Chapter I: Transition-Metal Based and Enzymatic C–H functionalization, Macrolide Natural Product Biosynthesis and the Cytochrome P450 PikC

1.1 Introduction

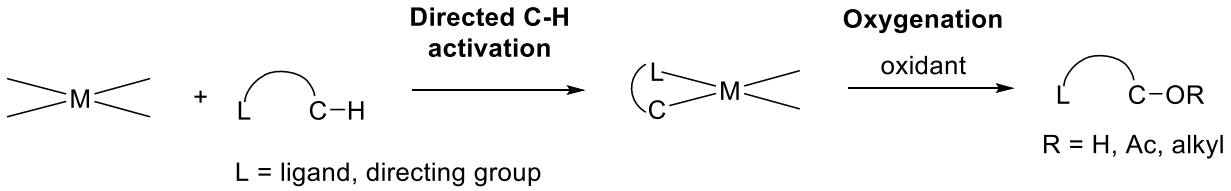
Carbon–hydrogen (C–H) bonds had been long considered inert in organic chemistry rather than as functional groups whose reactivity can be exploited in a selective manner. However, oxidation of unactivated C–H bonds is a key process in the biosynthesis of natural products and metabolism of xenobiotics.[1] Development of selective functionalization of C–H bonds can have broad impacts in streamlining the synthesis of pharmaceuticals and natural products. In fact, recent advances in the fields of transition metal- and bio-catalysis have significantly changed how the synthetic community views these bonds to the point that selective C–H functionalizations have been featured in tandem to construct complex structural frameworks[2] and have also been featured as a key step in the total synthesis of several natural products.[3-7] This chapter describes the transition metal-based and enzymatic-based C–H functionalization strategies as a prelude to developing the macrolide P450 mono-oxygenase PikC as a biocatalyst for the selective C–H hydroxylation.

1.2 Transition metal based C–H functionalization strategies

1.2.1 Directed C–H functionalization

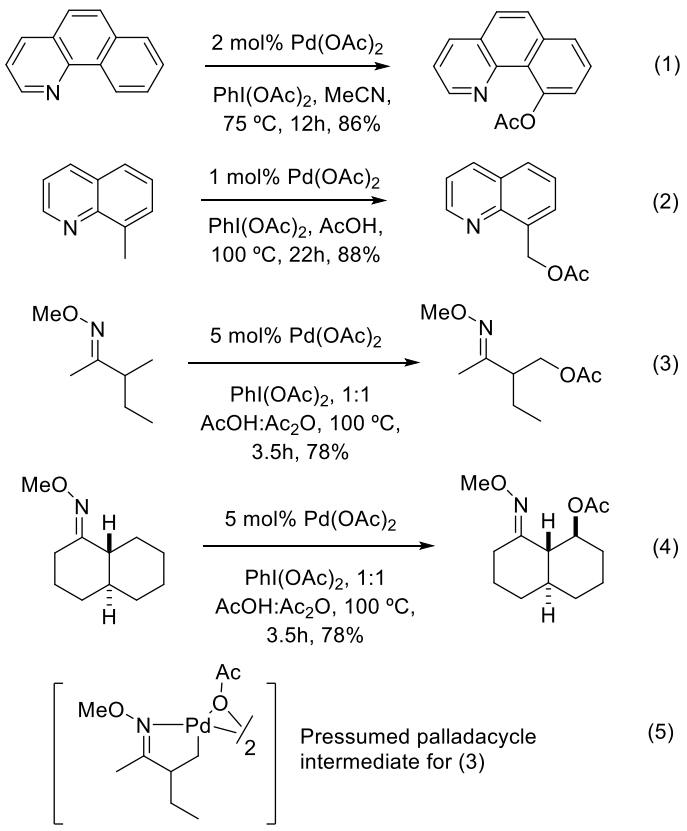
Metal-catalyzed C–H functionalization strategies can be divided into directed and non-directed approaches. In the latter, selectivity is achieved through reactivity differences between the substrates' C–H bonds. The former employ a coordinating group, permanently or temporarily

installed, in the substrate molecule that is proximal to the oxidation site (*Scheme 1.1*). The coordinating group, often a heteroatom, can bind a metal forming a 5- or 6-membered intermediate in the transition state which determines the selectivity in C–H bond functionalization.[8] An example of such reactions is the palladium-catalyzed oxygenation of C–H bonds pioneered by the Sanford group, where a nitrogen atom present in the substrate serves as a ligand for palladium.[9]



Scheme 1.1. Metal-catalyzed directed C–H oxygenation.

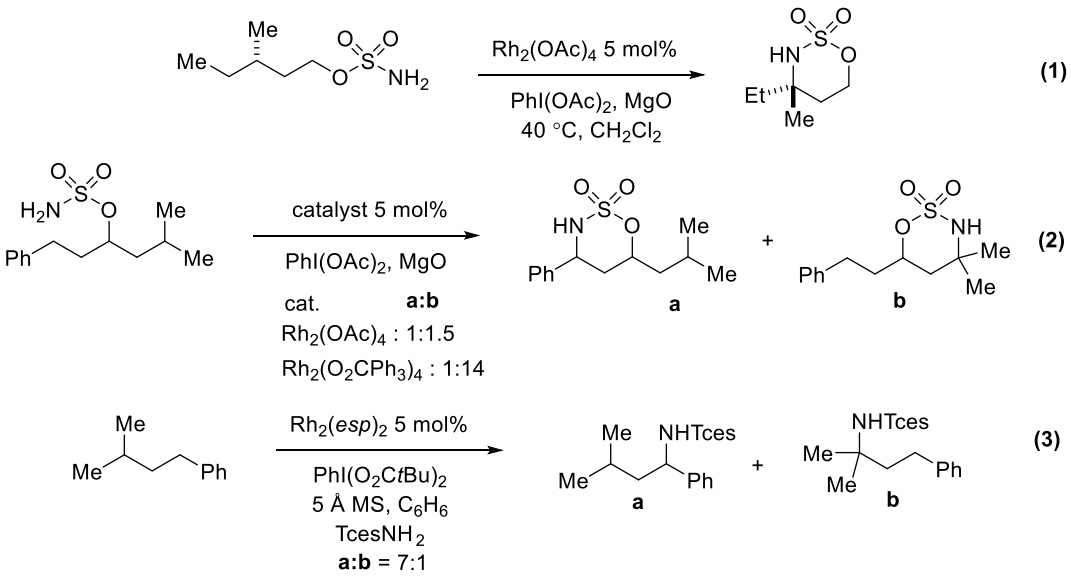
The examples shown in scheme 1.1 illustrate the scope of the aforementioned transformation.[21,22] Pyridine nitrogen serves as the coordinating ligand for Pd in the oxidation of proximal sp^2 C–H bonds as well as activated benzylic sp^3 C–H bonds (*Scheme 1.2 equations 1-2*). The nitrogen atom guides the insertion of Pd into the γ C–H bond, creating a 5-membered palladacycle (*Scheme 1.2 eq. 5*). In the case of unactivated sp^3 C–H bonds, both pyridine and oxime can serve as the ligand for Pd. The methodology prefers the sterically accessible primary C–H bonds; oxidation at secondary C–H bonds can occur only with proper stereoelectronic activation of the substrate (*Scheme 1.2 eqn. 4*).



Scheme 1.2. Pd-catalyzed directed C–H oxygenation examples.[10,11] (1) Pyridine directed oxidation of sp^2 C–H bonds. (2) Pyridine-directed oxidation of allylic/benzylic primary sp^3 C–H bonds. (3) Oxime directed oxidation of unactivated primary sp^3 C–H bonds. (4) Oxime directed oxidation of secondary sp^3 C–H bonds. (5) Palladacycle intermediate believed to reductively eliminate to give oxygenated products.

Other examples of directed C–H functionalization include the rhodium-catalyzed site-selective intramolecular aliphatic C–H amination by the DuBois group.[12] In these examples, a nitrene intermediate is first generated from a nitrogen source present in the substrate and an external oxidant. The rhodium-ligated nitrene then inserts into a proximal C–H bond as depicted in Scheme 1.3 eq 1. These types of catalysts prefer electronically activated C–H bonds such as tertiary C–H bonds, those α to oxygen and nitrogen, benzylic, and secondary aliphatic C–H bonds. In cases of competing reactivity for proximal C–H bonds, the catalyst prefers a tertiary

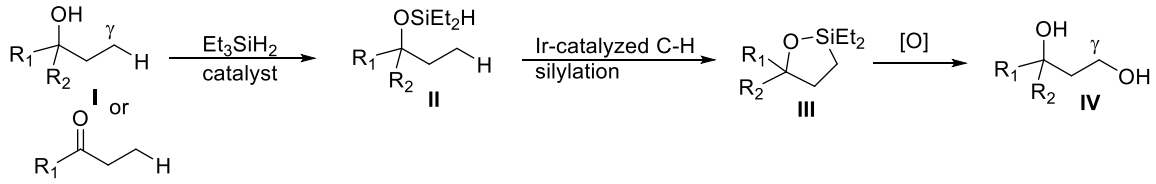
over a benzylic C–H bond (*Scheme 1.3 eq 2*).[13] The reaction has also been extended to a non-directed variant with $Rh_2(esp)_2$ as the catalyst and an external sulfamite source. In this case the intramolecular preference is reversed, favoring benzylic over tertiary C–H amination.



Scheme 1.3. Rhodium catalyzed C–H amination by the Du Bois group. (1) Intramolecular C–H amination. (2) Selectivity of intramolecular amination towards tertiary C–H bonds. (3) Selectivity of intermolecular amination towards benzylic C–H bonds.

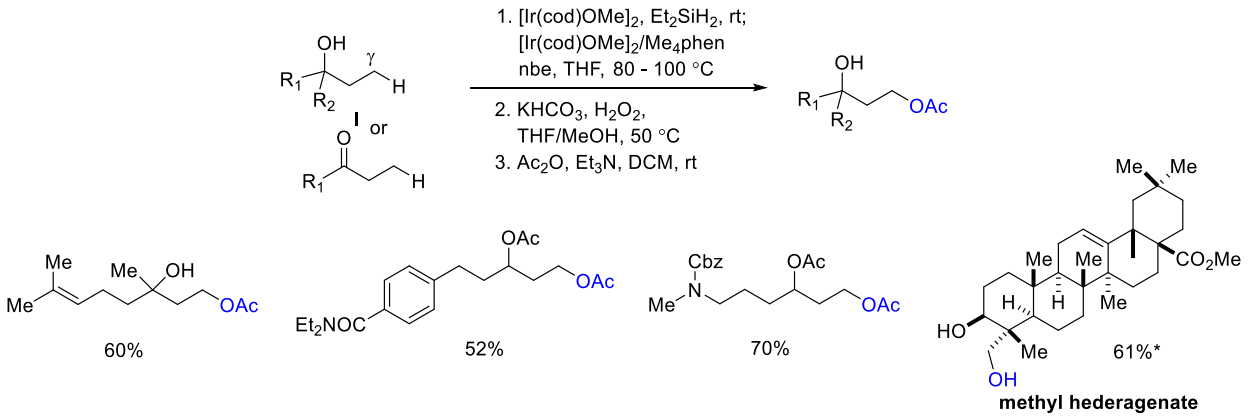
The Hartwig group has reported elegant approaches to directed functionalization of unactivated C–H bonds employing an alcohol as a directing group.[14] The authors achieved γ-functionalization at primary C–H bonds in molecules containing alcohols or ketones by combining iridium- phenanthroline catalysis and a dihydrosilane reagent (*Scheme 1.4*). The reaction proceeds first by dehydrogenative coupling of the silane to the oxygen functionality present in the substrate molecule forming hydridosilyl ether **II** which directs C–H functionalization (*Scheme 1.4*). The intermediate then undergoes selective dehydrogenative

functionalization to give a cyclic silyl ether intermediate **III**. Subsequent Tamao-Fleming oxidation yields 1,3-diol **IV**.



Scheme 1.4. Ir-catalyzed C–H functionalization of primary bonds directed by an alcohol.

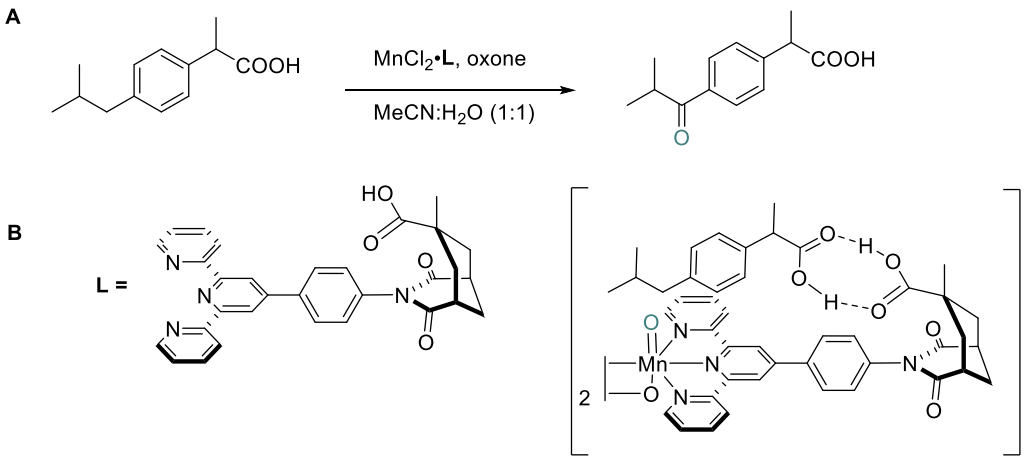
The catalyst was selective for primary oxidation in the presence of secondary C–H bonds, activated secondary (C–H bonds that are allylic or benzylic or adjacent to nitrogen), tertiary, and sp^2 C–H bonds (*Scheme 1.5*). However, when esters and amides were present in the substrate and accessible to the catalyst, competing carbonyl hydrosilylation was observed (*Scheme 1.5*). The catalyst was also employed selectively in complex systems. An example that demonstrates the power of the method was oxidation of methyl oleanate to methyl hederagenate in modest yields and high selectivity, as the same overall transformation was reported to require ten steps during the synthesis of a similar natural product.[15]



Scheme 1.5. Summary of the substrate scope of the Ir-catalyzed directed oxidation of primary C–H bonds. (*No acetylation step)

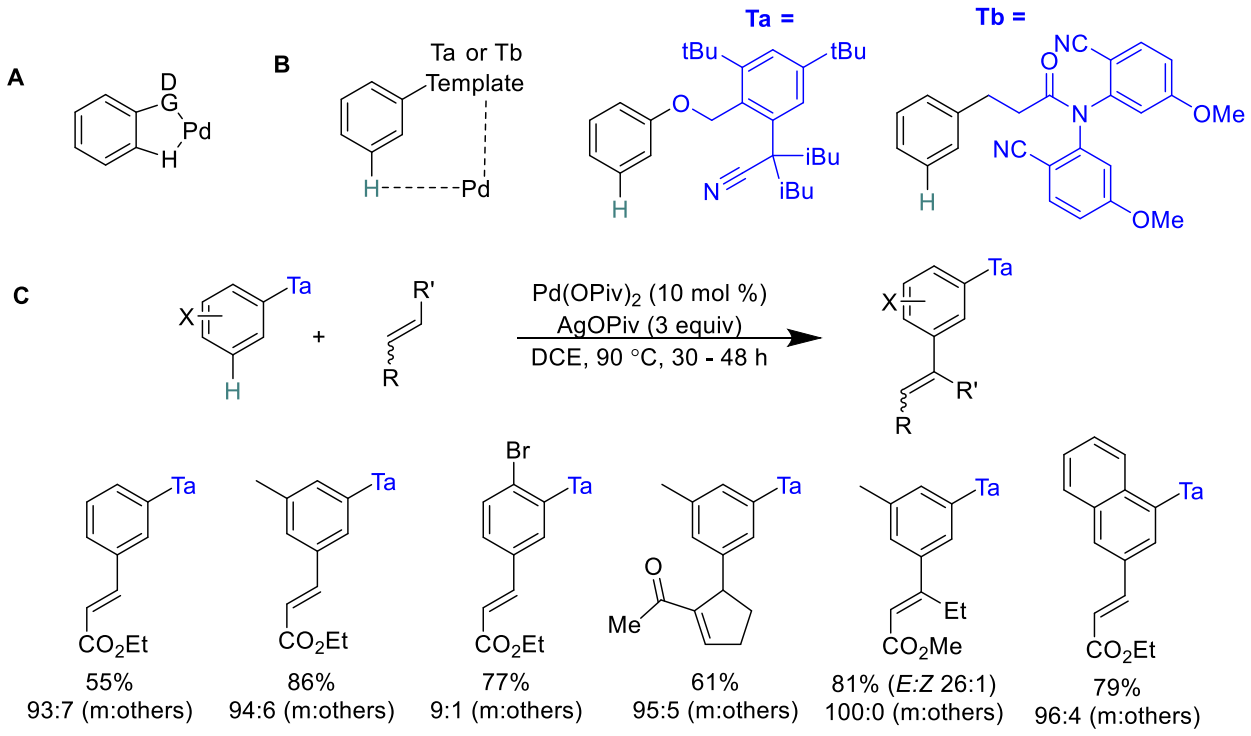
1.2.2 Remote directed C–H functionalization

Selective functionalization of stereoelectronically similar C–H bonds at positions that are distal from directing groups has been a challenge in the transition-metal-catalyzed field. A catalyst that not only can functionalize C–H bonds far from a directing functionality but also can override substrate biases is highly desirable. This important challenge has been recognized by Breslow with key studies developing engineered protecting groups and employing these for directed photochemical and free-radical reactions.[16] The aforementioned desirable transformation is one that enzymes perform with ease, and some of the work in directed functionalization of remote C–H bonds has drawn inspiration from enzymes and the molecular recognition strategies these employ to determine site-selectivity. Crabtree has employed molecular recognition through carboxylic acid (-COOH) mediated hydrogen bonding for the selective oxidation of sp^3 C–H bonds by a dimanganese catalyst (*Scheme 1.6*).[17] The ligand for Mn contains -COOH group that H-bonds with COOH functionality in the substrate and directs the C–H bonds for oxidation by a Mn-oxo. In the case of ibuprofen substrate (*Scheme 1.6A, and 1.6B*) a 2° benzylic C–H bond is placed favorably towards the Mn-oxo for oxidation.



Scheme 1.6. Carboxylic acid directed remote C–H oxidation. (A) Selective oxidation of ibuprofen (B) Ligand for Mn and presumed molecular recognition events that direct oxidation.

In another high-impact example, work by the Yu group has demonstrated directed activation of remote meta sp^2 C–H bonds.[18] As previously mentioned, most directed methods control selectivity by forming a 5-7 membered ring metallacycle intermediate that dictates selectivity (*Scheme 1.7A)*. Therefore, the use of σ-chelating groups for directing effects generates ortho-selectivity in the functionalization of sp^2 C–H bonds. The Yu group devised a solution by employing nitrile-containing removable templates that direct functionalization of meta C–H bonds at least ten atoms away from the directing group through a cyclophane-like intermediate (*Scheme 1.7B)*. The catalyst was remarkably effective in overriding ortho-directing effects and stereoelectronic substrate biases (*Scheme 1.7C).*

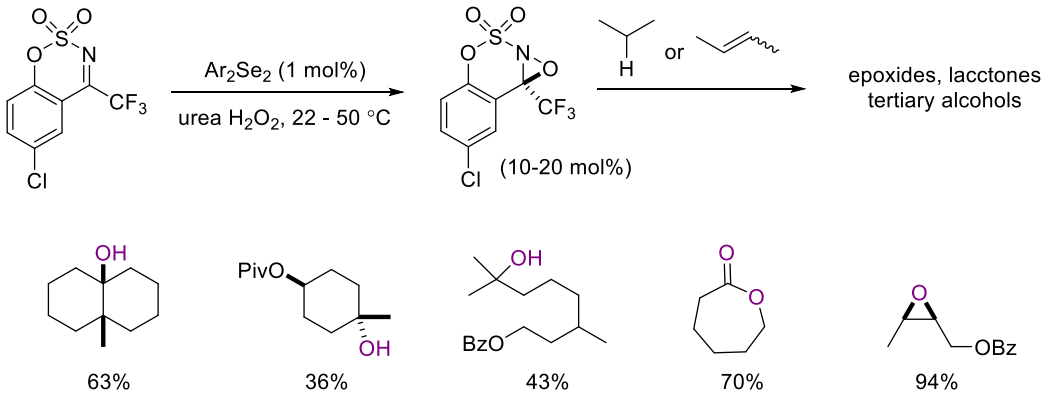


Scheme 1.7. Remote template-directed meta C–H functionalization. (A) Chelating directing

groups generate ortho selectivity. (B) Template-directed meta activation approach. (C) meta C–H activation substrate scope.

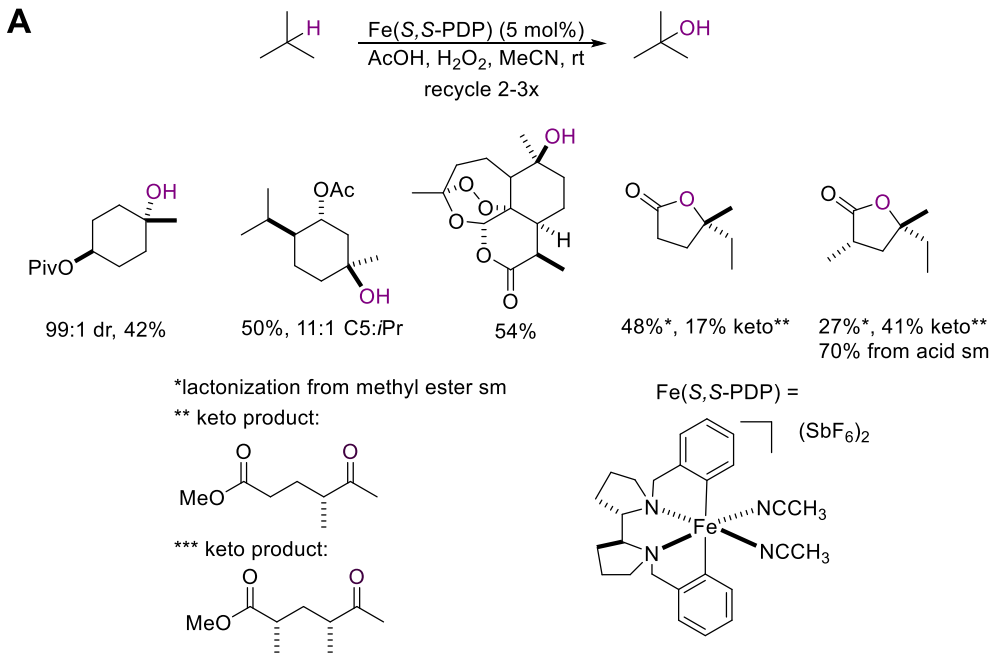
1.2.3 Non-directed C–H functionalization

Non-directed approaches rely on inherent substrate influences for differentiating the C–H bonds. These factors include steric effects, bond strengths, hybridization, conjugation, proximity to electron donating- and electron withdrawing- groups and stereochemical orientation.[19,20] The DuBois group has made important contributions to non-directed selective C–H functionalization as well. They have reported an oxaziridine-mediated catalytic hydroxylation of unactivated tertiary C–H bonds that employs a diaryl diselenide cocatalyst and urea·H_2O_2 as the terminal oxidant as an important development in selective C–H hydroxylation.[21] These perfluorinated oxaziridines prefer delivering oxygen to 3° site removed from electron-withdrawing groups. In addition, a cyclic secondary alcohol in which the ketone intermediate generated undergoes Baeyer-Villiger oxidation to yield a lactone, and C=C bonds are epoxidized under these reaction conditions (*Scheme 1.8*).[22]



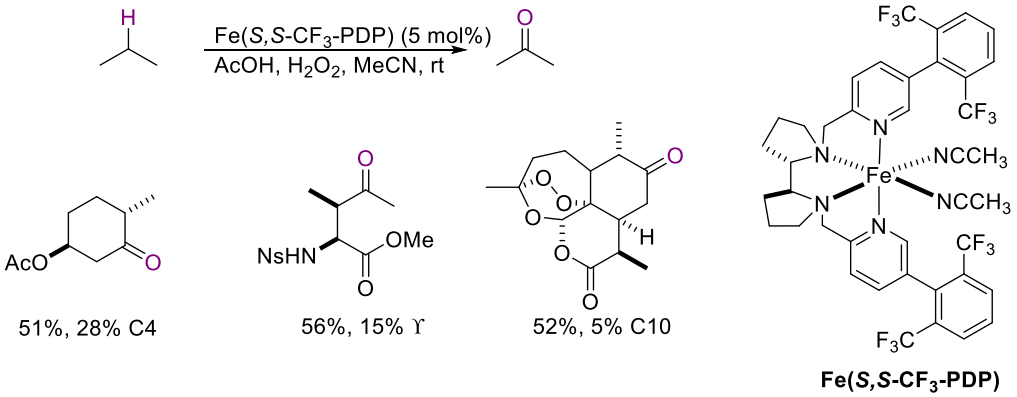
Scheme 1.8. Oxaziridine-mediated oxidation by the DuBois group.

Another example of non-directed approaches is the use of electrophilic iron catalysts for site-selective oxidations of unactivated sp^3 C–H bonds by the White group.[23,24] The employed Fe(*S,S*-PDP) catalyst selectively oxidizes electron-rich tertiary C–H bonds in the presence of other aliphatic C–H bonds, but when these were not present, methylenes were oxidized into ketone products by two rapid subsequent oxidations (*Scheme 1.9*). However, when multiple stereoelectronically similar 3° C–H bonds are present, poor selectivity was observed, although the catalyst does differentiate between sterically distinct tertiary sites that are electronically similar (*Scheme 1.9A*). The catalyst also demonstrated directed oxidation when carboxylic acid groups were present in the substrate and has been employed with useful levels of selectivity in complex molecule settings.



Scheme 1.9. Substrates for Fe(*S*,*S*-PDP) aliphatic C–H oxidation.

The same iron catalyst was also employed for methylene oxidation in both small molecules and complex settings. However, in these, like in the aforementioned examples of tertiary C–H oxidation employing for Fe(*S*,*S*-PDP) as the catalyst, the selectivity is dictated by the local chemical environment in the substrate (*Scheme 1.9B*). When multiple secondary sites were present, differentiation of the various C–H's is guided by stereoelectronic factors in the substrate. Less sterically hindered methylenes as well as methylenes proximal to electron-donating groups were preferred.

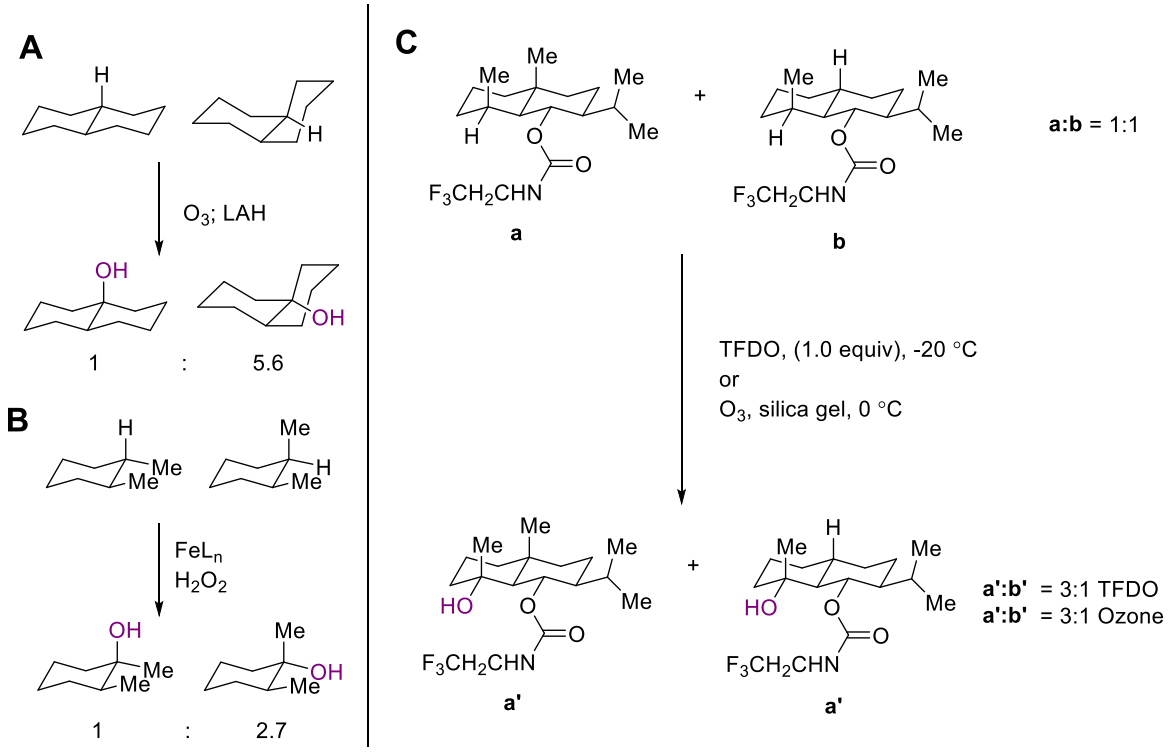


Scheme 1.10. Substrates for Fe(*S*,*S*-CF_3-PDP) aliphatic C–H oxidation.

Complementary reactivity was observed with a modified catalysts Fe(*S*,*S*-CF_3-PDP). The modification in the catalyst structure reduced the cone angle available for substrate approach due to the increased steric bulk around the iron provided by the additional Ph-CF_3 groups. This change made the catalyst the most influential of the parameters which dictate selectivity and switched the observed major products observed with certain previously examined substrates with Fe(*S*,*S*-PDP) (*Scheme 1.10*).[25] With this catalyst, methylene oxidation of artemisinin to the keto

product was achieved. Prior to White's work, a different electrophilic iron catalyst for methylene oxidation with similar selectivities but attenuated yields was reported by the Costas group.[26]

The chemistry literature has experienced a surge in the number of total syntheses that employ selective C–H functionalizations in complex molecule settings. This attests to the powerful impact these technologies currently have, and will continue to have as knowledge in the field continues to advance. The Baran group aims particularly to develop these reactions for applications in natural product and complex molecule synthesis. However, the successful implementation of these reactions in synthesis requires a thorough understanding of reactivity trends. One of such trends is that equatorial C–H bonds react faster than axial and, interestingly, this preference is observed regardless of the C–H activation method employed (*Scheme 1.11A and B*). In the case of tertiary C–H bonds, Baran illustrates that strain release acts in collaboration with steric hindrance and nucleophilicity of the C–H bond to account for the observed trends.[27] The strain release is due to the decrease in 1,3-diaxial interactions going from a sp^3 to an sp^2 hybridized carbon in the transition state. To support the theory the reaction rates between terpenes A and B were compared by subjecting 1:1 mixtures of A:B to TFDO and ozone-mediated oxidations (*Scheme 1.11C*).



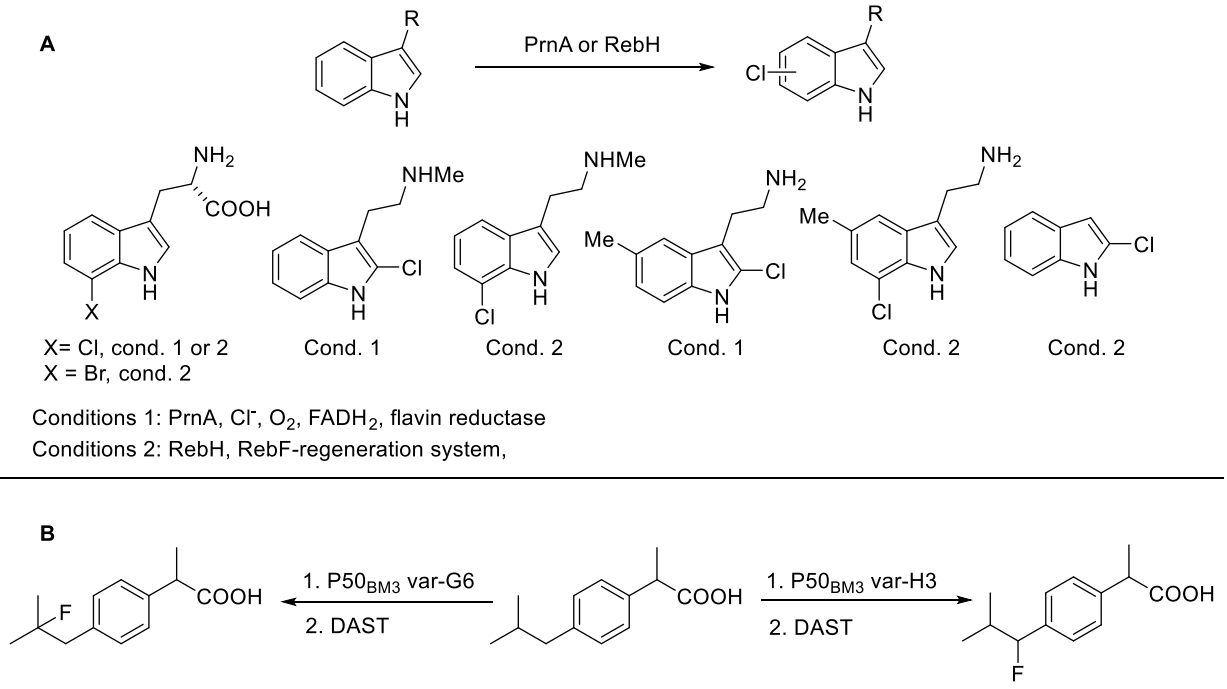
Scheme 1.11. Strain release in C–H functionalization.

1.3 Enzymes in organic synthesis and C–H functionalization

Many transition-metal catalyzed selective C–H functionalization methods have been developed, including both directed and non-directed approaches previously described. The lack of site-selectivity in the functionalization of C–H bonds that are not differentiated by steric or electronic factors and are not adjacent to directing groups is still challenging. Also a challenge in the development of such procedures is the difficulty in controlling over-oxidation. For example, oxidation at the same methylene site as observed with the White Fe(*S*,*S*-CF_3-PDP) catalyst (*Scheme 1.10*), but selectivity for hydroxylation was achieved by mutagenesis of a P450 enzyme.[28] Enzymes have been evolved by nature to accomplish these transformations on substrates while overriding all of the previously mentioned limitations. Therefore biological catalysts have the potential for selective oxidation of chemically similar bonds that are distant

from directing groups and present an opportunity to address these challenges.[29] Enzymes have inspired the design of biomimetic transition metal catalysts, however the potential of enzymes being used as a tool for the site-selective functionalization of C−H bonds continues unfulfilled. There have been great advances in the field of biocatalysis to develop enzymes as synthetic toolboxes, some of which will be described in this section. Challenges regarding enzymatic expression/purification, co-factor supply, organic solvent, oxygen tolerance, stability and substrate scope have been recognized and need to be addressed for the full success of enzymes as biocatalysts.[30,31]

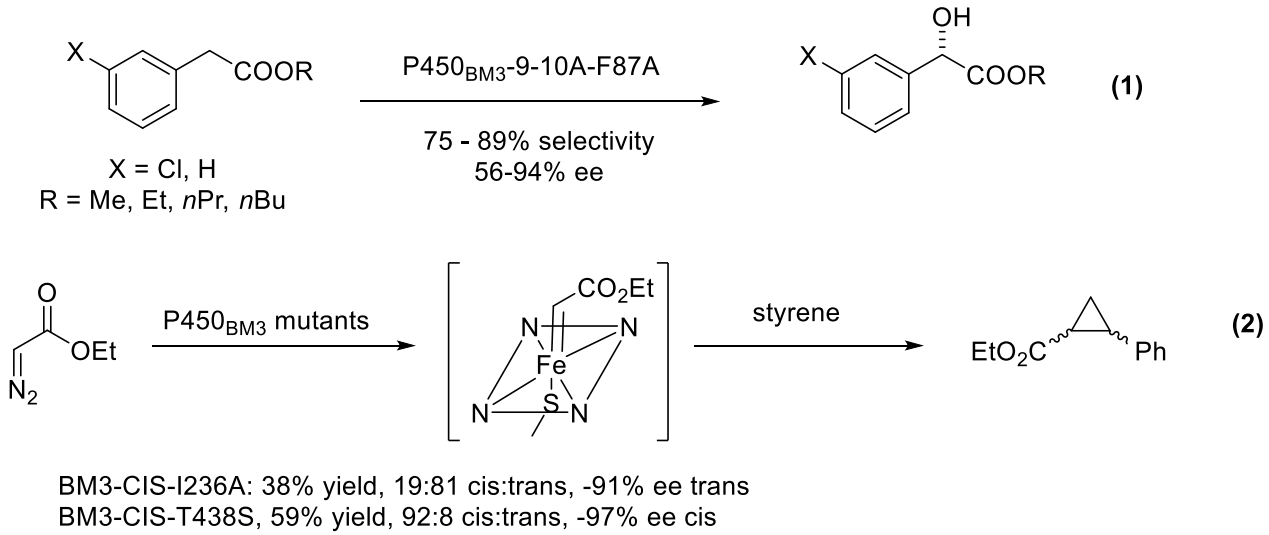
Flavin-adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are common redox cofactors for certain enzymes involved in C−H functionalization. FAD dependent hydroxylases have been utilized extensively for the hydroxylation of electron-rich aromatics that are structurally similar to endogenous substrates. FAD dependent halogenases had not enjoyed as much success until pioneering work for PrnA catalyzed selective indole halogenation by the van Peé[32] group, and more recently, regioselective arene halogenation employing RebH by the Lewis group.[33] These bacterial tryptophan-7-halogenase enzymes exhibited complementary reactivity in some substrates, some of which showed the same oxidation patterns previosly observed. (*Scheme 1.12A*) Halogenation reactions have attracted a lot of focus given the metabolic stability that halogens, particularly fluorine, can impart on a drug candidate. The well-studied fatty acid hydroxylase $P450_{BM3}$ enzyme[34] is employed in a prominent example towards selective halogenation through chemo-enzymatic functionalization of sp^3 C−H bonds. Different mutants of this enzyme displayed complementary reactivity towards functionalization of the distinct sp^3 C−H bonds in ibuprofen (*Scheme 1.12B*) and were also employed for selective fluorination of cyclopentenone scaffolds.[35]



Scheme 1.12. Chemo-enzymatic C−H halogenation. (A) Selective indole halogenation by tryptophan-7-halogenases PrnA and RebH. (B) Selective hydroxylation of ibuprofen by mutant $P450_{BM3}$ enzyme and selective fluorination employing diethylaminosulfur trifluoride (DAST).

The Arnold group has pioneered the field of enzymatic C−H functionalization employing variants of the self-sufficient cytochrome P450 (CYP) enzyme $P450_{BM3}$ for a diverse array of transformations (*Section 1.4 contains a detailed review of P450 enzymes*). Directed evolution approaches generated $P450_{BM3}$ variants capable of selective generation of 2-aryl-2-hydroxyacetic acid derivatives as single enantiomers which are useful building blocks for the synthesis of penicillins, cephalosphorin and anti-obesity agents (*Scheme 1.13A*).[36] No cytochrome P450 had accepted 2-arylacetic acids previous to this work. This enzyme has also been employed for cyclopropanation reactions, a reaction that has been heavily studied in the transition-metal catalysis field and the cyclopropane motif is present in natural products and pharmaceuticals.[37] The transition-metal catalyzed version of the reaction operates by transfer of a carbene to an

olefin, a mechanism very different from how nature carries out the reaction. In the $P450_{BM3}$ mutant, the heme-iron cofactor was engineered to bind the diazoacetate and generate an iron carbenoid and deliver it to an olefin moiety present in the substrate to generate cyclopropane derivatives with high diastereo- and enantio-selectivity (*Scheme 1.13B*).[37,38]



Scheme 1.13. Examples of chemo-enzymatic functionalization employing $P450_{BM3}$ by the Arnold group. (A) Enantioselective benzylic C−H hydroxylation. (B) Enantioselective and diastereoselective cyclopropanation.

