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Rhodium-catalyzed phosphorylation reaction of water-soluble disulfides using hypodiphosphoric acid tetraalkyl esters in water†

Mieko Arisawa, * Kohei Fukumoto and Masahiko Yamaguchi

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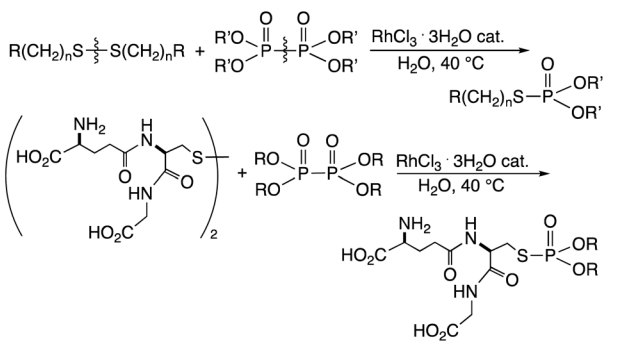
$RhCl_3$ catalyzed the exchange reaction of disulfides and hypodiphosphoric acid tetraalkyl esters in water under homogeneous conditions, which indicated the hypodiphosphoric acid tetraalkyl esters to be novel and efficient phosphorylation reagents in water. The reaction was used in the phosphorylation of unprotected glutathione disulfide.

Chemical modification of biomacromolecules such as peptides and proteins is critical to tuning their biological activity. Such modification should be conducted in water, because of the solubility of such biomacromolecules. The reaction should also proceed selectively in the presence of diverse heteroatom functional groups without requiring the use of protecting groups. Transition-metal-catalysis can be an effective method for chemical modification, although the availability of such reactions in homogeneous water has been limited. The click reaction has been used for such purposes, which, however, requires the introduction of an alkyne moiety into the biomacromolecules.[1a–c] Direct chemical modification of peptides and proteins is preferable, and reactions at tyrosine, tryptophan, and histidine residues have been examined.[2a–d] Disulfide RS–SR is an important sulfur functional group contained in cysteine residues and is involved in the construction of tertiary structures of proteins, which can exhibit chemical reactivities different from those of nitrogen and oxygen functional groups. Derivatization of disulfides is an interesting subject for the modification of the structure and function of biomacromolecules. Conventional methods for protein disulfide modification generally involve two-step procedures of reduction to thiol RS–H followed by and alkylation to form sulfides RS–R′.[3a–e] One-step procedures involving direct cleavage of the S–S bond in disulfides and transfer of the organothio group to other organic molecules are attractive for such modifications because of their simplicity. In addition, disulfides are more stable and easier to handle than thiols. Previously, we developed the S–S bond exchange reaction of glutathione and cystine in homogeneous water,[4] which was applicable to chemical modification of insulin.[5] The disulfide exchange reaction is in the equilibrium state; accordingly, it is necessary to use one of the substrates in large excess to effectively obtain the products.

It has also been described that a rhodium complex can cleave and exchange the P–P bond of tetraalkyldiphosphine disulfides with the S–S bond of disulfides in organic solvents.[6] This reaction using an equimolar amount of the substrates provides quantitative amount of phosphinothioates, because of the energetically favorable nature of the reaction. It was then considered that the phosphorylation could be used for the effective modification of peptide disulfides in water, provided that an appropriate water-soluble phosphorylation reagent could be developed. Described herein is the rhodium-catalyzed phosphorylation of water-soluble disulfides using hypodiphosphoric acid tetraalkyl esters in water (Scheme 1). $RhCl_3$ catalytically cleaved and exchanged S–S and P–P bonds, and the method was successfully applied to the phosphorylation of glutathione in water. The hypodiphosphoric acid tetraalkyl esters have not been used as phosphorylation reagents, and their high reactivity in the presence of rhodium catalyst is shown herein.



Scheme 1 Rhodium-catalyzed phosphorylation reaction of water-soluble disulfides using hypodiphosphoric acid tetraalkyl esters in water.

