Peptide Bond Cleavage

DOI: 10.1002/anie.201402618

Serine-Selective Aerobic Cleavage of Peptides and a Protein Using a Water-Soluble Copper–Organoradical Conjugate**

Yohei Seki, Kana Tanabe, Daisuke Sasaki, Youhei Sohma, Kounosuke Oisaki, and Motomu Kanai**

Abstract: *The site-specific cleavage of peptide bonds is an important chemical modification of biologically relevant macromolecules. The reaction is not only used for routine structural determination of peptides, but is also a potential artificial modulator of protein function. Realizing the substrate scope beyond the conventional chemical or enzymatic cleavage of peptide bonds is, however, a formidable challenge. Here we report a serine-selective peptide-cleavage protocol that proceeds at room temperature and near neutral pH value, through mild aerobic oxidation promoted by a water-soluble copper–organoradical conjugate. The method is applicable to the site-selective cleavage of polypeptides that possess various functional groups. Peptides comprising* D*-amino acids or sensitive disulfide pairs are competent substrates. The system is extendable to the site-selective cleavage of a native protein, ubiquitin, which comprises more than 70 amino acid residues.*

Chemical modifications of proteins[1,2] have a great impact on a broad range of research fields, such as chemical biology,[3] chemical genetics,[4] protein engineering,[5] proteomics,[6] and drug discovery.[7] Site-specific cleaving reactions of peptide bond are among such important chemical modifications. The method is routinely used when structural determination or fragmentation of peptides plays a pivotal role,[8] and it is also regarded as a new strategy for modulating the physiological functions of proteins for medical applications.[9,10] Although peptidases hydrolyze peptide bonds at specific sites under mild conditions with high fidelity,[11] the scope of the scissile substrates is restricted in principle to genetically encoded amino acid sequences. Unnatural or structurally modified peptides and proteins are out of the scope of peptidase digestion. Metal-catalyzed site-selective hydrolysis of peptides[12] has been intensively studied as a potential alternative to the enzymatic method. The establishment of practical protocols and generalization to protein cleavage, however, remain challenging because of the chemical robustness of amides.[13]

Herein we report a serine (Ser)-selective cleavage of peptides (including a native protein) through mild aerobic oxidation of the hydroxymethyl group of Ser, promoted by a water-soluble Cu/*N*-oxyl radical system.

We envisioned a Ser-selective cleavage of the peptide bond initiated by aerobic chemoselective oxidation of its hydroxymethyl moiety (see Scheme 1).[8c,d] Oxidation of the hydroxymethyl moiety of a Ser residue (**A**) to a formyl group would produce an α-formylglycineamide intermediate **B**. Further oxidation of **B** would produce oxalimide **C** through oxidative deformylation. Hydrolysis of the latent precursor **C** under mild conditions should then be possible, because the carbonyl groups of the oxalimide are more electrophilic than those of simple amides, giving peptide bond cleaved fragments **D** and **D′**. Using molecular oxygen as a terminal oxidant, water and a C_1 molecule (possibly HCO_2H) are stoichiometric side products. This strategy is distinct from Lewis acid promoted Ser-selective peptide hydrolysis through N-to-O rearrangement.[14]

To realize the designed reaction, we focused on an oxidation of a Ser hydroxymethyl moiety with molecular oxygen catalyzed by a Cu/*N*-oxyl radical.[15–17] This catalysis is tolerant to water and Lewis basic functionalities.[16b] The initial optimization was conducted using *N*-Cbz-serine *tert*-butylamide as a model substrate (0.1–0.2 M, see Tables S1 and S2 in the Supporting Information for details). Optimized catalytic conditions for the hydroxymethyl oxidation of Ser include the use of CuI, bathophenanthrolinedisulfonic acid disodium salt (bathophen salt), keto-ABNO[16b] (10 mol% each), and $NaNO_2$ (two additions: 15 mol% at the start of the reaction and 15 mol% after five hours) in CH_3CN/H_2O/AcOH (9/9/2) at room temperature under O_2 atmosphere (1 atm).

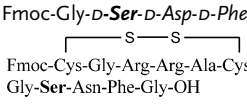
Next we applied the thus-optimized catalytic conditions to the Ser-selective cleavage of pentapeptide **1a** (Fmoc-Gly-Ser-Asn-Lys-Gly-OH, Fmoc was used for ease of analysis) in a diluted concentration (10 mol% of the catalyst and 5 mM of **1a**). Only small amounts of N-terminal fragments were produced as a mixture of Fmoc-Gly-OH and Fmoc-Gly-NH_2 (ca. 5% combined yield), but most of **1a** remained unchanged. Probably, the generation of the catalytically active species was inefficient because of the small concentrations of each catalytic component and the substrate. To overcome the low reactivity, we used stoichiometric condi-

