APPENDIX 6[†]

Synthetic Summary for Chapter 4

and Further C–H Functionalization Studies

A6.1 INTRODUCTION

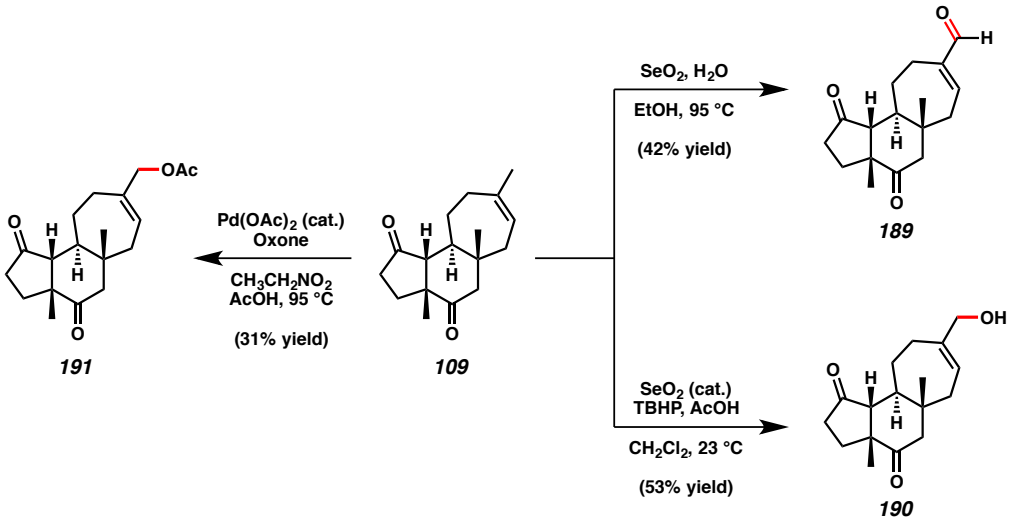
This Appendix summarizes the transformations of the cyanthiwigin core (**109**) and its hydrogenated counterpart (**193**) under the various conditions for intermolecular C–H functionalization detailed in Chapter 4. Additionally, efforts toward intramolecular C–H amination are presented, along with preliminary data from enzymatic oxidation studies.

A6.2 SUMMARY OF INTERMOLECULAR C–H FUNCTIONALIZATION

Overall, our investigations into the reactivity of the cyanthiwigin core (**109**) involved the formation of three allylic oxidation products over seven different conditions for oxidation examined. A summary of product formation from our allylic oxidation studies is presented in Scheme A6.1

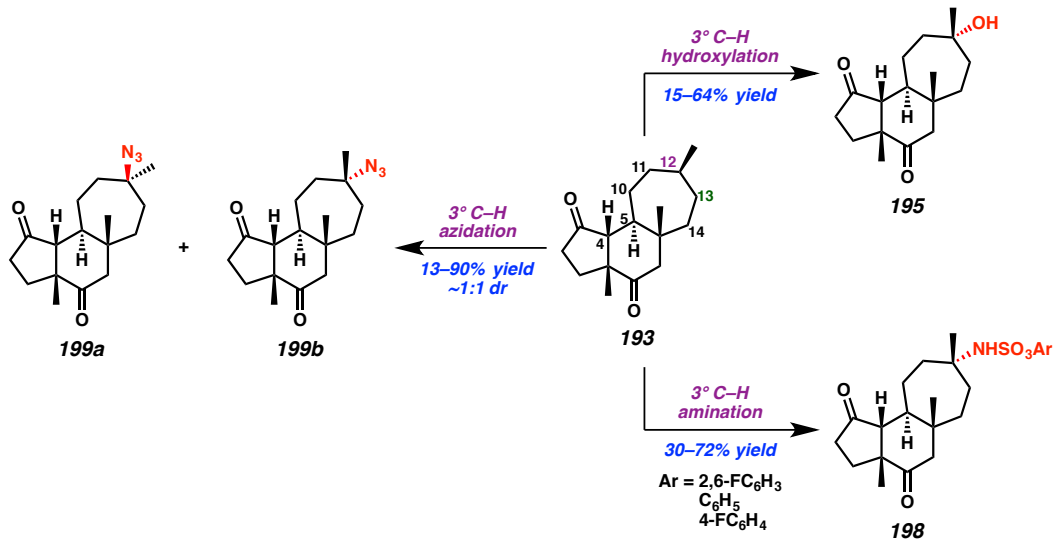
[†] The enzymatic oxidations described in this appendix were performed in collaboration with Dr. David Romney in the Arnold research group at Caltech.

*Scheme A6.1 Summary of the allylic C–H acetoxylation reactions of the cyanthiwigin core (**109**)*



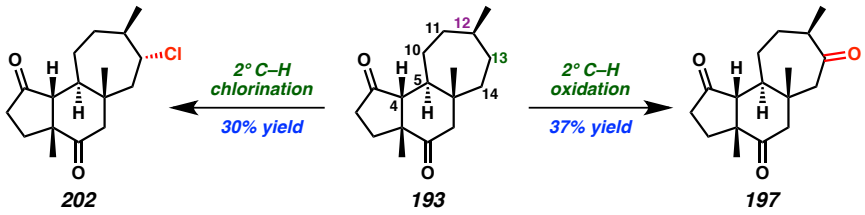
Explorations into the reactivity of the hydrogenated cyanthiwigin core (**193**) supplied six products resulting from tertiary C–H oxidation: hydroxylation (6 methods), amination (2 methods), and azidation (3 methods), which are depicted in Scheme A6.2. Efforts to apply the conditions for tertiary C–H azidation developed by Groves and co-workers proved inconclusive due to uncertainties about catalyst efficacy and purity.

