Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 46 (2005) 4479–4482
Tetrahedron Letters

Peptide ligation through click chemistry for the generation of assembled and scaffolded peptides

Raimo Franke, Christian Doll and Jutta Eichler*

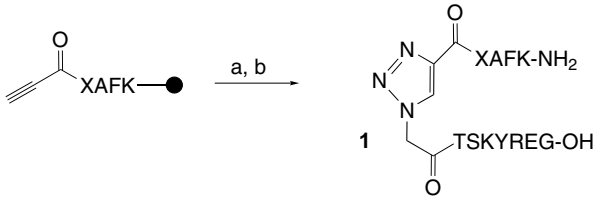
German Research Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany

Received 1 April 2005; revised 21 April 2005; accepted 25 April 2005
Available online 17 May 2005

Abstract—The synthesis of [1,2,3]-triazoles through copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition was examined for its utility to generate assembled and scaffolded peptides from peptide and scaffold precursors, which were N-terminally modified with azido and alkyne moieties, respectively.

A central step in convergent synthesis strategies for the generation of highly complex branched, assembled, or scaffolded peptides and proteins, is the site-selective ligation of peptide fragments with each other, or their attachment to defined sites of a molecular scaffold.[1] Such ligation reactions include the formation of thiazolidines[2] or oximes[3] from mutually reactive precursors, as well as native chemical ligation through reaction of a peptide thioester with an N-terminal cysteine[4] in aqueous buffers. Fully protected peptide acids, on the other hand, are often used for solid-phase fragment condensation.[5]

The generation of [1,2,3]-triazoles through 1,3-dipolar cycloadditions of alkynes to azides,[6] which belongs to a group of reactions referred to as click chemistry,[7] proceeds at room temperature in the presence of copper(I) as a catalyst.[8–10] This reaction has been used for the combinatorial synthesis of peptidotriazoles.[10,11] A highly potent acetylcholinesterase inhibitor was selectively assembled, without using a catalyst, in the presence of the enzyme as a template, in which the alkyne and azido precursors for the inhibitor were brought into close spatial proximity.[12] We have examined the copper(I)-catalyzed reaction for its utility to generate assembled and scaffolded peptides from peptide and scaffold precursors, which were N-terminally modified with azido and alkyne moieties, respectively. The strategy for the solid-phase ligation of assembled peptides is outlined in Scheme 1. In order to assess the compatibility of the method with different types of amino acids, 13 model peptides presenting all types of proteinogenic trifunctional amino acids were synthesized on solid phase, and their N-terminal amino group acylated with propiolic acid. The resin-bound peptides were ligated, in the presence of CuI as the catalyst, with the protected, azidoacetylated peptide N_3Ac-T(*t*-Bu)S(*t*-Bu)K(Boc)-Y(*t*-Bu)R(Pbf)E(O*t*-Bu)G-OH. Azidoacetic acid for the N-terminal acylation was generated in situ from bromoacetic acid and sodium azide. Upon cleavage from the resin, all assembled peptides **1** were obtained in high purity, as determined by HPLC with UV and ESI-MS detection (LC-MS, Fig. 1). The few impurities found



X = C, D, E, G, H, M, N, Q, R, S, T, W, Y

Scheme 1. Generation of assembled peptides **1** through formation of [1,2,3]-triazoles by ligating protected azido-peptides to resin-bound alkyne precursors. Reagents and conditions: (a) N_3Ac-T(*t*-Bu)S(*t*-Bu)K(Boc)Y(*t*-Bu)-R(Pbf)-E(O*t*-Bu)G-OH in the presence of CuI. (b) cleavage from the resin. See supplementary data for detail.

Keywords: Click chemistry; Ligation; Scaffolded peptides.
* Corresponding author. Tel.: +49 531 6181 793; fax: +49 531 6181 795; e-mail: jei@gbf.de

