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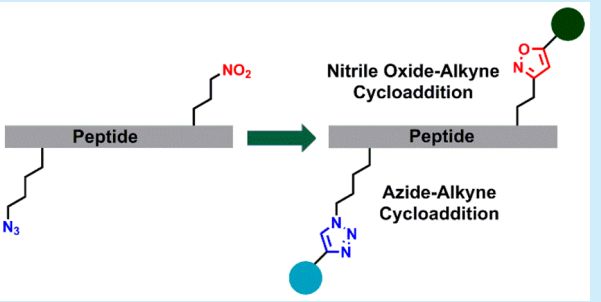
Chemoselective Nitrile Oxide–Alkyne 1,3-Dipolar Cycloaddition Reactions from Nitroalkane-Tethered Peptides

Rahi M. Reja, Sereena Sunny, and Hosahudya N. Gopi*

Department of Chemistry, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pune 411 008, India

Supporting Information

ABSTRACT: Synthesis and incorporation of a new amino acid with a nitroalkane side chain into peptides, in situ transformation of a nitroalkane side chain into nitrile oxide, and chemoselective 1,3-dipolar cycloaddition reactions between in situ generated nitrile oxide and different alkynes are reported. The nitroalkane-mediated nitrile oxide–alkyne cycloaddition was found to be orthogonal to the copper(I)-catalyzed azide–alkyne cycloaddition reaction. The combination of orthogonal nitrile oxide–alkyne and azide–alkyne cycloaddition reactions can be explored to tailor different 1,2,3-triazole and 3,5-isoxazoles, respectively, on the peptide backbone.



In his pioneering work, Hüisgen has established a wide range of three-atom, four-electron cycloaddition reactions with dipolarophiles.[1] The recent introduction of copper(I) as a catalyst in the azide–alkyne cycloaddition (CuAAC or click chemistry) reaction has exponentially increased its applications in chemistry, biology, and materials sciences.[2,3] The mild and broad substrate specificity of CuAAC has been extensively utilized in the conjugation of proteins, nucleic acids, and carbohydrates in vitro and in vivo.[4] In continuation, Bertozzi et al. developed a copper-free and strain-promoted azide–alkyne cycloaddition reaction (SPAAC) to avoid the toxicity of copper(I) in biological systems.[5] Besides the biomolecules, click chemistry has been widely utilized to functionalize small molecules and synthetic peptides. The click-conjugated peptides have found applications in therapeutics, diagnostics, tissue engineering, and drug delivery.[6] In spite of its widespread applications, click chemistry is also associated with inherent limitations. The selective azide–alkyne cycloaddition in a molecule containing two or more azides has been found to be a difficult task. Nevertheless, Wolfbeis and colleagues have demonstrated the dual labeling of biomolecules with and without copper using alkynes and strained alkynes, respectively, on a peptide.[7] In addition, Carell and colleagues demonstrated a triple modification of DNA by incorporating protected alkynes on the DNA backbone.[8] In addition to the double- and triple-click conjugations on peptides and nucleic acids, the click reaction was also found to be orthogonal to many other organic reactions. Recently, Boturyn and colleagues demonstrated the orthogonality of click reaction with oxime formation[9] as well as amide bond synthesis[10] involving thioacids[11] on a peptide template.