*Scheme A6.2 Summary of the tertiary C–H oxidation reactions of saturated tricycle **193***



Finally, while secondary C–H oxidation was observed far less frequently than tertiary oxidation, the regioselectivity and stereoselectivity of the two methodologies examined provided unique insights into the reactivity of the hydrogenated cyanthiwigin core (**193**). The products generated from these studies are summarized in Scheme A6.3

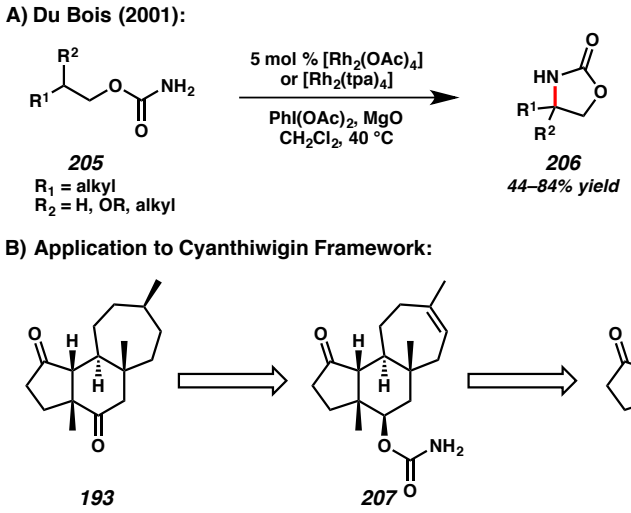
Scheme A6.3 Summary of the secondary C–H oxidation reactions of saturated tricycle ***193***



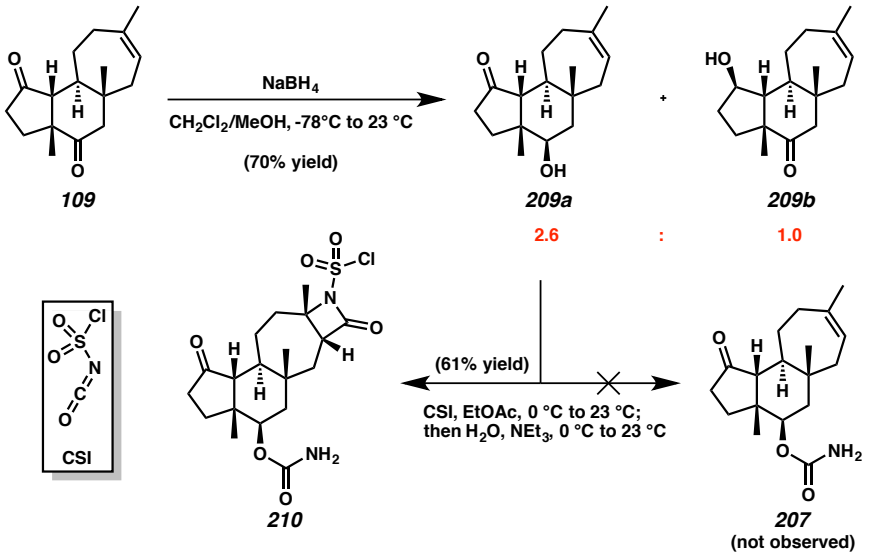
A6.3 EFFORTS TOWARD INTRAMOLECULAR C–H AMINATION

Having gained insight into the reactivity of the cyanthiwigin framework under various conditions for intermolecular C–H functionalization, we turned our attention to strategies for intramolecular C–H functionalization. In 2001, the Du Bois laboratory reported the conversion of carbamates (**205**) to oxazolidinones (**206**) via Rh-catalyzed intramolecular C–H amination (Scheme A6.4A).[1] To apply this approach to the cyanthiwigin core, we would first need to install the carbamate handle to generate a suitable substrate such as **207** for the Du Bois amination. Successful execution of the intramolecular C–H amination would subsequently furnish oxazolidinone **208** (Scheme A6.4B).

Scheme A6.4 Plan for intramolecular C–H amination



*Scheme A6.5 Unexpected reactivity of the cyanthiwigin core (**109**) with CSI*

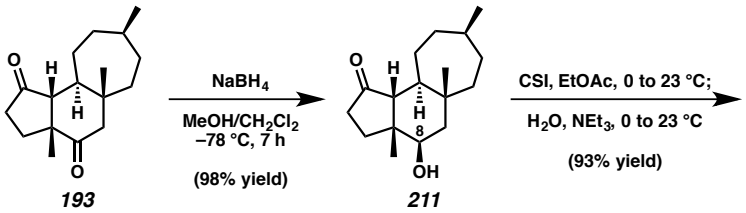


Treatment of tricycle **109** with $NaBH_4$ afforded a mixture of isomers **209a** and **209b** which were separable by column chromatography. We were surprised to find that the reaction of major product **209a** with chlorosulfonyl isocyanate (CSI) did not form the

expected carbamate (**207**). Instead, β-lactam **210** was isolated in 61% yield, indicating that CSI had undergone cycloaddition with the olefin moiety[2] in addition to installing the carbamate group at the secondary alcohol (Scheme A6.5).

Since tetracycle **210** was unreactive under Du Bois's conditions for C–H amination, we prepared a carbamate substrate that would preclude the possibility of cycloaddition with CSI. Treatment of hydrogenated tricycle **193** with $NaBH_4$ at –78 °C effected regioselective carbonyl reduction, furnishing C8 alcohol **211** as a single diastereomer in excellent yield (Scheme A6.6). Reaction of **211** with CSI afforded carbamate **212** which was unreactive under various C–H amination conditions employing different Rh catalysts, solvents, and temperatures. Efforts to effect intramolecular C–H amination using conditions reported by He and co-workers[3] were also unsuccessful, as were procedures employing elevated levels (up to 50 mol %) of Rh catalyst.

