J|A|C|S ARTICLES

Published on Web 01/20/2010

A Water-Soluble Ruthenium Glycosylated Porphyrin Catalyst for Carbenoid Transfer Reactions in Aqueous Media with Applications in Bioconjugation Reactions

Chi-Ming Ho,[†] Jun-Long Zhang,[‡] Cong-Ying Zhou,[†] On-Yee Chan,[†] Jessie Jing Yan,[†] Fu-Yi Zhang,[†] Jie-Sheng Huang,[†] and Chi-Ming Che*[,†]

Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Pokfulam Road, Hong Kong, P. R. China, and Department of Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China

Received September 11, 2009; E-mail: cmche@hku.hk

Abstract: Water-soluble [Ru^{II}(4-Glc-TPP)(CO)] (**1**, 4-Glc-TPP = *meso*-tetrakis(4-(β-D-glucosyl)phenyl)porphyrinato dianion) is an active catalyst for the following carbenoid transfer reactions in aqueous media with good selectivities and up to 100% conversions: intermolecular cyclopropanation of styrenes (up to 76% yield), intramolecular cyclopropanation of an allylic diazoacetate (68% yield), intramolecular ammonium/sulfonium ylide formation/[2,3]-sigmatroptic rearrangement reactions (up to 91% yield), and intermolecular carbenoid insertion into N−H bonds of primary arylamines (up to 83% yield). This ruthenium glycosylated porphyrin complex can selectively catalyze alkylation of the N-terminus of peptides (8 examples) and mediate N-terminal modification of proteins (four examples) using a fluorescent-tethered diazo compound (**15**). A fluorescent group was conjugated to ubiquitin via **1**-catalyzed alkene cyclopropanation with **15** in aqueous solution in two steps: (1) incorporation of an alkenic group by the reaction of *N*-hydroxysuccinimide ester **19** with ubiquitin and (2) cyclopropanation of the alkene-tethered Lys^6 ubiquitin (**23**) with the fluorescent-labeled diazoacetate **15** in the presence of a catalytic amount of **1**. The corresponding cyclopropanation product (**24**) was obtained with ∼55% conversion based on MALDI-TOF mass spectrometry. The products **23**, **24**, and the N-terminal modified peptides and proteins were characterized by LC-MS/MS and/or SDS-PAGE analyses.

Introduction

Metal-catalyzed carbenoid transfer reactions, such as alkene cyclopropanation, ylide formation/[2,3]-sigmatropic rearrangement, and carbenoid insertion into X−H (X = C, N, S) bonds, have emerged to become a useful means for the construction of organic molecules with complexity.[1] These reactions are usually performed in organic solvents, which are less favored than in aqueous media in the context of green chemistry.[2] Besides the environmentally benign nature, an organic reaction in aqueous media could also benefit from the hydrophobic effect.[3] However, a serious problem likely to be encountered is the facile reaction of metal carbenoids with water to give O−H insertion products[4] and/or the poor solubility/stability of the metal catalysts in aqueous solution. While metal-catalyzed carbenoid transfer reactions in aqueous or aqueous biphasic media are not unprecedented,[5−13] such reactions, since the studies by Nishiyama and co-workers,[6a] remain sparse in the literature. And the reported reactions include the following:

[†] The University of Hong Kong.
[‡] Peking University.

(1) (a) Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091. (b) Doyle, M. P.; Forbes, D. C. *Chem. Rev.* **1998**, *98*, 911. (c) Doyle, M. P.; Mckervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; Wiley: New York, 1998. (d) Davies, H. M. L.; Beckwith, R. E. J. *Chem. Rev.* **2003**, *103*, 2861. (e) Godula, K.; Sames, D. *Science* **2006**, *312*, 67. (f) Davies, H. M. L.; Manning, J. R. *Nature* **2008**, *451*, 417.
(2) (a) Herrmann, W. A.; Kohlpainter, C. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1524. (b) *Organic Synthesis in Water*; Grieco, P. A., Ed.; Blackie Academic & Professional: London, 1998. (c) Cornils, B.; Herrmann, W. A. *Aqueous Phase Organometallic Catalysis: Concept and Applications*, 2nd ed.; Wiley-VCH: Weinheim, 2004. (d) Li, C.-J.; Chan, T.-H. *Organic Reactions in Aqueous Media*; John Wiley & Sons: New York, 1997. (e) Lindström, U. M. *Chem. Rev.* **2002**, *102*, 2751.
(3) (a) Breslow, R. *Acc. Chem. Res.* **2004**, *37*, 471. (b) Lindström, U. M.; Andersson, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 548.
(4) Miller, D. J.; Moody, C. J. *Tetrahedron* **1995**, *51*, 10811.
(5) Nicolas, I.; Le Maux, P.; Simonneaux, G. *Coord. Chem. Rev.* **2008**, *252*, 727.
(6) (a) Iwasa, S.; Takezawa, F.; Tuchiya, Y.; Nishiyama, H. *Chem. Commun.* **2001**, 59. (b) Iwasa, S.; Tsushima, S.; Nishiyama, K.; Tsuchiya, Y.; Takezawa, F.; Nishiyama, H. *Tetrahedron: Asymmetry* **2003**, *47*, 855.
(7) Wurz, R. P.; Charette, A. B. *Org. Lett.* **2002**, *4*, 4531.
(8) (a) Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256. (b) Antos, J. M.; McFarland, J. M.; Iavarone, A. T.; Francis, M. B. *J. Am. Chem. Soc.* **2009**, *131*, 6301.
(9) (a) Candeias, N. R.; Gois, P. M. P.; Afonso, C. A. M. *Chem. Commun.* **2005**, 391. (b) Candeias, N. R.; Gois, P. M. P.; Afonso, C. A. M. *J. Org. Chem.* **2006**, *71*, 5489.
(10) Estevan, F.; Lloret, J.; Sanaú, M.; Úbeda, M. A. *Organometallics* **2006**, *25*, 4977.
(11) Liao, M.; Wang, J. *Green Chem.* **2007**, *9*, 184.
(12) Nicolas, I.; Le Maux, P.; Simonneaux, G. *Tetrahedron Lett.* **2008**, *49*, 5793.
(13) Aviv, I.; Gross, Z. *Chem.—Eur. J.* **2008**, *14*, 3995.

 10.1021/ja9077254

alkene cyclopropanation catalyzed by the complexes of ruthenium,[6,7,12] cobalt,[7] dirhodium,[7,10] and iron;[12] C−H insertion[8,9] and sulfonium ylide formation/[2,3]-sigmatropic rearrangement[11] catalyzed by dirhodium complexes; N−H insertion catalyzed by complexes of dirhodium[8] and iron.[13]

A formidable challenge is the application of metal-catalyzed carbenoid transfer in bioconjugation reactions such as site selective modification of proteins.[14,15] As an appealing strategy for modifying proteins, metal-catalyzed approaches can selectively target the natural amino acid side chains that are hardly modifiable by conventional methods and can also realize highly selective protein modification upon incorporating suitable unnatural functional groups into the amino acid side chains.[15] The challenges lie in that the bioconjugation of proteins not only has to be performed in aqueous media without being complicated by reactions with water but also should meet a number of requirements: (i) high efficiency at the protein concentration level (<100 μM); (ii) mild reaction conditions that allow proteins to retain their activity or structure features; (iii) selective modification of a single group out of many similar or the same groups in different sites, with tolerance to various functional groups present in the surface of the proteins.[14,15] Of the previously reported metal-catalyzed carbenoid transfer reactions in aqueous media,[6−13] $[Rh_2(OAc)_4]$-catalyzed carbenoid transfer reaction has been applied to site-selective modification of proteins,[8,16] which targets the indole group of internal tryptophan residues of proteins, as described in the work by Francis and Antos.[8]

In this paper, we report the site-selective modification of the N-terminus of proteins via metal-mediated carbenoid N−H insertion, together with the modification, via metal-catalyzed alkene cyclopropanation, of a protein prefunctionalized with a styrene moiety, using a water-soluble metalloporphyrin catalyst $[Ru^{II}$(4-Glc-TPP)(CO)] (**1**), wherein 4-Glc-TPP is the dianion of a previously reported glycosylated porphyrin ligand *meso*-tetrakis(4-(β-D-glucosyl)phenyl)porphyrin.[17] Also reported herein are **1**-catalyzed cyclopropanation of styrenes and allylic diazoacetate, ammonium/sulfonium ylide formation/[2,3]-sigmatropic rearrangement of diazoketones, and carbenoid N−H insertion reactions of primary arylamines in aqueous media. Prior to the present work, a myriad of metal-catalyzed alkene cyclopropanation reactions in organic solvents have been reported in literature.[1a−c] Metalloporphyrin-catalyzed carbenoid transfer reactions have been extensively studied in organic solvents,[18] examples of which performed in aqueous media are rare. During the preparation of this manuscript, Simonneaux and co-workers reported the intermolecular cyclopropanation of styrene in water catalyzed by water-soluble iron and ruthenium porphyrins;[12] Gross and Aviv reported intermolecular N−H insertion of anilines in THF/water catalyzed by myoglobin.[13] *To the best of our knowledge, the present work contributes the first example of metalloporphyrin-catalyzed carbenoid transfer for bioconjugation of proteins.*