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.04.107

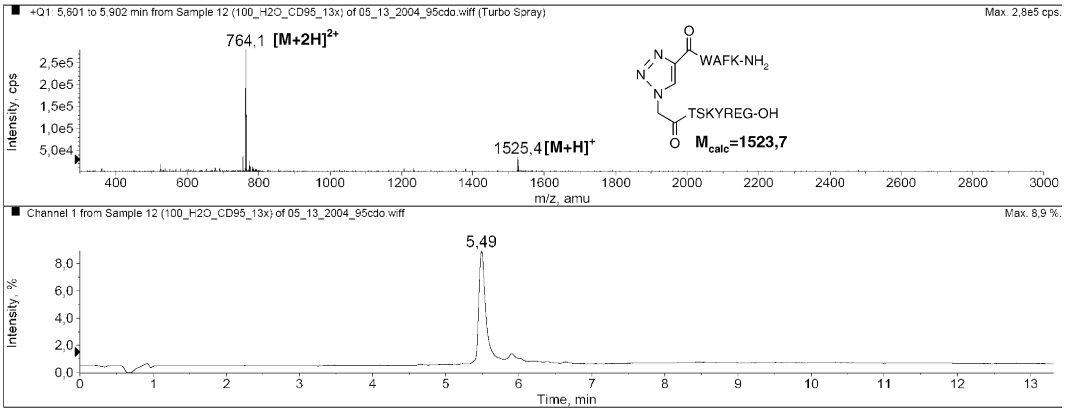


Figure 1. HPLC chromatogram (bottom) and ESI mass spectrum of the peak at 5.49 min (top) of crude **1** with X = W.

in some of the peptides, that is, the dimeric disulfide in X = C, the aspartimide peptide in X = D and the Met(O) peptide in X = M (see supplementary data) are the result of typical side reactions associated with these amino acids, and unlikely to be a side product of the cycloaddition reaction.

While the thermal, non-catalyzed 1,3-dipolar cycloaddition of azides to alkynes is a regio-unspecific reaction generating a mixture of the 1,4- and 1,5-substituted [1,2,3]-triazoles, the copper(I)-catalyzed reaction has been reported to yield selectively the 1,4-substituted triazole.[10] This could be confirmed through ^{1}H NMR spectroscopic analysis of assembled peptide **2**, which was generated through ligation of N_3Ac-Y(*t*-Bu)GGFLG-OH with resin-bound T(*t*-Bu)S(*t*-Bu)K(Boc)Y(*t*-Bu)R(Pbf)-E(O*t*-Bu)G (N-terminally acylated with propiolic acid), as described for **1**. The spin systems (amino acid residues and triazole system) were readily identified from the two-dimensional TOCSY and NOESY spectra (see supplementary data). The NOESY spectra enabled unambiguous sequential assignment of the amino acid residues from cross-peaks corresponding to (*i*, *i* + 1) $H^{N}H^{N}$ and (*i*, *i* + 1) $H^{N}H^{\alpha}$ NOEs. Strong NOE effects were observed between the triazole proton and the N-substituted methylene group, indicating a close proximity of the triazole proton and the N-substituent, thus providing strong evidence for the selective formation of the 1,4-substituted triazole (Fig. 2). This was further confirmed by the observation of a three-bond correlation between protons of the N-substituted methylene group and the protonated olefinic carbon of the triazole ring.

