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Design, Synthesis and Study of Potent Small Molecule Antifungal Synergizers and Palladium-Carbene Mediated C–C and C–N Bond Formations

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UNIVERSITY OF CALIFORNIA,
IRVINE

Design, Synthesis and Study of Potent Small Molecule Antifungal Synergizers

and

Palladium-Carbene Mediated C–C and C–N Bond Formations

DISSERTATION

Submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemistry

by

Ilandari Dewage Udara Anulal Premachandra

Dissertation Committee:
Professor David L. Van Vranken, Chair
Professor A. Richard Chamberlin
Professor Kenneth J. Shea

2016

DEDICATION

To

my grandmother

in recognition of her unconditional love and care

"The only real failure in life is one not learned from."

- Anthony J. D'Angelo

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ACKNOWLEDGMENTS

It's been almost five years since I started grad school and it still feels like yesterday. The time I spent as a grad student is filled with happiness, accomplishments, disappointments and a whole array of different emotions. However, I should say I enjoyed every single moment of it and this is the time to be thankful for everyone who contributed to this life changing learning experience.

I would have never accomplished the things I achieved in my life if it wasn't for my mom. I don't think I ever told you how grateful I am for everything that you have done for me. I understand it was very hard for you to be a single mother with three children and to have a minimum wage job. I want to say, you've done an outstanding job raising us to be productive members of the society and I am eternally grateful for all the sacrifices you've made to make my life a little easier. So thank you and I hope you'll be proud of what I'm going to do in life.

Next, I want to thank my grandmother who practically raised me in Sri Lanka when my mom was working in the United States. All the love and caring you've given me over the years made me the person I am today.

I'd like to credit my two sisters for being the best friends in my life. We always shared what life gave us, regardless of whether it was something good or bad. We never complained about our hard times, because we knew we had each other to share it with. Your love helped me to overcome so many obstacles in life and I know I can always count on you when I need you.

I am grateful to Professor David L. Van Vranken for his invaluable mentoring and being the best advisor that anyone could ever asked for. You taught me to solve problems not only in chemistry, but also in real life. I remember the time we wrote the patent application together. I spent countless hours in your office assisting you with the writing. After numerous hours of

work, when I got totally overwhelmed, I saw you still kept going like you just started working on it. That was one of those countless times I felt your infectious enthusiasm to learn new things and it made me to train myself to keep up with you. Over the past five years, your mentorship, patience and humility have been inspirational. I am extremely grateful to you for teaching me how to be a good scientist and hope I will have your continuous support for my future endeavors.

Professors Richard Chamberlin and Ken Shea, thank you for serving in my thesis committee. You've been extremely helpful and over the years our discussions have been valuable resources to my graduate career. The UCI chemistry department has been an excellent working environment and I will truly miss the entire faculty and staff that are dedicated to help anyone whenever they need it. The UCI is a very special place for me and I was blessed with making life long friendships at this place. Janine, Dan and Mariam thanks for making most of my disappointing days bearable. Thank you for your friendship and being the best people to hang out with. I enjoyed every single second we spent together.

I feel truly privileged to be a member of the Van Vranken group. Throughout this time I've been fortunate to collaborate with a number of co-workers in all my projects. Dr. Avinash Khanna's enormous support during the early years of my graduate career tremendously helped me to excel in later years. Our discussions over the years immensely helped me to think of new avenues in my projects. Thanks for being an exceptional mentor to me. My very motivated two undergraduate co-workers, Kevin and John were great resources for a major portion of my graduate career. I really appreciate you putting up with my demands and always supporting to reach our goals very quickly. I know you will achieve greater things in life and I am very happy I could play a small part of it. I also want to give a special thank to Vanessa for proofreading my thesis several times and being very helpful in all the technical issues I had with my computer

over the years. I am extremely fortunate to work side by side with all the other Van Vranken group members as well. Gene, Stan, Thi, Aaron, Paul, Nancy and Daniel, thanks for all the good times I had in the lab.

Finally, I want to thank the rest of my family who lives in different parts of the world. Your continuous love and support makes everything I do worthwhile.

CURRICULUM VITAE

ILANDARI DEWAGE UDARA ANULAL PREMACHANDRA

EDUCATION

2016 May Cambridge, MA	**Postdoctoral Fellow**, *beginning May 2016*, Harvard University Advisor: Professor Andrew G Myers
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2007 – 2011 Northridge, CA	**B.S. in Biochemistry**, with Honors, California State University, Northridge Advisor: Professor Joseph Hajdu

Awards:
Hypercube Scholar Award 2011

EXPERIENCE

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2012 – 2016 Irvine, CA	Mentor to Graduate and Undergraduate Researchers, UC Irvine - Direct mentor to one graduate student and three undergraduates.
2013 – 2016 Irvine, CA	Van Vranken Laboratory Safety Representative - Overlook the laboratory safety, SOPs and employee training.

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• Development of new metal-catalyzed carbene reactions
• Development of novel inhibitors of fungal transcription

PUBLICATIONS

1. **Premachandra, I. D. U. A.**; Mood, A. D.; Hiew, S.; Scott, K. A.; Wang, F.; Oldenhuis, N. J.; Liu, H.; Van Vranken, D. L. "Potent Antifungal Synergy of Phthalazinone and Isoquinolones with Azoles Against *C. Albicans*" *manuscript in preparation.*

2. **Premachandra, I. D. U. A.**; Nguyen, T. A.; Shen, C.; Gutman, E. S.; Van Vranken, D. L. "Stereoselectivity in the Aminocarbenylation of Vinyl Iodides involving Palladium Alkylidenes" *Org. Lett.* ***2015***, *17*, 5464–5467.

3. **Premachandra, I. D. U. A.**; Scott, K. A.; Shen, C.; Wang, F.; Lane, S.; Liu, H.; Van Vranken, D. L. "Potent Synergy between Spirocyclic Pyrrolidinoindolinones and Fluconazole against *Candida albicans*" *ChemMedChem.* **2015**, *10,* 1672–1686.

4. Van Vranken, D. L.; Liu, H.; **Premachandra, I. D. U. A.**; Wang, F.-Q.; Shen, C.; Scott, K. A.; Lane, S. R.-A. US Application No.62/175,202:"Spiroindolinones and Therapeutic Uses Thereof", filed on 12 June 2015.

5. Khanna, A.[†]; **Premachandra, I. D. U. A**.[†]; Sung, P. D.; Van Vranken, D. L. "Palladium-Catalyzed Catellani Aminocyclopropanation Reactions with Vinyl Halides" *Org. Lett.* **2013**, *15*, 3158–3161. [†] = Contributed equally

6. Khanna, A.; **Premachandra, I. D. U. A.**; Sung, P. D.; Van Vranken, D. L. "Palladium-Catalyzed Bis-cyclization/Dimerization Reactions of ω-Aminovinyl Halides" *Org. Lett.* **2013**, *15*, 3694–3697.

PRESENTATIONS

"Palladium-Catalyzed Cyclodimerization and Cyclopropanation Reactions of Vinyl Halides", **Kyoto University**, **Japan**, January 2014 – **Presentation**

"Harnessing Palladium-carbenoid reactivity." **Premachandra, I. D. U. A.**; Gutman, E. S.; Arredondo, V.; Van Vranken, D. L. Incoming Graduate Student Recruitment, March 2014 and 2015 (Poster)

"Palladium-Catalyzed Dimerization of Vinyl Halides." Khanna, A.; **Premachandra, I. D. U. A**.; Sung, P. D.; Van Vranken, D. L. Incoming Graduate Student Recruitment, March 2013. (Poster)

OUTREACH

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ABSTRACT OF THE DISSERTATION

Design, Synthesis and Study of Potent Small Molecule Antifungal Synergizers

and

Palladium-Carbene Mediated C–C and C–N Bond Formations

By

Ilandari Dewage Udara Anulal Premachandra

Doctor of Philosophy in Chemistry

University of California, Irvine, 2016

Professor David L Van Vranken, Chair

The doctoral studies described herein include two distinct aspects. One involves the design, synthesis and study of small molecule antifungal synergizers and the other consists of novel palladium-catalyzed C–C and C–N bond formations.

Spiroindolinones and dihydroisoquinolines were previously reported to enhance the antifungal effect of *Candida albicans*. A different diastereomer of a lead spiroindolinone, CID 6584729 was synthesized and shown to have synergy with fluconazole against *C. albicans*. Various other analogues of spiroindolinones and dihydroisoquinolines were designed, synthesized and studied. Many of the compounds were shown to enhance the antifungal activity of fluconazole against *C. albicans*, some with exquisite potency. One spirocyclic compound, which we have named synazo-1 and one dihydroisoquinoline analogue, compound **3-19** were shown to enhance fluconazole activity against several resistant clinical isolates with low EC_{50} values. Both of these compounds exhibit true synergy with fluconazole, with FIC indices below

0.5 in both susceptible and resistant strains. Synazo-1 and compound **3-19** also exhibited low intrinsic cytotoxicity in mammalian cells.

In a separate project, vinyl iodide were shown to be useful precursors in palladium-catalyzed transformations to gain access to η^3-allylpalladium intermediates that resist β-hydride elimination and to generate cyclodimerization and cyclopropanation adducts in synthetically useful yields.

Most palladium-catalyzed reactions involving insertion of alkylidenes with α-hydrogens undergo β-hydride elimination from alkylpalladium(II) intermediates to form alkenes. Vinyl iodides were utilized to generate η^3-allylpalladium intermediates and preserve the sp^3 center adjacent to the carbene. Acyclic stereocontrol (*syn/anti*) for carbenylative amination and alkylation reactions was found to be low, suggesting lack of control in the migratory insertion step. Highly hindered carbene precursors inexplicably led to formation of *Z*-alkenes with high levels of stereocontrol.

Vinyl iodides were shown to undergo palladium-catalyzed dimerizations and cyclization to generate pyrrolidine and piperidine dimers connected by a *trans*-ethylene bridge. The dimerization generates the skeleton of the alkaloid, hyalbidone in a single step. A crossover experiment is consistent with a Michael-type addition to vinylpalladium cation to generate Pd(0) alkylidene intermediate. A palladium-catalyzed intramolecular aminocyclopropanation of norbornenes with vinyl halides was shown to generate cycolpropylcarbinyl adducts in good yields. Stabilized enolate nucleophiles were also employed in these transformations.

Chapter 1:

Azole Synergizers

Introduction

Species of the yeast *Candida* are the most common cause of fungal infections[1] and account for 80% of major systemic fungal infections.[2] Among all the *Candida* species, *Candida albicans* is considered to be the most common infectious agent.[3] *C. albicans* normally exists within the gastrointestinal microbiome in a commensal relationship, but in immunocompromised patients it can become an invasive pathogen. Candidiasis is an infection of *Candida* and most commonly affects the areas such as skin, genitals, throat, mouth and/or blood. Candidemia, the most common form of invasive candidiasis is responsible for the high rates of morbidity and mortality of immunosuppressed or immunocompromised patients. According to recent United States Centers for Disease Control and Prevention surveillance data, mortality among people with candidemia is approximately 30% and overall fungal infections are responsible for increasing the cost of health care and the length of the hospital stay significantly.[4]

The antifungal drug market continues to be very profitable. Recent data suggest over $6 billion of annual sales and sales are predicted to grow annually. Several classes of antifungals are currently used to battle fungal infections (Table 1-1 and Scheme 1-1) and numerous other compounds are going through the FDA approval process to make it to the markets.

Major classes of antifungal drugs include azoles, polyenes, allylamines, antimetabolites, echinocandins and other compounds such as tolnaftate, cicloprimox, FK506 and ciclosporin. The potency and cytotoxicity of these drugs has significantly improved over the years and have noticeably changed the approach to antifungal therapy.[5]

Table 1-1: Antifungal Drugs and Their Targets

Compound Class	Example Drug	Target
Polyenes	Amphotericin B	Ergosterol
Azoles	Fluconazole	Ergosterol biosynthesis, lanosterol demethylase
Allylamines	Terbinafine	Ergosterol biosynthesis, squalene epoxidase
Antimetabolites	Flucytosine	Fungal nucleic acid (RNA and DNA)
Echinocandins	Caspofungin	Cell wall, β-1,3-glucan synthesis
Other	Griseofulvin	Fungal mitotic apparatus

Source: http://www.doctorfungus.org

Scheme 1-1. Chemical Structures of Representative Antifungal Drugs
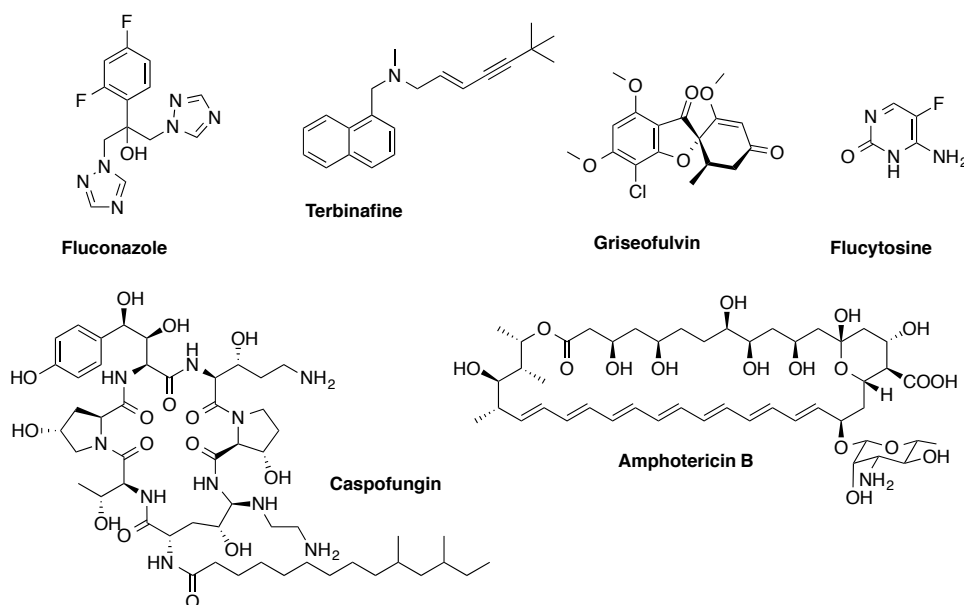
Role of Ergosterol and Biosynthesis of Ergosterol in Antifungal Drug Discovery

Several antifungal drugs' fungicidal or fungistatic activities involve targeting ergosterol or different stages of ergosterol biosynthesis (Table 1-1). Ergosterol, the functional fungal analogue of cholesterol, plays a vital role in maintaining membrane fluidity, nutrient transport and supporting cell wall biosynthesis.[21] Ergosterol also induces growth and proliferation of

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fungal cells due to its hormone-like function.[21,24] During the biosynthesis of either cholesterol or ergosterol, both humans and *Candida* produce zymosterol, a common intermediate, using homologous enzymes. Humans convert zymosterol into cholesterol using enzymes not found in *Candida* and conversely, *Candida* convert zymosterol into ergosterol using enzymes not found in humans (Scheme 1-2). These differences between fungal enzymes and human enzymes have created opportunities for drug development.

Scheme 1-2: Cholesterol vs. Ergosterol Biosynthesis
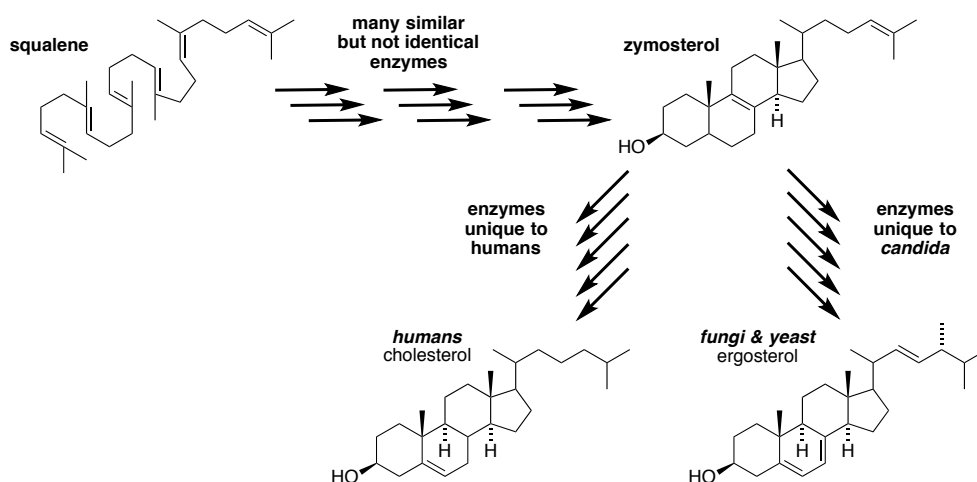
Polyenes as Antifungal Drugs

The polyene macrolide amphotericin B (Scheme 1-1) was the first commercially significant antifungal drug and it has been prescribed over 50 years as an effective treatment for most of the systemic fungal infections.[6] Amphotericin B is fungicidal due to its ability to interact with sterols present in the fungal cell wall, primarily ergosterol. These interactions result in the formation of pores in the cell membrane and subsequent leakage of cellular components. However, the drug's ability to interact with sterols has negative consequences on mammalian cells, since they are a vital part of mammalian cell membranes. In some cases the drug demonstrate significant cytotoxicity. Some adverse effects involve acute infusion reactions, neuropathy, gastrointestinal (GI) upset, renal failure, anemia, thrombophlebitis, hearing loss,

rash, hypokalemia and hypomagnesemia.[5] In order to alleviate the severe cytotoxicity in patients who do not respond well to the conventional formulation of amphotericin B, lipid-based formulations such as amphotericin B deoxycholate are designed and administered intravenously.[7]

Allylamines as Antifungal Drugs

Allylamine antifungals are both orally and topically active drugs and they are found to be fungicidal against most of the dermatophytes, *Aspergillus* species, *Candida parapsilosis, Scopulariopsis brevicaulis, Blastomyces dermatitidis* and *Histoplasma capsulatum*. Interestingly, it is only fungistatic against *C.albicans*.[8] Allylamine antifungals inhibit the enzyme squalene epoxidase that catalyzes the epoxidation of squalene to form 2,3-oxidosqualene, a transformation required in the biosynthesis of sterols. However, the potency of the drug towards the fungal enzyme is significantly higher than toward the mammalian squalene epoxidase. Consequently, the potential for the drug to interfere with the host sterol biosynthesis is very low.[8] Adverse effects of allylamines range from gastrointestinal problems such as diarrhea, constipation, and nausea to central nervous system, psychological problems. Minor side effects such as hair loss (alopecia), anemia, muscle pain and hepatitis were also reported.[9]

Antimetabolites as Antifungal Drugs

The cytosine analogue flucytosine (5-fluorocytosine, 5-FC), a compound that belongs to the general class of antimetabolites was first synthesized in 1957 as a potential anti-tumor agent (Scheme 1-1).[10] However, it was later discovered to be inadequately effective against tumors.[11] Flucytosine is a prodrug; once the susceptible fungal cell lines take up the flucytosine, the enzyme cytosine deaminase rapidly transforms the pro-drug to its active compound 5-fluorouracil (Scheme 1-3).[12] The resulting deaminated product is highly effective against fungi, because it inhibits the protein synthesis by incorporating into RNA.[13] Active intermediate 5-

fluorouracil is also responsible for inhibiting the DNA synthesis of the fungi by inhibiting the enzyme thymidylate synthase.[12] The active metabolite 5-fluorouracil can not be administered directly due to severe toxicity to mammalian cells and poor uptake by fungal cells.[14] Gaining resistance towards the monotherapy of flucytosine is a major drawback for the compound as an antifungal. This leads to administration of high and potentially cytotoxic doses to the patients. Consequently, 5-FC is currently used in combination with other antifungal drugs; mainly with amphotericin B.[15] Flucytosine demonstrates side effects such as nausea, diarrhea, hepatotoxicity and bone marrow depression.[16]

Scheme 1-3: Chemical Strucures of Cytosine, Flucytosine and 5-Fluorouracil

cytosine   flucytosine   5-fluorouracil
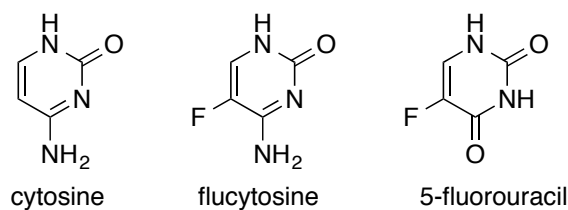
Echinocandins as Antifungal Drugs

Echinocandins are lipopeptides and the latest addition to the growing list of antifungals. Currently, there are three semi-synthetic echinocandins that have been approved for clinical use in the United States: caspofungin, micafungin, and anidulafungin. This is the first class of antifungals that attacks the fungal cell wall by inhibiting the enzyme β-1,3-D-glucan synthase, the key enzyme that is responsible for the synthesis of β-1,3-D-glucan. The combination of β-1,3-D-glucan and chitin is responsible for the integrity and shape of the cell wall.[17] Thus the inhibition of β-1,3-D-glucan synthesis leads to osmotic lysis of the fungal cell. Therefore, echinocandins act as fungicides. Similar to other lipopeptide antibiotics, echinocandins demonstrate limited oral bioavailability and therefore it is administered via intravenous infusion.[18] Echinocandins are now widely prescribed for treatment of systemic fungal infections

due to higher potency against azole-resistant *Candida* species such as *C. glabrata* and *C. krusei.* Also, echinocandins demonstrate significantly low cytotoxicity and drug-drug interactions. Minimal levels of adverse effects are reported including infusion-related reactions, such as rash and swelling and increased levels of hepatic transaminases were also observed.[19] However, one of the down sides to echinocandins is its ineffectiveness against *Cryptococcus* species.[20] This makes echinocandins a less attractive option in treating patients with compromised immune systems due to the challenges of identifying the responsible fungal strain in a reasonable time.

Scheme 1-4: Chemical Structures of Some of the Azole Antifungals

Ketoconazole

Itraconazole

Clotrimazole

Posaconazole

Miconazole
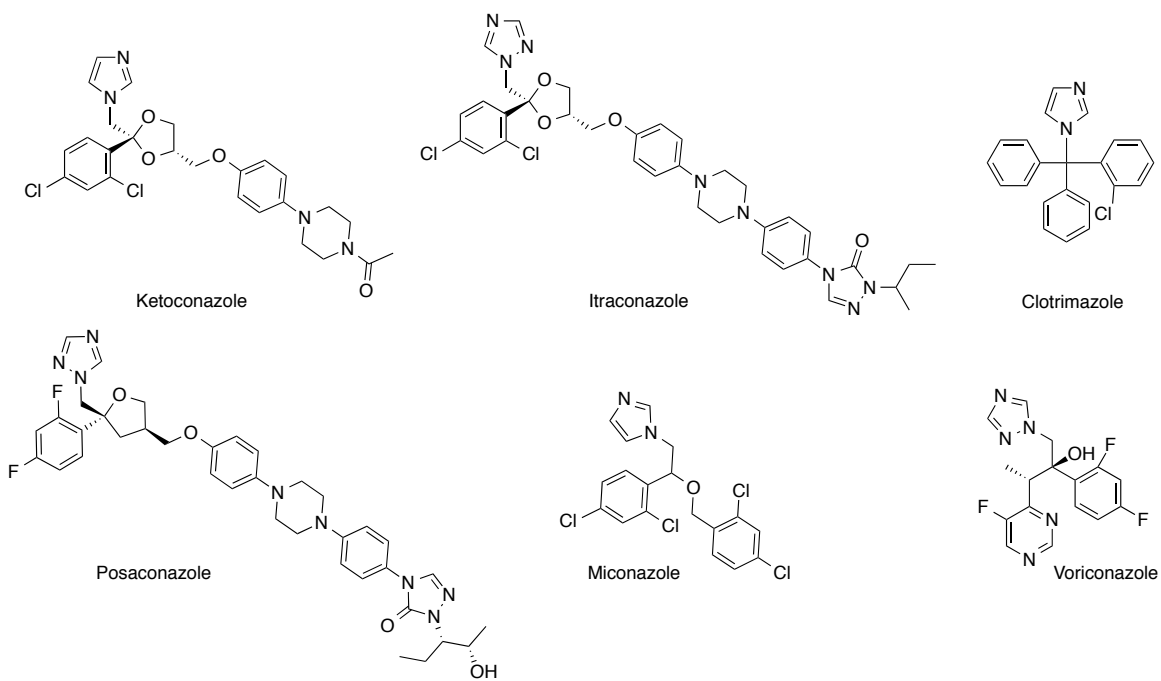
Voriconazole

Azoles as Antifungal Drugs

Azole antifungal drugs have evolved over the years, from less potent ketoconazole that exhibits severe adverse effects, to more potent and less cytotoxic fluconazole, itraconazole, posaconazole, and voriconazole (Scheme 1-4). Azole drugs can be further divided into imidazole and triazole based drugs. Some of the imidazole-based antifungals include ketoconazole,

clotrimazole, miconazole and econozole. More effective and less toxic triazoles include fluconazole, itraconazole, posaconazole, and voriconazole.

Azoles are fungistatic and they inhibit fungal cell growth by disrupting the ergosterol biosynthesis pathway. Particularly by inhibiting the cytochrome P-450-dependent enzyme, 14 α-sterol demethylase (P-450$_{DM}$) (Figure 1-1).[21,22,23,24] P-450$_{DM}$ has plays a vital role in mammalian cholesterol biosynthesis as well.[23] However, the efficacy of the azole drugs is attributed to their high affinity towards the fungal lanosterol 14 α-sterol demethylase than the corresponding mammalian enzyme.[23] Minimal reported side effects include GI discomfort and rash. Hepatic necrosis, Stevens-Johnson syndrome, anaphylaxis and alopecia were also rarely reported.[5]

Figure 1-1: Mechanism of Action of Azoles in Ergosterol Biosynthesis
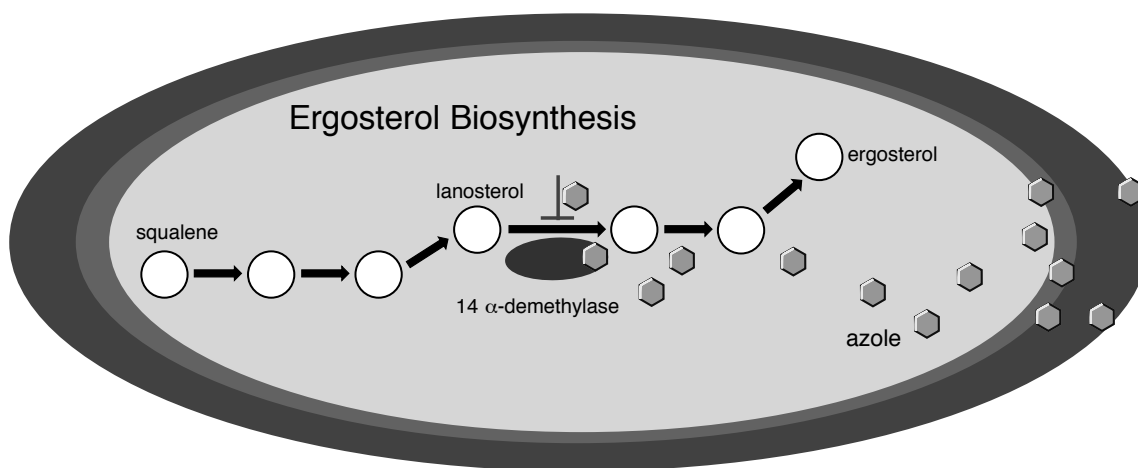
There are other antifungal drugs (e.g., griseofulvin, amorolfine) that are not frequently prescribed in the clinics. These drugs become either fungicidal or fungistatic by targeting several key aspects of the fungal cellular structures and mechanisms such as the mitotic apparatus and will not be discussed in this report.

Drug Synergy as a Solution to Drug-Resistance

Evolution of azole-resistant fungal strains is one of the major clinical challenges in the treatment of fungal infections.[25] Antifungal resistance has been classified in three different

categories: intrinsic, acquired and "clinical resistance".[26] Intrinsic resistance occurs before the exposure to antifungals, while acquired resistance develops after the exposure to the antifungals. Stable or transient genotypic alterations cause such acquired resistance. Finally, "clinical resistance, which encompasses progression or relapse of an infection by a fungal isolate that seems, in laboratory testing, to be fully susceptible to the antifungal used for the treatment of infection."[26] Clinical resistance is very common in patients with compromised immune systems.

One successful approach to treat patients with antifungal-resistant fungal infections is to utilize drugs that can act in a synrgestic fashion. This drug combination approach enhances each drug's effectiveness more than the effect from the sum of each drug's individual impact.[27] Such a treatment is thought to not only decrease the microorganism's resistance towards individual drugs but also increase the drug repertoire[27] and improve toxicities.[28]

Small Molecules Have Identified as Fluconazole Synergizers

A large number of studies have been carried out to test the synergy of small molecules with fluconazole against *Candida* spp. Varying levels of synergy were observed and compounds that synergize with fluconazole at low concentrations (e.g., MIC_{90} <0.1 µg/mL) are unusual. The antifungal agents flucytosine,[29,30,31,32] and fenpropimorph[33] have been shown to potently synergize with fluconazole against various strains of *C. albicans*. Micafungin[34,35,36] and caspofungin[33] are highly potent, but not synergistic with fluconazole, with FIC indices above 1.0. A number of drugs commonly used against non-fungal human diseases have also been shown to synergize with azoles against *C. albicans*. The calcineurin inhibiting drug tacrolimus[37,38] potently enhances the activity of fluconazole against *C. albicans*. Quite a few other compounds have been reported to inhibit the growth of *Candida* in synergy with fluconazole, with MIC_{90}s below 1 µg/mL, but not below 0.1 µg/mL: e.g., *T. broussonetii* extract,[39] terbinafine,[40] amlodarone,[41]

catechin, quercetin, epigallocatechin,[42] simvastatin,[43] tunicamycin,[33] cationic peptides IJ3, IJ4[44] and VS3,[45] ketorolac,[46] cyclosporin A,[37,47] nystatin,[48] sanguinarine,[49] allicin,[50] declofenac,[46] leaf extracts of *Lippia alba*,[51] diphenyldiselenide,[52] balcalein,[53] geldanamycin,[49] pseudolaric acid B,[54] and doxycycline.[55] Hundreds of other compounds have been reported to exhibit antifungal activity in concert with azoles but not below 1 μM. Chemical synthesis can be used to improve the potency of lead molecules; in a recent study, several analogues of the azole synergizer berberine (MIC_{80} 1.0 μg/mL) were identified with up to 8 times higher potency.[56,57]

Massive Screenings to Identify Small Molecule Fluconazole Synergizers

Several groups have screened large libraries of compounds in search of small molecules that synergize with azoles. In 2011, Spitzer and co-workers screened the Prestwick library of off-patent drugs.[58] During the initial high-throughput assay they screened 1120 off-patent drugs (30 μM [drug]) in the presence and absence of half of the minimal inhibitory concentration (MIC_{50}) of fluconazole. The assay primarily tested the antifungal activity of fluconazole and drug combination against four fungal species: *C.neoformans* (H99), *C.gattii* (R265), *C.albicans* (Caf2-1), *S.cerevisiae* (BY4741). A total of 148 compounds demonstrated to enhance the antifungal activity of fluconazole in at least one of the 4 fungal strains tested. Out of this pool of compounds, 12 compounds were selected to study the synergy with fluconazole depending on commercial availability of the compound, distinct chemical class, therapeutic importance and known mode of action of the compound. Interestingly, sertraline, a potent antidepressant and trifluoperazine, an antipsychotic have shown to synergize with fluconazole in all four strains. In addition, clofazimine, clomiphene, *L*-cycloserine and mitoxantrone synergized with fluconazole in *S.cerevisiae* and other antifungals, ketoconazole and caspofungin synergized with fluconazole in *C. albicans*. None of the compounds synergized with fluconazole in *Cryptococcus* species.

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Further analysis of some these drugs in genome-wide chemical–genetic screens revealed potential targets of the off-patent drugs in fungi. In these assays around 1000 deletion strains heterozygous for several essential genes were tested for drug sensitivity. Trifluoperazine, tamoxifen, clomiphene, sertraline and suloctidil were found to be effective due to the perturbation of cell membranes, interruption of the cellular vesicle trafficking and sterol biosynthesis, whereas *L*-cycloserine seems to disrupt the early stages of sphingolipid biosynthesis.[59] In this study the authors were able to establish the importance of systemic screens in dicovering new antifungal drug combinations.

Also in 2011, LaFleur and co-workers screened a library of 120,000 compounds in search of molecules that could act in synergy with clotrimazole against a variant of *C. albicans* CAF2-1 that forms biofilms.[60] The initial screen identified 19 hits and only 4 of those compounds were non-toxic to mammalian cells and all these compounds exhibit low antifungal activities in the presence of the azole drugs (3-140 μM) (Scheme 1-5).

Scheme 1-5: LaFleur Assay Summary
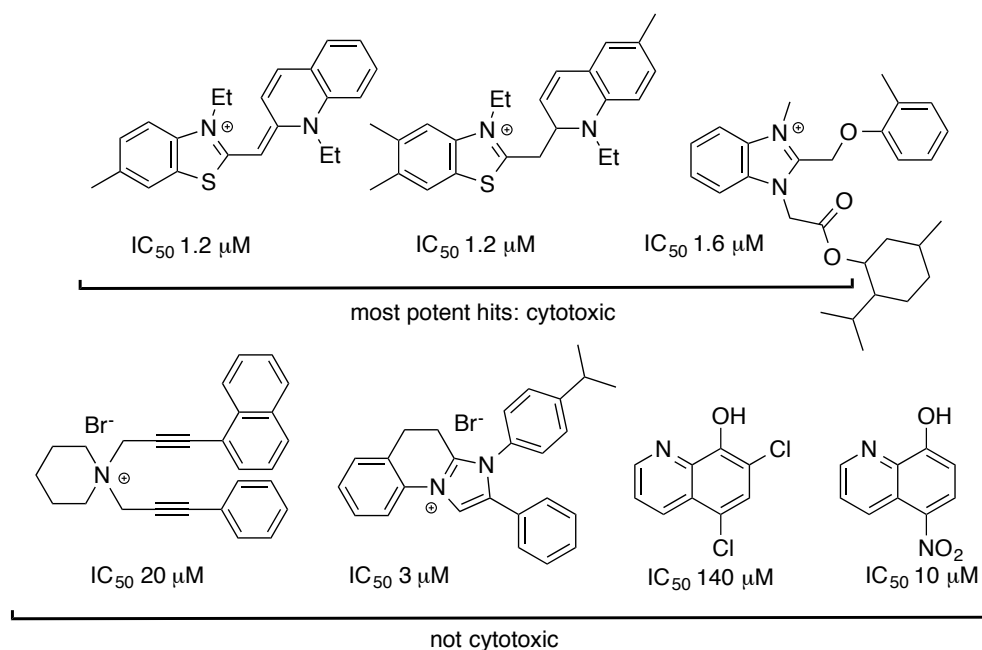
Starting in 2010 Lindquist, Schreiber, and others at the Broad Institute reported a massive screening of NIH's Molecular Libraries Small Molecule Repository (MLSMR)[61] to identify small molecules capable of inhibiting growth of *C. albicans* in synergy with fluconazole with a particular interest in Hsp90 and calcineurin pathways.[62,63]

Initially 302,509 compounds from MLSMR were screened for the growth inhibition of *C. albicans* clinical isolate CaCi-2 (MIC of fluconazole 2 μg/mL) in the presence of 8 μg/mL fluconazole (Figure 1-2). MIC is the minimum concentration of fluconazole required to completely inhibit the growth of microorganism. Interestingly, the Lindquist and co-workers utilized higher concentrations of fluconazole than its minimum inhibitory concentration against the corresponding clinical isolate, CaCi-2. However, according to the authors, "these strains continue to proliferate steadily (albeit at a reduced rate) when treated with fluconazole at or above the reported MIC. This behavior may contribute to the inability of fluconazole therapy to effectively clear the infection and allows for the further development of resistance."[64] Out of 1,893 total compounds that demonstrated >75% growth inhibition, 1,654 compounds were selected for further studies on the basis of availability. The selected molecules were subjected to three parallel assays that tested for: 1) growth inhibition of a slightly resistant strain CaCi-8 (MIC of fluconazole 8 μg/mL) in the presence of 8 μg/mL of fluconazole, 2) growth inhibition of CaCi-2 in the absence of fluconazole and 3) potency in a dose-response assay (the cut-off limits are indicated in Figure 1-2). Based on those assays, they identified 350 compounds that satisfied their selection criteria. A subsequent cytotoxicity assay against NIH 3T3 fibroblasts reduced the potential candidates to 296 compounds that did not demonstrate significant cytotoxicity below 26 μM. A total of 29 compounds were selected to be re-exposed to the screening tree once more.

Figure 1-2: Summary of the Broad assay

302,509 compounds (7.5 μM)
screened for CaCi-2 growth inhibition with
8 μg/mL fluconazole

> 75% inhibition

1893 active compounds
1654 available for retest

CaCi-8 growth inhibition
with 8 μg/mL fluconazole
$IC_{50} \leq 50$ μM

CaCi-2 growth inhibition
with no fluconazole
$IC_{50} \geq 26$ μM

dose-response
activities
$IC_{50} \leq 1$ μM

836 compounds   622 compounds   1012 compounds

meet all requirements

350 compounds
cytotoxicity against
3T3 fibroblasts
with no fluconazole

$IC_{50} \geq 26$ μM

296 compounds
29 compounds purchased/synthesized
re-exposed to the whole tree once more

best molecules for SAR

1.1

1.2

1.3

IC_{50} 0.7 μM
w/ fluconazole

IC_{50} 2.2 μM
w/ fluconazole

IC_{50} 3.2 μM
w/ fluconazole

Three of the most promising compounds[65,63] (Figure 1-2, compounds **1.1**, **1.2**, **1.3**) were
selected for further optimization. However, none of the resulting compounds ML189, ML212,

and ML229 respectively were active below 0.7 μM against CaCi-8 (Scheme 1-6). The many

active enhancers of fluconazole identified in this study provide a rich source of lead molecules

for further development.

Scheme 1-6: Best Molecules Identified After SAR Studies of the Probes

IC$_{50}$ 0.7 μM
ML189
(probe compound **1**)

IC$_{50}$ 0.7 μM
ML212

IC$_{50}$ 0.6 μM
ML229
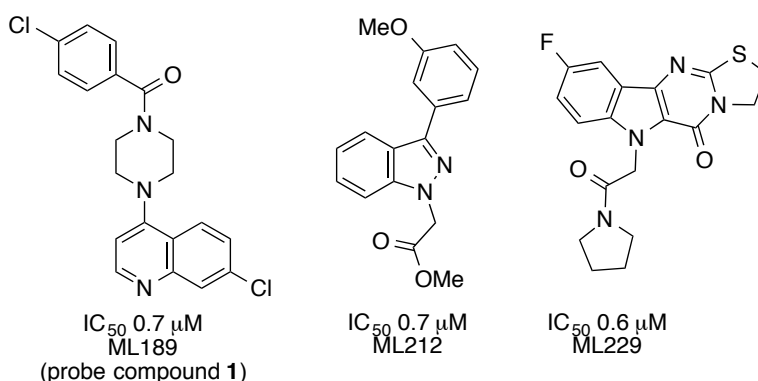
Conclusion:

Systemic fungal infections are serious and sometimes life threatening conditions that have to be

addressed immediately and efficiently. Several classes of antifungals are currently used in the

clinics as potent drugs. However, constant evolution of antifungal resistant strains is a major

clinical issue. Several groups have conducted massive screenings of small molecules to identify

potent compounds that can synergize with fluconazole and some small molecule synergizers are

shown to make the existing antifungals more potent against resistant cell lines. These small

molecules provide excellent probes for further optimizations to make very effective drugs that

can be combined with existing antifungals to combat resistant fungal infections.

Chapter 2

Small Molecule Fluconazole Synergizers - Spiroindolinones

Introduction

Small molecules have been demonstrated to enhance the antifungal activity of fluconazole by synergizing with the azole. Several high-throughput screening campaigns have identified numerous small molecules with the potential to be potent antifungal synergizers, but only with further optimization.[58,60,62,63,65] Consequently, we have decided to identify and optimize the small molecules that were identified in high-throughput screens, but were not further optimized by the corresponding research groups. Also, we were equally interested in identifying sites on the azole synergizers that can be biotinylated without losing their potency and eventually used for affinity isolation of proteins responsible for the antifungal activity/synergy.

Inhibition of Upc2 and Sensitizing Resistant Fungal Strains

Ergosterol and enzymes responsible of ergosterol biosynthesis are the most common targets of antifungal drugs. Upc2 is a transcriptional regulator that is responsible for controlling the expression of genes that are responsible for the ergosterol biosynthesis in *C. albicans*.[66] At the outset of this work, the mechanisms for regulation of these genes was poorly understood.[67] Upc2 is a zinc finger transcription factor. Recently, it was discovered that in Upc2 the ligand-binding domain is the C-terminus domain (CTD) and the nuclear localization domain to be the N-terminus domain.[67] Additionally, CTD is responsible for sensing ergosterol levels in the cell. According to the current model, ergosterol binding leads to sequestration of Upc2 in the cytosol. When ergosterol levels are low, the ergosterol-free form of Upc2 translocates from the cytosol to the nucleus (Figure 2-1). Once Upc2 enters the nucleus, it binds to DNA and activates transcription; but the downstream coactivators of Upc2 are unknown. The complexation of a co-

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activator with Upc2 triggers the signal for the transcription of *ERG* genes that are responsible for ergosterol biosynthesis.

One of the major resistant mechanisms of azole drugs involves overexpression of the enzymes that are inhibited by the antifungals in ergosterol biosynthesis. Therefore, as one strategy to overcome this mode of antifungal resistance, we were interested in small molecules that can potentially inhibit the activity of Upc2 and in turn prevent the upregulation of the genes responsible for the ergosterol biosynthesis. Potently interfering with Upc2 transcription factor binding to the DNA is expected to sensitize the resistant *C. albicans* to the antifungals.

Figure 2-1: Transcriptional Regulation of Upc2
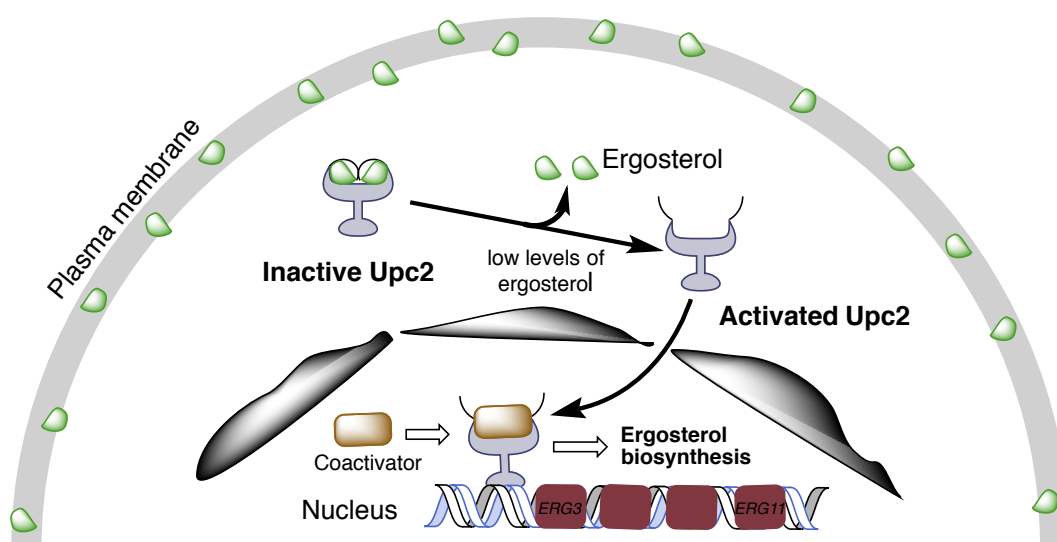
Intial Results and Attempts to Make Potent Analogues

Spitzer and co-workers have screened 1,120 off-patent drugs and identified 30 syncretic compounds that could enhance the activity of fluconazole against *C.albicans* CAF2-1.[58] We ordered 25 out of 30 compounds that were commercially available. Dr. Fu-Qiang Wang in Professor Haoping Liu's lab in the Department of Biological Chemistry at UC Irvine performed

the initial *ERG* expression and antifungal assays. I later carried the antifungal assays of my synthetic compounds.

ERG genes are responsible for the enzymes that catalyze ergosterol biosynthesis and these genes are under the control of transcription regulator Upc2. Mutations occurring in *ERG3* and *ERG11* are directly related to fluconazole drug resistance.[68] Therefore, the *C. albicans* strain, HLY4123 that was used as the susceptible laboratory strain in this study carries a GFP reporter for *ERG3* and *ERG11* expression. It was constructed by plasmid transformation of the commonly used laboratory *C. albicans* strain CAI4. Fluorescence-activated cell sorting (FACS) analysis was employed to monitor expression of *ERG* genes.

Several NSAIDs based on anthranilic acid such as flufenamic acid, mefenamic acid, tolfenamic acid, aceclofenac and isoxicam, which carried a benzothiazine core, were identified as downregulators of *ERG3* and *ERG11* genes at a single dose of 30 μM (Figure 2-2) in the presence of fluconazole. A potent selective serotonin reuptake inhibitor (SSRI) sertraline was also found to be an upregulator of *ERG3* and *ERG11* genes at 30 μM. It was reported by Zhai and co-workers that sertraline potently inhibits the translation of *Cryptococcus neoformans* by an unknown mechanism with EC_{50} of 1 nM.[69]

Interestingly, all the upregulators in this study inhibited the growth of *C. albicans* whereas all the downregulators except flufenamic acid showed no growth inhibition of *C. albicans* in the presence of fluconazole. Flufenamic acid indicated fungicidal activity even in the absence of fluconazole.

In order to optimize the potency and identify the site(s) for biotinylation, I initiated the synthesis of several different analogues of fenamic acid derivatives. Also I trained two

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undergraduate researchers (Kevin A. Scott and Chengtian (John) Shen) to assist me with synthesis and antifungal assays.

Figure 2-2: Initial Transcriptional Regulation Results for Spitzer Hits

flufenamic acid **(D)**

mefenamic acid **(D)**

tolfenamic acid **(D)**

aceclofenac **(D)**

isoxicam **(D)**

sertraline **(U)**

(D) downregulators of *ERG3* and *ERG 11*
(U) upregulators of *ERG3* and *ERG 11*
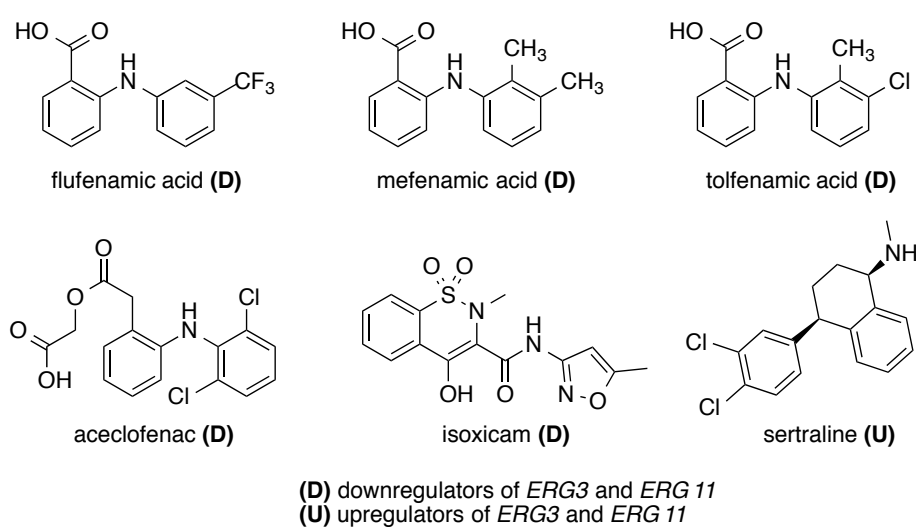
We synthesized 16 fenamic acid analogues and 8 aceclofenac analogues. Flufenamic acid demonstrated an EC_{50} of 2 μM in the presence of 0.25 μg/mL of fluconazole. Unfortunately, none of our analogs exhibited significantly better activities than the parent flufenamic acid. However, structure-activity relationships revealed that the fenamic acid core is essential for the activity (Figure 2-3). Also the fenamic acid core cannot be readily modified to improve or investigate the potency further.

Figure 2-3: Summary of SAR Study of Flufenamic Acid Analogues

	IC_{50}
flufenamic acid	2 μM
best analogue	1 μM
other analogues	>1 μM
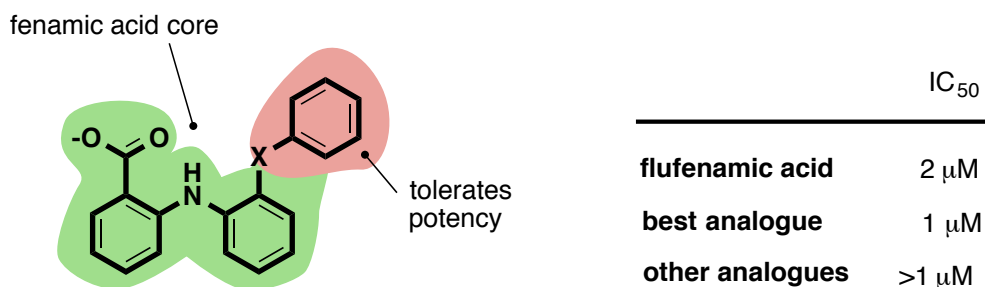
In 2010 Lindquist, Schreiber, and others at the Broad Institute reported a screen of 302,509 compounds searching for compounds that could enhance the antifungal activity of

fluconazole against a susceptible *C. albicans* isolate CaCi-2 (Fluconazole MIC 2 μg/mL). They identified a subset of 296 compounds that were active against susceptible CaCi-2, slightly resistant isolate CaCi-8 (Fluconazole MIC 8 μg/mL) and were non-toxic to NIH 3T3 fibroblasts. We ordered 51 of those 256 compounds that were inactive against Hsp90 or calcineurin from commercial suppliers and screened them for their effect on *ERG3* and *ERG11* transcription and discovered 20 compounds that affected the transcription of *ERG3* and *ERG11* at some level. In a secondary screen Dr. Fu-Qiang Wang discovered 5 compounds as potent downregulators, but none enhanced the antifungal activity of fluconazole against the non-resistant GFP-reporter strain, HLY4123 (Figure 2-4).

Figure 2-4: Downregulators Identified in the Initial Screen

CID 3833874   CID 3988428   CID 3235608

CID 16187539   CID 2263192
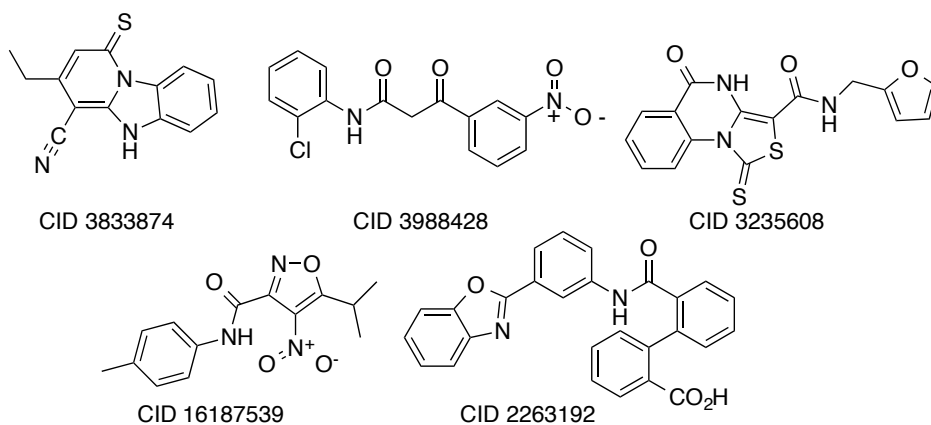
However, 6 of the 20 compounds were potent upregulators at 30 μM in the presence of 0.25 μg/mL fluconazole, and all six enhanced the antifungal effect of fluconazole against the non-resistant GFP-reporter strain (Figure 2-5). Since the upregulators exhibited antifungal activity in the presence of fluconazole, that made them very interesting candidates for further optimization.

Figure 2-5: Upregulators Identified in the Initial Screen

CID 2948951
108 nM

CID 6584729
11 nM

CID 22334057
15 nM

CID 2929781
64 nM

CID 4545471
839 nM

CID 3197668
14 nM
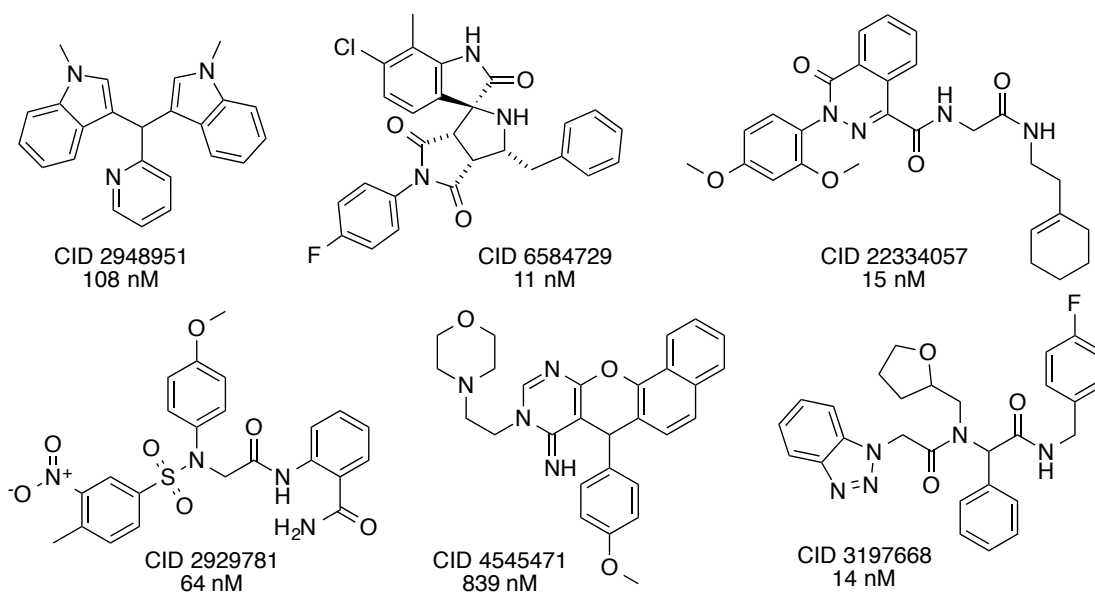
I attempted to synthesize and purify 8 different analogues of bisindolylpicoline, PubChem CID 2948951. Unfortunately, low solubility made chromatographic purification difficult and the oxidative sensitivity of the analogues caused them to readily form colored impurities. Only one minor variant of CID 2948951 was successfully purified and tested as an enhancer of fluconazole antifungal activity. However, it was slightly inferior to CID 2948951 (Scheme 2-6). Analogues of bisindolylpicoline CID 2948951 are unstable and difficult to purify and therefore made them unattractive candidates for further optimizations.

Figure 2-6: Attempts to Synthesize Analogues of CID 2948951

oxidatively
sensitive

AcOH
0 - 23 °C

8 analogous were attempted
1 was successfully purified

CID 2948951

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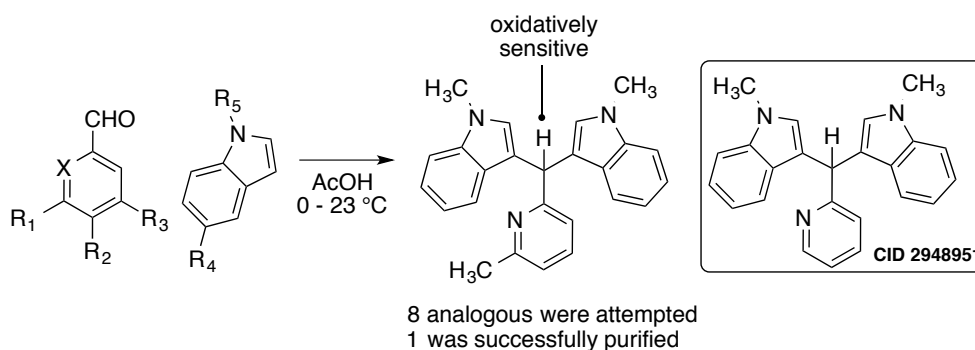
We then focused on the other potential analogs for optimization and were intrigued by the structurally interesting spirocyclic compound (1*S*,3*R*,3a*R*,6a*S*)-1-benzyl-6'-chloro-5-(4-fluorophenyl)-7'-methylspiro[1,2,3a,6a-tetrahydropyrrolo[3,4-*c*]pyrrole-3,3'-1H-indole]-2',4,6-trione (PubChem CID 6584729) and the potential activity of synthetically accessible diastereomer **2.1** and related analogues (Figure 2-7).

Figure 2-7: Fluconazole synergizer CID 6584729 and diastereomer **2.1**

CID 6584729   **2.1**
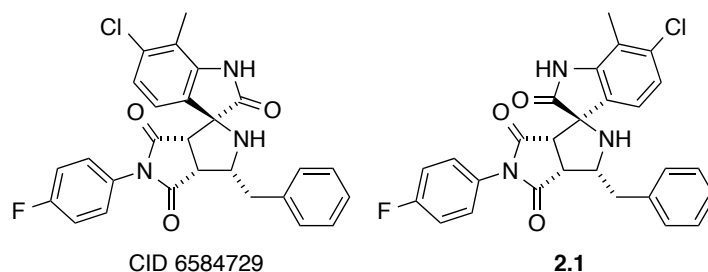
Results and Discussion

Chemistry

As shown in Scheme 2-1 *N*-phenylmaleimides **2.4a** and **2.4b** were synthesized through a two-step condensation of substituted anilines with maleic anhydride.[70] Anilines **2.2a** and **2.2b** were condensed with maleic anhydride to form the corresponding *N*-phenylmaleamic acids **2.3a** and **2.3b** that were cyclized using acetic anhydride in the presence of sodium acetate to afford the corresponding maleimides.

Scheme 2-1: Synthesis of Substituted *N*-Phenylmaleimides

	R	yield
a	4-F	95%
b	3,5-bis-(F$_3$C)	98%

2.2a, 2.2b **2.3a, 2.3b** **2.4a, 2.4b**
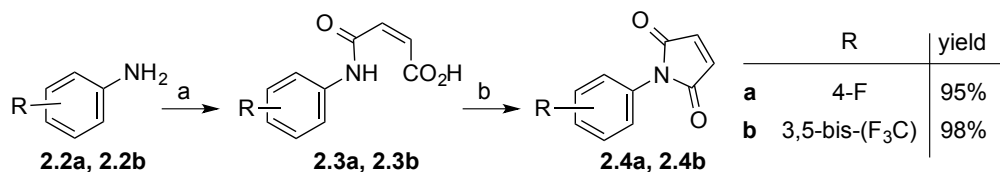
Reagents and conditions: (a) 1.0 equiv maleic anhydride, Et$_2$O, 23 °C, 15 min; (b) 0.7 equiv sodium acetate, (CH$_3$CO)$_2$O, 70 °C, 30 min.

Various substituted isatins were prepared from the corresponding anilines using the two-step Sandmeyer synthesis (Scheme 2-2).[71] Anilines were reacted with the oxime of chloral, generated in situ, to afford isonitrosoacetanilides, which were pure by TLC. The isonitrosoacetanilides were cyclized, without purification, through an intramolecular Friedel-Crafts reaction to afford the corresponding isatins **2.6a-2.6d** in good yield. *N*-Benzylisatins **2.7d** and **2.7e** were prepared by alkylation with benzyl bromide using sodium hydride as a base.[72]

Scheme 2-2: Synthesis of Substituted Isatins

	R4	R5	R6	yield
2.6a	OMe	H	H	65%
2.6b	H	Me	H	53%
2.6c	H	Me	Me	55%
2.6d	H	Cl	Me	72%
2.7d	H	Cl	Me	81%
2.7e	H	H	H	67%
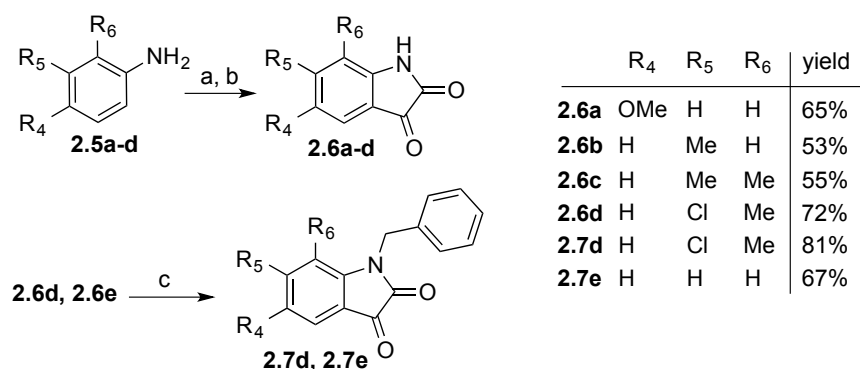
Reagents and conditions: (a) 1.1 equiv chloral hydrate, 3.0 equiv NH$_2$OH•HCl, 9.0 equiv Na$_2$SO$_4$, 1.1 equiv HCl, H$_2$O, 70 °C, 1 h; (b) H$_2$SO$_4$, 23 °C, 5 min, (c) 1.1 equiv benzyl bromide, 1.1 equiv NaH, DMF, 23 °C, 2 h.

Spiroindolinones are readily accessible through one-pot three-component coupling reactions of isatins, amino acids, and maleimides.[73,74,75] The reaction of isatin **2.6d**, L-phenylalanine and maleimide **2.4a** generated compound **2.1** as a single diastereomer in 74% yield (Scheme 2-3). The optically pure amino acid undergoes decarboxylation during the reaction; unless otherwise stated all spiroindolinones were isolated and tested as racemates.

The relative stereochemistry of compound **2.1** was secured through a NOESY experiment (Scheme 2-3) and shown to match that of related spiroindolinones prepared from isatins and maleimides under the same reaction conditions.[75] In particular, the strong nOe between protons on C3' and C6a' of the pyrrolidine ring indicate that they are on the same face and conversely

that the benzyl group and succinimide ring are both on the opposing face. Furthermore the strong nOe between the fluorophenyl proton and the proton on C4 of the indolone ring is consistent with the stereochemistry of compound **2.1**. Interestingly, the ^{1}H NMR spectra, ^{13}C NMR spectra and nOes for the compound sold as CID 6584729 (by Vitas-M) were indistinguishable from those of compound **2.1**. Thus commercial STK580951 is in fact the same as our synthetic compound **2.1** and does not match PubChem CID 6584729.

Scheme 2-3: Synthesis of Compound **2.1** and nOes Used in the Assignment of Relative Configuration
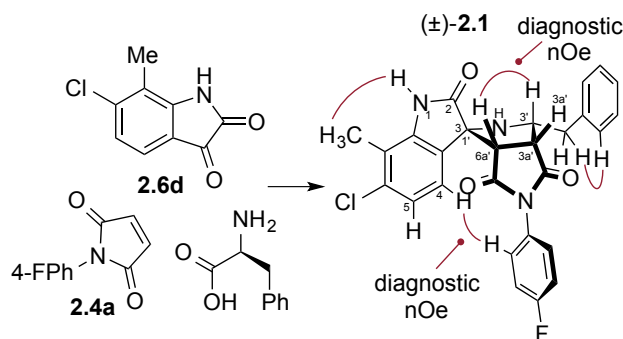
Reagents and conditions: 3:1 MeOH/H$_2$O, 65 °C, 16 h.

Other analogues of spirocycle **2.1** were synthesized (Scheme 2-4) using phenylalanine, tryptophan, and N_ε-Boc-Lysine. In all cases, the limiting reagent, isatin **2.6** or **2.7**, was completely consumed and the reaction gave the desired cycloadduct as a single diastereomer. The reactions of these amino acids were highly stereoselective, affording products with a relative configuration analogous to spiroindolinone **2.1**. We did not observe nor isolate other diastereomers of the spiroindolinones **2.8**-**2.13**.

The formation of diastereomer **2.1**, and **2.8**-**2.13** was anticipated based on the work of Pavlovskaya and co-workers,[74] but is best explained by examining a larger body of work involving reactions of amino acids, enones, and either isatins or phenylglyoxalate derivatives, which are essentially acyclic analogues of isatins. All known reactions of amino acids, isatins,

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and enones react to give products consistent with *syn-anti* azomethine ylides (Figure 2-8, configuration A).[76,77,78,79] However, in the corresponding reactions with phenylglyoxylate, the reactive configuration of the azomethine ylide seems to depend on the type of amino acid: proline gives products consistent with *syn-anti* azomethine ylides (Figure 2-8, configuration A),[80] whereas acyclic amino acids give products consistent with *anti-anti* azomethine ylides (Figure 2-8, configuration B).[81,82,83]

Scheme 2-4: Synthesis of Spirocyclic Pyrrolidines Through a Three-Component, 1-Pot [1,3]-Dipolar Cycloaddition with Amino Acids
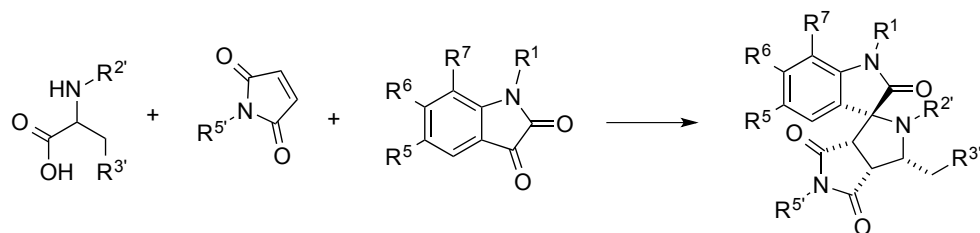
Compound	$R^{5'}$	$R^{2'}$	$R^{3'}$	R^5	R^6	R^7	R^1	Yield
2.1	4-fluorophenyl	H	Ph	H	Cl	CH_3	H	74%
2.8	Bn	H	Ph	H	H	H	H	60%
2.9	Bn	H	3 -indolyl	H	H	H	H	70%
2.10	Bn	H	$(CH_2)_3NHBoc$	H	H	H	H	33%
2.11	Bn	H	Ph	H	H	H	Bn	46%
2.12	Ph	H	Ph	H	H	H	Bn	57%
2.13	Ph	H	Ph	H	Cl	CH_3	H	26%
2.14	Ph	$CH_2CH_2N(Boc)$	H	H	Cl	CH_3	H	60%
2.15	4-fluorophenyl	$CH_2CH_2N(Boc)$	H	H	Cl	CH_3	H	49%
2.16	3,5-bis(F_3C)phenyl	$CH_2CH_2N(Boc)$	H	H	Cl	CH_3	H	12%
2.17	Ph	$CH_2CH_2N(Boc)$	H	H	Cl	CH_3	Bn	82%
2.18	Bn	$CH_2CH_2N(Boc)$	H	H	H	H	Bn	56%
2.19	Bn	$CH_2CH_2N(Boc)$	H	H	H	H	H	53%
2.20	Ph	$CH_2CH_2N(Boc)$	H	H	H	H	Bn	71%
2.21	Bn	$CH_2CH_2N(Boc)$	H	MeO	H	H	H	53%
2.22	Ph	$CH_2CH_2N(Boc)$	H	H	H	CH_3	H	60%
2.23	Ph	$CH_2CH_2N(Boc)$	H	H	CH_3	CH_3	H	61%

Reagents and conditions: 3:1 MeOH/H_2O, 65 °C, 4 h - 16 h.

Figure 2-8: Stereochemical Configurations of Azomethine Ylides
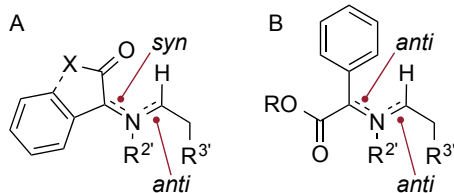
The 1,3-dipolar cycloaddition can proceed through either an *endo* or *exo* transition state. The products derived from all known reactions of amino acids, acyclic enones, and either isatins or phenylglyoxalates can be rationalized to arise through *endo* transition states (Scheme 2-5, path A).[77,78,79,80,81, 84] In contrast, reactions of amino acids, maleimides and either isatins or phenylglyoxalates can proceed through *endo* or *exo* transition states depending on the structure of the amino acid. Acyclic amino acids give products consistent with *endo* transition states (Scheme 2-5, paths B and C).[74,77,81,82,83,84] The only known reaction of a cyclic six-membered ring amino acid, pipecolic acid, with isatin and an acyclic dipolarophile also gives products consistent with an *endo* transition state (Scheme 2-5, path B);[78] however, the stereochemical outcome in the reaction of azomethine ylides with acyclic dipolarophiles cannot be extrapolated to reactions with maleimides.[75,79a] In contrast, the cyclic five-membered ring amino acid proline gives products consistent with an *exo* transition state (Scheme 2-5, path D).[75]

Similar ylides can be accessed from three-component reactions with amines instead amino acids, but there are cases where the trends in ylide configuration[85] and *endo/exo* selectivity[86] no longer hold. Notably, Ardil and co-workers showed that 1,3-dipoles derived from *N*-methylpiperazine aminals and related compounds favor *exo* adducts over *endo* adducts — sometimes exclusively *exo* — in refluxing toluene.[86]

The assumption that isatins would react through path C (Scheme 2-5) may have led to the misassignment of the compound CID 6584729 by the commercial supplier along with over 100

spiroindolinones in the PubChem database. We cannot be sure of the stereochemistry of the compound CID 6584729 that was tested by Lindquist and co-workers since the experimental data for compounds in PubChem assay IDs 1979, 2467, and 2423 were never reported. One cannot rule out the possibility that the compound CID 6584729 tested in those assays was correctly assigned and generated through a more lengthy synthetic route than the one-pot reaction used in this and related work.

Scheme 2-5: *Endo/Exo* Selectivity in Dipolar Cycloadditions of Azomethine Ylides
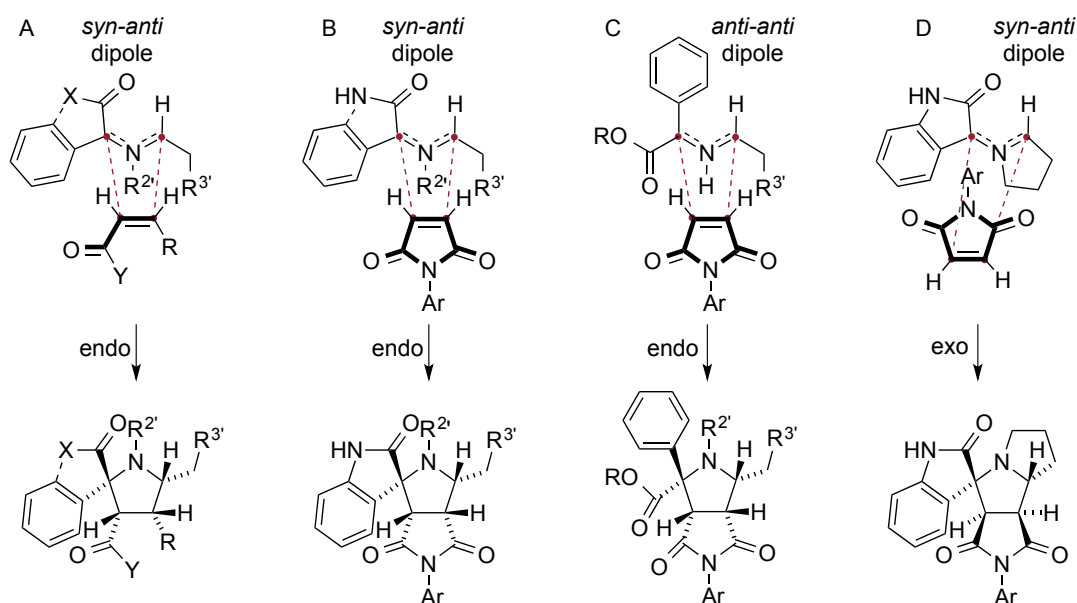
To provide access to diastereomeric spiroindolinones with defined absolute stereochemistries we carried out a three-component coupling with isatin **2.6d**, *N*-phenylmaleimide and (2*S*, 4*R*)-4-hydroxyproline (Scheme 2-6). After 16 h, the reaction generated an inseperable mixture of two spiroindolinones, **2.24a** and **2.24b**, in 30% yield along with unreacted isatin. The two optically pure stereoisomers were readily separated by silica gel chromatography after benzoylation of the hydroxy groups to afford esters **2.25a** and **2.25b**. The relative stereochemistry was assigned on the basis of diagnostic nOes. In particular, in both spiroindolinones **2.25a** and **2.25b**, there is an nOe between the bridgehead proton H^d and the

25

arene proton H^g on the indolone ring. In spiroindolinone **2.25a**, protons $H^{f'}$, H^a, and $H^{e'}$ on the β-face of the proline ring exhibit vicinal nOes between each other. Protons $H^{e'}$ and $H^{f'}$ on the β-face of the proline ring exhibit long-range nOes with protons H^c and H^d on the succinimide ring, respectively; and proton $H^{f'}$ exhibits highly diagnostic long-range nOes to the indolone aryl proton H^g. In spiroindolinone **2.25b**, protons $H^{f'}$, H^a, $H^{e'}$ and H^b on the β-face of the proline ring exhibit vicinal nOes between each other. Protons H^e and H^f on the α-face of the proline ring exhibit long-range nOes with protons H^c and H^d on the succinimide ring, respectively; and proton H^f exhibits a highly diagnostic long-range nOe to the indolone aryl proton H^g. Thus, the [3+2] cycloaddition of trans-hydroxyproline assembles proceeds via *exo* addition of maleimide to an azomethine ylide with a *syn-anti* configuration (Scheme 2-5, path D).

Scheme 2-6: Stereoselectivity in the Three-Component, 1-Pot [1,3]-Dipolar Cycloaddition with (2*S*, 4*R*)-4-Hydroxyproline. Stereochemistry was established by nOes (red lines)
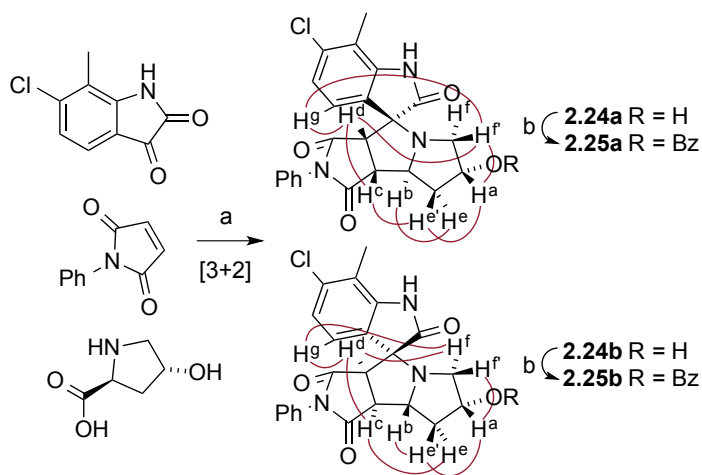
Reagents and conditions: a) 3:1 MeOH/H_2O, 90 °C, 16 h, b) 1.1 equiv BzCl, 1.2 equiv Et_3N, DMF, 23 °C, 20 h.

The reaction of the six-membered ring amino acid N_ε-Boc-piperazine-2-carboxylic acid proceeds in manner analogous with pipecolic acid (Scheme 2-5, path B) to afford spirocyclic indolinone **2.14** as a single diastereomer. The relative stereochemistry of spirocyclic piperazine **2.14** was secured with nOes after removal of the Boc and shown to match that of compound **2.1**.

Related spiroindolinones **2.15**-**2.23** were also prepared stereoselctively from N_ε-Boc-piperazine-2-carboxylic acid (Scheme 2-4).

Scheme 2-7: Synthesis of Pentacyclic Pyrrolidines Through Further Substitutions to Compound **2.26**
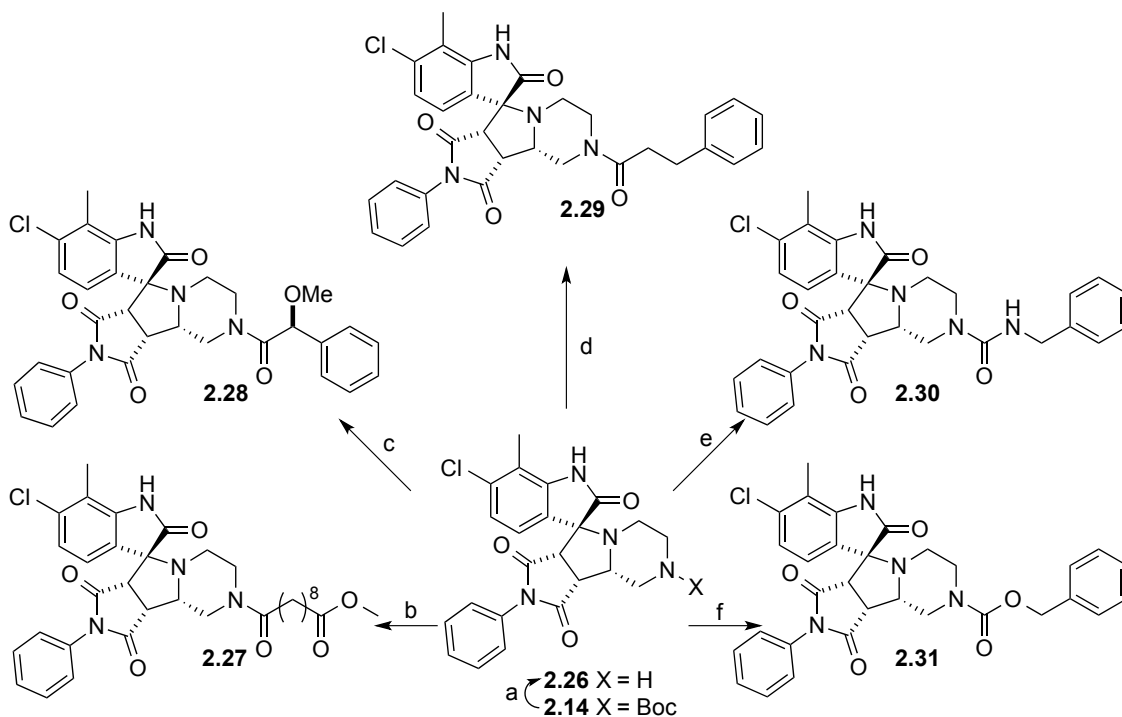
Reagents and conditions: (a) 1:1 TFA/CH$_2$Cl$_2$ 23 °C, 15 min, 90%, (b) 1.04 equiv methyl 10-chloro-10-oxodecanoate, 1.0 equiv Na$_2$CO$_3$, CH$_2$Cl$_2$, 23 °C, 30 min, 53%, (c) 1.3 equiv (*S*)-2-methoxy-2-phenylacetic acid, 2.1 equiv EDC, 3.5 equiv Et$_3$N, CH$_2$Cl$_2$, 23 °C, 20 min, 45%, (d) 1.05 equiv 3-phenylpropanoyl chloride, 1.1 equiv Et$_3$N, CH$_2$Cl$_2$, 23 °C, 3 h, 64%, (e) 1.1 equiv (isocyanatomethyl)benzene, CH$_2$Cl$_2$, 23 °C, 4 h, 75%, (f) 1.1 equiv benzyl chloroformate, 2.1 equiv DIPEA, CH$_2$Cl$_2$, 0-23 °C, 2.5 h, 54%.

The Boc group was removed from the spirocyclic piperazine **2.14** using trifluoroacetic acid to give a 90% yield of the piperazine **2.26** (Scheme 2-7). Piperazine **2.26** served as the precursor for various *N*-acyl derivatives **2.27**-**2.31** in the following reactions (Scheme 2-7). Compound **2.27** was synthesized by acylating **2.26** with methyl 10-chloro-10-oxodecanoate in the presence of sodium carbonate. Carbodiimide mediated coupling of (*S*)-2-methoxy-2-phenylacetic acid with the racemic piperazine **2.26** in the presence of triethylamine resulted in a mixture of diastereomers; only one diastereomer **2.28** was readily purified but the relative

stereochemistry was not assigned. Acylation of **2.26** with hydrocinnamoyl chloride in the presence of triethylamine afforded amide **2.29**. The reaction of piperazine **2.26** with benzyl isocyanate generated the urea **2.30**. Carbamate **2.31** was synthesized by acylation of piperazine **2.26** with benzyl chloroformate.

