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Total Synthesis and Stereochemical Assignment of Sunshinamide and Its Anticancer Activity

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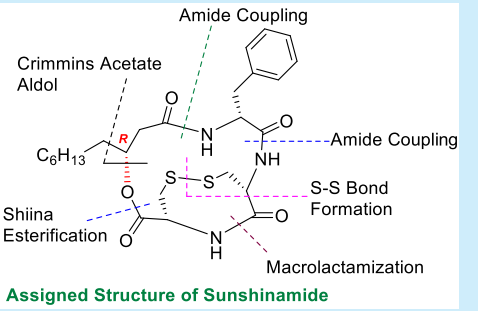
ABSTRACT: Total synthesis of cyclodepsipeptide sunshinamide has been achieved for the first time using a convergent approach. The key features of this synthesis comprise Crimmins acetate aldol, Shiina esterification, amide coupling, macrolactamization, and an I_2-mediated deprotection with concomitant 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.

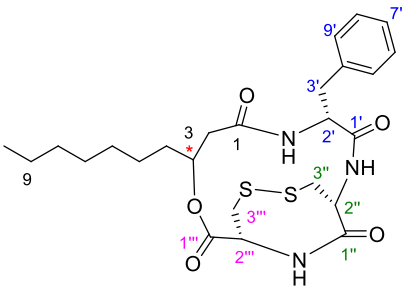


Figure 1. Proposed structure of sunshinamide (**1**).

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

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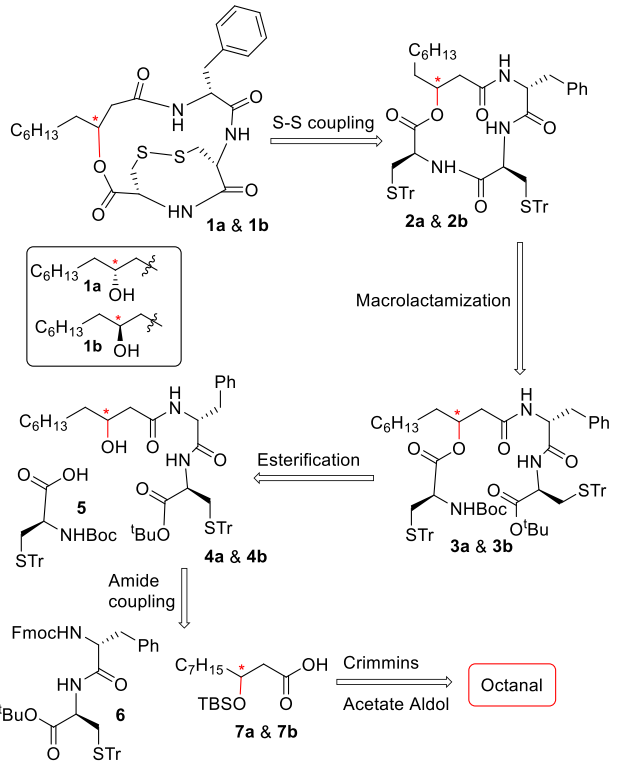
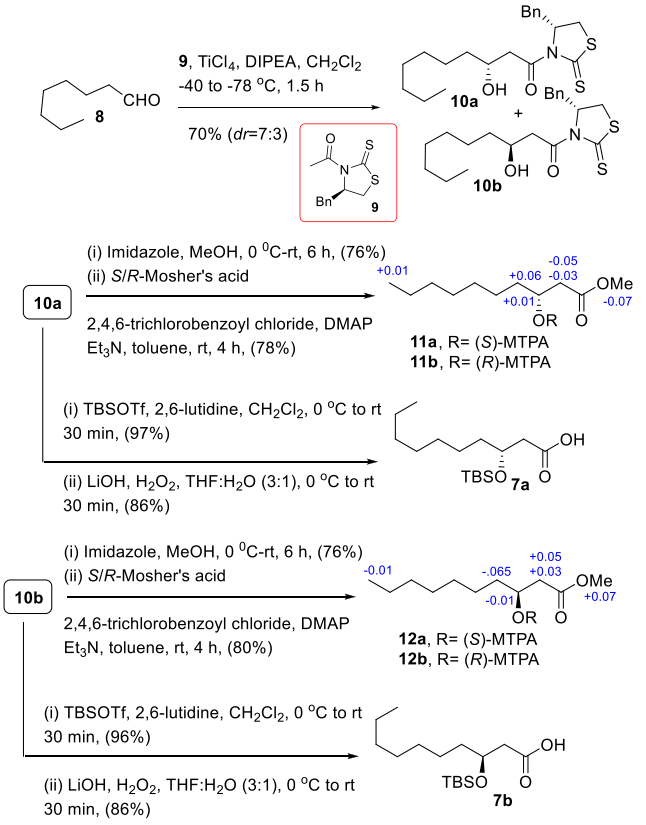
Scheme 1. Retrosynthetic Analysis of Sunshinamide



