Chemical Science

ROYAL SOCIETY OF CHEMISTRY

EDGE ARTICLE

View Article Online
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Cite this: *Chem. Sci.*, 2022, **13**, 13403

All publication charges for this article have been paid for by the Royal Society of Chemistry

Received 24th August 2022
Accepted 20th October 2022

DOI: 10.1039/d2sc04727f

rsc.li/chemical-science

Total synthesis of the antibacterial polyketide natural product thailandamide lactone†

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Stereoselective total synthesis of the structurally intriguing polyketide natural product thailandamide lactone was accomplished, and done so using a convergent approach for the first time to the best of our knowledge. The key features of this synthesis included use of a Crimmins acetate aldol reaction, Evans methylation, Urpi acetal aldol reaction, Sharpless asymmetric epoxidation and subsequent γ-lactonization for the installation of six asymmetric centers and the use of the Negishi reaction, Julia-Kocienski olefination, cross metathesis, HWE olefination and intermolecular Heck coupling for construction of a variety of unsaturated linkages. Pd(I)-based Heck coupling was introduced, for the first time to the best of our knowledge, quite efficiently to couple the major eastern and sensitive western segments of the molecule. The antibacterial activity of thailandamide lactone was also evaluated.

Introduction

During the mining of the genome of *Burkholderia thailandensis*, a bacterium isolated from rice paddies in central and north-eastern Thailand, Hertweck and co-workers[1] in 2008 first observed the labile polyene polyketide thailandamide A (**1**, Fig. 1), albeit in minute quantities. To better understand thailandamide biosynthesis, the silent *tha* PKS-NRPS gene cluster of *B. thailandensis* was activated by a research group in 2010 through manipulation of a quorum sensing (QS) regulatory system that produced a mutant with a dramatically altered metabolic profile.[2] This process resulted in the isolation of the structurally challenging new polyketide thailandamide lactone (**2**, Fig. 1), which was not detected in wild type broth initially. Moreover, the production of thailandamide A (**1**) was significantly greater here than when the wild type was used.[2] Later, the same group developed an elegant biosynthetic route yielding the first total synthesis of thailandamide A; this development enabled them to find another unstable metabolite, namely thailandamide B (**3**, Fig. 1), a geometrical isomer of thailandamide A.[3] Broad biological screening of thailandamide A revealed its selective and potential inhibitory activity against various pathogenic Gram-positive and Gram-negative bacteria with a specific mode of action.[4] However, the antibacterial activity of thailandamide lactone and thailandamide B remained undisclosed. The highly challenging architectural features and natural scarcity of thailandamide lactone and lack of a synthetic route to this lactone—together with our continual interest in natural products chemistry[5]—encouraged us to seek out its total synthesis. Structurally, thailandamide lactone[2] is a linear polyene polyketide where a tetraene conjugated with a γ-butyrolactone is fused with a conjugated triene through an enolized dione moiety. It consists of six asymmetric centers including a quaternary center at one terminus of the molecule and a phenolic moiety at the other terminus. Herein, we report a convergent and highly modular route for the first total synthesis of thailandamide lactone and report its antibacterial activity against various pathogenic and non-pathogenic bacterial strains.

Results and discussion

A retrosynthetic analysis of thailandamide lactone (**2**) is shown in Scheme 1. We envisioned that the target molecule could be constructed from vinyl iodide **4** and 1,3-dione-containing

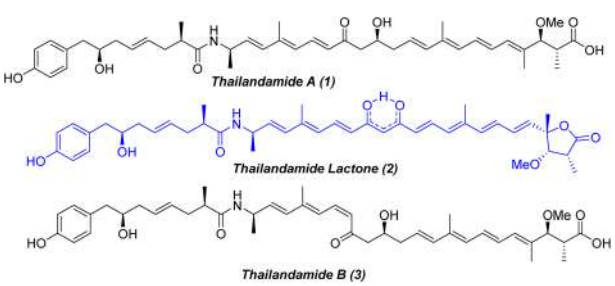

Fig. 1 Chemical structures of thailandamide family natural products.

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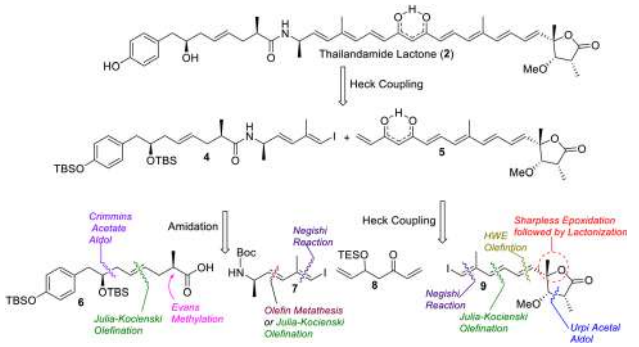
† Electronic supplementary information (ESI) available. CCDC 2184015 and 2184016. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d2sc04727f



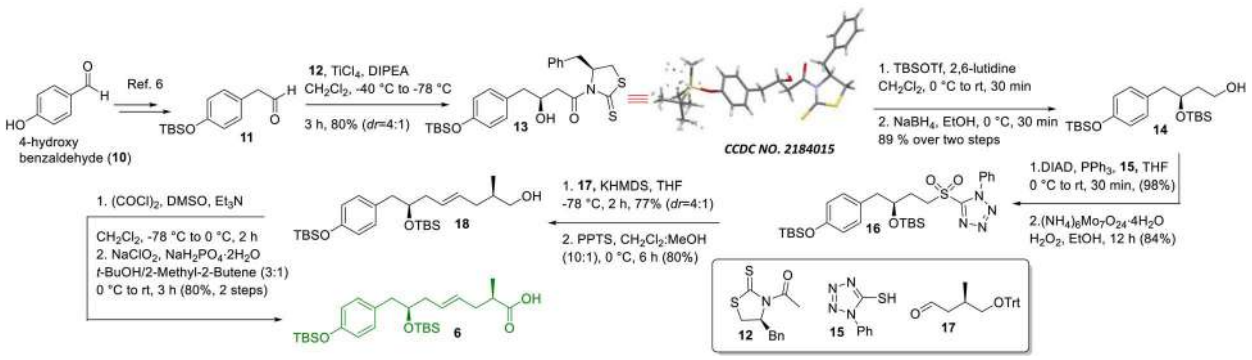
Scheme 1 Retrosynthetic analysis of thailandamide lactone (**2**).

polyene **5** adopting intermolecular Heck coupling as the key step. Vinyl iodide **4** could further be made from compounds **6** and **7** by performing amide coupling. Compound **6** could be assembled using the Crimmins acetate aldol reaction, Julia-Kocienski olefination and Evans methylation as the key steps, whereas compound **7** could be accessed utilizing cross olefin metathesis or Julia-Kocienski olefination and the Negishi reaction as the pivotal steps. On the other hand, keto-alkene **5** could be prepared from intermediates **8** and **9** using Heck coupling, whereas compound **9** could be synthesized using the Negishi reaction, Julia-Kocienski olefination, HWE olefination, Urpi acetal aldol reaction, Sharpless asymmetric epoxidation and subsequent γ-lactonization as the salient steps.

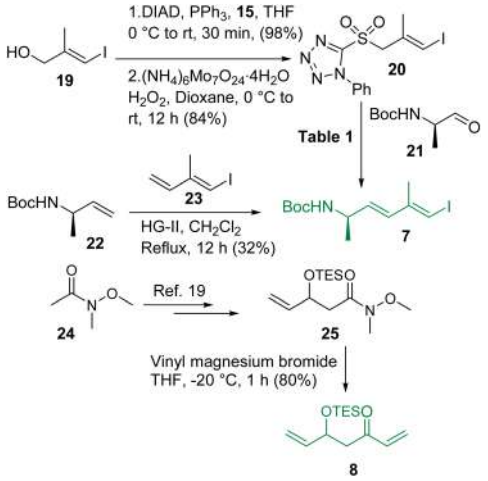
The synthesis of intermediate **6** was commenced with the known compound **11** (Scheme 2) prepared from commercially available 4-hydroxy benzaldehyde (**10**) following a literature procedure;[6] compound **11** was subjected to a Crimmins acetate aldol reaction[7] using the known auxiliary **12**[7b] in the presence of $TiCl_4$/DIPEA to obtain compound **13** as the major product in 80% yield along with its minor counterpart (dr = 4 : 1). The major aldol product was separated from other components using silica gel column chromatography and its structure was confirmed unambiguously using X-ray crystallographic analysis. Next, compound **13** was treated with TBSOTf/2,6-lutidine followed by $NaBH_4$ to access compound **14**, which was subjected to the Mitsunobu reaction[8] using 1-phenyl-5-thiotetrazole (**15**) in the presence of DIAD/PPh_3 and oxidized further using $(NH_4)_6Mo_7O_{24}\cdot 4H_2O/H_2O_2$ in ethanol[5c] to achieve sulfone **16** in very good overall yield. Next, sulfone **16** was subjected to Julia-Kocienski olefination[5c,9] with the known aldehyde **17**[10] using KHMDS to obtain the corresponding *E*-coupled isomer as the major product along with its minor *Z*-isomer (dr = 4 : 1). The purified major isomer was subsequently treated with PPTS to obtain alcohol **18**, which finally was oxidized to acid **6** using Swern oxidation followed by Pinnick oxidation.[11]