Structure-Activity Relationships

CID 6584729 was reported to enhance the effect of fluconazole against the partially resistant clinical isolate of *C. albicans* CaCi-8 at EC_{50} 0.12 μM.[87] We determined the antifungal potency of the new spiroindolinones in combination with fluconazole against a susceptible strain (HLY4123) derived from a commonly used laboratory strain of *C. albicans*. The activity of compound **2.1** was promising, with an EC_{50} of 0.011 μM. We then compared the activity of compound **2.1**, derived from phenylalanine with the activity of spiroindolinones derived from other amino acids (Table 2-1, compounds **2.1**, **2.8**, **2.9**, and **2.10**). Neither tryptophan nor N_{ε}-Boc-lysine derivatives were better than the parent compound **2.1** derived from phenylalanine. Regardless of the maleimide substituent, *N*-benzylisatin derivatives exhibited relatively low activity (compounds **2.11** and **2.12**). Compound **2.13** derived from 6-chloro-7-methylisatin but lacking the 4-fluoro substituent was exceedingly potent with an EC_{50} of 1 nM.

When we employed the non-natural amino acid N_{ε}-Boc-piperazine-2-carboxylic acid, the resulting spirocyclic piperazine **2.14** was still highly active with an EC_{50} of 5.6 nM. *N*-Benzylsuccinimide derivatives **2.18**, **2.19**, and **2.21** were not highly active. The substituents on the indolone ring were still important, even with the pentacyclic piperazine core (compounds **2.17**, **2.20**, **2.22**, **2.23**). We removed the Boc group from the piperazine ring of compound **2.14**, leading to a loss of potency (compound **2.26**).

Table 2-1: Structure-Activity Relationships for Polycyclic Pyrrolidines Against the Susceptible Strain HLY4123 of *C. albicans* in the Presence of Fluconazole (0.25 μg/mL)

Compound	$R^{5'}$	$R^{2'}$	$R^{3'}$	R^5	R^6	R^7	R^1	EC_{50} (μM)[a]
2.1	4-fluorophenyl	H	Ph	H	Cl	CH_3	H	0.011 ± 0.004
2.8	Bn	H	Ph	H	H	H	H	~ 10 ± 1.3
2.9	Bn	H	3-indolyl	H	H	H	H	~ 10 ± 1.8
2.10	Bn	H	$(CH_2)_3NHBoc$	H	H	H	H	>100
2.11	Bn	H	Ph	H	H	H	Bn	>10
2.12	Ph	H	Ph	H	H	H	Bn	>100
2.13	Ph	H	Ph	H	Cl	CH_3	H	0.001 ± 0.0005
2.14	Ph	$CH_2CH_2N(Boc)$		H	Cl	CH_3	H	0.0056 ± 0.003
2.15	4-fluorophenyl	$CH_2CH_2N(Boc)$		H	Cl	CH_3	H	0.037 ± 0.001
2.16	3,5-bis(F_3C)phenyl	$CH_2CH_2N(Boc)$		H	Cl	CH_3	H	0.0237 ± 0.01
2.17	Ph	$CH_2CH_2N(Boc)$		H	Cl	CH_3	Bn	>100
2.18	Bn	$CH_2CH_2N(Boc)$		H	H	H	Bn	0.0318 ± 0.4
2.19	Bn	$CH_2CH_2N(Boc)$		H	H	H	H	>100
2.20	Ph	$CH_2CH_2N(Boc)$		H	H	H	Bn	>100
2.21	Bn	$CH_2CH_2N(Boc)$		MeO	H	H	H	230 ± 5.7
2.22	Ph	$CH_2CH_2N(Boc)$		H	H	CH_3	H	0.213 ± 0.08
2.23	Ph	$CH_2CH_2N(Boc)$		H	CH_3	CH_3	H	0.0057 ± 0.006
2.26	Ph	CH_2CH_2NH		H	Cl	CH_3	H	~10 ± 1.5
2.27	Ph	$CH_2CH_2N[CO(CH_2)_8CO_2Me]$		H	Cl	CH_3	H	0.0379 ± 0.009
2.28	Ph	$CH_2CH_2N[COCH(OCH_3)C_6H_5]$		H	Cl	CH_3	H	0.035 ± 0.007
2.29	Ph	$CH_2CH_2N[CO(CH_2)_2Ph]$		H	Cl	CH_3	H	0.0181 ± 0.004
2.30	Ph	$CH_2CH_2N[CONHCH_2Ph]$		H	Cl	CH_3	H	0.256 ± 0.1
2.31	Ph	$CH_2CH_2N(Cbz)$		H	Cl	CH_3	H	0.0003 ± 0.00001

[a] Each value is the arithmetic mean ± SD of three independent experiments.

Surprisingly, the carboxyl oxygen of the carbamate moiety appears to be essential for high potency; because carbamates **2.14** and **2.31** were more potent than amides **2.27** and **2.28**. Even more revealing, the isosteric amide **2.29** and urea **2.30** were two and three orders of magnitude less active, respectively, than benzyloxycarbamate **2.31**. Ultimately, benzyloxycarbamate **2.31** proved to be exquisitely active in improving the efficacy of fluconazole with an EC_{50} of 300 pM. The two hydroxyproline adducts **2.25a** and **2.25b**, exhibited almost no activity under the conditions of the assay; those results are not surprising

given that the relative stereochemistry of those compounds was different from all the other compounds that were tested.

In general, substitution of small, hydrophobic groups on 6 and 7 positions of the indolone ring and benzyl substitution on the 3' position of the central pyrrolidine improves the antifungal activity. Also, a phenyl moiety not benzyl in the succinamide ring enhances the antifungal activity. When the piperazine is present in the polycyclic pyrrolidine, carbamoyl moiety but not the acyl groups on the piperazine ring significantly improves the antifungal activity. On the other hand, fluorinated substituents on the *para* and *meta* positions of the phenyl group in the succinamide ring as well as *N*-substitution of the indolone moiety diminish the activity of the spiroindolinones against *C. albicans*.

Activity Against Resistant Cell Lines

A variety of resistant clinical isolates of *C. albicans* were screened with 64 μg/ml fluconazole and compound **2.31** at a single dose (3 μM) in a broth microdilution assay (Table 2-2).[88] The strains grow at dramatically different rates. The published fluconazole minimum inhibitory concentrations (MICs) for these isolates convey the level of resistance.

Strains for which growth in the presence of compound **2.31** and fluconazole was less than 25% of growth in the presence of fluconazole alone (Isolates 17, 23, 26, 33, 36, and 45) were selected for determination of EC_{50}s. Compound **2.31** was particularly active against clinical isolates 17, 26 and 36 and exhibited good activity against the highly resistant isolate 45.

A checkerboard assay was used to determine the fractional inhibitory concentrations for compound **2.31** and fluconazole against the fluconazole-susceptible strain (HLY4123) and two fluconazole-resistant clinical isolates 26 and 45 (Table 2-3).[88]

Table 2-2: Effect of Compound **2.31** on the Growth of Resistant Clinical Strains in the Presence of Fluconazole

Isolate [a]	MIC$_{Flc}$[a]	Growth (OD$_{600}$) [b]		Potency
		+Fluc [c]	+Cpd **2.31** [d] +Fluc[c]	+Cpd **2.31** EC$_{50}$ (nM)[c,e]
17	32	3.96	0.76	5 ± 0.011
23	32	6.77	0.60	53 ± 0.005
26	32	6.44	0.83	2 ± 0.002
33	64	7.38	0.88	11 ± 0.008
36	64	5.08	1.07	5 ± 0.251
45	128	9.17	1.03	16 ± 0.002

[a] From reference 88. [b] Growth measured after 16 h in SC at 30°C. [c] [fluconazole]=64 µg/mL. [d] [compound **2.31**]=3 µM. [e] Each value is the arithmetic mean ± SD of three independent experiments.

Table 2-3: FIC Indices for Compound **2.31** and Fluconazole in Different Strains of *C. albicans* (MIC90 measured in µM)[a]

Strain	MIC$_{cpd}$	MIC$_{cpd}$(+Flc)	MIC$_{Flc}$	MIC$_{Flc}$(+cpd)	FICI
HLY4123	>300	0.03	0.5	0.125	0.25
26	>30	0.3	836	105	0.14
45	>30	0.03	836	105	0.13

[a] MIC data are derived from single replicates of two independent experiments; values were consistent between experiments.

In all of the strains tested, the FIC index was below 0.5, fitting the classical definition of synergy.[89] Compound **2.31** alone did not have measurable toxicity against any of the strains at the solubility limit (between 30 and 300 µM). In the strains tested, fluconazole dramatically enhances the activity of compound **2.31** against *C. albicans*. Conversely compound **2.31** makes fluconazole more potent against those same strains but the effect is less dramatic. We have named compound **2.31** as synazo-1 (Figure 2-9).

Greater than 90% inhibition of *C. albicans* sterol α-demethylase (a.k.a. Erg11 or CYP51) would be expected at ten times the K_i for fluconazole, which has been determined to be 0.03 µM.[90,91] When synazo-1 is present at 300 nM in the susceptible strain, the MIC_{90} for fluconazole is reduced from 0.5 µM to 0.125 µM, consistent with the theoretical limit for fluconazole potency of around 0.3 µM.

Figure 2-9: The Structure of Compound **2.31**, Renamed Synazo-1
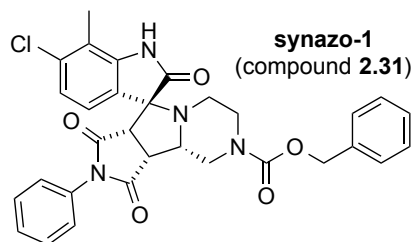
Cytotoxicity of Synazo-1 Against Mammalian Cells

We compared the cytotoxicity of spiroindolinone **2.1**, synazo-1 and compound **2.13** against NIH 3T3 cells at higher concentrations up to 15 mM (Figure 2-10).

Figure 2-10: Cytotoxicity of Compounds **2.1**, **2.13** and Synazo-1 Against NIH 3T3 Cells
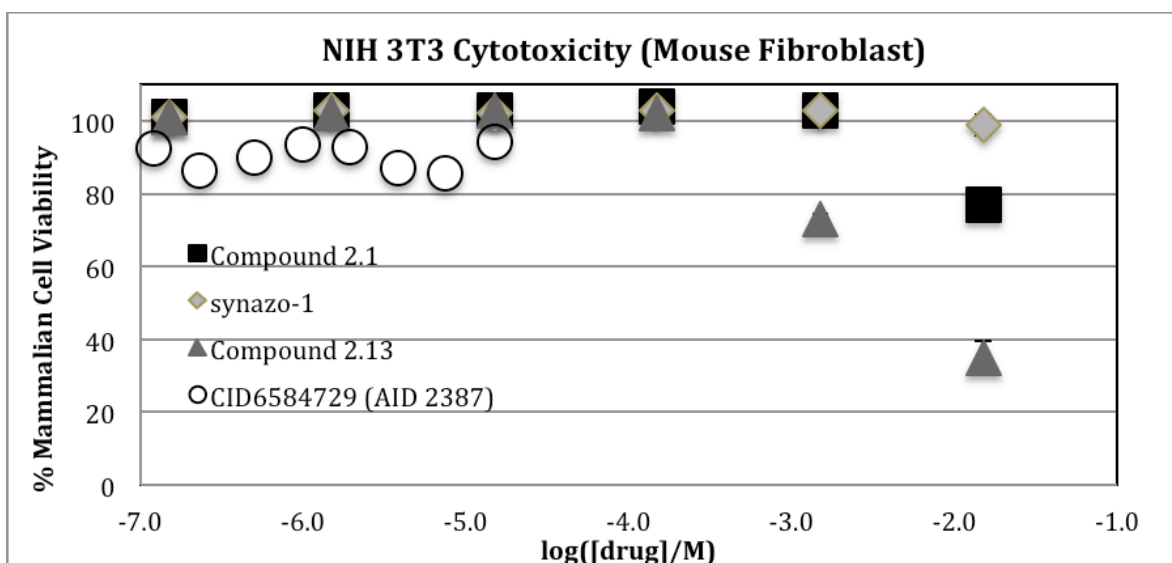
Data represent arithmetic means ± SD of three independent experiments.

All compounds exhibited only weak cytotoxicity. Compound **2.13** was slightly more cytotoxic, but not at the concentrations required for antifungal synergy. According to PubChem, CID 6584729 was previously tested for cytotoxicity against NIH 3T3 cells (PubChem AID 2387) but the EC50 was ≥160 μM, the limit of the assay. It is unclear how the biological activities reported for CID 6584729 should relate to that of the diastereomer **2.1** or whether they were even distinct compounds.

Drug-Like Parameters for Synazo-1

A wide range of readily calculated properties is often used as indicators of oral bioavailability. The calculated physicochemical properties of synazo-1 were compared with typical ranges for lead-like molecules.[92,93,94] Synazo-1 flags just one of the common warnings for drug lead-like properties (Table 2-4) — molecular weight. For comparison, the orally available azole posoconazole is outside the range on four of the parameters. It is widely recognized that the average molecular weight and complexity of newly approved oral drugs has been increasing with each year.[95,96]

Table 2-4: Calculated Physicochemical Properties of Synazo-1[a]

Property	Range	Synazo-1
milogP	-4.0 to 4.2	4.173
TPSA	≤120 Å^2	99.3 Å^2
molecular weight	≤460	571
N+O	≥1	9
H-bond donors	≤5	1
rotatable bonds	≤10	4
halogens	≤7	1
fraction sp^3	0.15-0.80	0.20
H-bond acceptors	≤9	5

[a] Calculated with Molinspiration property calculation service.
http://www.molinspiration.com/cgi-bin/properties.

Both the carbamate and imide moieties are potential liabilities for metabolism, yet when the stability of synazo-1 was tested in 10% FBS/phosphate buffered saline at 37 °C no decomposition was observed over 16 h.

Biological Studies

Inspired by the SAR studies, biological probes containing biotin and rhodamine were synthesized to use in pull-down and small molecule localization assays. Unfortunately, both molecules had no dose-dependent activity within the concentrations tested (30 μM, solubility limit) (Scheme 2-8). This is probably due to the decreasing cell permeation ability of molecules with the increasing values of molecular weight, rotatable bonds and fraction sp^3. In order to avoid this issue Shelley Lane in Liu lab carried out the pull-down assay with cell lysates instead of whole cell. However, after MS-MS studies, they were unable to pull-down interesting proteins that might be responsible for the synergistic activity of the synazo-1 and related analogues.

Scheme 2-8: Biotin and Rhodamine Attached Compound **2.18**

2.18-LC-LC-LC-biotin
EC$_{50}$ > 30 μM

2.18-LC-rhodamine
EC$_{50}$ > 30 μM
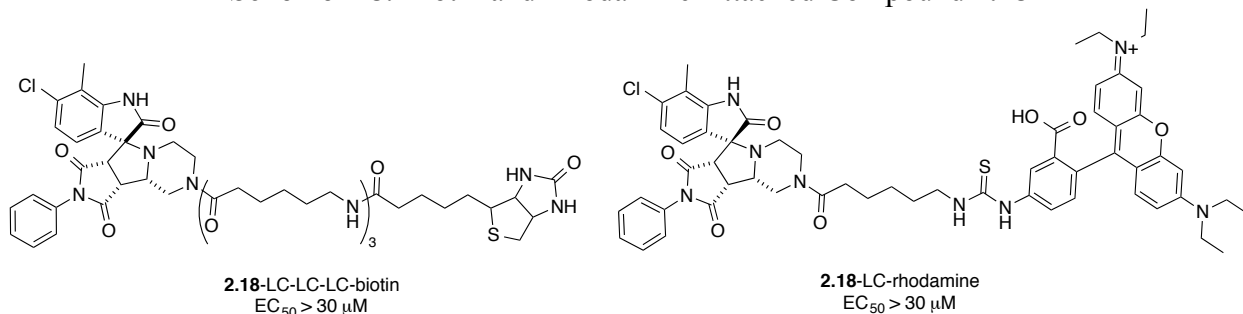
Conclusions

In conclusion, we have designed, synthesized and studied spiroindolinones inspired by CID 6584729, a screening hit that was previously reported to exhibit activity against *C. albicans* in combination with fluconazole. The relative stereochemistry of compound **2.1** and analogues was secured through 2D NMR experiments. The three-component, one-pot [3+2] dipolar cycloaddition of isatins, amino acids, and maleimides was found to proceed through *endo*

addition of maleimides to a *syn-anti* azomethine ylide in all cases except for a proline derivative. A number of the new spiroindolinones were substantially more potent against *C. albicans* than the original lead compound **2.1** when used in combination with fluconazole. In particular, synazo-1 was exquisitely potent with an EC_{50} of 300 pM against a susceptible strain. Synazo-1 also exhibited low nanomolar activity against a number of resistant isolates of *Candida*. When tested in both susceptible and resistant strains of *C. albicans*, synazo-1 was a true synergizer with an FIC index below 0.5. Synazo-1 has many of the calculated parameters associated with orally available drug molecules and represents a promising candidate for development as an antifungal synergizer.

Experimental Section

Chemistry

Analytical High-Performance Liquid Chromatography (HPLC)

The HPLC instrument consisted of an Agilent Technologies series 1200 autosampler, series 1200 UV/Vis detector, series 1100 pump, using ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The analytical column was a reverse-phase Waters Nova-pak® C18 150 mm × 3.9 mm column. A gradient elution was used (flow rate 0.2 mL/min), starting with 80% water and progressing to 100% acetonitrile over a period of 1 h, with both solvents containing 0.1% formic acid. All compounds have purity ≥95% (254 nm) by HPLC.

General Experimental Procedures

NMR spectral data were recorded at room temperature using a Bruker 500 or 600 MHz spectrometer unless stated otherwise. The NMR data are reported as follows: chemical shifts in ppm from an internal tetramethylsilane standard on the δ scale, multiplicity (br = broad, app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constants

(Hz), and integration. Analytical thin layer chromatography (TLC) was performed using EMD Reagents 0.25 mm silica gel 60-F plates. "Flash" chromatography on silica gel was performed using Silicycle silica gel (40-63 μm). All reactions were carried out under an atmosphere of nitrogen in glassware that was evacuated and back-filled with nitrogen three times. Reactions were carried out at room temperature unless otherwise indicated. Unless otherwise noted, all reagents were commercially obtained and, where appropriate, purified prior to use. THF, Et$_2$O, DMF and CH$_2$Cl$_2$ were dried by filtration through alumina according to the procedure of Grubbs and co-workers.[97] For final compounds the purity was determined by HPLC (Agilent Technologies series 1200).

General procedure for the synthesis of pyrrolidines through a three-component dipolar cycloaddition (2.1, 2.8-2.23, 2.24a and 2.24b):[73]
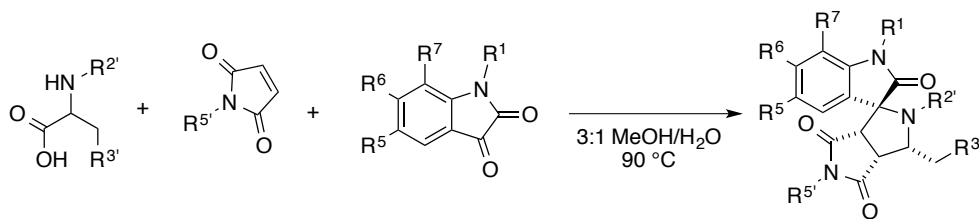
A 100 mL round bottom flask was charged with substituted isatin (1.0 equiv), *N*-substituted maleimide (1.1 equiv), the amino acid (1.1 equiv) and a stir bar. A 3:1 (v/v) mixture of water and methanol was added to the reaction flask such that the concentration of isatin was 0.25 M. The reaction was heated at reflux by immersing the reaction flask in a hot oil bath at 90 °C up to the level of the flask's contents. Initially a clear solution was obtained and CO$_2$ evolution was observed. However, after a few hours the reaction mixture became cloudy. The reaction was monitored for consumption of the substituted isatin by TLC (EtOAc/hex).

Upon consumption of the substituted isatin, the reaction was cooled to room temperature. Next, the reaction mixture was quenched by pouring it into a mixture of ice and sat. aq.

NaHCO₃. The resulting solid was filtered and washed thoroughly with water in the Büchner funnel to afford a grey solid. The solid was then dissolved in minimum amount of CH_2Cl_2 and purified by flash chromatography with EtOAc/hex (1:1) to afford the racemic substituted pyrrolidine.

(±)-(3R,3'R,3a'R,6a'S)-3'-benzyl-6-chloro-5'-(4-fluorophenyl)-7-methyl-2',3',3a',6a'-tetrahydro-4'H-spiro[indoline-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(5'H)-trione, 2.1.
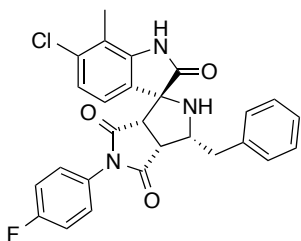
A 100 mL round bottom flask was charged with 1-(4-fluorophenyl)-1H-pyrrole-2,5-dione (0.50 g, 2.55 mmol, 1.0 equiv), p-fluoro-N-phenylmaleimide (0.53 g, 2.8 mmol, 1.1 equiv), L- phenylalanine (0.46 g, 2.8 mmol, 1.1 equiv) and a stir bar. A 3:1 mixture of water and methanol (11 mL) was added to the reaction flask. The content of the reaction flask was heated at reflux by immersing the reaction flask in a hot oil bath up to the level of the flask's contents. Initially a clear solution was obtained and CO_2 was expelled. After few hours a cloudy solution was observed.

Upon consumption of the substituted isatin (16 h), the reaction was allowed to cool to room temperature. Next, the reaction mixture was quenched by pouring it into a mixture of ice and sat. aq. NaHCO₃. The resulting solid was washed thoroughly with water in Büchner funnel to afford a grey solid. The solid was then dissolved in minimum amount of CH_2Cl_2 and purified by flash chromatography with different combinations of EtOAc/hex to afford the pyrrolidine **2.1** as a white solid (0.93 mg, 1.9 mmol, 74%). R$_f$ = 0.35 (1:1 EtOAc/hex); mp 212-215. The ¹H NMR chemical shifts were concentration-dependent in CDCl₃, particularly within the range 0.5-2 mM. ¹H NMR (600 MHz, CDCl₃) δ 8.00 (s, 1H), 7.41-7.38 (m, 2H), 7.27-7.16 (m, 6H), 7.02 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 8.5 Hz, 1H), 4.72-4.71 (m, 1H), 3.75-3.69 (m, 1H), 3.45 (dd, J = 14.0, 4.0 Hz, 1H), 2.73 (dd, J = 13.8, 10.5 Hz, 1H), 2.16 (s, 1H), 1.99 (s, 3H); ¹³C NMR (500

MHz, CDCl$_3$) δ 180.4, 175.1, 174.4, 163.4, 161.4, 140.5, 139.1, 136.2, 128.9, 128.8, 128.3, 127.6, 126.7, 124.6, 124.5, 123.5, 118.2, 116.6, 116.4, 68.2, 58.9, 51.6, 47.6, 37.9, 13.5; IR (thin film) 3201, 3065, 1710, 1696, 1623, 1601, 1510; HRMS (ESI): *m/z* calculated for C$_{28}$H$_{29}$ClN$_4$O$_5$Na [M+Na]$^+$ 559.1724, found 559.1743. HPLC purity: 95.76%.

(±)-(3*R*,3'*R*,3a'*R*,6a'*S*)-3',5'-Dibenzyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-*c*]pyrrole]-2,4',6'(5'*H*)-trione, 2.8.

Using the general procedure for the synthesis of pyrrolidines outlined above, indoline-2,3-dione (0.37 g, 2.5 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.8** as a white solid (0.65 mg, 1.5 mmol, 60%). R$_f$ = 0.50 (1:1 EtOAc/hex); mp 138-142; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.50-7.49 (m, 2H), 7.40-7.38 (m, 3H), 7.29 (br s, 1H), 7.26-7.25 (m, 1H), 7.24-7.22 (m, 3H), 7.18- 7.16 (m, 2H), 6.80 (t, *J* = 7.5 Hz, 1H), 6.69 (d, *J* = 7.8 Hz, 1H), 6.50 (d, *J* = 7.5 Hz, 1H), 4.83 (d, *J* = 14.0 Hz, 1H), 4.69 (d, *J* = 14.0 Hz, 1H), 4.68-4.67 (m, 1H), 3.58-3.56 (m, 1H), 3.42 (d, *J* = 7.6 Hz, 2H), 2.60 (dd, *J* = 13.8, 10.4 Hz, 1H), 2.01 (br s, 1H); ^{13}C NMR (500 MHz, CDCl$_3$, 313 K) δ 180.1, 175.8, 174.5, 140.3, 139.3, 135.8, 129.8, 129.0, 128.9, 128.8, 128.7, 128.1, 126.9, 126.5, 126.4, 122.6, 109.7, 67.6, 58.5, 51.5, 47.7, 42.7, 38.2; IR (thin film) 3850, 3646, 2971, 2843, 1697, 1619, 1054, 1032; HRMS (ESI): *m/z* calculated for C$_{27}$H$_{23}$N$_3$O$_3$Na [M+Na]$^+$ 460.1637, found 460.1620. HPLC purity: 100%.

(±)-(3*R*,3'*R*,3a'*R*,6a'*S*)-3'-((1*H*-Indol-3-yl)methyl)-5'-benzyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-*c*]pyrrole]-2,4',6'(5'*H*)-trione, 2.9.

Using the general procedure for the synthesis of pyrrolidines outlined above, indoline-2,3-dione (0.37 g, 2.5 mmol, 1.0 equiv) was used and the product was purified by flash
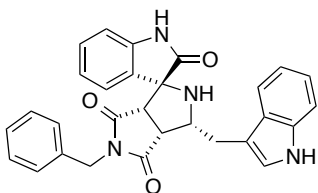
chromatography with EtOAc/hex to afford the pyrrolidine **2.9** as a yellow solid (0.83 mg, 1.7 mmol, 70%). R_f = 0.35 (1:1 EtOAc/hex); mp 130-142; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.96 (br s, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.52 (d, J = 7.3 Hz, 2H), 7.42-7.39 (m, 2H), 7.36-7.33 (m, 1H), 7.30- 7.29 (m, 1H), 7.17- 7.11 (m, 2H), 7.09- 7.06 (m, 2H), 6.80- 6.77 (m, 1H), 6.62 (d, J = 7.7 Hz, 1H), 6.49 (d, J = 7.5 Hz, 1H), 4.86 (d, J = 14.0 Hz, 1H), 4.80-4.76 (m, 1H), 4.70 (d, J = 14.0 Hz, 1H), 3.60 (t, J = 7.7 Hz, 1H), 3.49 (dd, J = 14.6, 3.7 Hz, 1H), 3.41 (d, J = 7.7 Hz, 1H), 2.82 (dd, J = 14.6, 10.3 Hz, 1H), 2.13 (br s, 1H); ^{13}C NMR (500 MHz, CDCl$_3$, 313 K) δ 180.3, 176.0, 174.8, 140.4, 136.2, 135.9, 129.6, 129.0, 128.8, 128.2, 127.5, 126.8, 126.5, 122.5, 122.4, 122.1, 119.5, 119.1, 113.6, 111.1, 109.7, 67.7, 58.1, 51.6, 47.7, 42.6, 27.7; IR (thin film) 3679, 2971, 2864, 2843, 1695, 1619, 1054, 1032; HRMS (ESI): m/z calculated for C$_{29}$H$_{24}$N$_4$O$_3$Na [M+Na]$^+$ 499.1746, found 499.1739. HPLC purity: 98.81%.

(±)-*tert*-Butyl (4-((3*R*,3'*R*,3a'*R*,6a'*S*)-5'-benzyl-2,4',6'-trioxo-3',3a',4',5',6',6a'-hexahydro-2'*H*-spiro[indoline-3,1'-pyrrolo[3,4-c]pyrrol]-3'-yl)butyl)carbamate, 2.10.

Using the general procedure for the synthesis of pyrrolidines outlined above, indoline-2,3-dione (0.37 g, 2.5 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.10** as a white solid (0.42 mg, 0.82 mmol, 33%). R_f = 0.35 (4:6 EtOAc/hex); mp 109-113; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.45-7.44 (m, 2H), 7.38-7.33 (m, 3H), 7.29 (br s, 1H), 7.20-7.17 (m, 1H), 6.79-6.76 (m, 2H), 6.36 (d, J = 7.4 Hz, 1H), 4.78 (d, J = 14.0 Hz, 1H), 4.70-4.63 (m, 1H), 4.61 (d, J = 14.0 Hz, 1H), 4.34-4.32 (m, 1H), 3.50 (t, J = 7.7 Hz, 1H), 3.42 (d, J = 7.7 Hz, 1H), 3.18-3.08 (m, 2H), 2.06-1.91 (m, 2H), 1.57-1.53 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (500 MHz, CDCl$_3$, 313 K) δ

180.15, 175.8, 174.5, 140.3, 135.8, 129.8, 129.2, 128.8, 128.2, 126.5, 126.4, 122.7, 109.8, 68.1, 58.3, 51.7, 48.1, 42.7, 40.1, 31.2, 30.0, 28.5, 24.6, 14.3; IR (thin film) 3707, 2971, 2843, 1345, 1054, 1032; HRMS (ESI): *m/z* calculated for $C_{29}H_{34}N_4O_5Na$ [M+Na]$^+$ 541.2427, found 541.2411. HPLC purity: 97.81%.

(±)-(3*R*,3'*R*,3a'*R*,6a'*S*)-1,3',5'-Tribenzyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-*c*]pyrrole]-2,4',6'(5'*H*)-trione, 2.11.

Using the general procedure for the synthesis of pyrrolidines outlined above, 1-benzylindoline-2,3-dione (0.07 g, 0.32 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.11** as a

white solid (0.08 mg, 0.15 mmol, 46%). R$_f$ = 0.60 (1:1 EtOAc/hex); mp 173-175; ^{1}H NMR (600 MHz, CDCl₃) δ 7.50-7.49 (m, 2H), 7.39-7.12 (m, 14H), 6.82-6.79 (m, 1H), 6.64 (d, *J* = 9.3 Hz, 1H), 6.54 (d, *J* = 8.7 Hz, 1H), 4.88-4.81 (m, 2H), 4.80-4.72 (m, 1H), 4.71-4.65 (m, 2H), 3.64-3.60 (m, 1H), 3.44-3.39 (m, 2H), 2.61 (dd, *J* = 16.6, 12.3 Hz, 1H), 2.01 (br s, 1H); ^{13}C NMR (500 MHz, CDCl₃, 313 K) δ 178.7, 175.9, 174.4, 142.6, 139.3, 135.8, 135.4, 129.8, 129.0, 128.9, 128.9, 128.8, 128.6, 128.1, 127.8, 127.3, 126.5, 126.4, 125.8, 122.6, 109.1, 67.5, 58.5, 51.8, 47.7, 43.6, 42.7, 38.2; IR (thin film) 3850, 3708, 2971, 2864, 1453, 1054, 1032; HRMS (ESI): *m/z* calculated for $C_{34}H_{29}N_3O_3Na$ [M+Na]$^+$ 550.2106, found 550.2108. HPLC purity: 98.45%.

(±)-(3*R*,3'*R*,3a'*R*,6a'*S*)-1,3'-Dibenzyl-5'-phenyl-2',3',3a',6a'-tetrahydro-4'*H*-spiro[indoline-3,1'-pyrrolo[3,4-*c*]pyrrole]-2,4',6'(5'*H*)-trione, 2.12.
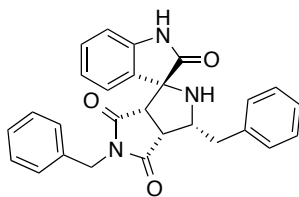
Using the general procedure for the synthesis of pyrrolidines outlined above, 1-benzylindoline-2,3-dione (0.30 g, 1.3 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.12** as a yellow solid (0.38
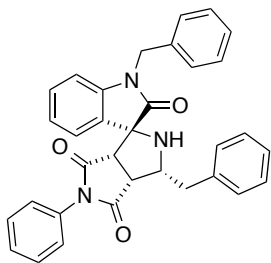
mg, 0.74 mmol, 57%). R_f = 0.60 (1:1 EtOAc/hex); mp 183-188; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.57-7.52 (m, 2H), 7.46-7.44 (m, 1H), 7.40-7.37 (m, 4H), 7.34-7.33 (m, 3H), 7.31-7.25 (m, 3H), 7.21-7.17 (m, 2H), 7.14 (dd, J = 7.4, 1.5 Hz, 1H), 6.97 (t, J = 7.5 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 4.90 (d, J = 15.6 Hz, 1H), 4.76 (d, J = 15.6 Hz, 1H), 4.52-4.50 (m, 1H), 3.73 (dd, J = 7.5, 1.8 Hz, 1H), 3.63 (dd, J = 7.8, 2.7 Hz, 1H), 2.61 (dd, J = 16.6, 12.3 Hz, 1H), 3.32 (br s, 1H), 2.82-2.78 (m, 1H); ^{13}C NMR (500 MHz, CDCl$_3$, 313 K) δ 178.7, 175.9, 174.4, 142.6, 139.3, 135.8, 135.4, 129.8, 129.0, 128.9, 128.9, 128.8, 128.6, 128.1, 127.8, 127.3, 126.5, 126.4, 125.8, 122.6, 109.1, 67.5, 58.5, 51.8, 47.7, 43.6, 42.7, 38.2; IR (thin film) 3680, 2966, 2865, 1706, 1054, 1032; HRMS (ESI): m/z calculated for C$_{34}$H$_{29}$N$_3$O$_3$Na [M+Na]$^+$ 550.2106, found 550.2108. HPLC purity: 95.07%.

(±)-(3R,3'R,3a'R,6a'S)-3'-Benzyl-6-chloro-7-methyl-5'-phenyl-2',3',3a',6a'-tetrahydro-4'H-spiro[indoline-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(5'H)-trione, 2.13.
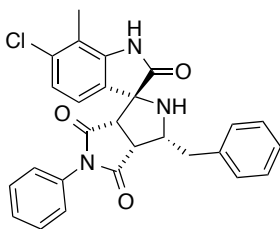
Using the general procedure for the synthesis of pyrrolidines outlined above, 6-chloro-7-methylindoline-2,3-dione (0.30 g, 1.7 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.13** as a white solid (0.21 g, 0.44 mmol, 26%). R_f = 0.60 (1:1 EtOAc/hex); mp 168-171; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 1H), 7.54-7.51 (m, 2H), 7.45-7.43 (m, 1H), 7.39-7.35 (m, 4H), 7.29-7.27 (m, 2H), 7.19-7.17 (m, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 4.55 (br s, 1H), 3.72 (t, J = 7.5 Hz, 1H), 3.53 (d, J = 7.8 Hz, 1H), 3.38-3.35 (m, 2H), 2.27 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 142.8, 140.6, 135.1, 134.5, 132.9, 129.4, 129.3, 128.7, 127.6, 127.3, 126.4, 125.0, 121.9, 117.3; IR (thin film) 3850, 3626, 2971, 2864, 1710, 1693, 1014,

1032; HRMS (ESI): *m/z* calculated for $C_{27}H_{22}ClN_3O_3Na$ $[M+Na]^+$ 494.1247, found 494.1255.

HPLC purity: 95.00%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.14.

Using the general procedure for the synthesis of pyrrolidines outlined above, 6-chloro-7-methylindoline-2,3-dione (0.81 g, 4.1 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.14** as

a light pink solid (1.3 g, 2.5 mmol, 60%). R_f = 0.35 (4:6 EtOAc/hexanes); mp 201-205; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.90 (s, 1H), 7.54-7.51 (m, 2H), 7.47-7.46 (m, 1H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.73 (d, *J* = 7.8 Hz, 1H), 4.31 (br s, 1H), 3.85 (t, *J* = 7.5 Hz, 2H), 3.64-3.65 (m, 1H), 3.55 (d, *J* = 7.8 Hz, 1H), 2.74-2.58 (m, 2H) 2.25 (app s, 4H), 2.13-2.08 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 313 K) δ 177.9, 174.7, 173.4, 153.6, 142.8, 134.6, 132.1, 128.9, 128.5, 126.9, 124.6, 123.2, 122.8, 117.3, 79.1, 71.7, 62.3, 58.7, 57.6, 50.4, 45.9, 44.9, 27.9, 13.7; IR (thin film) 3840, 3708, 3626, 2971, 2843, 1713, 1695, 1032; HRMS (ESI): *m/z* calculated for $C_{28}H_{29}ClN_4O_5Na$ $[M+Na]^+$ 559.1724, found 559.1743. HPLC purity: 96.96%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-6-chloro-2'-(4-fluorophenyl)-7-methyl-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.15.
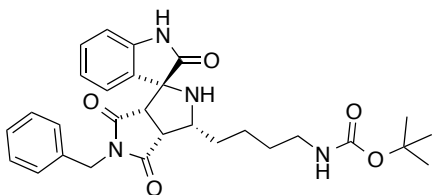
Using the general procedure for the synthesis of pyrrolidines outlined above, 6-chloro-7-methylindoline-2,3-dione (0.12 g, 0.63 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.15** as a white solid (0.17
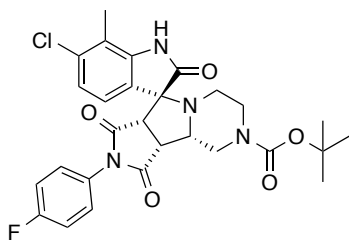
mg, 0.31 mmol, 49%). R_f = 0.35 (4:6 EtOAc/hex); R_f = 0.35 (4:6 EtOAc/hexanes); mp 246-248; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.42 (s, 1H), 7.39-7.35 (m, 2H), 7.32-7.29 (m, 2H), 7.02 (d, J = 7.8 Hz, 2H), 6.75 (d, J = 7.8 Hz, 1H), 4.35 (dd, J = 12.6, 1.8 Hz, 1H), 3.89-3.83 (m, 2H), 3.74-3.71 (m, 1H), 3.56 (d, J = 7.8 Hz, 1H), 2.74-2.58 (m, 2H) 2.77-2.73 (m, 1H), 2.74-2.54 (m, 1H), 2.50 (s, 3H), 2.29-2.09 (M, 1H), 1.44 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 313 K) δ 178.5, 175.3, 173.9, 161.0, 154.2, 143.3, 135.1, 129.7, 128.8, 125.1, 123.7, 122.6, 117.9, 116.6, 116.4, 79.6, 72.2, 58.1, 51.0, 46.6, 45.6, 45.9, 28.5, 14.3; IR (thin film) 3850, 3708, 2965, 2866, 1706, 1689, 1032; HRMS (ESI): m/z calculated for C$_{28}$H$_{28}$ClFN$_4$O$_5$Na [M+Na]$^+$ 577.1630, found 577.1644. HPLC purity: 100%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-2'-(3,5-bis(trifluoromethyl)phenyl)-6-chloro-7-methyl-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.16.
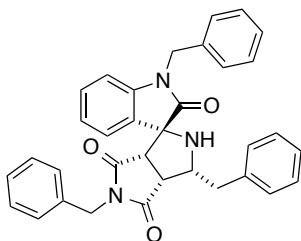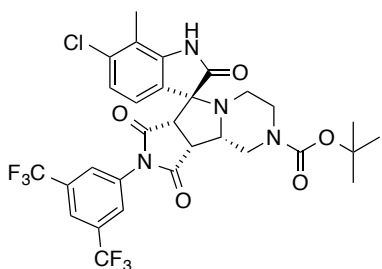

Using the general procedure for the synthesis of pyrrolidines outlined above, 6-chloro-7-methylindoline-2,3-dione (0.50 g, 2.6 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.16** as a white solid (0.13 mg, 0.30 mmol, 12%). R_f = 0.5 (2:3 EtOAc/hex); mp 199-201; ^{1}H NMR (600 MHz, CDCl$_3$, 320 K) δ 8.51 (s, 1H), 7.93-7.92 (m, 3H), 7.12 (d, J = 8.1 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 4.59 (br s, 1H), 4.11 (br s, 1H), 3.80-3.82 (m, 1H), 3.81-3.78 (m, 1H), 3.74-3.72 (m, 1H), 2.82-2.89 (m, 1H), 2.65-2.71 (m, 1H), 2.33-2.34 (m, 2H), 2.17 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 177.6, 174.2, 173.0, 154.4, 141.2, 136.8, 133.0, 132.7, 126.2, 124.5, 123.8, 123.7, 122.5, 118.4, 81.0, 72.9, 62.3, 58.3, 57.6, 50.5, 46.17, 45.6, 28.4, 13.8; IR (thin film) 3187, 1724, 1706, 1680,

1599, 1276, 1132; HRMS (ESI): *m/z* calculated for C$_{30}$H$_{27}$ClF$_6$N$_4$O$_5$Na [M+Na]$^+$ 695.1472, found 695.1450. HPLC purity: 98.46%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-1,2'-dibenzyl-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.18.
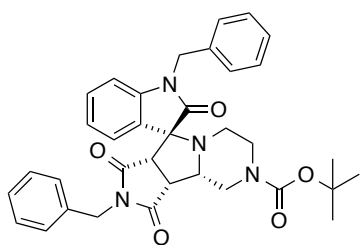
Using the general procedure for the synthesis of pyrrolidines outlined above, 1-benzylindoline-2,3-dione (0.30 g, 1.3 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.18**

as a white solid (0.39 mg, 0.66 mmol, 56%). R$_f$ = 0.8 (1:1 EtOAc/hex); mp 191-192; ^{1}H NMR (600 MHz, DMSO-*d$_6$*) δ 7.38-7.37 (m, 4H), 7.36-7.32 (m, 5H), 7.31-7.27 (m, 1H), 7.22-7.20 (m, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 6.82-6.79 (m, 1H), 6.50 (d, *J* = 7.2 Hz, 1H), 4.87 (s, 2H), 4.65 (AB q, *J* = 14.7 Hz, 2H), 4.36-4.34 (m, 1H), 3.85-3.83 (m, 1H), 3.79-3.76 (m, 1H), 3.72-3.71 (m, 1H), 3.49 (d, *J* = 7.8 Hz, 1H), 2.68-2.64 (m, 1H), 2.51-2.53 (m, 1H), 2.12-2.09 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (500 MHz, DMSO-*d$_6$*, 313 K) δ 176.1, 175.8, 174.5, 155.2, 143.7, 136.6, 136.4, 130.1, 129.2, 129.0, 128.2, 128.1, 127.9, 127.7, 126.7, 124.2, 122.7, 109.7, 79.7, 71.7, 58.2, 50.8, 46.3, 45.5, 43.1, 42.2, 28.5; IR (thin film) 3850, 3626, 2971, 2862, 1707, 1693, 1678, 1032; HRMS (ESI): *m/z* calculated for C$_{35}$H$_{36}$N$_4$O$_5$Na [M+Na]$^+$ 615.2584, found 615.2572. HPLC purity: 100%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-2'-benzyl-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.19.

Using the general procedure for the synthesis of pyrrolidines outlined above, indoline-2,3-dione (0.37 g, 2.5 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.19** as a white solid (0.66 mg, 1.3
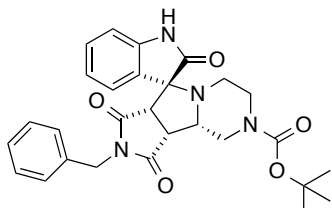
mmol, 53%). R_f = 0.45 (1:1 EtOAc/hex); mp 207-208; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.46-7.43 (m, 3H), 7.36-7.35 (m, 3H), 7.23-7.20 (m, 1H), 6.83-6.81 (m, 1H), 6.77 (d, J = 7.7 Hz, 1H), 6.32 (d, J = 6.8 Hz, 1H), 4.80 (d, J = 14.1 Hz, 1H), 4.63 (d, J = 14.1 Hz, 1H), 4.22-4.12 (m, 1H), 3.80-3.78 (m, 1H), 3.60-3.58 (m, 1H), 3.41 (d, J = 7.9 Hz, 1H), 2.79-2.45 (m, 2H), 2.27-2.18 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 310 K) δ 180.4, 175.8, 174.7, 156.1, 140.5, 135.8, 129.7, 129.2, 128.9, 128.8, 128.2, 126.5, 122.6, 109.9, 68.1, 58.3, 51.6, 48.1, 42.6, 40.1, 31.1, 30.0, 28.5, 24.6; IR (thin film) 3679, 2971, 2864, 1706, 1642, 1054, 1032, 1012; HRMS (ESI): m/z calculated for C$_{28}$H$_{30}$N$_4$O$_5$Na [M+Na]$^+$ 525.2114, found 525.2106. HPLC purity: 100%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-2'-benzyl-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.20.
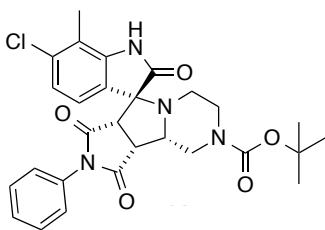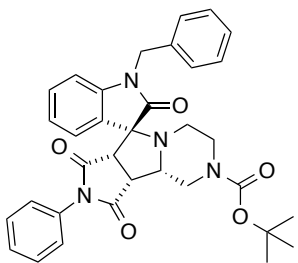
Using the general procedure for the synthesis of pyrrolidines outlined above, 1-benzylindoline-2,3-dione (0.30 g, 1.3 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.20** as a yellow solid (0.43 mg, 0.75 mmol, 71%). R_f = 0.75 (1:1 EtOAc/hex); mp 210-212; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 7.54-7.51 (m, 2H), 7.46-7.44 (m, 1H), 7.35-7.34 (m, 6H), 7.29-7.26 (m, 2H), 7.03-7.02 (m, 2H), 6.92 (d, J = 8.4 Hz, 1H), 4.91 (s, 2H), 4.40-4.38 (m, 1H), 3.92-3.89 (m, 2H), 3.81-3.77 (m, 1H), 3.61 (d, J = 7.8 Hz, 1H), 2.84-2.77 (m, 1H), 2.68-2.63 (m, 1H), 2.24-2.16 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 310 K) δ 176.7, 175.7, 174.3, 155.2, 144.2, 137.1, 133.1, 130.7, 130.0, 129.7, 129.5, 128.4, 128.1, 127.9, 126.9, 124.8, 123.4, 110.2, 80.1, 72.4, 58.8, 51.7, 47.0, 46.8, 43.6, 28.9; IR (thin film) 3850,

3671, 2972, 2843, 1712, 1690, 1135, 1032; HRMS (ESI): *m/z* calculated for C$_{34}$H$_{34}$N$_4$O$_5$Na

[M+Na]$^+$ 601.2427, found 601.2415. HPLC purity: 100%.

**(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-2'-benzyl-5-methoxy-1',2,3'-trioxo-2',3',3a',3b',4',6',7',9a'
-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylat
-e, 2.21.**
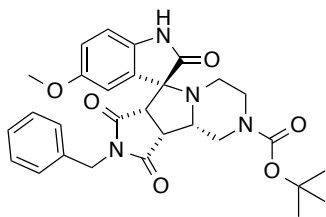
Using the general procedure for the synthesis of pyrrolidines

outlined above, indoline-2,3-dione (0.45 g, 2.5 mmol, 1.0 equiv)

was used and the product was purified by flash chromatography

with EtOAc/hex to afford the pyrrolidine **2.21** as a white solid

(0.66 mg, 1.3 mmol, 53%). R$_f$ = 0.35 (1:1 EtOAc/hex); mp 218-220; ^{1}H NMR (600 MHz,

DMSO-*d$_6$*) δ 10.43 (s, 1H), 7.38-7.36 (m, 2H), 7.33-7.32 (m, 2H), 7.29-7.27 (m, 1H), 6.77 (dd, *J*

= 8.7, 2.1 Hz, 1H), 6.73-6.71 (m, 1H), 6.07 (s, 1H), 4.64 (AB q, *J* = 15 Hz, 2H), 4.31 (br s, 1H),

3.87-3.83 (m, 1H), 3.75-3.73 (m, 1H), 3.68-3.64 (m, 1H), 3.45 (d, *J* = 7.8 Hz, 1H), 2.61-2.59 (m,

2H), 2.18-2.16 (m, 1H), 2.04 (td, *J* = 11.2, 3.0 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (500 MHz,

DMSO-*d$_6$*, 310 K) δ 177.7, 176.1, 174.7, 154.9, 153.8, 136.4, 129.1, 127.9, 127.5, 126.0, 115.4,

113.4, 110.5, 79.7, 72.3, 61.2, 57.9, 55.5, 50.6, 46.3, 45.2, 42.0, 28.5; IR (thin film) 3850, 3648,

2967, 1710, 1641, 1130, 1032; HRMS (ESI): *m/z* calculated for C$_{28}$H$_{30}$N$_4$O$_5$Na [M+Na]$^+$

555.2222, found 555.2239. HPLC purity: 96.22%.

**(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-
octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate,
2.22.**

Using the general procedure for the synthesis of pyrrolidines outlined above, 7-

methylindoline-2,3-dione (1.0 g, 6.2 mmol, 1.0 equiv) was used and the product was purified by

flash chromatography with EtOAc/hex to afford the pyrrolidine **2.22** as a white solid (2.0 g, 4.0

mmol, 60%). R$_f$ = 0.3 (1:1 EtOAc/hex); mp 212-214; ^{1}H NMR (600 MHz, DMSO-*d$_6$*, 398 K) δ

46
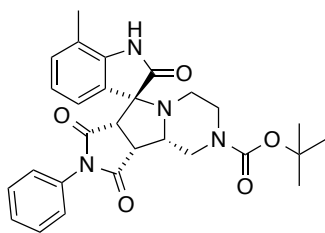
10.1 (s, 1H), 7.53-7.51 (m, 2H), 7.45-7.42 (m, 1H), 7.34-7.33 (m, 2H), 7.05 (d, J = 7.8 Hz, 1H), 6.88-6.86 (m, 1H), 6.76 (d, J = 7.2 Hz, 1H), 4.37-4.35 (m, 1H), 3.87-3.82 (m, 2H), 3.75-3.73 (m, 1H), 3.55-3.54 (m, 1H), 2.79-2.75 (m, 1H), 2.65-2.61 (m, 1H), 2.31-2.22 (m, 4H), 2.21-2.19 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 178.6, 175.5, 174.1, 154.5, 141.8, 132.7, 131.5, 129.6, 129.0, 127.5, 124.8, 123.9, 122.2, 119.5, 79.6, 72.3, 58.1, 50.9, 49.1, 46.7, 28.5, 16.7, 14.6; IR (thin film) 3229, 2973, 2360, 2340, 1714, 1696, 1391; HRMS (ESI): m/z calculated for $C_{28}H_{30}N_4O_5Na$ [M+Na]$^+$ 525.2114, found 525.2108. HPLC purity: 99.18%.

(±)-*tert*-Butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-6,7-dimethyl-1',2',3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.23.
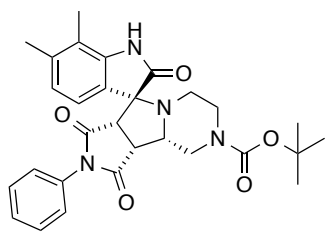
Using the general procedure for the synthesis of pyrrolidines outlined above, 6,7-dimethylindoline-2,3-dione (0.38 g, 2.2 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex to afford the pyrrolidine **2.23** as a white solid (0.60 g, 1.20 mmol, 61%). R$_f$ = 0.3 (1:1 EtOAc/hex); mp 215-218; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.6 (s, 1H), 7.53-7.52 (m, 2H), 7.50-7.44 (m, 1H), 7.29-7.28 (m, 2H), 6.77 (d, J = 7.5 Hz, 1H), 6.60 (d, J = 7.5 Hz, 1H), 4.29 (br s, 1H), 3.82-3.79 (m, 2H), 3.64-3.65 (m, 1H), 3.47-3.46 (m, 1H), 2.62 (br s, 2H), 2.19-2.17 (m, 4H), 2.09-2.07 (m, 4H), 1.39 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 320 K) δ 178.8, 175.5, 174.1, 141.8, 138.7, 132.7, 129.6, 129.0, 127.4, 123.6, 123.5, 122.4, 118.2, 79.6, 72.4, 58.1, 50.9, 46.7, 28.5, 20.1, 13.5; IR (thin film) 3739, 3244, 2976, 1712, 1696, 1417, 1390; HRMS (ESI): m/z calculated for $C_{29}H_{32}N_4O_5Na$ [M+Na]$^+$ 539.2271, found 539.2268. HPLC purity: 98.24%.

Synthesis of (3S,3a'S,7'R,8a'S,8b'R)-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',6',7',8',8a',8b'-octahydro-1'H-spiro[indoline-3,4'-pyrrolo[3,4-a]pyrrolizin]-7'-yl benzoate, 2.25a and (3R,3a'R,7'R,8a'R,8b'S)-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',6',7',8',8a',8b'-octahydro-1'H-spiro[indoline-3,4'-pyrrolo[3,4-a]pyrrolizin]-7'-yl benzoate, 2.25b.
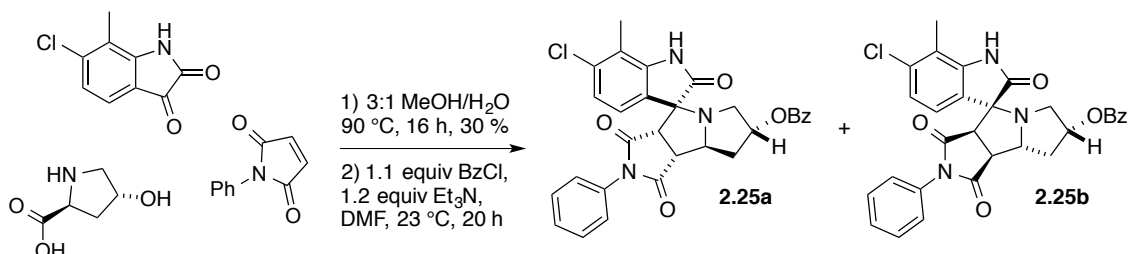

Using the general procedure for the synthesis of pyrrolidines outlined above, 6-chloro-7-methylindoline-2,3-dione (0.50 g, 2.6 mmol, 1.0 equiv) was used. The resulting crude reaction mixture was dissolved in cold DCM and the products precipitated as a yellow solid. Then the solid was collected by vacuum filtration and washed with cold DCM until the solid became white. The pyrrolidine products were collected as a mixture of two diastereomers. The resulting products were highly insoluble in most of the organic solvents and this made it very hard to separate the two diastereomers by flash column chromatography.

The mixture of two stereoisomers (0.030 g, 0.07 mmol, 1 equiv) from the above procedure was added to a 5 mL oven-dried round-bottom flask. The flask was fitted with a septum and purged with nitrogen. Next, 1.4 mL of DMF was added to the reaction flask such that the concentration of the mixture of two diastereomers was 0.05 M. Next, benzoyl chloride (9.0 mL, 0.075 mmol, 1.1 equiv) and triethylamine (0.01 mL, 0.08 mmol, 1.2 equiv) was added to the reaction flask and the reaction was allowed to stir at room temperature until the consumption of the alcohols as confirmed by TLC (9:1 DCM/MeOH).

Upon consumption of the alcohols, the reaction mixture was concentrated under vacuum to give a yellow solid. The solid was then purified by flash chromatography with DCM/Et$_2$O (20:1) to afford esters **2.25a** and **2.25b**.

The first stereoisomer **2.25a** (0.012 g, 0.02 mmol, 65%) was isolated as a white solid. R$_f$ = 0.55 (20:1 DCM/Et$_2$O); mp 236-239; ^{1}H NMR (600 MHz, Acetone-d_6) δ 9.67 (s, 1H), 7.97 (dd, J = 8.4, 1.2 Hz, 2H), 7.61-7.58 (m, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.48-7.37 (m, 5H), 7.28 (d, J = 7.8 Hz, 2H), 7.16 (d, J = 7.8 Hz, 1H), 5.63-5.61 (m, 1H), 4.71-4.69 (m, 1H), 4.35 (d, J = 9.6 Hz, 1H), 3.82 (dd, J = 10.2, 6.6 Hz, 1H), 3.52 (dd, J = 11.1, 5.1 Hz, 1H), 3.01 (d, J = 10.8 Hz, 1H), 2.76-2.72 (m, 1H), 2.56-2.52 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (500 MHz, Acetone-d_6) δ 177.4, 176.6, 174.9, 165.6, 135.6, 133.0, 130.2, 129.3, 128.6, 128.4, 128.1, 127.2, 125.6, 124.4, 122.4, 118.1, 74.7, 65.1, 55.3, 53.4, 53.1, 37.7, 13.3; IR (thin film) 3685, 2978, 2851, 1721, 1054, 1032, 1012; HRMS (ESI): m/z calculated for C$_{30}$H$_{24}$ClN$_3$O$_5$Na [M+Na]$^+$ 564.1302, found 564.1302. HPLC purity: 96.63%.

The second stereoisomer **2.25b** (0.015 g, 0.03 mmol, 81%) was also isolated as a white solid. R$_f$ = 0.48 (20:1 DCM/Et$_2$O); mp 221-224; ^{1}H NMR (600 MHz, Acetone-d_6) δ 9.69 (s, 1H), 8.09 (dd, J = 8.4, 1.2 Hz, 2H), 7.67 (t, J = 7.5 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.48-7.37 (m, 3H), 7.28 (d, J = 7.8 Hz, 2H), 7.13 (d, J = 8.4 Hz, 1H), 5.44-5.42 (m, 1H), 4.55-4.52 (m, 1H), 4.39 (d, J = 10.2 Hz, 1H), 4.00 (dd, J = 9.9, 6.9 Hz, 1H), 3.38 (dd, J = 10.2, 6.0 Hz, 1H), 3.28 (dd, J = 9.9, 6.3 Hz, 1H), 2.83-2.78 (m, 1H), 2.39-2.36 (m, 1H), 2.35 (s, 3H); ^{13}C NMR (500 MHz, Acetone-d_6) δ 177.4, 176.5, 174.8, 165.6, 135.6, 133.3, 133.0, 130.1, 129.5, 128.6, 128.2, 127.3, 125.5, 125.2, 124.6, 122.4, 74.7, 64.9, 54.4, 53.5, 53.3, 36.6, 13.3; IR (thin film) 3686, 2980, 2481, 1720, 1050, 1030, 1014; HRMS (ESI): m/z calculated for C$_{30}$H$_{24}$ClN$_3$O$_5$Na [M+Na]$^+$ 564.1302, found 564.1305. HPLC purity: 95.36%.

Synthesis of (±)-*tert*-butyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-1-benzyl-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.17. [98]
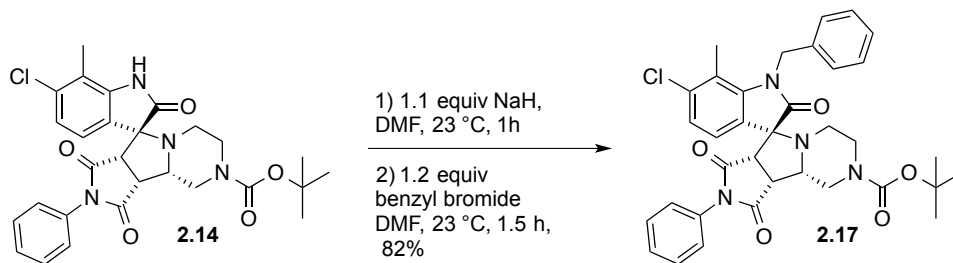
A 5 mL oven-dried round-bottom flask was charged with sodium hydride (60 wt % in mineral oil, 0.01 g, 0.37 mmol, 1.1 equiv) and the mineral oil dispersed in sodium hydride was removed by washing with hexanes (3 × 2 mL). The resulting white solid of sodium hydride was suspended in 0.2 mL of DMF. The suspension was cooled to 0 °C in an ice bath, after which pyrrolidines **2.14** (0.18 g, 0.34 mmol, 1.0 equiv) was added as a solid over the course of 15 min. After the addition was complete the ice bath was removed and the solution was stirred for 1 h at room temperature. Next, benzyl bromide (48 mL, 0.40 mmol, 1.2 equiv) was added to the reaction flask and the reaction was allowed to stir at room temperature until the consumption of **2.15** confirmed by TLC (2:3 EtOAc/hex).

Upon consumption of the **2.14**, the reaction mixture was concentrated under vacuum to give a yellow solid. The solid was then purified by flash chromatography with EtOAc/hex (4:1) to afford the pyrrolidine **2.17**, a white solid (0.17 g, 0.27 mmol, 82%). R_f = 0.65 (2:3 EtOAc/hex); mp 145-154; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 7.51-7.48 (m, 2H), 7.42-7.41 (m, 1H), 7.34-7.31 (m, 4H), 7.26-7.24 (m, 1H), 7.18-7.17 (m, 2H), 7.13 (d, *J* = 8.1 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 5.21-5.16 (m, 2H), 4.37-4.35 (m, 1H), 3.87-3.86 (m, 2H), 3.74-3.72 (m, 1H), 3.60 (d, *J* = 8.1 Hz, 1H), 2.80-2.78 (m, 1H), 2.67-2.65 (m, 1H), 2.34-2.32 (m, 1H), 2.27 (s, 3H), 2.19-2.17 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (500 MHz, DMSO-d_6, 310 K) δ 177.6, 175.2, 173.8, 154.2, 143.4, 137.8, 136.6, 132.6, 129.6, 129.5, 129.0, 127.7, 127.5, 125.8, 125.1, 124.5,

124.0, 118.6, 79.7, 71.0, 63.9, 58.6, 51.9, 46.5, 45.8, 45.1, 30.7, 28.5, 14.7; IR (thin film) 3680, 2971, 2843, 1713, 1054, 1032, 1012; HRMS (ESI): *m/z* calculated for $C_{35}H_{35}ClN_4O_5Na$ $[M+Na]^+$ 649.2194, found 649.2194. HPLC purity: 100%.

Synthesis of (±)-(3R,3a'R,3b'S,9a'S)-6-chloro-7-methyl-2'-phenyl-3a',4',5',6',7',9a'-hexahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-a]pyrazine]-1',2,3'(2'H,3b'H)-trione, 2.26.[99]
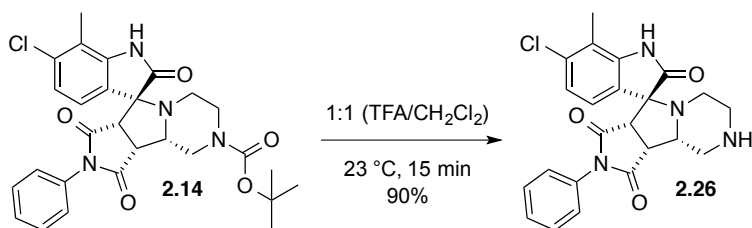
A 10 mL round bottom flask was charged with pyrrolidine **2.14** (0.57 g, 1.1 mmol, 1 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Next, 3.1 mL CH_2Cl_2 was added to the reaction flask such that the concentration of **2.14** was 0.34 M and stirred for 10 min. A pink color solution resulted. Then 3.1 mL trifluoroacetic acid was slowly added to the reaction mixture by syringe. A dark brown color solution resulted. The deprotection reaction was stirred until TLC indicated complete consumption of the pyrrolidine **2.14**, 15 min.

Upon consumption of the **2.14**, the reaction mixture was concentrated under vacuum to give a brown oil. 5 mL of saturated aqueous $NaHCO_3$ was added to the oil and the aqueous layer was then extracted with (3 × 5 mL) CH_2Cl_2. The combined organic layers were then washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated under vacuum to afford **2.26,** a pink solid, (0.42 g, 0.96 mmol, 87%). R_f = 0.81 (20:80:5 EtOAc/hex/Et$_3$N); mp 196-200; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.58-7.53 (m, 2H), 7.47-7.45 (m, 1H), 7.30 (d, *J* = 7.4 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 3.77-3.76 (m, 2H), 3.51-3.50 (m, 1H), 3.42 (br s, 1H), 3.30 (d, *J* = 11.9 Hz, 1H), 2.79 (app d, *J* = 12.1 Hz, 1H),

2.43-2.41 (m, 1H), 2.25 (s, 3H), 2.24-2.21 (m, 2H); ^{13}C NMR (500 MHz, CDCl$_3$, 310 K) δ 178.8,

175.5, 174.2, 143.4, 134.9, 132.7, 129.6, 128.9, 127.4, 125.2, 124.1, 122.5, 117.7, 72.6, 58.7,

50.7, 48.7, 47.1, 46.7, 44.9, 14.3; IR (thin film) 3850, 3671, 2971, 2864, 1709, 1054, 1032,

1013; HRMS (ESI): *m/z* calculated for C$_{23}$H$_{21}$ClN$_4$O$_3$Na [M+Na]$^+$ 459.1200, found 459.1190.

HPLC purity: 100%.

Synthesis of (±)-methyl 10-((3R,3a'R,3b'S,9a'S)-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazin]-5'(1'*H*)-yl)-10-oxodecanoate, 2.27.[100]

A 15 mL round bottom flask was charged with pyrrolidine **2.26** (0.13 g, 0.30 mmol, 1

equiv), Na$_2$CO$_3$ (0.03 g, 0.30 mmol, 1.0 equiv) and a stir bar. The flask was fitted with a septum

and purged with nitrogen. Then 6 mL of CH$_2$Cl$_2$ was added to the reaction flask such that the

concentration of **2.26** was 0.05 M. Next, methyl 10-chloro-10-oxodecanoate (0.07 mL, 0.31

mmol, 1.04 equiv) was added drop-wise to the reaction mixture by syringe. The reaction was

allowed to stir at room temperature until the consumption of **2.26** confirmed by TLC (20:80:5

EtOAc/hex/Et$_3$N).

Upon consumption of **2.26**, the reaction mixture was concentrated under vacuum to give

a yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et$_3$N (80:20:5)

to afford the pyrrolidine **2.27**, a yellow solid, (0.10 g, 0.16 mmol, 53%). R$_f$ = 0.75 (20:80:5

EtOAc/hex/Et$_3$N); mp 110-115; ^{1}H NMR (600 MHz, DMSO-*d$_6$*, 400 K) δ 10.42 (s, 1H), 7.53-

7.51 (m, 2H), 7.45-7.43 (m, 1H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J* =

7.8 Hz, 1H), 4.58 (br s, 1H), 4.08 (br s, 1H), 3.86 (t, J = 7.8 Hz, 1H), 3.73-3.72 (m, 1H), 3.61-

3.58 (m, 4H), 2.61-2.82 (m, 4H), 2.34-2.27 (m, 5H), 2.22-2.18 (m, 1H), 1.59-1.53 (m, 4H), 1.22-

1.42 (m, 9H); ^{13}C NMR (500 MHz, CDCl$_3$, 313 K) δ 178.2, 174.6, 174.3, 172.2, 171.7, 141.8,

136.6, 131.7, 129.4, 128.9, 126.3, 124.4, 123.5, 122.3, 118.7, 72.7, 58.7, 51.4, 50.4, 48.7, 46.2,

45.3, 44.5, 41.1, 34.1, 33.4, 29.7, 29.3, 29.2, 25.3, 24.9, 13.6; IR (thin film) 3817, 3671, 2921,

2863, 1710, 1617, 1054, 1032; HRMS (ESI): m/z calculated for C$_{34}$H$_{39}$ClN$_4$O$_6$Na [M+Na]$^+$

657.2456, found 657.2435. HPLC purity: 97.45%.

Synthesis of (±)-(3*R*,3a'*R*,3b'*S*,9a'*S*)-6-chloro-5'-((*S*)-2-methoxy-2-phenylacetyl)-7-methyl-2'-phenyl-3a',4',5',6',7',9a'-hexahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-1',2,3'(2'*H*,3b'*H*)-trione, 2.28.[101]

A 15 mL round bottom flask was charged with pyrrolidine **2.26** (0.20 g, 0.46 mmol, 1.0

equiv), (*S*)-2-methoxy-2-phenylacetic acid (0.10 g, 0.60 mmol, 1.3 equiv), N-(3-

dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.18 g, 0.96 mmol, 2.1 equiv) and a

stir bar. The flask was fitted with a septum and purged with nitrogen. Then 6 mL of CH$_2$Cl$_2$ was

added to the reaction flask such that the concentration of **2.26** was 0.07 M. Next triethylamine

(0.22 mL, 1.6 mmol, 3.5 equiv) was added to the reaction mixture and the reaction was allowed

to stir at room temperature until the consumption of **2.26** confirmed by TLC (20:80:5

EtOAc/hex/Et$_3$N).

Upon consumption of the **2.26**, the reaction mixture was quenched with 5 mL of 1 N aq.

HCl and the aqueous layer was then extracted with (3 × 5 mL) CH$_2$Cl$_2$. The combined organic

layers were then washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated under vacuum to give a yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et$_3$N (80:20:5) to afford the pyrrolidine **2.28**, a white solid, (0.12 g, 0.21 mmol, 45%). R_f = 0.65 (1:1 EtOAc/hex); ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.42 (s, 1H), 7.51-7.49 (m, 2H), 7.44-7.41 (m, 1H), 7.39-7.35 (m, 4H), 7.32-7.31 (m, 3H), 7.01 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.17-5.16 (m, 1H), 4.71-4.70 (m, 1H), 4.19-4.17 (m, 1H), 3.79-3.75 (m, 1H), 3.64-3.60 (m, 1H), 3.54 (app dd, J = 8.0, 2.9 Hz, 1H), 3.39-3.37 (m, 3H), 2.73-2.64 (m, 2H), 2.27-2.22 (m, 4H), 2.07-2.05 (m, 1H); ^{13}C NMR (500 MHz, DMSO-d_6, 310 K) (2 rotamers observed) δ 178.5, 175.3, 173.9, 154.9, 137.4, 135.2, 132.6, 129.5, 129.0, 128.9, 128.6, 127.6, 127.5, 127.3, 125.2, 123.6, 122.6, 117.8, 81.8, 81.0, 72.1, 68.9, 63.9, 58.6, 58.2, 57.3, 56.3, 50.9, 46.5, 45.3, 44.5, 41.7, 32.5, 30.6, 30.1, 21.8, 19.0, 17.1; IR (thin film) 3850, 3708, 2971, 2921, 1712, 1643, 1054, 1032, 1012; HRMS (ESI): m/z calculated for $C_{32}H_{29}ClN_4O_5Na$ [M+Na]$^+$ 607.1724, found 607.1736. HPLC purity: 97.04%.

Synthesis of (±)-(3R,3a'R,3b'S,9a'S)-6-chloro-7-methyl-2'-phenyl-5'-(3-phenylpropanoyl)-3a',4',5',6',7',9a'-hexahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-a]pyrazine]-1',2,3'(2'H,3b'H)-trione, 2.29.
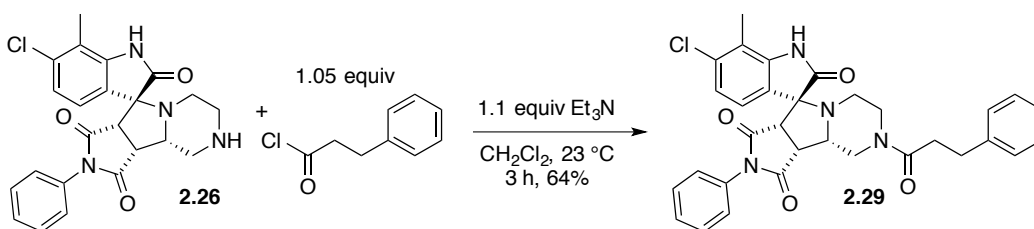
A 10 mL round bottom flask was charged with pyrrolidine **2.26** (0.05 g, 0.11 mmol, 1.0 equiv), triethylamine (18.0 mL, 0.13 mmol, 1.1 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Then 2.0 mL of CH_2Cl_2 was added to the round bottom flask by syringe. Then hydrocinnamoyl chloride (18.0 mL, 0.12 mmol, 1.05 equiv) was added to the

reaction flask with a syringe. The reaction mixture was allowed to stir for 3 h at room temperature. The reaction was monitored for consumption of **2.26** by TLC (100:5 EtOAc/Et₃N).

Upon consumption of **2.26**, the reaction mixture was quenched with 5 mL of saturated aqueous $NaHCO_3$ and the aqueous layer was then extracted with (3 × 5 mL) CH_2Cl_2. The combined organic layers were then washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated under vacuum to give a yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et₃N (60:40:5) to afford the pyrrolidine **2.29**, a white solid, (0.04 g, 0.07 mmol, 64%). R_f = 0.2 (60:40:5 EtOAc/hex/ Et₃N); mp 236-239; ¹H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.40 (s, 1H), 7.53-7.51 (m, 2H), 7.45-7.42 (m, 1H), 7.34-7.33 (m, 2H), 7.27-7.23 (m, 4H), 7.17-7.15 (m, 1H), 7.03 (d, J = 7.8 Hz, 1H), 6.78 (d, J = 8.4 Hz, 1H), 4.59 (s, 1H), 7.09-7.06 (m, 1H), 3.87-3.84 (m, 1H), 3.73-3.69 (m, 1H), 3.59-3.57 (m, 1H), 2.90-2.88 (m, 2H), 2.84-2.82 (m, 2H), 2.78-2.74 (m, 2H), 2.31-2.28 (m, 4H), 2.19-2.15 (m, 1H); ¹³C NMR (500 MHz, DMSO-d_6, 310 K) δ 178.6, 178.5, 175.4, 175.3, 174.0, 170.6, 143.4, 141.9, 135.2, 132.7, 129.6, 129.0, 128.8, 128.7, 127.5, 126.3, 125.2, 123.9, 122.6, 117.9, 72.2, 60.2, 58.9, 51.2, 48.5, 45.5, 44.9, 34.5, 31.2, 14.3; IR (thin film) 3423, 2917, 1708, 1633, 1620, 1384, 1204; HRMS (ESI): *m/z* calculated for $C_{32}H_{29}ClN_4O_4Na$ [M+Na]⁺ 591.1775, found 591.1763. HPLC purity: 98.20%.

Synthesis of (3R,3a'R,3b'S,9a'S)-N-benzyl-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-a]pyrazine]-5'(1'H)-carboxamide, 2.30.
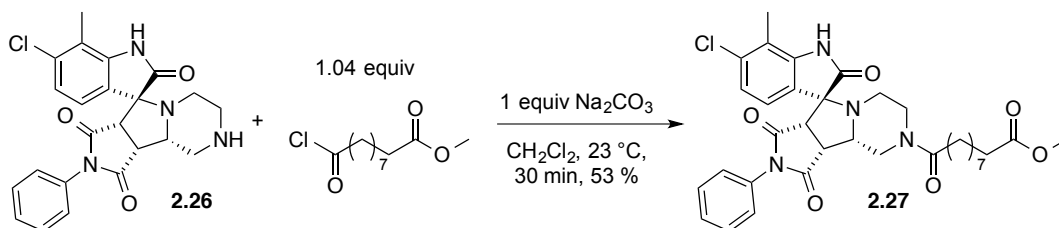

55
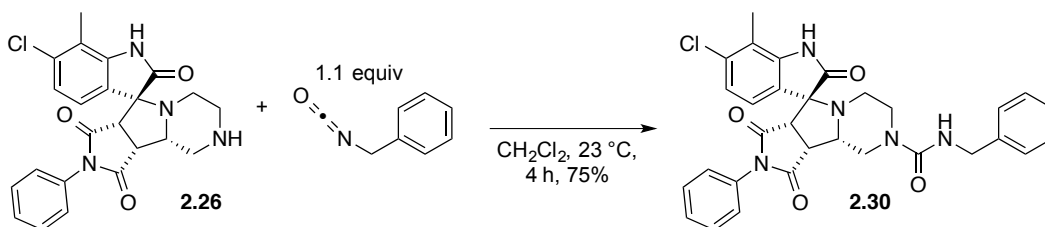
A 10 mL round bottom flask was charged with pyrrolidine **2.26** (0.06 g, 0.15 mmol, 1.0 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Then 3.0 mL of CH_2Cl_2 was added to the round bottom flask by syringe. Next, (isocyanatomethyl)benzene (0.02 mL, 0.16 mmol, 1.1 equiv) was added to the reaction flask by syringe. Then the reaction mixture was allowed to stir for 4 h at room temperature. The reaction was monitored for consumption of **2.26** by TLC (50:50:5 EtOAc/Hex/Et$_3$N).

Upon consumption of **2.26**, the resulting organic solution was concentrated under vacuum to give a yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et$_3$N (60:40:5) to afford the pyrrolidine **2.30**, a white solid, (0.63 g, 0.11 mmol, 75%). R$_f$ = 0.24 (50:50:5 EtOAc/Hex/Et$_3$N); mp 258-259; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.44 (s, 1H), 7.53-7.51 (m, 2H), 7.45-7.44 (m, 1H), 7.33-7.32 (m, 2H), 7.29-7.28 (m, 4H), 7.20 (m, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 4.47-4.45 (m, 1H), 4.29-4.27 (m, 2H), 3.95 (d, J = 14.1 Hz, 1H), 3.82-3.77 (m, 2H), 3.56 (d, J = 7.9 Hz, 1H), 2.75-2.71 (m, 1H), 2.64-2.58 (m, 1H), 2.28 (s, 3H), 2.25-2.23 (m, 2H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 178.7, 175.4, 174.1, 157.6, 143.4, 141.4, 135.1, 132.7, 129.6, 129.1, 128.6, 127.5, 126.9, 125.1, 123.9, 122.6, 117.9, 72.3, 58.3, 51.1, 47.5, 46.7, 45.7, 44.1, 43.6 ; IR (thin film) 3618, 2922, 2360, 2340, 1712, 1616, 1536, 1387; HRMS (ESI): m/z calculated for $C_{31}H_{28}ClN_5O_4Na$ [M+Na]$^+$ 592.1727, found 592.1724. HPLC purity: 97.18%.

Synthesis of (±)-benzyl (3*R*,3a'*R*,3b'*S*,9a'*S*)-6-chloro-7-methyl-1',2,3'-trioxo-2'-phenyl-2',3',3a',3b',4',6',7',9a'-octahydrospiro[indoline-3,9'-pyrrolo[3',4':3,4]pyrrolo[1,2-*a*]pyrazine]-5'(1'*H*)-carboxylate, 2.31.[102]
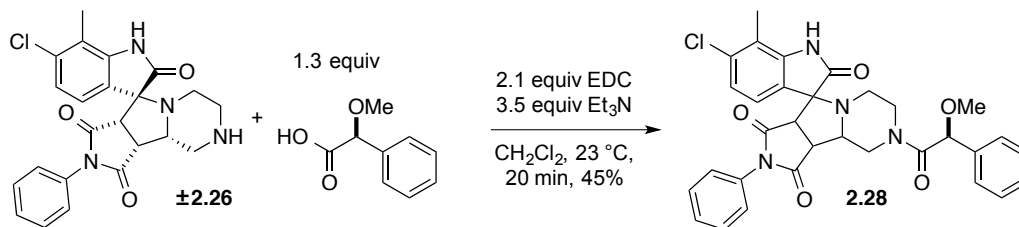
A 5 mL round bottom flask was charged with pyrrolidine **2.26** (0.20 g, 0.45 mmol, 1.0 equiv), *N*,*N*-Diisopropylethylamine (0.17 mL, 0.96 mmol, 2.1 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Then 0.92 mL of CH_2Cl_2 was added to the

round bottom flask by syringe. The reaction mixture was cooled to 0 °C with an ice bath. Meanwhile another 5 mL pear-shaped flask was charged with benzyl chloroformate (0.07 mL, 0.50 mmol, 1.1 equiv) and the flask was fitted with a septum and purged with nitrogen. Then 0.34 mL of CH_2Cl_2 was added to the pear shaped vial containing the benzyl chloroformate and stirred for 10 min. Next the contents of the 5 mL pear shaped vial was slowly added to the 5 mL round bottom flask containing **2.26**. Ice bath was removed and the reaction mixture was allowed to stir for 2.5 h at room temperature. The reaction was monitored for consumption of **2.26** by TLC (50:50:5 EtOAc/Hex/Et₃N).

