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Los Angeles

Computational Elucidation of Selectivities and

Mechanisms Performed by

Organometallic and Bioinorganic Catalysts

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Chemistry

by

Jessica Marie Grandner

2017

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ABSTRACT OF THE DISSERTATION

Computational Elucidation of Selectivities and

Mechanisms Performed by

Organometallic and Bioinorganic Catalysts

by

Jessica Marie Grandner

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2017

Professor Kendall N. Houk, Chair

Computational methods were used to determine the mechanisms and selectivities of organometallic-catalyzed reactions. The first half of the dissertation focuses on the study of metathesis catalysts in collaboration with the Grubbs group at CalTech. Chapter 1 describes the studies of the decomposition modes of several ruthenium-based metathesis catalysts. These studies were performed to better understand the decomposition of such catalysts in order to prevent decomposition (Chapter 1.2) or utilize decomposed catalysts for alternative reactions (Chapter 1.1). Chapter 2.1 describes the computational investigation of the origins of stereoretentive metathesis with ruthenium-based metathesis catalysts. These findings were then used to computationally design E-selective metathesis catalysts (Chapter 2.2). While the first half of the dissertation was centered around ruthenium catalysts, the second half of the dissertation pertains to iron-catalyzed reaction, in particular, iron-catalyzed reactions by P450 enzymes. The elements

of Chapter 3 concentrate on the stereo- and chemo-selectivity of P450-catalyzed C-H hydroxylations. By combining multiple computational methods, the inherent activity of the iron-oxo catalyst and the influence of the active site on such reactions were illuminated. These discoveries allow for the engineering of new substrates and mutant enzymes for tailored C-H hydroxylation. While the mechanism of C-H hydroxylations catalyzed by P450 enzymes has been well studied, there are several P450-catalyzed transformations for which the mechanism is unknown. The components of Chapter 4 describe the use of computations to determine the mechanisms of complex, multi-step reactions catalyzed by P450s. The determination of these mechanisms elucidates how these enzymes react with various functional groups and substrate architectures and allows for a better understanding of how drug-like compounds may be broken down by human P450s.

The dissertation of Jessica Marie Grandner is approved.

Jorge R Barrio

Yi Tang

Kendall N. Houk, Committee Chair

University of California, Los Angeles

2017

To my parents, family, friends, and loved ones,
thank you for always supporting and encouraging me.
This is for you, even though you won't understand a word.

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List of Frequently Used Acronyms

DFT = density functional theory

QM = quantum mechanics

MD = molecular dynamics

ATR = Atom Transfer Radical

ATRA = Atom Transfer Radical Addition

ATRP = Atom Transfer Radical Polymerization

MMA = Methyl Methacrylate

NHC = *N*-heterocyclic carbene

SIMes = 1,3-bis(2,4,6-trimethyl)phenyl-4,5-dihydroimidazol-2-ylidene

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Chapter 1.1 and related materials

This chapter is a version of a published work. Reprinted with permission from Lee, J.; Grandner, J. M.; Engle, K. M.; Houk, K. N.; Grubbs, R. H. *J. Am. Chem. Soc.* **2016**, *138*, 7171-7177 (DOI: 10.1021/jacs.6b03767). Copyright 2016 American Chemical Society. Lee and Engle performed experimental work. Grandner performed computational work. Houk was the PI for Grandner. Grubbs was the PI for Lee and Engle.

Chapter 1.2 and related materials

Chapter 1.2 is a version of Ahmed, T. S.;# Grandner, J. M.;# Taylor, B. L. H.; Houk, K. N.; Grubbs, R. H. “Metathesis and Decomposition of Fischer Carbenes of Cyclometalated Z-Selective Ruthenium Metathesis Catalysts” (in preparation). # indicates that Ahmed and Grandner contributed equally to this work. Ahmed performed experiments. Grandner performed all computations. Taylor was project supervisor for Grandner. Houk was the PI for Grandner. Grubbs was the PI for Ahmed.

Chapter 2.1 and related materials

Chapter 2.1 is a version of Grandner, J. M.; Shao, H.; Grubbs, R. H.; Liu, P.; Houk, K. N. “The Origins of the Stereoretentive Mechanism of Olefin Metathesis with Ru-Dithiolate Catalysts” (in preparation). Grandner performed all computations pertaining to transition states and their respective starting materials and intermediates. Shao performed computations pertaining to the

steric contour plots. Grubbs was a project advisor. Liu was the PI for Shao. Houk was the PI for Grandner.

Chapter 2.1 and related materials

This work was done in collaboration with the Grubbs group at CalTech. Grandner performed all computations. T. Patrick Montgomery performed experiments for dithiolate catalysts. Noah F. Fine Nathel performed experiments for amine-containing and amide-containing catalysts. Grubbs was the PI for Montgomery and Nathel. Houk was the PI for Grandner.

Chapter 3.1 and related materials

Chapter 3.1 is a collaborative project between the Sherman and Houk groups. The enclosed chapter will be incorporated into a manuscript not yet in preparation. As written, Grandner performed QM computations and Yang performed MD simulations (not discussed). Houk was the PI for Grandner and Yang.

Chapter 3.2 and related materials

Chapter 3.2 is a version of Gilbert, M. M.; DeMars, M. D.; Yang, S.; Grandner, J. M.; Wang, S.; Wang, H.; Narayan, A. R. H.; Sherman, D. H.; Houk, K. N.; Montgomery, J. “Regiodivergent Cyclization and Biocatalytic C-H Oxidation Enable Synthesis of Diverse 11- and 12-Membered Macrolatones from a Single Linear Substrate” (submitted to Science). Gilbert performed the synthesis and characterization. DeMars performed enzymatic experiments. Yang performed MD simulations. Grandner performed QM computations. S. Wang and H. Wang assisted with experimental portions of the project. Narayan was a project advisor/mentor. Sherman was PI for DeMars. Houk was PI for Yang and Grandner. Montgomery was PI for Gilbert.

Chapter 4.1 and related materials

Reprinted (adapted) with permission from Grandner, J. M.; Cacho, R. A.; Tang, Y.; Houk, K. N. *ACS Catal.* **2016**, 6, 4506-4511 (DOI: 10.1021/acscatal.6b01068). Copyright 2016 American Chemical Society. Grandner performed DFT computations and docking (with help from Blanton Martin for Docking). Cacho performed experiments and homology modeling. Tang was PI for Cacho. Houk was PI for Grandner.

Chapter 4.2 and related materials

Chapter 4.2 is a version of Grandner, J. M.; Aunins, T.; Giacometti, R. D.; Tang, Y.; Houk, K. N. “Unusual Oxidative Cascade Reactions by Cytochrome P450s in the Biosynthesis of Fumagillin” (in preparation). Grandner and Aunins performed in the manuscript. Giacometti performed related computations early on in project. Tang was project advisor. Houk was PI for Grandner, Aunins, and Giacometti at the time of research.

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Publications:

Grandner, J. M.; Cacho, R. A.; Tang, Y.; Houk, K. N. “Mechanism of the P450-Catalyzed Oxidative Cyclization in the Biosynthesis of Griseofulvin” *ACS Catal.* **2016**, 6, 4506-4511.

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Zhang, C.; Grandner, J.; Liu, R.; Lee, S. B.; Eichhorn, B. W. "Heterogeneous films of ordered CeO₂/Ni concentric nanostructures for fuel cell applications" *Phys. Chem. Chem. Phys.* **2010**, *12*, 4295-4300.

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Grandner, J. M.; Yang, S.; Houk, K. N. Computational modeling of PikC activity and selectivity for guided protein and substrate engineering. Presented at the ACS National Meeting, San Francisco, April 2017. (Poster)

Grandner, J. M. Computational Explorations of New Reactions and Useful Selectivities Catalyzed by P450 Enzymes. Presented at the ACS Division of Organic Chemistry Graduate Research Symposium, Bryn Mawr College, July 2016. (Lecture)

Grandner, J. M.;* Cacho, R. A.; Tang, Y.; Houk, K. N. Computational investigation of the P450-catalyzed oxidative cyclization step in the biosynthesis of griseofulvin. Presented at the ACS National Meeting, San Diego, March 2016. (Poster)

Grandner, J. M.;* Cacho, R. A.; Tang, Y.; Houk, K. N. Computational Investigation of the Key P450-Catalyzed Oxidation Step in the Biosynthesis of Griseofulvin. Presented at the 2015 National Organic Symposium, University of Maryland, July 01, 2015. (Poster)

Chapter 1.1

In Situ Catalyst Modification in Atom Transfer Radical Reactions with Ruthenium

Benzylidene Complexes

Juneyoung Lee, Jessica M. Grandner, Keary M. Engle, K. N. Houk, Robert H. Grubbs

Introduction

Since the first report of ruthenium-based catalysts in atom transfer radical addition (ATRA, also called Kharasch addition)¹ and atom transfer radical polymerization (ATRP),² this area of research has attracted widespread interest.^{3–14} Well defined ruthenium benzylidene complexes, commonly used as olefin metathesis catalysts, have also been reported to catalyze ATRA and ATRP.^{9,15–19} The ability of ruthenium benzylidene complexes to promote two reactions with such markedly different mechanisms has been utilized in various tandem reactions in which olefin metathesis and ATR reactions take place in one pot.^{20–22} Generally speaking, tandem catalysts, which catalyze multiple distinct reactions in one pot, are attractive synthetic tools that can simplify reaction procedures and reduce operational costs. An improved understanding of their mechanism can enable further catalyst development toward new applications. Among the many tandem catalysts that have been reported,²³ ruthenium benzylidene complexes have been a topic of interest to our research laboratory. For example, our group has reported ring opening metathesis polymerization (ROMP)-ATRP tandem catalysis for the preparation of block copolymers of 1,5-cyclooctadiene and methyl methacrylate (MMA).²⁰ Since the ROMP process was more rapid than ATRP, excess PCy₃ was added to the reaction and low-strain cycloolefins were employed to suppress the rate of ROMP. Using low-strain cycloolefins and excess phosphine, the rate of ROMP was suppressed to roughly the same rate as ATRP, allowing for productive tandem catalysis. While the mechanism by which ruthenium benzylidenes initiate and catalyze olefin metathesis has been

studied in great detail, little is known regarding the mechanism of ATR reactions promoted by these complexes. Herein, we present our findings regarding the mechanism of these reactions. We have performed kinetic studies of ATRA using various ruthenium benzylidene complexes. Under common ATRA conditions, these complexes were found to rapidly consume the alkene starting material, but not all of them promoted formation of the desired ATRA product. Our experimental results are consistent with a decomposed ruthenium species, rather than the ruthenium benzylidene, as the active ATRA catalyst in this system. These ATRA-active ruthenium complexes were further found to be inactive in olefin metathesis. We have attempted to identify the new ATRA-active ruthenium species. To do this, we employed NMR spectroscopy and X-ray crystallography. Finally, when this collection of ruthenium benzylidene complexes were tested in ATRP, we found that only the complexes that formed highly reactive ATRA catalysts were able to perform controlled polymerization, rather than redox-initiated free radical polymerization.

Experimental Section

Materials and Analytical Techniques. All reactions were carried out in dry vials with PTFE-faced silicone septa under an argon (Ar) atmosphere or in a Vacuum Atmospheres Glovebox under a nitrogen atmosphere, as specified. All solvents and reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. Fresh ampules of CDCl_3 (Sigma-Aldrich) were used in decomposition experiments of the ruthenium benzylidene catalysts. Complexes **1**, **2**, **3**, **6**, and **7** were obtained from Materia, Inc. Complexes **4** and **5** were prepared from **2** and **3**, respectively, following literature procedures.^{24,25} ^1H NMR spectra were recorded on one of the following instruments: Varian Mercury (300 MHz), Varian Inova (500 MHz), or Bruker Ascend with Prodigy broadband cryoprobe (400 MHz). Gel permeation chromatography (GPC) was conducted on two Agilent PLgel 10 μm MIXED-BLS 300 mm $\times$ 7.5 mm columns with Agilent

P260 series pump and autosampler with Wyatt Dawn Heleos-II multiangle static light scattering detector and Optilab T-rEX differential refractive index detector with THF as an eluent.

General Procedure for ATRA Catalyzed by Ruthenium Benzyldiene Complexes. To an 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 0.75×10^{-1} mmol), MMA (106.84 μ L, 9.99×10^{-1} mmol), and CHCl_3 (0.8 mL, 9.98 mmol) were added. Anisole (10 μ L, 9.20×10^{-2} mmol) was added as an internal standard. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to the specified temperature (65 or 40 °C). The reaction was kept under Ar (g), and aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. After 2 h, the solution was precipitated into petroleum ether and filtered to remove precipitated catalyst. Solvent and unreacted MMA were removed using a rotary evaporator. The yield of the product was calculated based on integration of ^1H NMR resonances at 6.01 ppm ($-\text{CCl}_2\text{H}$ from the product) and 1.84 ppm ($-\text{CH}_3$ from the product and byproducts). All of the ATRA reactions in this paper were performed following this general procedure using the same molar ratio of [catalyst]: [MMA]: [CHCl_3].

Decomposition Study of Ruthenium Benzyldiene Complexes. Inside the glovebox, an NMR tube was charged with the ruthenium complex and CDCl_3 in the same molar ratio as specified in the general ATRA procedure. The NMR tube was capped with a septum, removed from the glovebox, and heated to 65 °C. ^1H NMR spectra were collected at predetermined time points, and the integral of the benzyldiene resonance (16–20 ppm, ^1H) was plotted as a function of time.

General Procedure for ATRA Catalyzed by Activated Ruthenium Complexes. To an 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 0.75×10^{-1} mmol), anisole (10 μ L, 9.20×10^{-2} mmol), and CHCl_3 (0.8 mL, 9.98 mmol) were added. The solution was

degassed with Ar (g) for 10 min and then heated to 65 °C, until the benzyldiene ^1H NMR resonance had completely disappeared. The reaction vessel was allowed to cool to room temperature, and freshly degassed MMA (106.84 μL , 9.99×10^{-1} mmol) was added to the solution. The reaction was initialized by immersing the reaction vessel into an oil bath preheated to the specified temperature (65 or 40 °C) and was held under an Ar (g) atmosphere. Aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. All of the ATRA reactions in this report with preactivated ruthenium benzyldiene complexes were performed following this general procedure using identical concentrations.

General Procedure for ATRA Catalyzed by Ruthenium Benzyldiene Complexes with 5 equiv of PCy_3 . To an 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 7.53×10^{-2} mmol), MMA (106.84 μL , 9.99×10^{-1} mmol), anisole (10 μL , 9.20×10^{-2} mmol), and CHCl_3 (0.8 mL, 9.98 mmol) were added. PCy_3 (105.64 mg, 3.77×10^{-1} mmol) was then added, and the solution was degassed with Ar (g) for 10 min. The reaction was initialized by immersing the reaction vessel into an oil bath preheated to 65 °C and was held under an Ar (g) atmosphere. Aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. Experiments with **2** and **3** were performed following this general procedure using identical concentrations and reaction conditions.

*RCM Catalyzed by **3** and Benzyldiene-Decomposed (ATRA-Activated) **3**.* The reaction was performed following a literature procedure.²⁶ Complex **3** (7.47 mg, 8.01×10^{-3} mmol) was dissolved in degassed CDCl_3 (0.75 mL). For reactions catalyzed by decomposed **3**, the solution was then pretreated at 65 °C until the indicated level of benzyldiene decay (as monitored by ^1H NMR) was observed. The catalyst solution was cooled to room temperature, and diethyl diallylmalonate (19.3 μL , 7.98×10^{-2} mmol) was added. The reaction mixture was brought to a

temperature of 30 °C for 1 h, after which point an ^1H NMR spectrum was collected to calculate olefin conversion.

Crystallization of ATRA-Activated 1 with Bipy. Complex **1** (62 mg, 0.75×10^{-1} mmol) was dissolved into 0.8 mL of CHCl_3 (0.8 mL, 9.98 mmol) and was activated by heating at 65 °C until complete decay of the benzylidene peak in the ^1H NMR spectrum was observed. The solvent was removed under vacuum, and the resulting powder was redissolved in a minimal amount of DCM, prior to addition of bipy (58.83 mg, 0.38 mmol). Pentane was slowly added to make a layer above the DCM, and the solution was allowed to stand unperturbed at room temperature until crystals of the complex formed.

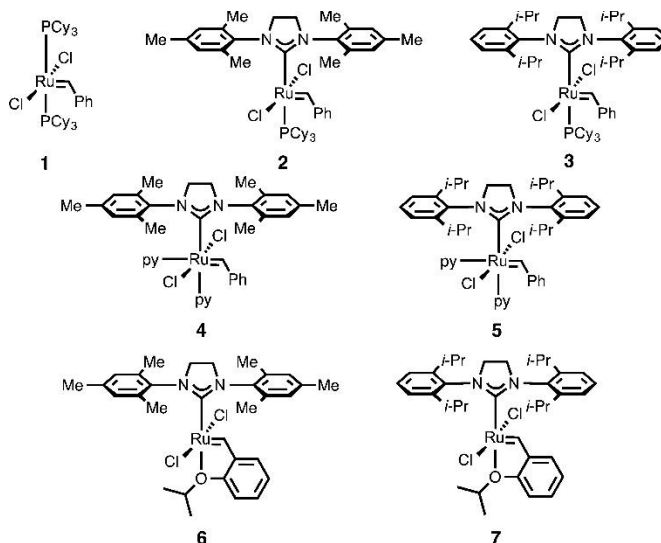
ATRA Catalyzed by Ru(III)Cl₃ and PCy₃ Complex. MeOH (0.8 mL) was added to Ru(III)Cl₃ (15.54 mg, 0.75×10^{-1} mmol) and PCy₃ (42.02 mg, 1.50×10^{-1} mmol), and the reaction mixture was heated to reflux overnight. The solvent was removed under vacuum. Benzene was added, and the solution was filtered through glass pipet with kimwipe plug. The filtrate was again concentrated under vacuum to give a dried powder. To this solid, were added MMA (106.84 μL , 9.99×10^{-1} mmol) and 0.8 mL of CHCl_3 (0.8 mL, 9.98 mmol), followed by anisole (10 μL , 9.20×10^{-2} mmol) as an internal standard. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to 65 °C.

ATRP Catalyzed by Ruthenium Benzylidene Complexes. To an 8 mL vial with silicone septum cap equipped with a magnetic stir bar, ethyl α -bromoisobutyrate (10 μL , 6.81×10^{-5} mmol), MMA (1.46 mL, 1.36×10^{-2} mmol), and complex **1** (56.07 mg, 6.81×10^{-5} mmol) were added. Toluene (681 μL) and anisole (10 μL) were added as the solvent and internal standard, respectively. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to 85 °C. Aliquots were removed at predetermined time

points and analyzed by ^1H NMR and GPC to monitor, MMA conversion M_n , and dispersity ($\bar{D}$) over time. All of the ATRP reactions in this report were performed following this same general procedure under identical reaction conditions.

Results and Discussion

Chart 1.1.1. Ruthenium Benzyldiene Complexes



This investigation was commenced by examining reaction kinetics of ATRA with a series of ruthenium benzyldienes commonly employed in olefin metathesis (Chart 1.1.1). All of these complexes have been previously reported to catalyze olefin metathesis, and **1** has been shown to be effective in ATR reactions.^{9,19} For ATRA, methyl methacrylate (MMA) was employed as a model substrate due to its well-established reactivity in ATRA and ATRP. Chloroform (CHCl_3) was chosen as the coupling partner and reaction solvent since ATRA using this halogen donor has been studied extensively (Scheme 1.1.1). Reactions with all of the complexes shown in Chart 1.1.1 were monitored over 2 h by measuring the MMA conversion at predetermined time points by ^1H NMR spectroscopy (Table 1.1.1). By monitoring MMA conversion over reaction time (Figure 1.1.2a and Table 1.1.1), it was found that five out of seven ruthenium benzyldiene catalysts in the

study led to consumption of MMA. Complexes **6** and **7** were found to be unreactive in ATRA. Complex **3** containing a SIPr ligand was the most active, followed in order by **1**, **4**, **2**, and **5**. The final yield of the desired product **8** was generally higher with faster ATRA catalysts. For example, with complexes **1** and **3**, >99% of consumed MMA was converted to ATRA product, whereas greater discrepancies between MMA conversion and product yield were observed with **2**, **4**, and **5**. In these cases, MMA may have been consumed in undesired oligomerization/polymerization, a well-known side reaction of ATRA. Notably, no relationship between metathesis activity (or metathesis initiation rate) and ATR rate was observed within this series. Density functional theory (DFT) calculations were performed assuming a general mechanistic paradigm involving inner-sphere electron transfer from an intact ruthenium benzyldiene moiety to homolyze the carbon–halogen bond (Figure 1.1.1). However, predicted relative catalyst activities from computed ΔG_{rxn} for this reaction with complexes **1–7** were not in agreement with the empirically observed reactivity trend. Additionally, the ΔG_{rxn} values for the halogen abstraction step with most complexes were too endergonic for effective catalysis. This inconsistency prompted us to consider the stability of complexes **1–7** under the reaction conditions.

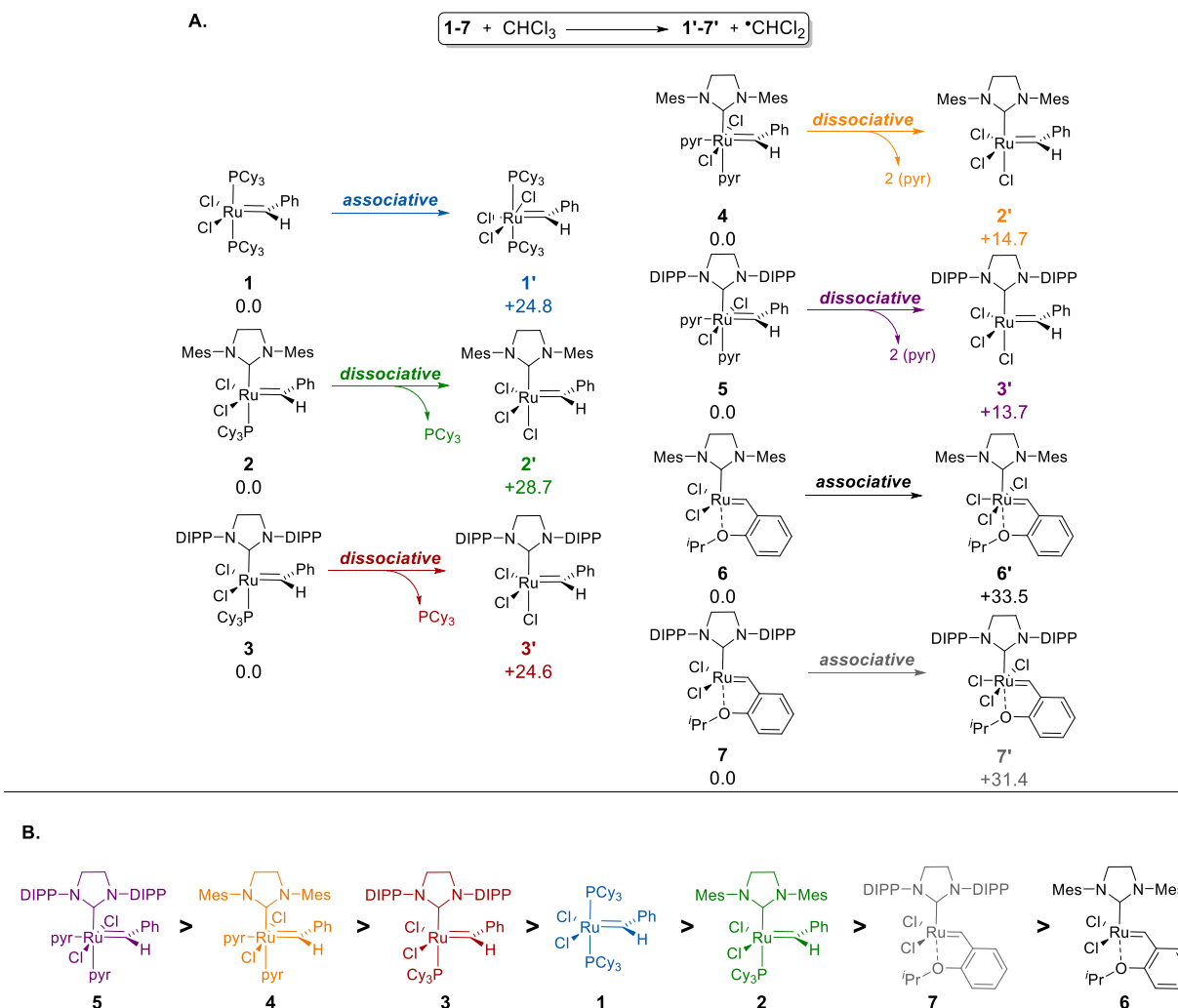
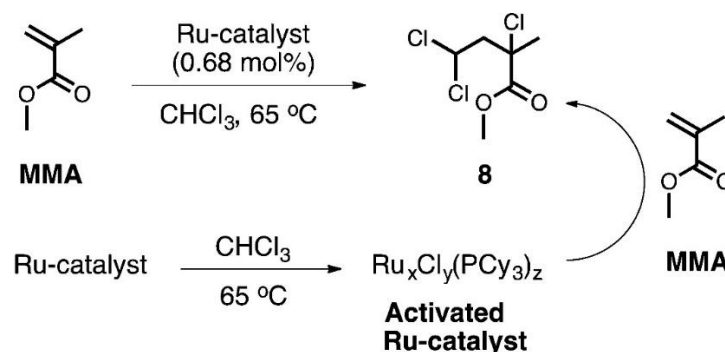


Figure 1.1.1. (a) Computed ΔG_{rxn} (kcal/mol) for chlorine atom transfer from CHCl_3 to complexes 1–7. (Note: loss of the first pyridine, anti to the benzylidene, from 4 and 5 is exergonic by 4.7 and 5.1 kcal/mol respectively) (b) Computationally predicted order of ATRA activity.

Scheme 1.1.1. ATRA of MMA Catalyzed by Ruthenium Benzylidenes of Activated Ruthenium Complexes



$$[\text{MMA}] : [\text{CHCl}_3] : [\text{catalyst}] = 1 : 10 : 0.075$$

Solutions of each complex in CDCl_3 without MMA were prepared at the same concentration used in the ATRA experiments. The diagnostic benzylidene proton peak was monitored by ^1H NMR (16–20 ppm) over time at the reaction temperature (65 $^\circ\text{C}$). Most of the catalysts were unstable in CDCl_3 , as evidenced by the disappearance of the benzylidene peak and appearance of new proton resonances far upfield of the benzylidene region. Complexes **6** and **7** were stable for >4 h under these conditions (Figure 1.1.2b, Figures A.S2–A.S12). This decomposition process was found to be highly temperature and solvent-dependent. For example, with catalyst **4**, no appreciable benzylidene decay was observed at a slightly reduced temperature of 55 $^\circ\text{C}$ for over 4 h. Complex **3** showed rapid benzylidene decay in CDCl_3 but did not show any benzylidene decay in C_6D_6 until subsequent addition of an alkyl halide (Figure 1.1.2c). This indicates that the alkyl halide triggers benzylidene decomposition. Furthermore, complex **3** exhibited a nearly identical benzylidene decay profile in CDCl_3 containing added K_2CO_3 . These results are consistent with the alkyl halide, rather than heat or trace HCl , as the component that drives benzylidene decomposition. Strikingly, the order of benzylidene decay rate in these stoichiometric experiments was the same order as MMA conversion in catalytic ATRA (Figures

1.1.2a and b). The correlation between benzylidene decay rate and ATRA rate prompted us to examine the extent to which the newly formed ruthenium species are active participants in ATRA reactions.

Table 1.1.1. MMA Conversion and Product Yield of ATRA (2 h) with Different Ruthenium Benzylidene Complexes

entry	catalyst	<i>T</i> (°C)	MMA conv (%) ^a	yield for 8 (%) ^b
1	1	65	89	89
2	2	65	45	7
3	3	65	95	94
4	4	65	79	61
5	5	65	17	~0
6	6	65	~0	~0
7	7	65	~0	~0
8	activated 1	65	97	86
9	activated 3	65	99	93
10	activated 4	65	86	68
11	1	40	20	~1
12	activated 1	40	41	28
13	3	40	32	~2
14	activated 3	40	85	70
15	1 + 5 equiv PCy ₃	65	67	16
16	2 + 5 equiv PCy ₃	65	40	~0
17	3 + 5 equiv PCy ₃	65	69	~2

^aMMA conversion was calculated from ¹H NMR integration using anisole as an internal standard.

^bProduct yield was calculated from ¹H NMR integration, after first removing the ruthenium catalyst by precipitation into petroleum ether.

To this end, the reactivity of the benzylidene-decomposed ruthenium species, formed from pretreatment of **1**, **3**, and **4** in CHCl₃, was investigated. First, solutions of these catalysts in CHCl₃ were heated at 65 °C under Ar (g) in the absence of MMA until no benzylidene peak was observed in the ¹H NMR spectrum. MMA was added, and reaction progress was monitored (Scheme 1.1.1). As shown in Table 1.1.1 and Figure 1.1.3a, the activated ruthenium complexes demonstrated faster

rates, providing equally high yield of the ATRA product. Given the pronounced temperature dependence of the stoichiometric benzylidene decay reactions, we next sought to determine the temperature dependence of ATRA reactivity. When ATRA reactions were run with **1** and **3** at 40 °C, where no benzylidene decay was observed, MMA consumption proceeded slowly and only trace product formation was observed. In contrast, preactivated, benzylidene-decayed **1** and **3** exhibited faster rate and provided greater product yields, even at 40 °C (Table 1.1.1).

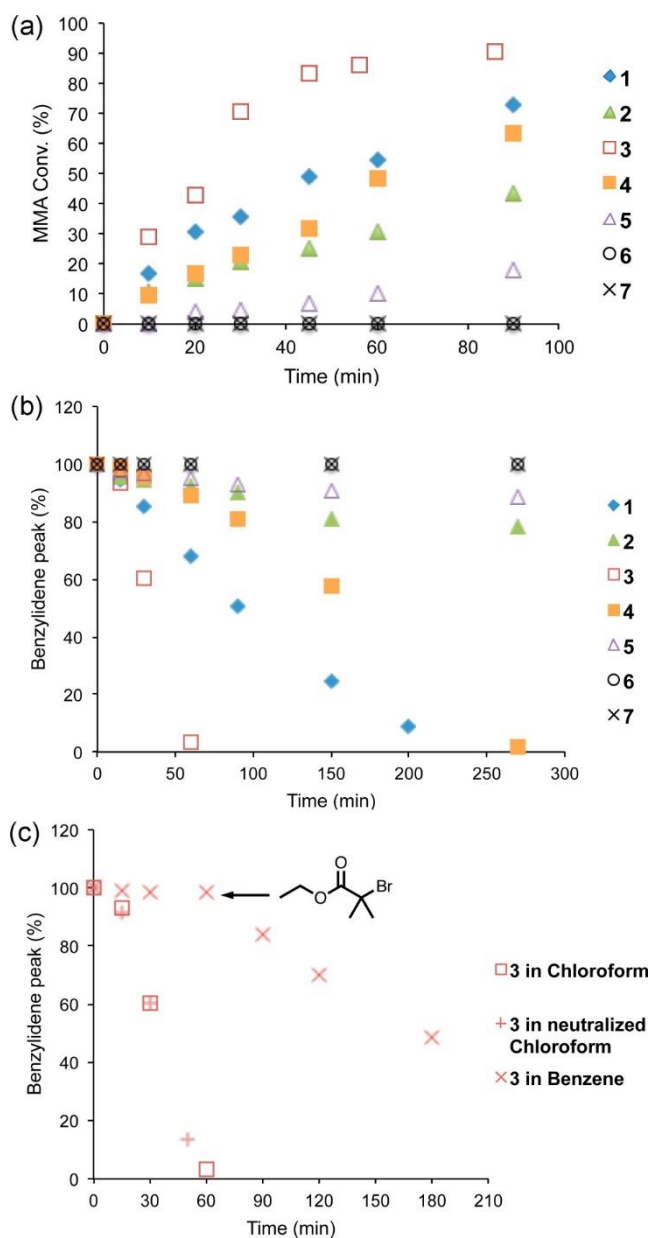


Figure 1.1.2. Rate profiles of (a) ATRA promoted by ruthenium benzylidene complexes. Reaction conditions as in Scheme 1.1.1. (b) Benzylidene ^1H NMR resonance decay of complexes **1–7** over time. Reaction conditions as in Scheme 1.1.1 in the absence of MMA. (c) Benzylidene ^1H NMR resonance decay of complex **3** in CDCl_3 , neutralized CDCl_3 , and C_6D_6 with addition of ethyl α -bromoisobutyrate after 1 h. In the neutralized experiment, excess K_2CO_3 solid was added to a freshly degassed CDCl_3 solution, and the resulting heterogeneous mixture was shaken vigorously prior to heating.

The data shown above indicate a reaction pathway for ATRA in which the ruthenium benzylidene is converted into one or more new ATRA-active ruthenium species under ATRA conditions. When activated, the new species exhibit superior reactivity in ATRA compared to the parent complexes, even at lower temperature. As is typical in ATR reactions, trials performed under our standard conditions were inhibited by O_2 . Consistent with this observation, the newly formed ruthenium species were found to be air-sensitive and were unreactive in ATRA after exposure to air.

The effect of excess phosphine ligand in catalytic ATRA was also studied. The addition of tricyclohexylphosphine (PCy_3 , 5 equiv relative to catalyst) to solutions of the complexes altered the benzylidene decomposition trends (Figure A.S13). With complexes **1** and **2**, the presence of additional PCy_3 increased the rate of benzylidene decay. However, complex **3** showed slower decay than **1** and **2** under the same conditions. The origins of these effects are still under investigation. The rates of catalytic ATRA reactions with additional PCy_3 using **1** and **2** were slightly inhibited by excess PCy_3 . The reaction with **3** became substantially slower, and the overall product yield was significantly reduced in all cases (Table 1.1.1 and Figure 1.1.3b).

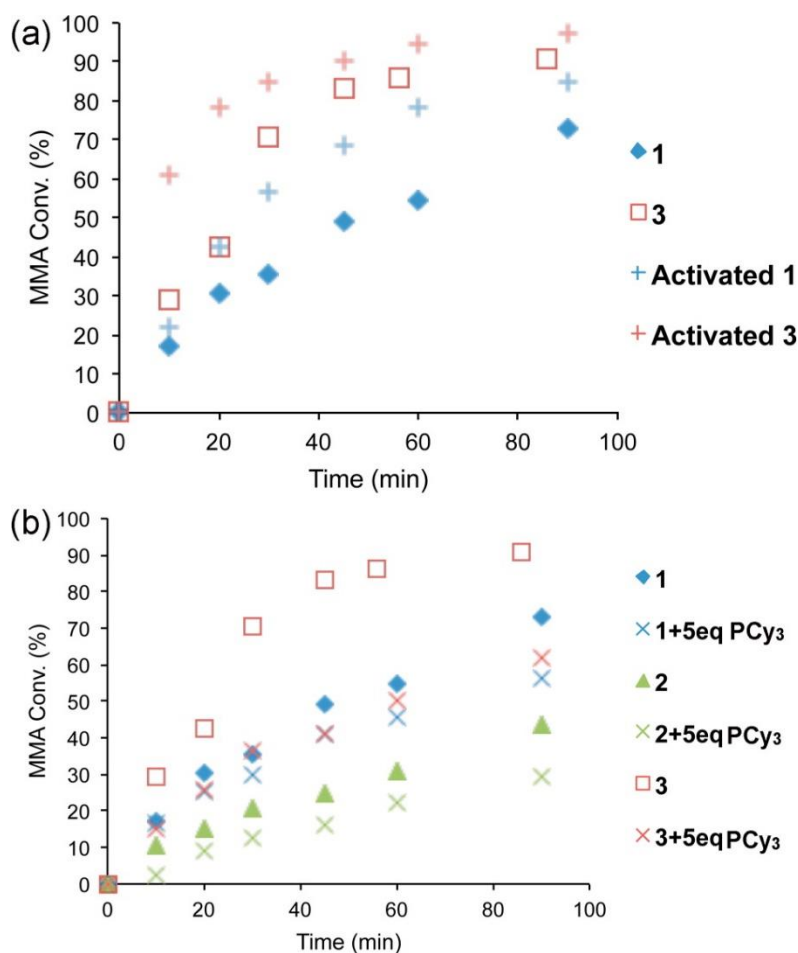
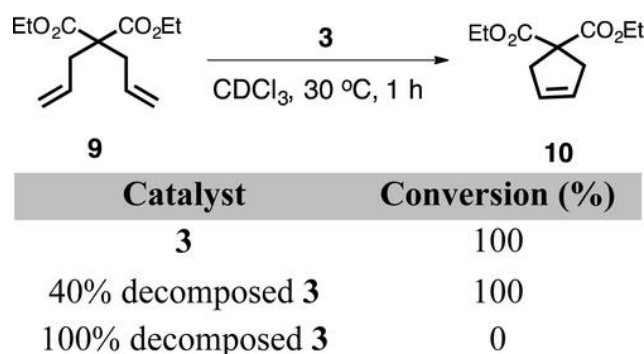


Figure 1.1.3. (a) Kinetic study of ATRA of MMA catalyzed by **1**, **3**, and their activated analogues. (b) Effect of adding PCy₃ (5 equiv) to ATRA catalyzed by **1**, **2**, and **3**. Reaction condition same as in Scheme 1.1.1.

The identity of the in situ generated ruthenium species was explored further. Complex **3** was decomposed in CDCl₃ to 40% completion and 100% completion (as measured by benzylidene ¹H NMR signal). It was found that an ATRA-activated sample of **3** with completely decayed benzylidene was inactive in ring-closing metathesis (RCM) of diethyl diallylmalonate (**9**), a highly reactive RCM substrate with complexes **1**–**7** (Scheme 1.1.2). In contrast, samples of untreated **3** and 40%-benzylidenedecayed **3** catalyzed RCM with **9**, providing 100% conversion to **10** after 1

h. These results, in conjunction with the ^1H NMR data, prove that the ATRA-active ruthenium species does not contain a benzylidene/alkylidene moiety.

Scheme 1.1.2. RCM Catalyzed by **3 and Benzylidene-Decomposed ATRA-Activated **3****



To gain more information regarding the structure of the ATRA-active species, we next turned to NMR spectroscopy and X-ray crystallography. As discussed above, upon decomposition of complexes **1** and **3** in CDCl_3 at $65\text{ }^\circ\text{C}$, the ^1H and ^{13}C NMR spectra revealed that the benzylidene moiety had fully dissociated. In the ^{31}P NMR spectra of ATRA-activated **1** and **3**, a substantial downfield shift of the major phosphine resonance was observed. In both cases, a major phosphine resonance at 108.10 ppm appeared upon decomposition (Figures A.S4 and A.S8). This peak is in an unusual region of the ^{31}P spectrum, and we suspect that it could represent the corresponding dichlorophosphonium salt.²⁷ The ^{31}P NMR results along with the data from excess PCy_3 experiments shown above (Table 1.1.1 and Figure 1.1.3b) with **1** and **3**, are consistent with a mechanism in which PCy_3 is partially or fully dissociated from the ruthenium center in the active form of the catalysts.

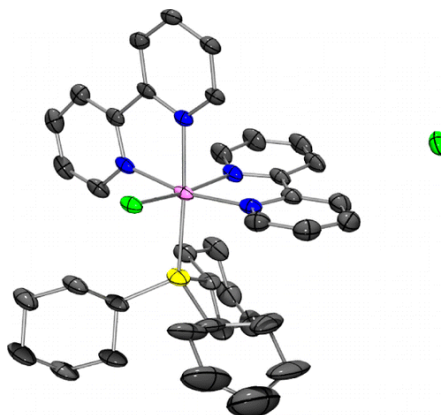


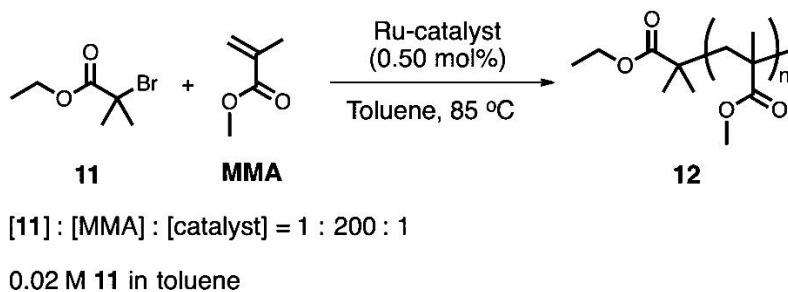
Figure 1.1.4. X-ray crystal structure of $\text{Ru(II)Cl(PCy}_3\text{)(bipy)}_2\text{Cl}^-$ formed from addition of bipy to ATRA-activated **1**. Hydrogen atoms and solvent molecules were omitted for clarity. Pink, Ru; gray, C; yellow, P; blue, N; green, Cl (CCDC 1473173).

In addition to NMR spectroscopy, we have attempted to obtain single crystals of ATRA-activated ruthenium complexes **1** and **3** that would be suitable for X-ray diffraction. Despite numerous attempts, we were unable to grow suitable crystals directly from the decomposed solutions. However, after extensive experimentation we found that the addition of 2,2'-bipyridine (bipy, 5 equiv) to a solution of ATRA-activated **1** led to formation of a new species, $\text{Ru(II)Cl(PCy}_3\text{)(bipy)}_2\text{Cl}^-$, which we were able to crystallize and characterize by X-ray diffraction (Figure 1.1.4). Interestingly preliminary X-ray crystal structure data of the analogous experiment with activated **3** allowed tentative identification of another new complex, $\text{Ru(III)Cl}_3\text{(PCy}_3\text{)(bipy)}$ (data not shown). In both cases, addition of bipy resulted in an upfield shift of the phosphine peak from 108.10 ppm (activated **1** and activated **3**) to 49.80 ppm (Figures A.S4 and A.S8). Moreover, during an attempt to obtain an X-ray crystal structure of activated **3**, we instead isolated and characterized the $\text{SIPr}\cdot\text{HCl}$ salt. In a separate experiment, when **3** was exposed to ethyl α -bromoisobutyrate in C_6D_6 (Figure 1.1.2c), we were able to obtain colorless crystal from this

reaction mixture, which turned out to be the $\text{SIPr}\cdot\text{HBr}$ salt. Both of these results suggest that the NHC ligands are labile under these reaction conditions.

Combining the insights from all of these experiments, we now suspect that the original ruthenium benzylidene complexes decompose under common ATRA reaction condition ($65\text{ }^{\circ}\text{C}$ in CHCl_3) through complete dissociation of all L-type ligands (benzylidene, PCy_3 , and NHC) from the ruthenium metal center. We propose that a simple ruthenium chloride complex, such as Ru(III)Cl_3 or Ru(II)Cl_2 or a Ru_xCl_y cluster, possibly containing one or more bound phosphine ligands, is the actual ATRA-active species. To explore this possibility further, we attempted to perform ATRA with Ru(III)Cl_3 , which we found to be completely insoluble in CHCl_3 even upon addition of MMA. To solubilize this complex, Ru(III)Cl_3 was refluxed with PCy_3 in MeOH overnight, concentrated in vacuo, suspended in benzene, filtered, washed with benzene and dried. The resulting ruthenium complex, presumably $\text{RuCl}_3(\text{PCy}_3)_n$, was soluble in CHCl_3 and successfully converted MMA to the ATRA product with 96% MMA conversion and 88% product yield (Figure A.S14). Also, the reaction kinetics with $\text{RuCl}_3(\text{PCy}_3)_n$ were faster than with **3** and were in perfect agreement with ATRA-activated **3**. The newly prepared $\text{RuCl}_3(\text{PCy}_3)_n$ complex, however, did not show the same peaks in the ^{31}P spectrum as activated **1** or activated **3** (108.10 ppm). This experiment confirms that the phosphine peak from 108.10 ppm in ATRA-activated **3** is a byproduct from the catalyst activation process and does not correspond to a ruthenium species that is involved in ATRA reactions.

Scheme 1.1.3. ATRP of MMA Catalyzed by Ruthenium Benzylidenes



Lastly, we performed a series of experiments to test whether insights gained from this investigation were relevant to ATRP. ATRP and ATRA have similar mechanisms involving active radicals generated by a reversible redox process of halogenated substrates and transition metal complexes.²⁸ In ATRP, a large excess of olefin leads to polymerization rather than a single radical addition. Ruthenium benzylidene complexes **1–5** converted MMA to polymer (Scheme 1.1.3, Figure 1.1.5). The order of reaction rates was similar to the previous catalytic ATRA, except that **4** was the slowest in ATRP. However, only **1** and **3** polymerized MMA in a controlled fashion to yield polymers with linear molecular weight increases and low dispersities. Polymerization with **2**, **4**, and **5** showed constant molecular weight, indicating early termination of the polymer chains and even undesired coupling reactions in the case of **5**. It has been reported that some ruthenium benzylidenes preferentially promote redox-initiated free radical polymerization over ATRP;¹⁸ however, no clear explanation has been put forward to rationalize the differences. The present work shows that complexes **1** and **3**, which exhibited rapid in situ conversion in CHCl₃ with high product yield in ATRA, also promoted efficient ATRP. A well-known side reaction of ATRA is polymerization/oligomerization, which can proceed via redox-initiated free radical polymerization. Thus, the ATRA data can be used to explain which ruthenium benzylidene precatalysts favors ATRP over free radical polymerization.

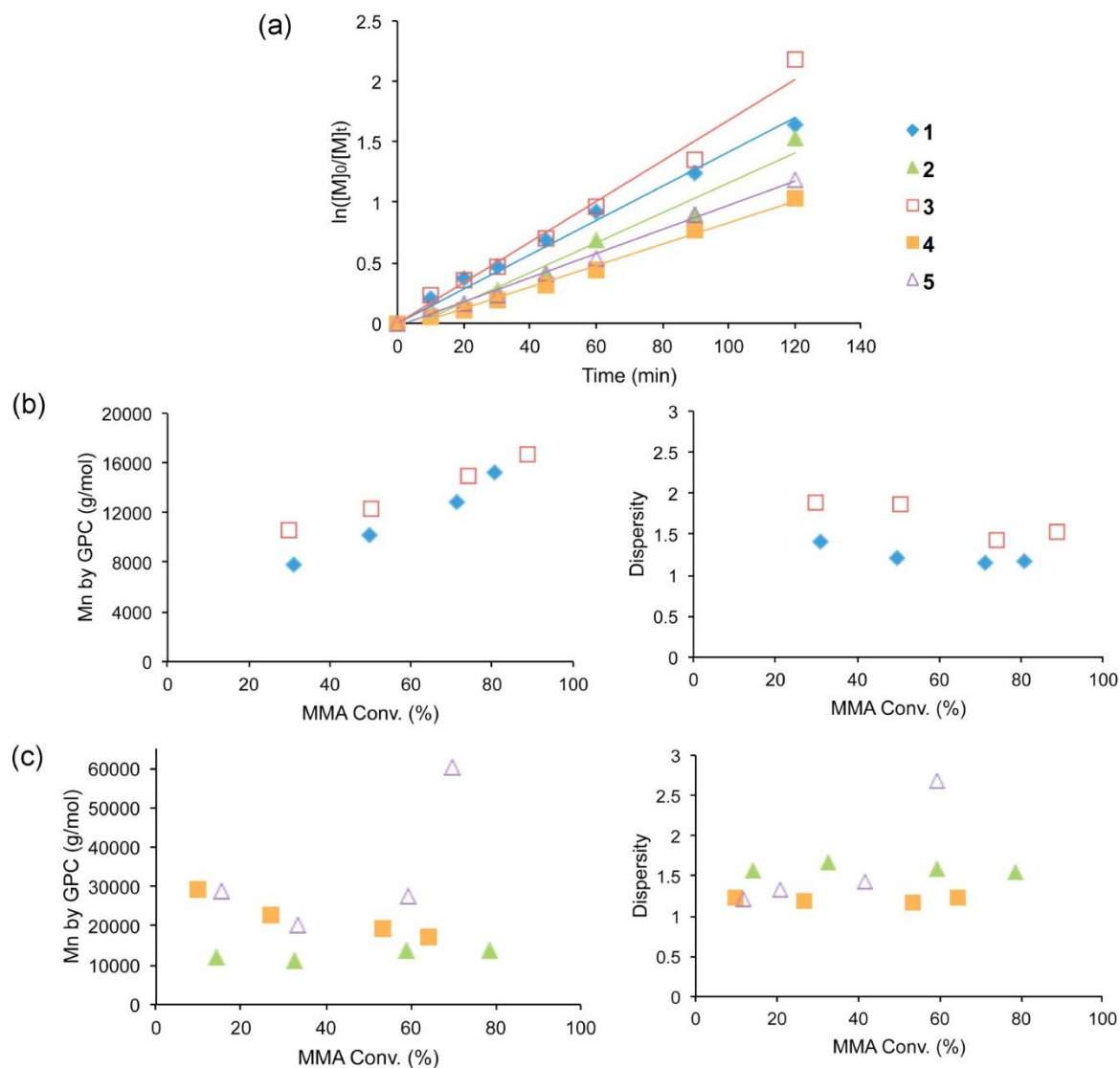


Figure 1.1.5. Kinetic studies of ATRP catalyzed by ruthenium benzylidene complexes. (a) $\ln([M]_0/[M]_t)$ over time. (b) M_n and $\mathcal{D}$ over MMA conversion with **1** and **3**. (c) M_n and $\mathcal{D}$ over MMA conversion with **2**, **4**, and **5**.

Conclusion

We have discovered that, under ATRA conditions, ruthenium benzylidene complexes are transformed into one or more new ATRA-active, metathesis-inactive ruthenium species, possibly a simple $\text{Ru}_x\text{Cl}_y(\text{PCy}_3)_z$ complex. The same complexes that give high yields and minimal

competing side reactions in ATRA also promote living ATRP over uncontrolled free radical polymerization. The results of this study showcase the importance of mechanistic inquiry as a means of better understanding and ultimately improving tandem catalytic reactions.

Supporting Information

Detailed experimental procedures, NMR spectra, and computational and crystal data are contained in Appendix A.

Notes

All experimental work was performed by Lee and Engle. All computational work was performed by Grandner.