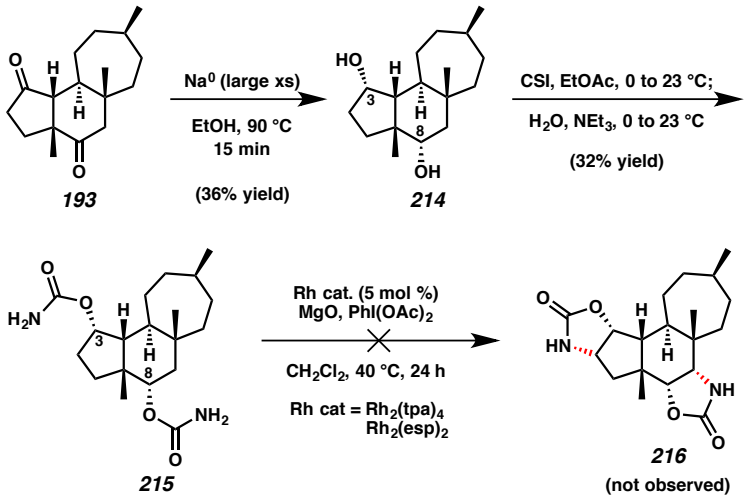
*Scheme A6.6 Efforts toward intramolecular C–H amination of carbamate **212***



Rh cat.	Solvent	Temperature
$Rh_2(OAc)_4$	CH_2Cl_2	40 °C
$Rh_2(tfa)_4$	CH_2Cl_2	40 °C
$Rh_2(esp)_2$	CH_2Cl_2	40 °C
$Rh_2(cap)_4$	CH_2Cl_2	40 °C
$Rh_2(tpa)_4$	CH_2Cl_2	40 °C
$Rh_2(esp)_2$	DCE	85 °C
$Rh_2(tpa)_4$	DCE	85 °C

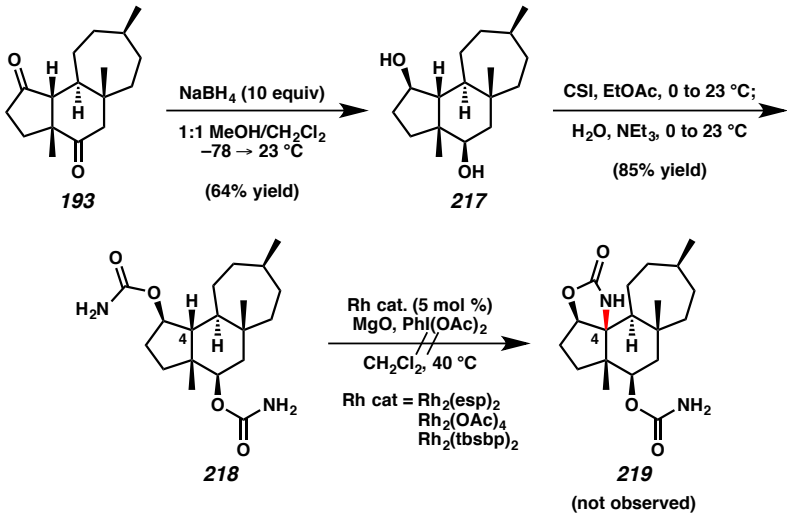
We hypothesized that the apparent lack of reactivity of carbamate **212** under intramolecular C–H amination conditions could be due to the stereochemical configuration of the molecule. Namely, the axial positioning of the C8 carbamate functionality in the six-membered B-ring of **212** could be hindering reactivity. To test this hypothesis, we designed a synthesis of a carbmate with the opposite stereochemistry at C8. After repeated efforts to effect carbonyl reduction from the β-face of **193** using L-selectride, K-selectride, and SmI_2 yielded exclusively α-face reduction, we finally discovered that treatment of tricycle **193** with a large excess (70 equiv) of sodium metal in boiling ethanol induced rapid reduction of both ketones from the β-face, generating diol **214** with the desired stereochemistry at C3 and C8. Subsequent reaction with CSI furnished bis-carbamate **215**, which, disappointingly, was also unreactive under Du Bois's conditions for Rh-catalyzed intramolecular C–H amination (Scheme A6.7).

*Scheme A6.7 Efforts toward intramolecular C–H amination of bis-carbamate **215***



Considering the lack of reactivity of both **212** and **215**, we surmised that the difficulty may be arising from the fact that in both cases a secondary C–H bond was targeted. As such, we reasoned that bis-carbamate **218** could undergo tertiary C–H activation at C4 to generate oxazolidinone **219**. Unfortunately, after preparation of **218** via borohydride reduction of **193** and subsequent carbamate formation, we found that bis-carbamate **218** was also unreactive under conditions for Rh-catalyzed C–H amination, returning unreacted starting material as was observed in all previous cases (Scheme A6.8).

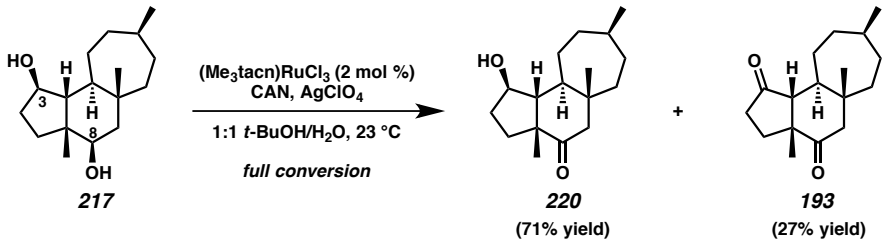
*Scheme A6.8 Efforts toward intramolecular C–H amination of bis-carbamate **218***



Subjection of diol **217** to Du Bois's Ru-catalyzed conditions for intermolecular tertiary C–H hydroxylation[4] resulted in re-oxidation of the secondary alcohols, furnishing alcohol **220** and diketone **193** (Scheme A6.9). The product distribution of this reaction indicates that the C8 hydroxyl is more readily oxidized than the C3 hydroxyl in diol **217**. Significantly, this transformation enables access to the C3-hydroxylated tricycle, which is

generally inaccessible from hydride reduction of diketone **193**, which tends to produce the C8-reduced alcohol product **211** (cf. Scheme A6.6).

