Multistage Antimalarial Inhibitors From Diversity-Oriented Synthesis and C-H Activation

Multistage antimalarial inhibitors from diversity-oriented synthesis and C-H activation

A dissertation presented

by

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to

The Department of Chemistry & Chemical Biology

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the subject of

Chemistry

Harvard University

Cambridge, Massachusetts

September 2017

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Abstract

The malaria parasite has three distinct life cycle stages in humans, yet most antimalarial drugs only target one of those stages: the symptomatic blood stage. Additionally, the emergence of parasite resistance has rendered many antimalarials ineffective in certain geographical contexts, underscoring the need for new, multistage antimalarials with novel mechanisms of action. This dissertation introduces three such compounds.

First, a series of spirocycles targeting PI(4)K with activity against three parasite stages is briefly described.

Next, a series of azetidine-2-carbonitriles targeting dihydroorotate dehydrogenase (DHODH) is described. This compound family displays activity against liver and blood stages of the *Plasmodium* life cycle. Analysis of structure-activity relationships identified BRD1331, which demonstrates excellent biochemical selectivity, potency against patient-derived strains of *P. falciparum* and *P. vivax*, and robust parasite clearance in two *in vivo* mouse models.

Finally, a series of bicyclic azetidines targeting phenylalanyl tRNA synthetase with activity against three parasite stages is introduced. Following a retrosynthetic analysis, a Pd-catalyzed C(sp^3)-H arylation of azetidines at the C3 position is described. This methodology exhibits broad substrate scope and provides C3-arylated azetidines, pyrrolidines, and piperidines. Application of this reaction gave access to valuable, stereochemically defined building blocks, enabling an efficient synthesis of antimalarial compound BRD3914. BRD3914 was evaluated in *P. falciparum*-infected mice, providing a cure after four oral doses.

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List of Abbreviations

AcCl	acetyl chloride
CQ	chloroquine
DBU	diazabicycloundecene
DCM	dichloromethane
DHODH	dihydroorotate dehydrogenase
DIPEA	*N,N*-Diisopropylethylamine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DOS	diversity-oriented synthesis
dppa	diphenylphosphoryl azide
EEF	exo-erythrocytic form
eEF2	translation elongation factor 2
EtOAc	ethyl acetate
EtOH	ethanol
EMA	European Medicines Agency
*Pf*CRT	*P. falciparum* chloroquine resistance transporter protein
FMN	flavin mononucleotide
FY	fiscal year
G6PD	glucose-6-phosphate dehydrogenase
HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HF	hydrofluoric acid

ITNs	insecticide-treated mosquito nets
IV	intravenous
IVIS	*in vivo* imaging system
KHMDS	potassium bis(trimethylsilyl)amide
LiHMDS	lithium bis(trimethylsilyl)amide
MeCN	acetonitrile
MEM	2-methoxyethoxymethyl ether
MeOH	methanol
MMV	Medicines for Malaria Ventures
MOM	methoxymethyl acetal
MsCl	methanesulfonylchloride
N-TFA	*N*-trifluoroacetamide
PK	pharmacokinetic
PMB	4-methoxybenzyl ether
PO	*per os* (Latin); oral dosing
RBCs	red blood cells
SAR	structure-activity relationships
SNP	single nucleotide polymorphism
SSAR	stereochemistry-based structure-activity relationships
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMDS	tetramethyldisiloxane
U.S.	United States

Acknowledgments

I was told once that the only part of my thesis that people will ever read is the Acknowledgments section. Irrespective of how much truth lies in that statement, I want to recognize and reflect on all of the people who helped me through this chapter of my education.

First and foremost, I'm grateful for my advisor, Stuart Schreiber, for enthusiastically welcoming me into his research group. The opportunity to work on such an exciting set of projects and the freedom to pursue my own scientific ideas was a blessing, and I can't imagine experiencing the frustrations and the exhilarations of science during my Ph.D. anywhere else. Stuart's ability to spark excitement and provide advice during the most difficult of experiments is second to none, and I'll always be appreciative of him taking time out of his busy schedule to talk about science, manuscripts, or life. There were no questions I was too afraid to ask, and Stuart's unwavering scientific and personal support were invaluable. From Stuart, I learned to question everything and be open to new ideas, and I'll carry this way of thinking for the rest of my career.

I would like to thank my committee members Dan Kahne and Emily Balskus for taking time out of their schedules to provide feedback and constructive criticism on my research. They were also willing and able to trek to the Broad for my committee meetings – efforts for which I am cognizant and appreciative – when they undoubtedly had more important things to do. Dan's looming philosophical questions and Emily's encouragement to think of unique solutions during my committee meetings helped shape the direction of my research projects, and I am grateful to have had their support.

My undergraduate research advisor at Stanford, Paul Wender, set the groundwork for my success in graduate school. Paul challenged me to be creative in solving problems and held me to the standards of one of his own graduate students, even if I wasn't ready for it. He deserves a lot

of credit for my work ethic and ability to think scientifically, and I have acquired hints of his thought processes along the way. Two former Wender group Ph.D. students, Lauren Sirois and Erika Geihe Stanzl, were instrumental during my undergraduate career with Paul. Lauren drilled lab techniques and taught me the basics of organometallic chemistry while making sure I didn't hurt myself (I did anyway). When she graduated, Erika encouraged me to think independently and analyze scientific literature critically in preparation for graduate school. Erika was always a fountain of useful and honest advice, and I look back to my undergraduate days thinking, "You were absolutely right." Without both of them, I certainly would not be writing this today.

I've had the privilege of working collaboratively on every project in this thesis. I'm grateful to Nobu Kato, an expert parasitologist with whom I worked with on all three projects I describe. Eamon Comer and Partha Nag welcomed and engaged in fruitful discussions with me around infectious disease, chemistry, and life. There was also a lot of project support for the malaria group, and I wouldn't have been able to push the work in Chapter 3 as far without the help of Christina Scherer, James Duffy, and Marshall Morningstar. My deskmates Jochen Zoller, Bruno Melillo, and Oscar Verho were also amazing and selfless coworkers that helped expedite the C-H activation efforts (Chapter 4). I learned a lot of chemistry from them, and I miss our discussions and sports outings already.

Frances Neville deserves special praise for holding the group (and Stuart's schedule) together like no other. Since she left, Allison Roach has done a tremendous job.

Perhaps the best thing about coming to Harvard was the tremendous amount of surrounding knowledge. When I first joined the Schreiber group, Leslie Aldrich, Max Majireck, Stephen Scully, and Zarko Boskovic entertained my petty "first year graduate student" questions and were patient in teaching me new approaches to synthesis. I also found myself speaking often to Jason

Law – my freshman year RA in college, my labmate in the Wender group, and my labmate in the Schreiber group. I valued his experience and opinions, and I've never met someone so energized by science (except maybe Stuart). Brinton Seashore-Ludlow taught me mechanisms I didn't know, and Shingo Yamasaki imparted valuable advice he learned from industry.

My classmates Bennett Meier, Enrique Garcia-Rivera, and Steven Poynter made life in the group fun. Bennett introduced me to new restaurants in Boston and the future of virtual reality. Late nights in lab were always entertaining with Enrique and Steven (and Sebastian Stark). Endless amounts of espresso, beer, and shenanigans make for a lot of excellent stories we'll recount in the future. I also miss strange conversations with Norah Liang, though it made me empathize with how Lauren felt when she was training me.

Some of the younger graduate students and friends in the group – Shawn Nelson Jr., Bruce Hua, and Chris Gerry – are now close to finishing, and it's crazy to think about. Chris' hood was directly next to my hood, and while I'll miss talking about sports with him on a daily basis, I won't miss losing money to him betting on football. At the risk of forgetting someone, I would also like to mention the group members that I had the extreme pleasure of working with: Sixun Chen, Matthias Leiendecker, Drew Adams, Giannina Schaefer, Stephanie Wang, Michelle Stewart, Matt Rees, Liza Leshchiner, Hiroshi Uehara, Josh Paulk, Fumika Yakushiji, Mengfei Zhang, Vasanthi Viswanathan, Jake Eaton, Denise Field, Tanaz Sharifnia, Elaine Oberlick, Anneke Kramm, Eric Minikel, Sonia Vallabh, Cristy Nonato, Emily Ricq, and Jannik Borghs.

One of my favorite parts of graduate school was meeting lifelong friends and scientists outside of the lab. I couldn't go through a weekend without my beer-drinking buddies Nicolas Sawaya, David Lee, and Fred Rubino, and we had hilariously fun times in Montreal and Chicago. Back when I did trivia on a weekly basis, Jack Nicoludis, Abraham Waldman, and Nitzan Koppel

got every pop culture question, and Chinatown lunches with Samantha Wellington and Angela She were something I always looked forward to. There was no better person to argue with about basketball or learn more about board games from than Sam Blau, and with him and Jeff Bessen, Pokemon Go was a much-needed escape from lab.

My closest college friends – Simon Ye, Alex Churchill, Manoli Liodakis, Ross Darwin, Dan Huang, Miriam Connor, Jane LePham, Amber Tariq, and Paul Iona – all kept in touch when I thought I was too busy, and each of them made visits to me in Boston. Simon and Manoli's eventual moves to the East Coast (Boston and New York, respectively) made for a lot of time spent outside of lab, and I enjoyed quick trips to visit Emilio Lopez and Eliana Arredondo. Two friends from home – Nathan Nakatsuka and Nolan Kamitaki – were always available to talk about science (and only science, because it's them) despite their commitment to their own research projects.

I also want to thank John Flygare, Sam Kintz, Joseph Barchi Jr., Robert Cooney, and Gail "Miss Ish" Ishimoto – all of whom made substantial contributions to my development as a scientist. In particular, Miss Ish encouraged me to pursue research when I didn't even know what it entailed, and I haven't looked back since.

Finally, I would not be here without the love and support of my wonderful family. Boston is a long way from Hawaii (a 13 hour flight with one connection and a five- or six-hour difference in time zone, but who's counting?), and my parents Mark and Pamela Maetani were somehow okay with me living that far from home to finish my Ph.D. I am forever indebted to them. The support from my brother Matt, my grandparents, and all of my cousins, aunts, and uncles made going home every December worth it. And to Enya – thanks for putting up with all nights and weekends spent in lab, and for all the long bus rides to/from New York. I wouldn't have it any other way.

Chapter 1

An Overview of Malaria & Strategies Toward New Medicines

1.1 Introduction

1.1.1 The epidemiology of malaria

Malaria is a deadly infectious disease caused by protozoan parasites of the genus *Plasmodium* and transmitted to humans by female *Anopheles spp.* mosquitoes.[1] There are four main *Plasmodium* species causing disease in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. *Plasmodium ovale* was recently shown to consist genetically of two subspecies, *P. ovale curtisi* and *P. ovale wallikeri*.[2] A fifth species, *Plasmodium knowlesi*, occasionally infects humans but primarily infects macaques.[3] This thesis will focus primarily on the two strains that pose the largest health consequences to humans (and thus are of significant interest to the broader scientific community), *P. falciparum* and *P. vivax*.

Malaria is transmitted in 97 countries worldwide. At the start of 2016, nearly half of the world's population was at risk of the disease (i.e. live in countries endemic for malaria), and the World Health Organization[4] estimates the 2016 global tally as 212 million cases and 429,000 deaths. Malaria disproportionally affects pregnant women and children, or individuals with

compromised or immature immune systems, and 85% of deaths occur in children under the age of 5 years.[1]

Notably, most malaria-associated deaths (90%) occur in sub-Saharan Africa for two complementary reasons: (1) the most effective malaria vector, *Anopheles gambiae*, is widespread in Africa and is difficult to control; and (2) the most infectious *Plasmodium* strain, *P. falciparum*, is widespread in Africa.[5] Fortunately, malaria mortality is changing. Since 2000, the malaria death rate in sub-Saharan Africa has declined by more than 57%,[6] largely due to the distribution of effective medications, increased malaria research and awareness worldwide, and improved vector control. For example, United States (U.S.) government funding for malaria, which includes malaria control efforts and research activities, increased from $146 million fiscal year (FY) 2001 to $861 in FY 2016[7] and does not include private funding (e.g. The Bill & Melinda Gates Foundation). In sub-Saharan Africa, it is estimated that 53% of the at-risk population slept under insecticide-treated mosquito nets (ITNs) in 2015, an increase from 5% in 2005.[4]

1.1.2 The life cycle of *Plasmodium* parasites

Plasmodium spp. have a complex life cycle that alternates between female *Anopheles* mosquitoes (the vector) and vertebrates (the host), both of which are necessary for progression of the disease (**Figure 1.1**). Broadly speaking, the parasites undergo three major stages in humans – the liver stage, the blood stage, and the gametocyte stage.

During a mosquito blood feed, *Plasmodium* sporozoites are injected into the host dermis, where they use gliding motility to reach and penetrate blood vessels to enter a bloodstream. The process from injection into the dermis to penetration of a blood vessel occurs in 1-3 hours, and

sporozoites that fail to reach the bloodstream are eventually destroyed by host immune response.[8]

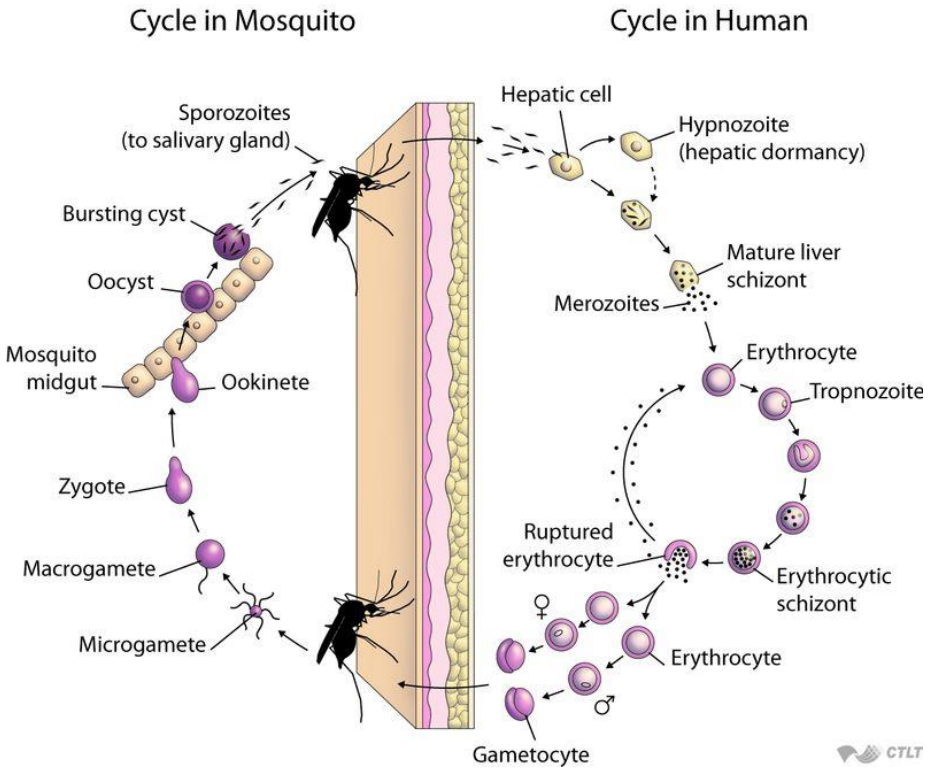

Figure 1.1. Schematic of the life cycle of *Plasmodium* parasites.[9]

Upon entrance into the bloodstream, sporozoites are transported to the liver, where they exit through the sinusoids and actively invade hepatic cells. The parasites transform over the next 2-16 days to an exo-erythrocytic form (EEF) that results in the development of up to 40,000 merozoites per cell. *Plasmodium* merozoites are released by the formation of merozoite-filled vesicles (merosomes), which bud off from the infected hepatocytes, enter the bloodstream, and eventually burst.[10] While this generally describes the "liver stage" phase for most *Plasmodium* species, a small population of *P. vivax* and *P. ovale* sporozoites (called hypnozoites) will remain dormant in hepatocytes and re-emerge months or years after initial infection. The reactivation

mechanism is poorly understood, and hypnozoites cannot be detected using currently available diagnostic tools.[11] Hypnozoites are problematic in malaria eradication efforts as infected individuals can unknowingly carry dormant parasites that, upon reactivation, introduce *P. vivax* or *P. ovale* to new geographical regions.

Once in the bloodstream, free merozoites attach to and invade erythrocytes, a process that is completed within two minutes.[12] Notably, *Plasmodium* strains infect and proliferate in different erythroid cell types. *P. vivax* exclusively invades reticulocytes, which represent only 1% of all red blood cells (RBCs) in circulation, whereas *P. falciparum* infects and grows in both reticulocytes and mature erythrocytes. This is thought to underlie the difference in virulence between *P. falciparum* and *P. vivax*, and thus why *P. falciparum* is responsible for most mortality associated with the disease.[13,14] Upon invasion, parasites then undergo a chronic 48 hour cycle of DNA replication, schizogeny, and merozoite re-invasion of a naïve erythrocyte. This process, termed the "blood stage" or "asexual blood stage", produces 8-32 new merozoites per red blood cell and can persist indefinitely in the absence of treatment.[1] This cycle of red blood cell rupture and subsequent re-invasion leads to many of the symptoms associated with the disease.

In a process that occurs simultaneously during the blood stage, a small population of blood stage parasites undergo gametocytogenesis, or sexual differentiation, via expression of *Pf*AP2-G.[15,16] Parasites mature to what is often referred to as the "gametocyte stage" or the "sexual blood stage." Secreted parasitic growth factors are thought to induce this transcriptional cascade,[17,18] and within days (15 for *P. falciparum*, 4 for *P. vivax*, respectively) the parasites sequester in the bone marrow, develop into stage V male or female gametocytes, and re-enter

peripheral circulation. Gametocytes are asymptomatic, non-replicating forms of the parasite that can persist in circulation for weeks.[1]

Within seconds of ingestion by a female mosquito during a blood meal, the gametocytes undergo gametogenesis, which is induced by a combination of (1) a rise in pH from 7.4 to 8.0 to 8.2; (2) a 5 °C drop in temperature from the host to the mosquito midgut; and (3) the presence of xanthurenic acid.[19] The male and female gametocytes differentiate into male and female gametes, respectively, which fuse in the mosquito midgut to form a zygote. Importantly, a single mosquito must ingest both male and female gametocytes for this process to occur, else the zygote will not form and propagation will not occur. Upon formation, the zygote undergoes meiosis, and within 24 hours transforms into an ookinete that migrates through the midgut epithelium and eventually forms an oocyst. In the oocyst, DNA replication produces thousands of sporozoites before oocyst rupture, and sporozoites migrate to the salivary glands where they can be injected into the next human host for the next round of the parasite life cycle.[1,8]

1.2 Therapeutic Strategies Toward Eradication of Malaria

1.2.1 Motivation

Malaria exacts an enormous toll on human health. Most deaths due to malaria occur in young children in sub-Saharan Africa, and very few people in these areas have access to resources or intensive care units to properly treat the disease. Those who do not succumb to malaria can face life-long complications such as intellectual disabilities directly as a result of the disease.[20,21] Additionally, malaria imposes heavy social and economic burdens in developing countries – between 1965 and 1990, countries with a large proportion of the population living in regions with *P. falciparum* experienced average growth in per-capita GDP of 0.4% per year

compared to 2.3% per year for other countries that did not have a large portion of the population living in infected areas.[22]

The development of diagnostics and the adoption of preventative measures such as ITNs are necessary and life-saving components in the fight against malaria. However, they are not sufficient if eradication of the disease is desired. The development of new therapeutics in the form of vaccines or drugs has shown in the past to play a critical role in these efforts.

1.2.2 Vaccine development

Malaria vaccination has been an interest to the scientific community since the 1960s, when it was determined that mice could attain protective immunity from malaria upon injection of irradiated sporozoites.[23] Studies in humans showed that volunteers could have a high level of protection against the parasite but required large numbers of bites by irradiated infectious mosquitos.[24] This led to enormous interest in the field, resulting in the identification of a numerous candidate antigens. A peptide-based candidate called SPf66 was the first vaccine to enter clinical trials but failed to demonstrate protection against the disease during field efficacy trials in Africa and in Asia.[25,26]

The most successful malaria vaccine to date, RTS,S (Mosquirix), was approved by the European Medicines Agency (EMA) in 2015 as the world's first licensed malaria vaccine. RTS,S consists of hepatitis B surface antigen virus-like particles that incorporate a portion of *P. falciparum*-derived circumsporozoite protein and a liposome-based adjuvant.[27] However, despite EMA approval, the World Health Organization did not recommend inclusion of the vaccine in the Expanded Programme of Immunisations due to declining efficacy in clinical trials.

Notably, over 15,000 infants and young children were enrolled in clinical trials in seven countries in sub-Saharan Africa to assess RTS,S efficacy. While the vaccine is estimated to have rates of protection of up to 45% among children 5 to 17 months of age and 27% for infants 6 to 12 weeks of age, efficacy is linked to number of vaccine doses and confers highest efficacy directly after vaccination. Unfortunately, efficacy wanes significantly over time: after seven years, the rate of protection drops to nearly 3%.[27,28] The underwhelming efficacy is partially linked to the epitope region of the circumsporozoite protein, where post-hoc analyses revealed significant polymorphisms on field-isolated parasites that are critical determinants of vaccine efficacy.[29,30] Additionally, the dose required to vaccinate adults is poorly established. The lack of long-term protective effects for RTS,S significantly devalues the vaccine, and without strong endorsement from the World Health Organization, it is unlikely that RTS,S will be widely adopted.[31] The development of new, efficacious vaccines is desperately needed and will play a key role in malaria elimination.

1.2.3 A brief history of small molecule therapeutics

Chemotherapy, primary in the form of herbal medicinal products, has been used to treat malaria for centuries. Quinine was the first natural product used in antimalarial chemotherapy and was isolated and identified from cinchona tree bark in 1820 (**Figure 1.2**). Attempts to synthesize quinine and analogues led to the development of methylene blue in 1876, which was fully synthetic and lacked supply problems that plagued quinine but was also less effective in antimalarial trials.[32]

Quinine and methylene blue became standard antimalarial treatments until the late 1920s, when Bayer initiated a campaign to the improve efficacy of these compounds. Quinacrine,

synthesized in 1931, became one of the most successful antimalarial drugs for nearly 15 years thereafter despite the discovery of chloroquine in 1934.[33] Chloroquine was initially rejected as being too toxic for human use but garnered significant interest after the United States government sponsored clinical trials for antimalarial drug development during World War II. In 1947, chloroquine was introduced into clinical practice as an antimalarial agent.[34]

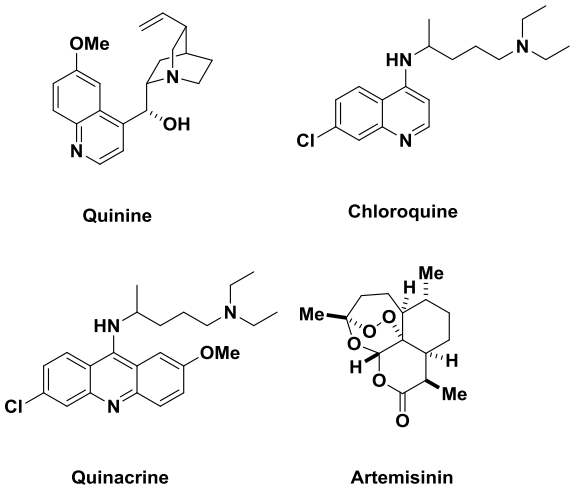

Figure 1.2. Key compounds in the history of antimalarial drug discovery.

Chloroquine is considered to be one of the most important drugs in the history of modern medicine – estimates suggest that it has saved hundreds of millions of lives since its inception. Unfortunately, within ten years after the wide adoption of chloroquine as an antimalarial drug, resistance was reported in the Cambodia-Thailand border (1957), followed by South America and Africa.[35] While chloroquine is now essentially useless against *P. falciparum* malaria in sub-Saharan Africa, it is still the recommended treatment for *P. vivax* infections in Southeast Asia and in South America because resistance has not fully developed in the latter species.[1]

After the emergence of chloroquine-resistant parasites and failure to eradicate the disease, a national project against malaria was set up in China in 1967. More than 2,000 Chinese herbs were extracted and tested for possible antimalarial properties with little success. While an extract from *Artemisia annua* L. (qinghao in Chinese) showed promising parasitic inhibition, the observation was not reproducible. After an intensive literature review, scientists proposed that the conventional extraction steps used on the herbs were destroying the active components. Indeed, a low temperature extraction procedure led to a neutral phase solution with 100% inhibition of parasitemia in mice. This led to the isolation (1972) and structural identification (1975 via crystallography) of artemisinin as the active compound.[36] Artemisinin and derivatives are now the first-line treatment for *P. falciparum* infections in regions where chloroquine-resistant parasites are present.[1]

Over the past decade, multiple reports have linked the reduced efficacy of artemisinin and derivatives to the emergence of parasite resistance.[37,38] New antimalarials are constantly needed to overcome multidrug-resistant parasites.

1.2.4 Considerations for next generation antimalarials

Because every widely adopted antimalarial drug to date has resulted in resistance in field-isolated parasites, new strategies are needed for next generation antimalarial drugs to prolong their lifespan and reduce the risk of future resistance.

The majority of current antimalarial drugs exclusively target the blood stage of *Plasmodium*, in which they parasitize and replicate within erythrocytes.[1] Alhough liver stage and gametocyte stage parasites are asymptomatic, prophylaxis and transmission-blocking drugs are an essential eradication strategy.[39] For example, asymptomatic patients can still be carriers of

Plasmodium gametocytes and thus help transmit parasites in regions endemic to *Anopheles* mosquitos. The identification and development of small molecule inhibitors that target multiple stages of the parasite life cycle would be a significant improvement over many current antimalarial drugs.

Next generation antimalarial drugs should also have a novel mechanism of action (i.e. not be an analogue of a current chemotype). Resistance to chloroquine, which inhibits crystallization of toxic hemozoin during parasitic restructuring of host erythrocytes,[40] developed within a decade of drug adoption by way of *P. falciparum* chloroquine resistance transporter protein (*Pf*CRT) that acts similarly to MDR1.[36] Artemisinin, whose mechanism of action remains under intense debate, almost certainly has multiple molecular targets within the blood stage of *P. falciparum*, and resistance "only" took 30 years to develop.[41] As one would expect, derivatives of these key drugs also retain the same mechanism of action and therefore pose no new value upon the emergence of resistance. Only 35% of the 5,300 genes in *Plasmodium falciparum* have a known function,[42,43] yet 46% of all gene products were detected in all stages of the parasite life cycle.[44] The identification of proteins that are necessary for all stages of the life cycle hold great promise and should help slow resistance mechanisms.

Hypnozoites also remain a formidable and unsolved challenge. Primaquine remains the only drug that can kill dormant liver stage *P. vivax* and *P. ovale* parasites, which constitutes a "radical cure" in the field. However, primaquine can cause fatal hemolysis in patients deficient in glucose-6-phosphate dehydrogenase (G6PD) and use is prohibited in groups such as pregnant women.[45] Because hypnozoites remain an important reservoir of parasites in endemic countries, eliminating the reservoir is crucial for eradication of the disease.

Finally, drug discovery in malaria is limited by our understanding of the malaria parasite. Among the invasive *Plasmodium* species to humans, only *P. falciparum* can be continuously cultured *in vitro*,[46] and *P. vivax* is limited to short-term culture. The lack of a continuous culture system for *P. vivax* necessitates constant access to freshly collected parasites from patients and remains a bottleneck in probing *P. vivax* biology, including hypnozoites.[47] Advances in our ability to culture parasites in particular stages of the *Plasmodium* life cycle will be needed to fully realize our goals.

1.2.5 Recent progress in therapeutics

In response to the threat of emerging resistance, Medicines for Malaria Ventures (MMV) was founded in Switzerland in 1999. The foundation's chief mission is to reduce the burden of malaria in disease-endemic countries by discovering, developing, and facilitating delivery of new, effective, and affordable antimalarial drugs. Prior to the launch of MMV, the pipeline for antimalarial drugs was essentially empty. Since 1999, MMV has partnered with and connected research labs across the globe to advance their mission.[48]

Significant advances in the understanding of malaria biology and the discovery of potential therapeutics have been made since the inception of MMV. One of the most promising compounds in development, KAE609 (also known as NITD609 and Cipargamin) is potent enough to cure *Plasmodium*-infected mice with a single 100 mg/kg oral dose and has activity in both the blood and gametocyte stages of the parasite life cycle.[49] Discovered in 2010, KAE609 targets the outer membrane transporter P-type ATPase4 (*Pf*ATP4), which is important for maintaining parasitic sodium homeostasis,[50] and is the first antimalarial in 20 years with a novel mechanism of action to enter clinical trials.[1] Other key parasitic targets that have been discovered

during this renaissance include dihydroorotate dehydrogenase (DHODH) (inhibited by DSM265; activity in liver and blood stages),[51] cytochrome bc1 Q_i site (inhibited by ELQ-300; activity in liver, blood, and gametocyte stages),[52] translation elongation factor 2 (eEF2) (inhibited by DDD107498; activity in liver, blood, and gametocyte stages),[53] prolyl tRNA synthetase (inhibited by febrifugine; activity in liver and blood stages),[54] and phenylalanyl tRNA synthetase (inhibited by BRD7929; activity in liver, blood, and gametocyte stages) (**Figure 1.3**).[39] Additionally, a number of antimalarial candidates have entered preclinical or clinical trials without having an established mechanism of action.[1]

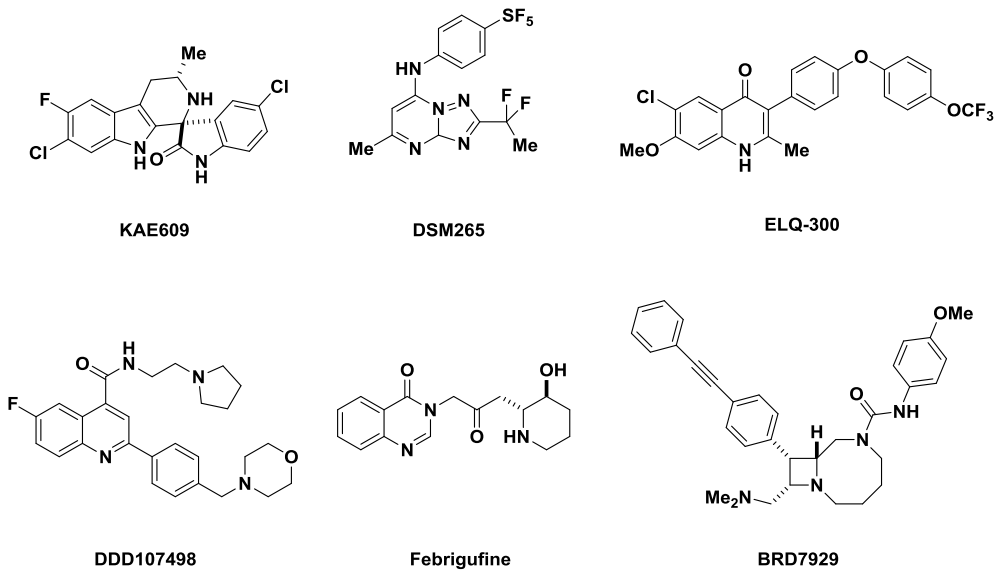

Figure 1.3. Structures of "next generation" antimalarial compounds.

1.3 Conclusions

Malaria is a disease that adversely affects human health and induces heavy social and economic burden. Additionally, *Plasmodium* has a complex life cycle, and scientists have an incomplete understanding of parasite biology that hinders therapeutic development.

Nevertheless, over the past two decades, significant advances and collaborations have enabled the discovery of new parasitic targets and candidate small molecules that have entered the clinic. While eradication will require multiple innovative ways of targeting the parasite, renewed interest in malaria drug discovery suggests an optimistic future.

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Chapter 2

Studies on Spirocycles as Inhibitors of *P. falciparum*

2.1 Introduction

2.1.1 Screening cascade against a diversity-oriented synthesis library

To identify antimalarial compounds with novel mechanisms of action, we implemented a screening strategy that differed from previous efforts.[1,2] First, the application of modern methods of asymmetric organic synthesis to create unique chemical matter was atypical of standard screening collections. Second, testing the resulting compounds in a series of phenotypic-based screens designed to uncover agents that act on targets essential for several stages of the parasite life cycle would help prioritize hit compounds. A small-scale pilot experiment that followed this blueprint performed by the Schreiber group had successfully yielded the antimalarial agent ML238, which inhibited cytochrome bc1 complex at the reduction (Q_i) site.[3,4,5] ML238 was the first known inhibitor at the Q_i site of cytochrome bc1.

We screened an approximately 100,000 compound library, which was synthesized at the Broad Institute using the build/couple/pair strategy[6,7] of diversity-oriented synthesis (DOS). The library was screened in high-throughput against a multidrug-resistant strain (*P. falciparum*, Dd2 strain) using a phenotypic growth-inhibition assay with infected erythrocytes that models a

human blood infection. Compounds scored as positives were counter-screened in parallel against a panel of parasite isolates and diverse drug-resistant clones to deprioritize compounds with previously identified mechanisms of action. Secondary screens against HepG2 (for toxicity) and liver and gametocyte stage parasites were conducted to facilitate the discovery of compounds that act through novel mechanisms of action and target multiple stages of malarial infection (**Figure 2.1**).

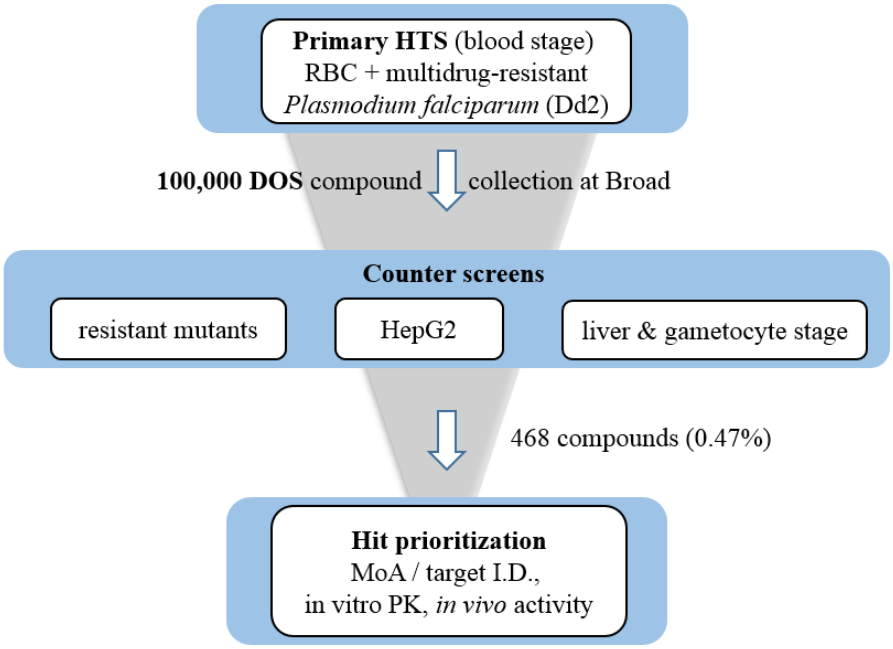


Figure 2.1. Summary of screening cascade. Identification of multistage, novel mechanism of action compounds.

After evaluating results from the cascading triage strategy, four promising chemical series yielded multistage activity and stereochemistry-based structure-activity relationships (SSAR), indicating the possibility of selective interactions with targets (**Figure 2.2**). Additionally, the screening process afforded other series not described but likely merit attention

in the future whose data are available at the Malaria Therapeutics Response Portal, http://portals.broadinstitute.org/mtrp/).[1]

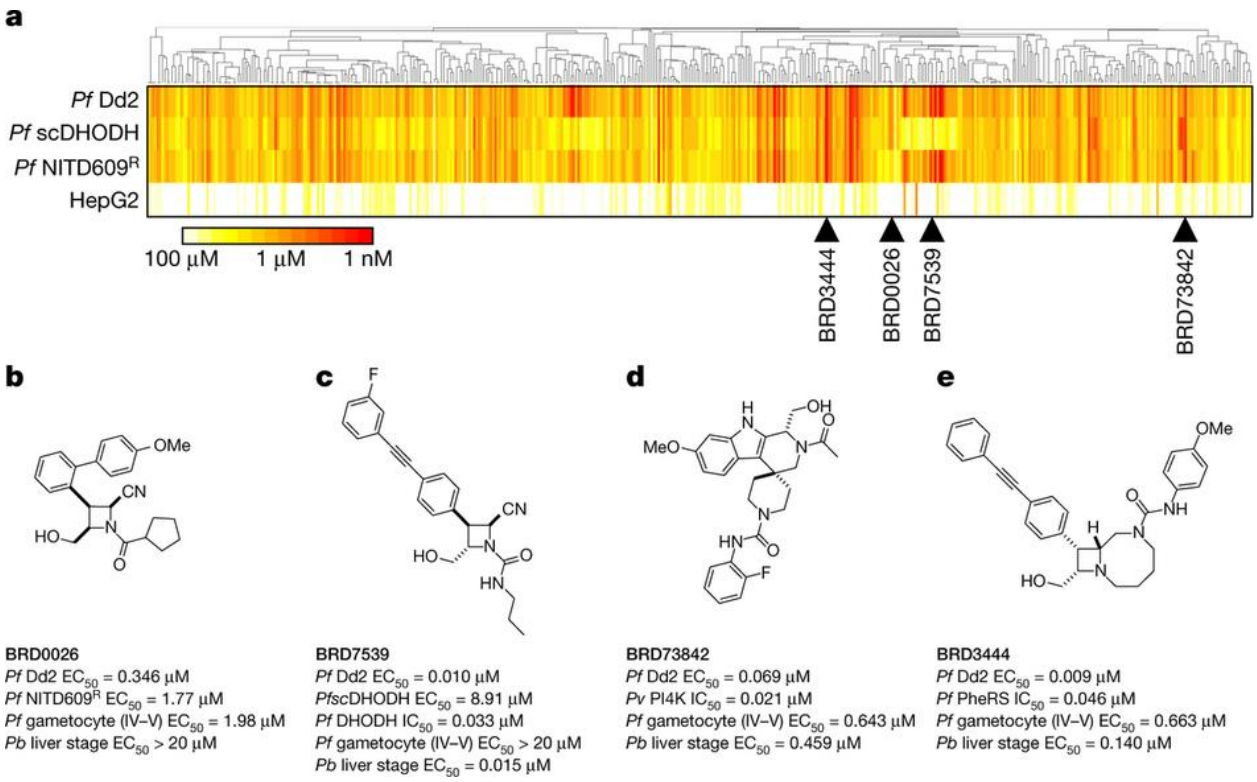


Figure 2.2. Cascading triage strategy reveals new compound scaffolds against known and then-unknown antimalarial targets.[1]

BRD0026 (**Figure 2.2b**) was a weak inhibitor of both blood stage (EC_{50} = 0.346 µM) and gametocyte stage (EC_{50} = 1.98 µM) parasites, and reduced activity against a *P. falciparum* strain resistant to NITD609 (EC_{50} = 1.77 µM) compared to wild type parasites implicated *Pf*ATP4 as the enzymatic target,[8,9] which was later confirmed. BRD7539 (**Figure 2.2c**) was a potent inhibitor of both blood stage (EC_{50} = 0.010 µM) and liver stage (EC_{50} = 0.015 µM) parasites, and reduced activity against a *P. falciparum* strain resistant to mitochondrial inhibitors (*Pfsc*DHODH, EC_{50} = 8.91 µM) as well as a strain resistant to dihydroorotate dehydrogenase

inhibitors implicated DHODH as the enzymatic target.[10] BRD7539 is the focus of Chapter 3 of this thesis.

BRD73842 (**Figure 2.2d**) exhibited activity in all stages of the parasite life cycle – blood stage (EC_{50} = 0.069 μM), liver stage (EC_{50} = 0.459 μM), and gametocyte stage (EC_{50} = 0.643 μM) – and was equipotent against a panel of resistant mutants, suggesting that it had an unknown mechanism of action. Indeed, during the course of our studies to identify the target of BRD73842, *Pf*PI(4)K was identified was a novel enzymatic target[11] of KAI407/KDU691. A brief structure-activity relationship (SAR) campaign on BRD73842 is described in this chapter.

Similarly, BRD3444 (**Figure 2.2e**) also exhibited activity in the blood, liver, and gametocyte stages (EC_{50} = 0.009 μM, 0.140 μM, and 0.663 μM, respectively) and equipotency against a panel of resistant mutants. BRD3444 and analogues were found to inhibit a new antimalarial target, phenylalanyl-tRNA synthetase, with curative ability in multiple *in vivo* efficacy models in mice. The development of a new Pd-catalyzed C(sp^3)-H methodology to access bicyclic azetidines such as BRD3444 is described in Chapter 4.

2.2 Resynthesis of BRD73842

2.2.1 Retrosynthetic analysis of BRD73842

A resynthesis of BRD73842 was necessary to validate its three-stage activity and to determine its mechanism of action. Following a retrosynthetic analysis (**Figure 2.3**), the key stereocenter could be formed using an enantioselective Pictet-Spengler reaction from a bifunctional aldehyde such as 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde. The intermediate spirocycle could arise from the nucleophilic C3 alkylation of 6-methoxy-1H-indole to an electron-withdrawing (EWG) exocyclic olefin from a piperidine-containing starting material.

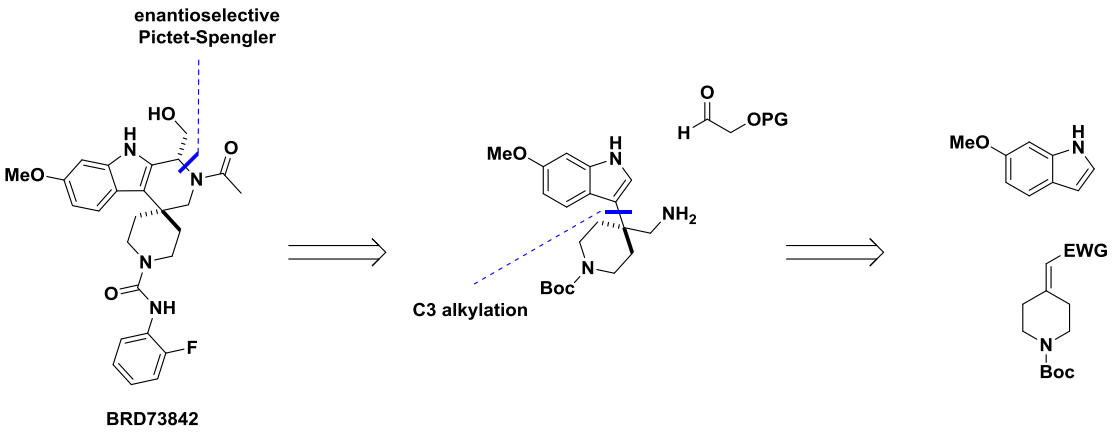
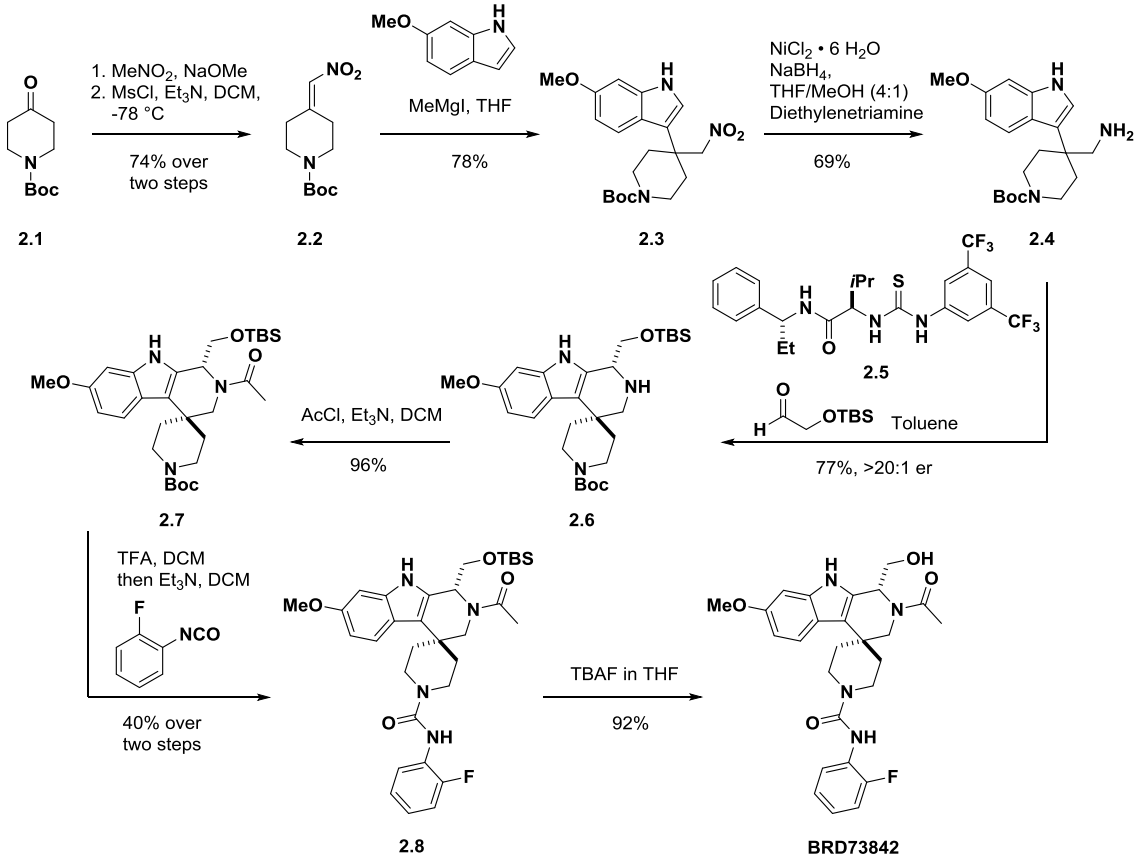


Figure 2.3. Retrosynthetic analysis of spirocycle BRD73842.

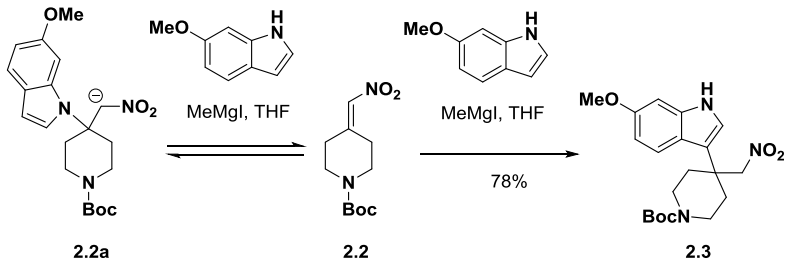
2.2.2 Synthesis of BRD73842

The synthesis of BRD73842 commenced from commercially-available *tert*-butyl 4-oxopiperidine-1-carboxylate (**2.1**) (**Scheme 2.1**). A Henry reaction using nitromethane followed by methanesulfonyl chloride-mediated elimination of the resulting β-nitro alcohol afforded trisubstituted olefin **2.2** in 74% yield over two steps. Next, slow addition of **2.2** in THF into a mixture of 6-methoxy-1H-indole and MeMgI gave C3-addition product **2.3** in 78% yield after 16 h. Of note, upon complete addition of **2.2**, an isomer of the product was observed by LCMS, presumably due to reversible formation of the kinetically-favored N1-addition side product **2.2a** (**Scheme 2.2**). Prolonged stirring of the reaction (> 12 h) followed by workup cleanly resulted in the thermodynamically stable **2.3**. Reduction of the nitroalkane with $NiCl_2 \cdot 6H_2O$ and $NaBH_4$ furnished primary amine **2.4** (69% yield).

The key stereocenter-generating step relied on an asymmetric Pictet-Spengler reaction using **2.4** as the key substrate and thiourea catalyst **2.5** (note: catalyst was generously gifted by the Jacobsen group at Harvard University). After significant optimization, modified conditions developed by the Jacobsen group[12,13] provided desired spirocycle **2.6** in 77% yield with >20:1 er. During the course of reaction optimization, numerous caveats were found: (1) 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde had to be freshly-distilled upon use, else the reaction yield failed to exceed 40% yield; (2) the reaction had to be maintained at -20 °C to prevent significant erosion to er; (3) two additional 0.08 equiv portions of catalyst **2.5** had to be added during the course of the reaction to encourage conversion; and (4) the reaction required 7+ days (> 168 h) to achieve maximum yield. Despite this, **2.6** could be reproducibly formed from **2.4** in gram scale.



Scheme 2.1. Synthetic route to spirocycle BRD73842.



Scheme 2.2. Proposed intermediate during indole addition to nitroolefin.

The remainder of the synthesis required a series of protecting group manipulations. Acylation of the secondary amine afforded **2.7** in excellent yield (96%). Boc deprotection with TFA followed by exposure of the free amine to 1-fluoro-2-isocyanatobenzene provided urea **2.8** in 40% yield over two steps. Finally, treatment with TBAF in THF delivered desired product BRD73842 in 9 steps and 11% overall yield.

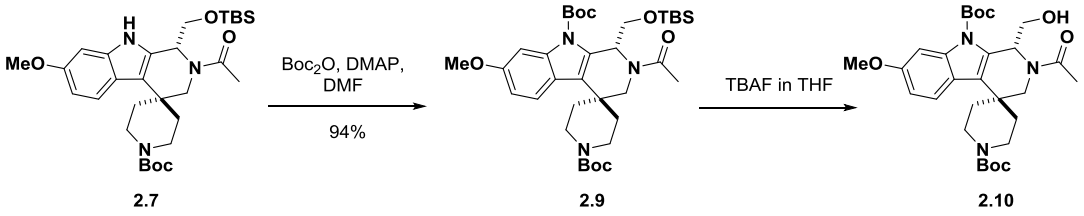
2.3 Synthesis of BRD73842 Analogues

In addition to resynthesizing BRD73842, we sought to synthesize and evaluate analogues to increase the potency of the spirocyclic series. This effort involved multiple chemists in the Schreiber group and at the Broad Institute of MIT & Harvard. Personal efforts toward this project are outlined below.

2.3.1 Synthesis of primary alcohol derivatives

The first region of BRD73842 we sought to explore was the primary alcohol, which we envisioned could be used as a handle for target identification. Protection of the indole nitrogen of

intermediate **2.7** followed by deprotection of the TBS group furnished crude **2.10** as a versatile handle for direct alkylation of the alcohol (**Scheme 2.3**).



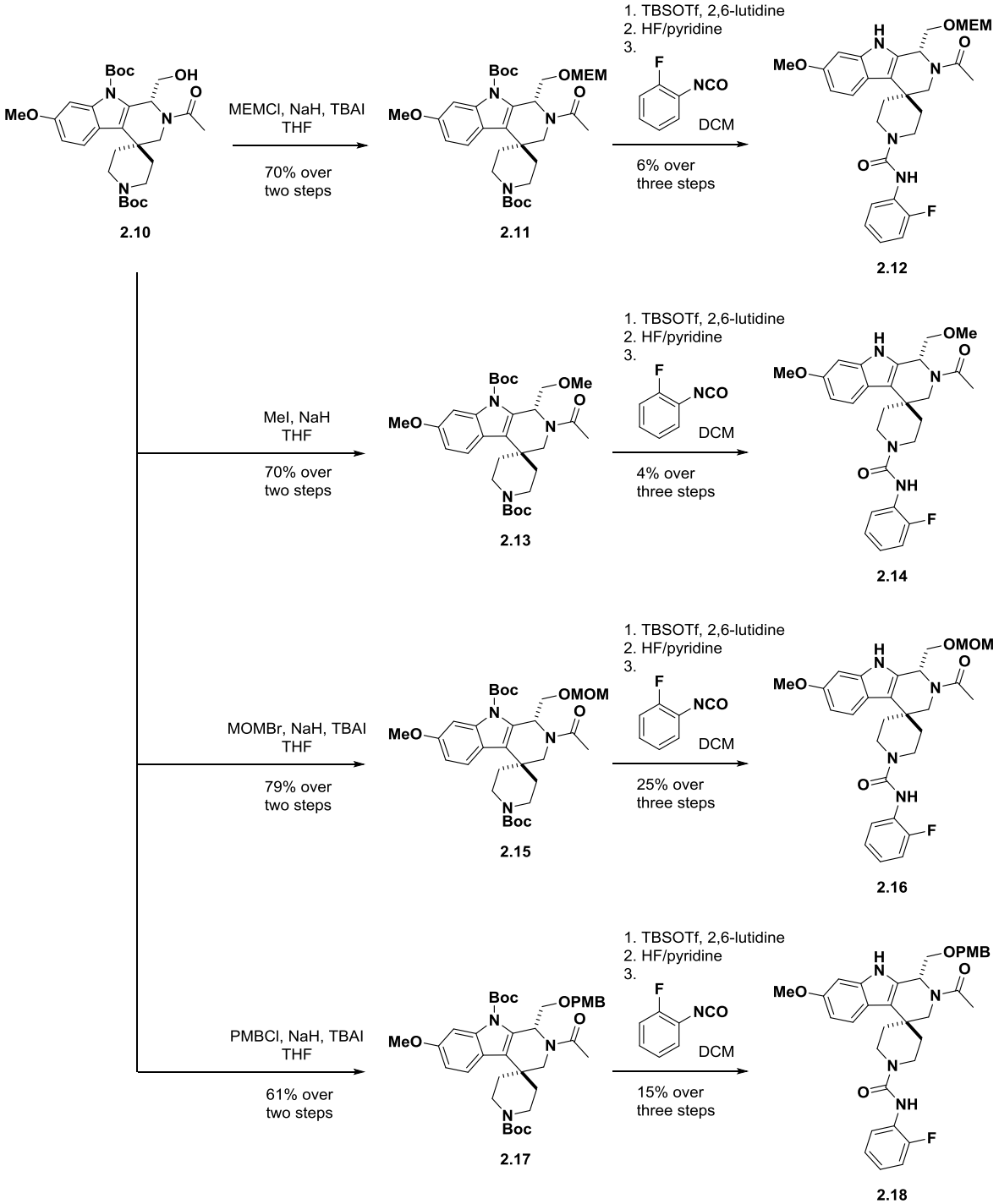
Scheme 2.3. Protecting group manipulation to access a versatile intermediate.

Diversification of **2.10** using alkyl electrophiles gave access to protected intermediates in 61-79% yield over two steps from **2.9** (**Scheme 2.4**). Alternative Boc deprotection condition using TBSOTf followed by HF/pyridine-mediated cleavage of the silyl carbamate were employed to prevent –OMEM and –OMOM deprotection under typical TFA conditions. Crude formation the urea furnished linker derivatives **2.12**, **2.14**, **2.16**, and **2.18** in 4-25% yield over three steps. The low yielding step was presumably due global Boc deprotection of the respective intermediates, though the reaction afforded enough material for biological evaluation.

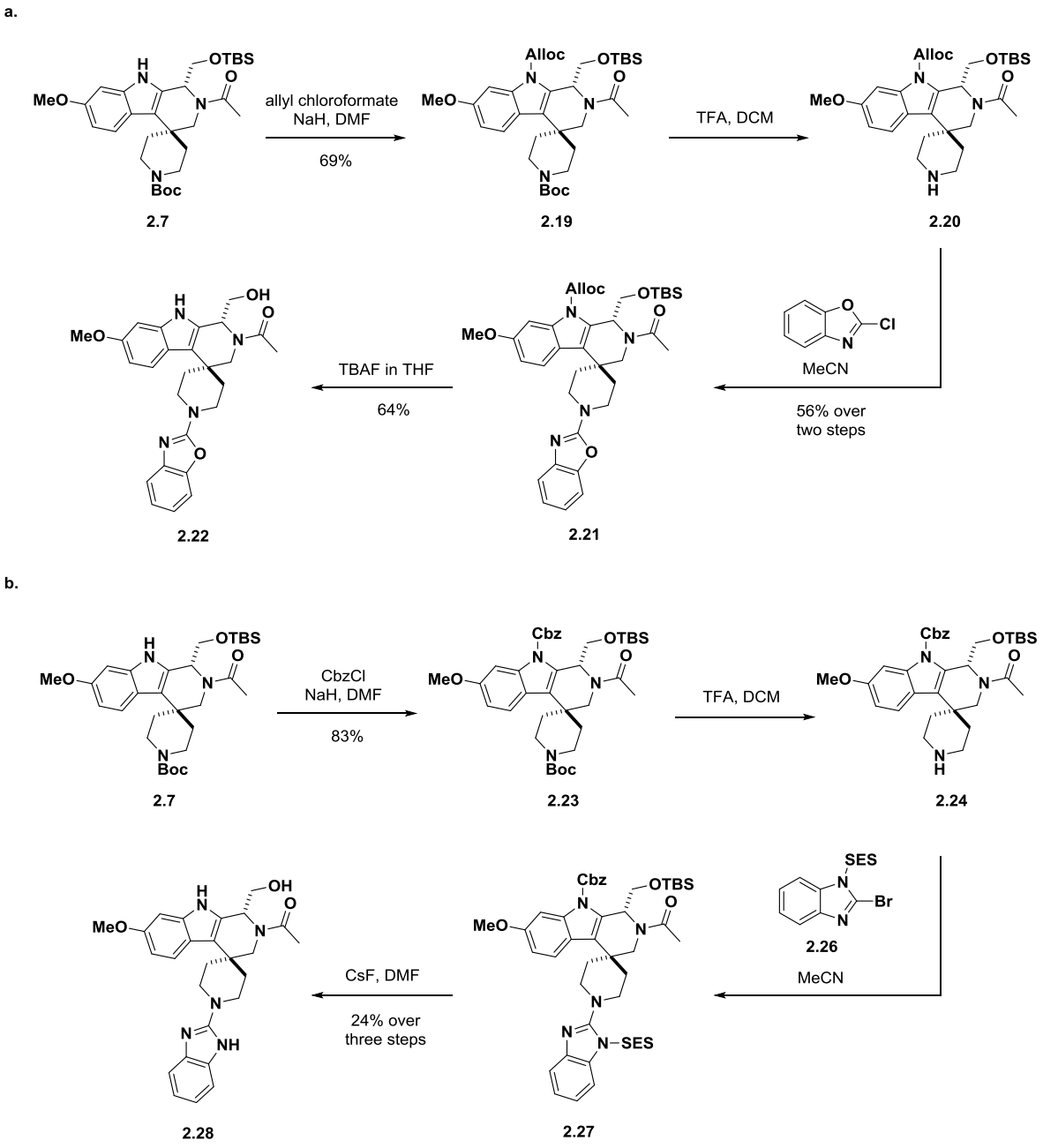
2.3.2 Synthesis of urea derivatives

The second region of BRD73842 we briefly sought to evaluate was the urea, which poses a potential solubility concern. Protection of the indole nitrogen of intermediate **2.7** with either allyl chloroformate (**Scheme 2.5a**) or CbzCl (**Scheme 2.5b**) furnished globally-protected **2.19** and **2.23**, respectively, in good yields (69-83%). TFA deprotection of Boc followed by SN_{Ar} with 2-chlorobenzoxazole gave access to **2.21**, upon which TBS and alloc deprotection afforded the

desired analogue **2.22**. A similar route employing 2-bromo-1-((2-(trimethylsilyl)ethyl)sulfonyl)-1H-benzo[d]imidazole (**2.26**)[14] as the SN_{Ar} partner[15] afforded desired analogue **2.28**.



Scheme 2.4. Diversification to access analogues of the primary alcohol.

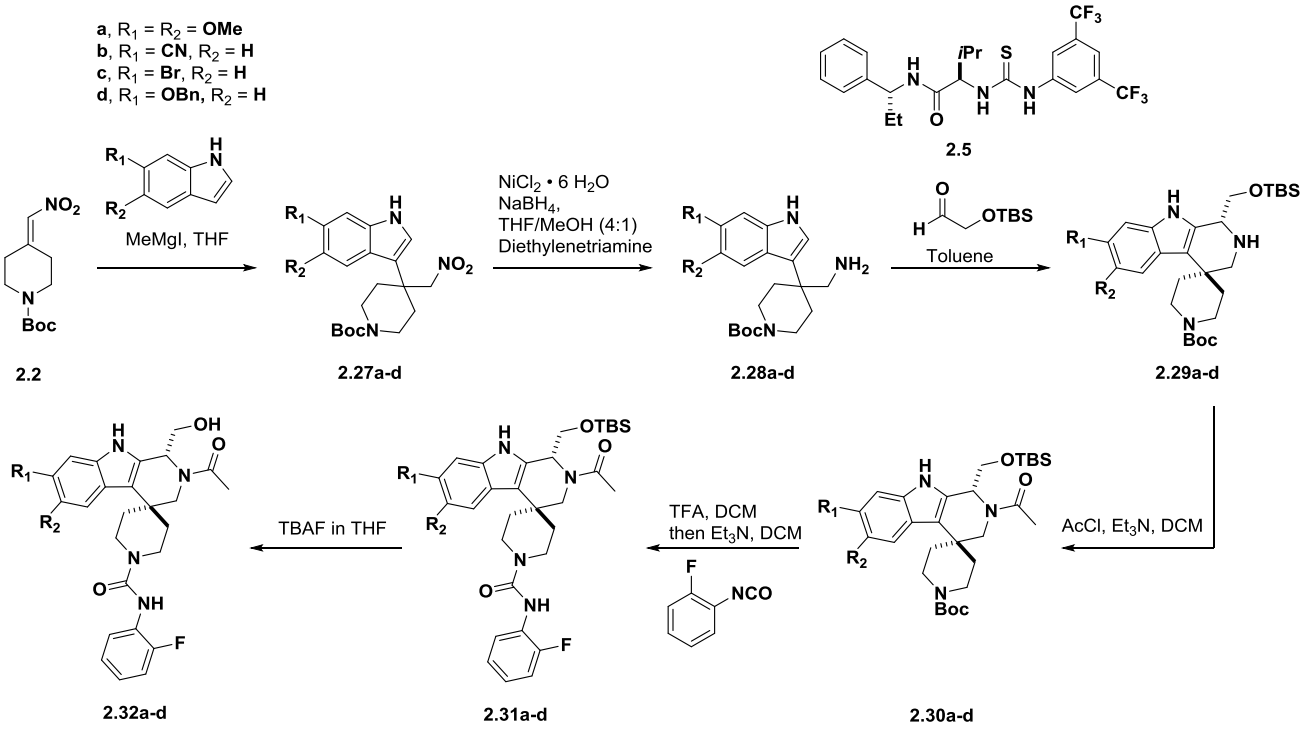


Scheme 2.5. Diversification to access analogues of the urea.

2.3.3 Synthesis of indole derivatives

A major limitation of the synthesis as outlined in **Scheme 2.1** is the inability to access analogues on the indole ring. Analogous syntheses were initiated to solve this problem as shown

in general **Scheme 2.6**. Disappointingly, the asymmetric Pictet-Spengler reaction did not succeed with indoles lacking strongly electron donating groups such as **2.28b** and **2.29c**, in agreement with previous observations with unsubstituted indoles.[12,13] Bis-methoxy substituted indole **2.28a** proceeded in 79% yield (18:1 er) whereas benzoxy-subsubstituted indole **2.28d** afforded product in 69% yield albeit much poorer enantioselectivity (4:1 er). Subsequently, this afforded products **2.32a** and **2.32d**, respectively, after completion of the synthetic route.

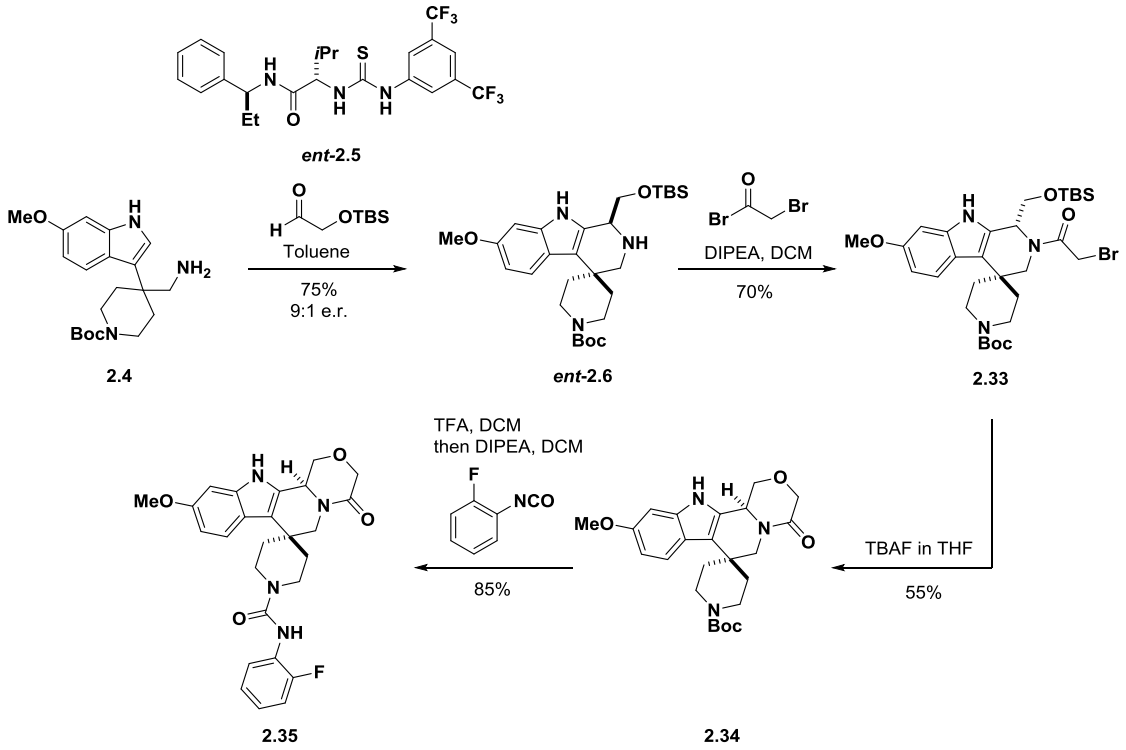


Scheme 2.6. Diversification of the indole ring.

2.3.4 Synthesis of a tricyclic derivative

An unusual analogue we sought to assess was tricycle **2.35** (**Scheme 2.7**), which we speculated would lock the analogue in a single conformation that could be favorable upon binding to its target (although it would lose hydrogen bond donation capabilities). From

intermediate **2.4**, an enantioselective Pictet-Spengler reaction using catalyst ***ent*-2.5** (also generously gifted by the Jacobsen group at Harvard University) gave ***ent*-2.6** in 75% yield with slightly lower enantioselectivity (9:1 er), and acylation with 2-bromoacetyl bromide allowed access to **2.33** in 70% yield. Simultaneous TBS deprotection and intramolecular cyclization under basic conditions afforded desired tricycle **2.34** in good yield (55%), and a one-pot Boc deprotection and urea formation delivered desired analogue **2.35** in 85% yield.



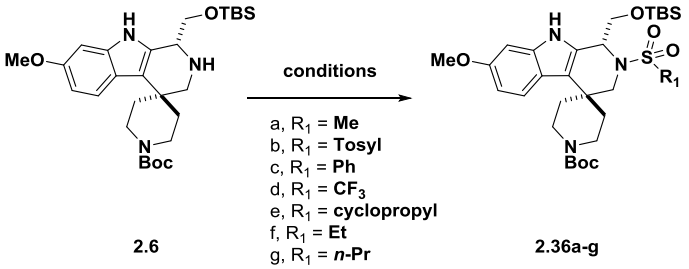
Scheme 2.7. Synthetic route to tricyclic derivative of BRD73842.

2.3.5 Synthesis of sulfonamide analogues

Finally, we sought to make sulfonamide analogues of BRD73842 as substitutes for the secondary amide. A series of sulfonylation reactions were performed on intermediate **2.6** to afford products **2.36a-g** using the respective sulfonyl chlorides. Standard conditions using 5-10

equiv of Et_3N in 0.05 M DCM at room temperature furnished desired products **2.36a-c** in high (79-96%) yield (**entries 1-3**), but related conditions at -78 °C resulted in decomposition for the trifluoromethyl variant (**entry 4**). Changing the electrophile from trifluoromethanesulfonyl chloride to triflic anhydride resulted in decomposition at 0 °C (**entry 5)**, but maintaining the reaction at -78 °C resulted in clean conversion to **2.36d** (**entry 6**).

Table 2.1. Screening of reaction conditions to access analogues **2.36a-g**.



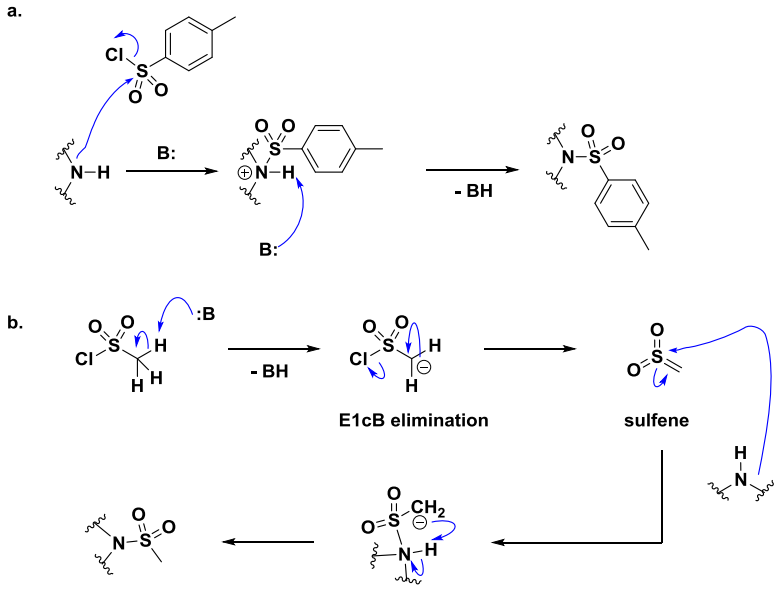
2.6 **2.36a-g**

Entry (DP)[a]	Reaction conditions[b]	Yield (%)[c]
1 (**2.36a**)	Sulfonyl chloride, Et_3N, DCM, 0 °C	96%
2 (**2.36b**)	Sulfonyl chloride, Et_3N, DCM	89%
3 (**2.36c**)	Sulfonyl chloride, Et_3N, DCM	79%
4 (**2.36d**)	Sulfonyl chloride, Et_3N, DCM, -78 °C	decomp
5 (**2.36d**)	Triflic anhydride, Et_3N, DCM, 0 °C	decomp
6 (**2.36d**)	Triflic anhydride, Et_3N, DCM, -78 °C	80%
7 (**2.36e**)	Sulfonyl chloride, Et_3N, DCM, 40 °C	0%
8 (**2.36e**)	Sulfonyl chloride, Et_3N, 2,6-lutidine, THF, 70 °C	54%
9 (**2.36f**)	Sulfonyl chloride, Et_3N, DCM	decomp
10 (**2.36f**)	Sulfonyl chloride, DBU, $CHCl_3$	17%
11 (**2.36f**)	Sulfonyl chloride, pyridine, $CHCl_3$	42%
12 (**2.36g**)	Sulfonyl chloride, Et_3N, DCM, -78 °C	decomp
13 (**2.36g**)	Sulfonyl chloride, Et_3N, DCM	decomp
14 (**2.36g**)	Sulfonyl chloride, Et_3N, DCM, 40 °C	decomp
15 (**2.36g**)	Sulfonyl chloride, pyridine, $CHCl_3$, 40 °C	37%

[a]DP refers to desired products **2.36a-g**. [b]Sulfonyl chloride refers to respective precursor (e.g. for **2.36a**, methane sulfonyl chloride, etc.). Reactions performed from **2.6** using 1-5 equiv. sulfonyl chloride, 5-10 equiv. of base in 0.05 M DCM at room temperature unless otherwise noted. See Chapter 5 for complete reaction conditions. [c]Decomp indicates decomposition observed with no measurable amounts of starting material or desired product formed.