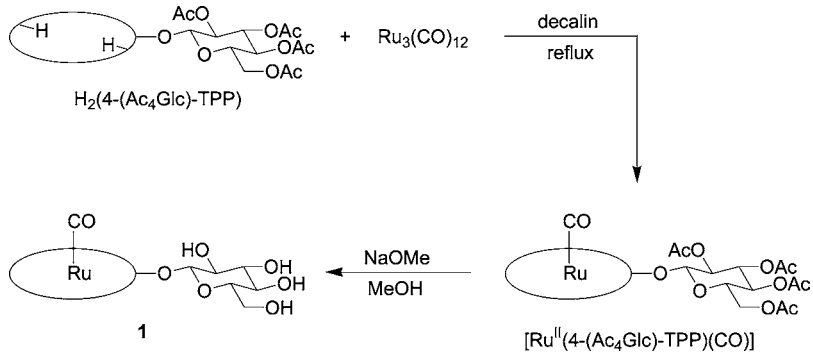
Results and Discussion

Synthesis of Water-Soluble Ruthenium Glycosylated Porphyrin $[Ru^{II}$(4-Glc-TPP)(CO)] (1). Despite the reports of a large variety of water-soluble porphyrin free bases,[19] only a few examples of water-soluble ruthenium porphyrins have been known in literature, including poly(ethylene glycol)-supported ruthenium porphyrins such as $[Ru^{II}$(4-Cl-TPP)(CO)]-PEG (**2**)[20a] reported in our previous work;[20] $[Ru^{II}$(TMPyP)(CO)]$^{4+}$ (**3**), $[Ru^{II}$(4-COOH-TPP)(CO)] (**4**), and $[Ru^{II}$(TPPS)(CO)]$^{4-}$ reported earlier by Meunier and co-workers;[21] and $[Ru^{II}(D_4$-PorS*)-(CO)]$^{4-}$ reported recently by Simonneaux and co-workers[12] (Chart S1 in Supporting Information). These water-soluble ruthenium porphyrins, except the PEG-supported ones, contain peripheral pyridinium, carboxyl, or sulfonato functional groups; such groups have been widely used to render metalloporphyrins soluble in aqueous media.[19]

A different approach to water-soluble ruthenium porphyrin was employed in this work, i.e. by covalently attaching sugar moieties to the *meso*-aryl rings of a porphyrin ligand. We envision that the sugar moieties, such as β-D-glucosyl (Glc), not only can enhance the solubility of ruthenium porphyrins in aqueous media but also might be able to direct the ruthenium catalyst to approach a specific site of biomolecules via hydrogen-bonding and dipole−dipole interactions. In literature, *acetylated* sugar moieties, including 2,3,4,6-tetraacetyl-β-D-glucosyl (Ac_4Glc), have been incorporated into the porphyrin ligands in a few iron and manganese catalysts for hydrocarbon oxidation

(14) Hermanson, G. T. *Bioconjugate Techniques*, 2nd ed.; Academic Press: San Diego, 2008.
(15) Antos, J. M.; Francis, M. B. *Curr. Opin. Chem. Biol.* **2006**, *10*, 253.
(16) Bao, Z.; Wang, S.; Shi, W.; Dong, S.; Ma, H. *J. Proteome Res.* **2007**, *6*, 3835.
(17) (a) Kohata, K.; Yamaguchi, Y.; Higashio, H.; Odashima, T.; Ishii, H. *Chem. Lett.* **1992**, 477. (b) Kohata, K.; Higashio, H.; Yamaguchi, Y.; Koketsu, M.; Odashima, T. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 668. (c) Oulmi, D.; Maillard, P.; Guerquin-Kern, J.-L.; Huel, C.; Momenteau, M. *J. Org. Chem.* **1995**, *60*, 1554.
(18) Selected examples: (a) Maxwell, J. L.; Brown, K. C.; Bartley, D. W.; Kodadek, T. *Science* **1992**, *256*, 1544. (b) Brown, K. C.; Kodadek, T. *J. Am. Chem. Soc.* **1992**, *114*, 8336. (c) Bartley, D. W.; Kodadek, T. *J. Am. Chem. Soc.* **1993**, *115*, 1656. (d) Smith, D. A.; Reynolds, D. N.; Woo, L. K. *J. Am. Chem. Soc.* **1993**, *115*, 2511. (e) Wolf, J. R.; Hamaker, C. G.; Djukic, J.-P.; Kodadek, T.; Woo, L. K. *J. Am. Chem. Soc.* **1995**, *117*, 9194. (f) Galardon, E.; Le Maux, P.; Simonneax, G. *Chem. Commun.* **1997**, 927. (g) Frauenkron, M.; Berkessel, A. *Tetrahedron Lett.* **1997**, *38*, 7175. (h) Gross, Z.; Galili, N.; Simkhovich, L. *Tetrahedron Lett.* **1999**, *40*, 1571. (i) Che, C.-M.; Huang, J.-S.; Lee, F.-W.; Li, Y.; Lai, T.-S.; Kwong, H.-L.; Teng, P.-F.; Lee, W.-S.; Lo, W.-C.; Peng, S.-M.; Zhou, Z.-Y. *J. Am. Chem. Soc.* **2001**, *123*, 4119. (j) Li, Y.; Huang, J.-S.; Zhou, Z.-Y.; Che, C.-M. *J. Am. Chem. Soc.* **2001**, *123*, 4843. (k) Mirafzal, G. A.; Cheng, G.; Woo, L. K. *J. Am. Chem. Soc.* **2002**, *124*, 176. (l) Li, Y.; Huang, J.-S.; Zhou, Z.-Y.; Che, C.-M.; You, X.-Z. *J. Am. Chem. Soc.* **2002**, *124*, 13185. (m) Chen, Y.; Fields, K. B.; Zhang, X. P. *J. Am. Chem. Soc.* **2004**, *126*, 14718. (n) Chen, Y.; Ruppel, J. V.; Zhang, X. P. *J. Am. Chem. Soc.* **2007**, *129*, 12074. (o) Zhu, S.; Ruppel, J. V.; Lu, H.; Wojtas, L.; Zhang, X. P. *J. Am. Chem. Soc.* **2008**, *130*, 5042. (p) Zhu, S.; Perman, J. A.; Zhang, X. P. *Angew. Chem., Int. Ed.* **2008**, *47*, 8460–8463. (q) Thu, H.-Y.; Tong, G. S.-M.; Huang, J.-S.; Chan, S. L.-F.; Deng, Q.-H.; Che, C.-M. *Angew. Chem., Int. Ed.* **2008**, *47*, 9747. (r) Wang, S. R.; Zhu, C.-Y.; Sun, X.-L.; Tang, Y. *J. Am. Chem. Soc.* **2009**, *131*, 4192.
(19) For reviews, see: (a) Bernadou, J.; Meunier, B. *Chem. Commun.* **1998**, 2167. (b) Purrello, R.; Gurrieri, S.; Lauceri, R. *Coord. Chem. Rev.* **1999**, *190−192*, 683. (c) Hambright, P. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, CA, 2000; Vol. 3, p 129. (d) Lang, K.; Mosinger, J.; Wagnerová, D. M. *Coord. Chem. Rev.* **2004**, *248*, 321. (e) Horváth, O.; Huszánk, R.; Valicsek, Z.; Lendvay, G. *Coord. Chem. Rev.* **2006**, *250*, 1792.
(20) (a) Zhang, J.-L.; Che, C.-M. *Org. Lett.* **2002**, *4*, 1911. (b) Zhang, J.-L.; Huang, J.-S.; Che, C.-M. *Chem.−Eur. J.* **2006**, *12*, 3020.
(21) Hartmann, M.; Robert, A.; Duarte, V.; Keppler, B. K.; Meunier, B. *J. Biol. Inorg. Chem.* **1997**, *2*, 427.

Scheme 1



in organic solvents.[22,23] However, concerning metal catalysts for organic transformations, we could find no literature examples, apart from **1**, that bear a water-soluble porphyrin ligand functionalized with sugar moieties (such as Glc).

