

Seongsanamides A–D: Antiallergic Bicyclic Peptides from *Bacillus safensis* KCTC 12796BP

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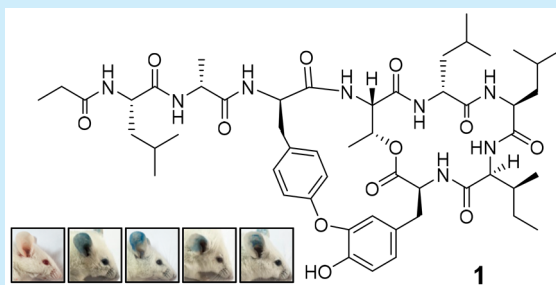
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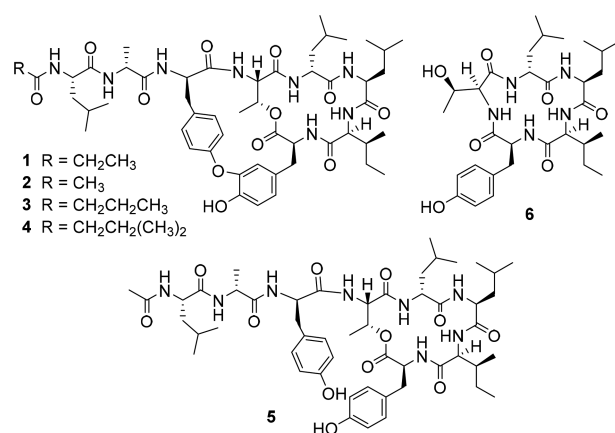
S Supporting Information

ABSTRACT: Six seongsanamides were isolated from the culture broth of *Bacillus safensis* KCTC 12796BP, and their structures were elucidated by spectroscopic data analysis combined with Marfey's method, electronic circular dichroism calculations, and biosynthetic gene cluster analysis. Compounds 1–4 were bicyclic peptides with isodityrosine residues; 5 and 6 were monocyclic peptides. Only the bicyclic seongsanamides inhibited degranulation and LTC₄/PGD₂ generation in IgE/Ag-stimulated bone marrow-derived mast cells. Oral administration of 1 suppressed mast cell-dependent passive cutaneous anaphylaxis reaction.



Cyclic peptides containing amide, ester, disulfide, thioester, and ether moieties have high conformational rigidity and resistance to peptidases and are therefore used widely as leads in drug discovery.¹ Bicyclic peptides possess even greater conformational rigidity and metabolic stability as well as high (antibody-like) affinity and specificity to drug targets; they are considered potential therapeutics.² Several bicyclic peptides have been reported from plants (moroidin³ and celogentins⁴), mushrooms (amatoxin⁵), marine sponges (theonellamide F⁶), and microorganisms (BI-32169⁷), which were revealed as a tubulin polymerization inhibitor,³ an antimetabolic agent,⁴ a RNA polymerase II inhibitor,⁵ an antifungal agent,⁶ and a glucagon receptor antagonist, respectively.⁷ The genus *Bacillus* in the marine environment is a representative producer of structurally diverse peptides, including lipopeptides⁸ and cyclic peptides.⁹ However, no bicyclic peptides have been reported from *Bacillus* sp.

Allergic diseases, such as allergic rhinitis, asthma, and atopic dermatitis, have emerged as common diseases that afflict more than 20% of the world's population.¹⁰ As the incidence of allergic diseases increases, the demand for antiallergic compounds has also increased. Therefore, we screened a number of microbial culture broth extracts for their antiallergic activities on bone marrow-derived mast cells (BMMCs) and found that the EtOAc extract of *Bacillus safensis* KCTC



12796BP exhibited antiallergic activity. In this study, the structural elucidation and antiallergic activity of bi- and monocyclic peptides isolated from *B. safensis* KCTC 12796BP have been described.

A bacterial strain isolated from a marine sponge collected in the waters of Seongsan in Jeju Island was identified as *B.*

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Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data for Seongsanamide A (1) in $\text{DMSO}-d_6$

	position	δ_{C} , mult.	δ_{H} (J in Hz)		position	δ_{C} , mult.	δ_{H} (J in Hz)		position	δ_{C} , mult.	δ_{H} (J in Hz)
propanoic acid	1	9.7, CH ₃	0.96, t (7.6)	3-Tyr	C4	155.8, C		6-Leu	δ	21.4, CH ₃	0.81 ^a
	2	28.1, CH ₂	2.15, q (7.6)		C5	120.2, CH	6.98, brs		δ'	22.8, CH ₃	0.89 ^a
	3	173.3, C			C6	130.9, CH	7.21, brs		CO	171.7, C	
1-Leu	NH		8.05, d (7.1)	4-Thr	CO	171.3, C		7-Ile	NH		7.89, brs
	α	51.5, CH	4.21 ^a		NH		8.14 ^a		α	59.7, CH	3.71, d (8.7)
	β	40.3, CH ₂	1.44, ^a 1.37 ^a		α	55.6, CH	4.27, d (7.2)		β	35.2, CH	1.92, m
	γ	24.2, CH	1.58, m		β	69.9, CH	5.15, q (6.6)		γ	24.3, CH ₂	1.32, m 0.91, m
	δ	21.4, CH ₃	0.81 ^a		γ	16.6, CH ₃	0.38, d (6.5)		δ	22.8, CH ₃	0.80 ^a
	δ'	22.8, CH ₃	0.89 ^a		CO	167.9, C			γ'	10.4, CH ₃	0.78 ^a
	CO	171.7, C			5-Leu	NH	7.52, d (8.4)		CO	170.4, C	
2-Ala	NH		8.14 ^a		α	50.5, CH	4.51, dd (8.0, 5.8)	8-DOPA	NH		8.32, d (3.2)
	α	47.3, CH	4.36, q (6.6)		β	41.3, CH ₂	1.44, ^a 1.36 ^a		α	52.6, CH	4.19 ^a
	β	17.8, CH ₃	1.16, d (7.1)		γ	24.0, CH	1.37 ^a		β	35.7, CH ₂	2.98, ^a 2.77 ^a
	CO	171.8, C			δ	22.2, CH ₃	0.79 ^a		C1	128.3, C	
3-Tyr	NH		8.17, d (5.8)		δ'	21.3, CH ₃	0.81 ^a		C2	115.9, CH	6.40, brs
	α	55.8, CH	4.59, m		CO	171.2, C			C3	145.8, C	
	β	37.2, CH ₂	3.03, ^a 2.79 ^a		6-Leu	NH	8.21, brs		C4	146.3, C	
	C1	130.7, C			α	52.9, CH	3.99, m		C5	116.9, CH	6.77, d (8.2)
	C2	130.9, CH	7.21, brs		β	40.2, CH ₂	1.52, ^a 1.37 ^a		C6	124.5, CH	6.62, dd (8.2, 1.5)
	C3	120.2, CH	6.98, brs		γ	24.3, CH	1.62, m		CO	169.8, C	

^aOverlapped signals.

safensis KCTC 12796BP (GenBank accession no. NZ_CP018197) by 16S rRNA gene sequence analysis. The EtOAc extract of the bacterial culture broth, treated at 100 $\mu\text{g}/\text{mL}$ in BMMCs, was found to inhibit the production of LTC₄ and PGD₂ by 98.6% and 40.2%, respectively. Bioactivity-guided isolation using silica gel vacuum liquid chromatography (VLC), and reversed-phase HPLC led to the isolation of seongsanamides A–F (1–6).

Seongsanamide A (1) was isolated as a colorless oil. Its molecular formula was deduced as C₅₂H₇₆N₈O₁₂ based on the exact mass value of the protonated molecule, by HR-FAB-MS (obsd. $[\text{M} + \text{H}]^+$ of m/z 1005.5666; calcd. for C₅₂H₇₇N₈O₁₂⁺, 1005.5655), together with NMR data (Table 1). The 1D NMR spectra of 1 showed the characteristic features of a peptide, which are eight amide proton peaks (δ_{H} 8.32, 8.21, 8.17, 8.14, 8.14, 8.05, 7.89, and 7.52) and nine amide/ester carbon peaks (δ_{C} 173.3, 171.8, 171.7, 171.7, 171.3, 171.2, 170.4, 169.8, and 167.9). In addition, HSQC showed eight methine signals at the α -positions of the amino acids ($\delta_{\text{H}}/\delta_{\text{C}}$ 4.59/55.8, 4.51/50.5, 4.36/47.3, 4.27/55.6, 4.21/51.5, 4.19/52.6, 3.99/52.9, and 3.71/59.7). Further 1D and 2D NMR data analyses enabled us to assign the nine constituent units of 1: a propionamide group and eight amino acids (three Leu, one Ala, one Thr, one Ile, one Tyr, and a 3-O-substituted Tyr residue) as shown in Figure 1. The connectivity of amino acids was identified by HMBC and ROESY correlations. The isodityrosine unit was established by the strong ROESY correlation between the C3 proton in Tyr (δ_{H} 6.98) and the meta-substituted proton (δ_{H} 6.40) in DOPA, which disturbed the free rotation of Tyr and DOPA. These observations led to the construction of the planar structure of 1 as a bicyclic peptide with an ester linkage and an isodityrosine residue.

Seongsanamide B (2) was identified with the molecular formula of C₅₁H₇₄N₈O₁₂ on the basis of HR-FAB-MS (obsd. $[\text{M} + \text{H}]^+$ of m/z 991.5504; calcd. for C₅₁H₇₅N₈O₁₂⁺, 991.5499), together with NMR data (Table S1). The NMR spectra of 2 were similar to those of 1 but showed a singlet methyl, rather than a triplet methyl and the quartet methylene

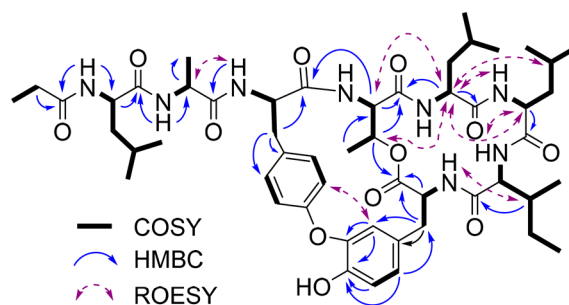


Figure 1. Key COSY, HMBC, and ROESY correlations of 1.

signal of propionamide. Extensive NMR data analyses indicated that 2 was a derivative of 1 containing acetamide, instead of propionamide.

Likewise, the comparison of the spectroscopic data of 3 and 4 with those of 1 and 2 indicated that all four compounds (1–4) possessed identical peptide sequences. The differences were found in the acyl groups on the N-terminus; compounds 3 and 4 possessed butyric acid and 3-methylbutanoic acid, respectively, rather than the propionamide found in 1.

The exact mass value of protonated seongsanamide E (5) was recorded at m/z 993.5661, whereas that of protonated 2 was observed at m/z 991.5504. The ^1H and ^{13}C NMR spectra of 5 displayed the resonances of two sets of 1,4-substituted benzene rings (Table S1; $\delta_{\text{H}}/\delta_{\text{C}}$ 7.03/130.5, 6.91/130.5, 6.66/115.4, and 6.64/115.4). These observations and further NMR data led to the elucidation of 5 as a monocyclic peptide that lacks an ether linkage between the two Tyr units of 1–4.

Seongsanamide F (6) was also identified as a derivative of seongsanamide owing to the similarity in NMR spectra. The exact mass value of the protonated molecule of 6 was observed at m/z of 604.3712, and the resonances of one Thr, one Ile, one Tyr, and two Leu residues were found in the NMR spectra of 6 (Table S1). Through careful analysis of the spectroscopic data, an amide bond between Thr and Tyr was assigned, whereas in 1–5 it is an ester linkage. The planar structure of 6

was elucidated as a monocyclic peptide with no ester or ether linkages.

The absolute configurations of the amino acids in **1–6** were first analyzed by flash acid hydrolysis followed by C3 Marfey's method (Figure S1 and Table S2).¹¹ Compound **6** was found to include L-Thr, D-Leu, L-Leu, L-Ile, and L-Tyr. These analyses and the similarity of the amino acid sequences between **1–5** and **6** led to speculation of the amino acid sequences in **1–5** as 1-L-Leu, 2-D-Ala, 3-D-Tyr, 4-L-Thr, 7-L-Ile, and 8-L-Tyr. However, the absolute configurations of 5-Leu and 6-Leu in **1–6** could not be determined by using acid hydrolysis.

The exact positions of the remaining L-Leu and D-Leu residues were deduced by the calculated distance between protons that showed strong ROESY correlations. The computational energy-minimized models of two possible stereoisomers of **1** (case-I, 5-D-Leu-6-L-Leu; case-II, 5-L-Leu-6-D-Leu) were calculated by using Turbomole¹² (Figure S2). The distance between the α proton of 5-Leu and the γ proton of 6-Leu (ROESY correlation 2) in case-II did not support the ROESY correlation between the two protons (Figure S2), whereas the protons with strong ROESY correlations were located within 5 Å in case-I, as shown in Figure 2 [calculated

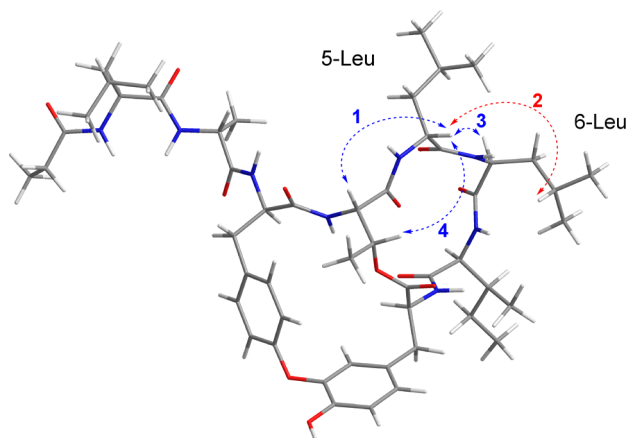


Figure 2. Key ROESY correlations (1–4) of the 5-Leu α proton with 3D structure of **1** in case-I.

H–H distance of ROESY correlation 2:4.541 Å (case-I) and 6.616 Å (case-II)]. The calculated electronic circular dichroism (ECD) spectra of the energy-minimized models also supported case-I, based on a similar pattern that showed a negative Cotton effect at 240–250 nm and a positive Cotton effect at 260–300 nm between the calculated ECD spectrum for case-I and the observed ECD spectrum for **1** (Figure S3A). The exact positions of D-Leu and L-Leu in **1** were speculated as 5-D-Leu and 6-L-Leu. From the similarity in the observed ECD spectra of **1–4**, it was speculated that they possessed identical absolute configurations and sequences of amino acids (Figure S3B).

The absolute configuration of 5-Leu and 6-Leu in compounds **5** and **6** were speculated on the basis of structural similarity with **1–4**, but the observed ECD spectra of **5** and **6** did not supply critical evidence. Therefore, the whole genome of *B. safensis* KCTC 12796BP was sequenced by using Pac-Bio next-generation sequencing. The acquired sequences were de novo assembled into two circular contigs, comprising a chromosome and a plasmid (BioProject PRJNA353573). Analysis using the PKS/NRPS Web site (<http://nrps.igs.umaryland.edu/>) revealed that two nonribosomal peptide synthetase (NRPS) genes (BSL056_RS13630 and BSL056_RS13635) were responsible for the biosynthesis of the seongsanamides A–F (Figure 3, red arrow). One NRPS gene encoded five modules for the biosynthesis of a Leu-Ala-Tyr-Thr-Leu fragment; the other encoded three modules that were implicated in the biosynthesis of a Leu-Ile-Tyr fragment. In these two NRPS genes, three epimerization domains were found in modules 2, 3, and 5, which corresponded to the position of the aforementioned D-amino acids. In addition, the production of the isodityrosine unit was related to the oxidation process by cytochrome P450 in the adjacent gene cluster. The production of **6** was speculated as a truncated form in the middle of the biosynthetic pathway. In conclusion, the absolute configurations of **1–5** were elucidated as 1-L-Leu, 2-D-Ala, 3-D-Tyr, 4-L-Thr, 5-D-Leu, 6-L-Leu, 7-L-Ile, and 8-L-Tyr, and the absolute configurations of **6** were elucidated as 1-L-Thr, 2-D-Leu, 3-L-Leu, 4-L-Ile, and 5-L-Tyr.

The antiallergic activities and cytotoxicities of **1–6** were evaluated in BMMCs. Only bicyclic peptides **1–4** dose-dependently inhibited β -hexosaminidase (β -Hex) release and

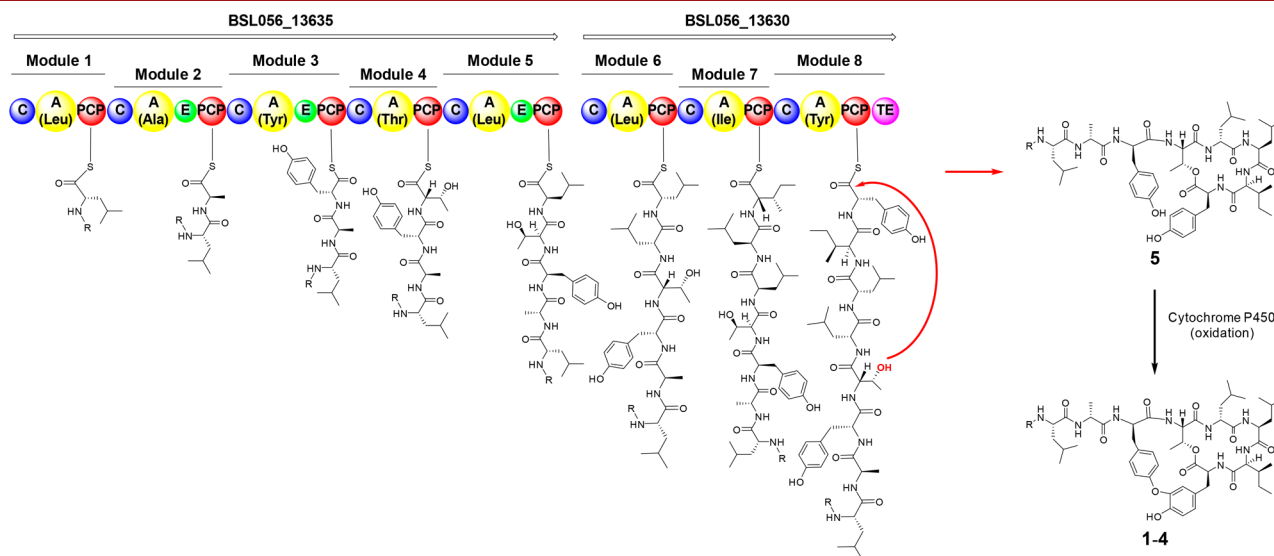


Figure 3. Putative NRPS gene clusters for the biosynthesis of **1–6**.

the generation of leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂); monocyclic compounds **5** and **6** exhibited no antiallergic activity at 10 μ M (Table 2). In addition, all

Table 2. Effects of **1**–**6** on IgE/Ag-Stimulated Mast Cells

compound	IC ₅₀ (μ M)		
	β -Hex	LTC ₄	PGD ₂
1	8.2 $\pm$ 0.1	5.5 $\pm$ 1.4	41.6 $\pm$ 0.5
2	12.6 $\pm$ 0.2	8.2 $\pm$ 3.7	60.4 $\pm$ 0.4
3	9.3 $\pm$ 0.5	10.1 $\pm$ 0.6	5.8 $\pm$ 0.1
4	49.0 $\pm$ 0.4	8.2 $\pm$ 1.8	66.6 $\pm$ 1.6
5	ND ^a	ND ^a	ND ^a
6	ND ^a	ND ^a	ND ^a
PP2 ^b	<10.0 ^c	<10.0 ^c	<10.0 ^c

^aNot determined; no inhibitory activities at 10 μ M. ^bSrc kinase inhibitor (positive control). ^c95% inhibition at 10 μ M. All experiments were performed in triplicate.

seongsanamides at 20 μ M did not exert cytotoxicity to BMMCs in the MTT assay; in particular, the four bicyclic seongsanamides did not show any cytotoxicity, even at 100 μ M. Among bicyclic seongsanamides, **1** exhibited the most potent inhibitory activity on LTC₄ production and β -Hex release with an IC₅₀ value of 5.5 and 8.2 μ M, respectively, and inhibition of PGD₂ production with an IC₅₀ value of 41.6 μ M. The in vitro results led us to evaluate the antiallergic activity of **1** in a passive cutaneous anaphylaxis (PCA) mouse model. The oral administration of compound **1** dose-dependently suppressed dye extravasation and serum levels of LTC₄ and PGD₂ (Figures 4 and S4). The antiallergic activity of **1** was

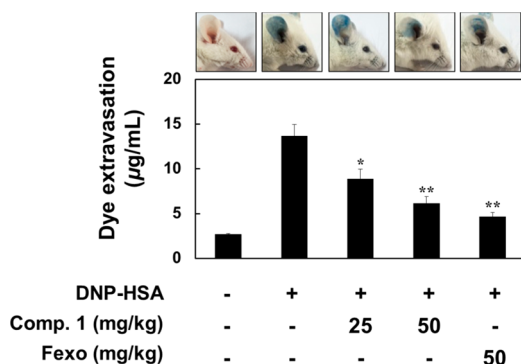


Figure 4. Dye extravasation after treatment with **1** and representative photos of ears with dye extravasation. The data show the mean $\pm$ SEM ($n = 6$ per group). The levels of significance were * $p < 0.05$ and ** $p < 0.01$.

comparable with that of fexofenadine-HCl (Fexo), a known H-1 blocker. Interestingly, compound **1** exhibited similar antiallergic activities in both the in vitro assay and the in vivo assay after oral administration, whereas most of the peptidic compounds did not exhibit in vivo bioactivity after oral administration similar to the in vitro test owing to the degradation by digestive enzymes, such as peptidases.

