Article

Evolution of Biocatalytic and Chemocatalytic C–H Functionalization Strategy in the Synthesis of Manzacidin C

Christian R Zwick, and Hans Renata

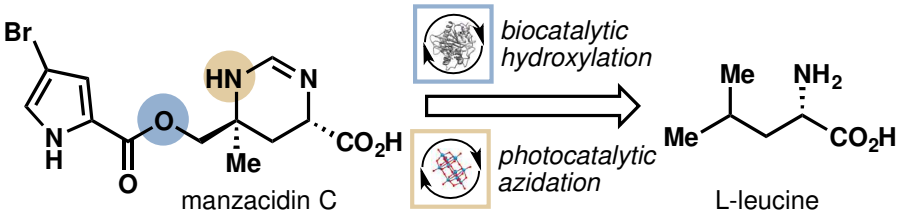
J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.8b00248 • Publication Date (Web): 17 May 2018

Evolution of Biocatalytic and Chemocatalytic C–H Functionalization Strategy in the Synthesis of Manzacidin C

Christian R. Zwick, III, Hans Renata*

Department of Chemistry, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458

hrenata@scripps.edu



Abstract

Due to their unique molecular architecture, the manzacidins have been the subject of intense synthetic efforts in the past two decades. Here, we describe two synthetic approaches towards manzacidin C that center on the enzymatic hydroxylation of unprotected L-leucine. This study also resulted in the discovery of novel synthetic methodologies, including a photocatalytic C–H azidation of unprotected amino acids. Additionally, we describe the use of hydroxylated L-leucine in the preparation of various densely-substituted pyrrolidines.

Introduction

Commonly isolated from marine organisms, the bromopyrrole alkaloids[1] have long fascinated chemists due to their intricately complex structures. Three members of this family, manzacidins A–C were isolated by Kobayashi and co-workers in 1991 from the Okinawan sponge *Hymeniacidon* sp.[2] Although small, the manzacidins exhibit dense structural complexity (Figure 1A). Their core structure contains a 2,4-disubstituted bromopyrrole ester, as well as a

tetrahydropyrimidine motif featuring a carboxylic acid and two stereocenters, including a nitrogen-containing quaternary center.[3] In 2000, Ohfune and co-workers established the first total syntheses of manzacidin A and C, sparking what has become a thriving area in the development of unique methodologies over the past eighteen years.[4] Their landmark strategy established lactone **5** as an advanced intermediate in thirteen steps, which can be converted to the natural product through a highly efficient 54% two-step protocol involving formation of the tetrahydropyrimidine motif, followed by bromopyrrole ester installation (Figure 1B). Since this seminal work, many approaches have developed or applied new methodology towards **5**, constituting a formal synthesis of manzacidin C (the γ epimer of **5** targeted for manzacidin A).[5] Although **5** appears structurally underwhelming, a literature survey suggests that approaches to this target remain non-trivial and typically require thirteen to fifteen steps. Alternative approaches that avoid the intermediacy of lactone **5** have also been developed, and in some cases, have resulted in some of the most step-efficient syntheses of the manzacidins.[6] Of note, Maruoka and co-workers quickly constructed manzacidin A with high stereocontrol via an enantioselective 1,3-dipolar cycloaddition, resulting in 17% overall yield in five steps.[6a]

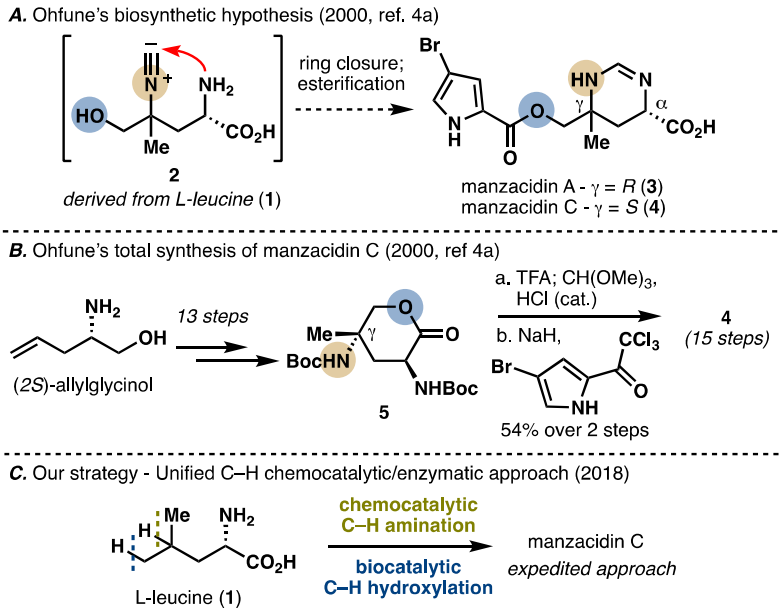


Figure 1. Sequential C–H functionalization approach for the synthesis of manzacidin C: biosynthetic inspiration and generalized synthetic strategy.

Ohfune's seminal publication briefly proposed that the manzacidins might have arisen from a hydroxylated isonitrile variant of L-Leu (**2**, Figure 1A). Isonitrile containing natural products are present in large abundance in marine sponges and tunicates.[7] In sponges, previous biosynthetic studies confirmed that inorganic cyanide is incorporated enzymatically to generate the isonitrile functionality, contrasting terrestrial isonitrile formation via α-amino acids (AAs).[7] Isonitrile incorporation likely occurs through a transiently-formed carbocation starting from an alkene or tertiary alcohol. Biosynthetically, intermediate **2** may be constructed from L-Leu through a tandem C–H dihydroxylation sequence, followed by a Ritter-type reaction with inorganic cyanide at the γ carbon to install the isonitrile. In contrast to isonitrile functionality, hydroxyl group incorporation via biocatalytic C–H hydroxylation is well-documented. Of note, iron- and α–ketoglutarate-dependent dioxygenases (Fe/αKGs) are prolific in their ability to perform site- and stereospecific hydroxylation of AAs.[8] However, investigations into the synthetic potential of these hydroxylases have remained severely overlooked.[9] The ability to manipulate and repurpose Nature's repertoire of oxidation biocatalysts for synthetic transformations can result in a highly enabling approach towards constructing complex molecules.[9] To demonstrate this idea with the Fe/αKGs, our laboratory chose to embark on a chemoenzymatic synthesis of manzacidin C, employing Ohfune's proposal as inspiration. This full account traces the evolution of our chemoenzymatic approach towards this alkaloid and the subsequent application of enzymatic amino acid hydroxylation in the preparation of novel scaffolds.

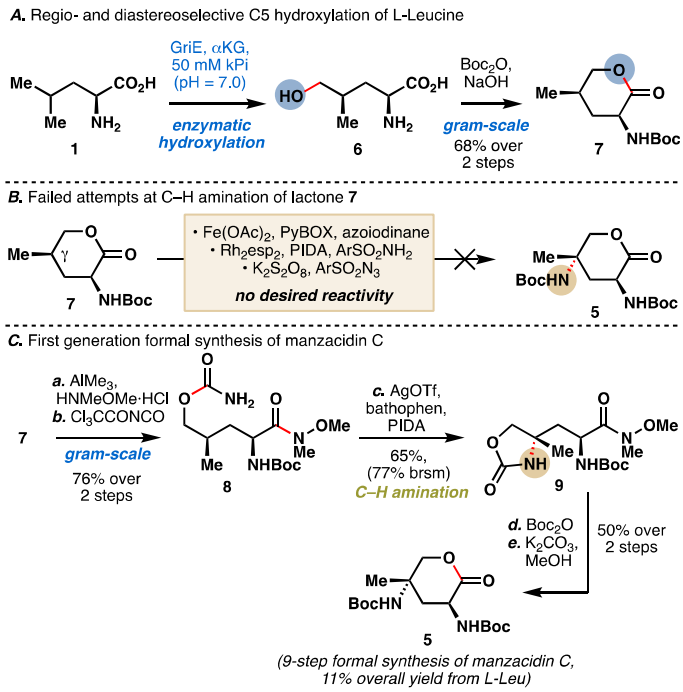
Results and Discussions

Towards the synthesis of manzacidin C, we envisioned a C–H functionalization strategy to access Ohfune's hypothetical intermediate **2** (Figure 1C). Our strategy centered on efficient C–H amination/hydroxylation transformations from the cheap and abundant starting material, L-Leu (**1**). Although C–H functionalization strategies have become ubiquitous in complex molecule synthesis, examples demonstrating remote/distal C–H functionalization are rare; even more so for AAs (Figure S1).[10] Site specific C–H functionalization largely depends on the steric and electronic features present in a molecule.[11] Selective tertiary C–H bond functionalization is largely precedented, as electron deficient intermediates are stabilized via hyperconjugation, yielding a high level of site selectivity. Transition metal catalyzed C–H functionalization of AAs utilizing directing groups has recently been reported, but typically results in β functionalization due to preferential formation of a 5-membered metallacycle intermediate.[12] Hofmann-Löffler-Freytag (HLF) chemistry has previously been developed for δ functionalization of AAs, but displays poor regio/stereocontrol and limited substrate scope.[10c] Known methods for C–H functionalization of AAs also require prudent protecting group strategies to mask highly reactive amine/carboxylic acid functional moieties.[10,12] Unlike traditional chemical methods, enzymatic C–H functionalization of AAs displays excellent regio/chemoselectivity and is able to operate on unprotected AAs.[8] Based on this information, our approach hinges on developing chemistry to selectively functionalize the γ and δ positions of L-Leu, ideally without the addition of protecting groups.

