Design, Synthesis, and Study of Lincosamide Antibiotics Containing a Bicyclic Amino Acid Moiety

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Design, Synthesis, and Study of Lincosamide Antibiotics

Containing a Bicyclic Amino Acid Moiety

A dissertation presented by
Katherine J. Silvestre
to
The Department of Chemistry and Chemical Biology
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
in the subject of
Chemistry

Harvard University
Cambridge, Massachusetts
November 2018
Design, Synthesis, and Study of Lincosamide Antibiotics

Containing a Bicyclic Amino Acid Moiety

Abstract

The lincosamide class has a small but important place in the armament of current antibiotics. Clindamycin, a semi-synthetic derivative of the natural product lincomycin, was first approved for clinical use in 1970 and has found continuous utility against Gram-positive bacterial infections, including Streptococi and methicillin-resistant S. aureus (MRSA). However, clinical usage of clindamycin is limited by its narrow spectrum of activity, increasingly prevalent antibacterial resistance, and its documented propensity to promote secondary C. difficile infections. To address these concerns, a fully synthetic, convergent approach to the development of next-generation lincosamide antibiotics was begun, with the hope of discovering a new candidate for the treatment of bacterial infections.

Prior work on the lincosamide project established bicyclic amino acids as a novel scaffold with promising antibacterial activity. These bicyclic amino acid scaffolds relied on a β-hydroxy-γ-allyl proline derivative (1.16) as a key synthetic intermediate. This dissertation presents a novel chiral pool strategy to the synthesis of 1.16, starting with the naturally occurring amino acid trans-γ-hydroxyproline (2.1). This chiral pool route enabled the preparation of 1.16 from 2.1 in 5 steps on multigram-scale, reducing the longest linear sequence from 11 steps to 5 steps while also enabling late-stage diversification at the γ-position.
This dissertation also describes a variety of chemical methods for diversification and exploration of the bicyclic scaffold 1.18 with the goal of improving its antibacterial activity. First, a pyrrolidino-oxazepane scaffold (3.6) was synthesized through an ozonolysis–reductive amination sequence, enabling direct substitution on the amine functionality. Next, a vinyl triflate was formed on the pyrrolidino-oxepine to establish a powerful handle for diversification. The vinyl triflate (4.2a) could be substituted through either palladium- or iron-catalyzed cross-coupling reactions to access a wide range of aryl and alkyl substituents. Through transformations of the vinyl triflate, structure–activity relationships around the bicycle were developed and refined. Many of these substituted bicyclic derivatives demonstrate superior antibacterial activity to clindamycin, and several of them show significant efficacy against clindamycin-resistant bacterial strains. The lincosamide analog FSA-513018b has emerged as a promising lead compound with excellent *in vitro* activity against multiple drug-resistant clinical isolates.
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Acknowledgments

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I am thankful to my undergraduate mentors for encouraging me in the beginning of my research career. Professor John M. Essigmann welcomed me into his research laboratory as an inexperienced first year and gave me the freedom to explore my growing interest in synthetic chemistry, while always making me feel like a valued individual. Professor Timothy F. Jamison was the best academic advisor I could have asked for, providing me with sage advice on problems both chemical and otherwise throughout my undergraduate years and even opening his laboratory to me to help further the synthetic side of my research project.

I am grateful to Professor Andrew G. Myers for his mentorship over the past four years. During my first year of graduate school, I felt woefully underqualified to join the Myers laboratory, with relatively little synthetic experience. Yet I saw something in Professor Myers’s lectures and in his research that deeply interested and inspired me, and I hoped that I could learn and grow under his tutelage. Thank you for giving me the opportunity to join your group, and for your mentorship and guidance throughout my graduate studies. I would also like to thank Professor
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My experience in the Myers group has also been shaped by my colleagues, who have all contributed to my learning and influenced the course of my graduate studies. I am especially thankful to Dr. Pavol Jakubec, for his unfailing support and encouragement during my first year while I was still getting my bearings; Dr. Udara Premachandra, for sharing his passion about reaction mechanisms with me and providing honest and constructive feedback on my work; and Finn Burg, for his inspiring chemistry and for bringing much needed laughter to our bay. In addition, I thank the members of the lincosamide team, Dr. Matt Mitcheltree, Jack Stevenson, and Dr. Amarnath Pisipati, for their collaborative contributions to this project. The lincosamide project truly was a team effort, and I cannot imagine this team without any one of you.

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List of Abbreviations

A adenosine
Å ångstrom
Ac acetyl
acac acetylacetonate
AIBN azobisisobutyronitrile
Alloc allyloxy carbonyl
arm aminoglycoside resistance methyltransferase gene
B. fragilis Bacteroides fragilis
Bn benzyl
Boc tert-butoxy carbonyl
BQ benzoquinone
BSTFA N,O-bis(trimethylsilyl)trifluoroacetamide
Bu butyl
C cytosine
°C degrees Celsius
calc’d calculated
C. difficile Clostridium difficile
C. scindens Clostridium scindens
dba dibenzylideneacetone
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DCE 1,2-dichloroethane
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<th>Full Form</th>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
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<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
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<td>DMDO</td>
<td>dimethyldioxirane</td>
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<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess–Martin periodinane</td>
</tr>
<tr>
<td>DMS</td>
<td>dimethyl sulfide</td>
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<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
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<tr>
<td>E. faecalis</td>
<td>Enterococcus faecalis</td>
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<tr>
<td>E. faecium</td>
<td>Enterococcus faecium</td>
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<tr>
<td>ee</td>
<td>enantiomeric excess</td>
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<tr>
<td>equiv.</td>
<td>molar equivalent</td>
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<tr>
<td>erm</td>
<td>erythromycin ribosome methyltransferase genes</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>FIA</td>
<td>flow injection analysis</td>
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<tr>
<td>FSA</td>
<td>fully synthetic antibiotic</td>
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<tr>
<td>FTIR</td>
<td>Fourier-transform infrared spectroscopy</td>
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<td>g</td>
<td>gram</td>
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<tr>
<td>G</td>
<td>guanosine</td>
</tr>
<tr>
<td>GI₅₀</td>
<td>50% growth inhibition</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>HATU</td>
<td>1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate</td>
</tr>
<tr>
<td>H–G I</td>
<td>Hoveyda–Grubbs catalyst, 1st generation</td>
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<td><em>H. influenzae</em></td>
<td><em>Haemophilus influenzae</em></td>
</tr>
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<td><em>H. marismortui</em></td>
<td><em>Haloarcula marismortui</em></td>
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<td>HMDS</td>
<td>hexamethyldisilazane</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-pressure liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>hVISA</td>
<td>heterogeneous vancomycin-intermediate <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
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<tr>
<td><em>i</em></td>
<td>iso</td>
</tr>
<tr>
<td><em>J</em></td>
<td>coupling constant</td>
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<td>KHMDS</td>
<td>potassium hexamethyldisilazide</td>
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<td>KPC</td>
<td><em>Klebsiella pneumoniae</em> carbapenemase gene</td>
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<td><em>K. pneumoniae</em></td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>lithium hexamethyldisilazide</td>
</tr>
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<td>LiTMP</td>
<td>lithium 2,2,6,6-tetramethylpiperidinide</td>
</tr>
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<td>LptD</td>
<td>lipopolysaccharide assembly protein D</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
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<tr>
<td><em>m</em>-CPBA</td>
<td><em>meta</em>-chloroperbenzoic acid</td>
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mef  macrolide efflux gene
Mes  mesityl (2,4,6-trimethylphenyl)
mg  milligram
MHz  megahertz
MIC  minimal inhibitory concentration
min  minute
MLSB  macrolide, lincosamide, and streptogramin B
mol  mole
MRSA  methicillin-resistant *Staphylococcus aureus*
MS  mass spectrometry
Ms  methanesulfonyl
msr  macrolide–streptogramin resistance gene
MTBE  methyl tert-butyl ether
MTL  methylthiolincosamine
MW  molecular weight
n  normal
NaHMDS  sodium hexamethyldisilazide
NBS  *N*-bromosuccinimide
NMR  nuclear magnetic resonance
NMP  *N*-methyl-2-pyrrolidone
NPET  nascent peptide exit tunnel
NT  not tested
*P. falciparum*  *Plasmodium falciparum*
<table>
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<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>pH</td>
<td>decimal negative logarithm of proton activity</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Pr</td>
<td>propyl</td>
</tr>
<tr>
<td>PTC</td>
<td>peptidyltransferase center</td>
</tr>
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<td>Pyr</td>
<td>pyridine</td>
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<tr>
<td>Rf</td>
<td>retention factor</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
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<td>s</td>
<td>secondary</td>
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<td>S. aureus</td>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td>S. pneumoniae</td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td><em>Streptococcus pyogenes</em></td>
</tr>
<tr>
<td>T</td>
<td>thymidine</td>
</tr>
<tr>
<td>t</td>
<td>tertiary</td>
</tr>
<tr>
<td>t-AmylOH</td>
<td>*tert-*amyl alcohol</td>
</tr>
<tr>
<td>TBHP</td>
<td>*tert-*butyl hydroperoxide</td>
</tr>
<tr>
<td>TBS</td>
<td>*tert-*butyldimethylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyle</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>TMP</td>
<td>2,2,6,6-tetramethylpiperidine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
<td>------------------------------------------------</td>
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<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>tRNA</td>
<td>transfer ribonucleic acid</td>
</tr>
<tr>
<td>U</td>
<td>uridine</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VISA</td>
<td>vancomycin-intermediate <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>VRE</td>
<td>vancomycin-resistant <em>Enterococcus</em></td>
</tr>
<tr>
<td>z</td>
<td>charge</td>
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<tr>
<td>δ</td>
<td>chemical shift</td>
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Chapter 1.

Introduction
History of the lincosamide class

The titular member of the lincosamide class of antibiotics is lincomycin (1.1), a natural product produced by the soil bacterium *Streptomyces lincolnensis* (*S. lincolnensis*) (Figure 1.1). In 1963, scientists at The Upjohn Company isolated lincomycin from fermentation broths of *S. lincolnensis*. Lincomycin was found to have good antibacterial activity against Gram-positive bacteria *in vitro*, including drug-resistant clinical strains of *S. aureus*, as well as low acute toxicity in rats and mice. In 1964, Upjohn scientists correctly assigned the structure of lincomycin and found it to be structurally unrelated to any major antibiotic known to date. Lincomycin is composed of the methyl thioglycoside of an aminooctose sugar, dubbed methylthiolincosamide, linked via an amide bond to 4'-n-propylhygric acid. That same year, lincomycin hydrochloride was approved by the U.S. Food and Drug Administration (FDA) for clinical use.

![Lincomycin and Clindamycin](image)

**Figure 1.1.** Clinically approved lincosamide antibiotics.

Having established the chemical structure of lincomycin, The Upjohn Company began work on semi-synthetic modifications. As early as 1965, Upjohn scientists reported that invertive replacement of the 7-hydroxyl group with chlorine significantly improved the *in vitro* and *in vivo* antibacterial activity of lincomycin and 4'-alkyl analogs. Compared to lincomycin, the 7-deoxy-7(S)-chloro analog clindamycin (1.2) demonstrated improved potency against Gram-positive and Gram-negative bacteria, even lower acute toxicity in rats, and more rapid oral absorption in
humans. Additionally, clindamycin showed slight activity against *P. falciparum*, suggesting potential for use as an antimalarial therapeutic. Clindamycin hydrochloride was approved by the U.S. FDA in 1970 for clinical use. Almost half a century later, clindamycin remains the only clinically relevant antibiotic in the lincosamide class.

In the decades following clindamycin’s approval, the lincosamide class has been subject to additional research aimed at further improvements to clindamycin’s therapeutic profile. The Upjohn Company continued their semi-synthetic work and replaced the 4’-propylhygric acid portion of clindamycin with azetidine, piperidine, and azepane amino acids. Upjohn scientists found that the cis-4’-ethylpiceolic acid derivative pirlimycin (1.3) had similar *in vitro* activity to clindamycin against susceptible bacteria, with improved *in vivo* potency and lower acute toxicity in mice. Ultimately, however, The Upjohn Company diverted pirlimycin into veterinary medicine, where it is used for the treatment of bovine mastitis.

In the early 2000s, Vicuron Pharmaceuticals began a renewed exploration of the lincosamide class, with the goal of bringing a next-generation lincosamide to the clinic. Their approach explored modifications on the C1 and C6 positions of the sugar moiety, as well as revisiting the azetidine, piperidine, and azepane-amino acid scaffolds first investigated by
Vicuron scientists discovered that, while the unsubstituted azepane amino acid had already been synthesized and evaluated at Upjohn,\(^9\) the addition of a cis-5’-substituent to the azepane yielded remarkable improvements in antibacterial activity. Additionally, Vicuron’s most active analog (1.4) displayed unprecedented activity in the Gram-negative pathogen *H. influenzae*.\(^{12}\) The expanded spectrum of activity against *H. influenzae* would give these lincosamide analogs potential clinical utility in the treatment of community-acquired bacterial pneumonia (CAP), most commonly caused by *S. pneumoniae* and *H. influenzae*.\(^{11,13}\) However, Vicuron was acquired by Pfizer in 2005, and their promising azepane lincosamide analogs never made it to the clinic.

In the 2010s, Meiji Seika Pharma Co. has re-examined the C7-position of the lincosamides, in a targeted effort to overcome the macrolide, lincosamide, streptogramin B (MLS\(_B\))-resistance phenotype.\(^{14-24}\) The rise of MLS\(_B\)-resistance is of particular concern in Asia, where the prevalence of antimicrobial-resistant *S. pneumoniae* is increasing.\(^{25}\) Scientists at Meiji Seika systematically explored 7-deoxy-7(S)-thiolincomycin derivatives, including aryl- and azetidinyl-sulfide substituents. Through rigorous iteration and optimization at the C7-position, multiple compounds with excellent activity against MLS\(_B\)-resistant *S. pneumoniae* and *S. pyogenes* were identified, and two lead compounds (1.5 and 1.6) were further evaluated in a rat pulmonary infection model.\(^{20}\) Despite these promising *in vitro* and *in vivo* data, it remains unclear if Meiji Seika intends to bring a lincosamide analog into clinical development.

**Mechanism of action**

The molecular target of the lincosamide class was initially established via chemical footprinting. Both lincomycin and clindamycin were found to bind to the 50S subunit of the
bacterial ribosome. As a class, the lincosamides are bacteriostatic, as they prevent bacterial reproduction through ribosome inhibition but do not directly kill bacteria. The lincosamide antibiotics bind specifically at the peptidyl transferase center (PTC) of 23S rRNA, where the peptide bond between the incoming amino acid and the nascent peptide is formed. These findings were later confirmed by X-ray crystallographic studies of lincosamide antibiotics bound to bacterial ribosomes (Figure 1.3). The octose moiety of clindamycin binds to the nascent peptide exit tunnel (NPET), overlapping with the binding site of the desosamine sugar of macrolide antibiotics such as erythromycin. The hydroxyl groups of the octose and the amide bond form an extensive hydrogen bonding network with the 23S rRNA (Figure 1.4). These hydrogen bonds are essential for target engagement, as modification of either the sugar hydroxyl groups or the residues engaged in hydrogen bonding has deleterious effects on transcription inhibition. The \( n \)-propylhygic acid moiety occupies the PTC, overlapping with the binding site of other antibiotics such as linezolid and chloramphenicol. The \( n \)-propyl chain extends into the A-site, making additional van der Waals interactions within a hydrophobic cleft formed by A2451 and C2452. These hydrophobic contacts also play an important role in the activity of lincosamide antibiotics, as removal of the \( n \)-propylhygic acid moiety greatly reduces transcription inhibition activity \textit{in vitro}.\textsuperscript{33}
Figure 1.3. Crystal structure of clindamycin bound to the ribosome of *H. marismortui*. Data from PDB entry 1YJN.²⁹

Figure 1.4. Schematic representation of important interactions between clindamycin and its ribosomal binding site. Dashed lines represent hydrogen bond contacts; curved lines represent van der Waals interactions.

**Clinical utility and limitations**

Since clindamycin’s approval by the U.S. FDA in 1970, it has maintained a presence in human medicine and is listed as an essential medicine by the World Health Organization. Clindamycin has activity against a variety of Gram-positive pathogens such as *S. aureus*, *S. pneumoniae*, and *S. pyogenes*, as well as certain Gram-negative anaerobes such as *B. fragilis*.³⁴
However, it is ineffective against many important Gram-negative pathogens, such as *E. coli* and *K. pneumoniae*. Clindamycin can be administered orally or parenterally, with good bioavailability\(^{35-36}\) and excellent penetration into soft tissue\(^{36}\) and bone\(^{37}\). Additionally, clindamycin is available as two pro-drug forms: clindamycin palmitate, an oral suspension formulation that reduces the unpleasant taste of clindamycin hydrochloride,\(^{38}\) and clindamycin-2-phosphate, a formulation for parenteral administration.\(^{39}\) Clindamycin has seen clinical use for a number of indications, including skin and soft tissue infections, osteomyelitis, dental infections, and gynecological infections.\(^{34}\) Clindamycin is a good alternative to β-lactams for patients who are allergic to penicillin and is commonly used in the treatment of methicillin-resistant *S. aureus* (MRSA), although mounting resistance has reduced its utility for this indication.\(^{40}\) The bacteriostatic properties of lincosamide antibiotics can be advantageous for clinical applications, as clindamycin treatment can reduce toxin production by *S. aureus* and streptococci, even when administered at sub-inhibitory concentrations.\(^{34}\)

However, acquired resistance to clindamycin has become increasingly reported in the clinic. The majority of clindamycin-resistant strains show the macrolide, lincosamide, streptogramin B (MLS\(_B\))-resistance phenotype. MLS\(_B\)-resistance is conferred by erythromycin resistance methyltransferase (*erm*) genes, which have been found in a variety of Gram-positive and Gram-negative pathogens. This resistance can either be inducible or constitutive. In cases of inducible MLS\(_B\)-resistance, *erm* expression is activated by the presence of a macrolide antibiotic.\(^{41}\) The encoded Erm enzyme dimethylates N\(^6\) of A2058 in the 23S rRNA. Dimethylation of A2058 eliminates the ability of this exocyclic amine to hydrogen bond with the lincosamide sugar, thereby disrupting the key hydrogen bonding network essential for clindamycin’s activity (Figure 1.5).\(^{29}\)
As A2058 is involved in binding macrolide and streptogramin B antibiotics as well, Erm confers cross-resistance to these classes of antibiotics.\textsuperscript{42}

![Figure 1.5](image_url)

**Figure 1.5.** Schematic representation of hydrogen bond interactions between clindamycin and A2058 (A). These hydrogen bonds are disrupted by N\textsuperscript{6}-dimethylation of A2058 by Erm (B).

One of the main clinical limitations of clindamycin is its propensity to promote secondary *C. difficile* infections (CDI). This issue was first noted in 1974, in an anonymous account of acute ulcerative colitis observed upon treatment with clindamycin.\textsuperscript{43} A study of clindamycin-induced colitis in hamsters identified the causative agent as a clindamycin-resistant species of *Clostridium*, named *C. difficile*.\textsuperscript{44} A variety of bacterial species in the human microbiome suppress *C. difficile* infection in healthy individuals. However, treatment with certain antibiotics such as clindamycin can severely disrupt the distribution of species in the microbiome, allowing *C. difficile* to overgrow and produce toxins.\textsuperscript{45-46} While nearly all antibiotics have some innate risk of causing CDI, clindamycin, third-generation cephalosporins, penicillins, and fluoroquinolones are considered to be particularly high risk.\textsuperscript{47} The concern over clindamycin’s propensity to cause secondary CDI is such that the FDA gave clindamycin a boxed warning for the risk of *C. difficile* associated diarrhea (CDAD).\textsuperscript{8} Thus, the risk of life-threatening CDI as an adverse effect may limit clinical use of clindamycin on a prescriber level.

**Previous work on this project**

In 2013, the Myers lab began a project aimed at the discovery of new lincosamide antibiotics, analogous to earlier work on the tetracycline\textsuperscript{48-49} and macrolide\textsuperscript{50} classes of antibiotics.
The lincosamide project sought to develop a convergent, fully synthetic platform that would allow for rapid diversification and exploration of novel scaffolds. While previous efforts at the development of new lincosamide antibiotics have been limited by the constraints of semi-synthesis, a fully-synthetic approach would enable the discovery of scaffolds that would be inaccessible via semi-synthesis and could create new opportunities to build structure–activity relationships. The goal of the lincosamide project was to discover novel lincosamide analogs that addressed the key limitations of clindamycin in clinical medicine: overcoming the MLSB resistance phenotype that has hindered its use in Gram-positive bacteria, expanding the spectrum of antibiotic activity into Gram-negative bacteria, and reducing activity against commensal bacteria to ameliorate the C. difficile liability.

The early stages of the lincosamide project, pioneered by Dr. Matthew Mitcheltree, focused on exploration of the amino acid moiety via a novel β-hydroxy-γ-allyl proline derivative.\textsuperscript{51} Previous work in the Myers laboratory established a diastereoselective aldol reaction between pseudoephenamine glycinamide and aldehyde or ketone coupling partners to yield β-hydroxy-α-amino acid derivatives (Figure 1.6).\textsuperscript{52} Relatedly, earlier research had shown that the related pseudoephedrine glycinamide could undergo sequential C- and N-alkylation to form cyclic amino acid.\textsuperscript{53} Based on these results, Dr. Mitcheltree selected β-hydroxy-γ-allyl proline as an initial synthetic target, with the β-hydroxy established by a diastereoselective aldol and the pyrrolidine ring formed by \textit{in situ} cyclization of the linear aldol coupling product to give 1.15. The β-hydroxyl substituent provided a versatile synthetic handle on an unexplored position of the proline moiety, while the γ-allyl group allowed for either diversification or simple conversion to the parent n-propyl chain via hydrogenation.
A variety of lincosamide analogs were prepared from this β-hydroxy-γ-allyl proline derivative; however none of them showed improved activity over clindamycin. In order to restrict the conformation of the $n$-propyl chain, the β-hydroxy-γ-allyl proline 1.16 was converted into a bicyclic pyrrolidinoxepine through $O$-allylation and ring-closing metathesis (Figure 1.7). The resulting bicyclic analog, FSA-24035, demonstrated nearly equipotent activity to clindamycin against Gram-positive bacteria. This novel scaffold represented an early lead that was believed to have significant potential for further optimization. By computationally docking FSA-24035 and overlaying it with the published X-ray crystal structure of clindamycin bound to the bacterial ribosome, it appeared that there was space within the hydrophobic wedge to further expand off the oxepine ring. This structural observation is supported by early SAR work at The Upjohn Company, which found that extending the $n$-propyl chain of clindamycin yields improved activity up to a maximum of an $n$-pentyl chain. The concurrence of the structural space in the binding site and the chemical versatility of the well-positioned olefin in FSA-24035 provided a fortuitous opportunity to extend the bicyclic scaffold and develop new SAR, within the overarching goal of
improving antibacterial activity. The synthetic work on the bicyclic scaffold and the activity of the resultant antibiotics form the subject of this dissertation.

![Synthesis of FSA-24035](image)

**Figure 1.7. Synthesis of FSA-24035.**

The second chapter of this dissertation describes the development of a second-generation route to β-hydroxy-γ-allyl proline 1.16 that leverages a chiral pool strategy to shorten and simplify preparation of this key intermediate. The third chapter details exploration of a bicyclic pyrrolidinooxazepane series of analogs and initial attempts at extending the canonical lincosamide binding pocket further into the A-site through an endocyclic nitrogen handle. The fourth and fifth chapters describe the use of a vinyl triflate formed at the 7'-position to branch off the oxepine ring, the former focusing on aryl substituents via Suzuki–Miyaura and Sonogashira cross-coupling reactions and the latter detailing an iron-catalyzed cross-coupling reaction to access alkyl substituents. Through the work described herein, new structure–activity relationships around the amino acid moiety of clindamycin has been developed and a lead candidate with excellent activity against a variety of MLSB-resistant Gram-positive bacteria has been identified.
Chapter 2.

A chiral pool approach to β-hydroxy-γ-allyl proline
Introduction

In the first year of the lincosamide project, Dr. Mitcheltree designed and synthesized a β-hydroxy-γ-allylproline derivative (1.16) as a key intermediate for exploration of the amino acid moiety. The initial route to 1.16 utilized a stereoselective aldol coupling between (R,R)-pseudoephenamine glycinamide 1.13 and the chirally enriched aldehyde 1.14 to establish the α- and β-stereocenters (Figure 2.1). The linear aldol product then cyclizes in situ to establish the pyrrolidine ring. The key intermediate 1.16 is obtained after hydrolysis of the pseudoephenamine auxiliary and protection of the amine and carboxylate functionalities.51

Efforts to scale the initial route to synthesize multigram quantities of 1.16 revealed several shortcomings. First, while the aldol reaction is a convergent coupling, each of the precursors for the aldol reaction requires several steps to prepare (Appendix Figure 1, 2).51, 54 As a result, the longest linear sequence in the preparation of 1.16 is 11 steps. Next, the γ-allyl substituent is set from the very first step of the sequence and its chirality is established via an enzymatic desymmetrization. Thus, alteration of the γ-substituent would have to be done early in the sequence and a modified substrate might not be compatible with the enzyme previously used for
desymmetrization, requiring additional optimization for any modification to the γ-substituent. Finally, the aldol reaction itself became operationally difficult to scale up beyond a 1–2 g scale. The reaction requires rigorously anhydrous conditions and is run at cryogenic temperatures, which became increasingly challenging to maintain at multigram scale. Additionally, a method to follow the course of the reaction had not been reported, and monitoring consumption of the aldehyde coupling partner 1.14 was not suitable due to its inherent instability. Given these shortcomings and the need for larger quantities of 1.16 to fuel downstream scaffold exploration, a shorter, simpler route to the key intermediate 1.16 was desired.

**Retrosynthetic analysis**

In considering an alternate route to 1.16, a chiral pool approach to this synthesis was examined for the first time (Figure 2.2). By using a natural L-proline derivative, the stereochemistry at the α-position would be established from the start, eliminating the need for the pseudoephedrine chiral auxiliary. The readily available, nonproteinogenic amino acid *trans-γ*-hydroxyproline had good potential as a chiral pool precursor; the desired stereochemistry at the α-position would be set from the outset, while the hydroxyl group could facilitate transformations at both the β- and γ-positions. The initial retrosynthesis from 1.16 to the protected γ-hydroxyproline 2.1 was straightforward and had the potential to substantially reduce the longest linear sequence. A regioselective opening of the corresponding *cis*-epoxide 2.3 could install the γ-allyl substituent. The *cis*-epoxide 2.3 could be made from the β,γ-unsaturated proline 2.2, which could be prepared by dehydration of γ-hydroxyproline 2.1. Thus, exploration of the proposed alternate route was begun with this framework in mind.
Development of new route

There was literature precedent for the dehydration of $\gamma$-hydroxyproline 2.1 to 2.2.\textsuperscript{55} Initial attempts at a Mitsunobu-type iodination of the hydroxyl group followed by elimination upon heating with DBU were successful, and isolated yields were similar to those reported (Figure 2.3). However, the use of DBU under harsh conditions raised concerns about the potential for epimerization of the labile $\alpha$-proton. To determine whether the enantiopurity of the starting material was maintained, a sample of 2.2 prepared by this method was analyzed using Mosher’s ester analysis. The ester was reduced to the corresponding alcohol, the prolinol derivative was coupled to Mosher’s acid chloride, and the $N$-Boc protecting group was removed to simplify interpretation of the resultant NMR spectra. The resulting proton and fluorine NMR spectra showed evidence of substantial epimerization (Appendix Figure 3). As enantiopurity at the $\alpha$-position is essential for lincosamide activity, this method would not be suitable for further synthesis.

An alternative strategy for the dehydration of $\gamma$-hydroxyproline was also found in the literature.\textsuperscript{56} In this route, the hydroxyl group was activated by formation of the mesylate 2.5, then
displaced by diphenyl diselenide. The resulting selenide 2.6 could be oxidized by hydrogen peroxide and underwent elimination to give 2.2 (Figure 2.4). Mosher’s ester analysis was performed on the material prepared by this method, and no sign of epimerization was present (Appendix Figure 3). While this method gave the desired product as a single enantiomer in moderate yield, the toxicity of organoselenides and the need for multiple reactions and purifications for the dehydration step alone were serious limitations for its scalability and utility.

![Figure 2.4. Secondary strategy for dehydration of γ-hydroxyproline.](image)

Having exhausted the apparent literature precedent for this transformation, a shorter and simpler method than those published was desired. Martin’s sulfurane was tested in the hopes that this reagent would accomplish activation of the alcohol and dehydration in a single step under mild conditions to avoid epimerization. Fortunately, Martin’s sulfurane provided the desired product in excellent yield (Figure 2.5). Thus, by using Martin’s sulfurane, a single-step method for the regioselective dehydration of γ-hydroxyproline was established.

![Figure 2.5. Martin’s sulfurane for the dehydration of γ-hydroxyproline.](image)

The next step in the proposed route was epoxidation of 2.2 to give the cis-epoxide 2.3. In agreement with literature precedent, the unsaturated proline 2.2 was found to undergo epoxidation with m-CPBA by refluxing in dichloroethane with Kishi’s radical inhibitor to prevent m-CPBA
decomposition (Figure 2.6). However, as reported, these epoxidation conditions favored the undesired trans-epoxide 2.7 over the cis-epoxide 2.3 in a 2:1 trans:cis ratio. Therefore, a new method of epoxidation was needed to favor the cis-epoxide.

![Figure 2.6. Initial attempts at epoxidation of 2.2.](image)

A variety of epoxidation conditions were screened on 2.2 (Figure 2.7). A Jacobsen–Katsuki epoxidation was attempted with (S,S)-Jacobsen’s catalyst, in the hope that the exogenous salen-like ligand would control facial selectivity; however, no product formation was observed and starting material was recovered. Vanadium and molybdenum catalysts were employed to try to leverage a directing effect from the α-methyl ester or carboxylate, as these catalysts have been reported to perform directed epoxidations on homoallylic alcohols. Similarly, no product formation was observed with these catalysts. Treatment with DMDO yielded a mixture of cis- and trans-epoxides, as well as the allylic oxidation byproduct 2.8. Other enantioselective epoxidation conditions reported in the literature relied on an allylic alcohol to exert a directing effect or had poor precedent for 1,2-cis-disubstituted olefins, and thus were not attempted.
As an alternative to direct epoxidation of the olefin, **2.2** was treated with *N*-bromosuccinimide (NBS) to give the bromohydrin product **2.9**.\(^{62}\) Due to the size of bromine, the bromonium ion intermediate is formed *trans* to the α-substituent. When the bromonium ion is opened via anti addition of water, the bromohydrin product has the hydroxyl group *cis* to the methyl ester. The bromohydrin product **2.9** can then be treated by potassium carbonate to eliminate bromide and form the desired *cis*-epoxide **2.3** in good yield (Figure 2.8).\(^{63}\)

The final step was regioselective opening of **2.3** to install an allyl substituent at the \(\gamma\)-position. Initial attempts at direct epoxide opening were unsuccessful (Figure 2.9). Allyl Grignard reagents were found to react preferentially with the ester over the epoxide, forming the allyl ketone **2.10**. Addition of copper cyanide to catalyze Grignard addition\(^{64}\) did not improve selectivity. A higher-order allylcuprate was prepared *in situ*,\(^{65}\) but failed to undergo addition to either the ester or the epoxide. Attempts at a Sakurai allylation also resulted in no observed product formation.
Figure 2.9. Epoxide-opening conditions screened on 2.3.

However, treatment of 2.3 with magnesium iodide selectively opened the epoxide to give the iodohydrin 2.12. The iodohydrin successfully underwent a Keck allylation, in which the stereochemistry at the γ-position was determined by steric effects from the adjacent α- and β-substituents, and provided the key intermediate 1.16 with good diastereoselectivity and yield (Figure 2.10).

Figure 2.10. Alternative route to β-hydroxy-γ-allylproline 1.16 via iodination and Keck allylation.

The new route shortened the longest linear sequence in the synthesis of 1.16 from 11 steps to 5 steps and all the transformations in the new route have been successfully run on >2 g scale with consistent yields (Figure 2.11). Additionally, the modified route allows for late stage incorporation of alternative γ-substituents without increasing the step count or requiring re-optimization of later steps, something that was not possible with the previous route. As a
demonstration of this capability, the 2-chloroallyl variant 2.13 was synthesized through an analogous Keck allylation with 2-chloroallyl-tri-n-butylstannane.67

Figure 2.11. Completed route to 1.16 and example of late-stage diversification to make 2.13.