Unlike in **1**–**4**, 3-Tyr of **5** does not bind to DOPA unit and possesses a free hydroxy group, which is a potential H-donor. Also, the ether linkage between 3-Tyr and DOPA in **1**–**4** increases molecular rigidity and possibly enhances resistance against enzymatic degradation. Therefore, the ether linkage formed by cytochrome P450 might play a vital role in the

antiallergic activity of seongsanamides. Additional medicinal chemistry studies are on the way to reveal the structure–activity relationship and to increase efficacy and druglikeness.

Previously, the isolation of a few linear and monocyclic peptides containing isodityrosine units has been reported from marine sponges, microorganisms, and plants. Bastadins found in marine sponges have cytotoxicity and δ -opioid receptor binding affinity.^{13,14} A bacterial secondary metabolite, K-13, was reported as angiotensin I converting enzyme inhibitor,¹⁵ and a fungal metabolite OF4949 was revealed as an aminopeptidase inhibitor.¹⁶ A series of bicyclohexapeptides containing isodityrosine, such as bouvardin¹⁷ and RA alkaloids,¹⁸ were reported with antitumor activities from the plants of the genus *Rubiaceae*. However, the antiallergic activity of isodityrosine-containing compounds has not been previously reported. Bicyclic seongsanamides are depsipeptides with rare isodityrosine residues, and they are biosynthesized through NRPS pathways and cyclized by thioesterases for off-loading and cytochrome P450; in contrast, most other bicyclic peptides are formed by sulfide or disulfide bonds.

In conclusion, seongsanamides A–D (**1**–**4**) are the first bicyclic depsipeptides identified from the marine-derived genus of *Bacillus*. Their bicyclic depsipeptide residues containing isodityrosine offer a novel and promising scaffold for the development of antiallergic drugs.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03293.

1D and 2D NMR and HR-FAB-MS data for isolated compounds **1**–**6** with experimental procedures (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting Information

Seongsanamides A–D: Anti-Allergic Bicyclic Peptides from *Bacillus safensis* KCTC 12796BP

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General experimental procedures

HPLC was performed on Shimadzu prep HPLC system and Waters series (996 PDA, pump) with a Shim-pack ODS column (20 × 250 mm) to isolate compounds. Specific rotations were measured by using a JASCO DIP-1000 spectrometer (Tokyo, Japan). UV spectra were obtained by JASCO V-550 UV/Vis spectrophotometer (Tokyo, Japan). ECD spectra were recorded using an Applied Photophysics Chirascan-Plus circular dichroism spectrometer. Low resolution ESI-MS were performed with an Agilent 6120 single-quadrupole mass spectrometer with a reversed-phase C18 column (Phenomenex Luna 3 μ C18(2) 100 Å, 4.6 × 150 mm). High resolution FAB-MS spectra were measured on JMS-700 (Jeol, Tokyo, Japan) mass spectrometer. NMR spectra were recorded on Bruker (Billerica, USA) AVANCEIII HD 700 (¹H, 700 MHz, ¹³C, 175 MHz), 600 (¹H, 600 MHz, ¹³C, 150 MHz), and DMX 250 (¹H, 250 MHz, ¹³C, 63 MHz), Varian (CA, USA) VNS 600 (¹H, 600 MHz, ¹³C, 150 MHz), and Jeol (Tokyo, Japan) ECA-500 (¹H, 500 MHz, ¹³C, 125 MHz) spectrometers with DMSO-*d*₆.

Isolation, identification and cultivation of the bacterial strain

The bacterial strains were obtained from marine sponge (*Mycale* sp.) collected on Je-ju Island, South Korea in 2012. Collected marine sponge was dried and cut into small pieces under sterilized condition. The dried crude sample was given a heat shock at 50 °C for 5 minutes and stamped on SYP (per liter of seawater : soluble starch, 10 g; yeast extract, 4 g; peptone, 2 g; bacto agar, 15 g) agar medium plate with 75% seawater. The plate was placed in 27 °C incubator and monitored for 1-2 weeks to obtain colonies. After serial subculture on the SYP agar medium, L056 strain was peaked as pure white colony. The 16S rRNA gene sequence was performed to identify L056 as *Bacillus safensis* with 98.75% (1402/1413) similarity. The bacterial strain of *Bacillus safensis* KCTC 12796BP was cultured into 50 mL centrifuge tube containing 10 mL of SYP liquid medium for 3 days for seed culture. The seed cultured broth of L056 was inoculated into 1 L of SYP liquid medium in 2.5 L baffled Erlenmeyer flasks (1 L × 24) at 25 °C with 150 rpm for 10 days.

Extraction of bacterial strain and isolation of seongsanamides (1–6)

The 24 L cultured broth of *Bacillus safensis* KCTC 12796BP was extracted twice with ethyl acetate (EtOAc). The EtOAc extract of L056 was evaporated to get 2.2 g of dried extract. The dried extract was fractionated into seven fractions (Fr. A–G) by normal phase silica gel vacuum liquid chromatography using step-gradient elution of MC and MeOH. Fr. E (642.0 mg) was subjected to reverse phased prep HPLC with following condition; Shim-pack ODS, 20 × 250 mm; eluting with a gradient solvent system [ACN:H₂O=53:47 (20 min)→68:32 (60 min); 6.0 mL/min] to give **2** (24.9 mg, t_R =28.7 min), **1** (50.1 mg, t_R =35.8 min), **3** (4.8 mg, t_R =41.7 min), and **4** (7.9 mg, t_R =47.6 min). Fr. F (896.3 mg) was applied to Sephadex LH20 column chromatography using gradient mixtures of MeOH in H₂O from 30% to 100% to obtain six fractions (Fr. F1 – Fr. F6). Fr. F1 (216.8 mg) was purified by prep-HPLC [Shim-pack ODS, 20 × 250 mm; eluting with ACN:H₂O=15:85→15:85 (20 min)→35:65 (70 min)→70:30 (90 min); 6.0 mL/min] to get nine sub-fractions (Fr. F1-A – Fr. F1-I). Fr. F1-I (2.1 mg) was purified by HPLC (Luna 5 μ C18(2), 4.6 × 250 mm, flow rate of 1 mL/min, 40% to 60% aqueous ACN gradient) to yield **6** (1.1 mg, t_R =8.6 min). Fr. F4 (108.6 mg) was subjected to reverse phase HPLC [Shim-pack ODS, 20 × 250 mm; eluting with ACN:H₂O=10:90→30:70 (60 min)→55:45 (80 min); 6.0 mL/min] to obtain thirteen fractions (Fr. F4-A – Fr. F4M). Fr. F4-H (4.3 mg) was further purified by HPLC (Penomenex Luna 5 μ C18(2), 10 × 250 mm; eluting with a 42% aqueous ACN isocratic solvent system); 2.0 mL/min] to afford **5** (1.3 mg, t_R =11.9 min).

Seongsanamide A (1): Colorless oil; $[\alpha]_D^{25}$ -10.7 (c 0.1, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 215 (-9.2), 233 (1.9), 246 (-4.1), 287 (2.4) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.6), 275 (3.5); ¹H and ¹³C NMR data, see Table S1; HRFABMS m/z 1005.5666 [M+H]⁺ (calcd for C₅₂H₇₇N₈O₁₂⁺, 1005.5655).

Seongsanamide B (2): Colorless oil; $[\alpha]_D^{25}$ -33.5 (c 0.1, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 216 (-9.9), 234 (0.6), 246 (-3.3), 285 (1.5) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.5), 276 (3.4); ¹H and ¹³C NMR data, see Table S1; HRFABMS m/z 991.5504 [M+H]⁺ (calcd for C₅₁H₇₅N₈O₁₂⁺, 991.5499).

Seongsanamide C (3): Colorless oil; $[\alpha]_D^{25}$ -23.5 (c 0.1, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 215 (-7.8), 234 (0.6), 246 (-2.9), 285 (1.5) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.3), 277 (3.1); ¹H and ¹³C NMR data, see Table S1; HRFABMS m/z 1019.5811 [M+H]⁺ (calcd for C₅₃H₇₉N₈O₁₂⁺, 1019.5812).

Seongsanamide D (4): Colorless oil; $[\alpha]_D^{25}$ -27.5 (c 0.08, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 213 (-12.7), 233 (1.9), 246 (-5.0), 286 (2.4) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.5), 276 (3.4); ¹H and ¹³C NMR data, see Table S1; HRFABMS m/z 1033.5964 [M+H]⁺ (calcd for C₅₄H₈₁N₈O₁₂⁺, 1033.5968).

Seongsanamide E (5): Colorless oil; $[\alpha]_D^{25} -50.4$ (c 0.1, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 204 (–24.4), 219 (–9.2), 246 (–3.3) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.3), 224 (4.1), 279 (3.2); ^1H and ^{13}C NMR data, see Table S1; HRFABMS m/z 993.5661 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{51}\text{H}_{77}\text{N}_8\text{O}_{12}^+$, 993.5655).

Seongsanamide F (6): Colorless oil; $[\alpha]_D^{25} -61.7$ (c 0.06, MeOH); CD(MeOH); $\lambda_{\max}$ ($\Delta\epsilon$) 201 (–12.3), 224 (–2.7) nm; UV(MeOH) $\lambda_{\max}$ (log ϵ) 205 (4.1), 224 (3.9), 279 (2.4); ^1H and ^{13}C NMR data, see Table S1; HRFABMS m/z 604.3712 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{31}\text{H}_{50}\text{N}_5\text{O}_7^+$, 604.3712).

Acid hydrolysis and C3 Marfey's analysis

Compounds **1–6** (100 μg) were hydrolyzed with 6 N HCl (100 μL) at 110 $^\circ\text{C}$ for 30 min with stirring. The hydrolysates were dried under the N_2 gas stream and then resuspended in H_2O (100 μL). These treated hydrolysates were concentrated again under the N_2 gas stream. Each hydrolysates was added to 1 N NaHCO_3 (100 μL) and 1% D-FDLA (1-fluoro-2,4-dinitrophenyl-5-D-leucine amide) in acetone (20 μL) in reaction vial. The reaction vials were incubated with stirring for 30 min at 40 $^\circ\text{C}$. The reactions were quenched with 100 μL of 1 N HCl. D- and L- authentic standard amino acids (100 μg) were also prepared by same protocol. For analysis of Marfey's reaction product with hydrolysates and standard amino acids, the following chromatographic method was used in LC-MS; Agilent SB-C3 Zorbax (5 μm , 4.6 $\times$ 150 mm), solvent A (100% 3DW + 0.02% formic acid), solvent B (MeOH), A:B=48:52 $\rightarrow$ 48:52(8 min) $\rightarrow$ 47.5:52.5(35 min) $\rightarrow$ 38:62(55 min) $\rightarrow$ 0:100(60 min) $\rightarrow$ 0:100(70 min) $\rightarrow$ 48:52(72 min) $\rightarrow$ 48:52(82 min), 1 mL/min, 50 $^\circ\text{C}$. The reaction products with FDLA-derivatized hydrolysates were analyzed on negative mode with a LC-MS system.

Computational analysis

The ground-state geometries were optimized by DFT calculations with Turbomole 6.5. as well as defSV(P) basis set for all atoms at the B3LYP/DFT functional level. The ground states were further confirmed by harmonic frequency calculations. The calculated ECD data of optimized structures were acquired by TD-DFT (time dependent density functional theory) at the basis set def2-TZVPP with B3LYP functional level. The ECD spectra were simulated by overlapping for each transition.

Whole genome sequence analysis

The genomic DNA of *Bacillus safensis* L056 was sequenced by Macrogen (Seoul, Korea) using PacBio_10K next generation sequencing system. Through *de novo* assembly using FALCON software (Pacific Biosciences, USA), two circular sequence each corresponding to chromosome and plasmid were obtained. The open reading frames (ORFs) were identified using a prokka pipeline (<http://www.vicbioinformatics.com/software/prokka.shtml>), and the gene cluster for the biosynthesis of secondary metabolites were analyzed using Antismash (<http://antismash.secondarymetabolites.org/protein>) and PKS/NRPS Analysis (<http://nrps.igs.umaryland.edu/>), in order to deduce the substrate specificity of each module.

MTT assay

Mouse bone marrow derived mast cells (BMMCs) were cultivated in 96-well plates and incubated for 24 h after treatment of compounds **1–6**. MTT (5 mg/mL) was added to each well. Treated plate was incubated with an additional 4 h at 37 °C. The medium was removed and formazan blue obtained from the cells was dissolved in DMSO. The optical density was measured at 540 nm.

In vitro anti-allergic activity assay

BMMCs were prepared by 6 to 7 week old male Balb/cJ mice. For mast cell stimulation, 1×10^6 cells/mL were sensitized with 500 ng/mL mouse anti-DNP IgE for overnight and stimulated with 25 ng/mL DNP-HSA for 15 min. When compounds treated with cells, compounds **1–6** were added 1 h before addition of DNP-HSA. It was evaluated at 15 min that releases of β -Hex, and production of LTC₄, and PGD₂ by using immunoassay kits (Cayman Chemicals, MI USA). In the primary screening, compounds **1–6** at the concentration of 10 μ M were evaluated their anti-allergic activities. Among them, **5–6** showed no inhibition to allergic mediator in BMMCs. On the basis of the primary results, anti-allergic activities of compounds **1–4** measured in dose dependent manners. Each test was performed in triplicate.

In vivo anti-allergic activity assay with IgE-mediated passive cutaneous anaphylaxis (PCA) in mice

Mouse anti-dinitrophenyl (DNP) IgE 80 ng was intradermally injected into one ear of 6 week old male ICR mice. After 24 h later, 25 and 50 mg/kg of compound **1** were orally administrated. 50 mg/kg of fexofenadine-HCl, a selective peripheral H1-blocker, was also orally administrated as positive control. One hour later, the mice were intravenously injected with 60 µg of DNP-HSA (human serum albumin) as antigen in 200 µL of PBS containing 1% (w/v) Evans blue. After treated antigen with 1 h, the ears of mice were removed and dissolved with 400 µL of formamide at 63 °C overnight. The quantity of dye extravasation was evaluated by absorbance at 630 nm. The amount of LTC₄ and PGD₂ in serum was measured by using immunoassay kits (Cayman Chemicals, MI, USA).

Statistic analysis

Data calculation and statistical analysis were obtained by using GraphPad Prism 3.0 software. The statistical significance was calculated by unpaired Student's *t* test and multiple comparisons were analyzed using one-way ANOVA. Data are presented as means ± S.E.M. Differences were considered statistically significant at $p < 0.05$.

