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Transformation of thiols to disulfides by epolactaene and its derivatives

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ABSTRACT

In this paper we report a disulfide formation of thiols induced by epolactaene and its derivatives. We previously reported the disulfide formation of *N*-acetylcysteine methyl ester by epolactaene in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution. The present studies reveal that the disulfide formation proceeds under mild conditions such as in PBS at pH 7.3, suggesting that epolactaene may induce disulfide formation of cellular thiols. This compound induces the disulfide formation of several thiols in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution at room temperature. Moreover, our results show that the acyl side-chain of epolactaene greatly influences the products of the reaction. We analyzed the reaction mechanism by using thiolysis products of epolactaene derivatives and propose a new reaction mechanism.

1. Introduction

Disulfide bonds play an important role in stabilizing protein structures and mediating the biological functions of proteins.[1–3] Reversible disulfide bond formation and the associated conformation changes are likely to play an important role in cellular redox regulation.[4–6] In eukaryotic cells, disulfide formation usually occurs in the lumen of the endoplasmic reticulum (ER) and the intermembrane space of mitochondria, but not in the cytosol.[7] The disulfide formation can proceed spontaneously in the presence of sufficient oxidant.[8,9] Recent evidence indicates that reactive oxygen species can act as signaling molecules by promoting the formation of disulfide bonds within or between select redox-sensitive proteins.[10]

Epolactaene (**1a**), a microbial metabolite isolated from *Penicillium* sp., has several attractive biological activities such as promotion of neurite outgrowth and induction of G0/G1 cell cycle arrest in a human neuroblastoma cell line SH-SY5Y,[11–14] an apoptosis-inducing activity in a human leukemia B cell line BALL-1,[15,16] inhibition of mammalian DNA polymerase and DNA topoisomerase activities,[17,18] and an anti-inflammatory activity on TPA (12-*O*-tetradecanoylphorbol 13-acetate)-induced inflammation.[19] We established an efficient synthesis of epolactaene via an oxiranyl anion derived from the α,β-epoxy-γ-lactone.[20–23] Using this synthetic methodology, we prepared several epolactaene derivatives to investigate the structure–activity relationship as well as its biological mechanism of action and to further examine potential new biological activities. In the course of our studies on the chemistry and biology of epolactaene and its derivatives, we found that epolactaene (**1a**) induce the disulfide formation of *N*-acetylcysteine methyl ester (**2**) (Scheme 1).[24] Treatment of **1a** and **2** (1.5 mol equiv) in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution gave the disulfide **3** in 77% yield and the carboxylic acid **4a** in 72% yield. This disulfide bond formation is unique in the following respect. (i) Epolactaene (**1a**) acts as an oxidant to induce the disulfide formation. (ii) This reaction is mechanistically interesting because the side-chain ketone of **1a** is converted into the corresponding carboxylic acid **4a** under mildly basic conditions. (iii) Epolactaene and its derivative might induce the disulfide formation of specific cellular thiols, which may account for their biological activities.[25–29]

In this paper, we report the disulfide formation of epolactaene and its derivatives.

2. Results

2.1. Reaction of epolactaene with thiols

We first examined the reaction of epolactaene (**1a**) and *N*-acetylcysteine methyl ester (**2**; 2.0–2.3 mol equiv) in various media

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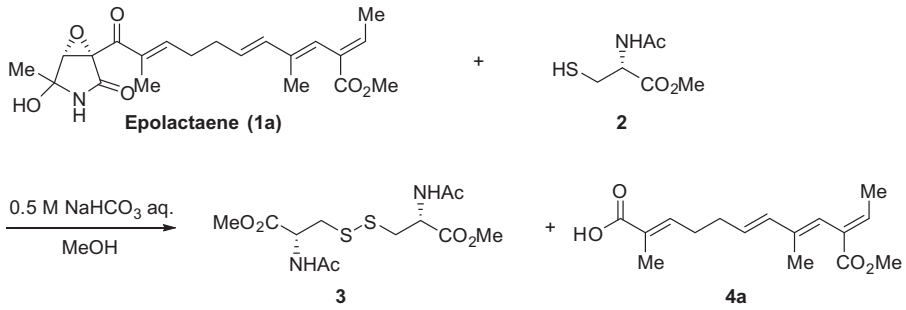


Scheme 1. Reaction of epolactaene (**1a**) with *N*-acetylcysteine methyl ester (**2**).

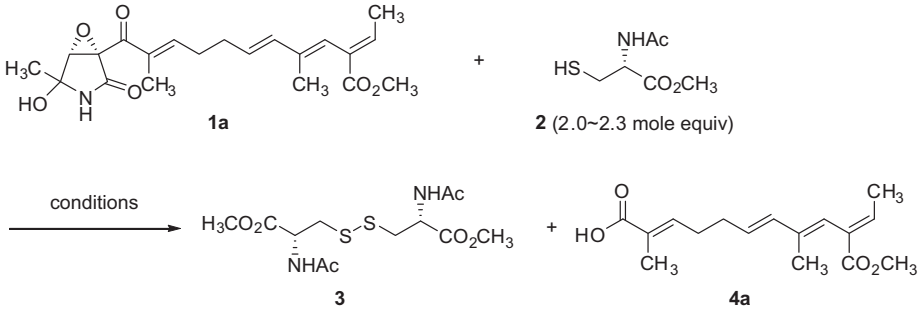
(Table 1). Treatment of **1a** and **2** in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution gave the disulfide **3** in 75% yield and the carboxylic acid **4a** in 68% yield (entry 1). This reaction proceeded smoothly and was complete in 10 min at room temperature. The reaction of **1a** and **2** with solid $NaHCO_3$ in unpurified MeOH gave **3** in 55% yield and **4a** in 33% yield (entry 2). The reaction was slow and the yield of **3** was moderate. In this reaction the methyl ester of **4a** was not obtained at all, indicating that water present as a contaminant in the MeOH was involved in the transformation of **1a** into **4a**. Thus, it was reasoned that water might be implicated in the reaction.[30,31] Indeed, disulfide formation did not proceed in absolute MeOH, but was observed in water (see entries 3 and 4). The reaction in water was slow; after 24 h, the disulfide **3** was obtained in 25% yield and the starting thiol **2** was recovered in 72% yield. We next examined the effect of pH on the disulfide formation of **2** induced by **1a** (entries 5–7). The reaction of **1a** and **2** in 0.2 M phosphate buffer at pH 6.0 for 24 h gave **3** in 49% yield and recovered **2** in 39% yield. By contrast, reactions carried out at pH 7.0 and 8.0 were complete within 24 h, in each case affording **3** in good yield. The low solubility of **1a** and **2** in water or buffer is one of the reasons why the reaction proceeds slowly. However, the reaction in phosphate buffer at pH 7.0 was complete within 24 h, but not in water. This result can be accounted for by the decrease in pH of the reaction solution due to the production of the carboxylic **4a**. The disulfide formation also proceeded in phosphate buffered saline at pH 7.3 (entry 8), suggesting that **1a** induces intramolecular or intermolecular disulfide bond formation in cells.

We next examined the reaction of **1a** with various thiols (Table 2). The reaction was performed using a 1:2.2 mole ratio of **1a** and thiol in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution at room temperature. Both aromatic and primary thiols were converted to the corresponding disulfides in good yield (entries 1–4). However, the reaction of **1a** and *tert*-dodecyl mercaptan did not proceed, and both reactants were recovered (entry 5). The bulky thiols, such as tertiary thiols, do not react with **1a** presumably due to steric hindrance.