The preparation of **1** did not require isolation of the porphyrin free base H_2(4-Glc-TPP), which could be obtained by treatment of H_2(4-(Ac_4Glc)-TPP) (*meso*-tetrakis(4-(2,3,4,6-tetraacetyl-β-D-glucosyl)phenyl)porphyrin) with NaOMe.[17] Reaction of H_2(4-(Ac_4Glc)-TPP) with $Ru_3(CO)_{12}$ in refluxing decalin afforded [Ru^{II}(4-(Ac_4Glc)-TPP)(CO)]. Deprotection of the acetyl groups of the porphyrin ligand in [Ru^{II}(4-(Ac_4Glc)-TPP)(CO)] with a catalytic amount of NaOMe in methanol gave **1** in 50% yield (Scheme 1).

Complex **1** shows a UV−vis spectrum with Soret and β bands at 412 and 527 nm, respectively, and an IR spectrum with an intense ν(CO) band at 1940 cm^{-1}, along with two sets of *ortho* proton resonances of the *meso*-phenyl groups (δ 8.05, 7.95 ppm). These spectral features are similar to those of other carbonylruthenium(II) *meso*-tetraarylporphyrins reported in literature.[24] The positive-ion mass spectrum of **1** shows a cluster peak at *m*/*z* 1455.2 attributed to the parent ion M^+.

Carbenoid Transfer Reactions Catalyzed by [Ru^{II}(4-Glc-TPP)(CO)] (1) in Aqueous Media. The chemistry of ruthenium porphyrin-catalyzed reactions is dominated by the catalysts bearing hydrophobic porphyrin ligands such as TPP (*meso*-tetraphenylporphyrinato dianion) and its derivatives,[25] which are difficult to be applied to reactions in aqueous media. We and others have reported a considerable number of metalloporphyrin-catalyzed carbenoid transfer reactions in organic solvents, including alkene cyclopropanation,[18a−j,l−p] carbenoid X−H (X = C, N, S) insertions,[13,18q,r,26] and ammonium/sulfonium ylide formation/[2,3]-sigmatropic rearrangements.[27] Ruthenium porphyrin-catalyzed ammonium ylide formation/[2,3]-sigmatropic rearrangement reaction in CH_2Cl_2 has been applied to the synthesis of alkaloid (±)-platynecine.[27b] Carbonylruthenium(II) porphyrins [Ru^{II}(Por)(CO)] are well-documented to catalyze these types of carbenoid transfer reactions.[18f−i,26a−c,27] Of particular interest is the air and moisture stability of isolated ruthenium porphyrin carbene complexes,[18i,28] quite a few of which have been structurally characterized by X-ray single crystal analysis.[18i,28c,e−j] Thus, water-soluble [Ru^{II}(Por)(CO)] catalysts, such as **1**, are likely to be converted to a ruthenium porphyrin carbene intermediate that has a sufficiently long lifetime to allow for carbenoid transfer to organic substrates in aqueous media (see also experimental results described in ref 29).

Indeed, complex **1** catalyzed the following carbenoid transfer reactions in aqueous media (Scheme 2; detailed results are depicted in Tables S1−S5 in Supporting Information): (i) intermolecular cyclopropanation of styrenes (**5**) with EDA (ethyl diazoacetate) to give **6** (R = H, Me, Cl; 70−76% yields; *trans*/*cis* (4−5):1), (ii) intramolecular cyclopropanation of allylic diazoacetates (**7**) to form **8** (R^t = R^c = Me; 68% yield), (iii)

(22) For reviews, see: (a) Aksenova, A. A.; Sebyakin, Y. L.; Mironov, A. F. *Russ. J. Bioorg. Chem.* **2003**, *29*, 201. (b) Cavaleiro, J. A. S.; Tomé, J. P. C.; Faustino, M. A. F. *Top. Heterocycl. Chem.* **2007**, *7*, 179.

(23) Selected examples: (a) Maillard, P.; Guerquin-Kern, J. L.; Momenteau, M. *Tetrahedron Lett.* **1991**, *32*, 4901. (b) Vilain, S.; Maillard, P.; Momenteau, M. *J. Chem. Soc., Chem. Commun.* **1994**, 1697. (c) Vilain-Deshayes, S.; Robert, A.; Maillard, P.; Meunier, B.; Momenteau, M. *J. Mol. Catal., A: Chem.* **1996**, *113*, 23. (d) Zhang, X.-B.; Guo, C.-C.; Xu, J.-B.; Yu, R.-Q. *J. Mol. Catal., A: Chem.* **2000**, *154*, 31.

(24) Selected examples: (a) Tsutsui, M.; Ostfeld, D.; Hoffman, L. M. *J. Am. Chem. Soc.* **1971**, *93*, 1820. (b) Bonnet, J. J.; Eaton, S. S.; Eaton, G. R.; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 2141. (c) Brown, G. M.; Hopf, F. R.; Ferguson, J. A.; Meyer, T. J.; Whitten, D. G. *J. Am. Chem. Soc.* **1973**, *95*, 5939. (d) Eaton, S. S.; Eaton, G. R. *J. Am. Chem. Soc.* **1975**, *97*, 3660. (e) Rillema, D. P.; Nagle, J. K.; Barringer, L. F., Jr.; Meyer, T. J. *J. Am. Chem. Soc.* **1981**, *103*, 56. (f) Collman, J. P.; Barnes, C. E.; Swepston, P. N.; Ibers, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 3500. (g) Collman, J. P.; Barnes, C. E.; Brothers, P. J.; Collins, T. J.; Ozawa, T.; Gallucci, J. C.; Ibers, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 5151.

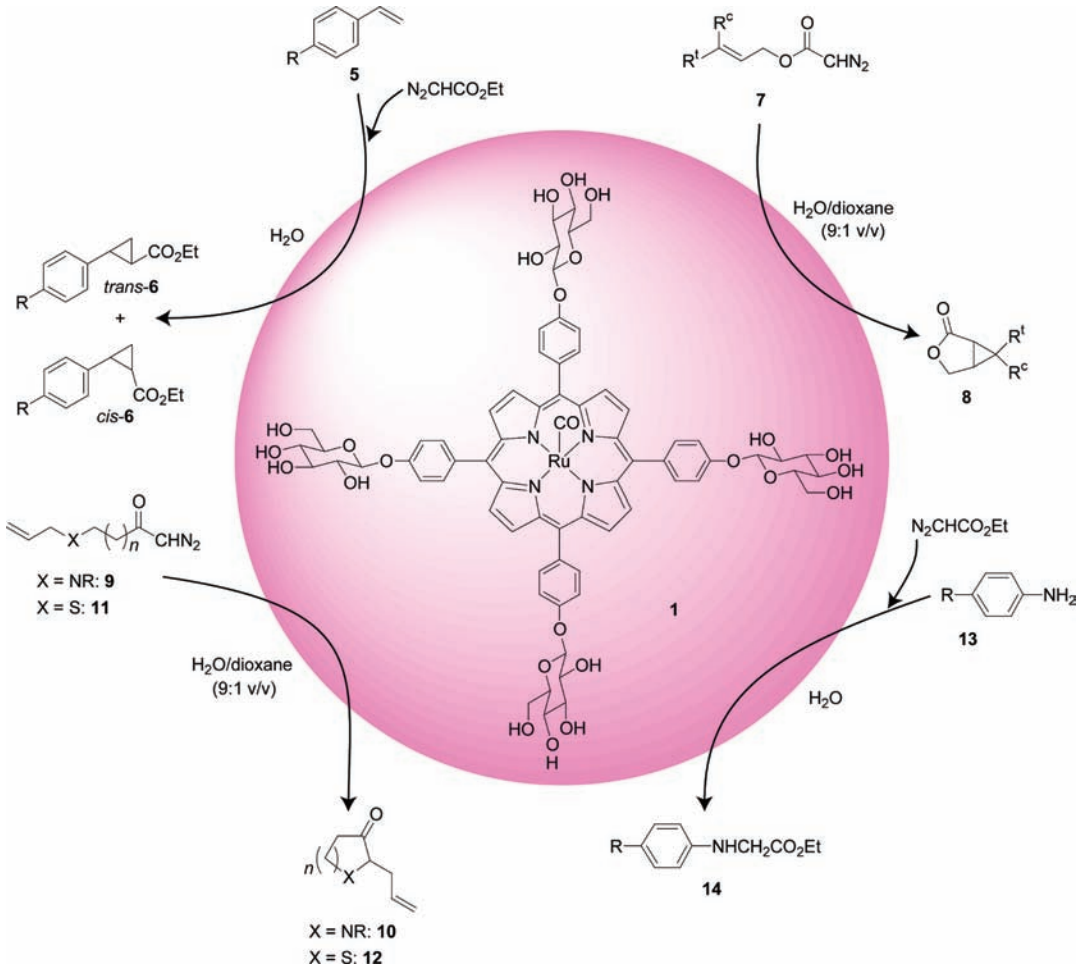
(25) For reviews, see: (a) Simonneaux, G.; Le Maux, P. *Coord. Chem. Rev.* **2002**, *228*, 43. (b) Ezhova, M. B.; James, B. R. In *Advances in Catalytic Activation of Dioxygen by Metal Complexes*; Simándi, L. I., Ed.; Kluwer Academic Publishers: London, 2003; p 1. (c) Simonneaux, G.; Le Maux, P.; Ferrand, Y.; Rault-Berthelot, J. *Coord. Chem. Rev.* **2006**, *250*, 2212. (d) Che, C.-M.; Huang, J.-S. *Chem. Commun.* **2009**, 3996.

