5-(chloromethyl)-3,7-dimethoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (2.14c).

Procedure: To a solution of bicycle 2.13c (348.9 mg, 0.8753 mmol) in methanol (43 mL) was added 4-dimethylaminopyridine (106.9 mg, 0.8753 mmol) in a sealed tube. After heating at 150 °C for 20 min in a silicon oil bath, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (30 CV)). Product fractions were concentrated to yield 2.14c as a clear oil (161.1 mg, 61% yield). 

\[ R_f = 0.24 \text{ in 25\% EtOAc in hexanes.} \]

IR (thin film, KBr) 2980 (w), 1714 (s), 1690 (m), 1637 (m), 1607 (m), 1464 (w), 1380 (w), 1224 (m), 1133 (m), 1072 (w), 983 (w), 725 (w) cm\(^{-1}\).

\[ ^1H \text{NMR (400 MHz, CDCl}_3\text{)} \delta 6.13 \text{ (s, 1H), 5.11 (s, 1H), 4.21 (qd, } J = 7.1, 2.8 \text{ Hz, 2H), 4.14 - 4.05 (m, 2H), 3.98 (s, 3H), 3.57 (s, 3H), 1.28 (t, } J = 7.1 \text{ Hz, 3H).} \]

\[ ^{13}C \text{NMR (100 MHz, CDCl}_3\text{)} \delta 188.0 \text{ (s), 168.7 \text{ (s), 162.9 \text{ (s), 146.2 \text{ (s), 117.3 \text{ (s), 111.8 \text{ (s), 87.9 \text{ (s), 83.6 \text{ (s), 61.0 \text{ (s), 60.9 \text{ (s), 55.2 \text{ (s), 46.3 \text{ (s), 14.5 \text{ (s).}}} \]

HRMS (ESI+) m/z calc’d for C\(_{13}\)H\(_{16}\)ClO\(_6\)+: 303.0630. Found: 303.0634.

### 2.6.4. Synthesis and Characterization of 3,7-Dimethoxytropolones via BCl\(_3\)

![Diagram of the reaction]
Methyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.15a).

**Procedure:** To a solution of bicyclic compound 2.14a (6.0 mg, 0.0236 mmol) in CH$_2$Cl$_2$ (1.69 mL) was added a 1M solution of BCl$_3$ in CH$_2$Cl$_2$ (165.2 μL, 0.1652 mmol). The reaction was allowed to stir at room temperature for 10 minutes before being quenched to pH 7 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.15a as a red solid (5.8 mg, 97% yield). Melting point (mp) = 93-97 °C. **IR (thin film, KBr)** 3192 (br), 2950 (w), 1734 (m), 1554 (w), 1459 (w), 1329 (s), 1268 (s), 1217 (s), 1138 (m), 1078 (w), 923 (w), 796 (w). **$^1$H NMR** (400 MHz, CDCl$_3$) δ 6.89 (s, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 2.37 (s, 3H). **$^{13}$C NMR** (100 MHz, CDCl$_3$) δ 168.6 (s), 167.7 (s), 158.3 (s), 155.2 (s), 150.1 (s), 134.0 (s), 131.1 (s), 119.5 (s), 61.4 (s), 56.8 (s), 52.8 (s), 24.9 (s). **HRMS (ESI+)** m/z calc’d for C$_{12}$H$_{14}$NaO$_6$+: 277.0683. Found: 277.0689.

Ethyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.15b).

**Procedure:** To a solution of bicyclic compound 2.14b (20.9 mg, 0.0780 mmol) in CH$_2$Cl$_2$ (5.6 mL) was added a 1M solution of BCl$_3$ in CH$_2$Cl$_2$ (546 μL, 0.546 mmol). The reaction was allowed to stir at room temperature for 10 minutes before being quenched to pH 7 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.15b as a yellow oil (16.9 mg,
81% yield). IR (thin film, KBr) 3734 (w), 2940 (w), 1731 (s), 1553 (s), 1454 (w), 1328 (m), 1267 (m), 1217 (s), 1138 (s), 1017 (w), 901 (w), 669 (s). $^1\text{H NMR (200 MHz, CDCl}_3\text{)}$ δ 6.89 (s, 1H), 4.41 (q, $J = 7.1$ Hz, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 2.39 (s, 3H), 1.39 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}$ δ 168.3 (s), 167.0 (s), 158.0 (s), 155.1 (s), 149.9 (s), 134.1 (s), 130.9 (s), 119.3 (s), 61.8 (s), 61.2 (s), 56.6 (s), 24.6 (s), 14.2 (s). HRMS (ESI+) $m/z$ calc’d for C$_{13}$H$_{17}$O$_6$+: 269.1020. Found: 269.1016.

**Ethyl 2-(chloromethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.15c).**

**Procedure:** To a solution of 1M BCl$_3$ in CH$_2$Cl$_2$ (80.6 μL, 0.0806 mmol) was added a solution of bicyclic compound 2.14c (6.1 mg, 0.0202 mmol) in CH$_2$Cl$_2$ (2.88 mL) at 0 °C. After stirring for 6 minutes, the reaction was slowly added to 6 mL of pH 5 phosphate buffer in a separatory funnel. After shaking, the pH of the aqueous layer was further adjusted to pH 4 via the gradual addition of an additional 12 mL of pH 5 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 5 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.15c as a brown oil (4.7 mg, 77% yield) which was immediately taken on to the next step. IR (thin film, KBr) 2924 (w), 2851 (w), 1731 (s), 1558 (m), 1464 (w), 1335 (m), 1268 (s), 1218 (m), 1135 (w), 1047 (w), 941 (w), 669 (w). $^1\text{H NMR (400 MHz, CDCl}_3\text{)}$ δ 7.07 (s, 1H), 4.48 (s, 2H), 4.45 (q, $J = 7.2$ Hz, 2H), 4.04 (s, 3H), 3.98 (s, 3H), 1.42 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}$ δ 169.4 (s), 166.0 (s), 158.7 (s), 155.8 (s), 149.0 (s), 134.7 (s), 129.1 (s), 117.6 (s), 62.4 (s), 61.3 (s), 56.8 (s), 47.2 (s), 14.2 (s). HRMS (ESI+) $m/z$ calc’d for C$_{13}$H$_{15}$ClNaO$_6$+: 325.0449. Found: 325.0443.
Ethyl 2-(acetoxyethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.15d).

**Procedure:** To a solution of dimethoxytropolone 2.15c (16.8 mg, 0.055 mmol) in acetic acid (5.55 mL) was added sodium acetate (91.05 mg, 1.11 mmol). The reaction was allowed to stir at room temperature for 15 hours before being quenched to pH 3 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.15d as a brown/yellow oil (14.7 mg, 93% yield). IR (thin film, KBr) 3734 (w), 2950 (br), 1737 (s), 1558 (w), 1462 (w), 1366 (w), 1333 (w), 1221 (s), 1139 (w), 1073 (w), 669 (w). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.14 (s, 1H), 5.03 (s, 2H), 4.43 (q, $J$ = 7.1 Hz, 2H), 4.02 (s, 3H), 3.98 (s, 3H), 2.12 (s, 3H), 1.40 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.6 (s), 168.8 (s), 166.2 (s), 158.4 (s), 156.8 (s), 149.8 (s), 135.2 (s), 128.2 (s), 118.0 (s), 66.9 (s), 62.4 (s), 61.5 (s), 57.0 (s), 21.0 (s), 14.3 (s). HRMS (ESI+) m/z calc’d for C$_{15}$H$_{18}$NaO$_8$: 349.0894. Found: 349.0898.

7-Hydroxy-5,8-dimethoxy-1H-cyclohepta[c]furan-1,6(3H)-dione (2.15e).

**Procedure A:** A solution of dimethoxytropolone 2.15d (12.9 mg, 0.034 mmol) in 2N aqueous NaOH (4.78 mL) was allowed to stir at room temperature for 3 hours before being diluted with 10 mL of CH$_2$Cl$_2$ and quenched to pH 3 with pH 3 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.15e as a yellow solid (9.1 mg, 81% yield).
**Procedure B:** Dimethoxytropolone 2.15c (4.6 mg, 0.015 mmol) was subjected to reverse phase column chromatography conditions (Biotage Isolera Prime, SNAP 12g C18 silica gel column, solvent gradient: 5% acetonitrile in water (3 CV); 5-100% acetonitrile in water (35 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were concentrated to yield 2.15e as a yellow solid (2.9 mg, 81% yield). Melting point (mp) = 180-184 °C. Rf= 0.36 in 10% methanol in dichloromethane. IR (thin film, KBr) 3734 (w), 3217 (br), 2945 (w), 1760 (s), 1573 (s), 1457 (w), 1338 (m), 1284 (m), 1122 (w), 1051 (s), 868 (w), 668 (s). H NMR (400 MHz, CDCl$_3$) δ 6.83 (s, 1H), 5.17 (s, 2H), 4.11 (s, 3H), 4.09 (s, 3H). C NMR (100 MHz, CDCl$_3$) δ 171.7 (s), 168.5 (s), 162.4 (s), 154.7 (s), 149.6 (s), 144.6 (s), 119.2 (s), 106.6 (s), 69.8 (s), 62.1 (s), 57.4 (s). HRMS (ESI+) m/z calc’d for C$_{11}$H$_{11}$O$_6$: 239.0550. Found: 239.0558.

**2.6.5. Synthesis and Characterization of α-Methoxytropolone via TfOH**

![Diagram](image.png)

**Methyl 6-hydroxy-4-methoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.27).**

To a solution of methyl 3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate$^{34}$ (484 mg, 2.16 mmol) in CH$_2$Cl$_2$ (22 mL) was added triflic acid (763 μL, 8.65 mmol). The reaction was allowed to stir for 30 min at rt before quenching with pH 7 phosphate buffer. The reaction mixture was then extracted with CH$_2$Cl$_2$ (3 x 20 mL), and the combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.27 as a brown solid (377 mg, 78% yield). Product can be further
purified via crystallization in MeOH to yield a yellow solid (233 mg, 48% yield). Melting point (mp) = 168-171 °C. **IR (thin film, KBr)** 3251 (br), 2918 (w), 2848 (w), 1717 (s), 1554 (m), 1483 (w), 1457 (m), 1335 (s), 1297 (m), 1220 (s), 1139 (w), 1053 (s), 907 (w), 788 (m) cm⁻¹. **1H NMR** (400 MHz, CDCl₃) δ 7.56 (s, 1H), 6.99 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H), 2.53 (s, 3H). **13C NMR** (100 MHz, CDCl₃) δ 171.0, 169.1, 159.6, 158.3, 137.4, 131.5, 121.5, 117.4, 56.6, 52.9, 26.0. **HRMS (ESI+) m/z** calc’d for C₁₁H₁₃O₅⁺: 225.0757. Found: 225.0753.

**2.6.6. Synthesis and Characterization of 3,7-Dihydroxytropolones**

Methyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.16a).

**Procedure:** To dimethoxytropolone 2.15a (3.3 mg, 0.0130 mmol) was added 145 μL of 33% HBr/AcOH. The reaction was heated to reflux at 120 °C for 35 minutes before being quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous later was extracted with CH₂Cl₂ (5 x 10 mL). Combined organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield 2.16a as a red/brown solid (1.6 mg, 55%). Melting point (mp) = 131-135 °C. **IR (thin film, KBr)** 3198 (br), 2925 (w), 1733 (s), 1586 (w), 1525 (m), 1431 (s), 1325 (s), 1201 (s), 1057 (w), 878 (w), 796 (w). **1H NMR** (400 MHz, CD₃OD) δ 7.00 (s, 1H), 3.91 (s, 3H), 2.32 (s, 3H). **13C NMR** (100 MHz, CDCl₃) δ 167.2 (s), 157.3 (s), 154.5 (s), 153.4 (s), 152.3 (s), 136.8 (s), 125.0 (s), 120.8 (s), 52.1 (s), 24.7 (s). **HRMS (ESI+) m/z** calc’d for C₁₀H₁₁O₆⁺: 227.0550. Found: 227.0556.
Ethyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (2.16b).

**Procedure:** To dimethoxytropolone 2.15b (15.5 mg, 0.0578 mmol) was added 682 μL of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux at 120 °C for 45 minutes in a silicon oil bath before being quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.16b as a red/brown oil (12.0 mg, 87%). IR (thin film, KBr) 3210 (br), 2929 (w), 1730 (s), 1581 (w), 1445 (w), 1326 (w), 1193 (s), 1056 (m), 1012 (w), 860 (w), 779 (w) cm$^{-1}$. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.02 (s, 1H), 4.39 (q, $J = 6.8$ Hz, 2H), 2.33 (s, 3H), 1.37 (t, $J = 6.8$ Hz, 3H). $^{13}$C NMR (100 MHz, CD$_3$OD) δ 169.3 (s), 158.7 (s), 157.5 (s), 157.4 (s), 153.2 (s), 137.0 (s), 126.9 (s), 121.0 (s), 62.8 (s), 24.4 (s), 14.4 (s). HRMS (ESI+) m/z calc’d for C$_{11}$H$_{12}$NaO$_6^+$: 263.0526. Found: 263.0528.

2,3,7-Trihydroxy-5-methylcyclohepta-2,4,6-trien-1-one (2.16c).

**Procedure:** To dimethoxytropolone 2.15a (6.5 mg, 0.0256 mmol) was added 4.6 μL (10 equiv.) of water and 284 μL of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux at 120 °C for 35 minutes. The membrane of the sealed vessel was punctured with an 18G needle to release gas buildup approximately 10 minutes after subjecting reaction to heat. Upon completion, the reaction was quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous later was extracted with CH$_2$Cl$_2$ (5 x 10 mL). Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 2.16c as a red/brown oil (1.8 mg, 42%). IR (thin film, KBr) 3509 (br), 3218 (br), 2918 (m), 1591 (w), 1517 (m), 1434 (s), 1394 (w), 1339 (w), 1199 (s), 1092 (m),
1068 (w), 668 (m) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 7.01 (s, 2H), 2.40 (s, 3H). \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) 158.2 (s), 156.6 (s), 141.2 (s), 120.3 (s), 27.0 (s). HRMS (ESI+) \(m/z\) calc’d for C\(_8\)H\(_9\)O\(_4\)^+: 169.0495. Found: 169.0492.

**5,7,8-Trihydroxy-1H-cyclohepta[c]furan-1,6(3H)-dione (2.16d).**

**Procedure:** To dimethoxytropolone 2.15e (10.0 mg, 0.042 mmol) was added 453 \(\mu\)L of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux in a silicon oil bath at 120 °C for 30 minutes before being quenched to pH 1.5 with pH 5 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3 x 2 mL). Combined organics were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure to yield 2.16d as a yellow solid (5.0 mg, 57%). IR (thin film, KBr) 3502 (br), 3215 (br), 2962 (w), 2918 (s), 2849 (m), 1747 (m), 1622 (w), 1517 (w), 1260 (s), 1096 (m), 1022 (s), 799 (s) cm\(^{-1}\). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 6.83 (s, 1H), 5.17 (s, 2H). \(^{13}\)C NMR (150 MHz, DMSO-\(d_6\)) \(\delta\) 171.0 (s), 164.7 (s), 159.9 (s), 154.3 (s), 150.9 (s), 149.1 (s), 109.8 (s), 105.1 (s), 69.7 (s). HRMS (ESI+) \(m/z\) calc’d for C\(_9\)H\(_7\)O\(_6\)^+: 211.0237. Found: 211.0231.
2.6.7. NMR Spectra of Synthesized Compounds
$^1$H-NMR of 2.13b
$^1$H-NMR of 2.14c
$^{13}$C-NMR of 2.14c
\(^1\)H-NMR of 2.15d
$^{13}$C-NMR of 2.15d
$^1$H-NMR of 2.16a
$^{13}$C-NMR of 2.16a
$^1$H-NMR of 2.16b
$^{13}$C-NMR of 2.16c
2.6.8. Supplementary References


Chapter III

Studies on Configurational Stability of Troplone-Amide Aryl-CO Bonds

3.1 Introduction

A fundamental goal of drug discovery is to develop safe and effective compounds with specificity towards their intended disease targets. In order to achieve this objective, it is crucial to consider the three-dimensional properties of drugs. One such characteristic is the handedness, or chirality, of a molecule since different spatial orientations of drugs with identical connectivity can have drastically differing effects in the body. While chirality is typically thought of in terms of asymmetry about a single atom or point, axial chirality (Figure 3.1) is a critically understudied form of chirality in which the spatial arrangement of substituents about an axis renders the molecule non-superimposable on its mirror image.\(^1\) Atropisomerism arises from restricted rotation about this axis, thus allowing for isolatable enantiomers. This type of isomerism is often exemplified by the biaryl scaffold where restricted rotation about the stereogenic \(C_{Ar}-C_{Ar}\) axis is responsible for the presence of enantiomers (Figure 3.1C). Separable atropisomers can generally be observed if the energetic barrier to rotation \((\Delta G^\ddagger)\) about the chiral axis is \(> 22\) kcal/mol, corresponding to a half-life to racemization of \(>1000\) seconds at room temperature.\(^2\) It is an issue of current interest in the development of ligands,\(^3\) molecular devices,\(^4\) and pharmaceutical drugs.\(^5\)

Atropisomerism is perhaps most widely appreciated within the field of synthetic organic chemistry as the source of chirality in numerous chiral biaryl ligands for asymmetric catalysis. Axially chiral bidentate ligands, such as BINAP, are most famously recognized for their use in

![Figure 3.1](image-url)

Figure 3.1. (A) Hands are a relatable example of chirality. (B) Point chirality refers to asymmetry about a single point or atom. (C) Axial chirality results from restricted bond rotation and exists around a plane.
Noyori’s asymmetric hydrogenation work on olefins and carbonyls (Figure 3.2A) for which he was awarded the 2001 Nobel Prize. BINAP ligands have also been used extensively in atropselective cross-couplings. The importance of atropisomerism can also be found in other areas such as unidirectional molecular devices and switches, the development of which won Feringa the 2016 Nobel Prize (Figure 3.2B).

![Figure 3.2. (A) Ruthenium-BINAP dihalide complexes were shown to be valuable catalysts for asymmetric hydrogenation of carbonyls. This work was awarded the 2001 Nobel Prize in Chemistry. (B) Photoisomerization of helical thioxanthenes was shown to be reversible at specific wavelengths. This represents early efforts towards molecular switch-based optical data storage devices, which was ultimately awarded the 2016 Nobel Prize in Chemistry.](image)

While numerous axially chiral drugs are commercially available, many exist as rapidly interconverting atropisomers with only one enantiomer possessing the desired activity. Single-enantiomer drugs have long been considered of paramount importance; perhaps most infamously, the antiemetic racemate thalidomide caused devastating birth defects in more than 10,000 babies in the 1950s owing to toxicity associated with the (S)-enantiomer as well as facile in vivo racemization. Consequently, the development of efficient synthetic strategies to access single enantiomers is a major theme of modern synthetic organic chemistry.
The thalidomide tragedy was not a standalone incident. Enantiomers of numerous drugs possessing point chirality have been shown to demonstrate drastically differing effects in the body.\textsuperscript{12} Similarly, it has been found that the presence of the opposite atropisomer can result in off-target binding, and preorganizing (or locking) a freely rotating axis into the relevant configuration can increase target selectivity.\textsuperscript{10,13} Single-atropisomer drugs are available (Figure 3.3), though their locked configuration is rarely deliberate. For instance, drugs such as Viagra (sildenafil) and Imatinib, while not displaying obvious chirality, will bind to their receptors in an enantiospecific form.

![Diagram of drugs]

Meanwhile, configurationally stable scaffolds such as colchicine,\textsuperscript{14} gossypol\textsuperscript{15} and vancomycin\textsuperscript{16} exist, but are frequently discovered as natural products and their high rotational barriers are as such not a design element. This is important because, as in the case of thalidomide, crucial differences in biology have been observed between separated atropisomers.\textsuperscript{10,13} (+)\textsuperscript{-}Telenzepine, for example, is 500 times more potent than (−)\textsuperscript{-}Telenzepine,\textsuperscript{17} while the S atropisomer of colchicine is the only active
The design of single-atropisomer drugs would therefore represent a paradigm shift away from point chirality towards axial chirality for drug discovery. Two major hurdles have impeded these efforts thus far: 1) Exceptionally high rotational barriers (>28 kcal/mol) in vivo are needed to ensure long-term biological configurational stability, and 2) there exists a dearth of efficient synthetic methods to access such molecules.