[*] Y. Seki, Dr. K. Tanabe, Dr. D. Sasaki, Dr. Y. Sohma, Dr. K. Oisaki, Prof. Dr. M. Kanai
Graduate School of Pharmaceutical Sciences
The University of Tokyo
Bunkyo-ku, Tokyo 113-0033 (Japan)
E-mail: oisaki@mol.f.u-tokyo.ac.jp
kanai@mol.f.u-tokyo.ac.jp
Dr. K. Tanabe, Dr. D. Sasaki, Dr. Y. Sohma, Prof. Dr. M. Kanai
Japan Science and Technology Agency (JST), ERATO
Kanai Life Science Catalysis Project
Bunkyo-ku, Tokyo 113-0033 (Japan)

[**] This work was supported by a Grant in-Aid for Young Scientist B and Scientific Research C from JSPS (for K.O.), and ERATO from JST (for M.K.). We thank T. Sonobe, Dr. Y. Aoi, and Dr. S. Kawashima for fruitful discussions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201402618.

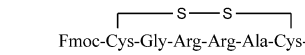
Table 1: Cu/keto-ABNO/NO_x/O_2-promoted Ser-selective chain cleavage.[a]

Entry	Substrate		Yield [%][b]
1	Fmoc-Gly-**Ser**-Asn-Lys-Gly-OH	**1a**	94
2	Fmoc-Gly-**Ser**-Asn-Arg-Gly-OH	**1b**	quant.
3	Fmoc-Gly-**Ser**-Asn-Asp-Gly-OH	**1c**	quant.
4	Fmoc-Gly-**Ser**-Asn-Cys-Gly-OH	**1d**	77
5	Fmoc-Gly-**Ser**-Asn-Met-Gly-OH	**1e**	93
6	Fmoc-Gly-**Ser**-Asn-Tyr-Gly-OH	**1f**	24
7	Fmoc-Gly-**Ser**-Asn-Trp-Gly-OH	**1g**	47
8	Fmoc-Gly-**Ser**-Asn-His-Gly-OH	**1h**	81
9	Fmoc-Gly-**Ser**-Asn-Thr-Gly-OH	**1i**	quant.
10[c]	Fmoc-Gly-Thr-Asn-**Ser**-Gly-OH	**1j**	52[d]
11	Fmoc-Gly-**Ser**-Gln-Phe-Gly-OH	**1k**	quant.
12	Fmoc-Ile-**Ser**-Asn-Lys-Gly-OH	**1l**	92
13	Fmoc-Ile-Gly-**Ser**-Asn-Lys-Gly-OH	**1m**	98
14	Fmoc-Gly-Ile-**Ser**-Asn-Lys-Gly-OH	**1n**	97
15	Fmoc-Aβ (21-30)-OH: Fmoc-Ala-Glu-Asp-Val-Gly-**Ser**-Asn-Lys-Gly-Ala-OH	**1o**	93
16	Fmoc-Bradykinin-OH: Fmoc-Arg-Pro-Pro-Gly-Phe-**Ser**-Pro-Phe-Arg-OH	**1p**	96
17	Fmoc-Gly-*D*-***Ser***-*D*-*Asp*-*D*-*Phe*-Gly-OH	**1q**	quant.
18[e]	 Fmoc-Cys-Gly-Arg-Arg-Ala-Cys-Gly-**Ser**-Asn-Phe-Gly-OH	**1r**	68
19[f]	Fmoc-Gly-**Ser**-Gly-Asn-Gly-Lys(ω-FmocNH)-Gly-OH	**1s**	83 (**2s**)[b] 82 (**3s**)[g]

[a] Standard conditions: CuI, bathophen salt, keto-ABNO (100 mol% each), and $NaNO_2$ (150 mol% at the start of the reaction and 150 mol% after 5 h) in CH_3CN/H_2O/AcOH (9/9/2, 5 mM) at room temperature for 20 hours under O_2 atmosphere (1 atm). [b] Combined yield of N-terminal fragments (Fmoc-peptide-NH_2 and Fmoc-peptide-OH) calculated from the absorbance at 301 nm (maximum absorbance of the Fmoc group) using reverse-phase HPLC analysis. [c] Reaction time was 3 h. [d] N-terminal fragments possessing oxidized Thr were observed in 5% yield. [e] Reaction was conducted in the presence of Me_2S (100 mol%). [f] CuI, bathophen salt, keto-ABNO (300 mol% each), and $NaNO_2$ (450 mol% at the start of the reaction, 450 mol% after 5 h) in CH_3CN/H_2O/AcOH (9/9/2, 5 mM) at 37°C for 51 h under O_2 atmosphere (1 atm). [g] Combined yield of C-terminal fragments (**3s**: X-COCO-Gly-Asn-Gly-Lys(ω-FmocNH)-Gly-OH; X = NH_2 and OH) calculated from the absorbance at 301 nm in reverse-phase HPLC analysis. keto-ABNO = 9-azabicyclo[3.3.1]nonan-3-one *N*-oxyl.