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 $\sim 10^{-3}$ 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

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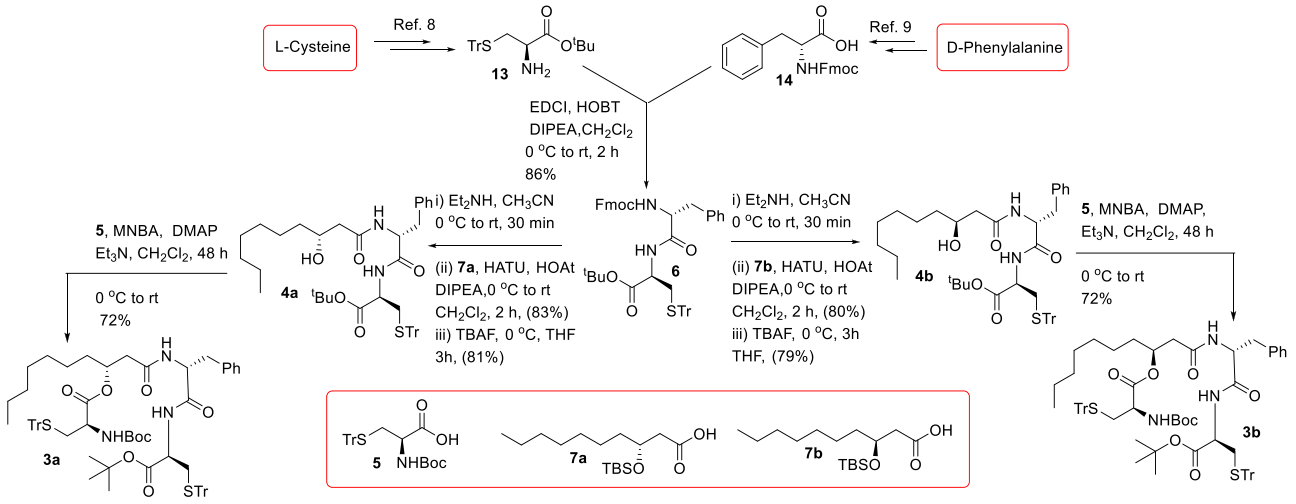
Scheme 3. Synthesis of Compounds 3a and 3b