Conclusions

A second-generation route to 1.16 was developed to facilitate scale-up of this key intermediate. During the route scouting process, alternate methodology for the dehydration of γ-hydroxyproline was discovered to preserve the enantiopurity of the starting material. The new route reduced the longest linear sequence from 11 steps to 5 steps, while also allowing for late-stage diversification of the γ-position, something not possible in the previous route.
Experimental section

General experimental procedures

All reactions were performed in oven- or flame-dried glassware fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless-steel cannula. Organic solutions were concentrated by rotary evaporation at 23–35 °C. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60-Å pore size, 230–400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet (UV) light, then were stained by submersion in a basic aqueous solution of potassium permanganate (KMnO₄), followed by brief heating on a hot plate. Reaction monitoring by mass spectrometry was carried out by analytical liquid chromatography–mass spectrometry (LCMS) or by flow-injection analysis–high-resolution mass spectrometry (FIA-HRMS). Flash column chromatography was performed with silica gel (60-Å pore size, 230–400 mesh, Agela Technologies) according to the method of Still and co-workers.⁶⁸

Materials

Commercial reagents and solvents were used as received, with the following exceptions. Dichloromethane, diethyl ether, tetrahydrofuran, 1,4-dioxane, N,N-dimethylformamide, toluene, and benzene were purified by passage through alumina (Al₂O₃) under argon according to the method of Pangborn and co-workers.⁶⁹ Magnesium iodide (MgI₂) was prepared in situ by refluxing magnesium turnings and iodine in diethyl ether. Hexamethyldisilazane (HMDS), N,N-diisopropylamine, trimethylsilyl chloride (TMSCl), and methanol were distilled from calcium hydride under an atmosphere of dinitrogen at 760 mmHg. 7-Deoxy-7-chloromethylthiolincosamine (7-CI-MTL) was prepared according to the method of Lewis and co-
The molarity of $n$-butyllithium solutions was determined by titration against diphenylacetic acid as an indicator in triplicate according to the method of Kofron and co-workers.\textsuperscript{70}

**Instrumentation**

Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on Varian INOVA 500 (500 MHz/125 MHz) or Varian INOVA 600 (600 MHz) NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, $\delta$ scale) and are referenced to residual protium in the NMR solvent (CHCl$_3$, $\delta$ 7.26; CHD$_2$OD, $\delta$ 3.31). Carbon chemical shifts are expressed in parts per million (ppm, $\delta$ scale) and are referenced to the carbon resonance of the NMR solvent (CDCl$_3$, $\delta$ 77.00; CD$_3$OD, $\delta$ 49.15). Data are represented as follows: chemical shift, multiplicity ($s =$ singlet, $d =$ doublet, $t =$ triplet, $q =$ quartet, $dd =$ doublet of doublets, $td =$ triplet of doublets, $m =$ multiplet), integration, and coupling constant ($J$) in hertz (Hz). Infrared (IR) transmittance spectra were obtained using a Shimadzu 8400S FTIR spectrophotometer referenced to a polystyrene standard or a Bruker ALPHA FTIR spectrophotometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm$^{-1}$) and intensity of absorption ($s =$ strong, $m=$ medium, $w =$ weak, $br =$ broad). High-resolution mass spectrometry (HRMS) was performed at the Harvard University Mass Spectrometry Facility using a Bruker micrOTOF-QII mass spectrometer. Analytical LCMS was performed using an Agilent Technologies 1260-series analytical HPLC system equipped with a Zorbax Eclipse Plus reverse-phase C$_{18}$ column (2.1 x 50 mm, 1.8 $\mu$m pore size) in tandem with an Agilent Technologies 6120 Quadrupole mass spectrometer. Preparative high-pressure liquid chromatography (HPLC) was performed using a Waters SunFire prep C$_{18}$ column (5 $\mu$m, 250 x 19 mm) eluting with a gradient of acetonitrile in water (flow rate 15 mL/min),
with both solvents containing 0.1% trifluoroacetic acid or formic acid, monitoring by UV absorbance at 210 and 254 nm.

**Biological evaluation**

Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method previously described by the Clinical and Laboratory Standards Institute for aerobic and anaerobic bacteria. The test organisms were strains provided by ATCC, Micromyx LLC, or Macrolide Pharmaceuticals.
A solution of 2.1 (2.88 g, 11.7 mmol, 1.0 equiv.) in DCM (59 mL) was added via cannula over 10 min to a suspension of Martin’s sulfurane (11.85 g, 17.6 mmol, 1.5 equiv.) in DCM (59 mL) at 0 °C. The resultant faintly orange solution was stirred under argon, and the reaction was allowed to warm to ambient temperature as the ice-water bath expired. Consumption of 2.1 was monitored by TLC analysis (50% EtOAc in hexanes). After starting material was completely consumed by TLC analysis (16 hours), the solution was concentrated under reduced pressure and purified directly by column chromatography (10–20–30% EtOAc in hexanes) to yield 2.2 (2.41 g, 10.6 mmol, 90%) as a colorless oil.

R_f = 0.55 (50% EtOAc in hexanes, KMnO4).

^1^H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl_3) δ 6.02, 5.97* (m, 1H), 5.77*, 5.73 (m, 1H), 5.04*, 4.97 (m, 1H), 4.24 (m, 2H), 3.75 (s, 3H), 1.50*, 1.45 (s, 9H).

^1^3^C NMR (asterisk denotes minor rotamer peaks,126 MHz, CDCl_3) δ 170.91, 170.59*, 153.69*, 153.19, 129.23, 129.14*, 124.50, 79.99, 66.40, 66.05*, 53.35*, 53.11, 52.09*, 51.98, 28.24*, 28.11.

FTIR (neat, cm^-1): 2976 (w), 1756 (m), 1699 (s), 1391 (s), 1169 (s), 1124 (s), 990 (m), 768 (m), 686 (m).

HRMS (ESI+, m/z): [M+H]^+ calc’d for C_{11}H_{17}NO_4, 227.1158; found 227.1175.
Procedure for Mosher’s ester analysis of 2.2

Lithium aluminum hydride (1.0 N in diethyl ether, 0.34 mL, 0.344 mmol, 1.1 equiv.) was added dropwise by syringe to a stirring solution of 2.2 (0.071 g, 0.312 mmol, 1.0 equiv.) in diethyl ether (1.6 mL) at 0 °C. The resultant solution was stirred at ambient temperature under argon. Consumption of 2.2 was monitored by TLC (30% EtOAc in hexanes). After 1 hour, the reaction solution was cooled to 0 °C and quenched by sequential dropwise addition of 0.1 mL of water, 0.1 mL of 1.0 N aq. sodium hydroxide, and 0.2 mL of water. The solution was stirred while warming to ambient temperature and filtered through a fritted funnel, the filter cake was washed with ether, and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (30–40% EtOAc in hexanes) to yield 2.14 (0.053 g, 0.266 mmol, 85%). This material was divided into two portions and the subsequent steps were carried out in parallel with the (R)- and (S)-enantiomers of Mosher’s acid chloride.

(R)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.03 mL, 0.144 mmol, 1.1 equiv.) was added to a stirring solution of 2.14 (0.026 g, 0.130 mmol, 1.0 equiv.) and pyridine (0.02 mL, 0.261 mmol, 2.0 equiv.) in DCM (0.7 mL). The resultant solution was stirred at ambient temperature under argon. Consumption of 2.14 was monitored by TLC (30% EtOAc in hexanes). After 1 hour, the reaction was concentrated under reduced pressure to give 2.15 as a white solid. To remove carbamate rotamerism and simplify interpretation of the NMR spectra, the material was taken forward without purification to N-Boc deprotection.
Trifluoroacetic acid (0.5 mL, 6.50 mmol, 50.0 equiv.) was added dropwise by syringe to a stirring solution of **2.15** (0.054 g, 0.130 mmol, 1.0 equiv.) in DCM (0.7 mL) at 0 °C. The resultant solution was stirred at 0 °C under argon. After 1 hour, the reaction solution was diluted with 1 mL of toluene and concentrated under reduced pressure to give **2.16**. The crude product was analyzed by $^1$H and $^{19}$F NMR spectroscopy to determine if epimerization had occurred.
N-bromosuccinimide (2.83 g, 15.9 mmol, 1.5 equiv.) was added in a single portion to a stirring solution of 2.2 (2.41 g, 10.6 mmol, 1.0 equiv.) in THF (53 mL) and water (53 mL) at 0 °C. The resulting yellow solution was stirred under air, and the reaction was allowed to warm to ambient temperature as the ice-water bath expired. Consumption of 2.2 was monitored by TLC analysis (50% EtOAc in hexanes). After starting material was completely consumed by TLC analysis (16 hours), the reaction solution was diluted with water and extracted with ether (3 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give an orange oil. The crude product was purified by column chromatography (20–30–40% EtOAc in hexanes) to yield 2.9 (2.60 g, 8.01 mmol, 76%) as a colorless oil.

R<sub>f</sub> = 0.20 (50% EtOAc in hexanes, KMnO<sub>4</sub>).

<sup>1</sup>H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl<sub>3</sub>) δ 4.66*, 4.54 (s, 1H), 4.42*, 4.35 (m, 2H), 3.94 (m, 1H), 3.79 (s, 3H), 3.59 (dd, <i>J</i> = 19.5, 11.8 Hz, 1H) 1.50*, 1.44 (s, 9H).


FTIR (neat, cm<sup>-1</sup>): 3400 (br), 2977 (w), 1753 (m), 1673 (s), 1593 (s), 1367 (s), 1159 (s), 730 (s).

HRMS (ESI+, <i>m/z</i>): [M+H]+ calc’d for C<sub>11</sub>H<sub>18</sub>BrNO<sub>5</sub>, 323.0368; found 323.0382.
Potassium carbonate (1.66 g, 12.0 mmol, 1.5 equiv.) was added in one portion to a stirring solution of 2.9 (2.60 g, 8.02 mmol, 1.0 equiv.) in DMF (27 mL) and THF (54 mL). The resulting colorless solution was stirred at ambient temperature under argon and gradually became cloudy and faintly violet in color. Consumption of 2.9 was monitored by mass spectrometry. After 16 hours, the reaction solution was filtered through a pad of Celite, the filter cake was washed with ether, and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (30–40–50% EtOAc in hexanes) to yield 2.3 (1.50 g, 6.18 mmol, 77%) as a colorless oil.

$R_f = 0.40$ (50% EtOAc in hexanes, UV+KMnO₄).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl₃) δ 4.37*, 4.32 (s, 1H), 3.92 (s, 1H), 3.85–3.76 (m, 2H), 3.80 (s, 3H), 3.50 (dd, $J = 19.0, 12.7$ Hz, 1H), 1.40*, 1.38 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl₃) δ 168.59, 167.91*, 154.28*, 153.67, 80.63*, 80.57, 60.12, 59.84*, 57.81, 57.13*, 55.88*, 55.28, 52.44*, 52.32, 47.84*, 47.57, 28.23*, 28.05.

FTIR (neat, cm⁻¹): 2977 (w), 1743 (m), 1690 (s), 1524 (s), 1365 (s), 1156 (s), 1109 (s), 914 (m), 727 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C₁₁H₁₇NO₅, 243.1107; found 243.1128.
A 0.3 M solution of magnesium iodide (30.8 mL, 9.25 mmol, 1.12 equiv.) in diethyl ether was added dropwise by syringe to a stirring solution of 2.3 (2.00 g, 8.22 mmol, 1.0 equiv.) in toluene (164 mL) at 0 °C. The resultant cloudy orange solution was stirred at 0 °C under argon. Consumption of 2.3 was monitored by TLC analysis (50% EtOAc in hexanes). After complete starting material was completely consumed by TLC analysis (2 hours), the reaction was quenched with saturated aq. sodium thiosulfate and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a faintly yellow oil. The crude product was purified by column chromatography (20–30% EtOAc in hexanes) to yield 2.12 (2.91 g, 7.84 mmol, 95%) as a faintly yellow oil.

\[ R_f = 0.55 \text{ (50\% EtOAc in hexanes, UV+KMnO}_4). \]

\[^1\text{H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl}_3\text{) } \delta 4.60 \text{ (m, 2H), 4.52 (dd, } J = 11.6, 6.2 \text{ Hz, 1H)}, 4.16 \text{ (m, 1H), 4.06 (ddd, } J = 11.7, 11.1, 5.9 \text{ Hz, 1H)}, 3.69 \text{ (m, 1H), 3.69 (s, 3H)}, 1.40^*, 1.35 \text{ (s, 9H)}. \]

\[^{13}\text{C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl}_3\text{) } \delta 169.95, 169.90^*, 153.94^*, 153.54, 80.79, 79.22, 78.34^*, 62.06, 61.49^*, 54.32^*, 53.69, 52.16^*, 51.97, 28.09^*, 27.95, 24.32^*, 23.87. \]

FTIR (neat, cm\(^{-1}\)): 3399 (br), 2979 (w), 1745 (m), 1677 (s), 1394 (s), 1160 (s), 906 (s), 726 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C\(_{11}\)H\(_{18}\)INO\(_5\), 371.023; found 371.0253.
AIBN (0.134 g, 0.814 mmol, 0.2 equiv.) was added to a stirring solution of 2.12 (1.51 g, 4.07 mmol, 1.0 equiv.) and allyltri-n-butyltin (12.6 mL, 40.7 mmol, 10.0 equiv.) in toluene (40.7 mL). The resultant colorless solution was heated to 80 °C and stirred at 80 °C under argon. Consumption of 2.12 was monitored by mass spectrometry. After 12 hours, the reaction solution was concentrated under reduced pressure and purified directly by column chromatography (5–20–30–40–50% EtOAc in hexanes to yield 1.16 (0.915 g, 3.21 mmol, 79%) as a colorless oil.

R$_f$ = 0.30 (40% EtOAc in hexanes, KMnO$_4$).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 5.73 (m, 1H), 5.02 (d, $J=17.1$ Hz, 1H), 4.97 (m, 1H), 4.36*, 4.29 (d, $J=7.6$ Hz, 1H), 4.12 (m, 1H), 3.70 (m, 1H), 3.68 (s, 3H), 3.63 (m, 1H), 2.97 (m, 1H), 2.30 (m, 2H), 1.96 (m, 1H), 1.39*, 1.34 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 171.04, 154.20*, 153.69, 153.6*, 153.24, 116.62, 116.55*, 80.11, 80.04*, 75.40, 74.67*, 63.31, 62.71*, 51.87*, 51.72, 48.91*, 48.42, 43.10*, 42.45, 34.79, 28.17*, 28.04.

FTIR (neat, cm$^{-1}$): 3428 (br), 2979 (w), 1744 (m), 1677(s), 1401 (s), 1174 (s), 907 (s), 727 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{14}$H$_{23}$NO$_5$, 285.1576; found 285.1601.
AIBN (10.6 mg, 0.065 mmol, 0.3 equiv.) was added to a stirring solution of 2.12 (0.080 g, 0.216 mmol, 1.0 equiv.) and 2-chloroallyltrin-butylin (0.79 g, 2.16 mmol, 10.0 equiv.) in toluene (2.2 mL). The resultant colorless solution was heated to 80 °C and stirred at 80 °C under argon. Consumption of 2.12 was monitored by mass spectrometry. After 12 hours, the reaction solution was concentrated under reduced pressure and purified directly by column chromatography (5–20–30–40–50% EtOAc in hexanes to yield 2.13 (0.041 g, 0.129 mmol, 60%) as a colorless oil.

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 5.19–5.13 (m, 2H), 4.38*, 4.31 (d, $J = 7.6$ Hz, 1H), 4.14 (m, 1H), 3.79*, 3.71 (dd, $J = 10.8$, 7.9 Hz, 1H), 3.69 (s, 3H), 3.54 (d, $J = 5.3$ Hz, 1H), 3.51*, 3.01 (m, 1H), 2.64–2.53 (m, 2H), 2.20 (m, 1H), 1.38*, 1.33 (s, 9H).
Chapter 3.

Synthesis and study of pyrrolidinooxazepane lincosamide analogs
Introduction

With a new, shorter route to the key intermediate 1.16 in hand, focus was shifted to the synthesis of lincosamide analogs in the hopes of further exploring the pyrrolidinoxepine scaffold discovered by Dr. Mitcheltree. The goal of this effort was to develop structure–activity relationships around this novel bicyclic scaffold and to improve activity in clindamycin-resistant bacteria. One early synthetic target was the corresponding pyrrolidinoxazepane scaffold 3.1, formed by replacement of one of the carbon atoms in the oxepine ring with a nitrogen atom (Figure 3.1). The amine would provide a highly versatile handle for further functionalization and could facilitate extension of the canonical lincosamide binding pocket. Additionally, incorporation of a second basic amine in the amino acid moiety would significantly alter the physicochemical properties of the finished analogs in a way that might influence antibacterial activity. The isosteric replacement of a carbon with a nitrogen would increase the polarity of the finished lincosamide analogs, and the amine could potentially bear a second positive charge at physiological pH, factors that might expand the spectrum of activity into Gram-negative bacteria.

Figure 3.1. Pyrrolidinoxepine FSA-24035 inspired the design and synthesis of pyrrolidinoxazepane derivatives (3.1).

Synthesis of pyrrolidinoxazepane derivatives

To incorporate this amine functionality, Dr. Mitcheltree’s method for synthesis of pyrrolidinodihydropyran 3.2 was leveraged (Figure 3.2). Through a palladium-catalyzed olefin
isomerization, the allyl substituent in 1.16 can be converted to the corresponding disubstituted olefin 3.3. Subsequent O-allylation and ring-closing metathesis provide the [5,6]-bicyclic scaffold 3.2.\textsuperscript{51} In order to expand the dihydropyran and incorporate a nitrogen atom, the olefin was cleaved by ozonolysis to give a bis-aldehyde intermediate. Immediate reductive amination of the unstable bis-aldehyde with benzylamine gave the N-benzyl azepane 3.5.\textsuperscript{71} Hydrogenation was used to cleave the N-benzyl protecting group and provide the highly diversifiable secondary amine 3.6.

**Figure 3.2.** Synthesis of pyrrolidinoazepane 3.6.

Two strategies were possible for diversification of the oxazepane amine. First, the amine in 3.6 could be directly substituted, and the corresponding products taken forward to saponification, amide coupling with the clindamycin sugar (7-Cl-MTL), and deprotection (Figure 3.3). In cases whether the conditions for amine substitution would be incompatible with functionalities on the sugar, such as guanidine formation, this early-stage diversification was preferable.
Alternatively, the oxazepane amine could be orthogonally protected by formation of the allyl carbamate (N-Alloc). The N-Alloc-protected oxazepane 3.9 can then be taken forward and the N-Alloc protecting group can be selectively removed for late-stage diversification in the presence of the clindamycin sugar (Figure 3.4). These two strategies enabled preparation of a wide range of substituted oxazepane analogs bearing a variety of functionalities.
Figure 3.4. N-Alloc protection as a strategy for late-stage diversification of oxazepane amine.

Results

Unfortunately, many of the analogs containing the oxazepane scaffold had poor activity against clindamycin-susceptible Gram-positive strains, and none of these analogs surpassed the pyrrolidinooxepine analog FSA-24035 in activity (Figure 3.5). The isosteric replacement of the methylene in FSA-24035 with an amine in FSA-503001 is highly deleterious to antibacterial activity. Previously, the part of the A-site occupied by the n-propyl chain of clindamycin had been characterized as a predominantly hydrophobic pocket. The sharp decrease in activity observed with the isosteric replacement of a carbon atom with a nitrogen atom suggests that this hydrophobic pocket does not tolerate highly polar functionality, such as an amine. Addition of a methyl (FSA-503002), n-propyl (FSA-503003), or benzyl (FSA-502002) substituent to the amine slightly improves activity, but these oxazepane analogs remain inferior to FSA-24035.
A variety of oxazepane derivatives were explored, to see if modification of the amine improved activity (Figure 3.6). Urea and carbamate derivatives were made to see if there were benefits to forming a polar but non-basic amine. However, neither the urea (FSA-503004) nor the carbamate (FSA-503073) showed promising activity. Two aminopyridine derivatives (FSA-504049 and FSA-504050) were synthesized in the hopes that these heterocycles could form hydrogen bonds with nucleobase residues around the A-site; however, these compounds were worse than FSA-502002. Conversion of the amine into a guanidine (FSA-504057) or an amino acid (FSA-504063) caused deteriorations in activity relative to the secondary amine FSA-503001. These results provide further evidence to suggest that the A-site binding pocket adjacent to clindamycin is predominantly hydrophobic and does not tolerate polar functionality.
Despite these disappointing results, an interesting and informative outcome is observed with the N-benzylamine, FSA-502002. While FSA-502002 is significantly less potent than FSA-24035, it is substantially more active than either the secondary amine FSA-503001 or the n-propylamine FSA-503003 (Figure 3.5). The phenyl ring may partially recover the activity of the parent pyrrolidinoxepine through added hydrophobic interactions or through aromatic ring stacking effects. These findings suggested that, while incorporation of a nitrogen into the 7-membered ring was not in and of itself beneficial, the ability to substitute the 7’-position of the pyrrolidinoxepine, particularly with aryl groups, could potentially yield improvements in antibacterial activity.

Conclusions
A novel method to incorporate an amine into the bicyclic amino acid scaffold was developed, utilizing a telescoped ozonolysis and reductive amination sequence. A variety of analogs were developed using the resultant pyrrolidinoazepane scaffold; however, their activity was inferior to both clindamycin and the parent pyrrolidinoazepine FSA-24035. Interestingly, the N-benzyl-substituted pyrrolidinoazepane FSA-502002 showed significantly better activity than either the corresponding secondary amine or the N-methyl counterpart. Thus, further exploration of the 7’-position on the bicyclic scaffold seemed promising.
A 1.0 M solution of tri-$t$-butylphosphane (0.14 mL, 0.136 mmol, 0.03 equiv.) in toluene and a solution of isobutyryl chloride (0.014 g, 0.136 mmol, 0.03 equiv.) in toluene were added sequentially to a stirring solution of 1.16 (1.29 g, 4.53 mmol, 1.0 equiv.) and bis(dibenzylideneacetone)palladium (0.078 g, 0.136 mmol, 0.03 equiv.) in toluene (11 mL). The resultant dark red solution was sparged with argon for 10 min, heated to 80 °C, and stirred at 80 °C under argon. The reaction progress was monitored by aliquot NMR. After 6 hours, the reaction solution was concentrated under reduced pressure and purified directly by column chromatography (30–40% EtOAc in hexanes) to yield 3.3 (1.15 g, 4.02 mmol, 89%) as a colorless oil. $^1$H NMR spectra were consistent with those previously reported for 3.3.\(^{51}\)

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 5.65 (m, 1H), 5.32 (m, 1H), 4.44*, 4.37 (d, $J = 7.6$ Hz, 1H), 4.25–4.17 (m, 1H), 3.81–3.72 (m, 1H), 3.75 (s, 3H), 3.56, 3.50* (m, 1H), 3.12 (dd, $J = 10.8, 9.1$ Hz, 1H), 3.05–2.85 (m, 1H), 1.69 (d, $J = 6.5$ Hz, 3H), 1.45*, 1.41 (s, 9H).
A solution of palladium acetate (0.045 g, 0.201 mmol, 0.05 equiv.) and triphenylphosphine (0.26 g, 1.00 mmol, 0.25 equiv.) in THF (20 mL) was transferred via cannula into a stirring solution of 3.3 (1.15 g, 4.02 mmol, 1.0 equiv.) and allyl ethyl carbonate (1.05 g, 8.04 mmol, 2.0 equiv.) in THF (10 mL), quantitating transfer with additional THF (10 mL). The resultant yellow solution was shielded from light, heated to reflux, and stirred while refluxing under argon. Consumption of 3.3 was monitored by TLC analysis (30% EtOAc in hexanes). After 2 hours, the reaction solution was cooled to ambient temperature and filtered through a pad of silica gel, washing with MTBE, and concentrated to give an orange oil. The crude product was purified by column chromatography (5–10–20% EtOAc in hexanes) to yield 3.4 (1.113 g, 3.42 mmol, 85%) as a yellow oil. \(^1\)H NMR spectra were consistent with those previously reported for 3.4.\(^{51}\)

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 5.84 (m, 1H), 5.62 (m, 1H), 5.31–5.25 (m, 2H), 5.18 (dt, \(J = 10.3, 2.0\) Hz, 1H), 4.55*, 4.46 (d, \(J = 7.6\) Hz, 1H), 4.17–4.10 (m, 1H), 4.05, 4.02* (d, \(J = 5.8\) Hz, 1H), 3.92–3.83 (m, 1H), 3.79–3.70 (m, 1H), 3.74*, 3.73 (s, 3H), 3.09–2.92 (m, 2H), 1.68 (d, \(J = 6.4\) Hz, 3H), 1.44*, 1.40 (s, 9H).
Hoveyda–Grubbs 1st generation catalyst (0.10 g, 0.168 mmol, 0.05 equiv.) was added to a stirring solution of 3.4 (1.10 g, 3.37 mmol, 1.0 equiv.) in DCM (67 mL). The resultant dark red solution was heated to reflux and stirred while refluxing under argon. Consumption of 3.4 was monitored by TLC analysis (30% EtOAc in hexanes). After 6 hours, the reaction solution was concentrated under reduced pressure and purified directly by column chromatography (10–20–30% EtOAc in hexanes) to yield 3.2 (0.844 g, 3.37 mmol, 88%) as a dark brown oil. 1H NMR spectra were consistent with those previously reported for 3.2.51

1H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl3) δ 6.00 (dd, J = 18.7, 10.0 Hz, 1H), 5.71 (dt, J = 10.0, 2.6 Hz, 1H), 4.49*, 4.40 (d, J = 8.0 Hz, 1H), 4.41 (s, 2H), 3.85–3.75 (m, 2H), 3.78 (s, 3H), 2.95–2.87 (m, 2H), 1.47*, 1.42 (s, 9H).
Ozone was bubbled through a stirring solution of 3.2 (0.577 g, 2.04 mmol, 1.0 equiv.) in DCM (41 mL) at –78 °C until a blue color persisted for 2 min. Nitrogen was bubbled through the solution until the color subsided, and dimethyl sulfide (4.25 mL, 57.1 mmol, 28 equiv.) was added to the solution. The resultant solution was stirred at ambient temperature and consumption of the ozonide intermediate was monitored by aliquot NMR. After 2 hours, the reaction solution was concentrated to give the bis-aldehyde intermediate, which was taken forward without further purification.

Benzyllamine (0.23 mL, 2.12 mmol, 1.04 equiv.) and sodium triacetoxyborohydride (0.949 g, 4.48 mmol, 2.20 equiv.) were added sequentially to a stirring solution of the bis-aldehyde prepared above (0.642 g, 2.04 mmol, 1.0 equiv.) in DCE (41 mL). The resultant solution was stirred at ambient temperature under argon. Consumption of the bis-aldehyde intermediate was monitored by mass spectrometry. After 3 hours, the reaction solution was concentrated under reduced pressure, the residue was partitioned between water and DCM, and extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to give a yellow oil. The crude product was purified by column chromatography (5–10% i-PrOH + 1% aq. NH₄OH in hexanes) to yield 3.5 (0.313 g, 0.802 mmol, 39% over 2 steps).

1H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl₃) δ 7.36–7.27 (m, 5H), 4.52*, 4.43 (d, J = 8.3 Hz, 1H), 4.44–4.39 (m, 1H), 3.97–3.87 (m, 2H), 3.83–3.78 (m, 1H), 3.78*, 3.77 (s, 3H), 3.72–3.65 (m, 1H), 3.68 (t, J = 6.1 Hz, 1H), 3.05–2.97 (m, 1H), 2.95–2.90
(m, 1H), 2.83 (dd, $J = 14.2$, 6.8 Hz, 1H), 2.71 (ddd, $J = 14.8$, 9.0, 7.3 Hz, 1H), 2.48 (br, 1H), 2.39–2.33 (m, 1H), 1.47*, 1.43 (s, 9H).
Palladium on carbon (10% wt, 0.126 g, 0.118 mmol, 0.2 equiv.) was added to a stirring solution of 3.5 (0.231 g, 0.592 mmol, 1.0 equiv.) in methanol (0.8 mL). The resultant black suspension was stirred at ambient temperature under an atmosphere of hydrogen (1 atm). Consumption of 3.5 was monitored by mass spectrometry. The reaction solution was filtered through a pad of Celite, washing with methanol, and concentrated to yield 3.6 (0.157 g, 0.523 mmol, 88%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 4.39*, 4.31 (d, $J = 8.2$ Hz, 1H), 4.23 (m, 1H), 3.81–3.66 (m, 3H), 3.65 (s, 3H), 3.17 (ddd, $J = 16.9$, 12.5, 5.0 Hz, 1H), 2.98–2.80 (m, 3H), 2.74–2.60 (m, 1H), 2.48 (dd, $J = 12.6$, 10.4 Hz, 1H), 2.35 (br, 1H), 1.34*, 1.30 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 171.16*, 170.95, 153.72*, 153.28, 81.04, 80.63*, 80.41*, 80.11, 62.17, 61.61*, 55.01, 54.95*, 52.52*, 51.73, 49.19, 49.14*, 48.30*, 48.23, 47.64, 44.06*, 43.29, 28.19*, 28.09.

FTIR (neat, cm$^{-1}$): 2950 (w), 1745 (m), 1691 (s), 1397 (s), 1177 (s), 1131 (s), 913 (m), 728 (s).
Di-tert-butyl dicarbonate (0.02 mL, 0.095 mmol, 1.1 equiv.) and triethylamine (0.02 mL, 0.173 mmol, 2.0 equiv.) were added sequentially to a stirring solution of 3.6 (0.026 g, 0.087 mmol, 1.0 equiv.) in DCM (0.2 mL). The resultant solution was stirred at ambient temperature. Consumption of 3.6 was monitored by mass spectrometry. After 16 hours, the reaction solution was quenched with saturated aq. ammonium chloride and extracted five times with DCM. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield 3.12 (0.034 g, 0.085 mmol, 98%).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 4.50*, 4.42 (t, \(J = 7.8\) Hz, 1H), 4.03 (m, 1H), 3.94–3.79 (m, 3H), 3.73*, 3.72 (s, 3H), 3.71 (m, 1H), 3.59 (dd, \(J = 13.4, 6.0\) Hz, 1H), 3.54 (m, 1H), 3.23 (m, 1H), 2.97 (m, 1H), 2.82–2.71 (m, 1H), 1.42*, 1.40*, 1.38 (s, 9H), 1.37 (s, 9H).

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta\) 170.98*, 170.81, 155.08, 154.94*, 153.66, 153.23*, 82.49*, 82.04*, 80.34, 80.08, 70.57, 70.13, 62.07, 61.46, 52.05*, 51.87, 48.88, 48.32, 48.04, 47.21, 42.29*, 41.46, 28.29, 28.21*, 28.10.
Formaldehyde (37% aq. solution, 0.03 mL, 0.346 mmol, 2.0 equiv.) and sodium cyanoborohydride (0.022 g, 0.346 mmol, 2.0 equiv.) were added to a stirring solution of 3.6 (0.052 g, 0.173 mmol, 1.0 equiv.) in methanol (0.9 mL). The resultant solution was stirred at ambient temperature. Consumption of 3.6 was monitored by mass spectrometry. After 1 hour, the reaction was concentrated, re-dissolved in DCM, filtered through a plug of sodium sulfate, and concentrated under reduced pressure. The crude product was purified by column chromatography (2–5% methanol + 1% aq. ammonium hydroxide in DCM) to yield 3.13 (0.037 g, 0.118 mmol, 68%).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta 4.46^*, 4.38 (d, J = 8.2 \text{ Hz, } 1\text{H}), 4.33 (m, 1\text{H}), 3.94 (dd, J = 8.1, 1.7 \text{ Hz, } 1\text{H}), 3.87–3.79 (m, 2\text{H}), 3.73^*, 3.72 (s, 3\text{H}), 2.95–2.88 (m, 2\text{H}), 2.80–2.74 (m, 2\text{H}), 2.67–2.62 (m, 1\text{H}), 2.38 (s, 3\text{H}), 2.24 (dd, J = 11.9, 9.8 \text{ Hz, } 1\text{H}), 1.42^*, 1.38 (s, 9\text{H}).

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta 171.34^*, 171.09, 153.87^*, 153.47, 118.29, 81.09^*, 80.44, 69.63, 69.53^*, 62.68, 62.10^*, 59.98, 57.78, 57.71^*, 52.09^*, 51.90, 48.57^*, 47.99, 46.63, 46.55^*, 40.78^*, 40.14, 28.29^*, 28.19.

