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The effect of sulfur stereochemistry of L-β,β-dimethylmethionine *S*-oxide on the physicochemical properties of truncated polytheonamides

Shigeru Matsuoka, Yuki Mizoguchi, Hiroaki Itoh, Ken Okura, Naoki Shinohara, Masayuki Inoue *

Graduate School of Pharmaceutical Sciences, The University of Tokyo, and PRESTO, Japan Science and Technology Agency, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 0033, Japan

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ABSTRACT

L-β,β-Dimethylmethionine *S*-oxide is a unique amino acid found in polytheonamides. Four designed hexapeptides containing the sulfide, (S_R)-sulfoxide, (S_S)-sulfoxide, and sulfone derivatives of L-dimethylmethionine were synthesized and functionally analyzed. Our results indicate that the sulfoxide stereochemistry of the peptides controls their overall physicochemical properties.

Polytheonamides A (**1**) and B (**2**), isolated from the marine sponge *Theonella swinhoei*, exhibit antitumor activities against P388 murine leukemia cells at concentrations of less than 100 pg/mL.[1,2] Compounds **1** and **2** are the largest non-ribosomal peptides identified to date (Fig. 1).[3] Their linear 48 residues with alternating D- and L-amino acids include 13 non-proteinogenic amino acids and an N-terminal cap. The sequences are also rich in β-branched amino acids, which are generally known to contribute to low conformational flexibility of the peptide backbone.[4,5]

Among the non-proteinogenic amino acid residues, the 44th L-β,β-dimethylmethionine *S*-oxide [L-Me_2Met(O)] is a unique amino acid only found in polytheonamides. Sulfoxides are a stereogenic functionality, and compounds **1** and **2** were in fact shown to be a pair of sulfoxide stereoisomers of the 44th residues.[3] Most recently, the absolute configuration of the sulfoxide of polytheonamide B (**2**) was determined to be *R* by our total synthesis.[6] Interestingly, distinct retention times for these isomers (**1**: T_r = 24.75 min; **2**: T_r = 29.03 min) were observed upon reversed-phase HPLC analyses (Inertsil C8 column with *n*-PrOH/H_2O solvent system),[6] suggesting that a single stereochemical change in the side chains affects the hydrophobicity of the entire molecule.

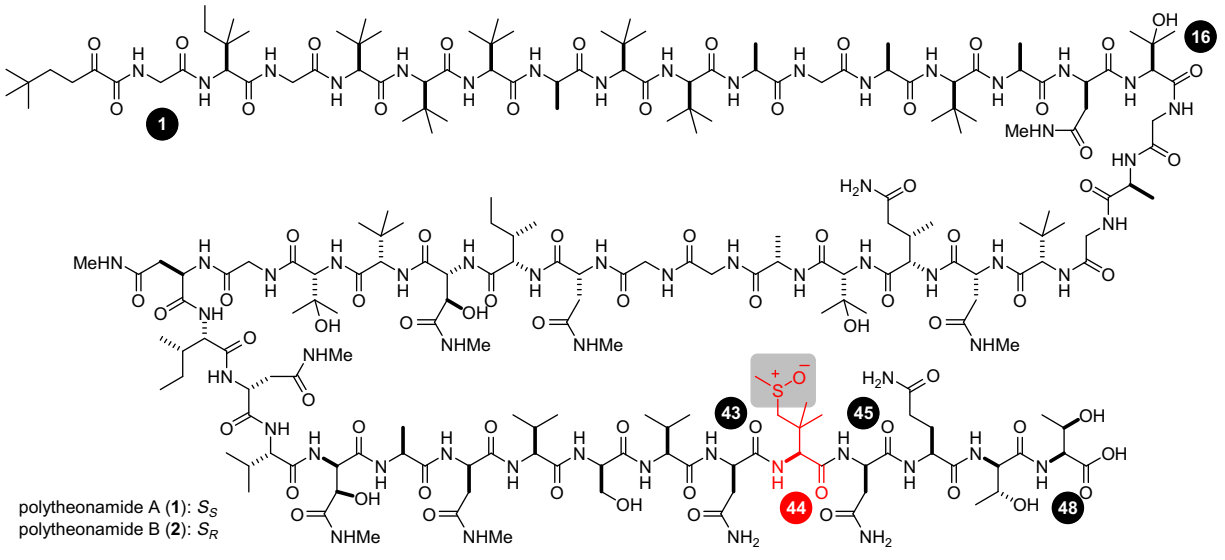

Figure 1. Structures of polytheonamides A (**1**) and B (**2**), which are sulfoxide epimers at the 44th residue. The S_R stereochemistry of **2** was determined by our total synthesis.

