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Three-Component Synthesis of Neoglycopeptides Using a Cu(II)-Triggered Aminolysis of Peptide Hydrazide Resin and an Azide–Alkyne Cycloaddition Sequence

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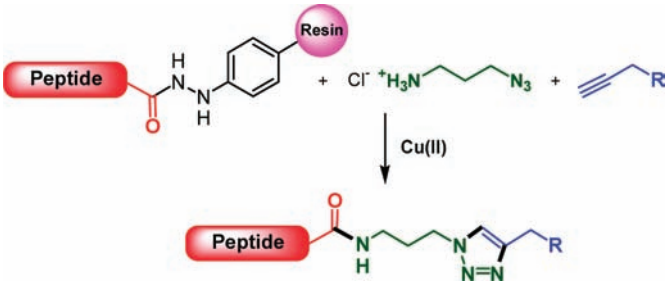
ABSTRACT



Copper(II)-induced oxidative aminolysis of hydrazides generates Cu(I), the catalyst of the azide–alkyne cycloaddition. This feature was exploited to design a novel solid phase detaching three-component reaction permitting the conversion of supported peptide hydrazides into 1,2,3-triazole linked C-terminal neoglycopeptides.

Multicomponent reactions (MCRs) represent a chemical process involving at least three reactants for the inherent formation of several covalent bonds in one operation.[1] By definition, MCRs are chemo- and regioselective, convergent step-efficient procedures and take place with high atom economy.[2] Another important feature implies the diminution of waste production due to the decrease of synthetic or isolation steps along with saving time.[3]

Since its discovery reported by Meldal[4] and Sharpless,[5] the copper(I)-catalyzed Huisgen[6] 1,3-dipolar cycloaddition of organic azides and terminal alkynes (CuAAC) has been playing an outstanding role in various fields of chemistry and biochemistry[7] research. The resulting 1,4-disubstituted 1,2,3-triazoles are obtained in high yields with exclusive regioselectivity under mild reaction conditions. However, only few reports describe the implementation of CuAAC in MCRs.[8,9] Most of these MCRs were designed to circumvent the handling of azides, whose synthesis and isolation can be problematic due to their potential explosive or unstable nature. For example, three-component

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(2) (a) Bienaymé, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem.—Eur. J.* **2000**, *6*, 3321–3329. (b) Bienaymé, H.; Bouzid, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 2234–2237. (c) Trost, B. M. *Angew. Chem., Int. Ed.* **1995**, *34*, 259–281.

(3) Lalli, C.; Bouma, J. M.; Bonne, D.; Masson, G.; Zhu, J. *Chem.—Eur. J.* **2011**, *17*, 880–889.

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

(5) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–259.

(6) (a) Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1965**, *98*, 4014–4021. (b) Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613–628.

(7) For recent reviews, see: (a) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015. (b) Debola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 1006–1017. (c) Amblard, F.; Cho, J. H.; Schinazi, R. F. *Chem. Rev.* **2009**, *109*, 4207–4220. (d) Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, *13*, 2399–2411.

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(9) (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897–3899. (b) Ackerman, L.; Potukuchi, H. K.; Landsberg, D.; Vicente, R. *Org. Lett.* **2008**, *10*, 3081–3084.

