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Mohangamides A and B, New Dilactone-Tethered Pseudo-Dimeric Peptides Inhibiting *Candida albicans* Isocitrate Lyase

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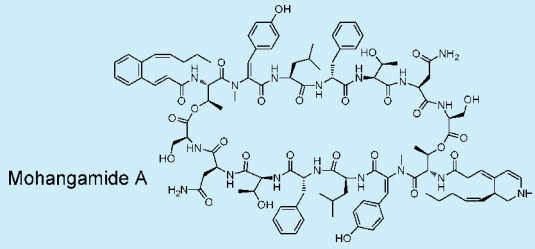
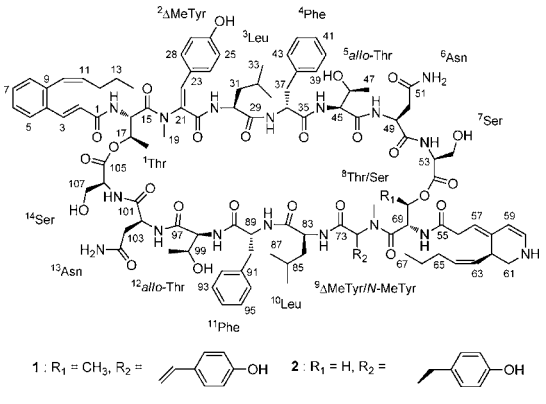
Supporting Information

ABSTRACT: Mohangamides A and B (**1**–**2**) were discovered from a marine *Streptomyces* sp. collected in an intertidal mud flat. The structures of the compounds were elucidated as novel dilactone-tethered pseudodimeric peptides bearing two unusual acyl chains and 14 amino acid residues based on comprehensive spectroscopic analysis. The absolute configurations of the mohangamides were determined by chemical derivatizations, followed by chromatographic and spectroscopic analyses. Mohangamide A displayed strong inhibitory activity against *Candida albicans* isocitrate lyase.



Candida albicans (*C. albicans*) is the most serious known pathogenic fungus, opportunistically causing candidiasis in humans, of which the mortality rate can be as high as 50%.[1] During the past two decades, the incidence of candidiasis in healthy populations has significantly increased.[2] Therefore, novel therapeutic agents that can overcome resistance or regulate the virulence of this pathogenic fungus are urgently required. Isocitrate lyase (ICL) is a key enzyme of the glyoxylate cycle that enables microorganisms to grow on acetate, ethanol, or fatty acids in host environments.[3] This enzyme is important in controlling microbial pathogens, as it is upregulated during *Mycobacterium tuberculosis* infection[4] and is also required for full virulence expression of *C. albicans*.[5] Because this metabolic cycle does not exist in humans, ICL has been considered to be a promising antimicrobial drug target. Several small molecule ICL inhibitors such as nitropropionate and itaconate have been identified;[6] however, no current drug in clinical use specifically targets ICL. Thus, the discovery of new structural ICL inhibitors is in high demand.

In our continuous search for novel ICL inhibitors, we have focused on marine actinomycetes, which were recently highlighted as a valuable source of bioactive compounds for drug discovery.[7] We discovered the first macrolide-class ICL inhibitor, which inhibits the mRNA expression of ICL in *C. albicans* under C_2-carbon-utilizing conditions, from a tropical marine actinomycete.[8,9] Our continuous screening for ICL inhibitors from marine actinomycetes led us to find out that the strain *Streptomyces* sp. SNM55 extract, collected from the Mohang mud flat in Buan, Republic of Korea, displayed a distinctively significant inhibitory effect (IC_{50} = 13.7 μg/mL). Large-scale cultivation of the strain and subsequent analyses of its chemical components revealed that the bacterium produced a major compound, named mohangamide A (**1**), which possesses a molecular ion $[M + Na]^+$ at m/z 2101 by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS) and a cyclic peptide WS9326A originally derived from *Streptomyces violaceoniger*.[10] We further purified mohangamide A and its analogue, mohangamide B (**2**), and determined their structures.