The following chapter will describe a solution to these challenges in the study of the rotational barriers and physical properties of troponoids. Owing to their ring size and increased bond angles, troponoids are predicted to have exceptionally high rotational barriers (Scheme 3.1). They also have exceptional bioactivity (see Chapters 1 and 2). The combination of these properties makes troponoids ideal candidates for the development of single-atropisomer drugs. However, they have historically represented a significant synthetic challenge; as such, there exists a scarcity of troponoid structure-function studies, with most current synthetic methods stifled by an inability to functionalize more than one position on the ring. Thus, the remainder of this chapter will discuss efforts to leverage ongoing synthetic studies in the Murelli lab towards the synthesis of configurationally stable troponoids.

3.2. Discovery of $\alpha$-Hydroxytropolone Atropisomerism

3.2.1. $^1$H-NMR Profiles of a Troponoid and Benzenoid Thiazolidine

As part of our ongoing medicinal chemistry studies, we synthesized thiazolidine $\alpha$-hydroxytropolone 3.4 via the oxidopyrylum cycloaddition/ring-opening route described in
Chapters 1 and 2 towards carboxylic acid 3.3 (Figure 3.4). Upon observing a remarkable degree of atropdiastereoselectivity by $^1$H-NMR, we became curious as to whether the analogous benzenoid would demonstrate similar properties at room temperature and thus carried out a similar amide coupling on benzoic acid 3.5. The resulting room temperature $^1$H-NMRs displayed very different profiles with the troponoid (Figure 3.4C, bottom, red) showing a clean set of AB quartets while the analogous thiazolidine signal on the benzenoid (Figure 3.4C, top, blue) presents as a set of singlets.

![Syntheses of (A) troponoid and (B) benzenoid thiazolidines via PyBOP-mediated amidation. (C) Differences observed at room temperature in $^1$H-NMR profiles of both compounds' thiazolidine moieties. * denotes Z amide rotamer, ** denotes E amide rotamer.](image)

3.2.2. *Experimental Calculation of Thiazolidine Rotational Barriers*

The reduced symmetry of the amide functionality in thiazolidines 3.4 and 3.6 enabled us to obtain kinetic parameters for Ar-CO atropisomerization via variable temperature $^1$H-NMR spectroscopy (Figure 3.5). Two moieties were investigated: the methylene in between the N and S (purple orb, Figure 3.4), and the methylene on the other side of the N (blue orb, Figure 3.4; see Supporting Information for details). The amide rotamers remained resolved over the course of these experiments, and we therefore were able to obtain rotational barrier measurements for each of the E/Z amide isomers. Benzenoid 3.6 was found to exist in rapid atropisomeric equilibrium at
25 °C, showing a single set of peaks for each E/Z amide rotamer with no atropdiastereotopic splitting. Therefore, the coalescence point of the benzenoid was met by cooling the sample down, while the troponoid required heating in order to observe coalescence. From this alone, a clear qualitative conclusion is evident: the benzenoid signals coalesce well before the troponoid signals (see Figure 3.5 and the Supporting Information). This pattern is evident across all signals belonging to both sets of molecules except for the most downfield methylene peak corresponding to the Z rotamer of the benzenoid. Initially, it was suspected that this rotamer may have a higher C-C rotational barrier; however, given that the other signals all coalesce, it can be concluded that the chemical shifts of the Z rotamers’ atropdiastereotopic protons are coincidentally equivalent.

Since E/Z amide isomerization occurs at a significantly higher barrier than C-C isomerization, the ΔG‡ between the two atropisomeric forms (aS and aR) in solution were determined by lineshape simulation of VT-NMR spectra using iNMR modeling software. These lineshape simulations were based on the experimental spectra obtained for both compounds, specifically on the chemical shifts and coupling constants, and gave a rate constant (k) for each temperature. This data was converted into a barrier to rotation
at a given temperature by plotting $1/T$ vs $\ln(k/T)$ and performing an Eyring plot (see Supporting Information).\(^{21}\) This plot gives a straight line, the slope of which gives the enthalpy of rotation ($\Delta H^\ddagger$) and the $y$-intercept of which gives the entropy of rotation ($\Delta S^\ddagger$). Inserting these values into Gibbs’ equation for free energy gives the rotational energy ($\Delta G^\ddagger$). In each case, the linear regression performed was obtained from a set of 5 data points with correlation coefficients between 0.9855 and 0.9984, demonstrating a high degree of linearity for the data obtained.

In the case of the benzenoid, C-N kinetic parameters were also analyzed by heating the sample in DMSO-$d_6$ (Figure 3.5A). Troponoid C-N rotation remained too high in energy to be measurable within the temperature limitations of the NMR, but benzenoid C-N isomerization could be observed ($\Delta G^\ddagger = 16.4$ kcal/mol, see Supporting Information for details). This alone speaks to the levels of ancillary rigidity that can be provided by restricted C-C rotation in troponoids. All in all, the difference in rotational barriers between the 6- and 7-membered substrates is considerably large. To gain a better understanding of the physical basis of these differences, we turned to molecular modeling to assess the barriers of a small library of benzenoid and troponoid derivatives.

3.3. Computational Modeling of Troponoids

3.3.1. Preliminary Substrate Scope

This work was performed in collaboration with Dr. Anthony Metrano at Yale University and the approach used in these studies had been optimized for benzenoids\(^{22}\) and quinazolinones.\(^{23}\) This work represents the first instance of a head-to-head comparison of benzenoid and troponoid side-chain rotational barriers via computational modeling, and one of the first extensive examples of troponoid rotational energy modeling entirely.\(^{24}\) Rotational barriers about the aryl/amide axis were performed using the OMEGA and GRACE supercomputer clusters provided by the Yale
University Faculty of Arts and Sciences High Performance Computing Center. All calculations were carried out using the Gaussian 09 suite. Ground state geometries were first optimized using density functional theory (DFT) at the B3LYP/6-31+G(d,p) level of theory. Harmonic vibrational frequencies were simultaneously calculated. The optimized structures were then used as foundations for a restricted torsional potential energy scan (PES) at the same level of theory. The scan was conducted by first defining the dihedral angle in question (CO-C\textsubscript{A}) as the constrained coordinate and performing a scan of that angle through 360° in 10° increments using the same method and basis set.

The resulting torsional profile was subsequently used as a starting point from which to narrow in on the single-point energies. The geometries of the scan maxima (transition states) were subjected to further optimization using B3LYP/6-31+G(d,p). The frozen single-point output geometries of these calculations, as well as the stationary ground state points on the torsional energy profile, were subjected to further optimization with no dihedral restrictions in place at the M06-2X/6-311++G(2d,3p) level of theory and basis set. All calculations were performed at the gas phase. The output from these calculations were inspected, and in the case of the transition states, the imaginary frequency was verified as a vibration along the reaction coordinate of interest. The M06-2X total electronic energies were converted to Gibbs free energies and the barrier height \( \Delta G^\ddagger \) values were converted to kcal/mol from Hartrees using a conversion factor of 627.509 a.u. = 1 kcal/mol. The Arrhenius equation was then used to calculate rate constants for each barrier of the rotation (\( k_1 \) and \( k_2 \)). These rate constants were then added to provide an observed rate constant, \( k_{obs} \). This value was used to calculate the observed barrier, \( \Delta G^{\ddagger}(obs) \) using the following equation:

\[
\Delta G^{\ddagger}(obs) = -RT \cdot \ln\left( \frac{k_B T}{\hbar k_{obs}} \right) \tag{Eq. 3.1}
\]
where \( R \) is the ideal gas constant \( [1.99 \times 10^{-3} \text{ kcal/(molK)}] \), \( T \) is the temperature in Kelvin, \( k_B \) is the Boltzmann constant \( [1.38 \times 10^{-23} \text{ (m}^2\text{kg)/(s}^2\text{K)}] \), and \( h \) is Planck’s constant \( [6.63 \times 10^{-34} \text{ (m}^2\text{kg)/(s}^2\text{)}] \).

This approach was used to calculate rotational barriers for a series of methoxytropolones and their corresponding benzenoids. In order to minimize complicating factors such as tautomerization, we chose to focus on methoxytropolones as they are key intermediates in our synthetic route towards \( \alpha \)HTs with only two possible tautomers. Given previous 2D-NMR\(^{29}\) and x-ray crystallographic\(^{30}\) evidence that the 7-methoxytropolone is the predominating tautomer, all troponoids were modeled in this form. These results are summarized in Table 3.1.

![Diagram](image)

**Table 3.1: Computed Rotational Barriers**

<table>
<thead>
<tr>
<th>Entry</th>
<th>( R^1 )</th>
<th>( R^2 )</th>
<th>( R^3 )</th>
<th>Troponoids ( \Delta G^f ) (kcal/mol)</th>
<th>( t_{1/2} ) (s)</th>
<th>Benzenoids ( \Delta G^f ) (kcal/mol)</th>
<th>( t_{1/2} ) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \text{N(CH}_2\text{)}_3 )</td>
<td>H</td>
<td>H</td>
<td>3.7</td>
<td>12.3</td>
<td>1.20 \times 10^{-4}</td>
<td>3.12</td>
</tr>
<tr>
<td>2</td>
<td>( \text{N(CH}_2\text{)}_3 )</td>
<td>Me</td>
<td>H</td>
<td>3.8</td>
<td>16.9</td>
<td>0.262</td>
<td>3.13</td>
</tr>
<tr>
<td>3</td>
<td>( \text{N(\text{i-Pr})}_2 )</td>
<td>Me</td>
<td>H</td>
<td>3.9</td>
<td>17.5</td>
<td>0.686</td>
<td>3.14</td>
</tr>
<tr>
<td>4</td>
<td>( \text{OMe} )</td>
<td>Me</td>
<td>H</td>
<td>3.10</td>
<td>1.7</td>
<td>1.98 \times 10^{-12}</td>
<td>3.15</td>
</tr>
<tr>
<td>5</td>
<td>( \text{Ph} )</td>
<td>Me</td>
<td>H</td>
<td>3.11</td>
<td>6.9</td>
<td>1.31 \times 10^{-9}</td>
<td>3.16</td>
</tr>
</tbody>
</table>

\(^{a}\) Barrier describes a different rotational mechanism, in which the orthogonal, rather than the co-planar, geometry is the transition state.

During the course of these studies, a remarkable trend was observed: the troponoid substrates consistently displayed drastically increased rotational barriers when compared to their analogous benzenoids, with observed increases in half-life to racemization of up to 4 orders of magnitude. While we had anticipated that the decreased external bond angles of the troponoids would result in increased rotational barriers, the extent to which this effect was observed was particularly dramatic. Interestingly, the methyl ester (entry 4, Table 3.1) represents the sole exception to this trend - the transition state in the benzenoid isomerization pathway is orthogonal as opposed to co-planar, thus
losing the stabilizing effect of extended conjugation in the transition state. Nonetheless, in the interest of studying a biologically relevant system, we subsequently modeled thiazolidines 3.4/3.6.

3.3.2. Computational Modeling of Benzenoid and Troponoid Thiazolidines

The asymmetry of the amide moiety resulted in several complicating factors. The presence of non-degenerate E/Z amide rotamers gave rise to 4 unique ground states, each proceeding through one of three pathways to interconversion: rotation about the C-C axis, C-N axis, or a concerted mechanism (see Figure 3.6). Independent C-N bond rotation was computed to proceed through one of two degenerate transition states, depending on the direction of rotation: when the thiazolidine sulfur atom is proximal to the methyl group, the energy is increased and independent C-C rotation is not possible.

Additionally, computations assessing the energy of C-C bond rotation lead to a concerted,\textsuperscript{31} simultaneous rotation about the atropisomeric C-C axis and C-N axis.\textsuperscript{22b} Compared to independent C-N rotation, which is a significantly higher energy process, this concerted C-N/C-C rotation represents the lowest energy pathway to amide isomerization (Figure 3.6). These computational

---

Figure 3.6. Proposed pathways to racemization (C-C rotation) and amide isomerization (C-N rotation) in a dual-axis, differentially substituted (A) benzenoid and (B) troponoid system. Barriers were calculated at the M06-2X/6-311++G(2d,3p) level of theory using the Gaussian 09 suite.
results imply that $E/Z$ isomerization for a given enantiomer most likely involves first, a concerted rotation of the C-C and C-N axes, and second, an independent C-C rotation. Regardless, the lowest energy atropisomeric racemization pathway was found to proceed via independent C-C rotation, as observed by $^1$H-NMR. The presence of DMSO in the troponoid experimental measurement likely accounts for the small differences between its calculated and experimental barriers. Even so, the benzenoid computed barriers were found to be in very good accord with the experimentally determined value. This data is summarized in Table 3.2.

<table>
<thead>
<tr>
<th></th>
<th>$E$</th>
<th>$Z$</th>
<th>$E$</th>
<th>$Z$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental $\Delta G^i$ (kcal/mol)</strong></td>
<td>16.7</td>
<td>16.5</td>
<td>12.2</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>Calculated $\Delta G^i$ (kcal/mol)</strong></td>
<td>15.8</td>
<td>15.1</td>
<td>12.6</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Ultimately, the differences in rotational barriers between the 6- and 7-membered substrates are notably large. We thus became curious if there was any way we could further exacerbate these differences, while simultaneously synthesizing high-rotational barrier troponoids. Given the vast literature precedence for employing halogenation on 6-membered homologs as a means of achieving configurational stability,\textsuperscript{22,23} we thought this seemed like feasible chemistry we could undertake on a troponoid. Prior to this synthetic work, we modeled brominated variants of the 7-methoxytropolones shown in Table 3.1 to see if we could verify this hypothesis.
3.3.3. Modeling of Brominated 7-Methoxytroponoids and Benzenoids

The same approach as described in Section 3.2.1 was utilized in the rotational barrier calculations on the brominated benzenoids and troponoids; the results are summarized in Table 3.3.

![Diagram of brominated troponoids and benzenoids](image)

Table 3.3: Computed Rotational Barriers of Brominated Analogs

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>R&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Troponoids</th>
<th>Benzenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>ΔG&lt;sup&gt;2&lt;/sup&gt; (kcal/mol)</td>
</tr>
<tr>
<td>1</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>H</td>
<td>Br</td>
<td>3.17</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Me</td>
<td>Br</td>
<td>3.18</td>
<td>31.2</td>
</tr>
<tr>
<td>3</td>
<td>N(i-Pr)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Me</td>
<td>Br</td>
<td>3.19</td>
<td>33.2</td>
</tr>
<tr>
<td>4</td>
<td>OMe</td>
<td>Me</td>
<td>Br</td>
<td>3.20</td>
<td>14.6</td>
</tr>
<tr>
<td>5</td>
<td>Ph</td>
<td>Me</td>
<td>Br</td>
<td>3.21</td>
<td>20.9</td>
</tr>
<tr>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Me</td>
<td>H</td>
<td>3.8</td>
<td>17.6</td>
</tr>
<tr>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Me</td>
<td>Br</td>
<td>3.18</td>
<td>31.9</td>
</tr>
<tr>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Me</td>
<td>H</td>
<td>3.8b</td>
<td>18.6</td>
</tr>
<tr>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Me</td>
<td>Br</td>
<td>3.18b</td>
<td>34.2</td>
</tr>
</tbody>
</table>

*<sup>a</sup> Computed at 125 °C. *<sup>b</sup> Tautomer form of entry 6 computed at 25 °C. *<sup>c</sup> Tautomer form of entry 2.

As expected, the brominated troponoids maintained dramatically higher barriers with respect to their respective benzenoids. This disparity is well exemplified by the piperidinyl-based scaffolds 3.18 and 3.23 (entry 2, Table 3.3), in which the half-life of racemization changes from 1.4 hours to over 200 years. Even more astounding, however, is the difference between the brominated diisopropyl-based scaffolds 3.19 and 3.24 (entry 3), where the half-life of racemization increases from ~2 days to 7593 years!

This intriguing disparity made us curious about the rotational energies of brominated but less sterically demanding substrates such as esters and ketones, which we subsequently modeled. As expected, they demonstrated low barriers to racemization even post-bromination, although the troponoids remained consistently higher. In all cases excluding R<sup>1</sup> = OMe and R<sup>2</sup> = R<sup>3</sup> = H (entry
1, Table 3.1), the pathway of isomerization involves geared rotation about the C\textsubscript{Ar}-CO and R\textsubscript{1}-CO axes in both the troponoids and the benzenoids.

From this aggregate data, two key trends can be observed (Figure 3.7). Within the brominated molecules (R\textsuperscript{3} = Br), the troponoids were all computed at ~8 kcal/mol higher in energy than their analogous benzenoids. As well, the difference in rotational energies between non-brominated and brominated substrates is considerably higher for the tropolones than for the respective benzenoids (15.7 vs 11.7 kcal/mol, where a bond rotation energy of >28 kcal/mol is necessary for configurational stability).\textsuperscript{18} As it appears that troponoids are more sensitive to structural changes than their respective benzenoid systems, our synthetic method towards αHTs could be utilized in the development of highly rigid atropisomeric drugs with critical implications on our ongoing medicinal chemistry studies.

An eventual aim of this work is to develop single-atropisomer αHT derivatives for medicinal chemistry pursuits. The calculated troponoids are only a demethylation away from containing the active pharmacophore, and furthermore, contain an extremely useful handle for cross-coupling and further derivatization. As our standard demethylation conditions are run in
refluxing hydrobromic acid, and cross-coupling reactions will likely need to be run at elevated temperatures, we computed rotational barriers for 3.8 and 3.18 at 125 °C (entries 6 and 7, Table 3.3). The barriers increase slightly at elevated temperatures due to increases in the entropic component of $\Delta G^\ddagger$. However, the half-life does decrease substantially. Thus, the temperatures of future functionalization reactions will need to be kept in mind.

While these results are highly promising, the calculations are not without limitations. The approach employed was developed for benzenoids$^{22a}$ and quinazolinones,$^{23a}$ and troponoid characteristics such as tautomerization,$^{32}$ puckering,$^{33}$ and electronic properties such as decreased aromaticity$^{34}$ can all influence the actual rotational barrier. Furthermore, even small changes in high-value free energy calculations can produce large rate effects since rate increases exponentially with $\Delta G$.

With this in mind, we modeled the tautomeric forms of 3.8 and 3.18 (3.8b and 3.18b; entries 8 and 9, Table 3.3) and found that while the two sets of tautomers have nearly identical respective ground state energies, the second tautomer enantiomerizes through a considerably higher energy pathway (i.e. 3.18 versus 3.18b, entries 2 versus 9, Table 3.3). This may result from repulsion between the carbonyl oxygen (which has anionic character resulting from troponoid aromaticity, requiring a formal positive charge on the carbonyl carbon) and the adjacent bromide. Regardless, we reasoned that racemization occurs predominantly via the first tautomer, which was the same tautomeric form assessed in all of the computations described above. We then set about confirming these higher rotational barriers experimentally.

3.4. Synthesis of Bromotropolones: Experimental Confirmation of Higher Barrier Molecules

Bromotroponoids had previously shown utility in some of our own medicinal chemistry pursuits.$^{35}$ In several cases, adding a halogen has increased the bioactivity over the parent scaffold,
and the added electron density provided by the halogens can yield higher quality x-ray crystal structures by increasing the atomic scattering factor. In addition, there are many examples in the literature of halogens increasing barriers to rotation about an adjacent axis with biomedical benefits. For example, this strategy has been leveraged by the Gustafson lab in the synthesis of a series of chlorinated kinase inhibitors, where it was found that the different atropisomers had drastically different biological activity (Scheme 3.2). Given these factors, we set out to develop a synthetic route to halotropolones. Early synthetic efforts utilizing bromomethylpropiolate lead to αHT, which has subsequently proven to be a highly valuable intermediate in a variety of pursuits.