Upon consumption of **2.26**, the reaction mixture was quenched with 5 mL of saturated aqueous $NaHCO_3$ and the aqueous layer was then extracted with (3 × 5 mL) CH_2Cl_2. The combined organic layers were then washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated under vacuum to give a yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et₃N (60:40:5) to afford the pyrrolidine **2.31**, a white solid, (0.14 g, 0.25 mmol, 54%). R_f = 0.81 (1:1 EtOAc/hex); mp 209-211; ^{1}H NMR (600 MHz, DMSO-d_6, 400 K) δ 10.40 (s, 1H), 7.53-7.51 (m, 2H), 7.45-7.42 (m, 1H), 7.37-7.35 (m, 6H), 7.32-7.31 (m, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 5.16 (s, 2H), 4.48 (d, J = 12.7 Hz, 1H), 3.99 (d, J = 13.1 Hz, 1H), 3.89-3.87 (m, 1H), 3.82-3.79 (m, 1H), 3.61 (d, J = 8.0 Hz, 1H), 2.91-2.86 (m, 2H), 2.78-2.75 (m, 1H), 2.34-2.25 (m, 5H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 178.5, 175.3, 173.9, 154.9, 143.4, 137.3, 135.2, 132.6, 129.5, 129.0, 128.9, 128.3, 128.1, 127.4, 72.3, 66.9, 58.2, 51.0, 47.2, 43.5, 30.9, 21.1, 14.3 ; IR (thin film) 3850, 3678, 3262, 2966,

2864, 1715, 1694, 1032; HRMS (ESI): *m/z* calculated for $C_{31}H_{27}ClN_4O_5Na$ [M+Na]$^+$ 593.1567, found 593.1584. HPLC purity: 97.60%.

Biological Evaluation

Strains, media, and compounds

The *C. albicans* strain HLY4123 was used as the susceptible laboratory strain for the antifungal evaluation in this study. HLY4123 carries a GFP reporter for ERG3 expression and was constructed by plasmid transformation of the commonly used laboratory *C. albicans* strain CAI4. Selected resistant *C. albicans* strains with different mechanisms of becoming drug resistance were obtained from Dr. David Rogers.[88] The strains were cultured at 30 °C under constant shaking (200 rpm) in synthetic complete (SC) medium containing 2% glucose. The stock solution of fluconazole (Sigma-Aldrich, USA) was prepared in sterile water (0.1 mg/mL), whereas the other test compounds were prepared in DMSO. The commercial sample sold as CID 6584729 was obtained from Vitas-M (supplier number STK 580951).

Dose-Response Curves for Test Compounds Against *C. albicans* with and without Fluconazole:

C. albicans was grown in SC medium overnight and then diluted to an effective OD600 of 0.0625. Serial ten-fold dilutions of the test compounds (0.15-1500 μM) were prepared in DMSO in 1.5 mL Eppendorf tubes. To each well in columns B-D (triplicate analysis) of a 24-well Palcon plate was added 2.5 μL of fluconazole solution. To each well in all four columns of the plate was added 1 mL of cells in SC medium such that the column A served as a control to assess the EC$_{50}$ of the compound in the absence of fluconazole. Then to each well in rows 2-5 was added a solution of the compound in DMSO (2 μL each) such that the final solution fluconazole in columns 2-4 was 0.25 μg/mL and the concentration of compound in each row varied from 0.003 μM to 30 μM. The plates were incubated in a rotary shaker/incubator at 30 °C

for 16 h. The contents of each well were re-suspended with a micropipettor and a 20 μL aliquot was added to a polystyrene cuvette and diluted with 680 μL of deionized water. The suspension was triturated again immediately before measuring the absorbance at 600 nm (OD600) for cell densities. EC_{50} values were determined by fitting to a standard curve using the Excel-based tool ED50PLUS v1.0 (Mario H. Vargas).

Determination of FIC90s with a Checkerboard Assay:

Checkerboard assays were carried out using four 24-well plates. The results on each plate were normalized by duplication of one row and one column with a row and column on another plate. *C. albicans* was grown in SC medium overnight and then diluted to an effective OD600 of 0.0625. Serial ten-fold dilutions of the test compounds (150 mM) were prepared in DMSO in 1.5 mL Eppendorf tubes. Serial two-fold dilutions of the fluconazole (6.53 μM) were prepared in sterile water in 1.5 mL Eppendorf tubes. To each well was added 2 mL of a stock solution of compound in DMSO and 2.5 μL of a stock solution of fluconazole in water. Concentrations of compound in rows 1-11 varied from 300 μM to 0.0003 nM; the last row 12 contained no compound. Concentrations of fluconazole in each column B-H varied from 0.0625 to 2 μM; the first column A contained no fluconazole. The plates were incubated in a rotary shaker/incubator at 30 °C for 16 h. The contents of each well was resuspended with a micropipettor and a 20 μL aliquot was added to a polystyrene cuvette and diluted with 680 μL of deionized water. The suspension was triturated again immediately before measuring the absorbance at 600 nm. The MIC90 values were determined as the lowest concentrations of the drugs (alone or in combination) that inhibited fungal growth by 90% compared with that of the drug-free wells.

Molecular Properties

Physicochemical properties were calculated from the SMILES representation of synazo-1 using

the Molinspiration Property Calculation Service at www.molinspiration.com.

Chapter 3

Small Molecule Fluconazole Synergizers - Dihydroisoquinolines

Introduction

Inspired by the highly potent analogues of spiroindolinone CID 6584729 (Scheme 2-5), we set out to optimize another molecule, dihydrophthalazine CID 22334057 from the Broad screening[62] that is also an active hit in our initial assays. I trained a first year graduate student Aaron Mood to work with me on this project. With my initial planning and designing of the analogues, my co-worker Aaron performed the major portion of the synthesis.

Schreiber and others at the Broad institute screened over 60 dihydrophthalazine analogues.[103] Yet only 4 analogues exhibited measurable antifungal activities against a susceptible *C.albicans* clinical isolate, CaCi-2 (fluconazole MIC 2 μg/mL) in the presence of 8 μg/mL fluconazole (Figure 3-1). However, some of the inactive molecules provide valuable insights of the structure activity relationships. Substitution pattern on the arene ring fused to the N3 of the dihydrophthalazine core seems to play a key role in the activity. Especially the *ortho*-substitution that provides a rotational barrier to the arene ring is vital to the potency of the molecule (compounds **3.1** and **3.1d** and compounds **3.1c** and **3.1g**). When atoms N_a and N_b are part of a piparazine ring and N_b is part of an aniline, the arrangement of aniline substituents plays an important role in synergistic activity. Groups on the *ortho*- position are detrimental to the activity (compounds **3.1a**, **3.1b** and **3.1f**). Substitution on the amide N_a is also key to the antifungal enhancement activity of these molecules. It is evident that not every non-polar alkyl chain provides the required activity to the molecule. The cyclohexenethyl group on N_a is specifically important for the activity and substituting it with other groups diminish the antifungal enhancement activity (compounds **3.1**, **3.1e**, **3.1h** and **3.1i**).

Figure 3-1: Representative Examples of Dihydrophthalazine Tested in Broad Screen (Activities against *C.albicans*, CaCi-2 in the presence of 8 μg/mL fluconazole)

CID 22334057, **3.1**
EC$_{50}$ 0.469 μM

3.1a
EC$_{50}$ 0.819 μM

3.1b
EC$_{50}$ 0.642 μM

3.1c
EC$_{50}$ 1.551 μM

3.1d
EC$_{50}$ >38 μM
INACTIVE

3.1e
EC$_{50}$ >38 μM
INACTIVE

3.1f
EC$_{50}$ >38 μM
INACTIVE

3.1g
EC$_{50}$ >38 μM
INACTIVE

3.1h
EC$_{50}$ >38 μM
INACTIVE

3.1i
EC$_{50}$ >38 μM
INACTIVE
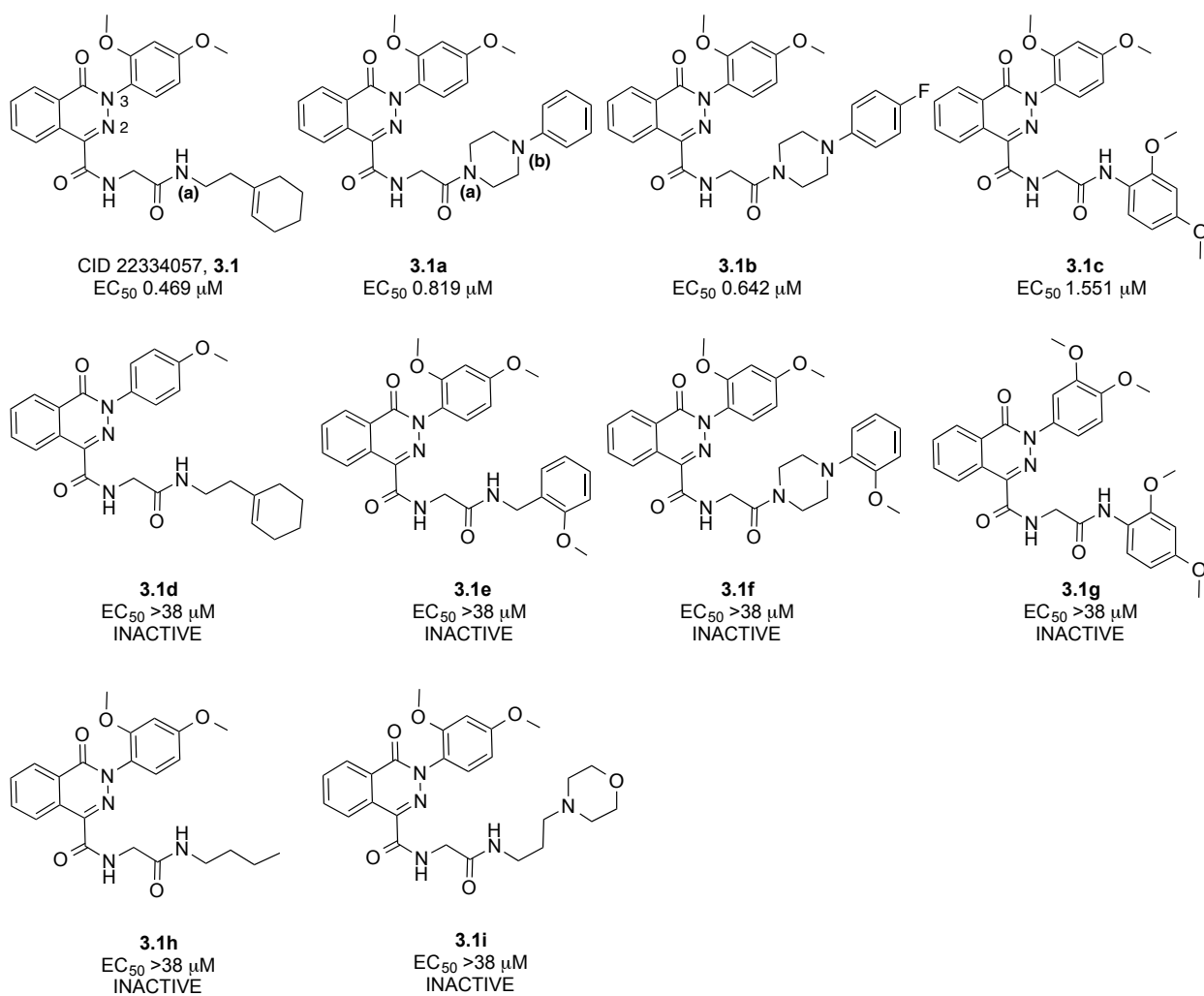
Early attempts to synthesize analogues of CID 22334057 by my undergraduate co-worker Kevin Scott resulted in unsuccessful condensation of dicarboxylic acid **3.2** and phenylhydrazine **3.3** (Figure 3-2).

Figure 3-2: Initial Plan to Synthesize Dihydrophthalazine Core
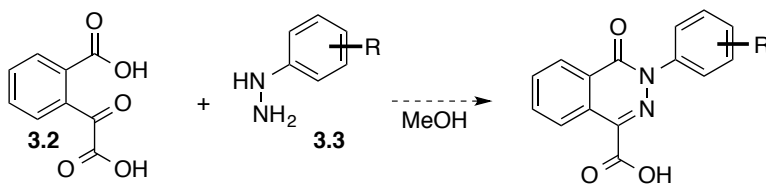
Handling of free base of the electron-rich arylhydrazine was also problematic. The synthesis was carried out with the initial diazotization of the aniline derivative by nitrous acid (Figure 3-3). Then subsequent reduction of the diazonium ion by tin(II) chloride in the presence of hydrochloric acid resulted in the HCl salt of the corresponding arylhydrazine product.[104] The diazotation and reduction steps were carried out successfully and the resulting arylhydrazine•HCl salt was characterized by ^{1}H NMR. However, the attempts to prepare the free base of the phenylhydrazine analogues were hampered by the instability of the electron-rich arylhydrazine.[105] Therefore, the condensation of dicarboxylic acid **3.2** and the arylhydrazine•HCl was attempted in the presence of various bases (Na_2CO_3, $NaHCO_3$, Et_3N and K_3PO_4). Yet, none of the combinations resulted in the expected dihydrophthalazine adduct and we only observed the starting material by TLC during the reaction and by ^{1}HNMR.

Figure 3-3: Synthesis of Arylhydrazine
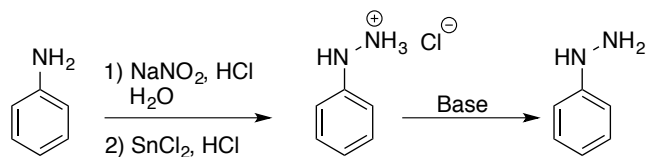
The difficulties arising while designing a convenient and efficient synthetic route to make the dihydrophthalazine core led us to focus on synthesizing the dihydroisoquinoline core containing isosteric methine moiety at the N2 position (Figure 3-4).

Figure 3-4: Fluconazole Synergizer CID 22334057 and Dihydroisoquinoline Analogues
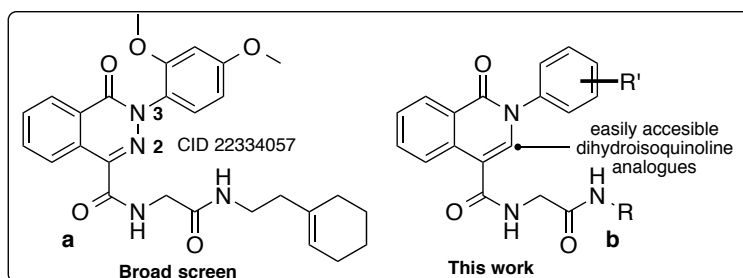
During the massive screening for antifungal synergizers Schreiber and others at the Broad Institute tested 10 other analogues of dihydroisoquinolines that bears the core depicted in Figure 3-4 (structure **b**). Interestingly, structurally similar compound dihydrophthalazine **3.1a** (Figure 3-1) and dihydroisoquinolines, CID 3245992 (Figure 3-5) displayed significantly different results. Compound **3.1a** had antifungal activity at EC_{50} 0.819 μM in the presence of fluconazole whereas CID 3245992 demonstrated no antifungal enhancer activity in the initial high throughput assay. Instead, compound CID 5224943 that lacks the glycine moiety of the parent compound **3.1b** displayed a comparable EC_{50} value of 0.79 μM.[87]

Figure 3-5: Dihydroisoquinolines Screened in Broad Assay
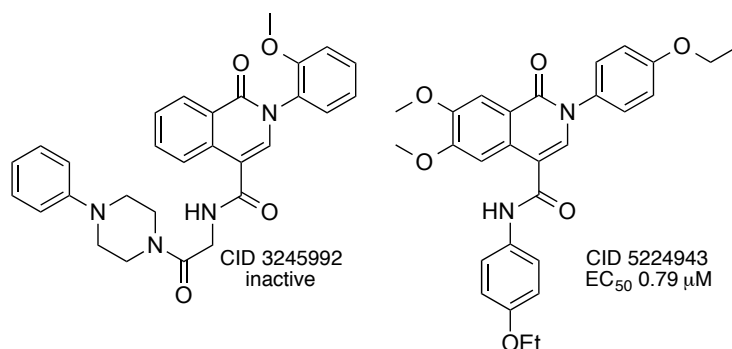
With this information in hand, we set out to optimize the antifungal enhancer activity of lead compound CID 22334057 in the presence of fluconazole and also to identify the sites for attachment of biological tags.

Results and Discussion

The overall synthesis strategy of isosteric dihydroisoquinoline analogues of CID 22334057 involved two aspects: dihydroisoquinoline acid chlorides and various amines. Final coupling was expected to result in different combinations of two fragments and therefore new analogues of the parent compound.

Synthesis of dihydroisoquinoline acid chloride fragment

As shown in Scheme 3-1, dihydroisoquinoline acid chlorides were synthesized by initial formylative condensation followed by cyclization of homophthalic acid **3.4** to afford enol ether **3.5** (Scheme 3-1).[106] Then conjugate substitution of the methoxy group with an aniline resulted in enamine adduct **3.6**. The presence of ethanol under basic conditions facilitated the final rearrangement of **3.6** to afford dihydroisoquinoline **3.7**.[107] Under Vilsmeier conditions acid chloride **3.8** was synthesized from the corresponding carboxylic acid.[108]

Scheme 3-1: Synthesis of Dihydroisoquinoline Acid Chloride Analogues*

Ar	-ortho	-para
a	OMe	OMe
b	OMe	Me
c	OMe	Br
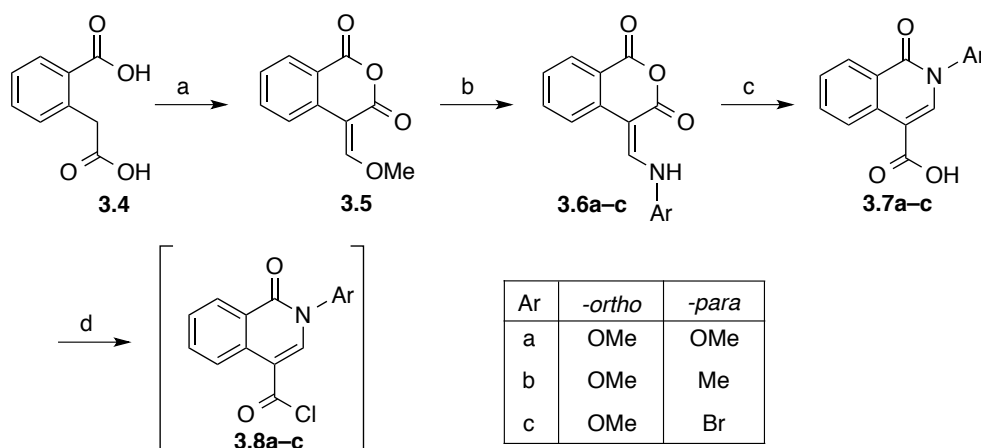
Reagents and conditions: (a) 1.0 equiv CH(OCH$_3$)$_3$, Ac$_2$O, 140 °C, 1 h, 65%, (b) 1.0 equiv ArNH$_2$, 1,4-dioxane, 23 °C, 1-4 h, 35-70%, (c) 5.0 equiv NaOH, EtOH, 80 °C, 1-4 h, 47-77%, (d)1.5 equiv oxalyl chloride, 10 mol% DMF, CH$_2$Cl$_2$, 0 °C, 2 h, 64%. *Performed by Aaron Mood.

In order to evaluate the electronic effects on overall antifungal enhancement, three analogues of dihydroisoquinoline acid chlorides (**3.8a-c**) were synthesized. Compound **3.8c** allowed further derivatization of the aryl ring via various cross coupling reactions in later stages.

We were able to successfully attach an alkynyl substituent to the benzo ring of the bicyclic dihydroisoquinoline. This modification allowed us to determine the substituent tolerance of the benzo ring. Overall synthesis includes an initial bromination of homophthalic acid **3.4** to

result in 5-bromo-2-(carboxymethyl)benzoic acid adduct **3.9**.[109] Subsequent Presser esterification

of **3.9** with trimethylsilyldiazomethane afforded the dimethyl ester **3.10** in excellent yield.[110]

Sonogashira coupling with TBDPS-protected 4-pentyn-1-ol was carried out before the

introduction of bromoaniline.[111] Diester **3.11** was saponified to afford the carboxylic acid **3.12**.[112]

Final cyclization to form the dihydroisoquinoline core was successfully achieved by following

the same methods as in Scheme 3-1, steps a-d. Deprotection of the TBDPS group with

tetrabutylammonium fluoride afforded dihydroisoquinoline analogue **3.8d**. However, earlier

attempts to synthesize **3.8d** by eliminating protection/deprotection steps of the alcohol was

unsuccessful due to the inability of the corresponding TBDPS free alcohol analogue of **3.12** to

take part in the formylation/cyclization step to form the enol ether (Scheme 3-1, step a).

Scheme 3-2: Synthesis of Dihydroisoquinoline Analogue **3.8d***
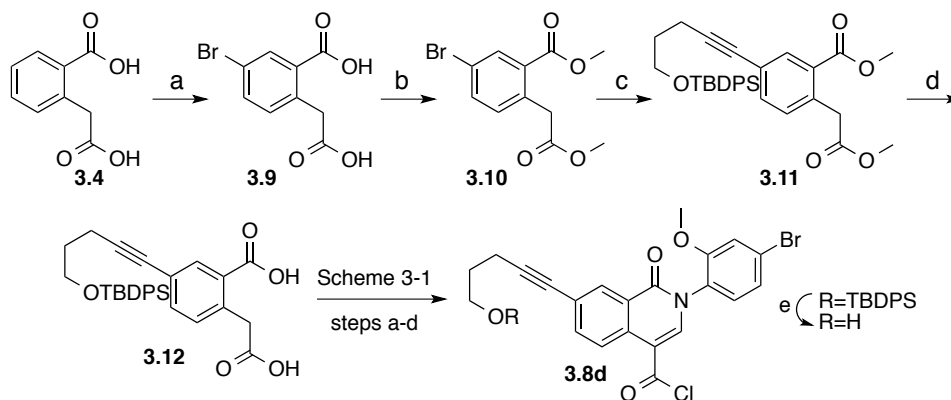
Reagents and conditions: (a) 1.5 equiv KBrO$_3$, H$_2$O/H$_2$SO$_4$, 90 °C, 4 h, 39%, (b) 2.8 equiv
TMSCHN$_2$, 2:1 benzene/MeOH, 23 °C, 2 h, 99%, (c) 5 mol% Pd$_2$Cl$_2$(PPh$_3$)$_2$, 5 mol% CuI, 3:2
DMF/Et$_3$N, 100 °C, 45 min, 85%, (d) 3.8 equiv LiOH, 3:1 THF/H$_2$O, 23 °C, 16 h, 90%.
*Conducted by Aaron Mood.

Synthesis of Amine Fragment

Amines, **3.16a-d** were synthesized for subsequent coupling with dihydroisoquinoline acid

chlorides **3.8a-d** (Scheme 3-3). *N*-Boc amino acids, **3.13a-c** were coupled with amines **3.14a-b**

through carbodiimide coupling reactions to afford amides **3.15a-d** in good yields.[113] However,

deprotection of the Boc group in the presence of the acid-sensitive cyclohexenyl group was found to be challenging. Common reagents such as trifuoroacetic acid resulted in complete degradation of the starting material due to protonation of the alkene. Consequently, thermal deprotection stratergy was employed to generate the amines **3.16a-d**. Compounds such as **3.15a** and **3.15b** were heated under reflux in water to remove the Boc group.[114] However, compounds with very limited solubility in water such as **3.15c** and **3.15d** were heated under reflux in the presence of ethylene glycol.[115]

Scheme 3-3: Synthesis of Amines **3.16a-d***

BocHN—[R]—OH   +   H₂N——R'   →ᵃ   BocHN—[R]—N(H)——R'   →ᵇ

3.13a R = H
3.13b R = CH₂CH₂CH₂CH₂NH-Cl-Z
3.13c R = CH₂CH₂CH₂CH₂NH-Alloc

3.14a R' = 1-cyclohexenyl
3.14b R' = phenyl

3.15a R = H, R' = 1-cyclohexenyl
3.15b R = H, R' = phenyl
3.15c R = CH₂CH₂CH₂CH₂NH-Cl-Z, R' = 1-cyclohexenyl
3.15d R = CH₂CH₂CH₂CH₂NH-Alloc, R' = 1-cyclohexenyl

H₂N—[R]—N(H)——R'

3.16a R = H, R' = 1-cyclohexenyl
3.16b R = H, R' = phenyl
3.16c R = CH₂CH₂CH₂CH₂NH-Cl-Z, R' = 1-cyclohexenyl
3.16d R = CH₂CH₂CH₂CH₂NH-Alloc, R' = 1-cyclohexenyl

Reagents and conditions: (a) 1.2 equiv HOBt, 1.2 equiv EDC•HCl, 1.5 equiv DIPEA, CH₂Cl₂, 0-23 °C, 4 h, 71-74%, (b) H₂O, 100 °C or ethylene glycol, 200 °C, 74-99%. *Aaron Mood performed all steps leading to **3.16b** and **3.16d**.

Coupling of the dihydroisoquinoline acid chloride and amine fragments and further modifications:

Dihydroisoquinoline acid chlorides **3.8a**, **3.8b** and **3.8d** were coupled with amine **3.16a**. Acid chloride **3.8c** was coupled with phenethylamine and amines **3.16a-d**. The reactions afforded carbon analogues of the lead dihydrophthalazine CID 22334057 in good yields (Scheme 3-4).[116]

Dihydroisoquinoline **3.19** was further functionalized by utilizing the Sonogashira coupling to form compound **3.24** with another rigid linker to test the SAR and also to explore a possible site for the attachment of biological tags (Scheme 3-5).

Scheme 3-4: Coupling of Dihydroisoquinoline Acid Chloride and Amine Fragments*
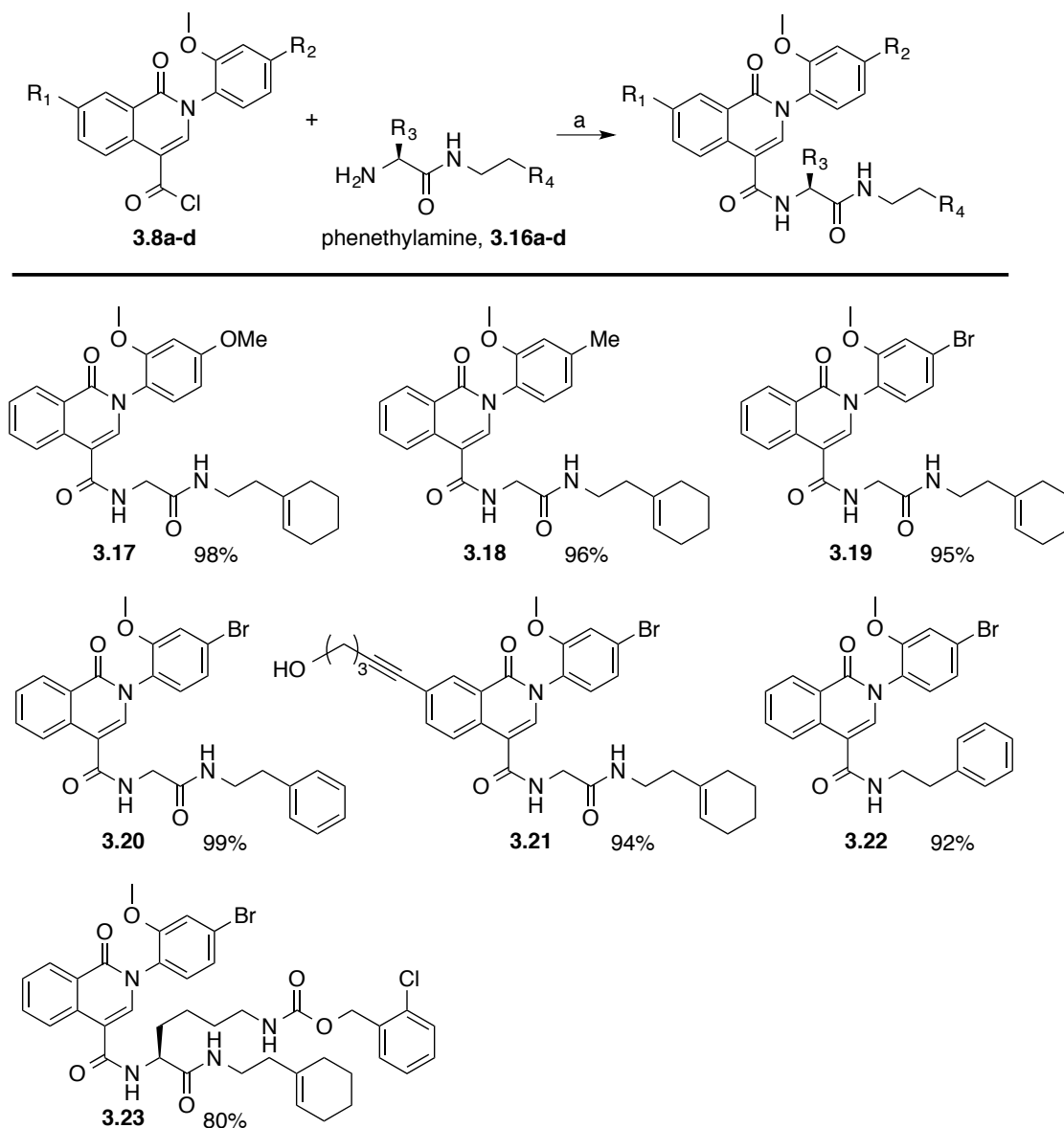
Reagents and conditions: (a) 1.2 equiv Et$_3$N, CH$_2$Cl$_2$, 0-23 °C, 30 min. * Performed by Aaron Mood.

Scheme 3-5: Synthesis of Dihydroisoquinoline Analogue **3.24**

3.19 → a → **3.24**
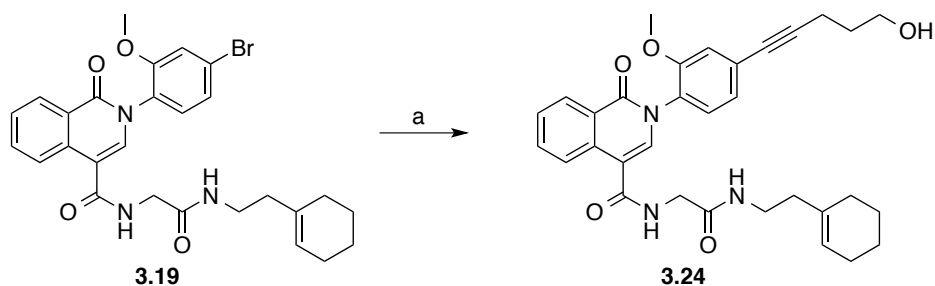
Reagents and conditions: (a) 1.2 equiv 4-pentyn-1-ol, 5 mol% $Pd_2Cl_2(PPh_3)_2$, 5 mol% CuI, 3:2 DMF/Et_3N, 100 °C, 45 min, 68%.

Structure-Activity Relationships

Lead molecule CID 22334057 was reported to synergize with fluconazole in a partially resistant clinical isolate of *C. albicans* CaCi-8 at EC_{50} 0.23 µM.[87] We tested our compounds against a susceptible strain (HLY4123) derived from a commonly used *C. albicans* strain. CID 22334057 demonstrated better and promising activity in the cell line that we used in our study (Scheme 3-6). Replacing the imino nitrogen of the dihydrophthalazine core with a methine group did not affect the synergistic activity of the analogues significantly (CID 22334057 and compound **3.17**). Substitution on the phenyl moiety proved to be essential to the biological activity of the molecules (molecules **3.17**, **3.18** and **3.19**). Specifically, replacing the 4-position with a bromo substituent showed the para bromo group to be superior to both methoxy and methyl substituents. As mentioned above deprotection of the Boc group from the amine moiety was problematic due to the presence of cyclohexene group. Therefore, we synthesized phenethyl homologue **3.20** by replacing the cyclohexene with a phenyl group. Interestingly, the substitution of cyclohexene with a phenyl moiety did not affect the synergistic activity significantly (Compound **3.19** and **3.20**). In addition, the inactivity of alkynyl analogues **3.21** and **3.24** show that the *N*-phenyl substituent and the benzo fragment of the dihydroisoquinoline cannot tolerate bulky groups, suggesting that these two sites play important roles in binding to the biological

69

target. Attempting to bypass the glycine moiety in the amine fragment negatively affected the antifungal synergistic activity of the molecules (Compound **3.19** and **3.22**). Finally, the potent activity of ClCbz-lysine derivative **3.23** revealed a site where a large functional tag could be affixed without compromising antifungal activity. This short list of structure-activity relationship data allowed us to not only identify molecules that demonstrate comparable antifungal activities to the lead molecule but also to locate a site that can tolerate a bulky substituent and allow the attachment of a biological tag. However, additional work is required to make analogues that display sub-nanomolar potent antifungal activity in the presence of azole drugs.

Scheme 3-6: Structure-Activity Relationship of Dihydroisoquinoline Analogues

CID 22334057 EC_{50} 11 nM

3.17 EC_{50} 33 nM

3.18 EC_{50} 65 nM

3.19 EC_{50} 3 nM

3.20 EC_{50} 20 nM

3.21 EC_{50} > 100,000 nM

3.22 EC_{50} 221 nM

3.23 EC_{50} 23 nM

3.24 EC_{50} > 100,000 nM
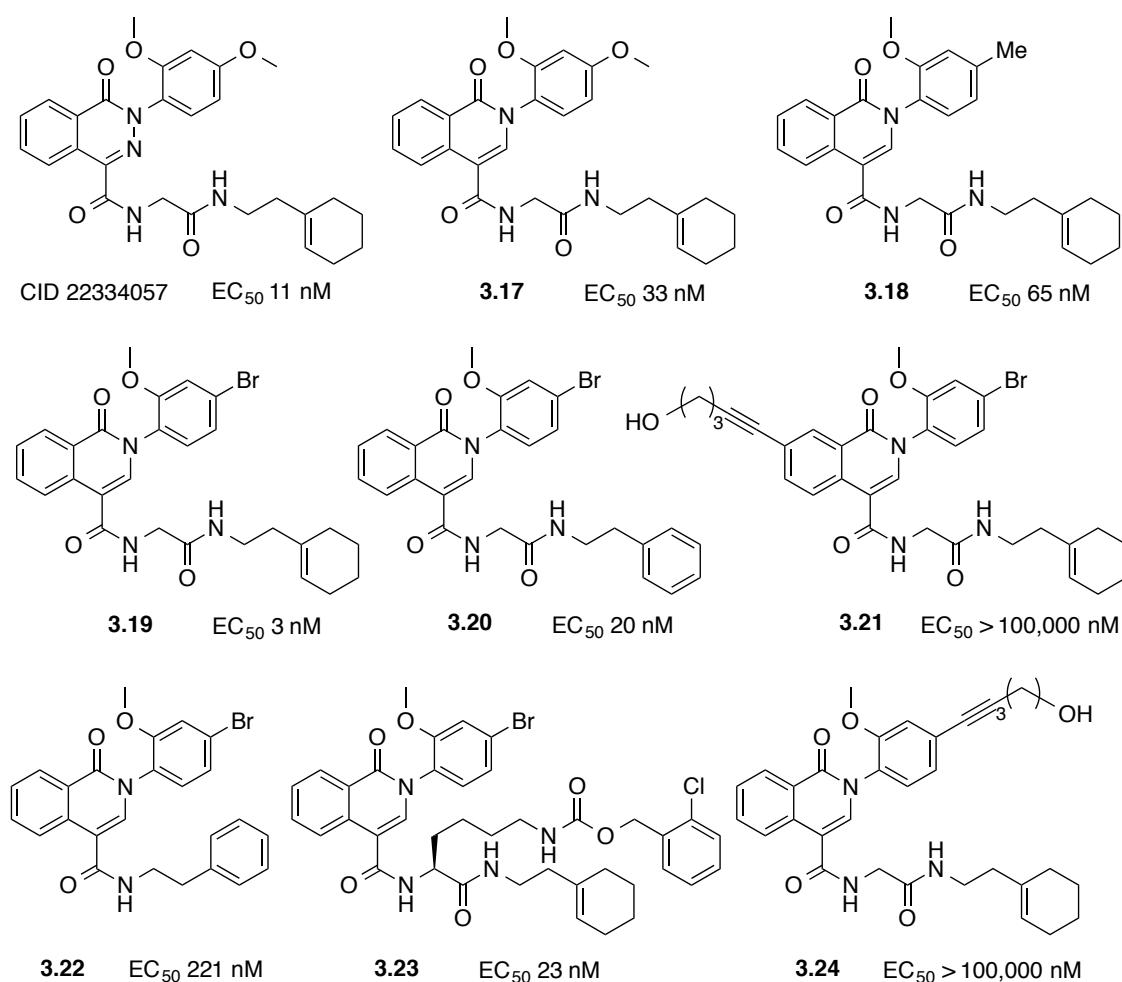
Synthesis and Activity of Functional Biological Probes:

Taking advantage of the information revealed during the SAR studies we set out to synthesize compounds that carry biological probes such as biotin. Deprotection of 2-ClCbz in compound **3.23** requires acidic conditions, which can lead to the complications with the cyclohexene moiety. Therefore, we decided to synthesize an analogue that contains an alloc-protecting group that could be removed without affecting the acid-sensitive and hydrogenation prone cyclohexene ring (Scheme 3-7, compound **3.25**).[117] Dihydroisoquinoline **3.25** was synthesized by utilizing the same steps described in Schemes 3-2, 3 and 4. Alloc group was deprotected with phenylsilane and catalytic palladium.

Scheme 3-7: Synthesis of Biotinylated Analogue **3.27**
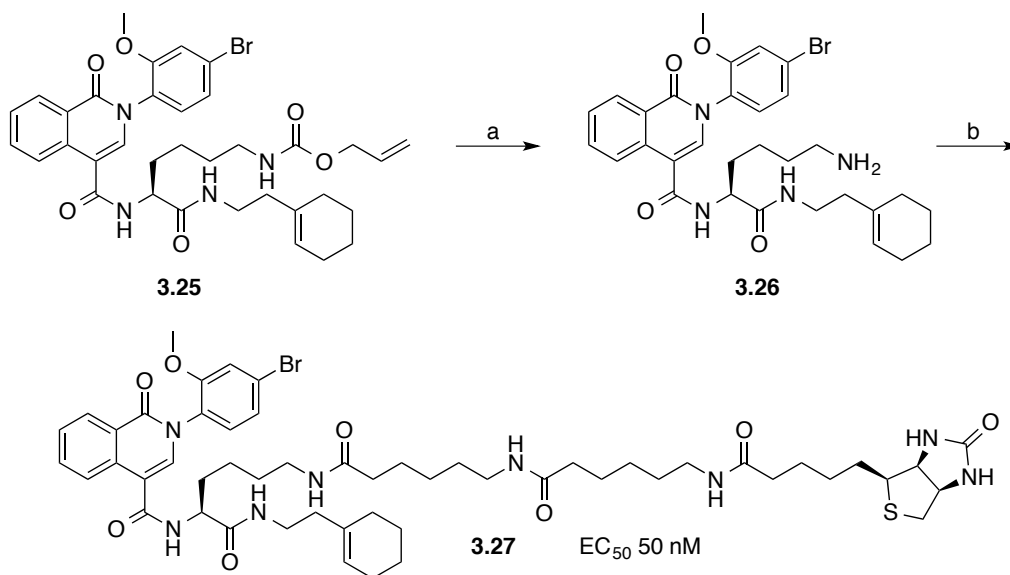
Reagents and conditions: (a) 100 equiv PhSiH$_3$, 20 mol% Pd(PPh)$_3$, 3:1 CH$_2$Cl$_2$/DMF, 23 °C, 1 h, 70%, (b) 1.1 equiv NHS-LC-LC-Biotin, 2.0 equiv DIPEA, DMF, 23 °C, 24 h, 53%.

The resulting amine **3.26** was biotinylated with a longer spacer to afford biotinylated dihydroisoquinoline **3.27**.[118] The space between the pharmacophore and the biological tag is important for the binding and potency of the compound. Longer linkers correlate with better binding due to the less interference of the tag. Biotin analogue **3.27** had an EC$_{50}$ value of 50 nM,

suggesting that the biotin does not significantly affect binding to the target. Shelley Lane at Liu lab has conducted the pull-down assay with *C.albicans* cell lysates. However, they did not pursue the results of the MS-MS results to identify a potential target that is responsible for the antifungal activity in the presence of fluconazole.

A checkerboard assay was used to determine the fractional inhibitory concentration for the synergistic activity of compound **3.19** and fluconazole against the fluconazole-susceptible strain (HLY4123). Compound **3.19** proved to be synergistic with fluconazole with the FIC index of 0.06.

Conclusions:

In conclusion, we have designed, synthesized and studied dihydroisoquinolines inspired by CID 22334057 that was previously reported to exhibit activity against *C. albicans* in combination with fluconazole. Substituting imino N2 in the dihydrophthalazine with a methine group did not significantly affect the antifungal activity and replacement of a 4-methoxy group with a bromine atom in the aniline moiety increased the activity. Ultimately, the best analogue compound **3.19** (EC_{50} 3 nM) demonstrated more potent activity than the lead compound CID 22334057 (EC_{50} 11 nM). A biotinylated dihydroisoquinoline analogue **3.27** was synthesized and hopefully the Liu lab will be able to identify a potential target that is responsible for the antifungal activity in the presence of fluconazole. Also, compound **3.19** was found to be a true synergizer of fluconazole with the FIC index of 0.06.

Experimental Section

Chemistry

General procedure for the synthesis of enamines, 3.6a–c.[119]

3.5 → **3.6a–c** (1.0 equiv ArNH₂, dioxane, 23 °C, 0.1-4 h)
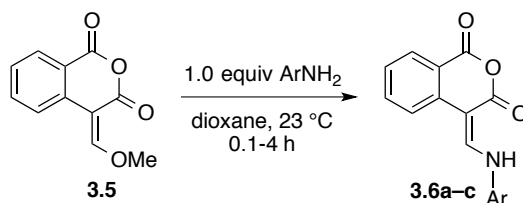
An oven–dried round bottom flask was charged with anhydride **3.5** (1.0 equiv) and a stir bar. Next, dioxane (0.50 M with respect to **3.5**) was added to the reaction flask. The resulting mixture turned into a cloudy white solution. Meanwhile, a separate oven–dried round bottom flask was charged with the substituted aniline (1.0 equiv) and a stir bar. Addition of dioxane (0.50 M with respect to the aniline) to the flask containing the aniline resulted in a deep red solution. Then, the contents of the flask with the aniline were transferred to the flask containing **3.5** via syringe. The reaction mixture gradually became a heterogenous green solution. The reaction was monitored until **3.5** was no longer detected by TLC. Upon consumption of **3.5**, the resulting green solid was filtered in a Büchner funnel. Then, the solid was washed with cold dioxane (3 x 15 mL) and dried under vacuum to obtain the corresponding pure enamine adduct, **3.6a–c**.

4–(((2,4–Dimethoxyphenyl)amino)methylene)isochromane–1,3–dione, 3.6a.
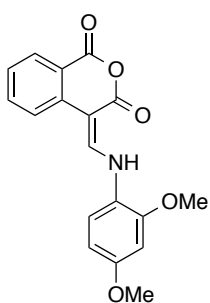
An oven–dried round bottom flask was charged with enol ether **3.5** (0.50 g, 2.5 mmol, 1.0 equiv) and a stir bar. Next, dioxane (5.0 mL) was added to the reaction flask. The resulting mixture turned into a cloudy white solution. Meanwhile, a separate oven–dried round bottom flask was charged with 2,4–dimethoxyaniline (0.38 g, 2.5 mmol, 1.0 equiv) and a stir

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bar. Addition of dioxane (5.0 mL) to the flask containing the aniline resulted in a deep red solution. Immediately after, the contents of the flask with the aniline were transferred to the flask containing **3.5** via syringe. The reaction mixture gradually became a heterogeneous green solution. The reaction was monitored until **3.5** was no longer detected by TLC (10 min). Upon consumption of **3.5**, the resulting green solid was filtered in a Büchner funnel. Then, the green solid was washed with cold dioxane (3 x 15 mL) to give enamine **3.6a** (0.53 g, 1.6 mmol, 70%). R_f = 0.44 (1:1:0.05 EtOAc:hex:Et$_3$N). [1H] NMR (500 MHz, CDCl$_3$) δ 11.64 (d, J = 13.2 Hz, 1H), 8.41 (d, J = 13.8 Hz, 1H), 8.20 (d, J = 8.1 Hz, 1H), 7.61 (t, J = 7.3 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.27–7.21 (m, 2H), 6.56–6.54 (m, 2H), 3.94 (s, 3H), 3.84 (s, 3H); [13C] NMR (500 MHz, CDCl$_3$) δ 163.5, 161.9, 158.6, 150.7, 144.2, 137.5, 135.0, 131.0, 124.9, 121.9, 117.7, 117.3, 116.3, 104.8, 99.5, 91.1, 56.1, 55.7; HRMS (ESI): m/z calculated for C$_{18}$H$_{15}$NO$_5$Na [M+Na]$^+$ 348.0848, found 348.0854.

4–(((2–Methoxy–4–methylphenyl)amino)methylene)isochromane–1,3–dione, 3.6b.
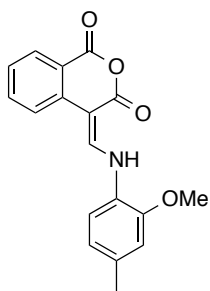
Using the general procedure for enamine formation outlined above, enol ether **5** (0.50 g, 2.5 mmol, 1.0 equiv) was used to give enamine **3.6b** (0.27 g, 0.86 mmol, 35%) as a green solid. R_f = 0.52 (1:1 EtOAc:hex). [1H] NMR (500 MHz, CDCl$_3$) δ 11.68 (d, J = 13.5 Hz, 1H), 8.46 (d, J = 13.6, 1H), 8.20 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 8.1 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 6.80 (s, 1H), 3.95 (s, 3H), 2.38 (s, 3H); [13C] NMR (500 MHz, CDCl$_3$) δ 163.5, 161.8, 149.3, 143.9, 137.4, 136.5, 135.0, 131.1, 125.7, 125.0, 121.7, 117.8, 117.4, 114.9, 112.5, 91.5, 56.0, 21.5; HRMS (ESI): m/z calculated for C$_{18}$H$_{15}$NO$_4$Na [M+Na]$^+$ 332.0899, found 348.0907.

4–(((4–Bromo–2–methoxyphenyl)amino)methylene)isochromane–1,3–dione, 3.6c.
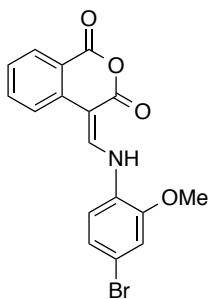
Using the general procedure for enamine formation outlined above, enol ether **3.5** (0.50 g, 2.5 mmol, 1.0 equiv) was used to give enamine **3.6c** (0.97 g, 2.6 mmol, 70%) as a green solid. R_f = 0.53 (1:1:0.05 EtOAc:hex:Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 11.65 (d, J = 13.3 Hz, 1H), 8.43 (d, J = 13.5 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.64 (td, J = 7.5 Hz, 1.3, 1H), 7.58 (d, J = 8.0 Hz, 1H) 7.30 (t, J = 7.8 Hz, 1H), 7.18 (br s, 2H), 7.11 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 163.5, 161.5, 149.8, 143.1, 136.9, 135.2, 131.2, 127.5, 125.5, 124.2, 118.4, 118.0, 117.6, 115.8, 115.2, 92.7, 56.4; HRMS (ESI): m/z calculated for C$_{17}$H$_{12}$BrNO$_4$Na [M+Na]$^+$ 395.9847, found 395.9850

General procedure for the synthesis of dihydroisoquinolines, 3.7a–c.[120]
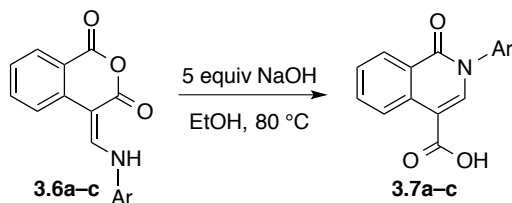
An oven–dried round bottom flask was charged with the corresponding enamine **3.6a–c** (1.0 equiv), anhydrous NaOH (5.0 equiv), and a stir bar. Next, EtOH (0.26 M with respect to **3.6**) was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 1.5 h. The reaction was monitored by TLC for disappearance of the enamine. Upon consumption of the enamine, the reaction mixture was cooled to room temperature. Then, the resulting mixture was treated with 3 N HCl to afford a solution with a pH of 1–2. The resulting solid was filtered with cold water (3 x 20 mL) in a Büchner funnel and dried under vacuum to obtain the corresponding pure dihydroisoquinoline adduct, **3.7a–c**.

2–(2,4–Dimethoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxylic acid, 3.7a.
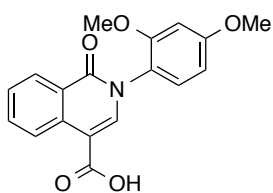
An oven–dried round bottom flask was charged with enamine **3.6a** (1.5 g, 4.6 mmol, 1.0 equiv), anhydrous NaOH (0.91 g, 23 mmol, 5.0 equiv), and a stir bar. Next, EtOH (18 mL) was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 1.5 h. The reaction was monitored by TLC for disappearance of the enamine. Upon consumption of the enamine, the reaction mixture was cooled to room temperature. Then, the resulting mixture was treated with 3 N HCl to afford a solution with a pH of 1–2. The resulting solid was filtered with cold water (3 x 20 mL) in a Büchner funnel to give dihydroisoquinoline **3.7a** (1.1 g, 3.5 mmol, 75%) as a purple solid. R_f = 0.60 (4:1 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, DMSO) δ 12.86 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 7.2 Hz, 1H), 8.02 (s, 1H), 7.85 (td, J = 7.7 Hz, 1.3, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1H), 6.78 (d, J = 2.6 Hz, 1H) 6.66 (d, J = 2.6 Hz, 1H), 6.64 (d, J = 2.7 Hz, 1H), 3.84 (s, 3H) 3.75 (s, 3H); ^{13}C NMR (500 MHz, DMSO) δ 166.3, 161.0, 160.9, 155.1, 142.0, 134.5, 133.2, 129.3, 127.7, 127.2, 125.2, 125.2, 122.0, 105.7, 105.1, 99.4, 56.0, 55.6; HRMS (ESI): m/z calculated for C$_{18}$H$_{15}$NO$_5$Na [M+Na]$^+$ 348.0848, found 348.0858.

2–(2–Methoxy–4–methylphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxylic acid, 3.7b.
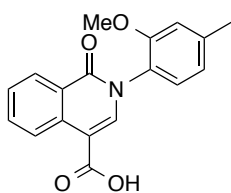
Using the general procedure for dihydroisoquinoline formation outlined above, enamine **3.6b** (0.20 g, 0.81 mmol, 1.0 equiv) was used to give dihydroisoquinoline **3.7b** (0.12 g, 0.38 mmol, 47%) as a brown solid. R_f = =0.57 (17:3 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, DMSO) δ 12.86 (br s, 1H), 8.86 (d, J = 8.3 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.02 (s, 1H), 7.85 (t, J = 7.8 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.08 (s, 1H), 6.91 (d, J = 7.9 Hz, 1H), 3.75 (s,

3H), 2.41 (s, 3H); ^{13}C NMR (500 MHz, DMSO) δ 166.3, 160.9, 153.8, 141.7, 140.5, 134.5, 133.3, 128.4, 127.7, 127.3, 126.4, 125.2, 125.16, 121.1, 113.2, 105.7, 55.8, 21.3; HRMS (ESI): *m/z* calculated for C$_{18}$H$_{15}$NO$_4$Na [M+Na]$^+$ 332.0899, found 348.0904.

2–(4–Bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxylic acid, 3.7c.
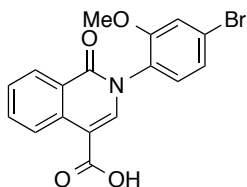
Using the general procedure for dihydroisoquinoline formation outlined above, enamine **3.6c** (0.23 g, 0.61 mmol, 1.0 equiv) was used to give dihydroisoquinoline **3.7c** (0.18 g, 0.47 mmol, 77% yield) as a brown solid. R$_f$ = 0.35 (17:3 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, DMSO) δ 12.90 (s, 1H), 8.85 (d, *J* = 8.4 Hz, 1H), 8.28 (d, *J* = 8.0 Hz, 1H), 8.06 (s, 1H), 7.86 (t, *J* = 7.7 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.47–7.43 (m, 2H), 7.31 (dd, *J* = 8.2, 1.6 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (500 MHz, DMSO) δ 166.2, 160.7, 155.1, 141.1, 134.5, 133.4, 133.4, 130.6, 128.2, 127.7, 127.4, 125.3, 125.0, 123.6, 123.0, 116.0, 106.1, 56.6; HRMS (ESI): *m/z* calculated for C$_{17}$H$_{12}$BrNO$_4$Na [M+Na]$^+$ 395.9847, found 395.9842.

2–Amino–*N*–(2–(cyclohex–1–en–1–yl)ethyl)acetamide, 3.16a.[121]
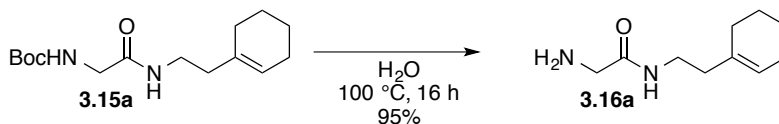
A round bottom flask was charged with Boc–protected amine **3.15a** (1.5 g, 5.3 mmol, 1.0 equiv) and a stir bar. Next, water (110 mL, 0.048 M with respect to **3.15a**) was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 16 h and monitored by TLC for disappearance of **3.15a**. Upon consumption of **3.15a**, the reaction mixture was cooled to room temperature. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 100 mL). Then, the combined organic layers were concentrated *in vacuo* to give amine **3.16a** (0.91 g, 5.0 mmol, 95%) as a yellow oil. R$_f$ = 0 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.21 (br

s, 1H), 5.48 (s, 1H), 3.38–3.34 (m, 4H), 2.15 (t, J = 6.7 Hz, 2H), 2.0–1.94 (m, 4H), 1.65–1.51

(m, 4H), 1.42 (br s, 2H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 172.5, 134.7, 123.4, 44.8, 37.7, 36.8,

28.0, 25.3, 22.9, 22.4; HRMS (ESI): *m/z* calculated for C$_{10}$H$_{18}$N$_2$ONa [M+Na]$^+$ 205.1317, found

205.1312.

General procedure for synthesis of amides via acid chlorides, 3.17–3.20, 3.22, 3.23, 3.25.[122,123]

3.7a-c   **3.8a-c**   **3.17-3.20, 3.22, 3.23, 3.25**
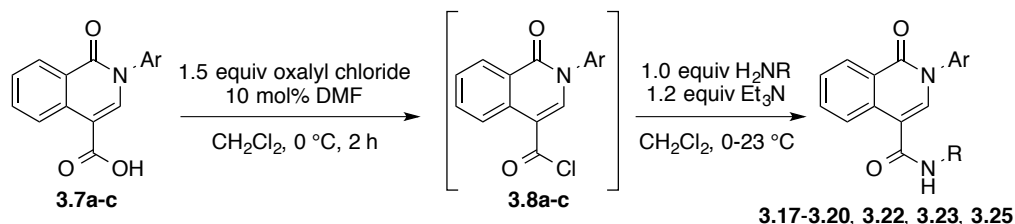
A flame–dried round bottom flask was charged with the corresponding carboxylic acid

3.7a–c (1.0 equiv) and a stir bar. Next, CH$_2$Cl$_2$ (0.077 M with respect to the carboxylic acid) was

added to the flask and the reaction mixture was cooled to 0 °C with an ice bath. Subsequently,

oxalyl chloride (1.5 equiv) and DMF (10 mol%) were added sequentially. The reaction mixture

was stirred at 0 °C for 2 h. The resulting solution was clear. Next, the reaction mixture was

concentrated *in vacuo* to yield the corresponding acid chloride, **3.8a–c**.

A separate flame–dried round bottom flask was charged with the corresponding amine

(**3.15aa–3.15ca** and phenethylamine) (1.0 equiv) and a stir bar. Next, CH$_2$Cl$_2$ (0.040 M with

respect to amine) and Et$_3$N (1.2 equiv) were added to the flask via syringe and the flask was

cooled to 0 °C with an ice bath. Next, the acid chloride was dissolved in CH$_2$Cl$_2$ (0.036 M with

respect to acid chloride) and transferred dropwise to the reaction mixture via syringe over 10

min. Afterwards, the reaction vessel was warmed to room temperature. The reaction mixture was

stirred until the acid chloride was no longer detected by TLC (30 min). Upon consumption of the

acid chloride, the reaction mixture was washed with 10% K$_2$CO$_3$ and extracted with CH$_2$Cl$_2$ (3 ×

20 mL). The combined organic layers were washed with brine and dried over Na$_2$SO$_4$. The

resulting organic solution was concentrated *in vacuo*. The crude material was purified by flash chromatography to afford the amide.

N–(2–((2–(cyclohex–1–en–1–yl)ethyl)amino)–2–oxoethyl)–2–(2,4–dimethoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.17.
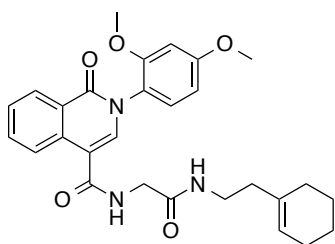
A flame–dried round bottom flask was charged with carboxylic acid **3.7a** (0.050 g, 0.15 mmol, 1.0 equiv) and a stir bar. Next, CH_2Cl_2 (2.0 mL) was added to the flask and the reaction mixture was cooled to 0 °C with an ice bath. Subsequently, oxalyl chloride (20 μL, 0.23 mmol, 1.5 equiv) and DMF (1.2 μL, 0.015 mmol, 10 mol%) were added sequentially. The reaction mixture was stirred at 0 °C for 2 h. The resulting solution was clear. Next, the reaction mixture was concentrated *in vacuo* to yield the acid chloride **3.8a**.

A separate flame–dried round bottom flask was charged with amine **3.16a** (0.028 g, 0.15 mmol, 1.0 equiv) and a stir bar. Next, CH_2Cl_2 (4.0 mL) and Et_3N (0.025 mL, 0.18 mmol 1.2 equiv) were added to the flask via syringe and the flask was cooled to 0 °C with an ice bath. Afterwards, the acid chloride was dissolved in CH_2Cl_2 (6.0 mL) and transferred drop-wise to the reaction mixture via syringe over 10 min. Then, the reaction vessel was warmed to room temperature. The reaction mixture was stirred until the acid chloride was no longer detected by TLC (30 min). Upon consumption of the acid chloride, the reaction mixture was washed with 10% K_2CO_3 and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated *in vacuo* to afford a purple solid. The crude material was purified by flash chromatography (CH_2Cl_2/Et_2O) to afford amide **3.17** (0.074 g, 0.15 mmol, 98%) as a white solid. R_f = 0.17 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.5 Hz,

1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.51 (s, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.84 (t, *J* = 4.9 Hz, 1H), 6.57

(d, *J* = 2.6 Hz, 1H), 6.53 (dd, *J* = 9.6, 2.5 Hz, 1H), 6.06 (t, *J* = 5.3 Hz, 1H), 5.46 (s, 1H), 4.08 (d,

J = 5.0 Hz, 2H), 3.85 (s, 3H), 3.75 (s, 3H), 3.56 (q, *J* = 5.6 Hz, 2H), 2.14 (t, *J* = 7.0 Hz, 2H),

1.96–1.90 (m, 4H), 1.62–1.50 (m, 4H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 168.4, 168.8, 162.0,

161.4, 155.5, 135.7, 134.4, 134.2, 133.1, 129.3, 128.8, 127.6, 126.3, 124.8, 124.0, 122.4, 112.8,

104.7, 99.8, 56.0, 55.8, 43.6, 37.6, 37.5, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): *m/z* calculated for

C$_{28}$H$_{31}$N$_3$O$_5$Na [M+Na]$^+$ 512.2161, found 512.2150

N–(2–((2–(cyclohex–1–en–1–yl)ethyl)amino)–2–oxoethyl)–2–(2–methoxy–4–methylphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.18.
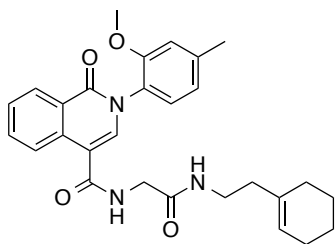
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid 6b (0.051 g, 0.17 mmol, 1.0 equiv) and amine **3.16a** (0.031 g, 0.17 mmol, 1.0 equiv) were used. The crude material was purified by flash chromatography using CH$_2$Cl$_2$:Et$_2$O to give amide **3.18** (0.075 g, 0.16 mmol, 96%)

as a white solid. R$_f$ = 0.18 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.47 (d, *J* = 8.0 Hz,

1H), 8.23 (d, *J* = 8.2 Hz, 1H), 7.72 (t, *J* = 7.1 Hz, 1H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.51 (s, 1H), 7.13

(d, *J* = 8.3 Hz, 1H), 6.86 – 6.83 (m, 3H), 6.08 (br s, 1H), 5.45 (s, 1H), 4.07 (d, *J* =5.0 Hz, 2H),

3.77 (s, 3H), 3.35 (q, *J* = 6.7 Hz, 2H), 2.41 (s, 3H), 2.13 (t, *J* = 6.8 Hz, 2H), 1.97 – 1.86 (m, 4H),

1.62 – 1.49 (m, 4H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 168.4, 166.8, 161.9, 154.3, 141.0, 135.5,

134.4, 134.2, 133.1, 128.8, 128.4, 127.6, 126.6, 126.3, 124.8, 124.0, 121.7, 113.1, 112.8, 55.9,

43.6, 37.6, 37.5, 28.0, 25.3, 22.9, 22.4, 21.9; HRMS (ESI): *m/z* calculated for C$_{28}$H$_{31}$N$_3$O$_4$Na

[M+Na]$^+$ 496.2212, found 496.2229.

2–(4–Bromo–2–methoxyphenyl)–N–(2–((2–(cyclohex–1–en–1–yl)ethyl)amino)–2–oxoethyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.19.
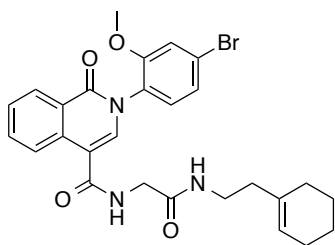
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid 6c (0.059 g, 0.15 mmol, 1.0 equiv) and amine **3.16a** (0.029 g, 0.16 mmol, 1.0 equiv) were used. The crude material was purified by flash chromatography using CH_2Cl_2:Et_2O to give amide **3.19** (0.078 g, 0.14 mmol, 95%) as a white solid. R_f = 0.16 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, J = 8.1 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.46 (s, 1H), 7.19–7.14 (m, 3H), 6.89 (br s, 1H), 6.05 (br s, 1H), 5.46 (s, 1H), 4.08 (d, J = 4.8 Hz, 2H), 3.79 (s, 3H), 3.35 (q, J = 6.2 Hz, 2H), 2.14 (t, J = 6.72 Hz, 2H), 1.97–1.90 (m, 4H), 1.62–1.50 (m, 4H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 168.4, 166.6, 161.6, 155.3, 134.7, 134.4, 134.2, 133.3, 130.1, 128.8, 128.3, 127.9, 126.2, 124.9, 124.2, 124.1, 123.9, 116.1, 113.3, 56.4, 43.5, 37.57, 37.56, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): m/z calculated for $C_{27}H_{28}BrN_3O_4Na$ [M+Na]$^+$ 560.1161, found 560.1158.

2–(4–Bromo–2–methoxyphenyl)–1–oxo–N–(2–oxo–2–(phenethylamino)ethyl)–1,2–dihydroisoquinoline–4–carboxamide, 3.20.
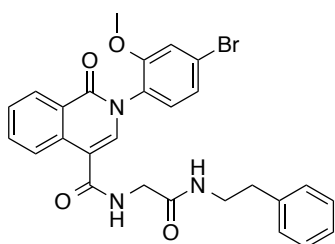
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid 6c (0.059 g, 0.16 mmol, 1.0 equiv) and amine **3.16b** (0.029 g, 0.16 mmol, 1.0 equiv) were used. The crude material was purified by flash chromatography using EtOAc:hex to give amide **3.20** (0.087 g, 0.16 mmol, >99%) as a white solid. R_f = 0.28 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, DMSO) δ 8.58 (t, J = 5.6 Hz, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.05 (t, J = 5.5 Hz, 1H), 7.81 (t, J = 8.1 Hz, 1H), 7.71 (s, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.48 (d, J = 1.6 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H),

7.35 (dd, *J* = 8.2 Hz, 1.8, 1H), 7.26 (t, *J* = 7.6 Hz, 2H), 7.22–7.16 (m, 3H), 3.82–3.78 (m, 5H), 3.3 (q, *J* = 7.0 Hz, 2H), 2.72 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (500 MHz, DMSO δ 168.8, 165.8, 160.2, 155.4, 139.4, 135.3, 134.6, 133.0, 130.6, 128.7, 128.3, 127.5, 127.4, 126.1, 125.3, 125.1, 123.6, 122.8, 115.9, 112.0, 79.2, 56.5, 42.4, 40.3, 35.2 HRMS (ESI): *m/z* calculated for C$_{27}$H$_{24}$BrN$_3$O$_4$Na [M+Na]$^+$ 556.0848, found 556.0844.

2–(4–Bromo–2–methoxyphenyl)–1–oxo–N–phenethyl–1,2–dihydroisoquinoline–4–carboxamide, 3.22.
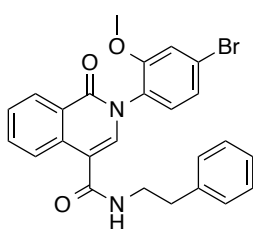
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid **3.7c** (0.058 g, 0.16 mmol, 1.0 equiv) and phenetyhlamine (0.019 g, 0.16 mmol, 1.0 equiv) were used. The crude material was purified by flash chromatography using EtOAc:hex to give amide **3.22** (0.067 g, 0.14 mmol, 92%) as a white solid. R$_f$ = 0.75 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.42 (d, *J* = 8.1, 1H), 8.0 (d, *J* = 8.2, 1H), 7.7 (t, *J* = 7.3, 1H), 7.52 (t, *J* = 7.8, 1H), 7.30–7.25 (m, 3H), 7.25–7.15 (m, 5H), 7.11 (d, *J* = 8.3, 1H), 5.94 (t, *J* = 5.5, 1H), 3.76–3.70 (m, 5H), 2.95 (t, 6.9, 2H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 166.3, 161.4, 155.2, 138.7, 134.1, 133.9, 133.2, 130.0, 128.9, 128.8, 128.6, 128.3, 127.7, 126.7, 126.1, 124.7, 124.1, 123.8, 116.0, 114.4, 56.2, 41.0, 35.5; HRMS (ESI): *m/z* calculated for C$_{25}$H$_{21}$BrN$_2$O$_3$Na [M+Na]$^+$ 499.0633, found 499.0637.

2–Chlorobenzyl (*S*)–(5–(2–(4–bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamido)–6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexyl)carbamate, 3.23.
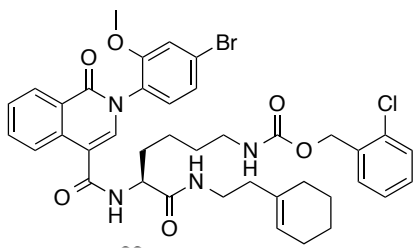
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid **3.7c** (0.058 g, 0.16 mmol, 1.0 equiv) and amine **3.16c** (0.068 g, 0.16 mmol, 1.0 equiv) were used. The crude material was

purified by flash chromatography using EtOAc:hex to give amide **3.23** (0.097 g, 0.12 mmol, 80%) as a white solid. R_f = 0.57 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.44 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 7.3 Hz, 1H), 7.53 (t, J =7.7 Hz, 1H), 7.41 (s, 1H), 7.35–7.32 (m, 2H), 7.26–7.13 (m, 5H), 6.80 (d, J = 7.8 Hz, 1H), 6.15 (t, J = 5.6 Hz, 1H), 5.44 (s, 1H), 5.12 (q, J = 9.3 Hz, 2H), 4.95 (s, J = 5.1 Hz, 1H), 4.57 (q, J = 6.5 Hz, 1H), 3.78 (s, 3H), 3.42–3.34 (m, 1H), 3.31–3.25 (m, 1H), 3.20 (q, J = 6.5 Hz, 2H), 2.13 (t, J = 6.5 Hz, 2H), 1.98–1.86 (m, 5H), 1.88–1.70 (m, 1H), 1.62–1.39 (m, 8H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 171.3, 166.3, 161.5, 156.5, 155.3, 134.43, 134.37, 134.27, 134.17, 133.6, 133.3, 130.1, 129.8, 129.6, 129.5, 128.8, 128.3, 127.8, 127.0, 126.1, 124.8, 124.2, 124.0, 116.1, 113.6, 64.1, 56.3, 53.4, 40.5, 37.6, 37.5, 32.7, 29.7, 28.0, 25.4, 22.9, 22.7, 22.4; HRMS (ESI): *m/z* calculated for C$_{39}$H$_{42}$BrClN$_4$O$_6$Na [M+Na]$^+$ 799.1874, found 799.1873.

Allyl (S)–(5–(2–(4–bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxa--mido)–6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexyl)carbamate, 3.25.
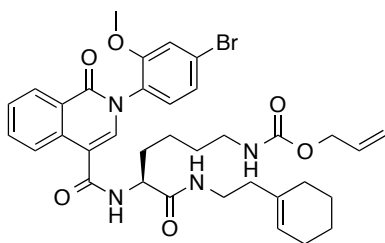
Using the general procedure for the formation of amides via acid chlorides outlined above, carboxylic acid **3.7c** (0.21 g, 0.55 mmol, 1.0 equiv) and amine **3.16d** (0.18 g, 0.55 mmol, 1.0 equiv) were used. The crude material was purified by flash chromatography using EtOAc:hex to give amide **3.25** (0.37 g, 0.53 mmol, 97%) as a white solid. R_f = 0.44 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.46 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.55 (t, J = 7.7 Hz, 1H), 7.41 (s, 1H), 7.22–7.15 (m, 3H), 6.83 (d, J = 7.6 Hz, 1H), 6.19 (br s, 1H), 5.87 – 5.78 (m, 1H), 5.45 (s, 1H), 5.23 (d, J = 17.0 Hz, 1H), 5.15 (d, J = 10.6 Hz, 1H), 4.86 (br s, 1H), 4.57 (q, J = 6.1 Hz, 1H), 4.49–4.39 (m, 2H), 3.80 (s, 3H), 3.45–3.35 (m, 1H), 3.31–3.25 (m, 1H), 3.20–3.14 (m, 2H), 2.13 (t, J = 6.6 Hz, 2H), 1.98–1.86 (m, 5H), 1.78–1.70 (m, 1H), 1.63–1.36

(m, 8H); ^{13}C 171.3, 166.3, 161.5, 156.6, 155.3, 134.42, 134.37, 134.2, 133.3, 132.9, 130.1, 128.8, 128.3, 127.8, 126.1, 124.8, 124.2, 124.0, 117.7, 116.1, 113.6, 65.6, 56.4, 53.4, 40.4, 37.6, 37.5, 32.6, 29.7, 27.9, 25.3, 22.9, 22.6, 22.4; NMR (500 MHz, CDCl$_3$) δ HRMS (ESI): *m/z* calculated for C$_{35}$H$_{41}$BrN$_4$O$_6$Na [M+Na]$^+$ 715.2107, found 715.2101.

N–(2–((2–(cyclohex–1–en–1–yl)ethyl)amino)–2–oxoethyl)–2–(4–(5–hydroxypent–1–yn–1–yl)–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.24.[124]
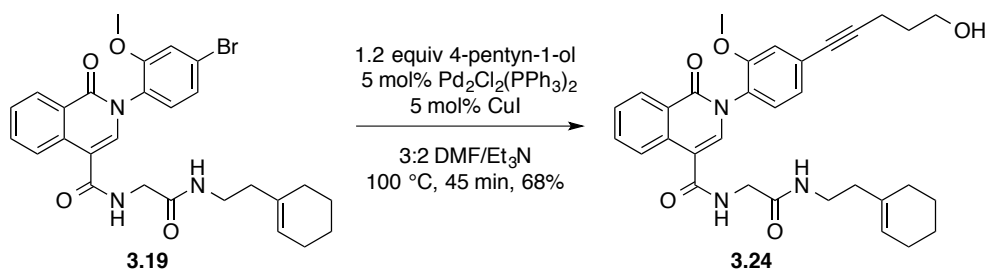
A flame–dried round bottom flask was charged with aryl bromide **3.19** (0.051 g, 0.095 mmol, 1.0 equiv). Next, 3:2 DMF/Et$_3$N (1 mL, 0.095 M with respect to **3.19**) and 4–pentyn–1–ol (0.010 mL, 0.11 mmol, 1.2 equiv) were added to the reaction flask via syringe and the reaction mixture was stirred for 3 min. Afterwords, CuI (0.90 mg, 0.0046 mmol, 5 mol%) and Pd$_2$Cl$_2$(PPh$_3$)$_2$ (3.3 mg, 0.0046 mmol, 5 mol%) were added quickly by lifting up the septum. Next, the flask was fitted with a reflux condenser and heated at reflux for 45 min. The reaction was monitored by TLC for disappearance of the aryl bromide. Upon consumption of **3.19**, the reaction mixture was cooled to room temperature and diluted with CH$_2$Cl$_2$ (10 mL). The organic layer was washed with water (5 x 10 mL) followed by brine and dried over Na$_2$SO$_4$. The resulting solution was concentrated *in vacuo* to give a red solid. The crude material was purified by flash chromatography (EtOAc:hex) to yield carboxamide **3.24** (0.035 g, 0.065 mmol, 68%) as a white solid. R$_f$ = 0.23 (4:1 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.46 (d, *J* = 8.3 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 8.1 Hz, 1H), 7.49 (s, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.06–7.04 (m, 2H), 6.93 (t, *J* = 5.0 Hz, 1H), 6.12 (t, *J* = 5.1 Hz, 1H), 5.45 (s,

1H), 4.07 (d, J = 5.4 Hz, 2H), 3.82 (t, *J* = 6.6 Hz, 2H), 3.77 (s, 3H), 3.35 (q, *J* = 5.9 Hz, 2H), 2.56

(t, 7.2, 2H), 2.14 (t, *J* = 6.9 Hz, 2H), 1.98–1.70 (m, 7H), 1.63–1.49 (m, 4H); ^{13}C 168.4, 166.7,

161.6, 154.3, 135.0, 134.4, 134.2, 133.2, 128.8, 128.73, 128.67, 127.8, 126.24, 126.18, 124.9,

124.4, 124.0, 115.4, 113.1, 91.0, 80.6, 61.8, 56.1, 43.6, 37.55, 37.53, 31.40, 28.0, 25.3, 22.9,

22.4, 16.1; NMR (500 MHz, CDCl$_3$) δ HRMS (ESI): *m/z* calculated for C$_{32}$H$_{35}$N$_3$O$_5$Na [M+Na]$^+$

564.2474, found 564.2480.

Methyl 5–bromo–2–(2–methoxy–2–oxoethyl)benzoate, 3.10.[125]
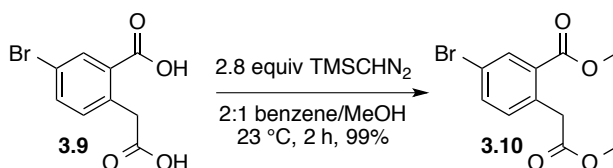
An oven–dried round bottom flask was charged with dicarboxylic acid **3.9** (0.99 g, 3.9

mmol, 1.0 equiv) and a stir bar. Next, 2:1 Benzene/MeOH (29 mL, 0.13 M with respect to **3. 9**)

was added to the reaction flask via syringe. Afterwords, TMSCHN$_2$ (2.0 M in Et$_2$O, 5.5 mL, 11

mmol, 2.8 equiv) was added drop wise to the reaction flask via syringe over 2 h. The reaction

was monitored by TLC for disappearance of **3.9**. Upon consumption of the dicarboxylic acid, the

reaction mixture was concentrated *in vacuo* to afford a pale yellow oil. The crude material was

purified by flash chromatography (EtOAc:hex) to yield diester **3.10** (1.1 g, 3.8 mmol, >99%) as a

clear oil. R$_f$ = 0.86 (9:1 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.15 (s, 1H), 7.61 (d, *J* =

8.2 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 3.97 (s, 2H), 3.88 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (500

MHz, CDCl$_3$) δ 171.6, 166.3, 135.4, 135.1, 134.1, 134.0, 131.4, 121.3, 52.5, 52.2, 40.0; HRMS

(ESI): *m/z* calculated for C$_{11}$H$_{11}$O$_4$Na [M+Na]$^+$ 308.9738, found 308.9739.

Methyl 5-(5-hydroxypent-1-yn-1-yl)-2-(2-methoxy-2-oxoethyl)benzoate.[126]
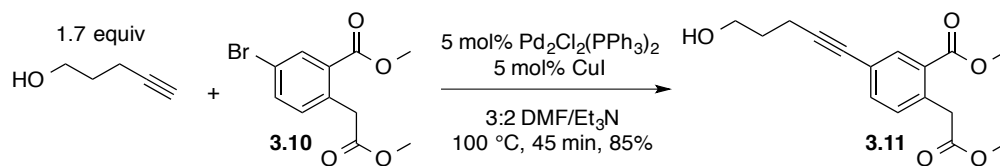
A flame–dried round bottom flask was charged with diester **3.10** (0.050 g, .17 mmol, 1.0 equiv) and a stir bar. Next, 3:2 DMF/Et$_3$N (1.9 mL, 0.095 M with respect to **3.9**) and 4–pentyn–1–ol (0.019 mL, 0.21 mmol, 1.2 equiv) were added to the reaction flask via syringe. Then, the reaction mixture was stirred for 3 min. Afterwords, CuI (0.017 g, 0.0087 mmol, 5 mol%) and Pd$_2$Cl$_2$(PPh$_3$)$_2$ (0.0061 g, 0.0087 mmol, 5 mol%) were added quickly by lifting up the septum. Next, the flask was fitted with a reflux condenser and heated at reflux for 45 min. The reaction mixture was monitored by TLC for disappearance of **3.10**. Upon consumption of **3.10**, the reaction mixture was cooled to room temperature and diluted with CH$_2$Cl$_2$ (10 mL). The organic layer was washed with water (5 x 10 mL) followed by brine and dried over Na$_2$SO$_4$. The resulting solution was concentrated *in vacuo* to afford a red oil. The crude material was purified by flash chromatography (EtOAc:hex) to yield alkyne product (0.041 g, 0.14 mmol, 81%) as a red oil. R$_f$ = 0.43 (1:1 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.04 (d, *J* = 1.7 Hz, 1H), 7.48 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 3.99 (s, 2H), 3.87 (s, 3H), 3.82 (br s, 2H), 3.69 (s, 3H), 2.55 (t, *J* = 7.0 Hz, 2H), 1.87 (quin, *J* = 6.5 Hz, 2H), 1.57 (br s, 1H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 171.9, 167.1, 135.4, 135.2, 134.3, 132.4, 129.8, 123.4, 90.7, 80.0, 61.8, 52.3, 52.2, 40.41, 31.4, 16.1; HRMS (ESI): *m/z* calculated for C$_{16}$H$_{18}$O$_5$Na [M+Na]$^+$ 313.1052, found 313.1060.

