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Chemoselective Formation of Successive Triazole Linkages in One Pot: "Click–Click" Chemistry

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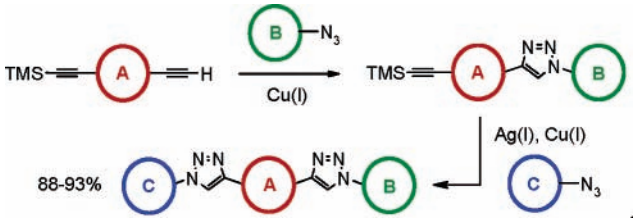
ABSTRACT



A methodology for the successive regiospecific "clicking" together of three components in one pot via two triazole linkages is reported. The protocol utilizes copper(I)-mediated alkyne–azide cycloaddition reactions combined with a silver(I)-catalyzed TMS-alkyne deprotection under mild hydroalcoholic conditions. We exemplify the approach with peptide-based components to illustrate its compatibility with polyfunctionalized biomolecules. The method constitutes a promising tool for peptide ligation. We also provide a procedure for directly using TMS-alkynes as the cycloaddition partner in classical "click" chemistry.

Since the independent discovery of the copper(I)-catalyzed variant of the Huisgen terminal alkyne−azide 1,3-cycloaddition[1] by the Meldal[2] and Sharpless[3] groups, hundreds of papers have appeared describing the use of this simple "click"[4] methodology to link together polyfunctionalized building blocks.[5] Its application in numerous areas has highlighted the value of its benign reaction conditions and simple workup and purification procedures. Here we report a one-pot procedure for the successive copper- and copper-and-silver-mediated chemoselective formation of two distinct triazole linkages using the trimethylsilyl (TMS) group as a temporary masking group for one of two alkyne moieties. We believe this "click−click" approach could be a potentially useful variation on standard click chemistry. As well as being a convenient tool for the selective ligation of multiple components in a biological environment, it could be used to generate a library of products whose population grows according to the square of the number of available azide building blocks.

Taking note of recent reports on the Ag(I)-mediated deprotection of TMS-alkynes under semiaqueous or alcoholic conditions,[6] we wondered whether TMS-alkynes could be unmasked and directly used for triazole formation without interim workup or purification. To test this idea, we

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(1) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984.

(2) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064.

(3) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. (b) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210−216.

(4) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021. (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128−1137.

(5) For a recent review, see: Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51−68.

(6) (a) Orsini, A.; Vitérisi, A.; Bodlenner, A.; Weibel, J.-M.; Pale, P. *Tetrahedron Lett.* **2005**, *46*, 2259−2262. (b) Carpita, A.; Mannocci, L.; Rossi, R. *Eur. J. Org. Chem.* **2005**, 1859−1864.

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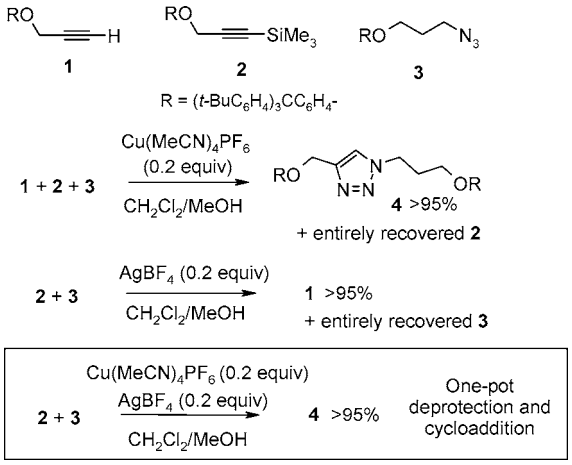
conducted some preliminary experiments using alkyne **1**, TMS-alkyne **2**, and azide **3** (Scheme 1).[7,8] An equimolar

