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Highly Convergent Total Synthesis and Assignment of Absolute Configuration of Majusculamide D, a Potent and Selective Cytotoxic Metabolite from *Moorea sp.*

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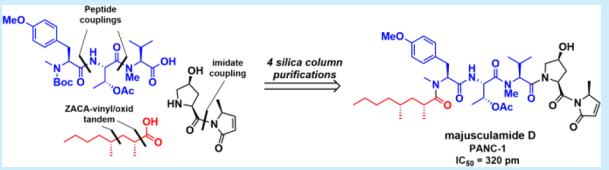
Supporting Information

ABSTRACT: The total synthesis of majusculamide D (MJS-D) is described, a lipopentapeptide originally isolated from *Lyngbya majuscula* and reisolated from a *Moorea* sp. MJS-D possesses selective and potent cancer cell toxicity. A scalable and convergent strategy with a minimal number of purifications produced significant quantities of MJM-D for in vivo evaluations. The absolute configuration of the 1,3-dimethyl-octanamide motif was determined by synthesis of this fragment via ZACA chemistry.



Natural products (NPs) continue to be an important source of novel chemical structures that have directly yielded or inspired a majority of our currently approved drugs.[1] Marine cyanobacteria, especially *Moorea sp.*,[2] are prolific producers of bioactive secondary metabolites.[3] Many of these cyanobacterial NPs have promising antitumor properties in vitro.[4] Our continued exploration of these life forms for their bioactive components has been productive in both finding new molecules as well as new biological activities for known ones.[5] In the current case, evaluation of the extract of a *Moorea* sp. sample collected from Papua New Guinea in a solid-tumor cell assay revealed promising selectivity and exquisite potency against pancreatic and glioblastoma cell lines. This activity is notable, given the acute increase in the mortality rate of pancreatic cancer that is projected to surpass breast, colon, and prostate cases in the near future.[6] Investigation of the active fraction revealed majusculamide D (MJS-D, **1**, Figure 1), a lipopentapeptide isolated by Moore and Entzerof in 1988,[7] to be responsible for the in vitro bioactivity. MJS-D is structurally related to the microcolins, a class of metabolites also isolated from cyanobacteria (Figure 1)[8] which show interesting biological properties. This has prompted total syntheses[9,10] as well as SAR studies of the microcolins.[11] Interestingly, the absolute configuration of both stereocenters within the 2,4-dimethyloctanoate side chain of **1** were never unequivocally determined in the original isolation work.[7] Given the promising in vitro biological profile and uncertainty about the two stereocenters in **1**, we designed a total synthesis that would allow access to all possible stereoisomers of **1** and provide more compounds for further anticancer investigations.

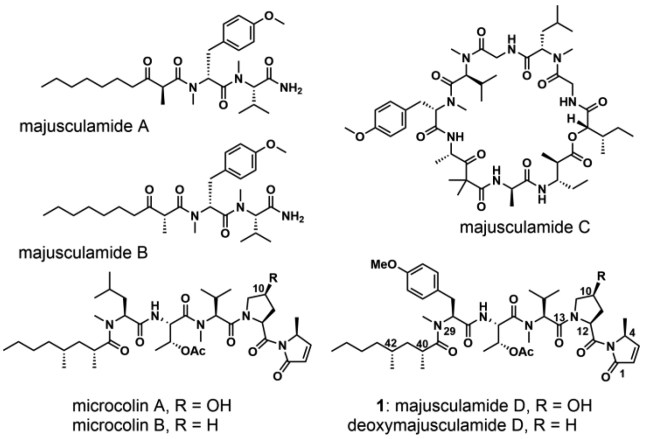


Figure 1. Structure of majusculamide D (MJS-D) and related analogues.