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Chapter 1.2

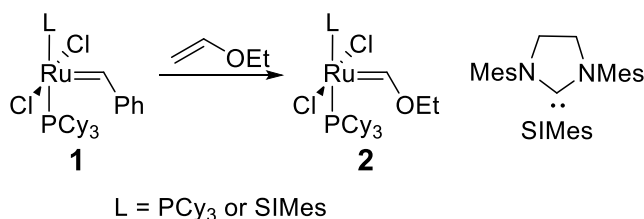
Metathesis and Decomposition of Fischer Carbenes of Cyclometalated Z-Selective Ruthenium Metathesis Catalysts

Tonia S. Ahmed,[#] Jessica M. Grandner,[#] Buck L. H. Taylor, Robert H. Grubbs, K. N. Houk

[#] Equal contribution

Olefin metathesis has become a favored method for the generation of carbon-carbon double bonds. Ruthenium-based catalysts exhibit excellent stability, functional group tolerance, and general ease of use.¹ Metathesis has been implemented in several fields including green chemistry,² organic synthesis,³ materials science,⁴ and pharmaceuticals.⁵

Scheme 1.2.1. Formation of Fischer Carbene Complexes by Reaction of Ethyl Vinyl Ether with Olefin Metathesis Catalysts.



Reactions utilizing these catalysts are often quenched by the addition of an excess of vinyl ether.⁶ The vinyl ether reacts with the active catalyst to form a Fischer carbene ruthenium complex (**2**, Scheme 1.2. 1). Due to their stabilities, Fischer carbenes of this type are considered metathesis-inactive. These Fischer carbenes have previously been isolated and structurally characterized.^{7,8} While not active under standard metathesis conditions, Fischer carbenes of this type are active at elevated temperatures and with specific substrates.^{9,10} For example, Takahira and Morizawa recently demonstrated the ability of **2**, containing the SIMes ligand, to catalyze productive metathe-

sis using heavily fluorinated olefins, albeit with very low catalyst turnover. The unexpected activity of these ruthenium complexes is due to the relative thermodynamic stability of the fluoro-Fischer carbene formed by metathesis, or Fischer carbene exchange, with **2**.¹⁰

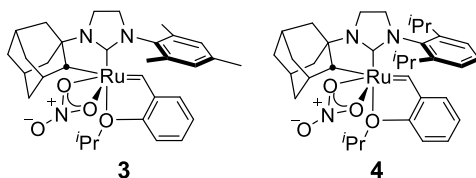
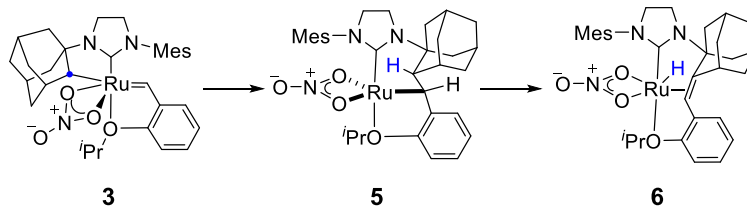


Figure 1.2.1. Prominent Z-selective catalysts.

In 2011, kinetically Z-selective ruthenium-based catalysts were first reported bearing an adamantyl-chelated NHC ligand and pivalate X-type ligand.¹¹ Many analogs have now been synthesized, including the highly active and Z-selective catalysts **3**¹² and **4**.¹³ Mechanistic and decomposition studies of these types of cyclometalated complexes have been carried out with both experiment and theory.^{14,15} Decomposition of these catalysts proceeds via irreversible insertion of the alkylidene into the chelating ruthenium-carbon bond to produce a ruthenium alkyl intermediate (**5**, Scheme 1.2. 2). A subsequent β -hydride elimination reaction gives ruthenium-hydride **6**.¹⁵

Reactions using these chelated metathesis catalysts are also quenched by vinyl ethers.¹⁶ It was expected that the Fischer carbenes derived from the cyclometalated catalysts would of similar stability to **2**. Fischer carbenes of type **2** can degrade to ruthenium hydrides.⁹ Ruthenium hydrides are known olefin hydrogenation and isomerization catalysts.¹⁷ In order to understand the decomposition and activity of chelated Fischer carbenes, we have studied the reactions of **3** and **4** with vinyl ethers and have identified a ruthenium hydride product potentially capable of causing olefin isomerization. Computations were used to explore the decomposition and, surprisingly, we have found that metathesis with the Fischer carbene is an integral part of the decomposition pathway.

Scheme 1.2.2. Decomposition Pathway of Cyclometalated Ruthenium Catalysts^{14,15}



The reactions of phenyl vinyl ether with chelated catalysts **3** and **4** were performed in THF-*d*₈ and monitored with ¹H NMR. Generation of species thought to be a Fischer carbene was observed by the appearance of a peak shifted upfield with respect to the alkylidene.⁹ Subsequent formation of a hydride species was observed by the appearance of ¹H NMR signals at -12.16 ppm and -11.97 ppm, respectively, consistent with chemical shifts of known ruthenium hydrides.¹⁵ Full assignment of the structure by NMR spectroscopy was challenging due to significant overlap of aromatic ¹H and ¹³C signals. Consequently, the reaction of butyl vinyl ether with **3** in THF-*d*₈ was studied in order to facilitate analysis of the complex by NMR spectroscopy. The reaction mirrored the results obtained using phenyl vinyl ether. The disappearance of the signal corresponding to the benzylidene proton of **3** and the appearance of a broad peak at 13.83 ppm was observed, consistent with previously reported Fischer carbenes.⁹ The subsequent disappearance of this signal and concurrent appearance of a new signal at -12.62 ppm indicated the formation of the hydride species **7** (Figure 1.2.2).

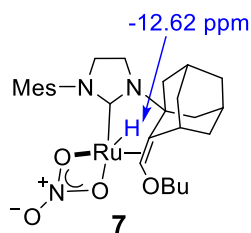
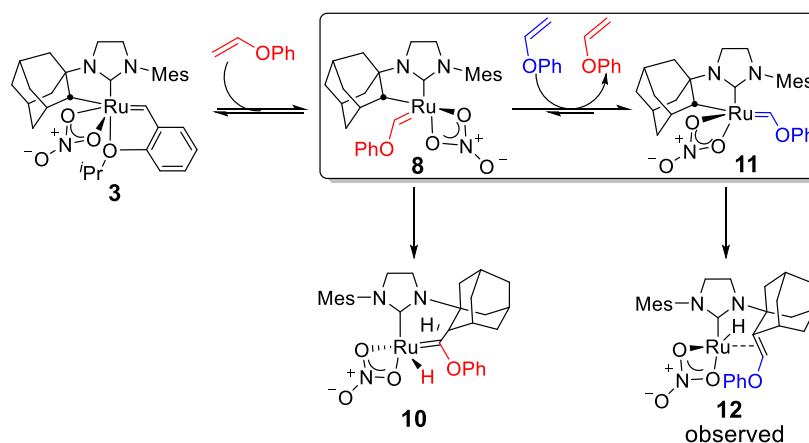


Figure 1.2.2. Structure of ruthenium hydride, **7**.

In the ^1H -NMR spectrum of this reaction mixture, a singlet corresponding to a single proton appears at 5.12 ppm, consistent with that of the alkene proton of known beta-hydride decomposition products of *Z*-selective catalysts.¹⁵ ^1H - ^{13}C HMBC studies show correlations between the methylenes of the butyl group to the aforementioned alkenyl singlet at 5.12 ppm, which furthermore shows correlations with the protons of the adamantyl group. ^{13}C -DEPT experiments showed the existence of 4 methyl groups, 10 methylene groups, 6 methine groups, 7 quaternary carbons in the structure of this complex. High-resolution mass spectrometry confirmed the mass corresponding to 7.

Scheme 1.2.3. Isomers of Fischer Carbene and Respective Hydrides



Density functional calculations were performed to determine the decomposition pathways available to Fischer carbenes derived from complex **3**. Reaction of **3** with phenyl vinyl ether leads to formation of Fischer carbene **8**. However, **8** cannot lead to the observed product **12** via migratory insertion and β -hydride elimination.¹⁵ Figure 1.2.3 is a top view of **8** and the subsequent insertion intermediate, **9**. The β -hydrogen of **8**, highlighted in green, is on the same side as the Fischer carbene and far from the ruthenium center. After migratory insertion of the carbene to **9**, the β -hydrogen is pushed even further from the ruthenium center, to a distance of 3.84Å. The structural

images in Figure 1.2.3 show that the highlighted hydrogen is not activated for elimination due to this distance. Therefore, diastereomer **8** is prevented from direct degradation to the observed product **12**. DFT results (Figure B.S17) indicate that migratory insertion followed by alpha-hydride elimination could occur to give **10** with a rate-determining step of 25.5 kcal/mol. This product has not been observed experimentally.

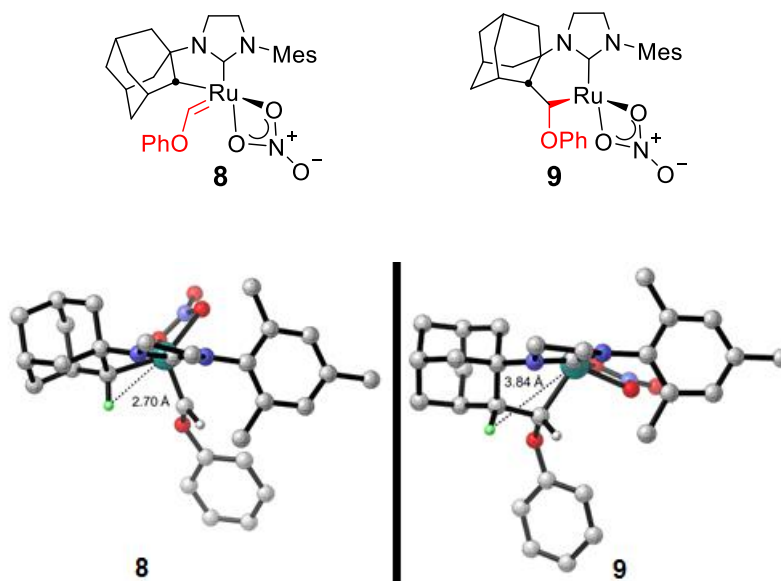


Figure 1.2.3. View of **8** and **9**, looking down on the NHC. β -hydrogen highlighted in green.

Therefore, there must be an alternative, lower energy path to decomposition of **8** to the experimentally observed **12**. Based on the recent literature on Fischer carbene exchange,¹⁰ we propose epimerization of Fischer carbene **8** to **11** via metathesis with excess vinyl ether.¹⁸ Complex **11** could then decompose to experimentally observed hydride **12** via the pathway previously reported and shown in Scheme 1.2.2.

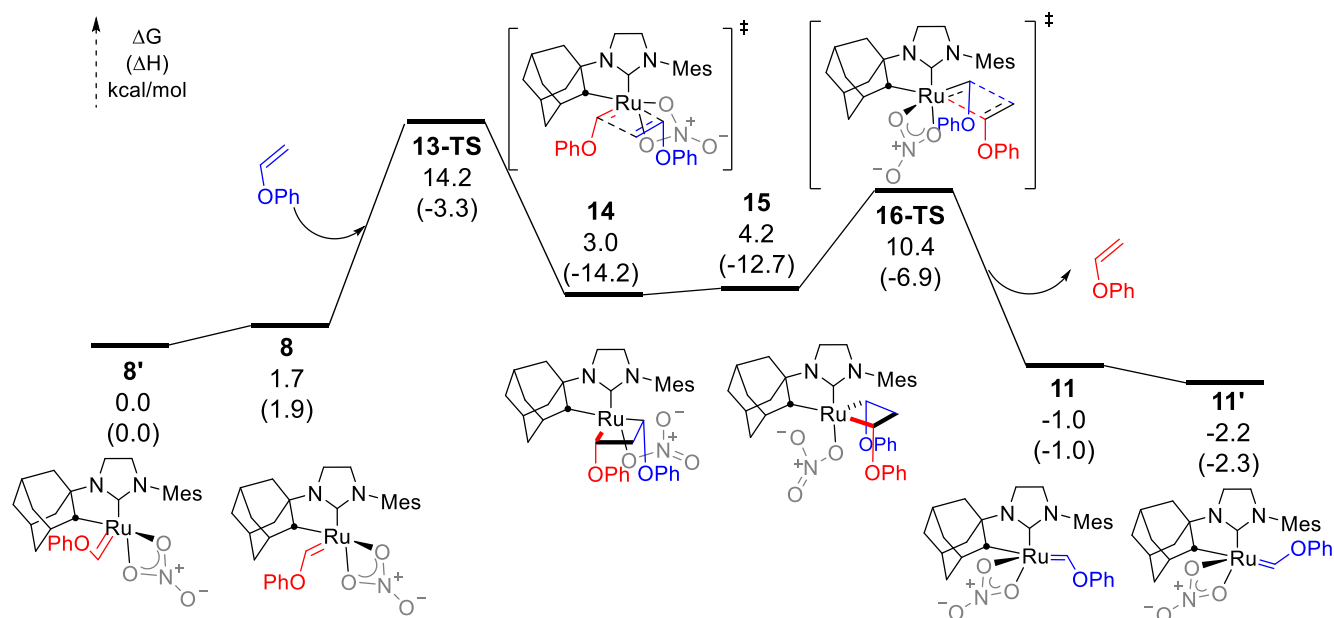


Figure 1.2.4. Metathesis of Fischer carbene **8** with phenyl vinyl ether to form the thermodynamically more stable diastereomer **11'**.

The free energy surface for Fischer carbene exchange is shown in Figure 1.2.3. The [2+2] cycloaddition of **8** with phenyl vinyl ether has a barrier of only 14.2 kcal/mol to form metallacycle **14**. Isomerization of **14** to **15** followed by retro-[2+2] via **16-TS** leads to the new diastereomer **11**. Carbene rotation leads to the more stable **11'**. Calculated barriers for the homodimerization of olefins with catalyst **3** and analogues range from ~11-15 kcal/mol and are comparable to the barrier for Fischer carbene exchange.^{14,19} Metathesis with **8** with vinyl ethers is therefore both kinetically and thermodynamically feasible.

The decomposition pathways of **11** and **11'** were also calculated (Figure 1.2.4). Decomposition of **11** leads to the more thermodynamically stable hydride and is shown in Figure 1.2.5.²⁰ Carbene insertion, via **17-TS**, has a barrier of 25 kcal/mol. This barrier is similar to that reported in the carbene insertion of benzylidene **3**. β -hydride elimination from **18** is nearly barrierless and leads directly to hydride **12**, with the vinyl ether acting as a chelating π -ligand.

Experiments with 0.1 equivalents of butyl vinyl ether and 1 equivalent of catalyst lead to quantitative conversion to hydride. This result is consistent with our predicted metathesis-dependent decomposition pathway if formation of **8**, or initiation, is slower than Fischer carbene exchange of **8** to **11**. Wang et al. previously computed initiation of **3** with styrene. The rate limiting step of the initiation is the retro-[2+2] to form the free Hoveyda chelate. The computed barrier for this transition state for reaction of **3** with phenyl vinyl ether is 23.4 kcal/mol. Therefore, initiation is significantly slower than carbene exchange. During the degradation process, a small portion of the catalyst will be initiated to **8/8'** and subsequently converted to **11/11'** via fast exchange and regenerate an equivalent of vinyl ether.

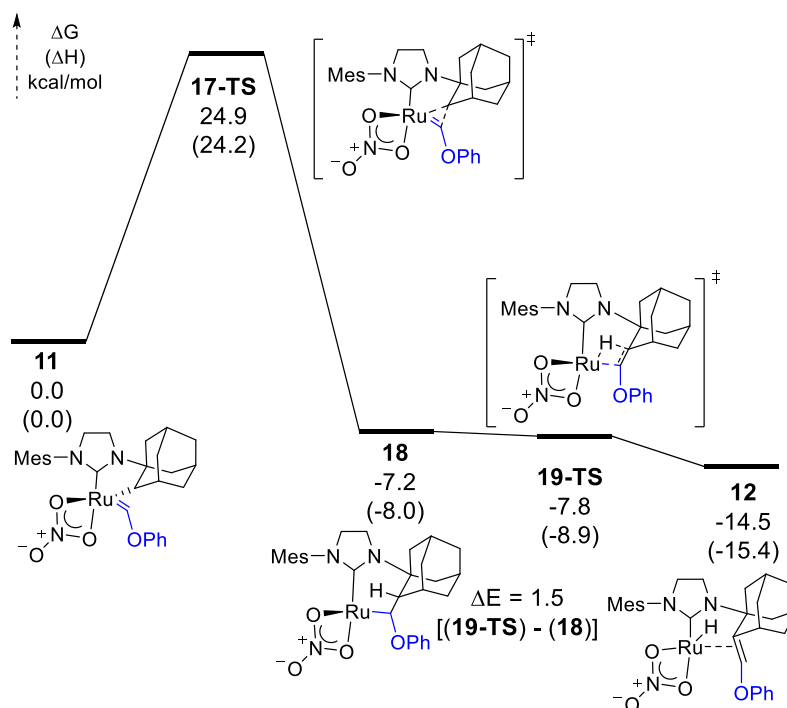


Figure 1.2.5. Decomposition of Fischer carbene **11** to hydride **12**.

Computations and experiment support the decomposition of the cyclometalated Fischer carbenes to hydrides and show that Fischer carbenes are not inert to metathesis if a Fischer carbene of similar stability can be generated. These results have an important impact for future use of vinyl ethers to quench reactions involving cyclometalated Z-selective catalysts. When a vinyl ether is used to quench a metathesis reaction, ruthenium hydrides can form rapidly in the reaction mixture if the Fischer carbene is not

separated promptly. The presence of hydrides can potentially lead to degradation of the Z-olefin content or to olefin walking.

In conclusion, we have demonstrated that Fischer carbenes can be formed from reactions of vinyl ethers with cyclometalated Z-selective ruthenium metathesis catalysts. These Fischer carbenes degrade to ruthenium hydrides rapidly under the reaction conditions as shown by ^1H NMR experiments. We have also shown that Fischer carbenes such as **8** and **11** are not metathesis inactive if carbenes of similar stability result. The mechanism of the decomposition has been elucidated by DFT. Experiments to determine how these hydrides affect internal olefins are currently underway.

Supporting Information

Experimental procedures, detailed NMR studies, and additional computational details are available in Appendix B.

Author Contributions

Ahmed and Grandner contributed equally to this work. Ahmed performed all experiments and Grandner performed all computations. The authors declare no competing financial interests.

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- (20) Decomposition of **11'** is shown in the supporting information as Figure B.S18.

Chapter 2.1

The Origins of the Stereoretentive Mechanism of Olefin Metathesis with Ru-Dithiolate

Catalysts

Jessica M. Grandner, Huiling Shao, Robert H. Grubbs, Peng Liu, K. N. Houk

Introduction

Since the advent of olefin metathesis catalysts, control of product olefin stereochemistry (Figure 2.1.1) has been an elusive goal. In the cross-metathesis of two alkenes, *E*-olefins are frequently obtained as the major product due to the thermodynamic preference for *E*- over *Z*-isomers. This thermodynamic favoring of *E*-olefin formation often leads to only moderate selectivity that varies from product to product, exemplified by ring-closing metathesis.¹ Thus, kinetic control of olefin stereochemistry is highly desirable. Recently, catalysts have been developed that favor kinetically the formation of thermodynamically unfavorable *Z*-olefins with >95% selectivity. These catalysts have been successfully applied to various *Z*-selective metathesis reactions including cross-metathesis, ring-closing metathesis, ring-opening cross-metathesis (ROCM) and ring-opening metathesis polymerization (ROMP).² However, ruthenium-based catalysts that provide the same high kinetic selectivity for formation of *E*-olefins have not yet been developed.³ In 2013, Hoveyda and coworkers synthesized dithiolate ligated Ru-based catalysts that demonstrated high *Z*-selectivity in ROCM and ROMP.⁴ Their computational studies revealed ring-opening cross metathesis via a side-bound mechanism that kinetically favors formation of *Z*-olefins. They subsequently demonstrated the ability of these catalysts to perform *Z*-selective cross metathesis of acyclic olefins.⁵

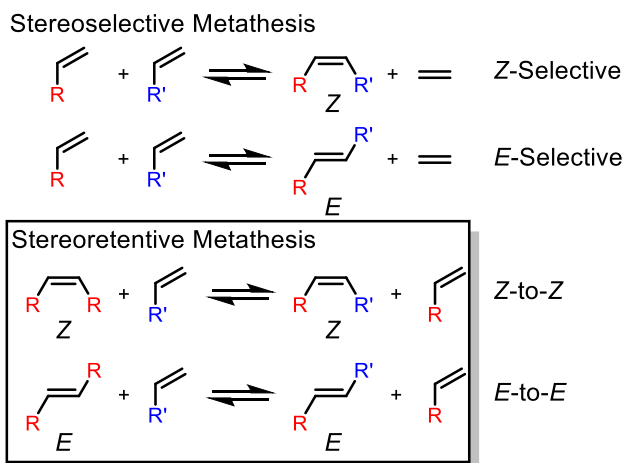


Figure 2.1.1. Models of stereoselective olefin metathesis (other work) and stereoretentive olefin metathesis (this work).

In 2016, a series of ruthenium-based dithiolate catalysts, **1-4** in Figure 2.1.2a, were synthesized and tested by the Grubbs laboratory.⁶ These complexes were able to catalyze the cross-metathesis of internal olefins with retention of starting olefin stereochemistry. *Z*-olefins were selectively converted to new *Z*-olefins while *E*-olefins were selectively converted into new *E*-olefins.⁶ This stereoretentive transformation provided the first example of kinetically controlled *E*-selective olefin cross-metathesis with Ru-based catalysts.

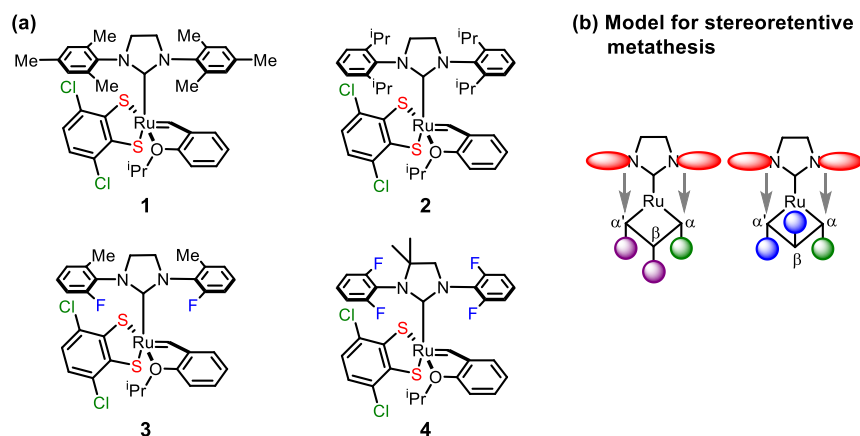
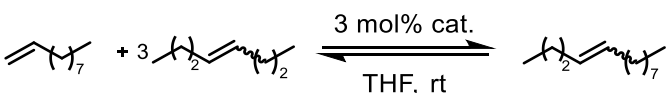


Figure 2.1.2. (a) Catalysts examined by the Grubbs group for stereoretentive metathesis; (b) Models for stereoretentive metathesis with *Z*-olefins (purple) and *E*-olefins (blue).

The report from Grubbs examined the reactivity and selectivity of these catalysts to form *E*-olefins.⁶ While catalyst **1** showed low yields for most cross-metathesis reactions with *E*-internal olefins, the reactions produced new products with complete retention of the starting olefin stereochemistry (Table 2.1.1). The yields of cross-metathesis reactions were improved by altering the NHC structure to that of catalyst **4**.⁶ While the yields are modest, the >99:1 selectivity of **4** for stereoretention with *E*-alkenes is unprecedented. The proposed model that explains the retention of stereochemistry is shown in Figure 2.1.2b. For these dithiolate containing catalysts, a side-bound mechanism is proposed.^{4,7} Therefore, the plane of the metallacycle (in the ruthenacyclobutane intermediate) is perpendicular to the NHC ligand and the substituents at the α - and α' -positions of the metallacycle are forced down to avoid steric repulsions with the *N*-aryl groups. Due to these ligand-metallacycle steric interactions, if a *Z*-olefin reacts, the substituent at the β -position also points down (i.e. away from the NHC) and a new *Z*-olefin is generated. If an *E*-olefin reacts, the substituent at the β -position points up and a new *E*-olefin is generated. There is presumably no intrinsic preference for the β -substituent to be up or down based on this model.

Table 2.1.1. Stereoretentive Cross-Metathesis Using Ruthenium Catalysts **1** and **4**.⁶

			
Stereochemistry of Starting Olefin	Catalyst	Yield	Product <i>E:Z</i>
<i>Z</i>	1	55%	<1:99
<i>E</i>	1	7%	>99:1
<i>Z</i>	4	42%	<1:99
<i>E</i>	4	19%	>99:1

We examined the origins of stereoretentive metathesis with catalyst **4** using computational methods. We have probed the ligand effects on stereoselectivity and examined the steric environment of the NHC ligand and the steric repulsions of the NHC ligand with substituents at both the α - and β -positions of the metallacycle. Since catalyst **4** is a promising prototype of a kinetically *E*-selective catalyst, these computational insights can assist in the design of a kinetically *E*-selective metathesis catalyst.

Computational Methods

All calculations were performed using Gaussian 09.⁸ Geometry optimizations and frequency calculations were performed at the B3LYP⁹ level using LANL2DZ for ruthenium and 6-31G(d) for all other atoms. Zero point vibrational energies, thermal corrections, and entropies were computed from frequency calculations with a standard state of 298 K and 1 atm. Quasiharmonic oscillator approximations were used to compute the entropic contributions to the Gibbs free energies, as discussed by Truhlar.¹⁰ Single point energy calculations were performed at the M06¹¹ level using SDD for ruthenium and 6-311+G(d,p) for other atoms with the SMD¹² continuum solvent model for THF.

Results and Discussion

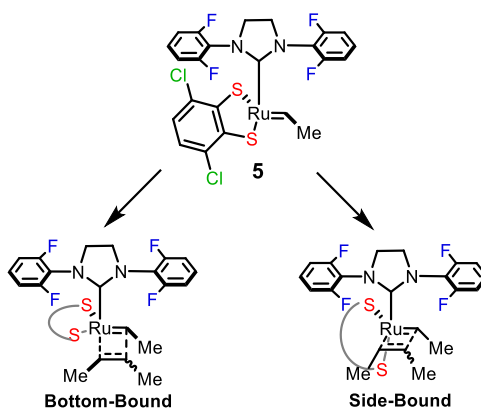


Figure 2.1.3. Possible mechanistic pathways for reactions of **5** with butene.

Our first goal was to determine if the metathesis with catalyst **4** proceeds through a bottom-bound mechanism, in which the olefin approaches *trans* to the NHC ligand, or a side-bound mechanism, in which the olefin approaches *cis* to the NHC (Figure 2.1.3).¹³ While Hoveyda and coworkers explored the side-bound mechanism in references 4a and 5, a direct comparison of the bottom-bound and side-bound mechanisms has not been performed for this class of catalysts. Using **5** as a model for the active ethylidene complex of catalyst **4**, and *E*- and *Z*-butene as substrates, we calculated all isomeric transition states for both bottom-bound and side-bound mechanisms. A graphical representation of the computed activation free energies is shown in Figure 2.1.4. In all cases, the side-bound transition states (blue) are lower in energy than the bottom-bound transition states (red). To adopt a bottom-bound mechanism, the ortho-dithiolate ligand must orient one of the sulfur atoms *trans* to the alkylidene. Such geometry is strongly destabilized due to *trans*-influence of the thiolate on the alkylidene.¹⁴ A large distortion of the ligand sphere is also required to accommodate the incoming olefin in the bottom-bound mechanism. In typical bottom-bound metathesis transition states (e.g. with the second generation Grubbs catalyst),¹⁵ the alkylidene prefers to be positioned directly under the *N*-alkyl group. In the bottom-bound transition states (**5Db** and **5Fb**), the $C_{ipso}-N-Ru-C_{alkylene}$ dihedrals (red-

highlighted atoms in **5Db** and **5Fb**) are $>50^\circ$. The bottom-bound pathway is disfavored in all cases due to a combination of *trans* influences and steric effects.

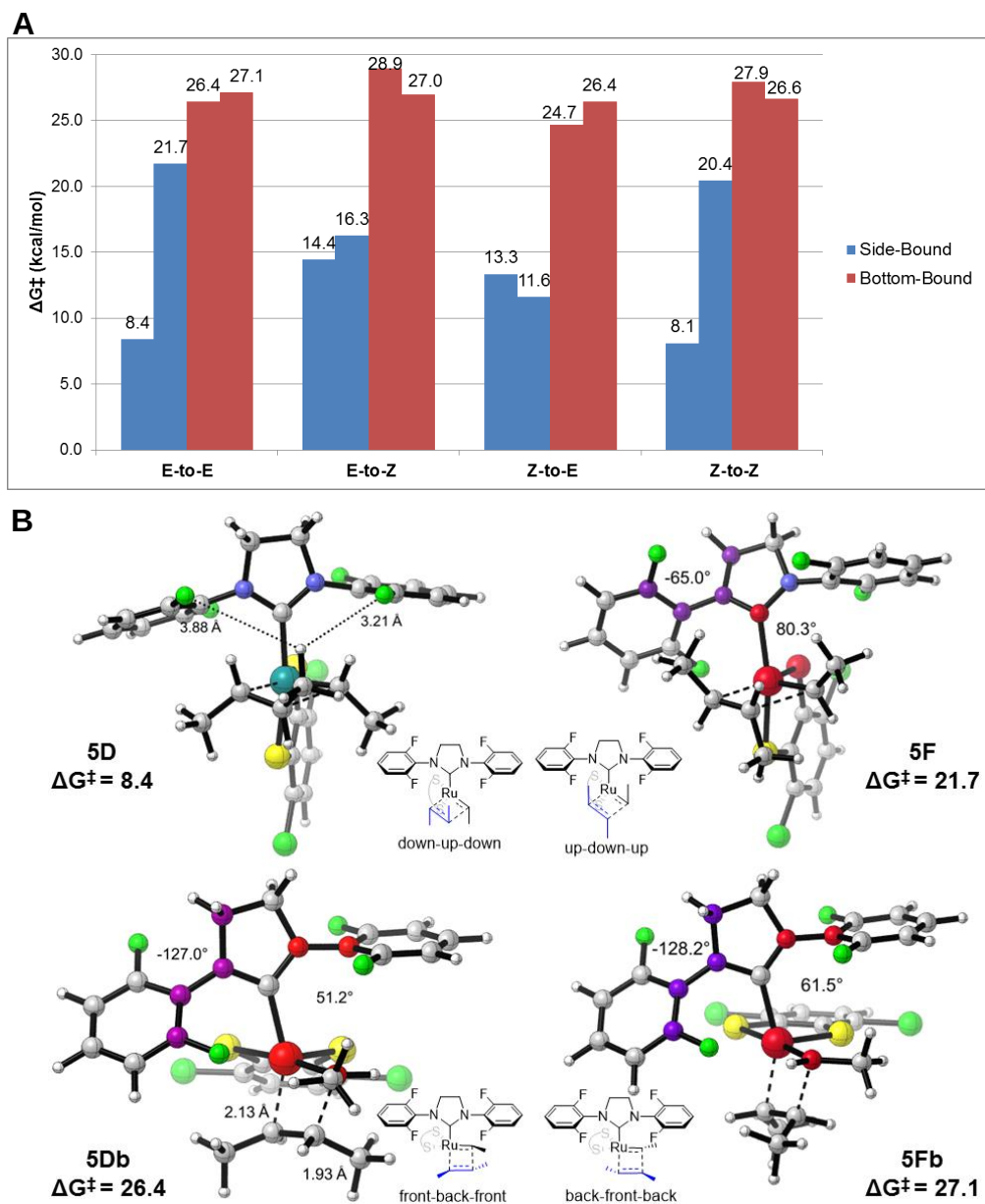


Figure 2.1.4. (A) Barriers to formation of ruthenacyclobutane (TS1). There are 2 retention pathways (E-to-E and Z-to-Z) and 2 inversion pathways (E-to-Z and Z-to-E) which are represented on the X-axis. Each pathway has 4 possible approaches, 2 side-bound (blue columns) and 2

bottom-bound (red columns). (B) Transition structures of the 4 approaches for E-to-E metathesis. Side-bound pathways (**5D** and **5F**) are shown on the top while bottom-bound pathways (**5Db** and **5Fb**) are shown on the bottom.

In the favored side-bound pathway, Figure 2.1.4 shows that the stereoretentive pathways for both *E*- and *Z*-olefins are lower in activation free energy than the corresponding stereoinversion pathways by >3-6 kcal/mol. For the reaction of *Z*-butene with **5**, the preferred mode of addition is to have all substituents on the forming metallacycle pointing down, away from the sterically demanding NHC (**5A**, Figure 2.1.5). After the retro-[2+2] cycloaddition of the trisubstituted metallacycle, this process leads to retention of the starting *Z*-olefin stereochemistry. As shown in Figure 2.1.5, the two possible pathways leading to *E*-butene both require much higher activation energies. If the *Z*-butene adds with both substituents “up” (**5C**), this costs an additional +3.5 kcal/mol compared to **5A** and induces significant steric repulsion between the *N*-aryl substituent on the NHC and the α' substituent on the forming metallacycle. The left *N*-aryl group must distort out of the way of the incoming alkene. If the olefin adds “down” and the alkylidene is pointing up (**5B**), there is a +5.2 kcal/mol activation free energy penalty compared to **5A** due to the direct steric clash of the alkylidene with the *N*-aryl group, evidenced by the short distance between the H atom on the alkylidene and the ortho-F atom on the NHC *N*-aryl group (2.29 Å). Due to these steric penalties, the retentive pathway is strongly favored, leading to exclusive formation *Z*-olefins.

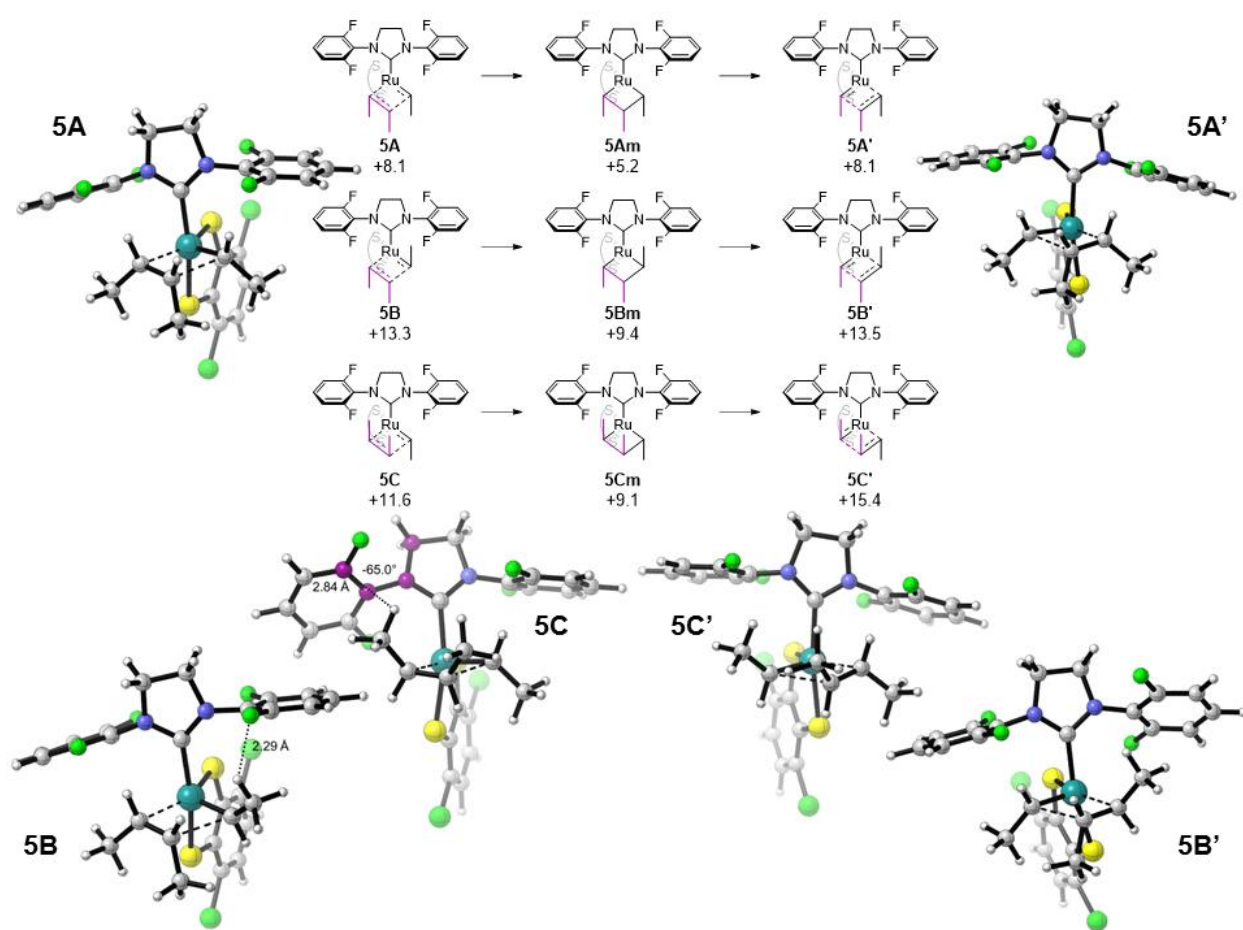


Figure 2.1.5. [2+2] cycloaddition (5A-C) and retro-[2+2] cycloaddition (5A'-C') transition structures and ruthenacyclobutane intermediates (5Am-5Cm) and respective Gibbs free energies of activation for the lowest energy Z-retentive pathway (A) and 2 possible stereoinversion pathways (B, C) for the reaction of 5 with Z-butene. All energies are Gibbs free energies in kcal/mol with respect to the separated reactants 5 and Z-butene. The other Z-retentive pathway with all three metallacycle substituents pointing up requires much higher activation energy ($\Delta G^\ddagger = 20.4$ kcal/mol) and is not shown.

In the reaction with *E*-olefin, at least one substituent on the forming metallacycle must point up towards the NHC. The least sterically demanding position for a substituent to point up towards the NHC is the β -position of the forming metallacycle (shown in blue spheres in Figure

2.1.2b). Figure 2.1.6 shows the computed reaction pathways for *E*-butene. The lowest energy pathway (**5D**) is one in which there is only one substituent, at the β -position, points towards the NHC and leads to retention of olefin stereochemistry. Due to similar steric effects as in the reactions with *Z*-olefins, pointing the alkylidene up (**5C'**) or reversing the olefin approach (**5B'**) incurs activation free energy penalties. This leads to a 6.0 kcal/mol preference for retention and exclusive formation of a new *E*-olefin when reacting with an *E*-olefin.

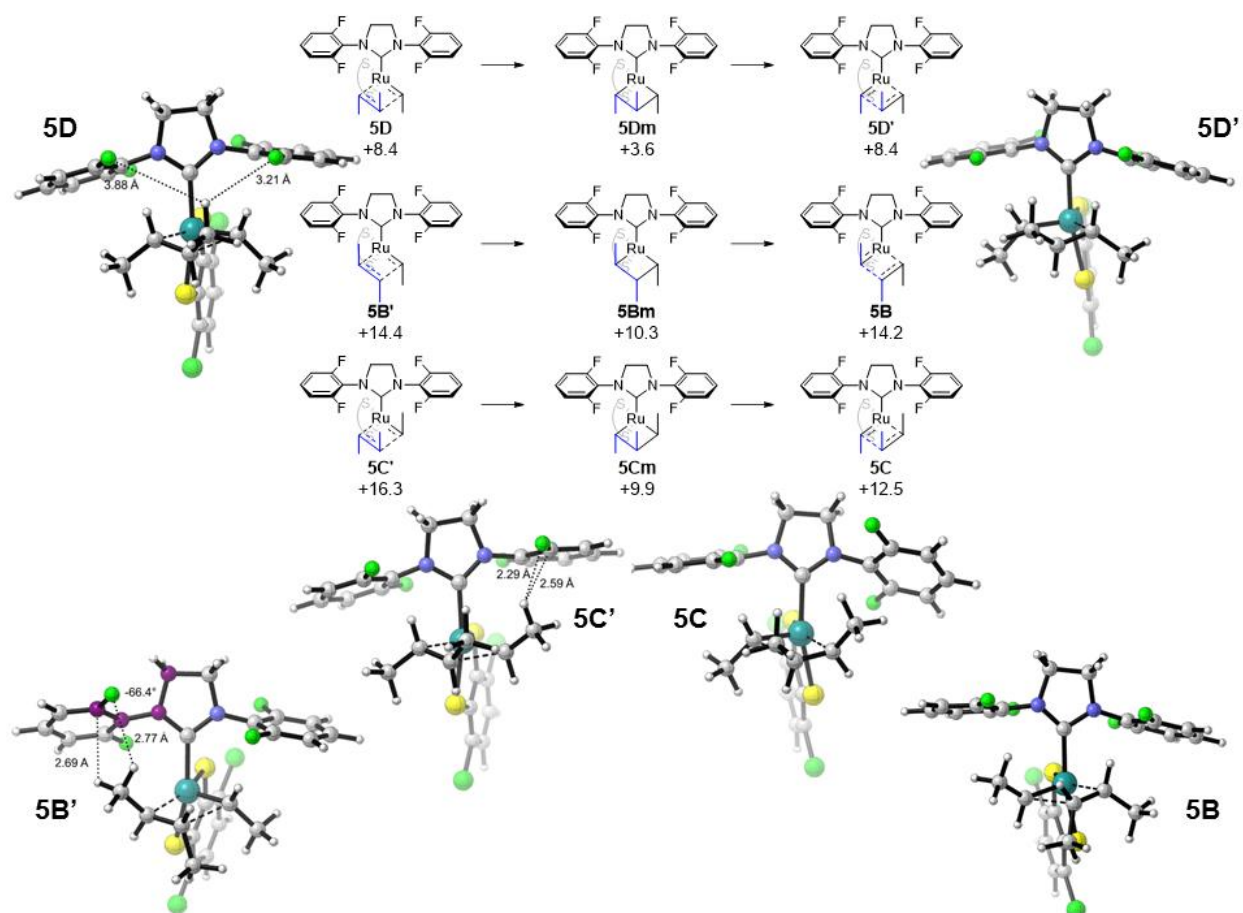


Figure 2.1.6. [2+2] cycloaddition (**5D**, **5B'**, **5C'**) and retro-[2+2] cycloaddition (**5D'**, **5B**, **5C**) transition structures and ruthenacyclobutane intermediates (**5Dm**, **5Bm**, **5Cm**) and respective Gibbs free energies of activation for the lowest energy *E*-retentive pathway (**D**) and 2 possible stereoinversion pathways (**B**, **C**) for the reaction of **5** with *E*-butene. All energies are Gibbs free

energies in kcal/mol with respect to the separated reactants **5** and *E*-butene. The other *E*-retentive pathway with the α and α' substituents of the metallacycle substituents pointing up requires a much higher activation energy ($\Delta G^\ddagger = 21.7$ kcal/mol) and is not shown.

The analysis of transition state isomers in Figure 2.1.6 revealed the design principles for *E*-selective metathesis catalyst: the *N*-aryl substituents on the NHC ligand should maximize the steric repulsions with the α -substituent on the metallacycle while still allowing the β -substituent to point up towards the NHC ligand. Thus, a few factors, including the steric properties of the *N*-aryl group as well as the NHC backbone substituents, are expected to affect the *E/Z* selectivity. To gain deeper insights into the steric environment of the NHC ligand and the steric interactions with the olefin and alkylidene substituents, we plotted the 2D steric contour maps¹⁶ of catalyst **4**. The ligand steric contour map is derived from the van der Waals surface of the NHC ligand from the optimized structures of the ethylidene complex **6** and the lowest-energy ruthenacyclobutane intermediates **6Dm** and **6Am** in the lowest energy *E*- and *Z*-retentive pathways, respectively. The contour map was created following the previously reported procedure.^{16g} The NHC ligand is rotated and translated so that the Ru atom is placed at the origin of the Cartesian coordinate system and the *z*-axis is oriented along the Ru–C(carbene) bond. The contour line of zero is drawn through all points on the van der Waals surface having the same *z* coordinate as the Ru atom. The positive contour lines (colored in green and blue) indicate regions on the ligand van der Waals surface having a positive *z* coordinate, *i.e.* more distant from the plane of the ruthenacyclobutane. Yellow and red indicate regions closer to the ruthenacyclobutane where more significant ligand-substrate steric clashes are expected.

To examine the conformational change of the NHC ligand along the *E*- and *Z*-retentive pathways, steric contour maps of the same NHC ligand in the ethylidene complex **6** and the two

ruthenacyclobutanes featuring a “down-up-down” (**6Dm**) and “down-down-down” (**6Am**) substitution patterns on the metallacycle are plotted in Figure 2.1.7. In ethylidene complex **6**, one of the *N*-2,6-difluorophenyl groups is significantly tilted due to flexibility of the *N*-aryl bond.¹⁷ This tilted *N*-aryl conformation creates a relatively sterically demanding pocket between the two ortho-F substituents (see the red and orange regions near the “closed” pocket on the contour map of **6**). In the metallacycle intermediates, the *N*-aryl group rotates away from the metallacycle, as indicated by the disappearance of the red and orange regions on the contour plot. This conformational change creates a much larger “open” pocket between the two ortho-F substituents to accommodate the β -substituent. To our surprise, the ligand conformation in **6Dm** and **6Am** are remarkably similar. The “open” pocket above the β -substituent is present regardless if the β -substituent is pointing up or down. A closer inspection of the geometries of the metallacycles revealed that the *N*-aryl groups in both **6Dm** and **6Am** are slightly tilted away from the metallacycle due to the steric repulsions with the hydrogen atoms at the α - and α' -positions. This steric effect also distorts the NHC ligand towards the dithiolate: the S–Ru–C(carbene) bond angle is decreased from 94.8° in ethylidene **6** to 87.7° and 86.8° in **6Dm** and **6Am** respectively. This distortion also contributes to the formation of the “open” pocket above the β -substituent in **6Dm** and **6Am**. Due to the formation of this “open” pocket, the steric repulsions between the NHC ligand and the β -substituent in **6Dm** are diminished. The “down-down-down” isomer **6Am** becomes 3.6 kcal/mol less stable than the “down-up-down” isomer **6Dm** because of the repulsion between the adjacent methyl groups on the metallacycle in **6Am**.

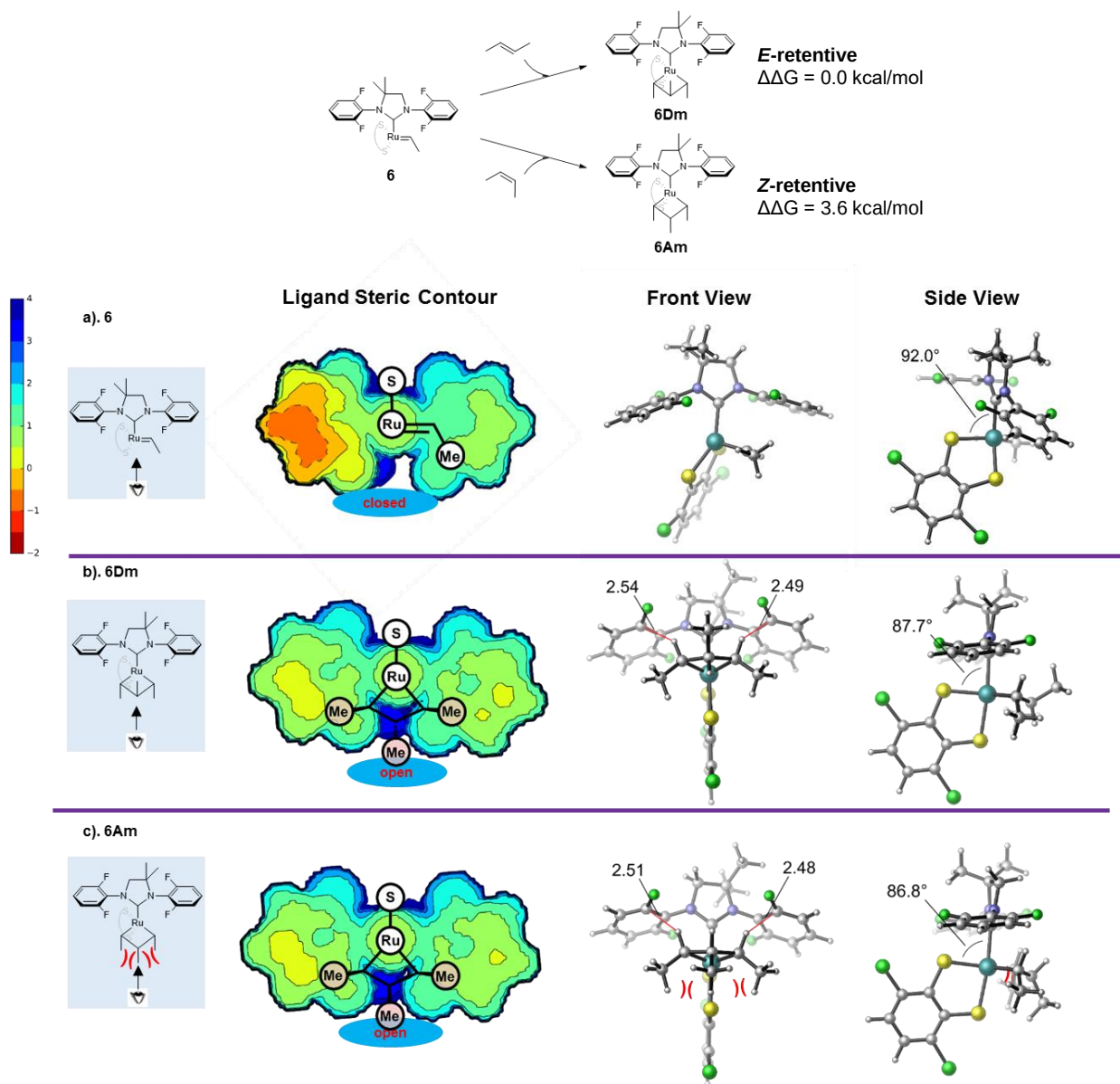
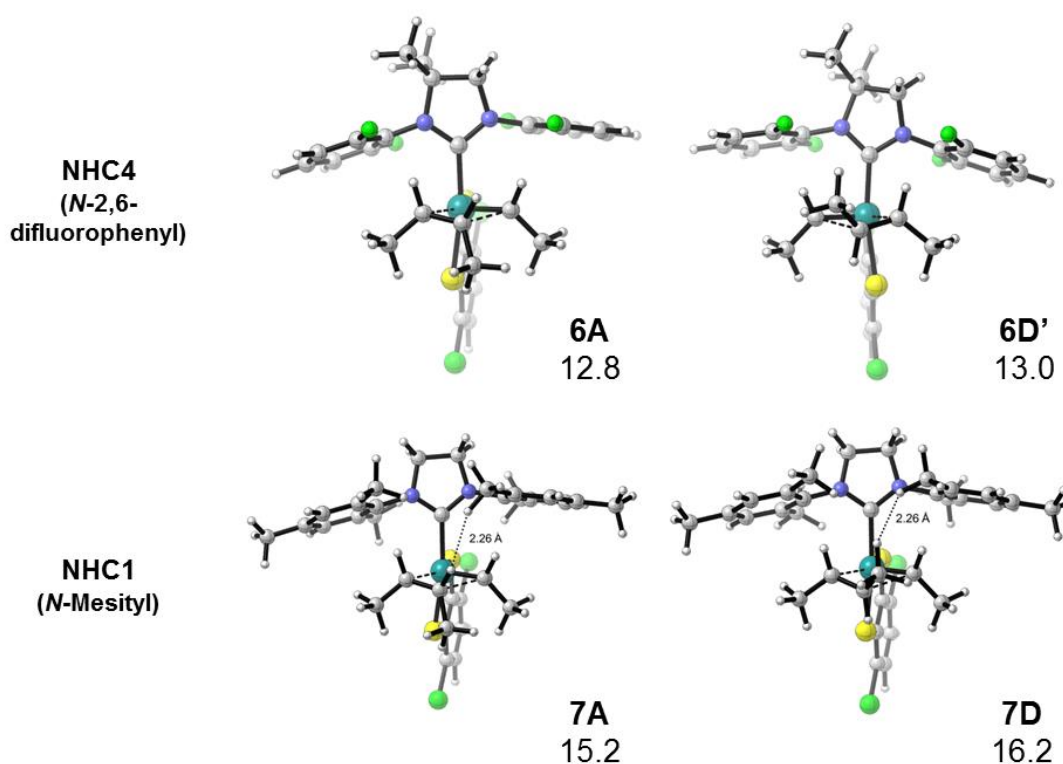


Figure 2.1.7. Ligand steric contour maps of (a) ethylidene complex **6**, ruthenacyclobutane intermediates (b) **6Dm** and (c) **6Am**. The open pocket above the β -Me substituent promotes both *E*- and *Z*-retentive pathways.