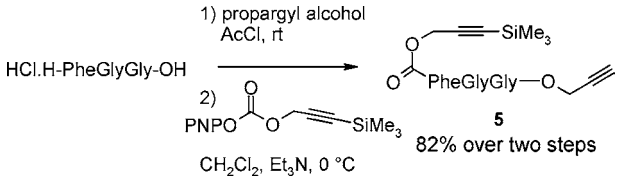
Scheme 1. In Situ Ag(I)-Catalyzed Unmasking and Cu(I)-Catalyzed 1,3-Cycloaddition of a TMS-Alkyne and an Azide



mixture of the three compounds was reacted in a CH_2Cl_2/MeOH mixture in the presence of a catalytic amount (0.2 equiv) of Cu(I). This furnished the expected triazole **4**, resulting from the cycloaddition of **1** and **3**, leaving unreacted TMS-protected **2**. Reaction of **2** and **3** in the presence of $AgBF_4$ led to the unmasked terminal alkyne **1** without affecting azide **3**.[9] Finally, simple mixing of **2** and **3** in the presence of catalytic quantities (0.2 equiv) of both Cu(I) and Ag(I) salts gave the click product from a one-pot deprotection/cycloaddition process. This not only indicates the potential of silyl-protected alkynes for a click−click strategy but also demonstrates a way of directly employing TMS-alkynes as a cycloaddition partner in classical click reactions.[10]

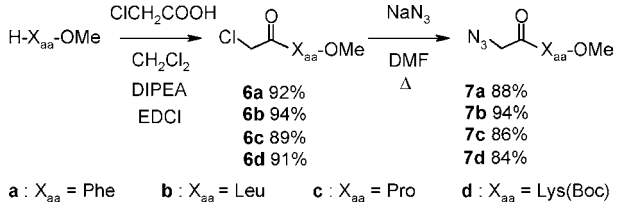
A molecule was then constructed with both a terminal alkyne and a TMS-protected one to act as the central scaffold for attempts to use this methodology to chemoselectively form successive triazole linkages in one pot. In view of the need to develop efficient selective ligation tools for the production of synthetic proteins and glycoproteins, we decided to focus on small peptide derivatives. Such synthons are easy to prepare, producing polyfunctional building blocks that could test the functional group tolerance of the desired click−click protocol.[11] Commercially available methyl phenylalanylglycylglycinate hydrochloride was esterified with propargyl alcohol under acidic conditions and then converted to the TMS-propargyloxycarbonyl derivative **5** using a tailor-made (see Supporting Information) 4-nitrophenol (PNP) carbonate reagent (Scheme 2).

Scheme 2. Synthesis of a TMS-Alkyne−Terminal Alkyne Bisfunctionalized Tripeptide



A series of azide-containing pseudodipeptides (**7a**−**d**) were synthesized via the EDCI-mediated amide coupling of commercial amino acid methyl esters and chloroacetic acid (**6a**−**d**), followed by nucleophilic displacement of the chloride by an azide. This two-step strategy was used to avoid the use of potentially hazardous[12] azidoacetic acid or one of its activated derivatives (Scheme 3).

Scheme 3. Synthesis of Azide-Containing Dipeptide Analogues[a]



[a] See Supporting Information for experimental details.

Clearly, strict respect of reactant stoichiometry and near-quantitative conversion during the first cycloaddition is

(7) Aucagne, V.; Hänni, K. D.; Leigh, D. A.; Lusby, P. J.; Walker, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 2186−2187.

(8) Most low molecular weight azides and alkynes are rather volatile, and we found it convenient to use **1**−**3**, which we had in hand from previous studies.[7]

(9) The mechanism of Ag(I)-mediated desilylation of TMS−alkynes involves a silver−alkyne species structurally related to the alkyne cuprates thought to be an early intermediate in the Cu(I)-catalyzed alkyne−azide cycloaddition.[3b,5] We added azide **3** to the alkyne unmasking reaction to see if this putative silver intermediate would undergo a copper-free triazole-forming reaction. However, no traces of triazole products were detected (^{1}H NMR, ESI-MS).

