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Site-Specific Modification of Amino Acids and Peptides by Aldehyde–Alkyne–Amine Coupling under Ambient Aqueous Conditions

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ABSTRACT

R = guanidine, disulfide, thioether, phenol, alcohol
R' = aryl, alkyl, TMS

A highly efficient method for the direct, site-specific functionalization of amino acids and peptides, under ambient conditions, is described. In aqueous, nearly solvent-free conditions, copper(I) chloride catalyzed the aldehyde–alkyne–amine (A^3) coupling of amino acids to form dipropargylated products in moderate to excellent yields. The propargylamine functionality provides a convenient handle for further structural modifications, demonstrated by a subsequent one-pot deprotection and "click" reaction and a solution-phase peptide coupling.

The selective and bio-orthogonal functionalization of biomolecules is a highly desirable tool for chemists and biologists alike. Peptides, in particular, are frequent targets of modification, as a method for alteration of their chemical and pharmacological properties for use as therapeutic agents.[1] In the past century, this field has seen impressive growth.[2] From simple modifications of nucleophilic residues such as lysine and cysteine, there now exist methods for functionalizing the carboxylic acid functionalities of glutamic and aspartic acid,[3] rare aromatic moieties such as tryptophan,[4] as well as tyrosine,[5] phenylalanine,[6] and arginine residues.[7] The site-selective C-functionalization of glycine redisues in peptides has also been explored.[8] New methods for the functionalization of N-terminal and lysine side-chain amines have also been developed, most of which rely on direct or reductive alkylation.[9]

The use of alkynes as a coupling partner for imines—an intermediate in the reductive alkylation of amines with aldehydes—allows an equivalent reductive alkylation of

(1) (a) De Filippis, V.; Quarzago, D.; Vindigni, A.; Di Cera, E.; Fontana, A. *Biochemistry* **1998**, *37*, 13507. (b) Jungheim, L. N.; Shepperd, T. A.; Baxter, A. J.; Burguess, J.; Hatch, S. D.; Lubbehusen, P.; Wiskerchen, M.; Muesing, M. A. *J. Med. Chem.* **1996**, *39*, 96. (c) Nakatani, S.; Hidaka, K.; Ami, E.; Nakahara, K.; Sato, A.; Nguyen, J.-T.; Hamada, Y.; Hori, Y.; Ohnishi, N.; Nagai, A.; Kimura, T.; Hayashi, Y.; Kiso, Y. *J. Med. Chem.* **2008**, *51*, 2992.

(2) For recent reviews, see: (a) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974. (b) Gauthier, M. A.; Klok, H.-A. *Chem. Commun.* **2008**, 2591. (c) Hackenberger, C. P. R.; Schwarzer, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 10030. (d) Levesque, G.; Arsène, P.; Fanneau-Bellenger, V.; Pham, T.-N. *Biomacromolecules* **2000**, *1*, 387.

(3) Hermanson, G. T. *Bioconjugate Techniques*, 2nd ed.; Academic Press: San Diego, 2008.

(4) (a) Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256. (b) Ruiz-Rodríguez, J.; Albericio, F.; Lavilla, R. *Chem.—Eur. J.* **2010**, *16*, 1124.

(5) (a) Joshi, N. S.; Whitaker, L. R.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 15942. (b) Romanini, D. W.; Francis, M. B. *Bioconjugate Chem.* **2008**, *19*, 153. (c) Tilley, S. D.; Francis, M. B. *J. Am. Chem. Soc.* **2006**, *128*, 1080.

(6) Espuña, G.; Arsequell, G.; Barluenga, J.; Alvarez-Gutiérrez, J. M.; Ballesteros, A.; González, J. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 325.

(7) Gauthier, M. A.; Klok, H.-A. *Biomacromolecules* **2011**, *12*, 482.

(8) (a) Zhao, L.; Baslé, O.; Li, C.-J. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 4106. (b) Ooi, T.; Tayama, E.; Maruoka, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 579.

(9) (a) McFarland, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 13490. (b) Chan, A. O.-Y.; Ho, C.-M.; Chong, H.-C.; Leung, Y.-C.; Huang, J.-S.; Wong, M.-K.; Che, C.-M. *J. Am. Chem. Soc.* **2012**, *134*, 2589. (c) Monfregola, L.; De Luca, S. *Amino Acids* **2011**, *41*, 981. (b) Monfregola, L.; Leone, M.; Calce, E.; De Luca, S. *Org. Lett.* **2012**, *14*, 1664. (d) Demmer, O.; Dijkgraaf, I.; Schottelius, M.; Wester, H.-J.; Kessler, H. *Org. Lett.* **2008**, *10*, 2015–2018.

