Published in final edited form as:

J Am Chem Soc. 2020 September 23; 142(38): 16161–16166. doi:10.1021/jacs.0c04917.

Synthesis of 9-Dechlorochrysopaentin A Enables Studies Revealing Bacterial Cell Wall Biosynthesis Inhibition Phenotype in *B. subtilis*

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Abstract

Chrysopaentin A is an antimicrobial natural product isolated from the marine alga *C. taylori* in milligram quantity. Structurally, chrysopaentin A features a macrocyclic biaryl ether core incorporating two trisubstituted chloroalkenes at its periphery. A concise synthesis of iso- and 9-dechlorochrysopaentin A enabled by a *Z*-selective ring-closing metathesis (RCM) cyclization followed by an oxygen to carbon ring contraction is described. Fluorescent microscopy studies

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The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c04917>.

Experimental details, additional biological data, and spectral data (PDF)

Time-lapse imaging of FtsZ localization in *B. subtilis*: Untreated (AVI)

Time-lapse imaging of FtsZ localization in *B. subtilis*: DMSO control (AVI)

Time-lapse imaging of FtsZ localization in *B. subtilis*: 9-Dechlorochrysopaentin A (VU0849855) (AVI)

Time-lapse imaging of FtsZ localization in *B. subtilis*: Iso-9-dechlorochrysopaentin A (VU0849838) (AVI)

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revealed 9-dechlorochrysopaentins leads to inhibition of bacterial cell wall biosynthesis by disassembly of key divisome proteins, the cornerstone to bacterial cell wall biosynthesis and division.

Graphical Abstract

Microbial secondary metabolites (natural products) have a rich history in the discovery and development of antibiotics for the clinical treatment of infections.¹ Not only have these antimicrobial natural products revealed new antibiotic leads, but they have also identified new antimicrobial targets and provided insight into fundamental microbiology by study of their mechanism of action. For example, our understanding of bacterial cell wall biosynthesis has benefited from the discovery and study of β -lactam and glycopeptide natural products.² From the perspective of human health, the emergence of antibiotic resistance remains problematic and further enforces the need for the continued identification and study of antimicrobial natural products.^{1b}

In 2010, Bewley and co-workers reported on the isolation, structure elucidation, and antimicrobial activity of chrysopaentins A-H (Figure 1).³ Isolated from the marine alga *Chrysophaeum taylori*, the structures of chrysopaentins A-D and F-H were determined to consist of common halogenated macrocyclic biaryl ethers, while chrysopaentins E was assigned an open-chain structure. Chrysopaentins A-D and F-H are presumably derived from chrysopaentins E by oxidative cyclization of the C ring phenol at B ring carbons C16 and C14, respectively (Figure 1). The most prevalent metabolite, chrysopaentins A, displayed antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. faecium*, and vancomycin-resistant *E. faecium* (VREF). Based on its structural similarity to zantrin Z1, a reported inhibitor of the bacterial cytoskeletal protein FtsZ,^{4,5} the mode of action of chrysopaentins A was hypothesized to operate by the same mechanism.^{3,6} Indeed, chrysopaentins A was shown to inhibit *S. aureus* and *E. coli* FtsZ GTPase activity and polymerization.^{3,6} Further study of chrysopaentins A was hindered due to unreliable supply from the producing marine alga (*C. taylori*) as well as failed attempts to produce chrysopaentins by in lab culturing of *C. taylori*.⁷ To date, synthetic studies have yielded only chrysopaentins fragments devoid of the macrocyclic core.^{8,9} In one exception, Harrowven and co-workers¹⁰ recently described an approach to chrysopaentins F that produced an inseparable mixture of isomeric macrocyclic products.

From a retrosynthetic perspective, the macrocyclic biaryl ether core structure of chrysopaentins A presented an interesting synthetic challenge, as it offers no obvious bond disconnection (Figure 2). Closure of the macrocyclic biaryl ether by way of a ring-closing metathesis (RCM) reaction was anticipated to be difficult as chloro alkenes remain

problematic substrates in RCM reactions.¹¹ However, a total synthesis of 9-dechlorochrysosphaentin A would momentarily avoid the chloro alkene RCM and reduce the problem to a *Z*-selective RCM closure (Figure 2).¹² Following RCM mediated ring closure, a Lewis acid mediated O to C alkyl migration¹³ would likely bifurcate between C5' and C3' leading to iso- and 9-dechlorochrysosphaentin A, respectively (Figure 2). Herein, we describe the total synthesis of iso- and 9-dechlorochrysosphaentin A, their antimicrobial activity, and preliminary mechanism of action studies revealing a potentially novel mode of inhibition of bacterial cell wall biosynthesis.

