Rhodium-Catalyzed Synthesis of Peptide Polysulfides by Insertion of Sulfur into Unprotected Peptide Disulfides

Fukumoto, Kohei
Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Yazaki, Masana
Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Arisawa, Mieko
Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

出版情報：Organic Letters. 24 (44), pp.8176-8179, 2022-11-01. American Chemical Society (ACS)
バージョン：

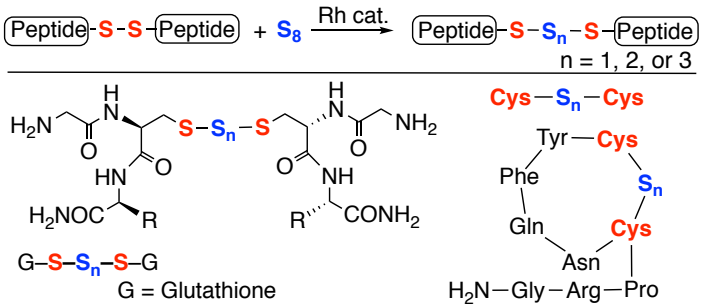
Rhodium-Catalyzed Synthesis of Peptide Polysulfides by Sulfur Insertion Reaction into Unprotected Peptide Disulfides

Kohei Fukumoto[a,b], Masana Yazaki[a], Mieko Arisawa[a]*

[a]*Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, 819-0395, Japan*

[b]*Department of Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Sendai, 980-8578, Japan*

Supporting Information Placeholder



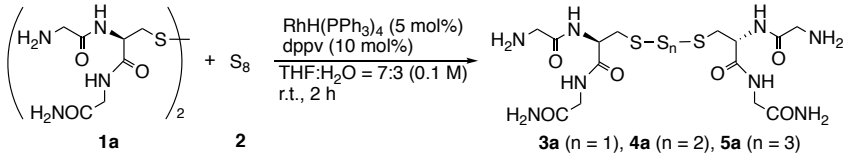
ABSTRACT: A rhodium-catalyzed sulfur insertion reaction into unprotected peptide disulfides in aqueous solvents has been developed, which yields mixtures of trisulfides and tetrasulfides. This method can be applied to peptides containing amino acids such as Gly, Phe, Tyr, Ser, Met, Asp, Gln, and Lys, and provides polysulfides with various amino acid residues without being affected by functional groups. A reaction can be conducted on a gram scale. Vasopressin can also be converted into its corresponding polysulfides.

Diverse chemical structures of organosulfur compounds containing sulfur atoms with redox states between –2 and +6 are distributed in nature, which exhibit various chemical reactivities.[1] An interesting property of sulfur is its ability to undergo catenation to form polysulfides, and sulfur S_8 with an eight-membered ring structure is a well-known example. It has become evident with the progress of analytical technology that polysulfides formed by sulfur catenation are common in biological systems. Peptides and proteins containing polysulfides occur in cells as sulfur metabolites, which are collectively called supersulfides, and their functions in cell signaling involving hydrogen sulfide H_2S and iron–sulfur cluster have attracted much interest.[2,3] Recently, it has also been suggested that supersulfides are responsible for energy metabolism (sulfur respiration) in mitochondria.[3]

Unlike disulfides, which have electrophilic sulfur atoms, the multiple sulfur atom groups in a polysulfide (RSS_nSR) are relatively unstable in living cells and react with coexisting nucleophiles, electrophiles, and reducing/oxidizing reagents, resulting in migration, recombination, catenation, reduction, and oxidation. For example, a polysulfide in water under basic conditions exists in equilibrium with polysulfide thiolates (GSS^-) and sulfenic acids (GSOH). The former reacts with acids and electrophiles E^+ to give polysulfide thiols (RSSH) and unsymmetrical disulfides (GSSE), respectively, and the latter react with thiols to give disulfides (GSSR) and water.[4] The chemical reactivity of polysulfides is affected by environmental conditions such as pH, temperature, and medium. In living cells, polysulfide thiols formed from polysulfides, play a pivotal role in cell signaling.[2] It is therefore necessary to understand the chemical and biological properties of synthetic peptide polysulfides, and synthetic organic chemistry can be important as a means of supplying diverse compounds in large quantities; this however, has not been extensively studied.