FTIR (neat, cm\(^{-1}\)): 2974 (w), 1748 (m), 1697 (s), 1383 (s), 1207 (m), 1179 (m), 1142 (s).

HRMS (ESI+, m/z): [M+H]\(^+\) calc’d for C\(_{15}\)H\(_{26}\)N\(_2\)O\(_5\), 315.1914; found 315.1914.
Propionaldehyde (0.025 mL, 0.346 mmol, 2.0 equiv.) and sodium cyanoborohydride (0.016 g, 0.260 mmol, 1.5 equiv.) were added to a stirring solution of 3.6 (0.052 g, 0.173 mmol, 1.0 equiv.) in methanol (1.7 mL). The resultant solution was stirred at ambient temperature. Consumption of 3.6 was monitored by TLC (10% methanol + 1% aq. ammonium hydroxide in DCM). After 4 hours, the reaction was concentrated under reduced pressure. The crude product was purified by column chromatography (2−5% methanol + 1% aq. ammonium hydroxide in DCM) to yield 3.14 (0.035 g, 0.102 mmol, 59%).

R<sub>f</sub> = 0.70 (10% methanol + 1% aq. ammonium hydroxide in DCM, KMnO<sub>4</sub>).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.48*, 4.40 (d, J = 8.2 Hz, 1H), 3.93 (m, 1H), 3.86 (dd, J = 10.4, 8.7 Hz, 1H), 3.80 (m, 1H), 3.75*, 3.74 (m, 1H), 3.73 (s, 3H), 2.95 (m, 2H), 2.83 (m, 2H), 2.68 (m, 1H), 2.45 (m, 2H), 2.31 (m, 1H), 1.46 (m, 2H), 1.44*, 1.39 (s, 9H), 0.88 (t, J = 7.4 Hz, 3H).

Isocyanatoethane (0.02 mL, 0.260 mmol, 1.5 equiv.) was added via syringe to a stirring solution of 3.6 (0.052 g, 0.173 mmol, 1 equiv.) in DCM (1.7 mL) at 0 °C. The resultant solution was stirred under argon, gradually warming to ambient temperature as ice-water bath expired. Consumption of 3.6 was monitored by TLC (10% methanol + 1% aq. ammonium hydroxide in DCM). After 14 hours, the reaction was quenched with brine and extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated over reduced pressure. The crude product was purified by column chromatography (75–90% EtOAc in hexanes) to yield 3.15 (0.026 g, 0.070 mmol, 40%).

Rf = 0.45 (10% methanol + 1% aq. ammonium hydroxide in DCM, KMnO4).

1H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl3) δ 4.54*, 4.46 (d, J = 8.2 Hz, 1H), 4.40 (m, 1H), 4.34*, 4.19 (m, 1H), 4.12*, 4.06 (m, 1H), 4.02–3.89 (m, 2H), 3.75*, 3.74 (s, 3H), 3.62–3.51 (m, 2H), 3.29–3.15 (m, 4H), 3.04–2.97 (m, 1H), 2.88 (m, 1H), 1.43*, 1.39 (s, 9H), 1.13 (td, J = 7.2, 2.5 Hz, 3H).

13C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl3) δ 171.07*, 170.83, 157.52, 157.48*, 153.67*, 153.29, 82.92, 82.23*, 80.62, 80.53*, 71.33, 71.10*, 62.37, 61.76*, 52.19*, 52.01, 49.00, 48.51, 47.66, 47.53*, 46.74*, 46.57, 42.08*, 41.28, 35.89, 28.29*, 28.18, 15.55.
FTIR (neat, cm⁻¹): 3366 (br), 2976 (w), 1748 (m), 1697 (s), 1628 (m), 1533 (m), 1398 (s), 1134 (s), 729 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C₁₇H₂₉N₅O₆, 371.2056; found 371.2085.
Pyridine (0.08 mL, 0.959 mmol, 3.0 equiv.) was added to a stirring solution of 3.6 (0.096 g, 0.320 mmol, 1 equiv.) in DCM (0.8 mL). The solution was cooled to 0 °C and allyl chloroformate (0.04 mL, 0.384 mmol, 1.20 equiv.) was added dropwise by syringe. The resultant solution was stirred at 0 °C for 10 minutes and then stirred at ambient temperature under argon. Consumption of 3.6 was monitored by mass spectrometry. After 2 hours, the reaction was concentrated, and the residue was re-dissolved in 1 N aq. hydrochloric acid and EtOAc. The product was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with saturated aq. sodium bicarbonate, washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (40% EtOAc in hexanes) to yield 3.9 (0.081 g, 0.210 mmol, 66%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 5.90 (m, 1H), 5.26 (dd, $J = 16.9$, 10.8 Hz, 1H), 5.19 (m, 1H), 4.57 (d, $J = 5.5$ Hz, 2H), 4.50*, 4.42 (dd, $J = 8.7$ Hz, 1H), 4.05 (m, 2H), 3.99–3.85 (m, 3H), 3.72*, 3.71 (s, 3H), 3.71–3.66 (m, 1H), 3.55 (m, 1H), 3.42–3.27 (m, 2H), 2.99 (m, 1H), 2.81 (m, 1H), 1.41*, 1.37 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 Hz, CDCl$_3$) δ 170.96*, 170.76, 155.62, 155.42*, 153.66*, 153.21, 132.68, 117.64, 82.53, 82.16*, 81.89, 81.45, 80.47, 80.42, 70.49*, 70.46, 70.16, 66.31, 66.24*, 62.17, 62.08*, 61.66, 61.47, 52.11*, 51.93, 48.80, 48.28, 48.23*, 47.85*, 47.77, 47.64*, 47.57, 42.10*, 41.91, 41.34*, 41.19, 28.24*, 28.13.
Mercuric oxide (0.041 g, 0.190 mmol, 1.10 equiv.) and triethylamine (0.07 mL, 0.519 mmol, 3.00 equiv.) were added sequentially to a stirring solution of 3.6 (0.052 g, 0.173 mmol, 1 equiv.) and 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.053 g, 0.182 mmol, 1.05 equiv.) in DCM (1.2 mL). The resulting suspension was stirred at ambient temperature under argon with exclusion of light. Consumption of 3.6 was monitored by TLC (20% isopropanol + 1% aq. ammonium hydroxide in hexanes). After 20 hours, the reaction was filtered through a plug of Celite, washed with DCM, and concentrated under reduced pressure. The crude product was purified by column chromatography (5–10% isopropanol + 1% aq. ammonium hydroxide in hexanes) to yield 3.7 (0.063 g, 0.117 mmol, 68%).

\[ R_f = 0.40 \text{ (20\% isopropanol + 1\% aq. ammonium hydroxide in hexanes, UV+KMnO}_4\text{).} \]

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta \) 9.99 (br, 1H), 4.52*, 4.44 (d, \(J = 8.2\) Hz, 1H), 4.05 (m, 1H), 4.00 (dd, \(J = 13.2, 4.4\) Hz, 1H), 3.92 (m, 1H), 3.85 (m, 1H), 3.75 (m, 1H), 3.74*, 3.73 (s, 3H), 3.49 (m, 1H), 3.38 (m, 1H), 2.98 (m, 1H), 2.93–2.85 (m, 1H), 1.48 (s, 9H), 1.46 (s, 9H), 1.42*, 1.38 (s, 9H).

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 Hz, CDCl\(_3\)) \(\delta \) 170.88*, 170.63, 162.63, 155.29, 153.66*, 153.22, 150.73, 148.75, 83.24, 82.25, 80.55, 79.72*, 69.41, 62.22, 61.66*, 52.16*, 51.99, 50.16, 28.27, 28.17*, 28.12, 28.02, 27.93.
General procedure for saponification

A 1.0 N aq. solution of lithium hydroxide (2.0 equiv.) was added to a solution of 3.16 (1.0 equiv.) in methanol and THF in a 1:1 ratio such that the concentration of 3.16 was 0.2 M. The resultant solution was stirred at ambient temperature until complete consumption of the methyl ester was observed by mass spectrometry. If the 7’-position nitrogen was non-basic, the reaction solution was acidified with 1 N aq. hydrochloric acid until it reached pH 2 and extracted five times with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to yield 3.17, which was taken forward without further purification. If the 7’-position nitrogen was basic, the reaction was concentrated directly to yield 3.17 as the carboxylate, which was taken forward without further purification.
General procedure for coupling to 7-Cl-MTL

Triethylamine (3.2 equiv.) was added to a solution of 3.17 (1.0 equiv.) and 7-Cl-MTL (1.1 equiv.) in DMF such that the concentration of 3.17 was 0.35 M at 0 °C. The resultant colorless solution was stirred for 10 min at 0 °C, and HATU (1.3 equiv.) was added in a single portion. The resultant bright yellow solution was stirred at ambient temperature under argon. Consumption of 3.17 was monitored by mass spectrometry. After complete consumption of 3.17 was observed by mass spectrometry, the reaction solution was concentrated under reduced pressure and directly purified by column chromatography (methanol in DCM) to yield 3.18. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
Tetrakis(triphenylphosphine)palladium (0) (0.014 g, 0.012 mmol, 0.1 equiv.) and morpholine (0.022 g, 0.249 mmol, 2.0 equiv.) were added sequentially to a solution of 3.10 (0.106 g, 0.124 mmol, 1.0 equiv.) in DCM (2.5 mL) at 0 °C. The resultant solution was stirred at ambient temperature under argon. Consumption of 3.10 was monitored by TLC (50% iPrOH + 1% aq. ammonium hydroxide in hexanes). After 5 hours, the reaction solution was concentrated under reduced pressure and purified by column chromatography (20–50% iPrOH + 1% aq. ammonium hydroxide in hexanes) to yield 3.11 (0.064 g, 0.083 mmol, 67%). Due to amide and carbamate rotamerism, this intermediate was taken forward without full characterization and the subsequent analogs were purified after N-Boc deprotection.
Sodium cyanoborohydride (0.004 g, 0.070 mmol, 2.0 equiv.) was added to a solution of 3.11 (0.027 g, 0.035 mmol, 1.0 equiv.) and glyoxylic acid monohydrate (0.016 g, 0.176 mmol, 5.0 equiv.) in a mixture of methanol (0.32 mL) and acetic acid (0.03 mL). The resultant solution was stirred at ambient temperature under argon. Consumption of 3.11 was monitored by mass spectrometry. After 3 hours, another portion each of glyoxylic acid and sodium cyanoborohydride were added. After 16 hours, the reaction solution was concentrated under reduced pressure to yield 3.19. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
Sodium triacetoxyborohydride (0.008 g, 0.036 mmol, 1.4 equiv.) was added to a solution of 3.11 (0.020 g, 0.026 mmol, 1.0 equiv.) and 3.20 (0.006 g, 0.026 mmol, 1.0 equiv.) in DCE (0.2 mL). The resultant solution was stirred at ambient temperature under argon. Consumption of 3.11 was monitored by mass spectrometry. After 16 hours, the reaction solution was quenched with saturated aq. sodium bicarbonate and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (2% methanol + 1% aq. ammonium hydroxide in DCM) to yield 3.21. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
Using the procedure for the synthesis of 3.21, 3.11 (0.020 g, 0.026 mmol, 1.0 equiv.) and 3.22 (0.006 g, 0.026 mmol, 1.0 equiv.) were used to prepare 3.23. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
General procedure for N-Boc deprotection

Trifluoroacetic acid (16.6 equiv.) was added dropwise by syringe to a solution of 3.18 (1.0 equiv.) and dimethyl sulfide (10.0 equiv.) in DCM such that the concentration of 3.18 was 0.1 M at 0 °C. The resultant solution was allowed to warm to ambient temperature and stirred at ambient temperature under argon. Consumption of 3.18 was monitored by mass spectrometry. After complete consumption of 3.18 was observed, the reaction solution was diluted with toluene and concentrated under reduced pressure. The residue was purified by either column chromatography (methanol + aq. ammonium hydroxide in DCM) or HPLC (acetonitrile in water) to yield 3.24.
Using the general procedures for saponification, amide bond coupling, and deprotection, 3.12 (0.034 g, 0.085 mmol, 1.0 equiv.) was used to prepare **FSA-503001** (0.012 g, 0.027 mmol, 32% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.29 (d, $J$ = 5.7 Hz, 1H), 4.60 (q, $J$ = 6.8 Hz, 1H), 4.40 (d, $J$ = 10.0 Hz, 1H), 4.30 (t, $J$ = 9.0 Hz, 1H), 4.19 (d, $J$ = 10.0 Hz, 1H), 4.08 (dd, $J$ = 10.4, 5.6 Hz, 1H), 4.01–3.95 (m, 2H), 3.89 (d, $J$ = 8.5 Hz, 1H), 3.79 (dd, $J$ = 13.1, 6.8 Hz, 1H), 3.58 (dd, $J$ = 10.2, 3.4 Hz, 1H), 3.33–3.27 (m, 1H), 3.23–3.15 (m, 2H), 2.74 (t, $J$ = 12.0 Hz, 1H), 2.63 (t, $J$ = 10.4 Hz, 1H), 2.57–2.50 (m, 1H), 2.14 (s, 3H), 1.51–1.47 (m, 1H), 1.49 (d, $J$ = 6.9 Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 174.70, 89.77, 84.53, 72.06, 71.27, 70.29, 69.84, 69.72, 63.88, 59.27, 54.46, 46.13, 24.95, 22.83, 13.45.

FTIR (neat, cm$^{-1}$): 3327 (br), 2928 (w), 2477 (br), 2361 (w), 1672 (s), 1360 (s), 976 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{17}$H$_{30}$ClN$_3$O$_6$S, 439.1544; found 439.1585.
Using the general procedures for saponification, amide bond coupling, and deprotection, \textbf{3.13} (0.037 g, 0.118 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-503002} (0.011 g, 0.024 mmol, 20% over 3 steps).

\textsuperscript{1}H NMR (600 MHz, CD\textsubscript{3}OD) \(\delta\) 5.29 (d, \(J = 5.6\) Hz, 1H), 4.59 (qd, \(J = 6.8, 1.6\) Hz, 1H), 4.40 (dd, \(J = 10.0, 1.6\) Hz, 1H), 4.35 (t, \(J = 9.0\) Hz, 1H), 4.18 (d, \(J = 10.0\) Hz, 1H), 3.98–3.94 (m, 2H), 3.89 (d, \(J = 3.5\) Hz, 1H), 3.84 (dd, \(J = 12.6, 6.4, 2.0\) Hz, 1H), 3.57 (dd, \(J = 10.2, 3.4\) Hz, 1H), 3.26 (dd, \(J = 10.0, 7.2\) Hz, 1H), 2.95 (dd, \(J = 10.0, 4.3\) Hz, 1H), 2.86 (ddt, \(J = 14.8, 6.3, 1.6\) Hz, 1H), 2.78 (ddd, \(J = 14.8, 8.1, 2.0\) Hz, 1H), 2.59 (dd, \(J = 10.9, 10.0\) Hz, 1H), 2.49 (m, 1H), 2.41 (s, 3H), 2.31 (dd, \(J = 11.8, 10.7\) Hz, 1H), 2.14 (s, 3H), 1.51–1.48 (m, 1H), 1.49 (d, \(J = 6.8\) Hz, 3H).

\textsuperscript{13}C NMR (126 MHz, CD\textsubscript{3}OD) \(\delta\) 175.08, 89.76, 83.87, 72.04, 71.30, 69.88, 69.73, 64.25, 60.28, 59.28, 58.38, 54.42, 46.92, 44.74, 22.81, 13.45.

FTIR (neat, cm\textsuperscript{-1}): 3339 (br), 2924 (w), 2479 (br), 2361 (m), 2068 (m), 1659 (m), 1452 (m), 1094 (s), 976 (s).

HRMS (ESI\textsuperscript{+}, m/z): [M+H]+ calc’d for C\textsubscript{18}H\textsubscript{32}ClN\textsubscript{3}O\textsubscript{6}S, 453.17; found 453.1699
Using the general procedures for saponification, amide bond coupling, and deprotection, \(3.14\) (0.035 g, 0.102 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-503003} (0.011 g, 0.023 mmol, 23% over 3 steps).

\(^1\)H NMR (600 MHz, CD\textsubscript{3}OD) \(\delta\) 5.29 (d, \(J = 5.6\) Hz, 1H), 4.59 (qd, \(J = 6.8, 1.7\) Hz, 1H), 4.39 (dd, \(J = 10.0, 1.7\) Hz, 1H), 4.34 (t, \(J = 9.1\) Hz, 1H), 4.18 (dd, \(J = 10.0, 1.2\) Hz, 1H), 4.07 (dd, \(J = 10.2, 5.6\) Hz, 1H), 3.96–3.92 (m, 2H), 3.90 (d, \(J = 3.5\) Hz, 1H), 3.80 (ddd, \(J = 12.4, 6.9, 1.9\) Hz, 1H), 3.57 (dd, \(J = 10.2, 3.4\) Hz, 1H), 3.25 (dd, \(J = 10.0, 7.2\) Hz, 1H), 2.98 (m, 1H), 2.90 (dd, \(J = 14.9, 6.8\) Hz, 1H), 2.77 (dd, \(J = 15.0, 7.7\) Hz, 1H), 2.58 (t, \(J = 10.4\) Hz, 1H), 2.51–2.42 (m, 3H), 2.36 (t, \(J = 11.1\) Hz, 1H), 2.13 (s, 3H), 1.55–1.48 (m, 2H), 1.49 (d, \(J = 6.9\) Hz, 3H), 0.91 (td, \(J = 7.4, 1.9\) Hz, 3H).

\(^{13}\)C NMR (126 MHz, CD\textsubscript{3}OD) \(\delta\) 175.25, 89.76, 83.90, 72.04, 71.35, 70.54, 69.84, 69.77, 64.23, 61.42, 59.28, 57.83, 55.94, 54.45, 45.13, 22.82, 21.33, 13.45, 12.24.

FTIR (neat, cm\(^{-1}\)): 3322 (br), 2930 (w), 2481 (br), 1661 (m), 1456 (m), 1188 (s).

HRMS (ESI+, \(m/z\)): \([\text{M+H}]^+\) calc’d for C\textsubscript{20}H\textsubscript{36}ClN\textsubscript{3}O\textsubscript{6}S, 481.2013; found 481.2012.
Using the general procedures for saponification, amide bond coupling, and deprotection, 3.5 (0.022 g, 0.056 mmol, 1.0 equiv.) was used to prepare **FSA-502002** (0.007 g, 0.013 mmol, 23% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.32 (m, 4H), 7.24 (m, 1H), 5.29 (d, $J = 5.6$ Hz, 1H), 4.59 (qd, $J = 6.8$, 1.6 Hz, 1H), 4.39 (m, 2H), 4.18 (dd, $J = 10.0$, 1.1 Hz, 1H), 4.07 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.93 (d, $J = 9.0$ Hz, 1H), 3.91–3.88 (m, 1H), 3.74 (ddd, $J = 12.2$, 7.2, 1.9 Hz, 1H), 3.69 (s, 2H), 3.57 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.34 (s, 2H), 3.23 (dd, $J = 9.7$, 6.9 Hz, 1H), 2.96 (dd, $J = 11.2$, 4.5 Hz, 1H), 2.85 (dd, $J = 14.9$, 6.8 Hz, 1H), 2.74 (ddd, $J = 14.9$, 7.2, 1.9 Hz, 1H), 2.54 (dd, $J = 9.8$, 9.1 Hz, 1H), 2.38 (m, 1H), 2.13 (s, 3H), 1.49 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 175.19, 139.94, 130.35, 129.51, 128.44, 89.75, 83.90, 72.04, 71.35, 71.21, 69.84, 69.79, 64.25, 63.59, 59.29, 57.85, 55.58, 54.46, 50.00, 45.50, 22.81, 13.45.

FTIR (neat, cm$^{-1}$): 3351 (br), 2925 (w), 1671 (s), 1200 (s), 1134 (s), 722 (m).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{24}$H$_{36}$ClN$_3$O$_6$S, 530.2086; found 530.2062.
Using the general procedures for saponification, amide bond coupling, and deprotection, **3.15** (0.026 g, 0.070 mmol, 1.0 equiv.) was used to prepare **FSA-503004** (0.010 g, 0.019 mmol, 27% over 3 steps).

\[^{1}\text{H NMR (600 MHz, CD}_{3}\text{OD)} \delta 5.29 (d, J = 5.6 Hz, 1H), 4.59 (qd, J = 6.8, 1.7 Hz, 1H), 4.40 (dd, J = 10.0, 1.6 Hz, 1H), 4.18 (d, J = 10.0 Hz, 1H), 4.07 (m, 1H), 4.03 (d, J = 8.7 Hz, 1H), 3.99–3.97 (m, 3H), 3.92 (d, J = 3.4 Hz, 1H), 3.57–3.48 (m, 3H), 3.37 (dd, J = 10.0, 7.2 Hz, 1H), 3.32–3.28 (m, 1H), 3.28–3.23 (m, 2H), 3.19 (q, J = 7.2 Hz, 2H), 2.63 (t, J = 10.3 Hz, 1H), 2.54 (m, 1H), 2.13 (s, 3H), 1.49 (d, J = 6.9 Hz, 3H), 1.11 (t, J = 7.2 Hz, 3H).\]

\[^{13}\text{C NMR (126 MHz, CD}_{3}\text{OD)} \delta 174.72, 160.44, 89.76, 85.83, 72.03, 71.82, 71.34, 69.85, 69.77, 64.32, 59.29, 54.48, 49.91, 48.06, 47.76, 46.66, 36.81, 22.81, 16.08, 13.44.\]

FTIR (neat, cm\(^{-1}\)): 2980 (w), 1732 (m), 1587 (m), 1514 (m), 1358 (s), 1152 (s), 947 (m), 727 (s).

HRMS (ESI+, m/z): [M+H]⁺ calc’d for C\(_{20}\)H\(_{35}\)ClN\(_4\)O\(_7\)S, 510.1915; found 510.1956.
Using the general procedure for deprotection, **3.10** (0.039 g, 0.040 mmol, 1.0 equiv.) was used to prepare **FSA-503073** (0.008 g, 0.015 mmol, 38%).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.96 (m, 1H), 5.32–5.28 (m, 1H), 5.29 (d, $J$ = 5.6 Hz, 1H), 5.21 (m, 1H), 4.59 (dt, $J$ = 5.4, 1.6 Hz, 3H), 4.40 (dt, $J$ = 10.1, 2.0 Hz, 1H), 4.19 (d, $J$ = 10.0 Hz, 1H), 4.09–4.05 (m, 2H), 4.02–3.96 (m, 3H), 3.92 (d, $J$ = 3.5 Hz, 1H), 3.65 (m, 1H), 3.56 (dd, $J$ = 10.3, 3.5 Hz, 2H), 3.45–3.34 (m, 3H), 2.64 (dd, $J$ = 12.9, 10.4 Hz, 1H), 2.51 (m, 1H), 2.14 (s, 3H), 1.49 (d, $J$ = 6.9 Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 174.53, 134.38, 117.99, 111.55, 89.75, 85.77, 85.38, 72.03, 71.31, 69.84, 69.77, 67.61, 67.55, 59.29, 54.48, 48.36, 46.36, 22.81, 13.44.

FTIR (neat, cm$^{-1}$): 2926 (w), 2361 (w), 1670 (s), 1464 (m), 1202 (m), 1138 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{21}$H$_{34}$ClN$_3$O$_8$S, 523.1755; found 523.1785.
Using the general procedure for deprotection, 3.21 (0.029 g, 0.030 mmol, 1.0 equiv.) was used to prepare **FSA-504049** (0.011 g, 0.019 mmol, 63%).

$^{1}$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.76 (d, $J$ = 6.6 Hz, 1H), 7.02 (s, 1H), 6.91 (dd, $J$ = 6.6, 1.6 Hz, 1H), 5.30 (d, $J$ = 5.6 Hz, 1H), 4.69 (t, $J$ = 9.0 Hz, 1H), 4.59 (d, $J$ = 10.0 Hz, 1H), 4.57 (dd, $J$ = 6.8, 1.6 Hz, 1H), 4.54 (m, 1H), 4.29 (d, $J$ = 10.0 Hz, 1H), 4.09 (dd, $J$ = 10.2, 5.6 Hz, 1H), 3.96 (ddd, $J$ = 12.1, 6.8, 2.3 Hz, 1H), 3.84 (m, 2H), 3.79 (s, 2H), 3.66 (dd, $J$ = 11.4, 7.7 Hz, 1H), 3.59 (dd, $J$ = 10.3, 3.2 Hz, 1H), 3.06–3.01 (m, 2H), 2.94 (dd, $J$ = 14.1, 6.3 Hz, 1H), 2.87 (ddd, $J$ = 15.0, 7.1, 2.2 Hz, 1H), 2.66 (m, 1H), 2.58 (t, $J$ = 11.0 Hz, 1H), 2.15 (s, 3H), 1.54 (d, $J$ = 6.8 Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 167.65, 159.52, 156.01, 136.26, 113.77, 112.72, 89.79, 80.53, 72.07, 71.39, 70.69, 62.16, 61.64, 59.04, 56.28, 55.02, 44.30, 22.64, 13.44.

FTIR (neat, cm$^{-1}$): 2928 (w), 2361 (w), 1668 (s), 1202 (s), 1132 (s), 800 (m), 721 (m).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{23}$H$_{36}$ClN$_{5}$O$_{6}$S, 545.2075; found 545.2082.
Using the general procedure for deprotection, 3.23 (0.012 g, 0.016 mmol, 1.0 equiv.) was used to prepare **FSA-504050** (0.005 g, 0.009 mmol, 56%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.80 (s, 1H), 7.71 (dd, $J = 14.8, 8.2$ Hz, 1H), 6.76 (d, $J = 8.6$ Hz, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.59–4.55 (m, 1H), 4.57 (q, $J = 6.8$ Hz, 1H), 4.50 (d, $J = 9.8$ Hz, 1H), 4.26 (d, $J = 9.9$ Hz, 1H), 4.08 (dd, $J = 10.2, 5.6$ Hz, 1H), 3.92 (m, 1H), 3.84 (m, 1H), 3.80 (m, 1H), 3.65 (s, 2H), 3.57 (d, $J = 10.0$ Hz, 2H), 3.19 (s, 1H), 3.02 (d, $J = 10.1$ Hz, 1H), 2.89 (m, 3H), 2.60 (s, 1H), 2.52 (m, 1H), 2.14 (s, 3H), 1.51 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 163.21, 143.42, 142.48, 123.80, 123.80, 112.38, 89.77, 72.05, 71.00, 70.78, 69.76, 69.55, 59.29, 59.07, 55.97, 55.34, 54.89, 49.85, 46.66, 44.22, 30.76, 22.63, 13.42.

FTIR (neat, cm$^{-1}$): 2926 (w), 2361 (w), 1672 (s), 1204 (s), 1130 (s), 837 (m), 800 (m), 723 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{23}$H$_{36}$ClN$_{5}$O$_{6}$S, 545.2075; found 545.2083.
Using the general procedures for saponification, amide bond coupling, and deprotection, 3.7 (0.043 g, 0.073 mmol, 1.0 equiv.) was used to prepare FSA-504057 (0.017 g, 0.035 mmol, 48% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.6$ Hz, 1H), 4.67 (d, $J = 8.7$ Hz, 1H), 4.58–4.53 (m, 2H), 4.44 (t, $J = 9.0$ Hz, 1H), 4.30 (d, $J = 10.0$ Hz, 1H), 4.08 (ddd, $J = 10.2$, 5.2, 2.3 Hz, 2H), 3.88–3.76 (m, 4H), 3.74–3.58 (m, 2H), 3.58 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.39 (t, $J = 12.2$ Hz, 1H), 3.16 (t, $J = 11.6$ Hz, 1H), 2.80 (m, 1H), 2.14 (s, 3H), 1.51 (d, $J = 6.6$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 166.91, 158.67, 129.90, 122.16, 89.78, 81.74, 72.07, 70.62, 69.75, 69.53, 61.99, 59.07, 55.02, 50.00, 47.57, 43.88, 22.65, 13.44.

FTIR (neat, cm$^{-1}$): 2926 (w), 2502 (br), 2353 (w), 1667 (s), 1576 (m), 1186 (s), 1136 (s), 721 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{18}$H$_{32}$ClN$_5$O$_6$S, 481.1762; found 481.1783.
Using the general procedure for deprotection, 3.19 (0.021 g, 0.035 mmol, 1.0 equiv.) was used to prepare **FSA-504063** (0.017 g, 0.035 mmol, 99% over 2 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.6$ Hz, 1H), 4.71–4.65 (m, 2H), 4.58–4.52 (m, 2H), 4.30 (d, $J = 9.7$ Hz, 1H), 4.25–4.19 (m, 3H), 4.16–4.12 (m, 1H), 4.08 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.85 (d, $J = 3.4$ Hz, 1H), 3.79–3.75 (m, 4H), 3.58 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.50 (t, $J = 12.3$ Hz, 1H), 3.17 (t, $J = 11.8$ Hz, 1H), 2.98 (m, 1H), 2.15 (s, 3H), 1.52 (d, $J = 6.7$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 168.37, 167.06, 161.96, 89.78, 81.53, 72.04, 70.62, 69.75, 69.53, 66.31, 61.89, 58.90, 58.10, 57.78, 55.11, 46.94, 40.78, 22.78, 13.44.

FTIR (neat, cm$^{-1}$): 2486 (br), 2362 (w), 1734 (w), 1663 (s), 1464 (m), 1142 (s), 972 (m), 723 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{19}$H$_{32}$ClN$_3$O$_8$S, 497.1599; found 497.1613.
Chapter 4.

Synthesis and study of aryl-substituted pyrrolidinoxepane analogs
Introduction

In light of the moderate activity observed with the $N$-benzylpyrrolidinoxazepane FSA-502002, developing a strategy to access facile substitution on the 7'-position of the bicycle seemed valuable. However, the oxazepane scaffold was not ideal to continue exploration of this position. The replacement of the 7'-carbon in the oxepine with an amine had a negative impact on antibacterial activity that was difficult to counteract via amine substitution. While addition of nonpolar substituents to the amine partially restored activity, finding a way to create a diversifiable handle directly on the oxepine seemed more opportune.

Synthesis of a diversifiable vinyl triflate

Previously, Dr. Mitcheltree had discovered conditions for a Wacker–Tsuji oxidation\textsuperscript{72} that regioselectively oxidized 1.18 into the oxepanone 4.1.\textsuperscript{51} Transforming the ketone in 4.1 into the corresponding vinyl triflate seemed like an opportune way to create a versatile handle for diversification and substitution. The triflation was found to proceed in high yield with Comins’ reagent and lithium HMDS (LiHMDS), providing the vinyl triflate as a 1:1 mixture of the olefin regioisomers, 4.2a and 4.2b (Figure 4.1).
A variety of parameters were screened to arrive at these reaction conditions (Figure 4.2). With \( N \)-phenyltriflate as the triflation agent, using LiHMDS as the amide base gave the cleanest conversion and highest yield. Switching from \( N \)-phenyltriflate to Comins’ reagent greatly increased the yield of both regioisomers. Changing the amide base counterion from lithium to potassium favored formation of 4.2a, but significantly decreased the overall yield. Thus, conditions to obtain a single regioisomer in good yield were not identified. However, the two regioisomers were separable by flash column chromatography and were both able to undergo subsequent cross-coupling reactions.
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Outcome</th>
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<tr>
<td>N-Phenyltriflate, LiHMDS</td>
<td>9% 4.2a, 8% 4.2b</td>
</tr>
<tr>
<td>N-Phenyltriflate, LDA</td>
<td>9% 4.2a, 5% 4.2b</td>
</tr>
<tr>
<td>N-Phenyltriflate, LiTMP</td>
<td>8% 4.2a, 7% 4.2b</td>
</tr>
<tr>
<td>Comins’ reagent, LiHMDS</td>
<td>39% 4.2a, 57% 4.2b</td>
</tr>
<tr>
<td>Comins’ reagent, NaHMDS</td>
<td>10% 4.2a, 13% 4.2b</td>
</tr>
<tr>
<td>Comin’s reagent, KHMDS</td>
<td>17% 4.2a, 0% 4.2b</td>
</tr>
</tbody>
</table>

Figure 4.2. Conditions screened for the triflation of 4.1.