Preparation of intermediates **2.36e-g** proved much more difficult than expected. Heating of **2.6** to 40 °C with cyclopropane sulfonyl chloride, Et_3N, and DCM resulted in no conversion to

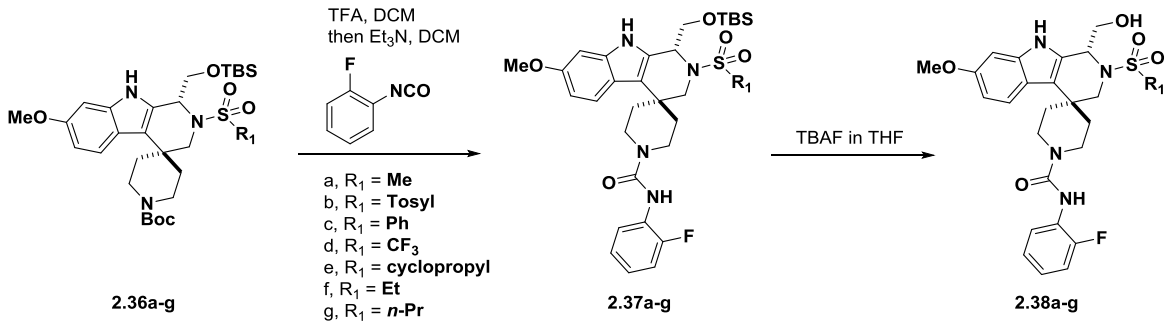
product (**entry 7**), but modified conditions using 2,6-lutidine as secondary base in THF at 70 °C provided desired **2.36e** in modest (54%) yield (**entry 8**). Decomposition was observed using standard sulfonylation conditions to prepare **2.36f** and **2.36g** (**entries 9**, **12-14**). However, switching Et_3N to DBU afforded **2.36f** in 17% yield (**entry 10**). Subsequent screening of reaction conditions revealed that the use of pyridine at either room temperature or at 40 °C in $CHCl_3$ was able to provide desired **2.36f** and **2.36g** in 42% and 37% yields, respectively (**entries 11, 15**).



Scheme 2.8. Proposed sulfonylation reaction mechanisms explain difference in reactivity for (a) aryl sulfonyl chlorides and (b) branched alphatic sulfonyl chlorides.[16]

The differences in reactivity for aryl sulfonyl chorides vs. methanesulfonyl chloride vs. branched sulfonyl chlorides can likely be attributed to reaction mechanism. The formation of aryl sulfonamides (i.e. tosyl sulfonamide) from amines and aryl sulfonyl chlorides is thought to proceed by the amine acting as a nucleophile toward the electrophilic sulfonyl chloride (**Scheme**

2.8a). Proton exchange with base affords the aryl sulfonamide. By contrast, aliphatic sulfonyl chlorides are postulated to react by an E1cb mechanism, whereby the acidic α-proton is removed by base followed by elimination of chloride to form a sulfene (**Scheme 2.8b**). Nucleophilic attack by the amine at the electrophilic sulfur generates a carbanion that undergoes proton transfer to give the desired aliphatic sulfonamide.[16] Thus, ethyl and *N*-propyl sulfonyl chlorides likely reacted more favorably to pyridine, despite the latter being a much weaker base (pK_b = 5.21), than Et_3N (pK_b = 11.01) because of accessibility to the α-proton (pK_a ~ 6.7) of the sulfonyl chloride.[17] Presumably, the use of a stronger, non-sterically hindered base should promote elimination and therefore increase the yield and decrease the reaction time of these reactions.



Scheme 2.9. Completion of synthesis for sulfonamide analogues.

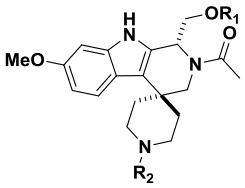
Having successfully accessed intermediates **2.36a-g**, a one-pot Boc deprotection/urea formation followed by TBS removal provided desired analogues **2.38a-g** in 53-80% yield over two steps (**Scheme 2.9**).

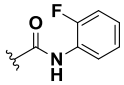
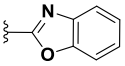
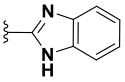
2.4 Biological Evaluation of BRD73842 and Analogues

2.4.1 *In vitro* results

Resynthesized BRD73842 and analogues were assessed in an *in vitro* phenotypic growth-inhibition assay using infected erythrocytes against a multidrug-resistant strain (*P. falciparum*, Dd2 strain) of parasites. This assay models a human blood-stage infection and is equivalent to the high-throughput assay used in the original screening cascade.[1]

Table 2.2. Evaluation of analogues at the alcohol and urea positions of BRD73842.



Cmpd	R_1	R_2	EC_{50} (μM)[a]
BRD73842			0.048
2.12	MEM	"	3.620
2.14	Me	"	9.051
2.16	MOM	"	3.037
2.18	PMB	"	0.422
2.22			8.500
2.28			7.019

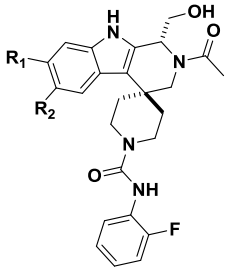
[a]EC_{50} (μM) against *P. falciparum* (Dd2 strain). Values are the mean of at least one independent experiment in triplicate.

Resynthesized BRD73842 (**Table 2.2**) was found to be a potent inhibitor of Dd2 parasites (EC_{50} = 0.048 uM), consistent with previous results.[1] Unfortunately, analogues at the primary alcohol position, such as methylated (**2.14**) and –OMEM (**2.12**) and –OMOM (**2.16**) derivatives

were inactive (EC_{50} > 5 μM). Intriguingly, **2.18**, which bears an –OPMB group, was moderately active against parasites (EC_{50} = 0.422 uM) though still nearly an order of magnitude less potent than the original hit compound.

The two indole analogues of BRD73842, **2.32a** and **2.32b**, were inactive against Dd2 parasites (EC_{50} > 5 μM, **Table 2.3**). While **2.32b**, which bears a 6-benzoxy group, is significantly larger than parent BRD73842, **2.32a** differs by the addition of a single methoxy group at the 5-position of the indole ring. These results suggest that the indole may play a crucial role in binding of BRD73842 to the target protein.

Table 2.3. Evaluation of indole analogues of BRD73842.



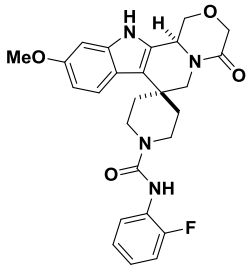
Cmpd	R_1	R_2	EC_{50} (μM)[a]
2.32a	OMe	OMe	5.760
2.32b	OBn	H	19.500

[a]EC_{50} (μM) against *P. falciparum* (Dd2 strain). Values are the mean of at least one independent experiment in triplicate.

Tricyclic analogue **2.35** (**Scheme 2.10**), which forms a C-O bond between the primary alcohol and the α-carbon of the amide to lock the conformation of the molecule, is significantly less potent than BRD73842.

Unlike analogues at the indole, alcohol, and urea positions, sulfonamide analogues show a weak (but demonstrable) structure-activity relationship (**Table 2.4**). Methyl sulfonamide **2.38a**

(EC_{50} = 0.059 μM) is equipotent to BRD73842 (EC_{50} = 0.048 μM) while the ethyl analogue is one of the most potent analogues (EC_{50} = 0.015 μM) in the series. Interestingly, trifluoromethyl sulfonamide **2.38d** is more than 4x less potent (EC_{50} = 0.269 μM) than **2.38a**. While cyclopropyl sulfonamide **2.38e** is still active against Dd2 parasites, a drop-off in potency is observed as substituent size increases from **2.38g** to **2.38c** to **2.38b.**



2.35
EC_{50} = 2.470 μM

Scheme 2.10. Evaluation of tricyclic analogue **2.35** against malaria parasites.

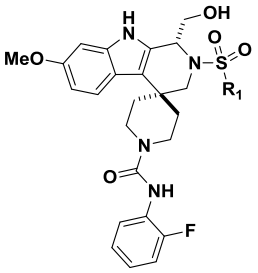
2.4.2 Target validation and medicinal chemistry summary

We were initially intrigued with BRD73842 because the compound exhibited activity in all three stages of the parasite life cycle and had a putative novel mechanism of action. In parallel with medicinal chemistry, resistant lines of *P. falciparum* were evolved against BRD73842 after more than three months of continuous drug pressure. During this time, a research group at the Genomics Institute of the Novartis Research Foundation published a research article on the discovery of *Plasmodium* PI(4)K as the novel target of small molecule KAI407/KDU691.[11]

After obtaining *P. falciparum* cultures with >3 fold loss in potency (EC_{50}) compared to BRD73842, sequencing of the parasite genome revealed single nucleotide polymorphisms (SNPs) at the loci encoding for *Plasmodium* PI(4)K. This was validated via biochemical assays

performed against purified human (IC_{50} > 10 μM) and *P. vivax* (IC_{50} = 0.021 μM) PI(4)K protein as well as biphasic dose-response curves characteristic of *P. falciparum* PI(4)K inhibitors (**Figure 2.4**).[1] Recently, second small molecule, MMV390048, was found to also inhibit *Plasmodium* PI(4)K,[18,19] making BRD73842 the third known inhibitor of this target.

Table 2.4. Evaluation of sulfonamide analogues of BRD73842.



Cmpd	R_1	EC_{50} (μM)[a]
2.38a		0.059
2.38b		1.080
2.38c		0.391
2.38d		0.269
2.38e		0.113
2.38f		0.015
2.38g		0.221

[a]EC_{50} (μM) against *P. falciparum* (Dd2 strain). Values are the mean of at least one independent experiment in triplicate.

The medicinal chemistry results of the analogues described herein as well as analogues synthesized by others as part of a large group effort (data not shown) demonstrated no noticeable improvement in compound potency. Notably, few SAR trends could be deduced from a large

medicinal chemistry campaign, and BRD73842 and **2.38f** remain among the most potent analogues in the spirocyclic series. Additionally, BRD73842 exhibited poor *in vitro* stability and mouse pharmacokinetic (PK) data. These results, in combination with the publication of other small molecules with superior *in vitro* and *in vivo* profiles targeting *Plasmodium* PI(4)K, led to a general deprioritization of this compound series.

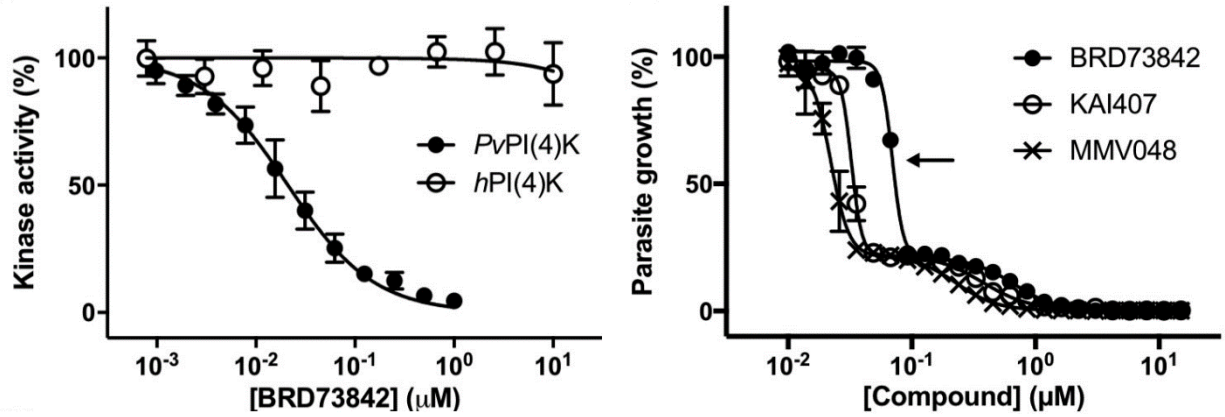


Figure 2.4. BRD73842 target identification. Inhibitory activity against *P. vivax* and human PI(4)K (left) and characteristic dose-response curves for PI(4)K inhibition (right).

2.5 Conclusions

Screening a diversity-oriented synthesis collection (~100,000 compounds) and employing counterscreens to unveil new chemotypes with novel mechanisms of actions led to the identification of at least four promising scaffolds, two of which exhibited activity in all stages of the *Plasmodium* parasite life cycle. A medicinal chemistry campaign on the first of these scaffolds, spirocycle BRD73842, failed to result in significant increase in potency, and the lead compound exhibited poor *in vitro* and *in vivo* pharmacokinetic profiles. PI(4)K, which was

unveiled as a novel *Plasmodium* target by another group during the course of these studies, was eventually found to also be the target of BRD73842.

2.6 Contributions

Nobutaka Kato performed the high-throughput screen of the diversity-oriented synthesis collection and *in vitro* assays against Dd2 parasites. Byung-Chul Suh designed the library employing the Pictet-Spengler-type reactions. Tomoyo Sakata-Kato generated BRD73842-resistant parasites, and Gillian Dornan, Jacob McPhail, and John Burke performed the biochemical assay against *P. vivax* PI(4)K. Eamon Comer led the overall medicinal chemistry efforts for BRD73842 – these results are not shown in this chapter. All other data, including the design and synthesis of all analogues in this chapter, were generated by the author.

2.7 References

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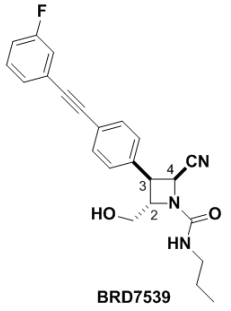
Chapter 3

Azetidine-2-carbonitriles That Inhibit *P. falciparum* Dihydroorotate Dehydrogenase

3.1 Introduction

A second compound identified from the high-throughput screen of interest to us was BRD7539, an azetidine carbonitrile with three contiguous stereocenters (2*S*,3*S*,4*S*). Stereochemistry-based structure-activity relationships (SSAR) showed that only two of eight possible stereoisomers were active (**Figure 3.1**), hinting at the possibility of selective interactions with its target.[1]



BRD7539

Stereochemistry C_2,C_3,C_4 *Pf* (Dd2) EC_{50}	
R, S, S 35 nM	S, R, R >5,000 nM
R, R, S >5,000 nM	S, S, R >5,000 nM
R, S, R 5,000 nM	S, R, S >5,000 nM
R, R, R 3,005 nM	S, S, S 10 nM

Figure 3.1. Activity of all eight diastereomers of BRD7539 against Dd2 parasites.

Table 3.1. Activity of BRD7539 against resistant lines and genotypes of respective parasites.

Assay	Resistant against	Resistant gene	EC_{50} (μM)[a]
Pf, Dd2	CQ, QN, PY, SDX	-	0.010
*Pf*scDHODH	ECT inhibitors	scdhodh	8.917
*Pf*CYTb:G33V	IDI5994	pfcytb-Qi site	0.006
Pf, TM90C6B	ATV	pfcytb-Qo site	0.011
HepG2	-	-	>26.000
*Pf*DHODH:E182D	Genz-666136	pfdhodh	0.637

CQ, chloroquine; QN, quinine; PY, pyrimethamine; SDX, sulphadoxine; ATV; atovaquone. [a]EC_{50} (μM) against *P. falciparum* (Dd2 strain). Values are the mean of at least one independent experiment in triplicate.

BRD7539 was a potent inhibitor of both blood stage (*P. falciparum,* Dd2 strain, EC_{50} = 0.010 μM) and liver stage (*P. berghei*, ANKA strain, EC_{50} = 0.015 μM) parasites but had no activity against gametocyte stage parasites (*P. falciparum,* stages IV-V, EC_{50} > 20 μM). Profiling against a panel of drug-resistant parasites revealed that BRD7539 had reduced activity (EC_{50} = 8.917 μM) against a strain of parasite expressing transgenic *Saccharomyces cerevisiae* dihydroorotate dehydrogenase (*Pfsc*DHODH); this strain is resistant to all electron transport chain inhibitors, implicating a mitochondrial target.[2] Cytochrome B (Q_0 and Q_i sites) and dihydroorotate dehydrogenase (DHODH) remain the only known *Plasmodium* mitochondrial targets,[3] and BRD7539 was assessed in parasites with mutations at these binding sites (**Table 3.1**). BRD7539 was equipotent against *P. falciparum* strains with mutations in the cytochrome B Q_0 site and Q_i sites,[1,4] and >30 fold reduced potency in parasites with mutations in the DHODH quinone binding site[5] suggested that the compound inhibited DHODH.

DHODH catalyzes the flavin mononucleotide (FMN)-dependent oxidation of L-dihydroorotate to orotate as the fourth and rate-limiting step in *de novo* pyrimidine biosynthesis (**Figure 3.2**). Most organisms use both *de novo* and salvage pathways to generate pyrimidines, which are required for the biosynthesis of DNA, RNA, glycoproteins, and phospholipids.[5] However, *Plasmodium* parasites lack the necessary genes for the latter, making *de novo* pyrimidine synthesis an essential pathway for the parasite.[6] Human DHODH and *Pf*DHODH show considerable variability in sequence and residue alignment, and the known development of selective inhibitors makes *Pf*DHODH an attractive antimalarial target.[7,8]

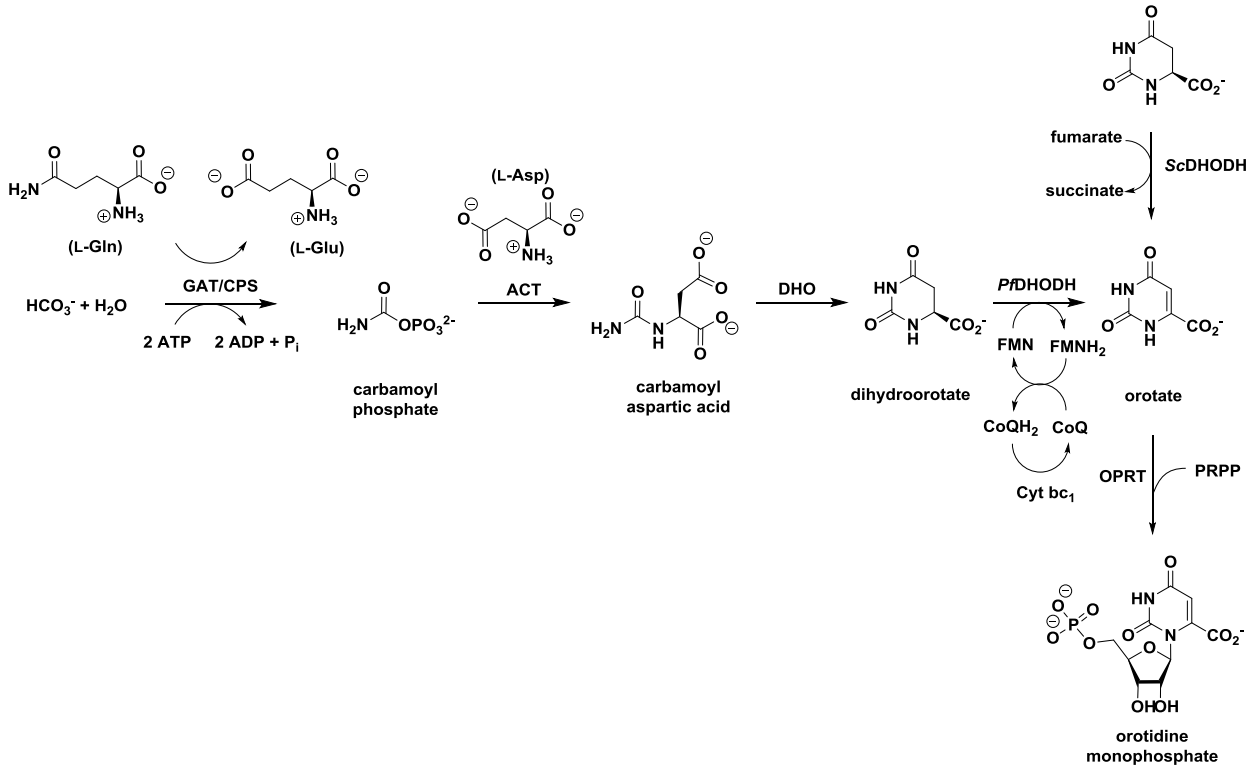


Figure 3.2. *De novo* pyrimidine synthesis pathway in *Plasmodium*.

One compound in the anti-malarial pipeline, DSM265, has progressed to phase-II clinical trials[9,10] and has activity against both asexual blood stage and liver stage parasites.[8] DSM265 and secondary candidate DSM421 (**Figure 3.3**) comprise a class of selective and potent antimalarial

DHODH inhibitors arising from a biochemical screen[5,11] and focused medicinal chemistry effort.[12,13,14,15,16,17,18] These triazolopyrimidines remain the most well-studied and clinically relevant antimalarial DHODH inhibitors to date, but 5-benzimidazolyl-thiophene-2-carboxamides[5,19] and 7-arylaminopyrazolo[1,5-α]pyrimidines have also been reported.[20]

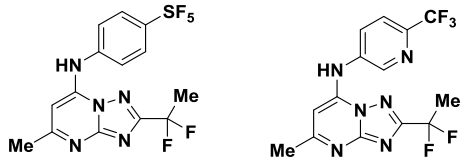


Figure 3.3. Structures of DSM265 and DSM421. These compounds are in clinical and pre-clinical trials, respectively.

BRD7539 was subsequently confirmed to inhibit *Pf*DHODH (IC_{50} = 0.033 μM) selectively over human DHODH (*Hs*DHODH, IC_{50} > 25 μM) (**Figure 3.4**). The clinical relevance of *Pf*DHODH inhibitors and the selectivity and potency of BRD7539 arising directly from a high-throughput screen encouraged us to pursue this series further.

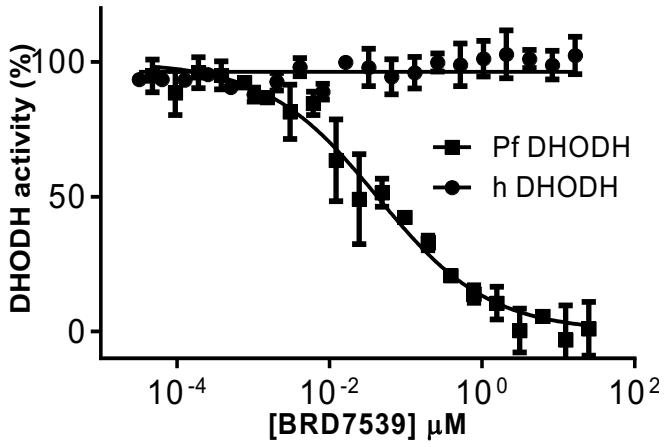
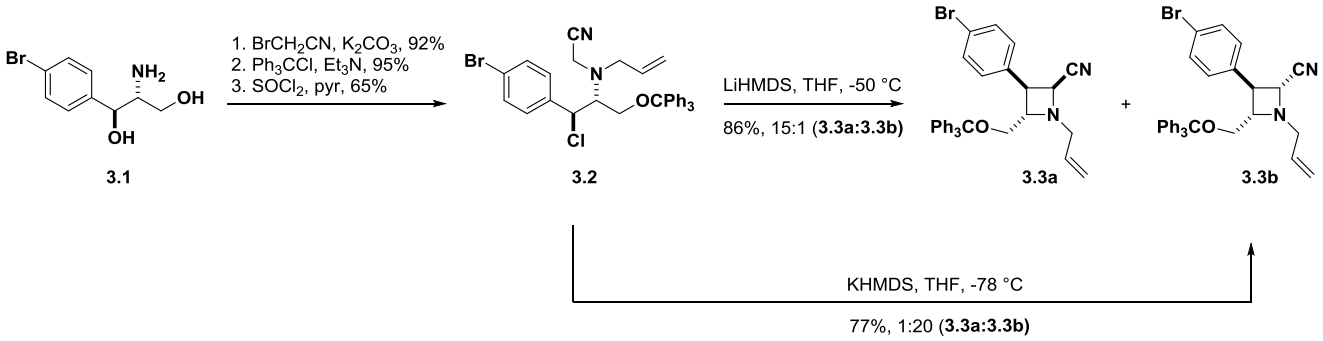


Figure 3.4. Biochemical potency of BRD7539 against human and *Plasmodium* DHODH.

3.2 Structure-Activity Relationships

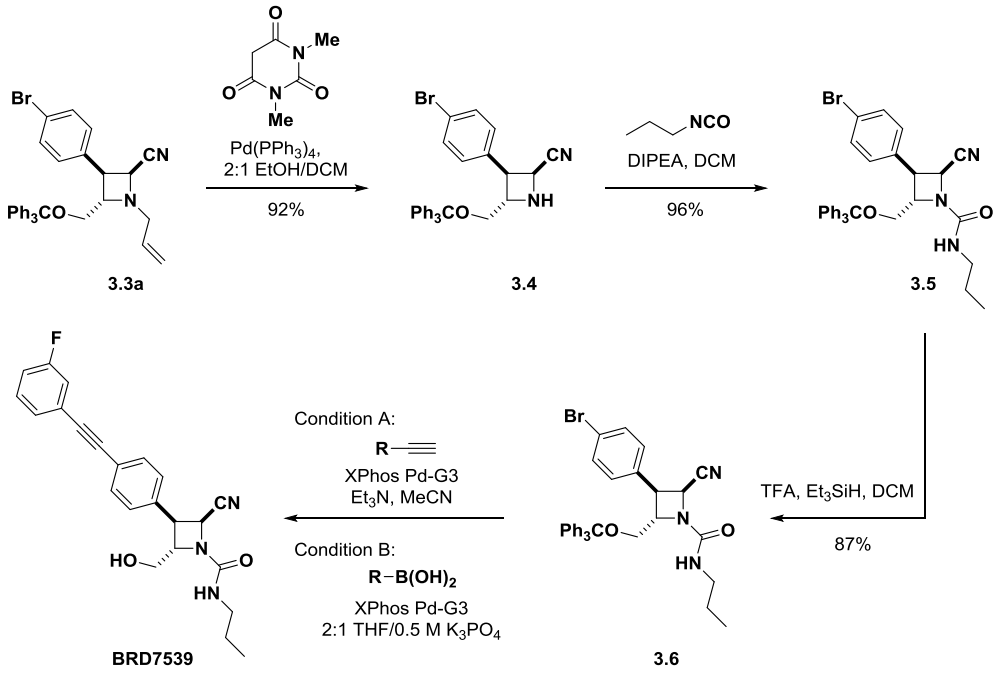
3.2.1 BRD7539 resynthesis and synthetic route toward aryl analogues

To confirm the biological activity of BRD7539 and to explore structure–activity relationships (SAR) of the scaffold, core structure **3.3a** (**Scheme 3.1**) was resynthesized as reported by Lowe and colleagues.[21] Notably, upon trityl protection of the primary alcohol in **3.1**, treatment with thionyl chloride provides intermediate **3.2** with retention of stereochemistry at the benzylic position. This is thought to occur via intramolecular aziridinium formation followed by Cl^--mediated ring opening (i.e. double S_N2). Ring closing with LiHMDS provides desired **3.3a** with excellent diastereoselectivity (15:1 dr of **3.3a** to **3.3b**). The use of KHMDS as base provides diastereomer **3.3b** with >20:1 dr, suggesting a cation-controlled transition state. All steps can be performed on gram scale, demonstrating the utility and versatility of this methodology.



Scheme 3.1. Synthesis of core structure **3.3a**. Scheme was adapted from Lowe *et al*.[21]

Upon access to core structure **3.3a**, deallylation and sequential capping of the nitrogen with propyl isocyanate gave urea **3.5** in high yields (**Scheme 3.2**). Trityl deprotection using TFA/Et_3SiH afforded **3.6**, a versatile intermediate for late-stage Suzuki cross-coupling or Heck alkynylation. This served as a general route to most analogues.



Scheme 3.2. General route to BRD7539 and cross-coupling analogues.

Biological activity of BRD7539 and analogues were confirmed in 20-point dose ($n \geq 2$ independent experiments in triplicate) against a multidrug-resistant strain (*P. falciparum*, Dd2 strain) using a phenotypic blood stage growth inhibition assay that models a human blood stage infection. Additionally, *in vitro* stability against mice and human microsomes for 1 h was used as a guide to identify analogues with potential *in vivo* stability.

3.2.2 Initial *in vivo* evaluation of BRD7539

Resynthesized BRD7539 was evaluated in two *in vivo P. berghei* models to assess initial activity of the compound. The *in vivo P. berghei* blood stage assay (**Figure 3.5a**) requires blood from "donor mouse" to infect a healthy mouse at t = -24 h. At t = 0, mice are treated with a single oral dose of BRD7539 or vehicle solution, and mice are monitored by parasite-associated bioluminescent imaging. The *in vivo P. berghei* liver stage assay (**Figure 3.5b**) requires dissection of sporozoites from infected mosquitos followed by the simultaneous injection of sporozoites and oral dosing of BRD7539.

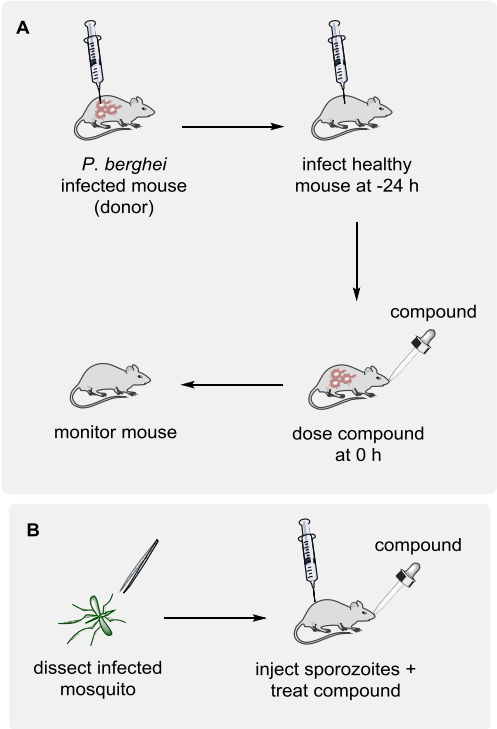


Figure 3.5. General schematic of *in vivo P. berghei* (a) blood stage and (b) liver stage models.

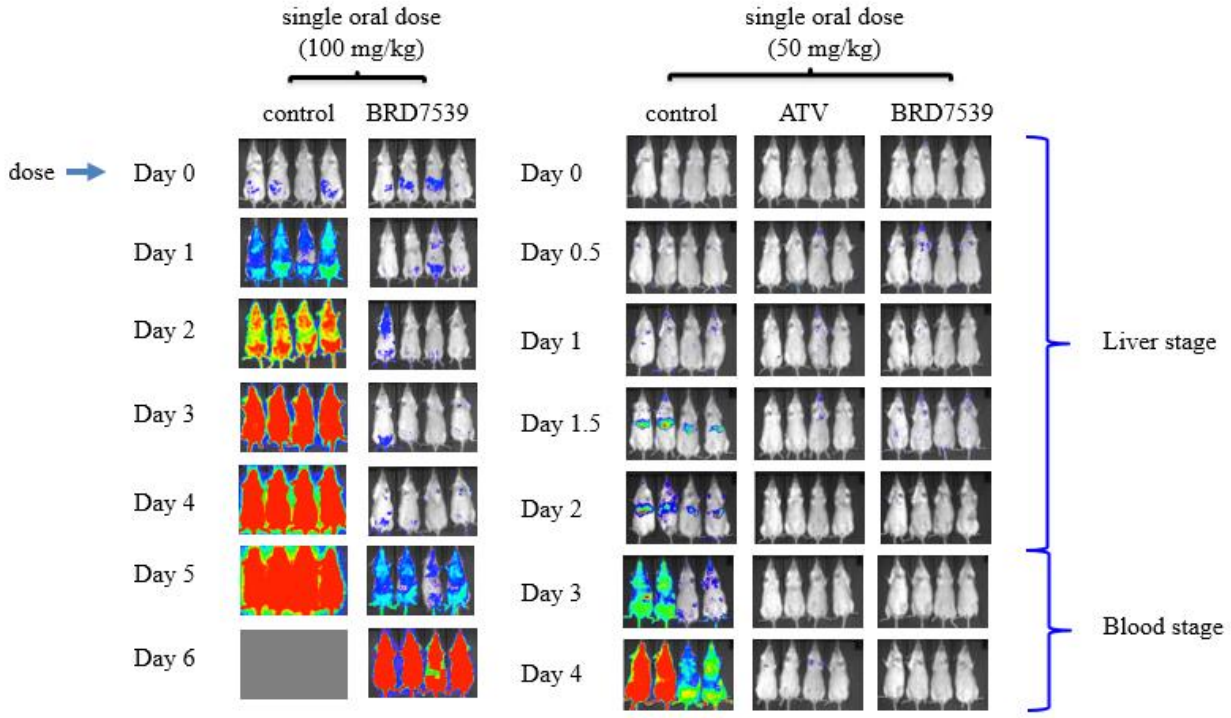
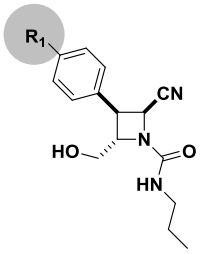
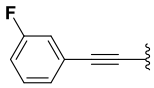
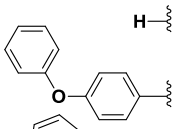
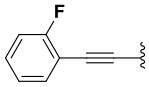
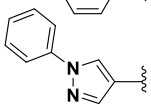
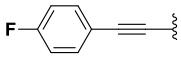
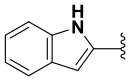
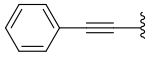
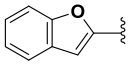
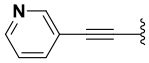
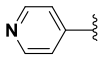
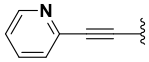
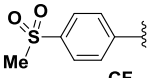
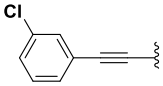
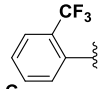
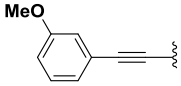
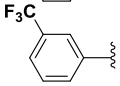
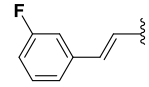
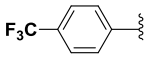
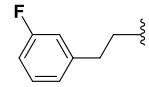
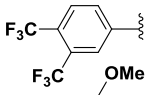
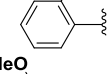
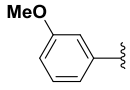
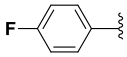
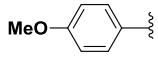
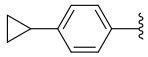


Figure 3.6. Efficacy of BRD7539 in *in vivo P. berghei* models. Blood stage (left) and liver stage (right) models.

Encouragingly, BRD7539 displayed modest activity in both models (**Figure 3.6**). In the *in vivo* blood stage model, a single oral 100 mg/kg dose of BRD7539 cleared parasites for four days when compared to control mice, though recrudescence is observed around day 5. In the liver stage model, a single oral 50 mg/kg dose of BRD7539 is able to prevent a liver stage infection and the resulting blood stage infection, thereby acting as a prophylactic. BRD7539 behaves similarly to known prophylactic drug and positive control atovaquone (ATV).

Table 3.2. Activity of BRD7539 and phenyl analogues against Dd2 parasites.

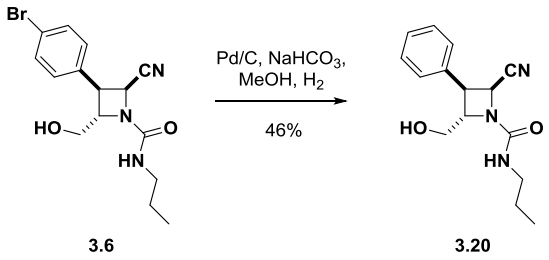


Cmpd	R_1	EC_{50} (μM)	M/H	Cmpd	R_1	EC_{50} (μM)	M/H
BRD7539		0.010	86/99	**3.20**		8.375	-
3.6		0.249	-	**3.21**		0.005	8/51
3.7		0.083	85/85	**3.22**		0.010	34/74
3.8		0.013	100/100	**3.23**		12.630	-
3.9		0.016	-	**3.24**		0.139	-
3.10		5.640	-	**3.25**		5.541	-
3.11		0.427	-	**3.26**		4.390	91/90
3.12		0.020	85/96	**3.27**		0.097	0/16
3.13		0.016	45/94	**3.28**		0.012	39/96
3.14		0.035	56/87	**3.29**		0.039	81/91
3.15		0.046	0/14	**3.30**		0.016	95/90
3.16		0.019	-	**3.31**		0.352	-
3.17		0.051	-	**3.32**		0.015	0/74
3.18		0.106	-	**3.33**		0.015	0/7
3.19		0.019	-	**3.34**		0.041	50/66

Mouse (M) and human (H) microsomal stability (% remaining after 1 hour).

3.2.3 Synthetic routes toward acetylene analogues

Having demonstrated promising *in vivo* results, initial SAR efforts focused primarily on the acetylene region of BRD7539 as this was the most facile point of diversification and a possible toxicity concern (**Table 3.2**).[22] Indeed, BRD7539 displays modest inhibition of the hERG channel (EC_{50} = 10 μM), a result likely due to the diphenyl acetylene. *In vitro* activity was maintained with a wide variety of hydrophobic acetylenes (**3.7-3.9**, **3.12-3.13**). Heteroaromatic 2- and 3-pyridyl analogues (**3.10-3.11**) showed significant loss in activity compared to aromatic analogues. Interestingly, cis-alkene (**3.14**) and alkane (**3.15**) derivatives of BRD7539 showed only a slight loss in activity, suggesting that the acetylene was not necessary. Unsubstituted biaryl **3.16** is essentially equipotent to BRD7539 while removing the distal ring (**3.17**, **Scheme 3.3**) abolished activity, indicating the need for a large hydrophobic region in the scaffold. Having removed the acetylenic toxicity concern, this region was used to modulate mouse and human microsomal stability while maintaining activity. Improved stability should correlate with favorable pharmacokinetic (PK) properties. Analogues bearing a 4-pyridyl (**3.25**) and 4-methanesulfonyl (**3.26**) distal aryl were synthesized in an effort to improve solubility but were inactive *in vitro*. CF_3-substitution (**3.27-3.30**) was found to impart greater in vitro microsomal stability than methoxy substituents (**3.31-3.33**). Ultimately, addition of two $–CF_3$ groups on the distal phenyl ring (**3.30**, BRD9185) were comparable in activity and microsomal stability to BRD7539.



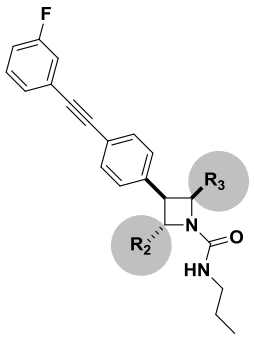
3.6 3.20

Scheme 3.3. Synthesis of analogue **3.20**.

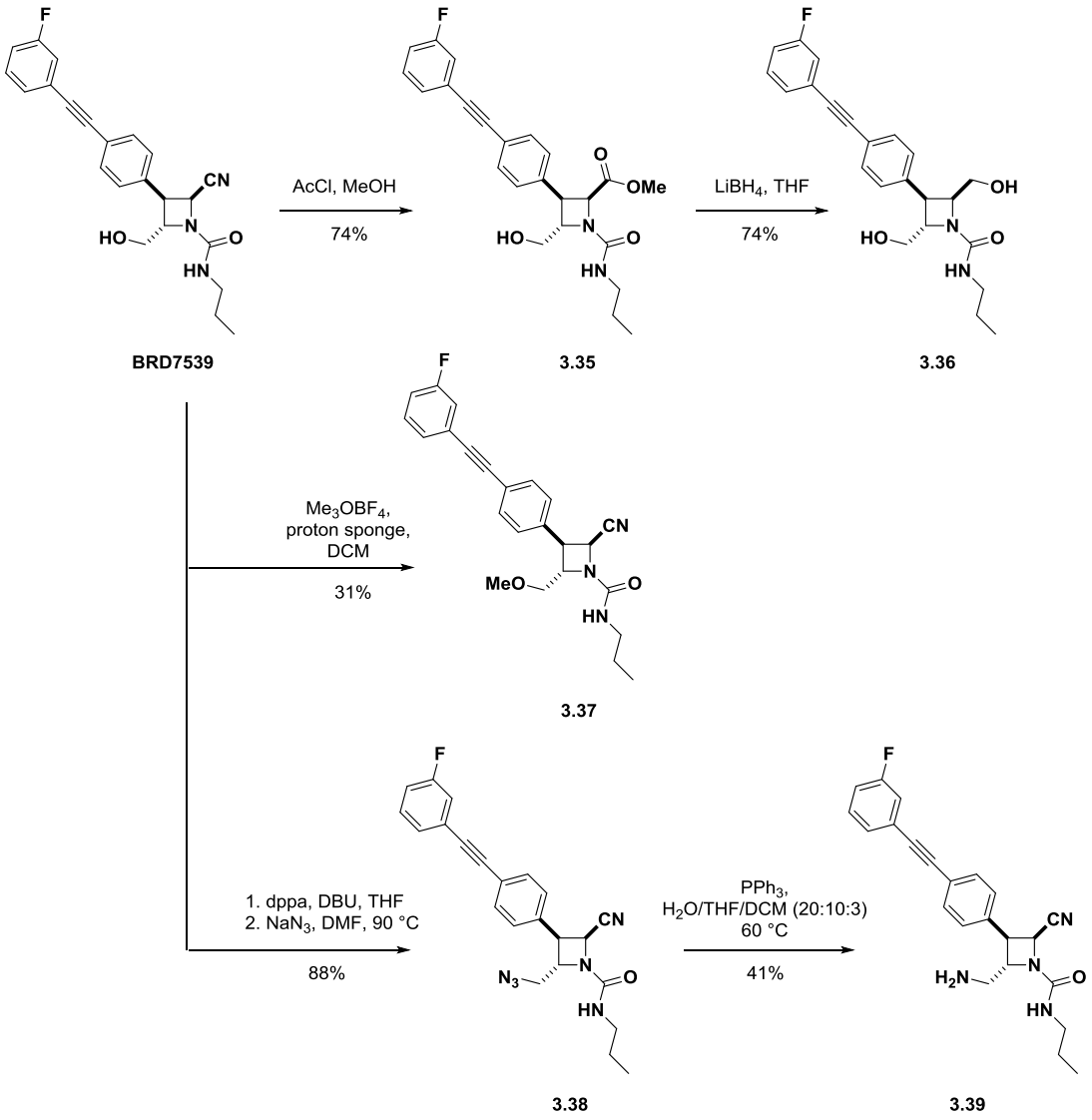
3.2.4 Synthetic routes toward alcohol and nitrile analogues

The roles of the primary alcohol (R_2) and secondary nitrile (R_3) on activity were briefly assessed (**Table 3.3**). Analogues were synthesized from BRD7539 in 1-3 steps or from commercially available starting materials (**Schemes 3.4-3.6**). Modifying the nitrile to a methyl ester (**3.35**) or alcohol (**3.36**) abolished activity. This is unsurprising given our previous result that the 2*S*,3*S*,4*R* diastereocenter – which only differs from BRD7539 at the nitrile-bearing stereocenter – is inactive, hinting at the importance of this functional group. This also illustrates the subtle but significant role stereochemistry can have on small molecule–protein interactions and highlights the strength of diversity-oriented synthesis in identifying these key interactions. Any modification of the primary alcohol, including methylation (**3.37**) or conversion to a primary amine (**3.39**), resulted in large loss in activity. **3.42**, which lacks the C2 stereocenter altogether, is also inactive, suggesting that the primary alcohol plays a key role in its interaction with DHODH.

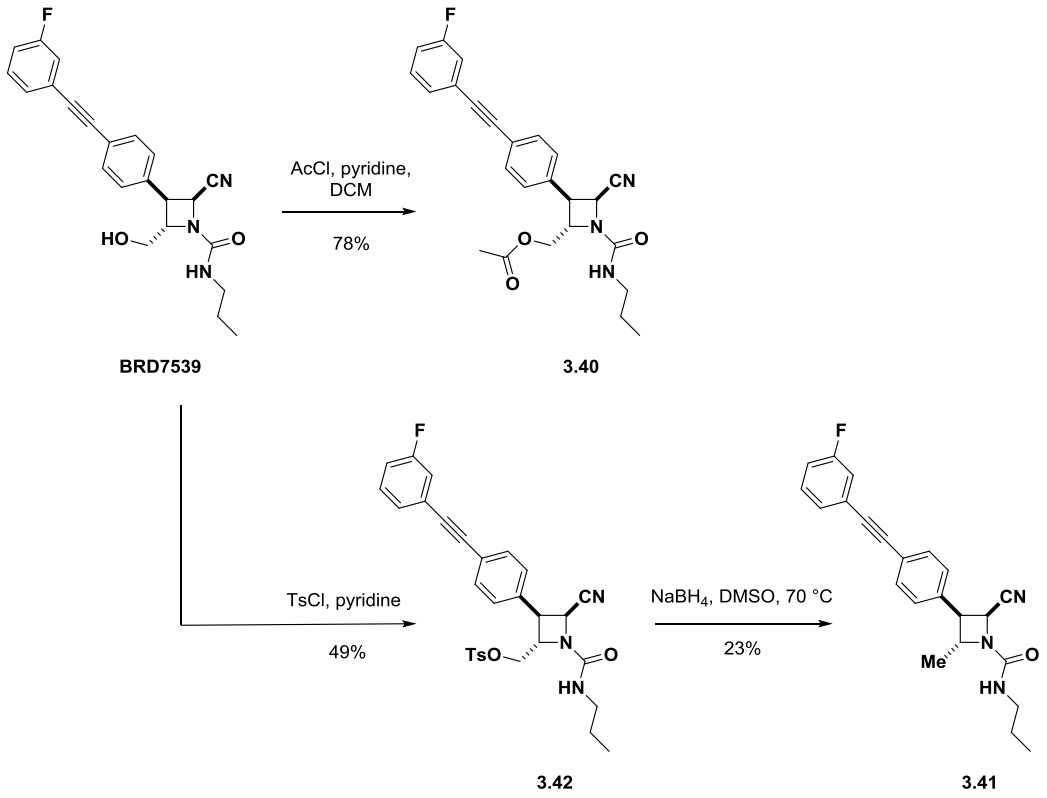
Table 3.3. Activity of nitrile and alcohol analogues of BRD7539 against Dd2 parasites.



Cmpd	R_2	R_3	EC_{50} (μM)
3.35			1.629
3.36	"		16.200
3.37			1.613
3.38		"	0.390
3.39		"	4.440
3.40		"	0.102
3.41		"	0.307
3.42		"	10.508

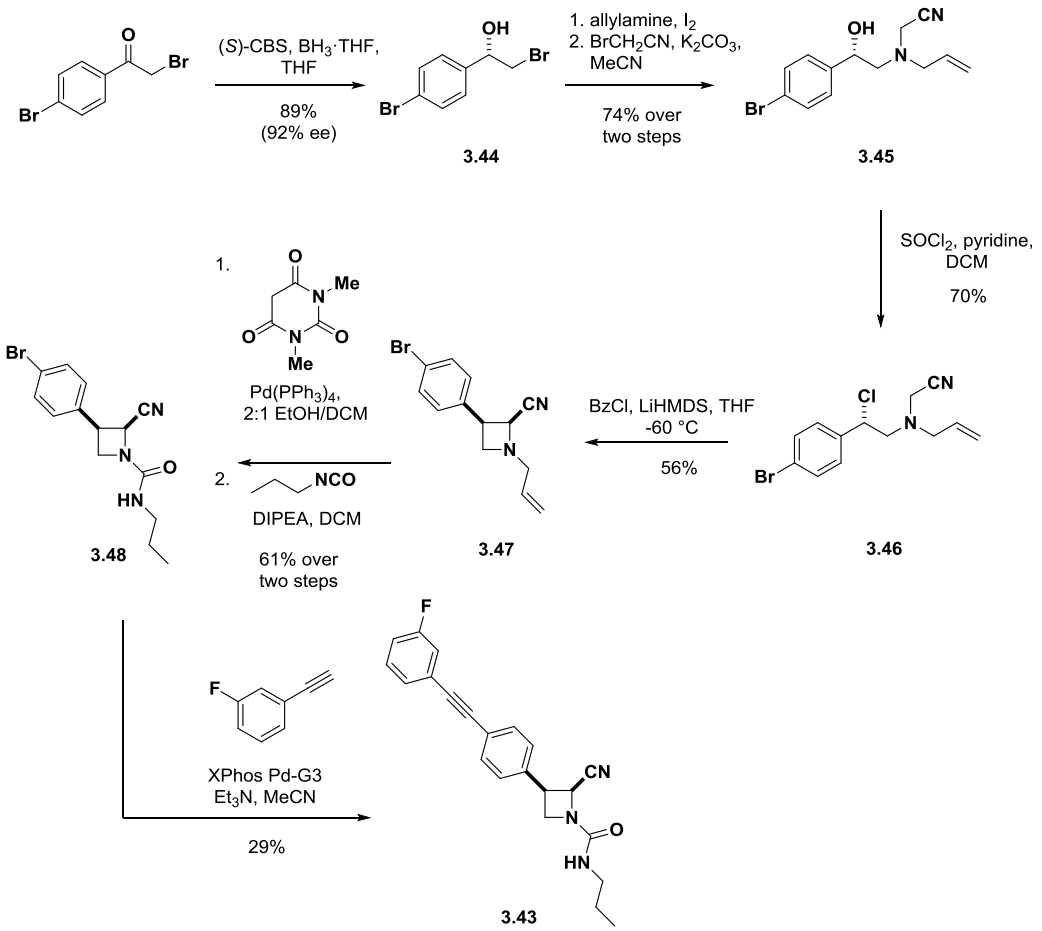


Scheme 3.4. Synthesis of analogues **3.35-3.39** from BRD7539.



Scheme 3.5. Synthesis of analogues **3.40** and **3.41** from BRD7539.

Of note, the synthesis of **3.39** proved difficult as dppa was unable to effect the desired transformation. The reaction stalled at the diphenyl phosphate intermediate even upon prolonged heating at elevated temperatures (up to 80 °C). Purification by column chromatography followed by subjection of the intermediate phosphate to excess NaN_3 in DMF at 90 °C smoothly converted the intermediate to the desired azide. The subsequent Staudinger reduction also proved sluggish at room temperature and required heating to 60 °C to afford **3.39**. These observations can perhaps be explained by the hindered trajectories of nucleophilic N_3^- and H_2O.



Scheme 3.6. Synthesis of analogue **3.43** from 2-bromo-1-(4-bromophenyl)ethanone.

To confirm that lead compound BRD9185 inhibits *Pf*DHODH despite removal of the acetylene motif, biochemical assays were performed against both recombinant *P. falciparum* and human DHODH enzyme. Similar to hit compound BRD7539, BRD9185 is a potent inhibitor of *Pf*DHODH (IC_{50} = 0.012 μM) but not *Hs*DHODH (IC_{50} > 50 μM), suggesting that this class of DHODH inhibitors provides selectivity between orthologues.

3.2.5 *In vivo* evaluation of BRD9185

To assess the suitability of BRD9185 further for *in vivo* use, we measured plasma protein binding and obtained mouse PK data (**Table 3.4**). BRD9185 is highly protein bound in both

mouse and human plasma (> 99%) and is a highly bioavailable (94%), long half-life (15 h) compound in mice (PO 5 mg/kg; IV 1 mg/kg) with low clearance (0.40 mL/min/kg). Notably, the $DNAUC_{0\text{-}inf}$ of BRD9185 is > 54.3 μM·h, higher than the EC_{50} *in vitro*. The PK profile of BRD9185 is noticeably superior to hit compound BRD7539.

Table 3.4. Key properties of BRD9185 vs. BRD7539.

***in vitro* enzyme inhibition, IC_{50}[a]**	**BRD9185**	**BRD7539**
*Pf*DHODH (μM)	0.012	0.033
*Hs*DHODH (μM)	>50	>25
plasma protein binding[b]		
mouse (%)	99.3	99.8
human (%)	>99.0	99.6
mouse PK[c]		
$t_{1/2}$ (h)	15.2	4.1
C_0 (μM)	4.9	16.7
C_{max} (μM)	16.9	18.2
$DNAUC_{0\text{-}inf}$ (μM·h)	>54.3	34.3
V_{ss} (L/kg)	0.37	0.53
CL (mL/min/kg)	0.40	1.58
F (%)	94	124

$t_{1/2}$, terminal half-life; C_0, initial serum concentration at t = 0; C_{max}, peak serum concentration; $DNAUC_{0\text{-}inf}$, dose-normalized area under the plasma concentration vs. time curve following PO dosing; V_{ss}, volume of distribution at steady state; CL, plasma clearance; F%, bioavailability. IV dosing in 5% DMSO/10% cremophor/85% H_2O at 0.25 mg/mL (1 mg/kg). PO dosing in 70% PEG300/30% (5% dextrose in H_2O) at 0.50 mg/mL (5 mg/kg). n = 3 mice in both IV and PO groups.

Based on the promising PK, we were interested in evaluating the efficacy of BRD9185 *in vivo* (**Figures 3.7** and **3.8**). We used a blood-stage model with the rodent malaria parasite *P. berghei* that expresses luciferase and treated infected CD-1 mice for three days with 66.6 mg/kg of BRD9185 or vehicle (70% PEG300, 30% solution of 5% dextrose in H_2O). Bioluminescence intensity was used to measure parasite growth, and artesunate was used as a positive control. No parasites were detected after 30 days in mice treated with BRD9185, suggesting that the analogue achieved a sterile cure for *P. berghei*, and BRD9185 outperforms artesunate in this model.

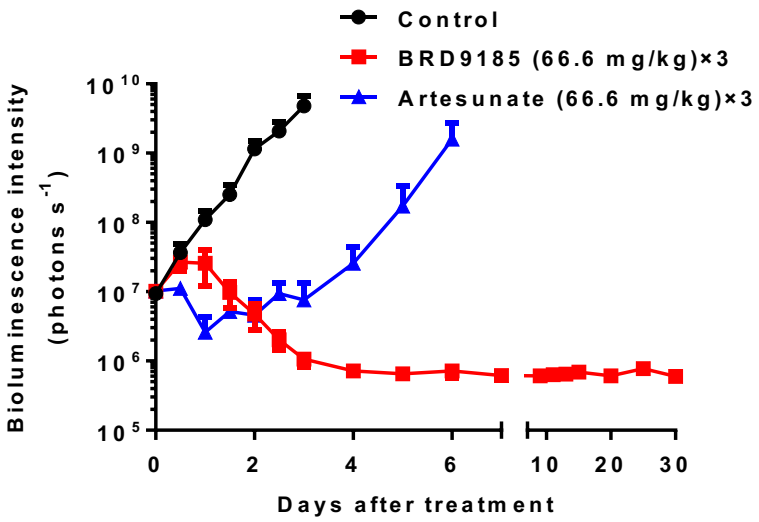


Figure 3.7. *In vivo* efficacy studies of BRD9185 using *P. berghei* mouse model. CD-1 mice were infected with *P. berghei* (ANKA GFP-luc) at -24 h (Day -1) and dosed orally at 0, 24, and 48 h with BRD9185, artesunate, or vehicle solution. Infections were monitored using the *in vivo* imaging system (IVIS), and bioluminescence intensity was quantified from each mouse and plotted against time. Animals with parasitemia exceeding 25% were humanely euthanized.

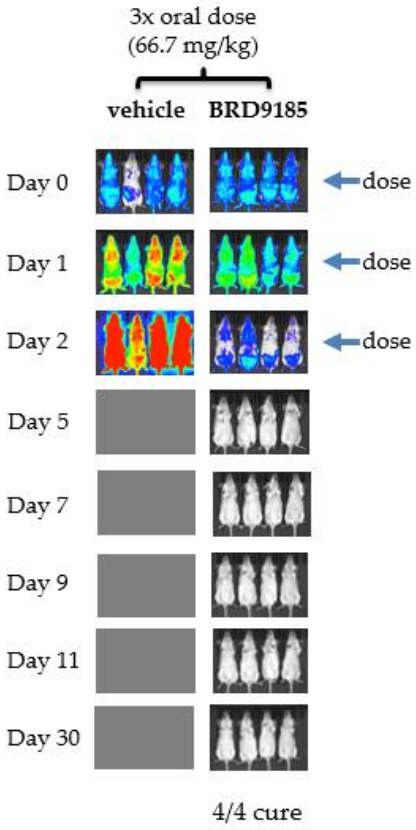


Figure 3.8. Corresponding mouse images for vehicle and BRD9185-treated mice in **Figure 3.7**. Mice were injected with luciferin and imaged using the *in vivo* imaging system (IVIS). Animals with parasitemia exceeding 25% were humanely euthanized.

These *in vivo* results are particularly interesting in light of the properties of DSM265 and the triazolopyrimidine series, which are not effective in the *P. berghei* (*Pb*) model due to poor binding to the *Pb*DHODH enzyme.[12,17] This raises the possibility that BRD9185 has a different mechanism of action from DSM265 on *Pf*DHODH. However, BRD9185 binds competitively with decylubiquinone (**Figure 3.9**), the same proposed binding site as DSM265.[14] X-ray crystallography studies are underway to gain insights into binding features of these compounds.

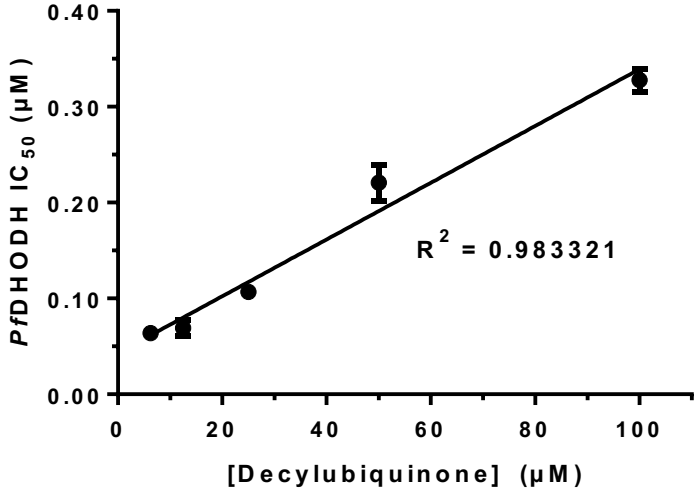
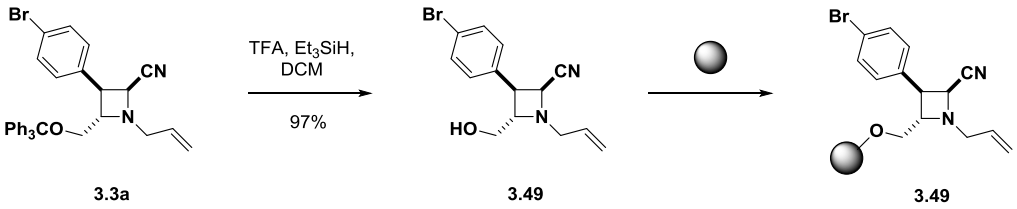


Figure 3.9. BRD9185 IC_{50} vs. varying concentrations of decylubiquinone.

3.2.6 Solid phase synthesis of BRD7539 urea analogues

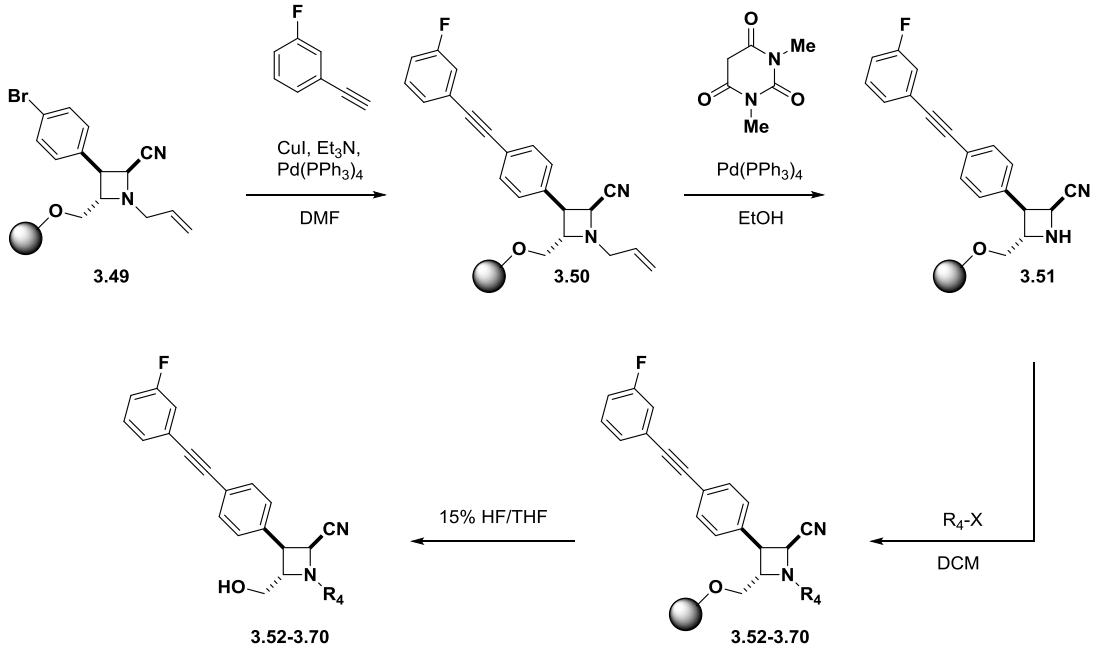
Structural variation of BRD7539 at the urea position has thus far remained unexplored. However, based on the synthetic route to analogues as described in **Scheme 3.2**, variation at this position would require three steps per analogue. Ideally, late-stage amine functionalization would occur at the last step of analogue synthesis, but this requires selective functionalization over the primary alcohol.

We hypothesized that a solid-phase synthesis approach might provide analogues at the azetidine nitrogen as the last step in analogue synthesis. Split-pool methods, which use polystyrene beads to tether compounds, are known for the efficient synthesis of structurally complex and diverse compounds.[23,24] To apply these known methods to a diverse intermediate, **3.3a** was deprotected using TFA to liberate the free alcohol **3.49**, which was tethered to a polystyrene bead (**Scheme 3.7**).



Scheme 3.7. Attachment of azetidine carbonitrile intermediates to polystyrene beads.

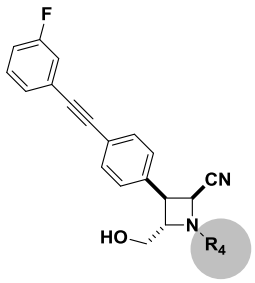
Sonogashira coupling with 1-ethynyl-3-fluorobenzene, allyl deprotection, and functionalization of the free amine with various electrophiles afforded analogues **3.52a-o** on solid-phase support (**Scheme 3.8**). Cleavage from the polystyrene bead using 15% HF in THF solution provided the desired analogues in solution phase. Of note, while beads are generally washed liberally with solvent after each transformation, which renders purification by column chromatography unnecessary,[23,24] analogues **3.52a-o** were subject to chromatography to ensure purity. Overall, this strategy successfully provided 15 analogues with last-step azetidine functionalizations, reducing number of steps per analogues, number of chromatographic separations, and time, albeit at the expense of super stoichiometric amounts of reagents such as $Pd(PPh_3)_4$ (see Chapter 5 for complete experimental details).



Scheme 3.8. Synthetic route to last-step functionalization on solid-phase.

In vitro activity of azetidine analogues emphasized the importance of the urea motif. The corresponding free azetidine **3.52** was inactive, and while small alkyl substitutions such as ethyl (**3.53**), butyl (**3.54**), cyclopropyl (**3.56**), and cyclobutyl (**3.57**) remained potent, activity against Dd2 parasites was reduced when substituent size was increased (**3.58-3.63**). Attempts to replace the urea with amides (**3.64, 3.68-3.70**), carbamates (**3.65**), and sulfonamides (**3.66, 3.67**) resulted in inactive compounds (note: **3.67-3.70** were obtained from the diversity-oriented synthesis library at the Broad Institute of MIT & Harvard; these compounds were not synthesized according to **Scheme 3.8**).

Table 3.5. Activity of BRD7539 and urea analogues against Dd2 parasites.



Cmpd	R_1	EC_{50} (μM)
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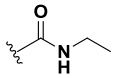
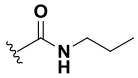
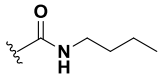
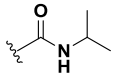
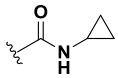
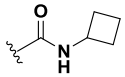
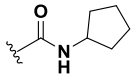
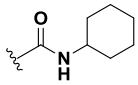
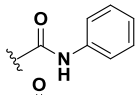
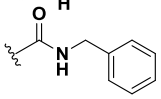
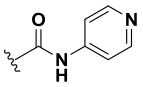
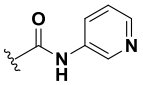
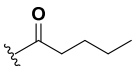
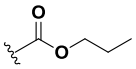
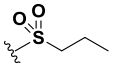
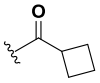
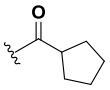
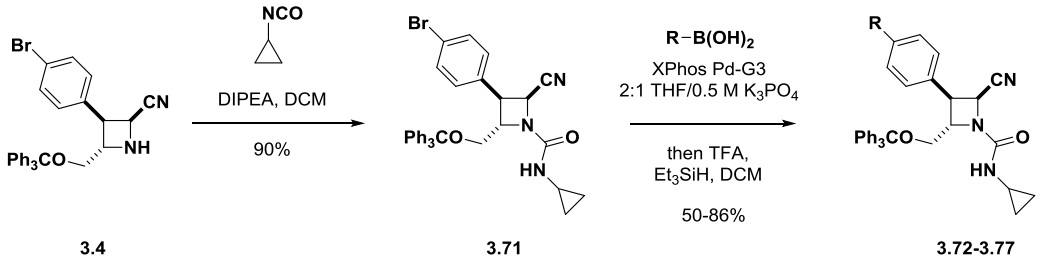
3.53		0.051
BRD7539		0.010
3.54		0.088
3.55		0.456
3.56		0.011
3.57		0.079
3.58		0.126
3.59		5.583
3.60		5.000
3.61		3.480

Cmpd	R_1	EC_{50} (μM)
3.62		2.315
3.63		7.457
3.64		5.000
3.65		19.500
3.66		12.250
3.67		10.732
3.68		5.000
3.69		9.696
3.70		21.000

3.2.7 Strategies to improve on BRD9185

While BRD9185 remains a promising compound with *in vivo* efficacy and favorable pharmacokinetic properties, it suffers from poor solubility (0.5 μM in PBS solution). During the course of these studies, we identified the need to improve on the physicochemical properties of

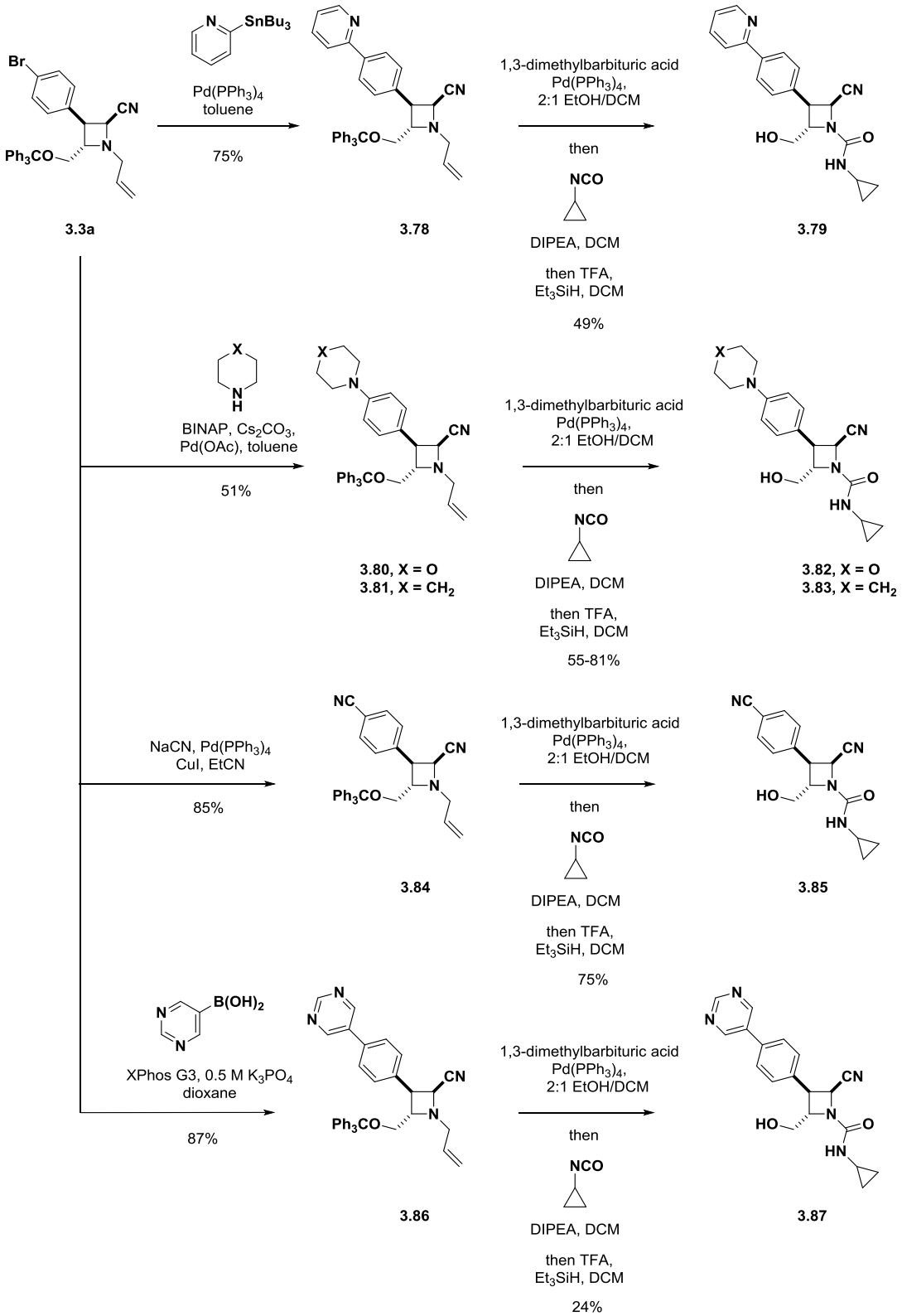
this azetidine carbonitrile series, including the removal of the bis-CF_3 motif on BRD9185. The identification of cyclopropyl urea analogue **3.56**, which was equipotent to BRD7539 but was predicted to be more soluble and metabolically stable *in vitro*,[25] led to a series of cyclopropyl urea analogues with various R_1 substitutions (**Schemes 3.9-3.11).**



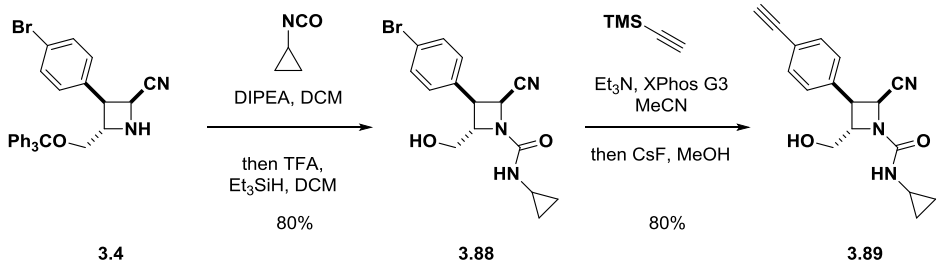
3.4 **3.71** **3.72-3.77**

Scheme 3.9. Synthetic route to analogues **3.72 – 3.77.**

Most analogues with substituents at the distal phenyl ring lost potency with replacement of one CF_3 group with a polar motif such as a nitrile (**3.72**) or heteroatom (**3.76**, **3.77**). Replacement of the second phenyl ring with a morpholine (**3.82**), piperidine (**3.83**), nitrile (**3.86**), pyrimidine (**3.87**), or alkyne (**3.89**) resulted in substantial loss of *in vitro* activity. However, trifluorobiphenyl analogue **3.73** (BRD1331) demonstrated equipotent activity compared to BRD9185. This compound was subsequently profiled for biochemical selectivity, *in vitro* stability, *in vivo* efficacy, and physicochemical properties.

