Protocol

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Side chain-to-side chain cyclization by click reaction

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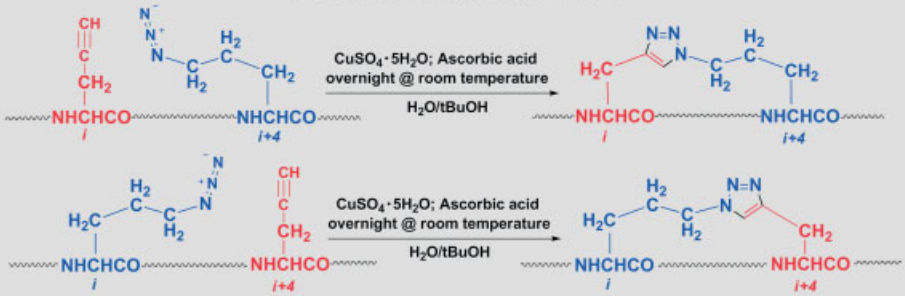
Cu^{I}-catalyzed azide-alkyne 1,3-dipolar Huisgen's cycloaddition (CuAAC) is a click reaction that has drawn a lot of attention, in general, and in the field of peptide and protein sciences, in particular. Among several reported applications, the preparation of novel heterodetic cyclopeptides by an intramolecular side chain-to-side chain CuAAC, forming a 1,4-disubstituted[1,2,3]triazolyl-containing bridge, is of great interest. Herein, we provide a detailed protocol for the syntheses of model heterodetic cyclopeptides as a prototypical intramolecular CuAAC, using as a model a sequence derived from parathyroid hormone-related protein.

Supporting information may be found in the online version of this article

Keywords: click reaction; Huisgen's cycloaddition; cyclopeptide; parathyroid hormone-related protein

REACTION SCHEME



GENERAL OPTIMIZED PROCEDURE

Cyclization by Cu(I)-catalyzed azide-alkyne Huisgen reaction. To a solution of the pure linear nonapeptide precursor containing the ω-azido and ω-alkynyl amino acyl residues (3.1 μmol) in H_2O/tBuOH (2 : 1 v/v) (4 ml) were added $CuSO_4 \cdot 5H_2O$ (43.4 μmol) and ascorbic acid (40.3 μmol). The mixture was stirred overnight at room temperature, then concentrated and lyophilized. The clicked heterodetic cyclo-nonapeptide was purified by reverse-phase solid-phase extraction on a RP-18 LiChroprep column pre-washed with H_2O and then eluted with a linear gradient of 0–50% of CH_3CN in H_2O, both phases containing 0.1% TFA.

Scope and Comments

Cu^{I}-catalyzed azide-alkyne 1,3-dipolar Huisgen's cycloaddition (CuAAC) [1,2] is the prototypic click reaction [3] and its applications in the fields of peptide and protein biomedical and material sciences is growing in an accelerating rate. Importantly, the following attributes make the CuAAC and the consequential 1,4-disubstituted-[1,2,3]triazolyl attractive tools in peptide chemistry: (i) the isosterism of the [1,2,3]triazolyl moiety to an amide bond makes it an interesting peptide bond surrogate [4], (ii) the bioorthogonal properties of the ω-azido and ω-alkynyl functions minimize side reactions and simplify protection schemes, enable a straightforward incorporation of the ω-azido- and ω-alkynyl-N^{α}-Fmoc protected amino acids and the isolation and purification

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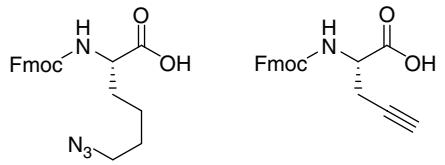

N^{α}–Fmoc-L-Nle(ε-N_3)-OH N^{α}–Fmoc-L-Pra-OH

Figure 1. The unnatural α-amino acids were introduced in positions 13 and 17 of N^{α}-Ac[Nle^{13}(ε-N_3),Pra^{17}]PTHrP(11–19)NH_2 by stepwise assembly through SPPS [14].

of the fully deprotected linear precursor, (iii) the mild and regioselective Cu^{I}-catalyzed cyclization can be performed in a aqueous or aqueous/organic media leading to high yields of the [1,2,3]triazolyl-bridged heterodetic cyclopeptide [1,2] with minimal side reactions [5], and therefore it is very appealing to both stepwise- and convergence-peptide synthetic strategies, and last but not least (iv) the resistance of the [1,2,3]triazolyl moiety to proteolysis and the reduced proteolytic susceptibility of the [1,2,3]triazolyl-bridged cyclopeptide [6]. Taken together, the fast growing number of different applications of the CuAAC in peptide sciences indicates extensive interest and great potential of this modification. For example, the 1,4-disubstituted-[1,2,3]triazolyl was introduced as a peptide bond surrogate generating potent inhibitors of cysteine protease [6] and HIV-1 protease [7]. It was used as an intramolecular disulfide bond replacement [8] and as a linker enabling formation of glycopeptides conjugates [2,9] and interpeptide side chain-to-side chain conjugates [10]. In addition, it was used to assemble protein-like oligomers and non-peptidic protein mimetic foldamers [11,12]. Moreover, 1,4-disubstituted-[1,2,3]triazolyl moieties were introduced as stabilizing elements of secondary structures including β-turns [13] and four helix bundles [4].

