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RhCl₃-catalyzed disulfide exchange reaction using water solvent in homogeneous and heterogeneous systems

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Abstract

RhCl₃ catalyzed the alkylthio exchange reaction of hydrophilic disulfides in water under homogeneous conditions, and equilibrium was attained in several hours. The reaction was applied to the exchange of unprotected glutathione disulfide. The reaction of dimethyl disulfide and hydrophilic disulfides under heterogeneous conditions also proceeded effectively. The mechanism turned out to be dependent on the water solubility of the substrates: The reaction of bis(3-hydroxypropyl) disulfide took place in the dimethyl disulfide phase, whereas the reaction of bis(6-aminohexyl) disulfide dihydrochloride proceeded in the water phase.
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1. Introduction

Disulfides R–S–S–R are an important functional group for the construction of the tertiary structures of peptides and proteins; therefore, their derivatization is an interesting subject of modifying their biological activities. Several methods, such as reduction to thiols R–S–H or alkylation to sulfides R–S–R′, have been examined [1]. Disulfide exchange, which converts R–S–S–R to another disulfide R–S–S–R′, is more attractive for such construction. Disulfides are more stable than thiols and the original compound can be regenerated by a reverse reaction. The arrangement of disulfide bond in peptides and proteins can be modified by intramolecular disulfide exchange, and intermolecular exchange can change the partial structures of the disulfide moiety, thus forming a number of alkylthio derivatives. Another interesting subject is regioselectivity in the disulfide exchange, which reflects the three-dimensional structures of peptides and proteins.

The conventional methods of exchanging peptide or protein disulfide unfortunately have not been efficient. Sanger exchanged cystine or insulin disulfide with N,N′-bis(2,4-dinitrophenyl)cystine in concentrated hydrochloric acid [2], and Glazer and Pescatore extended the method to several proteins [3,4]. Lawrence reported the catalysis of Na₂SeO₄ in concentrated hydrochloric acid [5]. Although the exchange is slow under weakly basic conditions, the presence of thiol promotes the reaction [6]. The reactions have generally been conducted under strong-acid or base conditions at a relatively low rate. During our investigations of the use of transition-metal catalysis for the synthesis of organosulfur compounds [7,8], we showed that RhH(PPh₃)₄ catalyzes the disulfide exchange reaction in refluxing acetone, which reaches equilibrium within 15 min, and the method was applied to a protected glutathione disulfide. This method, however, does not work with unprotected glutathione disulfide **8** in acetone because of solubility problems. We therefore studied the metal-catalyzed reaction in water, and described here the finding

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$$(R^1S)_2 + (R^2S)_2 \xrightleftharpoons[H_2O]{Rh\ cat.} 2\ R^1SSR^2$$
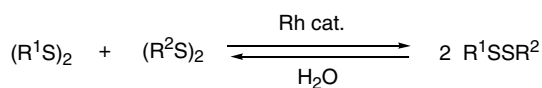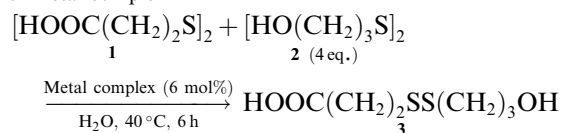
Scheme 1.

that $RhCl_3 \cdot 3H_2O$ is effective for this transformation (Scheme 1).

The development of transition-metal-catalyzed reactions in water has attracted considerable interest [9]. Such reactions very often proceed under heterogeneous conditions, in which the substrate and/or catalyst is not soluble in water [10,11]. Alternatively, they are conducted under homogeneous conditions using mixtures of water and polar organic solvents in order to dissolve the substrate and/or catalyst [12]. Since the reaction mechanisms can be quite different between homogeneous and heterogeneous reactions, it would be important to understand the two systems separately. In this regard, several advantages of the study of homogeneous reactions in water without using organic cosolvents are [13]: (1) The heterogeneous reaction mechanism can be complex compared with the homogeneous reaction, thereby making analysis difficult. (2) Heterogeneous reactions are generally less efficient than homogeneous reactions, since the transfer of the catalyst or substrate is quite often the rate-determining step. (3) Primary importance is that biological substances such as a peptides and proteins are soluble only in water. On the basis of understanding of the homogeneous reactions, heterogeneous reactions with water could be compared. As will be described later, heterogeneous reactions are more complex than homogeneous reactions in the disulfide exchange. This is another reason for studying the disulfide exchange reaction in water.

Initially, bis(2-carboxyethyl) disulfide **1** and bis(3-hydroxypropyl) disulfide **2** were selected as substrates, since both are soluble in water. $RhH(PPh_3)_4$ (6 mol%) was added to a homogeneous solution of **1** and **2** (4 equiv.) in water, and the mixture was heated at 40 °C for 6 h. The rhodium complex was removed by subjecting the mixture to reversed-phase chromatography without concentration, since the concentration process in the presence of metal complexes could cause secondary reactions. 2-Carboxylethyl 3-hydroxypropyl disulfide **3** was obtained in 42% yield (Table 1, entry 2). The rhodium complex was essential for this reaction, and no reaction occurred in its absence (entry 1). Although trifluoromethanesulfonic acid promoted the exchange reaction in acetone [8a], it was not the case in water (entry 3). The yield of **3** increased to 78% after the reaction for 9 h, which coincided with the calculated yield of 80% assuming equilibrium (entry 4). Although the reaction in acetone reached equilibrium within 15 min, the reaction in water was slow (entry 4) [8a]. The catalyst, however, was not deactivated for several hours. The rhodium complex was not soluble in water, which may be one of the reasons for the low catalytic activity. Several organometallic Rh(I) complexes, [Rh(cod)-

Table 1
Effect of metal complex

$$[HOOC(CH_2)_2S]_2 + [HO(CH_2)_3S]_2$$
$$\underset{1}{} \quad \underset{2\ (4\,eq.)}{}$$

$$\xrightarrow[H_2O,\ 40\,°C,\ 6\,h]{Metal\ complex\ (6\ mol\%)} \underset{3}{HOOC(CH_2)_2SS(CH_2)_3OH}$$

Entry	Metal complex	Yield of 3[a] (%)
1	None	ND
2	$RhH(PPh_3)_4$	42
3[b]	$RhH(PPh_3)_4$	35
4[c]	$RhH(PPh_3)_4$	78
5	$[Rh(OAc)_2]_2$	14
6	$RhCl_3 \cdot 3H_2O$	80
7	$PdCl_2 \cdot 2NaCl \cdot 3H_2O$	20

[a] Isolated yield based on $HOOC(CH_2)_2S$ group. ND indicates not detected by ^1H NMR.
[b] In the presence of CF_3SO_3H (18 mol%).
[c] Reaction time: 9 h.

$(NH_3)_2]PF_6$, $[RhCl(cod)]_2$, $[RhCl(nbd)]_2$, $Rh(acac)(C_2H_4)_2$, $Rh(acac)(CO)_2$, and $[Rh(cod)_2]BF_4$, exhibited similar activities with $RhH(PPh_3)_4$. Since all these complexes were not soluble in water and the ligand effect was small, it was considered that a small amount of a common rhodium species was involved. Solid-supported Rh–C and Rh–alumina were not effective at all, and water-insoluble Rh(III) complexes, namely, $Rh(acac)_3$ and $[RhCl_2Cp]_2$, did not exhibit activity.