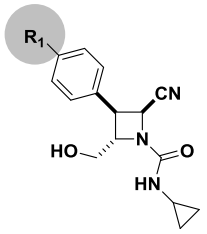


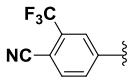
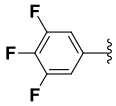
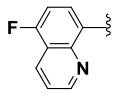
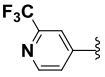
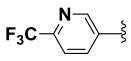
Scheme 3.10. Synthetic route to analogues **3.79**, **3.82**, **3.83**, **3.85**, and **3.87**.



Scheme 3.11. Synthetic route to analogue **3.89**.

Table 3.6. Activity of cyclopropyl analogues of BRD7539.



Cmpd	R_1	EC_{50} (μM)
3.72		0.424
3.73		0.009
3.74		0.113
3.75		0.160
3.76		0.624
3.77		0.171

Cmpd	R_1	EC_{50} (μM)
3.79		1.361
3.82		5.715
3.83		1.018
3.86		19.500
3.87		12.700
3.89		0.762

3.3 Profiling of BRD1331

3.3.1 Biochemical data

Table 3.7. Phenotypic and biochemical comparison of BRD1331 to DSM265.

Whole-cell EC_{50} (μM)	BRD1331	DSM265	DSM421
Dd2 strain	0.009	0.024	0.023
Biochemical IC_{50} (μM)			
*Pf*DHODH	0.038	0.014	0.053
*Pv*DHODH	0.0061	0.022	0.022
Human DHODH	> 100	~ 100	> 100
Mouse DHODH	> 100	2.3	18
Rat DHODH	> 100	2.7	22
Dog DHODH	> 100	16	35
Rabbit DHODH	> 100	> 100	> 100
Minipig DHODH	> 100	~ 100	> 100
Monkey DHODH	> 100	~ 100	> 100

BRD1331 was found to be highly selective for *Plasmodium* DHODH (**Table 3.7**). BRD1331 was a potent inhibitor of both *P. falciparum* (IC_{50} = 0.038 μM) and *P. vivax* (IC_{50} = 0.006 μM) DHODH but did not bind to any mammalian DHODH tested (IC_{50} > 100 μM, human, mouse, rat, dog, rabbit, minipig, and monkey). This compares favorably to clinical compound DSM265, which exhibits moderate (IC_{50} < 20 μM, mouse, rat, dog) to weak (IC_{50} ~ 100 μM, human, minipig, monkey) binding to the mammalian isoforms.

3.3.2 *Ex vivo* data

Table 3.8. Activity of BRD1331 against *ex vivo* parasites.

	P. falciparum		*P. vivax*	
	Number of isolates	**Median (range) (μM)**	**Number of isolates**	**Median (range) (μM)**
BRD1331	10	0.038 (0.022 – 0.061)	5	0.023 (0.016 – 0.026)
DSM265	12	0.190 (0.070 – 0.670)	16	0.918 (0.451 – 1.790)
DSM421	9	0.151 (0.085 – 0.281)	13	0.175 (0.056 – 0.373)

For *ex vivo* experiments, parasites derived from patients in Indonesia were cultured *in vitro* and treated with BRD1331. Gratifyingly, BRD1331 maintained potency in this *ex vivo* assay with EC_{50} = 0.022 – 0.061 μM against 10 isolated *P. falciparum* strains and EC_{50} = 0.016 – 0.026 μM against 5 isolated *P. vivax strains.* Of note, DSM265 and DSM421 perform less well in these assays; DSM265 has a 10x loss and 50x loss in activity against *P. falciparum* and *P. vivax*, respectively, when compared to its biochemical potency. These data are consistent with a lack of clinical efficacy against this species[26] and are alarming because *P. vivax* is prevalent on multiple continents.

3.3.3 *In vivo* efficacy and pharmacokinetic data

BRD1331 was assessed in two *in vivo* models. The first model exploited the rodent malaria parasite *P. berghei* in the blood stage, the same *in vivo* model used to assess BRD7539 and BRD9185 as shown previously. Infected CD-1 mice were treated with a *single dose* of BRD1331 or vehicle (70% PEG300, 30% solution of 5% dextrose in H_2O) at Day 0 (24 h following infection), and parasite-associated bioluminescence was monitored for 30 days (**Figure 3.10**). A mouse treated with BRD1331 (n = 1) exhibited parasite clearance for 9 days

after a single dose, although recrudescence occurs around Days 10 or 11. While these data cannot be directly compared to BRD9185 due to the different dosing regimen and lack of statistical power (n = 1 for this experiment), it provided evidence that BRD1331 was a promising compound that warranted additional follow up.

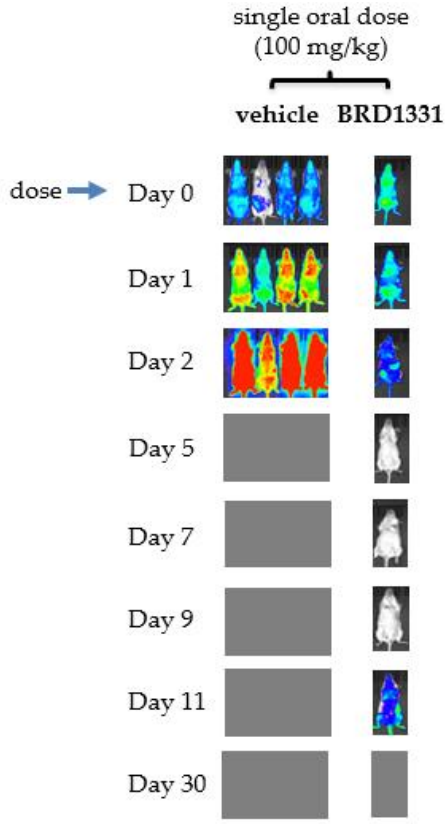


Figure 3.10. *In vivo* efficacy studies of BRD1331 using *P. berghei* mouse model. CD-1 mice were infected with *P. berghei* (ANKA GFP-luc) at -24 h (Day -1) and dosed orally at 0 h with BRD1331 or vehicle solution. Infections were monitored using the *in vivo* imaging system (IVIS), and bioluminescence intensity was measured at each time point specified.

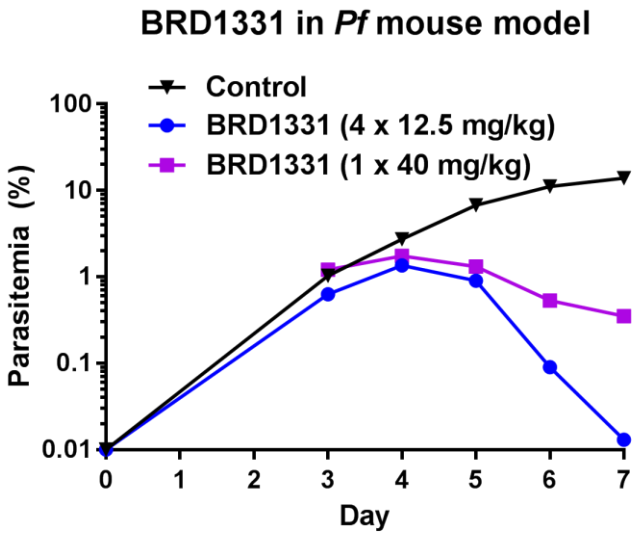


Figure 3.11. *In vivo P. falciparum* efficacy studies of BRD1331. *P. falciparum Pf3D7*$^{0087/N9}$ in NODscidIL2Rγ^{null} mice engrafted with human erythrocytes. Mice (n = 2 for all treatment arms) were infected at Day 0 and dosed orally at either Day 3 (single dose, 40 mg/kg) or Days 3, 4, 5, and 6 (4x dose, 12.5 mg/kg or vehicle). Infections were monitored via FACS analysis.

BRD1331 was also assessed in a *P. falciparum* model exploiting non-obese diabetic/severe combined immunodeficiency (NODscidIL2Rγ^{null}) mice engrafted with human erythrocytes (**Figure 3.11**). This model correlates well with human challenge models[27,28] despite the use of immunodeficient mice. Mice (n = 2 for all treatment arms) were dosed orally at either Day 3 (single dose, 40 mg/kg) or Days 3, 4, 5, and 6 (4x dose, 12.5 mg/kg or vehicle), and infections were monitored via FACS analysis until Day 7, at which point mice were humanely euthanized. Mice treated with 4×12.5 mg/kg displayed substantial reduction in parasitemia, including one mouse that displayed undetectable levels of parasite. The single 40 mg/kg dosing regimen was less efficacious, although a > 90% reduction in parasites was still observed, and parasitemia was ca. 1% on Day 7.

Table 3.9. Pharmacokinetic properties of BRD1331 in mice and rats.

	Mouse PK	Rat PK
$t_{1/2}$ (h)	17.6	16.3
C_0 (μM)	10.3	2.5
C_{max} (μM)	29.8	6.3
$DNAUC_{0\text{-}inf}$ (μM·h)	152	209
V_{ss} (L/kg)	0.27	1.54
CL (mL/min/kg)	0.22	1.09
F (%)	85	82

$t_{1/2}$, terminal half-life; C_0, initial serum concentration at t = 0; C_{max}, peak serum concentration; $DNAUC_{0\text{-}inf}$, dose-normalized area under the plasma concentration vs. time curve following PO dosing; V_{ss}, volume of distribution at steady state; CL, plasma clearance; F%, bioavailability. IV dosing in 5% DMSO/10% cremophor/85% H_2O at 0.25 mg/mL (1 mg/kg). PO dosing in 70% PEG300/30% (5% dextrose in H_2O) at 0.50 mg/mL (5 mg/kg). n = 3 mice in both IV and PO groups.

In light of the efficacy of BRD1331 in both *P. berghei* and *P. falciparum* models, pharmacokinetic data was obtained in both mice and rats (**Table 3.9**). BRD1331 has high bioavailability (> 80%) and long half-lives (> 16 h) in both animals, and PK data matches or exceeds that of BRD9185. Clearance is 5x higher in rats than in mice but is generally low.

3.3.4 *In vitro* profiling of BRD1331

BRD1331 was next profiled in a variety of *in vitro* assays to gauge its potential as a preclinical candidate and to identify potential toxicity issues. A large motivation of moving away from BRD9185 was aimed at enhancing the physicochemical properties (namely, solubility) of

analogues in this series. In three kinetic solubility assays (**Table 3.10**), BRD1331 proved as or much more soluble than both BRD9185 and DSM265.

Table 3.10. Solubility and plasma protein binding data for BRD1331.

Solubility	BRD1331	BRD9185	DSM265
PBS (μM)	58	0.5	2.3
FaSSIF (μM)	115.4	3.3	16.3
FeSSIF (μM)	67.4		88.5

In vitro stability assays showed that BRD1331 is expected to have moderate microsomal clearance and high hepatocytes clearance, especially in humans (**Table 3.11**). Interestingly, the *in vitro* mouse microsomal clearance is higher than the observed *in vivo* mouse microsomal clearance. This is likely a result of the high plasma protein binding of BRD1331: high plasma protein binding (99.8%) would shield BRD1331 from intrinsic clearance mechanisms as only 0.2% of BRD1331 is circulating in blood, which would artificially lower the observed clearance values. In fact, in the *in vitro* assay, BRD1331 is 38.5% unbound (61.5% bound), and higher clearance values are observed.

Table 3.11. *In vitro* stability and protein binding data for BRD1331.

In vitro data	BRD1331
Mouse microsomes CL_{int} (μL/min/mg)	25.2
Human microsomes CL_{int} (μL/min/mg)	21.4
Microsomal binding (mouse/human) (%)	61.5/80.4
Mouse/rat/dog hepatocytes CL_{int} (μL/min/10^6)	<6.4
Monkey hepatocytes CL_{int} (μL/min/10^6)	8.1
Human hepatocytes CL_{int} (μL/min/10^6)	14.0
Plasma protein binding (mouse/human) (%)	99.8/98.5

Finally, BRD1331 was profiled in a handful of assays to identify any off-target toxicity. BRD1331 displayed CC_{50} > 50 μM in both HepG2 and A549 human cell lines and was non-mutagenic in an Ames test.[29,30] However, BRD1331 was found to bind weakly to hERG (K_V11.1), COX1, COX2, and Na^+ channels (IC_{50} ~ 10 μM for all four). Efforts to reduce high predicted clearance and remove off-target binding are ongoing.

Table 3.12. Limited safety profiling of BRD1331.

Safety profiling	
Dd2 EC_{50} (μM)	0.015
HepG2 CC_{50} (μM)	>50
A549 CC_{50} (μM)	>50
hERG QPatch IC_{50} (μM)	~ 10
AMES test (+/- S9)	Non-mutagenic
CEREP panel (10 μM)	COX1, COX2, Na^+ channel All others (42) no inhibition

3.4 Conclusions

BRD7539 was identified as having *in vitro* and *in vivo* activity in both blood and liver stage parasites. Biochemical assays confirmed that BRD7539 targets dihydroorotate

dehydrogenase (DHODH), and a medicinal chemistry campaign identified BRD9185, which cured *P. berghei*-infected mice after three doses. However, BRD9185 had unfavorable physicochemical properties, which led to the identification of BRD1331. BRD1331 exhibits robust *in vivo* activity in both *P. berghei* and *P. falciparum* models. Unlike clinical candidate DSM265, BRD1331 displays high selectivity for *Plasmodium* DHODH over the mammalian DHODH and potency against both *P. falciparum* and *P. vivax* in *ex vivo* assays. However, *in vitro* profiling identified the potential for high clearance *in vivo* and weak off-target binding to enzymes and ion channels. Ongoing and future work seeks to address these issues and identify an inhibitor with the potential to advance to preclinical studies.

3.5 Contributions

Nobutaka Kato performed all *in vitro* assays against parasites, including those shown in Table 3.1, as well as all *in vivo* experiments with the exception of Figure 3.11. Competitive binding experiments were performed by Felipe Calil. Initial target validation with BRD7539 was performed by the author, but biochemical data for BRD1331 against *Plasmodium* and mammalian DHODH was performed by Meg Phillips. *Ex vivo* data was generated by Griffith University (Australia). *In vivo P. falciparum* data in Figure 3.11 was generated by Swiss Tropical and Public Health Institute. Pharmacokinetic data was performed by WuXi. *In vitro* stability and solubility assays were performed by The Broad Institute, WuXi, or EuroFins. All other data, including the design and synthesis of all analogues in this chapter, were generated by the author.

3.6 References

[1] Kato, N. *et al*. "Diversity-oriented synthesis yields novel multistage antimalarial inhibitors." *Nature* **2016**, 538, 344-349.

[2] Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B. "Specific role of mitochondrial electron transport in blood-stage Plasmodium falciparum." *Nature* **2007**, 446, 88-91.

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Chapter 4

Stereospecific, directed C(sp^3)-H arylation on azetidines

4.1 Introduction

A third compound identified from the high-throughput screen of interest to us was bicyclic azetidine BRD7929, which we found to inhibit novel target phenylalanyl tRNA synthetase.[1] BRD7929 exhibits activity in all stages of the parasite life cycle and is curative in multiple *in vivo* mouse models. However, a lengthy synthetic route to bicyclic azetidine BRD7929 (21 steps) hampers the ability to evaluate analogues systematically *in vivo.* Analysis of stereochemistry-based structure-activity relationships revealed that two of eight possible stereoisomers of parent compound BRD3444 – (2*S*,3*R*,4*R*) and (2*R*,3*R*,4*R*) – were active against a multidrug-resistant strain of malaria (*P. falciparum*, strain Dd2) (**Figure 4.1**). This observation suggested that substituents at one of the stereocenters (C2) were not necessary for activity. Indeed, BRD3914, an analogue lacking substitution at C2, was found to retain substantial antimalarial activity *in vitro* (EC_{50} = 13 nM, Dd2 strain).[2,3]

Production cost constitutes an important consideration in the development of antimalarial therapeutics. For example, Medicines for Malaria Ventures (MMV) notes that antimalarial drugs should ideally cost <$0.25 per dose for adults and <$0.05 per dose for infants.[4] We recognized in BRD3914 an opportunity to achieve an efficient synthesis of this class of antimalarials that proceeds with high regio- and stereoselectivity of key transformations in fewer steps than the

previous synthesis, and that minimizes the number of chromatographic purifications, which are known to drive both cost and waste output.[5] To this end, we developed a stereospecific C(sp^3)-H arylation of azetidines, enabling the preparation of all four stereoisomers of members of this series. The (2*R*,3*S*) stereoisomer of this building block led to an expeditious synthesis of antimalarial BRD3914. Subsequent *in vivo* evaluation in *P. falciparum*-infected humanized mice showed parasite clearance and lack of recrudescence after four oral doses, resulting in a durable cure.

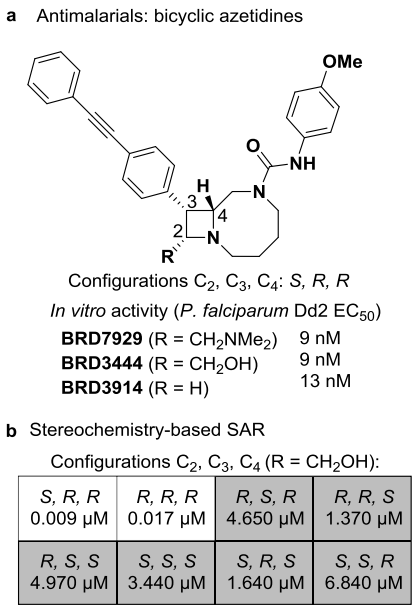
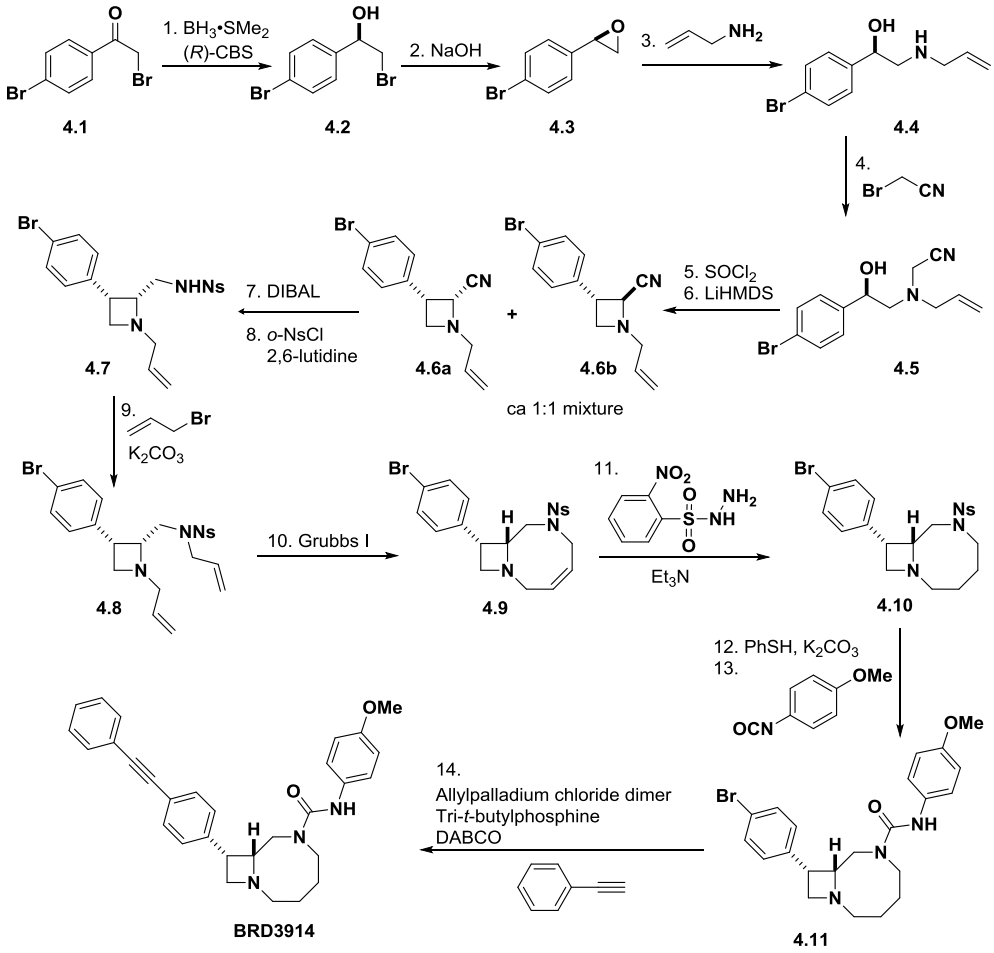


Figure 4.1. Overview of bicyclic azetidines. **a,** Structure and *in vitro* antiplasmodial activities of representative bicyclic azetidines. **b,** Stereochemical-based structure-activity relationships.

A key step in the original synthesis of BRD3914 (14 steps) and other bicyclic azetidines comprises a base-mediated intramolecular cyclization from **1** that affords azetidine-2-carbonitrile **2** with little or no diastereoselectivity (*ca*. 1:1, **Scheme 4.1**).[2,6] To date, intramolecular

cyclization from complex starting materials remains the predominant approach to access substituted azetidines.[7,8] Following azetidine ring formation of **4.6a**, nitrile reduction with DIBAL, protection with *o*-nitrobenzenesulfonyl chloride, and allylation gives precursor **4.8** for the key ring-closing metathesis to generate **4.9**. While the previous DOS route provided a versatile starting point for an initial SAR assessment focused, for example, on the 4-methoxyurea region, it limited the ability to assess other regions of the scaffold. Notably, various aryl and heteroaryl derivatives at the C3 position of the azetidine require the execution of the entire 14-step synthesis, and the introduction of functional groups on the eight-membered ring relies on olefin chemistry and thus presents regio- and stereoselectivity challenges.



Scheme 4.1. Scheme for previous synthesis of BRD3914.

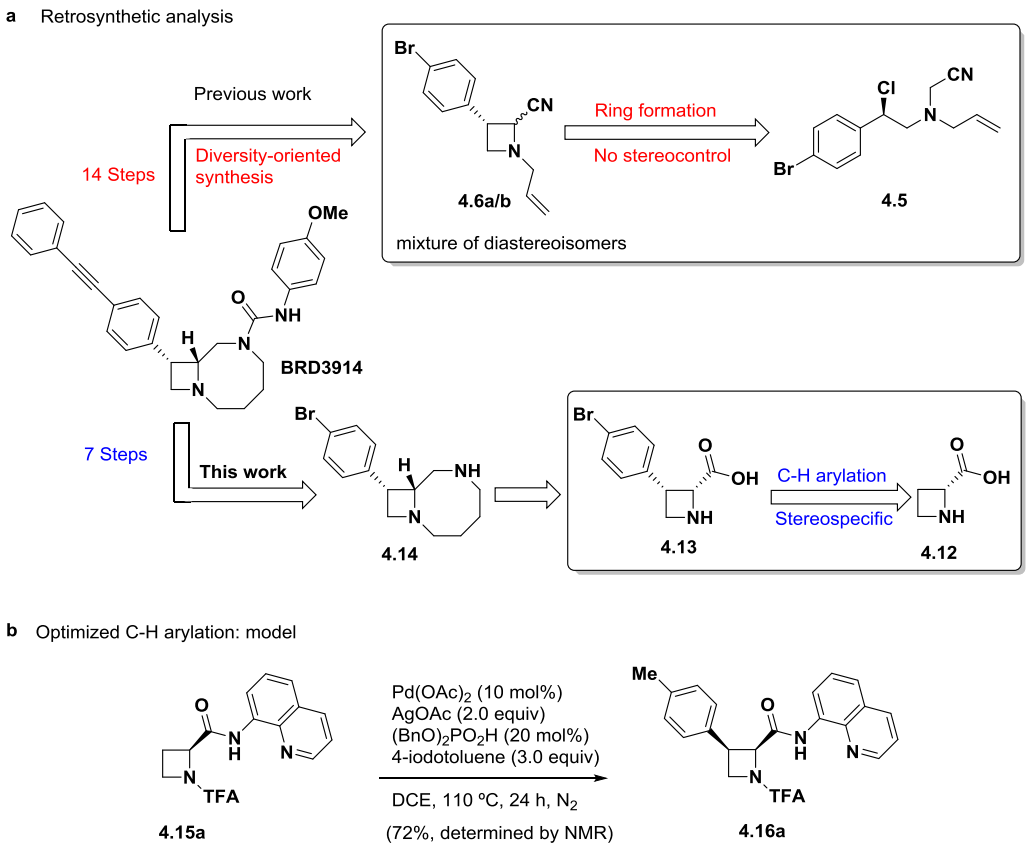


Figure 4.2. Overview of C-H arylation strategy. **a,** Comparison of approaches to BRD3914. **b,** Model system and optimized protocol for the C-H arylation of azetidines.

In a revised retrosynthetic analysis (**Figure 4.2a**), we reasoned that a bifunctional linker could be used in an advantageous way to form the eight-membered ring (diamine **4.14**). This approach would rely on *cis*-substituted azetidine **4.13** as a precursor, which we envisioned arising from a directed C-H arylation using commercially-available D-azetidine-2-carboxylic acid **4.12**. Since the first examples exploiting 8-aminoquinoline as a directing group by Daugulis and co-workers,[9,10] C(sp^3)-H functionalization methods have grown[11,12,13,14] and been applied to a number of challenging scaffolds, including cyclopropanes,[15,16,17,18,19,20,21]

cyclobutanes,[15,16,17,22,23,24,25,26,27] cyclopentanes,[10,28,29] pyrrolidines,[30,31,32] and piperidines.[33,34,35] There are few reports of the use of azetidines as a substrate[36] or product[37,38] in C-H functionalization, and to our knowledge the C3 arylation of azetidines has not been described. In this chapter, I describe the synthesis and *in vivo* evaluation of BRD3914, which was enabled by C3 arylation on a chiral azetidine building block. Notably, this C(sp^3)-H arylation gives access to chiral, disubstituted azetidines in a controlled manner from commercially available L- and D-azetidine-2-carboxylic acid.

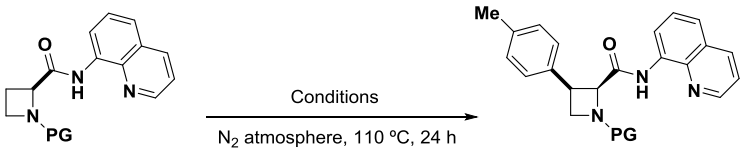
4.2 Method Development

4.2.1 Reaction screening

Pyrrolidines have been shown to be substrates for directed C3 arylation by multiple groups. [30,31,32] Unfortunately, initial attempts to extend the known methods to azetidines resulted in 8-9% yield by NMR (**Table 4.1**). Additional attempts to extend known methods using cyclobutanes[22,23,24] as substrates resulted in decomposition (not shown). Of note, the reaction conditions and substrate protecting group vary significantly between published methods, suggesting that screening reaction components might increase the yield of the desired reaction.

Following substantial optimization on a model system (**Tables 4.2-4.9**), we found that an unconventional *N*-trifluoroacetamide (*N*-TFA) protecting group[39] and the additive $(BnO)_2PO_2H$[15] were critical to reaction efficiency. Under the optimized conditions – aryl iodide (3 equiv), $Pd(OAc)_2$ (10 mol%), $(BnO)_2PO_2H$ (20 mol%), AgOAc (2 equiv), DCE (1.0 M), 110 °C – model product **4.16a** was formed in 72% yield as determined by ^{1}H NMR with internal standard. The reaction exhibited optimal performance under N_2 atmosphere, though it afforded product in 52-56% yield under air or O_2.

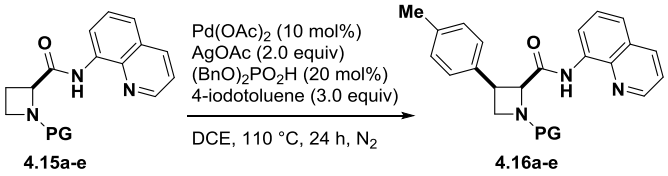
Table 4.1. Testing of literature C-H arylation conditions on an azetidine scaffold.



Entry	PG	Conditions	Yield [%][a]
1	Cbz	AgOAc (1.8 equiv), 4-iodotoluene (1.8 equiv), $Pd(OAc)_2$ (5 mol%), neat[b]	8
2	Piv	$(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene (5.0 equiv), $Pd(OAc)_2$ (10 mol%), toluene (0.1 M)[c]	9

[a]NMR yields are based on ^{1}H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). Reaction performed on 0.2 mmol scale. [b]Conditions reported in *Org. Lett.* **16**, 4956-4959 (2014). [c]Conditions reported in *Eur. J. Org. Chem.* 142-151 (2015).

Table 4.2. Development of a C-H arylation method for an azetidine scaffold (key examples).

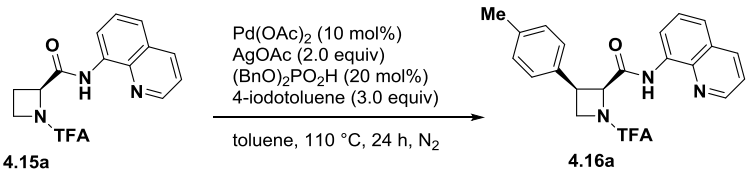


4.15a-e **4.16a-e**

Entry	PG (Substrate)	Variation from optimized conditions[a]	Yield (%)[b]
1	TFA (**4.15a**)	none	72
2	Boc (**4.15b**)	toluene, air atm.	n.d.
3	Cbz (**4.15c**)	toluene, air atm.	6
4	Piv (**4.15d**)	toluene, air atm.	30
5	TCA (**4.15e**)	toluene, air atm.	39
6	TFA (**4.15a**)	toluene, air atm.	52
7	TFA (**4.15a**)	toluene, O_2 atm.	56
8	TFA (**4.15a**)	toluene	65
9	TFA (**4.15a**)	no $(BnO)_2PO_2H$	31
10	TFA (**4.15a**)	no $Pd(OAc)_2$	n.d.
11	TFA (**4.15a**)	no AgOAc	n.d.
12	TFA (**4.15a**)	NaOAc (2 equiv) instead of AgOAc	n.d.

[a]Optimized reaction conditions: 0.2 mmol of substrate, $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), aryl iodide (3.0 equiv), and $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, 24 h. [b]Yields are based on ^{1}H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). TFA: Trifluoroacetyl, n.d.: not detected.

Table 4.3. Screening of reaction concentrations.

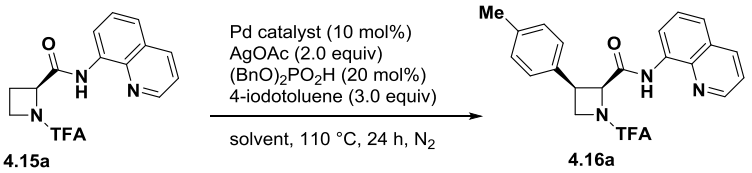


4.15a **4.16a**

Entry	c [M]	Yield [%][a]
1	neat	60
2	2.0	62
3	1.0	65
4	0.25	35

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), toluene (different concentrations), N_2 atmosphere, 110 ºC, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene).

Table 4.4. Screening of Pd catalysts and solvents.

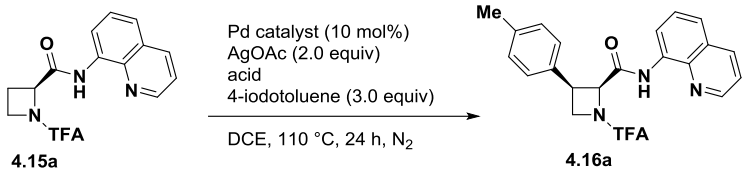


4.15a **4.16a**

Entry	Pd catalyst	solvent	Yield [%][a]
1	no Pd catalyst	toluene	n.d.
2	$Pd(TFA)_2$	toluene	47
3	$PdCl_2$	toluene	66
4	$Pd(OAc)_2$	toluene	65
5	$PdCl_2$	DCE	67
6	$Pd(OAc)_2$	DCE	72
7	$Pd(OAc)_2$	chlorobenzene	58
8	$Pd(OAc)_2$	trifluorotoluene	56
9	$Pd(OAc)_2$	*m*-xylene	53
10	$Pd(OAc)_2$	mesitylene	37
11	$Pd(OAc)_2$	1,4-dioxane	41
12	$Pd(OAc)_2$	*t*-AmylOH	47
13	$Pd(OAc)_2$	*t*-BuOH	n.d.
14	$Pd(OAc)_2$	9:1 toluene/*t*-AmylOH	63

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene (3.0 equiv), Pd catalyst (10 mol%), solvent (1.0 M), N_2 atmosphere, 110 ºC, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). n.d.: not detected.

Table 4.5. Screening of acids.

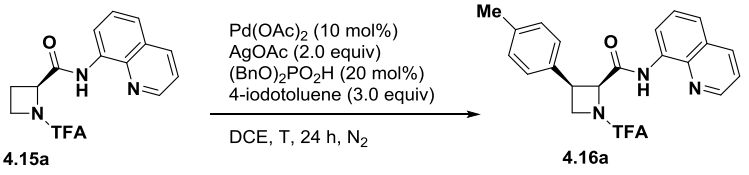


4.15a **4.16a**

Entry	acid	[mol%] acid	Yield [%][a]
1	no acid	-	31
2	PivOH	100	60
3	$(BnO)_2PO_2H$	10	60
4	$(BnO)_2PO_2H$	20	72
5	$(BnO)_2PO_2H$	30	56
6	$(BnO)_2PO_2H$	40	50
7	$(BnO)_2PO_2H$	50	68
8	$(BnO)_2PO_2H$	100	51
9	$(PhO)_2PO_2H$	20	n.d.

*Reaction conditions: 0.2 mmol of **4.15a,** acid, AgOAc (2.0 equiv), 4-iodotoluene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). n.d.: not detected.

Table 4.6. Screening of temperatures.

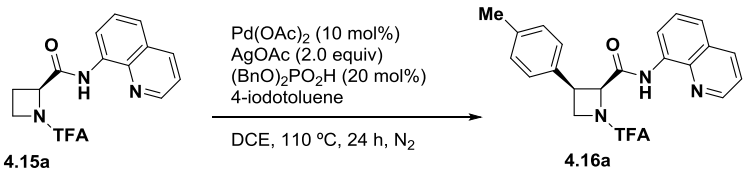


4.15a **4.16a**

Entry	T [°C]	Yield [%][a]
1	90	52
2	110	72
3	120	65
4	130	57

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, temperature, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene).

Table 4.7. Screening of aryl iodide stoichiometry.

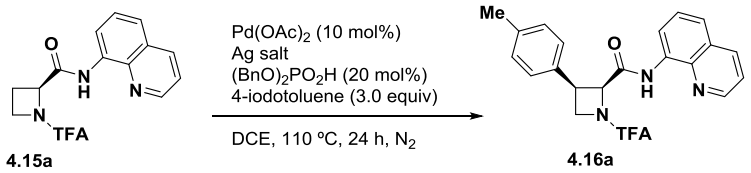


4.15a **4.16a**

Entry	4-iodotoluene [equiv]	Yield [%][a]
1	2	57
2	3	72
3	5	71
4	5[b]	72

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene, $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene), [b]Reaction performed without solvent.

Table 4.8. Screening of Ag salts.

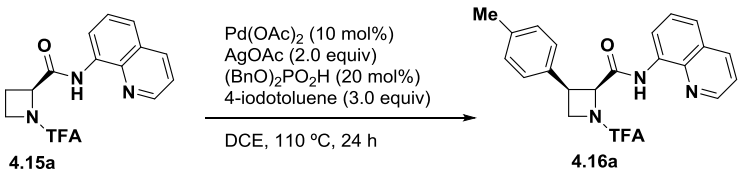


4.15a **4.16a**

Entry	Ag salt	amount [equiv]	Yield [%][a]
1	no Ag salt	-	n.d.
2	AgOAc	1.1	58
3	AgOAc	1.5	69
4	AgOAc	2	72
5	AgOAc	3	66
6	Ag_2CO_3	2	n.d.

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), Ag salt, 4-iodotoluene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, 24 h. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). n.d.: not detected.

Table 4.9. Screening of reaction time.



4.15a 4.16a

Entry	time [h]	Yield [%][a]
1[c]	0.5	26
2[c]	1	37
3[c]	2	46
4[c]	4	54
5	8	61
6	16	66
7	24	72
8	40	70

*Reaction conditions: 0.2 mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 4-iodotoluene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, time. [a]NMR yields are based on ^1H NMR integration relative to an internal standard (1,3,5-trimethoxybenzene). [b]Conversion is based on ratio of desired product to desired product plus starting material. [c]**ent-4.15a** was used as the substrate.

4.2.2 Reaction scope and directing group cleavage

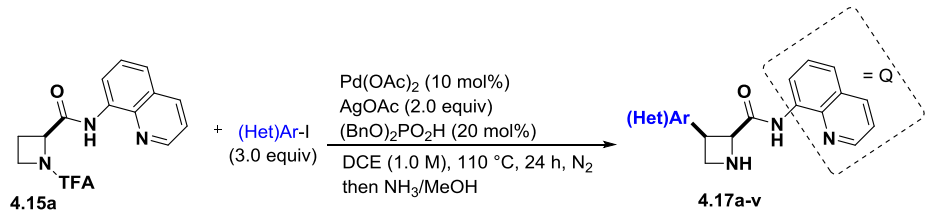
N-TFA is known to undergo hydrolysis readily, releasing the corresponding amine in mild alkaline solutions,[40] raising the possibility that the azetidine could be deprotected directly after the arylation. We were delighted to find that upon completion of the C-H arylation, the *N*-TFA group could be cleaved in the crude reaction mixture by the addition of 7.0 N NH_3 in MeOH to afford the free azetidine. The one-pot C-H arylation/deprotection sequence was performed on a range of aryl iodides to demonstrate the broad applicability of this method (**Table 4.10**). The reaction tolerated a variety of 3- and 4-substituted aryl iodides (**4.17a-m**), including both electron-donating and electron-withdrawing groups. Halogenated aryl-iodide derivatives performed well and provide sites for further diversification. Exceptions include 2-fluoro-iodobenzene and 2-methyliodobenzene, which gave 32% isolated product (**4.17n**) and no product (**4.17o**), respectively, likely due to steric congestion around a palladacycle intermediate

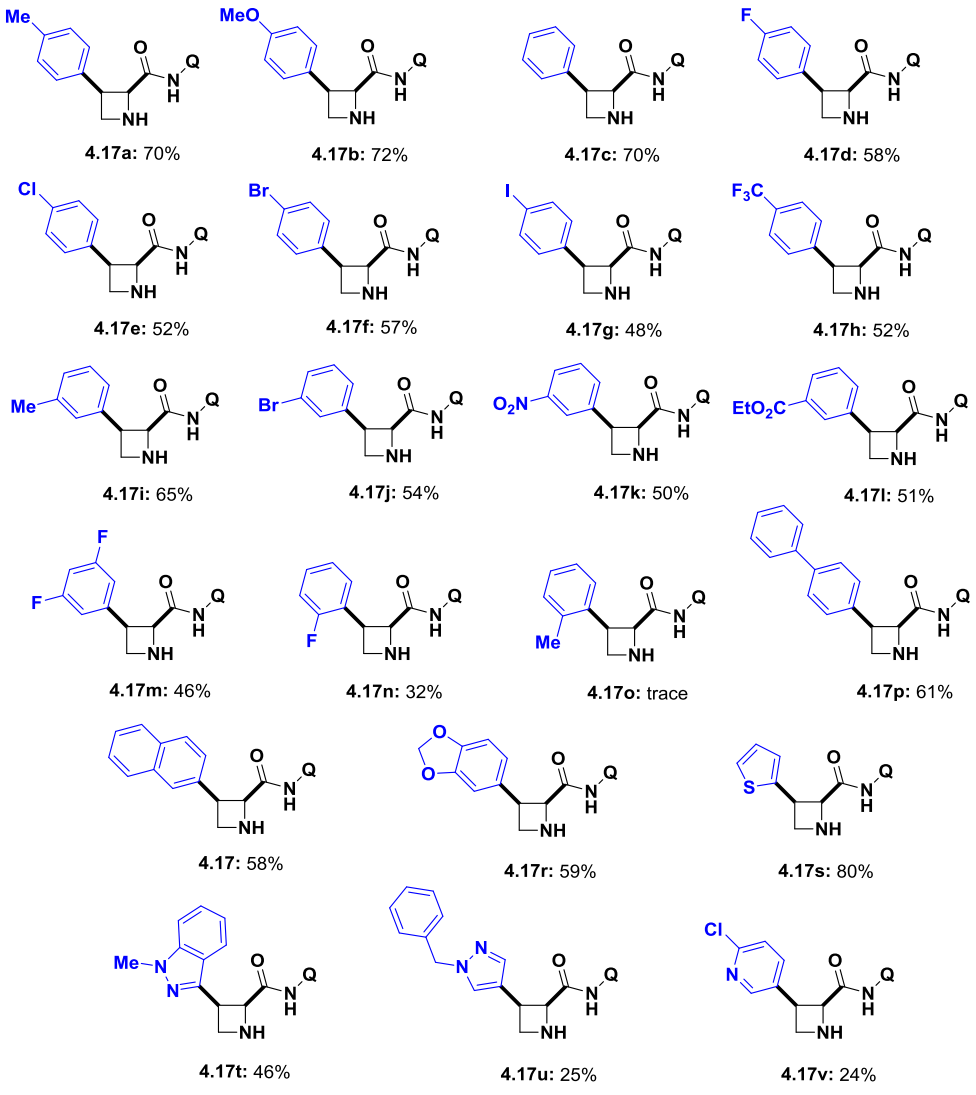
as proposed in previous studies.[30] Increased yields were obtained with 4-iodobiphenyl (**4.17p**) and 2-iodonaphthalene (**4.17q**). Arylation with 2-iodothiophene (**4.17s**) proceeded in 80% yield while other heterocyclic iodides were found to react less efficiently (**4.17t-v**). In particular, no *ee* erosion is observed during the course of the reaction, and products are isolated as single stereoisomers.

Encouragingly, the homologous pyrrolidine (**4.18**) and piperidine (**4.20**) analogues yielded products **4.19** and **4.21** in 95% and 72% isolated yields, respectively (**Figure 4.3a**), demonstrating the utility of these reaction conditions in the installation of C3 aryl groups on other cyclic amines.

Next, we explored reaction conditions to remove the directing group (**Figure 4.3b**). This would enable access to broad, well-defined chemical space in only a few steps from commercial material.[22,41] We chose aryl bromide **4.17f** as the substrate for these diversification studies given the possibility for further elaboration of the aromatic ring. Global Boc installation on (+)-**4.17f** followed by a $LiOH/H_2O_2$-mediated cleavage[23,42] of the 8-aminoquinoline provided *cis*-(+)-**4.22** in 69% yield with retention of stereochemistry at the α-position without need for purification by column chromatography. Subjecting the reaction to NaOH/EtOH at 110 °C afforded *trans*-(−)-**4.23** in 81% yield, thus achieving both removal of the auxiliary and epimerization at the α-carbon. These conditions were then applied to (−)-**4.17f** and provided (−)-**4.22** and (+)-**4.23** in 72% and 77% yield, respectively. Thus, any one of the four stereoisomers could be prepared in three steps with the right choice of substrate (L- or D-azetidine-2-carboxylic acid) and auxiliary removal conditions.

Table 4.10. Substrate scope of C-H arylation method.





The values beneath each structure indicate the isolated yields after column chromatography.

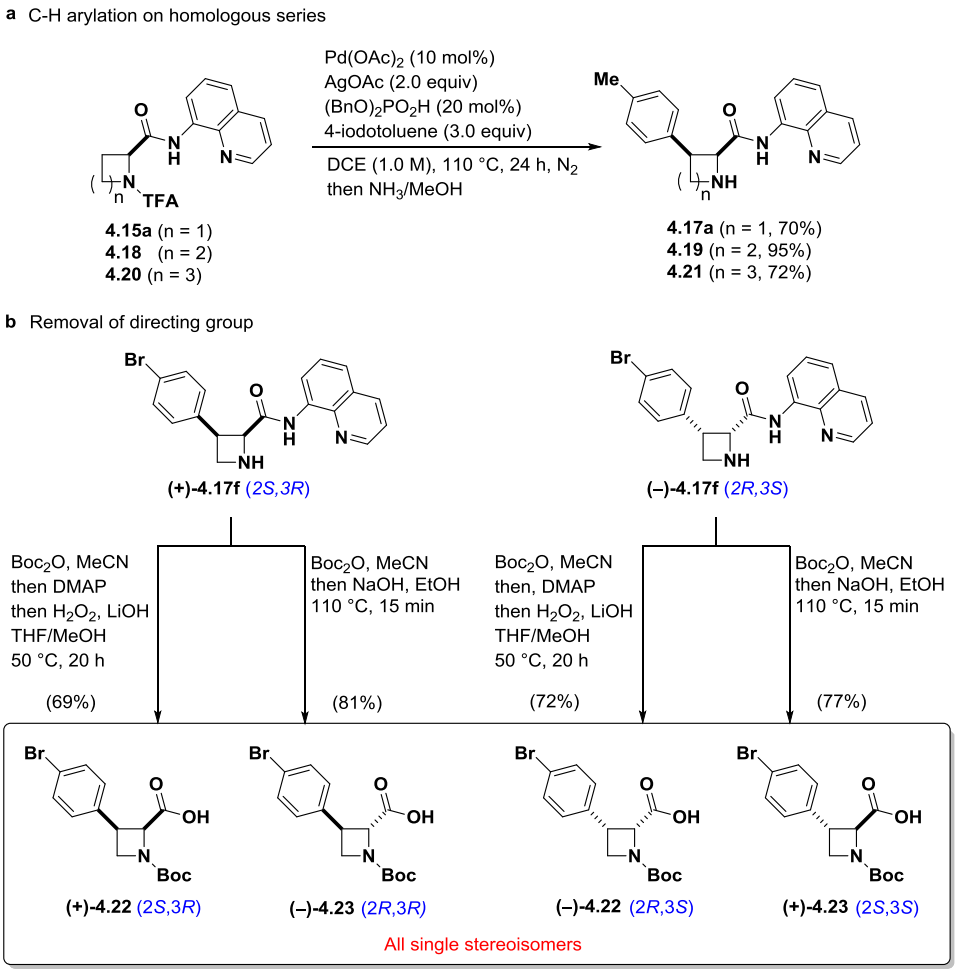


Figure 4.3. Versatility of the C-H arylation method. **a,** C-H arylation method to access homologous series of C-H arylation products. **b,** Varying conditions for directing group removal to access selectively *cis* and *trans* key building blocks; all compounds are isolated as single stereoisomers with dr >20:1 and ee >99%.

4.2.3 Proposed mechanism

We propose, like others,[30] that the reaction proceeds through a Pd(II)/Pd(IV) cycle (**Figure 4.4**) whereby the 8-aminoquinoline serves as a bidendate ligand in the reaction. Following concerted metalation-deprotonation (C-H palladation), insertion of the aryl iodide to

form the highly reactive Pd(IV) species may be assisted by a silver dibenzylphosphate salt formed *in situ*. Rapid reductive elimination followed by protonation/Pd(II) dissociation would give the desired product. As mentioned previously, we hypothesize that *ortho*-substituted aryl iodides may interfere with oxidative addition, resulting in poor or no conversion to product.

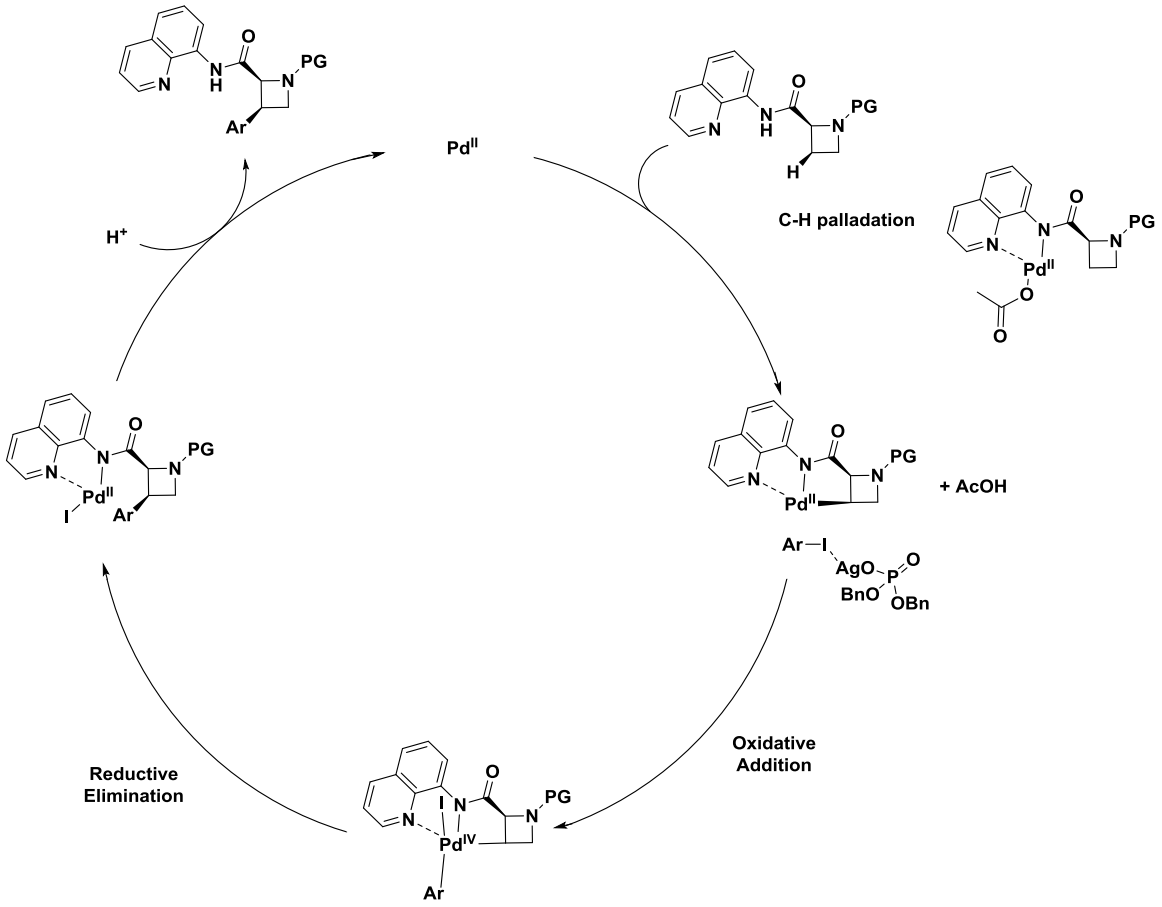


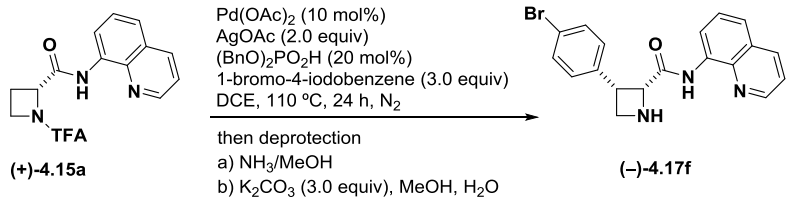
Figure 4.4. Proposed reaction mechanism for C-H arylation reaction.

4.3 Synthesis of BRD3914 using C-H arylation

Having established conditions for the C-H arylation and removal of the directing group, we turned our attention to the synthesis of BRD3914. TFA-protection and installation of 8-aminoquinoline on D-azetidine-2-carboxylic acid (+)-**4.12** (10.0 g) afforded 26.3 g of (+)**-4.15a**

in 82% yield and could be achieved in a single reaction vessel (**Figure 4.5**). The use of only 0.95 equivalents of DIPEA appeared critical to avoid epimerization at the α-position during the HATU-mediated amide coupling. The key one-pot arylation and deprotection sequence was performed on 10-g scale with 1-bromo-4-iodobenzene and furnished (−)-**4.17f** in 52% yield with both relative and absolute stereochemistry confirmed by X-ray crystallography. Of note, we found that the reaction performed equally well on 0.3, 3.0, and 30.1 mmol scale (100 mg to 10.0 g, **Table 4.11**), although incomplete TFA-deprotection under standard 7.0 N NH_3 in MeOH conditions was observed on ≥3.0 mmol scale. In these cases, modified conditions using K_2CO_3 in 9:1 MeOH/H_2O completely removed the protecting group**.** Directing group cleavage from (−)-**4.17** with retention of *cis*-stereochemistry afforded gram quantities of the synthetically useful carboxylic acid building block (−)-**4.22**.

Table 4.11. Effect of reaction scale on C-H arylation reaction.



Entry	[mmol] of **6f**	mass [g] of **6f**	Yield [%]
1[a]	0.310	0.100	55
2[b]	3.095	1.000	50
3[b]	30.95	10.00	52

*Reaction conditions: mmol of **4.15a,** $(BnO)_2PO_2H$ (20 mol%), AgOAc (2.0 equiv), 1-bromo-4-iodobenzene (3.0 equiv), $Pd(OAc)_2$ (10 mol%), DCE (1.0 M), N_2 atmosphere, 110 ºC, 24 h then deprotection [a]NH_3/MeOH (7.0 N, 3.0 mL) or [b]K_2CO_3 (3.0 equiv), 9:1 MeOH/H_2O.

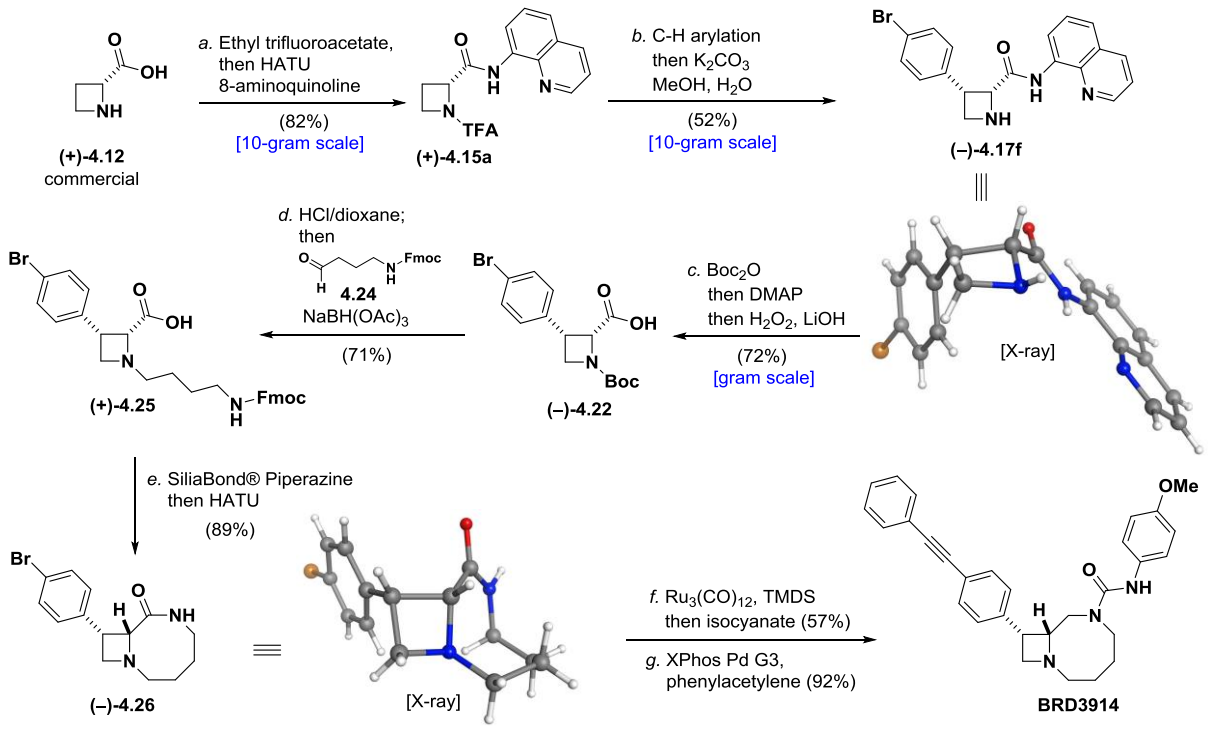


Figure 4.5. Stereocontrolled synthesis of BRD3914. Conditions: ***a.*** Ethyl trifluoroacetate, DIPEA, DMF, 50 °C, 15 h; evaporate volatile materials, then 8-aminoquinoline, HATU, DIPEA, DMF, 0 °C, 2 h (82%); ***b.*** $Pd(OAc)_2$ (10 mol%), AgOAc (2.0 equiv), $(BnO)_2PO_2H$ (20 mol%), 1-bromo-4-iodobenzene (3.0 equiv), DCE, 110 °C, 24 h; evaporate volatile materials, then K_2CO_3 (3.0 equiv), 9:1 MeOH/H_2O (52%); ***c.*** Boc_2O (3.0 equiv), MeCN, 50 °C, 15 min; then DMAP (0.1 equiv), 2 h; evaporate volatile materials, then H_2O_2 (10.0 equiv), LiOH (6.0 equiv), THF/MeOH (2:1), 0 °C, then 50 °C, 20 h (72%); ***d.*** HCl (4.0 M in dioxane, 15.0 equiv), DCM, 2 h; evaporate volatile materials, then **4.24** (2.5 equiv), $NaBH(OAc)_3$ (3.0 equiv), DCM, 12 h (71%); ***e.*** SiliaBond® Piperazine (3.0 equiv.), DMF, 50 °C, 2 h; then DIPEA (5.0 equiv), HATU (1.5 equiv), 20 min (89%); ***f.*** $Ru_3(CO)_{12}$ (10 mol%), 1,1,3,3-tetramethyldisiloxane (15.0 equiv), toluene, 90 °C, 24 h; then 4-methoxyphenylisocyanate (1.0 equiv), THF, 10 min (57%); ***g.*** XPhos Pd G3 (10 mol%), phenylacetylene (5.0 equiv), Et_3N (4.0 equiv), MeCN, 70 °C, 2 h (92%).

Next, deprotection of (−)-**4.17** using 4.0 M HCl in dioxane followed by reductive amination with bifunctional linker **4.24** (ref 43, 44) proceeded smoothly in a single flask, delivering (+)-**4.25** in 71% yield. Fmoc was removed using SiliaBond®-Piperazine, and sequential HATU-mediated intramolecular amide bond coupling led to lactam (−)-**4.26** (confirmed by X-ray crystallography) in 89% isolated yield in a single flask. The lactam reduction proved remarkably difficult as conventional reagents such as $LiAlH_4$ or $BH_3 \cdot Me_2S$ gave either competing protodebromination or adducts formation. Ultimately, we found that modified conditions reported by Reeves and colleagues[45] using $Ru_3(CO)_{12}$ and 1,1,3,3-tetramethyldisiloxane (TMDS) in toluene at 90 °C reduced the lactam, with the resulting diamine being trapped *in situ* with 4-methoxyphenyl isocyanate to afford the corresponding urea in 57% yield. Finally, a Pd-catalyzed Heck alkynylation with phenylacetylene delivered BRD3914 in 92% isolated yield. The synthesis of BRD3914 required 7 single-flask transformations (5 chromatographic separations) and proceeded in 10% overall yield from commercially available (+)-**4.12**.

4.4 *In vivo* evaluation of BRD3914

The short synthesis permitted further evaluation of the biological activity of BRD3914. First, the compound was confirmed to be potent *in vitro* (EC_{50} = 15 nM) against *P. falciparum* parasites (Dd2 strain), in line with previously measured activity.[2] Pleasingly, BRD3914 exhibited low *in vitro* cytotoxicity to human cell lines (A549 CC_{50} = 38 μM, HEK293 CC_{50} > 50 μM, HepG2 CC_{50} >50 μM). We then sought to evaluate the *in vivo* efficacy of this compound for the first time against blood-stage *P. falciparum* $3D7^{HLH/BRD}$ (expressing firefly luciferase) in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) $Il2r\gamma^{-/-}$ mice engrafted with

human erythrocytes (huRBC NSG). This mouse model has been shown to correlate well with human malaria challenge models.[4647] huRBC NSG mice were inoculated with parasites (-48 h) before administration with either 25 mg/kg or 50 mg/kg BRD3914 (single dose at 0 h, or multiple dosing at 0, 24, 48, and 72 h, formulated in 70% PEG300/30% solution of 5% dextrose/H_2O)[5] and monitored for 30 days (**Figures 4.6, 4.7**). Chloroquine was used as a positive control. After a single 50 mg/kg dose of BRD3914, a reduction in parasite-associated bioluminescence was observed in mice, but recrudescence occurred around day three. Gratifyingly, huRBC NSG mice treated with four q.d. (*quaque die*) doses[48] (25 mg/kg or 50 mg/kg) of BRD3914 were parasite-free after 30 days based on bioluminescent imaging. These results are striking compared to chloroquine treatment (4 × 25 mg/kg), where recrudescence of infection was observed in one of two mice.

While BRD3914 has a dosing shortcoming *in vivo* when compared to BRD7929, overall the compound succeeds in curing mice under a traditional Peters' test,[48] retains *in vitro* activity against *P. falciparum* parasites, and, most importantly, exhibits a more attractive safety profile.[1] In our *in vivo* studies, BRD3914 outperformed the antimalarial chloroquine, one of the most successful antimalarial agents to date.[49]

The biological studies presented herein were facilitated by the development of a Pd-catalyzed C(sp^3)-H arylation strategy, which allowed the efficient preparation of BRD3914 in seven steps with five chromatographic separations. This represents a significant improvement over previous syntheses of this class of inhibitors with respect to step count, chromatographic separations, and waste minimization. Notable features of the synthetic approach are the stereospecificity of the arylation and the controlled cleavage of the directing group, which affords any one of four C3-arylated azetidine carboxylic acids with judicious choice of substrate

and reaction conditions. The C-H arylation was performed on up to a 10-gram scale without a significant decrease in yield and provides a general strategy to C3-arylated azetidines, pyrrolidines, and piperidines. Given the substrate scope and the ability to explore stereochemical space, we envision that this method can be applied to the synthesis of other promising azetidine-containing antimalarials[50],[51] and antimicrobials[52] in addition to finding general use for the preparation of diverse fragments for chemical libraries[41] or peptidomimetics.[7,8]

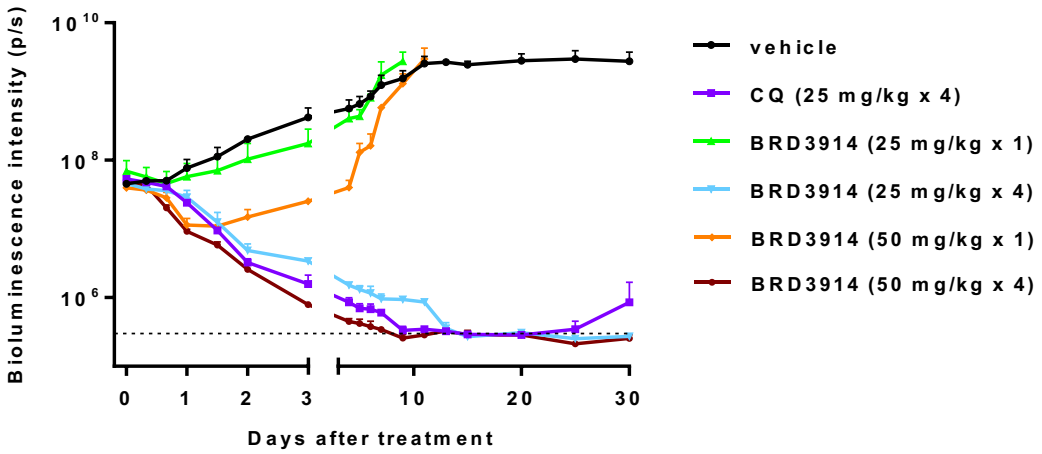


Figure 4.6. *In vivo* efficacy study of BRD3914 using *P. falciparum* and humanized mouse model. huRBC NSG mice were inoculated with *P. falciparum* (3D7$^{HLH/BRD}$) blood stage parasites 48 h before treatment, and BRD3914 was administered as a single dose (25 mg/kg or 50 mg/kg) at 0 h, or multiple dosing at (25 mg/kg or 50 mg/kg) at 0, 24, 48, and 72 h (n = 2 for each group, this study was conducted once). Chloroquine (CQ) was used as a positive control. Infections were monitored using the *in vivo* imaging system (IVIS). Bioluminescent intensity was quantified from each mouse and plotted against time. The dotted horizontal line represents the mean bioluminescence intensity level obtained from all the animals before the parasite inoculation.

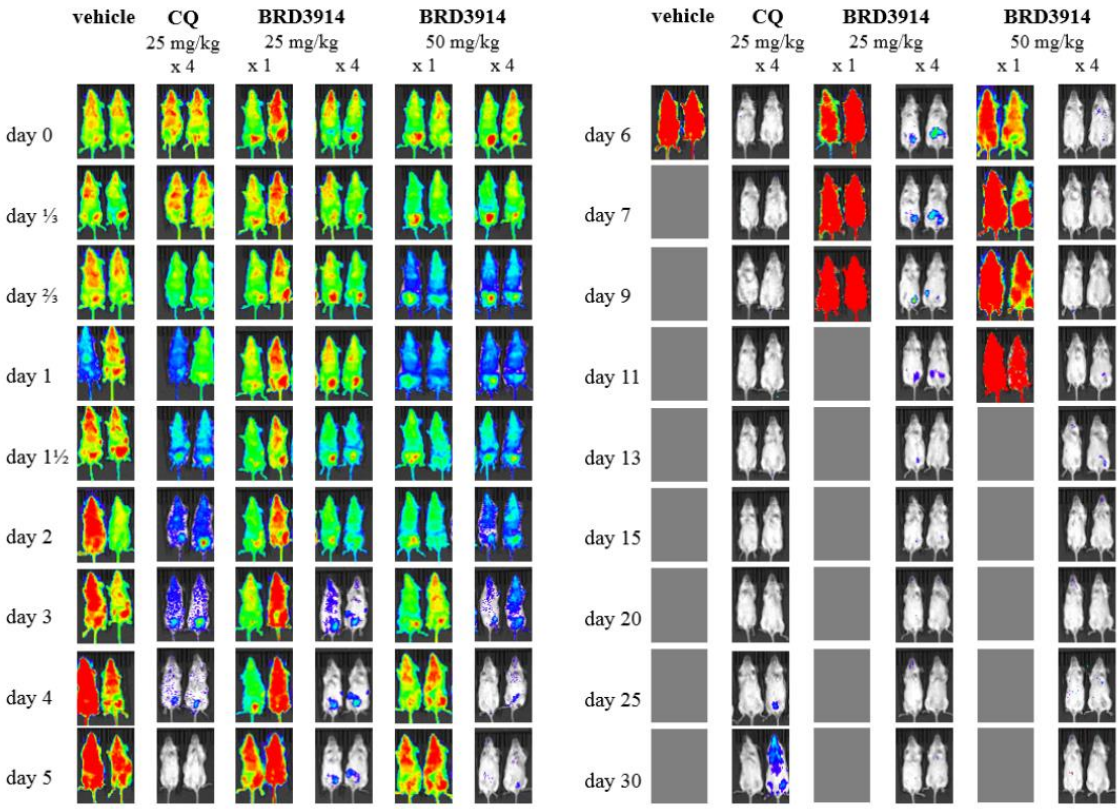


Figure 4.7. Corresponding images obtained from the *in vivo* imaging system (IVIS) for mice in Figure 4.6. Bioluminescent intensity after injection with luciferin is shown. Time refers to total elapsed time after first treatment. CQ = chloroquine.