Scheme 3.2. (A) Halogenation and subsequent deracemization of promiscuous scaffolds can be an effective method of increasing target selectivity. (B) Racemic synthesis and biochemical assessment of a brominated αHT.
As this ester was not computationally predicted to be configurationally stable, we turned our attention to piperidinyl amide 3.18. This molecule was first synthesized via an oxidopyrylium cycloaddition using bromoalkyne 3.34b to access bicyclic intermediate 3.35b, which was subsequently converted to methoxytropolone 3.18 via a triflic acid-mediated ring opening. Unfortunately, attempts to demethylate the analogous iodo-based methyl ester methoxytropolone lead to a mixture of products, while the strong lachrymator properties of bromopropiolates posed technical challenges. To overcome these difficulties, we developed a regioselective electrophilic bromination that allowed us to convert tropolones directly to bromotropolones (i.e. 3.8 → 3.18, Scheme 3.3).

### Scheme 3.3. Syntheses of atropisomeric tropolone 3.18.

3.5. **Experimental Validation of Bromotropolone Rotational Barriers**

In order to obtain experimental rotational barrier measurements of 3.18, we attempted to separate the enantiomers via HPLC. Unfortunately, while we were able to obtain analytical chromatographic conditions to resolve the atropisomers of 3.18, attempts to separate the enantiomers via preparatory scale HPLC proved problematic owing to the low separation factor of the peaks.

Instead, we sought to obtain enantioenriched material and monitor thermal racemization, and so we turned our attention to a dynamic, kinetic peptide-catalyzed atroposelective halogenation.
strategy developed by the Miller group \textit{(i.e. 3.36a $\rightarrow$ 3.36b, Scheme 3.4A)}.\textsuperscript{22a} This approach, first published in \textit{Science} in 2010,\textsuperscript{41} exploits the differences in rotational barriers around a chiral axis before and after bromination and has been applied to biaryls,\textsuperscript{41,42} quinazolinones,\textsuperscript{23a} and benzamides.\textsuperscript{22a} To the best of our knowledge, the only atropselective halogenation on a non-phenolic system was reported in 2016 towards the groundbreaking asymmetric total synthesis of natural product marinopyrrole A.\textsuperscript{43} Unfortunately, this route suffered from low selectivity (11\% ee); as such, Miller’s work represents the current state-of-the-art in atropselective halogenation.

The selectivity of Miller’s work, delivered by the peptide catalyst, is provided through a hydrogen bond network involving an H-bond donor and acceptor flanking the bromination site on the axially chiral substrate (see Scheme 3.4). As these features are shared by our troponoid scaffolds, we became intrigued by the possibility of using this method to halogenate troponoids. Thus, we initiated a collaboration with the Miller group to develop the first published method for catalytic, atropselective troponoid synthesis. After some optimization (see Chapter 4 for details), we were able to identify conditions that provided methoxytropolone 3.18 in 75:25 er via catalyst 3.38. While

\begin{center}
\includegraphics[width=\textwidth]{scheme3.12.png}
\end{center}

\textbf{Scheme 3.12.} (A) Peptide-catalyzed asymmetric transformation of 3.36a to 3.36b and associated pre-transition state. (B) Peptide 3.38 was found to deliver enantioenriched 3.18, presumably by operating on the substrate through a similar H-bond network.
efforts remain underway to increase selectivity, this method proved sufficient for obtaining optically enriched material for measuring experimental rotational energy barriers.

### 3.5.1. Determination of Rotational Barrier via Thermal Erosion of Enantiopurity

Racemization studies were performed on 3.18 to obtain its barrier to rotation (Figure 3.8).44 A solution (5.6 mM) of 3.18 (50% ee) in triethylene glycol dimethyl ether (triglyme) was heated in an oil bath at 145 °C. The enantiomeric ratio was measured by reversed phase chiral HPLC at time points spanning 2 to 60 minutes. Plots of ee versus time, and of ln(1/ee) versus time resulted in the respective graphs shown below. Using the slope of these lines and the Eyring equation, the free activation energy was determined and it was found that 3.18 has a barrier to rotation of 30.1 kcal/mol at 145 °C. Owing presumably to hydrogen bonding effects with the glyme solvent, the experimental value is slightly lower than that computed at 145 °C ($\Delta G^\dagger = 32.7$ kcal/mol), though certainly within the range of experimental error. Thus, experimental data clearly indicates that 3.18 has exceptionally high configurational stability and helps further illustrate the role that troponoids could play in designing molecules with enhanced conformational rigidity.
3.6. Resolution of a Single-Atropisomer α-Hydroxytropolone

Given the generally high rotational barriers of troponoids, we became interested in leveraging this quality towards developing molecules with biomedical utility. Recently, there has been a growing appreciation for atropisomerism in drug development and for designing atropisomerism in order to increase target specificity. Given the wealth of biological targets known to be accessible to αHTs, we decided to resolve optically pure αHTs for future biological testing. Simple demethylation using our standard conditions (refluxing HBr/AcOH) was effective for the piperidinyl substrate. The enantiomers were successfully resolved via preparatory CSP-HPLC to a high degree of optical purity and their relative stereochemistry was determined (MPLC ChiralFlash IC: Ent-1: (+)-3.39, >99:1 er; Ent-2: (-)-3.39, 97:3 er), setting the stage for future biological testing. Additionally, (-)-3.39 was found to be stable at physiological conditions (in a 4.4 mM solution of PBS buffer, pH 7.4, at 37 °C) for 24 hours with no loss of enantioenrichment.
observed over this timeframe, further demonstrating the potential value of rotationally restricted troponoids within biological applications.

3.7. Optical Assignment of Bromotroponoid Enantiomers

With optical rotation measurements of enantiopure [(+)-3.39] and enantioenriched [(-)-3.39] in hand, we set about determining the relative stereochemistry of 3.18 in order to determine the preference of peptide 3.38 for formation of (+) or (-) product. To this end, we subjected enantioenriched 3.18 to our demethylation conditions. With a computed $t_{1/2}$ of ~7 hours at the demethylation temperature of 125 °C, we anticipated seeing erosion of enantioenrichment – though not full racemization – within 30 minutes. Thus, we subjected 3.18 to these conditions, and gratifyingly found some retention of enantioenrichment (50% ee → 28% ee). From the HPLC trace of enantioenriched 3.39 as well as analogy to a previously reported atropselective halogenation (3.36a → 3.36b vs. 3.8 → 3.18, Scheme 3.12), we were able to infer that peptide 3.38 favors formation of the second-eluting (-) enantiomer (Figure 3.9). Depending on the relative
bioactivities of (+)- and (-)-3.39, future atropselective synthetic efforts towards single-enantiomer troponoids may benefit from this knowledge.

![Figure 3.9. Determination of optical rotation of enantioenriched 3.18 via demethylation and comparison of HPLC traces.](image)

### 3.8. Conclusions

Atropisomerism has an established and valuable role in a variety of scientific pursuits and troponoids possess unique structural properties resulting in increased rotational barriers that are of high interest in related studies. As such, computational and experimental rotational barriers measured on tropamides revealed that troponoid-based chiral axes have substantially higher rotational barrier measurements when compared to analogous benzenoid systems. Critical to the achievement of this discovery was the computational modeling of a library of axially chiral aryl systems, as well as the development of a catalytic atropselective halogenation on a novel troponoid substrate. Optimization of this reaction will allow us to aid in an emerging paradigm shift towards studying designed and restricted axial chirality in drug development.

### 3.9. References

1 (a) Wencel-Delord, J.; Panossian, A.; Leroux, F.R.; Colobert, F. Chem. Soc. Rev. 2015, 11, 3418-3430. For a recent example of further discoveries in spatial non-point chirality, see: (b) Canfield, P. J.; Blake,


26 CYLview was used to render the computational outputs. See: CYLview, 1.0b, Legault, C. Y. L., Universite de Sherbrooke, 2009 (http://www.cylview.org).


3.10. Supplementary Experimental Details

Computational studies on the methoxytropolones and analogous benzenoids were performed in collaboration with Dr. Anthony Metrano. Elizabeth Stone and Dr. Golo Storch performed the computational studies on the thiazolidines. All of these computational details can be found in the Supporting Information for this manuscript.\textsuperscript{S1}

3.10.1. General Information

All starting materials and reagents were purchased from commercially available sources and used without further purification, with exception of CH\textsubscript{2}Cl\textsubscript{2}, which was purified on a solvent purification system prior to the reaction. All reactions were performed in oven- or flame-dried glassware. \textsuperscript{1}H-NMR shifts are measured using the solvent residual peak as the internal standard (CHCl\textsubscript{3} δ 7.26, MeOH δ 3.31, CH\textsubscript{2}Cl\textsubscript{2} δ 5.32, DMSO δ 2.50), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, dq = doublet of quartet, ABq = AB quartet), coupling constant (Hz), and integration. \textsuperscript{13}C NMR shifts are measured using the solvent residual peak as the internal standard (CHCl\textsubscript{3} δ 77.2, MeOH δ 49.0, DMSO δ 39.5), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Microwave reactions were performed via the Biotage Initiator 2.5. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F254 pre-coated plates (0.25 mm thickness). TLC R\textsubscript{f} values are reported, with visualization accomplished by irradiation with a UV lamp or appropriate TLC stain. Purification via normal phase column chromatography was performed on the Biotage Isolera Prime, with Biotage SNAP 10g or 25g cartridges, in a solvent system of ethyl
acetate and hexanes. Reversed phase chromatography was performed on the Biotage Isolera Prime with Biotage SNAP C18 12 g cartridges, in a solvent system of water and acetonitrile with a 0.05% trifluoroacetic acid additive. Column gradients are measured in terms of column volumes (CV). Mass spectra were recorded on a spectrometer by the electrospray ionization (ESI) technique with a time-of-flight (TOF) mass analyzer. Commercially available dibromodimethylhydantoin (DBDMH) and N-bromosuccinimide (NBS) were purified by recrystallization from hot water. Optical rotations were recorded on a Perkin-Elmer Polarimeter 341 at the sodium D line (1.0 dm path length). Reverse-phase HPLC analysis was conducted with an Agilent 1100 series instrument equipped with a diode array detector (λ = 265 nm) and columns (chiral supports) from Daicel Chemical Industries (Chiralpak IA and Chiralpak IC) at ambient temperature. Reverse phase preparatory HPLC separation was performed on the Biotage Isolera Prime with a 30 x 100 mm (20 µm particle size) ChiralFlash IC column.

3.10.2. Synthesis and characterization of 3-bromo-1-(piperin-1-yl)prop-2-yn-1-one (3.34b)

![Chemical Structure]

**Procedure:** To a solution of 1-(piperin-1-yl)prop-2-yn-1-one 3.34a\(^{82}\) (563.9 mg, 4.11 mmol) in acetone (8.22 mL) was added N-bromosuccinimide (804.6 mg, 4.52 mmol). After stirring for 5 minutes, silver nitrate (69.63 mg, 0.411 mmol) was added slowly ambient temperature. The reaction was allowed to stir for 1.5 hours in the dark before being quenched by 10 mL of water. The reaction mixture was added to a separatory funnel containing 20 mL of CH\(_2\)Cl\(_2\). The organic layer was isolated and the
aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 10 mL). Combined organics were extracted with 20 mL of water, 20 mL of saturated sodium bicarbonate solution, and 20 mL of aqueous sodium chloride. Combined organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to yield 3.34b as a light yellow solid (774.6 mg, 87% yield). Melting point (mp) = 54-56 °C. Rf= 0.35 in 25% EtOAc in hexanes. IR (thin film, KBr) 3443 (br), 2941 (w), 2857 (w), 2098 (m), 1628 (s), 1441 (m), 1268 (w), 1223 (w), 1142 (w), 1014 (w) cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) δ 3.76 – 3.62 (t, 2H), 3.62 – 3.48 (t, 2H), 1.73 – 1.59 (m, 4H), 1.59 – 1.49 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 151.7 (s), 73.7 (s), 55.3 (s), 48.3 (s), 42.7 (s), 26.6 (s), 25.5 (s), 24.6 (s). HRMS (ESI+) m/z calc’d for C$_8$H$_{11}$BrNO$: 216.0019$. Found: 216.0025.

3.10.3. Synthesis and characterization of 8-oxabicyclo[3.2.1]octene (3.35a)

![Diagram](image)

3-methoxy-5-methyl-6-(piperidine-1-carbonyl)-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (3.35a). To a solution of dimer 3.28b (325 mg, 1.16 mmol) in DCM (4.63 mL) was added 1-(piperidin-1-yl)prop-2-yn-1-one 3.34a (954.3 mg, 6.96 mmol). After heating in an oil bath at 100 °C for 90 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column capped with triethylamine, solvent gradient: 2% EtOAc in hexanes (3 CV); 2-10% EtOAc in hexanes (5 CV); 15-25% EtOAc in hexanes (15 CV); 25-35% EtOAc in hexanes (8 CV); 35-60% (8 CV)). Product fractions were concentrated to yield 3.35a as a yellow solid that melts at
122-124 °C (417.9 mg, 65% yield). Rf= 0.32 in 25% EtOAc in hexanes. IR (thin film, KBr)
2937 (s), 2858 (s), 1708 (s), 1605 (s), 1443 (s), 1375 (w), 1347 (w), 1286 (m), 1179 (m), 1130
(m), 1078 (m), 988 (m), 863 (w) cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 6.26 (d, J = 2.4 Hz, 1H),
6.23 (s, 1H), 5.07 (d, J = 2.4 Hz, 1H), 3.68 – 3.59 (m, 2H), 3.57 (s, 3H), 3.54 – 3.44 (m, 2H),
1.74 – 1.64 (m, 2H), 1.61 (s, 3H), 1.59 – 1.49 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 188.6,
163.6, 150.6, 144.9, 127.5, 121.4, 87.2, 86.7, 54.6, 47.7, 42.6, 26.8, 25.6, 24.5, 20.5. HRMS
(ESI+) m/z calc’d for C₁₅H₂₀NΟ₄⁺: 278.1387. Found: 278.1390.

3.10.4. Synthesis and Characterization of Troponoids via Triflic Acid

2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one
(3.8). To a solution of 8-oxabicyclo[3.2.1]octane intermediate 3.35a (100.4 mg, 0.362 mmol) in
dried, deacidified DCM (3.60 mL) was added triflic acid (128.1 μL, 1.5 mmol). The reaction was allowed to stir for 35 minutes at rt before
quenching with pH 7 phosphate buffer and extracting with CH₂Cl₂. Combined organics were dried over Na₂SO₄, filtered, and
concentrated under reduced pressure to yield 3.8 as a pale green/brown solid that melts at 166-
169 °C (97.3 mg, 97% yield). IR (thin film, KBr) 3447 (br), 2938 (m), 2856 (m), 1735 (w),
1620 (s), 1560 (m), 1446 (m), 1325 (w), 1257 (s), 1136 (w), 1152 (w), 1026 (w), 996 (w). ¹H
NMR (400 MHz, CDCl₃) δ 7.15 (s, 1H), 7.02 (s, 1H), 4.00 (s, 3H), 3.85 – 3.57 (m, 2H), 3.28 –
3.10 (m, 2H), 2.41 (s, 3H), 1.67 (d, \( J = 4.7 \) Hz, 4H), 1.61 – 1.37 (m, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\) \( \delta 170.5, 168.7, 159.6, 158.6, 136.7, 132.9, 121.6, 115.9, 56.5, 47.7, 42.5, 26.4, 25.5, 24.4, 24.3. \) HRMS (ESI+) \( m/z \) calc’d for C\(_{13}\)H\(_{20}\)NO\(_4\)\(^+\): 278.1387. Found: 278.1380.

3-bromo-2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (3.18). To a solution of dimer 3.28b (45.8 mg, 0.163 mmol) in DCM (577 μL) was added 3-bromo-1-(piperidin-1-yl)prop-2-yn-1-one (3.34b) (312.1 mg, 1.44 mmol). After microwave irradiation at 100 °C for 35 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-15% EtOAc in hexanes (15 CV); 15-25% EtOAc in hexanes (15 CV); 25-35% EtOAc in hexanes (8 CV); 35-50% (5 CV)). Product fractions were concentrated to yield 3.35b as a semi-pure product that was used without further purification. To a solution of 8-oxabicyclo[3.2.1]octane intermediate 3.35b (10.9 mg, 0.031 mmol) in dried, deacidified DCM (305 μL) was added triflic acid (10.8 μL, 0.122 mmol). The reaction was allowed to stir for 30 minutes at rt before quenching with pH 7 phosphate buffer and extracting with CH\(_2\)Cl\(_2\). Combined organics were dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure to yield 3.8 as a pale yellow/brown waxy solid (6.3 mg, 50% yield over two steps). IR (thin film, KBr) 3424 (br), 2936 (w), 2854 (w), 1632 (w), 1571 (m), 1445 (w), 1352 (m), 1265 (m), 1247 (w), 1169 (w), 1136 (w), 1077 (w), 787 (w). \(^1\)H NMR (400 MHz, CDCl\(_3\) \( \delta 7.02 (s, 1H), 4.01 (s, 3H), 3.90 – 3.56 (m, 2H), 3.32 – 3.10 (m, 2H), 2.48 (s, 3H), 1.86 – 1.57 (m, 4H), 1.57 – 1.41 (m, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\) \( \delta 167.4, 166.4, 159.1, 157.2, 139.5, 131.3, 121.7, 118.8, 56.9, 47.3,
42.4, 26.1, 26.0, 25.2, 24.5. **HRMS (ESI+) m/z** calc’d for C_{15}H_{19}BrNO_{4}: 356.0492. Found: 356.0502.

### 3.10.5. Synthesis and Characterization of Troponoids via Electrophilic Aromatic Substitution

3-bromo-2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (3.18). Procedure A: To a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one 3.8 (10.8 mg, 0.039 mmol) in CDCl_{3} (1.30 mL) was added dibromodimethylhydantoin (DBDMH) (14.25 mg, 0.05 mmol), followed by triethylamine (5.4 μL, 0.039 mmol). The reaction was allowed to stir for 2 hours at rt before quenching with pH 3 phosphate buffer and extracting with CH_{2}Cl_{2}. Combined organics were dried over Na_{2}SO_{4}, filtered, and concentrated under reduced pressure to yield 3.18 as a pale yellow/brown waxy solid (5.6 mg, 88% yield) with \textsuperscript{1}H-NMR data consistent with previously reported data. See Section 3.10.4 for characterization data.
Procedure B: *N*-Bromosuccinimide (NBS, 1.1 equiv.) was added to a 0.02 M solution of tropamide 3.8 (17.3 mg, 0.062 mmol, 1 equiv) and catalyst 3.38 (3.5 mg, 0.006 mmol, 0.1 equiv) in CDCl₃ (3.12 mL) at 0 °C. The reaction was allowed to stir for 72 hours. The reaction was then diluted with 4 mL of DCM, transferred to a separatory funnel, and quenched with an equivalent volume of a pH 3 phosphate buffer (made by diluting 1.7 mL of 2.0 M aqueous sulfuric acid with 12 mL of 1.0 M pH 7 phosphate buffer), and extracted 3x. The combined organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure. Reversed phase chromatography of the crude residue with water/acetonitrile was accomplished on a Biotage Isolera Prime (SNAP 12g C18 silica gel column, solvent gradient: 10% acetonitrile in water (3 CV); 10-23% (8 CV), 23-35% (8 CV), 35-100% (6 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were combined, extracted with excess DCM (3x, Σ = 60 mL), filtered through Na₂SO₄ and concentrated at 30 °C *in vacuo*, yielding 3.18 as a waxy brown/yellow solid (11.2 mg, 50% yield) with ¹H-NMR data consistent with previously reported data. See Section 3.10.4 for characterization data. HPLC 75:25 er (Chiralpak IA, 1.5 mL/min, 35% acetonitrile in water with 0.1% formic acid): R<sub>T(minor)</sub> = 7.2 min, R<sub>T(major)</sub> = 8.1 min.
3.10.6. Synthesis and Chiral Resolution of αHT 3.39

3-bromo-2,7-dihydroxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (3.39). Procedure: To methoxylropolone 3.18 (20.3 mg, 0.057 mmol) was added 892 μL of 33% HBr/AcOH. The reaction was heated in a sealed tube to reflux at 120 °C for 30 minutes before being quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH₂Cl₂ (5 x 10 mL). Combined organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield 3.39 as a waxy brown solid (14.6 mg, 75%). HPLC 55:45 er (Chiralpak IC, 1.5 mL/min, 35% acetonitrile in water with 0.1% formic acid): Rₜ(Ent-1) =
11.0 min, R\textsubscript{T(Ent-2)} = 13.7 min; \textbf{IR (thin film, KBr)} 3420 (br), 2936 (m), 2856 (m), 1633 (s), 1542 (m), 1445 (s), 1270 (w), 1230 (w), 1137 (w), 1067 (s), 810 (w), 735 (w). \textbf{\textsuperscript{1}H NMR (200 MHz, CDCl\textsubscript{3})} \textsuperscript{δ} 7.43 (s, 1H), 3.92 – 3.54 (m, 2H), 3.23 – 3.05 (m, 2H), 2.47 (s, 3H), 1.83 – 1.56 (m, 4H), 1.56 – 1.38 (m, 2H). \textbf{\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3})} \textsuperscript{δ} 167.2, 165.3, 157.0, 156.3, 139.8, 134.9, 123.8, 119.6, 47.2, 42.3, 25.9, 25.7, 25.2, 24.5. \textbf{HRMS (ESI\textsuperscript{+}) \textit{m/z} calc’d for C\textsubscript{14}H\textsubscript{17}NO\textsubscript{4}\textsuperscript{+}: 342.0335. Found: 342.0390.