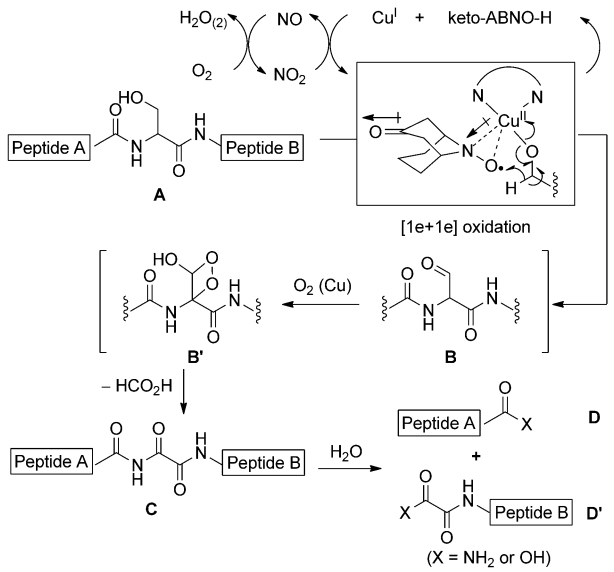
tions (CuI, bathophen salt, keto-ABNO (100 mol% each), and $NaNO_2$ (150 mol% at the start of the reaction and 150 mol% after five hours) in CH_3CN/H_2O/AcOH (9/9/2, 5 mM of **1a**) at room temperature under O_2 atmosphere (1 atm)), and the expected cleaved products were predominantly obtained in 94% combined yield (Table 1, entry 1).

We then investigated the substrate scope of the cleavage reaction (Table 1). Pentapeptides **1a**–**k**, which contain various amino acids, were investigated to assess the functional-group tolerance of this reaction (entries 1–11). Substrates that contain Lys, Arg (amine), Asp (carboxylic acid), Cys (thiol), Met (sulfide), or His (Lewis basic heterocycle) produced the N-terminal fragments (Fmoc-Gly-X: X = NH_2 and OH) with yields ranging from 77% to quantitative (entries 1–5, 8, and 9). In contrast, substrates that contain Tyr or Trp residues (i.e., electron-rich aromatic rings) gave the cleaved products in moderate yields, and some unidentified by-products were observed by high-performance liquid chromatography (HPLC) analysis (entries 6 and 7). In the case of Met-containing peptides (entry 5), oxidized C-terminal fragments (+16 Da) were detected as major products by liquid chromatography/mass spectroscopy (LC/MS). Tyr- and Trp-containing peptides (entries 6 and 7) produced complex mixtures of C-terminal-fragment derivatives as a result of overoxidation.[17e] We speculate that side reactions consumed active components and decreased the efficiency of the cleavage. The reaction was also applicable to substrates that contain Thr (secondary alcohol; entries 9 and 10). The oxidation of a Thr side chain was much slower (5% yield) than the oxidative cleavage at Ser (52% yield, entry 10). Other pentapeptides with greater steric hindrance around the Ser residue also provided good results (entries 11 and 12). Longer hexapeptides **1m** and **1n**, which contain a Ser residue at an internal position, gave comparable results (entries 13 and 14). Decapeptide **1o**, a fragment of the Alzheimer disease associated amyloid-β peptide, produced the cleaved chain in 93% yield (entry 15). A biorelevant oligopeptide, bradykinin, was cleaved in 96% yield (entry 16). Substrate **1q**, which contains D-amino acids and is out of the scope for enzymatic digestion, was successfully cleaved (entry 17). This method is compatible with disulfide pairings.[18] Peptide **1r**, which contains an intramolecular disulfide bond, afforded (X = NH_2 and OH)



with the disulfide bond remaining intact (entry 18). To achieve a higher and cleaner conversion, the addition of Me_2S as a peroxide scavenger was essential. Time-course HPLC analysis of the oxidative cleavage reaction of heptapeptide **1s**, which contains two Fmoc groups at both the N- and C-terminal sides of the Ser residue, demonstrated that the C-terminal fragments were recovered in equal combined amounts to the sum of N-terminal fragments (entry 19 and Figure S20 in the Supporting Information). This result supports our reaction design (Scheme 1), in which the N-terminal fragments **2s** and C-terminal fragments **3s** were simultaneously produced in a 1:1 ratio from the same precursor (oxalimide **C**).