In summary, enzymes have received considerable attention in the field of C−H functionalization but have not yet been realized to their full potential. P450 enzymes show promise in the biocatalysis field as exemplified by the work employing $P450_{BM3}$. The following section provides detail on cytochrome P450s, the reactions they can perform and the mechanism by which they are believed to operate.

1.4 Introduction to P450 Enzymes

The CYP super family of heme enzymes catalyzes a wide variety of reactions including hydroxylation, epoxidation, dealkylation, ring formation and expansion, dehydration and others;

therefore, P450s are described as "the most versatile biological catalyst in nature".[39] CYP enzymes are found across all forms of life, from human P450s involved in steroid hormone synthesis and metabolism to bacterial P450s such as the ones responsible for the final steps in the synthesis of macrolide antibiotics and many other natural products. P450 enzymes share some common features that enable the conserved oxygen activation method for catalysis. These contain the heme cofactor with the iron(III)protoporphyrin-IX prosthetic group that is covalently linked to the protein by a sulfur atom from a conserved cysteine residue. The enzymes require redox partners to deliver electrons for dioxygen activation. The similarities and the three-dimensional structures of this class of enzymes arise from well-conserved helices despite the low sequence similarity displayed by P450 enzymes (<20%).[40]

P450 catalyzed oxidation is believed to occur through a generally accepted hydrogen atom abstraction/oxygen rebound mechanism (*Figure 1.1*).[41] When substrate is not present, water usually serves as the sixth ligand of iron (low spin state that is favored in absence of the substrate). Once a substrate binds, water is displaced (high spin state), which changes the redox potential of the iron center (*Figure 1.1, transition from **A** to **B***) and triggers electron transfer from the reductase protein to give iron(II). Molecular oxygen then binds, and one of these oxygen atoms will be eventually inserted into the substrate. This is represented by intermediate **D**, which gains a second electron to give the iron(III)peroxo complex **E**, which is quickly protonated by amino acids in the active site to give the hydroperoxo complex **E'**. This intermediate undergoes a second protonation step that releases water to give the iron(V)oxo species **F**, which is the species believed to deliver the oxygen atom to the alkane to give the alcohol. **F** abstracts a hydrogen atom from a C–H bond in the substrate to generate an iron hydroxyl radical and a carbon radical, which combine in a rebound step to give the product.[42]

Although debate about the actual oxidizing species is still ongoing, the iron(V)oxo species **F** is generally accepted as the oxidant in a large number of cases, however it is possible that other iron-oxygen species present from the cycle could also act as oxidants such as hydroperoxo species **E'** and ferric peroxy anion **E**.[42,43] For example a recent co-crystal structure of the pimarycin pathway P450 PimD with its endogenous substrate 4,5-desepoxypimaricin which is epoxidized by the enzyme suggests that based on the olefin position and angle to the porphyrin plane oxygen delivery is only possible with hydroperoxo species **E'** as the oxidant.[44]

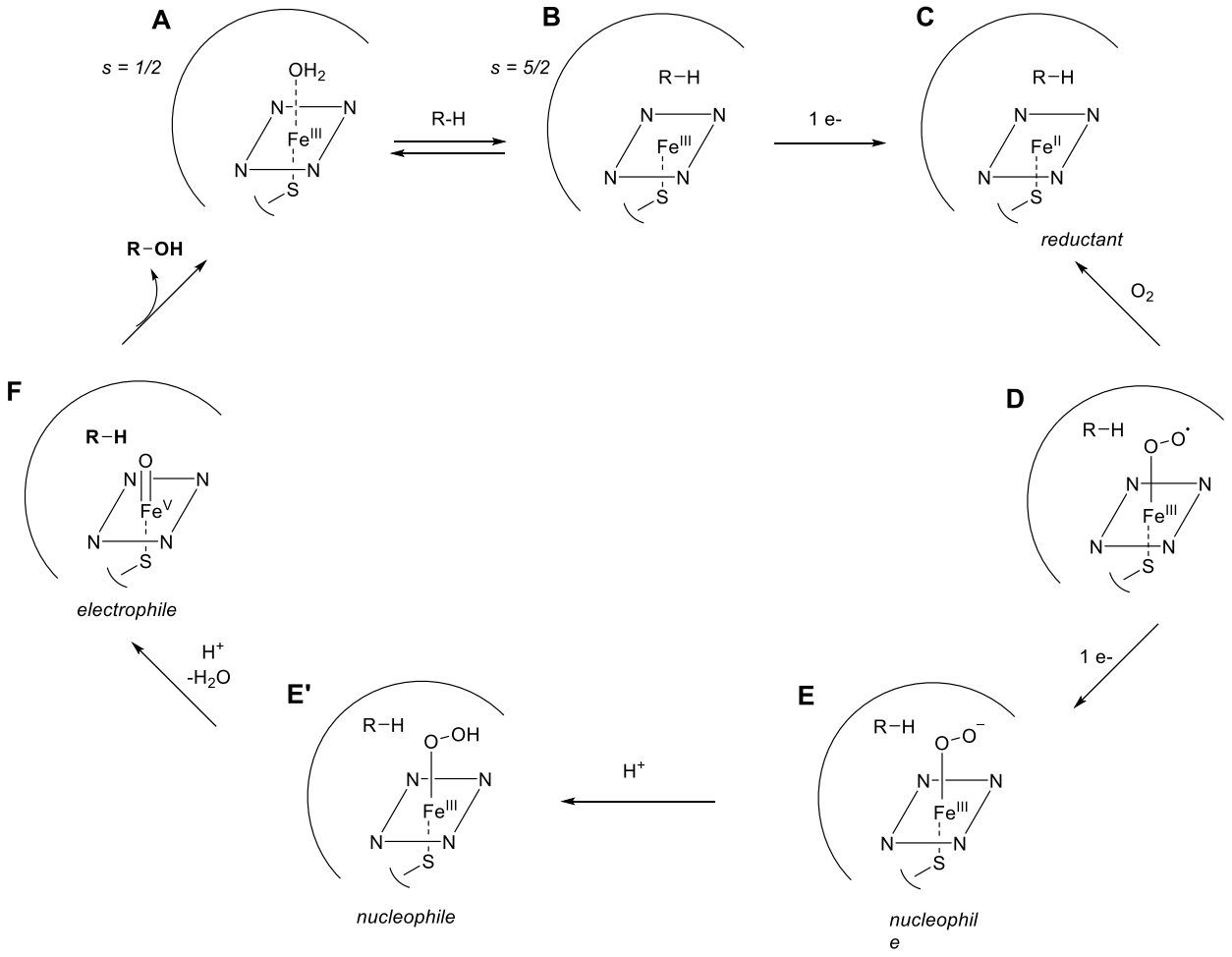


Figure 1.1. P450 Oxidation general mechanism (from review by Meunier et al).[41]

This heme-iron cofactor-bound P450 enzymes catalyze late-stage oxidations in the biosynthesis of many pharmaceutically relevant natural products with a diverse array of bioactivities. The following section describes macrolides and the enzymes involved in the biosynthesis of pikromycin, a natural product member of this class. In particular, the cytochrome P450 PikC responsible for a final hydroxylation step in the pathway will be discussed.

1.5 Biosynthesis of macrolide natural products, introduction to the pikromycin pathway and the cytochrome P450 PikC

1.5.1 Macrolide natural products

The first macrolactone glycoside antibiotics discovered were pikromycin and erythromycin A in the 1950's. R.B. Woodward proposed the term "macrolide" to describe this class of polyketide natural product antibiotics with structures consisting of a macrolactone, generally 12-, 14- or 16-membered rings, to which one or more deoxysugar residues may be attached (*Figures 1.2, 1.3*).[45] To further increase the chemical diversity and bioactivity of these polyketide natural products, most macrolides also possess oxygen-containing functionalities commonly in the form of hydroxyl, aldehyde or epoxide groups installed by cytochrome P450 enzymes at late stages in the biosynthetic process.[46]

Macrolide antibiotics are generally used to treat upper and lower respiratory tract infections and their mechanism of action involves inhibition of protein translation by binding reversibly to the 50S subunit of the bacterial ribosome. The particular site of binding depends on the macrolactone ring size of the macrolide.[45] Although the first macrolide discovered was pikromycin, erythromycin A was the first of this class of natural product antibiotic to be used clinically. However, due to poor oral bioavailability and side effects, first generation macrolides such as erythromycin A were replaced by the second and third generation semi-synthetic

macrolides including clarithromycin and azithromycin. Following the third generation macrolides the ketolides emerged; the first of these being telithromycin, which was approved by the FDA in 2004 and is effective against macrolide resistant *S. pneumonia* (*Figure 1.2*).[45]

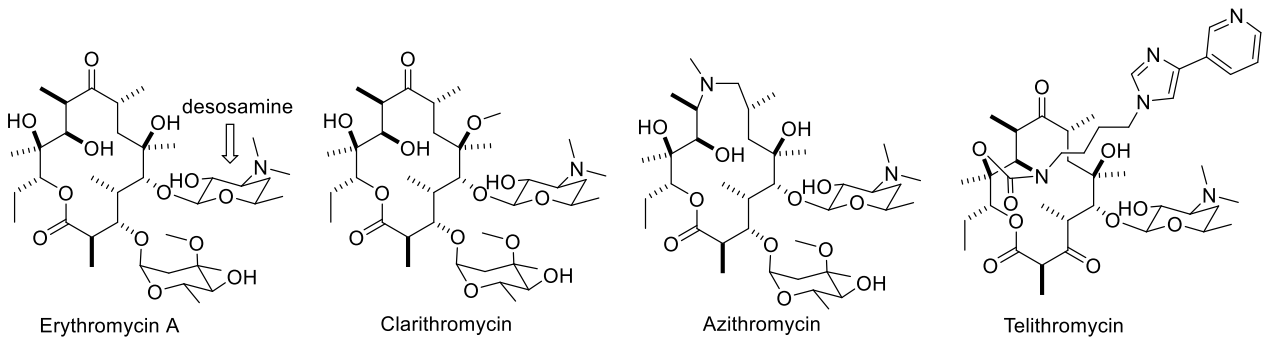


Figure 1.2. Chemical structures of clinically employed macrolide antibiotics.

To date, macrolides remain a very important class of antibiotics; however, due to increasing resistance issues associated with bacterial infections, the need for new antibiotics grows stronger despite the large number that are clinically available.[47] Natural products and their synthetic analogues provide a unique opportunity for the development of novel bioactive substances. In addition, the enzymes involved in macrolide biosynthesis can be transformed into catalysts for the generation of novel antibiotics. These studies focus on the Pikromycin pathway, which produces macrolide natural product antibiotics methymycin, neomethymycin and pikromycin, isolated from the soil bacteria *Streptomyces venezuelae* (*Figure 1.3*).[48]

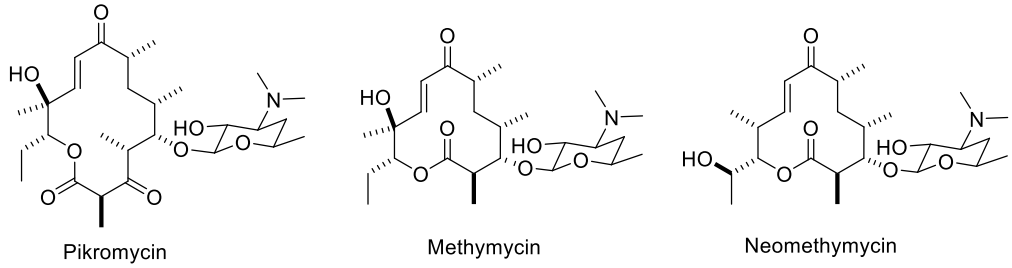


Figure 1.3. Major final products of the pikromycin pathway.

1.5.2 Pre-oxidation enzymatic tailoring of the pikromycin pathway natural products

The structural complexity of the macrolide structure stems from three distinct steps in the biosynthesis. The first step is polyketide synthesis and macrolactonization, mediated by modular polyketide synthases (PKS).[46] Following PKS processing, one or more sugars are attached to the macrolactone by the action of glycosyltransferases. Then at a late stage in the biosynthesis, additional oxygen functionalities, generally in the form of hydroxyl, aldehyde or epoxide groups, are added by P450 monooxygenases. The Pik gene cluster encodes for the enzymes involved in the biosynthesis of the pikromycin pathway natural products (*Figure 1.4*). Within Pik, the PikA cluster codes for the PKS system responsible for the construction of the aglycone ring systems. The PikB or Des cluster encodes for the enzymes responsible for the biosynthesis of the desosamine sugar these macrolactones are attached to and the glycosyltransferases that mediate the glycosylation step. PikC encodes for a P50 monooxygenase responsible for hydroxylation and lastly, PikD is a regulatory gene.[49]

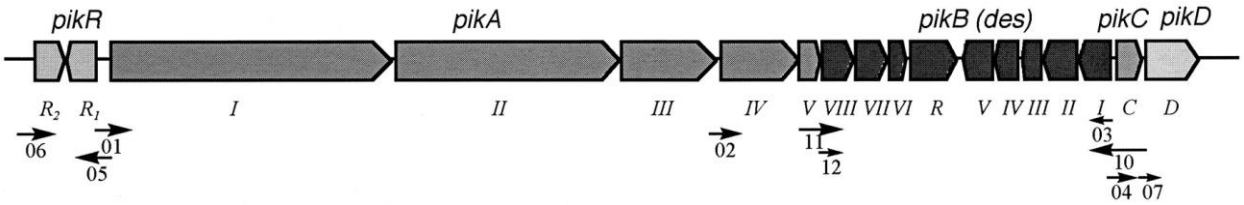


Figure 1.4. The Pik gene cluster.[49]

PikA is the PKS system responsible for the synthesis of the macrolactone rings 10-deoxymethynolide and narbomycin (*Figure 1.5*). Modular PKS are large multifunction and multienzyme complexes that catalyze the synthesis of the carbon backbone of polyketide natural products from activated derivatives of keto-containing acetate, propionate and butyrate building blocks, giving this class of natural products its name.[50,51] The first four polypeptides of the PikA PKS (PikAI-PikAIV) are divided into modules and are responsible for elongation of the linear

polyketide chain. The thioesterase (TE) at the end of PikAIV mediates the final macrocyclization step in the synthesis of the macrolactones. The last polypeptide, PikAV consists of a second thioesterase (TEII) whose suggested function is to remove aberrant chain units, to prevent blockage of the PKS assembly line. The PikA system produces two different macrolactone rings, the 14-membered narbonolide and the 12-membered 10-deoxymethynolide (*Figure 1.5*), which makes it unique from other PKS systems.

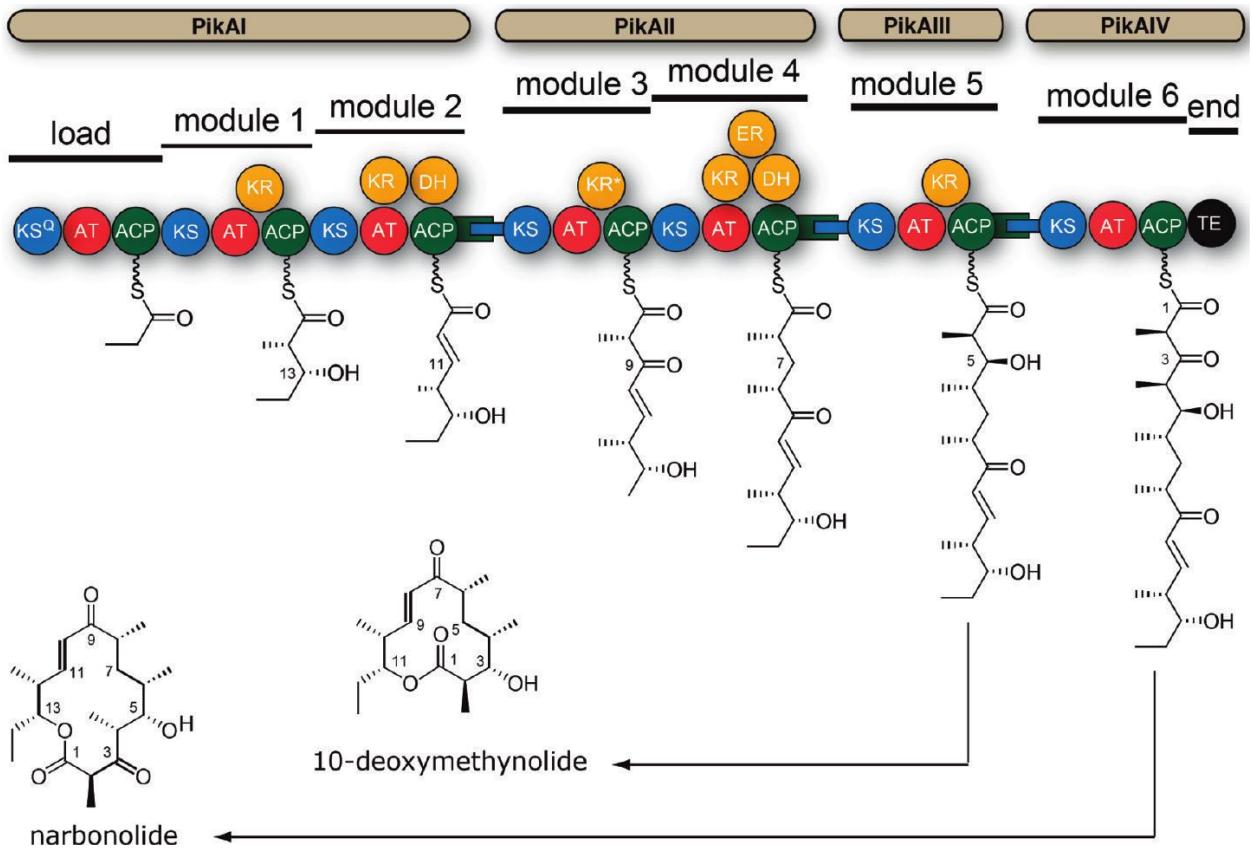


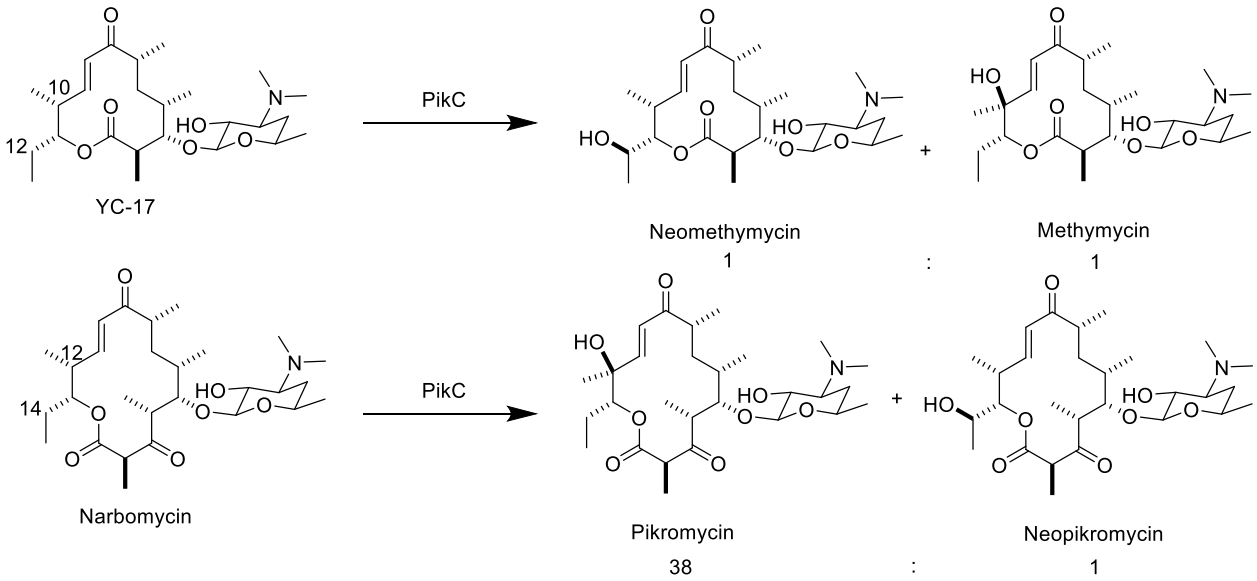
Figure 1.5. Pikromycin polyketide synthase.[52]

The step following the ring formation is the appendage of the sugar, desosamine. The Des gene cluster codes for the desosamine biosynthesis genes and the enzymes responsible for glycosylation. The final desosamine donor, thymidine diphosphate D-desosamine is synthesized from glucose-6-phosphate.[53] The promiscuous glycosyl transferase DesVII, which bound to its

auxiliary partner protein DesVIII, glycosylates both 10-deoxymethynolide and narbonolide to give YC-17 and pikromycin respectively.[54] The last step in the biosynthesis is the PikC-catalyzed hydroxylation that is described in the following sections.

1.5.3 PikC mediated oxidation of pikromycin and YC-17

PikC is the enzyme responsible for adding the hydroxyl functionality in the pathway following successful glycosylation.[48] PikC stereo- and regioselectively oxidizes 12-membered endogenous substrate YC-17 at the allylic tertiary C-10 to give methymycin, or it can oxidize the exocyclic methylene position from C-12 to give neomethymycin in a 1:1 ratio. PikC also oxidizes 14-membered endogenous substrate narbomycin at the allylic tertiary C-12 to give pikromycin or it can oxidize the exocyclic methylene from C-14 to give neopikromycin. However, production of pikromycin is highly favored over neopikromycin in a ratio of 38:1 (*Scheme 1.11*).[55] PikC shows greater substrate tolerance than most known P450's involved in macrolide hydroxylation because of its capability of oxidizing both 12- and 14-membered macrolides at different positions within the ring system.



Scheme 1.14 Hydroxylated products of YC-17 and narbomycin upon PikC oxidation.

PikC anchors its substrates by engaging in salt-bridge interactions through glutamate residues within the active site and the protonated dimethylamino group from desosamine.[56] Co-crystal structures of PikC with endogenous substrates YC-17 and narbomycin revealed that these substrates bind to two distinct buried and surface-exposed pockets within PikC (*Figure 1.6*). In the narbomycin structure, the protonated dimethylamino group is positioned between Glu-85 and Asp-50 in the exposed pocket, whereas with YC-17 it is positioned between Glu-85 and Glu-94 in the buried pocket.[56] The crystal structures also reveal that the interactions between the macrolactone rings within the PikC active site are entirely hydrophobic and non-specific, which can account for the substrate flexibility exhibited by PikC. These structures also place the sites of oxidation C10 and C12 positioned at a reasonable distance from the heme iron. Replacing Asp-50 within the PikC binding site with asparagine (D50N mutant) enhances catalytic activity because this residue functions as a gate to access the buried catalytically active pocket.[57]

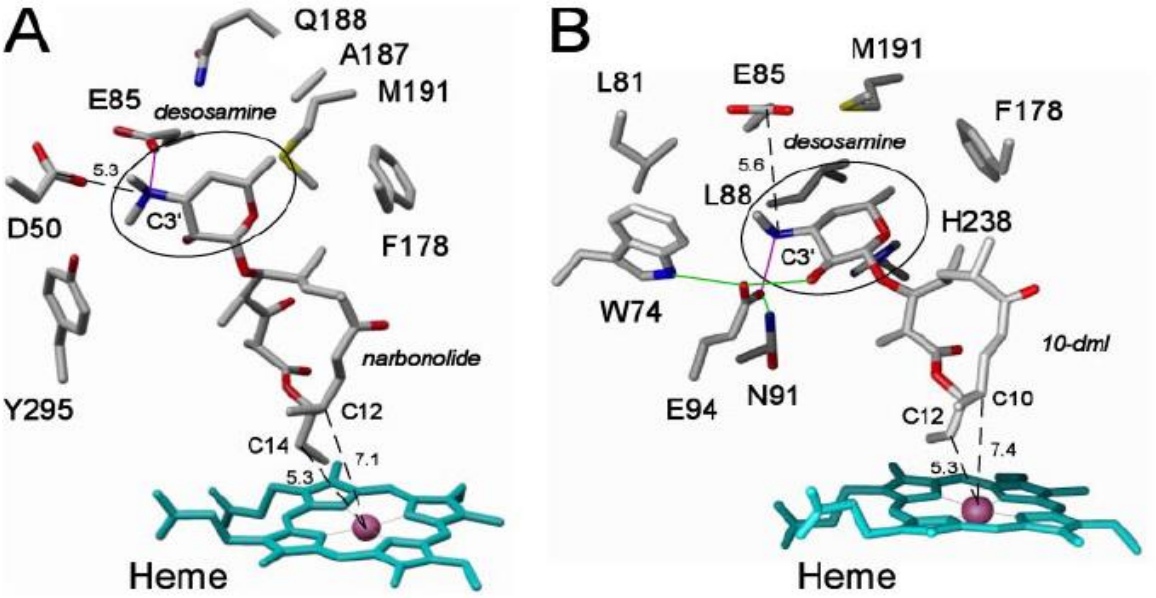


Figure 1.6. Co-crystal structures of YC-17 and narbomycin in the PikC active site.[56]

1.5.4 Replication of PikC activity *in vivo* and *in vitro*

The D50N mutant was chosen to replicate PikC activity for further *in vitro* experiments as it increases binding affinity of narbomycin and YC-17.[57] In order to use PikC *in vitro* in the laboratory, expensive exogenous spinach ferrodoxin and spinach ferrodoxin reductase were needed, as PikC's endogenous reductase partners were unknown. The discovery of a novel class of bacterial P450's that are fused to reductase partners inspired a solution to make the use of PikC *in vitro* more cost-effective. The cytochrome P450RhF from *Rhodococcus sp.* is fused by a single polypeptide chain to a reductase domain comprised of a flavin mononucleotide containing reductase, NADPH binding domains Fe_2S_2 ferrodoxin like center (RhFRED).[58] $PikC_{D50N}$ was fused to the aforementioned reductase domain RhFRED from *Rhodococcus sp.* which resulted in similar substrate binding affinity and increased catalytic activity for endogenous substrates, the latter due to stabilization of the interaction between PikC and the reductase partner which in turn enhanced electron transfer efficiency.[59]

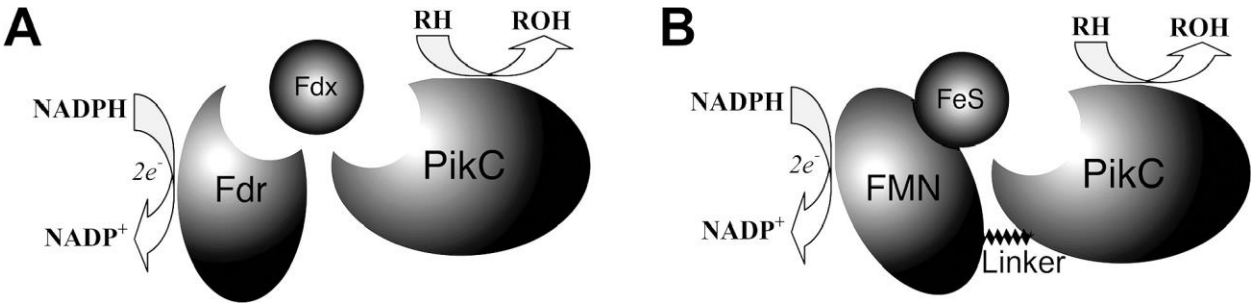


Figure 1.7. A three-component PikC system B. One-component PikC system.[59]

1.6 Summary and outlook

Chemists have worked extensively in developing transition metal catalysts to perform selective functionalization reactions of unactivated sp^3 C–H bonds. Nature's catalysts, the previously described cytochrome P450 enzymes, catalyze such reactions with high levels of regio- and stereo-selectivity. In the case of unactivated methylenes, enzymes remain unchallenged though these are arguably the most difficult bonds to functionalize in a selective manner. This thesis describes efforts towards the development of the macrolide P450 $PikC_{D50N}$-RhFRED as a biocatalyst for the selective hydroxylation of sp^3 C–H bonds.

Chapter II describes desosamine as an anchoring group for the moderately regioselective hydroxylation of carbolides (desosamine linked carbocyclic rings) from previous efforts from collaboration between the Podust-Montgomery-Sherman research groups. Replacement of desosamine with linkers containing other alkyl amine-containing anchors for the selective hydroxylation of unnatural substrates follows. In addition, the chapter describes how modifications in the linker to the amine group can influence the regioselectivity of hydroxylation on the 10-dml core.

Chapter III documents the efforts to determine the minimal structural requirements in the aglycone that facilitate highly regioselective and stereoselective hydroxylation of macrolide

analogues and how combined with modifications in the linker to the amine group the selectivity of hydroxylation can be modulated. A sample of macrolactones with varying degrees of functionality and anchoring groups are evaluated against $PikC_{D50N}$-RhFRED.

Finally, chapter IV describes the expansion of the $PikC_{D50N}$-RhFRED alkyl amine anchor mediated hydroxylation methodology substrate scope to include small rings. The active site of PikC was naturally engineered for 12- and 14-membered macrolactones but with appropriate selection of linker size, small bicylic systems and 6-membered cycloalkane substrates can be oxidized in high yields and with high selectivities.

Chapter II: Replacing desosamine with simplified removable anchoring groups for unnatural substrates

2.1 Introduction: PikC mediated oxidation of carbolides

The PikC catalyzed hydroxylation of YC-17 and narbomycin exemplifies one of the most challenging reactions for synthetic organic chemists: regio- and stereoselective oxidation of unactivated C–H bonds. A promising approach for the development of useful C−H oxidation procedures involves employing cytochrome P450 monooxygenases as bio-catalysts given their capacity to perform difficult oxidations in a regio- and stereoselective manner. Due to its innate substrate flexibility and distinct substrate anchoring mode, PikC promised to be an excellent candidate for bio-catalysis. PikC has been engineered as a self-sufficient macrolide biosynthetic P450 monooxygenase ($PikC_{D50N}$-RhFRED).

To probe $PikC_{D50N}$-RhFRED's ability to oxidize unnatural substrates, the desosamine anchor present in PikC's endogenous substrates YC-17 and narbomycin, was attached to a variety of carbocyclic and linear alcohols. These substrates were evaluated against $PikC_{D50N}$-RhFRED for hydroxylation reactions.[60] A number of these carbolides (desosamine linked carbocyclic alcohols) were oxidized to multiple monohydroxylated products in moderate yields. As in the case of the endogenous substrates, the hydroxylation occurred at positions most distal from the desosamine anchor. Figure 2.1 illustrates the substrate scope of the reaction.

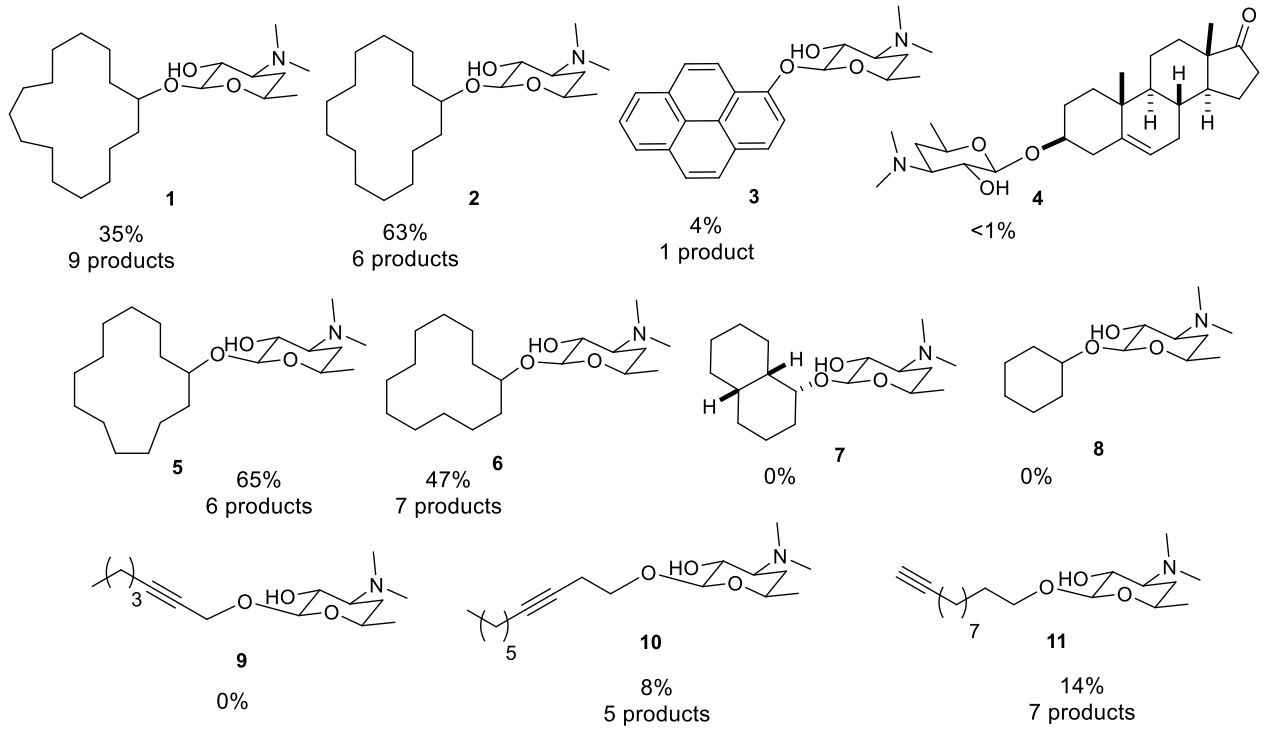
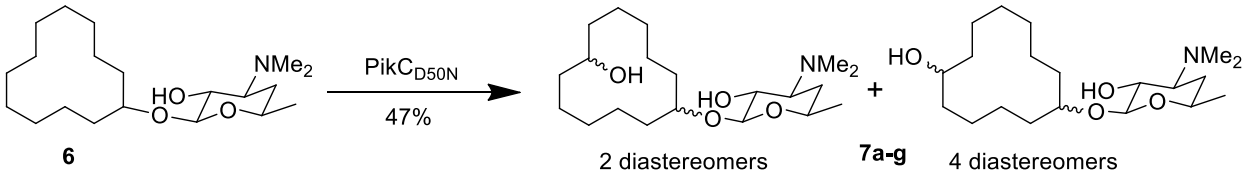


Figure 2.1. Carbolide substrates initially evaluated for $PikC_{D50N}$-RhFRED oxidation.

Monocyclic carbolides similar in size to PikC's endogenous substrates achieved the highest levels of oxidation (substrates **1, 2, 5, 6**). Large bicycles such as steroid derivative **4** and pyrene derivative **3** showed poor reactivity. Linear derivatives **9-11** displayed low levels of conversion upon treatment with $PikC_{D50N}$-RhFRED. Desosaminyl derivatives of smaller ring systems, such as cis-decalin and cyclohexanol substrates, did not undergo oxidation with $PikC_{D50N}$-RhFRED. We hypothesized these substrates were not large enough to reach the heme iron and undergo oxidation.

Achieving high levels of regioselectivity and stereoselectivity for hydroxylation of unnatural substrates has been challenging. Oxidation of a single compound generally occurred at several closely spaced sites, and most unnatural substrates yielded a number of different products in the $PikC_{D50N}$-RhFRED oxidation, indicating that the hydroxylation is not limited to

one site. It was determined that the region of carbolide **6** in which oxidation occurs is similar to that of YC-17, but yielding seven products as analyzed by LC-MS (six isomeric products were confirmed). The reason for this large number of products is believed to be due to the non-specific interactions inside the binding pocket and the greater degree of conformational freedom that this unnatural substrate displays at the binding site since it is composed entirely of sp^3-hybridized centers. A co-crystal structure of this substrate within the $PikC_{D50N}$ active site was obtained, it revealed carbons C-5, C-6, C-7 and C-8 to be closest to the heme iron center and suggests these are likely hydroxylation sites. The hydroxylation was confirmed to occur at the C-6, C-7 and C-8 positions by synthesis of authentic samples and comparison of retention times and co-injection of the authentic sample with the LC-MS $PikC_{D50N}$-RhFRED product mixture.



Scheme 2.1. Major monohydroxylated products of the $PikC_{D50N}$-RhFRED reaction with carbolide **6**.

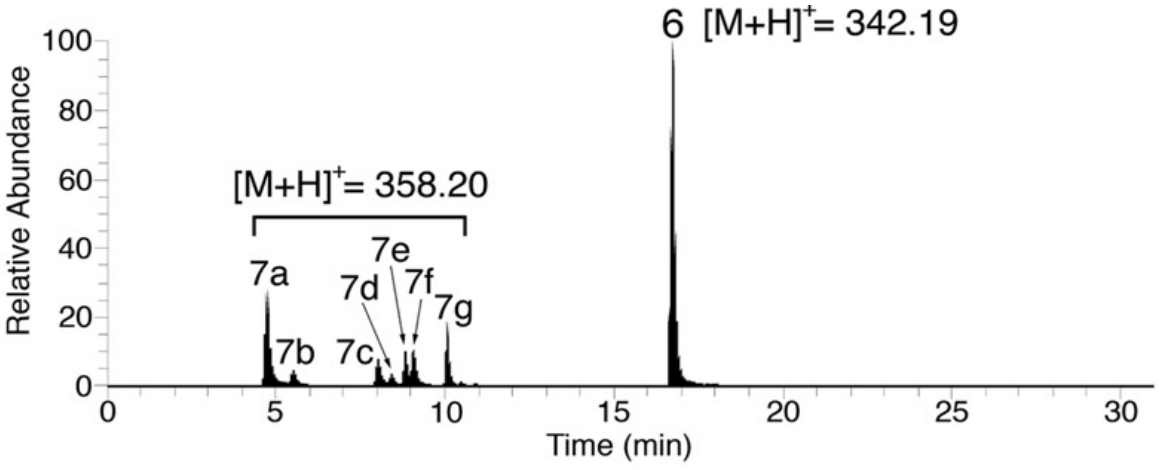
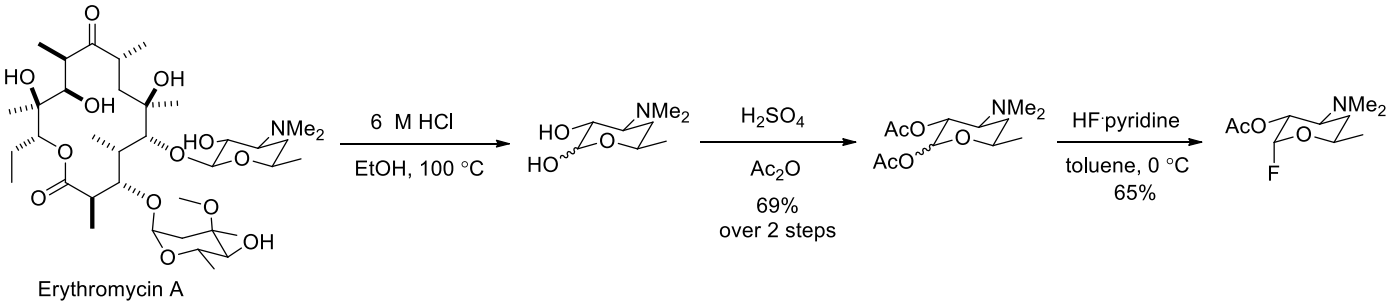


Figure 2.2. LC-MS analysis of the $PikC_{D50N}$-RhFRED reaction with carbolide substrate **6**.[60]

The goals of the work in this thesis are aimed towards expanding the substrate scope of the $PikC_{D50N}$-RhFRED hydroxylation of unnatural substrates, applying this enzymatic reaction as a synthetic tool for the selective oxidation of C–H bonds. Through substrate engineering we hoped to optimize the regioselectivity and stereoselectivity of the oxidation and also explore substrate anchoring groups that are easier to synthesize and cleave from the substrates in order to expand the usefulness of the procedure. This chapter describes the engineering of unnatural substrates for oxidation containing the same amino anchor but employing simpler linkers to the anchor amine instead of the desosamine sugar and also investigates the effects that the linkers have in the reaction selectivity.