Our synthetic strategy involved two key disconnections between positions C13−N12 and C39−N29, resulting in three fragments of similar molecular weight that converge at a late stage via standard peptide coupling reactions. We also sought to manage our reaction sequences by carefully removing unnecessary purifications and using simple aqueous workups and filtration over silica plugs so that solvent and labor were minimized. Furthermore, in our synthetic design of the 2,4-dimethyloctanoic acid fragment, we required a concise set of

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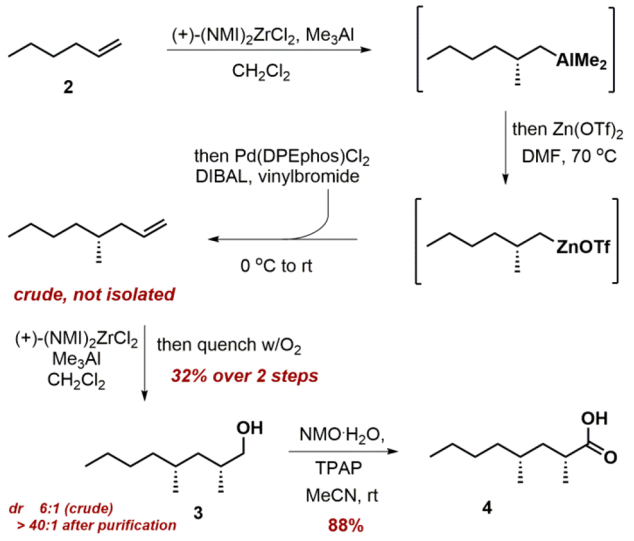
reactions that could access all possible stereoisomers, to establish the absolute configuration of C40 and C42.

Based on the absolute configuration established for microcolin A and B,[9,10b] we inferred that the correct configuration of **1** at the unassigned carbon centers C40 and C42 should be *R,R*. To accomplish the synthesis of this octanoic acid fragment, we took advantage of the zirconium-catalyzed asymmetric carboalumination (ZACA) chemistry described by Negishi and co-workers.[12] Beginning with commercially available 1-hexene, a catalyst/reagent controlled carboalumination reaction was performed with this alkene using Me_3Al and (+)-NMI_2ZrCl_2[13] (Scheme 1). The resulting

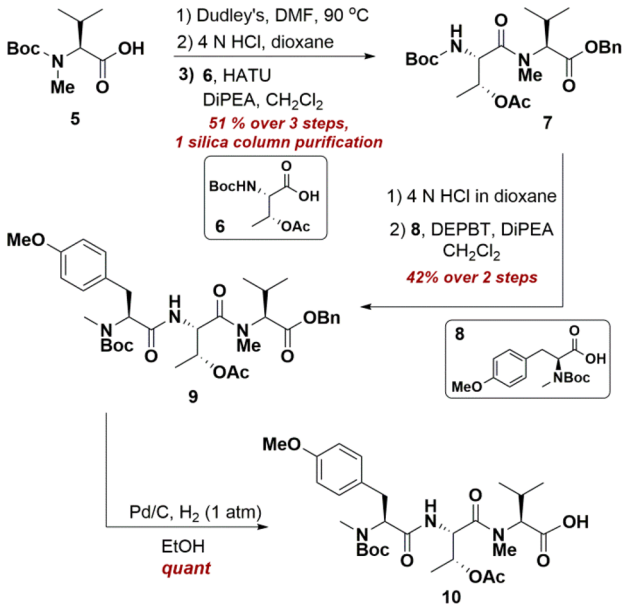
Scheme 1. Synthesis of (2*R*, 4*R*)-2,4-Dimethyl Octanoic Acid via ZACA Vinylation/Oxidation Sequence



organoaluminum intermediate was trans-metalated in situ with $Zn(OTf)_2$ and coupled with a freshly prepared vinyl-Pd species. The crude (*R*)-4-methyl-1-octene was moved forward with minimal purification and subjected to a second carboalumination reaction; upon exposure to a stream of O_2 and workup, crude **3** was obtained in a modest diasteromeric ratio (dr) ratio (6:1, ^{13}C NMR).[12b] Fortunately, the undesired diastereomer was largely removed via silica gel chromatography providing enantiomerically enriched **3** in 32% yield. Octanol **3** was then mildly oxidized[14] to the corresponding acid in good yield.