The synthesis of intermediates **7** and **8** is depicted in Scheme 3. The known vinyl iodide **19**,[12] prepared from propargylic alcohol using the Negishi reaction as the key step, was subjected to the Mitsunobu reaction using 1-phenyl-5-thiotetrazole (**15**) and the resultant sulfide was oxidized using $(NH_4)_6Mo_7O_{24}\cdot 4H_2O/H_2O_2$ in dioxane[13] to access sulfone **20**. Notably, the production of sulfone **20** from its corresponding sulfide was found to be much more efficient in dioxane than in the commonly used ethanol. Sulfone **20** was then reacted with the known aldehyde **21**[14] following the Julia-Kocienski olefination protocol.[9,15] Several conditions were screened for synthesizing compound **7** (Table 1) and the use of KHMDS in DME (entry-4) was found to be the best (*E*/*Z* = 3 : 1). In parallel, the cross metathesis[16] between the known alkenes **22**[17] and **23**[18] was also investigated and it was observed that HG-II produced compound **7** in 32% yield with much better selectivity (*E* : *Z* = 10 : 1) compared to Julia-Kocienski olefination whereas G-II and HG-I functioned ineffectively, leaving a trace amount of the desired product. However, the geometrical isomers remained inseparable at this stage. On the other hand, commercially available Weinreb amide **24** was transformed to the known compound **25** following a literature procedure[19] and subjected further to a reaction with vinyl magnesium bromide to access intermediate **8** in very good overall yield.

The synthesis of aldehyde **34** is described in Scheme 4. The known aldehyde **27** prepared from prenol (**26**) following a literature method[20] was converted to the corresponding acetal using $(MeO)_3CH$/CSA, which was subjected further to the Urpi acetal aldol reaction[5b,21] in the presence of $TiCl_4$/DIPEA/$SnCl_4$ to access compound **29** with excellent selectivity (dr = 20 : 1). The purified compound was then treated with $LiOH\cdot H_2O/H_2O_2$ followed by NaOMe/MeOH to obtain compound **30** in 72% yield. The stereochemistry of asymmetric centers newly generated using Urpi acetal aldol reaction was confirmed further from an X-ray



Scheme 2 Synthesis of intermediate **6**.

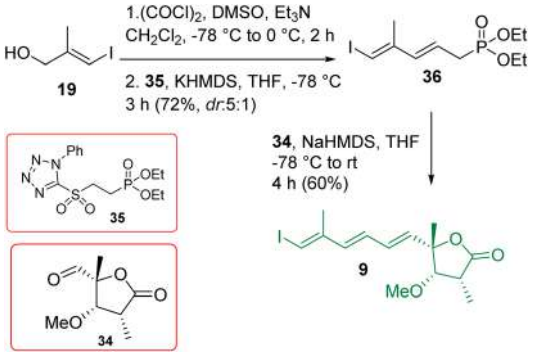


Scheme 3 Synthesis of intermediates 7 and 8.

Table 1 Efforts to optimize Julia-Kocienki olefination

Entry	Conditions	Yield ($E:Z$)
1	NaHMDS, THF, −78 °C	66% (1 : 1.2)
2	KHMDS, THF, −78 °C	70% (1 : 1)
3	LiHMDS, DME, −60 °C	62% (1.5 : 1)
4	KHMDS, DME, −60 °C	68% (3 : 1)

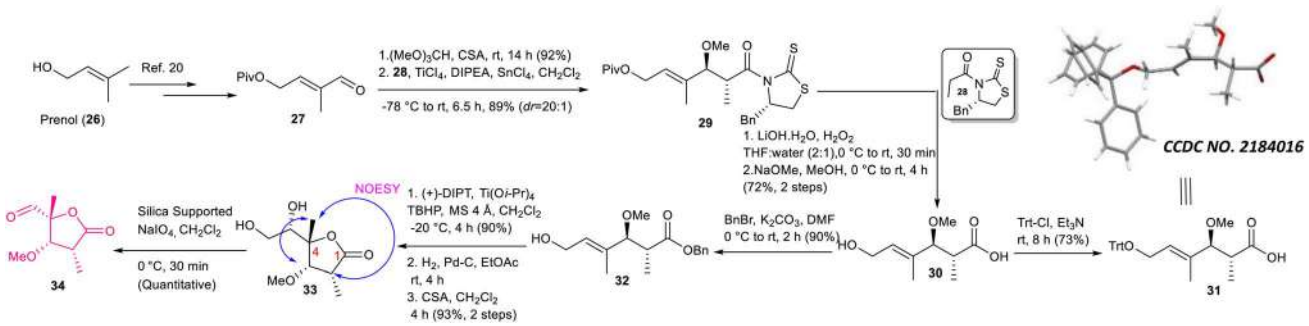
crystallographic analysis of compound **31**, which was synthesized from compound **30** by performing tritylation. Next, compound **30** was reacted with $BnBr/K_2CO_3$ to obtain benzyl ester **32**, which was subjected to Sharpless asymmetric epoxidation[22] followed by hydrogenation to produce the corresponding epoxy acid. The stage was set for γ-lactonization.[23] The corresponding epoxy acid was treated with CSA/CH_2Cl_2 to access the 5-exo cyclized product **33** exclusively. The characteristic NOESY correlation of C_4–Me with C_2–H and C_3–H confirmed its structure unambiguously. We did not observe the formation of any other possible γ-lactone originating *via* 6-endo cyclization followed by concomitant acyl migration.[23] Our exhaustive efforts for achieving oxidative cleavage of the diol moiety of compound **33** using either $NaIO_4$ or $NaIO_4/NaHCO_3$ did not produce aldehyde **34** in an isolable yield due to its rapid decomposition. Delightfully, silica-supported $NaIO_4$[24] provided considerable relief here, where the required aldehyde was obtained quantitatively.



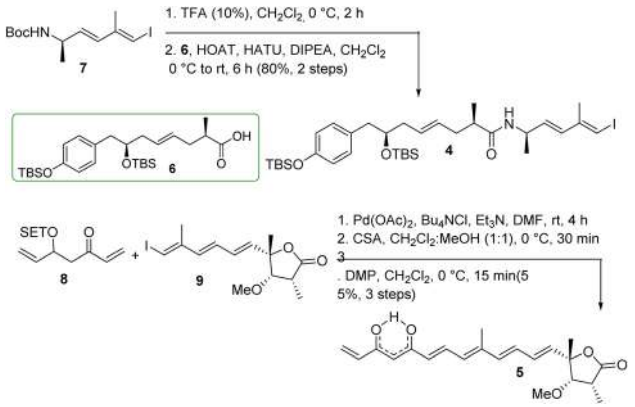
Scheme 5 Synthesis of intermediate 9.

The construction of compound **9** is shown in Scheme 5. Alcohol **19** was oxidized using the Swern condition and subjected to Julia-Kocienski olefination[9] with the known sulfone **35**[25] to access compound **36** as a major product (dr = 5 : 1). The purified major isomer was reacted further with aldehyde **34** in the presence of NaHMDS/THF following the HWE olefination protocol[25] to achieve intermediate **9** exclusively. The initially encountered isomerization problem with the α-methyl center was overcome by performing a controlled addition of NaHMDS and also by reducing the reaction time (see the optimization in Table S1 in ESI†).

The synthesis of major coupling partners (**4** and **5**) of thailandamide lactone is described in Scheme 6. Compound **7** was treated with 10% TFA/CH_2Cl_2 and the resultant Boc-deprotected amine was coupled with acid **6** to access the western segment **4** in 80% yield (over two steps). On the other hand, vinyl ketone **8** was subjected to intermolecular Heck coupling with compound **9** in the presence of $Pd(OAc)_2/Bu_4NCl/Et_3N$ in DMF[26] to obtain the corresponding coupled product in complete regioselectivity, and this coupled species was further treated with CSA to obtain the corresponding β-hydroxy ketone in 77% yield after two steps. Substantial trials have been conducted to optimize its conversion to the eastern segment **5**. Most of the oxidizing agents including $DMP/NaHCO_3$ did not function properly as



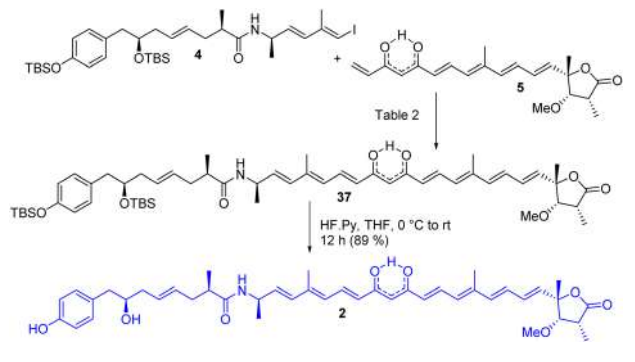
Scheme 4 Synthesis of aldehyde 34.