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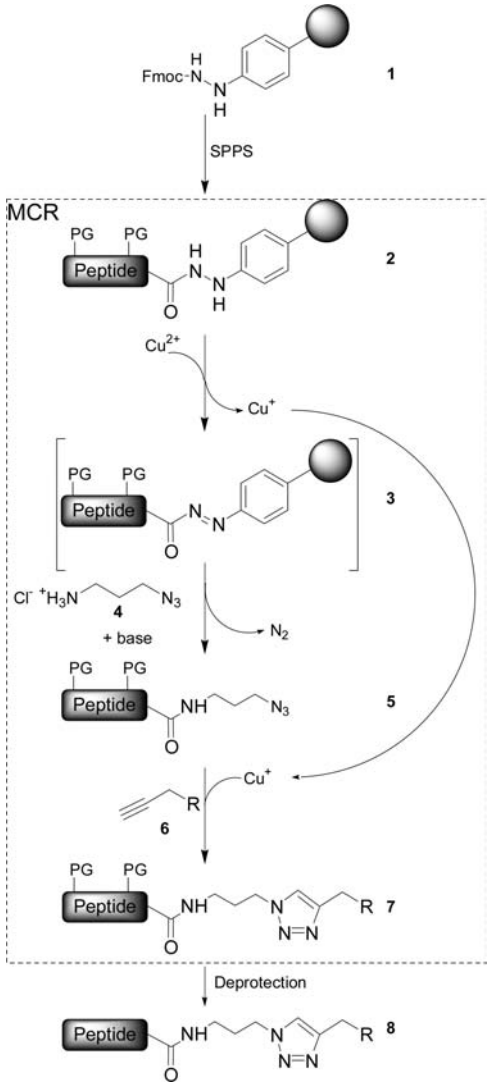
copper catalyzed reactions in which azides are generated *in situ* from corresponding alkyl[8] or aryl[9] halides and sodium azide in the presence of terminal alkynes give access to diversely substituted 1,2,3-triazoles.

We describe hereinafter a novel three-component reaction based on a Cu(II)-triggered aminolysis of peptide hydrazide resin and an azide−alkyne cycloaddition sequence. This process is delimited by the dashed square in Scheme 1.

Scheme 1. Principle of Three-Component Reaction



One of the components of this MCR is the protected peptidyl resin **2**, which is obtained from arylhydrazine resin **1** using standard solid phase peptide synthesis (SPPS). Copper(I), the catalyst of CuAAC, is generated *in situ* during oxidation of arylhydrazine resin **2** by copper(II). Aminolysis of the resulting supported peptidyl diazene resin **3** by azido amine **4** releases the protected azido peptide **5** in solution. Cu(I) formed in the first step of the MCR catalyzes the CuAAC between azido peptide **5** and alkyne **6** to give triazole **7**. Finally, removal of peptide protecting groups furnishes target peptide **8**. Overall, this MCR encompasses four chemical transformations: the oxidation of hydrazide **2** into diazene **3**, the reduction of Cu(II) into Cu(I), the diazene aminolysis which results in the detachment of the peptide chain from the resin, and finally the CuAAC.

This proof of concept study is illustrated with the synthesis of neoglycopeptides, for which the R group is a carbohydrate or carbohydrate mimic in the general formula **8** (Scheme 1). Several reports describe the application of CuAAC for the synthesis of these biologically significant conjugates[10] using either alkynyl[11] or azido[12] oligosaccharides. Recently, Brimble et al.[13] has combined Native Chemical Ligation and CuAAC in a one-pot process leading to neoglycopeptides from unprotected propargylpeptides and azido glycans.[14] However, the strategy depicted in Scheme 1 constitutes the first MCR approach to neoglycopeptides.

Neoglycoconjugates **8** (R = carbohydrate/carbohydrate mimic) feature a sugar moiety directly linked to the C-terminus of the peptide chain. The preparation of such neoglycoconjugates has not been reported before. Consequently, as a step toward the study of the MCR, we undertook their synthesis by a stepwise approach as shown in Scheme 2. Model sequence Ac-ILKEPVYX (X = Gly, Ala, Ser, Val, or His) was assembled on resin **1**, which allows Fmoc-SPPS and racemization-free synthesis of peptide amides by mild Cu(II)-oxidative aminolysis of the hydrazinocarbonyl bond.[15] 3-Azidopropylamine hydrochloride **4** required for the oxidative aminolysis reaction was obtained in two steps starting from commercially available *N*-(3-bromopropyl)phtalimide (see Supporting Information). The use of this ammonium salt avoids the handling of 3-azidopropylamine, which is volatile.[16] Formation of acyldiazene intermediate **3** (Scheme 1) from protected peptide hydrazide resin **2** requires 2 equiv of Cu(II). We used for this step 0.5 equiv of $Cu(OAc)_2$ with air bubbling to allow the recycling of the formed Cu(I) into Cu(II) by molecular oxygen (Scheme 2). Oxidative aminolysis of model peptidyl resins **2a**−**e** followed by

(10) (a) Pérez-Balderas, F.; Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asín, Isac-García, J.; Santoyo-González, F. *Org. Lett.* **2003**, *5*, 1951–1954. (b) Wittmann, V.; Seeberger, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 900–903. (c) Tejler, J.; Tullberg, E.; Frejd, T.; Leffer, H.; Nilsson, U. J. *Carbohydr. Res.* **2006**, *341*, 1353–1362.