Department of Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Sendai, 980-8578, Japan. E-mail: arisawa@m.tohoku.ac.jp

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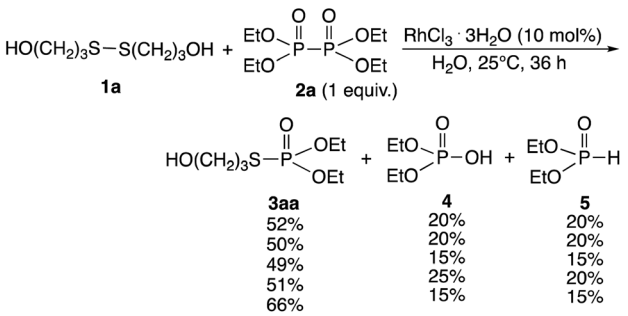
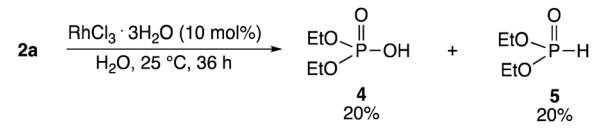
Diphosphine and diphosphine disulfides with aryl and alkyl groups that we previously studied are water-insoluble.[6,7a–c] In contrast, hypodiphosphoric acid tetraalkyl esters containing methyl, ethyl, butyl, and cyclic alkyl groups were found to be water-soluble and could be used as phosphorylation reagents in water in the presence of a rhodium catalyst. The hypodiphosphoric acid tetraalkyl esters were synthesized by copper-catalyzed dehydrogenative couplings of diethyl phosphonate.[8] A reaction of hypodiphosphoric acid tetraalkyl esters involving P–P bond cleavage was reported, in which hypodiphosphoric acid tetraethyl ester **2a** thermally isomerized to diphosphoric(III, V) acid tetraethyl ester at 190–200 °C.[9] We here employed the water-solubled hypodiphosphoric acid tetraalkyl esters for the phosphorylation reaction of disulfides in water. There have been no reports of direct phosphorylation of organodisulfides using hypodiphosphoric acid tetraalkyl esters. The thiophosphorylation reaction by P–S bond formation has generally been conducted in organic solvents.[10a–d,11a–c,12a–c] Phosphorylation reactions of cysteine peptides at SH groups and thiophosphorylation to α,β-unsaturated carbonyl units of peptides in water were reported.[13a–c]

Rhodium-catalyzed phosphorylation of di(hydroxyalkyl) disulfides using hypodiphosphoric acid tetraalkyl esters as reagents was examined in a homogeneous water solution. When di(3-hydroxypropyl)disulfide **1a** was treated with **2a** (1 equiv.) in the presence of $RhCl_3 \cdot 3H_2O$ (10 mol%) in water at 25 °C for 36 h, *O*,*O*-diethyl *S*-(3-hydroxypropyl)phosphorothioate **3aa** (52%) was obtained, which was accompanied by diethyl phosphate **4** (20%) and phosphorous acid diethyl ester **5** (20%) and the recovery of **1a** (40%) (Scheme 2). **2a** was not recovered, and the formation of **4** and **5** revealed the competitive reaction of **2a** with water. Increasing the amount of **2a** from 1 to 3 equiv. did not change the yield of **3aa** (51%). The yield of **3aa** was improved to 66% using 25 mol% of the catalyst. It was noted that these mixtures provided pH 3. Reactions in phosphate buffer (pH 7.4) and aqueous ammonia (pH 10.0) gave **3aa** in 50%, 49% yields, respectively. The formation of **3aa** was not much affected by pH. When the reaction was conducted at 40 °C for 20 h and at reflux for 3 h, the yield of **3aa** decreased to 28% and 38%, respectively. No reaction occurred in the absence of $RhCl_3 \cdot 3H_2O$. Other metal complexes such as $[Rh(OAc)_2]_2$ (7%), $[Rh(cod)_2]^+BF_4^-$ (5%), and $PdCl_2 \cdot 2NaCl \cdot 3H_2O$ (5%) were ineffective even when 40 mol% of the catalyst at reflux for 3 h was used.