(26) (a) Galardon, E.; Le Maux, P.; Simonneaux, G. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2455. (b) Galardon, E.; Le Maux, P.; Simonneaux, G. *Tetrahedron* **2000**, *56*, 615. (c) Cheung, W.-H.; Zheng, S.-L.; Yu, W.-Y.; Zhou, G.-C.; Che, C.-M. *Org. Lett.* **2003**, *5*, 2535. (d) Aviv, I.; Gross, Z. *Chem. Commun.* **2006**, 4477. (e) Aviv, I.; Gross, Z. *Synlett* **2006**, 951. (f) Baumann, L. K.; Mbuvi, H. M.; Du, G.; Woo, L. K. *Organometallics* **2007**, *26*, 3995.

(27) (a) Simonneaux, G.; Galardon, E.; Paul-Roth, C.; Gulea, M.; Masson, S. *J. Organomet. Chem.* **2001**, *617−618*, 360. (b) Zhou, C.-Y.; Yu, W.-Y.; Chan, P. W. H.; Che, C.-M. *J. Org. Chem.* **2004**, *69*, 7072.

(28) (a) Collman, J. P.; Brothers, P. J.; McElwee-White, L.; Rose, E.; Wright, L. J. *J. Am. Chem. Soc.* **1985**, *107*, 4570. (b) Collman, J. P.; Rose, E.; Venburg, G. D. *J. Chem. Soc., Chem. Commun.* **1993**, 934. (c) Galardon, E.; Le Maux, P.; Toupet, L.; Simonneaux, G. *Organometallics* **1998**, *17*, 565. (d) Simonneaux, G.; De Montigny, F.; Paul-Roth, C.; Gulea, M.; Masson, S. *Tetrahedron Lett.* **2002**, *43*, 3685. (e) Kawai, M.; Yuge, H.; Miyamoto, T. K. *Acta Crystallogr., Sect. C* **2002**, *58*, m581. (f) Wada, S.; Yuge, H.; Miyamoto, T. K. *Acta Crystallogr., Sect. C* **2003**, *59*, m369. (g) Harada, T.; Wada, S.; Yuge, H.; Miyamoto, T. K. *Acta Crystallogr., Sect. C* **2003**, *59*, m37. (h) Li, Y.; Chan, P. W. H.; Zhu, N.-Y.; Che, C.-M.; Kwong, H.-L. *Organometallics* **2004**, *23*, 54. (i) Li, Y.; Huang, J.-S.; Xu, G.-B.; Zhu, N.; Zhou, Z.-Y.; Che, C.-M.; Wong, K.-Y. *Chem.—Eur. J.* **2004**, *10*, 3486. (j) Le Maux, P.; Roisnel, T.; Nicolas, I.; Simonneaux, G. *Organometallics* **2008**, *27*, 3037.

Scheme 2



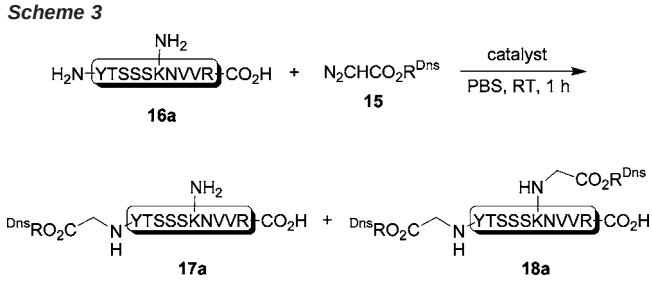
intramolecular ammonium ylide formation/[2,3]-sigmatropic rearrangement of diazoketones **9** to afford **10** (R = Bn, Me, CH_2=$CHCH_2$; n = 1, 2; 83−89% yields), (iv) intramolecular sulfonium ylide formation/[2,3]-sigmatropic rearrangement of diazoketones **11** to produce **12** (n = 1, 2; 90−91% yields), and (v) intermolecular carbenoid N−H insertion of primary arylamines **13** with EDA to afford **14** (R = H, Me, Br, OMe; 76−83% yields). The substrate conversions were 85−92% for the cyclopropanation of **5** and 100% for the other reactions.

Metal-catalyzed intramolecular ammonium/sulfonium ylide formation/[2,3]-sigmatropic rearrangement reactions, also known as a Doyle−Kirmse reaction in the sulfonium case, are synthetically useful transformations for the construction of C−C bonds[1b,c,30] and have been widely studied in organic solvents using catalysts such as $[Rh_2(OAc)_4]$ and $[Cu(acac)_2]$.[1b,c,30] Carbenoid insertion of diazo compounds into N−H bond is an attractive method for the synthesis of α-amino carboxylic derivatives.[31] For the carbenoid N−H insertion reactions catalyzed by **1**, a simultaneous addition of amine and EDA to avoid catalyst poisoning is not necessary, unlike those catalyzed by $[Ru^{II}(TMP)(CO)]$ (TMP = *meso*-tetramesitylporphyrinato dianion) in benzene.[26a,b] The reaction of **9** (R = Bn; n = 1) catalyzed by $[Rh_2(OAc)_4]$ or $[Cu(acac)_2]$ in aqueous media gave **10** in 21% and 31% yields, respectively (Table S3 in Supporting Information), and the main product (isolated in 62% yield) in the $[Rh_2(OAc)_4]$-catalyzed reaction resulted from carbenoid insertion into the O−H bond of water. In contrast, *no such O−H insertion product was detected in the same reaction catalyzed by **1***. Wang and Liao recently reported intermolecular $[Rh_2(OAc)_4]$-

(29) Taking into account the well-documented preparation of carbene complexes $[Ru(Por)(CR^1R^2)]$ from $[Ru^{II}(Por)(CO)]$ and diazo compounds $N_2CR^1R^2$ in organic solvents,[18i,28c−j] we made attempts to prepare and isolate a ruthenium porphyrin carbene complex by treating **1** (0.1 mM) with N_2CHCO_2Et (EDA) or N_2CPh_2 (100 equiv) in aqueous media at room temperature but have not been successful. Probably, the corresponding carbene complex generated in water is not sufficiently stable for isolation. The formation of a metal carbene intermediate from the reaction of **1** with EDA in water is inferred by the treatment of the reaction mixture with $PhNH_2$ (100 equiv), which gave $PhNHCH_2CO_2Et$ in 53% yield (detected by GC, conversion of EDA: 100%). This metal carbene species, however, escaped detection by spectroscopic means including MALDI-TOF MS. By treating $[Ru^{II}(4\text{-}(Ac_4Glc)\text{-}TPP)(CO)]$ (the acetylated form of **1**) with N_2CPh_2 in CH_2Cl_2, we observed the formation of a new species that showed a MALDI-TOF MS peak at m/z 2265 attributable to the carbene complex $[Ru(4\text{-}(Ac_4Glc)\text{-}TPP)(CPh_2)]^+$, along with a UV−vis spectrum featuring two Soret bands at 397 and 412 nm and a β band at 534 nm, similar to that of $[Ru(D_4\text{-}Por^*)(CPh_2)]$ reported in our previous work (Soret: 397 and 432 nm, β: 536 nm).[18i]

(30) For reviews, see: (a) Trost, B. M.; Melvin, L. S., Jr. *Sulfur Ylides*; Academic Press: New York, 1975; Chapter 7. (b) Vedejs, E. *Acc. Chem. Res.* **1984**, *17*, 358. (c) Pawda, A.; Hornbuckle, S. F. *Chem. Rev.* **1991**, *91*, 263. (d) Markó, I. E. In *Comprehensive Organic Synthesis*; Pergamon: Oxford, 1991; Vol. 3,Chapter 3.10, p 913. (e) Li, A.-H.; Dai, L.-X.; Aggarwal, V. K. *Chem. Rev.* **1997**, *97*, 2341. (f) Hodgson, O. M.; Pierard, F. Y. T. M.; Stupple, P. A. *Chem. Soc. Rev.* **2001**, *30*, 50. (g) Clark, J. S. In *Nitrogen, Oxygen and Sulfur Ylides Chemistry. A Practical Approach in Chemistry*; Clark, J. S., Ed.; Oxford University Press: Oxford, 2002. (h) Sweeney, J. B. *Chem. Soc. Rev.* **2009**, *38*, 1027.