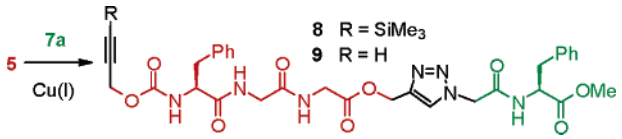
(10) In many reports, the alkyne moiety is introduced as its TMS derivative, which is deprotected, worked up, and purified prior to clicking. See, for example: (a) Malkoch, M.; Thibault, R. J.; Drockenmuller, E.; Messerschmidt, M.; Voit, B.; Russell, T. P.; Hawker, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 14942−14949. (b) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2005**, *127*, 15020−15021. (c) Suh, B.-C.; Jeon, H. B.; Posner, G. H.; Silverman, S. M. *Tetrahedron Lett.* **2004**, *45*, 4623−4625.

(11) Several recent articles illustrate the efficacy of click chemistry for the ligation of peptide building blocks. See, for example: (a) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366−15367. (b) Rijkers, D. T. S.; van Esse, G. W.; Merkx, R.; Brouwer, A. J.; Jacobs, H. J. F.; Pieters, R. J.; Liskamp, R. M. J. *Chem. Commun.* **2005**, *36*, 4581−4583. (c) Franke, R.; Doll, C.; Eichler, J. *Tetrahedron Lett.* **2005**, *46*, 4479−4482. (d) Dirks, A. J. T.; van Berkel, S. S.; Hatzakis, N. S.; Opsteen, J. A.; van Delft, F. L.; Cornelissen, J. J. L. M.; Rowan, A. E.; van Hest, J. C. M.; Rutjes, F. P. J. T.; Nolte, R. J. M. *Chem. Commun.* **2005**, *33*, 4172−4174. (e) Jang, H.; Fafarman, A.; Holub, J. M.; Kirshenbaum, K. *Org. Lett.* **2005**, *7*, 1951−1954. (f) Musiol, H.-J.; Dong, S.; Kaiser, M.; Bausinger, R.; Zumbusch, A.; Bertsch, U.; Moroder, L. *Chem. Bio. Chem.* **2005**, 625−628. (g) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Biomol. Chem.* **2006**, *4*, 1497−1502. (h) Bock, V. D.; Perciaccante, R.; Jansen, T. P.; Hiemstra, H.; van Maarseveen, J. H. *Org. Lett.* **2006**, *8*, 919−922.

(12) CAUTION: Low molecular weight azides can be explosive. For a recent review covering various aspects of azide chemistry, see: Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5188−5240.

crucial for a one-pot click−click strategy as any remaining azide or alkyne could react in the second step, complicating purification and lowering the overall yield.[13] We first focused on the chemoselective cycloaddition of dialkynes **5** and **7a** (Scheme 4). Hydroalcoholic conditions were used to ensure

Scheme 4. Chemoselective Click Cycloaddition[a]



[a] See Table 1 for reaction details and yields.

a general procedure that would be compatible with a wide range of substrates, including complex biomacromolecules. Although it had proved effective in CH_2Cl_2/MeOH mixtures (Scheme 1), $Cu(MeCN)_4PF_6$ proved to be a poor catalyst in *t*BuOH/water (Table 1, entry 2). However, the widely used

Table 1. Optimization of Conditions for the First Cycloaddition[a]

				yield (%)[c]		
entry	method[b]	temp (°C)	duration	**5**	**8**	**9**
1	**A**	20	18 h	61	39	0
2	**B**	20	18 h	85	15	0
3	**C**	20	18 h	8	92	0
4	**C**	20	72 h	2	98	0
5	**D**	20	18 h	5	75	17
6	**C**	100[e]	10 min	12	55	15
7	**C**	35	18 h	2	98[d]	0

[a] Reactions were carried out using a 1:1 mixture of **6** and **8a** at 0.05 M under a nitrogen athmosphere. [b] **A**: $Cu(CH_3CN)_4PF_6$ (0.1 equiv), CH_2Cl_2/MeOH 4:1. **B**: $Cu(CH_3CN)_4PF_6$ (0.1 equiv), *t*BuOH/water 95:5. **C**: $CuSO_4$ (0.1 equiv), sodium ascorbate (0.2 equiv), *t*BuOH/water 95:5. **D**: $CuSO_4$ (0.5 equiv), sodium ascorbate (1 equiv), *t*BuOH/water 95:5. [c] Conversions determined by ^1H NMR. [d] 94% isolated yield. [e] Microwave irradiation; see Supporting Information for details.