Notably, water-soluble $RhCl_3 \cdot 3H_2O$ effectively catalyzed the reaction, and gave **3** in 80% yield after 6 h (entry 6). Various other water-soluble transition-metal chlorides, namely, $CoCl_2 \cdot 6H_2O$, $IrCl_3 \cdot 3H_2O$, $NiCl_2 \cdot 6H_2O$, K_2PtCl_4, $FeCl_3 \cdot 6H_2O$, $RuCl_3 \cdot nH_2O$, $OsCl_2 \cdot 3H_2O$, $MnCl_2 \cdot 4H_2O$, $YbCl_3 \cdot 6H_2O$, $EuCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, and $ZnCl_2$, were not effective at all with the exception of $PdCl_2 \cdot 2NaCl \cdot 3H_2O$ (entry 7). Note that the rhodium complex specifically catalyzed the disulfide exchange reaction. This observation is in accordance with our previous observations of the transformation of organosulfur compounds that used rhodium complexes [7,8] and in some cases palladium complexes [14]. Certain characteristic feature of the Rh–S bond may be reflected in the activity of the rhodium complexes.

The examination of the $RhCl_3 \cdot 3H_2O$ reaction course revealed that the yield of **3** reached 80% after 3 h (Table 2, entries 1–4). Analogously, the exchange reactions of bis(carboxymethyl) disulfide **4** and bis(6-aminohexyl) disulfide dihydrochloride **6** with **2** (4 equiv.) in water gave unsymmetrical disulfides **5** and **7** in 80% yields in 3 h (entries 5 and 7).

The disulfide exchange reaction was confirmed to be under equilibrium (Scheme 2). When **2** (1 mmol) and **4** (1 mmol) were reacted with $RhCl_3 \cdot 3H_2O$ (0.06 mmol) at 40 °C for 1 h in water, carboxymethyl 3-hydroxypropyl disulfide **5** (1 mmol) was obtained with recovered **2** (0.5 mmol) and **4** (0.5 mmol). The treatment of the product

Table 2
RhCl$_3$-catalyzed homogeneous disulfide exchange reactions in water

$$\underset{\substack{0.25\,mmol}}{(RS)_2} + \underset{\substack{\textbf{2}\ (4\,eq.)}}{[HO(CH_2)_3S]_2} \xrightarrow[\text{H}_2\text{O (15 mL), 40 °C}]{\text{RhCl}_3\cdot3\text{H}_2\text{O (6 mol\%)}} RSS(CH_2)_3OH$$

Entry	R	Reaction time (h)	Yield (%)
1	HOOC(CH$_2$)$_2$ (**1**)	1	60
2		2	72
3		3	80
4		6	80
5	HOOCCH$_2$ (**4**)	3	80
6		6	78
7[a]	HCl · H$_2$N(CH$_2$)$_6$ (**6**)	3	80
8[a]		6	77

[a] Isolated as BocNH(CH$_2$)$_6$SS(CH$_2$)$_3$OH.

5 (2 mmol) with RhCl$_3$ · 3H$_2$O (0.06 mmol) at 40 °C in water for 1 h gave **2** (0.5 mmol) and **4** (0.5 mmol).

The rhodium catalyst has relatively long lifetime (Table 3). A mixture of RhCl$_3$ · 3H$_2$O (6 mol%), **2** (1 mmol), and **4** (1 mmol) in water was heated at 40 °C for 24 h, which gave **5** (0.98 mmol) with recovered **2** (0.46 mmol) and **4** (0.5 mmol): The equilibrium constant $K = [5]^2/[2] \cdot [4] = 4.1$. Then, another portion of **4** (3 mmol) was added, and the mixture was heated at 40 °C for 3 h giving **5** (1.55 mmol) with recovered **2** (0.20 mmol) and **4** (3.07 mmol); next, the addition of **4** (3 mmol) gave **5** (1.75 mmol) after 3 h with recovered **2** (0.14 mmol) and **4** (6.12 mmol). The experimental results were compared with

the calculated amounts of **2**, **4**, and **5** assuming $K = 4.0$. Starting from **2** (1 mmol) and **4** (b mmol), the product **5** ($2a$ mmol) with recovered **2** ($1 - a$ mmol) and **4** ($b - a$ mmol) should be formed at equilibrium, in which a (mmol) is the amount of the reacted **2** and b (mmol) is the total amount of **4**. Then, equation (1), $K = [2a]^2/[1 - a] \cdot [b - a] = 4$, was obtained, which could be transformed to equation (2), $a = b/(b + 1)$. For $b = 1$ (mmol), equation (2) gave $a = 0.5$ (mmol), and the amounts of **5**, **2**, and **4** were 1, 0.5, and 0.5 (mmol), respectively (Table 3). For $b = 4$ (mmol), $a = 0.8$ (mmol) was obtained, and the calculated amounts of **5**, **2**, and **4** were 1.6, 0.2, and 3.2 (mmol). For $b = 7$ (mmol), equation (2) gave $a = 0.88$ (mmol), and the calculated amounts of **5**, **2**, and **4** should be 1.75, 0.12, and 6.12 (mmol). The calculated results coincided well with the experimental results. Thus, the catalyst was active after 24 h, and the addition of a substrate shifted the equilibrium.

One benefit to this methodology is that the exchange reaction could be applied to an unprotected tripeptide, glutathione disulfide **8**, in water (Table 4). When **8** and **4** (4 equiv.) were treated in water for 1 h in the presence of RhCl$_3$ · 3H$_2$O (10 mol%) at 40 °C, carboxymethyl N-(N-γ-glutamylcysteinyl)glycyl disulfide **9** was obtained in 80% yield after reversed-phase chromatography (Table 4, entries 1 and 2). The reaction of **8** with bis(6-aminohexyl) disulfide dihydrochloride **6** also gave a glutathione derivative **10** in 80% yield after 3 h (entries 3–5). It is notable that the amino and carboxyl groups do not interfere with the reaction, and

$$\begin{array}{c}
\underset{\substack{\textbf{2}\\(1.0\ mmol)}}{[HO(CH_2)_3S]_2} + \underset{\substack{\textbf{4}\\(1.0\ mmol)}}{(HOOCCH_2S)_2} \\[2em]
\underset{\substack{\textbf{5}\\(2.0\ mmol)}}{HO(CH_2)_3SSCH_2COOH}
\end{array}
\xrightarrow[\text{H}_2\text{O, 40 °C, 1 h}]{\text{RhCl}_3\cdot3\text{H}_2\text{O (0.06 mmol)}}
\underset{\substack{(0.5\ mmol)\quad(0.5\ mmol)\quad(1.0\ mmol)}}{\textbf{2} + \textbf{4} + \textbf{5}}$$
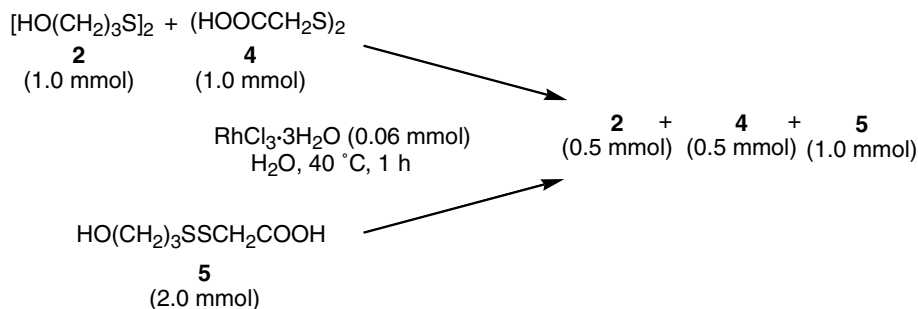
Scheme 2.