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amines while at the same time appending an alkyne—a highly useful moiety in bioorthogonal chemistry (scheme 1).[10] This three-component coupling is commonly referred to as the alkyne–aldehyde–amine or A^3 coupling.[11] The A^3 coupling has been accomplished with a very broad range of transition metals, including silver,[12] gold,[13] ruthenium/copper,[14] cobalt,[15] copper,[16] indium,[17] iridium,[18] and iron.[19] We envisioned that the A^3 coupling could act as a process that combines the selectivity of reductive alkylation with the utility of alkynylation in a single reaction, preferably under mild conditions and with inexpensive catalysts.

Herein, we report such a method for the site- selective modification of peptides and amino acids via copper(I)-catalyzed A^3 coupling under ambient, aqueous conditions.

Initial studies began based on a five-component, ruthenium/copper-catalyzed variety of the A^3 coupling.[14b] The reaction of glycine methyl ester **1a**, with phenylacetylene and formaldehyde to produce amino acid derivative **4a** was used as the testing model (Table 1).

Scheme 1. Direct Alkynylation of Free Amines Using the A^3 Reaction Represents an Interesting Alternative to Alkylation

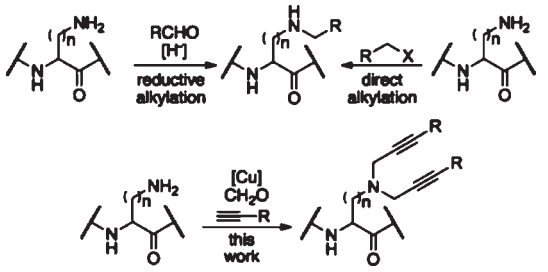
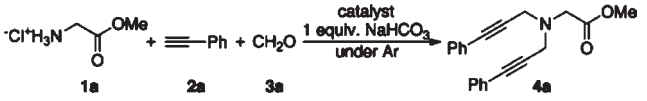


Table 1. Catalyst and Condition Screening for the Reaction of **1a**, **2a**, and **3a** To Create Propargylamine **4a**[a]



entry	catalyst[b] (%)	solvent, temp (°C)	time (h)	yield[c] (%)
1	$RuCl_3$/CuBr (5/15)	H_2O, rt	48	43
2	$RuCl_3$/CuBr (5/15)	H_2O, rt	96	61
3	$RuCl_3$/CuBr (5/15)	H_2O, 40	48	55
4	CuOTf (10)	MeCN, 60	48	47
5	CuOTf/bipy (10/10)	MeCN, 60	48	80
6	CuOTf/4,4′-MeO-bipy	MeCN, 60	48	70
7	CuOTf/phen (10/10)	MeCN., 60	48	67
8	CuOTf/terpyr (10/10)	MeCN, 60	48	85
9	CuBr (10)	MeCN, 60	48	80
10	CuI (10)	MeCN, 60	48	95
11	CuI (10)	H_2O, 60[d]	48	54
12	CuI (10)	H_2O, 35[d]	48	42
13	**CuCl (10)**	**H_2O, 35[d]**	**18**	**>95**
14	**CuCl (10)**	**H_2O, 35[d,e]**	**18**	**>95**
15	CuCl (10)	H_2O, rt[d]	18	78
16	$CuCl_2$–H_2O (10)	H_2O, 35[d]	18	85

[a] Conditions: glycine methyl ester hydrochloride (0.2 mmol), formaldehyde (0.5 mmol, 37% in water), phenylacetylene (0.5 mmol), CuCl (0.02 mmol), and $NaHCO_3$ (0.2 mmol), 0.5 mL of solvent, under Ar. [b] CuOTf = copper(I) triflate toluene complex; bipy = 2,2′-bipyridine; 4,4′-MeO-bipy = 4,4′-dimethoxy-2,2′-bipyridine; phen = 1,10-phenanthroline; terpyr = 2,2′:6′,2″-terpyridine. [c] Yields were determined by NMR spectroscopy using mesitylene as an internal standard. [d] Reaction was run with no additional solvent; only phenylacetylene and water from the formaldehyde solution were present as liquids in the vessel. [e] Reaction was run under air atmosphere.