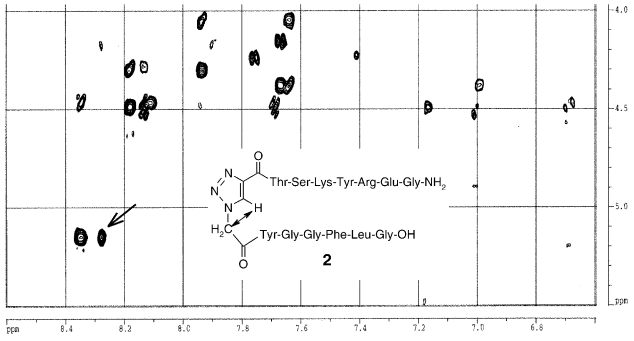
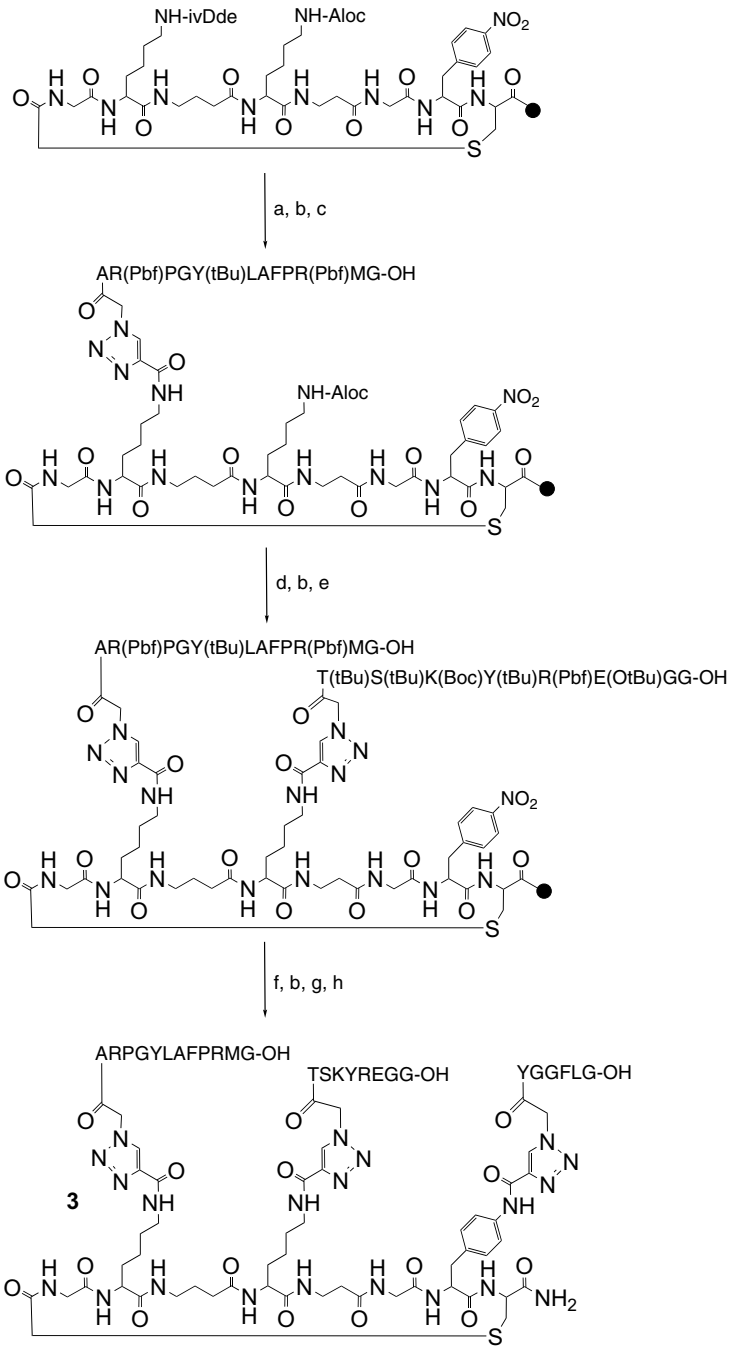


Figure 2. Structure and section of the 2D NOESY spectrum of **2**. The cross-peak marked with an arrow corresponds to the NOE between the triazole proton and the N-substituted methylene group.

The triazole ligation method was further tested for its utility to generate scaffolded peptides, in which peptide fragments are presented in a conformationally constrained, discontinuous fashion through a molecular scaffold.[13,14] Three different protected peptide acids, which were synthesized on 2-chlorotrityl resin, and N-terminally acylated with azidoacetic acid, were attached in a site-selective fashion to three sites of the scaffold, affording scaffolded peptide **3**. The synthesis is outlined in Scheme 2.

A resin-bound cyclic scaffold molecule containing two orthogonally protected lysine residues, as well as a 4-nitrophenylalanine residue, as selectively addressable attachment points for protein fragments, was synthesized on solid phase. The scaffold was cyclized through intramolecular thioether formation between the N-terminal bromoacetyl moiety and the SH group of the C-terminal cysteine residue. After removal of the ivDde group from the ε-amino group of one scaffold lysine residue, the free amino group was acylated with the symmetrical anhydride of propiolic acid, followed by ligation with N_3Ac-AR(Pbf)PGY(*t*-Bu)-LAFPR(Pbf)MG-OH in the presence of CuI. The Aloc group was removed from the second scaffold lysine residue, and the free amino group acylated with propiolic acid, followed by ligation with N_3Ac-T(*t*-Bu)S(*t*-Bu)K(Boc)Y(*t*-Bu)R(Pbf)E(O*t*-Bu)GG-OH. After reducing the nitro group with tin(II) chloride,[13] and acylating the resulting aromatic amino group with propiolic acid, the third triazole was formed through ligation with N_3Ac-Y(*t*-Bu)GGFLG-OH. The resulting scaffolded peptide was cleaved from the resin, purified by preparative HPLC, and analyzed by LC-MS (Fig. 3).