Scheme 2 Rhodium-catalyzed phosphorylation reaction of disulfide **1a** using hypodiphosphoric acid tetraethyl esters **2a**. [a]Under phosphate buffer (pH 7.4). [b]Under aqueous ammonia (pH 10.0). [c]Use of **2a** (3 equiv.). [d]Use of $RhCl_3$ (25 mol%).

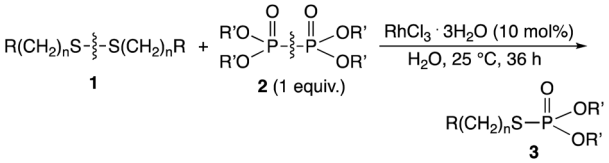


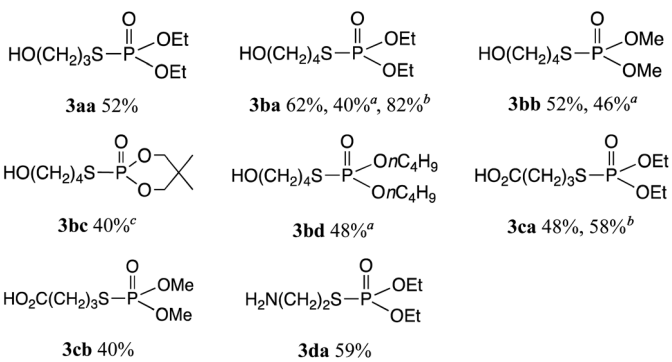
Scheme 3 Formation of diethyl phosphate **4** and phosphorous acid diethyl ester **5** from **2a** in water.

It was determined that **4** and **5** were formed by the reaction of **2a** and water in the presence of $RhCl_3 \cdot 3H_2O$. When **2a** was reacted in excess water in the presence of $RhCl_3 \cdot 3H_2O$ (10 mol%) at 25 °C for 36 h, **4** (20%) and **5** (20%) were obtained (Scheme 3). The hydrolytic reaction rate was considerably lower than the phosphorylation reaction rates of **1a** and **2a**. Neither **4** nor **5** was formed in the absence of $RhCl_3 \cdot 3H_2O$. **3aa** was not formed by the reaction of **4** or **5** with **1a**, which confirmed that **3aa** was directly formed from **2a**.

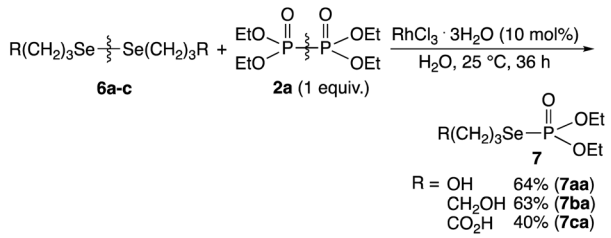
The phosphorylation of water-soluble disulfides was examined (Table 1). The reaction of di(4-hydroxybutyl)disulfides **1b** and **2a** proceeded in water at 25 °C for 36 h, and **3ba** was obtained in 62% yield. The reaction of hypodiphosphoric acid tetramethyl esters **2b** and **1b** gave **3bb** in 52% yield. The yields of **3ba** (40%) and **3bb** (46%) decreased when the reaction was conducted at 40 °C for 20 h. The reaction of **1b** with hypodiphosphoric acid tetrabutyl ester **2c** and 5,5,5′,5′-tetramethyl-bis-(1,3,2)-dioxaphosphinane 2,2′-dioxide **2d** gave the corresponding products **3bc** (40%) and **3bd** (48%) at elevated temperatures of 40 °C and 70 °C, respectively. The reaction did not proceed at 25 °C because of the lower solubility of **2c** and **2d** in water: **2c** was not soluble in water at 25 °C but became soluble at 70 °C. Analogously, the reactions of bis(3-carboxybutyl)

Table 1 Rhodium-catalyzed phosphorylation reaction of water-soluble disulfides using hypodiphosphoric acid tetraalkyl esters





[a] At 40 °C for 36 h. [b] Use of $RhCl_3$ $3H_2O$ (20 mol%). [c] At 70 °C for 36 h.