Bis(2-amino-2-carbonylethyl) trisulfide was obtained by hydrolysis of wool and other proteins.[5a] Cystine and glutathione polysulfides were synthesized in Tris-HCl buffer (pH 7.4) in the presence of iodine by the oxidative reaction of cysteine/glutathione thiols with NaSH.[2a] It has, however, been observed that residual iodide compounds cannot be easily removed completely and can promote decomposition of polysulfide peptides. A protected cysteine derivative was reacted with sulfur (10 equivalents) in $EtOH/CHCl_3/CS_2/NH_4OH$ (v/v = 4.5/5/2/2) at pH 10, and a protected trisulfide among several polysulfides was isolated by HPLC, although its yield was not reported.[5b] A general method of synthesizing peptide polysulfides from unprotected peptides is desired, one that does not use a H_2S derivative under iodine oxidation condition.

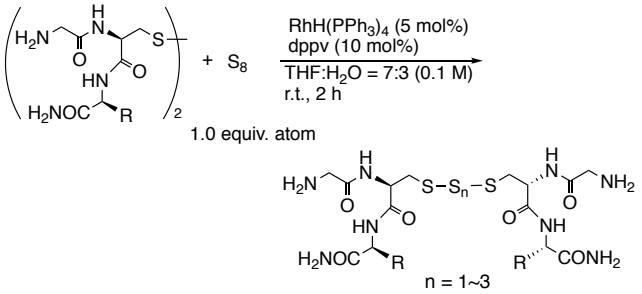
Table 1. Rhodium-catalyzed sulfur insertion reaction into disulfide of Gly–Cys–Gly 1a



Entry	Conditions	Yield of **3a**	Yield of **4a**	Yield of **5a**	ΔS
1	Use of S_8 (1.0 equiv. atom)	30%[b]	22%[b]	8%[a]	98%
2	Use of S_8 (2.0 equiv. atom)	31%[b]	27%[b]	7%[a]	53%
3	Use of S_8 (0.5 equiv. atom)	40%[b]	20%[b]	n.d.[c]	80%
4	THF	n.d.[c]	n.d.[c]	n.d.[c]	-
5	DMSO	n.d.[c]	n.d.[c]	n.d.[c]	-
6[a]	DMSO:H_2O = 7:3	16%	4%	trace	24%
7[a]	DMF:H_2O = 7:3	35%	10%	2%	61%
8[a]	acetone:H_2O = 7:3	24%	13%	n.d.[c]	50%
9[a]	CH_3CN:H_2O = 7:3	22%	trace	n.d.[c]	22%
10[a]	EtOH:H_2O = 7:3	9%	8%	n.d.[c]	25%
11[a]	1,4-dioxane:H_2O = 7:3	10%	trace	n.d.[c]	10%
12[a]	Without $RhH(PPh_3)_4$	n.d.[c]	n.d.[c]	n.d.[c]	-
13[a]	Without dppv	n.d.[c]	n.d.[c]	n.d.[c]	-
14[a]	Use of $RhCl_3{\cdot}3H_2O$ 5 mol%	10%	4%	n.d.[c]	18%

[a]Yield of polysulfide **3a**, **4a**, or **5a** were calculated by HPLC. [b]Isolated yields of **3a** and **4a**. [c]n.d. = not detected.

Scheme 1. Rhodium-catalyzed sulfur insertion reaction into unprotected peptide disulfides

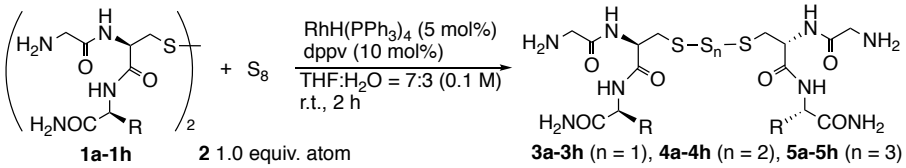


We previously reported the sulfur insertion reaction between simple organic disulfides and trisulfides catalyzed by rhodium phosphine complexes in an organic solvent.[6,7] Sulfur is an attractive reagent for the synthesis of peptide polysulfides, because it is stable, easy to handle, and non-acidic. In addition, the use of sulfur does not require oxidative reaction conditions. In this paper, we describe a rhodium-catalyzed sulfur insertion reaction into unprotected peptide disulfides in aqueous solvents, which efficiently provides peptide trisulfides, tetrasulfides, and pentasulfides (Scheme 1). The rhodium-catalyzed method employs a stoichiometric amount of sulfur and provides various peptide polysulfides at room temperature under neutral and acidic conditions. The reaction is also be applied to glutathione and cystine under modified conditions. Sulfur is also inserted into the disulfide of vasopressin, a cyclic nanopeptide.