We also investigated the origin of the reactivity difference between catalysts **1** and **4** in the *Z*- and *E*-retentive metathesis. In these studies, we used the complete NHC ligand for catalyst **4** with the germinal dimethyl substituted backbone (as in **6**). The rate-limiting transition states for

the *Z*- and *E*-retentive pathways of both catalysts are shown in Table 2.1.2. The rate-limiting steps for both pathways are more than 2 kcal/mol lower in activation free energy with catalyst **4** (**6A** & **6D**) than with **1** (**7A** & **7D**), suggesting **4** is a more reactive catalyst in reactions with both *E*- and *Z*-olefins. The lower reactivity of **1** is due to the presence of the more hindering ortho-methyl groups of the SIMes ligand which cause more steric repulsion with the incoming olefins than the ortho-F groups on catalyst **4**.

Table 2.1.2. Rate-limiting transition structures for the retention pathways with catalysts **4** and **1**.



To examine the ligand steric environment in catalyst **1**, we plotted the steric contour maps of the active alkylidene complex **7** and ruthenacyclobutane intermediates **7Dm** and **7Am** in the *E*- and *Z*-retentive pathways (Figure 2.1.8). The steric contour maps show that, similar to the reactions with complex **6** in Figure 2.1.7, an “open” pocket is created above the β -substituent in both ruthenacyclobutane intermediates **7Dm** and **7Am**. Again, the formation of this open pocket is due

to the steric repulsions with the hydrogen atoms at the α - and α' -positions of the metallacycle. Compared to the ortho-F substituted catalyst **1**, the ortho-Me groups on catalyst **4** lead to greater repulsions with the metallacycles in both **7Dm** and **7Am**. This is evidenced by the greater distortion of the NHC ligand towards the dithiolate: the S–Ru–C(carbene) bond angle is 84.2° and 83.2° in **7Dm** and **7Am**, respectively, even smaller than the S–Ru–C(carbene) angle in **6Dm** and **6Am** (87.7° and 86.8°, respectively). These results are in agreement with transition structures above that catalyst **1** is highly *E*- and *Z*-retentive due to the open pocket above the β -position on the metallacycle. However, the reactivity of catalyst **1** is lower than that of **4** due to stronger steric repulsions with the metallacycle in the side-bound pathways.

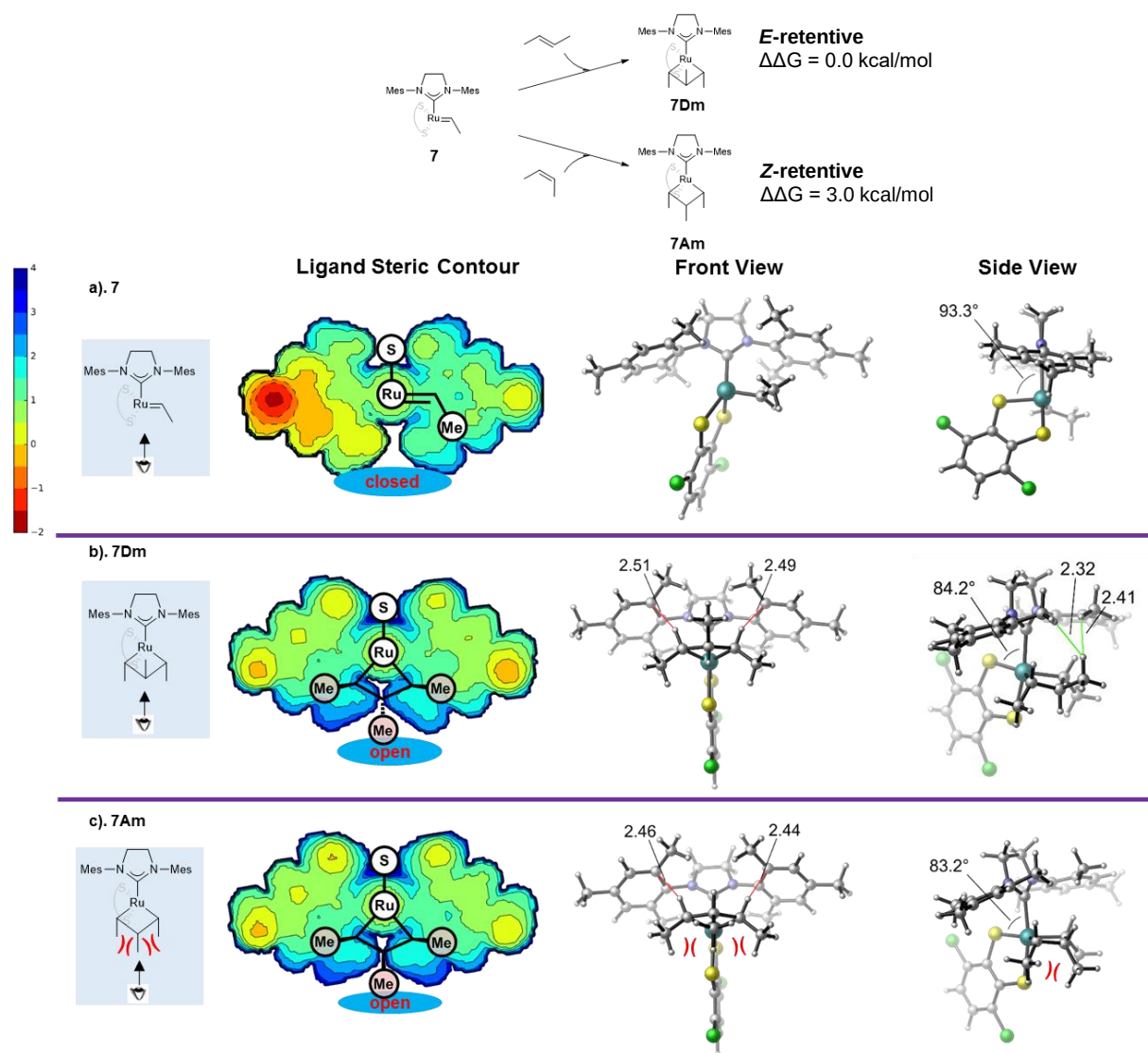


Figure 2.1.8. Ligand steric contour maps of (a) ethylidene complex **7**, ruthenacyclobutane intermediates (b) **7Dm** and (c) **7Am**.

Conclusion

In conclusion, we have performed computational studies on the stereoretentive olefin metathesis using dithiolate-ligated ruthenium catalysts **1** and **4** to understand their reactivity and selectivity in the cross-metathesis with *E*- and *Z*-olefins. We have confirmed that the substituents at the α -positions of the forming metallacycle prefer to point away from the NHC ligand, while

the β -substituents may point either away or towards the NHC without incurring significant steric clashes with the NHC ligand, as proposed by Grubbs and co-workers.⁶ These ligand-controlled steric interactions enforce the retention of the original olefin stereochemistry in metathesis. DFT-optimized transition structures and ligand steric contour maps revealed the important role of the steric interactions between the NHC ligand and the hydrogen atoms at the α - and α' -positions of the metallacycle. Such interactions tilt the *N*-aryl group on the NHC ligand and create an “open” pocket above the β -position of the metallacycle, which can accommodate a substituent pointing towards the NHC ligand. This “open” pocket is critical for the stereoselectivity in the *E*-retentive metathesis. The DFT calculations also revealed the effects of ligand-metallacycle steric interactions on reactivity. The *N*-mesityl substituted catalyst **1** is slightly less reactive than the 2,6-difluorophenyl substituted catalyst **4** due to the unfavorable steric repulsions between the bulkier *N*-mesityl groups and the metallacycle in the side-bound metathesis pathway.

Author Contribution and Notes

Grandner performed DFT computations on all transition structures and related intermediates for Figures 2.1.4, 2.1.5, & 2.1.6 and Table 2.1.2. Shao performed computations on the structures related to Figure 2.1.7 & 2.1.8.

Acknowledgements

All 3-dimensional images were made using CYLview.¹⁸

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Chapter 2.2

Design of a Kinetically E-Selective Metathesis Catalyst

We have built on the results from our computational investigation into the stereoretentive metathesis catalysts in Chapter 2.1 to design a catalyst which disfavors metathesis with Z-olefins while facilitating metathesis with E-olefins. We've shown that the bis(dithiolate) ligand forces a side bound mechanism which is critical for stereoretention. The Grubbs group hypothesized that adding steric bulk to the bis(dithiolate) would enforce stereoretentive metathesis but block interaction with Z-olefins. They synthesized phenanthrene- and naphthalene-based bis(dithiolate) ligands with the goal of having the arene point into the β position of the forming metallacycle.¹ The ligands, catalysts, and metallacycle model are shown in Figure 2.2.1.

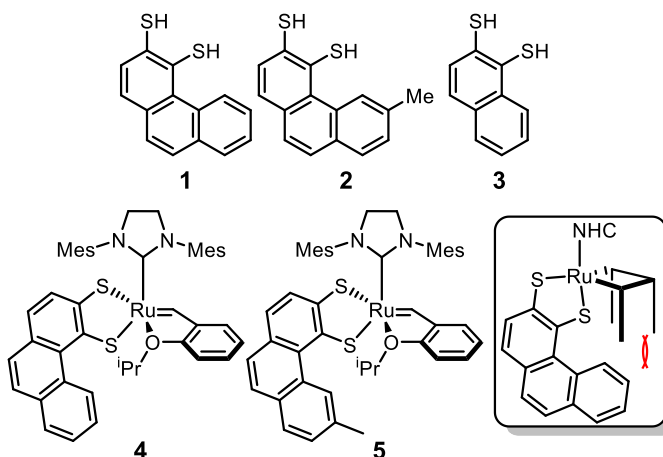


Figure 2.2.1. Structures of dithiolate ligands and the metallated catalysts. The model proposed for the metallacycle is shown in the box.

Once metallated, NMR spectra revealed that two isomers of the catalysts had been formed. These isomers are the possible ligation modes of the thiolate, arene down or arene up (Figure 2.2.2). Based on the NMR results, there is a ratio of 2.8:1 ratio for catalyst **4** and a 1.5:1 ratio for catalyst **5**, but the favored isomer of each catalyst was unknown.¹ We performed computational

studies² of the conformers of **4** and **5** to determine which conformer is favored and the likelihood that this catalyst would induce E-selective metathesis.

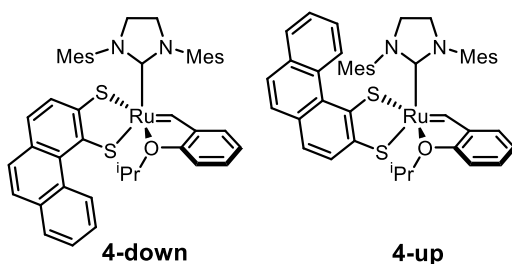


Figure 2.2.2. Stereoisomers of catalyst **4**.

The computed structures of catalyst **4** are shown in Figure 2.2.3A. The up conformer is favored by 0.8 kcal/mol. This equates to a up:down ratio of 3.4:1 which is very consistent with the experimental ratio of 2.8:1. The computed structures of catalyst **5** are shown in Figure 2.2.3B. The up and down conformers are isoenergetic and lead to a 1:1 ratio which is consistent with the observed 1.5:1 ratio. These computations indicate that the predominant catalyst isomer is the up isomer which does not impose any steric influence on the face of the catalyst which performs metathesis.

A front view of **5-down** is shown in Figure 2.2.4. The methyl substituent is far from the isopropyl group of the Hoveyda chelate and indicates that even the down isomer of the catalyst would impose little-to-no steric influence on the β position of a metallacycle. To test this, we optimized an unsubstituted metallacycle of **5-down** (**5-mcb** in Figure 2.2.4). As shown in **5-mcb**, the methyl group is too far from the ‘active site’ of the catalyst to impose a steric influence on the metallacycle.

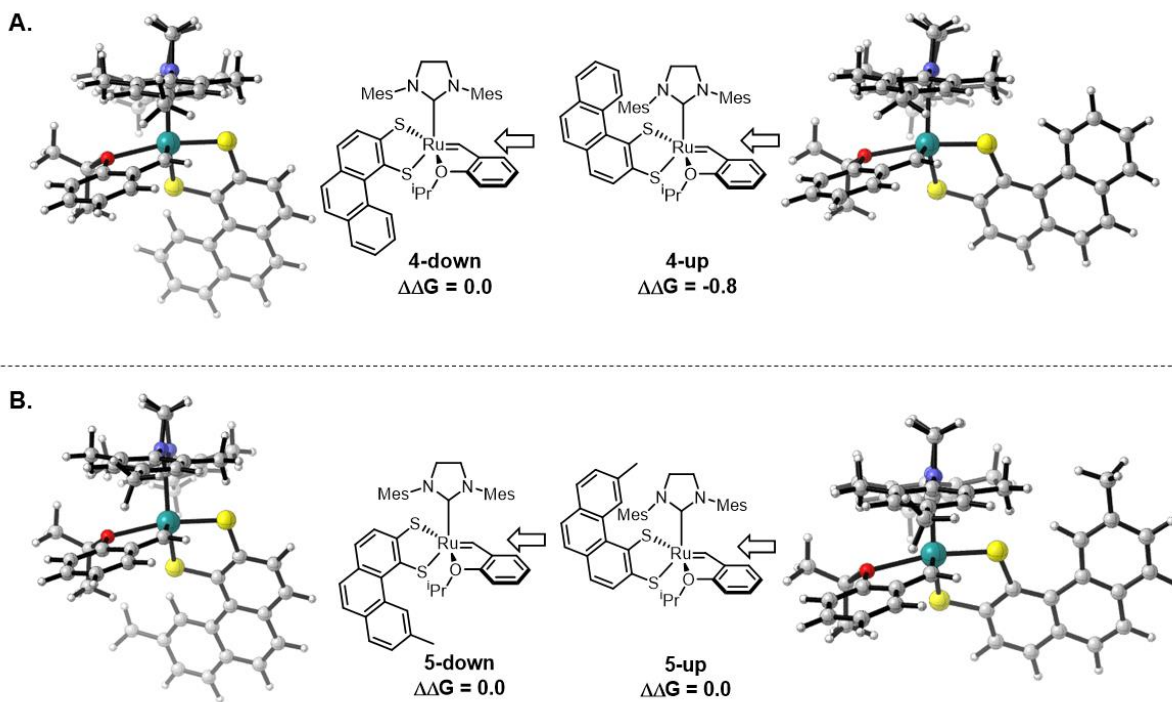


Figure 2.2.3. A. 3D side views of **4-down** (left) and **4-up** (right); B. 3D side views of **5-down** (left) and **5-up** (right) with relative free energy difference indicated in 2D structures (arrows in 2D indicate the point of view in 3D images).

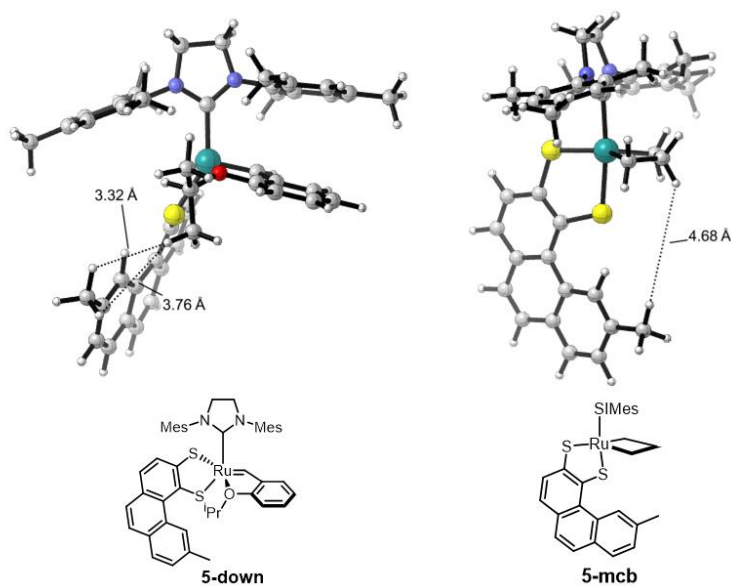


Figure 2.2.4. Front view of catalyst **5-down** and side view of the metallacycle **5-mcb**.

Through these computations, it is clear that substituents on dithiolate ligands are too remote to have an influence on metallacycle formation. Alternatively, if the nature of the thiolate could be altered to have a substituent directly off the axial, X-type ligand, this substituent may be close enough to the forming metallacycle. This thought process is illustrated in Figure 2.2.5.

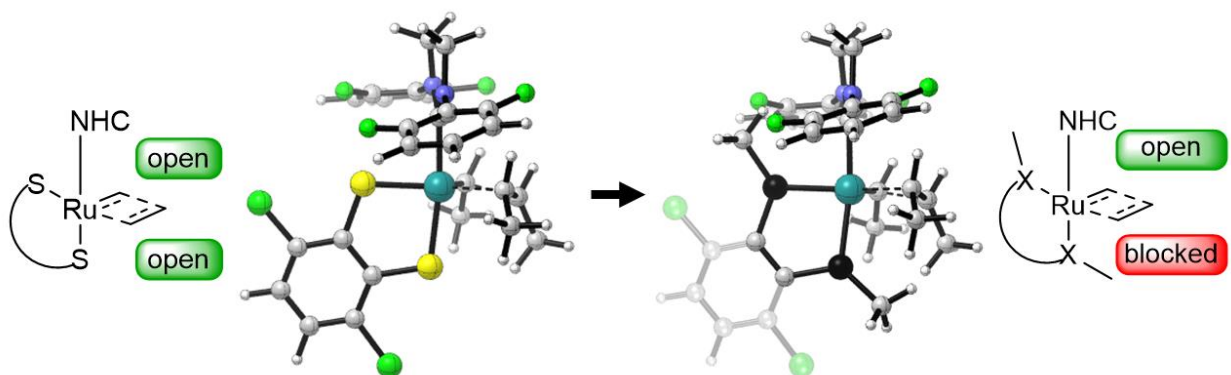


Figure 2.2.5. Schematic for design of E-selective catalysts. On the left is a side view of metathesis with Z-butene with dithiolate ligand from Chapter 2.1. The top and bottom of the metallacycle plane are open, allowing for metathesis with Z-olefins. On the right is an image of a catalyst with a new x-type ligand which has a substituent pointing directly into the bottom plane of the metallacycle. This may block metathesis with Z-olefins but allow for metathesis with E-olefins.

We obtained a list of synthesizable benzenediamine-based ligands shown in Figure 2.2.6. The computed the barriers to metallacycle formation (TS1) for E-to-E metathesis and Z-to-Z metathesis with each ligand are shown in Table 2.2.1. As with the dithiolate ligands, **NHC4** is predicted to perform faster metathesis than **NHC1**. For amines without conformational flexibility, such as **amine2**, TS1 (2+2) and TS2 (retro-2+2) are the same. **Amine1** and **amine2** were designed to be similar in structure to the dithiolate ligands in Chapter 2.1. **Amine3** through **amine5** were designed to reduce the basicity of the amines and avoid undesired catalyst decomposition.³ While **amine2** is conformationally rigid at the nitrogen substituent, **amine3** through **amine5** have

different conformations which could be accessed during catalysis. These conformations were examined for each amine and the lowest energy conformations of TS1 are given in Table 2.2.1. Although the combination of **NHC4** and **amine5** appears promising, the Grubbs group was unable to metallate **amine5**.

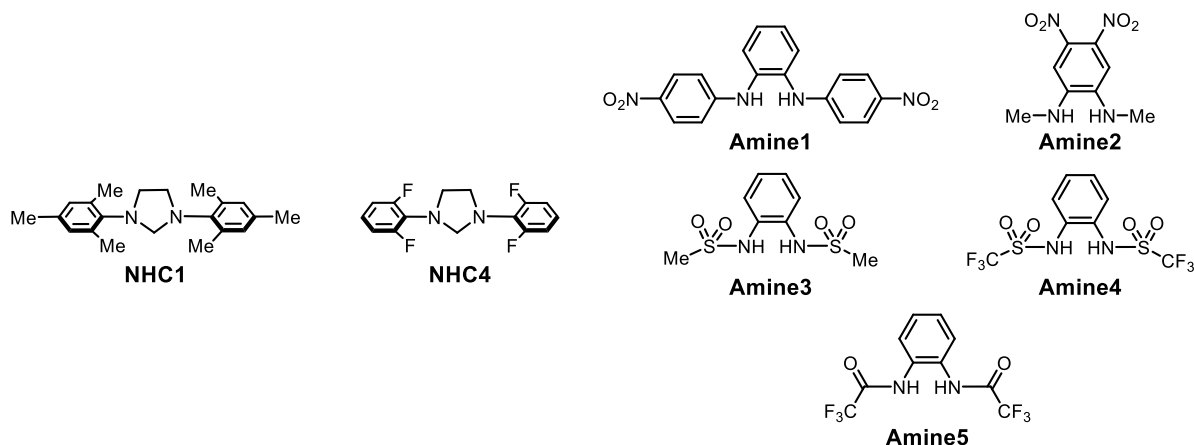


Figure 2.2.6. NHC and amine ligands examined for E-selective metathesis with computations.

Table 2.2.1. Barriers to stereoretentive metathesis with NHC diamine ligand combinations

<i>NHC/Amine combination</i>	E-to-E TS1	Z-to-Z TS1
<i>NHC1/Amine1</i>	29.0	27.1
<i>NHC4/Amine1</i>	20.0	20.6
<i>NHC1/Amine2</i>	15.1	17.3
<i>NHC4/Amine2</i>	12.7	14.9
<i>NHC1/Amine3</i>	19.1	21.1
<i>NHC4/Amine3</i>	13.6	18.4
<i>NHC4/Amine4</i>	12.9	16.4
<i>NHC4/Amine5</i>	7.8	11.7

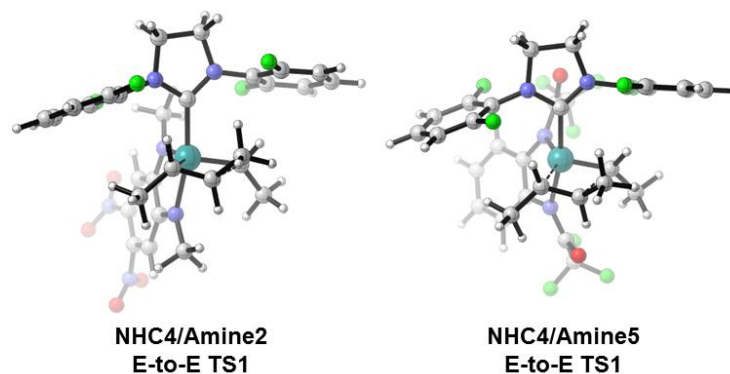


Figure 2.2.7. Transition structures with rigid amine ligands (left) and flexible amine ligands (right).

While many factors may have caused the unsuccessful metalation of **amine5**, the bulkiness of the nitrogen substituents that were designed to reduce basicity could be hindering metalation. We then designed new ligands shown in Figure 2.2.8, which contain functional groups that are able to reduce the basicity of the nitrogens but are not bulky and flexible. The ligands we designed incorporated aromaticity and carbonyls in the backbone of the ligand rather than on the N-substituents. Table 2.2.2 shows the results of the DFT metathesis calculations. **amide1** and **amine6** are predicted to have good E-selectivity and reasonable barriers to metallacycle formation. Alternatively, the methylene linker of **amine7** caused the pyrroles to adopt a distorted binding pose compared to **amine6** and thus caused an increase in barriers to metallacycle formation. As a result of these computational results, the Grubbs group is currently attempting to metallate **amide1** and **amine6** in combination with commercially available **NHC1**.

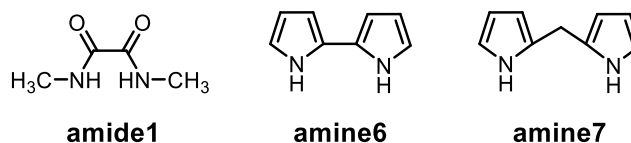


Figure 2.2.8. Second round of amine/amide ligands designed for E-selective metathesis.

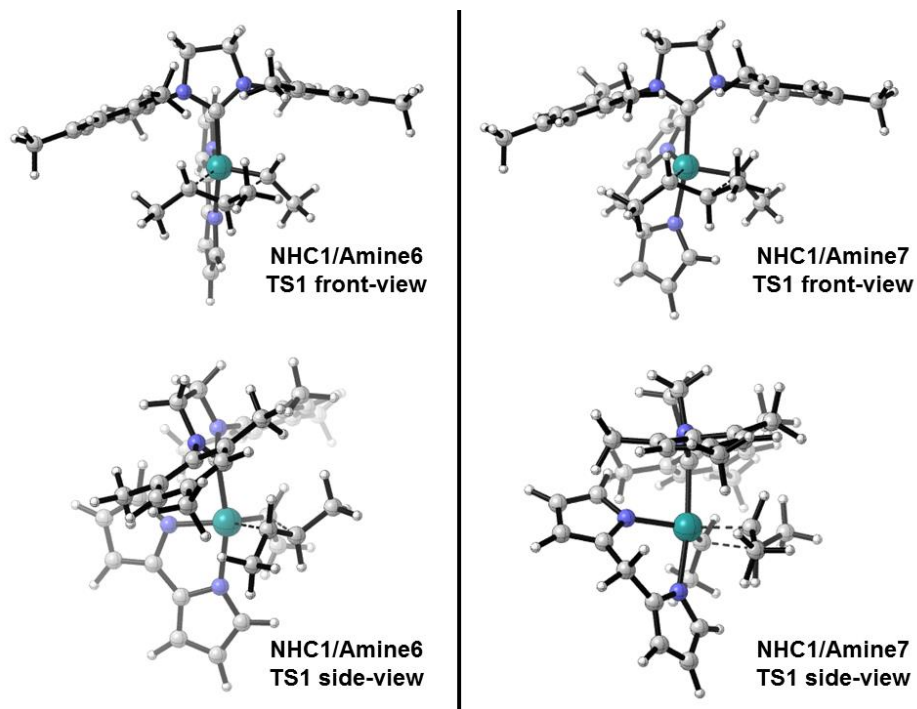


Figure 2.2.9. Transition structures for E-to-E metathesis with **NHC1/Amine6** and **NHC1/Amine7**. The methylene spacer of **amine7** forces improper ligation of the nitrogens.

Table 2.2.2. Barriers to stereoretentive metathesis with NHC diamine ligand combinations

<i>NHC/Amine Combination</i>	E-to-E TS1	Z-to-Z TS1
<i>NHC1/Amide1</i>	17.1	18.6
<i>NHC4/Amide1</i>	12.8	15.3
<i>NHC1/Amine6</i>	10.7	12.7
<i>NHC1/Amine7</i>	19.1	21.6

In conclusion, we have used computations to identify two di-nitrogen ligands, **amide1** and **amine6**, which are predicted to facilitate E-selective cross metathesis. In doing so, we have identified characteristics of di-nitrogen ligands. The ligand needs to contain functional groups that reduce the basicity of the nitrogens, such as carbonyls or aromatic groups. The ligand also needs to be rigid and not contain large groups as nitrogen substituents.

Supporting Information

Additional spectral data and computational information is provided in Appendix C.

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(1) Unpublished results from Grubbs group. See Supplemental Information for experimental data.

(2) Computational methods are the same as in Chapter 2.1.

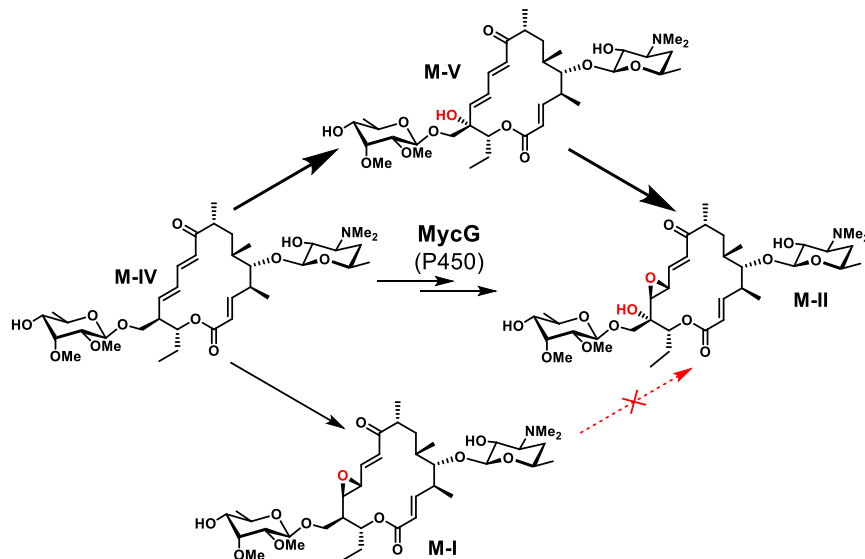
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Chapter 3.1

Studying the Substrate Specificity and Reactivity of MycG: A Multifunctional Enzyme

P450 enzymes catalyze a wide array of reactions including, but not limited to, hydroxylation, epoxidation, C-C coupling, N-dealkylation, sulfoxidation, and arene oxidation.¹ While enzymes have evolved to catalyze reactions with excellent selectivity and efficiency, a few are able to catalyze multiple oxidative steps involving separate reaction mechanisms.² MycG is one such enzyme which catalyzes the sequential hydroxylation and epoxidation of **M-IV** to **M-II**. The order of these oxidative steps is critical. **M-I** is a side product in which the epoxide is installed first. If **M-I** is fed back to MycG, no hydroxylation to **M-II** is observed.³

Scheme 3.1.1. MycG catalyzed oxidation of **M-IV**.



In addition to selectively performing 2 sequential and distinct oxidative steps, MycG is highly substrate specific. **M-IV**, **M-III**, and **M-VI** are identical in macrocyclic structure and differ only in extent of methylation of the mycinose sugar moiety. Despite having ≥ 110 atoms in common, $>94\%$ identical to **M-IV**, **M-III** and **M-VI** experience very low levels of oxidation when tested with MycG. Based on LC-MS and MS-MS data, it is believed that these minor products are

hydroxylation of the respective macrocycles but the exact structure of the oxidized products could not be determined since there was such a small amount of product formed.³

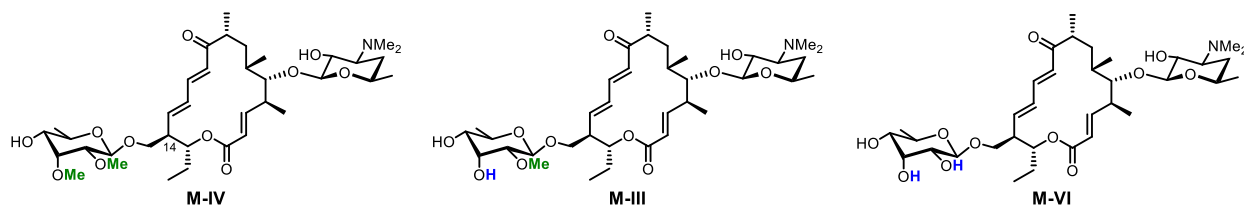


Figure 3.1.1. Macrocycles in the biosynthesis of **M-II** which differ in the extent of methylation of deoxysugar at C14. Only **M-IV** is effectively oxidized by MycG.

We set out on a computational investigation of the profound substrate selectivity and reactivity preferences of MycG. Molecular dynamics (MD) simulations and MetaDynamics were used to determine how these minor differences in the sugar at C14, remote from the sites of oxidation, have an impact on activity. Molecular dynamics were performed by Song Yang in the Houk group and will not be discussed in this dissertation. To determine why the order of oxidation of **M-IV** is so critical, we used density functional theory (DFT) calculations. Collectively, these methods complement each other to fully understand this complex, multi-functional enzyme.

Computational Methods

Quantum Mechanics. DFT calculations were performed using the Gaussian09 suite.⁴ Conformational sampling of substrates and transition states were performed using Spartan.⁵ The default settings for conformer distribution calculations were used. For transition states, the O_{Fe}-H_{substrate} and H_{substrate}-C_{substrate} bonds were frozen and other substrate torsions were sampled, including rotation about the C-H bond being abstracted. Due to the large number of conformers of each substrate and transition state, the iron-oxo model and transition structures were computed at the quartet spin state. Geometry optimizations and frequency calculations were performed at the

B3LYP level.⁶ The 6-31G(d) basis set was used on all atoms except Fe, for which the LANL2DZ pseudopotential was used. Saddle point transition structures were confirmed by the presence of one imaginary frequency corresponding to the desired transformation. Thermal corrections were computed at 1 atm and 298.15 K. The quasi-harmonic correction, as described by Truhlar, was used to adjust the Gibbs free energy.⁷ Single point calculations were performed at the B3LYP-D3(BJ)⁸ level with CPCM⁹ implicit solvation for water. LANL2DZ was used for Fe and 6-311+G(d,p) was used for all other atoms.

Results and Discussion

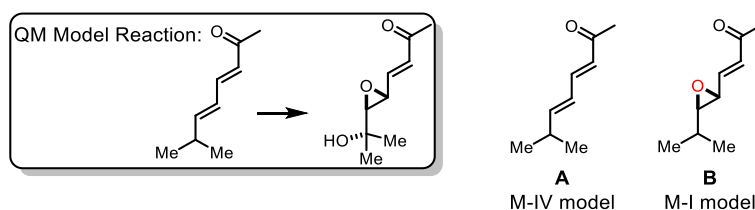


Figure 3.1.2. Model reaction used to study the activity of MycG with DFT.

Using **A** as a model for **M-IV** and **B** as a model for **M-I**, we performed density functional calculations to determine the barriers to C-H abstraction. With respect to separated reactants, the free energy barrier to C-H abstraction from **A** (**A-TS**) is 14.6 kcal/mol. The barrier to abstraction from **B** (**B-TS**) is 20.1 kcal/mol. This 5.5 kcal/mol increase for **B-TS** means that abstraction from the epoxidized substrate is 10,000 times slower than **A-TS**. This drastic difference in activity is in accord with the lack of activity toward **M-I**. The presence of the epoxide in **M-I** decreases the inherent reactivity, so that MycG is unable to hydroxylate this intermediate.

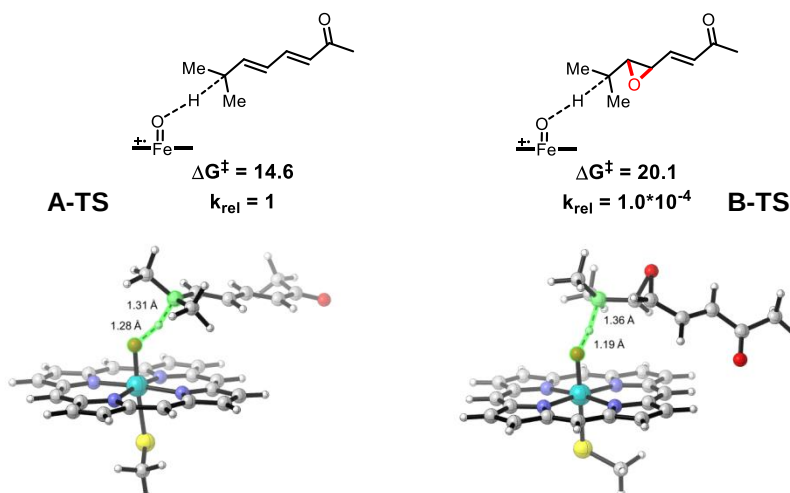


Figure 3.1.3. Free energies and transition structures of the C-H abstraction from **A** and **B**.

There are 2 attributes of the installed epoxide which could destabilize the developing radical and be the origin of the barrier increase: 1) the lack of allylic stabilization; 2) the electron-withdrawing nature of the oxygen. To probe these two properties of the epoxide independently we computed the barriers to C-H of two additional substrate models. Model **C** mimics the electron withdrawing nature of the oxygen in **M-I** but still has the conjugation present as in **M-IV**. Model **D** mimics the loss of conjugation due to the epoxide but no electron withdrawing groups are present. The barriers to C-H abstraction from **C** and **D** are 15.9 (**C-TS**) and 18.9 (**D-TS**) kcal/mol, respectively. In comparison to **A-TS**, the barrier increases by +1.3 kcal/mol for **C-TS** and +4.3 kcal/mol for **D-TS**. Both factors therefore contribute to the barrier increase in **B-TS**, but the lack of allylic stabilization is the most significant factor.

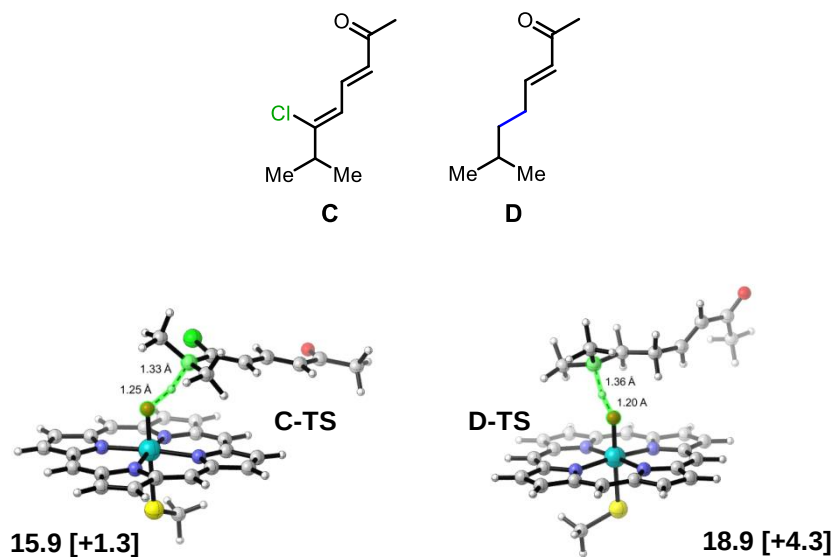


Figure 3.1.4. Top: Substrate models **C** and **D**; Bottom: $\Delta G^\ddagger$ [difference from **A**] for **C-TS** (left) and **D-TS** (right).

Conclusion

We have used multiple computational methods to investigate the selectivity of the multi-functional P450 MycG. In the mycinamicin biosynthetic pathway to **M-II**, **M-IV** undergoes sequential hydroxylation to **M-V** then epoxidation to **M-II**.³ Density function theory calculations prove that the order of oxidation of **M-IV** is due to the need for stabilization on the C-H abstraction transition state. When the epoxide is installed first, leading to **M-I**, the presence of the epoxide has removed the allylic stabilization of **M-IV** and is inductively withdrawing. By using substrate analogues, we determined that the lack of resonance stabilization of the developing radical is the lead cause of the inactivity of MycG towards **M-I**.

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Chapter 3.2

Regiodivergent Cyclization and Biocatalytic C-H Oxidation Enable Synthesis of Diverse 11- and 12-Membered Macrolactones from a Single Linear Substrate

Michael M. Gilbert, Matthew D. DeMars, Song Yang, Jessica M. Grandner, Shoulei Wang, Hengbin Wang, Alison R. H. Narayan, David H. Sherman, K. N. Houk, John Montgomery

In traditional linear synthetic approaches, complexity in a structure is assembled in a stepwise manner to obtain a single desired product. In this approach, a new synthesis must be devised for each structural analogue. As a method to streamline efficient access to analogues of a structural motif, late-stage diversification of an advanced synthetic intermediate often involves the interchange of functional groups after a fully functionalized core structure is accessed. A disadvantage of this approach is that assembly of the late-stage common intermediate itself often requires a lengthy linear synthesis and provides structures that are closely related with an identical core framework. An appealing strategy for more substantial late-stage diversification involves diversifying the framework itself (1). Through this approach, accessing two or more structural frameworks from a single intermediate is typically best achieved through processes such as accessing multiple cycloaddition pathways available to a single substrate or through catalyzing a thermodynamically driven rearrangement such as transesterification, sigmatropic rearrangement, or ring expansion or contraction (2-4). A second attractive strategy for achieving late-stage diversification begins from a single core structure followed by installation of various substituents on the periphery through site-selective C-H functionalization (5-11). While C-H functionalization strategies hold enormous promise, limitations in both site-selectivity and functional group compatibility (i.e. alkenes or basic amines) remain a considerable challenge in complex settings.

Currently, a significant gap exists in synthetic strategies that involve late-stage scaffold diversification combined with a versatile site-selective C-H functionalization approach. By first converting a single substrate into two or more molecular scaffolds and then accomplishing site-selective diversification of each of the obtained structural motifs through C-H functionalization, the rapid assembly of highly diverse analogues differing in both the core structural scaffold and peripheral functionality would become possible. (Figure 3.2.1A). Such an approach offers the simplicity of accessing a linear substrate with minimal functionality as the point of divergence and would provide a powerful strategy for the rapid assembly of molecular diversity and complexity.

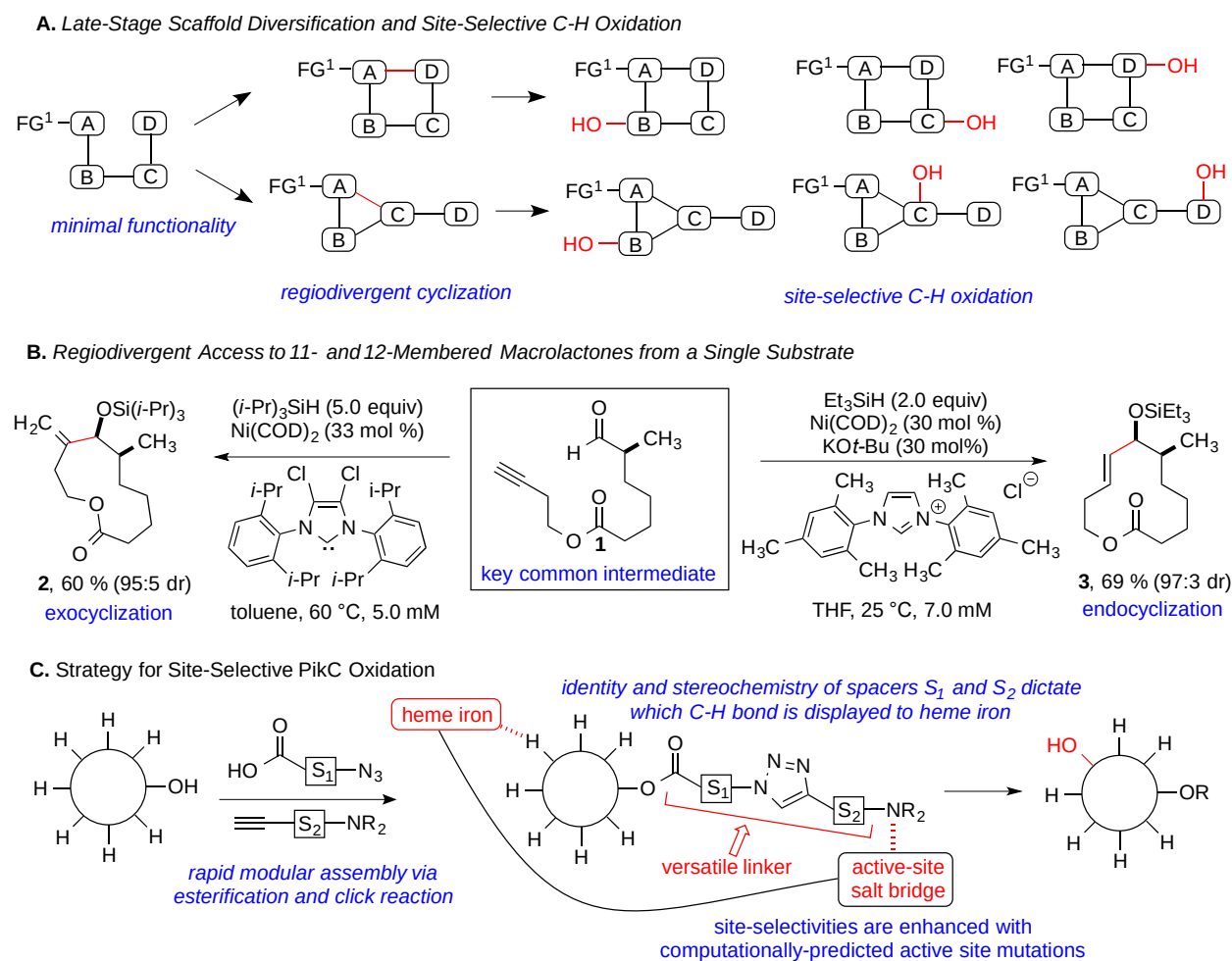


Figure 3.2.1. Approach to late-stage diversification. (A) Conceptual framework for late-stage diversification by regiodivergent scaffold assembly followed by site-selective C-H oxidation. **(B)** Regiodivergent reductive macrocyclizations. **(C)** General strategy for enabling site-selective biocatalytic oxidation.

The focus of this study is to utilize a simple linear ynal as a test substrate for assembly of 11- and 12-membered macrolactones followed by late-stage, site-selective oxidations to access a collection of functionalized macrolide analogues. Preparation of these test substrates by a nickel-catalyzed reductive macrocyclization process allows access to either regiochemical outcome (i.e. endo- or exocyclization) in macrolactone assembly by tailoring the ligand structure and reaction conditions (Figure 3.2.1B) (12-13). This regiodivergent catalytic process provides an ideal approach for accessing different ring sizes of macrolactone substrates that possess multiple unactivated methylene (CH₂) groups for exploration of new strategies for site-selective C-H oxidation. While impressive strides have been made in strategies that enable site-selective oxidations in multi-ring structures with well-defined conformations (14-17), selective access to multiple patterns of oxidation in macrocyclic compounds represents a challenge unmet by previous approaches. In order to address this limitation, the straightforward modular assembly of an enantiopure ynal precursor **1** was followed by regiodivergent and highly diastereoselective reductive macrocyclization to afford macrocycles **2** and **3**. This approach, governed largely by steric properties of the *N*-heterocyclic carbene (NHC) ligand, enables access to a large array of linear and macrocyclic structures through predictable reversals of regiochemistry in the C-C bond-forming step.

Macrocyclic substrates **2** and **3** provide a challenging context for developing site-selective C-H oxidations on different scaffolds that possess a multitude of similarly reactive C-H bonds. To

address this challenge, we examined the utility of an engineered fusion protein of the bacterial cytochrome P450 enzyme PikC. In prior work, engineered mutants of the PikC enzyme displayed excellent site-selectivity in oxidations of amine-containing substrates closely related to the endogenous macrolide substrate YC-17 (18) or of smaller, structurally-compact substrates such as simple terpenes (e.g., menthol) (19). However, the conformational flexibility of macrocycles that possess six or more methylene groups poses a tremendous challenge in site-selective oxidation that has not yet been efficiently addressed by chemical or biological catalysis. With such subtle differences in the reactivity of similar methylene groups, the use of directing groups presents the best opportunity for achieving tunable site-selectivity. While recent developments in enzyme engineering have demonstrated remarkable advances in organic synthesis (20-22), the use of substrate engineering as a synergistic approach to achieving site-selectivity in enzymatic transformations is greatly underutilized. Structural variations in rigid directing groups have recently demonstrated enormous promise in remote C-H functionalizations using small molecule catalysts (23-24). Despite pioneering early work from Breslow in designing biomimetic site-selective functionalizations based on template design (25-26), this approach has not yet been developed as a strategy to enable multiple selective outcomes in an enzyme-catalyzed process.