Synthesis of aryl-substituted bicyclic derivatives

Given the promising activity of the N-benzyloxazepane derivative FSA-502002, aryl substituents were initially targeted. The vinyl triflates 4.2a and 4.2b were found to undergo Suzuki–Miyaura cross-coupling reactions\(^7\) with substituted aryl boronic acids in good to excellent yields (Figure 4.3). The broad substrate scope of the Suzuki–Miyaura reaction allowed for rapid diversification at the 7’-position, incorporating a variety of functional groups on the arene to develop structure–activity relationships. Additionally, Sonogashira cross-coupling reactions could be used to incorporate a 2-carbon linker between the oxepine and aryl substituent to investigate whether this linker improved activity (Figure 4.3). Together, these transformations enabled exploration of the extended binding pocket first identified by FSA-502002.
Figure 4.3. Diversification of vinyl triflate via Suzuki–Miyaura and Sonogashira cross-coupling reactions.

The Suzuki–Miyaura and Sonogashira coupling products were brought forward to saponification, coupling with the sugar moiety of clindamycin, and deprotection. The resulting pyrrolidinoxepine analogs could be tested as is or hydrogenated to give the corresponding pyrrolidinoxepanes. In some cases, a single diastereomer was isolated from the hydrogenation. The absolute stereochemistry at the benzylic position was assigned via a diagnostic nuclear Overhauser effect (nOe) observed between protons on the 4-fluorophenyl and on the sugar (Figure 4.4, Appendix Figure 4). Interestingly, isolation of the saturated cis-substituted derivative upon hydrogenation was also observed by Vicuron scientists during their work on azepane scaffolds. In cases where both diastereomers at the benzylic position were formed in appreciable quantities, the diastereomers were separable by HPLC and absolute stereochemistry was assigned based on order of elution as well as proton NMR spectra.
Figure 4.4. Hydrogenation of pyrrolidinoxepines and assignment of stereochemistry by nuclear Overhauser effect (nOe).

Results

The position of the olefin in the oxepine was found to have virtually no effect on activity, as otherwise identical analogs prepared with different regioisomers of the vinyl triflate were nearly identical in activity (Figure 4.5). However, the saturated pyrrolidinoxepanes were universally superior in antibacterial activity compared to their corresponding unsaturated oxepines, regardless of aryl substituent. One possible explanation for this trend is that the increased rigidity of the oxepine restricts the aryl ring into a less optimal conformation for binding the ribosome, while the saturated oxepane allows for more flexibility in positioning the aryl substituent in the binding pocket to maximize hydrophobic contacts.
Boronic acids containing a variety of 4-substituted arenes were coupled to the vinyl triflates in order to gauge what functional groups on the arene were beneficial for antibacterial activity and how much polarity the extended A-site binding pocket would tolerate (Figure 4.6). The phenyl-substituted derivative **FSA-509018** had similar activity to clindamycin and **FSA-24035**, with improved activity against MLSB-resistant *S. pneumoniae* and reduced activity against MLSB-resistant *S. pyogenes*. Interestingly, a substantial improvement in activity is observed against the fastidious *E. faecalis* strain, against which both clindamycin and **FSA-24035** show mediocre activity. *E. faecalis* shows intrinsic resistance to clindamycin due to the lincosamide and streptogramin A resistance (*lsa*) gene, which encodes an ABC transporter homologue protein.\textsuperscript{74} However, the aryl-substituted pyrrolidinoxepanes appears to overcome the intrinsic resistance of *E. faecalis*. Inclusion of polar functionality through 4-methoxy- (**FSA-512080b**) or 4-cyanophenyl...

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**Figure 4.5.** MIC values (µg/mL) of aryl-substituted pyrrolidinoxepine/oxepane analogs. MIC data by Micromyx LLC and Dr. Amarnath Pisipati.
(FSA-512081a) derivatives appears to be moderately tolerated, although a slight decrease in activity against *S. aureus* is observed relative to FSA-509018. However, the 4-fluorophenyl derivative (FSA-507061) showed similar activity against clindamycin-susceptible strains, excellent activity against *E. faecalis*, and significant improvement against MLSB-resistant strains. FSA-507061 showed good activity against MLSB-resistant Streptococci and limited but measurable activity against MLSB-resistant *S. aureus* and *E. faecalis*. Additionally, slight improvements were observed against the Gram-negative bacteria *E. coli* and *K. pneumoniae*, although activity against *H. influenzae* is lower than FSA-24035. From these results, further exploration of fluorophenyl-substituted derivatives was warranted.

![Figure 4.6](image)

**Figure 4.6.** MIC values (µg/mL) of aryl-substituted pyrrolidinoxepane analogs. MIC data by Micromyx LLC and Dr. Amarnath Pisipati.

A variety of fluoro- and difluorophenyl boronic acids were coupled to the vinyl triflate to refine structure–activity relationships (Figure 4.7). Most of the fluorophenyl derivatives displayed
similar activity to clindamycin and **FSA-24035** against clindamycin-susceptible strains, with varying degrees of activity against MLSB-resistant Gram-positive bacteria. All of these derivatives showed improved activity against *E. faecalis* relative to clindamycin, although **FSA-507061** had the highest potency against this intrinsically resistant species. In one case, an *N*-methyl side-product, **FSA-511100**, was formed during hydrogenation, and this unexpected derivative was tested. The *N*-methyl derivative **FSA-511100** showed a significant decrease in activity against MLSB-resistant Streptococci and against Gram-negative bacteria compared to related *N*-desmethyfluorophenyl derivatives. Neither the 3-fluorophenyl nor the 3,5- or 2,4-difluorophenyl derivatives showed improved activity compared to the initial 4-fluorophenyl derivative **FSA-507061**.

![Chemical structures of FSA-507061, FSA-511077, FSA-511100, FSA-511080, and FSA-511078](image)

**Figure 4.7.** MIC values (µg/mL) of fluorophenyl-substituted pyrrolidinoxepane analogs. MIC data by Micromyx LLC and Dr. Amarnath Pisipati.
The activity of the 4-fluorophenyl-substituted pyrrolidinooxepane FSA-507061 was a significant improvement over the parent pyrrolidinooxepine. Like FSA-24035, FSA-507061 maintained similar activity against clindamycin-susceptible Gram-positive strains. In addition, FSA-507061 is superior to both clindamycin and FSA-24035 against all MLSB-resistant strains tested, with good activity against MLSB-resistant *S. pneumoniae* and *S. pyogenes* and measurable activity against MLSB-resistant *S. aureus* and *E. faecalis*. Against Gram-negative strains, FSA-507061 has limited activity, albeit slightly better than either clindamycin or FSA-24035. These initial results suggested that the 4-fluorophenyl moiety in FSA-507061 made additional contacts in the ribosome binding site that led to improved activity against MLSB-resistant Gram-positive bacteria.

Efforts to extend beyond a single arene ring were made, through Suzuki–Miyaura couplings with bis-aryl boronic acids and Sonogashira couplings with phenylacetylene derivatives (Figure 4.8). The bis-aryl-containing analog (FSA-510001) tested showed diminished activity across all strains tested compared to phenyl-based derivatives. These results suggest that a bis-aryl substituent is too large to fit into the binding pocket and thus binding and activity is reduced. The phenylacetylene derivatives (FSA-510002, FSA-510003) and their saturated counterparts (FSA-510021, FSA-510022) showed excellent activity against Gram-positive and Gram-negative strains, including MLSB-resistant strains. However, secondary testing of these analogs indicated significant concentration-dependent hemolysis, suggesting that these compounds cause membrane disruption and would likely show toxicity in human cells. The issue of membrane disruption emerges when the amino acid moiety has an extended hydrophobic chain; the resulting analogs have a polar head attached to a nonpolar tail and show detergent-like properties against both
bacterial and eukaryotic cells. As a result, these phenylacetylene derivatives were not pursued further.

While addition of the aryl-substituent to the pyrrolidinoxepane significantly improved in vitro activity against MLSB-resistant Gram-positive bacteria, the aryl motif had the potential to decrease metabolic stability relative to clindamycin. Two aryl-substituted analogs (FSA-509018 and FSA-507061) were evaluated by Professor Michael Cameron of the Scripps Research Institute, Florida to determine their half-life in human hepatic microsomes (Figure 4.9). Clindamycin and lead compounds from Meiji Seika21 and Vicuron12, synthesized in the Myers laboratory according to published procedures, were included as comparators. The assay found that FSA-509018 (T_{1/2} =
20 min) and FSA-507061 \((T_{1/2} = 22 \text{ min})\) have significantly longer half-lives than clindamycin \((T_{1/2} = 13 \text{ min})\). However, the semi-synthetic lincosamide analog reported by Meiji Seika displayed a shorter half-life than clindamycin \((T_{1/2} = 3 \text{ min})\), potentially due to metabolism of the 7-arylthio substituent. The azepane analog reported by Vicuron had a longer half-life than either clindamycin or the aryl-substituted oxepane analogs \((T_{1/2} = 35 \text{ min})\). These data suggest that the aryl-substituted pyrrolidinooxepane scaffold is not a metabolic liability and that these compounds may in fact have better \textit{in vivo} metabolic stability than clindamycin.

![Chemical structures](image)

**Figure 4.9.** Half-life (min) of lincosamide analogs in 1 mg/mL human hepatic microsomes. Meiji Seika compound was prepared according to published procedures in collaboration with Dr. Mitcheltree;\(^{21}\) Vicuron compound was prepared by Dr. Mitcheltree.\(^{51}\) Data by Professor Michael Camerson, the Scripps Research Institute, Florida.
Conclusions

Conditions were found to convert the pyrrolidinooxepanone 4.1 into the vinyl triflates 4.2a and 4.2b. The vinyl triflate proved a valuable intermediate for substitution via Suzuki–Miyaura and Sonogashira cross-coupling reactions. Structure–activity relationships around aryl substituents on this site were investigated, and a 4-fluorophenyl-substituted pyrrolidinooxepane, FSA-507061, was discovered to have improved activity against MLSB-resistant Gram-positive strains compared to clindamycin.
Experimental section

A solution of palladium acetate (0.057 g, 0.255 mmol, 0.05 equiv.) and triphenylphosphine (0.33 g, 1.28 mmol, 0.25 equiv.) in THF (30 mL) was transferred via cannula to a stirring solution of 1.16 (1.46 g, 5.10 mmol, 1.0 equiv.) and allyl ethyl carbonate (1.33 g, 10.2 mmol, 2.0 equiv.) in THF (11 mL); the transfer was quantitated with additional THF (10 mL). The resultant yellow solution was shielded from light, heated to reflux, and stirred while refluxing under argon. Consumption of 1.16 was monitored by TLC analysis (30% EtOAc in hexanes). After 2 hours, the reaction solution was cooled to room temperature and filtered through a pad of silica gel, washing with MTBE, and concentrated to give an orange oil. The crude product was purified by column chromatography (5–10–20% EtOAc in hexanes) to yield 1.17 (1.33 g, 4.09 mmol, 80%) as a yellow oil. \(^1\)H NMR spectra were consistent with those previously reported for 1.17.\(^{51}\)

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) δ 5.91 (m, 1H), 5.81 (m, 1H), 5.31 (dt, \(J = 17.3, 1.7\) Hz, 1H), 5.21 (d, \(J = 10.4\) Hz, 1H), 5.11 (d, \(J = 17.0\) Hz, 1H), 5.05 (m, 1H), 4.61*, 4.53 (d, \(J = 7.6\) Hz, 1H), 4.22 (m, 1H), 4.15 (m, 1H), 4.07, 4.05* (d, \(J = 5.8\) Hz, 1H), 3.90 (m, 1H), 3.82–3.71 (m, 1H), 3.75 (s, 3H), 3.02 (m, 1H), 2.53–2.39 (m, 2H), 1.48*, 1.44 (s, 9H).
Hoveyda–Grubbs 1st generation catalyst (0.123 g, 0.205 mmol, 0.05 equiv.) was added to a stirring solution of 1.17 (1.33 g, 4.09 mmol, 1.0 equiv.) in DCM (82 mL). The resultant dark red solution was heated to reflux and stirred while refluxing under argon. Consumption of 1.17 was monitored by TLC analysis (30% EtOAc in hexanes). After 2 hours, the reaction solution was concentrated under reduced pressure and purified directly by column chromatography (10–20–30% EtOAc in hexanes) to yield 1.18 (1.18 g, 3.97 mmol, 97%) as a dark brown oil. \(^1\)H NMR spectra were consistent with those previously reported for 1.18.\(^{\text{51}}\)

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 5.85 (m, 1H), 5.75 (m, 1H), 4.54*, 4.46 (d, \(J = 8.3\) Hz, 1H), 4.34*, 4.30 (m, 1H), 4.08 (m, 1H), 3.98–3.93 (m, 1H), 3.92–3.87 (m, 1H), 3.85–3.79 (m, 1H), 3.76 (s, 3H), 2.94 (m, 1H), 2.56–2.44 (m, 2H), 1.47*, 1.42 (s, 9H).
An aqueous solution of fluoroboric acid (48%, 0.20 mL, 1.51 mmol, 0.5 equiv.) was added to a stirring solution of 1.18 (0.900 g, 3.03 mmol, 1.0 equiv.), palladium acetate (0.034 g, 0.151 mmol, 0.05 equiv.), and benzoquinone (0.327 g, 3.03 mmol, 1.0 equiv.) in acetonitrile (13 mL) and water (1.9 mL). The resultant yellow solution was stirred at ambient temperature. Consumption of 1.18 was monitored by TLC (50% EtOAc in hexanes). After 4 hours, the reaction solution was concentrated under reduced pressure to remove acetonitrile, diluted with brine, and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to give an orange oil. The crude product was purified by column chromatography (20–30–40–50% EtOAc in hexanes) to yield 4.1 (0.690 g, 2.20 mmol, 73%) as a yellow oil. \(^1\)H NMR spectra were consistent with those previously reported for 4.1.\(^{51}\)

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 4.55*, 4.47 (d, \(J = 8.2\) Hz, 1H), 4.15–4.11 (m, 1H), 3.98–3.85 (m, 2H), 3.77*, 3.76 (s, 3H), 3.70–3.65 (m, 1H), 2.98–2.91 (m, 2H), 2.89–2.71 (m, 2H), 2.58 (dd, \(J = 15.8, 4.9\) Hz, 1H), 2.44 (dd, \(J = 17.9, 12.1\) Hz, 1H), 1.44*, 1.40 (s, 9H).
A 1.00 M solution of lithium bis(trimethylsilyl)amide (2.64 mL, 2.64 mmol, 1.2 equiv.) in THF was added dropwise by syringe to a stirring solution of 4.1 (0.690 g, 2.20 mmol, 1.0 equiv.) in THF (22 mL) at −78 °C. The resultant solution was stirred at −78 °C under argon for 1 hour, then Comins’ reagent (1.04 g, 2.64 mmol, 1.2 equiv.) was added in a single portion and stirring was continued at −78 °C. Consumption of 4.1 was monitored by TLC (60% EtOAc in hexanes). After 3 hours, the reaction solution was quenched with saturated aq. ammonium chloride, warmed to ambient temperature while stirring, and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (20–30–40–50% ether in hexanes) to yield 4.2a (0.556 g, 1.25 mmol, 57%) and 4.2b (0.316 g, 0.711 mmol, 32%).

4.2a

\[ R_f = 0.55 \text{ (60\% EtOAc in hexanes, UV+KMnO}_4\text{).} \]

\[ ^1H \text{NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl}_3\text{)} \delta 5.83, 5.81^* \text{ (s, 1H), 4.46^*, 4.38 (d, } J = 8.1 \text{ Hz, 1H), 4.08–3.91 (m, 3H), 3.77–3.64 (m, 1H), 3.74^*, 3.73 (s, 3H), 3.42–3.22 (m, 1H), 3.13–3.02 (m, 2H), 2.42 (d, 17.1, 1H), 1.42^*, 1.37 (s, 9H).} \]

\[ ^{13}C \text{NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl}_3\text{)} \delta 170.44^*, 170.28, 153.60^*, 153.11, 149.49, 149.39^*, 122.14^*, 122.02, 119.60, 117.50, 84.99, 84.34^*, 80.54, 64.75, 61.89, 61.37^*, 52.14^*, 51.97, 49.35^*, 48.73, 37.71^*, 37.01, 34.70^*, 34.63, 28.18^*, 28.08. \]

\[ ^{19}F \text{NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl}_3\text{)} \delta \text{ –73.92^*, –73.94.} \]
FTIR (neat, cm\(^{-1}\)): 2978 (w), 1748 (m), 1697 (s), 1402 (s), 1206 (s), 1138 (s), 860 (m).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{16}\)H\(_{22}\)F\(_3\)NO\(_8\)S, 445.1018; found 445.1026.

4.2b

R\(_f\) = 0.60 (60% EtOAc in hexanes, UV+KMnO\(_4\)).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 5.84 (s, 1H), 4.49*, 4.41 (d, \(J = 8.2\) Hz, 1H), 4.36*, 4.33 (d, \(J = 6.8\) Hz, 1H), 4.19, 4.16* (s, 1H), 3.95–3.83 (m, 2H), 3.71*, 3.70 (s, 3H), 2.91 (m, 1H), 2.71–2.56 (m, 3H), 1.40*, 1.36 (s, 9H)

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta\) 170.78*, 170.57, 153.60*, 153.15, 151.50, 121.54, 121.43*, 119.69, 117.14, 82.20, 81.58*, 80.71, 66.94, 61.54, 60.99*, 52.24*, 52.06, 48.51*, 47.86, 38.50*, 37.85, 36.03, 35.97*, 29.62, 28.24*, 28.13.

\(^{19}\)F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl\(_3\)) \(\delta\) –73.58, –73.62*.

FTIR (neat, cm\(^{-1}\)): 2978 (w), 1749 (m), 1705 (s), 1402 (s), 1207 (s), 1142 (s), 885 (m).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{16}\)H\(_{22}\)F\(_3\)NO\(_8\)S, 445.1018; found 445.1035.
General procedure for Suzuki–Miyaura cross-coupling

A 2.0 M aq. solution of sodium carbonate (0.64 equiv.) was added to a stirring solution of 4.2a (1.0 equiv.), the boronic acid (1.5 equiv.), and triphenylphosphine palladium (II) chloride (0.05 equiv.) in THF and DMF in a 1:1 ratio such that the concentration of 4.2a was 0.2 M. The resultant yellow solution was heated to 50 °C and was stirred at 50 °C under argon. Consumption of 4.2a was monitored by mass spectrometry. After complete consumption of 4.2a was observed, the reaction solution was diluted with saturated aq. sodium bicarbonate and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc in hexanes) to yield 4.5.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2a (0.054 g, 0.121 mmol, 1.0 equiv.) was used to prepare 4.3 (0.032 g, 0.085 mmol, 70%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 7.33–7.26 (m, 5H), 6.01 (m, 1H), 4.58*, 4.49 (d, $J = 8.2$ Hz, 1H), 4.46 (d, $J = 7.1$ Hz, 1H), 4.42*, 4.22 (m, 1H), 4.05 (m, 1H), 3.94–3.90 (m, 1H), 3.87–3.80 (m, 1H), 3.79*, 3.78 (s, 3H), 2.99 (m, 1H), 2.87 (m, 1H), 2.63 (m, 1H), 1.47*, 1.43 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 170.99*, 170.84, 153.95*, 153.50, 151.52, 144.35, 144.26*, 143.95, 128.29, 127.41*, 127.26, 125.90, 87.52, 86.98*, 80.32, 67.96, 66.98*, 62.19, 61.61*, 52.20*, 52.02, 49.44*, 48.78, 40.13*, 39.51, 34.82, 28.35*, 28.25.

FTIR (neat, cm$^{-1}$): 2976 (w), 2359 (w), 1749 (m), 1402 (s), 1209 (s), 1173 (m), 1140 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{21}$H$_{27}$NO$_5$, 373.1889; found 373.1899.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2a (0.054 g, 0.121 mmol, 1.0 equiv.) was used to prepare 4.6 (0.031 g, 0.079 mmol, 66%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 7.31 (m, 2H), 7.04 (t, $J = 8.6$ Hz, 2H), 6.00 (m, 1H), 4.61*, 4.52 (d, $J = 8.1$ Hz, 1H), 4.49 (m, 1H), 4.25 (m, 1H), 4.22*, 4.08 (m, 1H), 3.95 (m, 1H), 3.88*, 3.82*, 3.81 (s, 3H), 3.02 (m, 1H), 2.85 (m, 1H), 2.72–2.62 (m, 2H), 1.50*, 1.46 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 170.09*, 170.83, 163.07, 161.11, 153.92*, 153.47, 143.36, 140.02*, 127.50, 115.00, 87.48, 86.95*, 80.35, 67.86, 62.15, 61.57*, 52.21*, 52.03, 49.39*, 48.74, 40.05*, 39.43, 34.94, 28.34*, 28.24.

$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ –115.32.

FTIR (neat, cm$^{-1}$): 2976 (w), 2861 (w), 1748 (m), 1699 (s), 1508 (m), 1402 (s), 1209 (m), 1171 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{21}$H$_{26}$FNO$_5$, 391.1795; found 391.1808.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.055 g, 0.123 mmol, 1.0 equiv.) was used to prepare 4.7 (0.036 g, 0.091 mmol, 73%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 7.26 (m, 2H), 7.01 (t, $J = 8.6$ Hz, 2H), 5.89 (s, 1H), 5.87*, 4.53*, 4.44 (d, $J = 8.2$ Hz, 1H), 4.14 (m, 1H), 4.07*, 3.99 (m, 2H), 3.81*, 3.80 (s, 3H), 3.63 (m, 1H), 3.56–3.45 (m, 1H), 3.21 (m, 1H), 3.01 (m, 1H), 2.68 (d, $J = 5.1$ Hz, 1H), 2.65*, 1.48*, 1.43 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 171.15*, 170.96, 163.07, 161.11, 153.91*, 153.45, 143.76, 139.00, 127.60*, 127.31, 115.23, 82.02, 81.37*, 80.40, 68.68, 68.57*, 62.15, 61.59*, 52.17*, 51.97, 49.42*, 48.74, 42.92*, 42.26, 36.16*, 35.94, 28.34*, 28.23.

$^{19}$F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl$_3$) δ −115.20*, −115.34.

FTIR (neat, cm$^{-1}$): 2976 (w), 2363 (w), 1748 (m), 1701 (s), 1508 (m), 1398 (s), 1207 (s), 1142 (s), 733 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{21}$H$_{26}$FNO$_5$, 391.1795; found 391.1812.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2a (0.062 g, 0.139 mmol, 1.0 equiv.) was used to prepare 4.8 (0.034 g, 0.088 mmol, 63%).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 7.21 (d, \(J = 8.1\) Hz, 2H), 7.14 (d, \(J = 7.9\) Hz, 2H), 5.91 (m, 1H), 5.90*, 4.54*, 4.45 (d, \(J = 8.2\) Hz, 1H), 4.15 (dd, \(J = 11.0, 4.1\) Hz, 1H), 4.08 (t, \(J = 9.8\) Hz, 1H), 3.99 (m, 1H), 3.81 (s, 3H), 3.64 (td, \(J = 11.4, 4.1\) Hz, 1H), 3.55*, 3.49 (m, 1H), 3.21 (m, 1H), 3.00 (m, 1H), 2.72 (d, \(J = 5.1\) Hz, 1H), 2.69*, 2.35 (s, 3H), 2.28*, 1.49*, 1.44 (s, 9H).

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta\) 171.25*, 171.03, 153.99, 153.76*, 153.56, 144.51, 139.93, 137.03, 129.90, 129.01, 128.21, 126.79*, 125.50, 115.07, 82.02, 81.39*, 80.47, 80.39*, 68.80, 62.21, 61.63*, 52.17*, 51.98, 49.49*, 48.82, 42.93*, 42.26, 35.71, 28.35*, 28.23, 20.99.

FTIR (neat, \(\text{cm}^{-1}\)): 2924 (w), 1748 (s), 1702 (s), 1514 (m), 1397 (s), 1178 (s), 1139 (s), 810 (m).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{22}\)H\(_{29}\)NO\(_5\), 387.2046; found 387.2049.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.063 g, 0.141 mmol, 1.0 equiv.) was used to prepare 4.9 (0.048 g, 0.123 mmol, 87%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 7.20 (m, 2H), 7.14 (d, $J$ = 7.8 Hz, 2H), 5.91 (m, 1H), 5.90*, 4.53*, 4.44 (d, $J$ = 8.1 Hz, 1H), 4.14 (dd, $J$ = 12.9, 5.0 Hz, 1H), 4.07 (t, $J$ = 9.8 Hz, 1H), 3.99 (m, 1H), 3.81*, 3.80 (s, 3H), 3.63 (td, $J$ = 11.6, 4.1 Hz, 1H), 3.55*, 3.48 (m, 1H), 3.22 (m, 1H), 3.01 (m, 1H), 2.72 (d, $J$ = 5.2 Hz, 1H), 2.69*, 2.35 (s, 3H), 1.48*, 1.43 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 171.16*, 170.98, 153.92*, 153.44, 144.49, 139.93, 137.00, 129.00, 126.80, 125.49, 125.47*, 82.02, 81.37, 80.30, 80.28*, 68.79, 62.18, 61.61*, 52.12*, 51.93, 49.47*, 48.80, 42.92*, 42.24, 35.71, 35.67*, 28.33*, 28.21, 20.97.

FTIR (neat, cm$^{-1}$): 2975 (w), 2860 (w), 1747 (s), 1696 (s), 1397 (s), 1178 (s), 1139 (s), 908 (m), 728 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{22}$H$_{29}$NO$_5$, 387.2046; found 387.2057.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2a (0.054 g, 0.121 mmol, 1.0 equiv.) was used to prepare 4.10 (0.035 g, 0.080 mmol, 66%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 7.58 (d, $J = 8.1$ Hz, 2H), 7.40 (dd, $J = 7.8$, 5.2 Hz, 2H), 6.06 (m, 1H), 4.58*, 4.50 (d, $J = 8.2$ Hz, 1H), 4.49 (m, 1H), 4.25 (m, 1H), 4.22*, 4.06 (td, $J = 9.1$, 3.6 Hz, 1H), 3.93 (dd, $J = 10.4$, 7.7 Hz, 1H), 3.86*, 3.79*, 3.78 (s, 3H), 3.01 (m, 1H), 2.83 (m, 1H), 2.72–2.60 (m, 1H), 1.47*, 1.43 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 170.96*, 170.81, 153.90*, 153.45, 147.51, 143.06, 142.97*, 129.42, 129.30, 126.24, 125.29*, 87.38, 86.84*, 80.41, 67.85, 62.13, 61.57*, 52.23*, 52.04, 49.39*, 48.74, 40.14*, 39.51, 34.76, 28.33*, 28.23.

$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ –62.53.

FTIR (neat, cm$^{-1}$): 2976 (w), 2849 (w), 1748 (m), 1697 (s), 1402 (s), 1323 (s), 1111 (s), 1069 (s), 1017 (m), 731 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{22}$H$_{26}$F$_3$NO$_5$, 441.1763; found 441.1767.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, **4.2b** (0.055 g, 0.123 mmol, 1.0 equiv.) was used to prepare **4.11** (0.043 g, 0.097 mmol, 79%).

\( ^1H \) NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \( \delta \) 7.58 (d, \( J = 8.2 \text{ Hz}, 2\text{H} \)), 7.40 (d, \( J = 7.9 \text{ Hz}, 2\text{H} \)), 6.01 (s, 1H), 4.55*, 4.47 (d, \( J = 8.2 \text{ Hz}, 1\text{H} \)), 4.17*, 4.15 (m, 1H), 4.10*, 4.03 (m, 2H), 3.81 (s, 3H), 3.80*, 3.65 (m, 1H), 3.61–3.50 (m, 1H), 3.25 (m, 1H), 3.05 (m, 1H), 2.72 (d, \( J = 5.0 \text{ Hz}, 1\text{H} \)), 2.69*, 1.49*, 1.44 (s, 9H).

\( ^{13}C \) NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \( \delta \) 171.23*, 170.98, 159.21, 154.04*, 153.65, 146.34, 143.74, 135.00, 129.65, 128.04*, 126.97, 125.97, 125.32, 115.46, 81.88, 81.27*, 80.80, 80.71*, 68.63, 68.52*, 62.14, 61.57*, 52.56*, 52.09, 49.34*, 48.66, 43.10*, 42.44, 35.89*, 35.66, 28.34*, 28.23.

\( ^{19}F \) NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl\(_3\)) \( \delta \) –61.42*, –62.53.

FTIR (neat, cm\(^{-1}\)): 2976 (w), 2868 (w), 1749 (m), 1701 (m), 1400 (m), 1323 (s), 1111 (s), 1067 (s), 841 (m).

HRMS (ESI+, \( m/z \)): [M+H]+ calc’d for C\(_{22}\)H\(_{26}\)F\(_3\)NO\(_5\), 441.1763; found 441.1771
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, \( \textbf{4.2a} \) (0.062 g, 0.139 mmol, 1.0 equiv.) was used to prepare \( \textbf{4.12} \) (0.036 g, 0.089 mmol, 64%).

\( ^1\text{H} \text{ NMR} \) (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \( \delta \): 7.24 (d, \( J = 8.7 \) Hz, 2H), 6.86 (d, \( J = 8.7 \) Hz, 2H), 5.87 (m, 1H), 5.84*, 4.51*, 4.43 (t, \( J = 7.6 \) Hz, 1H), 4.14*, 4.08–3.97 (m, 2H), 3.80*, 3.79 (s, 3H), 3.78*, 3.77 (s, 3H), 3.63 (td, \( J = 11.3, 4.6 \) Hz, 1H), 3.20 (m, 1H), 3.11 (m, 1H), 2.99 (dd, \( J = 16.3, 10.7 \) Hz, 1H), 2.70 (d, \( J = 5.1 \) Hz, 1H), 2.67*, 2.47 (d, \( J = 4.0 \) Hz, 1H), 2.44*, 1.47*, 1.46*, 1.43 (s, 9H), 1.41*.

\( ^{13}\text{C} \text{ NMR} \) (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \( \delta \): 171.45*, 171.26, 171.07*, 170.85, 159.19, 154.20*, 153.74, 151.76, 144.35, 135.59, 127.02, 126.29*, 121.81, 121.71*, 116.21, 114.96, 113.95, 82.49, 81.86, 81.01, 80.60*, 69.04, 67.22, 62.46, 61.89*, 61.81, 61.26, 55.54, 52.55*, 52.36, 52.20, 49.78*, 49.10, 48.79*, 48.15, 43.14*, 42.47, 38.78*, 38.13, 36.32, 36.25*, 36.01, 28.60*, 28.54*, 28.48, 28.43.

\( \text{FTIR (neat, cm}^{-1}) \): 2974 (w), 1748 (m), 1699 (s), 1399 (s), 1205 (s), 1139 (s), 885 (m), 732 (m).

\( \text{HRMS (ESI+, m/z): [M+H]+ calc'd for C}_{22}\text{H}_{29}\text{NO}_{6}, 403.1995; \text{found 403.199.} \)
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.063 g, 0.141 mmol, 1.0 equiv.) was used to prepare 4.13 (0.044 g, 0.110 mmol, 78%).

$^{1}$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 7.24 (m, 2H), 6.86 (d, $J$ = 8.2 Hz, 2H), 5.86 (m, 1H), 5.84*, 4.52*, 4.43 (d, $J$ = 8.1 Hz, 1H), 4.13 (dd, $J$ = 11.8, 4.8 Hz, 1H), 4.06 (t, $J$ = 9.7 Hz, 1H), 3.98 (m, 1H), 3.81 (s, 3H), 3.80*, 3.79 (s, 3H), 3.62 (td, $J$ = 11.4, 4.4 Hz, 1H), 3.54*, 3.47 (m, 1H), 3.21 (m, 1H), 2.99 (dd, $J$ = 15.3, 11.2 Hz, 1H), 2.70 (d, $J$ = 4.9 Hz, 1H), 2.67*, 1.48*, 1.43 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 171.16*, 170.98, 158.91, 153.92*, 153.45, 144.07, 135.31, 126.74, 126.01*, 113.67, 82.04, 81.40*, 80.31, 68.75, 62.18, 61.62*, 55.26, 52.11*, 51.92, 49.50*, 48.82, 42.86*, 42.19, 35.73, 28.32*, 28.21.

FTIR (neat, cm$^{-1}$): 2953 (w), 1747 (s), 1695 (s), 1511 (s), 1397 (s), 1245 (s), 1177 (s), 1139 (s), 1031 (m), 910 (m), 728 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{22}$H$_{29}$NO$_6$, 403.1995; found 403.1972.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, **4.2a** (0.062 g, 0.139 mmol, 1.0 equiv.) was used to prepare **4.14** (0.036 g, 0.090 mmol, 64%).