A disulfide derivative of Gly–Cys–Gly **1a** (0.1 mmol) was treated with sulfur **2** (1 equivalent atom, 0.0125 mmol) in the presence of $RhH(PPh_3)_4$ (5 mol%) and 1,2-bis(diphenylphosphino)ethylene (dppv, 10 mol%) in a 7:3 mixed solution (0.1 M) of tetrahydrofuran (THF) and water at room temperature for 2 h. Then, trisulfide **3a** (0.03 mmol, 30%), tetrasulfide **4a** (0.022 mmol, 22%), and pentasulfide **5a** (0.008 mmol, 8%) were obtained with the recovery of **1a** (0.03 mmol, 30%) (Table 1, entry 1). **3a** and **4a** were isolated by reverse-phase column chromatography, and structures were determined by NMR, IR, and MS. **5a** was not isolated because of its instability, and the structure and yield of **5a** were determined by LCMS-IT-TOF. No hexasulfide or heptasulfide was detected by the LCMS. At this stage, **3a** contains less than 0.1% of rhodium metal as indicated by ICP OES analysis. Amorphous powders of **3a** can be stably stored at –5 °C for several months. **3a** is stable also in water at room temperature for several days and at –5 °C for over a month without decomposition. However, **4a** is relatively unstable in water (0.025 M) at room temperature and is converted to a mixture of **1a**, **3a**, and **4a** at a 2:2:1 ratio after 1 h. **5a** is less stable than **4a**, and a very rapid disproportionation occurs in D_2O.

The exchange efficiency ΔS is defined by $\{[\mathbf{3a}] + 2[\mathbf{4a}] + 3[\mathbf{5a}]\}/[\mathbf{2}]_0$, in which [**3a**], [**4a**], and [**5a**] are the yields (mmol) of products and $[\mathbf{2}]_0$ is the initial amount (mmol atom) of **2**.[6] ΔS indicates the amount of sulfur atoms in the polysulfides derived from sulfur **2**, and ΔS = 100% indicates that all the sulfur atoms of sulfur **2** are transferred into polysulfides. This reaction proceeds at a high efficiency ΔS = 98%, which enables the use of a stoichiometric amount of sulfur **2** (entry 1). Increasing the amount of sulfur to 2 equivalents provided **3a** (31%), **4a** (27%), and **5a** (7%) (entry 2) with ΔS = 53%. When the amount was reduced to 0.5 equivalents, **3a** and **4a** were formed in 40% and 20% yields based on sulfur, respectively, with ΔS = 80%, and

Table 2. Rhodium-catalyzed synthesis of unprotected peptide polysulfides using sulfur 2



1a-1h **2** 1.0 equiv. atom **3a-3h** (n = 1), **4a-4h** (n = 2), **5a-5h** (n = 3)

Entry	Conditions	Yield of **3**[a]	Yield of **4**[a]	Yield of **5**[b]	ΔS
1	-H (**a**)	30%	22%	8%	98%
2	$-CH_2C_6H_5$ (**b**)	30%	15%	5%	75%
3	$-CH_2C_6H_4OH$ (**c**)	25%	11%	3%	56%
4	$-CH_2OH$ (**d**)	35%	13%	4%	73%
5	$-(CH_2)_2SCH_3$ (**e**)	28%	17%	7%	83%
6	$-CH_2CO_2H$ (**f**)	38%	20%	5%	93%
7	$-(CH_2)_2CONH_2$ (**g**)	33%	15%	4%	75%
8	$-(CH_2)_4NH_2$ (**h**)	36%	12%	4%	72%

[a]Isolated yields of **3** and **4**. [b]Yields of **5** were obtained by HPLC.

no **5a** was detected (entry 3). Changing in the amount of sulfur does not significantly affect yields, which is possibly due to the low solubility of sulfur **2** in the solvent, which enables the gradual dissolution of sulfur **2** as the reaction progress and minimizes the concentration of sulfur **2** in solution, thereby reducing undesired side reactions.