Scheme 3

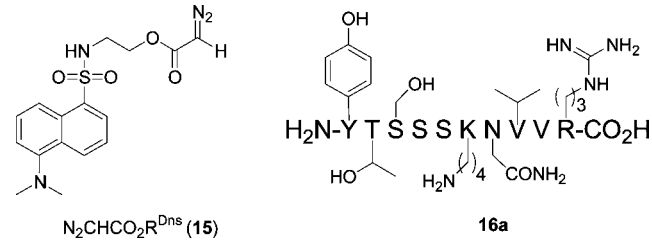


catalyzed Doyle−Kirmse reactions in water.[11] To the best of our knowledge, the **1**-catalyzed reactions of **9** and **11** are the first examples of the following types of metal-catalyzed reactions in aqueous media: (i) intramolecular Doyle−Kirmse reaction and (ii) ammonium ylide formation/[2,3]-sigmatropic rearrangement reaction.

To compare the catalytic properties of **1** with those of previously reported water-soluble ruthenium porphyrins, we examined the above carbenoid transfer reactions using catalysts **2**−**4** (the results are included in Tables S1−S5 in Supporting Information). A comparison of the catalytic properties among **1**−**4**, $[Ru^{II}(D_4\text{-PorS*})(CO)]^{4-}$,[12] and other related metal catalysts is described in the Supporting Information.

Peptide Modification through Carbenoid Transfer Reactions Catalyzed by [Ru^{II}(4-Glc-TPP)(CO)] (1) in Aqueous Media. Metal-catalyzed carbenoid insertion into X−H (X = C, N, O, S) bonds is possibly applicable for the modification of peptides that contain amino acid residues such as tyrosine (Y), tryptophan (W), lysine (K), cysteine (C), aspartic acid (D), glutamic acid (E), threonine (T), and serine (S). A challenge for this type of catalytic bioconjugation reactions is to achieve high site selectivity and chemoselectivity for the reaction of a diazo compound with peptides that contain multiple reactive X−H (X = C, N, O, S) sites. We prepared diazo compound $N_2CHCO_2R^{Dns}$ (**15**, R^{Dns} = dansylaminoethyl), which contains a dansyl fluorophore, by treating dansyl chloride with 2-aminoethanol followed by sequential reactions with diketene, MsN_3, and LiOH (Scheme S1 in Supporting Information). YTSSSKNVVR (**16a**) was chosen as a model peptide substrate for catalyst screening, since it has various types of O−H (primary, secondary, and phenolic) and N−H (N-terminal, ε-, and guanidinyl) sites possibly available for carbenoid insertion, allowing examination of site selectivity.

(i) N−H Insertion. Reaction of **16a** (100 μM) with **15** (1.1 equiv) in a solution of phosphate-buffered saline (PBS, pH 7.4) containing a catalytic amount of **1** (10 mol %) at room temperature for 1 h afforded **17a** (Scheme 3) with 93% substrate conversion (entry 2 in Table 1), as revealed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (Figure S1 in Supporting Information). Only a trace amount of **18a** (Scheme 3) was detected in the reaction mixture, with a **17a**/**18a** ratio of >100:1 (entry 2 in Table 1). Neither other N−H insertion products nor O−H insertion products were detected by LC-MS/MS analysis of the reaction mixture. In the absence of catalyst **1**, no substrate conversion was observed (entry 1 in Table 1). *Changing the catalyst from* **1** to **2**−**4** all resulted in *a* < 5% substrate conversion (entries 10−12 in Table 1). The same reaction catalyzed by $[Ru^{II}(Por)(CO)]$ (Por = TPP, TTP, TMP, 3,4,5-MeO-TPP) in PBS solution containing 1% dioxane led to 0% or <10% substrate conversion (entries 13−16 in Table 1). When $[Rh_2(OAc)_4]$ was used as catalyst, no modified peptide product was observed (entry 17 in Table 1), and an O−H insertion product $HOCH_2CO_2R^{Dns}$ was detected by mass spectrometry (*m*/*z* 352).



With **1** as the catalyst, slightly raising the temperature to 37 °C increased the substrate conversion to >99%, and no **18a** was detected (entry 3 in Table 1). Upon reducing the loading of **1** from 10 mol % to 5 mol %, the substrate conversion decreased to 52% (entry 4 in Table 1), with a product turnover of ∼10. A marked decrease in substrate conversion was also found by lowering the pH value of the reaction mixture from 7.4 to 6.4 and 5.0; further reduction of the pH value to 4.0 resulted in no substrate conversion (entries 5−7 in Table 1). When the pH value was increased from 7.4 to 8.4, the substrate conversion slightly decreased to 97% (entry 8 in Table 1).

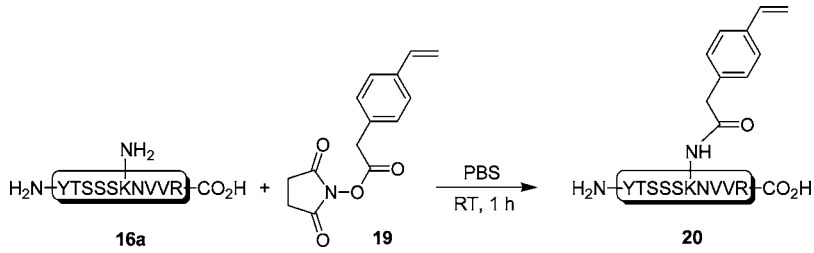
Table 1. Modification of Peptide YTSSSKNVVR (**16a**) by **1**-Catalyzed Carbenoid Transfer Reactions under Various Conditions[a] (Results Using Catalysts **2**−**4** and Other Metal Complexes Are Included for Comparison)

entry	pH	temp (°C)	metal catalyst	conv (%)	selectivity (**17a**/**18a**)[b]
1	7.4	RT	-	0	-
2	7.4	RT	**1**	93	>100:1
3	7.4	37	**1**	>99	**17a** only
4[c]	7.4	37	**1**	52	100:1
5	4.0	37	**1**	0	-
6	5.0	37	**1**	19	**17a** only
7	6.4	37	**1**	46	**17a** only
8	8.4	37	**1**	97	100:1
9[d]	7.4	37	**1**	81	**17a′** only[d]
10	7.4	RT	**2**	<5	-
11	7.4	RT	**3**	<5	-
12	7.4	RT	**4**	<5	-
13[e]	7.4	RT	$[Ru^{II}(TPP)(CO)]$	<5	-
14[e]	7.4	RT	$[Ru^{II}(TTP)(CO)]$[f]	<5	-
15[e]	7.4	RT	$[Ru^{II}(TMP)(CO)]$	0	-
16[e]	7.4	RT	$[Ru^{II}(3,4,5\text{-MeO-TPP})(CO)]$[g]	9	-
17	7.4	RT	$[Rh_2(OAc)_4]$	0	-

[a] Reaction conditions: **16a** (100 μM), **15** (110 μM), catalyst (10 μM), PBS (pH 7.4, 100 mM NaCl), 1 h. Product and substrate conversion were determined by LC-MS/MS. [b] Based on ion count of the peaks in LC-MS. [c] Metal catalyst (5 μM). [d] EDA (instead of **15**) was used; **17a′** differs from **17a** in that the former bears an Et group, instead of an R^{Dns} group. [e] PBS (pH 7.4, 100 mM NaCl) containing 1% dioxane. [f] TTP = *meso*-tetrakis(*p*-tolyl)porphyrinato dianion. [g] 3,4,5-MeO-TPP = *meso*-tetrakis(3,4,5-trimethoxyphenyl)porphyrinato dianion.

(31) Selected examples: (a) Cama, L. D.; Christensen, B. G. *Tetrahedron Lett.* **1978**, *19*, 4233. (b) Aller, E.; Buck, R. T.; Drrysdale, M. J.; Ferris, L.; Haigh, D.; Moody, C. J.; Pearson, N. D.; Sanghera, J. B. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2879. (c) Bolm, C.; Kasyan, A.; Drauz, K.; Günther, K.; Raabe, G. *Angew. Chem., Int. Ed.* **2000**, *39*, 2288. (d) Morilla, M. E.; Díaz-Requejo, M. M.; Belderrain, T. R.; Nicasio, M. C.; Trofimenko, S.; Pérez, P. J. *Chem. Commun.* **2002**, 2998. (e) Matsushita, H.; Lee, S.-H.; Yoshida, K.; Clapham, B.; Koch, G.; Zimmermann, J.; Janda, K. D. *Org. Lett.* **2004**, *6*, 4627. (f) Davies, J. R.; Kane, P. D.; Moody, C. J. *J. Org. Chem.* **2005**, *70*, 7305. (g) Aviv, I.; Gross, Z. *Chem. Commun.* **2006**, 4477. (h) Liu, B.; Zhu, S.-F.; Zhang, W.; Chen, C.; Zhou, Q.-L. *J. Am. Chem. Soc.* **2007**, *129*, 5834. (i) Lee, E. C.; Fu, G. C. *J. Am. Chem. Soc.* **2007**, *129*, 12066. (j) Taber, D. F.; Berry, J. F.; Martin, T. J. *J. Org. Chem.* **2008**, *73*, 9334.