**1H NMR** (asterisk denotes minor rotamer peaks, 500 MHz, CDCl3) δ 7.61 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 6.05 (m, 1H), 6.03*, 4.53*, 4.44 (d, J = 8.2 Hz, 1H), 4.15 (ddd, J = 12.2, 5.1, 2.2 Hz, 1H), 4.08 (m, 1H), 4.01 (m, 1H), 3.80*, 3.79 (s, 3H), 3.63 (m, 2H), 3.52*, 3.23 (m, 1H), 3.05 (dd, J = 16.7, 11.5 Hz, 1H), 2.67 (dd, J = 16.4, 5.0 Hz, 1H), 1.47*, 1.42 (s, 9H).

**13C NMR** (asterisk denotes minor rotamer peaks, 126 MHz, CDCl3) δ 171.04*, 170.85, 153.82*, 153.37, 147.27, 143.39, 132.20, 130.83, 126.30, 118.70, 110.75, 81.81, 81.17*, 80.50, 68.50, 62.04, 61.48*, 52.19*, 52.00, 49.22*, 48.55, 43.17*, 42.50, 35.41, 28.31*, 28.20.

**FTIR** (neat, cm⁻¹): 2976 (w), 2226 (w), 1745 (s), 1695 (s), 1397 (s), 1179 (s), 1140 (s), 913 (m), 730 (s).

**HRMS** (ESI+, m/z): [M+H]+ calc’d for C_{22}H_{26}N_{2}O_{5}, 398.1842; 398.1854.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, \(4.2b\) (0.063 g, 0.141 mmol, 1.0 equiv.) was used to prepare \(4.15\) (0.048 g, 0.120 mmol, 85%).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 7.60 (d, \(J = 8.1\) Hz, 2H), 7.39 (m, 2H), 6.04 (m, 1H), 6.03*, 4.52*, 4.44 (d, \(J = 8.2\) Hz, 1H), 4.15 (dd, \(J = 11.3, 4.0\) Hz, 1H), 4.08 (t, \(J = 9.8\) Hz, 1H), 4.00 (m, 1H), 3.80*, 3.79 (s, 3H), 3.62 (m, 1H), 3.51 (m, 1H), 3.22 (m, 1H), 3.04 (dd, \(J = 15.4, 11.5\) Hz, 1H), 2.69 (d, \(J = 4.8\) Hz, 1H), 2.65*, 1.46*, 1.42 (s, 9H).

\(^{13}\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta\) 171.02*, 170.83, 153.80*, 153.34, 147.26, 143.38, 132.19, 130.82, 126.29, 118.68, 110.73, 81.80, 81.15*, 80.46, 80.42*, 68.48, 62.02, 61.46*, 52.17*, 51.98, 49.20*, 48.54, 43.15*, 42.48, 35.40, 35.35*, 28.30*, 28.18.

FTIR (neat, cm\(^{-1}\)): 2976 (w), 2227 (w), 1745 (m), 1694 (s), 1397 (s), 1178 (s), 1139 (s), 910 (m), 727 (s).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{22}\)H\(_{26}\)N\(_2\)O\(_5\), 398.1842; found 398.1855.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, **4.2b** (0.052 g, 0.117 mmol, 1.0 equiv.) was used to prepare **4.16** (0.031 g, 0.079 mmol, 68%).

\(^1\)H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\(_3\)) \(\delta\) 7.29 (m, 1H), 7.08 (m, 1H), 7.01 (m, 1H), 6.96 (m, 1H), 6.04 (m, 1H), 4.58*, 4.49 (d, \(J = 8.1\) Hz, 1H), 4.48 (m, 1H), 4.23 (m, 1H), 4.20*, 4.05 (td, \(J = 8.7, 3.0\) Hz, 1H), 3.93 (dd, \(J = 10.6, 7.4\) Hz, 1H), 3.86*, 3.79*, 3.78 (s, 3H), 3.00 (m, 1H), 2.82 (m, 1H), 2.69–2.58 (m, 2H), 1.47*, 1.43 (s, 9H).

\(^13\)C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\(_3\)) \(\delta\) 170.95*, 170.79, 163.64, 161.68, 153.90*, 153.45, 146.24, 143.14, 129.77, 128.40*, 128.30, 121.54, 114.11, 112.86, 87.39, 86.86*, 80.35, 80.30*, 67.82, 62.14, 61.56*, 52.21*, 52.03, 49.38*, 48.73, 40.07*, 39.45, 34.71, 28.33, 28.23.

\(^19\)F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl\(_3\)) \(\delta\) –113.23, –113.26*.

FTIR (neat, cm\(^{-1}\)): 2975 (w), 1745 (m), 1695 (s), 1400 (s), 1165 (s), 1129 (s), 903 (m), 731 (m).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{21}\)H\(_{26}\)FNO\(_5\), 391.1795; found 391.1811.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.052 g, 0.117 mmol, 1.0 equiv.) was used to prepare 4.17 (0.047 g, 0.120 mmol, 99%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 600 MHz, CDCl$_3$) $\delta$ 7.24 (m, 1H), 7.19 (td, $J = 7.7, 1.8$ Hz, 1H), 7.08 (td, $J = 7.5, 1.2$ Hz, 1H), 7.02 (dd, $J = 10.9, 8.2$ Hz, 1H), 5.93 (m, 1H), 4.57*, 4.48 (d, $J = 8.2$ Hz, 1H), 4.44 (ddd, $J = 15.1, 7.2, 2.1$ Hz, 1H), 4.22 (m, 1H), 4.19*, 4.03 (ddd, $J = 9.6, 8.0, 4.2$ Hz, 1H), 3.89 (dd, $J = 10.9, 7.6$ Hz, 1H), 3.82*, 3.79*, 3.78 (s, 3H), 2.95 (dd, $J = 10.4, 9.1$ Hz, 1H), 2.72–2.59 (m, 3H), 1.45*, 1.42 (s, 9H).


$^{19}$F NMR (471 MHz, CDCl$_3$) $\delta$ –114.52.

FTIR (neat, cm$^{-1}$): 2975 (w), 1746 (m), 1694 (s), 1400 (s), 1129 (s), 910 (m), 728 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{21}$H$_{26}$FNO$_5$, 391.1795; found 391.1805.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.052 g, 0.117 mmol, 1.0 equiv.) was used to prepare 4.18 (0.045 g, 0.109 mmol, 94%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 7.16 (m, 1H), 6.80 (m, 2H), 5.90 (m, 1H), 4.56*, 4.48 (d, $J = 8.2$ Hz, 1H), 4.43 (ddd, $J = 15.1$, 7.2, 1.8 Hz, 1H), 4.20 (m, 1H), 4.17*, 4.02 (ddd, $J = 9.5$, 8.0, 3.3 Hz, 1H), 3.88 (dd, $J = 10.8$, 7.4 Hz, 1H), 3.81*, 3.78*, 3.77 (s, 3H), 2.94 (m, 1H), 2.61 (m, 3H), 1.45*, 1.41 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 170.93*, 170.79, 163.20, 161.22, 160.47, 158.48, 153.90*, 153.43, 139.91, 130.51*, 130.41, 130.06, 128.33, 111.16, 103.97, 87.62, 87.09*, 80.29, 80.24*, 67.71, 62.11, 61.54*, 52.17*, 51.99, 49.28*, 48.62, 40.09, 39.47, 35.47, 28.30*, 28.21.

$^{19}$F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl$_3$) $\delta$ –110.37, –110.41*, –111.50.

FTIR (neat, cm$^{-1}$): 2976 (w), 1745 (m), 1695 (s), 1437 (s), 1208 (m), 1097 (s), 911 (m), 729 (s).

HRMS (ESI+, $m/z$): [M+H]+ calc’d for C$_{21}$H$_{25}$F$_2$NO$_5$, 409.1701; found 409.1729.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2b (0.052 g, 0.117 mmol, 1.0 equiv.) was used to prepare 4.19 (0.040 g, 0.098 mmol, 84%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 600 MHz, CDCl$_3$) δ 6.81 (m, 2H), 6.70 (td, $J = 8.9$, 2.3 Hz, 1H), 6.05 (m, 1H), 4.57*, 4.48 (d, $J = 8.2$ Hz, 1H), 4.47 (m, 1H), 4.21 (m, 1H), 4.19*, 4.04 (m, 1H), 3.92 (m, 1H), 3.86*, 3.78*, 3.77 (s, 3H), 2.99 (m, 1H), 2.77 (m, 1H), 2.67–2.58 (m, 2H), 1.46*, 1.42 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 170.89*, 170.74, 163.83*, 163.72*, 161.85*, 161.75*, 153.86*, 153.41, 147.24, 142.17, 142.10*, 129.34*, 129.25, 108.96*, 108.81*, 102.46, 87.25, 86.73*, 80.39, 67.68, 62.09, 61.52*, 52.20*, 52.02, 49.33*, 48.68, 40.01*, 39.39, 34.54, 28.30*, 28.20.

$^{19}$F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl$_3$) δ –109.91, –109.94*.

FTIR (neat, cm$^{-1}$): 2976 (w), 1745 (m), 1695 (s), 1590 (m), 1400 (s), 1117 (s), 988 (s), 730 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{21}$H$_{25}$F$_2$NO$_5$, 409.1701; found 409.1707.
Using the general procedure for Suzuki–Miyaura cross-coupling outlined above, 4.2a (0.040 g, 0.090 mmol, 1.0 equiv.) was used to prepare 4.20 (0.032 g, 0.071 mmol, 80%).

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) δ 7.61 (d, $J = 8.0$ Hz, 2H), 7.57 (d, $J = 8.1$ Hz, 2H), 7.46 (t, $J = 7.6$ Hz, 2H), 7.39 (m, 3H), 6.09 (m, 1H), 4.60*, 4.51 (d, $J = 8.2$ Hz, 1H), 4.50 (m, 1H), 4.24 (m, 1H), 4.08 (m, 1H), 3.95 (m, 1H), 3.87 (m, 1H), 3.80*, 3.79 (s, 3H), 3.03 (m, 1H), 2.92 (m, 1H), 2.72–2.62 (m, 2H), 1.48*, 1.44 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) δ 170.99*, 170.83, 153.94*, 153.50, 143.91, 143.82*, 142.78, 140.53, 140.13, 128.76, 127.40*, 127.31, 126.98*, 126.94, 126.32, 87.53, 86.99*, 80.32, 67.96, 62.19, 61.61*, 52.21*, 52.02, 49.44*, 48.78, 40.12*, 39.50, 34.71, 28.35*, 28.25.

FTIR (neat, cm$^{-1}$): 2976 (w), 2870 (w), 2251 (w), 1748 (m), 1697 (s), 1400 (s), 1169 (s), 908 (m), 729 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{27}$H$_{31}$NO$_5$, 449.2202; found 449.2179.
General procedure for Sonogashira cross-coupling

\[
\text{4.2b} \xrightarrow{\text{CuI, DIPEA, THF}} \text{4.21}
\]

\(N,N\)-Diisopropylethylamine (2.0 equiv.) and the alkyne (1.5 equiv.) were added sequentially to a stirring solution of \(\text{4.2b}\) (1.0 equiv.), copper (I) iodide (0.10 equiv.), and triphenylphosphine palladium (II) chloride (0.05 equiv.) in THF such that the concentration of \(\text{4.2b}\) was 0.1 M. The resultant black solution was stirred at ambient temperature under argon. Consumption of \(\text{4.2b}\) was monitored by mass spectrometry. After complete consumption of \(\text{4.2b}\) was observed, the reaction solution was diluted with ether, filtered through a plug of Celite, washing with ether, and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc in hexanes) to yield \(\text{4.21}\).
Using the general procedure for Sonogashira cross-coupling outlined above, 4.2b (0.040 g, 0.090 mmol, 1.0 equiv.) was used to prepare 4.4 (0.034 g, 0.086 mmol, 95%).

$^{1}$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl$_3$) $\delta$ 7.35–7.24 (m, 5H), 6.17, 6.14* (s, 1H), 4.43*, 4.35 (d, $J = 8.1$ Hz, 1H), 4.00–3.95 (m, 2H), 3.92–3.86 (m, 2H), 3.72*, 3.71 (s, 3H), 3.56 (m, 1H), 3.11 (m, 1H), 2.83 (m, 1H), 2.41 (dd, $J = 16.4$, 4.9 Hz, 1H), 1.41*, 1.36 (s, 9H).

$^{13}$C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl$_3$) $\delta$ 171.07*, 170.87, 153.75*, 153.40, 136.78, 131.36, 128.29, 128.22*, 126.65, 122.96, 90.75, 89.17, 89.11*, 81.94, 81.29*, 80.49, 68.56, 68.46*, 61.95, 61.41*, 52.18*, 52.00, 48.83*, 48.20, 43.67*, 43.00, 37.21, 28.32*, 28.20.

FTIR (neat, cm$^{-1}$): 2974 (w), 2864 (w), 2359 (w), 1749 (m), 1701 (s), 1398 (s), 1180 (s), 1128 (s), 756 (m), 691 (m).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{23}$H$_{27}$NO$_5$, 397.1889; found 397.1907.
Using the general procedure for Sonogashira cross-coupling outlined above, \textit{4.2b} (0.040 g, 0.090 mmol, 1.0 equiv.) was used to prepare \textit{4.22} (0.036 g, 0.087 mmol, 96%).

\textit{^1}H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.24 (m, 1H), 7.14 (m, 1H), 7.06 (d, \(J = 9.4\) Hz, 1H), 6.98 (m, 1H), 6.22 (m, 1H), 6.19*\textsuperscript{*}, 4.46*, 4.38 (d, \(J = 8.2\) Hz, 1H), 3.99 (m, 1H), 3.92 (m, 1H), 3.75*, 3.74 (s, 3H), 3.57 (m, 1H), 3.13 (m, 1H), 2.85 (m, 1H), 2.44 (d, \(J = 4.7\) Hz, 1H), 2.41*, 1.70 (m, 1H), 1.58 (m, 1H), 1.43*, 1.38 (s, 9H).

\textit{^13}C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl\textsubscript{3}) \(\delta\) 171.05*, 170.86, 163.29, 161.33, 153.86*, 153.41, 137.58, 129.90, 129.83*, 127.25, 126.33, 124.80, 118.20, 115.46, 91.60*, 91.58, 87.88, 81.90, 81.25*, 80.56, 68.52, 61.93*, 61.39, 52.20*, 52.01, 48.79*, 48.16, 43.73*, 43.06, 37.06, 28.31*, 28.20.

\textit{^19}F NMR (asterisk denotes minor rotamer peaks, 471 MHz, CDCl\textsubscript{3}) \(\delta\) –112.94*, –112.95.

FTIR (neat, cm\textsuperscript{-1}): 2976 (w), 2866 (w), 1749 (s), 1699 (s), 1398 (s), 1180 (s), 1140 (s), 912 (m), 731 (s).

HRMS (ESI+, \textit{m/z}): [M+H]\textsuperscript{+} calc’d for C\textsubscript{23}H\textsubscript{26}FNO\textsubscript{5}, 415.1795; found 415.1796.
A 1.0 N aq. solution of lithium hydroxide (2.0 equiv.) was added to a solution of 4.23 (1.0 equiv.) in methanol and THF in a 1:1 ratio such that the concentration of 4.23 was 0.2 M. The resultant solution was stirred at room temperature until complete consumption of the methyl ester was observed by mass spectrometry. The reaction solution was acidified with 1 N aq. hydrochloric acid until it reached pH 2 and extracted five times with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to yield 4.24, which was taken forward without further purification.
General procedure for coupling to 7-Cl-MTL

Triethylamine (3.2 equiv.) and bis(trimethylsilyl)trifluoroacetamide (1.5 equiv.) were added sequentially to a solution of 7-Cl-MTL (1.1 equiv.) in DMF such that the concentration of 4.24 was 0.35 M at 0 °C. The resultant colorless solution was stirred for 10 min at 0 °C, stirred for 1 hour at room temperature, then transferred via cannula into a flask charged with 4.24 (1.0 equiv.). HATU (1.3 equiv.) was added in a single portion and the resultant yellow solution was stirred at ambient temperature under argon. After complete consumption of 4.24 was observed by mass spectrometry, the reaction solution was concentrated under reduced pressure and directly purified by column chromatography (methanol in DCM) to yield 4.25. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
General procedure for \textit{N}-Boc deprotection

![Chemical structures and reaction scheme]

Bis(trimethylsilyl)trifluoroacetamide (3.5 equiv.) was added by syringe to a solution of \textbf{4.25} (1.0 equiv.) in acetonitrile such that the concentration of \textbf{4.25} was 0.1 M at 0 °C. The resultant solution was stirred at 0 °C for 5 min, stirred at ambient temperature for 15 min, then cooled back to 0 °C. Trimethylsilyl iodide (1.0 equiv.) was added dropwise by syringe to the reaction solution at 0 °C. The resultant solution was stirred at 0 °C until complete \textit{N}-Boc deprotection was observed by mass spectrometry. The reaction solution was quenched with dropwise addition of methanol and concentrated under reduced pressure. The crude product was purified on reverse-phase HPLC (acetonitrile in water) to yield \textbf{4.26}. 
General procedure for hydrogenation

A suspension of 4.26 (1.0 equiv.) and palladium hydroxide (20% on carbon, 1.0 equiv.) in anhydrous methanol such that the concentration of 4.26 was 0.1 M was stirred under an atmosphere of hydrogen (1 atm) until complete hydrogenation was observed by mass spectrometry. The reaction solution was filtered through a pad of Celite, washing with methanol, and concentrated. The crude product was purified on reverse-phase HPLC (acetonitrile in water) to yield 4.27a, either as a single diastereomer or alongside the 7’-epimer 4.27b.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.3 (0.032 g, 0.086 mmol, 1.0 equiv.) was used to prepare FSA-507056 (0.013 g, 0.025 mmol, 29% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.34 (m, 4H), 7.27 (m, 1H), 6.07 (s, 1H), 5.34 (d, $J = 5.3$ Hz, 1H), 4.68 (d, $J = 8.0$ Hz, 1H), 4.59–4.49 (m, 3H), 4.33 (m, 3H), 4.12 (m, 1H), 3.88 (s, 1H), 3.74 (t, $J = 9.6$ Hz, 1H), 3.62 (d, $J = 9.9$ Hz, 1H), 3.10 (t, $J = 12.2$ Hz, 1H), 2.96 (d, $J = 15.2$ Hz, 1H), 2.74 (t, $J = 13.6$ Hz, 1H), 2.34 (m, 1H), 2.17 (s, 3H), 2.06 (d, $J = 3.8$ Hz, 1H), 1.54 (d, $J = 6.6$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 168.50, 145.28, 129.40, 128.41, 127.05, 89.75, 87.17, 72.07, 70.78, 69.75, 69.56, 68.73, 61.75, 59.10, 55.03, 42.07, 34.18, 22.60, 13.42.

FTIR (neat, cm$^{-1}$): 2924 (w), 2479 (br), 2361 (w), 2070 (w), 1676 (m), 1586 (s), 1466 (m), 1327 (m), 1256 (m), 1090 (s), 1030 (s), 976 (s), 638 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{33}$ClN$_2$O$_6$S, 512.1748; found 512.1776.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.6 (0.031 g, 0.079 mmol, 1.0 equiv.) was used to prepare FSA-507057 (0.019 g, 0.035 mmol, 44% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.35 (dd, $J = 8.6$, 5.4 Hz, 2H), 7.03 (t, $J = 8.7$ Hz, 2H), 6.00 (m, 1H), 5.30 (d, $J = 5.5$ Hz, 1H), 4.57 (dd, $J = 7.1$, 6.4 Hz, 1H), 4.53–4.47 (m, 2H), 4.34–4.29 (m, 1H), 4.27 (d, $J = 9.9$ Hz, 1H), 4.24–4.16 (m, 2H), 4.08 (dd, $J = 10.2$, 5.5 Hz, 1H), 3.88–3.81 (m, 1H), 3.59 (m, 2H), 3.01–2.95 (m, 1H), 2.90–2.82 (m, 1H), 2.73–2.61 (m, 1H), 2.32 (m, 1H), 2.15 (s, 3H), 1.52 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 144.25, 129.00, 128.58, 116.08, 89.76, 87.46, 72.05, 70.82, 69.78, 69.57, 68.65, 61.95, 59.08, 54.97, 42.21, 34.37, 22.61, 13.42.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –117.62.

FTIR (neat, cm$^{-1}$): 2924 (w), 2072 (w), 1678 (m), 1593 (s), 1225 (s), 1159 (s), 1030 (s), 638 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{32}$ClFN$_2$O$_6$S, 530.1654; found 530.1677.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.7 (0.036 g, 0.092 mmol, 1.0 equiv.) was used to prepare **FSA-507052** (0.004 g, 0.008 mmol, 9% over 3 steps).

\[ \text{H NMR (500 MHz, CD}_3\text{OD)} \delta 7.40 (dd, J = 8.6, 5.4 Hz, 2H), 7.07 (t, J = 8.7 Hz, 2H), 6.01 (s, 1H), 5.33 (d, J = 5.6 Hz, 1H), 4.62 (d, J = 8.4 Hz, 1H), 4.61–4.57 (m, 2H), 4.32 (d, J = 10.0 Hz, 1H), 4.23–4.16 (m, 2H), 4.11 (dd, J = 10.2, 5.6 Hz, 1H), 3.84 (m, 2H), 3.71 (t, J = 11.5 Hz, 1H), 3.60 (dd, J = 10.2, 3.4 Hz, 1H), 3.01 (m, 1H), 2.80 (dd, J = 16.5, 4.8 Hz, 1H), 2.18 (s, 3H), 1.62 (d, J = 6.8 Hz, 3H). \]

\[ \text{C NMR (126 MHz, CD}_3\text{OD)} \delta 164.55, 145.92, 128.68, 115.91, 111.38, 89.77, 72.12, 70.95, 69.80, 69.51, 59.06, 37.06, 30.75, 22.99, 13.41. \]

\[ \text{F NMR (471 MHz, CD}_3\text{OD)} \delta -117.76. \]

\[ \text{FTIR (neat, cm}^{-1}\): 2970 (w), 2928 (w), 1682 (m), 1601 (s), 1381 (m), 1256 (s), 1167 (s), 1034 (s), 640 (s). \]

\[ \text{HRMS (ESI+), m/z): [M+H]}^+ \text{ calc’d for C}_{24}\text{H}_{32}\text{ClFNO}_6\text{S, 530.1654; found 530.1627.} \]
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.8 (0.034 g, 0.088 mmol, 1.0 equiv.) was used to prepare FSA-511019 (0.014 g, 0.027 mmol, 31% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 7.23 (d, $J = 7.9$ Hz, 2H), 7.12 (d, $J = 7.9$ Hz, 2H), 5.97 (s, 1H), 5.31 (d, $J = 5.5$ Hz, 1H), 4.59 (q, $J = 7.8$, 6.9 Hz, 1H), 4.53 (d, $J = 10.0$ Hz, 1H), 4.41 (d, $J = 8.8$ Hz, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 4.17 (ddd, $J = 12.1$, 5.1, 2.3 Hz, 1H), 4.11–4.06 (m, 2H), 3.91 (m, 1H), 3.69–3.59 (m, 3H), 3.20 (t, $J = 9.0$ Hz, 1H), 3.14 (t, $J = 11.8$ Hz, 1H), 2.95 (dd, $J = 16.4$, 10.9 Hz, 1H), 2.76 (dd, $J = 16.4$, 4.8 Hz, 1H), 2.30 (s, 3H), 2.15 (s, 3H), 1.57 (d, $J = 6.7$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 170.37, 146.92, 141.23, 138.25, 130.01, 126.69, 126.00, 89.70, 83.38, 72.03, 70.92, 69.75, 62.19, 59.22, 54.85, 45.25, 36.81, 22.69, 21.07, 13.39.

FTIR (neat, cm$^{-1}$): 3340 (br), 2915 (w), 1694 (m), 1585 (s), 1346 (m), 1081 (s), 1058 (s), 986 (m), 808 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{35}$ClN$_2$O$_6$S, 526.1904; found 526.1943.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.9 (0.048 g, 0.124 mmol, 1.0 equiv.) was used to prepare FSA-512075 (0.020 g, 0.039 mmol, 31% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 7.23 (d, $J$ = 8.1 Hz, 2H), 7.12 (d, $J$ = 8.0 Hz, 2H), 5.97 (s, 1H), 5.31 (d, $J$ = 5.6 Hz, 1H), 4.58 (m, 1H), 4.54 (d, $J$ = 9.5 Hz, 1H), 4.50 (d, $J$ = 7.7 Hz, 1H), 4.29 (d, $J$ = 10.0 Hz, 1H), 4.17 (ddd, $J$ = 12.1, 4.9, 2.3 Hz, 1H), 4.09 (m, 2H), 3.89 (br, 1H), 3.74 (m, 1H), 3.66 (t, $J$ = 11.4 Hz, 1H), 3.60 (dd, $J$ = 10.2, 2.9 Hz, 1H), 3.22 (m, 2H), 2.95 (dd, $J$ = 16.2, 11.3 Hz, 1H), 2.77 (dd, $J$ = 16.4, 4.7 Hz, 1H), 2.31 (s, 3H), 2.15 (s, 3H), 1.58 (d, $J$ = 6.8 Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 169.33, 147.12, 141.13, 138.32, 130.02, 126.70, 125.54, 89.72, 82.96, 72.04, 70.84, 69.78, 69.73, 69.57, 61.94, 59.22, 54.95, 44.99, 36.78, 22.68, 21.07, 13.41.

FTIR (neat, cm$^{-1}$): 3344 (br), 2915 (w), 1691 (m), 1581 (s), 1512 (m), 1345 (s), 1059 (m), 807 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{35}$ClN$_2$O$_6$S, 526.1904; found 526.1943.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.10 (0.035 g, 0.079 mmol, 1.0 equiv.) was used to prepare FSA-507060 (0.014 g, 0.023 mmol, 29% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) δ 7.61 (d, $J = 8.2$ Hz, 2H), 7.52 (d, $J = 8.1$ Hz, 2H), 6.15 (s, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.59–4.51 (m, 4H), 4.34 (d, $J = 15.5$ Hz, 1H), 4.29–4.24 (m, 2H), 4.09 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.87 (s, 1H), 3.63 (dd, $J = 11.0$, 7.3 Hz, 1H), 3.59 (dd, $J = 10.2$, 3.1 Hz, 1H), 3.01 (t, $J = 11.8$ Hz, 1H), 2.93 (d, $J = 16.0$ Hz, 1H), 2.74 (t, $J = 13.7$ Hz, 1H), 2.34 (m, 1H), 2.15 (s, 3H), 1.53 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 169.01, 149.26, 143.74, 130.76, 127.72, 126.30, 89.75, 87.25, 72.06, 70.80, 69.74, 69.57, 68.69, 61.90, 59.11, 54.97, 42.36, 34.13, 22.62, 13.42.

$^{19}$F NMR (471 MHz, CD$_3$OD) δ –64.04.

FTIR (neat, cm$^{-1}$): 2924 (w), 2361 (w), 1678 (m), 1598 (s), 1325 (s), 1117 (s), 1071 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{32}$ClF$_3$N$_2$O$_6$S, 580.1622; found 580.1644.
Using the general procedures for saponification, amide bond coupling, and deprotection, \textbf{4.11} (0.043 g, 0.097 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-507053} (0.017 g, 0.029 mmol, 30\% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.64 (d, $J =$ 8.1 Hz, 2H), 7.57 (d, $J =$ 8.2 Hz, 2H), 6.17 (s, 1H), 5.35 (d, $J =$ 5.5 Hz, 1H), 4.65 (m, 2H), 4.59 (m, 2H), 4.33 (d, $J =$ 10.0 Hz, 1H), 4.22 (m, 2H), 4.12 (m, 2H), 3.89 (s, 2H), 3.73 (t, $J =$ 11.5 Hz, 1H), 3.63 (d, $J =$ 10.1 Hz, 1H), 3.04 (m, 1H), 2.83 (dd, $J =$ 16.6, 4.5 Hz, 1H), 2.17 (s, 3H), 1.61 (d, $J =$ 6.7 Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.14, 147.92, 146.01, 128.95, 127.47, 126.37, 89.73, 82.73, 72.05, 70.83, 69.74, 69.58, 61.90, 59.20, 54.98, 45.14, 36.60, 22.69, 13.41.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –64.03.

FTIR (neat, cm$^{-1}$): 2922 (w), 2490 (br), 2070 (w), 1678 (m), 1583 (s), 1464 (m), 1325 (s), 1121 (s), 1069 (s), 976 (m).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{25}$H$_{32}$ClF$_3$N$_2$O$_6$S, 580.1622; found 580.166.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.12 (0.036 g, 0.089 mmol, 1.0 equiv.) was used to prepare **FSA-511020** (0.011 g, 0.020 mmol, 22% over 3 steps).

**1H NMR** (600 MHz, CD$_3$OD) $\delta$ 7.28 (d, $J = 8.8$ Hz, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 5.93 (s, 1H), 5.31 (d, $J = 5.7$ Hz, 1H), 4.59 (q, $J = 6.8$ Hz, 1H), 4.52 (d, $J = 10.0$ Hz, 1H), 4.40 (d, $J = 8.9$ Hz, 1H), 4.27 (d, $J = 10.2$ Hz, 1H), 4.17 (ddd, $J = 12.0$, 5.0, 2.3 Hz, 1H), 4.09 (m, 2H), 3.90 (s, 1H), 3.77 (s, 3H), 3.69–3.57 (m, 3H), 3.19 (m, 1H), 3.13 (m, 1H), 2.94 (ddd, $J = 16.8$, 10.5 Hz, 1H), 2.76 (dd, $J = 16.5$, 4.8 Hz, 1H), 2.15 (s, 3H), 1.57 (d, $J = 6.8$ Hz, 3H).

**13C NMR** (126 MHz, CD$_3$OD) $\delta$ 170.43, 160.62, 146.56, 136.51, 127.96, 125.13, 114.74, 89.71, 83.45, 72.09, 70.93, 69.77, 62.22, 59.22, 55.71, 54.85, 45.23, 36.83, 22.69, 13.39.

FTIR (neat, cm$^{-1}$): 3325 (br), 2918 (w), 1690 (m), 1586 (s), 1510 (s), 1347 (m), 1246 (s), 1035 (m).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{25}$H$_{35}$ClN$_2$O$_7$S, 542.1853; found 542.1895.
Using the general procedures for saponification, amide bond coupling, and deprotection, \textbf{4.13} (0.044 g, 0.109 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-512076} (0.016 g, 0.030 mmol, 28% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.32 (d, $J = 8.6$ Hz, 2H), 6.89 (d, $J = 8.6$ Hz, 2H), 5.96 (s, 1H), 5.33 (d, $J = 5.6$ Hz, 1H), 4.66 (d, $J = 8.9$ Hz, 1H), 4.60 (m, 2H), 4.32 (d, $J = 10.1$ Hz, 1H), 4.20 (m, 2H), 4.11 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.86 (m, 2H), 3.80 (s, 3H), 3.70 (t, $J = 11.4$ Hz, 1H), 3.61 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.32 (m, 2H), 2.97 (dd, $J = 16.2$, 11.1 Hz, 1H), 2.81 (dd, $J = 16.5$, 4.7 Hz, 1H), 2.17 (s, 3H), 1.61 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.29, 160.67, 146.77, 136.38, 127.98, 124.61, 114.75, 89.73, 82.99, 72.04, 70.84, 69.75, 69.58, 61.95, 59.22, 55.71, 54.96, 44.95, 36.80, 22.68, 13.41.

FTIR (neat, cm$^{-1}$): 3342 (br), 2916 (w), 1695 (m), 1584 (s), 1511 (s), 1465 (m), 1346 (m), 1246 (s), 1059 (m).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{25}$H$_{35}$ClN$_2$O$_7$S, 542.1853; found 542.188.
Using the general procedures for saponification, amide bond coupling, and deprotection, \(4.14\) (0.036 g, 0.090 mmol, 1.0 equiv.) was used to prepare **FSA-511033** (0.014 g, 0.027 mmol, 30% over 3 steps).

\(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 7.67 (d, \(J = 8.4\) Hz, 2H), 7.54 (d, \(J = 8.3\) Hz, 2H), 6.19 (s, 1H), 5.31 (d, \(J = 5.6\) Hz, 1H), 4.59 (qd, \(J = 6.8, 1.6\) Hz, 1H), 4.53 (d, \(J = 11.6\) Hz, 1H), 4.43 (d, \(J = 8.8\) Hz, 1H), 4.28 (d, \(J = 10.0\) Hz, 1H), 4.18 (ddd, \(J = 12.2, 5.0, 2.3\) Hz, 1H), 4.14–4.08 (m, 2H), 3.91 (s, 1H), 3.75–3.65 (m, 2H), 3.60 (dd, \(J = 10.2, 3.3\) Hz, 1H), 3.30–3.25 (m, 1H), 3.19 (t, \(J = 11.6\) Hz, 1H), 3.02 (m, 1H), 2.78 (dd, \(J = 16.5, 4.7\) Hz, 1H), 2.15 (s, 3H), 1.57 (d, \(J = 6.8\) Hz, 3H).