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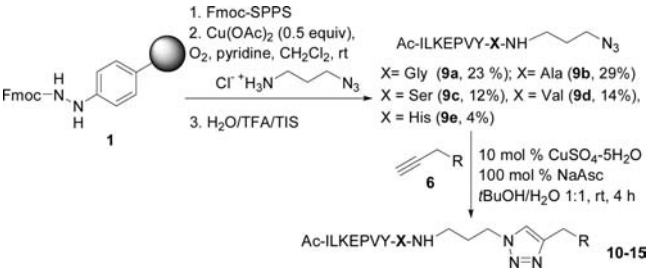
Scheme 2. Stepwise Synthesis of 1,2,3-Triazole Peptides **10**–**15**



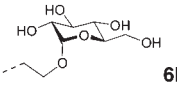
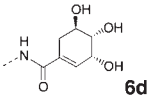
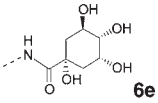
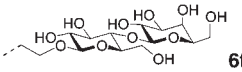
deprotection in TFA and reversed-phase (RP) HPLC purification successfully furnished C-terminal azido peptides **9a**–**d** with 12–29% overall yields starting from resin **1**. Synthesis of peptide **9e** with the C-terminal His residue was problematic whose yield was only 4%. This is probably due to the capacity of the imidazole group to chelate with copper.

The Cu(I)-catalyzed 1,3-azide–alkyne cycloaddition was optimized using azido peptide **9b** (X = Ala) and 4-phenyl-1-butyne **6a** (R = CH_2Ph) as a model alkyne (Scheme 2). Several solvents and catalysts were screened at room temperature. The CuI/DIEA system in DMF[4] was unsatisfactory. Likewise, no product formation was observed with $CuSO_4 \cdot 5H_2O$/sodium ascorbate (NaAsc) 10/100 mol % catalyst in aqueous acetonitrile. Alternately, this catalyst successfully yielded 1,3-triazole **8a** in 4 h using *t*BuOH/H_2O as solvent (99% convertion, 89% isolated, entry 1, Table 1). Decreasing $CuSO_4 \cdot 5H_2O$/ascorbate loading to 5/50 mol % led to a conversion of 93%, but the reaction time needed to be extended to 20 h. Consequently, optimized reaction conditions corresponded to $CuSO_4 \cdot 5H_2O$/NaAsc 10/100 mol % in *t*BuOH/H_2O 1/1 by vol.

The utility of this procedure was confirmed by examining a series of alkyne monosaccharides **6b,c** and mannose mimics[17] **6d,e** along with C-terminal azido peptides **9a**–**d** (72–82% isolated yields, entries 2–5, Table 1). Cycloaddition of His peptide **9e** with lactose derivative **6f** permitted the isolation of neoglypeptide **15** albeit with a lower yield (entry 5, Table 1).

These preliminary experiments set the stage for studying the MCR between protected peptide hydrazide resin **2**,