2.2. Reaction of epolactaene derivatives with thiols

We examined the reaction of epolactaene (**1a**) and its derivatives (**1b–d**) with 1.2–2.0 mol equiv of *N*-acetylcysteine methyl ester (**2**) (Table 3). Treatment of **1a** with **2** gave the disulfide **3** in 63%

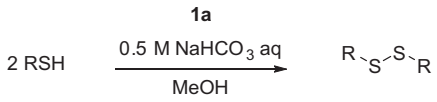
Table 1
Disulfide formation of **2** induced by epolactaene (**1a**)



Entry	Conditions	Yield (%)	
		3[a]	**4a**[b]
1	MeOH:0.5 M $NaHCO_3$ aq = 1:1, 10 min	75 (88)	68
2	MeOH + $NaHCO_3$ (10 equiv), 6 h	55 (58)	33
3	MeOH, 24 h	No reaction	
4[c]	H_2O, 24 h	25 (27)	—
5[d]	Phosphate buffer (pH 6.0), 24 h	49 (52)	—
6	Phosphate buffer (pH 7.0), 24 h	82 (83)	47
7	Phosphate buffer (pH 8.0), 24 h	73 (76)	38
8	PBS (pH 7.3), 24 h	77 (81)	42

[a] Yields based on **2**. The yields in parentheses are those based on **1a**.
[b] Yields based on **1a**.
[c] 72% of **2** was recovered.
[d] 39% of **2** was recovered.

Table 2
Disulfide formation of various thiols induced by epolactaene (**1a**)



Entry[a]	Thiol	Time (h)	Yield[b] (%)
1	PhSH	0.5	89 (98)
2	2-Mercaptopyridine	4	86 (95)
3	$CH_3(CH_2)_5SH$	0.5	89 (98)
4	$CH_3(CH_2)_{11}SH$	0.5	73 (80)
5	$CH_3(CH_2)_8C(CH_3)_2SH$	24	No reaction

[a] The reaction was performed using a 1:2.2 mole ratio of **1a** and thiol.
[b] Yields of disulfides based on thiols. The yields in parentheses are those based on **1a**.

yield and the carboxylic acid **4a** in 60% yield (entry 1). As compared with entry 1 in Table 1, the yield of **3** and **4a** increased by using 2.2 mol equiv of **2**. In both reactions, only disulfide **3** and carboxylic acid **4a** were the products identified. Uniquely, reaction of Epo-C12 (**1b**) with 1.2 mol equiv of **2** gave the adduct **5b** as a sole product in 82% yield (entry 2).[24] However, reaction of **1b** with excess **2** (2.0 mol equiv) afforded the disulfide **3**, dodecanoic acid **4b** and **5b** in 64%, 42% and 13% yield, respectively (entry 3). Similarly, reaction of **1c**,[22] a C6-acyl derivative, with **2** (1.2 mol equiv) afforded **3**, hexanoic acid **4c** and the adduct **5c** in 48%, 29% and 25% yield, respectively (entry 4). However, reaction of **1d**, a phenyl derivative, with **2** gave **3** and benzoic acid **4d** in 56% and 58% yield, respectively (entry 5).

These results show that the acyl side-chain of **1** strongly influences the reaction between **1** and **2**. Both **1a** and **1d**, which have an unsaturated acyl side-chain, reacted with **2** to give the disulfide **3** and carboxylic acid **4**. Meanwhile, the adduct **5** was obtained by the reaction of **2** with **1b** or **1c**, which has a saturated acyl group.

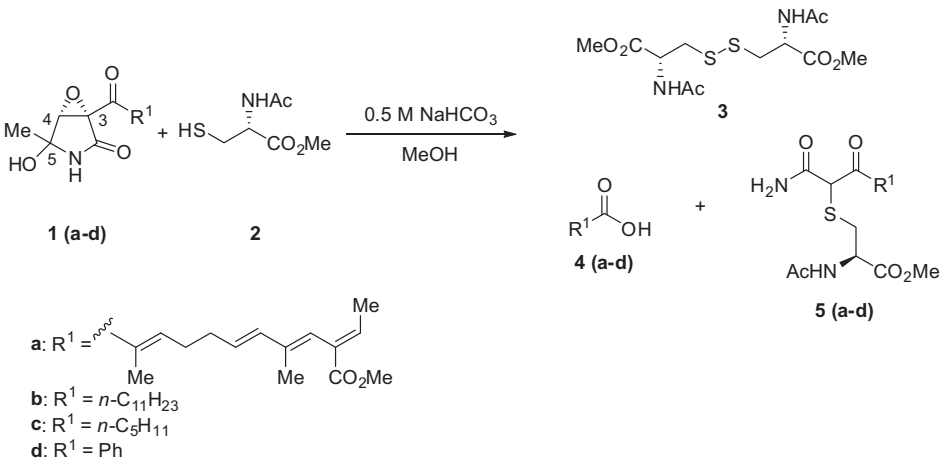
We further investigated the effect of the amount of thiol on the reaction between **1b** and thiophenol (Table 4). Treatment of **1b** and thiophenol (1.0 mol equiv) gave diphenyl disulfide, **4b**, the adduct **6b** and γ-lactam **8-Ph** in 56%, 49%, 18% and 13% yield, respectively, accompanied by a trace amount of β-ketoamide **7b** (entry 1). The use of 2.0 mol equiv of thiophenol gave the disulfide, **4b** and **6b** in 85%, 20% and 9% yield, respectively (entry 2). In contrast to entry 1, compound **7b** and **8-Ph** were not obtained in entry 2. These results indicate that the amount of thiol influences the product of the reaction between **1b** and thiophenol.

2.3. Studies on the reaction mechanism

We previously proposed the mechanism of the disulfide formation of **2** induced by **1b** (Scheme 2).[24] The proposed reaction mechanism may involve the initial formation of the α-sulfanylketoamide (**5b**), followed by a retro-Claisen reaction to give carboxylic acid **4b**. Excess **2** then might react with methyl *N*-acetyl-*S*-(2-amino-2-oxoethyl)cysteinate **9**, the intermediate of the retro-Claisen reaction, yielding **3**.

To clarify that the retro-Claisen reaction actually occurs, compound **5b** was subjected to basic conditions in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution (Scheme 3A). However, the proposed retro-Claisen reaction did not proceed and only racemization at the cysteine moiety was observed. Next, to determine whether **5b** was the

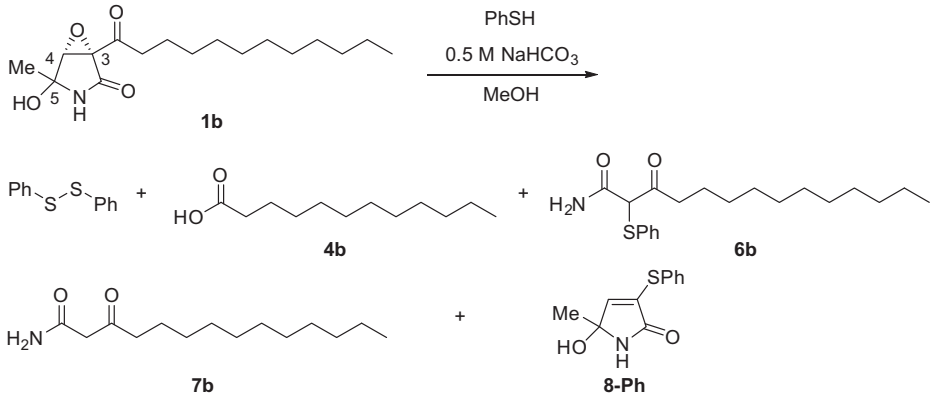
Table 3
Reaction of epolactaene and its derivatives **1 (a–d)** with **2**