* Corresponding author. Tel.: +81 3 5841 1354; fax: +81 3 5841 0568.
E-mail address: inoue@mol.f.u-tokyo.ac.jp (M. Inoue).

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Oxidation of methionine to methionine *S*-oxide in proteins has been correlated with the changes in peptide conformation,[7] association properties[8–12] and biological functions,[13,14] presumably due to the marked difference in polarity. However, precise structural role of methionine *S*-oxide in such functional changes is still unclear. In this context, we are interested in the effects of the stereochemistry and oxidation state of the unique dimethylmethionine on the overall properties of the compounds. As a preliminary study, the physicochemical and biological properties of the L-Me_2Met derivatives were evaluated at both amino acid and peptide levels.

The two stereoisomers of L-Me_2Met(O) could be generated by non-stereoselective oxidation of L-β,β-dimethylmethionine (L-Me_2Met). Further oxidation of L-Me_2Met(O) gives the sulfone derivative [L-Me_2Met(O_2)]. Therefore, the 44th residue of polytheonamide is capable of generating four analogues in terms of stereochemistry and oxidation state. Accordingly, we designed, synthesized, and functionally analyzed the four protected amino acids and the four model hexapeptides, which contain the dimethylmethionine derivatives, L-Me_2Met, L-(S_R)-Me_2Met(O), L-(S_S)-Me_2Met(O), and L-Me_2Met(O_2), in their structures.

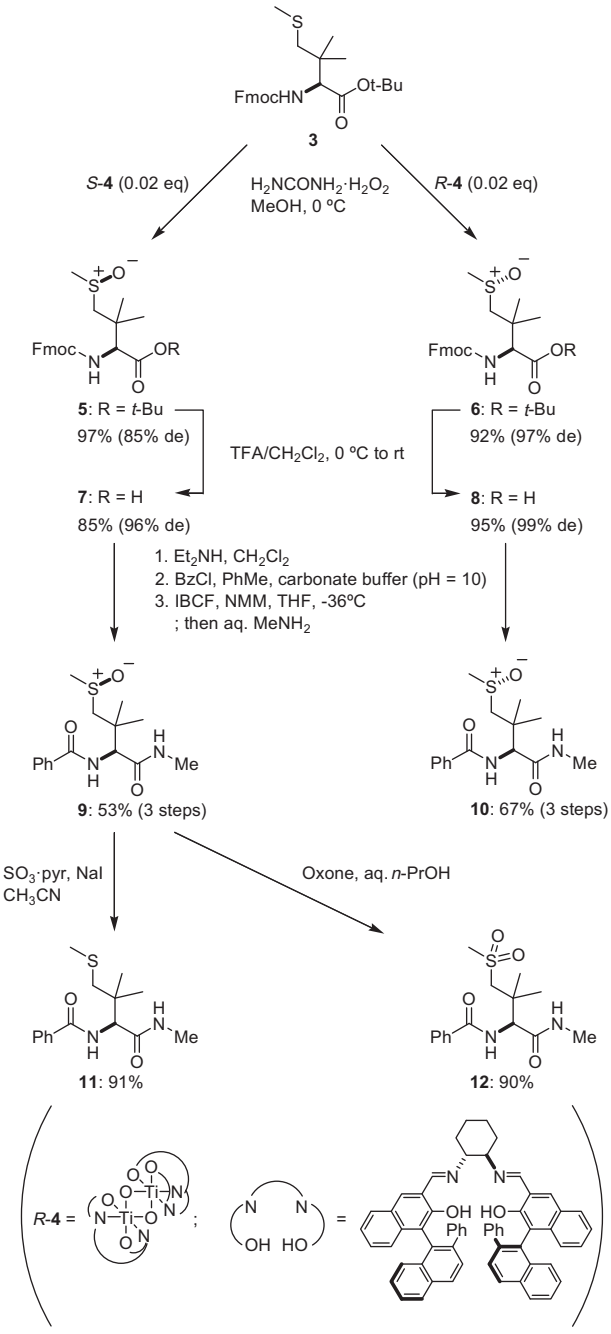
For experiments at the amino acid level, we selected an *N*-benzoyl, *C*-methylamide-capped structure to evaluate the properties of the single residual side chain. Scheme 1 shows syntheses of the four analogues of N- and C-protected L-Me_2Met with the specific sulfur stereochemistries and oxidation states. Sulfide **3**, which was previously synthesized from L-aspartic acid,[6] was stereo- and chemoselectively oxidized to (S_R)-sulfoxide **5** and (S_S)-sulfoxide **6** by using Katsuki catalysts *S*-**4** and *R*-**4**, respectively.[15] For the purpose of further derivatization, **5** and **6** were treated with TFA/CH_2Cl_2 for removal of the *t*-Bu group to afford diastereomeric carboxylic acids **7** and **8**, respectively.