**Preparatory Chromatography.** 10.0 mg of \textbf{3.39} were loaded onto a Chiralflash IC column (100 mm, i.d. 30 mm, particle size 20 \textmu m) fitted with adaptors for use with an MPLC Biotage Isolera Prime. Conditions: water/acetonitrile/formic acid (70:30:0.1, v/v/v), 12 mL/min, direct load in water/acetonitrile (30:70, v/v). Fractions containing product for both enantiomers were combined separately, extracted with excess DCM (3x, Σ = 300 mL), filtered through Na\textsubscript{2}SO\textsubscript{4} and concentrated at 30 °C \textit{in vacuo}, yielding a total of 2.3 mg of \textbf{3.39-(Ent-1)} and 4.5 mg \textbf{3.39-(Ent-2)}. Optical rotation of Ent-1: [\alpha]_{D}^{20.0} +0.114 (c 1.0, CH\textsubscript{2}Cl\textsubscript{2}); Ent-2: [\alpha]_{D}^{20.0} -0.079 (c 1.0, CH\textsubscript{2}Cl\textsubscript{2}).
Assessment of chemical purity. Racemic material (red trace, bottom) and isolated enantiomers (Ent-1 = green trace, middle; Ent-2 = purple trace, top) were compared via UPLC-MS. Calculated m/z for C_{14}H_{17}NO_4^+: 342.03. Found: 342.12 (Ent-1); 342.16 (Ent-2).

Assessment of enantiopurity. Each sample was assessed via the reversed phase CSP-HPLC conditions previously described. 3.39-(Ent-1): >99:1 er. 3.39-(Ent-2): 97:3 er.

Racemic Material: t_{ret}^1 = 11.0 min, t_{ret}^2 = 13.7 min.
3.39-(Ent-1): >99:1 e.r.

![Graph](image1.png)


![Graph](image2.png)

3.10.7. *Synthesis and Characterization of Thiazolidines*

![Diagram](image3.png)

2,7-dihydroxy-4-methyl-5-(thiazolidine-3-carbonyl)cyclohepta-2,4,6-trien-1-one (3.4).

**Procedure:** To a solution of carboxylic acid\(^3 \) 3.3 (25.0 mg, 0.127 mmol) in THF (3.18 mL) was added 2,6-lutidine (33 μL, 0.280 mmol) and benzotriazol-1-yl-oxytripyrrolidinophosphonium
hexafluorophosphate (PyBOP) (73 mg, 0.140 mmol). The mixture was allowed to stir for 15 min in the dark at rt under an atmosphere of argon gas. Thiazolidine (11.1 μL, 0.140 mmol) was then added to the solution. The reaction was subjected to microwave irradiation at 85 °C for 10 min, concentrated in vacuo, and dissolved in 800 μL of DMSO for purification via reverse phase chromatography (Biotage Isolera Prime, SNAP 12g C18 silica gel column, solvent gradient: 0% acetonitrile in water (3 CV); 0-15% (15 CV); 15-25% (20 CV); 25-50% (8 CV); 50-100% (10 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were concentrated in vacuo to remove acetonitrile, and the remaining aqueous solution was extracted with DCM (3 x 15 mL). The combined organics were dried over sodium sulfate, filtered, and concentrated in vacuo to yield 3.4 as a yellow oil (11.8 mg, 35% yield). IR (thin film, KBr) 3442 (br), 2075 (w), 1637 (m), 1545 (w), 1488 (w), 1302 (w), 1145 (w), 1056 (w), 1033 (w), 781 (w), 537 (w) cm⁻¹. ¹H NMR (400 MHz, MeOD) δ 7.45 (s, 2H), 7.23 (d, J = 2.0 Hz, 2H), 4.71 (ABq, 2H, JAB = 10.3 Hz), 4.29 (ABq, 2H, JAB = 9.5 Hz), 4.02 – 3.84 (m, 2H), 3.63 – 3.43 (m, 2H), 3.23 – 3.12 (m, 2H), 3.11 – 3.00 (m, 2H), 2.36 (d, J = 1.4 Hz, 6H). ¹³C NMR (100 MHz, DMSO) δ 168.3, 168.3, 167.7, 167.5, 159.3, 159.2, 158.2, 158.2, 135.8, 135.5, 134.7, 134.4, 123.5, 123.4, 117.6, 117.6, 50.1, 49.3, 47.3, 47.0, 30.34, 29.3, 22.9, 22.8. For 2D NMR experiments, see Section 3.10.10. HRMS (ESI+) m/z calc’d for C₁₂H₁₄NO₄S⁺: 268.0638. Found: 268.0639.

(5-hydroxy-2-methylphenyl)(thiazolidin-3-yl)methanone (3.6).

Procedure: To a solution of carboxylic acid 3.5 (19.3 mg, 0.127 mmol) in THF (3.18 mL) was added 2,6-lutidine (33 μL, 0.280 mmol) and benzotriazol-1-yl-oxytripyrrolidinophosphonium
hexafluorophosphate (PyBOP) (73 mg, 0.140 mmol). The mixture was allowed to stir for 15 min at rt under an atmosphere of argon gas. Thiazolidine (11.1 µL, 0.140 mmol) was then added to the solution. After microwave irradiation at 85 °C for 10 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-25% EtOAc in hexanes (12 CV); 25-30% EtOAc in hexanes (5 CV); 30-50% EtOAc in hexanes (10 CV); 50-100% (20 CV)). Product fractions were concentrated to yield 3.6 as a white solid (16.2 mg, 57% yield). Rf= 0.22 in 50% EtOAc in hexanes Melting point (mp) = 139-141 °C. IR (thin film, KBr) 3423 (br), 1608 (s), 1450 (s), 1336 (w), 1291 (m), 1261 (w), 1236 (m), 1095 (w), 822 (w), 713 (w) cm⁻¹. ¹H NMR (400 MHz, CDCl₃) ¹H NMR (400 MHz, CDCl₃) δ 7.51 – 7.28 (bs, 2H), 7.01 (dd, J = 8.3, 3.9 Hz, 2H), 6.76 – 6.68 (m, 2H), 6.61 (dd, J = 8.0, 2.3 Hz, 2H), 4.75 (s, 2H), 4.20 (s, 2H), 3.99 (t, J = 6.5 Hz, 2H), 3.49 (t, J = 6.2 Hz, 2H), 3.11 (t, J = 6.5 Hz, 2H), 2.95 (t, J = 6.2 Hz, 2H), 2.19 (d, J = 4.3 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 170.4, 170.2, 154.7, 136.9, 136.6, 132.0, 131.9, 125.1, 124.7, 117.4, 117.4, 113.0, 112.9, 51.2, 50.4, 47.9, 47.6, 30.9, 30.3, 18.1. For 2D NMR experiments, see Section 3.10.10. HRMS (ESI+) m/z calc’d for C₁₁H₁₄NO₂S⁺: 224.0740. Found: 224.0778.

3.10.8. Determination of Kinetic Parameters via Dynamic NMR Studies

Lineshape simulation of the spectra was performed using the iNMR software (Version 6), which gave a rate constant (k) at a range of temperatures. These were converted into rotational barriers using the Eyring equation:

$$
k = \frac{k_B T}{h} e^{-\frac{\Delta G^\ddagger}{RT}}$$

Eq. S3.1

This equation can be rearranged into the form:
The plot of ln(k/T) vs 1/T gives a straight line fitting the form y=mx+b. The slope (m) gives the enthalpy of rotation (ΔH‡):

$$\Delta H^\ddagger = -mR$$  \textbf{Eq. S3.2}

The y-intercept (b) gives the entropy of rotation (ΔS‡):

$$\Delta S^\ddagger = R \left[ b - \ln \left( \frac{k_B}{h} \right) \right]$$  \textbf{Eq. S3.3}

Inserting these values into Gibbs’ equation for free energy (ΔG‡ = ΔH‡ – TΔS‡) gives an estimation of ΔG‡_{298K}. (k_B – Boltzmann constant, h – Planck’s constant, R – universal gas constant, T = 298 K).
A) Determination of C-C Barriers

For troponoid 3.4, lineshape fitting was performed on peaks $A_Z$, $B_Z$, $C_Z$, $A_E$, and $B_E$. Due to its complex splitting pattern, accurate simulation of troponoid signal $C_E$ proved problematic. The reported kinetic parameters (Figure S3.1) represent averages of the values obtained for these signals. Fitting $^1$H-NMR data at temperatures above 353 K was impractical due the amide rotamer signals beginning to coalesce. In the case of benzenoid 3.6, lineshape fitting was performed on $A_E$, $B_Z$, $B_E$, and $C_E$. Simulation of peak $C_Z$ proved similarly challenging, and resolution of peak $A_Z$ was not observed. While it was initially suspected that this effect may be indicative of rotamer Z possessing a higher energy C-C barrier, given that the other Z signals all coalesce, it can be concluded that the chemical shifts of the Z rotamer’s atropdiastereotopic protons are coincidentally equivalent.

Figure S3.1. VT $^1$H-NMR spectra of thiazolidines 3.4 (DMSO-d$_6$, 298-363K) and 3.6 (CD$_2$Cl$_2$, 213-298K). Amide E and Z rotamers are denoted by the subscripts in the peak labels.
Troponoid 3.4: Peak $A_Z$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
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</thead>
<tbody>
<tr>
<td>298</td>
<td>0.003354</td>
<td>4.5</td>
<td>-4.194</td>
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<tr>
<td>323</td>
<td>0.003095</td>
<td>21</td>
<td>-2.734</td>
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<tr>
<td>333</td>
<td>0.003002</td>
<td>45</td>
<td>-2.002</td>
</tr>
<tr>
<td>343</td>
<td>0.002914</td>
<td>89</td>
<td>-1.349</td>
</tr>
<tr>
<td>353</td>
<td>0.002832</td>
<td>167.3</td>
<td>-0.747</td>
</tr>
</tbody>
</table>

Table S3.1. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $A_Z$ of troponoid 3.4.

Troponoid 3.4: Peak $A_E$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
</tr>
</thead>
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Table S3.2. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $A_E$ of troponoid 3.4.

Troponoid 3.4: Peak $B_E$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
</tr>
</thead>
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<td>5.5</td>
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<td>323</td>
<td>0.003095</td>
<td>17.5</td>
<td>-2.916</td>
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<td>333</td>
<td>0.003002</td>
<td>36.5</td>
<td>-2.211</td>
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<td>0.002914</td>
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<td>-1.649</td>
</tr>
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<td>353</td>
<td>0.002832</td>
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<td>-1.202</td>
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</table>

Table S3.3. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $B_E$ of troponoid 3.4.
Troponoid 3.4: Peak B\textsubscript{Z}

<table>
<thead>
<tr>
<th>T (K)</th>
<th>1/T (K\textsuperscript{-1})</th>
<th>k (s\textsuperscript{-1})</th>
<th>ln(k/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>0.003354</td>
<td>5</td>
<td>-4.088</td>
</tr>
<tr>
<td>323</td>
<td>0.003095</td>
<td>25.5</td>
<td>-2.539</td>
</tr>
<tr>
<td>333</td>
<td>0.003002</td>
<td>45</td>
<td>-2.002</td>
</tr>
<tr>
<td>343</td>
<td>0.002914</td>
<td>89</td>
<td>-1.350</td>
</tr>
<tr>
<td>353</td>
<td>0.002832</td>
<td>152.73</td>
<td>-0.838</td>
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</table>

Table S3.4. Rate constants \(k\) for isomerization at given temperatures T, with Eyring plot used to determine kinetic parameters from peak B\textsubscript{Z} of troponoid 3.4.

Troponoid 3.4: Peak C\textsubscript{Z}

<table>
<thead>
<tr>
<th>T (K)</th>
<th>1/T (K\textsuperscript{-1})</th>
<th>k (s\textsuperscript{-1})</th>
<th>ln(k/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>0.003354</td>
<td>5</td>
<td>-4.088</td>
</tr>
<tr>
<td>323</td>
<td>0.003095</td>
<td>26</td>
<td>-2.520</td>
</tr>
<tr>
<td>333</td>
<td>0.003002</td>
<td>63.5</td>
<td>-1.658</td>
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<tr>
<td>343</td>
<td>0.002914</td>
<td>92</td>
<td>-1.316</td>
</tr>
<tr>
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<td>0.002832</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table S3.5. Rate constants \(k\) for isomerization at given temperatures T, with Eyring plot used to determine kinetic parameters from peak C\textsubscript{Z} of troponoid 3.4.

Benzenoid 3.6: Peak A\textsubscript{E}

<table>
<thead>
<tr>
<th>T (K)</th>
<th>1/T (K\textsuperscript{-1})</th>
<th>k (s\textsuperscript{-1})</th>
<th>ln(k/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>213</td>
<td>0.004692</td>
<td>5</td>
<td>-3.752</td>
</tr>
<tr>
<td>223</td>
<td>0.004481</td>
<td>19.7</td>
<td>-2.427</td>
</tr>
<tr>
<td>233</td>
<td>0.004289</td>
<td>62.7</td>
<td>-1.313</td>
</tr>
<tr>
<td>243</td>
<td>0.004113</td>
<td>144.08</td>
<td>-0.523</td>
</tr>
<tr>
<td>258</td>
<td>0.003874</td>
<td>421.5</td>
<td>0.490</td>
</tr>
</tbody>
</table>

Table S3.6. Rate constants \(k\) for isomerization at given temperatures T, with Eyring plot used to determine kinetic parameters from peak A\textsubscript{E} of benzenoid 3.6.
Benzenoid 3.6: Peak $B_E$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>213</td>
<td>0.004692</td>
<td>13.5</td>
<td>-2.759</td>
</tr>
<tr>
<td>223</td>
<td>0.004481</td>
<td>34</td>
<td>-1.881</td>
</tr>
<tr>
<td>233</td>
<td>0.004289</td>
<td>87.5</td>
<td>-0.980</td>
</tr>
<tr>
<td>243</td>
<td>0.004113</td>
<td>197.34</td>
<td>-0.209</td>
</tr>
<tr>
<td>258</td>
<td>0.003874</td>
<td>476.2</td>
<td>0.612</td>
</tr>
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</table>

Table S3.7. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $B_E$ of benzenoid 3.6.

Benzenoid 3.6: Peak $B_Z$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
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<tbody>
<tr>
<td>213</td>
<td>0.004692</td>
<td>5</td>
<td>-3.752</td>
</tr>
<tr>
<td>223</td>
<td>0.004481</td>
<td>19.5</td>
<td>-2.437</td>
</tr>
<tr>
<td>233</td>
<td>0.004289</td>
<td>54.5</td>
<td>-1.454</td>
</tr>
<tr>
<td>243</td>
<td>0.004113</td>
<td>69</td>
<td>-1.260</td>
</tr>
<tr>
<td>258</td>
<td>0.003874</td>
<td>600</td>
<td>0.843</td>
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</table>

Table S3.8. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $B_Z$ of benzenoid 3.6.

Benzenoid 3.6: Peak $C_E$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>213</td>
<td>0.004692</td>
<td>12</td>
<td>-2.877</td>
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<td>223</td>
<td>0.004481</td>
<td>29</td>
<td>-2.051</td>
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<tr>
<td>233</td>
<td>0.004289</td>
<td>61</td>
<td>-1.341</td>
</tr>
<tr>
<td>243</td>
<td>0.004113</td>
<td>162.22</td>
<td>-0.405</td>
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<tr>
<td>258</td>
<td>0.003874</td>
<td>617.14</td>
<td>0.872</td>
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</table>

Table S3.9. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $C_E$ of benzenoid 3.6.
B) Determination of Benzenoid C-N Barrier

For benzenoid 3.6, lineshape fitting to determine the C-N amide isomerization barrier was performed on peaks A_E and A_Z. Since there is a slightly higher population of the Z rotamer as visible by ^1H-NMR (integration of A_E:A_Z = 1.0:1.1), separate analyses were carried out for each isomer (Figure S3.2). As this effect is minor, the reported rotational barrier (see main text) represents an average of these two values.

![Figure S3.2. VT ^1H-NMR spectra of thiazolidine 3.6 (DMSO-d_6, 298-373K). Amide E and Z rotamers are denoted by the subscripts in the peak labels. Kinetic parameters calculated by analysis of 'A' peaks.](image)

Analysis of the ‘A’ peaks gave rate constants k at temperatures spanning 298-373 K (Tables S3.10-S3.11). Using the method described above, these rate constants were used to calculate the reported kinetic parameters.
Benzenoid 3.6: Peak $A_Z$ (representing $Z \rightarrow E$ isomerization)

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
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<td>6.50</td>
<td>-3.826</td>
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<tr>
<td>303</td>
<td>0.003299</td>
<td>7.25</td>
<td>-3.733</td>
</tr>
<tr>
<td>313</td>
<td>0.003193</td>
<td>18.25</td>
<td>-2.842</td>
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<tr>
<td>323</td>
<td>0.003095</td>
<td>36.75</td>
<td>-2.174</td>
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<td>333</td>
<td>0.003002</td>
<td>81.75</td>
<td>-1.405</td>
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<td>343</td>
<td>0.002914</td>
<td>157.70</td>
<td>-0.777</td>
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<td>353</td>
<td>0.002832</td>
<td>307.54</td>
<td>-0.138</td>
</tr>
<tr>
<td>363</td>
<td>0.002754</td>
<td>627.12</td>
<td>0.546</td>
</tr>
<tr>
<td>373</td>
<td>0.002680</td>
<td>1027.00</td>
<td>1.012</td>
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</table>

Table S3.10. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $A_Z$ of benzenoid 3.6.

Benzenoid 3.6: Peak $A_E$ (representing $E \rightarrow Z$ isomerization)

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$1/T$ (K$^{-1}$)</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\ln(k/T)$</th>
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<td>303</td>
<td>0.003299</td>
<td>8.25</td>
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<td>313</td>
<td>0.003193</td>
<td>23.75</td>
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<td>0.003095</td>
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<td>0.546</td>
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<tr>
<td>373</td>
<td>0.002680</td>
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<td>1.012</td>
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</table>

Table S3.11. Rate constants $k$ for isomerization at given temperatures $T$, with Eyring plot used to determine kinetic parameters from peak $A_E$ of benzenoid 3.6.
3.10.9. Experimental Barrier Determination of 3.18 via Thermal Racemization

To an oven-dried 1 dram vial equipped with a stir bar was added a solution (5.6 mM) of 3.18 (5.0 mg, 50% ee) in triethylene glycol dimethyl ether (triglyme, 2 mL). The vial was sealed with a puncturable septa-lined cap and heated in an oil bath (equipped with a temperature probe and a thermometer to confirm temperature) at 145 °C. The enantiomeric ratio was measured by reversed phase CSP-HPLC (I-A column, 35% acetonitrile in water with 0.1% formic acid additive, 5 µL injection volume, 1.5 mL/min; monitored at 265 nm) at time points spanning 1 to 90 minutes. Aliquots of 60 µL were withdrawn from the reaction vessel and quickly diluted in 40 µL of stock 3:1 ACN:H₂O solution at 0 °C before analysis (see Figure S3.4 for a representative example). Plots of ee versus time, and of ln(1/ee) versus time were generated from the data described in Tables S3.12-S3.14, and resulted in the respective graphs shown below (Figure S3.4).