Our synthesis started with *O*-alkylation of resorcinol **1** (Scheme 1) followed by a Dakin oxidation of the remaining aromatic aldehyde to afford the corresponding phenol. The latter was alkylated with allyl bromide, and the resulting allyl aryl ether was subjected to a Claisen rearrangement to afford phenol **2**.⁸ A solution of phenol **2** and 5-bromo-2-fluoronitrobenzene were subjected to nucleophilic-aromatic substitution reaction conditions to yield biaryl ether **3** in 93%. The C-ring aryl bromide **3** was fashioned to corresponding phenol by way of cross-coupling with (BPin)₂ followed by an oxidative workup. Finally, the C-ring nitro group was reduced to the corresponding aniline and chlorinated under Sandmeyer¹⁴ reaction conditions to complete the Northern BC biaryl ether fragment (**4**).

The synthesis of the Southern AD biaryl ether (**10**), its merger with biaryl **4**, and the completion of 9-dechlorochrysosphaentins are shown in Scheme 2. First, phenol **5**, available in two steps from 4-bromo-3,5-resorcylic acid, was coupled with 3-chloro-4-fluorobenzaldehyde by way of a second S_NAr reaction. Baeyer-Villiger oxidation of the A ring aldehyde of the coupled product afforded momentarily an aryl formate that upon treatment with acidic methanol provided phenol **6** in 45–50% yield. Bromination of phenol **6** (NBS, *p*-TSA) occurred selectively at the C5' carbon, and following phenol protection (*i*-PrBr, K₂CO₃), the derived aryl bromide was coupled with allyl pinacol boronate ester to give **7**.¹⁵ Attention was then turned to D ring functionalization starting with reduction of the ester group with DIBAL, followed by chlorination (TPP·Cl₂) to give an intermediate benzyl chloride that was homologated to alkyne **8** in two steps.⁹ Alkyne **8** was advanced to trisubstituted allyl alcohol **10** starting with homologation to the corresponding alkynoate. Alkynoates derived by direct acylation of alkyne **8** with chloroformates tended to undergo isomerization to the corresponding allenolate. The undesired isomerization was avoided by Fisher esterification of the corresponding alkynoic acid, the latter derived from alkyne **8** by deprotonation followed by carbon dioxide quench. Stannyl cupration¹⁶ of the derived methyl alkynoate proceeded in 60–70% yield to afford enoate **9** as a single geometric isomer. Reduction of enoate **9** to the corresponding allylic alcohol followed by tin-chloride exchange¹⁷ completed assembly of biaryl ether **10**. Coupling of phenol **4** and allyl alcohol **10** under Mitsunobu conditions resulted in the smooth merger of the BC and AD fragments. The coupled product underwent cyclization to macrocycle **11** on treatment with a Grubbs *Z*-selective catalyst (**C633**)^{12,18} in yields of 65% to 70%. In contrast, ring closure using a second-generation Grubbs catalyst resulted in an equal mixture of *Z*- and *E*-isomeric products. Heating a dichloroethane solution of aryl ether **11** and BF₃·OEt₂ afforded a near 1:1 inseparable mixture of isomeric products from nonselective O to C3'/C5' alkyl migration. Removal of the isopropyl protecting groups proceeded smoothly on treatment

with boron trichloride to yield 9-dechlorochrysopaentin A and iso-9-dechlorochrysopaentin A following HPLC assisted separation. The isomeric 9-dechlorochrysopaentins were assigned compound registry numbers **VU0849855** and **VU0849838**, respectively. In the course of our synthetic studies, we accessed two additional chrysopaentin congeners, **VU0848355** and **VU0848354**, that incorporate D ring methyl ethers (Scheme 2) and proved useful in preliminary antimicrobial and phenotypic studies.

Aside from a slight loss of potency against VRE, the bacterial growth inhibition of 9-dechlorochrysopaentins and congeners compared favorably to chrysopaentin A when evaluated across a panel of Gram-positive bacteria (Table 1, Figures S3 and S4). Unexpectedly, iso-chrysopaentins (iso-9-dechlorochrysopaentin A and **VU0848354**), possessing an unnatural C ring substitution pattern, showed approximately 2-fold greater potency relative to their naturally substituted C ring counterparts (9-dechlorochrysopaentin A and **VU0848355**).