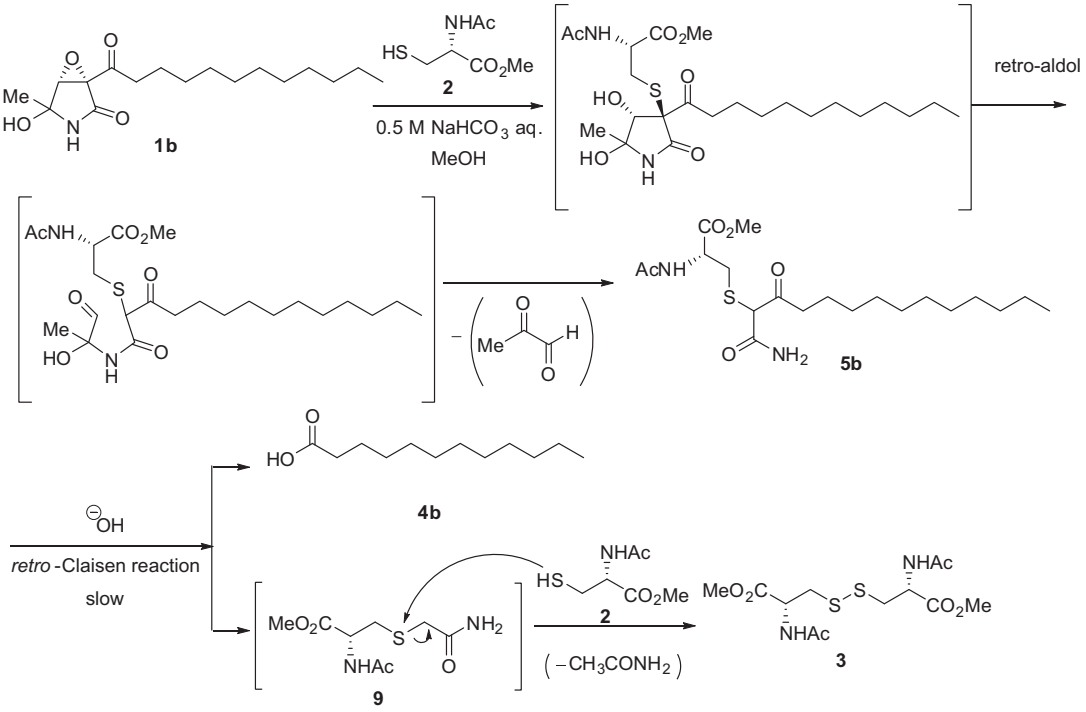
Entry	**1**	**2** (mol equiv)	Yield (%)		
			3[a]	**4**[b]	**5**[b]
1	**1a**	1.2	63 (75)	**4a**: 60	–
2	**1b**	1.2	–	–	**5b**: 82
3	**1b**	2.0	64	**4b**: 42	**5b**: 13
4	**1c**	1.3	48 (62)	**4c**: 29	**5c**: 25
5	**1d**	1.2	56 (68)	**4d**: 58	–

[a] Yields based on **2**. The yields in parentheses are those based on **1**.
[b] Yields based on **1**.

Table 4
Reaction of **1b** with thiophenol



Entry	Thiol (mol equiv)	Time (h)	Disulfide	Yield (%)			
				4b	**6b**	**7b**	**8-Ph**
1	Thiophenol (1.0)	12	56	49	18	3	13
2	Thiophenol (2.0)	0.5	85	20	9	—	—

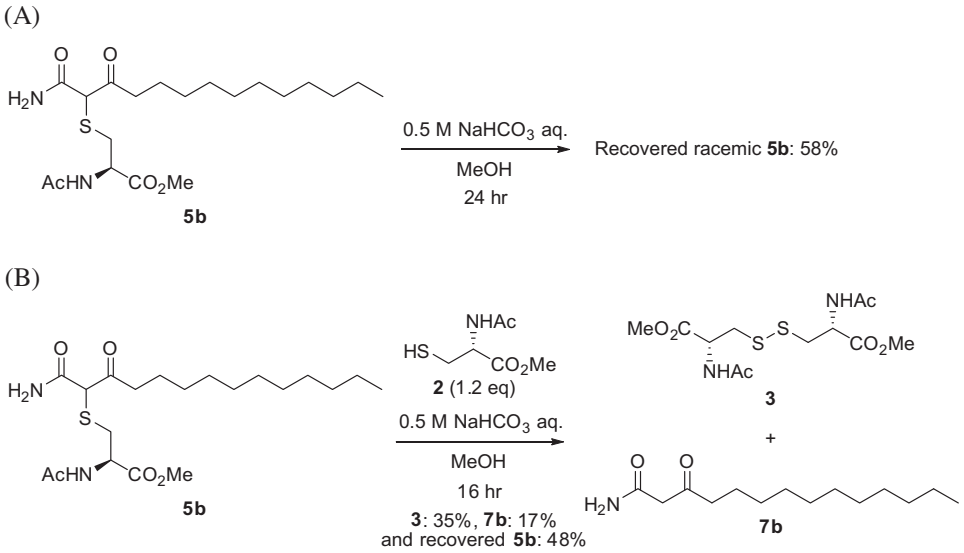


Scheme 2. The previously proposed mechanism.

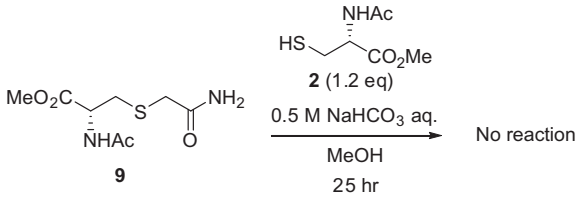
intermediate for the disulfide formation of **2** by **1b**, we examined the reaction of **5b** with **2** (Scheme 3B). Treatment of **5b** with *N*-acetylcysteine methyl ester (**2**; 1.1 mol equiv) in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution for 16 h gave the disulfide **3** in 35% yield and the β-ketoamide **7b** in 17% yield, and the unreacted **5b** was recovered in 48% yield. Although the disulfide **3** was obtained in this reaction, no dodecanoic acid was generated. This result also indicates that the proposed retro-Claisen reaction did not proceed by the reaction of **5b** and **2** under the basic conditions. We also found that the reaction of **9** with **2** did not proceed (Scheme 4).

In addition, we checked whether the retro-Claisen reaction occurred during the reaction of **6b** with thiophenol (Scheme 5). Treatment of **6b** with thiophenol in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution for 21 h gave diphenyl disulfide and **7b** in 74% and 71% yield, respectively. Carboxylic acid **4b** was not obtained in this reaction, indicating that the retro-Claisen reaction did not proceed.

These results suggest that our proposed mechanism shown in Scheme 2 is probably incorrect and should be further examined. We considered that a retro-Claisen reaction of β-hydroxy-γ-lactam derivative **10** or **11** might occur under the basic conditions



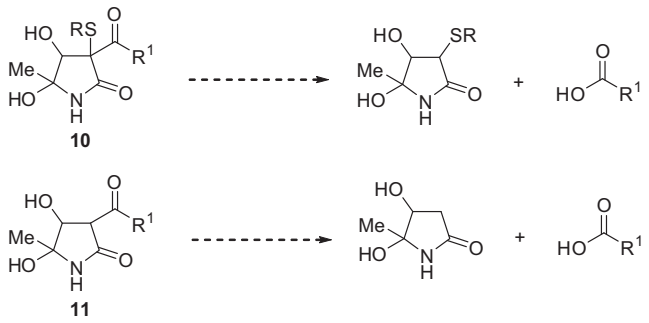
Scheme 3. Treatment of **5b** with a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution in the absence (A) or presence (B) of **2**.



Scheme 4. Treatment of **9** with **2** in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution.

(Scheme 6).[32,33] We decided to prepare each compound by a coupling of methyl glyoxal and β-ketoamide derivatives.