Even without ruthenium, the use of terpyridine or bipyridines with copper(I) triflate afforded good yields in acetonitrile solvent (Table 1, entries 4–8). However, by using only copper(I) chloride and neat conditions, a dramatic increase in yield was observed (entry 13).

Interestingly, $CuCl_2$ provided yields nearly as high as CuCl. The high effective concentration of these reactions would allow reduction of the aqueous Cu^{2+} to the active Cu^{1+} species by the alkyne (via oxidative dimerization) or by methanol (present in the formaldehyde solution) to occur quickly. The rapid formation of a canary-yellow precipitate in these reactions, characteristic of polymeric Cu(I)–ladderane complexes,[20] supports this theory. Exclusion of oxygen was not necessary for excellent yields (entry 14) but precluded formation of small quantities of the alkyne homocoupling product.

Encouraged by this promising result, the scope of the reaction was tested with a number of alkynes (Scheme 2).

(10) For a recent review of alkyne chemistry in biological contexts, see:Uhlig, N.; Li, C.-J. *Chem. Sci.* **2011**, *2*, 1241.

(11) For recent reviews of the A^3 reaction, see: (a) Li, C.-J. *Acc. Chem. Res.* **2010**, *43*, 581. (b) Yoo, W.-J.; Zhao, L.; Li, C.-J. *Aldrichimica Acta* **2011**, *44*, 43. (c) Zani, L.; Bolm, C. *Chem. Commun.* **2006**, 4263–4275. (d) Peshkov, V. A.; Pereshivko, O. P.; Van der Eycken, E. V. *Chem. Soc. Rev.* **2012**, *41*, 3702–3702.

(12) (a) Wei, C.; Li, Z.; Li, C.-J. *Org. Lett.* **2003**, *5*, 4473. (b) Li, Z.; Wei, C.; Chen, L.; Varma, R. S.; Li, C.-J. *Tetrahedron Lett.* **2004**, *45*, 2443. (c) Huang, B.; Yao, X.; Li, C.-J. *Adv. Syn. Catal.* **2006**, *348*, 1528. (d) Li, Y.; Chen, X.; Song, Y.; Fang, L.; Zou, G. *Dalton Trans.* **2011**, *40*, 2046. (e) Li, P.; Wang, L.; Zhang, Y.; Wang, M. *Tetrahedron Lett.* **2008**, *49*, 6650.

(13) (a) Lo, V.K.-Y.; Liu, Y.; Wong, M.-K.; Che, C.-M. *Org. Lett.* **2006**, *8*, 1529. (b) Wei, C.; Li, C.-J. *J. Am. Chem. Soc.* **2003**, *125*, 9584. (c) Cheng, M.; Zhang, Q.; Hu, X.; Li, B.; Ji, J.; Chan, A. S. C. *Adv. Syn. Catal.* **2011**, *353*, 1274. (d) Lo, V. K.-Y.; Kung, K. K.-Y.; Wong, M.-K.; Che, C.-M. *J. Organomet. Chem.* **2009**, *694*, 583.

(14) (a) Li, C.-J.; Wei, C. *Chem. Commun.* **2002**, 268. (b) Bonfield, E. R.; Li, C.-J. *Org. Biomol. Chem.* **2007**, *5*, 435.

(15) Chen, W.-W.; Bi, H.-P.; Li, C.-J. *Synlett* **2010**, 475.

(16) (a) Kabalka, G. W.; Wang, L.; Pagni, R. M. *Synlett* **2001**, 676. (b) Leadbeater, N. E.; Torenius, H. M.; Tye, H. *Mol. Divers.* **2003**, *7*, 135. (c) Choudary, B. M.; Sridhar, C.; Kantam, M. L.; Sreedhar, B. *Tetrahedron Lett.* **2004**, *45*, 7319. (d) Shi, L.; Tu, Y.-Q.; Wang, M.; Zhang, F.-M.; Fan, C.-A. *Org. Lett.* **2004**, *6*, 1001. (e) Wei, C.; Mague, J. T.; Li, C.-J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5749. (f) Sreedhar, B.; Reddy, P. S.; Prakash, B. V.; Ravindra, A. *Tetrahedron Lett.* **2005**, *46*, 7019. (g) Kidwai, M.; Bansal, V.; Kumar Mishra, N.; Kumar, A.; Mozumdar, S. *Synlett* **2007**, 1581. (h) Madhav, J. V.; Kuarm, B. S.; Someshwar, P.; Rajitha, B.; Reddy, Y. T.; Crooks, P. *Synth. Commun.* **2008**, *38*, 3215. (i) Zeng, T.; Yang, L.; Hudson, R.; Song, G.; Moores, A. R.; Li, C.-J. *Org. Lett.* **2011**, *13*, 442. (j) Wei, C.; Li, C.-J. *J. Am. Chem. Soc.* **2002**, *124*, 5638. (k) Gommermann, N.; Koradin, C.; Polborn, K.; Knochel, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 5763.