Scheme 6 Synthesis of coupling partners **4** and **5**.

their use ended up with complete decomposition of the product. However, DMP without $NaHCO_3$ produced the required product **5** in 73% yield. The appearance of a signal at a δ of 15.6 ppm in the ^{1}H NMR spectrum of compound **5** and two carbonyl carbons at δ 183.0, 183.9 ppm in its ^{13}C NMR spectrum clearly ascertained its existence as keto–enol tautomeric mixtures.

The completion of the total synthesis of thailandamide lactone is depicted in Scheme 7 where the stage was set for the crucial coupling between the western (**4**) and eastern (**5**) segments. Extensive efforts were made to optimize the Heck coupling (Table 2). Trials with $PdCl_2(MeCN)_2/Et_3N/HCO_2H$ in MeCN (entry-1)[27] ended up with complete decomposition of staring materials, whereas those with $Pd(PPh_3)_4/Et_3N/Bu_4NCl$ in



Scheme 7 Completion of the synthesis of thailandamide lactone (**2**).

DMF (entry-2)[27] provided the coupled product **37** in a trace amount. Use of $Pd(PPh_3)_2Cl_2/K_2CO_3/Bu_4NCl$ in DMF (entry-3), $Pd(OAc)_2/Et_3N/Bu_4NCl$ in DMF (entry-4)[27] and $Pd(OAc)_2/K_3PO_4$ in DMF (entry-5)[27c] resulted in the required product in 10%, 45% and 40% yields, respectively. A mixture of some unidentified compounds was formed along with the required compound **37** in most of the cases. Having moderate success in the transformation of compound **37** using either Pd(0) or Pd(II), we then turned our attention towards Pd(I)-catalyzed Heck coupling as it has provided excellent results in some cases.[28] Thus, $[Pd(\mu\text{-}I)(P\textit{t}\text{-}Bu_3)]_2$ (entry-6),[28b] prepared from PdI_2/P^tBu_3 following a literature report,[28a] was then screened in the presence of DIPEA/toluene to furnish the coupled product in an improved yield (58%). Later, an equimolar mixture of $Pd(OAc)_2$ and $Pd(PPh_3)_4$ in the presence of K_3PO_4/DMF (entry-7)[28e] was tested. Delightfully, this reaction was found to proceed in a considerably cleaner manner than those with all the other tested conditions, and the coupled product **37** was obtained in 77% yield. All the reactions were performed at room temperature to reduce the rate of decomposition. A detailed NMR study unambiguously confirmed the identity of compound **37** (see the 2D spectra in ESI†). Notably the attempted synthesis of the corresponding compound requisite for an alternative Heck coupling with compound **9** was not successful—because the corresponding β-hydroxy ketone obtained from the Heck coupling between compounds **4** and **8** followed by subsequent TES ether deprotection was found to be very sensitive to various oxidizing agents including DMP. Next, compound **37** was subjected to global deprotection using HF Py to access compound **2**[29] in 89% yield. ^{1}H and ^{13}C NMR data (see comparison Table S2 in ESI†), optical rotation results {observed $[\alpha]_D^{28} = -43.20$ (*c* 0.24, methanol); reported $[\alpha]_D = -45.76$}, and HRMS, FT-IR and UV-visible spectra (see ESI†) of synthesized compound **2** were found to be in good agreement with reported data of the isolated thailandamide lactone, which unambiguously confirmed its first total synthesis.

Having thailandamide lactone in hand, we then screened its antibacterial activity against different non-pathogenic and pathogenic Gram-positive bacteria such as *Bacillus subtilis* (PY79), *Bacillus megaterium* (2G), *Staphylococcus aureus* as well as Gram-negative bacteria such as *Vibrio cholerae* (N16961), *Enteropathogenic Escherichia coli* (EPEC e2348/69), and *Escherichia coli* (MC1061)]. This screening revealed its moderate to potent antibacterial activity (Table 3). The efficacies of

Table 2 Optimization of final Heck coupling

Entry	[Pd] (mol%)	Condition	Yield (%)
1	$PdCl_2(MeCN)_2$ (10)	Et_3N, HCO_2H, MeCN, rt, 3 h	Decomposition
2	$Pd(PPh_3)_4$ (5)	Et_3N, Bu_4NCl, DMF, rt, 6 h	Trace
3	$PdCl_2(PPh_3)_2$ (10)	K_2CO_3, Bu_4NCl, DMF, rt, 12 h	10
4	$Pd(Oac)_2$ (5)	Et_3N, Bu_4NCl, DMF, rt, 4 h	45′
5	$Pd(Oac)_2$ (5)	K_3PO_4, DMF, rt, 12 h	40
6	$[Pd(\mu\text{-}I)(P^tBu_3)]_2$ (7.5)	DIPEA, toluene, rt, 9 h	58
7	$Pd(Oac)_2$, (10) $Pd(PPh_3)_4$ (10)	K_3PO_4, DMF, rt, 18 h	77

Table 3 Antibacterial activities of thailandamide lactone

Staining type	Strains	MIC (μg ml^{-1})
Gram negative	*V. cholerae* (N16961) (pathogenic)	71.3
	EPEC (e2348/69) (pathogenic)	71.3
	E. coli (MC1061)	53.5
Gram positive	*B. subtilis* (PY79)	57.0
	B. megaterium (2G)	53.5

thailandamide lactone even against Gram-negative strains were found to be promising.

Conclusions

In summary, we have developed a convergent route for the first total synthesis of a structurally challenging and labile polyketide natural product, namely thailandamide lactone, starting from the known compound **26** in 17 LLS with 8.5% overall yield. Our synthesis includes several coupling operations including two intermolecular Heck reactions. Notably, Pd(I)-based Heck coupling has been introduced for the first time, to the best of our knowledge, in the total synthesis of natural product. The possible site to couple the highly sensitive eastern and western polyene segments of thailandamide lactone was determined. The antibacterial activities of thailandamide lactone against different bacterial strains have been disclosed. Importantly, our modular strategy is expected to be amenable to thailandamide A, another member of this family as well as to structurally simplified designed analogues for further antibacterial study.

Data availability

Please see the ESI† for the data related to the manuscript.

Author contributions

R. K. G. conceived the idea, designed the hypothesis and managed overall manuscript preparation. R. R. P. planned and supervised the biological testing and assisted with manuscript preparation. H. S. and J. M. completed the total synthesis, A. K. G. was responsible for biological testing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

H. S., J. M. and A. K. G. thank the Council of Scientific and Industrial Research (CSIR), New Delhi and the Indian Association for the Cultivation of Science, respectively, for their research fellowship. The financial support from the Science and Engineering Research Board (Project no-CRG/2019/001664) of India to carry out this work is gratefully acknowledged.

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29 Compound **2** was found highly sensitive to light as well as quite unstable. It decomposed to a mixture of unidentified compounds. The colour changed from yellow to dark red. It was observed that use of Silica gel for purification and glass vessel for storing was not suitable as it underwent faster decomposition. Notably, the major part of compound stuck to reverse phase C18 column (Xbridge RP18) during purification.

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Late-Stage Functionalization: Total Synthesis of Beauveamide A and Its Congeners and Their Anticancer Activities

Sanu Saha, Sourya Shankar Auddy, Akash Chatterjee, Prosenjit Sen, and Rajib Kumar Goswami*

Cite This: *Org. Lett.* 2022, 24, 7113−7117 Read Online

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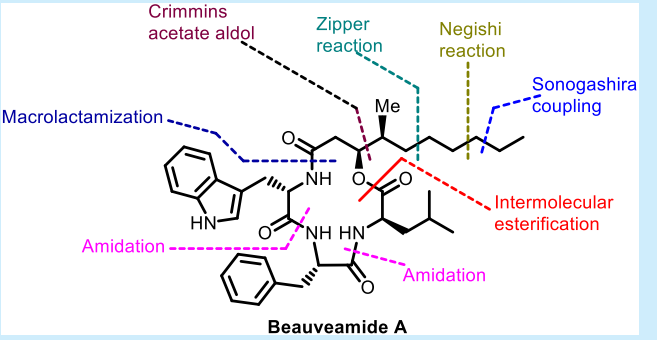
ABSTRACT: Asymmetric total synthesis of cyclotetradepsipeptide beauveamide A has been achieved for the first time. A macrolactamization strategy involving two possible sites has been explored to find the most effective route for cyclization. A late-stage functionalization approach has been adopted for easy access of non-natural analogues of beauveamide A for further biological evaluation. Interestingly, the anticancer activity of one of the synthesized analogues was better than that of the parent natural product.