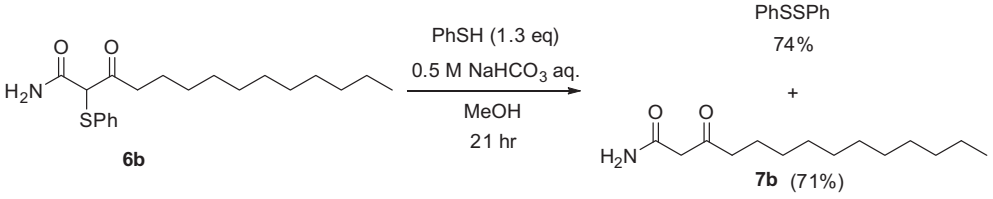
Reaction of **6b** with methyl glyoxal in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution gave dodecanoic acid **4b** in quantitative yield (Scheme 7A). Although the desired β-hydroxy-γ-lactam derivative **10b** was not isolated, compound **4b** should be formed by retro-Claisen reaction of **10b** and **10b′**. We modified the reaction conditions to obtain β-hydroxy-γ-lactam derivatives (Scheme 7B). Treatment of **6b** and methyl glyoxal with sodium bicarbonate (3.2 equiv) in THF/MeOH/H_2O (4:1:1) gave **4b** in 86% yield and β-hydroxy-γ-lactam derivatives **12** as a 4:1 diastereomeric mixture at C-5, **13**, **14** and **15** in 36%, 17%, 5% and 4% yield, respectively. The relative stereochemistry of **14** and **15** were deduced by NOE experiments shown in Figure 1. Formation of these β-hydroxy-γ-lactams shows that the initial formation of **10b** and **10b′** followed by a retro-Claisen reaction certainly occurred in the reaction of **6b** and methyl glyoxal. These results (described in Scheme 7) demonstrate that a retro-Claisen reaction of α-sulfanyl-β-hydroxy-γ-lactam derivative **10** will proceed under the conditions of disulfide formation of thiols by **1**.



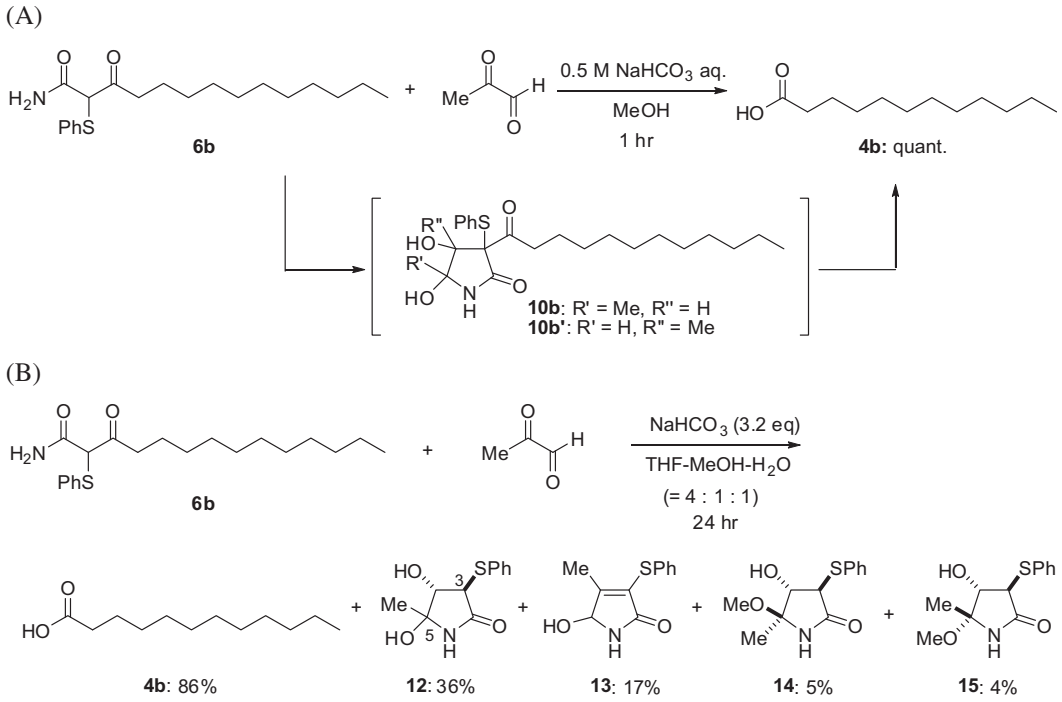
Scheme 6. Possible retro-Claisen reaction of **10** and **11**.

We assumed that the disulfide formation of thiophenol by **1** might proceed via compound **12**, **14** or **15**. However, treatment of **12** with thiophenol gave only **16** as a single diastereomer, and the disulfide formation was not observed in this reaction (Scheme 8). Treatment of **14** or **15** with thiophenol also afforded **16**. The relative stereochemistry of **16** (as well as **12**) was deduced by NOE correlations (Fig. 2). Furthermore, no reaction took place by treatment of **16** with thiophenol. Taken together, our results suggest that compounds **12**, **14** and **15** do not constitute key reaction intermediates for disulfide formation of thiophenol by **1**.

Next, we tried to prepare β-hydroxy-γ-lactam **11b** by a coupling of **7b** and methyl glyoxal.[34] We obtained α,β-unsaturated γ-lactam **17** by a coupling of **7b** with methyl glyoxal (1.1 equiv) in the presence of sodium bicarbonate (1.1 equiv) in THF/MeOH/H_2O (4:1:1) (Scheme 9). Compound **17** should be formed via **11b**. However, compounds **4b** and **11b** were not isolated in this reaction and only



Scheme 5. Reaction of **6b** with thiophenol.



Scheme 7. Reaction of **6b** with methyl glyoxal in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution (A) and in 4:1:1 THF/MeOH/H_2O in the presence of $NaHCO_3$ (B).

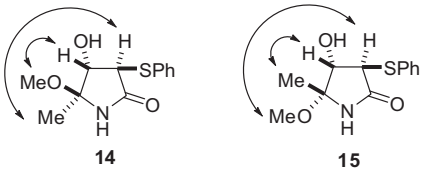


Figure 1. NOE correlations in compounds **14** and **15**.

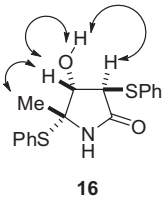


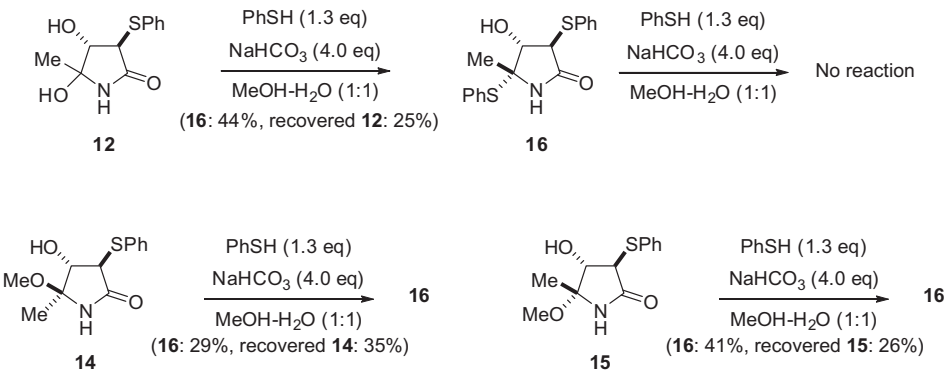
Figure 2. NOE correlations in compound **16**.

a couple of unidentified products were obtained. We attempted to conduct the reaction of **7b** with methyl glyoxal under various different conditions, but neither **11b** nor **4b** could be obtained (data not shown). Thus, we could not confirm whether the retro-Claisen reaction of **11b** actually proceeds.