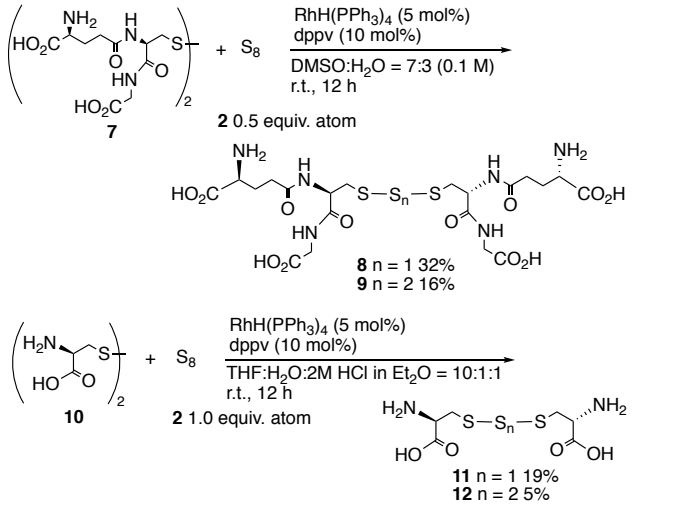
Note that the reaction smoothly proceeded in an aqueous solvent, in which **1a** and the catalyst were both soluble. **1a** did not react in THF because of its low solubility (entry 4). No reaction occurred in DMSO, in which **1a**, the catalyst, and sulfur **2** are soluble (entry 5). Aqueous solvents (v/v = 7/3) containing DMSO, *N,N'*-dimethylformamide (DMF), acetone, and acetonitrile gave comparable yields and suppressed the formation of higher polysulfides, whereas those containing ethanol and 1,4-dioxane gave reduced yields (entries 6–11). The gradual dissolution of sulfur **2** may be an important characteristic of a 7:3 THF–water solution (entry 1). No reaction occurs in the absence of $RhH(PPh_3)_4$ or dppv (entries 12 and 13). $RhCl_3{\cdot}3H_2O$[8] can be used in the presence of dppv, although the yield is low (entry 14).

The reaction has been applied to several unprotected peptides **1a**–**1h** containing disulfides (Table 2). When a disulfide derivative of Gly–Cys–Phe **1b** (0.1 mmol) and **2** (1.0 equiv. atom) were reacted in a 7:3 THF–water solution (0.1 M) at room temperature for 2 h in the presence of both $RhH(PPh_3)_4$ (5 mol%) and dppv (10 mol%), trisulfide **3b**, tetrasulfide **4b**, and pentasulfide **5b** were obtained in 30%, 15%, and 5% yields, respectively, with ΔS = 75%. The reaction can be applied to disulfide derivatives of Gly–Cys–X with X of tyrosine (Tyr), serine (Ser), methionine (Met), aspartic acid (Asp), glutamine (Gln), and lysine (Lys) at ΔS = 56–98% without being affected by phenol, hydroxyl, sulfide, carboxylate, amide, or amino groups. The method enables the synthesis of a range of pure peptide polysulfides.

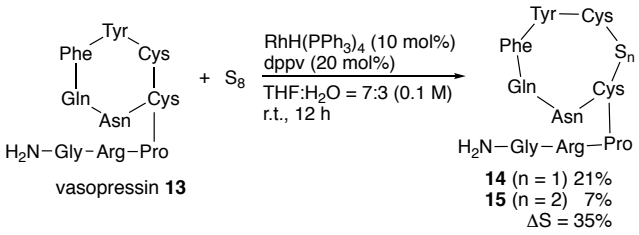
Glutathione disulfide **7** provides the corresponding trisulfide **8** (32%) and tetrasulfide **9** (16%) when reacted with sulfur **2** in 7:3 DMSO–water solution at room temperature for 12 h (Scheme 2). **7** is insoluble in a 7:3 THF–water solution and the reaction does not proceed. A scale-up experiment was conducted for the synthesis of **8** and **9**, and the reaction of **7** (7 mmol) and sulfur **2** (1 equivalent atom) gave **8** and **9** in 25% (1.15 g, 0.24 mmol) and 7% (0.33 g, 0.08 mmol) isolated yields, respectively (Scheme 2). At this stage, 99.9% of the rhodium metal in **8** and **9** were removed as indicated by ICP OES analysis. When a 10:1:1 solution of tetrahydrofuran, water, and 2 M hydrochloric acid in diethyl ether was used, cystine trisulfide **11** and tetrasulfide **12** were obtained in 19% and 5% yield, respectively. Cystine is soluble in acidic aqueous solutions but not in neutral ones. Thus, the composition and pH of aqueous solutions can be adjusted to dissolve peptides, which is an advantage of the present method. This study also provides the full characterization of **8**, **9**, **11**, and **12**, which were previously analyzed only by MS.[2a]

The peptide hormone vasopressin **13**, a cyclic nanopeptide disulfide, was subjected to the sulfur insertion reaction (Scheme 3). When **13** (22 mg, 0.02 mmol) was reacted with sulfur **2** (1.0 equiv. atom) under the standard conditions for 12 h, the corresponding trisulfide **14** (21%) and tetrasulfide **15** (7%) were obtained at ΔS = 35% as indicated by LCMS-IT-TOF. The results

Scheme 2. Rhodium-catalyzed sulfur insertion reaction into glutathione/cystine disulfides



Scheme 3. Rhodium-catalyzed sulfur insertion reaction into disulfide of vasopressin 13



show that this method can be used for modification of biologically active peptides with relatively large molecular weight.