Finally, to demonstrate the substrate scope of the present method, we extended the current conditions to the scission of a native protein, ubiquitin (UQ(1-76), exact mass = 8559.6 Da; Figure 1), which comprises 76 amino acid residues, including three serines (Ser^{20}, Ser^{57}, Ser^{65}).[19] The conversion of UQ was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Figure 1, inset). UQ was consumed completely after five hours under conditions A (CuI, bathophen salt, keto-ABNO (500 mol% each) and $NaNO_2$ (750 mol% at the start of the reaction and 750 mol% after five hours) in CH_3CN/H_2O/AcOH (9/9/2, 1 mM) at room temperature under O_2 atmosphere (1 atm)). Coomassie Brilliant Blue staining of the gel showed three distinct bands that were different from that of UQ. On the other hand, UQ remained unchanged under conditions B in the absence of keto-ABNO and conditions C in the absence of CuI, bathophen salt, and keto-ABNO. The reaction was



Scheme 1. A plausible mechanism of Ser-selective chain cleavage.

slower with the decreased loading of the Cu/keto-ABNO conjugate (Figure S1 in the Supporting Information).

Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis of the crude reaction mixture obtained under conditions A showed the presence of a UQ(58-64) fragment in a monooxidized form ($[M+O+H]^+$; observed $m/z = 996.8$). However, no other fragments were detected, probably because of their poor ionizability. To gain more insight into the product components, we extracted the products obtained under conditions A from the SDS-PAGE bands and conducted MALDI-TOF-MS analysis (Figure 1). The extract of band 1 (>7 kDa by SDS-PAGE) contained the UQ(1-64) fragments ($[M+2O+H]^+$; $m/z = 7289.8$ by MS). The extract of band 2 (<7 kDa by SDS-PAGE) contained the UQ(1-56) ($[M+O+H]^+$; $m/z = 6295.4$ by MS) and UQ(21-56) ($[M+H]^+$; $m/z = 4132.4$ by MS) fragments as an inseparable mixture because of the resolution limit of the employed gel. The extract of band 3 (<4 kDa by SDS-PAGE) contained the UQ(1-19) fragments ($[M+O+H]^+$; $m/z = 2164.1$ by MS). Mass peaks that correspond to mono- and dioxidized fragments were observed,

