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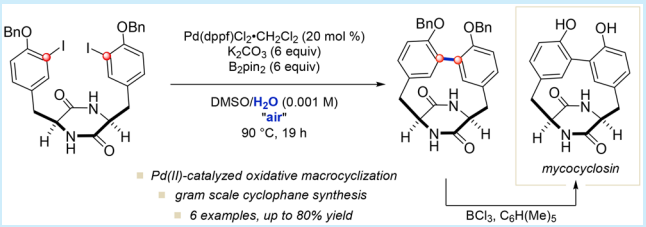
Scalable Synthesis of Mycocyclosin

Xu Zhu, Christopher C. McAtee, and Corinna S. Schindler*

Department of Chemistry, Willard Henry Dow Laboratory, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109, United States

Supporting Information

ABSTRACT: We report herein the scalable total synthesis of the secondary metabolite, mycocyclosin, initially isolated from *Mycobacterium tuberculosis*. Mycocylosin bears a highly strained 3,3′-dityrosine biaryl system which arises biosynthetically from an intramolecular oxidative dehydrogenative cross-coupling of cyclo(L-Tyr-L-Tyr) (cYY) catalyzed by the P450 enzyme CYP121. CYP121 is found exclusively in *M. tuberculosis*. Scalable access to mycocyclosin and related derivatives via a Pd(II)-catalyzed macrocyclization is anticipated to facilitate the biological evaluation of these compounds as novel tuberculosis antimicrobials.



The human pathogen *Mycobacterium tuberculosis* is the organism responsible for the development of tuberculosis (TB), a chronic but curable infectious disease that is associated with up to 3 million deaths annually.[1] This incongruity between treatment and global health threat can be attributed to the rise of drug-resistant and multidrug-resistant strains for which the current front- and second-line antitubercular treatments have proven ineffective.[2] The genome of *M. tuberculosis* encodes a significant amount of cytochrome P450 enzymes, while only a select few are essential for *M. tuberculosis* virulence.[3] Furthermore, interest in these enzymes was enhanced by studies implicating them as targets for several azole-derived compounds that were previously identified as effective antimicrobial molecules.[4] Unfortunately, the use of azole-derived pharmacaphores often results in significant toxicity due to cross-reactivity with other cytochrome P450 enzymes within the host.

The gene rv2276 encodes CYP121, a cytochrome P450 enzyme with a distinct metabolic role found exclusively in strains of *M. tuberculosis*, thereby making it one of the most logical candidates for evaluation as a potential drug target.[4a,5,6] Specifically, CYP121 converts cyclic dipeptide cYY (**1**) in a dehydrogenative cross-coupling reaction of the tyrosine subunits, accessing a highly strained 3,3′-dityrosine biaryl system, to form mycocyclosin (**2**) (Figure 1).[5] Moreover, cYY (**1**) is the only known substrate of CYP121 activity. As a result, the distinct scaffold of mycocyclosin can be used as a platform to design selective inhibitors of CYP121 that exhibit low toxicity to the host.[7] The first total synthesis of mycocyclosin (**2**) was reported by Hutton in 2012, in which the target molecule was assembled in eight synthetic transformations.[8] In the key step, a Suzuki−Miyaura cross-coupling effected the desired macrocyclization in 42% yield on a 50 mg scale.[8] With an interest in developing potent inhibitors of CYP121 based on structural analogy to mycocyclosin, we planned to take advantage of this previously reported route. To facilitate the biological evaluation of mycocyclosin and related derivatives as small-molecule drug candidates for the inhibition of CYP121, we required a scalable and robust synthesis to access the constrained cyclophane. Unfortunately, when employing previously reported macrocyclization conditions, we observed difficulties executing the Suzuki−Miyaura cross-coupling on scales larger than 50 mg. Herein, we report a synthesis of mycocyclosin and derivatives relying on a Pd(II)-catalyzed cross-coupling which can be carried out on up to gram scale.