![Trial 1 (Representative trial, 418K)](image)

Figure S3.3. Representative example (trial 1) of HPLC traces monitoring thermal racemization of 3.18 at 418K in triglyme.
Table S3.12. Enantiomeric excess measured at various timepoints with HPLC spectra, trial 1.

<table>
<thead>
<tr>
<th>Trial 1</th>
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<td>Entry</td>
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<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Time (s)</td>
<td></td>
<td>69.00</td>
<td>185.00</td>
<td>310.00</td>
<td>725.00</td>
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<td>ee (x 10^{-2}, %)</td>
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<td>0.356</td>
<td>0.188</td>
<td>0.118</td>
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<tr>
<td>ln(1/ee)</td>
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<td>0.705</td>
<td>1.03</td>
<td>1.67</td>
<td>2.14</td>
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Table S3.13. Enantiomeric excess measured at various timepoints with HPLC spectra, trial 2.

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<td></td>
<td>Entry</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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</tr>
<tr>
<td>Time</td>
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<td>247.00</td>
<td>361.00</td>
<td>479.00</td>
<td>602.00</td>
<td>1204.00</td>
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<td>ee (x 10^{-2}, %)</td>
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<td>0.246</td>
<td>0.178</td>
<td>0.148</td>
<td>0.118</td>
<td>0.118</td>
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<tr>
<td>ln(1/ee)</td>
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<td>1.73</td>
<td>1.91</td>
<td>2.14</td>
<td>2.14</td>
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</table>

Table S3.14. Enantiomeric excess measured at various timepoints with HPLC spectra, trial 3.

<table>
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<td>Entry</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
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<td>Time</td>
<td></td>
<td>90.00</td>
<td>166.00</td>
<td>234.00</td>
<td>300.00</td>
<td>363.00</td>
<td>425.00</td>
</tr>
<tr>
<td>ee (x 10^{-2}, %)</td>
<td></td>
<td>0.458</td>
<td>0.336</td>
<td>0.226</td>
<td>0.176</td>
<td>0.154</td>
<td>0.120</td>
</tr>
<tr>
<td>ln(1/ee)</td>
<td></td>
<td>0.781</td>
<td>1.09</td>
<td>1.49</td>
<td>1.73</td>
<td>1.87</td>
<td>2.12</td>
</tr>
</tbody>
</table>
Racemization rate constants \( (k_{\text{rac}}) \) were derived from the slopes of the lines shown in Figure S3.4. The rate constants to enantiomerization, \( k_{\text{enant}} \), were obtained by halving \( k_{\text{rac}} \) values. Using a rearranged form (Eq. S3.1B) of the Eyring equation described above (Eq. S3.1), the free activation energies of racemization (\( \Delta G^{f}_{\text{rac}} \)) and enantiomerization (\( \Delta G^{f}_{\text{enant}} \)) were determined. These values are summarized in Table S3.15.
\[ \Delta G^\ddagger_{\text{enant}} = RT \times \left[ \ln \left( \frac{k_{\text{enant}} \hbar}{k_B T} \right) \right] \]

**Eq. S3.1B**

Table S3.15. Experimentally determined rate constants and rotational barriers of 3.18 at 145 °C.

<table>
<thead>
<tr>
<th>Trial</th>
<th>(k_{\text{rac}}) (s(^{-1}))</th>
<th>(\Delta G^\ddagger_{\text{rac}}) (kcal/mol)</th>
<th>(k_{\text{enant.}}) (s(^{-1}))</th>
<th>(\Delta G^\ddagger_{\text{enant.}}) (kcal/mol)</th>
<th>Average (\Delta G^\ddagger_{\text{enant.}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00402</td>
<td>29.372</td>
<td>0.00201</td>
<td>29.948</td>
<td>30.03 kcal/mol</td>
</tr>
<tr>
<td>2</td>
<td>0.00300</td>
<td>29.615</td>
<td>0.00150</td>
<td>30.192</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.00400</td>
<td>29.377</td>
<td>0.00200</td>
<td>29.954</td>
<td></td>
</tr>
</tbody>
</table>
3.10.10. NMR Spectra of New Compounds

$^1$H-NMR of 3.34b
$^{13}\text{C-NMR of 3.34b}$
$^{13}$C-NMR of 3.35a
$^{13}$C-NMR of 3,8
$^{1}H$-NMR of 3.18
$^{13}$C-NMR of 3.39
\[^1\text{H-NMR of 3.4}\]
$^{13}$C-NMR of 3.4
COSY NMR of 3.6
3.10.11. Supplementary References


Chapter IV

Atropselective Halogenation of Troponoids

4.1. Introduction

Atropisomerism, a form of chirality arising from restricted rotation about a chiral axis, plays an important role in a number of scientific pursuits including ligand development (e.g. 4.1, Figure 4.1), molecular devices, and drug discovery. While such rotationally restricted scaffolds are ubiquitous among modern therapeutics, single-atropisomer drugs are rarely created by design, and are instead frequently based on natural products (e.g. 4.2 and 4.3, Figure 4.1). Given the critical importance of chirality in drug development, and the potential target selectivity increases of single-atropisomer drugs, they are also becoming increasingly prevalent in de novo drug design (4.4, Figure 4.1). As was discussed in Chapter 3, this paradigm shift has the potential to usher in a new wave of atropisomerically pure drugs that have enhanced selectivity profiles over analogous racemic or racemizable compounds. Synthetic efforts towards selective formation of single-atropisomer bioactive molecules therefore constitute an essential area of study that is rapidly garnering increasing attention from medicinal, synthetic, and physical chemists alike (Scheme 4.1).

![Figure 4.1](image-url)

**Figure 4.1.** Examples of highly valuable molecules that possess atropisomerism. BINOL is used in a wide variety of asymmetric catalysis reactions, while gossypol, colchicine, and the pictured kinase inhibitor are all under investigation for the differential bioactivities of their respective atropisomers.
Approaches involving applications of various chiral reagents are described in the literature. One classic example of this strategy is the work of Bringmann, who was able to perform an asymmetric CBS reduction of lactone 4.5 using a chiral reducing agent (S)-oxazaborolidine 4.6 to access 4.7 (a precursor to natural product dioncopeltine A) with excellent selectivity (Figure 4.2A). Chiral auxiliaries have also found utility in this regard. In 2012, Colobert and coworkers

Scheme 4.1. Methods of atroposelective synthesis. Highlighted approaches feature key steps involving (A) asymmetric reagents, (B) chiral auxiliaries in cross coupling reactions, and (C-D) chiral ligands in cross coupling reactions.
reported a highly atropselective synthesis of the biaryl moiety (4.10) of vancomycin using a β-hydroxysulfoxide auxiliary (Scheme 4.1B). More widely studied, however, are techniques for atropselective cross-coupling, which by nature are more efficient transformations since no attachment/removal of an auxiliary is required. The first reported example of an atropselective cross-coupling was published by Makoto Kumada in 1975 (Scheme 4.1C). Using ligands 4.14 and 4.15 he was able to access binaphthyl compound 4.13 via a chiral phosphine-nickel catalyst. While this transformation was achieved with modest selectivity, it represents a breakthrough in atropselective synthesis and has set the stage for numerous advancements in this field. One such example comes from the laboratory of Buchwald and coworkers, who reported efficient syntheses of axially chiral biaryl amides (such as 4.18, Scheme 4.1D). These were reported in high yields and enantioselectivities via an asymmetric Suzuki coupling with KenPhos 4.19 as a ligand.

Despite these advancements, atropselective synthetic methods are often stifled by the exceptionally high rotational barriers (>28 kcal/mol) needed to maintain configurational stability. This is especially problematic in the development of atropselective transformations for biological studies, where physiological conditions and metabolic processes can further influence these barriers. The following chapter describes a solution to these challenges by outlining a new synthetic strategy to access enantioenriched troponoids. Troponoids are bioactive non-benzenoid aromatic molecules that are predicted to have remarkably high barriers compared to the 5- and 6-membered rings commonly found in drugs (see Chapter 3 for more details). This is due to their decreased external bond angles and increased sensitivity to the so-called “buttressing effect” (Figure 4.2A). However, other features such as tropylium characteristics, ring puckering, and decreased aromaticity could influence the rotational barriers as well. To the best of our knowledge, the only troponoid known to be atropisomeric is colchicine (4.3, Figure 4.1A, ΔG‡ =
22 kcal/mol) and related analogs. Interestingly, while it would be expected to racemize within minutes at room temperature, an added stereocenter renders the aS form more thermodynamically favorable.\textsuperscript{6} The only other troponoid known to possess any degree of atropisomerism is bistropone homodimer 4.20, which has a relatively low barrier to isomerization ($\Delta G^\ddagger = 20.7$ kcal/mol) such that it can only undergo chiral resolution at decreased temperatures (Figure 4.2B).\textsuperscript{21} Given the growing interest in troponoid drug development by our group\textsuperscript{22} and others,\textsuperscript{23} as well as the importance of atropisomerism throughout the field of chemistry, the development of an asymmetric, catalytic method of single-atropisomer troponoid synthesis would significantly advance biological studies on these molecules. To date, no such methods exist.

![Figure 4.2](image)

**Figure 4.2.** (A) Owing to their decreased external bond angles, larger aryl rings are predicted to have higher rotational energies based on the increased proximity of their substituents and a greater sensitivity to the "buttressing effect". (B) Bistropone homodimer with experimental rotational barrier measurement, shown with accompanying HPLC traces at 2-30 °C.

To address this limitation, we turned our attention to atropselective halogenation. This technique has recently emerged as a viable synthetic strategy towards accessing enantioenriched benzamides,\textsuperscript{24} biaryls,\textsuperscript{25} quinazolinones,\textsuperscript{26} quinolines,\textsuperscript{27} and other scaffolds (Scheme 4.2A).\textsuperscript{28} These transformations are typically dynamic, kinetic resolutions that are mediated by bifunctional organocatalysts capable of recognizing specific substrate conformations through hydrogen-bonding interactions.\textsuperscript{29} However, these methods have found limited application in the synthesis of single-atropisomer drugs and drug candidates.\textsuperscript{30} We therefore began our studies by investigating
atropselective troponoid synthesis via a peptide-catalyzed halogenation strategy developed by Scott Miller’s group at Yale University.\textsuperscript{24-26} This approach exploits differences in rotational barriers around a chiral axis before and after bromination and has been applied to a range of scaffolds including benzamides (\textit{i.e.} \textbf{4.25} $\rightarrow$ \textbf{4.26}, Scheme 4.2B),\textsuperscript{24} which are close troponoid homologs. Prior studies indicate that effective catalysis is contingent on certain structural features of the peptide (\textit{e.g.} \textbf{4.29I}),\textsuperscript{26} primarily revolving around functional groups capable of forming H-bond contacts between the catalyst and the substrate.\textsuperscript{31} These include the basic $\beta$-dimethylaminoalanine (Dmaa) residue (which is capable of targeting acidic moieties on the substrate) as well as the capacity to exist in a $\beta$-hairpin structure (Scheme 4.2B).\textsuperscript{32}

![Diagram](image_url)
More recent $^1$H-NMR and DFT studies further support this hypothesis. Furthermore, selectivity is likely provided by substrate H-bond donor and acceptor groups flanking the bromination site (see Chapter 3 for details). As these features are shared by our synthetic troponoid scaffolds, we became intrigued by the possibility of using these peptides to halogenate troponoids (i.e. 4.27 $\rightarrow$ 4.28), and thus initiated a collaboration with the Miller group to develop the first ever method for atropselective troponoid synthesis.

4.2. Catalyst Identification

Our studies began with a screen of peptide-based catalysts for the bromination of amidotroponoid 4.27 using the conditions described in Scheme 4.3. Our choice of catalysts was guided by previous reports from the Miller group on analogous scaffolds, wherein the Dmaa residue was embedded into a sequence that was capable of adopting a β-turn geometry (i.e., H-bonding between the $i$ and $i+3$ residues, Scheme 4.2, shown in red). These characteristics were predicted to facilitate selective complexation with one atropisomer of $(\pm)$-4.27 over the other.

In the absence of catalyst, bromination of 4.27 was nonselective and sluggish (Scheme 4.3). In the presence of triethylamine as a catalyst, however, the reaction provided 50% conversion of racemic 4.28. Site-selectivity was confirmed by synthesis of the authentic isomer via the regioselective oxidopyrylium cycloaddition/ring-opening strategy discussed in Chapter 3. Upon observing identical $^1$H-NMR profiles, we began to assess peptide-based catalysts for this transformation at a variety of temperatures. Where possible, the reactions were monitored by $^1$H-NMR and run to full conversion. The results of this optimization are summarized in Table 4.1 below.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Peptide Sequence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time</th>
<th>Temp.</th>
<th>Quench</th>
<th>Conversion&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Yield&lt;sup&gt;c&lt;/sup&gt;</th>
<th>ee&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc-Dmaa-0-Pro-Alb-Leu-OMe (4.29a)</td>
<td>2 hr</td>
<td>RT</td>
<td>DMAP/AC&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>100%</td>
<td>53%</td>
<td>40%</td>
</tr>
<tr>
<td>2</td>
<td>Boc-Dmaa-0-Pro-Apc-Leu-OMe (4.29b)</td>
<td>98 hr</td>
<td>RT</td>
<td>DMAP/AC&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>100%</td>
<td>41%</td>
<td>6%</td>
</tr>
<tr>
<td>3</td>
<td>Boc-Dmaa-0-Pro-Acbc-Leu-OMe (4.29c)</td>
<td>46 hr</td>
<td>RT</td>
<td>DMAP/AC&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>100%</td>
<td>6%</td>
<td>31%</td>
</tr>
<tr>
<td>4</td>
<td>Boc-Dmaa-0-Pro-Aic-Leu-OMe (4.29d)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>15%</td>
<td>24%</td>
</tr>
<tr>
<td>5</td>
<td>Boc-Dmaa-0-Pro-Achc-Leu-OMe (4.29e)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>56%</td>
<td>28%</td>
</tr>
<tr>
<td>6</td>
<td>Boc-Dmaa-0-Pro-Phe-Leu-OMe (4.29f)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>n/a</td>
<td>28%</td>
</tr>
<tr>
<td>7</td>
<td>Boc-Dmaa-0-Pro-Gly-Leu-OMe (4.29g)</td>
<td>17 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>30%</td>
<td>22%</td>
</tr>
<tr>
<td>8</td>
<td>Boc-Dmaa-0-Pro-Gly-Leu-OMe (4.29h)</td>
<td>3 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>21%</td>
<td>33%</td>
</tr>
<tr>
<td>9</td>
<td>Boc-Dmaa-0-Pro-Phe-Leu-OMe (4.29i)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>n/a</td>
<td>52%</td>
<td>20%</td>
</tr>
<tr>
<td>10</td>
<td>Boc-Dmaa-0-Pro-Alb-[αMe, αPhe]-OMe (4.29j)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>81%</td>
<td>43%</td>
<td>54%</td>
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<tr>
<td>11</td>
<td>Boc-Dmaa-0-Pro-Alb-[αααα, αPhe]-OMe (4.29k)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>69%</td>
<td>32%</td>
</tr>
<tr>
<td>12</td>
<td>Boc-Tmga-0-Pro-Alc-Phe-OMe (4.29l)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>90%</td>
<td>66%</td>
<td>3%</td>
</tr>
<tr>
<td>13</td>
<td>Boc-Dmaa-0-Pro-Alb-Phe-OMe (4.29m)</td>
<td>24 hrs</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>47%</td>
<td>40%</td>
</tr>
<tr>
<td>14</td>
<td>Boc-Dmaa-0-Pro-Alb-Leu-OMe (4.29n)</td>
<td>4.5 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>33%</td>
<td>-6%</td>
</tr>
<tr>
<td>15</td>
<td>Boc-Dmaa-0-Pro-Ac7c-Leu-OMe (4.29o)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>61%</td>
<td>41%</td>
<td>18%</td>
</tr>
<tr>
<td>16</td>
<td>Boc-Dmaa-0-Pro-Acoc-Leu-OMe (4.29p)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>84%</td>
<td>59%</td>
<td>40%</td>
</tr>
<tr>
<td>17</td>
<td>Boc-Dmaa-0-Pro-Cpg-Leu-OMe (4.29q)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>45%</td>
<td>36%</td>
</tr>
<tr>
<td>18</td>
<td>Boc-Dmaa-0-Pro-Alb-Val-OMe (4.29r)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>19</td>
<td>Boc-Dmaa-0-Pro-Alb-Ile-OMe (4.29s)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>45%</td>
<td>32%</td>
</tr>
<tr>
<td>20</td>
<td>Boc-Dmaa-0-Pro-Alb-Tle-OMe (4.29t)</td>
<td>6 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>91%</td>
<td>49%</td>
<td>2%</td>
</tr>
<tr>
<td>21</td>
<td>Boc-[Phe-F&lt;sub&gt;3&lt;/sub&gt;]-0-Pro-Alb-Phe-OMe (4.29u)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>87%</td>
<td>39%</td>
<td>7%</td>
</tr>
<tr>
<td>22</td>
<td>Boc-Phe-0-Pro-Alb-Leu-OMe (4.29v)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>70%</td>
<td>23%</td>
<td>4%</td>
</tr>
<tr>
<td>23</td>
<td>Boc-[3Pall]-0-Pro-Alb-Leu-OMe (4.29w)</td>
<td>24 hr</td>
<td>RT</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>68%</td>
<td>31%</td>
<td>6%</td>
</tr>
<tr>
<td>24</td>
<td>Boc-Dmaa-0-Pro-Alb-2Nap-OMe (4.29x)</td>
<td>24 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>38%</td>
<td>34%</td>
</tr>
<tr>
<td>25</td>
<td>Boc-Dmaa-0-Pro-Alb-2Nap-Pyr (4.29y)</td>
<td>24 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>44%</td>
<td>38%</td>
</tr>
<tr>
<td>26</td>
<td>Boc-Dmaa-0-Pro-[αMe, Val]-2Nap-OMe (4.29z)</td>
<td>24 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>45%</td>
<td>34%</td>
</tr>
<tr>
<td>27</td>
<td>Boc-α-Me-Hyp(But)-Cl-Leu-OMe (4.29aa)</td>
<td>24 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>19%</td>
<td>2%</td>
</tr>
<tr>
<td>28</td>
<td>Fmoc-0-Pro-Alb-Phe-2Nap-OMe (4.29ab)</td>
<td>24 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>44%</td>
<td>4%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peptide abbreviations: Dmaa, β-dimethylaminoalanine; Alb, 2-aminobutryic acid; Cle, cycloleucine (1-aminocyclopentane carboxylic acid); Apc, 1-aminocyclopropane carboxylic acid; Acbc, 1-aminocyclobutyl-1-carboxamide; Alic, 2-aminodinane carboxylic acid; Aqh, 1-aminoclohexane-1-carboxamide; Phe, phenylalanine; Gly, glycine; Ac7c, 1-aminocloheptane-1-carboxylic acid; Acoc, 1-aminoclooctane-1-carboxylic acid; Tmga, tetramethylguanidine; Png, phenylglycine; Chg, cyclohexylglycine; 2nap, β-naphthylalanine; Hyp(But), O-butyl-d-hydroxyproline; Ile, isoleucine; Tle, t-butyl-leucine; Phe-F<sub>5</sub>, pentafluorophenylalanine; 3-Pal, 3-β-3-pyridylalanine; Pyrr, pyrroldinyl; αmth, α-methyl histidine; Thr, threonine. <sup>b</sup> Approximation of conversion based on 4.27:4.28<sup>1</sup> H-NMR ratios post-quench. <sup>c</sup> Isolated yield following flash chromatography. <sup>d</sup> Determined by CSP-HPLC using an AS-H column for normal phase and an IA column for reversed phase chromatography. <sup>e</sup> Used 2.2 eq NBS, 20 mol% peptide.
Gratifyingly, peptides 4.29a, 4.29i, 4.29l, 4.29o, and 4.29q (highlighted in blue in Table 4.1) were discovered to promote conversion to bromotroponoid 4.28 in roughly 40-50% ee. Consistent with previous reports, these catalysts are known to be capable of adopting β-turn geometries that have been demonstrated to be necessary for enantioselectivity in related scaffolds.33,35 Effective catalysts were found to share several other structural characteristics, including a Dmaa residue at the i position. A preliminary investigation into the impact of the i residue provided some insights into the mechanism of bromination (Figure 4.3). It was found that increasing the pKa of this functionality (as in the case of the tetramethyl guanidine (Tmga), Entry 12) resulted in a nonselective reaction. Similarly, π-methyl histidine- and threonine-based catalysts 4.29z and 4.29aa (Entries 27 and 28, respectively) also resulted in racemic reactions. New synthetic constructs with aryl rings at the i position (4.29t, 4.29u, 4.29v; Entries 21-23) were synthesized in hopes of engaging in favorable π/π stacking interactions between the catalyst and substrate. This proved not to be the case. The nature of substrate/catalyst complexation is contingent on many factors, and is the result of multiple dynamic processes and interactions. The change in both steric and electronic factors may both be responsible for these peptides adopting an unfavorable geometry.