Table 1. Yields for 1,2,3-Triazole Peptides **10**–**15**

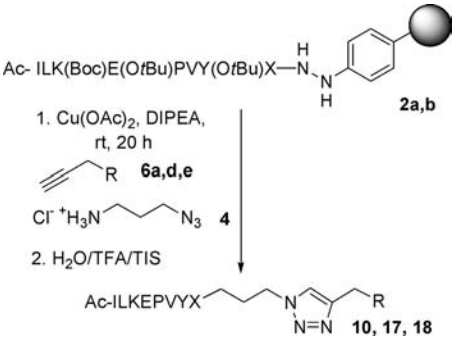
entry	peptide **9** (X)	R	1,2,3-triazole	yield (%)[a]
1	**9b (Ala)**	CH_2Ph **6a**	**10**	89
2	**9b (Ala)**	 **6b**	**11**	82
3	**9d (Val)**	 **6c**	**12**	78
4	**9a (Gly)**	 **6d**	**13**	79
5	**9c (Ser)**	 **6e**	**14**	72
6	**9e (His)**	 **6f**	**15**	36

[a] Isolated yields. Products were purified by RP-HPLC.

3-azidopropyl amine hydrochloride **4**, and alkyne **6**. As previously indicated, the oxidative aminolysis of the hydrazide bond requires 2 equiv of Cu(II). The use of substoichiometric amounts of Cu(II) for this step must be compensated by the introduction of an oxidant in the process, such as molecular oxygen, which oxidizes Cu(I) back to Cu(II). However, azide–alkyne cycloaddition requires maintenance of a high concentration of Cu(I) in solution. This is usually achieved by using Cu(II) and a reductant such as sodium ascorbate. At first glance, these two chemical reactions appear incompatible. But this apparent problem can be resolved by using stoichiometric amounts of Cu(II) for the hydrazide oxidation step and by working rigorously under an inert atmosphere (O_2 < 1 ppm), thereby avoiding the oxidation of Cu(I) to Cu(II).

Optimization of experimental conditions was performed using protected peptide hydrazide resin **2b** (X = Ala), 2

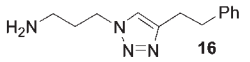
Scheme 3. Study of the MCR–Deprotection Sequence



(16) Stability of **4** at rt was confirmed by thermogravimetric analysis (see Supporting Information). Synthesis of 3-azidopropyl amine as a free base is reported in several papers: (a) Carboni, B.; Benalil, A.; Vaultier, M. *J. Org. Chem.* **1993**, *58*, 3736–3741. (b) Knör, S.; Modlinger, A.; Poethko, T.; Schottelius, M.; Wester, H.-J.; Kessler, H. *Chem.—Eur. J.* **2007**, *13*, 6082–6090. (c) Tamanini, E.; Rigby, S. E. J.; Motevalli, M.; Todd, M. H.; Watkinson, M. *Chem.—Eur. J.* **2009**, *15*, 3720–3728. For a review on azides, see: Scriven, E. F. V.; Turnbull, K. *Chem. Rev.* **1988**, *88*, 297–368.

(17) (a) Grandjean, C.; Rommens, C.; Gras-Masse, H.; Melnyk, O. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2967–2975. (b) Grandjean, C.; Gras-Masse, H.; Melnyk, O. *Chem.—Eur. J.* **2001**, *7*, 230–239. (c) Chenevier, P.; Grandjean, C.; Loing, E.; Malingue, F.; Angyalosi, G.; Gras-Masse, H.; Roux, D.; Melnyk, O.; Bourel-Bonnet, L. *Chem. Commun.* **2002**, 2446–7. (d) Angyalosi, G.; Grandjean, C.; Lamirand, M.; Auriault, C.; Gras-Masse, H.; Melnyk, O. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2723–2727. (e) Grandjean, C.; Gras-Masse, H.; Melnyk, O. *Chemistry* **2001**, *7*, 230–9.

Table 2. Results for the MCR−Deprotection Sequence

entry	resin **2** X	alkyne **6** R	solvent	metal	product	overall isolated yield (%)
1	Ala[a]	**6a**	THF	$Cu(OAc)_2$	**10**	6
2	Ala[a]	**6a**	DMF	$Cu(OAc)_2$	**10**	13
3	Ala[a]	**6a**	CH_2Cl_2	$Cu(OAc)_2$	**10**	14
4	Ala[a]	**6a**	CH_2Cl_2/pyridine (5 equiv)	$Cu(OAc)_2$	**10**	18
5	Ala[a]	**6a**	CH_2Cl_2/pyridine (5 equiv)	Cu(OAc)	 **16**	84
6	Ala[b]	**6d**	CH_2Cl_2/pyridine (5 equiv)	$Cu(OAc)_2$	**17**	14
7	Gly[b]	**6e**	CH_2Cl_2/pyridine (5 equiv)	$Cu(OAc)_2$	**18**	11