Methyl 5–(5–((tert–butyldimethylsilyl)oxy)pent–1–yn–1–yl)–2–(2–methoxy–2–oxoethyl)benzoate, 3.11.
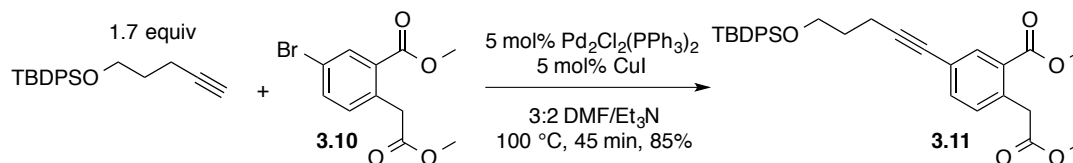
A flame–dried round bottom flask was charged with diester **3.10** (0.75 g, 2.6 mmol, 1.0 equiv) and a stir bar. Next, 3:2 DMF/Et₃N (28 mL, 0.095 M with respect to **3.10**) and tert–butylpent–4–ynyloxydiphenylsilane (1.4 g, 4.3 mmol, 1.7 equiv) were added to the reaction flask via syringe. Then, the reaction mixture was stirred for 3 min. Afterwards, CuI (0.025 g, 0.13 mmol, 5 mol%) and Pd₂Cl₂(PPh₃)₂ (0.092 g, 0.13 mmol, 5 mol%) were added quickly by lifting up the septum. Next, the flask was fitted with a reflux condenser and heated at reflux for 45 min. The reaction mixture was monitored by TLC for disappearance of **3.10**. Upon consumption of **3.10**, the reaction mixture was cooled to room temperature and diluted with CH₂Cl₂ (50 mL). The organic layer was washed with water (5 x 50 mL) followed by brine and dried over Na₂SO₄. The resulting solution was concentrated *in vacuo* to afford a red oil. The crude material was purified by flash chromatography (EtOAc:hex) to yield alkyne **3.11** (1.2 g, 2.2 mmol, 85%) as a red oil. R_f = 0.49 (1:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl₃) δ 8.02 (d, J =1.7 Hz, 1H), 8.03–8.00 (m, 4H), 7.45–7.35 (m, 7H), 7.2 (d, J = 8.1 Hz, 1H), 3.99 (s, 2H), 3.87 (s, 3H), 3.81 (t, J = 6.1 Hz, 2H), 3.70 (s, 3H), 2.57 (t, J = 7.2 Hz, 2H), 1.85 (quin, J = 7.2 Hz, 2H), 1.06 (s, 9H); ^{13}C NMR (500 MHz, CDCl₃) δ 171.9, 167.1, 135.7, 135.24, 135.22, 134.3, 133.9, 132.4, 129.74, 129.71, 127.8, 123.7, 91.3, 79.7, 62.5, 52.3, 52.2, 40.4, 31.6, 27.0, 19.4, 16.1; HRMS (ESI): *m/z* calculated for C₃₂H₃₆O₅SiNa [M+Na]⁺ 551.2230, found 551.2228.

2-(Carboxymethyl)-5-(5-hydroxypent-1-yn-1-yl)benzoic acid, 3.12a.[127]
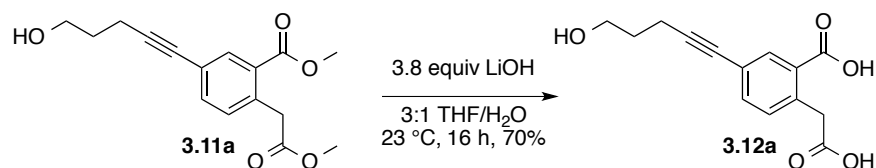
A round bottom flask was charged with alkyne **3.11a** (0.040 g, 0.14 mmol, 1.0 equiv) and

a stir bar. Next, THF (1.3 mL, 0.11 M with respect to **3.10a**) and LiOH (1.4 M in water, 0.39

mL, 0.53 mmol, 3.8 equiv) was added to the flask via syringe. The reaction mixture was

monitored by TLC for disappearance of **3.11a**. After consumption of **3.11a** overnight, 4 mL of 1

N HCl and 6.5 mL of EtOAC were added to the reaction mixture. The mixture was extracted

with EtOAc (3 x 10 mL). The combined organic layers were washed with brine and dried with

Na_2SO_4. The resulting solution was concentrated *in vacuo* to yield dicarboxylic acid **3.12a** (0.025

g, 0.098 mmol, 70 %) as a brown solid. R_f = 0.30 (8:2:1 CH_2Cl_2:MeOH:AcOH). ^{1}H NMR (500

MHz, DMSO) δ 12.64 (br s, 2H), 7.86 (d, *J* = 1.7 Hz, 1H), 7.52 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.33

(d, *J* = 8.1 Hz, 1H), 4.58 (s, 1H), 3.95 (s, 2H), 3.54 (t, *J* = 6.2 Hz, 2H), 2.50 (t, *J* = 7.1 Hz, 2H),

1.71 (quin, *J* = 6.6 Hz, 2H); ^{13}C NMR (500 MHz, DMSO) δ 172.2, 167.6, 136.2, 134.1, 133.0,

132.8, 131.0, 122.1, 91.4, 79.4, 59.4, 31.5, 15.3; HRMS (ESI): *m/z* calculated for $C_{14}H_{13}O_5$ [M −

H]$^-$ 261.0763, found 261.0769.

5–(5–(((*Tert*–butyldimethylsilyl)oxy)pent–1–yn–1–yl)–2–(carboxymethyl)benzoic acid, 3.12b.
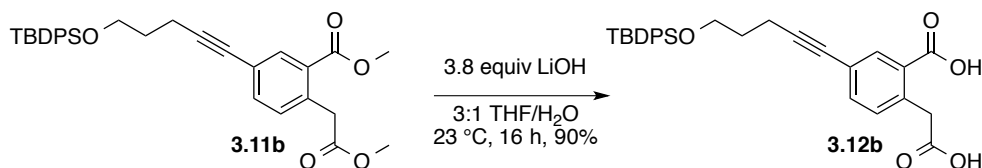
A round bottom flask was charged with alkyne **3.11b** (0.73 g, 1.4 mmol, 1.0 equiv) and a

stir bar. Next, THF (13 mL, 0.11 M with respect to **3.11b**) and LiOH (1.4 M in water, 3.9 mL,

5.3 mmol, 3.8 equiv) was added to the flask via syringe. The reaction mixture was monitored by TLC for disappearance of **3.11b**. After consumption of **3.11b** overnight, 40 mL of 1 N HCl and 65 mL of EtOAC were added to the reaction mixture. The mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine and dried with Na_2SO_4. The resulting solution was concentrated *in vacuo* to yield dicarboxylic acid **3.12b** (0.62 g, 1.2 mmol, 90%) as a brown solid. R_f = 0.46 (17:3 CH_2Cl_2:MeOH). ^{1}H NMR (500 MHz, DMSO) δ 13.2–12.0 (br s, 2H), 7.82 (s, 1H), 7.66–7.61 (m, 4H), 7.46–7.36 (m, 7H), 7.30 (d, J = 7.8 Hz, 1H), 3.93 (s, 2H), 3.79 (t, J = 6.0 Hz, 2H), 2.57 (t, J = 7.0 Hz, 2H), 1.81 (quin, J = 6.6 Hz, 2H), 1.01 (s, 9H); ^{13}C NMR (500 MHz, DMSO) δ 172.2, 167.6, 136.3, 135.0, 134.5, 134.1, 133.1, 133.0, 132.8, 129.9, 127.9, 121.9, 90.7, 79.7, 62.0, 30.9, 26.7, 18.8, 15.2; HRMS (ESI): *m/z* calculated for $C_{30}H_{31}O_5Si$ [M − H]$^-$ 499.1941, found 499.1953.

Tert–butyl (2–oxo–2–(phenethylamino)ethyl)carbamate, 3.15b.[128]
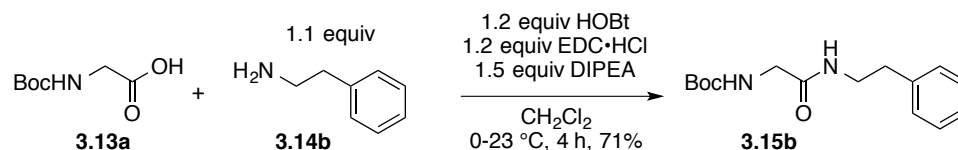
An oven–dried round bottom flask was charged with Boc–glycine **3.13a** (0.64 g, 3.7 mmol, 1.0 equiv), HOBt (0.59 g, 4.3 mmol, 1.2 equiv) and a stir bar. Next, CH_2Cl_2 (90 mL, 0.41 M with respect to **3.13a**) was added to the reaction vessel followed by phenethylamine (0.50 mL, 3.97 mmol, 1.1 equiv). The reaction vessel was cooled to 0 °C with an ice bath and then EDC•HCl (0.83 g, 4.33 mmol, 1.2 equiv) was added by lifting the septum. Next, DIPEA (0.95 mL, 5.6 mmol, 1.5 equiv) was added to the reaction vessel via syringe and the reaction mixture was stirred for 1 h. Afterwards, the reaction vessel was warmed to room temperature. The reaction was monitored for disappearance of **3.13a** by TLC (4 h). Upon consumption of **3.13a**, the reaction mixture was washed with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 100

mL). The combined organic layers were washed with brine and dried with Na_2SO_4. The resulting

solution was concentrated *in vacuo* to afford a yellow oil and purified with flash chromatography

(EtOAc/hex) to yield amide **3.15b** (0.11 g 71%) as a yellow oil. R_f = 0.57 (17:3 CH_2Cl_2:MeOH).

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.31 (t, *J* = 7.3 Hz, 2H), 7.25–7.18 (m, 3H), 6.12 (s, 1H), 5.09 (s,

1H), 3.74 (d, *J* = 5.7 Hz, 2H), 3.54 (q, *J* = 6.6 Hz, 2H), 2.82 (t, *J* = 7.1 Hz, 2H), 1.43 (s, 9H);

HRMS (ESI): *m/z* calculated for $C_{15}H_{22}N_2O_3Na$ [M+Na]$^+$ 301.1528, found 301.1531.

2–Amino–*N*–phenethylacetamide, 3.16b.
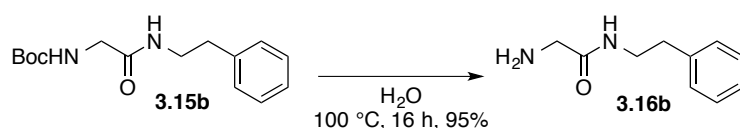
A round bottom flask was charged with Boc–protected amine **3.15b** (0.25 g, 0.89 mmol,

1 equiv) and a stir bar. Next, water (20 mL, 0.045 M with respect to **3.15b**) was added to the

flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux

for 16 h and monitored by TLC for disappearance of **3.15b**. Upon consumption of **3.15b**, The

reaction mixture was cooled to room temperature. Next, the aqueous layer was extracted with

CH_2Cl_2 (3 x 20 mL) and the combined organic layers were concentrated *in vacuo* to give amine

3.16b (0.91 g, 0.85 mmol, 95%) as a yellow oil. R_f = 0 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz,

CDCl$_3$) δ 7.34–7.18 (m, 6H), 3.55 (q, *J* = 6.7 Hz, 2H), 3.32 (s, 2H), 2.84 (t, *J* = 7.1 Hz, 2H), 1.40

(s, 2H); HRMS (ESI): *m/z* calculated for $C_{10}H_{14}N_2ONa$ [M+Na]$^+$ 201.1004, found 201.1002.

2–Chlorobenzyl (S)–(5–amino–6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexyl)carba-
-mate, 3.16c.[129]
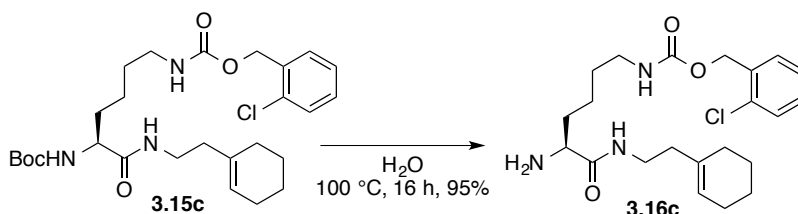
An oven–dried round bottom flask was charged with the Boc–protected amine **3.15c** (0.66 g, 1.3 mmol, 1 equiv) and a stir bar. Next, ethylene glycol (28 mL, 0.046 M with respect to **3.15c**) was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 16 h and monitored by TLC for disappearance of the Boc–protected amine. Upon consumption of the Boc–protected amine (20 min), the reaction mixture was cooled to room temperature and diluted with CH_2Cl_2 (50 mL). Next, the organic layer was washed with water (5x100 mL) followed by brine and dried over Na_2SO_4. The resulting solution was concentrated *in vacuo* to give amine **3.16c** (0.39 g, 0.96 mmol, 74%) as a yellow oil. R_f = 0 (6:4 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.43–7.35 (m, 2H), 7.26–2.20 (m, 3H), 5.45 (s, 1H), 5.25–5.17 (m, 3H), 4.92 (br s, 1H), 3.34–3.29 (m, 2H), 3.21 (q, J = 6.5 Hz, 2H), 2.13 (t, J = 6.8 Hz, 2H), 2.02–1.90 (m, 4H), 1.85–1.78 (m, 1H), 1.64–1.39 (m, 11H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 174.8, 156.4, 134.8, 134.5, 133.6, 129.8, 129.6, 129.4, 127.0, 123.6, 64.0, 55.2, 40.9, 37.9, 37.0, 34.8, 29.9, 28.0, 25.4, 23.0, 22.9, 22.5; HRMS (ESI): *m/z* calculated for $C_{22}H_{32}ClN_3O_3H$ [M + H]$^+$ 422.2210, found 422.2216.

Allyl *tert*–butyl (6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexane–1,5–diyl) (*S*)–dicarbamate, 3.15d.
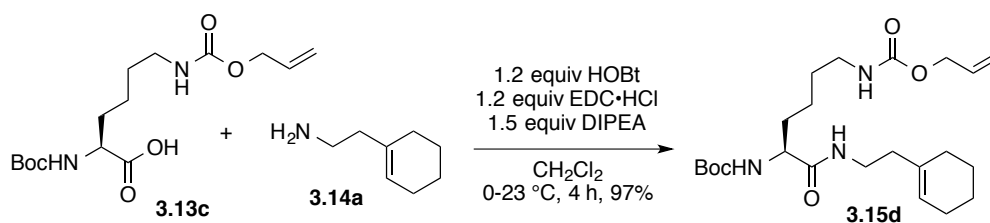
Following the same procedure for the amide synthesis of **3.16b**, carboxylic acid **3.13c** (1.0 g, 3.0 mmol, 1.0 equiv) and amine **3.14a** (0.42 g, 3.32 mmol, 1.1 equiv) were used. The crude material was purified by flash chromatography using EtOAc:hex to give amide **3.15d** (1.17 g, 2.9 mmol, 97%) as a yellow oil. R_f = 0.76 (7:3 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ

6.03 (br s, 1H), 5.96–5.87 (m, 1H), 5.46 (s, 1H), 5.30 (dd, J = 17.2, 1.6 Hz, 1H), 5.25 (dd, J = 10.3, 1.1, 1H), 5.09 (s, 1H), 4.81 (s, 1H), 4.55 (d, J = 4.7 Hz, 2H), 4.01 (s, 1H), 3.38–3.24 (m, 2H), 3.18 (d, J = 6.1 Hz, 2H), 2.12 (t, 7.0, 2H), 2.01–1.88 (m, 4H), 1.86–1.78 (m, 1H), 1.64–1.48 (m, 7H), 1.44 (s, 9H), 1.40–1.33 (m, 2H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 171.9, 156.6, 155.9, 134.6, 133.1, 123.8, 117.8, 80.16, 65.6, 54.5, 40.5, 37.7, 37.3, 32.2, 29.7, 28.5, 28.0, 25.4, 22.9, 22.6, 22.5; HRMS (ESI): m/z calculated for C$_{23}$H$_{39}$N$_3$O$_5$Na [M+Na]$^+$ 460.2787, found 460.2790.

Allyl (*S*)–(5–amino–6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexyl)carbamate, 3.16d.
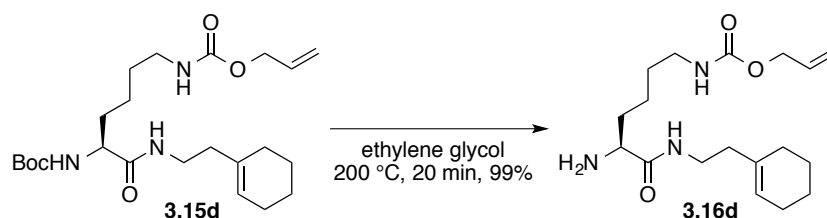
Following the same procedure for the amine synthesis of **3.16c**, Boc-protected amine **3.15d** (0.69 g, 1.6 mmol, 1.0 equiv) and ethylene glycol (27 mL, 0.046 M with respect to **3.15d**) were used. The amine product **3.16d** (0.43 g, 1.6 mmol, 99%) was isolated as a yellow oil. R$_f$ = 0.36 (1:1 EtOAc:hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.22 (br s, 1H), 5.94–5.89 (m, 1H), 5.46 (s, 1H), 5.30 (d, J = 17.2 Hz, 1H), 5.21 (d, J = 10.4 Hz, 1H), 4.90 (s, 1H), 4.55 (d, J = 5.3 Hz, 2H), 3.36–3.28 (m, 3H), 3.19 (q, J = 6.4 Hz, 2H), 2.13 (t, J = 6.4 Hz, 2H), 2.01–1.90 (m, 4H), 1.85–1.78 (m, 1H), 1.65–1.35 (m, 11H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 174.8, 156.5, 134.8, 133.1, 123.5, 117.7, 65.5, 55.2, 40.7, 37.8, 36.9, 34.8, 29.9, 28.0, 25.4, 22.94, 22.92, 22.5; HRMS (ESI): m/z calculated for C$_{18}$H$_{31}$N$_3$O$_3$Na [M+Na]$^+$ 360.2263, found 360.2259.

(*S*)–*N*–(6–amino–1–((2–(cyclohex–1–en–1–yl)ethyl)amino)–1–oxohexan–2–yl)–2–(4–bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.26.[130]
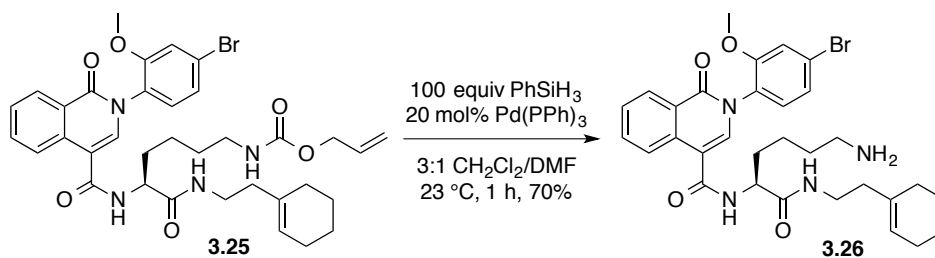
A flame–dried round bottom flask was charged with alloc protected amine **3.25** (0.20 g, 0.29 mmol, 1.0 equiv) and a stir bar. Next, 3:1 CH$_2$Cl$_2$/DMF (40 mL, 0.0073 M with respect to **3.25**) was added to the flask via syringe. Afterwords, phenylsilane (3.6 mL, 2.9 mmol, 100 equiv) was added to the flask drop wise via syringe over 3 min. After 15 min, Pd(PPh$_3$)$_4$ (0.067 g, 0.060 mmol, 20 mol%) was added by lifting up the septum. The reaction was monitored by TLC for disappearance of **3.25**. Upon consumption of the **3.25** (1 h), the reaction mixture was cooled to room temperature. Next, the organic layer was washed with water (5x100 mL) followed by brine and dried over Na$_2$SO$_4$. The resulting solution was concentrated *in vacuo* and purified with flash chromatography (CH$_2$Cl$_2$:MeOH) to yield amine **3.26** (0.12g, 0.20 mmol, 70%) as a brown solid. R$_f$ = 0.75 (5:1 Acetone: CH$_2$Cl$_2$). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.42 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.70, (t, *J* = 7.7 Hz, 1H), 7.54–7.32 (m, 4H), 7.25–7.11 (m, 3H), 5.40 (s, 1H), 4.70 (q, *J* = 5.8 Hz, 1H), 3.77 (s, 3H), 3.35–3.16 (m, 2H), 2.95 (br s, 3H), 2.64 (d, *J* = 14.4 Hz, 1H), (br s, 2H), 1.94–1.43 (m, 14H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 171.5, 166.8, 161.5, 155.3, 134.8, 134.6, 134.1, 133.3, 130.2, 128.8, 128.3, 127.8, 2.10 126.0, 124.7, 124.3, 124.0, 123.4, 116.1, 113.0, 56.4, 53.2, 39.4, 38.0, 37.7, 32.6, 28.1, 26.9, 25.4, 23.0, 22.6, 22.4; HRMS (ESI): *m/z* calculated for C$_{31}$H$_{37}$BrN$_4$O$_4$H [M + H]$^+$ 609.2076, found 609.2076.

**Tert–butyl (*S*)–(6–((6–((5–(2–(4–bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquin-
-oline–4–carboxamido)–6–((2–(cyclohex–1–en–1–yl)ethyl)amino)–6–oxohexyl)amino)–6–
oxohexyl)amino)–6–oxohexyl)carbamate, 3.29.**
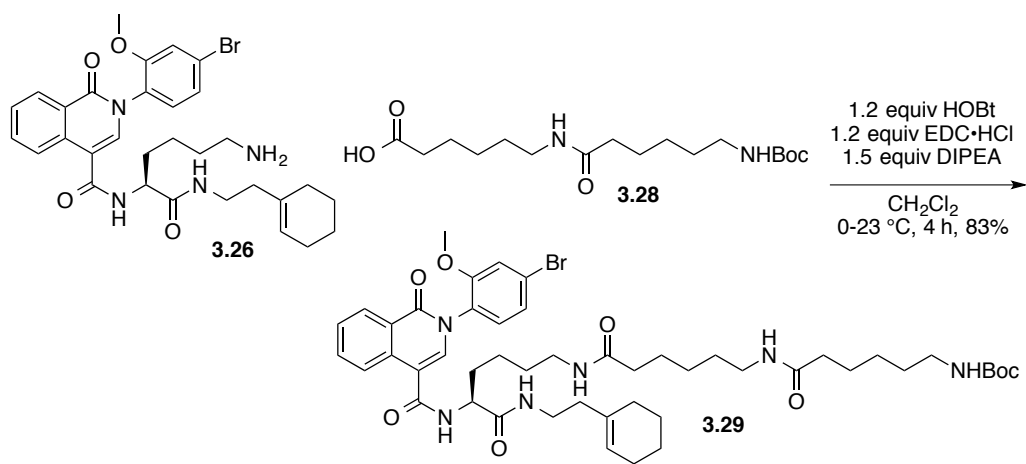
Following the same procedure for the synthesis of amide **3.16b**, amine **3.26** (0.050 g, 0.082 mmol, 1.0 equiv) and carboxylic acid **3.28** (0.026 g, 0.075 mmol, 1.0 equiv), were used. The crude material was purified by flash chromatography using CH$_2$Cl$_2$:MeOH to give amide **3.29** (0.058 g, 0.068 mmol, 83%) as a yellow solid. R$_f$ = 0.37 (9:1 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.44 (d, *J* = 8.2 Hz, 1H), 8.20 (br s, 1H), 7.73 (t, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.46 (s, 1H), 7.25–7.15 (m, 3H), 6.73 (br s, 1H), 6.19 (br s, 1H), 6.02 (br s, 1H), 5.42 (s, 1H), 4.70 (s, 1H), 4.57 (s, 1H), 3.79 (s, 3H), 3.41 – 3.03 (m, 8H), 2.15–1.12 (m, 42); ^{13}C NMR (500 MHz, CDCl$_3$) δ 173.5, 173.2, 171.6, 165.5, 161.6, 156.2, 155.3, 134.6, 134.3, 133.3, 130.0, 128.6, 128.4, 127.9, 127.8, 126.0, 124.9, 124.2, 123.9, 123.6, 116.1, 114.0, 133.6, 79.2, 56.3, 53.5, 40.5, 39.1, 37.7, 37.6, 36.6, 36.4, 29.9, 29.8, 29.2, 29.1, 28.5, 28.0, 26.5, 26.3, 25.5, 25.3, 25.2, 22.9, 22.7, 22.5; NMR (500 MHz, CDCl$_3$) δ HRMS (ESI): *m/z* calculated for C$_{48}$H$_{67}$BrN$_6$O$_8$Na [M+Na]$^+$ 957.4102, found 957.4105.

(S)–N–(6–(6–(6–aminohexanamido)hexanamido)–1–((2–(cyclohex–1–en–1–yl)ethyl)amino)–1–oxohexan–2–yl)–2–(4–bromo–2–methoxyphenyl)–1–oxo–1,2–dihydroisoquinoline–4–carboxamide, 3.30.
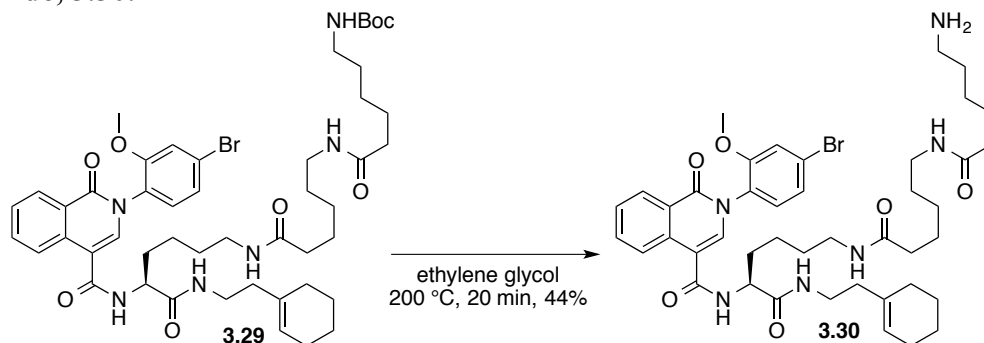
Following the same procedure for the synthesis of amine **3.15ba**, Boc–protected amine **3.29** (0.050 g, 0.053 mmol, 1.0 equiv) and ethylene glycol (1.2 mL, 0.044 M with respect to 32) were used. The amine product **3.30** (0.019 g, 0.023 mmol, 44%) was isolated as a yellow solid. R_f = 0.32 (47:3 CH$_2$Cl$_2$:MeOH). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.45 (d, J = 7.6 Hz, 1H), 8.2 (br s, 1H), 7.74 (br s, 1H), 7.55 (br s, 1H), 7.46 (s, 1H), 7.26–7.10 (m, 3H), 6.81 (br s, 1H), 6.72 (br s, 1H), 6.07 (br s, 1H), 5.98 (br s, 1H), 5.44 (s, 1H), 4.57 (br s, 1H), 4.10 (br s, 1H), 3.80 (s, 1H), 3.80–3.10 (m, 8H), 2.70–1.10 (m, 33); ^{13}C NMR (500 MHz, CDCl$_3$) δ 174.3, 171.6, 168.8, 161.6, 155.4, 153.4, 134.6, 134.3, 133.3, 130.1, 128.7, 128.4, 127.9, 127.8, 124.9, 124.3, 124.0, 123.7, 116.1, 114.0, 56.4, 53.4, 39.1, 37.7, 37.6, 36.7, 36.5, 32.1, 31.1, 29.84, 29.80, 29.3, 28.0, 26.5, 26.3, 25.5, 25.3, 25.2, 22.9, 22.8, 22.4; HRMS (ESI): *m/z* calculated for C$_{43}$H$_{59}$BrN$_6$O$_6$H [M + H]$^+$ 835.3758, found 835.3785.

Biological evaluations

Strains, media, and compounds: The *C. albicans* strain HLY4123 was used as the susceptible laboratory strain for the antifungal evaluation in this study. HLY4123 carries a GFP reporter for ERG3 expression and was constructed by plasmid transformation of the commonly used laboratory *C. albicans* strain CAI4. The strains were cultured at 30 °C under constant shaking (200rpm) in synthetic complete (SC) medium containing 2% glucose. The stock solution

of fluconazole (Sigma–Aldrich, USA) was prepared in sterile H_2O (0.1 mg/mL), whereas the other test compounds were prepared in DMSO.

Dose–response curves for test compounds against *C. albicans* with and without fluconazole:

C. albicans was grown in SC medium overnight and then diluted to an effective OD600 of 0.0625. Serial ten- fold dilutions of the test compounds (0.15–1500 μm) were prepared in DMSO in 1.5 mL Eppendorf tubes. To each well in columns B–D (triplicate analysis) of a 24-well Palcon plate was added 2.5 μL of fluconazole solution. To each well in all four columns of the plate was added 1 mL of cells in SC medium such that column A served as a control to assess the EC50 value of the compound in the absence of fluconazole. Then to each well in rows 2–5 was added a solution of the compound in DMSO (2 μL each) such that the final fluconazole concentration in columns 2–4 was 0.25 μg/mL, and the concentration of compound in each row varied from 0.003 to 30 μm. The plates were incubated in a rotary shaker/incubator at 30 °C for 16 h. The contents of each well were re-suspended with a micropipettor and a 20 μL aliquot was added to a polystyrene cuvette and diluted with 680 μL deionized water. The suspension was triturated again immediately before measuring the absorbance at λ = 600 nm (OD600) for cell densities. EC50 values were determined by fitting to a standard curve using the Excel- based tool ED50PLUS v. 1.0 (Mario H. Vargas).

Chapter 4

Reactivity of Palladium-Carbene Intermediates

Introduction

Transition metal-catalyzed reactions play a vital role in synthetic organic chemistry as one of the most powerful and direct ways to access complex molecules. Among many transition metals, palladium catalysts and reagents have proved to be more useful and versatile in organic synthesis.[131] The facility of oxidative addition under mild reaction conditions, the tolerance of many polar functional groups and the high degree of chemo-, regio- and steroselectivity makes Pd-catalysis uniquely suitable for an array of organic transformations.[131] Therefore, novel reactive intermediates involving palladium catalysis allow unique bond disconnections that will be enormously useful in complex molecule synthesis.[132] In this regard, palladium-carbene intermediates provide a very interesting starting point to explore.

Palladium-carbenes can potentially be coupled with a variety of other reactions accessible to Pd, such as oxidative addition, reductive elimination, and migratory insertion.[131] This provides an opportunity to incorporate the carbene units, during other palladium mediated transformations in modular synthesis of complex molecules.[132] Recent work in this research area utilizes such transient carbene units in multi-component coupling reactions as cross-coupling partners.[133]

This chapter discusses the synthetic methods and reactivity of palladium-carbenes and their utility in organic synthesis.

Palladium-Carbenes

Metal-carbenes are organometalic complexes that consist of divalent carbon ligands. There are two extremes of metal-carbenes: Fischer carbenes and Schrock carbenes (Figure 4-1). Fisher carbenes arise from two different bonding interactions. One interaction is between a filled

d_{yz} orbital of the metal and an empty p-orbital of the carbon and the second interaction occurs between a filled sp^2 orbital of the carbon and an empty d_z^2 orbital of the metal. Middle to late transition metals with low oxidation states prefer to make Fisher carbenes and they are generally eloctrophilic in the carbene carbon. The π-donor substituents on the carbene carbon geneally stabilize the metal-carbene complex in fisher carbenes. Conversely, Schrock carbenes are nucleophilic in the carbene center.

Figure 4-1: Depictions of Fischer and Schrock Carbenes

Fischer carbenes

low oxidation state metals
middle to late transition metals
π–acceptor metal ligands
π–donor substituents on the carbene C

Schrock carbenes

High oxidation state metals
early transition metals
non π–acceptor metal ligands
non π–donor substituents on the carbene C
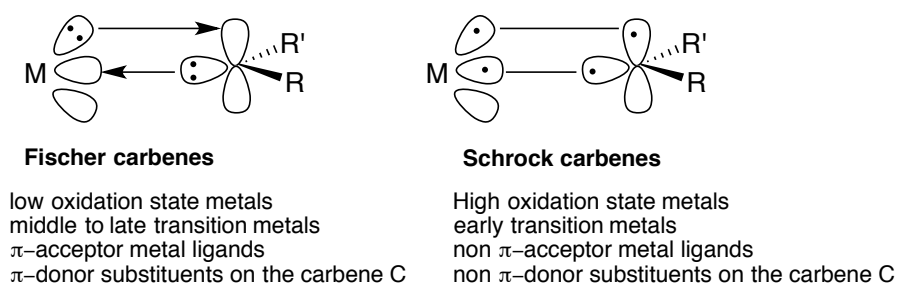
Palladium carbenes are classified as a Fischer carbene because palladium is a late transition metal and exhibit electrophilic character at the carbene carbon. Carbenes arising from palladium(0) intermediates show distinct reactivities from alkylpalladium(II) carbenes. Palladium(0) carbene intermediates efficiently participate in cyclopropanation reactions,[134] ylide formations,[135] and C–H insertions (Figure 4-2).[136] Palladium-catalyzed cyclopropanation reactions that proceed via carbene intermediates involve an initial palladacyclobutane formation followed by a reductive elimination to generate a cyclopropane. The cyclopropanation reaction is generally stereospecific. Unlike rhodium(II) acetate-catalyzed cyclopropanation reactions palladium-catalyzed reactions go through unimolecular migratory insertion. Therefore the efficiency of the reaction depends on the ligand's ability to ligate to palladium(0).

Figure 4-2: Pd(0)-Carbene Catalyze Cyclopropanations , C–H insertions and Ylide Formations
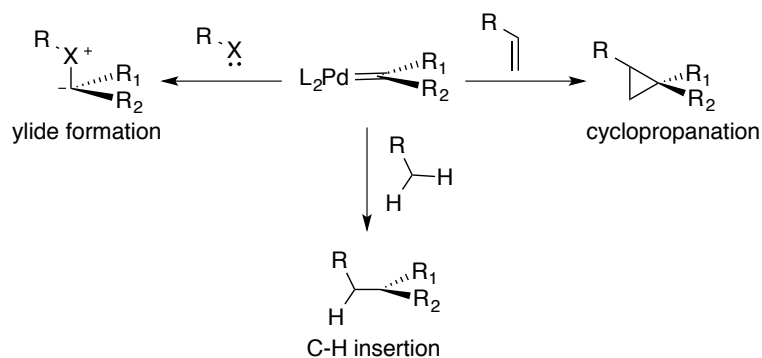
Palladium(II) carbene intermediates comprise of a more electrophilic carbene carbon than the carbene carbon in a palladium(0) carbene intermediates. Additionally, palladium(II)-carbene complexes generally contain anionic ligands that rapidly migrate to the electron-deficient carbene carbon. The resulting alkylpalladium complex can be trapped with nucleophiles or undergo β-hydride elimination. For the past ten years reactions exploiting the migratory insertions in alkylpalladium(II) complexes have become a very exciting field of exploration.[137]

Palladium-Catalyzed Carbenylation

Migratory insertion is one of the most important elementary reactions in organomettalic chemistry, especially in Pd-catalyzed transformations. In carbopalladation, the key step in Heck reaction, a two-carbon unit inserts into a Pd–C bond, whereas in carbonylation with CO and carbenylation a one-carbon unit inserts into a Pd–C bond (Figure 4-3). Carbonylation is utilized as a powerful tool for inserting an sp^2 carbon during related transformations. Carbenylation also offers a way to insert an sp^3 carbon; unlike carbonylation, carbenylation provides a new and powerful mode of enantioselective insertion of the one-carbon unit. Therefore, exploiting new methods of carbenylation that leads to C–C bond formation would tremendaously benefit synthetic organic chemistry.[138]

Figure 4-3: Comparison of Migratory Insertions
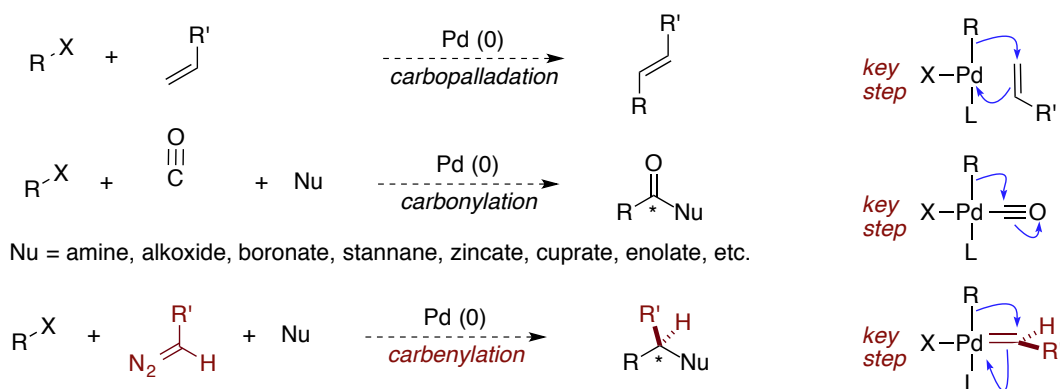
In 2001, Van Vranken and co-workers demonstrated the first example of catalytic cross-coupling reaction involving Pd-carbenes (Figure 4-4).[139] It is hypothesized that the formation of benzylpalladium(II) carbene intermediate **b** is initiated by the oxidative addition of the benzyl halide to form **a** followed by the trapping by trimethylsilyldiazomethane. Intermediate **b** undergoes migratory insertion to give alkylpalladium **c**, which readily undergoes β-hydride elimination to form the palladium•olefin complex **d**. subsequent hydride transfer and protodesilylation leads to the styrene adduct. Although the reaction has a few limiations with the scope and the efficiency, it provided an excellent avenue to explore effective transformations involving migratory insertions into palladium-carbenes.[133]

Figure 4-4: Pd-Catalyzed Carbenylative Coupling Between Aryl Halides and TMSCHN$_2$
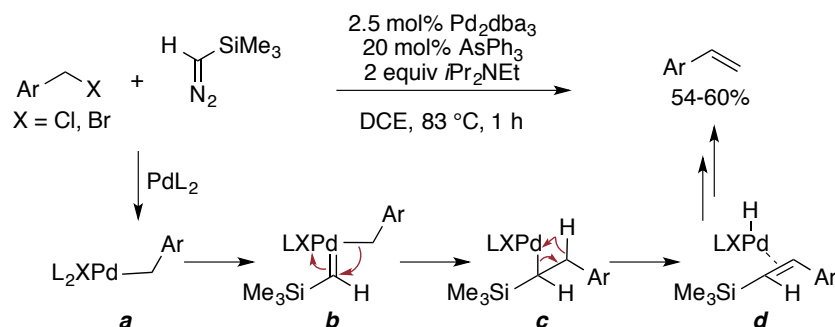
Palladium-Carbenes to π-Allylpalladium Intermediates

The Van Vranken group has discovered that when a vinyl moiety migrates to a carbene center (intermediate f) it gives rise to an η^1-allylpalladium intermediate (intermediate g). Upon isomerizing to η^3-allylpalladium intermediate (intermediate h) it can be trapped with nucleophiles to afford chiral vinylsilane adducts in a single step.[140,141]

Figure 4-5: Proposed Mechnism for the Nucleophilic Trapping after Carbenylative Insertion
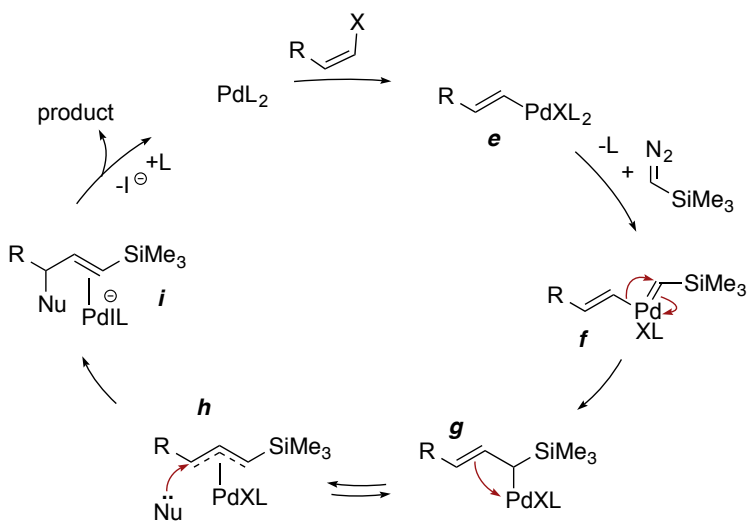
Trapping of π-Allylpalladium Intermediates Generated from Palladium-Carbenes

The nucleophilic trapping of η^3-allylpalladium intermediates generated from palladium carbenes was successfully utilized to synthesize a variety of vinylsilanes from different nucleophiles (Figure 4-6). During the initial optimization, when E-styrylbromide and arylstannane were used as coupling partners, palladium catalyzed homocoupling of E-styrylbromide was observed. This was attributed to the carbapalladation of the styryl bromide by the vinylpalladium bromide, followed by β-elimination of $PdBr_2$, which is reduced back to palladium(0) by the arylstannane. Therefore, to avoid this competitive Heck-dimerization Van Vranken group utilized vinyl halides with aliphatic substituents and enolate nucleophiles instead of aryl-substituted alkenes and arylstannane nucleophiles.

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Cyclic amine nucleophiles were superior to the acyclic amines such as benzylamine (compounds **4.2a-d**). When pyrrolidine was included as the nucleophile, formation of catalytically inactive palladium(II) salts were noted. Addition of phenylboronic acid effectively regenerated active palladium(0) catalyst and afforded the corresponding vinylsilane adduct, **4.2c** in good yield. Utilizing (*E*)- **4.1** resulted in reduced 11% yield, probably due to the decreased rate of migratory insertion in a sterically unenumbered environment. Stabilized carbon necleophiles required larger amounts of the corresponding nucleophile (4 equiv of amines vs. 12 equiv of malonates) and elevated temperatures (46 °C for amines vs. 66 °C for malonates) to afford products in reasonable yields.

Figure 4-6: Pd-Catalyzed Three-Component Coupling
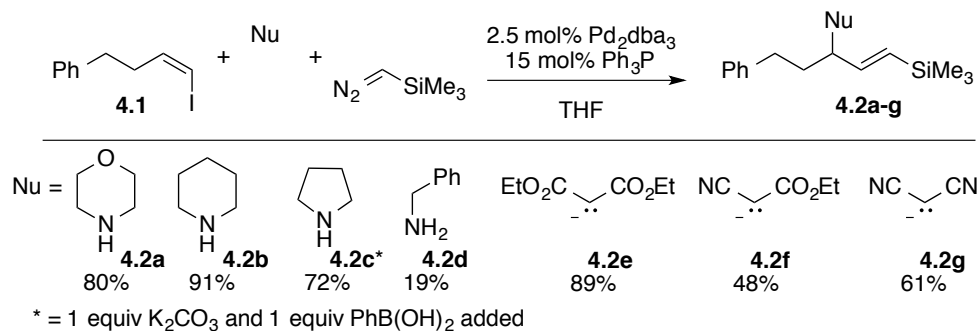
The scope of the vinyl iodide was analyzed in the Pd-catalyzed carbenylative coupling reaction. Under the optimized reaction conditions both vinyl iodides and vinyl bromides resulted in comparable yields suggesting similar efficiencies (Figure 4-6, compound **4.2e** and Figure 4-7, compound **4.3a**). Cyano substituents and silyl protected hydroxyl groups were tolerated under the reaction conditions (compounds **4.3b** and **4.3c**). Both stabilized carbon nucleophiles and amines participated in the three-component coupling with geminally-substituted vinyl iodides to afford **4.3d** and **4.3e** in slightly lower efficiencies. Finally a reaction with an internal vinyl iodide resulted in a sluggish reaction to afford vinyl silane **4.3f** in 18% yield.

Figure 4-7: Scope of Vinyl Halide in Carbenylative Three-Component Coupling

* = vinyl bromide was used
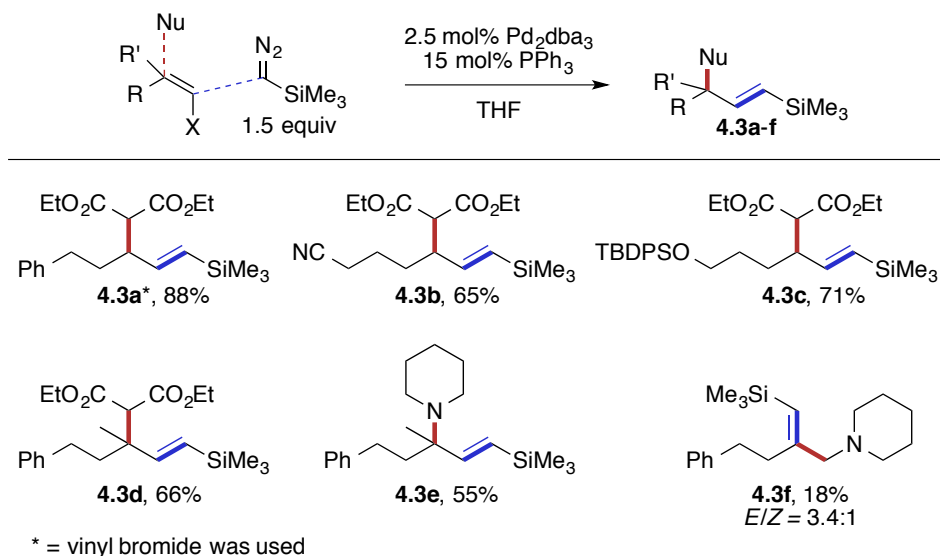
Next, trimethylsilyldaiazomethane was replaced with ethyl diazoacetate (EDA) as the carbene precursor to gain access to α,β-unsaturated γ-amino esters, common pharmocophores in covalent cysteine protease inhibitors (Figure 4-8).[142] Morpholine reacted more efficiently compared to the other cyclic amines to afford **4.4a** in 94% yield (compounds **4.4a-c**). Secondary amines were superior to the primary amine counterparts, probably due to the more nucleophilic character on N atom in secondary amines (compounds **4.4d-g**). Addition of triethylamine as a sacrificial amine increased the yields from 42% to 62% in formation of **4.4c**. In subsequent reactions that are not described in this report, Kudrika and co-workers discovered that *Z*-vinyl iodides participate in the more efficient coupling reactions compared to *E*-vinyl iodides.

Overall, the pioneering work by the Van Vranken group paved the way to understanding the utility of palladium-catalyzed carbenylation in useful transformation. Followed by these initial findings, the Van Vranken group and several other groups have reported new transformations that resulted from trapping carbene insertion intermediates arising from diazo compounds with nucleophiles other than amines and stabilized anions. These reactions include

incoporating carbenylation with a heck reaction to form indenes and indenyl amines.[143] Also, Wang and co-workers were able to trap the intermediates of their carbenylation reaction with aryl boronic acids.[144]

Figure 4-8: Pd-Catalyzed Three-Component Coupling with EDA
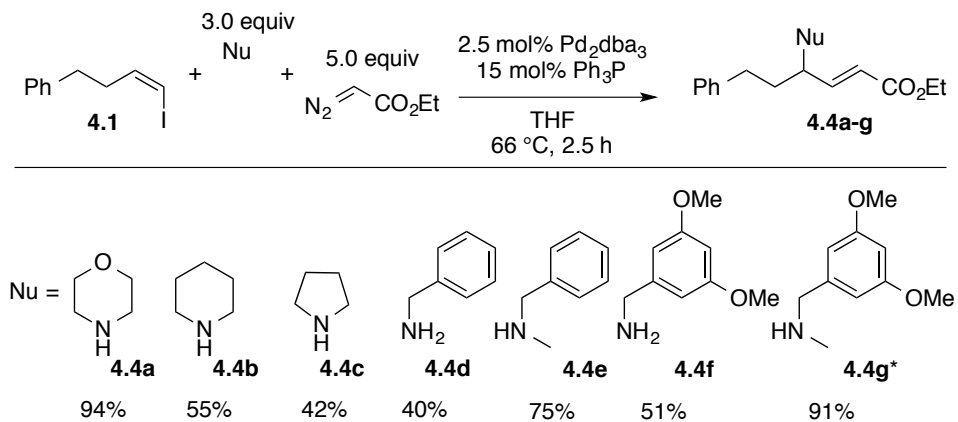
Conclusion

In this chapter I described the general reactivity of metal-carbenes with emphasis on Pd-carbenes. Mechanistically, palladium(0)-carbenes show distinct reactivities compared to Pd(II)-carbenes. Migration of anionic ligands in palladium(II) to the carbene center opens up a new avenue to explore new C–C and C–heteroatom bond formations. During the past 10 years, the carbenylation process was exploited successfully and as a future prospect, controlling the migration to form enantioenriched adducts will provide a powerful tool in organic synthesis.

Chapter 5

Carbenylative Coupling Involving Palladium Alkylidene Intermediates

Alkylidine Precursors in Palladium-Catalyzed Carbenylative Coupling Reactions

Metal-carbene intermediates are involved in a wide variety of transformations.[145] During the early work, diazo compounds served as the sole carbene precursors. Generally, diazo compounds are stabilized anions and the π-acceptor groups such as carbonyl or phenyl moieties are present to stabailize the anion. Therefore, compounds that do not present π-acceptor groups such as diazoalkanes (alkylidines) are highly unattractive starting meterials due to their explosive nature and toxicity.[146]

The discovery of the utility of *N*-tosylhydrazone as carbene precursors tremendously increased the substrate scope of the carbenylative coupling reactions. In the presence of base, *N*-tosylhydrazones undergo Bamford-Stevens reaction to form corresponding diazo compounds *in situ*.[147] Aggarwal and co-workers successfully utilized *N*-tosylhydrazones to deliver unstabilized diazo compounds in iron- and rhodium-catalyzed cyclopropanations and olefination reactions.[148] In 2007, Barluenga and co-workers efficaciously implemented *N*-tosylhydrazones in Pd-catalyzed carbenylations to synthesize poly-substituted olefins.[149] Several other less common classes of compound such as tethered alkynes,[150] diazirines,[151] Fischer carbenes,[152] and chloroform[153] were also employed as carbene precursors in several other transformations.

β-Hydride Elimination in Processes Involving Pd-Alkylidene Intermediates

Palladium(II) alkylidenes that possess hydrogens adjacent to the carbene center rapidly undergo β-hydride elimination causing the carbene center to end up as an sp^2 center (Figure 5-1). Such a process can be exploited as a way to regenerate active Pd(0) catalyst upon reductively eliminating HX. However, β-hydride elimination takes away the opportunity to preserve the

stereochemical information gained from the migratory insertion of anionic ligand to the carbene center.

Figure 5-1: Palladium(II) Alkylidene Leads to β-Hydride Eliminations
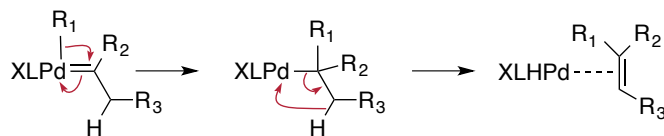
β-hydride Elimination With Pd-alkylidenes Generated from Ketone Hydrazones

Ketone *N*-tosylhydrazone and ArPdX from Arylhalides/pseudohalides in Pd-catalyzed carbenylative coupling

Barluenga and co-workers reported the first example of a palladium-catalyzed carbenylative coupling with *N*-tosylhydrazones (Scheme 5-2).[149] The scope of the alkylidienes was previously limited by the stability of the diazo precursor. However, employing *N*-tosylhydrazones as carbene precursors greatly increased the scope of carbenylative coupling reactions. Many groups have demonstrated the ability to utlize several R-X functional groups such as aryl halides[154] and pseudohalides such as nonaflates[155] as well as alkenyl pseudohalides such as alkenyl tosylates[156] as coupling partners in such transformations.

The proposed mechanism for the palladium-catalyzed carbenylative coupling with ketone *N*-tosylhydrazone involves an initial oxidative addition of an aryl/alkyl halide to palladium(0) to form RPdX intermediate **b**. *N*-tosylhydrazone reacts with the base to form the corresponding diazo compound *in situ*. Incorporation of the diazo moiety results in palladium alkylidene intermediate **c**. Migration of the aryl/alkyl group to the carbene center generates alkylpalladium halide intermediate **d**, which then undergoes rapid β-hydride elimination to yield alkene product. Reductive elimination of HX from palladium(II) hydride regenerates the active palladium(0) catalyst.

Figure 5-2: Representative Examples of Pd-Catalyzed Carbenylative Coupling with Ketone *N*-Tosylhydrazone and ArPdX from Arylhalides/pseudohalides
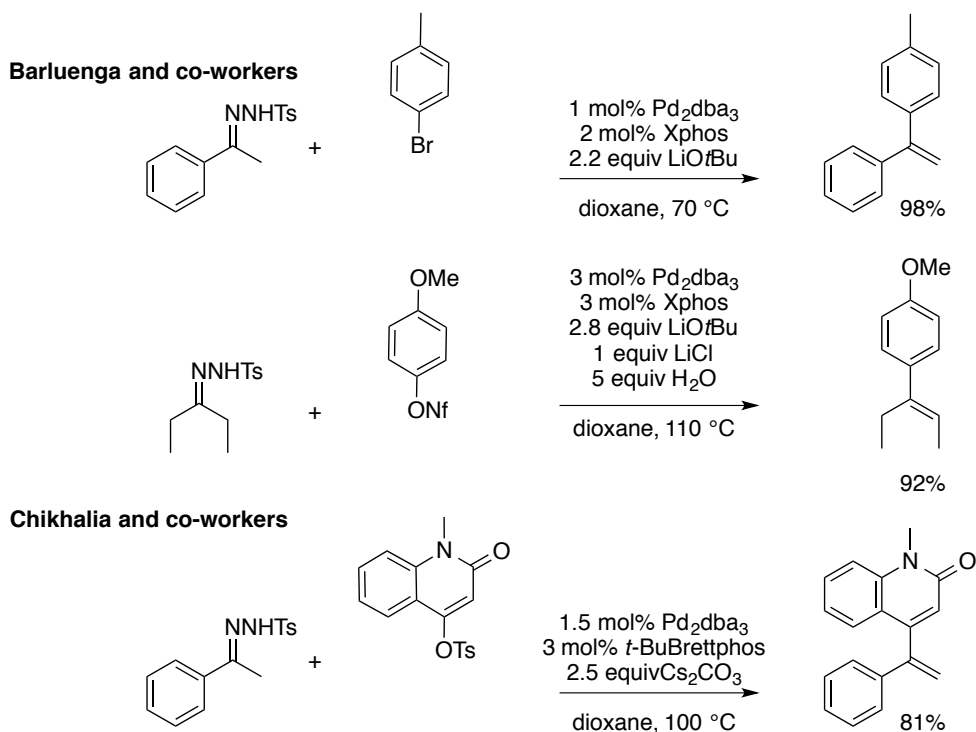
Figure 5-3: Proposed Reaction Mechanism for the Palladium-Catalyzed Carbenylative Coupling with Ketone *N*-Tosylhydrazone
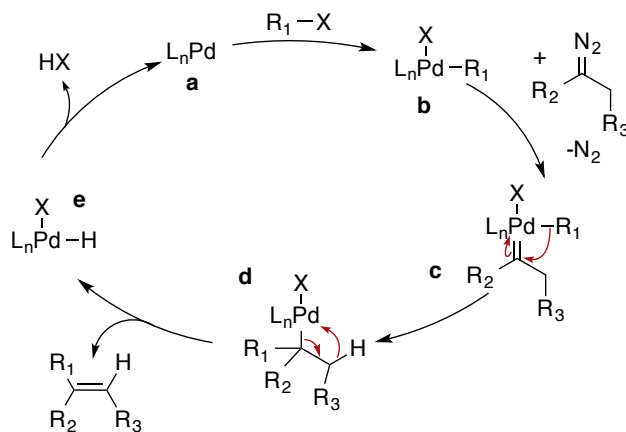
Synthesis of combrestatin A-4 (CA-4), a potent cytotoxic agent and its analogues have been readily accessed by palladium-catalyzed carbenylative coupling reactions (Figure 5-4). Petit and co-workers isolated combrestatin A-4, a *Z*-stilbene from an Eastern Cape South African bushwillow tree, *Combretum caffrum*.[157] It has been shown that combrestatin A-4 is potently

cytotoxic to a variety of cancer cells and it is the most studied example of vascular disrupting agents that bind on β-tubulin at the colchicine binding site.[158] However, over time combrestatin A-4 isomerizes into its less cytotoxic (*E*)-isomer. Several analogues of CA-4, such as *isocombretastatin A-4*, (*iso*CA-4), *iso*NH₂CA-4, *iso*FCA-4 have been shown to be active in nanomolar concentrations against several cancer cell lines. The palladium-catalyzed carbenylative coupling reactions have been utilized to readily access the 1,1-diarylethylene motifs in CA-4 and its analogues.[159]

Figure 5-4: Synthesis of *iso*CA-4 and the Structure of *Combretastatin A-4* (CA-4)
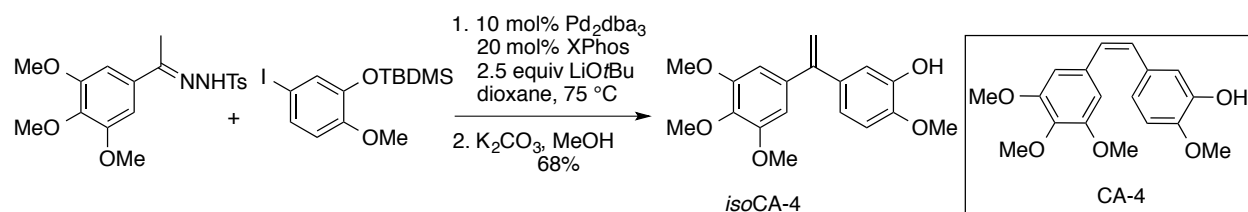
Ketone *N*-tosylhydrazones and ArPdX derived from Nucleophiles and Oxidants in Pd-catalyzed Carbenylative Coupling

Under oxidative conditions palladium-alkylidene intermediates react with boronic acids[144,160] and indoles and other nitrogn-containing heterocycles such as carbazole, 1,2,3,4-tetrahydrocarbazole, pyrrole, 1,5,6,7-tetrahydro-4H-indol-4-one, benzimidazole, and imidazole [161] to afford corresponding substituted olefin products (Figure 5-5). Mechanistically, these transformations are similar to the reactions shown in Figure 5-2, except palladium(II) intermediates were accessed via oxidation of palladium(0) by utilizing various different oxidants such as molecular oxygen,[146b,d,147a] benzoquinone,[144,146b] and iodobenzene.[147b,c]

Terminal alkynes were also used in palladium-catalyzed coupling reactions under oxidative conditions (Figure 5-6).[162] Weak electron-rich tris-(2-furyl)phosphine ligands were used to facilitate the formation of alkynylpalladium(II) intermediate. The reaction tolerates a

wide variety of *N*-tosylhydrazones and terminal alkynes. Conjugated enynes were also synthesized with high >20:1 *Z/E* selectivity.

Figure 5-5: Representative Examples of Palladium-Catalyzed Oxidative Carbenylative Couplings
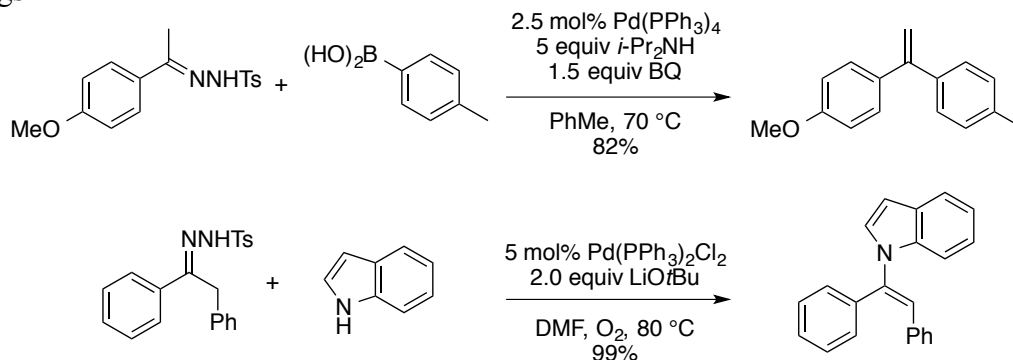
Figure 5-6: Pd-Catalyzed Oxidative Coupling of Terminal Alkynes with *N*-Tosylhydrazone
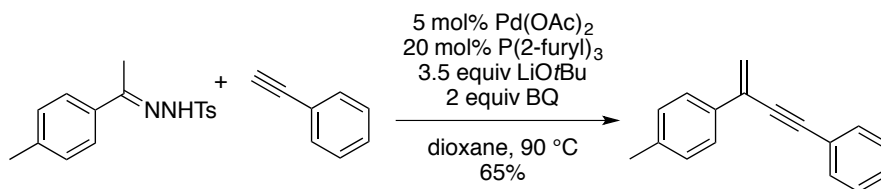
Ketone *N*-tosylhydrazone and ArPdX or ArCOPdX Derived from Tandem Reactions in Pd-catalyzed Carbenylative Coupling

In 2010, Wang and co-workers demonstrated that *N*-tosylhydrazones could participate in tandem carbonylative cross-coupling reactions.[163] Upon oxidative addition of the aryl iodide the aryl group on the palladium intermediate migrates to a carbon monoxide ligand to result in a benzoyl group. The benzoyl group can then migrate to the alkylidene moiety to form an alkylpalladium intermediate (Figure 5-7). Consequent β-hydride elimination results in the enone product **5.1**. Also the alkylpalladiumm intermediate can be trapped with Et$_3$SiH to give the ketone adduct **5.2**. The reaction can also be tuned to afford either the enone **5.1** or the ketone **5.2** products by changing the palladium pre-catalyst and the ligand.

Figure 5-7: Pd-Catalyzed Carbonylative Cross-Coupling with *N*-Tosylhydrazone and Aryl Halide
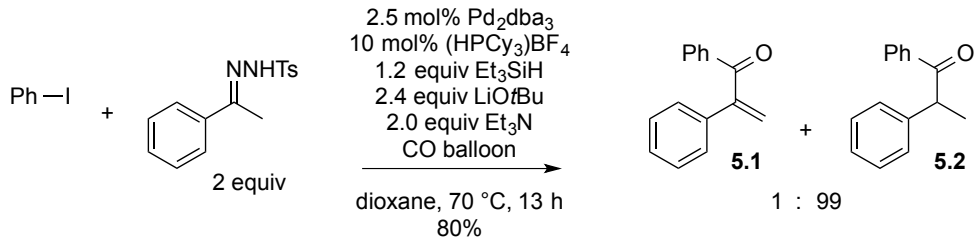
Figure 5-8: Palladium-Catalyzed Ring Opening of Norbornene
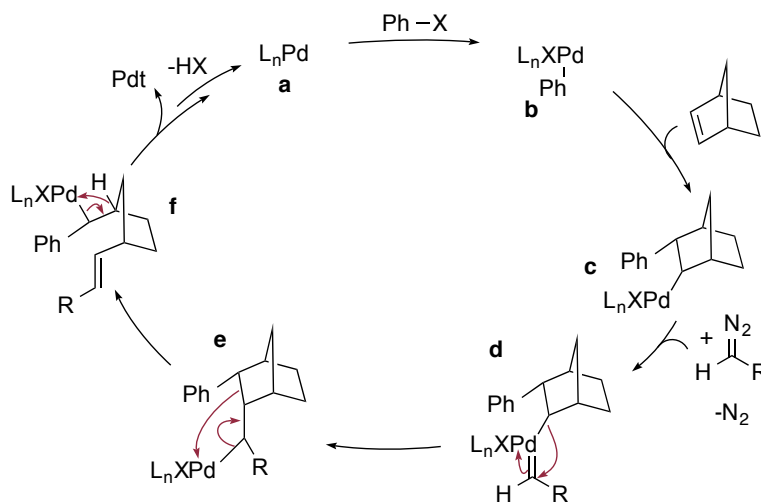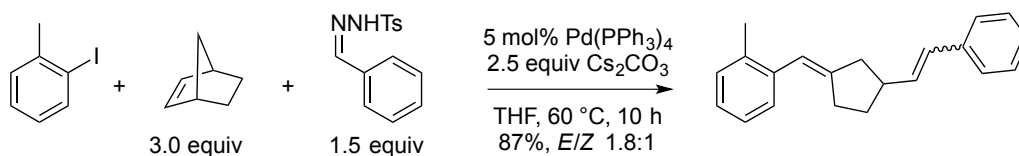
Recently, Liang and co-workers have established that *N*-tosylhydrazone could be involved in palladium-catalyzed ring opening of norbornene to synthesize methylenecyclopentane derivatives (Scheme 5-8).[164] This multi-step domino reaction involves the oxidative addition of aryl halide to afford arylpalladium halide intermediate **b**. Carbopalladation across aryl palladium results in intermediate **c**. Palladium(II) benzylidine intermediate **d** forms upon incorporation of the diazo compound and alkyl migration to the carbene carbon yields alkyl palladium adduct **e**. C–C bond cleavage followed by β-hydride elimination affords the desired methylenecyclopentane product. A wide range of aryl iodides and

110

aryl aldehyde *N*-tosylhydrazones tolerated the reaction conditions. Ketone *N*-tosylhydrazones gave lower yields. The authors attributed the lower yields to the less reactive nature of the ketone *N*-tosylhydrazones compared to the corresponding aldehyde substrates and the formation of undesired β-hydride eliminating byproducts.

β-hydride Elimination With Pd-alkylidenes Generated from Diazo Compounds

Diazo compounds are not an ideal carbene precursor in carbenylative couplings due to their high reactivity. However, in 2008, Wang and co-workers reported the first example of α-diazocarbonyl compounds with arylbornic acids to access α-aryl substituted α,β-unsaturated carbonyl compounds (Figure 5-9).[144] The reaction tolerates a wide variety of α-diazocarbonyl compounds such as α-alkyl substituted diazocarbonyl compounds and cyclic and acyclic α-diazoketones. Electron rich aryl groups on the aryl boronic acid facilitate product formation in good yields where electron deficient groups afforded the products in lower yields. Also, a tetra-substituted olefin, which is difficult to access, can be obtained in moderate yields. The same group has shown the utility of palladium-catalyzed carbenylative cross coupling of cyclic α-diazoketones with vinyl boronic acids to synthesize 1,3-diene compounds bearing a ring structure in good yields.[146b] The reaction with β-substituted vinyl boronic acids proceeded smoothly and α-aryl vinyl boronic acid gave the corresponding products in diminished yields.

Figure 5-9: Palladium-Catalyzed Carbenylative Coupling of α-Diazocarbonyl Compounds with Arylbornic Acids
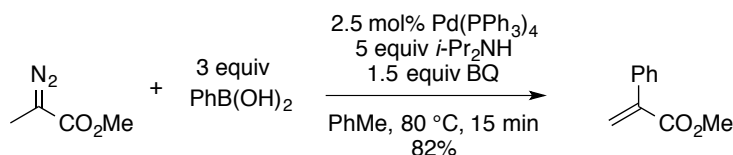
β-hydride Elimination With Pd-alkylidenes Generated from Aldehyde Hydrazones

Aldehyde *N*-tosylhydrazones are shown to be more reactive than ketone substrates and aldehyde *N*-tosylhydrazones are very rarely used in carbenylative coupling processes compared to the corresponding ketone *N*-tosylhydrazones.[165] Barluenga and co-workers have demonstrated that aldehyde *N*-tosylhydrazones can engage in palladium-catalyzed carbenylative coupling reactions with aryl halides to afford the corresponding elimination products in high efficiency (Figure 5-10). Aldehyde *N*-tosylhydrazones have shown to give coupling products with both aryl halides and pseudohalides such as triflates and nonaflates with similar efficiencies as their ketone counterparts.

Figure 5-10: Palladium-Catalyzed Carbenylative Coupling of Aldehyde *N*-Tosylhydrazones with Aryl Halides
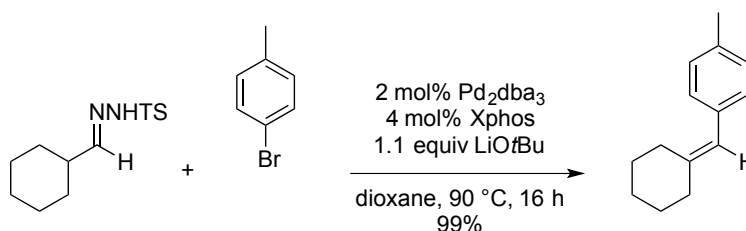
Palladium-catyalyzed Carbenylative Coupling Processes with Cyclopropyl Ketone N-Tosylhydrazones

Methods to synthesize 1,3-butadiene adducts via cyclopropylmethyl palladium species have been reported. Wang and Yu have independatly shown that cyclopropyl *N*-tosylhydrazone with aryl/alkyl halide or ketone *N*-tosylhydrazone with cyclopropyl halide can afford the corresponding 1,3-butadiene products in good to moderate yields (Figure 5-11).[166] When bromocyclopropane was used as the coupling partner the elimination product **5.3** gave significantly lower yields. The transformation demonstrates that when a cyclopropyl group and hydrogen occupy the beta position of the cyclopropylmethyl palladium species the β-alkyl

elimination of a strained C–C bond in the 3-membered ring is much faster than the β-hydride elimination.

Figure 5-11: Synthesis of 1,3-Butadiene via Cyclopropylcarbenylpalladium Species
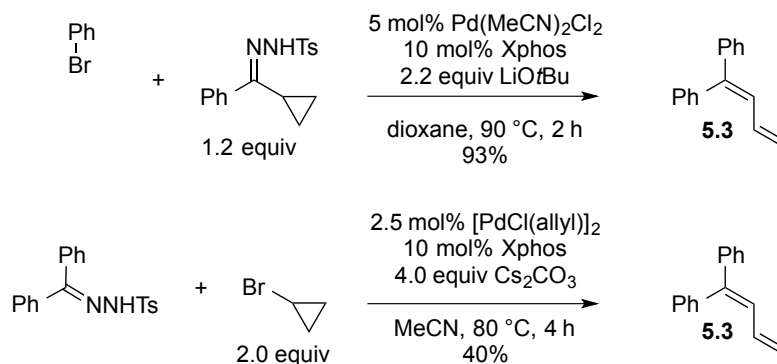
Mechanisiacally, the initial oxidative addition of aryl halide forms allylpalladium(II) halide intermediate **b**. In the presence of a base *N*-tosylhydrazone decomposes to diazo compound *in situ* and the diazo compound reacts with **b** to afford cyclopropylcarbenylpalladium intermediate **c**. Migratory insertion of the aryl moiety generates cyclopropylcarbinylpalladium intermediate **d**. Upon β-alkyl elimination, homoallylpallaium intermediate **e** undergoes β-hydride elimination to form 1,3 diene product. Final reductive elimination of HX regenerates the active palladium(0) catalyst.

Figure 5-12: Proposed Reaction Mechanism for The Synthesis of 1,3-Butadiene via Cyclopropylcarbenylpalladium Species
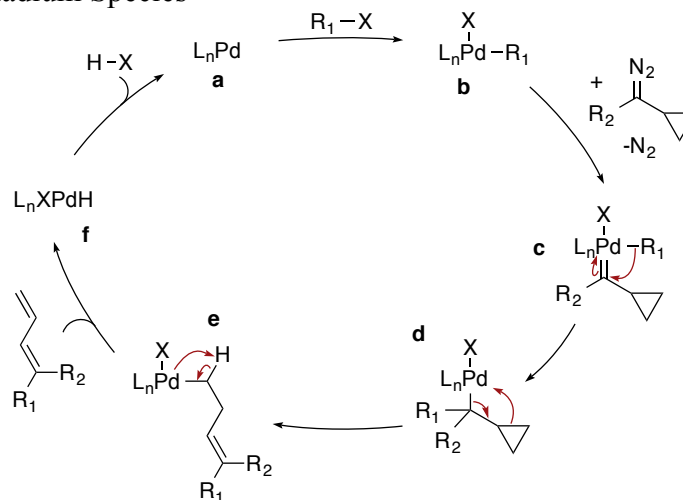
Carbenylative Couplings Involving β-Hydride Elimination Toward the Alkyl Halide

Benzyl and allyl halides have been shown to engage in carbenylative coupling processes. Such elecrophiles had been utilized in several palladium-catalyzed reactions [167] and in carbenylative coupling reactions the β-hydride elimination occurs on the electrophile in the absence of hydrogens adjacent to the carbene carbon. In 2001, Van Vranken and co-workers have reported the first example of synthesizing styrene derivatives by using (trimethylsilyl)diazomethane (TMSD), benzyl halides and catalytic palladium.[168] Nucleophilic phosphine ligands were avoided in order to prevent substitutuions on benzyl bromides.[169] Allyl halides were also shown to participate in the homologation processes with β-hydride elimination on the electrophile.[170]

Figure 5-13: Palladium-Catalyzed Homologation of Benzyl Halides with TMSD
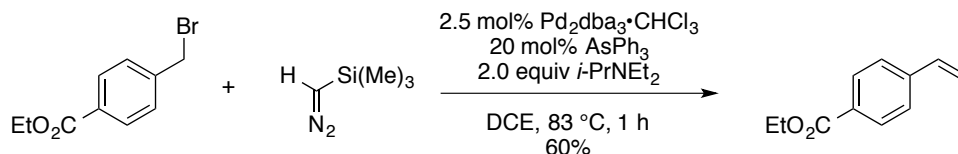
Palladium-Catalyzed Homo-coupling Processes Involving β-hydride Elimination

In 2013, both Jiang and Prabhu independently showed that ketone *N*-tosylhydrazone can undergo oxidative homo-coupling in the presence of a palladium catalyst to form 2,3-substituted-1,3-butadiene adducts (Figure 5-14).[171,172] The reaction tolerated a wide variety of substitutions on both the aryl ring and the α-carbon. Electron donating moieties on the aryl ring tended to give relatively higher yields and *vice versa*. Also α-substitution showed good levels of *E/Z* selectivities on the resulting breanched dienes.

Figure 5-14: Pd-Catalyzed Oxidative Homo-coupling of *N*-Tosylhydrazones
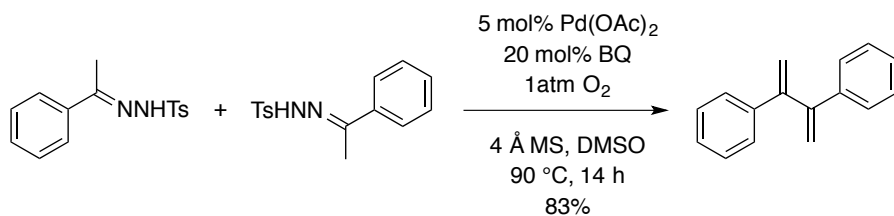
A plausible mechaninsm for the palladium-catalyzed oxidative homo-coupling of *N*-tosylhydrazones is proposed in Figure 5-15. Diazo compounds that formed via Bamford-Stevens reaction of *N*-tosylhydrazone reacted with the palladium(II) active catalyst to form palladium-carbene intermediate **b**. Elimination of the α-proton affords vinylpalladium adduct **c** that combines with another diazo compound to form vinylpalladium alkylidene intermediate **d**. Upon migration of the vinyl group to the carbene carbon, allylpalladim intermediate **e** undergoes β-hydride elimination to generate the branched alkene product. Finally the resulting palladium(0) intermediate is oxidized by molecular oxygen into the active palladium(II) in the reaction.

Figure 5-15: Prposed Mechanism for the Palladium-Catalyzed Oxidative Homo-coupling of *N*-Tosylhydrazones.
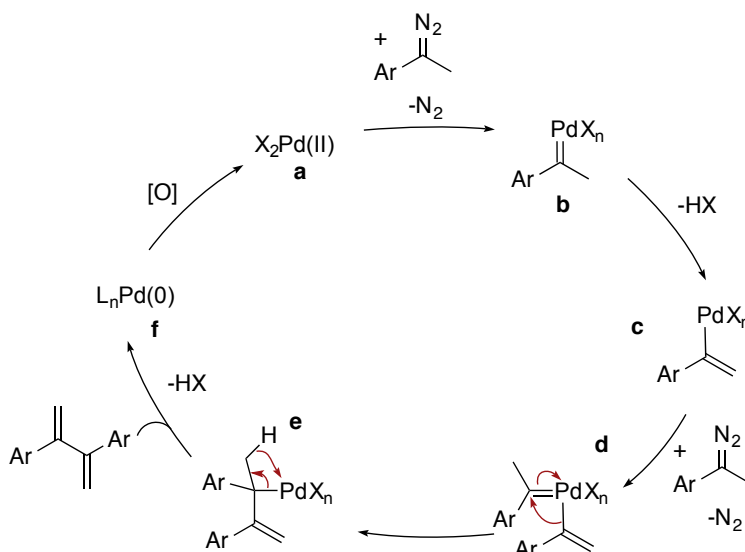
Carbenylative Coupling Processes without β-Elimination

A few reports have demonstrated that other processes could avoid competitive β-hydride elimination. These reports can be categorized into two general classes of reactions that occur prior to β-hydride elimination; formation of π-allyl/oxa-allyl palladium intermediate and formation of ketene/ketenamine formation.

Formation of π-allyl/Oxa-Allyl Palladium Intermediates Prior to β-Hydride Elimination

In 2009, Van Vranken and co-workers reported the first example of bypassing β-hydride elimination to form η^1-allylpalladium intermediates that can be trapped with various nucleophiles upon isomerization to η^3-allylpalladium intermediates.[173] Palladium-catalyzed three-component coupling of ethyl α-diazopropionate, morpholine and vinyl iodide was used to generate γ-aminoester, **5.4** in 2:1 *E/Z* selectivity (Figure 5-16).

Figure 5-16: Three-Component Coupling Reaction to Generate γ-Aminoesters
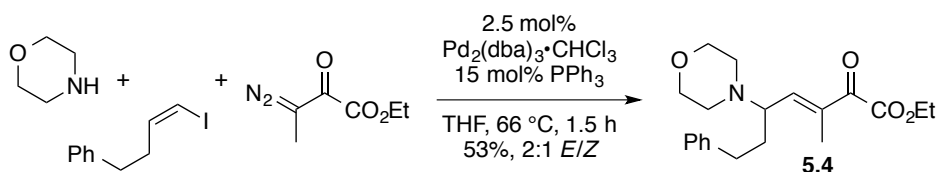
The reaction is expected to start with oxidative addition of palladium to the vinyl iodide to generate vinylpalladium halide **b** (Figure 5-17). Subsequent incorporation of the diazo compound formed vinylpalladium carbene intermediate **c**. Vinyl group migration to the carbene ligand generates η^1-allylpalladium complex **d** that isomerizes to an η^3-allylpalladium intermediate **e**. The η^3-allylpalladium intermediate can be trapped with nucleophiles to afford γ-aminoesters without β-hydride elimination.

Figure 5-17: Proposed Mechanism for the Pd-Catalyzed Three-Component Coupling Reaction to Generate γ-Aminoesters
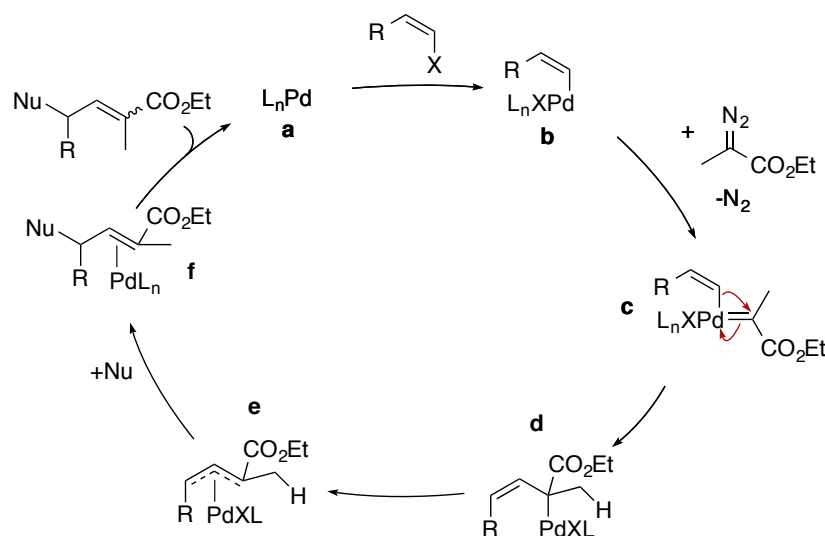
In a similar transformation Wang and co-workers[163] and Zhou and co-workers[174] have independently reported that oxa-allyl intermediates can also outcompete the β-hydride elimination. In their seminal work Wang and co-workers reported the first example of palladium-catalyzed tandem migratory insertion with both CO and a carbene (Figure 5-18). In this example, formation of O-palladium enolate **b** prior to the β-hydride elimination from the C- palladium enolate **a**, generates the expected ketone products in high yields.