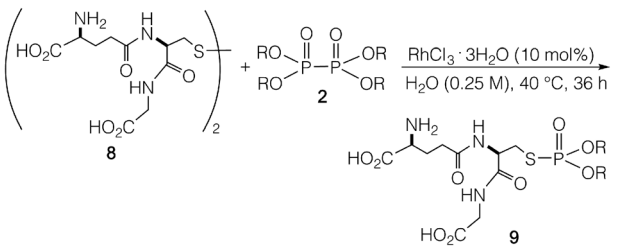
Scheme 4 Rhodium-catalyzed phosphorylation reaction of water-soluble diselenides using hypodiphosphoric acid tetraethyl ester **2a**.

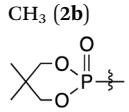
disulfide **1c** with **2a** and **2b** in water gave the phosphorylated products **3ca** and **3cb** in 48% and 40%, respectively. The reaction of bis(2-aminoethyl)disulfide **1d** and **2a** also proceeded to give the corresponding product **3da** (59%).

Water-soluble diselenides **6a–6c** was reacted with **2a** at 25 °C for 36 h in water, and the phosphorylated **7aa–7ac** with the P–Se bonds were obtained in good yields (Scheme 4). The cleavage and exchange of S–S/Se–Se bonds and P–P bonds effectively using the rhodium complex provided P–S/P–Se bonds.

The reaction was applied to unprotected glutathione disulfide **8** (Table 2). When **8** and **2a** (1 equiv.) were reacted in water at 40 °C for 36 h in the presence of $RhCl_3 \cdot 3H_2O$ (10 mol%), carboxymethyl *N*-[*N*-L-γ-glutamyl-*S*-(dimethoxyphosphinyl)-L-cysteinyl]glycine **9a** was obtained in 77% yield; **9a** was isolated by reversed-phase chromatography. When the reaction was conducted at concentrations of 0.125 M, 0.25 M, and 0.5 M at 25 °C in the presence of $RhCl_3 \cdot 3H_2O$ (20 mol%), **9a** was obtained in 28%, 48%, and 55% yields, respectively. The yield of **9a** improved with increasing concentration. The rhodium catalyst is essential in the phosphorylation reaction of **8**. The reaction of

Table 2 Rhodium-catalyzed phosphorylation reaction of glutathione disulfide using hypodiphosphoric acid tetraalkyl esters



Entry	R	Yield
1	CH_3CH_2 (**2a**)	77%, 64%[a], 72%[b], 28%[c,d], 48%[b,c], 55%[b,e], n.d.[f] (**9a**)
2	CH_3 (**2b**)	66% (**9b**)
3	 (**2c**)	64%[g] (**9c**)

[a] Reaction concentration: 0.5 M. Reaction time: 24 h. [b] Use of $RhCl_3 3H_2O$ (20 mol%). Reaction time: 24 h. [c] Reaction temperature: 25 °C. [d] Reaction concentration: 0.125 M. [e] Reaction concentration: 0.5 M. [f] Without $RhCl_3 \cdot 3H_2O$. [g] Reaction temperature: 70 °C.