Table S1. ¹H and ¹³C NMR data of **2–6** in DMSO-*d*₆

Position		2^a		3^b		4^c	
		δ_{C} , mult.	δ_{H} (J in Hz)	δ_{C} , mult.	δ_{H} (J in Hz)	δ_{C} , mult.	δ_{H} (J in Hz)
	1	22.8, CH ₃	1.86, s	22.4, CH ₃	0.84 ^d	15.5, CH ₃	0.87 ^d
	2	169.8, C		25.6, CH ₂	1.28, m	22.2, CH ₃	1.98, m
	3			44.2, CH ₂	1.98, t (6.2)	25.7, CH ₂	2.02 ^d
	4			172.0, C		44.5, CH ₂	0.85, d (6.5)
	5					172.7, C	
1-Leu	NH		8.10, d (7.5)		8.01, d (7.1)		8.10, d (6.3)
	α	52.9, CH	4.21 ^d	51.3, CH	4.21 ^d	51.7, CH	4.21 ^d
	β	40.7, CH ₂	1.51 ^d , 1.36 ^d	40.1, CH ₂	1.47, m 1.44, m	40.2, CH ₂	1.43 ^d , 1.41 ^d
	γ	24.0, CH	1.59, m	24.0, CH	1.57, m	24.2, CH	1.58, m
	δ	22.7, CH ₃	0.81 ^d	21.5, CH ₃	0.83 ^d	22.9, CH ₃	0.83, d (6.4)
	δ'	22.2, CH ₃	0.90 ^d	22.6, CH ₃	0.89 ^d	21.7, CH ₃	0.89 ^d
	CO	170.1, C		176.3, C		172.1, C	
2-Ala	NH		8.14 ^a		8.11, d (7.7)		8.12, d (8.1)
	α	47.7, CH	4.37, q (7.3)	47.2, CH	4.36, quint (7.4)	47.4, CH	4.35, quint (7.2)
	β	18.3, CH ₃	1.16, d (7.1)	17.6, CH ₃	1.16, d (6.5)	18.0, CH ₃	1.15, d (7.0)
	CO	171.8, C		172.0, C		172.0, C	
3-Tyr	NH		8.23 ^d		8.20 ^d		8.25 ^d
	α	55.3, CH	4.58, m	55.4, CH	4.60, m	56.1, CH	4.56, m
	β	39.6, CH ₂	2.77 ^d , 3.02 ^d	37.0, CH ₂	2.79 ^d , 3.03 ^d	37.1, CH ₂	2.77 ^d , 3.07 ^d
	C1	130.1, C		130.5, C		131.9, C	
	C2	129.8, CH	7.03, brs	130.8, CH	7.23, brs	131.0, CH	7.21, brs
	C3	124.2, CH	6.66 ^d	120.1, CH	6.99, brs	120.4, CH	6.97, brs
	C4	156.0, C		156.4, C		155.8, C	
	C5	124.2, CH	6.66 ^d	120.1, CH	6.99, brs	120.4, CH	6.97, brs
	C6	129.8, CH	7.03, brs	130.8, CH	7.23, brs	131.0, CH	7.21, brs
	CO	171.4, C		171.3, C		171.5, C	
4-Thr	NH		8.17 ^d		8.18 ^d		8.23 ^d
	α	55.6, CH	4.28, d (10.7)	55.5, CH	4.26, d (9.5)	55.8, CH	4.28, d (10.1)
	β	70.4, CH	5.16, q (6.3)	69.7, CH	5.12, m	70.1, CH	5.17, q (6.4)
	γ	16.9, CH ₃	0.39, d (6.5)	16.3, CH ₃	0.39, d (6.0)	16.5, CH ₃	0.38, d (6.4)
	CO	168.0, C		168.3, C		168.3, C	
5-Leu	NH		7.51, d (8.7)		7.50, d (8.2)		7.57, d (8.3)
	α	51.0, CH	4.52, m	50.3, CH	4.50, m	50.7, CH	4.51, m
	β	41.1, CH ₂	1.44 ^d , 1.36 ^d	40.9, CH ₂	1.41, m 1.37, m	41.2, CH ₂	1.51 ^d , 1.36 ^d
	γ	24.0, CH	1.39 ^d	24.2, CH	1.36, m	24.2, CH	1.37 ^d
	δ	24.0, CH ₃	0.79 ^d	21.9, CH ₃	0.78 ^d	22.2, CH ₃	0.78 ^d
	δ'	21.4, CH ₃	0.81 ^d	21.7, CH ₃	0.84 ^d	23.0, CH ₃	0.80 ^d
	CO	171.4, C		170.8, C		171.8, C	
6-Leu	NH		8.27, brs		8.44 ^d		8.30, brs
	α	52.3, CH	3.99, m	52.8, CH	3.99, m	53.2, CH	3.97, quint (4.4)
	β	39.6, CH ₂	1.53, m 1.44 ^d	40.1, CH ₂	1.52, m 1.38, m	40.4, CH ₂	1.49 ^d , 1.35 ^d
	γ	23.8, CH	1.61, m	24.0, CH	1.62, m	24.3, CH	1.31 ^d
	δ	22.4, CH ₃	0.81 ^d	21.4, CH ₃	0.81 ^d	21.4, CH ₃	0.81 ^d
	δ'	22.2, CH ₃	0.89 ^d	22.7, CH ₃	0.89 ^d	21.3, CH ₃	0.89 ^d
	CO	172.2, C		171.8, C		172.3, C	
7-Ile	NH		7.87, brs		8.06, brs		7.80, brs
	α	60.1, CH	3.73, t (9.5)	59.4, CH	3.73, brs	59.7, CH	3.77, brs
	β	34.7, CH	1.88, m	35.1, CH	1.91, brs	35.5, CH	1.88, brs
	γ	22.9, CH ₂	1.33, m 0.96, m	24.4, CH ₂	1.33, m 0.97, m	25.7, CH ₃	0.98, m 0.95, m
	δ	22.9, CH ₃	0.82 ^d	22.1, CH ₃	0.82 ^d	24.3, CH ₂	0.80 ^d
	γ'	15.6, CH ₃	0.79 ^d	13.5, CH ₃	0.85 ^d	10.3, CH ₃	0.78 ^d
	CO	170.5, C		172.0, C		170.7, C	
8-DOPA	NH		8.19, d (3.4)		8.37, brs		8.26 ^d
	α	52.8, CH	4.18 ^d	52.3, CH	4.20 ^d	52.8, CH	4.18 ^d
	β	36.0, CH ₂	2.96 ^d , 2.79 ^d	35.5, CH ₂	2.98 ^d , 2.77 ^d	35.5, CH ₂	2.98 ^d , 2.80 ^d
	C1	124.3, C		127.2, C		128.3, C	
	C2	116.2, CH	6.44, d (2.0)	115.7, CH	6.44, brs	115.9, CH	6.43, brs
	C3	145.9, C		145.9, C		145.8, C	
	C4	146.7, C		146.6, C		146.4, C	
	C5	117.4, CH	6.80, d (8.2)	116.9, CH	6.81, d (8.1)	117.1, CH	6.80, d (8.2)
	C6	114.8, CH	6.64 ^d	124.4, CH	6.67, d (8.1)	124.6, CH	6.66, d (8.3)
	CO	169.8, C		170.2, C		170.0, C	

(continued)

Position		5 ^b		Position		6 ^c	
		δ_{C} , mult.	δ_{H} , (J in Hz)			δ_{C} , mult.	δ_{H} , (J in Hz)
1-Leu	1	22.75, CH ₃	1.86, s				
	2	170.08, C					
	NH		8.10, d (7.5)				
	α	51.56, CH	4.21 ^d				
	β	41.03, CH ₂	1.51 ^d , 1.36 ^d				
	γ	24.60, CH	1.59, m				
	δ	22.03, CH ₃	0.81 ^d				
	δ'	22.99, CH ₃	0.90 ^d				
2-Ala	CO	172.75, C					
	NH		8.14 ^a				
	α	48.12, CH	4.37, q (7.3)				
	β	18.37, CH ₃	1.16, d (7.1)				
3-Tyr	CO	171.38, C					
	NH		8.23 ^d				
	α	55.15, CH	4.58, m				
	β	36.46, CH ₂	2.77 ^d , 3.02 ^d				
	C1	127.23, C					
	C2	130.48, CH	7.03, brs				
	C3	115.37, CH	6.66 ^d				
	C4	150.36, C					
4-Thr	C5	115.37, CH	6.66 ^d				
	C6	130.48, CH	7.03, brs				
	CO	172.34, C					
	NH		8.17 ^d				
	α	56.10, CH	4.28, d (10.7)				
	β	70.04, CH	5.16, q (6.3)				
	γ	15.97, CH ₃	0.39, d (6.5)				
	CO	168.63, C					
5-Leu	NH		7.51, d (8.7)				
	α	51.56, CH	4.52, m				
	β	41.03, CH ₂	1.44 ^d , 1.36 ^d				
	γ	24.60, CH	1.39 ^d				
	δ	22.03, CH ₃	0.79 ^d				
	δ'	22.99, CH ₃	0.81 ^d				
6-Leu	CO	172.55, C					
	NH		8.27, brs				
	α	52.90, CH	3.99, m				
	β	40.33, CH ₂	1.53, m 1.44 ^d				
	γ	24.47, CH	1.61, m				
	δ	21.83, CH ₃	0.81 ^d				
7-Ile	δ'	23.13, CH ₃	0.89 ^d				
	CO	172.51, C					
	NH		7.87, brs				
	α	59.51, CH	3.73, t (9.5)				
	β	35.98, CH	1.88, m				
	γ	24.43, CH ₂	1.33, m 0.96, m				
8-Tyr	δ	22.10, CH ₃	0.82 ^d				
	γ'	10.58, CH ₃	0.79 ^d				
	CO	170.21, C					
	NH		8.19, d (3.4)				
	α	55.15, CH	4.18 ^d				
	β	36.83, CH ₂	2.96 ^d , 2.79 ^d				
	C1	127.68, C					
	C2	130.48, CH	6.44, d (2.0)				
	C3	115.37, CH					
	C4	150.36, C					
	C5	115.37, CH	6.80, d (8.2)				
	C6	130.48, CH	6.64 ^d				
	CO	169.84, C					
1-Thr	NH		8.17 ^d				
	α	57.93, CH	4.21 ^d				
	β	67.28, CH	3.89, m				
	γ	20.41, CH ₃	1.02, d (6.2)				
	CO	171.48, C					
	NH		8.52, d (6.7)				
	α	52.05, CH	4.20 ^d				
	β	39.27, CH ₂	1.53 ^d , 1.38 ^d				
2-Leu	γ	24.65, CH	1.46, m				
	δ	22.93, CH	0.83, d (6.5)				
	δ'	22.61, CH	0.89, d (6.5)				
	CO	171.90, C					
	NH		8.58, d (8.5)				
	α	52.83, CH	4.15, m				
3-Leu	β	39.28, CH ₂	1.55 ^d , 1.49 ^d				
	γ	24.64, CH	1.57, m				
	δ	21.31, CH ₃	0.77 ^d				
	δ'	23.39, CH ₃	0.87, d (6.4)				
	CO	172.30, C					
	NH		7.68, brs				
4-Ile	α	58.62, CH	3.93, t (9.7)				
	β	36.01, CH	1.78, m				
	γ	24.61, CH ₂	1.34, m 0.89, m				
	δ	11.13, CH ₃	0.78 ^d				
	γ'	15.59, CH ₃	0.61, d (6.3)				
	CO	170.82, C					
5-Tyr	NH		8.67, brs				
	α	59.30, CH	4.05, m				
	β	36.00, CH ₂	3.05, d (7.8)				
	C1	128.38, C					
	C2	130.46, CH	7.02, d (8.3)				
	C3	115.48, CH	6.63, d (8.3)				
	C4	156.39, C					
	C5	115.48, CH	6.63, d (8.3)				
	C6	130.46, CH	7.02, d (8.3)				
	CO	172.68, C					

^a ¹H and ¹³C NMR data were acquired at 500, and 125 MHz, ^b 600, and 150 MHz, and ^c 700, and 175 MHz, respectively, ^d overlapped signals

Table S2. Absolute configurations of composition amino acids of **1–6** by C3 Marfey's analysis with D-FDLA

Amino acids		<i>m/z</i> range	Retention time (min)				Absolute configurations
			D-AA-D-FDLA	D- <i>allo</i> -AA-D-FDLA	L- <i>allo</i> -AA-D-FDLA	L-AA-D-FDLA	
Standard AA	Leu	423-425	16.7			53.1	
	Ile	423-425	16.3	15.2	50.1	52.0	
	Ala	381-383	9.6			18.4	
	Tyr	473-475	9.3			17.9	
	Thr	411-413	6.2	7.0	9.5	12.5	
1	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Ala	381-383	9.6				D
	Tyr	473-475					
	Thr	411-413				12.5	L
2	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Ala	381-383	9.6				D
	Tyr	473-475					
	Thr	411-413				12.5	L
3	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Ala	381-383	9.6				D
	Tyr	473-475					
	Thr	411-413				12.5	L
4	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Ala	381-383	9.6				D
	Tyr	473-475					
	Thr	411-413				12.5	L
5	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Ala	381-383	9.6				D
	Tyr	473-475	9.3			17.9	D/L
	Thr	411-413				12.5	L
6	Leu	423-425	16.7			53.1	D/L
	Ile	423-425				52.0	L
	Tyr	473-475				17.9	L
	Thr	411-413				12.5	L

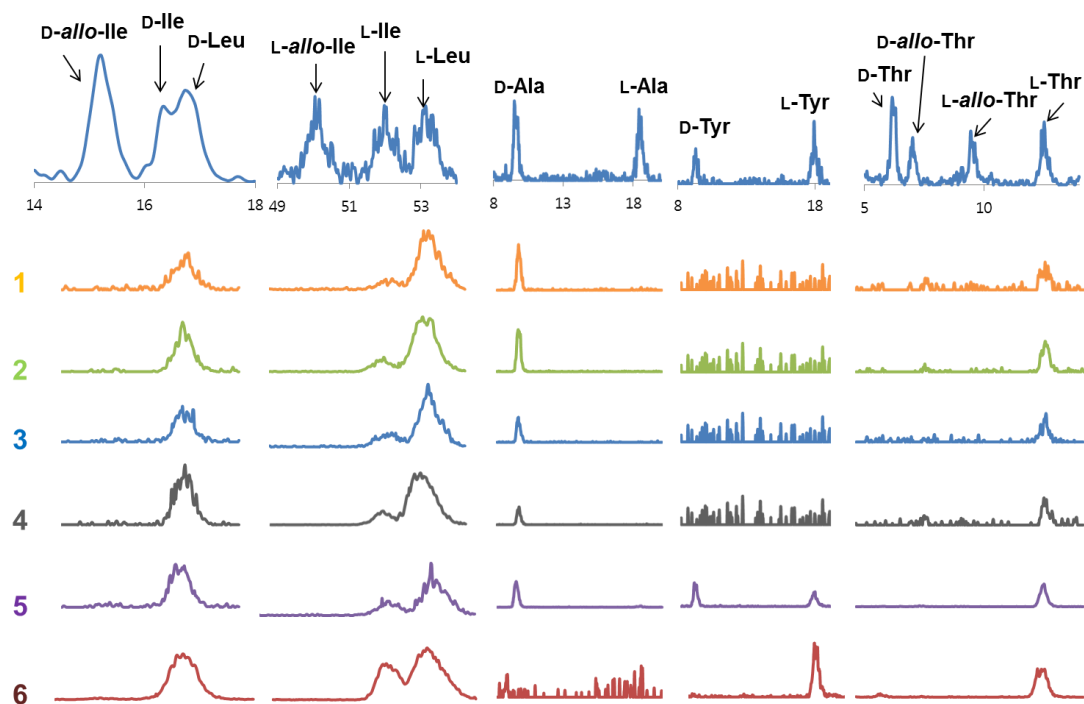
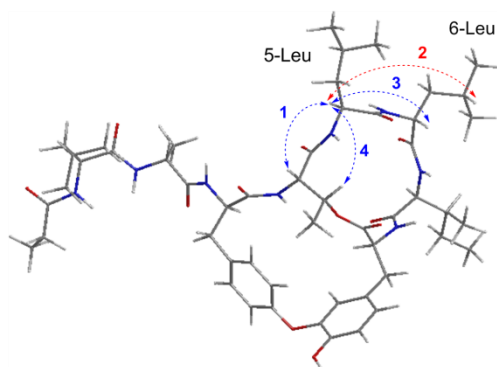


Figure S1. LC-MS chromatograms of C3 Marfey's products with 1–6



< Distance between corresponding protons >

ROESY Correlation	H-H distance (Å)	
	Case-I	Case-II
1	4.452	4.474
2	4.541	6.616
3	4.362	4.517
4	4.439	4.686

Figure S2. Energy minimized 3D structure of case-II in 1 with key ROESY correlations and H-H distance between 5-Leu α proton and correlated protons

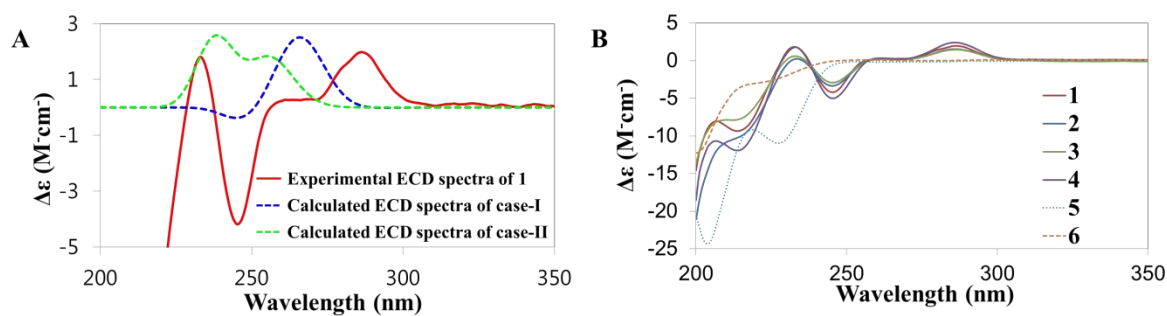


Figure S3. Spectroscopic analysis by ECD (A) ECD spectra of experimental and calculated model of 2. (B) Experimental ECD spectra of 1–6

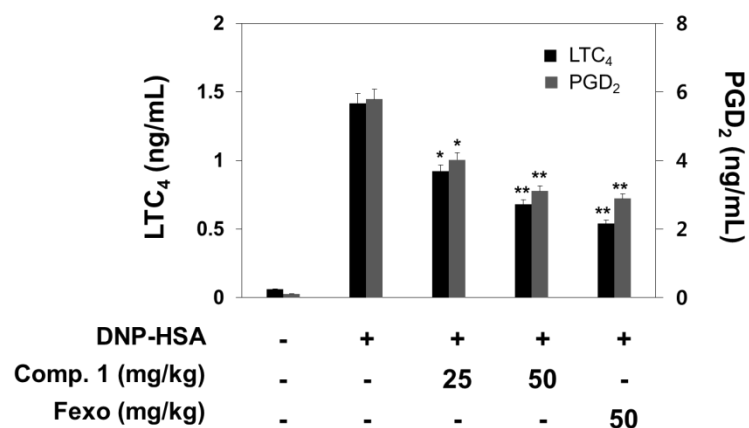


Figure S4. The levels of serum LTC₄ and PGD₂ treated with 1 in PCA model. The statistical significance of data were as follows **p* < 0.05, and ***p* < 0.01

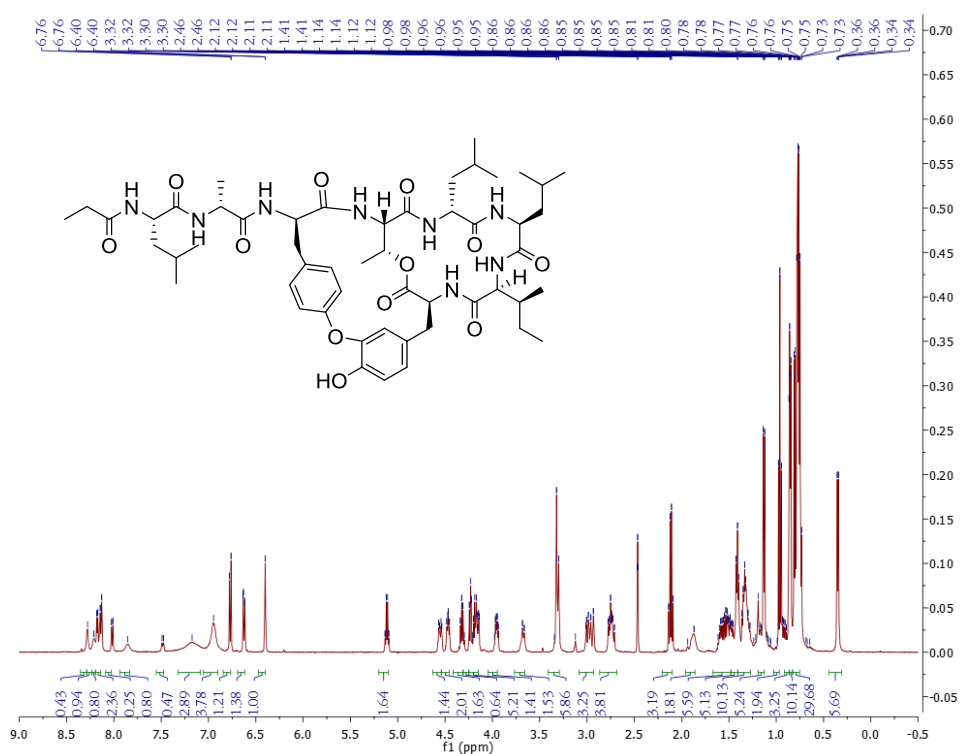


Figure S5. ^1H NMR spectrum (500 MHz) of seongsanamide A (**1**) in $\text{DMSO}-d_6$