*Scheme A6.9 Re-oxidation of diol **217** using Du Bois's Ru-catalyzed C–H hydroxylation conditions*



217 ***220*** (71% yield) ***193*** (27% yield)

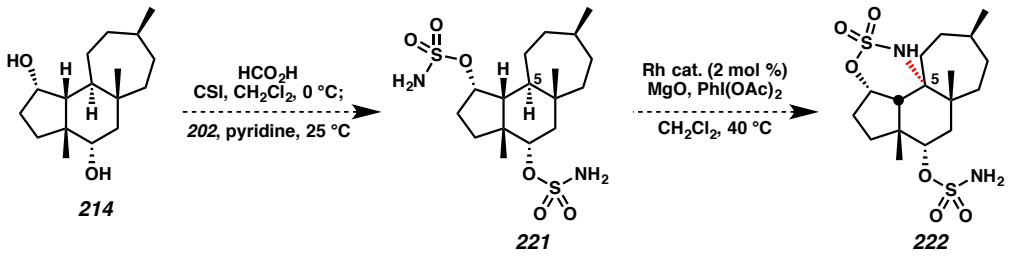
A6.4 FUTURE DIRECTIONS

These investigations into intramolecular C–H amination of the cyanthiwigin core demonstrate that C–H functionalization of molecules with complex three-dimensional architectures remains a challenging research goal. We believe that much of the difficulties encountered in our studies toward intramolecular C–H amination can be attributed to steric factors, given the compactness of the cyanthiwigin core and the density of tertiary and quaternary stereocenters around the potential sites of reactivity. The two methyl substiuents at the A–B and B–C ring junctures contribute significantly to steric deactivation of the β-face of the cyanthwigin core, as observed in the facial selectivity exhibited by carbonyl reduction reactions.

A6.4.1 INTRAMOLECULAR C–H AMINATION

We anticipate that a potential future direction for this project could entail intramolecular C–H amination at the C5 tertiary C–H bond on the α-face of the molecule, thereby avoiding the steric influence of the β-face methyls. This reactivity could be studied using bis-sulfamate **221**, which would be accessible from diol **214** (Scheme A6.10). The Du Bois group showed previously that sulfamates could undergo intramolecular C–H amination in the presence of a Rh catalyst, oxidant, and additive, generating a six-membered ring in the product which could be hydrolyzed to generate a 1,3-functionalized amine derivative.[5] This is a critical difference from the carbamate reactivity because the formation of a six-membered cycle enables access to sites in the cyanthiwigin core previously unreachable using the carbamate handles at C3 and C8.

Scheme A6.10 Future directions toward intramolecular C–H amination

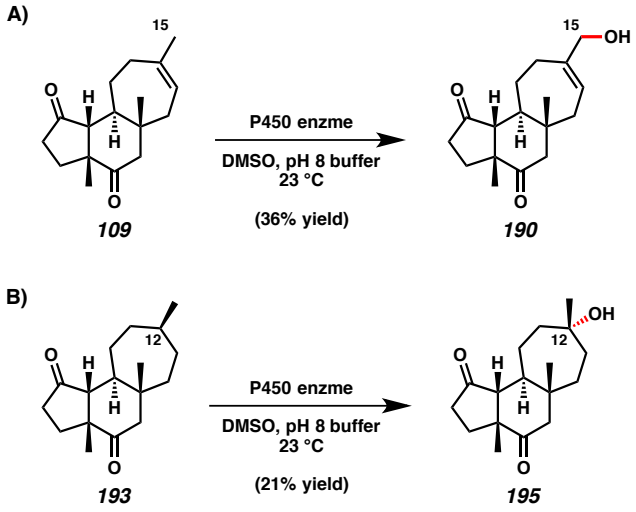


A6.4.2 ENYZMATIC C–H OXIDATION

Another frontier for C–H oxidation includes hydroxylation by enzymatic catalysts. Preliminary studies show that treatment of the cyanthiwigin core (**109**) with a mutated P450 enzyme catalyst[6] results in allylic oxidation at the C15 position, as was observed in the selenium dioxide studies, along with multiple other unidentified products (Scheme

A6.11A). Likewise, hydrogenated tricycle **193** reacts in a familiar fashion with the enzyme catalyst, furnishing the C12 alcohol **195** under the reaction conditions (Scheme A6.11B), the same product as was observed in methods employing synthetic catalysts for C–H hydroxylation (see Chapter 4). Future studies in this research area would involve exploring the reactivity of these two scaffolds with various other enzyme catalysts that have been prepared out of directed evolution studies.

*Scheme A6.11 Preliminary data toward enzymatic oxidation of tricycles **109** and **193***



A6.5 EXPERIMENTAL SECTION

A6.5.1 MATERIALS AND METHODS

Unless noted in the specific procedure, reactions were performed in flame-dried glassware under argon atmosphere. Dried and deoxygenated solvents (Fisher Scientific) were prepared by passage through columns of activated aluminum before use.[7] Methanol (Fisher Scientific) was distilled from magnesium methoxide immediately prior to use. Triethylamine (Oakwood Chemicals) was distilled from calcium hydride immediately prior to use. Anhydrous ethanol, *tert*-butanol, and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich in sure-sealed bottles and used as received unless otherwise noted. Commercial reagents (Sigma Aldrich, Alfa Aesar, or Oakwood Chemicals) were used as received. Catalysts $(Me_3tacn)RuCl_3$ and $Rh_2(esp)_2$ were donated by the Du Bois group (Stanford) and used without further purification. The $Rh_2(tbsbp)_2$ was donated by the Davies group (Emory) and used without further purification. Brine is defined as a saturated aqueous solution of sodium chloride. Reactions requiring external heat were modulated to the specified temperatures using an IKAmag temperature controller. Reaction progress was monitored by thin-layer chromatography (TLC) or Agilent 1290 UHPLC-LCMS. TLC was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching, potassium permanganate, or *p*-anisaldehyde staining. Silia*Flash* P60 Academic Silica gel (particle size 0.040–0.063 mm) was used for flash chromatography. NMR spectra were recorded on a Varian Mercury 300 spectrometer (at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR), a Varian Inova 500 spectrometer (at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR), or a Bruker AV III HD spectrometer equipped with a Prodigy liquid nitrogen

temperature cryoprobe (at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR), and are reported in terms of chemical shift relative to residual $CHCl_3$ (δ 7.26 and δ 77.16 ppm, respectively). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Abbreviations are used as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet. Infrared (IR) spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer using thin film samples on KBr plates, and are reported in frequency of absorption (cm^{-1}). High-resolution mass spectra (HRMS) were obtained from the Caltech Mass Spectral Facility using a JEOL JMS-600H High Resolution Mass Spectrometer with fast atom bombardment (FAB+) ionization mode or were acquired using an Agilent 6200 Series TOF with an Agilent G1978A Multimode source in electrospray ionization (ESI+) mode. Optical rotations were measured with a Jasco P-1010 polarimeter at 589 nm using a 100 mm path-length cell.