In summary, a rhodium-catalyzed method has been developed to insert sulfur atom into disulfides of unprotected peptides in aqueous solutions to provide mixtures of peptide trisulfides and tetrasulfides. Peptide trisulfides and tetrasulfides can be isolated by reverse phase chromatography. Sulfur is inserted into peptide disulfides without been affected by phenol, hydroxyl, sulfide, carboxylate, amide, or amino groups, and the method can be applied to acyclic and cyclic peptides containing various amino acid residues. The reaction can also be conducted on a gram scale. This method provides a diversity of peptide trisulfides and tetrasulfides for chemical and biological studies of supersulfur compounds, the biological activities of which have recently attracted much interest.[2,3]

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online supplementary material.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedure, characterization data, and NMR spectra for products (PDF)

AUTHOR INFORMATION

Corresponding Author

*Mieko Arisawa–*Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, 819-0395, Japan;*
Email: arisawa@agr.kyushu-u.ac.jp

ORCID

Mieko Arisawa: 0000-0002-9561-2653

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This research was supported by JSPS KAKENHI (Grant Numbers 21K18947), JST Moonshot R&D (Grant Number JPMJMS2033-13), JST FOREST Program (Grant Number JPMJFR205X, Japan), the Uehara Memorial Foundation, Kobayashi Foundation, and Takahashi Industrial and Economic Research Foundation. K.F. and M.Y. thanks the JST FOREST Program (Grant Number JPMJFR205X, Japan) for a Fellowship for Young Scientists.

ABBREVIATIONS

HPLC, High Performance Liquid Chromatography; LCMS-IT-TOF, Liquid Chromatography-Mass spectrometry; NMR, Nuclear Magnetic Resonance; IR, Infrared Spectroscopy.

REFERENCES

(1) Reusch W. Available online: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Thiols_and_Sulfides

(2) (a) Ida, T.; Sawa, T.; Ihara, H.; Tsuchiya, Y.; Watanabe, Y.; Kumagai, Y.; Suematsu, M.; Motohashi, H.; Fujii, S.; Matsunaga, T.; Yamamoto, M.; Ono, K.; Devarie-Baez, N. O.; Xian, M.; Fukuto, J. M.; Akaike, T. Reactive Cysteine Persulfides and *S*–Polythiolation Regulate Oxidative Stress and Redox Signaling. *Proc. Natl. Acad. Sci. USA*, **2014**, *111*, 7606–7611. (b) McLaughlin, M. I.; Lanz, N. D.; Goldman, P. J.; Lee, K.-H.; Booker, S. J.; Drennan, C. L. Crystallographic Snapshots of Sulfur Insertion by Lipoyl Synthase. *Proc. Natl. Acad. Sci. USA*, 2016, 113, 9446–9450. (c) Yang, C.-T.; Devarie-Baez, N. O.; Hamsath, A.; Fu, X.-D.; Xian, M. *S*–Persulfidation: Chemistry, Chemical Biology, and Significance in Health and Disease. *Antioxid. Redox Signal*. **2020**, *33*, 1092–1114. (d) Dóka, E.; Ida, T.; Dagnell, M.; Abiko, Y.; Luong, N. C.; Balog, N.; Takata, T.; Espinosa, B.; Nishimura, A.; Cheng, Q.; Funato, Y.; Miki, H.; Fukuto, J. M.; Prigge, J. R.; Schmidt, E. E.; Arnér, E. S. J. Kumagai, Y.; Akaike, T.; Nagy, P. Control of Protein Function Though Oxidation and Reduction of Persulfidated States. *Sci. Adv.* **2020**, *6*, eaax8358. (e) Ishima, Y.; Yoshida, F.; Kragh-Hansen, U.; Watanabe, K.; Katayama, N.; Nakajou, K.; Akaike, T.; Kai, T.; Maruyama, T.; Otagiri, M. Cellular Uptake Mechanisms and Responses to NO Transferred from Mono– and Poly–*S*–nitrosated Human Serum Albumin. *Free Radic. Res*. **2011**, *45*, 1196–1206.