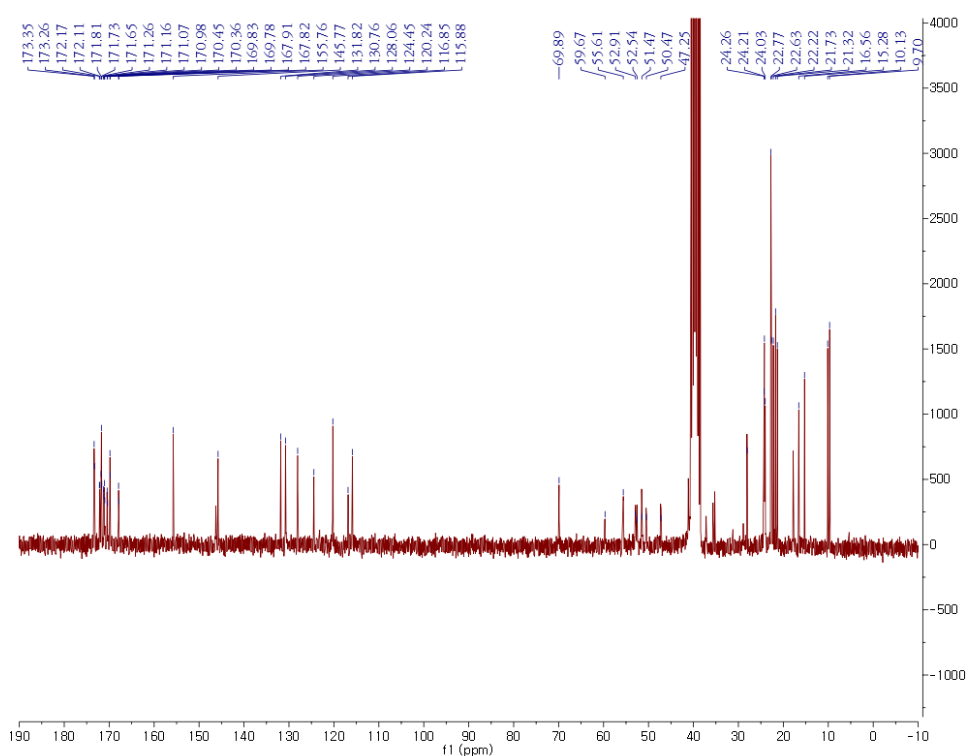


Figure S6. ^{13}C NMR spectrum (63 MHz) of seongsanamide A (**1**) in $\text{DMSO}-d_6$

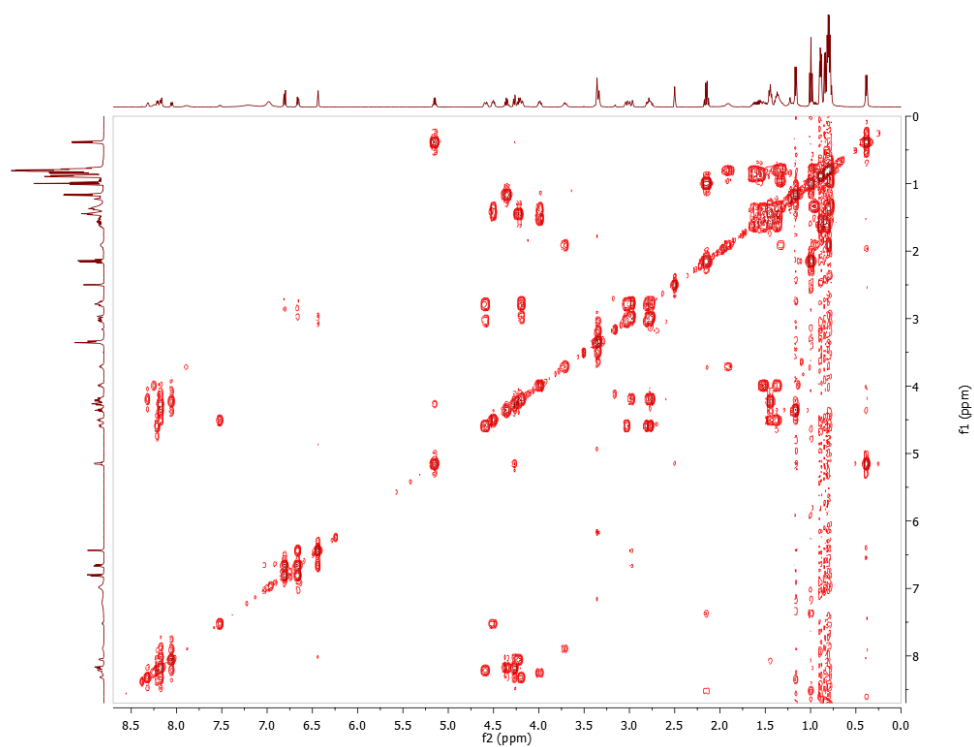


Figure S7. COSY spectrum (500 MHz) of seongsanamide A (**1**) in DMSO- d_6

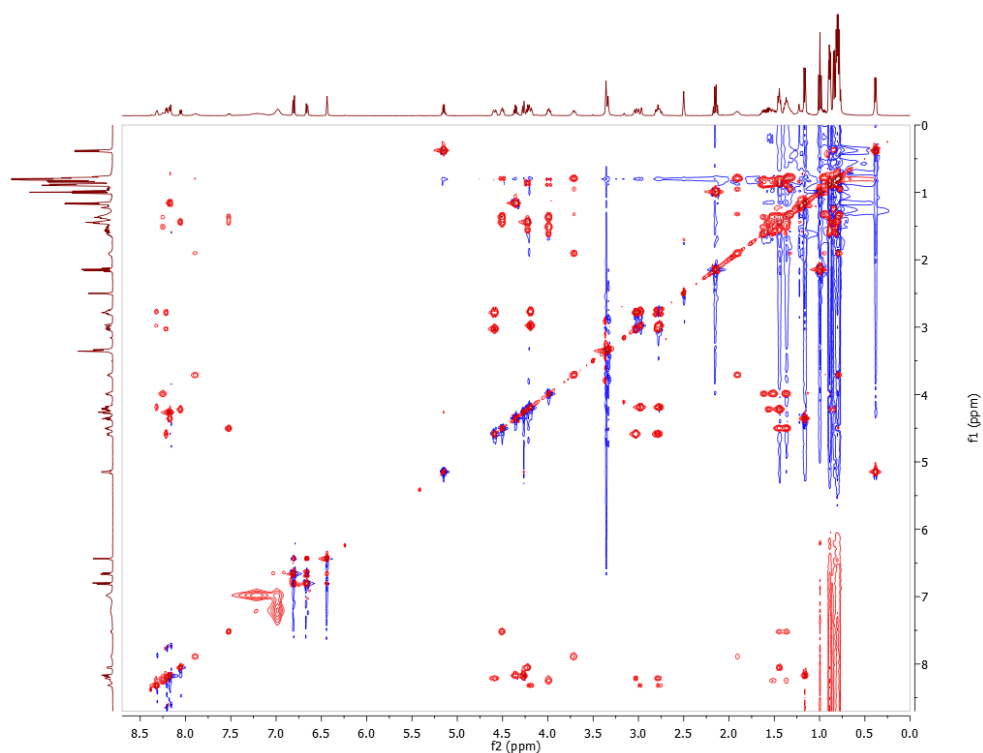


Figure S8. TOCSY spectrum (500 MHz) of seongsanamide A (**1**) in DMSO- d_6

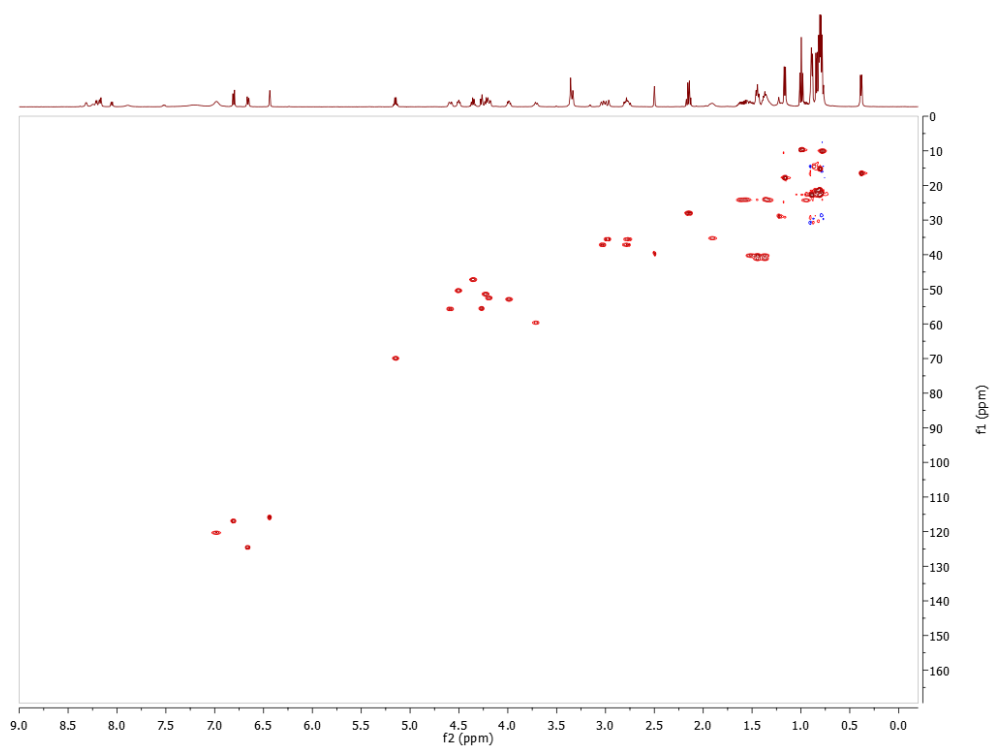


Figure S9. HMBC spectrum (500 MHz) of seongsanamide A (**1**) in DMSO- d_6

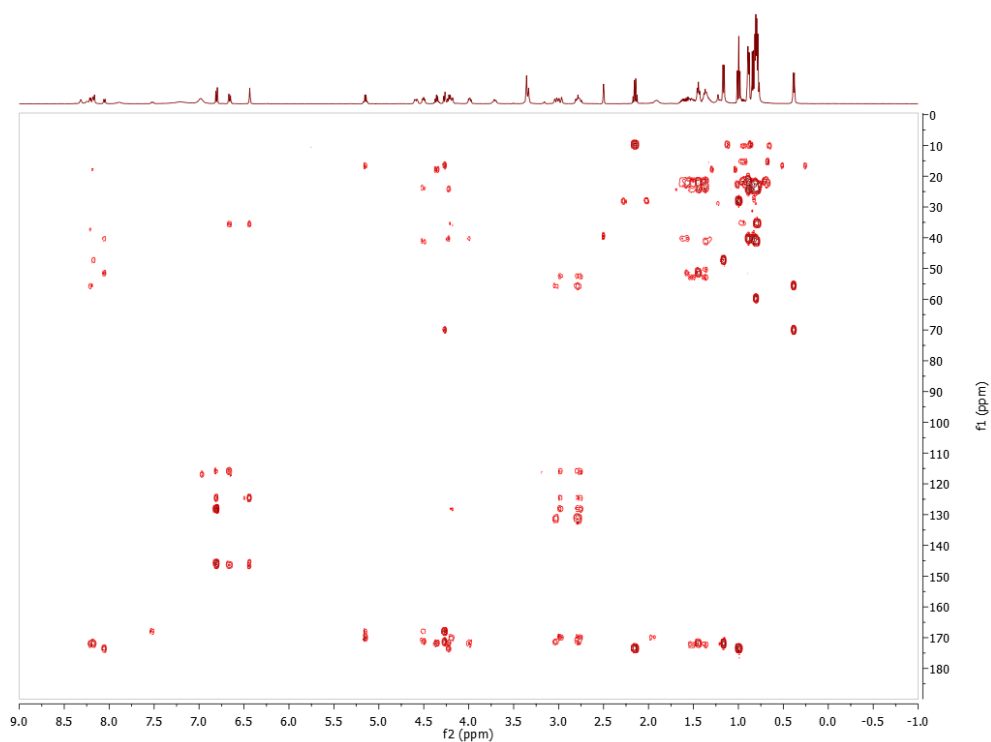


Figure S10. HMBC spectrum (500 MHz) of seongsanamide A (**1**) in DMSO- d_6

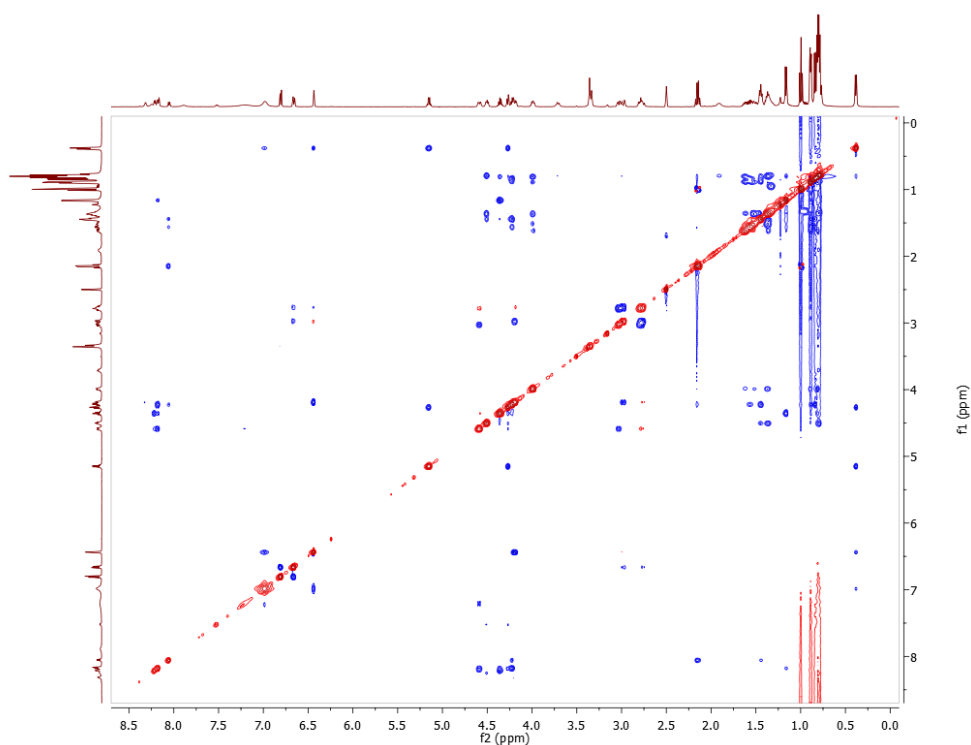


Figure S11. ROESY spectrum (500 MHz) of seongsanamide A (**1**) in DMSO-*d*₆

[Elemental Composition]
 Data : FAB-L782
 Sample: 2
 Note : m-NBA
 Inlet : Direct
 RT : 0.26 min
 Elements : C 100/0, H 100/0, N 10/0, O 15/5
 Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000
 Unsaturation (U.S.) : -0.5 - 100.0
 Date : 28-Apr-2015 15:43
 Ion Mode : FAB+
 Scan#: (7,19)

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
1005.5666	100.0	+2.3 / +2.3	31.5	C 64 H 73 N 6 O 5
		+1.0 / +1.0	31.0	C 66 H 75 N 3 O 6
		-4.8 / -4.9	27.5	C 58 H 73 N 10 O 6
		-0.3 / -0.3	30.5	C 68 H 77 O 7
		-6.2 / -6.2	27.0	C 60 H 75 N 7 O 7
		+6.3 / +6.4	27.5	C 59 H 73 N 8 O 7
		-7.5 / -7.6	26.5	C 62 H 77 N 4 O 8
		+5.0 / +5.0	27.0	C 61 H 75 N 5 O 8
		-8.8 / -8.9	26.0	C 64 H 79 N O 9
		+3.7 / +3.7	26.5	C 63 H 77 N 2 O 9
		-2.2 / -2.2	23.0	C 55 H 75 N 9 O 9
		-3.5 / -3.5	22.5	C 57 H 77 N 6 O 10
		+9.0 / +9.0	23.0	C 56 H 75 N 7 O 10
		-4.8 / -4.9	22.0	C 59 H 79 N 3 O 11
		+7.7 / +7.7	22.5	C 58 H 77 N 4 O 11
		-6.2 / -6.2	21.5	C 61 H 81 O 12
		+6.3 / +6.4	22.0	C 60 H 79 N O 12
		+0.5 / +0.5	18.5	C 52 H 77 N 8 O 12
		-0.8 / -0.8	18.0	C 54 H 79 N 5 O 13
		-2.2 / -2.2	17.5	C 56 H 81 N 2 O 14
		-8.0 / -8.1	14.0	C 48 H 79 N 9 O 14
		+4.5 / +4.5	14.5	C 47 H 77 N 10 O 14
		+9.0 / +9.0	17.5	C 57 H 81 O 15
		-9.4 / -9.4	13.5	C 50 H 81 N 6 O 15
		+3.2 / +3.2	14.0	C 49 H 79 N 7 O 15

Figure S12. HR-FAB-MS data of **1**

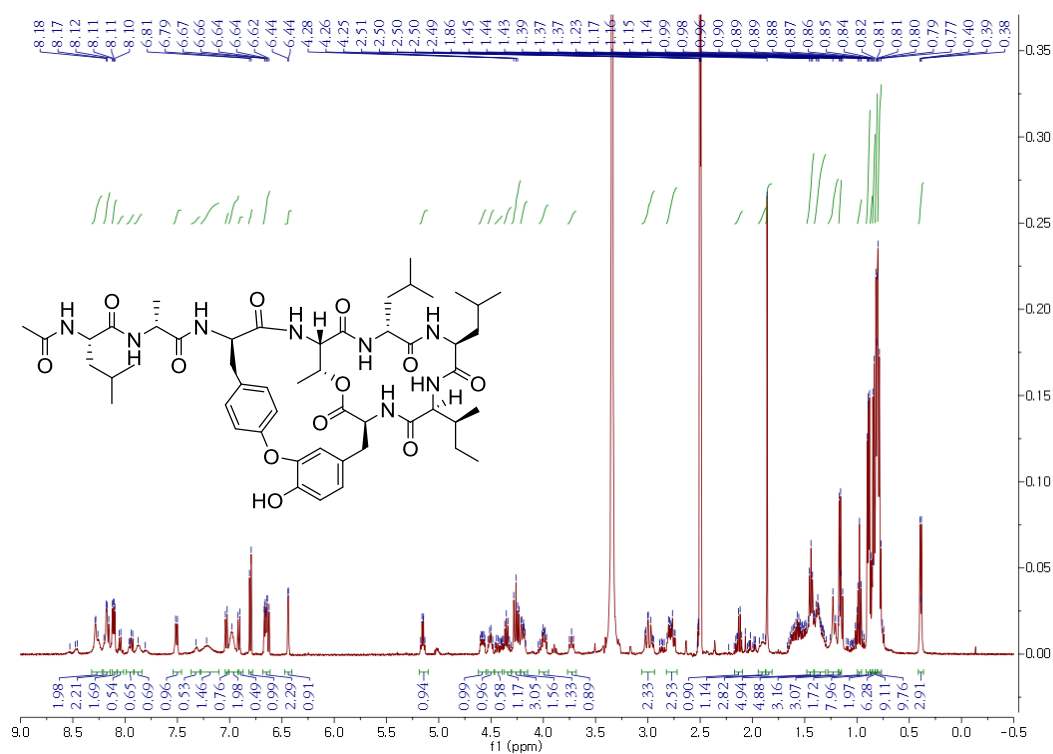


Figure S13. ^1H NMR spectrum (500 MHz) of seongsanamide B (2) in $\text{DMSO}-d_6$