(17) Zhang, Y.; Li, P.; Wang, M.; Wang, L. *J. Org. Chem.* **2009**, *74*, 4364.

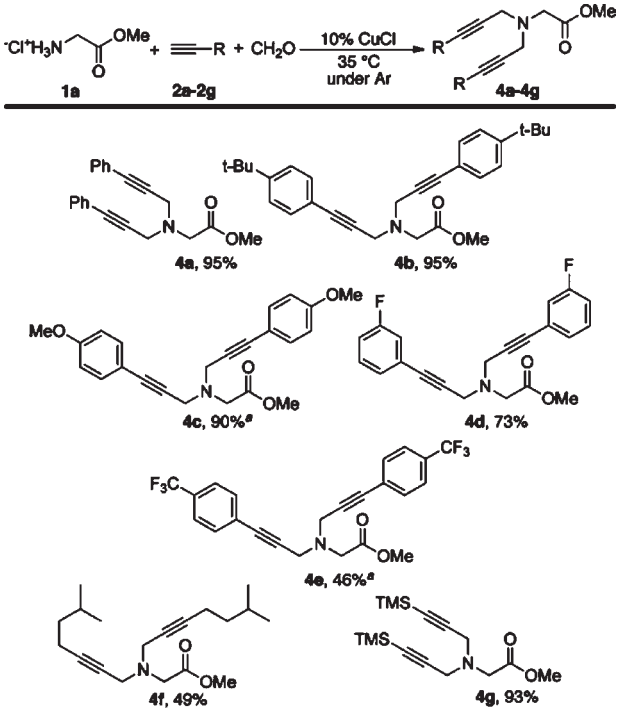
(18) (a) Sakaguchi, S.; Kubo, T.; Ishii, Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 2534. (b) Fischer, C.; Carreira, E. M. *Org. Lett.* **2001**, *3*, 4319. (c) Chen, W. W.; Nguyen, R. V.; Li, C.-J. *Tetrahedron Lett.* **2009**, *50*, 2895.

(19) Li, P.; Zhang, Y.; Wang, L. *Chem.—Eur. J.* **2009**, *15*, 2045.

(20) (a) Buckley, B. R.; Dann, S. E.; Heaney, H. *Chem.—Eur. J.* **2010**, *16*, 6278. (b) Buckley, B. R.; Dann, S. E.; Heaney, H.; Stubbs, E. C. *Eur. J. Org. Chem.* **2011**, 770. (c) Chui, S. S. Y.; Ng, M. F. Y.; Che, C. *Chem.—Eur. J.* **2005**, *11*, 1739.

To our delight, every alkyne substrate tested produced moderate to excellent yields of the desired products, though alkynes containing highly electron-withdrawing substituents gave less favorable results (**4d**, **4e**). Aliphatic alkynes also gave poorer yield (**4f**), though TMS-acetylene gave excellent yield under the same conditions (**4g**).

Scheme 2. A^3 Coupling of Alkynes, Formaldehyde, and Glycine Methyl Ester[a]