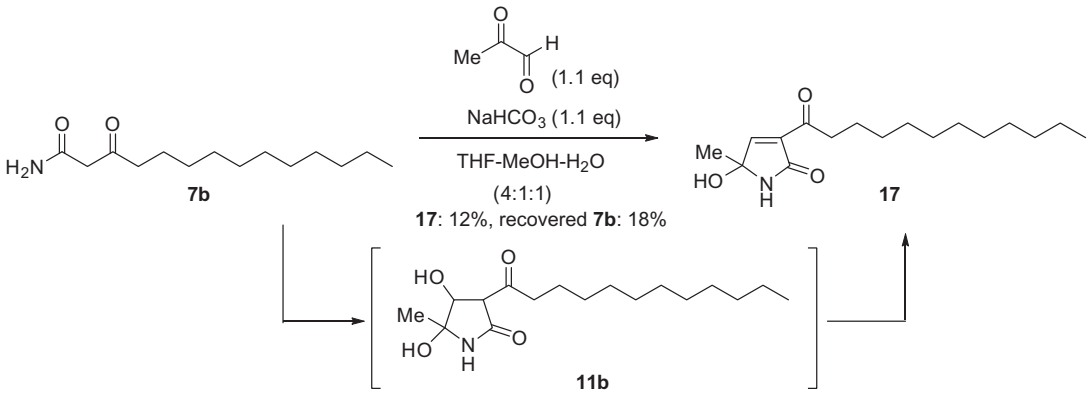
However, treatment of **7d** with methyl glyoxal in a 1:1 MeOH/0.5 M $NaHCO_3$ aq solution gave benzoic acid **4d** in 21% yield, indicating that formation of **11d** or **11d′** followed by retro-Claisen reaction afforded **4d** (Scheme 10). This result leaves open the possibility that a retro-Claisen reaction of β-hydroxy-γ-lactam derivatives **11** may take place under the conditions of the disulfide formation of thiols by **1**.

3. Discussion

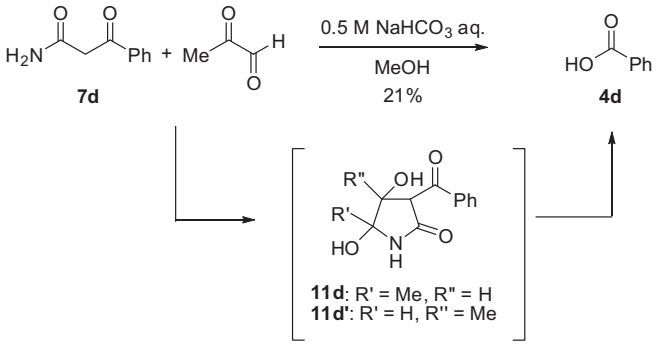
Epolactaene (**1a**) reacts with various thiols and induces disulfide formation. Given that disulfide formation is favored under basic conditions (Table 1) and that sterically bulky *tert*-dodecyl mercaptan does not react with **1a** (Table 2, entry 5), the reaction



Scheme 8. Reaction of **12**, **14**, **15** or **16** with thiophenol.



Scheme 9. Reaction of **7b** with methyl glyoxal.



Scheme 10. Reaction of **7d** with methyl glyoxal.

must involve a nucleophilic attack of the thiolate anion on the epoxide in **1a**. Water might be involved in the series of reactions. One possibility is that the hydrogen bond between the epoxide oxygen and water might activate the reactivity of the epoxide in **1a**.[31] It has been reported that cerulenin, a related natural product containing an α,β-epoxy-γ-lactam, reacts with thiol to form the cerulenin-thiol adduct in protic solvents (Scheme 11).[35,36] Cerulenin did not convert thiol into the corresponding disulfide, indicating that the acyl side-chain in epolactaene and its derivatives (**1**) is essential for disulfide formation.

The reactivity of **1** strongly depends on the acyl side-chain (Table 3). Reaction of **2** with **1a** and **1d**, which contain the unsaturated acyl side-chain gave disulfide **3** and carboxylic acid **4** (entries 1 and 5). Meanwhile, reaction of **2** with **1b**, which has a *n*-dodecanoyl side-chain, only gave the adduct **5b** (entry 2). Furthermore, reaction of **2** with **1c**, which has a *n*-hexanoyl side-chain, gave disulfide **3** and hexanoic acid **4c** as well as the adduct **5c** (entry 4). The difference of the reactivity might be explained by the solubility of the side-chain of **1** in polar solvent. The low solubility derived from the hydrophobic side-chain in the polar solvent might cause the sequential reactions to stop and thereby lower the production of the final disulfide. In the reaction of **1b** and **2**, the use of excess **2** recovered the production of **3** (entry 3). Because the adduct **5** was obtained in the reaction of **2** with **1b** or **1c** (Table 3, entries 3 and 4), the thiolate anion selectively attack the C-3 position on the epoxide in **1**. The regioselective epoxide opening might be due to the steric hindrance between the attacking thiolate and the substituent (methyl or hydroxyl group) at the C-5 position in the lactam ring.

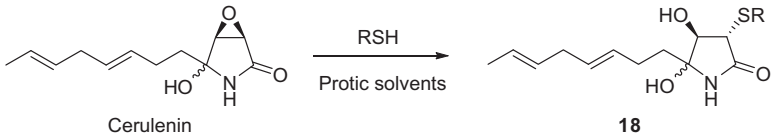
The reaction of **1b** with 1.2 mol equiv of **2** gave the adduct **5b** as a sole product (entry 2 in Table 3). However, the reaction of **1b** with 1.0 equiv of thiophenol afforded the adduct **6b**, diphenyl disulfide, carboxylic acid **4b**, β-ketoamide **7b** and γ-lactam **8-Ph** (entry 1 in Table 4). These results indicate that the products of the reaction of **1b** with an almost equimolar amount of thiol depend on the thiol used in the reaction. By contrast, similar products were obtained when the thiol was present in excess (entry 3 in Table 3 and entry 2 in Table 4). The products obtained by the reaction of **1b** with 2.0 equiv of **2** or thiophenol were the disulfide, **4b** and the adduct (**5b** or **6b**).

Previously, we proposed the mechanism of the reaction of **1b** with **2** as shown in Scheme 2.[24] However, the present studies reveal that the proposed mechanism is incorrect because a retro-Claisen reaction of **5b** and the reaction of **9** with **2** under basic conditions did not proceed (Schemes 3 and 4). Thus, we have reexamined the mechanism of the reaction of **1** and thiol.

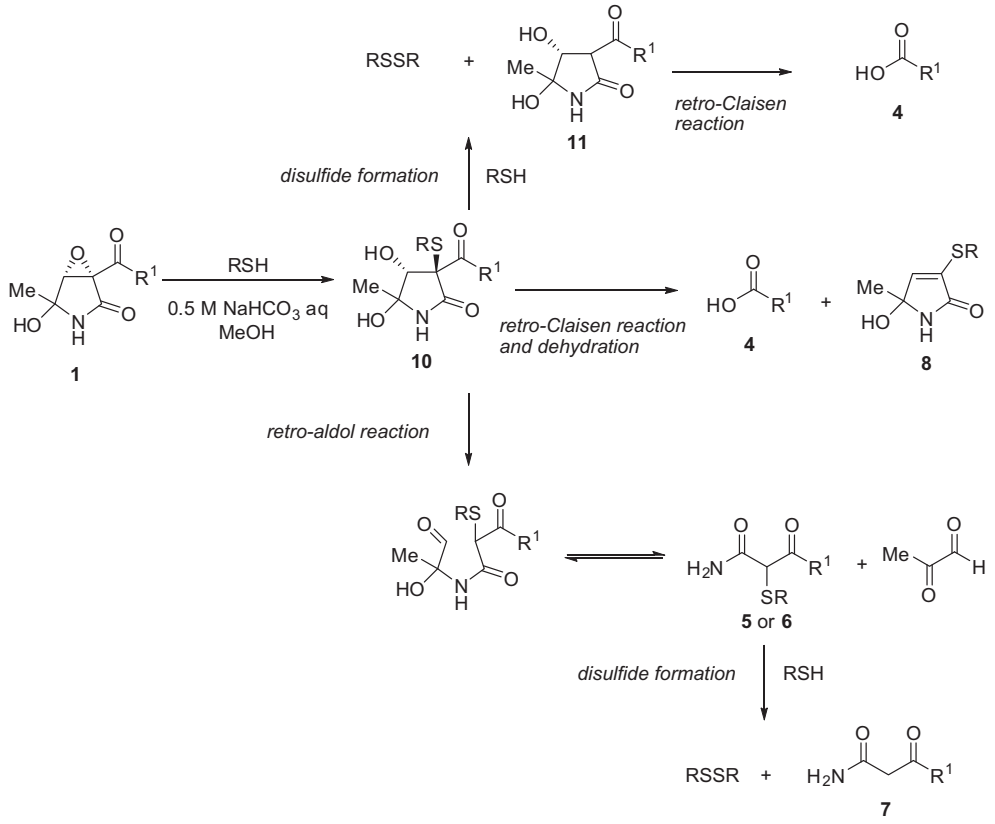
We found that reaction of the α-sulfanyl-β-ketoamide **6b** with methyl glyoxal under basic conditions afforded carboxylic acid **4b** and γ-lactams **12**, **14** and **15** (Scheme 7). This result indicates that compound **10** is liable to cleavage by a retro-Claisen reaction (Scheme 6). The formation of **8-Ph** in the reaction between **1b** and thiophenol also supports this conclusion (Table 4, entry 1).