\(^{13}\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 170.25, 148.78, 145.57, 133.39, 130.44, 127.77, 119.68, 111.77, 89.70, 83.10, 72.03, 70.89, 69.58, 62.13, 59.21, 54.85, 45.49, 36.35, 22.70, 13.39.

FTIR (neat, cm\(^{-1}\)): 3338 (br), 2918 (w), 2226 (w), 1690 (m), 1602 (s), 1347 (m), 1080 (m).

HRMS (ESI+, \(m/z\)): [M+H]\(^+\) calc’ed for C\(_{25}\)H\(_{32}\)ClN\(_3\)O\(_6\)S, 537.17; found 537.1737.
Using the general procedures for saponification, amide bond coupling, and deprotection, **4.15** (0.048 g, 0.120 mmol, 1.0 equiv.) was used to prepare **FSA-512077** (0.014 g, 0.025 mmol, 21% over 3 steps).

**H NMR** (600 MHz, CD$_3$OD) δ 7.70 (d, $J = 8.5$ Hz, 2H), 7.56 (d, $J = 8.5$ Hz, 2H), 6.21 (s, 1H), 5.33 (d, $J = 5.6$ Hz, 1H), 4.61–4.56 (m, 3H), 4.31 (d, $J = 10.0$ Hz, 1H), 4.20 (m, 1H), 4.12 (m, 1H), 3.90 (br, 1H), 3.81 (m, 1H), 3.71 (m, 1H), 3.61 (m, 1H), 3.36–3.29 (m, 2H), 3.03 (m, 1H), 2.17 (s, 3H), 2.04 (d, $J = 11.4$ Hz, 2H), 1.60 (d, $J = 6.7$ Hz, 3H).

**C NMR** (126 MHz, CD$_3$OD) δ 148.78, 145.61, 133.40, 130.35, 127.78, 111.80, 89.72, 83.06, 72.03, 70.89, 69.76, 69.58, 62.11, 59.21, 54.87, 45.47, 36.35, 22.70, 13.39.

**FTIR** (neat, cm$^{-1}$): 3339 (br), 2918 (w), 2226 (w), 1690 (m), 1586 (s), 1503 (m), 1346 (m), 1080 (s), 820 (m).

**HRMS** (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{32}$ClN$_3$O$_6$S, 537.17; found 537.1736.
Using the general procedures for saponification, amide bond coupling, and deprotection, **4.16** (0.031 g, 0.079 mmol, 1.0 equiv.) was used to prepare **FSA-511071** (0.021 g, 0.040 mmol, 51% over 3 steps).

^{1}H NMR (600 MHz, CD$_3$OD) $\delta$ 7.32 (dd, $J = 8.0, 6.0$ Hz, 1H), 7.15 (d, $J = 7.6$ Hz, 1H), 7.08 (dt, $J = 10.5, 2.2$ Hz, 1H), 6.98 (td, $J = 8.6, 2.6$ Hz, 1H), 6.09 (m, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.65 (dd, $J = 9.0, 3.7$ Hz, 1H), 4.57–4.49 (m, 3H), 4.33–4.26 (m, 3H), 4.09 (dd, $J = 10.2, 5.6$ Hz, 1H), 3.87 (s, 1H), 3.70 (dd, $J = 10.8, 7.1$ Hz, 1H), 3.59 (dd, $J = 10.2, 3.2$ Hz, 1H), 3.07 (t, $J = 11.9$ Hz, 1H), 2.91 (dd, $J = 16.0, 2.6$ Hz, 1H), 2.71 (td, $J = 16.2, 11.3$ Hz, 1H), 2.34 (m, 1H), 2.15 (s, 3H), 1.53 (d, $J = 6.8$ Hz, 3H).

^{13}C NMR (126 MHz, CD$_3$OD) $\delta$ 167.92, 166.80, 147.68, 143.76, 131.17, 129.65, 122.97, 115.06, 113.96, 89.73, 86.76, 72.06, 70.74, 69.55, 68.67, 61.57, 59.11, 55.05, 42.02, 33.97, 22.60, 13.43.

^{19}F NMR (471 MHz, CD$_3$OD) $\delta$ –115.39.

FTIR (neat, cm$^{-1}$): 3341 (br), 1677 (m), 1582 (s), 1467 (m), 1374 (m), 1148 (m), 1088 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{32}$ClFN$_{2}$O$_{6}$S, 530.1654; found 530.1689.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.17 (0.047 g, 0.120 mmol, 1.0 equiv.) was used to prepare **FSA-511073** (0.046 g, 0.086 mmol, 72% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 7.28 (dd, $J = 7.9$, 5.1 Hz, 1H), 7.22 (td, $J = 7.8$, 1.7 Hz, 1H), 7.11 (td, $J = 7.6$ Hz, 1H), 7.05 (dd, $J = 10.8$, 8.2 Hz, 1H), 5.97 (m, 1H), 5.31 (d, $J = 5.7$ Hz, 1H), 4.63 (m, 1H), 4.58–4.53 (m, 2H), 4.48 (dd, $J = 15.2$, 7.0 Hz, 1H), 4.31–4.25 (m, 3H), 4.09 (dd, $J = 10.2$, 5.7 Hz, 1H), 3.88 (m, 1H), 3.64–3.59 (m, 2H), 3.01 (td, $J = 11.9$, 4.8 Hz, 1H), 2.75–2.67 (m, 2H), 2.36 (m, 1H), 2.15 (s, 3H), 1.54 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 168.40, 141.45, 131.60, 130.65, 125.44, 116.44, 89.71, 87.31, 72.06, 70.76, 69.55, 68.50, 61.61, 59.12, 55.01, 41.89, 34.98, 22.60, 13.43.

$^{19}$F NMR (471 MHz, CD$_3$OD) δ −116.95.

FTIR (neat, cm$^{-1}$): 3337 (br), 2920 (w), 1680 (m), 1585 (s), 1449 (m), 1348 (m), 1056 (m), 758 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{31}$ClF$_2$N$_2$O$_6$S, 548.1559; found 548.1587.
Using the general procedures for saponification, amide bond coupling, and deprotection, \textbf{4.18} (0.045 g, 0.110 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-511072} (0.029 g, 0.053 mmol, 48\% over 3 steps).

\textbf{1}H NMR (600 MHz, CD\textsubscript{3}OD) \(\delta\) 7.25 (dd, \(J = 9.2, 8.7\) Hz, 1H), 6.93–6.90 (m, 2H), 5.96 (m, 1H), 5.31 (d, \(J = 5.6\) Hz, 1H), 4.66 (m, 1H), 4.57–4.53 (m, 2H), 4.48 (dd, \(J = 15.3, 7.0\) Hz, 1H), 4.31–4.26 (m, 3H), 4.09 (dd, \(J = 10.2, 5.6\) Hz, 1H), 3.88 (s, 1H), 3.65 (t, \(J = 9.1\) Hz, 1H), 3.60 (d, \(J = 10.1\) Hz, 1H), 3.05 (t, \(J = 11.9\) Hz, 1H), 2.73–2.67 (m, 2H), 2.37 (m, 1H), 2.15 (s, 3H), 1.53 (d, \(J = 6.7\) Hz, 3H).

\textbf{13}C NMR (126 MHz, CD\textsubscript{3}OD) \(\delta\) 167.88, 140.29, 132.06, 112.20, 104.78, 89.70, 87.01, 72.06, 70.72, 69.54, 68.47, 61.48, 59.11, 55.04, 41.79, 34.86, 22.59, 13.43.

\textbf{19}F NMR (471 MHz, CD\textsubscript{3}OD) \(\delta\) –112.57, –113.36.

FTIR (neat, cm\textsuperscript{-1}): 3367 (br), 2921 (w), 1689 (s), 1590 (s), 1499 (s), 1367 (m), 1290 (m), 1213 (s), 1213 (s), 851 (m).

HRMS (ESI+, \textit{m/z}): [M+H]+ calc’\d for C\textsubscript{24}H\textsubscript{31}ClF\textsubscript{2}N\textsubscript{2}O\textsubscript{6}S, 548.1559; found 548.1587.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.19 (0.040 g, 0.098 mmol, 1.0 equiv.) was used to prepare FSA-511074 (0.022 g, 0.040 mmol, 41% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 6.96 (dt, $J = 7.3$, 2.2 Hz, 2H), 6.84 (tt, $J = 9.0$, 2.3 Hz, 1H), 6.14 (m, 1H), 5.30 (d, $J = 5.8$ Hz, 1H), 4.64 (d, $J = 8.9$ Hz, 1H), 4.57–4.50 (m, 3H), 4.33–4.25 (m, 3H), 4.09 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.86 (m, 1H), 3.70 (dd, $J = 11.3$, 7.4 Hz, 1H), 3.59 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.06 (dd, $J = 12.5$, 11.4 Hz, 1H), 2.88 (dd, $J = 16.2$, 2.7 Hz, 1H), 2.69 (tt, $J = 16.3$, 11.2, 2.6 Hz, 1H), 2.34 (m, 1H), 2.15 (s, 3H), 1.52 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 167.97, 130.77, 110.22, 110.01, 103.27, 89.74, 86.68, 72.07, 70.74, 69.56, 68.60, 61.61, 59.11, 55.05, 42.12, 33.82, 22.62, 13.43.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –111.91.

FTIR (neat, cm$^{-1}$): 3338 (br), 1692 (m), 1590 (s), 1470 (m), 1345 (m), 1120 (m), 1077 (m), 988 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{31}$ClF$_2$N$_2$O$_6$S, 548.1559; found 548.1574.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.20 (0.032 g, 0.071 mmol, 1.0 equiv.) was used to prepare FSA-510001 (0.013 g, 0.022 mmol, 31% over 3 steps).

\(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\) 7.59 (m, 4H), 7.43 (m, 4H), 7.33 (m, 1H), 6.12 (m, 1H), 5.30 (d, \(J = 5.6\) Hz, 1H), 4.58 (qd, \(J = 6.7, 1.6\) Hz, 1H), 4.54–4.50 (m, 2H), 4.47 (d, \(J = 8.7\) Hz, 1H), 4.32 (ddd, \(J = 15.2, 4.2, 2.0\) Hz, 1H), 4.27 (d, \(J = 10.0\) Hz, 1H), 4.23 (t, \(J = 7.7\) Hz, 1H), 4.09 (dd, \(J = 10.2, 5.7\) Hz, 1H), 3.86 (dd, \(J = 3.4, 1.2\) Hz, 1H), 3.61–3.57 (m, 2H), 2.96 (m, 2H), 2.71 (m, 1H), 2.33–2.26 (m, 1H), 2.15 (s, 3H), 1.54 (d, \(J = 6.8\) Hz, 3H).

\(^13\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 144.96, 144.23, 141.84, 141.43, 129.87, 1288.41, 127.90, 127.79, 127.55, 111.40, 89.74, 87.92, 72.04, 70.88, 69.76, 69.59, 68.72, 62.14, 59.12, 54.90, 42.42, 34.32, 22.64, 13.41.

FTIR (neat, cm\(^{-1}\)): 3348 (br), 2923 (w), 1686 (s), 1585 (s), 1349 (m), 1081 (m), 764 (s).

HRMS (ESI+, \(m/z\)): [M+H]\(^+\) calc’d for C\(_{30}\)H\(_{37}\)ClN\(_2\)O\(_6\)S, 588.2061; found 588.2086.
Using the general procedure for hydrogenation, **FSA-507056** (0.008 g, 0.016 mmol, 1.0 equiv.) was used to prepare **FSA-509018** (0.007 g, 0.013 mmol, 81%).

**1H NMR** (600 MHz, CD$_3$OD) $\delta$ 7.28 (t, $J = 7.6$ Hz, 2H), 7.24 (d, $J = 6.7$ Hz, 2H), 7.16 (t, $J = 7.2$ Hz, 1H), 5.31 (d, $J = 5.6$ Hz, 1H), 4.58 (q, $J = 6.5$ Hz, 1H), 4.52 (d, $J = 10.3$ Hz, 1H), 4.43 (m, 1H), 4.38 (m, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 4.14–4.08 (m, 2H), 3.90 (br, 1H), 3.69 (t, $J = 11.6$ Hz, 1H), 3.60 (dd, $J = 10.0$, 3.3 Hz, 1H), 3.53 (m, 1H), 3.25 (m, 1H), 2.89 (m, 1H), 2.64 (m, 1H), 2.27 (m, 1H), 2.15 (s, 3H), 2.04 (m, 1H), 1.90 (m, 1H), 1.83 (dd, $J = 15.2$, 4.3 Hz, 1H), 1.54 (d, $J = 6.8$ Hz, 3H).

**13C NMR** (126 MHz, CD$_3$OD) $\delta$ 169.64, 149.08, 129.67, 127.63, 127.22, 89.73, 83.11, 72.58, 72.05, 70.89, 69.72, 69.58, 63.18, 59.20, 54.80, 50.66, 45.37, 44.74, 38.68, 36.07, 22.62, 13.40.

**FTIR** (neat, cm$^{-1}$): 3369 (br), 2923 (w), 1682 (m), 1585 (s), 1350 (m), 1081 (m), 1056 (m), 701 (m).

**HRMS** (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{35}$ClN$_2$O$_6$S, 514.1904; found 514.1931.
Using the general procedure for hydrogenation, **FSA-507057** (0.012 g, 0.023 mmol, 1.0 equiv.) was used to prepare **FSA-507061** (0.006 g, 0.011 mmol, 48%).

\(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\) 7.27 (dd, \(J = 8.5, 5.3\) Hz, 2H), 7.02 (td, \(J = 8.8\) Hz, 1.2 Hz, 2H), 5.32 (d, \(J = 5.5\) Hz, 1H), 4.60 (q, \(J = 6.8\) Hz, 1H), 4.53 (d, \(J = 9.9\) Hz, 1H), 4.47–4.35 (m, 2H), 4.29 (d, \(J = 10.0\) Hz, 1H), 4.16–4.09 (m, 2H), 3.90 (s, 1H), 3.70 (t, \(J = 11.6\) Hz, 1H), 3.60 (d, \(J = 10.1\) Hz, 1H), 3.54 (m, 1H), 3.28 (m, 1H), 2.89 (m, 1H), 2.64 (s, 1H), 2.27 (dd, \(J = 12.3, 11.6\) Hz, 1H), 2.17 (s, 3H), 2.02 (dt, \(J = 15.8, 7.9\) Hz, 1H), 1.91 (m, 1H), 1.82 (d, \(J = 11.4\) Hz, 1H), 1.55 (d, \(J = 6.7\) Hz, 3H).

\(^{13}\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 169.58, 163.69, 161.76, 145.02, 129.36, 116.25, 89.75, 83.05, 72.52, 72.05, 70.88, 69.77, 69.58, 63.17, 59.18, 54.81, 50.67, 44.62, 38.74, 36.12, 22.62, 13.40.

\(^{19}\)F NMR (471 MHz, CD\(_3\)OD) \(\delta\) –119.26.

FTIR (neat, cm\(^{-1}\)): 3329 (br), 2926 (w), 2363 (w), 1684 (m), 1593 (s), 1350 (m), 1138 (m), 1088 (m), 1030 (s).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{24}\)H\(_{34}\)ClFN\(_2\)O\(_6\)S 532.181, found 532.182.
Using the general procedure for hydrogenation, **FSA-512075** (0.010 g, 0.019 mmol, 1.0 equiv.) was used to prepare **FSA-512079b** (0.004 g, 0.008 mmol, 42%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.11 (d, $J = 8.3$ Hz, 2H), 7.08 (d, $J = 8.2$ Hz, 2H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.59 (dd, $J = 6.8$, 1.6 Hz, 1H), 4.46 (d, $J = 10.1$ Hz, 1H), 4.29 (t, $J = 8.9$ Hz, 1H), 4.12–4.06 (m, 3H), 3.91 (m, 1H), 3.64 (t, $J = 11.6$ Hz, 1H), 3.58 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.41 (m, 1H), 3.18 (m, 1H), 2.73 (t, $J = 11.2$ Hz, 1H), 2.56 (m, 1H), 2.28 (s, 3H), 2.25–2.21 (m, 2H), 2.15 (s, 3H), 1.99 (m, 1H), 1.85 (m, 1H), 1.77 (m, 1H), 1.52 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 146.20, 136.68, 130.20, 127.50, 89.70, 83.73, 72.58, 72.00, 70.98, 69.74, 69.60, 63.54, 59.18, 54.69, 51.08, 45.06, 38.83, 36.32, 22.63, 21.00, 13.37.

FTIR (neat, cm$^{-1}$): 3346 (br), 2922 (s), 2867 (w), 1663 (m), 1592 (s), 1453 (m), 1352 (m), 1088 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{37}$ClN$_2$O$_6$S, 528.2061; found 528.2078.
Using the general procedure for hydrogenation, **FSA-512075** (0.010 g, 0.019 mmol, 1.0 equiv.) was used to prepare **FSA-512079c** (0.005 g, 0.009 mmol, 47%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.11 (d, $J = 8.2$ Hz, 2H), 7.08 (d, $J = 8.2$ Hz, 2H), 5.30 (d, $J = 5.7$ Hz, 1H), 4.60 (m, 1H), 4.46 (d, $J = 10.0$ Hz, 1H), 4.42 (t, $J = 9.1$ Hz, 1H), 4.24 (d, $J = 9.8$ Hz, 1H), 4.16 (d, $J = 9.0$ Hz, 1H), 4.10–4.05 (m, 2H), 3.92 (dd, $J = 11.6$, 3.0 Hz, 1H), 3.89 (d, $J = 3.0$ Hz, 1H), 3.59 (dd, $J = 10.3$, 3.4 Hz, 1H), 3.38 (dd, $J = 10.5$, 7.4 Hz, 1H) 2.82 (m, 1H), 2.71 (m, 1H), 2.36–2.26 (m, 2H), 2.28 (s, 3H), 2.15 (s, 3H), 2.05 (m, 1H), 1.87 (m, 1H), 1.54 (d, $J = 6.8$ Hz, 3H). 1.50–1.44 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 145.78, 136.73, 130.14, 127.43, 89.69, 82.18, 72.01, 71.04, 69.74, 69.61, 69.19, 63.32, 59.16, 54.63, 50.57, 45.85, 44.73, 39.98, 37.40, 22.67, 21.01, 13.36.

FTIR (neat, cm$^{-1}$): 3354 (br), 2922 (s), 2859 (m), 1664 (s), 1592 (s), 1448 (m), 1352 (m), 1256 (w), 1088 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{25}$H$_{37}$ClN$_2$O$_6$S, 528.2061; found 528.2087.
Using the general procedure for hydrogenation, **FSA-512076** (0.010 g, 0.018 mmol, 1.0 equiv.) was used to prepare **FSA-512080b** (0.006 g, 0.011 mmol, 61%).

**H NMR (600 MHz, CD$_3$OD)** $\delta$ 7.14 (d, $J = 8.7$ Hz, 2H), 6.83 (d, $J = 8.7$ Hz, 2H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.59 (dd, $J = 6.8$, 1.6 Hz, 1H) 4.45 (d, $J = 9.9$, 1.6 Hz, 1H), 4.27 (t, $J = 9.0$ Hz, 1H), 4.23 (d, $J = 10.2$ Hz, 1H), 4.19 (d, $J = 8.8$ Hz, 1H), 4.12 (m, 1H), 4.08 (dd, $J = 10.3$, 5.7 Hz, 1H), 3.93 (m, 1H), 3.75 (s, 3H), 3.64 (m, 1H), 3.58 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.38 (m, 1H), 3.17 (m, 1H), 2.71 (t, $J = 11.1$ Hz, 1H), 2.54 (m, 1H), 2.23 (m, 1H), 2.15 (s, 3H), 1.98 (m, 1H), 1.84 (m, 1H), 1.77 (m, 1H), 1.52 (d, $J = 6.8$ Hz, 3H).

**$^{13}$C NMR (126 MHz, CD$_3$OD)** $\delta$ 159.46, 141.40, 128.54, 114.97, 89.69, 83.86, 72.57, 71.99, 71.00, 69.74, 69.60, 63.62, 59.19, 55.66, 54.66, 51.18, 45.08, 44.67, 39.01, 36.48, 22.64, 13.36.

**FTIR (neat, cm$^{-1}$):** 3324 (br), 2925 (m), 2858 (w), 1677 (m), 1584 (s), 1513 (s), 1353 (m), 1249 (s), 1119 (m), 1055 (m).

**HRMS (ESI+, m/z):** [M+H$^+$] calc’d for C$_{25}$H$_{37}$ClN$_2$O$_7$S, 544.201; found 544.2034.
Using the general procedure for hydrogenation, FSA-512076 (0.010 g, 0.018 mmol, 1.0 equiv.) was used to prepare FSA-512080c (0.004 g, 0.007 mmol, 39%).

$\text{H NMR (600 MHz, CD}_3\text{OD)} \delta 7.14 (d, J = 8.7 \text{ Hz, 2H}), 6.83 (d, J = 8.7 \text{ Hz, 2H}), 5.30 (d, J = 5.6 \text{ Hz, 1H}), 4.60 (dd, J = 6.7, 1.6 \text{ Hz, 1H}), 4.47 (d, J = 9.9 \text{ Hz, 1H}), 4.44 (t, J = 9.2 \text{ Hz, 1H}), 4.25 (d, J = 10.1 \text{ Hz, 1H}), 4.22 (d, J = 9.2 \text{ Hz, 1H}), 4.10–4.05 (m, 2H), 3.93 (dd, J = 11.6, 3.1 \text{ Hz, 1H}), 3.89 (d, J = 3.4 \text{ Hz, 1H}), 3.75 (s, 3H), 3.59 (dd, J = 10.2, 3.4 \text{ Hz, 1H}), 3.42 (m, 1H), 2.82 (t, J = 11.3 \text{ Hz, 1H}), 2.75 (t, J = 11.2 \text{ Hz, 1H}), 2.34 (m, 1H), 2.29–2.23 (m, 1H), 2.15 (s, 3H), 2.05 (m, 1H), 1.88 (m, 1H), 1.54 (d, J = 6.8 \text{ Hz, 3H}), 1.47 (m, 1H).

$\text{C NMR (126 MHz, CD}_3\text{OD)} \delta 128.44, 114.94, 89.73, 81.59, 72.03, 70.95, 69.76, 69.59, 69.22, 62.93, 59.14, 55.66, 54.76, 45.21, 44.49, 37.56, 22.66, 13.39.$

FTIR (neat, cm$^{-1}$): 3370 (br), 2924 (m), 1682 (m), 1593 (s), 1513 (s), 1353 (m), 1248 (s), 1086 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{37}$ClN$_2$O$_7$S, 544.201; found 544.2022.
Using the general procedure for hydrogenation, **FSA-512077** (0.010 g, 0.019 mmol, 1.0 equiv.) was used to prepare **FSA-512081a** (0.004 g, 0.007 mmol, 40%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.65 (d, $J = 8.4$ Hz, 2H), 7.44 (d, $J = 8.4$ Hz, 2H), 5.30 (d, $J = 5.7$ Hz, 1H), 4.59 (m, 1H), 4.46 (d, $J = 10.0$ Hz, 1H), 4.30 (t, $J = 8.9$ Hz, 1H), 4.24 (d, $J = 9.7$ Hz, 2H), 4.14 (ddd, $J = 12.4$, 4.6, 2.4 Hz, 1H), 4.08 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.92 (m, 1H), 3.66 (t, $J = 11.6$ Hz, 1H), 3.58 (dd, $J = 10.1$, 3.4 Hz, 1H) 3.42 (m, 1H), 3.34 (m, 1H), 2.75 (t, $J = 11.1$ Hz, 1H), 2.59 (m, 1H), 2.29 (m, 1H), 2.14 (s, 3H), 1.99 (m, 1H), 1.90 (m, 1H), 1.79 (d, $J = 14.2$ Hz, 1H), 1.52 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 155.06, 133.80, 129.09, 119.93, 111.16, 89.86, 83.75, 72.50, 72.16, 71.10, 69.89, 69.74, 63.62, 59.34, 54.86, 51.07, 45.60, 45.18, 38.17, 35.90, 22.79, 13.52.

FTIR (neat, cm$^{-1}$): 3343 (br), 2923 (m), 2226 (w), 1675 (m), 1590 (s), 1351 (m), 1258 (w), 1084 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{34}$ClN$_3$O$_6$S, 539.1857; found 539.1875.
Using the general procedure for hydrogenation, FSA-512077 (0.010 g, 0.019 mmol, 1.0 equiv.) was used to prepare FSA-512081b (0.006 g, 0.011 mmol, 58%).

$^1$H NMR (600 MHz, CD$_3$OD) δ 7.66 (d, $J$ = 8.1 Hz, 2H), 7.45 (d, $J$ = 8.3 Hz, 2H), 5.30 (d, $J$ = 5.6 Hz, 1H), 4.59 (m, 1H), 4.49 (t, $J$ = 9.4 Hz, 2H), 4.35 (d, $J$ = 8.9 Hz, 1H), 4.27 (d, $J$ = 10.0 Hz, 1H), 4.09 (m, 2H), 3.95 (td, $J$ = 11.7, 3.4 Hz, 1H), 3.87 (m, 1H), 3.59 (dd, $J$ = 10.2, 2.4 Hz, 1H), 3.52 (dd, $J$ = 11.0, 7.4 Hz, 1H), 3.00 (t, $J$ = 11.2 Hz, 1H), 2.85 (t, $J$ = 11.5 Hz, 1H), 2.41 (m, 1H), 2.34 (m, 1H), 2.15 (s, 3H), 2.09 (d, $J$ = 13.4 Hz, 1H), 1.92 (d, $J$ = 15.3 Hz, 1H), 1.55 (d, $J$ = 6.6 Hz, 3H), 1.53 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 154.40, 133.77, 129.01, 119.90, 111.33, 89.92, 80.70, 72.22, 70.96, 69.90, 69.71, 69.16, 62.31, 59.28, 55.09, 45.58, 44.44, 38.82, 36.89, 22.82, 13.57.

FTIR (neat, cm$^{-1}$): 3340 (br), 2924 (m), 2228 (w), 1687 (m), 1592 (s), 1347 (m), 1137 (m), 1084 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{34}$ClN$_3$O$_6$S, 539.1857; found 539.1888.
Using the general procedure for hydrogenation, **FSA-507060** (0.012 g, 0.021 mmol, 1.0 equiv.) was used to prepare **FSA-509019** (0.005 g, 0.009 mmol, 43%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.59 (d, $J = 8.0$ Hz, 2H), 7.45 (d, $J = 8.1$ Hz, 2H), 5.31 (d, $J = 5.5$ Hz, 1H), 4.57 (dd, $J = 7.4$, 6.8 Hz, 1H), 4.52 (d, $J = 10.0$ Hz, 1H), 4.50–4.45 (m, 1H), 4.44–4.38 (m, 1H), 4.28 (d, $J = 10.1$ Hz, 1H), 4.14 (ddd, $J = 12.4, 4.4, 2.2$ Hz, 1H), 4.09 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.90 (s, 1H), 3.71 (t, $J = 11.6$ Hz, 1H), 3.60 (m, 1H), 3.55 (m, 1H), 3.37 (q, $J = 9.4$ Hz, 1H), 2.93 (s, 1H), 2.68 (s, 1H), 2.30 (m, 1H), 2.15 (s, 3H), 2.03 (dd, $J = 13.3, 9.3$, 6.8 Hz, 1H), 1.94 (m, 1H), 1.84 (dd, $J = 15.2$, 4.3 Hz, 1H), 1.52 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.42, 153.61, 128.59, 126.69, 89.89, 83.04, 72.56, 72.20, 71.00, 69.88, 69.73, 63.24, 59.33, 54.98, 50.68, 45.23, 44.81, 38.35, 35.85, 22.77, 13.56.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –63.90.

FTIR (neat, cm$^{-1}$): 3368 (br), 2924 (w), 1677 (m), 1585 (s), 1325 (s), 1121 (s), 1069 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{25}$H$_{34}$ClF$_3$N$_2$O$_6$S, 582.1778; found 582.1804.
Using the general procedure for hydrogenation, **FSA-511071** (0.015 g, 0.028 mmol, 1.0 equiv.) was used to prepare **FSA-511077** (0.007 g, 0.013 mmol, 46%).

\(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 7.29 (dd, \(J = 8.0, 6.1\) Hz, 1H), 7.06 (d, \(J = 7.1\) Hz, 1H), 7.00 (dt, \(J = 10.2, 2.1\) Hz, 1H), 6.90 (m, 1H), 5.31 (d, \(J = 5.6\) Hz, 1H), 4.59–4.49 (m, 3H), 4.40 (t, \(J = 8.9\) Hz, 1H), 4.29 (d, \(J = 9.9\) Hz, 1H), 4.14–4.08 (m, 3H), 3.91 (m, 1H), 3.69 (t, \(J = 11.6\) Hz, 1H), 3.61–3.55 (m, 1H), 3.31–3.26 (m, 1H), 2.94 (m, 1H), 2.66 (m, 1H), 2.25 (dd, \(J = 13.4, 10.9\) Hz, 1H), 2.15 (s, 3H), 2.02 (m, 1H), 1.94–1.88 (m, 1H), 1.83 (dd, \(J = 15.4, 3.6\) Hz, 1H), 1.53 (d, \(J = 6.7\) Hz, 3H).

\(^{13}\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 169.09, 164.58 (d, \(J = 244.5\) Hz), 151.91, 131.49, 123.70, 114.57 (d, \(J = 21.4\) Hz), 114.02 (d, \(J = 21.3\) Hz), 89.87, 82.88, 72.55, 72.23, 70.98, 69.81, 69.72, 63.13, 59.37, 55.00, 50.56, 45.13, 44.65, 38.51, 35.87, 22.78, 13.56

\(^{19}\)F NMR (471 MHz, CD\(_3\)OD) \(\delta\) –115.23.

FTIR (neat, cm\(^{-1}\)): 3308 (br), 2924 (w), 1677 (m), 1588 (s), 1452 (m), 1351 (m), 1257 (m), 1084 (m).

HRMS (ESI+, \(m/z\)): \([M+H]^+\) calc’d for C\(_{24}\)H\(_{34}\)ClFN\(_2\)O\(_6\)S, 532.181; found 532.1851.
Using the general procedure for hydrogenation, **FSA-511072** (0.017 g, 0.031 mmol, 1.0 equiv.) was used to prepare **FSA-511078** (0.008 g, 0.014 mmol, 45%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.34 (ddd, $J = 8.8, 8.3, 6.3$ Hz, 1H), 6.94–6.87 (m, 2H), 5.31 (d, $J = 5.6$ Hz, 1H), 4.58 (q, $J = 6.9$ Hz, 1H), 4.51 (d, $J = 10.0$ Hz, 1H), 4.41 (d, $J = 9.2$ Hz, 1H), 4.35 (t, $J = 8.9$ Hz, 1H), 4.27 (d, $J = 10.0$ Hz, 1H), 4.14 (ddd, $J = 12.4, 4.5, 2.3$ Hz, 1H), 4.09 (dd, $J = 10.1, 5.5$ Hz, 1H), 3.91 (d, $J = 3.4$ Hz, 1H), 3.67 (t, $J = 11.6$ Hz, 1H), 3.59 (dd, $J = 10.1, 3.5$ Hz, 1H), 3.54–3.49 (m, 2H), 2.87 (t, $J = 11.6$ Hz, 1H), 2.63 (m, 1H), 2.32 (m, 1H), 2.15 (s, 3H), 2.04–1.99 (m, 1H), 1.94–1.88 (m, 1H), 1.77 (dd, $J = 15.1, 4.3$ Hz, 1H), 1.55 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.35, 130.42, 112.46*, 112.29*, 104.61, 89.73, 83.12, 72.69, 72.06, 70.86, 69.71, 69.58, 63.19, 59.20, 54.80, 50.55, 44.70, 38.55, 37.57, 34.29, 22.63, 13.40.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –115.42, –116.18.