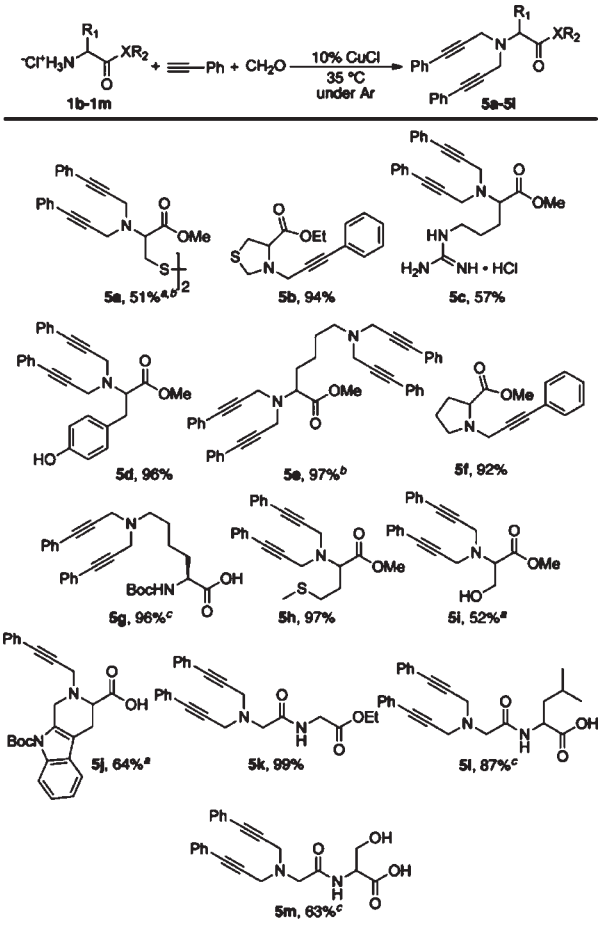


[a] Reaction conditions: glycine methyl ester hydrochloride, sodium bicarbonate (1 equiv), 37% formaldehyde (2.5 equiv as 37% aqueous solution), alkyne (2.5 equiv), argon, 35 °C, 18 h. Isolated yields, based on glycine methyl ester hydrochloride. [b] Product was purified by preparatory thin-layer chromatography.

For the functionalization of various amino acids and dipeptides, a large variety of functional groups (disulfides, esters, alcohols, phenols, guanidines, secondary amides, thioethers, and carboxylic acids) proved to be tolerated by the reaction, and very little purification was required (Scheme 3). In most cases, only evaporation of the excess reagents under high vacuum was necessary to yield a pure product. The exceptions to this were products **4e** (Scheme 2) and **5a**, **5c**, and **5i** (Scheme 3). The reaction of cystine dimethyl ester gave the desired tetrafunctionalized dimer product in moderate yield but also yielded a small amount of a possible monomer cyclized product. Cysteine ethyl ester gave nearly quantitative yield of this cyclized thiazolidine product **5b**, the precursor to which is known to be produced upon reaction of cysteine with formaldehyde.[21] Boc-protected tryptophan gave a similar result, producing the interesting tetrahydrocarboline

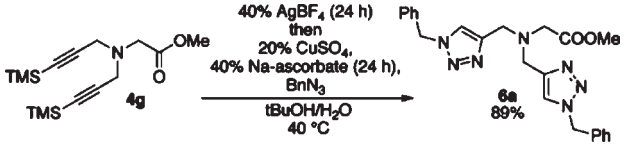
(21) (a) Tam, J. P.; Yu, Q.; Miao, Z. *Biopolymers* **1999**, *51*, 311. (b) Tam, J. P.; Miao, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9013.

Scheme 3. A^3 Coupling of Amino Acids and Dipeptides with Phenylacetylene and Formaldehyde[a]



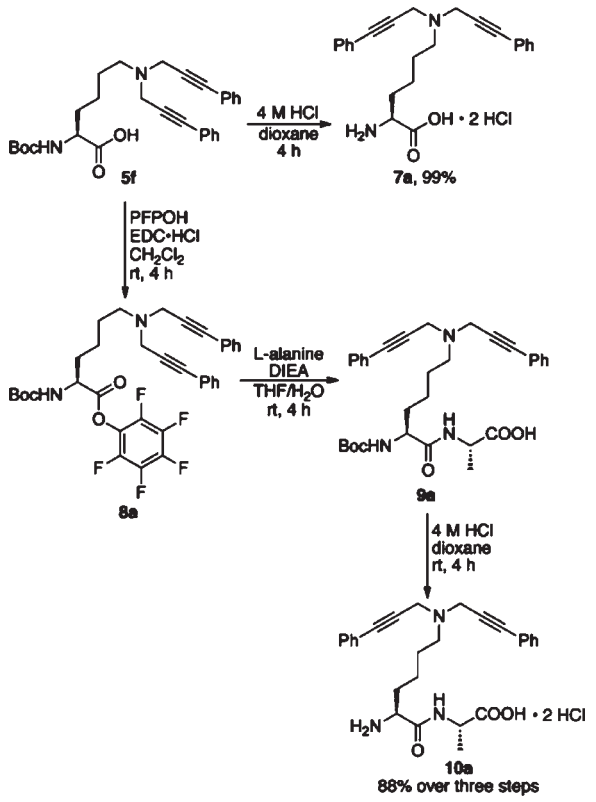
[a] Reaction conditions: amino acid, sodium bicarbonate (1 equiv), formaldehyde (2.5 equiv as 37% aqueous solution), phenylacetylene (2.5 equiv), copper(I) chloride (10 mol %), under argon, 35 °C, 18 h. Isolated yields based on the amino acid. [b] Product was purified by preparatory thin-layer chromatography. [c] Dihydrochloride salts were used; 5 equiv each of formaldehyde and phenylacetylene and 2 equiv of $NaHCO_3$ were used. [d] No sodium bicarbonate was added.