We also found that reaction of the β-ketoamide **7d** with methyl glyoxal under basic conditions afforded carboxylic acid **4d** (Scheme 10). The formation of **4d** should come from the initial formation of **11d** or **11d′**, followed by a retro-Claisen reaction. Although the formation of **4b** was not observed in the reaction of **7b** and methyl glyoxal (Scheme 9), the result obtained by the reaction of **7d** with methyl glyoxal suggests that the formation of carboxylic acid **4** in the reaction of **1** with thiol may be derived from the retro-Claisen reaction of **11** (Scheme 6).

As a result of these findings, we have revised the mechanism of the disulfide formation of thiol induced by **1** (Scheme 12). The reaction involves nucleophilic attack of the initial thiolate anion



Scheme 11. Reported reaction between cerulenin and thiols to form the cerulenin-thiol adduct.



Scheme 12. Revision of our proposed mechanism according to the results obtained in this study.

on the epoxide in **1** to give the adduct **10**. Then the subsequent attack of the second thiolate anion on the sulfur atom in **10** may produce the disulfide, accompanied by the formation of **11**. Compound **11** may be cleaved by a retro-Claisen reaction to yield the carboxylic acid **4**. The formation of the adduct **5** or **6** is explained by a retro-aldol reaction of **10** followed by elimination of methyl glyoxal. The alternative disulfide may be caused by the reaction of **5** or **6** with the second thiolate anion. One of the side reactions is a retro-Claisen reaction of **10** to give **4** and the γ-lactam **8**.

4. Conclusion

In this paper, we investigated the thiolysis and disulfide formation of epolactaene and its derivatives. We examined the disulfide formation using various thiols and epolactaene derivatives as well as the mechanistic studies using thiolysis products. As a result of these studies, we have revised our proposed mechanism of disulfide formation induced by epolactaene and its derivatives. We believe that our present studies will be invaluable not only for analyzing the molecular and cellular biological activities of epolactaene and its derivatives, but also in the development of new reagents for disulfide bond formation.

5. Experimental procedure

5.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a JEOL 270 MHz spectrometer (EX-270W) or a Bruker 600 MHz spectrometer (Avance DRX-600). Chemical shifts are expressed in δ (ppm) relative to Me_4Si or the residual solvent resonance, and coupling constants (J) are expressed in Hertz. Melting point (Mp) data were determined with a Yanaco MP-3S instrument and were uncorrected. Optical rotation data were recorded on a Jasco P-1030 digital polarimeter. Infrared (IR) spectra were recorded on a Jasco FT/IR-410 spectrometer, using NaCl (neat) or KBr pellet (solid). Mass spectra (MS) were obtained on an Applied Biosystems mass spectrometer (API QSTAR pulsar i) under high-resolution conditions or Varian 910-MS Fourier transform mass spectrometer. Analytical thin-layer chromatography was performed on Silica Gel 60 F254 plates (Merck, Germany). Flash chromatography was carried out on PSQ 100B (Fuji Silysia Co., Japan).

5.2. General method for the disulfide formation of *N*-acetyl-L-cysteine methyl ester (2) induced by epolactaene (1a) (Table 1)

A degassed solution of **1** (1.0 equiv) and **2** (2.0–2.3 mol equiv) in the solvent indicated in Table 1 was stirred at room temperature under a nitrogen atmosphere until no further change in TLC was observed. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc = 1:1, then $CHCl_3$/MeOH = 20:1) to give **3** and **4a**. The structures of **3** and **4a** were confirmed by comparison of the ^{1}H NMR data with those reported.

5.3. General procedure for the formation of disulfides (Table 2)

To a solution of **1** (10 mg, 26 μmol) and thiol (2.2 mol equiv) in degassed MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere until no further

change in TLC was observed. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography to give the corresponding disulfide. The structures of diphenyl disulfide, 2,2′-dipyridyl disulfide, *n*-dihexyl disulfide and *n*-didodecyl disulfide were confirmed by MS analyses as well as comparison of the ^{1}H NMR data with those of the authentic disulfides.

5.4. (*1R,5R*)-1-Benzoyl-4-hydroxy-4-methyl-6-oxa-3-azabicyclo-[3.1.0]hexan-2-one (1d)

The titled compound was prepared according to the bridgehead oxiranyl anion strategy previously reported.[24] Compound **1d** was obtained as white solid. Mp = 137–139 °C. $[\alpha]_D^{23} = -54.7$ (*c* 0.20, MeOH). IR (KBr): 3286, 3062, 2990, 2922, 2853, 1691, 1651, 1540, 1486, 1447, 1409, 1354, 1266, 1177, 1110, 1050, 937, 879, 851, 787. 690 cm^{-1}. NMR data: compound **1d** was observed as a 5:1 diastereomeric mixture in CD_3OD. Peaks derived from the major isomer were indicated. ^{1}H NMR (600 MHz, CD_3OD): δ = 8.06 (2H, d, *J* = 7.9 Hz), 7.67 (1H, t, *J* = 7.9 Hz), 7.53 (2H, d, *J* = 7.9 Hz), 4.17 (1H, s), 1.56 (3H, s). ^{13}C NMR (125 MHz, CD_3OD): δ = 191.2, 171.8, 136.3, 135.5, 130.2 (×2), 129.9 (×2), 84.8, 66.2, 64.4, 22.2. HRMS (ESI): calcd for $C_{12}H_{11}NO_4Na$ ([M+Na]$^+$) 256.0580, found 256.0568.

5.5. General procedure for the reaction of 1 with 2 (Table 3)

To a solution of **1** (1.0 equiv) and **2** (1.2–2.0 mol equiv) in degassed MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere until no further change in TLC was observed. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The products were purified by column chromatography. The structures of **3**, **4a** and **5b** were confirmed by comparison of the ^{1}H NMR data with those reported. The structures of **4b**, **4c** and **4d** by MS analyses as well as comparison of the ^{1}H NMR data with those of the authentic carboxylic acids.

5.5.1. *N*-Acetyl-*S*-[1-(aminocarbonyl)-2-oxooctanyl]-L-cysteinate (5c)

Colorless oil. $[\alpha]_D^{23} = -12.3$ (*c* 0.21, MeOH). IR (neat): 3444, 3325, 3018, 2929, 2861, 1739, 1670, 1585, 1438, 1304, 1039, 667 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$ with 0.03% TFA): δ = 6.58 (1H, br s), 5.92 (1H, br s), 4.82 (1H, m), 3.82 (3H, s), 3.13 (1H, m), 2.98 (1H, m), 2.17 (3H, s), 1.63 (2H, m), 1.36–1.25 (8H, br m), 0.88 (3H, t, *J* = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$ with 0.03% TFA): δ = 187.4, 175.6, 171.4, 170.7, 53.2, 52.8, 38.8, 34.2, 31.5, 29.7, 26.4, 22.8, 22.3, 13.8. HRMS (ESI): calcd for $C_{14}H_{24}N_2O_5NaS$ ([M+Na]$^+$) 355.1298, found 355.1298.