Cyclodepsipeptides make up a large family of natural products having diverse architectural features. Many of them possess potential pharmaceutical and agrochemical value, which has attracted researchers worldwide to envisage their chemical synthesis.[1] During the search for novel secondary metabolites from endolichenic fungi, Puno and co-workers in 2021 discovered a family of seven new cyclotetradepsipeptides beauveamides A−G (**1**−**7**, respectively) along with the known cyclodepsipeptide beauverolide Ka (**8**) (Figure 1) using the cultures of endolichenic *Beauveria* sp. isolated from *Gypsoplaca macrophylla* (Zahlbr.) Timdal.[2] The structures of these natural products were determined using detailed spectroscopic analysis as well as Marfey's and NMR computational methods.[2] Structurally, beauveamides A−G **1**−**7** are 13-membered macrocycles bearing a common fatty acid moiety, 3-hydroxy-4-methyldecanoic acid (HMDA). There are three amino acids in the peptide segment that varies among the members. Beauveamides A (**1**) and B (**2**) exhibited protective effects on a mouse auditory cell line (HEI-OC1) at micromolar concentrations, whereas beauveamides A (**1**), D (**4**), and E (**5**) stimulated glucose uptake in cultured rat L6 myoblasts. Bioactivities, natural scarcity, and interesting structural features together with our[3] interest in natural product chemistry prompted us to embark on the chemical synthesis of beauveamide A (**1**), the most active member of this family, where the peptide part comprises D-leucine, L-phenylalanine, and L-tryptophan. Herein, we report the first total synthesis of beauveamide A (**1**) following a late-stage functionalization approach.[4] A common intermediate that could provide a diverse set of analogues having modification in the HMDA unit suitable to drive biological study has been designed. The anticancer activities of beauveamide A and two of its non-natural variants have been evaluated against human metastatic breast adenocarcinoma (MDA-MB-231) and cervical cancer (HeLa) cell lines in which one of analogues was found to be more effective than the parent natural product.

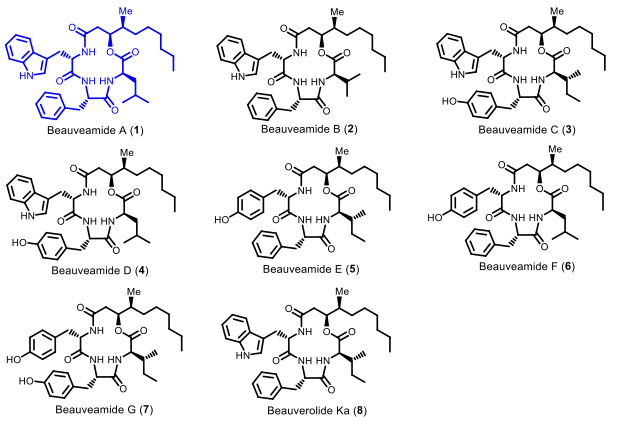


Figure 1. Chemical structures of beauveamides A−G (**1**−**7**) and beauverolide Ka (**8**).

The retrosynthetic analysis of beauveamide A (**1**) is depicted in Scheme 1, in which a late-stage functionalization approach has been adopted to diversify the synthetic route. The target

Received: August 10, 2022
Published: September 23, 2022

https://doi.org/10.1021/acs.orglett.2c02699
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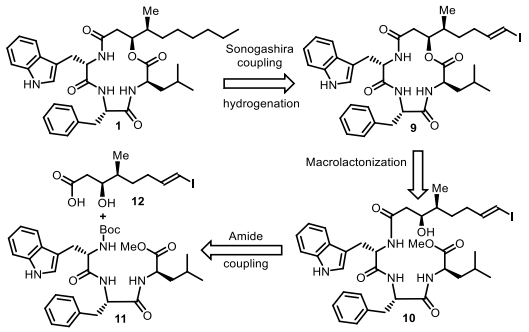
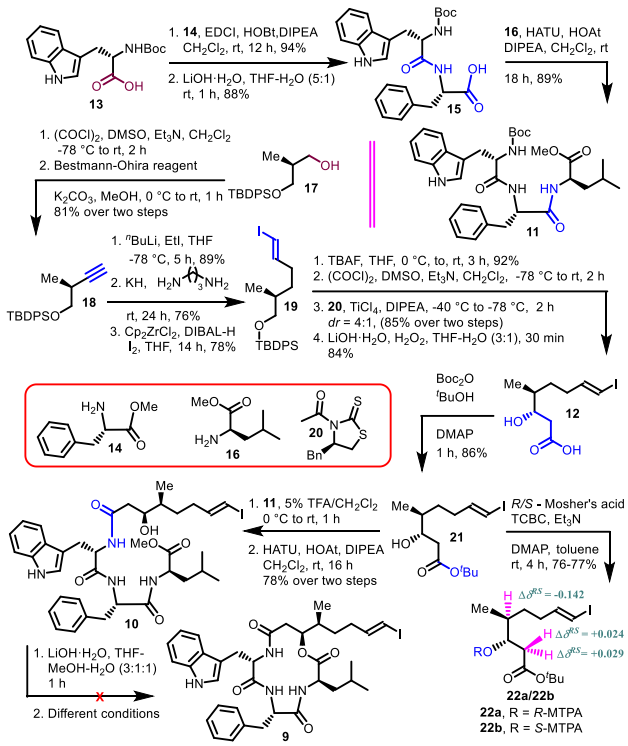
Scheme 1. Retrosynthesis of Beauveamide A (1)



natural product could be synthesized from key precursor **9** following Sonogashira coupling followed by hydrogenation. Notably, the introduction of vinyl iodide **9** would enable us to test the efficacy of the metal-mediated cross coupling in a peptide context. Moreover, designing this intermediate could provide biologically active unnatural analogues having unsaturation in the HMDA unit as it is known[5] that saturated lipidic amino acid is not very important to show activity. A macrolactonization approach could be planned to access compound **9** from the corresponding seco acid derived from compound **10**, which in turn could be prepared from tripeptide **11** and acid **12** using amidation chemistry.

The effort toward the synthesis of vinyl iodide **9** adopting a macrolactonization approach is depicted in Scheme 2. Commercially available Boc-protected tryptophan **13** was subjected to amidation with the known methyl ester of L-phenylalanine **14**[6] using EDCI/HOBt/DIPEA to obtain the corresponding coupled product, which was then hydrolyzed in the presence of $LiOH·H_2O$ to obtain dipeptide **15**. It was then

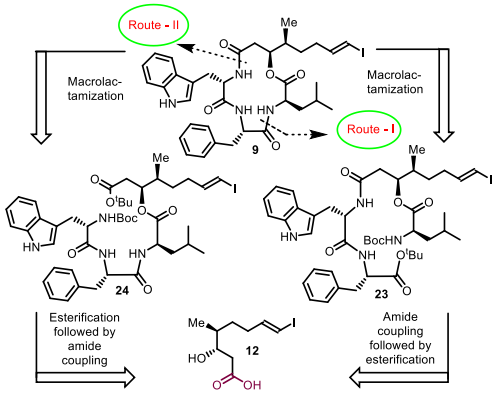
Scheme 2. Effort toward the Synthesis of Vinyl Iodide 9 following a Macrolactonization Approach



coupled with methyl D-leucinate **16**[7] using HATU/HOAt/DIPEA to access tripeptide **11** in very good overall yield. We then concentrated on the synthesis of the β-hydroxy γ-methyl aliphatic acid part following a route other than the existing approach.[8] The known alcohol **17**[9] was oxidized under Swern conditions and subjected further to Bestmann−Ohira reaction[10] to yield alkyne **18**. This was reacted with *n*-BuLi/EtI to obtain the corresponding ethylated product and subsequently exposed to zipper reaction conditions[11] to transform it into the corresponding terminal alkyne, which was finally reacted with Cp_2ZrCl/DIBAL-H/I_2 following the Negishi protocol[12] to access vinyl iodide **19** in 59% overall yield. The TBDPS ether of vinyl iodide **19** was then cleaved, and the resultant alcohol was oxidized using Swern conditions and concomitantly subjected to Crimmins acetate aldol[13] using the known *N*-acetylthiazolidinethione **20**[13a] to obtain the corresponding required aldol product (dr = 4:1). The major aldol isomer was separated from its minor counterpart and hydrolyzed using $LiOH·H_2O/H_2O_2$ to afford acid **12**, which was treated further with Boc_2O/*t*-BuOH to provide *tert*-butyl ester **21**. The absolute stereochemistry of the hydroxy center generated in the acetate aldol reaction was confirmed further as *R* by NMR analysis of synthesized Mosher's esters **22a** and **22b**.[14] Next, tripeptide **11** was treated with 5% TFA/CH_2Cl_2 and subsequently subjected to amidation with acid **12** in the presence of HATU/HOAt/DIPEA to obtain compound **10** in 78% yield (over two steps). The methyl ester of compound **10** was hydrolyzed to the corresponding seco acid, which was subjected to macrolactonization.[15] A number of conditions, including Steglich (DIPC/DMAP),[15a] modified Steglich (EDCI·HCl/collidine/DMAP),[15b] Shiina (2-methyl-6-nitrobenzoic anhydride/Et_3N/DMAP),[15c] and Yamaguchi (2,4,6-trichlorobenzoyl chloride/Et_3N/DMAP)[15d] protocols, have been screened under different conditions. Unfortunately, none of these conditions provided the required key intermediate **9**, which compelled us to search for a different strategy.