(3) Akaike, T.; Ida, T.; Wei F.-Y.; Nishida, M.; Kumagai, Y.; Alam, M. M.; Ihara, H.; Sawa, T.; Matsunaga, T.; Kasamatsu, S.; Nishimura, A.; Morita, M.; Tomizawa K.; Nishimura, A.; Watanabe S.; Inaba, K.; Shima, H.; Tanum, N.; Jung, M.; Fujii, S.; Watanabe, Y.; Ohmuraya, M.; Nagy, P.; Feelisch, M.; Fukuto, J. M.; Motohashi, H. Cysteinyl–tRNA Synthetase Governs Cysteine Polysulfidation and Mitochondrial Bioenergetics. *Nat. Commun*. **2017**, *8*, 1177.

(4) (a) Sawa, T.; Takata, T.; Matsunaga, T.; Ihara, H.; Motohashi, H.; Akaike, T. Chemical Biology of Reactive Sulfur Species: Hydrolysis–Driven Equilibrium of Polysulfides as a Determinant of Physiological Functions. *Antioxid. Redox Signal.* **2022**, *36*, 327–336. (b) Hamid, H. A.; Tanaka, A.; Ida, T.; Nishimura, A.; Matsunaga, T.; Fujii, S.; Morita, M.; Sawa, T.; Fukuto, J. M.; Nagy, P.; Tsutsumi, R.; Motohashi, H.; Ihara, H.; Akaike, T. Polysulfid Stabilization by Tyrosine and Hydroxyphenyl–Containing Derivatives that is Important for a Reactive Sulfur Metabolomics Analysis. *Redox Biology*, **2019**, *21*, 101096. (c) Rudyk, O.; Rowan, A.; Prysyazhna, O.; Krasemann, S.; Hartmann, K.; Zhang, M.; Shah, A. M.; Ruppert, C.; Weiss, A.; Schermuly, R. T.; Ida, T.; Akaike, T.; Zhao, L.; Eaton, P. Oxidation of PKGIα Mediates an Endogenous Adaptation to Pulmonary Hypertension. *Proc. Natl. Acad. Sci. USA*, **2019**, *116*, 13016–13025.

(5) (a) Fletcher. J. C.; Robson. A. The Occurrence of Bis-(2-Amino-2-Carboxyethyl) Trisulphide in Hydrolysates of Wool

and other Proteins. *Biochem. J.* **1963**, 87, 553–559. (b) Parmentier, B.; Moutiez, M.; Tartar, A.; Sergheraert, C. Preparation of Trisulfide Derivatives of Cystine and Their Formation as By-products during Peptide Synthesis. *Tetrahedron Lett*., **1994**, *35*, 3531–3534.

(6) Arisawa, M.; Tanaka, K.; Yamaguchi, M. Rhodium–Catalyzed Sulfur Atom Exchange Reaction Between Organic Polysulfides and Sulfur. *Tetrahedron Lett*., **2005**, *46*, 4797–4800.

(7) *Review* (a) Arisawa, M.; Yamaguchi, M. Rhodium–Catalyzed Synthesis of Organosulfur Compounds Involving S–S Bond Cleavage of Disulfides and Sulfur. *Molecules*, **2020**, *25*, 3595. (b) Arisawa, M. Yamaguchi, M. Rhodium–Catalyzed Synthesis of Organosulfur Compounds Using Sulfur. *Synlett*, **2019**, *30*, 1621–1631. (c) Arisawa, M. Synthesis of Organosulfides using Transition–metal–catalyzed Substitution Reactions: to Construct Exergonic Reactions Employing Metal Inorganic and Organic Co–substrate/Co–product methods. *Tetrahedron Lett.*, **2014**, *55*, 3391–3399. *Use of sulfur* (d) Arisawa, M.; Ichikawa, T.; Yamaguchi, M. Rhodium–Catalyzed Synthesis of Diaryl Sulfides Using Aryl Fluorides and Sulfur/Organopolysulfides. *Org. Lett.*, **2012**, *14*, 5318–5321.

(8) Arisawa, M.; Fukumoto, K.; Yamaguchi, M. Rhodium–Catalyzed Oxidation of Unprotected Peptide Thiols to Disulfides with Oxygen in Water. *ACS Catal*. **2020**, *10*, 15060–15064.