In addition to azides, nitrile oxides have also been extensively explored as precursors in the 1,3-dipolar cycloaddition reactions to derive medicinally important isoxazoles.[12] Mukaiyama and Hoshino demonstrated the transformation of alkyl nitro compounds into nitrile oxides mediated by phenyl isocyanate.[13] However, 1,3-dipolar cycloaddition reactions between nitrile oxide and dipolarophiles on the peptide backbone have scarcely been studied. In addition, orthogonality of 1,3-dipolar cycloaddition reaction between in situ generated nitrile oxide and azide has not been systematically investigated. Nitroalkanes are neutral and versatile functional groups.[14] Besides their transformation into nitrile oxides, the nitroalkanes can be transformed into various other functional groups such as amines, carboxylic acids, aldehydes, and ketones. We hypothesized that the introduction of a neutral nitroalkane functionality on peptide backbone could be immediately utilized for 1,3-dipolar cycloaddition reaction with alkynes through in situ generated reactive nitrile oxide. In addition, as the reaction conditions for azide–alkyne and nitrile oxide–alkyne cycloaddition are quite different, they may be orthogonal to one another. Nevertheless, no amino acids with nitroalkane side chains are commercially available to test our hypothesis. In this context, we sought to investigate the introduction of amino acids with nitroalkane side chains into peptides, generation of nitrile oxide on the peptide backbone, and the possibility of 1,3-dipolar cycloaddition reactions with alkynes. Herein, we report the synthesis and incorporation of nitroalkane-functionalized new amino acid into peptides and its utility in efficient and chemoselective 1,3-dipolar nitrile oxide–alkyne cycloaddition reactions in the presence of azide functionality on peptides. The nitroalkane mediated nitrile oxide–alkyne cycloaddition reactions were found to be compatible in the solution phase as well as on resin. Importantly, the nitroalkane functionality was found to unaffected while performing CuAAC reactions.

To introduce nitroalkane functionality on the peptide backbone, we have synthesized a new nitroalkane-function-

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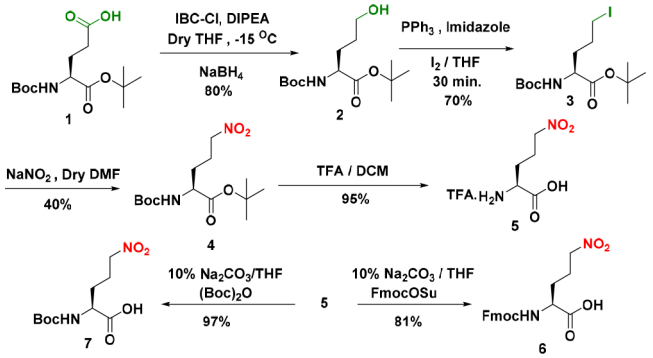
alized amino acid **4** starting from the commercially available α-*tert*-butyl ester of Boc-Glu(COOH)-OH (**1**). The schematic representation of the reaction is shown in Scheme 1. The

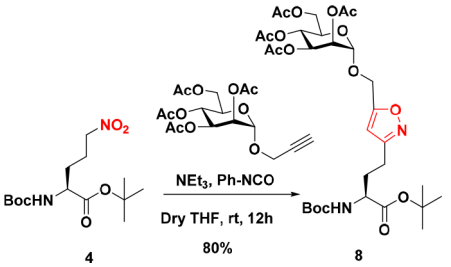
Scheme 1. Synthesis of Amino Acid with a Nitroalkane Side Chain from Protected Glutamic Acid



unprotected γ-carboxylic acid (**1**) was reduced to alcohol (**2**) through a mixed anhydride method using $NaBH_4$. The alcohol was transformed to iodide (**3**) through a modified Apple reaction.[15] The alkyl iodide was finally subjected to a substitution reaction with $NaNO_2$ in DMF[16] to obtain amino acid **4**, which was further transformed to TFA salt **5** (δ-nitro ornithine) through the deprotection of Boc and *tert*-butyl esters.[17] The free amine of amino acid **5** was again protected with Fmoc and Boc groups to obtain amino acids **6** and **7**, respectively. Synthetic details are given in the Supporting Information. The X-ray structure of protected amino acid **4** is shown in Figure S1. We adopted a *tert*-butyl ester strategy instead of ethyl/methyl esters to avoid the complications of nitro functionality during the saponification of esters.[18]

To understand whether the nitroalkane amino acid can undergo a 1,3-dipolar cycloaddition reaction with alkyne through in situ generated nitrile oxide, the amino acid **4** was treated with protected mannose alkyne in the presence of phenyl isocyanate and triethylamine (Scheme 2). The reaction