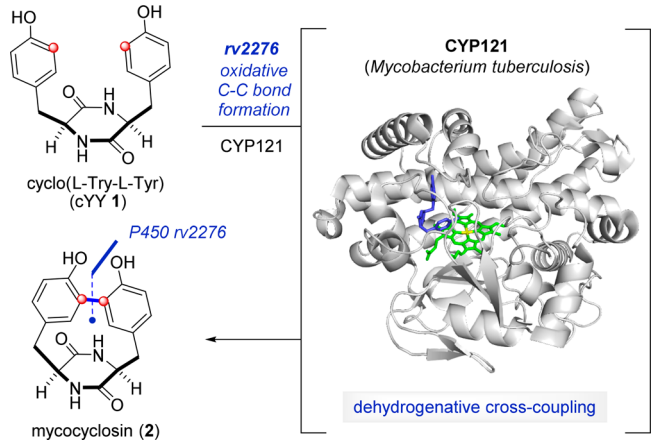


Figure 1. Biogenesis of mycocyclosin (**2**) from cYY (**1**).

Benzylated diketopiperazine (DKP) **3** is readily accessible from 3-iodo- L-tyrosine and was chosen as our initial substrate to evaluate reaction conditions for the scalable synthesis of strained cyclophane **4** via a Pd(II)-catalyzed macrocyclization (see the Supporting Information for a complete summary of reaction conditions). When **3** was subjected to reaction

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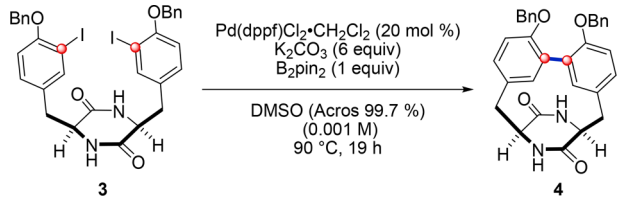
parameters initially reported by Hutton ($Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (20 mol %), K_2CO_3 (6 equiv), B_2pin_2 (1 equiv) and DMSO at 90 °C), the anticipated biaryl product **4** was isolated in 39% yield on 50 mg scale in accordance with the literature (Table 1, entry 1).[8] Interestingly, switching the DMSO source from Acros (anhydrous 99.7%) to DMSO dried over alumina via a solvent-dispensing system led to no isolable yield of the desired product in our hands (Table 1, entry 2). Nevertheless, the yield of **4** could be further improved to 61% by increasing the amount of B_2pin_2 (Table 1, entries 3–4). While increasing the B_2pin_2 ratio proved beneficial on smaller scale (50 mg), carrying out the biaryl coupling on 100 and 500 mg scales, under otherwise identical reaction conditions, led to diminished yields of **4** of 44% and 25%, respectively (Table 1, entries 5 and 6). Inspired by Denmark's mechanistic insight into the Suzuki–Miyaura transmetalation step,[9] we hypothesized that water may play a critical role in facilitating the biaryl coupling. Karl Fischer titration of our reaction solvent provided additional support for this proposal. Specifically, we observed a stark difference in water content of Acros DMSO (anhydrous 99.7%) and that from our solvent system (464.8 and 197.2 ppm, respectively). Based on this analysis, we evaluated the mixed solvent system $DMSO/H_2O$ (100:1). Water proved to be beneficial and allowed for consistent formation of **4** in 63% isolated yield on 50 mg scale (Table 1, entry 7). Moreover, an identical yield was obtained on half gram scale with 6 equiv of B_2pin_2 (Table 1, entry 8).

Observing that excess B_2pin_2 leads to increased reaction efficiency, we considered that an alternative reaction pathway to the Suzuki–Miyaura coupling may be plausible.[10] Recently, Jasti elegantly demonstrated that preformed diboronic esters readily undergo Pd(II)-catalyzed intramolecular homocoupling in air to form strained macrocycles.[11] Similarly, we hypothesized that under our optimized reaction conditions for accessing **4** the transformation may be proceeding via a diboronate in which trace contamination of air allows for productive catalysis. In an attempt to improve cross-coupling efficiency, we next evaluated the addition of air to the reaction. Notably, under aerobic conditions, catalysis was not inhibited as **3** converted to **4** in an identical yield of 63% as under an inert nitrogenous atmosphere on 500 mg scale (Table 2, entry 2). In comparison, biaryl substrate **5** underwent the macrocyclization in a modest 51% yield under inert conditions (Table 2, entry 3). Examination of the crude reaction mixture after 30 min by HRMS led us to observe the *m*/*z* ratio consistent with diboronate **7** (see the Supporting Information for details). Subsequently, subjecting **5** to the cross-coupling conditions with air as exogenous oxidant, under otherwise identical conditions, afforded **6** in an enhanced 80% isolated yield. Thus, depending on the biaryl substrate being evaluated under the cross-coupling conditions, the addition of air to the reaction can be advantageous.