We were initially drawn to the aforementioned Fe/αKG family of enzymes due to their known activity in the hydroxylation of AAs en route to the biogenesis of nonribosomal peptides (NRPs) .[8,13] Prior to our studies, C5 hydroxylation of L-leucine has previously been established

in the biosynthesis of the 4-methylproline motif of the nostopeptolides and the echinocandins, and the corresponding enzymes (LdoA and EcdK, respectively) have been biochemically characterized.[14] Due to previously reported limitations of LdoA and EcdK, we chose to characterize a putative leucine hydroxylase called GriE, which is implicated in the biosynthesis of griselimycin (Figure S2).[15] To this end, we first heterologously overexpressed GriE in *E*. coli as the C-His_6-tagged protein, resulting in protein yield as high as 100mg/L. Combining GriE with L-Leu, α-ketoglutarate, iron(II) sulfate, and L-ascorbic acid yielded 4-(OH)-L-Leu with complete conversion and high total turnover number (TTN = 7800). In tandem with our studies, Müller characterized GriE using a combination of *in vitro* studies and gene inactivation.[16] Further biochemical characterization showed that GriE displays a superior kinetic profile with its native substrate relative to previously reported Fe/αKG enzymes.[8b] Encouraged by these observations, we conducted gram-scale hydroxylation of L-Leu with GriE, which following Boc protection and facile intramolecular cyclization, provided lactone **7** in 68% yield (Scheme 1A).

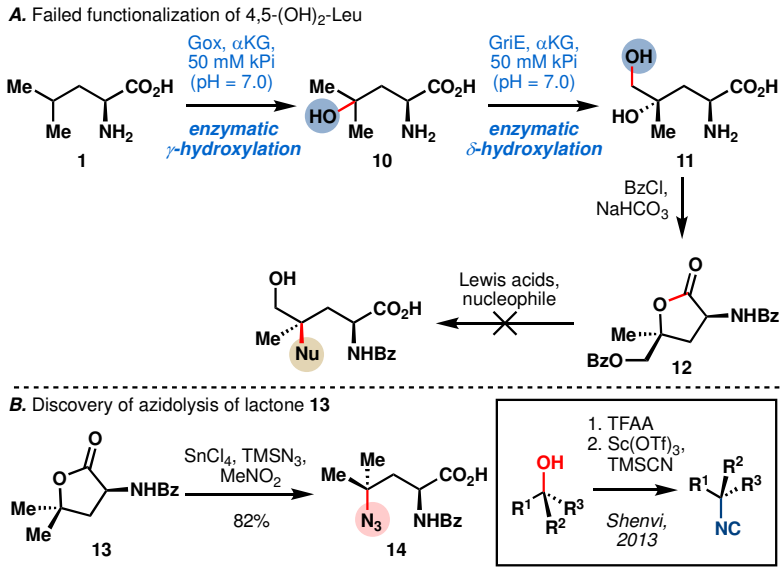
Scheme 1. C5 hydroxylation of L-Leu (**1**) en route to our first generation formal synthesis of manzacidin C.



With an efficient protocol for producing gram quantities of **7** in hand, we next targeted an appropriate C–H amination transformation towards manzacidin C (Scheme 1B). Though intermolecular amination or azidation of tertiary C–H bonds is well represented in the literature,[17] established procedures to effect this transformation showed no reaction on **7**. This observation is attributed to a combination of steric hindrance (axial C–H bond) and electronic deactivation (proximal to ester oxygen).[11a] We next sought a more robust strategy to install the critical C–N bond. Intramolecular nitrene insertion has seen extensive use in natural product synthesis.[18] Of note, rhodium and silver mediated strategies have become particularly powerful approaches for effecting late-stage C–H amination. This disconnection has been applied in the syntheses of nitrogen-containing natural products such as the welwitindolinones, tetrodotoxin, pactamycin, and most notably manzacidin A and C by Du Bois.[18,19] Mechanistic studies indicate that a carbamate/sulfamate is first converted to an iminoiodinane (formally a nitrene) by the hypervalent iodine reagent (e.g., phenyliododiacetate (PIDA) or iodosobenzene (PhIO)). Subsequently, the metal catalyst (Ag(I) or Rh(II)) decomposes the iminoiodinane, yielding an

electrophilic metal nitrenoid, which performs insertion at proximal/electronically rich C–H bonds.[18] Our revised strategy involved installation of a carbamate moiety, which would participate in the proposed C–H amination (Scheme 1C). Towards carbamate **8**, we first converted lactone **7** to the Weinreb amide using a combination of trimethylaluminum and *N*,*O*-dimethylhydroxylamine hydrochloride, followed by carbamate formation with trichloroacetylisocyanate, resulting in 76% two-step yield on gram scale.[19c] At this stage, the critical C–H insertion could be tested. While catalytic rhodium(II) acetate dimer ($Rh_2(OAc)_4$) gave no reaction, the more active catalyst Rh_2esp_2 yielded the desired product in 53% yield.[20] Previously reported conditions utilizing silver(I) triflate, bathophenanthroline, and PIDA in MeCN at 82 °C yielded oxazolidinone **9** in 65% (77% brsm).[19a] A two-step procedure involving Boc protection of the oxazolidinone, followed by methanolysis and lactonization yielded **5** in 50% yield over two steps. This sequence constitutes a nine-step formal synthesis of manzacidin C (11% total yield), exhibiting complete stereocontrol through sequential C–H functionalizations.

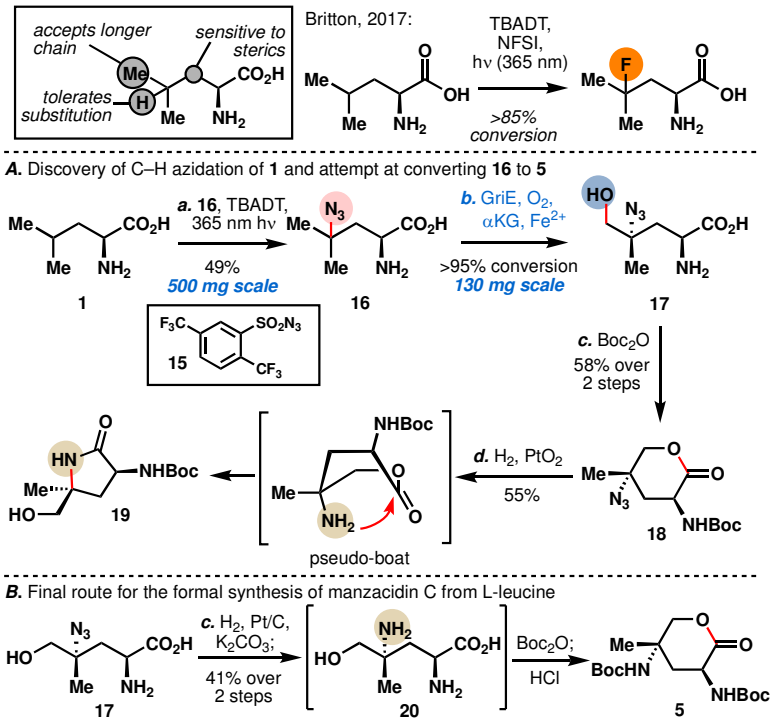
Scheme 2. Failed attempts to convert 4,5-dihydroxyleucine (**11**) to a key intermediate towards manzacidin C.



Although our formal synthesis has improved step count relative to those previously reported, it lacks optimal synthetic ideality.[21] We identified that our critical C–N bond disconnection necessitated several functional group interconversions (FGIs) and protecting group manipulations (PGMs), leading to the poor ideality and step count. For this reason, a more efficient strategy to access **5** was sought. Concurrent to our effort in developing a chemoenzymatic synthesis of manzacidin C, we were also able to establish the substrate scope of the enzymatic hydroxylation.[9a] A key observation derived from the substrate profiling of GriE is that substitution at the γ position of the amino acid substrate is tolerated. In particular, 4-(OH)-Leu[22] could be oxidized to the corresponding dihydroxy amino acid (**11**) in moderate TTN (Scheme 2A). Elaboration of this compound to lactone **5** was next investigated. In particular, displacement of the tertiary alcohol with an appropriate nitrogenous nucleophile would closely mimic our postulated biosynthesis of the manzacidins. Inspired by the work of Shenvi and co-workers (Scheme 2B), as well as related work on Lewis/Brønsted Acid-mediated opening of butyrolactones, we reasoned prudent combination of Lewis Acid and nucleophile may yield our desired C–N bond.[23] This process likely proceeds through the intermediacy of a contact ion pair,

dictating stereochemical inversion during isonitrile formation. If stereoinvertive, this would allow the synthesis of manzacidin A, which is epimeric at the γ position with respect to manzacidin C (Figure 1A). Unfortunately, lactone **12** showed no reaction under Shenvi's conditions. After screening various Lewis Acids in combination with azidotrimethyl silane ($TMSN_3$), we discovered combination of $SnCl_4$ in a 1:1 mixture of $TMSN_3$:$MeNO_2$ resulted in epimerization at the γ carbon (no other conditions showing any reaction). While disheartening, this result suggested that a carbocation was transiently formed under the reaction conditions. If this were the case, generating a more persistent/electron rich carbocation may yield the desired reactivity. To test this hypothesis, lactone **13** was subjected to the reaction conditions (Scheme 2B, see Experimental Procedures for preparation of **13**), which resulted in the formation of the desired azidocarboxylic acid **14**. Subsequent optimization of the reaction conditions improved the yield of **14** to 82% yield (see Table S1). Attempting the azidolysis reaction in other solvents besides $MeNO_2$ resulted in no reaction (see Table S1). Strict $MeNO_2$ requirement with $SnCl_4$ has similarly been reported for intramolecular Freidel-Crafts reactions with cyclopropylesters, wherein a carbocation intermediate was also proposed as a transient intermediate.[24] Similarly, hydrobromic acid has been shown to open α-tertiary lactones yielding a tertiary bromocarboxylic acid, also likely proceeding via a carbocation intermediate.[25] Interestingly, other azide sources such as sodium azide or tetrabutylammonium azide showed no reaction, suggesting $TMSN_3$ is required for the reaction to proceed. Though fruitless towards the synthesis of manzacidin A, this chemistry is currently under further synthetic and mechanistic investigations.