Previous studies of PikC-catalyzed oxidations illustrated that substrates possessing numerous contiguous methylene groups led to unselective oxidation when substrate engineering approaches were attempted (27). Thus, although the presence of the aminosugar desosamine is essential for binding and selectivity of the PikC enzyme toward its native macrolide substrates, at least seven oxidized products were generated when using a desosaminylated, but unfunctionalized, twelve-membered macrocyclic substrate. While the use of simple aromatic spacers avoids the cumbersome installation of aminosugars (18), we recognized the need for a more versatile strategy

that enables an anchoring basic amine to be covalently attached through a linker. The method should provide a rapid and high-throughput synthesis, facile length and shape variations of the linker component, and efficient chemoselective removal of the linker following C-H oxidation. These criteria can all be addressed by the modular connection of hydroxyl-containing substrates, amino acid-derived azido acids, and acetylenic amines (Figure 3.2.1C). Through the appropriate choice of spacer elements S_1 and S_2 and access to regiochemistry reversal in azide-alkyne click cycloadditions (28-29), a large number of linker structures are readily available. Notably, click reactions are typically utilized as a simple means to connect two structures without regard to the precise structural features of the linking functionality. However, in our approach, the precise shape and geometry of the unit assembled by the azide-alkyne cycloaddition is an integral feature required for success of the strategy.

Derivatives of 11-membered substrate **2** were first examined in the click anchor strategy for site-selective biocatalytic oxidation (Table 3.2.1). The engineered PikC fusion protein PikCD50ND176QE246A-RhFRED, which was designed by molecular dynamics-guided analysis (19), was used in our studies. The active site residues that were predicted to lead to unproductive substrate binding orientations were mutated in order to promote formation of the closed, active form of the enzyme. A collection of 13 triazole anchors was then assembled from seven azido acids and two amines. Triazoles examined include those derived from azido alkynes possessing and lacking chirality, *ortho*, *meta*, and *para*-spaced benzene spacers, and both 1,4- and 1,5-triazole regioisomers. Following installation of the triazole anchor to substrate **2**, analytical scale biocatalytic oxidations on derivatives of **4** were performed, and promising anchors were selected from LCMS analysis based on percent conversion and selectivity for a major product. Representative cases were then conducted on a 30-60 mg preparative scale, products were isolated

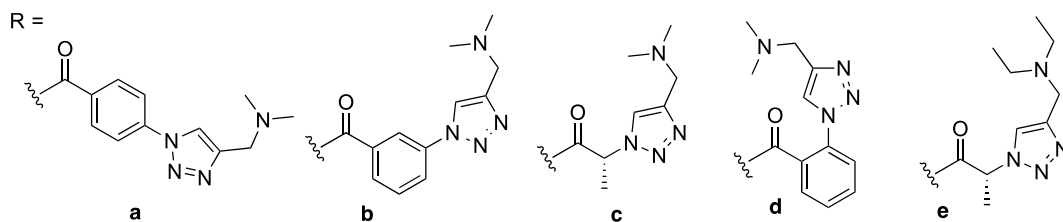
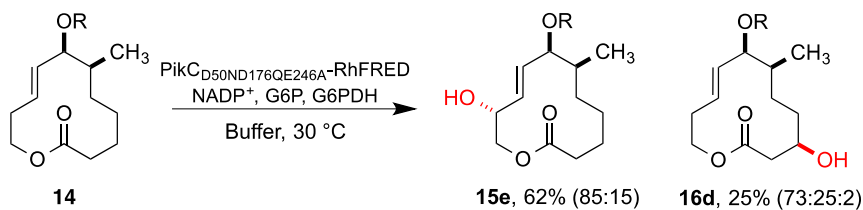
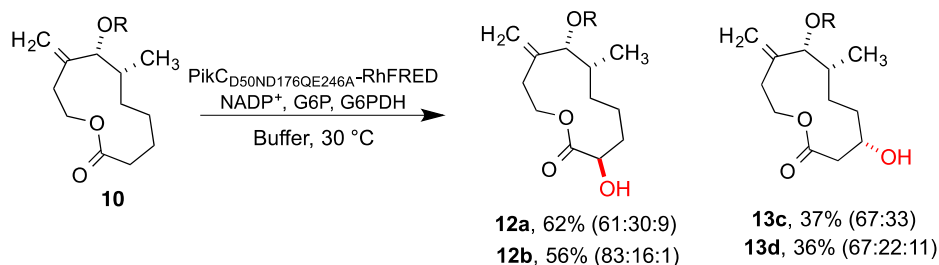
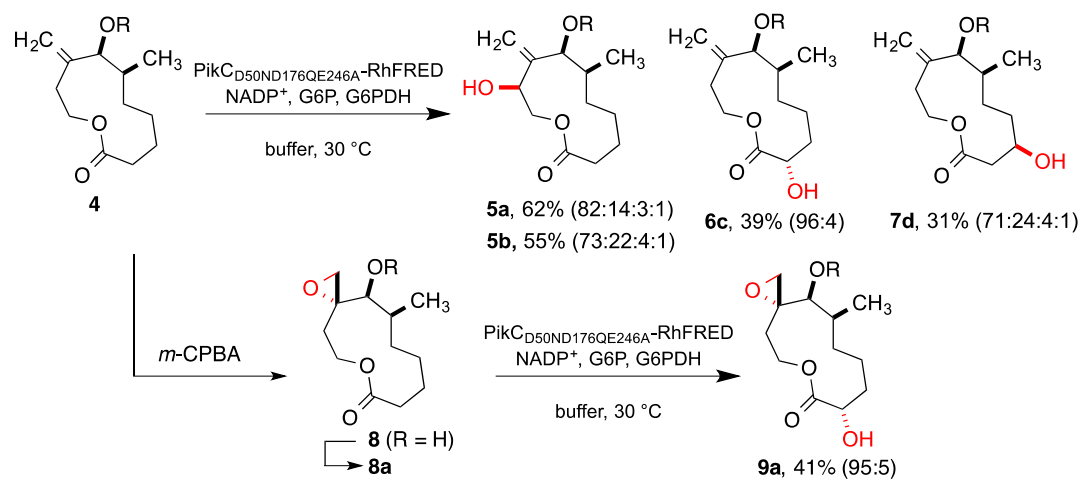
by preparative HPLC, and the structure and stereochemistry of major products were elucidated by NMR. Isolated monohydroxylated products were then used as internal standards in the analysis of additional analytical scale experiments in order to identify anchors that enable synthesis of a different hydroxylated product.

Using this approach, three different monohydroxylated products (**5**, **6**, and **7**) were obtained from substrate **4**. Notably, triazoles possessing *para*- or *meta*-substituted benzene spacers **a** or **b** enable oxidation of allylic protons proximal to the point of anchor connection to provide product **5**. Alternatively, selection of anchors that possess a shorter linker motif enables oxidation of the distal region of the substrate. For example, using alanine-derived anchor **c** leads to the production of product **6c** with oxidation α - to the carbonyl. In contrast, anchor **d** with an *ortho*-substituted benzene spacer leads to product **7d** with oxidation β - to the carbonyl. No evidence for epoxidation or amine oxidation was observed in PikC-catalyzed oxidations, demonstrating desirable chemoselectivity features of C-H oxidation. Epoxide **8** is cleanly obtained (when R = Ac) in reactions with either *m*-CPBA as the electrophilic oxidant, or by using the White C-H oxidation catalyst (17), demonstrating orthogonal chemoselectivity of the PikC oxidation method. By installing anchor **a**, biocatalytic oxidation of **8a** then cleanly affords epoxy alcohol **9a**. The site-selective oxidation to provide **5**, **6**, **7** or **9** demonstrates that this method is useful for oxidizing C-H bonds that are either proximal or distal to the directing group. The versatile directing capability of the triazole linkers paired with PikC catalysis thus does not directly correlate with C-H bond strength, acidity, inductive influences, steric accessibility, or immediate proximity to the directing group. Instead, the enzyme active site conformation matched with the structure, stereochemistry, and conformation of both the triazole anchor and the substrate overrides these influences.

To probe the interrelationship of the stereochemical features of the active site residues, triazole anchor, and macrocyclic substrate, the enantiomeric form of the macrocycle (substrate **10**) was examined with the same biocatalyst and triazole anchors **a-d**. By using the *meta*- and *para*-substituted anchors **a** and **b** that enabled oxidation of the proximal allylic methylene in substrate **4**, these same anchors instead led to oxidation of distal protons α - to the carbonyl to provide products **12a** and **12b**. Use of an alanine-derived anchor **c** or the *ortho*-substituted anchor **d** afforded **13c** and **13d** as the major product via oxidation β - to the carbonyl. These results demonstrate that optimal anchor structure will vary from substrate to substrate, even within an enantiomeric series, thus adding appeal to the simple and potentially high throughput access to linkers in the approach described.

In taking advantage of the regiodivergent access to macrocycles described, the 12-membered structure derived from endocyclization was also examined in biocatalytic oxidations. In this case, two different oxidized products were obtained from substrate **14**. The alanine anchor **e** provided access to allylic oxidation product **15e**, and a diethylamine anchor was employed in this case to minimize *N*-demethylation, which was observed as a minor byproduct in some instances. Alternatively, using the *ortho*-linked benzene spacer **d**, major product **16d** was obtained via oxidation β - to the carbonyl. These results collectively illustrate that a variety of monohydroxylated compounds of macrocycles varying in ring size may be efficiently obtained starting from a single, easily accessible ynal substrate.

Table 3.2.1. Scope of biocatalytic macrocycle oxidation. R groups are depicted by the letters a-e shown at bottom. Major product is depicted in the table. Percent yield refers to conversion to monohydroxylated products, and ratios of monohydroxylated products are given in parentheses.



As described above, the structure, stereochemistry, and conformation of the substrate, linker, and enzyme active site all play important roles in determining site selectivity of the C-H oxidations. In order to understand these influences and to add a predictive component to the strategy, a combined density functional theory (DFT) and molecular dynamics (MD) computational study was undertaken. DFT was used to compute the intrinsic reaction barriers to C-H abstraction at C3 allylic and C10 alpha positions of **4**. The R group shown in **4** was truncated to a methyl, and conformations of the macrocycle were explored with MMFF in Spartan (30). These conformers were then optimized, and single point energy calculations were performed with DFT in Gaussian09. The lowest energy conformer was identified along with a second low energy conformer only 0.9 kcal/mol higher in energy. All other conformers were >2 kcal/mol higher in energy than conformer 1. The barriers to C-H abstraction at C3 and C10 were computed for these two low energy conformers and are shown in Figure 3.2.2A. The barriers to abstraction of the hydrogens shown in purple and green are the lowest for each site of both conformers, and correspond to the hydrogens that are abstracted to form **5** and **6**. Figures 3.2.2B and 3.2.2C show the transition structures for abstraction of the C3 purple (equatorial) and C3 yellow (axial) hydrogens, respectively, from conformer 1. It is clear from close inspection of the transition structures that the abstraction of the equatorial hydrogen (purple) benefits from developing conjugation with the neighboring exocyclic alkene. The axial hydrogen (yellow) is perpendicular to the π -system and cannot benefit from the allylic stabilization. A similar conjugation effect is seen with the C-H abstractions at C10. The green hydrogen is in conjugation with the carbonyl group (Figure 3.2.2D), but the ring must distort for the blue hydrogen to benefit from the same stabilization. The substrate prefers a dihedral of 16.1° between the exocyclic alkene and the

carbonyl. In Figure 3.2.2E, this dihedral expanded to 55.6° in the transition structure for the abstraction of the blue hydrogen.

Conformer 1 was used for molecular dynamics simulations. Linker **a** and linker **c** were each attached to the macrocycle and docked into the active site of the PikC_{D50N/D176Q/E246A}, and 500ns trajectories were run on each of these complexes using Amber. Over the course of the simulations, both conformer 1 and conformer 2 are observed. As shown in the snapshot of Figures 3.2.3A and 3.2.3B, linker **a** places C3 closest to the active iron-oxo. C2 is also placed close to the iron-oxo (3.7 Å averaged over the 500ns simulation), but the computed barriers to abstraction at C2 are +5 kcal/mol higher in energy than at C3 (Figure 3.2.4). Alternatively, linker **c** places C10 closest to the iron-oxo species. Figures 3.2.3C and 3.2.3E show the average distance of each hydrogen to the iron-oxo oxygen over the course of the 500ns simulations. Figures 3.2.3D and 3.2.3F are plots of the H_{substrate}-O_{iron} distances vs. C-H-O angles over the course of the trajectories with linkers **a** and **c** respectively. Figures 3.2.3C and 3.2.3D reveal that the purple hydrogen is closest to the DFT-computed transition state geometry. Collectively these data show that the purple, equatorial hydrogen of C3 is the most accessible to the iron-oxo in the enzyme and intrinsically more reactive. Figures 3.3.3E and 3.2.3F show that the blue hydrogen is closest to the DFT-computed transition state geometry, but abstraction of this hydrogen is disfavored by up to 1.1 kcal/mol. While linker **c** places C10 closest to the iron-oxo, the intrinsic reactivity of the equatorial hydrogen (green) overrides the proximity of the axial hydrogen (blue). MD simulations of **4d** and **14e** were able to help understand the preference of the corresponding major products, **7d** and **15e** respectively (Figures D.S1-D.S3). The combined DFT and MD study provides an explanation for the observed regiochemistry and stereoselectivity of these PikC-catalyzed hydroxylations.

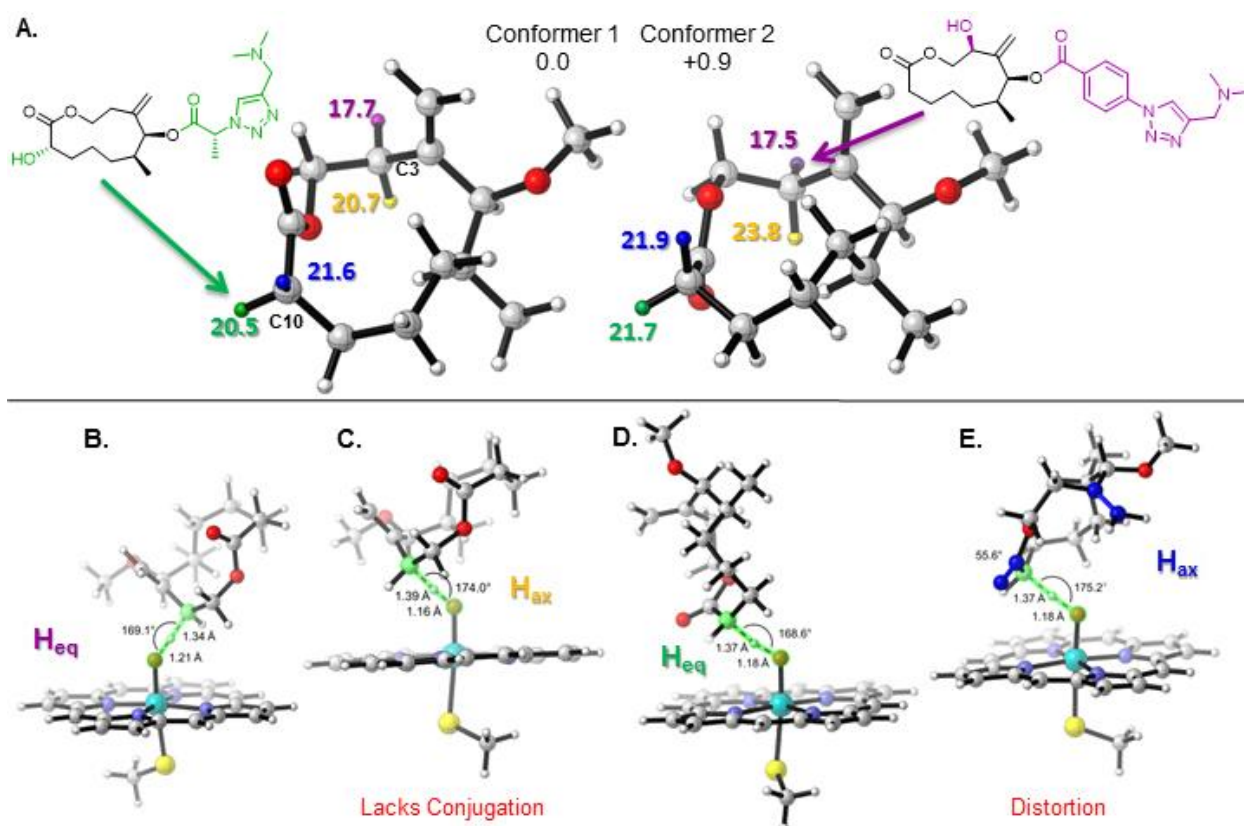


Figure 3.2.2. (A) Lowest energy conformers of a model of structure **4**, with DFT barriers (kcal/mol) to C-H abstraction at C3 and C10; (B) Transition structure of C3 (purple) hydrogen abstraction; (C) Transition structure of C3 (yellow) hydrogen; (D) Transition structure of C10 (green) hydrogen abstraction; (E) Transition structure of C10 (blue) hydrogen abstraction.

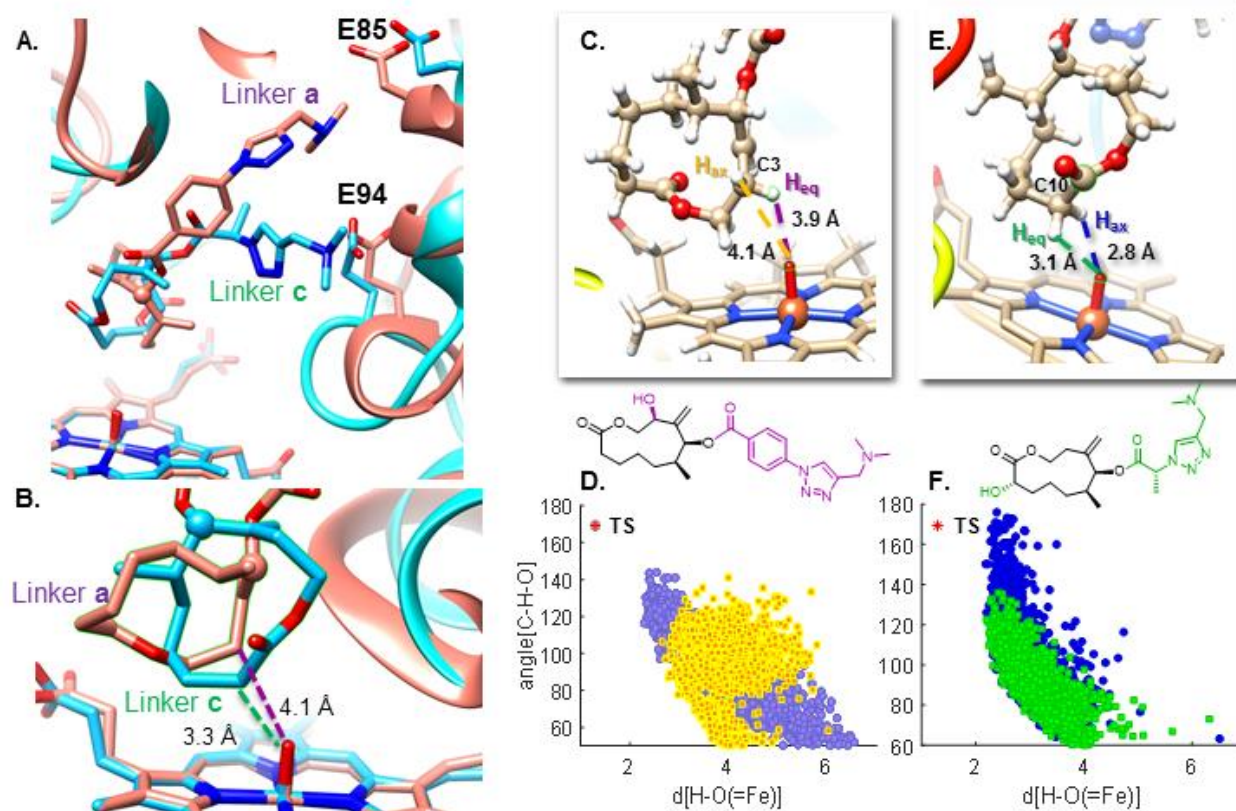


Figure 3.2.3. (A) Snapshot of MD trajectory of **4** with linker **a** overlaid with a snapshot of **4** with linker **c**. (B) Closeup of Figure 3.2.3A snapshot with average C-O_{Fe} distances shown. (C) Snapshot of **4** with linker **a** with average H-O distances shown. (D) Plot of hydrogen (of substrate C3) to oxygen (of iron-oxo) distances vs C-H-O angles throughout the MD trajectory, with TS geometry shown in red; (E) Snapshot of **4** with linker **c** with average H-O distances shown; (F) Plot of hydrogen (of substrate C10) to oxygen (of iron-oxo) distances and C-H-O angles throughout the MD trajectory, with TS geometry shown in red.

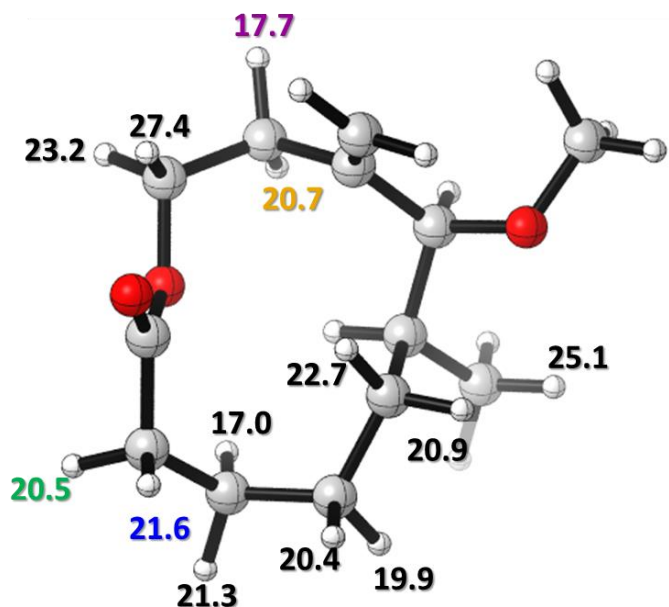


Figure 3.2.4. DFT computed barriers to C-H abstraction from conformer 1 of model **4**.

In summary, this study describes a versatile strategy for the rapid generation of a collection of macrocyclic compounds that differ in ring size and oxidation pattern. Starting from a single linear substrate, catalyst control in a regiodivergent macrocyclization is paired with site-selective C-H oxidations enabled by the synergy of a computationally-designed engineered cytochrome P450 catalyst and a tailored amine-containing directing group. Computational analysis uncovers a complex synergy of macrocycle conformation, inherent reactivity of various C-H bonds, linker structure, and substrate-active site interactions in controlling site selectivity of C-H oxidation. This work provides a general strategy and new opportunities for diversification through late-stage functionalization of a broad array of substrate classes.

Supporting Information

Details on how computations were performed and additional computational figures are given in Appendix D.

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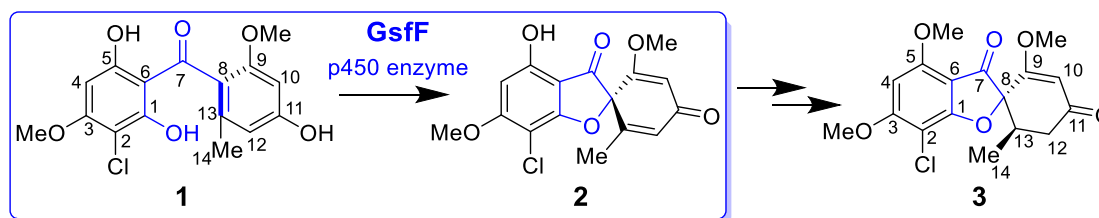
Chapter 4.1

Mechanism of the P450-Catalyzed Oxidative Cyclization in the Biosynthesis of Griseofulvin

Jessica M. Grandner, Ralph A. Cacho, Yi Tang, K. N. Houk

Introduction

Scheme 4.1.1. Griseofulvin (**3**) and biosynthetic scheme highlighting the role of P450 GsfF.



Griseofulvin (**3**), originally of interest for its antifungal activity, has more recently been reported to have both anticancer¹ and antiviral² activity in mammals.³ The important biological applications and intriguing spirocyclic core of **3** make it a compound of interest to biochemists and synthetic chemists alike. The Tang group reported the genes in *Penicillium aethiopicum* responsible for the biosynthesis of **3** in 2010,⁴ and subsequently determined the full biosynthetic pathway in 2013.⁵ One important discovery made during the elucidation of this pathway was that a P450 enzyme (herein referred to as GsfF, as it is encoded in the *gsf* gene cluster) is responsible for the important oxidative cyclization of griseophenone B (**1**) to desmethyl-dehydro griseofulvin A (**2**), shown in Scheme 4.1.1. Intermediate **2** only requires methylation and stereoselective reduction by two subsequent enzymes to afford the natural product **3**.⁵ The mechanism by which this P450 produces **2** has been explored with a combination of density functional theory (DFT), homology modeling, docking, and experiment to determine how this stereoselective oxidative cyclization occurs.

Computational Methods

DFT calculations were performed using Gaussian 09.⁶ Geometry optimizations and frequency calculations were performed using unrestricted B3LYP (UB3LYP)⁷ with the LANL2DZ basis set for Fe and 6-31G(d) on all other atoms. Transition states had one negative force constant corresponding to the desired transformation. Enthalpies and entropies were calculated for 1 atm and 298.15 K. A correction to the harmonic oscillator approximation, as discussed by Truhlar and co-workers, was also applied to the entropy calculations by raising all frequencies below 100 cm⁻¹ to 100 cm⁻¹.⁸ Single point energy calculations were performed using the functional (U)B3LYP-D3(BJ)⁹ with the LANL2DZ basis set on iron and 6-311+G(d,p) on all other atoms. Where relevant, single points with solvent corrections were performed using the PCM continuum model with both water and chlorobenzene.¹⁰ Transition structures were connected to intermediates via IRC calculations.

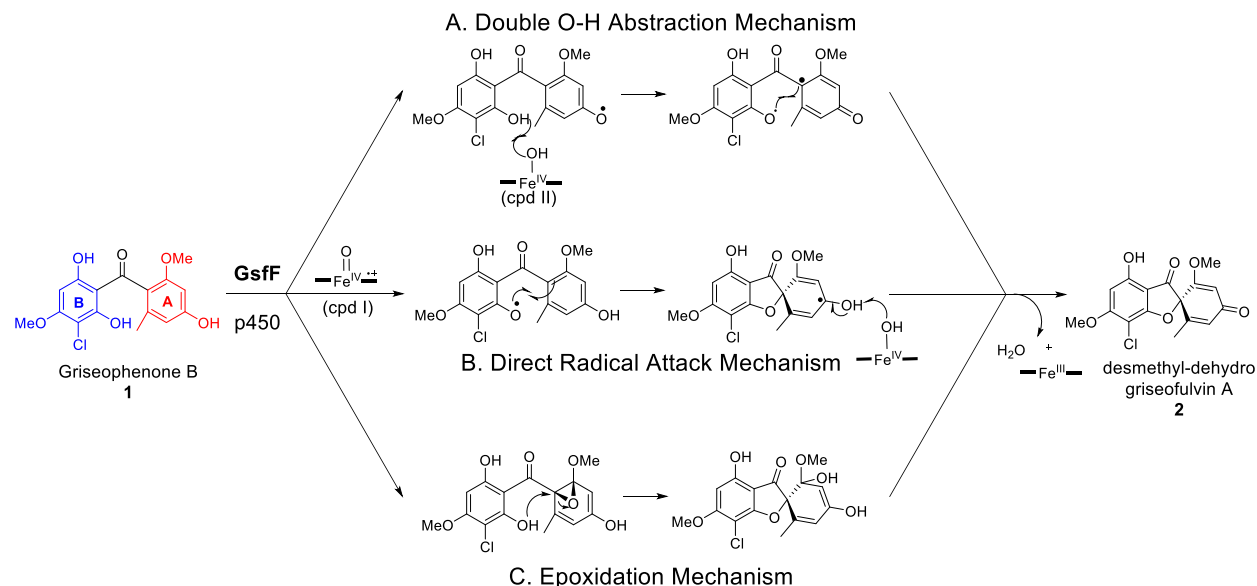
Homology models for GsfF were generated using the Robetta online server (<http://rosetta.bakerlab.org/>).¹¹ The Rosetta fragment insertion method was used to provide both *ab initio* and comparative models of the target protein. The resulting homology model, following truncation of the putative membrane-anchoring N-terminal helix (first 53 residues of GsfF), was subjected to full-atom structural refinement using the Rosetta “relax” application with the iron atom of the heme ligand constrained to within 2.3 Å of the catalytic Cys450.¹² A total of 300 refined models were generated from the Rosetta “relax” protocol with the 20 highest scoring models within a root mean square deviation (RMSD) of 0.356 Å from the top scoring model. Autodock Vina¹³ was used to perform docking on the highest-ranked, refined homology model obtained above, without membrane-anchoring N-terminal helix. Residues TYR49, TRP52, LEU159, ALA165, THR253, ALA254, ASP257, ALA258, TYR434, PHE435 were allowed to be flexible, along with the substrate, during the docking procedure. The box within which the ligand

was allowed to be position contained the flexible residues and was positioned above the heme iron. Nine poses were obtained as a result of the docking, and all are given in the Supporting Information.

Results and Discussion

Three possible mechanisms, Scheme 4.1.2, were explored computationally to determine how GsfF catalyzes the transformation of griseophenone B (**1**) to desmethyl-dehydro griseofulvin A (**2**). Pathways A and C were originally proposed in Cacho et al.'s paper in 2013.⁵ In pathway A, initial O-H abstraction from ring A is followed by a second phenolic abstraction from ring B and radical coupling to yield **2**. In pathway C, the enzyme could catalyze the epoxidation of ring A to form an arene oxide intermediate. Nucleophilic opening of the epoxide by the neighboring phenol then yields a hemiacetal, which then rearomatizes through the loss of water. Pathway B was newly proposed in this manuscript as a way the enzyme could perform O-H abstraction but avoid diradical formation. Initial phenolic O-H abstraction from ring B could be followed directly by ring closure. Subsequent phenolic O-H abstraction from ring A of the preformed spirocycle would also lead to **2**.

Scheme 4.1.2. Three possible mechanisms for the P450-catalyzed transformation of 1 to 2.



Initially, the epoxidation of ring A of **1** was explored, following the studies of arene oxidations that have been examined computationally by Shaik and others.^{14,15,16,17,18} Many arene oxidation reactions, including epoxidation, are said to go through a common intermediate involving a tetrahedral, iron-oxo bound arene.¹⁶ Two orientations for the formation of this tetrahedral intermediate have been determined, a “face-on”¹⁷ approach and a “side-on”^{16,18} approach.¹⁹ For productive arene oxide formation, the iron-oxo attack must occur at either carbon 8, 9, or 13 ring A, which all have non-hydrogen substituents. For this reason, a “face-on” approach was studied.

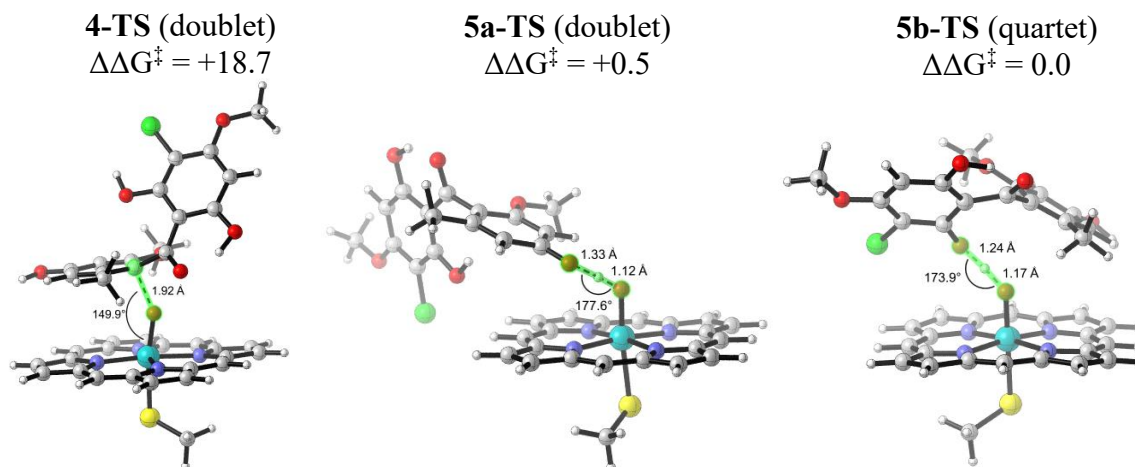


Figure 4.1.1. Lowest energy transition states²⁰ for the three possible pathways outlined in Scheme 4.1.2. Forming bonds are dashed and highlighted in green.

The lowest energy transition structure for the formation of the tetrahedral intermediate is shown in Figure 4.1.1, **4-TS**. This transition state was then compared to the two possible O-H abstraction transition states, **5a-TS** and **5b-TS**, to determine the likelihood of epoxidation. As shown in Figure 4.1.1, O-H abstraction from either ring is substantially favored over tetrahedral intermediate formation. Transition state **4-TS** can also have mixed radical/cationic character, which varies with substituents.¹⁵⁻¹⁹ Using NBO charges,²¹ there is a change in the total charge of ring A from isolated reactant to the transition state **4-TS** equal to +0.44. Therefore, single points calculations were repeated using the PCM model for both water and chlorobenzene.¹⁰ **4-TS** is stabilized by both solvents, 1.7 kcal/mol in water and 0.9 kcal/mol in chlorobenzene. This small stabilization from implicit solvent does not compensate for the inherent preference for O-H abstraction. Due to the large difference in energy between **4-TS** the O-H abstraction mechanisms, an epoxidation mechanism as outlined in Scheme 4.1.2 is unlikely.

The oxa-spiro ring of griseofulvin is also formed nonenzymatically by radical oxidation of similar substrates catalyzed by iron. In the first synthesis of griseofulvin, Day and co-workers

utilized aqueous potassium hexacyanoferrate(III)²² to cyclize the 5-methoxy analogue of **1**, or griseophenone A, to the respective analogue of **2** (5-methoxy-**2**).²³ Kuo et al. used a similar transformation in their total synthesis of **3**.²⁴ The preference for O-H abstraction over epoxidation is supported by these total syntheses.^{23,24} Schyman, Shaik, and co-workers discuss the discovery that in the biosynthesis of dopamine by Cytochrome P450 enzyme CYP2D6, the formation of the covalent iron-oxo complex is much higher in energy than a mechanism involving phenolic O-H abstraction and subsequent rebound.^{10,25} Several others have proposed similar dual abstraction mechanisms. Gesell et al. propose a phenolic coupling by the CYP719B1 to form salutaridine.²⁶ Holding and Spencer also propose similar phenolic coupling mechanisms in the biosynthesis of chloroeremomycin.²⁷ Yong, Wang, and co-workers computationally demonstrated that a “reversed dual hydrogen abstraction” (R-DHA) mechanism, which involves a rate limiting O-H abstraction from the hydroxyl group of ethanol and subsequent C-H abstraction from the neighboring carbon, can be the predominant mode of oxidation depending on the environment.²⁸ The overall $\Delta G^\ddagger$ of abstraction ring A is 7.1 kcal/mol and 2.9 kcal/mol for ring B, with respect to each corresponding prereaction complex. These barriers are fairly low but are comparable to previous calculated barriers from the literature referenced above. For the oxidation of tyramine, the B3LYP portion of the QM/MM calculations gives a $\Delta E^\ddagger$ for O-H abstraction of 10.4 kcal/mol with respect to the pre-reaction complex.¹⁰ When the D3 dispersion correction is applied, the $\Delta E^\ddagger$ value decreases to 9.7 kcal/mol. From the respective complex, the $\Delta E^\ddagger$ of **5b-TS** is 6.1 kcal/mol when the D3(BJ) dispersion correction is included and 8.3 kcal/mol without dispersion. This barrier is very comparable to that of the tyramine O-H abstraction. The O-H abstraction of the R-DHA mechanism has a computed $\Delta E^\ddagger$ of 16.3 kcal/mol, with no dispersion corrections included.²⁸ The substituents of ring B in **1** are able to stabilize the developing radical character in the transition

state via resonance and thereby slightly lower the barrier to abstraction, compared to that of ethanol or even tyramine. Initial O-H abstraction from ring B is favored by +0.5 kcal/mol over abstraction from ring A. The inherent preference for O-H abstraction from ring B is due to a greater number of favorable dispersion interactions. When the D3(BJ) correction is not applied, initial O-H abstraction from ring A is favored by 3 kcal/mol.

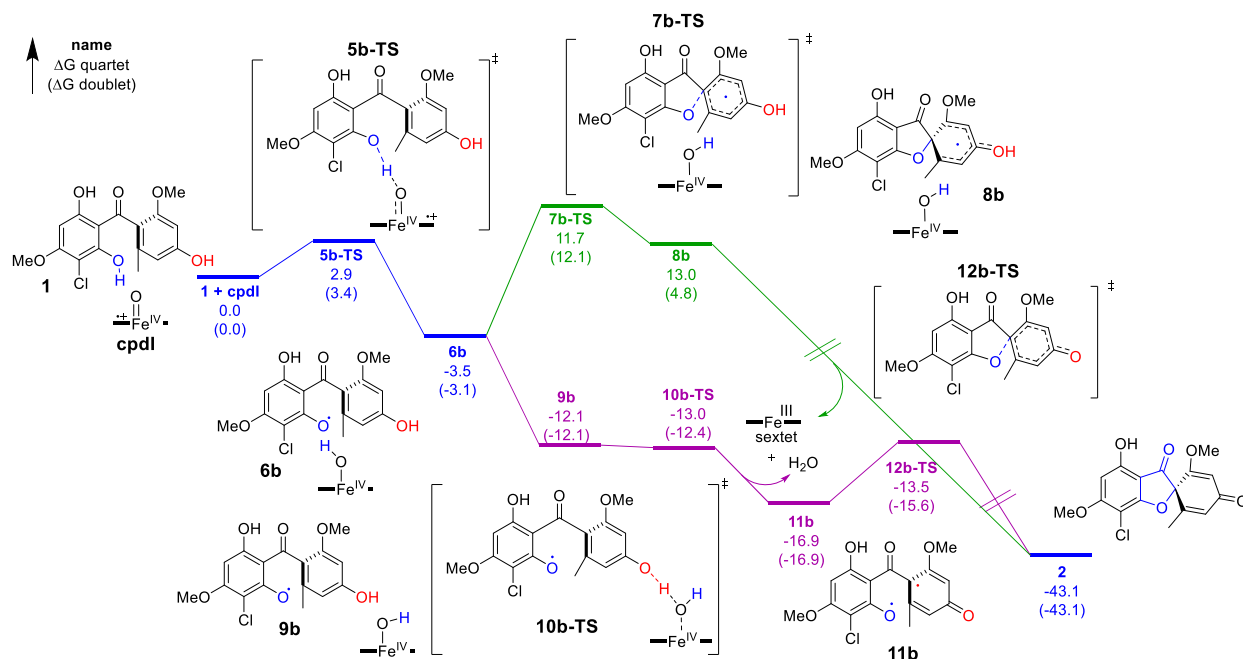


Figure 4.1.2. Iron-oxo catalyzed formation of **2** with initial O-H abstraction from ring B (energies given in kcal/mol).

Figure 4.1.2 shows the energy profile following O-H abstraction from ring B.²⁹ Initial hydrogen abstraction from ring B (**5b-TS**) has a low barrier of 2.9 kcal/mol. After formation of **6b**, the newly formed oxy-radical could directly attack ring A via **7b-TS** with a barrier of 15 kcal/mol, shown in green. This transition state leads directly to intermediate complex **8b**, shown in Figure 4.1.3. A scan of the $O_{Fe}-H_{ringA}$ distance reveals no barrier to the second O-H abstraction.³⁰ Alternatively, **6b** could undergo a mechanism similar to pathway A of Scheme 4.1.2. Reorientation

of the substrate to **9b** involves a shift in substrate binding to the iron-hydroxo, or cpdII. Product complex **6b** involves a O_{ringB}-H_{cpdII} hydrogen bond while pre-reaction complex **9b** involves an H_{ringA}-O_{cpdII} hydrogen bond. This reorientation is likely not feasible without deleterious side reactions with either highly reactive intermediate. Additionally, while the barrier could from **6b** to **9b** could not be calculated for this QM model, it is highly likely that the barrier to reorientation is higher in energy than the barrier to direct attack via **7b-TS**. If the reorientation could occur, O-H abstraction from ring A via **10b-TS** is essentially barrierless ($\Delta E^\ddagger = 2.3$ kcal/mol from **9b** in the quartet state), shown in purple. Diradical intermediate **11b** has a low coupling barrier **12b-TS** of 3.4 kcal/mol. This reaction is very favorable with at ΔG_{rxn} of -43 kcal/mol. The exergonic nature of this reaction is similar to other reported oxidations involving O-H abstractions. For the R-DHA mechanism, the ΔE_{rxn} is downhill by -60 kcal/mol.²⁸

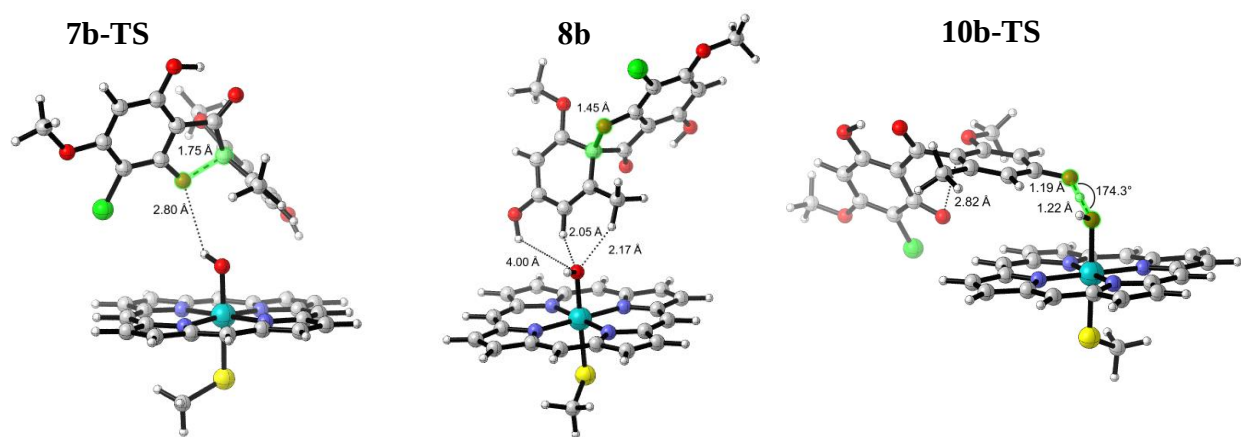


Figure 4.1.3. Transition structures for **7b-TS** (left), **8b**, and **10b-TS** (right). Forming bonds are dashed and highlighted in green.

The lowest energy conformation of the substrate, as shown in the transition states of Figure 4.1.1, is preorganized to undergo ring coupling. Interactions of side chain in the binding site are not required to orient the substrate in a way that favors reaction. The homology model shown in

Figure 4.1.4 reveals a highly hydrophobic active site above the iron-heme. The hydrophobicity of the active site suggests that there will be a low concentration of water within the binding pocket and little involvement of the side chains in stabilization of radical transition states. The neutral substrate studied here does not require stabilization within the active site. The hydrophobicity of the active site, preorganization of the substrate, and neutral character of the substrates/transition states all validate our use of the QM model in the gas phase. Figure 4.1.5a shows a chemically relevant binding mode for abstraction from ring A, ranked first among all binding poses, and Figure 4.1.5b is a chemically relevant binding mode for abstraction from ring B. The establishment of these two binding poses indicates the ability of the active site to accommodate the necessary substrate in orientations for both O-H abstractions. While these binding poses do not exactly resemble the corresponding prereaction complexes calculated by QM, the docked poses show that the substrate does fit in the homology model in modes primed for O-H abstraction.

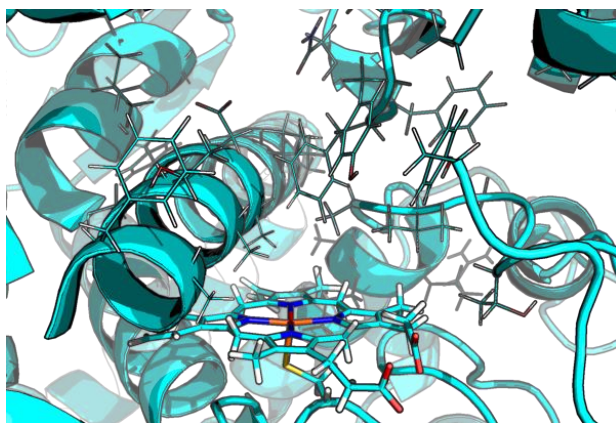


Figure 4.1.4. Apo homology model of GsFf. Active site residues are shown as lines, while iron-heme and axial cysteine are shown as sticks.

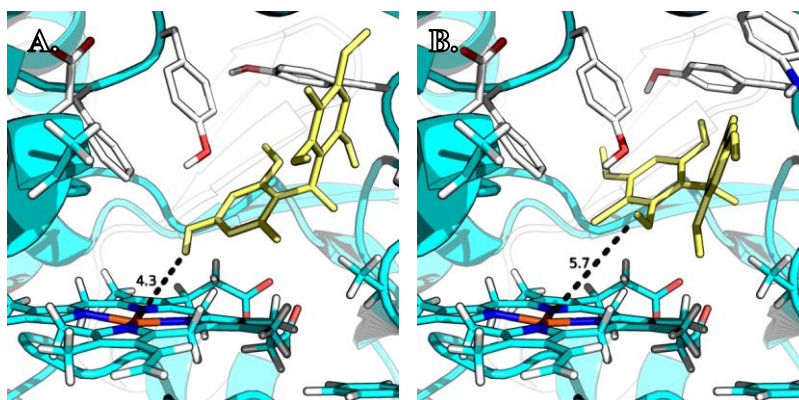


Figure 4.1.5. Docked poses for (A) abstraction from ring A and (B) abstraction from ring B. Substrate is shown in yellow, flexible residues are shown in silver, and other active site residues are shown in blue (including iron-heme).

Shaik and co-workers found that proton-coupled electron transfer between active site residues/water molecules was essential for the C2-C2 bond coupling of two linked indoles. While the C2-H bonds of indole have a calculated bond dissociation enthalpy (BDE) of 117.5 kcal/mol,³¹ the calculated phenolic O-H BDE is 82.9 kcal/mol.³² For comparison, the BDE of the benzylic C-H in toluene is calculated to be 89.8 kcal/mol.³³ The bond enthalpy indicates that C2-H abstraction from indole is inaccessible and an alternative path to C2-C2 coupling is operative. On the other hand, abstraction of the phenolic O-H atoms from **1** is feasible and can proceed through the calculated pathway.

In either pathway shown in Figure 4.1.2, the C-O coupling is the highest energy barrier. For the case of cholesterol 7 α -hydroxylation, the first reduction of Fe³⁺ to Fe²⁺ during catalyst regeneration was postulated to be rate-limiting.^{34,35} For many other P450 enzymes, the second reduction of the dioxygen complex is rate-limiting.^{14,36,37} GsfF is a member of the eukaryotic membrane-bound cytochrome P450 family, which uses a separate enzyme, the cytochrome P450 reductase, to donate electrons from NADPH to the heme cofactor during the catalytic cycle.³⁸ It is

likely that, as with the other P450 enzymes, one of the reduction steps is rate-limiting and, therefore, observation of a ^{13}C or ^{18}O kinetic isotope effect is unlikely.

In the discussion above, we have proposed a likely mechanism for the oxidation of griseophenone **B** based on DFT model calculations. We conclude that a hydrogen-atom abstraction-based mechanism occurs rather than epoxidation, due to the large energy difference in the computed transition states. We propose that O-H abstraction at ring B is followed directly by spirocyclic ring formation then subsequent abstraction from ring A to yield **2**. We do acknowledge that the intricate mechanistic detail following O-H abstraction is ultimately determined by the enzymatic environment. The overall barriers for O-H abstraction from ring A and ring B are very close in free energy and, therefore, binding of the substrate will determine which hydrogen is abstracted first. If abstraction from ring A occurs first, the resulting radical cannot undergo direct spirocyclic ring formation, as shown in Figure 4.1.2, and reorientation of the active radical would be required. As with the reorientation of **6b** to **9b**, we believe that the reorientation of the radical resulting from abstraction of ring A is unlikely to occur without deleterious side reactions. Quantum mechanics/molecular mechanics (QM/MM) calculations can address these minute details, but are best obtained based on a crystallographic structure, which is not available at this time.

We have explored the abstraction of the phenolic hydrogen of ring B which leads to the observed product, but there is an alternative phenolic hydrogen present at C5. There were no shunt products observed that would have been derived from abstraction of this hydrogen.⁵ We postulate that this is due to the strong hydrogen bond that forms between the C5-hydroxyl and the bridging carbonyl. This hydrogen bond is present in the lowest energy conformation of the substrate, as seen in the transition state structures of Figure 4.1.1. The presence of this hydrogen bond helps

preorganize the substrate for attack of ring B onto ring A. Methylation of the C5-hydroxyl group occurs after the cyclization step in the biosynthesis of griseofulvin,⁵ and we postulate that preorganization of the substrate plays a role in the feasibility of the cyclization. It has also been shown that griseophenone A, is not cyclized to the natural product.³⁹ Figure 4.1.6 shows that the lowest energy conformation of griseophenone A, **13a**, is 8.0 kcal/mol lower in energy than the “active” conformation, **13b**, which is similar to **1**.

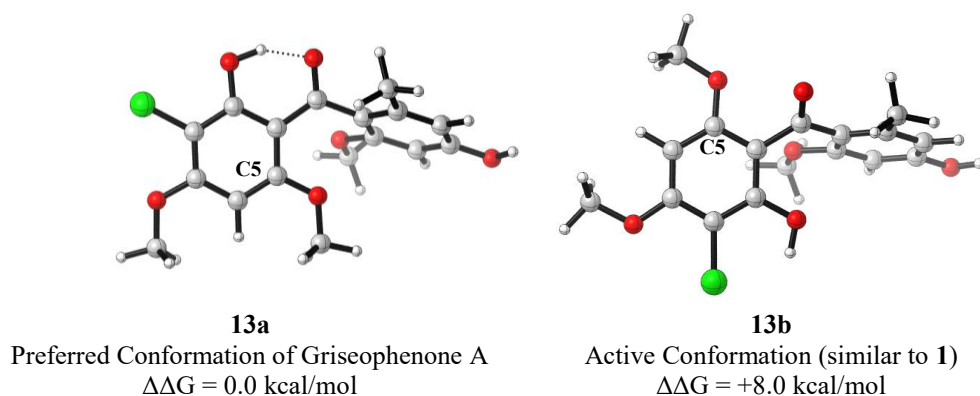


Figure 4.1.6. Conformations and relative free energies of griseophenone A.