With suitable reaction conditions in hand to access the desired cyclophane motif on half gram scale, we next sought to investigate the functional group tolerance on the DKP and aryl subunits (Table 3). While the OBn-NH DKP scaffold (**3**) proved to be a viable cross-coupling substrate under either aerobic or anaerobic atmosphere, the corresponding OMe-NH DKP **8** proved to be sluggish under either set of reaction conditions (Table 3, entries 3 and 4 versus 5 and 6).[12] Conversely, when substrates bearing acid-sensitive MOM ethers (**12**) or oxidatively labile PMB groups (**14**) were exposed to the Pd(II)-catalyzed cross-coupling reaction, the isolated yield of the anticipated [8.2.2] polycycle (**13** and **15**, respectively) were only modestly affected upon the incorporation of air (Table 3, entries 9–12). As a means to provide a metric for molecular strain, the arene displacement angles (α), which depict how distorted a benzene ring is from planarity, were determined for cyclophane **11** by acquiring a single-crystal

Table 1. Reaction Optimization for the Conversion of 3 to 4.[a]

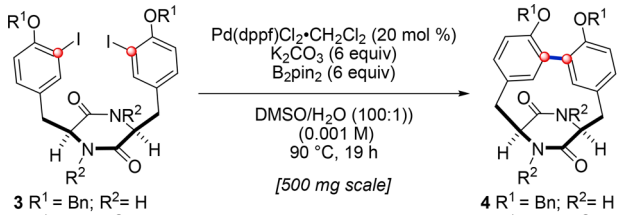


entry	deviation from above	yield **4** (%)
1	n/a	39
2[b]	DMSO (solvent dispensing system)	0
3	B_2pin_2 (2 equiv)	45
4	B_2pin_2 (4 equiv)	61
5	B_2pin_2 (4 equiv) [100 mg scale]	44
6	B_2pin_2 (4 equiv) [500 mg scale]	25
7[c]	B_2pin_2 (4 equiv), $DMSO/H_2O$ (100:1) [50 mg scale]	63
8[c]	B_2pin_2 (6 equiv), $DMSO/H_2O$ (100:1) [500 mg scale]	63

Karl Fischer Titration	***a.*** DMSO (Acros 99.7%) **464.8 ppm**	***b.*** DMSO (solvent system) **197.2 ppm**

[a]Conditions: biaryl **3** (50 mg, 0.066 mmol to 500 mg, 0.66 mmol), $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (20 mol %) K_2CO_3 (6 equiv), B_2pin_2 (1.0–6.0 equiv as noted) and DMSO (N_2 sparged, ACROS Chemicals, 99.7%, 0.001 M) at 90 °C for 19 h. [b]DMSO from solvent dispensing system. [c]$DMSO/H_2O$ = 100:1.

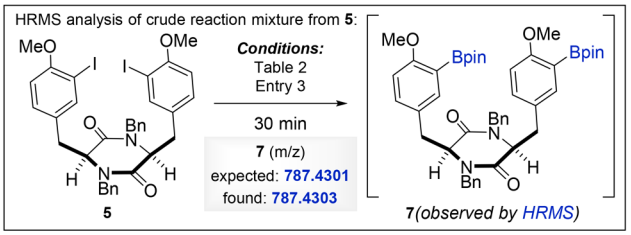
Table 2. Evaluating the Addition of Exogenous Air[a]



3 R^1 = Bn; R^2= H
5 R^1 = Me; R^2= Bn

4 R^1 = Bn; R^2= H
6 R^1 = Me; R^2= Bn

entry	deviation from above	R^1	R^2	yield (%)
1	N_2 atmosphere	Bn	H	63
2[b]	***air*** added after 30 min	Bn	H	63
3	N_2 atmosphere	Me	Bn	51
4[b]	***air*** added after 30 min	Me	Bn	80



[a]Conditions: biaryl **3** or **5** (500 mg,1 equiv), $Pd(dppf)_2Cl_2{\bullet}CH_2Cl_2$ (20 mol %) K_2CO_3 (6 equiv), B_2pin_2 (6.0 equiv) and $DMSO/H_2O$ = 100:1 (N_2 sparged, ACROS Chemicals, 99.7%, 0.001 M) at 90 °C for 19 h. [b]5 mL air added after 30 min.