4.5 Conclusions

In summary, this chapter a Pd-catalyzed, directed C(sp^3)-H arylation of azetidines, which exhibited a broad substrate scope and permitted the preparation of stereochemically defined, synthetically useful carboxylic acid building blocks. This method was applied to the short synthesis of antimalarial compound BRD3914, which cured *P. falciparum* infection in a mouse model after four oral doses. Collectively, these results arise from interplay between chemical

methodology, stereochemically-oriented diversification, synthesis of a structurally complex scaffold, and biological studies.

4.6 Contributions

Extensive reaction condition screening, reaction scope, and directing group cleavage was performed jointly with Jochen Zoller, Bruno Melillo, and Oscar Verho. The total synthesis of BRD3914 was performed with Jochen Zoller and Bruno Melillo. Nobutaka Kato performed the *in vivo* experiments. All other data shown, including project idea and initial screening of literature conditions, were performed by the author.

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Chapter 5

Experimental Procedures

5.1 General Methods

5.1.1 Laboratory procedures and reaction set up

Oxygen- and/or moisture-sensitive reactions were carried out in oven (>110 °C) or flame-dried glassware capped with a rubber septa or Teflon-coated cap under a positive pressure of dry nitrogen or argon from a manifold or balloon. Likewise, air- and moisture-sensitive reagents, solvents, and solutions were transferred via syringe or stainless steel cannula under a dry inert atmosphere. Stirring was achieved using oven (>110 °C) or flame-dried Teflon-coated magnetic stir bars cooled under a stream of dry nitrogen or argon.

Oxygen- and/or moisture-insensitive reactions were carried out in ambient temperature-stored glassware or 1- and 2-dram vials capped with a rubber septa or Teflon-coated cap. Stirring was achieved using Teflon-coated magnetic stir bars.

Reagents and substrates were added in single portions and were transferred in a time frame of less than ca. 15 seconds unless otherwise indicated. Reaction temperatures greater than ambient temperature refer to the external temperature of an IKA stir plate and respectively-sized heating blocks. Temperatures of 0 °C and -78 °C refer to bath temperatures achieved with an ice/water slurry and a dry ice/acetone slurry, respectively, unless otherwise noted. Temperatures

between 0 °C and -78 °C were maintained with a dry ice/acetone slurry via continuous monitoring by the addition of dry ice as appropriate or through use of a CryoCool using *i*-PrOH as a solvent. Ambient or room temperature is defined as 22 °C-25 °C.

5.1.2 Reagents and solvents

All reagents were purchased and used as received from Aldrich Chemical Co., VWR International, Oakwood Chemicals, or Fisher Scientific/Acros unless otherwise noted or synthesized according to cited procedures. In particular, all palladium catalysts were purchased from Strem Chemicals Inc.; L-, D-, and racemic azetidine-2-carboxylic acid were purchased from Combi-Blocks; 8-aminoquinoline, ethyl trifluoroacetate, and HATU were purchased from Oakwood Chemical; AgOAc and *N,N*-diisopropylethylamine were purchased from Aldrich Chemical Co.; and dibenzyl phosphate was purchased from AKSci. All metal catalysts were stored in a room temperature desiccator. All other reagents were stored as instructed by the manufacturer.

All dry solvents were purchased from Aldrich Chemical Co. and stored under a SureSeal crimp top. Non-dry solvents were purchased from Fisher Scientific/Acros, VWR International, or EMD Millipore as 4 L containers.

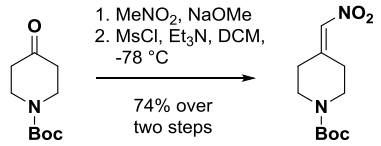
5.1.3 Chromatography and physical/spectroscopic data

Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Flash chromatography was performed using 20-40 μm silica gel (60Å mesh) on a Teledyne Isco Combiflash Rf. Analytical thin layer chromatography (TLC) was performed on

0.25 mm silica gel 60-F plates and visualized by UV light (254 nm). Melting points were recorded on an OptiMelt Automated Melting Point System from Stanford Research Systems. Optical rotations were measured on an Autopol IV automatic polarimeter from Rudolph Research Analytical. NMR spectra were recorded on Bruker 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers at 300 K unless otherwise noted. NMR solvents were purchased from Cambridge Isotope Laboratories, Inc., and NMR data were collected in $CDCl_3$, CD_3OD, $(CD_3)_2SO$, or C_5D_5N. Chemical shifts are reported in parts per million (ppm) relative to the appropriate solvent (for ^{1}H, $CDCl_3$ = δ 7.26, CD_3OD = δ 3.31, $(CD_3)_2SO$ = δ 2.50, or C_5D_5N = δ 1.94; for ^{13}C, $CDCl_3$ = δ 77.16, CD_3OD = δ 49.00, $(CD_3)_2SO$ = δ 39.52, or C_5D_5N = δ 118.26). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet), coupling constants, and integration. Infrared spectra were obtained with a Nicolet IR100 FTIR from Thermo Scientific. Tandem liquid chromatography/mass spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. High-resolution mass-spectra were acquired on an Agilent 1290 Infinity separations module coupled to a 6230 time-of-flight (TOF) mass detector operating in ESI+ or ESI- mode. Masses were confirmed using the "Find by Formula" feature in MassHunter Qualitative Analysis vB.06.00. All values are averages of three independent measurements. X-ray crystallography experiments were performed at the Massachusetts Institute of Technology, Department of Chemistry, X-Ray Diffraction Facility with a Bruker X8 Dual IμS diffractometer and APEX II CCD detector. Enantiopurity of selected compounds were determined by analytical supercritical fluid chromatography (SFC-MS) on a Waters UPC2 convergence chromatography system connected to a QDa single quadrupole mass

spectrometer using chiral stationary phase with solvent mixtures and temperatures as indicated in each entry. The methods were calibrated with the corresponding racemic mixtures.

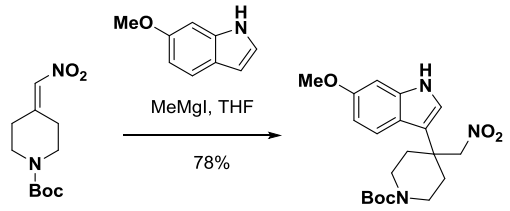
5.2 Supporting Data for Chapter 2



2.1 2.2

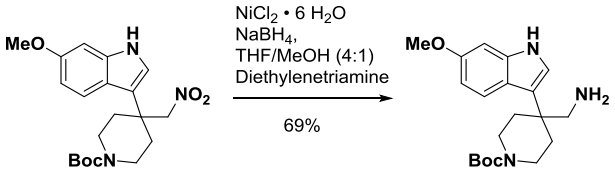
tert-butyl 4-(nitromethylene)piperidine-1-carboxylate (**2.2**). To a solution of 1-boc-4-piperidone (**2.1**) (1.0 g, 5.02 mmol) in MeOH (10 mL) was added CH_3NO_2 (2.71 mL, 50.2 mmol) and NaOMe (136 mg, 2.51 mmol) at room temperature. The reaction was stirred for 15 h before being quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to yield crude product as slightly off-white solid (1.23 g).

The resulting crude product was dissolved in DCM (23 mL), cooled to -78 °C, and Et_3N (2.87 mL, 20.62 mmol) and MsCl (1.166 mL, 15 mmol) were added in sequence. The solution was stirred at -78 °C for 30 min and then warmed to -20 °C and stirred for an additional 2 h. The reaction was quenched with saturated $NaHCO_3$ and warmed slowly to room temperature before being extracted 3x with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude residue was purified by column chromatography (EtOAc/hexanes) to afford product **2.2** (899 mg, 74% over two steps) as a light yellow solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.99 (s, 1 H), 3.55 (q, 4 H, J=6 Hz), 2.98 (t, 2 H, J=6 Hz), 2.32 (t, 2 H, J=6 Hz), 1.47 (s, 9 H). **m/z** 241 $(M-H)^-$.



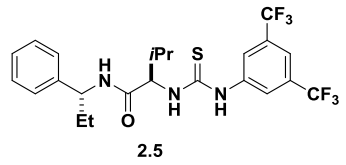
2.2 2.3

tert-butyl 4-(6-methoxy-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.3**). To a solution of 6-methoxyindole (601 mg, 4.08 mmol) in anhydrous THF (9 mL) was added CH_3MgI (1.422 mL, 4.27 mmol, 3.0 M in anhydrous diethyl ether) dropwise over 5 min at room temperature. The mixture was stirred for 30 min before being cooled to 0 °C. A solution of **2.2** (1.2 g, 4.69 mmol) in 28 mL anhydrous THF was added dropwise to the first mixture over 5 min. The reaction was stirred at 0 °C for 15 min, then stirred for 16 h while allowed to warm to room temperature. After complete consumption of starting material, the reaction was quenched with saturated NH_4Cl and stirred for 30 minutes. The resulting mixture was extracted 3x with EtOAc, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude brown residue was purified by column chromatography (EtOAc/hexanes) to afford product **2.3** (1.12 g, 78%) as a light brown foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.12 (s, 1 H), 7.52 (d, 1 H, J=9 Hz), 6.90 (dd, 2 H, J=3 Hz, 9 Hz), 6.79 (dd, 2 H, J=3 Hz, 9 Hz), 4.69 (s, 1 H), 3.90 (m, 1 H), 3.86 (s, 3 H), 3.11 (dt, 2 H, J=3 Hz, 13.5 Hz), 2.43 (d, 2 H, J=13.5 Hz), 1.94 (dt, 2 H, J=3 Hz, 13.5 Hz), 1.60 (s, 1 H), 1.44 (s, 9 H). **m/z** 390 $(M+H)^+$.

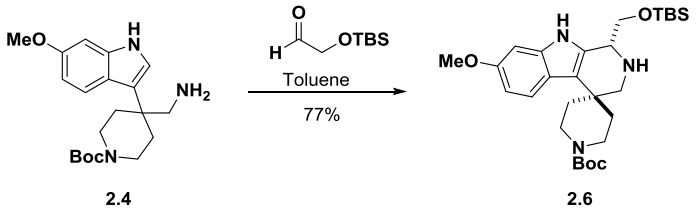


2.3 2.4

tert-butyl 4-(aminomethyl)-4-(6-methoxy-1H-indol-3-yl)piperidine-1-carboxylate (**2.4**). To a solution of **2.3** (7.77 g, 19.95 mmol) in THF (320 mL) was added $NiCl_2 \cdot 6\ H_2O$ (4.74 g, 19.95 mmol) and $NaBH_4$ (3.02 g, 80 mmol). The solution was cooled to 0 °C before MeOH (80 mL) was added dropwise over 2 min (*note: vigorous bubbling*). The resulting solution was stirred at room temperature for 4 h. Next, diethylenetriamine was added, and the mixture was stirred for 1 h at room temperature before concentration *in vacuo*. The crude concentrate was reconstituted with EtOAc and washed 2x with $NaHCO_3$ and once with brine. The resulting organic layer was dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude residue was purified via column chromatography (MeOH/DCM) to afford product **2.4** (4.928 g, 69%) as foaming white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.72 (s, 1 H), 7.55 (d, 1 H, J=9 Hz), 6.85 (s, 2 H), 6.74 (dd, 1 H, J=3 Hz, 9 Hz), 3.82 (s, 3 H), 3.77 (m, 2 H), 3.12 (t, 2 H, J=11.1 Hz), 2.93 (s, 2 H), 2.24 (d, 2 H, J=13.5 Hz), 1.67 (m, 2 H), 1.44 (s, 9). **m/z** 360 $(M+H)^+$.



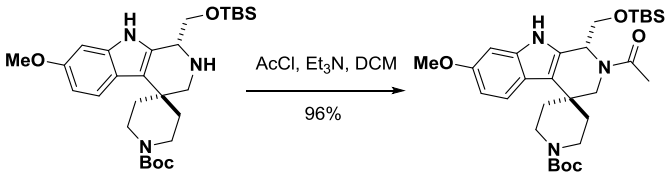
2.5



2.4 2.6

(*R*)-*tert*-butyl 1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.6**). To a solution of **2.4**

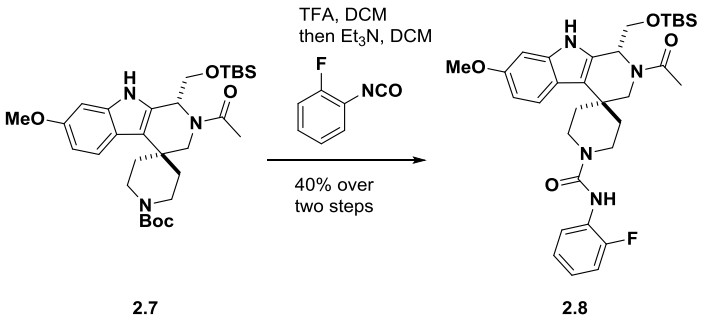
(2.678 g, 7.45 mmol) in toluene (135 mL) at -20 °C was added (*R*)-2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-N,3-dimethyl-N-((R)-1-phenylpropyl)butanamide catalyst **2.5**, 581 mg, 1.118 mmol) (*Note: **2.5** was graciously donated by the Jacobsen group at Harvard University*). The solution was stirred for 5 minutes before freshly-distilled 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (1.558 g, 8.94 mmol) in toluene (14 mL) was added. The solution was maintained at -20 °C using a CryoCool™ and stirred for 24 h before additional **2.5** (270 mg, 0.596 mmol) was added. At 114 h, additional **2.5** (270 mg, 0.596 mmol) was added into the reaction mixture. At 138 h, the reaction was brought to room temperature and solvent removed *in vacuo*. Crude residue was purified via column chromatography (EtOAc/hexanes) to yield product **2.6** (2.956 g, 77%) as a light yellow foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.44 (s, 1 H), 7.53 (d, 1 H, J=9 Hz), 6.80 (d, 1 H, 2.4 Hz), 6.73 (dd, 1 H, J=2.4 Hz, 9 Hz), 4.11 (m, 2 H), 3.90 (dd, 1 H, J=6 Hz, 9 Hz), 3.84 (s, 3 H), 3.67 (t, 1 H, 9 Hz), 3.48 (d, 1 H, 9 Hz), 2.93 (m, 2 H), 2.82 (d, 1 H, 12.6 Hz), 2.53 (m, 1 H), 2.16 (m, 1 H), 1.72 (m, 3 H), 1.50 (s, 9 H), 0.98 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H). **m/z** 516 $(M+H)^+$. ***>20:1 er by SFC***.



2.6 **2.7**

(*R*)-*tert*-butyl 2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.7**). To a solution of **2.6** (862 mg, 1.671 mmol) in DCM (7 mL) at -78 °C was added triethylamine (1.158 mL, 8.36 mmol) followed by acetyl chloride (119 µL, 1.671 mmol). The reaction was stirred for 15 minutes at -78 °C before the addition of saturated NH_4Cl. The resulting solution was brought to

room temperature and extracted 3x with DCM. Combined organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified via column chromatography (EtOAc/hexanes) to afford product **2.7** (893 mg, 96%) as a white foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.41 (s, 1 H), 7.52 (dd, 1 H, J=2.4 Hz, 9 Hz), 6.80 (d, 1 H, 2.4 Hz), 6.74 (dd, 1 H, J=2.4 Hz, 9 Hz), 5.54 (dd, 1 H, J=4.5 Hz), 5.30 (d, 1 H, J=13.5 Hz), 4.97 (t, 1 H, J=7.5 Hz), 4.12 (m, 1 H), 3.98 (m, 1 H), 3.92 (dd, 2 H, J=2.1 Hz, 6 Hz), 3.82 (d, 3 H, J=6 Hz), 3.28 (m, 1 H), 2.72 (m, 1 H), 2.58 (d, 1 H, J=13.5 Hz), 2.24 (d, 3 H, J=9 Hz), 1.89 (m, 1 H), 1.58 (m, 1 H), 1.50 (d, 9 H, J=4.5 Hz), 0.92 (d, 9 H, J=2.4 Hz), 0.08 (s, 3 H), 0.07 (s, 3 H). **m/z** 558 $(M+H)^+$.

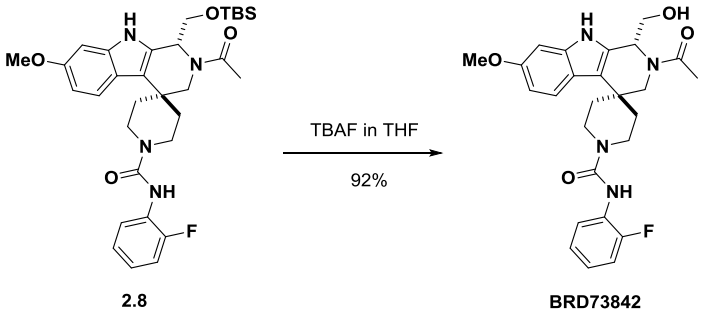


2.7 **2.8**

(*R*)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-N-(2-fluorophenyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.8**). To a solution of **2.7** (100 mg, 0.179 mmol) in DCM (2 mL) was added TFA (100 µL, 1.306 mmol) (20:1 DCM/TFA v/v). The solution was stirred at room temperature for 3 h before being quenched with saturated $NaHCO_3$. The resulting mixture was extracted 3x with DCM, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to afford crude intermediate.

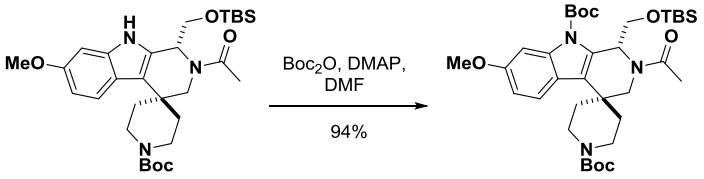
Crude intermediate was reconstituted in DCM (1 mL), followed by the addition of 2-fluorophenylisocyanate (6 µL, 0.051 mmol). After 1 h at room temperature, 50 µL of triethylamine was added to the mixture. At 3 h, the reaction was quenched with deionized water

and extracted 3x with dichloromethane. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.8** (19 mg, 40%) as an off-white crystal. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.13 (m, 1 H), 7.54 (dd, 1 H, J=4.5 Hz, 9 Hz), 7.09 (m, 2 H), 6.99 (m, 1 H), 6.80 (m, 1 H), 6.74 (m, 2 H), 5.30 (d, 1 H, J=13.5 Hz), 4.96 (t, 1 H, J=7.5 Hz), 4.26 (d, 1 H, J=13.5 Hz), 3.96 (d, 1 H, J=7.5 Hz), 3.82 (d, 3 H, J=3 Hz), 3.56 (dt, 1 H, J=3 Hz, 13.5 Hz), 3.19 (dt, 2 H, J=3 Hz, 13.5 Hz), 2.83 (m, 1 H), 2.63 (d, 1 H, J=13.5 Hz), 2.25 (d, 3 H, J=6 Hz), 1.58 (m, 4 H), 1.40 (s, 1 H), 0.91 (m, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H). **m/z** 596 $(M+H)^+$.



(*R*)-2'-acetyl-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**BRD73842**). To a solution of **2.8** (87 mg, 0.156 mmol) in THF (2 mL) at was added TBAF in THF (1.0 M, 234 uL, 0.234 mmol). The reaction was allowed to stir at room temperature. After 5 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (MeOH/DCM) to afford product **BRD73842** (63.7 mg, 92%) as a pale yellow residue/solid. **^{1}H NMR** (300 MHz, $(CD_3)_2SO$) δ 10.78 (s, 1H), 8.39 (s, 1H), 7.52 – 7.33 (m, 2H), 7.30 – 7.10 (m, 3H), 6.91-6.77 (m, 1H), 6.69-6.55 (m, 1H), 5.50 – 5.30 (m, 1H), 5.19 – 5.07 (m, 1H), 5.03 – 4.91 (m, 1H), 4.16 – 3.90 (m, 2H), 3.74 (s, 4H), 3.39 – 3.28 (m, 1H), 3.29 – 2.97 (m,

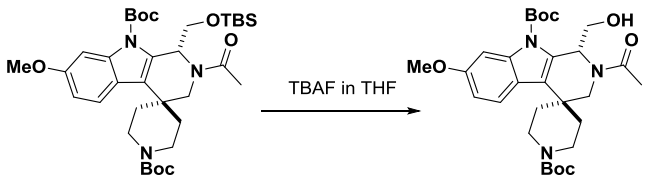
3H), 2.77 – 2.54 (m, 1H), 2.18 (s, 3H), 1.56 – 1.20 (m, 2H). Mixture of rotamers – major rotamer is shown. **^{13}C NMR** (101 MHz, $(CD_3)_2SO$) δ 169.92, 169.29, 157.18, 155.91, 155.86, 155.55, 155.42, 154.75, 137.75, 137.55, 131.44, 130.33, 130.18, 128.42, 128.31, 126.68, 126.56, 125.30, 124.48, 124.45, 119.99, 119.76, 119.11, 115.05, 108.72, 108.49, 95.58, 95.45, 62.52, 56.27, 55.68, 50.81, 48.47, 41.82, 41.59, 40.96, 35.49, 35.30, 34.63, 34.14, 32.80, 32.17, 32.15, 22.23. Mixture of rotamers – all carbon peaks are shown. **HRMS** (ESI) calculated for $C_{26}H_{30}FN_4O_4$ $[M+H]^+$: 481.2251. Found: 481.2255.



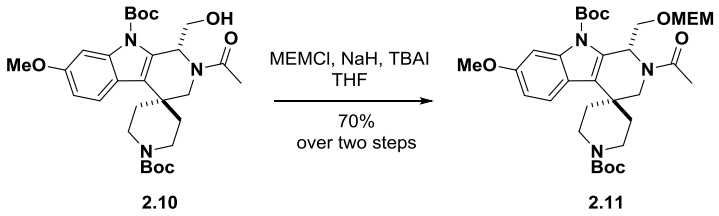
2.7 2.9

di-*tert*-butyl (*R*)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.9**). To a solution of **2.7** (200 mg, 0.359 mmol) in DMF (4 mL) was added Boc_2O (157 mg, 0.717 mmol). The solution was stirred for 12 h at 65°C, after which point additional Boc_2O (80 mg, 0.359 mmol) and catalytic DMAP (7 mg, 0.054 mmol) were added. 1 h after the addition, the reaction was quenched with H_2O and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified by column chromatography (MeOH/DCM) to afford product **2.9** (221 mg, 94%) as a yellow foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.80 (d, 1 H, J=2 Hz), 7.49 (d, 1 H, J=9 Hz), 6.83 (dd, 1 H, J=3 Hz, 9 Hz), 5.67 (dd, 1 H, J=3 Hz, 9 Hz), 5.29 (m, 1 H), 4.10 (m, 2 H), 4.01 (dd, 1 H, J=3 Hz, 13.5 Hz), 3.84 (s, 3 H), 3.78 (t, 1 H, J=3 Hz), 3.30 (m, 1 H), 3.02 (m, 1 H), 2.94 (s, 2 H), 2.86 (s,

2 H), 2.65 (m, 2 H), 2.27 (s, 3 H), 1.89 (s, 1 H), 1.70 (s, 9 H), 1.50 (s, 11 H [9+2]), 0.89 (s, 9 H), 0.06 (s, 3 H), 0.05 (s, 3 H). **m/z** 659 $(M+H)^+$.

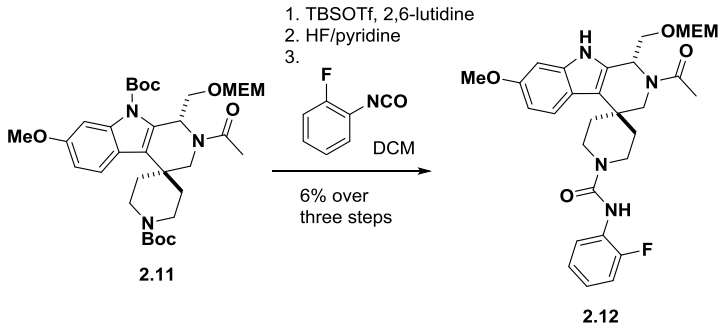


di-*tert*-butyl (*R*)-2'-acetyl-1'-(hydroxymethyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.10**). To a solution of **2.9** (221 mg, 0.336 mmol) in THF (1.7 mL) at room temperature was added TBAF in THF (1.0 M, 1.008 mL, 1.008 mmol). After 2 h at room temperature, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to afford crude product **2.10** as a yellow foaming solid. Crude **2.10** was used as an intermediate in additional reactions without purification or characterization. **m/z** 542 $(M-H)^-$.



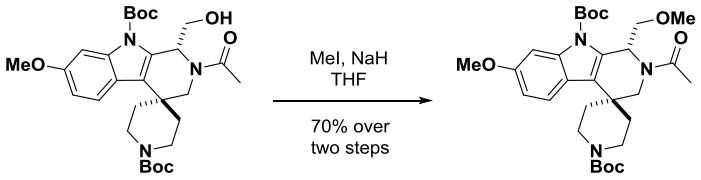
di-*tert*-butyl (*R*)-2'-acetyl-7'-methoxy-1'-(((2-methoxyethoxy)methoxy)methyl)-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.11**). To a solution of crude **2.10** (44 mg, 0.081 mmol) in anhydrous THF (1 mL) at 0 °C was added NaH (5 mg, 0.121 mmol), 2-methoxyethoxymethyl chloride (14 µl, 0.121 mmol), and TBAI (6 mg, 0.016 mmol) in sequence. The solution was stirred at 0 °C for 1 h, then brought to room temperature for 1 h,

before being quenched with saturated NH_4Cl. The mixture was extracted 3x with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (MeOH/DCM) to afford product **2.11** (35 mg, 70% over two steps) as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.51 (dd, 1 H, J=3 Hz, 9 Hz), 6.95 (dd, 1 H, J=2.1 Hz, 13.5 Hz), 6.76 (m, 1 H), 5.58 (t, 2 H, J=13.5 Hz), 5.32 (m, 1 H), 4.82 (m, 1 H), 4.51 (m, 1 H), 4.40 (m, 1 H), 4.12 (m, 2 H), 3.85 (s, 3 H), 3.70 (m, 2 H), 3.55 (m, 3 H), 3.38 (d, 3 H, J=1.8 Hz), 3.35 (d, 2 H, J=9 Hz), 2.99 (m, 1 H), 2.70 (m, 2 H), 2.20 (d, 3 H, J=6 Hz), 1.90 (m, 1 H), 1.47 (m, 18 H). **m/z** 676 $(M+HCO_2^-)^-$.



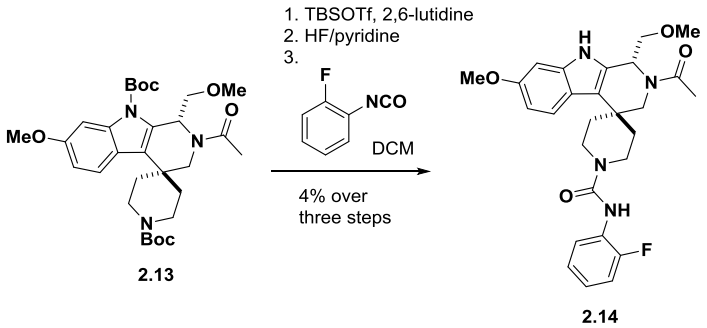
(*R*)-2'-acetyl-N-(2-fluorophenyl)-7'-methoxy-1'-(((2-methoxyethoxy)methoxy)methyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.12**). To a solution of **2.11** (35 mg, 0.055 mmol) in DCM (1 mL) at room temperature was added 2,6-lutidine (51.6 uL, 0.443 mmol) and TBSOTf (71.6 uL, 0.332 mmol). After 1 h, the mixture was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude silyl carbamate, which was subsequently dissolved in THF (1 mL) at room temperature. HF/pyridine (7.13 uL, 0.055 mmol) was then added to the solution. After 45 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude intermediate.

Crude intermediate was reconstituted in DCM (1 mL) at room temperature followed by the addition of 2-fluorophenylisocyanate (6.17 uL, 0.055 mmol). The solution was stirred for 48 h, after which point methanol was added to quench the reaction. Solvent was evaporated *in vacuo*, and crude residue was purified by column chromatography (MeOH/DCM) to afford product **2.12** (1.90 mg, 6% over three steps) as a colorless residue. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.15 (m, 1 H), 7.57 (dd, 1 H, J=2.4 Hz, 9 Hz), 7.04 (m, 3 H), 6.79 (dd, 1 H, J=2.4 Hz, 9 Hz), 6.71 (s, 1 H), 5.83 (dd, 1 H, J=2.4 Hz, 6 Hz), 5.52 (m, 2 H), 5.32 (d, 1 H, J=13.5 Hz), 5.12 (t, 1 H, J=7.5 Hz), 4.22 (m, 4 H), 3.86 (s, 3 H), 3.59 (m, 4 H), 3.40 (m, 1 H), 3.33 (s, 1 H), 3.27 (s, 1 H), 3.16 (m, 2 H), 2.84 (m, 3 H), 2.26 (d, 3 H, J=9 Hz), 1.60 (m, 3 H). **m/z** 569 $(M+H)^+$.



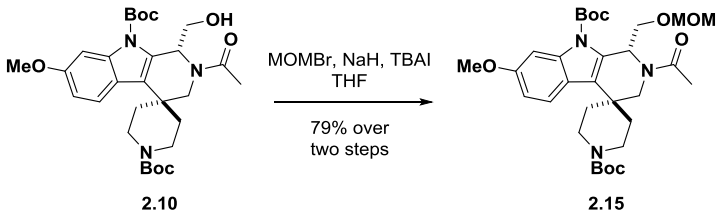
2.10 **2.13**

di-*tert*-butyl (*R*)-2'-acetyl-7'-methoxy-1'-(methoxymethyl)-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.13**). To a solution of crude **2.10** (50 mg, 0.092 mmol) in anhydrous THF (1 mL) at 0 °C was added NaH (3.3 mg, 0.138 mmol) and MeI (8.6 uL, 0.138 mmol) in sequence. After 15 min, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.13** (36 mg, 70% over two steps) as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.47 (m, 1 H), 6.70 (m, 2 H), 5.25 (m, 1 H), 4.30 (m, 2 H), 4.00 (m, 2 H), 3.81 (s, 3 H), 3.69 (s, 3 H), 3.33 (m, 1 H), 2.93 (m, 2 H), 2.64 (m, 2 H), 2.15 (d, 3 H, J=2.4 Hz), 1.84 (m, 1 H), 1.68 (s, 2 H), 1.38-1.48 (18 H). **m/z** 558 $(M+H)^+$.

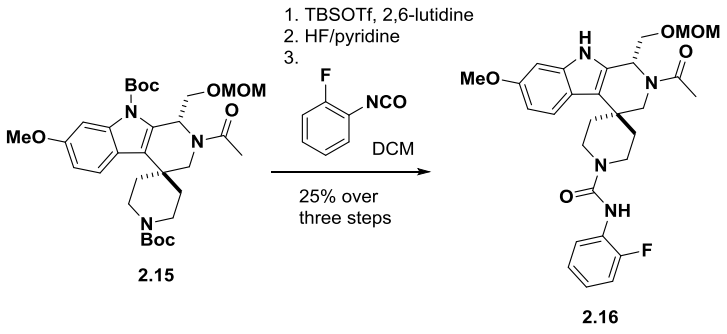


(*R*)-2'-acetyl-N-(2-fluorophenyl)-7'-methoxy-1'-(methoxymethyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.14**). To a solution of **2.13** (45 mg, 0.081 mmol) in DCM (1 mL) at room temperature was added 2,6-lutidine (75 uL, 0.646 mmol) and TBSOTf (104 uL, 0.484 mmol) in sequence. After 1 h, the mixture was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude silyl carbamate, which was subsequently dissolved in THF (1 mL) at room temperature. HF/pyridine (16 uL, 0.121 mmol) was then added to the solution. After 45 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude intermediate.

Crude intermediate was reconstituted in DCM (1 mL) at room temperature, followed by the addition of 2-fluorophenylisocyanate (9.1 uL, 0.081 mmol). After 17 h, the mixture was quenched with MeOH and solvent removed *in vacuo*. Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.14** (1.5 mg, 4% over three steps) as a colorless residue. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.15 (m, 1 H), 7.57 (m 1 H), 7.05 (m, 3 H), 6.76 (m, 2 H), 5.33 (s, 1 H), 4.17 (s, 1 H), 3.91 (s, 3 H), 3.68 (s, 3 H), 3.19 (s, 2 H), 2.86 (m, 1 H), 2.75 (m, 1 H), 2.31 (d, 3 H, J=15 Hz), 1.25 (s, 9 H). **m/z** 495 $(M+H)^+$.



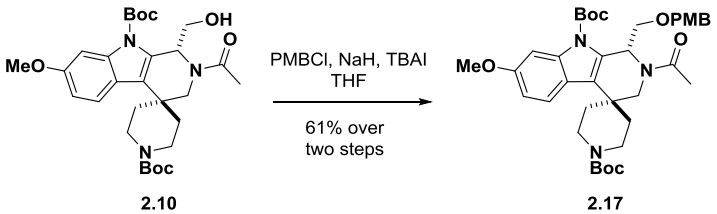
di-*tert*-butyl (*R*)-2'-acetyl-7'-methoxy-1'-(methoxymethyl)-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.15**). To a solution of crude **2.10** (50 mg, 0.092 mmol) in THF (1 mL) at 0 °C was added NaH (3.3 mg, 0.138 mmol), bromomethyl methyl ether (12.5 uL, 0.138 mmol), and TBAI (6.8 mg, 0.018 mmol) in sequence. After 1 h, the mixture was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.15** (43 mg, 79% over two steps) as a white foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.52 (m, 1 H), 6.91 (m, 1 H), 6.80 (m, 1 H), 5.47 (m, 2 H), 5.28 (m, 1 H), 4.57 (dt, 1 H, J=3 Hz, 12 Hz), 4.37 (m, 1 h), 4.05 (m, 2 H), 3.88 (s, 3 H), 3.33 (m, 4 H [3+1]), 3.00 (m, 2 H), 2.67 (m, 2 H), 2.21 (m, 3 H), 1.92 (m, 1 H), 1.70 (s, 2 H), 1.50 (m, 18 H). **m/z** 632 $(M+HCO_2^-)^-$.



(*R*)-2'-acetyl-N-(2-fluorophenyl)-7'-methoxy-1'-(methoxymethyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.16**). To a solution of **2.15** (43 mg, 0.073 mmol) in DCM (1 mL) at room temperature was added 2,6-lutidine (68.2 uL,

0.585 mmol) and TBSOTf (94 uL, 0.439 mmol). After 1 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude silyl carbamate, which was subsequently dissolved in THF (1 mL) at room temperature. HF/pyridine (38 uL, 0.293 mmol) was then added to the solution. After 45 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude intermediate.

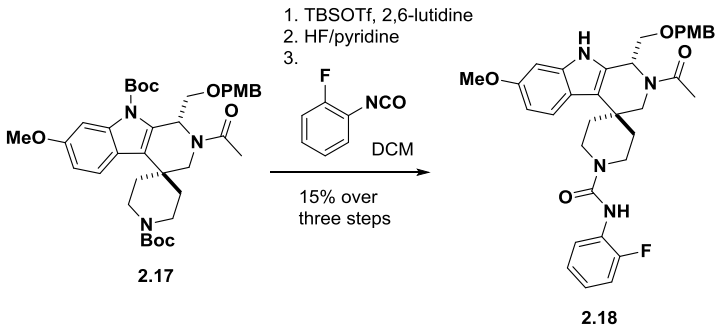
Crude intermediate was reconstituted in DCM (1 mL) at room temperature followed by the addition of 2-fluorophenylisocyanate (8.2 uL, 0.073 mmol) and one drop of EtOAc. The solution was stirred for 22 h, after which point MeOH was added to quench the reaction. Solvent was evaporated *in vacuo*, and crude residue was purified by column chromatography (MeOH/DCM) to afford product **2.16** (9.6 mg, 25% over three steps) as an off-white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.12 (t, 1 H, J=9 Hz), 7.55 (dd, 1 H, J=9 Hz, 4.5 Hz), 6.91-7.16 (m, 4 H), 6.78 (m, 2 H), 5.38 (m, 2 H), 5.30 (m, 1 H), 4.20 (m, 3 H), 3.97 (m, 1 H), 3.87 (s, 3 H), 3.50 (m, 2 H), 3.36 (d, 3 H, J=9 Hz), 3.22 (m, 1 H), 2.79 (m, 2 H), 2.27 (d, 3 H, J=11.5 Hz), 2.06 (m ,1 H), 1.62 (m, 3 H). **m/z** 525 $(M+H)^+$.



2.10 2.17

di-*tert*-butyl (*R*)-2'-acetyl-7'-methoxy-1'-(((4-methoxybenzyl)oxy)methyl)-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.17**). To a solution of crude **2.10** (50 mg, 0.092 mmol) in anhydrous THF (1 mL) at 0 °C was added NaH (5.5 mg,

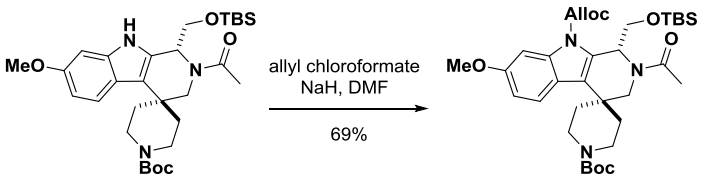
0.128 mmol), 4-methoxybenzyl chloride (19 uL, 0.138 mmol), and TBAI (5 mg, 0.014 mmol) in sequence. The solution was stirred for 1 h at 0 °C before being warmed to room temperature. After 7 h, additional NaH (5.5 mg, 0.128 mmol) and 4-methoxybenzyl chloride (19 uL, 0.138 mmol) were added to the mixture. After 22 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude material was purified twice by column chromatography (MeOH/DCM, then EtOAc/hexanes) to afford **2.17** (37 mg, 61% over two steps) as off-white foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.57 (dd, 1 H, J=9 Hz, 13.5 Hz), 6.79 (m, 6 H), 5.31 (m, 3 H), 4.15 (m, 4 H), 3.79 (s, 3 H), 3.75 (s, 3 H), 3.36 (m, 1 H), 3.00 (m, 1 H), 2.74 (m, 2 H), 2.04-2.18 (m, 3 H), 1.47 (m, 22 H). **m/z** 664 $(M+H)^+$.



(*R*)-2'-acetyl-N-(2-fluorophenyl)-7'-methoxy-1'-(((4-methoxybenzyl)oxy)methyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.18**). To a solution of **2.17** (37 mg, 0.056 mmol) in DCM (1 mL) at room temperature was added 2,6-lutidine (52 uL, 0.446 mmol) and TBSOTf (72 uL, 0.334 mmol). After 1 h, the mixture was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude silyl carbamate, which was subsequently dissolved in THF (1 mL) at room temperature. HF/pyridine (29 uL, 0.223 mmol) was then added to the

solution. After 45 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to give crude intermediate.

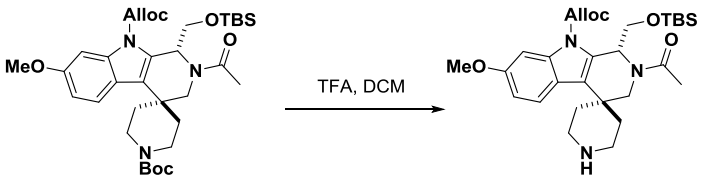
Crude intermediate was reconstituted in DCM (1 mL) at room temperature, followed by the addition of 2-fluorophenylisocyanate (6.3 uL, 0.056 mmol). After 16 h, the mixture was quenched with MeOH and solvent removed *in vacuo*. Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.18** (5.2 mg, 15% over three steps) as a colorless residue. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.17 (m, 1 H), 7.61 (dd, 1 H, J=9 Hz, 6 Hz), 6.91-7.19 (m, 4 H), 6.66-6.84 (m, 6 H), 5.29 (m, 3 H), 4.92 (dd, 1 H, J=3 Hz, 9 Hz), 4.30 (m, 1 H), 3.90 (m, 1 H), 3.78 (m, 6 H), 3.56 (m, 2 H), 3.24 (m, 2 H), 2.86 (m, 2 H), 2.06 (m, 3 H), 1.66 (m, 4 H). **m/z** 601 $(M+H)^+$.



2.7 2.19

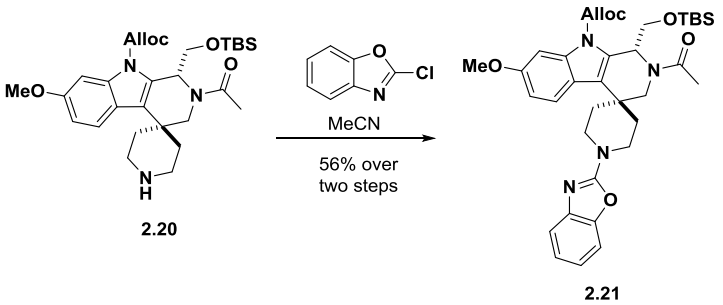
9'-allyl 1-(*tert*-butyl) (R)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.19**). To a solution of **2.7** (170 mg, 0.305 mmol) in DMF (6 mL) at 0 °C was added NaH (19 mg, 0.457 mmol) followed by allylchloroformate (50 uL, 0.457 mmol). The solution was stirred for 1 h 0 °C before being brought to room temperature. After 24 h, additional NaH (19 mg, 0.457 mmol) and allylchloroformate (50 uL, 0.457 mmol) were added to the mixture. After 36 h, the reaction was quenched with water and stirred for 30 min. The resulting mixture was extracted 3x with Et_2O, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.19** (135

mg, 69%) as a white foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.76 (d, 1 H, J=2.1 Hz), 7.50 (d, 1 H, J=9 Hz), 6.84 (dd, 1 H, J=2.4 Hz, 9 Hz), 6.11 (m, 1 H), 5.67 (dd, 1 H, J=2.4 Hz, 9 Hz), 5.50 (dd, 1 H, J=1.2 Hz, 16.7 Hz), 5.38 (dd, 1 H, J=0.9 Hz, 13.5 Hz), 5.29 (d, 1 H, J=13.5 Hz), 4.93 (d, 2 H, J=6 Hz), 4.07 (m, 3 H), 3.79 (m, 4 H [3+1]), 3.19 (m, 1 H), 2.99 (m, 1 H), 2.64 (d, 2 H, J=13.5 Hz), 2.25 (s, 3 H), 1.86 (m, 1 H), 1.49 (s, 11 H [9+2]), 0.89 (s, 9 H), 0.06 (s, 3 H), 0.03 (s 3 H).). **m/z** 642 $(M+H)^+$.

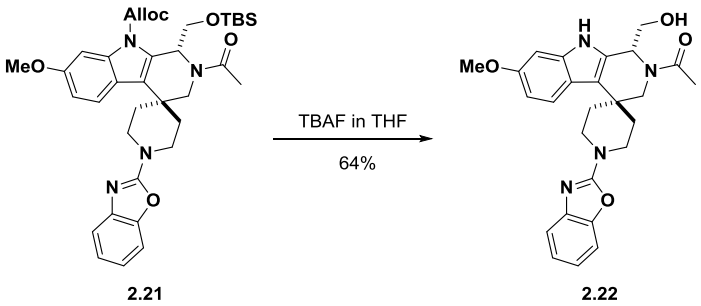


2.19 2.20

allyl (*R*)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-9'(1'H)-carboxylate (**2.20**). To a solution of **2.19** (41 mg, 0.064 mmol) in DCM (1 mL) was added TFA (50 uL) (20:1 DCM/TFA v/v) at room temperature. The solution was stirred for 4 h before being quenched with saturated $NaHCO_3$. The resulting mixture was extracted 3x with DCM, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to afford crude product **2.20** (quantitative) as a white foaming solid. Crude **2.20** was used as an intermediate in additional reactions without purification or characterization. **m/z** 542 $(M+H)^+$.

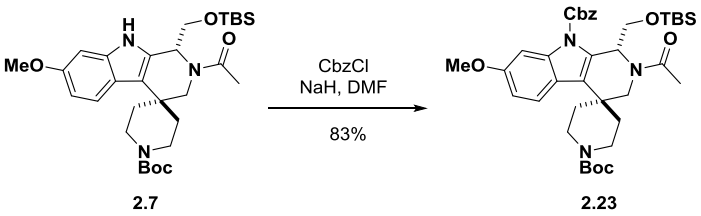


allyl (*R*)-2'-acetyl-1-(benzo[d]oxazol-2-yl)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-9'(1'H)-carboxylate (**2.21**). To a solution of **2.20** (34 mg, 0.063 mmol) in anhydrous CH_3CN (1 mL) at 0 °C was added 2-chlorobenzoxazole (7.2 uL, 0.063 mmol) and one drop of Et_3N. The solution was stirred for 1 h before being brought to room temperature. After 60 h, the reaction was quenched with crushed ice and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.21** (23 mg, 56% over two steps) as a thick, colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.75 (d, 1 H, J=2.4 Hz), 7.46 (d, 1 H, J=9 Hz), 7.38 (d, 1 H, 7.5 Hz), 7.28 (d, 1 H, J=9 Hz), 7.18 (dt, 1 H, J=7.5 Hz, 0.9 Hz), 7.04 (dt, 1 H, J=7.5 Hz, 0.9 Hz), 6.80 (dd, 1 H, J=2.4 Hz, 9 Hz), 6.12 (m, 1 H), 5.70 (dd, 1 H, J=2.4 Hz, 9 Hz), 5.51 (dt, 1 H, J=1.2 Hz, 17.4 Hz), 5.40 (m, 2 H), 4.95 (d, 2 H, 6 Hz), 4.39 (dd, 1 H, J=4.5 Hz, 13.5 Hz), 4.27 (dd, 1 H, J=4.5 Hz, 13.5 Hz), 4.09 (dd, 1 H, J=2.4 Hz, 13.5 Hz), 3.82 (s, 4 H), 3.72 (m, 1 H), 3.40 (m, 1 H), 2.88 (dt, 1 H, J=4.5 Hz, 13.5 Hz), 2.75 (d, 1 H, J=13.5 Hz), 2.28 (s, 3 H), 2.11 (dt, 1 H, J=4.5 Hz, 13.5 Hz), 1.61 (m, 2 H), 0.90 (s, 9 H), 0.09 (s, 3 H), 0.6 (s, 3 H). **m/z** 659 $(M+H)^+$.



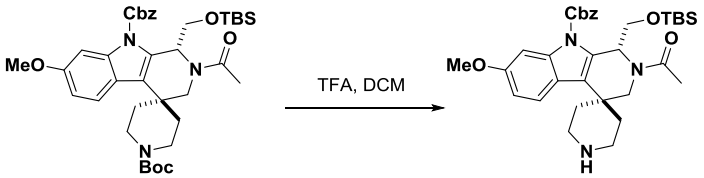
2.21 **2.22**

(*R*)-1-(1-(benzo[d]oxazol-2-yl)-1'-(hydroxymethyl)-7'-methoxy-1',9'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indol]-2'(3'H)-yl)ethan-1-one (**2.22**). To a solution of **2.21** (23 mg, 0.035 mmol) in THF (700 uL) at room temperature was added TBAF in THF (1.0 M, 244 uL, 0.244 mmol). The reaction was allowed to stir for 16 h, after which point the mixture was then quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.22** (10.3 mg, 64%) as a white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.77 (s, 0.5 H), 8.48 (s, 0.5 H), 7.46 (dd, 1 H, J=2.4 Hz, 9 Hz), 7.28-7.41 (m, 2 H), 7.19 (m, 1 H), 7.05 (dt, 1 H, J=7.5 Hz, 13.5 Hz), 6.84 (t, 1 H, J=3 Hz), 6.72 (d, 1 H, J=9 Hz), 5.71 (dd, 0.5 H, J=6.9 Hz, 6.9 Hz), 5.02 (t, 0.5 H, J=6.9 Hz), 4.30 (m, 2 H), 4.00 (m, 1 H), 2.89 (m, 1 H), 3.79 (s, 3 H), 3.62 (s, 1 H), 3.30 (m, 2 H), 2.89 (m, 2 H), 2.61 (d, 1 H, J=13.5 Hz), 2.23 (s, 3 H), 1.72 (m, 2 H). **m/z** 461 $(M+H)^+$.



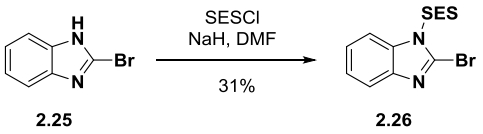
2.7 **2.23**

9'-benzyl 1-(*tert*-butyl) (*R*)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1,9'(1'H)-dicarboxylate (**2.23**). To a solution of **2.7** (295 mg, 0.529 mmol) in DMF (5 mL) at 0 °C was added NaH (15 mg, 0.608 mmol). The solution was allowed to stir for 30 min before the addition of benzylchloroformate (87 uL, 0.608 mmol). After 1 h, the mixture was quenched with water and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.23** (305 mg, 83%) as a white foaming solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.69 (d, 1 H, J=2.1 Hz), 7.50 (m, 3 H), 7.41 (m, 3 H), 6.82 (dd, 1 H, J=2.4 Hz, 9 Hz), 5.63 (dd, 1 H, J=2.7 Hz, 9 Hz), 5.51 (d, 1 H, J=12 Hz), 5.42 (d, 1 H, J=12 Hz), 4.07 (m, 3 H), 3.80 (t, 1 H, J=9.9 Hz), 3.68 (s, 3 H), 3.37 (m, 1 H), 3.97 (m, 1 H), 2.65 (d, 2 H, J=13.5 Hz), 2.18 (s, 3 H), 1.86 (m, 1 H), 1.47 (s, 11 H [9+2]), 0.92 (s, 9 H), 0.06 (s, 3 H), 0.03 (s, 3 H). **m/z** 692 $(M+H)^+$.



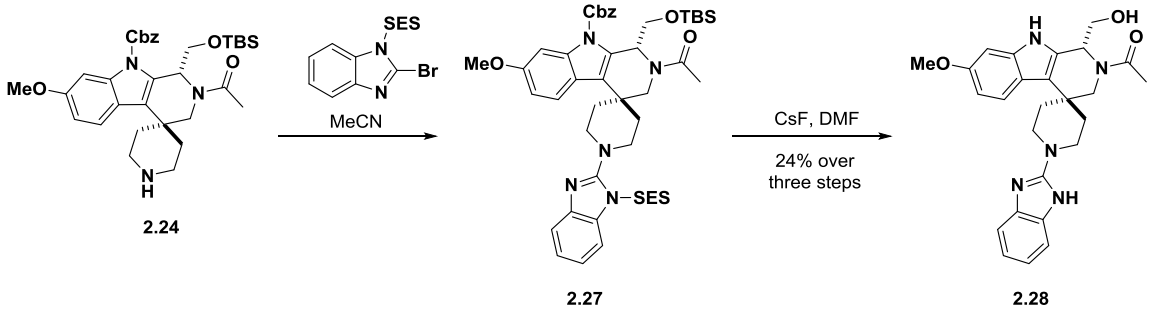
2.23 2.24

benzyl (*R*)-2'-acetyl-1'-(((*tert*-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2',3'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-9'(1'H)-carboxylate (**2.24**). To a solution of **2.23** (300 mg, 0.434 mmol) in DCM (4.5 mL) was added TFA (225 uL) (20:1 DCM/TFA v/v). The solution was stirred for 4 h before being quenched with saturated $NaHCO_3$. The resulting mixture was extracted 3x with DCM, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to afford crude **2.24** (quantitative) as a white foaming solid. Crude **2.24** was used as an intermediate in additional reactions without purification or characterization.



2.25 2.26

2-bromo-1-((2-(trimethylsilyl)ethyl)sulfonyl)-1H-benzo[d]imidazole (**2.26**). To a solution of 2-bromo-1H-benzimidaole **2.25** (500 mg, 2.54 mmol) in THF (5 mL) at 0 °C was added NaH (73 mg, 3.05 mmol) followed by 2-(trimethylsilyl)ethanesulfonyl chloride (577 uL, 3.05 mmol). The reaction was then allowed to warm slowly to room temperature. After 18 h, the mixture was quenched with water and extracted 3x with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.26** (326 mg, 31%) as an off-white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.92 (m, 1 H), 7.71 (m, 1 H), 7.35 (m, 2 H), 3.44 (m, 2 H), 0.94 (m, 2 H), 0.01 (s, 9 H). **m/z** 362 $(M+H)^+$.

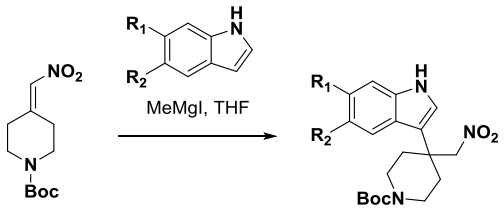


2.24 2.27 2.28

(*R*)-1-(1-(1H-benzo[d]imidazol-2-yl)-1'-(hydroxymethyl)-7'-methoxy-1',9'-dihydrospiro[piperidine-4,4'-pyrido[3,4-b]indol]-2'(3'H)-yl)ethan-1-one (**2.28**). To a solution of crude **2.24** (35 mg, 0.059 mmol) in toluene (1 mL) was added **2.26** (23.5 mg, 0.065 mmol), $Pd_2(dba)_3$ (5.4 mg, 0.006 mmol), DavePhos (2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, 4 mg, 0.010 mmol), and potassium *tert*-butoxide (8 mg, 0.071 mmol)

in sequence. The solution was heated at 60 °C for 24 h before water was added to the reaction mixture. The resulting solution was extracted 3x with EtOAc, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was subject to a short column chromatography (EtOAc/hexanes) to afford "crude" **2.27**.

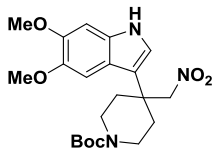
To a solution of **2.27** in DMF (1 mL) was added CsF (54 mg, 0.354 mmol) at room temperature. After 6 h, additional CsF (43 mg, 0.295 mmol) was added to the mixture, which was subsequently heated to 45 °C. After 24 h, MeOH was added to the reaction and solvent removed *in vacuo*. Crude material was purified by column chromatography (MeOH/DCM) to afford product **2.28** (6.5 mg, 24% over three steps) as an off-white solid. **^{1}H NMR** (300 MHz, CD_3OD): δ 7.32 (m, 3 H), 7.05 (m, 2 H), 6.88 (m, 1 H), 6.60 (m, 1 H), 5.36 (d, 1 H, J=13.5 Hz), 5.15 (m, 1 H), 4.16 (m, 1 H), 3.99 (m, 1 H), 3.80 (s, 3 H), 3.61 (M, 1 h), 3.41 (m, 1 H), 2.38 (m, 2 H), 2.93 (m, 2 H), 2.34 (m, 3 H), 2.12 (m, 1 H), 1.66 (m, 2 H). **m/z** 460 $(M+H)^+$.



2.2 2.27a-d

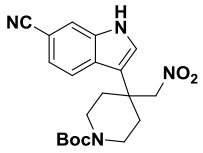
General Procedure A: To a solution of **indole** (1.1 equiv) in anhydrous THF (0.5 M) was added CH_3MgI (1.15 equiv, 3.0 M in anhydrous diethyl ether) dropwise over 5 min at room temperature. The mixture was stirred for 30 min before being cooled to 0 °C. A solution of **2.2** (1.0 equiv) in anhydrous THF (0.5 M) was added dropwise to the first mixture over 5 min. The

reaction was stirred at 0 °C for 15 min, then stirred for 16 h while allowed to warm to room temperature. After complete consumption of starting material, the reaction was quenched with saturated NH_4Cl and stirred for 30 minutes. The resulting mixture was extracted 3x with EtOAc, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. Crude brown residue was purified by column chromatography (EtOAc/hexanes) to afford desired product.



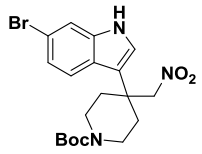
2.27a

tert-butyl 4-(5,6-dimethoxy-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.27a**). Synthesized according to **General Procedure A** from 5,6-dimethoxy-1H-indole and 620 mg of **2.2** to afford product **2.27a** (387 mg, 36% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.91 (s, 1 H), 7.01 (s, 1 H), 2.03 (s, 1 H), 4.64 (s, 2 H), 3.83 (m, 5 H), 3.78 (s, 3 H), 3.12 (t, 2 H, J=11.4 Hz), 2.33 (d, 2 H, J=13.2 Hz), 1.89 (m, 2 H), 1.42 (s, 9 H). **m/z** 419 $(M-H)^-$.



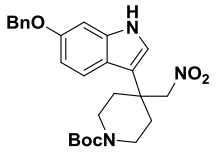
2.27b

tert-butyl 4-(6-cyano-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.27b**). Synthesized according to **General Procedure A** from 1H-indole-6-carbonitrile and 775 mg of **2.2** to afford product **2.27b** (250 mg, 20% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 9.96 (s, 1 H), 7.74 (t, 2 H, J=3.9 Hz), 7.33 (m, 1 H), 7.26 (d, 1 H, J=2.7 Hz), 4.74 (s, 2 H), 3.85 (m, 2 H), 3.13 (t, 2 H, J=11.1 Hz), 2.46 (d, 2 H, J=13.5 Hz), 1.98 (m, 2 H), 1.47 (s, 9 H). **m/z** 383 $(M-H)^-$.



2.27c

tert-butyl 4-(6-bromo-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.27c**). Synthesized according to **General Procedure A** from 6-bromo-1H-indole and 900 mg of **2.2** to afford product **2.27c** (595 mg, 64% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.81 (s, 1 H), 7.49 (m, 2 H), 7.20 (dd, 1 H, J=1.5 Hz, 8.4 Hz), 6.95 (d, 1 H, J=2.4 Hz), 4.68 (s, 2 H), 3.86 (d, 2 H, J=13.8 Hz), 3.08 (m, 2 H), 2.35 (d, 2 H, J=13.8 Hz), 1.94 (m, 2 H), 1.46 (s, 9 H). **m/z** 438 $(M-H)^-$



2.27d

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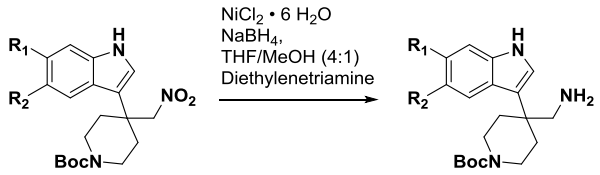
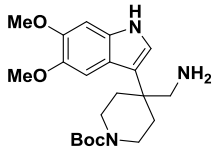
tert-butyl 4-(6-(benzyloxy)-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.27d**). Synthesized according to **General Procedure A** from 6-(benzyloxy)-1H-indole and 1.50 g of **2.2** to afford product **2.27d** (2.03 g, 70% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.54 – 8.37 (m, 1H), 7.59 – 7.31 (m, 6H), 6.98 – 6.81 (m, 3H), 5.07 (s, 2H), 4.69 (s, 2H), 3.89 (dt, J = 14.0, 4.1 Hz, 2H), 3.12 (ddd, J = 13.7, 11.1, 2.7 Hz, 2H), 2.43 (d, J = 13.8 Hz, 2H), 1.94 (ddd, J = 14.3, 11.0, 4.1 Hz, 2H), 1.49 (s, 9H). **m/z** 464 $(M-H)^-$.



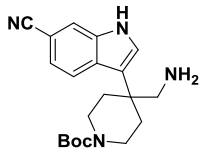
2.27a-d **2.28a-d**

General Procedure B: To a solution of **2.27** derivative (1.0 equiv) in THF (0.06 M) was added $NiCl_2 \cdot 6\ H_2O$ (1.0 equiv) and $NaBH_4$ (4.0 equiv). The solution was cooled to 0 °C before MeOH (0.25 M) was added dropwise over 2 min (*note: vigorous bubbling*). The resulting solution was stirred at room temperature for 4 h. Next, diethylenetriamine was added, and the mixture was stirred for 1 h at room temperature before concentration *in vacuo*. The crude concentrate was reconstituted with EtOAc and washed 2x with $NaHCO_3$ and once with brine. The resulting organic layer was dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude residue was purified via column chromatography (MeOH/DCM) to afford desired product.



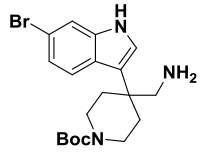
2.28a

tert-butyl 4-(5,6-dimethoxy-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.28a**). Synthesized according to **General Procedure B** from **2.27a** (700 mg) to afford product **2.28a** (372 mg, 57% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 9.03 (s, 2 H), 7.07 (s, 1 H), 6.83 (s, 1 H), 6.82 (s, 1 H), 3.88 (s, 3 H), 3.82 (s, 3 H), 3.74 (m, 2 H), 3.11 (t, 2 H, J=11.1 Hz), 2.91 (s, 2 H), 2.21 (d, 2 H, J=13.5 Hz), 1.94 (s, 2 H), 1.66 (m, 2 H), 1.42 (s, 9 H). **m/z** 390 $(M+H)^+$.



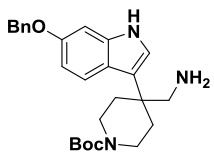
2.28b

tert-butyl 4-(6-cyano-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.28b**). Synthesized according to **General Procedure B** from **2.27b** (225 mg) to afford product **2.28b** (85 mg, 41% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 9.98 (s, 1 H), 7.78 (d, 1 H, J=8.4 Hz), 7.70 (s, 1 H), 7.28 (s, 1 H), 7.23 (s, 1 H), 3.78 (m, 2 H), 3.12 (t, 2 H, J=11.1 Hz), 3.01 (s, 2 H), 2.37 (m, 2 H), 2.16 (s, 2 H), 1.77 (m, 2 H), 1.46 (s, 9 H). **m/z** 353 $(M-H)^-$.



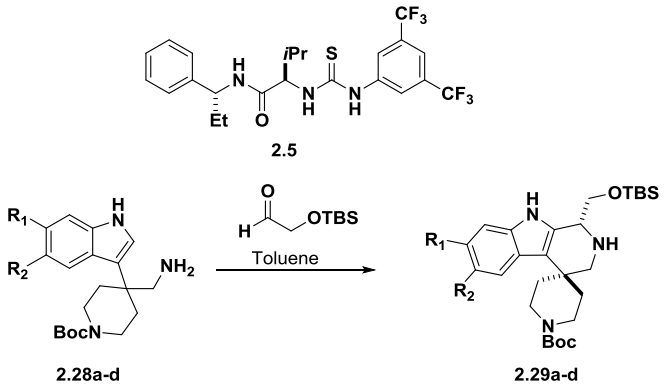
2.28c

tert-butyl 4-(6-bromo-1H-indol-3-yl)-4-(nitromethyl)piperidine-1-carboxylate (**2.28c**). Synthesized according to **General Procedure B** from **2.27c** (990 mg) to afford product **2.28c** (595 mg, 65% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 10.05 (s, 1 H), 7.37 (m, 2 H), 6.97 (m, 1 H), 6.81 (m, 1 H), 3.67 (s, 2 H), 3.00 (m, 2 H), 2.82 (s, 2 H), 2.86 (m, 2 H), 1.72 (m, 2 H), 1.37 (s, 9 H). **m/z** 408 $(M-H)^-$.

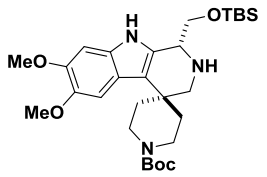


2.28d

tert-butyl 4-(aminomethyl)-4-(6-(benzyloxy)-1H-indol-3-yl)piperidine-1-carboxylate (**2.28d**). Synthesized according to **General Procedure B** from **2.27d** (2.02 g) to afford product **2.28d** (1.00 g, 53% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.98 (s, 1 H), 7.55 (d, 1 H, J=9.4 Hz), 7.31-7.45 (m, 5 H), 6.89 (d, 1 H, J=2 Hz), 6.82 (m, 2 H), 5.05 (s, 2 H), 3.83 (m, 2 H), 3.11 (t, 2 H, J=10.8 Hz), 2.91 (s, 2 H), 2.21 (d, 2 H, J=13.2 Hz), 1.81 (s, 2 H), 1.66 (dt, 2 H, J=3.8 Hz, 12.4 Hz), 1.46 (s, 9 H). **m/z** 353 $(M-H)^-$.

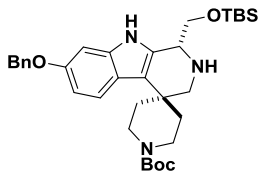


General Procedure C: To a solution of **2.28a** derivative (1.0 equiv) in toluene (0.02 M) at -20 °C was added (*R*)-2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-N,3-dimethyl-N-((R)-1-phenylpropyl)butanamide catalyst **2.5**, 581 mg, 1.118 mmol, 0.15 equiv) (*Note: **2.5** was graciously donated by the Jacobsen group at Harvard University*). The solution was stirred for 5 minutes before freshly-distilled 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (1.2 equiv) in toluene (2 M) was added. The solution was maintained at -20 °C using a CryoCool™ and stirred for 24 h before additional **2.5** (0.08 equiv) was added. At 114 h, additional **2.5** (0.08 equiv) was added into the reaction mixture. At 138 h, the reaction was brought to room temperature and solvent removed *in vacuo*. Crude residue was purified via column chromatography (EtOAc/hexanes) to yield desired product.



2.29a

tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-6',7'-dimethoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.29a**). Synthesized according to **General Procedure C** from **2.28a** (372 mg) to afford product **2.29a** (412 mg, 79% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.63 (s, 1 H), 7.09 (s, 1 H), 6.79 (s, 1 H), 4.07 (m, 3 H), 3.87 (s, 5 H [3+2]), 3.83 (s, 3 H), 3.73 (t, 1 H, J=8.4 Hz), 3.43 (d, 1 H, J=12.6 Hz), 2.90 (m, 2 H), 2.82 (d, 1 H, J=12.9 Hz), 2.47 (m, 1 H), 2.24 (m, 2 H), 1.75 (d, 1 H, J=13.5 Hz), 1.53 (s, 9 H), 0.94 (s, 9 H), 0.11 (s, 6 H). **m/z** 548 $(M+H)^+$. ***18:1 er by SFC***.

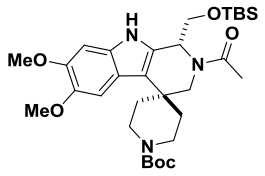


2.29d

tert-butyl (*R*)-7'-(benzyloxy)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.29d**). Synthesized according to **General Procedure C** from **2.28d** (1.20 g) to afford product **2.29d** (1.13 g, 69% yield). **^{1}H NMR** (300 MHz, $(CD_3)_2SO$): δ 8.46 (s, 1 H), 7.54 (d, 1 H, J=8.8 Hz), 7.29-7.48 (m, 5 H), 6.88 (d, 1 H, J=2.2 Hz), 6.82 (dd, 1 H, J=2.2 Hz, 8.8 Hz), 5.09 (s, 2 H), 4.10 (m, 3 H), 3.91 (dd, 1 H, J=5.0 Hz, 9.4 Hz), 3.69 (t, 1 H, J=9.2 Hz), 3.48 (d, 1 H, J=12.9 Hz), 2.90 (m, 3 H), 2.53 (m, 1 H), 2.24 (m, 1 H), 1.75 (d, 1 H, J=14.4 Hz), 1.52 (s, 9 H), 0.94 (s, 9 H), 0.11 (s, 6 H). **m/z** 592 $(M+H)^+$. ***4:1 er by SFC***.

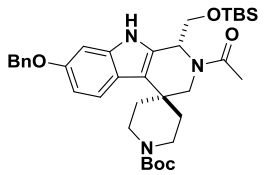
2.29a-d 2.30a-d

General Procedure D: To a solution of **2.29** derivative (1.0 equiv) in DCM (0.1 M) at -78 °C was added triethylamine (5 equiv) followed by acetyl chloride (1.1 equiv). The reaction was stirred for 15 minutes at -78 °C before the addition of saturated NH_4Cl. The resulting solution was brought to room temperature and extracted 3x with DCM. Combined organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* Crude material was purified via column chromatography (EtOAc/hexanes) to afford desired product.



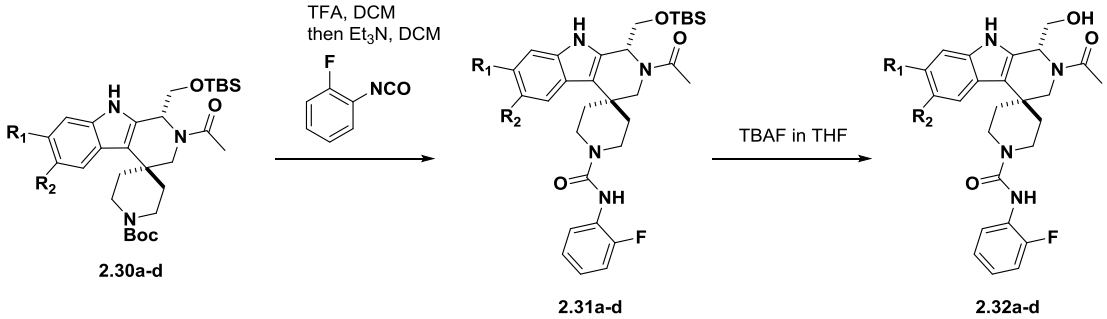
2.30a

tert-butyl (*R*)-2'-acetyl-1'-(((tert-butyldimethylsilyl)oxy)methyl)-6',7'-dimethoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.30a**). Synthesized according to **General Procedure D** from **2.29a** (190 mg) to afford product **2.30a** (195 mg, 95% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.64 (s, 1 H), 7.04 (s, 1 H), 6.80 (s, 1 H), 4.10 (m, 3 H), 3.95 (m, 2 H), 3.88 (t, 6 H, J=3.6 Hz), 3.82 (s, 3 H), 3.21 (m, 1 H), 2.98 (m, 2 H), 2.64 (m, 2 H), 2.32 (s, 3 H), 1.84 (m, 1 H), 1.48 (s, 9 H), 0.91 (s, 9 H), 0.04 (s, 6 H). **m/z** 587 $(M-H)^-$.



2.30d

tert-butyl (*R*)-2'-acetyl-7'-(benzyloxy)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.30d**). Synthesized according to **General Procedure D** from **2.29d** (1.11 g) to afford product **2.30d** (1.19 mg, quantitative). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.62 (s, 1 H), 7.55 (d, 1 H, J=9.2 Hz), 7.30-7.46 (m, 5 H), 6.86 (ddd, 2 H, J=2.2 Hz, 6.3 Hz, 16.3 Hz), 5.31 (d, 1 H, J=13.9 Hz), 5.08 (m, 2 H), 4.98 (t, 1 H, J= 7.1 Hz), 3.91-4.28 (m, 4 H), 3.24 (m, 1 H), 2.98 (m, 1 H), 2.73 (m, 1 H), 2.59 (d, 1 H, J=12.8 Hz), 2.23 (m, 3 H), 1.93 (m, 1 H), 1.54 (m, 11 H [9+2]), 0.92 (m, 9 H), 0.07 (m, 6 H). **m/z** 634 $(M+H)^+$.