Table 3
Equilibrium shift experiment

$$\underset{\substack{\textbf{2}\\1.0\ mmol}}{[HO(CH_2)_3S]_2} + \underset{\substack{\textbf{4}\\1.0\ mmol}}{(HOOCCH_2S)_2} \xrightarrow[\text{H}_2\text{O, 40 °C, 24 h}]{\text{RhCl}_3\cdot3\text{H}_2\text{O (6 mol\%)}} \left(\underset{\substack{\text{H}_2\text{O, 40°C, 3 h}}}{\xrightarrow{\quad\downarrow\quad\textbf{4}\ 3.0\ mmol\quad}} \right)_n \underset{\substack{\textbf{5}}}{HO(CH_2)_3SSCH_2COOH}$$

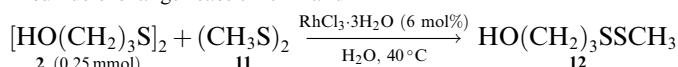| n	Yield/mmol		Recovery/mmol			
	5	Calcd.[a]	**2**	Calcd.[a]	**4**	Calcd.[a]
0	0.98	1.00	0.46	0.50	0.50	0.50
1	1.55	1.60	0.20	0.20	3.07	3.20
2	1.75	1.75	0.14	0.12	6.12	6.12

[a] See text.

RhCl$_3$ selectively affects the disulfide moiety. It is shown that RhCl$_3 \cdot 3H_2O$ effectively catalyzes the disulfide exchange in water under homogeneous conditions, and this encouraging result may be applicable to complex proteins.

At this stage, it was considered interesting to examine dimethyl disulfide **11** as a reagent for the disulfide exchange in water. The volatile nature (bp 109 °C) of **11** can be utilized in the modification of peptide or protein disulfides, since excess **11** can readily be removed after the reaction. Dimethyl disulfide **11**, however, is insoluble in water, and has been surmised whether the RhCl$_3$ method can be applied to a heterogeneous system. It was also interesting to compare heterogeneous reactions with homogeneous reactions. It is shown below that the RhCl$_3$ method was effective for the heterogeneous system as well, even though heterogeneous reactions were more complex than homogeneous reactions. Behaviors were observed depending on the substrates, bis(3-hydroxypropyl) disulfide **2** for example was quite different from bis(6-aminohexyl)disulfide dihydrochloride **6**, presumably due to the different solubilities of substrates in water.

When **2** was treated with **11** (1 equiv.) in the presence of RhCl$_3 \cdot 3H_2O$ (6 mol%) at 40 °C in water for 15 min, 3-hydroxypropyl methyl disulfide **12** was obtained in 28% yield (Table 5, entry 1). This was an equilibrated state, and the yield remained unchanged after 60 min (entry 2). When the amount of **11** was increased from 1 to 4 and 15 equivalents, the yield of **12** increased 50% and 72%, respectively (entries 3 and 5). These yields did not change after 15 min (entries 4 and 6). Further increase in the amount of **11** to 30 equivalents improved the yields to 88% after 60 min (entries 7–9). In contrast, the change in the amount of water did not affect the yield of **12** (entries 10–15). These results suggest that the exchange reaction proceeded in the dimethyl disulfide phase and not in the water phase. Thus, the presence of a large amount of **11** shifts the equilibrium to the exchanged product **12**; moreover, the amount of water has little effect, since it does not largely affect the amount of **11** in the organic phase. Also note that the present heterogeneous reaction proceeded much faster that the homogeneous reaction

Table 4
Exchange reaction of glutathione **8** and hydrophilic disulfides

8 (0.25 mmol)　　　4 eq.　　　　　　　　　　　**9** R = CH$_2$COOH
　　　　　　　　　　　　　　　　　　　　　　　　　10 R = (CH$_2$)$_6$NH$_2$

Entry	R	Time (h)	Yield (%)
1	HOOCCH$_2$ (**4**)	1	81
2		3	80
3	HCl · H$_2$N(CH$_2$)$_6$ (**6**)	1	55
4		3	80
5		8	79

Table 5
Disulfide exchange reaction of **2** and **11**

$$[HO(CH_2)_3S]_2 + (CH_3S)_2 \xrightarrow[H_2O, 40\,°C]{RhCl_3 \cdot 3H_2O\ (6\ mol\%)} HO(CH_2)_3SSCH_3$$

2 (0.25 mmol)　　**11**　　　　　　　　　　　　　　　**12**

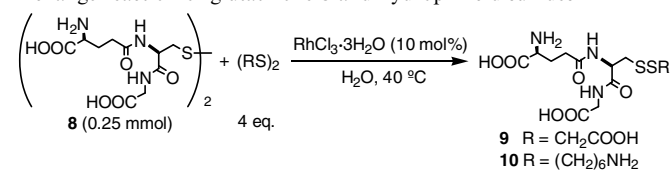| Entry	(CH$_3$S)$_2$ (equiv.)	H$_2$O (mL)	Time (min)	Yield of **12** (%)
1	1	2	15	28
2	1	2	60	22
3	4	2	15	50
4	4	2	60	50
5	15	2	15	72
6	15	2	60	77
7	30	2	15	77
8	30	2	60	88
9	30	2	75	88
10	4	5	15	50
11	4	5	60	49
12	4	8	15	50
13	4	8	60	49
14	4	10	15	50
15	4	10	60	48

described before, and the rate was similar to the reaction rate in acetone. This supports the occurrence of the reaction in the organic phase.

The heterogeneous reaction was confirmed to be under equilibrium (Scheme 3). When **2** (0.25 mmol) and **11** (0.25 mmol) were reacted with RhCl$_3 \cdot 3H_2O$ (0.015 mmol) at 40 °C in water (2 mL) for 15 min, **12** (0.14 mmol) was obtained with recovered **2** (0.17 mmol). The treatment of the product **12** (0.5 mmol) in the presence of RhCl$_3$ (0.015 mmol) at 40 °C in water (2 mL) for 15 min gave **2** (0.16 mmol) with recovered **12** (0.15 mmol).
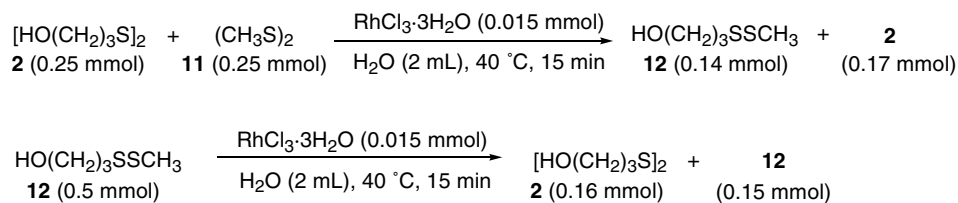
The steady state could be shifted by the addition of **11** as indicated by the following results (Table 6). A mixture of RhCl$_3 \cdot 3H_2O$ (6 mol%), **2** (0.25 mmol), and **11** (0.25 mmol) in water (2 mL) was heated at 40 °C for 10 min giving **12** (0.13 mmol) with recovered **2** (0.17 mmol), reaction (a). A mixture of RhCl$_3 \cdot 3H_2O$ (6 mol%), **2** (0.25 mmol), and **11** (0.25 mmol) in water (2 mL) was heated at 40 °C for 10 min. Then, **11** (0.75 mmol) was added, and the mixture was heated at 40 °C for 10 min giving **12** (0.21 mmol) with recovered **2** (0.13 mmol), reaction (b). A mixture of RhCl$_3 \cdot 3H_2O$ (6 mol%), **2** (0.25 mmol), and **11** (0.25 mmol) in water (2 mL) was heated at 40 °C for 10 min. Then, **11** (0.75 mmol) was added, and the mixture was heated at 40 °C for 10 min. The next addition of **11** (2.75 mmol) gave **12** (0.36 mmol) after 10 min at 40 °C with recovered **2** (0.05 mmol), reaction (c). The amount of **12** increased as **11** was added, which also showed the robustness of the catalyst.