As mentioned above, chrysopaentin A has been proposed to act as an inhibitor of the cytoskeletal protein FtsZ. As a central player in cell division, inhibition of FtsZ has been considered an untapped target in the development of novel antimicrobial agents.^{5,19} The synthesis described here allows us to investigate in detail the mechanism by which the chrysophantins perturb FtsZ.

FtsZ is a GTP-dependent protein shown to play a critical role in bacterial cell division.^{19,20} Only recently has the central role of FtsZ and other proteins in the orchestration of cell division been fully appreciated.²¹ It is known that, in preparation for cell division, FtsZ, along with FtsA, forms filaments at the new division site that recruit the peptidoglycan-synthesizing enzymes required for construction of the septal disk.²² High-resolution microscopy, along with single-molecule tracking and real-time, temporal visualization, of bacterial cell wall synthesis has revealed FtsZ's dynamic role in guiding the multiprotein complex called the divisome.²³ Super-resolution techniques have revealed FtsZ filaments display a circumferential, inward movement that correlates with the progressive insertion of new cell wall material.²⁴ FtsZ "treadmilling", powered by its own GTPase activity, controls both the rate of new cell wall synthesis and the rate of cell division. This dynamic process has been visualized in real-time using high-resolution microscopy with orthogonally labeled fluorescent protein fusions and fluorescent d-amino acids (FDAAs).²⁵

Chrysopaentin A was shown to inhibit FtsZ mediated polymerization *in vitro* by microscopy and was shown to bind recombinant FtsZ at the GTP binding site by Saturation Transfer Difference (STD) NMR.³ We compared chrysopaentin congeners **VU0848354** and **VU0848355** to the well-studied FtsZ inhibitor PC1907723 (Figure 3A).²⁶ Inhibition of FtsZ by PC1907723 is known to produce an elongated phenotype of rod-like bacteria such as *B. subtilis*. We observed cell lengthening when *B. subtilis* was treated with PC190723 at a concentration of 5 $\mu\text{g/mL}$ ($10 \times \text{MIC}$) (Figure 3B). In contrast, concentrations of **VU0848354** and **VU0848355** up to 20 μM ($10 \times \text{MIC}$) did not lead to cell lengthening. Next, we examined the effect of PC190723 and 9-dechlorochrysopaentins on cell wall biosynthesis using FDAAs. Much to our surprise 9-dechloro- and iso-9-dechlorochrysopaentin (a.k.a. **VU0849855** and **VU0849838**) (Figures 3C and S1C) and

congeners **VU0848354** and **VU0848355** (Figure S1A, B) inhibited incorporation of FDAAs at 10 μM ($5 \times \text{MIC}$), comparable to the well-known peptidoglycan inhibitor ampicillin (10 $\times \text{MIC}$) (Figure S1A, B). In contrast, FtsZ inhibitor PC190723 showed minimal inhibition of FDAA incorporation up to 10 times its MIC as reported earlier (Figure S1A, B).²⁴ We next examined, the effect of 9-dechlorochrysopaentins on the localization of FtsZ using fluorescently labeled FtsZ protein fusion (mNeonGreen). Treatment with 30 μM ($5 \times \text{MIC}$) **VU0849855** or 10 μM ($5 \times \text{MIC}$) **VU0849838** lead to complete delocalization of FtsZ as observed by high-resolution microscopy (Figure 3D; SI Movies S1 (untreated), S2 (**VU0849855**), and S3 (**VU0849838**)). In parallel microscopy experiments, we observed the delocalization of mNeonGreen fluorescent protein fusions of FtsZ, FtsA, and PBP2B from the division plane upon treatment with natural (isolated from *C. taylori*) chrysopaentin A (Figure S2). In contrast to the 9-dechlorochrysopaentins and chrysopaentin A, PC190723 leads not to the dispersion of FtsZ but dislocation away from the cell midline, as reported earlier,²⁶ and in agreement with continued cell wall synthesis as observed using FDAA labeling (Figure S1B).

In conclusion, we report a concise and cost-effective chemical synthesis of iso- and 9-dechlorochrysopaentin A (a.k.a. **VU0849855** and **VU0849838**) and confirmed their antimicrobial activity as equal or greater to that of chrysopaentin A itself. In experiments aimed at defining the mechanism of their antimicrobial activity, we discovered a novel mode of inhibition of bacterial cell wall biosynthesis. Our data suggest that the treatment of *B. subtilis* cells with chrysopaentin A and congeners results in the disassembly of the Z-ring and dispersion of FtsZ from the division plane in cells preparing for division. Interestingly, cells treated with 9-dechlorochrysopaentins when in their early growth stages do not appear to be able to localize FtsZ at the new division site. Furthermore, diminished FDAA incorporation in treated cells suggests that new cell wall synthesis and/or cell wall modification are being suppressed.