To efficiently build the tripeptide backbone of **1**, we sought to use a peptide coupling reagent that could be removed via aqueous workup, would provide acceptable yields, and, most importantly, avoid epimerization of the α-carbons during reaction. Thus, we protected commercially available Boc-N-Me-L-valine with Dudley's reagent,[15] which after filtration through a silica plug, was directly coupled with N-Boc-OAc-L-threonine **6** (Scheme 2) using hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) as the coupling reagent. A single-column purification was used in this three-reaction sequence. This was followed by quantitative acidic conditions removal of the N-Boc protecting group from dipeptide **7** and subsequent coupling to tyrosine derivative **8**, this time with Goodman's[16] reagent. Debenzylation by standard hydrogenation conditions quantitatively provided tripeptide acid **10**. This six-step reaction sequence required only two silica

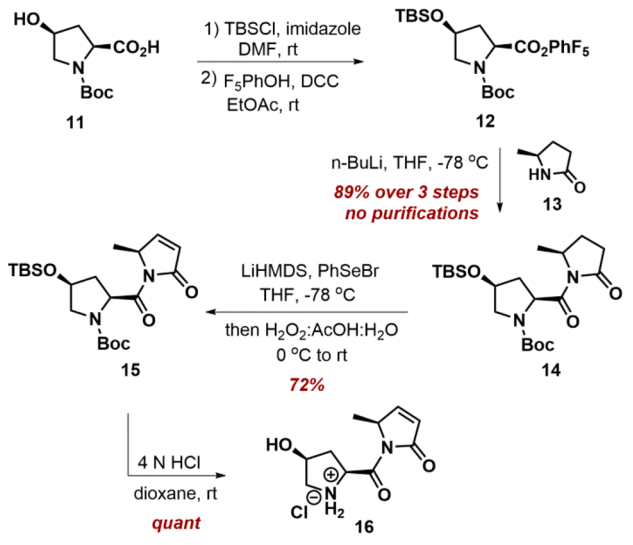
Scheme 2. Synthesis of Tripeptide Backbone of MJS-D



column purifications and provided gram-scale quantities of intermediate **10**.

For the synthesis of the pyrrolylproline fragment of **1**, we sought to improve upon the established chemistry[10,11] to access these types of motifs. Beginning with commercially available N-Boc-*cis*-4-hydroxy-L-proline, we distinguished the two functional groups via TBS protection of the alcohol and pentafluorophenol DCC coupling to the acid (Scheme 3). A

Scheme 3. Synthesis of Pyrrolylproline Fragment via Imide Coupling



careful aqueous workup of both reactions gave high-purity TBS-protected perfluorester **12** without the need for purification. Imide coupling with chiral pyrrolidone **13** and *n*-BuLi at low temperature smoothly provided imide **14**, which could be oxidized to enone **15** under well-established selenoxide elimination conditions.[14] Simultaneous removal of the N-Boc and *O*-TBS protecting groups under strongly acidic conditions yielded the appropriate pyrrolylproline HCl salt **16** in quantitative fashion. Overall, synthesis of this fragment was

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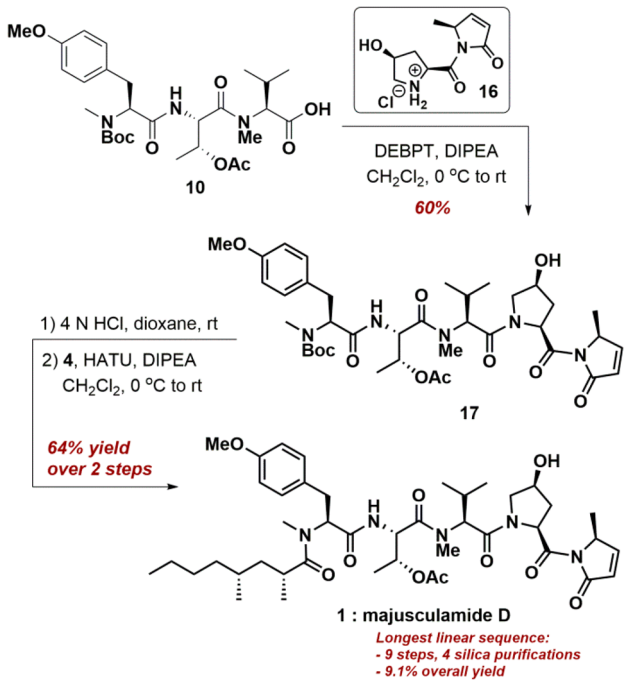
achieved in five synthetic steps, with only two silica column purifications and an overall yield of 64%.