The distributions of **2** and **12** in water and dimethyl disulfide phase were examined (Scheme 4). The compound **2** (1 mmol) was treated with **11** (30 equiv.) in the presence of RhCl$_3 \cdot 3H_2O$ (6 mol%) at 40 °C in water (8 mL) for 75 min, and the water and dimethyl disulfide phases were separated. From the water phase, **12** (0.46 mmol) and **2**
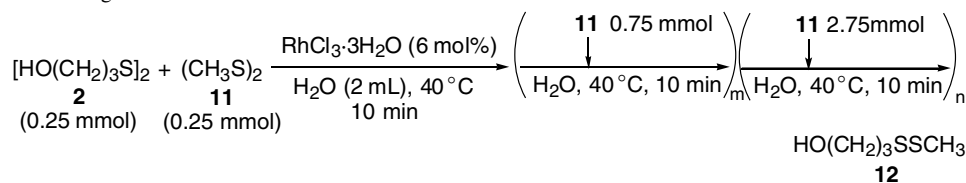
$[HO(CH_2)_3S]_2$ + $(CH_3S)_2$ $\xrightarrow[\text{H}_2\text{O (2 mL), 40 °C, 15 min}]{\text{RhCl}_3 \cdot 3\text{H}_2\text{O (0.015 mmol)}}$ $HO(CH_2)_3SSCH_3$ + **2**

2 (0.25 mmol) **11** (0.25 mmol)   **12** (0.14 mmol)   (0.17 mmol)

$HO(CH_2)_3SSCH_3$ $\xrightarrow[\text{H}_2\text{O (2 mL), 40 °C, 15 min}]{\text{RhCl}_3 \cdot 3\text{H}_2\text{O (0.015 mmol)}}$ $[HO(CH_2)_3S]_2$ + **12**

12 (0.5 mmol)   **2** (0.16 mmol)   (0.15 mmol)

Scheme 3.

Table 6
Equilibrium experiment in heterogeneous reaction

$[HO(CH_2)_3S]_2$ + $(CH_3S)_2$ $\xrightarrow[\substack{\text{H}_2\text{O (2 mL), 40 °C} \\ \text{10 min}}]{\text{RhCl}_3 \cdot 3\text{H}_2\text{O (6 mol\%)}}$ $\left(\substack{\textbf{11} \ 0.75 \ \text{mmol} \\ \downarrow \\ \text{H}_2\text{O, 40 °C, 10 min}} \right)_m \left(\substack{\textbf{11} \ 2.75 \ \text{mmol} \\ \downarrow \\ \text{H}_2\text{O, 40 °C, 10 min}} \right)_n$

2 **11**
(0.25 mmol) (0.25 mmol)

$HO(CH_2)_3SSCH_3$
12

	m and n	Yield of **12** (mmol)	Recovery of **2** (mmol)
Reaction (a)	$m = 0, n = 0$	0.13	0.18
Reaction (b)	$m = 1, n = 0$	0.21	0.13
Reaction (c)	$m = 1, n = 1$	0.36	0.05

$[HO(CH_2)_3S]_2$ + $(CH_3S)_2$ $\xrightarrow[\text{H}_2\text{O (8 mL), 40 °C, 75 min}]{\text{RhCl}_3 \cdot 3\text{H}_2\text{O (6 mol\%)}}$ $HO(CH_2)_3SSCH_3$

2 **11** **12**
(1 mmol) (30 eq.) (1.76 mmol)

H_2O

$(CH_3S)_2$

→ **12**: 0.46 mmol, **2**: 0.04 mmol
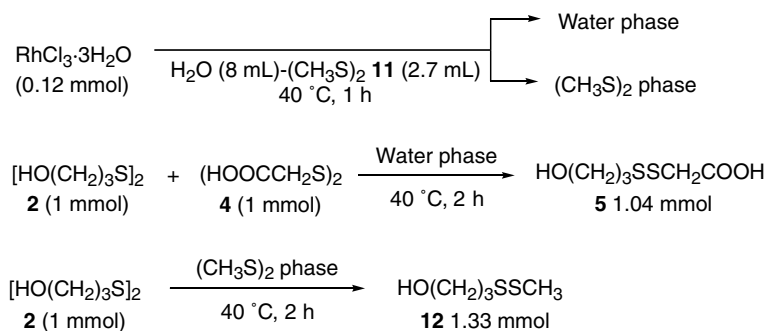→ **12**: 1.30 mmol, **2**: 0.04 mmol
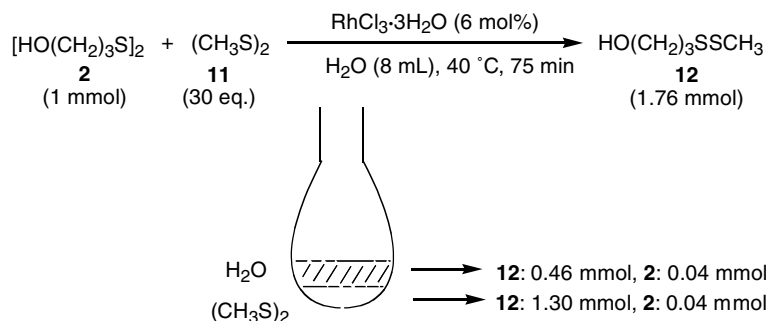
Scheme 4.

(0.04 mmol) were obtained, and from the dimethyl disulfide phase **12** (1.30 mmol) and **2** (0.04 mmol) were obtained. This indicates that **2** and **12** existed in both the water and dimethyl disulfide phases.

The distribution of the rhodium complex in the water and dimethyl disulfide phases was also examined (Scheme 5). $RhCl_3 \cdot 3H_2O$ (0.12 mmol) was treated with a mixture of water (8 mL) and **11** (30 mmol, 2.7 mL) at 40 °C for 1 h, and the two phases were separated. Then, **2** (1.0 mmol) and **4** (1.0 mmol) were added to the water phase, and the mixture was reacted at 40 °C for 2 h giving **5** (1.04 mmol). Compound **2** (1.0 mmol) was added to the dimethyl disulfide phase, and the mixture was reacted at 40 °C for 2 h giving **12** (1.33 mmol). The results indicated that the rhodium catalyst existed in both the water and dimethyl disulfide phases.
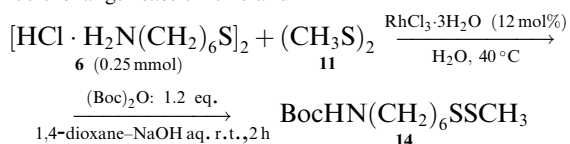
Bis(6-aminohexyl) disulfide dihydrochloride **6** behaved considerably different from **2** in the exchange reaction (Table 7). The compound **6** (0.25 mmol) was treated with **11** (4 equiv.) in the presence of $RhCl_3 \cdot 3H_2O$ (12 mol%) at 40 °C in water (5 mL) for 3 h, and the crude products containing $HCl \cdot H_2N(CH_2)_6SSCH_3$ **13** were treated with Boc_2O in 1 M NaOH and 1,4-dioxane at room temperature for 2 h giving 6-(tert-butoxycarbonylamino)hexyl methyl disulfide **14** in 55% yield (entry 1). The yield did not change after 5 h (entry 2), and the reaction reached a steady state within 3 h. When the amount of **11** was increased to 8, 15 and 30 equivalents, the yield of **14** did not change (entries 3–8). The results are highly in contrast to those of the reactions of **2** and **11** (Table 5). The amount of water had an effect on the yield of **14**, which also differed from the reaction of **2**. When the amount of water was changed to 2, 5, 8, and 10 mL, the yield of **14** after 3 h increased from 43% to 67% (entries 7–14). These are the steady states as confirmed by the reactions conducted for 5 h. The heterogeneous reaction using **6** therefore was concluded to occur in the water phase. In such a case, the amount of **11** in the water phase increases with increasing amount of water,