Mohangamide A (**1**) was isolated as a white powder (yield: 0.7 mg/L). The molecular formula of **1** was determined to be $C_{107}H_{139}N_{17}O_{26}$ by ^{1}H and ^{13}C nuclear magnetic resonance

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(NMR) (Table 1) and high-resolution fast atom bombardment MS (HRFAB-MS) data (observed $[M]^+$ ion at m/z 2078.0071, calculated $[M]^+$ ion at 2078.0077). Careful analysis of the complicated 2D NMR spectra, including COSY, TOCSY, HSQC, and HMBC, clarified the existence of ΔMe tyrosine,[11]

Table 1. ^{1}H and ^{13}C NMR Data for 1 in DMSO-d_6[a]

no.	δ_H, mult (J in Hz)	δ_C	no.	δ_H, mult (J in Hz)	δ_C
1	–	171.5, s	55	–	171.6, s
2	6.94, 1H, d (15.0)	125.8, d	56	2.83, 1H, m	34.5, t
3	7.30, 1H, m	129.3, d		3.08, 1H, m	
4	–	141.5, s	57	5.10, 1H, m	119.5, d
5	7.28, 1H, m	126.7, d	58	–	137.2, s
6	7.15, 1H, t (7.5)	126.8, d	59	6.39, 1H, d (8.5)	124.2, d
7	7.09, 1H, t (7.5)	126.0, d	60	5.83, 1H, d (8.5)	132.8, d
8	6.99, 1H, d (7.5)	129.7, d	61	2.88, 2H, brs	36.5, t
9	–	137.1, s	62	3.03, 1H, m	37.5, d
10	6.31, 1H, d (10.5)	129.3, d	63	5.17, 1H, m	132.3, d
11	5.63, 1H, m	132.7, d	64	5.18, 1H, m	129.2, d
12	1.95, 1H, m	30.5, t	65	1.85, 2H, m	29.3, t
	2.03, 1H, m		66	1.23, 2H, m	22.5, t
13	1.35, 2H, m	22.8, t	67	0.77, 3H, t (7.5)	22.3, q
14	0.83, 3H, t (7.5)	14.3, q	68	–	169.7, s
15	–	169.7, s	69	5.19, 1H, d (8.0)	53.5, d
16	5.22, 1H, d (8.0)	53.4, d	69-NH	8.37, 1H, brs	
16-NH	8.39, 1H, brs		70	4.91, 1H, m	73.6, d
17	4.97, 1H, m	73.7, d	71	1.08, 3H, d (6.0)	17.2, t
18	1.11, 3H, d (6.0)	17.0, q	72	2.98, 3H, s	34.5, t
19	2.97, 3H, s	34.7, q	73	–	166.1, s
20	–	166.2, s	74	–	129.0, s
21	–	128.9, s	75	6.00, 1H, s	132.2, d
22	5.99, 1H, s	132.0, d	76	–	123.5, s
23	–	123.3, s	77	7.24, 1H, d (8.0)	132.4, d
24	7.35, 1H, d (8.0)	132.3, d	78	6.62, 1H, d (8.0)	115.4, d
25	6.60, 1H, d (8.0)	115.5, d	79	–	158.4, s
26	–	158.5, s	80	6.62, 1H, m	115.4, d
27	6.60, 1H, m	115.5, d	81	7.24, 1H, m	132.4, d
28	7.35, 1H, m	132.3, d	82	–	172.4, s
29	–	172.6, s	83	4.04, 1H, m	54.2, d
30	4.07, 1H, m	54.0, d	83-NH	9.22, 1H, brs	
30-NH	9.23, 1H, brs		84	1.25, 2H, m	39.6, t
31	1.26, 2H, m	39.5, t	85	0.87, 1H, m	23.4, d
32	0.87, 1H, m	23.6, d	86	0.67, 3H, d (7.0)	23.4, q
33	0.65, 3H, d (7.0)	23.2, q	87	0.74, 3H, d (7.5)	22.4, q
34	0.75, 3H, d (7.5)	22.2, q	88	–	170.6, s
35	–	170.7, s	89	4.33, 1H, m	56.4, d
36	4.35, 1H, m	56.5, d	89-NH	9.13, 1H, brs	
36-NH	9.18, 1H, brs		90	2.75, 1H, m	36.7, t
37	2.72, 1H, m	36.9, t		3.28, 1H, m	
	3.26, 1H, m		91	–	139.3, s
38	–	139.5, s	92	7.31, 1H, m	129.6, d
39	7.32, 1H, m	129.5, d	93	7.26, 1H, m	128.6, d
40	7.27, 1H, m	128.5, d	94	7.19, 1H, m	126.3, d
41	7.20, 1H, m	126.5, d	95	7.26, 1H, m	128.6, d
42	7.27, 1H, m	128.5, d	96	7.31, 1H, m	129.6, d
43	7.32, 1H, m	129.5, d	97	–	170.4, s
44	–	170.5, s	98	4.27, 1H, m	57.5, d
45	4.32, 1H, m	57.7, d	98-NH	7.61, 1H, brs	
45-NH	7.59, 1H, brs		99	4.14, 1H, m	68.5, d
46	4.20, 1H, m	68.6, d	100	0.58, 3H, m	22.2, q
47	0.56, 3H, m	22.3, q	101	–	171.9, s
48	–	172.0, s	102	4.41, 1H, m	51.2, d
49	4.46, 1H, m	51.3, d	102-NH	8.27, 1H, brs	
49-NH	8.32, 1H, brs		103	2.40, 2H, m	36.9, t
50	2.42, 2H, m	37.2, t	104	–	171.7, s
51	–	171.6, s	105	–	169.3, d
52	–	169.5, s	106	4.29, 1H, m	56.0, d
53	4.38, 1H, m	55.7, d	106-NH	8.26, 1H, brs	
53-NH	8.44, 1H, brs		107	2.97, 2H, m	61.1, t
54	3.26, 2H, m	61.4, t			