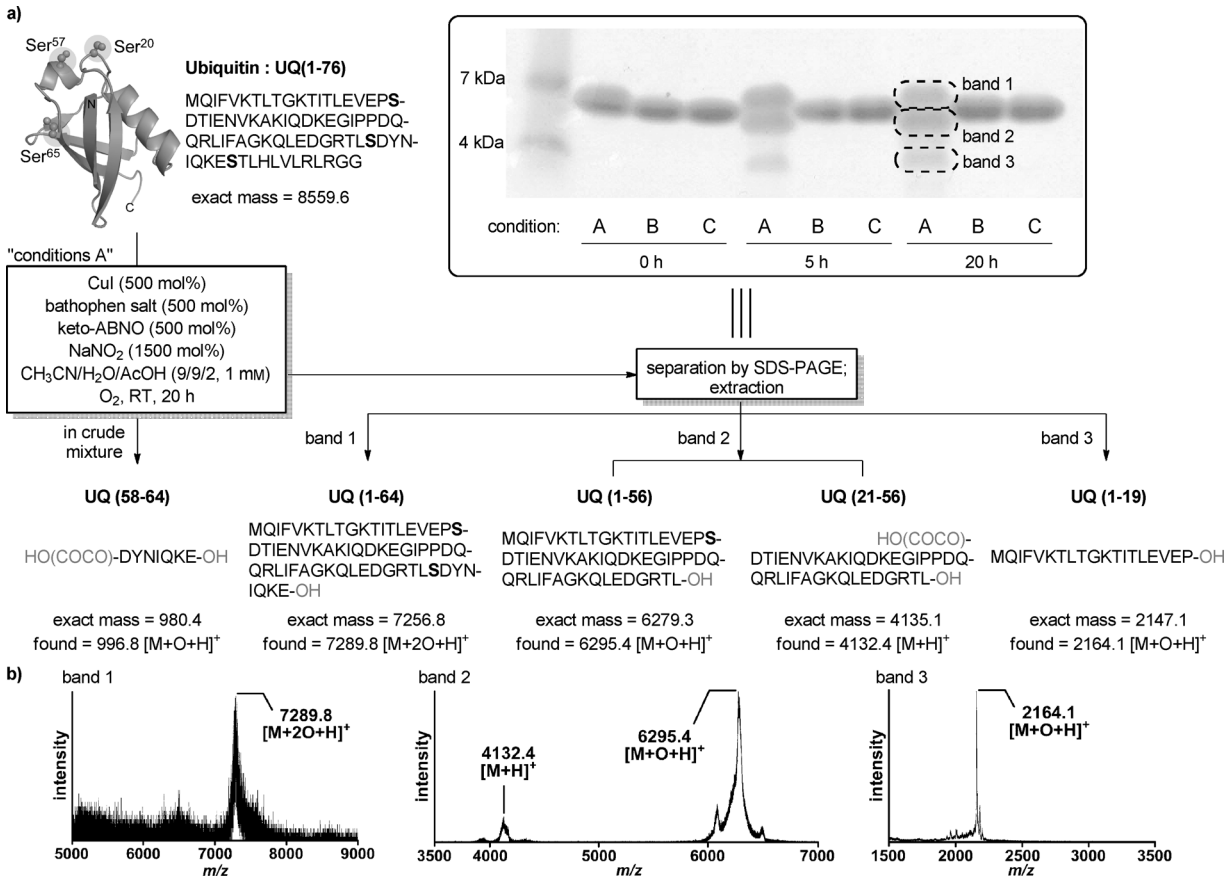


Figure 1. a) Ser-selective peptide chain cleavage of ubiquitin (UQ). Inset shows time-course of SDS-PAGE analysis of the reaction with the Coomassie Brilliant Blue stain. SeeBlue pre-stained standard was used as the molecular weight marker in the left lane. Conditions A = CuI, bathophen salt, keto-ABNO (500 mol% each), and $NaNO_2$ (750 mol% at 0 h and 750 mol% at 5 h) in CH_3CN/H_2O/AcOH (9/9/2, 1 mM) at room temperature under O_2 (1 atm); conditions B = conditions A without keto-ABNO; conditions C = conditions A without catalyst components (CuI, bathophen salt, and keto-ABNO). Primary sequence and 3D crystallographic structure of UQ are available from the Protein Data Bank (ID: 1UBQ). b) MALDI-TOF-MS spectra corresponding to each fragment in the gel extract.

likely because of oxidation at the Met and/or Tyr residues. Although we could not detect the UQ(66-76) fragment, predominant production of the three expected fragments (UQ(1-19), UQ(21-56), and UQ(58-64)) clearly demonstrated that the Ser selectivity of the present method was reliable when using native protein as the substrate.

A plausible mechanism for the Ser-selective chain cleavage is depicted in Scheme 1.[20] First, an oxidatively active complex of Cu^{II} and keto-ABNO is generated from Cu^{I} and keto-ABNO under oxygen atmosphere. Oxidation of the Ser residue proceeds through the formation of Cu^{II}-alkoxide species, single electron oxidation of the alkoxide species by Cu^{II}, and concomitant abstraction of a hydrogen radical from the α-carbon atom by keto-ABNO ([1e + 1e] oxidation process),[21] affording aldehyde **B** (α-formylglycineamide) and reduced Cu^{I} + keto-ABNO−H. Regeneration of the oxidatively active complex (Cu^{II} + keto-ABNO) is promoted by NO_2, generated from $NaNO_2$ and acetic acid. The resulting NO is reconverted to NO_2 through aerobic oxidation.[22] Oxidative deformylation of α-formylglycineamide **B** proceeds through presumed intermediate **B′** to produce oxalimide **C**.[8c,d,23] This process is likely also promoted by Cu catalysis.[23e,f] Oxalimide **C** is hydrolyzed under mild conditions to produce fragments **D** and **D′**.

In conclusion, we developed a Ser-selective cleavage of peptide/protein chains through aerobic oxidation promoted by a Cu/keto-ABNO/NO_x/O_2 system in aqueous media at ambient temperature and near neutral pH value. This is the first example of the site-selective cleavage of peptides and proteins using molecular oxygen as a stoichiometric oxidant. Because of its broad substrate scope, including peptides comprising unnatural D-amino acids or disulfide pairing, this method will be a useful complement to enzymatic methods for the structural determination of peptides. This system is also applicable to the site-selective scission of native proteins, which are considered the most challenging substrates for any artificial catalysis.[1,2] Site-selective, artificial chemical transformation (including scission) of biomolecules will be a novel approach for modulating their physiological functions. Research in this direction is ongoing in our group.

Received: February 20, 2014
Published online: May 14, 2014

Keywords: aerobic oxidation · copper · peptide bonds · proteins · serine

[1] Selected reviews: a) J. M. Antos, M. B. Francis, *Curr. Opin. Chem. Biol.* **2006**, *10*, 253 – 262; b) J. P. Pellois, T. W. Muir, *Curr. Opin. Chem. Biol.* **2006**, *10*, 487 – 491; c) C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem.* **2008**, *120*, 10182 – 10228; *Angew. Chem. Int. Ed.* **2008**, *47*, 10030 – 10074; d) W. P. Heal, E. W. Tate, *Org. Biomol. Chem.* **2010**, *8*, 731 – 738; e) Y. W. Wua, R. S. Goody, *J. Pept. Sci.* **2010**, *16*, 514 – 523; f) Z. Hao, S. Hong, S. Chen, P. R. Chen, *Acc. Chem. Res.* **2011**, *44*, 742 – 751; g) Y. Takaoka, A. Ojida, I. Hamachi, *Angew. Chem.* **2013**, *125*, 4182 – 4200; *Angew. Chem. Int. Ed.* **2013**, *52*, 4088 – 4106.