RhCl$_3$·3H$_2$O
(0.12 mmol)
$\xrightarrow[\text{40 °C, 1 h}]{\text{H}_2\text{O (8 mL)-(CH}_3\text{S)}_2 \text{ } \mathbf{11} \text{ (2.7 mL)}}$
→ Water phase
→ (CH$_3$S)$_2$ phase

[HO(CH$_2$)$_3$S]$_2$ + (HOOCCH$_2$S)$_2$ $\xrightarrow[\text{40 °C, 2 h}]{\text{Water phase}}$ HO(CH$_2$)$_3$SSCH$_2$COOH
2 (1 mmol)   **4** (1 mmol)   **5** 1.04 mmol

[HO(CH$_2$)$_3$S]$_2$ $\xrightarrow[\text{40 °C, 2 h}]{\text{(CH}_3\text{S)}_2 \text{ phase}}$ HO(CH$_2$)$_3$SSCH$_3$
2 (1 mmol)   **12** 1.33 mmol

Scheme 5.

Table 7
Disulfide exchange reaction of **6** and **11**

[HCl·H$_2$N(CH$_2$)$_6$S]$_2$ + (CH$_3$S)$_2$ $\xrightarrow[\text{H}_2\text{O, 40 °C}]{\text{RhCl}_3\cdot\text{3H}_2\text{O (12 mol\%)}}$
6 (0.25 mmol)   **11**

$\xrightarrow[\text{1,4-dioxane–NaOH aq. r.t., 2 h}]{\text{(Boc)}_2\text{O: 1.2 eq.}}$ BocHN(CH$_2$)$_6$SSCH$_3$
14

Entry	(CH$_3$S)$_2$ (equiv.)	H$_2$O (mL)	Time (h)	Yield of **14** (%)
1	4	5	3	55
2	4	5	5	51
3	8	5	3	55
4	8	5	5	54
5	15	5	3	53
6	15	5	5	53
7	30	5	3	55
8	30	5	5	55
9	30	2	3	43
10	30	2	5	42
11	30	8	3	58
12	30	8	5	63
13	30	10	3	67
14	30	10	5	68

which shifts the equilibrium to **14**. An increase in the amount of **11** should not affect the equilibrium, since the amount of **11** in the water phase does not change.
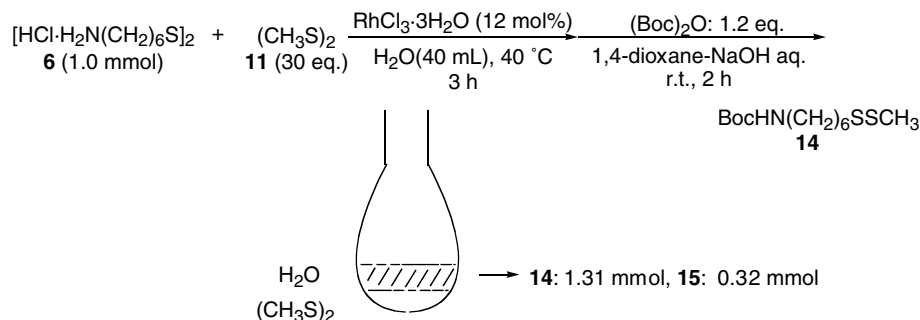
The distributions of **6** and **13** in water and dimethyl disulfide were examined (Scheme 6). The compound **6** (1 mmol) was treated with **11** (30 equiv.) in the presence of RhCl$_3$ · 3H$_2$O (12 mol%) at 40 °C in water (40 mL) for

3 h, and the water and dimethyl disulfide phases were separated. Each phase was treated with Boc$_2$O in 1 M NaOH and 1,4-dioxane for 2 h at room temperature. From the water phase, **14** (1.31 mmol) and [BocHN(CH$_2$)$_6$S]$_2$ (0.32 mmol) **15** were obtained, while **14** and **15** were not detected in the dimethyl disulfide phase. Thus, **6** and **13** existed in only the water phase, which is consistent with the disulfide exchange reaction taking place in the water phase. The rate of this heterogeneous system being comparable to that of the homogeneous reaction of **1** and **2** also supported the interpretation.

The equilibrium in the water phase was examined using the solubility of **11**, that is 0.038 mmol/mL at 40 °C [15]. The reaction of **6** (0.25 mmol) and **11** (7.5 mmol) in water (10 mL) gave **15** (0.083 mmol) and **14** (0.334 mmol) (Table 7, entry 13). The amount of **11** in water can be calculated as 0.38 mmol, and a reasonable equilibrium constant $K = [\mathbf{13}]^2/[\mathbf{6}] \cdot [\mathbf{11}] = 3.5$ was obtained.

This heterogeneous method using **11** can be applied to **8** (Table 8), the reaction of which was presumed to proceed in the water phase analogous to **6**. The reaction of **8** with **11** (30 equiv.) in water in the presence of RhCl$_3$ (12 mol%) gave **16** in 40% yield after 3 h (Table 8, entry 1). The yield did not change after 12 h (entry 2). The reaction with **8** gave higher yields of **16** at higher temperatures (entry 3), which may be due to the higher solubility of **11** in water at high temperatures.

The RhCl$_3$-catalyzed disulfide exchange reactions of **11** under heterogeneous conditions are shown to be rather complex, and highly dependent on the solubility of

[HCl·H$_2$N(CH$_2$)$_6$S]$_2$ + (CH$_3$S)$_2$ $\xrightarrow[\text{H}_2\text{O(40 mL), 40 °C}]{\text{RhCl}_3\cdot\text{3H}_2\text{O (12 mol\%)}}$ $\xrightarrow[\text{1,4-dioxane-NaOH aq.}]{\text{(Boc)}_2\text{O: 1.2 eq.}}$
6 (1.0 mmol)   **11** (30 eq.)   3 h   r.t., 2 h

BocHN(CH$_2$)$_6$SSCH$_3$
14

H$_2$O
(CH$_3$S)$_2$
→ **14**: 1.31 mmol, **15**: 0.32 mmol

Scheme 6.

Table 8
Exchange reaction of glutathione disulfide **8** and **11**

Entry	Temperature (°C)	Time (h)	Yield of **16** (%)
1	40	3	40
2	40	12	41
3	65	3	61
4	65	12	62

substrates and products. The reaction takes place in the dimethyl disulfide phase for **2** being soluble in dimethyl disulfide, and in the water phase for **8** being insoluble in dimethyl disulfide. This is an example that the analysis of heterogeneous reaction using water solvent is complex. We need to know more about homogenous reactions in water before the heterogeneous system could be understood. It should also be emphasized that an equilibrium reaction can be used as a probe for examining the heterogeneous reaction.