[a] ^{1}H and ^{13}C NMR were recorded at 900 and 225 MHz, respectively.

two leucine, two phenylalanine, two asparagine, and two serine residues.

The amino acid composition was identical to that of WS9326A but was doubled, indicating that mohangamide A (**1**) possibly possessed a dimeric feature of WS9326A. Despite its dimeric feature, the ^{1}H and ^{13}C NMR spectra of **1** indicated asymmetric portions of the molecule because the numbers of ^{1}H and ^{13}C peaks were not half of the numbers of atoms indicated in its molecular formula. One of the asymmetric portions was elucidated as 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid.[12] The last partial structure, which is an unusual acyl chain, was established by further 2D NMR analyses. First, based on COSY, a C_5 (C-63 to C-67) chain was easily constructed. Additional ^{1}H−^{1}H correlations between H-63 and H-62 secured the connectivity of the chain to C-62. The H-61/H-62 correlation extended this partial structure to C-61. These connectivities were also supported by TOCSY from H-61 to H-67. The COSY correlations between H-56 and H-57 verified that C-56 is located adjacent to C-57. The ^{1}H−^{1}H coupling constant (8.5 Hz) of the doublet olefinic signals (H-59 and H-60) secured their connectivity and indicated that they belong to a six-membered ring, not a linear chain. Further analysis of the HMBC spectrum assigned the dihydropyridine moiety and connected the amide carbon (C-55) to the ring through C-56 and C-57 (Figure 1). The double bond geometries in the acyl chain were established as 57*E*, 59*Z*, and 63*Z* by H-57/H-59 and H-63/H-64 NOESY and ROESY correlations.[13]

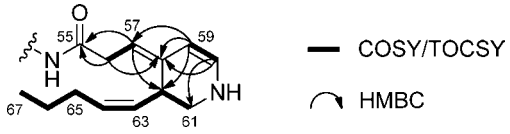

Figure 1. Key ^{1}H−^{1}H COSY/TOCSY and HMBC correlations of the acyl chain bearing dihydropyridine.

Once the partial structures were elucidated, we analyzed the HMBC correlations to connect the amino acids and the acyl chains. The sequence of the amino acids, -Thr-ΔMeTyr-Leu-Phe-Thr-Asn-Ser-, was repeated. The first acyl chain, 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid, was connected to ^{1}Thr by the HMBC correlation from 16-NH to C-1. Similarly to the sequence of the first molecule half, the other acyl chain bearing dihydropyridine was assigned next to ^{8}Thr by the HMBC correlation from 69-NH to C-55, which completed two acyl chain-Thr-ΔMeTyr-Leu-Phe-Thr-Asn-Ser- substructures. These two substructures were assembled through two ester linkages at ^{1}Thr-^{14}Ser and ^{7}Ser-^{8}Thr based on the H-17/C-105 and H-70/C-52 HMBC couplings, thus finalizing the planar structure of mohangamide A (**1**). The structure was further confirmed by full NMR assignment of the methanolysis products, **3a** and **3b** (see Supporting Information). The absolute configurations of the amino acids in **1** were determined using the advanced Marfey's method.[14] Phenylalanine possessed a D-configuration, whereas the other residues (Thr, Leu, Asn, and Ser) corresponded to L-amino acids. Futher analysis by 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) derivatization[15] of **1** disclosed that mohangamide A contains two *allo*-L-Thr and L-Thr units (see Supporting Information Table S4). These L-Thr and *allo*-L-Thr units were distinguished by *J*-based configuration analyses (see Supporting Information Figure S33).[16]

Although we determined the absolute configuration of the amino acids of **1**, the stereochemistry of the asymmetric carbon