Conclusion

A computational investigation of the oxidation of griseophenone B (**1**) to desmethyl-dehydro griseofulvin A (**2**) by a P450 enzyme (GsfF) has been performed. We have determined that the phenolic coupling occurs through initial phenolic O-H abstraction rather than arene oxidation, as shown in Figure 4.1.1. After O-H abstraction, the oxygen radical of ring B can directly attack the neighboring arene to form the spirocycle. This finding is supported docking structures and recent computational investigations by Shaik and co-workers.¹⁰ This type of O-H abstraction is quite facile and is likely a common mode of phenolic oxidation because it avoids the high barriers involved in arene oxide formation.

SUPPORTING INFORMATION

Experimental procedures and details, computational details, and additional figures are given in Appendix E.

Author Contribution and Notes

QM calculations were performed by Grandner. Homology modeling and experimental work was performed by Cacho. Autodock Vina docking was performed by Grandner with the assistance of Blanton Martin. Images of homology modeling and docked poses were made in PyMol.⁴⁰ Images of transition states were made using CYLview.⁴¹

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(29) Energy profile starting with O-H abstraction from ring A is shown in Figure E.S4.

(30) Doublet scan shown in Figure E.S5 and Table E.S3. The C-O bond in **8b** for the quartet state is 1.55 Å in quartet vs 1.45 Å in the doublet state. From the gas phase optimization energies, the **8b** (quartet) is 2.5 kcal/mol lower than **7b-ts** (quartet).

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Chapter 4.2

Unusual Oxidative Cascade Reactions by Cytochrome P450s in the Biosynthesis of Fumagillin

Jessica M. Grandner, Thomas Aunins, Robert D. Giacometti, Yi Tang, K. N. Houk

Introduction

Fumagillin (**1**) is a fungal-derived secondary metabolite.¹ The Tang group identified the *fma* gene cluster and the P450 encoded within the cluster that is responsible for several oxidative steps in the biosynthesis of fumagillin.² Af510 or Fma-P450 performs three sequential oxidative steps in the biosynthetic pathway.³ The mechanisms of the first C-H hydroxylation (**2** to **3**) and last olefin epoxidation (**4** to **5**) in this sequence have been thoroughly investigated.⁴ The second step of the P450 sequence is a unique oxidative C-C cleavage (**3** to **4**) responsible for revealing the core of the metabolite. While the role of this enzyme in the synthesis of the biologically active compound has been elucidated, the mechanism of the oxidative ring-opening has only been postulated.^{3,5} Understanding the mechanism is not only critical to determining synthetic approaches to similarly functionalized cores, but also to understanding possible modes of metabolism by human P450s.

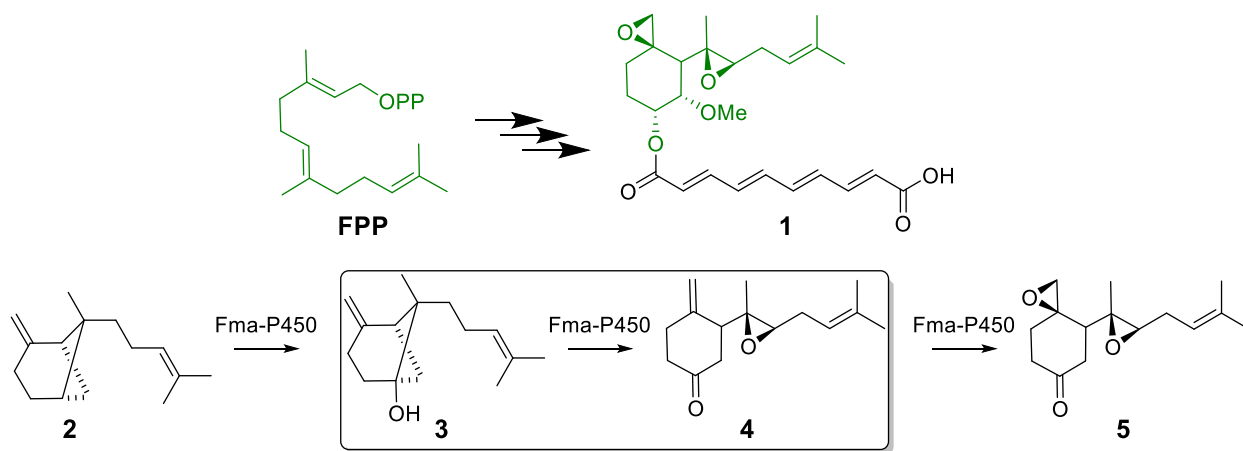


Figure 4.2.1. Fumagillin (**1**) and the role of Fma-P450 in the biosynthesis.

A proposed mechanism for the C-C cleavage is shown in Figure 4.2.2.³ An initial C-H abstraction would lead to radical **6**, which could then be further oxidized to cation **7**. Trapping of the cation by the iron-peroxo intermediate, naturally present in the P450 catalytic cycle, would lead to **8** which could convert to **4** as shown. A modified version of this mechanistic pathway has been proposed and discussed by Ortiz de Montellano.⁶

Path A

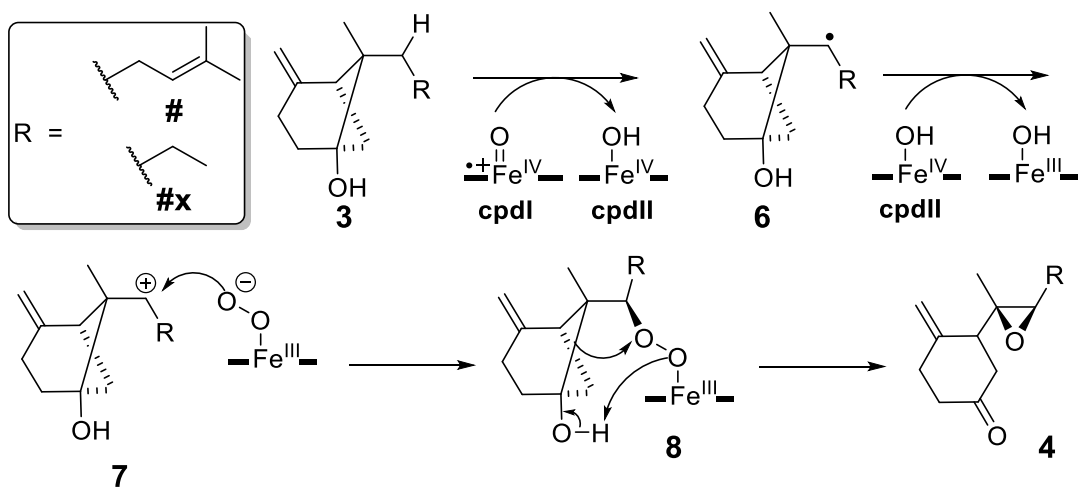


Figure 4.2.2. Proposed mechanism (Path A) of formation of 4 from 3.³ Plain numbers (#) represent the native compounds (R = prenyl group) whereas numbers with an ‘x’ (#x) indicate truncated computational model compounds (R= ethyl).

Alcohols can be converted into higher order oxidized products by P450s.⁷ Computational studies into the mechanisms of these oxidations by Grandner et al.⁸ and Shaik et al.^{9,10} have shown that O-H abstractions are facile for certain substrates and can compete with C-H abstraction barriers. Two alternative mechanisms, (B and C; Figure 4.2.3) begin with an O-H abstraction from the bridgehead hydroxyl of 3.⁵ The oxygen centered radical **9** could cleave the neighboring C-C bridge to form a carbonyl and 3° radical **10**. The two alternative mechanisms diverge from intermediate **10**. In path B, cpdII abstracts a hydrogen next to the radical to form alkene **11**. Subsequent alkene epoxidation would lead to **4**. In C-H oxidation mechanisms, the hydroxyl of **cpdII** rebounds with little or no barrier onto the developing radical.^{4a} A similar rebound may occur onto the 3° radical of **10**, although it would be remote from the site of the initial hydrogen atom abstraction. In path C, a “remote-rebound” onto **10** could occur leading to **12**. Sequential C-H and O-H abstractions, for a net loss of H₂, could then lead to **4**. We now report computational studies

that show which of these mechanisms for conversion of **3** to **4** is most likely. There have been similar experimental studies of such radical-based ring opening reactions by Ortiz de Montellano and coworkers. They used α - and β -thujone as a radical clock to distinguish between radical and cationic mechanisms¹¹ and explored the mechanisms of oxidation by P450_{cam} and P450_{BM3}.¹² Our computational studies will elucidate how P450s can perform complex oxidative ring-opening reactions.

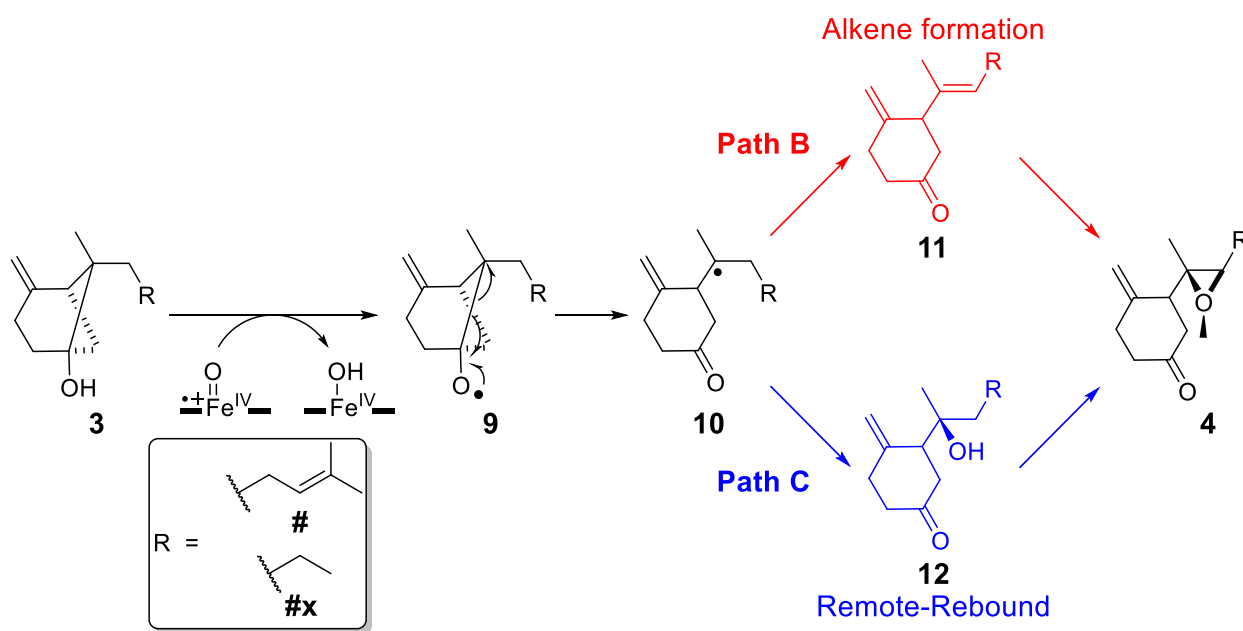


Figure 4.2.3. Proposed O-H abstraction mechanisms for oxidation of **3** to **4**. Plain numbers (#) represent the native compounds (R = prenyl group) whereas numbers with an 'x' (**3x**) indicate truncated computational model compounds (R= ethyl).

Computational Methods

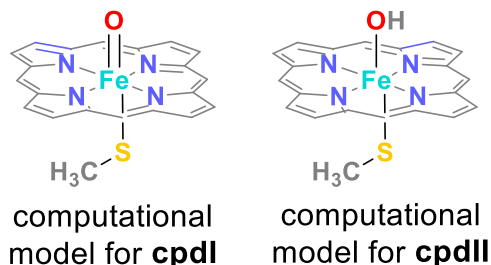


Figure 4.2.4. Computational models used for **cpdI** and **cpdII**

The prenyl group of all relevant compounds was truncated to an ethyl group, as noted in Figures 4.2.2 and 4.2.3. Computational models for **cpdI** and **cpdII** are shown in Figure 4.2.4. Conformational searches of substrate and organic intermediates were performed using the MMFF force field in Spartan.¹³ Conformational searches were not performed on the transition structures or iron-bound intermediates unless otherwise noted. Iron-containing intermediates and transition states were found from IRC calculations, scans, or using the lowest energy conformers of the respective organic compounds. All density functional calculations were performed using Gaussian09.¹⁴ Geometry optimizations and frequency calculations were performed at the B3LYP¹⁵ level with LANL2DZ for Fe and 6-31G(d) for all other atoms. Frequency calculations were used to identify local minima (0 negative frequencies) and transition structures (1 negative frequency). Enthalpies and Gibbs free energies were also computed from frequency calculations at a standard state of 1 atm and 298.15 K. A correction was applied to the Gibbs free energy by adjusting all frequencies below 100 cm⁻¹ to 100 cm⁻¹.¹⁶ Single point energy calculations were performed at the B3LYP-D3(BJ)¹⁷ level with LANL2DZ for Fe and 6-311+G(d,p) for all other atoms. All energies reported within are Gibbs free energies (kcal/mol) unless otherwise noted.

Results and Discussion

We first explored the feasibility of the formation and oxidation of radical **6**. An IRC of the transition state for C-H abstraction (**13x**) shows direct and barrierless formation of the hydroxylated product (**15x**) rather than a radical intermediate (**14x**) (Figure 4.2.5). Radical **6x** does not form as a result of C-H abstraction in the gas phase and thereby does not lead to product by path A. If the enzyme could somehow stop this barrierless rebound, oxidation of **6** would require the reduction of **cdpII**. According to Rydberg et al., the reduction potential of **cdpII** is -2.11 V (+48.7 kcal/mol) in vacuum and -0.15 V (+3.5 kcal/mol) in water.¹⁸ Lastly, as shown in Figure 4.2.6, attempts to optimize most conformers of cation **7x** led to cleavage of one of the bridging bonds to form a more stable oxonium (**16x**) or allylic radical (**17x**).¹⁹ These data indicate that C-H abstraction leads to formation of the hydroxylated shunt product, but not to the observed product **4**.

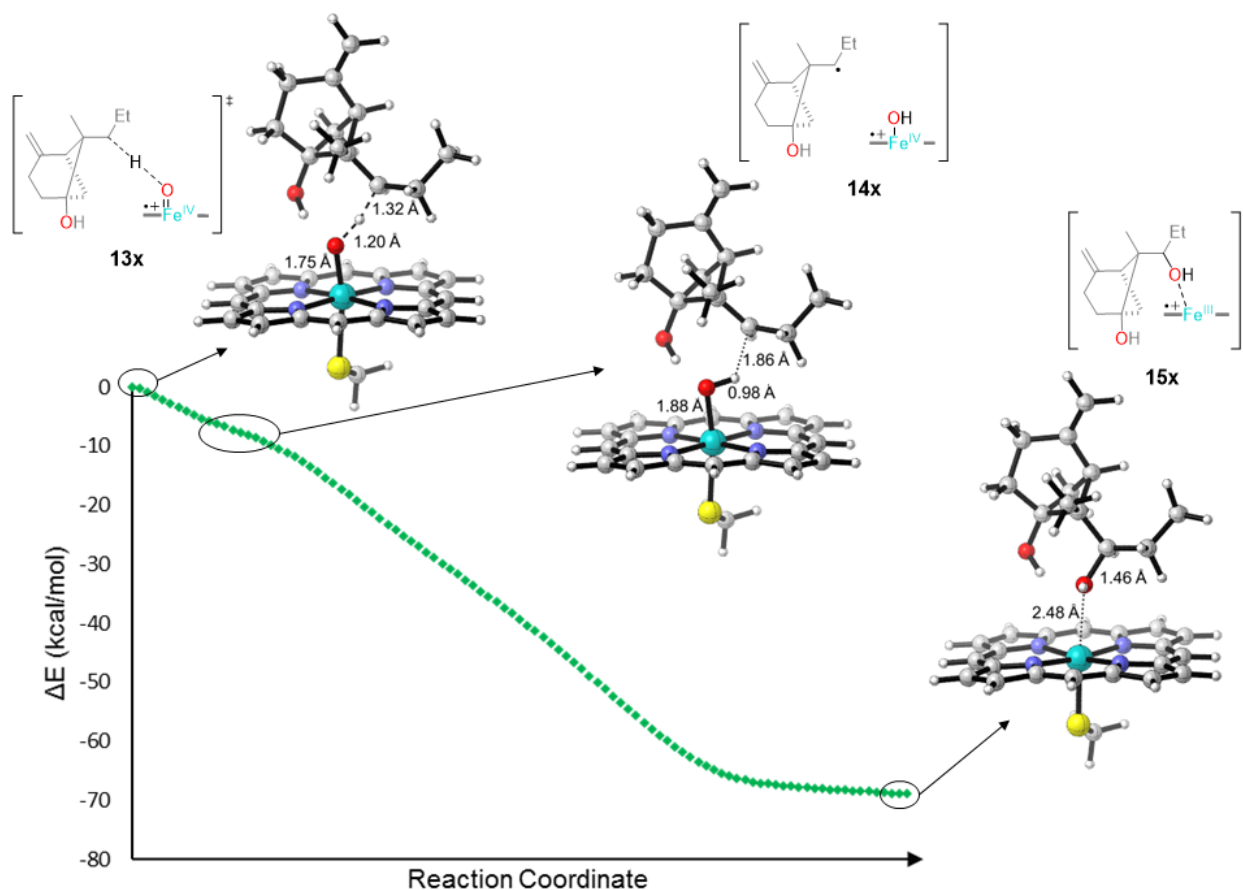


Figure 4.2.5. Intrinsic reaction coordinate (IRC) diagram from the C-H abstraction transition state **13x**.

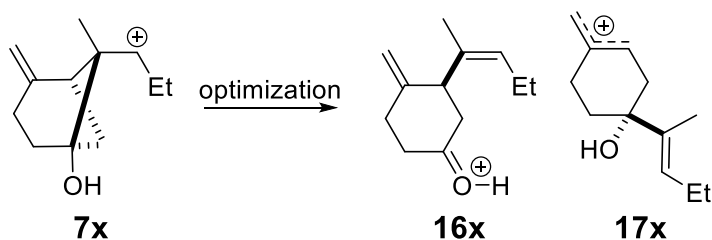


Figure 4.2.6. Optimization of cation **7x** does not maintain bridgehead bonds and instead leads to oxonium **16x** or allylic cation **17x**.

The barrier to O-H abstraction from **3x** is 14.0 kcal/mol. An IRC of the transition state reveals that O-H abstraction and C-C cleavage are concerted, albeit highly asynchronous. The free

energy diagrams for paths B and C are shown in Figures 4.2.7 and 4.2.8, respectively. A transition state for the remote rebound (**18x** to **10x**) could not be obtained. In both pathways, O-H abstraction with simultaneous C-C cleavage is either rate-limiting or isoenergetic with subsequent steps. It is reasonable to expect that no intermediates along these pathways will be observed. Additionally, since the two pathways have identical rate-determining steps, either or both mechanisms could be operative within Fma-P450.

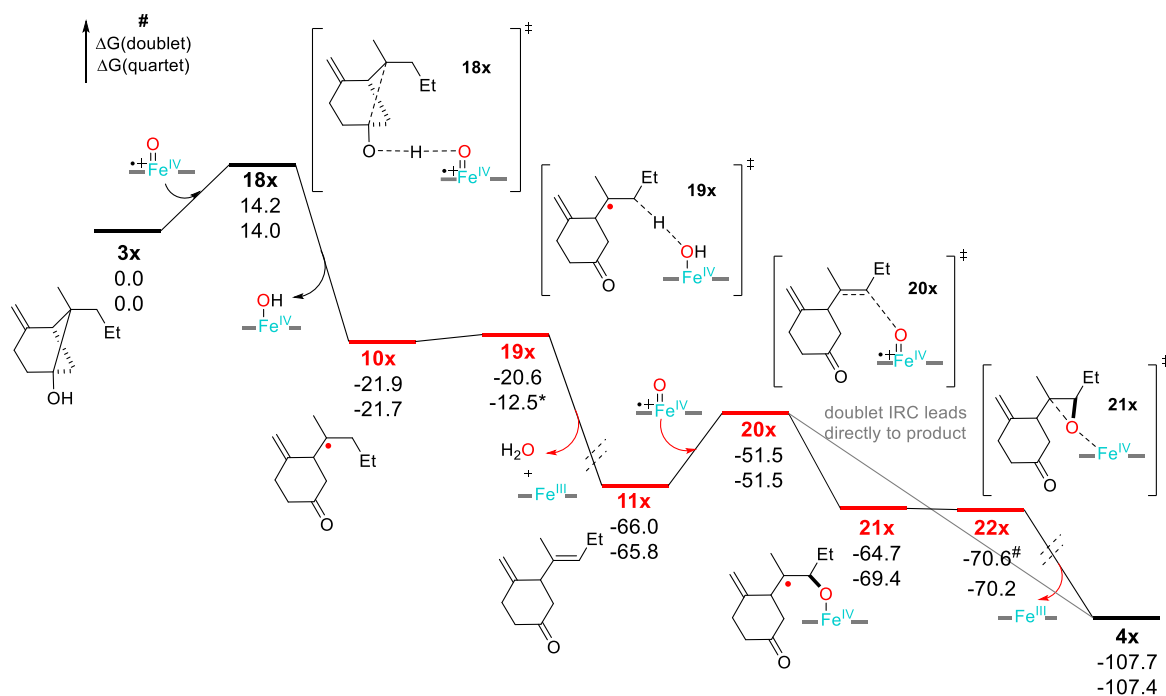


Figure 4.2.7. Free energy diagram for Path B involving alkene formation. Numbers with '*' are quartet single points on doublet geometries. Numbers with '#' are doublet single points on quartet geometries.

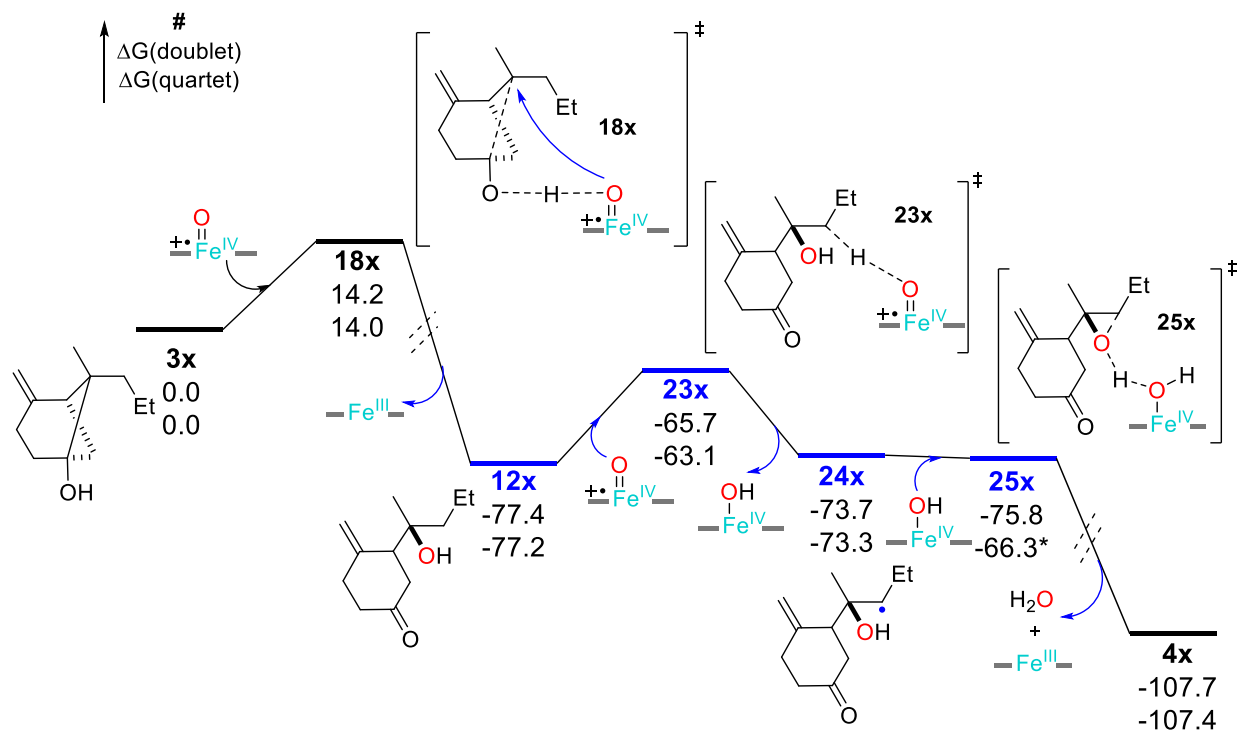


Figure 4.2.8. Free energy diagram for Path C involving remote-rebound. Numbers with ‘*’ are quartet single points on doublet geometries.

Previous experimental studies Rettie et al.²⁰ and Guan et al.²¹ have shown that P450s are capable of desaturation. Jennewein et al. found that a P450 catalyzes a remote-rebound onto an intermediate in the biosynthesis of Taxol.²² The radical clock studies by Ortiz de Montellano and coworkers have demonstrated both mechanisms can be performed on a single substrate.¹²

The mechanism for epoxidation in Path C is an unusual proposal but energetically feasible.²³ As shown in Figure 4.2.5, **13x** avoids formation of the radical intermediate and leads directly to the hydroxylated product **15x**. Alternatively, the IRC of **23x** (Figure 4.2.9) reveals that rebound is not spontaneous and a radical intermediate is formed. The computed barrier to rebound from **24x**, to form a syn-diol, is 1.2 kcal/mol higher in energy than **25x**.²⁴ There is a hydrogen bond

between the hydroxyl of **24x** and compound II, shown in Figure 4.2.9, which likely raises the barrier to rebound and increases the probability of competitive O-H abstraction.

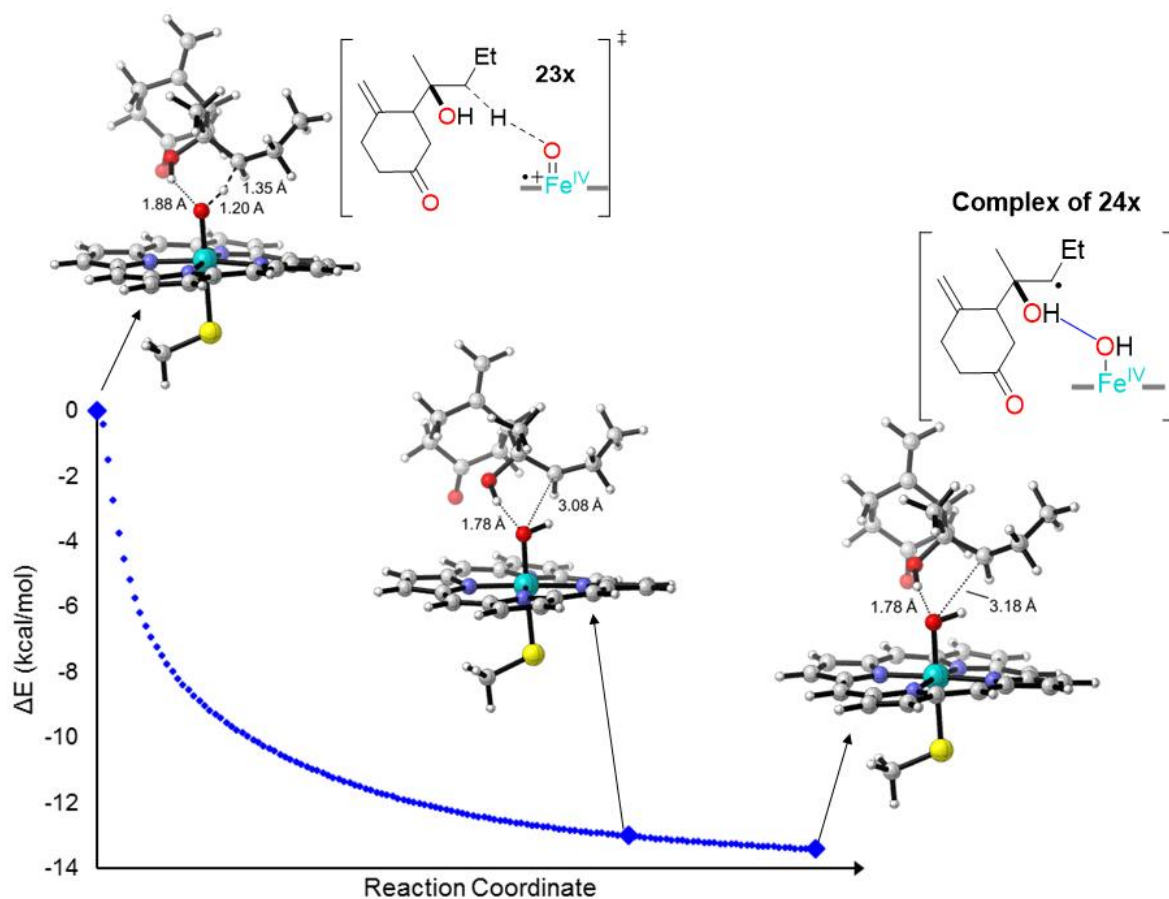


Figure 4.2.9. IRC of **23x** which shows that rebound is not spontaneous.

Conclusion

In conclusion, we have determined that the Fma-P450-catalyzed ring-opening oxidation in the biosynthesis of fumagillin begins with an atypical O-H abstraction. This O-H abstraction causes the spontaneous ring-opening of the bicycle to reveal the carbocyclic core of fumagillin. There are two mechanistic possibilities that could occur following the ring opening: 1) alkene formation and epoxidation or 2) remote-rebound and C-H/O-H abstraction. These findings

illustrate and support the novel mechanisms outside of C-H abstraction which can be performed by P450s.

Supporting Information

Additional figures are given in Appendix F.

Acknowledgements

All 3-dimensional images were made using CYLview.²⁵

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Appendix A

Supporting Information for Chapter 1.1

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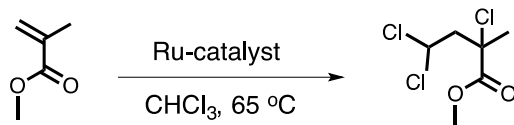
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MATERIALS AND ANALYTICAL TECHNIQUES

All reactions were carried out in dry vials with PTFE-faced silicone septum under an argon (Ar) atmosphere or in a Vacuum Atmospheres Glovebox under a nitrogen atmosphere, as specified. All solvents and reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. Fresh ampules of CDCl₃ (Sigma-Aldrich) were used in decomposition experiments of the ruthenium benzylidene catalysts. Complexes **1**, **2**, **3**, **6**, and **7** were generously donated by Materia, Inc. Complexes **4** and **5** were prepared from **2** and **3**, respectively, following a literature procedures.^{1,2} ¹H NMR spectra were recorded on one of the following instruments: Varian Mercury (300 MHz), Varian Inova (500 MHz), or Bruker Ascend with Prodigy broadband cryoprobe (400 MHz). Gel permeation chromatography (GPC) was conducted on two Agilent PLgel 10 μm MIXED-BLS 300 mm × 7.5 mm columns with Agilent P260 series pump and autosampler with Wyatt Dawn Heleos-II multi-angle static light scattering detector and Optilab T-rEX differential refractive index detector with THF as an eluent.

EXPERIMENTAL PROCEDURES

General procedure for ATRA catalyzed by ruthenium benzylidene complexes.



$$[\text{MMA}] : [\text{CHCl}_3] : [\text{catalyst}] = 1 : 10 : 0.075$$

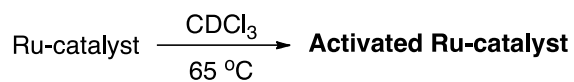
To a 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 0.75×10^{-1} mmol), MMA (106.84 μL , 9.99×10^{-1} mmol), and 0.8 mL of CHCl_3 (0.8 mL, 9.98 mmol) were added. Anisole (10 μL , 9.20×10^{-2} mmol) was added as an internal standard. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to the specified temperature ($65\text{ }^\circ\text{C}$ or $40\text{ }^\circ\text{C}$). The reaction was kept under Ar (g), and aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. After 2 h, the solution was precipitated into petroleum ether and filtered to remove precipitated catalyst. Solvent and unreacted MMA were dried using rotary evaporator. The yield of the product was calculated using ^1H NMR integration at 6.01 ppm ($-\text{CCl}_2\text{H}$ from the product) and 1.84 ppm ($-\text{CH}_3$ from the product and by-products). All of the ATRA reactions in this report were performed following this general procedure using the same molar ratio of [catalyst] : [MMA] : [CHCl_3].

Computed ΔG_{rxn} (kcal/mol) for ATRA reactions.

Geometry optimizations and frequency calculations were performed at the (U)B3LYP level³⁻⁵ using LANL2DZ for Ru and 6-31G(d) on all other atoms. Thermal corrections were calculated from vibrational frequencies using a standard state of 1 atm and 298.15 K. All

frequencies below 100 cm⁻¹ were raised to 100 cm⁻¹ to correct entropies for the breakdown of the harmonic oscillator approximation as discussed by Truhlar.⁶ Subsequent single point energy calculation were performed at the (U)M06-L⁷ level using SDD for Ru and 6-311+G(d,p) on all other atoms and including the SMD⁸ (chloroform) solvent model. All calculations were performed using Gaussian 09.⁹

Decomposition study of ruthenium benzylidene complexes.



$$[\text{CDCl}_3] : [\text{catalyst}] = 10 : 0.075$$

Inside the glovebox, an NMR tube was charged with the ruthenium complex and CDCl₃ in the same molar ratio as specified in the general ATRA procedure. The NMR tube was capped with a septum, removed from the glovebox, and heated at 65 °C. ¹H NMR spectra were collected at predetermined time points, and the integral of the benzylidene resonance (16–20 ppm, 1H) was plotted as a function of time.

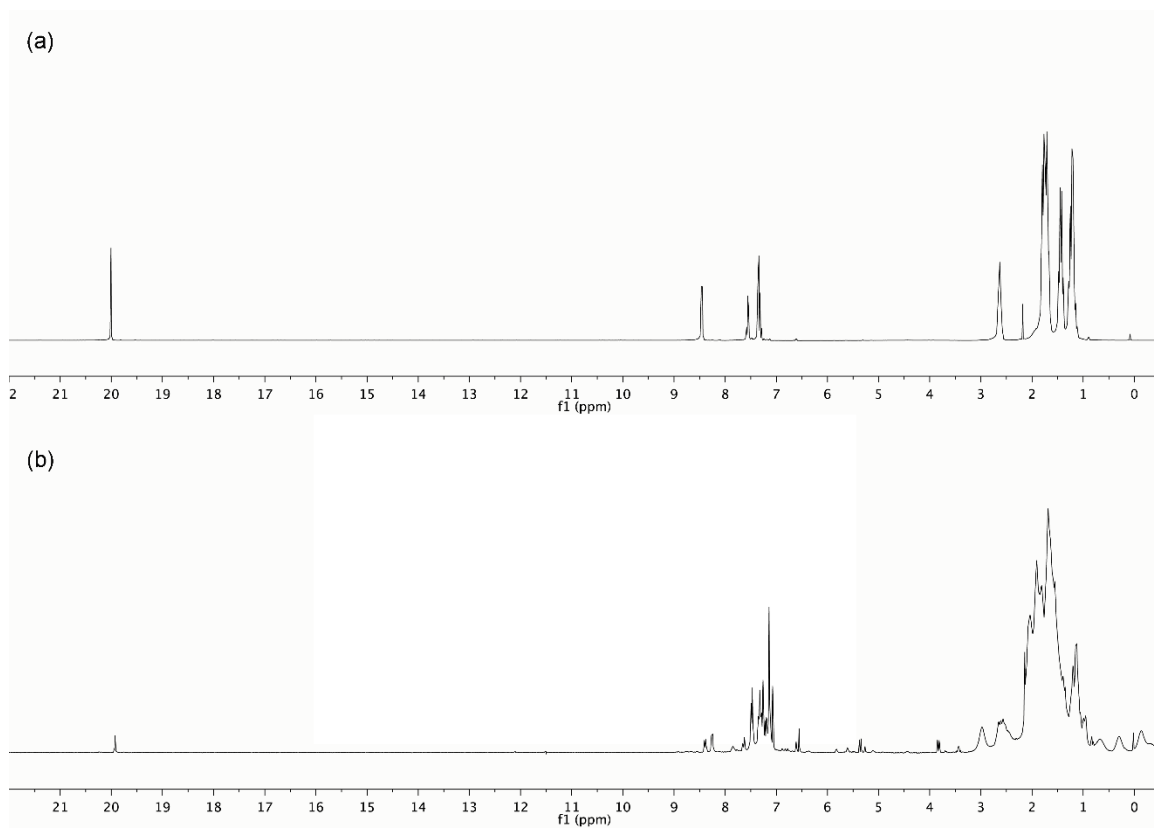


Figure A.S2. ^1H NMR spectra of **1** at (a) 0 min and (b) 200 min after activation in CDCl_3 at 65°C .

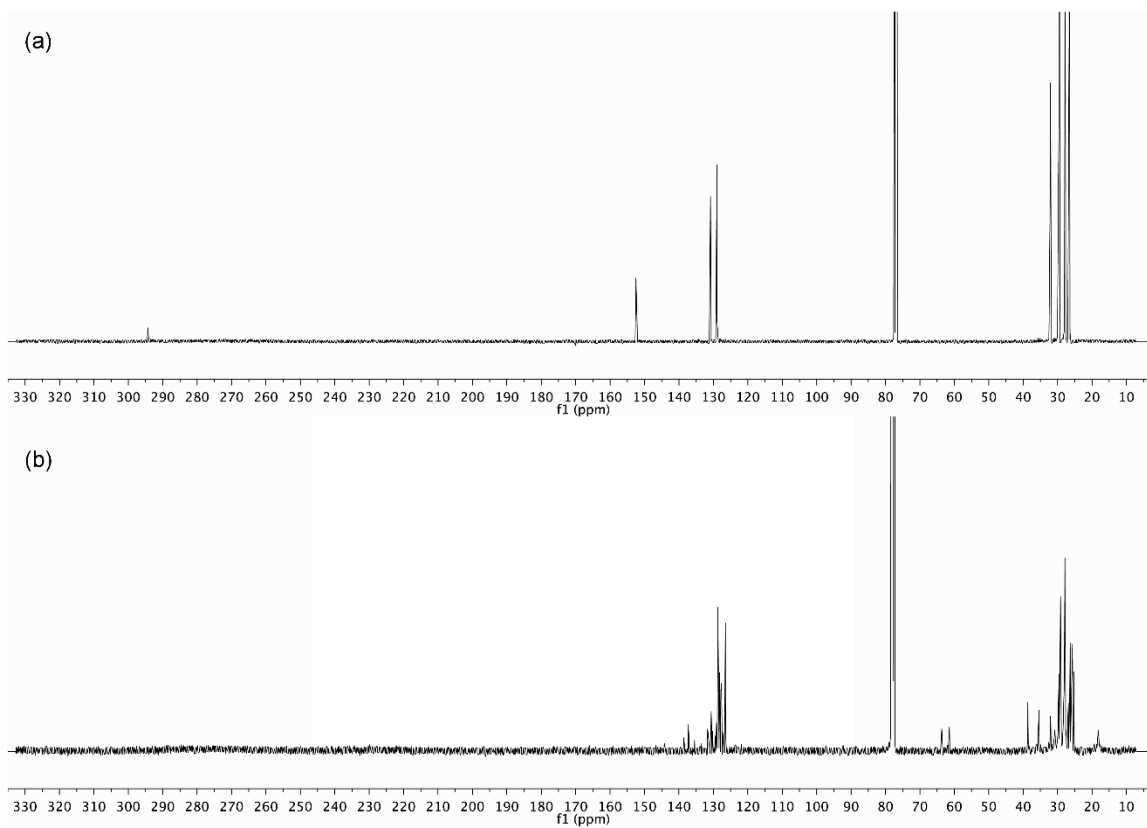


Figure A.S3. ^{13}C NMR spectra of **1** at (a) 0 min and (b) 200 min after activation in CDCl_3 at 65°C .

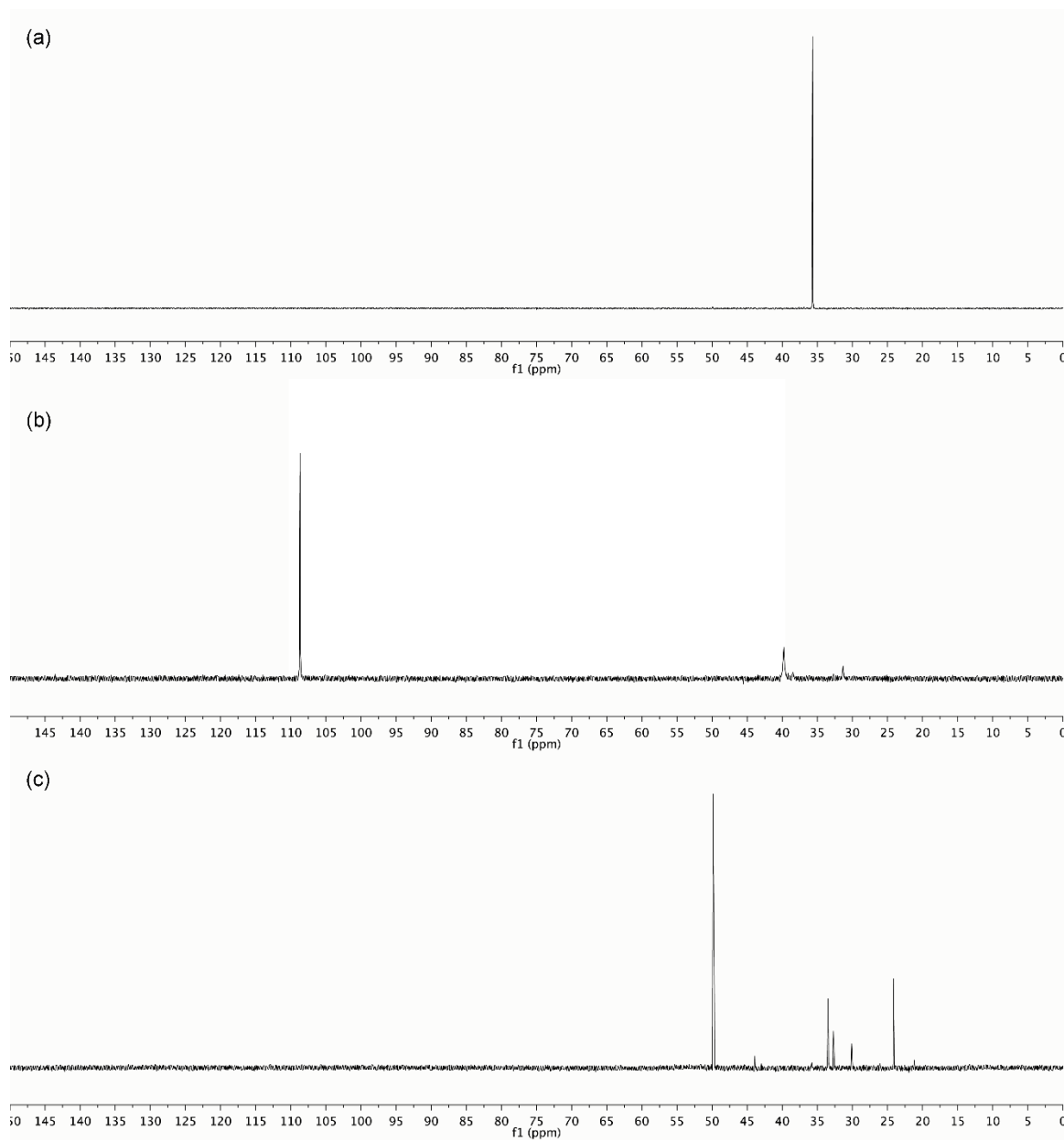


Figure A.S4. ^{31}P NMR spectra of **1** at (a) 0 min, (b) 200 min after activation in CDCl_3 at 65°C , and (c) bipy added after the activation.