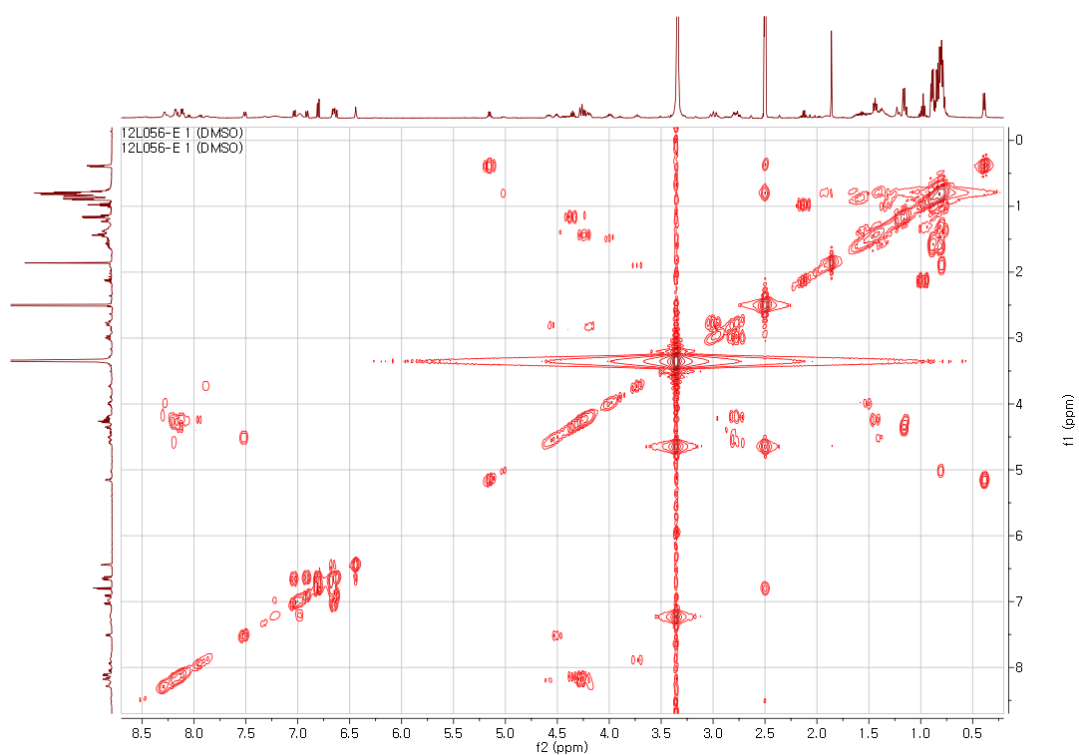


Figure S14. COSY spectrum (250 MHz) of seongsanamide B (2) in $\text{DMSO}-d_6$

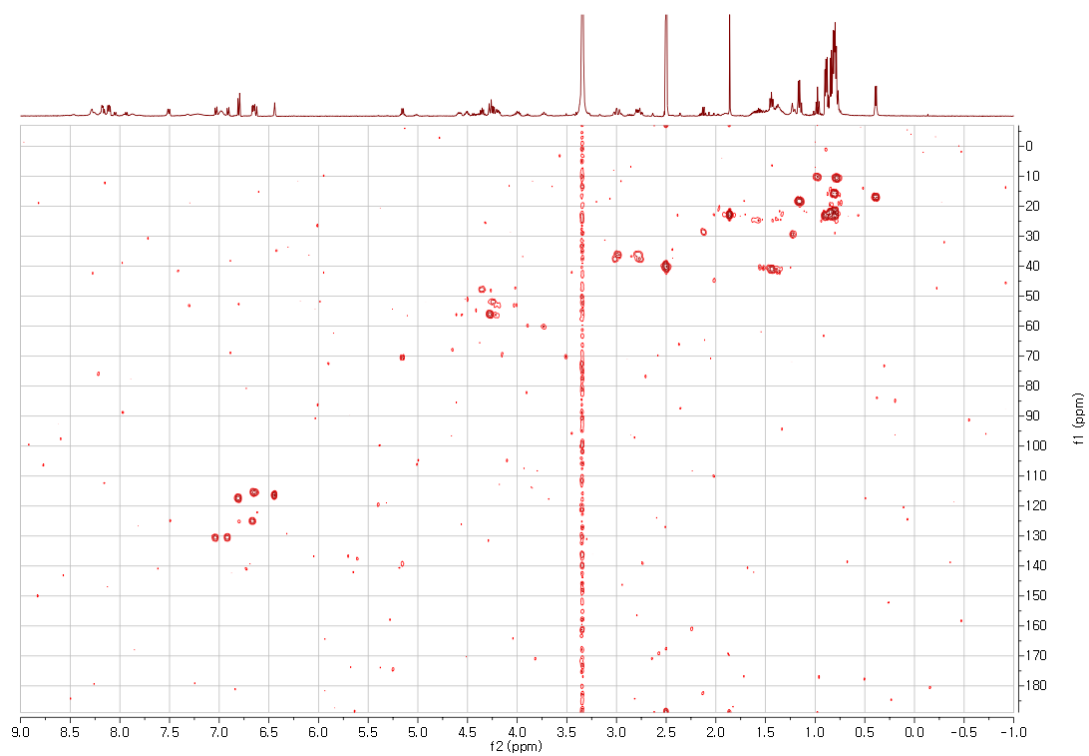


Figure S15. HMBC spectrum (500 MHz) of seongsanamide B (**2**) in DMSO- d_6

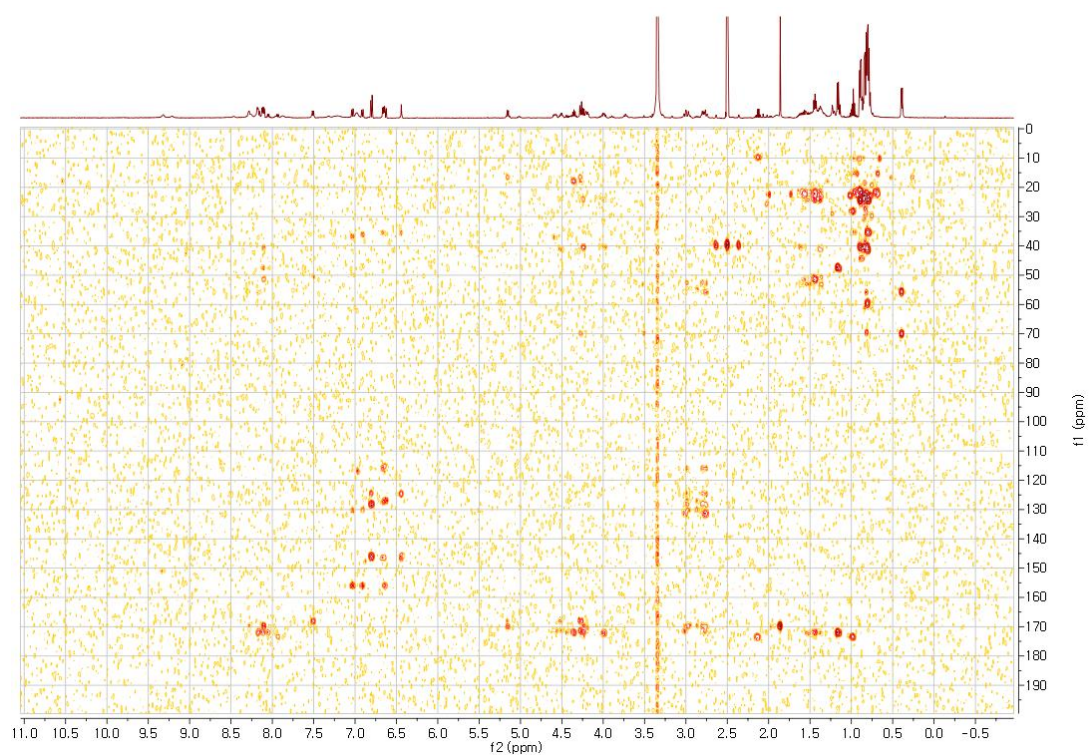


Figure S16. HMBC spectrum (500 MHz) of seongsanamide B (**2**) in DMSO- d_6

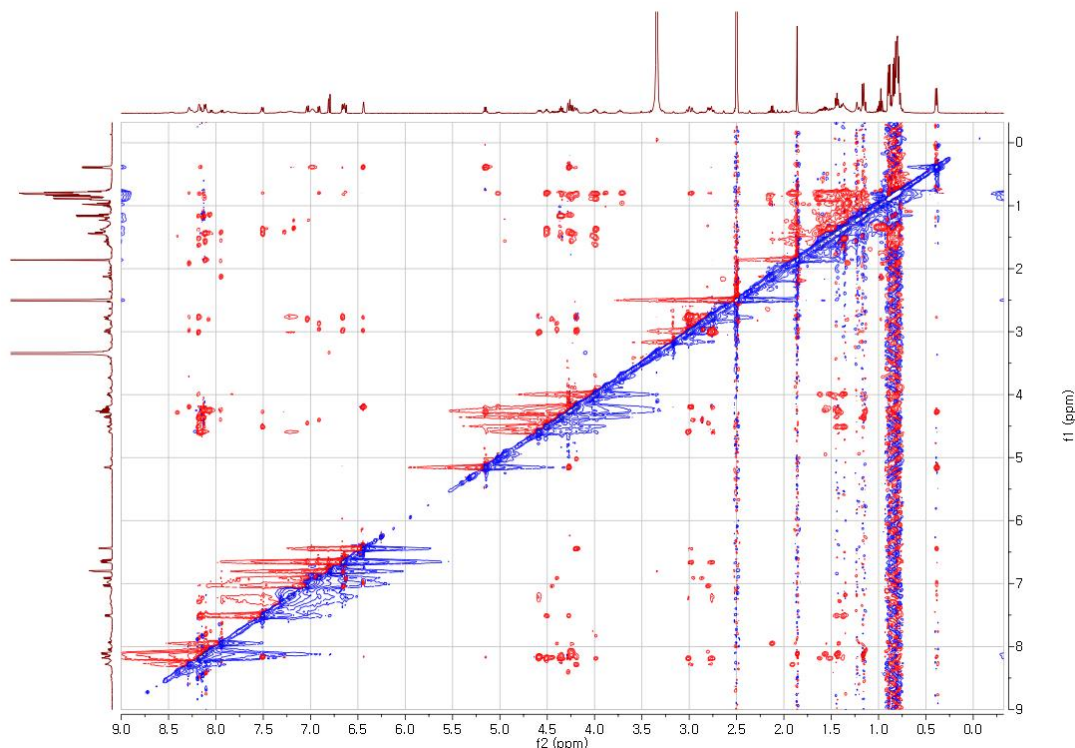


Figure S17. ROESY spectrum (600 MHz) of seongsanamide B (2) in DMSO- d_6

[Elemental Composition]
 Data : FAB-L780
 Sample: 1
 Note : m-NBA
 Inlet : Direct
 RT : 0.10 min
 Elements : C 100/0, H 100/0, N 10/0, O 15/5
 Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000
 Unsaturation (U.S.) : -0.5 - 100.0
 Date : 28-Apr-2015 15:38
 Ion Mode : FAB+
 Scan#: (1,10)

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
991.5504	100.0	+1.8 / +1.8	31.5	C 63 H 71 N 6 O 5
		+0.5 / +0.5	31.0	C 65 H 73 N 3 O 6
		-5.4 / -5.4	27.5	C 57 H 71 N 10 O 6
		-0.9 / -0.9	30.5	C 67 H 75 O 7
		-6.8 / -6.7	27.0	C 59 H 73 N 7 O 7
		+5.9 / +5.8	27.5	C 58 H 71 N 8 O 7
		-8.1 / -8.1	26.5	C 61 H 75 N 4 O 8
		+4.5 / +4.5	27.0	C 60 H 73 N 5 O 8
		-9.5 / -9.4	26.0	C 63 H 77 N O 9
		+3.2 / +3.2	26.5	C 62 H 75 N 2 O 9
		-2.7 / -2.7	23.0	C 54 H 73 N 9 O 9
		+9.9 / +9.9	23.5	C 53 H 71 N 10 O 9
		-4.1 / -4.1	22.5	C 56 H 75 N 6 O 10
		+8.6 / +8.5	23.0	C 55 H 73 N 7 O 10
		-5.4 / -5.4	22.0	C 58 H 77 N 3 O 11
		+7.2 / +7.2	22.5	C 57 H 75 N 4 O 11
		-6.8 / -6.7	21.5	C 60 H 79 O 12
		+5.9 / +5.8	22.0	C 59 H 77 N O 12
		+0.0 / +0.0	18.5	C 51 H 75 N 8 O 12
		-1.4 / -1.4	18.0	C 53 H 77 N 5 O 13
		-2.7 / -2.7	17.5	C 55 H 79 N 2 O 14
		+9.9 / +9.9	18.0	C 54 H 77 N 3 O 14
		-8.7 / -8.6	14.0	C 47 H 77 N 9 O 14
		+4.0 / +4.0	14.5	C 46 H 75 N 10 O 14
		+8.6 / +8.5	17.5	C 56 H 79 O 15
		+2.7 / +2.6	14.0	C 48 H 77 N 7 O 15

Figure S18. HR-FAB-MS data of 2

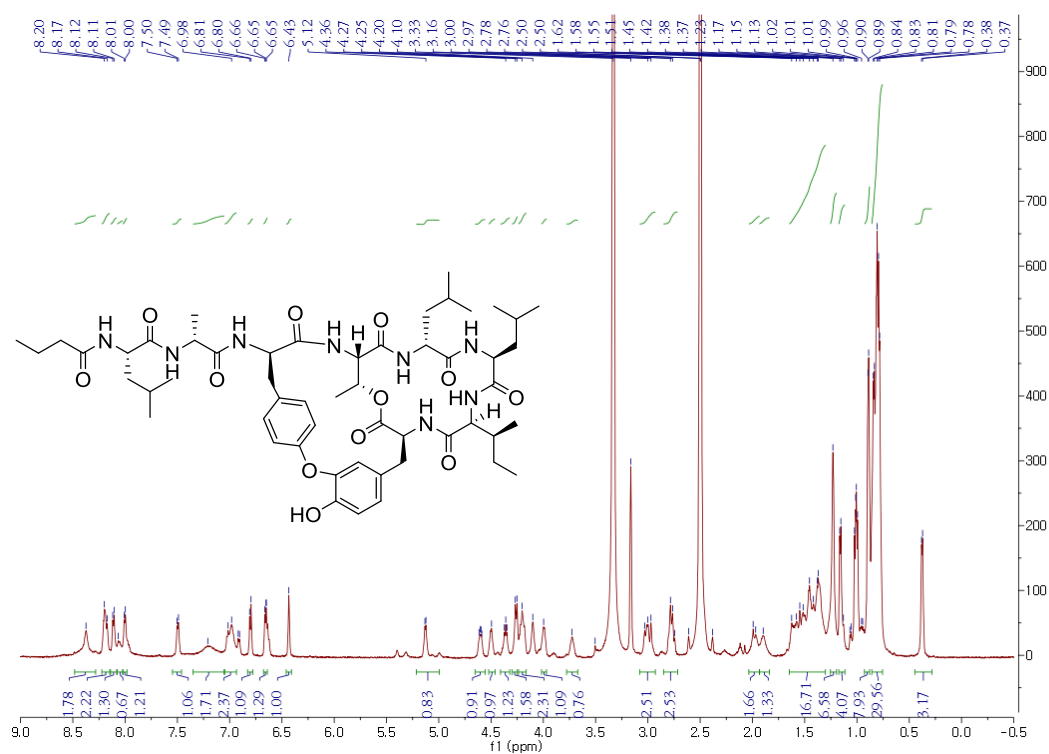


Figure S19. ^1H NMR spectrum (600 MHz) of seongsanamide C (3) in $\text{DMSO}-d_6$