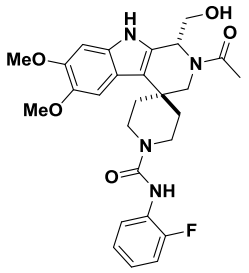


2.30a-d **2.31a-d** **2.32a-d**

General Procedure E: To a solution of **2.30** derivative (1.0 equiv) in DCM (0.1 M) was added TFA (2.0 M) (20:1 DCM/TFA v/v). The solution was stirred at room temperature for 3 h before being quenched with saturated $NaHCO_3$. The resulting mixture was extracted 3x with DCM, and the organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo* to afford crude intermediate.

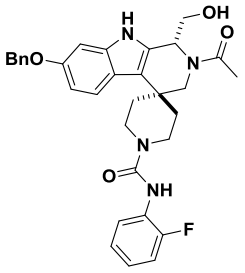
Crude intermediate was reconstituted in DCM (0.1 M), followed by the addition of 2-fluorophenylisocyanate (1.0 equiv). After 1 h at room temperature, 50 μL of triethylamine was added to the mixture. At 3 h, the reaction was quenched with deionized water and extracted 3x with dichloromethane. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified *briefly* by column chromatography (EtOAc/hexanes) to afford "crude" **2.31** derivative.

To a solution of crude **2.31** derivative (1.0 equiv) in THF (0.1 M) at was added TBAF in THF (3.0 equiv). The reaction was allowed to stir at room temperature. After 5 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (MeOH/DCM) to afford desired product.



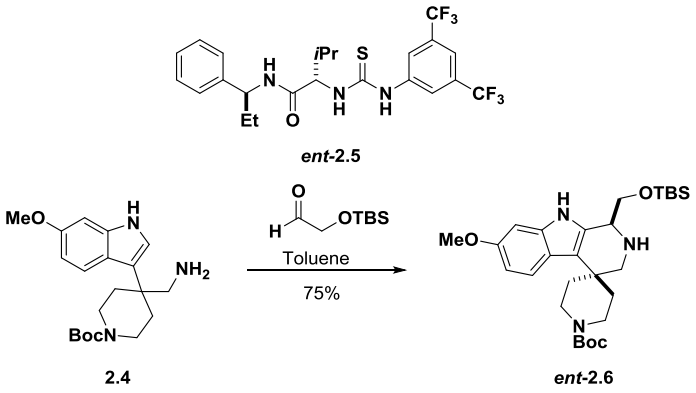
2.32a

(*R*)-2'-acetyl-N-(2-fluorophenyl)-1'-(hydroxymethyl)-6',7'-dimethoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.32a**). Synthesized according to **General Procedure E** from **2.30a** (88 mg) to afford product **2.32a** (11 mg, 14% yield over three steps). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.80 (s, 0.5 H), 8.60 (s, 0.5 H), 8.07 (q, 1 H, J=8.4 Hz), 7.03 (m, 3 H), 6.82 (m, 2 H), 5.70 (s, 0.5 H), 5.01 (s, 0.5 H), 4.17 (m, 2 H), 3.97 (m, 1 H), 3.87 (m, 6 H), 3.20 (m, 2 H), 2.76 (m, 3 H), 2.21 (s, 4 H), 2.06 (m, 2 H), 1.65 (m, 2 H). **m/z** 509 $(M-H)^-$.



2.32d

(*R*)-2'-acetyl-7'-(benzyloxy)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.32d**). Synthesized according to **General Procedure E** from **2.30d** (80 mg) to afford product **2.32d** (11 mg, 23% yield over three steps). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.91 (s, 0.5 H), 8.73 (s, 0.5 H), 8.06 (q, 1 H, J=8.2 Hz), 7.50 (m, 1 H), 7.26-7.41 (m, 4 H), 6.94-7.14 (m, 2 H), 6.73-6.87 (m, 3 H), 5.01 (s, 2 H), 4.18 (m, 1 H), 3.94 (m, 3 H), 2.48 (m, 1 H), 3.21 (d, 1 H, J=13.7 Hz), 3.06 (m, 2 H), 2.72 (m, 2 H), 2.18 (m, 3 H). **m/z** 557 $(M+H)^+$.

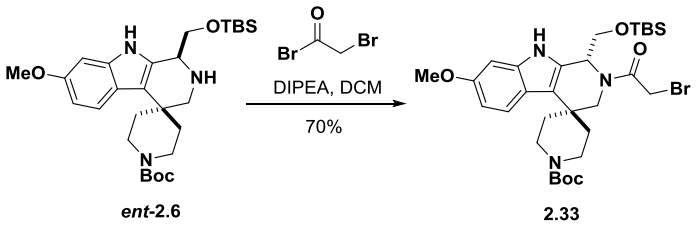


ent-2.5

2.4

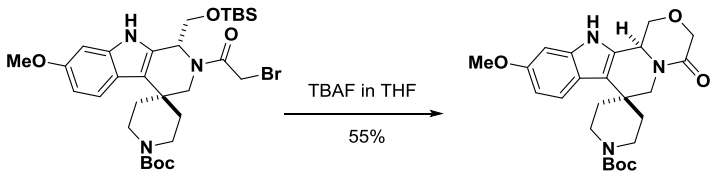
ent-2.6

tert-butyl (*S*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (***ent*-2.6**). Performed as reported for the synthesis of **2.6** from starting material **2.4** (200 mg) and ***ent*-2.5** ((*S*)-2-(3-(3,5-bis(trifluoromethyl)phenyl)thioureido)-*N*,3-dimethyl-*N*-((*R*)-1-phenylpropyl)butanamide catalyst) to afford product ***ent*-2.6** (214 mg, 75% yield). ***9:1 er by SFC***.



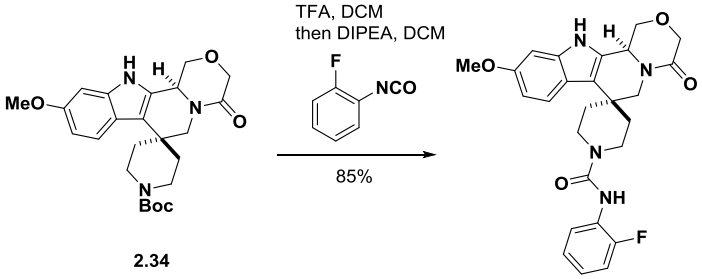
ent-2.6 2.33

<u>*tert*-butyl (*R*)-2'-(2-bromoacetyl)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate</u> (**2.33**). To a solution of ***ent*-2.6** (190 mg, 0.368 mmol) in DCM (3.6 mL, 0.1 M) at -78 °C was added DIPEA (385 uL, 2.21 mmol) followed by bromoacetyl bromide (40 uL, 0.460 mmol). The reaction was maintained at -78 °C. After 2 h, another portion of bromoacetyl bromide (40 uL, 0.460 mmol) was added to the reaction mixture. After 3.5 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.33** (164 mg, 70% yield). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.82 (s, 1 H), 7.53 (d, 1 H, J=9 Hz), 6.83 (d, 1 H, J=1.8 Hz), 6.73 (dd, 1 H, J=8.7 Hz, 1.8 Hz), 5.25 (d, 1 H, J=13.2 Hz), 5.14 (dd, 1 H, J=4.5 Hz, 4.5 Hz), 4.34 (d, 1 H, J=10.5 Hz), 4.10 (m, 4 H0, 3.87 (m, 3 H), 3.81 (s, 3 H), 3.28 (t, 1 H, J=14.1 Hz), 3.02 (m, 1 H), 2.70 (t, 2 H, J=13.5 Hz), 1.91 (m, 1 H), 1.52 (s, 9 H), 0.91 (s, 9 H), 0.08 (s, 3 H), 0.07 (s, 3 H). **m/z** 636 $(M-H)^-$.



2.33 2.34

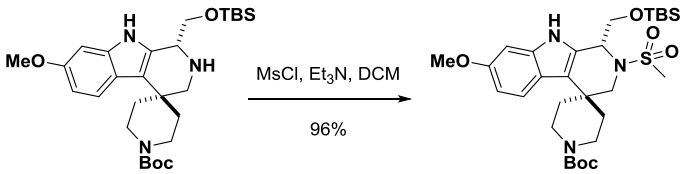
tert-butyl (*S*)-10'-methoxy-4'-oxo-3',4',12',12b'-tetrahydro-1'H,6'H-spiro[piperidine-4,7'-[1,4]oxazino[4',3':1,2]pyrido[3,4-b]indole]-1-carboxylate (**2.34**). To a solution of **2.33** (110 mg, 0.173 mmol) in THF (1.7 mL, 0.1 M) at room temperature was added 1.0 M TBAF in THF solution (518 uL, 3.0 equiv.). After 6 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (MeOH/DCM) to afford product **2.34** (42 mg, 55% yield). **^{1}H NMR** (300 MHz, $(CD_3)_2SO$): δ 10.86 (s, 1 H), 7.41 (d, 1 H, J=9 Hz), 6.83 (d, 1 H, J=2.1 Hz), 6.63 (dd, 1 H, J=2.1 Hz, 9 Hz), 5.75 (s, 1 H), 5.13 (d, 1 H, J=12.9 Hz), 5.00 (dd, 1 H, J=4.5 Hz, 9.6 Hz), 4.55 (dd, 1 H, J=4.5 Hz, 11.4 Hz), 4.14 (q, 2 H, J=16.2 Hz), 3.86 (m, 2 H), 3.74 (s, 3 H), 3.46 (t, 1 H, J=10.5 Hz), 3.16 (d, 1 H, J=5.1 Hz), 3.00 (m, 2 H), 2.55 (m, 2 H), 1.72 (m, 1 H), 1.42 (s, 9 H). **m/z** 440 $(M-H)^-$.



(*S*)-*N*-(2-fluorophenyl)-10'-methoxy-4'-oxo-3',4',12',12b'-tetrahydro-1'H,6'H-spiro[piperidine-4,7'-[1,4]oxazino[4',3':1,2]pyrido[3,4-b]indole]-1-carboxamide (**2.35**). To a solution of **2.34** (40 mg, 0.091 mmol) in DCM (900 uL, 0.1 M) was added TFA (91 uL, 910 mmol) (10:1 DCM/TFA v/v). The solution was stirred at room temperature for 1 h before volatiles were removed *in vacuo* and azeotroped 3x with toluene.

The resulting crude material was reconstituted in DCM (900 uL, 0.1 M), followed by the addition of DIPEA (80 uL, 0.455 mmol), 2-fluorophenylisocyanate (10 uL, 0.091 mmol), and a

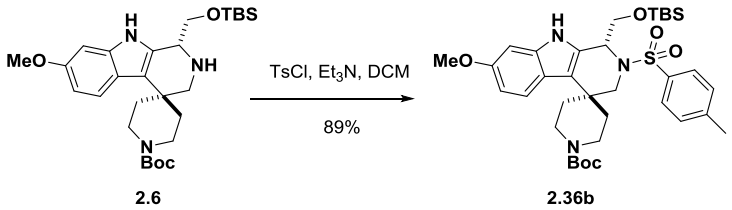
drop of EtOAc (for solubility). After 16 h at room temperature, the reaction was quenched with MeOH and solvent removed *in vacuo*. The crude material was purified by column chromatography (MeOH/DCM) to afford product **2.35** (37 mg, 85% yield). **^{1}H NMR** (300 MHz, DMSO): δ 8.97 (s, 1 H), 8.05 (dt, 1 H, J=1.2 Hz, 8.1 Hz), 7.53 (d, 1 H, J=8.7 Hz), 7.07 (m, 2 H), 6.96 (m, 1 H), 6.78 (d, 2 H, J=2.1 Hz), 6.73 (dd, 1 H, J=2.1 Hz, 8.7 Hz), 5.35 (d, 1 H, J=12.9 Hz), 4.99 (dd, 1 H, J=4.5 Hz, 10.2 Hz), 4.55 (dd, 1 H, J=4.5 Hz, 11.7 H), 4.37 (d, 1 H, J=16.5 Hz), 4.25 (d, 1 H, J=16.5 Hz), 4.16 (d, 1 H, J=16.8 Hz), 3.92 (d, 1 H, J=10.8 Hz), 3.78 (s, 3 H), 3.54 (t, 1 H, J=13.5 Hz), 3.47 (t, 1 H, J=11.1 Hz), 3.18 (t, 1 H, J=13.2 Hz), 2.80 (dt, 1 H, J=4.5 Hz, 13.5 Hz), 2.60 (d, 1 H, J=13.2 Hz), 2.10 (dt, 1 H, J=4.2 Hz, 12.9 Hz), 1.64 (d, 2 H, J=13.5 Hz). **m/z** 479 $(M+H)^+$.



2.6 2.36a

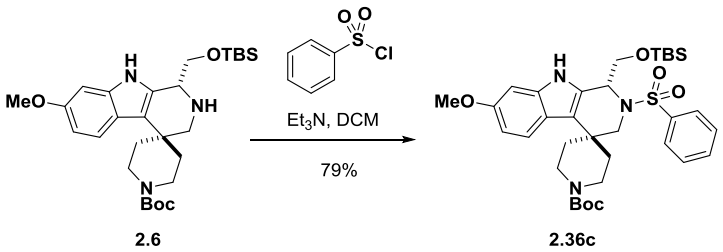
tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2'-(methylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36a**). To a solution of **2.6** (125 mg, 0.242 mmol) in DCM (2.4 mL, 0.1 M) at 0 °C was added Et_3N (101 uL, 0.727 mmol) followed by methanesulfonyl chloride (21 uL, 0.267 mmol). After 2 h at 0 °C, the reaction was quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36a** (138 mg, 96% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.53 (d, J = 8.7 Hz, 1H), 6.86 – 6.71 (m, 2H), 4.98 (t, J = 6.5 Hz, 1H), 4.37 (d, J

= 13.6 Hz, 1H), 4.25 – 3.99 (m, 5H), 3.97 – 3.79 (m, 5H), 3.24 – 2.97 (m, 6H), 2.95 – 2.83 (m, 1H), 2.69 (td, J = 13.6, 4.7 Hz, 1H), 1.51 (s, 12H), 1.12 – 0.84 (m, 12H), 0.07 (d, J = 2.4 Hz, 6H). **m/z** 592 $(M-H)^-$.



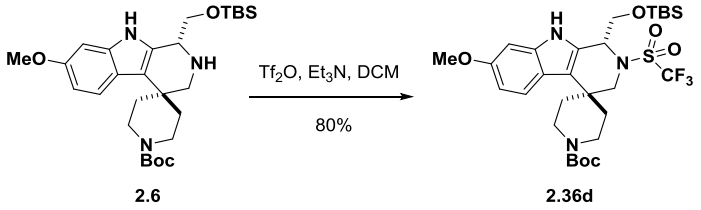
2.6 2.36b

tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2'-tosyl-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36b**). To a solution of **2.6** (20 mg, 0.039 mmol) in DCM (0.76 mL, 0.05 M) at room temperature was added Et_3N (16 uL, 0.116 mmol) followed by 4-toluenesulfonyl chloride (8 mg, 0.043 mmol). After 16 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36b** (23 mg, 89% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 6.78 (d, *J* = 2.2 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.97 (dd, *J* = 9.6, 4.4 Hz, 1H), 4.37 (d, *J* = 13.7 Hz, 1H), 4.17 – 4.03 (m, 1H), 3.95 – 3.84 (m, 2H), 3.82 (s, 3H), 3.67 (t, *J* = 9.3 Hz, 1H), 3.01 (d, *J* = 13.5 Hz, 1H), 2.95 – 2.70 (m, 2H), 2.63 (td, *J* = 13.8, 4.9 Hz, 1H), 2.40 (s, 3H), 1.84 (td, *J* = 13.7, 5.1 Hz, 1H), 1.50 (s, 9H), 1.47 – 1.37 (m, 2H), 0.89 (s, 9H), 0.00 (d, *J* = 13.6 Hz, 6H). **m/z** 668 $(M-H)^-$.



2.6 2.36c

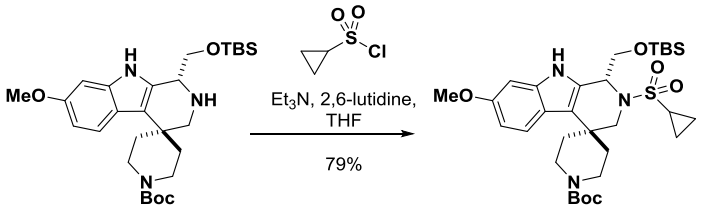
tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2'-(phenylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36c**). To a solution of **2.6** (15 mg, 0.029 mmol) in DCM (0.60 mL, 0.05 M) at room temperature was added Et_3N (21 uL, 0.145 mmol) followed by benzenesulfonyl chloride (4.5 uL, 0.035 mmol). After 5 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36c** (15 mg, 79% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.57 (t, *J* = 7.0 Hz, 1H), 7.54 – 7.45 (m, 3H), 6.79 (d, *J* = 2.6 Hz, 1H), 6.76 – 6.69 (m, 1H), 5.00 (dd, *J* = 9.5, 4.4 Hz, 1H), 4.40 (d, *J* = 13.7 Hz, 1H), 4.16 – 4.05 (m, 1H), 3.83 (s, 5H), 3.67 (q, *J* = 6.6, 3.9 Hz, 1H), 3.03 (d, *J* = 13.7 Hz, 1H), 2.94 – 2.75 (m, 2H), 2.65 (dq, *J* = 13.7, 5.0 Hz, 1H), 1.85 (td, *J* = 13.5, 5.1 Hz, 1H), 1.42 (t, *J* = 11.7 Hz, 2H), 0.89 (s, 9H). **m/z** 654 $(M-H)^-$.



2.6 2.36d

tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2'-((trifluoromethyl)sulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-

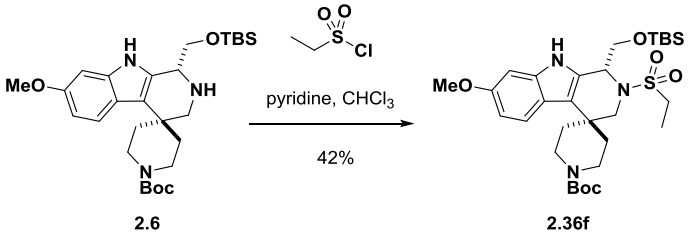
carboxylate (**2.36d**). To a solution of **2.6** (16 mg, 0.031 mmol) in DCM (0.60 mL, 0.05 M) at -78 °C was added Et_3N (45 uL, 0.310 mmol) followed by triflic anhydride (6.3 mg, 0.037 mmol). After 2 h at -78 °C, the reaction was quenched with saturated NH_4Cl, warmed to ambient temperature, and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36d** (16 mg, 80% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 2.5 Hz, 1H), 6.77 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.11 – 4.97 (m, 1H), 4.42 (d, *J* = 13.9 Hz, 1H), 4.11 (dt, *J* = 33.4, 9.3 Hz, 3H), 3.91 (d, *J* = 8.9 Hz, 1H), 3.84 (s, 3H), 3.25 (d, *J* = 14.0 Hz, 1H), 3.08 (s, 1H), 2.93 – 2.67 (m, 2H), 2.05 (s, 2H), 1.79 (s, 1H), 1.61 (t, *J* = 7.1 Hz, 1H), 1.51 (s, 9H), 0.91 (s, 9H), 0.06 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -75.67 (s). **m/z** 646 $(M-H)^-$.



2.6 **2.36e**

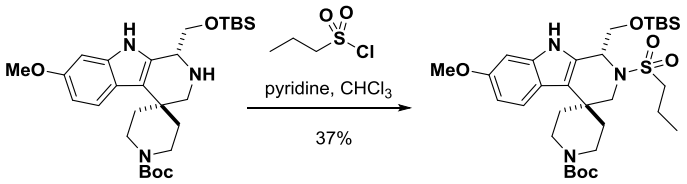
tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-2'-(cyclopropylsulfonyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36e**). To a solution of **2.6** (23 mg, 0.045 mmol) in THF (0.90 mL, 0.05 M) at room temperature was added Et_3N (19 uL, 0.134 mmol) and 2,6-lutidine (15.5 uL, 0.134 mmol) followed by cyclopropanesulfonyl chloride (5.0 uL, 0.049 mmol). The reaction was heated to 70 °C. After 2 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36e** (15 mg, 54%

yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 6.75 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.00 – 4.90 (m, 1H), 4.36 (d, *J* = 13.8 Hz, 1H), 4.20 – 4.05 (m, 2H), 3.88 (d, *J* = 9.0 Hz, 1H), 3.84 (s, 3H), 3.16 – 3.02 (m, 2H), 2.85 (d, *J* = 15.2 Hz, 1H), 2.70 (td, *J* = 13.6, 4.6 Hz, 1H), 2.46 (tt, *J* = 7.8, 4.6 Hz, 1H), 2.01 (d, *J* = 4.1 Hz, 1H), 1.51 (s, 9H), 1.33 – 1.18 (m, 4H), 1.06 – 0.97 (m, 2H), 0.93 (s, 9H), 0.07 (d, *J* = 7.9 Hz, 6H). **m/z** 618 $(M-H)^-$.



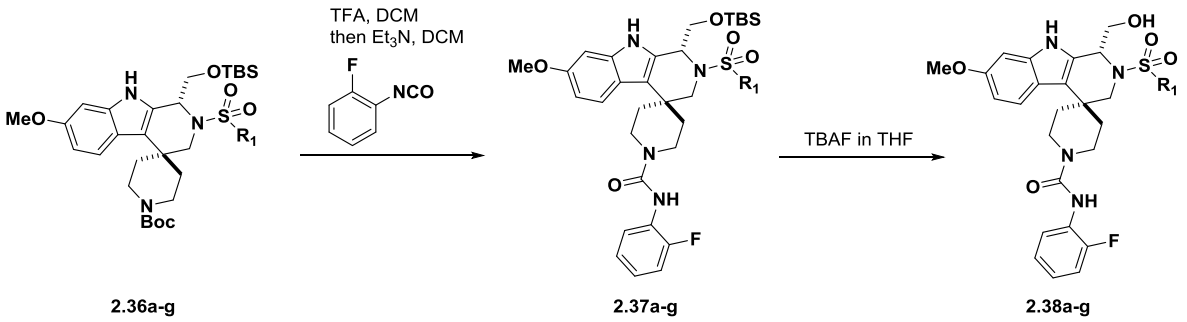
2.6 **2.36f**

tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-2'-(ethylsulfonyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36f**). To a solution of **2.6** (20 mg, 0.039 mmol) in $CHCl_3$ (0.78 mL, 0.05 M) at room temperature was added pyridine (31 uL, 0.388 mmol) followed ethanesulfonyl chloride (5.5 uL, 0.058 mmol). The reaction was maintained at room temperature for 2 h 30 min, then heated to 40 °C for 4 h. The reaction was then quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36f** (10 mg, 42% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 6.86 – 6.79 (m, 1H), 6.74 (dd, *J* = 8.5, 2.6 Hz, 1H), 4.95 (t, *J* = 6.4 Hz, 1H), 4.37 (d, *J* = 13.9 Hz, 1H), 4.19 – 3.98 (m, 3H), 3.84 (d, *J* = 6.6 Hz, 5H), 3.24 – 3.00 (m, 4H), 2.88 (t, *J* = 13.8 Hz, 1H), 2.69 (td, *J* = 13.8, 4.9 Hz, 1H), 2.01 (dd, *J* = 16.7, 11.1 Hz, 1H), 1.82 (d, *J* = 14.1 Hz, 1H), 1.47 (s, 9H), 1.39 (t, *J* = 7.3 Hz, 3H), 0.92 (s, 9H), 0.07 (d, *J* = 6.7 Hz, 6H). **m/z** 606 $(M-H)^-$.



2.6 2.36g

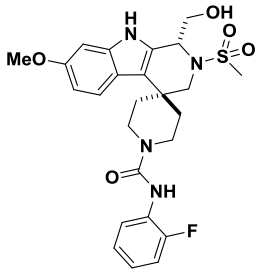
tert-butyl (*R*)-1'-(((tert-butyldimethylsilyl)oxy)methyl)-7'-methoxy-2'-(propylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxylate (**2.36g**). To a solution of **2.6** (20 mg, 0.039 mmol) in $CHCl_3$ (0.78 mL, 0.05 M) at room temperature was added pyridine (31 uL, 0.388 mmol) followed 1-propanesulfonyl chloride (6.6 uL, 0.058 mmol). The reaction was maintained at room temperature for 16 h, then heated to 40 °C for 24 h. The reaction was then quenched with saturated NH_4Cl and extracted 3x with DCM. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (EtOAc/hexanes) to afford product **2.36g** (9 mg, 37% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 6.81 (s, 1H), 6.79 – 6.65 (m, 1H), 4.94 (t, *J* = 6.2 Hz, 1H), 4.36 (d, *J* = 13.9 Hz, 1H), 4.23 – 3.96 (m, 3H), 3.84 (d, *J* = 5.8 Hz, 4H), 3.19 – 2.96 (m, 4H), 2.88 (t, *J* = 13.7 Hz, 1H), 2.70 (dt, *J* = 13.9, 7.0 Hz, 1H), 2.06 – 1.77 (m, 5H), 1.51 (s, 9H), 1.06 (t, *J* = 7.4 Hz, 3H), 0.93 (s, 9H), 0.07 (d, *J* = 7.5 Hz, 6H). **m/z** 620 $(M-H)^-$.



General Procedure F: To a solution of **2.36** derivative (1.0 equiv) in DCM (0.1 M) was added TFA (2.0 M) (20:1 DCM/TFA v/v). The solution was stirred at room temperature for 3 h before solvent was removed *in vacuo*.

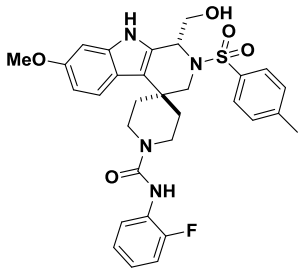
Crude intermediate was reconstituted in DCM (0.1 M), followed by the addition of 2-fluorophenylisocyanate (1.0 equiv). After 1 h at room temperature, 50 μL of triethylamine was added to the mixture. At 3 h, the reaction was quenched with deionized water and extracted 3x with dichloromethane. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified *briefly* by column chromatography (EtOAc/hexanes) to afford "crude" **2.37** derivative.

To a solution of crude **2.37** derivative (1.0 equiv) in THF (0.1 M) at was added TBAF in THF (3.0 equiv). The reaction was allowed to stir at room temperature. After 5 h, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The organic layers were dried over Na_2SO_4, filtered, and solvent removed *in vacuo.* The crude material was purified by column chromatography (MeOH/DCM) to afford desired product.



2.38a

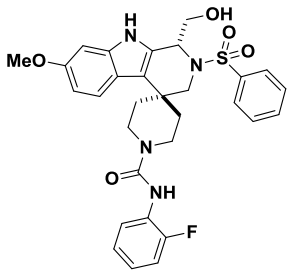
(*R*)-*N*-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-2'-(methylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38a**). Synthesized according to **General Procedure F** from **2.36a** (138 mg) to afford product **2.38a** (64 mg, 53% yield over two steps). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ 10.79 (s, 1 H), 8.37 (s, 1 H), 7.43 (m, 2 H), 7.21 (m, 1 H), 7.15 (m, 2 H), 6.85 (s, 1 H), 6.64 (d, 1 H, J= 8.8 Hz), 5.36 (s, 1 H), 4.91 (s, 1 H), 4.35 (d, 1 H, J=13.9 Hz), 4.04 (dd, 2 H, J=13.3 Hz, 14.6 Hz), 3.81 (s, 2 H), 3.75 (s, 3 H), 3.21 (s, 3 H), 3.10 (m, 3 H), 2.59 (t, 1 H, J=13.0 Hz), 1.85 (s, 1 H), 1.75 (d, 1 H, J=13.4 Hz), 1.47 (d, 1 H, J=13.4 Hz). **m/z** 517 $(M+H)^+$.



2.38b

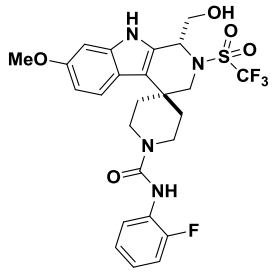
(*R*)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-2'-tosyl-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38b**). Synthesized according to **General Procedure F** from **2.36b** (23 mg) to afford product **2.38b** (13 mg, 65% yield over two steps). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.29 (s, 2H), 8.11 (td, *J* = 8.2, 1.6 Hz, 2H), 7.81 – 7.74 (m, 4H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 4H), 7.17 – 6.94 (m, 6H), 6.81

(d, *J* = 2.3 Hz, 2H), 6.73 (dd, *J* = 8.7, 2.3 Hz, 2H), 6.67 (d, *J* = 4.0 Hz, 2H), 5.11 (dd, *J* = 7.3, 5.6 Hz, 2H), 4.37 (d, *J* = 13.9 Hz, 2H), 4.13 (d, *J* = 13.8 Hz, 2H), 3.97 (dd, *J* = 10.6, 5.7 Hz, 2H), 3.83 – 3.73 (m, 10H), 3.17 – 2.98 (m, 6H), 2.73 (td, *J* = 13.6, 4.8 Hz, 2H), 2.41 (s, 6H), 2.20 (s, 1H), 1.97 (td, *J* = 13.7, 4.9 Hz, 3H), 1.30 (d, *J* = 30.1 Hz, 3H). **m/z** 593 $(M+H)^+$.



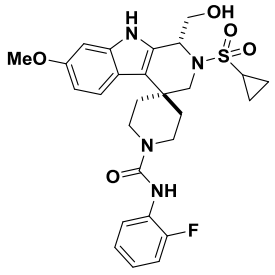
2.38c

(*R*)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-2'-(phenylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38c**). Synthesized according to **General Procedure F** from **2.36c** (15 mg) to afford product **2.38c** (9 mg, 68% yield over two steps). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ 10.66 (s, 1H), 8.36 (s, 1H), 7.97 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.0 Hz, 1H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.49 – 7.36 (m, 2H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.13 (dd, *J* = 6.5, 3.6 Hz, 2H), 6.90 – 6.85 (m, 1H), 6.62 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.94 (dt, *J* = 17.7, 5.3 Hz, 2H), 4.43 (d, *J* = 13.8 Hz, 1H), 4.11 – 3.99 (m, 1H), 3.91 (d, *J* = 13.5 Hz, 1H), 3.74 (s, 3H), 3.63 (dq, *J* = 11.1, 6.2 Hz, 2H), 3.05 (t, *J* = 13.4 Hz, 2H), 1.80 (t, *J* = 12.9 Hz, 1H), 1.44 (d, *J* = 13.7 Hz, 2H). **m/z** 579 $(M+H)^+$.



2.38d

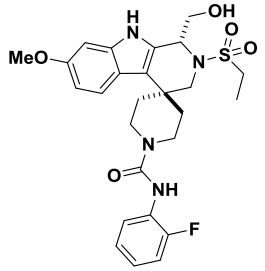
(*R*)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-2'-((trifluoromethyl)sulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38d**). Synthesized according to **General Procedure F** from **2.36d** (16 mg) to afford product **2.38d** (9 mg, 63% yield over two steps). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ 10.84 (s, 1H), 8.39 (s, 1H), 7.45 (dd, *J* = 7.4, 4.4 Hz, 2H), 7.21 (td, *J* = 8.5, 6.7, 4.2 Hz, 1H), 7.14 (dd, *J* = 6.3, 3.3 Hz, 2H), 6.88 (d, *J* = 2.5 Hz, 1H), 6.66 (dd, *J* = 8.8, 2.7 Hz, 1H), 5.35 (t, *J* = 5.2 Hz, 1H), 4.94 (s, 1H), 4.42 (d, *J* = 14.3 Hz, 1H), 4.07 (dd, *J* = 14.7, 9.3 Hz, 2H), 3.87 (q, *J* = 7.5, 6.4 Hz, 2H), 3.75 (s, 2H), 3.65 (d, *J* = 14.5 Hz, 1H), 3.17 (d, *J* = 5.5 Hz, 1H), 3.07 (q, *J* = 13.3, 12.2 Hz, 2H), 1.96 (d, *J* = 13.6 Hz, 1H), 1.65 (d, *J* = 13.7 Hz, 1H), 1.52 (d, *J* = 13.8 Hz, 1H). ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ -74.29. **m/z** 571 $(M+H)^+$.



2.38e

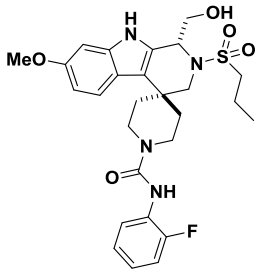
(*R*)-2'-(cyclopropylsulfonyl)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38e**). Synthesized

according to **General Procedure F** from **2.36e** (15 mg) to afford product **2.38e** (9 mg, 68% yield over two steps). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.10 (td, *J* = 8.2, 1.7 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.17 – 6.92 (m, 3H), 6.82 (d, *J* = 2.4 Hz, 1H), 6.79 – 6.63 (m, 2H), 5.08 (t, *J* = 6.7 Hz, 1H), 4.34 (d, *J* = 13.8 Hz, 1H), 4.21 (d, *J* = 13.5 Hz, 1H), 4.07 (dd, *J* = 11.0, 6.9 Hz, 1H), 4.00 – 3.76 (m, 5H), 3.34 (td, *J* = 13.1, 3.3 Hz, 1H), 3.17 – 2.96 (m, 2H), 2.78 (dt, *J* = 13.7, 6.8 Hz, 1H), 2.65 (tt, *J* = 8.1, 4.7 Hz, 1H), 2.06 (td, *J* = 13.3, 4.7 Hz, 1H), 1.97 – 1.86 (m, 1H), 1.57 (d, *J* = 13.4 Hz, 1H), 1.30 – 1.17 (m, 3H), 1.03 (qd, *J* = 9.9, 7.7 Hz, 2H). **m/z** 541 $(M-H)^-$.



2.38f

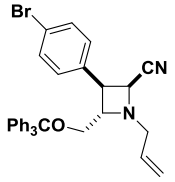
(*R*)-2'-(ethylsulfonyl)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38f**). Synthesized according to **General Procedure F** from **2.36f** (15 mg) to afford product **2.38f** (10.4 mg, 80% yield over two steps). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ 10.80 (s, 1H), 8.37 (s, 1H), 7.44 (dt, *J* = 18.3, 6.8 Hz, 2H), 7.27 – 7.17 (m, 1H), 7.14 (dq, *J* = 6.6, 4.2, 3.2 Hz, 2H), 6.84 (d, *J* = 2.5 Hz, 1H), 6.63 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.76 (s, 1H), 4.86 (t, *J* = 5.7 Hz, 1H), 4.35 (d, *J* = 14.1 Hz, 1H), 4.13 – 3.95 (m, 2H), 3.85 – 3.78 (m, 2H), 3.75 (s, 3H), 3.36 (dq, *J* = 21.0, 7.1 Hz, 2H), 3.19 (d, *J* = 13.6 Hz, 3H), 3.04 (t, *J* = 13.6 Hz, 1H), 1.90 – 1.81 (m, 1H), 1.76 (d, *J* = 13.6 Hz, 1H), 1.46 (d, *J* = 13.6 Hz, 1H), 1.30 (t, *J* = 7.5 Hz, 3H). **m/z** 531 $(M+H)^+$.



2.38g

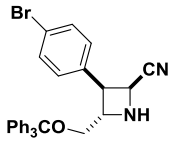
(*R*)-N-(2-fluorophenyl)-1'-(hydroxymethyl)-7'-methoxy-2'-(propylsulfonyl)-1',2',3',9'-tetrahydrospiro[piperidine-4,4'-pyrido[3,4-b]indole]-1-carboxamide (**2.38g**). Synthesized according to **General Procedure F** from **2.36g** (15 mg) to afford product **2.38g** (10.4 mg, 80% yield over two steps). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ 10.78 (s, 1H), 8.36 (s, 1H), 7.44 (dt, *J* = 17.9, 6.8 Hz, 2H), 7.25 – 7.17 (m, 1H), 7.13 (dq, *J* = 6.9, 4.3, 3.4 Hz, 2H), 6.84 (d, *J* = 2.6 Hz, 1H), 6.63 (dd, *J* = 8.9, 2.7 Hz, 1H), 5.33 (t, *J* = 4.7 Hz, 1H), 4.86 (t, *J* = 5.7 Hz, 1H), 4.34 (d, *J* = 14.1 Hz, 1H), 4.14 – 3.94 (m, 2H), 3.79 (dd, *J* = 7.9, 4.2 Hz, 2H), 3.74 (s, 3H), 3.23 – 3.10 (m, 2H), 3.03 (t, *J* = 13.6 Hz, 1H), 2.58 (dd, *J* = 13.4, 4.8 Hz, 1H), 1.86 – 1.73 (m, 3H), 1.50 – 1.41 (m, 1H), 1.00 (t, *J* = 7.4 Hz, 3H). **m/z** 545 $(M+H)^+$.

5.3 Supporting Data for Chapter 3



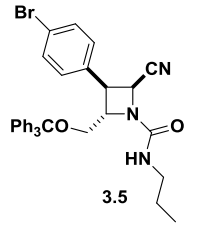
3.3a

(2*S*,3*S*,4*S*)-1-allyl-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.3a**) was prepared according to the method of Lowe *et al*.[1] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.43 – 7.23 (m, 16H), 7.20 – 7.15 (m, 2H), 5.77 (dddd, J = 17.2, 10.3, 7.0, 5.1 Hz, 1H), 5.34 (d, J = 16.8 Hz, 1H), 5.18 (dq, J = 10.2, 1.3 Hz, 1H), 4.64 (d, J = 6.7 Hz, 1H), 3.88 – 3.74 (m, 2H), 3.53 (ddt, J = 13.8, 5.2, 1.7 Hz, 1H), 3.41 – 3.20 (m, 3H). Molecular formula: $C_{33}H_{29}BrN_2O$. Calculated mass: 548.15. **ESIMS m/z** 549.0 $(M+H)^+$.



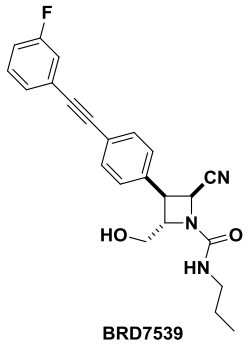
3.4

(2*S*,3*S*,4*S*)-3-(4-bromophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.4**) was prepared according to the method of Kato *et al*.[2] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.44 – 7.38 (m, 6H), 7.34 – 7.23 (m, 9H), 7.21 – 7.15 (m, 2H), 5.77 (dddd, J = 17.2, 10.3, 7.0, 5.1 Hz, 1H), 5.34 (dq, J = 17.2, 1.6 Hz, 1H), 5.23 – 5.15 (m, 1H), 4.64 (d, J = 6.7 Hz, 1H), 3.87 – 3.77 (m, 2H), 3.53 (ddt, J = 13.8, 5.2, 1.7 Hz, 1H), 3.39 – 3.31 (m, 2H), 3.31 – 3.24 (m, 1H). Molecular formula: $C_{30}H_{25}BrN_2O$. Calculated mass: 508.12. **ESIMS m/z** 553.4 $(M+HCOO^-)^-$.



3.5

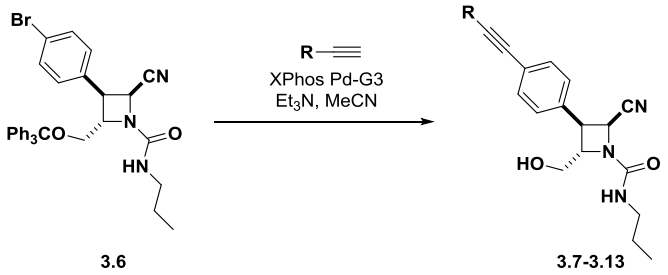
(2*S*,3*S*,4*S*)-3-(4-bromophenyl)-2-cyano-N-propyl-4-((trityloxy)methyl)azetidine-1-carboxamide (**3.5**) was prepared according to the method of Kato *et al*.[2] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51 (d, J = 7.8 Hz, 2H), 7.20 (d, J = 7.7 Hz, 2H), 6.54 (s, 1H), 4.97 (d, J = 8.4 Hz, 1H), 4.89 – 4.70 (m, 1H), 4.70 – 4.61 (m, 1H), 3.96 – 3.62 (m, 3H), 3.13 (m, 2H), 1.57 – 1.40 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). Molecular formula: $C_{34}H_{32}BrN_3O_2$. Calculated mass: 593.17. **ESIMS m/z** 638.8 $(M+ HCOO^-)^-$.



BRD7539

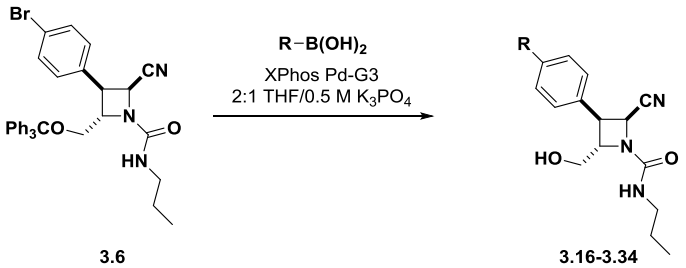
(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**BRD7539**) was prepared according to the method of Kato *et al*.[2] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 (d, J = 7.7 Hz, 2H), 7.38 – 7.28 (m, 4H), 7.21 (dd, J = 9.5, 3.0 Hz, 1H), 7.04 (dq, J = 7.8, 3.7 Hz, 1H), 6.27 (s, 1H), 5.01 (d, J = 8.4 Hz, 1H), 4.74 (t, J = 7.8 Hz, 1H), 4.02 (s, 1H), 3.92 (d, J = 10.9 Hz, 1H), 3.88 – 3.80 (m, 1H), 3.74 (t, J = 8.1 Hz, 1H), 3.18 (m, 2H), 1.51 (d, J = 7.2 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.73, 161.28, 158.06, 134.35, 132.25, 130.10 (d, J = 8.6 Hz), 129.48, 128.51, 127.66 (d, J = 3.0 Hz), 124.92 (d, J = 9.5 Hz), 123.45, 118.62, 118.39, 116.05, 115.79 (d, J = 10.1 Hz), 89.59,

89.29 (d, J = 3.4 Hz), 68.12, 65.81, 52.73, 42.29, 39.54, 29.82, 23.27, 11.46. **ESIMS m/z** 392 $(M+H)^+$. **HRMS** (ESI) calculated for $C_{23}H_{22}FN_3O_2$ $[M+H]^+$: 392.1774. Found: 392.1781.



General Procedure G: Heck alkynylation.

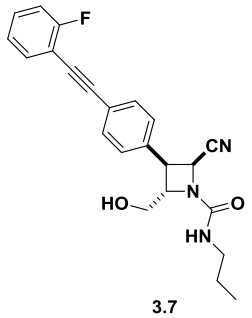
Aryl bromide **3.6** (1.0 equiv) and appropriate alkyne (5.0 equiv) were dissolved in degassed MeCN (0.1 M). XPhos Pd-G3 (0.15 equiv) and Et_3N (20 equiv) were added in sequence, and the reaction was heated to 70 ^oC for 2-3 h or until completion. The reaction was then cooled to ambient temperature, diluted with pH 7 buffer, and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM).



General Procedure H: Suzuki coupling.

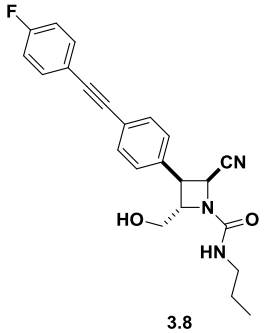
Aryl bromide **3.6** (1.0 equiv) and appropriate boronic acid (3.0 equiv) were dissolved in degassed 2:1 THF/0.5 M K_3PO_4 (0.17 M) before the addition of XPhos Pd-G3 (0.15 equiv). The

reaction was heated to 50 °C for 2-5 h or until completion, at which point the mixture was cooled to ambient temperature, diluted with pH 7 buffer, and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM).



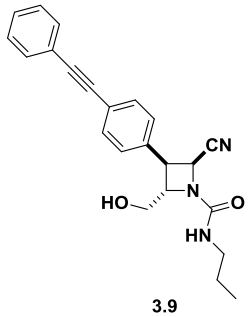
3.7

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((2-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.7**). Prepared from **3.6** following **General Protocol G** (41% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.7 Hz, 2H), 7.51 (td, *J* = 7.4, 1.8 Hz, 1H), 7.39 – 7.28 (m, 3H), 7.12 (q, *J* = 8.5 Hz, 2H), 5.03 (d, *J* = 8.3 Hz, 1H), 4.76 (t, *J* = 7.3 Hz, 1H), 3.98 – 3.91 (m, 1H), 3.85 (dd, *J* = 11.4, 7.6 Hz, 1H), 3.76 (d, *J* = 8.1 Hz, 1H), 3.21 (dtd, *J* = 27.1, 13.4, 7.0 Hz, 2H), 1.54 (p, *J* = 7.0 Hz, 2H), 0.99 – 0.86 (m, 3H). Molecular formula: $C_{23}H_{22}FN_3O_2$. Calculated mass: 391.17. **ESIMS m/z** 392.5 $(M+H)^+$.



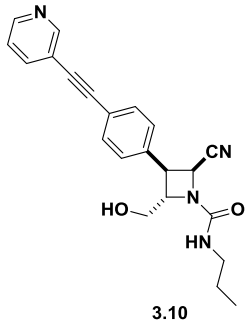
3.8

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((4-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.8**). Prepared from **3.6** following **General Protocol G** (34% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.61 – 7.46 (m, 4H), 7.40 – 7.29 (m, 2H), 7.10 – 6.99 (m, 2H), 6.14 (t, J = 5.6 Hz, 1H), 5.01 (d, J = 8.5 Hz, 1H), 4.74 (td, J = 8.0, 2.2 Hz, 1H), 3.97 – 3.69 (m, 3H), 3.31 – 3.06 (m, 2H), 1.54 (p, J = 7.3 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{22}FN_3O_2$. Calculated mass: 391.17. **ESIMS m/z** 392.2 $(M+H)^+$.



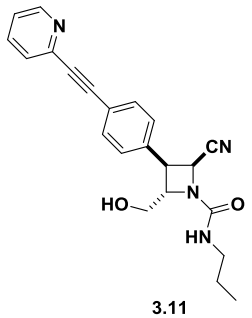
3.9

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(4-(phenylethynyl)phenyl)-N-propylazetidine-1-carboxamide (**3.9**). Prepared from **3.6** following **General Protocol G** (96% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.60 – 7.54 (m, 2H), 7.54 – 7.50 (m, 2H), 7.39 – 7.29 (m, 5H), 6.08 (t, *J* = 5.6 Hz, 1H), 5.00 (d, *J* = 8.5 Hz, 1H), 4.74 (td, *J* = 8.2, 2.2 Hz, 1H), 4.00 – 3.78 (m, 3H), 3.74 (t, *J* = 8.1 Hz, 1H), 3.30 – 3.08 (m, 2H), 1.54 (h, *J* = 7.3 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{23}N_3O_2$. Calculated mass: 373.18. **ESIMS m/z** 374.8 $(M+H)^+$.



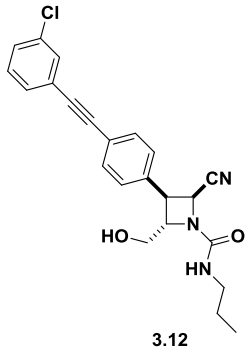
3.10

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-3-ylethynyl)phenyl)azetidine-1-carboxamide (**3.10**). Prepared from **3.6** following **General Protocol G**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.57 (d, J = 4.9 Hz, 1H), 7.90 (dt, J = 8.0, 1.8 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.38 (dd, J = 8.0, 4.5 Hz, 3H), 5.58 (s, 1H), 5.05 (d, J = 8.5 Hz, 1H), 4.77 (t, J = 7.2 Hz, 1H), 3.99 – 3.74 (m, 4H), 3.24 (ddd, J = 13.9, 12.2, 6.7 Hz, 2H), 1.58 (p, J = 7.2 Hz, 4H), 0.98 – 0.91 (m, 3H). Molecular formula: $C_{22}H_{22}N_4O_2$. Calculated mass: 374.17. **ESIMS m/z** 375.2 $(M+H)^+$.



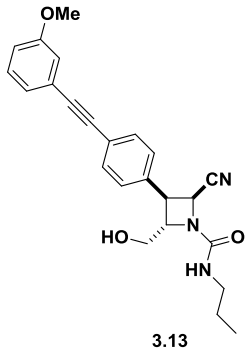
3.11

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-2-ylethynyl)phenyl)azetidine-1-carboxamide (**3.11**). Prepared from **3.6** following **General Protocol G**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.58 (dt, *J* = 5.0, 1.3 Hz, 1H), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H), 7.56 (td, *J* = 8.1, 1.4 Hz, 3H), 7.36 – 7.27 (m, 3H), 6.28 (t, *J* = 5.6 Hz, 1H), 5.01 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.2, 2.1 Hz, 1H), 3.98 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.85 (dd, *J* = 11.4, 8.4 Hz, 1H), 3.74 (t, *J* = 8.2 Hz, 1H), 3.30 – 3.11 (m, 2H), 1.54 (h, *J* = 7.3 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}N_4O_2$. Calculated mass: 374.17. **ESIMS m/z** 375.2 $(M+H)^+$.



3.12

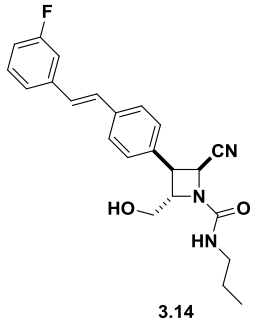
(2*S*,3*S*,4*S*)-3-(4-((3-chlorophenyl)ethynyl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.12**). Prepared from **3.6** following **General Protocol G** (63% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 2H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.33 (dt, *J* = 7.2, 1.5 Hz, 1H), 7.31 – 7.18 (m, 4H), 5.76 (t, *J* = 5.8 Hz, 1H), 4.95 (d, *J* = 8.5 Hz, 1H), 4.68 (td, *J* = 8.0, 2.1 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.78 (dd, *J* = 11.4, 8.1 Hz, 1H), 3.69 (t, *J* = 8.1 Hz, 1H), 3.24 – 3.04 (m, 2H), 1.46 (d, *J* = 7.2 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{22}ClN_3O_2$. Calculated mass: 407.14. **ESIMS m/z** 408.3 $(M+H)^+$.



3.13

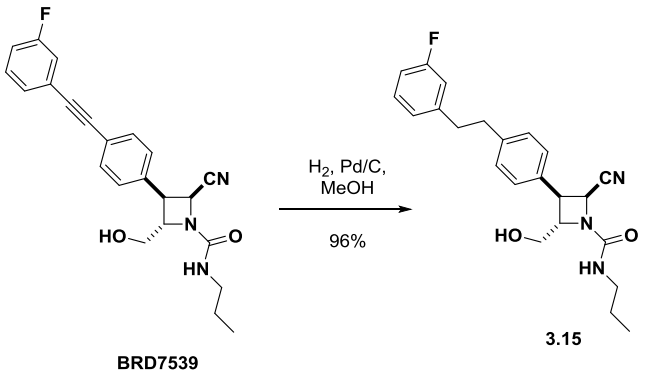
(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3,4-difluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.13**). Prepared from **3.6** following **General Protocol G** (42% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 – 7.52 (m, 2H), 7.42 – 7.30 (m, 3H), 7.25 (dd, *J* = 3.8, 1.8 Hz, 1H), 7.14 (dt, *J* = 10.3, 8.3 Hz, 1H), 5.84 (s, 1H), 5.03 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.0, 2.1 Hz, 1H), 3.94 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.86 (dd, *J* = 11.4, 8.1 Hz, 1H), 3.77 (t, *J* =

8.1 Hz, 1H), 3.21 (dtd, *J* = 27.4, 13.4, 7.0 Hz, 2H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{21}F_2N_3O_2$. Calculated mass: 409.16. **ESIMS m/z** 410.3 $(M+H)^+$.



3.14

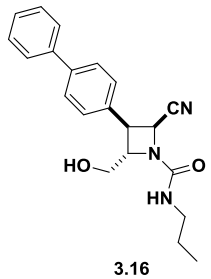
(2*S*,3*S*,4*S*)-2-cyano-3-(4-((E)-3-fluorostyryl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.14**). Prepared from **3.6** following **General Protocol H** (45% yield). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ 7.62 – 7.56 (m, 2H), 7.42 – 7.37 (m, 2H), 7.37 – 7.29 (m, 2H), 7.25 (ddd, *J* = 10.5, 2.5, 1.4 Hz, 1H), 7.14 (s, 2H), 6.98 (dddd, *J* = 8.7, 7.5, 2.6, 1.5 Hz, 1H), 5.94 – 5.82 (m, 1H), 5.01 (d, *J* = 8.5 Hz, 1H), 4.75 (td, *J* = 8.1, 2.3 Hz, 1H), 3.97 – 3.81 (m, 2H), 3.77 (t, *J* = 8.1 Hz, 1H), 3.45 – 3.36 (m, 1H), 3.20 (qtd, *J* = 13.0, 7.0, 5.6 Hz, 2H), 1.54 (p, *J* = 7.2 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{24}FN_3O_2$. Calculated mass: 393.19. **ESIMS m/z** 394.2 $(M+H)^+$.



BRD7539 **3.15**

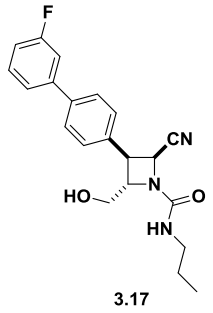
(2*S*,3*S*,4*S*)-2-cyano-3-(4-(3-fluorophenethyl)phenyl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.15**). To a solution of **BRD7539** (12.0 mg, 0.031 mmol) in MeOH (310 μL) was

added 10% Pd/C (w/w). The atmosphere was purged 3×15 seconds with a H_2 balloon, and the reaction was maintained under a H_2 atmosphere for 16 h. Upon completion, the reaction mixture was filtered over Celite to afford pure product as a white solid (11.6 mg, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34 – 7.20 (m, 4H), 7.00 – 6.85 (m, 3H), 5.95 (d, *J* = 5.9 Hz, 1H), 5.03 (d, *J* = 8.5 Hz, 1H), 4.78 (t, *J* = 7.5 Hz, 1H), 4.00 – 3.84 (m, 3H), 3.75 (t, *J* = 8.2 Hz, 1H), 3.34 – 3.13 (m, 2H), 2.94 (s, 4H), 1.58 (h, *J* = 7.3 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{23}H_{26}FN_3O_2$. Calculated mass: 395.20. **ESIMS m/z** 396.9 $(M+H)^+$.



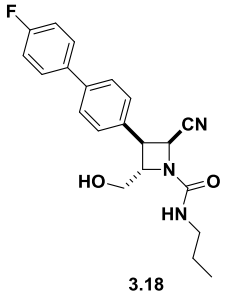
3.16

(2*S*,3*S*,4*S*)-3-([1,1'-biphenyl]-4-yl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.16**). Prepared from **3.6** following **General Protocol H** (87% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 – 7.61 (m, 2H), 7.61 – 7.55 (m, 2H), 7.48 – 7.40 (m, 4H), 7.40 – 7.34 (m, 1H), 5.84 (s, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.80 (td, *J* = 8.0, 2.2 Hz, 1H), 3.95 (dd, *J* = 11.4, 2.2 Hz, 1H), 3.86 (dd, *J* = 11.4, 8.2 Hz, 1H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.21 (ddt, *J* = 27.6, 13.5, 6.8 Hz, 3H), 1.56 (q, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{23}N_3O_2$. Calculated mass: 349.18. **ESIMS m/z** 350.2 $(M+H)^+$.



3.17

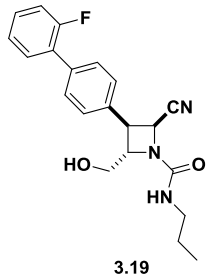
(2*S*,3*S*,4*S*)-2-cyano-3-(3'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.17**). Prepared from **3.6** following **General Protocol H** (57% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.51 – 7.33 (m, 4H), 7.29 (t, *J* = 5.4 Hz, 1H), 7.11 – 7.02 (m, 1H), 5.67 – 5.56 (m, 1H), 5.05 (d, *J* = 8.4 Hz, 1H), 4.80 (t, *J* = 7.1 Hz, 1H), 4.01 – 3.93 (m, 1H), 3.88 (dd, *J* = 11.4, 7.9 Hz, 1H), 3.81 (t, *J* = 8.1 Hz, 1H), 3.24 (dp, *J* = 26.0, 6.5 Hz, 2H), 1.57 (h, *J* = 7.1 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. **ESIMS m/z** 368.6 $(M+H)^+$.



3.18

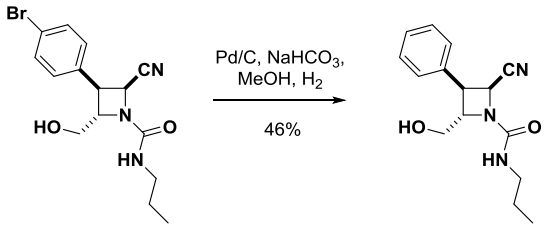
(2*S*,3*S*,4*S*)-2-cyano-3-(4'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.18**). Prepared from **3.6** following **General Protocol H** (77% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.7 Hz, 2H), 7.51 – 7.33 (m, 4H), 7.29 (t, *J* = 5.4 Hz, 1H), 7.11 – 7.02 (m, 1H), 5.67 – 5.56 (m, 1H), 5.05 (d, *J* = 8.4 Hz, 1H), 4.80 (t, *J* = 7.1 Hz, 1H), 4.01 – 3.93 (m, 1H), 3.88 (dd, *J* = 11.4, 7.9 Hz, 1H), 3.81 (t, *J* = 8.1 Hz, 1H), 3.24 (dp, *J* = 26.0, 6.5 Hz,

2H), 1.57 (h, *J* = 7.1 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. **ESIMS m/z** 368.6 $(M+H)^+$.



3.19

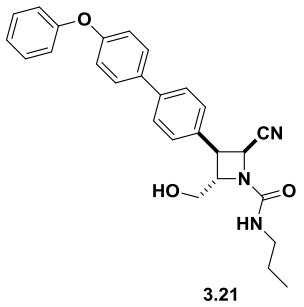
(2*S*,3*S*,4*S*)-2-cyano-3-(2'-fluoro-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.19**). Prepared from **3.6** following **General Protocol H** (80% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 7.6 Hz, 2H), 7.47 (d, *J* = 7.4 Hz, 3H), 7.41 – 7.31 (m, 1H), 7.24 (t, *J* = 7.4 Hz, 1H), 7.18 (dd, *J* = 10.9, 8.0 Hz, 1H), 5.74 (s, 1H), 5.08 (d, *J* = 8.4 Hz, 1H), 4.89 – 4.77 (m, 1H), 3.98 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.89 (dd, *J* = 11.5, 8.0 Hz, 1H), 3.82 (t, *J* = 8.1 Hz, 1H), 3.25 (dt, *J* = 23.8, 8.8 Hz, 2H), 1.66 – 1.52 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{21}H_{22}FN_3O_2$. Calculated mass: 367.17. **ESIMS m/z** 368.8 $(M+H)^+$.



3.6 3.20

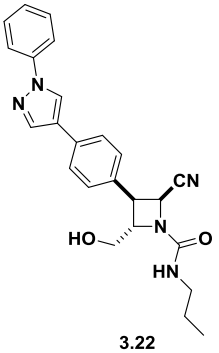
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-phenyl-N-propylazetidine-1-carboxamide (**3.20**). To a flask containing aryl bromide **3.6** (12 mg, 0.034 mmol), 10% Pd/C (w/w), and sodium bicarbonate (17 mg, 0.204 mmol) was added a solution of MeOH (340 μL). The atmosphere was purged 3×15 seconds with a H_2 balloon, and the reaction was maintained under a H_2 atmosphere for 2 h. The reaction was then filtered over Celite and purified via flash column chromatography

to afford product as a colorless residue (4.2 mg, 46%). **^{1}H NMR** (400 MHz, CD_3OD) δ 7.42 (d, *J* = 4.4 Hz, 4H), 7.34 (ddd, *J* = 8.5, 5.1, 3.8 Hz, 1H), 5.14 (d, *J* = 8.6 Hz, 1H), 4.67 (td, *J* = 6.9, 2.7 Hz, 1H), 3.98 (dd, *J* = 8.6, 7.2 Hz, 1H), 3.91 (dd, *J* = 11.8, 2.7 Hz, 1H), 3.74 (dd, *J* = 11.8, 6.4 Hz, 1H), 3.21 (dt, *J* = 13.7, 7.0 Hz, 1H), 3.11 (dt, *J* = 13.3, 6.9 Hz, 1H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{15}H_{19}N_3O_2$. Calculated mass: 273.15. **ESIMS m/z** 274.1 $(M+H)^+$.



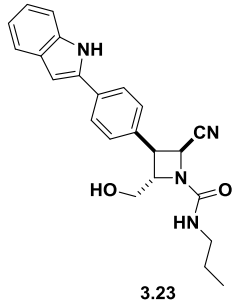
3.21

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(4'-phenoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (**3.21**). Prepared from **3.6** following **General Protocol H**. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 2H), 7.58 – 7.52 (m, 2H), 7.46 – 7.33 (m, 4H), 7.19 – 7.01 (m, 5H), 5.70 (s, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.80 (td, *J* = 7.9, 2.2 Hz, 1H), 4.00 – 3.73 (m, 3H), 3.22 (s, 2H), 1.56 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{27}H_{27}N_3O_3$. Calculated mass: 441.41. **ESIMS m/z** 442.2 $(M+H)^+$.



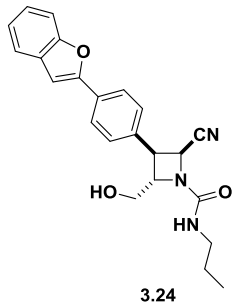
3.22

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(4-(1-phenyl-1H-pyrazol-4-yl)phenyl)-N-propylazetidine-1-carboxamide (**3.22**). Prepared from **3.6** following **General Protocol H** (31% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.00 (s, 1H), 7.74 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 7.7 Hz, 2H), 7.50 (q, *J* = 8.0, 6.9 Hz, 2H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 5.52 (s, 1H), 5.04 (d, *J* = 8.3 Hz, 1H), 4.79 (t, *J* = 7.6 Hz, 1H), 4.00 – 3.91 (m, 1H), 3.87 (dd, *J* = 11.5, 7.9 Hz, 1H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.35 – 3.15 (m, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{24}H_{25}N_5O_2$. Calculated mass: 415.20. **ESIMS m/z** 417.6 $(M+2H)^{2+}$.



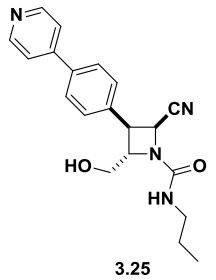
3.23

(2*S*,3*S*,4*S*)-3-(4-(1H-indol-2-yl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.23**). Prepared from **3.6** following **General Protocol H**. **^{1}H NMR** (300 MHz, CD_3OD) δ 7.53 (dd, *J* = 8.4, 2.5 Hz, 2H), 7.31 – 7.24 (m, 1H), 7.17 – 6.96 (m, 4H), 6.86 (ddt, *J* = 8.4, 6.8, 1.4 Hz, 1H), 6.75 (tdd, *J* = 8.7, 5.6, 3.4 Hz, 1H), 6.58 – 6.53 (m, 1H), 4.00 (dt, *J* = 8.0, 3.9 Hz, 1H), 3.96 – 3.86 (m, 1H), 3.79 – 3.59 (m, 3H), 3.01 – 2.88 (m, 2H), 2.75 (ddd, *J* = 11.0, 8.3, 5.7 Hz, 2H), 1.01 – 0.92 (m, 2H), 0.31 (td, *J* = 6.8, 6.2, 2.1 Hz, 3H). Molecular formula: $C_{23}H_{24}N_4O_2$. Calculated mass: 388.19. **ESIMS m/z** 389.2 $(M+H)^+$.



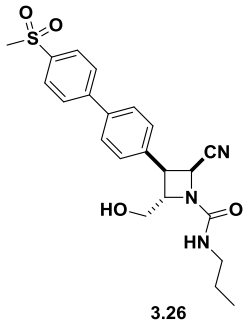
3.24

(2*S*,3*S*,4*S*)-3-(4-(benzofuran-2-yl)phenyl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.24**). Prepared from **3.6** following **General Protocol H** (80% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.0 Hz, 2H), 7.63 – 7.57 (m, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.34 – 7.27 (m, 1H), 7.23 (d, *J* = 7.3 Hz, 1H), 7.06 (d, *J* = 0.9 Hz, 1H), 5.51 (s, 1H), 5.05 (d, *J* = 8.5 Hz, 1H), 4.85 – 4.76 (m, 1H), 4.00 – 3.77 (m, 4H), 3.25 (tq, *J* = 13.3, 6.5 Hz, 2H), 1.57 (q, *J* = 7.3 Hz, 3H), 0.96 (dd, *J* = 7.9, 6.9 Hz, 3H). Molecular formula: $C_{23}H_{23}N_3O_2$. Calculated mass: 389.19. **ESIMS m/z** 390.2 $(M+H)^+$.



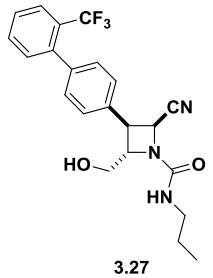
3.25

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4-(pyridin-4-yl)phenyl)azetidine-1-carboxamide (**3.25**). Prepared from **3.6** following **General Protocol H** (80% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.72 – 8.63 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.60 – 7.47 (m, 4H), 5.67 (s, 1H), 5.07 (d, *J* = 8.5 Hz, 1H), 4.85 – 4.75 (m, 1H), 4.02 – 3.78 (m, 4H), 3.25 (dp, *J* = 18.9, 6.3 Hz, 2H), 1.57 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{20}H_{22}N_4O_2$. Calculated mass: 350.17. **ESIMS m/z** 351.2 $(M+H)^+$.



3.26

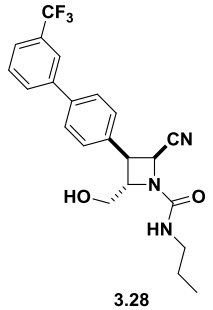
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (**3.26**). Prepared from **3.6** following **General Protocol H** (43% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.04 – 7.98 (m, 2H), 7.82 – 7.73 (m, 2H), 7.69 – 7.63 (m, 2H), 7.53 – 7.45 (m, 2H), 5.64 (t, *J* = 5.7 Hz, 1H), 5.07 (d, *J* = 8.6 Hz, 1H), 4.80 (td, *J* = 7.9, 2.2 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.93 – 3.87 (m, 1H), 3.87 – 3.79 (m, 1H), 3.48 (t, *J* = 3.4 Hz, 1H), 3.33 – 3.16 (m, 2H), 3.10 (s, 3H), 1.57 (q, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{23}N_3O_2S$. Calculated mass: 427.16. **ESIMS m/z** 428.1 $(M+H)^+$.



3.27

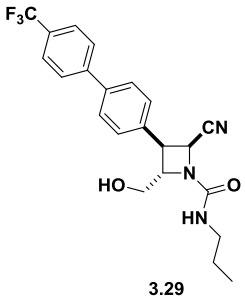
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(2'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (**3.27**). Prepared from **3.6** following **General Protocol H** (32% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 – 7.72 (m, 1H), 7.61 – 7.54 (m, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.40 (s, 4H), 7.34 (d, *J* = 7.6 Hz, 1H), 5.87 (s, 1H), 5.10 (d, *J* = 8.5 Hz, 1H), 4.84 (td, *J* = 7.9, 2.0 Hz, 1H), 4.04 (dd, *J* = 11.6, 2.1 Hz, 1H), 3.92 (dd, *J* = 11.4, 8.2 Hz, 1H), 3.84 (t, *J* =

8.1 Hz, 1H), 3.24 (tp, *J* = 13.4, 7.0 Hz, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. **ESIMS m/z** 462.3 $(M+HCOO^-)^-$.



3.28

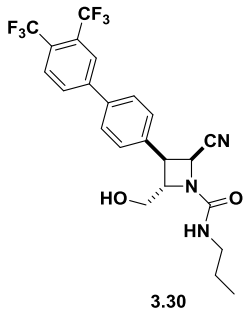
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (**3.28**). Prepared from **3.6** following **General Protocol H** (59% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.76 (d, *J* = 7.5 Hz, 1H), 7.70 – 7.44 (m, 7H), 5.61 (t, *J* = 5.5 Hz, 1H), 5.06 (d, *J* = 8.5 Hz, 1H), 4.81 (t, *J* = 7.7 Hz, 1H), 4.04 – 3.94 (m, 1H), 3.94 – 3.87 (m, 1H), 3.83 (q, *J* = 7.9 Hz, 1H), 3.23 (dh, *J* = 25.7, 6.4 Hz, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. **ESIMS m/z** 418.8 $(M+H)^+$.



3.29

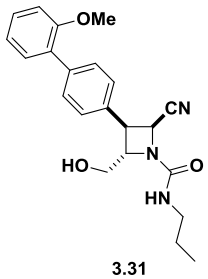
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-N-propyl-3-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (**3.29**). Prepared from **3.6** following **General Protocol H** (35% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.69 (s, 2H), 7.68 – 7.61 (m, 2H), 7.52 – 7.45 (m, 2H), 5.63 (s, 1H), 5.06 (d, *J* = 8.6 Hz, 1H), 4.81 (td, *J* = 7.8, 2.2 Hz, 1H), 4.02 – 3.78 (m, 3H), 3.35 –

3.15 (m, 2H), 1.57 (h, *J* = 7.3 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{22}F_3N_3O_2$. Calculated mass: 417.17. **ESIMS m/z** 418.5 $(M+H)^+$.