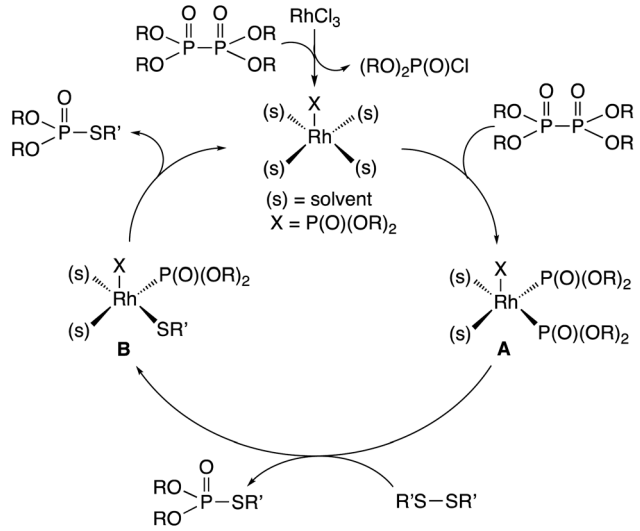


Fig. 1 Proposed reaction mechanism.

8 and the hypodiphosphoric acid tetramethyl ester **2b** also gave the glutathione derivative **9b** in 66% yield. The reaction of 5,5,5′,5′-tetramethyl-bis-(1,3,2)-dioxaphosphinane 2,2′-dioxide **2c** proceeded at an elevated temperature of 70 °C to give the product **9c** at 64% yield. Phosphorylation of glutathione (reduced form) in water did not form **9a.** In addition, reactions of **2a** with methionine, tyrosine, histidine, and tryptophan under rhodium-catalyzed condition did not proceed. **2a** and $RhCl_3 \cdot 3H_2O$ can likely phosphorylate disulfides in peptides without being affected by hydroxy, carboxy, amino, sulfide, phenol, thiol, imidazolyl, and indolyl groups.

A similar reaction mechanism for rhodium-catalyzed phosphorylation in organic solvents may be considered involving a rhodium(I) phosphonate intermediate, which is formed from hypodiphosphoric acid tetraalkyl esters and $RhCl_3$ (Fig. 1).[7a,b] Oxidative addition of hypodiphosphoric acid tetraalkyl esters to rhodium(I) phosphonate provides a rhodium(III) triphosphonate intermediate, which undergoes an organothio exchange reaction with the disulfide R′SSR′ forming the R′S–Rh(III)–$P(O)(OR)_2$ complex and a thiophosphorylated product. Another thiophosphorylated product may be liberated by reductive elimination with the regeneration of the rhodium(I) phosphonate.

Conclusions

In summary, $RhCl_3$ catalyzed the phosphorylation reaction of water-soluble disulfides, including unprotected glutathione disulfide, with hypodiphosphoric acid tetraalkyl esters in water under homogeneous conditions. We have shown that $RhCl_3$ effectively activates disulfides in water without being affected by presence of the amino and carboxy groups. This method may be applicable to complex proteins. This reaction is of additional interest because phosphorylation of serine and tyrosine is a critical process for signal transduction in biological cells, and cysteine phosphorylation can exhibit interestingly biological phenomena.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 For examples, (*a*) S. Li, L. Wang, F. Yu, Z. Zhu, D. Shobaki, H. Chen, M. Wang, J. Wang, G. Qin, U. J. Erasquin, L. Ren, Y. Wang and C. Cai, *Chem. Sci.*, 2017, **8**, 2107; (*b*) J. Martell and E. Weerapana, *Molecules*, 2014, **19**, 1378; (*c*) S. I. Presolski, V. P. Hong and M. G. Finn, *Curr. Protoc. Chem. Biol.*, 2011, **3**, 153.

2 For examples, (*a*) J. Ohata, M. B. Minus, M. E. Abernathy and Z. T. Ball, *J. Am. Chem. Soc.*, 2016, **138**, 7472; (*b*) S. D. Tilley and M. B. Francis, *J. Am. Chem. Soc.*, 2006, **128**, 1080; (*c*) J. M. Antos and M. B. Francis, *J. Am. Chem. Soc.*, 2004, **126**, 10256; (*d*) Review: E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974.