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

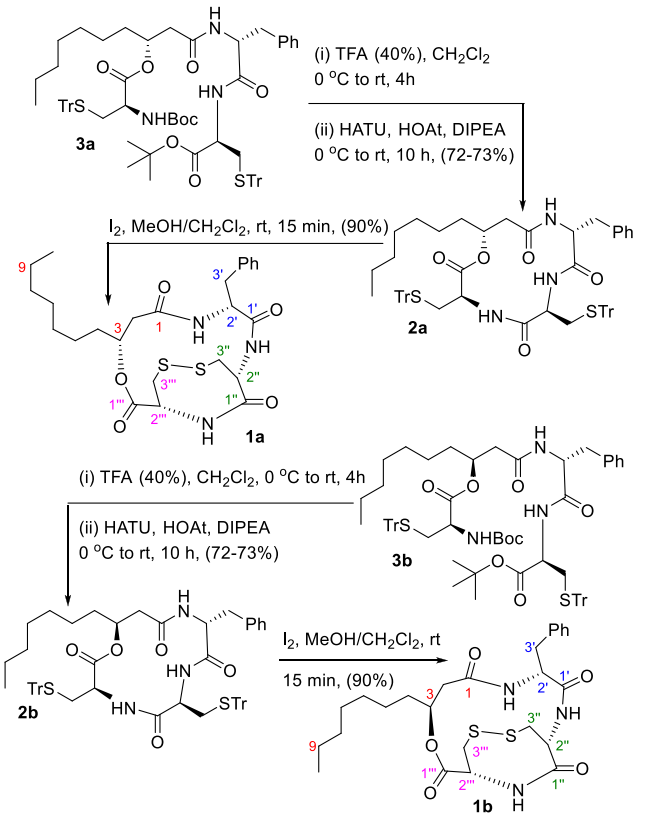


Table 1. Optimization of Macrolactamization for Compound 2a

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

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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

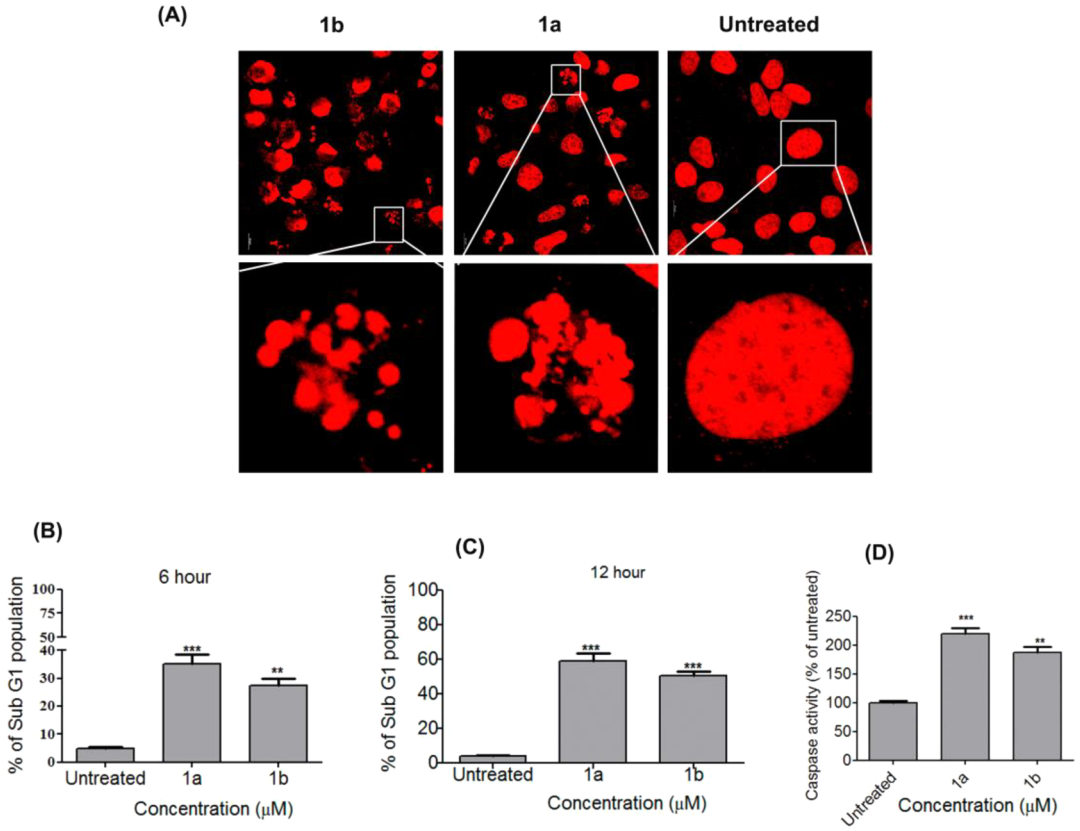

Figure 2. Induction of apoptosis by the synthesized compounds **1a** and **1b**. (A) MDA-MB-231 cells were incubated with IC_{50} doses of compounds **1a** and **1b** for 6 h and then stained with PI. Images were captured by confocal microscopy. Images are representative of three independent experiments. (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)

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Notes

The authors declare no competing financial interest.

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