3.30

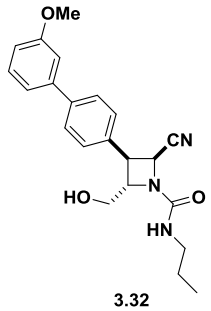
(2*S*,3*S*,4*S*)-3-(3',4'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-2-cyano-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.30**, **BRD9185**). Prepared from **3.6** following **General Protocol H** (84% yield). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ 8.08 (d, *J* = 1.6 Hz, 2H), 7.90 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.56 – 7.51 (m, 2H), 5.98 (s, 1H), 5.07 (d, *J* = 8.6 Hz, 1H), 4.79 (td, *J* = 8.0, 2.2 Hz, 1H), 3.98 – 3.82 (m, 3H), 3.20 (qq, *J* = 12.8, 6.6 Hz, 2H), 1.56 (p, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 157.72, 142.39, 138.36, 135.04 (d, J = 7.3 Hz), 132.12, 131.79, 130.79, 129.31, 127.44 (d, J = 26.5 Hz), 124.78, 122.07, 121.66 – 121.08 (m), 115.75, 67.89, 65.81, 53.33, 53.13, 52.86, 52.55, 42.11, 39.13, 23.21, 11.07. **HRMS** (ESI) calcd for $C_{23}H_{21}F_6N_3O_2$ $[M+H]^+$: 486.1608. Found: 486.1618.



3.31

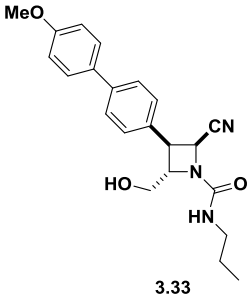
(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(2'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (**3.31**). Prepared from **3.6** following **General Protocol H** (70% yield). **^{1}H NMR**

(400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 8.5 Hz, 2H), 7.09 – 6.94 (m, 2H), 5.74 – 5.62 (m, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.87 – 4.73 (m, 1H), 3.94 (d, *J* = 12.4 Hz, 1H), 3.90 – 3.84 (m, 1H), 3.81 (s, 4H), 3.24 (dh, *J* = 25.6, 6.5 Hz, 2H), 1.56 (h, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. **ESIMS m/z** 380.8 $(M+H)^+$.



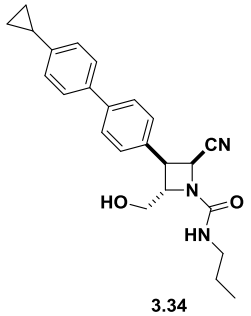
3.32

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(3'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (**3.32**). Prepared from **3.6** following **General Protocol H** (58% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 7.7 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.35 (q, *J* = 6.7, 5.6 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 3.0 Hz, 1H), 6.91 (dd, *J* = 8.4, 2.9 Hz, 1H), 5.71 – 5.53 (m, 1H), 5.04 (d, *J* = 8.5 Hz, 1H), 4.86 – 4.76 (m, 1H), 3.95 (d, *J* = 10.8 Hz, 1H), 3.87 (s, 4H), 3.79 (t, *J* = 8.1 Hz, 1H), 3.23 (dh, *J* = 27.1, 7.2, 6.7 Hz, 2H), 1.56 (h, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. **ESIMS m/z** 379.6 $(M+H)^+$.



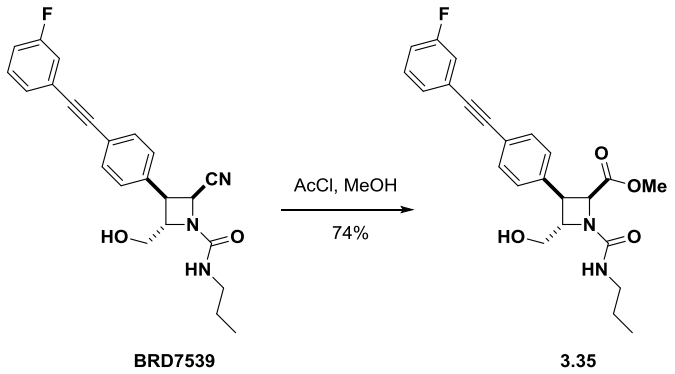
3.33

(2*S*,3*S*,4*S*)-2-cyano-4-(hydroxymethyl)-3-(4'-methoxy-[1,1'-biphenyl]-4-yl)-N-propylazetidine-1-carboxamide (**3.33**). Prepared from **3.6** following **General Protocol H** (61% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 7.7 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 5.71 – 5.62 (m, 1H), 5.03 (d, *J* = 8.4 Hz, 1H), 4.79 (td, *J* = 8.0, 7.4, 2.6 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.86 (d, *J* = 4.1 Hz, 4H), 3.78 (t, *J* = 8.1 Hz, 1H), 3.63 (d, *J* = 31.9 Hz, 1H), 3.23 (dp, *J* = 26.3, 6.5 Hz, 2H), 1.56 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). Molecular formula: $C_{22}H_{25}N_3O_2$. Calculated mass: 379.19. **ESIMS m/z** 380.2 $(M+H)^+$.



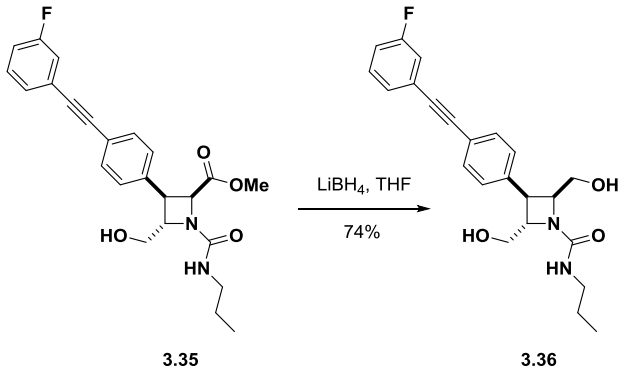
3.34

(2*S*,3*S*,4*S*)-2-cyano-3-(4'-cyclopropyl-[1,1'-biphenyl]-4-yl)-4-(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.34**). Prepared from **3.6** following **General Protocol H** (31% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.64 – 7.59 (m, 2H), 7.51 – 7.46 (m, 2H), 7.44 – 7.39 (m, 2H), 7.17 – 7.13 (m, 2H), 5.51 (t, *J* = 5.7 Hz, 1H), 5.04 (d, *J* = 8.6 Hz, 1H), 4.80 (td, *J* = 8.0, 2.1 Hz, 1H), 3.95 (dd, *J* = 11.2, 4.5 Hz, 1H), 3.91 – 3.82 (m, 1H), 3.79 (t, *J* = 8.1 Hz, 1H), 3.45 (s, 1H), 3.32 – 3.17 (m, 2H), 1.94 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.62 – 1.51 (m, 3H), 1.02 – 0.98 (m, 2H), 0.95 (td, *J* = 7.4, 4.1 Hz, 4H), 0.74 (dt, *J* = 6.7, 4.7 Hz, 2H). Molecular formula: $C_{24}H_{27}N_3O_2$. Calculated mass: 389.21. **ESIMS m/z** 390.4 $(M+H)^+$.



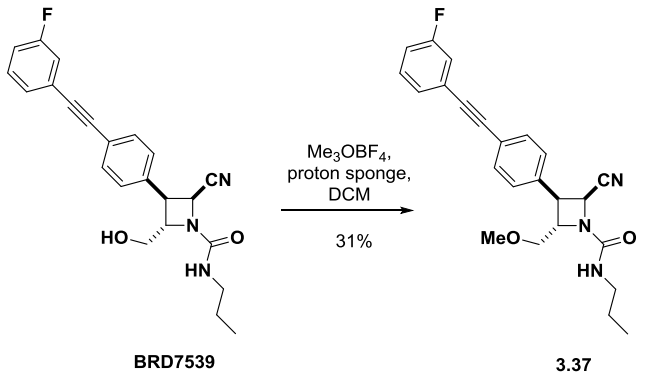
BRD7539 3.35

(2*S*,3*R*,4*S*)-methyl 3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-1-(propylcarbamoyl)azetidine-2-carboxylate (**3.35**). To a vial containing MeOH (80 µL, 0.1 M) at 0 °C was added AcCl (6.5 µL, 0.92 mmol) and stirred for 10 min. The resulting solution was transferred into a secondary vial containing BRD7539 (3 mg, 0.008 mmol). The reaction was stirred for 22 h at ambient temperature, after which point the temperature was raised to 50 °C. After 3 h, the solvent was removed *in vacuo* and crude residue purified via flash column chromatography (MeOH/DCM) to afford product as white, waxy residue (2.4 mg, 74%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 – 7.45 (m, 2H), 7.33 – 7.28 (m, 2H), 7.28 – 7.19 (m, 3H), 7.05 (ddq, *J* = 11.0, 6.0, 3.2, 2.6 Hz, 1H), 5.45 (d, *J* = 6.1 Hz, 1H), 4.96 (dd, *J* = 9.3, 1.7 Hz, 1H), 4.82 – 4.74 (m, 1H), 4.38 – 4.26 (m, 1H), 3.98 – 3.88 (m, 2H), 3.71 – 3.63 (m, 1H), 3.35 (d, *J* = 1.7 Hz, 3H), 3.24 (dtd, *J* = 13.1, 6.6, 6.1, 1.9 Hz, 1H), 3.19 – 3.08 (m, 1H), 1.53 (qd, *J* = 7.4, 1.7 Hz, 2H), 0.93 (td, *J* = 7.4, 1.7 Hz, 3H). Molecular formula: $C_{24}H_{25}FN_2O_4$. Calculated mass: 424.18. **ESIMS m/z** 425.3 $(M+H)^+$.



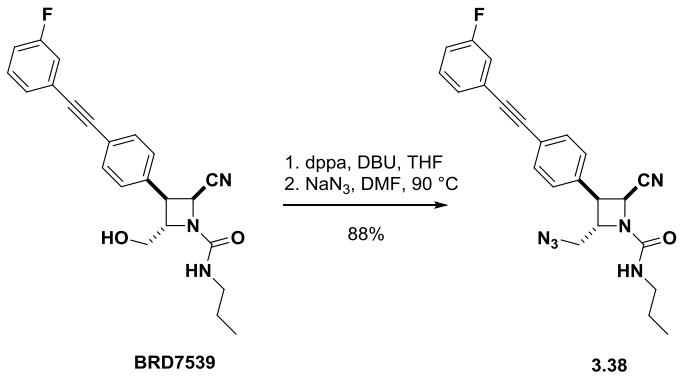
3.35 3.36

(2*S*,4*S*)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-2,4-bis(hydroxymethyl)-N-propylazetidine-1-carboxamide (**3.36**). To a solution of **3.35** (10 mg, 0.024 mmol) in THF (200 µL) was added $LiBH_4$ (1.3 mg, 0.059 mmol). The reaction was heated to 40 °C for 3 h, after which point one drop of water was added to quench the reaction. The mixture was extracted 3x with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue (4.5 mg, 49%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.9 Hz, 2H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 2H), 7.05 (tq, *J* = 10.4, 3.8, 3.1 Hz, 1H), 6.55 (d, *J* = 4.7 Hz, 1H), 4.74 (t, *J* = 7.5 Hz, 1H), 4.56 (td, *J* = 9.8, 2.0 Hz, 1H), 3.97 (t, *J* = 10.2 Hz, 1H), 3.84 – 3.77 (m, 1H), 3.60 (t, *J* = 10.6 Hz, 1H), 3.46 (dd, *J* = 9.5, 6.3 Hz, 1H), 3.27 – 3.20 (m, 1H), 3.14 (dq, *J* = 16.5, 6.7 Hz, 2H), 1.56 – 1.46 (m, 2H), 0.92 (td, *J* = 7.6, 2.7 Hz, 3H). Molecular formula: $C_{23}H_{25}FN_2O_3$. Calculated mass: 396.18. **ESIMS m/z** 397.6 $(M+H)^+$.



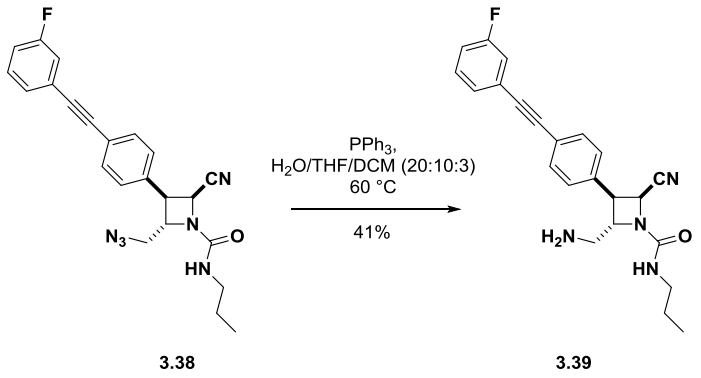
BRD7539 3.37

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(methoxymethyl)-N-propylazetidine-1-carboxamide (**3.37**). To a solution of **BRD7539** (26 mg, 0.066 mmol) in DCM (266 μL) was added 1,8-bis(dimethylamino)naphthalene (28 mg, 0.133 mmol) and trimethyloxonium tetrafluoroborate (12 mg, 0.078 mmol). The mixture was stirred for ambient temperature for 16 h. The solvent was then removed *in vacuo* and the crude residue was purified by flash column chromatography (MeOH/DCM) to afford product as a pale yellow residue (8.2 mg, 31%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 – 7.54 (m, 2H), 7.38 – 7.28 (m, 4H), 7.25 – 7.20 (m, 1H), 7.05 (dtd, *J* = 9.1, 4.8, 2.6 Hz, 1H), 6.23 (s, 1H), 4.96 (d, *J* = 8.4 Hz, 1H), 4.71 (td, *J* = 7.9, 2.6 Hz, 1H), 3.78 – 3.60 (m, 3H), 3.44 (s, 3H), 3.33 – 3.21 (m, 1H), 3.21 – 3.12 (m, 1H), 1.53 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{24}H_{24}FN_3O_2$. Calculated mass: 405.19. **ESIMS m/z** 406.2 $(M+H)^+$.



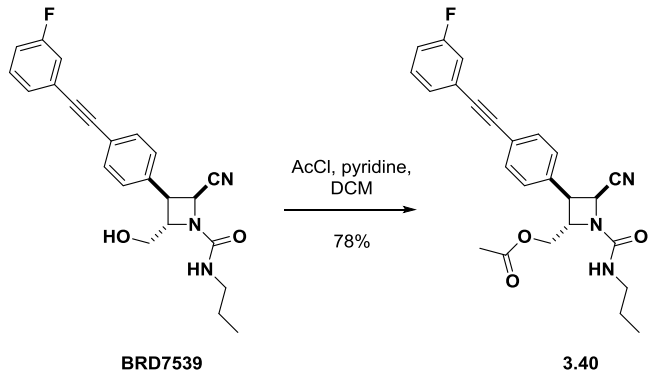
BRD7539 **3.38**

(2*S*,3*R*,4*S*)-2-(azidomethyl)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (**3.38**). To a solution of **BRD7539** (11 mg, 0.028 mmol) in THF (300 µL) was added DBU (22 µL, 0.225 mmol), then diphenylphosphoryl azide (30 µL, 0.141 mmol). The mixture was stirred at ambient temperature for 16 h. The reaction solvent was then removed in vacuo, and crude material purified *briefly* via flash column chromatography (MeOH/DCM) to afford *intermediate ((2S,3S,4S)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl diphenyl phosphate.* The intermediate was dissolved in DMF (300 µL), and NaN_3 (8.9 mg, 0.137 mmol) was added in one portion. The mixture was stirred at 90 °C for 3 h, after which point the reaction was quenched with H_2O. The resulting mixture was extracted 3x with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a pale yellow solid (8.3 mg, 88%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.28 (m, 2H), 7.25 – 7.19 (m, 1H), 7.09 – 7.02 (m, 1H), 5.09 (d, *J* = 8.5 Hz, 1H), 5.01 (t, *J* = 5.8 Hz, 1H), 4.64 (ddd, *J* = 6.5, 4.7, 3.0 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.90 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.56 (dd, *J* = 13.1, 4.7 Hz, 1H), 3.34 – 3.16 (m, 2H), 1.59 (dt, *J* = 14.6, 7.5 Hz, 2H), 0.99 – 0.92 (m, 3H). Molecular formula: $C_{23}H_{21}FN_6O$. Calculated mass: 416.18. **ESIMS m/z** 417.7 $(M+H)^+$.



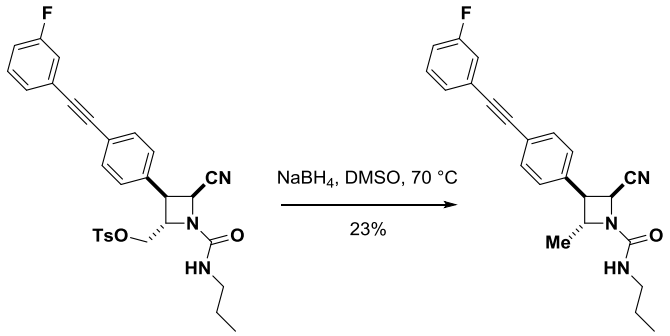
3.38 3.39

(2*S*,3*R*,4*S*)-2-(aminomethyl)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (**3.39**). To a solution of **3.38** (6.7 mg, 0.016 mmol) in DCM (300 μL) was added triphenylphosphine (7.0 mg, 0.024 mmol). The mixture was stirred at ambient temperature for 4 h, after which point 2 mL of H_2O and 1 mL of THF were added to the reaction. The reaction was then heated to 60 °C for 24 h. Upon completion, solvent was removed *in vacuo* and crude residue purified by flash column chromatography (MeOH/DCM) to afford product as colorless solid (2.6 mg, 41%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.61 – 7.53 (m, 2H), 7.42 – 7.29 (m, 4H), 7.24 – 7.19 (m, 1H), 7.05 (ddt, *J* = 9.2, 8.1, 3.2 Hz, 1H), 4.94 (d, *J* = 8.4 Hz, 1H), 4.49 (ddd, *J* = 9.9, 8.2, 1.8 Hz, 1H), 3.69 (t, *J* = 8.2 Hz, 1H), 3.21 (dddd, *J* = 18.6, 16.8, 9.8, 4.0 Hz, 3H), 2.94 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.66 – 1.50 (m, 5H), 0.98 – 0.89 (m, 3H). Molecular formula: $C_{23}H_{23}FN_4O$. Calculated mass: 390.19. **ESIMS m/z** 391.9 $(M+H)^+$.



BRD7539 3.40

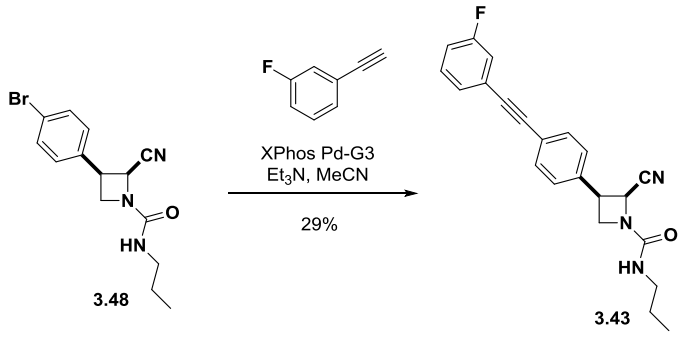
((2*S*,3*S*,4*S*)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl acetate (**3.40**). To a solution of **BRD7539** (6 mg, 0.015 mmol) in DCM (300 µL) was added pyridine (1.6 µL, 0.020 mmol) and acetyl chloride (1.4 µL, 0.018 mmol) in sequence. The reaction was stirred at ambient temperature for 30 min. Upon completion, the solvent was removed *in vacuo* and crude residue purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue (5.2 mg, 78%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.2 Hz, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.28 (m, 2H), 7.25 – 7.20 (m, 1H), 7.15 – 7.02 (m, 1H), 5.05 (dd, *J* = 7.5, 4.6 Hz, 2H), 4.71 (ddd, *J* = 7.0, 5.4, 3.1 Hz, 1H), 4.59 (dd, *J* = 12.3, 3.3 Hz, 1H), 4.23 (dd, *J* = 12.3, 5.4 Hz, 1H), 3.89 (dd, *J* = 8.5, 7.0 Hz, 1H), 3.35 – 3.14 (m, 2H), 2.12 (s, 3H), 1.69 – 1.51 (m, 3H), 0.96 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{25}H_{24}FN_3O_3$. Calculated mass: 433.18. **ESIMS m/z** 435.0 $(M+2H)^{2+}$.



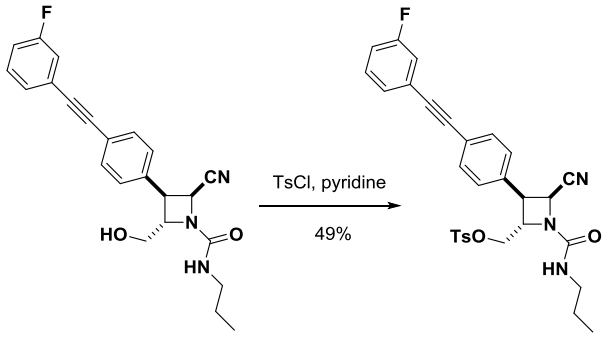
3.42 **3.41**

(2*S*,3*R*,4*R*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-methyl-N-propylazetidine-1-carboxamide (**3.41**). To a solution of **3.42** (6 mg, 0.011 mmol) in DMSO (220 µL) was added $NaBH_4$ (1.6 mg, 0.044 mmol). The reaction was heated to 70 °C and stirred for 1 h. Upon completion, the mixture was quenched with H_2O and extracted 4x with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (MeOH/DCM) to afford product as a colorless residue

(1.0 mg, 23%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 8.1, 5.9 Hz, 2H), 7.40 – 7.34 (m, 2H), 7.34 – 7.29 (m, 2H), 7.22 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.09 – 7.02 (m, 1H), 5.11 (d, *J* = 8.5 Hz, 1H), 5.00 (d, *J* = 9.3 Hz, 1H), 4.89 (t, *J* = 8.0 Hz, 1H), 4.62 (p, *J* = 6.1 Hz, 1H), 4.45 (t, *J* = 8.7 Hz, 1H), 4.28 (t, *J* = 5.7 Hz, 1H), 3.75 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.62 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.48 (dd, *J* = 10.2, 2.0 Hz, 1H), 3.40 – 3.14 (m, 3H), 1.60 – 1.55 (m, 2H), 0.96 (td, *J* = 7.4, 4.1 Hz, 3H). Molecular formula: $C_{23}H_{22}FN_3O$. Calculated mass: 375.17. **ESIMS m/z** 376.0 $(M+H)^+$.

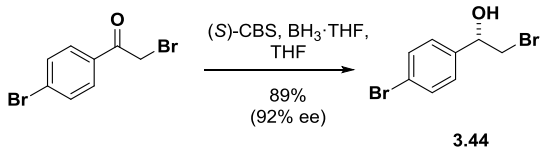


(2*S*,3*R*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-N-propylazetidine-1-carboxamide (**3.43**). Prepared from **3.48** following **General Protocol G** (29% yield). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.63 – 7.56 (m, 2H), 7.47 – 7.41 (m, 2H), 7.35 – 7.29 (m, 2H), 7.25 – 7.18 (m, 1H), 7.11 – 7.00 (m, 1H), 5.19 (d, *J* = 8.3 Hz, 1H), 4.48 (t, *J* = 5.9 Hz, 1H), 4.30 (dd, *J* = 8.4, 7.2 Hz, 1H), 4.14 – 3.97 (m, 2H), 3.22 (dt, *J* = 7.8, 6.3 Hz, 2H), 1.55 (h, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{22}H_{20}FN_3O$. Calculated mass: 361.16. **ESIMS m/z** 362.5 $(M+H)^+$.



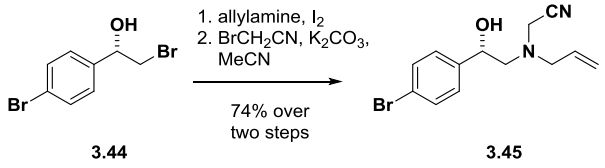
BRD7539 3.42

((2*S*,3*S*,4*S*)-4-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylcarbamoyl)azetidin-2-yl)methyl 4-methylbenzenesulfonate (**40**). To a solution of **BRD7539** (25 mg, 0.064 mmol) in pyridine (650 µL) was added 4-toluenesulfonyl chloride (18 mg, 0.096 mmol). The reaction was heated to 40 ºC for 8 h. Upon completion, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a pale yellow residue (17 mg, 49%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 – 7.74 (m, 2H), 7.60 – 7.54 (m, 2H), 7.41 – 7.28 (m, 6H), 7.24 – 7.19 (m, 1H), 7.05 (ddt, *J* = 9.3, 8.2, 3.3 Hz, 1H), 5.08 (d, *J* = 8.5 Hz, 1H), 4.81 (t, *J* = 5.7 Hz, 1H), 4.69 (ddd, *J* = 6.7, 4.1, 2.8 Hz, 1H), 4.50 (dd, *J* = 11.1, 2.9 Hz, 1H), 4.21 (dd, *J* = 11.1, 4.1 Hz, 1H), 3.98 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.28 – 3.07 (m, 2H), 2.46 (s, 3H), 1.52 (q, *J* = 7.3 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). Molecular formula: $C_{30}H_{28}FN_3O_4S$. Calculated mass: 545.18. **ESIMS m/z** 546.3 $(M+H)^+$.



3.44

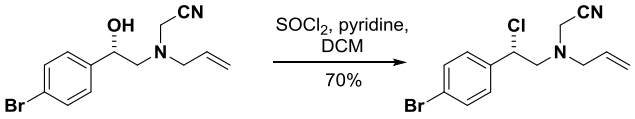
(*S*)-2-bromo-1-(4-bromophenyl)ethanol (**3.44**). To a solution of (*S*)-(−)-2-Methyl-CBS-oxazaborolidine (250 mg, 0.90 mmol) in THF (3 mL) was added borane tetrahydrofuran complex solution (9.0 mL, 1.0 M in THF). After 5 min, a solution of 2-bromo-1-(4-bromophenyl)ethanone (5.00 g, 17.99 mmol) in THF (15 mL) was added dropwise over 10 min. The reaction was stirred at ambient temperature for 30 min. Upon completion, the reaction was quenched with MeOH and solvent was removed *in vacuo*. Crude residue was purified by flash column chromatography (EtOAc/hexanes) to afford product as a white crystalline solid (4.51 g, 89%, 92% ee by chiral HPLC). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 2H), 4.91 (dd, *J* = 8.9, 3.5 Hz, 1H), 3.63 (dd, *J* = 10.6, 3.5 Hz, 1H), 3.51 (t, *J* = 9.7 Hz, 1H). Molecular formula: $C_8H_8Br_2O$. Calculated mass: 277.89. **ESIMS m/z** 278.7 $(M+H)^+$.



3.44 **3.45**

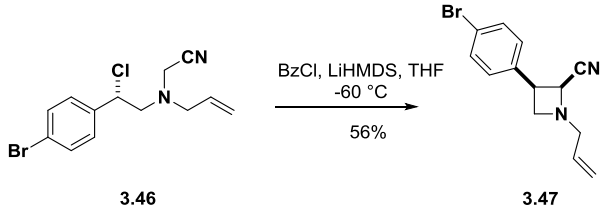
(*S*)-2-(allyl(2-(4-bromophenyl)-2-hydroxyethyl)amino)acetonitrile (**3.45**). To a flask containing **3.44** (1.00 g, 3.57 mmol) was added neat allylamine (5.35 mL, 71.4 mmol) and I_2 (54 mg, 0.36 mmol). The reaction was stirred at ambient temperature for 16 h. Upon completion, allylamine was removed *in vacuo*. The resulting crude residue was dissolved in MeCN (18 mL), and bromoacetonitrile (746 µL, 10.71 mmol) and K_2CO_3 (740 mg, 5.36 mmol) were added in sequence. The reaction was heated at 85 ^oC for 40 h. Upon completion, the solvent was removed *in vacuo* and the resulting crude residue was suspended in H_2O. The aqueous layer was extracted 3x with Et_2O, and the combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography

(EtOAc/hexanes) to afford product as a yellow crystalline solid (778 mg, 74% over two steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 4.2 Hz, 2H), 5.81 (ddt, *J* = 16.9, 10.1, 6.5 Hz, 1H), 5.34 (dd, *J* = 21.8, 13.7 Hz, 2H), 4.74 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.68 (q, *J* = 17.4 Hz, 2H), 3.38 (dd, *J* = 13.6, 6.2 Hz, 1H), 3.27 – 3.16 (m, 2H), 2.82 (dd, *J* = 13.3, 3.4 Hz, 1H), 2.63 (dd, *J* = 13.3, 10.0 Hz, 1H). Molecular formula: $C_{13}H_{15}BrN_2O$. Calculated mass: 294.04. **ESIMS m/z** 296.5 $(M+H)^+$.



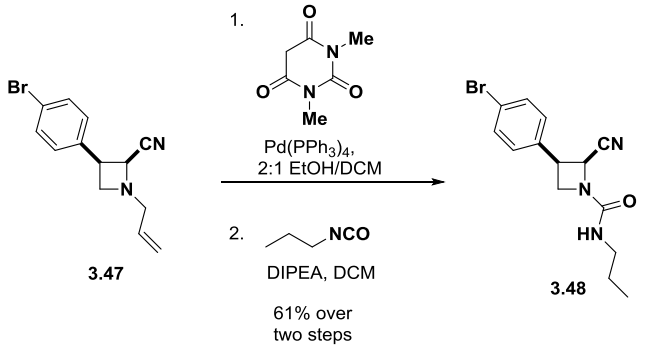
3.45 **3.46**

(*S*)-2-(allyl(2-(4-bromophenyl)-2-chloroethyl)amino)acetonitrile (**3.46**). To a solution of pyridine (1.06 mL, 13.09 mmol) in DCM (26 mL) was added thionyl chloride (380 μL, 5.24 mmol). The mixture was cooled to 0 °C, and a solution of **3.45** (773 mg, 2.62 mmol) in DCM (2 mL) was added in dropwise over ten minutes. After 15 min, the reaction was quenched with saturated $NaHCO_3$ and extracted 3x with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a yellow oil (572 mg, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 4.1 Hz, 2H), 5.73 (ddt, *J* = 16.6, 9.8, 6.2 Hz, 1H), 5.37 – 5.21 (m, 2H), 4.86 (t, *J* = 7.0 Hz, 1H), 3.60 (d, *J* = 17.6 Hz, 1H), 3.49 (d, *J* = 17.6 Hz, 1H), 3.23 (dd, *J* = 6.6, 2.9 Hz, 2H), 3.15 (dd, *J* = 14.1, 7.5 Hz, 1H), 3.05 (dd, *J* = 14.1, 6.5 Hz, 1H). Molecular formula: $C_{13}H_{14}BrClN_2$. Calculated mass: 312.00. **ESIMS m/z** 315.3 $(M+H)^+$.



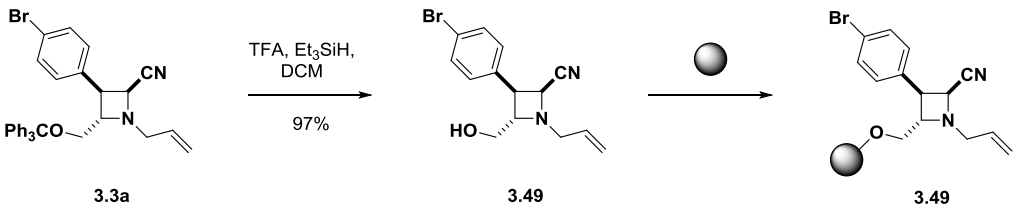
3.46 3.47

(2*S*,3*R*)-1-allyl-3-(4-bromophenyl)azetidine-2-carbonitrile (**3.47**). A solution of benzyl chloride **3.46** (572 mg, 1.824 mmol) in THF (18 mL) was cooled to -60 °C, and lithium bis(trimethylsilyl)amide (2.2 mL, 1.0 M in THF) was added dropwise over 25 minutes. After 60 min, the reaction was quenched with saturated NH_4Cl and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and removed *in vacuo*. The crude product was purified by flash column chromatography (EtOAc/hexanes) to afford product as a separable mixture of diastereomers (1.8:1). (2*S*,3*R*) - 282 mg, 56%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 5.83 (ddt, *J* = 16.5, 9.9, 4.9 Hz, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.24 (d, *J* = 10.2 Hz, 1H), 4.37 (d, *J* = 7.8 Hz, 1H), 3.84 (td, *J* = 7.7, 4.8 Hz, 1H), 3.53 (dt, *J* = 23.0, 6.8 Hz, 2H), 3.28 (d, *J* = 6.1 Hz, 2H). Molecular formula: $C_{13}H_{13}BrN_2$. Calculated mass: 276.03. **ESIMS m/z** 276.7 $(M+H)^+$. (2*R*,3*R*) - 158 mg, 31%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.1 Hz, 2H), 7.17 (s, 2H), 5.84 (ddt, *J* = 16.6, 10.0, 6.1 Hz, 1H), 5.28 (dd, *J* = 28.5, 13.7 Hz, 2H), 3.93 (q, *J* = 7.5 Hz, 1H), 3.87 – 3.74 (m, 2H), 3.26 (d, *J* = 6.2 Hz, 2H), 3.18 (t, *J* = 7.2 Hz, 1H). Molecular formula: $C_{13}H_{13}BrN_2$. Calculated mass: 276.03. **ESIMS m/z** 277.0 $(M+H)^+$.



3.47 3.48

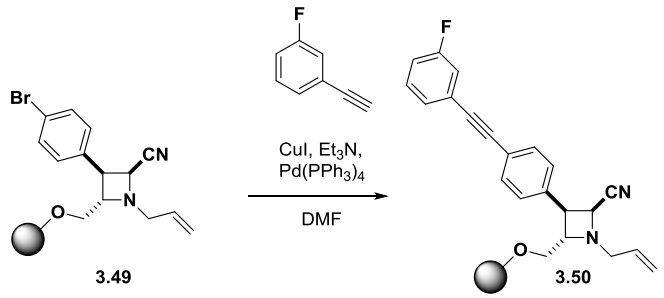
(2*S*,3*R*)-3-(4-bromophenyl)-2-cyano-N-propylazetidine-1-carboxamide (**3.48**). To a solution of **3.47** (154 mg, 0.556 mmol) in EtOH (3.7 mL) and DCM (1.85 mL) was added 1,3-dimethylbarbituric acid (130 mg, 0.833 mmol) and $Pd(PPh_3)_4$ (64 mg, 0.056 mmol). The reaction was heated to 40 ^oC for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated intermediate. This material was then directly dissolved in DCM (5.56 mmol), followed by the addition of DIPEA (968 µL, 5.56 mmol) and propylisocyanate (78 µL, 0.834 mmol). The reaction was allowed to stir at ambient temperature. After 30 min, solvent was removed *in vacuo* and crude material purified via flash column chromatography to afford product as a yellow solid (110 mg, 61% over two steps). Molecular formula: $C_{14}H_{16}BrN_3O$. Calculated mass: 321.05. **ESIMS m/z** 362.6 $(M+HCOOH)^+$.



3.3a 3.49 3.49

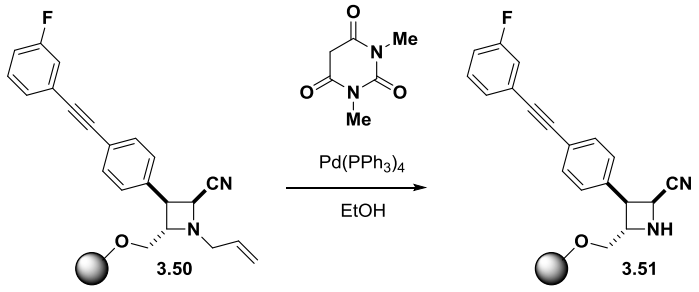
(2S,3S,4S)-1-allyl-3-(4-bromophenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile (**3.49**). To a solution of **3.3a** (1.84 g, 3.35 mmol) in DCM (0.1 M) was added TFA (2.56 mL, 33.5 mmol), followed by Et_3SiH (0.802 mL, 5.02 mmol). After 15 min, solvent was removed *in vacuo* and product was purified by flash column chromatography (EtOAc/hexanes) to provide the desired product as a thick yellow oil (1.00 g, 97%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 5.83 (ddt, *J* = 16.6, 9.9, 6.1 Hz, 1H), 5.43 (d, *J* = 17.2 Hz, 1H), 5.31 (d, *J* = 10.2 Hz, 1H), 4.73 (d, *J* = 7.9 Hz, 1H), 4.11 – 3.92 (m, 2H), 3.81 (dd, *J* = 12.7, 2.9 Hz, 1H), 3.60 (dd, *J* = 12.6, 2.8 Hz, 1H), 3.49 (t, *J* = 6.8 Hz, 2H). **m/z** 351.0 $(M+HCO_2^-)^-$.

3.49 was attached to silicon-based lanterns as described previously.[3]



General Procedure I-1: Solid Phase Synthesis of 3.50

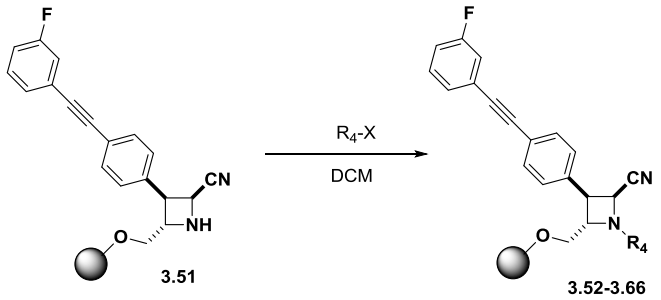
To lanterns **3.49** (0.0139 mmol/lantern) in DMF (0.018 M) was added Et_3N (30 equiv), CuI (3 equiv), 1-ethynyl-3-fluorobenzene (20 equiv), and $Pd(PPh_3)_4$ (2 equiv). The mixture was purged 3x with Ar, capped with Teflon, and heated in a 50 °C shaker for 72 h. The mixture was then decanted and washed for 20-30 min by each of the following solvent combinations: (1) DMF x 2; (2) 1:1 THF/H_2O; (3) 1:1 THF/*i*PrOH; (4) THF; (5) DCM x 2. Lanterns were then dried *in vacuo* for 5-16 h. Lantern and reaction purity was assessed by cleavage in 15% HF/THF.



General Procedure I-2: Solid Phase Synthesis of 3.51

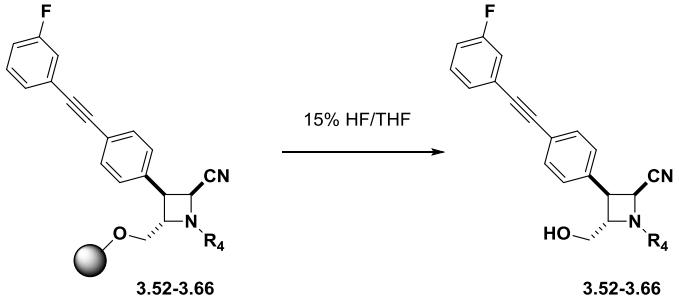
To lanterns **3.50** (0.0139 mmol/latern) in EtOH (0.018 M) was added 1,3-dimethylbarbituric acid (10 equiv) and $Pd(PPh_3)_4$ (2 equiv). The mixture was purged 3x with Ar, capped with Teflon, and heated in a 40 °C shaker for 72 h. The mixture was then decanted and

washed for 20-30 min by each of the following solvent combinations: (1) DMF x 7; (2) 1:1 THF/H_2O; (3) 1:1 THF/*i*PrOH; (4) THF; (5) DCM x 2. Lanterns were then dried *in vacuo* for 5-16 h. Lantern and reaction purity was assessed by cleavage in 15% HF/THF.



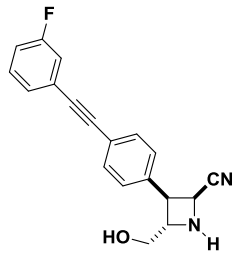
General Procedure I-3: Solid Phase Synthesis of 3.52-3.66

To lanterns **3.51** (0.0139 mmol/latern) in DCM (0.018 M) was added appropriate electrophile (R-NCO, R-COCl, R-SO_2Cl, R-CH_2Br) (20 equiv). The mixture was purged 3x with Ar, capped with Teflon, shaken at ambient temperature for 24 h. The mixture was then decanted and washed for 20-30 min by each of the following solvent combinations: (1) DMF x 7; (2) 1:1 THF/H_2O; (3) 1:1 THF/*i*PrOH; (4) THF; (5) DCM x 2. Lanterns were then dried *in vacuo* for 5-16 h.



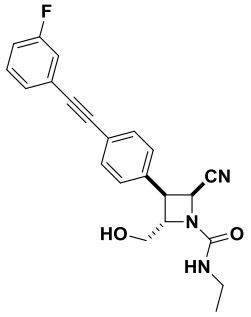
General Procedure I-4: Cleavage of 3.52a-o

To lanterns **3.52-3.66** was added 15% HF/THF (0.015 M) for 3 h. Upon complete cleavage, TMSOMe (2 equiv) was added to quench excess HF, and resulting solutions were removed *in vacuo*. Products were purified by column chromatography (EtOAc/hexanes or MeOH/DCM) as appropriate to ensure purity despite frequent washing steps as described above.



3.52

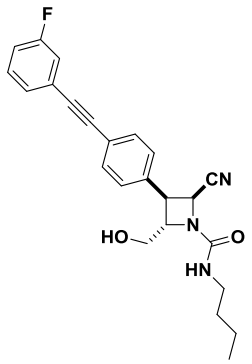
(2*S*,3*S*,4S)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile (**3.52**). Synthesized according to General Procedures **I-1, I-2, and I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 4.4 Hz, 2H), 7.25 – 7.18 (m, 1H), 7.05 (ddt, *J* = 11.7, 8.1, 4.1 Hz, 1H), 4.58 (dd, *J* = 24.0, 7.5 Hz, 2H), 4.23 (d, *J* = 8.2 Hz, 1H), 3.74 (d, *J* = 10.8 Hz, 1H), 3.61 (d, *J* = 12.1 Hz, 1H), 2.44 (s, 2H). **m/z** 306.7 $(M+H)^+$.



3.53

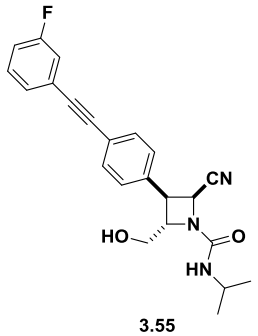
(2*S*,3*S*,4*S*)-2-cyano-N-ethyl-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.53**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400

MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.6 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 4.1 Hz, 2H), 7.27 – 7.21 (m, 1H), 7.08 (dq, *J* = 7.7, 4.3, 3.9 Hz, 1H), 5.50 – 5.36 (m, 1H), 5.06 (d, *J* = 8.4 Hz, 1H), 4.79 (t, *J* = 7.2 Hz, 1H), 4.02 – 3.94 (m, 1H), 3.89 (dd, *J* = 11.5, 7.8 Hz, 1H), 3.81 (t, *J* = 8.0 Hz, 1H), 3.41 – 3.26 (m, 2H), 1.21 (t, *J* = 7.3 Hz, 3H). **m/z** 379.4 $(M+H)^+$.



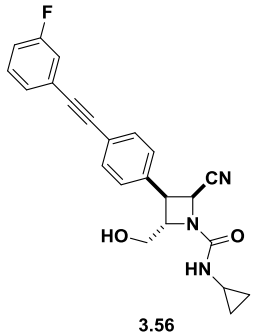
3.54

(2*S*,3*S*,4*S*)-N-butyl-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.54**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.7 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.25 (d, *J* = 10.5 Hz, 2H), 7.18 – 7.12 (m, 1H), 6.98 (h, *J* = 3.9, 3.4 Hz, 1H), 5.43 (s, 1H), 4.97 (d, *J* = 8.4 Hz, 1H), 4.75 – 4.65 (m, 1H), 3.93 – 3.85 (m, 1H), 3.80 (dd, *J* = 11.5, 7.8 Hz, 1H), 3.71 (t, *J* = 8.0 Hz, 1H), 3.20 (dtd, *J* = 20.1, 13.3, 7.0 Hz, 2H), 1.45 (p, *J* = 7.1 Hz, 2H), 1.30 (h, *J* = 7.2 Hz, 2H), 0.87 (t, *J* = 7.2 Hz, 3H). **m/z** 406.5 $(M+H)^+$.



3.55

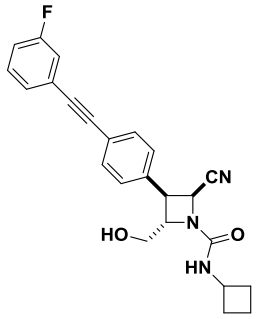
(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-isopropylazetidine-1-carboxamide (**3.55**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 2H), 7.37 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 4.1 Hz, 2H), 7.24 – 7.20 (m, 1H), 7.05 (dq, *J* = 7.9, 3.7 Hz, 1H), 5.25 (s, 1H), 5.02 (d, *J* = 8.3 Hz, 1H), 4.76 (t, *J* = 7.5 Hz, 1H), 3.97 (dd, *J* = 18.9, 9.3 Hz, 2H), 3.86 (dd, *J* = 11.6, 7.7 Hz, 1H), 3.83 – 3.73 (m, 1H), 1.23 – 1.14 (m, 6H). **m/z** 392.8 $(M+H)^+$.



3.56

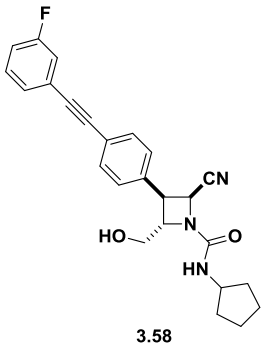
(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.56**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.7 Hz, 2H), 7.36 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 4.0 Hz, 2H), 7.25 – 7.19 (m, 1H), 7.05 (dd, J = 9.3, 5.3 Hz, 1H), 5.70 (s, 1H), 5.03 (d, J = 8.5 Hz, 1H), 4.76 (t, J = 7.6 Hz, 1H), 4.00 – 3.90 (m, 1H), 3.90 – 3.82 (m, 1H), 3.80 (q, J = 8.0,

7.0 Hz, 1H), 2.67 (td, J = 6.8, 3.3 Hz, 1H), 0.83 – 0.72 (m, 2H), 0.62 – 0.52 (m, 2H). **m/z** 390.78 $(M+H)^+$.



3.57

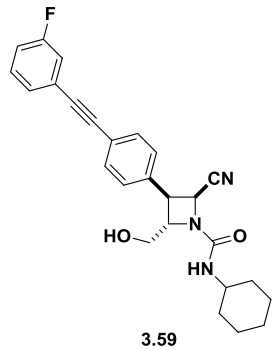
(2*S*,3*S*,4*S*)-2-cyano-N-cyclobutyl-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.57**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.24 (t, *J* = 2.8 Hz, 2H), 7.16 (dd, *J* = 9.8, 2.8 Hz, 1H), 7.03 – 6.95 (m, 1H), 5.58 (s, 1H), 4.97 (d, *J* = 8.4 Hz, 1H), 4.69 (t, *J* = 7.3 Hz, 1H), 4.23 (s, 1H), 3.90 (d, *J* = 11.1 Hz, 1H), 3.80 (dd, *J* = 11.4, 7.9 Hz, 1H), 3.71 (t, *J* = 7.9 Hz, 1H), 1.81 (p, *J* = 8.3 Hz, 2H), 1.63 (dd, *J* = 11.3, 3.0 Hz, 2H). **m/z** 448.3 $(M+HCO_2^-)^-$.



3.58

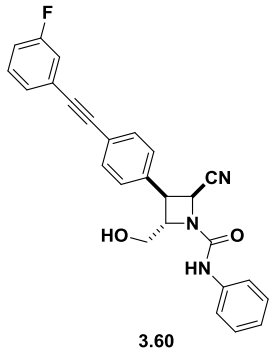
(2*S*,3*S*,4*S*)-2-cyano-N-cyclopentyl-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.58**). Synthesized according to General Procedures

I-1 – I-4. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 2H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.31 (d, *J* = 4.2 Hz, 2H), 7.25 – 7.19 (m, 1H), 7.05 (dt, *J* = 8.3, 5.2 Hz, 1H), 5.41 (s, 1H), 5.02 (d, *J* = 8.4 Hz, 1H), 4.75 (d, *J* = 8.1 Hz, 1H), 4.21 – 4.07 (m, 1H), 4.02 – 3.90 (m, 1H), 3.86 (dd, *J* = 11.5, 7.8 Hz, 1H), 3.77 (t, *J* = 8.2 Hz, 1H), 1.99 (dt, *J* = 13.7, 6.9 Hz, 2H), 1.68 (t, *J* = 11.9 Hz, 4H), 1.43 (dt, *J* = 13.1, 6.8 Hz, 2H). **m/z** 418.8 $(M+H)^+$.



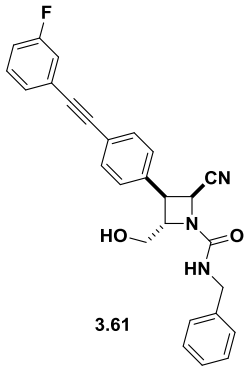
3.59

(2*S*,3*S*,4*S*)-2-cyano-N-cyclohexyl-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.59**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.6 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 4.2 Hz, 2H), 7.18 – 7.13 (m, 1H), 6.98 (dq, *J* = 7.8, 4.2, 3.5 Hz, 1H), 5.25 (d, *J* = 19.2 Hz, 1H), 4.95 (d, *J* = 8.3 Hz, 1H), 4.69 (t, *J* = 7.7 Hz, 1H), 3.92 – 3.84 (m, 1H), 3.78 (dd, *J* = 11.7, 7.7 Hz, 1H), 3.70 (t, *J* = 8.0 Hz, 1H), 3.66 – 3.54 (m, 1H), 1.96 – 1.82 (m, 2H), 1.63 (d, *J* = 6.1 Hz, 2H), 1.55 (t, *J* = 4.8 Hz, 1H), 1.31 (q, *J* = 13.1 Hz, 2H), 1.12 (d, *J* = 11.7 Hz, 3H). **m/z** 433.4 $(M+H)^+$.



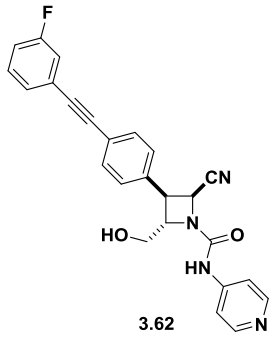
3.60

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-phenylazetidine-1-carboxamide (**3.60**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.9 Hz, 2H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.41 – 7.30 (m, 5H), 7.27 – 7.22 (m, 1H), 7.09 (d, *J* = 7.7 Hz, 2H), 5.09 (d, *J* = 8.4 Hz, 1H), 4.97 – 4.88 (m, 1H), 4.12 – 3.97 (m, 2H), 3.77 (t, *J* = 8.1 Hz, 1H). **m/z** 426.7 $(M+H)^+$.



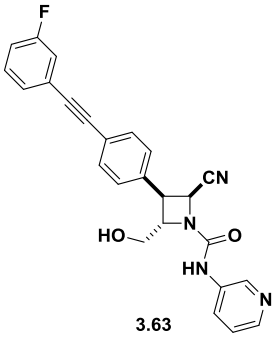
3.61

(2*S*,3*S*,4*S*)-N-benzyl-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.61**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 7.7 Hz, 2H), 7.36 (p, *J* = 6.8, 5.4 Hz, 8H), 7.26 – 7.20 (m, 1H), 7.08 (dd, *J* = 8.8, 5.1 Hz, 1H), 6.03 (s, 1H), 5.06 (d, *J* = 8.4 Hz, 1H), 4.81 (t, *J* = 7.8 Hz, 1H), 4.57 – 4.40 (m, 2H), 4.02 – 3.93 (m, 1H), 3.89 (dd, *J* = 11.5, 7.8 Hz, 1H), 3.81 (t, *J* = 8.1 Hz, 1H). **m/z** 440.4 $(M+H)^+$.



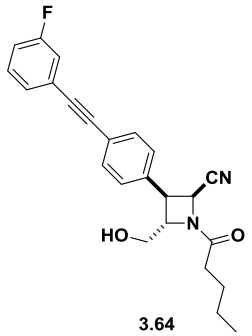
3.62

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-(pyridin-4-yl)azetidine-1-carboxamide (**3.62**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.63 – 8.55 (m, 1H), 8.49 (s, 1H), 8.11 (s, 1H), 7.52 (d, *J* = 7.6 Hz, 2H), 7.41 (s, 1H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 4.1 Hz, 2H), 7.18 – 7.12 (m, 1H), 7.03 – 6.94 (m, 1H), 5.13 (d, *J* = 8.5 Hz, 1H), 4.96 (d, *J* = 8.8 Hz, 1H), 4.07 (d, *J* = 10.9 Hz, 1H), 3.97 (t, *J* = 10.1 Hz, 1H), 3.79 (t, *J* = 8.2 Hz, 1H). **m/z** 426.5 $(M+H)^+$.



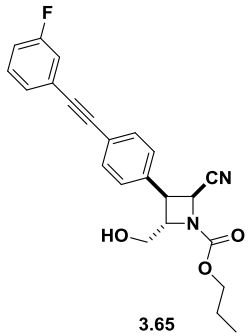
3.63

(2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-N-(pyridin-3-yl)azetidine-1-carboxamide (**3.63**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, CD_3OD) δ 8.56 (d, *J* = 6.7 Hz, 2H), 8.04 (d, *J* = 6.5 Hz, 2H), 7.62 (d, *J* = 7.7 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.39 (dt, *J* = 23.1, 7.1 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.14 (t, *J* = 8.3 Hz, 1H), 5.42 (d, *J* = 8.7 Hz, 1H), 5.05 (t, *J* = 6.0 Hz, 1H), 4.12 (t, *J* = 7.4 Hz, 1H), 4.04 (d, *J* = 11.6 Hz, 1H), 3.92 (dd, *J* = 12.2, 6.5 Hz, 1H). **m/z** 426.9 $(M+H)^+$.



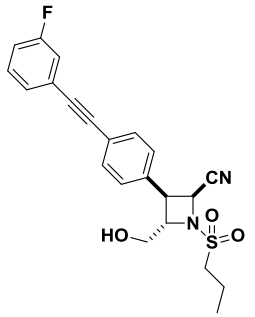
3.64

(2*S*,3*S*,4*S*)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)-1-pentanoylazetidine-2-carbonitrile (**3.64**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.7 Hz, 2H), 7.32 (d, *J* = 4.0 Hz, 2H), 7.28 (s, 2H), 7.24 – 7.20 (m, 1H), 7.07 (dd, *J* = 8.8, 5.1 Hz, 1H), 4.78 (d, *J* = 6.1 Hz, 1H), 4.62 (s, 1H), 3.98 (d, *J* = 12.5 Hz, 1H), 3.90 (dd, *J* = 13.4, 6.9 Hz, 2H), 2.46 – 2.32 (m, 1H), 2.27 (dt, *J* = 15.3, 7.5 Hz, 1H), 1.69 (hept, *J* = 7.4 Hz, 2H), 1.40 (dd, *J* = 14.6, 7.3 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). **m/z** 390.6 $(M+H)^+$.



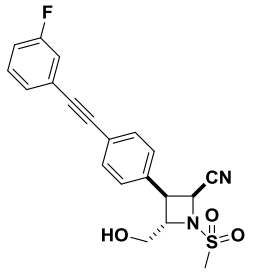
3.65

propyl (2*S*,3*S*,4*S*)-2-cyano-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxylate (**3.65**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 4.3 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 5.12 (d, *J* = 8.6 Hz, 1H), 4.77 – 4.60 (m, 1H), 4.20 (q, *J* = 7.6, 5.7 Hz, 1H), 4.11 (td, *J* = 14.1, 11.5, 6.9 Hz, 2H), 3.98 (s, 1H), 3.86 – 3.74 (m, 1H), 1.73 (q, *J* = 6.9 Hz, 2H), 1.25 (s, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). **m/z** 393.1 $(M+H)^+$.



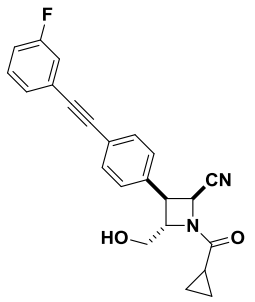
3.66

((2S,3S,4S)-4-((λ^2-azaneylidene)-λ^3-methyl)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(propylsulfonyl)azetidin-2-yl)methanol (**3.66**). Synthesized according to General Procedures **I-1 – I-4**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.7 Hz, 2H), 7.44 (d, *J* = 7.9 Hz, 2H), 7.34 (d, *J* = 4.2 Hz, 2H), 7.25 (dd, *J* = 9.8, 3.2 Hz, 1H), 7.08 (dq, *J* = 7.5, 3.3 Hz, 1H), 5.32 (d, *J* = 8.7 Hz, 1H), 4.63 (dd, *J* = 5.9, 3.2 Hz, 1H), 4.31 – 4.22 (m, 1H), 4.11 (dd, *J* = 9.0, 5.4 Hz, 1H), 3.78 (dd, *J* = 13.1, 3.3 Hz, 1H), 3.28 – 3.17 (m, 2H), 2.01 (h, *J* = 7.3 Hz, 2H), 1.17 (t, *J* = 7.4 Hz, 3H). **m/z** 413.2 $(M+H)^+$.



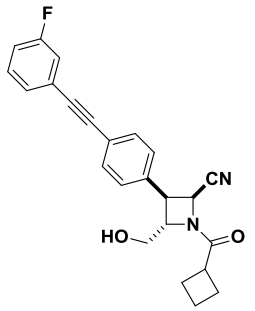
3.67

((2*S*,3*S*,4*S*)-4-((λ^2-azaneylidene)-λ^3-methyl)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-1-(methylsulfonyl)azetidin-2-yl)methanol (**3.67**). Compound was picked from diversity-oriented synthesis library and synthesis is publicly available on PubChem; no NMR available.



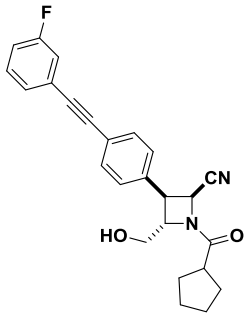
3.68

(2*S*,3*S*,4*S*)-1-(cyclopropanecarbonyl)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile (**3.68**). Compound was picked from diversity-oriented synthesis library and synthesis is publicly available on PubChem; no NMR available.



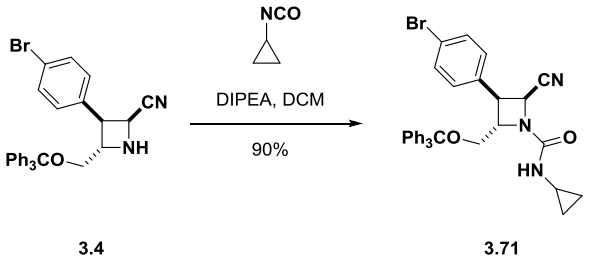
3.69

(2*S*,3*S*,4*S*)-1-(cyclobutanecarbonyl)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile (**3.69**). Compound was picked from diversity-oriented synthesis library and synthesis is publicly available on PubChem; no NMR available.



3.70

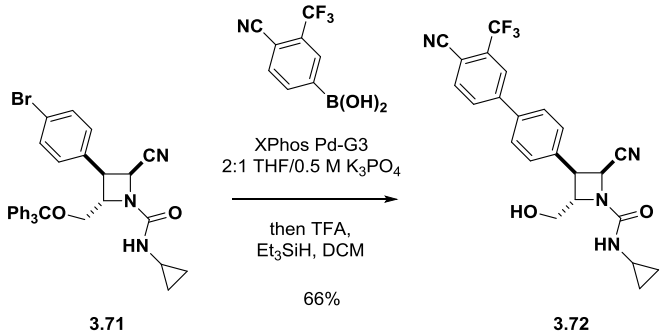
(2*S*,3*S*,4*S*)-1-(cyclopentanecarbonyl)-3-(4-((3-fluorophenyl)ethynyl)phenyl)-4-(hydroxymethyl)azetidine-2-carbonitrile (**3.70**). Compound was picked from diversity-oriented synthesis library and synthesis is publicly available on PubChem; no NMR available.



3.4 3.71

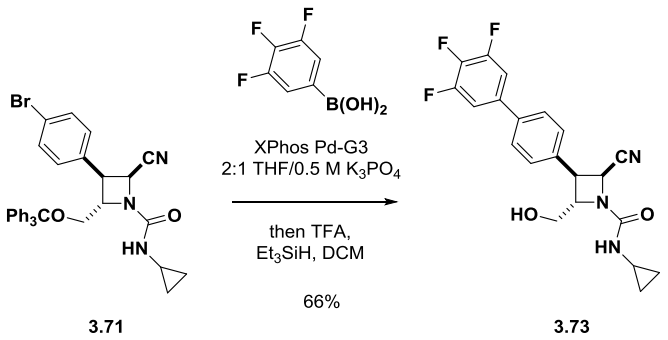
(2*S*,3*S*,4*S*)-3-(4-bromophenyl)-2-cyano-N-cyclopropyl-4-((trityloxy)methyl)azetidine-1-carboxamide (**3.71**). To a solution of **3.4** (450 mg, 0.883 mmol) in DCM (11.8 mL, 0.075 M) at was added DIPEA (0.308 mL, 1.77 mmol) followed by cyclopropylisocyanate (88 mg, 1.06 mmol). The mixture was stirred at ambient temperature for 4 h, after which point volatiles were removed *in vacuo* and crude material purified by column chromatography (EtOAc/hexanes) to afford desired product as white foaming solid (470 mg, 90%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 – 7.47 (m, 2H), 7.43 – 7.28 (m, 15H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.47 (s, 1H), 4.90 (d, *J* = 8.5

Hz, 1H), 4.46 (td, *J* = 8.1, 2.1 Hz, 1H), 3.62 (dd, *J* = 10.9, 2.2 Hz, 1H), 3.59 – 3.44 (m, 2H), 2.59 (ttd, *J* = 7.2, 3.7, 2.1 Hz, 1H), 0.67 – 0.53 (m, 2H), 0.40 – 0.18 (m, 2H). **m/z** 638.1 $(M+HCO_2^-)^-$.



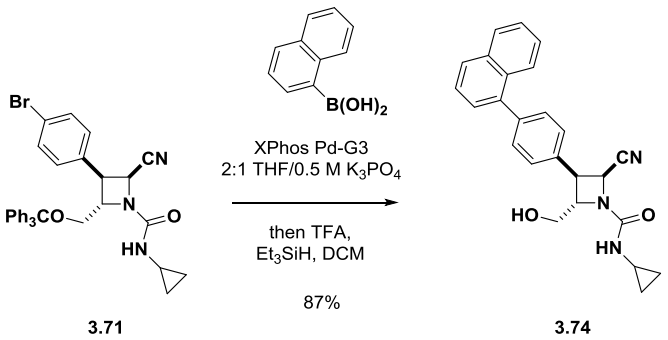
3.71 3.72

(2*S*,3*S*,4*S*)-2-cyano-3-(4'-cyano-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-N-cyclopropyl-4-(hydroxymethyl)azetidine-1-carboxamide (**3.72**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (66%). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ 8.04 (s, 1H), 7.94 (d, *J* = 1.7 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.58 – 7.50 (m, 2H), 5.98 (s, 1H), 5.05 (d, *J* = 8.6 Hz, 1H), 4.76 (td, *J* = 7.9, 2.3 Hz, 1H), 3.96 – 3.78 (m, 3H), 2.65 (tdd, *J* = 6.8, 4.7, 2.6 Hz, 1H), 0.73 (ddd, *J* = 7.7, 5.2, 3.1 Hz, 2H), 0.53 (dq, *J* = 4.0, 1.9 Hz, 2H). **m/z** 441.5 $(M+H)^+$.



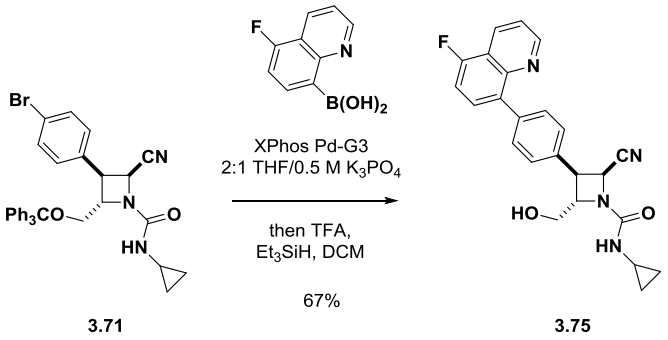
3.71 3.73

(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)azetidine-1-carboxamide (**3.73**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.18 (dd, *J* = 8.5, 6.3 Hz, 2H), 6.52 (s, 1H), 5.07 (d, *J* = 8.6 Hz, 1H), 4.79 (td, *J* = 8.1, 2.2 Hz, 1H), 3.96 (dd, *J* = 11.5, 2.2 Hz, 2H), 3.92 – 3.77 (m, 2H), 2.65 (dh, *J* = 6.8, 3.3 Hz, 1H), 0.74 (dt, *J* = 7.0, 3.6 Hz, 2H), 0.60 – 0.51 (m, 2H). **m/z** 402.4 $(M+H)^+$.



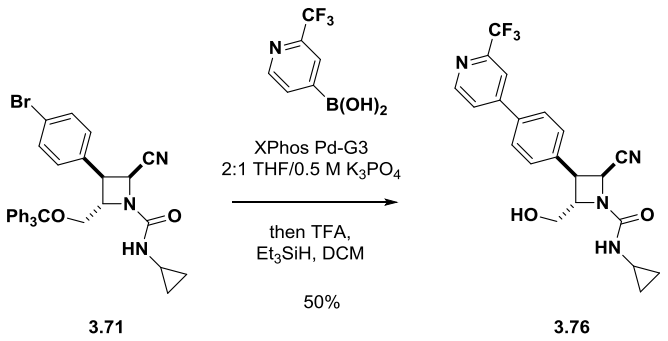
3.71 3.74

(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(naphthalen-1-yl)phenyl)azetidine-1-carboxamide (**3.74**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (87%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.96 – 7.81 (m, 3H), 7.59 – 7.37 (m, 8H), 5.96 (s, 1H), 5.09 (d, *J* = 8.6 Hz, 1H), 4.84 (td, *J* = 8.0, 2.1 Hz, 1H), 4.00 (dq, *J* = 7.8, 3.0, 2.3 Hz, 1H), 3.94 – 3.80 (m, 2H), 3.67 (q, *J* = 3.6, 3.1 Hz, 1H), 2.69 (dddd, *J* = 10.6, 6.7, 3.6, 2.1 Hz, 1H), 0.77 (ddd, *J* = 7.3, 4.5, 3.0 Hz, 2H), 0.57 (dtd, *J* = 6.6, 3.6, 1.4 Hz, 2H). **m/z** 397.8 $(M+H)^+$.

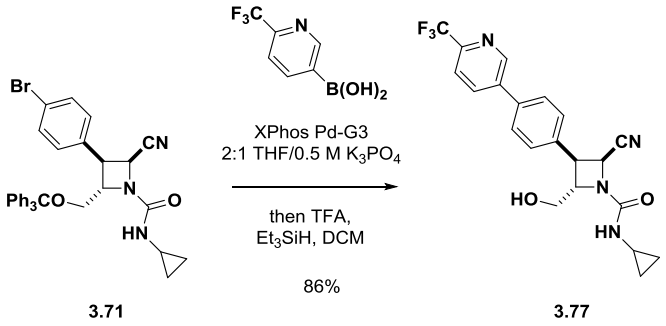


(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-3-(4-(4-fluoroquinolin-8-yl)phenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.75**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.97 (dd, *J* = 4.3, 1.8 Hz, 1H), 8.56 (dd, *J* = 8.4, 1.7 Hz,

1H), 7.74 – 7.64 (m, 3H), 7.54 (dd, *J* = 8.5, 4.2 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.32 (dd, *J* = 9.3, 8.1 Hz, 1H), 6.23 (s, 1H), 5.06 (d, *J* = 8.5 Hz, 1H), 4.83 – 4.63 (m, 2H), 3.90 – 3.70 (m, 3H), 2.69 (q, *J* = 6.1 Hz, 1H), 0.80 – 0.71 (m, 2H), 0.61 – 0.51 (m, 2H). **m/z** 417.4 $(M+H)^+$.

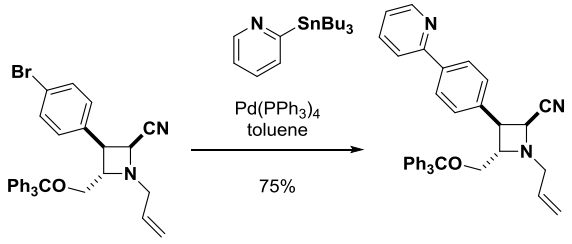


(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(2-(trifluoromethyl)pyridin-4-yl)phenyl)azetidine-1-carboxamide (**3.76**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (50%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.79 (d, *J* = 5.1 Hz, 1H), 7.91 – 7.86 (m, 1H), 7.76 – 7.67 (m, 3H), 7.53 (d, *J* = 8.0 Hz, 2H), 6.06 (s, 1H), 5.10 (d, *J* = 8.5 Hz, 1H), 4.80 (t, *J* = 7.9 Hz, 1H), 3.99 (d, *J* = 12.3 Hz, 1H), 3.87 (q, *J* = 8.7, 7.6 Hz, 2H), 2.67 (s, 1H), 0.80 – 0.72 (m, 2H), 0.61 – 0.52 (m, 2H).). ^{19}F NMR (376 MHz, $CDCl_3$) δ -67.96. **m/z** 417.2 $(M+H)^+$.



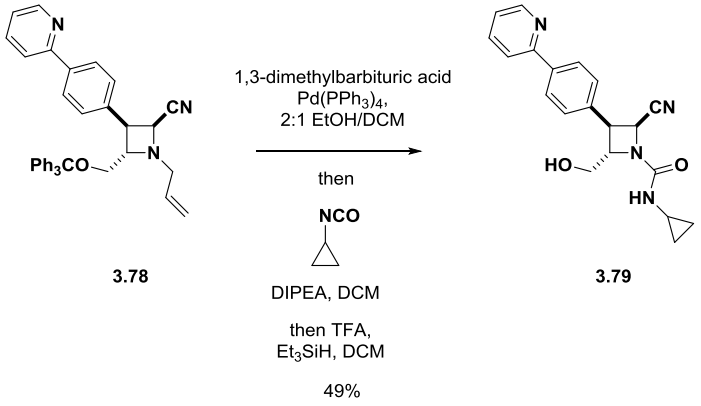
3.71 3.77

(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(6-(trifluoromethyl)pyridin-3-yl)phenyl)azetidine-1-carboxamide (**3.77**). Prepared according to modified **General Procedure H** from **3.4**. Following extraction with EtOAc and removal *in vacuo*, crude material was reconstituted in DCM (0.05 M), and TFA (10 equiv) and Et_3SiH (1.5 equiv) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (86%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 2.2 Hz, 1H), 8.04 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.55 – 7.47 (m, 2H), 6.24 (s, 1H), 5.09 (d, *J* = 8.5 Hz, 1H), 4.87 – 4.73 (m, 1H), 4.35 (s, 1H), 3.98 (d, *J* = 11.4 Hz, 1H), 3.87 (dt, *J* = 16.1, 8.0 Hz, 2H), 2.66 (dq, *J* = 10.2, 5.9, 4.6 Hz, 1H), 0.80 – 0.68 (m, 2H), 0.60 – 0.49 (m, 2H). **m/z** 416.8 $(M+H)^+$.