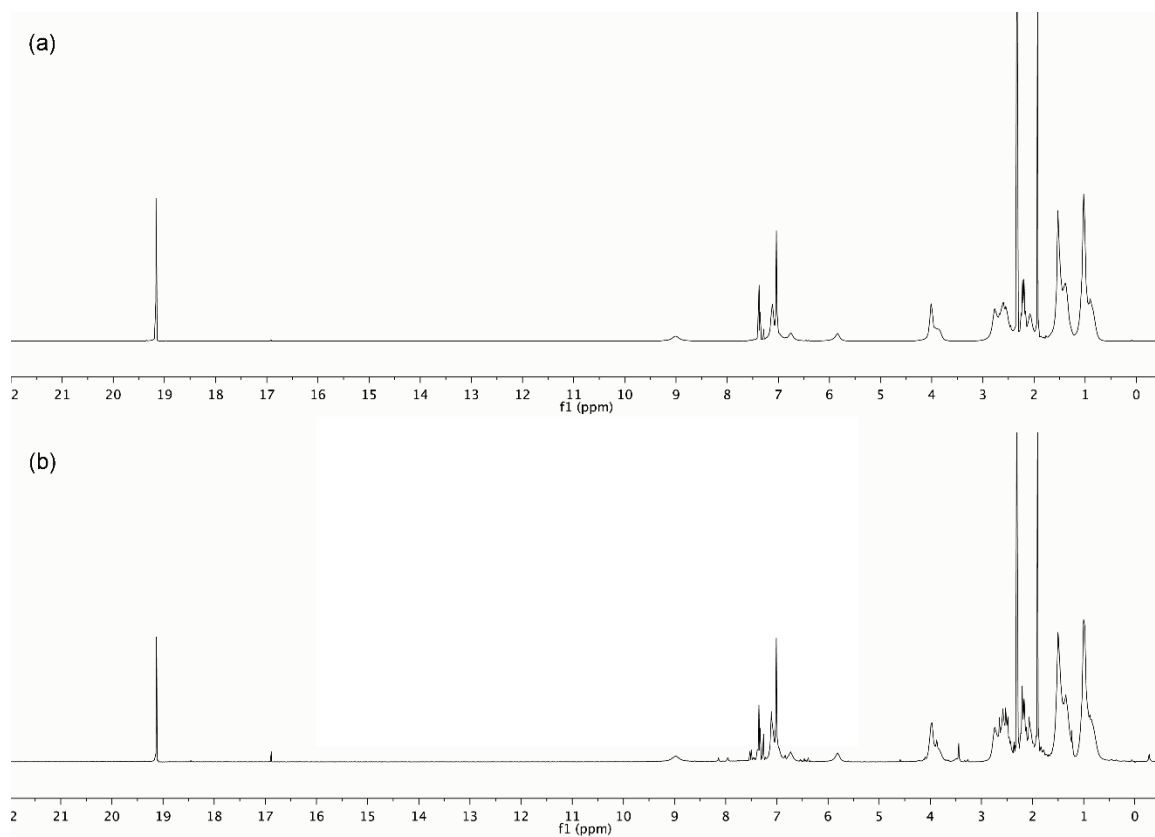


Figure A.S5. ^1H NMR spectra of **2** at (a) 0 min and (b) 270 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

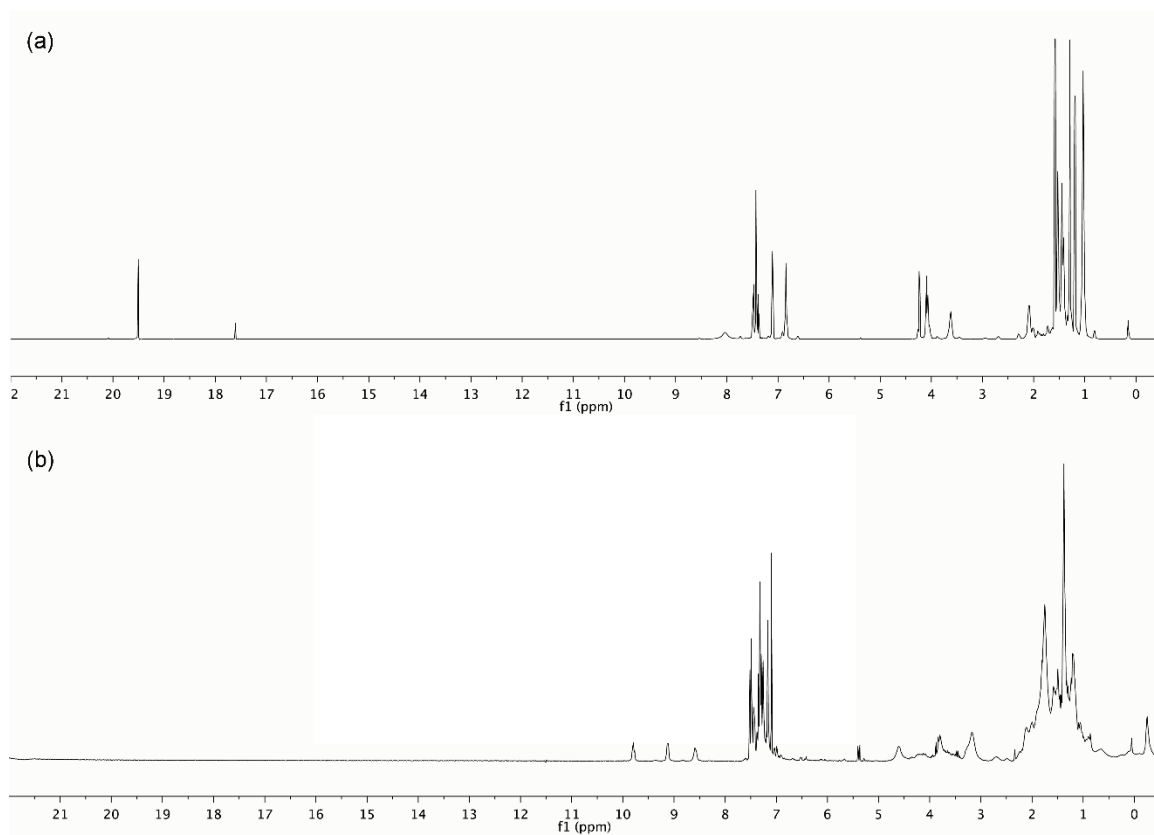


Figure A.S6. ^1H NMR spectra of **3** at (a) 0 min and (b) 60 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

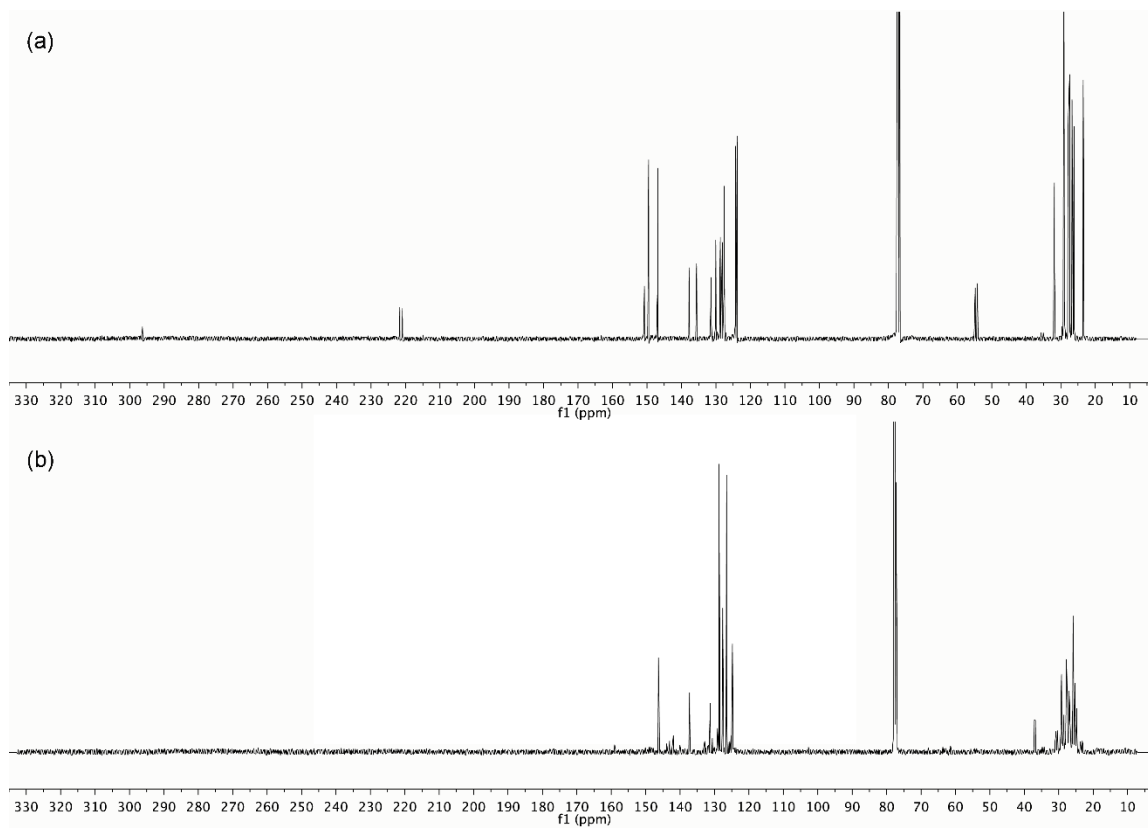


Figure A.S7. ^{13}C NMR spectra of **3** at (a) 0 min and (b) 60 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

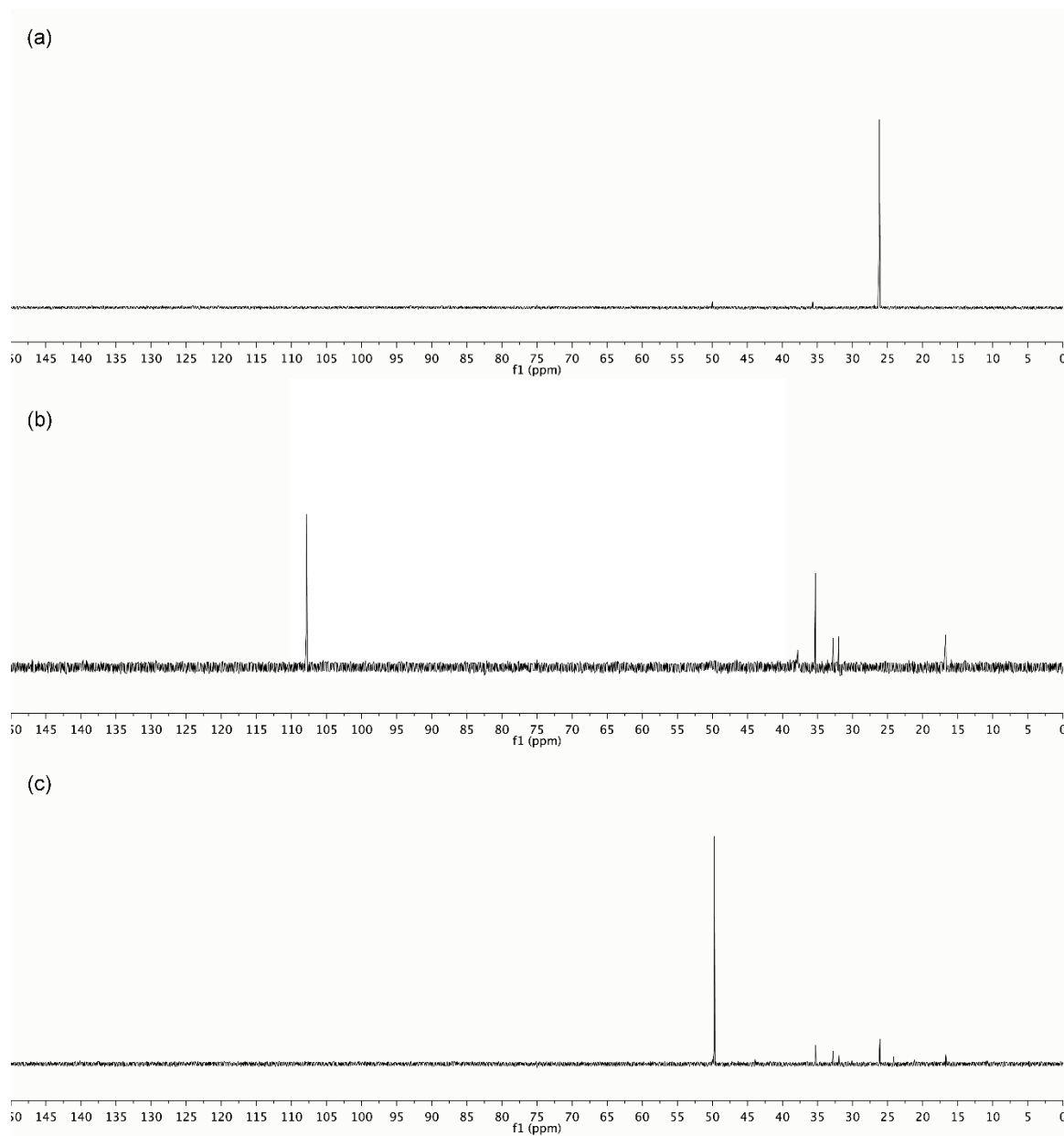


Figure A.S8. ^{31}P NMR spectra of **3** at (a) 0 min and (b) 60 min after activation in CDCl_3 at 65 $^\circ\text{C}$, and (c) bipy added after the activation.

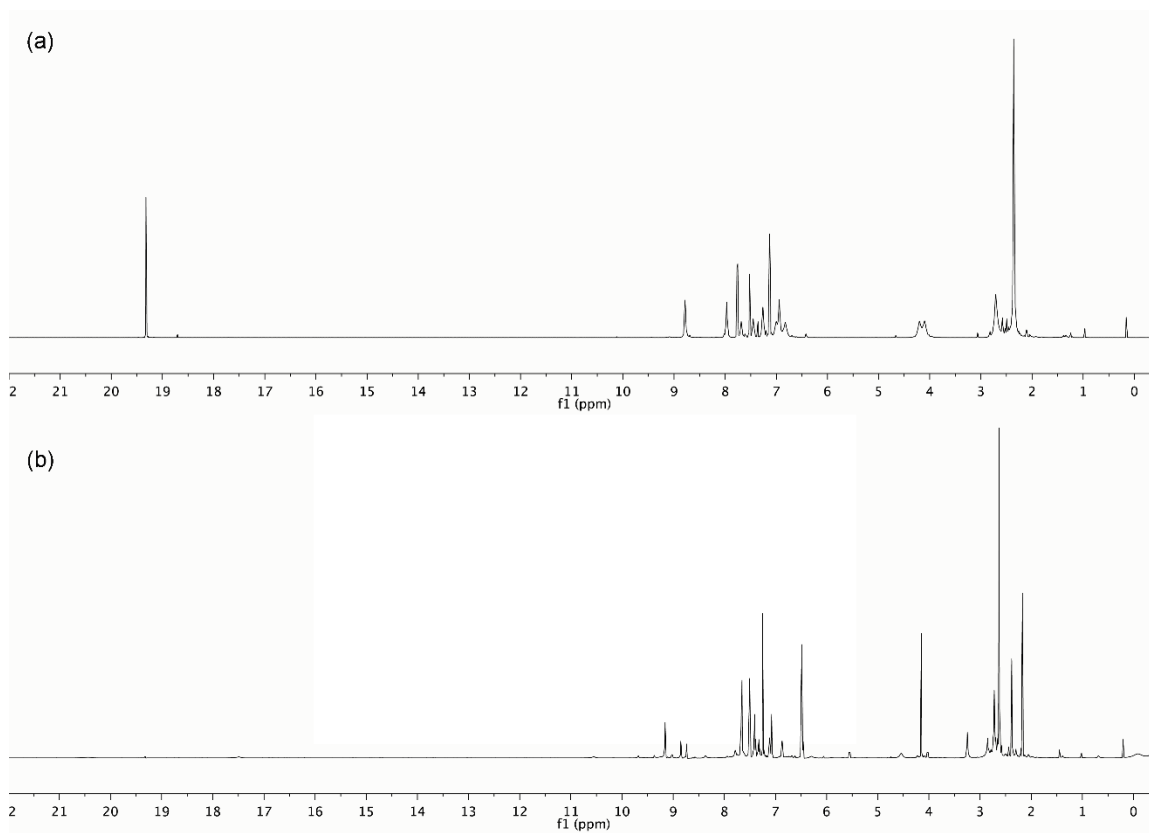


Figure A.S9. ^1H NMR spectra of **4** at (a) 0 min and (b) 270 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

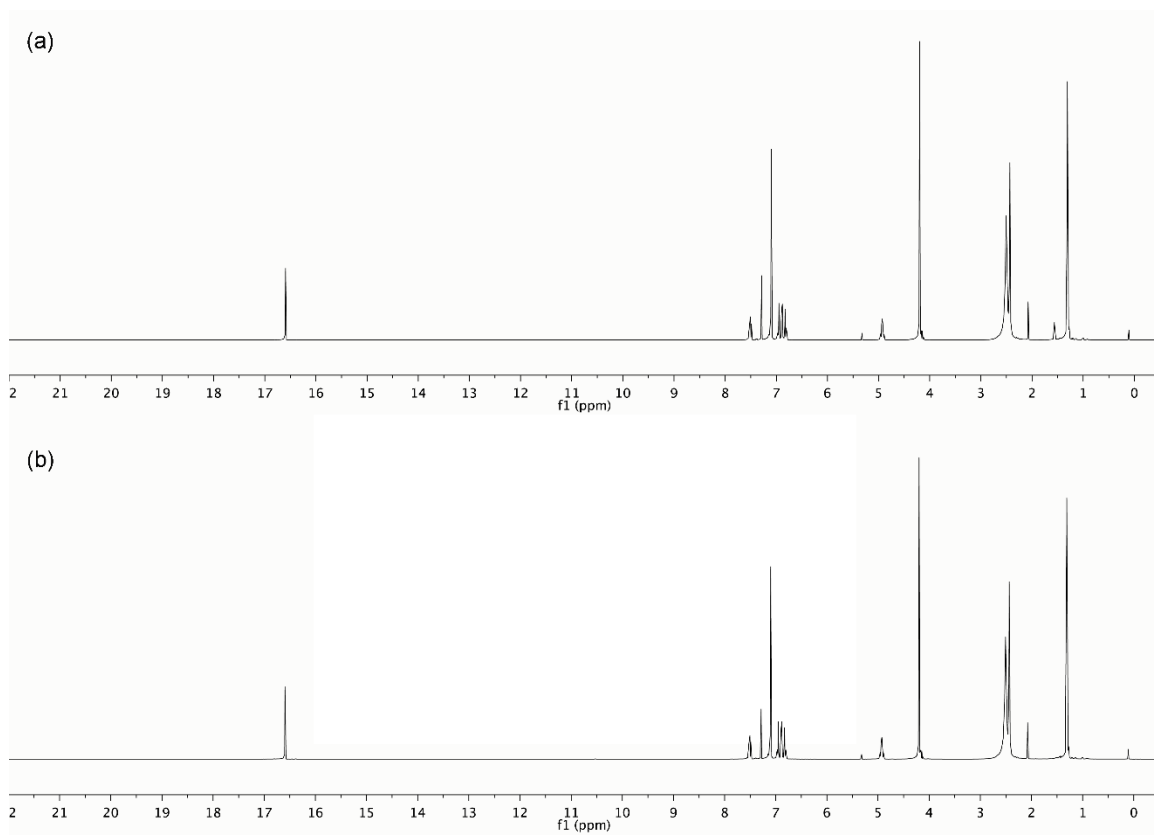


Figure A.S10. ^1H NMR spectra of **5** at (a) 0 min and (b) 270 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

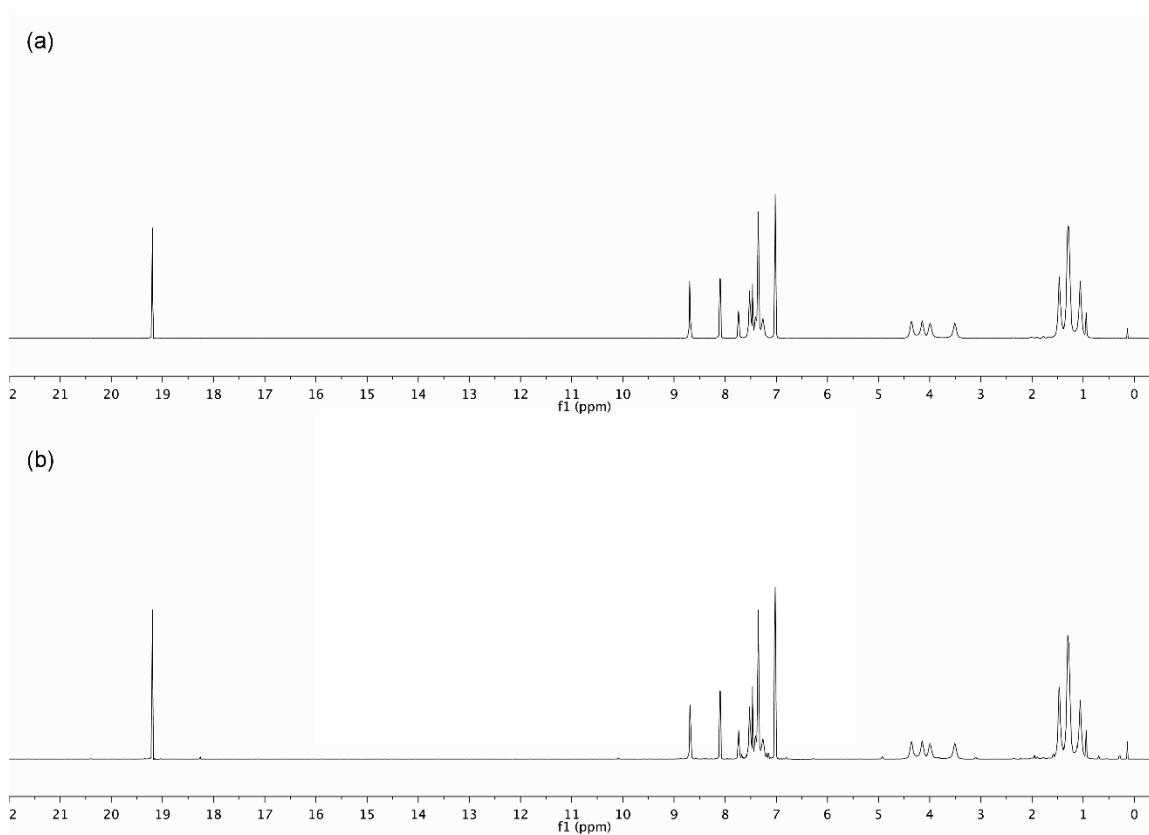


Figure A.S11. ^1H NMR spectra of **6** at (a) 0 min and (b) 270 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

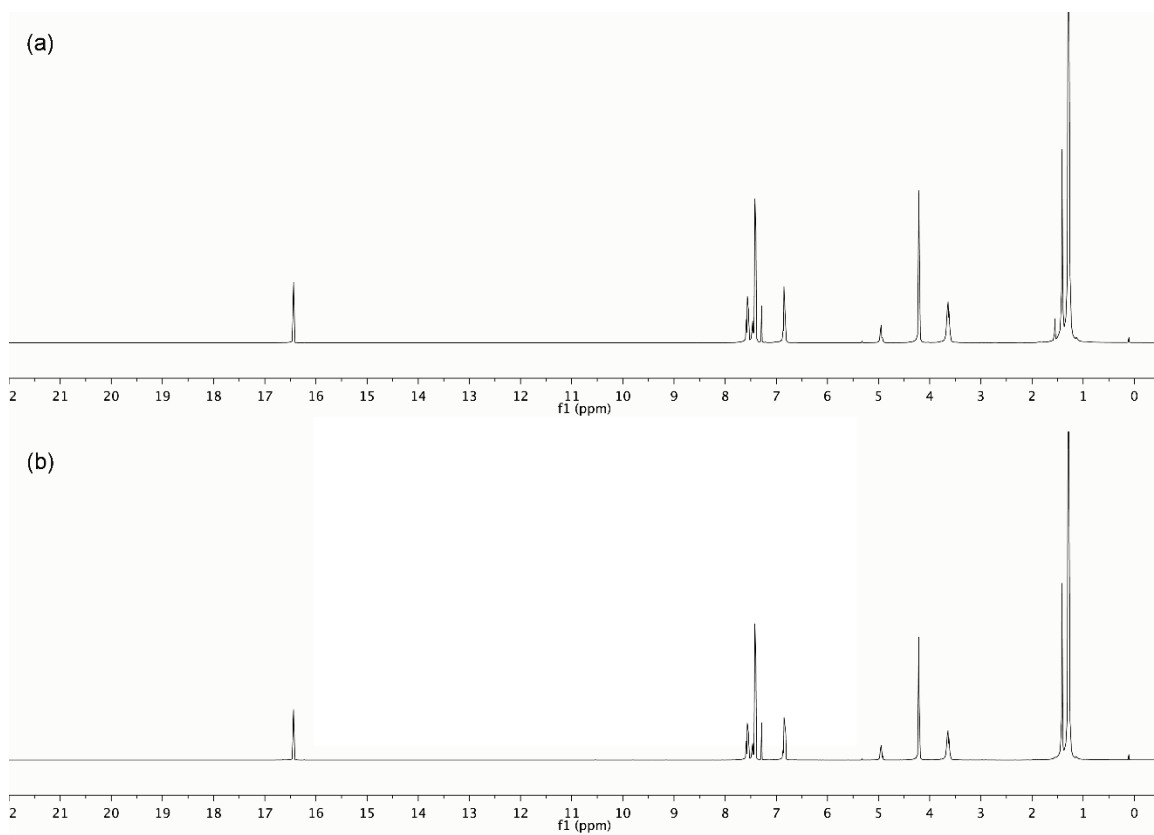
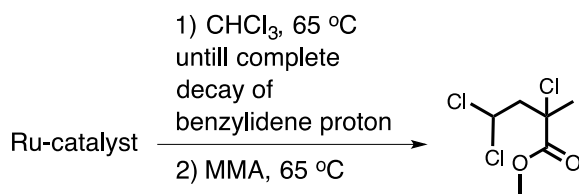


Figure A.S12. ^1H NMR spectra of **7** at (a) 0 min and (b) 270 min after activation in CDCl_3 at 65 $^\circ\text{C}$.

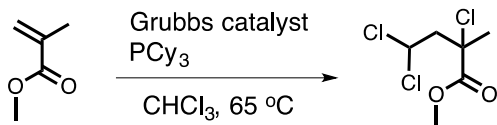
General procedure for ATRA catalyzed by activated ruthenium complexes.



$$[\text{MMA}] : [\text{CHCl}_3] : [\text{catalyst}] = 1 : 10 : 0.075$$

To a 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 0.75×10^{-1} mmol), anisole (10 μL , 9.20×10^{-2} mmol), and CHCl_3 (0.8 mL, 9.98 mmol) were added. The solution was degassed with Ar (g) for 10 min and then heated at 65 $^{\circ}\text{C}$ until complete disappearance of benzylidene ^1H NMR resonance. The reaction vessel was allowed to cool to room temperature, and freshly degassed MMA (106.84 μL , 9.99×10^{-1} mmol) was added to the solution. The reaction was initialized by immersing the reaction vessel into an oil bath preheated to the specified temperature (65 $^{\circ}\text{C}$ or 40 $^{\circ}\text{C}$) and was held under an Ar (g) atmosphere. Aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. All of the ATRA reactions in this report with pre-activated ruthenium benzylidene complexes were performed following this general procedure using identical concentrations.

General procedure for ATRA catalyzed by ruthenium benzylidene complexes with 5 equivalent of PCy_3 .



$$[\text{MMA}] : [\text{CHCl}_3] : [\text{catalyst}] : [\text{PCy}_3] = 1 : 10 : 0.075 : 0.375$$

To a 8 mL vial with silicone septum cap equipped with a magnetic stir bar, complex **1** (62 mg, 7.53×10^{-2} mmol), MMA (106.84 μ L, 9.99×10^{-1} mmol), anisole (10 μ L, 9.20×10^{-2} mmol) and CHCl_3 (0.8 mL, 9.98 mmol) were added. PCy_3 (105.64 mg, 3.77×10^{-1} mmol) was then added, and the solution as degassed with Ar (g) for 10 min. The reaction was initialized by immersing the reaction vessel into an oil bath preheated to 65 °C and was held under an Ar (g) atmosphere. Aliquots were removed at predetermined time points and analyzed by ^1H NMR to monitor reaction progress over time. Experiments with **2** and **3** were performed following this general procedure using identical concentrations and reaction conditions.

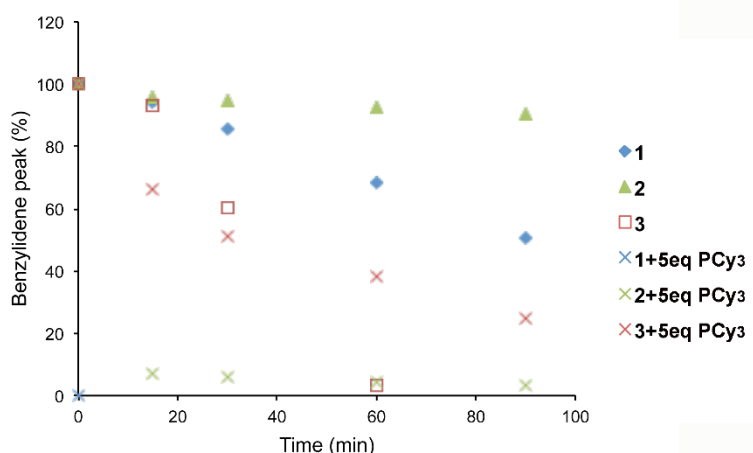
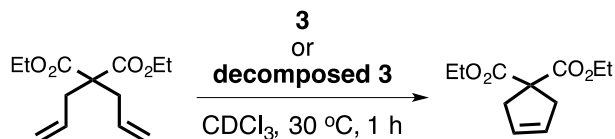


Figure A.S13. Rate profile of benzylidene ^1H NMR resonance decay in CDCl_3 with 5 equivalent PCy_3 relative to the catalysts.

RCM catalyzed by 3 and benzylidene-decomposed (ATRA-activated) 3.



The reaction was performed following a literature procedure.¹⁰ Complex **3** (7.47 mg, 8.01×10^{-3} mmol) was dissolved in degassed CDCl_3 (0.75 mL). For reactions catalyzed by decomposed **3**, the solution was then pre-treated at 65 °C until the indicated level of decay of benzylidene ^1H NMR resonance was observed. After preparing the appropriate catalyst solution, the solution was cool to room temperature and diethyl diallylmalonate (19.3 μL , 7.98×10^{-2} mmol) was added and a ^1H NMR spectrum was collected after 1 h at 30 °C to calculate olefin conversion.

Crystallization of ATRA-activated 1 with bipy.

Complex **1** (62 mg, 0.75×10^{-1} mmol) was dissolved into 0.8 mL of CHCl_3 (0.8 mL, 9.98 mmol) and activated by heating at 65 °C until the complete decay of the benzylidene peak from ^1H NMR was observed. The solvent was dried and powder was re-dissolved into minimal amount of DCM with addition of bipy (58.83 mg, 0.38 mmol). Pentane was added for the crystallization at room temperature.

X-Ray structure determination.

Low-temperature diffraction data (ϕ - and ω -scans) were collected on a Bruker AXS D8 VENTURE KAPPA diffractometer coupled to a PHOTON 100 CMOS detector with Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) from an $\text{I}\mu\text{S}$ micro-source for the structure of compound ATRA-activated **1** with bipy. The structure was solved by direct methods using SHELXS¹¹ and refined against F^2 on all data by full-matrix least squares with SHELXL-2014¹² using established refinement techniques.¹³ All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms they are linked to (1.5 times for methyl

groups). All disordered atoms were refined with the help of similarity restraints on the 1,2- and 1,3-distances and displacement parameters as well as enhanced rigid bond restraints for anisotropic displacement parameters. The compound crystallizes in the triclinic space group *P*-1 with one molecule in the asymmetric unit along with 3.3 molecules of dichloromethane. Two of the cyclohexane moieties on the phosphine were disordered over two positions with appropriate restraints. The dichloromethane solvent is located in two cavities. One was modeled as a mixture of three solvent positions and the other over four positions.

Table A.S1. Crystal data and structure analysis details for *of* ATRA-activated 3 with bipy (CCDC 1473173).

Identification code	P15507	
Empirical formula	C41.26 H55.51 Cl8.51 N4 P Ru	
Formula weight	1041.00	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.1461(8) Å	a = 95.620(3)°.
	b = 13.3816(11) Å	b = 94.475(3)°.
	c = 18.3452(14) Å	g = 104.537(3)°.
Volume	2385.8(3) Å ³	
Z	2	
Density (calculated)	1.449 Mg/m ³	
Absorption coefficient	0.872 mm ⁻¹	
F(000)	1069	
Crystal size	0.100 x 0.100 x 0.100 mm ³	
Theta range for data collection	2.244 to 30.603°.	
Index ranges	-14 ≤ h ≤ 11, -19 ≤ k ≤ 18, -26 ≤ l ≤ 26	
Reflections collected	54887	
Independent reflections	14596 [R(int) = 0.0377]	

Completeness to theta = 25.242°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7461 and 0.6938
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	14596 / 1155 / 719
Goodness-of-fit on F ²	1.047
Final R indices [I>2sigma(I)]	R1 = 0.0597, wR2 = 0.1609
R indices (all data)	R1 = 0.0807, wR2 = 0.1749
Extinction coefficient	n/a
Largest diff. peak and hole	1.869 and -0.896 e.Å ⁻³

ATRA catalyzed by Ru(III)Cl₃ and PCy₃ complex.

First, 0.8 mL MeOH was added to Ru(III)Cl₃ (15.54 mg, 0.75×10⁻¹ mmol) and PCy₃ (42.02 mg, 1.50×10⁻¹ mmol) and refluxed overnight. The solution was then dried and filtered with benzene and filtrate was dried again. To the dried powder MMA (106.84 μL, 9.99×10⁻¹ mmol) and 0.8 mL of CHCl₃ (0.8 mL, 9.98 mmol) were added. Anisole (10 μL, 9.20×10⁻² mmol) was added as an internal standard. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to 65 °C.

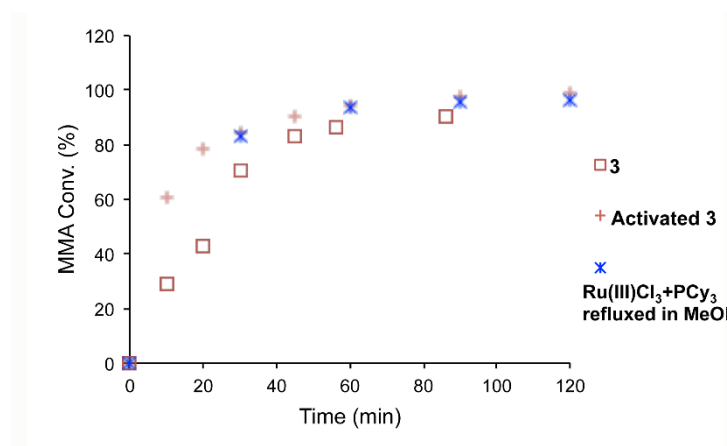
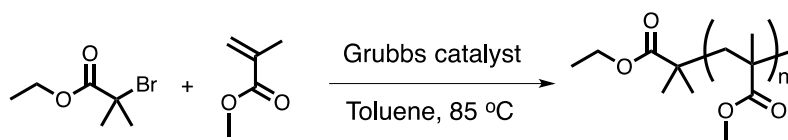


Figure A.S14. Kinetic study of ATRA of MMA catalyzed by **3**, **activated 3**, and Ru(III)Cl₃ refluxed with PCy₃ (2 equiv).

ATRP catalyzed by ruthenium benzylidene complexes.



[Initiator] : [MMA] : [catalyst] = 1 : 200 : 1

0.02 M Initiator in toluene

To a 8 mL vial with silicone septum cap equipped with a magnetic stir bar, ethyl α -bromoisobutyrate (10 μ L, 6.81×10^{-5} mmol), MMA (1.46 mL, 1.36×10^{-2} mmol), and complex **1** (56.07 mg, 6.81×10^{-5} mmol) were added and dissolved into toluene (681 μ L). Anisole (10 μ L) was added as an internal standard. The solution was degassed with Ar (g) for 10 min, and the reaction was initialized by immersing the reaction vessel into an oil bath preheated to 85 $^\circ$ C. Aliquots were removed at predetermined time points and analyzed by ^1H NMR and GPC to monitor, MMA conversion, M_n , and dispersity ($\mathcal{D}$) over time. All of the ATRP reactions in this report were performed following this same general procedure under identical reaction conditions.

Initial Mechanistic Studies of ATRA/ATRP with W, X, Y, and Z

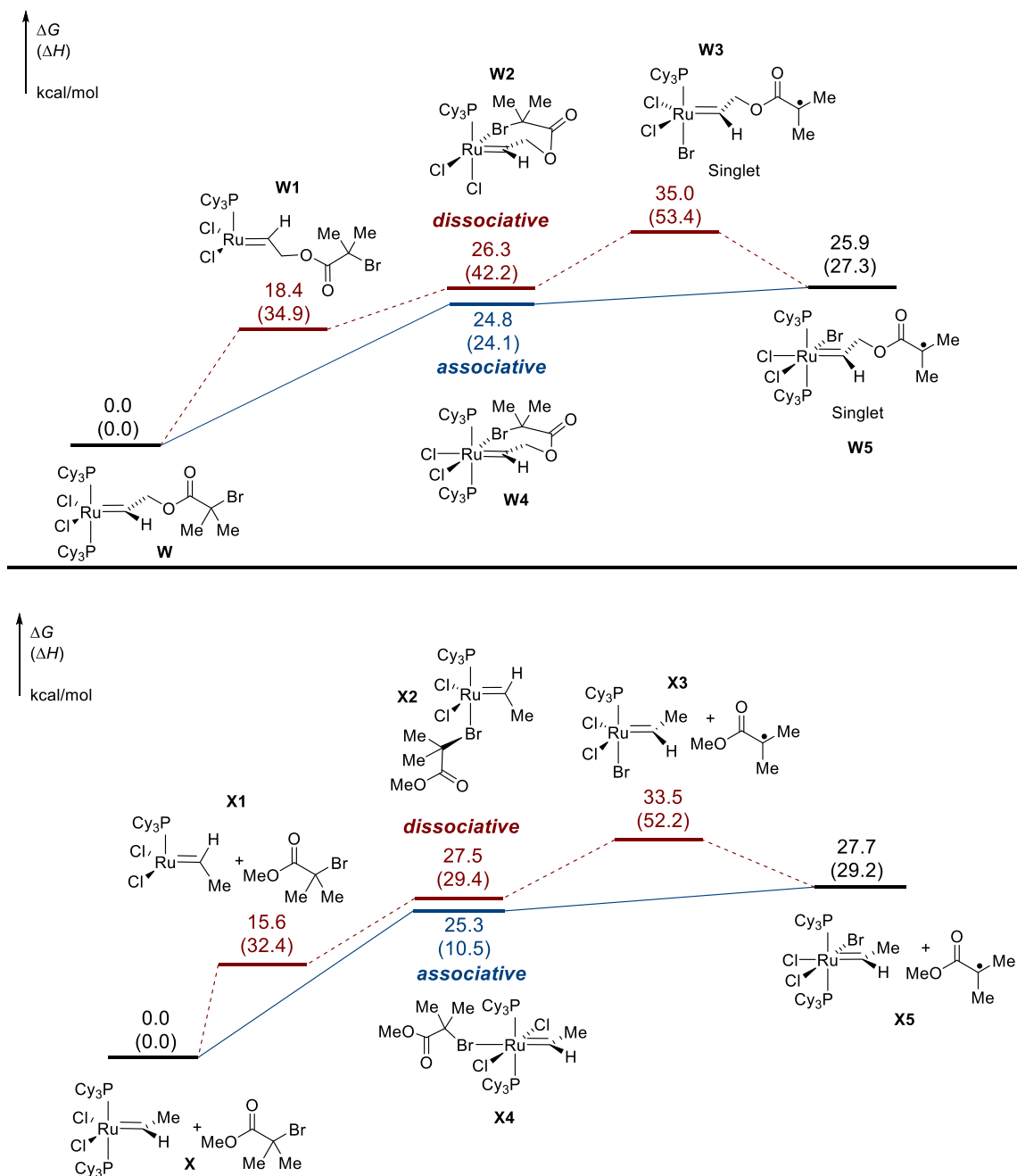


Figure A.S15. Computed pathways for intra- (top) and intermolecular (bottom) inner-sphere electron transfer with **W** (from reference 20 in Chapter 1.1).

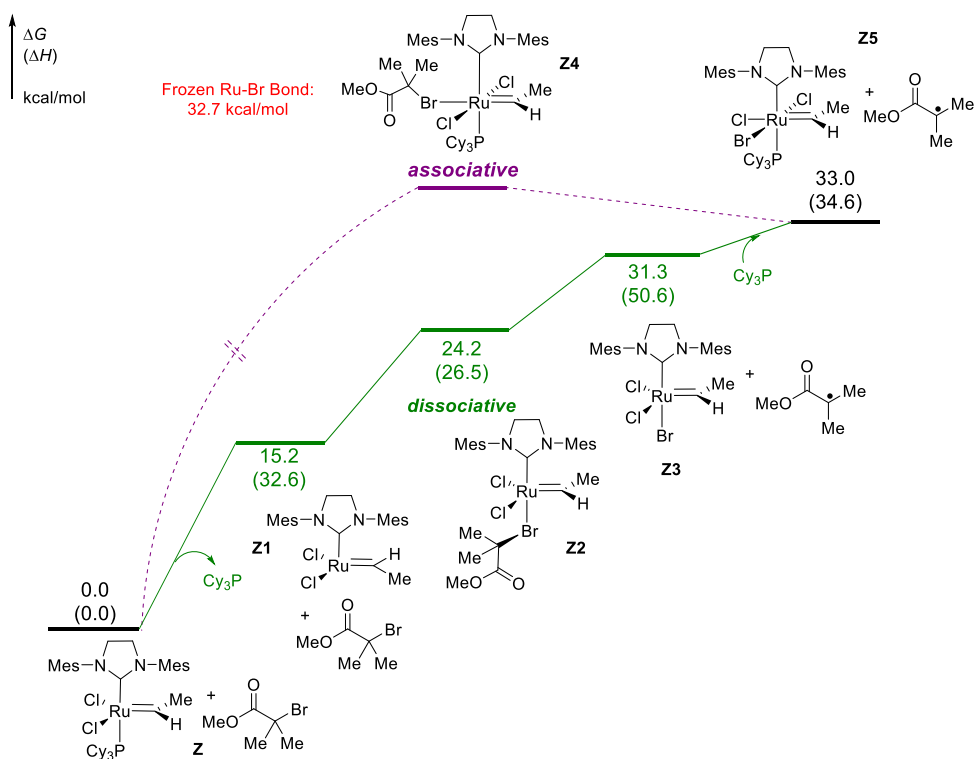
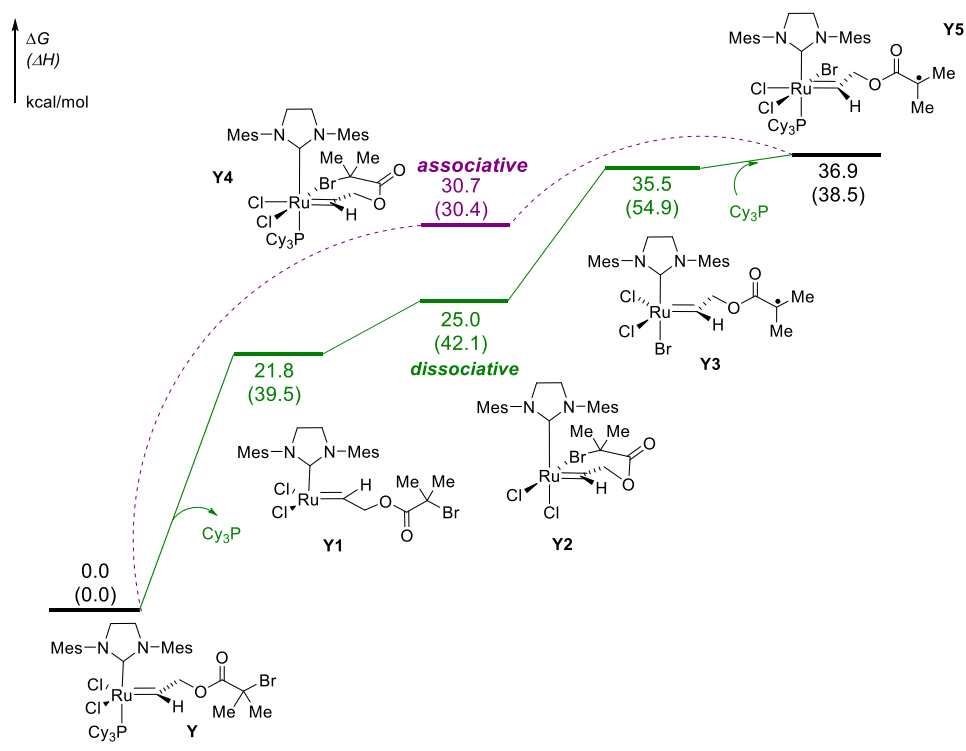


Figure A.S16. Computed barriers to intra- (top) and intermolecular (bottom) inner-sphere electron transfer with second generation version of **W** (from reference 20 in Chapter 1.1).

These initial mechanistic studies were performed to determine which mechanism was operative for ATRP with **W** in reference 20 in Chapter 1.1 and if a second generation version **X** would improve catalysis. These preliminary computations showed that it was unlikely an intact catalyst was performing ATRP catalysis. These led to the further experimental and computational exploration contained in Chapter 1.1. These preliminary computations were performed similarly to those discussed above and in the text except single points were performed with M06¹⁴/SDD/6-311+G(d,p) (SMD: Toluene)

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Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision D.01; Gaussian, Inc.: Wallingford, CT, USA, **2013**.

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Appendix B

Supporting Information for Chapter 1.2

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These authors contributed equally to this work

Experimental Details

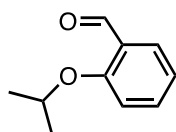
General Information.

Unless otherwise specified, all reactions were carried out under a nitrogen atmosphere in a Vacuum Atmospheres Glovebox in dry glassware. Solvents were purified by passage through solvent purification columns and sparged with argon. THF-*d*₈ was dried over Na/benzophenone, vacuum transferred into a dried Schlenk flask, and subsequently degassed by methods of freeze-pump-thaw. Phenyl vinyl ether was prepared by literature procedure.¹ Phenyl vinyl ether and butyl vinyl ether (Sigma-Aldrich) were sparged with argon and filtered over neutral alumina (Brockmann I) prior to use. Catalysts **3** and **4** were provided by Materia, Inc.

Standard NMR experiments were conducted using a Bruker 400 MHz instrument and a Varian Inova 400 MHz instrument unless otherwise specified. Chemical shifts are reported in ppm downfield using the residual solvent peak as a reference. NMR spectra were analyzed and processed using MestReNova version 8.1.2-11880.

A JEOL MSRoute mass spectrometer was used to obtain high-resolution mass-spectrometry data using FAB+ ionization.

Synthesis of 2-isopropoxybenzaldehyde



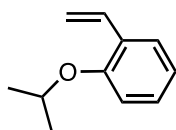
S1

To a Schlenk flask charged with a stir bar was added potassium carbonate (4.54 g, 32.8 mmol). After evacuating and refilling the flask with argon three times, 15 mL dry DMF, salicaldehyde (1.00 mL, 9.38 mmol), and 2-iodopropane (1.12 mL, 11.2 mmol) was added. After stirring at 45 °C overnight, the reaction was quenched with water. The aqueous phase was

extracted with ether (3×150 mL). The organic layer was then washed with water (3×100 mL) and dried over anhydrous MgSO₄, filtered, and solvents were removed *in vacuo* (1.12 g, 72%).

Spectroscopic data was in accordance with those provided previously in the literature.² ¹H NMR (400 MHz, chloroform-*d*₁) δ 10.51 (d, *J* = 0.8 Hz, 1H), 7.85 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.54 (ddd, *J* = 8.4, 7.3, 1.9 Hz, 1H), 7.01 (dddd, *J* = 7.9, 3.9, 2.5, 0.9 Hz, 2H), 4.70 (heptd, *J* = 6.1, 0.6 Hz, 1H), 1.42 (d, *J* = 6.1 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 190.38, 160.74, 135.90, 128.42, 125.80, 120.51, 114.09, 71.20, 22.12.

Synthesis of 2-isopropoxystyrene.

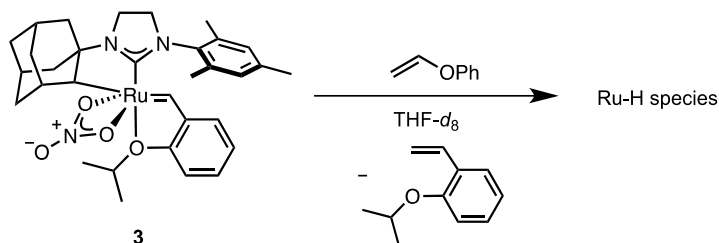


S2

To a Schlenk flask charged with a stir bar was added methyltriphenylphosphonium bromide (652.6 mg, 1.827 mmol) and potassium *tert*-butoxide (205.0 mg, 1.827 mmol). After evacuating and refilling the flask with argon three times, 25 mL of dry diethyl ether was added, and the reaction mixture was stirred for 1 hour at 0 °C. 2-isopropoxybenzaldehyde (100.0 mg, 0.6096 mmol) was then added, and the reaction mixture was stirred for an additional hour at 0°C. Saturated aqueous NH₄Cl solution was then added to the mixture, and the aqueous phase was extracted with Et₂O (3×10 mL). The organic layer was dried over anhydrous MgSO₄, filtered, and solvents were removed *in vacuo*. The crude product was purified by column chromatography on silica using pentane as the eluent, giving the pure product (79.0 mg, 80%) as a colorless oil. Spectroscopic data was in accordance those provided previously in the literature.³ ¹H NMR (400 MHz, THF-*d*₈) δ, 7.45 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.14 (ddd, *J* = 8.7, 7.4, 1.7 Hz, 1H), 7.04 (dd, *J* = 17.8, 11.2 Hz, 1H), 6.91 (dd, *J* = 8.3, 1.1 Hz, 1H), 6.83 (td, *J* = 7.4, 1.1 Hz, 1H), 5.69 (dd, *J* = 17.9,

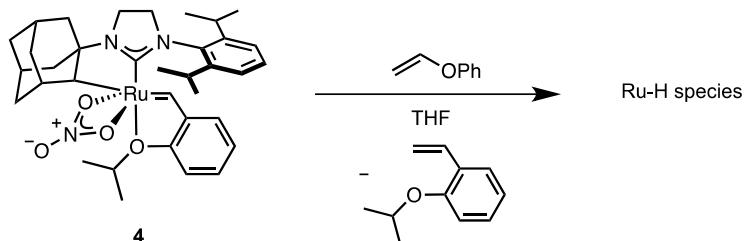
1.7 Hz, 1H), 5.13 (dd, $J = 11.2, 1.7$ Hz, 1H), 4.57 (hept, $J = 6.1$ Hz, 1H), 1.30 (d, $J = 6.0$ Hz, 6H).
 ^{13}C NMR (101 MHz, THF) δ , 155.94, 132.82, 129.23, 128.32, 126.84, 120.93, 114.47, 113.23, 70.90, 22.22.

Synthesis of Ru-Hydride Species From Reaction of 3 with Phenyl Vinyl Ether (Reaction 1)



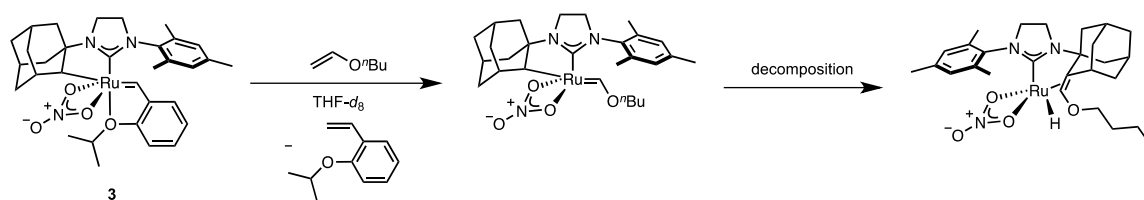
To a 4 mL vial charged with a stir bar was added catalyst **3** (2.2 mg, 0.0035 mmol), 0.65 mL $\text{THF-}d_8$, and phenyl vinyl ether (1.1 μL , 0.010 mmol). After 5 hours, a ruthenium-hydride species could be seen by the appearance of a singlet in the ^1H NMR (400 MHz) at -12.16 ppm.

Synthesis of Ru-Hydride Species From Reaction of 4 with Phenyl Vinyl Ether (Reaction 2)



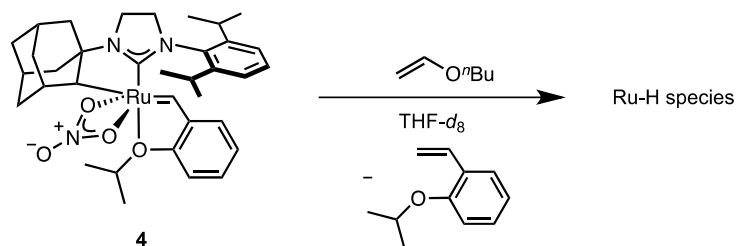
To a 4 mL vial charged with a stir bar was added catalyst **4** (2.4 mg, 0.0035 mmol), 0.25 mL of THF, and phenyl vinyl ether (9.2 μL , 0.0875 mmol). After 4 hours, a 100.0 μL aliquot of the reaction mixture was added to 0.6 mL $\text{THF-}d_8$ in a NMR tube. The formation of the ruthenium-hydride species was seen by the appearance of a singlet in the ^1H NMR (400 MHz) at -11.97 ppm.

Synthesis of Ru-Hydride Species From Reaction of 3 with Butyl Vinyl Ether (Reaction 3)



To a J. Young tube was added catalyst **3** (60.0 mg, 0.0711 mmol), 0.65 mL of THF-*d*₈, and butyl vinyl ether (9.21 μ L, 0.0711 mmol). After taking a ¹H NMR spectrum after 10 minutes to see the initial formation of the Fischer carbene, the reaction to form complex was completed overnight at room temperature. Analysis by ¹H, ¹³C, ¹H-¹H COSY, ¹H-¹³C HSQC, and ¹H-¹³C HSQC NMR spectroscopy were conducted using a Bruker 400 MHz instrument. ¹³C-DEPT NMR studies were performed using a Varian Inova 400 MHz instrument. ¹H NMR (400 MHz, THF-*d*₈) δ , 6.81 (d, 2H), 5.11 (s, 1H), 3.94 (dt, *J* = 9.8, 6.2 Hz, 1H), 3.66 (m, 1H), 3.52 (t, 1H), 3.41 (td, *J* = 10.0, 4.2 Hz, 1H), 3.30-3.15 (m, 2H), 2.58 (dt, *J* = 11.9, 3.0 Hz, 1H), 2.32-2.17 (m, 6H, overlapping), 2.22 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 1.94-1.75 (m, 6H), 1.62-1.49 (m, 2H), 1.45-1.35 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H), -12.63 (s, 1H). ¹³C NMR (101 MHz, THF) δ 218.56, 137.64, 137.40, 136.89, 136.52, 129.57, 129.02, 93.09, 71.16, 65.38, 60.43, 51.93, 44.88, 41.52, 38.67, 38.33, 38.29, 35.16, 33.14, 32.92, 31.61, 30.85, 21.01, 20.14, 18.13, 17.75, 14.20. HRMS (FAB⁺): Calculated – 570.1906, Found – 570.1896.

Synthesis of Ru-Hydride Species From Reaction of **4** with Butyl Vinyl Ether (Reaction 4)



To a J. Young tube was added catalyst **4** (5.3 mg, 0.0079 mmol), 0.65 mL of THF-*d*₈, and phenyl vinyl ether (20.4 μ L, 0.158 mmol). Decomposition to the ruthenium-hydride species was

completed overnight at room temperature as seen by the appearance of a singlet in the ^1H NMR (400 MHz) at -12.50 ppm.