An alternative strategy for the synthesis of key intermediate **9** is depicted in Scheme 3, in which we relied on a macrolactamization approach. There are three sites for macrolactamization. To search for the shortest synthetic path, we planned to test the feasibility of this strategy in two possible sites using intermediates **23** (route I) and **24** (route II), which could be synthesized from acid **12** and the dipeptide

Scheme 3. Alternative Retrosynthetic Approach for Vinyl Iodide 9



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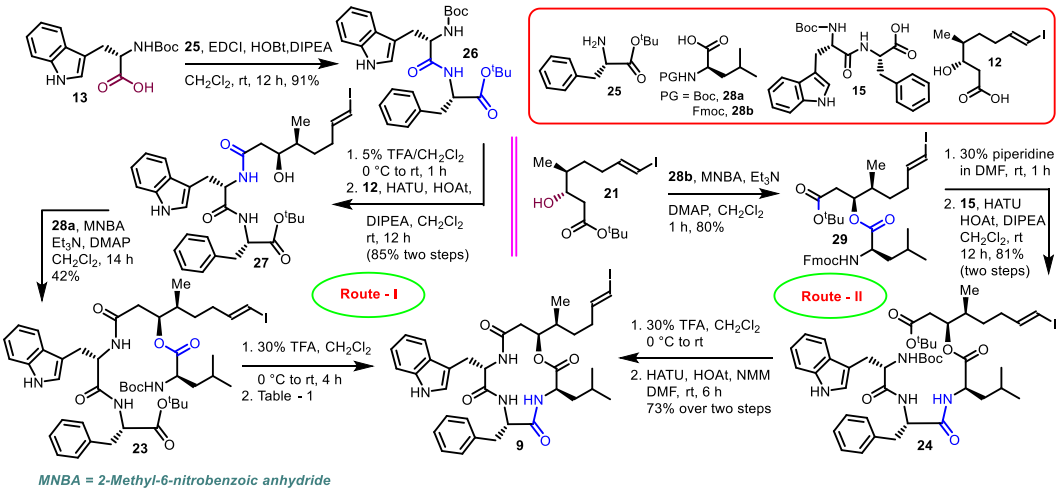
Scheme 4. Synthesis of Vinyl Iodide 9 Using a Macrolactamization Approach



of L-tryptophan-L-phenylalanine and D-leucine using amidation and esterification chemistry, respectively.

The synthesis of vinyl iodide **9** is depicted in Scheme 4. Suitably protected dipeptide **26** was prepared from commercially available Boc-protected L-tryptophan (**13**) and the known *tert*-butyl-L-phenyl alaninate (**25**)[16] in the presence of EDCI/HOBt/DIPEA, which was treated with 5%TFA/CH_2Cl_2 and subsequently coupled with acid **12** in the presence of HATU/HOAt/DIPEA to access compound **27** in 85% overall yield. The free hydroxy of compound **27** was then subjected to esterification using commercially available Boc-D-leucine (**28a**). Different protocols involving modified Steglich,[17] Yamaguchi,[18] and Shiina[19] conditions have been tested to obtain compound **23** in trace amounts, in 36% and 42% yields. Disappointingly, none of these conditions enhanced the yield of the esterification to a satisfactory level. Starting material **27** was recovered to different extents in all of the cases mentioned above. Next, the Boc and *tert*-butyl groups of compound **23** were deprotected using 30% TFA/CH_2Cl_2, and the resultant compound was subjected to macrolactamization. Different conditions have been screened (Table 1). HATU/HOAt/NMM conditions (entry 3) were found to be the best for producing vinyl iodide **9**. On the contrary, previously synthesized compound **21** was esterified with commercially available Fmoc-protected D-leucine (**28b**) following the Shiina conditions (MNBA/Et_3N/DMAP)[19] to produce compound **29** in 80% yield. It was then treated with 30% piperidine, and the resultant Fmoc-deprotected counterpart was coupled with dipeptide **15** in the presence of HATU/HOAt/DIPEA to obtain compound **24**. The Boc and *tert*-butyl groups of compound **24** were deprotected using 30% TFA/CH_2Cl_2 and subsequently subjected to macrolactamization in the presence of HATU/HOAt/NMM to afford vinyl iodide **9** in 73% yield.

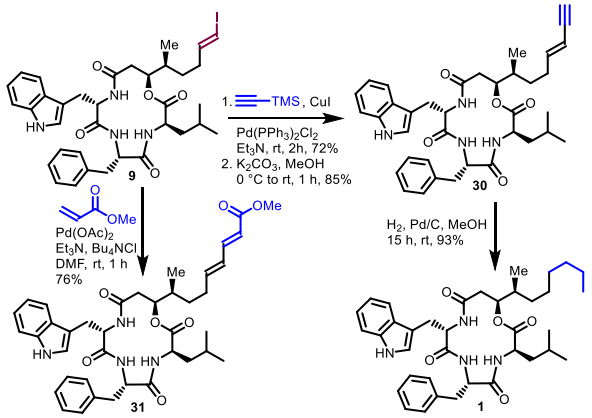
Table 1. Optimization of the Macrolactamization of Compound 23

entry	reagents and conditions	yield (%)
1	EDCI/HOBt/DIPEA, CH_2Cl_2, 0 °C to rt, 14 h	48
2	HATU/HOAt/DIPEA, DMF, 0 °C to rt, 10 h	72
3	HATU/HOAt/NMM, DMF, 0 °C to rt, 6 h	78
4	PyBOP/DIPEA, DMF, 0 °C to rt	65
5	COMU/DIPEA, DMF 0 °C to rt	59

Notably, the macrolactamization via intermediate **24** (route II) was found to be much more efficient than that via intermediate **23** (route I) as the esterification was found to be the determining step.

The completion of the total synthesis of beauveamide A (**1**) is shown in Scheme 5. A late-stage functionalization approach

Scheme 5. Completion of the Total Synthesis of Beauveamide A (1) and Its Congeners



was adopted in which vinyl iodide **9** was subjected to Sonogashira coupling[20] using TMS-acetylene in the presence of $Pd(Ph_3P)_2Cl_2/Et_3N$, and the coupled product was treated with K_2CO_3 in MeOH to furnish compound **30**, which was finally hydrogenated to compound **1** in 57% yield over three steps. The spectroscopic data of compound **1** were recorded and compared with the literature data. The ^1H and ^{13}C NMR data (see the comparison in Table S1 and Figures S2−S4) and specific rotation $[[\alpha]_D^{25} = -14.66$ (c 0.27, MeOH); reported $[\alpha]_D^{22} = -15.81$ (c 0.04, MeOH)] of synthesized compound **1** were in good agreement with the data of isolated beauveamide A, which unambiguously established the first total synthesis of the title natural product. The initial success in the diversification of vinyl iodide **9** following Sonogashira coupling prompted us to test the feasibility of the Heck coupling.[21] Thus, vinyl iodide **9** was treated with methyl acrylate in the presence of $Pd(Ph_3P)_2Cl_2/Et_3N/Bu_4NCl$ to access compound **31** in very good yield. Cyclodepsipeptide-based natural products are known to exhibit anticancer activities,[1,22] which

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made us curious to evaluate the anticancer efficacies of beauveamide A and its analogues **30** and **31** as no such literature precedents were available in this direction for this family of cyclodepsipeptides. The *in vitro* cytotoxic effects of synthesized compound **1** and its congeners **30** and **31** were determined by the MTT assay using HeLa and MDA-MB-231 cancer cell lines. The results suggested (Table 2) that the cytotoxicity of compound **31** was better than that of beauveamide A (**1**), whereas compound **30** was found to be the least active. Next, the mode of killing of cancer cells by all of the synthesized compounds was evaluated using flow cytometric analysis (Figure 2). The cell cycle of the MDA-MB-231 cells was measured. All of the synthesized compounds have shown a significant increase in DNA content at the sub-G0/G1 position, which is considered the signature peak for apoptosis. The untreated control set has very insignificant (5.6%) DNA content at the sub-G0/G1 position, whereas DNA contents of 45.6%, 33.5%, 53.7%, and 60.5% were observed with a distinct apoptotic peak for beauveamide A (**1**), compound **30**, compound **31**, and doxorubicin, respectively. FACS analysis was found to be consistent with the MTT assay in which the extent of inducing apoptosis of compound **31** was comparable to that of the known chemotherapeutic drug doxorubicin.

Table 2. Estimation of Cytotoxic Potentials of Beauveamide A (1) and Its Congeners (30 and 31) against Cancer Cell Lines

	IC_{50} (μM)	
compound	HeLa	MDA-MB-231
beauveamide A (**1**)	13.6	16.2
30	21.2	25.7
31	7.6	9.6
doxorubicin	5.5	8.0

In summary, we have achieved the first total synthesis of beauveamide A from known compound **17** in 18 linear steps with a 5.2% overall yield in which the applicability of both macrolactamization and macrolactonization approaches has been studied. The adopted late-stage functionalization approach diversified the synthesis, which has provided initially two analogues of the parent natural product quite efficiently. The anticancer activities of beauveamide A and two of its analogues have been evaluated for the first time. Interestingly, the effect of modification in the HMDA part of the target natural product is reflected in its bioactivity. The analogue having an enyne moiety (**30**), however, was found to be less active than the parent molecule, whereas analogues embedded with a conjugated ester (**31**) exhibited the best apoptotic results. It could be possible that the presence of a Michael acceptor in the HMDA part is responsible for the difference. This study left an opportunity to explore different beauveamide A variants by coupling a variety of suitable partners with key vinyl intermediate **9** to understand their structure−activity relationships. A related study is in progress in our laboratory and will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c02699.