2.2 Replacing desosamine with simple ethers and esters with a terminal dimethylamino group

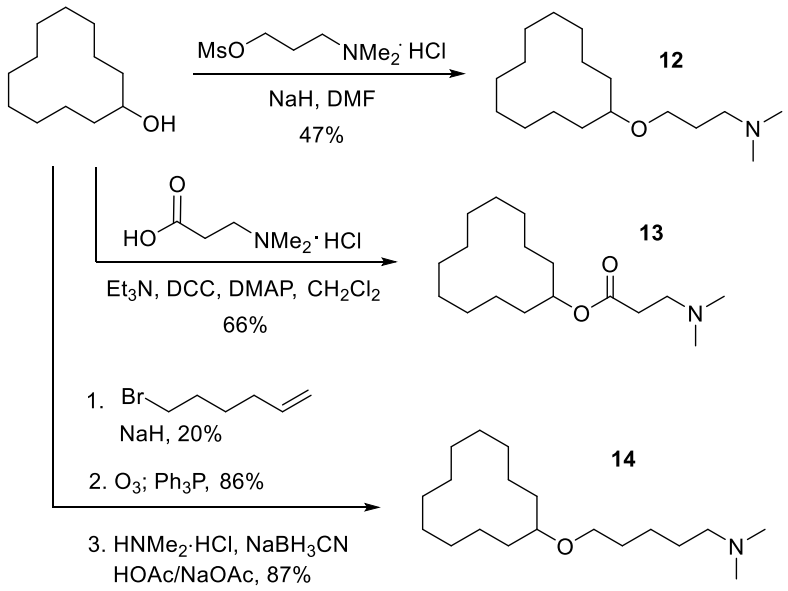
Desosamine served as an effective anchoring group for the hydroxylation of unnatural substrates, however, it limited the substrate scope to molecules containing this moiety as removal of the glycosidic bond with desosamine is challenging. Also the use of desosamine requires harvesting of the sugar from erythromycin A, acetylation and fluorination to generate the glycosyl donor desosamine fluoride (DesF, *scheme 2.2*).[61,62] Alternatively, desosamine could also be synthesized in nine steps from commercially available materials.[63] DesF was coupled to macrocyclic alcohols with $BF_3{\cdot}OEt_2$ as a promoting Lewis acid in moderate yields.[60] Therefore increasing generality and utility of the PikC-catalyzed hydroxylation methodology required simpler, removable anchoring groups that additionally possess characteristics that facilitate substrate binding.



Scheme 2.2. Synthesis of desosamine from erythromycin A.

2.2.1 Synthetic attachment of unnatural anchors

Substrates containing anchoring groups other than desosamine have been evaluated for $PikC_{D50N}$ oxidation. Compound **12** was synthesized through an S_N2 reaction of cyclododecanol with 3-(dimethylamino)propyl methanesulfonate. It contains an *N*,*N*-dimethylaminopropanoxy moiety as the anchoring group (*Scheme 2.3*). To increase length of the linkage between the *N*,*N*-dimethylamino moiety and the substrate, compound **14** containing a longer ether linker was synthesized in three steps. The first step is an alkylation of cyclododecanol with 6-bromo-1-hexene. The terminal olefin of the ether was oxidized to an aldehyde through an ozonolysis and the resulting aldehyde transformed to the *N*,*N*-dimethylamino containing substrate through a reductive amination. Finally, a third linker was employed to produce substrates in which the cleavage of the anchoring group from the oxidized products would be easier, moving towards the goal of making the PikC oxidation of unnatural substrates a useful C−H oxidation procedure. An example of such substrates is **13**, which was synthesized through a dicyclohexylcarbodiimide (DCC) mediated coupling between cyclododecanol and *N*,*N*-dimethyl-β-alanine.



Scheme 2.3. Forward syntheses of unnatural linker substrates.

2.2.2 Results from $PikC_{D50N}$-RhFRED oxidation of cycloalkyl ether and alkyl ester derivatives

Several of these unnatural linker substrates showed promising results upon $PikC_{D50N}$-RhFRED oxidation (*see table 2.1*). A range of substrate conversions and varying degrees of regioselectivities were observed using ether- and ester-based attachment of linkers to the terminal *N,N*-dimethylamino anchor in unnatural substrates. Compared to the desosamine-fused cyclododecane **6**, we observed a decrease in yield for the oxidation reaction of cyclododecanol linked to a 3-*N,N*-dimethylaminopropanoxy group in substrate **12** (*Figure 2.1 and table 2.1*). While with the same anchor, cyclopentadecanol derivative **15** afforded improved yields in comparison to its desosamine counterpart (*Figure 2.1 and table 2.1*). Therefore the effects of reaction efficiency with this 3-*N,N*-dimethylaminopropanoxy anchor is dependent on cycloalkane size. Also linker length within the same macrocycle scaffold had a considerable effect on conversion as evidenced by **14** that has a 5-*N,N*-dimethylaminopentanoxy anchor (*see*

table 2.1). With the longer linker between the *N*,*N*-dimethylamino anchor in **14**, high reactivity was achieved in comparison to **12** and is now comparable to the levels exhibited by desosaminyl derivative **6**. This result indicates that we can influence the efficiency of the oxidation by modifying the linker length.

In terms of regioselectivity, these substrates appear to show decreased number of products in comparison to desosamine derivatives. Whereas seven products were observed in oxidations of **6**, comparisons must consider that diastereotopic protons in **6** (due to desosamine chirality) become enantiotopic when achiral linkers are employed. This indicates that the hydroxylation reactions with the unnatural anchors might be less selective. Decreased conversion and regioselectivity of oxidations of the simple cycloalkane-derived substrate **12** compared to the macrolactone system was likely attributed to suboptimal substrate binding affinity in the PikC active site due to a loss of interactions between the PikC active site and desosamine, while retaining the salt-bridge between E94 and the dimethylamino group necessary for oxidation.

The presence of an ester group linking the dimethylamino moiety and the substrate, increased yield moderately in the case of cyclododecane derivative **13** in comparison to the ether substrate **12** with the same linker length. This ester did not appear to have an effect on substrate conversion for cyclopentadecane derivative **15**. However, the ester group did decrease the number of products. A more rigid ester such as *p*-benzylic amine substituted benzoate also displayed a very mild reduction in the number of oxidation sites (substrate **13** compared to **17**). This result shows that the desosamine sugar moiety is not essential for PikC to be able to bind its substrates. In this case a linear chain linker still containing the a terminal *N*,*N*-dimethylamino group, which is necessary to form a salt-bridge interaction at the $PikC_{D50N}$-RhFRED active site, was sufficient for oxidation.

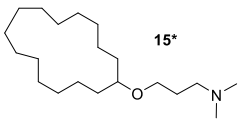
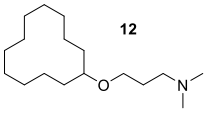
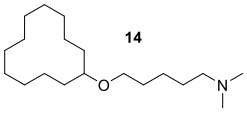
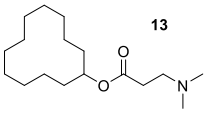
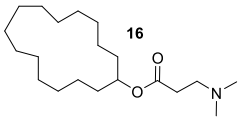
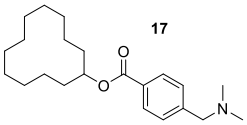
Substrate	Yield (%)	Number of products	TTN
 15*	52	7	397
 12	25	5	134
 14	56	5	578
 13	45	5	385
 16	52	5	519
 17	44	4	195

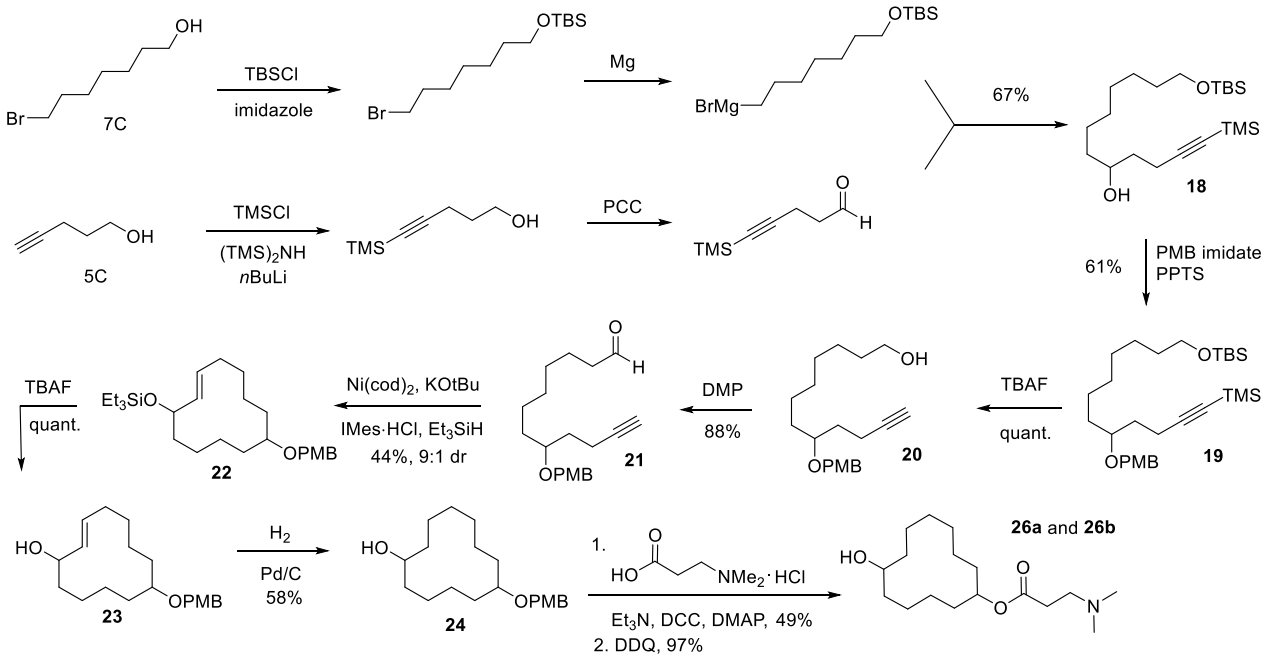
Table 2.1. Unnatural linker substrates evaluated against $PikC_{D50N}$-RhFRED. (*Compound originally synthesized by Allison, R. Knauff, Enzymatic reactions and TTN by Alison R.H. Narayan)

To measure $PikC_{D50N}$-RhFRED efficiencies with these unnatural substrates, total turnover number (TTN) measurements were carried out. The 5 μM concentration of the enzyme in standard reaction conditions was reduced to 1 μM. The TTN's correlated very well with the percent conversions observed. The reacting sites of hydroxylation were presumed to be similar, if not the same, to those of desosaminyl derivatives (sites most distal from the dimethylamino

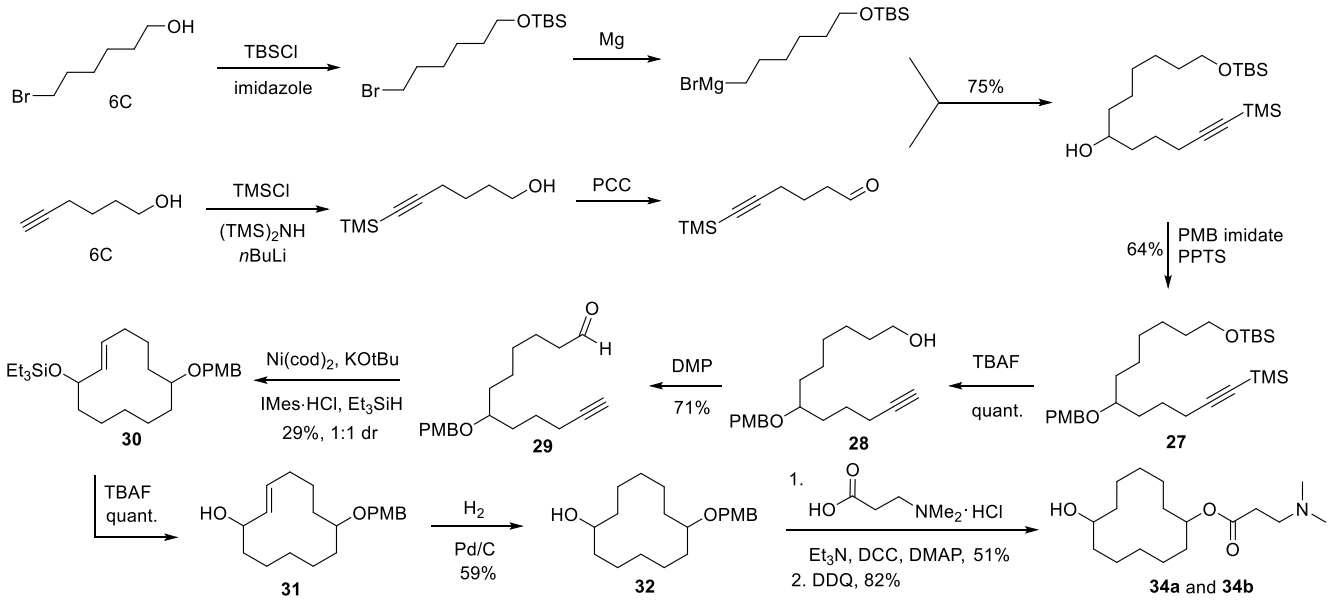
anchor). Characterization of reaction products with select substrates is described within the next section.

2.2.3 Characterization of PikC$_{D50N}$-RhFRED reaction products

For product confirmation of the cyclododecanol unnatural ester derivative **13,** authentic samples of the likely hydroxylated products were synthesized for LC-MS co-injection with the PikC$_{D50N}$-RhFRED reaction mixture The synthesis of these diols draws from the work carried out by previous Montgomery lab member Mani Chaulagain for the identification of carbolide **6** hydroxylated products.[62] Scheme 2.4 shows the forward synthesis for C6/C8 monohydroxylated products. The synthesis began with acid-promoted *p*-methoxybenzyl (PMB) protection of the racemic alcohol **18**, synthesized by a Grignard reaction.[64] It was accomplished with pyridinium *p*-toluenesulfonate (PPTS) and PMB trichloroacetimidate and generated PMB ether **19**.[60] Global silyl deprotection with tertabutylammonium fluoride (TBAF) generates alcohol **20**. The subsequent Dess-Martin periodinane (DMP) oxidation generates the aldehyde. The resulting ynal **21** was cyclized regioselectively employing Ni/ IMes catalyzed aldehyde-alkyne reductive coupling to generate a 9:1 mixture of diastereomers.[65,66] The diastereomers were not separated and carried through as a mixture for the remaining steps. Allylic silyl-ether **22** was treated with TBAF, followed by a hydrogenation to yield reduced monoprotected alcohol **24**, which was coupled with *N*,*N*-dimethyl-β-alanine to generate ester **25**. Finally, the PMB ether was cleaved using DDQ as the oxidant to yield hydroxylated standards **26a** and **26b** (*cis* and *trans* pair). The synthesis of the C7 monohydroxylated product series only differs in that 6-bromo-1-hexanol and 5-hexynyn-1-ol were employed as the initial starting materials instead of the staring materials shown in Scheme 2.5 and generates a diastereomeric pair of hydroxylated standards **34a** and **34b**.



Scheme 2.4. Forward synthesis for C6/C8 monohydroxylated products from PikC$_{D50N}$-RhFRED oxidation of substrate **13**.



Scheme 2.5. Forward synthesis for C7 monohydroxylated products from PikC$_{D50N}$-RhFRED oxidation o substrate **13**.

The identity of the hydroxylated products from the PikC$_{D50N}$-RhFRED reaction of substrate **13** is confirmed by comparison of their LC-MS retention times of these with the retention time of the authentic hydroxylated samples as well as co-injections with the PikC reaction mixture. The retention times of the authentic hydroxylated standards correlated with three of the four observed peaks in the LC-MS (*Figure 2.3*). The compounds were synthesized as diastereomeric pairs bearing the cis/trans relationship between the ester and hydroxyl substituents. One of the diastereomers from the C6/C8 set co-eluted with one of the C7 diastereomers. This was similar to the regioselectivity of hydroxylation in the corresponding desosaminyl analog, in which six of the seven hydroxylated products corresponded to oxidation at C6, C7 and C8. The unaccounted for peak in the LC-MS trace probably corresponds to another regioisomer, possibly for oxidation at C5.

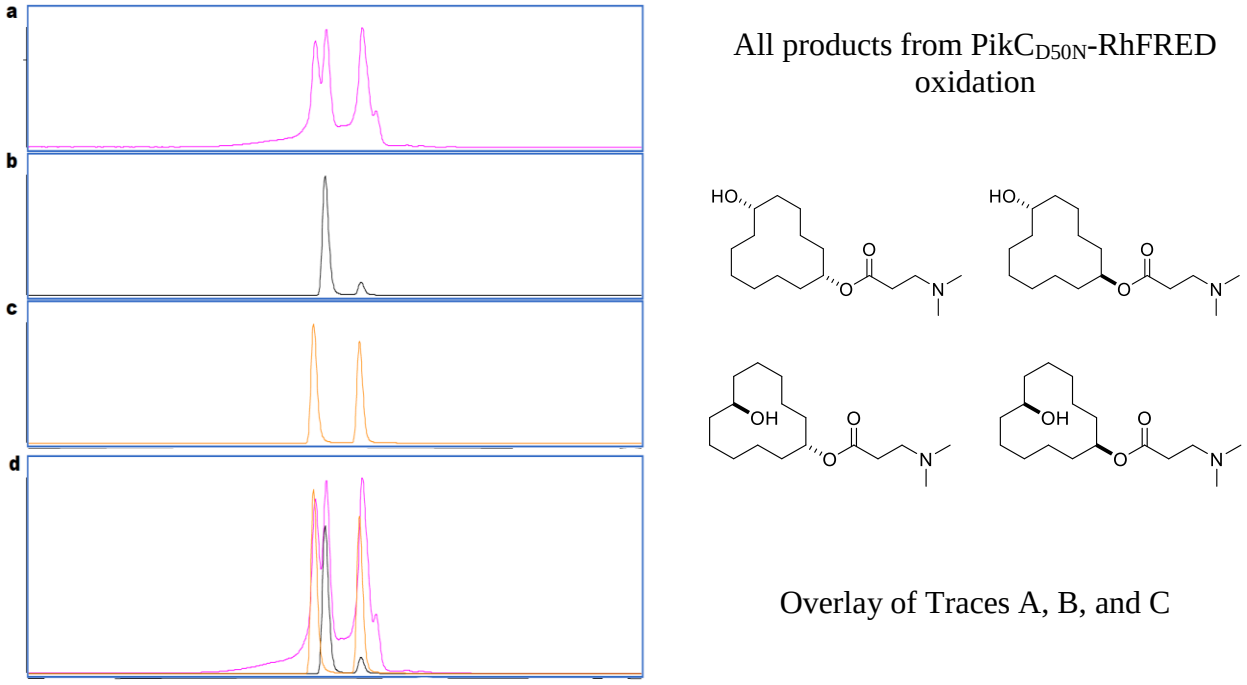


Figure 2.3. Structural determination of monohydroxylated products of PikC$_{D50N}$-RhFRED reaction with substrate **13**. (a) Product profile from PikC$_{D50N}$-RhFRED oxidation (b) Authentic standard of pair of C-6/C-8 hydroxylated diastereomers (c) Authentic standard pair of C-7

hydroxylated diastereomers (d) Overlay of hydroxylated diastereomers and product profile. (With Karoline C. Chiou)

2.2.4 Co-crystal structure of cycloalkyl derivative in the Pik_{D50N} active site

The only cycloalkane derivative that was able to be structurally characterized within the enzyme active site was the five carbon ether derivative of cyclododecanol **14**, which was represented by a 2.7 Å structure (PDB ID 4BF4, *figure 2.4*). A co-crystal structure of substrate **14** with $PikC_{D50N}$ revealed an electrostatic salt-bridge interaction between the Glu-94 residue in the active site and the protonated dimethyl amino group from the substrate. The carbocycle part of the molecule appears poorly defined due to the ring sampling several different conformations in the active site, as was observed in the co-crystal structure of carbolide **6**, and the C-H bonds more distal from the anchoring group locate themselves 3-4 Å away from the heme iron, in a position favorable for oxidation, which suggests C6, C7 and C8 monohydroxylated compounds as likely products from the oxidation.

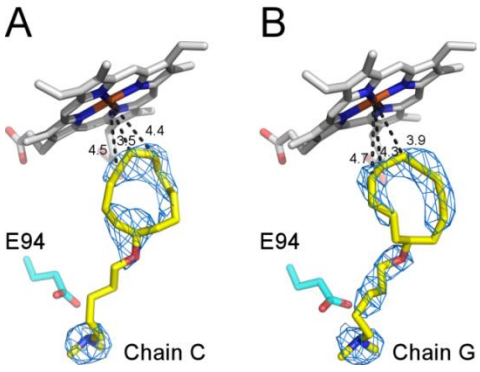


Figure 2.4. Co-crystal structure of ether **14** within the Pik_{D50N} active site.

Our studies on the carbocyclic substrates above demonstrated that the ester functionality offered an attractive alternative to desosamine attachment by providing an easily attachable,

chemically labile and effective anchoring group. However these esters did not have a substantial effect in the selectivity of the reaction. Thus, we pursued ester-containing linkers to probe the effect of anchoring group structure on the regioselectivity of targeted C-H bonds in the endogenous macrolactone systems of the PikC pathway.

2.3 10-deoxymethynolide and narbonolide core substrates with unnatural anchoring groups

2.3.1 Isolation of narbonolide and 10-deoxymethynolide

During a sabbatical stay in the Sherman laboratory, part of the proposed work finished was the isolation of the macrolide biosynthetic intermediates narbonolide and 10-deoxymethynolide (10-dml) which are generated after the chain elongation and cyclization steps catalyzed by the Pik-PKS and thioesterase respectively (*Figure 1.5*).[46] These intermediates contain the free hydroxyl group to which the desosamine sugar is attached in a subsequent glycosylation step by DesVII/DesVIII and can serve as useful starting materials for further transformations, such as attachment of different anchoring groups other than desosamine. The intermediates narbonolide and 10-dml were isolated from a *S. venezuelae* mutant Kdes1, in which the genes DesI is deleted, inhibiting desosamine biosynthesis. Without this gene, attaching desosamine to these macrolactone intermediates is not possible, as desosamine biosynthesis is disrupted.[67] This deletion renders the organism practically incapable of modifying narbonolide and 10-deoxymethynolide any further because PikC cannot oxidize the unglycosylated macrolactones, and inhibiting the completion of the synthesis of the final products, therefore making it possible to isolate the desired aglycone intermediates upon growing the Kdes1 strain of the organism.

2.3.2 Hydroxylation of unnatural YC-17 and pikromycin derivatives by PikC

Both YC-17 and narbomycin derivatives containing ester anchoring groups were synthesized through DCC couplings with the corresponding amino acid. The LC-MS trace of the $PikC_{D50N}$-RhFRED reaction of narbonolide derivative **35** showed good conversion and similar product ratio to oxidation of pikromycin (*results in table 2.2*). 10-dml derivatives **36**, **37** and **38**, employing *N*,*N*-dimethyl glycine, *N*,*N*-dimethyl-β-alanine and 4- *N*,*N*-dimethylamino butanoate respectively as the anchors, showed that these compounds were almost entirely converted into products. Only two peaks were observed in the LC-MS trace of these reactions, as is the case for endogenous substrate YC-17. However the ratio of these products shifted to approximately 1:1.6 for substrate **36**, 1:2.1 for substrate **37** and 1.8:1 for substrate **38**, whereas in YC-17 the ratio is 1:1. The products were determined to be methymycin and neomethymycin analogs, with hydroxylation occurring at the tertiary allylic position (C-10) and secondary exocyclic methylene (C-12), respectively. In **36** and **37** the major product was the neomethynolide analog whereas the longer linear linker in substrate **38** switched the regioselectivity to oxidation primarily at C-10 to give the methymycin analog as the major product (*details on the identification of reaction products will be provided in the following section*). These results highlight the importance of the anchoring group on the selectivity of the oxidation and presented opportunities to further exploit this strategy.

Evaluation of these endogenous rings containing unnatural linkers in the $PikC_{D50N}$-RhFRED oxidation assays yielded insights into the role of desosamine in the selectivity of the oxidation of the endogenous substrates. The C–C bonds present in these linear linkers are able to rotate freely, unlike those in desosamine which are constrained in a ring. In the case of the 10-deoxymethynolide ring system, the product ratio was moderately affected by these ester

anchoring group substrates, proving the different conformation this linear linkers adopt can have an effect on selectivity.

To further expand the substrate scope and the ability to manipulate the hydroxylation sites substrates containing aromatic anchoring groups were synthesized. We envisioned being able to change the substitution pattern of an *N*,*N*-dimethylamino functionality on a benzene ring, and therefore modify the regioselectivity of C–H oxidation. We were pleased to find a more dramatic enhancement of the hydroxylation regioselectivity by employing differentially substituted ester-linked benzylic amines as the anchors. The aforementioned derivatives, **39**-**40** were converted into products in excellent yields. Interestingly *ortho* benzylic amine derivative **39**, in which the ester carbonyl group is the same number of carbon atoms away from the *N*,*N*-dimethylamino as in derivative **38**, afforded a reversal of regioselectivity to favor C-12 hydroxylation. Increasing the distance between the ester carbonyl and the terminal *N*,*N*-dimethylamino with *meta* and *para* derivatives **40** and **41** afforded highly regioselective hydroxylation at C-10 with up to 20:1 selectivity for derivative **40**. We hypothesize that these more rigid anchors restrict the conformational freedom of the substrate in the active site thereby enhancing the selectivity of the oxidation, as evidenced by the >10:1 product ratio exhibited by these longer linkers in derivatives **40** and **41**. These anchors place the more favorable oxidation site, the electronically activated 3° allylic C-H bond at C-10 much closer to the heme iron, compared to **36**, **37** and **39**, even though C-12 of compounds **40** and **41** may be closer at such distance from the iron(IV)oxo, stereoelectonic effects in the substrate predominate in determining the site of hydroxylation.

Additionally, 10-dml derivatives attached to both enantiomers of *N*-methylproline **42** and **43** also confirm that not only the linker length and rigidity but also stereochemical configuration

affects the selectivity of the oxidation. These derivatives afforded C-10 hydroxylation as the major product contrasting the observation that for substrate **36**, containing a methylene spacer between the ester carbon and the dimethylamino group, hydroxylation of C-12 was slightly favored over C-10 hydroxylation. Also we were very surprised to find that these derivatives afforded a third novel site of hydroxylation, presumably methyl oxidation on the vicinity of the C-10/C-12 region. Efforts to characterize this third, novel oxidation product are still underway. Finally, a 10-dml was attached to nicotinic acid and evaluated against PikC_{D50N}-RhFRED to yield predominantly one monohydroxylated product in modest yields (around 50%), suggesting that pyridine can also serve as an anchor but not as effectively as alkyl amines.

The efficiency of PikC_{D50N}-RhFRED with these unnatural 10-dml derivatives was also assessed by TTN measurements and dissociation constant (K_d) measurements. The TTN values observed were lower than YC-17 (896) but much higher than cyloalkane substrates (*table 2.1 and 2.2*), which indicates reduced efficiency of the enzyme with unnatural substrates, but some was recovered with addition of the 10-dml core. Additionally K_d values observed for 10-dml derivatives were higher than YC-17 as expected.

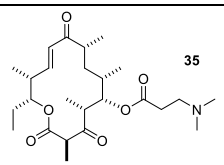
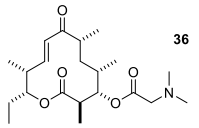
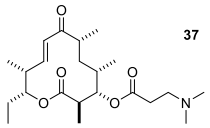
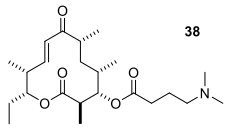
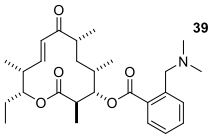
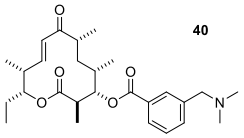
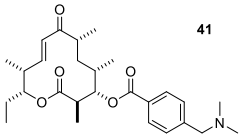
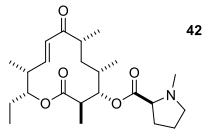
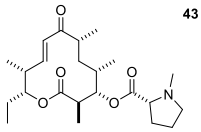
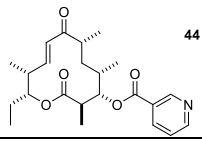
Substrate	Yield (%)	Isolated Yield (%)	Number of products	C10:C12:other	TTN	K_d
 35	76	-	2	20:1	-	-
 36	80	46	2	1:1.6	260	118
 37	99	63	2	1:2.1	544	51.6
 38	91	89	2	1.8:1	352	143
 39	99	43	2	1:4	152	ND
 40	99	74	2	20:1	580	ND
 41	99	63	2	10:1	602	ND
 42	94	58	3	3.7:1.9:1	485	46.2
 43	94	56	3	2.5:1:1.3	456	37.6
 44		~50	1	-	-	-

Table 2.2. Endogenous aglycone unnatural anchoring group derivatives evaluated against $PikC_{D50N}$-RhFRED. (With Alison R.H. Narayan and Jessica Stachowski)

Furthermore, 10-dml attached to linkers that do not contain the amino group were not accepted as substrates for $PikC_{D50N}$-RhFRED, confirming the indispensability of the salt-bridge interaction in the enzyme active site for efficient regioselective hydroxylation. Additionally, 3-*N*,*N*-dimethyl benzoate was coupled to 10-dml and did not show conversion upon treatment with $PikC_{D50N}$-RhFRED confirming that aromatic amines are incapable of serving as anchors. We hypothesized that the reduced basicity of the aniline due to the electron-withdrawing character of the benzoate moiety inhibited protonation under physiological conditions and in turn binding and productive reaction with PikC. Also, most aniline nitrogens do not exhibit the sp^3 hybridization common for alkyl amines as re-hybridization to sp^2 orbitals is required so that the nitrogen lone-pair can participate in the stabilizing electron delocalization within the benzene ring. It is possible also that the sp^2 hybridized nitrogen is placed in an unfavorable orientation with respect to the glutamate residues that participate in the salt-bridge interaction, which can explain the reduced efficiency of pyridine as an anchor.

2.3.3 Crystallographic data of 10-dml derivatives

To establish the substrate binding orientation within the active site, X-ray co-crystal structures of **36**-**38** within the active site of $PikC_{D50N}$ were sought. Structures of **36** and **37** were successfully obtained. We observed the characteristic salt bridge between the *N*,*N*-dimethylamino group of the anchoring group and Glu-94 . Substrate binding modes of **36** and **37** in the active site were comparable to one another as well as to binding observed for YC-17. However, there were important differences in the observed binding modes of our semisynthetic compounds, such as an enzyme-substrate interaction between residue His-238 and the ester carbonyl of the linker in **37** in addition to the salt-bridge . This influenced the orientation of the macrolactone of **37** as it shifted 1.4 Å in the active site compared to YC-17, which we presumed

explained the preference for oxidation of the methylene carbon over the allylic carbon. Also, this methylene C-H is now closer to the heme iron than in the YC-17 structure.

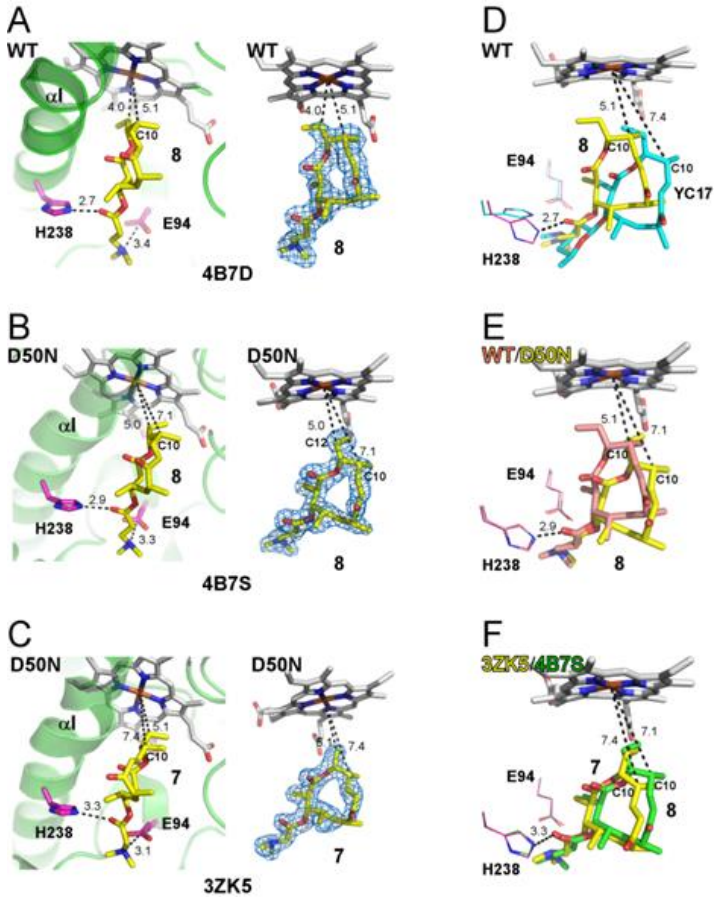


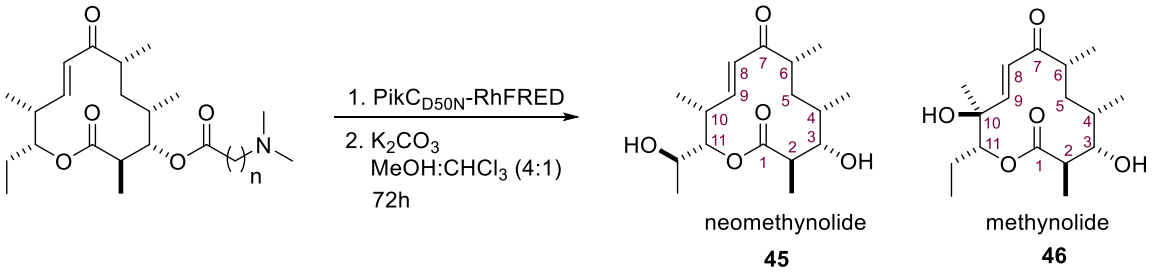
Figure 2.5. Co-crystal structures of **36-37** in the active site of $PikC_{D50N}$/$PikC_{WT}$. (With Larissa M. Podust). (A) Compound **37** with $PikC_{WT}$. (B) Compound **37** with $PikC_{D50N}$. (C) Compound **36** with $PikC_{D50N}$. (D) Superimposition of **37** (yellow)/YC-17(cyan) with $PikC_{WT}$. (E) Superimposition of **37**/ $PikC_{D50N}$ (pink) and **37**/ $PikC_{WT}$ (yellow). (F) Superimposition of **37**/ $PikC_{D50N}$ (green) and **36**/ $PikC_{D50N}$ (yellow).

Based on the hydrogen bond observed in the co-crystal structures of PikC with **36** and **37** between H238 and the carbonyl oxygen of the anchoring group, we were motivated to biochemically probe the role of H238 in PikC catalysis. We hypothesized that if the H-bond

between H238 and the anchoring group carbonyl aided substrate binding, mutation of H238 to a residue unable to participate in hydrogen bonding would increase the K_d's and decrease the TTNs of our unnatural substrates with PikC. The double mutant $PikC_{D50NH238A}$-RhFRED was generated to test this hypothesis, TTN and binding constants for the 10-dml analogs were also assessed (data not shown). However, all TTNs were lower and most K_d's were higher suggesting that H-bond between H238 and the anchoring group carbonyl could exist and exert a stabilizing effect on the substrate-enzyme complexes but it does not play an enhancing role in catalysis.

2.3.4 Product characterization

For the YC-17 and derivatives, ^{1}H-NMR was used to characterize the monohydroxylated products from preparative scale enzymatic reactions. The products of **42** and **43** were assessed by ^{1}H-NMR analysis of the crude $PikC_{D50N}$-RhFRED reaction mixtures. The products from the $PikC_{D50N}$-RhFRED oxidation of the 10-dml esters **36-41** were subjected to ester hydrolysis conditions to yield primarily mixtures of methynolide and neomethynolide, whose structures have been previously obtained by total synthesis, and ^{1}H-NMR and ^{13}C-NMR data for these compounds is available in the literature (*Scheme 2.6*).[63,68-70] The ratios of methynolide and neomethynolide varied according to the linker employed in the substrate. The ^{1}H-NMR spectra stacked plot shows alkene proton signals from the hydrolyzed products corresponding to protons of C8 and C9 (*Figure 2.6*). For the methynolide analog, the characteristic doublet of doublets from C9 disappeared after oxidation at the tertiary allylic position. In the neomethynolide analog, the C9–H signal remained as a doublet of doublets. The C8–H signal corresponded to the C-H adjacent to the carbonyl group and was split into a doublet in both cases. These signals were both present in the ^{1}H-NMR spectra of hydrolyzed PikC reaction products and are shown in a stacked plot format for substrates **36-38**.



Scheme 2.6. Hydroxylated aglycones obtained from hydrolysis of PikC$_{D50N}$-RhFRED reaction mixtures.