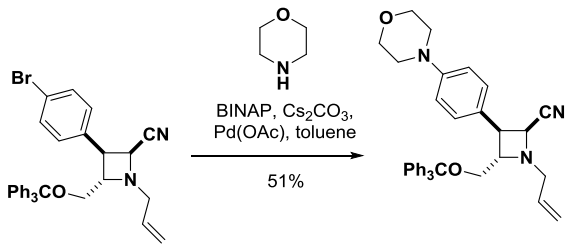
3.3a 3.78

(2*S*,3*S*,4*S*)-1-allyl-3-(4-(pyridin-2-yl)phenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.78**). To a solution of **3.3a** (20 mg, 0.036 mmol) and 2-(tributylstannyl)pyridine (15 µL, 0.040 mmol) in toluene (1.2 mL, 0.3 M) was added $Pd(PPh_3)_4$ (6.3 mg, 0.005 mmol). The mixture was heated and stirred at 110 °C for 24 h, upon which it was quenched with DCM. The organic layer was washed 3x with H_2O and the resulting organic layer was removed *in vacuo* and purified using column chromatography (EtOAc/hexanes) to afford the desired product as a colorless residue (15 mg, 75%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.72 (dt, *J* = 4.9, 1.3 Hz, 1H), 8.09 – 7.98 (m, 2H), 7.80 – 7.73 (m, 2H), 7.47 – 7.37 (m, 8H), 7.35 – 7.24 (m, 10H), 5.80 (dddd, *J* = 17.2, 10.2, 7.0, 5.0 Hz, 1H), 5.37 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dt, *J* = 10.1, 1.5 Hz, 1H), 4.73 – 4.66 (m, 1H), 3.94 (t, *J* = 4.3 Hz, 2H), 3.59 (ddt, *J* = 13.9, 5.0, 1.7 Hz, 1H), 3.44 – 3.27 (m, 3H). **m/z** 548.6 $(M+H)^+$.



(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(pyridin-2-yl)phenyl)azetidine-1-carboxamide (**3.79**). To a solution of **3.78** (28 mg, 0.051 mmol) in EtOH (0.68 mL) and DCM (0.34 mL) was added 1,3-dimethylbarbituric acid (20 mg, 0.128 mmol) and $Pd(PPh_3)_4$ (6 mg, 0.005 mmol). The reaction was heated to 40 °C for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give

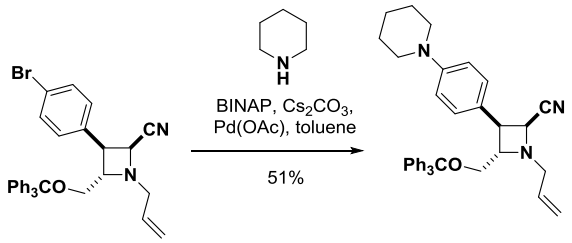
deallylated product. This material was then directly dissolved in DCM (0.71 mL, 0.05 M), followed by the addition of DIPEA (31 µL, 0.177 mmol) and cyclopropylisocyanate (3.5 mg, 0.043 mmol). The reaction was allowed to stir at ambient temperature. After 4 h, solvent was removed *in vacuo*, and crude material was reconstituted in DCM (0.71 mL, 0.05 M), and TFA (27 µL, 0.355 mmol) and Et_3SiH (8.5 µL, 0.053 mmol) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a colorless residue (6.4 mg, 49% over three steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.67 (dt, *J* = 4.9, 1.3 Hz, 1H), 8.03 – 7.94 (m, 2H), 7.79 (td, *J* = 7.7, 1.8 Hz, 1H), 7.73 (dt, *J* = 8.1, 1.2 Hz, 1H), 7.45 – 7.36 (m, 2H), 7.30 – 7.26 (m, 1H), 6.26 (s, 1H), 5.01 (d, *J* = 8.5 Hz, 1H), 4.69 (td, *J* = 8.1, 2.1 Hz, 1H), 3.86 (dd, *J* = 11.7, 2.2 Hz, 1H), 3.77 (ddd, *J* = 10.3, 8.3, 2.0 Hz, 2H), 2.67 (tdd, *J* = 6.8, 4.8, 2.8 Hz, 1H), 0.80 – 0.70 (m, 2H), 0.60 – 0.50 (m, 2H). **m/z** 349.2 $(M+H)^+$.



3.3a **3.80**

(2*S*,3*S*,4*S*)-1-allyl-3-(4-morpholinophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.80**). To a vial was charged **3.3a** (33 mg, 0.060 mmol), $Pd(OAc)_2$ (2.7 mg, 0.012 mmol), racemic BINAP (7.5 mg, 0.012 mmol), and Cs_2CO_3 (39 mg, 0.120 mmol). The vial was purged 3x with N_2 before the addition of toluene (0.601 mL, 0.1 M) and morpholine (10.4 µL, 0.120 mmol), the vial was heated and stirred at 90 °C for 90 min. Upon completion, the mixture was quenched

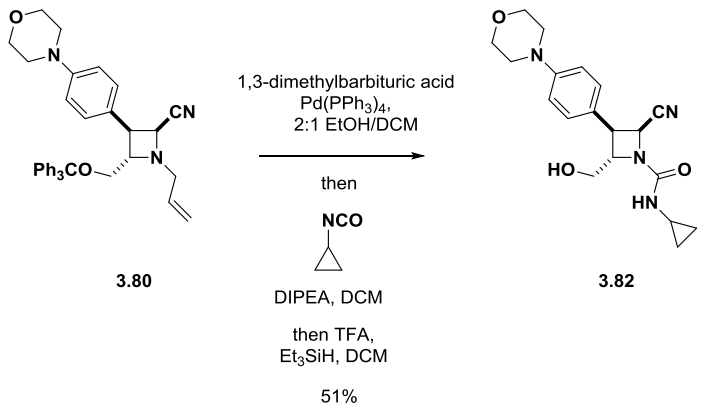
with saturated brine, extracted 3x with EtOAc, dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified by flash column chromatography (EtOAc/hexanes) to provide desired product as colorless residue (17 mg, 51%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49 – 7.39 (m, 6H), 7.33 – 7.19 (m, 11H), 6.94 – 6.85 (m, 2H), 5.80 (dddd, *J* = 17.3, 10.2, 7.0, 5.0 Hz, 1H), 5.36 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.19 (dq, *J* = 10.2, 1.4 Hz, 1H), 4.62 (d, *J* = 7.6 Hz, 1H), 3.96 – 3.84 (m, 5H), 3.80 (t, *J* = 8.3 Hz, 1H), 3.60 (ddt, *J* = 14.0, 5.0, 1.7 Hz, 1H), 3.39 (dd, *J* = 13.9, 7.1 Hz, 1H), 3.35 – 3.23 (m, 2H), 3.20 – 3.17 (m, 3H). **m/z** 556.4 $(M+H)^+$.



3.3a **3.81**

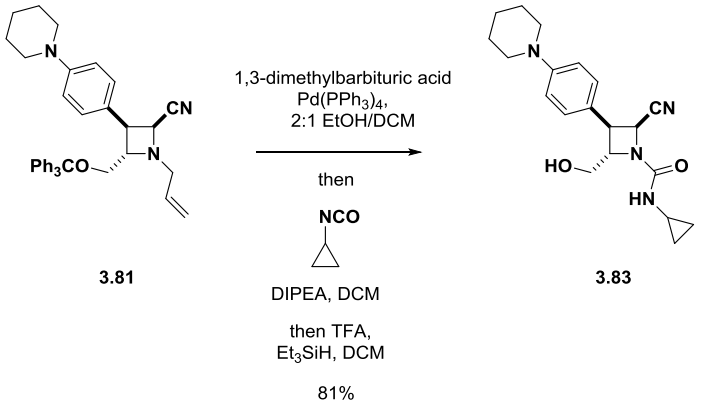
(2*S*,3*S*,4*S*)-1-allyl-3-(4-(piperidin-1-yl)phenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.81**). To a vial was charged **3.3a** (33 mg, 0.060 mmol), $Pd(OAc)_2$ (2.7 mg, 0.012 mmol), racemic BINAP (7.5 mg, 0.012 mmol), and Cs_2CO_3 (39 mg, 0.120 mmol). The vial was purged 3x with N_2 before the addition of toluene (0.601 mL, 0.1 M) and piperidine (11.9 μL, 0.120 mmol), the vial was heated and stirred at 90 °C for 16 h. Upon completion, the mixture was quenched with saturated brine, extracted 3x with EtOAc, dried over Na_2SO_4, filtered, and solvent removed *in vacuo*. The crude material was purified by flash column chromatography (EtOAc/hexanes) to provide desired product as colorless residue (17 mg, 51%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.46 – 7.40 (m, 6H), 7.34 – 7.22 (m, 9H), 7.21 – 7.16 (m, 2H), 6.95 – 6.90 (m, 2H), 5.80 (dddd, *J* = 17.2, 10.2, 7.1, 5.0 Hz, 1H), 5.36 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.18 (dq, *J* = 10.3, 1.3 Hz, 1H), 4.61 (d, *J* = 7.7 Hz, 1H), 3.92 – 3.84 (m, 1H), 3.78 (t, *J* = 8.3 Hz, 1H), 3.61

(ddt, *J* = 13.9, 4.9, 1.7 Hz, 1H), 3.39 (ddt, *J* = 14.0, 7.2, 1.2 Hz, 1H), 3.28 (qd, *J* = 10.1, 4.9 Hz, 2H), 3.22 – 3.14 (m, 4H), 1.76 – 1.69 (m, 4H), 1.64 – 1.58 (m, 2H). **m/z** 554.9 $(M+H)^+$.



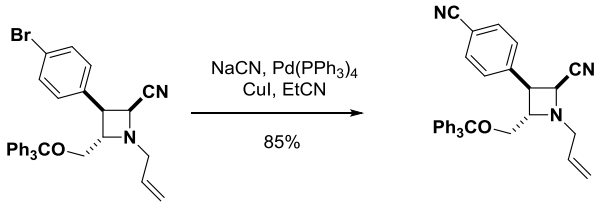
(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-morpholinophenyl)azetidine-1-carboxamide (**3.82**). To a solution of **3.80** (17 mg, 0.031 mmol) in EtOH (0.413 mL) and DCM (0.207 mL) was added 1,3-dimethylbarbituric acid (7.3 mg, 0.047 mmol) and $Pd(PPh_3)_4$ (3.6 mg, 0.003 mmol). The reaction was heated to 40 °C for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated product. This material was then directly dissolved in DCM (0.310 mL, 0.1 M), followed by the addition of DIPEA (54 µL, 0.311 mmol) and cyclopropylisocyanate (3.9 mg, 0.047 mmol). The reaction was allowed to stir at ambient temperature. After 5 h, solvent was removed *in vacuo*, and crude material was reconstituted in DCM (0.310 mL, 0.1 M), and TFA (24 µL, 0.311 mmol) and Et_3SiH (7.5 µL, 0.047 mmol) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a colorless residue (6.1 mg, 55% over three steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 6.6 Hz, 2H), 6.97 – 6.90 (m, 2H), 5.90 – 5.78 (m, 1H), 4.97 (d, *J* = 8.5 Hz, 1H), 4.75 – 4.67 (m, 1H), 3.90 – 3.84 (m, 5H), 3.84 –

3.78 (m, 1H), 3.68 (t, J = 8.1 Hz, 2H), 3.23 – 3.15 (m, 4H), 2.66 (tt, J = 6.8, 3.5 Hz, 1H), 0.75 (ddd, J = 7.0, 3.9, 2.7 Hz, 2H), 0.56 – 0.51 (m, 2H). **m/z** 357.2 $(M+H)^+$.



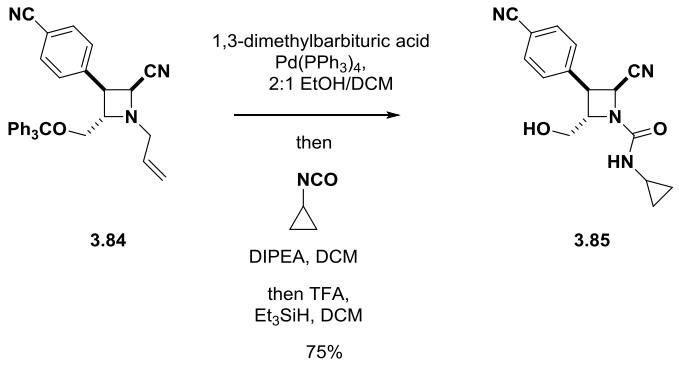
(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(piperidin-1-yl)phenyl)azetidine-1-carboxamide (**3.83**). To a solution of **3.80** (17 mg, 0.031 mmol) in EtOH (0.413 mL) and DCM (0.207 mL) was added 1,3-dimethylbarbituric acid (7.3 mg, 0.047 mmol) and $Pd(PPh_3)_4$ (3.6 mg, 0.003 mmol). The reaction was heated to 40 °C for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated product. This material was then directly dissolved in DCM (0.310 mL, 0.1 M), followed by the addition of DIPEA (54 μL, 0.311 mmol) and cyclopropylisocyanate (3.9 mg, 0.047 mmol). The reaction was allowed to stir at ambient temperature. After 5 h, solvent was removed *in vacuo*, and crude material was reconstituted in DCM (0.310 mL, 0.1 M), and TFA (24 μL, 0.311 mmol) and Et_3SiH (7.5 μL, 0.047 mmol) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a colorless residue (8.9 mg, 81% over three steps). ^{1}H NMR (400 MHz, CD_3OD) δ 5.18 (d, J = 8.6 Hz, 1H), 4.63 (td, J = 6.3, 2.9 Hz, 1H), 4.05 (dd, J = 8.7, 6.8 Hz, 1H), 3.90 (dd, J = 11.8, 2.9 Hz, 1H), 3.71 (dd, J = 11.8, 5.8

Hz, 1H), 3.64 – 3.54 (m, 4H), 3.35 (s, 1H), 2.59 (tt, *J* = 7.1, 3.7 Hz, 1H), 2.00 (h, *J* = 5.6, 5.0 Hz, 4H), 1.84 – 1.73 (m, 2H), 0.71 (qd, *J* = 5.1, 1.1 Hz, 2H), 0.55 – 0.47 (m, 2H). **m/z** 355.3 $(M+H)^+$.

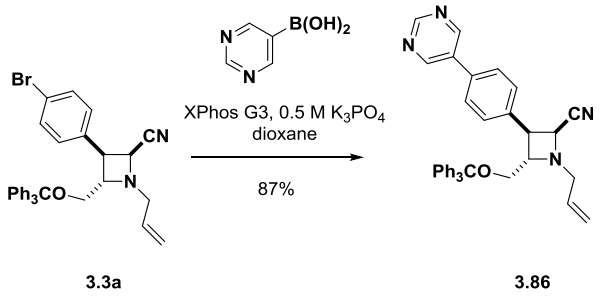


3.3a **3.84**

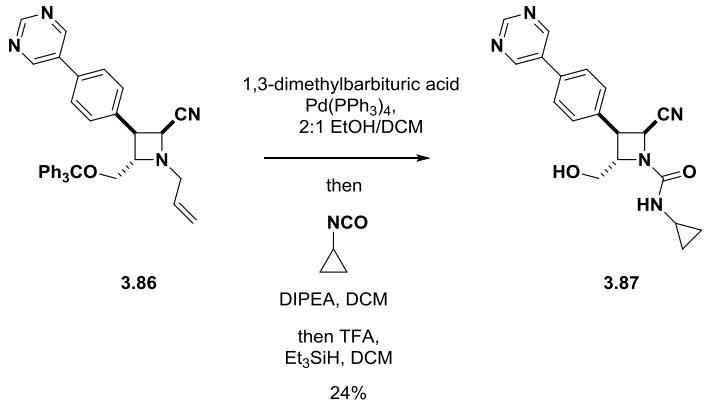
(2*S*,3*S*,4*S*)-1-allyl-3-(4-cyanophenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.84**). To a vial was charged **3.3a** (20 mg, 0.036 mmol), NaCN (3.6 mg, 0.073 mmol), $Pd(PPh_3)_4$ (8.4 mg, 0.007 mmol), and CuI (2.8 mg, 0.015 mmol). The vial was purged 3x with N_2 before the addition of propionitrile (91 µL, 0.4 M), and the mixture was heated at reflux (98 °C) for 24 h. Upon completion, the mixture was quenched by the addition of EtOAc, and solvent was removed *in vacuo*. Purification by column chromatography afforded desired product as a white foaming solid (19 mg, 85%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.71 – 7.66 (m, 2H), 7.44 – 7.37 (m, 8H), 7.35 – 7.24 (m, 9H), 5.76 (dddd, *J* = 17.2, 10.2, 7.0, 5.2 Hz, 1H), 5.34 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dq, *J* = 10.2, 1.3 Hz, 1H), 4.68 (d, *J* = 7.5 Hz, 1H), 3.94 – 3.81 (m, 2H), 3.51 (ddt, *J* = 13.6, 5.2, 1.6 Hz, 1H), 3.44 – 3.27 (m, 3H). **m/z** 496.5 $(M+H)^+$.



(2*S*,3*S*,4*S*)-2-cyano-3-(4-cyanophenyl)-N-cyclopropyl-4-(hydroxymethyl)azetidine-1-carboxamide (**3.85**). To a solution of **3.84** (19 mg, 0.038 mmol) in EtOH (0.511 mL) and DCM (0.256 mL) was added 1,3-dimethylbarbituric acid (9 mg, 0.058 mmol) and $Pd(PPh_3)_4$ (4.4 mg, 0.008 mmol). The reaction was heated to 40 °C for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated product. This material was then directly dissolved in DCM (0.380 mL, 0.1 M), followed by the addition of DIPEA (33 μL, 0.190 mmol) and cyclopropylisocyanate (3.8 mg, 0.046 mmol). The reaction was allowed to stir at ambient temperature. After 4 h, solvent was removed *in vacuo*, and crude material was reconstituted in DCM (0.380 mL, 0.1 M), and TFA (29 μL, 0.381 mmol) and Et_3SiH (9 μL, 0.057 mmol) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a colorless residue (8.4 mg, 75% over three steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.74 – 7.69 (m, 2H), 7.51 – 7.45 (m, 2H), 6.24 (s, 1H), 5.07 (d, J = 8.6 Hz, 1H), 4.76 – 4.67 (m, 1H), 4.01 – 3.91 (m, 1H), 3.89 – 3.67 (m, 4H), 2.63 (dh, J = 6.7, 3.2 Hz, 1H), 0.77 – 0.70 (m, 2H), 0.57 – 0.50 (m, 2H). **m/z** 296.9 $(M+H)^+$.

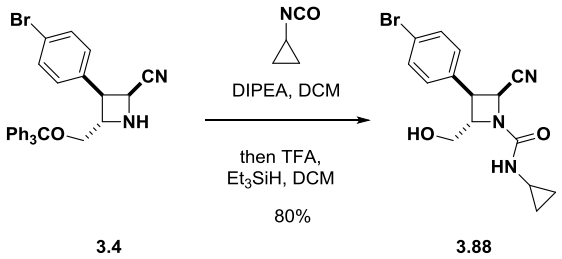


(2*S*,3*S*,4*S*)-1-allyl-3-(4-(pyrimidin-5-yl)phenyl)-4-((trityloxy)methyl)azetidine-2-carbonitrile (**3.86**). Prepared according to modified **General Procedure H** from **3.3a** (15 mg). Dioxane replaced THF as solvent, and mixture was heated to 100 °C for 1 h. Purification by column chromatography provided product as a white foaming solid (13 mg, 87%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.98 (s, 2H), 7.64 – 7.57 (m, 2H), 7.50 – 7.45 (m, 2H), 7.45 – 7.39 (m, 6H), 7.34 – 7.23 (m, 10H), 5.79 (dddd, *J* = 17.2, 10.3, 7.0, 5.1 Hz, 1H), 5.36 (dq, *J* = 17.1, 1.6 Hz, 1H), 5.20 (dd, *J* = 10.5, 1.7 Hz, 1H), 4.75 – 4.65 (m, 1H), 3.94 (t, *J* = 4.4 Hz, 2H), 3.57 (ddt, *J* = 13.9, 5.0, 1.7 Hz, 1H), 3.43 – 3.28 (m, 3H). **m/z** 548.9 $(M+H)^+$.



(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)-3-(4-(pyrimidin-5-yl)phenyl)azetidine-1-carboxamide (**3.87**). To a solution of **3.86** (13 mg, 0.024 mmol) in EtOH (0.316 mL) and DCM (0.158 mL) was added 1,3-dimethylbarbituric acid (5.6 mg, 0.036 mmol) and $Pd(PPh_3)_4$ (2.4 mg,

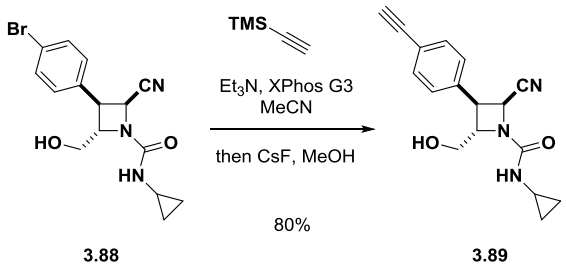
0.002 mmol). The reaction was heated to 40 °C for 16 h. Upon completion, the solvent was removed *in vacuo* and crude material briefly purified by flash column chromatography to give deallylated product. This material was then directly dissolved in DCM (0.240 mL, 0.1 M), followed by the addition of DIPEA (21 μL, 0.120 mmol) and cyclopropylisocyanate (2.4 mg, 0.029 mmol). The reaction was allowed to stir at ambient temperature. After 6 h, solvent was removed *in vacuo*, and crude material was reconstituted in DCM (0.240 mL, 0.1 M), and TFA (18 μL, 0.240 mmol) and Et_3SiH (6 μL, 0.036 mmol) were added to the solution. After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a colorless residue (2.0 mg, 24% over three steps). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ 9.17 (s, 1H), 8.98 (s, 2H), 7.71 – 7.66 (m, 2H), 7.57 – 7.50 (m, 2H), 5.97 (s, 1H), 5.04 (d, *J* = 8.6 Hz, 1H), 4.75 (td, *J* = 7.9, 2.3 Hz, 1H), 3.92 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.88 – 3.80 (m, 2H), 2.64 (tdd, *J* = 6.8, 4.8, 2.8 Hz, 1H), 0.75 – 0.69 (m, 2H), 0.52 (dq, *J* = 3.8, 1.9 Hz, 2H). **m/z** 350.4 $(M+H)^+$.



3.4 3.88

(2*S*,3*S*,4*S*)-3-(4-bromophenyl)-2-cyano-N-cyclopropyl-4-(hydroxymethyl)azetidine-1-carboxamide (**3.88**). To a solution of **3.4** (125 mg, 0.245 mmol) in DCM (3.3 mL, 0.075 M) was added DIPEA (0.427 mL, 2.454 mmol) and cyclopropylisocyanate (25 mg, 0.294 mmol). The reaction was stirred at ambient temperature for 2 h. Upon completion, volatiles were removed *in*

vacuo, and crude material was reconstituted in DCM (3.3 mL, 0.075 M), followed by the addition of TFA (0.188 mL, 2.454 mmol) and Et_3SiH (59 µL, 0.368 mmol). After 15 min at ambient temperature, solvent was removed *in vacuo* and crude material purified by column chromatography (MeOH/DCM) to afford desired product as a white foaming solid (69 mg, 80% over two steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 – 7.52 (m, 2H), 7.26 – 7.22 (m, 2H), 5.75 (s, 1H), 5.01 (d, *J* = 8.6 Hz, 1H), 4.70 (td, *J* = 7.7, 2.1 Hz, 1H), 3.92 (dd, *J* = 11.6, 2.2 Hz, 1H), 3.83 (dd, *J* = 11.5, 7.8 Hz, 1H), 3.73 (t, *J* = 8.0 Hz, 1H), 2.71 – 2.61 (m, 1H), 0.77 (dtd, *J* = 8.1, 4.6, 3.9, 2.6 Hz, 2H), 0.59 – 0.51 (m, 2H). **m/z** 352.4 $(M+H)^+$.



(2*S*,3*S*,4*S*)-2-cyano-N-cyclopropyl-3-(4-ethynylphenyl)-4-(hydroxymethyl)azetidine-1-carboxamide (**3.89**). To a solution of **3.88** (8 mg, 0.023 mmol) in MeCN (0.228 mL, 0.1 M) was added trimethylsilylacetylene (16 µL, 0.115 mmol), XPhos Pd G3 (2.9 mg, 0.003 mmol), and Et_3N (0.080 mL, 0.571 mmol). The mixture was heated to and stirred at 70 °C for 3 h. Upon completion, the mixture was quenched with saturated brine and extracted 3x with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting crude material was reconstituted in MeOH (70 µL, 0.25 M) before the addition of CsF (3.9 mg, 0.026 mmol). The mixture was heated to and stirred at 40 °C for 2 h. Upon completion, solvent was removed *in vacuo*, and crude material purified by column chromatography

(MeOH/DCM) to afford desired product as a white foaming solid (4.1 mg, 80%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.51 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 5.15 (d, J = 8.6 Hz, 1H), 4.62 (td, J = 6.4, 2.7 Hz, 1H), 3.99 (dd, J = 8.6, 7.0 Hz, 1H), 3.89 (dd, J = 11.9, 2.8 Hz, 1H), 3.70 (dd, J = 11.9, 5.9 Hz, 1H), 3.52 (s, 1H), 3.31 (p, J = 1.5 Hz, 1H), 2.59 (tt, J = 7.2, 3.8 Hz, 1H), 0.71 (dt, J = 6.6, 3.2 Hz, 2H), 0.54 – 0.46 (m, 2H). **m/z** 295.8 $(M+H)^+$.

5.4 Supporting data for Chapter 4

5.4.1 Reaction screening

General Procedure J: Synthesis of Directing Group Substrate

To a reaction vessel containing carboxylic acid (1.0 equiv, >99% ee) in DMF (0.4 M) was added *N,N*-diisopropylethylamine (2.0 equiv), followed by ethyl trifluoroacetate (5.0 equiv). The reaction mixture was heated to 40 ºC for 16 h, after which point excess reagents were removed *in vacuo*. The resulting crude material was dissolved in DMF (0.25 M) and cooled to 0 ºC before the addition of HATU (1.0 equiv), 8-aminoquinoline (1.0 equiv), and *N,N*-diisopropylethylamine (0.95 equiv) in sequence. The reaction mixture was maintained at 0 º C for 2 h. Upon completion, the reaction mixture was diluted with EtOAc and washed with saturated brine (1x) and with water (2x). The resulting organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided the desired directing group substrate. The products **(−)-4.15a** and **(+)-4.15a** were examined by analytical supercritical fluid chromatography (SFC) to ensure retention of enantiopurity.

SFC Method 1

Mobile phase A consisted of supercritical carbon dioxide, while mobile phase B consisted of isopropanol. The gradient ran from 3% to 50% mobile phase B over 5.0 min at 1.5 mL/min, followed by a 1.5 minute hold at 50% B before returning to starting conditions. A ChiralPak IC, 4.6x250 mm column was used with column temperature maintained at 45 °C. 1-2 mg of the appropriate compound was dissolved in 1 mL methanol, and 5 μL of this solution was injected.

General Procedure K: C-H Arylation

GENERAL NOTE: A sealed one dram vial was used for reactions ~0.3 mmol (reflux condenser is not necessary). A 250 mL round bottom flask was used for reactions ~ 3.0 mmol and a 500 mL round bottom flask was used for 30 mmol scale reactions (all reactions were under a reflux condenser).

A reaction vessel was charged with directing group substrate (1.0 equiv), dibenzyl phosphate (20 mol%), AgOAc (2.0 equiv), aryl iodide (3.0 equiv), and $Pd(OAc)_2$ (10 mol%). The reaction vessel was evacuated and refilled with inert atmosphere (N_2, 3x) before the addition of DCE (1.0 M), then placed in a pre-heated 110 ºC heat plate for 24 h. The reaction mixture was then allowed to cool to ambient temperature before the addition of 7 N NH_3 in MeOH solution (3 mL of solution per 0.2 mmol reactant). The mixture was allowed to stir at ambient temperature for 3 h before after which the reaction was concentrated *in vacuo.* Purification by flash column chromatography (EtOAc/hexanes) to afforded the desired product.

NOTE: filtering over Celite is an optional step to monitor conversion after C-H arylation reaction; crude reaction after 24 h can immediately be subjected to sequence (single flask) below.

For reactions containing < 3.0 mmol of reactant, crude arylation material from above was dissolved in 7 N NH_3 in MeOH solution (3 mL of solution per 0.2 mmol reactant). The mixture was allowed to stir at ambient temperature for 3 h before solvent removal *in vacuo*, and crude material was purified by flash column chromatography under the specified conditions.

For reactions containing > 3.0 mmol of reactant, crude arylation material from above was dissolved in 9:1 MeOH/H_2O solution (8 mL of solution per 1 mmol reactant), followed by the addition of K_2CO_3 (3.0 equiv). The mixture was allowed to stir at ambient temperature for up to 16 h before solvent removal *in vacuo*. The resulting crude material was dissolved in dichloromethane, filtered over Celite and concentrated *in vacuo.* The resulting crude material was dissolved in 3:7 *i*-PrOH/$CHCl_3$, washed with brine (1x) and with water (1x). The resulting organic layer was dried over Na_2SO_4, filtered, concentrated *in vacuo* before purification by flash column chromatography under the specified conditions.

General Procedure L: Functionalization of C-H arylation products for determination of enantiopurity. Racemic samples (internal standard) of C-H arylation products were inseparable on SFC, so they were functionalized further to enable separation.

Derivatization of racemic C-H arylation products for SFC:

Racemic starting material (1.0 equiv) was added to a reaction vessel and dissolved in dichloromethane (0.05 M), then ethyl isocyanate (1.2 equiv) was added and the reaction mixture was stirred for 1 h at room temperature. Methanol was added to the reaction mixture and the resulting crude material was concentrated *in vacuo* before purification by flash column chromatography under the specified conditions.

Derivatization of enantiopure C-H arylation products for SFC:

Enantiopure starting material (1-2 mg, 1.0 equiv) was added to a reaction vessel and dissolved in dichloromethane (~ 100 μL), then ethyl isocyanate (~1.2 equiv) was added and the reaction mixture was stirred for 20 minutes at room temperature. Methanol was added to the reaction mixture and the resulting crude material was concentrated *in vacuo*. The resulting functionalized CH arylation products **4.17a-Urea** and **4.17f-Urea** were examined by analytical supercritical fluid chromatography (SFC) in order to ensure retention of enantiopurity.

SFC Method 2

Mobile phase A consisted of supercritical carbon dioxide, while mobile phase B consisted of 0.05% triethylamine in methanol. The gradient ran from 3% to 50% mobile phase B over 5.0 min at 1.5 mL/min, followed by a 1.5 minute hold at 50% B before returning to starting conditions. A ChiralPak AS-H, 4.6×250 mm column was used with column temperature maintained at 45 °C. 1-2 mg of the appropriate compound was dissolved in 1 mL MeOHl, and 5 μL of this solution was injected.

General Procedure M: Directing Group Cleavage (*cis*-Stereochemistry)

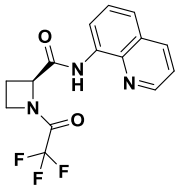
4.17f (1.0 equiv) was added to a round bottom flask and dissolved in MeCN (0.2 M). Boc_2O (3 equiv) was added, and the reaction mixture was heated to 50 ºC and stirred for 15 minutes, then DMAP (0.1 equiv) was added, and the reaction mixtures was stirred for another 2 h at 50 ºC. The solvent was removed *in vacuo* and the residue was dissolved in THF/H_2O (2:1, 0.1 M). After cooling the biphasic mixture to 0 ºC, 30% aq. H_2O_2 (10 equiv) was added followed by LiOH (6 equiv). The reaction mixture was allowed to reach ambient temperature, stirred for 1 h and then

heated to 50 ºC for 20 h. The reaction was allowed to reach ambient temperature and diluted with EtOAc, saturated aqueous sodium sulfite was added (reaction mixture was tested by peroxide test strips) and stirred for 15 min. The reaction mixture was separated, and the aqueous layer was washed with EtOAc (2x). The resulting aqueous solution was acidified with 1 M HCl, exhaustively extracted with 3:7 *i*-PrOH/$CHCl_3$ (5x) and dried over sodium sulfate. Concentration *in vacuo* gave **4.22** as a colorless solid which was used directly in the following step without further purification.

General Procedure N: Directing Group Cleavage (*trans*-Stereochemistry)

4.17f (1.0 equiv) was added to a round bottom flask and dissolved in MeCN (0.5 M). Boc_2O (1.2 equiv) was added, and the reaction mixture was heated to 50 ºC and stirred for 15 min. Upon complete Boc protection, the solvent was removed *in vacuo* and residue was reconstituted in EtOH (0.2 M) before the addition of solid NaOH (10 equiv). The reaction was heated to 110 ºC and stirred for 15 min (*note: reaction time is critical*). The mixture was then diluted with water and washed with DCM (2x). The resulting aqueous solution was acidified with 1 M HCl, exhaustively extracted with 3:7 *i*-PrOH/$CHCl_3$ (5x), and dried over sodium sulfate. Concentration *in vacuo* gave trans acid **4.23** as a pale brown residue.

Preparation of Directing Group Substrates



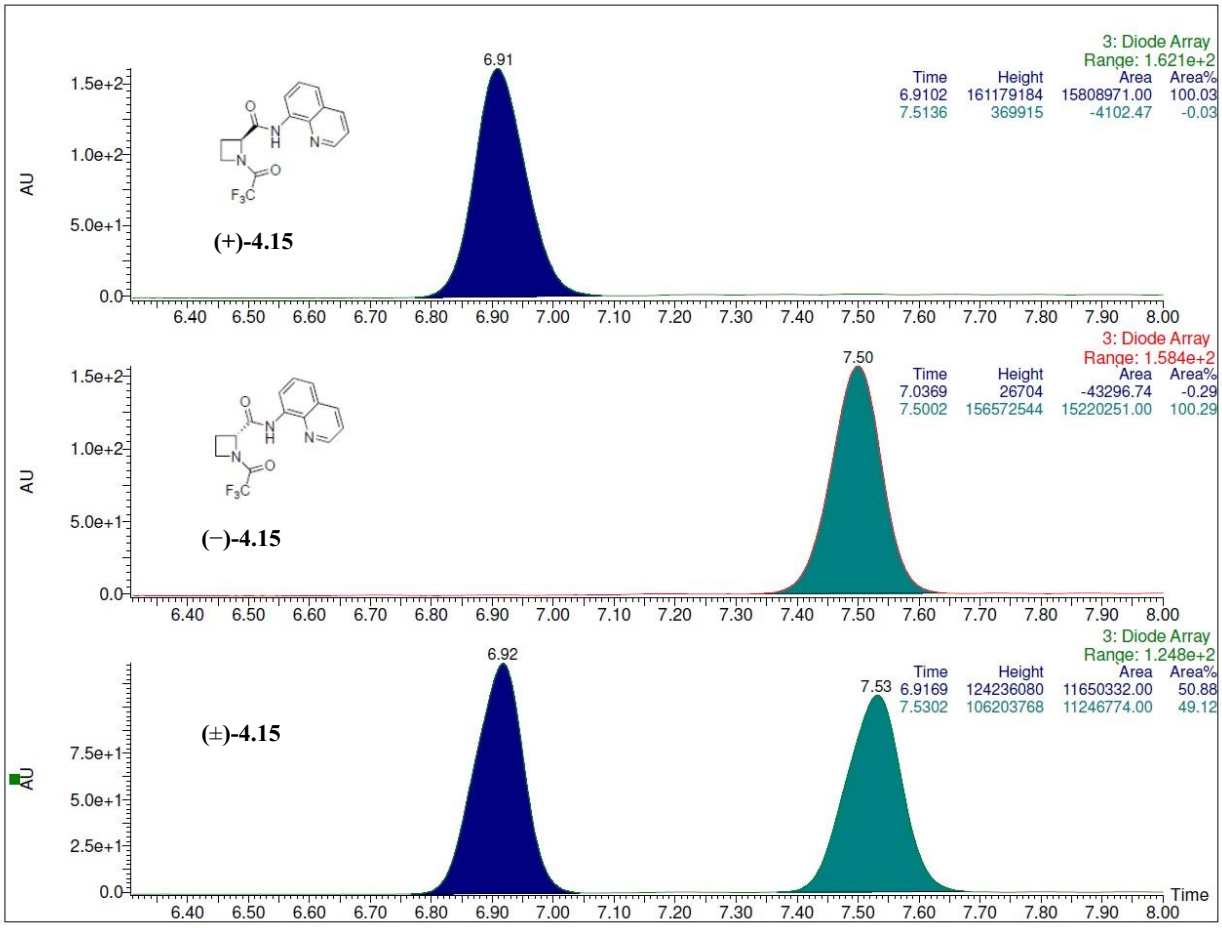
4.15a

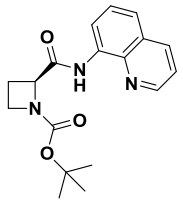
(*S*)-*N*-(quinolin-8-yl)-1-(2,2,2-trifluoroacetyl)azetidine-2-carboxamide (−)-(**4.15a**)

(*R*)-*N*-(quinolin-8-yl)-1-(2,2,2-trifluoroacetyl)azetidine-2-carboxamide (+)-(**4.15a**)

Prepared according to **General Procedure J** from 1.00 g of *(S)*-azetidine-2-carboxylic acid or 10.0 g of *(R)*-azetidine-2-carboxylic acid. Purification by flash column chromatography (EtOAc/hexanes) provided 2.34 g (73%) of the desired product (−)-**4.15a** and 26.3 g (82%) of the desired product (+)-**4.15a** as brown amorphous solids (~4.4:1 mixture of rotamers in $CDCl_3$). **R_f** 0.21 (35% EtOAc in hexanes). **$[\alpha]^{22}_D$** = −123.2 (c = 1.0, $CHCl_3$) (−)-**4.15a**. **$[\alpha]^{23}_D$** = +121.9 (c = 1.0, $CHCl_3$) (+)-**4.15a**. **IR** (thin film) ν_{max} 3319, 1690, 1533, 1488, 1426, 1323, 1246, 1198, 1152, 827, 792, 756. **^{1}H NMR** (400 MHz, $CDCl_3$, both rotamers) δ 10.55 (s, 1H), 8.82 (ddd, *J* = 8.6, 4.2, 1.7 Hz, 1H), 8.79 – 8.72 (m, 1H), 8.16 (ddd, *J* = 15.1, 8.3, 1.7 Hz, 1H), 7.60 – 7.39 (m, 3H), 5.36 – 5.11 (m, 1H), 4.65 – 4.18 (m, 2H), 3.03 – 2.44 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$, both rotamers) δ 167.3, 166.7, 157.5 (q, *J* = 38.0 Hz), 148.8, 148.7, 138.8, 138.5, 136.6, 136.3, 134.0, 133.5, 128.1, 127.4, 127.3, 122.7, 122.6, 122.0, 121.9, 117.6, 117.2, 117.1, 114.7, 66.1, 63.2, 51.0, 50.93, 50.90, 50.88, 48.2, 22.9, 20.8. **^{19}F NMR** (376 MHz, $CDCl_3$, both rotamers) δ -72.1, -72.7. **HRMS** (ESI^+) **m/z** calculated for $C_{15}H_{13}F_3N_3O_2$ $[M+H]^+$ 324.0960; Found 324.0958

Retention of enantiopurity was determined by SFC Method 1, λ = 210 nm, T_{rac} = 6.92 and 7.53 min.



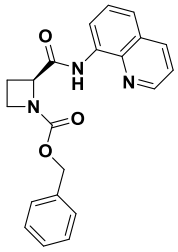


4.15b

(*S*)-tert-butyl 2-(quinolin-8-ylcarbamoyl)azetidine-1-carboxylate (**4.15b**). To a reaction vessel containing *(S)*-azetidine-2-carboxylic acid (50 mg, 0.50 mmol) in saturated $NaHCO_3$ (0.74 mL) at 0 ºC was added a solution of Boc_2O (119 mg, 0.54 mmol) in THF (0.25 mL). The reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. The mixture was brought to pH = 1 with 1 M HCl, further diluted with brine, and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The resulting residue was dissolved in DMF (2.5 mL, 0.2 M) before the addition of *N,N*-diisopropylethylamine (0.26 mL, 3.0 equiv), HATU (207 mg, 1.2 equiv), and 8-aminoquinoline (78 mg, 1.1 equiv). The reaction mixture was stirred at ambient temperature for 2 h. Upon completion, the reaction mixture was diluted with brine (3x) and extracted with EtOAc (3x). The combined organic layers were washed with brine (3x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided 150 mg (87%) of the desired product as a light brown oil. **R_f** 0.44 (35% EtOAc in hexanes). **$[\alpha]^{25}_D$** = −217.9 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3333, 2975, 1685, 1530, 1488, 1389, 1366, 1258, 1131, 855, 792, 758. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 8.94 – 8.69 (m, 2H), 8.16 (d, *J* = 8.2 Hz, 1H), 7.55 (d, *J* = 6.0 Hz, 2H), 7.45 (dd, *J* = 8.4, 4.2 Hz, 1H), 4.85 (dd, *J* = 9.7, 6.7 Hz, 1H), 4.09 – 3.91 (m, 2H), 2.65 (tt, *J* = 9.6, 5.0 Hz, 1H), 2.56 – 2.43 (m, 1H), 1.49 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.34, 156.98, 148.40, 138.85, 136.17, 134.22, 128.00,

127.23, 122.03, 121.66, 116.65, 80.96, 63.29, 46.86, 28.25, 21.09. **HRMS** (ESI^+) **m/z** calculated for $C_{18}H_{21}N_3O_3$ $[M+H]^+$ 328.1661; Found 328.1656

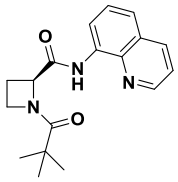


4.15c

(*S*)-benzyl 2-(quinolin-8-ylcarbamoyl)azetidine-1-carboxylate (**4.15c**). To a reaction vessel containing *(S)*-azetidine-2-carboxylic acid (200 mg, 1.97 mmol) in a 1:1 mixture of THF and saturated aqueous $NaHCO_3$ (4 mL) at 0 ºC was added benzyl chloroformate (0.34 mL, 1.2 equiv) dropwise. The reaction mixture was allowed to warm to ambient temperature and stirred for 3 h. The mixture was brought to pH = 1 with 1 M HCl, further diluted with saturated brine, and extracted with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The resulting residue was dissolved in DMF (5 mL, 0.4 M) before the addition of *N,N*-diisopropylethylamine (1.03 mL, 3.0 equiv), HATU (970 mg, 1.3 equiv), and 8-aminoquinoline (369 mg, 1.3 equiv). The reaction mixture was stirred at ambient temperature for 3 h. Upon completion, the reaction mixture was diluted with brine and extracted with EtOAc (3x). The combined organic layers were washed with brine (3x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided 392 mg (55%) of the desired product as a light brown oil. $\mathbf{R_f}$ 0.46 (35% EtOAc in hexanes). $\mathbf{[\alpha]^{23}_D}$ = −259.77 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3331, 2962, 1711, 1686, 1531, 1488, 1400, 1344, 1129, 827, 792, 755, 698. ^{1}**H NMR** (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 8.82 (dd, *J* =

5.9, 3.1 Hz, 1H), 8.73 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.15 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.43 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.38 – 7.27 (bs, 2H), 7.19 (bs, 3H), 5.33 – 5.14 (m, 2H), 4.94 (dd, *J* = 9.5, 6.4 Hz, 1H), 4.18 – 3.99 (m, 2H), 2.68 (dtd, *J* = 11.8, 9.2, 5.6 Hz, 1H), 2.56 (ddt, *J* = 11.8, 8.8, 6.8 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 157.3, 148.5, 138.8, 136.2, 136.2, 134.1, 128.4, 128.03, 127.96, 127.9, 127.2, 122.1, 121.6, 116.7, 67.3, 63.5, 47.5, 21.2. **HRMS** (ESI^+) **m/z** calculated for $C_{21}H_{20}N_3O_3$ $[M+H]^+$ 362.1505; Found 362.1501

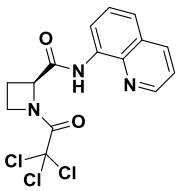


4.15d

(*S*)-1-pivaloyl-*N*-(quinolin-8-yl)azetidine-2-carboxamide (4.15d). To a reaction vessel containing *(S)*-azetidine-2-carboxylic acid (50 mg, 0.50 mmol) in deionized H_2O (0.11 mL) at 0 ºC was added a solution of NaOH (40 mg) in H_2O (0.22 mL), followed by tetra-*n*-butylammonium bromide (8.0 mg, 0.05 equiv). This mixture was stirred for 10 min before the addition of a solution of pivaloyl chloride (0.067 mL, 1.1 equiv) in DCM (0.17 mL) was added dropwise. The reaction was allowed to warm to ambient temperature and stirred for 18 h. Upon completion, the reaction was diluted with DCM and brought to pH = 1 with 1 M HCl. The mixture was extracted with DCM (3x), organic layers were combined, and solvent removed *in vacuo*.

The resulting residue was dissolved in DMF (2.5 mL, 0.2 M) before the addition of *N,N*-diisopropylethylamine (0.26 mL, 3.0 equiv), HATU (207 mg, 1.2 equiv), and 8-aminoquinoline (78 mg, 1.1 equiv). The reaction mixture was stirred at ambient temperature for 2 h. Upon completion, the reaction mixture was diluted with brine and extracted with EtOAc (3x). The combined organic layers were washed with brine (3x), dried over Na_2SO_4, filtered, and

concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided 95 mg (62%) of the desired product as a white solid. $\mathbf{R_f}$ 0.27 (35% EtOAc in hexanes). $\mathbf{[\alpha]^{24}_D}$ = −195.13 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3333, 2968, 1678, 1630, 1530, 1485, 1426, 1403, 1377, 1362, 1328, 1240, 831, 796, 758. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.66 (s, 1H), 8.77 – 8.70 (m, 2H), 8.06 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.51 – 7.41 (m, 2H), 7.36 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.00 (dd, *J* = 9.7, 5.9 Hz, 1H), 4.38 (dtd, *J* = 28.8, 8.5, 6.1 Hz, 2H), 2.63 (dtd, *J* = 11.8, 9.4, 6.0 Hz, 1H), 2.50 (ddt, *J* = 11.9, 9.0, 6.1 Hz, 1H), 1.29 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 179.4, 169.5, 148.2, 138.8, 136.1, 134.3, 127.9, 127.1, 121.8, 121.5, 116.6, 63.0, 51.5, 38.8, 27.2, 20.9. **HRMS** (ESI^+) **m/z** calculated for $C_{14.17h22}N_3O_2$ $[M+H]^+$ 312.1712; Found 312.1709

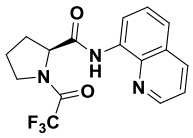


4.15e

(*S*)-*N*-(quinolin-8-yl)-1-(2,2,2-trichloroacetyl)azetidine-2-carboxamide (**4.15e**). To a reaction vessel containing *(S)*-azetidine-2-carboxylic acid (100 mg, 0.99 mmol) and DMAP (190 mg, 1.6 equiv) in 2:1 mixture of MeCN/DMF (6.0 mL, 0.2 M) was added trichloroacetyl chloride (0.22 mL, 2.0 equiv) dropwise at ambient temperature. After 3 h, the mixture was diluted with EtOAc, brought to pH = 1 with 1 M HCl, and extracted with EtOAc (3x). The combined organic layers were washed with brine (3x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The resulting residue was dissolved in DMF (3.0 mL, 0.3 M) before the addition of *N,N*-diisopropylethylamine (0.25 mL, 1.3 equiv), HATU (382 mg, 1.2 equiv), and 8-aminoquinoline (466 mg, 1.3 equiv). The reaction mixture was stirred at ambient temperature for 3 h. Upon completion, the reaction mixture was diluted with brine and extracted with EtOAc (3x). The

combined organic layers were washed with brine (3x), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided 141 mg (38%) of the desired product as a white solid. **R_f** 0.31 (35% EtOAc in hexanes). **$[\alpha]^{24}_D$** = −85.33 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3322, 1678, 1532, 1487, 1393, 1324, 843, 827, 811, 792, 758, 668. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.60 (s, 1H), 8.82 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.78 (q, *J* = 4.5 Hz, 1H), 8.16 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.59 – 7.53 (m, 2H), 7.46 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.19 (dt, *J* = 10.6, 3.8 Hz, 1H), 4.72 (dq, *J* = 32.9, 8.4, 7.9 Hz, 2H), 2.91 – 2.62 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 167.9, 161.9, 149.2, 139.4, 136.9, 134.6, 128.6, 127.9, 123.0, 122.5, 117.6, 92.0, 64.9, 54.4, 21.5. **HRMS** (ESI^+) **m/z** calculated for $C_{15}H_{13}Cl_3N_3O_2$ $[M+H]^+$ 372.0073; Found 372.0071



4.18

(*S*)-*N*-(quinolin-8-yl)-1-(2,2,2-trifluoroacetyl)pyrrolidine-2-carboxamide (**4.18**). Prepared according to **General Procedure J** from 0.50 g of *(S)*-pyrrolidine-2-carboxylic acid (L-proline). Purification by flash column chromatography (EtOAc/hexanes) provided 1.02 g (70%) of the desired product as a brown amorphous solid (~5.7:1 mixture of rotamers in $CDCl_3$).

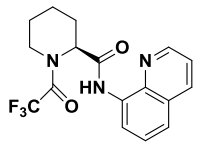
R_f 0.25 (35% EtOAc in hexanes)

$[\alpha]^{25}_D$ = −50.5 (c = 1.0, $CHCl_3$)

IR (thin film) ν_{max} 3331, 2958, 1686, 1530, 1487, 1457, 1426, 1350, 1234, 1205, 1147, 827, 792, 758.

^{1}H NMR (400 MHz, $CDCl_3$, both rotamers) δ 10.26 – 10.09 (m, 1H), 8.78 (m, 1H), 8.68, (m, 1H), 8.12 (m, 1H), 7.53 – 7.40 (m, 3H), 4.92 – 4.82 (m, 1H), 3.97 (m, 1H), 3.82 (m, 1H), 2.36 –

2.23 (m, 3H), 2.08 (m, 1H).**^{13}C NMR** (100 MHz, $CDCl_3$, both rotamers) δ 168.8, 168.2, 156.4 (q, *J* = 37.6 Hz), 148.6, 148.4, 138.5, 138.4, 136.4, 136.3, 133.9, 133.4, 127.90, 127.87, 127.18, 127.15, 122.3, 122.1, 116.7, 116.6, 116.1 (q, *J* = 287 Hz), 62.7, 61.9, 48.4, 47.5 (q, *J* = 3.4 Hz), 32.5, 28.8, 25.1, 21.3.**^{19}F NMR** (376 MHz, $CDCl_3$, both rotamers) δ -72.4, -71.4. **HRMS** (ESI^+) **m/z** calculated for $C_{16}H_{15}F_3N_3O_2$ $[M+H]^+$ 338.1116; Found 338.1114

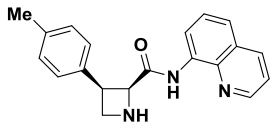


4.20

(*S*)-*N*-(quinolin-8-yl)-1-(2,2,2-trifluoroacetyl)piperidine-2-carboxamide (**4.20**). A reaction vessel containing *(S)*-piperidine-2-carboxylic acid (250 mg, 1.0 equiv) in MeOH (2 M) was added *N,N*-diisopropylethylamine (2.0 equiv), followed by ethyl trifluoroacetate (5.0 equiv). The reaction mixture was heated to 40 ºC for 16 h, after which point excess reagents were removed *in vacuo*. The resulting crude oil was dissolved in DMF (0.25 M) and cooled to 0 ºC before the addition of HATU (1.0 equiv), 8-aminoquinoline (1.0 equiv), and *N,N*-diisopropylethylamine (0.95 equiv) in sequence. The reaction mixture was maintained at 0 ºC for 2 h. Upon completion, the reaction mixture was diluted with EtOAc and washed with brine (1x), water (2x), and saturated sodium bicarbonate (1x). The resulting organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexanes) provided 0.450 g (70%) of the desired product as a pale yellow amorphous solid (~4.9:1 mixture of rotamers in $CDCl_3$). **R_f** 0.39 (35% EtOAc in hexanes). **$[\alpha]^{25}_D$** = −124.0 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3331, 2946, 1686, 1531, 1487, 1449, 1327, 1210, 1192, 1145, 1007, 849, 827, 757. **^{1}H NMR** (400 MHz, $CDCl_3$, both rotamers) δ 10.31 (s, 1H), 8.83 – 8.64 (m, 2H), 8.13 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.43 (dt, *J* = 8.2, 4.0 Hz, 1H), 5.59 – 4.93 (m, 1H), 4.07 (ddd, *J* = 13.3,

4.1, 2.0 Hz, 1H), 3.50 (td, *J* = 14.1, 13.5, 2.7 Hz, 1H), 2.73 – 2.49 (m, 1H), 1.96 – 1.46 (m, 5H). **^{13}C NMR** (100 MHz, $CDCl_3$, both rotamers) δ 167.07, 166.71, 157.3 (q, *J* = 37.6 Hz), 148.63, 148.42, 138.34, 136.45, 136.33, 133.68, 133.53, 127.85, 127.15, 122.31, 122.05, 121.82, 121.76, 118.06, 116.60, 116.45, 115.20, 58.10, 55.14, 44.35, 44.31, 44.27, 44.24, 41.92, 27.46, 26.00, 25.32, 24.61, 20.42, 20.32. **^{19}F NMR** (376 MHz, $CDCl_3$, both rotamers) δ -68.4, -68.8. HRMS (ESI+) **m/z** Calculated for $C_{17}H_{17}F_3N_3O_2$ $[M+H]^+$ 352.1273; Found 352.1267.

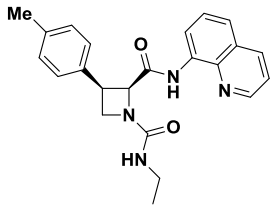
5.4.2 Reaction scope



4.17a

(2*S*,3*R*)-*N*-(quinolin-8-yl)-3-(*p*-tolyl)azetidine-2-carboxamide (**4.17a**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a** for product **4.17a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 44.8 mg (70%) of the desired product **4.17a** as an amorphous solid. **R_f** 0.23 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24}_D$** = +162.7 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3286, 2878, 1676, 1523, 1486, 1460, 1425, 1325, 826, 792, 756. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.32 (s, 1H), 8.92 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.50 (dd, *J* = 7.2, 1.5 Hz, 1H), 8.11 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.45 – 7.36 (m, 5H), 6.90 (d, *J* = 7.9 Hz, 2H), 4.93 (d, *J* = 9.1 Hz, 1H), 4.29 – 4.18 (m, 2H), 3.73 (dd, *J* = 7.4, 4.0 Hz, 1H), 2.61 (s, 1H), 2.11 (s, 3H). Azetidine NH not visible. **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.0, 148.6, 139.2, 136.5, 136.2, 135.9, 134.0, 129.0, 128.1, 127.9, 127.3, 121.7, 121.5, 116.6, 65.0, 50.2, 43.0, 21.0. **HRMS** (ESI^+) **m/z** calculated for $C_{20}H_{20}N_3O$ $[M+H]^+$ 318.1606; Found 318.1603

Functionalization for SFC

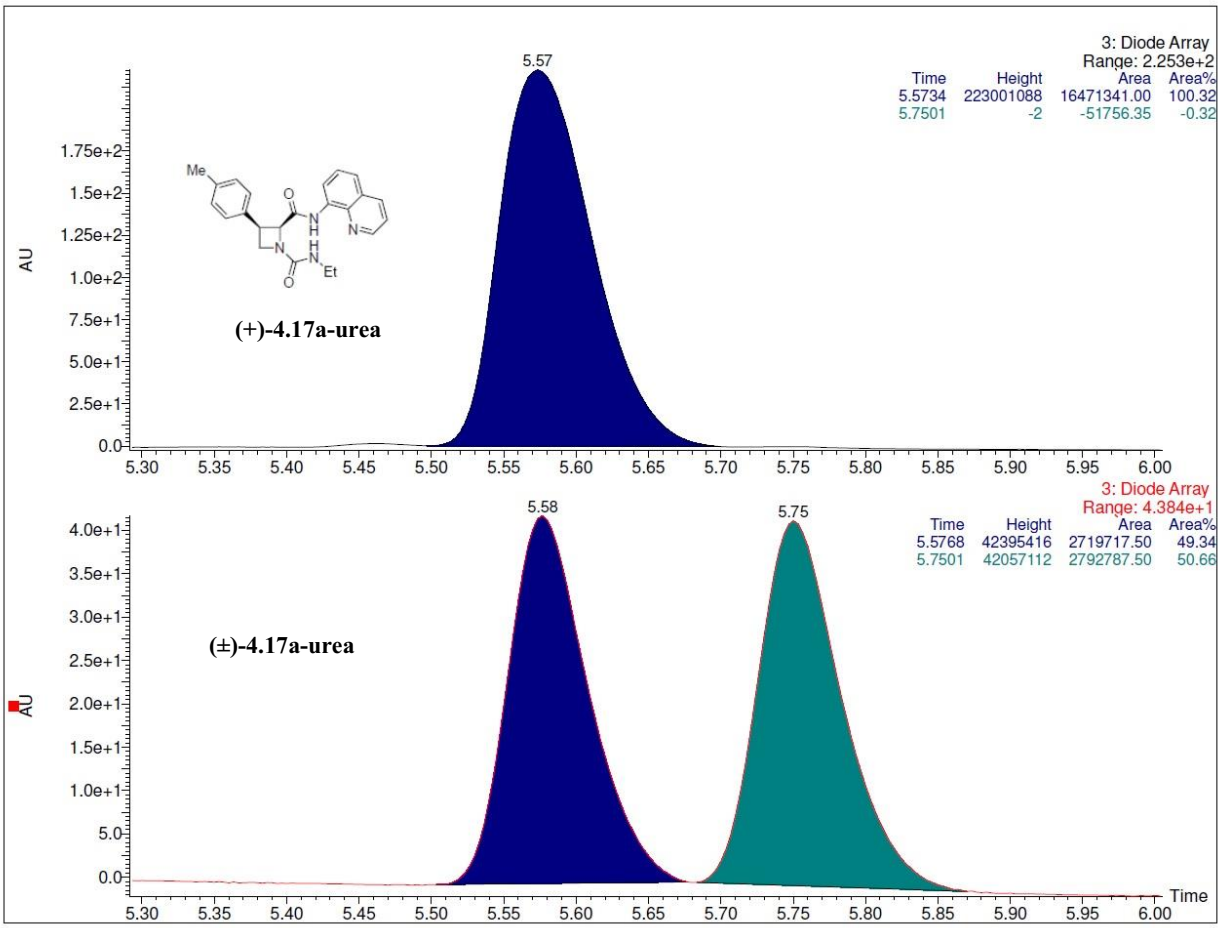


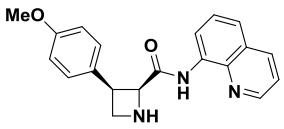
4.17a-urea

(±)-(Cis)-N^1-ethyl-N^2-(quinolin-8-yl)-3-(*p*-tolyl)azetidine-1,2-dicarboxamide (**(±)-4.17a-Urea**).

Prepared according to **General Procedure L** from 15.8 mg of racemic **4.17a** to give racemic **4.17a-Urea**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 14.7 mg (76%) of the desired product **4.17a-Urea** as residue. $\mathbf{R_f}$ 0.72 (10% MeOH in CH_2Cl_2). **IR** (thin film) ν_{max} 3324, 1654, 1524, 1486, 1425, 1369, 1326, 826, 792, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.49 (s, 1H), 8.81 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.41 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.12 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.50 – 7.37 (m, 3H), 7.31 (d, *J* = 8.1 Hz, 2H), 6.94 (d, *J* = 7.8 Hz, 2H), 5.14 (d, *J* = 9.2 Hz, 1H), 4.99 (t, *J* = 5.7 Hz, 1H), 4.55 – 4.45 (m, 1H), 4.15 – 4.05 (m, 2H), 3.40 – 3.25 (m, 2H), 2.13 (s, 3H), 1.17 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 167.8, 160.7, 148.6, 138.9, 137.3, 136.2, 134.6, 133.5, 129.4, 127.98, 127.9, 127.2, 122.2, 121.7, 116.9, 68.4, 53.4, 38.1, 35.5, 21.1, 15.7. **HRMS** (ESI^+) **m/z** calculated for $C_{23}H_{25}N_4O_2$ $[M+H]^+$ 389.1978; Found 389.1986

Retention of enantiopurity was determined by SFC Method 2, λ = 210 nm, T_{rac} = 5.57 and 5.73 min.

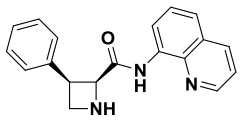




4.17b

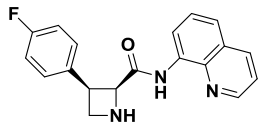
(2*S*,3*R*)-3-(4-methoxyphenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17b**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 48.0 mg (72%) of the desired product **4.17b** as an amorphous solid. $\mathbf{R_f}$ 0.19 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{24}_D}$ = +185.5 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3286, 2876, 1675, 1523, 1486, 1460, 1425, 1326, 1248, 1179, 1033, 828, 792, 759. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.30 (s, 1H), 8.94 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.51 (dd, *J* =

7.3, 1.7 Hz, 1H), 8.15 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.64 – 7.34 (m, 5H), 6.82 – 6.43 (m, 2H), 4.94 (d, *J* = 9.3 Hz, 1H), 4.42 – 4.14 (m, 2H), 3.75 (dd, *J* = 7.7, 4.3 Hz, 1H), 3.62 (s, 3H), 2.63 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.0, 158.5, 148.5, 139.1, 136.1, 133.9, 131.0, 129.1, 128.0, 127.2, 121.6, 121.4, 116.5, 113.7, 65.0, 55.0, 50.2, 42.6. **HRMS** (ESI^+) **m/z** calculated for $C_{20}H_{20}N_3O_2$ $[M+H]^+$ 334.1556; Found 334.1551.



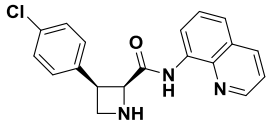
4.17c

(2*S*,3*R*)-3-phenyl-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17c**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 42.3 mg (70%) of the desired product **4.17c** as an amorphous solid. **R_f** 0.25 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24}_D$** = +186.7 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3282, 2879, 1677, 1523, 1486, 1459, 1425, 1325, 826, 792, 754, 699. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.28 (s, 1H), 8.91 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.46 (dd, *J* = 7.4, 1.6 Hz, 1H), 8.12 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.53 – 7.35 (m, 5H), 7.09 (dd, *J* = 8.2, 6.6 Hz, 2H), 7.05 – 6.98 (m, 1H), 4.96 – 4.90 (m, 1H), 4.34 – 4.19 (m, 2H), 3.83 – 3.72 (m, 1H), 2.63 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 148.5, 139.0, 138.8, 136.1, 133.8, 128.2, 128.02, 127.98, 127.2, 127.0, 121.6, 121.4, 116.4, 64.9, 49.8, 43.4. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{14.17n3}O$ $[M+H]^+$ 304.1450; Found 304.1447.



4.17d

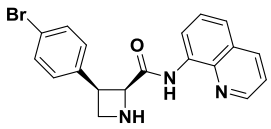
(2*S*,3*R*)-3-(4-fluorophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17d**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 37.0 mg (58%) of the desired product **4.17d** as an amorphous solid. $\mathbf{R_f}$ 0.33 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{25}_D}$ = +211.3 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3282, 2880, 1674, 1524, 1486, 1460, 1425, 1326, 1223, 1160, 1102, 826, 791, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.91 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.46 (dd, *J* = 7.4, 1.5 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.49 – 7.38 (m, 5H), 6.77 (t, *J* = 8.7 Hz, 2H), 4.93 (d, *J* = 9.2 Hz, 1H), 4.30 (t, *J* = 8.3 Hz, 1H), 4.20 (td, *J* = 9.0, 4.1 Hz, 1H), 3.71 (dd, *J* = 7.9, 4.1 Hz, 1H), 2.58 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 161.8 (d, *J* = 245 Hz), 148.5, 139.0, 136.2, 134.6 (d, *J* = 3.1 Hz), 133.7, 129.6 (d, *J* = 8.1 Hz), 128.0, 127.2, 121.8, 121.4, 116.5, 115.0 (d, *J* = 21.4 Hz), 64.8, 50.0, 42.6. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -116.0. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}FN_3O$ $[M+H]^+$ 322.1356; Found 322.1353.



4.17e

(2*S*,3*R*)-3-(4-chlorophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17e**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 35.4 mg (52%) of the desired product

4.17e as an amorphous solid. **R_f** 0.5 (10% MeOH in CH_2Cl_2). **$[\alpha]^{25}_D$** = +199.1 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3281, 2879, 1677, 1523, 1487, 1425, 1325, 1094, 1014, 826, 791, 756. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.31 (s, 1H), 8.93 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.50 (dd, *J* = 7.4, 1.6 Hz, 1H), 8.15 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.55 – 7.35 (m, 5H), 7.08 (d, *J* = 8.4 Hz, 2H), 4.96 (d, *J* = 9.3 Hz, 1H), 4.31 (t, *J* = 8.3 Hz, 1H), 4.21 (td, *J* = 8.9, 4.1 Hz, 1H), 3.71 (dd, *J* = 7.9, 4.1 Hz, 1H), 2.60 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 148.7, 139.2, 137.6, 136.3, 133.8, 132.9, 129.5, 128.5, 128.12, 127.3, 122.0, 121.6, 116.6, 64.8, 50.0, 42.8. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}ClN_3O$ $[M+H]^+$ 338.1060; Found 338.1058



4.17f

(2*R*,3*S*)-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**(−)-4.17f**).

(2*S*,3*R*)-3-(4-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**(+)-4.17f**).

Prepared according to **General Procedure K** from 64.6 mg of starting material (−)-**4.15a** for product (+)-**4.17f** or 10 g starting material (+)-**4.15a** for product (−)-**4.17f**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 43.8 mg (57%) of the desired product (+)-**4.17f** as an amorphous solid and 43.8 g (52%) of the desired product (−)-**4.17f** as an amorphous solid. **R_f** 0.43 (10% MeOH in CH_2Cl_2). **$[\alpha]^{25}_D$** = +226.3 (c = 1.0, $CHCl_3$), (+)-**4.17f**. **$[\alpha]^{25}_D$** = −243.8 (c = 1.0, $CHCl_3$), (−)-**4.17f**. **IR** (thin film) ν_{max} 3284, 2878, 1676, 1523, 1487, 1425, 1325, 1064, 825, 791, 756. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.29 (s, 1H), 8.93 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.50 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.17 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.53 – 7.42 (m, 3H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.5 Hz, 2H + $CHCl_3$), 4.97 (d, *J* = 9.3 Hz, 1H), 4.33 (t, *J* = 8.3 Hz, 1H), 4.21 (td, *J* = 9.0, 4.2 Hz, 1H), 3.72 (dd, *J* = 8.0, 4.2 Hz, 1H). Azetidine NH not visible.

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 148.7, 139.1, 138.1, 136.3, 133.7, 131.4, 129.9, 128.1, 127.3, 122.0, 121.6, 121.0, 116.6, 64.7, 49.9, 42.8. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}BrN_3O$ $[M+H]^+$ 382.0555; Found 382.0552.

A portion of the chromatographed material (−)-**4.17f** was crystallized from a mixture of toluene/hexane. The crystals obtained (mp 152-155 ºC) were characterized by X-ray diffraction.

X-ray crystallographic data for (−)-**4.17f**

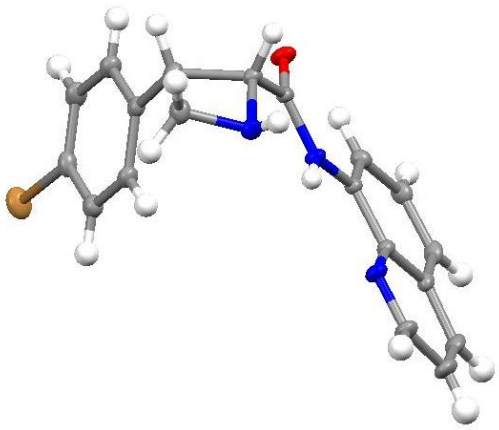


Table 1. Crystal data and structure refinement for X16226.

Identification code	X16226
Empirical formula	C19 H16 Br N3 O
Formula weight	382.26
Temperature	100(2) K
Wavelength	0.71073 Å

Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 7.7531(14) Å	α= 90°.
	b = 6.6125(12) Å	β= 94.153(3)°.
	c = 16.284(3) Å	γ = 90°.
Volume	832.7(3) $Å^3$	
Z	2	
Density (calculated)	1.525 Mg/m^3	
Absorption coefficient	2.479 mm^{-1}	
F(000)	388	
Crystal size	0.560 x 0.102 x 0.015 mm^3	
Theta range for data collection	2.508 to 30.989°.	
Index ranges	-11<=h<=11, -9<=k<=9, -23<=l<=23	
Reflections collected	49337	
Independent reflections	5301 [R(int) = 0.0360]	
Completeness to theta = 25.242°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7462 and 0.5456	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5301 / 3 / 223	
Goodness-of-fit on F^2	1.067	

Final R indices [I>2sigma(I)]	R1 = 0.0231, wR2 = 0.0570
R indices (all data)	R1 = 0.0248, wR2 = 0.0577
Absolute structure parameter	-0.007(3)
Extinction coefficient	n/a
Largest diff. peak and hole	0.304 and -0.339 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for X16226. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	1114(1)	7968(1)	555(1)	30(1)
O(1)	4961(2)	2780(3)	3502(1)	22(1)
N(1)	8199(2)	976(3)	2138(1)	18(1)
N(2)	7549(2)	4107(2)	3173(1)	15(1)
N(3)	10455(2)	6211(3)	3154(1)	19(1)
C(1)	6287(2)	2680(3)	3140(1)	15(1)
C(2)	6643(2)	872(3)	2613(1)	15(1)
C(3)	5481(2)	598(3)	1791(1)	18(1)

C(4)	7199(3)	105(3)	1403(1)	22(1)
C(11)	4477(2)	2430(3)	1484(1)	17(1)
C(12)	5222(3)	4041(3)	1087(1)	19(1)
C(13)	4235(3)	5700(3)	808(1)	21(1)
C(14)	2485(3)	5730(3)	944(1)	20(1)
C(15)	1711(3)	4180(4)	1349(1)	22(1)
C(16)	2709(3)	2542(3)	1617(1)	21(1)
C(21)	7647(2)	5867(3)	3650(1)	14(1)
C(22)	6367(3)	6573(3)	4116(1)	18(1)
C(23)	6619(3)	8362(3)	4583(1)	20(1)
C(24)	8122(3)	9446(3)	4578(1)	21(1)
C(25)	9455(3)	8774(3)	4095(1)	19(1)
C(26)	11034(3)	9829(3)	4035(1)	24(1)
C(27)	12254(3)	9062(4)	3559(2)	27(1)
C(28)	11920(3)	7228(4)	3132(1)	24(1)
C(29)	9237(2)	6972(3)	3631(1)	16(1)

Table 3. Bond lengths [Å] and angles [°] for X16226.