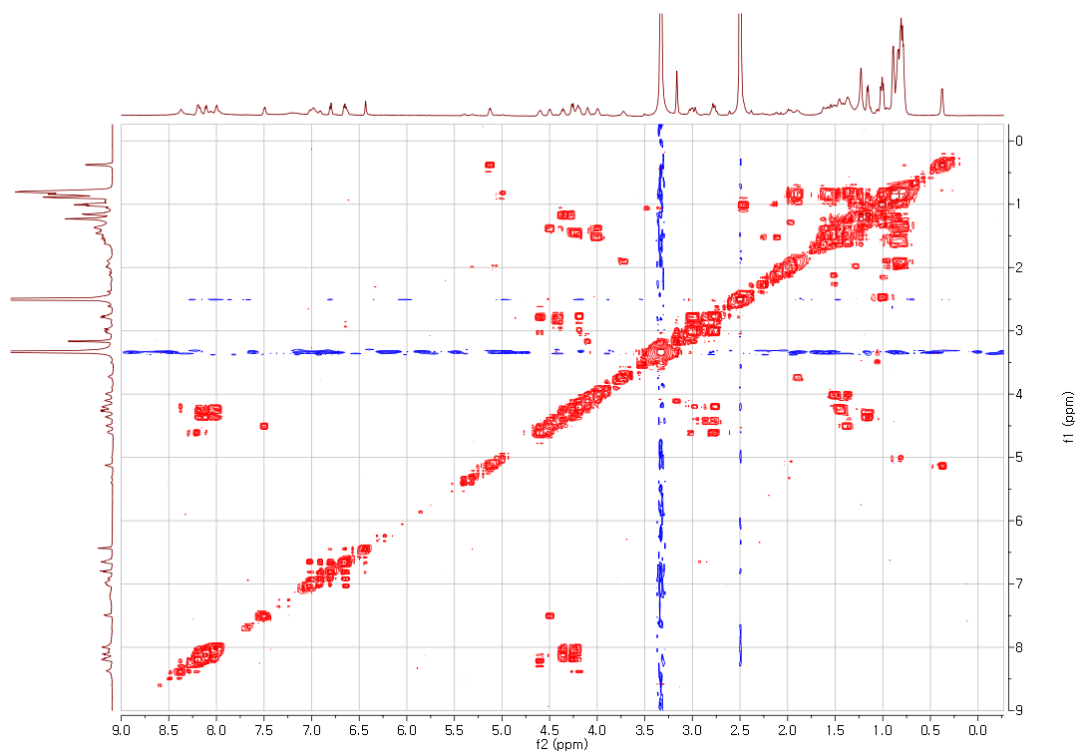


Figure S20. COSY spectrum (600 MHz) of seongsanamide C (3) in $\text{DMSO}-d_6$

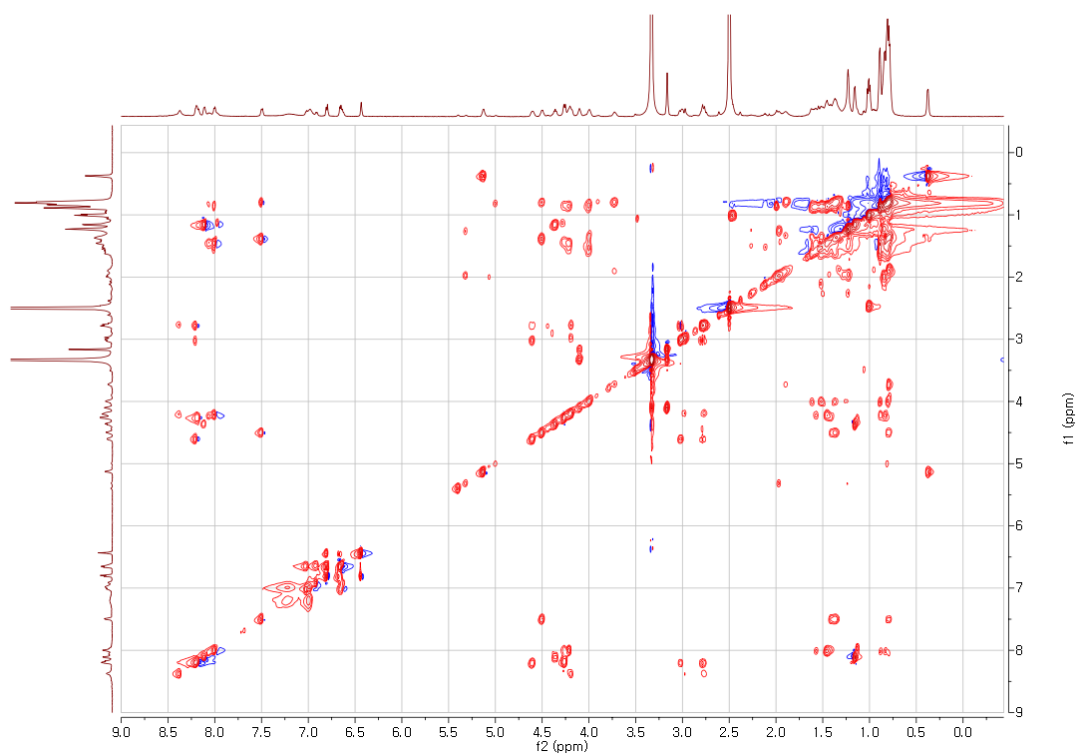


Figure S21. TOCSY spectrum (600 MHz) of seongsanamide C (**3**) in DMSO- d_6



Figure S22. HSQC spectrum (600 MHz) of seongsanamide C (**3**) in DMSO- d_6

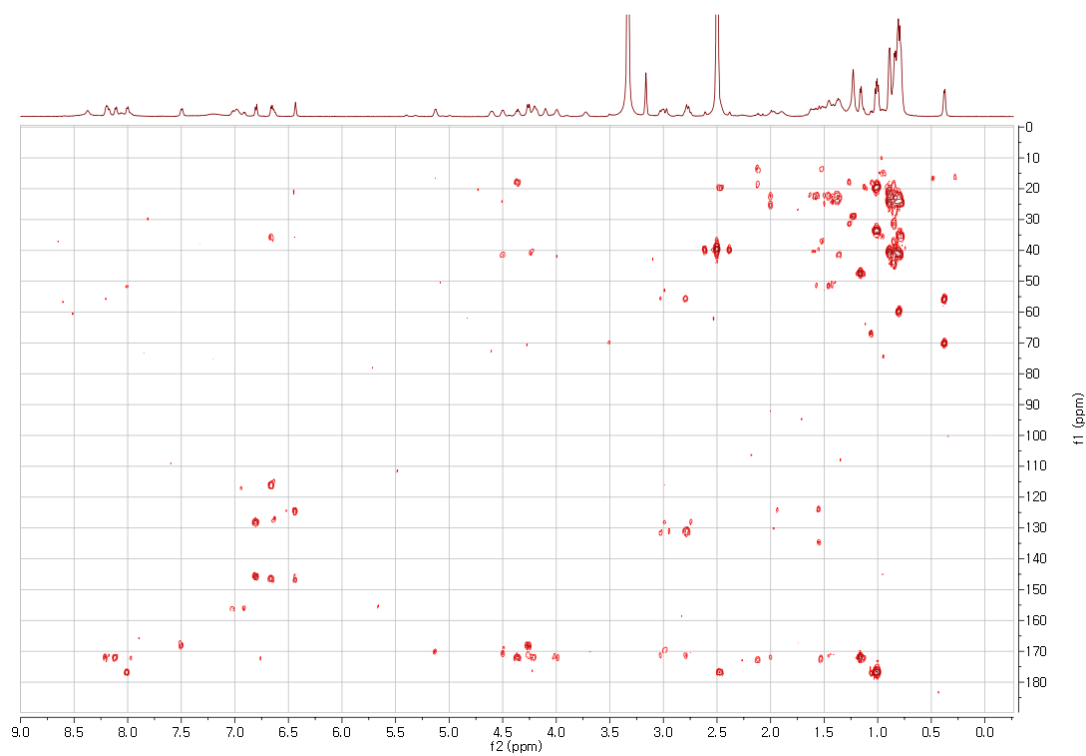


Figure S23. HMBC spectrum (600 MHz) of seongsanamide C (**3**) in DMSO- d_6

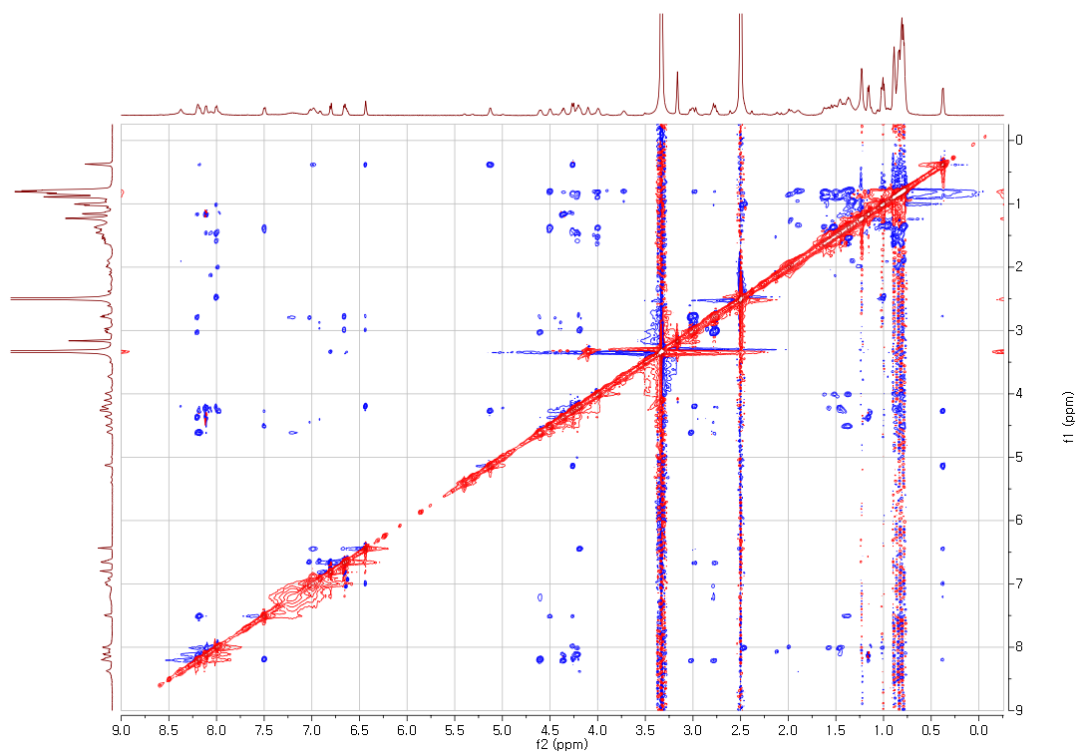


Figure S24. ROESY spectrum (600 MHz) of seongsanamide C (**3**) in DMSO- d_6

[Elemental Composition]
 Data : FAB-L783 Date : 28-Apr-2015 15:45
 Sample: 3
 Note : m-NBA
 Inlet : Direct Ion Mode : FAB+
 RT : 0.21 min Scan#: (4,17)
 Elements : C 100/0, H 100/0, N 10/0, O 15/5
 Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000
 Unsaturation (U.S.) : -0.5 - 100.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
1019.5811	90.4	+1.2 / +1.2	31.5	C 65 H 75 N 6 O 5
		-0.1 / -0.1	31.0	C 67 H 77 N 3 O 6
		-5.9 / -6.0	27.5	C 59 H 75 N 10 O 6
		-1.4 / -1.5	30.5	C 69 H 79 O 7
		-7.2 / -7.3	27.0	C 61 H 77 N 7 O 7
		+5.1 / +5.2	27.5	C 60 H 75 N 8 O 7
		-8.5 / -8.7	26.5	C 63 H 79 N 4 O 8
		+3.8 / +3.9	27.0	C 62 H 77 N 5 O 8
		-9.8 / -10.0	26.0	C 65 H 81 N O 9
		+2.5 / +2.6	26.5	C 64 H 79 N 2 O 9
		-3.2 / -3.3	23.0	C 56 H 77 N 9 O 9
		+9.1 / +9.3	23.5	C 55 H 75 N 10 O 9
		-4.6 / -4.7	22.5	C 58 H 79 N 6 O 10
		+7.8 / +7.9	23.0	C 57 H 77 N 7 O 10
		-5.9 / -6.0	22.0	C 60 H 81 N 3 O 11
		+6.5 / +6.6	22.5	C 59 H 79 N 4 O 11
		-7.2 / -7.3	21.5	C 62 H 83 O 12
		+5.1 / +5.2	22.0	C 61 H 81 N O 12
		-0.6 / -0.6	18.5	C 53 H 79 N 8 O 12
		-1.9 / -2.0	18.0	C 55 H 81 N 5 O 13
		-3.3 / -3.3	17.5	C 57 H 83 N 2 O 14
		+9.1 / +9.3	18.0	C 56 H 81 N 3 O 14
		-9.0 / -9.2	14.0	C 49 H 81 N 9 O 14
		+3.3 / +3.4	14.5	C 48 H 79 N 10 O 14
		+7.8 / +7.9	17.5	C 58 H 83 O 15
		+2.0 / +2.1	14.0	C 50 H 81 N 7 O 15

Figure S25. HR-FAB-MS data of 3

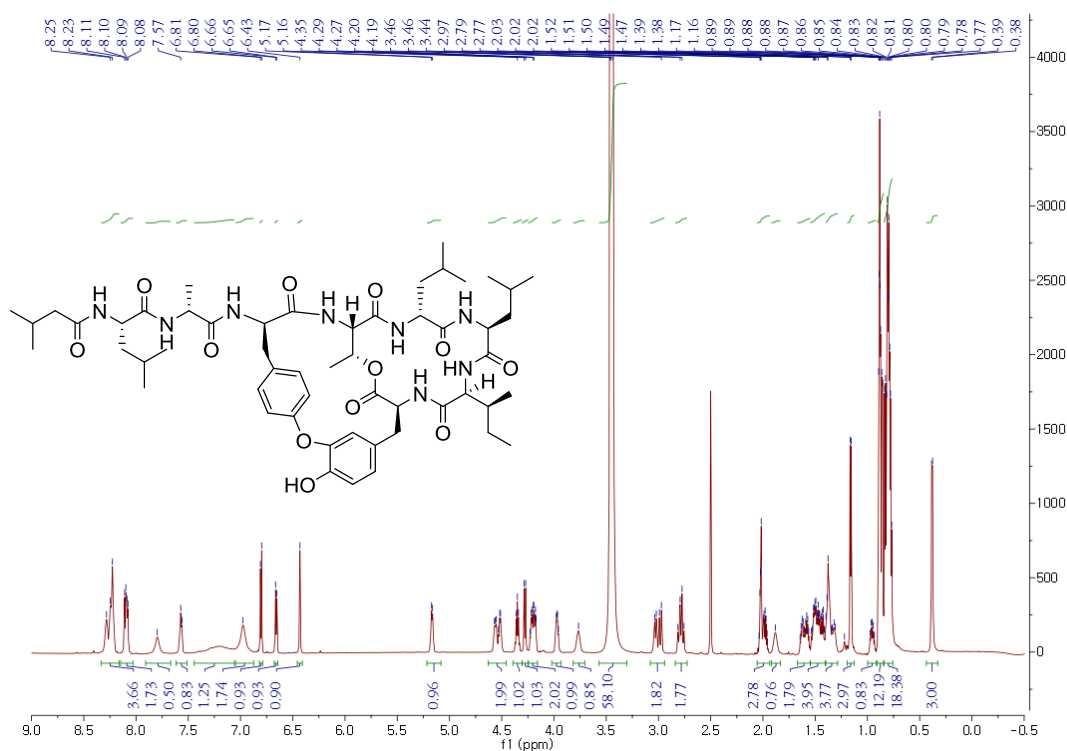


Figure S26. ^1H NMR spectrum (700 MHz) of seongsanamide D (4) in $\text{DMSO}-d_6$

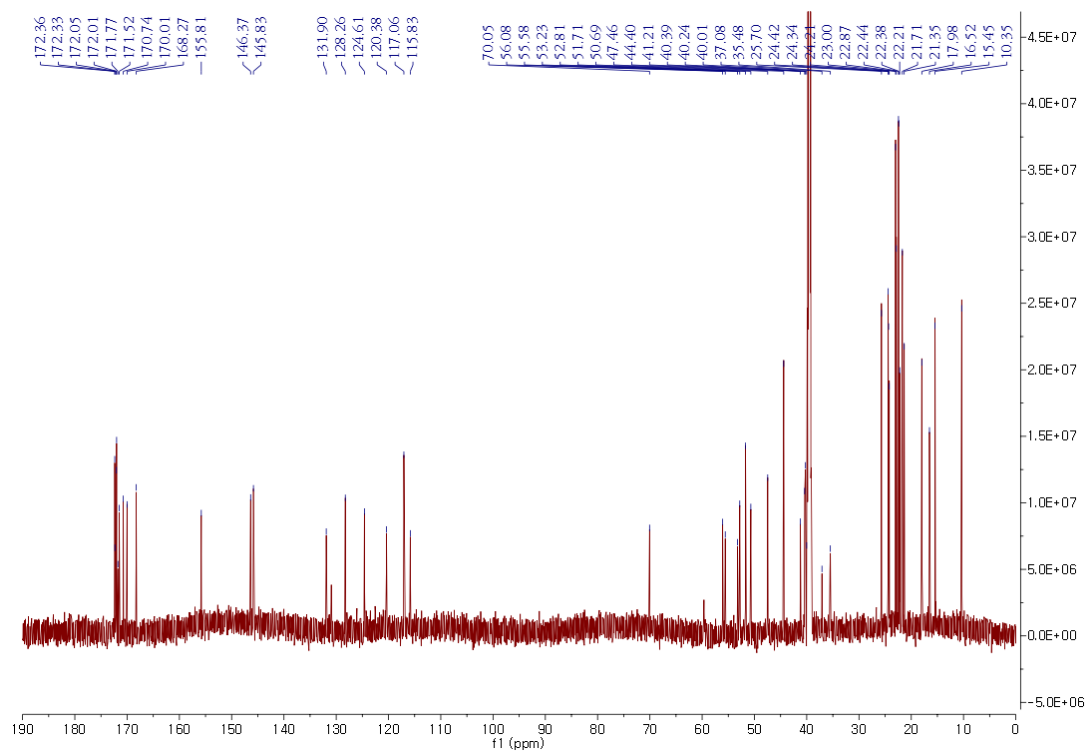


Figure S27. ^{13}C NMR spectrum (175 MHz) of seongsanamide D (**4**) in $\text{DMSO}-d_6$

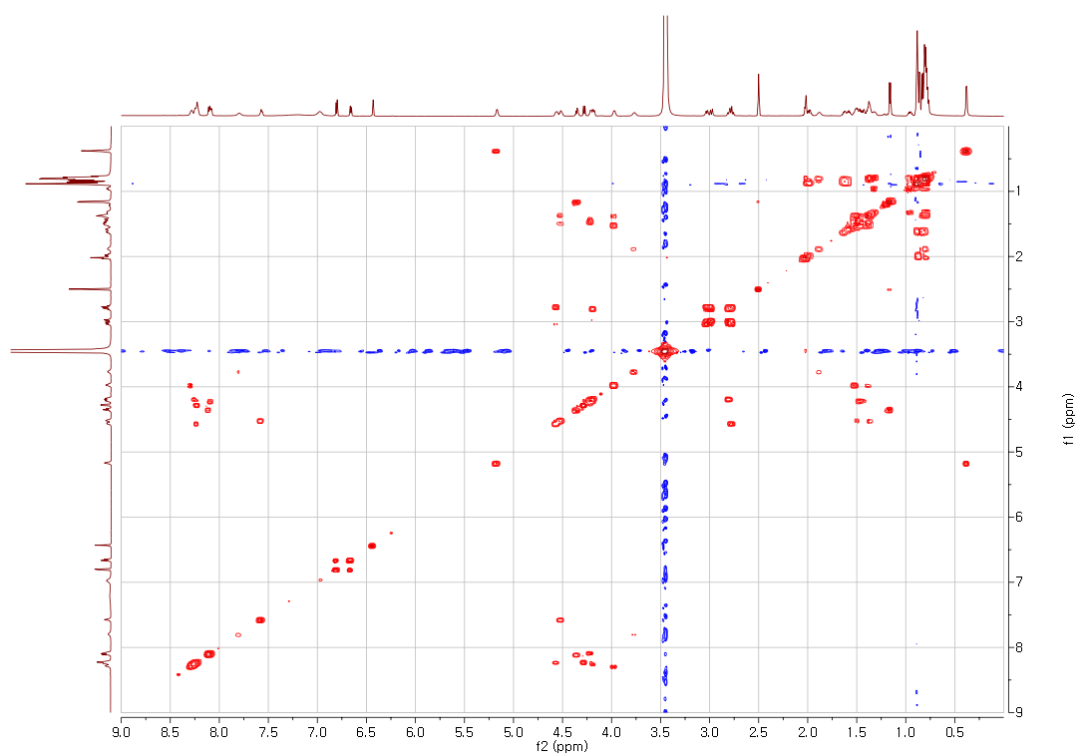


Figure S28. COSY spectrum (700 MHz) of seongsanamide D (**4**) in $\text{DMSO}-d_6$