Experimental section (chemistry and biology), NMR comparison (Table S1 and Figures S2−S4), two-dimensional (2D) NMR correlation (Figure S1), copies of NMR spectra (^{1}H, ^{13}C, and 2D), and HRMS spectra of representative compounds (PDF)

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Notes

The authors declare no competing financial interest.

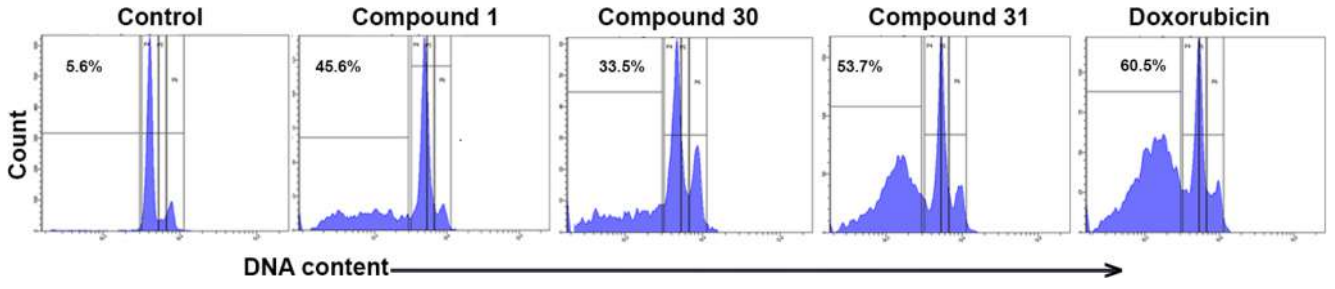

Figure 2. Compounds reduce cancer cell viability by triggering apoptosis. The apoptotic cell population was measured by flow cytometry analysis. MDA-MB-231 cells were treated with compounds **1**, **30**, and **31** at 25 μM for 48 h and stained with PI, and cell cycle analysis was performed in the flow cytometer. The percentages of apoptotic cells are given.

https://doi.org/10.1021/acs.orglett.2c02699
Org. Lett. 2022, 24, 7113−7117

■ ACKNOWLEDGMENTS

S.S., S.S.A., and A.C. thank the University Grants Commission (UGC), the Council of Scientific and Industrial Research (CSIR), and the Indian Association for the Cultivation of Science (IACS), respectively, for their research fellowships. Financial support from the Science and Engineering Research Board (Projects CRG/2019/001664 and STR/2021/000002), India, is gratefully acknowledged.

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Total Synthesis and Stereochemical Assignment of Sunshinamide and Its Anticancer Activity

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Cite This: *Org. Lett.* 2020, 22, 1188–1192 Read Online

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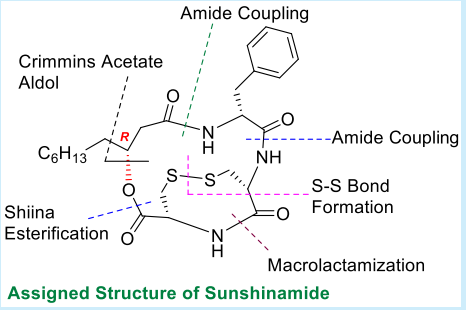
ABSTRACT: Total synthesis of cyclodepsipeptide sunshinamide has been achieved for the first time using a convergent approach. The key features of this synthesis comprise Crimmins acetate aldol, Shiina esterification, amide coupling, macrolactamization, and an I_2-mediated deprotection with concomitant disulfide-bridge formation. This synthetic study enabled the unambiguous determination of the stereochemistry of the unassigned stereocenter of the isolated sunshinamide. The cytotoxicity of sunshinamide and one of its analogues was evaluated against different cancerous and noncancerous human cell lines, which revealed their attractive and selective activities toward cancer cells at very low concentrations.



Bicyclic natural products containing a disulfide linkage make up an important class of molecules that exhibit a broad range of biological activities and pharmacological properties. Many of these natural products showed striking anticancer[1] and immunosuppressant[2] activities. Considerable efforts have been made toward the synthesis of this class of natural products and their analogues by the synthetic chemistry community,[3] which led some of them into a very advanced stage of a drug discovery program.[1g] Thus, searching for new members of this class of natural products and chemical synthesis and evaluation of their biological efficacies is a subject of great importance. Piel and co-workers in 2018 discovered first the disulfide-containing cyclodepsipeptide sunshinamide (**1**) (Figure 1) from a plant-associated marine bacterium *Gynuella sunshinyii* YC6258, using a genome-based identification method.[4] Sunshinamide showed potent cytotoxicity against HeLa cells with an IC_{50} value of 0.59 μM. The structure of the molecule was proposed on the basis of spectroscopic investigations that revealed that it comprises two cyclic scaffolds: one 15-membered and the other 8-membered. D-Phenylalanine and two consecutive L-cysteines are embedded in the peptide backbone, whereas the nonpeptidic part is 3-hydroxydecanoic acid.[4] The stereochemistry of the hydroxy center remained unestablished. Sunshinamide is architecturally novel; the consecutive L-cysteines are connected through a disulfide bond. The unique architectural features and promising bioactivity of sunshinamide together with our interest[5] in the chemical synthesis of bioactive natural products prompted us to develop a total synthesis. In this work, we report a convergent and flexible synthetic route for sunshinamide and one of its analogues for the first time. We also disclose herein their cytotoxicity against different human cancerous and noncancerous cell lines with the aim of determining their pharmaceutical relevance.

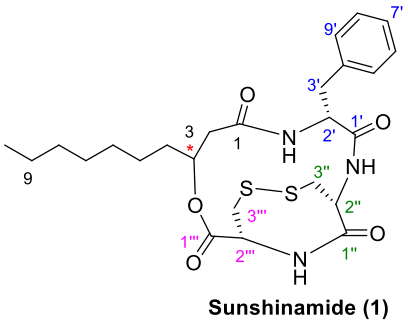


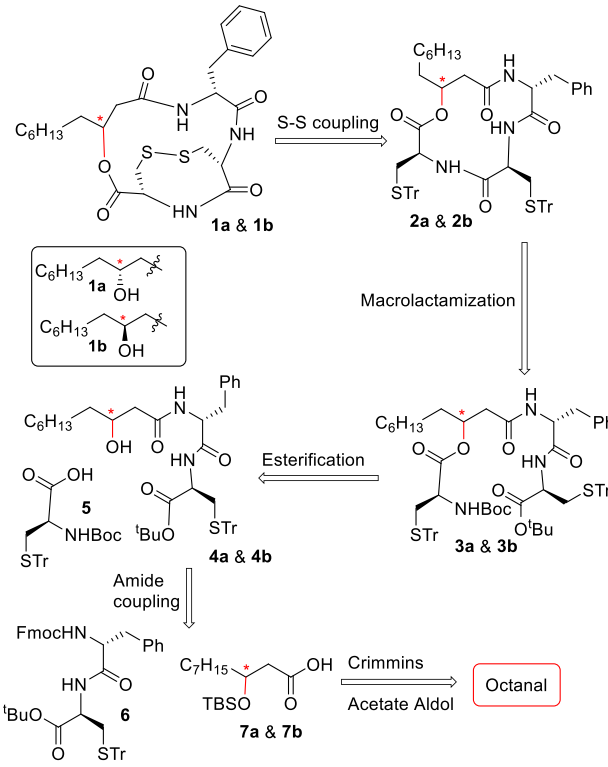
Figure 1. Proposed structure of sunshinamide (**1**).

The retrosynthesis analysis of sunshinamide is shown in Scheme 1. The stereochemistry of the hydroxy center in the nonpeptidic segment of sunshinamide remained undisclosed during the determination of the structure. Thus, we planned to synthesize both possible stereoisomers **1a** and **1b** to compare them with the reported data of the isolated natural product. Compounds **1a** and **1b** could be constructed from compounds **2a** and **2b**, respectively, by S−S bond formation. There are several possible sites in compounds **1a** and **1b** for macrocyclization. We relied on macrolactamization and planned to disconnect compounds **2a** and **2b** between two cysteine residues to realize compounds **3a** and **3b**, respectively, which

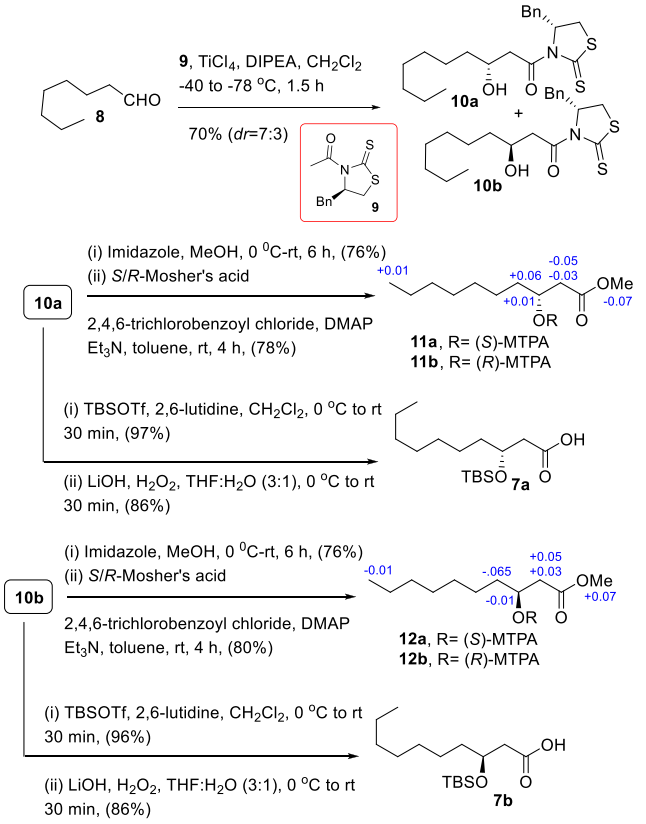
Received: January 7, 2020
Published: January 22, 2020

https://dx.doi.org/10.1021/acs.orglett.0c00070
Org. Lett. 2020, 22, 1188–1192

Scheme 1. Retrosynthetic Analysis of Sunshinamide



Scheme 2. Synthesis of Compounds 7a and 7b



could further be made by esterification of compound **5** with compounds **4a** and **4b**, respectively. Compounds **4a** and **4b** would be constructed separately from compounds **7a** and **7b**, respectively, by amide coupling with compound **6**. Compounds **7a** and **7b** could be synthesized from octanal using Crimmins acetate aldol as one of the key steps.