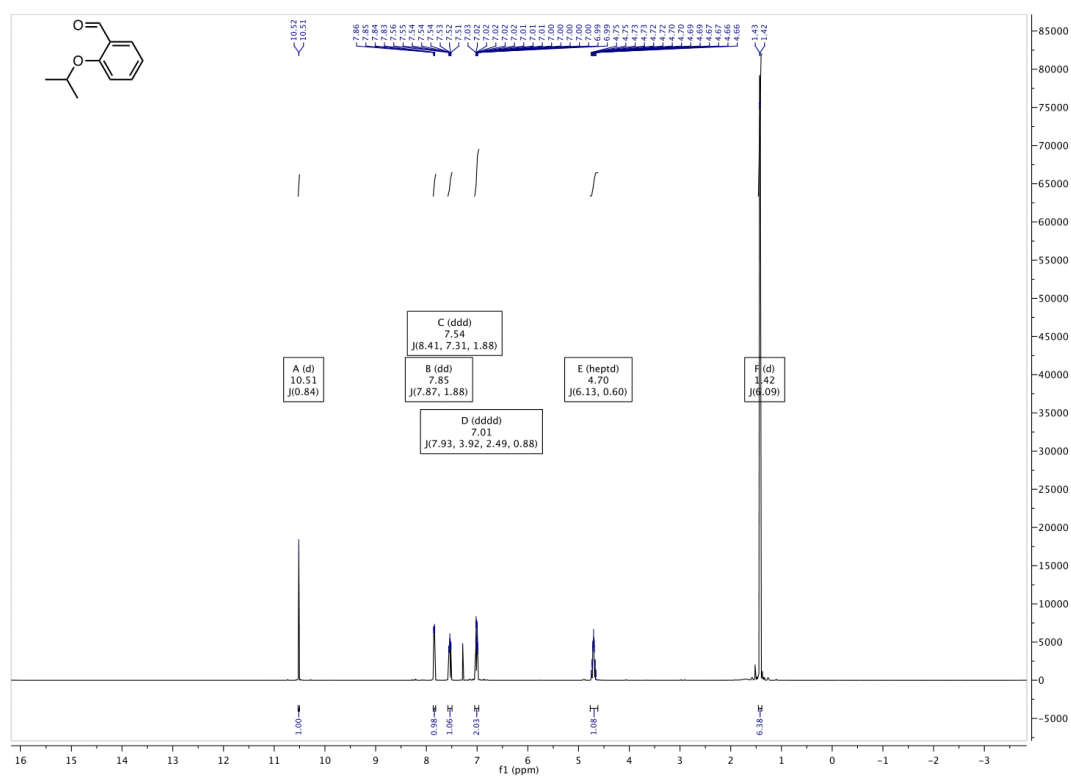


Figure B.S1. ¹H NMR (CDCl₃, 400 MHz) Spectrum of S1.

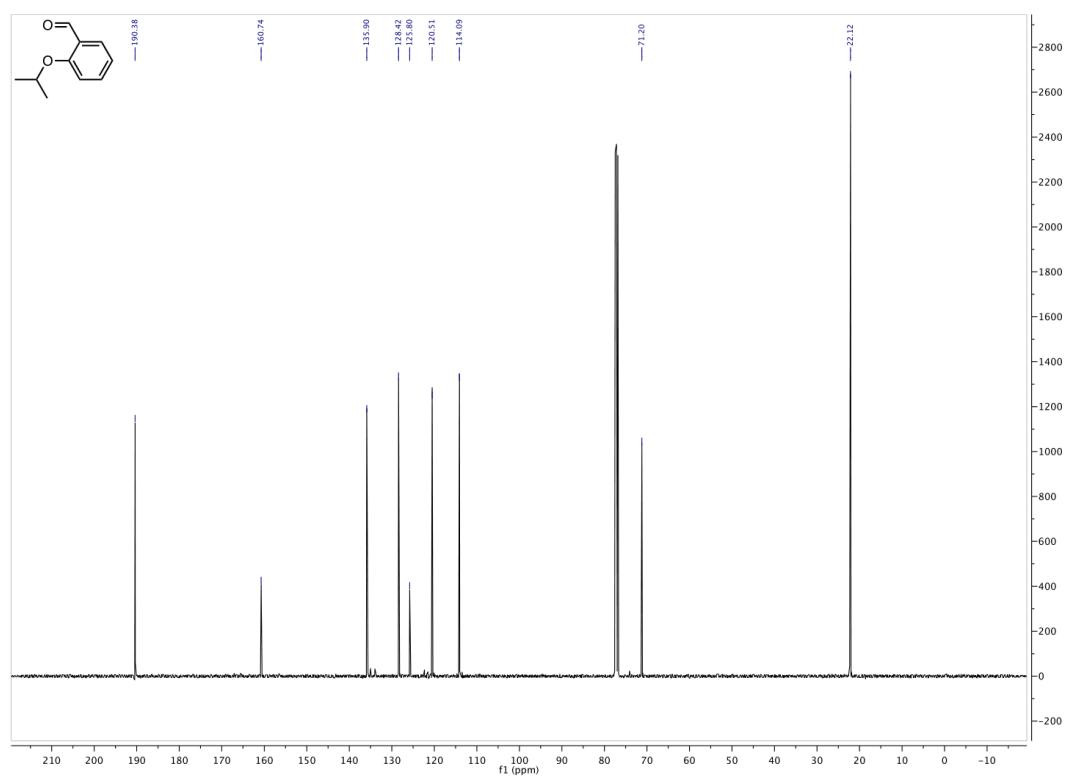


Figure B.S2. ^{13}C NMR (CDCl_3 , 101 MHz) Spectrum of **S1**.

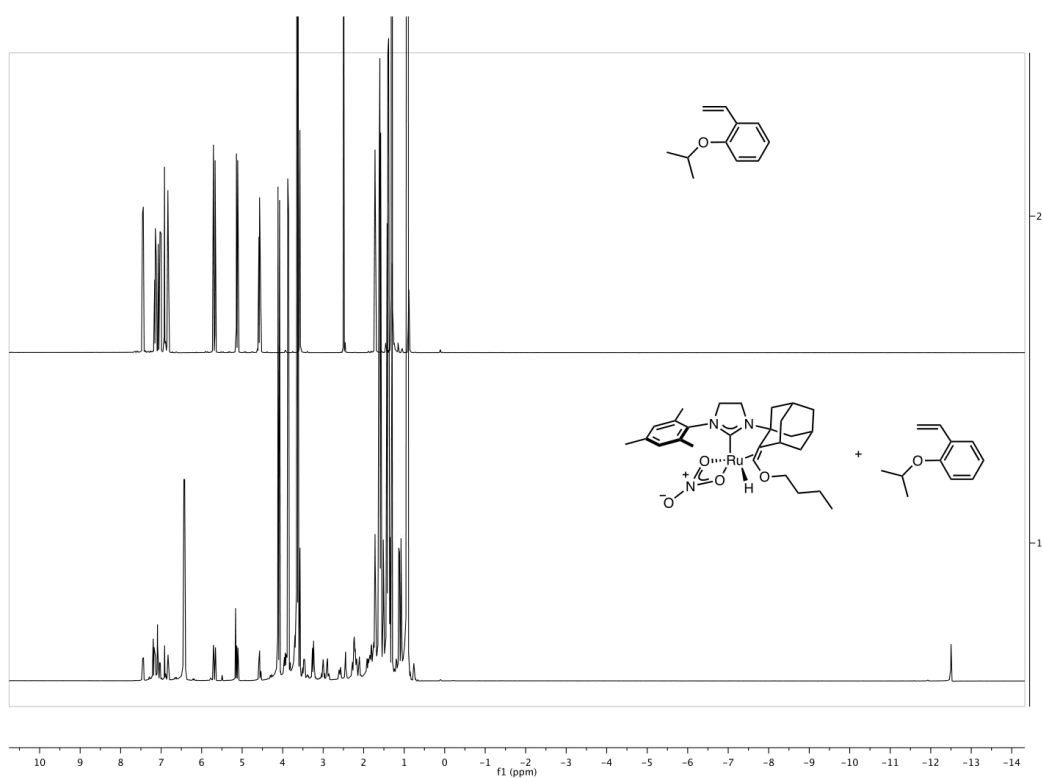


Figure B.S3. Stacked ^1H NMR (THF- d_8 , 400 MHz) Spectrum of S2 and Reaction 3.

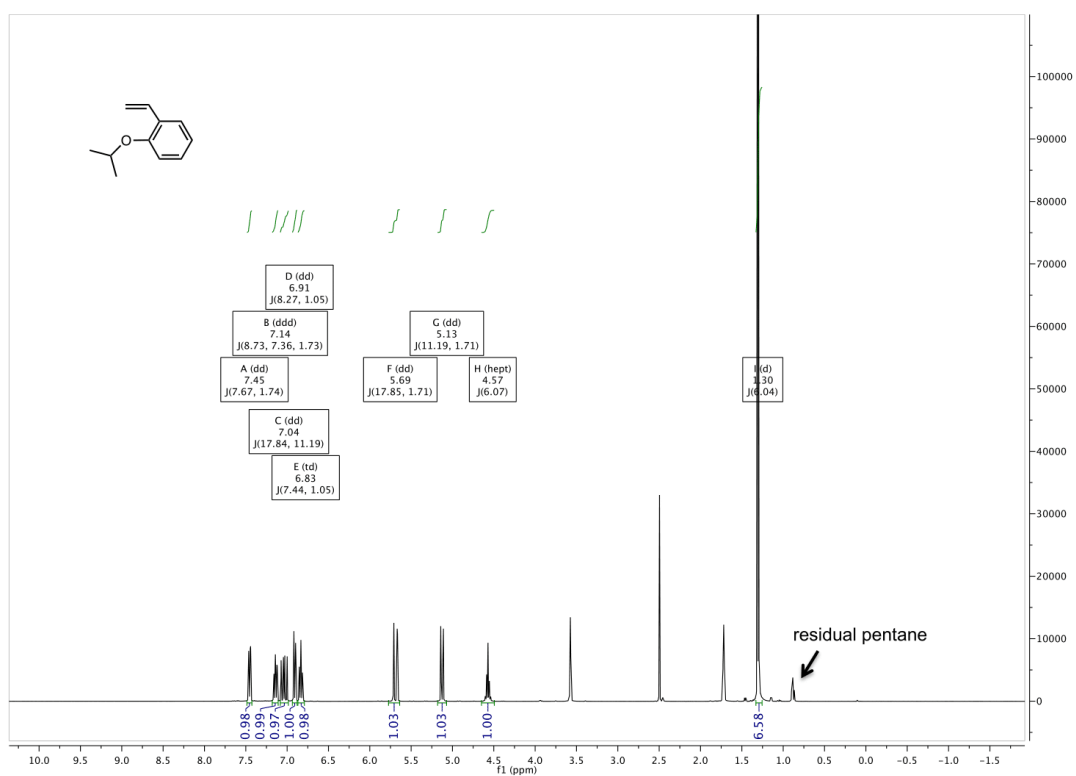


Figure B.S4. ^1H NMR ($\text{THF-}d_8$, 400 MHz) Spectrum of S2.

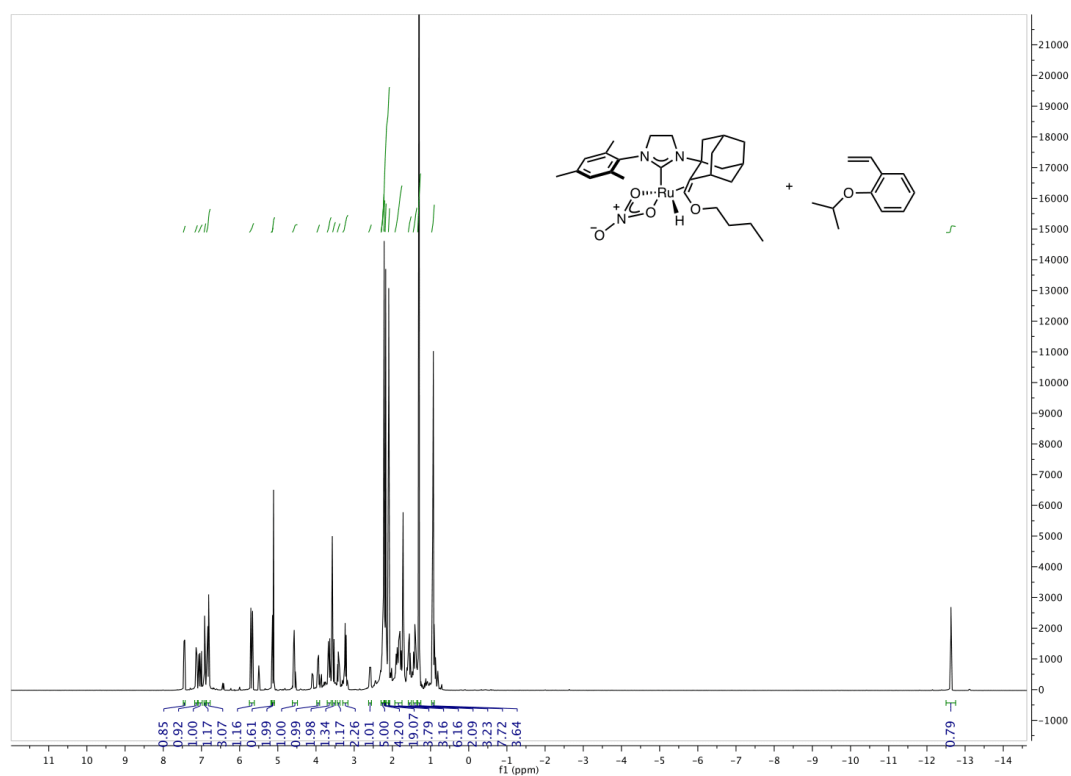


Figure B.S5. ^1H NMR (THF-d_8 , 400 MHz) Spectrum of **Reaction 3**.

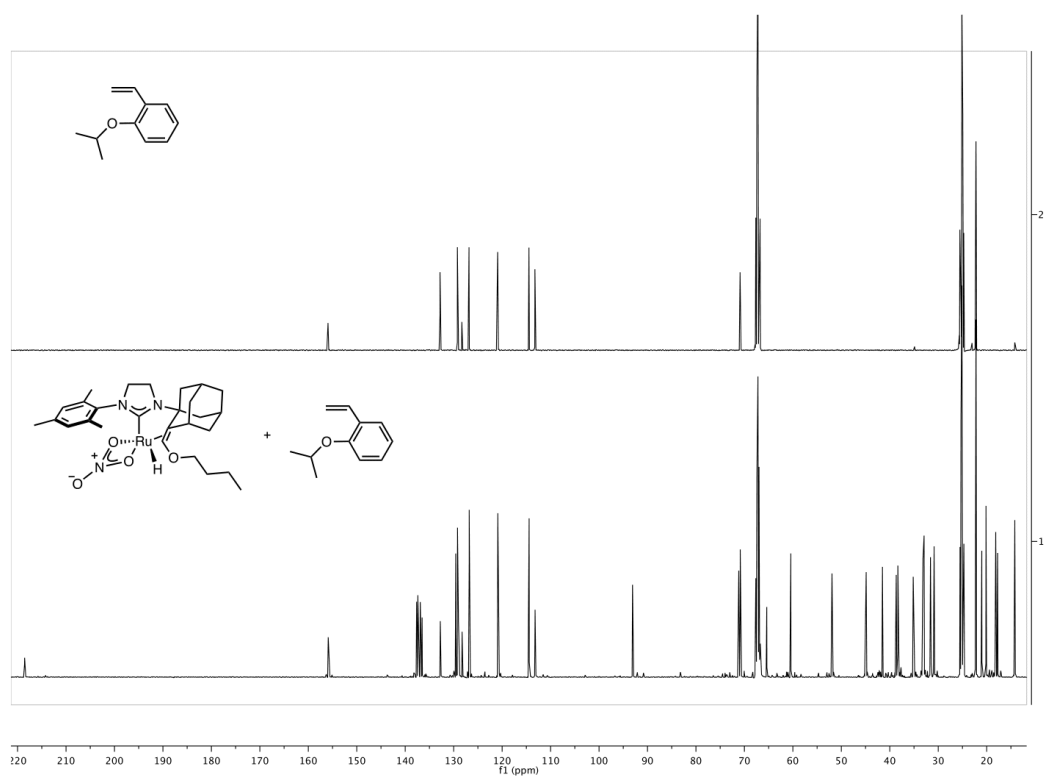


Figure B.S6. Stacked ^{13}C NMR (THF- d_8 , 101 MHz) Spectrum of **2** and **Reaction 3**.

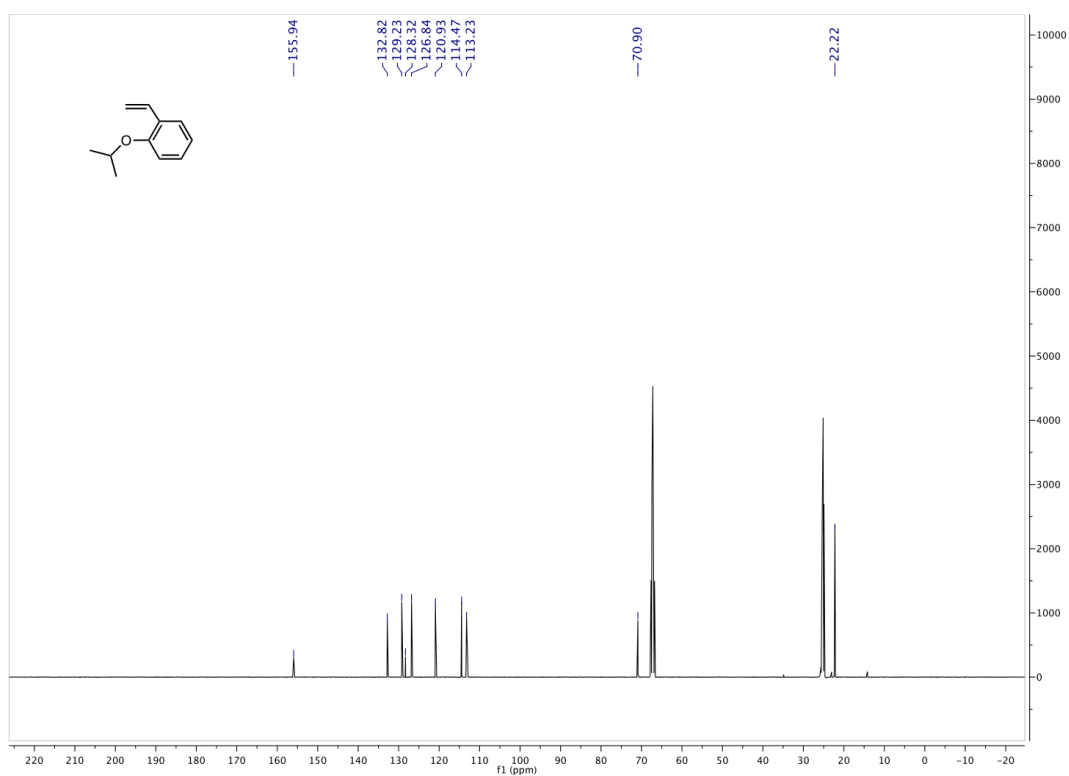


Figure B.S7. ¹³C NMR (THF-*d*₈, 101 MHz) Spectrum of 2.

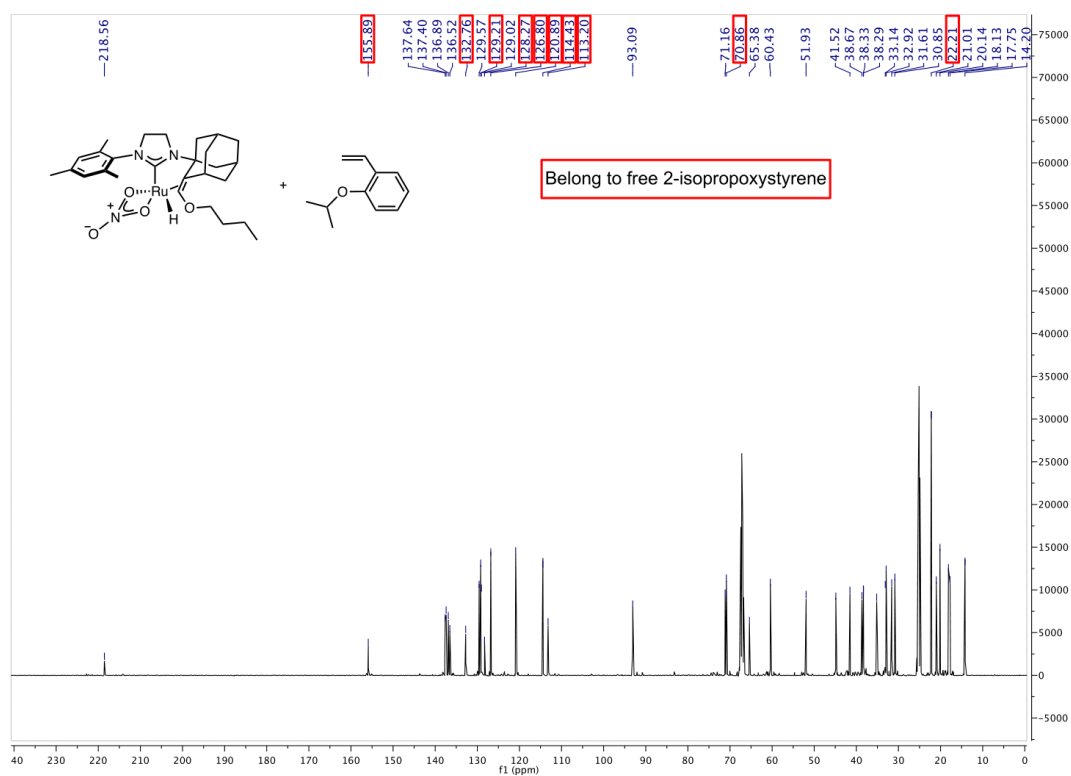


Figure B.S8. ^{13}C NMR ($\text{THF-}d_8$, 101 MHz) Spectrum of **Reaction 3**.

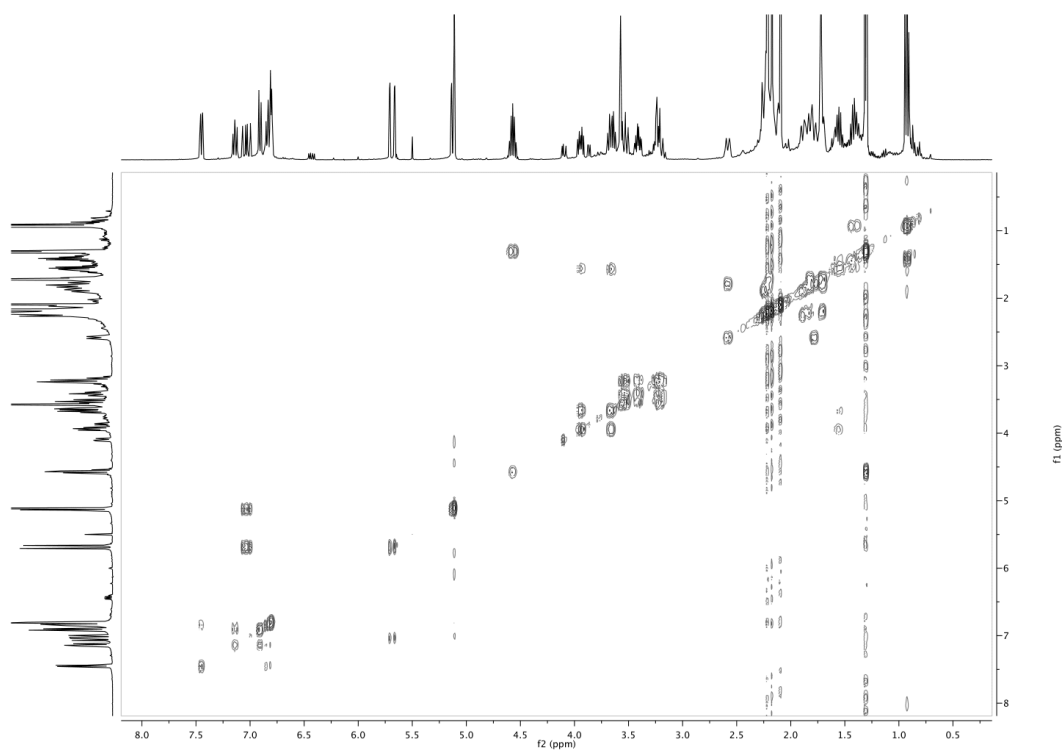


Figure B.S9. ^1H - ^1H COSY NMR ($\text{THF-}d_8$) Spectrum of **Reaction 3**.

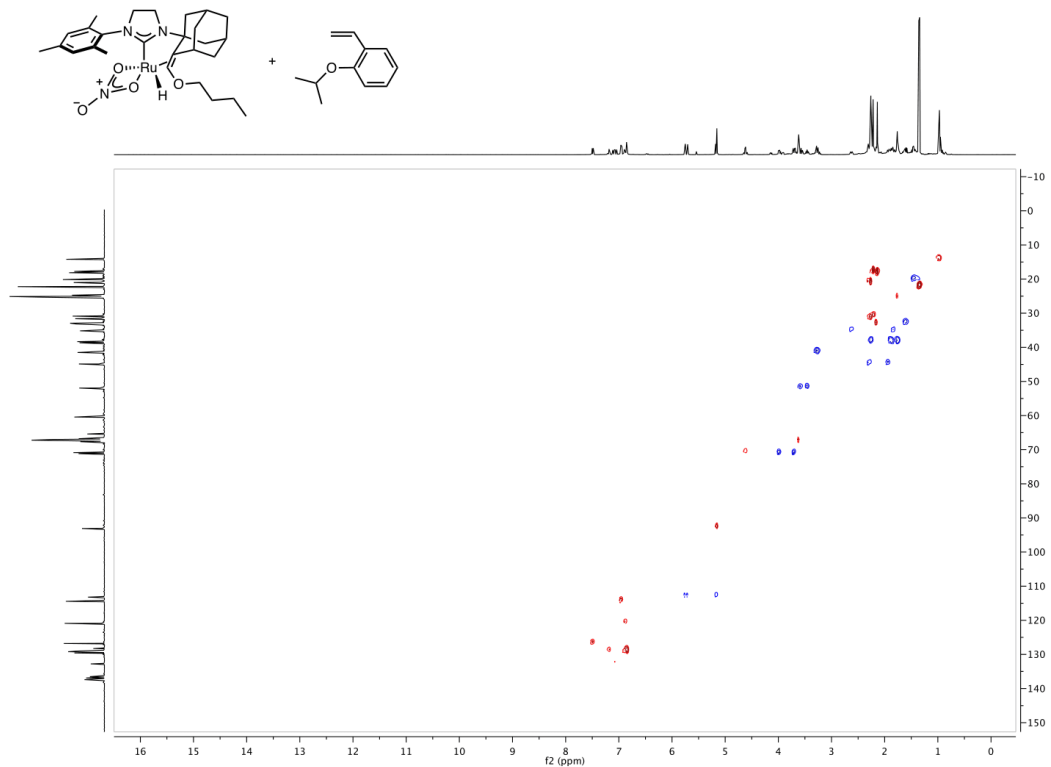


Figure B.S10. ^1H - ^{13}C HSQC NMR (THF- d_8) Spectrum of **Reaction 3**.

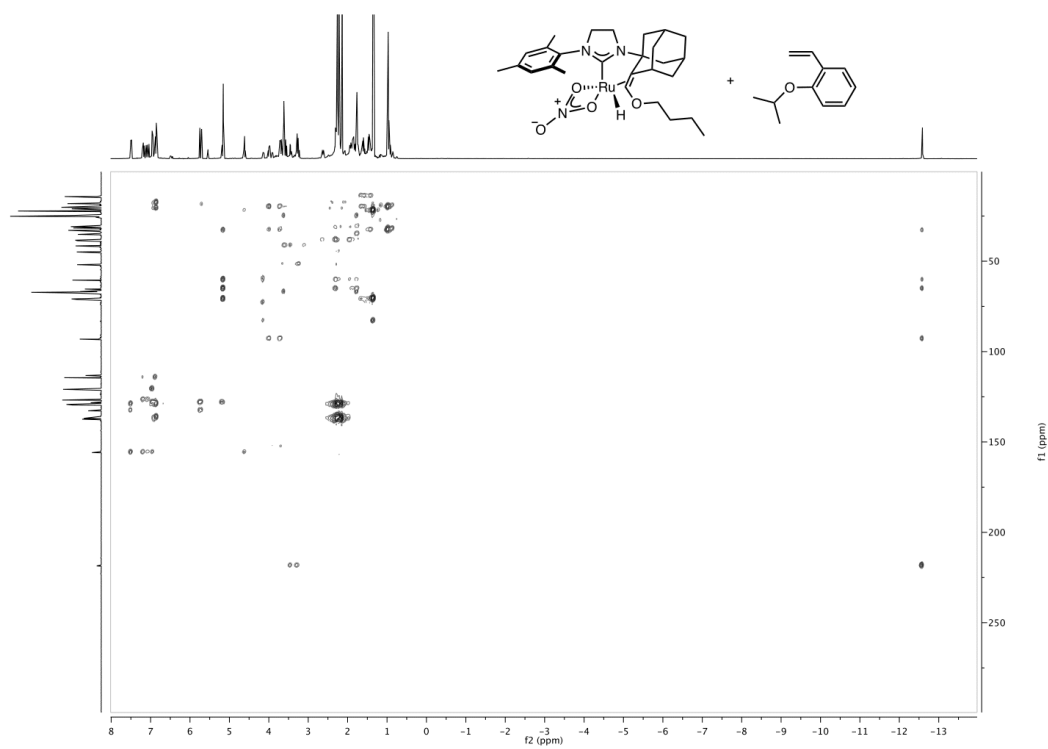


Figure B.S11. ^1H - ^{13}C HMBC NMR ($\text{THF-}d_8$) Spectrum of **Reaction 3**.

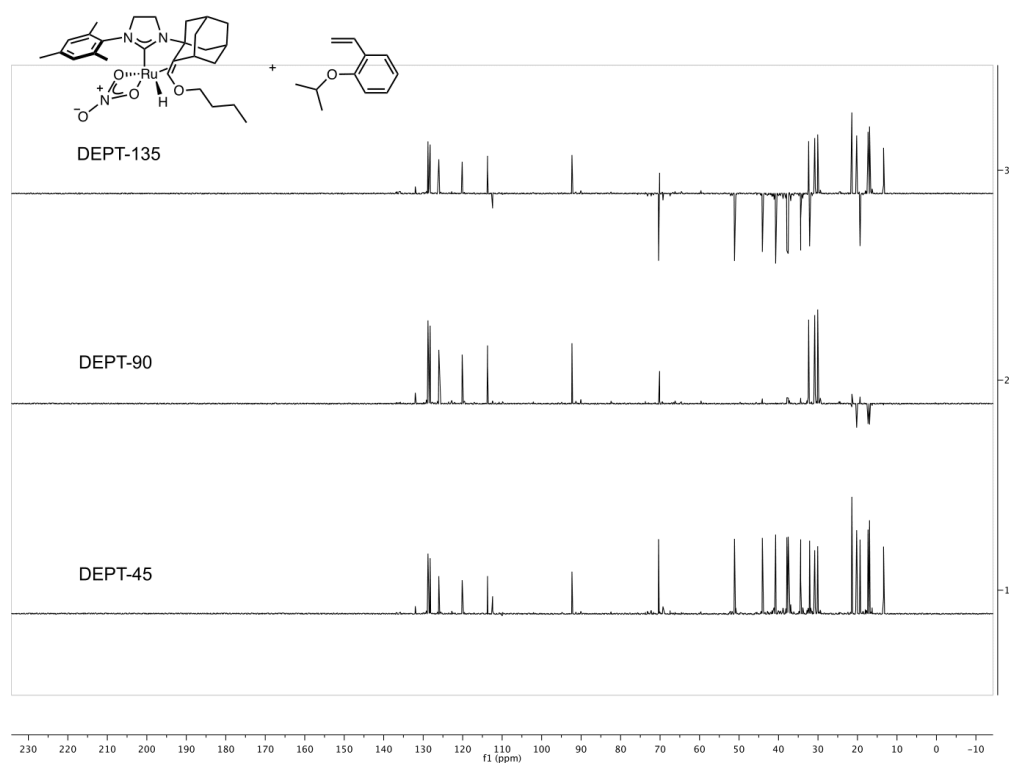


Figure B.S12. ^{13}C -DEPT (THF- d_8 , 101 MHz) Spectrum of **Reaction 3**.

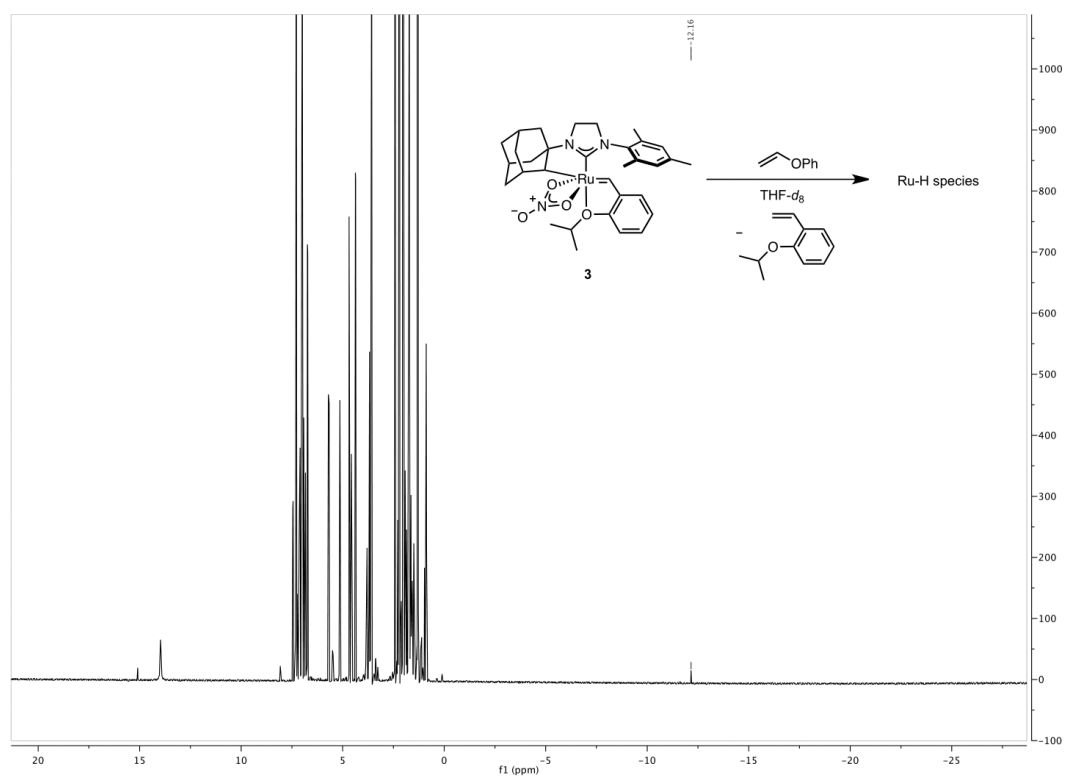


Figure B.S13. ^1H NMR (THF-d_8 , 400 MHz) Spectrum of **Reaction 1**.

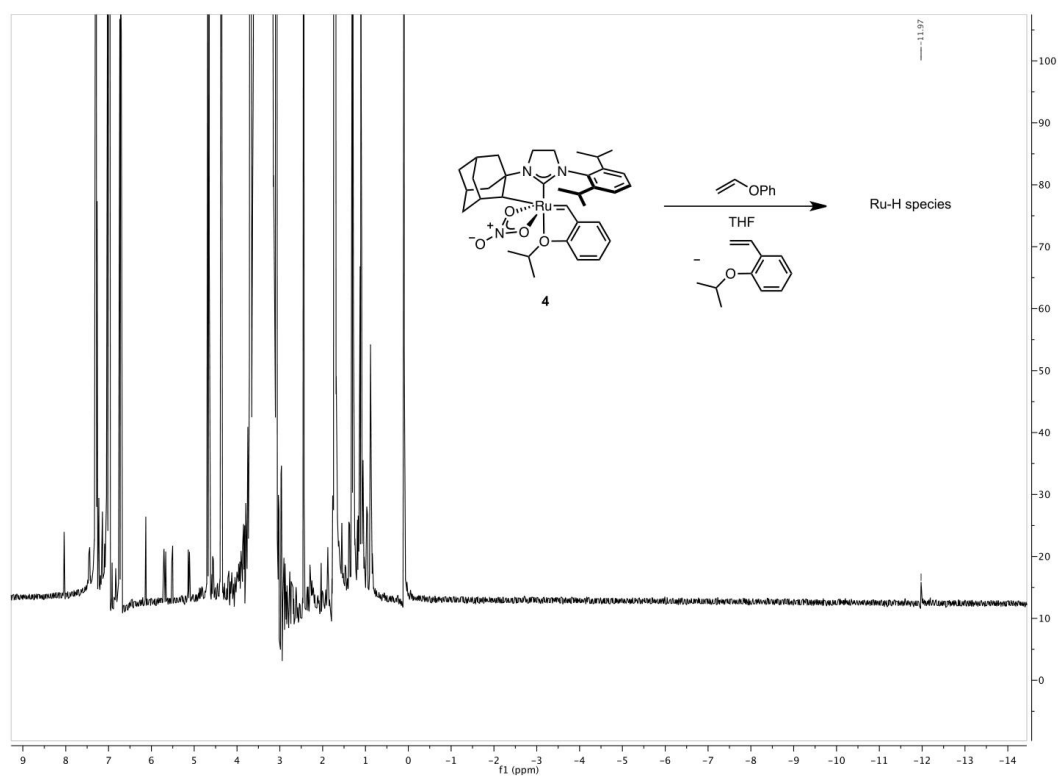


Figure B.S14. ^1H NMR (THF- d_8 , 400 MHz) Spectrum of **Reaction 2**.

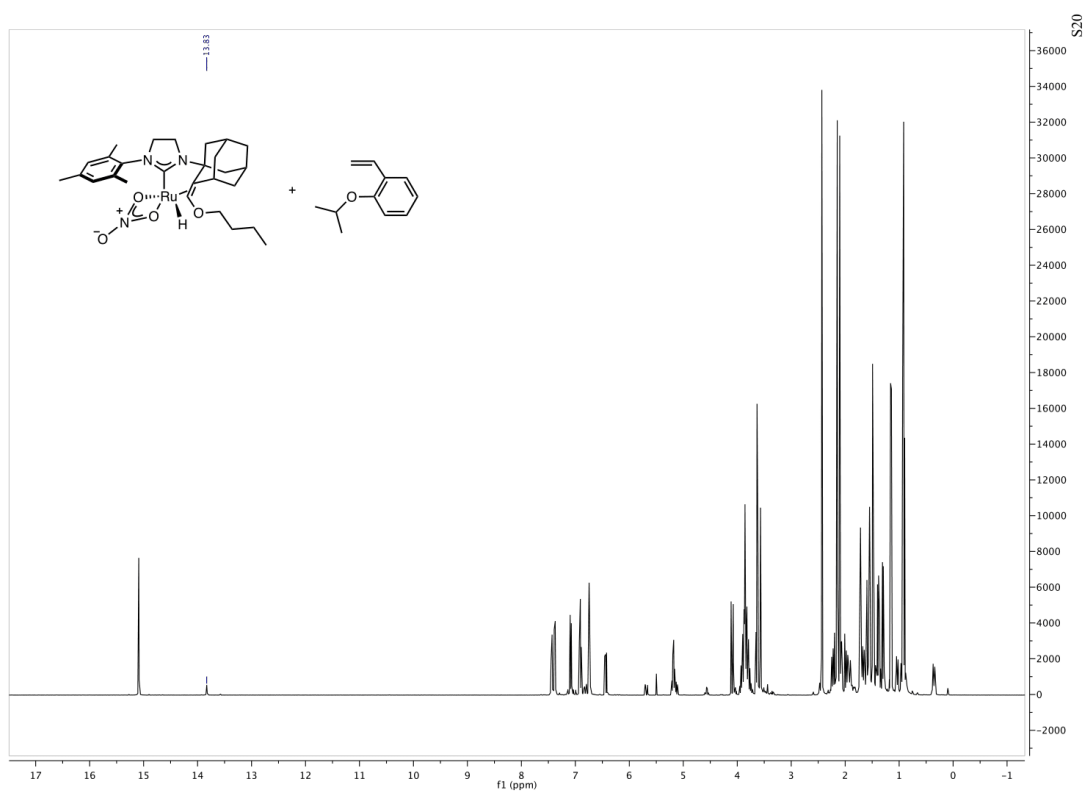


Figure B.S15. ^1H NMR ($\text{THF-}d_8$, 400 MHz) Spectrum of **Reaction 3** (after 10 min).

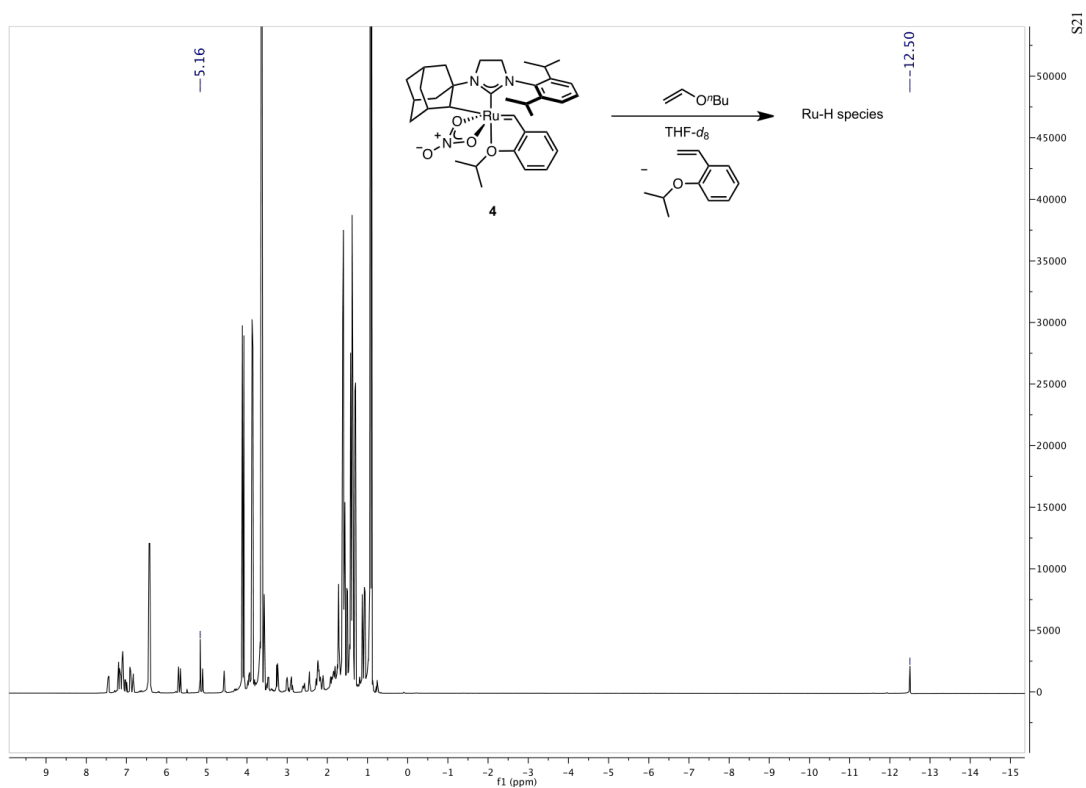


Figure B.S16. ^1H NMR ($\text{THF-}d_8$, 400 MHz) Spectrum of **Reaction 4**.

Computational Details

Geometry optimizations on all intermediates and transition states were performed using the B3LYP⁴ method of density functional theory (DFT) in the gas phase with a mixed basis set using LANL2DZ for ruthenium and 6-31G(d) for all other atoms. Frequency calculations were performed on all optimizations to confirm the location of relative minima (zero negative frequencies) and transition states (one negative frequency). Thermal corrections were computed from frequency calculations at the standard state of 1 atm and 298 K. All frequencies below 100 cm⁻¹ were manually adjusted to 100 cm⁻¹ to account for the breakdown of the harmonic oscillator approximation, as discussed by Truhlar and coworkers.⁵ Intrinsic reaction coordinate (IRC) calculations were performed at the same level of theory on most transition states to confirm the connection of the transition states to the calculated intermediates. Single point energy calculations were performed on all optimized structures using the M06⁶ functional and a mixed basis set using SDD for ruthenium and 6-311+G(d,p) for all other atoms. The SMD⁷ solvation model for tetrahydrofuran as employed for all single point calculations. Electrostatic potential maps were generated from the respective optimizations of the 2 structures. All calculations were performed using the Gaussian 09 software.⁸ All 3D structures were rendered using CYLView.⁹

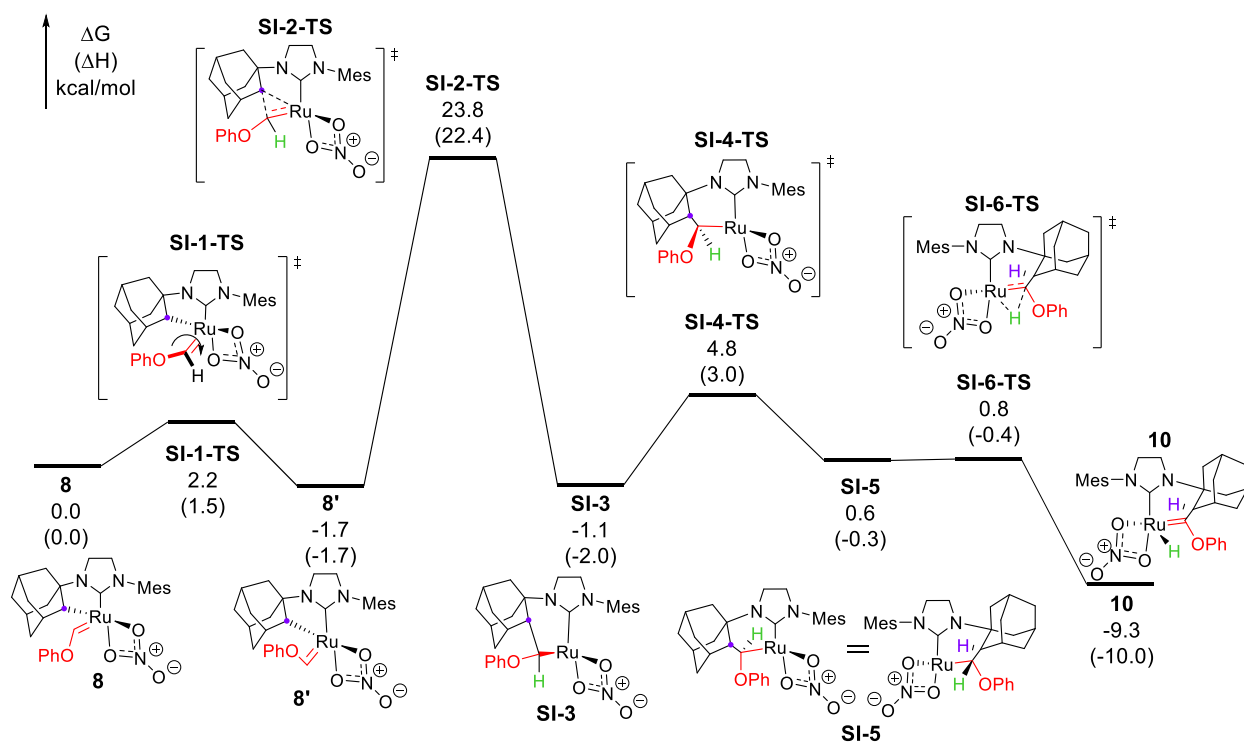


Figure B.S17. Decomposition pathway of **8** leading to hydride **10**.

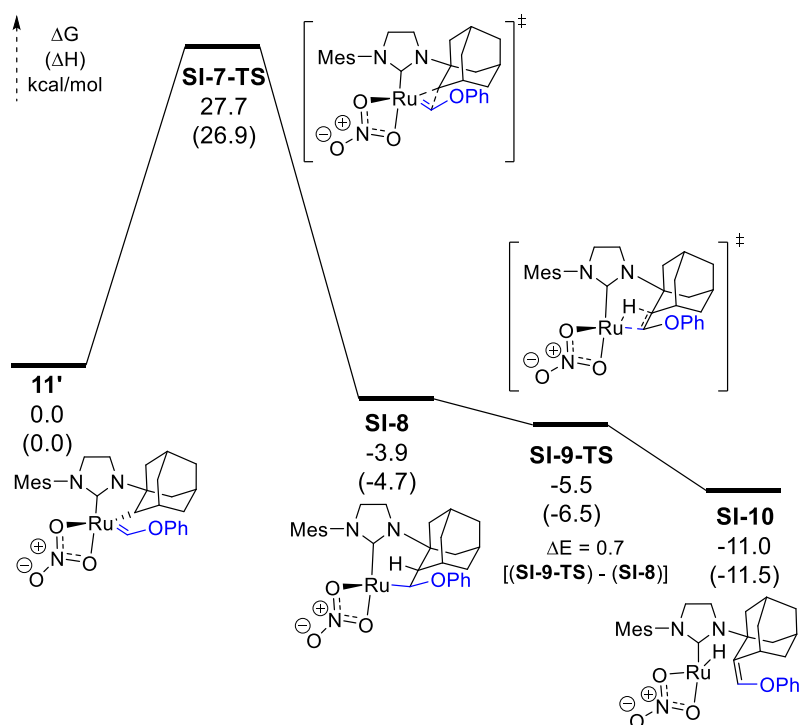


Figure B.S18. Decomposition pathway of **11'** to hydride **SI-10**.