To complete the synthesis of MJS-D, the three fragments constructed above were coupled together in two moderate yielding condensation reactions. Given the chemo-differentiation of the protecting groups on tripeptide **9**, we could selectively reveal the appropriate functional group for coupling. Hence, acid **10** was coupled to the pyrrolylproline fragment **16**, using Goodman's reagent[16] to provide pentapeptide **17** (Scheme 4). N-Boc removal and subsequent coupling with

Scheme 4. Late-Stage Coupling of Key Fragments and Completion of the Synthesis of 1



dimethyl octanoic acid **4** provided MJS-D (**1**) in acceptable yield. This synthetic material perfectly matched HPLC retention time, MS/MS fragmentation, ^{1}H and ^{13}C NMR spectra, and 2D NMR data sets (COSY, HSQC), with those of an authentic sample of natural MJS-D. Our comparison of natural and synthetic **1** usng ^{1}H NMR is possible given that MJS-D does not have concentration-dependent NMR shifts and is observed as a single rotamer (see SI-14 in the Supporting Information). Furthermore, close inspection of previously published NMR data for the total synthesis of microcolin A showed substantial differences in the shift associated with other diastereomers when other octanoic acid stereoisomers are installed.[10] Upon completion of the total synthesis of **1**, we submitted this material, along with natural MJS-D for cancer cell cytotoxicity evaluation. We initially utilized a solid-tumor disk-diffusion assay (DDA),[4a] where a panel of cancer cell lines are grown on agar under conditions that simulate anchorage independency.[17] The synthetic and naturally occurring samples of MJS-D had essentially identical biological profiles exhibiting selective cytotoxicity toward both pancreatic (PANC-1) and glioblastoma (U251N) cancer cell lines. Furthermore, **1** showed a range of potencies (IC_{50} values; see Table 1) demonstrating a >4000-fold difference between PANC-1 cells and HepG2 cells

Table 1. In Vitro IC_{50} Values of Majusculamide D (MJS-D, 1) to a Panel of Cancel Cell Lines

cancer cell line	tissue type	IC_{50} (nM)
PANC-1	pancreatic	0.32
U251N	brain	36.8
HepG2	liver	1,396
H125	lung	147

(Table 1 and Figure 2). These results have stimulated additional ongoing SAR studies in the MJS-D structure class.

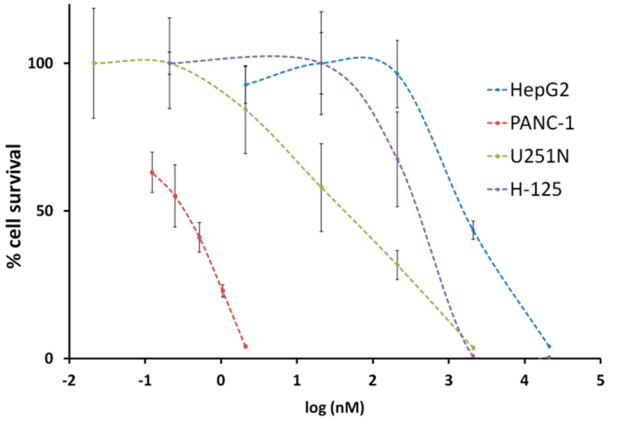


Figure 2. In vitro concentration–response curves for MJS-D (**1**) to a panel of cancer cell lines

In conclusion, we report here a highly convergent and efficient approach for the synthesis of the cyanobacterial lipopeptide majusculamide D (**1**). Our synthetic strategy highlights the use of ZACA chemistry to rapidly build the chirally methylated octanoic acid side chain. We also carefully selected reagents and conditions that facilitated convenient workups and bypassed purifications of the synthetic intermediates; this resulted in a chemical synthesis with significant scale-up potential and thereby enables future biological investigations. Finally, we determined the absolute configuration of the C40 and C42 methyl groups (Figure 1) within the octanoic acid side chain of **1** to be *R*,*R*, and showed that the synthetic material is equipotent and similarly selective to the natural product in its cytotoxicity profiles toward PANC-1 and U251N cell lines *in vitro*. We envision utilizing this efficient and scalable chemical synthesis to further explore Structure–Activity-Relationships (SARs) of majusculamide D as well as creation of derivatives that will allow determination of its biochemical target and mechanism of action.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b04050.

Synthetic procedures and spectroscopic data (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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