[2] Selected examples of chemical modification of native proteins: a) J. M. Antos, M. B. Francis, *J. Am. Chem. Soc.* **2004**, *126*, 10256 – 10257; b) D. Bang, B. Pentelute, S. B. H. Kent, *Angew. Chem.* **2006**, *118*, 4089 – 4092; *Angew. Chem. Int. Ed.* **2006**, *45*, 3985 – 3988; c) G. J. L. Bernardes, J. M. Chalker, J. C. Errey, B. G. Davis, *J. Am. Chem. Soc.* **2008**, *130*, 5052 – 5053; d) S. Tsukiji, M. Miyagawa, Y. Takaoka, T. Tamura, I. Hamachi, *Nat. Chem. Biol.* **2009**, *5*, 341 – 343; e) H. Ban, J. Gavrilyuk, C. F. Barbas III, *J. Am. Chem. Soc.* **2010**, *132*, 1523 – 1525; f) B. V. Popp, Z. T. Ball, *J. Am. Chem. Soc.* **2010**, *132*, 6660 – 6662; g) S. Sato, H. Nakamura, *Angew. Chem.* **2013**, *125*, 8843 – 8846; *Angew. Chem. Int. Ed.* **2013**, *52*, 8681 – 8684.

[3] S. L. Schreiber, *Nat. Chem. Biol.* **2005**, *1*, 64 – 66.

[4] C. J. O'Conner, L. Laraia, D. R. Spring, *Chem. Soc. Rev.* **2011**, *40*, 4332 – 4345.

[5] a) M. Leisola, O. Turunen, *Appl. Microbiol. Biotechnol.* **2007**, *75*, 1225 – 1232; b) B. A. Smith, M. H. Hecht, *Curr. Opin. Chem. Biol.* **2011**, *15*, 421 – 426.

[6] a) R. Aebersold, M. Mann, *Nature* **2003**, *422*, 198 – 207; b) R. S. Morrison, Y. Kinoshita, M. D. Johnson, T. P. Conrads, *Adv. Protein Chem.* **2003**, *65*, 1 – 23.

[7] a) M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor, E. M. Gordon, *J. Med. Chem.* **1994**, *37*, 1233 – 1251; b) A. Cruz-Migoni, N. Fuentes-Fernandez, T. H. Rabbitts, *Med. Chem. Commun.* **2013**, *4*, 1218 – 1221.

[8] Selected examples of the chemical cleavage of peptides targeting native amino acid residues: a) E. Gross, B. Witkop, *J. Biol. Chem.* **1962**, *237*, 1856 – 1860; b) A. Patchornik, M. Sokolovsky, *J. Am. Chem. Soc.* **1964**, *86*, 1206 – 1212; c) D. Ranganathan, S. Saini, *J. Am. Chem. Soc.* **1991**, *113*, 1042 – 1044; d) D. Ranganathan, N. K. Vaish, K. Shah, *J. Am. Chem. Soc.* **1994**, *116*, 6545 – 6557; e) E. J. Corey, L. F. Haefele, *J. Am. Chem. Soc.* **1959**, *81*, 2225 – 2228; f) G. L. Schmir, L. A. Cohen, B. Witkop, *J. Am. Chem. Soc.* **1959**, *81*, 2228 – 2233; g) N. M. Alexander, *J. Biol. Chem.* **1974**, *249*, 1946 – 1952; h) A. R. Ekkati, J. J. Kodanko, *J. Am. Chem. Soc.* **2007**, *129*, 12390 – 12391; i) A. I. Abouelatta, A. A. Campanali, A. R. Ekkati, M. Shamoun, S. Kalapugama, J. J. Kodanko, *Inorg. Chem.* **2009**, *48*, 7729 – 7739; j) S. Murahashi, A. Mitani, K. Kitao, *Tetrahedron Lett.* **2000**, *41*, 10245 – 10249; k) K. Tanabe, A. Taniguchi, T. Matsumoto, K. Oisaki, Y. Sohma, M. Kanai, *Chem. Sci.* **2014**, DOI: 10.1039/C3SC53037J.

[9] a) T. Y. Lee, J. Suh, *Chem. Soc. Rev.* **2009**, *38*, 1949 – 1957; b) J. Prakash, J. J. Kodanko, *Curr. Opin. Chem. Biol.* **2013**, *17*, 197 – 203.