Next, Fmoc-protected acids **7** and **8** were derivatized into the corresponding model amides **9** and **10**, respectively, through base-mediated Fmoc removal, Bz protection, and condensation with methyl amine. To prepare the analogues at different oxidation states, compound **9** was reduced by SO_3·pyr-NaI to generate sulfide **11**[16] or oxidized by Oxone to generate sulfone **12**.[17]

The hydrophobicities of the synthesized four protected amino acids were evaluated next. Namely, log k_w, a hydrophobic parameter,[18,19] was deduced from the retention times on reversed-phase HPLC for the four protected peptides **9**–**12**. First, each amino acid was eluted with a solvent system of CH_3CN/H_2O at three different mixing ratios. The resulting three retention times for each amino acid were then analyzed according to the method reported by Braumann to calculate its log k_w (Table 1).[19] It was found that sulfide **11** had the largest value of log k_w, and thus possessed the highest hydrophobicity among the four protected amino acids, while sulfone **12** had the lowest hydrophobicity. The log k_w difference between **9** and **10**, the sulfoxide stereoisomers, was negligible. Thus, only the oxidation states of sulfur affected the hydrophobicities of the protected mono-amino acids [sulfide > (S_R)-sulfoxide = (S_S)-sulfoxide > sulfone].

To investigate the physicochemical influence of the sulfur state on the peptide backbone, we designed four truncated hexapeptide models of polytheonamides containing the L-Me_2Met analogues (**14**–**17**, Scheme 2). The amino acid sequences of the peptides are composed of the seven units, which are characteristic structures in the polytheonamide sequence, that is, the N-terminal cap, glycine-1, L-β-hydroxyvaline-16, D-asparagine-43, an analogue of L-Me_2Met(O)-44, D-asparagine-45, and glycine as the C-terminal carboxylic acid (Fig. 1).

The hexapeptide sequences of **14** and **15**, bearing L-(S_R)-Me_2Met(O) and L-(S_S)-Me_2Met(O), respectively, were synthesized from commercially available Fmoc-Gly-Wang resin (**13**) by Fmoc chemistry on an automatic solid-phase peptide synthesizer.[20] After elongation of the peptide using the corresponding *N*-Fmoc-amino acids,



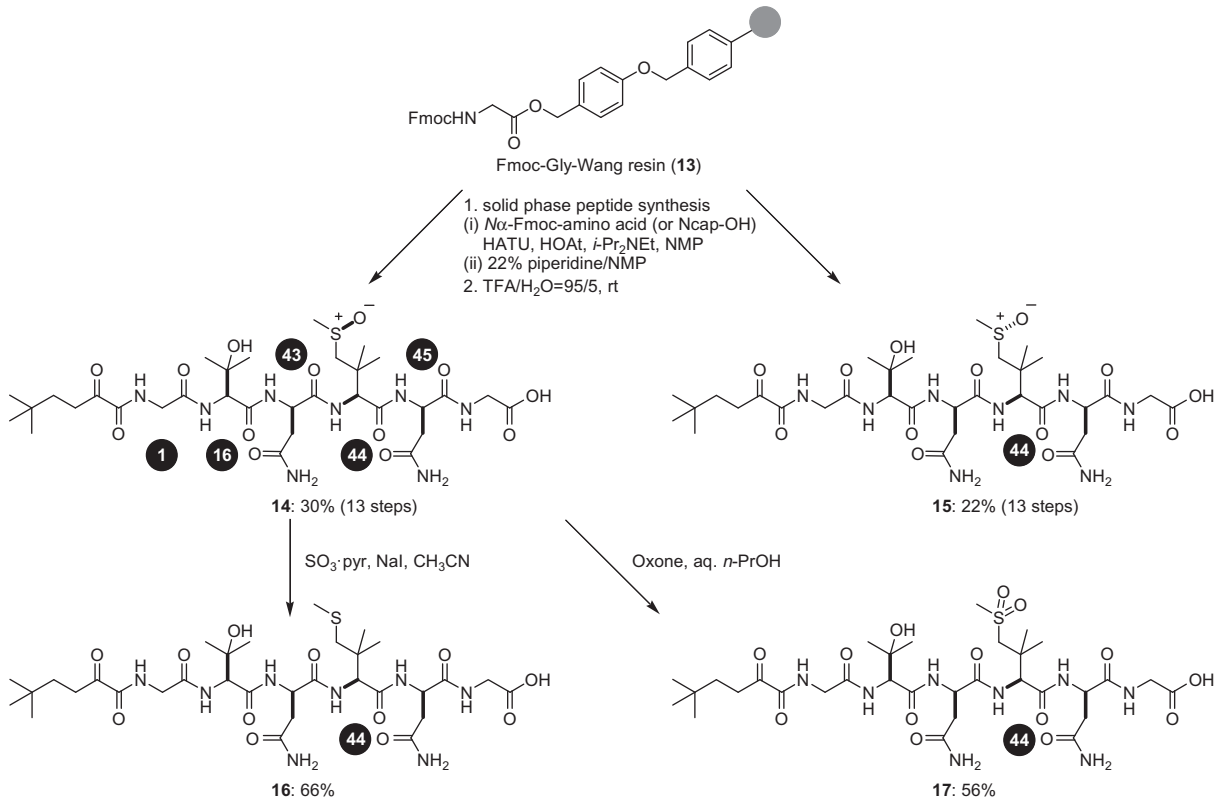
Scheme 1. Chemical structures and reduction/oxidation of N- and C-protected L-Me_2Met(O), (S_R)-sulfoxide **9**, and (S_S)-sulfoxide **10**. Sulfide **11** and sulfone **12** were obtained by single-step reactions from **9**. IBCF = isobutyl chloroformate; NMM = *N*-methylmorpholine.