Scheme 3. Second generation formal synthesis of manzacidin C utilizing C–H azidation and biocatalytic C–H hydroxylation.

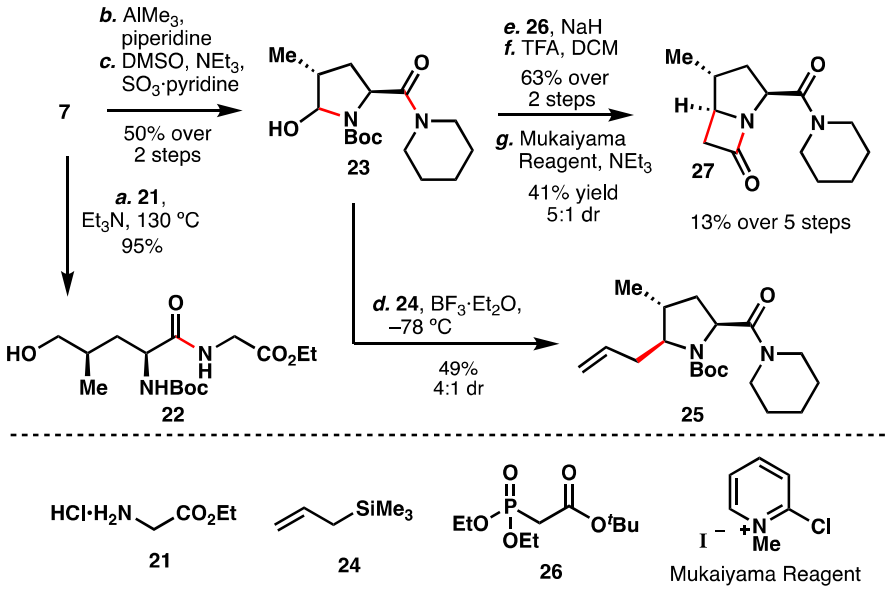


Based on the above foray, we realized the strategic benefit of using a pre-functionalized substrate for the enzymatic hydroxylation in improving the step efficiency of our synthesis. Specifically, early installation of the γ C–N bond in the synthesis would avoid subsequent concession steps, including the tethering strategy for a stereospecific intramolecular C–H amination. The proposed strategy would also dovetail nicely with GriE-mediated δ hydroxylation, which based on our substrate scope model accepts substrates with additional substitution at the γ position. This strategy is not without its challenge, as methods to effect direct C–H amination on unprotected AAs are not well-established. However, Britton's recent work on the γ selective C–H fluorination of unprotected L-Leu with tetrabutylammonium decatungstate (TBADT) as photocatalyst served as an inspiration to develop an analogous protocol for amination.[26] In the proposed catalytic cycle,[27] TBADT selectively performs hydrogen atom abstraction on the substrate to generate a persistent tertiary carbon centered radical. Subsequent trapping of this species by N-fluorobenezesulfonimide (NFSI) results in C–F

bond formation at the γ position. We reasoned that a similar electrophilic azide source may prove amenable to form our desired C–N bond.[10b,17a,b] Indeed, when L-Leu•TFA was stirred with 5 mol% TBADT and 3 equiv. of azide **15**, azidoleucine **16** was obtained; albeit as a 1:2.5 SM:PD mixture, which was separated by preparative HPLC (Scheme 3A). This reaction proved scalable, resulting in 49% yield using 500 mg of L-Leu•TFA (SM was not recovered). This is the first example of TBADT mediated azidation, as well as the first C–H azidation protocol on unprotected AAs. Due to the unique reactivity of the catalytic manifold, extension of this photoreaction is currently under further investigation in our group. Biocatalytic hydroxylation of **16** with GriE yielded difunctionalized amino acid **17** in >95% conversion on 130 mg scale. This sequence is remarkable, as two sequential C–H functionalizations on unprotected L-Leu could be effected. At this stage, **17** was Boc protected and lactonized to **18** in 58% yield over two steps. All that remained was a simple reduction and Boc protection to finish our synthesis. To our surprise, submitting **18** to reductive conditions (H_2/PtO_2) resulted in complete conversion to lactam **19** in 55% yield. A quick literature search revealed that the same by-product was previously obtained during reduction of a structurally similar nitrolactone.[28] Presumably, after azide reduction the molecule can adopt a pseudo boat conformation, which allows the tertiary amine to participate in an intramolecular addition-elimination with the lactone carbonyl to afford **19**. This pathway also occurred in the presence of triphenylphosphine (PPh_3), presumably through a Staudinger reaction. No desired product was ever formed, even when the reaction was run in the presence of Boc_2O or Boc-ON to capture the transiently formed amine after reduction. Though **19** was considered to complete the synthesis of manzacidin C via Boc protection and methanolysis, it would only improve our previous synthesis by one step. Nevertheless, the facile lactamization provided a key insight to choreograph an alternative sequence to access **5** from **17**

(Scheme 3B). We reasoned that under basic pH, the carboxylic acid would exist as the carboxylate, which would have a much-reduced propensity to undergo lactamization during the azide reduction and Boc protection sequence. Thus, a one-pot procedure was developed wherein **17** was first reduced, the resulting free amines (**20**) subsequently Boc protected, and alcohol/carboxylic acid motifs lactonized to provide **5** in 41% yield over two steps. This constitutes a five-step formal synthesis of manzacidin C in 11% overall yield. Although displaying equivalent overall yield to our first generation synthesis, it is remarkably shorter and displays significantly improved ideality over the first three transformations.

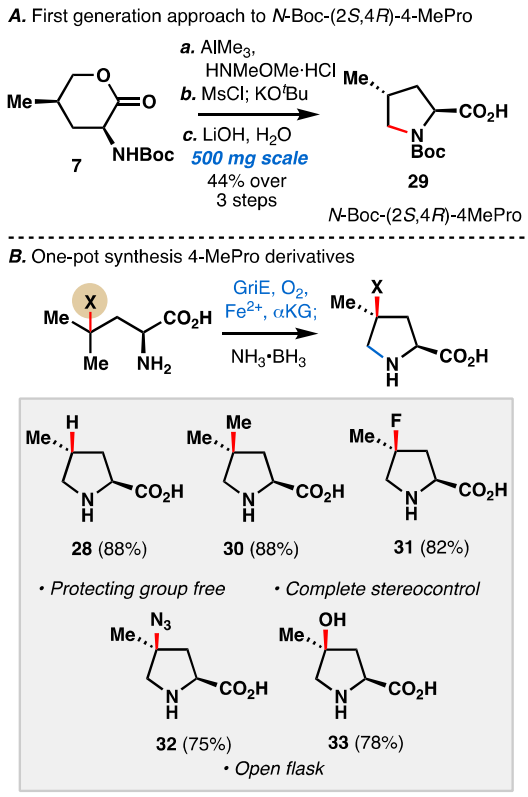
Scheme 4. Synthetic access to complex pyrrolidine scaffolds starting from lactone **7**.



During the course of our studies, we also sought to demonstrate the utility of GriE in the synthesis of other complex and biologically relevant molecules (Scheme 4). Since hydroxylated AAs are prevalent in nonribosomal peptides (NRPs), **7** was submitted to a peptide coupling by simple heating with L-glycine ethyl ester (**21**) to provide dipeptide **22** in excellent yield.[29] Standard peptide coupling requires the use of carboxylic acid activating agents, whereas lactones

represent a form of protected and pre-activated carboxylic acids, obviating the use of external coupling reagents. Lactone **7** was converted to the piperidyl amide, followed by Parikh-Doering oxidation to yield hemiaminal **23**. This functionalized proline variant features increased oxidation state at the δ position, serving as a handle for further derivatization. Synthetic manipulation of similarly oxidized pyrrolidines—albeit lacking methylation at the δ position—has recently been reported in the literature.[30] To demonstrate this, **23** was reacted with allyltrimethylsilane (**24**) in the presence of BF_3•Et_2O to yield **25** in 49% yield as a 4:1 mixture of diastereomers.[31] Carbapenam β-lactams represent structurally intricate scaffolds *en route* to carbapenem antibiotics.[13b] Since hemiaminal **23** exists in an equilibrium with the free aldehyde, Horner-Wadsworth-Emmons (HWE) reaction yielded a mixture of the homologated pyrrolidine (**S-03**) and its open chain tautomer (**S-04**) (Figure S3).[30a] Submitting the mixture directly to trifluoroacetic acid resulted in complete conversion to the corresponding amino acid (**S-05**) in 63% yield over two steps. Dehydration with Mukaiyama reagent (2-chloro-1-methylpyridinium iodide) afforded γ-methylated carbapenam **27** in 41% yield as a 5:1 mixture of diastereomers.[32] This sequence resulted in 13% yield over five steps from **7**.

Scheme 5. Chemoenzymatic synthesis of *N*-protected and free (2*S*,4*R*)-4MePro.