[a] 2 equiv of amine **4**. [b] 1.5 equiv of amine **4**.

equiv of ammonium salt **4**, 2 equiv of 4-phenyl-1-butyne **6a**, and 2.5 equiv of copper(II) acetate in the presence of *N, N*-diisopropylethylamine (DIPEA) (Scheme 3, Table 2). We first examined the influence of the solvents on the MCR efficiency. Dichloromethane (entry 3, Table 2) proved superior to THF (entry 1) and DMF (entry 2); this may be due to the better swelling of the polystyrene resin in the former solvent. In each case, C-terminal azido peptide **9b** was identified as a major side product (~30% by RP-HPLC). Using a mixture of pyridine and dichloromethane for the MCR improved the isolation of target peptide **10** (entry 4) and decreased the proportion of **9b** 2-fold. In a control experiment, we replaced Cu(II) by Cu(I) using pyridine/dichloromethane as solvent. Formation of peptide **10** was not observed, but the 1,2,3-triazole product **16** formed from the cycloaddition of azidopropylamine **4** and 4-phenyl-1-butyne **6a** could be isolated with a 84% yield (entry 5). This experiment shows the importance of Cu(II) for triggering the MCR. Note that 1,2,3-triazole product **16** can be formed during the MCR once Cu(I) has been generated during the hydrazide oxidation step. **16** can participate also in the aminolysis of diazene resin **3** (Scheme 1). Peptide **7** is thus potentially the end product of two different chemical pathways.

The application of the MCR to peptidyl resin **2b** (X = Ala) and shikimic derivative **6d** successfully furnished neoglycopeptide **17**. We used only 1.5 equiv of amine **4** for this reaction to further minimize the formation of azido peptide **9b**, which was indeed barely detected in the crude product. The RP-HPLC chromatogram shown in Figure 1 highlights the good purity of the crude neoglycopeptide **17** released during the MCR. A similar result was obtained for the MCR involving resin **2a** (X = Gly) and quinic derivative **6e** (entry 7, Table 2).

In conclusion, we have described a novel Cu(II)-triggered MCR based on an oxidative aminolysis 1,3-cycloaddition sequence. The MCR process requires a peptide hydrazide resin, an amino azide linker, and an alkyne, resulting in the formation of peptides modified at the C-terminus through an amino 1,2,3-triazole linker. Cu(I), the catalyst of the 1,3-cycloaddition reaction, is formed during the oxidative aminolysis step, which also allows the cleavage of the peptide from the solid support. The MCR was applied to the synthesis of a novel family of neoglycopeptides. This reaction can potentially be used for the synthesis of a large variety of peptide derivatives starting from Fmoc-SPPS assembled peptidyl resins.

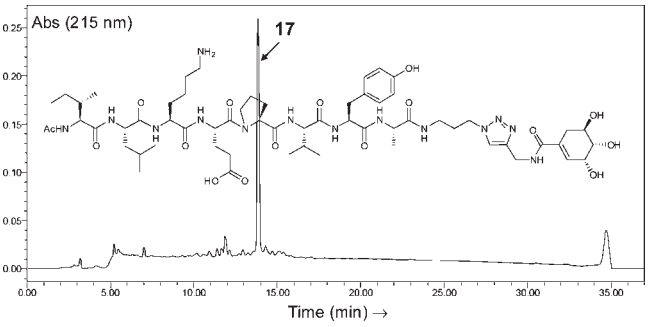


Figure 1. RP-HPLC chromatogram of the crude MCR with peptidyl resin **2b** (X = Ala) and shikimic derivative **6d**.

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Supporting Information Available. Experimental procedures and characterization data for all peptides. This material is available free of charge via the Internet at http://pubs.acs.org.