A6.5.2 PREPARATIVE PROCEDURES

A6.5.2.1 GENERAL PROCEDURES

General Procedure A. *Sodium borohydride reduction*. To a solution of tricyclic diketone **109** or saturated tricyclic diketone **193** (1.0 equiv) in 1:1 CH_2Cl_2/MeOH (0.02 M) was added a solution of sodium borohydride (5.0 equiv for mono-reduction, 10 equiv for bis-reduction) in 1:1 CH_2Cl_2/MeOH (0.02 M) at –78 °C. The reaction mixture was allowed to warm to 23 °C over the course of six hours. When TLC analysis indicated full consumption of starting material, the reaction was quenched with acetone and 2N NaOH.

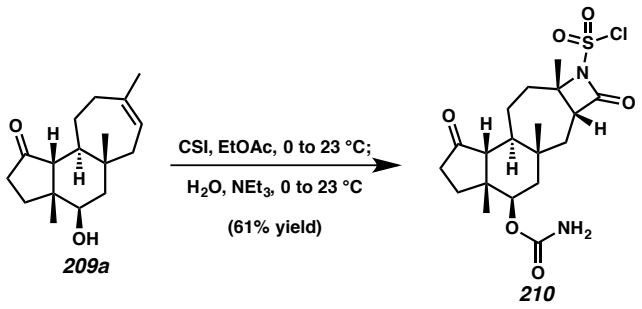
The phases were separated, and the organic layer was immediately washed with brine and dried over sodium sulfate. After filtration and concentration under reduced pressure, the crude residue was purified by silica gel column chromatography (ethyl acetate/hexanes).

General Procedre B. *Reaction with CSI*. To a solution of alcohol **209a** or **211** or diol **214** or **217** (1.0 equiv) in ethyl acetate (0.31 M) at 0 °C was added dropwise chlorosulfonyl isocyanate (1.33 equiv for **209a** or **211**, 2.66 equiv for **214** or **217**). The resulting mixture was stirred at 0 °C for 10 minutes, after which time the ice/water bath was removed, and the reaction allowed to warm to 23 °C. After 10 hours, the reaction mixture was cooled to 0 °C once more, and water (3 mL) was added dropwise, followed by dropwise addition of triethylamine (2.03 equiv for **209a** or **211**, 4.06 equiv for **214** or **217**). The resulting mixture was stirred at 23 °C for 24 hours, after which time the phases were separated, and the aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography, (ethyl acetate/hexanes).

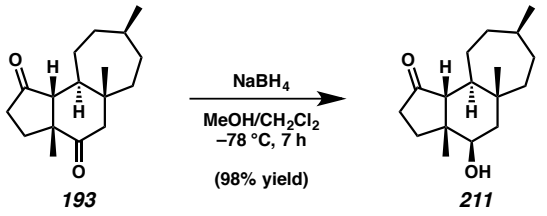
General Procedure C. *Rh-catalyzed intramolecular C–H amination*. A flame-dried 1-dram vial under argon was charged with carbamate **212** or bis-carbamates **215** or **218** (1.0 equiv), magnesium oxide (2.3 equiv), (diacetoxyiodo)benzene (1.4 equiv), and Rh catalyst (0.05 equiv), and the resulting mixture was diluted with dichloromethane (0.02 M in substrate). The vial was sealed with a Teflon-lined cap and heated to 40 °C. After 24 hours, heating was discontinued, and the reaction mixture was diluted with

dichloromethane (2 mL) and filtered through a pad of Celite, rinsing the filter cake with dichloromethane (2x). The filtrate was concnetrated in vacuo, and the crude residue was purified by silica gel column chromatography (ethyl acetate/hexanes).

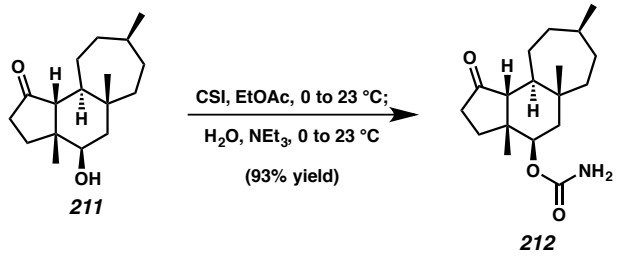
A6.5.2.2 SUBSTRATE PREPARATION FOR INTRAMOLECULAR C–H AMINATION STUDIES



***β*-Lactam 210.** Prepared using General Procedure B (3.0 mg, 61% yield). Column eluent: 20% to 30% to 40% to 75% ethyl acetate in hexanes. Partial characterization data is as follows: R*f* = 0.20 (75% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.82 (dd, *J* = 3.9, 2.3 Hz, 1H), 4.56 (br s, 2H), 3.20 (dd, *J* = 10.9, 7.7 Hz, 1H), 2.44–2.32 (m, 3H), 2.07–2.00 (m, 2H), 1.96 (m, 1H), 1.84 (d, *J* = 4.1 Hz, 1H), 1.82 (s, 3H), 1.79 (d, *J* = 3.3 Hz, 2H), 1.74–1.67 (m, 3H), 1.23–1.17 (m, 2H), 0.97 (s, 3H), 0.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 219.0, 164.6, 155.9, 74.1, 72.6, 57.8, 57.3, 46.4, 41.9, 41.3, 39.4, 35.5, 35.1, 33.3, 30.3, 23.8, 23.3, 22.6, 17.3; HRMS (EI+) *m/z* calc'd for $C_{19}H_{28}N_2O_6SCl$ $[M+H]^+$: 447.1357, found 447.1353; $[a]^{25}_D$ –13.3 (*c* 0.20, $CHCl_3$).