Due to the ubiquity of (2*S*,4*R*)-4-MePro (**28**) in a variety of biologically interesting NRPs, a scalable and efficient synthesis of this motif is highly desirable.[13-15] A previous approach to **28** requires an alcohol directing group and uses expensive Crabtree's catalyst to perform asymmetric reduction of an exocyclic olefin, yielding the desired stereochemistry with 40:1 dr.[33] Towards (2*S*,4*R*)-4-MePro, **7** was converted to the Weinreb amide as previously described (*vide supra*). The resulting alcohol was then mesylated and cyclized in one-pot upon addition of KO^{t}Bu. Hydrolysis of the Weinreb amide with LiOH provided Boc-(2*S*,4*R*)-4-MePro (**29**) in 44% over three steps on 500 mg scale and 30% overall yield from L-Leu (Scheme 5A). Our synthesis compares favorably to the hydrogenation route as it proceeds with complete stereocontrol and avoids directing groups and expensive catalysts. An observation during our substrate scope examination of GriE spurred further work in this area. It was observed that hydroxylation of γ-Me-L-Leu with GriE resulted in minor amounts of over oxidation to an

aldehyde, which is present in solution as the cyclic imine. This was similarly observed with L-Leu as well as other γ-substituted L-Leu variants with increased enzyme loading. Drawing inspiration from (*2S,4R*)-4-MePro's biosynthesis (see Supporting Information), we reasoned that conducting the reaction with sufficiently high GriE concentration, followed by addition of a mild reductant could yield γ-substituted L-Pro derivatives in a single step (Scheme 5B).[34] To our delight, this chemoenzymatic cascade could be performed on L-Leu and its derivatives—commercially available or synthesized from L-Leu in one step utilizing TBADT mediated photochemistry or Gox hydroxylation—, resulting in five unique proline derivatives in one step.

Conclusion

During the course of this work we demonstrated the unique advantage of combining enzymatic and traditional chemical methods in complex molecule synthesis.[8b] Our initial attempts to emulate the biogenesis of manzacidin C in the laboratory resulted in the characterization of a leucine hydroxylase, GriE, that can be used for preparative scale hydroxylation of a wide range of AAs. Additionally, our efforts to address the construction of the quaternary center of the target molecule yielded interesting new methodologies for nucleophilic opening of lactones with azides and photocatalytic C–H azidation of unprotected AAs. These discoveries culminated in an efficient formal synthesis of manzacidin C in just five steps. We further demonstrated the synthetic utility of amino acid oxidation with GriE by developing a chemoenzymatic approach to a variety of structurally and biologically interesting molecules, including novel proline analogs. Further applications of this synthetic paradigm[35] in the preparation of bioactive natural products are on-going and will be reported in due course.

Experimental Procedures

Compounds **5**, **6**, **7**, **10**, **12**, **15**, **16**, **17**, **20**, **22**, **28**, **30**, **31**, **32**, and **33** were previously synthesized and characterized.[9a]

tert-butyl ((2*S*,4*R*)-5-(carbamoyloxy)-1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (8)

A flame-dried 100 mL round bottom (w/ stir bar) was sequentially charged with *N*,*O*-dimethylhydroxylamine hydrochloride (1.21 g, 12.44 mmol, 3.0 eq) and 12.4 mL anhydrous THF. The suspension was stirred under Ar and cooled to 0 °C using an ice bath. $AlMe_3$ (2 M in PhMe, 6.2 mL, 12.44 mmol, 3.0 eq) was added via syringe pump over 30 min. The resulting solution was stirred for 1h at 23 °C, then cooled to –30 °C using a dry ice/acetone bath. Lactone **7** (0.951 g, 4.14 mmol, 1.0 eq, 0.5 M in THF) was added to a dry scintillation vial and dissolved in anhydrous THF (8.3 mL). The resulting solution was added dropwise over 10 min to the round bottom at –30 °C. After 30 min, the reaction was then quenched at –30 °C with 20 mL 10% (w/w) aq. Rochelle's salt (20 mL) and Et_2O (20 mL). The resulting mixture was allowed to stir at 23 °C for 30 min, diluted with H_2O and filtered over celite. The aq. layer was then extracted with Et_2O (3 x 20 mL). The organics were washed with sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated by air stream. The resulting oil was immediately transferred to a 250 mL round bottom flask, placed under Ar, and diluted with anhydrous DCM (83 mL). This solution was cooled to –40 °C, and trichloroacetyl isocyanate (740 µL, 6.22 mmol, 1.2 eq) was added dropwise with stirring. The reaction was stirred for 30 min at this temperature, then warmed to 23 °C and concentrated *in vacuo*. The resulting oil was diluted with MeOH (56 mL) and K_2CO_3 (860 mg, 6.22 mmol, 1.5 eq) was added. The resulting slurry was stirred for 30 min, filtered over celite, and concentrated *in vacuo.* The crude oil was purified by silica gel

chromatography via gradient elution (1:1 EtOAc:hexanes to 100% EtOAc), affording **8** (1.11 g, 76% yield) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 (d, *J* = 9.8 Hz, 1H), 4.92 (bs, 1H), 4.07 (dd, *J* = 10.6, 4.8 Hz, 1H), 3.85 (dd, *J* = 10.6, 7.4 Hz, 1H), 3.79 (s, 3H), 3.19 (s, 3H), 2.05 – 1.94 (m, 1H), 1.71 (ddd, *J* = 13.3, 8.8, 4.2 Hz, 1H), 1.49 – 1.42 (m, 1H), 1.42 (s, 9H), 0.96 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.6, 157.1, 155.3, 79.9, 69.8, 61.8, 48.9, 38.0, 32.2, 29.5, 28.5, 17.8; R_f = 0.30 (2:8 EtOAc:hexanes); $[\alpha]_D^{23}$ = 1.64º (c = 0.3, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{27}N_3O_6H^+$ 334.1978; Found 334.1977.

***tert*-butyl ((*S*)-1-(methoxy(methyl)amino)-3-((*S*)-4-methyl-2-oxooxazolidin-4-yl)-1-oxopropan-2-yl)carbamate (9)**

Two 20 mL Biotage® microwave vials were flame dried (w/ stir bars). To each vial was added 225 µL of 1 M stock solution of **8** in DCM. The vials were placed on high vacuum for 30 min, resulting in immediate formation of a yellow foam. After refilling with Ar, the vials were moved to a glovebox, wherein AgOTf (35 mg, 0.18 mmol, 0.25 eq), bathophenanthrolene (0.182 g, 0.55 mmol, 0.5 eq), and anhydrous/degassed MeCN (4 mL) was added to each vial. The vials were covered with foil and stirred for 30 min. Subsequently, 4 mL MeCN and PIDA (0.182 g, 0.55 mmols, 1.0 eq) were added to each vial. Each vial was sealed, wrapped in foil, removed from the glovebox, and heated to 82 °C (with stirring) in an oil bath for 24 h. The reactions were cooled to 23 °C, combined, filtered over a plug of silica with EtOAc, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography via gradient elution (1:1 EtOAc:hexanes to EtOAc), affording **8** (22 mg, 12% yield) and **9** (0.117 g, 65%, white solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.11 (s, 1H), 5.46 (d, *J* = 9.0 Hz, 1H), 4.81 (t, *J* = 9.7 Hz, 1H), 4.11 (d, *J* = 8.4 Hz, 1H), 4.03 (d, *J* = 8.4 Hz, 1H), 3.78 (s, 3H), 3.21 (s, 3H), 2.03 (dd, *J* = 14.0, 3.0

Hz, 1H), 1.69 (dd, *J* = 13.9, 10.5 Hz, 1H), 1.44 (s, 9H), 1.41 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.0, 158.3, 155.89, 80.8, 62.02, 57.0, 48.0, 42.1, 32.4, 28.5, 25.6; R_f = 0.20 (2:8 EtOAc:hexanes). $[\alpha]_D^{20}$ = –28.5º (c = 0.1, $CHCl_3$); mp = 94 °C; HRMS (ESI-TOF) m/z: $[M+H]^+$ - $[CO_2 + CH_2C(CH_3)_2]$ Calcd for $C_9H_{17}N_3O_4H^+$ 232.1297; Found 232.1287.

di-*tert*-butyl ((3*S*,5*S*)-5-methyl-2-oxotetrahydro-2*H*-pyran-3,5-diyl)dicarbamate (5)

Carbamate **9** (0.104 g, 0.31 mmol, 1.0 eq) was added to a scintillation vial (w/ stir bar) and dissolved in 3.1 mL THF (0.1M) with stirring. The solution was subsequently treated with TEA (174 µL, 1.25 mmol, 4.0 eq), DMAP (76 mg, 0.62 mmol, 2.0 eq), and Boc_2O (0.273 g, 1.25 mmol, 4.0 eq). After stirring 1.5 h, the reaction was quenched with sat. aq. NH_4Cl (3.0 mL), extracted with EtOAc (3 x 10 mL), and washed with sat. aq. NaCl. The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The resulting material was diluted with 6.3 mL MeOH (0.05 M), cooled using an ice bath (0 °C), and K_2CO_3 (86 mg, 0.62 mmol, 2.0 eq) was added. The resulting slurry was warmed to 23 °C and stirred for 12 h. After concentrating *in vacuo*, the mixture was dissolved in 10 mL H_2O and 10 mL EtOAc. The pH of this mixture was adjusted to 3 using AcOH. The aq. layer was extracted with EtOAc (3 x 10 mL) and concentrated *in vacuo.* The resulting oil was diluted with 10 mL PhMe. $MgSO_4$ (~0.5 g) was then added, and the resulting slurry was stirred vigorously with heating for 2 h at 70 °C using an oil bath. The slurry was cooled to 23 °C, filtered, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography via gradient elution (dry loaded) (2:98 MeOH:DCM to 5:95 MeOH:DCM), affording **5** (54 mg, 50% yield) as a white solid. ^{1}H NMR spectrum matches previous report.[9a] ^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.30 (bs, 1H), 4.71 (bs, 1H), 4.57 (bs, 2H), 4.25 (bs, 1H), 2.75 (bs, 1H), 1.66 (t, *J* = 13.0 Hz, 1H), 1.46 (s, 9H), 1.44 (s, 9H), 1.39 (s, 3H).