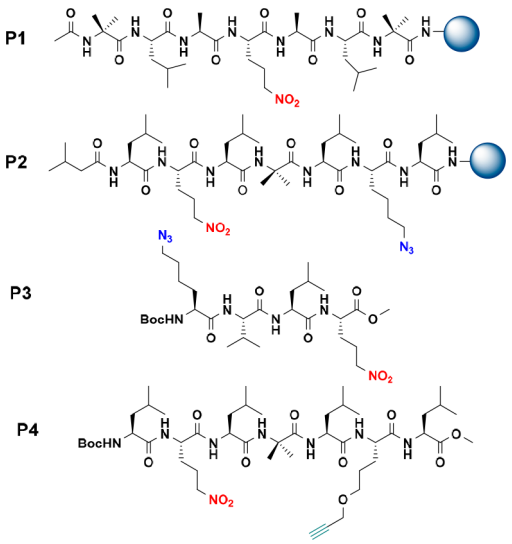
Scheme 2. 1,3-Dipolar Cycloaddition Reaction on Amino Acid 4



was monitored using TLC, and complete transformation of nitro amino acid **4** into its isoxazole derivative **8** was achieved in 12 h at room temperature. The 1,3-dipolar cycloaddition product was isolated in excellent yield. The reaction was found to be regioselective, and we isolated only 3,5-disubstituted isoxazole derivative.[19]

Inspired by the mild cycloaddition reaction between alkyne and amino acid **4**, we sought to investigate the compatibility of nitroalkane-mediated nitrile oxide−alkyne cycloaddition reaction on peptide backbone as well as its chemoselectivity with azide functionality on peptides. In this context, we designed peptides **P1**−**P4** by incorporating either nitro amino acid alone or along with azide as well as alkyne. The sequences of the peptides are shown in Scheme 3.

Scheme 3. Sequences of Peptides



First to understand whether the nitro amino acids can be incorporated into peptides through solid-phase peptide synthesis, we synthesized peptide **P1** on Rink amide resin using nitro amino acid **6**. All coupling reactions were performed using HBTU/HOBt conditions. The Fmoc group was deprotected using 20% piperidine in DMF. All coupling reactions were monitored using the Kaiser test. The mass spectral analysis of the final peptide, cleaved from the resin using TFA, suggests the incorporation of nitro amino acid into the peptide. The resin-bound peptide **P1** was treated with phenylacetylene in the presence of phenyl isocyanate and triethylamine to understand the feasibility of 1,3-dipolar cycloaddition reaction between the in situ generated nitrile oxide and phenylacetylene. The progress of the reaction was monitored using MALDI-TOF. Complete transformation of the primary nitroalkane group into the isoxazole derivative (**P5**) was achieved in 12 h with slow stirring of the resin beads at room temperature. The excess phenylacetylene and urea byproducts[20] were removed by washing the resin with DMF. The schematic representation of the reaction is shown in Scheme 4. Peptide **P5** was cleaved from the resin and purified by RP-HPLC. The HPLC analysis suggested the neat and quantitative transformation of **P1** to **P5**.

Motivated by the mild 1,3-dipolar cycloaddition on solid support, we synthesized peptide **P2** by incorporating both nitroamino acid **5** and azido lysine to understand the orthogonality of 1,3-dipolar cycloadditions with alkynes. The resin-bound peptide **P2** was subjected to nitroalkane-mediated nitrile oxide−alkyne cycloaddition reaction. In the process of in situ generation of nitrile oxide through the dehydration using phenyl isocyanate and subsequent 1,3-dipolar cycloaddition with *N*-Cbz-propargylamine, we observed no cycloaddition product from azide functionality on peptide. The formation of isoxazole and unreacted azide on peptide **P6** was confirmed by the mass spectral analysis. Concurrently, we subjected **P2** to CuAAC with phenylacetylene in the presence of nitroalkane functionality. We observed no cycloaddition from the nitro functionality while performing the click reaction on azide (**P7**).