Table 1
Hydrophobic parameters (log k_w) of protected amino acids **9–12** and hexapeptides **14–17**

Oxidation states	Compound	Log k_w[a]	Compound	Log k_w[a]
(S_R)-Sulfoxide	**9**	2.29	**14**	1.91
(S_S)-Sulfoxide	**10**	2.30	**15**	3.88
Sulfide	**11**	2.93	**16**	ND[b]
Sulfone	**12**	2.03	**17**	1.36

[a] Values were determined from retention times in reversed-phase HPLC. Analyses were performed at 30 °C for **9–12** and at 40 °C for **14–17**. Detailed experimental conditions are described in the Supplementary data.

[b] Compound **16** failed to give clear peaks to calculate a log k_w value due to its high hydrophobicity (ND = not determined).



Scheme 2. Structures of truncated model peptides of polytheonamides. N-capped hexapeptides with a specific stereochemistry at sulfoxide, (S_R)-sulfoxide **14** and (S_S)-sulfoxide **15**, were obtained by SPPS. Sulfide **16** and sulfone **17** were derived from **14** according to the same methods used for the preparation of **11** and **12**, respectively. HATU = *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; NMP = *N*-methylpyrrolidone.

the N-terminal cap was manually introduced, because the activated carboxylate intermediate of the dicarbonyl unit was chemically sensitive to the automated coupling conditions. Finally, the peptides were cleaved from the Wang resin under acidic conditions, and subjected to HPLC purification to generate **14** and **15**. Similar to the syntheses of the protected amino acid derivatives, **14** was then reduced to produce sulfide **16** or oxidized to produce sulfone **17**.

The four model peptides synthesized with the different stereochemistries and sulfur oxidation states (**14**–**17**) were subjected to reversed-phase HPLC analysis to estimate the hydrophobic parameter, log k_w. The analysis conditions were the same as described above for protected amino acids, except for column temperature. The resulting log k_w values are shown in Table 1. Although sulfide **16** was too hydrophobic to give a precise log k_w value in the CH_3CN/H_2O solvent system, the order of hydrophobicities among the three oxidation states in model peptides (sulfide > sulfoxide > sulfone) coincided with that of the protected amino acids. The significant difference in log k_w for **14** and **15**, the pair of sulfoxide stereoisomers, was remarkable. Log k_w of (S_S)-sulfoxide **15** was about twice as large as that of (S_R)-sulfoxide **14** [(S_S)-sulfoxide > (S_R)-sulfoxide]. The effect of the stereochemistry of the sulfoxide on the overall hydrophobicity was clearly enhanced in hexapeptides **14** and **15** in comparison to mono-amino acids **9** and **10**. These results should reflect the large structural difference between **14** and **15** induced by the single stereochemistry of the sulfoxide.

The profound effects of the sulfoxide stereochemistries were also observed in the hemolytic activities of the model peptides. The potency of hemolytic activity has a positive correlation with the hydrophobic parameter, because membrane disruption of erythrocytes requires partitioning of amphiphilic compounds from water to the hydrophobic membrane.[21,22] Hemolytic activities of the three truncated model peptides, **14**, **15**, and **17**, were evaluated against rabbit erythrocytes at 37 °C for 15 h (Table 2). Both (S_S)-sulfoxide **15** and sulfone **17** showed no activities at concentrations up to 1 mM. On the other hand, (S_R)-sulfoxide **14** displayed hemolytic activity with an EC_{50} of 0.38 mM. Curiously, the activity order [**14** > **15**, **17** (Table 2)] did not reflect the hydrophobic order [**15** > **14** > **17** (Table 1)]. These findings indicated that **14** and **15** differ in molecular properties in addition to hydrophobicity.