(*S*)-*N*-(5,5-dimethyl-2-oxotetrahydrofuran-3-yl)benzamide (13)

Following previously reported procedure,[9a] *E. coli* expressing Gox were grown on 400 mL scale. After harvest, the cells were resuspended to OD_{600} = 45 in 50 mM kPi buffer (pH 7.0). Cells were disrupted by sonication (3x1 min, 50% duty cycle) and pelleted by centrifugation at 4,000 rpm for 15 min at 4 ºC. A 250 mL Erlenmeyer flask was charged with L-leucine (328 mg, 2.5 mmol, 1.0 equiv), L-ascorbic acid (440 mg, 2.5 mmol, 1.0 equiv), α-ketoglutaric acid (disodium salt dihydrate, 1.69 g, 7.5 mmol, 3.0 equiv), and $FeSO_4$ (heptahydrate, 28 mg, 0.1 mmol, 0.04 equiv). After addition of clarified Gox lysate (50 mL), the flask was shaken at 20 ºC for 7 h at 250 rpm. Reaction was quenched by addition of 1 M HCl (20 mL) and centrifuged at 4,000 rpm for 15 min at 4 ºC. The supernatant was collected and concentrated to dryness and was used without further purification. The crude material was resuspended in sat. aq. $NaHCO_3$ (~ 75 mL). To this suspension was added a solution of BzCl (1.16 mL, 10 mmol, 4.0 equiv) in 75 mL DCM and the mixture was stirred vigorously at room temperature overnight. The pH of the mixture was adjusted to 2–3 and the layers were separated. The aqueous phase was extracted with DCM (2 x 50 mL), and the combined organic layers were washed with sat. aq. NaCl (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography via gradient elution (1:4 EtOAc:hexanes to 1:1 EtOAc:hexanes) to afford lactone **13** (269 mg, 46% yield over 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, *J* = 7.2, 2H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 6.63 (s, 1H), 4.92 (ddd, *J* = 11.7, 8.7, 5.4 Hz, 1H), 2.90 (dd, *J* = 12.6, 8.6 Hz, 1H), 2.08 – 1.97 (t, *J* = 12.6 1H), 1.56 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.4, 167.7, 133.1, 132.2, 128.7, 127.2, 83.4, 50.8, 42.5, 29.1, 27.1; R_f =

0.40 (1:1 EtOAc:hexanes); $[\alpha]_D^{20}$ = 47.0º (c = 0.3, $CHCl_3$); mp = 121 °C; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{15}NO_3H^+$ 234.1130; Found 234.1121.

(*S*)-4-azido-2-benzamido-4-methylpentanoic acid (14)

To a flame-dried 10 mL round bottom flask (w/ stir bar) under Ar, was added **13** (30 mg, 0.13 mmol, 1.0 eq). The flask was charged with 220 µL $MeNO_2$ and 220 µL $TMSN_3$ (both dried over 4Å molecular sieves prior to use). The resulting solution was treated dropwise with anhydrous $SnCl_4$ (1 M in heptane) (424 µL, 0.42 mmol, 3.3 eq) at 0 ºC. The reaction was stirred at 0 °C for 1 h, immediately warmed to 23 °C, and stirred at this temperature for 48 h. The reaction was diluted with 1 mL EtOAc and quenched with 1 mL H_2O with vigorous stirring. The resulting solution was adjusted to pH = 1 using 1 N aq. HCl. The aq. layer was extracted with EtOAc (3 x 5 mL) and the combined organics were washed with sat. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude oil was then purified by silica gel chromatography (1:1 EtOAc:hexanes, then gradient elution 1:99 MeOH:DCM 1% AcOH to 3:97 MeOH:DCM 1% AcOH), affording **14** (29 mg, 82% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 – 7.78 (m, 2H), 7.59 – 7.50 (m, 1H), 7.46 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.27 (s, 1H), 4.75 (ddd, *J* = 9.9, 6.2, 4.2 Hz, 1H), 2.20 – 1.99 (m, 2H), 1.43 (d, *J* = 19.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.7, 175.3, 168.2, 133.1, 132.4, 128.9, 127.3, 60.9, 51.1, 41.6, 27.2, 25.3, 20.8; R_f = 0.15 (5:95 MeOH:DCM 1% AcOH); $[\alpha]_D^{20}$ = – 11.8º (c = 0.3, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{16}N_4O_3H^+$ 277.1301; Found 277.1300.

***tert*-butyl ((3*S*,5*S*)-5-azido-5-methyl-2-oxotetrahydro-2*H*-pyran-3-yl)carbamate (18)**

A scintillation vial was charged with 4-azidoleucine (TFA salt, 22 mg, 0.08 mmol, 1.0 equiv), L-ascorbic acid (7.0 mg, 0.04 mmol, 0.5 equiv), α-ketoglutaric acid (disodium salt dihydrate, 18 mg, 0.08 mmol, 1.0 equiv), followed by 4.0 mL of 50 mM kPi buffer (pH 7.5). After addition of 20 µL of 200 mM aq. $FeSO_4$ solution (4.0 µmol, 0.05 equiv), the reaction was started by the addition of GriE stock solution (final concentration = 0.015 mM, 0.00075 equiv). The mixture was shaken at 20 ºC, 250 rpm. After 2 h, L-ascorbic acid (7.0 mg, 0.04 mmol, 0.5 equiv), α-ketoglutaric acid (disodium salt dihydrate, 18 mg, 0.08 mmol, 1.0 equiv), 20 µL of 200 mM aq. $FeSO_4$ solution (4.0 µmol, 0.05 equiv), and GriE stock solution (0.00075 equiv) were added and the mixture was shaken further for 3 h. The reaction was acidified with 1 M HCl (1 mL) and centrifuged at 15,000 rpm for 15 min. The supernatant was collected and lyophilized. The resulting off-white powder was treated with 2 M NaOH until pH ~ 9.0 and H2O was added to adjust the volume to ~ 2.5 mL. To this suspension was added a solution of Boc_2O (61 mg, 0.28 mmol, 3.5 equiv) in 2.5 mL EtOH and the mixture was stirred at room temperature overnight. The pH of the solution was adjusted to 2–3 and the mixture was concentrated *in vacuo* to remove EtOH. To the resulting brown slurry was then added DCM (4 mL) and the mixture was stirred overnight at room temperature. The layers were separated, the aqueous phase was extracted with DCM (2 x 2 mL), and the combined organic layers were washed with sat. aq. NaCl (5 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Purification by silica gel chromatography (1:5 to 1:1 EtOAc:hexanes) afforded lactone **18** (12.5 mg, 58% yield over 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.30 (bs, 1H), 4.31 (dt, *J* = 12.8, 6.7 Hz, 1H), 4.20 (s, 2H), 2.50 (ddt, *J* = 13.7, 6.9, 1.2 Hz, 1H), 1.98 (t, *J* = 13.0 Hz, 1H), 1.45 (d, 12H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3, 155.5, 80.8, 74.2, 58.7, 47.8, 38.6, 28.4, 22.9; R_f = 0.53 (1:1 EtOAc:hexanes). $[\alpha]_D^{20}$ = 14.0º (c =

1.1, $CHCl_3$); mp = 94 °C; HRMS (ESI-TOF) m/z: $[M+H]^+$ - $[CO_2 + CH_2C(CH_3)_2]$ Calcd for $C_6H_{10}N_4O_2H^+$ 171.0882; Found 171.0879.

***tert*-butyl ((3*S*,5*S*)-5-(hydroxymethyl)-5-methyl-2-oxopyrrolidin-3-yl)carbamate (19)**

To a scintillation vial (w/ stir bar) was added PtO_2 (1.6 mg, 0.007 mmol, 0.2 eq), **18** (9.4 mg, 0.035 mmol, 1.0 eq) and 500 µL MeOH (0.07 M). The solution was degassed by bubbling with H_2 for 15 min (during this time solution went from brown to black). The reaction was left under an atmosphere of H_2 and stirred for 3 h. The slurry was filtered over celite, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography using gradient elution (5:95 MeOH:DCM to 10:90 MeOH:DCM), affording **19** (4.7 mg, 55% yield) as a white film. ^{1}H NMR spectrum matches that reported in the literature.[28] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (s, 1H), 5.53 (d, *J* = 7.0 Hz, 1H), 4.39 (d, *J* = 9.0 Hz, 1H), 3.52 (d, *J* = 11.5 Hz, 1H), 3.39 (d, *J* = 11.5 Hz, 1H), 2.27 (t, *J* = 11.4 Hz, 1H), 1.98 (dd, *J* = 13.1, 8.2 Hz, 1H), 1.44 (s, 9H), 1.25 (s, 3H).