Figure 5-18: Palladium-Catalyzed reaction of Phenyl Iodide and Ketone *N*-Tosylhydrazone in the presence of CO
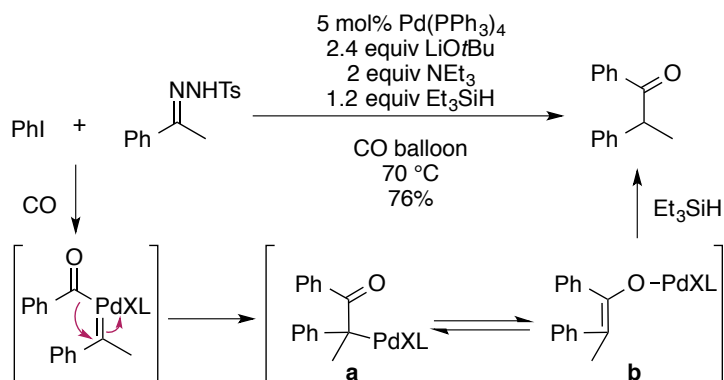
Ketene/Ketenamine Formation Prior To β-Hydride Elimination

In palladium-catalyzed carbonylation, insertion of a CO ligand to Pd–C bond results in acyl-palladium intermediate. However, CO insertion into a palladium-carbene ligand generates ketene intermediates that can be trapped by different nucleophiles in subsequent steps. Wang and co-workers have reported that when (1-diazoethyl)benzene is reacted with aniline in a CO atmosphere it generates the corresponding amide product **5.7** in moderate efficiency (Figure 5-19). Mechanistically, CO ligand in intermediate **5.5** migrates to the carbene center and generates the ketene intermediate **5.6**. Migration of CO is expected to be faster than the competitive β-hydride elimination.

Figure 5-19: Palladium-Catalyzed Three-Component Coupling of Aniline, CO and Diazo Compounds
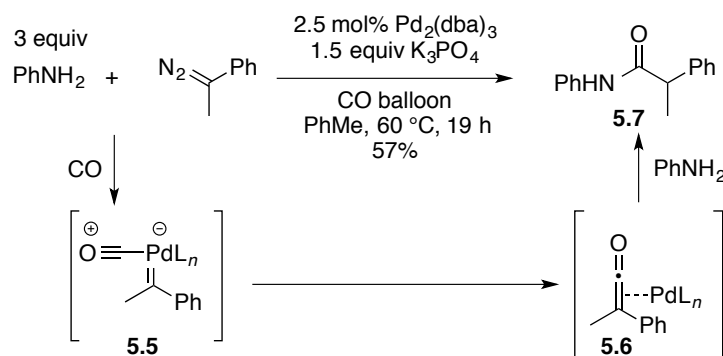
Similarly, Zhou and co-workers used isocyanides that are isoelectronic to carbon monoxides in similar carbenylative coupling reactions to generate ketenimine intermediates.[175] Isocyanide insertion is analogous to the previously described CO insertion and in the presence of hydrogens next to the carbene reactions leading to amides are faster than the β-hydride elimination (Figure 5-20). The resulting ketenimine intermediate **5.8** was trapped with water to afford amide products. The reaction tolerated a variety of aldehydes and ketones derived *N*-tosylhydrazones. However, *N*-tosylhydrazones derived from alkyl aldehydes resulted in reduced

yields. Authors have attributed this to the weaker stabilizing ability of the alkyl groups of the

resulting carbene intermediates.

Figure 5-20: Palladium-Catalyzed Amidation of *N*-Tosylhydrazones with Isocyanides
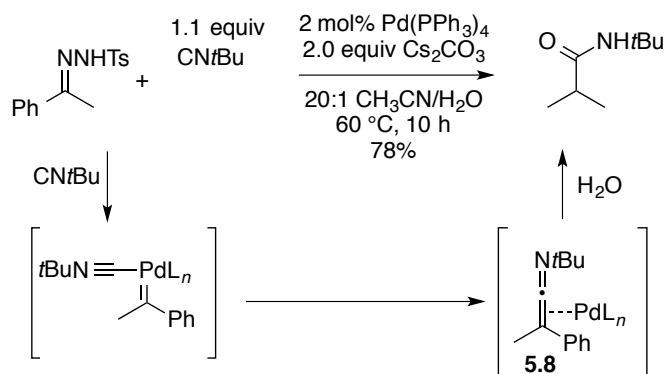
As a result, they speculated that palladium-carbene species are more reactive and

decompose faster under the reaction conditions. Optimal conditions for the amidation reaction

with aryl aldehydes/ketones derived *N*-tosylhydrazones resulted in lower yields of reactions with

N-tosylhydrazones derived from alkyl ketones. By switching the solvent, CH$_3$CN with less polar

1,4-dioxane generated the desired products from alkyl ketones in moderate yields.

Chapter 6

Carbenylative Amination and Alkylation of Vinyl Iodides via Palladium Alkylidene Intermediates

Introduction

Palladium-catalyzed carbenylative insertion processes are gaining increasing attention, as they are analogous to widely used carbonylative insertion processes. Three-component carbenylative cross-coupling reactions offer a powerful method for joining molecular fragments through one-carbon units, similar to three-component carbonylative cross-coupling reactions. In initial applications, diazo compounds served as the major carbene precursors, but more recently *N*-tosylhydrazone anions have been used to expand the scope of carbene precursors to include benzylidene and alkylidene derivatives.[149,141c,176] When there are hydrogens adjacent to the carbene center, carbene insertion is usually followed by β-hydride elimination which out-competes nucleophilic trapping and erases any stereochemical information created in the carbene insertion step (Scheme 6-1).

Scheme 6-1: β-Hydride Elimination Out-Competes Nucleophilic Trapping
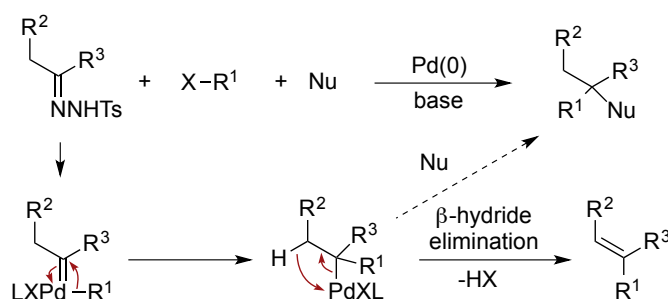
Most palladium-catalyzed carbene insertion processes involve RPdX complexes derived from oxidative addition of aryl (pseudo)halides,[177] benzylic and allylic halides.[178] In other processes, RPdX complexes arise from addition of nucleophiles to palladium(II).[179] Regardless of how the migratable group ends up on the palladium(II) intermediate, migration to the

alkylidene ligand ultimately results in substituted olefins due to the susceptibility to β-hydride eliminations. Two-component reactions of palladium(0) alkylidenes with carbon monoxide or isonitriles lead to ketenes or ketenimines, respectively, without β-hydride elimination.[180,181]

In rare instances, three-component carbenylative insertions have been observed to out-compete β-hydride elimination using η^1-to η^3-allyl or oxa-allyl transitions,[142,182,183] but the generality of this approach has not previously been demonstrated. Palladium catalysts have been used to unite vinyl iodides with nucleophiles and carbene precursors incapable of undergoing hydride elimination: trimethylsilyl, carboxyalkyl, aryl, and vinyl (Figure 6-1).[142,184] In this work, we demonstrate that simple alkylidene groups can efficiently engage in three-component carbenylative cross-coupling reactions of vinyl iodides without β-hydride elimination. Furthermore, we demonstrate the utility of *N*-trisylhydrazones as alkylidene precursors that react faster than the corresponding *N*-tosylhydrazones.

Results and Discussion

Figure 6-1: Carbenylative Amination and Alkylation with Alkylidene Carbenes without β-Hydride Elimination
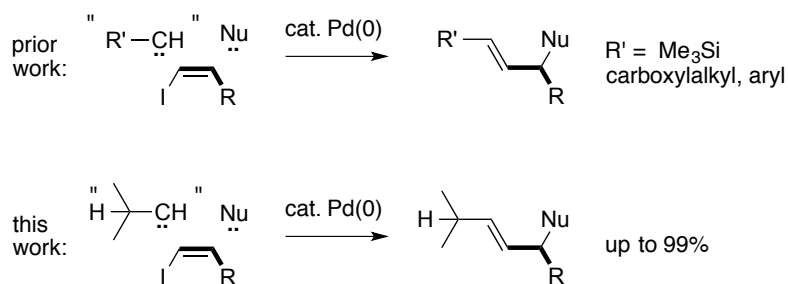
We initiated the investigation of carbenylative insertion reactions of ω-aminovinyl iodide **6.1** and isobutyraldehyde *N*-tosylhydrazone **6.2a** using conditions similar to our previous work on carbenylative amination of benzaldehyde tosylhydrazones.[171b] The only product isolated from the reaction was known dimer **6.4** (31%)[171b,185] and a large amount of unreacted vinyl iodide **6.1**

(68%) was recovered. We speculated that the low solubility of the lithiated *N*-tosylhydrazone was responsible for its failure to engage in the reaction. When the corresponding *N*-trisylhydrazone **6.3** was employed as the alkylidene precursor, the lithiated *N*-trisylhydrazone exhibited better solubility in the reaction and afforded the desired pyrrolidine **6.5** in 15% yield. From there the conditions were optimized to afford pyrrolidine **6.5** in 91% yield, and no evidence of dimer **6.4** or products resulting from elimination of the alkylidene α proton (Scheme 6-2).

Scheme 6-2: Intramolecular Carbenylative Amination with an Alkylidene Precursor
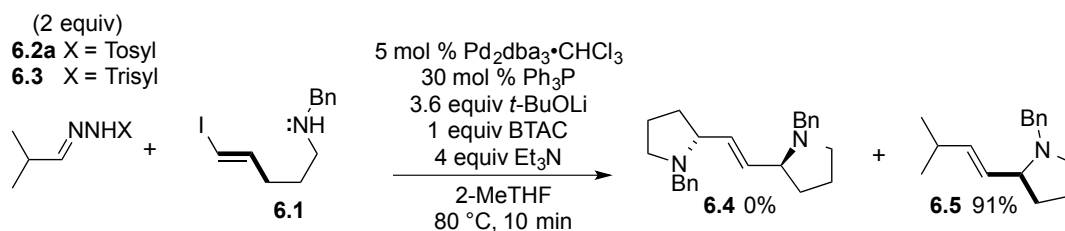
Alkenylcyclopentanes are found in a variety of natural products, such as brefeldin C, doproston B, isopulo'upone and amaminol A. To test the potential for carbon nucleophiles in the intramolecular carbenylative insertion reaction, substrate **6.6** was synthesized and subjected to the optimized reaction conditions (Scheme 6-3). The lithium enolate of malonate **6.6**, produces the corresponding alkenylcyclopentane **6.7** in 57% yield. The low yield is probably attributable to the sensitivity of the substrate. Srivastava and co-workers have previously shown related ε-iodovinylmalonates to be highly sensitive to alkoxides.[186] When malonate **6.6** was exposed to potassium carbonate, lithium *tert*-butoxide, or potassium *tert*-butoxide at 80 °C for 1 h, increasing levels of decomposition (17%, 52%, and 100%, respectively) were observed.

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Scheme 6-3: Intramolecular Carbenylative Alkylation with an Alkylidene Precursor
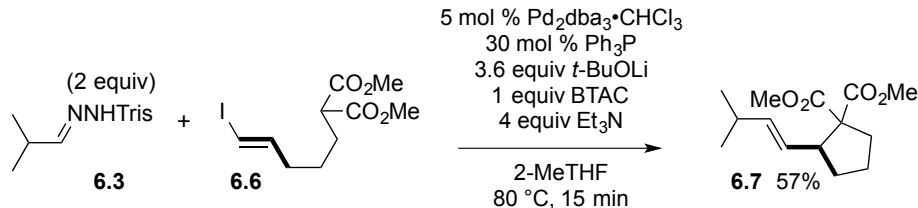
A three-component version of the reaction was tested using piperidine as the external nucleophile and *Z*-vinyl iodide **6.8a** (Scheme 6-4). Under the conditions optimized for intramolecular trapping with vinyl iodide **6.1**, none of the desired allylamine **6.9** was observed. Under these conditions trisylhydrazone **6.3** was too reactive as a carbene source. When *N*-tosylhydrazone **6.2a** was used along with four equivalents of piperidine, the desired allylamine **6.9** was obtained in 44% yield. The triethylamine additive can be omitted from the reaction conditions. Under optimized conditions, 5 equiv of piperidine was used and the amount of *N*-tosylhydrazone and lithium *tert*-butoxide was increased, leading to a 75% isolated yield of the carbenylative amination product **6.9**. When the *E* isomer of vinyl iodide **6.8a** was employed in the reaction, the product was obtained in lower yield (55%). Previously, it had been shown that *Z*-vinyl iodides and *E*-vinyl iodides give comparable yields in intramolecular carbenylative aminations.[184b]

Scheme 6-4: Intermolecular Carbenylative Amination with Alkylidene Precursors
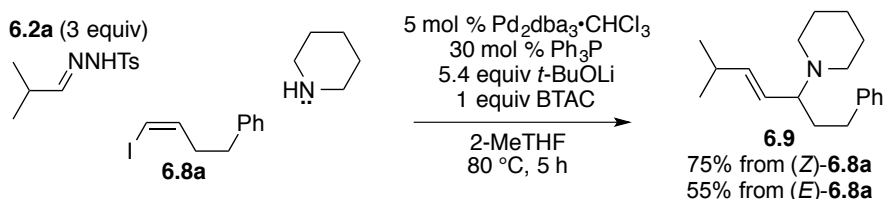
The reaction is believed to involve intermolecular attack of piperidine on an η^3-allylpalladium intermediate on the least hindered side of the allyl fragment.[187] When fewer equivalents of lithium *tert*-butoxide were used, allylamine **6.9** (55%) was accompanied by the

allylic regioisomer **6.10** (25%). The poor regioselectivity is probably attributable to the faster

palladium-catalyzed equilibration of the protonated forms of allylic amines **6.9** and **6.10** (Scheme

6-5).[188] To test this hypothesis, we exposed product **6.9** to the less basic conditions, without the

vinyl iodide starting material, for 20 h and found it to produce an 80:20 mixture of allylamines

6.9 and **6.10**.

Scheme 6-5: Allylamines Slowly Isomerize Under the Conditions of the Reaction
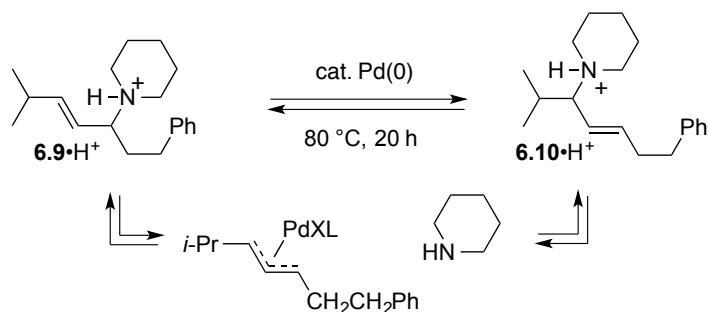
In theory, regioisomer **6.9** should be highly favored under kinetic conditions regardless of

how one accesses the η^3-allylpalladium intermediate. When the vinyl iodide, rather than the *N*-

tosylhydrazone, is substituted with a secondary alkyl group, the amine still prefers to attack at

the least hindered side of the allylic system. Reaction of vinyl iodide **6.11** with *N*-tosylhydrazone

6.2b generated allylamine **6.12** (Scheme 6-6), analogous to the preferred formation of

regiosomer **6.9** over **6.10**. The net transformation is a carbenylative cross-coupling, similar to a

carbonylative cross-coupling reaction with carbon monoxide.

Scheme 6-6: Carbenylative Cross-Coupling with a Hindered Vinyl Iodide
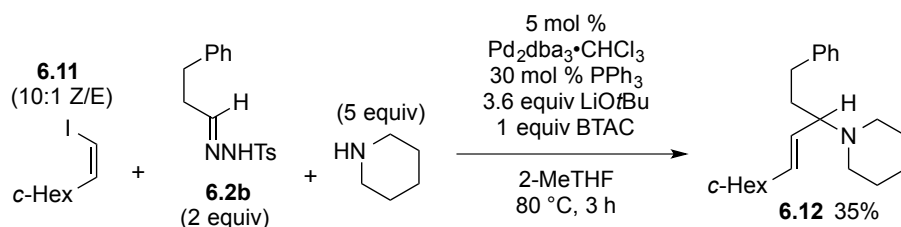
With optimized conditions for the intermolecular carbenylative cross coupling reaction in hand, we next set out to explore variations in the alkylidene precursor **6.2a-d**, the vinyl iodide, **6.8a** and **6.8b**, and the nucleophile (Scheme 6-7). The sulfonylhydrazone anions compete with other nucleophiles in the reaction by attacking the η^3-allylpalladium intermediate;[189] and formation of *N*-allylated hydrazone **6.23** accounts for 20-30% of the mass balance based on NMR of the crude reaction mixtures.

Scheme 6-7: Scope of Intermolecular Carbenylative Alkylation and Amination with Alkylidene Precursors
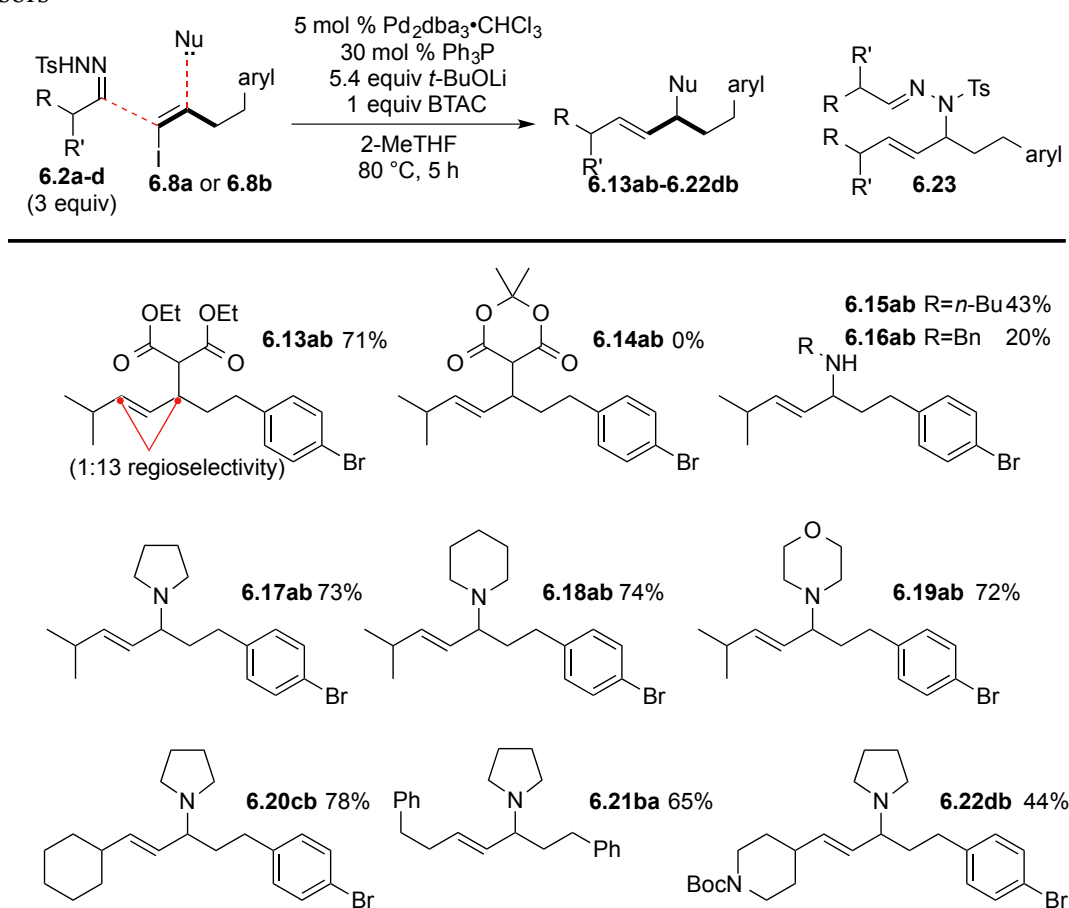
In the absence of a nucleophile a mixture of diene products, resulting from β-hydride elimination, was observed along with adduct **6.23** (22%). Diethyl malonate afforded comparable yields and resulted in a 13:1 regioisomeric mixture of allylic alkylation products **(6.13ab).** Not surprisingly, when Meldrum's acid was utilized as the nucleophile, none of the desired adduct

6.14ab was obtained, probably due to the weaker nucleophilicity of the conjugate base (pK_a' = 4.97). Butylamine and benzylamine gave modest yields of the desired coupling products **6.15ab** and **6.16ab** respectively. The cyclic secondary amines, pyrrolidine, piperidine and morpholine gave good yields (**6.17ab-6.19ab**). The superiority of cyclic amines in three-component carbenylative amination reactions was demonstrated in previous studies.[142,184a] The carbenylative amination and alkylation reactions proceed with high chemoselectivity; oxidative addition across the Ar–Br bond was not observed. We next explored the tolerance of different alkyltosylhydrazones, and the coupling reactions furnished yields up to 78% (**6.20cb, 6.21ba, 6.22db**).
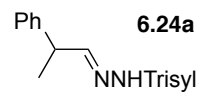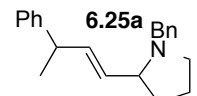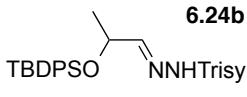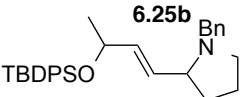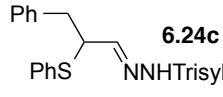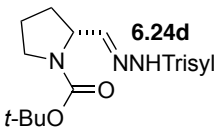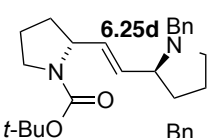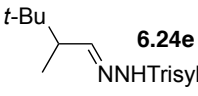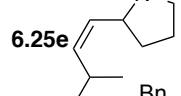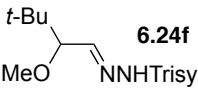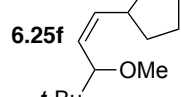
Valdés and co-workers have previously shown that palladium-catalyzed reactions of *N*-tosylhydrazones derived from α-chiral ketones proceed with preservation of stereochemistry.[154c] Since carbenylative amination reactions create new stereogenic centers it is possible to assess the potential for acyclic stereocontrol. The Felkin-Anh model reliably predicts the acyclic stereocontrol in nucleophilic additions to carbonyls with α-chiral centers (Figure 6-2a). Chiral centers might also affect 1,2-migration reactions in alkylpalladium carbene complexes, but it has never been studied. There have been surprisingly few studies of asymmetric 1,2-migrations to discrete acyclic carbocations, which are structurally analogues to late metal carbenes; none involve an adjacent stereogenic center.[190]

Figure 6-2: Acyclic Stereocontrol. a. Nucleophilic Addition to Carbonyls, b. 1,2-Migration to Palladium Carbenes
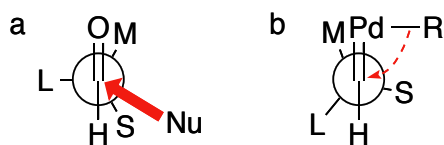
The effect of adjacent stereogenic centers in migratory carbene insertions was evaluated by utilizing chiral alkyl *N*-trisylhydrazones **6.24a-f** in intramolecular carbenylative aminations (Table 6-1). Unfortunately, the products were obtained as nearly equal mixtures of *syn* and *anti* diastereomers. *N*-Trisylhydrazones **6.24a** and **6.24b** afforded pyrrolidines **6.25a** and **6.25b**, respectively, in good yields but thioether **6.24c** gave none of the desired product, and 85% of the vinyl iodide was recovered. *N*-Boc-pyrrolidine **6.24d** gave a slight preference for the one diastereomer of **6.25d**.

Table 6-1: Stereoselectivity in Carbenylative Amination

entry	starting material	product	yield (%)	dr
1	**6.24a** Ph / NNHTrisyl	Ph **6.25a** Bn-N	72	55:45
2	**6.24b** TBDPSO / NNHTrisyl	**6.25b** TBDPSO Bn-N	77	58:42
3	**6.24c** Ph / PhS NNHTrisyl	—	0	—
4	**6.24d** N-NNHTrisyl, *t*-BuO—C=O	**6.25d** Bn-N, *t*-BuO—C=O	75	65:35 *anti:syn*
5	**6.24e** *t*-Bu / NNHTrisyl	**6.25e** Bn-N *t*-Bu	72	56:44
6	**6.24f** *t*-Bu / MeO NNHTrisyl	**6.25f** Bn-N OMe *t*-Bu	99	50:50

The stereochemistry of the major diastereomer of **6.25d** was assigned as *anti* by converting the inseparable mixture to the corresponding bis-*N*-benzyl-bis-pyrrolidines; the major diastereomer was shown to be identical to the known *meso* (*anti*) isomer **6.26** (Scheme 6-8).[185] To our surprise, when sterically encumbered *N*-trisylhydrazones **6.24e** and **6.24f** were employed the products were obtained as the *Z* alkenes ($J \leq 7.9$ Hz) with none of the expected *E* alkene products (Table 6-1, entries 5 and 6). To test the effect of steric encumbrance on alkene geometry, the hindered *N*-tosylhydrazone **6.27** was tested and shown to give only the *E* product **6.28** (Scheme 6-9). Thus sterics alone is not sufficient to explain formation of *Z* products **6.25e** and **6.25f**.

Scheme 6-8: Assignment of Relative Stereochemistry by Conversion to Known Bis-Pyrrolidine
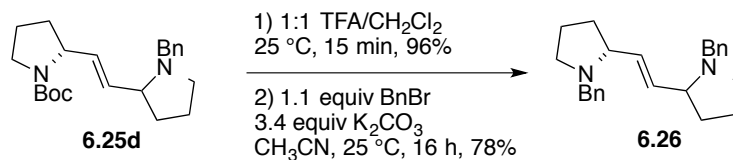
Scheme 6-9: Sterically Encumbered *N*-Tosylhydrazone **6.27** Shown to Give Only the *E* Product **6.28**
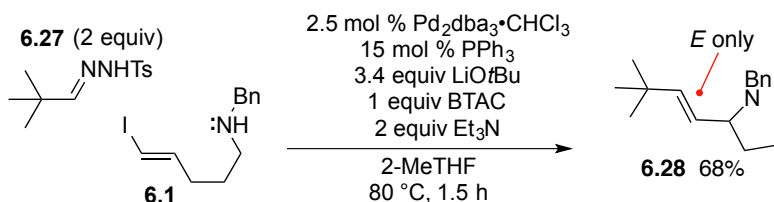
Adjacent stereogenic centers seem to exert much less influence in migration to palladium carbenes than they do in corresponding nucleophilic addition to carbonyls. This may be due to the elevated temperatures used for the palladium reactions and/or the difference in preferred angles for 1,2-migration processes versus carbonyl additions.

Conclusions

In conclusion, unstabilized alkylidene groups are shown to participate in palladium-catalyzed carbenylative amination and carbenylative alkylation reactions in high efficiency for both intramolecular and intermolecular processes. Good yields are obtained under conditions that minimize a number of competing processes such as palladium-catalyzed ionization of allylic amines, competing addition of metalated hydrazones to η^3-allylpalladium complexes, and base-promoted decomposition of vinyl iodides with pendant malonate groups. *N*-Trisylhydrazones are shown to give superior results relative to *N*-tosylhydrazones when faster rates of participation are needed from the alkylidene precursor. When there is a stereogenic center adjacent to the metal carbene carbon, the resulting products are obtained with low levels of *syn/anti* stereocontrol but high levels of *E* or *Z* selectivity.

Experimental Section

Scheme 6-10. Proposed Catalytic Cycle of Carbenylative Amination and Alkylation of Vinyl Iodide.
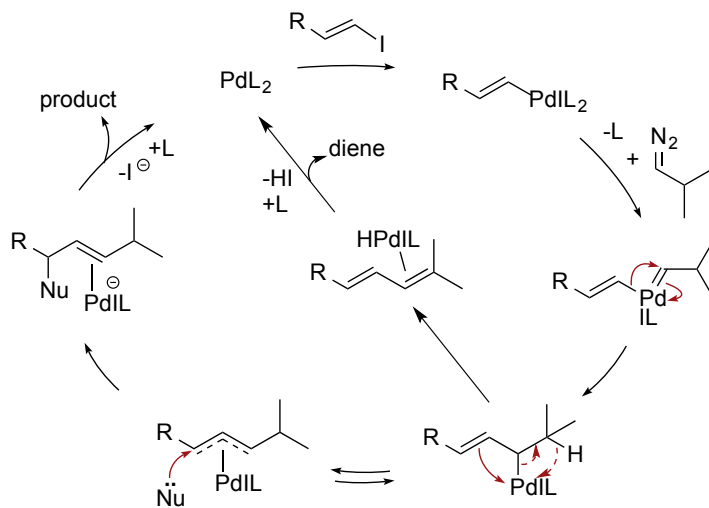
Table 6-2. Optimization of Conditions for Intramolecular Carbenylative Amination with *N*-Trisylhydrazone **6.3**[a]
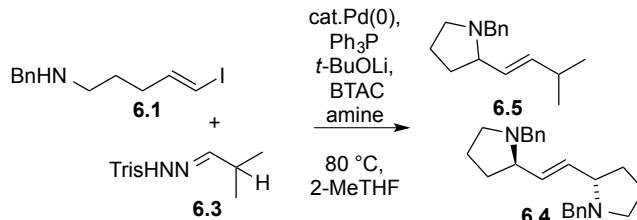
entry	equiv of BTAC	amine	equiv of amine	yield of **6.5**
1[b]	1	Et₃N	2	31% **6.4** + 68% **6.1**
2	1	Et₃N	2	15%
3	1	Et₃N	4	46%
4	1	Et₃N	6	30%
5[c]	1	Et₃N	4	33%
6	1	*i*PrNH₂	4	36%
7	1	Et₂NH	4	30%
8	0	Et₃N	4	26%
9	2	Et₃N	4	48%
10[d]	1	Et₃N	4	91%

[a] Conditions: 2.5 mol% Pd₂dba₃CHCl₃, 15 mol% Ph₃P, 2 equiv of **6.3**, 3.6 equiv *t*-BuOLi. [b] 2 equiv of tosylhydrazone instead of trisyl hydrazone. [c] 20 equiv H₂O. [d] 5 mol Pd₂dba₃CHCl₃, 30 mol% Ph₃P.

Table 6-3. Optimization of Conditions for Intermolecular Carbenylative Amination with *N*-Tosylhydrazone **6.2a**
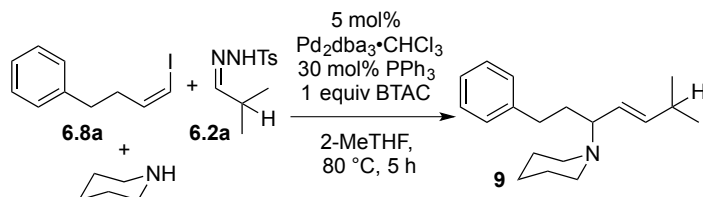
entry	equiv of **6.2a**	equiv of LiO*t*Bu	equiv of piperidine	yield of **9**
1[a]	2	3.6	4	0%
2[b]	2	3.6	4	44%
3	2	3.6	4	56%
4	2	3.6	5	66%
5	2	3.6	10	68%
6[c]	2	3.6	5	29%
7[d]	2	3.6	5	41%
8[e]	2	3.6	5	54%
9	3	5.4	5	75%
10	4	7.2	5	60%

Conditions: [a] 2 equiv isobutyraldehyde trisylhydrazone and 4 equiv Et₃N was utilized. [b] 4 equiv Et₃N added. [c] no BTAC used, [d] temperature 60 °C, [e] 20 equiv of H₂O added

Experimental

Synthesis of (*Z*)-1-bromo-4-(4-iodobut-3-en-1-yl)benzene, 6.8b.[191]
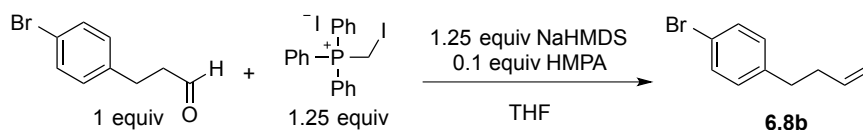
An oven-dried 200 mL round-bottom flask was charged with iodomethylphosphonium iodide (5.07 g, 9.56 mmol, 1.25 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Tetrahydrofuran (30 mL) was added and the yellow slurry was stirred at room temperature. A solution of NaHMDS (1.0 M in THF) (9.56 mL, 9.56 mmol, 1.25 equiv) was slowly added to the slurry. The reaction mixture was cooled to -60 ºC, and then HMPA (133 μL, 0.765 mmol, 0.1 equiv) was added. The reaction mixture was cooled to -78 ºC, and then 3-(4-bromophenyl)propanal (1.63 g, 7.65 mmol, 1.0 equiv) was added drop-wise via a syringe. The reaction was stirred at -78 ºC for 30 min and let warm to room temperature over an hour. The reaction mixture was poured into brine (12 mL) and extracted with pentane (3x30 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo* to give orange oil. The oil was purified by flash chromatography with hexanes to afford *Z*-isomer **6.8b** as a colorless liquid with 10:1 *Z/E* isomer (1.63 g, 4.32 mmol, 56 %). R_f = 0.59 (hexanes); ¹H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.25 (d, *J* = 7.4 Hz, 1H), 6.17 (q, *J* = 7.0 Hz, 1H), 2.69 (t, *J* = 7.5 Hz, 2H), 2.44 (q, *J* = 7.3 Hz, 2H); ¹³C NMR (125 MHz, $CDCl_3$) δ 140.0, 139.8, 131.5, 130.3, 119.9, 83.6, 36.2, 33.5; IR (thin film) 2925, 1608, 1487, 1283, 1071, 1011 cm⁻¹; HRMS (ESI): m/z calculated for $C_{10}H_{10}BrI$ [M]⁺ 335.9011, found 335.9022.

General procedure for the synthesis of *N*-sulfonylhydrazones:
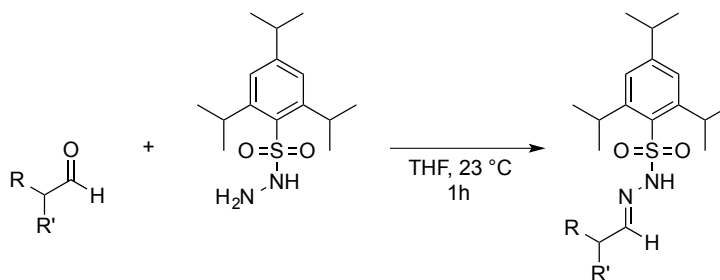
A 50 mL round-bottom flask was charged with 2,4,6-triisopropylbenzenesulfonyl hydrazide (1.0 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. THF (0.5 M) was added and the reaction flask was cooled to 0 °C. The corresponding aldehyde (1.0 equiv) was added drop-wise to the flask containing the trisyl hydrazide and THF. Reaction mixture was stirred at 23 °C and was monitored for consumption of the starting material by TLC (10:90 EtOAc/hex).

Upon consumption of the starting materials, the crude reaction mixture was concentrated *in vacuo* to give a white solid. The solid was then purified by flash chromatography with EtOAc/hex to afford the corresponding hydrazones.

(*E*)-2,4,6-triisopropyl-*N*'-(2-phenylpropylidene)benzenesulfonohydrazide, 6.24a.
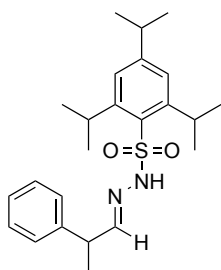
A 50 mL round-bottom flask was charged with 2,4,6-triisopropylbenzenesulfonyl hydrazide (2.22 g, 7.48 mmol, 1.0 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Next, 14 mL of THF was added and the reaction flask was cooled to 0 °C. Then 2-phenylpropanal (0.15 mL, 7.45 mmol, 1.0 equiv) was added drop-wise to the flask containing the trisyl hydrazide and THF. Reaction mixture was stirred at 23 °C and was monitored for consumption of the starting material by TLC (10:90 EtOAc/hex).

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Upon consumption of the starting materials, the crude reaction mixture was concentrated *in vacuo* to give a white solid. The crude mixture was then purified by flash chromatography with EtOAc/hex to afford the hydrazone **6.24a** (2.94 g, 7.09 mmol, 95%) as a white solid, mp: 109-112 $^{\circ}$C. R$_f$ = 0.48 (1:9 EtOAc/hex); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.46 (s, 1H), 7.24-7.19 (m, 4H), 7.16 (d, J = 5.0 Hz, 1H), 6.99 (d, J = 7.1 Hz, 2H), 4.19 (sep, J = 6.7 Hz, 2H), 3.60-3.56 (m, 1H), 2.93 (sep, J = 6.8 Hz, 1H), 1.33 (d, J = 7.0 Hz, 3H), 1.28 (d, J = 6.9 Hz, 6H), 1.25 (dd, J = 6.7, 1.4 Hz, 12H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 153.4, 152.8, 151.3, 141.5, 131.2, 128.8, 127.5, 127.0, 123.8, 42.6, 34.2, 30.0, 24.9, 24.8, 23.6, 18.3; IR (thin film) 3185, 2954, 1598, 1450, 1322, 1165, 1024 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{24}$H$_{34}$N$_2$O$_2$SNa [M+Na]$^+$ 437.2239, found 437.2238.

(*E*)-*N'*-(2-((tert-butyldiphenylsilyl)oxy)propylidene)-2,4,6-triisopropylbenzenesulfonohydr--azide, 6.24b.
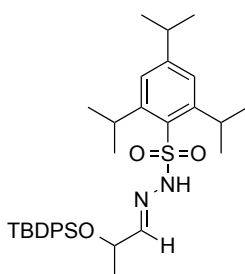
Using the general procedure for condensation outlined above, 2-((tert-butyldiphenylsilyl)oxy)propanal (0.50 g, 1.60 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex (1:9) to afford the trisylhydrazone **6.24b** (0.87 g, 1.47 mmol, 92%) as a white solid, mp: 61-63 $^{\circ}$C. R$_f$ = 0.55 (1:9 EtOAc/hex); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.59-7.52 (m, 4H), 7.40-7.24 (m, 8H), 4.32-4.30 (m, 1H), 4.13-4.10 (m, 2H), 2.91-2.89 (m, 1H), 1.26 (d, J = 6.5 Hz, 6H), 1.20 (d, J = 5.5 Hz, 12H), 1.14 (d, J = 6.2 Hz, 3H), 0.97 (s, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 153.4, 151.6, 151.4, 135.7, 134.8, 133.7, 133.3, 131.1, 129.8, 129.7, 127.7, 127.6, 123.8, 69.1, 34.2, 29.9, 26.8, 24.8, 24.7, 23.6, 21.6, 19.1; IR (thin film) 2982, 1634, 1383 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{34}$H$_{48}$N$_2$O$_3$SSiNa [M+Na]$^+$ 615.3052, found 615.3053.

(*E*)-2,4,6-triisopropyl-*N'*-(3-phenyl-2-(phenylthio)propylidene)benzenesulfonohydrazide, 6.24c.
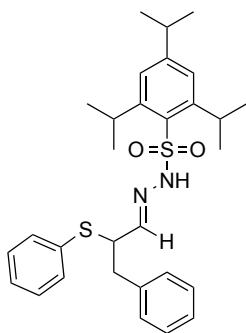
Using the general procedure for condensation outlined above, 3-phenyl-2-(phenylthio)propanal (0.12 g, 0.50 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex (1:9) to afford the trisylhydrazone **6.24c** (0.23 g, 0.44 mmol, 88%) as a white solid, mp: 48-50 °C. R_f = 0.35 (2:8 EtOAc/hex); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.18 (s, 1H), 7.17-7.14 (m, 7H), 7.07-6.97 (m, 6H), 4.11-4.05 (m, 2H), 3.89 (q, *J* = 3.9 Hz, 1H), 3.06 (dd, *J* = 14.1, 7.5 Hz, 1H), 2.96-2.89 (m, 2H), 1.29 (d, *J* = 6.9 Hz, 6H), 1.19 (d, *J* = 6.7 Hz, 6H), 1.13 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 153.4, 151.5, 147.5, 137.5, 132.7, 132.2, 131.2, 129.3, 128.8, 128.4, 127.3, 126.7, 123.9, 50.5, 37.8, 34.3, 29.9, 24.9, 24.8, 23.8, 23.7; IR (thin film) 3193, 2958, 1599, 1455, 1316, 1151 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{30}$H$_{38}$N$_2$O$_2$S$_2$Na [M+Na]$^+$ 545.2272, found 545.2258.

tert-Butyl (*E*)-2-((2-((2,4,6-triisopropylphenyl)sulfonyl)hydrazono)methyl)pyrrolidine -1-carboxylate, 6.24d.
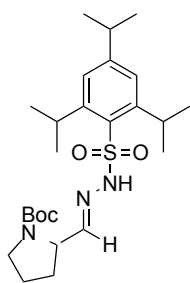
Using the general procedure for condensation outlined above, *tert*-butyl 2-formylpyrrolidine-1-carboxylate (0.50 g, 2.51 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex (1:9) to afford the trisylhydrazone **6.24d** (1.14 g, 2.38 mmol, 95%) as a white solid, mp: 72-75 °C. R_f = 0.31 (2:8 EtOAc/hex); ^{1}H NMR (600 MHz, DMSO-*d$_6$*, 380 K) δ 10.8 (s, 1H), 7.26 (d, *J* = 4.5 Hz, 1H), 7.20 (br s, 2H), 4.26-4.21 (m, 2H), 4.19-4.17 (m, 1H), 3.27-3.23 (m, 1H), 3.17-3.13 (m, 1H), 2.95-2.91 (m, 3H), 1.93-1.87 (m, 1H), 1.82-1.77 (m, 1H), 1.70-1.66 (m, 1H), 1.60-1.52 (m, 1H), 1.29 (br s, 8H), 1.23-1.19 (m, 18H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 151.3, 123.9, 34.3, 29.9, 28.4, 28.3, 24.9, 24.8, 23.6; IR (thin

film) 2959, 1667, 1392, 1163 cm^{-1}; HRMS (ESI): m/z calculated for C$_{25}$H$_{41}$N$_3$O$_4$SNa [M+Na]$^+$ 502.2715, found 502.2705.

(*E*)-2,4,6-Triisopropyl-*N'*-(2,3,3-trimethylbutylidene)benzenesulfonohydraziden, 6.24e.
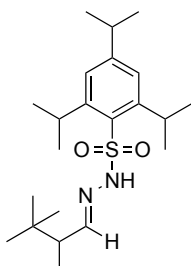
Using the general procedure for condensation outlined above, 2,3,3-trimethylbutanal (2.23 g mL, 7.48 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex (1:9) to afford the trisylhydrazone **6.24e** (2.81 g, 7.12 mmol, 95%) as a white solid, mp: 118-121 $^{\circ}$C. R$_f$ = 0.42 (1:9 EtOAc/hex); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.28 (s, 1H), 7.15 (s, 1H), 7.05 (d, J = 7.0 Hz, 1H), 4.20-4.16 (m, 2H), 2.91-2.87 (m, 1H), 2.09-2.05 (m, 1H), 1.27 (d, J = 2.9 Hz, 12H), 1.25 (d, J = 2.9 Hz, 6H), 0.92 (d, J = 7.0 Hz, 3H), 0.71 (s, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 154.4, 153.4, 151.0, 131.1, 123.8, 123.7, 46.4, 34.2, 33.0, 29.9, 27.2, 24.9, 24.8, 23.6, 12.6; IR (thin film) 3209, 2958, 1599, 1460, 1321, 1163 cm^{-1}; HRMS (ESI): m/z calculated for C$_{22}$H$_{38}$N$_2$O$_2$SNa [M+Na]$^+$ 417.2552, found 417.2539.

tert-Butyl (E)-4-((2-tosylhydrazono)methyl)piperidine-1-carboxylate, 6.2d.
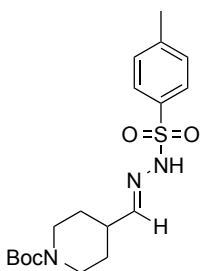
Using the general procedure for condensation outlined above, *tert*-butyl 4-formylpiperidine-1-carboxylate (0.97 g, 4.55 mmol, 1.1 equiv) and tosyl hydrazide (0.77 g, 4.14 mmol, 1.0 equiv) were used to afford the tosylhydrazone **6.2d** (1.48 g, 3.89 mmol, 94%) as a white solid, mp: 48-49 $^{\circ}$C. R$_f$ = 0.48 (1:9 EtOAc/hex); ^{1}H NMR (600 MHz, DMSO-d_6, 380 K) δ 8.32 (s, 1H), 7.78 (d, J = 9.8 Hz, 2H), 7.31 (d, J = 9.8 Hz, 2H), 7.11 (d, J = 5.9 Hz, 1H), 3.98 (br s, 3H), 2.91-2.67 (m, 3H), 2.43 (s, 3H), 2.34-2.30 (m, 1H), 1.69-1.65 (m, 2H), 1.45-1.43 (m, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 178.2, 154.8, 154.0, 144.2, 136.2, 129.6, 127.9, 79.7, 38.8, 28.8,

28.4, 21.7; IR (thin film) 2975, 1658, 1426, 1365, 1234, 1158 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{18}$H$_{27}$N$_3$O$_4$SNa [M+Na]$^+$ 404.1620, found 404.1618.

General procedure for intramolecular carbenylation:
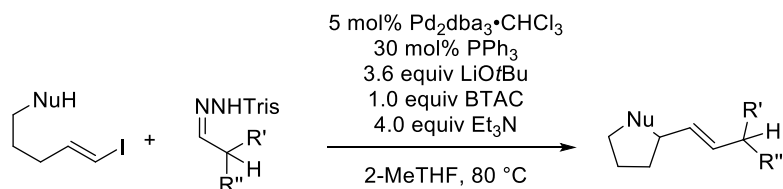
An oven-dried 5 mL pear-shaped flask was charged with Pd$_2$dba$_3$•CHCl$_3$ (5.0 mol%), PPh$_3$ (30 mol%), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-Methyltetrahydrofuran (2-MeTHF) was added to make a 0.01 M solution, and the brown slurry was then stirred for 20 min at room temperature to give a clear yellow catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*- trisylhydrazone (2.0 equiv), benzyltriethylammonium chloride (1.0 equiv), lithium *tert*-butoxide base (3.6 equiv), and a stir bar. The reaction vessel was evacuated and back-filled with N$_2$ three times, and then capped with a septum. A solution of the vinyl iodide (1.0 equiv, 0.5 M in 2-MeTHF) was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 **x** 0.15 mL). Triethylamine (4.0 equiv) was added to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred 2-MeTHF (2 **x** 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in an 80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature.

The reaction was monitored by thin layer chromatography (20:79:1 EtOAc/hex/Et₃N) to check for depletion of the vinyl iodide. The reactions reached completion between ca. 5 and 30 min depending on *N*-trisylhydrazone. Upon consumption of the vinyl iodide, the reaction was allowed to cool to room temperature and 1% (w/v) aq. NaOH was added to the reaction vessel. The mixture was extracted with EtOAc three times and the combined organic extracts were washed with brine, dried with Na$_2$SO$_4$, and concentrated *in vacuo*. The pyrrolidine was purified by silica gel chromatography.

(*E*)-1-benzyl-2-(3-methylbut-1-en-1-yl)pyrrolidine, 6.5.

An oven-dried 5 mL pear-shaped flask was charged with Pd$_2$dba$_3$•CHCl$_3$ (5.4 mg, 0.0071 mmol), PPh$_3$ (11.0 mg, 0.042 mmol), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-MeTHF (0.2 mL) was added, and the brown slurry was then stirred for 20 min at room temperature to give a clear yellow catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*-trisylhydrazone (98.6 mg, 0.280 mmol), benzyltriethylammonium chloride (31.9 mg, 0.140 mmol), lithium *tert*-butoxide base (40.4 mg, 0.504 mmol), and a stir bar. The reaction vessel was evacuated and back-filled with N$_2$ three times, and then capped with a septum. A solution of the vinyl iodide **6.1** (42.1 mg, 0.140 mmol) in 0.2 mL of 2-MeTHF was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 x 0.15 mL). Triethylamine (78 mL, 0.560 mmol) was added to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred 2-MeTHF (2 x 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in a

80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature. The reaction reached completion within 10 min and was allowed to cool to room temperature; then 2 mL 1% (w/v) aq. NaOH was added to the reaction vessel. The mixture was extracted with 3x5 mL EtOAc and the combined organic extracts were washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The crude reaction mixture was purified by silica gel chromatography (10:85:5 EtOAc/Hex/Et$_3$N) to provide pyrrolidine **6.5** (29.0 mg, 91%), as a brown oil. R_f = 0.6 (20:79:1 EtOAc/Hexanes/Et$_3$N). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.43-7.07 (m, 5H), 5.59 (dd, J = 15.3, 6.4 Hz, 1H), 5.33 (dd, J = 15.3, 8.3 Hz, 1H), 4.01 (d, J = 13 Hz, 1H), 3.04 (d, J = 12.9 Hz, 1H), 2.93 (t, J = 8.4, 1H), 2.70 (dd, J = 16.0, 8.0 Hz, 1H), 2.32-2.29 (m, 1H), 2.09-2.06 (m, 1H), 1.92-1.89 (m, 1H), 1.76-1.60 (m, 3H), 1.00-0.99 (m, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 132.5, 129.3, 129.0, 128.7, 128.2, 128.1, 127.0, 67.8, 57.7, 53.1, 31.6, 30.9, 22.6, 22.5, 21.8; IR (thin film) 2959, 1681, 1455, 1364 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{16}$H$_{23}$NH (M+H)$^+$ 230.1909, found 230.1902.

Dimethyl (*E*)-2-(3-methylbut-1-en-1-yl)cyclopentane-1,1-dicarboxylate, 6.7.
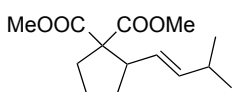
Following the general procedure for intramolecular carbenylation, vinyl iodide, dimethyl (*E*)-2-(5-iodopent-4-en-1-yl)malonate **6.6** (40.8 mg, 0.125 mmol) gave compound **6.7** (18.1 mg, 57%) as a brown oil. R_f = 0.5 (5:95 EtOAc/Hex). ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.46 (dd, J = 15.4, 6.7 Hz, 1H), 5.30 (dd, J = 15.4, 8.2 Hz, 1H), 3.72 (s, 3H), 3.63 (s, 3H), 3.21 (q, J = 7.5 Hz, 1H), 2.57-2.33 (m, 1H), 2.31-2.15 (m, 1H), 2.12-2.00 (m, 1H), 2.00-1.90 (m, 1H), 1.90-1.77 (m, 1H), 1.71-1.49 (m, 1H), 0.94 (dd, J = 6.7, 1.0 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.9, 171.3, 139.3, 126.1, 64.6, 52.5, 52.0, 48.8, 33.8, 31.3, 31.0, 29.7, 23.2, 22.6, 22.5; IR (thin film) 2954, 1729, 1433, 1264, 1212 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{14}$H$_{22}$O$_4$Na (M+Na)$^+$ 277.1416, found 277.1413.

(*E*)-1-benzyl-2-(3-phenylbut-1-en-1-yl)pyrrolidine, 6.25a.
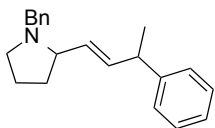
Following the general procedure for intramolecular carbenylation, vinyl iodide **6.1** (44.3 mg, 0.147 mmol) gave pyrrolidine **6.25a** (30.9 mg, 72%) as a brown oil and a mixture of 2 diastereomers that were inseparable by flash column chromatography. R_f = 0.8 (20:79:1 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.31-7.11 (m, 10H), 5.84-5.76 (m, 1H), 5.50-5.40 (m, 1H), 4.04 (d, J = 13.0 Hz, 1H), 3.57-3.43 (m, 1H), 3.07 (d, J = 12.9 Hz, 1H), 2.95-2.90 (m, 1H), 2.77 (q, J = 8.2 Hz, 1H), 2.10 (q, J = 8.8 Hz, 1H), 1.99-1.86 (m, 1H), 1.83-1.59 (m, 3H), 1.37 (dd, J = 7.0, 3.0 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 146.1, 139.7, 137.9, 131.4, 129.2, 128.5, 128.2, 127.3, 126.8, 126.1, 67.7, 58.3, 53.4, 42.2, 31.8, 22.0, 21.6; IR (thin film) 3026, 2964, 2787, 1601, 1493, 1452, 1370 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{21}$H$_{25}$NH (M+H)$^+$ 292.2065, found 292.2057.

(*E*)-1-benzyl-2-(3-((*tert*-butyldiphenylsilyl)oxy)but-1-en-1-yl)pyrrolidine, 6.25b.
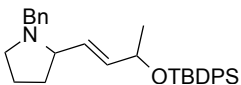
Following the general procedure for intramolecular carbenylation, vinyl iodide **6.1** (34.5 mg, 0.115 mmol) gave pyrrolidine **6.25b** (41.5 mg, 77%) as a brown oil. R_f = 0.9 (20:79:1 EtOAc/Hex/Et$_3$N). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.71-7.64 (m, 4H), 7.46-7.17 (m, 11H), 5.69 (dd, J = 15.5, 6.3 Hz, 1H), 5.44 (dd, J = 15.4, 8.3 Hz, 1H), 4.42-4.27 (m, 1H), 3.93 (d, J = 13.0 Hz, 1H), 3.01 (d, J = 13.0 Hz, 1H), 2.91-2.85 (m, 1H), 2.74-2.65 (m, 1H), 2.12-2.01 (m, 1H), 1.94-1.79 (m, 1H), 1.77-1.62 (m, 1H), 1.57-1.43 (m, 1H), 1.16 (d, J = 6.3 Hz, 3H), 1.08-1.04 (m, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 139.8, 136.8, 132.4, 130.2, 129.7, 129.3, 72.7, 67.8, 58.1, 53.1, 31.0, 32.1, 30.6, 27.5, 26.8; IR (thin film) 2982, 1734, 1373 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{31}$H$_{40}$NOSiH (M+H)$^+$ 470.2879, found 470.2881.

tert-butyl (_E_)-2-(2-(1-benzylpyrrolidin-2-yl)vinyl)pyrrolidine-1-carboxylate, 6.25d.
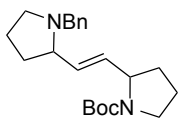
Following the general procedure for intramolecular carbenylation, vinyl iodide **6.1** (41.4 mg, 0.138 mmol) gave pyrrolidine **6.25d** (36.8 mg, 75%) as a brown oil. R_f = 0.8 (20:79:1 EtOAc/Hex/Et$_3$N). ^{1}H NMR (600 MHz, DMSO-_d6_, 400 K) δ 7.31-7.23 (m, 4H), 7.21-7.18 (m, 1H), 5.58 (dd, _J_ = 15.3, 6.0 Hz, 1H), 5.41 (m, 1H), 4.35-4.14 (m, 1H), 3.93 (d, _J_ = 13.6 Hz, 1H), 3.47-3.24 (m, 2H), 3.21 (d, _J_ = 13.4 Hz, 1H), 3.10-2.72 (m, 2H), 2.19 (q, _J_ = 8.5 Hz, 1H), 2.07-1.86 (m, 2H), 1.94-1.79 (m, 1H), 1.78-1.76 (m, 2H), 1.73-1.59 (m, 3H), 1.55-1.52 (m, 1H), 1.43-1.34 (m, 9H); ^{13}C NMR (125 MHz, DMSO-_d6_) δ 139.4, 132.9, 131.7, 128.2, 128.1, 127.9, 126.4, 77.9, 66.2, 57.6, 57.0, 52.5, 45.8, 31.4, 28.0, 21.6; IR (thin film) 2970, 1692, 1392, 1249, 1167, 1112 cm^{-1}; HRMS (ESI): _m/z_ calc'd for C$_{22}$H$_{32}$N$_2$O$_2$Na (M+Na)$^+$ 379.2361, found 379.2360.

(_Z_)-1-benzyl-2-(3,4,4-trimethylpent-1-en-1-yl)pyrrolidine, 6.25e.
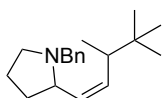
Following the general procedure for intramolecular carbenylation, vinyl iodide **6.1** (39.7 mg, 0.132 mmol) gave pyrrolidine **6.25e** (25.8 mg, 72%) as a brown oil. R_f = 0.8 (20:79:1 EtOAc/Hex/Et$_3$N). Two diastereomers were observed. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.34-7.27 (m, 4H), 7.23 (dd, _J_ = 13.0, 4.1 Hz, 1H), 5.59-5.54 (m, 1H), 5.34 (dd, _J_ = 8.4, 4.1 Hz, 1H), 4.05 (dd, _J_ = 20.1, 12.8 Hz, 1H), 3.03 (dd, _J_ = 12.7, 9.7, 1H), 2.96-2.85 (m, 1H), 2.75-2.71 (m, 1H), 2.09-2.07 (m, 1H) 1.99-1.87 (m, 1H), 1.85-1.51 (m, 1H), 1.03-0.92 (m, 4H), 0.87 (d, _J_ = 9.9, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 139.7, 139.6, 136.8, 136.6, 132.2, 132.0, 129.2, 129.1, 128.3, 128.2, 126.8, 126.7, 68.1, 67.9, 58.2, 58.1, 53.4, 53.3, 47.1, 46.9, 32.9, 32.8, 31.8, 31.7, 27.6, 27.5, 22.0, 21.9, 15.8, 15.7; IR (thin film) 2960, 1685, 1454, 1364 cm^{-1}; HRMS (ESI): _m/z_ calc'd for C$_{19}$H$_{29}$NH (M+H)$^+$ 272.2378, found 272.2369.

(*Z*)-1-benzyl-2-(3-methoxy-4,4-dimethylpent-1-en-1-yl)pyrrolidine, 6.25f.
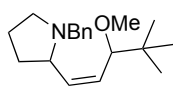
Following the general procedure for intramolecular carbenylation, vinyl iodide **6.1** (45.9 mg, 0.152 mmol) gave pyrrolidine **6.25f** (43.4 mg, 99%) as a brown oil. R_f = 0.7 (20:79:1 EtOAc/Hex/Et$_3$N). Two diastereomers were observed. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.30-7.29 (m, 4H), 7.23-7.22 (m, 1H), 5.64 (dd, *J* = 7.9, 3.5 Hz, 1H), 5.60 (d, *J* = 8.1 Hz, 1H), 4.05 (dd, *J* = 20.1, 12.8 Hz, 1H), 3.03 (dd, *J* = 12.7, 9.7, 1H), 2.96-2.85 (m, 1H), 2.75-2.71 (m, 1H), 2.09-2.07 (m, 1H) 1.99-1.87 (m, 1H), 1.85-1.51 (m, 1H), 1.03-0.92 (m, 4H), 0.87 (d, *J* = 9.9, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 139.7, 139.6, 136.8, 136.6, 132.2, 132.0, 129.2, 129.1, 128.3, 128.2, 126.8, 126.7, 68.1, 67.9, 58.2, 58.1, 53.4, 53.3, 47.1, 46.9, 32.9, 32.8, 31.8, 31.7, 27.6, 27.5, 22.0, 21.9, 15.8, 15.7; IR (thin film) 2954, 1695, 1454, 1362, 1179, 1092 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{19}$H$_{29}$NOH (M+H)$^+$ 288.2327, found 288.2324.

(*E*)-1-Benzyl-2-(3,3-dimethylbut-1-en-1-yl)pyrrolidine, 6.28.
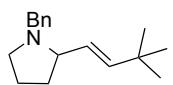
Following the procedure for intramolecular carbenylation by Khanna, *et al*,[3] vinyl iodide **6.1** (45.2 mg, 0.150 mmol) and *N*-tosylhydrazone of pivalaldehyde (0.130 g, 0.420 mmol) were used to give pyrrolidine **6.28** (24.9 mg, 68%) as a yellow oil. R_f = 0.24 (10:90 EtOAc/Hex). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.62 (app d, *J* = 4.4 Hz, 3H), 7.23 (sext, J = 4 Hz, 1H), 5.63 (d, *J* = 15.6 Hz, 1H), 5.28 (dd, *J* = 15.6, 8.4 Hz, 1H), 3.98 (d, *J* = 12.9 Hz, 1H), 3.04 (d, *J* = 12.9 Hz, 1H), 2.93 (dt, *J* = 9.2, 2.2 Hz, 1H), 2.69 (q, *J* = 8.4 Hz, 1H), 2.09 (q, *J* = 8.85 Hz, 1H), 1.95-1.90 (m, 1H), 1.80-1.72 (m, 1H), 1.71-1.58 (m, 3H), 1.02 (s, 9H); ^{13}C NMR (125 MHz) δ 144.6, 139.8, 129.1, 128.2, 127.1, 126.7, 67.9, 58.1, 53.4, 32.9, 31.8, 29.8, 21.9; IR (thin film) 2957, 2786, 1454, 1362 cm^{-1}; HRMS (ESI): m/z calc'd for C$_{17}$H$_{25}$NH (M+H)$^+$ 244.2065, found 244.2074.

General procedure for intermolecular carbenylation:
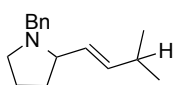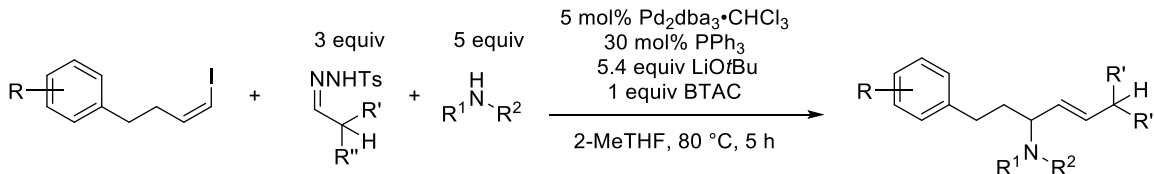

An oven-dried 5 mL pear-shaped flask was charged with Pd$_2$dba$_3$•CHCl$_3$ (5.0 mol%), PPh$_3$ (30 mol%), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-MeTHF was added to make a 0.01 M solution, and the brown slurry was then stirred for 20 min at room temperature to give a clear yellow catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*-tosylhydrazone (3.0 equiv), benzyltriethylammonium chloride (1.0 equiv), lithium *tert*-butoxide base (5.4 equiv), and a stir bar was evacuated and back-filled with N$_2$ three times, and then capped with a septum. A solution of the vinyl iodide (1.0 equiv, 0.5 M in 2-MeTHF) was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 x 0.15 mL). Next, amine (5.0 equiv) was added to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred using 2-MeTHF (2 x 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in an 80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature.

The reaction was monitored by thin layer chromatography (20:79:1 EtOAc/hex/Et$_3$N) to check for depletion of the vinyl iodide. The reactions reached completion between ca. 3 and 5 h depending on *N*-tosylhydrazone. Upon consumption of the vinyl iodide, the reaction was allowed

to cool to room temperature and 1% (w/v) aq. NaOH was added to the reaction vessel. The mixture was extracted with EtOAc three times and the combined organic extracts were washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The pyrrolidine was purified by silica gel chromatography.

**(*E*)-1-(6-methyl-1-phenylhept-4-en-3-yl)piperidine, 6.9.**
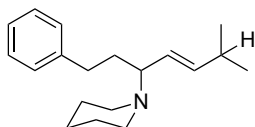
An oven-dried 5 mL pear-shaped flask was charged with $Pd_2dba_3 \cdot CHCl_3$ (8.6 mg, 0.008 mmol), PPh_3 (13.1 mg, 0.050 mmol), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-MeTHF (0.2 mL) was added, and the brown slurry was then stirred for 20 min at room temperature to give a clear yellow catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*-tosylhydrazone (0.12 g, 0.499 mmol), benzyltriethylammonium chloride (46.2 mg, 0.166 mmol), lithium *tert*-butoxide base (72.2 mg, 0.899 mmol), and a stir bar was evacuated and back-filled with N_2 three times, and then capped with a septum. A solution of the (*Z*)-(4-iodobut-3-en-1-yl)benzene **6.8a** (43.0 mg, 0.167 mmol) in 0.2 mL of 2-MeTHF was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 x 0.15 mL). Next, piperidine (82 mL, 0.833 mmol) was added to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred 2-MeTHF (2 x 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in a 80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature. The reaction reached completion within 10 min and was allowed to cool to room

temperature; then 2 mL 1% (w/v) aq. NaOH was added to the reaction vessel. The mixture was extracted with 3x10 mL EtOAc and the combined organic extracts were washed with brine, dried with Na$_2$SO$_4$, and concentrated *in vacuo*. The pyrrolidine was purified by silica gel chromatography (10:85:5 EtOAc/Hex/Et$_3$N) to provide of pyrrolidine **6.9** (29.0 mg, 91%), as a yellow oil. R$_f$ = 0.45 (10:90:5 EtOAc/Hexanes/Et$_3$N). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.37-7.21 (m, 2H), 7.20-7.08 (m, 3H), 5.47 (dd, *J* = 15.4, 6.6 Hz, 1H), 5.30 (dd, *J* = 15.4, 8.9 Hz, 1H), 2.73-2.69 (m, 1H), 2.65-2.59 (m, 1H), 2.56-2.45 (m, 3H), 2.42-2.26 (m, 3H), 2.07-1.82 (m, 1H), 1.83-1.64 (m, 1H), 1.64-1.47 (m, 4H), 1.46-1.33 (m, 2H), 1.02 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 142.8, 141.5, 128.5, 128.3, 125.6, 125.5, 67.5, 50.6, 34.4, 33.0, 31.2, 26.4, 24.9, 22.9, 22.8; IR (thin film) 2929, 2856, 2791, 1495, 1453, 1101 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{19}$H$_{29}$NH (M+H)$^+$ 272.2378, found 272.2384.

(*E*)-1-(1-cyclohexyl-5-phenylpent-1-en-3-yl)piperidine, 6.12.
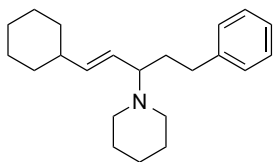
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.11** (38.5 mg, 0.163 mmol) gave **6.12** (14.5 mg, 35%) as a yellow oil. R$_f$ = 0.45 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.27-7.24 (m, 2H), 7.17-7.14 (m, 3H), 5.44 (dd, *J* = 15.5, 6.6 Hz, 1H), 5.30 (ddd, *J* = 15.5, 9.0, 0.9 Hz, 1H), 2.71-2.59 (m, 2H), 2.54-2.48 (m, 3H), 2.38-2.96 (m, 2H), 2.03-1.92 (m, 2H), 1.76-1.53 (m, 7H), 1.60-1.50 (m, 4H), 1.43-1.39 (m, 2H), 1.33-1.24 (m, 3H), 1.22-1.07 (m, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 142.8, 140.3, 128.5, 128.3, 126.1, 125.6, 67.6, 50.6, 40.8, 34.4, 33.4, 33.3, 33.0, 26.5, 26.3, 26.1, 24.9; IR (thin film) 2922, 2850, 1730, 1495, 1449, 1271, 1116 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{22}$H$_{33}$NH (M+H)$^+$ 312.2691, found 312.2698.

Diethyl (*E*)-2-(1-(4-bromophenyl)-6-methylhept-4-en-3-yl)malonate, 6.13ab.
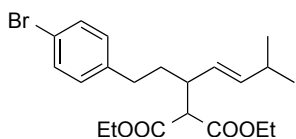
Diethyl malonate (0.23 mL, 1.52 mmol, 12 equiv) was slowly added to a suspension of NaH (0.055 g, 1.38 mmol, 11 equiv) in THF (1.0 mL) at 0 °C. The mixture was stirred at room temperature for 10 min and set aside.

An oven-dried 5 mL pear-shaped flask was charged with $Pd_2dba_3 \cdot CHCl_3$ (6.5 mg, 0.006 mmol, 0.05 equiv), PPh_3 (9.4 mg, 0.038 mmol, 0.3 equiv), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-MeTHF (0.3 mL) was added, and the brown slurry was then stirred for 20 min at room temperature to give a clear orange catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*-tosylhydrazone (91.2 mg, 0.380 mmol, 3.0 equiv), benzyltriethylammonium chloride (35.1 mg, 0.126 mmol, 1.0 equiv), lithium *tert*-butoxide base (54.8 mg, 0.683 mmol, 5.4 equiv), and a stir bar. The reaction vessel was evacuated and back-filled with N_2 three times, and then capped with a septum. A solution of the vinyl iodide **6.8b** (47.7 mg, 0.126 mmol, 1.0 equiv) in 0.2 mL of 2-MeTHF was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 x 0.15 mL). Sodium malonate solution was added via syringe to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred 2-MeTHF (2 x 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in a 80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature. The reaction reached completion within 3 h. The resulting orange mixture was cooled to room temperature and quenched with a saturated NH_4Cl solution. The resulting

solution was extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation to give an orange oil.

The crude reaction mixture was purified by silica gel chromatography (1:9 EtOAc/Hex) to afford **6.13ab** (24.3 mg, 71%), as a colorless oil. R_f = 0.56 (10:90 EtOAc/Hex). Isolated as a mixture of 2 regioisomers, 13% of the minor regioisomer was inseparable by column chromatography. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.38 (d, *J* = 7.8 Hz, 2H), 7.02 (d, *J* = 7.9 Hz, 1H), 5.48 (dd, *J* = 15.3, 6.7 Hz, 1H), 5.21 (dd, *J* = 15.3, 9.6 Hz, 1H), 4.22-4.04 (m, 4H), 3.30 (d, *J* = 8.9, 1H), 2.76-2.39 (m, 2H), 2.27 (dq, *J* = 13.5, 6.6 Hz, 1H), 1.83-1.48 (m, 1H), 1.29-1.20 (m, 6H), 0.97 (dd, *J* = 10.9, 6.8 Hz, 6H), ^{13}C NMR (125 MHz, CDCl$_3$) δ 168.4, 168.2, 141.8, 140.9, 131.4, 131.3, 130.3, 130.2, 125.9, 119.5, 61.3, 61.2, 57.5, 42.6, 34.2, 32.9, 31.2, 25.8, 22.6, 22.5, 14.2, 14.2; IR (thin film) 2958, 1731, 1488, 1367, 1242, 1149 cm^{-1}; HRMS (ESI): *m/z* calc'd for $C_{21}H_{29}BrO_4Na$ (M+Na)$^+$ 447.1147, found 447.1154.

**(*E*)-1-(4-bromophenyl)-*N*-butyl-6-methylhept-4-en-3-amine, 6.15ab.**
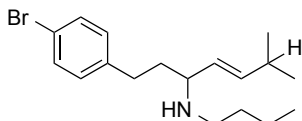
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (53.9 mg, 0.143 mmol) gave **6.15ab** (20.9 mg, 43%) as a yellow oil. R_f = 0.41 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.38 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 7.9 Hz, 1H), 5.49 (dd, *J* = 15.4, 6.6 Hz, 1H), 5.14 (dd, *J* = 15.4, 8.4 Hz, 1H), 2.93-2.88 (m, 1H), 2.67-2.49 (m, 2H), 2.46-2.41(m, 2H), 2.36-2.27 (m, 1H), 1.81-1.74 (m, 1H), 1.68-1.60 (m, 1H), 1.47-1.38 (m, 2H), 1.35-1.29 (m, 2H), 1.01 (d, *J* = 6.6 Hz, 6H), 0.9(t, J = 7.2 Hz, 4H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 141.2, 131.4, 130.3, 119.4, 66.9, 60.6, 46.7, 31.8, 31.0, 22.69, 22.64, 20.5, 14.0; IR (thin film) 2956, 2926, 2867, 1488, 1464, 1072, 1012 cm^{-1}; HRMS (ESI): *m/z* calc'd for $C_{18}H_{28}NBrH$ (M+H)$^+$ 338.1483, found 338.1496.

(*E*)-*N*-benzyl-1-(4-bromophenyl)-6-methylhept-4-en-3-amine, 6.16ab.
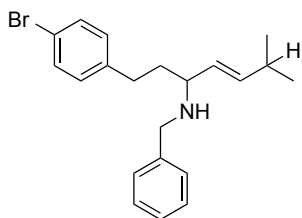
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (148 mg, 0.440 mmol) gave **6.16ab** (33.7 mg, 21%) as a colorless oil. R_f = 0.9 (20:79:1 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.36 (d, *J* = 8.4 Hz, 2H), 7.32-7.22 (m, 5H), 7.23 (d, *J* = 8.4 Hz, 2H), 5.50 (dd, *J* = 15.4, 6.7 Hz, 1H), 5.19 (ddd, *J* = 15.4, 8.5, 1 Hz, 1H), 3.80 (d, *J* = 13.2 Hz, 1H), 3.62 (d, *J* = 13.2, 1H), 2.96 (m, 1H), 2.57 (m, 2H), 2.37-2.30 (m, 1H), 1.81-1.74 (m, 1H), 1.71-1.64 (m, 1H), 1.02 (dd, *J* = 6.8, 3.2 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 141.4, 140.8, 140.7, 131.4, 130.2, 129.2, 128.4, 128.3, 126.9, 119.4, 59.7, 51.2, 37.5, 31.8, 31.0, 22.74, 22.70; IR (thin film) 2923, 1727, 1488, 1454, 1288, 1105, 1072, 1011 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{21}$H$_{26}$BrNH (M+H)$^+$ 372.1327, found 372.1328.

**(*E*)-1-(1-(4-bromophenyl)-6-methylhept-4-en-3-yl)pyrrolidine, 6.17ab.**
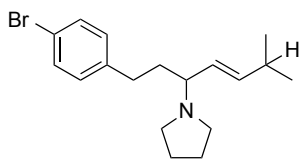
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (51.2 mg, 0.136 mmol) gave **6.17ab** (32.0 mg, 70 %) as a yellow oil. R_f = 0.43 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.38 (d, *J* = 8.3 Hz, 2H), 7.04 (d, *J* = 8.3 Hz, 2H), 5.50 (dd, *J* = 15.4, 6.6 Hz, 1H), 5.31(ddd, *J* = 15.4, 8.9, 0.6 Hz, 1H), 2.64-2.41 (m, 6H), 2.35-2.29 (m, 1H), 2.01-1.94 (m, 1H), 1.75-1.65 (m, 6H), 1.01 (dd, *J* = 6.8, 3.3 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 141.6, 140.7, 131.3, 130.3, 127.9, 119.4, 67.1, 51.6, 35.7, 31.9, 31.0, 23.2, 22.7; IR (thin film) 2957, 2867, 2782, 1487, 1458, 1361, 1121, 1071, 1011 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{18}$H$_{26}$BrNH (M+H)$^+$ 336.1327, found 336.1330.

(*E*)-1-(1-(4-bromophenyl)-6-methylhept-4-en-3-yl)piperidine, 6.18ab.
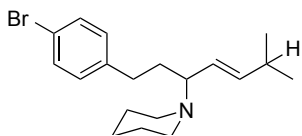
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (49.7 mg, 0.132 mmol) gave **6.18ab** (34.0 mg, 74%) as a yellow oil. R_f = 0.45 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.42-7.33 (m, 2H), 7.04 (d, *J* = 8.3 Hz, 2H), 5.44 (dd, *J* = 15.4, 6.6 Hz, 1H), 5.31-5.25 (m, 1H), 2.68-2.65 (m, 1H), 2.61-2.56 (m, 1H), 2.50-2.45 (m, 3H), 2.33-2.29 (m, 3H), 1.94-1.86 (m, 1H), 1.71-1.61 (m, 1H), 1.61-1.48 (m, 4H), 1.43-1.35 (m, 2H), 1.01 (dd, *J* = 6.7, 2.1 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 141.7, 141.6, 131.3, 130.3, 125.4, 119.3, 67.2, 50.6, 34.2, 32.4, 31.2, 26.5, 24.9, 22.9, 22.8; IR (thin film) 2929, 2855, 2791, 1660, 1487, 1452, 1095, 1071, 1011 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{19}$H$_{28}$BrNH (M+H)$^+$ 350.1483, found 350.1482.

(*E*)-4-(1-(4-bromophenyl)-6-methylhept-4-en-3-yl)morpholine, 6.19ab.
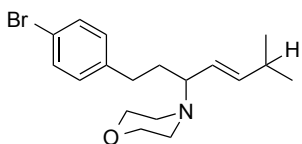
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.14** (47.6 mg, 0.126 mmol) gave **6.19ab** (31.9 mg, 72%) as a colorless oil. R_f = 0.41 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.40-7.36 (m, 2H), 7.04 (d, *J* = 8.3 Hz, 2H), 5.44 (dd, *J* = 15.5, 6.6 Hz, 1H), 5.31-5.25 (ddd, *J* = 15.5, 6.6, 1.2 Hz, 1H), 3.76-3.62 (m, 4H), 2.68-2.58 (m, 2H), 2.58-2.46 (m, 3H), 2.43-2.37 (m, 2H), 2.36-2.29 (m, 1H), 1.97-1.87 (m, 1H), 1.70-1.61 (m, 1H), 1.01 (dd, *J* = 6.8, 1.1 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 142.4, 141.4, 131.4, 130.3, 125.0, 119.4, 67.4, 67.0, 50.1, 33.6, 32.0, 31.2, 22.8, 22.7; IR (thin film) 2954, 2853, 2810, 1487, 1452, 1117, 1071, 1011 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{18}$H$_{26}$BrNOH (M+H)$^+$ 352.1276, found 352.1278.

(*E*)-1-(5-(4-bromophenyl)-1-cyclohexylpent-1-en-3-yl)piperidine, 6.20cb.
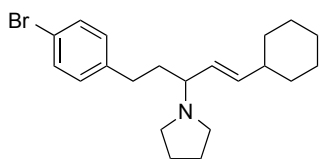
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (53.9 mg, 0.160 mmol) gave **6.20cb** (47.0 mg, 78%) as a yellow oil. R_f = 0.59 (5:95:3 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 (d, *J* = 8.2 Hz, 2H), 7.33-7.24 (d, *J* = 8.1 Hz, 2H), 5.48 (dd, *J* = 15.5, 6.6 Hz, 1H), 5.31 (dd, *J* = 15.4, 8.9 Hz, 1H), 2.70-2.31 (m, 8H), 1.99-1.94 (m, 2H), 1.80-1.60 (m, 9H), 1.35-1.00 (m, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 141.6, 139.6, 131.3, 130.3, 128.3, 119.3, 67.2, 51.6, 40.6, 35.7, 33.2, 33.3, 31.9, 26.2, 26.1, 23.2; IR (thin film) 2921, 1652, 1487, 1447, 1122, 1072, 1011 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{21}$H$_{30}$BrNH (M+H)$^+$ 376.1640, found 376.1637.

(*E*)-1-(1,8-diphenyloct-4-en-3-yl)piperidine, 6.21ba.
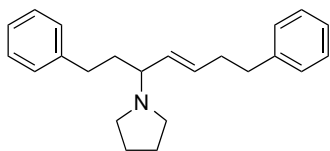
Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8a** (49.0 mg, 0.190 mmol) gave **6.21ba** (34.3 mg, 57%) as a yellow oil. R_f = 0.39 (10:90:5 EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.28-7.25 (m, 4H), 7.20-7.13 (m, 6H), 5.56 (dt, *J* = 15.4, 6.7 Hz, 1H), 5.41-5.37 (m, 1H), 2.75-2.72 (m, 2H), 2.62-2.56 (m, 2H), 2.48-2.39 (m, 5H), 2.00-1.94 (m, 1H), 1.73-1.67 (m, 4H), 1.63 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 142.7, 141.8, 132.2, 128.5, 128.4, 128.33, 128.30, 125.8, 125.6, 67.3, 51.6, 35.83, 35.80, 34.1, 32.4, 23.2; IR (thin film) 2925, 2782, 1603, 1495, 1454 cm^{-1}; HRMS (ESI): *m/z* calc'd for C$_{23}$H$_{29}$NH (M+H)$^+$ 320.2378, found 320.2379.

