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Oxidative Amide Synthesis and N-Terminal α-Amino Group Ligation of Peptides in Aqueous Medium

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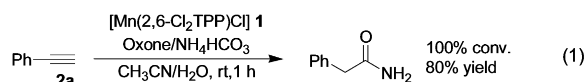
Selective modification of amino groups is an important strategy in bioconjugation.[1] In particular, N-terminal α-amino group ligation of peptides is of significance in positional proteomics studies,[2] construction of bioconjugates with amino acid side chains unmodified,[3] and peptide microarray fabrication.[4] Despite the recent advances in bioconjugation reactions,[5] it remains a significant challenge to selectively modify the N-terminal α-amino groups of peptides with other nucleophilic side chain residues, such as the lysine ϵ-amino group remaining intact.[1,3b,c]

The amide functionality is commonly found in natural products, therapeutic drugs, and biomolecules.[6] Despite the significant applications of copper-catalyzed cycloaddition of alkynes with azides in bioconjugation,[7] alkyne conjugation via amide bond formation remains sparse.[8,9] Oxidation of alkynes generally affords mixtures of products, including cleavage products, 1,2-dicarbonyl compounds, and/or carboxylic acids/esters.[10] Recently, we reported that manganese porphyrins are efficient catalysts for diastereoselective alkene epoxidations.[11] During the course of our studies, we found that oxidation of phenylacetylene using the "[Mn(2,6-Cl_2TPP)Cl] **1** (H_2(2,6-Cl_2TPP) = *meso*-tetrakis(2,6-dichlorophenyl)porphyrin)/Oxone/NH_4HCO_3" protocol afforded phenyl acetamide in 80% isolated yield (eq 1).



To investigate the amide synthesis reaction with amines, $NaHCO_3$ was used instead of NH_4HCO_3. Treatment of a CH_3CN solution of alkyne **2a** (0.3 mmol), amine **3a** (0.45 mmol), and [Mn(2,6-Cl_2TPP)Cl] **1** (1 μmol) with Oxone (0.45 mmol) and $NaHCO_3$ (1.4 mmol) afforded amide **4a** in 96% yield (Table 1, entry 1).[12] Without catalyst **1**, <5% conversion of **2a** was observed. Apart from Oxone, H_2O_2 could also be used as a terminal oxidant for this reaction (22% conversion, 93% yield). Using the "$MnSO_4/H_2O_2$" protocol developed by Burgess and co-workers, **4a** was obtained in 34% yield based on 16% conversion.[13] No conversion of **2a** was observed using dioxirane generated in situ from methyl pyruvate (50 mol %) and Oxone.[14]

Oxidative coupling of arylalkynes **2b**−**e** with hexyl amine **3a** afforded the corresponding amides **4b**−**e** (Table 1). For **2f** bearing two alkyne groups, a diamide **4f** was obtained. For benzyl amine **3b**, cyclohexyl amine **3c**, and serine derivative **3d**, amides **4g**−**j** were obtained (Table 2). Coupling of 1-octyne with NH_4HCO_3 and **3b** gave amides **4k** and **4l**, respectively. Coupling of dihexylamine with **2a** gave nitrone **5**[15] in 53% isolated yield (<5% conversion of **2a**). For coupling of aniline with **2a**, azoxybenzene was obtained in 83% yield based on 91% aniline conversion with no amide product detected by GC−MS analysis.

Using 1.0 g of phenylacetylene (**2a**), a one-pot synthesis of phenyl acetamide in gram-scale (eq 1) (1.1 g, 84% isolated yield) has been achieved in 2 h.

Table 1. Oxidative Amide Synthesis Using Aromatic Alkynes[a]

Ar−≡ (**2a-f**) + H_2N-hexyl (**3a**) → ([Mn(2,6-Cl_2TPP)Cl] **1**, Oxone/$NaHCO_3$, CH_3CN/H_2O, rt,1 h) → $ArCH_2C(O)NH$-hexyl (**4a-f**)

entry	alkyne	product	% yield[b]
1	**2a** (R = H)	**4a** (R = H)	96[c]
2	**2b** (R = CH_3)	**4b** (R = CH_3)	72
3	**2c** (R = F)	**4c** (R = F)	70
4[d]	**2d** (R = NHAc)	**4d** (R = NHAc)	48[e]
5[d]	**2e**	**4e**	46[e]
6[f]	**2f**	**4f**	52[g]

[a] Alkyne **2** (0.3 mmol), amine **3a** (0.45 mmol), **1** (1 μmol), Oxone (0.45 mmol), and $NaHCO_3$ (1.4 mmol) in CH_3CN (1.5 mL) and H_2O (1 mL), 1 h. [b] Isolated yield based on complete alkyne consumption. [c] Determined by GC. [d] Alkyne/amine/Oxone/**1** = 1.5/1/1.5/0.003. [e] Isolated yield based on amine used. [f] Double amount of Oxone, $NaHCO_3$, hexyl amine **3a**, and **1** was used. [g] Determined by ^{1}H NMR with internal standard.

Table 2. Oxidative Amide Synthesis Using Alkynes (**2a**, **2b**, and 1-Octyne)[a]

entry	amine	product	% conv.[b]	% yield[c]
1	**3b**	**4g**	81	84
2	**3c**	**4h**	92	81
3	**3d**	**4i** (R = H)	92	76
4[d]	**3d**	**4j** (R = CH_3)	100	64
5[e]	NH_4HCO_3	**4k**	71	31
6[e]	**3b**	**4l**	67	22

[a] Alkyne **2a** (0.3 mmol), amine **3** (0.45 mmol), **1** (1 μmol), Oxone (0.45 mmol), and $NaHCO_3$ (1.4 mmol) in CH_3CN (1.5 mL) and H_2O (1 mL), 1 h. [b] Determined by GC. [c] Isolated yield based on conversion. [d] **2b** was used instead of **2a**. [e] 1-Octyne was used instead of **2a**.