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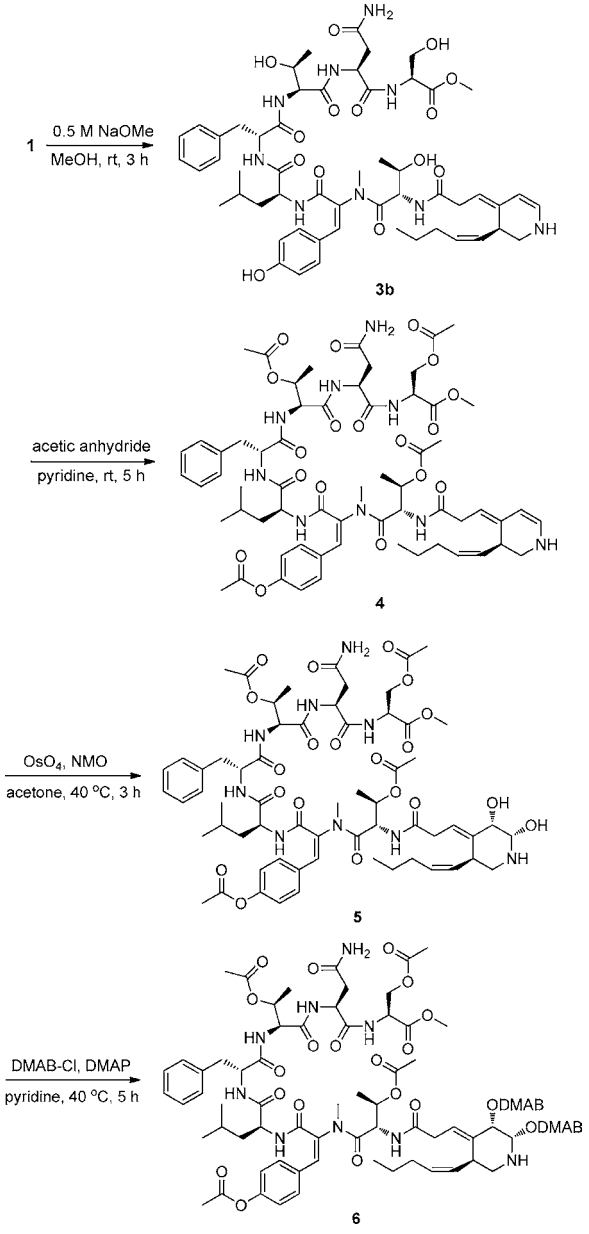
at C-62 in the dihydropyridine ring required further identification. Thus, we designed a four-step derivatization procedure of **1** (Scheme 1). First, mohangamide A (**1**) was

Scheme 1. A Four-Step Derivatization Scheme To Determine the Absolute Configuration of Dihydropyridine in 1



subjected to methanolysis to yield products **3a** and **3b**, which were utilized to confirm the planar structure of **1** *vide supra*. Product **3b**, which bears the C-62 stereogenic center, was acetylated. The major product, tetraacetate **4**, deprived of hydroxyl groups, was then oxidized by using OsO_4[17] to introduce vicinal diol groups into the double bond in the dihydropyridine ring. After 3 h, the major product (compound **5**) was obtained, in which original olefinic methines in the dihydropyridine ring were replaced with vicinal hydroxyl groups [δ_{C59} 71.4-δ_{H59} 3.91; δ_{C60} 73.4-δ_{H60} 3.78].

The relative configurations of the ring in **5** were established as 59*S**, 60*S**, and 62*S** by analyzing the ROESY data (Figure 2). Finally, we derivatized the introduced diol in **5** with 4-

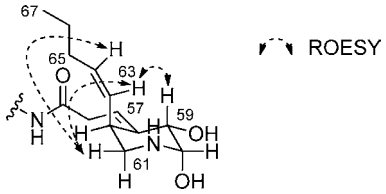


Figure 2. Key ROESY correlations of piperadine in **5**.

(dimethylamino) benzoyl chloride (DMAB-Cl) to form a DMAB diester and induce exciton coupling in circular dichroism (CD).[18] The CD spectrum of **6** exhibited a negative bisignate signal and established the anticlockwise configuration of the diol (see Supporting Information and Figure 3). Therefore, the absolute configurations of the stereogenic centers in the ring of **5** were determined as 59*S*, 60*S*, and 62*S* and were finally assigned as 62*S* in mohangamide A (**1**).