The efficacy of peptide-catalyzed reactions is often particularly sensitive to functionality at the i+2 position.26 This position plays a key role in determining certain structural attributes of
the peptide. Upon examining this residue, we observed pronounced differences in selectivity resulting from seemingly minor alterations (as in 4.29a, 4.29b, 4.29c; see Figure 4.4). Indeed, in prior desymmetrization bromination studies, enantiodivergent catalysis has been observed using similar catalysts, featuring either a cyclopropyl or an aminoisobutyramide residue which differ by only 2 amu. While this effect is not fully understood, a linear correlation has previously been observed between enantioselectivity and the τ(i+2) angle in the bromination of axially chiral quinazolinones. This angle is a function of the dihedral φ and ψ angles of the i+2 position and impacts the nature of the β-turn geometry. Accordingly, a difference of 36% ee was observed (Entries 2 and 3). Additionally, a similar effect was observed when comparing cycloheptyl- and cyclooctyl-substituted catalysts 4.29n and 4.29o (Entries 15 and 16), wherein selectivity was doubled with addition of a single methylene unit in a substituent with existing steric bulk.

![Figure 4.4. Effects of i+2 substitution on enantioselectivity of troponoid bromination.](image)

Having identified the Boc-Dmaa-dPro-Aib sequence to be optimal, we began to assess i+3 substituents. In so doing, we again observed a high degree of sensitivity of catalytic activity towards seemingly subtle structural changes to the peptide scaffold. In addition to changes in peptidic secondary structural attributes, a more obvious steric element may also be in effect. While the degree of enhancement observed across the span of several i+2 residues (Acpc, Acbc, Aib) is likely too large to be explained by stericities alone, an interesting trend was observed between valine, isoleucine, and tert-leucine-substituted peptides. While valine at the i+3 position was found to
deliver bromotroponoid 4.28 in up to 50% ee, isoleucine substitution decreased catalytic activity by nearly half (32% ee, Entry 19), and tert-butyl leucine was nonselective (Entry 20; see Figure 4.5). This trend is reflected in the reported A-values of isopropyl and tert-butyl substituents, potentially suggesting a steric component to achieving favorable substrate-catalyst interactions.\textsuperscript{37} Moreover, a preliminary screen of various C-terminal functionality did not yield any improvement over peptide leads 4.29a, 4.29i, 4.29l, 4.29o, or 4.29q, highlighting the methyl ester endcap as a suitable protecting group and thus setting the stage for further reaction optimization.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure45.png}
\caption{Effects of i+3 substitution on enantioselectivity of troponoid bromination.}
\end{figure}

4.3. Optimization of the Reaction Conditions

Having identified catalysts 4.29a/4.29l and 4.29q as capable of delivering 4.28 in 40% and 50% ee respectively, we looked more closely at probing reaction conditions. These results are summarized in Table 4.2. Of note, while it was discovered that catalyst 4.29q was capable of delivering comparable selectivity at 5 mol% as compared to 10 mol% (Entries 28 and 27, Table 4.2), the majority of these studies were performed using 10% catalyst loading. Interestingly, it was found that stoichiometric peptide proved deleterious to the reaction (Entry 23), and that more dilute concentrations – while not substantially affecting selectivity – proved detrimental to the isolated yield (Entry 31).
Table 4.2. Reaction optimization studies.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Br&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solvent</th>
<th>Time</th>
<th>Temp.</th>
<th>Quench</th>
<th>Conversion&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Yield&lt;sup&gt;c&lt;/sup&gt;</th>
<th>ee&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td><strong>Boc-Dmaa-dPro-Alb-Leu-OMe (4.29a)</strong></td>
<td></td>
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<tr>
<td>1</td>
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<td>24 hr</td>
<td>RT</td>
<td>HCl</td>
<td>100%</td>
<td>54%</td>
<td>28%</td>
</tr>
<tr>
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<td>2 hr</td>
<td>RT</td>
<td>DMAP/Ac&lt;sub&gt;2&lt;/sub&gt;O</td>
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<td>53%</td>
<td>40%</td>
</tr>
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<td>3</td>
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<td>2 min</td>
<td>RT</td>
<td>NEt&lt;sub&gt;3&lt;/sub&gt;</td>
<td>100%</td>
<td>11%</td>
<td>40%</td>
</tr>
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<td>4</td>
<td>NBS</td>
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<td>3 hr</td>
<td>-40 °C</td>
<td>NEt&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>11%</td>
<td>36%</td>
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<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>4 days</td>
<td>-10 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>3 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>48%</td>
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<td>NBS</td>
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<td>3 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>3 days</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>CDCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
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<td>3 hr</td>
<td>0 °C</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100%</td>
<td>26%</td>
<td>50%</td>
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</table>

<sup>a</sup> Approximation of conversion based on 4.27.1-28 <sup>1</sup>H-NMR ratios post-quench.  
<sup>b</sup> Isolated yield after flash chromatography.  
<sup>c</sup> Determined by chiral stationary phase HPLC using an IA column.  
<sup>d</sup> Solvent: 30% CO(CD<sub>3</sub>)<sub>2</sub> in CDCl<sub>3</sub>.  
<sup>e</sup> Solvent: 55% THF-d<sub>8</sub> in CDCl<sub>3</sub>.  
<sup>f</sup> Solvent: 60% Tol-d<sub>8</sub> in CO(CD<sub>3</sub>)<sub>2</sub>.  
<sup>g</sup> Solvent: 70% EtOAc in CDCl<sub>3</sub>.  
<sup>h</sup> Used stoichiometric (100 mol%) catalyst loading.  
<sup>i</sup> Used 5 mol% catalyst loading.  
<sup>j</sup> Used 2 mol% catalyst loading.  
<sup>k</sup> Used 5 mol% catalyst loading with no aqueous quench prior to reversed phase MPLC purification.  
<sup>l</sup> Reaction run at 0.01 M concentration.  
<sup>m</sup> N-Chlorosuccinimide, NCS; dichlorodiethylhydantoin, DCDMH.  

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We next turned our attention to a screen of bromination reagents. Previous reports from the Miller lab demonstrate profound selectivity differences depending on the bromonium source. \(^{24-26}\) We thus compared bromination reagents \(N\)-bromophthalimide (NBP), \(N\)-bromoacetamide (NBA), and \(N\)-bromosaccharin (NBSac) to NBS. Brominating reagent NBS was found to outperform the others in terms of conversion, yield, and selectivity (Entries 8, 10, 12 and 13). However, NBP demonstrated comparable selectivity. We hypothesize that this might be an effect of the dibrominated amide byproduct (Figure 4.6). Whereas succinimide and phthalimide have similar pK\(_a\)s (9.6\(^{38a}\) and 8.3, \(^{38b}\) respectively), acetamide (pK\(_a\) = 17)\(^{39}\) and saccharin (pK\(_a\) = 1.6)\(^{40}\) are well outside of this range, which may facilitate non-selective peptide/substrate contacts, or alter their protonation states altogether. Notably, chlorination reagents were also briefly assessed (Entries 14 and 15) and found to deliver substantially lowered selectivities and decomposition of substrate to an unidentifiable reaction mixture. However, further investigation of chlorination is warranted.

Temperature effects were also examined. While colder conditions proved beneficial, NBS dissolution was poor at temperatures below 0 °C. A similar issue was encountered while investigating solvent effects, where both NBS and substrate solubility were significantly impacted by decreases in temperature. Ultimately, CDCl\(_3\) proved to be the most effective solvent. Interestingly, impacts on conversion were observed when CHCl\(_3\) was used in place of CDCl\(_3\) (not
shown). Side reactions, potentially with chloroform additive amylene were observed, although the specific nature of these decomposition pathways is unclear at present.

Deuterated solvents served an additional purpose in allowing us to monitor the progress of these reactions by $^1$H-NMR. As such, the reactions were quenched when they reached full conversion, or when conversion appeared to plateau. Such quenches ranged from basic (Entries 2-4) to acidic in nature. DMAP/Ac$_2$O quenches were employed prior to procurement of reversed phase chiral HPLC supports, as a means of acetylating 4.28 for ease of normal phase purification. Head-to-head assessments (not shown) of this quench and an acid quench proved comparable in terms of selectivity. Acidic quenches were conducted by adding acidic solutions to a pre-made 1.0 M pH 7 phosphate buffer, to reach an ultimate pH of 3. Curiously, the nature of this quench in certain instances lead to different outcomes in terms of selectivity (vide infra), prompting further mechanistic investigations.

### 4.4. Mechanism-Driven Experiments

Examination of certain elements of the reaction optimization inspired us to investigate mechanistic aspects of this reaction. This was largely borne out of the discovery of tentatively-assigned intermediate 4.34 upon monitoring the reactions by $^1$H-NMR. The intermediate appears to be related to the mechanism of bromination (see Scheme 4.4) and is characterized by long-range W-coupling between H$_C$ and the proximal methyl group. Further $^{13}$C-NMR and HSQC studies, though not definitive, support this structural hypothesis (see Supporting Information). The intermediate was observed in all reactions that demonstrated selectivity, and was absent from the nonselective runs. It was also observed, fleetingly and at trace
levels, in a $^1$H-NMR study of background, uncatalyzed bromination (see Supporting Information for details).

![Scheme 4.4](image)

**Scheme 4.4.** Hypothesized mechanism showing formation and rearomatization of a potentially stereodetermining intermediate 4.34. $^1$H-NMRs show presence of starting material, immediate full conversion to 4.34, and slow rearomatization to bromotroponoid 4.28. Stereochemistry of 4.34 is shown arbitrarily.

In cases where intermediate 4.34 was observed, its formation was rapid (immediate), while rearomatization to product was a slower process (in some cases taking up to a day to convert fully; see Scheme 4.4). This is particularly perplexing given the increased electrophilicity of troponoids with respect to benzenoids. In all cases in which this intermediate was observed, consumption of NBS was observed almost instantly, while formation of brominated product was observed over significantly longer timeframes (3 hours – 6 days; see Tables 4.1 and 4.2), thus indicating that NBS is not the direct brominating reagent in this reaction. Overall, these initial observations
suggest that the sluggishness of the electrophilic troponoids have allowed us to observe phenomena that would be challenging to observe on a more reactive benzenoid.

4.4.1. Characterization Attempts

Characterization of this intermediate is complicated by its low stability and resulting high reactivity. These traits coupled with the fact that it exists only fleetingly when in the absence of catalyst render definitive characterization a challenge. Attempts to isolate it via recrystallization were unsuccessful, and the molecule predictably proved unstable to a variety of chromatography conditions.

$^{13}$C and HSQC NMR experiments, though not definitive, support the proposed structure of 4.34. Of particular note, the $^{13}$C-NMR spectra of 4.34 in comparison to that of 4.28 shows three new signals characteristic of carbonyls (see Supporting Information). DEPT experiments are also under investigation to specifically study the nature of the putative sp3 carbon. Stoichiometrically, it makes sense that this sp3 carbon would be bonded to one proton and one bromine, and while proton $H_e$ remains difficult to see by $^1$H-NMR, there is potential evidence of its existence in the HSQC spectra (see Supporting Information for details). However, treatment of bromotroponoid 4.28 to excess NBS and 20 mol% peptide 4.29a was found to lead to immediate formation of the intermediate by $^1$H-NMR. Identical conditions with succinimide in place of NBS lead to no reaction (Scheme 4.5). While it is possible that 4.34 and a gem-dibromo variant of 4.34 (as in 4.34a) could coincidentally have identical $^1$H-NMR profiles, this remains unlikely. Stoichiometrically speaking, exclusive formation of gem-dibromo variant 4.34a directly from 4.27 with only 1.1 equivalents of NBS (such as described in Scheme 4.3) would be impossible. Attempts to definitively characterize this intermediate remain underway.
4.4.2. Quench Studies

Upon further examination, it was found that in the presence of base, intermediate 4.34 is immediately quenched. However, quenching of the intermediate did not always lead to formation of bromotropolone product (Scheme 4.6). Upon allowing the reaction to run to full conversion of starting material – while in the presence of intermediate – it was observed that addition of base could lead to reversion back to starting material. This may potentially be explained by an interaction between intermediate 4.34 and product 4.28. However, both the choice of base and the reaction time preceding base addition appear to impact the degree to which the reversion occurs. For instance, in a head-to-head comparison, triethylamine was found to effect a higher degree of reversion than DMAP. As well, when peptide/substrate/NBS were allowed only 10 minutes to react prior to base addition, no base-catalyzed reversion to starting material was observed. While the origin of this effect remains unclear, it appears that the presence of bromotropolone 4.28 is necessary for reversion to starting material. It also remains a possibility that an early base quench may take place while some NBS is still present in the reaction. This residual NBS may serve to buffer adventitious water, which suggests that water may play a role in this reversion mechanism.
An additional quench that was investigated was previously-reported methyl ketone-containing troponoid \textbf{4.35b} (Scheme 4.7). As the brominated variant of this molecule had been synthesized and characterized,^4^ any bromination of this compound could be reliably detected. Therefore, \textbf{4.27} was pushed to full conversion to \textbf{4.34} (with total consumption of NBS) before treatment with one equivalent of methyl ketone \textbf{4.35b} (hypothesized to be capable of existing as \textbf{4.35a} in the presence of succinimide). While sluggish, bromination of \textbf{4.35b} was observed to begin within 55 min, although no reversion of either tropolone to starting material was observed. Remarkably, this quench also appeared to lead to full racemization of bromotropolone \textbf{4.28}, potentially through a racemization equilibrium as outlined in Scheme 4.7. While concrete conclusions cannot necessarily be drawn from these experiments, it is clear that intermediate \textbf{4.34} is serving as a brominating reagent and is potentially responsible for the stunted selectivities observed in Tables 4.1 and 4.2.
4.4.3. Halogen Exchange

Given the inconsistency of the base quenches, we elected to reinvestigate acidic quenches instead. Remarkably, crude material showing no evidence of chlorotropolone 4.36 by $^1$H-NMR, upon treatment with an HCl-containing buffer, underwent conversion (to varying degrees) to aforementioned chlorotropolone 4.36 (Table 4.3).
Table 4.3. Halogenation exchange upon aqueous workup.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.29a: [i=2] = Aib</td>
<td>3h</td>
<td>r.t.</td>
<td>HCl</td>
<td>1 : 0.15</td>
<td>5 : 1</td>
</tr>
<tr>
<td>2b</td>
<td>4.29a: [i=2] = Aib</td>
<td>3h</td>
<td>0 °C</td>
<td>HCl</td>
<td>1 : 0.8</td>
<td>1 : 6</td>
</tr>
<tr>
<td>3</td>
<td>4.29g: [i=2] = Gly</td>
<td>3h</td>
<td>r.t.</td>
<td>HCl</td>
<td>1 : 0.24</td>
<td>2 : 1</td>
</tr>
<tr>
<td>4</td>
<td>4.29g: [i=2] = Gly</td>
<td>3h</td>
<td>r.t.</td>
<td>H₂SO₄</td>
<td>1 : 1.68</td>
<td>1 : 0</td>
</tr>
<tr>
<td>5</td>
<td>4.29g: [i=2] = Gly</td>
<td>17h</td>
<td>r.t.</td>
<td>H₂SO₄</td>
<td>1 : 0.08</td>
<td>1 : 0</td>
</tr>
<tr>
<td>6</td>
<td>4.29c: [i=2] = Acbc</td>
<td>24 h</td>
<td>r.t.</td>
<td>HCl</td>
<td>1 : 0.08</td>
<td>16 : 1</td>
</tr>
<tr>
<td>7</td>
<td>4.29a: [i=2] = Aib</td>
<td>24 h</td>
<td>r.t.</td>
<td>HCl</td>
<td>1 : 0</td>
<td>1 : 0</td>
</tr>
<tr>
<td>8</td>
<td>4.29f: [i=2] = Phe</td>
<td>24 h</td>
<td>r.t.</td>
<td>H₂SO₄</td>
<td>1 : 0</td>
<td>1 : 0</td>
</tr>
<tr>
<td>9</td>
<td>4.29c: [i=2] = Achc</td>
<td>24 h</td>
<td>r.t.</td>
<td>H₂SO₄</td>
<td>1 : 0</td>
<td>1 : 0</td>
</tr>
<tr>
<td>10c</td>
<td>4.29a: [i=2] = Aib</td>
<td>3h</td>
<td>0 °C</td>
<td>HCl</td>
<td>1 : 7</td>
<td>1 : 5</td>
</tr>
</tbody>
</table>

* Ratio based on ¹H-NMR integrations. a This run resulted in 52% ee of chlorotropolone 4.36. c Excess of intermediate upon quenching lead to unwanted byproducts, data point not included in Figure 4.7.

Halogen exchange studies revealed that this conversion did not take place via direct substitution on the intermediate. While a linear relationship between intermediate and chlorotropolone may be inferred from Entry 1 (Table 4.3), closer examination reveals that this is not the case. Instead, it was observed that increasing amounts of intermediate lead to increasing chlorination in an exponential trend (Figure 4.7). For example, when a nearly 1:1 product:intermediate (4.28 : 4.34) ratio is observed pre-quench, a 6:1 chloro:bromotropolone ratio is observed post-quench (Entry 2). A similar effect is observed in Entry 3. Plotting of these ratios gave a second order polynomial fit (R² = 0.9999), while a plot of the log gave a straight line with a very high degree of linearity (R² = 0.9993). While the mechanistic underpinnings behind these observations at present remain unclear, further investigation may uncover the relationship between
intermediate 4.34 and the enantioselectivity of the peptide-catalyzed reaction. Interestingly, the enantioinduction of chlorotropolone 4.36 was found to be comparable to the most selective bromination reaction (52% ee, Table 4.3), though its presence creates purification challenges given the similar polarities of 4.28 and 4.36. Ultimately, it was discovered that changing the acid additive to H$_2$SO$_4$, as well as increasing the reaction time such that all intermediate had reacted, bypassed this issue.

![Figure 4.7](image)

**Figure 4.7.** Plots of chloro- to bromotropolone (4.36:4.28) ratios vs intermediate to bromotropolone (4.34:4.28) ratios.