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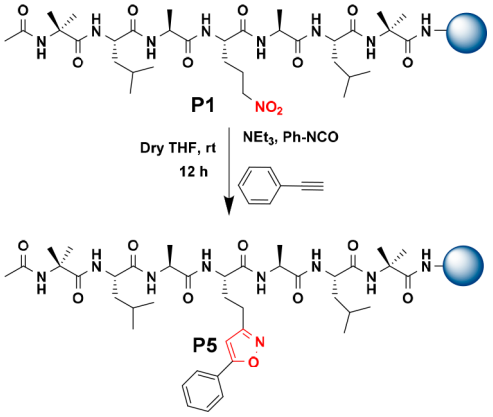
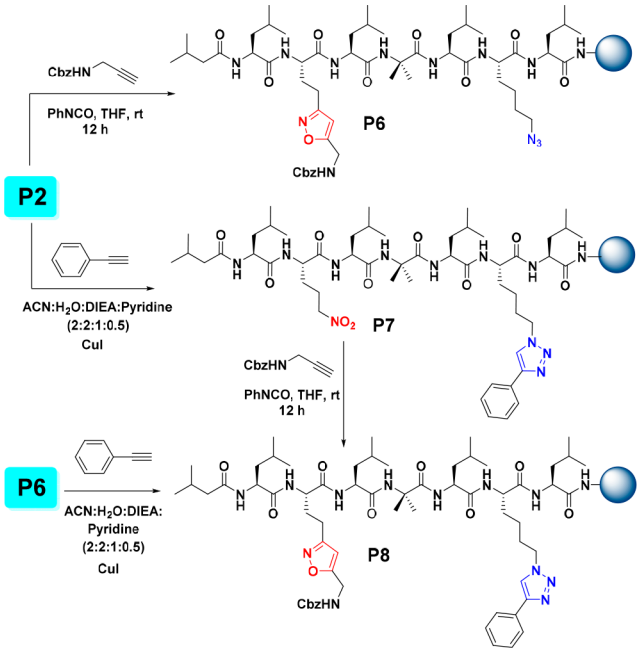
Scheme 4. 1,3-Dipolar Cycloaddition Reaction of the Peptide on Solid-Phase Support



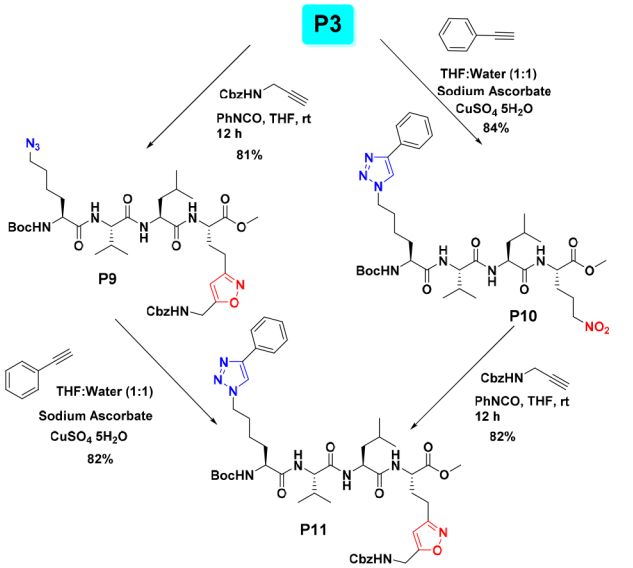
The cycloaddition reactions on **P2** are shown in Scheme 5. Both peptides **P6** and **P7** containing nitro and azide functional groups, respectively, were further subjected to cycloaddition reactions separately with alkynes to obtain doubly conjugated product **P8** on resin. The RP-HPLC and mass spectral analysis suggested the quantitative conversion of both nitro and azide into isoxazole and 1,2,3-triazole products, respectively. The peptide **P8** was cleaved from the resin, purified by RP-HPLC, and characterized.