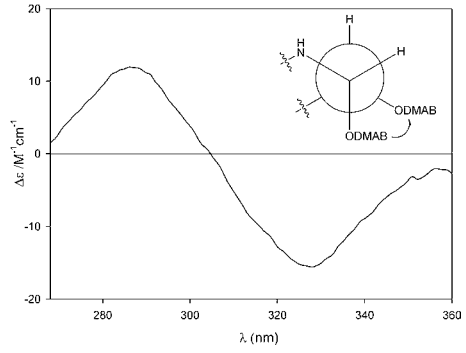


Figure 3. Exciton coupling CD spectrum of **6** to determine the absolute configuration of C-62 in **1**.

Mohangamide B (**2**) was obtained as a white powder (yield: 0.4 mg/L). The molecular formula, $C_{106}H_{139}N_{17}O_{26}$, was determined based on ^1H and ^{13}C spectroscopic data (Table S1) and HRFAB-MS (observed $[M + Na]^+$ ion at m/z 2088.9971, calculated $[M + Na]^+$ at 2088.9975). The planar structure of **2** was determined by careful comparison of the 1D and 2D NMR data of **1** and **2**, which revealed that the ^{8}Thr and $^9\Delta$MeTyr residues in **1** were respectively replaced by ^{8}Ser and ^9N-MeTyr in **2**. Determination of the absolute configuration of the amino acids in **2** was conducted using the same procedure as that for **1**. The absolute configurations of the amino acid units commonly existing in **1** and **2** were identical. Different units, such as ^{8}Ser and ^9N-MeTyr, were determined to be the L-form. The absolute configuration of the dihydropyridine ring was determined to be the same as that for **1** based on the identical ^1H and ^{13}C NMR chemical shifts, $^1H-^1H$ coupling constants, and their common biosynthetic origin.

The structures of the mohangamides are unique in several ways. In our comprehensive literature search, comparable or similar structures to that of the unusual acyl chain-bearing dihydropyridine discovered in **1** and **2** could not be found. Moreover, the dilactone-tethered, pseudodimeric feature is extremely rare. To our best knowledge, the only class of peptide dimer natural products connected with dilactone is echinomycin and its analogues.[19] Furthermore, the mohangamides bear 14 amino acid units, whereas the echinomycin class incorporates 10 amino acids in total. Therefore, the mohangamides are the largest characterized dilactone-tethered, dimeric cyclic peptides. For echinomycin, its biosynthetic modules iterate the synthesis of

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two peptide chains, and the terminal thioesterase domain catalyzes the dimerization of the peptide chain and concomitant cyclization.[20] Therefore, dimeric peptides have not been detected together with their monomers. Surprisingly, the mohangamides were discovered with their monomer WS9326A, which is unprecedented and intriguing regarding the specificity of iteration and the function of thioesterase in nonribosomal peptide biosynthesis.

The biological activities of the mohangamides were primarily evaluated against *C. albicans* ICL. Mohangamide A (**1**) displayed significant inhibition against ICL with an IC_{50} value of 4.4 μM, which was three times more potent than the positive control, 3-nitropropionate (IC_{50} = 14.1 μM). Mohangamide B (**2**) exhibited moderate ICL inhibition (IC_{50} = 20.5 μM). Interestingly, the monomeric compound WS9326A, methanolysis products **3a** and **3b**, and tetraacetate (**4**) did not inhibit ICL (IC_{50} > 100 μM), indicating that the dimeric feature is required for ICL inhibition. Overall, our evaluation suggests that the mohangamides could represent the first peptide class of ICL inhibitors, as peptide-derived compounds have not been reported to inhibit ICL.

We further investigated the biological activities of **1** and **2** in cell-based antifungal assays against *C. albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, and *T. mentagrophytes*. However, these compounds did not show significant antifungal activity when the fungi were fed glucose. Interestingly, mohangamide A (**1**) inhibited *C. albicans* grown on acetate, which indicates that the ICL is crucial in the proliferation of the fungus on C_2 substrates (see Supporting Information Figure S34).

The mohangamides did not display antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Kocuria rhizophila*, *Salmonella enterica*, *Proteus hauseri*, or *Escherichia coli*. In addition, the mohangamides showed no cytotoxicity against the tested human carcinoma cell lines, A549, HCT116, SNU638, K562, SK-HEP1, or MDA-MB231.

In conclusion, we discovered mohangamides A and B (**1**–**2**) as inhibitors of *C. albicans* ICL from a marine actinomycete *Streptomyces* sp. derived from an intertidal mud flat. Mohangamides are structurally novel and biosynthetically interesting because they possess a unique dihydropyridine acyl chain and a dilactone-tethered, dimeric cyclic peptide structure discovered along with a monomeric structure.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, ^1H, ^{13}C NMR and 2D NMR spectra of **1** and **2**, and their chemical derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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