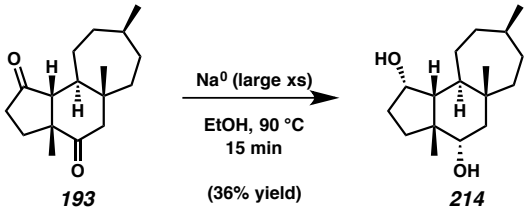


Tricyclic Alcohol 211. Prepared using General Procedure A (10.9 mg, 98% yield). Column eluent: 10% to 20% ethyl acetate in hexanes. Full characterization data is as follows: R*f* = 0.24 (25% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.69 (t, *J* = 3.4 Hz, 1H), 2.37 (dddd, *J* = 19.6, 10.8, 2.3, 0.8 Hz, 1H), 2.32–2.22 (m, 1H), 2.02–1.94 (m, 1H), 1.75–1.67 (m, 3H), 1.62 (dd, *J* = 14.8, 3.2 Hz, 1H), 1.58 (m, 2H), 1.57–1.49 (m, 3H), 1.42–1.35 (m, 3H), 1.27–1.19 (m, 3H), 1.01 (s, 6H), 0.88 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 220.3, 72.6, 57.7, 45.9, 45.4, 43.4, 41.1, 35.2, 34.9, 33.7, 32.8, 30.9, 30.4, 24.5, 23.5, 23.2, 20.4; IR (Neat Film, KBr) 3462, 2950, 2925, 2867, 1715, 1468, 1385, 1180, 734 cm^{-1}; HRMS (FAB+) *m/z* calc'd for $C_{17}H_{29}O_2$ $[M+H]^+$: 265.2168, found 265.2178; $[a]^{25}_D$ –11.3 (*c* 0.35, $CHCl_3$).

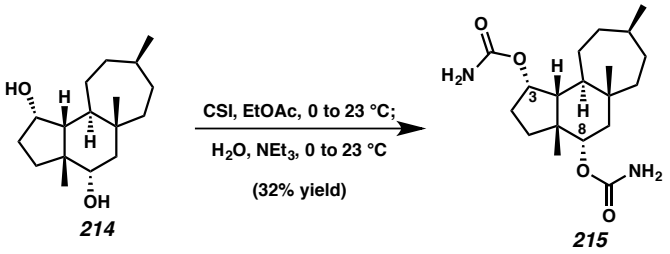


Carbamate 212. Prepared using General Procedure B (2.7 mg, 93% yield). Column eluent: 30% to 50% to 70% ethyl acetate in hexanes. Partial characterization data is as follows: R*f* = 0.34 (50% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.75 (t, *J* = 3.2 Hz, 1H), 4.54 (br s, 2H), 2.44–2.35 (m, 1H), 2.33–2.23 (m, 1H), 2.10–2.00 (m,

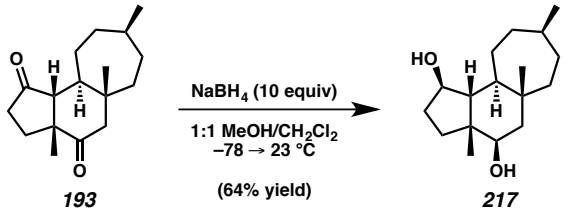
1H), 1.76–1.73 (m, 1H), 1.71 (m, 2H), 1.66–1.61 (m, 2H), 1.54–1.48 (m, 2H), 1.43–1.39 (m, 1H), 1.39–1.35 (m, 2H), 1.34 (m, 1H), 1.25–1.17 (m, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.88 (d, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 215.4, 156.3, 74.8, 57.8, 45.5, 42.1, 40.8, 34.8, 34.7, 33.3, 32.7, 30.6, 29.9, 29.7, 24.3, 23.3, 23.0, 19.7; HRMS (EI+) *m/z* calc'd for $C_{18}H_{30}NO_3$ $[M+H]^+$: 308.2226, found 308.2210; $[a]^{25}_D$ –14.5 (*c* 0.27, $CHCl_3$).



Tricyclic Diol 214. A flame-dried two-necked round-bottom flasked fitted with a reflux condenser and magnetic stir bar was charged with a solution of tricyclic diketone **193** (15 mg, 0.0572, 1.0 equiv) in absolute ethanol (6 mL) and heated to reflux. Once the solution had reached reflux (90 °C), small chunks (~10 mg) of freshly cut sodium metal (90 mg total, 3.94 mmol, 69.0 equiv) were added carefully through the open second neck of the flask. Pieces were added one at a time, waiting for each chunk to dissolve fully before addition of the next. After the last piece of sodium metal had dissolved, the reaction was removed from heat, quenched with ice water (10 mL), and extracted with Et_2O (2 x 10 mL). The combined organic layers were washed with brine and dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (10% to 20% ethyl acetate in hexanes) to afford diol **214** as a white amorphous solid (5.5 mg, 36% yield). Characterization was hampered by persisting impurities.

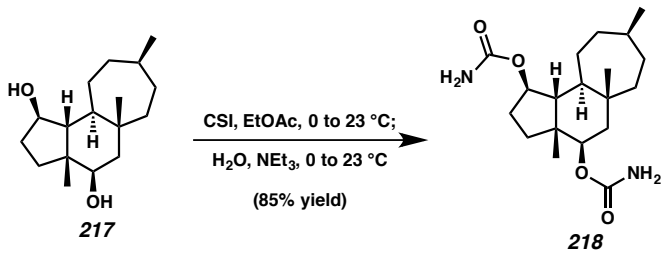


Bis-carbamate 215. Prepared using General Procedure B (2.1 mg, 32% yield). Column eluent: 40% ethyl acetate in hexanes. Characterization was hampered by persisting impurities.