2. Experimental

2.1. 2-Carboxyethyl 3-hydroxypropyl disulfide (3)

In a flask were placed $RhCl_3 \cdot 3H_2O$ (6 mol%, 3.9 mg, Wako Pure Chemical Industries, Ltd.), bis(2-carboxyl-ethyl) disulfide **1** (0.25 mmol, 52.5 mg), and bis(3-hydroxy-propyl) disulfide **2** (1.0 mmol, 182 mg) in degassed water (15 mL) under an argon atmosphere, and the solution was heated at 40 °C for 3 h. Then, the mixture without concentration was subjected to short reversed phase column chromatography [Wakogel® 50C18 (38–63 μm), MeOH/water, 1:1] to remove the Rh complex. After concentration, the residue was purified by silica gel chromatography (AcOH/Et₂O/hexane, 1:10:10) to give **3** (78.4 mg, 80%) with recovered **1** (8.4 mg, 16%) and **2** (144.4 mg, 79%). Mp. 72–73 °C (CHCl₃). ¹H NMR (400 MHz, CD₃OD₃) δ 1.89 (2H, quintet, $J = 7.2$ Hz), 2.70 (2H, t, $J = 7.2$ Hz), 2.78 (2H, t, $J = 7.2$ Hz), 2.90 (2H, t, $J = 7.2$ Hz), 3.62 (2H, t, $J = 6.4$ Hz). ¹³C NMR (100 MHz, CD₃OD) δ 32.9, 34.2, 34.8, 35.9, 61.1, 175.4. IR (KBr) 3416, 2947, 1703, 1538, 1261 cm⁻¹. MS (EI) m/z 196 (M⁺, 100). HRMS: Calcd. for $C_6H_{12}O_3S_2$: 196.0228. Found: 196.0203. Anal. Calc. for $C_6H_{12}O_3S_2$: C, 36.71; H, 6.16; S, 32.67. Found: C, 36.47; H, 5.96; S, 32.79%.

2.2. Carboxymethyl 3-hydroxypropyl disulfide (5)

¹H NMR (400 MHz, CD₃OD₃) δ 1.90 (2H, quintet, $J = 6.8$ Hz), 2.84 (2H, t, $J = 7.2$ Hz), 3.47 (2H, s), 3.63 (2H, t, $J = 6.8$ Hz). ¹³C NMR (100 MHz, CD₃OD) δ 32.8, 35.5, 42.2, 61.1, 173.3. IR (neat) 3343, 2940, 1714,

1284 cm⁻¹. MS (EI) m/z 182 (M⁺, 100), 106 (M⁺−76, 82). HRMS: Calcd. for $C_5H_{10}O_3S_2$: 182.0071. Found: 182.0085.

2.3. 6-(tert-Butoxycarbonylamino)hexyl 3-hydroxyprpyl disulfide (7)

In a flask were placed $RhCl_3 \cdot 3H_2O$ (6 mol%, 3.9 mg), bis(6-aminohexyl) disulfide dihydrochloride **6** (0.25 mmol, 84 mg), and bis(3-hydroxypropyl) disulfide **2** (1.0 mmol, 182 mg) in degassed water (15 mL) under an argon atmosphere, and the solution was heated at 40 °C for 3 h. Then, the mixture without concentration was subjected to short flash column chromatography (AcOEt/Et₃N, 1:1) to remove Rh complex, and the eluents were concentrated. The residue was dissolved in 1 M NaOH (0.6 mL), to which was added Boc₂O (0.60 mmol, 131.0 mg) in 1,4-dioxane (2 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, and was subjected to flash column chromatography without concentration to give **7** (128.5 mg, 80%) and **15** (15.0 mg, 13%) with recovered **2** (143.7 mg, 79%). ¹H NMR (CDCl₃, 400 MHz) δ 1.34 (2H, q, $J = 7.6$ Hz), 1.44 (9H, s), 1.37–1.52 (4H, m), 1.68 (2H, quintet, $J = 7.6$ Hz), 1.94 (2H, quintet, $J = 6.8$ Hz), 2.68 (2H, t, $J = 7.2$ Hz), 2.77 (2H, t, $J = 7.2$ Hz), 3.10 (2H, q, $J = 6.4$ Hz), 3.73 (2H, t, $J = 6.0$ Hz), 4.70 (1H, br). ¹³C NMR δ 26.1, 27.9, 28.3, 28.8, 29.7, 31.7, 35.1, 38.6, 40.3, 60.7, 78.9, 155.9. IR (neat) 3355, 2931, 1688, 1523, 1365, 1252, 1171 cm⁻¹. MS (EI) m/z 323 (M⁺, 100), 177 (M⁺−146, 64), 132 (M⁺−191, 67), 57 (M⁺−266, 83). HRMS. Calc. for $C_{14}H_{29}NO_3S_2$: 323.1589. Found: 323.1577.

2.4. Homogeneous equilibrium experiments of 2 and 4

Reaction (a): A mixture of $RhCl_3 \cdot 3H_2O$ (6 mol%, 15.8 mg), **2** (1.0 mmol, 182 mg), and **4** (1.0 mmol, 182 mg) in degassed water (15 mL) under an argon atmosphere was heated at 40 °C for 1 h. The mixture without concentration was purified by reversed phase column chromatography (MeOH/water, 1:1) to give **5** (182.3 mg, 1.0 mmol) with recovered **2** (91.0 mg, 0.5 mol) and **4** (91.2 mg, 0.5 mmol). *Reaction (b):* A mixture of $RhCl_3 \cdot 3H_2O$ (3 mol%, 15.8 mg) and **5** (2.0 mmol, 364 mg) in degassed water (15 mL) under an argon atmosphere was heated at 40 °C for 1 h. The mixture without concentration was purified by reversed phase column chromatography to give **2** (90.6 mg, 0.5 mmol) and **4** (90.5 mg, 0.5 mmol) with recovered **5** (181.8 mg, 1.0 mmol).

2.5. Equilibrium shift experiment of 2 and 4

A mixture of $RhCl_3 \cdot 3H_2O$ (6 mol%, 15.8 mg), **2** (1.0 mmol, 182 mg), **4** (1.0 mmol, 182 mg), and 1,4-dioxane (0.25 mmol, 0.0213 mL, internal standard) in degassed water (15 mL) under an argon atmosphere was heated at 40 °C for 24 h. A small amount of sample was taken, and ¹H NMR (CD₃OD) analysis indicated the presence of **5** (0.98 mmol) with recovered **2** (0.50 mmol) and **4**

(0.46 mmol). Then, another portion of **4** (3.0 mmol, 546 mg) was added, and the mixture was heated at 40 °C for 3 h. ^{1}H NMR (CD$_3$OD) analysis showed the presence of **5** (1.55 mmol) with recovered **2** (0.20 mmol) and **4** (3.07 mmol). Next addition of **4** (3.0 mmol, 546 mg) followed by heating at 40 °C for 3 h gave **5** (1.75 mmol) with recovered **2** (0.14 mmol) and **4** (6.12 mmol) by ^{1}H NMR (CD$_3$OD). The mixture without concentration was subjected to reversed phase column chromatography (MeOH/water, 1:1) to give **5** (314 mg, 1.73 mmol) with recovered **2** (20.3 mg, 0.11 mmol) and **4** (1110 mg, 6.10 mmol).