5.6. The reaction of 1b with thiophenol (Table 4)

To a solution of **1b** (34.5 mg, 0.11 mmol for entry 1) or (16.3 mg, 52.3 μmol for entry 2) and thiophenol (11 μL, 0.11 mmol) in degassed MeOH (2.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere until no further change in TLC was observed. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The products were purified by PTLC (hexane/EtOAc = 4:1). In entry 1, diphenyl disulfide (6.6 mg, 56%), **4b** (10.9 mg, 49%), **6b** (7.0 mg, 18%), **7b** (0.8 mg, 3%) and **8-Ph** (3.2 mg, 13%) were obtained. In entry 2, diphenyl disulfide (10.0 mg, 85%), **4b** (2.1 mg, 20%) and **6b** (1.7 mg, 9%) were obtained. The structures of diphenyl disulfide and dodecanoic acid **4b** were confirmed by MS analyses as well as comparison of the ^{1}H NMR data with those of the authentic samples.

5.6.1. 3-Oxo-2-phenylsulfanyltetradecanamide (6b)

Yellow oil. IR (neat): 3452, 3309, 3234, 3176, 2925, 2856, 1722, 1637, 1585, 1468, 1412, 1329, 957 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.29 (2H, d, *J* = 7.6 Hz), 7.14 (2H, d, *J* = 7.6 Hz), 7.14 (1H, t, *J* = 7.6 Hz), 6.72 (1H, br s), 5.27 (1H, br s), 2.62 (2H, t, *J* = 7.6 Hz), 1.58 (2H, m), 1.21 (16H, br m), 0.88 (3H, t, *J* = 6.8 Hz), −4.14 (1H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ = 188.5, 174.8, 137.0, 129.0 (×2), 125.4, 124.8 (×2), 89.4, 34.1, 32.0, 29.7 (×2), 29.5, 29.4, 29.3, 29.3, 26.7, 22.8, 14.2. HRMS (ESI): calcd for $C_{20}H_{31}NO_2NaS$ ([M+Na]$^+$) 372.1973, found 372.1968.

5.6.2. 3-Oxotetradecanamide (7b)

White solid. Mp = 117 °C. IR (KBr): 3377, 3186, 2920, 2852, 1707, 1651, 1635, 1431, 1130, 721 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.07 (1H, br s), 5.59 (1H, br s), 3.43 (2H, s), 2.53 (2H, t, *J* = 7.3 Hz), 1.59 (2H, m), 1.26 (16H, br s), 0.88 (3H, t, *J* = 7.0 Hz). ^{13}C NMR (68 MHz, $CDCl_3/CD_3OD$ = 4:1): δ = 205.9, 169.2, 43.3, 31.7, 29.4 (×3), 29.2, 29.1, 29.1, 28.8, 23.1, 22.4, 14.8. HRMS (ESI): calcd for $C_{14}H_{27}NO_2Na$ ([M+Na]$^+$) 264.1934, found 264.1940.

5.6.3. 5-Hydroxy-5-methyl-3-phenylsulfanyl-3-pyrrolin-2-one (8-Ph)

Amorphous solid. IR (film): 3384, 3219, 3018, 2978, 2929, 1700, 1577, 1477, 1440, 1419, 1377, 1356, 1151, 1097, 1026, 1003, 943, 831 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.56 (2H, m), 7.42 (3H, m), 6.68 (1H, br s), 6.00 (1H, s), 2.96 (1H, br s), 1.55 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.3, 138.8, 137.9, 134.3 (×2), 129.8 (×2), 129.41, 129.37, 86.5, 24.9. HRMS (ESI): calcd for $C_{11}H_{11}NO_2NaS$ ([M+Na]$^+$) 244.0403, found 244.0401.

5.7. Treatment of 5b in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 3A)

To a solution of **5b** (10.3 mg, 24.7 μmol) in degassed MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 24 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc = 3:1, then $CHCl_3$/MeOH = 20:1) to give the recovered **5b** (5.8 mg, 58%). The specific rotation of **5b** decreased from $[\alpha]_D^{21} = -12.8$ (*c* 0.50, MeOH) to $[\alpha]_D^{21} = -0.9$ (*c* 0.29, MeOH) after treatment of **5b** under the basic conditions for 24 h

5.8. Treatment of 5b with 2 in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 3B)

To a solution of **5b** (5.8 mg, 13.9 μmol) and **2** (3.0 mg, 16.9 μmol) in degassed MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 16 h. The reaction was quenched by the addition of 1 M HCl

aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 20:1) to give **3** (1.7 mg, 35%), **7b** (0.6 mg, 17%) and the recovered **5b** (2.8 mg, 48%).

5.9. Methyl *N*-acetyl-*S*-(2-amino-2-oxoethyl)-L-cysteinate (9)

To a solution of **2** (26.7 mg, 0.15 mmol) in THF (2 mL) was added NaH (7.0 mg, 60% dispersion in mineral oil, 0.18 mmol) at 0 °C and the mixture was stirred at 0 °C for 10 min. To the solution was added 2-iodoacetamide (28.8 mg, 0.16 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was saturated with NaCl and extracted with $CHCl_3$ (×13 times). The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 20:1–10:1) to give **9** (7.8 mg, 22%) as white solid. Mp = 154–155 °C. $[\alpha]_D^{23} = -39.6$ (*c* 0.47, MeOH). IR (KBr): 3359, 3178, 3022, 2927, 1738, 1637, 1533, 1425, 1387, 1128, 1088 cm^{-1}. ^1H NMR (270 MHz, CD_3OD): δ = 4.66 (1H, dd, *J* = 8.4 Hz, 5.1 Hz), 3.73 (3H, s), 3.24 (1H, d, *J* = 14.9 Hz), 3.21 (1H, d, *J* = 14.9 Hz), 3.09 (1H, dd, *J* = 14.0 Hz, 5.1 Hz), 2.93 (1H, dd, *J* = 14.0 Hz, 8.4 Hz), 1.99 (3H, s). ^{13}C NMR (68 MHz, CD_3OD): δ = 174.6, 173.1, 172.3, 53.5, 52.9, 36.0, 34.9, 22.4. HRMS (ESI): calcd for $C_8H_{14}N_2O_4NaS$ ($[M+Na]^+$) 257.0566, found 257.0559.

5.9.1. Reaction of 9 with 2 in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 4)

To a solution of **9** (7.8 mg, 33.3 μmol) and **2** (7.1 mg, 40.1 μmol) in degassed MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 25 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 20:1) to give the recovered **2** (4.9 mg, 69%).

5.10. Reaction of 6b with thiophenol in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 5)

To solution of **6b** (17.9 mg, 51.2 μmol) and thiophenol (7.0 μL, 68.4 μmol) in degassed MeOH (0.5 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (0.5 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 21 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc = 20:1–10:1–4:1–1:1) to give diphenyl disulfide (8.3 mg, 74%) and **7b** (8.8 mg, 71%).

5.11. Reaction of 6b with methyl glyoxal in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 7A)

To solution of **6b** (5.9 mg, 16.9 μmol) and methyl glyoxal (12.0 μL, 40% solution in water, 66.6 μmol) in MeOH (0.5 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (0.5 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 1 hour. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane/EtOAc = 2:1) to give **4b** (3.4 mg, quant).

5.12. Reaction of 6b with methyl glyoxal in the presence of $NaHCO_3$ in a 4:1:1 THF/MeOH/H_2O solution (Scheme 7B)

To solution of **6b** (74.2 mg, 0.21 mmol) and methyl glyoxal (150 μL, 40% solution in water, 0.83 mmol) in THF/MeOH/H_2O (4:1:1, 6 mL) was added $NaHCO_3$ (56.3 mg, 0.67 mmol) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 24 hours. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (EtOAc/MeOH = 20:1) and preparative TLC (hexane/EtOAc = 1:3) to give **4b** (36.6 mg, 86%), **12** (18.2 mg, 36%), **13** (7.8 mg, 17%), **14** (2.5 mg, 5%) and **15** (2.3 mg, 4%).