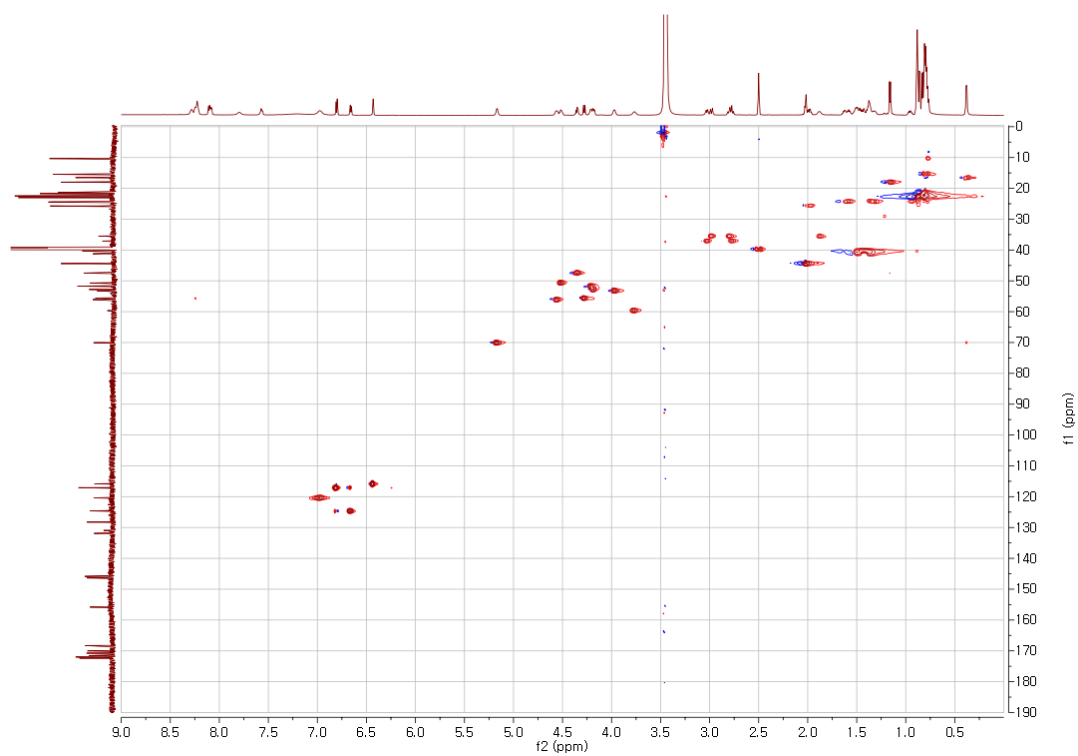


Figure S29. HSQC spectrum (700 MHz) of seongsanamide D (**4**) in DMSO- d_6

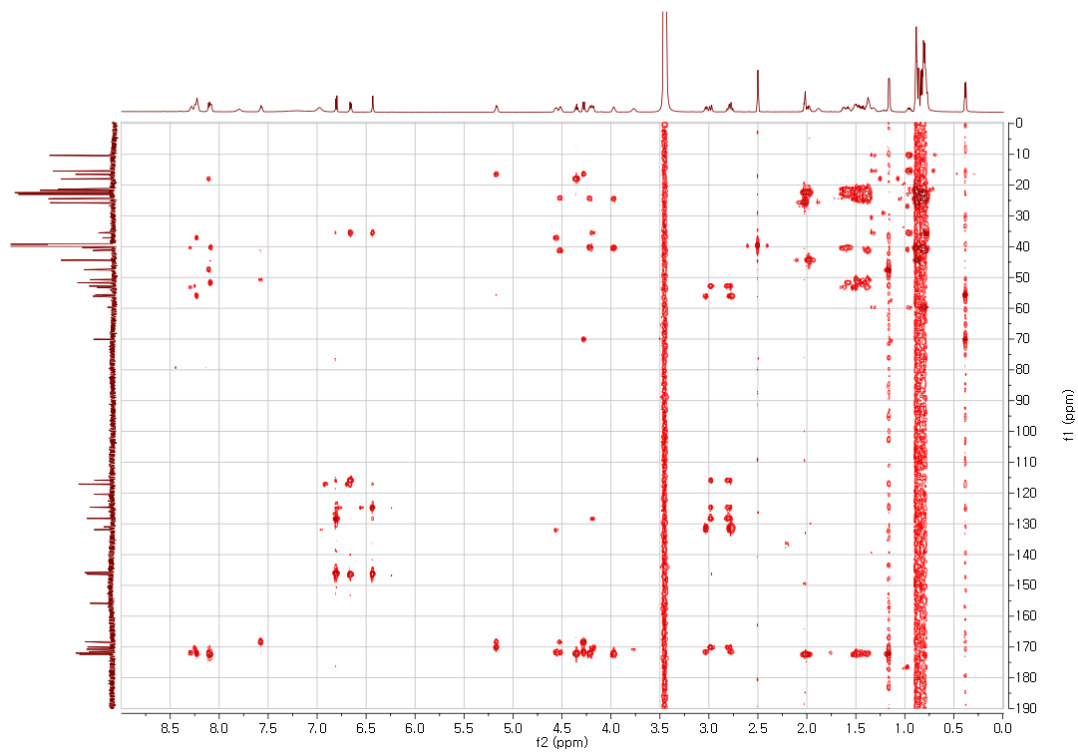


Figure S30. HMBC spectrum (700 MHz) of seongsanamide D (**4**) in DMSO- d_6

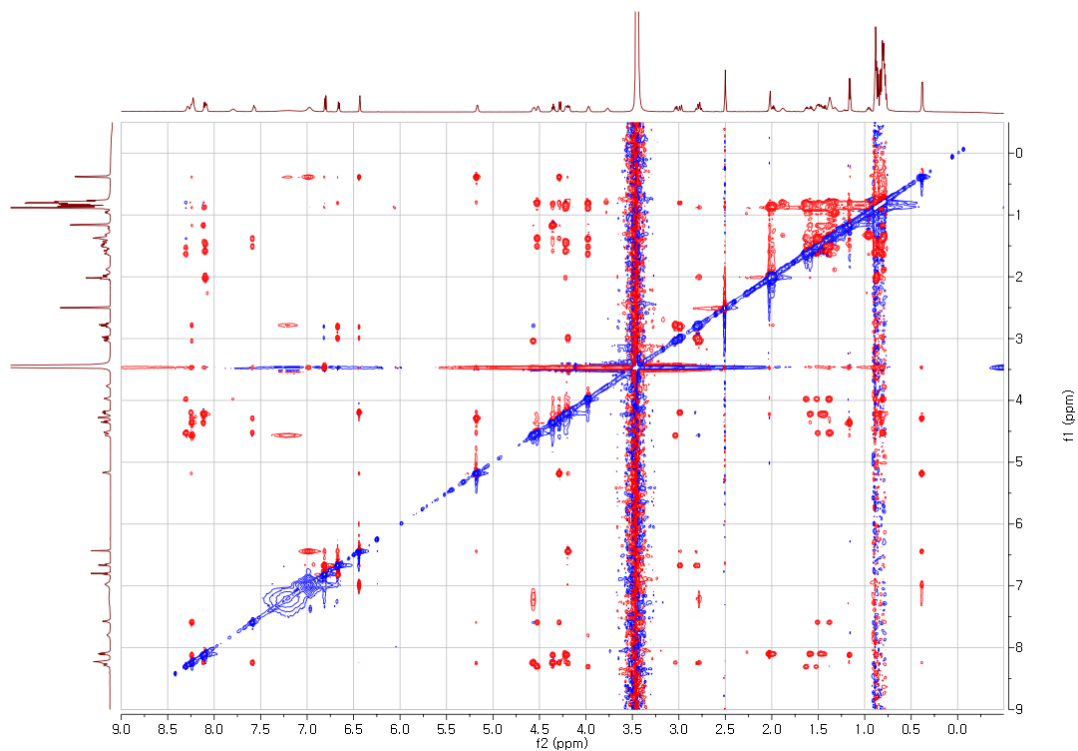


Figure S31. ROESY spectrum (700 MHz) of seongsanamide D (**4**) in DMSO- d_6

```
[ Elemental Composition ]
Data : FAB-L784
Sample: 4
Note : m-NBA
Inlet : Direct
RT : 0.66 min
Elements : C 100/0, H 100/0, N 10/0, O 15/5
Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000
Unsaturation (U.S.) : -0.5 - 100.0
Date : 28-Apr-2015 15:50
Ion Mode : FAB+
Scan#: (1,62)
```

Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
1033.5964	100.0	+0.9 / +0.9	31.5	C 66 H 77 N 6 O 5
		-0.4 / -0.5	31.0	C 68 H 79 N 3 O 6
		-6.1 / -6.3	27.5	C 60 H 77 N 10 O 6
		-1.7 / -1.8	30.5	C 70 H 81 O 7
		-7.4 / -7.7	27.0	C 62 H 79 N 7 O 7
		+4.8 / +4.9	27.5	C 61 H 77 N 8 O 7
		-8.7 / -9.0	26.5	C 64 H 81 N 4 O 8
		+3.5 / +3.6	27.0	C 63 H 79 N 5 O 8
		+2.2 / +2.2	26.5	C 65 H 81 N 2 O 9
		-3.5 / -3.6	23.0	C 57 H 79 N 9 O 9
		+8.6 / +8.9	23.5	C 56 H 77 N 10 O 9
		-4.8 / -5.0	22.5	C 59 H 81 N 6 O 10
		+7.3 / +7.6	23.0	C 58 H 79 N 7 O 10
		-6.1 / -6.3	22.0	C 61 H 83 N 3 O 11
		+6.0 / +6.3	22.5	C 60 H 81 N 4 O 11
		-7.4 / -7.7	21.5	C 63 H 85 O 12
		+4.7 / +4.9	22.0	C 62 H 83 N O 12
		-0.9 / -1.0	18.5	C 54 H 81 N 8 O 12
		-2.2 / -2.3	18.0	C 56 H 83 N 5 O 13
		+9.9 / +10.3	18.5	C 55 H 81 N 6 O 13
		-3.5 / -3.6	17.5	C 58 H 85 N 2 O 14
		+8.6 / +8.9	18.0	C 57 H 83 N 3 O 14
		-9.2 / -9.5	14.0	C 50 H 83 N 9 O 14
		+3.0 / +3.1	14.5	C 49 H 81 N 10 O 14
		+7.3 / +7.6	17.5	C 59 H 85 O 15
		+1.7 / +1.7	14.0	C 51 H 83 N 7 O 15

Figure S32. HR-FAB-MS data of **4**

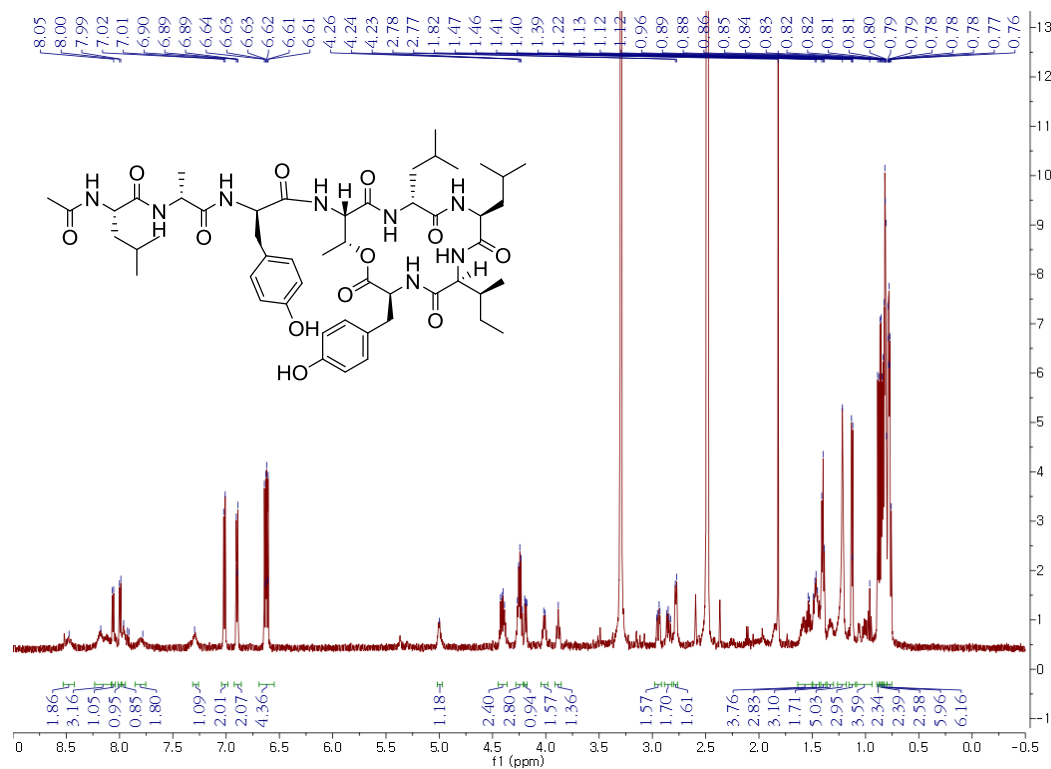


Figure S33. ^1H NMR spectrum (600 MHz) of seongsanamide E (5) in $\text{DMSO-}d_6$

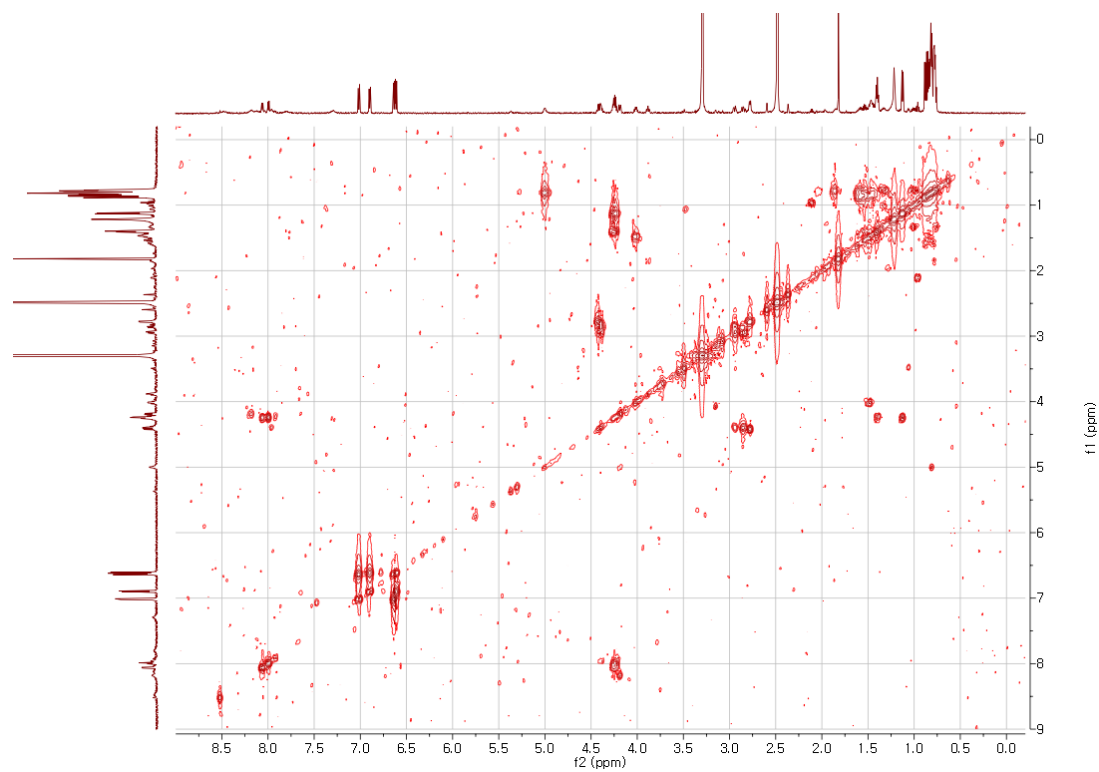


Figure S34. COSY spectrum (600 MHz) of seongsanamide E (5) in $\text{DMSO-}d_6$

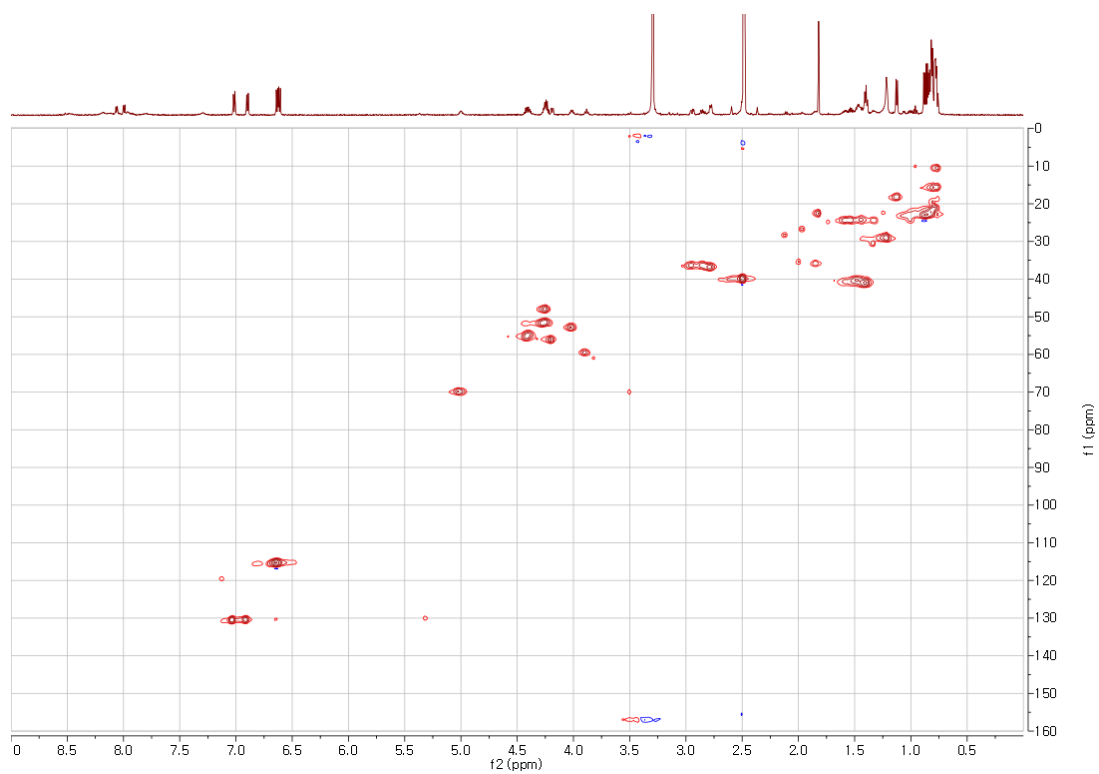


Figure S35. HMQC spectrum (700 MHz) of seongsanamide E (5) in DMSO- d_6



Figure S36. HMBC spectrum (700 MHz) of seongsanamide E (5) in DMSO- d_6

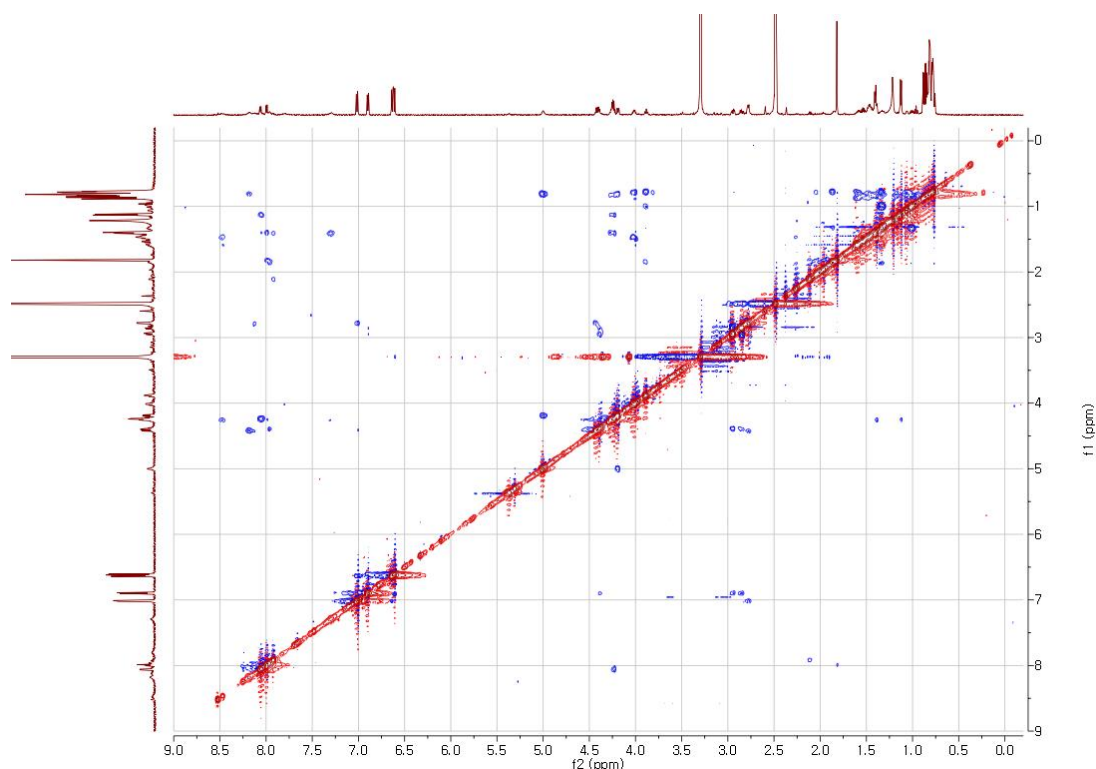


Figure S37. ROESY spectrum (600 MHz) of seongsanamide E (5) in DMSO- d_6

[Elemental Composition]

Data : FAB-L777 Date : 28-Apr-2015 15:21

Sample: 5

Note : m-NBA

Inlet : Direct Ion Mode : FAB+

RT : 3.85 min Scan#: (177,180)