The synthesis of compounds **7a** and **7b** is depicted in Scheme 2. Octanal was subjected to Crimmins acetate aldol,[5a−c,6] with the known thiazolidinethione **9**[6b,c] in the presence of $TiCl_4$/DIPEA to obtain aldol adducts **10a** and **10b**, respectively, in 70% yield (dr = 7:3), which were separated by silica gel column chromatography. The absolute stereochemistry of the newly generated hydroxy center of compound **10a** was determined by converting it to (*S*)- and (*R*)-MTPA esters **11a** and **11b**, respectively.[5c,7] All of the protons of the pairs of Mosher's esters were assigned by ^{1}H NMR. The negative $\Delta\delta$ values ($\Delta\delta = \delta S - \delta R$) (Scheme 2) found for H-2 protons from esters **11a** and **11b** clearly confirmed the desired (*R*)-configuration of the originated hydroxy center. Compound **10a** was then reacted with TBSOTf/2,6-lutidine followed by LiOH/H_2O_2 to obtain the known acid **7a**.[8] Similarly, the absolute stereochemistry of the hydroxy center of compound **10b** was established as the (*S*)-configuration from (*S*)- and (*R*)-MTPA esters **12a** and **12b**, respectively [$\Delta\delta = (\delta S - \delta R) = +$ve], which was transformed finally to the known acid **7b**.[8]

The synthesis of compounds **3a** and **3b** is described in Scheme 3. Known amine **13**[9] and acid **14**[10] prepared from L-cysteine and D-phenylalanine, respectively, following literature procedures, were coupled together using EDCI/HOBT/DIPEA[11] to realize compound **6** in 86% yield.

Compound **6** was then treated with Et_2NH[5d] to obtain the corresponding Fmoc-deprotected amine that was then coupled with acid **7a**. EDCI/HOBt/DIPEA and HATU/HOAt/DIPEA[11] coupling conditions were screened. HATU/HOAt/DIPEA was found to function efficiently to produce the corresponding coupling product that was subsequently treated with TBAF to obtain compound **4a** in 67% yield over three steps. Compound **4b** was also synthesized from compound **6** using acid **7b** in very good overall yield following exactly the same chemistry. Next, both compounds **4a** and **4b** were esterified separately with the known acid **5**[12] in the presence of MNBA (2-methyl-6-nitrobenzoic anhydride)/DMAP/Et_3N following the Shiina protocol[13,5b] to obtain compounds **3a** and **3b**, respectively

The completion of the total synthesis of compounds **1a** and **1b** is depicted in Scheme 4. Compound **3a** was treated separately with TFA (40% in CH_2Cl_2) to obtain the corresponding Boc- and *tert*-butyl-deprotected product. The stage was now set to perform the crucial macrolactamization. Different conditions have been screened at a concentration of ~10^{-3} M at this stage (Table 1). HATU/HOAt/DIPEA was found to be the best condition in our case to obtain compound **2a** in 72% yield. No epimerization or cyclodimerization was observed during this reaction. Compound **2b** was also prepared with a similar yield from compound **3a** following the chemistry of compound **2a**. Both compounds **2a** and **2b** were then reacted with I_2/MeOH-CH_2Cl_2[3a,b] to access the disulfide bridge-containing targeted compounds **1a** and **1b**, respectively, in excellent yield (90%). The spectroscopic data of both compounds **1a** and **1b** were recorded. ^{1}H and ^{13}C NMR data of compound **1a** were in very good agreement with the data of isolated natural products, whereas discrepancies in chemical shifts between the synthesized compound **1b** and isolated sunshinamide were observed (see the NMR comparisons in Tables S1 and S2). The major anomalies

https://dx.doi.org/10.1021/acs.orglett.0c00070
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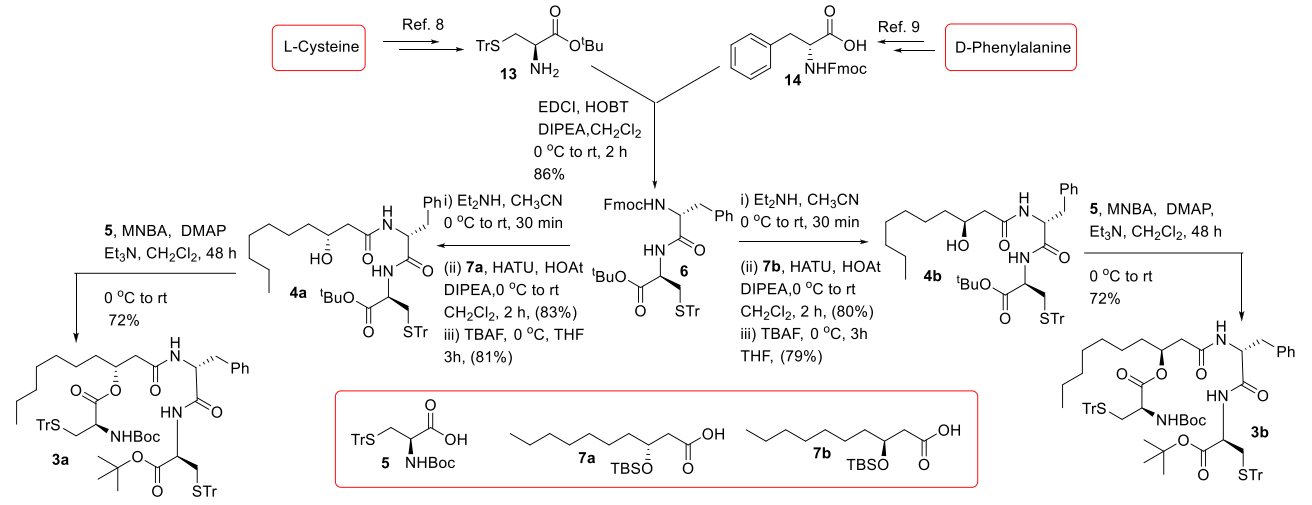
Scheme 3. Synthesis of Compounds 3a and 3b



Scheme 4. Completion of the Synthesis of Compounds 1a and 1b

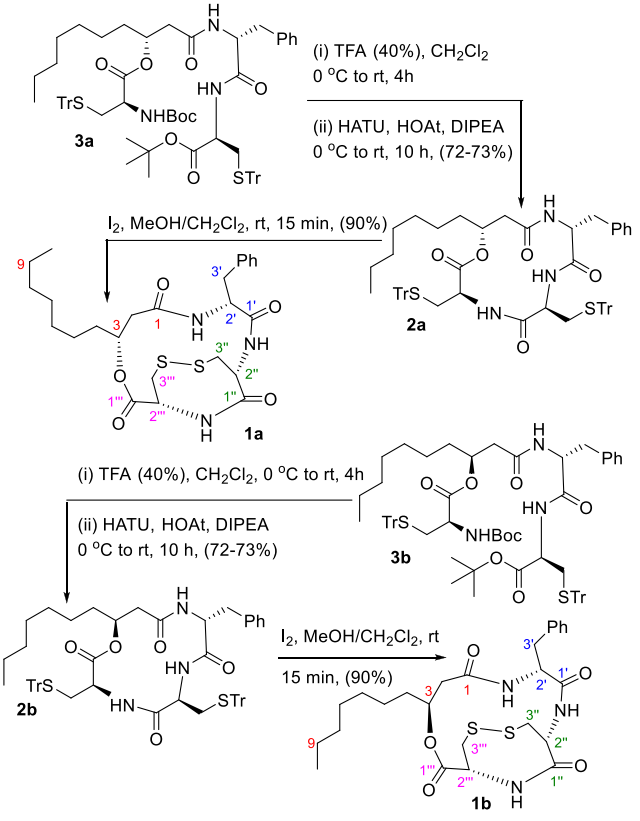


Table 1. Optimization of Macrolactamization for Compound 2a

entry	reagents	conditions	time (h)	yield
1	EDCI/HOBt, CH_2Cl_2	0 °C to rt	14	14
2	HATU, HOAt/DIPEA, CH_2Cl_2	0 °C to rt	16	72
3	PyBOP/DIPEA, CH_2Cl_2	0 °C to rt	14	9
4	COMU/DIPEA, DMF	0 °C to rt	3	15

were observed in the ^{1}H NMR signals of H_2-2, H_2-4, 2′-NH, H-2″, 2″-NH, 2‴-NH, and H_2-3″ of the synthesized compound **1b** with respect to the reported values. Moreover, the splitting patterns of H_2-2 and H_2-4 were also quite different from the reported spectra. The ^{13}C signals of C-1, C-3, C-1′, C-1‴, and C-2‴ of compound **1b** also differ from the isolated values. The 2D NMR correlations of compound **1a** were also in accordance with the reported data. These observations clearly confirmed that the structures of isolated sunshinamide and synthesized compound **1a** were identical. However, the observed difference in the specific rotation [observed $[\alpha]^{26}_D$ −37.1 (*c* 0.015, MeOH); reported $[\alpha]^{26}_D$ −1.5 (*c* 0.015, MeOH)] of synthesized sunshinamide could not be reconciled at this point.

