DEVELOPING A BACTERIAL P450 AS A GENERAL AND REGIOSELECTIVE CATALYST FOR C–H BOND OXIDATION

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DEDICATION

This dissertation is dedicated to my parents Juan Carlos and Angelina.

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LIST OF ABBREVIATIONS

Ac acetyl

Ar aryl

atm atmosphere

aq aqueous

BDE Bond dissociation energy

Bn benzyl

***n*-Bu** butyl

***t*-Bu** *tert*-butyl

°C temperature in degrees centigrade

CAM Cerium Ammonium Molybdate

cod 1,5-cyclooctadiene

10-CSA 10- Camphorsulfonic acid

CYP Cytochrome P450

DAST Diethylaminosulfur trifluoride

DCC dicyclohexylcarbodiimide

DCM dichloromethane

DDQ 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DesF Desosamine fluoride

DIPEA diisopropylethylamine

DMAP 4-dimethylaminopyridine

DMF *N*,*N*-dimethyl formamide

10-dml 10-deoxymethynolide

DMP Dess-Martin Periodinane

DMSO dimethyl sulfoxide

DP-IPr (rac)-(*4S*,*5S*)-1,3-bis(2,6-diisopropylphenyl)-4,5-diphenylimidazolidin-2-ylidene

dr diastereomeric ratio

EDG Electron donating groups

EWG Electron withdrawing groups

Et ethyl

equiv. equivalent

FAD Flavin-adenine dinucleotide

FMN Flavin mono-nucleotide

FDR ferrodoxin reductase

FDX ferrodoxin iron–sulfur-cluster

FMN Flavine mononucleotide

GCMS gas chromatography mass spectrometry

h hour(s)

HMDS Hexamethyldisilazide

HRMS high resolution mass spectrometry

IMes 1,3-bis-(1,3,5-trimethylphenyl)imidazol-2-ylidene

IPr 1,3-bis-(2,6-diisopropylphenyl)imidazol-2-ylidene

kcal kilocalories

K_d dissociation constant

LC-MS liquid chromatography mass spectrometry

LRMS low resolution mass spectrometry

Me methyl

Ms methanesulfonyl (mesyl)

$NADP^+$ Nicotinamide adenine dinucleotide phosphate

NADPH Reduced form of $NADP^+$

nbe norborene

NHC *N*-heterocyclic carbene

Pd/C palladium on carbon

Ph phenyl

phen phenantroline

PKS Polyketide Synthase

PMA Phosphomolybdic acid

PMB *p*-methoxy benzyl

PPTS Pyridinium *p*-toluenesulfonate

***i*-Pr** isopropyl

***i*-Pr-BAC** 2,3-bis(diisopropylamino)cycloprop-2-en-1-ylium

pyr pyridine

rac racemic

rbf round-bottom flask

RhFRED

rsm recovered starting material

rt room temperature

sat'd saturated

sm starting material

soln solution

TBAF tetrabutylammonium fluoride

TBS tert-butyldimethylsilyl

TE Thioesterase

Tf trifluoromethanesulfonate

TFDO Methyl(trifluoromethyl)dioxirane

THF tetrahydrofuran

TLC thin layer chromatography

TTN Total turnover number

ABSTRACT

The biosynthetic macrolide P450 monooxygenase $PikC_{D50N}$-RhFRED has been employed as a biocatalyst for the stereo- and regioselective oxidation of C–H bonds. Previous results from collaborative work between the Montgomery, Podust and Sherman groups reported employing $PikC_{D50N}$-RhFRED for the moderately regioselective hydroxylation of several desosamine linked carbocyclic rings, proving PikC to be capable of oxidizing unnatural substrates. The *N*,*N*-dimethylamino group in desosamine anchors the substrates in the active site and enables hydroxylation.

This thesis describes the expansion the substrate scope of the $PikC_{D50N}$-RhFRED hydroxylation of unnatural substrates, by probing structural modifications in the aglycone and replacing desosamine with anchoring groups that are easier to synthesize and cleave from the substrates to develop this enzymatic reaction as a synthetic tool for the selective oxidation of C–H bonds. Through substrate engineering, optimized the regioselectivity and stereoselectivity of the oxidation and insights into the factors site-selectivity of PikC catalyzed hydroxylation were gained. A benzylic *N*,*N*-dimethylamino anchoring group effectively controlled the regioselectivity of the reaction of 10-deoxymethynolide derivatives while unnatural anchors had little effect on the regioselectivity of the enzymatic oxidation of simple carbocycles.

The degree of structural complexity necessary for selective oxidation for 12-membered ring macrolides was probed by synthesis of variety of 12-membered macrolide analogues that more closely resemble the endogenous substrates from PikC. We found it is possible to modestly

control selectivity by the choice of anchor, in systems with moderate functionality. Finally, $PikC_{D50N}$-RhFRED was employed as a bio-catalyst for the selective hydroxylation of small-molecules by coupling of alcohols with larger linkers containing the dimethylamino anchor.