5.12.1. (3*R**,4*S**,5*RS*)-4,5-Dihydroxy-5-methyl-3-phenylsulfanyl-2-pyrrolidinone (12)

Colorless oil. IR (film): 3282, 3008, 2929, 2862, 1705, 1583, 1471, 1429, 1383, 1128, 1053, 997 cm^{-1}. Compound **12** was observed as a 4:1 diastereomeric mixture in DMSO-d_6. The data for major diastereomer was indicated. ^1H NMR (270 MHz, DMSO-d_6): δ = 8.59 (1H, s), 7.46 (2H, m), 7.31 (2H, m), 7.24 (1H, m), 5.58 (1H, d, *J* = 7.0 Hz), 5.46 (1H, s), 3.82 (1H, d, *J* = 8.9 Hz), 3.63 (1H, dd, *J* = 8.9 Hz, 7.0 Hz), 1.27 (3H, s). ^{13}C NMR (68 MHz, DMSO-d6): δ = 170.9, 135.0, 130.3 (×2), 128.9 (×2), 126.6, 83.9, 77.3, 53.3, 25.2. HRMS (ESI): calcd for $C_{11}H_{13}NO_3NaS$ ($[M+Na]^+$) 262.0508, found 262.0510.

5.12.2. 5-Hydroxy-4-methyl-3-phenylsulfanyl-3-pyrrolin-2-one (13)

White solid. Mp = decomposition with browning at 130 °C. IR (film): 3298, 3066, 3016, 2925, 2858, 1697, 1579, 1469, 1339, 1218, 1184, 1084, 1034, 883 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ = 7.33 (1H, s), 7.30–7.17 (5H, m), 5.34 (1H, br s), 4.23 (1H, br s), 2.06 (3H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ = 170.6, 133.4, 133.1, 129.1 (×2), 129.0 (×3), 126.6, 81.2, 13.3. HRMS (ESI): calcd for $C_{11}H_{11}NO_2NaS$ ($[M+Na]^+$) 244.0403, found 244.04010.

5.12.3. (3*R**,4*S**,5*S**)-4-Hydroxy-5-methoxy-5-methyl-3-phenylsulfanyl-2-pyrrolidinone (14)

Amorphous solid. IR (film): 3398, 3276, 3008, 2935, 1705, 1585, 1468, 1427, 1389, 1352, 1302, 1176, 1107, 918, 876, 833 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ = 7.63–7.59 (2H, m), 7.34–7.29 (3H, m), 6.36 (1H, br s), 4.24 (1H, dd, *J* = 6.2 Hz, 4.9 Hz), 3.60 (1H, d, *J* = 6.2 Hz), 3.06 (3H, s), 2.50 (1H, d, *J* = 4.9 Hz), 1.45 (3H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ = 171.4, 133.0 (2C), 131.5, 129.1 (2C), 128.1, 91.3, 76.5, 55.1, 49.4, 20.2. HRMS (ESI): calcd for $C_{12}H_{15}NO_3NaS$ ($[M+Na]^+$) 276.0665, found 276.0668.

5.12.4. (3*R**,4*S**,5*R**)-4-Hydroxy-5-methoxy-5-methyl-3-phenylsulfanyl-2-pyrrolidinone (15)

Amorphous solid. IR (film): 3410, 3248, 3012, 2931, 1711, 1583, 1468, 1429, 1394, 1346, 1174, 1112, 1058, 920 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ = 7.62–7.57 (2H, m), 7.32–7.27 (3H, m), 7.08 (1H, br s), 3.88 (1H, dd, *J* = 10.0 Hz, 8.6 Hz), 3.73 (1H, d, *J* = 8.6 Hz), 3.31 (3H, s), 2.84 (1H, d, *J* = 10.0 Hz), 1.45 (3H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ = 172.7, 133.3 (2C), 132.9, 128.9 (2C),

128.0, 86.8, 79.2, 55.1, 49.6, 19.8. HRMS (ESI): calcd for $C_{12}H_{15}NO_3$-NaS ([M+Na]$^+$) 276.0665, found 276.0665.

5.12.5. (3*R**,4*R**,5*R**)-4-Hydroxy-5-methyl-3,5-bis(phenylsulfanyl)-2-pyrrolidinone (16)

To a solution of **12** (13.0 mg, 54.3 μmol) and thiophenol (7.0 μL, 68.4 μmol) in MeOH/H_2O (1:1, 4 mL) was added $NaHCO_3$ (18.3 mg, 0.22 mmol) at room temperature, and the mixture was stirred at room temperature for 24 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by preparative TLC (hexane/EtOAc = 1:3) to give **16** (8.0 mg, 44%) as amorphous solid and recovered **12** (3.3 mg, 25%). According to the same procedure, compound **16** was obtained from **14** or **15**. **16**: IR (film): 3406, 3068, 3016, 2927, 1709, 1581, 1475, 1437, 1387, 1336, 1176, 1078, 1037, 924 cm^{-1}. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 8.73 (1H, s), 7.58−7.16 (10H, m), 6.42 (1H, d, J = 5.7 Hz), 3.90 (1H, dd, J = 10.0 Hz, 5.7 Hz), 3.36 (1H, d, J = 10.0 Hz), 1.46 (3H, s). ^{13}C NMR (68 MHz, $CDCl_3$): δ = 169.9, 137.7 (×2), 134.7, 130.7, 130.1 (×2), 129.1, 128.90 (×2), 128.87 (×2), 126.7, 79.6, 74.6, 54.0, 26.7. HRMS (ESI): calcd for $C_{17}H_{17}NO_2$-NaS_2 ([M+Na]$^+$) 354.0593, found 354.0592.

5.13. 3-Dodecanoyl-5-hydroxy-5-methyl-3-pyrrolin-2-one (17)

To solution of **7b** (50.0 mg, 0.21 mmol) and methyl glyoxal (41 mg, 40% solution in water, 0.23 mmol) in THF/MeOH/H_2O (4:1:1, 4.5 mL) was added $NaHCO_3$ (19.7 mg, 0.23 mmol) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 24.5 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by preparative TLC (hexane/EtOAc = 1:3) to give **17** (7.4 mg, 12%) as colorless oil and recovered **7b** (8.9 mg, 18%). **17**: IR (film): 3298, 2954, 2925, 2854, 1711, 1618, 1466, 1412, 1379, 1136, 1119, 943, 667 cm^{-1}. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 8.66, (1H, br s), 7.52 (1H, d, J = 1.6 Hz), 6.01 (1H, s), 2.80 (2H, t, J = 7.0 Hz), 1.48 (2H, m), 1.41 (3H, s), 1.24 (16H, m), 0.86 (3H, t, J = 6.2 Hz). ^{13}C NMR (68 MHz, DMSO-d_6): δ = 197.0, 167.1, 156.5, 134.0, 83.8, 41.2, 31.5, 29.2 (2C), 29.1, 29.0, 28.9, 28.7, 25.0, 23.2, 22.3, 14.2. HRMS (ESI): calcd for $C_{17}H_{29}NO_3Na$ ([M+Na]$^+$) 318.2040, found 318.2039.

5.14. Reaction of 7d with methyl glyoxal in a 1:1 MeOH/0.5 M $NaHCO_3$ aqueous solution (Scheme 10)

To solution of **7d** (22.6 mg, 0.14 mmol) and methyl glyoxal (29.7 mg, 40% solution in water, 0.16 mmol) in MeOH (1.0 mL) was added 0.5 M aqueous solution of $NaHCO_3$ (1.0 mL) at room temperature. The mixture was stirred at room temperature under a nitrogen atmosphere for 22.5 h. The reaction was quenched by the addition of 1 M HCl aqueous solution, and the mixture was diluted with EtOAc. The layers were separated, the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by preparative TLC (hexane/EtOAc = 1:3) to give **4d** (3.6 mg, 21%).

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