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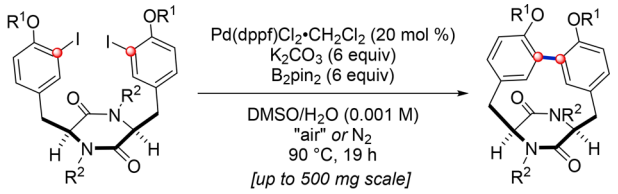
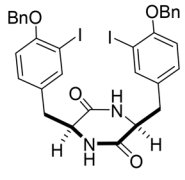
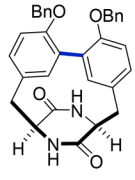
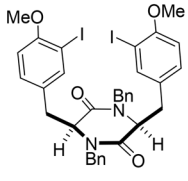
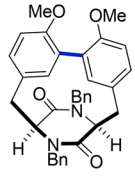
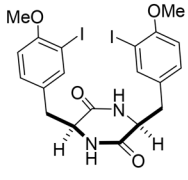
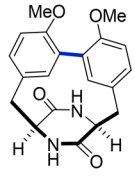
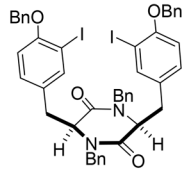
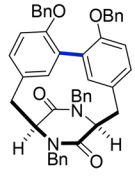
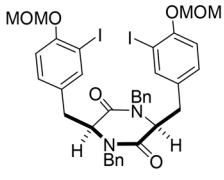
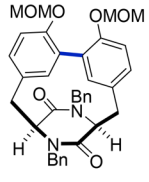
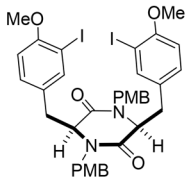
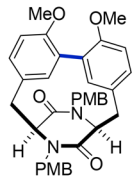
Table 3. Functionalized Mycocyclosin Derivatives Obtained



entry[a]	substrate	product	yield (%)
1			63% (N_2)
2			63% (air)[b]
	3	**4**	
3			51% (N_2)
4			80% (air)[b]
	5	**6**	
5			16% (N_2)
6			20% (air)[b]
	8	**9**	
7			29% (N_2)
8			64% (air)[b]
	10	**11**	
9			74% (N_2)
10			79% (air)[b]
	12	**13**	
11			62% (N_2)
12			61% (air)[b]
	14	**15**	

[a]Conditions: biaryl (1.0 equiv), $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (20 mol %) K_2CO_3 (6 equiv), B_2pin_2 (6.0 equiv) and DMSO/H_2O = 100:1 (N_2 sparged, ACROS Chemicals, 99.7%, 0.001 M) at 90 °C for 19 h. [b]5 mL of air added via syringe after 30 min.

X-ray structure (Figure 2).[11b] Indeed, there proved to be significant variation between α_1, α_2, and α_3, which ranged from 5.7° to 9.7°.

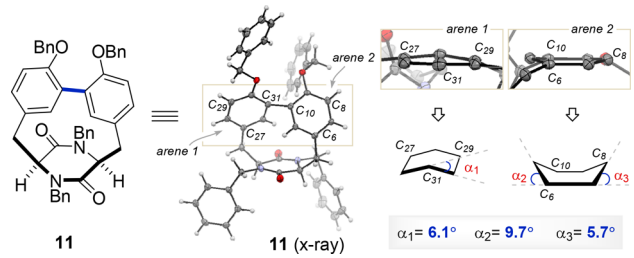


Figure 2. Arene displacement angles (α) of **11**.

Intrigued by our observation that H_2O and air are critical reaction additives in order to obtain reproducible yields for the Pd(II)-catalyzed macrocyclization on scale, we considered two complementary mechanistic regimes generalized in Figure 3.