Next, we employed the oxidative amide synthesis method for the ligation of unprotected peptides. Oxone could be used as an oxidant for peptide modification (see Supporting Information), but H_2O_2 was subsequently employed to reduce salt concentration and simplify the sampling procedure for mass spectrometric analysis. Peptide **6a** (100 μM) in an aqueous CH_3CN solution with H_2O_2 (30% v/v, 10 mM), **2a** (5 mM), $NaHCO_3$ (1 mM), and **1** (20 μM) was kept at room temperature for 6 h to give modified peptide **7aa** with complete conversion (eq 2). The pH of the $NaHCO_3$ buffer (4 mg/mL in H_2O) used was 8.3. LC-MS/MS analysis confirmed that the N-terminal α-amino group of **6a** was acylated without modification of the side chains of tyrosine, threonine, serine, and lysine. In contrast, by treatment of **6a** with an excess amount of *N*-hydroxysuccinimide ester[1] (a well-known agent for acylation of

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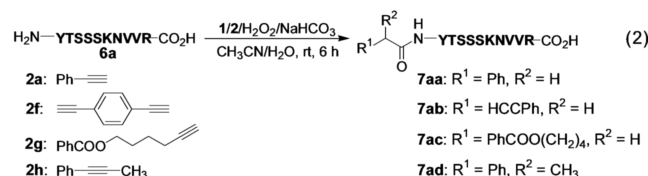
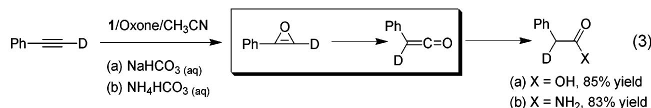
amino groups) in the same solvent system, both the α-amino group and lysine residue were acylated (see Supporting Information). Using a stoichiometric amount of *N*-hydroxysuccinimide ester, only the lysine residue was acylated. To the best of our knowledge, the present method is the first example of N-terminal α-amino group acylation of peptides without lysine modification in aqueous $NaHCO_3$ buffer. Furthermore, **2f**, aliphatic **2g**, and internal **2h** alkynes were found to couple at the N-terminal α-amino group of **6a** to give **7ab**−**ad** (eq 2).



N-Terminal acylations of five other peptides [GEQRKDVYVQLYL, HDMNKVLDL, TYGPVFMSL, STSSSCNLSK, and SSCSSCPLSK] at 100 μM scale with phenylacetylene (**2a**) have also been achieved (see Supporting Information). With these five peptides, inter- and intramolecular disulfide bond formations were observed at cysteine residues, and oxidation of methionine residues to sulfoxides was observed. Nevertheless, the disulfide-bonded cysteines and oxidized methionines could be reduced back to free cysteines (this work) and methionines by treatment with dithiothreitol and *N*-methylmercaptoacetamide,[1a] respectively. The present method could be scaled up; for example, 5.3 mg of **2a**-modified SSCSSCPLSK [purified by preparative reversed-phase HPLC in 65% isolated yield based on 81% conversion and confirmed by MS/MS] was obtained through a one-pot reaction; see Supporting Information.

Ketenes are widely regarded as intermediates in alkyne oxidation to carboxylic acids/esters.[10,16] In this work, deutero-1-phenylacetylene was oxidized under (a) aqueous $NaHCO_3$ and (b) aqueous NH_4HCO_3 conditions to afford deutero-2-phenyl acetic acid and deutero-2-phenyl acetamide in 85 and 83% isolated yields, respectively (eq 3). No crossover of deuterium in the amide products was observed by GC−MS when a 1:1 ratio of deutero-1-phenylacetylene and **2b** was oxidized under aqueous NH_4HCO_3 conditions (see Supporting Information). ESI-MS analysis of **1** in CH_3CN solution in the presence of Oxone/$NaHCO_3$ showed ion cluster peaks centered at $m/z = 958.9$, which matched the $[Mn(2,6\text{-}Cl_2TPP)(O)]^+$ formulation.[17] These findings are consistent with the mechanism of inhibition of cytochrome P450 activities by alkynes, which was proposed to occur through alkyne oxidation to generate oxirene and ketene intermediates.[10d,18]



We have independently generated PhCH=C=O from photo-Wolff rearrangement of $PhC(O)CHN_2$. The same N-terminal selectivity was observed in the coupling reactions of PhCH=C=O with **6a**, GEQRKDVYVQLYL, and HDMNKVLDL, and the lysine residues remained intact (see Supporting Information). CD studies (using the CONTINLL program for deconvolution) estimated the α-helical, β-sheet, and turn content of **6a** in solution to be 21.7, 6.6, and 12.6%, respectively, with 59.1% of random coil. Two-dimensional NOESY analysis revealed an inter-residue NOE signal between the K6 NH proton and the Y1 phenyl ring proton of **6a**, thus suggesting that the N-terminal selectivity in peptide ligation may be in part due to the solution conformation of **6a**.

In summary, a new method for oxidative amide synthesis and peptide ligation using the "**1** + Oxone/H_2O_2" protocol has been developed.

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Supporting Information Available: Full experimental procedures, characterization data, mass spectra, CD and 2D NMR studies, and ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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