[10] A. Taniguchi, D. Sasaki, A. Shiohara, T. Iwatsubo, T. Tomita, Y. Sohma, M. Kanai, *Angew. Chem.* **2014**, *126*, 1406 – 1409; *Angew. Chem. Int. Ed.* **2014**, *53*, 1382 – 1385.

[11] a) J. Arnau, C. Lauritzen, G. E. Petersen, *Protein Expression Purif.* **2006**, *48*, 1 – 13; b) *New Methods in Peptide Mapping for the Characterization of Proteins* (Ed.: W. S. Hancock), CRC, Boca Raton, FL, **1996**.

[12] Selected examples of metal-catalyzed hydrolytic cleavage of peptides: a) L. Zhu, N. M. Kostić, *J. Am. Chem. Soc.* **1993**, *115*, 4566 – 4570; b) L. Zhu, L. Qin, T. N. Parac, N. M. Kostić, *J. Am. Chem. Soc.* **1994**, *116*, 5218 – 5224; c) N. V. Kaminskaia, T. W. Johnson, N. M. Kostić, *J. Am. Chem. Soc.* **1999**, *121*, 8663 – 8664; d) N. M. Milović, N. M. Kostić, *J. Am. Chem. Soc.* **2002**, *124*, 4759 – 4769; e) A. Krężel, E. Kopera, A. M. Protas, J. Poznański, A. Wysłouch-Cieszyńska, W. Bal, *J. Am. Chem. Soc.* **2010**, *132*, 3355 – 3366; f) D. Hoyer, H. Cho, P. G. Schultz, *J. Am. Chem. Soc.* **1990**, *112*, 3249 – 3250; g) E. L. Hegg, J. N. Burstyn, *J. Am. Chem. Soc.* **1995**, *117*, 7015 – 7016; h) M. A. Smith, M. Easton, P. Everett, G. Lewis, M. Payne, V. Riveros-Moreno, G. Allen, *Int. J. Peptide Protein Res.* **1996**, *48*, 48 – 55; i) T. Takarada, M. Yashiro, M. Komiyama, *Chem. Eur. J.* **2000**, *6*, 3906 – 3913; j) K. Stroobants, E. Moelants, H. G. T. Ly, P. Proost, K. Bartik, T. N. Parac-Vogt, *Chem. Eur. J.* **2013**, *19*, 2848 – 2858; k) J. P. Collman, D. A. Buckingham, *J. Am. Chem. Soc.* **1963**, *85*, 3039 – 3040; l) A. Schepartz, R. Breslow, *J. Am. Chem. Soc.* **1987**, *109*, 1814 – 1826;

m) A. Schepartz, B. Cuenoud, *J. Am. Chem. Soc.* **1990**, *112*, 3247 – 3249; n) N. Ettner, W. Hillen, *J. Am. Chem. Soc.* **1993**, *115*, 2546 – 2548.

[13] A. Radzicka, R. Wolfenden, *J. Am. Chem. Soc.* **1996**, *118*, 6105 – 6109.

[14] Y. Kita, Y. Nishii, T. Higuchi, K. Mashima, *Angew. Chem.* **2012**, *124*, 5821 – 5824; *Angew. Chem. Int. Ed.* **2012**, *51*, 5723 – 5726.

[15] Aerobic oxidation of alcohols by Cu or Fe/*N*-oxyl radical catalysis: a) M. F. Semmelhack; C. R. Schmid, D. A. Cortés, C. S. Chou, *J. Am. Chem. Soc.* **1984**, *106*, 3374 – 3376; C. R. Schmid, D. A. Cortés, C. S. Chou, *J. Am. Chem. Soc.* **1984**, *106*, 3374 – 3376; b) N. Wang, R. Liu, J. Chen, X. Liang, *Chem. Commun.* **2005**, 5322 – 5324; c) M. H. Jessica, S. S. Stahl, *J. Am. Chem. Soc.* **2011**, *133*, 16901 – 16910; d) J. E. Steves, S. S. Stahl, *J. Am. Chem. Soc.* **2013**, *135*, 15742 – 15745, and references therein.

[16] Aerobic oxidation of amines by Cu/*N*-oxyl radical catalysis: a) Z. Hu, F. M. Kerton, *Org. Biomol. Chem.* **2012**, *10*, 1618 – 1624; b) T. Sonobe, K. Oisaki, M. Kanai, *Chem. Sci.* **2012**, *3*, 3249 – 3255; c) B. Huang, H. Tian, S. Lin, M. Xie, X. Yu, Q. Xu, *Tetrahedron Lett.* **2013**, *54*, 2861 – 2864; d) J. Kim, S. S. Stahl, *ACS Catal.* **2013**, *3*, 1652 – 1656.