The synthesized sunshinamide (**1a**) and its configurational isomer (**1b**) were evaluated for their *in vitro* cytotoxic effects against MDA-MB-231 (human metastatic breast adenocarcinoma), MCF7 (human breast adenocarcinoma), HeLa (human cervical cancer), and HepG2 (human liver cancer) cells using the MTT reduction assay. The effects of both of the compounds were also evaluated on noncancerous cell lines, specifically CHOK1 (Chinese hamster ovary), WI38 (human lung fibroblast) cells, to check whether they have differential cytotoxic effects on cancer and noncancer cells. The results are listed in Table 2, which revealed that the synthesized compounds are selective toward cancer cell lines and possessed attractive cytotoxic activity. Next, to study the mechanism behind the cytotoxic effects, we have treated the cancer cells with synthesized compounds (**1a** and **1b**) and systematically analyzed the mode of killing by measuring several cellular assays. Confocal microscopic examination of the cancer cells (MDA-MB-231) treated with the compounds and nuclear DNA stained with propidium iodide showed typical apoptotic features, like fragmented nuclei, chromatin condensation, and formation of apoptotic bodies (Figure 2A). This study also showed that the exposure of the synthesized compounds (**1a** and **1b**) to the cancer cells increased the activity of the caspase 3 (Figure 2D), which is known as the key signature regulator of the apoptotic process. To further confirm apoptosis, treated MDA-MB-231 cells were subjected to flow cytometric analysis to evaluate the fragmented apoptotic DNA. Exposure of MDA-MB-231 cancer cells to sunshinamide (**1a**) and its analogue (**1b**) caused significant accumulation of fragmented DNA at the sub G0/G1 phase of the cell cycle (Figure 2B,C), which is also a characteristic feature of apoptosis. Overall, these results

https://dx.doi.org/10.1021/acs.orglett.0c00070
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Table 2. Evaluation of the Cytotoxic Activities of Sunshinamide (1a) and Its Congener (1b) with Respect to Cancer and Noncancerous Human Cell Lines

	IC_{50} value (μM)					
	MDA-MB-231	MCF-7	HeLa	HepG2	CHOK1	WI38
1a	0.11 ± 0.041	0.08 ± 0.019	0.11 ± 0.005	0.10 ± 0.018	0.72 ± 0.099	0.36 ± 0.031
1b	0.10 ± 0.087	0.09 ± 0.013	0.13 ± 0.017	0.13 ± 0.20	0.47 ± 0.008	1.05 ± 0.645

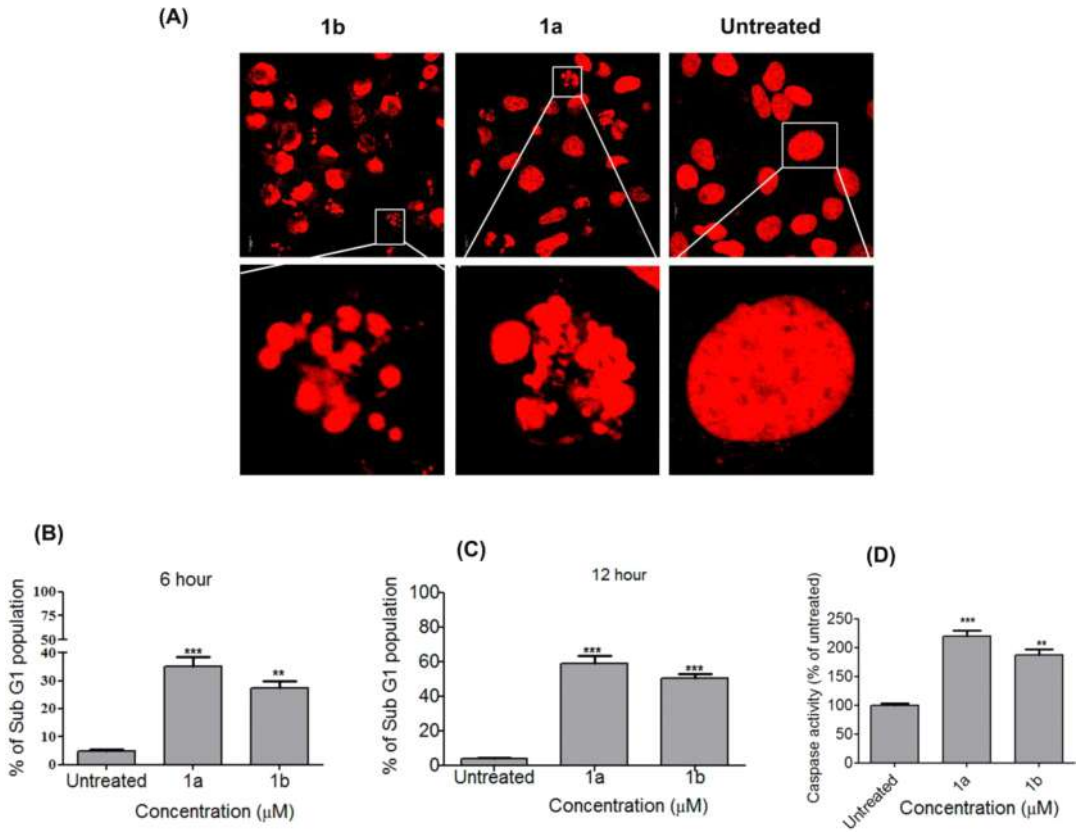

Figure 2. Induction of apoptosis by the synthesized compounds **1a** and **1b**. (A) MDA-MB-231 cells were incubated with IC_{50} doses of compounds **1a** and **1b** for 6 h and then stained with PI. Images were captured by confocal microscopy. Images are representative of three independent experiments. (B and C) MDA-MB-231 cells were treated with IC_{50} doses of the compounds for 6 and 12 h and subjected to cell cycle analysis by flow cytometry, following staining with PI. The percentage of the sub G1 phase is graphically represented. Values are expressed as the means ± the standard deviation (SD) of three independent experiments. (D) MDA-MB-231 cells were exposed to IC_{50} doses of the compounds, and caspase 3 activities were assessed. Values are expressed as the means ± the SD of triplicate samples. The scale bar is 10 μM.

suggest that sunshinamide showed cytotoxicity by inducing apoptosis in the cancer cells.

In summary, we have achieved the first total synthesis of sunshinamide in nine linear steps from octanal with an overall yield of 13.7%. The previously unassigned C-3 stereocenter of sunshinamide has been established unambiguously, and the absolute stereochemistry was determined as *R*. The cytotoxicity of synthesized sunshinamide and that of its C-3 epimer were evaluated against a number of human cancerous and noncancerous cell lines, which revealed their promising and selective activities with respect to cancer cells with very encouraging IC_{50} values. Notably, the stereochemistry of the C-3 center of sunshinamide has no such differential cytotoxic effect against human cancerous (MDA-MB-231, MCF-7, HeLa, and HepG2) cell lines, but such differences were observed in the case of human noncancerous (CHOK1 and WI38) cell lines. Further study revealed that sunshinamide (**1a**) and its analogue (**1b**) have cytotoxic effects on human cancer cells through the induction of apoptosis. The convergent synthetic route we have developed allows easy access to a large array of analogues of sunshinamide. Evaluation of the detailed signaling cascades involved in inducing apoptosis by sunshinamide and its structure–activity relationship studies are in progress and will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00070.

Experimental procedure, spectroscopic data, Tables S1 and S2, copies of NMR (^{1}H and ^{13}C) and HRMS spectra of representative compounds, and 2D NMR data (COSY, HSQC, HMBC, NOESY, and ROESY) of compounds **1a** and **1b** (PDF)

https://dx.doi.org/10.1021/acs.orglett.0c00070
Org. Lett. 2020, 22, 1188–1192

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

J.M. and R.S. thank the Council of Scientific and Industrial Research, New Delhi, and DST for the research fellowship. Financial support from the Science and Engineering Research Board (Project EMR/2016/000988) and the Council of Scientific & Industrial Research [Project 02(0294)/17/EMR-II], India, to carry out this work is gratefully acknowledged.

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