Scheme 4



The results depicted in Table 1 reveal that the ruthenium glycosylated porphyrin **1** is a highly selective catalyst for modification of the N-terminus of **16a** in an aqueous medium via carbenoid insertion into the N−H bond. In a previous work, we demonstrated a selective modification of the N-terminus of peptides (including **16a**) by a different type of metal-catalyzed reaction, namely, N-terminal acylation with alkynes in aqueous $NaHCO_3$ buffer using the "[Mn(2,6-Cl_2TPP)Cl] + H_2O_2" oxidation system (2,6-Cl_2TPP = *meso*-tetrakis(2,6-dichlorophenyl)porphyrinato dianion).[32]

Interestingly, the **1**-catalyzed carbenoid N−H insertion reaction of **16a** occurred predominately at the N-terminal amine (pK_a 7.6−8.0[14]) but not at the more basic internal lysine ε-amine (pK_a 9.3−9.5[14]); this contrasts with the modification of the same peptide under similar conditions (37 °C, PBS, pH 7.4) using *N*-hydroxysuccinimide (NHS) ester **19** (Scheme 4; NHS ester is a widely used acylation agent for amines), in which case the major product was the lysine modified peptide **20** (Figure S2 in Supporting Information), instead of the corresponding N-terminal modified peptide. A rationalization is that most of the lysine ε-amine groups are protonated at pH 7.4−8.4 in aqueous media, rendering these amine groups not available for **1**-catalyzed carbenoid N−H insertion under these pH conditions. This is supported by a dramatic decrease in substrate conversion upon lowering the pH from 7.4 to 5.0 (Table 1), as most of the N-terminal amines would also be protonated at pH 5.0 (no reaction was observed when the pH was lowered to 4.0). The reason for the absence of the O−H insertion product in the **1**-catalyzed reaction might be due to a lower nucleophilicity of the OH groups compared with the N-terminal amine group.

The **1**-catalyzed carbenoid insertion reaction is useful for selective modification of peptides not only at N-terminal tyrosine (in **16a**) but also at other N-terminal amino acids such as glycine, alanine, and histidine. Under the optimum conditions (37 °C, pH 7.4), a variety of peptides YLSGANLNL (**16b**), PPGFSPFR (**16c**), GGG (**16d**), ALILTLVS (**16e**), HDMNKVLDL (**16f**), TYGPVFMSL (**16g**), and DRVYIHPFHL (**16h**) reacted with **15** in the presence of catalyst **1**, resulting in modification of the N-terminus of **16b**−**g** (Figures S3−S8 in Supporting Information) with 21−95% substrate conversions (Table 2). Excellent substrate conversions of 95% and 93% were obtained for **16b** and **16c**, respectively (entries 1, 2 in Table 2).

(ii) Competition between N−H and S−H Insertion. The competition between N−H and S−H bonds for a carbenoid insertion reaction is commonly encountered in peptide modification. Treatment of peptide AYEMWCFHQK (**16i**), which contains both N-terminal amine and internal SH groups, with **15** in PBS (pH 7.4) in the presence of catalyst **1** (10 mol %) at room temperature for 1 h afforded the S−H insertion product **21** exclusively (Scheme 5 and Figure S9 in Supporting Information) with complete substrate conversion, indicating that a S−H insertion reaction is more rapid than a N−H insertion reaction. The thioether group of methionine in the peptide remained intact throughout the reaction. Under the same conditions, **1** catalyzed the reaction of **15** with SSCSSC-PLSSK (**16j**), a peptide containing a disulfide group and both N-terminal and internal amine groups, to give the N-terminal N−H insertion product **17j** only, with 73% substrate conversion (Scheme 5 and Figure S10 in Supporting Information), and no cleavage of the disulfide bond was observed.

Table 2. Modification of Peptides (**16b**−**h**) by **1**-Catalyzed Carbenoid Transfer Reactions[a]

entry	peptide	conv (%)[b]
1	YLSGANLNL (**16b**)	95
2	PPGFSPFR (**16c**)	93
3	GGG (**16d**)	∼80
4	ALILTLVS (**16e**)	46
5	HDMNKVLDL (**16f**)	34
6	TYGPVFMSL (**16g**)	21
7	DRVYIHPFHL (**16h**)	5

[a] Conditions: peptide (100 μM), **15** (110 μM), **1** (10 μM), PBS (pH 7.4, 100 mM NaCl), 37 °C, 1 h. [b] Determined by LC-MS/MS.

Modification of N-Terminus of Proteins through Carbenoid Transfer Reactions Mediated by [Ru^{II}(4-Glc-TPP)(CO)] (1) in Aqueous Media. Bioconjugation of proteins is a useful tool in biological studies. There is an increasing need to develop new synthetic technologies for the bioconjugation reaction of proteins,[14] and metal-catalyzed site-selective modification of proteins has attracted considerable interest in recent years.[15] Despite the wide applications of metal-catalyzed carbenoid transfer reactions in synthetic organic chemistry,[1] modification of proteins by this type of metal catalysis has previously been confined to [$Rh_2(OAc)_4$]-catalyzed carbenoid transfer to the indole group of internal tryptophan residues of proteins including myoglobin,[8a] β-lactoglobulin,[16] subtilisin,[8b] lysozyme,[8b] and mutants of FKBP.[8b] In contrast to the [$Rh_2(OAc)_4$]-catalyzed modification of proteins under denaturing conditions (at pH ∼3.5 in the presence of $HONH_2$·HCl additive[8a] or at pH 6−7 in the presence of ^{t}BuNHOH at 75−95 °C[8b]), the protein modification reactions mediated by **1** can be conducted at 37 °C in a pH range of 5.0−8.4 in the absence of an additive, without causing the proteins to denature.

Reaction of an N-terminal lysine-containing protein, RNase A (10 μM), with **15** (1.1 equiv) in PBS (pH 7.4) solution in the presence of **1** (1 equiv) at 37 °C for 1 h increased the mass of RNase A by ∼334 amu on the basis of liquid chromatography-mass spectrometry (LC-MS) analysis (Figure S11 in Supporting Information), indicating that one dansyl carbene unit $CHCO_2R^{Dns}$ (mass: 334 amu) had been incorporated into this protein. The substrate conversion based on MS analysis was 65% (entry 1 in Table 3). LC-MS/MS analysis of the modified protein, after

(32) Chan, W.-K.; Ho, C.-M.; Wong, M.-K.; Che, C.-M. *J. Am. Chem. Soc.* **2006**, *128*, 14796.

Scheme 5

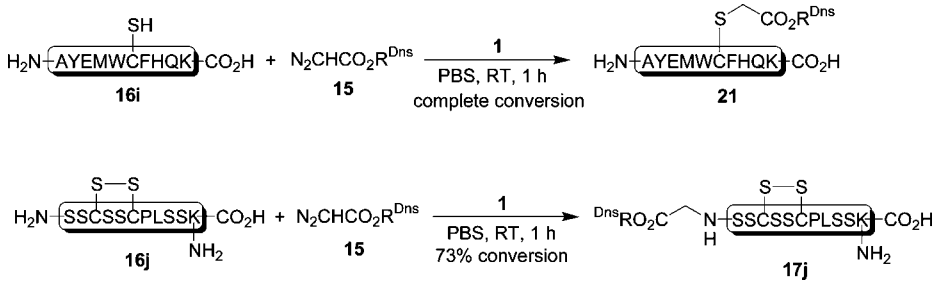


Table 3. Alkylation of N-Terminus of Proteins by **1**-Catalyzed Carbenoid Transfer Reactions[a]

entry	protein	N-terminal amino acid	conv (%)
1	RNase A	lysine	65
2	insulin	glycine (A-chain) and phenylalanine (B-chain)	54[b]
3	myoglobin	glycine	trace
4	ubiquitin	methionine	trace

[a] Conditions: protein (10 μM), **15** (11 μM), **1** (10 μM), PBS (pH 7.4, NaCl, 100 mM), 37 °C, 1 h. [b] Total conversion (singly modified 48%, doubly modified 6%).

digestion with trypsin, revealed that only the N-terminal lysine of RNase A was modified (Figure S12 in Supporting Information), consistent with the formation of **22** (Scheme 6). This "**15** + **1**" protocol was also effective for insulin, giving singly modified insulin with 48% substrate conversion; a doubly modified insulin was also detected, with a low substrate conversion of 6% (Figure S13 in Supporting Information and entry 2 in Table 3). The doubly modified insulin species is probably due to acylation of both N-termini of A- and B-chains of the insulin molecule. However, attempts to apply this protocol to myoglobin and ubiquitin, which contain 17 and 7 lysine residues, respectively, besides N-terminal glycine or methionine, were not successful (see Figures S14 and S15 in Supporting Information).