Scheme 5. Orthogonal Cycloaddition Reaction with Azide on Solid-Phase Support



Inspired by the orthogonal cycloaddition of nitro and azide on a solid support, we sought to investigate whether the same reaction can be performed in solution. In this context, we synthesized peptide **P3** by incorporating both azido lysine and nitro amino acid **5** at the *N*- and *C*-terminus, respectively. The nitro amino acid **7** was used in the solution-phase synthesis. Peptide **P3** was subjected to chemoselective azide−alkyne and nitrile oxide−alkyne 1,3-dipolar cycloaddition reactions separately. Monoconjugated product **P9** was isolated through selective 1,3-dipolar cycloaddition with *N*-Cbz-propargylamine with a nitro group in the presence of phenyl isocyanate and Et_3N. Separately, **P3** was also subjected to copper-catalyzed click reaction with phenylacetylene to obtain monoconjugated peptide **P10**. Finally, both **P9** and **P10** were separately subjected to cycloaddition reactions with phenylacetylene and *N*-Cbz-propargylamine, respectively, to obtain double-conjugated peptide **P11**. The selective cycloaddition reactions are shown in the Scheme 6. All three peptides were purified by RP-HPLC and characterized by ^1H NMR and mass spectral analysis.

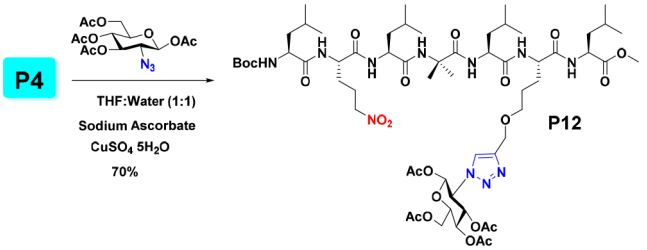
Scheme 6. Orthogonal Cycloaddition Reaction with Azide in Solution Phase



Finally, to understand whether the nitro group is compatible with alkyne functionality as well as alkyne−azide cycloaddition, we have synthesized peptide **P4** by incorporating both nitro and alkyne functionalities. Pure **P4** gave X-ray quality single crystals after slow evaporation in aqueous MeOH solution, and its X-ray structure is shown in Figure S2.

The peptide **P4** was subjected to copper(I)-catalyzed 1,3-dipolar cycloaddition reaction with 2-deoxyazido-1,3,4,6-tetraacetyl glucopyranoside in the presence of the nitro functionality on peptide. Selective azide−alkyne cycloaddition product **P12** was achieved without affecting the nitro functionality (Scheme 7). The peptide was purified by RP-HPLC and characterized by MALDI-TOF mass spectral analysis. These results suggest that alkynes can also be used along with the nitro alkane functional groups for orthogonal

Scheme 7. Orthogonal Cycloaddition Reaction with Alkyne in Solution Phase



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cycloaddition reactions. Nevertheless, to avoid intramolecular cyclization, the alkyne must be subjected to cycloaddition reaction before the nitro group.

In conclusion, we have demonstrated the synthesis and incorporation of nitroalkane-functionalized new amino acid into peptides and their chemoselective 1,3-dipolar cycloaddition reactions with alkynes through in situ generated nitrile oxide. The cycloaddition reaction between nitrile oxide and alkyne was found to be compatible with both solution- and solid-phase methods. More importantly, the nitrile oxide−alkyne cycloaddition is orthogonal to azide−alkyne and alkyne−azide cycloaddition reactions. In combination with azides and alkynes, the amino acid with primary nitroalkane side chain can be explored to functionalize small molecules and peptides. As phenyl isocyanate is required to transform the nitro group into nitrile oxide, reactive functional groups, particularly amines, must be protected during the nitroalkane−alkyne cycloaddition reactions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01498.

^{1}H and ^{13}C NMR spectra, mass spectra, synthetic details of amino acids and peptides, and crystallographic data of amino acid **4** and **P4** (PDF)

X-ray data for compound **4** (CIF)

X-ray data for compound **P4** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hn.gopi@iiserpune.ac.in.

ORCID

Hosahudya N. Gopi: 0000-0001-8156-9271

Notes

The authors declare no competing financial interest.

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