When compared to data obtained with PC190723, our data are suggestive of a unique mechanism of action. For example, *B. subtilis* cells treated with PC190723 show some dislocation of FtsZ from the division plane whereas those treated with chrysopaentin A and congeners show complete dispersion of FtsZ from the division site. Moreover, PC190723-treated cells are still able to incorporate new cell wall material whereas those treated with the chrysopaentins show no evidence for incorporation of new cell wall material. Interestingly, as noted above, cells treated with the chrysopaentins in early growth stages appear unable to localize FtsZ at the new division site. It is tempting to speculate that the chrysopaentins may inhibit the ability of FtsZ to bind GTP, which is required for proper FtsZ polymerization for treadmilling and consistent with the GTP-competitive binding originally reported for chrysopaentin A.³ This might explain the inability of cells in early growth stages to localize FtsZ to the new division site. The inability to reacquire GTP after its hydrolysis may also explain the dispersion of FtsZ from the division site after chrysopaentin treatment.

Regardless, *B. subtilis* cells treated with the structurally interesting chrysopaentins produce a novel phenotype that is distinct from the well-studied FtsZ inhibitor PC190723. This family of compounds may reveal a novel target(s) for antibacterial intervention or serve as

valuable tools to further elucidate key elements of bacterial cell division. Toward this end, additional experiments designed to further elucidate the unique mechanism(s) of action of these novel agents are being designed and results from these studies will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We gratefully acknowledge Professor Robert Grubbs (Caltech) for supplying catalyst C633 and Professor Jared Shaw (UC-Davis) for helpful discussions. C.R.F. acknowledges the support of the Vanderbilt Chemistry Biology Interface (V-CBI) training program (T32 GM065086). M.S.V. gratefully acknowledges support from the National Institutes of Health (GM113172 and GM136365), and C.A.B. is supported by the Intramural Research Program of the National Institutes of Health (NIDDK).

ABBREVIATIONS

RCM	ring-closing metathesis
FtsZ	Filamenting temperature-sensitive mutant Z
(BPin)₂	Bis(pinacolato)diboron
NBS	N-bromosuccinimide
p-TSA	p-Toluenesulfonic acid
iPr-Br	isopropyl bromide
DIABL	diisobutylaluminum hydride
TPP·Cl₂	triphenylphosphine dichloride
STD NMR	saturation transfer difference nuclear magnetic resonance
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
VRE	vancomycin-resistant <i>Enterococcus faecalis</i>
MIC	minimum inhibitory concentration
FDAAs	fluorescently labeled D-amino acids

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Figure 1.

Bio-oxidative cyclization of chrysosphaentin E and structure of chrysosphaentin A.

Figure 2.
Retrosynthetic analysis of 9-dechlorochrysosphaentins.

Figure 3.
Bacillus subtilis phenotype in response to antimicrobials. (A) Structure and activity of the FtsZ inhibitor PC190723. (B) Cell lengthening in response to PC190723 and chrysopaentin congeners **VU0848354** and **VU0848355**. (C) Quantification of FDAA incorporation is inhibited in cells treated with **VU0849838** and **VU0849855**. (D) *B. subtilis* cells treated with **VU0849838** or **VU0849855** leads to the dispersion of FtsZ.

Scheme 1.
Synthesis of BC Biaryl Ether 4

Scheme 2.
Synthesis of 9-Dechlorochrysopaentins

Table 1.Antimicrobial Activities of Chrysopaentins^a

	MIC ($\mu\text{g/mL}$)				
	<i>S. aureus</i>	MRSA	<i>E. faecalis</i>	VRE	<i>B. subtilis</i>
chrysopaentin A	2	2	2	2	1.4
9-dechlorochrysopaentin A	2.5	2.5	2.5	5	4
iso-9-dechlorochrysopaentin A	1.1	1.1	0.3	2.2	1.4
VU0848355	4.2	4.2	4.2	8.4	4.2
VU0848354	2.1	2.1	4.2	4.2	1.4

^aBacterial strains were obtained from the ATCC and have the following identifiers: pathogenic strains: *S. aureus* 25913; MRSA, methicillin-resistant *S. aureus* 43300; *Enterococcus faecalis* 29212; VRE, vancomycin-resistant-*E. faecalis* 51299; nonpathogenic strain: *B. subtilis*. Each assay was performed in triplicate.