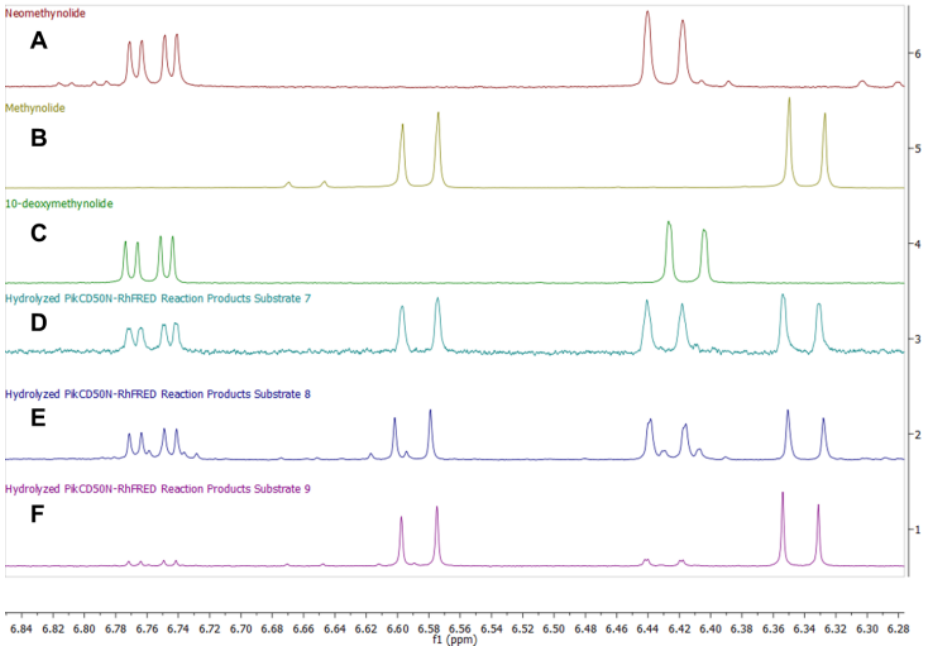


Figure 2.6. Stacked plot of ^{1}H-NMR of hydrolyzed reaction products from substrates 36-38. From top to bottom (1 to 6): A. Neomethynolide, B. Methynolide, C. 10-deoxymethynolide, D. Hydrolyzed PikC$_{D50N}$-RhFRED Reaction Products from substrate **36**, E. Hydrolyzed PikC$_{D50N}$-RhFRED Reaction Products from substrate **37**, F. Hydrolyzed PikC$_{D50N}$-RhFRED Reaction Products from substrate **38**. From left to right: C9–H signal, C8–H signal.

2.4 Conclusions and outlook

The inherent substrate flexibility of PikC was exploited to expand substrate scope using amine containing anchoring groups and the effect of these in controlling the regioselectivity of

the hydroxylation was studied. Moderately regioselective oxidation of remote sites was observed for simple cycloalkane derivatives with unnatural anchors. In the cases of more functionalized systems such as the 10-dml core, anchor size, linear length, rigidity and stereochemistry produced meaningful changes in regioselectivity of C-H bond oxidation, substantiating the substrate-engineering strategy as an effective means of expanding the scope of this P450. By this approach, regions of substrates distal to the alkyl amino functionality were oxidized; rendering the regioselectivity patterns orthogonal to that what can be achieved by either directed or non-directed transition-metal catalyzed oxidations.

Replacement of desosamine in semi-synthetic substrates with a simple and removable group eliminates a number of labor-intensive steps for harvesting and synthetic attachment of desosamine, and enables facile recovery of the oxidized product(s) for additional chemical or enzymatic elaboration. The substrate-engineering approach offers a foothold towards gaining valuable insight into the rules that govern productive substrate binding and conversion of potential P450 substrates, and also towards developing a general method for regio- and stereoselective oxidation of highly complex molecules, including secondary metabolites. This is possible because the products of the enzymatic reactions are readily recoverable and further elaborated towards the development of novel compounds.

Finally, these results underscore the potential of bacterial biosynthetic P450s, such as PikC, to be developed as biocatalysts for the selective oxidation of C-H bonds for diversification of natural products and creation of a broad range of bioactive synthetic molecules. Since this chapter focuses on simple cycloalkanes and the heavily substituted aglycones from the pikromycin pathway, it allowed us to study solely the effects of the anchoring group on

selectivity. The following chapter aims to understand the effects of increasing aglycone functionality in the PikC oxidation.

2.5. Collaborator acknowledgements

Karoline C. Chiou, Ph.D. and Alison R.H. Narayan, Ph.D., from the Sherman laboratory, performed the enzymatic reactions of compounds synthesized. A.R.H.N. initially synthesized compounds **36** and **39**. Jessica L. Stachowski, from the Montgomery laboratory, initially synthesized compound **41**. Allison R. Knauff, from the Montgomery laboratory, initially synthesized compound **15**. Petrea M. Kells, from the Podust laboratory, collected crystallographic data of compounds in the enzyme active site.

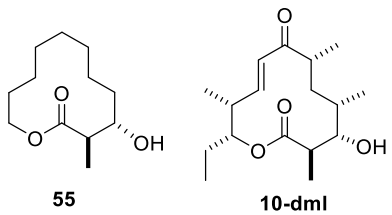
Chapter III: Exploring the structural requirements for selectivity in 12-membered macrolide hydroxylation

3.1 Introduction

In order to effectively employ PikC as a biocatalyst for the hydroxylation of unnatural substrates, a deeper understanding of the factors within the substrate molecule that determine the selectivity is of utmost importance, as it will aid future endeavors of substrate design and will facilitate pairing of a target molecule with the appropriate anchoring group to achieve selective hydroxylation. The previous chapter dealt with the effects the anchoring groups have on the efficiency and selectivity of the oxidation. Anchoring group size, rigidity and stereochemistry had profound effects in the regioselectivity of 10-dml scaffold oxidation but little effect in the case of simple carbocycles. The minimal structural elements within the macrolactone required to induce a high level of regioselectivity had not been yet determined. In a step towards this important understanding, this chapter herein reports progress towards the synthesis of variety of 12-membered macrolide analogues with varying levels of functionality that more closely resemble the endogenous substrate YC-17, along with enzymatic oxidation against $PikC_{D50N}$-RhFRED when available.

3.2 Macrolactone 55

3.2.1 Rationale

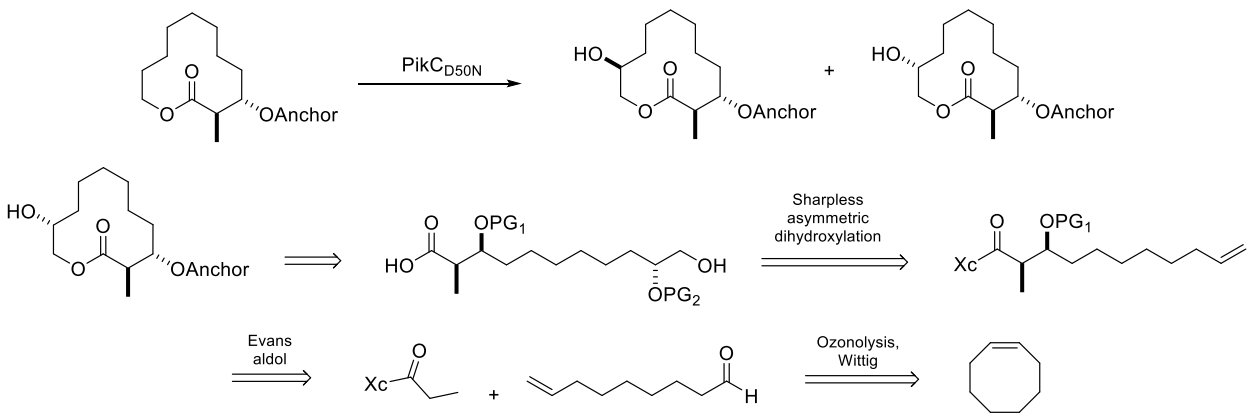


55 10-dml

Figure 3.1. Initial target macrolactone structures and endogenous aglycone 10-dml.

This study started with the synthesis of macrolactone **55**. We envisioned replacing the carbocyclic ring of **6** with the more polar and slightly more rigid lactone, which could be easily achieved through a macrolactonization.[71] The stereochemistry of the hydroxyl and the α-methyl substitution to the ester carbonyl could be easily established through the powerful asymmetric Evans aldol reaction.[72,73] This first macrolactone scaffold displayed more resemblance to endogenous substrate at the anchoring center, but unlike the endogenous substrate YC-17, **55** does not possess any sp^2-hybridized carbon centers near the hydroxylation site. The olefin moiety highlights important differences in the bond dissociation energies (BDE) of the oxidation sites of the endogenous substrate YC-17 and the expected oxidation site on macrolactone **55**. On YC-17 it is a tertiary allylic C-H bond (C-10) that is oxidized, whereas in macrolactone **55**, it is a secondary C-H bond. C-10 in YC-17 is more reactive towards this oxidation because of its increased capacity of stabilizing the carbon radical formed during the reaction, therefore having a lower BDE. Additionally, compound **55** does not have the secondary exocyclic oxidation C-12 site in YC-17 is expected to be more activated as it can be more proximal to the heme iron.

Additionally, we chose this macrolactone scaffold to help us explore the stereoselectivity of oxygen delivery in methylene hydroxylation by PikC. Likely products of the reaction between PikC and macrolactone **55** coupled with the appropriate anchor are C-11 hydroxylated macrolides (*Scheme 3.1*). These hydroxylated sites in the products correspond to the allylic position oxidized in YC-17 and the C-6/C-8 oxidized products of 12-membered carbolide substrate **6**. Both stereo configurations of the hydroxyl group can be easily accessed through the Sharpless asymmetric dihydroxylation of olefins and employed for synthesis of authentic hydroxylated standards for product characterization.[74]

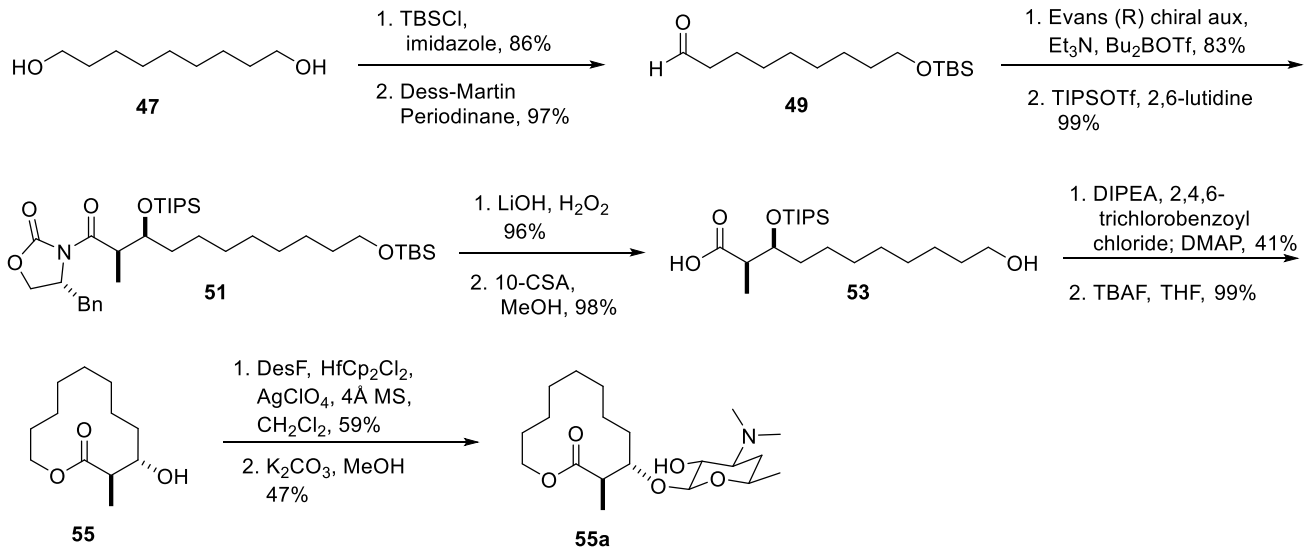


Scheme 3.1. Possible hydroxylated products of macrolactone and retrosynthesis of authentic hydroxylated samples.

3.2.2 Synthesis

The synthesis of the macrolactone **55** began with the *tert*-butyldimethylsilyl (TBS) monoprotection of commercially available 1,9-nonanediol **47**, followed by Dess-Martin periodinane oxidation to give aldehyde **49**. The subsequent Evans aldol reaction with a chiral auxiliary afforded the aldol adduct with the desired stereochemistry in good yields as a single diastereomer (*Scheme 3.2*). The hydroxyl group of the aldol adduct **50** was protected as the triisopropylsilyl (TIPS) ether and the Evans chiral auxiliary was cleaved to yield protected acid **52**. A variety of deprotection conditions were tested to achieve selective cleavage of the terminal TBS group, surprisingly nucleophilic fluoride sources such as TBAF and HF·pyridine deprotected the more bulky TIPS group over the TBS group. We hypothesized that the carboxylic acid hydrogen could participate from an internal H-bond with the TIPS-ether oxygen thereby activating it towards deprotection. We then explored mildly acidic methods, of which 10-CSA afforded selective quantitative deprotection of the acid-sensitive TBS ether without any erosion in the diastereomeric ratio. The resulting hydroxy acid **53** was cyclized through a

Yamaguchi macrolactonization in a 41% yield. The modest yield in the macrolactonization can be attributed to the lack of substitution around the alkyl chain, as this can aid the molecular organization in solution.[71] The TIPS-protected hydroxyl was revealed to yield **55**. In the more oxygen rich macrolactone **55**, $BF_3{\cdot}OEt_2$ was an unselective Lewis acid for DesF and yielded only 10% of the glycosylated product (data not shown). Silver perchlorate reaction with *bis*(cyclopentadienyl)hafnium dichloride ($HfCp_2Cl_2$) generates the more fluorophilic Lewis acid $HfCp_2^{2+}$, which was much more effective by generating the desired glycosylated macrolactone in a 59% yield.[75,76] The final deprotection step yielded macrolide **55a**. Linear ester anchors and benzylic amine anchors described in the previous chapter were attached through DCC mediated couplings to give compounds **55b**, **55c** and **55d** (*Figure 3.2*).



Scheme 3.2. Forward synthesis of macrolactone substrate **55**.

3.2.3 PikC$_{D50N}$-RhFRED oxidation studies

Due to the closer resemblance of this macrolactone to YC-17 we expected improvement in the regioselectivity and stereoselectivity of the oxidation of **55a** upon treatment with PikC$_{D50N}$-RhFRED, in comparison with carbolide **6**. However, the LC-MS trace of the reaction

between substrate **55a** and PikC_{D50N}-RhFRED showed that it was a poor substrate for this enzyme, only providing a slight improvement in the selectivity with a considerable decrease in the yield (*Figure 3.2*). Additional substrates with this core and ester anchoring groups were evaluated as well. The reaction of *N*,*N*-dimethyl-β-alanine linked **55b** showed poor yields and regioselectivity but with larger anchors such as 4-*N*,*N*-dimethylamino butanoate linked **55c** showed good conversion and a decrease in product ratio in comparison with its desosamine counterpart. Finally the reaction of **57d**, employing benzylic amine anchors resulted in excellent conversion and reasonable selectivity.

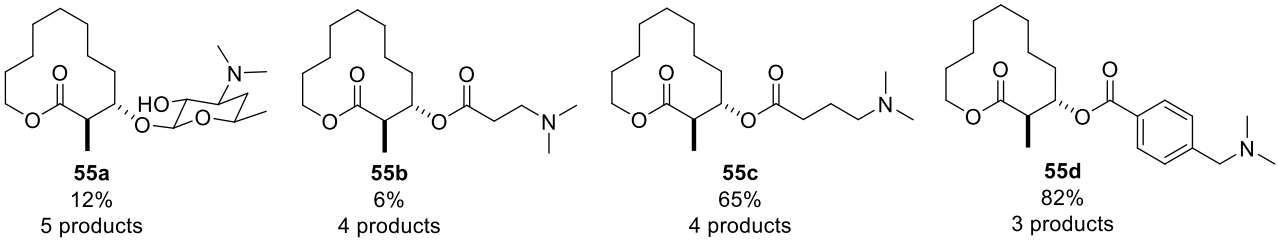


Figure 3.2. Substrates containing the macrolactone core **25**. (Enzymatic reactions by Karoline C. Chiou and Alison R.H Narayan)

3.3 Increasing functionality in macrolactone analogues

3.3.1 Rationale

We hypothesized the poor selectivity in the oxidation of **55a** is because there is no tertiary allylic oxidation site or a secondary exocyclic oxidation site corresponding to the reactive C-H bonds in YC-17. This lack of any highly favored pathway on macrolide **55a** can explain why the oxidation was so low yielding and unselective. We started synthesizing structures that contain either of these sites separately to assess their individual contributions to the reaction selectivity (*Figure 3.3*). The macrolactone **63** possesses the secondary exocyclic oxidation site in an environment that is electronically similar to the corresponding C_{12}–H in YC-

17. Substrate **75** contains the electronically favored allylic oxidation site that resembles C_{10}–H in YC-17. We also envisioned being able to synthesize regioisomer 11-membered macrolactone **78**, from the same ynal intermediate for the synthesis of **75**, to help us probe additional ring sizes as substrates for PikC as well as the effect of the present exocyclic olefin in PikC's ability to carry out different oxidative transformations. The following section describes the synthesis of ethyl-substituted macrolactone **63**.

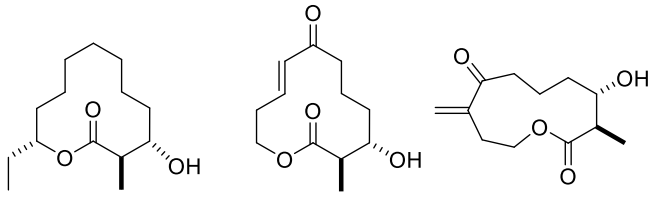
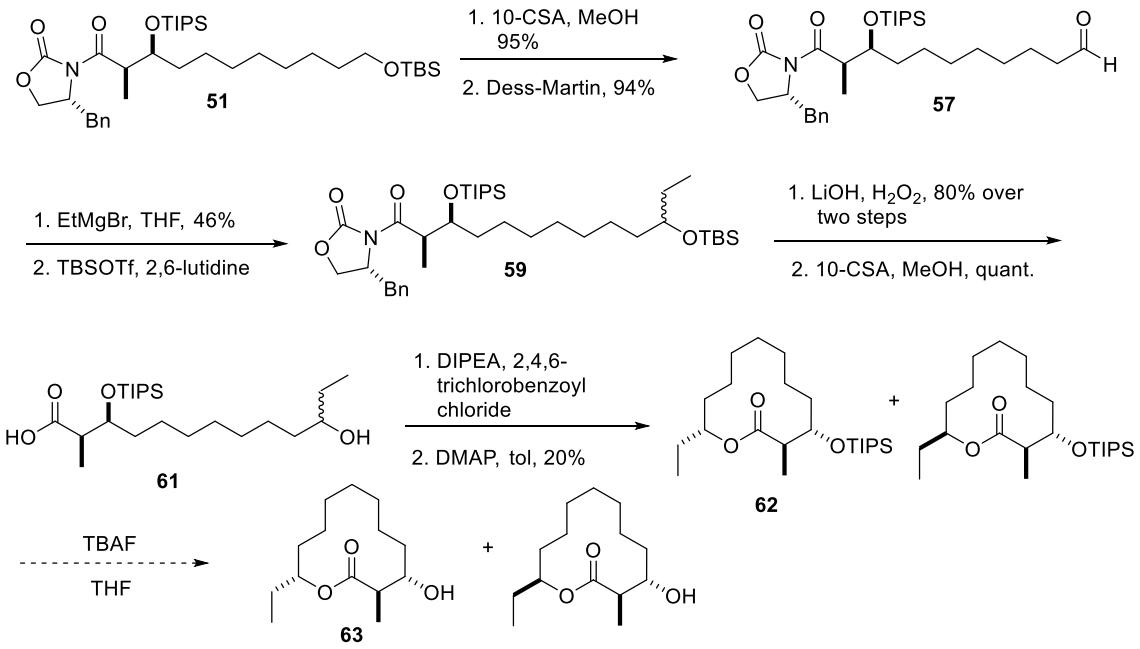


Figure 3.3. Additional proposed macrolactone substrates.

3.3.2 Synthesis of ethyl-substituted macrolactone

Macrolactone **63** is synthesized from protected aldol adduct **55** from the macrolactone **55** synthesis (*Scheme 3.3*). From this intermediate, the more labile TBS ether was selectively unmasked to the hydroxyl using 10-camphor sulphonic acid and then converted to aldehyde **57** through a Dess-Martin oxidation. Addition to the aldehyde using ethyl Grignard reagent was then employed followed by protection of the alcohol to yield an inseparable pair of diastereomers **59**. We believe this is because the newly added stereocenter is too distant from the other sources of chirality around the molecule. The chiral auxiliary group was cleaved and the TBS ether cleaved as described before. The resulting hydroxyacid **61** was cyclized through a Yamaguchi macrolactonization. The lower yield of the macrolactonization in comparison with **54** is due to the increased steric bulk of the Et substituent around the esterification site. At the macrocycle stage, the diastereomers are separable, and to conclude the synthesis the TIPS ether can then be

removed and the resulting hydroxyl bearing macrolactone **63** can be glycosylated and coupled with a variety of *N*,*N*-dimethylamino containing carboxylic acids. Additionally, the synthesis generates the opposite configuration at the ethyl-substituent as well, which allows exploration of the effects of proximity to the heme-iron oxo combined with stereochemical configuration.

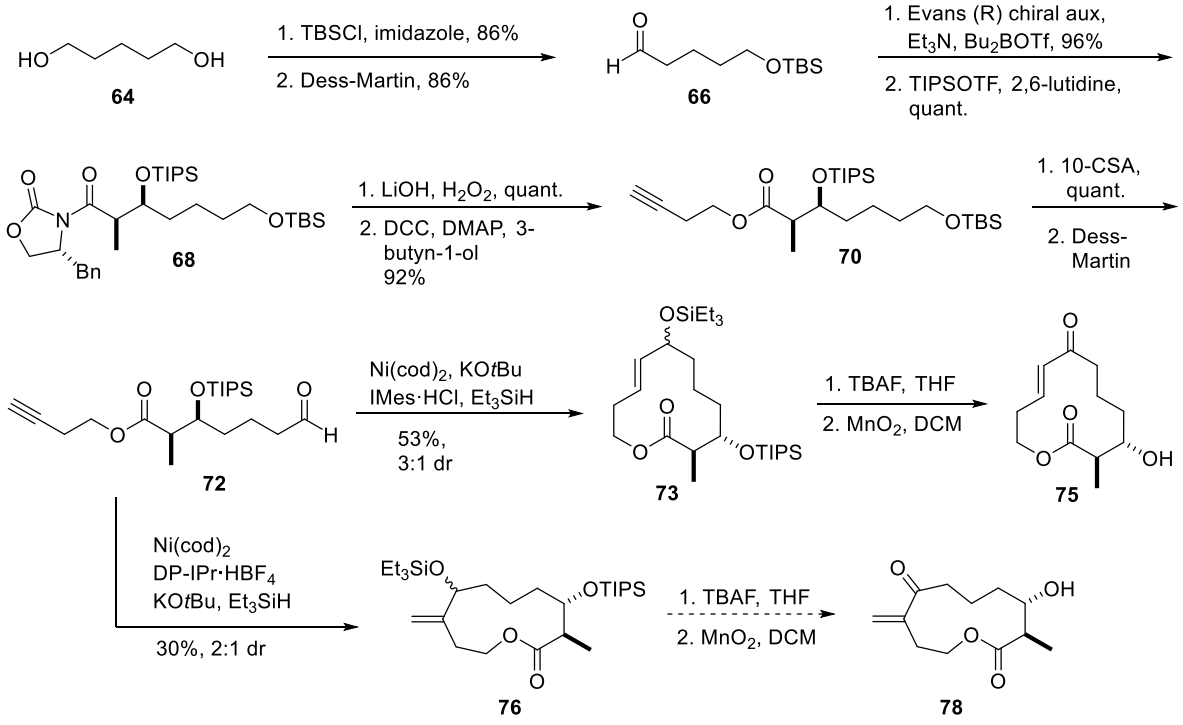


Scheme 3.3. Forward synthesis for macrolactone **63**.

3.3.3 Synthesis of enone-substituted macrolactones

The synthesis of substrate **75** began with TBS monoprotection of 1,5-pentanediol **64**, followed by Dess-Martin oxidation (*Scheme 3.4*). The resulting aldehyde **66** served as the starting material for the Evans aldol reaction with the chiral oxazolidinone. The resulting hydroxyl of the aldol adduct **67** was protected as the TIPS ether **68**, the Evans chiral auxiliary was then cleaved to give the acid **69**, which was then coupled with 3-butyn-1-ol to yield ester **70**. The terminal TBS ether was deprotected using 10-CSA and oxidized to the ynal **72**. We

envisioned employing a regiodivergent Ni-catalyzed ynal macrocyclization, which was demonstrated by former colleague Dr. Rafay Shareef, in the total synthesis of methymycin.[66] This methodology originates from the discovery that the NHC ligand employed in the aldehyde-alkyne intermolecular reductive coupling can override electronic effects within the substrate and dictate the regioselectivity in C-C bond formation.[77] Since the regioselectivity relies on the choice of ligand, the reaction conditions can be manipulated to give either the endocyclization product, the 12-membered macrolactone **73** employing the small NHC ligand IMes, or the exocyclization product, an 11-membered macrolactone **75** employing the large ligand DP-IPr. With 11- and 12-membered macrolactones in hand, global deprotection and selective allylic alcohol oxidation affords macrolactone structures **75** and **78**.



Scheme 3.4. Forward synthesis for macrolactones **75** and **78.**

3.4 Additional determination of bio-activity

Given that the pikromycin pathway produces both 12-membered macrolides methymycin and neomethymycin, and 14-membered pikromycin, PikC is not the only promiscuous enzyme in this pathway. The Sherman lab has explored the promiscuity of the PKS multi-enzyme domain and the glycosyl transferases involved in the pathway.[46] Additionally, a mutant version of *Streptomyces venezuelae* was employed for bio-catalytic production of methymycin, neomethymycin, and pikromycin from 10-dml and narbonolide.[78] We envision feeding these macrolactones **55, 63**, **75** and **78** to DHS2001 or YJ112 for biotransformation into glycosylated and hydroxylated YC-17 analogs. DHS2001 and YJ112 are PKS knockout mutants; they cannot produce aglycones 10-dml and narbonolide but can glycosylate these if they are externally fed to the bacterial culture. These macrolactones give the opportunity to explore the promiscuity of DesVII/DesVIII and also for more efficient hydroxylation by PikC since *in vivo* PikC is constantly being produced. The biotransformation strategy has the potential to generate libraries of novel bio-active macrolides.

3.5 Conclusions and outlook

With these three new macrolactones substrates we are gaining a better understanding of the factors within the ring system that govern the selectivity of the oxidation, whether it is proximity to the heme-iron center, or BDE's in the C–H bonds in question that plays a more important role in influencing selectivity. Simple macrolactone **55** shows that additional functionality around the anchoring site is not productive towards a more selective hydroxylation when additional functionality is not present close to the oxidation sites. However, paired with the appropriate anchoring group, simple macrolactone derivatives can yield excellent conversion and moderate selectivity. These results highlight the possibility of modest control on selectivity by the choice of anchor, in systems with moderate functionality. To increase the selectivity of the

oxidation more biased macrolides are necessary. Future directions include the evaluation of **73** and **75** coupled with a variety of anchors against PikC_{D50N}-RhFRED as well as the evaluation of the free hydroxyl macrolactones against biotransformation strains.

3.6. Collaborator acknowledgements

Karoline C. Chiou, Ph.D. and Alison R.H. Narayan, Ph.D., from the Sherman laboratory, performed the enzymatic reactions of compounds synthesized.

Chapter IV: Expanding the substrate scope of the PikC catalyzed hydroxylation to include small ring systems

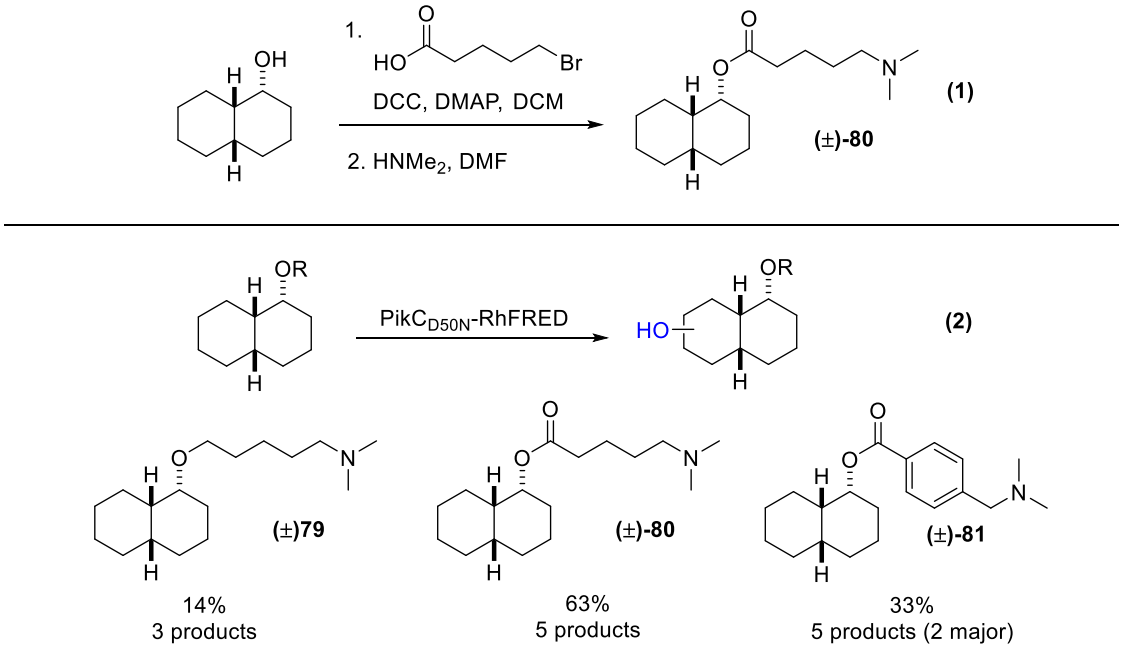
4.1 Introduction

To accomplish the goal of a more useful C–H bond functionalization procedure it would be desirable to be able to employ $PikC_{D50N}$-RhFRED as a synthetic tool to oxidize small ring systems. This was limitation encountered early on, as carbolide substrates from cyclohexanol and *cis*-decahydronaphthol (*substrates **7** and **8** respectively*) failed to yield hydroxylated products upon treatment with $PikC_{D50N}$-RhFRED (*Figure 2.1*). Even though the substrates contained the desosamine anchor, which is capable of engaging in the salt-bridge interaction necessary for anchoring, these carbocycles were not large enough to reach the heme iron(V)-oxo. The promising results of substantial increase in substrate conversion caused by an increment in linker length (*observed for substrates **12** vs. **14***) and of anchor controlled selectivity for 10-dml derivatives encouraged us to re-visit looking at $PikC_{D50N}$-RhFRED as a bio-catalyst for hydroxylation of small-molecules by coupling of alcohols with ester and ether linkers containing the dimethylamino anchor.

4.2 Moderately regioselective hydroxylation of small bicyclic systems

We first decided to re-visit the *cis*-decalin core (*Scheme 4.1*). The five carbon ether linker employed for substrate **14** was attached to racemic *cis*-decahydronaphthol to yield substrate **(±)-79**. We were gratified to find that **(±)-79** was a substrate for $PikC_{D50N}$-RhFRED catalyzed

oxidation, albeit in low yield. *Cis*-decalin ester derivative **(±)-80** was synthesized from DCC mediated coupling of *cis*-decahydronaphthol and 5-bromovaleric acid (*Scheme 4.1 eq 1*). The resulting bromide was displaced by dimethylamine in S_N2 fashion to yield derivative **(±)-80**. Upon treatment of this substrate with $PikC_{D50N}$-RhFRED we were pleased to find hydroxylated products were generated in moderate yield and regioselectivity. Additionally, we explored the effect of the more rigid *p*-benzylic amine anchor and synthesized substrate **(±)-81**. Although this anchor in **(±)-81** did not increase the yield of the oxidation reaction or reduce the overall number of peaks present in the LC-MS trace when compared to **(±)-80**, it did however cause the two major products to account for over 85% of the monohydroxylated material, showing that the rigid anchors can have an effect on the selectivity in the oxidation of this simple small bicyclic compound.



Scheme 4.1. *cis*-Decalin derivatives with unnatural anchors evaluated against $PikC_{D50N}$-RhFRED. (1) Synthesis of substrate **(±)-80**. (2) Enzymatic oxidation of *cis*-decalin derivatives

(±)-79, **(±)-80** and **(±)-81** (Enzymatic reactions by Dr. Karoline C. Chiou and Dr. Alison R.H. Narayan)

Due to the successful results obtained with the small decalin system, we then decided to explore the even smaller and highly prevalent 6-membered ring systems with the larger anchoring groups against PikC. Considering the inherent chirality of the PikC enzyme we hypothesized that a kinetic resolution may be in effect when these racemic decalin derivatives are fed to the enzyme. It would be interesting to test the reactivity of each enantiomer of *cis*-decahydronaphthol. However, since *cis*-decahydronaphthol is only commercially available as the racemate, we decided to prove the hypothesis with different single-enantiomer small-molecule substrates whose enantiomers can be easily obtained.

4.3 PikC catalyzed hydroxylation of 6-membered ring systems

4.3.1 Menthol series

As a test-substrate to probe PikC's ability to oxidize small molecules, we chose the menthol scaffold because it contains primary, secondary and tertiary C–H bonds in close proximity, which allowed us to evaluate PikC's ability to distinguish between these different C–H bonds. Additionally, its well-defined chair with predictable *J* values makes structural elucidation easier. PikC showed anchor-controlled regioselectivity in oxidizing tertiary versus secondary C–H bonds but within the 10-dml core. It is of interest is to determine whether $PikC_{D50N}$-RhFRED can act with the high level of regiocontrol observed for 10-dml derivatives for small-molecules. Finally, these study whether $PikC_{D50N}$-RhFRED reacts differently to enantiomers of the same molecule, and for menthol they are easily available. To this end, (-)-

menthol and (+)-menthol was coupled with a variety of amino-containing acid anchors through DCC-mediated couplings to yield ester substrates **82-90** as shown in table 4.1.

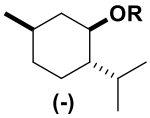
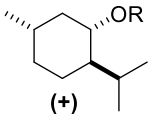
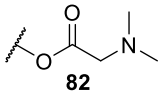
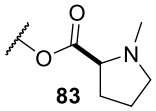
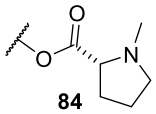
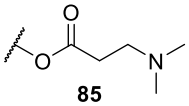
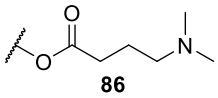
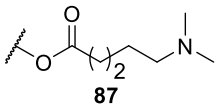
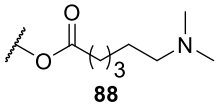
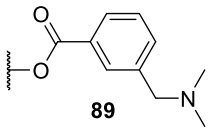
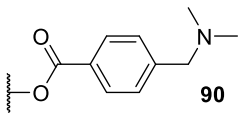
Menthol enantiomer/ Anchor	 **(-)**	 **(+)**
 **82**	8%, 1 product	0%
 **83**	7%, 1 product	0%
 **84**	trace	0%
 **85**	16% 1 product	trace
 **86**	24% 3 products 6:1:1	5% 4 products
 **87**	40% 3 products 5:1:1	25% 4 products
 **88**	51% 2 products 3:2	43% 4 products
 **89**	32% 2 products 8:1	N.A.
 **90**	93% 2 products 3.2:1	5% 3 products

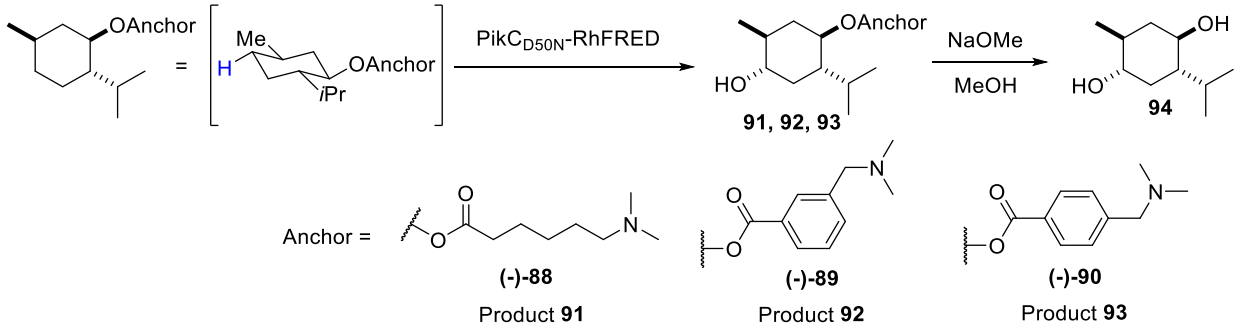
Table 4.1. (+)-Menthol and (-)-menthol substrates with different anchoring groups evaluated against PikC_{D50N}-RhFRED. (With Dr. Alison Narayan and Michael Gilbert)

Across the board, (-)-menthol derivatives performed far better upon treatment with $PikC_{D50N}$-RhFRED than (+)-menthol substrates containing the same anchor (*table 4.1, middle column vs. right column*). These results show that there is a matched and mismatched case between the enantiomers of the substrate and $PikC_{D50N}$-RhFRED. Also important to note, is that in an enantiomeric series, the retention times in LC-MS traces of the $PikC_{D50N}$-RhFRED reaction did not generally match, indicating that the enantiomers of menthol yielded different diastereomers upon oxidation. Additionally, for menthol matched/mismatched reactivity is observed when chirality is present in the anchoring group. The preferred (-)-menthol core coupled to L-*N*-methyl proline (natural proline enantiomer) in substrate **(-)-83** is converted into products albeit in low yield. However, (-)-menthol core coupled to D-*N*-methyl proline in substrate **(-)-84** displays no reactivity in the presence of $PikC_{D50N}$-RhFRED. This contrasts the observation of no difference in reactivity between 10-dml *N*-methyl proline derivatives **42** and **43** (*see chapter II, table 2.2*). We hypothesize that in the case of **42** and **43**, the effect of anchor chirality is diminished due to the increased binding affinity between substrates with the endogenous 10-dml core and $PikC_{D50N}$-RhFRED.

The results also highlight the importance of anchor controlled efficiency and selectivity. Smaller anchoring group substrates **(-)-81** to **(-)-86** were converted into products in poor yields. This small anchors placed the target C–H bonds at suboptimal distance from the iron(V)oxo but at which hydroxylation was still possible. However, larger anchoring groups coupled to (-)-menthol display moderate to excellent conversion towards the $PikC_{D50N}$-RhFRED catalyzed oxidation, substrate **(-)-90** is converted to exclusively two monohydroxylated products with synthetically useful 3.2:1 regioisomer ratio and in excellent yields. In the (-)-menthol series generally, an increase in the anchor size brought the C–H bonds closer to the iron(V)oxo and

more efficient oxidation occurred, with the exception being *m*-benzylic amine derivative **(-)-89**. The difference in reactivity between **(-)-89** and **(-)-90**, provides insight into the effect the substitution pattern has in substrate orientation in the active site, which in turn influences the selectivity of the hydroxylation of these small molecule substrates. Also, the more rigid benzylic amine derivatives provided the better regioselectivity observed in the oxidation reaction. We hypothesize the more rigid benzene ring restricts conformational sampling in the active site and is therefore better at directing bonds to the iron(V)oxo.