Br(1)-C(14)	1.904(2)

O(1)-C(1)	1.223(2)
N(1)-C(2)	1.481(2)
N(1)-C(4)	1.494(3)
N(1)-H(1)	0.87(2)
N(2)-C(1)	1.358(2)
N(2)-C(21)	1.399(2)
N(2)-H(2)	0.88(2)
N(3)-C(28)	1.323(3)
N(3)-C(29)	1.361(3)
C(1)-C(2)	1.509(3)
C(2)-C(3)	1.569(3)
C(2)-H(2A)	1.0000
C(3)-C(11)	1.506(3)
C(3)-C(4)	1.550(3)
C(3)-H(3)	1.0000
C(4)-H(4A)	0.9900
C(4)-H(4B)	0.9900
C(11)-C(12)	1.393(3)
C(11)-C(16)	1.405(3)
C(12)-C(13)	1.395(3)
C(12)-H(12)	0.9500

C(13)-C(14)	1.391(3)
C(13)-H(13)	0.9500
C(14)-C(15)	1.379(3)
C(15)-C(16)	1.383(3)
C(15)-H(15)	0.9500
C(16)-H(16)	0.9500
C(21)-C(22)	1.374(3)
C(21)-C(29)	1.435(2)
C(22)-C(23)	1.412(3)
C(22)-H(22)	0.9500
C(23)-C(24)	1.369(3)
C(23)-H(23)	0.9500
C(24)-C(25)	1.414(3)
C(24)-H(24)	0.9500
C(25)-C(29)	1.415(3)
C(25)-C(26)	1.418(3)
C(26)-C(27)	1.364(4)
C(26)-H(26)	0.9500
C(27)-C(28)	1.413(3)
C(27)-H(27)	0.9500
C(28)-H(28)	0.9500

C(2)-N(1)-C(4)	90.29(15)
C(2)-N(1)-H(1)	115.7(19)
C(4)-N(1)-H(1)	110.9(19)
C(1)-N(2)-C(21)	127.60(16)
C(1)-N(2)-H(2)	114.0(18)
C(21)-N(2)-H(2)	118.3(18)
C(28)-N(3)-C(29)	117.42(18)
O(1)-C(1)-N(2)	124.87(19)
O(1)-C(1)-C(2)	120.80(17)
N(2)-C(1)-C(2)	114.33(15)
N(1)-C(2)-C(1)	116.70(15)
N(1)-C(2)-C(3)	89.95(14)
C(1)-C(2)-C(3)	117.33(16)
N(1)-C(2)-H(2A)	110.5
C(1)-C(2)-H(2A)	110.5
C(3)-C(2)-H(2A)	110.5
C(11)-C(3)-C(4)	118.22(17)
C(11)-C(3)-C(2)	116.23(16)
C(4)-C(3)-C(2)	85.12(14)
C(11)-C(3)-H(3)	111.6

C(4)-C(3)-H(3)	111.6
C(2)-C(3)-H(3)	111.6
N(1)-C(4)-C(3)	90.22(14)
N(1)-C(4)-H(4A)	113.6
C(3)-C(4)-H(4A)	113.6
N(1)-C(4)-H(4B)	113.6
C(3)-C(4)-H(4B)	113.6
H(4A)-C(4)-H(4B)	110.9
C(12)-C(11)-C(16)	118.28(18)
C(12)-C(11)-C(3)	123.17(17)
C(16)-C(11)-C(3)	118.54(17)
C(11)-C(12)-C(13)	121.07(19)
C(11)-C(12)-H(12)	119.5
C(13)-C(12)-H(12)	119.5
C(14)-C(13)-C(12)	118.5(2)
C(14)-C(13)-H(13)	120.7
C(12)-C(13)-H(13)	120.7
C(15)-C(14)-C(13)	122.0(2)
C(15)-C(14)-Br(1)	119.02(16)
C(13)-C(14)-Br(1)	119.01(17)
C(14)-C(15)-C(16)	118.65(19)

C(14)-C(15)-H(15)	120.7
C(16)-C(15)-H(15)	120.7
C(15)-C(16)-C(11)	121.49(19)
C(15)-C(16)-H(16)	119.3
C(11)-C(16)-H(16)	119.3
C(22)-C(21)-N(2)	125.28(17)
C(22)-C(21)-C(29)	119.65(18)
N(2)-C(21)-C(29)	115.07(16)
C(21)-C(22)-C(23)	120.31(18)
C(21)-C(22)-H(22)	119.8
C(23)-C(22)-H(22)	119.8
C(24)-C(23)-C(22)	121.35(19)
C(24)-C(23)-H(23)	119.3
C(22)-C(23)-H(23)	119.3
C(23)-C(24)-C(25)	119.70(19)
C(23)-C(24)-H(24)	120.2
C(25)-C(24)-H(24)	120.2
C(24)-C(25)-C(29)	119.85(18)
C(24)-C(25)-C(26)	123.6(2)
C(29)-C(25)-C(26)	116.6(2)
C(27)-C(26)-C(25)	119.6(2)

C(27)-C(26)-H(26)	120.2
C(25)-C(26)-H(26)	120.2
C(26)-C(27)-C(28)	119.30(19)
C(26)-C(27)-H(27)	120.4
C(28)-C(27)-H(27)	120.4
N(3)-C(28)-C(27)	123.3(2)
N(3)-C(28)-H(28)	118.3
C(27)-C(28)-H(28)	118.3
N(3)-C(29)-C(25)	123.71(18)
N(3)-C(29)-C(21)	117.17(17)
C(25)-C(29)-C(21)	119.13(17)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for X16226. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	31(1)	31(1)	28(1)	0(1)	0(1)	15(1)
O(1)	16(1)	23(1)	26(1)	-4(1)	8(1)	-6(1)

N(1)	15(1)	20(1)	20(1)	-1(1)	3(1)	1(1)
N(2)	13(1)	14(1)	19(1)	-2(1)	4(1)	-4(1)
N(3)	14(1)	21(1)	23(1)	-2(1)	2(1)	-6(1)
C(1)	15(1)	14(1)	15(1)	2(1)	0(1)	-3(1)
C(2)	13(1)	14(1)	19(1)	1(1)	1(1)	-2(1)
C(3)	16(1)	17(1)	20(1)	-3(1)	-1(1)	-1(1)
C(4)	21(1)	24(1)	20(1)	-5(1)	1(1)	4(1)
C(11)	16(1)	19(1)	16(1)	-3(1)	0(1)	-1(1)
C(12)	14(1)	23(1)	21(1)	-1(1)	1(1)	0(1)
C(13)	22(1)	21(1)	20(1)	-1(1)	1(1)	1(1)
C(14)	19(1)	23(1)	18(1)	-3(1)	-2(1)	7(1)
C(15)	15(1)	29(1)	22(1)	-3(1)	3(1)	3(1)
C(16)	15(1)	26(1)	22(1)	-1(1)	3(1)	0(1)
C(21)	15(1)	13(1)	15(1)	0(1)	0(1)	-2(1)
C(22)	18(1)	18(1)	18(1)	2(1)	4(1)	0(1)
C(23)	25(1)	19(1)	17(1)	-1(1)	4(1)	4(1)
C(24)	28(1)	16(1)	19(1)	-3(1)	-4(1)	2(1)
C(25)	22(1)	16(1)	17(1)	1(1)	-5(1)	-3(1)
C(26)	27(1)	18(1)	26(1)	1(1)	-8(1)	-8(1)
C(27)	20(1)	28(1)	32(1)	3(1)	-5(1)	-12(1)
C(28)	15(1)	29(1)	27(1)	0(1)	2(1)	-7(1)

C(29)	14(1)	15(1)	17(1)	1(1)	-1(1)	-2(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for X16226.

	x	y	z	U(eq)
H(1)	9050(30)	180(40)	2307(17)	22
H(2)	8420(30)	3820(40)	2875(15)	18
H(2A)	6652	-397	2948	19
H(3)	4722	-621	1810	21
H(4A)	7395	-1360	1328	26
H(4B)	7352	854	887	26
H(12)	6423	4009	1005	23
H(13)	4747	6786	531	25
H(15)	516	4236	1442	26
H(16)	2188	1469	1898	25
H(22)	5307	5855	4124	21
H(23)	5728	8823	4907	24

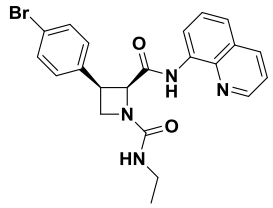
H(24)	8270	10645	4897	25
H(26)	11240	11063	4324	29
H(27)	13318	9754	3515	32
H(28)	12793	6698	2812	29

Table 6. Hydrogen bonds for X16226 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(2)-H(2)...N(1)	0.88(2)	2.23(3)	2.739(2)	117(2)
N(2)-H(2)...N(3)	0.88(2)	2.26(3)	2.650(2)	107(2)

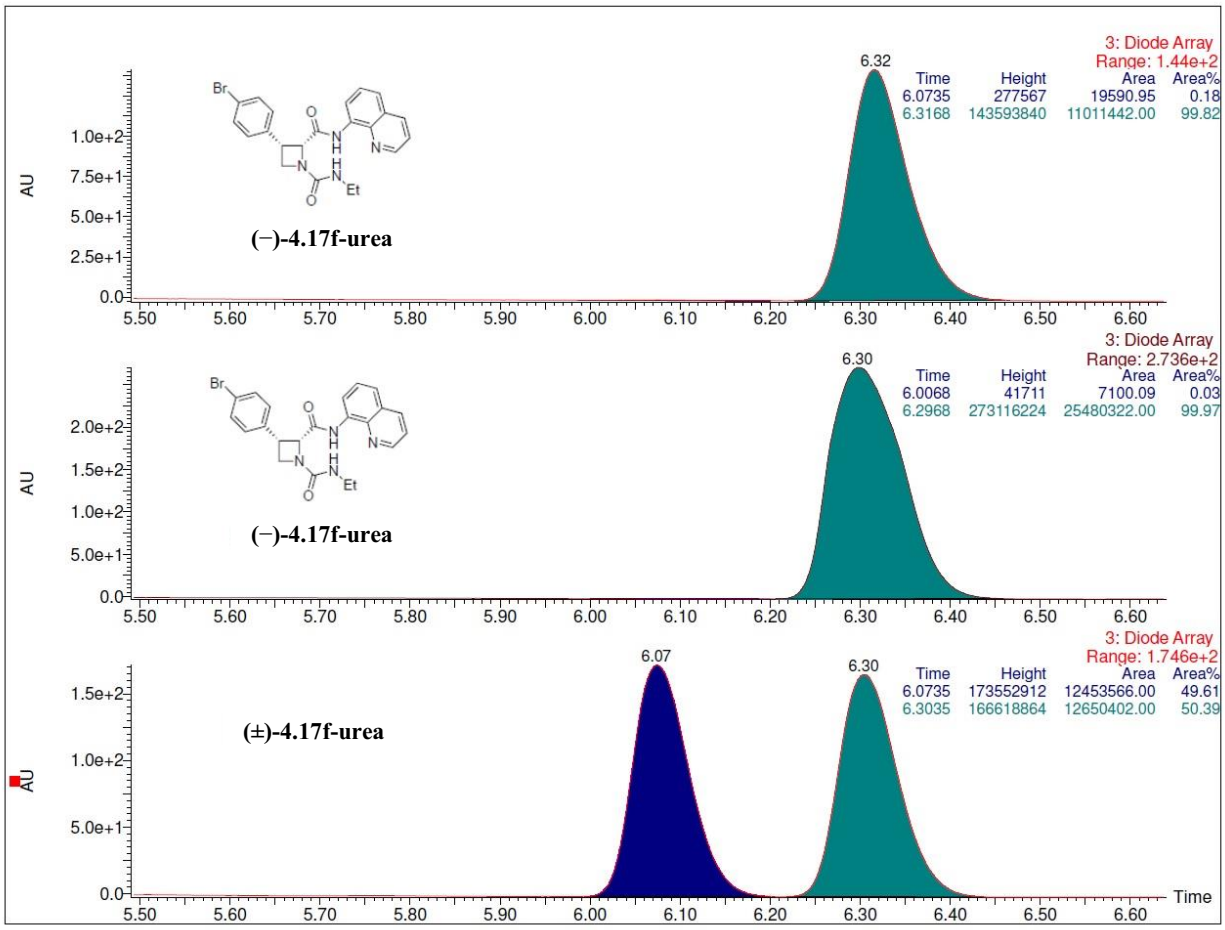
Symmetry transformations used to generate equivalent atoms:

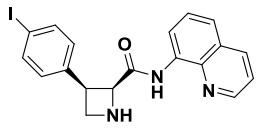
Functionalization for SFC



4.17f-urea

(±)-(Cis)-3-(4-bromophenyl)-N^1-ethyl-N^2-(quinolin-8-yl)-azetidine-1,2-dicarboxamide (**(±)-4.17f-Urea**). Prepared according to **General Procedure L** from 19.1 mg of racemic **4.17f** to give racemic **4.17f-Urea**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 19.3 mg (85%) of the desired product (±)-**4.17f-Urea** as a residue. $\mathbf{R_f}$ 0.72 (10% MeOH in CH_2Cl_2). **IR** (thin film) ν_{max} 3319, 1648, 1526, 1487, 1425, 1370, 1326, 826, 791, 755. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.48 (s, 1H), 8.81 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.40 (dd, *J* = 7.5, 1.3 Hz, 1H), 8.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.53 – 7.40 (m, 3H), 7.32 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.5 Hz, 2H + $CHCl_3$), 5.15 (d, *J* = 9.3 Hz, 1H), 4.98 (t, *J* = 5.8 Hz, 1H), 4.51 (t, *J* = 8.3 Hz, 1H), 4.14 – 4.00 (m, 2H), 3.33 (qd, *J* = 7.2, 5.6 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 167.4, 160.6, 148.7, 138.9, 136.9, 136.4, 133.2, 131.8, 129.8, 128.0, 127.3, 122.5, 121.8, 121.8, 117.0, 68.0, 53.1, 37.9, 35.6, 15.7. **HRMS** (ESI^+) **m/z** calculated for $C_{22}H_{22}BrN_4O_2$ $[M+H]^+$ 453.0926; Found 453.0936. Retention of enantiopurity was determined by SFC, λ = 210 nm, T_{rac} = 6.07 and 6.30 min.

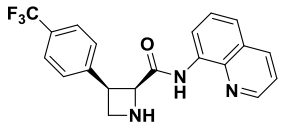




4.17g

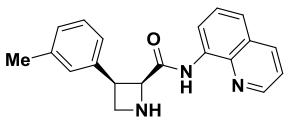
(2*S*,3*R*)-3-(4-iodophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17g**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 41.1 mg (48%) of the desired product **4.17g** as an amorphous solid. **R_f** 0.44 (10% MeOH in CH_2Cl_2). **$[\alpha]^{25}_D$** = +224.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3289, 2880, 1674, 1523, 1486, 1425, 1325, 1006, 825, 790, 754. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.90 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.47 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.15 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.52 – 7.34 (m, 5H), 7.23 (d, *J* = 8.4 Hz, 2H), 4.94 (d, *J* = 9.4 Hz, 1H), 4.29

(t, *J* = 8.3 Hz, 1H), 4.16 (td, *J* = 9.0, 4.2 Hz, 1H), 3.68 (dd, *J* = 7.9, 4.2 Hz, 1H), 2.64 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 148.7, 139.2, 138.9, 137.4, 136.3, 133.8, 130.2, 128.2, 127.4, 122.0, 121.6, 116.7, 92.7, 64.7, 49.9, 42.9. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}IN_3O$ $[M+H]^+$ 430.0416; Found 430.0411.



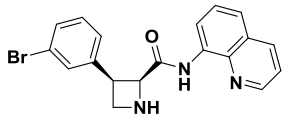
4.17h

(2*S*,3*R*)-*N*-(quinolin-8-yl)-3-(4-(trifluoromethyl)phenyl)-azetidine-2-carboxamide (**4.17h**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 38.5 mg (52%) of the desired product **4.17h** as an amorphous solid. **R_f** 0.48 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24}_D$** = +187.8 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3284, 2884, 1679, 1526, 1488, 1425, 1326, 1164, 1114, 1070, 1018, 825, 792, 760. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.26 (s, 1H), 8.90 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.41 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.60 (m, 2H), 7.45 (m 2 H), 7.40 (m, 1H), 7.33 (m, 2H), 4.98 (d, *J* = 9.4 Hz, 1H), 4.34 – 4.24 (m, 2H), 3.73 (m, 1H), 2.60 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3, 148.6, 143.0, 139.0, 136.2, 133.5, 129.1 (q, *J* = 32.3 Hz), 128.4, 128.1, 127.1, 125.1 (q, *J* = 3.7 Hz), 124.0 (q, *J* = 272 Hz), 121.7, 121.5, 116.5, 64.6, 49.5, 43.0. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -62.6. **HRMS** (ESI^+) **m/z** calculated for $C_{20}H_{17}F_3N_3O$ $[M+H]^+$ 372.1324; Found 372.1320.



4.17i

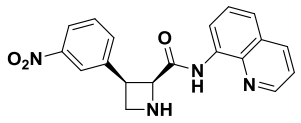
(2*S*,3*R*)-*N*-(quinolin-8-yl)-3-(*m*-tolyl)azetidine-2-carboxamide (**4.17i**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 41.0 mg (65%) of the desired product **4.17i** as an amorphous solid. $\mathbf{R_f}$ 0.27 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{25}_D}$ = +193.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3287, 2878, 1676, 1525, 1487, 1460, 1425, 1325, 826, 792, 757, 700. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.30 (s, 1H), 8.93 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.47 (dd, *J* = 7.3, 1.7 Hz, 1H), 8.12 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.46 – 7.38 (m, 3H), 7.34 (m, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 4.94 (d, *J* = 9.3 Hz, 1H), 4.28 (m, 1H), 4.19 (td, *J* = 8.9, 4.2 Hz, 1H), 3.76 (dd, *J* = 7.7, 4.2 Hz, 1H), 2.44 (bs, 1H), 1.97 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 148.5, 139.1, 138.8, 137.6, 136.1, 133.8, 128.7, 128.1, 128.0, 127.7, 127.2, 125.2, 121.6, 121.4, 116.4, 65.0, 50.0, 43.3, 21.0. **HRMS** (ESI^+) **m/z** calculated for $C_{20}H_{20}N_3O$ $[M+H]^+$ 318.1606; Found 318.1605.



4.17j

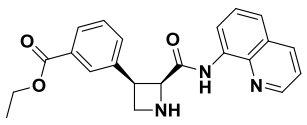
(2*S*,3*R*)-3-(3-bromophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17j**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 41.2 mg (54%) of the desired product **4.17j** as an oil. $\mathbf{R_f}$ 0.34 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{25}_D}$ = +221.8 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3289, 2879, 1676, 1525, 1486, 1425, 1325, 825, 790, 756. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.29 (s, 1H), 8.94 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.45 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.68 (s, 1H), 7.59 – 7.26 (m, 4H), 7.11 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 6.94 (t, *J* = 7.8 Hz, 1H), 4.95 (d, *J* = 9.3 Hz, 1H), 4.30 (t, *J* = 8.3 Hz, 1H), 4.17 (td, *J* = 8.9, 4.1 Hz, 1H), 3.72

(dd, *J* = 8.0, 4.1 Hz, 1H), 2.67 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.4, 148.8, 141.3, 139.2, 136.2, 133.7, 131.1, 130.2, 129.8, 128.1, 127.23, 127.0, 122.4, 121.9, 121.6, 116.5, 64.9, 49.8, 43.1. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}BrN_3O$ $[M+H]^+$ 382.0555; Found 382.0551.



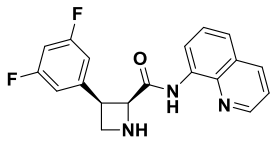
4.17k

(2*S*,3*R*)-3-(3-nitrophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17k**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 35.0 mg (50%) of the desired product **4.17k** as an amorphous solid. **R_f** 0.32 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24.15d}$** = +185.1 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3284, 2882, 2360, 1677, 1525, 1487, 1426, 1348, 1326, 1099, 945, 827, 793, 758, 733, 684. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.91 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.42 (t, *J* = 2.0 Hz, 1H), 8.37 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.11 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.85 – 7.74 (m, 2H), 7.51 – 7.41 (m, 2H), 7.39 – 7.33 (m, 1H), 7.23 (t, *J* = 7.9 Hz, 1H), 5.00 (d, *J* = 9.0 Hz, 1H), 4.36 (t, *J* = 8.2 Hz, 1H), 4.29 (td, *J* = 8.7, 3.6 Hz, 1H), 3.77 (dd, *J* = 7.8, 3.5 Hz, 1H), 2.70 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 168.9, 148.7, 148.1, 140.9, 138.9, 136.1, 134.5, 133.3, 129.1, 128.0, 127.0, 122.9, 122.07, 122.06, 121.6, 116.3, 64.6, 49.2, 42.9. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}N_4O_3$ $[M+H]^+$ 349.1301; Found 349.1298.



4.17l

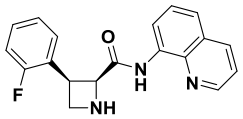
(2*S*,3*R*)-3-(3-(ethoxycarbonyl)phenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17l**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 38.0 mg (51%) of the desired product **4.17l** as an amorphous solid. $\mathbf{R_f}$ 0.23 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{24.15d}}$ = +184.9 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3286, 2880, 1714, 1677, 1525, 1487, 1461, 1426, 1325, 1276, 1258, 1198, 1108, 1088, 1023, 826, 793, 751, 694. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.31 (s, 1H), 8.90 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.41 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.11 (m, 2H), 7.71 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.43 (m, 2H), 7.37 (m, 1H), 7.15 (t, *J* = 7.7 Hz, 1H), 4.97 (m, 1H), 4.34 – 4.26 (m, 2H), 4.18 – 4.06 (m, 2H), 3.81 – 3.75 (m, 1H), 2.67 (bs, 1H), 1.14 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.4, 166.4, 148.5, 139.2, 139.0, 136.1, 133.7, 132.4, 130.4, 129.1, 128.31, 128.26, 128.0, 127.2, 121.7, 121.4, 116.4, 64.8, 60.6, 49.8, 43.0, 14.1. **HRMS** (ESI^+) **m/z** calculated for $C_{22}H_{21}N_3NaO_3$ $[M+Na]^+$ 398.1481; Found 398.1475.



4.17m

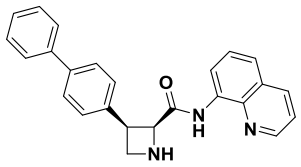
(2*S*,3*R*)-3-(3,5-difluorohenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17m**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 31.1 mg (46%) of the desired product **4.17m** as an oil. $\mathbf{R_f}$ 0.49 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{25}D}$ = +196.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3286, 2884, 1676, 1625, 1594, 1526, 1486, 1459, 1324, 1117, 826, 791, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.23 (s, 1H), 8.91 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.47 (dd, *J* = 7.4, 1.6 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.52 – 7.35 (m, 3H), 7.04 (dd, *J* = 8.6, 2.1 Hz, 2H), 6.43 (tt, *J* = 8.9, 2.3 Hz, 1H), 4.95 (d, *J* = 9.2 Hz, 1H), 4.30 (t, *J* = 8.2 Hz, 1H), 4.16 (td, *J* = 8.8, 4.0 Hz, 1H),

3.68 (dd, J = 8.0, 4.0 Hz, 1H), 2.63 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.1, 162.8 (dd, J = 248.0, 12.9 Hz), 148.7, 142.9 (t, J = 9.2 Hz), 139.1, 136.3, 133.7, 128.1, 127.2, 122.1, 121.6, 116.7, 111.2 (dd, J = 18.4, 6.8 Hz), 102.6 (t, J = 25.3 Hz), 64.7, 49.6, 43.1. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -110.5. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{14.15f2}N_3O$ $[M+H]^+$ 340.1261; Found 340.1258.



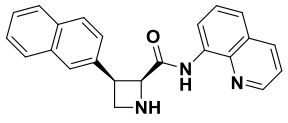
4.17n

(2*S*,3*R*)-3-(2-fluorophenyl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17n**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 20.5 mg (32%) of the desired product **4.17n** as an amorphous solid. **R_f** 0.30 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24.15d}$** = +155.2 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3285, 2883, 1677, 1525, 1488, 1457, 1426, 1325, 1234, 1105, 826, 792, 755. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.19 (s, 1H), 8.90 (dd, J = 4.3, 1.7 Hz, 1H), 8.46 (dd, J = 7.4, 1.6 Hz, 1H), 8.12 (dd, J = 8.3, 1.7 Hz, 1H), 7.67 (td, J = 7.7, 1.7 Hz, 1H), 7.47 – 7.36 (m, 3H), 6.98 (m, 1H), 6.91 (m, 1H), 6.80 (m, 1H), 4.96 (d, J = 9.6 Hz, 1H), 4.66 (td, J = 9.1, 5.1 Hz, 1H), 4.25 (t, J = 8.4 Hz, 1H), 3.83 (dd, J = 8.1, 5.1 Hz, 1H), 2.57 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 160.7 (d, J = 246 Hz), 148.5, 139.0, 136.1, 133.8, 128.6 (d, J = 8.3 Hz), 128.4 (d, J = 3.6 Hz), 127.2, 125.7 (d, J = 14.6 Hz), 123.8 (d, J = 3.7 Hz), 121.6, 121.4, 116.4, 115.0 (d, J = 22.5 Hz), 64.5, 48.9, 35.0 (d, J = 3.9 Hz). **^{19}F NMR** (376 MHz, $CDCl_3$) δ -117.3. **HRMS** (ESI^+) **m/z** calculated for $C_{19}H_{17}FN_3O$ $[M+H]^+$ 322.1356; Found 322.1348.



4.17p

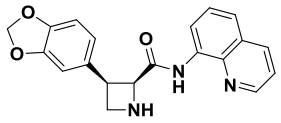
(2*S*,3*R*)-3-([1,1'-biphenyl]-4yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17p**). Prepared according to **General Procedure K** from 316 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 228 mg (61%) of the desired product **4.17p** as an amorphous solid. $\mathbf{R_f}$ 0.2 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{23}_D}$ = +246.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3281, 2923, 1672, 1518, 1485, 1459, 1424, 1324, 825, 791, 755, 698. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.29 (s, 1H), 8.92 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.47 (dd, *J* = 7.5, 1.6 Hz, 1H), 8.11 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.46 – 7.20 (m, 10H), 4.99 (d, *J* = 9.0 Hz, 1H), 4.37 – 4.25 (m, 2H), 3.84 – 3.79 (m, 1H), 2.76 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 148.6, 141.0, 139.9, 139.2, 138.1, 136.3, 133.9, 128.6, 128.6, 128.1, 127.3, 127.1, 127.0, 121.8, 121.5, 116.6, 65.1, 50.1, 43.1. **HRMS** (ESI^+) **m/z** calculated for $C_{25}H_{22}N_3O$ $[M+H]^+$ 380.1763; Found 380.1774.



4.17q

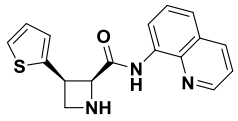
(2*S*,3*R*)-3-(naphthalen-2-yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17q**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 41.0 mg (58%) of the desired product **4.17q** as an amorphous solid. $\mathbf{R_f}$ 0.25 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{24.15d}}$ = +291.8 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3283, 2927, 2880, 1676, 1523, 1486, 1460, 1424, 1376, 1034, 824,

791, 753, 619. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.34 (s, 1H), 8.94 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.38 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.08 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.91 (s, 1H), 7.67 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.57 (m, 3H), 7.43 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.37 (m, 1H), 7.35 – 7.24 (m, 3H), 5.02 (d, *J* = 9.0 Hz, 1H), 4.43 – 4.32 (m, 2H), 3.87 (dd, *J* = 7.6, 4.0 Hz, 1H), 2.73 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 148.5, 139.0, 136.5, 136.1, 133.7, 133.2, 132.5, 127.9, 127.7, 127.4, 127.1, 127.0, 126.2, 125.6, 125.3, 121.6, 121.4, 116.4, 65.0, 50.1, 43.5. **HRMS** (ESI^+) **m/z** calculated for $C_{23}H_{20}N_3O$ $[M+H]^+$ 354.1606; Found 354.1602.



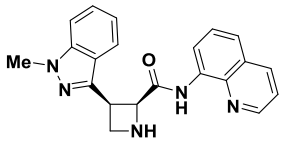
4.17r

(2*S*,3*R*)-3-(benzo[d][1,3]dioxol-5-yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17r**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 41.0 mg (59%) of the desired product **4.17r** as an amorphous solid. **R_f** 0.21 (5% MeOH in CH_2Cl_2). **$[\alpha]^{24}_D$** = +168.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3283, 2882, 1676, 1526, 1487, 1444, 1324, 1238, 1101, 1039, 935, 826, 792, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.24 (s, 1H), 8.92 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.52 (dd, *J* = 7.1, 1.9 Hz, 1H), 8.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.48 – 7.41 (m, 3H), 7.02 (d, *J* = 1.8 Hz, 1H), 6.92 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 5.73 (d, *J* = 1.5 Hz, 1H), 5.61 (d, *J* = 1.6 Hz, 1H), 4.90 (d, *J* = 9.3 Hz, 1H), 4.27 (t, *J* = 8.3 Hz, 1H), 4.16 (td, *J* = 9.0, 4.3 Hz, 1H), 3.70 (dd, *J* = 7.9, 4.3 Hz, 1H), 2.59 (bs, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 148.6, 147.5, 146.4, 139.1, 136.1, 133.9, 132.8, 128.0, 127.2, 121.7, 121.39, 121.36, 116.5, 108.5, 108.0, 100.6, 65.0, 50.2, 43.2. **HRMS** (ESI^+) **m/z** calculated for $C_{20}H_{18}N_3O_3$ $[M+H]^+$ 348.1348; Found 348.1346.



4.17s

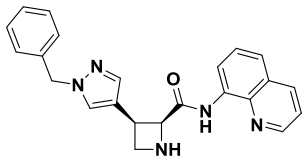
(2*S*,3*R*)-*N*-(quinolin-8-yl)-3-(thiophen-2-yl)azetidine-2-carboxamide (**4.17s**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 49.5 mg (80%) of the desired product **4.17s** as an amorphous solid. $\mathbf{R_f}$ 0.29 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{24}_D}$ = +121.6 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3285, 2879, 1675, 1524, 1487, 1460, 1425, 1325, 826, 791, 756, 699. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.26 (s, 1H), 8.89 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.59 (dd, *J* = 6.6, 2.5 Hz, 1H), 8.11 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.47 – 7.41 (m, 3H), 7.06 (d, *J* = 3.3 Hz, 1H), 6.98 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.76 (dd, *J* = 5.1, 3.5 Hz, 1H), 4.89 (d, *J* = 9.2 Hz, 1H), 4.53 (td, *J* = 8.9, 4.7 Hz, 1H), 4.32 (t, *J* = 8.4 Hz, 1H), 3.78 (dd, *J* = 8.0, 4.7 Hz, 1H), 2.59 (bs, 1H). **^{13}C NMR** (100 MHz, $(CD_3)_2SO$) δ 170.0, 149.6, 143.5, 138.5, 137.0, 134.2, 128.3, 127.4, 127.0, 126.0, 125.0, 122.6, 122.2, 115.6, 64.1, 51.4, 38.2. **HRMS** (ESI^+) **m/z** calculated for $C_{17}H_{16}N_3OS$ $[M+H]^+$ 310.1014; Found 310.1012.



4.17t

(2*S*,3*R*)-3-(1-methyl-1*H*-indazol-3-yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17t**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 33.0 mg (46%) of the desired product **4.17t** as an amorphous solid. $\mathbf{R_f}$ 0.60 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{22}_D}$ = +160.9

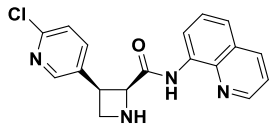
(c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3290, 2882, 1676, 1525, 1487, 1460, 1425, 1325, 826, 792, 747. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.20 (s, 1H), 8.87 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.36 (dd, *J* = 7.7, 1.4 Hz, 1H), 8.17 – 7.88 (m, 2H), 7.50 – 7.20 (m, 4H), 7.09 (dt, *J* = 16.4, 8.3 Hz, 2H), 6.80 (ddd, *J* = 8.0, 6.5, 1.1 Hz, 1H), 5.03 (d, *J* = 9.5 Hz, 1H), 4.75 (td, *J* = 9.1, 5.1 Hz, 1H), 4.32 (t, *J* = 8.3 Hz, 1H), 4.20 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.89 – 3.76 (m, 1H), 3.74 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.0, 148.5, 142.1, 141.1, 139.0, 136.1, 134.0, 128.0, 127.2, 126.0, 122.5, 121.5, 121.4, 121.2, 119.2, 116.4, 108.7, 64.3, 48.9, 36.5, 35.2. **HRMS** (ESI^+) **m/z** calculated for $C_{21}H_{20}N_5O$ $[M+H]^+$ 358.1668; Found 358.1658.



4.17u

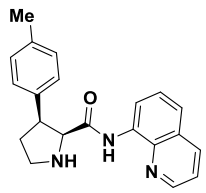
(2*S*,3*R*)-3-(1-benzyl-1*H*-pyrazol-4-yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17u**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 19.0 mg (25%) of the desired product **4.17u** as an amorphous solid. **R_f** 0.42 (10% MeOH in CH_2Cl_2). **$[\alpha]^{24}_D$** = +69.5 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3287, 2928, 1673, 1525, 1487, 1425, 1325, 997, 827, 759, 727, 696, 669. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.23 (s, 1H), 8.87 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.61 (dd, *J* = 6.8, 2.2 Hz, 1H), 8.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.56 (d, *J* = 0.8 Hz, 1H), 7.52 – 7.41 (m, 4H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.96 (t, *J* = 7.6 Hz, 2H), 6.81 (d, *J* = 7.5 Hz, 2H), 5.01 (s, 2H), 4.83 (d, *J* = 8.9 Hz, 1H), 4.27 (dd, *J* = 8.6, 7.5 Hz, 1H), 4.15 (td, *J* = 8.7, 4.1 Hz, 1H), 3.59 (dd, *J* = 7.5, 4.2 Hz, 1H), 2.54 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.0, 148.6, 139.04, 138.98, 136.4, 136.2, 133.8, 128.4, 128.3, 128.1, 127.5, 127.3, 127.1, 121.8, 121.5, 119.9, 116.5, 64.8,

55.8, 51.1, 33.9. **HRMS** (ESI^+) **m/z** calculated for $C_{23}H_{22}N_5O$ $[M+H]^+$ 384.1824; Found 384.1817.



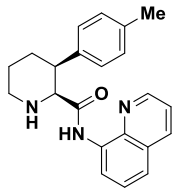
4.17v

(2*S*,3*R*)-3-(6-chloropyridin-3-yl)-*N*-(quinolin-8-yl)azetidine-2-carboxamide (**4.17v**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.15a**. Purification by flash column chromatography ($MeOH/CH_2Cl_2$) provided 16.0 mg (24%) of the desired product **4.17v** as an amorphous solid. $\mathbf{R_f}$ 0.53 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{23}_D}$ = +139.0 (c = 0.5, $CHCl_3$). **IR** (thin film) ν_{max} 3279, 2882, 1671, 1562, 1525, 1487, 1459, 1325, 1108, 826, 792, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.30 (s, 1H), 8.90 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.50 – 8.40 (m, 2H), 8.15 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.86 (dd, *J* = 8.3, 2.6 Hz, 1H), 7.50 – 7.41 (m, 3H), 7.03 (d, *J* = 8.3 Hz, 1H), 4.99 (d, *J* = 9.3 Hz, 1H), 4.35 (t, *J* = 8.4 Hz, 1H), 4.22 (td, *J* = 8.9, 4.1 Hz, 1H), 3.68 (dd, *J* = 8.2, 4.1 Hz, 1H). Azetidine NH not visible. **^{13}C NMR** (100 MHz, $CDCl_3$) δ 168.8, 150.2, 149.4, 148.7, 139.0, 138.2, 136.3, 133.6, 133.3, 128.1, 127.2, 123.8, 122.2, 121.6, 116.6, 64.3, 49.5, 40.0. **HRMS** (ESI^+) **m/z** calculated for $C_{14.17h14.15c}lN_4O$ $[M+H]^+$ 339.1013; Found 339.1005.



4.19

(2*S*,3*S*)-*N*-(quinolin-8-yl)-3-(*p*-tolyl)pyrrolidine-2-carboxamide (**4.19**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.18**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 63.0 mg (95%) of the desired product **4.19** as an amorphous solid. $\mathbf{R_f}$ 0.25 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{23}_D}$ = +8.96 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3295, 2922, 2362, 1682, 1518, 1485, 1438, 1425, 1384, 1324, 825.8, 792, 757. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.91 (s, 1H), 8.84 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.46 (dd, *J* = 6.5, 2.6 Hz, 1H), 8.11 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.43 – 7.38 (m, 3H), 7.14 (d, *J* = 8.1 Hz, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 4.22 (d, *J* = 8.8 Hz, 1H), 3.74 (q, *J* = 8.1 Hz, 1H), 3.58 (m, 1H), 3.25 (m, 1H), 2.64 (bs, 1H), 2.31 – 2.15 (m, 2H), 2.08 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.4, 148.3, 139.0, 137.4, 136.0, 135.9, 134.1, 128.9, 128.1, 127.9, 127.2, 121.31, 121.29, 116.4, 67.1, 48.6, 46.4, 30.7, 20.9. **HRMS** (ESI^+) **m/z** calculated for $C_{21}H_{22}N_3O$ $[M+H]^+$ 332.1763; Found 332.1758,

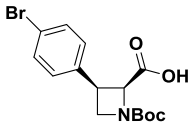


4.21

(2*S*,3*S*)-*N*-(quinolin-8-yl)-3-(*p*-tolyl)piperidine-2-carboxamide (**4.21**). Prepared according to **General Procedure K** from 64.6 mg of starting material **4.20**. Purification by flash column chromatography (MeOH/CH_2Cl_2) provided 50.0 mg (72%) of the desired product **4.21** as an amorphous solid. $\mathbf{R_f}$ 0.10 (5% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{23}_D}$ = +36.3 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3303, 2930, 2856, 1683, 1524, 1486, 1425, 1323, 825, 791, 755. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.45 (s, 1H), 8.78 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.66 (dd, *J* = 6.0, 3.1 Hz, 1H), 8.09 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.46 – 7.37 (m, 5H), 6.98 (d, *J* = 7.8 Hz, 2H), 3.99 (d, *J* = 4.2 Hz, 1H), 3.49

(q, *J* = 4.8 Hz, 1H), 3.42 (dt, *J* = 12.2, 4.8 Hz, 1H), 3.02 (s, 1H), 2.96 (ddd, *J* = 12.5, 9.2, 3.6 Hz, 1H), 2.15 (s, 4H), 2.06 – 1.99 (m, 1H), 1.89 (ddt, *J* = 13.6, 9.7, 5.4 Hz, 1H), 1.58 (dp, *J* = 15.4, 5.7, 4.8 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.2, 148.1, 138.9, 138.7, 136.0, 135.5, 134.2, 128.8, 128.7, 127.8, 127.2, 121.3, 116.4, 64.1, 45.4, 41.8, 29.6, 22.9, 20.9. **HRMS** (ESI^+) **m/z** calculated for $C_{22}H_{24}N_3O$ $[M+H]^+$ 346.1919; Found 346.1914.

5.4.3 Directing group cleavage



4.22

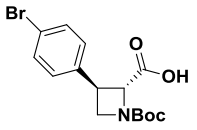
(2*R*,3*S*)-3-(4-bromophenyl)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (**(−)-4.22**)

(2*S*,3*R*)-3-(4-bromophenyl)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (**(+)-4.22**)

Conditions were modified from previous literature.[4,5]

Prepared according to **General Procedure M** from 3.25 g of starting material (−)-**4.17f** for product (−)-**4.22** or 65 mg starting material (+)-**4.17f** for product (+)-**4.22**. Purification by aqueous work-up provided 2.16 g (72%) of the desired product (−)-**4.22** as an amorphous solid and 47.9 mg (69%) of the desired product (+)-**4.22** as an amorphous solid. **$[\alpha]^{25}_D$** = −34.0 (c = 1.0, DMSO), (−)-**4.22**. **$[\alpha]^{25}_D$** = +31.8 (c = 1.0, DMSO), (+)-**4.22**. **IR** (thin film) ν_{max} 2980, 1728, 1629, 1477, 1450, 1152, 1105. **^{1}H NMR** (400 MHz, DMSO) δ 12.44 (bs, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 4.80 (d, *J* = 9.0 Hz, 1H), 4.25 – 3.98 (m, 3H), 1.37 (s, 9H). **^{13}C NMR** (100 MHz, DMSO, 353 K) δ 168.9, 154.6, 136.2, 130.5, 129.8, 119.9, 78.6, 65.6, 51.7,

35.3, 27.6 (3 C). **HRMS** (ESI^+) **m/z** Calculated for $C_{15}H_{14.17b}rNNaO_4$ $[M+Na]^+$ 378.0317; Found 378.0328



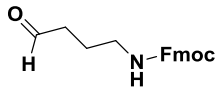
4.23

(2*S*,3*S*)-3-(4-bromophenyl)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (**(+)-4.23**)

(2*R*,3*R*)-3-(4-bromophenyl)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (**(−)-4.23**)

Prepared according to **General Procedure N** from 130 mg of starting material (−)-**4.17f** for product (+)-**4.23** or 75 mg starting material (+)-**4.17f** for product (−)-**4.23**. Purification by aqueous work-up provided 93 mg (77%) of the desired product (+)-**4.23** as a pale brown residue and 57 mg (81%) of the desired product (−)-**4.23** as amorphous solid. $\mathbf{[\alpha]^{25}_D}$ = +43.5 (c = 1.0, DMSO), (+)-**4.23**. $\mathbf{[\alpha]^{25}_D}$ = −43.6 (c = 1.0, DMSO), (−)-**4.23**. **IR** (thin film) ν_{max} 2977, 1746, 1707, 11490, 1477, 1420, 1394, 1367, 1166, 1142, 1074. **^{1}H NMR** (400 MHz, CD_3OD) δ 7.54 (d, *J* = 8.1 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.53 (d, *J* = 5.4 Hz, 1H), 4.38 (t, *J* = 8.4 Hz, 1H), 3.92 (dd, *J* = 8.2, 5.6 Hz, 1H), 3.71 (dt, *J* = 8.7, 5.3 Hz, 1H), 1.47 (s, 9H). **^{13}C NMR** (100 MHz, CD_3OD) δ 173.6, 157.5, 141.0, 133.0, 129.8, 122.2, 81.9, 39.4, 28.5. **HRMS** (ESI^+) **m/z** calculated for $C_{15}H_{14.17b}rNNaO_4$ $[M+Na]^+$ 378.0317. Found 378.0316.

5.4.4 Synthesis of BRD3914

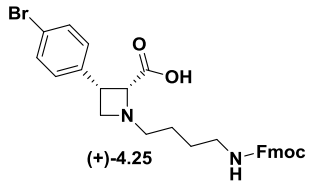


4.24

(9*H*-fluoren-9-yl)methyl (4-oxobutyl)carbamate (**4.24**). To a precooled (–78 ºC) solution of oxalyl chloride (296 µL, 3.50 mmol, 1.25 equiv) in DCM (28 mL) was added dropwise dimethyl sulfoxide (496 µL, 7.00 mmol, 2.5 equiv). The resulting mixture was stirred at –78 ºC for 10 min.

A solution of (9*H*-fluoren-9-yl)methyl (4-hydroxybutyl)carbamate[6] (871 mg, 2.80 mmol, 1 equiv) in DCM (25 mL + 3×1 mL rinse) was then added slowly via syringe and the resulting mixture was stirred at –78 ºC for 1 h. Triethylamine (1.94 mL, 14.0 mmol, 5 equiv) was added, the cold bath was removed and the reaction was stirred allowing to warm to ambient temperature for 30 min.

The reaction mixture was concentrated *in vacuo* leading to the formation of a white solid. Acetone was added, and the resulting slurry was filtered over cotton and concentrated to afford a clear, pale yellow oil that crystallized upon standing. The spectral data obtained were consistent with those found in the literature,[7] albeit showing contamination with DMSO and Et_3N•HCl. The crude material obtained was used without further purification in the 2-3 h following isolation.

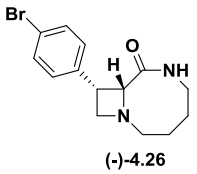


(+)-4.25

(2R,3S)-1-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)butyl)-3-(4-bromophenyl)azetidine-2-carboxylic acid (**(+)-4.25)**. To a precooled (0 °C) solution of (−)-**4.22** (400 mg, 1.12 mmol, 1 equiv) in DCM (11 mL) was added hydrochloric acid (4 M solution in dioxane, 4.20 mL, 16.8 mmol, 15 equiv). The reaction was allowed to reach ambient temperature and stirred for 2 h, upon which complete consumption of starting material was observed. Next, the reaction mixture was concentrated under a stream of N_2 (to remove excess HCl) then *in vacuo*. A solution of crude aldehyde **4.24** (866 mg, 2.80 mmol, 2.5 equiv) in DCM (11 mL) was then added and the mixture cooled to 0 ºC. $NaBH(OAc)_3$ (712 mg, 3.36 mmol, 3 equiv) was added gradually and the reaction was allowed to reach ambient temperature (without removing the ice bath) and stirred overnight. The reaction was quenched by addition of saturated NH_4Cl (30 mL). The resulting aqueous layer was exhaustively extracted with 3:7 *i*-PrOH:$CHCl_3$ (4x). Organic layers were combined, dried over anhydrous Na_2SO_4, filtered over cotton, and concentrated over SiO_2. Column chromatography (EtOAc/hexane gradient, followed by MeOH/DCM) afforded (+)-**4.25** (439 mg, 71%) as a white solid.

Note: this compound exhibited extremely low solubility or provided spectra of difficult interpretation in several routine NMR solvents (deuterated chloroform, acetonitrile, methanol, benzene, DMSO) at various temperatures. Interpretable spectral data were recorded employing deuterated pyridine as NMR solvent, with albeit also poor solubility and therefore moderate signal-to-noise ratio. Structural proof was established using ^1H NMR, COSY and HSQC experiments tabulated below, in addition to the other methods hereby indicated.

R_f 0.33 (10% MeOH in CH_2Cl_2). **$[\alpha]^{22}_D$** = +85.9 (c = 0.30, DMSO). **IR** (solid) ν_{max} 3304, 3041, 2929, 1714, 1651, 1448, 1398, 1258, 1091, 1010, 839, 738, 507. **^{1}H NMR** (300 MHz, C_5D_5N + 0.05% v/v TMS, 300 K) δ 8.23 (t, *J* = 5.6 Hz, 1H, NH), 7.86 (d, *J* = 7.7 Hz, 2H), 7.78 – 7.65 (m, 4H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 4.69 (d, *J* = 6.9 Hz, 2H), 4.36 (t, *J* = 6.8 Hz, 1H), 4.21 (d, *J* = 8.6 Hz, 1H), 3.76 (td, *J* = 8.2, 2.4 Hz, 1H), 3.48 (q, *J* = 6.7 Hz, 2H), 3.38 (dd, *J* = 6.5, 1.9 Hz, 1H), 3.18 (t, *J* = 7.2 Hz, 1H), 2.85 (dt, *J* = 11.7, 7.4 Hz, 1H), 2.45 (dt, *J* = 11.7, 6.7 Hz, 1H), 1.89 – 1.75 (m, 2H), 1.67 – 1.52 (m, 2H). H_{COOH} not observed. **^{13}C NMR** (151 MHz, C_5D_5N + 0.05% v/v TMS, 300 K, from HSQC) δ 138.0, 131.5, 130.6, 127.4, 125.4, 120.1, 70.4, 66.2, 57.9, 57.0, 47.7, 41.0, 39.2, 28.4, 24.8. 8 C_q not observed **HRMS** (ESI^+) **m/z** Calculated for $C_{29}H_{30}BrN_2O_4$ $[M+H]^+$ 549.1384; Found 549.1401.



(-)-4.26

(8*R*,9*S*)-9-(4-bromophenyl)-1,6-diazabicyclo[6.2.0]decan-7-one (**(−)-4.26**). To a solution of **(+)-4.25** (398 mg, 0.724 mmol, 1 equiv) in DMF (72 mL) was added Silia-Bond® piperazine (2.58 g, 0.84 mmol/g, 2.17 mmol, 3 equiv) and the resulting mixture was warmed to 50 ºC and vigorously stirred for 2 h, upon which complete consumption of starting material was observed. The reaction was then cooled to 0 ºC, HATU (410 mg, 1.08 mmol, 1.5 equiv) and DIPEA (0.63 mL, 3.61 mmol, 5 equiv) were added, and the mixture was warmed to ambient temperature and stirred for 20 min. MeOH (1 mL) was added and mixture was concentrated on SiO_2 (approx. 5 g) and transferred to fritted funnel. The lactam-adsorbed SiO_2 was first washed with hexane to remove any dibenzofulvene, then with 10% MeOH/DCM to recover the desired product. The MeOH fraction was concentrated *in vacuo*, taken up in EtOAc (50 mL), washed with sat. aq.

$NaHCO_3$ (3x), dried over anhydrous Na_2SO_4, filtered and concentrated to afford (−)-**17** as a pale yellow amorphous solid (198 mg, 89%). $\mathbf{R_f}$ 0.63 (10% MeOH in CH_2Cl_2). $\mathbf{[\alpha]^{24}_D}$ = −28.3 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3271, 2929, 2820, 1648, 1487, 1408, 1365, 1011, 846. **^{1}H NMR** (400 MHz, $CDCl_3$, 300 K) δ 7.48 – 7.35 (m, 4H), 5.57 (d, *J* = 7.7 Hz, 1H), 4.93 – 4.71 (m, 1H), 4.09 (d, *J* = 8.3 Hz, 1H), 3.67 (t, *J* = 8.1 Hz, 1H), 3.46 (d, *J* = 6.8 Hz, 1H), 3.13 (td, *J* = 7.2, 2.3 Hz, 1H), 3.00 – 2.85 (m, 2H), 2.47 (d, *J* = 10.7 Hz, 1H), 1.78 – 1.47 (m, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$, 300 K) δ 172.7, 139.0, 131.5, 129.8, 120.8, 69.1, 56.8, 56.2, 41.5, 39.7, 29.2, 21.0. **HRMS** (ESI^+) **m/z** Calculated for $C_{14}H_{14.17b}rN_2O$ $[M+H]^+$ 309.0603; Found 309.0604.

A portion of the material obtained was chromatographed, dissolved in dichloromethane and crystallized by evaporation under a stream of N_2. The crystals thus obtained (mp 63-65 ºC) were characterized by X-ray diffraction.

X-ray crystallographic data for (−)-**4.26** (crystallized solvent dichloromethane was removed for clarity in the schematic representation)

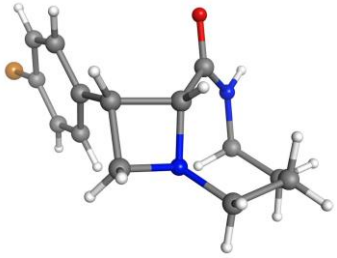


Table 1. Crystal data and structure refinement for X17072.

Identification code	X17072
Empirical formula	C15 H19 Br Cl2 N2 O
Formula weight	394.13

Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.071(2) Å	α= 90°.
	b = 10.749(3) Å	β= 90°.
	c = 19.914(5) Å	γ = 90°.
Volume	1727.7(8) $Å^3$	
Z	4	
Density (calculated)	1.515 Mg/m^3	
Absorption coefficient	2.688 mm^{-1}	
F(000)	800	
Crystal size	0.508 x 0.224 x 0.138 mm^3	
Theta range for data collection	2.045 to 30.027°.	
Index ranges	-11<=h<=11, -15<=k<=15, -28<=l<=28	
Reflections collected	83926	
Independent reflections	5053 [R(int) = 0.0554]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7461 and 0.5360	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5053 / 92 / 221	
Goodness-of-fit on F^2	1.025	
Final R indices [I>2sigma(I)]	R1 = 0.0321, wR2 = 0.0748	
R indices (all data)	R1 = 0.0447, wR2 = 0.0808	
Absolute structure parameter	0.028(3)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.280 and -0.603 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for X17072. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	6395(1)	7535(1)	3335(1)	72(1)
O(1)	5815(2)	2480(2)	5245(1)	46(1)
N(1)	5482(3)	4744(2)	6626(1)	42(1)
N(2)	3499(3)	3443(2)	5579(1)	34(1)
C(1)	7128(4)	5335(4)	6650(2)	60(1)
C(2)	7570(3)	4687(3)	5986(2)	44(1)
C(3)	6144(3)	3776(3)	6183(1)	36(1)
C(4)	5112(3)	3206(2)	5636(1)	31(1)
C(5)	4662(4)	4347(4)	7244(1)	58(1)
C(6)	3292(4)	3425(3)	7101(2)	55(1)
C(7)	1929(4)	3882(3)	6638(2)	46(1)
C(8)	2485(3)	4336(3)	5949(1)	36(1)
C(9)	7286(3)	5417(3)	5352(1)	38(1)
C(10)	6138(4)	6379(3)	5311(2)	45(1)
C(11)	5886(4)	7026(3)	4722(2)	51(1)
C(12)	6777(4)	6697(3)	4161(2)	47(1)
C(13)	7917(4)	5741(3)	4179(2)	47(1)
C(14)	8168(3)	5112(3)	4776(2)	42(1)
C(1S)	3870(30)	4280(30)	3993(8)	49(2)
Cl(1)	4365(19)	4031(14)	3161(4)	85(2)
Cl(2)	2290(12)	5363(14)	4069(4)	85(2)

C(1SA)	3720(40)	4410(30)	3951(10)	50(3)
Cl(1A)	4577(16)	4274(12)	3136(4)	64(2)
Cl(2A)	2371(12)	5688(15)	4005(5)	68(2)

Table 3. Bond lengths [Å] and angles [°] for X17072.

Br(1)-C(12)	1.901(3)
O(1)-C(4)	1.239(3)
N(1)-C(5)	1.462(4)
N(1)-C(3)	1.465(3)
N(1)-C(1)	1.473(4)
N(2)-C(4)	1.332(3)
N(2)-C(8)	1.460(3)
N(2)-H(2)	0.92(2)
C(1)-C(2)	1.536(4)
C(1)-H(1A)	0.9900
C(1)-H(1B)	0.9900
C(2)-C(9)	1.505(4)
C(2)-C(3)	1.561(4)
C(2)-H(2A)	1.0000
C(3)-C(4)	1.503(3)
C(3)-H(3)	1.0000
C(5)-C(6)	1.512(5)
C(5)-H(5A)	0.9900
C(5)-H(5B)	0.9900
C(6)-C(7)	1.517(5)
C(6)-H(4.15a)	0.9900

C(6)-H(4.15b)	0.9900
C(7)-C(8)	1.522(4)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-H(4.17a)	0.9900
C(8)-H(4.17b)	0.9900
C(9)-C(14)	1.390(4)
C(9)-C(10)	1.391(4)
C(10)-C(11)	1.379(5)
C(10)-H(10)	0.9500
C(11)-C(12)	1.375(5)
C(11)-H(11)	0.9500
C(12)-C(13)	1.379(4)
C(13)-C(14)	1.383(4)
C(13)-H(13)	0.9500
C(14)-H(14)	0.9500
C(1S)-Cl(1)	1.727(13)
C(1S)-Cl(2)	1.730(12)
C(1S)-H(1S1)	0.9900
C(1S)-H(1S2)	0.9900
C(1SA)-Cl(2A)	1.758(15)
C(1SA)-Cl(1A)	1.768(15)
C(1SA)-H(1S3)	0.9900
C(1SA)-H(1S4)	0.9900

C(5)-N(1)-C(3)	117.7(3)
C(5)-N(1)-C(1)	120.4(2)
C(3)-N(1)-C(1)	89.8(2)

C(4)-N(2)-C(8)	129.1(2)
C(4)-N(2)-H(2)	115(2)
C(8)-N(2)-H(2)	116(2)
N(1)-C(1)-C(2)	89.2(2)
N(1)-C(1)-H(1A)	113.8
C(2)-C(1)-H(1A)	113.8
N(1)-C(1)-H(1B)	113.8
C(2)-C(1)-H(1B)	113.8
H(1A)-C(1)-H(1B)	111.0
C(9)-C(2)-C(1)	116.8(3)
C(9)-C(2)-C(3)	115.2(2)
C(1)-C(2)-C(3)	84.1(2)
C(9)-C(2)-H(2A)	112.6
C(1)-C(2)-H(2A)	112.6
C(3)-C(2)-H(2A)	112.6
N(1)-C(3)-C(4)	121.6(2)
N(1)-C(3)-C(2)	88.5(2)
C(4)-C(3)-C(2)	118.8(2)
N(1)-C(3)-H(3)	108.7
C(4)-C(3)-H(3)	108.7
C(2)-C(3)-H(3)	108.7
O(1)-C(4)-N(2)	121.0(2)
O(1)-C(4)-C(3)	117.3(2)
N(2)-C(4)-C(3)	121.7(2)
N(1)-C(5)-C(6)	111.3(2)
N(1)-C(5)-H(5A)	109.4
C(6)-C(5)-H(5A)	109.4
N(1)-C(5)-H(5B)	109.4

C(6)-C(5)-H(5B)	109.4
H(5A)-C(5)-H(5B)	108.0
C(5)-C(6)-C(7)	115.6(3)
C(5)-C(6)-H(4.15a)	108.4
C(7)-C(6)-H(4.15a)	108.4
C(5)-C(6)-H(4.15b)	108.4
C(7)-C(6)-H(4.15b)	108.4
H(4.15a)-C(6)-H(4.15b)	107.4
C(6)-C(7)-C(8)	115.9(2)
C(6)-C(7)-H(7A)	108.3
C(8)-C(7)-H(7A)	108.3
C(6)-C(7)-H(7B)	108.3
C(8)-C(7)-H(7B)	108.3
H(7A)-C(7)-H(7B)	107.4
N(2)-C(8)-C(7)	114.2(2)
N(2)-C(8)-H(4.17a)	108.7
C(7)-C(8)-H(4.17a)	108.7
N(2)-C(8)-H(4.17b)	108.7
C(7)-C(8)-H(4.17b)	108.7
H(4.17a)-C(8)-H(4.17b)	107.6
C(14)-C(9)-C(10)	117.9(3)
C(14)-C(9)-C(2)	119.5(3)
C(10)-C(9)-C(2)	122.6(3)
C(11)-C(10)-C(9)	121.5(3)
C(11)-C(10)-H(10)	119.2
C(9)-C(10)-H(10)	119.2
C(12)-C(11)-C(10)	118.9(3)
C(12)-C(11)-H(11)	120.5

C(10)-C(11)-H(11)	120.5
C(11)-C(12)-C(13)	121.3(3)
C(11)-C(12)-Br(1)	119.7(2)
C(13)-C(12)-Br(1)	118.9(2)
C(12)-C(13)-C(14)	119.0(3)
C(12)-C(13)-H(13)	120.5
C(14)-C(13)-H(13)	120.5
C(13)-C(14)-C(9)	121.3(3)
C(13)-C(14)-H(14)	119.4
C(9)-C(14)-H(14)	119.4
Cl(1)-C(1S)-Cl(2)	111.1(9)
Cl(1)-C(1S)-H(1S1)	109.4
Cl(2)-C(1S)-H(1S1)	109.4
Cl(1)-C(1S)-H(1S2)	109.4
Cl(2)-C(1S)-H(1S2)	109.4
H(1S1)-C(1S)-H(1S2)	108.0
Cl(2A)-C(1SA)-Cl(1A)	111.3(11)
Cl(2A)-C(1SA)-H(1S3)	109.4
Cl(1A)-C(1SA)-H(1S3)	109.4
Cl(2A)-C(1SA)-H(1S4)	109.4
Cl(1A)-C(1SA)-H(1S4)	109.4
H(1S3)-C(1SA)-H(1S4)	108.0

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for X17072. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	98(1)	50(1)	70(1)	18(1)	0(1)	0(1)
O(1)	40(1)	42(1)	55(1)	-16(1)	13(1)	2(1)
N(1)	39(1)	58(1)	30(1)	-12(1)	-4(1)	0(1)
N(2)	29(1)	41(1)	34(1)	-10(1)	-1(1)	-1(1)
C(1)	48(2)	81(2)	50(2)	-18(2)	-16(1)	-12(2)
C(2)	25(1)	57(2)	49(2)	-4(1)	-6(1)	-1(1)
C(3)	28(1)	45(1)	35(1)	0(1)	-3(1)	5(1)
C(4)	31(1)	29(1)	33(1)	-2(1)	4(1)	1(1)
C(5)	58(2)	88(2)	28(1)	-5(1)	-1(1)	7(2)
C(6)	67(2)	64(2)	36(1)	12(1)	15(1)	4(2)
C(7)	42(1)	51(2)	46(1)	-2(1)	10(1)	-3(1)
C(8)	27(1)	42(1)	40(1)	-5(1)	1(1)	4(1)
C(9)	27(1)	38(1)	49(1)	-7(1)	-3(1)	-7(1)
C(10)	42(2)	38(1)	56(2)	-8(1)	4(1)	1(1)
C(11)	50(2)	32(1)	71(2)	-4(1)	1(2)	4(1)
C(12)	50(2)	33(1)	57(2)	2(1)	0(1)	-8(1)
C(13)	40(1)	48(2)	53(2)	-3(1)	6(1)	0(1)
C(14)	31(1)	40(2)	55(2)	-7(1)	1(1)	1(1)
C(1S)	55(5)	50(5)	42(4)	8(3)	-11(4)	-9(4)
Cl(1)	140(5)	60(4)	54(2)	6(2)	7(2)	23(3)
Cl(2)	59(2)	99(4)	96(2)	25(3)	16(2)	18(2)
C(1SA)	61(7)	52(7)	38(5)	3(4)	-10(5)	-10(5)
Cl(1A)	107(3)	53(3)	33(2)	1(2)	4(1)	5(2)
Cl(2A)	56(2)	77(4)	72(3)	-10(3)	6(2)	7(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for X17072.

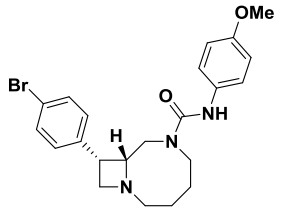
	x	y	z	U(eq)
H(2)	2950(40)	2970(30)	5270(14)	41
H(1A)	7814	5063	7035	71
H(1B)	7094	6253	6618	71
H(2A)	8685	4282	5999	52
H(3)	6616	3094	6466	44
H(5A)	4193	5083	7475	70
H(5B)	5486	3961	7547	70
H(4.15a)	2783	3178	7533	66
H(4.15b)	3791	2671	6900	66
H(7A)	1338	4571	6865	55
H(7B)	1125	3198	6573	55
H(4.17a)	1491	4534	5678	44
H(4.17b)	3125	5114	6006	44
H(10)	5512	6595	5698	54
H(11)	5110	7690	4704	61
H(13)	8520	5519	3787	57
H(14)	8959	4458	4792	50
H(1S1)	4860	4587	4236	59
H(1S2)	3519	3490	4202	59
H(1S3)	3115	3638	4063	61
H(1S4)	4631	4512	4281	61

Table 6. Hydrogen bonds for X17072 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(2)-H(2)...O(1)#1	0.92(2)	2.06(2)	2.894(3)	150(3)
C(7)-H(7B)...Cl(1)#1	0.99	2.83	3.775(12)	158.8
C(8)-H(4.17b)...N(1)	0.99	2.30	2.803(3)	110.3

Symmetry transformations used to generate equivalent atoms:

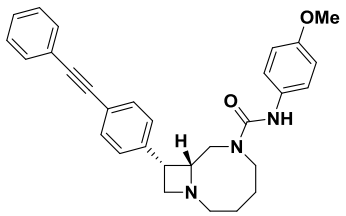
#1 x-1/2,-y+1/2,-z+1



(+)-4.27

(8*R*,9*S*)-9-(4-bromophenyl)-*N*-(4-methoxyphenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (**(+)-4.27**). In a sealable vial, $Ru_3(CO)_{12}$ (10 mg, 0.016 mmol, 0.1 equiv) was added to (−)-**4.26** (50 mg, 0.16 mmol, 1.0 equiv). The vial was evacuated and backfilled with N_2 (3x). Toluene (80 µL) was added, and the vial was vortexed for 30 s. TMDS (425 µL, 2.41 mmol, 15 equiv) was finally added, and the reactor was sealed and heated to 90 ºC. The initially orange heterogenous mixture turned a clear red upon heating. After 24 h, the reaction was allowed to reach ambient temperature and concentrated *in vacuo*. The resulting crude residue was dissolved in THF (0.64 mL) and 4-methoxyphenylisocyanate (21 µL, 0.16 mmol, 1.0 equiv) was added slowly. The

resulting solution was stirred at ambient temperature for 10 min, following which the reaction was quenched by addition of MeOH, and concentrated *in vacuo*. The crude residue was purified by chromatography (MeOH/DCM) to furnish (+)-**4.27** (41 mg, 57%) as an off-white solid. **R_f** 0.58 (10% MeOH in CH_2Cl_2). **$[\alpha]^{25}_D$** = +36.9 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 2928, 1636, 1511, 1238, 1035, 804. **^{1}H NMR** (400 MHz, $CDCl_3$, 300 K) δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 9.0 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.03 (s, 1H), 3.77 (s, 3H), 3.74 – 3.60 (m, 4H), 3.53 (td, *J* = 8.3, 2.6 Hz, 1H), 3.47 – 3.36 (m, 1H), 3.30 (t, *J* = 8.0 Hz, 1H), 2.92 – 2.80 (m, 1H), 2.47 (ddd, *J* = 11.9, 5.4, 3.1 Hz, 1H), 2.32 (dd, *J* = 14.5, 11.2 Hz, 1H), 1.91 – 1.78 (m, 1H), 1.70 (qd, *J* = 10.3, 7.0 Hz, 2H), 1.63 – 1.51 (m, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$, 300 K) δ 155.7, 155.0, 138.8, 132.2, 131.4 (2C), 130.4, 122.0, 120.4, 114.1, 65.9, 59.0, 58.7, 55.5, 51.2, 47.7, 37.2, 27.0, 24.3. **HRMS** (ESI^+) **m/z** Calculated for $C_{22}H_{27}BrN_3O_2$ $[M+H]^+$ 444.1287; Found 444.1298.



BRD3914

(8*R*,9*S*)-*N*-(4-methoxyphenyl)-9-(4-(phenylethynyl)phenyl)-1,6-diazabicyclo[6.2.0]decane-6-carboxamide (BRD3914). Acetonitrile (0.3 mL), phenylacetylene (33 µL, 0.30 mmol, 5.0 equiv), and triethylamine (34 µL, 0.24 mmol, 4.0 equiv), all previously sparged with N_2 for 60 min, were added to a vial containing (+)-**4.27** (27 mg, 0.061 mmol, 1.0 equiv). XPhos-Pd-G3 (5.1 mg, 6.1 µmol, 10 mol%) was added, and the vial was sealed and heated to 70 ºC. After 2 h, the reaction was allowed to cool to ambient temperature, saturated $NaHCO_3$ (1 mL) was added, and the mixture was extracted with DCM (3x). The combined organic layers were dried over

anhydrous Na_2SO_4, filtered over cotton and concentrated *in vacuo*. The residue was purified by chromatography (MeOH/DCM) to afford BRD3914 (26 mg, 92%) as a pale brown oil. **R_f** 0.61 (10% MeOH in CH_2Cl_2). **$[\alpha]^{25}_D$** = +81.2 (c = 1.0, $CHCl_3$). **IR** (thin film) ν_{max} 3317, 2926, 2845, 1636, 1511, 1232, 1178, 1036, 827, 756. **^{1}H NMR** (400 MHz, $CDCl_3$, 300 K) δ 7.59 – 7.48 (m, 4H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 6.1 Hz, 3H), 7.29 – 7.19 (m, 2H + $CHCl_3$), 6.82 (d, *J* = 8.5 Hz, 2H), 6.03 (s, 1H), 3.77 (s, 3H), 3.75 – 3.65 (m, 3H), 3.59 (t, *J* = 8.5 Hz, 1H), 3.50 – 3.37 (m, 1H), 3.32 (t, *J* = 8.0 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.55 – 2.44 (m, 1H), 2.36 (dd, *J* = 14.2, 10.9 Hz, 1H), 1.91 – 1.78 (m, 1H), 1.71 (t, *J* = 9.2 Hz, 2H), 1.64 – 1.52 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$, 300 K) δ 155.7, 155.0, 140.2, 132.2, 131.59, 131.56, 128.7, 128.3, 128.2, 123.4, 121.9, 121.5, 114.1, 89.33, 89.31, 66.1, 58.9, 58.7, 55.5, 51.2, 47.6, 37.6, 26.9, 24.3. **HRMS** (ESI^+) **m/z** Calculated for $C_{30}H_{32}N_3O_2$ $[M+H]^+$ 466.2495; Found 466.2503.

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