***tert*-butyl (3*R*,5*S*)-2-hydroxy-3-methyl-5-(piperidine-1-carbonyl)pyrrolidine-1-carboxylate (23)**

To a flame-dried 50 mL round bottom flask (w/ stir bar) under Ar was added piperidine (204 µL, 2.06 mmol, 1.2 eq) and 4.1 mL anhydrous PhMe. The solution was cooled to 0 °C using an ice bath, and $AlMe_3$ (2 M solution in PhMe, 1.03 mL, 2.06 mmol, 1.2 eq) was added dropwise. The reaction was immediately warmed to 23 °C and stirred for 45 min. Lactone **7** (0.394 g, 1.72 mmol, 1.0 eq) was added to a flame-dried scintillation vial under Ar and dissolved in 6.9 mL anhydrous PhMe (0.25M). The resulting solution was added to the round bottom flask dropwise

at 23 °C. The reaction was stirred for 2 hours and quenched with 30% (w/w) aq. Rochelle's salt (20 mL), stirred for 30 min, then transferred to a separatory funnel. Following dilution with DCM (20 mL), the aq. layer was subsequently extracted with DCM (3 x 10 mL). The organics were washed with sat. aq. NaCl (20 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude alcohol was dissolved in anhydrous DCM (17 mL, 0.1M). The resulting solution was subsequently treated with DMSO (1.2 mL, 17.2 mmol, 10.0 eq), TEA (1.2 mL, 8.62 mmol, 5.0 eq), and cooled to 0 °C using an ice bath. SO_3•pyr (0.823 g, 5.17 mmol, 3.0 eq) was added and the reaction was allowed to warm slowly to 23 °C over 16 h. The reaction was quenched with H_2O (1 mL) and the aq. layer was extracted with DCM (3 x 10 mL). The combined organics were washed with sat. aq. NaCl (3 x 5 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography using gradient elution (10:90 EtOAc:hexanes to 50:50 EtOAc:hexanes), affording **23** (0.269 g, 50% over two steps, isolated as a mixture diastereomers and rotamers) as a colorless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52 (d, *J* = 5.7 Hz, 1.4H), 5.38 (d, *J* = 5.5 Hz, 1H), 5.29 (s, 1.7H), 5.19 (d, *J* = 1.7 Hz, 1.6H), 5.06 (d, *J* = 1.6 Hz, 2.2H), 4.88 (dd, *J* = 9.0, 5.3 Hz, 2.5H), 4.78 – 4.67 (m, 2.8H), 4.64 – 4.56 (m, 1.7H), 3.84 – 3.75 (m, 2H), 3.66 (ddt, *J* = 29.7, 10.5, 6.0 Hz, 7H), 3.57 – 3.36 (m, 18H), 3.28 (td, *J* = 9.1, 8.7, 4.4 Hz, 3.3H), 2.49 – 2.23 (m, 6.8H), 2.15 – 1.93 (m, 6H), 1.88 – 1.70 (m, 9H), 1.71 – 1.53 (m, 30H), 1.47 (s, 31H), 1.42 (d, *J* = 3.4 Hz, 29H), 1.05 – 0.95 (m, 17H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.4, 172.0, 169.8, 169.6, 169.4, 154.5, 154.1, 153.7, 125.4, 125.1, 115.2, 88.2, 87.9, 83.4, 83.1, 80.9, 80.7, 80.6, 80.4, 80.3, 80.2, 56.8, 56.7, 56.6, 56.2, 53.6, 47.0, 46.6, 46.4, 44.0, 43.7, 43.4, 42.2, 40.8, 39.0, 36.3, 35.5, 35.3, 35.1, 34.7, 31.1, 28.7, 28.6, 28.5, 28.5, 28.5, 26.7, 26.5, 26.4, 25.8, 25.6, 24.7, 24.6, 18.4, 18.1, 13.5, 12.8, 12.7;

R_f = 0.30 (1:1 EtOAc:hexanes); $[\alpha]_D^{20}$ = – 70.5º (c = 0.4, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ - [CO_2 + $CH_2C(CH_3)_2$] - [OH] Calcd for $C_{11}H_{18}N_2OH^+$ 195.1497; Found 195.1497.

***tert*-butyl (2*S*,3*R*,5*S*)-2-allyl-3-methyl-5-(piperidine-1-carbonyl)pyrrolidine-1-carboxylate (25)**

To a flame-dried 10 mL round bottom flask under Ar (w/ stir bar) was added **23** (28 mg, 0.090 mmol, 1.0 eq) followed by 900 µL anhydrous DCM (0.1M). The reaction was cooled to –78 °C using a dry ice acetone bath, at which point allyltrimethylsilane (70 µL, 0.45 mmol, 5.0 eq) and BF_3•Et_2O (17 µL, 0.14 mmol, 1.5 eq) were sequentially added dropwise to the solution. After 1 h the reaction was quenched at – 78 °C with sat. aq. $NaHCO_3$ (1 mL). The resulting solution was warmed to 23 °C and diluted with 5 mL DCM. The aq. layer was extracted with DCM (3 x 5 mL). The organics were then washed with sat. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography using gradient elution (3:7 EtOAc:hexanes to 4:6 EtOAc:hexanes), affording **25** (14.9 mg, d.r. = 3.6:1.0, 49%, mixture of rotamers) as a colorless oil. Characterization data for β-epimer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88 (dddd, *J* = 16.6, 10.2, 8.2, 6.2 Hz, 2H), 5.07 (d, *J* = 17.1 Hz, 2H), 5.01 (d, *J* = 10.2 Hz, 2H), 4.79 – 4.70 (m, 1H), 4.62 (t, *J* = 7.8 Hz, 1H), 3.76 – 3.61 (m, 2H), 3.58 – 3.40 (m, 6H), 2.80 (bs, 1H), 2.69 (bs, 1H), 2.45 – 2.27 (m, 2H), 2.19 (s, 2H), 1.98 (dt, *J* = 13.1, 6.8 Hz, 2H), 1.75 (ddd, *J* = 13.4, 8.7, 5.0 Hz, 2H), 1.70 – 1.52 (m, 14H), 1.43 (d, *J* = 21.0 Hz, 18H), 1.01 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.9, 170.7, 154.7, 154.0, 136.3, 136.2, 116.4, 79.7, 79.6, 65.6, 65.1, 56.7, 46.6, 46.4, 43.5, 43.3, 39.3, 38.2, 37.2, 36.2, 35.8, 28.7, 28.6, 26.7, 25.7, 24.7, 19.9; R_f = 0.30 (4:6 EtOAc:hexanes); $[\alpha]_D^{20}$ = – 6.5º (c =

0.3, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{32}N_2O_3H^+$ 337.2491; Found 337.2491.

2-((3*R*,5*S*)-3-methyl-5-(piperidine-1-carbonyl)pyrrolidin-2-yl)acetic acid (S-05)

To a flame-dried 10 mL round bottom flask under Ar (w/ stir bar) was added NaH (22 mg, 0.55 mmol, 2.0 eq) followed by 3.0 mL anhydrous THF (0.2M). *tert*-Butyl diethylphosphonoacetate (130 µL, 0.55 mmol, 2.0 eq) was added dropwise, and the resulting solution was stirred for 1 h. Amide **23** (86 mg, 0.27 mmol, 1.0 eq) was added to a flame-dried scintillation vial and diluted with 550 µL anhydrous THF (0.1 M final concentration). The resulting solution was added to the round bottom flask dropwise. After stirring for 5 h, the reaction was quenched with sat. aq. NH_4Cl (2 mL). The resulting mixture was diluted with 10 mL EtOAc. The aq. layer was extracted with EtOAc (3 x 5 mL). The organics were then washed with sat. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was then dissolved in 800 µL DCM (0.3M). The solution was cooled to 0 °C using an ice bath, and treated with 270 µL TFA (1.0 M final TFA concentration). The reaction was heated at 80 °C using an oil bath for 12 h. The reaction was concentrated *in vacuo* and purified using Dowex 50WX8 resin (5 grams). The resin was charged with 1 N HCl (until pH = 1) and washed with H_2O (until pH = 6–7). The crude material was added to the resin in H_2O, washing with MeCN then H_2O. 1 N NH_4OH was then used to wash the resin until **S-05** eluted completely as judged by TLC (Note: TLC plates were run in 2:8 DCM:MeOH and dried for 1 min to remove NH_4OH prior to staining with ninhydrin). The fractions were concentrated *in vacuo* affording **S-05** (44 mg, d.r. = 1.7:1.0, 63% over two steps) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 4.75 (dd, *J* = 9.7, 6.4 Hz, 2H), 4.70 – 4.61 (m, 1H), 3.96 (dt, *J* = 9.6, 5.7 Hz, 1.6H), 3.67 – 3.51 (m, 6H), 3.45 (t, *J* = 5.7 Hz, 5H), 2.71

(dd, *J* = 16.8, 3.9 Hz, 1H), 2.62 – 2.54 (m, 1H), 2.53 – 2.36 (m, 5H), 2.27 (ddd, *J* = 13.1, 9.7, 7.3 Hz, 1.7H), 2.19 – 2.00 (m, 5H), 1.75 – 1.52 (m, 16H), 1.12 (d, *J* = 5.1 Hz, 3H), 1.06 (d, *J* = 7.0 Hz, 5H); ^{13}C NMR (101 MHz, CD_3OD): δ 177.6, 176.9, 168.3, 167.9, 64.7, 62.0, 57.8, 57.1, 47.4, 47.3, 44.8, 44.7, 37.5, 37.4, 37.0, 36.9, 36.0, 33.2, 27.2, 26.5, 25.3, 16.2, 13.3; $[\alpha]_D^{20}$ = – 53.1° (c = 0.3, MeOH); mp = 59 °C; HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{22}N_2O_3H^+$ 255.1709; Found 255.1719.