Not only the high conversion and selectivity were exciting results, but also the major product generated during the reaction was surprising. Preparative scale enzymatic reactions of selected substrates were carried out and the major product was isolated and characterized by ^{1}H-^{1}H-COSY. In the cases of substrates **(-)-88**, **(-)-89** and **(-)-90**, methylene oxidation afforded products **91**, **92** and **93** as a single diastereomer for each (*Scheme 4.2*). A larger scale-up of the enzymatic reaction is required for characterization of minor products. Rapid assessment for methylene hydroxylation can be achieved by sodium methoxide hydrolysis of the ester anchor to yield diol **94**, which can be employed as a standard in GC-MS analysis of hydrolyzed crude PikC$_{D50N}$-RhFRED reaction extracts. Removing the anchor makes the major product obtained from the PikC$_{D50N}$-RhFRED oxidation reaction the same compound, regardless of the anchor employed, therefore GC-MS retention time and ionization profile can be used to identify the presence of this product in crude hydrolyzed PikC$_{D50N}$-RhFRED reaction extracts. Also, our results are in accordance to previously described observation of faster reaction rates for equatorial C–H bonds than axial C–H bonds in C–H functionalization reactions (*chapter I*).[27] Additionally, the secondary C–H oxidation observed is complementary to what is observed from transition-metal based approaches.[21,23]



Scheme 4.2. Characterization of hydroxylated products from PikC$_{D50N}$-RhFRED oxidation of menthol derivatives.

4.3.2 Additional 6-membered ring substrates to understand hydroxylation selectivity

To probe the limits of selective hydroxylation of small molecules, cyclohexanol ether derivative **95** and ester derivative **96** were evaluated (*Figure 4.1*). The highly flexible cyclohexyl ether derivative **95** did not show any conversion with PikC$_{D50N}$-RhFRED, while the slightly larger, more rigid ester derivative **96** afforded moderate conversion to three monohydroxylated products, a decrease in regioselectivity compared to substrate **(-)-90**. These results highlight the effects of the substituents within the cyclohexane ring on restricting conformational freedom. The all-equatorial substituents in the chair conformer that these menthol substrates adopt is highly stable and creates a barrier greater than 5 kcal/mol to chair-chair interconversions and thus provided the hydroxylated products with higher regioselectivity (*Scheme 4.2*). α-Terpineol derivatives **97**-**98** afforded only one monohydroxylated product in the PikC$_{D50N}$-RhFRED catalyzed hydroxylation reaction, but only the reaction of derivative **98**, containing the *p*-benzylic amine anchor, resulted in useful substrate conversion. This result again highlights the importance of the rigid benzene ring in directing the cyclohexyl C–H bonds towards the iron(V)oxo. In this series, the major product of PikC$_{D50N}$-RhFRED oxidation of α-terpineol derivative **98** characterized from a preparative scale enzymatic reaction and was determined to

be methyl oxidation to give alcohol **102**. This result is consistent with PikC$_{D50N}$-RhFRED catalyzed hydroxylation preference for positions most distal from the amine anchor. (*S*)-perillyl alcohol derivatives **99** and **100** afforded multiple products due to the large number of activated allylic C–H bonds present in the substrate. Additionally, it is possible that the (*S*) enantiomer is not the correct match for the PikC enzyme. Finally, (-)-isopulegol coupled to the *p*-benzylic amine anchor in derivative **101** afforded primarily one monohydroxylated product in good yields. We are interested in probing whether PikC$_{D50N}$-RhFRED will still oxidize the methylene position analogous to **(-)-90** in the presence of the allylic site. The characterization of the oxidation product is ongoing in the Montgomery group.

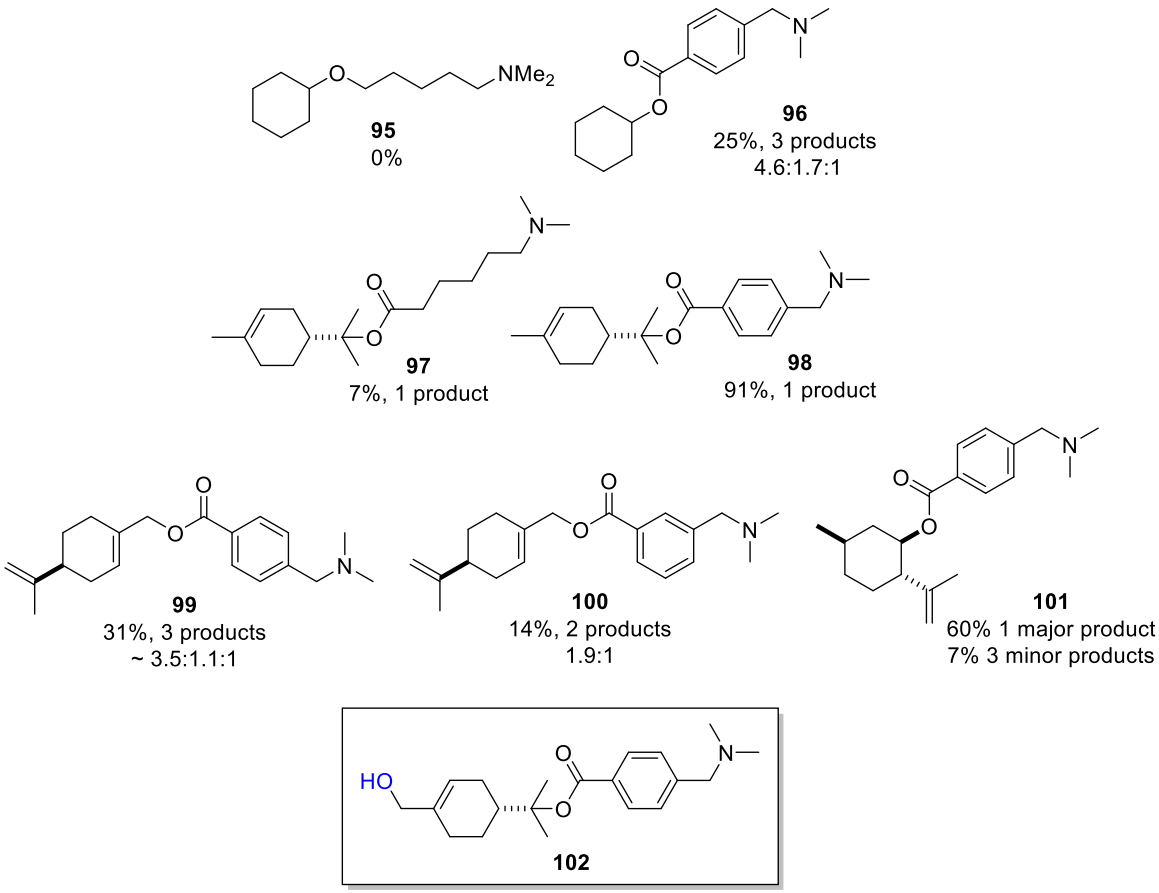


Figure 4.1. Results from the reaction of PikC$_{D50N}$-RhFRED and additional 6-membered carbocycle substrates. (Enzymatic reactions by Alison R.H. Narayan, Ph.D.)

4.4 Conclusions and outlook

A number of different small-molecule carbocycle substrates containing the amine anchor, attached through ester and ether groups with different lengths, overall size, and rigidity afforded products with varying degrees of efficiency upon reaction with $PikC_{D50N}$-RhFRED. These results bring us closer to the goal of developing a more useful C–H bond oxidation procedure for a broad range of substrates. Additionally, these anchors are generally easier to synthesize and attach to the substrates than desosamine and are easily cleavable. Secondly, these larger anchors effectively position the C–H bonds closer to the heme iron center of PikC and improved yields of oxidation for small carbocycles that were previously evaluated and found to be poorly oxidized or unreactive upon reaction with $PikC_{D50N}$-RhFRED. Thirdly, this reaction can be carried out on a preparative scale, the products can be identified, and cleavage of the anchoring group generates a higher throughput method for product analysis. We have also been able to explore the role of these unnatural anchors the selectivity of the oxidation for the small ring systems. Finally, $PikC_{D50N}$-RhFRED catalyzed oxidation achieves complimentary results to transition-metal catalyzed reactions.

Among immediate future directions for this work is the characterization of hydroxylated products of 6-membered carbocycle substrates. Additionally, we envision a more thorough exploration of additional anchors for small molecules. We propose to further increase the linker size by employing the naphthalene core, different anchoring group attachments such as silyl ethers as well as click chemistry approaches with alkynes and azides of different lengths to unite the substrate and an *N*,*N*-dimethylamino moiety.

4.5. Collaborator acknowledgements

Alison R.H. Narayan, Ph.D., from the Sherman laboratory, performed the enzymatic reactions of compounds and synthesized **82**, **85**, **(+)-86** and **(+)-90**. Michael M. Gilbert, from the Montgomery laboratory, synthesized compounds **87** and **88**.

Chapter V: Conclusions and Future Directions

The substrate scope of the PikC-catalyzed hydroxylation has been extended from endogenous macrolides and synthetic carbolides to simple macrolides and most importantly, substrates replacing the desosamine anchor with simpler removable alkyl amine containing ethers and esters. Simple carbocycles coupled to *N*,*N*-dimethylamino anchors afforded moderate yields and poor regioselectivity to monohydroxylated products in the oxidation reaction. Moderate selectivity in the PikC-catalyzed hydroxylation was observed for 12-membered simple macrolactones, but with substrates featuring the endogenous macrolactone 10-dml, these rigid benzylic amine anchors afforded excellent regiocontrol in the production methymycin or neomethymycin analogs selectively. The minimal structural complexity in 12-membered macrolactone substrates for selective oxidation will be determined with macrolactones more similar to 10-dml than **55**. These macrolactones will also facilitate the evaluation of the whole *Streptomyces venezuelae* organism for the bio-catalytic production of novel glycosylated and hydroxylated macrolides and the evaluation of these as potential anti-bacterial agents.[78]

Not only was the need for desosamine-containing substrates eliminated with the work described in this thesis, but these novel larger alkyl amine anchors enabled small single enantiomer carbocyclic rings to be efficiently hydroxylated by the enzyme as well as controlling the regioselectivity of the oxidation. Benzylic amine derivatives of six-membered carbocyclic terpene cores such as (-)-menthol, (-)-isopulegol and α-terpineol afforded predominantly one major monohydroxylated product in good yields and selectivities. PikC also exhibited matched

and mismatched reactivity with enantiomers, as (+)-menthol derivatives showed inferior efficiency and selectivity in the oxidation reaction. In addition, racemic *cis*-decahydronaphthol core substrates afforded multiple major and minor products, presumably with one of the enantiomers reacting better with the enzyme. Exploration of macrolide, racemic bicycle and single enantiomer small-molecule substrates in the $PikC_{D50N}$-RhFRED reaction has allowed us to gain a better understanding of the different factors that govern the selectivity in the oxidation both within substrate and anchor moieties which will allow for more predictable reactivity of $PikC_{D50N}$-RhFRED with additional unnatural substrates. Further exploration of single enantiomer small-molecules and bicycles is among the immediate future directions of the project. These explorations include different anchors, such as amides, amino alcohols coupled to carboxylic acid substrates and triazoles. The latter class of click-linkers could allow for higher throughput screening of the appropriate anchor size for a particular substrate by reaction of an azide-containing substrate molecule with a variety of dimethylamino containing alkynes and evaluation of this crude mixture in the PikC oxidation reaction in a competition experiment.

In addition to continuing substrate engineering approaches as a means to control selectivity, future directions call for further engineering of the PikC enzyme. Molecular dynamics simulations from the co-crystal structures of substrates within the $PikC_{D50N}$ active site performed by the Houk group revealed additional salt-bridge interactions between the dimethyl amino group and other carboxylate containing residues within the active site. Double mutants that lacked these carboxylates showed increased substrate conversion serving as an example of a combination of molecular modeling guided site-directed mutagenesis as a tool for enhancing reaction efficiency. Additionally, these modeling experiments will enable easier predictions of the potential hydroxylation sites of substrates and enable the discovery of novel anchoring

groups for selective oxidation. Finally, the results from the oxidation of unnatural substrates by $PikC_{D50N}$-RhFRED highlight the potential of bacterial biosynthetic P450's, to be developed as biocatalysts for the selective oxidation of C-H bonds in both smaller and larger molecules by the appropriate matching of substrate size and anchor size.

Chapter VI:Experimental Supporting Information

6.1 General Protocols

All reagents were used as received unless otherwise noted. Solvents were purified under nitrogen using a solvent purification system (Innovative Technology, inc., Model # SPS-400-3 and PS-00-3. $Ni(COD)_2$ (Strem Chemicals, Inc., used as received), 1,3-*bis*(2,4,6-Trimethyl-phenyl)imidazolium chloride (IMes·HCl) and potassium *tert*-butoxide were stored and weighed in an inert atmosphere glovebox. All reactions were conducted in flame-dried glassware under nitrogen atmosphere unless otherwise specified. Reactions were monitored by thin layer chromatography using SiliCycle silica gel 60 F254 precoated plates (0.25 mm) which were visualized using UV light, *p*-anisaldehyde stain, $KMnO_4$, PMA or CAM stain. Flash column chromatography was performed using Kieselgel 60 (230-400 mesh) silica gel. Eluent mixtures are reported as v:v percentages of the minor constituent in the major constituent. All compounds purified by column chromatography were sufficiently pure for use in further experiments unless otherwise indicated. ^{1}H and ^{13}C spectra were obtained in $CDCl_3$ at rt (25 °C), unless otherwise noted, on a Varian vnmrs (500 MHz or 700 MHz), Varian MR400 or Varian Unity 500 MHz. Chemical shifts of ^{1}H NMR spectra were recorded in parts per million (ppm) on the δ scale from an internal standard of residual chloroform (7.26 ppm). Chemical shifts of ^{13}C NMR spectra were recorded in ppm from the central peak of $CDCl_3$ (77.0 ppm) on the δ scale. Low resolution electrospray mass spectra were obtained on a Micromass LCT spectrometer and high resolution

electrospray mass spectra were obtained on a Micromass AutoSpec Ultima spectrometer at the University of Michigan Mass Spectrometry Laboratory.

List of reagents prepared or purified:

3-(*N*,*N*-Dimethylamino)propyl methanesulfonate hydrochloride was synthesized following the procedure described by Nakazumi et al.[79]

3Å Molecular sieves were flame dried prior to use.

12-((tert-butyldimethylsilyl)oxy)-1-(trimethylsilyl)dodec-1-yn-5-ol was synthesized following the procedure described by Li et al.[60]

12-((tert-butyldimethylsilyl)oxy)-1-(trimethylsilyl)dodec-1-yn-6-ol was synthesized following the procedure described by Li et al.[60]

4-Methoxybenzyl 2,2,2-trichloroacetimidate was prepared as described in the procedure by Shareef et al.[66]

Dess Martin periodinane was synthesized following the procedure described by Boeckman et al. [80]

Triethylamine was distilled under calcium hydride.

Triethylsilane was filtered through a plug of basic alumina and degassed.

D-*N*-methylproline was synthesized following the procedure described by Daughtry et al.[81]

9-((*tert*-butyldimethylsilyl)oxy)nonanal and 5-((*tert*-butyldimethylsilyl)oxy)pentanal were synthesized following the procedure described by Li et al.[60]

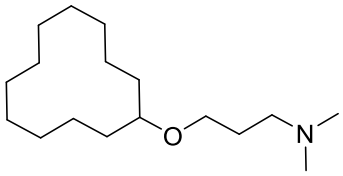
Desosamine fluoride was synthesized following the procedures described by Anzai et al.[62]

6.2 Chapter 2 Experimental

6.2.1 General procedure for ester hydrolysis

General Hydrolysis Procedure: The crude $PikC_{D50N}$-RhFRED reaction mixture was dissolved in a 4:1 mixture of MeOH:$CHCl_3$ (0.015 M) and 2.5 equiv of potassium carbonate were added. The reaction mixture was allowed to stir at rt for 72 hours or until all starting material was consumed. The reaction mixture was concentrated under reduced pressure, quenched by addition of saturated ammonium chloride and extracted thrice with portions of EtOAc. The combined organic layers were dried over Na_2SO_4, and filtered through a plug of silica gel and concentrated under reduced pressure. The products were analyzed by ^{1}H-NMR and determined to be a mixture of methynolide and neomethynolide (comparison to previously reported spectra), the ratio of the two components depended on the length of the ester anchoring group in the substrate.

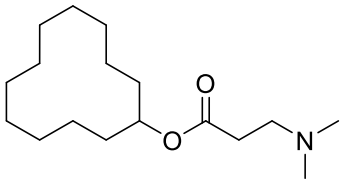
6.2.2 Experimental for PikC substrates and intermediates, hydroxylated standards and intermediates



3-(Cyclododecyloxy)-*N*,*N*-dimethylpropan-1-amine (12)

To a DMF (3.0 mL) suspension of NaH (57.0 mg, 2.26 mmol) was added a DMF solution (2.0 mL) of Cyclododecanol (200 mg, 1.07 mmol) dropwise at 0 °C. After the solution was stirred for 0.5 h at 0 °C, 3-(*N*,*N*-dimethylamino)propyl methanesulfonate hydrochloride (246 mg, 1.13 mmol) was added in three portions at 0 °C. The reaction mixture was gradually warmed to rt and stirred 12 h. The reaction mixture was diluted in water and extracted with EtOAc three times. The EtOAc extract was dried over anhydrous magnesium sulfate, filtered, evaporated and chromatographed (silica gel; eluent EtOAc) to give the title compound as a colorless oil (137 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46 (t, *J* = 6.6 Hz, 2H) 3.39 (tt, *J* = 7.2, 3.6 Hz, 1H) 2.33 (t, *J* = 7.4 Hz, 2H) 2.22 (s, 1H) 1.72 (quint, *J* = 6.8 Hz, 2 H) 1.63 – 1.33 (m, 22H). ^{13}C NMR

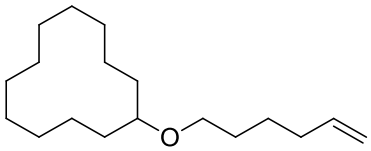
(125 MHz, $CDCl_3$) δ 77.0, 66.8, 56.9, 45.5, 28.9, 28.4, 25.7, 24.2, 23.2, 23.1, 20.7. IR (thin film, cm^{-1}) 2930, 2847, 2811, 2761, 1468, 1443, 1336. HRMS (ESI) *m/z* calculated for $[M+H]^+$, 270.2796 found 270.2823.



Cyclododecyl 3-(dimethylamino)propanoate (13)

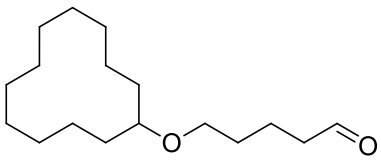
cyclododecanol (25 mg, 0.14 mmol) was dissolved in 1 mL of dichloromethane along with 21 mg 3-*N*,*N*-dimethylaminopropanoic acid hydrochloride (0.14 mmol, 1.1 equiv), 19 µL of freshly distilled triethylamine (0.14 mmol, 1.0 equiv), 28 mg of dicyclohexylcarbodiimide (0.24 mmol, 1.0 equiv) and 2 mg 4-dimethylaminopyridine (0.014 mmol, 0.1 equiv). The reaction mixture was allowed to stir at room temperature for 3 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was transferred to a separatory funnel by washing the flask with 0.1 M HCl and 30% EtOAc in hexanes. The acidified aqueous phase was extracted three times with 30% EtOAc in hexanes. Then the aqueous layer was basified with saturated aqueous $NaHCO_3$ solution until it reached a pH of about 8-9, measured by pH paper. The basified aqueous layer was extracted three times with dichloromethane. The dichloromethane organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) to yield 26 mg (66% yield) of the desired product as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 5.01 (m, 1H), 3.28 (t, J = 7.3 Hz, 2 H) 2.95 (t, J = 7.3 Hz, 2H) 2.76 (s, 6 H) 1.72 –1.23 (m, 22H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 73.9, 53.0, 43.1, 29.9, 29.0, 23.9, 23.7, 23.3, 23.1, 20.8. IR

(thin film, cm^{-1}) 2928, 2850, 2818, 2765, 1728, 1468, 1445. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 284.2590, found 284.2616



(Hex-5-en-1-yloxy)cyclododecane (int-14a)

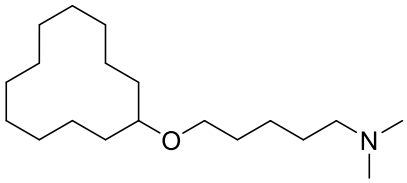
To a DMF (1.5 mL) suspension of NaH (15.1 mg, 0.597 mmol) was added a 1.0 mL DMF solution of cyclododecanol (100 mg, 0.543 mmol) dropwise at 0 °C. After the solution was stirred for 0.5 h at 0 °C, 6-bromo-1-hexene (99 µL, 0.464 mmol) was added in three portions at 0 °C. The reaction mixture was gradually warmed to rt and stirred 12 h. The reaction mixture was diluted in water and extracted with hexanes thrice. The hexanes extract was dried over anhydrous magnesium sulfate, filtered, evaporated and chromatographed (silica gel; eluent 2% EtOAc in hexanes) to give the title compound as a pale yellow oil (28 mg, 20%). ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H δ 5.81 (ddt, *J* = 15.6, 10.3, 6.7 Hz, 1H), 5.00 (dq, *J* = 17.2, 1.8 Hz, 1H), 4.97 – 4.86 (m, 1H), 3.41 (t, *J* = 6.5 Hz, 2H), 3.40 – 3.34 (m, 1H), 2.14 – 2.00 (m, 2H), 1.76 – 1.13 (m, 26H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 138.9, 114.4, 70.7, 68.3, 33.6, 29.7, 28.9, 25.6, 24.6, 24.2, 23.2, 23.1, 20.7. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 289.3, found 289.3



5-(Cyclododecyloxy)pentanal (int-14b)

Ozone was bubbled through a solution of (hex-5-en-1-yloxy)cyclododecane (27.3 mg, 0.102 mmol) in dichloromethane (5 mL) at –78 °C until a faint blue color persisted. Oxygen was

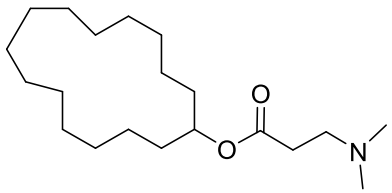
bubbled through the reaction mixture until the blue color dissipated; Nitrogen was then bubbled through the reaction mixture for 5 minutes. A solution of PPh_3 (40.7 mg, 0.154 mmol) in dichloromethane (3 mL) was then added. The mixture was allowed to gradually warm to rt over 12 h. The reaction mixture was diluted with ether and washed successively with water and brine. The organic extract was dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude residue was purified through flash chromatography (silica gel, 10 % EtOAc/hexanes), affording the aldehyde as a colorless oil (24 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 3.43 (t, *J* = 6 Hz, 2H), 3.38 (tt, *J* = 7.2, 3.6 Hz, 1H), 2.46 (td, *J* = 7.2, 1.2 Hz, 2H), 1.75 – 1.25 (m, 26H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 201.7, 77.1, 67.8, 43.7, 29.6, 28.8, 24.7, 24.3, 23.1, 23.0, 20.7, 19.1. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 291.4, found 291.4



5-(Cyclododecyloxy)-*N*,*N*-dimethylpentan-1-amine (14)

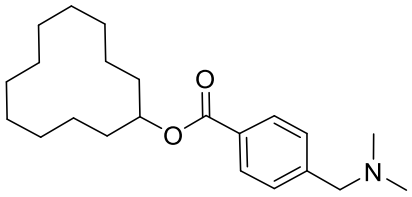
5-(cyclododecyloxy)pentanal (5.3 mg, 0.20 mmol), dimethylamine hydrochloride (4.9 mg, 0.059 mmol), sodium acetate (4.3 mg, 0.051 mmol) and 3Å powdered molecular sieves (10 mg) were combined in 1 mL of MeOH and stirred at rt for 30 mins before sodium cyanoborohydride (2.9 mg, 0.043 mmol) was added. The reaction mixture was allowed to stir at rt for 72h, periodically adjusting the pH of the reaction to be between 5-7 by the addition of drops of glacial acetic acid. The reaction mixture was then concentrated under reduced pressure, the crude residue acidified with 3 mL of 2M HCl aqueous solution and transferred to a separatory funnel. The aqueous phase was washed three times with 3 mL hexanes each time before it was basified with 5%

NaOH aqueous solution to a pH 9. The basified aqueous layer was extracted thrice with 4 mL EtOAc, the combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield 5-(cyclododecyloxy)-N,N-dimethylpentan-1-amine as a colorless oil (5.1 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.41 (t, *J* = 6.6 Hz, 2H), 3.39 – 3.35 (m, 1H), 2.32 – 2.26 (m, 2H), 2.24 (s, 6H), 1.66 – 1.23 (m, 26H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 76.9, 68.4, 59.9, 45.5, 30.2, 28.9, 27.6, 24.7, 24.2, 24.1, 23.2, 23.1, 20.7. IR (thin film, cm^{-1}) 2930, 2850, 2811, 2759, 1467, 1443. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 298.3110, found 298.3133.



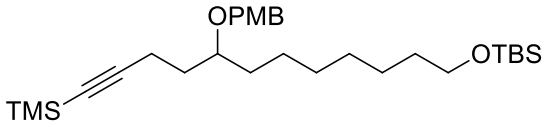
Cyclopentadecyl 3-(dimethylamino)propanoate (16)

Following the procedure employed to synthesize cyclododecyl 3-(dimethylamino)propanoate, cyclopentadecanol (50 mg, 0.22 mmol), 3-*N*,*N*-dimethylaminopropanoic acid hydrochloride (37 mg, 0.24 mmol), triethylamine (37 µL, 0.24 mmol), DCC (50 mg, 0.24 mmol), DMAP (30 mg, 0.24 mmol) in 1 mL of CH_2Cl_2 were employed to yield 22 mg (31% yield) of the desired product as a colorless oil after purification over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) ^{1}H NMR (700 MHz, $CDCl_3$) δ 4.90 (tt, *J* = 7.0, 5.6 Hz, 1H), 2.61 (t, *J* = 7.7 Hz, 2H), 2.45 (t, *J* = 7.7 Hz, 2H), 2.24 (s, 6H), 1.64 – 1.58 (m, 2H), 1.57 – 1.51 (m, 2H), 1.42 – 1.24 (m, 24H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.1, 73.7, 54.8, 45.2, 33.2, 31.8, 27.0, 26.8, 26.7, 26.6, 26.6, 23.1. IR (thin film, cm^{-1}) 2924, 2855, 2817, 2764, 1729, 1459. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 326.3059, found 326.3100



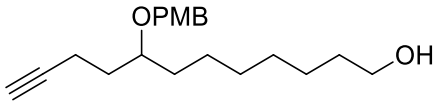
Cyclododecyl 4-((dimethylamino)methyl)benzoate (17)

cyclododecanol (25 mg, 0.14 mmol) was dissolved in 1.8 mL of dichloromethane along with 36 mg of 4-((dimethylamino)methyl)benzoic acid (0.20 mmol, 1.5 equiv), 42 mg of dicyclohexylcarbodiimide (0.20 mmol, 1.5 equiv) and 30 mg 4-dimethylaminopyridine (0.24 mmol, 1.5 equiv). The reaction mixture was allowed to stir at room temperature for 4 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was transferred to a separatory funnel by washing the flask with 0.1 M HCl and 30% EtOAc in hexanes. The acidified aqueous phase was extracted three times with 30% EtOAc in hexanes. Then the aqueous layer was basified with saturated aqueous $NaHCO_3$ solution until it reached a pH of about 8-9, measured by pH paper. The basified aqueous layer was extracted three times with dichloromethane. The dichloromethane organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) to yield 29 mg (62% yield) of the desired ester derivative as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.5 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 5.25 (tt, *J* = 6.5, 5.0 Hz, 1H), 3.46 (s, 2H), 2.24 (s, 6H), 1.87 – 1.78 (m, 2H), 1.69 – 1.60 (m, 2H), 1.54 – 1.28 (m, 18H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.2, 144.0, 129.8, 129.5, 128.8, 72.8, 64.0, 45.3, 29.1, 24.2, 24.0, 23.4, 23.2, 20.9. IR (thin film, cm^{-1}) 2932, 2851, 2809, 2761, 1701, 1611, 1467, 1412, 1324. HRMS (ESI) *m/z* calculated for $[M+H]^+$



***tert*-Butyl((8-((4-methoxybenzyl)oxy)-12-(trimethylsilyl)dodec-11-yn-1-yl)oxy)dimethylsilane (19)**

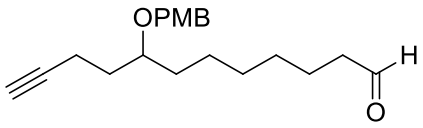
To a mixture of 12-((tert-butyldimethylsilyl)oxy)-1-(trimethylsilyl)dodec-1-yn-5-ol (364.1 mg, 0.946 mmol) in 8 mL of a 4:1 mixture of cyclohexane to dichloromethane was added pyridinium *p*-toluenesulfonate (24 mg, 0.0946 mmol) followed by 4-methoxybenzyl 2,2,2-trichloroacetimidate (0.295 mL, 1.42 mmol). The reaction mixture was allowed to stir at rt for 16 h before it was filtered through silica (20% EtOAc in hexanes as the eluent) and concentrated under reduced pressure. The crude residue was purified through column chromatography (SiO_2, 2.5% EtOAc in hexanes) to yield 291 mg (61%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, *J* = 6.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.46 (d, *J* = 11.0 Hz, 1H), 4.42 (d, *J* = 11.0 Hz, 1H), 3.80 (s, 3H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.48 (quint. *J* = 4.0 Hz, 1H), 2.32 (t, *J* = 7.25 Hz, 1H), 1.72 (d, *J* = 13.5 Hz, 1H), 1.71 (d, *J* = 13 Hz, 1H), 1.59 – 1.25 (m, 14H), 0.89 (s, 9H), 0.14 (s, 9H), 0.05 (s, 6H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 130.9, 129.4, 113.7, 107.5, 84.5, 77.3, 70.8, 63.3, 55.2, 33.7, 32.9, 29.8, 29.4, 26.0, 25.8, 25.2, 18.4, 16.1, 0.1, -5.3. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 527.4, found 527.2.



8-((4-Methoxybenzyl)oxy)dodec-11-yn-1-ol (20)

1.1 mL (1.1 mmol) of a 1.0 M solution of tetrabutylammonium fluoride (TBAF) in THF were added to a solution of tert-butyl((8-((4-methoxybenzyl)oxy)-12-(trimethylsilyl)dodec-11-yn-1-yl)oxy)dimethylsilane (266 mg, 0.527 mmol) in 10.5 mL of THF. The reaction mixture was

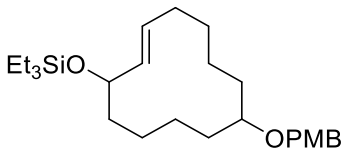
stirred for 2 h at rt before it was quenched with aqueous saturated ammonium chloride and transferred to a separatory funnel. The aqueous layer was extracted thrice with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2, 40% EtOAc in hexanes) to yield 165 mg (98%) of the desired product as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 7.2 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.74 (d, *J* = 10.8 Hz, 1H), 4.42 (d, *J* = 10.8 Hz, 1H), 3.89 (s, 3H), 3.64 (t, *J* = 5.8 Hz, 2H), 3.50 (quint, *J* = 6.0 Hz, 1H), 2.29 (td, *J* = 7.6, 2.4 Hz, 2H), 1.94 (m, 1H), 1.74 (d, *J* = 6.4, 1H), 1.71 (d, *J* = 7.2, 1H), 1.58 – 1.25 (m, 14H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 130.9, 129.3, 113.7, 84.4, 77.1, 70.8, 68.3, 62.8, 55.2, 33.6, 32.8, 32.6, 29.6, 29.3, 25.6, 25.1, 18.4, 14.6. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 341.2, found 341.2.



8-((4-Methoxybenzyl)oxy)dodec-11-ynal (21)

Dess-Martin periodinane (439 mg, 1.04 mmol) was added to a solution of 8-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol (165 mg, 0.518 mmol) in 5.2 mL of CH_2Cl_2 at 0 °C. The mixture was allowed to stir for 3.5 h as it cooled gradually to rt, then it was quenched with saturated sodium bicarbonate solution and stirred for an additional 10 min before it was transferred to a separatory funnel. The aqueous layer was washed thrice with EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2, 30% EtOAc in hexanes) to yield 134 mg (82%) of the aldehyde as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (s, 1H) 7.26 (d, *J* = 6 Hz, 2H), 6.87 (d, *J* = 8 Hz, 2H), 4.47 (d, *J* = 11 Hz,

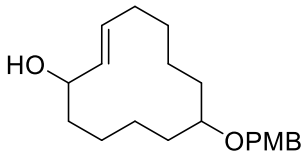
1H), 4.43 (d, *J* = 11 Hz, 1H), 3.8 (s, 3H), 3.50 (quint. *J* = 5.8 Hz, 1H), 2.42 (d, *J* = 7.5 Hz, 2H), 2.28 (d, *J* = 7.5 Hz, 2H), 1.94 (m, 1H), 1.75 – 1.25 (m, 12H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.7, 159.1, 130.8, 129.3, 113.7, 84.4, 77.0, 70.8, 68.3, 55.2, 43.8, 33.5, 32.8, 29.4, 29.0, 24.9, 21.9, 14.6. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 339.2, found 339.2.



(*E*)-Triethyl((8-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (22)

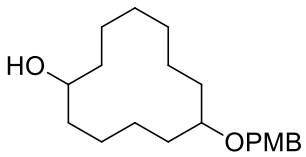
Tetrahydrofuran (25 mL) was injected to a mixture of $Ni(cod)_2$ (34 mg, 0.12 mmol), IMes·HCl (35 mg, 0.10 mmol), and potassium *tert*-butoxide (11 mg, 0.10 mmol) at rt and was stirred for 20 min. Triethylsilane (106 µL, 0.42 mmol) was added, followed by syringe drive addition of a 25 mL THF solution of 8-((4-methoxybenzyl)oxy)dodec-11-ynal (105 mg, 0.33 mmol) over 3 h. The reaction mixture was then stirred at rt for 8 h, then concentrated, filtered through a plug of silica eluting with 50% EtOAc in hexanes, re-concentrated and purified by column chromatography (SiO_2, 2.5% EtOAc in hexanes) to afford (*E*)-triethyl((8-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (57 mg, 40%) as a colorless oil (9:1 mixture of diastereomers, only major diastereomer reported in ^{13}C NMR) ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.27 (d, *J* = 7.7 Hz, 2H), 6.83 (d, *J* = 7.7 Hz, 2H), 5.44 (ddd, *J* = 15.4, 9.8, 5.6 Hz, 1H), 5.32 (dd, *J* = 15.4, 7.7 Hz, 0.9H), 5.15 (dd, *J* = 15.5, 7.5 Hz, 0.1H), 4.48 – 4.38 (m, 2H), 4.03 (td, *J* = 8.4, 4.2 Hz, 0.9 H), 3.96 – 3.91 (m, 0.1H), 3.80 (s, 3H), 3.40 (td, *J* = 6.7 4.3 Hz, 0.9H), 3.34 – 3.29 (m, 1H), 2.30 – 2.18 (m, 1H), 2.16 – 2.08 (m, 1H), 1.80 – 1.10 (m, 14H), 0.93 (t, *J* = 11.2 Hz, 9H), 0.56 (q, *J* = 11.2 Hz, 6H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 159.0, 133.9, 131.2, 131.1,

129.3, 113.7, 76.6, 74.5, 69.8, 55.2, 35.5, 30.3, 29.4, 27.2, 25.9, 25.7, 21.7, 21.1, 6.8, 4.9. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 455.3, found 455.3.



(*E*)-8-((4-Methoxybenzyl)oxy)cyclododec-2-enol (23)

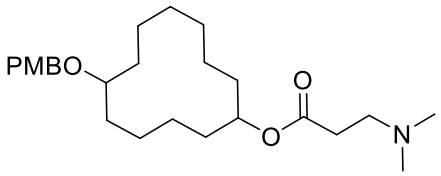
Following the procedure employed to synthesize 7-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol, (E)-triethyl((8-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (41 mg, 0.094 mmol) and 1.0 M TBAF (122 µL, 0.122 mmol) were employed to afford (E)-8-((4-methoxybenzyl)oxy)cyclododec-2-enol (30 mg, quantitative) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 5.6 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.56 (ddd, *J* = 15.2, 9.6, 5.2 Hz, 1H), 5.36 (dd, *J* = 15.2, 8.0 Hz, 0.9 H), 5.12 (dd, *J* = 15.2, 7.2 Hz, 0.1 H), 4.48 – 4.38 (m, 2H), 4.11 – 4.04 (m, 1H), 3.80 (s, 3H), 3.44 – 3.37 (m, 0.9H), 3.33 – 3.26 (m, 0.1H), 2.32 – 2.20 (m, 1H), 2.19 – 2.09 (m, 1H), 1.80 – 1.08 (m, 14H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 133.1, 132,9, 131.2, 129.3, 113.7, 76.5, 74.5, 69.8, 55.3, 34.5, 30.0, 29.3, 27.1, 26.1, 25.6, 21.8, 21.0. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 341.3, found 341.3



6-((4-Methoxybenzyl)oxy)cyclododecanol (24)

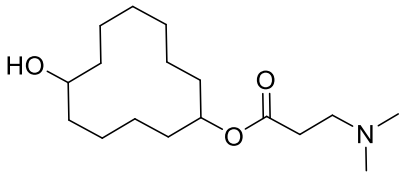
To a solution of (*E*)-8-((4-methoxybenzyl)oxy)cyclododec-2-enol (12 mg, 0.037 mmol) in methanol (12 mL) was added 10 % Pd/C (4 mg). The system was purged with a H_2 balloon for 20 min. The balloon was then refilled and the reaction was allowed to stir at rt under a H_2

atmosphere for 40 min. The reaction mixture was filtered through a plug of silica gel with 1:1 ethyl acetate: hexanes to afford 6-((4-methoxybenzyl)oxy)cyclododecanol (7 mg, 58%) after flash column chromatography (30% ethyl acetate/ hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.54 – 4.33 (m, 2H), 3.85 – 3.75 (m, 4H), 3.52 – 3.45 (m, 1H), 1.81 – 1.16 (m, 20H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 159.0, 131.2, 129.2, 113.7, 76.0, 69.8, 55.3, 33.4, 31.2, 28.7, 28.1, 24.8, 24.3, 22.6, 22.3, 19.7, 19.0. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 343.3, found 343.3.



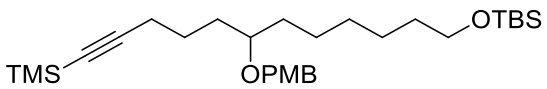
6-((4-Methoxybenzyl)oxy)cyclododecyl 3-(dimethylamino)propanoate (25)

Following the procedure employed to synthesize cyclododecyl 3-(dimethylamino)propanoate, 6-((4-methoxybenzyl)oxy)cyclododecanol (4.5 mg, 0.014 mmol), 3-*N*,*N*-dimethylaminobutyric acid hydrochloride (3.2 mg, 0.021 mmol), triethylamine (3.3 µL, 0.024 mmol), DCC (4.4 mg, 0.021 mmol), DMAP (2.6 mg, 0.021 mmol) in 0.56 mL of CH_2Cl_2 were employed to yield 2.9 mg (49% yield) of the desired product as a colorless oil after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.03 – 4.92 (m, 1H), 4.54 – 4.32 (m, 2H), 3.79 (s, 3H), 3.52 – 3.42 (m, 1H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.44 (t, *J* = 7.3 Hz, 2H), 2.23 (s, 6H), 1.82 – 1.18 (m, 20H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 159.0, 131.2, 129.2, 113.7, 75.9, 72.1, 69.8, 55.3, 54.8, 45.2, 33.2, 30.0, 28.7, 28.1, 27.8, 24.7, 24.1, 22.2, 22.1, 19.9, 18.9. LRMS (ESI) *m/z* calculated for $[M+H]^+$ 420.3, found 420.3.