FTIR (neat, cm$^{-1}$): 3335 (br), 2923 (w), 1677 (m), 1594 (s), 1503 (s), 1349 (m), 1138 (m), 1087 (m), 967 (m).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{24}$H$_{34}$ClFN$_2$O$_6$S, 550.1716; found 550.1739.
Using the general procedure for hydrogenation, \textbf{FSA-511074} (0.014 g, 0.025 mmol, 1.0 equiv.) was used to prepare \textbf{FSA-511080} (0.006 g, 0.011 mmol, 44%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 6.89–6.87 (m, 2H), 6.76–6.71 (m, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.58 (dd, $J = 6.9$, 6.3 Hz, 1H), 4.50 (d, $J = 10.0$ Hz, 1H), 4.37 (d, $J = 9.2$ Hz, 1H), 4.34 (t, $J = 8.8$ Hz, 1H), 4.26 (d, $J = 10.1$ Hz, 1H), 4.18–4.12 (m, 1H), 4.10–4.06 (m, 1H), 3.88 (d, $J = 3.3$ Hz, 1H), 3.67 (t, $J = 11.6$ Hz, 1H), 3.58 (dd, $J = 10.2$, 3.2 Hz, 1H), 3.50 (dd, $J = 10.9$, 7.4 Hz, 1H), 3.30–3.19 (m, 1H), 2.85 (t, $J = 11.6$ Hz, 1H), 2.69–2.57 (m, 1H), 2.34–2.20 (m, 1H), 2.14 (s, 3H), 2.00 (dd, $J = 9.2$, 6.8 Hz, 1H), 1.90 (m, 1H), 1.81 (dd, $J = 15.3$, 4.3 Hz, 1H), 1.53 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.24, 153.41, 110.55, 102.26, 89.73, 84.46, 82.83, 72.26, 70.84, 69.72, 69.57, 63.02, 59.20, 54.82, 54.13, 44.97, 44.49, 38.01, 35.46, 22.62, 13.40.

$^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ –107.91.

FTIR (neat, cm$^{-1}$): 3340 (br), 2923 (w), 1672 (m), 1595 (s), 1458 (m), 1350 (m), 1117 (s), 984 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{33}$ClF$_2$N$_2$O$_6$S, 550.1716; found 550.1742.
Using the general procedure for hydrogenation, **FSA-511079** (0.020 g, 0.038 mmol, 1.0 equiv.) was used to prepare **FSA-511100** (0.010 g, 0.018 mmol, 47%).

$^1$H NMR (500 MHz, CD$_3$OD) δ 7.31 (td, $J = 7.7$, 1.9 Hz, 1H), 7.19 (m, 1H), 7.11 (td, $J = 7.5$, 1.3 Hz, 1H), 7.02 (dd, $J = 10.8$, 8.1 Hz, 1H), 5.31 (d, $J = 5.6$ Hz, 1H), 4.65 (q, $J = 6.8$ Hz, 1H), 4.38 (d, $J = 9.9$ Hz, 1H), 4.25 (dd, $J = 9.7$, 8.1 Hz, 1H), 4.18 (d, $J = 10.8$ Hz, 1H), 4.14 (d, $J = 3.2$ Hz, 1H), 4.08 (dd, $J = 10.2$, 5.6 Hz, 1H), 3.60 (dd, $J = 10.2$, 3.6 Hz, 1H), 3.56–3.46 (m, 2H), 3.24 (d, $J = 9.8$ Hz, 1H), 3.19 (dd, $J = 9.1$, 6.3 Hz, 1H), 2.68 (m, 1H), 2.45–2.36 (m, 1H), 2.37 (s, 3H), 2.20 (dd, $J = 11.5$, 9.1 Hz, 1H), 2.14 (s, 3H), 2.00–1.85 (m, 2H), 1.67 (dd, $J = 15.1$, 4.2 Hz, 1H), 1.50 (d, $J = 6.8$ Hz, 3H), 1.29 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 173.87, 129.40, 128.81, 125.59, 116.42, 116.24, 89.47, 84.88, 74.23, 72.95, 71.65, 71.39, 69.66, 60.97, 59.12, 54.07, 46.88, 41.68, 39.35, 37.50, 34.74, 23.04, 13.18.

$^{19}$F NMR (471 MHz, CD$_3$OD) δ –120.75.

FTIR (neat, cm$^{-1}$): 3327 (br), 2926 (w), 1655 (m), 1596 (m), 1519 (m), 1083 (m), 1020 (s), 757 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{28}$H$_{36}$ClFN$_2$O$_6$S, 546.1967; found 546.2002.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.4 (0.034 g, 0.086 mmol, 1.0 equiv.) was used to prepare FSA-510002 (0.023 g, 0.042 mmol, 49% over 3 steps).

^1^H NMR (600 MHz, CD$_3$OD) δ 7.38 (m, 2H), 7.33 (m, 3H), 6.27 (s, 1H), 5.31 (d, $J = 5.6$ Hz, 1H), 4.58 (qd, $J = 6.7$, 1.5 Hz, 1H), 4.52 (dd, $J = 10.0$, 1.6 Hz, 1H), 4.45 (d, $J = 8.8$ Hz, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 4.09 (m, 3H), 3.89 (d, $J = 3.4$ Hz, 1H), 3.70–3.63 (m, 2H), 3.60 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.25 (m, 1H), 3.15 (t, $J = 11.8$ Hz, 1H), 2.87 (dd, $J = 15.2$, 11.0 Hz, 1H), 2.50 (dd, $J = 16.3$, 4.6 Hz, 1H), 2.15 (s, 3H), 1.56 (d, $J = 6.7$ Hz, 3H).

^1^3^C NMR (126 MHz, CD$_3$OD) δ 169.74, 136.09, 132.34, 129.52, 129.45, 128.75, 124.40, 91.54, 90.35, 89.70, 83.08, 72.03, 70.85, 69.71, 69.57, 69.41, 61.90, 59.19, 54.88, 45.76, 38.36, 22.68, 13.40.

FTIR (neat, cm$^{-1}$): 3342 (br), 2919 (w), 1681 (m), 1578 (s), 1347 (m), 1079 (s), 756 (s), 690 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{26}$H$_{33}$ClN$_2$O$_6$S; 536.1748; found 536.1778.
Using the general procedures for saponification, amide bond coupling, and deprotection, 4.22 (0.036 g, 0.087 mmol, 1.0 equiv.) was used to prepare **FSA-510003** (0.020 g, 0.035 mmol, 40% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.35 (td, $J = 8.0, 5.9$ Hz, 1H), 7.20 (dt, $J = 7.7, 1.2$ Hz, 1H), 7.12 (ddd, $J = 9.5, 2.7, 1.4$ Hz, 1H), 7.08 (tdd, $J = 8.7, 2.6, 1.0$ Hz, 1H), 6.31 (s, 1H), 5.31 (d, $J = 5.6$ Hz, 1H), 4.58 (qd, $J = 6.8, 1.6$ Hz, 1H), 4.51 (dd, $J = 10.0, 1.6$ Hz, 1H), 4.40 (d, $J = 8.8$ Hz, 1H), 4.27 (dd, $J = 10.0, 1.2$ Hz, 1H), 4.10–4.06 (m, 3H), 3.88 (d, $J = 3.4$ Hz, 1H), 3.66–3.62 (m, 2H), 3.59 (dd, $J = 10.2, 3.3$ Hz, 1H), 3.23 (m, 1H), 3.11 (dd, $J = 12.6, 10.8$ Hz, 1H), 2.88 (m, 1H), 2.50 (dd, $J = 16.4, 4.5$ Hz, 1H), 2.15 (s, 3H), 1.55 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 170.26, 164.83, 162.88, 137.29, 131.47, 128.43, 126.36, 118.89, 116.48, 92.50, 89.71, 88.86, 83.26, 72.02, 70.89, 69.75, 69.59, 69.39, 62.02, 59.18, 54.84, 45.97, 38.18, 22.68, 13.39.

FTIR (neat, cm$^{-1}$): 3339 (br), 2920 (w), 1682 (m), 1578 (s), 1348 (m), 1080 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{26}$H$_{32}$ClFN$_2$O$_6$S, 554.1654; found 554.169.
Using the general procedure for hydrogenation, FSA-510002 (0.013 g, 0.024 mmol, 1.0 equiv.) was used to prepare FSA-510021 (0.008 g, 0.014 mmol, 58%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.24 (m, 2H), 7.16 (m, 3H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.57 (m, 1H), 4.51 (m, 2H), 4.28 (m, 1H), 4.25–4.19 (m, 1H), 4.10–4.00 (m, 2H), 3.83–3.77 (m, 1H), 3.65–3.56 (m, 3H), 2.93 (m, 1H), 2.63 (m, 2H), 2.42 (m, 1H), 2.14 (s, 3H), 2.08–1.97 (m, 1H), 1.86 (m, 1H), 1.76 (m, 2H), 1.65 (m, 3H), 1.50 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 143.52, 129.38, 126.82, 89.77, 81.72, 72.10, 70.94, 70.73, 69.74, 69.54, 62.36, 59.17, 54.92, 43.39, 40.60, 39.07, 37.25, 35.83, 35.66, 34.49, 33.71, 22.60, 13.43.

FTIR (neat, cm$^{-1}$): 3349 (br), 2921 (w), 1677 (m), 1584 (s), 1455 (m), 1349 (m), 1056 (m), 700 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{26}$H$_{39}$ClN$_2$O$_6$S, 542.2217; found 542.2248.
Using the general procedure for hydrogenation, **FSA-510003** (0.010 g, 0.018 mmol, 1.0 equiv.) was used to prepare **FSA-510022** (0.006 g, 0.010 mmol, 56%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.25 (tdd, $J = 8.1, 6.0, 2.0$ Hz, 1H), 6.99 (dt, $J = 7.8, 1.9$ Hz, 1H), 6.92 (dt, $J = 10.1, 2.2$ Hz, 1H), 6.88 (m, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.57 (m, 1H), 4.51 (m, 1H), 4.43 (d, $J = 8.9$ Hz, 1H), 4.27 (dd, $J = 10.0, 1.3$ Hz, 1H), 4.09–3.99 (m, 3H), 3.82 (m, 1H), 3.63 (ddd, $J = 11.7, 8.7, 2.6$ Hz, 1H), 3.57 (m, 2H), 2.90 (m, 1H), 2.66 (m, 2H), 2.44–2.34 (m, 1H), 2.14 (s, 3H), 2.08–1.96 (m, 1H), 1.85 (m, 1H), 1.75 (m, 1H), 1.69–1.60 (m, 3H), 1.55–1.48 (m, 1H), 1.51 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 165.35, 131.06, 125.25, 115.87, 113.54, 89.76, 81.93, 72.06, 70.76, 69.75, 69.55, 62.47, 59.17, 54.89, 43.90, 43.48, 38.79, 37.28, 35.83, 33.69, 22.60, 13.42.

FTIR (neat, cm$^{-1}$): 3368 (br), 1669 (s), 1438 (m), 1197 (s), 1138 (s), 801 (m), 724 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{26}$H$_{38}$ClFN$_2$O$_6$S, 560.2123; found 560.2137.
Chapter 5.

Synthesis and study of alkyl-substituted pyrrolidinoxepane analogs
Introduction

Promising activity in MLS$_B$-resistant Gram-positive bacteria was observed with the aryl-substituted pyrrolidinooxepanes described in Chapter 4. This was thought to be a result of expanding the canonical binding site of lincosamide antibiotics further into the A-site pocket inhabited by the $n$-propyl chain on clindamycin. However, optimization of the aryl substituent appeared to plateau with the 4-fluorophenyl derivative FSA-507061, and further expansion of the aryl group either compromised antibacterial activity or caused toxicity associated with membrane disruption. Additionally, there was concern that the aryl functionality was a substrate for efflux pumps, reducing efficacy against Gram-negative bacteria. Thus, alkyl-substituted pyrrolidinooxepanes were of interest to maintain the additional contacts gained by the aryl group while reducing the liability of the aryl functionality, and an alternate methodology to easily install alkyl substituents was desired.

Synthesis of alkyl-substituted bicyclic derivatives

Initial attempts were made to utilize modified Suzuki–Miyaura cross-coupling conditions to couple the vinyl triflates to alkenyl boronic acids. However, the Suzuki–Miyaura methodology was limited by the availability of alkenyl boronic acids, and the resulting dienes showed instability during later steps in analog preparation. Fortunately, an iron-catalyzed cross-coupling reaction was found to readily couple the vinyl triflates 4.2a and 4.2b to a variety of alkyl Grignard reagents (Figure 5.1). This transformation proceeded in good to excellent yield under mild conditions and enabled straightforward syntheses of alkyl-substituted pyrrolidinooxepines.
Results

As with the aryl-substituted bicycles, the saturated pyrrolidinooxepanes were superior in activity to the corresponding unsaturated oxepines. Upon hydrogenation, a mixture of diastereomers at the 7'-position were formed. The diastereomers were separable via HPLC and were tested separately (Figure 5.2). N-methyl versions of some of the hydrogenated compounds were also isolated and tested, and the N-methyl compounds were universally inferior to their secondary amine counterparts. This is consistent with previous semi-synthethic work, which found that N-desmethylclindamycin has greater antibacterial activity than clindamycin7, as well as previous testing results (FSA-511100, Figure 4.7).
The most active of the alkyl-substituted pyrrolidinoxepanes was the sec-butyl analog, FSA-513018b (Figure 5.3). This compound displayed excellent activity against clindamycin-susceptible *S. aureus* and Streptococcal strains, as well as the more challenging *E. faecalis*. Additionally, FSA-513018b showed very good to excellent activity against MLSB-resistant Gram-positive strains, including the *S. aureus* and *E. faecalis* strains. There is also a marked increase in activity against *E. coli* strains, albeit not at therapeutic levels. FSA-513018b has good activity against *H. influenzae*, suggesting that it might have the spectrum of action needed to be an effective treatment for community-acquired bacterial pneumonia.\(^{13}\) Against every strain tested, FSA-513018b demonstrated equal or superior activity compared to clindamycin, FSA-24035, and FSA-507061, the previous 4-fluorophenyl lead compound.
The activity of clindamycin, the lead aryl-substituted analog **FSA-507061**, and two highly active alkyl-substituted analogs (**FSA-512078a** and **FSA-513018b**) were evaluated against three facultative anaerobic strains: *C. difficile*, *C. scindens*, and *B. fragilis* (Figure 5.4). While clindamycin and **FSA-507061** have limited activity against *C. difficile*, both alkyl-substituted derivatives show significantly improved activity against this *C. difficile* strain. In addition, **FSA-512078a** and **FSA-513018b** maintain similar potency to clindamycin against *C. scindens* and *B. fragilis*. The overall profile presented by **FSA-512078a** and **FSA-513018b** presents a double-edged sword: while their improved activity against *C. difficile* suggests a reduced liability for CDI compared to clindamycin, their activity against *C. scindens* and *B. fragilis* suggests that these lincosamide analogs may have a similar disruptive effect on the intestinal microbiome. Potency against *C. scindens* is of particular concern, as *C. scindens* has been shown to inhibit overgrowth...
of *C. difficile*. Further testing will be necessary to see if these lincosamide derivatives share the CDI liability issue that has limited the clinical utility of clindamycin.

![Structures of FSA-507061, FSA-512078a, and FSA-513018b](image)

**Figure 5.4.** Minimal inhibitory concentration (µg/mL) of pyrrolidinoxepanes against *C. difficile* and *B. fragilis*. MIC data by Dr. Amarnath Pisipati.

Comparing the activity of **FSA-513018b** to published lead compounds from Vicuron\(^{12}\) and Meiji Seika\(^{19}\), **FSA-513018b** shows comparable or improved antibacterial activity across the panel (Figure 5.5). **FSA-513018b** has similar activity to one of Meiji Seika’s lead compounds against MLS\(_B\)-resistant Gram-positive bacteria. However, **FSA-513018b** also has similar potency to Vicuron’s lead compound against the fastidious Gram-positive species *E. faecalis* and the Gram-negative species *E. coli* and *K. pneumoniae*. Thus, **FSA-513018b**’s spectrum of *in vitro* activity has similar promise to these preclinical candidates.
Given the exemplary activity of FSA-513018b against the primary and secondary panel, this compound was tested against an extended panel of Gram-positive and Gram-negative bacteria, including multiple clinical isolates with resistance phenotypes (Figure 5.6). A number of clinically relevant antibiotics were tested alongside FSA-513018b as comparators. The results of the extended panel further supported FSA-513018b’s promise as a lead candidate. FSA-513018b showed very good activity against vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant E. faecium (VRE). Based on these data, FSA-513018b has potential as an antibiotic for a...
variety of Gram-positive bacterial infections, including indications for which clindamycin is not appropriate.

Figure 5.6. MIC values (µg/mL) of FSA-513018b and comparator antibiotics against an extended panel of bacterial strains. MIC data by Dr. Amarnath Pisipati.

To better understand these gains in activity, an X-ray crystal structure of FSA-513018b bound to the bacterial ribosome was obtained in collaboration with Professor Yury Polikanov at the University of Illinois, Chicago (Figure 5.7). Professor Polikanov found that FSA-513018b binds to the same site on the bacterial ribosome as clindamycin, as anticipated. The sugar moiety of FSA-513018b binds in a similar conformation to that of clindamycin, preserving the key hydrogen bond contacts between the hydroxy groups and the nucleobases. The amide bond and the pyrrolidine ring of FSA-513018b also adopt a similar conformation to clindamycin.
Figure 5.7. X-ray crystal structure of FSA-513018b bound to the bacterial ribosome. Crystallographic studies and figure by Professor Yury Polikanov.

The oxepane ring extends to the second carbon of the $n$-propyl chain in clindamycin; however, the $sec$-butyl of FSA-513018b extends further into the A-site than clindamycin does. Additionally, Professor Polikanov found that the electron density of the A-site tRNA appeared to be poorly resolved, suggesting that the tip of the A-site tRNA is displaced by the $sec$-butyl end of
**FSA-513018b** when it binds to the ribosome. Comparing the apo form and the **FSA-513018b**-bound form, there is significant movement of A-site tRNA upon **FSA-513018b** binding (**Figure 5.8**). Clindamycin cannot physically reach the A-site tRNA enough to displace it in this manner. Thus, it seems that much of the increased antibiotic activity of **FSA-513018b** comes from the additional contacts made in the A-site by the sec-butyl extension off the oxepane ring.

**Figure 5.8.** Comparison of A- and P-tRNA position in the absence and presence of FSA-513018b, showing significant displacement of A-tRNA upon FSA-513018b binding. Crystallographic studies and figure by Professor Yury Polikanov.

Comparison of **FSA-513018b** with other antibiotics known to bind to the PTC or NPET provided further insight into its target engagement (**Figure 5.9**). **FSA-513018b** extends further into the A-site than clindamycin, reaching about as far as chloramphenicol and linezolid. Linezolid is a widely used antibiotic against Gram-positive bacterial infections. While linezolid has lower potency against clindamycin-susceptible strains, it maintains its activity against MLS<sub>B</sub>-resistant strains, against which clindamycin is ineffective (**Figure 5.6**). Linezolid’s efficacy against MLS<sub>B</sub>-resistant bacteria can be explained by the fact that linezolid does not bind to the pocket occupied...
by the sugar moiety of clindamycin. Thus, its binding is unaffected by dimethylation of A2058 N6 by Erm and the activity of linezolid is not compromised by the MLS$_B$-resistance phenotype. The superimposed structures of clindamycin, linezolid, and FSA-513018b suggests that FSA-513018b has incorporated key features from both clindamycin and linezolid to achieve its improved spectrum of activity (Figure 5.9). FSA-513018b retains the sugar moiety from clindamycin and key structural elements that may contribute to its high potency against clindamycin-susceptible Gram-positive strains. However, FSA-513018b makes additional contacts in the A-site, similar to linezolid, due to the oxepane ring and sec-butyl appendage, which likely results in better activity against clindamycin-resistant Gram-positive strains. As a result, FSA-513018b is effective against a wide range of Gram-positive bacteria and overcomes the MLS$_B$-resistance phenotype in the strains tested.
Further assays were conducted by Dr. Amarnath Pisipati to gain valuable information about the pharmacological properties of **FSA-513018b**. Dr. Pisipati conducted a time kill assay against a MLSB-resistant *S. aureus* strain. The time kill assay showed that **FSA-513018b** had a bacteriostatic effect against the *S. aureus* strain used, as anticipated for a lincosamide antibiotic. Unlike similarly potent analogs made via Sonogashira cross-coupling, **FSA-513018b** did not exhibit hemolysis against human erythrocytes up to 200 µM in concentration. This supports the theory that **FSA-513018b**’s improved activity is due to engagement with the A-site, not via membrane disruption. Cytotoxicity assays were performed by Dr. Pisipati and R. Porter Ladley against several immortalized cell lines to gauge toxicity against human cells (**Figure 5.10**). Exposure to clindamycin did not cause any significant growth inhibition up to 200 µM, while **FSA-**
513018b caused 50% growth inhibition (G_{50}) between 100–200 µM. While not acutely concerning, the toxicity observed at the highest concentrations of the cytotoxicity assays suggest possible off-target effects that should be the subject of further investigation during subsequent preclinical assays.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Clindamycin GI_{50} (µM)</th>
<th>FSA-513018b GI_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-549</td>
<td>&gt;200</td>
<td>155</td>
</tr>
<tr>
<td>K-562</td>
<td>&gt;200</td>
<td>158</td>
</tr>
<tr>
<td>HCT-116</td>
<td>&gt;200</td>
<td>161</td>
</tr>
<tr>
<td>HepG2</td>
<td>&gt;200</td>
<td>122</td>
</tr>
</tbody>
</table>

Figure 5.10. Cytotoxicity of clindamycin and FSA-513018b against human cancer cell lines, expressed as concentration required for 50% growth inhibition (G_{50}, µM) as calculated from the best fit trend line. Cytotoxicity assay data by Dr. Amarnath Pisipati and R. Porter Ladley.

Conclusions

In order to further improve the activity observed with the 4-fluorophenyl-substituted pyrrolidinooxepane FSA-507061, alkyl-substituted analogs were synthesized via an iron-catalyzed cross-coupling reaction. Of the compounds tested, FSA-513018b emerged as a promising lead, with excellent activity against a variety of Gram-positive bacteria, including MLS_{B}-resistant strains. Crystallography studies suggest that this improved activity comes from extension of the sec-butyl appendage into the A-site of the ribosome and displacement of the A-tRNA.
Experimental section

General procedure for iron-catalyzed cross-coupling

A solution of alkyl Grignard reagent (1.2 equiv.) in THF or diethyl ether was added dropwise by syringe to a stirring solution of 4.2a (1.0 equiv.), iron (III) acetylacetonate (0.05 equiv.), and N-methylpyrrolidone (10.0 equiv.) at –30 °C in THF such that the concentration of 4.2a was 0.05 M. The resultant solution immediately changed in color from red-orange to dark brown/black. The solution was stirred at –30 °C under argon. Consumption of 4.2a was monitored by TLC (EtOAc in hexanes). After complete consumption of 4.2a was observed, the reaction solution was quenched with saturated aqueous ammonium chloride and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc in hexanes) to yield 5.2.
Using the general procedure for iron-catalyzed cross-coupling outlined above, 4.2a (0.055 g, 0.123 mmol, 1.0 equiv.) was used to prepare 5.1 (0.042 g, 0.123 mmol, 99%).

\[ R_f = 0.70 \text{ (30\% EtOAc in hexanes, KMnO}_4 \] 

\[ ^1H \text{ NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl}_3 \] \[ \delta 5.56 (m, 1H), 4.49*, 4.41 (d, J = 8.2 Hz, 1H), 4.25 (ddd, J = 14.5, 7.2, 2.2 Hz, 1H), 4.01 (m, 1H), 3.98*, 3.87 (m, 2H), 3.78 (m, 1H), 3.75*, 3.73 (s, 3H), 2.35 (m, 1H), 2.26 (m, 2H), 2.09 (m, 1H), 2.01*, 1.43*, 1.39 (s, 9H), 0.97 (m, 6H). \]

\[ ^{13}C \text{ NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl}_3 \] \[ \delta 170.96*, 170.86, 153.89*, 153.45, 150.82, 150.62*, 121.24*, 121.06, 87.80, 87.25*, 80.11, 67.82, 62.03, 61.47*, 52.08*, 51.89, 49.34*, 48.64, 40.09*, 39.45, 37.42, 31.85, 28.29*, 28.17, 20.84*, 20.75. \]

FTIR (neat, cm\(^{-1}\)): 2959 (w), 2870 (w), 1748 (s), 1702 (s), 1400 (s), 1257 (m), 1169 (s).

HRMS (ESI+, \(m/z\)): [M+H]\(^+\) calc’d for C\(_{18}\)H\(_{29}\)NO\(_5\), 339.2046; found 339.2076.
Using the general procedure for iron-catalyzed cross-coupling outlined above, 4.2a (0.051 g, 0.115 mmol, 1.0 equiv.) was used to prepare 5.3 (0.039 g, 0.111 mmol, 97%).

R_f = 0.70 (30% EtOAc in hexanes, KMnO_4)

^1^H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl_3) δ 5.52 (m, 1H), 4.50*, 4.41 (d, J = 8.1 Hz, 1H), 4.25*, 4.22 (d, J = 6.7 Hz, 1H), 4.04 (m, 1H), 4.01*, 3.90 (t, J = 8.9 Hz, 1H), 3.83*, 3.75*, 3.74 (s, 3H), 2.87 (m, 1H), 2.45–2.33 (m, 2H), 2.24 (t, J = 16.9 Hz, 1H), 2.14–2.04 (m, 2H), 1.93–1.83 (m, 1H), 1.70 (m, 1H), 1.44*, 1.39 (s, 9H), 0.85 (dd, J = 12.8, 6.4 Hz, 6H).

^1^C NMR (asterisk denotes minor rotamer peaks, 126 MHz, CDCl_3) δ 170.94*, 170.82, 153.88*, 153.46, 143.78, 143.62*, 125.02*, 124.90, 87.44, 86.88*, 80.14, 80.06*, 67.73, 62.11, 61.54*, 52.07*, 51.88, 50.20, 49.44*, 48.75, 39.86*, 39.21, 34.14*, 34.06, 28.28*, 28.17, 26.10, 26.02, 22.52, 22.26*, 22.21.

FTIR (neat, cm\(^{-1}\)): 2953 (w), 1748 (m), 1698 (s), 1399 (s), 1257 (m), 1207 (s), 1137 (s), 987 (m).

HRMS (ESI+, m/z): [M+H]^+ calc’d for C_{19}H_{31}NO_5, 353.2202; found 353.2226.
Using the general procedure for iron-catalyzed cross-coupling outlined above, 4.2a (0.051 g, 0.115 mmol, 1.0 equiv.) was used to prepare 5.4 (0.036 g, 0.097 mmol, 84%).

R_f = 0.60 (30% EtOAc in hexanes, KMnO_4)

^1H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl_3) δ 5.53 (m, 1H), 4.51*, 4.42 (d, J = 8.2 Hz, 1H), 4.26*, 4.24 (d, J = 7.6 Hz, 1H), 4.05 (m, 1H), 4.02*, 3.91 (dd, J = 10.4, 8.3 Hz, 1H), 3.82 (dd, J = 10.2, 8.3 Hz, 1H), 3.77*, 3.75 (s, 3H), 2.85 (m, 1H), 2.47–2.34 (m, 1H), 2.27 (m, 2H), 1.95 (s, 2H), 1.94*, 1.45*, 1.40 (s, 9H), 0.90 (s, 9H).


FTIR (neat, cm⁻¹): 2950 (m), 2866 (w), 1749 (s), 1702 (s), 1400 (s), 1123 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C_{20}H_{33}NO_5, 367.2359; found 367.2378.

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Using the general procedure for iron-catalyzed cross-coupling outlined above, 4.2a (0.055 g, 0.123 mmol, 1.0 equiv.) was used to prepare 5.5 (0.045 g, 0.123 mmol, 99%).

$R_f = 0.55$ (30% EtOAc in hexanes, KMnO₄)

$^1$H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl₃) $\delta$ 5.61 (m, 1H), 4.50*, 4.41 (d, $J = 8.1$ Hz, 1H), 4.25 (dd, $J = 14.3$, 7.9 Hz, 1H), 4.02 (m, 1H), 3.99*, 3.89 (t, $J = 9.0$ Hz, 1H), 3.84 (m, 1H), 3.78*, 3.75*, 3.74 (s, 3H), 2.42 (m, 1H), 2.36–2.24 (m, 2H), 2.12 (m, 1H), 1.71 (m, 2H), 1.64 (m, 2H), 1.57 (m, 2H), 1.44*, 1.39 (s, 9H), 1.31 (m, 3H).


FTIR (neat, cm$^{-1}$): 2951 (w), 2868 (w), 1748 (s), 1698 (s), 1400 (s), 1207 (s), 1125 (s), 909 (m), 728 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{20}$H$_{31}$NO$_5$, 365.2202; found 365.2205.
Using the general procedure for iron-catalyzed cross-coupling outlined above, 4.2a (0.055 g, 0.123 mmol, 1.0 equiv.) was used to prepare 5.6 (0.029 g, 0.076 mmol, 61%).

R<sub>f</sub> = 0.65 (30% EtOAc in hexanes, KMnO<sub>4</sub>)

1H NMR (asterisk denotes minor rotamer peaks, 500 MHz, CDCl<sub>3</sub>) δ 5.55 (m, 1H), 4.51*, 4.42 (d, J = 7.7 Hz, 1H), 4.27 (dd, J = 14.5, 7.3 Hz, 1H), 4.03 (m, 1H), 4.00*, 3.88 (m, 2H), 3.76*, 3.75 (s, 3H), 2.90 (q, J = 10.0 Hz, 1H), 2.31 (m, 2H), 2.13 (m, 1H), 1.85 (m, 1H), 1.76 (m, 2H), 1.68 (m, 2H), 1.45*, 1.40 (s, 9H), 1.26 (m, 3H), 1.12 (m, 3H).


FTIR (neat, cm<sup>-1</sup>): 2924 (m), 2851 (w), 1749 (s), 1700 (s), 1399 (s), 1177 (s), 1123 (s), 731 (m).

HRMS (ESI+, m/z): [M+H]+ calc’d for C<sub>21</sub>H<sub>33</sub.NO<sub>5</sub>, 379.2359; found 379.2365.
General procedure for saponification

A 1.0 N aq. solution of lithium hydroxide (2.0 equiv.) was added to a solution of 5.2 (1.0 equiv.) in methanol and THF in a 1:1 ratio such that the concentration of 5.2 was 0.2 M. The resultant solution was stirred at ambient temperature until complete consumption of the methyl ester was observed by mass spectrometry. The reaction solution was acidified with 1 N aq. hydrochloric acid until it reached pH 2 and extracted five times with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to yield 5.7, which was taken forward without further purification.
General procedure for coupling to 7-Cl-MTL

Triethylamine (3.2 equiv.) was added to a solution of 5.7 (1.0 equiv.) and 7-Cl-MTL (1.1 equiv.) in DMF such that the concentration of 5.7 was 0.35 M at 0 °C. The resultant colorless solution was stirred for 10 min at 0 °C, and HATU (1.3 equiv.) was added in a single portion. The resultant bright yellow solution was stirred at ambient temperature under argon. Consumption of 5.7 was monitored by mass spectrometry. After complete consumption of 5.7 was observed by mass spectrometry, the reaction solution was concentrated under reduced pressure and directly purified by column chromatography (methanol in DCM) to yield 5.8. Due to amide and carbamate rotamerism, this intermediate was taken forward to N-Boc deprotection before full characterization.
Bis(trimethylsilyl)trifluoroacetamide (3.5 equiv.) was added by syringe to a solution of 5.8 (1.0 equiv.) in acetonitrile such that the concentration of 5.8 was 0.1 M at 0 °C. The resultant solution was stirred at 0 °C for 5 min, stirred at ambient temperature for 15 min, then cooled back to 0 °C. Trimethylsilyl iodide (1.0 equiv.) was added dropwise by syringe to the reaction solution at 0 °C. The resultant solution was stirred at 0 °C until complete N-Boc deprotection was observed by mass spectrometry. The reaction solution was quenched with dropwise addition of methanol and concentrated under reduced pressure. The crude product was purified on reverse-phase HPLC (acetonitrile in water) to yield 5.9.
Using the general procedures for saponification, amide bond coupling, and deprotection, 5.1 (0.042 g, 0.123 mmol, 1.0 equiv.) was used to prepare **FSA-512073** (0.021 g, 0.043 mmol, 35% over 3 steps).