The ineffective modification of myoglobin and ubiquitin by **1**-mediated N−H insertion could be due to the relatively small solvent-accessible areas of the nitrogen atoms of their N-terminal amine groups, which were calculated to be 29.06 and 18.68 Å^2, respectively, based on the crystal structure data of horse heart myoglobin (1YMB) and bovine ubiquitin (1AAR) (using GETAREA 1.4[33]), compared with the corresponding value of 40.20 Å^2 calculated for RNase A (1FS3). We reason that a larger solvent-accessible area favors the bioconjugation reaction, as the N−H site at which the reaction takes place is not bounded by the protein molecule. Insulin contains two N-termini: the one belonging to phenylalanine has a solvent-accessible area of 31.19 Å^2, larger than that of 18.31 Å for the one belonging to glycine; thus, the former is more exposed and more reactive, consistent with the observation described above. However, such rationalization should be taken with caution, as proteins have a dynamic structure in solution. Also, the carbenoid transfer reaction is dependent on the pK_a value of amines, which may vary with the amino acids, salts, buffer, temperature, and ionic strength. These factors may also affect the reactivity of carbenoid group transfer and should not be neglected.

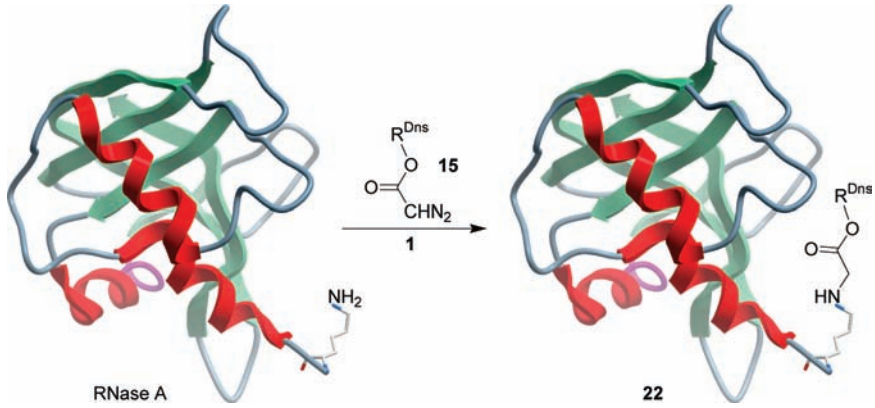
The water-soluble ruthenium porphyrin complex **1** could be used to mediate the conjugation of a fluorescent group, through carbenoid N−H insertion, to the N-terminus of proteins having different classes of N-terminal amino acids such as proline, threonine, tyrosine, alanine, histidine, glutamic acid, and glycine. Francis and co-workers recently reported N-terminal protein modification by introducing a ketone or aldehyde group.[34] Note that the **1**-mediated N−H insertion reaction would not be compatible with the free SH group of cysteine, as both thiolate (RS$^-$) and free SH are stronger nucleophiles than NH, which would render the SH group a more favorable site for carbenoid insertion (see Scheme 5). This limitation on N−H insertion could actually suggest another potential application of the **1**-mediated carbenoid transfer reactions, i.e. selective modification of proteins via S−H insertion.

Modification of Alkene-Tethered Protein through Carbenoid Transfer Reaction Mediated by [Ru^{II}(4-Glc-TPP)(CO)] (1) in Aqueous Media. As direct modification of ubiquitin by **1**-mediated N−H insertion was not effective, we performed a site-selective modification of this protein in two steps. The first step is to covalently tether an alkene group to the lysine residue Lys^6 of ubiquitin by treatment of this protein with **19** (Scheme 7) in PBS solution (pH 7.4), analogous to the previously reported reaction of ubiquitin with sulfosuccinimidobiotin to give Lys^6-biotinylated ubiquitin.[35] The alkene-tethered ubiquitin (**23**) purified by HPLC exhibited a peak at *m*/*z* 8710 (Figure S16 in Supporting Information) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). After tryptic digestion of **23**, the resulting peptides were analyzed by LC-MS/MS, and the results, as compared with those of ubiquitin, revealed attachment of the alkenic moiety ($C_{10}H_8O$, 144 amu) to the Met^1-Lys^{11} peptide (MQIFVKTLTGK) only (the details are given in Table S6 of Supporting Information). A doubly charged ion peak at *m*/*z* 705.4 attributable to this alkene-tethered peptide was observed by LC-MS for the trypsin-digested **23**. MS/MS analysis confirmed that the alkene was specifically incorporated at Lys^6.

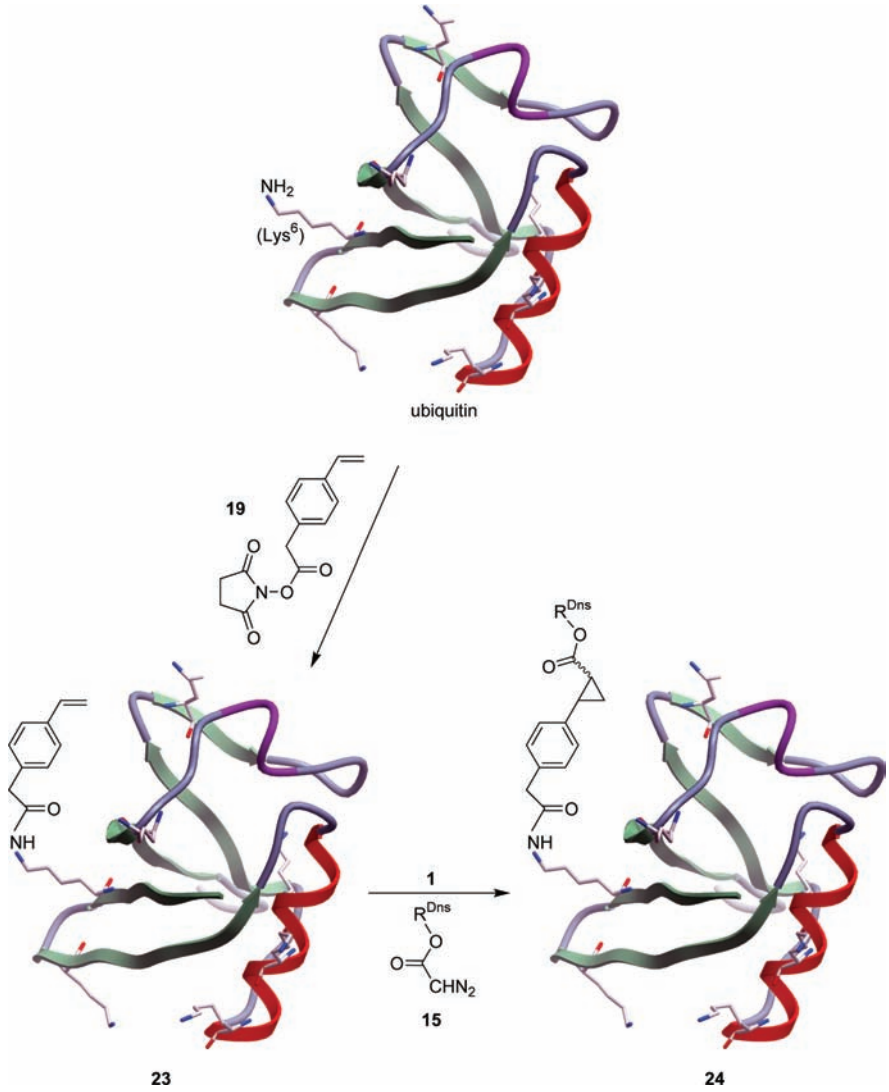
In the second step, reaction of **23** (100 μM) with **15** (1.1 equiv) in PBS (pH 7.4) containing **1** (10 mol %) at 25 °C for 1 h afforded the cyclopropanation product **24** (Scheme 7) with ~55% substrate conversion (~5.5 product turnovers). Similar selectivity and product yield were found for the reaction even when the concentration of **23** was lowered to 10 μM. MALDI-TOF MS analysis of the product, upon purification by HPLC, revealed a peak at *m*/*z* 9044 (Figure S16 in Supporting Information) attributed to **24**. This peak has an *m*/*z* value 334 larger than that observed for **23**, consistent with the incorporation of one dansyl carbene unit $CHCO_2R^{Dns}$ (334 amu) into **23**. Indeed, the dansyl-labeled protein **24** could be seen as a yellow fluorescent band under UV-irradiation by SDS-PAGE analysis (Figure S16 in Supporting Information). For the **1**-catalyzed reaction of **23** with **15**, other forms of modified **23**, such as

(33) Fraczhiewicz, R.; Braun, W. *J. Comput. Chem.* **1998**, *19*, 319.
(34) Gilmore, J. M.; Scheck, R. A.; Esser-Kahn, A. P.; Joshi, N. S.; Francis, M. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 5307.
(35) Shang, F.; Deng, G.; Liu, Q.; Guo, W.; Haas, A. L.; Crosas, B.; Finley, D.; Taylor, A. *J. Biol. Chem.* **2005**, *280*, 20365.