Table 2
Hemolytic activities of hexapeptides **14**, **15**, and **17**

Oxidation states	Compound	EC_{50}[a] (mM)
(S_R)-Sulfoxide	**14**	0.38
(S_S)-Sulfoxide	**15**	>1.0
Sulfone	**17**	>1.0

[a] Values are evaluated using 10^7 cells/mL of rabbit red blood cells in PBS at 37 °C for 15 h.

To investigate whether the sulfoxide moiety controlled the peptide structures, NMR and CD studies of hexapeptides **14** and **15** were carried out. ^{1}H NMR spectra were recorded at 2 mM in CD_3CN/H_2O (10:1) at 40 °C. A large shift in the amide proton signal of the L-Me_2Met(O) residue was observed between the model peptides **14** and **15** of −0.73 ppm (δ_{SR}–δ_{SS}). In addition, an obvious shift was observed for the amide proton of the adjacent residue, asparagine-5, of +0.34 ppm.[23]

Drastically different behavior between **14** and **15** was also observed by CD spectra. At 0.1 mM in CH_3CN/H_2O (10:1), the peak at 213 nm was increased in the spectrum of **14** compared to that of **15** (Fig. 2A). Interestingly, while the spectrum of **14** remained

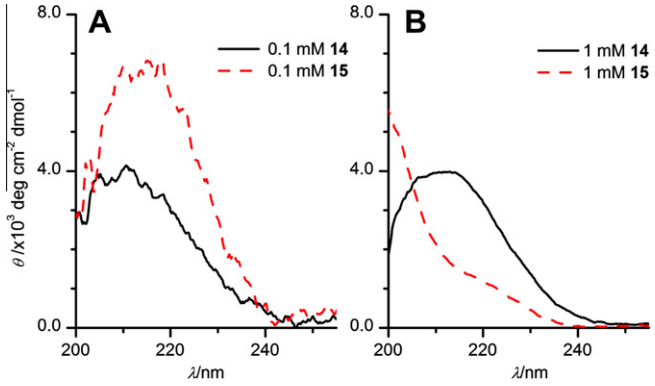


Figure 2. CD spectra of **14** and **15** recorded at room temperature in CH_3CN/H_2O (10:1) at concentrations of 0.1 mM (**A**) and 1 mM (**B**).

unchanged at higher concentrations (1 mM, Fig. 2B), the peak at 213 nm in **15** was diminished. It clearly suggests that **15** undergoes conformational change in a concentration-dependent fashion, most likely due to aggregation. This is consistent with the fact that **15** is more hydrophobic than **14**. Therefore, starkly different behaviors of **14** and **15** observed by ^{1}H NMR, CD spectra, and the hemolysis assay appear to originate in the different aggregation states of **14** and **15**.

Sulfoxides are known as strong hydrogen bond acceptors due to their substantial dipole moments.[24–26] The sulfoxide stereochemistry defines the orientation of its $S^+–O^-$ bond and confers the geometry of the hydrogen bond. Based on our results, it is highly likely that the chirality of sulfoxides in **14** and **15** serves as a controlling structural element at least at two levels; it controls the overall molecular shape by introducing intramolecular hydrogen bonds, thereby controlling the hydrophobicities, and it controls aggregation of the peptides. Peptide **15** with (S_S)-sulfoxide promotes aggregation through increased hydrophobicity,[27] whereas peptide **14** with (S_R)-sulfoxide disfavors aggregation, which is apparently critical in exerting its hemolytic activity.

In conclusion, we synthesized protected amino acids and model hexapeptides containing sulfide (L-Me_2Met), sulfoxides [L-(S_R)-Me_2Met(O), and L-(S_S)-Me_2Met(O)], or sulfone [L-Me_2Met(O_2)] moieties. The (S_R)- and (S_S)-stereochemistries of L-Me_2Met(O) were stereoselectively introduced by asymmetric oxidation of the sulfide. Spectral and biological analyses using model hexapeptides proved that the one stereochemical perturbation of the sulfoxide of the L-Me_2Met(O) moiety has an important structural role in controlling the overall physicochemical and therefore functional properties of the model hexapeptides. Further structural and biological analyses of the peptides and the parent polytheonamides are currently underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.126.

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