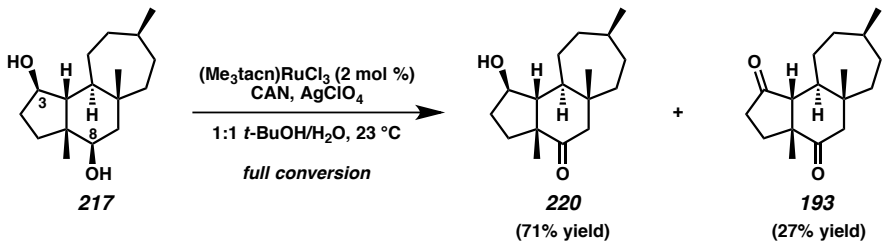
Tricyclic Diol 217. Prepared using General Procedure A (11.8 mg, 64% yield). Column eluent: 25% ethyl acetate in hexanes. Full characterization data is as follows: R*f* = 0.43 (50% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.97 (td, *J* = 6.3, 3.0 Hz, 1H), 3.64 (dd, *J* = 10.0, 4.1 Hz, 1H), 2.06–1.96 (m, 1H), 1.70–1.64 (m, 3H), 1.64–1.60 (m, 2H), 1.59–1.55 (m, 2H), 1.50 (d, *J* = 10.0 Hz, 1H), 1.48–1.43 (m, 2H), 1.43–1.38 (m, 3H), 1.34–1.29 (m, 1H), 1.29–1.25 (m, 2H), 1.24–1.20 (m, 1H), 1.18–1.13 (m, 1H), 1.11 (s, 3H), 0.95 (s, 3H), 0.88 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 80.8, 73.2, 58.7, 46.9, 46.2, 45.5, 42.8, 37.0, 36.7, 35.7, 33.6, 33.4, 32.5, 25.6, 24.9, 22.3, 22.2; IR (Neat Film, KBr) 3338 (br), 2909, 1458, 1376, 1026, 758 cm^{-1}; HRMS

(FAB+) *m/z* calc'd for $C_{17}H_{31}O_2$ $[M+H]^+$: 267.2324, found 267.2336; $[a]^{25}_D$ 7.57 (*c* 1.2, $CHCl_3$).



Bis-carbamate 218. Prepared using General Procedure B (11.8 mg, 85% yield). Column eluent: 50% ethyl acetate in hexanes. Partial characterization data is as follows: R*f* = 0.17 (50% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.91–4.85 (m, 1H), 4.72 (dd, *J* = 5.6, 3.7 Hz, 1H), 4.66 (br s, 4H), 2.27–2.18 (m, 1H), 1.75 (m, 1H), 1.72–1.67 (m, 2H), 1.60 (dd, *J* = 14.6, 5.5 Hz, 3H), 1.53–1.48 (m, 3H), 1.47–1.41 (m, 2H), 1.33 (td, *J* = 7.5, 4.2 Hz, 2H), 1.28–1.23 (m, 2H), 1.07 (s, 3H), 0.99 (ddd, *J* = 12.5, 9.1, 1.7 Hz, 1H), 0.92 (s, 3H), 0.87 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 157.0, 156.6, 83.2, 75.8, 54.3, 46.5, 44.4, 42.6, 42.2, 36.0, 35.8, 35.6, 32.9, 31.8, 30.1, 25.3, 24.6, 23.6, 21.0.

A6.5.2.3 RE-OXIDATION OF DIOL 217 UNDER Ru CATALYSIS



Tricyclic Alcohol 220. A 1-dram vial was charged with (1,4,7-trimethyl-1,4,7-triazacyclononane)ruthenium(III) trichloride (0.2 mg, 0.63 μmol, 0.020 equiv), silver perchlorate (0.3 mg, 1.5 μmol, 0.080 equiv), and water (0.5 mL). The vial was sealed with a Teflon-lined cap and heated to 80 °C with vigorous stirring for 5 minutes. The reaction mixture was then allowed to cool to 23 °C, and a solution of diol **217** (5.0 mg, 18.8 μmmol, 1.0 equiv) in *tert*-butanol (0.50 mL) was added, followed by ceric(IV) ammonium nitrate (30.9 mg, 56.4 μmol, 3.0 equiv). The resulting mixture suspension was stirred at 23 °C for 25 minutes, at which time a second portion of ceric(IV) ammonium nitrate (30.9 mg, 56.4 μmol, 3.0 equiv) was added. After 24 hours, the reaction was quenched with methanol (2 mL), diluted with water (5 mL), and extracted with ethyl acetate (3 x 5 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (10% to 20% to 50% ethyl acetate in hexanes), furnishing tricyclic alcohol **220** (3.6 mg, 71% yield) and tricyclic diketone (1.7 mg, 27% yield). Full characterization data for **220** is as follows: R*f* = 0.59 (50% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.20 (ddd, *J* = 7.0, 4.8, 1.5 Hz, 1H), 2.35 (d, *J* = 14.6 Hz, 1H), 2.32–2.25 (m, 1H), 1.99 (d, *J* = 14.7 Hz, 1H), 1.92–1.84 (m, 1H), 1.85–1.77 (m, 2H), 1.71–1.65 (m, 1H), 1.65–1.59 (m, 2H), 1.52–1.40 (m, 5H), 1.35–1.30 (m, 2H), 1.29 (s, 3H), 1.26–1.21 (m, 1H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 215.7, 80.7, 62.9, 53.7, 53.3, 48.9, 42.6, 40.0, 37.3, 35.3, 34.4, 32.0, 29.5, 24.5, 23.6 (x2), 19.1; IR (Neat Film, KBr) 3419 (br), 2918, 2869, 1697, 1456, 1384, 1269, 1021, 974 cm^{-1}; HRMS (EI+) *m/z* calc'd for $C_{17}H_{29}O_2$ $[M+H]^+$: 265.2168, found 265.2171; $[a]^{25}_D$ –58.6 (*c* 0.36, $CHCl_3$).