### 4.5. Mechanistic Hypotheses

At the outset of these studies, we hypothesized that reaction of troponoid substrate with the catalyst would proceed via a transition state analogous to a previously reported atropselective halogenation (Figure 4.8).$^{24,26}$ In such systems, $^1$H-NMR spectra of a 1:1 substrate/peptide complex shows evidence of such complexation in the form of chemical shift differences between the complex and both individual molecules. When such titration experiments were carried out on troponoid 4.27 with catalysts 4.29a, 4.29e, 4.29f and 4.29g, no significant chemical shifts were observed. While in and of itself not confirmation of the presence or absence of this binding mode,$^{42}$ it is perhaps indicative of a weaker binding interaction. This seems all the more likely when an equilibrium as described in Scheme 4.7 is invoked. Bromination at the substrate amide carbonyl...
would block one of the necessary points of contact between substrate and catalyst in this binding model. Additionally, it remains a strong possibility that succinimide (pKa 9.6)\(^{38}\) deprotonates the troponoid (pKa ~6-7),\(^{43}\) rendering it incapable of coordinating with the peptide for this second binding interaction. In spite of this hypothesis, NBS emerged as the lead brominating reagent in the optimization. However, due to commercial availability, pKas of the amide byproducts were only studied within certain pKa ranges and thus interference with the proposed binding model (Figure 4.8C) remains possible.

![Chemical structures and reactions](image)

**Figure 4.8.** (A) Peptide catalyzed atropselective halogenation on a benzenoid. (B) Analogous reaction on benzenoid structural homolog, troponoid 4.27. (C) Hypothesized enantiidetermining substrate/catalyst complex for both reactions. In the case of the benzenoids, this binding mode is supported by \(^1\)H-NMR experiments.

Thus, the impacts of acid additives were preliminarily assessed. Choice of additive was guided by three necessary characteristics, namely: low reactivity towards bromination, low nucleophilicity, and a pKa\(^{44}\) high enough to avoid protonating the catalytic tertiary amine of the peptide. To this end, nitrophenols 4.40 and 4.41 (Tables 4.4A and 4.4B) were investigated.
Unfortunately, these were found to have the opposite effect of what was anticipated, and in fact in the case of 4.40 the selectivity of the reaction was almost completely destroyed (Entry 3 vs Entry 4). Unforeseen complexation of these additives with catalyst, direct protonation of the catalyst, or π/π and/or π-cation interactions with the substrate may all be responsible for these effects.

![Chemical Structures](image)

Table 4.4A. Acid additive comparison using peptide sequence Boc-Dmaa-oPro-Alb-Phe-OMe (4.29l)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additive</th>
<th>Time</th>
<th>Temp.</th>
<th>Conversiona</th>
<th>Yieldb</th>
<th>ee2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>24 hr</td>
<td>RT</td>
<td>100%</td>
<td>47%</td>
<td>40%</td>
</tr>
<tr>
<td>2</td>
<td>4.39</td>
<td>24 hr</td>
<td>RT</td>
<td>71%</td>
<td>30%</td>
<td>14%</td>
</tr>
</tbody>
</table>

Table 4.4B. Acid additive comparison using peptide sequence Boc-Dmaa-oPro-Alb-Val-OMe (4.29q)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additive</th>
<th>Time</th>
<th>Temp.</th>
<th>Conversiona</th>
<th>Yieldb</th>
<th>ee2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>None</td>
<td>3 hr</td>
<td>0 °C</td>
<td>80%</td>
<td>36%</td>
<td>48%</td>
</tr>
<tr>
<td>4</td>
<td>4.40</td>
<td>3 hr</td>
<td>0 °C</td>
<td>n/a</td>
<td>49%</td>
<td>8%</td>
</tr>
</tbody>
</table>

a Approximation of conversion based on 1H-NMR ratio of 4.27:4.28. b Isolated yield after flash chromatography. c Determined by CSP-HPLC using an IA column for reversed phase chiral resolution.
These aggregate mechanistic observations lead us to the hypotheses outlined in Figure 4.9. In such a mechanistic model, nonlinear conversion of intermediate to chlorotropolone is explained, since intermediate formation is predicted to feed into formation of a chlorinated variant. The necessity of the presence of bromotropolone 4.28 alongside intermediate 4.34 for reversion to starting material in the presence of base may also be rationalized. Additionally, the stunted enantioselectivity of the reaction may be explained; if brominated product is indeed reacting with some version of this intermediate, lowered enantioinduction would be expected. Furthermore, if intermediate 4.34 is serving as a bromination reagent, it may simply be an ineffective one for atropselective halogenation.
4.6. Impeding Formation of Intermediate 4.34

Driven by the hypothesis that intermediate 4.34 is somehow contributing to enantioerosion, we sought to impede its formation. We speculated we might be able to accomplish this and thus boost enantioselectivity by changing the mode of NBS delivery. Under previous conditions, NBS was added in one portion at the beginning of the reaction. Therefore, we investigated a slow addition mode of delivery, where 1.1 equivalents of NBS were added over a 2.5-hour time period. Unfortunately, this did not prove effective in increasing enantioselectivity (Entries 1 and 2, Table 4.5).

We next sought to examine the effects of running the reaction under NBS-controlled low conversion, such that formation of an intermediate of the proposed structure 4.34 would be inhibited. Gratifyingly, a correlation with enantioselectivity was observed (Table 4.5). Under conditions where 1.1 equivalents of NBS provided 40% ee, it was discovered that 0.5 equivalents of NBS boosted the selectivity to 48%, a profound improvement. Further cutting the NBS down to 0.28 equivalents produced an additional enhancement to 52%, which remains one of our highest equivalences.

Table 4.5. Comparison of NBS mode of delivery and equivalents, using peptide 4.29a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time</th>
<th>Temp.</th>
<th>NBS Eq.</th>
<th>NBS Delivery</th>
<th>Conv.</th>
<th>Yield</th>
<th>ee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 hr</td>
<td>0 °C</td>
<td>1.1</td>
<td>At once</td>
<td>100%</td>
<td>13%</td>
<td>38%</td>
</tr>
<tr>
<td>2</td>
<td>3 hr</td>
<td>0 °C</td>
<td>1.1</td>
<td>Slow&lt;sup&gt;d&lt;/sup&gt;</td>
<td>95%</td>
<td>21%</td>
<td>28%</td>
</tr>
<tr>
<td>3</td>
<td>24 hr</td>
<td>RT</td>
<td>1.1</td>
<td>At once</td>
<td>100%</td>
<td>47%</td>
<td>40%</td>
</tr>
<tr>
<td>4</td>
<td>7 hr</td>
<td>RT</td>
<td>0.5</td>
<td>At once</td>
<td>24%</td>
<td>20%</td>
<td>48%</td>
</tr>
<tr>
<td>5</td>
<td>7 hr</td>
<td>RT</td>
<td>0.28</td>
<td>At once</td>
<td>25%</td>
<td>21%</td>
<td>52%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Approximation of conversion based on <sup>1</sup>H-NMR ratios of 4.27:4.28. <sup>b</sup> Isolated yield after flash chromatography. <sup>c</sup> Determined by CSP-HPLC using an IA column for reversed phase chiral resolution. <sup>d</sup> Slow addition of NBS over 2 hours. Reaction stirred for additional 3 hours.
ee’s thus far. These results shed light on the otherwise unclear nature of this reaction. While the resulting stifled conversions render reagent-controlled low conversion an impractical tactic for single-atropisomer troponoid synthesis, these results indicate that intermediate suppression is likely the path forward in optimizing this reaction.

4.7. Conclusions

Herein, the first reported efforts at development of an atropselective synthesis of an axially chiral troponoid are described. Asymmetric peptide-based catalysis was investigated and found to be capable of delivering a bromotroponoid in high levels of enantioinduction at 75:25 er. During the course of these studies, mechanistic discoveries unveiled the existence of a non-aromatic intermediate that may be simultaneously indicative of atropselectivity while also stimulating enantioerosion. This bromine-containing intermediate was also found to facilitate delivery of a chlorotroponoid with substantially higher selectivity than was able to be provided by running the reaction with a chlorinating agent. While we have made several significant discoveries in the study of this novel reaction, there remains much to be uncovered, and work is currently ongoing to ascertain more information on the nature of this intermediate. The highly unstable nature of the intermediate renders definitive characterization difficult, and speaks to the challenges associated with optimizing this reaction. Further mechanism-driven experiments may assist in the path forward.
4.8. References


7 Zask, A.; Murphy, J.; Ellestad, G. A. Chirality, 2013, 25, 265-274.


42 For an example of a highly selective peptide-catalyzed reaction where no significant chemical shifts were observed in such $^1$H-NMR titration experiments, see: Metrano, A. J.; Miller, S. J. *J. Org. Chem.*, **2014**, *79*, 1542–1554.


4.9. Supplementary Experimental Details

With the exception of those described in the sections below, all peptide catalysts were synthesized and provided by the Miller laboratory, and characterized by the Miller laboratory as previously published. HPLC access and assistance was also provided by the Miller laboratory.

4.9.1. General Information

All starting materials and reagents were purchased from commercially available sources and used without further purification, with the exception of CH₂Cl₂, which was purified on a solvent purification system prior to the reaction. All reactions were performed in oven- or flame-dried glassware. Commercially available dibromodimethylhydantoin (DBDMH), N-bromosuccinimide (NBS), and N-chlorosuccinimide (NCS) were purified by recrystallization from hot water.

¹H-NMR spectra were obtained on a Bruker 400 MHz spectrometer. 2D NMR spectra were recorded on Agilent 400, 500, or 600 MHz spectrometers. Chemical shifts are measured using the solvent residual peak as the internal standard (CHCl₃ δ 7.26, MeOH δ 3.31, CH₂Cl₂ δ 5.32, DMSO δ 2.50), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, dq = doublet of quartet, ABq = AB quartet), coupling constant (Hz), and integration. ¹³C NMR shifts are measured using the solvent residual peak as the internal standard (CHCl₃ δ 7.2, MeOH δ 49.0, DMSO δ 39.5), and reported as chemical shifts.

Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Microwave reactions were performed via the Biotage Initiator 2.5. Analytical thin-layer
chromatography (TLC) was performed using Silica Gel 60 Å F254 pre-coated plates (0.25 mm thickness). TLC Rf values are reported, with visualization accomplished by irradiation with a UV lamp or appropriate TLC stain. Purification via normal phase column chromatography was performed on the Biotage Isolera Prime, with Biotage SNAP 10g or 25g cartridges, in a solvent system of ethyl acetate and hexanes. Reversed phase chromatography was performed on the Biotage Isolera Prime with Biotage SNAP C18 12 g cartridges, in a solvent system of water and acetonitrile with a 0.05% trifluoroacetic acid additive. Column gradients are measured in terms of column volumes (CV). Mass spectra were recorded on a spectrometer by the electrospray ionization (ESI) technique with a time-of-flight (TOF) mass analyzer. HPLC analysis was conducted with an Agilent 1100 series instrument equipped with a diode array detector (λ = 265 nm) and columns (chiral supports) from Daicel Chemical Industries (Chiralpak AS-H for normal phase, and Chiralpak IA and Chiralpak IC for reversed phase purification) at ambient temperature unless otherwise noted.

4.9.2. Solution Phase Peptide Synthesis and Characterization

The majority of peptides used in these studies were synthesized and characterized as reported by the Miller laboratory. S1 Peptides 4.29t-v, however, were synthesized for this work. This was accomplished using the Boc protecting group strategy² outlined in aforementioned prior publications. S1 All amino acid residues and coupling reagents were purchased from commercial sources.
Representative Synthesis and Characterization of Peptide Catalyst 4.29u

Boc-Phe-dPro-Aib-Phe-OMe (4.29u).

Peptide S4.3 was synthesized as reported previously. To a flask containing trimer S4.3 (84 mg, 0.21 mmol), HOBt·H2O (38 mg, 0.28 mmol, 1.3 eq) and a stir bar was added N-Boc-L-phenylalanine (Boc-Phe-OH; 68 mg, 0.25 mmol, 1.2 eq). The solid mixture was dissolved in dry CH2Cl2 (1.05 mL, 0.20 M with respect to S4.3). EDC·HCl (54 mg, 0.28 mmol, 1.3 eq) was then added. The resulting solution was left to stir at RT as DIPEA (97 μL, 0.55 mmol, 2.6 eq with respect to S4.3) was slowly added. The solution was allowed to stir for 18 hours before being diluted with 20 mL CH2Cl2, poured into a separatory funnel, and washed with an equivalent volume of 10% aqueous (w/v) citric acid. The organic layer was separated and subsequently washed with an equivalent volume each of saturated aqueous NaHCO3 and brine. Organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to provide 4.29u as a white solid (105 mg, 74% yield). Rf = 0.76 in 10% MeOH in CH2Cl2. IR (thin film, KBr) 3323 (br), 2978 (w), 2360 (m),
2341 (m), 1745 (w), 1637 (w), 1454 (m), 1366 (m), 1270 (w), 1245 (m), 1170 (s), 701 (w), 669 (w) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.32 – 7.26 (m, 4H), 7.26 – 7.17 (m, 6H), 7.14 (d, \(J = 7.6\) Hz, 1H), 6.77 (s, 1H), 5.52 (d, \(J = 7.1\) Hz, 1H), 4.82 (dd, \(J = 14.1, 7.5\) Hz, 1H), 4.55 (q, \(J = 7.5\) Hz, 1H), 4.18 (dd, \(J = 8.0, 3.6\) Hz, 1H), 3.66 (s, 3H), 3.61 – 3.53 (m, 1H), 3.13 (ddd, \(J = 21.2, 13.8, 6.8\) Hz, 3H), 3.01 – 2.99 (bs, 1H), 2.99 – 2.96 (bs, 1H), 2.80 (dt, \(J = 9.6, 7.5\) Hz, 1H), 2.13 – 2.04 (m, 1H), 1.93 – 1.82 (m, 1H), 1.71 – 1.63 (m, 1H), 1.61 – 1.50 (m, 1H), 1.45 (s, 3H), 1.40 (s, 9H), 1.34 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 173.8, 172.4, 171.7, 170.5, 136.6, 136.4, 129.4, 129.4, 128.5, 128.4, 127.1, 126.8, 79.9, 60.9, 57.2, 54.0, 53.3, 52.1, 47.2, 39.1, 38.0, 28.4, 27.9, 25.9, 24.9, 24.6 HRMS (ESI+) \(m/z\) calculated for C\(_{33}\)H\(_{45}\)N\(_4\)O\(_7\): 609.3283. Found: 609.3221.

**Boc-[Phe-F\(_5\)]-dPro-Aib-Phe-OMe (4.29t).**

Peptide S\(_{4.3}\) was synthesized as reported previously.\(^{51}\) To a flask containing trimer S\(_{4.3}\) (84 mg, 0.21 mmol), HOBt \(\cdot\) H\(_2\)O (38 mg, 0.28 mmol, 1.3 eq) and a stir bar was added N-Boc-L-pentafluorophenylalanine (Boc-[Phe-F\(_5\)]-OH; 89 mg, 0.25 mmol, 1.2 eq). The solid mixture was dissolved in dry CH\(_2\)Cl\(_2\) (1.05 mL, 0.20 M with respect to S\(_{4.3}\)). EDC \(\cdot\) HCl (54 mg, 0.28 mmol, 1.3 eq) was then added. The resulting solution was left to stir at RT as DIPEA (97 \(\mu\)L, 0.55 mmol, 2.6 eq with respect to S\(_{4.3}\)) was slowly added. The solution was allowed to stir for 12 hours before being diluted with 20 mL CH\(_2\)Cl\(_2\), poured into a separatory funnel, and washed with an equivalent volume of 10% aqueous (w/v) citric acid. The organic layer was separated and subsequently washed with an equivalent volume each of saturated aqueous NaHCO\(_3\) and brine. Organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Reversed phase chromatography of the crude residue with water/methanol was accomplished on a Biotage
Isolera Prime (SNAP 12g C18 silica gel column, solvent gradient: 10% methanol in water (3 CV); 10-30% (4 CV), 30-100% (16 CV); methanol and water each contained 0.05% TFA; monitored at 210 nm and 254 nm). Product fractions were combined and concentrated at 30 °C in vacuo, yielding 4.29t as a yellow foam/oil (75 mg, 50% yield). Rf = 0.72 in 10% MeOH in CH₂Cl₂. IR (thin film, KBr) 3325 (br), 2980 (m), 1743 (w), 1639 (s), 1521 (s), 1504 (s), 1441 (m), 1366 (w), 1124 (m), 1002 (w), 974 (m), 701 (m), 607 (w) cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 7.24 (d, J = 3.5 Hz, 2H), 7.21 – 7.15 (m, 3H), 6.67 (s, 1H), 6.16 (d, J = 9.2 Hz, 1H), 4.92 (dd, J = 14.1, 8.2 Hz, 1H), 4.66 (td, J = 9.4, 4.2 Hz, 1H), 4.22 (dd, J = 7.2, 5.7 Hz, 1H), 3.71 (s, 3H), 3.67 – 3.54 (m, 2H), 3.17 (dd, J = 13.8, 5.6 Hz, 1H), 3.09 – 2.89 (m, 4H), 2.21 – 2.03 (m, 3H), 1.98 – 1.85 (m, 1H), 1.49 (s, 3H), 1.29 (s, 9H), 1.24 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.9, 172.7, 170.8, 170.1, 155.3, 146.9 (m), 144.4 (m), 141.4 (m), 138.6 (m), 136.3, 136.1 (m), 129.3, 128.4, 126.9, 110.9 (t), 79.9, 61.4, 57.3, 52.9, 52.3, 51.5, 47.5, 38.2, 28.4, 27.9, 27.2, 25.3, 25.2, 23.6. HRMS (ESI+) m/z calculated for C₃₃H₄₀F₅N₄O₇⁺: 699.2812. Found: 699.2852.

**Boc-3Pal-dPro-Aib-Phe-OMe (4.29v).**

**Peptide S4.3** was synthesized as reported previously.⁵¹ To a flask containing trimer S4.3 (50 mg, 0.13 mmol), HOBt·H₂O (28 mg, 0.21 mmol, 1.3 eq) and a stir bar was added N-Boc-D-3-pyridylalanine (Boc-3Pal-OH; 41 mg, 0.15 mmol, 1.2 eq). The solid mixture was dissolved in dry CH₂Cl₂ (1.05 mL, 0.20 M with respect to S4.3). EDC·HCl (32 mg, 0.17 mmol, 1.3 eq) was then added. The resulting solution was left to stir at RT as DIPEA (26 µL, 0.15 mmol, 1.2 eq with respect to S4.3) was slowly added. The solution was allowed to stir for 20 hours before being diluted with 20 mL CH₂Cl₂, poured into a separatory funnel, and washed with an equivalent
volume of 10% aqueous (w/v) citric acid. The organic layer was separated and subsequently washed with an equivalent volume each of saturated aqueous NaHCO₃ and brine. Organic layers were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Chromatography of the crude residue with dichloromethane/methanol was accomplished on a Biotage Isolera Prime (SNAP 10g silica gel column, solvent gradient: 0% methanol in dichloromethane (3 CV); 0-1% (3 CV), 1-2% (5 CV); 2-5% (10 CV); 5-10% (10 CV); 10% (3 CV); monitored at 210 and 254 nm). Product fractions were combined and concentrated at 30 °C under reduced pressure, yielding 4.29v as a clear foam/oil (29 mg, 38% yield). $^1$H NMR (400 MHz, CDCl₃) δ 8.51 – 8.44 (m, 1H), 8.44 – 8.40 (m, 1H), 7.55 – 7.49 (m, 1H), 7.23 – 7.17 (m, 1H), 7.12 (d, $J = 6.5$ Hz, 3H), 7.07 (d, $J = 6.8$ Hz, 3H), 6.90 (d, $J = 7.2$ Hz, 1H), 5.24 (d, $J = 8.9$ Hz, 1H), 4.80 (dd, $J = 13.2$, 6.1 Hz, 1H), 4.65 – 4.56 (m, 1H), 4.44 – 4.35 (m, 1H), 3.70 (s, 3H), 3.65 – 3.58 (m, 1H), 3.54 – 3.43 (m, 1H), 3.13 (ddd, $J = 33.9$, 13.9, 6.0 Hz, 2H), 2.92 (dd, $J = 14.0$, 5.0 Hz, 1H), 2.66 (dd, $J = 14.0$, 8.4 Hz, 1H), 2.29 – 2.18 (m, 1H), 1.96 – 1.83 (m, 3H), 1.51 (s, 3H), 1.39 (s, $J = 3.2$ Hz, 3H), 1.34 (s, 9H). $^{13}$C NMR (100 MHz, CDCl₃) δ 173.8, 172.5, 171.9, 170.4, 155.3, 150.9, 148.4, 137.2, 136.3, 132.3, 129.5, 129.3, 128.6, 128.5, 127.0, 123.5, 80.2, 60.9, 60.5, 57.3, 53.1, 52.4, 47.7, 37.7, 35.7, 28.4, 27.3, 26.5, 25.3, 24.5, 21.2, 14.3. HRMS (ESI+) m/z calculated for C₃₂H₄₄N₅O₇⁺: 610.3236. Found: 610.3271.
Note: Peptides 4.29i and 4.29j were synthesized by the Miller laboratory.