Elements : C 100/0, H 100/0, N 10/0, O 15/5

Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000

Unsaturation (U.S.) : -0.5 - 100.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
993.5663	100.0	+2.1 / +2.0	30.5	C 63 H 73 N 6 O 5
		+0.7 / +0.7	30.0	C 65 H 75 N 3 O 6
		-5.2 / -5.2	26.5	C 57 H 73 N 10 O 6
		-0.7 / -0.6	29.5	C 67 H 77 O 7
		-6.6 / -6.5	26.0	C 59 H 75 N 7 O 7
		+6.1 / +6.1	26.5	C 58 H 73 N 8 O 7
		-7.9 / -7.9	25.5	C 61 H 77 N 4 O 8
		+4.7 / +4.7	26.0	C 60 H 75 N 5 O 8
		-9.3 / -9.2	25.0	C 63 H 79 N O 9
		+3.4 / +3.4	25.5	C 62 H 77 N 2 O 9
		-2.5 / -2.5	22.0	C 54 H 75 N 9 O 9
		-3.9 / -3.8	21.5	C 56 H 77 N 6 O 10
		+8.8 / +8.7	22.0	C 55 H 75 N 7 O 10
		-5.2 / -5.2	21.0	C 58 H 79 N 3 O 11
		+7.4 / +7.4	21.5	C 57 H 77 N 4 O 11
		-6.6 / -6.5	20.5	C 60 H 81 O 12
		+6.1 / +6.1	21.0	C 59 H 79 N O 12
		+0.2 / +0.2	17.5	C 51 H 77 N 8 O 12
		-1.2 / -1.2	17.0	C 53 H 79 N 5 O 13
		-2.5 / -2.5	16.5	C 55 H 81 N 2 O 14
		-8.4 / -8.4	13.0	C 47 H 79 N 9 O 14
		+4.2 / +4.2	13.5	C 46 H 77 N 10 O 14
		+8.8 / +8.7	16.5	C 56 H 81 O 15
		-9.8 / -9.7	12.5	C 49 H 81 N 6 O 15
		+2.9 / +2.9	13.0	C 48 H 79 N 7 O 15

Figure S38. HR-FAB-MS data of 5

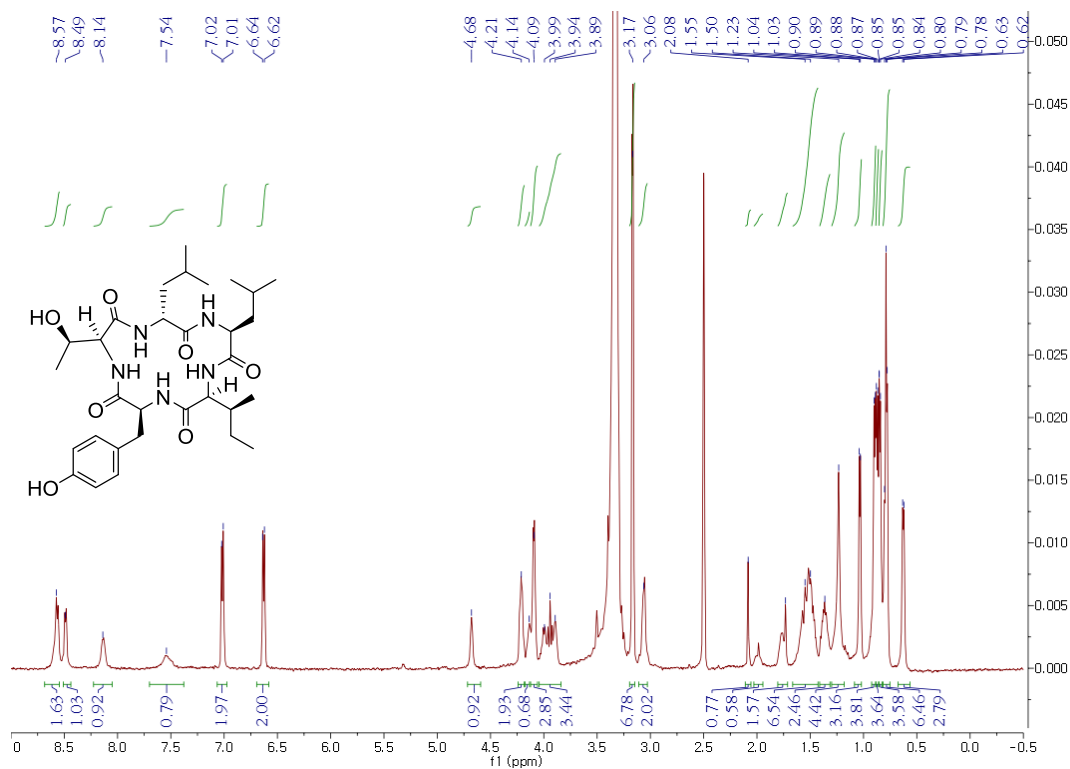


Figure S39. ¹H NMR spectrum (500 MHz) of seongsanamide F (6) in DMSO-*d*₆



Figure S40. COSY spectrum (700 MHz) of seongsanamide F (6) in DMSO-*d*₆

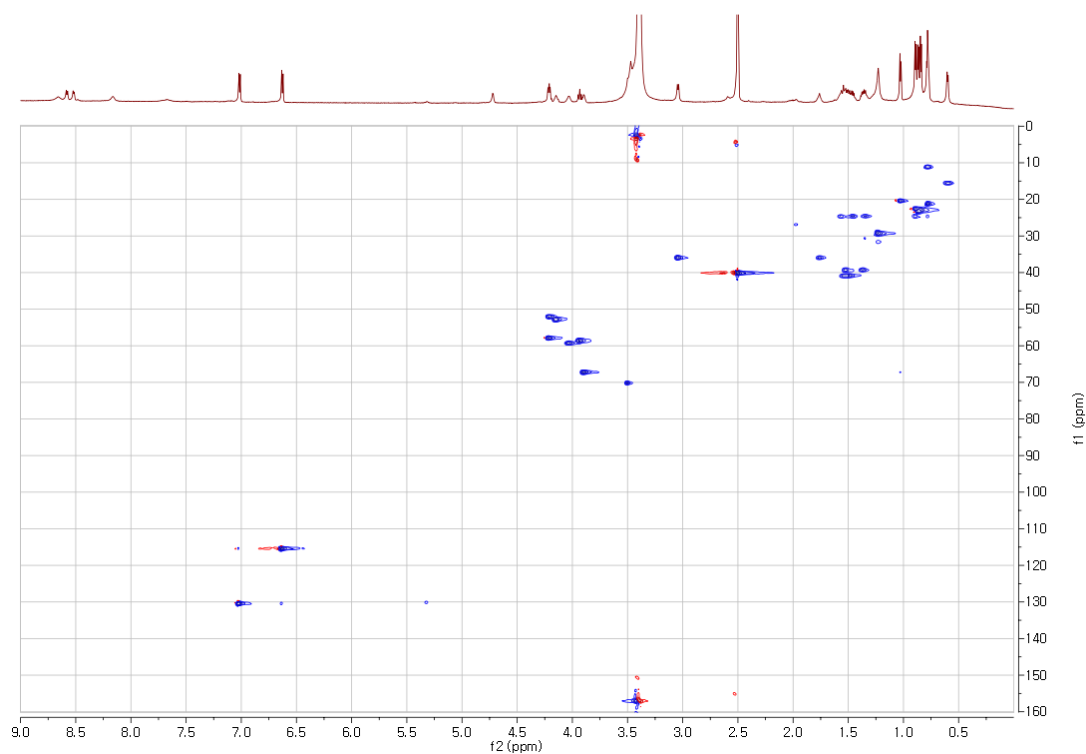


Figure S41. HMQC spectrum (700 MHz) of seongsanamide F (**6**) in DMSO- d_6

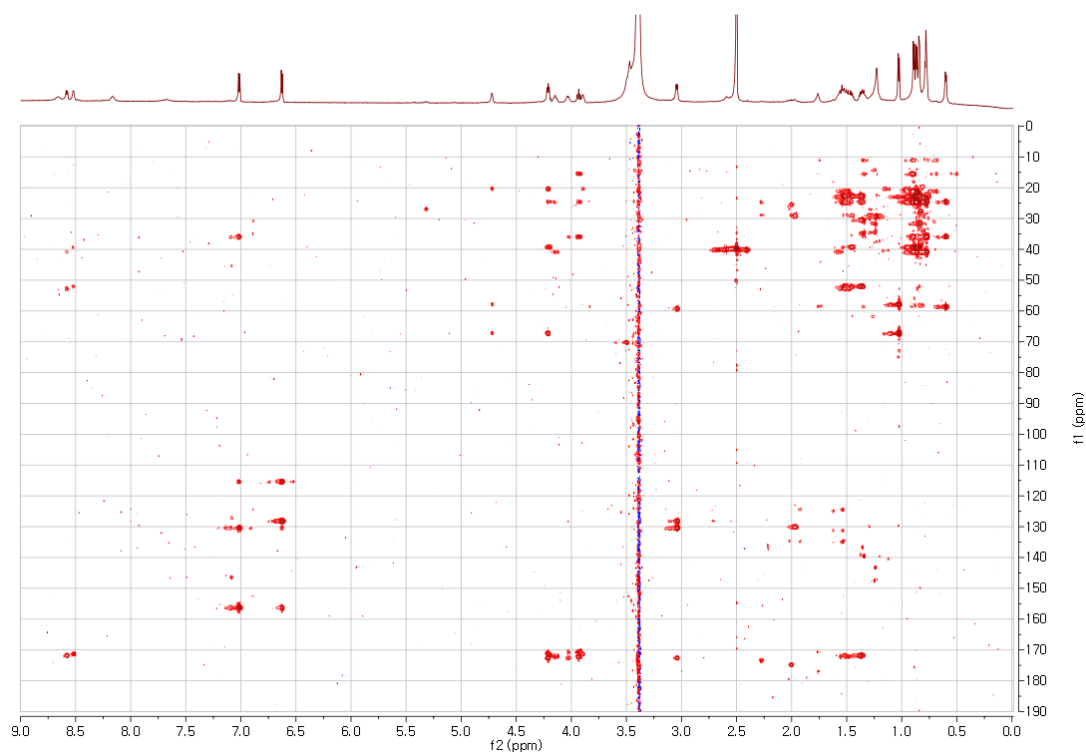


Figure S42. HMBC spectrum (700 MHz) of seongsanamide F (**6**) in DMSO- d_6

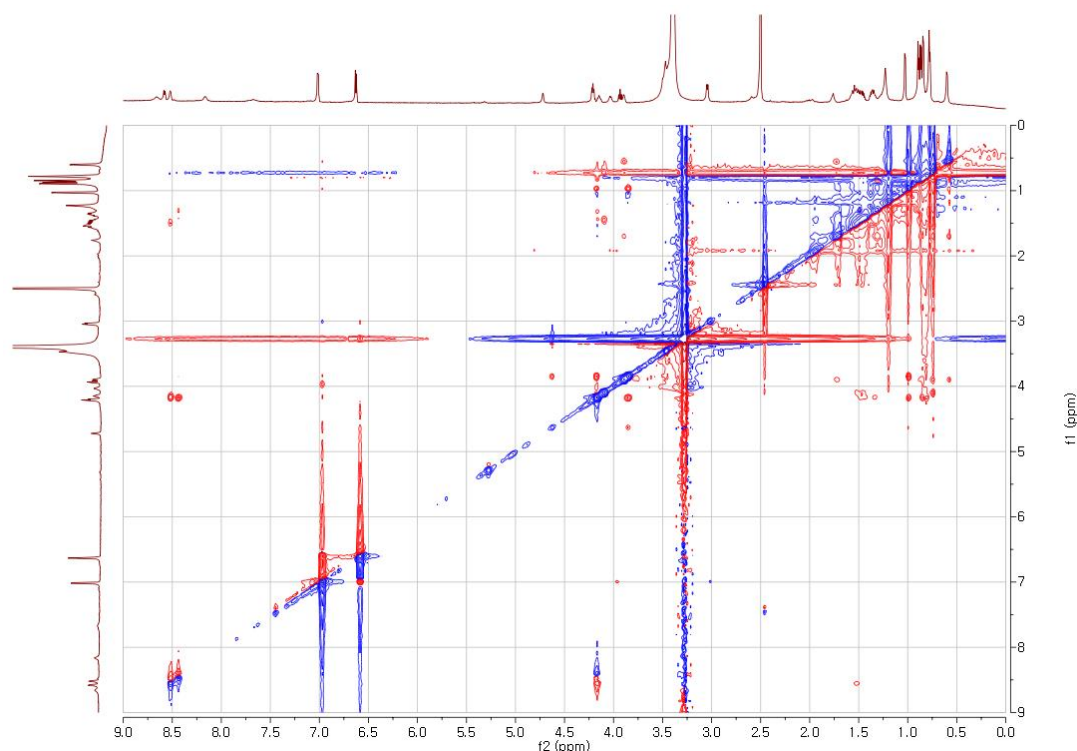


Figure S43. ROESY spectrum (700 MHz) of seongsanamide F (6) in DMSO- d_6

[Elemental Composition]
 Data : FAB-L775
 Sample: 6
 Note : m-NBA
 Inlet : Direct
 RT : 0.27 min
 Elements : C 100/0, H 100/0, N 10/0, O 10/0
 Mass Tolerance : 10ppm, 5mmu if m/z < 500, 20mmu if m/z > 2000
 Unsaturation (U.S.) : -0.5 - 100.0
 Date : 28-Apr-2015 15:08
 Ion Mode : FAB+
 Scan#: (1,26)

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
604.3712	100.0	+3.3 / +2.0	22.5	C 43 H 46 N 3
		-6.4 / -3.9	19.0	C 35 H 44 N 10
		+1.1 / +0.6	22.0	C 45 H 48 O
		-8.7 / -5.2	18.5	C 37 H 46 N 7 O
		+9.9 / +6.0	18.5	C 38 H 46 N 5 O 2
		+7.7 / +4.7	18.0	C 40 H 48 N 2 O 3
		-2.0 / -1.2	14.5	C 32 H 46 N 9 O 3
		-4.2 / -2.5	14.0	C 34 H 48 N 6 O 4
		-6.4 / -3.9	13.5	C 36 H 50 N 3 O 5
		-8.7 / -5.2	13.0	C 38 H 52 O 6
		+2.4 / +1.5	10.0	C 29 H 48 N 8 O 6
		+0.2 / +0.1	9.5	C 31 H 50 N 5 O 7
		-2.0 / -1.2	9.0	C 33 H 52 N 2 O 8
		+9.1 / +5.5	6.0	C 24 H 48 N 10 O 8
		+6.9 / +4.2	5.5	C 26 H 50 N 7 O 9
		+4.7 / +2.8	5.0	C 28 H 52 N 4 O 10

Figure S44. HR-FAB-MS data of 6

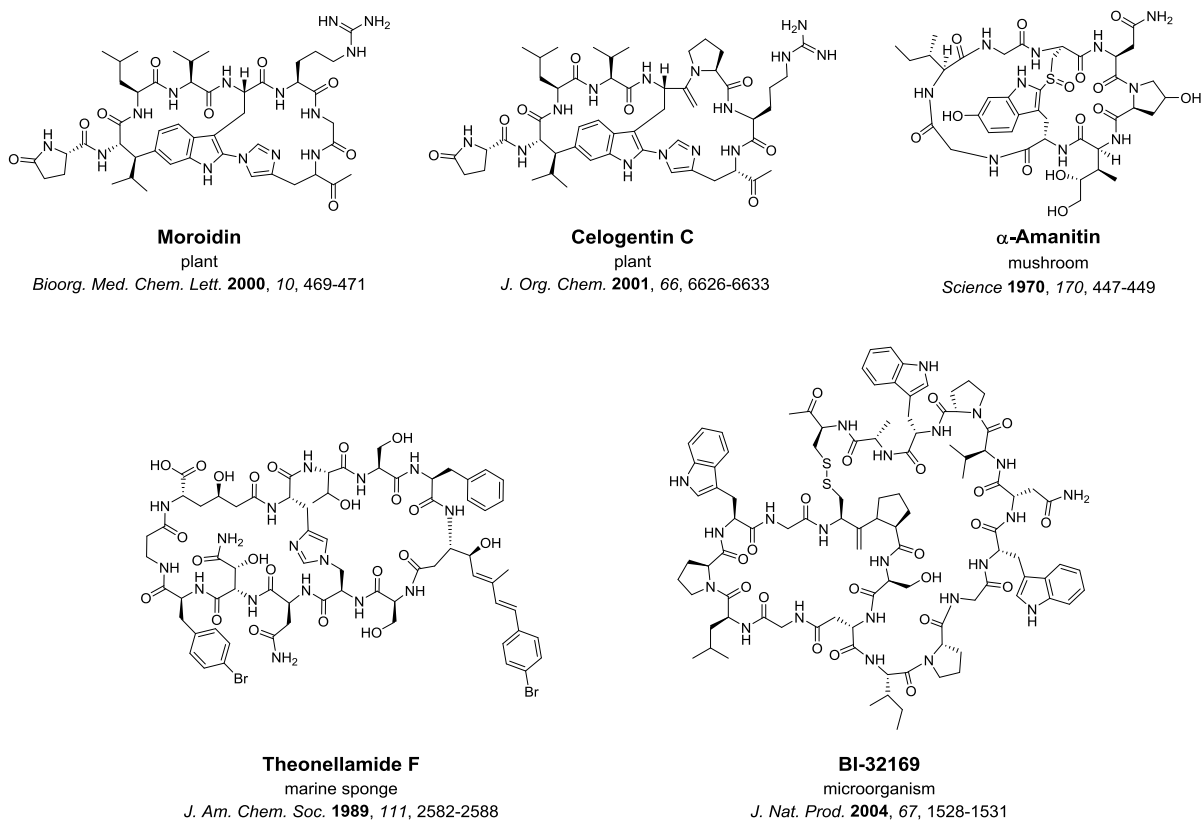


Figure S45. Previously reported bicyclic peptides isolated from natural sources

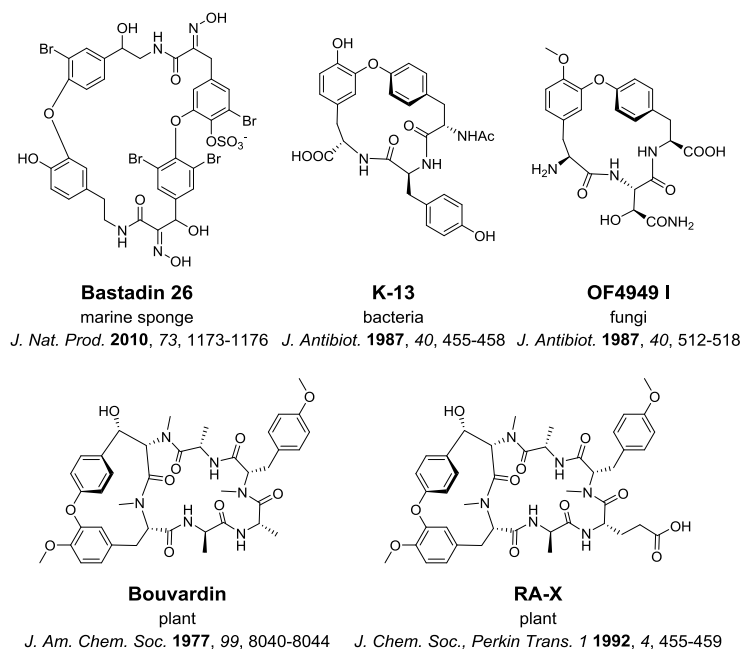


Figure S46. Previously reported monocyclic and bicyclic peptides containing isodityrosine residue