***tert*-Butyl (*E*)-4-(5-(4-bromophenyl)-3-(piperidin-1-yl)pent-1-en-1-yl)piperidine-1-carboxy--late, 6.22db.**

Following the general procedure for intermolecular carbenylation, vinyl iodide **6.8b** (49.9 mg, 0.148 mmol) gave **6.22db** (35.3 mg, 50%) as a yellow oil. R_f = 0.27 (10:90:5
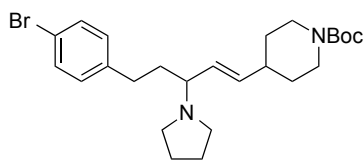
EtOAc/Hex/Et$_3$N). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 (d, $J =$ 8.4 Hz, 2H), 7.03 (d, $J =$ 8.4 Hz, 2H), 5.49 (dd, $J =$ 15.5, 6.4 Hz, 1H), 5.38 (dd, $J =$ 15.7, 8.7 Hz, 1H), 4.10 (m, 2H), 2.82-2.69 (m, 2H), 2.63-2.40 (m, 7H), 2.19-2.12 (m, 1H), 2.02-1.96 (m, 1H), 1.75-1.67 (m, 6H), 1.46 (s, 9H), 1.35-1.27 (m, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 154.9, 141.3, 137.5, 131.4, 130.2, 129.6, 119.4, 79.4, 67.0, 51.6, 38.8, 35.4, 32.0, 31.8, 29.8, 28.5, 23.2; IR (thin film) 2927, 2853, 1689, 1422, 1364, 1274, 1231, 1162 cm^{-1}; HRMS (ESI): m/z calc'd for C$_{25}$H$_{37}$BrN$_2$O$_2$H (M+H)$^+$ 477.2117, found 477.2126.

Control experiment: Carbenylation in the absence of the amine nucleophile.
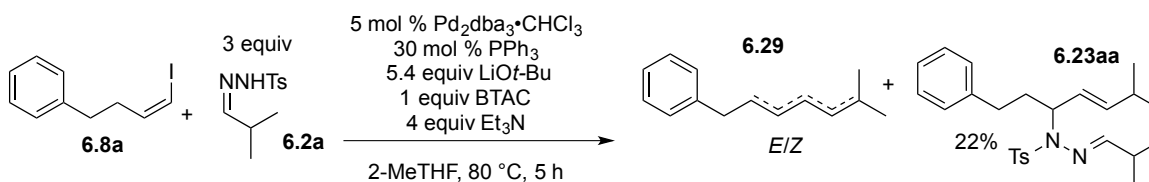
An oven-dried 5 mL pear-shaped flask was charged with Pd$_2$dba$_3$•CHCl$_3$ (7.6 mg, 0.007 mmol), PPh$_3$ (11.5 mg, 0.044 mmol), and a stir bar. The flask was fitted with a septum and purged with nitrogen. 2-MeTHF (0.2 mL) was added, and the brown slurry was then stirred for 20 min at room temperature to give a clear yellow catalyst solution.

Meanwhile, a separate oven-dried 5 mL round-bottom flask containing of *N*-tosylhydrazone **6.2a** (0.11 g, 0.441 mmol), benzyltriethylammonium chloride (40.8 mg, 0.147 mmol), lithium *tert*-butoxide base (63.6 mg, 0.793 mmol), and a stir bar was evacuated and back-filled with N$_2$ three times, and then capped with a septum. A solution of the (*Z*)-(4-iodobut-3-en-1-yl)benzene **6.8a** (37.9 mg, 0.147 mmol) in 0.2 mL of 2-MeTHF was transferred from a pear-shaped flask by syringe to the dry reagents in the round-bottom flask. The residual vinyl iodide

in the pear-shaped flask was transferred to the reaction vessel using 2-MeTHF (2 x 0.15 mL). Next, triethylamine (78 mL, 0.587 mmol) was added to the round-bottom flask.

Finally, the catalyst solution was transferred to the reaction vessel via syringe, the remaining catalyst solution was transferred using 2-MeTHF (2 x 0.2 mL). The reaction vessel was fitted with a reflux condenser and capped with a septum. The reaction vessel was immersed in a 80 °C oil bath up to the level of the flask contents, and the stirred slurry rapidly reached reflux temperature. The reaction reached completion within 3 h and was allowed to cool to room temperature; then 2 mL 1% (w/v) aq. NaOH was added to the reaction vessel. The mixture was extracted with 3x10 mL EtOAc and the combined organic extracts were washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The crude reaction mixture was analyzed by GC/MS (EI). In the absence of an amine nucleophile, the reaction conditions resulted in 43% of β-hydride eliminated diene **6.29**, 22% of **6.23aa** and 10% of remaining starting vinyl iodide **6.8a**.

Chapter 7

Palladium-Catalyzed Bis-cyclization/Dimerization Reactions

In 2012, we reported an intramolecular carbenylative amination reaction that employed *N*-tosylhydrazones as carbene precursors to generate 2-substituted pyrrolidines **7.2**.[184b] During optimization of the carbenylative amination, we noted that dimerization of the vinyl halide was competing with the desired carbenylation reaction to form bis-pyrrolidine **7.3** (Scheme 7-1). When the *N*-tosylhydrazone was omitted from the reaction, the bis-pyrrolidine dimer **7.3** was formed in 67% yield. Intrigued by this unprecedented dimerization, my coworker Avinash Khanna and I set out to explore the scope and potential applications of the reaction.

Scheme 7-1: Dimerization as a Competing Side Reaction in Carbenylative Amination
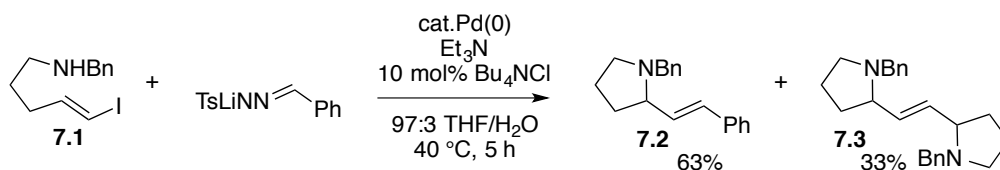
First we sought to identify which components of the heavily optimized carbenenylative insertion were necessary for the dimerization reaction and to improve the yield of the dimer (Table 7-1). Among the catalyst precursors that we examined $(Ph_3P)_4Pd$ proved to be more efficient than $Pd_2dba_3 \cdot CHCl_3$ or palladium(II) pre-catalysts (entries 1-5). Reducing the amount of lithium *tert*-butoxide to just 1.1 equivalents was beneficial (entries 1, 6 and 7), but surprisingly, lithium hydroxide was less efficient than lithium *tert*-butoxide, even though water is a cosolvent. Other metal alkoxides were less efficient (entries 7, 10 and 11). Substitution of lithium *tert*-butoxide with silver salts as bases led to a dramatic acceleration of the reaction rate, reducing the half-life to under an hour (entries 12-15). The triethylamine additive proved to be

dispensible (entries 18 and 20). Ultimately, the best yields with silver phosphate were obtained at 55 °C (entries 18 and 20).

Other experiments not shown in Table 1 revealed that the added water was essential for good yields and that other solvents were less efficient. Surprisingly, doubling the amount of palladium catalyst to 10 mol % reduced the yield of dimer **7.3**.

Table 7-1: Optimization of the Bis-cyclization/Dimerization of Vinyl Iodide **7.1** to Generate Dimer **7.3**
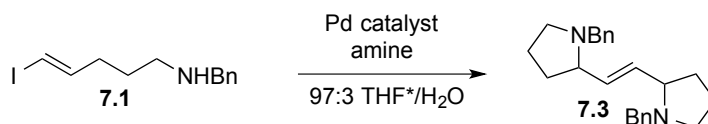
entry	Pd source (mol %)	phosphine (mol %)	base (equiv)	equiv Et$_3$N	temp	time	yield
1	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	t-BuOLi (2.2)	2	23 °C	24 h	61%
2	(Ph$_3$P)$_4$Pd (5.0%)	-	t-BuOLi (2.2)	2	23 °C	24 h	65%
3	(Ph$_3$P)$_2$PdCl$_2$ (5.0%)	-	t-BuOLi (2.2)	2	23 °C	24 h	47%
4	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	dppe (5%)	t-BuOLi (2.2)	2	23 °C	24 h	50%
5	Pd(OAc)$_2$ (5.0%)	Ph$_3$P (10%)	t-BuOLi (2.2)	2	23 °C	24 h	18%
6	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	t-BuOLi (4.4)	2	23 °C	24 h	33%
7	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	t-BuOLi (1.1)	2	23 °C	24 h	67%
8	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	-	2	23 °C	24 h	10%
9	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	LiOH (1.1)	2	23 °C	24 h	48%
10	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	t-BuONa(1.1)	2	23 °C	24 h	<20%
11	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	t-BuOK (1.1)	2	23 °C	24 h	55%
12	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	Ag$_2$CO$_3$ (1.1)	2	23 °C	3 h	51%
13	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	Ag$_3$PO$_4$ (1.1)	2	23 °C	3 h	61%
14	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	Ag$_3$PO$_4$ (0.37)	2	23 °C	3 h	65%
15	Pd$_2$dba$_3$•CHCl$_3$ (2.5%)	Ph$_3$P (15%)	Ag$_3$PO$_4$ (0.11)	2	23 °C	3 h	49%
16	(Ph$_3$P)$_4$Pd (5.0%)	-	Ag$_3$PO$_4$ (1.1)	2	23 °C	3 h	71%
17	(Ph$_3$P)$_4$Pd (5.0%)	-	Ag$_3$PO$_4$ (0.37)	2	23 °C	3 h	50%
18	(Ph$_3$P)$_4$Pd (5.0%)	-	Ag$_3$PO$_4$ (1.1)	2	55 °C	1 h	72%
19	(Ph$_3$P)$_4$Pd (5.0%)	-	Ag$_3$PO$_4$ (1.1)	2	80 °C	0.16 h	65%
20	(Ph$_3$P)$_4$Pd (5.0%)	-	Ag$_3$PO$_4$ (1.1)	0	55 °C	1 h	75%

* temperature dependence studies were carried out in 2-methyltetrahydrofuran

The optimized dimerization reaction generates only the trans alkene. The configuration of the double bond in **7.3** was established rigorously as E by the 15 Hz coupling constants in the ^{13}C satellites for the olefinic protons (Scheme 7-2). With alkoxide bases, dimer (E)-**7.3** was present as a mixture of two different stereoisomers in about 95:5 ratio, differing in configuration at the

stereogenic centers. With silver phosphate a single stereoisomer was obtained. The stereochemistry of the dimer **7.3** was rigorously established as *meso* by a crystal structure (Scheme 7-2). Of note, *meso* dimers of this kind cannot be efficiently made through olefin metathesis.

Scheme 7-2: NMR and X-ray Crystallography Establish the Configuration of the Double Bond and the Relative Configuration
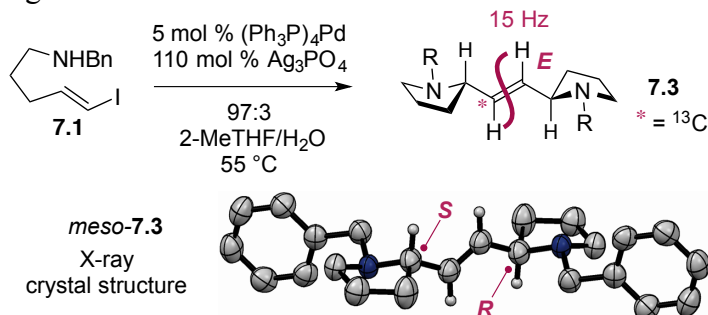
With optimized conditions for stereoselective formation of the dimer **7.3** we next assessed the tolerance of the reaction to variations of the *N*-alkyl substituent (Scheme 7-3). The reaction was chemoselective, tolerating terminal olefins and styryl groups to give **7.3a** and **7.3b** in 56% and 48% yield, respectively. The reaction tolerated electron rich furans to give dimer **7.3c** in good yield. Surprisingly, cylopentyl, and even 1-adamantyl substituents were well tolerated on the amino group. However, the *N*-adamantyl substrate required a slightly longer reaction time (3 h) to generate **7.3e**.

The bis-piperidines are formed less efficiently than bis-pyrrolidines under the optimized reaction conditions (Scheme 7-4). However the dimerization of **7.4** directly generates the skeleton of the bis-piperidine alkaloid hyalbidone in a single step. The natural product hyalbidone was isolated as mixture of *meso* and *d/l* isomers from the roots of *H. albus*.[192] It is not clear whether the mixture of stereoisomers occurs in Nature or was the result of epimerization during isolation.

Scheme 7-3: The Bis-cyclization/Dimerization Reaction Tolerates a Variety of *N*-Alkyl Groups

5 mol % (Ph₃P)₄Pd
110 mol % Ag₃PO₄

97:3 2-MeTHF/H₂O
55 °C, 0.3-3 h

7.1a

7.3a 56%

7.3b 48%   **7.3c** 61%   **7.3d** 56%   **7.3e** 65%
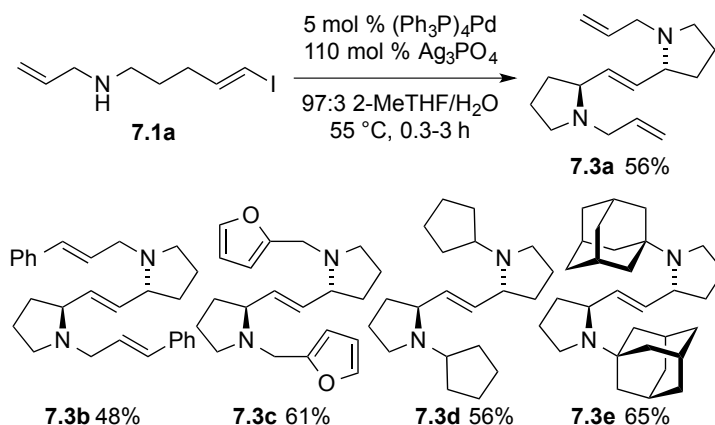
Scheme 7-4: One-Step Synthesis of the Hyalbidone Skeleton

5 mol % (Ph₃P)₄Pd
110 mol % Ag₃PO₄

97:3 2-MeTHF/H₂O
55 °C, 0.3-3 h

7.4

7.5a
47% 7:1 *dr*

7.5b
36% 9:1 dr*meso* / *d/l*

hyalbidone (*Hyoscyamus albus*)
1:1 dr
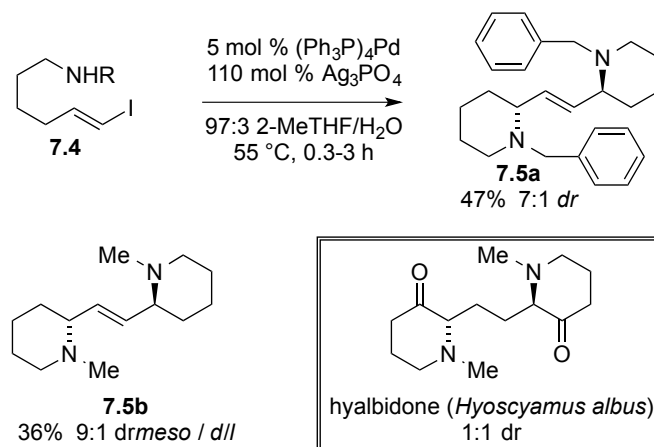
The dimeric products accessed by our reaction bear a remarkable resemblence to the dimeric products noted by Balme and co-workers in the palladium-catalyzed reactions of terminal ω-sulfonylalkyne **7.6** (Scheme 7-5). The products observed by Balme [193] were rationalized to arise from carbopalladation of a terminal alkyne by a hydridopalladium species leading to Pd(0) alkylidene intermediate **7.7**.[194]

Scheme 7-5: The Balme Dimerization of Terminal Alkynes is Distinct from Reactions of Vinyl Halides
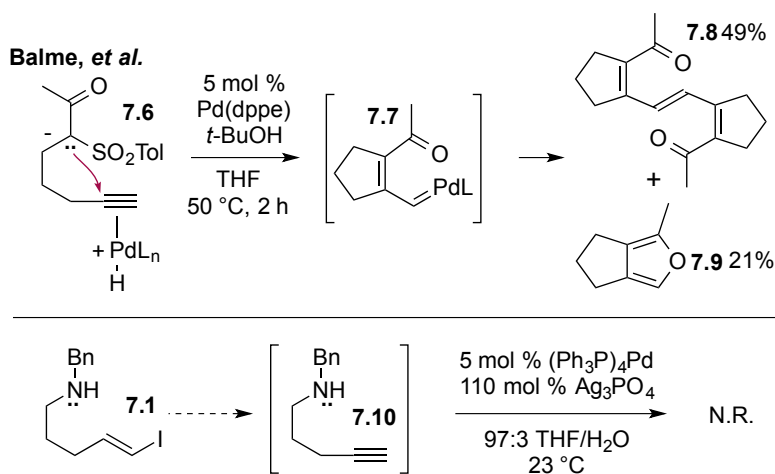
We do not observe alkyne intermediates in our dimerization reactions. To test whether our reaction was ocurring through a *syn* elimination to form a terminal alkyne intermediate, we synthesized the terminal alkyne **7.10** and subjected it to the bis-cyclization/dimerization reaction conditions. No dimer was formed and the alkyne starting material **7.10** was recovered. Thus, the bis-cyclization/dimerization reaction reported in this work is complementary to, yet distinct from, the desulfonylative dimerization of alkynes reported by Balme.

Scheme 7-6: Bis-Cyclization/Dimerization with a Carbon Nucleophile
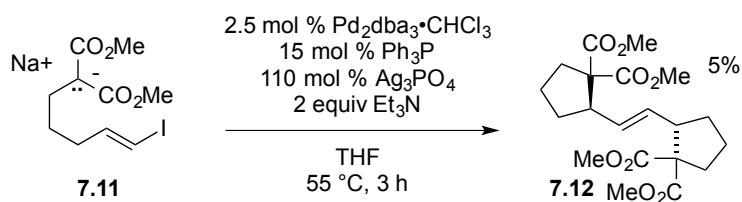
The Balme system exploited a carbon-centered nucleophile to forge the cyclodimer **7.8**. To test the potential for carbon nucleophiles in our cyclodimerization, substrate **7.11** was synthesized and subjected to the optimized conditions from Scheme 7-3. None of the desired dimer was formed even when water was omitted to prevent hydrolysis of the malonate ester.

Using slightly modified conditions, the hindered bis-cyclopentane **7.12** was formed in a modest 5% yield, representing a single turnover (Scheme 7-6).

The beneficial effect of silver is consistent with the involvement of a vinylpalladium(II) cation (Scheme 7-7). Inspired by the palladium carbenes proposed for the Balme reaction, we hypothesized that the ω-amino group on intermediate **a** might be poised to add to the vinylpalladium cation in a process resembling a Michael addition.[195] The resulting palladium(0) carbene *b* could then undergo oxidative addition, much like palladium(0) complexes with *N*-heterocyclic carbene ligands, to generate vinylpalladium carbene *c*. Migratory insertion would generate an η^1-allylpalladium intermediate *d* which could undergo an allylic alkylation through the η^3-allylpalladium intermediate *e*.

Alternatively, the first C–N bond might arise through an intramolecular aminopalladation reaction of *f* or *i* to generate α-iodopalladium carbenoid intermediate *g*. α-Elimination of the halide would produce the same palladium carbene intermediate *c* that was invoked for the Michael-type addition mechanism. Palladium carbenoid *g* is also set up for reductive elimination to generate an allyl iodide *h*, that could undergo substitution through an S_N2' reaction or a palladium-catalyzed allylic alkylation.

The starting materials in this bis-cyclization reaction bear a striking resemblence to *N*-Boc and *N*-aryl substrates used in the Wolfe reaction and related processes.[196] However, both Balme[197] and Wolfe[198] have independently noted the failure of *N*-alkylamines (and specifically *N*-benzylpent-4-en-1-amine) to engage in intramolecular *syn* aminopalladation reactions, presumably due to the difficulty in forming the amidopalladium intermediate *i* (X = H). Given the lack of precedence for Wolfe reactions of *N*-alkylamines, we are reluctant to invoke *syn* aminopalladation. Complexes such as *f* would be well-suited for an *anti* aminopalladation –

analogues to a Wacker reaction – leading to the palladium carbenoid **g**.[199] Given the potential for vicinal amino groups to promote substitution with double inversion,[200] the stereospecificity of the aminopalladation can not be used to rationalize formation of the *meso* bis-pyrrolidine **3** from either diastereomer of intermediate **g**.

Scheme 7-7: A Variety of Mechanistic Pathways Would Lead to Dimer
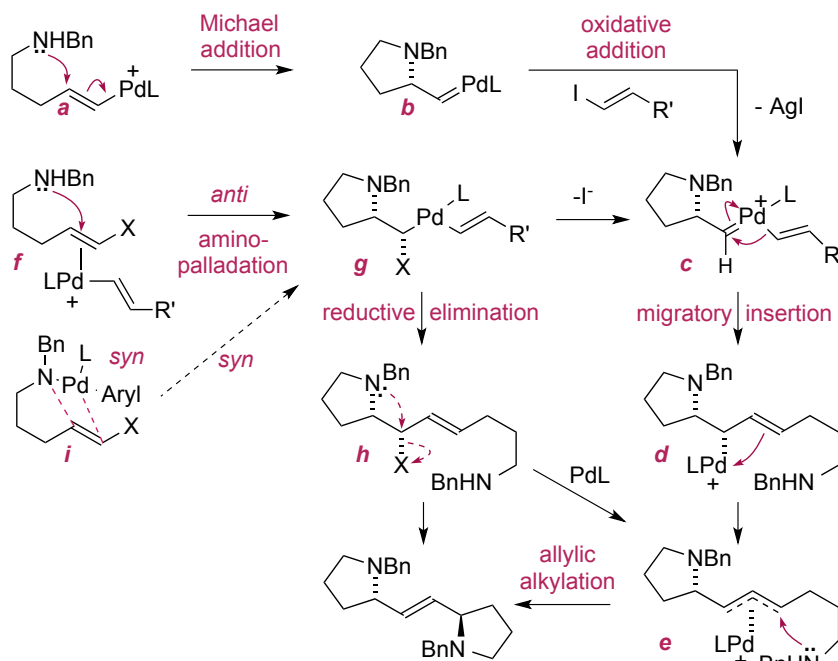
When vinyl iodide **7.1** was subjected to the dimerization reaction in the presence of norbornadiene, an 8:1 mixture of cyclopropane **7.13** and dimer **7.3** was obtained. The formation of cyclopropanes is consistent with Pd(0) alkylidene intermediates. We showed that vinyl iodide **7.1** and related compounds **7.1a-7.1e** could cyclopropanate norbornadiene (Scheme 7-8) consistent with a palladium-alkylidene intermediate **b** in Scheme 7-7.[201,193] Unfortunately, cyclopropanation of norbornenes is also consistent with a double carbopalladation sequence proposed by Catellani and co-workers or a carbopalladation/aminopalladation/reductive elimination sequence.[202,203] When styrene was added to the reaction mixture, no cyclopropanation was observed. Thus, it is unclear whether formation of cyclopropanes from

vinyl iodide **7.1** and norbornadiene is evidence for a Pd(0) alkylidene intermediate or merely symptomatic of the unique reactivity of norbornenes.

Scheme 7-8: A Cyclopropanation Experiment
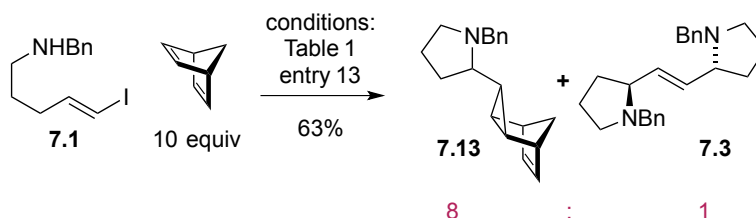
In order to gather mechanistic insight we set up a crossover experiment, taking advantage of the sluggish oxidative addition of vinyl bromides relative to vinyl iodides (Scheme 7-9). Vinyl bromide **7.14** gives little reaction at 55 °C over 3 h, conditions where vinyl iodide **7.1b** generates dimer **7.3b** in 48% yield. The vinyl bromide can be coaxed to form dimer **7.3** in 29% yield at higher temperature and with an extended reaction time. When 50 mol % of vinyl iodide **7.1b** and 50 mol % of vinyl bromide **7.14** were subjected to the reaction, the only dimer that formed was the bis-cinnamylamine dimer **7.3b**, isolated in 49% yield. None of the bis-benzylamine dimer **7.3** and none of the mixed dimer **7.15** were observed during the reaction or after workup. Some of the vinyl bromide (35%) starting material was recovered whereas the vinyl iodide was completely consumed.

Scheme 7-9: A Crossover Experiment

conditions: 5 mol % (Ph₃P)₄Pd, 110 mol % Ag₃PO₄, 97:3 solvent/water
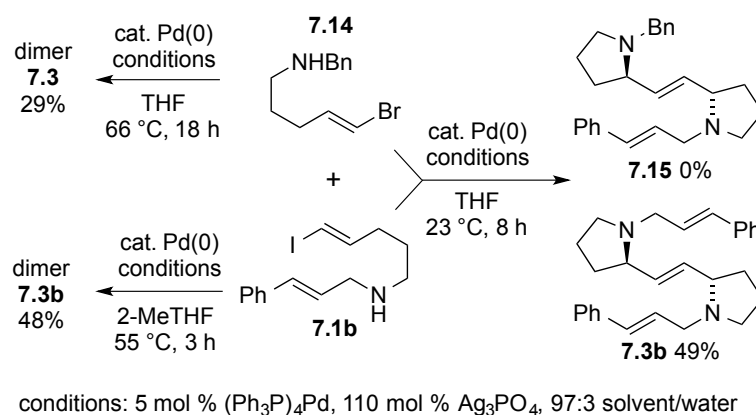
Based on the result of this crossover experiment, the aminopalladation pathway in Scheme 7-7 is untenable because the intermediate cationic Pd(II)•olefin complex *f* would be expected to undergo intramolecular aminopalladation at comparable rates regardless of whether the halide substituent X was bromide or iodide. The intermediate *g* (X=Br) would either generate mixed dimer **7.15**, or if unreactive, would reduce the catalytic turnover and yield; and neither of these results was observed. In contrast, the exclusive formation of dimer **7.3b** is consistent with the Michael-type addition/oxidative addition pathway in which both of the vinyl halides that are incorporated into the dimer participate through successive oxidative additions. The ability to access palladium(0) alkylidene intermediates such as *b* in Scheme 7-7 from vinyl halides would offer immense potential for construction of complex molecules.[204]

In conclusion we have described the first example of a bis-cyclization/dimerization reaction of vinyl iodides that generates *meso* bis-pyrrolidines and bis-piperidines. The reaction tolerates a range of *N*-alkyl substituents and was used to synthesize the skeleton of the alkaloid hyalbidone in a single step. A crossover experiment is consistent with a novel Michael-type addition of an amine to a vinylpalladium cation giving rise to a palladium(0) alkylidene intermediate.

Experimental Section

Vinyl iodides **7.1a-e** and **7.11** were synthesized by the method of Khanna and co-workers.[201]

Experimental

(*E*)-*N*-Allyl-5-iodopent-4-en-1-amine, 7.1a.
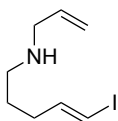
A flame dried 5 mL round bottom flask was charged with the (*E*)-5-iodopent-4-en-1-yl methanesulfonate[201] (0.29 g, 1.0 mmol, 1.0 equiv). Next, allyl amine (0.75 mL, 10 mmol, 10 equiv) and 0.8 mL DMSO were added by syringe. Then sodium

iodide (8.2 mg, 0.055 mmol, 0.055 equiv) was added to the reaction flask by quickly opening the septum. This mixture was heated at 55 ºC until the mesylate starting material was no longer detectable by TLC (EtOAc/hex 50:50), 3 h. Upon consumption of the mesylate, the reaction mixture was added to 1% (w/v) aq. NaOH (3 mL) and then extracted with (3 x 5 mL) Et$_2$O. The combined organic extracts were dried with Na$_2$SO$_4$ and concentrated *in vacuo* to give a yellow oil. The oil was purified by flash chromatography with EtOAc/hex/Et$_3$N (20:80:5) to afford the monoalkylated amine **7.1a** as a brown oil (0.24 g, 0.97 mmol, 97%). R$_f$ = 0.35 (20:80:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.51 (dt, *J* = 14.0, 7.5 Hz, 1H), 5.99 (d, *J* = 14.9 Hz, 1H), 5.88 (m, 1H), 5.18 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.08 (d, *J* = 10.0 Hz, 1H), 3.23 (d, *J* = 6.0 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.09-2.13 (m, 2H), 1.56-1.61 (m, 2H), 1.20 (br s, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 146.1, 136.9, 115.9, 74.9, 52.5, 48.5, 33.9, 28.8; IR (thin film) 2935, 2853, 2250, 1667, 1551; HRMS (ESI): *m/z* calculated for C$_8$H$_{13}$IN [M-H]$^-$ 250.0094, found 250.0093.

General procedure for the dimerization of ω-aminovinyliodides, 7.3, 7.3a-e:
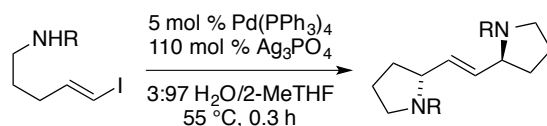
A 10 mL pear-shaped flask was charged with tetrakis(triphenylphosphine)palladium (0.050 equiv), silver(I) phosphate (1.1 equiv) and a stir bar. The flask was fitted with a septum and purged with nitrogen. Meanwhile, a separate 5 mL pear-shaped flask was charged with the ω-aminovinyliodide (1.0 equiv). Under a stream of nitrogen, the pear-shaped flask was fitted with a septum and 2-MeTHF (0.07 M with respect to the vinyl iodide) was added. The solution of vinyl iodide was added to the flask containing the tetrakis(triphenylphosphine)palladium and inorganic base, followed sequentially by water (20 equiv). Reaction mixture was heated to 55 °C

by immersing the reaction flask in a hot oil bath up to the level of the flask's contents. The reaction was monitored for consumption of the vinyl iodide by TLC (10:90:5 EtOAc/hex/Et$_3$N).

Upon consumption of the vinyl iodide, the reaction was cooled to room temperature, the crude reaction mixture was washed with 1% (w/v) aq. NaOH. The aqueous layer was then extracted with (3 x 10 mL) Et$_2$O. The combined organic layers were then washed with brine and dried over Na$_2$SO$_4$. The resulting organic solution was concentrated *in vacuo* to give dark brown oil. The oil was then purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the bis-pyrrolidine dimer.

(*E*)-1-((*R*)-1-Allylpyrrolidin-2-yl)-2-((*S*)-1-allylpyrrolidin-2-yl)ethane, 7.3a.
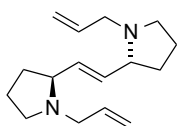
Using the general procedure for dimerization outlined above, (*E*)-*N*-allyl-5-iodopent-4-en-1-amine **7.1a** (29 mg, 0.11 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the bis-pyrrolidine dimer **7.3a** as a dark brown oil (7.8 mg, 0.032 mmol, 56%). R$_f$ = 0.78 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.94-5.86 (m, 2H), 5.49-5.48 (m, 2H), 5.19-5.16 (m, 2H), 5.11-5.09 (m, 2H), 3.45-3.39 (m, 2H), 3.18-3.09 (m, 4H), 2.66-2.62 (m, 2H), 2.18-2.13 (m, 2H), 1.97-1.82 (m, 4H), 1.76-1.71 (m, 2H), 1.61-1.54 (m, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 136.1, 136.0, 134.9, 134.6 117.1, 117.0, 61.2, 62.0, 57.0, 56.8, 53.4, 53.3, 31.7, 31.4, 22.0, 21.9; IR (thin film) 2968, 2792, 1683, 1386 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{16}$H$_{27}$N$_2$ [M+H]$^+$ 247.2174, found 247.2172.

(*E*)-1-((*R*)-1-(Furan-2-ylmethyl)pyrrolidin-2-yl)-2-((*S*)-1-(furan-2-ylmethyl)pyrrolidin-2-yl)ethane, 7.3c.

Using the general procedure for dimerization outlined above, (*E*)-*N*-(furan-2-ylmethyl)-5-iodopent-4-en-1-amine **7.1c** (29 mg, 0.10 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the bis-pyrrolidine
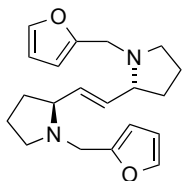
dimer **7.3c** as a dark brown oil (9.8 mg, 0.030 mmol, 61%). R$_f$ = 0.78 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.34 (app s, 2H), 6.28 (dd, *J* = 6.1, 1.9 Hz, 2H), 6.14 (d, *J* = 3.0 Hz, 2H), 5.57-5.51 (m, 2H), 3.87 (d, *J* = 14.1 Hz, 2H), 3.30 (d, *J* = 14.1 Hz, 2H), 3.04-3.00 (m, 2H), 2.82-2.78 (m, 2H), 2.27-2.23 (m, 2H), 1.99-1.91 (m, 2H), 1.84-1.77 (m, 2H), 1.66-1.62 (m, 4H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 152.9, 141.9, 134.9, 110.0, 108.0, 66.6, 53.4, 49.4, 31.9, 21.9; IR (thin film) 2982, 1734, 1373 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{20}$H$_{26}$N$_2$O$_2$Na [M+Na]$^+$ 349.1892, found 349.1898.

(*E*)-1-((*R*)-1-Cyclopentylpyrrolidin-2-yl)-2-((*S*)-1-cyclopentylpyrrolidin-2-yl)ethane, 7.3d.
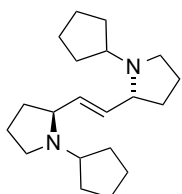
Using the general procedure for dimerization outlined above, (while obtaining TLC samples extra care was taken to prevent exposure to air), (*E*)-*N*-(5-iodopent-4-en-1-yl)cyclopentanamine **7.1d** (50 mg, 0.18 mmol, 1.0 equiv) and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the bis-pyrrolidine dimer **7.3d** as a dark brown oil (15 mg, 0.050 mmol, 56%). R$_f$ = 0.75 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.49-5.47 (m, 2H), 3.02-2.96 (m, 4H), 2.79-2.77 (m, 2H), 2.51-2.49 (m, 2H), 1.96-1.94 (m, 2H), 1.93-1.90 (m, 4H), 1.81-1.77 (m, 4H), 1.76-1.67 (m, 6H), 1.65-1.40 (m, 8H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 133.2, 66.3, 64.2, 51.2, 32.4, 32.2, 29.4, 24.3, 23.8, 22.5; IR (thin film) 2970, 1771, 1394. 1299 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{20}$H$_{34}$N$_2$Na [M+Na]$^+$ 325.2620, found 325.2608.

(*E*)-1-((*R*)-1-Adamantylpyrrolidin-2-yl)-2-((*S*)-1-adamantylpyrrolidin-2-yl)ethane, 7.3e.
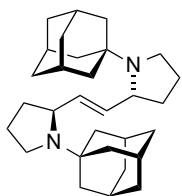
Using the general procedure for dimerization outlined above, (3*s*,5*s*,7*s*)-*N*-((*E*)-5-iodopent-4-en-1-yl)adamantan-1-amine **7.1e** (37 mg, 0.11 mmol, 1.0 equiv) was used and the product was purified by flash chromatography with

EtOAc/hex/Et$_3$N (10:90:5) to afford the bis-pyrrolidine dimer **7.3e** as a dark brown oil (15 mg, 0.035 mmol, 66%). The reaction required slightly longer reaction time (3 h). R$_f$ = 0.88 (10:90:5 EtOAc/hex/ Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.36-5.48 (m, 2H), 3.46-3.55 (m, 2H), 2.87-2.96 (m, 2H), 2.74-2.69 (m, 2H), 2.02 (br s, 6H), 1.78-1.76 (m, 10H), 1.66-1.54 (m, 22H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 134.7, 57.9, 54.3, 46.0, 40.1, 37.0, 33.4, 29.7, 23.7; IR (thin film) 2956, 2924, 2873, 2860, 1540, 1373 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{30}$H$_{47}$N$_2$ [M+H]$^+$ 435.3739, found 435.3737.

(2*R*,2*S*)-Tetramethyl 2,2'-((*E*)-ethene-1,2-diyl)bis(cyclopentane-1,1-dicarboxylate), 7.12.
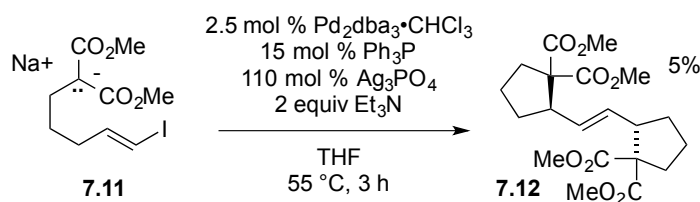
Sodium hydride (95 wt%, 6.7 mg, 0.26 mmol) was added to a 10 mL round bottom flask and suspended in 0.1 mL of THF. The suspension was cooled to 0 °C with an ice bath, after which dimethyl malonate vinyl iodide **7.11**[201] (82 mg, 0.25 mmol) was added via syringe over 15 min. After the addition was complete, the ice bath was removed and the solution was stirred for 75 min at room temperature.

A 5 mL pear-shaped flask was and charged with Pd$_2$dba$_3$•CHCl$_3$ (13 mg, 2.5 mol %) and Ph$_3$P (9.9 mg, 15 mol %), meanwhile another 5 mL round bottom flask was charged with silver(I) phosphate (0.12 g, 1.1 equiv) and a stir bar. 1.0 mL of THF was added to the pear-shaped flask containing Pd$_2$dba$_3$•CHCl$_3$ and Ph$_3$P and stirred (approximately 5 min). The solution of sodiomalonate vinyl iodide was added to the flask containing the silver(I) phosphate, followed by Et$_3$N (70 mL, 2.0 equiv). Lastly, the catalyst solution of Pd$_2$dba$_3$•CHCl$_3$ and Ph$_3$P in THF was added to the round bottom flask. The round bottom flask was heated to 55 °C by

immersing the reaction flask in a hot oil bath up to the level of the flask's contents. The reaction was monitored for consumption of the vinyl iodide by TLC (20:80 EtOAc/Hex).

The reaction reached completion at 4 h, and was then cooled to room temperature. The crude reaction mixture was washed with 1% (w/v) aq. NaOH. The aqueous layer was then extracted with (3 x 10 mL) Et$_2$O. The combined organic layers were then washed with brine and dried over Na$_2$SO$_4$. The resulting organic solution was concentrated *in vacuo* to give dark brown oil. Standard flash chromatography was unsuccessful for purification of cyclopentyl dimer **7.12**. Therefore, **7.12** was hydrolyzed and purified by extraction via the following procedure:[205]
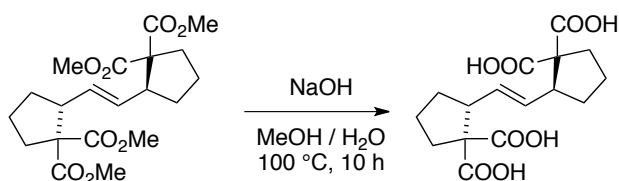
A solution of cyclopentyl dimer **7.12** (25 mg, 0.06 mmol, 1 equiv), sodium hydroxide (35 mg, 0.88 mmol, 15 equiv) in 0.2 mL of ethanol and 0.7 mL of water was heated at reflux for 10 h. The reaction was cooled to room temperature, and the crude reaction mixture was extracted with (3 x 2 mL) Et$_2$O. The aqueous layer was treated with 1N HCl to obtain a pH 3 solution. The resulting mixture was extracted again with (3 x 2 mL) Et$_2$O. The resulting organic solution was concentrated *in vacuo* to yield the pure diacid derivative of **7.12** as a white solid. (20 mg, 0.060 mmol, 5% from vinyl iodide **7.11**). R$_f$ = 0.70 (20:80 MeOH/CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 12.5 (s, 4H), 5.36 (dd, *J* = 5.1, 2.3 Hz, 2H), 2.91-3.03 (m, 2H), 2.20-2.29 (m, 2H), 1.90-1.98 (m, 2H), 1.87-1.75 (m, 2H), 1.77-1.65 (m, 2H), 1.49-1.44 (m, 4H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 174.1, 172.1, 131.1, 64.1, 48.1, 33.5, 31.8, 22.9; IR (thin film) 3354, 1645, 1635, 1046 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{16}$H$_{19}$O$_8$ [M-H]$^-$ 339.1080, found 339.1084.

Chapter 8

Palladium-Catalyzed Catellani Aminocyclopropanation Reactions with Vinyl Halides

Norbornenes are gaining increasing attention for their participation in metal-catalyzed reactions, for example as traceless participants in C–H activation reactions[206] and as ligands for asymmetric catalysis.[207] Norbornenes are also exceptional substrates for cyclopropanation. Palladium(0) can catalyze the cyclopropanation of norbornenes using traditional carbene precursors such as α-diazo esters[208] and various carbenoid precursors.[209]

Vinyl halides have received scant attention as reagents for cyclopropanation. In the 1980s Catellani and co-workers reported that tandem Heck reactions of vinyl bromides with norbornene generate three types of cyclopropane products (Scheme 8-1). In the presence of potassium acetate, 1-bromo-1-octene reacts via β-hydride elimination to generate vinylcyclopropane **8.1**.[210] β-Styryl bromide generates intermediates that can be trapped with hydride donors or secondary amines such as benzylcyclopropane **8.2** and cyclopropylcarbinylamine **8.3**.[211] It was proposed that all three products arise from a tandem reaction involving intermolecular carbopalladation of norbornene to give vinylnorbornane **8.4**, followed by intramolecular carbopalladation to give cyclopropylcarbinylpalladium intermediate **8.5**.[212]

Other than styryl bromide, no other vinyl halides have been shown to generate cyclopropylcarbinylamines, presumably due to facile β-hydride elimination that leads to formation of vinylcyclopropanes analogues to **8.1**. Together with my coworker Avinash Khanna we set out to explore this distinctive aminocyclopropanation reaction using vinyl halides other than styryl bromides.

Scheme 8-1: Common Reactive Intermediates in Catellani Cyclopropanations can Generate Three Different Products
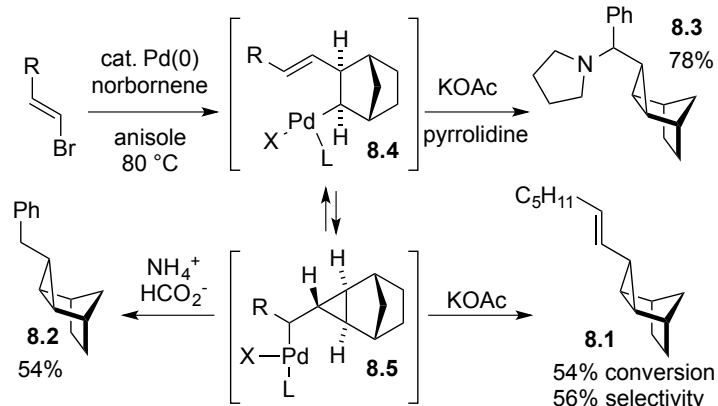
In order to favor this unique aminocyclopropanation reaction, we turned to a vinyl iodide substrate **8.6a** with a pendant secondary amine. Under the conditions reported for styryl bromide, none of the cyclopropylcarbinylamine **8.7a** was observed and we isolated only (*E*)-vinylcyclopropane **8.8a**, resulting from β-hydride elimination (Scheme 8-2).

Scheme 8-2: Aminocyclopropanation Reactions
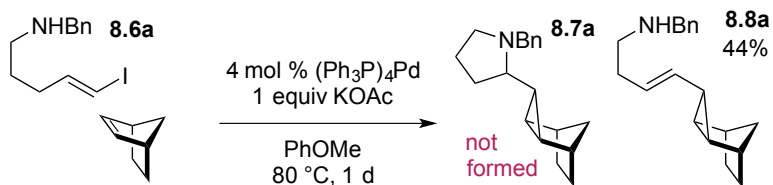
In order to promote the formation of pyrrolidine **8.7a**, we changed both the alkene acceptor and the reaction conditions. We substituted norbornadiene for norbornene since it has been reported to provide higher yields in palladium-catalyzed cyclopropanation reactions with diazo compounds.[208a] We also changed the reaction conditions to those reported by Torii for reductive trapping of a putative cyclopropylcarbinylpalladium intermediate using formic acid as a hydride source.[213] In a footnote, Torii and co-workers reported the isolation of a cyclopropane in 84% yield by reacting a vinyl iodide, norbornene, Ph₃P, Pd(OAc)₂, and Et₃N in DMF.

Therefore, we set out to optimize the aminocyclopropanation of norbornadiene under the Torii conditions (Table 8-1).

The reaction is complete in less than one hour with a large excess of norbornadiene and additional phosphine (Table 1, entries 1-3). The yields slightly improved in the presence of the phase transfer catalyst tetra-*n*-butylammonium chloride (entry 2 and 4), and the optimal temperature was 80 °C (entries 4-6). Unfortunately, the reaction was less efficient when the amount of triphenylphosphine was reduced (entries 5 and 7). Two equivalents of diethylamine were optimal over other stoichiometries, and secondary amine additives were superior to tertiary or primary amines (entries 8-15). To better accommodate the volatile reaction components, the reaction was carried out in sealed tubes, ultimately providing yields over 80% (entries 12 and 13). The optimized conditions (entry 12) favor the participation of an amine nucleophile in the cyclopropanation reaction as opposed to β-hydride elimination.

Table 8-1: Optimization of the Aminocyclopropanation of Norbornadiene
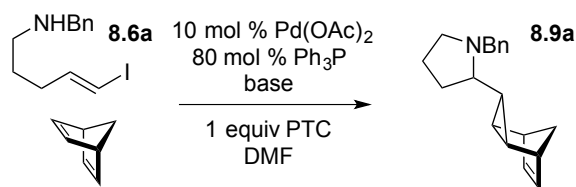
entry	equiv Ph$_3$P	equiv amine	equiv nbd	PTC	temp	vessel	yield
1	0.4	2 Et$_3$N	2		80 °C		39%
2	0.8	2 Et$_2$NH	5		80 °C		52%
3	0.8	2 Et$_2$NH	10		80 °C		64%
4	0.8	2 Et$_2$NH	10	Bu$_4$NCl	66 °C		73%
5	0.8	2 Et$_2$NH	10	Bu$_4$NCl	80 °C		79%
6	0.8	2 Et$_2$NH	10	Bu$_4$NCl	100 °C		57%
7	0.4	2 Et$_2$NH	10	Bu$_4$NCl	80 °C		56%
8	0.8	-	10	Bu$_4$NCl	80 °C		21%
9	0.8	2 Bu$_3$N	10	Bu$_4$NCl	80 °C		36%
10	0.8	2 Et$_3$N	10	Bu$_4$NCl	80 °C		56%
11	0.8	2 Et$_2$NH	10		80 °C	sealed	66%
12	0.8	2 Et$_2$NH	10	Bu$_4$NCl	80 °C	sealed	81%
13	0.8	3 Et$_2$NH	10	Bu$_4$NCl	80 °C	sealed	82%
14	0.8	1 Et$_2$NH	10	Bu$_4$NCl	80 °C	sealed	70%
15	0.8	2 *n*-PrNH$_2$	10	Bu$_4$NCl	80 °C	sealed	72%

Figure 8-1: Establishing the relative stereochemistry

8.9a
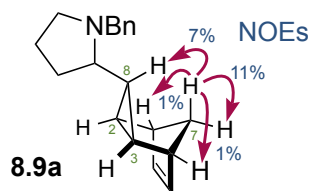
The relative stereochemistry of the amine product **8.9a** was established by the presence of

a positive steady state nOe from one of the protons on the norbornene bridge C7 to one of the

cyclopropane protons and an absence of nOes between the proton at C8 and the protons at C2

and C3 (Figure 8-1).

Scheme 8-3: Intramolecular Aminocyclopropanation with Variations in the Amine Substituent

8.6a-e   10 equiv

10 mol % Pd(OAc)$_2$
80 mol % Ph$_3$P
2 equiv Et$_2$NH

1 equiv *n*-Bu$_4$NCl
DMF, 80 °C

8.9a
81%

8.9b   **8.9c**   **8.9d**   **8.9e**   +   **8.9f**
60%   87%   61%   56% 2:1 (**8.9e/8.9f**)
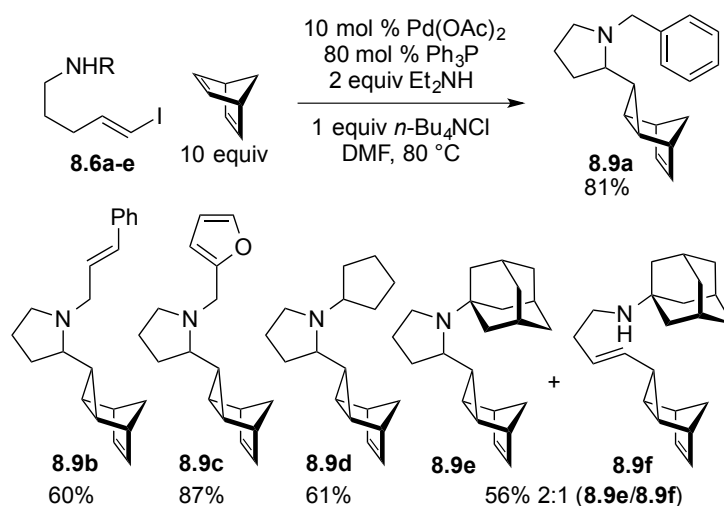
With optimized conditions in hand, we next explored the tolerance of the amine nitrogen

substituent to varying degrees of steric hindrance (Scheme 8-3). The reaction conditions led to

chemoselective cyclopropanation of the nobornadiene acceptor without a competing reaction of a

pendant cinnamyl group in **8.9b**. The reaction furnished cyclopropanes in yields up to 87%. As

expected, the bulky *N*-cyclopentylamine **8.9d** was formed in lower yield. The exceedingly

hindered adamantyl group of adamantylamine **8.6e** led to a slower reaction and an inseparable

2:1 mixture of pyrrolidine **8.9e** and vinylcylopropane **8.9f** in 56% yield. The *N*-benzyl vinyl bromide corresponding to **8.6a** also provided the cyclopropylcarbinylamine **8.9a** in 53% yield.

We next set out to explore variations in the alkene acceptor. Norbornene and dicyclopentadiene were slightly less efficient than norbornadiene (Scheme 8-4). The adduct of dicyclopentadiene **8.7b** was obtained as an inseparable 1:1 mixture of diastereomers. An oxabicyclic [2.2.1] substrate generated the aminocyclopropane **8.7c** in 69% yield.[214] The cyclic alkene acenaphthylene, which has been shown to resist β-hydride elimination, generated none of the cyclopropane **8.7d**.[215]

Scheme 8-4: Scope of Alkene Acceptor
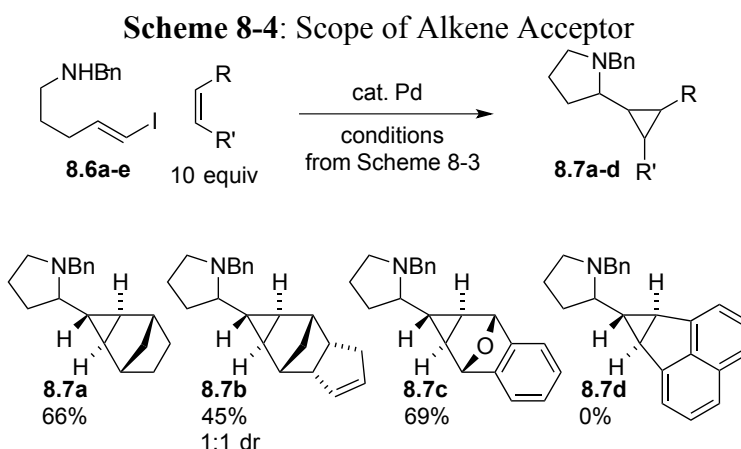
We set out to test carbon nucleophiles in the Catellani cyclopropanation. The malonate anion **8.10**, generated with sodium hydride, produces the corresponding cyclopropane adduct **8.11** in 60% yield under the optimized conditions (Scheme 8-5).

Scheme 8-5: Carbon Nucleophiles Generate Carbocyclic Rings in Conjunction with Cyclopropanation
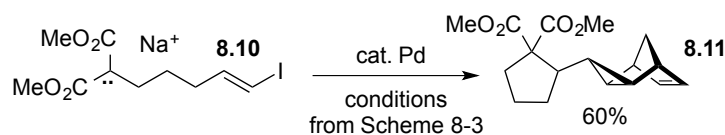
The mechanism of these unique cyclizations and cyclopropanations is still unclear. In their seminal report Catellani and co-workers proposed the intermediacy of

cyclopropylcarbinylpalladium intermediate **8.5** (Scheme 8-1) in the aminocyclopropanation reaction.[202] The first step involves an oxidative addition to form vinylpalladium halide **b** (Scheme 8-6), followed by intermolecular carbopalladation across the norbornene double bond to give *exo*-norbornylpalladium intermediate **c**. The *exo* palladium atom can not undergo *syn* β-hydride elimination but is poised to carbopalladate across the *exo* vinyl group to produce cyclopropylcarbinylpalladium intermediate **e**. Carbon-nitrogen bond formation could occur via a reductive elimination[202] or ionization of XPd⁻ to give a cyclopropylcarbinyl cation, both of which would generate the aminocyclopropane product. Reductive elimination seems less likely as a mechanism for C-N bond formation given the challenges that have been documented[216] with Buchwald-Hartwig aminations.

Scheme 8-6: Mechanistic Models for Intramolecular Aminocyclopropanation
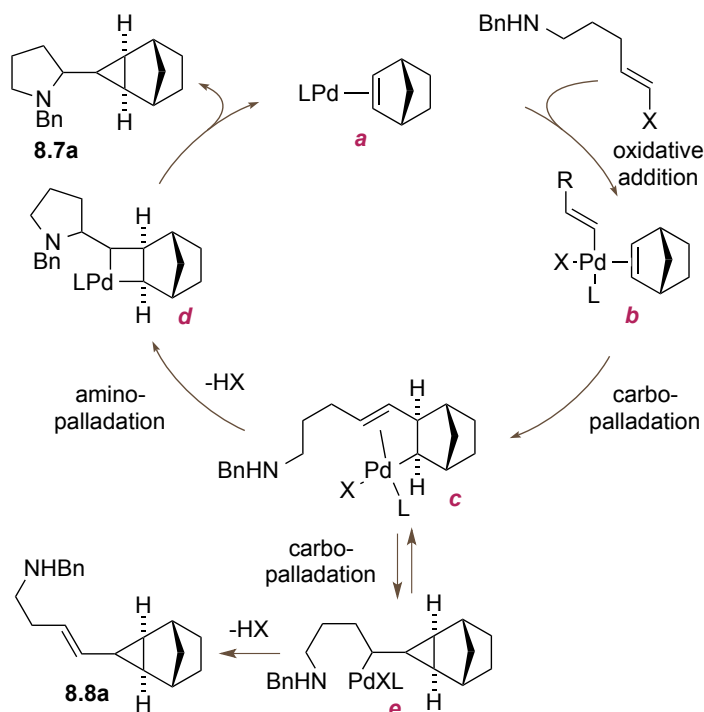
However, the olefin and palladium groups in norbornylpalladium intermediate **c** are poised for an aminopalladation to give palladacyclobutane **d**. Palladacyclobutanes have

previously been invoked in the mechanisms for palladium catalyzed cyclopropanation.[217, 209b] In previous studies of aminopalladations, *N*-alkylamines have been shown to aminopalladate *anti*[218] whereas *N*-aryl and *N*-sulfonylamines have been shown to aminopalladate *syn*.[219] Moreover, the participation of malonate anions is consistent with the well-accepted *anti*-carbopalladation mechanism.[220]

In summary, we show for the first time that the Catellani reaction can be applied to aliphatic vinyl halides – not just styryl bromide – and can even engage stabilized enolates as well as amine nucleophiles. The reaction is selective for norbornenes over other alkenes, even acenaphthylene, which, like norbornenes should generate palladium intermediates that resist β-hydride elimination. Ultimately, the mechanism of the reaction is unclear, but given the participation of both alkylamines and stabilized enolates as nucleophiles, we favor a mechanism involving palladacyclobutanes such as *d* in Scheme 8-6.

Experimental Section

Experimental

(*E*)-5-Iodopent-4-en-1-yl methanesulfonate, 8.12.[221]
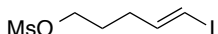
An oven-dried 50 mL round-bottom flask was charged with a stir bar, (*E*)-5-iodopent-4-en-1-ol[222] (0.514 g, 2.44 mmol), and dichloromethane (12 mL). Et$_3$N (0.540 mL, 3.90 mmol) was added to the solution of alcohol and then the reaction mixture was cooled to -10 °C. Subsequently, methanesulfonyl chloride (0.230 mL, 2.93 mmol) was added dropwise to the reaction flask. Soon after the addition of methanesulfonyl chloride, the reaction turned from a colorless clear to a pale yellow solution. Upon completion of the reaction, as observed by TLC (10 min), the crude mixture was washed with water (15 mL), saturated aq. NaHCO$_3$ (15 mL) and brine (15 mL) respectively. The organic phase was then

dried over Na_2SO_4 and concentrated *in vacuo* to furnish pale yellow oil. The oil was then purified by flash column chromatography (10:90 EtOAc/hex) to yield mesylate **8.12** as a yellow oil (0.64 g, 90% yield). R_f = 0.8 (1:1 EtOAc /hex); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.49 (dt, J = 14.4, 7.2 Hz, 1H), 6.11 (d, J = 14.5 Hz, 1H), 4.22 (t, J = 6.0 Hz, 2H), 3.01 (s, 3H), 2.21 (app q, J = 7.3 Hz, 2H), 1.86 (app p, J = 6.2 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9, 76.4, 68.6, 37.5, 32.0, 27.8; IR (thin film) 1606, 1350, 1332, 1172 cm^{-1}; HRMS (ESI): *m/z* calculated for $C_6H_{11}IO_3SNa$ $[M+Na]^+$ 312.9371, found 312.9361.

General procedure for alkylation of an amine with mesylate 8.12, 8.6b-e:

A flame dried 5 mL round bottom flask was charged with the (*E*)-5-iodopent-4-en-1-yl methanesulfonate **8.12** (1.0 equiv). Subsequently, the primary amine (10 equiv) and DMSO (0.8 M in vinyl iodide) were added by syringe. Lastly, sodium iodide (0.055 equiv) was added to the reaction flask by temporarily removing the septum. The reaction mixture was heated at 55 ºC until mesylate **8.12** was no longer detectable by thin layer chromatography (2-5 h). The reaction mixture was added to 1% (w/v) aq. NaOH and then extracted with (3 × 5 mL) Et_2O. The combined organic extracts were dried with Na_2SO_4 and concentrated under vacuum to give yellow oil. The oil was then purified by flash chromatography with EtOAc/hex/Et_3N (20:80:5) to afford the monoalkylated amine.

(*E*)-*N*-(Furan-2-ylmethyl)-5-iodopent-4-en-1-amine, 8.6c.

Using the general procedure for alkylation, furfurylamine was alkylated with mesylate **8.12** (29 mg, 0.11 mmol, 1.0 equiv) and the product was purified by flash chromatography with EtOAc/hex/Et_3N (20:80:5) to afford monoalkylated amine **8.6c** as a brown oil (29 mg, 0.10
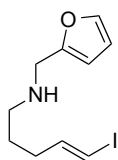
mmol, 98%). R_f = 0.54 (20:80:5 EtOAc/hex/Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 7.36 (dd, J = 13.4, 1.0 Hz, 1H), 6.49 (dt, J = 14.3, 7.2 Hz, 1H), 6.31 (dd, J = 3.0, 1.9 Hz, 1H), 6.16 (app d, J = 3.0 Hz, 1H), 6.00 (dt, J = 14.4, 1.2 Hz, 1H), 3.77 (s, 2H), 2.60 (t, J = 7.0 Hz, 2H), 2.08-2.12 (m, 2H), 1.56-1.60 (m, 2H), 1.36 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 153.9, 146.0, 141.9, 110.2, 106.9, 74.9, 48.2, 46.2, 33.8, 28.7; IR (thin film) 2938, 1669, 1456 cm⁻¹; HRMS (ESI): m/z calculated for C₁₀H₁₃INO [M-H]⁺ 290.0042, found 290.0042.

(E)-N-(5-Iodopent-4-en-1-yl)cyclopentanamine, 8.6d.
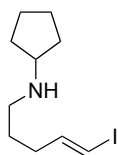
Using the general procedure for alkylation, cyclopentylamine was alkylated with mesylate **8.12** (29 mg, 0.11 mmol, 1.0 equiv) and the product was purified by flash chromatography with EtOAc/hex/Et₃N (20:80:5) to afford monoalkylated amine **8.6d** as a brown oil (0.27 mg, 0.11 mmol, 98%). R_f = 0.55 (20:80:5 EtOAc/hex/Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 6.51 (dt, J = 14.3, 7.2 Hz, 1H), 6.00 (d, J = 14.3 Hz, 1H), 3.04 (quintet, J = 6.8 Hz, 1H), 2.58 (t, J = 7.2 Hz, 2H), 2.12-2.08 (m, 2H), 1.84-1.82 (m, 2H), 1.62-1.71 (m, 2H), 1.60-1.52 (m, 4H), 1.30-1.27 (m, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 146.2, 74.8, 59.9, 47.9, 34.0, 33.3, 29.1, 24.1; IR (thin film) 2983, 1653, 1236 cm⁻¹; HRMS (ESI): m/z calculated for C₁₀H₁₇IN [M-H]⁺ 278.0406, found 278.0411.

(3s,5s,7s)-N-((E)-5-Iodopent-4-en-1-yl)adamantan-1-amine, 8.6e.
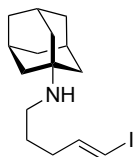
Using the general procedure for alkylation, 1-adamantylamine was alkylated with mesylate **8.12** (29 mg, 0.11 mmol, 1.0 equiv) and the product was purified by flash chromatography with EtOAc/hex/Et₃N (20:80:5) to afford monoalkylated amine **8.6e** as a white solid (0.16 mg, 0.10 mmol, 94%). R_f = 0.67 (20:80:5 EtOAc/hex/Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 6.50 (dt, J = 14.3, 7.2 Hz, 1H), 6.00 (d, J = 14.3 Hz, 1H), 2.57 (t, J =

7.2 Hz, 2H), 2.09-2.12 (m, 2H), 1.98 (app s, 3H), 1.68-1.59 (m, 12H), 1.56-1.53 (m, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 146.2, 74.8, 50.8, 42.8, 39.6, 36.8, 34.1, 29.8, 29.6; IR (thin film) 2980, 1660 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{15}$H$_{24}$IN [M+H]$^+$ 346.1032, found 346.1041.

(*E*)-Dimethyl 2-(5-iodopent-4-en-1-yl)malonate, 8.10.
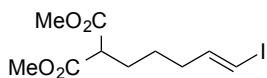
A 100 mL oven-dried round-bottom flask was charged with sodium hydride (60 wt % in mineral oil, 0.43 g, 10.8 mmol) and the mineral oil was removed by washing with hexanes (3 × 2 mL). The sodium hydride was suspended in DMF (54 mL). The suspension was cooled to 0 °C on an ice bath, after which dimethyl malonate (1.42 g, 10.8 mmol) was added via syringe over the course of 15 min. After the addition was complete, the ice bath was removed and the solution was stirred for 30 min at room temperature. Meanwhile, a solution of mesylate **8.12** (1.25 g, 4.31 mmol) was prepared by dissolving it in THF (22 mL). The solution of **8.12** was added to the reaction flask by a syringe followed by potassium iodide (0.716 g, 4.31 mmol). The reaction mixture was then allowed to stir at 80 °C for 11 h. Subsequently, saturated NH$_4$Cl solution (30 mL) was added to the reaction mixture, which was then extracted with EtOAc (3 × 30 mL). The combined organic layers were then washed with H$_2$O (5 × 30 mL). The resulting organic layer was then over MgSO$_4$ and concentrated *in vacuo* to deliver a pale yellow oil. The oil was purified by flash chromatography (10:90 EtOAc/hex) to yield the monoalkylated malonate, as a colorless oil (1.1 g, 79% yield). R_f = 0.30 (10:90 EtOAc/hex); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.47 (dt, *J* = 14.3, 7.1 Hz, 1H), 6.02 (dt, *J* = 14.4, 1.2 Hz, 1H), 3.74 (s, 6H), 3.35 (t, *J* = 7.5 Hz, 1H), 2.08 (br, 2H), 1.91 (dt, *J* = 8.1, 7.7, 2H), 1.42 (br, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 169.7, 145.5, 75.4, 52.6, 51.5, 35.6, 28.1, 26.1; IR (thin film) 3021, 2954, 1732, 1435 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{10}$H$_{15}$IO$_4$Na [M+Na]$^+$ 348.9913, found 348.9911.

General procedure for cyclopropanation:
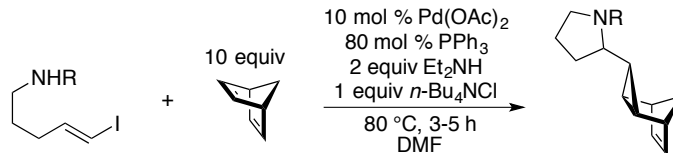
An oven-dried 10 mL conical vial with a stir bar was charged with tetra-*n*-butylammonium chloride (1.0 equiv). Then the conical vial was sealed and purged with nitrogen. A separate 5 mL pear-shaped flask with a stir bar was charged with palladium(II) acetate (0.10 equiv), Ph₃P (0.80 equiv) and DMF (0.10 M solution with respect to palladium). The catalyst mixture was stirred for 20-30 min resulting an orange solution. Meanwhile, a separate oven-dried 5 mL pear-shaped flask containing ω-aminovinyl iodide (1.0 equiv) and DMF (0.3 M with respect to vinyl iodide) was prepared. The solution of vinyl iodide was added to the 10 mL conical vial, followed by addition of diethylamine (2.0 equiv) and norbornadiene (10 equiv). Finally, the palladium catalyst solution was transferred to the reaction flask by syringe. Additional DMF was added to the conical vial by syringe, resulting in a 0.17 M reaction with respect to the ω-aminovinyl iodide. The reaction mixture was heated to 80 °C by immersing the reaction flask in a hot oil bath up to the level of the flask contents. The stirred reaction was then monitored by TLC (10:90:5 EtOAc/hex/Et₃N) to check for depletion of the vinyl iodide.

After cooling to ambient temperature, the crude reaction mixture was washed with 1% (w/v) aq. NaOH. The aqueous layer was then extracted with (3 × 10 mL) Et₂O. The combined organic layers were then washed with brine and dried over Na₂SO₄. The resulting organic solution was concentrated under vacuum to give dark brown oil. The oil was then purified by flash chromatography with EtOAc/hex/Et₃N (10:90:5) to afford the aminocyclopropanated product.

Sample procedure for cyclopropanation: 1-benzyl-2-((1*R*,2*R*,4*S*,5*S*)-tricycle[3.2.1.0²,⁴]oct-6-en-3-yl)pyrimidine, 8.9a.
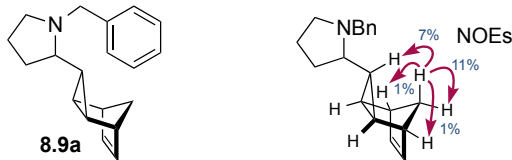
An oven-dried 10 mL conical vial with a stir bar was charged with tetra-*n*-butylammonium chloride (34 mg, 0.12 mmol, 1.0 equiv). Then the conical vial was sealed and purged with nitrogen. A separate 5 mL pear-shaped flask with a stir bar was charged with palladium(II) acetate and DMF (0.2 mL) was added to the pear-shaped flask and stirred for 20 min resulting an orange solution of the catalyst. Meanwhile, a separate oven-dried 5 mL pear-shaped flask containing reported ω-aminovinyl iodide **8.6a**[223] (37 mg, 0.12 mmol, 1.0 equiv) and DMF (0.1 mL) was prepared. The solution of vinyl iodide was added to the 10 mL conical vial, followed by diethylamine (25 mL, 0.25 mmol, 2.0 equiv), norbornadiene (0.12 mL, 1.2 mmol, 10 equiv). Finally, the palladium catalyst solution was transferred to the reaction flask by syringe. Additional DMF (0.6 mL) was added to the conical vial by syringe. The Reaction mixture was heated to 80 °C by immersing the reaction flask in a hot oil bath up to the level of the flask contents. The stirred reaction was monitored by TLC (10:90:5 EtOAc/hex/Et₃N) to check for depletion of the vinyl iodide.

After cooling to ambient temperature, the crude reaction mixture was washed with 1% (w/v) aq. NaOH. The aqueous layer was then extracted with (3 × 10 mL) Et₂O. The combined organic layers were then washed with brine and dried over Na_2SO_4. The resulting organic solution was concentrated under vacuum to give dark brown oil. The oil was then purified by flash chromatography with EtOAc/hex/Et₃N (10:90:5) to afford the aminocyclopropanated product **8.9a** as a dark brown oil (26 mg, 0.098 mmol, 81% (95% purity)). This product contained an impurity (< 5 mol%) that was difficult to remove by column chromatography. $R_f =$

0.81 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.36 (d, J = 6.1 Hz, 2H), 7.31 (t,

J = 6.2 Hz, 2H), 7.23 (t, J = 6.1 Hz, 1H), 6.38 (dd, J = 2.4, 4.7 Hz, 1H), 6.36 (dd, J = 2.4, 4.7 Hz,

1H), 4.29 (d, J = 10.7 Hz, 1H), 3.11 (d, J = 10.8 Hz, 1H), 2.88 (t, J = 7.5 Hz, 1H), 2.80 (s, 1H),

2.75 (s, 1H), 2.05 (q, 8.5 Hz, 1H), 1.94-1.88 (m, 2H), 1.78-1.60 (m, 4H), 1.17 (d, J = 9.0 Hz,

1H), 0.94 (d, J = 7.5 Hz, 1H), 0.85 (d, J = 9.0 Hz, 1H), 0.79 (d, J = 7.5 Hz, 1H); ^{13}C NMR (125

MHz, CDCl$_3$) δ 141.0, 140.8, 140.2, 129.0, 128.9, 126.7, 68.0, 59.1, 54.1, 41.8, 41.6, 39.1, 36.4,

30.8, 30.1, 25.3, 21.6; IR (thin film) 2906, 1736, 1457, 1371, 1239, 1041 cm^{-1}; HRMS (ESI): m/z

calculated for C$_{19}$H$_{24}$N [M+H]$^+$ 266.1909, found 266.1904.

1-Cinnamyl-2-((1R,2R,4S,5S)-tricyclo[3.2.1.0^{2,4}]oct-6-en-3-yl)pyrrolidine, 8.9b.
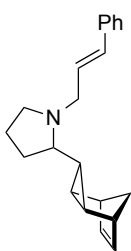
Using the general procedure for cyclopropanation, (*E*)-*N*-cinnamyl-5-iodopent-4-en-1-amine **8.6b** (89 mg, 0.27 mmol, 1.0 equiv) was reacted with norbornadiene and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the cyclopropane **8.9b** as a light brown solid (48 mg, 0.16 mmol, 60%). R_f = 0.84 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.38 (d, J = 7.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.21 (t, J = 7.0 Hz, 1H), 6.53 (d, J = 16.0 Hz,

1H), 6.41-6.35 (m, 3H), 3.88 (dd, J = 4.5, 13.0 Hz, 1H), 3.11 (t, J = 7.0 Hz, 1H), 2.88 (dd, J =

8.0, 13.0 Hz, 1H), 2.82 (s, 1H), 2.80 (s, 1H), 2.15 (q, J = 9.0 Hz, 1H), 1.93-1.89 (m, 1H), 1.86

(2.5, 6.0 Hz, 1 H), 1.82-1.75 (m, 1H), 1.71 (q, J = 7.5 Hz, 1H), 1.67-1.59 (m, 2H), 1.19 (d, J =

9.0 Hz, 1H), 0.96 (d, J = 7.0 Hz, 1H), 0.86 (d, J = 9.5 Hz, 1H), 0.78 (d, J = 7.0 Hz, 1H); ^{13}C

NMR (125 MHz, CDCl$_3$) δ 141.0, 140.8, 137.3, 131.8, 128.6, 128.2, 127.3, 126.3, 67.4, 56.8,

54.1, 41.8, 41.6, 39.1, 36.2, 30.9, 29.9, 25.4, 21.6; IR (thin film) 2970, 2908, 2786, 2359, 2336,

1494, 1314, 1153 cm^{-1}; HRMS (ESI): m/z calculated for C$_{21}$H$_{26}$N (M+H$^+$) 292.2065, found

292.2064.

1-(Furan-2-ylmethyl)-2-((1*R*,2*R*,4*S*,5*S*)-tricyclo[3.2.1.0²,⁴]oct-6-en-3-yl)pyrrolidine, 8.9c.
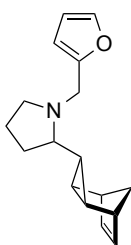
Using the general procedure for cyclopropanation, (*E*)-*N*-(furan-2-ylmethyl)-5-iodopent-4-en-1-amine **8.6c** (37 mg, 0.12 mmol, 1.0 equiv) was reacted with norbornadiene and the product was purified by flash chromatography with EtOAc/hex/Et₃N (10:90:5) to afford the cyclopropane **8.9c** as a dark brown oil (15 mg, 0.050 mmol, 87%). R_f = 0.82 (10:90:5 EtOAc/hex/Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 7.34 (app s, 1H), 6.38-6.34 (m, 1H), 6.28 (d, *J* = 1.0 Hz, 2H), 6.29-6.28 (m, 1H), 4.14 (d, *J* = 14.0 Hz, 1H), 3.37 (d, *J* = 14.0 Hz, 1H), 2.95-2.91 (m, 1H), 2.81 (s, 1H), 2.79 (s, 1H), 2.19 (q, *J* = 7.2 Hz, 1H), 1.91-1.84 (m, 2H), 1.78-1.70 (m, 2H), 1.67-1.55 (m, 2H), 1.17 (d, *J* = 9.5 Hz, 1H), 0.97 (d, *J* = 7.5 Hz, 1H), 0.85 (d, *J* = 9.0 Hz, 1H), 0.76 (d, *J* = 7.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 153.1, 141.8, 141.0, 140.9, 140.8, 140.8, 110.0, 107.9, 66.8, 53.8, 50.0, 41.8, 41.6, 39.1, 36.1, 30.8, 29.7, 25.2, 21.6; IR (thin film) 3498, 2997, 2919, 2363, 1352, 1224 cm⁻¹; HRMS (ESI): *m/z* calculated for C₁₇H₂₂NO [M+H]⁺ 256.1701, found 256.1708.

1-Cyclopentyl-2-((1*R*,2*R*,4*S*,5*S*)-tricyclo[3.2.1.0²,⁴]oct-6-en-3-yl)pyrrolidine, 8.9d.
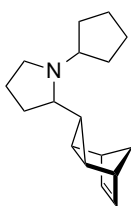
Using the general procedure for cyclopropanation, ((*E*)-*N*-(5-iodopent-4-en-1-yl)cyclopentanamine **8.6d** (50 mg, 0.18 mmol, 1.0 equiv) was reacted with norbornadiene and the product **8.9d** was purified by flash chromatography with EtOAc/hex/Et₃N (10:90:5) to afford the cyclopropane as a dark brown oil (27 mg, 0.11 mmol, 61%). R_f = 0.84 (10:90:5 EtOAc/hex/Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 6.40-6.34 (m, 2H), 3.26-3.24 (m, 1H), 3.00-2.92 (m, 1H), 2.80 (s, 1H), 2.77 (s, 1H), 2.44 (q, 7.1 Hz, 1H), 2.03-1.97 (m, 2H), 1.88-1.83 (m, 2H), 1.77-1.74 (m, 2H), 1.67-1.61 (m, 5H), 1.49-1.43 (m, 3H), 1.16 (d, *J* = 8.8 Hz, 1H), 0.95 (d, *J* = 7.5 Hz, 1H), 0.83 (d, *J* = 9.3 Hz, 1H), 0.74 (d, *J* = 7.6 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 140.9, 140.8, 62.3, 49.6, 41.8, 41.7, 39.0, 31.7, 31.1,

30.3, 27.5, 26.2, 24.6, 24.5, 21.9; IR (thin film) 3029, 2939, 2785, 1702, 1452, 907 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{17}$H$_{26}$N [M+H]$^+$ 244.2065, found 244.2070.

1-((3*S*,5*S*,7*S*)-Adamantan-1-yl)-2-((1*R*,2*R*,4*S*,5*S*)-tricyclo[3.2.1.0^{2,4}]oct-6-en-3-yl)pyrrolidines, 8.9e.
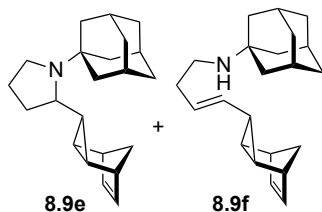
Using the general procedure for cyclopropanation, *N*-((*E*)-5-iodopent-4-en-1-yl)adamantan-1-amine **8.6e** (49 mg, 0.14 mmol, 1.0 equiv) was reacted with norbornadiene and the product **8.9e** and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (2:98:5) to afford an inseparable 2:1 mixture of amioncyclopropanated product **8.9e** and the vinylcyclopropane **8.9f** (25 mg, 56%). An analytical sample (3 mg) of pyrrolidine **8.9e** was isolated after three more rounds of flash chromatography: ^{1}H NMR (600 MHz, CDCl$_3$) δ 6.35 (br s, 2H), 2.91 (t, *J* = 7.8, 1H), 2.79-2.75 (m, 3H), 2.56 (t, *J* = 7.8 Hz, 1H), 2.06 (br s, 3H), 1.96 (d, *J* = 7.8 Hz, 1H), 1.88-1.80 (m, 1H), 1.74-1.60 (m, 11H), 1.53-1.48 (m, 1H), 1.17 (d, J = 9.6 Hz, 1H), 0.98 (d, *J* = 6.6 Hz, 1H), 0.79 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 140.9, 140.8, 58.0, 53.5, 46.4, 42.1, 41.8, 41.0, 40.1, 39.0, 37.0, 32.1, 30.3, 29.6, 28.2, 24.5; IR (thin film) 2902, 2848, 2363, 2328, 1451, 1312, 1096 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{22}$H$_{32}$N (M+H$^+$) 310.2535, found 310.2534. The residual signals in the ^{1}H NMR spectrum of the 2:1 mixture are consistent with vinylcyclopropane **8.9f**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.41-5.36 (m, 1H), 5.13-5.11 (m, 1H), 2.83-2.75 (m, 1H), 2.65-2.57 (m, 1H), 2.41-2.40 (m, 1H), 2.38-2.30 (m, 1H), 2.24-2.20 (m, 1H), 2.11-2.01 (m, 4H), 1.99-1.96 (m, 1H), 1.75-1.73 (m, 1H), 1.75-1.72 (m, 3H), 1.63-1.56 (m, 15H), 1.40-1.35 (m, 1H), 1.32-1.28 (m, 1H); R$_f$ = 0.84 (2:98:5 EtOAc/hex/Et$_3$N).

1-Benzyl-2-((1*R*,2*S*,3r,4*R*,5*S*)-tricyclo[3.2.1.0^{2,4}]octan-3-yl)pyrrolidine, 8.7a.
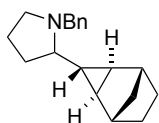
Using the general procedure for cyclopropanation, (*E*)-*N*-benzyl-5-iodopent-4-en-1-amine **8.6a** (36 mg, 0.14 mmol, 1.0 equiv) was reacted with norbornene (126 mg, 1.34 mmol, 10.0 equiv) and the product was purified by flash chromatography with EtOAc/hex/Et$_3$N (10:90:5) to afford the cyclopropane **8.7a** as a dark brown oil (24 mg, 0.089 mmol, 66%). R_f = 0.84 (10:90:5 EtOAc/hex/Et$_3$N); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.35 (d, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 7.0 Hz, 1H), 4.31 (d, *J* = 12.8 Hz, 1H), 3.06 (d, *J* = 12.6 Hz, 1H), 2.86 (t, *J* = 7.8 Hz, 1H), 2.27 (s, 1H), 2.24 (s, 1H), 2.03 (q, 8.6 Hz, 1H), 1.92-1.85 (m, 1H), 1.71-1.54 (m, 4H), 1.46-1.38 (m, 2H), 1.23-1.19 (m, 2H), 0.98 (d, *J* = 10.5 Hz, 1H), 0.73 (d, *J* = 8.3 Hz, 1H), 0.63-0.60 (m, 2H), 0.46 (d, *J* = 5.6 Hz, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 140.2, 129.0, 128.2, 126.7, 68.0, 59.1, 54.1, 36.0, 35.8, 31.0, 29.8, 29.7, 28.7, 23.5, 21.5, 18.7, 17.6; IR (thin film) 2938, 1736, 1469, 1371, 1233, 1044 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{19}$H$_{26}$N [M+H]$^+$ 268.2065, found 268.2060.