6-Hydroxycyclododecyl 3-(dimethylamino)propanoate (26)

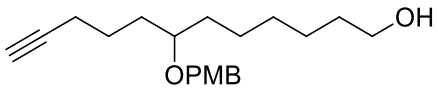
To a solution of 6-((4-methoxybenzyl)oxy)cyclododecyl 3-(dimethylamino)propanoate (2.9 mg, 0.007 mmol) in CH_2Cl_2:H_2O (10:1, 0.87 mL) cooled to 0°C was added 2,2-dchloro-5,6-dicyano-1,4-benzoquinone (4.8 mg, 0.021 mmol). The solution was stirred at 0°C for 30 mins before it was filtered through a pad of celite with CH_2Cl_2 as the eluent. The crude residue was chromatographed over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH) to yield 2.0 mg (97%) of 6-hydroxycyclododecyl 3-(dimethylamino)propanoate as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) 5.05 – 4.95 (m, 1H), 3.82 (qd, *J* = 6.5, 3.5 Hz, 1H), 2.60 (t, *J* = 7.3 Hz, 2H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.24 (s, 6H), 1.82 – 1.22 (m, 20H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.1, 72.2, 69.4, 54.8, 45.2, 33.2, 32.8, 31.1, 29.6, 27.8, 24.5, 24.3, 22.2, 22.1, 19.6, 19.2. IR (thin film, cm^{-1}) 3351, 2937, 2851, 1726, 1468. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 300.2539, found 300.2548 and 300.2542.



***tert*-Butyl((7-((4-methoxybenzyl)oxy)-12-(trimethylsilyl)dodec-11-yn-1-yl)oxy)dimethylsilane (27)**

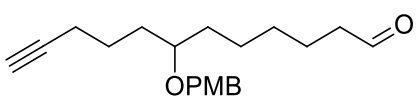
Following the procedure employed to synthesize tert-butyl((8-((4-methoxybenzyl)oxy)-12-(trimethylsilyl)dodec-11-yn-1-yl)oxy)dimethylsilane, 12-((tert-butyldimethylsilyl)oxy)-1-(trimethylsilyl)dodec-1-yn-6-ol (930 mg, 2.42 mmol), 20.6 mL of a 4:1 mixture of cyclohexane to dichloromethane, pyridinium *p*-toluenesulfonate (62 mg, 0.242 mmol) and 4-methoxybenzyl 2,2,2-trichloroacetimidate (0.753 mL, 3.63 mmol) were employed to yield the desired product as

a colorless oil (781 mg, 64%) purified through column chromatography (SiO_2, 5% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.43 (s, 2H), 3.80 (s, 3H), 3.60 (t, *J* = 6.8 Hz, 2H), 3.37 (quint. *J* = 5.3 Hz, 1H), 2.25 – 2.19 (m, 2H), 1.65 – 1.26 (m, 14H), 0.90 (s, 9H), 0.15 (s, 9H), 0.05 (s, 6H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 131.1, 129.3, 113.7, 107.4, 84.6, 77.9, 70.2, 63.2, 55.2, 33.7, 32.8, 32.6, 29.6, 26.0, 25.8, 25.3, 24.2, 19.9, 18.3, 0.1, -5.3. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 527.4, found 527.2.



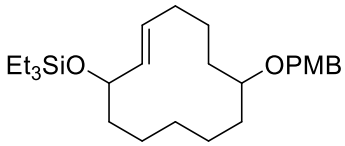
7-((4-Methoxybenzyl)oxy)dodec-11-yn-1-ol (28)

Following the procedure employed to synthesize 7-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol, 1.1 mL (1.1 mmol) of a 1.0 M solution TBAF in THF, tert-butyl(7-((4-methoxybenzyl)oxy)-12-(trimethylsilyl)dodec-11-yn-1-yl)oxy)dimethylsilane (781 mg, 1.55 mmol) and 20 mL of THF, were employed to yield the desired product (493 mg, quantitative) as a colorless oil after purification by column chromatography (SiO_2, 40% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.43 (s, 1H), 3.80 (s, 3H), 3.64 (t, *J* = 6.0 Hz, 2H), 3.37 (quint. *J* = 5.3 Hz, 1H), 2.19 (td, *J* = 6.0, 2.5 Hz, 2H), 1.94 (t, *J* = 2.5 Hz, 1H), 1.66 – 1.20 (m, 14H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 140.0, 129.3, 113.7, 84.4, 77.9, 70.3, 68.4, 62.9, 55.2, 33.7, 32.7, 29.5, 25.7, 25.2, 18.4. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 341.2, found 341.2.



7-((4-Methoxybenzyl)oxy)dodec-11-ynal (29)

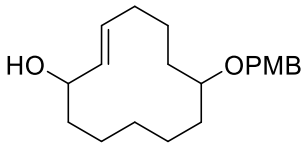
Following the procedure used to synthesize 6-((4-methoxybenzyl)oxy)dodec-11-ynal, Dess-Martin periodinane (984 mg, 2.32 mmol), 7-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol (493 mg, 1.55 mmol), 15.5 mL of CH_2Cl_2 were employed to yield 349 mg (71%) of the aldehyde as a colorless oil following purification by column chromatography (SiO_2, 30% EtOAc in hexanes) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 1H) 7.26 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.27 (s, 2H), 3.80 (s, 3H), 3.37 (quint. *J* = 5.2 Hz, 1H), 2.42 (t, *J* = 7.2 Hz, 2H), 2.23 – 2.15 (m, 2H), 1.96 – 1.93 (m, 1H), 1.68 – 1.25 (m, 12H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 202.7, 159.0, 130.9, 129.3, 113.7, 84.4, 77.7, 77.2, 70.3, 68.4, 55.2, 43.8, 33.5, 32.7, 29.2, 25.0, 24.1, 21.9, 18.4. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 339.2, found 339.2.



(*E*)-Triethyl((7-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (30)

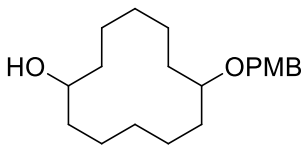
Following the procedure employed to synthesize (*E*)-triethyl((8-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane a mixture of $Ni(cod)_2$ (102 mg, 0.364 mmol), IMes·HCl 107 mg, 0.303 mmol), and potassium *tert*-butoxide (35 mg, 30 mmol) in 60 mL of THF to which was added Triethylsilane (323 µL, 2.02 mmol) and 75 mL THF solution of 7-((4-methoxybenzyl)oxy)dodec-11-ynal (320 mg, 1.01 mmol) were combined to afford (*E*)-triethyl((8-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (131 mg, 29%) as a colorless oil (1:1 mixture of diastereomers) after column chromatography (SiO2, 2.5 % EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 2H), 6.90 – 6.85 (m, 2H), 5.45 – 5.26 (m, 2H), 4.48 – 4.39 (m, 2H), 3.97 (td, *J* = 8.3, 3.3 Hz, 1H), 3.80 (s, 3H), 3.52 – 3.45 (m, 0.5H), 3.40 (quint. *J* = 5.3 Hz, 0.5H), 2.22 – 2.15 (m, 1H), 2.08 –1.93 (m, 1H), 1.82 – 1.13 (m, 14H), 0.93 (t, *J* = 8.0 Hz, 9H), 0.56 (q, *J* = 8.0 Hz, 6H) ^{13}CNMR (175 MHz, $CDCl_3$) δ 159.1, 159.0,

135.3, 134.9, 131.3, 131.2, 130.8, 130.2, 129.4, 129.4, 113.7, 113.6, 75.7, 74.8, 74.5, 73.4, 69.8, 69.7, 55.2, 55.2, 36.0, 35.9, 31.8, 31.3, 30.5, 29.9, 29.7, 29.6, 29.4, 26.4, 24.9, 24.8, 22.9, 22.0, 22.0, 21.9, 21.9, 6.8, 4.9. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 455.3, found 455.3.



(*E*)-7-((4-Methoxybenzyl)oxy)cyclododec-2-enol (31)

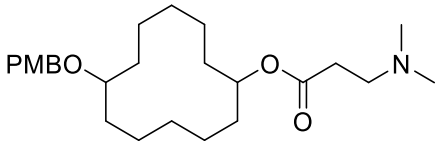
Following the procedure employed to synthesize 7-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol, (*E*)-triethyl((7-((4-methoxybenzyl)oxy)cyclododec-2-en-1-yl)oxy)silane (86 mg, 0.198 mmol) and 1.0 M TBAF (237 µL, 0.237 mmol) were employed to afford (*E*)-7-((4-methoxybenzyl)oxy)cyclododec-2-enol (63 mg, quantitative) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.58 – 5.45 (m, 1H), 5.42 – 5.28 (m, 1H), 4.48 – 4.38 (m, 2H), 4.08 – 3.99 (m, 1H), 3.81 (s, 3H), 3.52 – 3.45 (m, 0.4H), 3.42 – 3.37 (m, 0.6H), 2.25 – 1.28 (m, 14H) ^{13}CNMR (175 MHz, $CDCl_3$) δ 159.1, 134.5, 134.4, 132.3, 132.2, 131.2, 129.4, 129.4, 113.7, 75.8, 74.5, 74.5, 73.2, 69.8, 69.8, 55.3, 55.3, 34.9, 34.6, 31.9, 31.4, 30.4, 29.9, 29.8, 29.7, 29.6, 26.3, 24.8, 24.6, 23.1, 22.3, 22.0, 22.0. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 341.3, found 341.3.



7-((4-Methoxybenzyl)oxy)cyclododecanol (32)

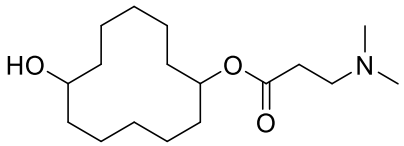
Following the procedure employed to synthesize 6-((4-methoxybenzyl)oxy)cyclododecanol, (E)-7-((4-methoxybenzyl)oxy)cyclododec-2-enol (22 mg, 0.068 mmol) in methanol (20 mL), 10 % Pd/C (7 mg) under H_2 atmosphere afforded 7-((4-methoxybenzyl)oxy)cyclododecanol (13 mg,

59%) after flash column chromatography (30% ethyl acetate/ hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 6.89 – 6.85 (m, 2H), 4.44 (s, 2H), 3.84 (tt, *J* = 8.0, 3.0 Hz, 0.5H), 3.80 (s, 3H), 3.78 – 3.72 (m, 0.5H), 3.52 – 3.41 (m, 1H), 1.74 – 1.20 (m, 20H) ^{13}CNMR (175 MHz, $CDCl_3$) δ 159.0, 159.0, 131.2, 131.2, 129.3, 129.0, 113.7, 77.1, 75.2, 70.9, 69.9, 69.6, 69.0, 55.3, 55.3, 32.4, 30.7, 28.9, 27.5, 26.0, 24.4, 20.8, 20.8, 19.1, 18.9. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 343.3, found 343.3.



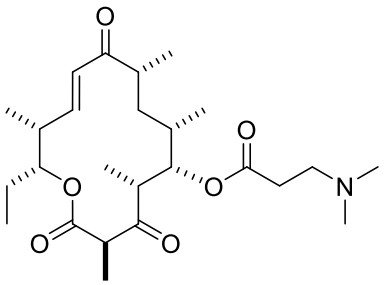
7-((4-MMethoxybenzyl)oxy)cyclododecyl 3-(dimethylamino)propanoate (33)

Following the procedure employed to synthesize cyclododecyl 3-(dimethylamino)propanoate, 7-((4-methoxybenzyl)oxy)cyclododecanol (4.6 mg, 0.014 mmol), 3-N,N-dimethylaminobutyric acid hydrochloride (3.3 mg, 0.022 mmol), triethylamine (3.4 µL, 0.024 mmol), DCC (4.5 mg, 0.022 mmol), DMAP (2.7 mg, 0.022 mmol) in 0.57 mL of CH_2Cl_2 were employed to yield 3.0 mg (51% yield) of the desired product as a colorless oil after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.29 – 7.24 (m, 2H), 6.88 – 6.85 (m, 2H), 4.97 (tt, *J* = 7.0, 2.8 Hz, 0.5H), 4.91 (tt, *J* = 8.1, 2.8 Hz, 0.5H), 4.44 (s, 2H), 3.80 (s, 3H), 3.55 – 3.46 (m, 1H), 2.65 – 2.60 (m, 2H), 2.50 – 2.40 (m, 2H), 2.25 (s, 6H), 1.72 – 1.22 (m, 20H) ^{13}CNMR (175 MHz, $CDCl_3$) δ 172.2, 171.8, 159.0, 159.0, 131.2, 129.3, 129.0, 113.7, 113.7, 77.1, 75.7, 73.7, 72.0, 70.2, 69.6, 55.3, 54.8, 45.2, 33.2, 33.2, 28.9, 28.9, 27.4, 27.3, 25.9, 24.4, 20.7, 20.7, 19.0, 18.9. LRMS (ESI) *m/z* calculated for $[M+H]^+$ 420.3, found 420.3.



7-hydroxycyclododecyl 3-(dimethylamino)propanoate (34)

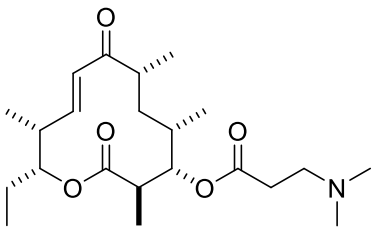
Following the procedure employed to synthesize 6-hydroxycyclododecyl 3-(dimethylamino)propanoate**,** 6-((4-methoxybenzyl)oxy)cyclododecyl 3-(dimethylamino)propanoate (4.8 mg, 0.011 mmol) in CH_2Cl_2:H_2O (10:1, 1.4 mL, DDQ (7.9 mg, 0.034 mmol) were combined to yield 7-hydroxycyclododecyl 3-(dimethylamino)propanoate (2.8 mg, 82%) as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH). ^{1}H NMR (700 MHz, $CDCl_3$) δ 4.99 (tt, *J* = 7.0, 4.9 Hz, 0.4H), 4.93 (tt, *J* = 8.1, 2.8 Hz, 0.4H), 3.89 – 3.83 (m, 1H), 2.66 (t, *J* = 7.4 Hz, 2H), 2.36 (td, *J* = 7.7, 4.2 Hz, 2H), 2.27 (s, 6H), 1.72 –1.22 (m, 20H) ^{13}CNMR (175 MHz, $CDCl_3$) δ 172.2, 171.8, 73.6, 71.8, 70.8, 68.7, 55.8, 55.8, 45.2, 45.2, 32.4, 30.8, 29.1, 27.5, 25.9, 24.1, 20.9, 20.9, 19.1, 18.9. IR (thin film, cm^{-1}) 3405, 2935, 2851, 1726, 1468. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 300.2539, found 300.2548 and 300.2547.



(*3R,5R,6S,7S,9R,13R,14R,E*)-14-ethyl-3,5,7,9,13-pentamethyl-2,4,10-trioxooxacyclotetradec-11-en-6-yl 3-(dimethylamino)propanoate (35)

17 mg of narbonolide, isolated from Kdes1 strain, (0.051 mmol, 1 equiv) were dissolved in 0.7 mL of dichloromethane along with 9.8 mg 3-*N*,*N*-dimethylaminopropanoic acid hydrochloride (0.064 mmol, 1.25 equiv), 11 µL of freshly distilled triethylamine (0.077 mmol, 1.50 equiv),

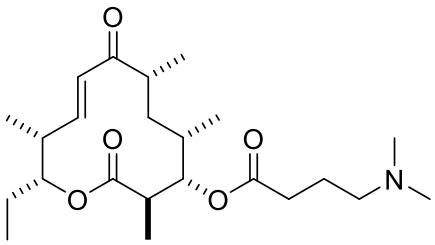
30.6 mg of dicyclohexylcarbodiimide (0.147 mmol, 1.25 equiv) and 7.9 mg 4-dimethylaminopyridine (0.064 mmol, 1.25 equiv). The reaction mixture was allowed to stir at room temperature for 6 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was transferred to a separatory funnel by washing the flask with 0.1 M HCl and 30% EtOAc in hexanes. The acidified aqueous phase was extracted thrice with 30% EtOAc in hexanes. Then the aqueous layer was basified with saturated aqueous $NaHCO_3$ solution until it reached a pH of about 8-9, measured by pH paper. The basified aqueous layer was extracted thrice with dichloromethane. The dichloromethane organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc) to yield 6.4 mg (28% yield) of the ester derivative. ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 15.4, 6.3 Hz, 1H), 6.16 (dd, *J* = 15.8, 1.8 Hz, 1H), 5.12 (d, *J* = 11.2, 1H), 5.07 – 4.98 (m, 1H), 3.77 (q, *J* = 7.0 Hz, 1H), 2.92 (dq, *J* = 7.0 Hz, 7.0 Hz, 1H), 2.74 – 2.66 (m, 2H), 2.65 – 2.57 (m, 2H), 2.53 – 2.46 (m, 2H), 2.23 (s, 6H), 1.72 – 1.68 (m, 1H), 1.64 – 1.57 (m, 3H), 1.35 (d, *J* = 6.3 Hz, 1H), 1.17 (d, *J* = 7.7 Hz, 3H), 1. 16 – 1.13 (m, 4H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 206.2, 202.8, 171.8, 169.1, 147.6, 126.9, 78.6, 75.1, 54.8, 50.6, 47.7, 45.2, 45.0, 38.4, 35.6, 34.8, 33.1, 23.0, 17.4, 16.4, 14.8, 13.6, 12.5, 10.5.



(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-(dimethylamino)propanoate (37)

8.0 mg of 10-deoxymethynolide, isolated from Kdes1 strain, (0.098 mmol, 1 equiv) were dissolved in 2 mL of dichloromethane along with 22.6 mg 3-*N*,*N*-dimethylaminopropanoic acid hydrochloride (0.147 mmol, 1.5 equiv), 23.2 µL of freshly distilled triethylamine (0.166 mmol, 1.7 equiv), 30.6 mg of dicyclohexylcarbodiimide (0.147 mmol, 1.5 equiv) and 18.1 mg 4-dimethylaminopyridine (0.147 mmol, 1.5 equiv). The reaction mixture was allowed to stir at room temperature for 6 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was transferred to a separatory funnel by washing the flask with 0.1 M HCl and 30% EtOAc in hexanes. The acidified aqueous phase was extracted thrice with 30% EtOAc in hexanes. The organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (30% EtOAc in hexanes) to recover the unreacted 10-deoxymethinolide. Then the aqueous layer was basified with saturated aqueous $NaHCO_3$ solution until it reached a pH of about 8-9, measured by pH paper. The basified aqueous layer was extracted thrice with dichloromethane. The dichloromethane organic layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc) to yield 13.3 mg (34% yield) of the 10-dml ester derivative. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 15.7, 5.5 Hz, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 5.12 (d, *J* = 11.1, 1H), 5.07 – 5.00 (m, 1H), 2.74 (dq, *J* = 10.8 Hz, 6.8 Hz, 1H), 2.68 – 2.58 (m, 3H), 2.57 – 2.46 (m, 3H), 2.25 (s, 6H), 1.80 – 1.64 (m, 2H), 1.60 – 1.51 (m, 1H), 1.44 – 1.35 (m, 1H), 1.28 – 1.22 (m, 3H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.88 (d,

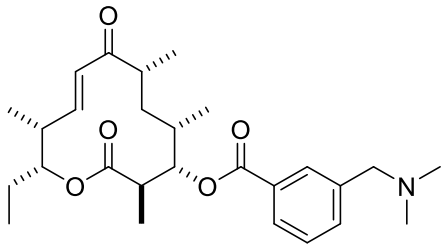
J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.6, 173.8, 171.9, 147.3, 125.4, 78.5, 74.0, 54.8, 45.1, 45.1, 41.7, 38.2, 34.1, 33.0, 32.7, 25.1, 17.7, 17.1, 15.9, 10.3, 9.5. IR (thin film, cm^{-1}) 2965, 2930, 1728, 1689, 1625, 1457, 1380, 1324. HRMS (ESI) *m/z* calculated for $[M+H]^+$, found HRMS (ESI) *m/z* calculated for $[M+H]^+$ 396.2750, found 396.2856



(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 4-(dimethylamino)butanoate (38)

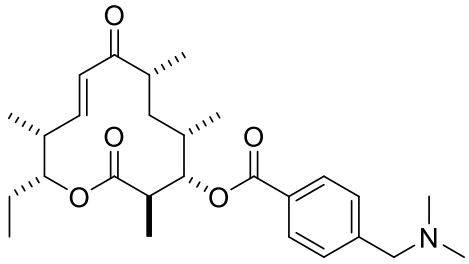
Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-(dimethylamino)propanoate, 10-dml (25 mg, 0.084 mmol), 3-*N*,*N*-dimethylaminobutyric acid hydrochloride (22 mg, 0.13 mmol), triethylamine (20 µL, 0.14 mmol), DCC (27 mg, 0.13 mmol), DMAP (16 mg, 0.13 mmol) in 1.7 mL of CH_2Cl_2 were employed to yield 14.5 mg (42% yield) of the desired product after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (dd, *J* = 15.8, 5.4 Hz, 1H), 6.41 (dd, *J* = 15.4, 1.0 Hz, 1H), 5.11 (dd, *J* = 10.8, 1.2 Hz, 1H), 5.03 (ddd, *J* = 8.4, 5.6, 2.4 Hz, 2H), 2.75 (dq, *J* = 11.0, 6.8 Hz 1H), 2.68 – 2.59 (m, 1H), 2.58 – 2.45 (m, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.31 (t, *J* = 7.2 Hz, 1H), 2.22 (s, 1H), 1.86 – 1.63 (m, 4H), 1.63 – 1.49 (m, 1H), 1.42-1.34 (m, 1H), 1.31–1.20 (m, 4H), 1.13 (d, *J* = 7.2 Hz, 3H), 1.11 (d, *J* = 7.2 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H) 0.86 (d, *J* = 6.4 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.6, 173.7, 173.0, 147.3, 125.4, 78.3, 74.0, 58.8, 45.3, 45.0, 41.7, 38.1, 34.0, 32.6, 31.9, 25.0, 23.0, 17.6, 17.1, 15.9, 10.3, 9.5. IR (thin film, cm^{-1})

2965, 2936, 2764, 1733, 1688, 1627, 1456. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 410.2906, found 410.3099.



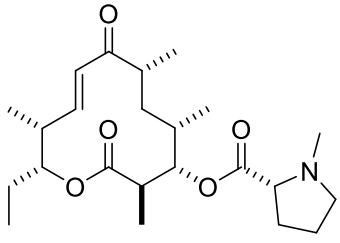
(*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-((dimethylamino)methyl)benzoate (40)

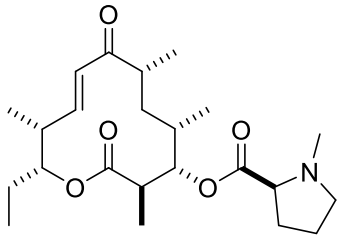
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate 10-deoxymethynolide (120 mg, 0.405 mmol), 109 mg of 3-((dimethylamino)methyl)benzoic acid (0.607 mmol, 1.5 equiv), 127 mg of dicyclohexylcarbodiimide (0.607 mmol, 1.5 equiv) and 75.0 mg 4-dimethylaminopyridine (0.607 mmol, 1.5 equiv) to yield 40.5 mg (22%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 6.80 (dd, *J* = 15.4, 5.6 Hz, 1H), 6.47 (dd, *J* = 15.4, 1.1 Hz, 1H), 5.37 (dd, *J* = 11.2, 1.1 Hz, 1H), 5.07 (ddd, *J* = 8.4, 5.6, 2.1 Hz, 1H), 3.52 (s, 2H), 2.93 (dq, *J* = 10.5, 7.0 Hz, 1H), 2.70 – 2.64 (m, 1H), 2.58 – 2.51 (m, 1H), 2.28 (s, 6H), 1.93 (t, *J* = 14.0 Hz), 1.77 – 1.68 (m, 1H), 1.66 – 1.57 (m, 3H), 1.42 – 1.34 (m, 1H), 1.31 – 1.22 (m, 4H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 7.0 Hz, 3H), 0.95 – 0.91 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.5, 173.8, 166.1, 147.4, 139.5, 133.8, 130.3, 129.8, 128.4, 128.3, 125.4, 79.1, 74.0, 63.8, 45.3, 45.1, 41.9, 38.2, 34.1, 33.0, 25.1, 17.7, 17.2, 16.0, 10.3, 9.5. IR (thin film, cm^{-1}) 2967, 2937, 2818, 2771, 1724, 1688, 1625, 1456.



(*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 4-((dimethylamino)methyl)benzoate (41)

Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate 10-deoxymethynolide (40.0 mg, 0.135 mmol), 36.3 mg of 4-((dimethylamino)methyl)benzoic acid (0.202 mmol, 1.5 equiv), 42.0 mg of dicyclohexylcarbodiimide (0.202 mmol, 1.5 equiv) and 25.0 mg 4-dimethylaminopyridine (0.202 mmol, 1.5 equiv) to yield 37.2 mg (60%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 6.80 (dd, *J* = 15.6, 5.2 Hz, 1H), 6.47 (d, *J* = 15.6 Hz, 1H), 5.36 (d, *J* = 10.8 Hz, 1H), 5.07 (ddd, *J* = 8.4, 5.6, 2.4 Hz, 1H), 3.48 (s, 2H), 2.92 (dq, *J* = 10.8, 6.8 Hz, 1H), 2.72 – 2.62 (m, 1H), 2.60 – 2.49 (m, 1H), 2.26 (s, 6H), 1.93 (t, *J* = 13.0 Hz), 1.80 – 1.66 (m, 1H), 1.64 – 1.46 (m, 2H), 1.44 – 1.34 (m, 1H), 1.26 (d, *J* = 7.2 Hz, 3H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 0.96 – 0.89 (m, 6H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.5, 173.8, 166.0, 147.4, 144.6, 129.7, 129.0, 128.7, 125.4, 79.0, 74.0, 63.9, 45.4, 45.1, 41.9, 38.2, 34.1, 33.0, 25.1, 17.7, 17.2, 16.0, 10.3, 9.5. IR (thin film, cm^{-1}) 2965, 2936, 2771, 1725, 1688, 1627, 1456.





(*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl methyl-L-prolinate (42)

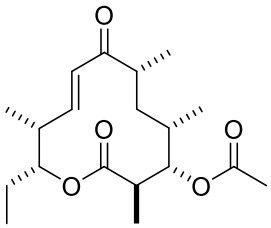
Following the procedure employed to synthesize (*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 4-((dimethylamino)methyl)benzoate, 10-dml (60 mg, 0. mmol), L-*N*-methylproline (39 mg, 0.30 mmol), DCC (63 mg, 0.30 mmol), DMAP (37 mg, 0.30 mmol) in 3 mL of CH_2Cl_2 were employed to yield 46 mg (56% yield) of the desired product after purification over silica gel (gradient from 10% EtOAc in hexanes to 70% EtOAC in hexanes) ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 15.8, 5.4 Hz, 1H), 6.41 (dd, *J* = 16.0, 0.8 Hz, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 5.04 (ddd, *J* = 8.2, 5.8, 2.4 Hz, 1H), 3.20 – 3.12 (m, 1H), 2.98 (t, *J* = 7.2 Hz), 2.79 (dq, *J* = 10.8, 2.8 Hz, 1H), 2.70 – 2.60 (m, 1H), 2.58 – 2.46 (m, 1H), 2.41 (s, 3H), 2.33 (dd, *J* = 15.4, 7.8 Hz), 2.22 – 2.10 (m, 1H), 2.00 – 1.89 (m, 2H), 1.88 – 1.50 (m, 4H), 1.46 – 1.36 (m, 1H), 1.32 – 1.20 (m, 4H), 1.14 (d, *J* = 7.2 Hz, 3H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.2 Hz, 3H) 0.88 (d, *J* = 6.4 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.6, 173.7, 173.1, 147.4, 125.4, 78.5, 74.0, 67.6, 56.0, 45.1, 41.6, 40.6, 38.2, 34.1, 32.6, 29.9, 25.0, 23.0, 17.6, 17.2, 15.8, 10.3, 9.5. IR (thin film, cm^{-1}) 2970, 2935, 1220, 2773, 1749, 1732, 1716, 1682, 1625, 1454, 1323.

(*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl methyl-D-prolinate (43)

Following the procedure used to synthesize (*3R,4S,5S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 4-((dimethylamino)methyl)benzoate, 10-dml (40

mg, 0.13 mmol), D-*N*-methylproline (26 mg, 0.20 mmol), DCC (42 mg, 0.20 mmol), DMAP (25 mg, 0.20 mmol) in 1.8 mL of CH_2Cl_2 were employed to yield 24 mg (44% yield) of the desired product after purification over silica gel (gradient from 10% EtOAc in hexanes to 70% EtOAC in hexanes) ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 16.1, 5.3 Hz, 1H), 6.41 (d, *J* = 16.1 Hz, 1H), 5.18 (d, *J* = 10.5 Hz, 1H), 5.04 (ddd, *J* = 8.1, 5.3, 2.1 Hz, 2H), 3.15 (td, *J* = 7.2, 2.1 Hz), 2.96 (t, *J* = 7.7 Hz), 2.78 (dq, *J* = 11.2, 7.0 Hz, 1H), 2.68 – 2.62 (m, 1H), 2.55 – 2.48 (m, 1H), 2.41 (s, 3H), 2.32 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.21 – 2.13 (m, 2H), 1.84 – 1.76 (m, 2H), 1.75 – 1.67 (m, 1H), 1.60 – 1.50 (m, 1H), 1.42 – 1.38 (m, 1H), 1.27 – 1.21 (m, 4H), 1.15 (d, *J* = 6.3 Hz, 3H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H) 0.88 (d, *J* = 6.3 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.5, 173.6, 173.1, 147.3, 125.5, 78.4, 74.0, 67.5, 56.1, 45.0, 41.6, 40.7, 38.1, 34.1, 32.6, 30.0, 25.0, 23.0, 17.6, 17.1, 15.9, 10.3, 9.5. IR (thin film, cm^{-1}) 2922, 2851, 1727, 1689, 1626, 1455, 1379, 1323.

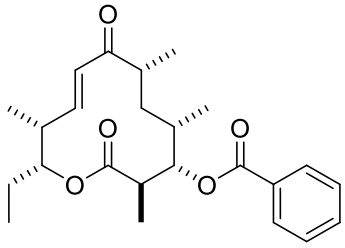
5.2.3 Experimental data for PikC control substrates



(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate (II-S1)

7.5 mg of 10-dml, (0.025 mmol) were dissolved in 0.75 mL of CH_2Cl_2 along with 2.2 µL glacial acetic acid (0.038 mmol), 7.9 mg of DCC (0.038 mmol) and 4.6 mg DMAP (0.038 mmol). The reaction mixture was allowed to stir at room temperature for 5 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (eluent 15% EtOAc in hexanes) to afford 6.7 mg (79% yield) of

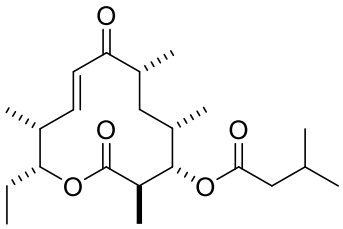
the desired compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.75 (dd, *J* = 15.8, 5.3 Hz, 1H), 6.41 (dd, *J* = 16.0, 1.3 Hz, 1H), 5.10 (dd, *J* = 10.8, 1.3 Hz, 1H), 5.03 (ddd, *J* = 8.3, 5.4, 2.5 Hz, 1H), 2.74 (dq, *J* = 11.0, 7.0 Hz, 1H), 2.68 – 2.61 (m, 1H), 2.55 – 2.47 (m, 1H), 2.08 (s, 3H), 1.78 – 1.63 (m, 2H), 1.62 – 1.49 (m, 1H), 1.44 – 1.35 (m, 1H), 1.29 – 1.21 (m, 4H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.6, 173.7, 170.5, 147.3, 125,4, 78.4, 74.0, 45.1, 41.7, 38.1, 34.0, 32.6, 25.1, 20.7, 17.6, 17.1, 15.9, 10.3, 9.5. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 339.2171, found 339.2167.



(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl benzoate (II-S2)

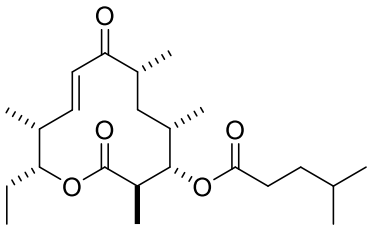
Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, 10-dml (5.7 mg, 0.019 mmol), DCC (6.0 mg, 0.029 mmol), DMAP (3.6 mg, 0.029 mmol., benzoic acid (3.5 mg, 0.029 mmol) in 0.75 mL of CH_2Cl_2 were employed to yield 5.0 mg (66%) of the desired product after it was chromatographed over silica gel (eluent 20% EtOAc in hexanes). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.7 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 6.80 (dd, *J* = 16.1, 5.6 Hz, 1H), 6.47 (d, *J* = 16.1 Hz, 1H), 5.37 (d, *J* = 10.5, 1H), 5.07 (ddd, *J* = 8.4, 5.6, 2.5 Hz, 1H), 2.92 (dq, *J* = 11.2, 7.0 Hz, 1H), 2.70 – 2.65 (m, 1H), 2.58 – 2.52 (m, 1H), 1.93 (t, *J* = 13.0 Hz, 1H), 1.76 – 1.69 (m, 1H), 1.61 – 1.48 (m, 2H), 1.43 – 1.38 (m, 1H), 1.26 (d, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 7.0 Hz, 3H), 0.94 – 0.91 (m, 6H) (175 MHz, $CDCl_3$) δ

204.6, 173.8, 166.1, 147.4, 133.1, 129.9, 129.7, 128.5, 125.4, 79.1, 74.0, 45.1, 41.9, 38.2, 34.1, 33.0, 25.1, 17.7, 17.2, 16.0, 10.3, 9.6. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 401.2328, found



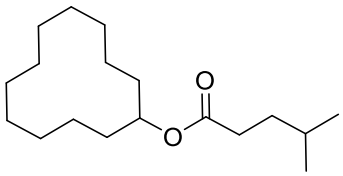
(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-methylbutanoate (II-S3)

Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, 10-dml (6.1 mg, 0.021 mmol), DCC (6.4 mg, 0.031 mmol), DMAP (3.8 mg, 0.031 mmol), isovaleric acid (3.4 µL, 0.031 mmol) in 0.60 mL of CH_2Cl_2 were employed to yield 7.0 mg (88%) of the desired product after it was chromatographed over silica gel (eluent 20% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 15.5, 5.5 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 5.12 (d, *J* = 11.0 Hz, 1H), 5.03 (ddd, *J* = 8.3, 5.5, 2.3 Hz, 1H), 2.74 (dq, *J* = 11.0 Hz, 7.0 Hz), 2.68 – 2.61 (m, 1H), 2.56-2.47 (m, 1H), 2.26 – 2.18 (m, 2H), 2.11 (sept., *J* = 6.8 Hz, 1H), 1.80 – 1.66 (m, 2H), 1.65 – 1.49 (m, 1H), 1.43-1.34 (m, 1H), 1.28 – 1.20 (m, 4H), 1.18 (d, *J* = 7.0 Hz), 1.11 (d, *J* = 7.0 Hz), 0.97 (d, *J* = 6.5 Hz), 0.91 (t, *J* = 7.3 Hz), 0.87 (d, *J* = 6.5 Hz) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.6, 173.8, 172.6, 147.3, 125.4, 78.1, 74.0, 45.1, 43.4, 41.7, 38.1, 34.1, 32.6, 25.5, 25.1, 22.5, 17.6, 17.2, 15.9, 10.3, 9.5. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 381.2641, found 381.2673.



(3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-Ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 4-methylpentanoate (II-S4)

Following the procedure employed to synthesize (*3R,4S*,5*S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, 10-dml (11 mg, 0.038 mmol), DCC (12 mg, 0.057 mmol), DMAP (7 mg, 0.057 mmol, 4-methylvaleric acid (7.2 µL, 0.057 mmol) in 0.80 mL of CH_2Cl_2 were employed to yield 8.7 mg (58%) of the desired product after it was chromatographed over silica gel (eluent 20% EtOAc in hexanes). ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 15.4, 5.3 Hz, 1H), 6.41 (d, *J* = 15.4 Hz, 1H), 5.11 (d, *J* = 10.5 Hz, 1H), 5.03 (ddd, *J* = 8.4, 5.6, 2.5 Hz, 1H), 2.74 (dq, *J* = 14.0, 11.2 Hz, 1H), 2.67-2.62 (m, 1H), 2.51 (qd, *J* = 6.7, 4.2 Hz, 1H), 2.33 (t, *J* = 7.7 Hz, 2H), 1.76 (t, *J* = 13.3 Hz, 1H), 1.73-1.67 (m, 1H), 1.60-1.50 (m, 4H), 1.42-1.35 (m, 1H), 1.27-1.21 (m, 4H), 1.12 (d, *J* = 7.0 Hz, 3H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.92-0.89 (m, 9H), 0.86 (d, *J* = 7.0 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.6, 173.8, 173.5, 147.2, 125.4, 78.1, 74.0, 45.1, 41.7, 38.2, 34.1, 34.0, 32.6, 32.3, 27.7, 25.1, 22.2, 17.7, 17.2, 15.9, 10.3, 9.5. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 395.2797, found 395.2773



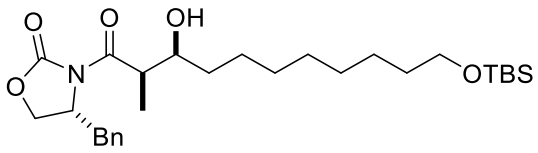
cyclododecyl 4-methylpentanoate (II-S5)

Following the procedure employed to synthesize (*3R,4S*,5*S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, cyclododecanol (25 mg, 0.14 mmol), DCC (42 mg, 0.20 mmol), DMAP (2.5 mg, 0.020 mmol, 4-methylvaleric acid (26 µL, 0.20 mmol) in 1.4 mL of CH_2Cl_2 were employed to yield 38 mg (100%) of the desired product as a colorless oil after it was chromatographed over silica gel (eluent 10% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.01 (tt, *J* = 7.0, 4.8 Hz, 1H), 2.27 (t, *J* = 7.8 Hz, 2H), 1.78 – 1.64 (m,

2H), 1.62 – 1.24 (m, 23H), 0.89 (d, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 71.9, 33.9, 32.7, 29.1, 27.7, 24.0, 23.8, 23.3, 23.2, 22.2, 20.9.