A6.5.2.4 ENZYMATIC C–H OXIDATION PROCEDURES

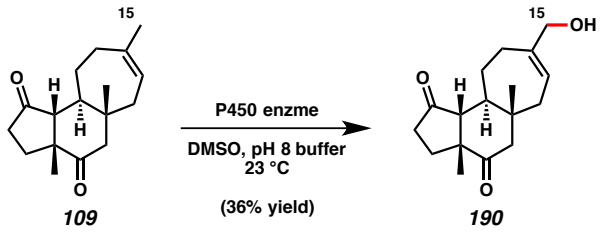
Protein Expression:

E. coli DH5α cells that harbored a pCWori plasmid encoding variant 8C7 under the control of the Plac promoter were stored as a glycerol stock. These cells were streaked onto a plate of LB_{amp}/agar, which was incubated at 37 °C. After 12 h, the plate was stored at 4 °C until further use.

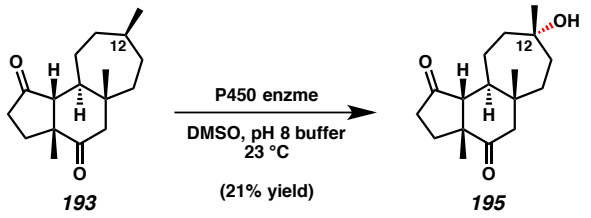
A 5-mL culture of LB_{amp} was inoculated with a single colony from the aforementioned agar plate, then shaken at 37 °C (220 RPM). After 12 hours, the culture was poured into a 1-L Erlenmeyer flask that contained 500 mL of TB_{amp} with 500 μL of trace metals mix. This new culture was shaken at 37 °C (220 RPM). After 3 hours, the culture was chilled in ice. After 20 minutes, 250 μL of IPTG and 500 μL of ALA were added to the culture, which was shaken at 220 RPM at 25 °C. After 17 hours, the culture was transferred into plastic bottles, then subjected to centrifugation at 5000×*g* at 4 °C for 10 minutes. The supernatant was discarded, and the combined cell pellet was stored at –30 °C.

Lysis:

After thawing, the cell pellet (2.6 g) was suspended in a lysis cocktail consisting of hen egg-white lysozyme (10.2 mg), bovine pancreas DNase (1 mg), BugBuster (1 mL) and potassium phosphate buffer (10 mL, pH 8, 100 mM phosphate). The cell pellet was suspended through vortexing, then the suspension was shaken at 37 °C (220 RPM). After 15 min, the culture was cooled on ice, and then subjected to centrifugation at 5000×*g* at 4 °C for 10 min. The supernatant was used directly in the biocatalytic transformation.



Enzymatic Oxidation of Tricycle 109. A 20-mL vial was charged with a solution of tricyclic diketone **109** (5.0 mg, 0.0192 mmol, 1.0 equiv) in DMSO (111 μL), followed by β-NADP disodium salt (1.8 mg, 0.1 equiv) and potassium phosphate buffer (3.4 mL, pH 8, 100 mM). The cell lysate (891 μL) was added, followed by *E. coli* alcohol dehydrogenase (17.8 μL). After addition of isopropanol (34.1 μL), the reaction vessel was wrapped in aluminum foil and shaken at 23 °C (230 RPM). After 14 hours, the product was extracted from the reaction mixture with ethyl acetate (3x). (If an emulsion formed, then the mixture was subjected to centrifugation at 4000×*g* for 2 minutes to separate the layers.) The combined organic portions were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (10% to 25% to 40% to 50% ethyl acetate in hexanes), affording unreacted starting material (2.1 mg, 42% recovery), a mixture of unidentified oxidation products (1.0 mg), and allylic alcohol **190** (1.9 mg, 36% yield), which matched previously reported characterization data (see Chapter 4).



Enzymatic Oxidation of Hydrogenated Tricycle 193. A 20-mL vial was charged with a solution of tricyclic diketone **193** (4.5 mg, 0.0171 mmol, 1.0 equiv) in DMSO (111 μL), followed by β-NADP disodium salt (1.8 mg, 0.1 equiv) and potassium phosphate buffer (3.4 mL, pH 8, 100 mM). The cell lysate (891 μL) was added, followed by *E. coli* alcohol dehydrogenase (17.8 μL). After addition of isopropanol (34.1 μL), the reaction vessel was wrapped in aluminum foil and shaken at 23 °C (230 RPM). After 14 hours, the product was extracted from the reaction mixture with ethyl acetate (3x). (If an emulsion formed, then the mixture was subjected to centrifugation at 4000×*g* for 2 minutes to separate the layers.) The combined organic portions were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (10% to 25% to 35% to 50% ethyl acetate in hexanes), affording unreacted starting material (2.5 mg, 56% recovery), a mixture of unidentified oxidation products (0.7 mg), and tertiary alcohol **195** (1.0 mg, 21% yield), which matched previously reported characterization data (see Chapter 4).

A6.6 NOTES AND REFERENCES

(1) Espino, C. G.; Du Bois, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 598–600.

(2) A literature search on the reactivity of CSI shows that examples of cycloaddition with alkenes are known. For a review on CSI, see: Dhar, D. N.; Murthy, K. S. K. *Synthesis* **1986**, *1986*, 437–449.

(3) Cui, Y.; He, C. *Angew. Chem., Int. Ed.* **2004**, *43*, 4210–4212.

(4) McNeill, E.; Du Bois, J. *Chem. Sci.* **2012**, *3*, 1810–1813.

(5) Espino, C. G.; Wehn, P. M.; Chow, J.; Du Bois, J. *J. Am. Chem. Soc.* **2001**, *123*, 6935–6936.

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(7) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.