3 For examples, (*a*) G. D'Alessio, M. C. Maloni and A. Parente, *Biochemistry*, 1975, **14**, 1116; (*b*) D. L. Sondack and A. Light, *J. Biol. Chem.*, 1971, **246**, 1630; (*c*) P. A. Price, W. H. Stein and S. Moore, Disulfide bridged modification of proteins *via* reduction, *J. Biol. Chem.*, 1969, **244**, 929; (*d*) Review: S. L. Kuan, T. Wang and T. Weil, *Chem.–Eur. J.*, 2016, **22**, 17112; (*e*) S. Brocchini, A. Godwin, S. Balan, J. Choi, M. Zloh and S. Shaunak, *Adv. Drug Delivery Rev.*, 2008, **60**, 3.

4 M. Arisawa, A. Suwa and M. Yamaguchi, *J. Organomet. Chem.*, 2006, **691**, 1159.

5 M. Arisawa, M. Kuwajima, A. Suwa and M. Yamaguchi, *Heterocycles*, 2010, **80**, 1239.

6 M. Arisawa, T. Ono and M. Yamaguchi, *Tetrahedron Lett.*, 2005, **46**, 5669.

7 For examples, (*a*) M. Arisawa, T. Tazawa, W. Ichinose, H. Kobayashi and M. Yamaguchi, *Adv. Synth. Catal.*, 2018, **360**, 3488; (*b*) M. Arisawa, T. Yamada, S. Tanii, Y. Kawada, H. Hashimoto and M. Yamaguchi, *Chem. Commun.*, 2016, **52**, 13580; (*c*) M. Arisawa and M. Yamaguchi, *Tetrahedron Lett.*, 2010, **51**, 4840.

8 Y. Zhou, S. Yin, Y. Gao, Y. Zhao, M. Goto and L.-B. Han, *Angew. Chem., Int. Ed.*, 2010, **49**, 6852.

9 J. Michalski, W. Stec and A. Zwierzak, *Chem. Ind.*, 1965, **8**, 347.

10 For examples of nucleophilic substitution, (*a*) C. M. Timperley, S. A. Saunders, J. Szpalek and M. J. Waters, *J. Fluorine Chem.*, 2003, **119**, 161; (*b*) P.-Y. Renard, H. Schwebel, P. Vayron, L. Josien, A. Valleix and C. Mioskowski, *Chem.–Eur. J.*, 2002, **8**, 2910; (*c*) T.-L. Au-Yeung, K.-Y. Chan, W.-K. Chan, R. K. Haynes, I. D. Williams and L. L. Yeung, *Tetrahedron Lett.*, 2001, **42**, 453; (*d*) R. G. Harvey, H. I. Jacobson and E. V. Jensen, *J. Am. Chem. Soc.*, 1963, **85**, 1623.

11 For examples of oxidative coupling of thiols and phosphonates, (*a*) H. Huang, J. Ash and J. Y. Kang, *Org. Biomol. Chem.*, 2018, **16**, 4236; (*b*) S. Song, Y. Zhang, A. Yeerlan, B. Zhu, J. Liu and N. Jiao, *Angew. Chem., Int. Ed.*, 2017, **56**, 2487; (*c*) Y. Moon, Y. Moon, H. Choi and S. Hong, *Green Chem.*, 2017, **19**, 1005.

12 Using phosphorothioates, (*a*) D. J. Jones, E. M. O'Leary and T. P. O'Sullivan, *Tetrahedron Lett.*, 2018, **59**, 4279; (*b*) X.-Y. Chen, M. Pu and H.-G. Cheng, *Angew. Chem., Int. Ed.*, 2019, **58**, 11395; (*c*) X. Zhang, Z. Shi, C. Shao, J. Zhao, D. Wang, G. Zhang and L. Li, *Eur. J. Org. Chem.*, 2017, 1884.

13 (*a*) M. J. Piggott and P. V. Attwood, *Amino Acids*, 2017, **49**, 1309; (*b*) J. Bertran-Vicente, M. Penkert, O. Nieto-Garcia, J.-M. Jeckelmann, P. Schmieder, E. Krause and C. P. R. Hackenberger, *Nat. Commun.*, 2016, **7**, 12703; (*c*) A. K. Buchowiecka, *Amino Acids*, 2019, **51**, 1365.