Recently, we have reported the introduction of an intramolecular i-to-i + 4 side chain-to-side chain CuAAC forming a 1,4-disubstituted[1,2,3]triazolyl-containing bridge as a new modality of heterodetic α-helical mimetic cyclopeptides [14]. Our protocol reports detailed experimental data on an efficient and facile procedure for the preparation of some model cyclo-nonapeptides derived from parathyroid hormone-related protein (PTHrP) as a prototypical intramolecular side chain-to-side chain CuAAC. Two examples of this application of the click reaction procedure have already been published [8,14]. The examples of 1,4- and 4-1-disubstituted-[1,2,3]triazolyl-containing side chain-to-side chain-bridged cyclo-nonapeptide in this study are closely related to the previously reported [15] i-to-i + 4 lactam-containing side chain-to-side chain cyclo-nonapeptide derived from hPTHrP, Ac-[Lys^{13}(&^{1}),Asp^{17}(&^{2})]hPTHrP(11–19)NH_2 [16]. In order to mimic the α-helical lactam-bridged cyclopeptide model, residues 13 and 17 in the linear precursor nonapeptide were replaced by unnatural amino acids in the form of the synthetic building blocks N^{α}-Fmoc-L-Nle(ε-N_3)-OH [17] and the commercially available propargylglycine (N^{α}-Fmoc-L-Pra-OH) (Figure 1).

Our protocol reports the synthesis, purification and characterization of two regioisomers of linear precursosrs **1** and **2**, containing either Nva(δ-N_3) and Pra or Pra and Nva(δ-N_3) in positions 13 and 17, respectively, (Figure 2) and their cyclization to the corresponding heterodetic cyclo-nonapeptide regioisomers containing either 1,4-disubstituted[1,2,3]triazolyl- or 4,1-disubstituted[1,2,3]triazolyl-modifications N^{α}–Ac-[Nva^{13}(&^{1}),Ala^{17}(&^{2})]hPTHrP(11–19)-NH_2][(&^{1}1,4-[1,2,3]triazolyl&^{2})] (**1**′) and N^{α}–Ac-[Ala^{13}(&^{1}),Nva^{17}(&^{2})]hPTHrP(11–19)-NH_2][(&^{1}4,1-[1,2,3]triazolyl&^{2})] (**2**′), respectively (Figure 3). The two regioisomers differ only in the bridges connecting the C^{α}s of the residues in positions 13 and 17. Both bridges are of the same length but differ in the location and orientation of the [1,2,3]triazolyl ring within the bridge. The [1,2,3]triazolyl is introduced in two opposite orientations, 1,4- and 4,-1, which are flanked by the reciprocal number of methylenes, 3 + 1 and 1 + 3 for the heterodetic cyclo-nonapeptides **1**′ and **2**′, respectively (Figure 3).

The preferred synthetic strategy includes stepwise solid phase assembly of the linear precursors **1** and **2**, followed by concomitant cleavage from resin and total deprotection. It concluded with solution phase CuAAC intramolecular cyclization generating the heterodetic[1,2,3]triazolyl-bridged cyclo-nonapeptides **1**′ and **2**′.

Experimental Procedure

SPPS of linear precursors 1 and 2

Peptides **1** and **2** were synthesized on a manual batch synthesizer (PLS 4 × 4, Advanced ChemTech) using a Teflon reactor (10 ml), following the Fmoc/tBu SPPS procedure. The coupling of each N^{α}-Fmoc-amino acid used TBTU/HOBt/NMM (2.5 eq.: 2.5 eq.: 3.5 eq.) and 2.5 eq. of the Fmoc protected amino acids, except for the unnatural amino acids N^{α}-Fmoc-L-Nva(δ-N_3)-OH and N^{α}-Fmoc-L-Pra-OH, for which only 1.5 eq. were used. The coupling was carried out in DMF (1 ml/100 mg of resin) for 40 min. Each coupling was monitored by Kaiser test, which were negative and therefore did not require recouplings. Importantly, incorporation of the unnatural amino acids into the peptide sequences was carried out in the standard fashion and was straightforward in both the coupling and the deprotection steps. The detailed protocol for the solid phase stepwise assembly of the linear peptide precursors and their deprotection and cleavage from resin are reported in the Supporting Information. The syntheses of the two linear precursors (**1** and **2**) afforded crude peptides, as shown by ultra performance liquid chromatography (UPLC) coupled with ESI-MS (see Supporting Information). The crude peptides were purified by semi-preparative RP-HPLC using a linear gradient of 10–60% of B in A for 20 min (A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN). The analytical characterization of final linear precursors is reported in the Supporting Information.