Scheme 6



Scheme 7



a doubly modified one, were not detected in the reaction mixture. No modification of **23** was observed in the absence of catalyst **1**.

Conclusion

We have synthesized a water-soluble glycosylated ruthenium porphyrin catalyst (**1**), which not only exhibits high activity and selectivity toward a number of carbenoid transfer reactions in aqueous media, including inter- and intramolecular alkene cyclopropanation, intermolecular carbenoid N−H insertion, and intramolecular ammonium/sulfonium ylide formation/[2,3]-sigmatropic rearrangement but also can be applied to site-selective modification of peptides and proteins. By using **1** as a catalyst, the N-terminus of a number of peptides can be modified through carbenoid N−H insertion in aqueous media

with high site selectivity and moderate to excellent substrate conversions. Proteins RNase A and insulin can also be modified at their N-terminus in a similar manner. The bioconjugation reaction is selective to protein structure. Site-selective modification of ubiquitin via metal catalysis has been achieved by introducing an alkene group to the protein followed by **1**-catalyzed cyclopropanation of the alkene-tethered ubiquitin with a fluorescent-labeled diazo compound in aqueous media. Modification of proteins by the **1**-mediated carbenoid transfer can be performed at pH 5.0−8.4, an optimum pH range for most proteins, with high chemoselectivity and without nonproductive binding to the protein surface. This ruthenium porphyrin-mediated bioconjugation method could be applicable for proteins containing various N-terminal amino acids. For peptides having the SH group, the modification would be dominated by carbenoid S−H insertion instead of N−H insertion reaction. The selective conjugation of the fluorescent dansyl probe to protein chains by the method reported in this work is potentially useful for biological studies.

The present work, to the best of our knowledge, contributes the first examples of (a) ruthenium porphyrin-catalyzed modification of peptides and proteins in aqueous media and (b) metal-mediated carbenoid transfer for modification of the N-terminus of proteins. The **1**-catalyzed carbenoid transfer to alkene-tethered protein in aqueous media confirmed by LC-MS and LC-MS/MS could open up an entry to new bioconjugation reactions for protein modifications using metalloporphyrins as catalysts.

Experimental Section

Synthesis of Glycosylated Ruthenium Porphyrin [Ru^{II}(4-Glc-TPP)(CO)] (1). A mixture of $Ru_3(CO)_{12}$ (200 mg) and H_2(4-(Ac_4Glc)-TPP) (200 mg) in decalin (50 mL) was refluxed under nitrogen for 24 h. The resulting red solution was loaded on an alumina column. Decalin and some impurities were removed with hexane as the eluent. The brick red band containing the desired product was eluted with hexane/EtOAc (1:2 v/v) and collected. After removal of solvent, a red solid of [Ru^{II}(4-(Ac_4Glc)-TPP)(CO)] was obtained (yield: 50%. IR: ν(CO) 1940 cm^{-1}). This complex was refluxed in methanol (50 mL) containing NaOMe (50 mg) for 30 min. Treatment of the mixture with ion-exchange resin to pH = 7 and concentration of the filtrate afforded the desired product **1** as a red solid. Yield: 50%. UV−vis (MeOH): λ_{max} (log ε) 412 (5.46), 527 nm (4.43); IR: ν(CO) 1940 cm^{-1}; ^{1}H NMR (CD_3OD): 8.58 (s, β-H, 8H), 8.05 (d, J = 6.3 Hz, 4H), 7.95 (d, J = 8.1 Hz, 4H), 7.41 (m, 8H), 5.18 (d, J = 6.6 Hz, 4 H), 3.97 (d, J = 10.8 Hz, 4H), 3.78 (m, 4H), 3.58 (m, 12H), 3.45 (m, 4H); ESI-MS: m/z 1455.2 (M^+).

Typical Procedure for 1-Catalyzed Intermolecular Cyclopropanation of Styrenes. To a vigorously stirred aqueous solution of **1** (0.01 mmol) in water (10 mL) was added styrene (1 mmol). An aqueous solution of EDA (1.2 mmol) in water (10 mL) was slowly added to the reaction mixture via a syringe pump over 24 h at RT. The reaction mixture was stirred at RT, with the reaction progress monitored by TLC. The aqueous phase was extracted with Et_2O (15 mL × 3), and the combined organic extracts were dried over anhydrous Na_2SO_4. Conversions, yields, and *trans*/*cis* ratios were determined by GC analysis using the internal standard method (1,4-dichlorobenzene).

Typical Procedure for 1-Catalyzed Intermolecular Carbenoid N−H Insertion of Amines. To a vigorously stirred aqueous solution of amine (1 mmol) and **1** (0.005 mmol) in water (5 mL) was slowly added an aqueous solution of EDA (0.5 mmol) in water (5 mL) via a syringe pump over 10 h at RT. The reaction mixture was stirred until all the substrate was consumed, as monitored by TLC. The aqueous phase was extracted with Et_2O (15 mL × 3), and the combined organic extracts were dried over anhydrous Na_2SO_4. Pure products were obtained by flash chromatography.

Typical Procedure for Modification of Peptides by 1-Catalyzed Carbenoid Transfer Reactions. A PBS solution (pH 7.4) containing peptide (100 μM) and **1** (10 μM) in a 1.5 mL eppendorf tube was treated with a solution of the diazo compound **15** (1.1 equiv) in dioxane (1 mg/mL). The solution was incubated at 37 °C for 1 h. The resulting N-terminal modified peptide was characterized by LC-MS/MS analysis.

Typical Procedure for Modification of N-Terminus of Proteins by 1-Mediated Carbenoid Transfer Reactions. A PBS solution (pH 7.4) containing protein (10 μM) and **1** (10 μM) in a 1.5 mL eppendorf tube was treated with a solution of the diazo compound **15** (1.1 equiv) in dioxane (1 mg/mL). The solution was incubated at 37 °C for 1 h. The resulting N-terminal modified protein was characterized by LC-MS/MS analysis.

Modification of Alkene-Tethered Lys^6 Ubiquitin (23) by 1-Catalyzed Carbenoid Transfer Reaction. 100 μL of alkene-tethered Lys^6 ubiquitin (100 μM) in PBS at pH 7.4 was added to a 1.5 mL vial, followed by adding 10 μL of an aqueous solution of **15** (110 μM). 10 mol % of **1** in 10 μL of dioxane was added, and the reaction mixture was generally mixed in a thermomixer (Eppendrof Thermomixer Comfort) at 25 °C for 1 h. After removal of small molecules by ultrafiltration (Millipore, Centricon, YM-3), the purified protein sample **24** was resuspended in 100 μL of PBS (pH 7.4) solution and then analyzed by SDS-PAGE and MALDI-TOF MS. Upon digestion of **24** with trypsin, the resulting peptides were analyzed by LC-MS/MS. A doubly charged peptide fragment at 873 m/z was identified. MS/MS analysis of this peptide fragment showed a full y-ion series that is consistent with the cyclopropanation of the alkene tethered to the Lys^6 residue of ubiquitin (Figure S17 in Supporting Information). The formulation of the cyclopropanation product **24** was also based on the well-documented cyclopropanation of alkenes with diazo compounds catalyzed by ruthenium porphyrins[25a,c] and the catalytic activity of **1** toward cyclopropanation of alkenes with diazoacetates (see, for example, Scheme 2).

Acknowledgment. This work was supported by The University of Hong Kong (University Development Fund), the University Grants Council of HKSAR (the Area of Excellence Scheme: AoE 10/01P), and the Hong Kong Research Grants Council (HKU 1/CRF/08).

Supporting Information Available: Experimental section including general, product identification, procedures for preparation of **15**, **19**, and **23**, Tables S1−S6, Chart S1, Scheme S1, and Figures S1−S17. This material is available free of charge via the Internet at http://pubs.acs.org.

JA9077254