In summary, the regioselective, copper(I)-catalyzed 1,3-dipolar cycloaddition of alkynes to azides, yielding 1,4-substituted [1,2,3]-triazoles, has been successfully used



Scheme 2. Generation of scaffolded peptide **3**. Reagents and conditions: (a) Removal of the ivDde group. (b) Acylation with propiolic acid. (c) Ligation with N_3Ac-AR(Pbf)PGY(*t*-Bu)LAFPR(Pbf)MG-OH. (d) Removal of the Aloc group. (e) Ligation with N_3Ac-T(*t*-Bu)S(*t*-Bu)K(Boc)Y (*t*-Bu)-R(Pbf)E(O*t*-Bu)GG-OH. (f) Reduction of the nitro group. (g) Ligation with N_3Ac-Y(*t*-Bu)GGFLG-OH. (h) Cleavage from the resin. See supplementary data for detail.

to generate assembled and scaffolded peptides from alkyne and azido peptide and scaffold precursors, respectively. This strategy is expected to be particularly useful for the generation of combinatorial libraries of assembled and scaffolded peptides through cross-combination of mutually reactive alkyne and azido peptide and scaffold precursors, respectively. Furthermore, the free carboxy groups of the resulting molecules are points for further chemical modification, for example, through the intra- or intermolecular formation of ester and amide bonds. The extension of this ligation method to the use of unprotected peptide precursors is in progress.

Acknowledgements

We thank Victor Wray for NMR analysis. This work was funded by BioFuture Grant 0311882 from the German Federal Ministry of Education and Research (BMBF).

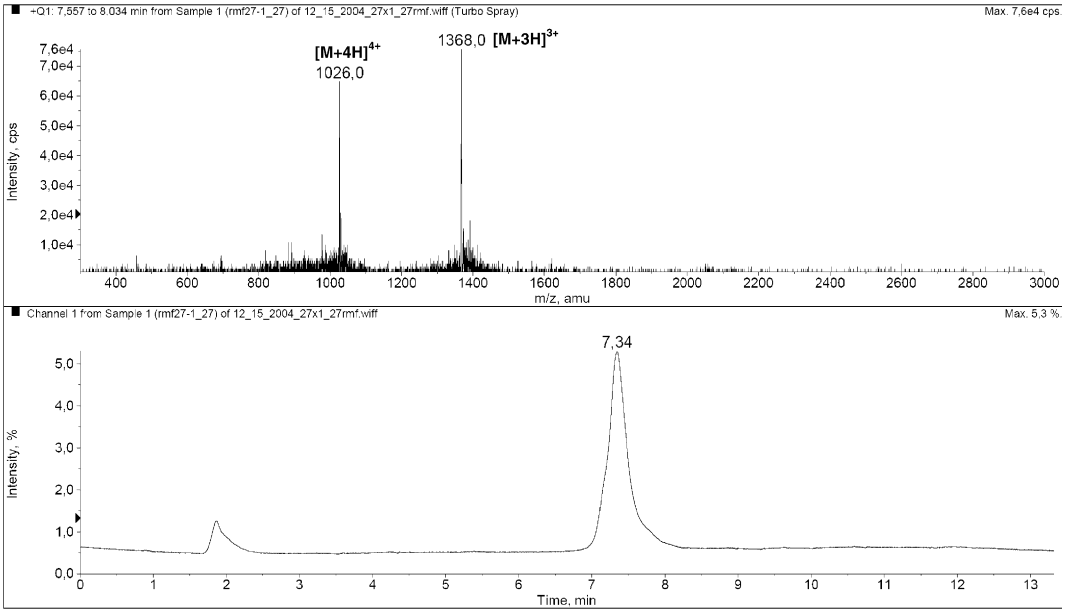


Figure 3. HPLC chromatogram (bottom) and ESI mass spectrum of the peak at 7.34 min (top) of purified **3**.

Supplementary data

Experimental detail of the syntheses, LC-MS data of **1**, NMR data of **2**, ESI-MS data of **3**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.04.107.

References and notes

1. Eichler, J. *Protein Pept. Lett.* **2004**, *11*, 281–290.
2. Liu, C. F.; Tam, J. P. *Proc. Natl. Acad. Sci.* **1994**, *91*, 6584–6588.
3. Rose, K. *J. Am. Chem. Soc.* **1994**, *116*, 30–33.
4. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776–779.
5. Albericio, F.; Lloyd-Williams, P.; Giralt, E. *Methods Enzymol.* **1997**, *289*, 313–336.
6. Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984; Vol. 1, pp 1–176.
7. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
8. L'abbe, G. *Bull. Soc. Chim. Belg.* **1984**, *93*, 579–592.
9. Rostovtsev, V. V.; Green, L. G.; Vokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
10. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
11. Tornoe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 312–324.
12. Warren, G. L.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053–1057.
13. Franke, R.; Doll, C.; Wray, V.; Eichler, J. *Prot. Peptide Lett.* **2003**, *10*, 531–539.
14. Franke, R.; Doll, C.; Wray, V.; Eichler, J. *Org. Biomol. Chem.* **2004**, *2*, 2847–2851.