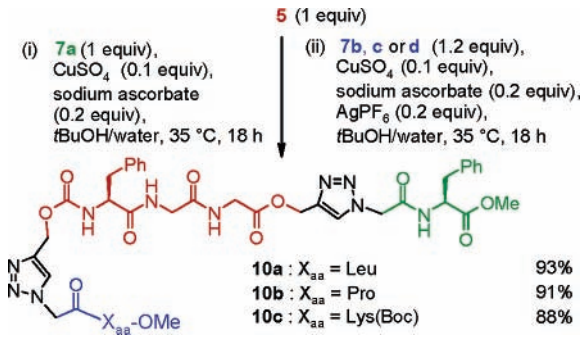
click conditions which utilize the in situ generation of Cu(I) (0.1 equiv of $CuSO_4$/sodium ascorbate) proved to be effective even if the reaction was sluggish (entries 3 and 4). Increasing the catalyst loading caused the partial deprotection of **8** leading to **9** (entry 5).[14] Attempts to use microwave-accelerated conditions led to the same two compounds, together with several byproducts, but gentle warming of the reaction overnight resulted in excellent conversion (entry 7).

(13) Tagged or solid-supported alkynyl "azide-quenching" agents could allow excess reactants to be used in both cycloaddition steps, probably enabling the overall yield to be increased still further.

(14) Cu(I)-mediated cleavage of an alkyne C−Si bond has been reported: Ito, H.; Arimoto, K.; Sensui, H-o; Hosomi, A. *Tetrahedron Lett.* **1997**, *38*, 3977−3980. We are currently developing more robust terminal alkyne protecting groups.

These optimized conditions for the first cycloaddition were used as the basis for a second successive triazole-forming reaction in the same reaction vessel (Scheme 5).

Scheme 5. Chemoselective Click−Click Chemistry: Successive Triazole Peptide Ligations in One Pot



Addition of the second azide (**7b**, **c**, or **d**) and a catalytic amount (0.2 equiv) of $AgBF_4$ to the product mixture from Table 1, entry 7, resulted in complete disappearance of **8** and formation of bistriazoles **10a**−**c** (Scheme 5). Additional $CuSO_4$ (0.1 equiv) and sodium ascorbate (0.2 equiv) were needed to drive the reaction to completion in a reasonable time. After 18 h, simple filtering through a silica plug removed excess azide and metal salts to give the desired bistriazole pseudononapeptides **10a**−**c** in good purity and yield (Scheme 5). LC-MS analysis of the crude material, **10a**−**c**, showed only traces (0−4%) of contamination from reaction of **5** with two molecules of the same azide.

In conclusion, we have described a methodology for the one-pot chemoselective ligation of three polyfunctionalized building blocks. The strategy exploits the Cu(I)-catalyzed alkyne−azide cycloaddition. This efficient reaction, combined with the one-pot Ag(I)-mediated deprotection of a TMS-protected alkyne moiety, allows for the synthesis of bistriazole products in high yields in good purity after simple filtration through a plug of silica. The ease of preparation (and, in many cases, commercial availability) of unprotected polyfunctionalized azides, alkynes, and silylated alkynes, together with the simple conditions for reaction and workup, suggest this click−click chemoselective ligation strategy could have wide potential. In particular, it appears promising as a tool for peptide ligation and, probably, combinatorial chemistry, allowing direct access to complex structures from simple building blocks under mild conditions.

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Supporting Information Available: General synthetic experimental procedure, characterization, and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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