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 5.66 (m, 1H), 5.31 (d, $J = 5.5$ Hz, 1H), 4.54 (m, 2H), 4.30 (m, 2H), 4.10 (m, 2H), 3.86 (br, 1H), 3.60 (m, 1H), 2.98 (t, $J = 9.6$ Hz, 1H), 2.44 (d, $J = 14.9$ Hz, 1H), 2.30 (m, 1H), 2.22 (m, 2H), 2.15 (s, 3H), 2.03 (m, 2H), 1.50 (d, $J = 6.7$ Hz, 3H), 1.24 (t, $J = 7.1$ Hz, 1H), 1.01 (m, 6H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 151.60, 122.70, 89.71, 87.53, 72.04, 70.78, 69.66, 69.56, 68.58, 61.56, 59.09, 54.96, 41.74, 38.92, 31.21, 22.54, 21.40, 21.26, 13.42.

FTIR (neat, cm$^{-1}$): 3370 (br), 2957 (w), 1686 (s), 1594 (s), 1466 (m), 1349 (m), 1079 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{21}$H$_{35}$ClN$_2$O$_6$S, 478.1904; found 478.1939.
Using the general procedures for saponification, amide bond coupling, and deprotection, 5.3 (0.039 g, 0.111 mmol, 1.0 equiv.) was used to prepare **FSA-512099** (0.014 g, 0.029 mmol, 26% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.60 (m, 1H), 5.30 (d, $J = 5.6$ Hz, 1H), 4.55 (q, $J = 6.7$ Hz, 1H), 4.51 (d, $J = 9.5$ Hz, 2H), 4.29 (m, 1H), 4.28 (m, 1H), 4.14–4.07 (m, 3H), 3.86 (m, 1H), 3.57 (m, 2H), 2.92 (t, $J = 11.7$ Hz, 1H), 2.36 (d, $J = 14.3$ Hz, 1H), 2.23 (m, 1H), 2.15 (s, 3H), 2.09 (m, 1H), 1.93 (d, $J = 7.1$ Hz, 2H), 1.75 (dt, $J = 13.5, 6.7$ Hz, 1H), 1.51 (d, $J = 6.8$ Hz, 3H), 0.88 (t, $J = 6.1$ Hz, 6H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 168.94, 144.46, 126.53, 89.72, 87.44, 72.04, 70.78, 69.70, 69.56, 68.54, 61.71, 59.11, 54.94, 51.41, 41.68, 33.53, 27.23, 22.77, 22.56, 13.42.

FTIR (neat, cm$^{-1}$): 3337 (br), 2954 (w), 2490 (br), 1677 (s), 1581 (s), 1464 (m), 1380 (m), 1256 (m), 1055 (s), 847 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{22}$H$_{37}$ClN$_2$O$_6$S, 492.2061; found 492.2028.
Using the general procedures for saponification, amide bond coupling, and deprotection, 5.4 (0.036 g, 0.097 mmol, 1.0 equiv.) was used to prepare FSA-512100 (0.009 g, 0.017 mmol, 18% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.62 (m, 1H), 5.30 (d, $J$ = 5.6 Hz, 1H), 4.56 (q, $J$ = 7.2, 6.8 Hz, 1H), 4.51 (d, $J$ = 10.0 Hz, 1H), 4.48 (d, $J$ = 8.6 Hz, 1H), 4.31 (m, 1H), 4.28 (t, $J$ = 9.2 Hz, 1H), 4.14–4.07 (m, 3H), 3.85 (m, 1H), 3.58 (dd, $J$ = 10.2, 3.0 Hz, 1H), 3.52 (dd, $J$ = 11.2, 7.4 Hz, 1H), 2.89 (t, $J$ = 11.8 Hz, 1H), 2.41–2.33 (m, 2H), 2.15 (s, 3H), 2.11 (m, 1H), 2.01 (s, 2H), 1.51 (d, $J$ = 6.8 Hz, 3H), 0.92 (s, 9H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 169.23, 144.39, 128.99, 89.72, 87.92, 72.03, 70.80, 69.72, 69.57, 68.45, 61.69, 59.11, 55.20, 54.94, 41.36, 36.16, 32.78, 30.30, 22.56, 13.41.

FTIR (neat, cm$^{-1}$): 3323 (br), 2950 (w), 1685 (m), 1585 (s), 1452 (m), 1348 (s), 1254 (m), 1059 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{23}$H$_{39}$ClN$_2$O$_6$S, 506.2217; found 506.2228.
Using the general procedures for saponification, amide bond coupling, and deprotection, 5.5 (0.045 g, 0.123 mmol, 1.0 equiv.) was used to prepare FSA-512088 (0.035 g, 0.069 mmol, 56% over 3 steps).

\[
\begin{align*}
\text{FSA-512088} & \\
\end{align*}
\]

\[
\begin{align*}
^1H \text{ NMR (600 MHz, CD}_3\text{OD}) \delta & 5.69 \text{ (m, 1H), } 5.31 \text{ (d, } J = 5.6 \text{ Hz, 1H), } 4.61 \text{ (m, 1H), } 4.54 \text{ (q, } J = 6.9 \text{ Hz, 1H), } 4.52 \text{ (d, } J = 10.2 \text{ Hz, 1H), } 4.31-4.28 \text{ (m, 2H), } 4.13-4.08 \text{ (m, 3H), } 3.87 \text{ (br, 1H), } 3.64 \text{ (m, 1H), } 3.59 \text{ (d, } J = 11.6 \text{ Hz, 1H), } 3.00 \text{ (t, } J = 11.6 \text{ Hz, 1H), } 2.48 \text{ (t, } J = 8.2 \text{ Hz, 1H), } 2.42 \text{ (d, } J = 14.6 \text{ Hz, 1H), } 2.24 \text{ (m, 1H), } 2.15 \text{ (s, 3H), } 2.06 \text{ (m, 1H), } 1.73 \text{ (m, 2H), } 1.68 \text{ (m, 2H), } 1.60 \text{ (m, 2H), } 1.51 \text{ (d, } J = 6.7 \text{ Hz, 3H), } 1.37 \text{ (m, 2H).}
\end{align*}
\]

\[
\begin{align*}
^13C \text{ NMR (126 MHz, CD}_3\text{OD}) \delta & 168.09, 148.70, 123.41, 89.69, 87.28, 72.03, 70.73, 69.65, 69.53, 68.59, 61.39, 59.06, 55.01, 51.01, 41.57, 31.85, 31.74, 31.54, 26.20, 22.53, 13.43.
\end{align*}
\]

FTIR (neat, cm\(^{-1}\)): 3343 (br), 2953 (w), 2489 (br), 1686 (s), 1581 (s), 1467 (m), 1346 (m), 1078 (s).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{23}\)H\(_{37}\)ClN\(_2\)O\(_6\)S, 504.2061; found 504.2104.
Using the general procedures for saponification, amide bond coupling, and deprotection, 5.6 (0.029 g, 0.076 mmol, 1.0 equiv.) was used to prepare **FSA-512089** (0.019 g, 0.037 mmol, 49% over 3 steps).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.63 (m, 1H), 5.30 (d, $J = 5.5$ Hz, 1H), 4.55 (m, 2H), 4.51 (d, $J = 10.1$ Hz, 1H), 4.33–4.27 (m, 2H), 4.12–4.07 (m, 3H), 3.85 (br, 1H), 3.59 (t, $J = 10.7$ Hz, 2H), 2.97 (t, $J = 11.5$ Hz, 1H), 2.43 (d, $J = 15.2$ Hz, 1H), 2.23 (m, 1H), 2.15 (s, 3H), 2.02 (m, 1H), 1.91 (t, $J = 12.8$ Hz, 1H), 1.78 (d, $J = 12.8$ Hz, 2H), 1.71–1.65 (m, 3H), 1.50 (d, $J = 6.8$ Hz, 3H), 1.30 (m, 2H), 1.22–1.10 (m, 3H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 168.25, 151.16, 123.03, 89.73, 87.36, 72.04, 70.75, 69.70, 69.55, 68.60, 61.43, 59.08, 55.02, 41.52, 32.58, 32.09, 27.60, 27.55, 27.29, 22.55, 13.42.

FTIR (neat, cm$^{-1}$): 3268 (br), 2922 (w), 2847 (w), 1689 (s), 1578 (s), 1447 (m), 1345 (s), 1059 (s), 985 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{39}$ClN$_2$O$_6$S, 518.2217; found 518.2259.
General procedure for hydrogenation

A suspension of 5.9 (1.0 equiv.) and palladium hydroxide (20% on carbon, 1.0 equiv.) in anhydrous methanol such that the concentration of 5.9 was 0.1 M was stirred under an atmosphere of hydrogen (1 atm) until complete hydrogenation was observed by mass spectrometry. The reaction solution was filtered through a pad of Celite, washing with methanol, and concentrated. The crude product was purified on reverse-phase HPLC (acetonitrile in water) to yield 5.10a, either as a single diastereomer or alongside the 7’-epimer 5.10b.

Note: In some cases, concomitant N-methylation was observed during hydrogenation. To obtain the desired N-desmethyl versions, the corresponding N-Boc oxepine was prepared and hydrogenation was carried out before N-Boc deprotection.
Using the general procedure for hydrogenation, **FSA-512088** (0.020 g, 0.040 mmol, 1.0 equiv.) was used to prepare **FSA-513001a** (0.009 g, 0.018 mmol, 45%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.6$ Hz, 1H), 4.62 (q, $J = 6.1$ Hz, 1H), 4.37 (d, $J = 10.8$ Hz, 1H), 4.16 (d, $J = 9.9$ Hz, 1H), 4.11–4.05 (m, 4H), 3.56 (dd, $J = 10.2$, 3.5 Hz, 1H), 3.46 (m, 1H), 3.27 (d, $J = 9.7$ Hz, 1H), 3.20 (dd, $J = 9.0$, 6.4 Hz, 1H), 2.42 (m, 1H), 2.40 (s, 3H), 2.23 (m, 1H), 2.13 (s, 3H), 1.76 (m, 4H), 1.70 (m, 3H), 1.62 (m, 4H), 1.55 (m, 3H), 1.49 (d, $J = 6.8$ Hz, 3H), 1.15 (m, 2H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.50, 83.79, 73.86, 72.32, 71.74, 71.26, 69.69, 60.92, 59.16, 54.20, 47.42, 44.60, 41.57, 35.30, 32.93, 31.75, 31.63, 26.49, 26.20, 22.94, 13.21.

FTIR (neat, cm$^{-1}$): 3344 (br), 2923 (s), 2862 (s), 1663 (s), 1592 (m), 1516 (s), 1450 (m), 1349 (m), 1102 (s), 1053 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{41}$ClN$_2$O$_6$S, 520.2374; found 520.24.
Using the general procedure for hydrogenation, **FSA-512088** (0.020 g, 0.040 mmol, 1.0 equiv.) was used to prepare **FSA-513001b** (0.004 g, 0.007 mmol, 18%).

$^{1}H$ NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.5$ Hz, 1H), 4.63 (m, 1H), 4.36 (d, $J = 9.9$ Hz, 1H), 4.24 (m, 1H), 4.16 (d, $J = 10.0$ Hz, 1H), 4.07 (dd, $J = 10.2, 5.6$ Hz, 1H), 4.02 (d, $J = 3.2$ Hz, 1H), 3.98 (m, 1H), 3.78 (m, 1H), 3.58 (dd, $J = 10.2, 3.5$ Hz, 1H), 2.37 (s, 3H), 2.27 (m, 1H), 2.17 (m, 1H), 2.13 (s, 3H), 2.02 (m, 1H), 1.82–1.77 (m, 4H), 1.73–1.66 (m, 3H), 1.64 (m, 2H), 1.54 (m, 2H), 1.48 (d, $J = 6.8$ Hz, 1H), 1.43 (m, 1H), 1.29 (m, 1H), 1.16 (m, 2H), 0.99 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.47, 83.29, 73.98, 71.72, 71.36, 69.69, 69.01, 60.31, 59.12, 53.99, 47.83, 46.41, 45.39, 41.69, 36.56, 33.57, 31.88, 31.69, 26.49, 26.22, 22.98, 13.17.

FTIR (neat, cm$^{-1}$): 3344 (br), 2924 (s), 2860 (m), 2529 (br), 1660 (s), 1587 (s), 1517 (s), 1450 (m), 1350 (m), 1054 (s), 1030 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{24}$H$_{41}$ClN$_{2}$O$_{8}$S, 520.2374, found 520.2413.
Using the general procedure for hydrogenation, **FSA-512089** (0.015 g, 0.029 mmol, 1.0 equiv.) was used to prepare **FSA-513002a** (0.005 g, 0.010 mmol, 34%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.5$ Hz, 1H), 4.62 (q, $J = 6.3$ Hz, 1H), 4.37 (d, $J = 9.9$ Hz, 1H), 4.16 (d, $J = 9.9$ Hz, 1H), 4.11–4.05 (m, 4H), 3.57 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.41 (t, $J = 11.2$ Hz, 1H), 3.24 (d, $J = 9.5$ Hz, 1H), 3.20 (m, 1H), 2.39 (s, 3H), 2.37 (m, 1H), 2.21 (m, 1H), 2.13 (s, 3H), 1.77–1.62 (m, 9H), 1.54 (dd, $J = 14.6$, 4.8 Hz, 1H), 1.49 (d, $J = 6.7$ Hz, 3H), 1.46 (m, 1H), 1.29–1.23 (m, 4H), 1.16 (m, 1H), 1.09 (m, 1H), 1.00 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.49, 84.03, 73.99, 72.92, 71.72, 71.28, 69.69, 69.65, 61.02, 59.15, 54.17, 46.57, 44.94, 44.36, 41.59, 33.96, 31.06, 30.48, 30.28, 27.88, 27.84, 22.95, 13.20.

FTIR (neat, cm$^{-1}$): 3359 (br), 2922 (s), 2851 (s), 1664 (s), 1592 (s), 1518 (s), 1449 (s), 1350 (m), 1104 (s), 1055 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{26}$H$_{45}$ClN$_2$O$_5$S, 534.253; found 534.2542.
Using the general procedure for hydrogenation, FSA-512089 (0.015 g, 0.029 mmol, 1.0 equiv.) was used to prepare FSA-513002b (0.004 g, 0.008 mmol, 28%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.31 (d, $J = 5.6$ Hz, 1H), 4.62 (m, 1H), 4.37 (m, 1H), 4.21 (d, $J = 9.9$ Hz, 1H), 4.12 (d, $J = 3.1$ Hz, 1H), 4.07 (dd, $J = 10.2$, 5.5 Hz, 1H), 4.02 (m, 1H), 3.77 (t, $J = 9.5$ Hz, 1H), 3.60 (dd, $J = 10.3$, 3.5 Hz, 1H), 3.24 (d, $J = 10.1$ Hz, 1H), 3.20 (m, 1H), 3.01 (m, 1H), 2.60 (m, 1H), 2.41 (s, 3H), 2.13 (s, 3H), 1.86 (m, 1H), 1.76 (m, 3H), 1.69 (m, 4H), 1.48 (d, $J = 6.7$ Hz, 2H), 1.29 (m, 6H), 1.18–1.09 (m, 4H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 151.59, 123.37, 89.46, 85.74, 72.56, 71.72, 71.46, 70.15, 69.80, 59.16, 58.29, 54.05, 47.08, 41.44, 35.62, 32.81, 32.63, 27.59, 27.39, 22.98, 13.16.

FTIR (neat, cm$^{-1}$): 3358 (br), 2923 (s), 2851 (s), 1664 (s), 1590 (s), 1517 (s), 1449 (m), 1348 (m), 1088 (s), 1054 (s).

HRMS (ESI+, m/z): [M+H]$^+$ C$_{25}$H$_{43}$ClN$_2$O$_6$S$_n$, 535.2603; found 535.2586.
Using the general procedure for hydrogenation, **FSA-512099** (0.010 g, 0.020 mmol, 1.0 equiv.) was used to prepare **FSA-513003a** (0.004 g, 0.008 mmol, 40%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.5$ Hz, 1H), 4.63 (q, $J = 6.8$ Hz, 1H), 4.36 (dd, $J = 9.8$ Hz, 1H), 4.15 (d, $J = 9.9$ Hz, 1H), 4.11–4.04 (m, 4H), 3.57 (dd, $J = 10.2$, 3.5 Hz, 1H), 3.47 (m, 1H), 3.22 (d, $J = 9.7$ Hz, 1H), 3.17 (dd, $J = 9.0$, 6.3 Hz, 1H), 2.42 (m, 1H), 2.38 (s, 3H), 2.19 (m, 1H), 2.13 (s, 3H), 1.98 (m, 1H), 1.68 (m, 1H), 1.64–1.57 (m, 3H), 1.55–1.48 (m, 1H), 1.49 (d, $J = 6.8$ Hz, 3H), 1.29 (br, 1H), 1.19 (m, 2H), 0.89 (t, $J = 7.2$ Hz, 6H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.48, 84.20, 73.98, 72.11, 71.69, 71.32, 69.64, 61.00, 59.13, 54.14, 48.25, 46.32, 41.63, 36.85, 36.25, 34.16, 26.33, 23.34, 22.97, 22.78, 13.19.

FTIR (neat, cm$^{-1}$): 3350 (br), 2925 (m), 1747 (m), 1700 (s), 1518 (m), 1403 (s), 1179 (m), 1141 (s), 1093 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{23}$H$_{41}$ClN$_2$O$_6$S, 508.2374; found 508.241.
Using the general procedure for hydrogenation, **FSA-512100** (0.007 g, 0.014 mmol, 1.0 equiv.) was used to prepare **FSA-513004a** (0.003 g, 0.006 mmol, 43%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.6$ Hz, 1H), 4.63 (q, $J = 6.8$ Hz, 1H), 4.35 (d, $J = 9.9$ Hz, 1H), 4.14 (m, 1H), 4.12 (m, 1H), 4.09 (d, $J = 3.6$ Hz, 1H), 4.06 (dd, $J = 10.2$, 5.6 Hz, 1H), 4.03 (m, 1H), 3.57 (dd, $J = 10.2$, 3.6 Hz, 1H), 3.51 (dd, $J = 12.0$, 10.1 Hz, 1H), 3.19 (d, $J = 9.8$ Hz, 1H), 3.15 (dd, $J = 9.0$, 6.3 Hz, 1H), 2.42 (m, 1H), 2.36 (s, 3H), 2.16 (dd, $J = 11.5$, 9.0 Hz, 1H), 2.13 (s, 3H), 2.02 (m, 1H), 1.80 (m, 1H), 1.62 (m, 3H), 1.49 (d, $J = 6.8$ Hz, 3H), 1.31 (dd, $J = 14.1$, 5.5 Hz, 1H), 1.29 (m, 1H), 1.25 (dd, $J = 14.0$, 4.9 Hz, 1H), 0.92 (s, 9H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.46, 84.08, 73.95, 71.67, 71.53, 71.35, 69.63, 60.92, 59.12, 54.11, 52.55, 46.20, 41.64, 39.05, 37.06, 35.33, 32.05, 30.35, 22.98, 13.18.

FTIR (neat, cm$^{-1}$): 3366 (br), 2923 (s), 1664 (s), 1594 (s), 1518 (s), 1451 (m), 1363 (m), 1100 (s), 1056 (s).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{24}$H$_{43}$ClN$_2$O$_6$S, 522.253; found 522.257.
Using the general procedure for hydrogenation, FSA-512073 (0.010 g, 0.021 mmol, 1.0 equiv.) was used to prepare FSA-512078a (0.005 g, 0.010 mmol, 48%).

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.30 (d, $J = 5.6$ Hz, 1H), 4.58 (q, $J = 6.9$ Hz, 1H), 4.47 (d, $J = 10.0$ Hz, 1H), 4.27 (d, $J = 9.0$ Hz, 1H), 4.24 (d, $J = 9.9$ Hz, 1H), 4.17 (t, $J = 8.9$ Hz, 1H), 4.08 (m, 2H), 3.88 (d, $J = 2.4$ Hz, 1H), 3.57 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.54 (m, 1H), 3.46 (dd, $J = 10.8$, 7.3 Hz, 1H), 2.78 (t, $J = 11.4$ Hz, 1H), 2.31 (m, 1H), 2.15 (s, 3H), 1.81 (m, 2H), 1.73–1.63 (m, 3H), 1.52 (d, $J = 6.8$ Hz, 3H), 1.48 (m, 1H), 0.90 (dd, $J = 6.8$, 3.3 Hz, 6H).

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 89.71, 82.85, 72.33, 72.03, 70.89, 69.72, 69.58, 63.28, 59.18, 54.74, 50.90, 44.63, 44.57, 33.83, 30.18, 22.59, 19.93, 19.36, 13.38.

FTIR (neat, cm$^{-1}$): 3351 (br), 2926 (s), 1676 (m), 1591 (s), 1463 (m), 1351 (m), 1085 (m).

HRMS (ESI+, m/z): [M+H]$^+$ calc’d for C$_{21}$H$_{37}$ClN$_2$O$_6$S, 480.2061; found 480.2075.
Using the general procedure for hydrogenation, FSA-512073 (0.010 g, 0.021 mmol, 1.0 equiv.) was used to prepare FSA-512078b (0.005 g, 0.010 mmol, 48%).

\(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 5.30 (d, \(J = 5.6\) Hz, 1H), 4.58 (q, \(J = 7.0\) Hz, 1H), 4.45 (d, \(J = 10.4\) Hz, 1H), 4.23 (d, \(J = 10.0\) Hz, 1H), 4.18–4.07 (m, 4H), 3.90 (m, 1H), 3.57 (dd, \(J = 10.1, 3.2\) Hz, 1H), 3.52 (dd, \(J = 11.9, 9.8\) Hz, 1H), 3.40 (m, 1H), 2.71 (t, \(J = 11.2\) Hz, 1H), 2.28 (m, 1H), 2.14 (s, 3H), 1.80–1.61 (m, 5H), 1.51 (d, \(J = 6.8\) Hz, 3H), 1.48 (m, 2H), 0.90 (dd, \(J = 6.8, 2.9\) Hz, 6H).

\(^{13}\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 89.68, 83.48, 72.37, 71.98, 71.00, 69.71, 59.19, 54.63, 51.28, 44.95, 33.86, 30.32, 22.60, 19.94, 19.58, 19.36, 13.35.

FTIR (neat, cm\(^{-1}\)): 3319 (br), 2924 (m), 2868 (w), 1594 (s), 1463 (m), 1352 (m), 1097 (m).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{21}\)H\(_{37}\)ClN\(_2\)O\(_6\)S, 480.2061; found 480.2084.
Using the general procedures for saponification, amide bond coupling, hydrogenation, and deprotection, 5.5 (0.058 g, 0.159 mmol, 1.0 equiv.) was used to prepare **FSA-513013a** (0.006 g, 0.012 mmol, 8% over 4 steps).

\(^1\)H NMR (600 MHz, CD\(_3\)OD) δ 5.30 (d, \(J = 5.7\) Hz, 1H), 4.57 (q, \(J = 6.8\) Hz, 1H), 4.50 (d, \(J = 10.0\) Hz, 1H), 4.40 (d, \(J = 9.0\) Hz, 1H), 4.27 (d, \(J = 10.0\) Hz, 1H), 4.23 (t, \(J = 9.0\) Hz, 1H), 4.08 (dd, \(J = 10.2, 5.6\) Hz, 1H), 4.05 (m, 1H), 3.85 (d, \(J = 2.5\) Hz, 1H), 3.63 (ddd, \(J = 11.7, 8.5, 2.5\) Hz, 1H), 3.58 (dd, \(J = 10.2, 3.2\) Hz, 1H), 3.52 (dd, \(J = 11.1, 7.4\) Hz, 1H), 2.87 (t, \(J = 11.7\) Hz, 1H), 2.38 (m, 1H), 2.15 (s, 3H), 1.86–1.77 (m, 6H), 1.73 (m, 1H), 1.65 (m, 2H), 1.60–1.53 (m, 3H), 1.52 (d, \(J = 6.8\) Hz, 3H), 1.14 (m, 2H).

\(^{13}\)C NMR (126 MHz, CD\(_3\)OD) δ 169.22, 89.74, 82.14, 72.05, 71.30, 70.83, 69.73, 69.57, 62.77, 59.17, 54.82, 50.49, 46.48, 43.93, 35.09, 33.00, 31.87, 31.73, 26.34, 26.18, 22.58, 13.40.

FTIR (neat, cm\(^{-1}\)): 3355 (br), 2944 (w), 2493 (br), 1686 (s), 1585 (s), 1465 (m), 1349 (m), 1063 (s).

HRMS (ESI+, \(m/z\)): [M+H]+ calc’d for C\(_{23}\)H\(_{39}\)ClN\(_2\)O\(_6\)S, 506.2217; found 506.2216.
Using the general procedures for saponification, amide bond coupling, hydrogenation, and deprotection, 5.5 (0.058 g, 0.159 mmol, 1.0 equiv.) was used to prepare FSA-513013b (0.010 g, 0.020 mmol, 13% over 4 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.31 (d, $J = 5.6$ Hz, 1H), 4.57 (q, $J = 6.8$ Hz, 1H), 4.51 (d, $J = 10.0$ Hz, 1H), 4.47 (m, 1H), 4.37 (t, $J = 9.2$ Hz, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 4.09 (dd, $J = 10.2$, 5.6 Hz, 1H), 4.02 (dt, $J = 11.7$, 4.0 Hz, 1H), 3.86 (br, 1H), 3.78 (td, $J = 11.5$, 3.5 Hz, 1H), 3.59 (m, 2H), 2.91 (t, $J = 11.7$ Hz, 1H), 2.25 (m, 1H), 2.15 (s, 3H), 2.08 (m, 1H), 1.88 (m, 1H), 1.80 (m, 3H), 1.73 (m, 1H), 1.63 (m, 2H), 1.55 (m, 2H), 1.53 (d, $J = 6.8$ Hz, 3H), 1.47 (m, 1H), 1.38 (d, $J = 6.6$ Hz, 1H), 1.18 (m, 2H), 1.08 (m, 1H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 168.96, 89.74, 80.69, 72.04, 70.77, 69.73, 69.54, 69.37, 62.22, 59.07, 54.93, 47.63, 44.52, 44.03, 35.96, 34.39, 31.81, 31.48, 26.53, 26.25, 22.58, 13.43.

FTIR (neat, cm$^{-1}$): 3369 (br), 2946 (s), 2865 (m), 1686 (s), 1581 (s), 1466 (m), 1348 (m), 1258 (m), 1059 (s), 849 (m).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{23}$H$_{39}$ClN$_2$O$_6$S, 506.2217; found 506.2171.
Using the general procedures for saponification, amide bond coupling, hydrogenation, and deprotection, 5.3 (0.050 g, 0.168 mmol, 1.0 equiv.) was used to prepare FSA-513018a (0.008 g, 0.016 mmol, 10% over 4 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.31 (d, $J = 5.6$ Hz, 1H), 4.57 (m, 1H), 4.52 (d, $J = 11.1$ Hz, 1H), 4.46 (d, $J = 9.0$ Hz, 1H), 4.28 (d, $J = 9.9$ Hz, 1H), 4.26 (m, 1H), 4.09 (dd, $J = 10.2$, 5.6 Hz, 1H), 4.02 (m, 1H), 3.86 (m, 1H), 3.65 (m, 1H), 3.58 (dd, $J = 10.2$, 3.3 Hz, 1H), 3.55 (m, 1H), 2.91 (t, $J = 11.8$ Hz, 1H), 2.40 (m, 1H), 2.15 (s, 3H), 2.07 (m, 1H), 1.76 (m, 1H), 1.64 (m, 4H), 1.53 (d, $J = 6.8$ Hz, 3H), 1.23 (t, $J = 7.2$ Hz, 2H), 0.90 (t, $J = 6.3$ Hz, 3H).

$^{13}$C NMR (600 MHz, CD$_3$OD) δ 169.33, 89.74, 82.08, 72.06, 71.01, 70.77, 69.73, 69.55, 62.58, 59.16, 54.86, 50.30, 46.77, 43.56, 36.24, 35.30, 33.95, 26.30, 23.17, 22.88, 22.59, 13.41.

FTIR (neat, cm$^{-1}$): 3343 (br), 2920 (m), 1681 (m), 1585 (s), 1466 (m), 1350 (m), 1255 (m), 1054 (s).

HRMS (ESI+, m/z): [M+H]+ calc’d for C$_{22}$H$_{35}$ClN$_2$O$_6$S, 494.2217; found 494.225.
Using the general procedures for saponification, amide bond coupling, hydrogenation, and deprotection, 5.3 (0.050 g, 0.168 mmol, 1.0 equiv.) was used to prepare **FSA-513018b** (0.036 g, 0.073 mmol, 43% over 4 steps).

$^1$H NMR (600 MHz, CD$_3$OD) δ 5.32 (d, $J = 5.6$ Hz, 1H), 4.59 (d, $J = 9.1$ Hz, 1H), 4.56 (m, 1H), 4.52 (d, $J = 10.0$ Hz, 1H), 4.42 (t, $J = 9.3$ Hz, 1H), 4.30 (d, $J = 9.8$ Hz, 1H), 4.09 (dd, $J = 10.2, 5.6$ Hz, 1H), 4.02 (m, 1H), 3.90 (d, $J = 3.0$ Hz, 1H), 3.82 (dd, $J = 11.3, 3.4$ Hz, 1H), 3.79 (m, 1H), 3.61 (m, 2H), 2.97 (t, $J = 11.9$ Hz, 1H), 2.28 (m, 1H), 2.16 (s, 3H), 2.00 (m, 1H), 1.76 (m, 2H), 1.73–1.63 (m, 2H), 1.54 (d, $J = 6.8$ Hz, 3H), 1.21 (m, 2H), 1.01 (m, 1H), 0.89 (d, $J = 6.6$ Hz, 6H).

$^{13}$C NMR (126 MHz, CD$_3$OD) δ 168.20, 89.70, 80.32, 72.00, 70.66, 69.68, 69.51, 69.13, 62.05, 58.99, 54.98, 48.07, 43.98, 36.98, 36.39, 35.94, 26.27, 23.16, 22.95, 22.56, 13.45.

FTIR (neat, cm$^{-1}$): 3372 (br), 2919 (m), 1676 (s), 1578 (m), 1465 (s), 1365 (m), 1255 (m), 1140 (s), 1049 (s).

HRMS (ESI+, $m/z$): [M+H]$^+$ calc’d for C$_{22}$H$_{39}$ClN$_2$O$_6$S, 494.2217; found 494.2258.
References


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Appendix A.

Previous route to β-hydroxy-γ-allyl proline
Appendix Figure 1. Synthesis of (R,R)-pseudoephenamine glycaminide 1.13.  

Appendix Figure 2. Synthesis of aldehyde 1.14.
Appendix B.

Configuration assignment by NMR data
Appendix Figure 3. Mosher ester analysis of 2.2 prepared by the method in Figure 2.3 (red, above) and in Figure 2.4 (blue, below).
Appendix Figure 4. NOESY experiment to determine stereochemistry of FSA-507061 at the 7’-position.
Appendix C.

Cytotoxicity assay data
Cytotoxicity assay data by Dr. Amarnath Pisipati and R. Porter Ladley.
Appendix D.

Catalog of MIC Data
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<th>Species Strain Description</th>
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**Species Strain Description**

- **S. aureus** ATCC 29213
- **S. aureus** BAA-977; iErmA
- **S. aureus** MMX-3035; cErmA
- **S. aureus** MP-549; MsrA
- **S. aureus** MMX USA 300; MRSA
- **S. pneumoniae** ATCC 49619
- **S. pneumoniae** MMX-3028; cErmB
- **S. pneumoniae** MMX-3031; cMeFA
- **S. pyogenes** ATCC 19615
- **S. pyogenes** MMX-946; cErmB
- **E. faecalis** ATCC 29212
- **E. faecalis** MP-9; ΔtolC
- **E. coli** ATCC 25922
- **E. coli** MP-9; ΔtolC
- **E. coli** MP-9; ΔtolC
- **K. pneumoniae** ATCC 10031
- **H. influenzae** ATCC 49247

**Clindamycin (μg/mL)**

- 0.12
- ≤0.06
- >64
- NT
- 0.06

**Gram-positive Gram-negative**

- **S. aureus** ATCC 29213
- **S. aureus** BAA-977; iErmA
- **S. aureus** MMX-3035; cErmA
- **S. aureus** MP-549; MsrA
- **S. aureus** MMX USA 300; MRSA
- **S. pneumoniae** ATCC 49619
- **S. pneumoniae** MMX-3028; cErmB
- **S. pneumoniae** MMX-3031; cMeFA
- **S. pyogenes** ATCC 19615
- **S. pyogenes** MMX-946; cErmB
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**Gram-positive**

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Appendix E.

Catalog of NMR Spectra
FSA-503004