(2*S*,4*R*,5*S*)-4-methyl-2-(piperidine-1-carbonyl)-1-azabicyclo[3.2.0]heptan-7-one (27)

To a flame-dried 10 mL round bottom flask (w/ stir bar) under Ar was added 2-chloro-1-methyl-pyridinium iodide (Mukaiyama reagent) (0.119 g, 0.47 mmol, 4.0 eq), 1.5 mL anhydrous MeCN, and TEA (130 µL, 0.93 mmol, 8 eq). The mixture was heated to 70 °C in an oil bath with stirring for 30 min. To a second flame-dried 10 mL round bottom flask (w/ stir bar) under Ar was added **S-05** (29.6 mg, 0.12 mmol, 1.0 eq) and 1.5 mL anhydrous MeCN. The resulting solution was subsequently heated to 70 °C in an oil bath with stirring for 30 min. The solution from the first round bottom flask was taken up in a syringe and added to the second round bottom flask dropwise. The first round bottom flask was rinsed with 3 mL anhydrous DCM, and the resulting solution was added dropwise to the second round bottom flask. After 15 min, the reaction was gradually cooled to 23 °C by turning off the heat but leaving the reaction in the oil bath. After stirring for 16 h, the reaction was concentrated *in vacuo*. The resulting oil was dilute with 10 mL EtOAc and 10 mL sat. aq. NaCl. The aq. layer was extracted with EtOAc (3 x 5 mL). The organics were then washed with sat. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography using gradient elution (6:4 EtOAc:hexanes to 7:3 EtOAc:hexanes), affording **27** (11 mg, d.r. = 5.0:1.0, 41%) as

a white film. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.66 (dd, *J* = 7.4, 5.0 Hz, 5H), 4.58 (dd, *J* = 8.8, 7.5 Hz, 1H), 3.92 (ddd, *J* = 6.7, 5.3, 2.6 Hz, 4H), 3.62 (ddd, *J* = 17.2, 8.7, 5.0 Hz, 8H), 3.57 – 3.45 (m, 17H), 3.43 – 3.35 (m, 3H), 3.23 – 3.16 (m, 1H), 3.08 – 2.99 (m, 5H), 2.76 (dd, *J* = 16.0, 2.6 Hz, 5H), 2.70 – 2.54 (m, 12H), 1.94 – 1.78 (m, 16H), 1.77 – 1.68 (m, 11H), 1.68 – 1.43 (m, 58H), 1.21 (d, *J* = 6.2 Hz, 2H), 1.15 (d, *J* = 6.5 Hz, 2H), 1.10 (d, *J* = 6.7 Hz, 2H), 1.01 (d, *J* = 7.1 Hz, 14H); ^{13}C NMR (101 MHz, $CDCl_3$): (*Note: Only the major epimer is clearly observable*) δ 177.3, 168.1, 56.7, 55.6, 46.7, 43.7, 40.8, 37.4, 36.8, 33.2, 26.5, 25.7, 24.8, 24.6, 23.5, 15.5; R_f = 0.30 (7:3 EtOAc:hexanes); $[\alpha]_D^{20}$ = – 24.6º (c = 0.5, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{20}N_2O_2H^+$ 237.1603; Found 237.1577.

(2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-methylpyrrolidine-2-carboxylic acid (29)

To a flame-dried 50 mL round bottom flask under Ar (w/ stir bar) was added *N*,*O*-dimethylhydroxylamine hydrochloride (0.64 g, 6.56 mmol, 3.0 eq) and 6.6 mL anhydrous THF. The suspension was cooled to 0 °C using an ice bath and $AlMe_3$ (2 M in PhMe, 3.3 mL, 6.56 mmol, 3.0 eq) was added via syringe pump over 30 min. The resulting solution was stirred for 1h at 23 °C, then cooled to –30 °C using a dry ice/acetone bath. Lactone **7** (0.501 g, 2.19 mmol, 1.0 eq) was added to a dry scintillation vial, diluted with 4.3 mL anhydrous THF (0.5M), and added dropwise over 10 min to the 50 mL round bottom at –30 °C. After stirring 30 min, the reaction was then quenched at –30 °C with 20 mL 10% (w/w) aq. Rochelle's salt (20mL) and Et_2O (20mL). The resulting mixture was allowed to stir at 23 °C for 30 min, diluted with H_2O and filtered over celite. The aq. layer was then extracted with Et_2O (3 x 20 mL). The organics were washed with sat. aq. NaCl (50 mL), dried over $MgSO_4$, filtered, and concentrated by air stream.

The resulting oil was placed under Ar, dissolved in 22 mL anhydrous THF (0.1 M), and cooled to –78 °C using a dry ice/acetone bath. After treating with TEA (610 µL, 4.37 mmol, 2.0 eq) and MsCl (186 µL, 2.40 mmol, 1.1 eq), dropwise, the reaction was stirred for 1 h at – 78 °C then allowed to warm to 23 °C. KO^{t}Bu (1.23 g, 10.93 mmol, 5.0 eq) was then added, and the reaction was allowed to stir 12 h. The reaction was filtered over celite and concentrated *in vacuo*. The resulting material was diluted with 11 mL H_2O (0.2 M final concentration) and treated with LiOH (0.523 g, 21.85 mmol, 10.0 eq). The resulting slurry was heated to 80 °C for 19 h. The material was then filtered over celite. The aq. layer was extracted with Et_2O and acidified to pH = 1 using aq. HCl (1N). The solution was then extracted with DCM (3 x 20 mL). The organics were washed with sat. aq. NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude material was purified by silica gel chromatography using gradient elution (DCM to 5:95 MeOH:DCM), affording **29** (0.222 g, 44% over three steps, mixture of rotamers) as a slightly yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ 4.31 – 4.20 (m, 2H), 3.64 (m, 2H), 3.31 (p, *J* = 1.7 Hz, 12H), 2.92 (m, 2H), 2.41 – 2.32 (m, 2H), 2.12 – 2.07 (m, 2H), 1.95 – 1.85 (m, 2H), 1.46 (s, 9H), 1.42 (s, 9H), 1.06 – 1.03 (m, 6H); ^{13}C NMR (101 MHz, CD_3OD): δ 155.9, 81.4, 81.2, 60.7, 54.2, 39.4, 38.7, 33.2, 32.5, 31.1, 28.7, 28.6, 17.6, 17.5; R_f = 0.30 (5:95 MeOH:DCM); $[\alpha]_D^{20}$ = – 60.8º (c = 0.4, MeOH); HRMS (ESI-TOF): $[M+H]^+$ - [CO_2 + $CH_2C(CH_3)_2$] Calcd for $C_6H_{11}NO_2H^+$ 130.0868; Found 130.0861.

(2*S*,4*R*)-4-methylpyrrolidinium-2-carboxylic acid 2,2,2-trifluoroacetate (S-06)

To a 1-dram vial (w/ stir bar) was added **30** (1.7 mg, 0.0074 mmol, 1.0 eq), 100 µL DCM, and 7.4 µL TFA. The reaction was stirred at 23 °C for 48 h. The reaction was then concentrated *in vacuo*. The resulting mass and yield were not obtained. ^{1}H NMR analysis of this crude material

shows only one compound. ^{1}H NMR (400 MHz, CD_3OD): δ 4.31 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.54 (dd, *J* = 11.3, 7.3 Hz, 1H), 2.83 (dd, *J* = 11.4, 8.8 Hz, 1H), 2.39 (ddt, *J* = 19.8, 11.9, 6.9 Hz, 2H), 2.06 – 1.91 (m, 1H), 1.13 (d, *J* = 6.4 Hz, 3H).

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supplementary figures and spectral data for all compounds

Crystallographic data for **19**

Acknowledgments

Financial support for this work was generously provided by The Scripps Research Institute. We thank C. A. Reiher and R. A. Shenvi for helpful discussions on tertiary lactone opening. We are grateful to A. L. Rheingold, C. E. Moore, and M. Gembicky (University of California, San Diego) for x-ray crystallographic analysis. We thank the Shen lab, the Disney lab, the Kodadek lab, and the Roush lab for generous access to their instrumentations.

References

1. Rane, R.; Sahu, N.; Shah, C.; Karpoormath, R. *Curr. Top. Med. Chem.* **2014**, *14*, 253–273.
2. Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574–4576.
3. Hager, A.; Vrielink, N.; Hager, D.; Lefranc, J.; Trauner, D. *Nat. Prod. Rep.* **2016**, *33*, 491–522.

4. (a) Namba, K.; Shinada, T.; Teramoto, T.; Ohfune, Y. *J. Am. Chem. Soc.* **2000**, 122, 10708−10709. (b) Hashimoto, T.; Maruoka, K. *Org. Biomol. Chem.* **2008**, *6*, 829−835. (c) Bretzke, S.; Scheeff, S.; Vollmeyer, F.; Eberhagen, F.; Rominger, F.; Menche, D. *Beilstein. J. Org. Chem.* **2016**, *12*, 1111–1121. (d) Nagatomo, M.; Nishiyama, H.; Fujino, H.; Inoue, M. *Angew. Chem., Int. Ed.* **2015**, *54*, 1537–1541. (e) Yoshimura, T.; Kinoshita, T.; Yoshioka, H.; Kawabata, T. *Org. Lett.* **2013**, *15*, 864–867. (f) Ohfune, Y.; Oe, K.; Namba, K.; Shinada, T. *Heterocycles* **2012**, *85*, 2617–2649.

5. (a) Tong, T. M. T.; Soeta, T.; Suga, T.; Kawamoto, K.; Hayashi, Y.; Ukaji, Y. *J. Org. Chem.* **2017**, *82*, 1969–1976. (b) Kudoh, T.; Araki, Y.; Miyoshi, N.; Tanioka, M.; Sakakura, A. *Asian. J. Org. Chem.* **2017**, *6*, 1760–1763.

6. (a) Kano, T.; Hashimoto, T.; Maruoka, K. *J. Am. Chem. Soc.* **2006**, *128*, 2174–2175. (b) Tran, K.; Lombardi, P. J.; Leighton, J. L. *Org. Lett*. **2008**, *10*, 3165–3167. (c) Nagatomo, M.; Nishiyama, H.; Fujino, H.; Inoue, M. *Angew. Chem., Int. Ed*. **2015**, *54*, 1537–1541.

7. (a) Schnermann, M. J.; Shenvi, R. A. *Nat. Prod. Rep.* **2015**, *32*, 543–577. (b) Garson, M. J.; Simpson, J. S. *Nat. Prod. Rep.* **2004**, *21*, 164–179. (c) Emsermann, J.; Kauhl, U.; Opatz, T. *Mar. Drugs*. **2016**, *14*, 1–83.

8. (a) Hausinger, R. P. *Crit. Rev. Biochem. Mol. Biol.* **2004***, 39,* 21–68. (b) Hibi, M.; Ogawa, *J. Appl. Microbiol. Biotechnol.* **2014**, *98*, 3869−3876.