2.6. Carboxymethyl N-(N-γ-glutamylcysteinyl)glycyl disulfide (9)

In a flask were placed RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), glutathione disulfide **8** (0.25 mmol, 152 mg), and **4** (1.0 mmol, 182 mg) in degassed water (5 mL) under an argon atmosphere, and the solution was heated at 40 °C for 1 h. The mixture without concentration was purified by reversed phase column chromatography (MeOH/water, 1:9) to give **9** (160.7 mg, 81%) with recovered **8** (28.7 mg, 0.047 mmol) and **4** (140.1 mg, 0.77 mmol). Mp. 103–104 °C (H$_2$O–EtOH–Et$_2$O). ^{1}H NMR (400 MHz, D$_2$O) δ 2.17 (2H, q, J = 7.2 Hz), 2.54 (2H, q, J = 7.6 Hz), 3.02 (1H, dd, J = 9.6, 14.0 Hz), 3.30 (1H, dd, J = 4.4, 14.0 Hz), 3.56 (2H, s), 3.83 (1H, t, J = 6.4 Hz), 3.98 (2H, s), 4.70–4.77 (1H, m). ^{13}C NMR (100 MHz, D$_2$O) δ 28.9, 34.1, 41.3, 43.9, 44.4, 55.6, 56.6, 175.5, 176.3, 176.4, 177.6, 177.8. IR (KBr) 3272, 1726, 1647, 1536, 1232 cm^{-1}. FABMS: *m/z* 398 (M$^+$+H). HRMS: Calcd. for C$_{12}$H$_{20}$N$_3$O$_8$S$_2$: 398.0692. Found: 398.0681. Anal. Calc. for C$_{12}$H$_{19}$N$_3$O$_8$S$_2$: C, 36.27; H, 4.82; N, 10.57; S, 16.14. Found: C, 36.56; H, 4.86; N, 10.27; S, 16.51%. $[\alpha]_D^{22}$ − 66.4 (c 1.0, H$_2$O).

2.7. 6-Aminohexyl N-(N-γ-glutamylcysteinyl)glycyl disulfide (10)

Mp 156–157 °C (H$_2$O–EtOH–Et$_2$O). ^{1}H NMR (400 MHz, D$_2$O) δ 1.36–1.49 (4H, m), 1.62–1.76 (4H, m), 2.16 (2H, q, J = 7.6 Hz), 2.55 (2H, t, J = 7.2 Hz), 2.76 (2H, q, J = 7.6 Hz), 2.93–2.30 (3H, m), 3.26 (1H, dd, J = 4.4, 14.0 Hz), 3.72–3.77 (3H, m), 4.73–4.84 (1H, m). ^{13}C NMR (100 MHz, D$_2$O) δ 27.9, 29.1, 29.3, 29.7, 30.7, 34.3, 40.8, 41.8, 42.2, 46.2, 55.6, 56.9, 174.7, 176.7, 177.8, 178.9. IR (KBr) 3411, 3264, 3058, 2931, 1648, 1540 cm^{-1}. FABMS: *m/z* 439 (M$^+$+H). HRMS: Calcd. for C$_{16}$H$_{31}$N$_4$O$_6$S$_2$: 439.1685. Found: 439.1687. $[\alpha]_D^{22}$ − 68.4 (c 1.0, H$_2$O). Anal. Calc. for C$_{16}$H$_{30}$N$_4$O$_6$S$_2$: C, 43.82; H, 6.89; N, 12.78; S, 14.62. Found: C, 43.73; H, 6.96; N, 12.45; S, 14.51%.

2.8. 3-Hydroxypropyl methyl disulfide (12) [16]

In a flask were placed RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), **2** (0.25 mmol, 45.5 mg), and dimethyl disulfide **11**

(7.5 mmol, 0.68 mL) in degassed water (2 mL) under an argon atmosphere, and the mixture was heated at 40 °C for 1 h. The mixture without concentration was subjected to flash column chromatography (AcOEt/hexane, 1:1) to give **12** (60.9 mg, 88%) with the recovered **2** (5.0 mg, 11%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.72 (1H, br), 1.97 (2H, quintet, J = 6.4 Hz), 2.41 (3H, s), 2.82 (2H, t, J = 6.4 Hz), 3.76 (2H, t, J = 6.4 Hz). ^{13}C NMR (100 MHz, CDCl$_3$) δ 23.0, 31.7, 34.3, 60.9. IR (neat) 3336, 2913, 1428, 1257, 1055 cm^{-1}. MS (EI) *m/z* 138 (M$^+$, 89), 80 (M$^+$−58, 100). HRMS: Calcd. for C$_4$H$_{10}$OS$_2$: 138.0173. Found: 138.0158.

2.9. Heterogeneous equilibrium experiments of 2 and 11

Reaction (a): A mixture of RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), **2** (0.25 mmol, 45.5 mg), and **11** (0.25 mmol, 0.023 mL) in degassed water (2 mL) under an argon atmosphere was heated at 40 °C for 15 min. The mixture without concentration was subjected to flash column chromatography to give **12** (0.14 mmol, 19.3 mg) with recovered **2** (0.17 mmol, 31.4 mg). *Reaction (b):* A mixture of RhCl$_3$ · 3H$_2$O (3 mol%, 3.9 mg) and **12** (0.5 mmol, 69 mg) in degassed water (2 mL) under an argon atmosphere was heated at 40 °C for 15 min. The mixture without concentration was subjected to flash column chromatography to give **2** (0.16 mmol, 29.2 mg) with recovered **12** (0.15 mmol, 20.7 mg).

2.10. Equilibrium shift experiment of 2 and 11

Reaction (a): A mixture of RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), **2** (0.25 mmol, 45.5 mg), and **11** (0.25 mmol, 0.023 mL) in degassed water (2 mL) under an argon atmosphere was heated at 40 °C for 10 min. The mixture without concentration was purified by flash column chromatography to give **12** (0.13 mmol, 17.9 mg) with recovered **2** (0.17 mmol, 31.1 mg). *Reaction (b):* A mixture of RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), **2** (0.25 mmol, 45.5 mg), and **11** (0.25 mmol, 0.023 mL) in degassed water (2 mL) under an argon atmosphere was heated at 40 °C for 10 min. Then, another portion of **11** (0.75 mmol, 0.068 mL) was added, and the mixture was heated at 40 °C for 10 min. The mixture without concentration was purified by flash column chromatography to give **12** (0.21 mmol, 29 mg) with recovered **2** (0.13 mmol, 23.6 mg). *Reaction (c):* A mixture of RhCl$_3$ · 3H$_2$O (6 mol%, 3.9 mg), **2** (0.25 mmol, 45.5 mg), and **11** (0.25 mmol, 0.023 mL) in degassed water (2 mL) under an argon atmosphere was heated at 40 °C for 10 min. Then, another portion of **11** (0.75 mmol, 0.068 mL) was added, and the mixture was heated at 40 °C for 10 min. Another portion of **11** (2.75 mmol, 0.25 mL) was added, and the mixture was heated at 40 °C for 10 min. The mixture without concentration was purified by flash column chromatography to give **12** (0.36 mmol, 49.7 mg) and recovered **2** (0.05 mmol, 9.1 mg).