Dimethyl 2-((2S,3R)-tricyclo[3.2.1.0²,⁴]octan-3-yl)cyclopentane-1,1-dicarboxylate, 8.11.
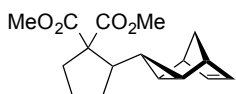
A 10 mL oven-dried round-bottom flask was charged with sodium hydride (95 wt%, 6.7 mg, 0.26 mmol) sodium hydride was suspended in 0.1 mL of DMF. The suspension was cooled to 0 °C with an ice bath, after which malonate derivative **8.10** (82 mg, 0.25 mmol) was added via syringe over 15 min. after the addition was done, ice bath was removed and the solution was stirred for 75 min at room temperature.

Using the general procedure for cyclopropanation, (*E*)-dimethyl 2-(5-iodopent-4-en-1-yl)sodiomalonate (82 mg, 0.25 mmol, 1.0 equiv) and the product was purified by flash chromatography with EtOAc/hex (10:90) to afford the cyclopropane **8.11** as a pale yellow oil (44 mg, 0.15 mmol, 60%). R_f = 0.64 (10:90 EtOAc/hex); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.32-6.29

(m, 2H), 4.10 (s, 3H), 3.72 (s, 3H), 2.72 (s, 1H), 2.65 (s, 1H), 2.51-2.46 (m, 1H), 2.21-2.17 (m, 1H), 2.07-2.03 (m, 1H), 1.91-1.86 (m, 1H), 1.81-1.76 (m, 1H), 1.69-1.67 (m, 1H), 1.54-1.48 (m, 1H), 1.46-1.41 (m, 1H), 1.08 (d, J = 9.2 Hz, 1H), 0.95 (d, J = 7.2 Hz, 1H), 0.82 (d, J = 6.6 Hz, 1H), 0.79 (d, J = 9.1 Hz, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.2, 171.7, 140.7, 140.6, 63.7, 52.6, 52.1, 48.3, 41.7, 38.8, 34.4, 34.3, 31.6, 29.7, 28.2, 23.3; IR (thin film) 2943, 2904, 1729, 1435, 1263 cm^{-1}; HRMS (ESI): *m/z* calculated for C$_{17}$H$_{22}$O$_4$Na [M+Na]$^+$ 313.1416, found 313.1419.

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Appendix A: Chapter 2 – HPLC Traces

Test of Chemical Stability

HPLC:
Stationary phase: reverse phase C18
Mobile phase: A: 0.1% aq. trifluoroacetic acid; B: acetonitrile

Synazo-1 (30 µM)
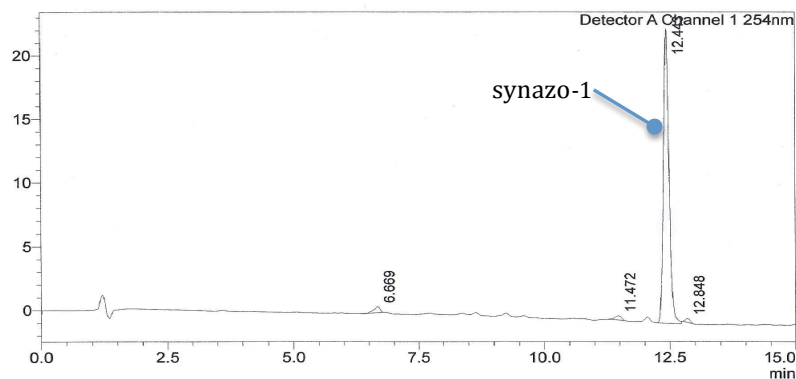
Internal Standard: 1-naphthylmethylamine (30 µM)
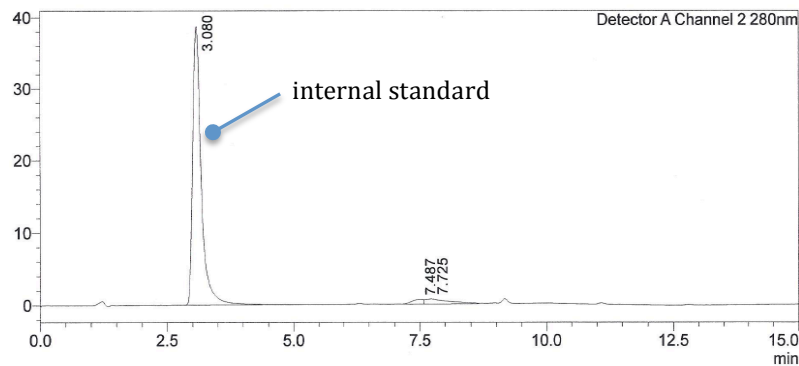
Test Medium: 10:90 FBS/10 mM phosphate, 100 mM NaCl, pH 7.2
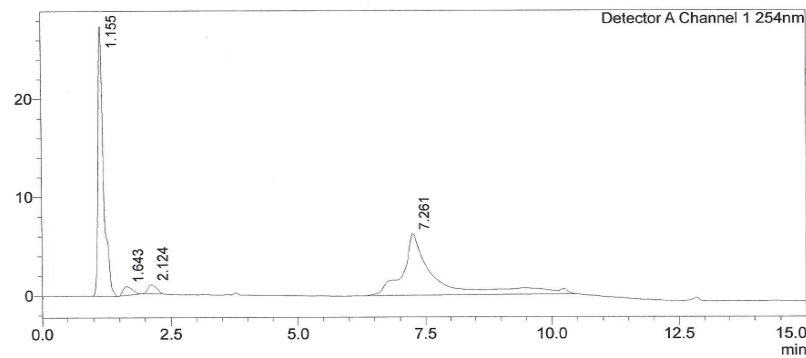
(The compounds in this chromatogram are present in the FBS.)

Synazo-1 (30 μM) + Internal Standard (30 μM):
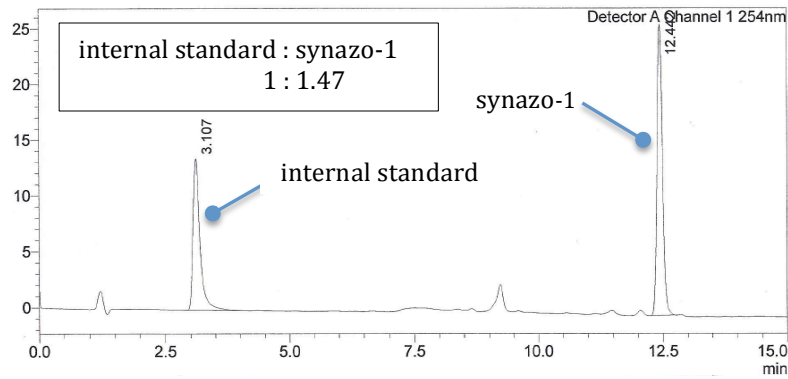
Synazo-1 (30 μM) + Internal Standard (30 μM) in 10:90 FBS/10 mM phosphate, 100 mM NaCl, pH 7.2 at 16 h:
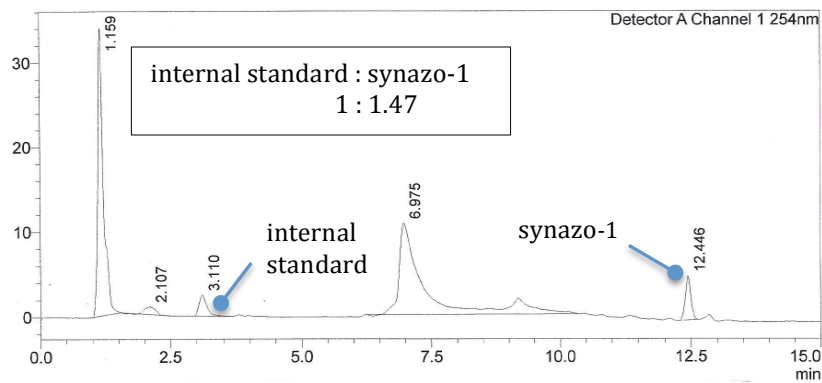
```
========================================================================
Acq. Operator   : UDARA                         Seq. Line :   7
Acq. Instrument : Instrument 1                   Location : Vial 15
Injection Date  : 5/20/2015 7:04:56 PM               Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\015-0701.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 7:00:40 PM by UDARA
```
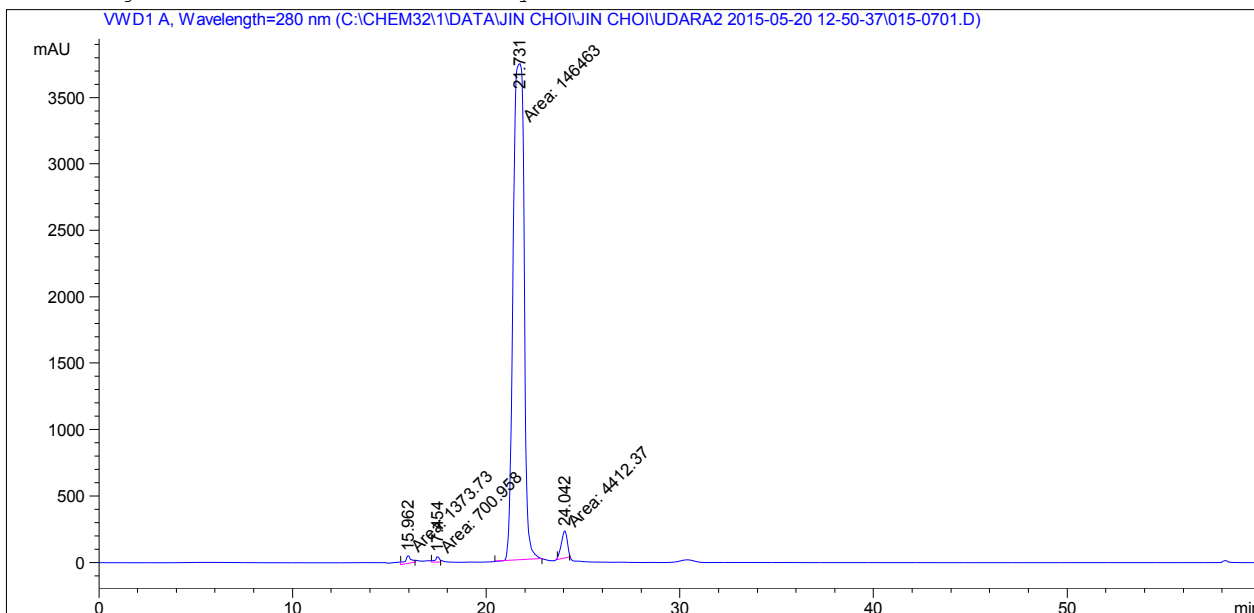

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\015-0701.D)

```
========================================================================
                        Area Percent Report
========================================================================

Sorted By             :        Signal
Multiplier            :        1.0000
Dilution              :        1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU   *s    [mAU    ]       %
----|-------|----|-------|----------|----------|--------|
   1  15.962 MM   0.3841 1373.73389   59.60886   0.8982
   2  17.454 MM   0.2723  700.95770   42.89745   0.4583
   3  21.731 MM   0.6536 1.46463e5  3735.03833  95.7587
   4  24.042 MM   0.3563 4412.36914  206.39709   2.8849
```

```
========================================================================
Acq. Operator   : UDARA                       Seq. Line :  14
Acq. Instrument : Instrument 1                  Location : Vial 22
Injection Date  : 5/21/2015 2:19:28 AM               Inj :   1
                                               Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\022-1401.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:41:38 AM by UDARA
```


VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\022-1401.D)

```
========================================================================
                        Area Percent Report
========================================================================

Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU  *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
   1  20.037 MM    0.9354 2.09562e5  3733.92871 100.0000

Totals :                  2.09562e5  3733.92871
```

```
=====================================================================
Acq. Operator   : UDARA                        Seq. Line :   1
Acq. Instrument : Instrument 1                  Location : Vial 1
Injection Date  : 5/19/2015 8:32:38 PM              Inj :   1
                                               Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA 2015-05-19 20-27-11\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\001-0101.D\DA.M (
                  UDARA.M)
Last changed    : 5/20/2015 10:07:18 AM by UDARA
                  (modified after loading)
```



```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By          :       Signal
Multiplier         :       1.0000
Dilution           :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]   mAU   *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  21.975 MM    1.4194 2.97796e5   3496.66187  98.8049
  2  23.155 MM    0.5826 3602.12891   103.05294   1.1951

Totals :                  3.01398e5   3599.71481
```

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\002-0201.D **Cpd 2.10**
Sample Name: KS-1-205

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   2
Acq. Instrument : Instrument 1                  Location : Vial 2
Injection Date  : 5/19/2015 9:34:36 PM             Inj :   1
                                               Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA 2015-05-19 20-27-11\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\002-0201.D\DA.M (
                  UDARA.M)
Last changed    : 5/20/2015 10:11:16 AM by UDARA
                  (modified after loading)
```



```
========================================================================
                       Area Percent Report
========================================================================

Sorted By            :       Signal
Multiplier           :       1.0000
Dilution             :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s   [mAU    ]       %
----|-------|----|-------|----------|----------|--------|
  1  20.112 VV   0.2418  2156.25171  115.13561   2.1874
  2  20.738 VV   0.4232  9.64191e4   3158.04980  97.8126

Totals :                 9.85753e4   3273.18542
```

Instrument 1 5/20/2015 10:11:28 AM UDARA   Page   1 of 2

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   4
Acq. Instrument : Instrument 1                  Location : Vial 26
Injection Date  : 5/21/2015 12:02:30 PM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\026-0401.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 1:09:43 PM by UDARA
```

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\026-0401.D)


```
========================================================================
                         Area Percent Report
========================================================================

Sorted By           :      Signal
Multiplier          :      1.0000
Dilution            :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  17.612 MM    0.3397 1799.95520    88.31090   1.5490
  2  23.186 MM    0.5225 1.14399e5   3649.06860  98.4510

Totals :                 1.16199e5   3737.37950
```
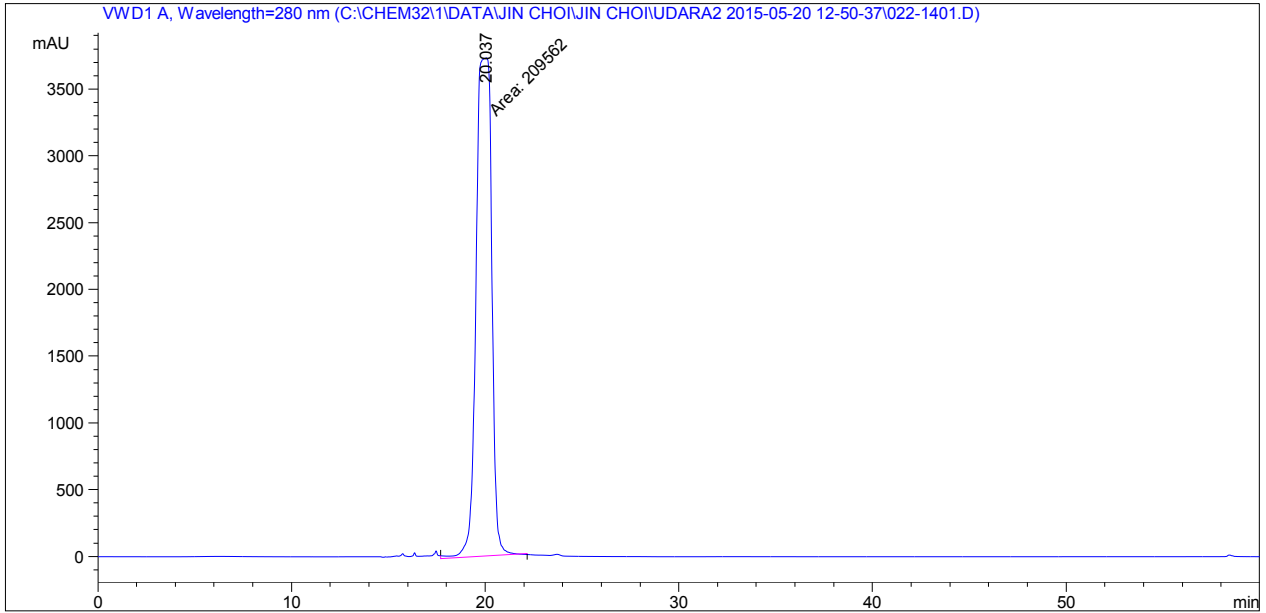
```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   5
Acq. Instrument : Instrument 1                  Location : Vial 5
Injection Date  : 5/20/2015 6:53:16 AM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA 2015-05-19 20-27-11\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\005-0501.D\DA.M (
                  UDARA.M)
Last changed    : 5/21/2015 7:35:13 PM by UDARA
```



```
========================================================================
                        Area Percent Report
========================================================================

Sorted By            :      Signal
Multiplier           :      1.0000
Dilution             :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
  1  17.855 MM   1.3946 4052.03711    48.42530   1.8326
  2  19.127 MM   0.9442 3453.91431    60.96648   1.5621
  3  20.292 MM   0.5955  958.41284    26.82559   0.4335
  4  23.092 MM   0.9361 2.10207e5   3742.41382  95.0716
  5  24.757 MM   0.5780 2432.46143    70.13708   1.1001
```
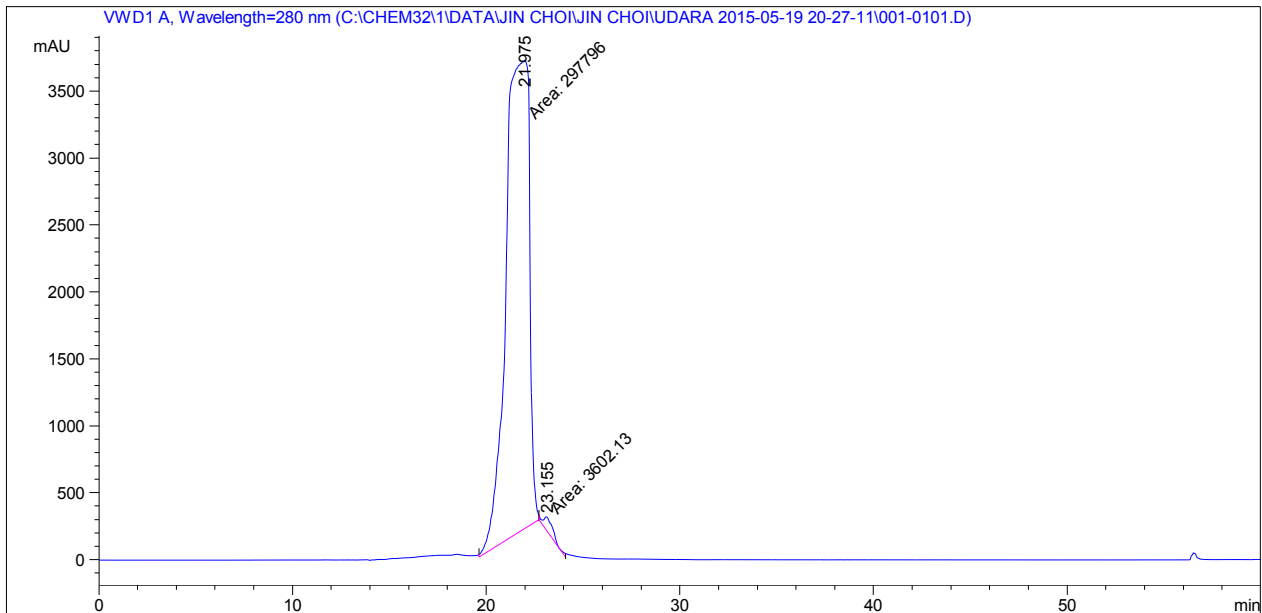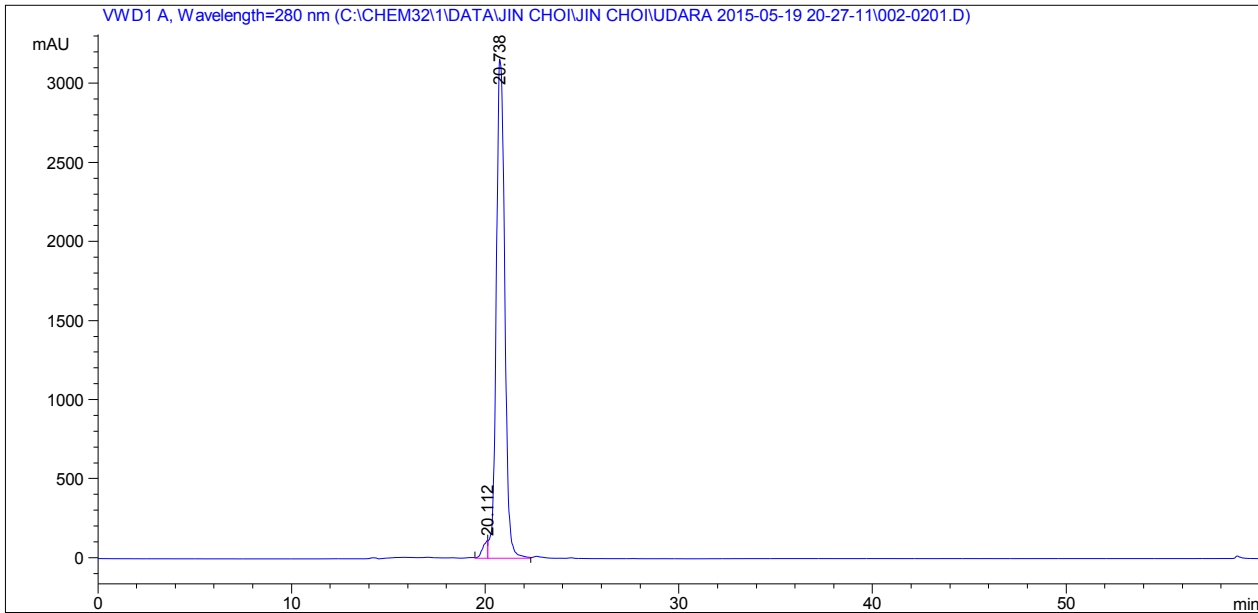
```
=====================================================================
Acq. Operator   : UDARA                       Seq. Line :  11
Acq. Instrument : Instrument 1                 Location : Vial 33
Injection Date  : 5/21/2015 7:17:01 PM              Inj :   1
                                              Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\033-1101.D\DA.M
                  (UDARA.M)
Last changed    : 5/19/2015 6:12:26 PM by UDARA
```
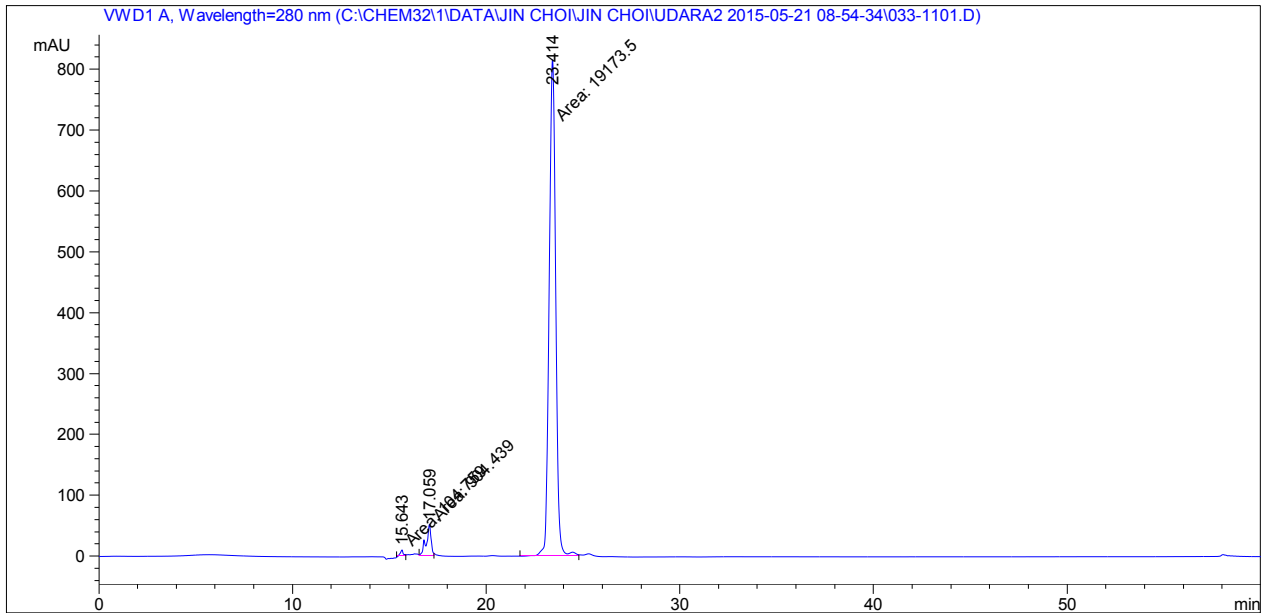

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\033-1101.D)

```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By           :       Signal
Multiplier          :       1.0000
Dilution            :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU   *s     [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
  1  15.643 MM    0.1787  104.75854    9.77096   0.5191
  2  17.059 MM    0.2919  904.43872   51.63951   4.4813
  3  23.414 MM    0.3919  1.91735e4  815.45270  94.9997

Totals :                  2.01827e4  876.86317
```
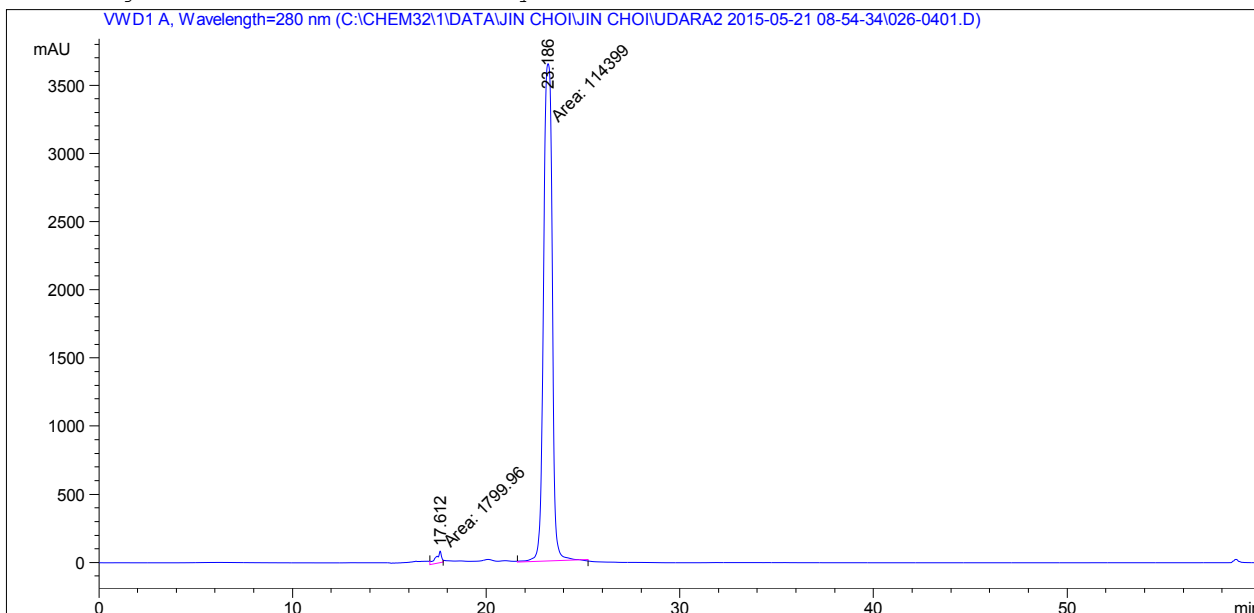
```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   8
Acq. Instrument : Instrument 1                  Location : Vial 16
Injection Date  : 5/20/2015 8:07:01 PM               Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\016-0801.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:31:21 AM by UDARA
```


VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\016-0801.D)

```
========================================================================
                        Area Percent Report
========================================================================

Sorted By          :        Signal
Multiplier         :        1.0000
Dilution           :        1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width      Area        Height       Area
  #   [min]        [min]   mAU   *s     [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  16.375 MM   0.1710   574.73639    56.00963   1.3931
  2  17.598 MM   0.2990   676.79230    37.72561   1.6405
  3  22.183 VB   0.4242 4.00043e4   1322.52563  96.9664

Totals :                  4.12558e4   1416.26087
```
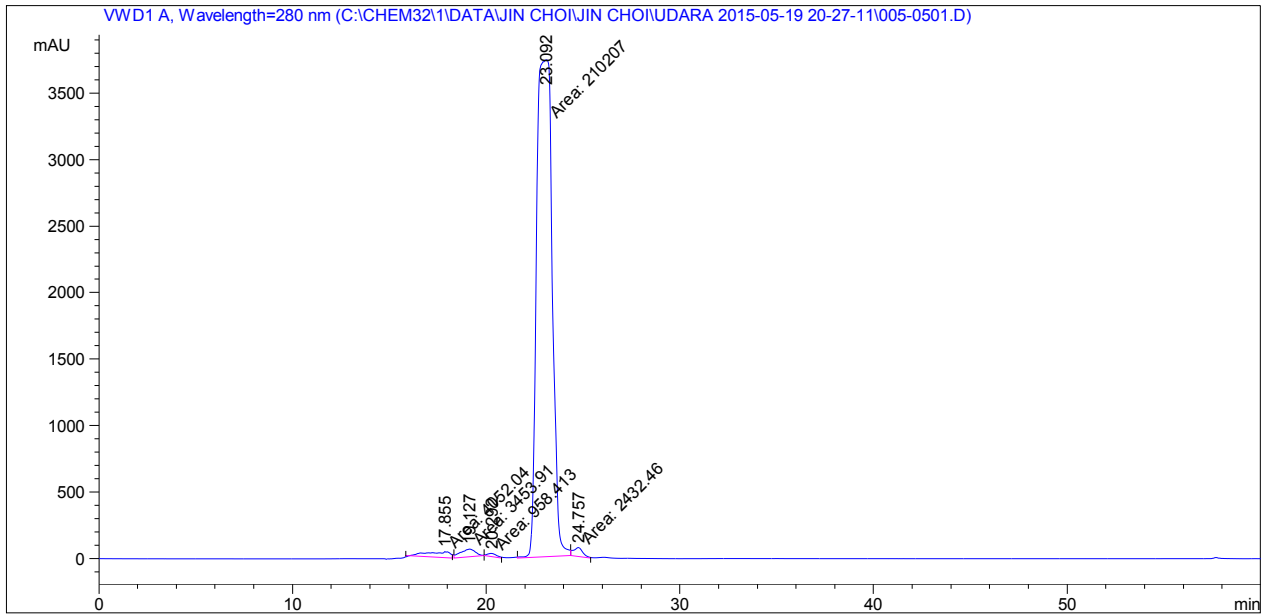
Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\013-0501.D **Cpd 2.15**
Sample Name: JS-1-152

```
=====================================================================
Acq. Operator   : UDARA                          Seq. Line :   5
Acq. Instrument : Instrument 1                   Location : Vial 13
Injection Date  : 5/20/2015 5:00:52 PM                 Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\013-0501.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:26:49 AM by UDARA
```



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By            :      Signal
Multiplier           :      1.0000
Dilution             :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
   1  22.196 VB    0.5143 1.22338e5  3709.80981 100.0000

Totals :                  1.22338e5  3709.80981
```
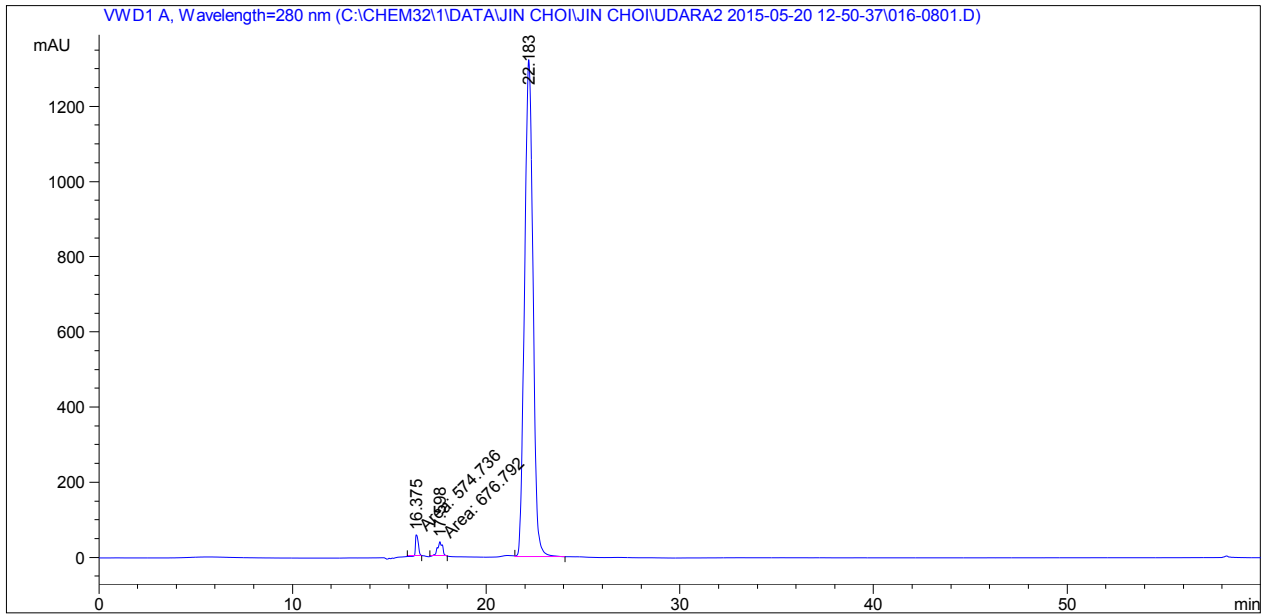
Instrument 1 5/21/2015 9:27:11 AM UDARA   Page   1 of 2

==
Acq. Operator   : UDARA   Seq. Line :   3
Acq. Instrument : Instrument 1   Location : Vial 11
Injection Date : 5/20/2015 2:56:33 PM   Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method   : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed   : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\011-0301.D\DA.M
                  (UDARA.M)
Last changed   : 5/21/2015 9:24:50 AM by UDARA
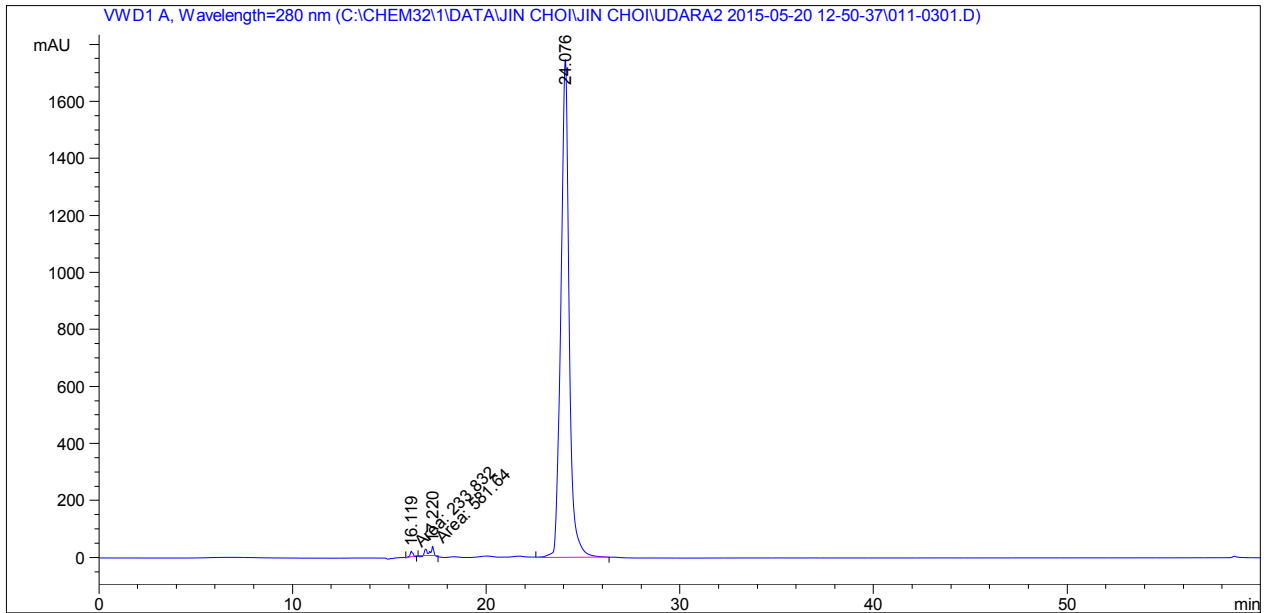
VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\011-0301.D)

==
                        Area Percent Report
==

Sorted By   :   Signal
Multiplier   :   1.0000
Dilution   :   1.0000
Use Multiplier & Dilution Factor with ISTDs
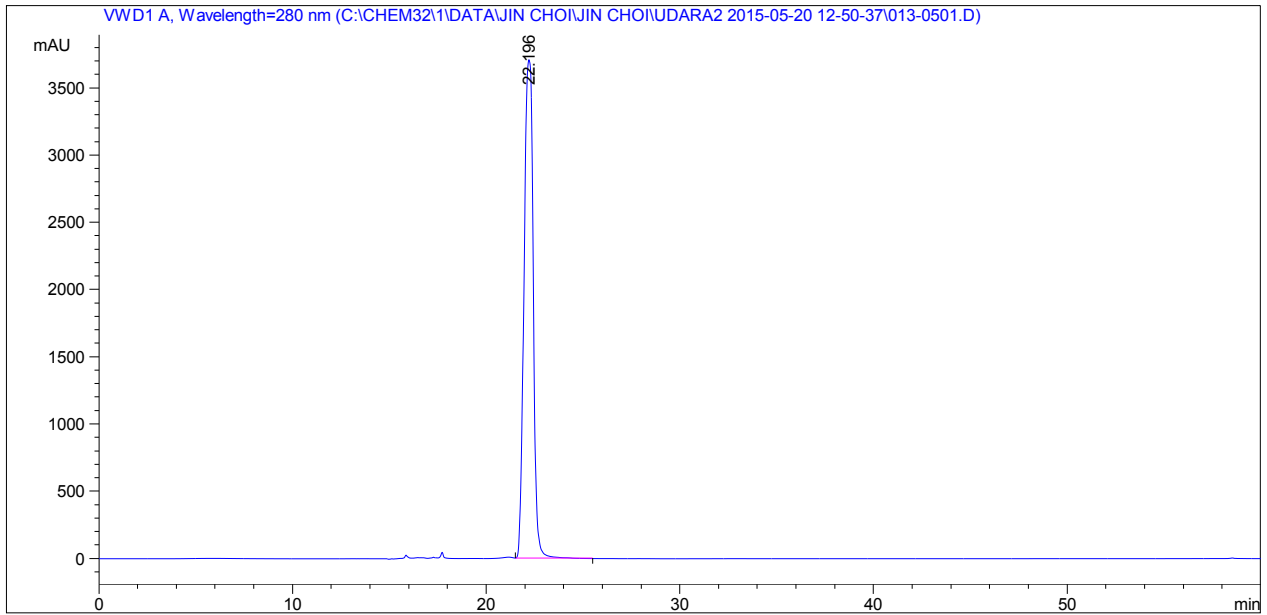

Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min] mAU   *s   [mAU   ]   %
----|-------|----|-------|----------|----------|--------|
  1 16.119 MM   0.1864 233.83177   20.90388   0.4420
  2 17.220 MM   0.2918 581.64020   33.21647   1.0995
  3 24.076 VB   0.4178 5.20829e4 1746.68005 98.4584

Totals :   5.28984e4 1800.80040

Instrument 1 5/21/2015 9:25:34 AM UDARA   Page   1 of 2

```
=====================================================================
Acq. Operator   : UDARA                         Seq. Line :   3
Acq. Instrument : Instrument 1                   Location : Vial 8
Injection Date  : 5/20/2015 11:45:57 AM              Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 09-40-00\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 09-40-00\008-0301.D\DA.M
                  (UDARA.M)
Last changed    : 5/20/2015 12:56:06 PM by UDARA
                  (modified after loading)
```


VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 09-40-00\008-0301.D)

```
=====================================================================
                       Area Percent Report
=====================================================================

Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  23.127 BB    0.4578 3.93470e4  1224.74646 100.0000

Totals :                  3.93470e4  1224.74646
```

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\012-0401.D **Cpd 2.18**
Sample Name: JS-1-125

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   4
Acq. Instrument : Instrument 1                  Location : Vial 12
Injection Date  : 5/20/2015 3:58:38 PM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\012-0401.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:25:55 AM by UDARA
```

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\012-0401.D)


```
========================================================================
                      Area Percent Report
========================================================================

Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s   [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  24.322 VV    0.4236 1.00716e5  3652.29980 100.0000

Totals :                  1.00716e5  3652.29980
```
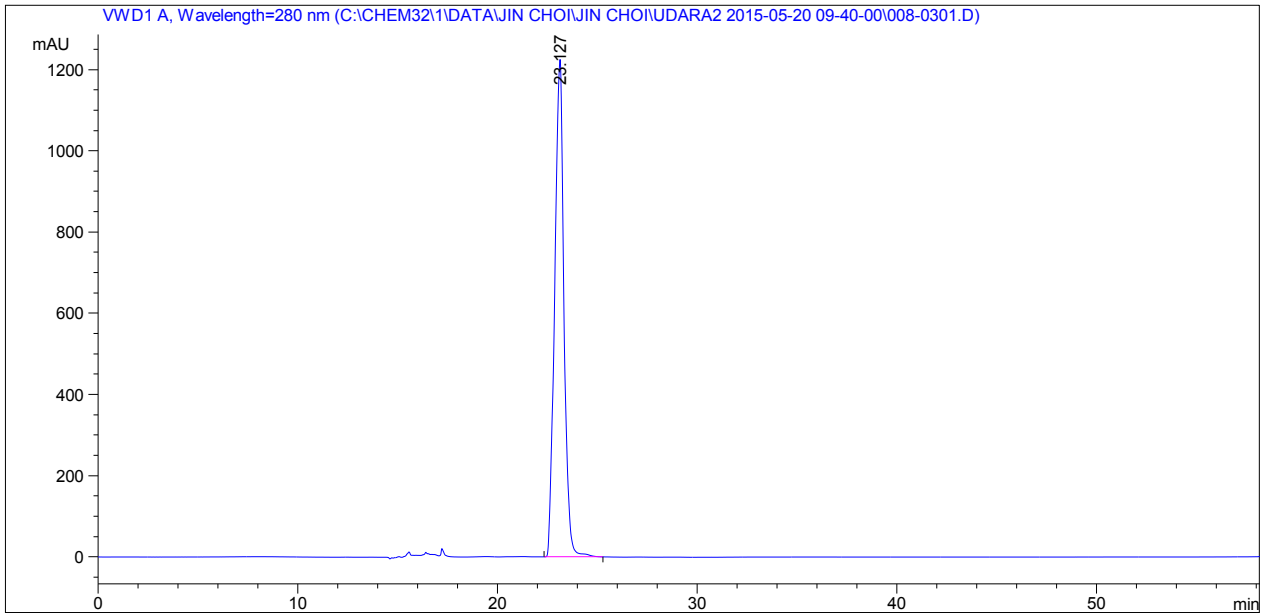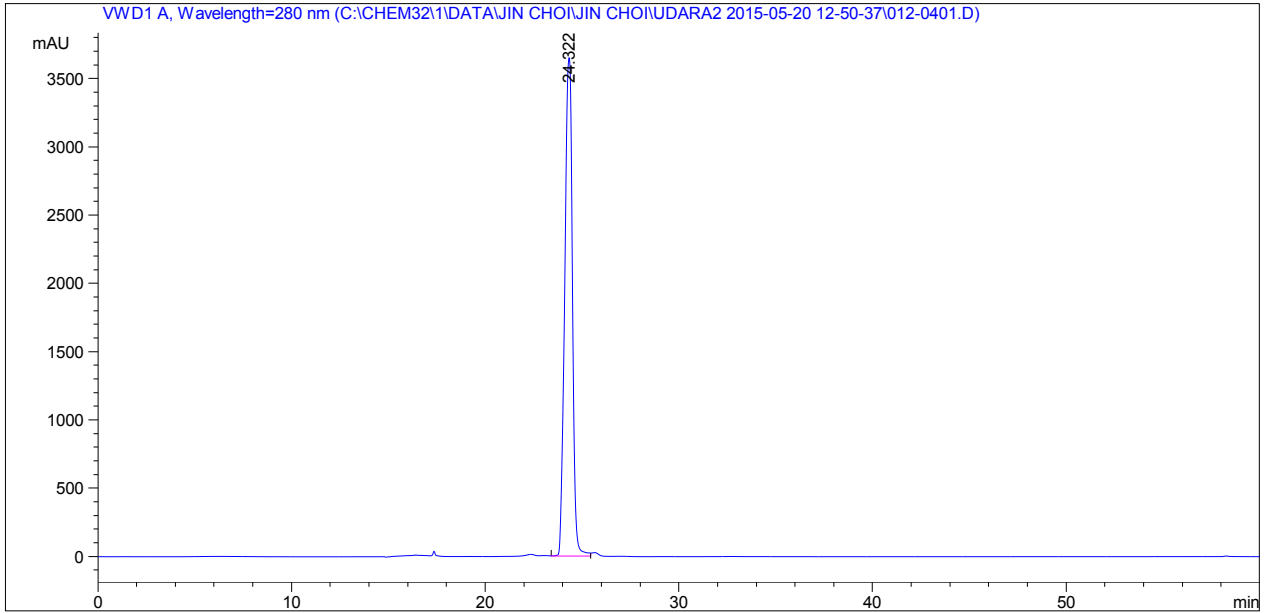
Instrument 1 5/21/2015 9:26:26 AM UDARA   Page   1 of 2

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\003-0301.D **Cpd 2.19**
Sample Name: KS-1-210

```
=====================================================================
Acq. Operator   : UDARA                        Seq. Line :   3
Acq. Instrument : Instrument 1                  Location : Vial 3
Injection Date  : 5/19/2015 11:38:51 PM              Inj :   1
                                             Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA 2015-05-19 20-27-11\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA 2015-05-19 20-27-11\003-0301.D\DA.M (
                  UDARA.M)
Last changed    : 5/20/2015 10:12:14 AM by UDARA
                  (modified after loading)
```



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=====================================================================
                    Area Percent Report
=====================================================================

Sorted By            :        Signal
Multiplier           :        1.0000
Dilution             :        1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU    *s    [mAU    ]       %
----|-------|----|-------|----------|----------|--------|
  1  22.099 BB    0.4564 1.27038e5  3735.68188 100.0000

Totals :                  1.27038e5  3735.68188
```
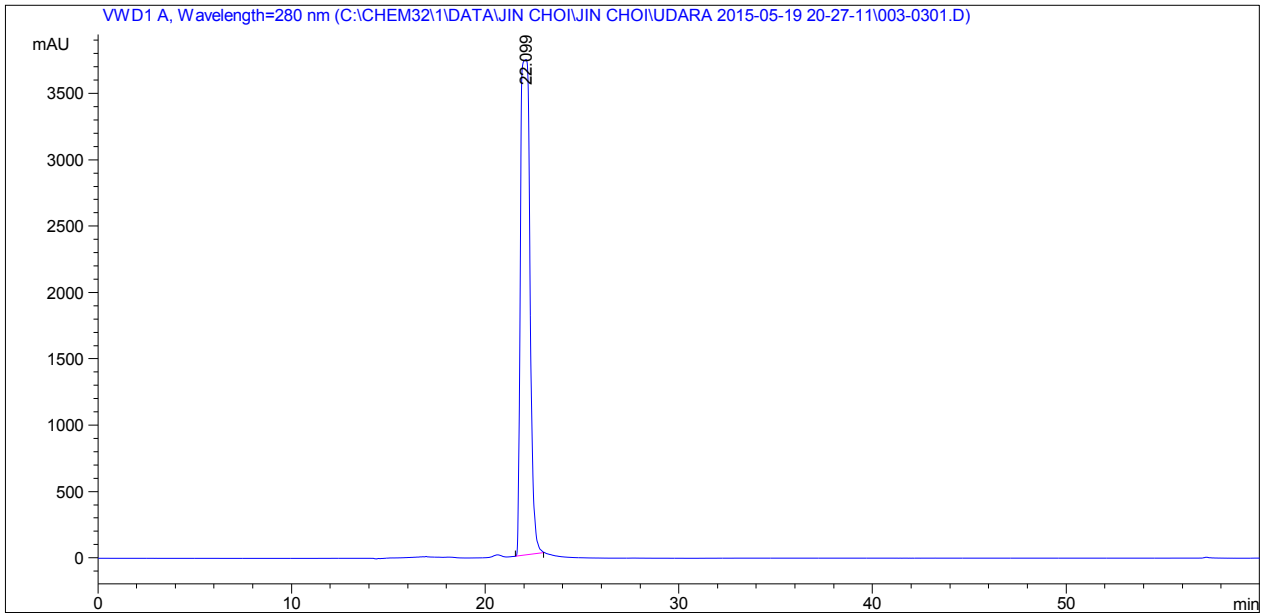
Instrument 1 5/20/2015 10:12:24 AM UDARA   Page   1 of 2

===
Acq. Operator   : UDARA   Seq. Line :   1
Acq. Instrument : Instrument 1   Location : Vial 9
Injection Date : 5/20/2015 12:52:35 PM   Inj :   1
                                              Inj Volume : 20 µl
Acq. Method   : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed   : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\009-0101.D\DA.M
                  (UDARA.M)
Last changed   : 5/21/2015 9:21:48 AM by UDARA
                  (modified after loading)

===
                     Area Percent Report
===

Sorted By   :   Signal
Multiplier   :   1.0000
Dilution   :   1.0000
Use Multiplier & Dilution Factor with ISTDs
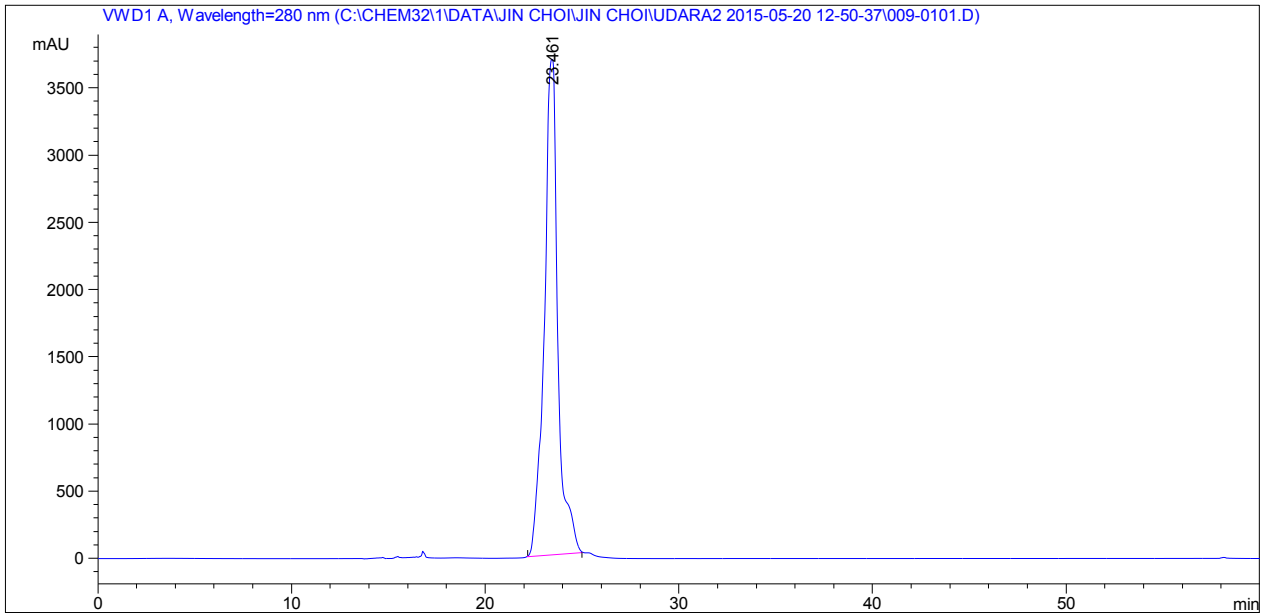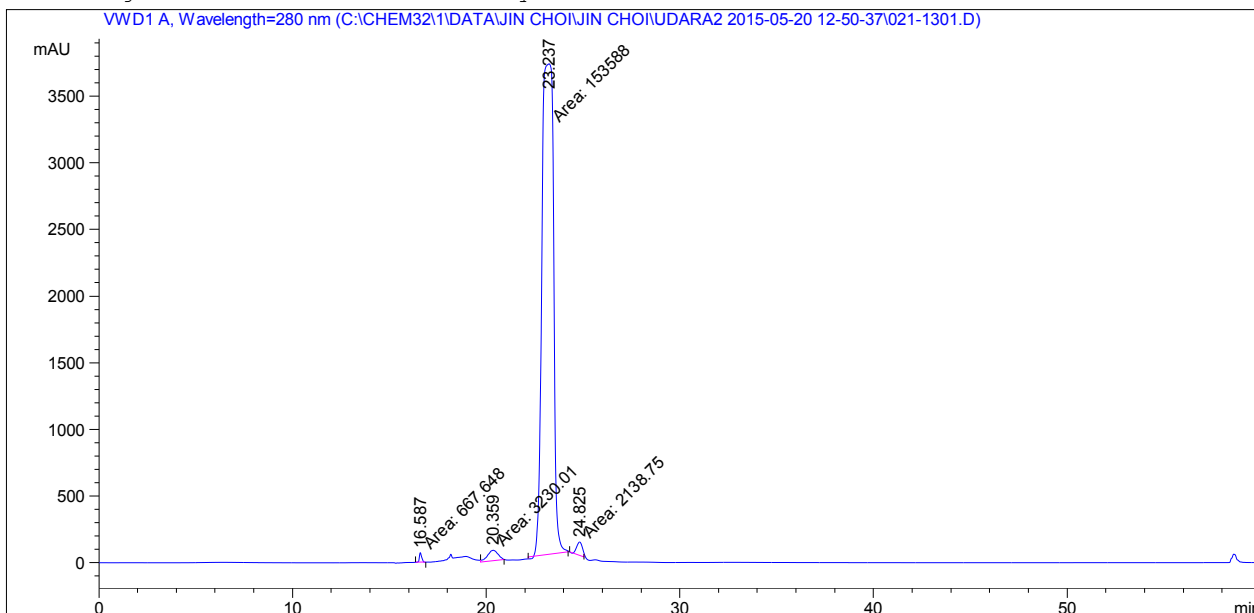

Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min] mAU *s   [mAU   ]   %
----|-------|----|-------|----------|----------|--------|
  1 23.461 BB   0.6200 1.78877e5 3686.57007 100.0000

Totals :   1.78877e5 3686.57007

Instrument 1 5/21/2015 9:22:01 AM UDARA   Page   1 of 2

220

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :  13
Acq. Instrument : Instrument 1                  Location : Vial 21
Injection Date  : 5/21/2015 1:17:27 AM               Inj :   1
                                               Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\021-1301.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:39:45 AM by UDARA
```



```
========================================================================
                       Area Percent Report
========================================================================

Sorted By            :      Signal
Multiplier           :      1.0000
Dilution             :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU   *s     [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  16.587 MM    0.1514   667.64807    73.47980   0.4183
  2  20.359 MM    0.6603  3230.01245    81.52984   2.0235
  3  23.237 MM    0.6943 1.53588e5    3687.11255  96.2184
  4  24.825 MM    0.3466  2138.75195   102.85317   1.3399
```
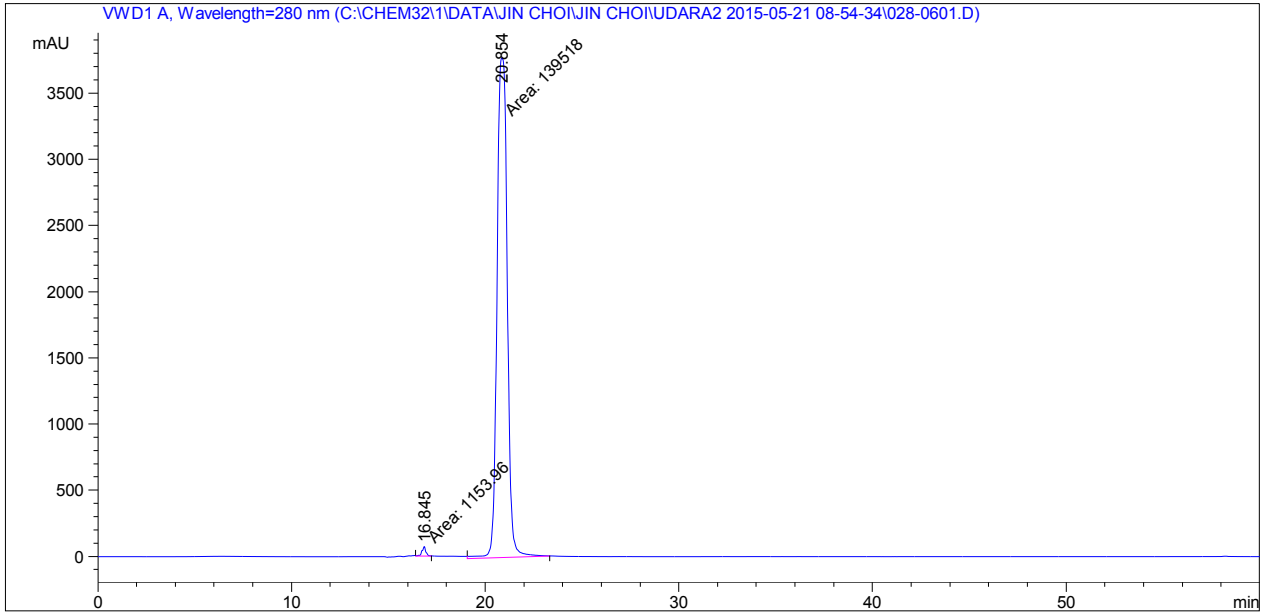
Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\028-0601.D **Cpd 2.22**
Sample Name: JS-1-157

```
=====================================================================
Acq. Operator   : UDARA                        Seq. Line :   6
Acq. Instrument : Instrument 1                  Location : Vial 28
Injection Date  : 5/21/2015 2:06:33 PM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\028-0601.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 3:10:16 PM by UDARA
```

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\028-0601.D)


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By           :      Signal
Multiplier          :      1.0000
Dilution            :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU  *s      [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  16.845 MM    0.2568 1153.96167    74.88272   0.8203
  2  20.854 MM    0.6152 1.39518e5   3779.73755  99.1797

Totals :                  1.40672e5   3854.62027
```
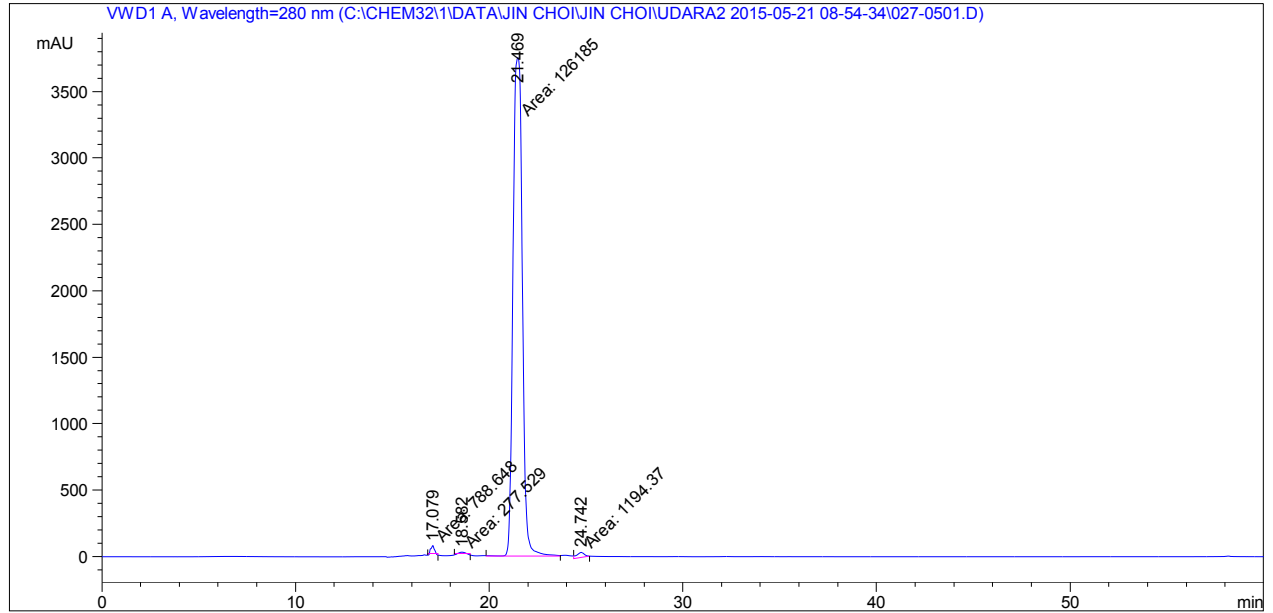
```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   5
Acq. Instrument : Instrument 1                  Location : Vial 27
Injection Date  : 5/21/2015 1:04:30 PM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\027-0501.D\DA.M
                  (UDARA.M)
Last changed    : 5/19/2015 6:12:26 PM by UDARA
```



```
========================================================================
                        Area Percent Report
========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
 #   [min]        [min]   mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
  1  17.079 MM   0.2093   788.64758   62.79678   0.6140
  2  18.582 MM   0.3490   277.52902   13.25263   0.2161
  3  21.469 MM   0.5601 1.26185e5   3754.53735  98.2401
  4  24.742 MM   0.4811  1194.37280   41.37428   0.9299
```

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\029-0701.D **Cpd 2.25a**
Sample Name: UP-2-88 D1

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   7
Acq. Instrument : Instrument 1                  Location : Vial 29
Injection Date  : 5/21/2015 3:08:37 PM              Inj :   1
                                             Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\029-0701.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 4:09:12 PM by UDARA
```
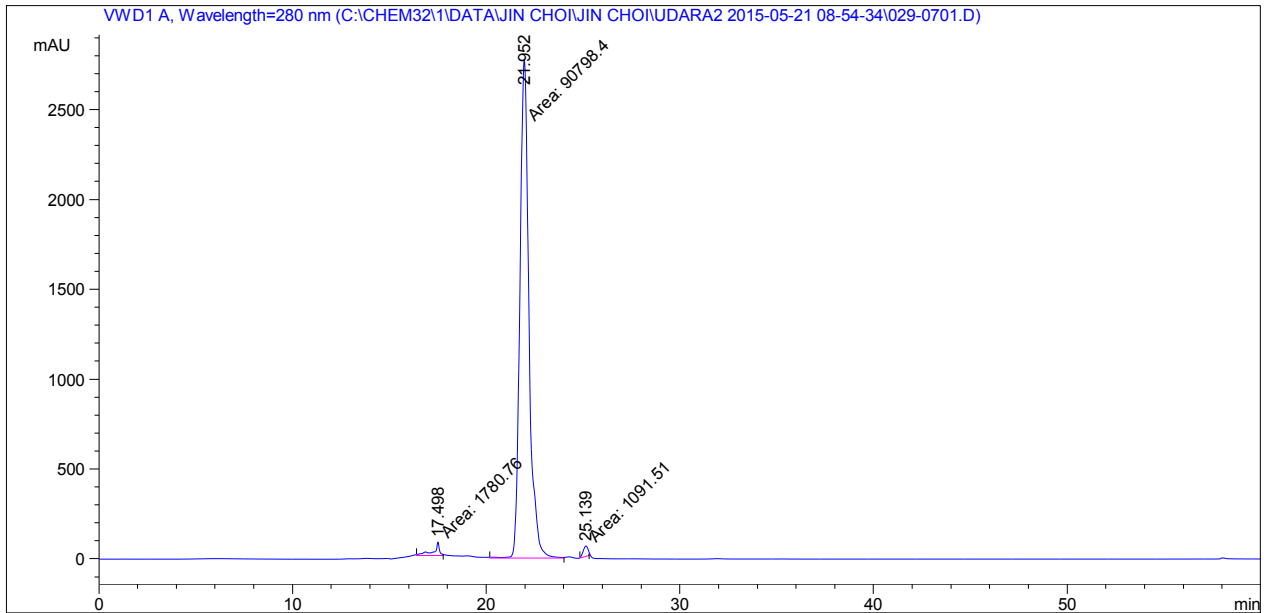


```
========================================================================
                        Area Percent Report
========================================================================

Sorted By            :       Signal
Multiplier           :       1.0000
Dilution             :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  17.498 MM   0.3853 1780.76465    77.02604   1.9011
  2  21.952 MM   0.5452 9.07984e4   2775.51880  96.9337
  3  25.139 MM   0.2988 1091.50671    60.89183   1.1653

Totals :                  9.36707e4   2913.43666
```
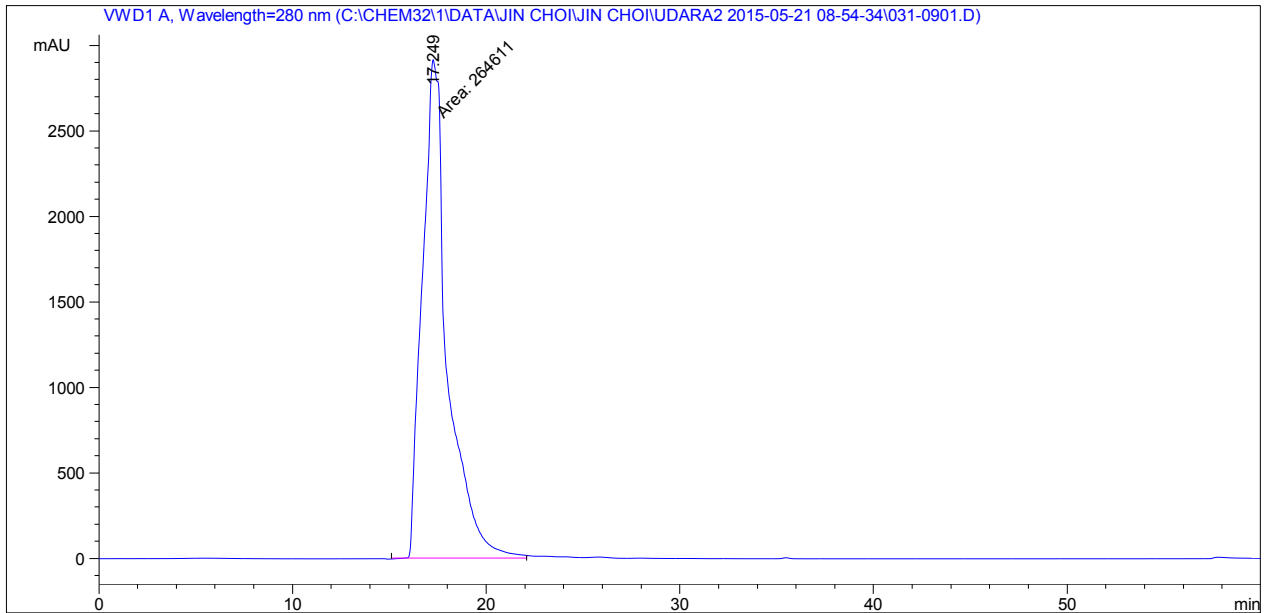
Instrument 1 5/21/2015 4:09:53 PM UDARA   Page   1 of 2

224

Sample Name: UP-2-88 D2

==
Acq. Operator   : UDARA   Seq. Line : 10
Acq. Instrument : Instrument 1   Location : Vial 32
Injection Date : 5/21/2015 6:14:52 PM   Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method   : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed   : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\032-1001.D\DA.M
                  (UDARA.M)
Last changed   : 5/19/2015 6:12:26 PM by UDARA
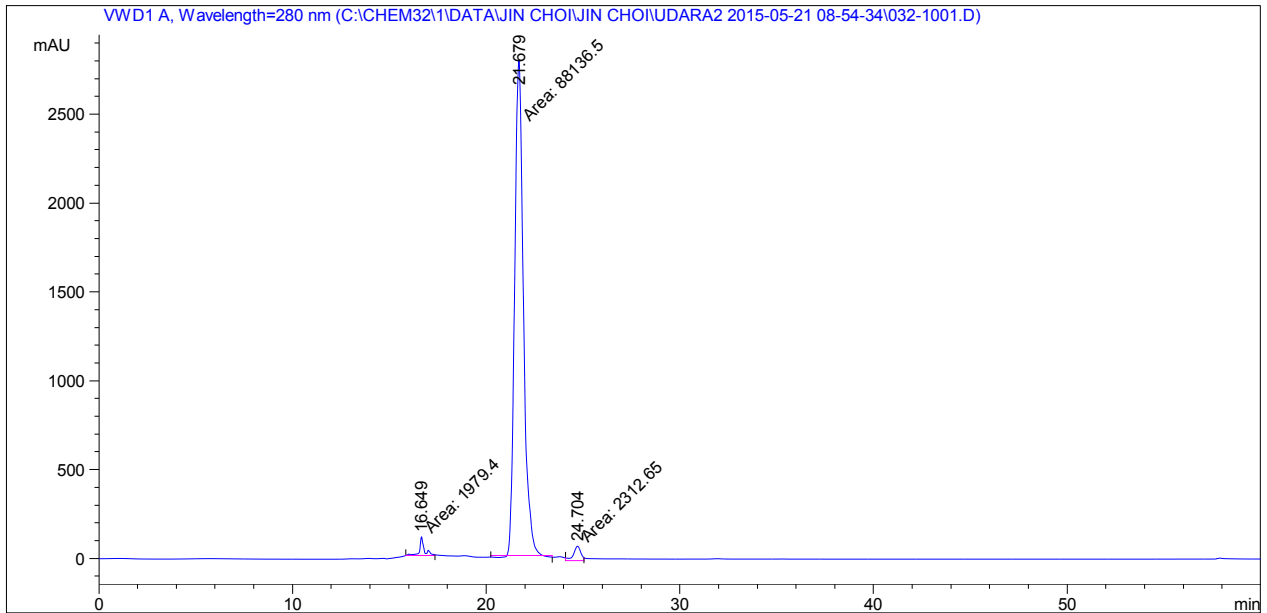
==
                         Area Percent Report
==

Sorted By   :   Signal
Multiplier   :   1.0000
Dilution   :   1.0000
Use Multiplier & Dilution Factor with ISTDs
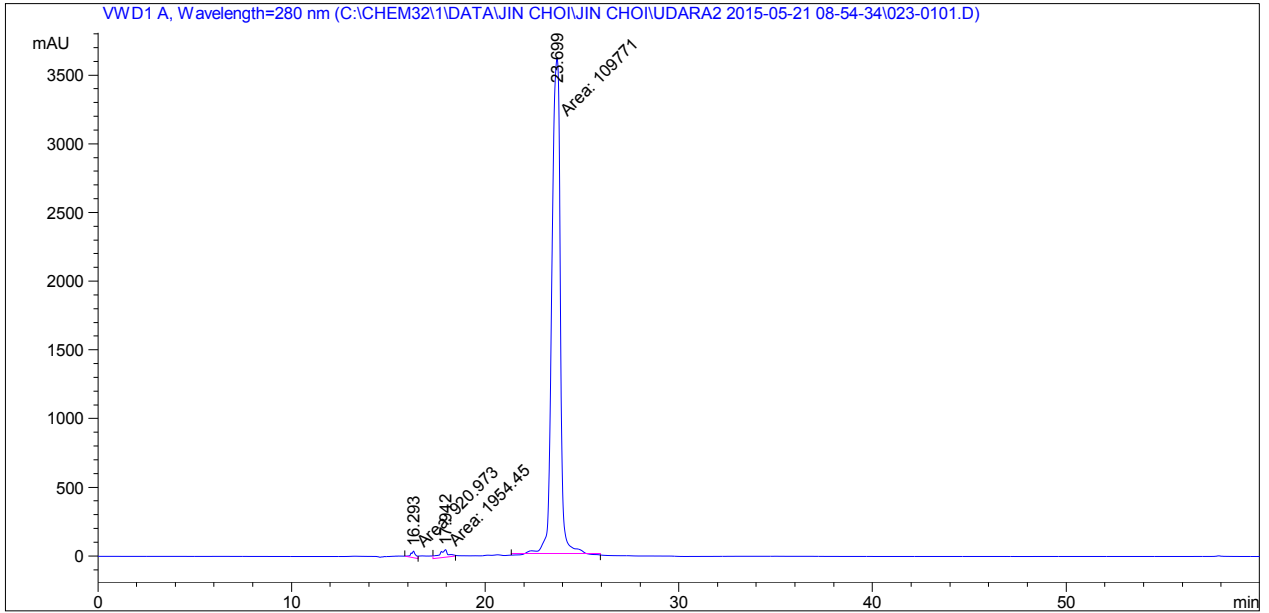

Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type Width   Area   Height   Area
  #   [min]   [min] mAU   *s   [mAU   ]   %
----|-------|----|-------|----------|----------|--------|
   1 16.649 MM   0.3074 1979.40442 107.31641   2.1415
   2 21.679 MM   0.5260 8.81365e4 2792.54321 95.3564
   3 24.704 MM   0.4618 2312.64844   83.45880   2.5021

Totals :   9.24286e4 2983.31842

Instrument 1 5/21/2015 7:33:48 PM UDARA   Page   1 of 2

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\031-0901.D **Cpd 2.26**
Sample Name: JS-1-126 rerun

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   9
Acq. Instrument : Instrument 1                 Location : Vial 31
Injection Date  : 5/21/2015 5:12:47 PM              Inj :   1
                                              Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\031-0901.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 6:19:47 PM by UDARA
```



```
========================================================================
                          Area Percent Report
========================================================================

Sorted By         :       Signal
Multiplier        :       1.0000
Dilution          :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s   [mAU    ]       %
----|-------|----|-------|----------|----------|--------|
  1  17.249 MM    1.5123 2.64611e5  2916.28052 100.0000

Totals :                 2.64611e5  2916.28052
```
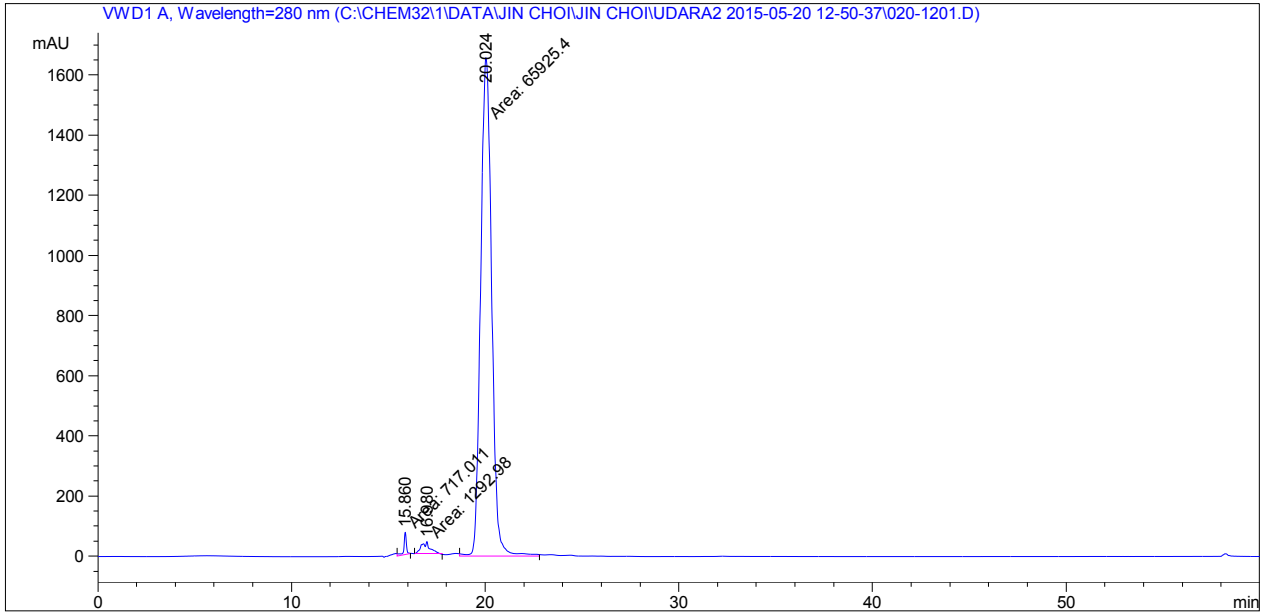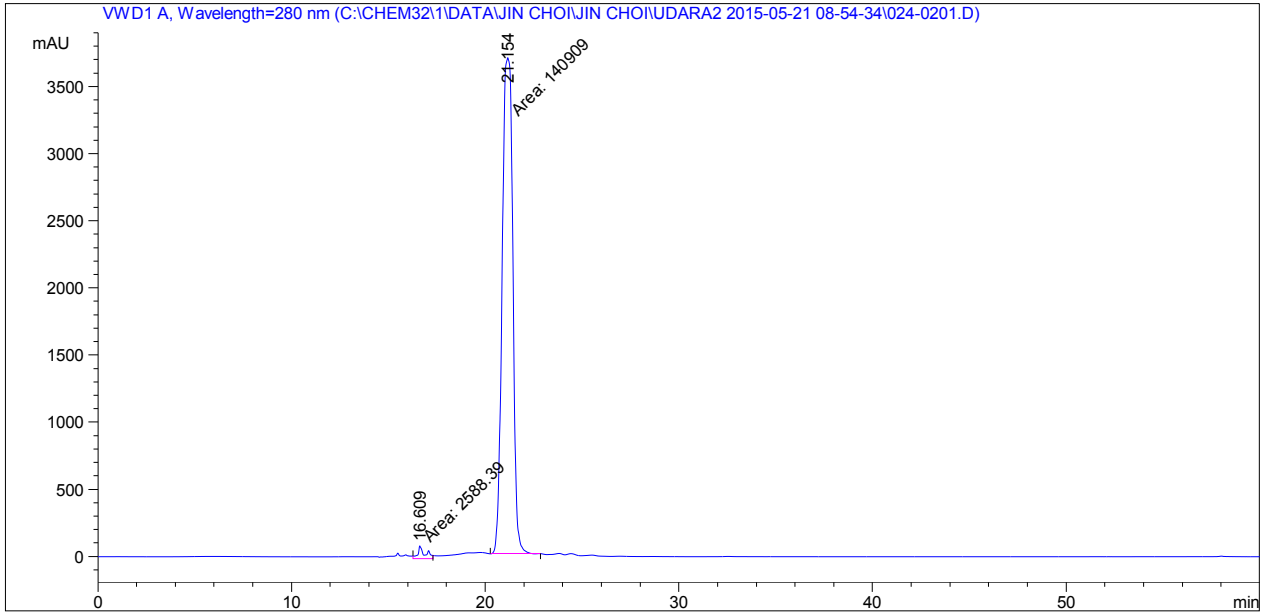
Instrument 1 5/21/2015 6:20:16 PM UDARA   Page   1 of 2

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\023-0101.D **Cpd 2.27**
Sample Name: UP-1-293

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   1
Acq. Instrument : Instrument 1                  Location : Vial 23
Injection Date  : 5/21/2015 8:56:53 AM              Inj :   1
                                              Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\023-0101.D\DA.M
                  (UDARA.M)
Last changed    : 5/19/2015 6:12:26 PM by UDARA
```

VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\023-0101.D)


```
========================================================================
                       Area Percent Report
========================================================================

Sorted By            :      Signal
Multiplier           :      1.0000
Dilution             :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
  1  16.293 MM    0.3038  920.97321    50.52233  0.8176
  2  17.942 MM    0.5627 1954.45496    57.89123  1.7350
  3  23.699 MM    0.5066 1.09771e5   3611.09277 97.4474

Totals :                 1.12647e5   3719.50634
```
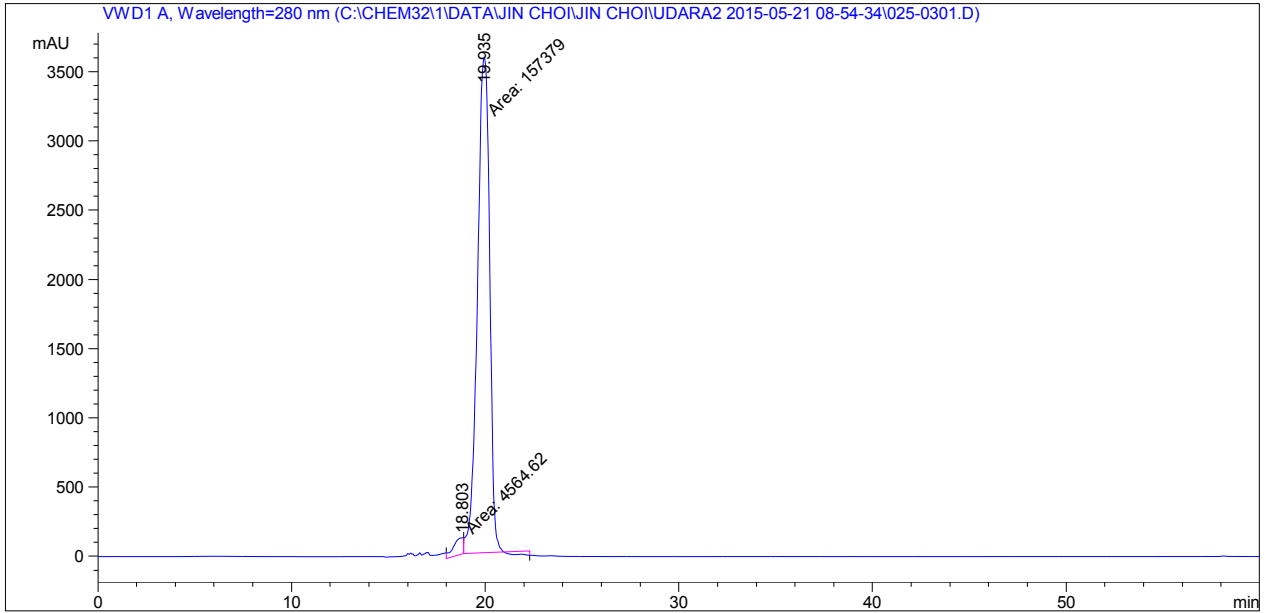
Instrument 1 5/21/2015 9:58:07 AM UDARA   Page   1 of 2

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```
=====================================================================
Acq. Operator   : UDARA                    Seq. Line :  12
Acq. Instrument : Instrument 1             Location : Vial 20
Injection Date  : 5/21/2015 12:15:26 AM         Inj :   1
                                           Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\020-1201.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 9:38:20 AM by UDARA
```



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By           :       Signal
Multiplier          :       1.0000
Dilution            :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
  1  15.860 MM   0.1591  717.01111    75.10148   1.0554
  2  16.980 MM   0.5235 1292.97766    41.16173   1.9032
  3  20.024 MM   0.6626  6.59254e4  1658.29358  97.0413

Totals :                 6.79354e4  1774.55679
```
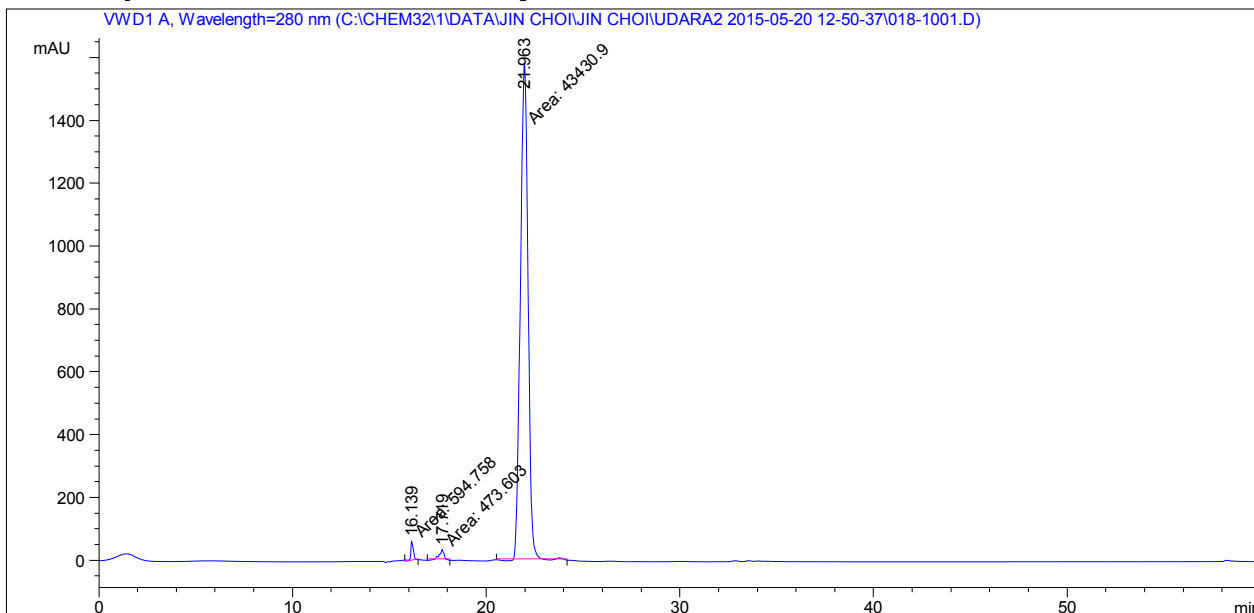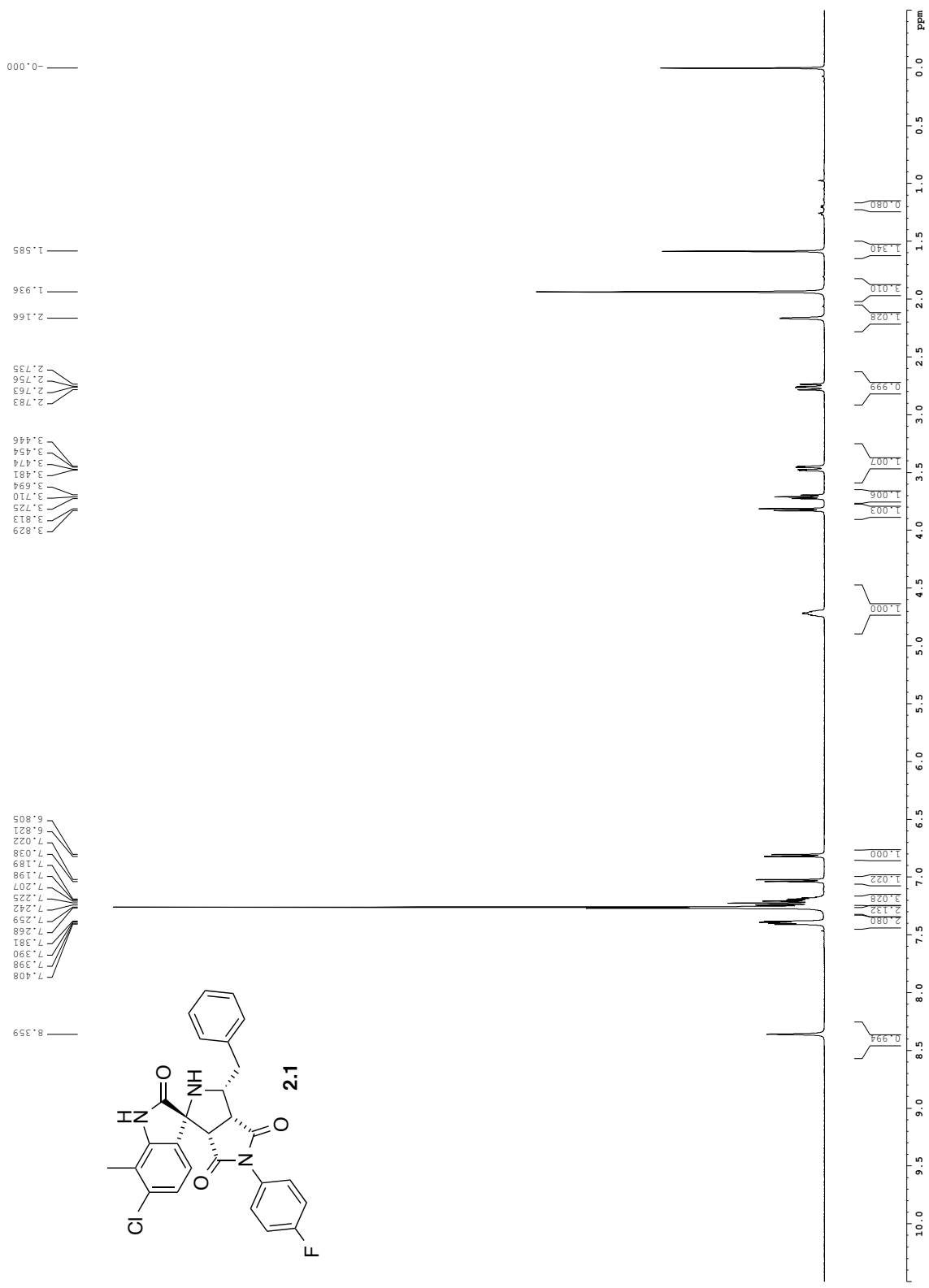
Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\024-0201.D **Cpd 2.29**
Sample Name: JS-1-160

```
========================================================================
Acq. Operator   : UDARA                        Seq. Line :   2
Acq. Instrument : Instrument 1                  Location : Vial 24
Injection Date  : 5/21/2015 9:58:22 AM              Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\024-0201.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 11:30:01 AM by UDARA
```


VWD1 A, Wavelength=280 nm (C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\024-0201.D)

```
========================================================================
                       Area Percent Report
========================================================================

Sorted By            :        Signal
Multiplier           :        1.0000
Dilution             :        1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]        %
----|-------|----|-------|----------|----------|--------|
  1  16.609 MM    0.4414 2588.38770    97.73698   1.8038
  2  21.154 MM    0.6355 1.40909e5   3695.72412  98.1962

Totals :                  1.43497e5   3793.46110
```
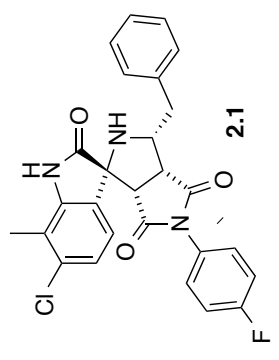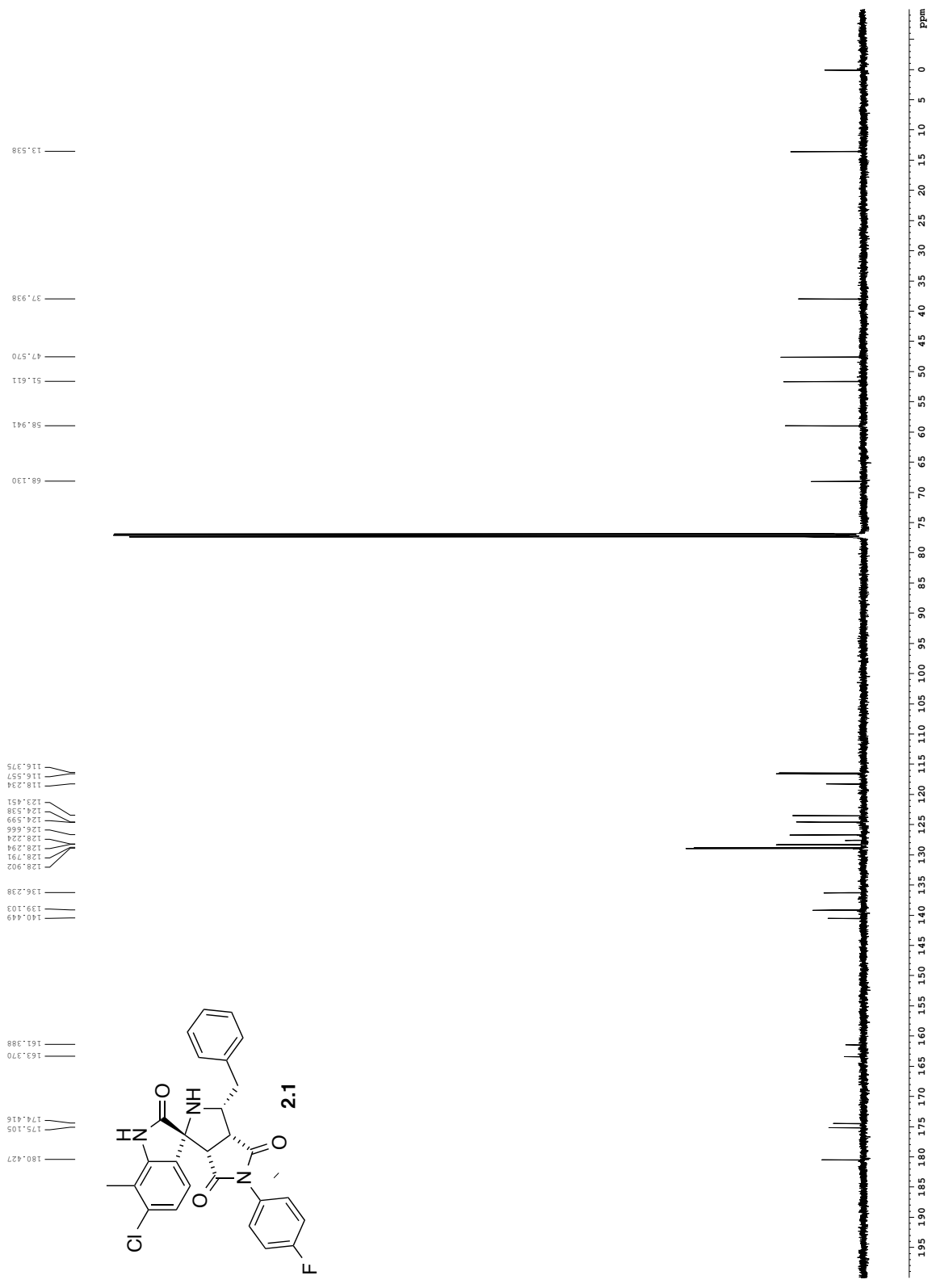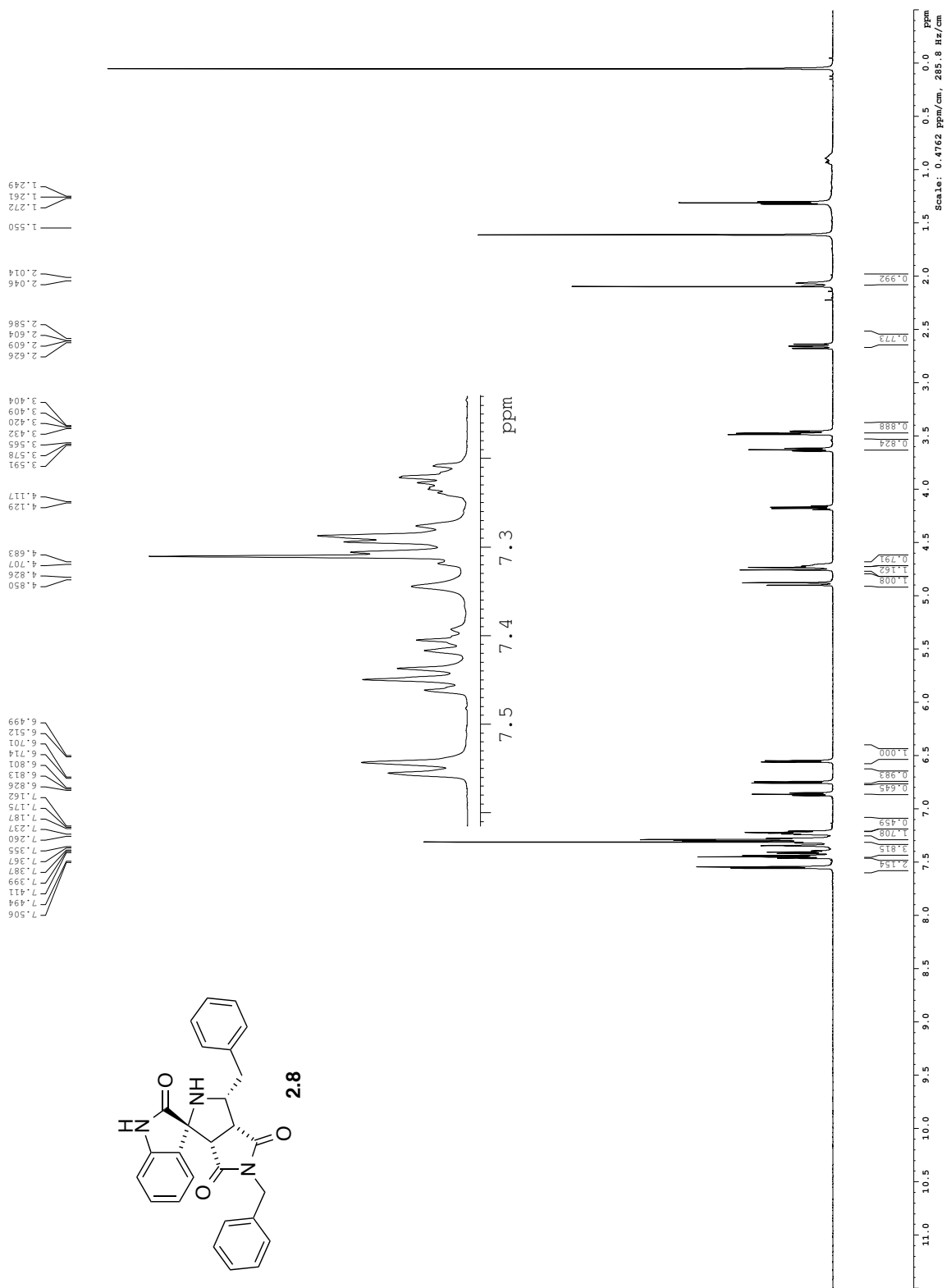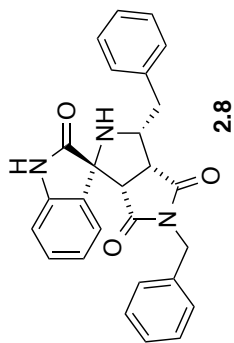
Instrument 1 5/21/2015 11:30:33 AM UDARA   Page   1 of 2

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\025-0301.D **Cpd 2.30**
Sample Name: JS-1-161

```
=====================================================================
Acq. Operator   : UDARA                        Seq. Line :   3
Acq. Instrument : Instrument 1                  Location : Vial 25
Injection Date  : 5/21/2015 11:00:29 AM             Inj :   1
                                                Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-21 08-54-34\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-21 08-54-34\025-0301.D\DA.M
                  (UDARA.M)
Last changed    : 5/21/2015 12:43:42 PM by UDARA
```



```
=====================================================================
                       Area Percent Report
=====================================================================

Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU   *s    [mAU    ]       %
----|-------|----|-------|----------|----------|--------|
  1  18.803 MM    0.4683 4564.62256   119.39576   2.8186
  2  19.935 MM    0.7331 1.57379e5   3577.96289  97.1814

Totals :                 1.61944e5   3697.35865
```

Instrument 1 5/21/2015 12:44:19 PM UDARA   Page   1 of 2

Data File C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\018-1001.D **Cpd 2.31**
Sample Name: JS-1-128

```
====================================================================
Acq. Operator   : UDARA                          Seq. Line :  10
Acq. Instrument : Instrument 1                    Location : Vial 18
Injection Date  : 5/20/2015 10:11:08 PM               Inj :   1
                                                 Inj Volume : 20 µl
Acq. Method     : C:\Chem32\1\DATA\Jin Choi\JIN CHOI\UDARA2 2015-05-20 12-50-37\UDARA.M
Last changed    : 5/19/2015 6:12:26 PM by UDARA
Analysis Method : C:\CHEM32\1\DATA\JIN CHOI\JIN CHOI\UDARA2 2015-05-20 12-50-37\018-1001.D\DA.M
                  (UDARA.M)
Last changed    : 5/19/2015 6:12:26 PM by UDARA
```



```
====================================================================
                         Area Percent Report
====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=280 nm

Peak RetTime Type  Width     Area        Height      Area
  #   [min]        [min]  mAU    *s     [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
  1  16.139 MM    0.1613   594.75769   61.46890   1.3366
  2  17.719 MM    0.2505   473.60257   31.51163   1.0643
  3  21.963 MM    0.4584 4.34309e4   1579.18811  97.5992

Totals :                  4.44993e4   1672.16865
```

Instrument 1 5/21/2015 9:36:13 AM UDARA   Page   1 of 2

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Appendix B: Chapter 2 – NMR Spectra

2.1

2.1

13.538

37.938

47.570
51.611

58.941

68.130

116.375
116.557
118.234
123.451
124.538
124.599
126.666
128.224
128.294
128.791
128.902

136.238
139.103
140.449

161.388
163.370

174.416
175.105

180.427

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

234

2.8

180.108
175.867
174.494

140.297
139.352
135.818
129.783
129.043
128.955
128.781
128.687
128.166
126.907
126.505
126.401
122.620

109.703

67.570

58.482

51.523
47.674
42.696
38.173

2.8

127 128 129 130 ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm
Scale: 8.300 ppm/cm, 1044 Hz/cm

27.711

42.652

47.698
51.615

58.098

67.649

109.691
111.108
113.634
119.108
119.497
122.072
122.402
122.485
126.488
126.780
127.466
128.154
128.791
129.026
129.621
135.918
136.203
140.419

174.781
176.033

180.271

ppm

122

124

126

128

130

2.9

-5 ppm
Scale: 8.300 ppm/cm, 1044 Hz/cm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

2.10

2.10

14.255

24.564
28.487
30.031
31.170

40.140
42.665

48.050
51.664

58.272

68.046

109.802

122.707
126.411
126.494
128.170
128.785
129.208
129.822
135.815

140.353

174.493
175.751
180.145

127 128 129 130 ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

Scale: 8.268 ppm/cm, 1040 Hz/cm

2.11

2.11

2.12

36.930

42.960

49.624
53.594

60.490

68.806

109.429

122.430
126.430
126.474
127.273
127.686
127.749
127.943
128.722
128.966
129.160
129.490
129.618
129.685
132.979
136.777
140.563
143.153

174.413
176.154
178.943

127

128

129

130

ppm

2.12

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 ppm

2.092
2.265
2.498
2.501
2.504
2.796
2.809
2.829
3.353
3.362
3.376
3.386
3.531
3.544
3.716
3.728
3.741

4.555

6.902
6.916
6.966
6.980
7.067
7.174
7.186
7.198
7.268
7.281
7.293
7.351
7.363
7.380
7.392
7.403
7.426
7.439
7.451
7.470
7.483
7.496
7.514
7.527
7.539

10.148

7.5 7.4 7.3 7.2 7.1 7.0 ppm

2.13

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

Scale: 0.4762 ppm/cm, 285.8 Hz/cm

1.000
1.003
1.016
0.613
0.984
2.024
5.005
1.043
0.703
2.019
3.050
1.958
1.015
1.013
1.022

14.256

36.970

49.470
53.225

60.281

69.016

117.347
121.925
125.053
126.407
127.281
127.620
128.211
128.659
128.886
129.353
129.419
129.430
132.939
134.494
135.151
140.557
142.767

170.396
174.409
176.080
181.206

2.13

ppm

124

126

128

130

132

134

136

ppm

-5 ppm
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195

Scale: 8.367 ppm/cm, 1052 Hz/cm

NMR spectrum

0.872
0.885
0.897
1.163
1.174
1.186
1.409

1.988
1.992
2.085
2.091
2.104
2.109
2.123
2.128
2.245
2.498
2.501
2.504
2.613
2.644

3.322
3.550
3.563
3.654
3.833
3.846
3.859
3.989
4.023
4.035
4.315

6.723
6.736
7.061
7.074
7.307
7.320
7.455
7.468
7.519
7.532
7.545

10.922

Integrals:
9.279
0.416
1.017
3.895
1.888
0.995
0.975
1.931
0.304
0.988
0.972
0.970
1.937
0.998
1.953
1.000

ppm
Scale: 0.4743 ppm/cm, 284.6 Hz/cm

2.14

13.687

27.941

45.025
45.979

50.425

57.634

63.367

71.682

79.074

117.303

122.072
123.187
124.565
126.857
128.449
128.952
132.050
134.553

142.773

153.641

173.382
174.718
177.953

2.14

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm
Scale: 8.300 ppm/cm, 1044 Hz/cm

2.15

Scale: 0.4743 ppm/cm, 284.6 Hz/cm

14.290

28.533

39.921
40.088
40.255

45.615
46.576

51.003

58.195

72.233

79.672

116.411
116.593
117.901

122.626
123.738
125.180
128.806
129.639
129.706

135.153

143.369

154.226

161.074
163.027

173.965
175.298
178.520

2.15

ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

Scale: 8.268 ppm/cm, 1040 Hz/cm

1.293
1.304
1.316
1.524
1.660

2.082
2.207
2.217
2.389
2.399

2.733
2.911

3.780
3.794
3.835
3.848
3.861
3.927
3.931
3.944
3.948
3.956
3.961
4.078
4.154
4.166
4.178
4.617

6.748
6.761

7.115
7.129
7.309

7.977
7.984

8.556

2.16

Scale: 0.4743 ppm/cm, 284.6 Hz/cm

ppm

0.587
9.090
0.945

0.574
3.187
1.978

0.962
0.961

1.023
1.027
1.051
0.892
0.423

0.930

1.000

1.014
0.519

3.043

0.839

251

2.16

13.775
28.439
45.581
46.166
50.519
58.313
72.910
118.346
122.504
123.669
123.819
124.477
126.185
132.726
133.001
136.819
141.219
154.399
173.002
177.604

ppm

Scale: 8.333 ppm/cm, 1048 Hz/cm

2.17

2.17

14.700

28.534

45.124
45.800
46.570

51.921

58.626

71.044

79.734

118.603
124.030
124.492
125.154
125.808
127.476
127.738
129.094
129.474
129.569
132.603
136.574
137.838

143.408

173.785
175.236
177.660

ppm

2.18

2.18

28.528

39.922
40.089
40.256
42.204
43.112
45.527
46.341
50.854

58.183

71.797

79.747

109.666

122.726
124.158
126.673
127.988
128.123
130.142
136.410
136.627

143.681

174.511
175.885
176.124

124 126 128 130 132 134 136 ppm

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

Scale: 9.523 ppm/cm, 1198 Hz/cm

−0.002

0.856
0.870
0.882
0.894
1.246
1.258
1.270
1.463
1.569

2.044
2.170
2.211
2.230
2.249
2.263
2.518

2.756

3.411
3.424
3.577
3.590
3.602
3.775
3.780
3.788
3.793
3.798
3.806
3.811
4.017
4.103
4.115
4.127
4.139
4.466
4.624
4.648
4.801
4.825

6.319
6.330
6.765
6.778
6.810
6.823
6.835
7.203
7.216
7.229
7.260
7.321
7.333
7.338
7.345
7.351
7.363
7.374
7.431
7.443
7.455

2.19

ppm
Scale: 0.4762 ppm/cm, 285.8 Hz/cm

8.878
1.915
0.918
0.880
0.981
0.980
1.044
0.729
1.098
0.994
0.917
1.000
1.014
1.014
3.037
3.005

24.559
28.489
30.025
31.141
40.150
42.662
48.101
51.648
58.282
68.108
109.947
122.627
126.294
126.544
128.169
128.783
129.210
129.769
135.804
140.539
156.126
174.665
175.799
180.414

2.19

ppm

129

130

−5 ppm
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195

Scale: 8.300 ppm/cm, 1044 Hz/cm

258

ppm

Scale: 0.4743 ppm/cm, 284.6 Hz/cm

1.450
2.156
2.162
2.175
2.180
2.193
2.199
2.222
2.227
2.242
2.245
2.494
2.497
2.500
2.503
2.506
2.633
2.639
2.655
2.658
2.661
2.674
2.680
2.773
2.791
2.794
2.813
2.839

3.607
3.620
3.772
3.777
3.785
3.790
3.795
3.803
3.808
3.866
3.890
3.903
3.916
4.379
4.397
4.400
4.909

6.919
6.932
7.017
7.021
7.026
7.257
7.262
7.267
7.272
7.275
7.280
7.286
7.344
7.352
7.444
7.456
7.514
7.527
7.539

9.158
2.058
1.019
1.118
0.986
1.017
2.024
1.027
2.046
1.000
2.011
2.006
5.929
1.036
2.016

2.20

259

28.964

43.600
46.044
47.017

51.693

58.835

72.390

80.131

110.251

123.432
124.805
126.977
127.906
128.091
128.426
129.488
129.648
130.000
130.713
133.102
137.065

144.172

154.657

174.339
175.735
176.699

2.20

138 136 134 132 130 128 126 ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

Scale: 8.333 ppm/cm, 1048 Hz/cm

260

2.21

28.522

41.994
45.565
46.306
50.568
55.518
57.874
60.215

72.338

79.692

110.495
113.402
115.363

126.015
127.489
127.930
129.110

136.365
136.457

154.155
154.951

174.686
176.080
177.674

2.21

2.22

1.443

2.192
2.198
2.211
2.216
2.239
2.608
2.614
2.630
2.633
2.636
2.649
2.655
2.750
2.768
2.771
2.789
3.226
3.542
3.555
3.734
3.739
3.752
3.757
3.824
3.837
3.850
3.869
3.888
3.891
4.349
4.369

6.760
6.772
6.855
6.868
6.880
7.056
7.069
7.326
7.339
7.341
7.436
7.448
7.506
7.519
7.531

10.182

9.236
5.094
1.015
1.178
0.672
1.209
1.032
2.055
1.021
0.998
1.028
1.026
2.036
1.049
2.086
1.000

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

2.22

2.23

13.457
20.094
28.523

46.691
50.975

58.090

72.445

79.625

118.244
122.432
123.537
123.588
127.448
129.019
129.574
132.682

138.745
141.827

174.111
175.511
178.810

2.23

ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

2.330
2.523
2.537
2.548
2.561
2.718
2.723
2.730
2.735
2.743
2.748
2.755
2.760
2.771
2.804
2.993
3.011
3.307
3.507
3.516
3.526
3.534
3.812
3.823
3.829
3.840
4.346
4.362
4.685
4.690
4.701
4.710
4.715

5.611
5.621
5.630

7.154
7.167
7.280
7.293
7.387
7.397
7.399
7.417
7.441
7.454
7.466
7.479
7.503
7.516
7.583
7.585
7.587
7.597
7.610
7.955
7.957
7.969
7.971

9.673

2.25a

3.001
1.058
1.874
1.688
1.341

1.124
1.068

1.000

1.000

1.012

0.998
1.830
1.587
3.678
0.977
0.971

1.883

0.815

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

ppm

205.232

176.619
174.848

165.637

135.575
133.013
130.229
129.282
128.583
128.435
128.127
127.251
124.407
122.386
118.141

74.723

65.140

55.339
53.357
53.052

37.713

13.247

2.25a

1.292
1.410

2.046
2.050
2.053
2.086
2.339
2.357
2.364
2.369
2.381
2.387
2.392
2.785
2.798
2.810
2.821
2.834
3.265
3.275
3.281
3.292
3.370
3.379
3.386
3.396
3.990
4.001
4.007
4.018
4.383
4.400
4.536
4.539

5.420
5.427
5.430
5.433
5.440

6.957
7.129
7.143
7.268
7.281
7.371
7.383
7.396
7.436
7.449
7.462
7.466
7.480
7.531
7.544
7.557
7.659
7.671
7.683
8.085
8.097

9.689

2.25b

3.082
1.023
1.023

2.062

1.186
1.024

1.063

0.964
0.978

1.039

1.000
2.067
3.208
1.896
0.934

1.827

0.790

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

2.25b

13.311

36.595

53.281
53.503
54.382

64.939

73.789
74.724

122.414
124.629
125.192
125.483
127.279
128.181
128.630
129.493
130.142
133.036
133.270
135.675

165.620

174.796
176.529

ppm

205 200 195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

2.26

1.187

2.001
2.216
2.219
2.255
2.421
2.425
2.511
2.514
2.517
2.788
2.809

3.292
3.312
3.504
3.516
3.750
3.759
3.762
3.769
3.773
3.781
4.036
4.048

5.767

6.715
6.729
7.064
7.077
7.303
7.315
7.317
7.454
7.467
7.479
7.533
7.546
7.558

10.898

0.337

0.385
1.868
3.069
0.997
4.478

1.010

1.449
1.411
1.203

2.120

0.442

0.777

1.000

0.996

1.989
1.030
2.017

1.053

Scale: 0.4762 ppm/cm, 285.8 Hz/cm

14.271

44.934
46.681
47.078
48.670
50.747

58.742

72.593

117.743

122.532
124.067
125.156
127.437
128.990
129.558
132.720
134.980

143.383

174.177
175.514

178.806

2.26

ppm
125
130
135
140

ppm
−5 ppm
Scale: 8.235 ppm/cm, 1036 Hz/cm

14.207

24.956
25.216
29.107
29.210
29.346
33.315
34.124

41.125
45.312
46.227
48.715
50.477
51.435

58.418
58.718
60.395

72.702

118.765
122.308
123.533
124.428
126.322
129.381
131.578

136.577

141.848

171.665
172.212
174.617
178.158

2.27

2.28

13.715
14.004
14.155
14.274
17.109
19.083
21.885

30.089
30.561
30.668
32.547

40.193
40.331
40.497
41.744
44.502
45.477
45.827
46.581
46.692
50.928
56.335
57.161
57.316
57.473
58.142
58.691
63.970

72.089

81.037
81.728
82.063

117.880
122.620
123.592
125.158
127.321
127.487
127.665
128.485
128.613
128.892
129.050
129.539
132.629
135.168
137.439

143.357

155.288

168.410
168.606

173.955
175.248

178.410

195.542

ppm

OMe

2.28

2.29

2.29

2.30

2.30

14.289

31.178
31.242
34.501
34.670

44.696
44.850
45.490
46.106
46.573
46.709
48.502
51.022
51.204

58.169
58.944
60.222

72.169

117.904
122.653
123.796
123.860
125.114
125.188
126.318
127.471
128.707
128.853
128.880
129.046
129.556
132.658
135.161

141.796
141.889
143.357

170.550

174.028
175.309
175.400
178.534
178.603

ppm

1.200
1.212
1.224
1.706
1.986
2.248
2.253
2.266
2.271
2.285
2.291
2.304
2.327
2.329
2.346
2.498
2.501
2.504
2.742
2.748
2.764
2.767
2.784
2.789
2.857
2.870
2.889
2.892
2.910
3.603
3.613
3.791
3.796
3.804
3.809
3.822
3.827
3.869
3.882
3.895
3.979
4.001
4.077
4.088
4.470
4.491
5.130
5.151
5.153
5.175

6.795
6.808
7.035
7.048
7.241
7.304
7.312
7.319
7.326
7.333
7.350
7.367
7.374
7.425
7.438
7.450
7.508
7.521
7.533

10.440

ppm
0.0
0.5
Scale: 0.4762 ppm/cm, 285.8 Hz/cm

2.31

Integrations:
5.053
0.991
1.684
0.766
0.968
0.984
0.994
0.975
0.322
0.976
1.956
0.980
0.982
0.282
1.199
0.994
5.808
1.042
2.049
1.000

281

14.302
14.556

21.201

30.880

43.555
45.595
46.587
47.282

51.032

58.259
60.227

66.958

72.329

117.961
121.141
122.654
123.733
125.190
127.459
128.064
128.219
128.348
128.903
129.041
129.540
132.646
135.236
137.314

143.399

154.885

173.985
175.312

178.555

2.31

ppm

128

129

130

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

Scale: 8.434 ppm/cm, 1061 Hz/cm

2.25a

2.25b

2.26

Compound 1

Vitas M-STK80951

Compound 1

2 mM

1 mM

0.7 mM

0.5 mM

Appendix C: Chapter 3 – NMR Spectra

3.845
3.945

6.545
6.562
7.208
7.224
7.242
7.272
7.542
7.558
7.597
7.613
7.627
8.190
8.206
8.397
8.424

11.629
11.656

3.168
2.967

1.941
4.027
1.122
1.104
1.000
1.081

1.110

3.6a

OMe

NH

OMe

ppm
-0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 12.5 13.0 13.5 14.0 14.5

55.702
56.059
91.081
99.481
104.799
116.329
117.282
117.723
121.913
124.866
131.063
135.002
137.501
144.255
150.727
158.576
161.897
163.526

3.6a

OMe
OMe
NH
O
O

291

2.380

3.947

6.795
6.829
6.845
7.183
7.199
7.247
7.277
7.558
7.574
7.601
7.615
7.617
7.631
8.186
8.202
8.451
8.478

11.668
11.694

OMe

NH

3.6b

3.098
3.157
1.017
1.011
1.055
2.308
1.071
1.036
0.948
1.018
0.955

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0

21.483

55.979

91.494

112.501
114.933
117.410
117.842
121.657
125.013
125.713
131.067
135.029
136.507
137.422
143.844
149.302
161.857
163.485

3.6b

ppm

11.634
11.660

8.201
8.217
8.415
8.442

7.117
7.180
7.288
7.303
7.318
7.575
7.591
7.626
7.640
7.657

3.978

1.154

1.097

1.000

1.050
1.086

0.999
1.911
1.103

2.985

3.6c

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5
12.0

294

56.418

92.691

115.180
115.768
117.623
118.007
118.414

124.156
125.519
127.515

131.158

135.149
136.862

143.059

149.824

161.490
163.519

OMe

NH

Br

3.6c

OMe

MeO

O

N

OH

3.7a

12.854

8.866
8.849
8.290
8.288
8.274
8.272
8.015
7.863
7.860
7.846
7.832
7.829
7.616
7.615
7.600
7.586
7.584
7.362
7.344
6.777
6.772
6.666
6.661
6.648
6.643

3.844
3.752

3.076
3.219

1.011
1.019

0.991
1.005
1.012
1.004
1.006
1.000

0.778

ppm

13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

55.601
55.947

99.377

105.126
105.698

121.944
125.167
125.229
127.216
127.705
129.340

133.242
134.486

141.980

155.117

160.902
161.040

166.317

3.7a

2.405

3.746

6.900
6.916
7.078
7.298
7.313
7.591
7.606
7.621
7.837
7.852
7.868
8.016
8.276
8.292

8.852
8.868

12.863

3.076

3.156

1.008
1.010
0.991
1.015
1.016
1.991
0.999

1.000

0.949

3.7b

21.257
55.777
105.727
113.168
121.127
125.160
125.233
126.345
127.245
127.690
128.413
133.269
134.462
140.452
141.652
153.840
160.860
166.288

3.7b

3.7c

3.7c

Peaks (rotated labels):
166.248
160.676
155.131
141.103
134.456
133.413
130.595
128.181
127.687
127.351
125.294
125.029
123.592
122.984
115.954
106.109
56.487

3.8e

16.074

31.402

40.407

52.149
52.276

61.833

80.031

90.687

123.438

129.761
132.424
134.288
135.161
135.378

167.051

171.847

3.8e

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

303

3.10

NMR spectrum (¹H).

Peak labels (ppm):
1.064
1.826, 1.840, 1.852, 1.865, 1.878
2.561, 2.575, 2.589
3.698, 3.703, 3.793, 3.805, 3.817, 3.868, 3.874, 3.993
7.161, 7.177, 7.346, 7.349, 7.362, 7.377, 7.390, 7.393, 7.396, 7.402, 7.408, 7.414, 7.423, 7.427, 7.439, 7.443, 7.674, 7.677, 7.690, 7.693, 8.014, 8.017

Integration values:
8.598, 2.154, 2.000, 3.193, 1.971, 3.133, 2.016, 1.053, 7.084, 3.964, 1.059

3.11

TBDPSO

16.074
19.413
26.997
31.628
40.430
52.156
52.260
62.462
79.705
91.263
123.699
127.796
129.707
129.736
132.367
133.943
134.301
135.223
135.712
167.109
171.877

3.11

TBDPSO

ppm

3.12

TBDPSO

308

15.146
18.838
26.671
30.860
62.015
79.741
90.727
127.913
129.848
132.764
133.034
133.125
134.072
134.462
135.029
136.275
167.594
172.221

TBDPSO

OH

O

OH

O

3.12

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

1.436

2.804
2.818
2.832

3.516
3.530
3.543
3.556
3.736
3.748

5.087

6.116

7.180
7.195
7.213
7.228
7.243
7.291
7.306
7.321

3.15b

9.207

2.018

1.955
2.000

0.845

0.917

4.247
1.887

ppm
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

3.15d

3.15d

171.862
156.587
155.865
134.585
133.070
123.842
117.769
80.124
65.635
54.509
40.481
37.690
37.265
32.199
29.646
28.459
27.965
25.359
22.925
22.610
22.457

3.16a

172.507

134.648

123.419

44.829

37.661
36.799

27.964
25.260
22.847
22.377

3.16a

ppm

3.16b

1.402

2.825
2.840
2.854

3.320
3.554
3.548
3.561
3.575

7.204
7.220
7.228
7.243
7.295
7.310
7.325

2.478
2.024
1.933
2.000
6.534

ppm

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

315

3.16c

22.513
22.938
22.973
25.396
28.030
29.899
34.814
36.948
37.859
40.861
55.228
63.982
123.559
126.973
129.441
129.622
129.803
133.695
134.081
134.793
156.353
174.808

ppm

3.16c

Cl

O

H
N

H
N

O

H₂N

3.16d

174.836

156.468

134.761
133.092

123.527

117.664

65.523

55.192

40.736
37.823
36.933
34.801

29.891
28.001
25.362
22.942
22.914
22.480

3.16d

1.513
1.523
1.535
1.590
1.602
1.613
1.624
1.899
1.955
2.125
2.138
2.152

3.336
3.349
3.361
3.374

3.753
3.846

4.074
4.084

5.455

6.064

6.524
6.529
6.541
6.546
6.569
6.574
6.842

7.166
7.183
7.507
7.521
7.535
7.551
7.705
7.719
7.736

8.218
8.234
8.460
8.476

4.050

3.836

1.898

2.027

2.952
2.909

1.914

1.039

1.074

0.999
1.032

1.033

1.097

1.982

1.060

1.030

1.000

3.17

22.397
22.877
25.304
27.978

37.522
37.552

43.569

55.785
55.950

99.819

104.738

112.817

122.362
123.986
124.777
126.249
127.612
128.744
129.275

133.080
134.242
134.384
135.720

155.541

161.405
162.041

166.834
168.438

3.17

1H NMR spectrum

1.510
1.521
1.532
1.587
1.599
1.610
1.622
1.895
1.951
2.120
2.133
2.147
2.409

3.329
3.343
3.354
3.368

3.761

4.069
4.079

5.450

6.083

6.826
6.838
7.127
7.144
7.508
7.518
7.534
7.550
7.705
7.719
7.733

8.220
8.236
8.461
8.477

3.18

Integrations: 4.013, 3.915, 1.910, 2.783, 1.914, 2.824, 1.913, 1.000, 1.034, 2.824, 1.031, 1.952, 1.055, 1.010, 1.000

ppm: 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

21.917
22.404
22.884
25.311
27.978
37.501
37.558
43.570
55.891
112.827
113.119
121.657
124.010
124.784
126.287
126.610
127.610
128.494
128.758
133.084
134.229
134.385
135.482
141.018
154.277
161.861
166.830
168.403

3.18

22.416
22.897
25.332
28.009
37.557
37.572
43.539
56.359
113.305
116.130
123.497
124.056
124.231
124.886
126.162
127.851
128.312
128.764
130.101
133.330
134.172
134.376
134.707
155.316
161.552
166.614
168.344

3.19

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

2.702
2.716
2.731

3.281
3.295
3.308
3.322

3.794
3.811
3.823

7.168
7.183
7.199
7.213
7.254
7.269
7.284
7.333
7.337
7.350
7.353
7.389
7.406
7.475
7.478
7.580
7.595
7.611
7.710
7.796
7.810
7.827
8.035
8.046
8.057
8.259
8.275
8.317
8.334
8.573
8.585
8.596

3.20

1.932

1.979

4.773

2.879
1.906
0.969
1.025
0.965
1.034
0.947
1.013

1.003

0.972
1.191

1.000

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

326

35.206

40.280
42.416

56.456

79.193

112.011

115.857

122.828
123.593
125.128
126.340
126.098
127.360
127.477
128.337
128.645
130.060
132.946
134.556
135.294

139.414

155.395

160.240

165.750

168.750

3.20

ppm 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

327

3.22

1.513
1.524
1.535
1.590
1.602
1.613
1.625
1.724
1.803
1.853
1.866
1.879
1.892
1.955
2.123
2.137
2.150
2.545
2.559
2.573

3.330
3.343
3.355
3.368
3.770
3.811
3.823
3.835
4.067
4.077

5.454

6.123

6.933
7.037
7.042
7.058
7.171
7.186
7.488
7.529
7.544
7.559
7.711
7.725
7.740
8.213
8.230
8.449
8.464

3.24

3.826
7.690
1.813
1.884
1.942
2.615
1.808
1.877
0.976
1.029
0.997
1.848
0.924
1.008
1.014
1.045
1.020
1.000

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

16.115
22.400
22.881
25.313
27.981
31.396
37.531
37.549
43.563
56.069
61.774
80.547
90.944
113.097
115.351
124.010
124.442
124.884
126.178
126.235
127.766
128.666
128.727
128.761
133.242
134.175
134.373
134.945
154.285
161.640
166.727
168.421

3.24

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

1.417
1.431
1.533
1.545
1.574
1.587
1.598
1.609
1.897
1.958
2.046
2.117
2.130
2.144

3.172
3.185
3.262
3.275
3.288
3.299
3.313
3.357
3.370
3.383
3.397
3.409
3.798
4.099
4.113
4.128
4.142
4.438
4.550
4.565
4.577
4.592
4.860

5.144
5.165
5.213
5.247
5.445

5.820

6.189

6.821
6.836
7.158
7.174
7.194
7.212
7.408
7.535
7.549
7.565
7.723
7.737
7.754

8.162
8.178
8.449
8.465

3.25

Integrations: 8.457, 2.239, 4.651, 2.000, 2.128, 1.178, 1.171, 3.037, 2.054, 1.259, 1.048, 1.016, 1.187, 1.136, 1.050, 1.048, 1.056, 2.992, 1.058, 1.111, 1.082, 1.086, 1.095

ppm: 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

334

22.417
22.623
22.888
25.336
27.963
29.707
32.560
37.498
37.636
40.344
53.418
56.347
65.563
113.598
116.118
117.706
123.948
124.243
124.817
126.128
127.830
128.315
128.755
130.093
132.909
133.333
134.183
134.365
134.419
155.342
156.602
161.505
166.299
171.288

3.25

22.432
22.566
22.944
25.361
26.940
28.101

32.616

37.672
37.970
39.435

53.183

56.389

113.041

116.055

123.434
124.003
124.253
124.733
126.040
127.871
128.285
128.790
130.159
133.321
134.141
134.547
134.839

155.342

161.448

166.772

171.441

3.26

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

15.322
31.491
59.420
79.403
91.351
122.072
131.006
132.820
133.028
134.098
136.228
167.599
172.240

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

339

8.229
8.147
8.143
8.128
7.962
7.957
7.945
7.940

4.303

3.023

1.095

1.942
1.000

ppm

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

340

169.529
161.802
160.192

137.856
132.757
131.663
127.526
121.234
119.522

99.384

65.380

9.074
9.054

8.169
8.061
7.943
7.883

7.371
7.274

3.969

2.922
2.064
3.937
1.000

ppm
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

342

162.830
160.343

149.103
145.906

137.435
136.666

131.348

126.981

123.799
122.769

118.666
117.623
117.423
117.203
115.055

91.661

56.886

175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm

343

22.331
22.807
25.260
27.909
37.458
37.471
43.331
56.297
111.608
116.085
121.936
124.050
124.099
124.209
126.833
127.488
127.917
129.852
131.177
132.897
134.241
134.943
136.318
155.111
160.254
166.094
168.193

ppm
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175

347

348

16.054
19.323

26.907

31.444

62.341
64.928

123.831
125.987
127.717
129.684
133.577
133.801
135.621
137.866

167.099

TBDPSO

OMe

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 ppm

349

350

352

353

354

355

Appendix D: Chapter 6 – NMR Spectra

6.8b

6.8b

33.512
36.182

76.823
77.077
77.331

83.647

119.920

130.285
131.510

139.826
140.008

ppm

358

1.253
1.255
1.264
1.266
1.276
1.288
1.326
1.337
1.556

2.045

2.913
2.924
2.936
2.947
2.959

3.561
3.572
3.582
3.593
3.604
4.104
4.116
4.128
4.140
4.167
4.178
4.189
4.200
4.211

6.990
7.002
7.154
7.162
7.183
7.201
7.215
7.228
7.239
7.260
7.466

19.391
3.093
0.686
0.680
1.021
1.013
0.468
2.029
2.076
1.111
3.140
2.057
0.999

6.24a

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

359

18.280

23.651
24.873
24.888

29.991

34.257

42.656

123.825
126.997
127.494
128.764
129.488
131.179

141.533

151.342
152.767
153.438

6.24a

ppm
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195

NMR peak labels (left side, rotated):

0.974
1.072
1.130
1.143
1.200
1.211
1.256
1.269
1.551

2.897
2.911
2.923

4.102
4.115
4.129
4.314

7.019
7.029
7.160
7.242
7.260
7.313
7.327
7.346
7.361
7.378
7.402
7.522
7.535
7.577
7.588

Integration labels (right side):
9.265
4.990
18.705
2.304
0.973
2.454
0.985
1.196
2.274
5.663
3.963

ppm axis: 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

6.24b

TBDPSO

361

19.143
21.607
23.630
24.774
24.841
26.862
29.923

34.251

69.128

123.847
127.661
127.690
128.791
129.810
131.086
133.311
133.740
134.847
135.719

151.425
151.650
153.408

ppm

6.24b

TBDPSO

6.24c

8.184

7.176
7.156
7.139
7.075
7.063
7.044
7.042
7.035
7.024
7.003
6.990
6.977

4.100
4.089
4.077
4.066
4.055
3.920
3.908
3.896
3.884

3.083
3.070
3.059
3.046
2.957
2.946
2.934
2.922
2.911
2.899
2.887

1.296
1.284
1.198
1.187
1.138
1.127

ppm

1.020
7.038
4.199
2.089
0.999
2.045
1.010
2.073
6.038
6.102
6.082

23.717
23.729
24.819
24.967
29.935
34.316
37.827
50.517
123.906
126.747
127.314
128.450
128.786
129.326
131.184
132.205
132.689
137.477
147.530
151.559
153.384

6.24c

6.24d

151.295

123.852

34.251
29.938
28.920
28.300
24.883
24.826
23.627

6.24d

ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

6.24e

0.719
0.912
0.924
1.231
1.243
1.259
1.264
1.270
1.275
1.538

2.045
2.059
2.070
2.082

2.866
2.878
2.889
2.901
2.912

4.159
4.170
4.181
4.193
4.204

7.042
7.054
7.154
7.278

8.910
3.049
18.550
1.041
0.999
2.039
0.989
2.026
1.110

ppm

10.0   9.5   9.0   8.5   8.0   7.5   7.0   6.5   6.0   5.5   5.0   4.5   4.0   3.5   3.0   2.5   2.0   1.5   1.0   0.5   0.0

6.24e

12.607

23.628
24.846
24.871
27.288
29.868
33.032
34.253

46.390

123.784

131.084

151.094
153.375
154.434

ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

368

6.2d

-0.001

1.654
1.681

2.330
2.339
2.427

2.753

3.982

7.107
7.117
7.277
7.297
7.314

7.777
7.793

8.320

12.078
2.298
1.151
3.449
2.838
2.949
0.967
2.053
2.017
1.000

ppm

21.664

27.863
28.470
28.757

38.810
40.698

76.856
77.110
77.313
77.565
79.721

127.913
129.631

135.228

144.180

154.012
154.813

178.191

ppm

6.2d

370

1.001
1.008
1.012
1.606
1.618
1.634
1.656
1.675
1.686
1.737
1.750
1.912
1.917
1.926
1.932
2.067
2.082
2.097
2.296
2.307
2.318
2.329
2.689
2.701
2.714
2.921
2.935
2.949
3.033
3.055

4.005
4.027

5.310
5.324
5.335
5.349
5.574
5.585
5.600
5.610

7.223
7.236
7.259
7.263
7.299

6.105
2.376
1.132
1.248
1.169
1.000
1.030
1.141
1.020
1.110
1.023
0.998
1.211
4.224

6.5

21.904
22.588
22.653

30.914
31.716

53.345

58.056

67.852

126.782
128.180
129.171

140.782

6.5

372

1H NMR spectrum

Peak list (ppm):
1.237, 1.239, 1.251, 1.260, 1.548, 1.562, 1.573, 1.583, 1.589, 1.593, 1.600, 1.604, 1.612, 1.618, 1.626, 1.639, 1.811, 1.818, 1.824, 1.827, 1.831, 1.838, 1.845, 1.852, 1.914, 1.919, 1.926, 1.931, 1.938, 1.946, 1.959, 2.037, 2.044, 2.050, 2.059, 2.067, 2.073, 2.081, 2.191, 2.203, 2.214, 2.225, 2.426, 2.440, 2.449, 2.454, 2.463, 2.477, 3.189, 3.201, 3.214, 3.225, 3.628, 3.715

5.275, 5.289, 5.301, 5.315, 5.436, 5.448, 5.462, 5.473

Integration values: 6.173, 2.581, 1.067, 1.075, 1.061, 0.999, 1.043, 0.999, 3.099, 3.074, 0.997, 0.998

Structure label: 6.7

MeOOC COOMe (cyclopentane with MeOOC, COOMe substituents and alkenyl side chain)

22.563
22.588
23.220

31.011
31.345
33.843

48.853
51.962
52.506

64.630

126.083

139.305

171.338
172.923

6.7

374

Bn
N

6.25a

1.361
1.367
1.375
1.381
1.585
1.655
1.667
1.679
1.737
1.936
1.949
2.070
2.087
2.105
2.123
2.140
2.756
2.772
2.789
2.931
3.020
3.046
3.057
3.082
3.473
3.487
3.501
3.516
3.961
3.987
4.032
4.058

5.420
5.437
5.451
5.467
5.487
5.772
5.787
5.802
5.818
5.832

7.186
7.201
7.214
7.228
7.246
7.277
7.292
7.300
7.309

1.609
4.225
1.069
1.305

1.166
1.035
1.017

1.193

1.259

1.000

1.128

2.259
2.990

ppm
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

21.282
21.556
22.009
22.022

31.720
31.777

42.008
42.176

53.406
53.429
58.216
58.257

67.687
67.723

77.096

126.102
126.122
126.771
126.811
127.257
127.277
128.177
128.224
128.457
128.493
128.828
129.131
129.156
131.312
131.388
137.824
137.885

146.011
146.083

6.25a

Bn
N

376

6.25b

Bn / N / OTBDPS (structure)

NBn

BocN

6.25d

ppm

22.166
28.573
28.611
46.394
53.116
57.615
57.776
58.262
66.751
66.804
78.479
127.043
128.490
128.817
128.872
132.359
140.060

ppm

6.25d

NBn

BocN

379

6.25e

0.970
1.275
1.323
1.323
1.609
1.613
1.618
1.622
1.626
1.637
1.646
1.651
1.655
1.660
1.665
1.671
1.675
1.680
1.685
1.696
1.732
1.738
1.745
1.751
1.765
1.912
1.917
1.924
1.928
1.933
1.945
1.954
1.958
1.965
1.968
1.980
1.995
2.072
2.078
2.087
2.092
2.101
2.727
2.741
2.755
2.908
2.920
2.933
2.936
3.016
3.032
3.037
3.053
4.024
4.046
4.058
4.079
5.319
5.326
5.333
5.340
5.345
5.351
5.359
5.365
5.544
5.559
5.572
5.586
5.598
5.612

7.222
7.230
7.258
7.287
7.294
7.298

9.341
3.834
0.865
2.235
1.185
2.226
1.121
1.040
1.091
0.985
1.034
1.000
1.025
1.265
4.232

ppm

380

6.25e

6.25f

22.142
22.154
22.575
22.724
25.000
25.171
26.168
26.197
31.868
31.949
34.594
34.633
35.321
35.447

53.293

56.805
56.871
58.315

67.427
68.436
68.642

90.502
90.661

126.842
126.865
128.236
129.029
129.094
129.945
130.009

137.528
137.561
139.473
139.502

ppm

NBn OMe

6.25f

383

6.28

21.926

29.803
31.793
32.984

53.442

58.106

67.950

76.816
77.070
77.324

126.718
127.063
128.158
129.146

139.754

144.590

6.28

6.9

6.9

0.897
0.921
1.077
1.099
1.121
1.152
1.160
1.184
1.209
1.249
1.255
1.274
1.280
1.299
1.396
1.408
1.540
1.552
1.560
1.573
1.637
1.683
1.711
1.719
1.733
1.917
1.930
1.938
1.951
1.965
1.977
1.986
2.342
2.507
2.519
2.527
2.540
2.594
2.605
2.615
2.626
2.643
2.653
2.663
2.672
2.682
2.691
2.700
2.709

5.272
5.290
5.303
5.321
5.417
5.431
5.448
5.462

7.144
7.162
7.177
7.243
7.260
7.273

3.381
2.923
2.609
4.346
7.586
2.198
2.110
2.853
2.304
1.112
1.145
3.000
2.846

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

6.12

24.907
26.102
26.258
26.454

33.039
33.320
33.435
34.433

40.815

50.645

67.622

76.819
77.013
77.327

125.614
126.060
128.272
128.534

140.283
142.831

6.12

389

6.13ab

390

14.160
14.173

22.522
22.627

31.155
32.853
34.241

42.626

57.545

61.161
61.347

119.527

125.927
130.251
130.294
131.327
131.389

140.911
141.513
141.773

166.076
168.193
168.397

6.13ab

6.15ab

6.15ab

393

6.16ab

6.16ab

22.706
22.737

31.033
31.791

37.549

51.169

59.726

76.818
77.071
77.325

119.399

126.865
128.269
128.430
129.234
130.244
131.355

140.703
140.802
141.378

ppm
195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

6.17ab

6.17ab

22.664
23.238
31.053
31.879
35.692
51.633
67.116
76.819
77.073
77.327
119.336
127.968
130.250
131.339
140.738
141.626

ppm
195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

1.532
1.537
1.543
1.549
1.554
1.560
1.565
1.571
1.577
1.582
1.587
1.593
1.639
1.642
1.649
1.652
1.659
1.665
1.674
1.680
1.690
1.888
1.894
1.902
1.906
1.909
1.911
1.916
1.919
1.923
1.933
1.942
2.290
2.301
2.312
2.323
2.334
2.343
2.453
2.463
2.469
2.476
2.480
2.487
2.492
2.503
2.557
2.567
2.574
2.583
2.590
2.597
2.607
2.645
2.654
2.660
2.668
2.675
2.689
5.257
5.259
5.272
5.274
5.283
5.284
5.299
5.421
5.432
5.447
5.458

7.035
7.048

7.359
7.373

6.18ab

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

5.883
2.058
4.255
1.097
1.097
2.929
2.953
1.127
1.077
1.000
1.007
2.061
2.053

6.18ab

22.766
22.884
24.891
26.465

31.209
32.361
34.216

50.561

67.189

76.835
77.089
77.343

119.300

125.367

130.337
131.284

141.554
141.720

ppm

195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

399

6.19ab

6.19ab

22.669
22.745

31.194
32.010
33.632

50.091

66.978
67.381

119.436

125.041

130.280
131.368

141.406
142.380

ppm

6.20cb

6.20cb

23.248
26.069
26.234
31.892
33.182
33.214
35.662
40.621
51.609
67.194
119.334
128.333
130.260
131.332
139.631
141.606

ppm

403

6.21ba

6.21ba

6.22db

6.22db

23.227

28.517
29.750
31.828
31.975
35.428
38.753

51.557

67.012

76.842
77.096
77.350
79.401

119.426

129.694
130.203
131.380

137.473

141.331

154.895

ppm

Appendix E: Chapter 7 – NMR Spectra

7.1a

409

7.1a

S11

410

7.3a

S16

21.990
22.078

31.448
31.734

53.297
53.328
56.855
56.997
62.023
67.175

76.821
77.075
77.329

116.999
117.069

134.549
134.894
136.069

7.3a

412

7.3c

S20

1.808
1.814
1.822
1.825
1.828
1.834
1.843
1.905
1.912
1.923
1.936
1.939
1.944
1.947
1.952
1.956
1.959
1.964
1.972
1.983
1.997
2.039
2.230
2.245
2.260
2.275
2.281
2.786
2.794
2.802
2.807
2.812
2.816
2.821
2.842
3.003
3.007
3.018
3.021
3.032
3.035
3.289
3.313
3.340
3.861
3.884
5.509
5.530
5.535
5.539
5.543
5.564
5.568
6.138
6.143
6.267
6.271
6.281
6.284
6.286
6.289
7.260
7.343
7.345
7.346

4.063
2.182
2.296
2.019
2.024
2.006
2.054
1.981
2.002
2.000
2.004
1.998

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

21.983

31.878

49.450
53.407

66.596
76.833
77.087
77.341

108.000
110.022

134.963

141.857

152.905

7.3c

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

S21

414

7.3d

S22

22.501
23.846
24.339
29.407
32.163
32.386

51.229

64.228
66.283

76.818
77.070
77.324

7.3d

0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

7.3e

-0.008

0.875
1.024
1.247
1.537
1.560
1.585
1.607
1.634
1.659
1.757
1.778
2.019
2.696
2.713
2.728
2.745
2.915

3.512

5.422

7.260

22.421
10.617
6.766

2.244
2.275

2.270

2.157

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

417

S24

23.698
29.642
29.683
29.712
33.392
37.022
40.039
40.123
45.996

57.952

76.823
77.076
77.330

7.3e

S25

7.12

S30

419

22.940
31.827
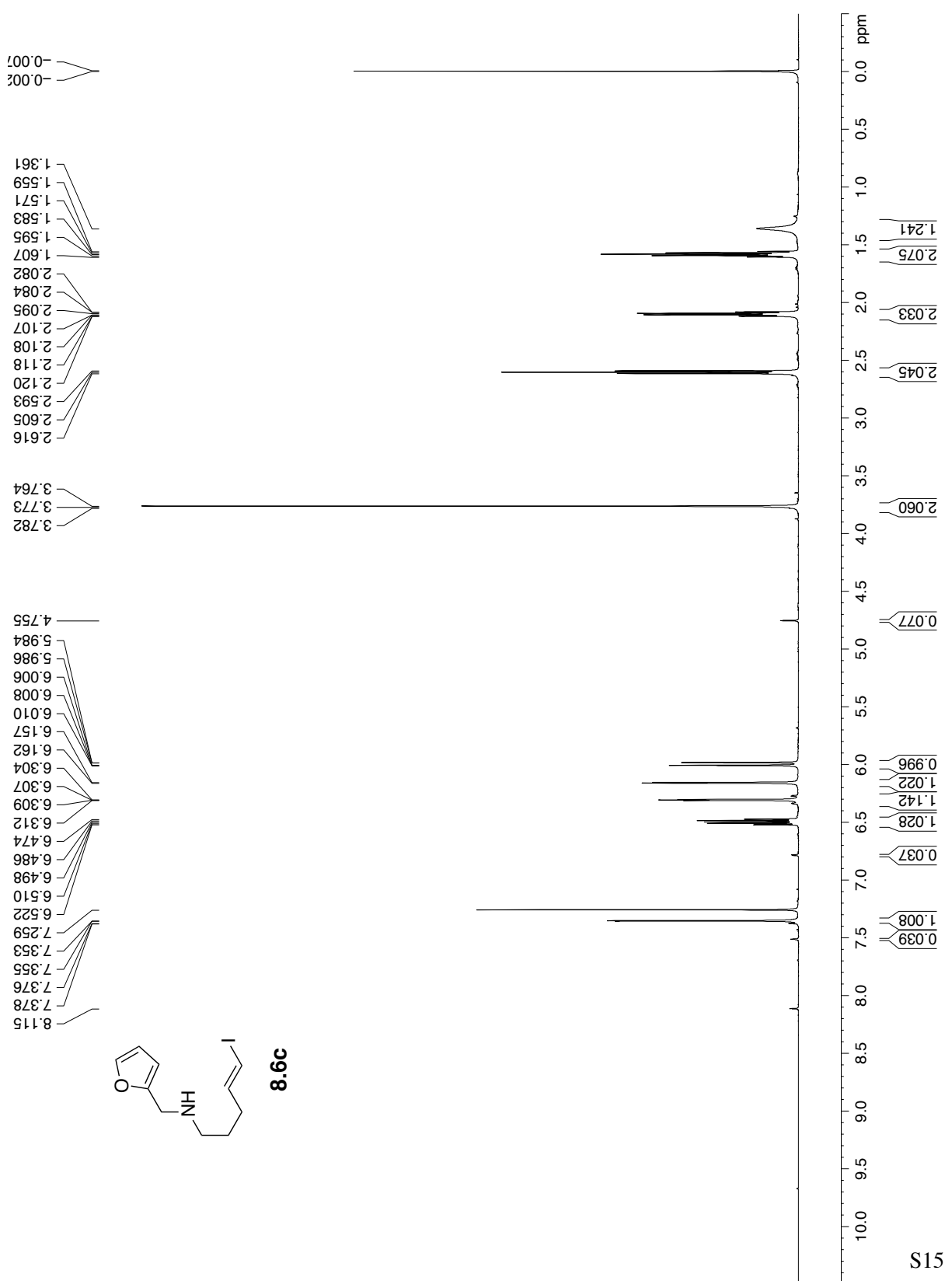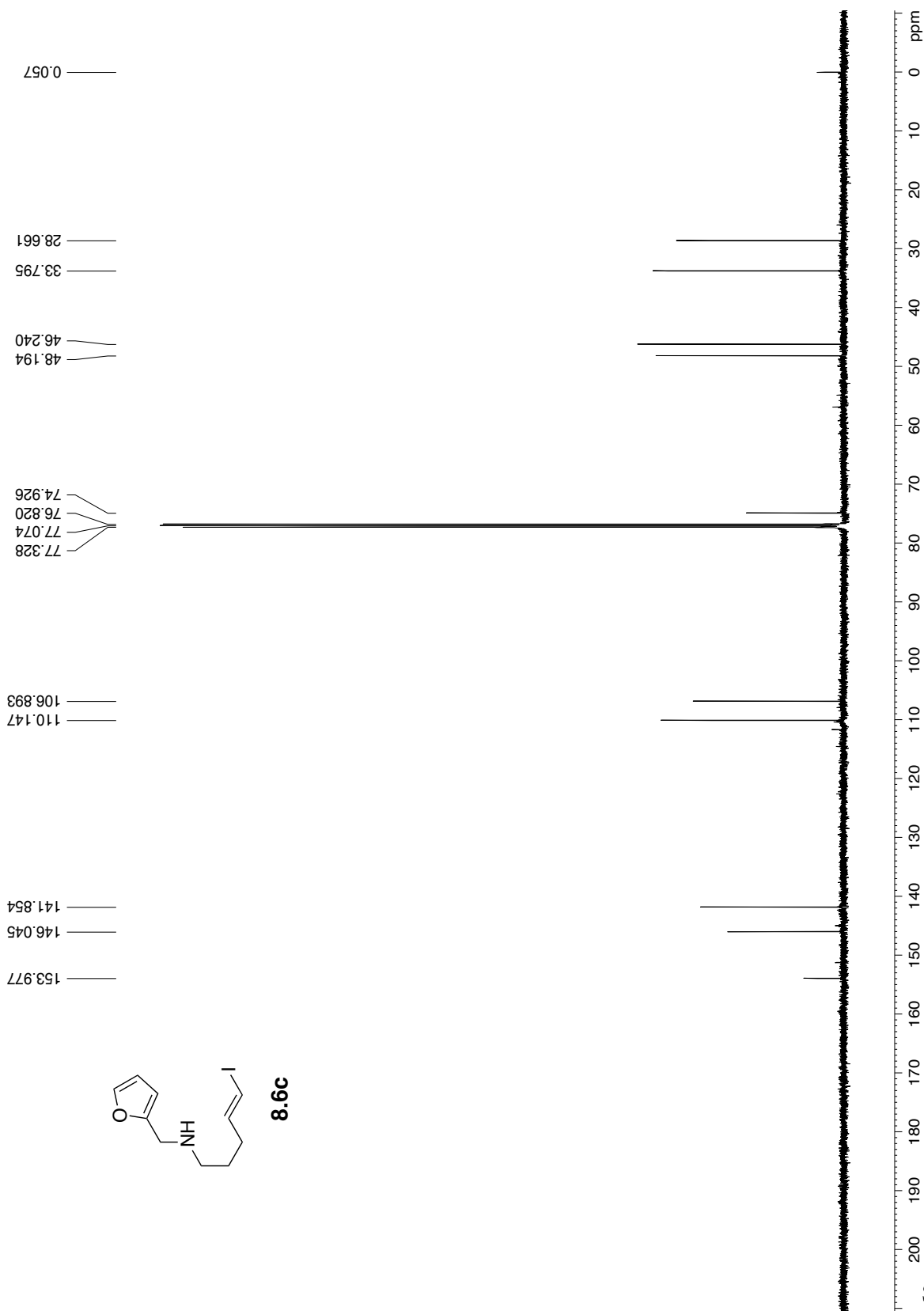33.529
39.485
39.652
39.819
39.986
40.153
40.319
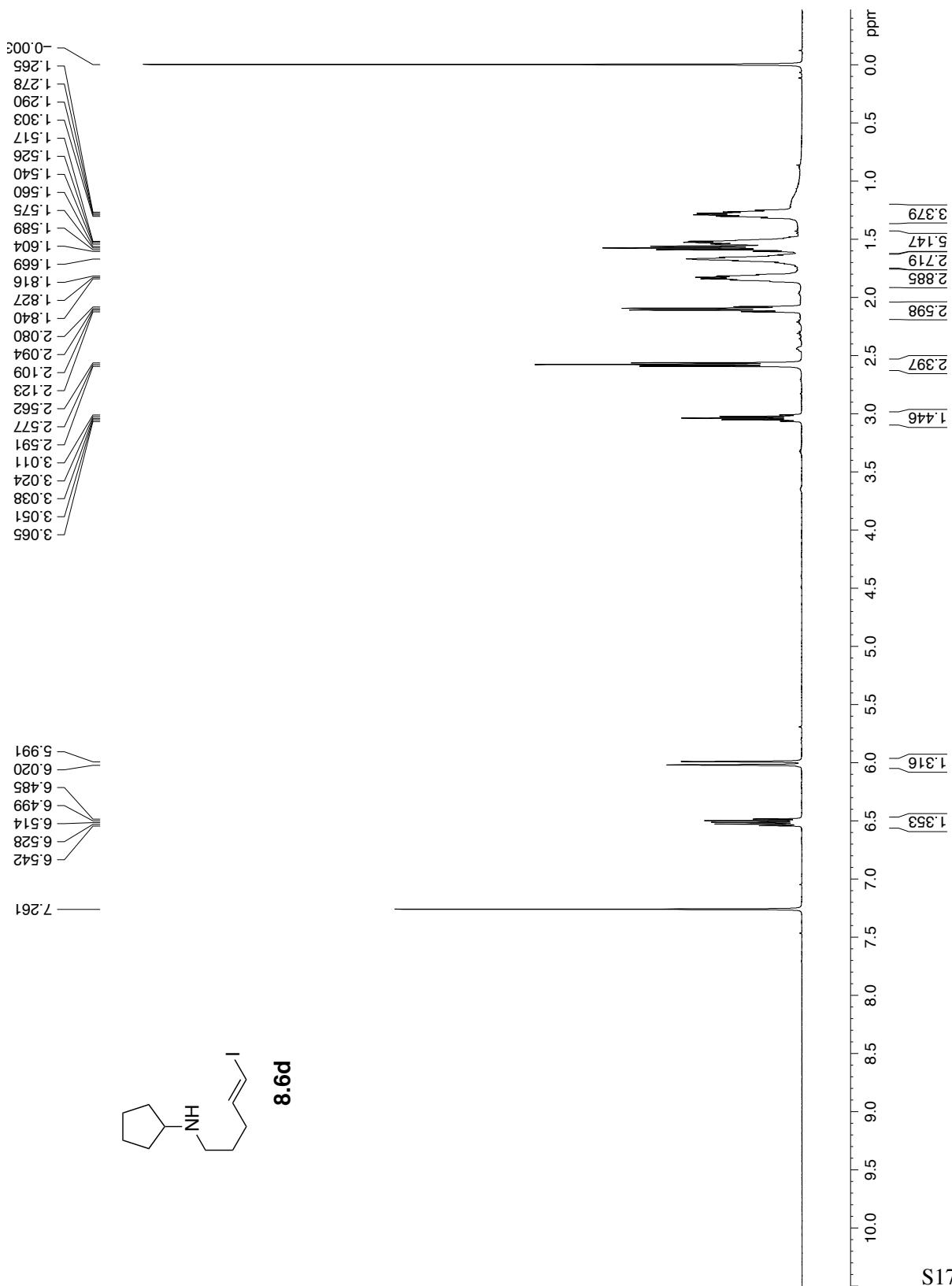40.486

131.137

7.12

COOH
HOOC
COOH
COOH

S31

420

Appendix F: Chapter 8 – NMR Spectra

8.12

S11

422

This is a ¹³C NMR spectrum. The peaks are labeled:

- 0.055
- 27.837
- 31.945
- 37.500
- 68.644
- 76.426
- 76.823
- 77.076
- 77.331
- 144.193

Structure label: **8.12**

Chemical structure: MsO—CH₂CH₂CH=CH—CH₂—I (drawn with MsO group and terminal I)

Inset expansion showing peaks: 76.426, 76.823, 77.076, 77.331 over the range 75.0–78.5 ppm.

x-axis: ppm (0–180)

S12

423

S15

8.6c

−0.007
−0.002

1.361
1.559
1.571
1.583
1.595
1.607
2.082
2.084
2.095
2.107
2.108
2.118
2.120
2.593
2.605
2.616

3.764
3.773
3.782

4.755
5.984
5.986
6.006
6.008
6.010
6.157
6.162
6.304
6.307
6.309
6.312
6.474
6.486
6.498
6.510
6.522
7.259
7.353
7.355
7.376
7.378
8.115

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

1.241
2.075
2.033
2.045
2.060
0.077
0.996
1.022
1.142
1.028
0.037
1.008
0.039

This is a ¹³C NMR spectrum with the following peak labels:

0.057

28.661
33.795

46.240
48.194

74.926
76.820
77.074
77.328

106.893
110.147

141.854
146.045

153.977

8.6c

ppm scale: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200

S16

425

7.261

6.542
6.528
6.514
6.499
6.485
6.020
5.991

3.065
3.051
3.038
3.024
3.011
2.591
2.577
2.562
2.123
2.109
2.094
2.080
1.840
1.827
1.816
1.699
1.604
1.589
1.575
1.560
1.540
1.526
1.517
1.303
1.290
1.278
1.265
-0.003

8.6d

3.379
3.375
5.147
2.719
2.885
2.598
2.397
1.446
1.316
1.353

ppm

8.6d

0.054

24.108
29.108
33.309
34.038

47.935

59.954

74.772
76.817
77.071
77.325

146.207

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

S18

427

8.6e

-0.002

1.253
1.529
1.543
1.558
1.589
1.614
1.655
1.679
2.063
2.089
2.103
2.118
2.558
2.573
2.587

5.990
6.019
6.483
6.497
6.512
6.526

7.260

1.472
1.939
11.806
2.836
2.061
2.010
0.980
0.996

ppm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0

8.6e

0.052

29.631
29.790
34.068
36.795
39.630
42.837

74.776
76.814
77.068
77.322

146.229

ppm

42 41 40 39 38 37 36 35 34 33 32 31 ppm

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

S20

8.10

EtO₂C
EtO₂C

S21

8.10

S22

431

1.692
1.697
1.706
1.710
1.718
1.731
1.733
1.741
1.755
1.767
1.780
1.881
1.885
1.891
1.895
1.899
1.903
1.907
1.911
1.915
1.919
1.923
1.927
1.938
2.032
2.046
2.061
2.075
2.316
2.752
2.796
2.865
2.869
2.882
2.894
2.897
3.077
3.098
3.119
3.357
3.724
3.773
4.247
4.254
4.275
4.296
5.124
6.348
6.353
6.358
6.362
6.374
6.378
6.383
6.388
7.221
7.233
7.245
7.260
7.298
7.310
7.323
7.334
7.354
7.366

8.9a

ppm

0.441

0.987
1.078
0.997
1.044

4.489
2.096
1.069

0.074

0.990
1.002
1.047
1.032

1.039

1.949

1.106
2.329
2.149

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

S23

432

gnoe

8.9a

8.9a

21.564
25.267
30.055
30.067
30.818
36.382
39.091
41.600
41.613
41.803
41.818
54.139
59.127

67.956

76.814
77.067
77.322

126.711
128.186
128.198
128.946
128.959
140.795
140.807
140.976
140.987

0.055

ppm

S25

8.9b

8.9b

21.614
25.391
29.942
30.858
36.248
39.111
41.613
41.806
54.066
56.756
67.439
76.849
77.103
77.357
126.308
127.313
128.176
128.581
131.806
137.315
140.812
140.993

ppm

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

S27

436

8.9c

S28

437

8.9c

21.573
25.235
29.757
30.811
36.126
39.099
41.598
41.822
50.008
53.774
66.795
76.847
77.101
77.355
107.905
110.000
140.814
140.824
140.979
140.996
141.838
153.172

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

438

8.9d

S30

439

8.9d

21.937
24.471
24.621
26.162
27.481
30.273
31.086
31.708
39.044
41.674
41.831
49.609
62.358
76.832
77.086
77.341
140.760
140.955

ppm
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220

S31

440

8.9e

S32

441

8.9e

24.462
28.211
29.646
29.783
30.325
32.118
36.959
38.951
40.115
41.036
41.823
42.108
46.377
58.013

76.847
77.101
77.355

140.800
140.895

ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

S33

442

8.9f

8.9e

+

0.974
1.000
1.146
1.162
1.245
1.307
1.376
1.501
1.579
1.613
1.637
1.728
1.751
1.960
2.003
2.053
2.112
2.126
2.140
2.213
2.337
2.401
2.418
2.570
2.584
2.598
2.635
2.650
2.749
2.793
2.825
2.969
5.078
5.096
5.109
5.126
5.350
5.364
5.379
5.395
5.409
6.039
6.342
6.356
7.260

ppm

0.856
1.121
0.350
1.676
1.625
0.880
0.681
0.664
0.915
13.322
1.717
0.819
0.300
0.639
3.696
1.270
0.701
0.511
0.689
0.199
1.728
0.691
1.197
1.187
0.490
0.222
0.145
0.212
0.584
0.679
0.755
2.000

S34

8.11

23.259
28.180
29.710
31.592
34.323
34.441
38.794
41.731
48.330
52.130
52.568
63.746
76.833
77.087
77.341
140.685
140.749
171.679
173.226

8.11

ppm

8.7a

1.575
1.587
1.599
1.613
1.618
1.623
1.629
1.638
1.647
1.653
1.657
1.661
1.674
1.694
1.703
1.711
1.717
1.730
1.745
1.771
1.784
1.808
1.853
1.868
1.882
1.894
1.902
1.906
1.923
2.002
2.020
2.037
2.054
2.239
2.271
2.844
2.848
2.863
2.878
3.039
3.065
3.356
3.414
4.299
4.325
7.213
7.228
7.242
7.260
7.290
7.304
7.319
7.343
7.357

1.050
2.023
1.053
1.094
2.866
2.413
4.565
1.220
1.131
2.118
1.020
1.019
1.002
1.044
4.220

ppm
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

446

8.7a

17.552
18.652
21.542
23.543
28.739
29.668
29.797
30.985
35.797
35.984

54.129

59.084

68.016

76.827
77.081
77.335

126.685
128.177
128.991

ppm 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

S38

447