[17] Selected reviews: a) I. W. C. E. Arends, Y.-X. Li, E. Aussan, R. A. Sheldon, *J. Mol. Catal. A* **2006**, *251*, 200 – 214; b) J. M. Bobbitt, C. Brückner, N. Merbouh, *Org. React.* **2009**, *74*, 103 – 424; c) L. Tebben, A. Studer, *Angew. Chem.* **2011**, *123*, 5138 – 5174; *Angew. Chem. Int. Ed.* **2011**, *50*, 5034 – 5068; d) A. E. Wendlandt, A. M. Suess, S. S. Stahl, *Angew. Chem.* **2011**, *123*, 11256 – 11283; *Angew. Chem. Int. Ed.* **2011**, *50*, 11062 – 11087; e) S. E. Allen, R. R. Walvoord, R. Padilla-Salinas, M. C. Kozlowski, *Chem. Rev.* **2013**, *113*, 6234 – 6458; f) Y. Iwabuchi, *Chem. Pharm. Bull.* **2013**, *61*, 1197 – 1213.

[18] J. J. Gorman, T. P. Wallis, J. J. Pitt, *Mass Spectrom. Rev.* **2002**, *21*, 183 – 216.

[19] S. Vijay-Kumar, C. E. Bugg, W. J. Cook, *J. Mol. Biol.* **1987**, *194*, 531 – 544.

[20] Recent mechanistic discussions of catalytic oxidation of alcohols by Cu/TEMPO/O_2 system: a) L. Cheng, J. Wang, M. Wang, Z. Wu, *Inorg. Chem.* **2010**, *49*, 9392 – 9399; b) P. Belanzoni, C. Michel, E. J. Baerends, *Inorg. Chem.* **2011**, *50*, 11896 – 11904; c) J. M. Hoover, B. L. Ryland, S. S. Stahl, *J. Am. Chem. Soc.* **2013**, *135*, 2357 – 2367; d) J. M. Hoover, B. L. Ryland, S. S. Stahl, *ACS Catal.* **2013**, *3*, 2599 – 2605. For amine oxidation, see ref. [16b].

[21] Discussions of [1e + 1e] oxidation mechanism in Cu-peroxide catalysis: a) E. Boess, D. Sureshkumar, A. Sud, C. Wirtz, C. Fares, M. Klussmann, *J. Am. Chem. Soc.* **2011**, *133*, 8106 – 8109; b) S. Hashizume, K. Oisaki, M. Kanai, *Chem. Asian J.* **2012**, *7*, 2600 – 2606; c) C. Zhang, C. Tang, N. Jiao, *Chem. Soc. Rev.* **2012**, *41*, 3464 – 3484.

[22] Acceleration effect of NO_x additive (acid + $NaNO_2$) in the oxidation catalyzed by *N*-oxyl radical: a) R. Liu, X. Liang, C. Dong, X. Hu, *J. Am. Chem. Soc.* **2004**, *126*, 4112 – 4113; b) Y. Xie, W. Mo, D. Xu, Z. Shen, N. Sun, B. Hu, X. Hu, *J. Org. Chem.* **2007**, *72*, 4288 – 4291; c) X. Wang, R. Liu, Y. Jin, X. Liang, *Chem. Eur. J.* **2008**, *14*, 2679 – 2685; d) A. Rahimi, A. Azarpira, H. Kim, J. Ralph, S. S. Stahl, *J. Am. Chem. Soc.* **2013**, *135*, 6415 – 6418, and references therein.

[23] We could not detect putative intermediates **B** or **B′** because the oxidation from **A** to **C** was very fast. Although a detailed reaction mechanism is still controversial, there are several reports documenting that easily enolizable α-(acylamino)aldehydes were rapidly converted to oxalimides through C–C bond cleavage through **B** and **B′**, even under mild oxidative conditions. For such precedents, see: a) W. von E. Doering, R. M. Haines, *J. Am. Chem. Soc.* **1954**, *76*, 482 – 486; b) P. Meffre, L. Gauzy, E. Branquet, P. Durand, F. Le Goffic, *Tetrahedron* **1996**, *52*, 11215 – 11238, and references therein; c) G. Cabarrocas, M. Ventura, M. Maestro, J. Mahía, J. M. Villalgordo, *Tetrahedron: Asymmetry* **2001**, *12*, 1851 – 1863; d) M. García-Valverde, R. Pedrosa, M. Vicente, *Synlett* **2002**, 2092 – 2094; e) L. M. Sayre, S.-J. Jin, *J. Org. Chem.* **1984**, *49*, 3498 – 3503; f) S.-J. Jin, P. K. Arora, L. M. Sayre, *J. Org. Chem.* **1990**, *55*, 3011 – 3018.