6.3 Chapter 3 Experimental

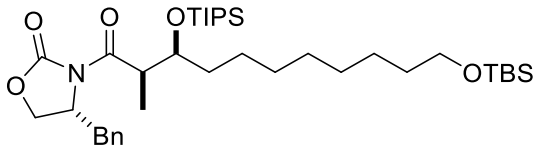
6.3.1 Experimental data for the synthesis of macrolactone 55 and analogues



(*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2-methylundecanoyl)oxazolidin-2-one (50)

To a solution of (*4R*)-3-propionyl-4-benzyl-2-oxazolidinone (1.02 g, 4.40 mmol) in CH_2Cl_2 (9 mL) was added dibutylboron triflate (1.1 mL, 4.59 mmol) followed by triethylamine (0.92 mL, 6.61 mmol) dropwise at 0 °C and stirred for 20 min. The solution was cooled down to -78 ºC. To this solution was added a solution of 9-((tert-butyldimethylsilyl)oxy)nonanal (1.00 g, 3.67 mmol) in CH_2Cl_2 (11 mL) at -78 °C. The resulting solution was stirred for 20 min at -78 ºC. The solution was then warmed to rt and stirred for 7 h. The reaction was terminated by adding 7 mL pH 7 aqueous phosphate buffer solution and methanol (21 mL) at 0 ºC. To this cloudy solution was added a solution of methanol and 30% hydrogen peroxide (2:1, 21 mL) and the resulting solution was stirred for 1 h at 0 ºC. Then the reaction mixture was concentrated to remove organic solvents and it was extracted thrice with diethyl ether. The organic layer was washed with saturated aqueous sodium bicarbonate and saturated aqueous NaCl, dried over Na_2SO_4 and concentrated. The crude residue was purified by silica column chromatography (30% EtOAc in hexanes) to yield 1.46 g (79%) of the desired aldol product as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (t, J =7.0 Hz, 2H), 7.28 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 7.0 Hz, 2H), 4.71 (dddd, J = 9.5, 7.5, 3.0, 3.0 Hz, 1H), 4.23 (dd, J = 8.3, 8.3 Hz, 1H), 4.19 (dd, J = 9.0, 3.0 Hz,

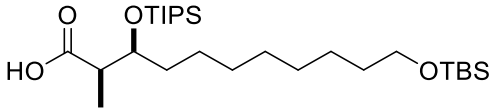
1H), 3.98 – 3.91 (m, 1H), 3.76 (qd, *J* = 7.0, 2.5 Hz, 1H), 3.59 (t, *J* = 6.8 Hz), 3.25 (dd, *J* = 13.5, 2.5 Hz, 1H), 2.86 (s, 1H), 2.79 (dd, *J* = 13.5, 9.5 Hz), 1.59 – 1.23 (m, 14H), 1.25 (d, *J* = 7.5 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 6H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 177.5, 153.0, 135.0, 129.4, 129.0, 127.4, 71.4, 66.2. 63.1, 55.1, 42.1, 37.8, 33.6, 32.7, 26.0, 22.3, 18.3, 10.4, -5.3. IR (thin film, cm^{-1}) 3514, 2926, 2853, 1779, 1692, 1455, 1383. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 506.3302, found 506.3316.



(*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one (51)

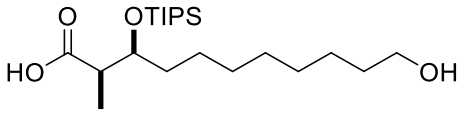
A solution of (*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2-methylundecanoyl)oxazolidin-2-one (1.45 g, 2.87 mmol) in CH_2Cl_2 (11.2 mL) was cooled to 0 °C before 2,6-lutidine (0.67 mL, 5.73 mmol) was added followed by TIPSOTf (1.03 mL, 8.61 mmol). The reaction solution was stirred from 0 °C to rt for 3 h, before it was quenched by the addition of saturated aqueous $NaHCO_3$ solution. The organic solution was separated, washed with saturated aqueous NaCl, and dried over Na_2SO_4. After concentration, the residue was chromatographed over silica gel (7% EtOAc in hexanes) to give 1.90 g of the desired silyl ether (100% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (t, *J* =7.5 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 7.0 Hz, 2H), 4.57 (dddd, *J* = 9.8, 7.5, 2.6, 2.3 Hz, 1H), 4.23 (ddd, *J* = 7.0, 5.0, 5.0 Hz, 1H), 4.16 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.11 (dd, *J* = 9.0, 7.5 Hz, 1H), 3.86 (qd, *J* = 7.0, 4.0 Hz, 1H), 3.59 (t, *J* = 6.8 Hz), 3.32 (dd, *J* = 13.3, 2.8 Hz, 1H), 2.77 (dd, *J* = 13.5, 9.5 Hz), 1.64 – 1.46 (m, 4H), 1.37 – 1.23 (m, 10H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.08 – 1.01 (m, 21H), 0.89 (s, 9H), 0.04 (s, 6H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.2, 153.1, 135.5, 129.5, 128.9, 127.3, 73.2, 66.3, 55.9,

42.5, 37.6, 35.8, 32.9, 29.9, 29.5, 29.4, 26.0, 24.8, 18.3, 18.2, 18.1, 13.0, 10.2, -5.3. IR (thin film, cm^{-1}) 2927, 2862, 1782, 1706, 1462, 1381, 1349. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 662.4636, found 662.4661.



(*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid (52)

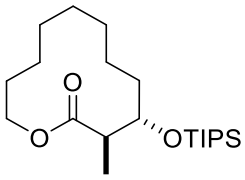
To an ice cooled solution of the aldol adduct (1.90 g, 2.87 mmol) in 21.4 mL of a 4:1 mixture of THF:H_2O was added 30% solution of H_2O_2 (2.34 mL, 23.0 mmol), followed by LiOH (210 mg, 8.61 mmol). The reaction mixture was stirred from 0 °C to rt for 12 h before it was quenched by the addition of 14.7 mL of saturated aqueous Na_2SO_3 solution and 14.7 mL of saturated aqueous NH_4Cl solution. The mixture was transferred to a separatory funnel and the aqueous layer extracted thrice with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated. The crude residue was chromatographed over silica gel (15% EtOAc in hexanes) to yield 1.37 g (95%) of the desired acid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.13 (td, *J* = 5.8, 4.0 Hz, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.71 (qd, *J* = 6.8, 4.0 Hz, 1H), 1.66 – 1.43 (m, 4H), 1.83 – 1.23 (m, 10H), 1.15 (d, *J* = 5.1 Hz, 3H), 0.93 (s, 9H), 0.06 (s, 6H). IR (thin film, cm^{-1}) 2928, 2855, 1706, 1467, 1253. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 503.3946, found 503.3931.



(*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid (53)

To an ice cooled solution of (*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid (295 mg, 0.587 mmol) in 11.7 mL of MeOH was added

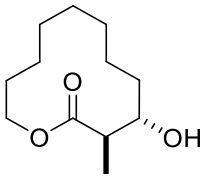
10-camphorsulphonic acid (34.4 mg, 0.147 mmol). The reaction mixture was allowed to stir at 0 °C for 1h and was quenched by the addition of saturated aqueous $NaHCO_3$ solution and transferred to a separatory funnel. The aqueous layer was extracted thrice with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated. The crude residue was used without further purification for macrolactonization (228 mg, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (td, *J* = 5.4, 4.6 Hz, 1H), 3.64 (t, *J* = 6.4 Hz, 2H), 2.70 (qd, 7.0, 4.4 Hz, 1H), 1.63 – 1.49 (m, 4H), 1.42 – 1.22 (m, 10H), 1.14 (d, *J* = 7.2 Hz, 3H), 1.12 – 1.05 (m, 21H) LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 411.3, found 411.2.



(*3R,4S*)-3-methyl-4-((triisopropylsilyl)oxy)oxacyclododecan-2-one (54)

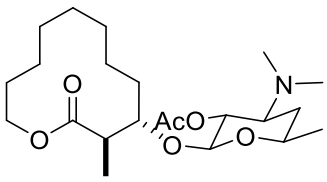
To a solution of (*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid (103 mg, 0.265 mmol) in THF (8.8 mL) was added added DIPEA (620 µL, 3.70 mmol) and 2,4,6-trichlorobenzoyl chloride (254 µL, 1.59 mmol) at 0 °C. The mixture was stirred gradually warming to room temperature for 8 h and diluted with toluene (150 mL) to give a solution of mixed anhydride. The mixed anhydride was added by syringe pump over 22 h to a solution of 4-(dimethylamino)pyridine (648 mg, 5.30 mmol) in toluene (100 mL). After stirring for an additional 13 h at room temperature, the mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with thrice with EtOAc. The combined organic extracts were washed thrice with pH 4 buffer and once with brine, dried over Na2SO4, and concentrated. The crude material was purified by silica gel column chromatography to yield 39.3 mg (40%) of the macrolactone as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.66 (ddd, *J* = 11.0, 6.5, 2.0 Hz, 1H), 4.02 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.76 (ddd, *J* = 11.1, 9.5, 2.0 Hz, 1H), 2.67 (dq, *J* = 9.5, 7.0, 1H), 1.84 – 1.27

(m, 14H), 1.26 (d, *J* = 6.5 Hz, 3H). 1.10 – 1.06 (m, 21H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.7, 74.6, 66.1, 45.5, 33.0, 26.7, 26.0, 25.9. 24.3, 22.9, 18.2, 18.2, 18.2, 15.8, 12.9. IR (thin film) 2945, 2867, 1732, 1464, 1120, 1098, 1058 cm^{-1}. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 393.3, found 393.2.



(*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (55)

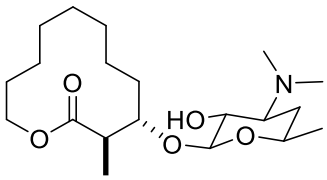
Following the procedure employed to synthesize 7-((4-methoxybenzyl)oxy)dodec-11-yn-1-ol, (*3R,4S*)-3-methyl-4-((triisopropylsilyl)oxy)oxacyclododecan-2-one (14 mg, 0.038 mmol) and TBAF·$3H_20$ (15 mg, 0.045 mmol) were employed to afford (*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (8.1 mg, 99%) as a white solid.^{1}H NMR (700 MHz, $CDCl_3$) δ 4.24 – 4.17 (m, 2H), 3.82 – 3.76 (m, 1H), 2.54 (dq, *J* = 9.8, 7.0 Hz, 1H), 1.74 – 1.24 (m, 17H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 174.7, 72.8, 65.2, 46.9, 32.6, 26.0, 25.0, 24.2, 23.0, 20.5, 14.9. IR (thin film, cm^{-1}) 3430, 2933, 2862 1730, 1709, 1456. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 215.1642, found 215.1632.



(*2S,3R,4S,6R*)-4-(dimethylamino)-6-methyl-2-(((*3R,4S*)-3-methyl-2-oxooxacyclododecan-4-yl)oxy)tetrahydro-2H-pyran-3-yl acetate (int-55a)

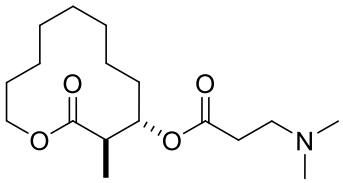
A solution of desosamine fluoride (15.3 mg, 0.070 mmol) in 0.5 mL of DCM was injected into a flame dried rbf containing $HfCp_2Cl_2$ (136 mg, 0.350 mmol), $AgClO_4$ (74.8 mg, 0.350 mmol), 4 Å MS (150 mg) previously weighed in a glovebox. The resulting mixture was allowed to stir at rt

for 20 min before it was cooled down to 0 °C and a solution of (*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (7.5 mg, 0.035 mmol) in 1.5 mL of DCM was added to the reaction mixture. The reaction mixture was allowed to stir under N_2 atm gradually warming to rt for 12 h before it was quenched by the addition of sat'd aq $NaHCO_3$ and extracted with thrice with EtOAc. The combined organic layers were washed with a mixture 75% sat'd aq NaCl and 25% aq $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified through silica column chromatography (1% MeOH in EtOAc) to yield 8.6 mg (59%, β-anomer only) of the glycosylated macrolactone. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82 (dd, *J* = 10.4, 7.6 Hz, 1H), 4.44 (ddd, *J* = 10.8, 6.8, 2.0 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.03 – 3.92 (m, 1H), 3.68 (dt, *J* = 10.0, 3.6 Hz, 1H), 3.58 – 3.46 (m, 1H) 2.80 – 2.66 (m, 2H), 2.26 (s, 6H), 2.06 (s, 3H) 1.80 – 1.20 (m, 22H). LRMS (ESI) *m/z* calculated for $[M+H]^+$ 414.3, found 414.2.



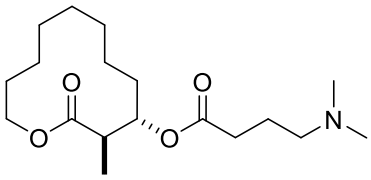
(*3R,4S*)-4-(((*2S,3R,4S,6R*)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-3-methyloxacyclododecan-2-one (55a)

To a solution of the acetate obtained above (x mg, mmol) in xmL of methanol K_2CO_3 () was added as the reaction mixture stirred at rt for 12. The reaction mixture was diluted with brine and extracted 3x with EtOAc. The combined organic layers were dried with Na2SO4, filtered, and concentrated to afford the final macrolide as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 – 4.28 (m, 2H) (ddd, *J* = 9.7, 8.0, 2.4 Hz, 1H), 3.78 (ddd, *J* = 10.4, 4.0, 4.0 Hz, 1H), 3.60 – 3.48 (m, 1H), 3.35 (br s, 1H), 3.28 (dd, *J* = 3.68 10.0, 7.2 Hz, 1H), 2.79 (dq, *J* = 10.0, 6.8 Hz, 1H), 2.58 – 2.47 (m, 1H), 2.28 (s, 6H), 1.79 – 1.18 (m, 22H). LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 394.3, found 394.2.



(*3R,4S*)-3-methyl-2-oxooxacyclododecan-4-yl 3-(dimethylamino)propanoate (55b)

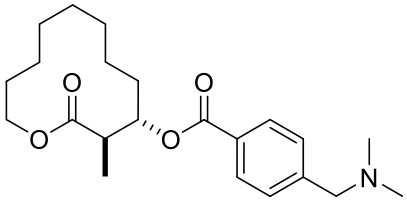
Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-(dimethylamino)propanoate, (*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (10 mg, 0.047 mmol), 3-*N*,*N*-dimethylaminopropanoic acid hydrochloride (8.6 mg, 0.056 mmol), triethylamine (7.8 µL, 0.056 mmol), DCC (11.7 mg, 0.056 mmol), DMAP (0.7 mg, 0.006 mmol) in 1.5 mL of CH_2Cl_2 were employed to yield 4.8 mg (27% yield) of the desired product after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (ddd, *J* = 9.5, 6.0, 3.5 Hz, 1H), 4.27 – 4.17 (m, 2H), 2.72 (dq, *J* = 10.5, 2.0 Hz, 1H), 2.63 (t, *J* = 7.8 Hz, 2H), 2.50 (t, *J* = 7.8 Hz, 2H), 2.25 (s, 6H), 1.76 – 1.19 (m, 14H), 1.16 (d, *J* = 7.0 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.9, 172.0, 74.0, 65.4, 54.8, 45.2, 44.7, 33.0, 29.9, 25.9, 25.0, 24.1, 23.8, 22.9, 20.8, 14.7.



(*3R,4S*)-3-methyl-2-oxooxacyclododecan-4-yl 4-(dimethylamino)butanoate (55c)

Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl 3-(dimethylamino)propanoate, (*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (10 mg, 0.047 mmol), 4-*N*,*N*-dimethylaminobutyric acid hydrochloride (12 mg, 0.070 mmol), triethylamine (11 µL, 0.079 mmol), DCC (15 mg, 0.070 mmol), DMAP (8.6 mg, 0.070 mmol) in 0.7 mL of CH_2Cl_2 were employed to yield 6.6 mg

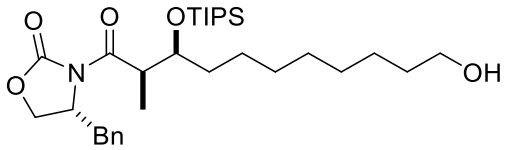
(43% yield) of the desired product as a colorless oil after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$)^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (ddd, *J* = 9.8, 6.5, 4.0 Hz, 1H), 4.28 – 4.17 (m, 2H), 2.71 (dq, *J* = 11.0, 7.0 Hz, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.30 (t, *J* = 7.0 Hz, 2H), 2.22 (s, 6H), 1.83 – 1.20 (m, 16H), 1.15 (d, *J* = 6.5 Hz, 3H). IR (thin film, cm^{-1}) 2926, 2816, 1734, 1460. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 328.2488, found 328.2487.



(*3R,4S*)-3-methyl-2-oxooxacyclododecan-4-yl 4-((dimethylamino)methyl)benzoate (55d)

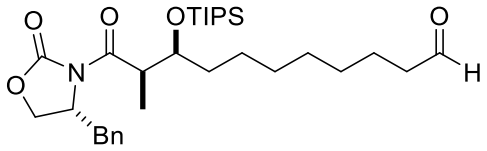
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate (*3R,4S*)-4-hydroxy-3-methyloxacyclododecan-2-one (22 mg, 0.098 mmol), 26 mg of 4-((dimethylamino)methyl)benzoic acid (0.15 mmol, 1.5 equiv), 31 mg of dicyclohexylcarbodiimide (0.15 mmol, 1.5 equiv) and 18 mg 4-dimethylaminopyridine (0.15 mmol, 1.5 equiv) to yield 8.6 mg (23%) of the desired ester derivative after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 5% MeOH in EtOAc) ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 5.51 (ddd, *J* = 9.1, 5.6, 3.5 Hz, 1H), 4.26 (t, *J* = 4.9 Hz, 2H), 3.50 (s, 2H), 2.89 (dq, *J* = 10.5, 7.0 Hz, 1H), 2.26 (s, 6H), 1.80 – 1.24 (m, 14H), 1.22 (d, *J* = 6.3 Hz, 3H). HRMS (ESI) *m/z* calculated for $[M+H]^+$ 376.2488, found 376.2487.

6.3.2 Experimental data for the synthesis of macrolactone 63



(*R*)-4-benzyl-3-((*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one (56)

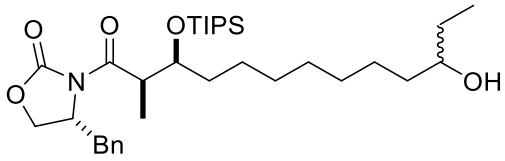
Following the procedure used to synthesize (*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid, (*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one (671 mg, 1.01 mmol), 10-camphorsulphonic acid (48 mg, 0.203 mmol) in MeOH (20 mL) were employed to yield 459 mg (83%) of the monoprotected diol as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (t, *J* = 7.3 Hz, 2H), 7.28 (t, *J* = 7.0 Hz, 1H), 7.22 (d, *J* = 7.0 Hz, 2H), 4.57 (tdd, *J* = 9.5, 5.0, 2.5 Hz, 1H), 4.23 (dt, *J* = 7.5, 4.5 Hz, 1H), 4.16 (dd, *J* = 9.3, 2.3 Hz, 1H), 4.11 (dd, *J* = 8.5, 7.5 Hz, 1H), 3.86 (qd, *J* = 7.0, 4.0 Hz, 1H), 3.64 (t, *J* = 6.5 Hz, 2H), 3.32 (dd, *J* = 13.5, 3.0 Hz, 1H), 2.77 (dd, *J* = 13.5, 10.0 Hz, 1H), 1.66 – 1.52 (m, 4H), 1.38 – 1.24 (m, 10H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.07 – 1.02 (m, 21H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.1, 153.1, 135.4, 129.5, 128.9, 127.3, 73.2, 66.0, 55.9, 42.5, 37.6, 35.7, 32.7, 29.8. 29.4, 29.3, 25.6, 24.8, 18.2, 18.1, 13.0, 10.1. IR (thin film, cm^{-1}) 3405, 2927, 2563, 1778, 1702, 1455, 1381, 1349. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 548.3771, found 548.3752



(*9S,10R*)-11-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-10-methyl-11-oxo-9-((triisopropylsilyl)oxy)undecanal (57)

Following the procedure used to synthesize 6-((4-methoxybenzyl)oxy)dodec-11-ynal, Dess-Martin periodinane (141 mg, 0.033 mmol), (*R*)-4-benzyl-3-((*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one (13 mg, 0.024 mmol), 1 mL of CH_2Cl_2 were employed to yield 12 mg (94%) of the aldehyde as a colorless oil following purification by

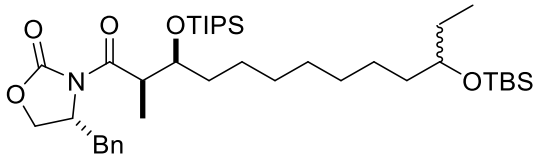
column chromatography (SiO_2, 30% EtOAc in hexanes).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.6 Hz, 1H), 7.34 (t, *J* = 7.2 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 2H), 4.57 (dddd, *J* = 10.0, 7.2, 3.2, 2.0 Hz, 1H), 4.23 (dt, *J* = 7.6, 4.0 Hz, 1H), 4.17 (dd, *J* = 9.2, 2.2 Hz, 1H), 4.11 (dd, *J* = 9.2, 7.2 Hz, 1H), 3.86 (qd, *J* = 6.8, 4.0 Hz, 1H), 3.31 (dd, *J* = 13.2, 2.8 Hz, 2H), 3.32 (dd, *J* = 13.5, 3.0 Hz, 1H), 2.77 (dd, *J* = 13.6, 9.6 Hz, 1H), 2.42 (td, *J* = 7.5, 2.0 Hz, 2H), 1.68 – 1.52 (m, 4H), 1.38 – 1.24 (m, 8H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.07 – 1.02 (m, 21H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.8, 175.1, 135.4, 129.4, 128.9, 127.3, 73.1, 66.0, 55.9, 43.8, 42.4, 37.5, 35.7, 29.6, 29.2, 29.0, 24.7, 22.0, 18.2, 18.1, 13.0, 10.2. IR (thin film, cm^{-1}) 2925, 2862, 1778, 1705, 1453, 1383, 1349.



(*4R*)-4-benzyl-3-((*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)tridecanoyl)oxazolidin-2-one (58)

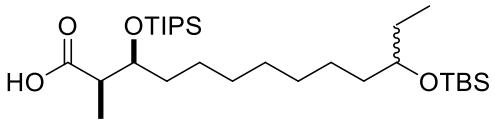
To an ice cooled stirred solution of the aldehdyde (13 mg, 0.023 mmol) in THF (1.25 mL) was added ethyl magnesium bromide (100 µL of 0.28 M in THF, 0.028 mmol) over 5 min. After 1 h, the reaction mixture was quenched by addition of a sat'd NH_4Cl soln., transferred to a separatory funnel and extracted thrice with EtOAc. The organic solutions were combined, dried (Na_2SO_4) and concentrated. The crude residue was purified by flash silica chromatography (30% EtoAc in Hexanes) to yield 6.1 mg (46%) of the desired product as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, *J* = 7.6 Hz, 2H), 7.27 (t, *J* = 6.8 Hz, 1H), 7.22 (d, *J* = 6.8 Hz, 2H), 4.57 (dddd, *J* = 9.6, 7.6, 3.2, 2.0 Hz, 1H), 4.23 (dt, *J* = 7.6, 4.0 Hz, 1H), 4.16 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.11 (dd, *J* = 8.8, 7.6 Hz, 1H), 3.86 (qd, *J* = 6.8, 4.0 Hz, 1H), 3.56 – 3.47 (m, 1H), 3.32 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.66 – 1.24 (m, 16H), 1.22 (d, *J* = 6.8 Hz, 3H), 1.08

– 1.02 (m, 21H), 0.94 (t, *J* = 7.4 Hz, 3H). LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 598.4, found 598.3.



(*4R*)-4-benzyl-3-((*2R*,*3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)tridecanoyl)oxazolidin-2-one (59)

Following the procedure used to synthesize (*R*)-4-benzyl-3-((*2R*,*3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one, (*4R*)-4-benzyl-3-((*2R*,*3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)tridecanoyl)oxazolidin-2-one (14 mg, 0.024 mmol), 2,6-lutidine (7.1 µL, 0.060 mmol), TBSOTf (9.6 µL, 0.041 mmol) in CH_2Cl_2 (2 mL) were employed to yield 17 mg of the desired silyl ether as a colorless oil (100% yield after chromatography over silica gel (7% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (t, *J* = 7.6 Hz, 2H), 7.28 (t, *J* = 6.8 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 2H), 4.61 – 4.53 (m, 1H), 4.26 – 4.19 (m, 1H), 4.17 (dd, *J* = 9.0, 1.8 Hz, 1H), 4.11 (dd, *J* = 8.8, 7.6 Hz, 1H), 3.86 (qd, *J* = 6.8, 4.0 Hz, 1H), 3.55 (quint, *J* = 5.8 Hz, 1H), 3.32 (dd, *J* = 13.4, 3.0 Hz, 1H), 2.77 (dd, *J* = 13.2, 9.4 Hz, 1H), 1.70 – 1.18 (m, 19H), 1.09 – 1.04 (m, 21H), 1.02 – 0.83 (m, 12H), 0.03 (s, 6H).

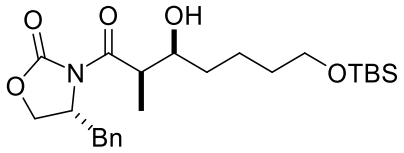


(*2R*,*3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)tridecanoic acid (60)

Following the procedure used to synthesize (*2R*,*3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid, aldol adduct (17 mg, 0.024 mmol), 30% solution of H_2O_2 (20 µL, 0.19 mmol), LiOH (1.8 mg, 0.072 mmol), in 0.9 mL of a 4:1 mixture of THF:H_2O

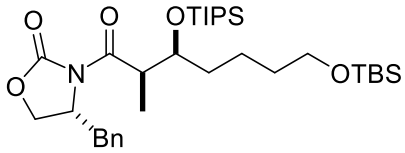
were employed to yield 10 mg (80%) of the desired acid as a colorless oil after chromatography over silica gel (15% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 – 1.11 (m, 1H), 3.56 (quint, *J* = 5.8 Hz, 1H), 2.70 (qd, *J* = 7.2, 4.0 Hz, 1H), 1.64 – 1.22 (m, 16H), 1.12 – 1.06 (m, 21H), 0.88 (s, 9H), 0.86 (t, *J* = , 3H), 0.04 (s, 6H).

6.3.3 Experimental data for the synthesis of macrolactones 75 and 78



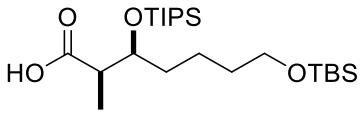
(*R*)-4-benzyl-3-((2*R*,3*S*)-7-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-2-methylheptanoyl)oxazolidin-2-one (67)

Following the procedure used to synthesize (*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2-methylundecanoyl)oxazolidin-2-one, (*4R*)-3-propionyl-4-benzyl-2-oxazolidinone (418 mg, 1.77 mmol), dibutylboron triflate (448 µL, 1.85 mmol) and triethylamine (309 µL, 2.22 mmol) in CH_2Cl_2 (3.3mL) and a solution of 5-((tert-butyldimethylsilyl)oxy)pentanal (320 mg, 1.48 mmol, 3.9 mL CH_2Cl_2) were employed to yield 635 mg (96%) of the desired aldol product as a colorless oil after chromatography over silica gel (30% EtOAc in hexanes) ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.34 (t, *J* =7.7 Hz, 2H), 7.29 (t, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 7.0 Hz, 2H), 4.73 – 4.68 (m, 1H), 4.23 (dd, *J* = 9.1, 9.1 Hz, 1H), 4.19 (dd, *J* = 9.1, 2.8 Hz, 3.98 – 3.93 (m, 1H), 3.76 (qd, *J* = 6.3, 2.5 Hz, 1H), ^{13}C NMR (175 MHz, $CDCl_3$) δ 177.5, 153.0, 135.0, 129.4, 128.9, 127.4, 71.4, 66.1, 63.1, 55.1, 42.1, 37.8, 33.5, 32.6, 25.9, 22.3, 18.3, 10.4, -5.3. IR (thin film) 3524, 2930, 2858, 1784, 1698, 1456, 1386, 1210, 1104 cm^{-1}. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 450.2676, found 450.2678.



(*R*)-4-benzyl-3-((2*R*,3*S*)-7-((*tert*-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)heptanoyl)oxazolidin-2-one (68)

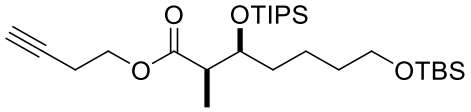
Following the procedure used to synthesize (*R*)-4-benzyl-3-((*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoyl)oxazolidin-2-one (*R*)-4-benzyl-3-((*2R,3S*)-7-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2-methylheptanoyl)oxazolidin-2-one (592 mg, 1.32 mmol), 2,6-lutidine (341 µL, 3.00 mmol), TIPSOTf (586 µL, 2.11 mmol) in CH_2Cl_2 (6.6 mL) were employed to yield 836 mg of the desired silyl ether as a colorless oil (100% yield after chromatography over silica gel (7% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, *J* = 6.8 Hz, 2H), 7.29 (t, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 6.8 Hz, 2H), 4.61 – 4.53 (m, 1H), 4.2X (1H) 4.16 (dd, *J* = 9.2, 2.9 Hz, 1H), 4.11 (dd, *J* = 8.2, 8.2 Hz, 1H), 3. 86 (qd, *J* = 6.0, 4.0 Hz, 1H), 3.59 (t, *J* = 6.6 Hz, 2H), 3.31 (dd, *J* = 13.4, 3.4 Hz, 1H), 2.77 (dd, *J* = 13.4, 9.8 Hz, 1H), 1.68 – 1.46 (m, 4H), 1.42 – 1.31 (m, 2H), 1.22 (d, *J* = 6.8 HZ, 3H), 1.10 – 1.01 (m, 21H), 0.89 (s, 9H), 0.04 (s, 6H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.1, 153.1, 135.5, 129.5, 128.9, 127.3, 73.2, 66.0, 63.0, 55.9, 42.5, 37.6, 35.6, 33.2, 25.9, 21.2, 18.3, 18.2, 18.1, 17.7, 13.0, 12.2, 10.2, -5.3. IR (thin film, cm^{-1}) 2942, 2864, 1782, 1705, 1462, 1381.



(2*R*,3*S*)-7-((*tert*-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)heptanoic acid (69)

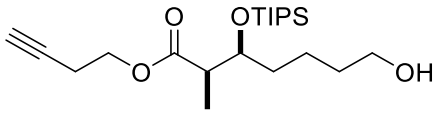
Following the procedure used to synthesize (*2R,3S*)-11-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid, aldol adduct (690 mg, 1.14 mmol), 30% solution of

H_2O_2 (930 µL, 9.10 mmol), LiOH (83.4 mg, 3.41 mmol), in 11.4 mL of a 4:1 mixture of THF:H_2O were employed to yield 508 mg (95%) of the desired acid as a colorless oil after chromatography over silica gel (15% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (td, *J* = 5.5, 4.5 Hz, 1H), 3.60 (t, *J* = 6.0 Hz, 2H), 2.72 (qd, *J* = 7.0, 4.0 Hz, 1H), 1.66 – 1.34 (m, 6H), 1.19 – 1.04 (m, 24 H), 0.89 (s, 9H), 0.04 (s, 6H). IR (thin film, cm^{-1}) 2945, 2867, 1464, 1387. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 447.3326, found 447.3338.



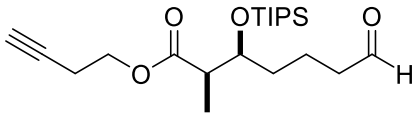
but-3-yn-1-yl (*2R,3S*)-7-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)heptanoate (70)

Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, (*2R,3S*)-7-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)heptanoic acid (508 mg, 1.14 mmol), DCC (261 mg, 1.25 mmol), DMAP (155 mg, 1.25 mmol), 3-butyn-1-ol (140 µL, 1.83 mmol) in 14 mL of CH_2Cl_2 were employed to yield 522 mg (92%) of the desired product as a colorless oil after it was chromatographed over silica gel (eluent 20% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.25 (ddd, *J* = 8.5, 8.5, 4.0 Hz, 1H), 4.21 (dt, *J* = 10.5, 7.0 Hz), 4.11 (dt, *J* = 10.5, 7.0 Hz, 1H), 3.60 (t, *J* =6.5 Hz, 2H), 2.58 (qd, *J* = 7.0, 4.0 Hz, 1H), 2.52 (td, *J* = 7.0, 2.5 Hz, 2H), 1.98 (t, *J* = 2.3 Hz), 1.65 – 1.47 (m, 4H), 1.39 – 1.29 (m, 2H), 1.15 (d, *J* = 7.0 Hz, 3H), 1.04 (s, 21H), 0.89 (s, 9H), 0.04 (s, 6H). IR (thin film, cm^{-1}) 2921, 2854, 1711, 1454.



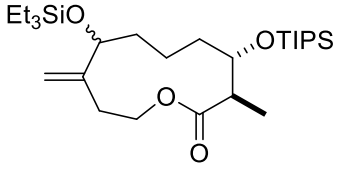
but-3-yn-1-yl (*2R,3S*)-7-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)heptanoate (71)

Following the procedure used to synthesize (*2R,3S*)-11-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)undecanoic acid, but-3-yn-1-yl (*2R,3S*)-7-((tert-butyldimethylsilyl)oxy)-2-methyl-3-((triisopropylsilyl)oxy)heptanoate (522 mg, 1.05 mmol), 10-camphorsulphonic acid (61.4 mg, 0.262 mmol) in MeOH (20 mL) were employed to yield 347 mg (86%) of the hydroxyl acid as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.43 (dt, *J* = 7.6, 4.8 Hz, 1H), 4.21 (dt, *J* = 10.8, 6.8 Hz), 4.12 (dt, *J* = 10.4, 6.8 Hz, 1H), 3.69 – 3.61 (m, 2H), 2.59 (qd, *J* = 6.8, 4.0 Hz, 1H), 2.53 (td, *J* = 6.8, 2.4 Hz, 2H), 1.99 (t, *J* = 2.4 Hz, 1H), 1.67 – 1.51 (m, 4H), 1.43 – 1.31 (m, 2H), 1.16 (d, *J* = 7.0 Hz, 3H), 1.28 – 1.19 (m, 3H), 1.05 (s, 18H).



but-3-yn-1-yl (*2R,3S*)-2-methyl-7-oxo-3-((triisopropylsilyl)oxy)heptanoate (72)

Following the procedure used to synthesize 6-((4-methoxybenzyl)oxy)dodec-11-ynal, Dess-Martin periodinane (46 mg, 0.11 mmol), but-3-yn-1-yl (*2R,3S*)-7-hydroxy-2-methyl-3-((triisopropylsilyl)oxy)heptanoate (28 mg, 0.072 mmol), 1 mL of CH_2Cl_2 were employed to yield 26 mg (95%) of the aldehyde as a colorless oil following purification by column chromatography (SiO_2, 30% EtOAc in hexanes). ^{1}H NMR (700 MHz, $CDCl_3$) δ 9.77 (s, 1H), 4.24 (dt, *J* = 7.0, 4.9 Hz, 1H), 4.24 (dt, *J* = 10.5, 7.0 Hz), 4.13 (dt, *J* = 10.5, 7.0 Hz, 1H), 2.61 (qd, *J* = 7.0, 4.2 Hz, 1H), 2.53 (td, *J* = 7.0, 2.8 Hz, 2H), 2.46 (t, *J* = 7.0 Hz, 2H), 1.99 (t, *J* = 2.8 Hz, 1H), 1.70 – 1.55 (m, 4H), 1.17 (d, *J* = 7.0 Hz, 3H), 1.08 – 1.02 (m, 21H).



(*3R,4S*)-3-methyl-9-methylene-8-((triethylsilyl)oxy)-4-((triisopropylsilyl)oxy)oxacycloundecan-2-one (76)

$Ni(cod)_2$ (20 mg, 0.071 mmol), DP-IPr (45 mg, 0.071 mmol), and KO-*t*-Bu (8.1 mg, 0.071 mmol), were charged to a round-bottom flask, in a glovebox, the flask was removed from the glovebox, THF (11 mL) was added and stirred for 20 min at rt. Triethylsilane (75 μL, 0.47 mmol) was added, followed by syringe drive addition of the ynal (90 mg, 0.24 mmol) in THF (2 mL) over three hours, the reaction was stirred for 12 h at rt. The reaction mixture was concentrated in vacuo, filtered through a plug of silica eluting with 50% EtOAc in hexanes, re-concentrated and purified by column chromatography (SiO_2, 2.5% EtOAc in hexanes) to afford (*3R*,*4S*)-3-methyl-9-methylene-8-((triethylsilyl)oxy)-4-((triisopropylsilyl)oxy)oxacycloundecan-2-one as a colorless oil (35 mg, 30%, 2:1 dr). Major diastereomer ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.22 (s, 1H), 5.12 (s, 1H) 4.24 (dt, *J* = 7.0, 4.9 Hz, 1H), 4.58 – 4.51 (m, 1H), 4.29 (dd, *J* = 10.0, 7.0, 3.0 Hz, 1H), 4.08 (d, *J* = 6.0 Hz, 1H), 3.99 (td, *J* = 9.0, 3.0 Hz, 1H), 2.48 – 2.36 (m, 2H), 2.14 (ddd, *J* = 15.0, 9.0, 3.0 Hz, 1H), 1.79 – 1.65 (m, 2H), 1.64 – 1.52 (m, 2H), 1.35 – 1.25 (m, 5H), 1.13 – 1.02 (m, 21H), 0.96 – 0.86 (m, 12H), 0.56 (q, *J* = 7.8 Hz, 6H). Minor diastereomer diagnostic signals 5.09 (s, 0.1H), 5.00 (s, 0.1H).

6.4 Chapter 4 Experimental

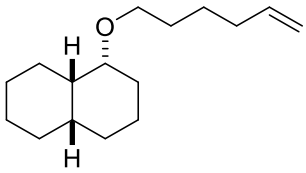
6.4.1 Scaled-up PikC reaction general procedure

$PikC_{D50N}$-RhFRED preparative-scale enzymatic reactions:

Preparative –scale enzymatic reactions were conducted on 20 mg of each substrate under the following conditions: 5 μM $PikC_{D50N}$-RhFRED, 1 mM substrate, 1 mM NADP+, 1 U/mL glucose-6-phosphate dehydrogenase, and 5 mM glucose-6-phosphate for NADPH generation in reaction buffer (50 mM NaH_2PO_4, pH 7.3, 1 mM EDTA, 0.2 mM dithoerythritol, and 10% glycerol). The reaction mixture was divided into 50 mL conical tubes in 7 mL aliquots. Each conical tube was loosely capped and transferred to a shaking incubator. The reaction was carried

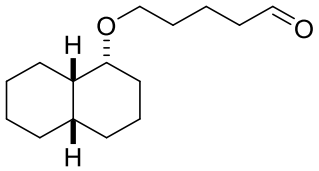
out at 30 °C for 12 h at 160 rpm. After 12 h, a 50 µL aliquot was removed and processed in an identical manner to the analytical-scale reactions described above to access the outcome of the reaction. The remaining reaction mixture was diluted with acetone (2 x total reaction volume) and cooled to 4 °C for two hours. The mixture was then filtered through celite and concentrated under reduced pressure until the acetone had been removed. The remaining solution was adjusted to pH 9, brined and extracted with EtOAc (3 x 200 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated to afford a crude yellow oil. The crude oil was purified through silica flash column chromatography (column conditions from the starting material purification) to afford the mixture of hydroxylated products.