9. (a) Zwick, C. R., III; Renata, H. *J. Am. Chem. Soc.* **2018**, *140*, 1165–1169. (b) Dockery, S. A. B.; Lukowski, A. L.; Becker, M. R.; Narayan, A. R. H. *Nature Chem.* **2018**, 10, 119–125. (c) King-Smith, E.; Zwick. C. R., III; Renata, H. *Biochemistry* **2018**, *57*, 403–412 and references cited therein. (d) Zhang, K.; Shafer, B. M.; Demars, M. D., II; Stern,

H. A.; Fasan, R. *J. Am. Chem. Soc*. **2012**, *134*, 18695–18704. (e) Kolev, N. J.; O'Dwyer, K. M.; Jordan, C. T.; Fasan, R. *ACS Chem. Biol*. **2014**, *9*, 164–173.

10. (a) Yamaguchi, J.; Yamaguchi, A. D.; Itami, K. *Angew. Chem., Int. Ed.* **2012**, *51*, 8960–9009. (b) Quinn, R. K.; Könst, Z. A.; Michalak, S. E.; Schmidt, Y.; Szklarski, A. R.; Flores, A. R.; Nam, S.; Horne, D. A.; Vanderwal, C. D.; Alexanian, E. J. *J. Am. Chem. Soc.* **2016**, *138*, 696–702. (c) Liu, T.; Myers, M. C.; Yu, J.-Q. *Angew. Chem., Int. Ed.* **2017**, *56*, 306–309. (d) Reddy, L. R.; Reddy, B. V. S.; Corey, E. J. *Org. Lett.* **2006**, *8*, 2819–2821.
11. (a) Newhouse, T.; Baran, P. S. *Angew. Chem., Int. Ed.* **2011**, *50*, 3362–3374. (b) Hartwig, J. F.; Larsen, M. A. *ACS Cent. Sci.* **2016**, *2*, 281–292.
12. He, J.; Li, S.; Deng, Y.; Fu, H.; Laforteza, B. N.; Spangler, J. E.; Homs, A.; Yu, J.-Q. *Science* **2014**, *343*, 1216–1220.
13. (a) Yin, X.; Zabriskie, T. M. *ChemBioChem.* **2004**, *5*, 1274–1277. (b) Hamed, R. B.; Gomez-Castellanos, J. R.; Henry, L.; Ducho, C.; McDonough, M. A.; Schofield, C. J. *Nat. Prod. Rep.* **2013**, *30*, 21–107. (c) Wu, L.-F.; Meng, S.; Tang, G.-L. *Biochim. Biophy. Acta.* **2016**, *1864*, 453-470.
14. (a) Hibi, M.; Kawashima, T.; Sokolov, P. M.; Smirnov, S. V.; Kodera, T.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2467–2472. (b) Jiang, W.; Cacho, R. A.; Chiou, G.; Garg, N. K.; Tang, Y.; Walsh, C. T. *J. Am. Chem. Soc.* **2013**, *135*, 4457–4466.
15. Kling, A.; Lukat, P.; Almeida, D. V.; Bauer, A.; Fontaine, E.; Sordello, S.; Zaburannyi, N.; Herrmann, J.; Wenzel, S. C.; Kö nig, C.; Ammerman, N. C.; Barrio, M. B.; Borchers, K.; Bordon-Pallier, F.; Brönstrup, M.; Courtemanche, G.; Gerlitz, M.; Geslin, M.;

Hammann, P.; Heinz, D. W.; Hoffmann, H.; Klieber, S.; Kohlmann, M.; Kurz, M.; Lair, C.; Matter, H.; Nuermberger, E.; Tyagi, S.; Fraisse, L.; Grosset, J. H.; Lagrange, S.; Müller, R. *Science* **2015**, *348*, 1106–1112.

16. Lukat, P.; Katsuyama, Y.; Wenzel, S.; Binz, T.; König, C.; Blankenfeldt, W.; Brönstrup, M.; Müller, R. *Chem. Sci.* **2017**, *8*, 7521–7527.
17. (a) Karimov, R. R.; Sharma, A.; Hartwig, J. F. *ACS Cent. Sci.* **2016**, *2*, 715–724. (b) Zhang, X.; Yang, H.; Tang, P. *Org. Lett.* **2015**, *17*, 5828–5831. (c) Bess, E. N.; DeLuca, R. J.; Tindall, D. J.; Oderinde, M. S.; Roizen, J. L.; Du Bois, J.; Sigman, M. S. *J. Am. Chem. Soc.* **2014**, *136*, 5783–5789. (d) Li, Z.; Capretto, D. A.; Rahaman, R.; He, C. *Angew, Chem., Int. Ed.* **2008**, *46*, 5184–5186. (e) Prier, C. K.; Zhang, R. K.; Buller, A. R.; Brinkmann-Chen, S.; Arnold, F. H. *Nature Chem.* **2017**, *9*, 629–634. (f) Williams, K.; Du Bois, J. *J. Am. Chem. Soc.* **2007**, *129*, 562–568. (g) Huang, X.; Groves, J. T. *ACS Catal.* **2016**, *6*, 751-759.
18. (a) Darses, B.; Rodrigues, R.; Neuville, L.; Mazurais, M.; Dauban, P. *Chem. Commun.* **2017**, *53*, 493–508. (b) Roizen, J. L.; Harvey, M. E.; Du Bois, J. *Acc. Chem. Res.* **2012**, *45*, 911-922.
19. (a) Quasdorf, K. W.; Huters, A. D.; Lodewyk, M. W.; Tantillo, D. J.; Garg, N. K. *J. Am. Chem. Soc.* **2012**, *134*, 1396-1399. (b) Hinman, A.; Du Bois, J. *J. Am. Chem. Soc.* **2003**, *125*, 11510–11511. (c) Wehn, P. M.; Du Bois, J. *J. Am. Chem. Soc.* **2002**, *124*, 12950–12951.
20. Zalatan, D. N.; Du Bois, J. *J. Am. Chem. Soc.* **2009**, *131*, 7558–7559.
21. Gaich, T.; Baran, P. S. *J. Org. Chem.* **2010**, *75*, 4657–4673.

22. Smirnov, S. V.; Sokolov, P. M.; Kodera, T.; Sugiyama, M.; Hibi, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. *FEMS Microbiol. Lett.* **2012**, *331*, 97−104.

23. (a) Pronin, S. V.; Reiher, C. A.; Shenvi, R. A. *Nature* **2013**, *501*, 195–199. (b) Lv, Z.; Li, Z.; Liang, G. *Org. Lett.* **2014**, *16*, 1653–1655. (c) Bowie, A. L.; Hughes, C. C.; Trauner, D. *Org. Lett.* **2005**, *7*, 5207–5209.

24. (a) Murphy, W. S.; Wattanasin, S. *J. Chem. Soc., Perkin Trans.* **1982**, *0*, 1029–1035. (b) Ivanova, O. A.; Budynina, E. M.; Chagarovskiy, A. O.; Rakhmankulov, E. R.; Trushkov, I. V.; Semeykin, A. V.; Shimanovskii, N. L.; Melnikov, M. Y. *Chem. - Eur. J.* **2011**, *17*, 11738–11742.

25. Siddiqui, A. U.; Satyanarayana, Y.; Ahmed, I.; Siddiqui, A. H. *Steroids* **1996**, *61*, 302–304.

26. (a) Nodwell, M. B.; Yang, H.; Čolović, M.; Yuan, Z.; Merkens, H.; Martin, R. E.; Bénard, F.; Schaffer, P.; Britton, R. *J. Am. Chem. Soc.* **2017**, *139*, 3595−3598. (b) Halperin, S. D.; Kwon, D.; Holmes, M.; Regalado, E. L.; Campeau, L.-C.; DiRocco, D. A.; Britton, R. *Org. Lett.* **2015**, *17*, 5200−5203.

27. Dondi, D.; Fagnoni, M.; Albini, A. *Chem. - Eur. J.* **2006**, *12*, 4153−4163.

28. Xiong, G.; Wei, M.; Zhou, Y.; Zhang, F.; Gong, Y. *Synthesis* **2011**, *21*, 3439−3446.

29. Aleiwi, B. A.; Schneider, C. M.; Kurosu, M. *J. Org. Chem.* **2012**, *77*, 3859−3867.

30. (a) Osberger, T. J. Rogness, D. C.; Kohrt, J. T.; Stepan, A. F.; White, C. *Nature* **2016**, *537*, 214−219. (b) Chen, W.; Ma, L.; Paul, A.; Seidel, D. *Nature Chem.* **2018**, 10, 165–169.

31. Kawasaki, M.; Shinada, T.; Hamada, M.; Ohfune, Y. *Org. Lett.* **2005**, *7*, 4165−4167.

32. Chang, W.-C.; Guo, Y.; Wang, C.; Butch, S. E.; Rosenzweig, A. C.; Boal, A. K.; Krebs, C.; Bollinger, J. M., Jr. *Science* **2014**, *343*, 1140−1144.

33. Del Valle, J. R.; Goodman, M. *J. Org. Chem.* **2003**, *68*, 3923–3931.

34. For a related use of $NH_3 \cdot BH_3$ as water-compatible reductant: Ghislieri, D.; Green, A. P.; Pontini, M.; Willies, S. C.; Rowles, I.; Frank, A.; Grogan, G.; Turner, N. J. *J. Am. Chem. Soc.* **2013**, *135*, 10863−10869.

35. (a) Turner, N. J.; O'Reilly, E. *Nature Chem. Biol.* **2013**, *9*, 285−288. (b) de Souza, R. O. M. A.; Miranda, L. S. M.; Bornscheuer, U. T. *Chem. - Eur. J.* **2017**, *23*, 12040−12063.