Synthesis of heterodetic cyclo-nonapeptides 1′ and 2′

Heterodetic cyclo-nonapeptides **1**′ and **2**′ were generated in solution by intramolecular CuAAC, in $tBuOH/H_2O$ as solvent mixture, in the presence of a large excess of ascorbic acid and Cu_2SO_4 generating *in situ* Cu(I). The crude cyclo-nonapeptides (**1**′ and **2**′) were analyzed by UPLC, showing complete conversion of the linear precursors (see Supporting Information). We obtained a very effective purification using solid phase extraction (SPE) on RP (C18) column, eluted with CH_3CN in H_2O. The copper salts were removed by elution with H_2O, whereas the desired heterodetic cyclo-nonapeptides were eluted with CH_3CN/H_2O mixture (Supporting Information).

Following this exemplary protocol, we were able to generate an extended series of i-to-i + 4 side chain-to-side chain [1,2,3]triazolyl-containing cyclo-nonapeptide by an efficient intramolecular CuAAC (Scrima M., Le Chevalier Isaad A, *et al*, in preparation).

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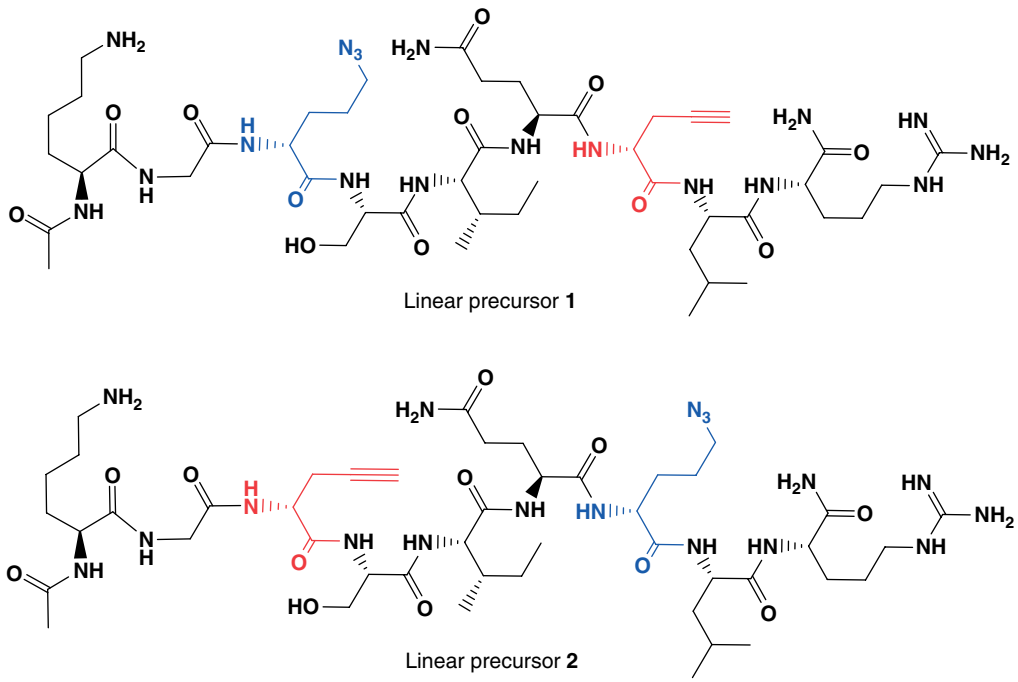

Figure 2. Peptide sequences of the linear precursors **1** and **2**.

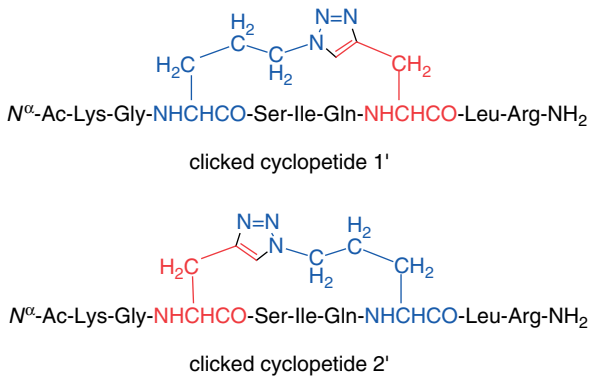

Figure 3. Peptide structures of the clicked heterodetic cyclo-nonapeptides **1**′ and **2**′.

This straightforward protocol is devoid of potential side products that can be formed through dimerizations and macrocyclizations, which are often observed in on-resin CuAAC, thus generating easy to purify crude products. We strongly endorse this protocol for a wide range of intramolecular side chain-to-side chain CuAAC aiming at the synthesis of [1,2,3]triazolyl-containing cyclopeptides that vary in bridge size, location and orientation of the [1,2,3]triazolyl moiety within the bridge.

Limitations

Although the biocompatibility of the [1,2,3]triazolyl moiety as a molecular scaffold and a pseudopeptidic modification in linear peptides has been demonstrated in numerous instances of bioactive small molecules and peptides, we have only very limited experience of it as a heterodetic side chain-to-side chain bridging moiety [[18–20] and references therein]. In addition, we have not used these reaction conditions in peptides containing cystinyl and histidyl residues. The former can be reduced in the presence of ascorbic acid, whereas the latter can chelate to the copper ions and require extra steps in isolation.

Acknowledgments

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Supporting information

Supporting information may be found in the online version of this article.

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