Scheme 4. One-Pot Deprotection and "Click" Functionalization of Dipropargylated Glycine Derivative **4g**



derivative **5j**. While several amino acids participate in this side reaction (including tryptophan, cysteine, serine, asparagine, and histidine),[20] it only occurs when said amino acid is present at the N-terminus. Serine methyl ester gave a somewhat decreased yield of the desired product **5i**, along with 6 mg of a second product which could not be conclusively identified at this time, but may be the

Scheme 5. Boc-Protected Lysine Derivative **5f** Can Be Easily Deprotected or Subjected to Solution-Phase Peptide Couplings To Produce Side-Chain Functionalized Lysine Derivatives (**8a**) or Peptides (**10a**) in Excellent Yield



oxazolidine product. The reaction was additionally tested on several dipeptides to examine its potential use for the direct functionalization of larger structures. Glycylglycine ethyl ester, glycylleucine, and glycylserine all gave good to excellent yields of the N-terminal difunctionalized products (**5j**, **5k**, and **5l**, respectively).

Due to ethynyltrimethylsilane's excellent yield in the functionalization of glycine methyl ester, it was envisioned that this substrate combination could provide a convenient route to the free alkyne-functionalized product, which could in turn be functionalized using the copper(I)-catalyzed azide–alkyne click (CuAAC) reaction.

A one-pot deprotection–CuAAC reaction was performed on compound **4g** using a silver-catalyzed deprotection of TMS-alkynes[22] followed by direct addition of benzyl azide and a copper catalyst to effect the CuAAC reaction.[23] This procedure afforded the bis-triazole product **6a** in excellent yield (Scheme 4).

In addition, the excellent tolerance of sensitive groups such as *tert*-butoxycarbonyl protecting groups suggested the possibility of incorporating side-chain-functionalized lysine residues into synthetic peptides. To this end, product **5g** was converted to the activated pentafluorophenyl ester **8a**, which was then used directly in a solution-phase peptide coupling (Scheme 5) with L-alanine to yield the N-protected dipeptide **10a** in excellent yield and with no epimerization of either stereocenter.[24] Both this resulting dipeptide and the original lysine derivative **5g** could be easily deprotected to their yield **10a** and **7a**, respectively (Scheme 5).

In summary, we have developed a highly efficient method for the direct functionalization of amino acids and peptides under ambient, aqueous conditions via the A^3 coupling, catalyzed by copper(I) chloride. The reaction showed tremendous scope, being compatible with thioethers, ethers, secondary amides, hydroxyl groups,esters, carboxylic acids, guanidine, and—to a lesser extent—disulfide groups, as well as a wide variety of alkynes. These results indicate great potential for the use of the A^3 reaction as a method to modify and conjugate because of its simplicity, low cost, tolerance of functional groups, exceedingly mild conditions, and ease of transformation of its products into further useful compounds. Studies aimed at reducing concentration and application to other biomolecules are ongoing.

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Supporting Information Available. Detailed experimental procedures and physical and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(22) (a) Carpita, A.; Mannocci, L.; Rossi, R. *Eur. J. Org. Chem.* **2005**, *2005*, 1859. (b) Orsini, A.; Vitérisi, A.; Bodlenner, A.; Weibel, J.-M.; Pale, P. *Tetrahedron Lett.* **2005**, *46*, 2259.

(23) (b) Aucagne, V.; Leigh, D. A. *Org. Lett.* **2006**, *8*, 4505.

(24) Meneses, C.; Nicoll, S. L.; Trembleau, L. *J. Org. Chem.* **2010**, *75*, 564.

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