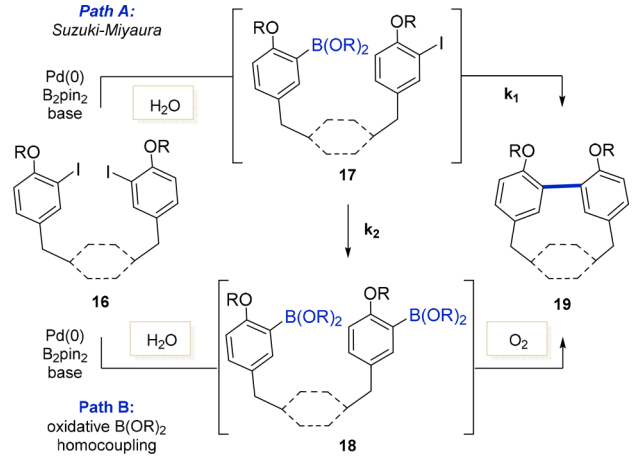


Figure 3. General formation of **19** from **16**.

Following initial Miyaura borylation of the bisiodide **16** leading to **17**, we hypothesize that $(Ar)Pd^{II}I$ can readily undergo anion metathesis with hydroxide to form $(Ar)Pd^{II}OH$, which allows for facile oxopalladium transmetalation with the aryl boronate (Figure 3, path A).[13] Additionally, two consecutive borylations of **16** can provide **18**, which under *oxidative conditions* can undergo Pd(II)-catalyzed homocoupling to form **19** (Figure 3, path B).[11] If the rate of Suzuki–Miyaura coupling (**17** → **19**, k_1) is faster than the second borylation event (**17** → **18**, k_2), then the addition of oxidant to the reaction will not affect the overall reaction yield (see Table 3, entries 11 and 12), Conversely, if k_2 is greater than k_1, exogenous oxidant should be advantageous (see Table 2, entries 7 and 8).

With these considerations in mind, we propose that two catalytic cycles are operative, and complementary, for the formation of macrocycle **19** (Scheme 1A). Under an anaerobic atmosphere (Scheme 1A, path A), monoboronate **17** can engage the Suzuki–Miyaura catalytic cycle by undergoing oxidative addition with $LPd^{(0)}$ (L= dppf) to **20**.[14] Conversion of **20** to palladium alkoxy **21** facilitates the incipient transmetalation event to **23** proceeding through a transient intermediate like **22**, initially identified by Denmark,[9] followed by reductive elimination to **19**. Additionally, on the basis of the experimentally and computationally proposed mechanism by Adamo,[15] under oxidative conditions (Scheme 1A, path B), $LPd^{(0)}$ may also be oxidized with O_2 to the corresponding palladium(II) peroxy intermediate **24**. Next, coordination of an aryl boronate ester (**18**) to **24** affords **25**, which facilitates the