Characterization of Boc-Dmaa-DPro-[α-Me, D-Phe]-Leu-OMe (4.29i): White, foamy solid, 15% overall yield. Rf = 0.59 in 10% MeOH in CH₂Cl₂. ¹H NMR (500 MHz, CDCl₃): δ 7.34 (d, J = 8.6 Hz, 1H), 7.30–7.20 (m, 3H), 7.14 (d, J = 7.0 Hz, 2H), 6.42 (s, 1H), 5.82 (d, J = 6.3 Hz, 1H), 4.75–4.62 (m, 1H), 4.44 (q, J = 6.9 Hz, 1H), 4.26 (dd, J = 7.6, 4.0 Hz, 1H), 3.87–3.79 (m, 1H), 3.72 (s, 3H), 3.66–3.58 (m, 1H), 3.56 (d, J = 13.7 Hz, 1H), 3.10 (d, J = 13.8 Hz, 1H), 2.63 (dd, J = 12.1, 7.6 Hz, 1H), 2.41 (dd, J = 12.2, 6.9 Hz, 1H), 2.27 (s, 6H), 2.25–2.18 (m, 1H), 2.13–2.01 (m, 2H), 2.01–1.87 (m, 2H), 1.77–1.60 (m, 4H), 1.41 (s, 9H), 1.38 (s, 3H), 0.98–0.85 (m, 6H). IR (thin film, KBr) 3324 (br), 2955 (br), 1748 (m), 1652 (s), 1522 (s), 1456 (m), 1367 (w), 1284 (w), 1163 (m), 860 (w), 708 (w) cm⁻¹. ¹³C NMR (150 MHz, CDCl₃): δ 174.0, 173.6, 171.3, 170.4, 155.8, 137.1, 130.8, 127.9, 126.6, 79.9, 61.7, 60.2, 53.4, 51.9, 51.0, 50.8, 47.5, 45.6, 40.8, 40.2, 28.8, 28.4, 24.6, 24.1, 23.1, 21.7. HRMS (ESI⁺) m/z calculated for C₃₂H₅₂N₅O₇⁺: 618.3561. Found: 618.3452.

Characterization of Boc-Dmaa-DPro-[α-Me, L-Phe]-Leu-OMe (4.29j): White, foamy solid, 12% overall yield. ¹H NMR (600 MHz, CDCl₃): δ 7.26–7.24 (m, 2H), 7.24–7.21 (m, 1H), 7.19 (d, J = 8.1 Hz, 1H), 7.16–7.12 (m, 2H), 6.32 (s, 1H), 5.58 (s, 1H), 4.57 (ddd, J = 9.6, 7.8, 4.9 Hz, 1H), 4.33–4.30 (m, 1H), 4.28 (dd, J = 9.7, 4.2 Hz, 1H), 3.84–8.79 (m, 1H), 3.72–3.69 (m, 1H), 3.69 (s, 3H), 3.49 (q, J = 8.6 Hz, 1H), 3.09 (d, J = 13.8 Hz, 1H), 2.55–2.48 (m, 1H), 2.47–2.42 (m, 1H), 2.24 (s, 6H), 2.21–2.15 (m, 1H), 2.15–2.06 (m, 1H), 1.94–1.87 (m, 1H), 1.87–1.79 (m, 1H), 1.79–1.70 (m, 2H), 1.63–1.57 (m, 1H), 1.41 (s, 3H), 1.38 (s, 9H), 0.94 (dd, J = 10.7, 6.5 Hz, 6H). ¹³C
NMR (150 MHz, CDCl₃): δ 173.9, 173.4, 171.2, 170.3, 155.7, 136.9, 130.8, 127.9, 126.5, 79.9, 61.6, 60.2, 59.6, 51.8, 50.9, 50.7, 47.4, 45.5, 40.7, 40.1, 28.8, 28.4, 24.5, 24.1, 23.0, 21.6. IR (thin film, KBr) 3750 (br), 2955 (br), 2360 (w), 1748 (m), 1652 (s), 1456 (m), 1367 (w), 1284 (w), 1163 (m), 860 (w), 708 (w) cm⁻¹. HRMS (ESI⁺) m/z calculated for C₃₂H₅₂N₅O₇⁺: 618.3561. Found: 618.3867.

4.9.3. Peptide Screening Protocols and Optimization of Reaction Conditions: Halogenation of 4.27

2-Bromo-6-methoxy-4-methyl-7-oxo-3-(piperidine-1-carbonyl)cyclohepta-1,3,5-trien-1-yl acetate (4.28a).

**General Procedure.** To an oven-dried 1-dram vial equipped with peptide catalyst 4.29d (1.84 mg, 0.003 mmol, 0.1 eq) was added troponoid 4.27 (8.3 mg, 0.300 mmol), a magnetic stir bar, and CDCl₃ (1.5 mL, 0.02 M). The solution was allowed to stir at ambient temperature. N-Bromosuccinimide (NBS, 5.9 mg, 1.1 eq) was then added (all at once) to the stirring solution at room temperature after the sides of the vial were wrapped in aluminum foil. The reaction was further protected from light by turning off the lights in the ventilation hood. Reaction progress was monitored by ¹H-NMR in an NMR tube wrapped in aluminum foil when not inside the NMR spectrometer. When the reaction reached full conversion
of starting material (26 hours), it was quenched by addition of a flake of DMAP and 3 drops of acetic anhydride and allowed to stir at ambient temperatures overnight. Acetylation progress was monitored by thin layer chromatography. The solvent was removed under reduced pressure, and the crude reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, elution solvents: dichloromethane with 2% acetic acid additive, and acetone. Solvent gradient: 0% acetone in dichloromethane (3 CV); 0-20% (10 CV); 20% (4 CV); 20-25% (8 CV); 25-30% (2 CV); 30-60% (5 CV)). Product fractions were concentrated to yield 4.28a as a yellow oil (5.1 mg, 43% yield). Rf = 0.32 in 20% acetone in dichloromethane. IR (thin film, KBr) 3545 (br), 2938 (m), 2855 (m), 1770 (s), 1633 (s), 1591 (w), 1446 (w), 1298 (m), 1269 (s), 1223 (m), 1184 (w), 1135 (s), 1073 (m), 873 (w) cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 6.73 (bs, 1H), 3.95 (s, 3H), 3.91 – 3.79 (m, 1H), 3.68 – 3.52 (m, 1H), 3.30 – 3.18 (m, 2H), 2.44 (s, 3H), 2.37 (s, 3H), 1.83 – 1.47 (m, 6H). ¹³C NMR (100 MHz, CDCl₃; partial) δ 166.9, 137.2, 57.1, 47.3, 42.3, 26.4, 26.0, 25.2, 24.6, 20.8. HRMS (ESI+) m/z calculated for C₁₇H₂₁BrNO₅⁺: 398.0598. Found: 398.0602. HPLC 70:30 er (Chiralpak AS-H, 1.5 mL/min, 8% ethanol in hexanes, regulated at 40 °C, 254 nm). Rₜ(major) = 22.8 min, Rₜ(minor) = 25.6 min.
Please see Table 4.1 in the main text for a summary of all peptides screened.

3-bromo-2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (4.28).

General Procedure A. N-Bromosuccinimide (NBS, 1.1 equiv.) was added to a 0.02 M solution of tropamide 4.27 (17.3 mg, 0.062 mmol, 1 equiv) and catalyst 4.29q (3.5 mg, 0.006 mmol, 0.1 equiv) in CDCl₃ (3.12 mL) at 0 °C (while shielded from light). The reaction was allowed to stir for 72 hours. The reaction was then diluted with 4 mL of DCM, transferred to a separatory funnel, and quenched with an equivalent volume of a pH 3 phosphate buffer (made by diluting 1.7 mL of 2.0 M aqueous sulfuric acid with 12 mL of 1.0 M pH 7 phosphate buffer), and extracted 3x. The combined organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure. Reversed phase chromatography of the crude residue with water/acetonitrile was accomplished on a Biotage Isolera Prime (SNAP 12g
C18 silica gel column, solvent gradient: 10% acetonitrile in water (3 CV); 10-23% (8 CV), 23-35% (8 CV), 35-100% (6 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were combined, extracted with excess DCM (3x, Σ = 60 mL), filtered through Na₂SO₄ and concentrated at 30 °C in vacuo, yielding 4.28 as a waxy brown/yellow solid (11.2 mg, 50% yield) with ¹H-NMR data consistent with previously reported data. See Section 3.10.4 in Chapter 3 for characterization data. **HPLC** 75:25 er (Chiralpak IA, 1.5 mL/min, 35% acetonitrile in water with 0.1% formic acid): \( R_T(\text{minor}) = 7.2 \text{ min}, R_T(\text{major}) = 8.1 \text{ min}. 

**General Procedure B.** The same procedure as outlined above was followed, but the reaction was run at ambient temperature. Such reactions were monitored for conversion by ¹H-NMR. Upon reaching full conversion, they were quenched according the procedure described above.

**General Procedure C.** The same procedure as outlined above (Procedure B), with N-chlorosuccinimide (NCS) used in place of N-bromosuccinimide (NBS). Additionally, the reaction
was instead quenched with a pH 3 phosphate buffer that was made by diluting 1.0 M phosphate buffer with enough 2.0 M HCl solution to bring the total solution to a pH of 3.

**General Procedure D.** The same procedure as outlined above was followed (A and B only), but the reaction was instead quenched with a pH 3 phosphate buffer that was made by diluting 1.0 M phosphate buffer with enough 2.0 M HCl solution to bring the total solution to a pH of 3.

3-chloro-2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (4.36).

**Procedure C.** To a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one **4.27** (8.0 mg, 0.029 mmol) and **4.29a** (1.6 mg, 0.0029 mmol) in CDCl$_3$ (1.44 mL, 0.02 M) was added N-chlorosuccinimide (NCS, 4.2 mg, 0.032 mmol) at ambient temperature at which point the reaction immediately turned red. The reaction was transferred to an oven-dried NMR tube for monitoring the progress of the reaction and was also shielded from light. The reaction proved sluggish, and NCS took 7 days to be fully consumed. After 9 days, **4.27** and **4.36** were present in a 1:0.78 ratio, and conversion appeared to have plateaued. The reaction was then diluted with 4 mL of DCM, transferred to a separatory funnel, and quenched with an equivalent volume of a pH 3 phosphate buffer (made by diluting 10 mL of 1.0 M pH 7 phosphate buffer with 2.0 M HCl until a pH of 3 was reached), and extracted 3x. The combined organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield a yellow oil. Reversed phase chromatography of the crude residue with water/acetonitrile was accomplished on a Biotage Isolera Prime (SNAP 12g...
C18 silica gel column, solvent gradient: 10% acetonitrile in water (3 CV); 10-23% (8 CV), 23-35% (8 CV), 35-100% (6 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were combined, extracted with excess DCM (3x, Σ = 50 mL), filtered through Na2SO4 and concentrated at 30 °C under reduced pressure to yield a yellow oil (1.0 mg, yield not calculated). **1H NMR (400 MHz, CDCl3)** δ 7.01 (s, 1H), 4.01 (s, 3H), 3.86 – 3.66 (m, 2H), 3.27 – 3.13 (m, 2H), 2.46 (s, 3H), 1.79 – 1.62 (m, 6H). **13C NMR (100 MHz, CDCl3; partial)** δ 157.2, 121.5, 56.9, 47.2, 42.3, 26.1, 25.7, 25.3, 24.6. **HR-LCMS (ESI+) m/z** calculated for C15H19ClNO4+: 312.0997. Found: 312.0999. **HPLC** 53:47 er (Chiralpak IC, 1.0 mL/min, 65% acetonitrile in water with 0.1% formic acid, monitored at 265 nm). R_T(minor) = 6.7 min, R_T(major) = 10.2 min.
Procedure D. To a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one \(4.27\) (11.2 mg, 0.040 mmol) and \(4.29a\) (2.18 mg, 0.004 mmol) in CDCl\(_3\) (2.02 mL, 0.02 M) was added N-bromosuccinimide (NBS, 7.9 mg, 0.044 mmol) at 0 °C. The reaction was transferred to an oven-dried NMR tube for monitoring the progress of the reaction and shielded from light. After allowing the reaction to stir at 0 °C for 3 hours, the reaction was then diluted with 4 mL of DCM, transferred to a separatory funnel, and quenched with an equivalent volume of a pH 3 phosphate buffer (made by diluting 10 mL of 1.0 M pH 7 phosphate buffer with 2.0 M HCl until a pH of 3 was reached), and extracted 3x. The combined organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield a mixture of \(4.28\) and \(4.36\) in a 1:6 ratio by \(^1\)H-NMR. Reversed phase chromatography of the crude residue with water/acetonitrile was accomplished on a Biotage Isolera Prime (SNAP 12g C18 silica
gel column, solvent gradient: 10% acetonitrile in water (3 CV); 10-23% (8 CV), 23-35% (8 CV), 35-100% (6 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were combined, extracted with excess DCM (3x, Σ = 60 mL), filtered through Na₂SO₄ and concentrated at 30 °C under reduced pressure to yield a yellow oil (2.2 mg, 17% yield) with ¹H-NMR data consistent with previously reported data. HPLC 76:24 er (Chiralpak IC, 1.0 mL/min, 65% acetonitrile in water with 0.1% formic acid, monitored at 265 nm). R<sub>T</sub>(minor) = 6.7 min, R<sub>T</sub>(major) = 10.2 min.

4.9.4. NMR Studies on Racemic and Uncatalyzed Conversion

Uncatalyzed Conversion. To a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one 4.27 (5.5 mg, 0.020 mmol) in CDCl₃ (1.0 mL, 0.02 M) was added N-bromosuccinimide (NBS, 3.9 mg, 0.022 mmol). The reaction was transferred to an oven-dried NMR tube for monitoring the progress of the reaction and shielded from light. For ¹H-NMR spectra showing conversion, see Section 4.9.6.
Racemic Conversion with 0.1 Equivalents of Base.

To an oven-dried 1-dram vial was added a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one 4.27 (8.3 mg, 0.030 mmol) in CDCl$_3$ (1.5 mL, 0.02 M) and N-bromosuccinimide (NBS, 5.9 mg, 0.033 mmol). The reaction was allowed to stir at ambient temperature before adding triethylamine (0.4 μL, 0.003 mmol; added as a stock solution in CDCl$_3$). The reaction was transferred to an oven-dried NMR tube for monitoring the progress of the reaction and shielded from light. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.15 (s, 1H), 7.03 (s, 1H), 6.99 (s, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.89 – 3.54 (m, 4H), 3.25 – 3.17 (m, 4H), 2.43 (s, 3H), 2.41 (s, 3H), 1.80 – 1.41 (m, 12H). ($^1$H-NMR of crude reaction. Triethylamine and succinimide peak values omitted for clarity. For $^1$H-NMR spectrum, see Section 4.9.6.)

Racemic Conversion with Stoichiometric Base.

3-bromo-2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one (4.28). In the presence of stoichiometric base, NBS was discovered to result in decomposition of the reaction. Accordingly, brominating agent dibromodimethylhydantoin was used instead. To a solution of 2-hydroxy-7-methoxy-5-methyl-4-(piperidine-1-carbonyl)cyclohepta-2,4,6-trien-1-one 4.27 (10.8 mg, 0.039 mmol) in CDCl$_3$ (1.30 mL) was added dibromodimethylhydantoin
(DBDMH, 14.25 mg, 0.05 mmol), followed by triethylamine (5.4 μL, 0.039 mmol). The reaction was allowed to stir for 2 hours at rt before quenching with pH 3 phosphate buffer and extracting with CH₂Cl₂. Combined organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield 4.28 as a pale yellow/brown waxy solid (5.6 mg, 88% yield) with ¹H-NMR data consistent with previously reported data. HPLC 51:49 er (Chiralpak IA, 1.5 mL/min, 35% acetonitrile in water with 0.1% formic acid). See Section 3.10.4 in Chapter 3 for characterization data.
4.9.5. NMR Studies on Intermediate 4.34

Synthesis and Tentative Characterization. To an oven-dried, 1-dram vial was added peptide catalyst 4.29a (2.0 mg, 0.004 mmol, 20 mol%), troponoid 4.27 (5.2 mg, 0.019 mmol) in CDCl₃ (0.937 mL, 0.02 M) and a magnetic stir bar. The solution was allowed to stir at ambient temperature. N-Bromosuccinimide (NBS, 7.3 mg, 0.041 mmol, 2.2 eq) was then added (all at once) to the stirring solution at room temperature after the sides of the vial were wrapped in aluminum foil. The reaction was immediately transferred to an oven-dried NMR tube and wrapped in aluminum foil. ¹H NMR (400 MHz, CDCl₃) δ 6.41 (q, J = 1.4 Hz, 1H), 3.63 (s, 3H), 3.62 (s, 1H), 2.12 (d, J = 1.3 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 180.1, 178.8, 177.4, 164.7, 135.2, 131.8, 57.6, 57.1, 46.6, 42.6, 28.4, 25.9, 25.3, 24.5. Not stable on LCMS, or long-lived enough to study by HMBC or NOE correlation experiments. For ¹³C and HSQC data, see Section 4.9.6.

Base Quench Study.
To an oven-dried, 1-dram vial was added peptide catalyst 4.29c (1.7 mg, 0.003 mmol), troponoid 4.27 (8.7 mg, 0.031 mmol) in CDCl3 (1.56 mL, 0.02 M) and a magnetic stir bar. The solution was allowed to stir at ambient temperature. N-Bromosuccinimide (NBS, 6.1 mg, 0.034 mmol) was then added (all at once) to the stirring solution at room temperature after the sides of the vial were wrapped in aluminum foil. Reaction was monitored by ¹H-NMR for 90 minutes to ensure full conversion of starting material. At this time, all NBS was fully converted, and the ratio of product 4.28 to intermediate 4.34 was 1:3.7.

**Reaction 1** (top, blue): 4-Dimethylaminopyrydine (DMAP, 3.8 mg, 0.031 mmol) was added. After 10 minutes, ¹H-NMR showed total disappearance of intermediate 4.34. The ratio of starting material 4.27 to product 4.28 was 2:3.

**Reaction 2** (bottom, red): Triethylamine (4.4 μL, 0.031 mmol) was added. After 10 minutes, ¹H-NMR showed total disappearance of intermediate 4.34. The ratio of starting material 4.27 to product 4.28 was 3:2.

**Halogenation Exchange Study.** Refer to Section 4.9.3, General Procedure C. These results are summarized in full in the main text.
4.9.6. NMR Spectra of New Compounds

$^1$H-NMR of 4.29t
1H-NMR of 4.29u
$^{13}$C-NMR of 4.29 u
$^1$H-NMR of 4.29v
$^1$H-NMR of 4.29
$^{13}$C-NMR of 4.29j
Uncatalyzed Conversion

4.28 - (OH)

4.34

NBS gone

4.27

$ t_0 $
Racemic conversion of 4.27 to 4.28 with 0.1 eq. of triethylamine
$^1$H-NMR of 4.34 (from crude reaction, contains peptide/succinimide impurities)
$^{13}$C-NMR of 4.34
Tentatively Assigned
(from crude reaction; contains some 4.28)
$^{13}$C-NMR of 4.28

$^{13}$C-NMR of 4.34 (crude; with 4.28)

3 characteristic CO peaks; signals shifted downfield from 4.28 spectra (above)
4.9.7. Supplementary References