6.4.2 Bicycle substrate synthesis experimental



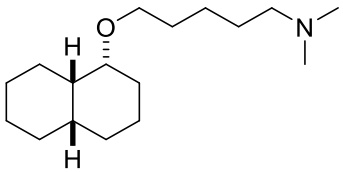
(*1R,4aS,8aS*)-1-(hex-5-en-1-yloxy)decahydronaphthalene (int1-(±)79)

Following the procedure used to synthesize 3-(cyclododecyloxy)-*N*,*N*-dimethylpropan-1-amine *cis*-decahydro-1-naphtol (150 mg, 0.972 mmol), NaH (24.8 mg, 1.02 mmol) and 6-bromo-1-hexene (178 µL, 1.27 mmol) in 4 mL of DMF were employed to yield the product (59.1 mg, 26%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.05 – 4.90 (m, 2H), 3.48 (dt, *J* = 9.2, 6.6 Hz, 1H), 3.43 – 3.29 (m, 1H), 3.23 (dt, *J* = 11.6, 4.6 Hz, 1H), 2.11 – 1.94 (m, 3H), 1.81 – 1.71 (m, 2H), 1.69 – 1.11 (m, 17H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 114.4, 80.8, 67.5, 39.7, 35.7, 33.6, 32.0, 29.6, 26.8, 26.4, 25.6, 25.0, 24.5, 21.6, 19.4. LRMS (EI) *m/z* calculated for $[M]^+$ = 236, found 236.



5-(((*1R,4aS,8aS*)-decahydronaphthalen-1-yl)oxy)pentanal (int2-(±)79)

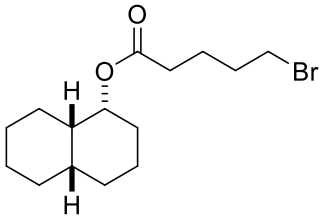
Following the procedure used to synthesize 5-(cyclododecyloxy)pentanal (1R,4aS,8aS)-1-(hex-5-en-1-yloxy)decahydronaphthalene (37.7 mg, 0.159 mmol) in 5 mL of anhydrous CH_2Cl_2, ozone and PPh_3 (63.4 mg, 0.239 mmol) in 2.3 mL of CH_2Cl_2 were employed to afford the product (25.2 mg, 67%) as a colorless oil which was purified through flash chromatography (silica gel, 10 % EtOAc/hexanes) ^{1}H NMR (500 MHz, $CDCl_3$) 9.76 (s, 1H), 3.49 (dt, *J* = 9.0, 6.3 Hz, 1H), 3.35 (dt, *J* = 9.0, 6.5 Hz, 1H), 3.22 (dt, *J* = 11.5, 4.8 Hz, 1H), 2.46 (td, *J* = 7.3 , 2.0 Hz, 2H), 2.00 – 1.94 (m, 1H), 1.79 – 1.12 (m, 15H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.7, 80.9, 67.1, 43.7, 39.7, 35.7, 32.0, 29.5, 26.7, 26.4, 24.9, 24.4, 21.6, 19.4, 19.1. LRMS (ESI) *m/z* calculated for $[M+Na]^+$ 261.2, found 261.2.



5-(((*1R,4aS,8aS*)-decahydronaphthalen-1-yl)oxy)-N,N-dimethylpentan-1-amine ((±)-79)

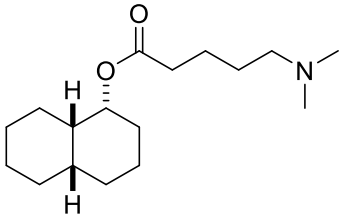
Following the procedure used to synthesize 5-(cyclododecyloxy)-*N*,*N*-dimethylpentan-1-amine 5-(((1R,4aS,8aS)-decahydronaphthalen-1-yl)oxy)pentanal (19 mg, 0.079 mmol), dimethylamine hydrochloride (20 mg, 0.24 mmol), sodium acetate (14 mg, 0.17 mmol), 3Å molecular sieves (40 mg) and $NaBH_3CN$ (14 mg, 0.21 mmol) in 3.9 mL of MeOH were employed to afford 5-(((1R,4aS,8aS)-decahydronaphthalen-1-yl)oxy)-N,N-dimethylpentan-1-amine as a colorless oil (17 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.48 (dt, *J* = 9.1, 6.6 Hz, 2H), 3.32 (dt, *J* = 9.1, 6.7

Hz, 1H), 3.23 (dt, *J* = 11.5, 4.6 Hz, 1H), 2.24 (s, 8H), 1.98 (dq, *J* = 13.4, 3.8 Hz, 1H), 1.76 (dq, *J* = 13.9, 3.3 Hz, 2H), 1.69 – 1.10 (m, 18H). HRMS (ESI) *m/z* calculated for $[M+H]^+$ 268.2640, found 268.2711



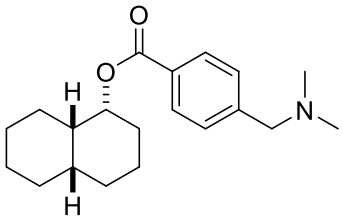
(*1R,4aS,8aS*)-decahydronaphthalen-1-yl 5-bromopentanoate (int-(±)-80)

Following the procedure employed to synthesize (3*R*,4*S*,5*S*,7*R*,11*R*,12*R*,*E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, cis-decahydronaphthol (50 mg, 0.32 mmol), DCC (101 mg, 0.49 mmol), DMAP (60 mg, 0.59 mmol), 5-bromovaleric acid (91 mg, 0.059 mmol) in 3.3 mL of CH_2Cl_2 were employed to yield 52 mg (51%) of the desired product after it was chromatographed over silica gel (eluent 7% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.80 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 2.01 – 1.94 (m, 1H), 1.93 – 1.86 (m, 2H), 1.82 – 1.71 (m, 5H), 1.65 – 1.28 (m, 10H), 1.26 – 1.12 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.4, 75.9, 39.9, 35.4, 33.6, 33.0, 32.0, 31.5, 26.0, 25.9, 24.3, 24.0, 23.6, 21.3, 19.8. IR (thin film, cm^{-1}) 2923, 2856, 1725, 1446. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 317.1111, found 317.1124.



(*1R,4aS,8aS*)-decahydronaphthalen-1-yl 5-(dimethylamino)pentanoate ((±)-80)

To a DMF (1.4 mL) solution of (*1R,4aS,8aS*)-decahydronaphthalen-1-yl 5-bromopentanoate (45 mg, 0.14 mmol) was added dimethyl amine 40% wt. solution in H_2O (72 µL, 0.57 mmol). The reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted in sat'd $NaHCO_3$ and extracted with EtOAc thrice. The EtOAc extracts were dried over anhydrous magnesium sulfate, filtered, evaporated and chromatographed (silica gel; eluent 10% MeOH in CH_2Cl_2) to give the title compound as a colorless oil (34 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.79 (dt, *J* = 12.0, 5.3 Hz, 1H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.32 (t, *J* = 7.0 Hz, 2H), 2.29 (s, 6H), 1.96 (ddt, (t, *J* = 11.5, 4.5, 4.5 Hz, 1H), 1.82 – 1.71 (m, 3H), 1.68 – 1.12 (m, 15H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 172.6, 75.9, 58.5, 44.0, 39.9, 35.4, 34.4, 31.5, 26.6, 26.1, 25.9, 24.3, 24.1, 22.7, 21.3, 19.9. IR (thin film, cm^{-1}) 2923, 2857, 2814, 2766, 1710, 1611, 1448, 1412. IR (thin film, cm^{-1}). HRMS (ESI) m/z calculated for [M+H]+ 282.2428, found 282.2434.

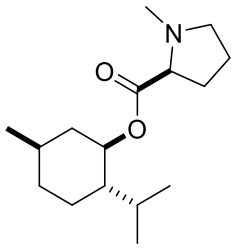


(*1R,4aS,8aS*)-decahydronaphthalen-1-yl 4-((dimethylamino)methyl)benzoate ((±)-80)

Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate, *cis*-decahydro-1-naphtol (50 mg, 0.324 mmol), 87 mg of 4-((dimethylamino)methyl)benzoic acid (0.486 mmol, 1.5 equiv), 101 mg of dicyclohexylcarbodiimide (0.486 mmol, 1.5 equiv) and 60 mg 4-dimethylaminopyridine (0.486 mmol, 1.5 equiv) to yield 69 mg (68%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8. Hz, 2H), 7.37 (d, *J* = 8.0 Hz), 5.04 (ddd, *J* = 12.0, 5.2, 5.2 Hz, 1H), 3.47 (s, 2H), 2.24 (s, 6H), 2.16 – 2.06 (m, 2H), 1.88 – 1.73 (m, 4H), 1.72 – 1.36 (m,

9H), 1.29 – 1.13 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.8, 144.3, 129.8, 129.5, 128.8, 76.3, 64.0, 45.4, 40.1, 35.5, 31.6, 26.1, 26.0, 24.8, 24.1, 21.4, 20.1. IR (thin film, cm^{-1}) 2923, 2856, 2815, 2766, 1710, 1611, 1448, 1412. HRMS (ESI) m/z calculated for [M+H]+ 316.2277, found 316.2287.

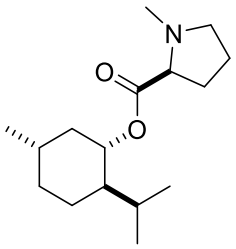
6.4.3 Six membered carbocycles experimental



(*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl methyl-L-prolinate ((-)-83)

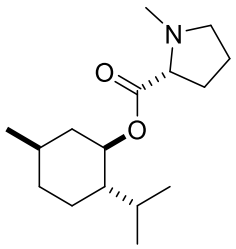
(-)-(*1R,2S,5R*)-menthol (25 mg, 0.16 mmol) was dissolved in 2.0 mL of dichloromethane along with 31 mg of L-*N*-methylproline (0.24 mmol, 1.5 equiv), 50 mg of dicyclohexylcarbodiimide (0.24 mmol, 1.5 equiv) and 30 mg 4-dimethylaminopyridine (0.24 mmol, 1.5 equiv). The reaction mixture was allowed to stir at room temperature for 4 days, and then the reaction mixture was filtered through cotton and evaporated to dryness in vacuo. The crude residue was transferred to a separatory funnel by washing the flask with 0.1 M HCl and 30% EtOAc in hexanes. The acidified aqueous phase was extracted three times with 30% EtOAc in hexanes. Then the aqueous layer was basified with saturated aqueous $NaHCO_3$ solution until it reached a pH of about 8-9, measured by pH paper. The basified aqueous layer was extracted three times with EtOAc. The EtOAc layers were combined, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness in vacuo. The crude residue was chromatographed over silica gel (eluent 60% EtOAc in hexanes) to yield 17 mg (40% yield) of the desired ester derivative as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (td, J = 10.8, 4.4 Hz, 1H), 3.14 (td, J =8.3, 2.3 Hz, 1H), 2.91 (t, J = 7.8 Hz, 1H), 2.38 (s, 3H), 2.29 (dd, J = 16.8, 8.4 Hz, 1H), 2.20 – 2.08 (m,

1H), 2.02 – 1.73 (m, 5H), 1.72 – 1.64 (m, 2H), 1.56 – 1.38 (m, 2H), 1.12 – 0.83 (m, 9H), 0.76 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.3, 68.0, 56.1, 46.8, 40.8, 40.5, 34.2, 31.3, 29.7, 26.2, 23.3, 23.0, 22.0, 20.7, 16.2. IR (thin film, cm^{-1}) 2950, 2867, 1731, 1456, 1369. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 268.2277, found 268.2275



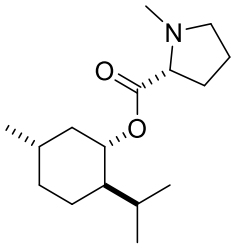
(*1S*,*2R*,5S)-2-isopropyl-5-methylcyclohexyl methyl-D-prolinate ((+)-83)

Following the procedure employed to synthesize (*1R*,*2S*,*5R*)-2-isopropyl-5-methylcyclohexyl methyl-L-prolinate, (+)-(*1S*,*2R*,*5S*)-menthol (25 mg, 0.16 mmol), L-*N*-methylproline (31 mg, 0.24 mmol), DCC (50 mg, 0.24 mmol), DMAP (30 mg, 0.24 mmol) in 2.0 mL of CH_2Cl_2 were employed to yield 12 mg (29% yield) of the desired product as a colorless oil after purification over silica gel (eluent 60% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (td, *J* = 10.8, 4.4 Hz, 1H), 3.17 – 3.10 (m, 1H), 2.91 (t, *J* = 7.8 Hz, 1H), 2.40 (s, 3H), 2.29 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.20 – 2.10 (m, 1H), 2.02 – 1.62 (m, 7H), 1.56 – 1.38 (m, 2H), 1.11 – 0.80 (m, 7H), 0.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.2, 74.3, 68.0, 56.1, 46.8, 40.8, 40.5, 34.2, 31.3, 29.7, 26.2, 23.3, 23.0, 22.0, 20.7, 16.2. IR (thin film, cm^{-1}) 2953, 2868, 1730, 1455, 1370. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 268.2277, found 268.2271



(*1R*,*2S*,*5R*)-2-isopropyl-5-methylcyclohexyl methyl-D-prolinate ((-)-84)

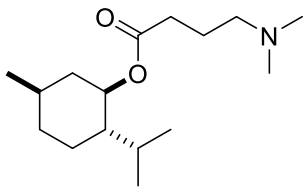
Following the procedure employed to synthesize (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl methyl-L-prolinate, (-)-(*1R,2S,5R*)-menthol (25 mg, 0.16 mmol), D-*N*-methylproline (31 mg, 0.24 mmol), DCC (50 mg, 0.24 mmol), DMAP (25 mg, 0.20 mmol) in 2.0 mL of CH_2Cl_2 were employed to yield 30 mg (71% yield) of the desired product as a colorless oil after purification over silica gel (eluent 60% EtOAc in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.75 (td, *J* = 10.8, 4.4 Hz, 1H), 3.17 – 3.10 (m, 1H), 2.91 (t, *J* = 7.8 Hz, 1H), 2.40 (s, 3H), 2.29 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.20 – 2.10 (m, 1H), 2.02 – 1.62 (m, 7H), 1.56 – 1.38 (m, 2H), 1.11 – 0.80 (m, 7H), 0.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.3, 68.0, 56.1, 46.8, 40.8, 40.5, 34.2, 31.3, 29.7, 26.2, 23.3, 23.0, 22.0, 20.7, 16.2. ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.2, 74.3, 68.0, 56.1, 46.8, 40.8, 40.5, 34.2, 31.3, 29.7, 26.2, 23.3, 23.0, 22.0, 20.7, 16.2. IR (thin film, cm^{-1}) 2953, 2868, 1730, 1455, 1370. IR (thin film, cm^{-1}) 2950, 2866, 1729, 1452, 1368. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 268.2277, found 268.2267.



(*1S,2R*,5S)-2-isopropyl-5-methylcyclohexyl methyl-L-prolinate ((+)-84)

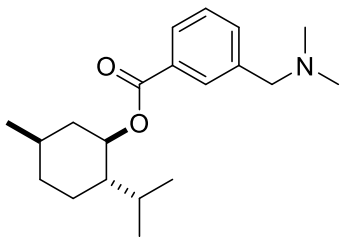
Following the procedure employed to synthesize (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl methyl-L-prolinate, (+)-(*1S,2R,5S*)-menthol (25 mg, 0.16 mmol), D-*N*-methylproline (31 mg, 0.24 mmol), DCC (50 mg, 0.24 mmol), DMAP (30 mg, 0.24 mmol) in 2.0 mL of CH_2Cl_2 were employed to yield 28 mg (65% yield) of the desired product as a colorless oil after purification over silica gel (eluent 60% EtOAc in hexanes) ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (td, *J* = 10.8, 4.4 Hz, 1H), 3.14 (td, *J* =8.3, 2.3 Hz, 1H), 2.91 (t, *J* = 7.8 Hz, 1H), 2.38 (s, 3H), 2.29 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.20 – 2.08 (m, 1H), 2.02 – 1.73 (m, 5H), 1.72 – 1.64 (m, 2H), 1.56 – 1.38 (m,

2H), 1.12 – 0.83 (m, 9H), 0.76 (d, J = 6.8 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.3, 74.3, 67.7, 56.2, 46.9, 40.9, 40.7, 34.2, 31.4, 29.7, 26.1, 23.2, 22.8, 22.0, 20.0, 16.0. IR (thin film, cm^{-1}) 2930, 2868, 1701, 1455, 1369. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 268.2277, found 268.2273.



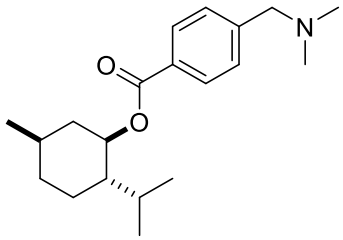
(*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 4-(dimethylamino)butanoate ((-)-86)

Following the procedure employed to synthesize cyclododecyl 3-(dimethylamino)propanoate, (-)-(*1R,2S,5R*)-menthol (200 mg, 1.28 mmol), 3-*N,N*-dimethylaminobutyric acid hydrochloride (325 mg, 1.92 mmol), triethylamine (304 µL, 2.18 mmol), DCC (400 mg, 1.92 mmol), DMAP (237 mg, 1.92 mmol) in 12.5 mL of CH_2Cl_2 were employed to yield 168 mg (49% yield) of the desired product as a colorless oil after purification over silica gel (gradient from 30% EtOAc in hexanes to 20% MeCN, 10% MeOH in EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.67 (ddd, J = 10.8, 10.8, 4.4 Hz, 2H), 2.35 – 2.24 (m, 4H), 2.22 (s, 6H), 2.06 – 1.93 (m, 1H), 1.91 – 1.74 (m, 3H), 1.72 – 1.62 (m, 2H), 1.54 – 1.42 (m, 1H), 1.41 – 1.31 (m, 1H), 1.11 – 0.80 (m, 9H), 0.75 (d, J = 6.8 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 173.1, 74.0, 58.9, 47.0, 45.4, 40.9, 34.2, 32.4, 31.3, 26.2, 23.4, 23.1, 22.0, 20.7, 16.3. IR (thin film, cm^{-1}) 2925, 2865, 2812, 2764, 1728, 1456 HRMS (ESI) *m/z* calculated for $[M+H]^+$ 270.2433, found 270.2431.



(*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 3-((dimethylamino)methyl)benzoate ((-)-89)

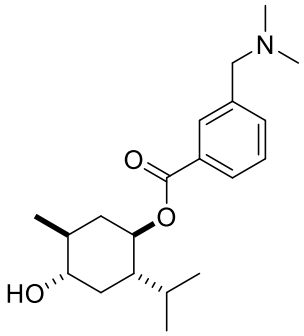
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate (-)-(*1R,2S,5R*)-menthol (160 mg, 1.02 mmol), 154 mg of 3-((dimethylamino)methyl)benzoic acid (1.54 mmol, 1.5 equiv), 320 mg of dicyclohexylcarbodiimide (1.54 mmol, 1.5 equiv) and 190 mg 4-dimethylaminopyridine (1.54 mmol, 1.5 equiv) to yield 105 mg (33%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) ^{1}H NMR (500 MHz, DCl_3) δ 7.97 – 7.92 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 2H), 4.94 (ddd, *J* = 11.0, 11.0, 4.5 Hz, 1H), 3.49 (d, *J* = 13.0 Hz, 1H), 3.45 (d, *J* = 13.0 Hz, 1H), 2.25 (s, 6H), 2.11 (dtd, *J* = 11.5, 3.5, 2.0 Hz, 1H), 1.96 (hd, *J* = 7.0, 2.5 Hz, 1H), 1.73 – 1.69 (m, 2H), 1.63 – 1.52 (m, 2H), 1.18 – 1.06 (m, 2H), 0.92 (t, *J* = 6.5 Hz, 6H), 0.79 (d, *J* = 7.5 Hz, 3H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.0, 139.2, 133.3, 130.8, 130.0, 128.3, 128.2, 74.7, 63.9, 47.1, 45.2, 40.9, 34.2, 31.4, 26.4, 23.6, 22.0, 20.7, 16.4. IR (thin film, cm^{-1}) 2949, 2864, 2815, 2767, 1711, 1455, 1360. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 318.2433, found 318.2440.



(*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 4-((dimethylamino)methyl)benzoate ((-)-90)

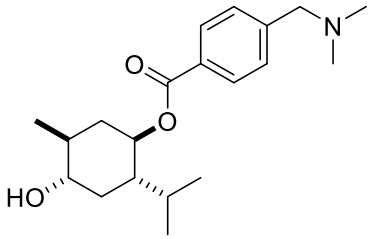
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate, (-)-(*1R,2S,5R*)-menthol (100 mg, 0.640 mmol), 172 mg of 4-((dimethylamino)methyl)benzoic acid (0.960 mmol, 1.5 equiv), 200 mg of dicyclohexylcarbodiimide (0.960 mmol, 1.5 equiv) and 118 mg 4-dimethylaminopyridine (0.960 mmol, 1.5 equiv) to yield 166 mg (82%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H

NMR (700 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 4.93 (ddd, *J* = 11.2, 11.2, 4.2 Hz, 1H), 3.47 (s, 2H), 2.25 (s, 6H), 2.12 (d, *J* = 11.9 Hz, 1H), 1.96 (hd, *J* = 7.0, 2.8 Hz, 1H), 1.75 – 1.70 (m, 2H), 1.61 – 1.53 (m, 2H), 1.14 (td, *J* = 13.7, 3.2 Hz, 1H), 1.09 (t, *J* = 12.0 Hz, 1H), 0.97 – 0.88 (m, 7H), 0.79 (d, *J* = 7.0 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0, 144.1, 129.6, 129.5, 128.8, 74.7, 64.0, 47.2, 45.3, 41.0, 34.3, 31.4, 26.5, 23.6, 22.0, 20.8, 16.5. IR (thin film, cm^{-1}) 2923, 2856, 2767, 1712, 1611, 1454. HRMS (ESI) *m/z* calculated for $[M+H]^+$ 318.2433, found 318.2420.



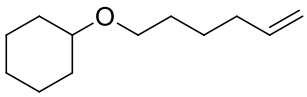
(*1R,2S,4S,5S*)-4-hydroxy-2-isopropyl-5-methylcyclohexyl 3-((dimethylamino)methyl)benzoate (92)

Following general procedure 5.4.1, 20 mg of (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 3-((dimethylamino)methyl)benzoate were employed to yield 3.0 mg of the monohydroxylayed product as a colorless oil.^1H NMR (500 MHz, DCl_3) δ 7.94 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 4.95 (ddd, *J* = 11.0, 11.0, 4.5 Hz, 2H), 3.53 (d, *J* = 13.5 Hz, 1H), 3.49 (d, *J* = 12.5 Hz, 1H), 3.27 (ddd, *J* = 10.5, 10.5, 4.0 Hz, 2H), 2.28 (s, 6H), 2.19 – 2.11 (m, 1H), 2.01 – 1.93 (m, 2H), 1.80 – 1.72 (m, 1H), 1.60 – 1.48 (m, 1H), 1.38 – 1.16 (m, 2H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.82 (d, *J* = 7.0 Hz, 3H).



(*1R,2S,4S,5S*)-4-hydroxy-2-isopropyl-5-methylcyclohexyl 4-((dimethylamino)methyl)benzoate (93)

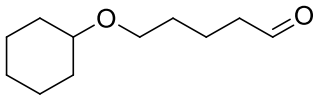
Following general procedure 5.4.1, 20 mg of (*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl 4-((dimethylamino)methyl)benzoate were employed to yield 15.6 mg of the monohydroxylayed product as a white solid. ^{1}H NMR (700 MHz, DCl_3) δ 7.98 (d, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.7 Hz, 2H), 4.93 (ddd, *J* = 10.9, 10.9, 4.2 Hz, 2H), 3.74 (s, 1H), 3.62 (ddd, *J* = 10.5, 10.5, 4.2 Hz, 2H), 2.24 (s, 6H), 2.14 (dt, *J* = 13.3, 3.9 Hz, 1H), 2.00 – 1.93 (m, 2H), 1.75 (ddt, *J* = 19.9, 10.9, 3.2 Hz, 1H), 1.58 – 1.51 (m, 1H), 1.31 – 1.17 (m, 2H), 1.04 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 5.6 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H). IR (thin film, cm^{-1}) 3405, 2931, 2868, 2815, 2765, 1710, 1455.



(Hex-5-en-1-yloxy)cyclohexane (int1-95)

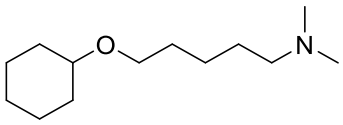
Following the procedure used to synthesize 3-(cyclododecyloxy)-N,N-dimethylpropan-1-amine cyclohexanol (100 mg, 0.998 mmol), NaH (27.7 mg, 1.10 mmol) and 6-bromo-1-hexene (183 µL, 1.30 mmol) in 4 mL of DMF were employed to yield the product (63 mg, 35%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 17.1, 10.4, 6.7 Hz, 1H), 5.00 (d, *J* = 17.1 Hz, 1H), 4.94 (dd, *J* = 10.2, 1.0 Hz, 1H), 3.44 (t, *J* = 6.5 Hz, 2H), 3.19 (p, *J* = 4.1 Hz, 1H), 2.16 – 1.99 (m, 2H), 1.99 – 1.82 (m, 2H), 1.72 (h, *J* = 4.3 Hz, 2H), 1.63 – 1.49 (m, 3H), 1.44 (p, *J* = 7.4

Hz, 2H), 1.31 – 1.12 (m, 4H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 138.9, 114.4, 77.4, 67.7, 33.6, 32.4, 29.7, 25.9, 25.6, 24.3. LRMS (EI) *m/z* calculated for $[M]^+$ = 182, found 182



5-(Cyclohexyloxy)pentanal (int2-95)

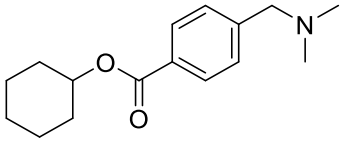
Following the procedure used to synthesize 5-(cyclododecyloxy)pentanal, (hex-5-en-1-yloxy)cyclohexane (62.8 mg, 0.344 mmol) in 10 mL of anhydrous CH_2Cl_2, ozone and PPh_3 (136 mg, 0.517 mmol) in 5 mL of CH_2Cl_2 were employed to afford the product (50 mg, 80%) as a colorless oil which was purified through flash chromatography (silica gel, 10 % ether/pentane) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 3.45 (t, *J* = 6.2, 2H), 3.23 – 3.15 (m, 1H), 2.47 (t, *J* = 7.3, 2H), 1.93-1.84 (m, 2H), 1.77 – 1.67 (m, 4H), 1.65 – 1.46 (m, 3H), 1.31 – 1.15 (m, 5H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.6, 77.5, 67.2, 43.7, 32.3, 29.6, 25.8, 24.2, 19.0. LRMS (EI) *m/z* calculated for $[M]^+$ = 184, found 184



5-(Cyclohexyloxy)-*N,N*-dimethylpentan-1-amine (95)

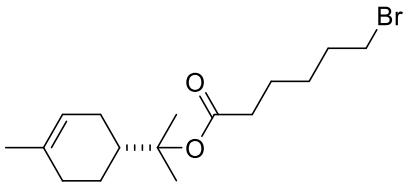
Following the procedure used to synthesize 5-(cyclododecyloxy)-N,N-dimethylpentan-1-amine 5-(cyclohexyloxy)pentanal (21 mg, 0.11 mmol), dimethylamine hydrochloride (28 mg, 0.34 mmol), sodium acetate (21 mg, 0.25 mmol), 3Å molecular sieves (60 mg) and $NaBH_3CN$ (14 mg, 0.29 mmol) in 5.6 mL of MeOH were employed to afford the product as a colorless oil (20 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.43 (t, *J* = 6.5 Hz, 2H), 3.18 (dt, *J* = 9.5, 4.8 Hz, 1H), 2.36 – 2.21 (m, 8H), 1.95 – 1.83 (m, 2H), 1.78 – 1.75 (m, 2H), 1.63 – 1.44 (m, 5H), 1.40 – 1.12 (m, 7H) ^{13}C NMR (125 MHz, $CDCl_3$) 77.5, 67.7, 59.6, 45.2, 32.3, 30.1, 27.2, 25.8, 24.3, 24.1.

IR (thin film, cm^{-1}) 2918, 2850, 1725, 1558, 1466. HRMS (ESI) *m/z* calculated for $[M+H]^+$, found HRMS (ESI) *m/z* calculated for $[M+H]^+$ 214.2170, found 214.2193



Cyclohexyl 4-((dimethylamino)methyl)benzoate (96)

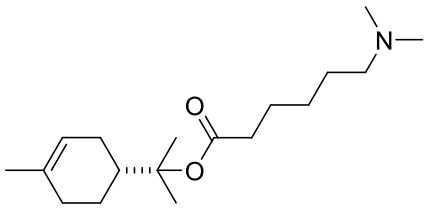
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate, cyclohexanol (50 mg, 0.499 mmol), 134 mg of 4-((dimethylamino)methyl)benzoic acid (0.749 mmol, 1.5 equiv), 156 mg of dicyclohexylcarbodiimide (0.749 mmol, 1.5 equiv) and 92 mg 4-dimethylaminopyridine (0.749 mmol, 1.5 equiv) to yield 77 mg (59%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 5.02 (tt, *J* = 8.8, 4.0 Hz, 1H), 3.47 (s, 2H), 2.24 (s, 6H), 1.98 – 1.90 (m, 2H), 1.82 – 1.74 (m, 2H) 1.68 – 1.53 (m, 2H), 1.50 – 1.40 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.8, 144.0, 129.8, 129.4, 128.7, 72.8, 63.9, 45.3, 31.5, 25.4, 23.6. IR (thin film, cm^{-1}) 2934, 2856, 2815, 2767, 1711, 1611, 1453, 1317. HRMS (ESI) m/z calculated for [M+H]+ 262.1807, found 262.1815.



(*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 6-bromohexatanoate (int1-97)

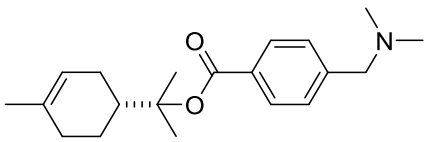
Following the procedure employed to synthesize (*3R,4S*,5*S,7R,11R,12R,E*)-12-ethyl-3,5,7,11-tetramethyl-2,8-dioxooxacyclododec-9-en-4-yl acetate, α-terpineol (150 mg, 0.972 mmol), DCC (304 mg, 1.46 mmol), DMAP (180 mg, 1.46 mmol, 6-bromohexanoic acid (293 mg, 1.46 mmol)

in 12 mL of CH_2Cl_2 were employed to yield 30.3 mg (9%) of the desired product as a colorless oil after it was chromatographed over silica gel (eluent 10% EtOAc in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.24 (t, *J* = 7.5 Hz, 2H), 2.07 – 1.74 (m, 7H), 1.67 – 1.56 (m, 5H), 1.50 – 1.40 (m, 8H), 1.29 (qd, *J* = 12.5, 5.5 Hz, 1H).



(*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 6-(dimethylamino)hexanoate (97)

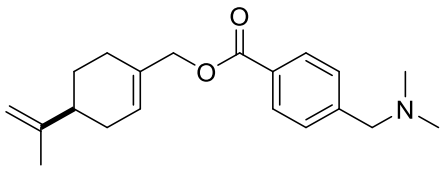
Following the procedure used to synthesize (*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 5-(dimethylamino)pentanoate, (*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 6-bromohexatanoate (30 mg, 0.092 mmol), and dimethyl amine 40% wt. solution in H_2O (46 µL, 0.37 mmol) in 1 mL of DMF were employed to yield the product (28 mg, 100%) as a colorless oil.^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 2.28 – 2.19 (m, 10H), 2.08 – 1.70 (m, 7H), 1.68 – 1.55 (m, 5H), 1.53 – 1.49 (m, 5H), 1.33 – 1.23 (m, 3H). IR (thin film, cm^{-1}) 2929, 2762, 1726, 1456, 1366. HRMS (ESI) m/z calculated for [M+H]+ 296.2590, found 296.2590.



(*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 4-((dimethylamino)methyl)benzoate (98)

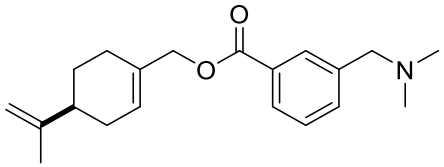
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate, α-terpineol (60 mg, 0.39 mmol), 98 mg of 4-((dimethylamino)methyl)benzoic acid (0.55 mmol, 1.4 equiv), 114 mg of DCC (0.55 mmol, 1.4 equiv) and 67 mg of DMAP (0.960 mmol, 1.5 equiv) to yield 10 mg (8%) of the desired ester

derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 7.7 Hz, 2H), 5.40 (s, 1H), 3.46 (s, 2H), 2.24 (s, 6H), 2.18 (tdd, *J* = 11.9, 4.6, 2.5 Hz, 1H), 2.12 – 2.04 (m, 2H), 2.03 – 1.86 (m, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.41 (qd, *J* = 12.6, 5.6 Hz, 1H) ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.6, 143.2, 134.0, 131.0, 129.4, 128.9, 120.4,35.4, 63.8, 45.2, 45.1, 30.9, 26.5, 24.0, 23.4, 23.3. IR (thin film, cm^{-1}) 2925, 2765, 1709, 1262, 1098 1018. HRMS (ESI) m/z calculated for [M+H]+ 316.2277, found 316.2269.



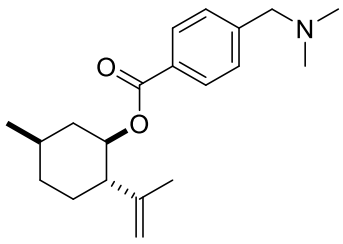
(*S*)-(4-(prop-1-en-2-yl)cyclohex-1-en-1-yl)methyl 4-((dimethylamino)methyl)benzoate (99)

Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate, (*S*)-(−)-perillyl alcohol (37 µL, 0.22 mmol), 60 mg of 4-((dimethylamino)methyl)benzoic acid (0.34 mmol, 1.5 equiv), 70 mg of dicyclohexylcarbodiimide (0.34 mmol, 1.5 equiv) and 41 mg 4-dimethylaminopyridine (0.34 mmol, 1.5 equiv) to yield 55 mg (79%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 5.84 (s, 1H), 4.74 (s, 1H), 4.73 (s, 1H), 4.71 (s, 2H), 3.47 (s, 2H), 2.25 (s, 6H), 2.22 – 2.13 (m, 4H), 2.04 – 1.97 (m, 1H), 1.90 – 1.85 (m, 1H), 1.75 (s, 1H), 1.57 – 1.49 (m, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.3, 149.5, 144.2, 132.7, 129.6, 129.2, 128.8, 125.5, 108.7, 68.7, 63.9, 45.4, 40.8, 30.4, 27.3, 26.4, 20.7. IR (thin film, cm^{-1}) 2925, 2735, 1714, 1610, 1454. HRMS (ESI) m/z calculated for [M+H]+ 314.2120, found 314.2139.



(*S*)-(4-(prop-1-en-2-yl)cyclohex-1-en-1-yl)methyl 3-((dimethylamino)methyl)benzoate (100)

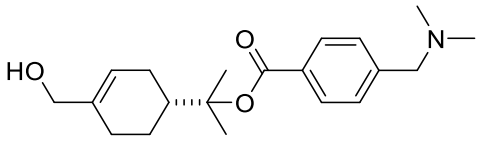
Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate (*S*)-(−)-Perillyl alcohol (104 mg, 0.656 mmol), 176 mg of 3-((dimethylamino)methyl)benzoic acid (0.985 mmol, 1.5 equiv), 205 mg of dicyclohexylcarbodiimide (0.985 mmol, 1.5 equiv) and 122 mg 4-dimethylaminopyridine (0.985mmol, 1.5 equiv) to yield 105 mg (51%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.94 (d, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 5.84 (s, 1H), 4.76 – 4.69 (m, 4H), 3.47 (s, 2H), 2.25 (s, 6H), 2.23 – 2.12 (m, 3H), 2.08 – 1.95 (m, 1H), 1.88 (d, *J* = 13.5 Hz, 1H), 1.75 (s, 3H), 1.66 – 1.46 (m, 2H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.4, 149.4, 139.3, 133.5, 132.6, 130.3, 130.0, 128.3, 128.2, 125.5, 108.7, 68.7, 63.8, 45.2, 40.7, 30.4, 27.2, 26.4, 20.6. IR (thin film, cm^{-1}) 2937, 2814, 2769, 1715, 1643, 1587, 1439, 1360. HRMS (ESI) m/z calculated for [M+H]+ 314.2120, found 314.2129.



(*1R,2S,5R*)-5-methyl-2-(prop-1-en-2-yl)cyclohexyl 4-((dimethylamino)methyl)benzoate (101)

Following the procedure employed to synthesize cyclododecyl 4-((dimethylamino)methyl)benzoate (−)-isopulegol (44 µL, 0.26 mmol), 70 mg of 4-

((dimethylamino)methyl)benzoic acid (0.39 mmol, 1.5 equiv), 81 mg of dicyclohexylcarbodiimide (0.39 mmol, 1.5 equiv) and 48 mg 4-dimethylaminopyridine (0.39 mmol, 1.5 equiv) to yield 47 mg (57%) of the desired ester derivative as a colorless oil after chromatography over silica gel (gradient from 30% EtOAc in hexanes to 100% EtOAc) ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 5.01 (ddd, *J* = 11.0, 11.0, 4.5 Hz, 1H), 4.79 (s, 1H), 4.71 (s, 1H), 3.45 (s, 2H), 2.29 (td, *J* = 11.5, 3.3 Hz, 1H), 2.24 (s, 6H), 2.15 (d, *J* = 12.0 Hz, 1H), 1.82 – 1.70 (m, 2H), 1.69 (s, 3H), 1.68 – 1.58 (m, 2H), 1.46 (qd, *J* = 13.0, 3.0 Hz, 1.14 (dt, *J* = 12.0, 11.5 Hz, 1H), 1.06 – 0.97 (m, 1H), 0.95 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.8, 146.1, 144.0, 129.6, 129.5, 128.7, 111.9, 74.2, 64.0, 50.8, 45.4, 40.5, 34.1, 31.4, 30.5, 22.0, 19.5. IR (thin film, cm^{-1}) 2924, 2856, 2816, 2767, 1709, 1647, 1610, 1454. HRMS (ESI) m/z calculated for [M+H]+ 316.2277, found 316.2286.



(*R*)-2-(4-(hydroxymethyl)cyclohex-3-en-1-yl)propan-2-yl 4-((dimethylamino)methyl)benzoate (102)

Following general procedure 5.4.1, 15 mg of (*R*)-2-(4-methylcyclohex-3-en-1-yl)propan-2-yl 4-((dimethylamino)methyl)benzoate were employed to yield 3.5 mg of the primary alcohol as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 5.71 (s, 1H), 4.04 (d, *J* = 13.0 Hz, 1H), 4.01 (d, *J* = 13.0 Hz, 1H), 3.47 (s, 2H), 2.28 – 2.08 (m, 10H), 2.04 – 1.94 (m, 2H), 1.55 (s, 3H), 1.53 (s, 3H), 1.42 (qd, *J* = 13.0, 6.0 Hz, 1H).

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