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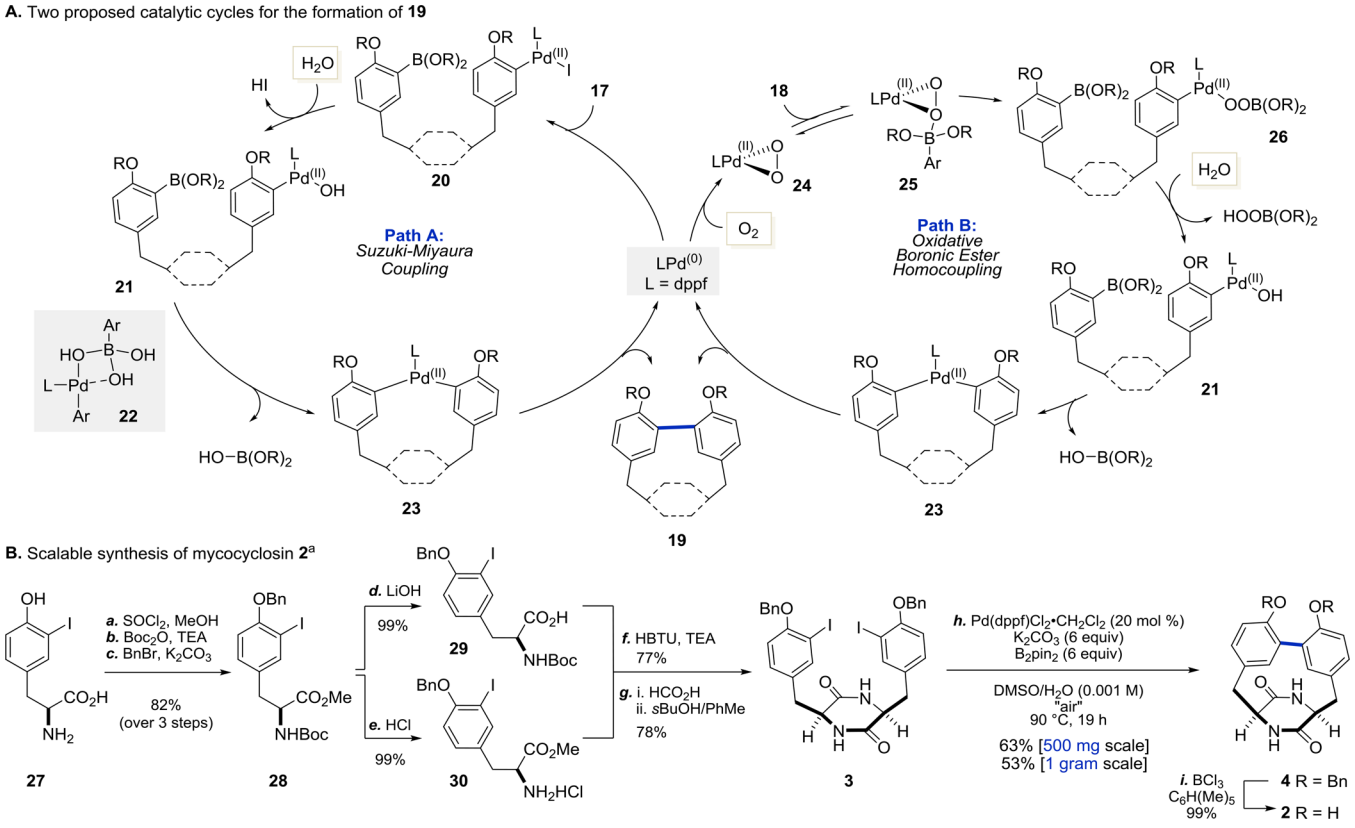
Scheme 1. (A) Proposed Catalytic Cycles for the Formation of 19. (B) Synthesis of Mycocyclsin[a]



[a]Conditions: see the Supporting Information for complete reaction details.

first transmetalation event leading to boronic peroxo **26**. Attack of water on peroxide **26** generates palladium(II) hydroxy complex **21**, which intercepts the Suzuki–Miyaura cycle (Scheme 1A, path A).

Careful optimization of the palladium(II)-catalyzed macrocyclization allowed for a scalable synthesis of mycocyclsin (Scheme 1B). 3-Iodo-L-tyrosine **27** was smoothly converted to **28** in three steps with no column chromatography. At this stage, ester hydrolysis or Boc deprotection afforded **29** or **30**, respectively, in quantitative yields. Next, peptide coupling of **29** and **30** with HBTU, followed by acid-promoted intramolecular cyclization, provided DKP **3** in up to eight grams.[8] Diiodide **3** was readily transformed to cyclophane **4** by employing the optimized Pd(II)-catalyzed oxidative coupling conditions on both half gram and gram scale in yields up to 63%. Finally, benzyl ether removal to expose the free phenol afforded mycocyclosin **2** in excellent yield.[16]

In conclusion, we have developed a scalable approach to the strained macrocycle, mycocyclosin. Careful evaluation of the reaction conditions for the palladium(II) catalyzed cross-coupling reaction led us to observe that both water and air are critical reaction additives to promote efficient reactivity. Experimental support for the formation of a diboronate intermediate suggests that oxidative homocoupling to the strained biaryl architecture is plausible. We anticipate that this scalable entry to mycocyclosin will facilitate expedient access to potential *M. tuberculosis* antimicrobials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b00894.

Experimental details and spectroscopic data for all intermediates, reactants, and products (PDF)

Accession Codes

CCDC 1837911 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: corinnas@umich.edu.

ORCID

Corinna S. Schindler: 0000-0003-4968-8013

Notes

The authors declare no competing financial interest.

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