2.11. 6-(tert-Butoxycarbonylamino)hexyl methyl disulfide (**14**)

In a flask were placed $RhCl_3 \cdot 3H_2O$ (12 mol%, 7.8 mg), bis(6-aminohexyl) disulfide dihydrochloride **6** (0.25 mmol, 84 mg), and dimethyl disulfide **11** (7.5 mmol, 0.68 mL) in degassed water (10 mL) under an argon atmosphere, and the mixture was heated at 40 °C for 3 h. The mixture without concentration was passed through reversed phase column chromatography (MeOH/0.1 M HCl, 1:1) to remove Rh complex. The eluent was neutralized to pH 8 by adding 1 M NaOH, and concentrated. The residue was dissolved in 1 M NaOH (0.6 mL), to which was added Boc_2O (0.6 mmol, 131.0 mg) in 1,4-dioxane (2 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, and was subjected without concentration to flash column chromatography (Et$_2$O/hexane, 1:5) to give **14** (93.2 mg, 67%) and bis[6-(*tert*-butoxycarbonylamino)hexyl] disulfide **15** (38.1 mg, 33%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.34 (2H, q, $J = 6.4$ Hz), 1.44 (9H, s), 1.40–1.52 (4H, m), 1.69 (2H, quintet, $J = 7.6$ Hz), 2.40 (3H, s), 2.69 (2H, t, $J = 7.2$ Hz), 3.10 (2H, q, $J = 7.2$ Hz), 4.50 (1H, br). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 23.2, 26.3, 28.0, 28.3, 29.0, 29.9, 38.0, 40.4, 78.9, 155.9. IR (neat) 3354, 2975, 2929, 2857, 1696, 1516, 1365, 1250, 1172 cm^{-1}. MS (EI) m/z 279 (M$^+$, 36), 176 (M$^+$−103, 66), 74 (M$^+$−74, 205), 57 (M$^+$−222, 100). HRMS: Calcd. for C$_{12}$H$_{25}$NO$_2$S$_2$: 279.1327. Found: 279.1302.

2.12. Experiment to examine the distribution of 2 and 12 in the heterogeneous system

A mixture of $RhCl_3 \cdot 3H_2O$ (6 mol%, 15.8 mg), **2** (1.0 mmol, 182 mg), and **11** (30 mmol, 2.7 mL) in degassed water (8 mL) under an argon atmosphere was heated at 40 °C for 75 min. Then, water and dimethyl disulfide phase were separated. The water phase without concentration was subjected to flash column chromatography (AcOEt/Hexane, 1:1) to give **12** (0.46 mmol, 63.4 mg) with recovered **2** (0.04 mmol, 7.3 mg). The dimethyl disulfide phase without concentration was subjected to flash column chromatography to give **12** (1.30 mmol, 179.5 mg) with recovered **2** (0.04 mmol, 7.2 mg).

2.13. Experiment to examine the distribution of 6 and 14 in the heterogeneous system

A mixture of $RhCl_3 \cdot 3H_2O$ (12 mol%, 31.2 mg), **6** (1.0 mmol, 336 mg), and **11** (30 mmol, 2.7 mL) in degassed water (40 mL) under an argon atmosphere was heated at 40 °C for 3 h. Then, water and dimethyl disulfide phase were separated. The water phase without concentration was passed through reversed phase column chromatography (MeOH/0.1 M HCl = 1:1) to remove Rh complex. The eluent was neutralized to pH 8 by adding 1 M NaOH, and concentrated. The residue was dissolved in 1 M NaOH (2.4 mL), to which was added Boc_2O (2.4 mmol, 524 mg) in 1,4-dioxane (8 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, and was subjected without concentration to flash column chromatography (Et$_2$O/hexane, 1:5) to give **14** (1.31 mmol, 365.3 mg) and **15** (0.32 mmol, 148.5 mg). The dimethyl disulfide phase without concentration was passed through reversed phase column chromatography (MeOH/0.1 M HCl = 1:1) to remove Rh complex. The residue was dissolved in 1 M NaOH (2.4 mL), to which was added Boc_2O (2.4 mmol, 524 mg) in 1,4-dioxane (8 mL) at 0 °C. The mixture was stirred at room temperature for 2 h. Compounds **13** and **15** were not detected by TLC.

2.14. Experiment to examine the distribution of RhCl$_3$ in the heterogeneous system

A mixture of $RhCl_3 \cdot 3H_2O$ (0.12 mmol, 31.2 mg) and **11** (30 mmol, 2.7 mL) in degassed water (8 mL) under an argon atmosphere was heated at 40 °C for 1 h. Then, water and dimethyl disulfide phases were separated. In a flask were placed **2** (1 mmol, 182 mg) and **4** (1 mmol, 182 mg) under an argon atmosphere, to which the water phase was added. The mixture was heated at 40 °C for 2 h, and was passed through reversed phase column chromatography to remove Rh complex. After concentration, the residue was purified by flash column chromatography to give **5** (1.04 mmol, 189.2 mg), **12** (0.10 mmol, 13.8 mg), and carboxymethyl methyl disulfide (0.10 mmol, 13.9 mg) with recovered **2** (0.43 mmol, 78.2 mg) and **5** (0.42 mmol, 76.4 mg). In another flask was placed **2** (1 mmol, 182 mg) under an argon atmosphere, to which the dimethyl disulfide phase was added. The mixture was heated at 40 °C for 2 h. The mixture without concentration was subject to flash column chromatography to give **12** (1.33 mmol, 183.5 mg) with recovered **2** (0.33 mmol, 60 mg).

2.15. Carboxymethyl methyl disulfide [17]

^{1}H NMR (400 MHz, CDCl$_3$) δ 2.49 (3H, s), 3.51 (2H, s). ^{13}C NMR (100 MHz, CDCl$_3$) δ 22.9, 40.6, 176.2. IR (neat) 3090, 2995, 1707, 1417, 1292 cm^{-1}. MS (EI) m/z 138 (M$^+$, 100), 93 (M$^+$−45, 49). HRMS: Calcd. for C$_3$H$_6$O$_2$S$_2$: 137.9809. Found: 137.9764.

2.16. N-(N-γ-Glutamylcysteinyl)glycyl methyl disulfide (**16**) [18]

Mp. 202–203 °C decomp. (H$_2$O–EtOH–Et$_2$O). lit. [18] 203–204 °C decomp. ^{1}H NMR (400 MHz, D$_2$O) δ 2.15 (2H, q, $J = 7.2$ Hz), 2.41 (3H, s), 2.53 (2H, q, $J = 6.4$ Hz), 2.98 (1H, dd, $J = 9.6$, 14.2 Hz), 3.25 (1H, dd, $J = 4.4$, 14.8 Hz), 3.80 (1H, t, $J = 6.4$ Hz), 3.95 (2H, s), 4.69–4.80 (1H, m). ^{13}C NMR (100 MHz, D$_2$O) δ 24.9, 29.0, 34.2, 40.7, 45.9, 55.5, 56.8, 174.6, 176.4, 177.5, 178.3. IR (KBr) 3374, 3351, 3000, 1676, 1643, 1513 cm^{-1}. FABMS: m/z 354 (M$^+$+H). HRMS: Calcd. for C$_{11}$H$_{20}$N$_3$O$_6$S$_2$: 354.0794. Found: 354.0806. Anal. Calc.

for $C_{11}H_{20}N_3O_6S_2$: C, 37.38; H, 5.42; N, 11.89; S, 18.15. Found: C, 37.08; H, 5.53; N, 11.86; S, 18.08%. $[\alpha]_D^{23} -82.8$ (*c* 1.0, H_2O).

2.17. The solubility of dimethyl disulfide 11 in water at 40 °C

In five screw vials with cap were placed dimethyl disulfide **11**, 0.033 mL (0.366 mmol), 0.034 mL (0.377 mmol), 0.035 mL (0.388 mmol), 0.036 mL (0.40 mmol), and 0.037 mL (0.411 mmol), to which degassed water (10 mL) was added. The mixtures were heated at 40 °C with ultrasonication. The first two vials contained clear water solution, and the rest of vials emulsion.

Acknowledgements

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