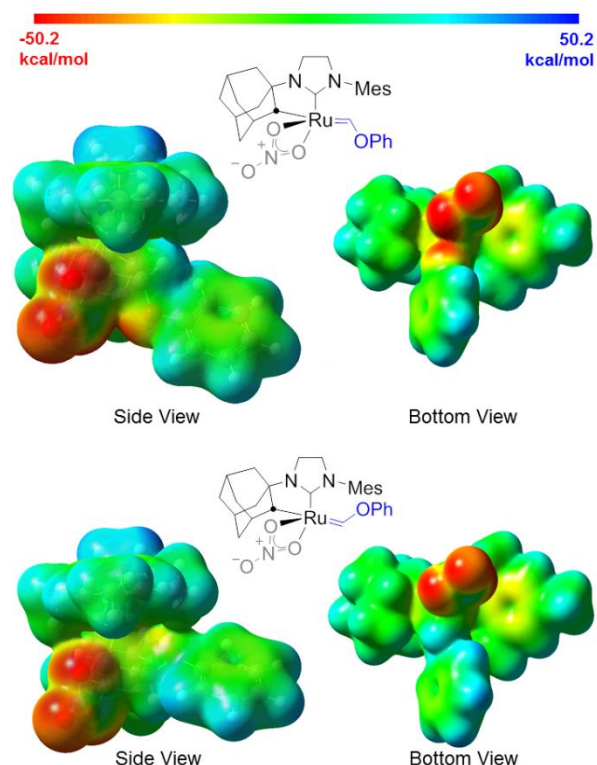


Figure B.S19. Electrostatic potential maps of **11** (top) and **11'** (bottom).

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(8) Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, **2013**.

(9) CYLview, 1.0b; Legault, C. Y., Université de Sherbrooke, **2009** (<http://www.cylview.org>).

Appendix C

Supplemental Information for Chapter 2.2

Jessica M. Grandner, T. Patrick Montgomery, Noah F. Fine Nathel, Robert H. Grubbs, K. N. Houk

(Experimental and spectral data provided by T. Patrick Montgomery)

Ruthenium Catalyst 4

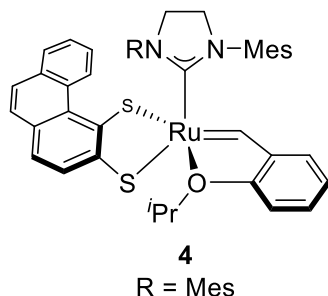


Figure C.S1. Catalyst **4-up**.

¹H NMR (400 MHz, CDCl₃): δ 14.05 (s, 0.28H), 13.97 (s, 0.72H), 11.72 (d, *J* = 8.6 Hz, 0.75H), 10.87 – 10.78 (m, 0.29H), 7.83 (dd, *J* = 7.8, 1.6 Hz, 0.83H), 7.71 (d, *J* = 8.2 Hz, 0.26H), 7.69 – 7.66 (m, 0.27H), 7.62 (td, *J* = 6.5, 3.5 Hz, 1H), 7.59 (d, *J* = 6.0 Hz, 0.85H), 7.56 – 7.49 (m, 2H), 7.38 (d, *J* = 8.6 Hz, 0.28H), 7.34 – 7.18 (m, 4H), 7.16 (d, *J* = 8.4 Hz, 0.83H), 6.98 (d, *J* = 31.5 Hz, 2H), 6.82 – 6.71 (m, 1H), 6.59 (dd, *J* = 7.5, 1.7 Hz, 0.94H), 6.55 (dd, *J* = 7.5, 1.6 Hz, 0.42H), 6.25 (s, 1H), 5.43 (hept, *J* = 6.6 Hz, 0.3H), 5.19 (hept, *J* = 6.5 Hz, 0.83H), 4.07–3.85 (m, 4H), 2.94 (s, 2H), 2.73 – 2.34 (m, 4H), 2.14 (d, *J* = 57.4 Hz, 10H), 1.77 (d, *J* = 6.5 Hz, 1H), 1.61 (dd, *J* = 6.5, 3.4 Hz, 4H), 1.47 (d, *J* = 6.4 Hz, 3H).

¹³C NMR (101 MHz, CDCl₃): δ 248.94, 248.61, 220.27, 220.06, 157.07, 155.71, 155.52, 151.58, 143.90, 143.01, 142.96, 139.15, 138.67, 137.85, 137.30, 136.99, 136.52, 134.51, 134.38, 133.40, 132.58, 131.44, 131.20, 131.07, 130.63, 130.56, 130.27, 130.00, 129.56, 129.19, 128.98, 128.95, 128.48, 128.29, 128.15, 128.11, 127.54, 127.46, 125.46, 125.37, 125.33, 125.14, 125.00, 124.32, 124.18, 124.08, 123.48, 122.90, 122.81, 122.39, 116.31, 116.05, 81.67, 80.92, 52.29, 52.24, 24.35, 24.09, 22.26, 22.17, 21.25, 21.20, 19.84, 18.04.

HRMS (FAB⁺): [M]⁺ C₄₅H₄₆N₂OS₂Ru Calculated: 796.2096, Found: 796.2104.

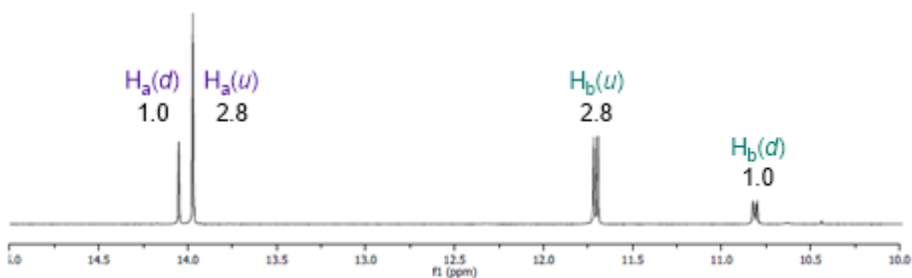


Figure C.S2. Portion of ^1H -NMR of **4**.

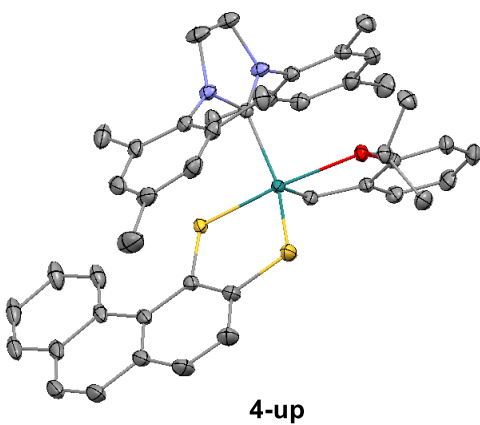


Figure C.S3. X-ray Crystal Structure of **4**.

Ruthenium Catalyst **5**

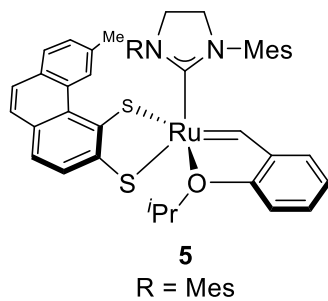


Figure C.S4. Catalyst **5-up**.

¹H NMR (400 MHz, THF-d₈): δ 14.04 (s, 0.4H), 13.99 (s, 0.6H), 11.57 (s, 0.6H), 10.74 (s, 0.4H), 7.73 (dd, *J* = 8.2, 1.6 Hz, 0.6H), 7.68 (dd, *J* = 8.2, 1.7 Hz, 0.4H), 7.57 (d, *J* = 8.0 Hz, 0.4H), 7.53 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 0.6H), 7.35 (d, *J* = 16.0 Hz, 0.6H), 7.33 (d, *J* = 20.0 Hz, 0.4H), 7.28 – 7.20 (m, 2H), 7.17 (dd, *J* = 8.2, 3.5 Hz, 2H), 7.04 – 6.84 (m, 2H), 6.75 (q, *J* = 7.2 Hz, 1H), 6.54 (ddt, *J* = 17.8, 8.9, 2.8 Hz, 1H), 6.43 (s, 1.4H), 6.15 (s, 0.6H), 5.49 – 5.40 (m, 0.4H), 5.40 – 5.29 (m, 0.6H), 4.05 (d, *J* = 9.4 Hz, 1.6H), 3.98 – 3.78 (m, 2.4H), 2.87 (s, 2H), 2.73 (s, 2H), 2.66 – 2.39 (m, 5H), 2.29 – 2.04 (m, 8H), 1.84 – 1.46 (m, 11H).

¹³C NMR (101 MHz, THF-d₈): δ 248.22, 247.39, 220.38, 220.01, 156.65, 155.51, 155.47, 151.73, 143.44, 143.02, 142.99, 139.11, 138.99, 138.57, 138.04, 137.70, 137.28, 136.92, 136.37, 136.04, 133.55, 133.49, 133.26, 132.57, 132.47, 132.36, 131.33, 131.28, 130.82, 130.72, 130.55, 130.36, 130.19, 130.04, 129.65, 129.15, 128.77, 128.23, 128.03, 127.99, 127.60, 127.40, 127.35, 127.05, 126.94, 125.14, 125.04, 124.98, 124.04, 123.54, 122.82, 122.26, 116.35, 116.26, 81.67, 81.33, 52.36, 52.23, 52.15, 24.28, 24.18, 22.89, 22.50, 22.18, 21.24, 21.18, 20.14, 19.80, 17.94.

HRMS (FAB⁺): [M]⁺ C₄₆H₄₈N₂OS₂Ru Calculated: 810.2252, Found: 810.2240.

NOE Spectrum of 4

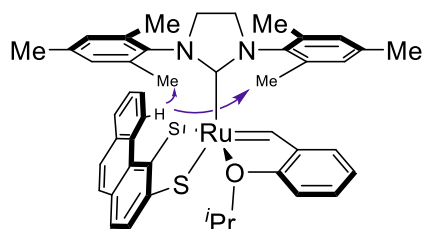


Figure C.S5. Proton at the 5-position of the phenanthrene dithiolate is at 11.72 ppm for the up isomer and 10.83 for the down isomer.

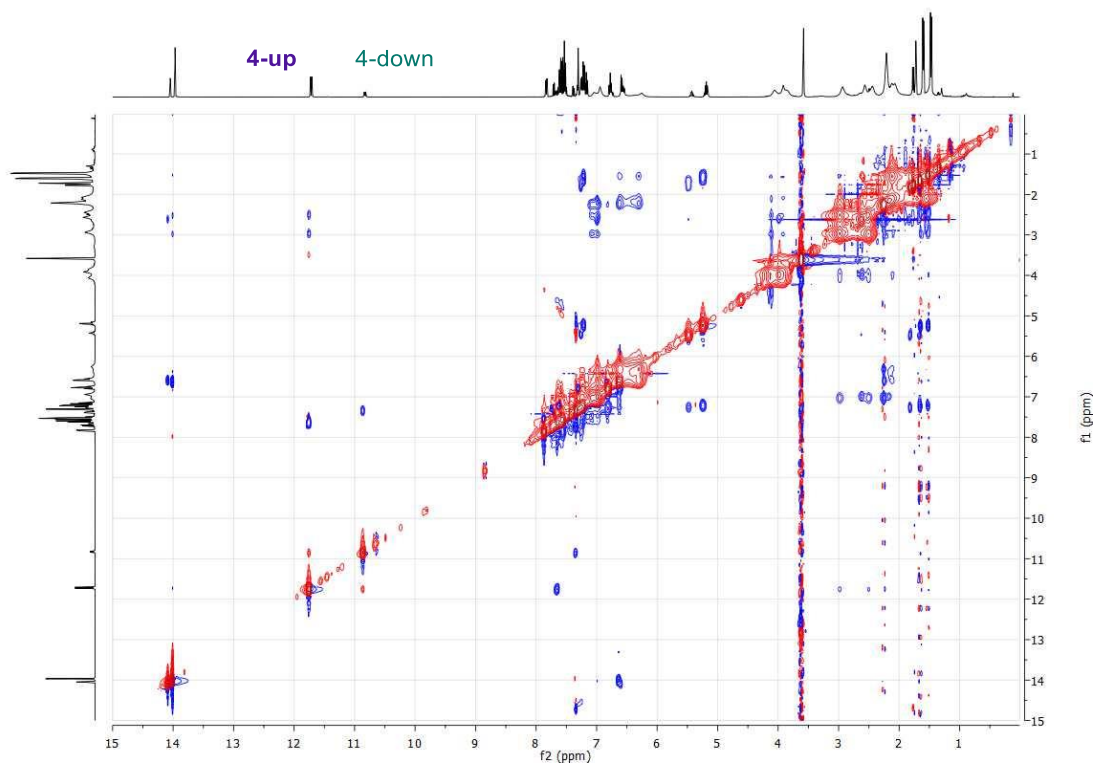


Figure C.S6. NOE Spectra of **4**.

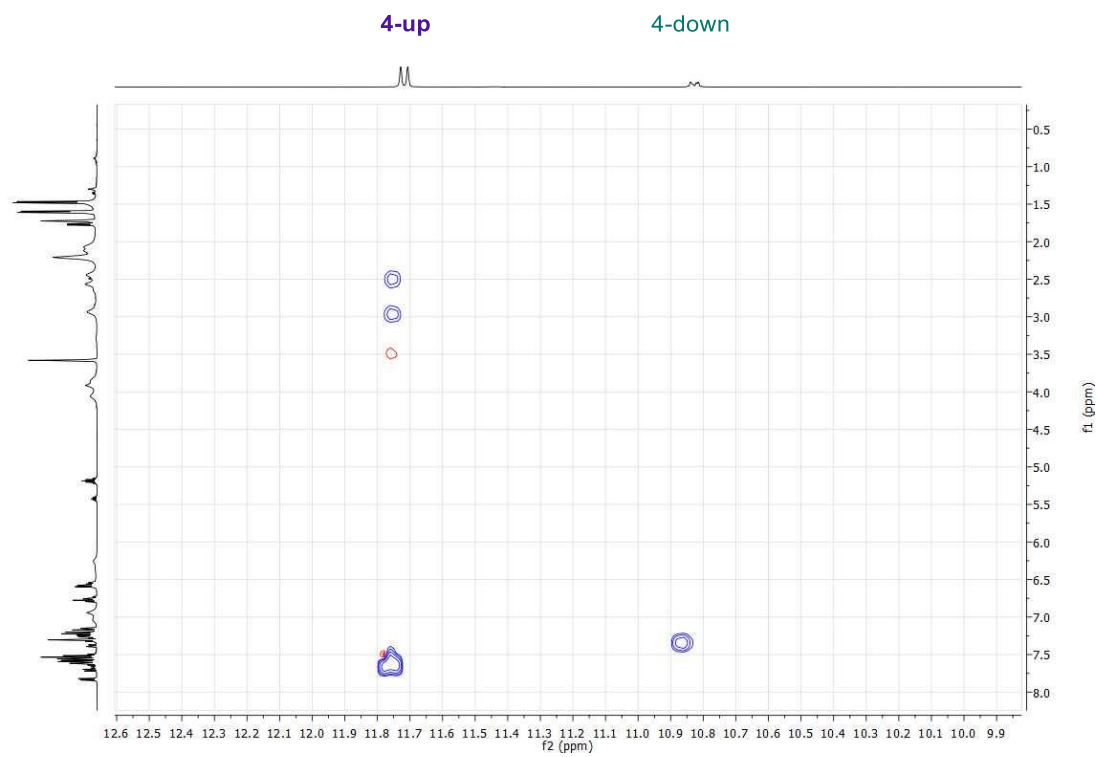


Figure C.S7. Portion of NOE Spectra of **4**.

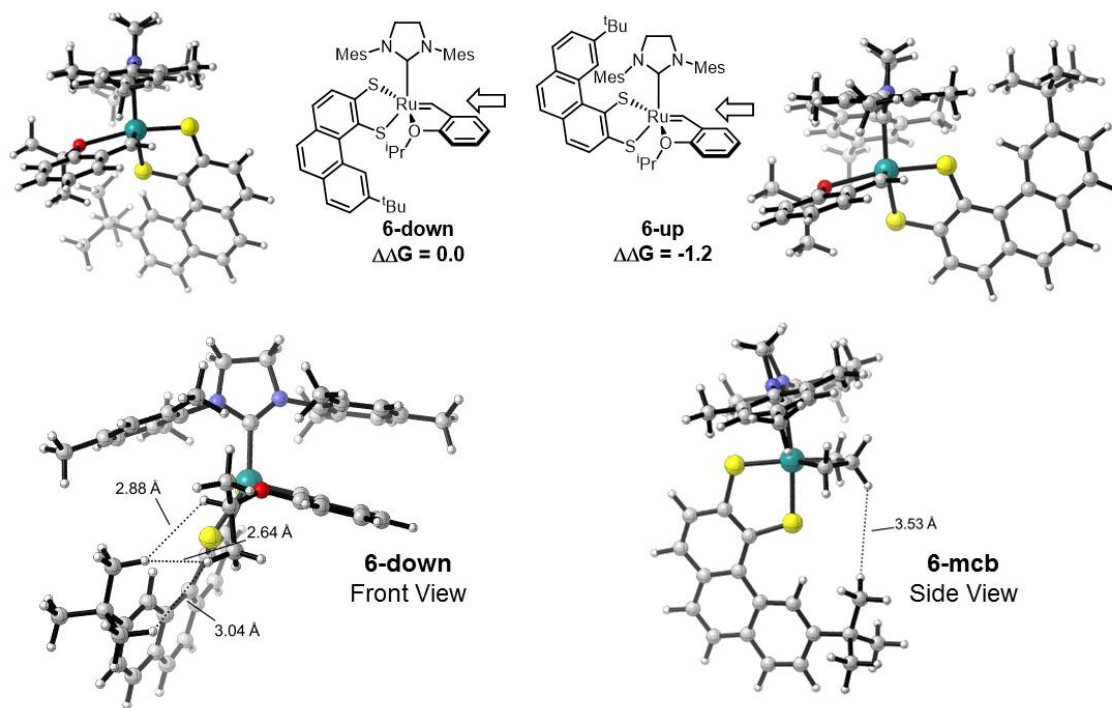


Figure C.S8. 3D side views of **6-down** (left) and **6-up** (right) with free energy difference shown with 2D images. Bottom left shows front view of **6-down** with distances to isopropyl group indicated. Bottom right shows a side view of **6-mcb** with distance of the tert-butyl to metallacycle β -substituent.

To see if a substituent larger than methyl would change the preference for the two isomers and have a greater influence on the metallacycle, we computed the structure of catalyst **6**. The up and down isomers are shown in Figure 2.2.5. The larger substituent was predicted to favor the up position by an even larger ratio (7:1) than previous catalysts. The tert-butyl group has a closer proximity to the isopropyl group of the Hoveyda chelate as shown in the front view. The metallacycle (**6-mcb**) also indicates that the tert-butyl group will still not likely significantly hinder Z-olefin metathesis.

Appendix D

Supplemental Computational Information for Chapter 3.2

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QM calculations

All QM calculations were performed with Gaussian09.¹ Conformational searches were performed using MMFF² in Spartan.³ Geometry optimizations and frequencies calculations were performed at the B3LYP⁴ level with LANL2DZ for iron and 6-31G(d) for all other atoms. Transition structures contained one negative frequency. Enthalpies and free energies were computed at 1atm and 298.15K. A correction to the entropy was applied in accordance with the work of Truhlar et al.⁵ Single point energy computations were performed at the B3LYP-D3(BJ)⁶ level with LANL2DZ for iron and 6-311+G(d,p) and CPCM⁷ for water.

MD simulations

The heme iron(IV)-oxo complex involved in the cytochrome-catalyzed oxidative hydroxylation cycle (compound I) was used to model the active form of the cofactor. Simulations were performed using the GPU code (pmemd)⁸ of the Amber 12 package.⁹ The Amber-compatible parameters developed by Cheatham et al.¹⁰ were used for compound I and its axial Cys ligand. Parameters for

macrolactone substrates were generated within the antechamber module using the general AMBER force field (gaff),¹¹ with partial charges set to fit the electrostatic potential generated at the HF/6-31G(d) level by the RESP model.¹² The charges were calculated according to the Merz-Singh-Kollman scheme¹³ using Gaussian 09.¹ Each protein was immersed in a pre-equilibrated truncated cuboid box with a 10 Å buffer of TIP3P¹⁴ water molecules using the tleap module, resulting in the addition of around 11370 solvent molecules. The systems were neutralized by addition of explicit counter ions (Na⁺ and Cl⁻). All subsequent calculations were done using the widely tested Sony Brook modification of the Amber 99 force field (ff99sb).¹⁵ The substrate and enzyme were optimized for total 1000000 steps, with 750000 steepest descent steps and 250000 conjugate gradient steps. The systems were gently heated using six 50 ps steps, incrementing the temperature by 50 K for each step (0-300 K) under constant-volume and periodic-boundary conditions. Water molecules were treated with the SHAKE algorithm such that the angle between hydrogen atoms were kept fixed. Long-range electrostatic effects were modelled using the particle-mesh-Ewald method.¹⁶ An 8 Å cutoff was applied to Lennard-Jones and electrostatic interactions. Harmonic restraints of 30 kcal/(mol Å²) were applied to the solute and the Andersen equilibration scheme was used to control and equalize the temperature. The time step was kept at 1 fs during the heating stages, allowing potential inhomogeneities to self-adjust. Each system was then equilibrated for 2 ns with a 2 fs time step at a constant volume. Production trajectories were then run for an additional 500 ns under the same simulation conditions.

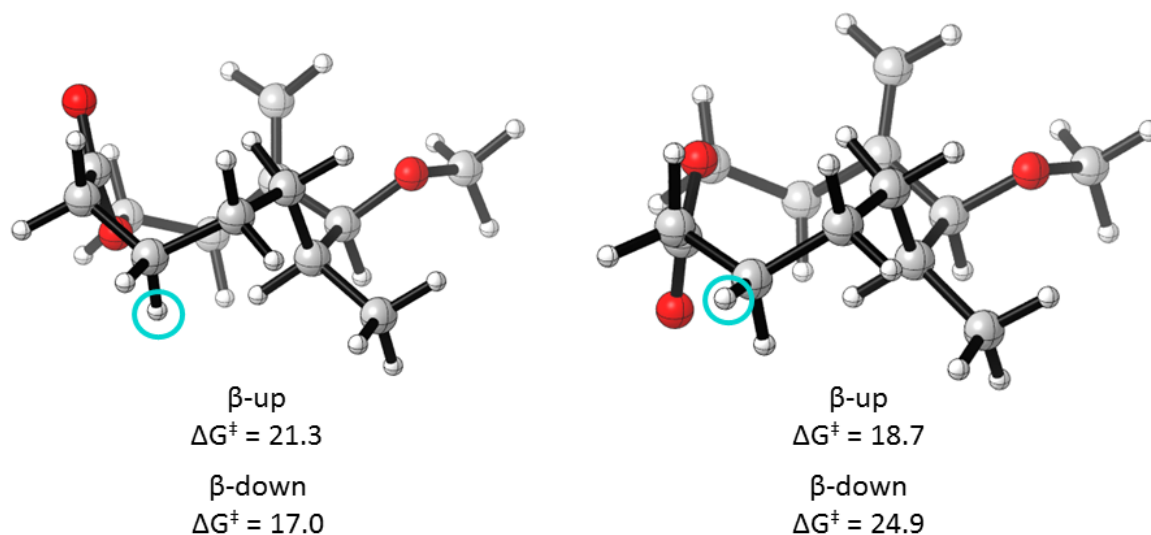


Figure D.S1. The two low energy conformers and the barriers to C-H abstraction of the two possible β -hydrogens. The favored hydrogen for oxidation is circled in each conformer.

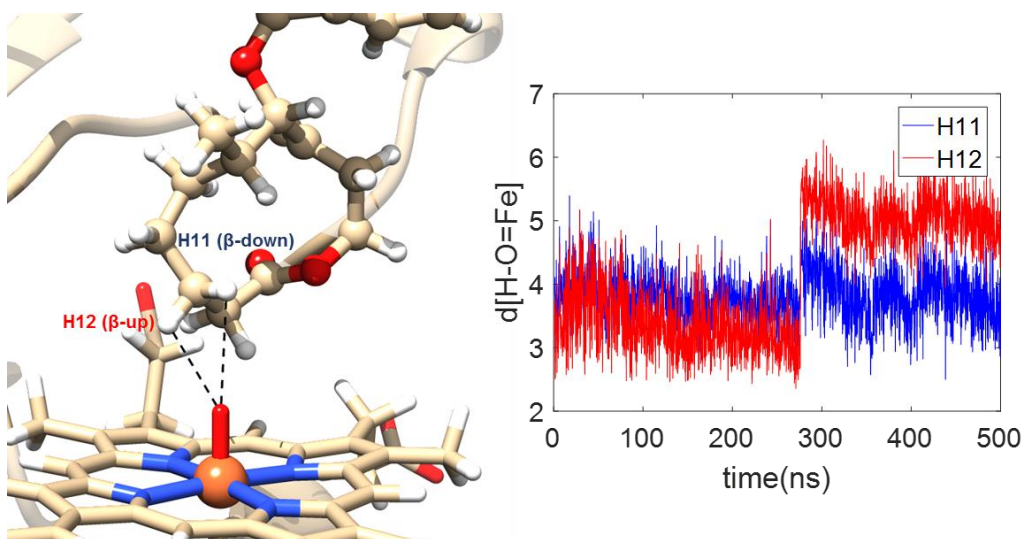


Figure D.S2. 500 ns MD simulation for substrate **4d**.

Analysis of β -oxidation

From the QM model computations, the two conformers of the model of **4** favor different hydrogens for abstraction, unlike the allylic and α -positions. Both hydrogens have low barriers to

abstraction when in one of the conformations. From the 500 ns MD simulation, the tether places C α 3.9 Å and C β 4.0 Å away from the iron-oxo. Comparison of the equatorial (H_{up}) and axial (H_{down}) hydrogens over the course of the 500ns simulation shows that H_{up} is the closest to the iron-oxo for nearly 300 ns before moving far from the catalytic center. H_{down} remains at a constant distance around 4Å, which is seemingly not close enough facilitate catalysis.

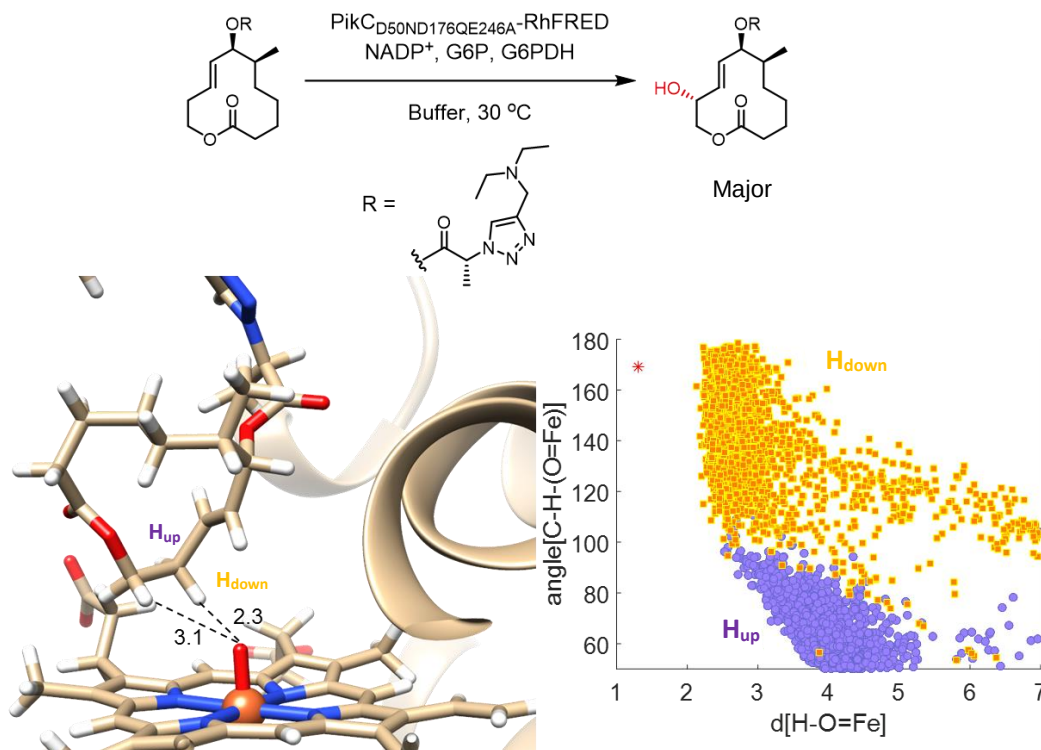


Figure D.S3. Results of 500 ns MD simulation for substrate **14e**. Average bond distances shown in snapshot on the left.

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Appendix E

Supplemental Information for Chapter 4.1

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Materials and Methods

Materials.

Nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) was purchased from Enzo BioSciences. Deuterated water (D₂O) was purchased from Sigma-Aldrich. The GsfF & *Aspergillus terreus* cytochrome P450 reductase co-expression plasmid pESC-GsfF/AtCPR was used previously and constructed as described in Cacho *et al.*¹ The *Penicillium aethiopicum* mutant strains Δ gsfF and Δ gsfD were obtained from previous study in Cacho *et. al.*¹ All other chemicals and media components were purchased from Fisher Scientific.

Experimental Procedures

Purification and characterization of Griseophenone B and desmethyl-dehydrogriseofulvin.

Griseophenone B and desmethyl-dehydrogriseofulvin were isolated and purified from Δ gsfF and Δ gsfD mutant strains of *Penicillium aethiopicum* as described in Cacho *et al.*¹ Briefly, a 3 L of mutant cultures of *P. aethiopicum* was extracted twice with ethyl acetate and evaporated to dryness. The dried extract was subjected to purification using Sephadex LH-20 and high performance liquid chromatography equipped with Phenomenex Luna 5 μ 250 x 10mm C18 reverse-phase column. NMR characterization (1D ¹H and ¹³C, 2D HSQC and HMBC) of the purified compound was then performed on a Bruker AV500 NMR (500 MHz) equipped with 5mm dual cryoprobe at the UCLA Molecular Instrumentation Center.

Isolation of yeast microsomes containing GsfF and *A. terreus* cytochrome P450 reductase.

Yeast microsome extraction was adapted from the method described by Ralston *et al* and Barriuso *et al* and was done as described in Cacho *et al.*¹⁻³ Overnight seed culture of *Saccharomyces*

cerevisiae strain BJ5464-NpgA⁴ harboring the expression plasmid *pESC-gsfF/AtCPR* was inoculated into 500 mL synthetic leucine dropout media with galactose (SGMM, -Leu) and grown at 28 °C with shaking speed at 250 rpm for 24 hours after induction and were pelleted by centrifugation at 4 °C. After washing and of the cell pellet in 100 mL TES buffer (50 mM Tris-HCl pH 7.5, 1 mM EDTA and 0.6 mM sorbitol) with 10 mL 2-mercaptoethanol, the cells were pelleted again and resuspended in 5 mL extraction buffer ((TES buffer supplemented with 1% bovine serum albumin and 2 mM β -mercaptoethanol and 1mM phenylmethylsulfonyl fluoride (Sigma-Aldrich)). The resuspended yeast cells were lysed using Zirconia/silica beads. Cellular debris was removed from the lysate by centrifugation at 10,000*g* and 4°C for 10 min. The microsomal fraction was isolated from the clarified lysate by centrifugation at 100,000*g* and 4 °C for 70 min. The microsomal pellets were weighed prior to resuspension in 1.5 mL of TEG-M buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 20% glycerol, and 1.5 mM 2-mercaptoethanol) and stored frozen at -80 °C. Final concentration of protein in microsome as measured by Bradford assay was 180 μ g per mL.

Assay of GsfF using griseophenone B as substrate: The GsfF in vitro assay was performed in a 100 μ L reaction volume and 30 °C by addition of a final concentration of microsomal protein of 0.18 μ g/ μ L to 100 mM Tris-HCl pH 7.5, 0.1 μ g/ μ L bovine serum albumin, 20 -100 μ M griseophenone B and 2 mM NADPH. Initial reaction velocities were obtained for all the substrate concentration using the given condition. Ten microliter aliquots of the reaction were taken at five minute intervals and quenched via addition of acetonitrile to a final volume of 80%.

The timepoint samples were subjected to LCMS analysis with a Shimadzu 2020 Liquid Chromatography Mass Spectrometer; with the mass spectrometer in a selective-ion-monitoring mode and set to specifically detect the substrate and product from the reaction. Samples were separated on a Phenomenex Kinetex (1.7 μ pore size, 100 Å particle size, 100 x 2 mm) C18 reverse-phase column using a flow rate of 0.3 mL/min on a linear gradient of 5-95% solvent B in 10 min followed by isocratic 95% solvent B for another 2.5 min (solvent A: water with 0.1% (v/v) formic acid, solvent B: acetonitrile with 0.1% (v/v) formic acid). The total yield of **14** was measured by comparing the area under the chromatogram peak of **14** against a standard curve of known amount of **14** injected and analyzed by LCMS.

The GsfF in vitro assay in D₂O was performed and analyzed as the same manner as above except the reaction was performed such that the final concentration of deuterium is ~96%.

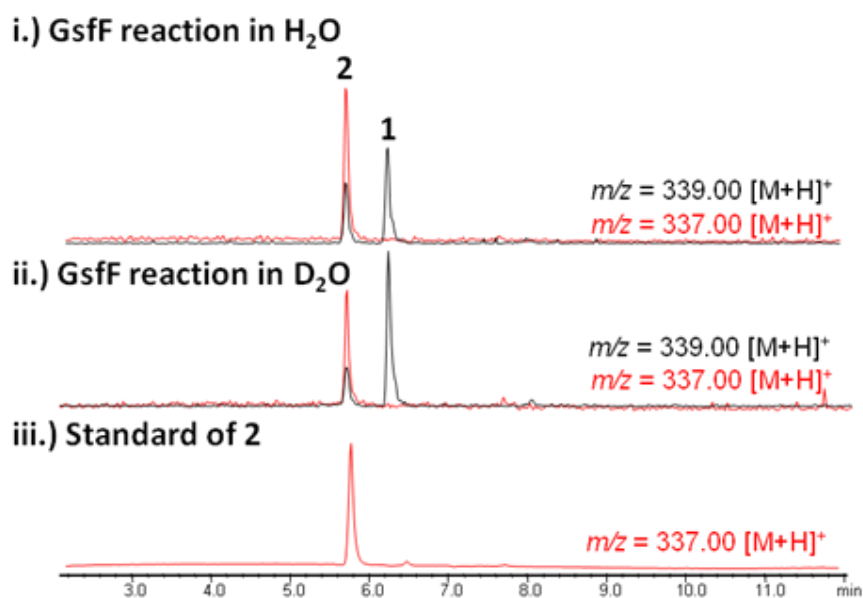
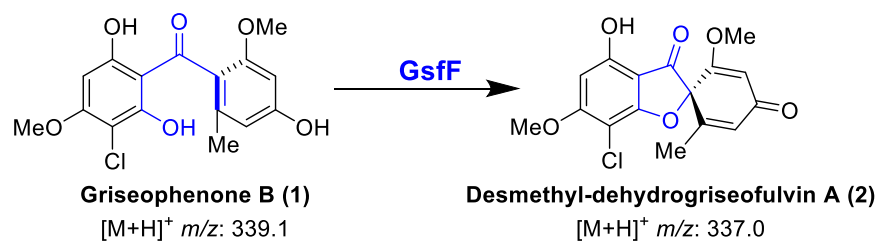


Figure E.S1. (Top) Conversion of **1** to **2** showing the expected mass-to-charge ratio (m/z) of the species in the reaction. (Bottom) Extracted ion chromatogram of **1** ($m/z = 339$, in black) and **2** ($m/z = 337$, in red) from the LCMS analysis of the GsfF reaction performed in water (trace i) and D₂O (trace ii) showing conversion of **1** to **2**. The LCMS trace for product **2** was also obtained as comparison (trace iii).

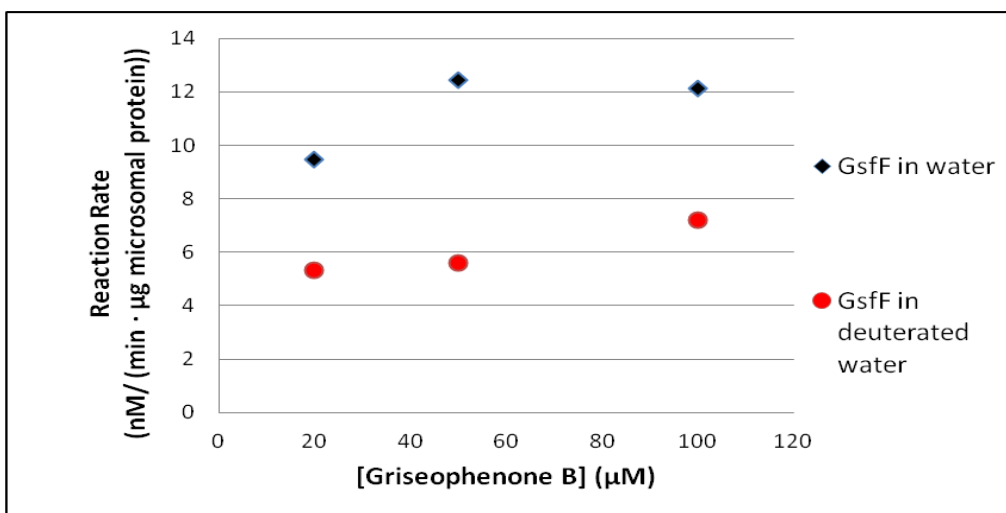


Figure E.S2. Reaction rates of GsfF in 20 µM, 50 µM and 100 µM **1** in water (black) and deuterated water (red). Taking the ratio of the rates in water and D₂O gives an average deuterium kinetic isotope effect of 1.90 ± 0.29 .

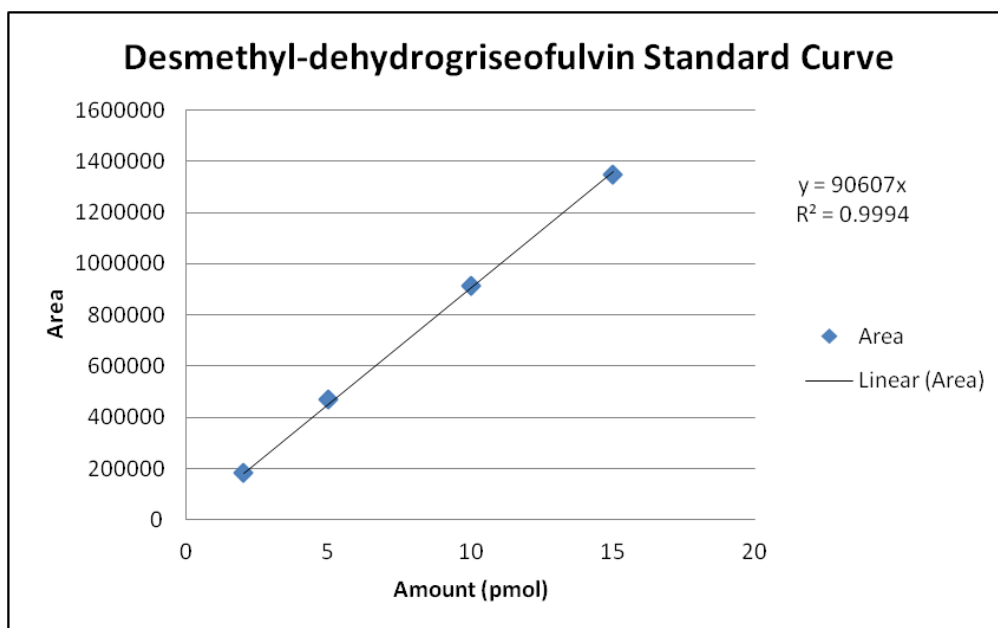


Figure E.S3. Standard curve used to quantify the amount of **14** in the injected samples from the quenched reaction.

Table E.S1. NBO Charges of 1

Atom	No	Charge
C	1	0.37089
C	2	-0.17044
C	3	0.37009
C	4	-0.3008
C	5	0.42256
C	6	-0.37147
H	7	0.23085
C	8	0.52987
C	9	-0.14937
C	10	0.00264
C	11	0.33847
C	12	-0.31237
C	13	-0.34459
C	14	0.35947
H	15	0.20597
H	16	0.23229
C	17	-0.59986
H	18	0.23666
H	19	0.21823

H	20	0.20706
O	21	-0.65528
H	22	0.49531
O	23	-0.53348
O	24	-0.66499
H	25	0.47362
O	26	-0.51327
O	27	-0.66798
H	28	0.50076
Cl	29	0.03474
O	30	-0.62381
C	31	-0.21133
H	32	0.17648
H	33	0.19689
H	34	0.17667
C	35	-0.20927
H	36	0.19836
H	37	0.17451
H	38	0.17776

Table E.S2. NBO Charges of 6-TS-doublet

Atom	No	Charge
Fe	1	0.34958
O	2	-0.42574
C	3	-0.18046
N	4	-0.44402
C	5	0.16449
C	6	0.15585
C	7	-0.21541
H	8	0.21471
C	9	-0.22745
H	10	0.21291
N	11	-0.43675
C	12	0.16216
C	13	0.16816
C	14	-0.20982
H	15	0.21579
C	16	-0.21633
H	17	0.21515
N	18	-0.42678
C	19	0.1629
C	20	0.14317
C	21	-0.24489

H	22	0.21521
C	23	-0.13683
H	24	0.20643
N	25	-0.43997
C	26	0.15929
C	27	0.16331
C	28	-0.22472
H	29	0.21515
C	30	-0.21746
H	31	0.21576
C	32	-0.19144
C	33	-0.18765
C	34	-0.18928
H	35	0.21397
H	36	0.21579
H	37	0.21217
H	38	0.21488
S	39	0.0576
C	40	-0.71547
H	41	0.20287
H	42	0.20274
H	43	0.2134
C	44	0.35391

C	45	-0.17045
C	46	0.32408
C	47	-0.15776
C	48	0.38634
C	49	-0.36609
H	50	0.23559
C	51	0.54493
C	52	-0.02164
C	53	0.07211
C	54	0.44658
C	55	-0.29733
C	56	-0.36048
C	57	0.39843
H	58	0.20804
H	59	0.23276
C	60	-0.59715
H	61	0.24903
H	62	0.21965
H	63	0.20351

O	64	-0.66915
H	65	0.48833
O	66	-0.51227
O	67	-0.6497
H	68	0.47722
O	69	-0.51952
O	70	-0.66622
H	71	0.50421
Cl	72	0.00518
O	73	-0.59009
C	74	-0.2115
H	75	0.17716
H	76	0.1966
H	77	0.1774
C	78	-0.21134
H	79	0.1964
H	80	0.19205
H	81	0.17271

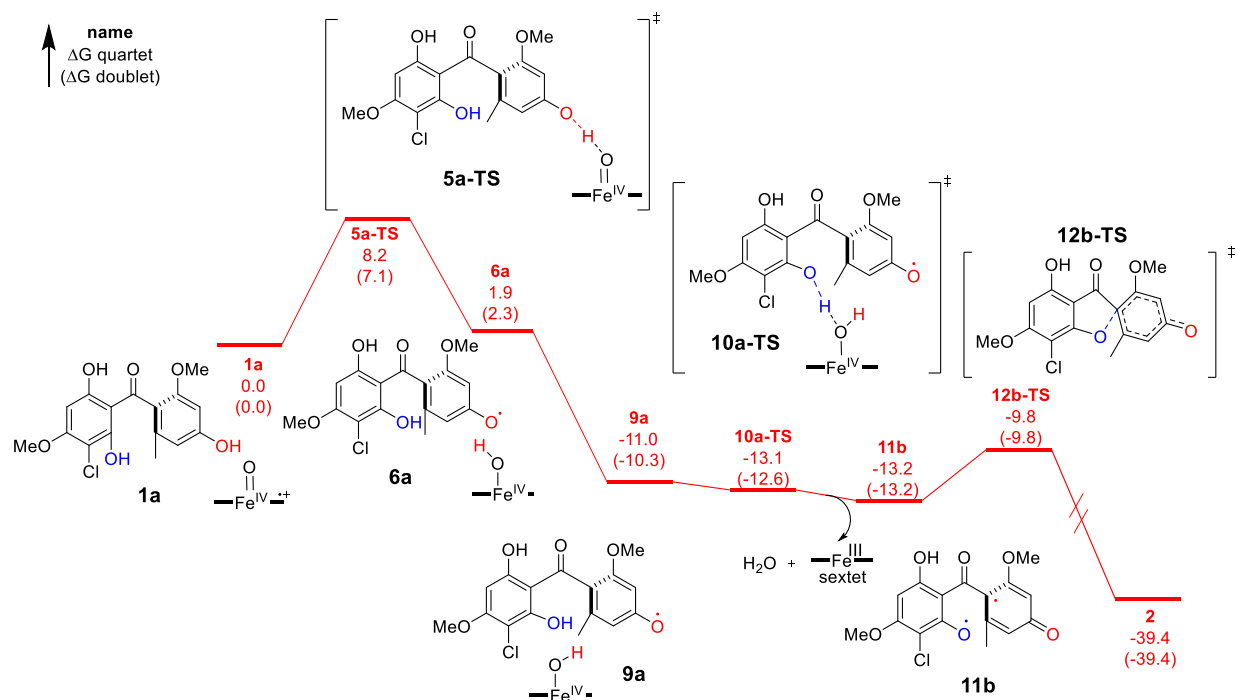


Figure E.S4. Energy Diagram for Initial Abstraction from Ring A

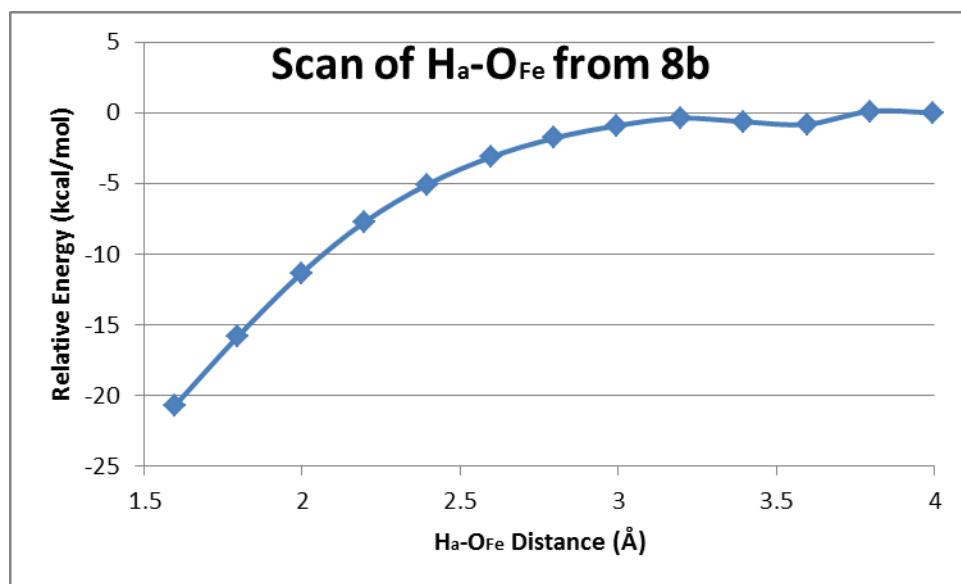


Figure E.S5. Scan of Second H-Abstraction from Intermediate 8b

Table E.S3. Bond distances and energies of scan in Figure E.S5

H_a-O_{Fe} Distance (Å)	Energy (Hartree)	Relative Energy (kcal/mol)
3.995985813	-3155.340032	0
3.795985813	-3155.339859	0.108584244
3.595985813	-3155.341336	-0.818623794
3.395985813	-3155.341036	-0.63017014
3.195985813	-3155.340629	-0.375018503
2.995985813	-3155.341476	-0.906562975
2.795985813	-3155.342887	-1.79200398
2.595985813	-3155.345042	-3.143879071
2.395985813	-3155.348113	-5.07126195
2.195985813	-3155.35237	-7.742607542
1.995985813	-3155.35817	-11.38214382
1.795985813	-3155.365253	-15.82676223
1.595985813	-3155.37309	-20.74472361

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Appendix F

Supplemental Information for Chapter 4.2

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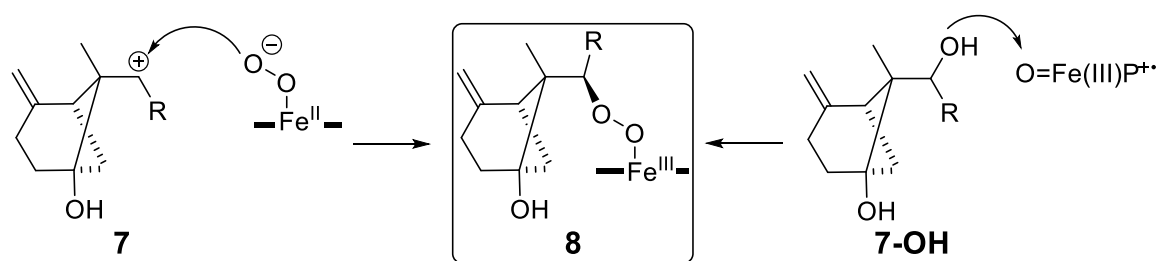


Figure F.S1. Ortiz de Montellano proposed alternate mode for formation of **8**.

Ortiz de Montellano¹ proposes that **7-OH** could be converted to **8** as shown but Tang and coworkers have shown that **7-OH** is not converted to **4**.²

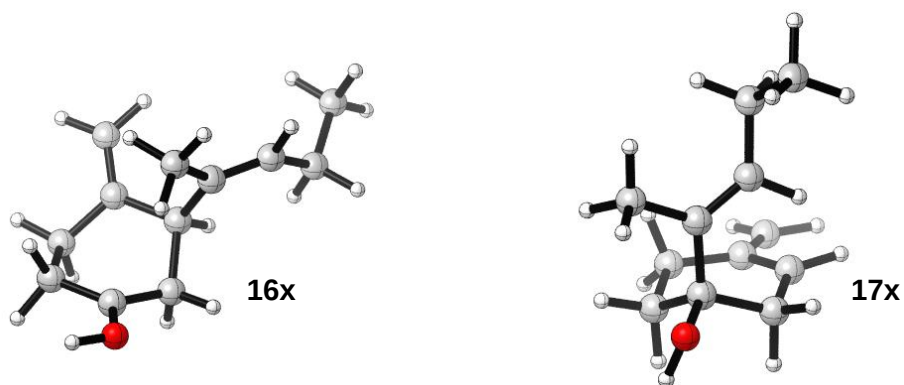


Figure F.S2. CYLview images of **16x** (left) and **17x** (right). Both structures were formed spontaneously upon optimization of various conformers of **7x**. XYZ coordinates are made available below.

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