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Synthesis of Oligo-(alkyne-triplet)peptide Constructs

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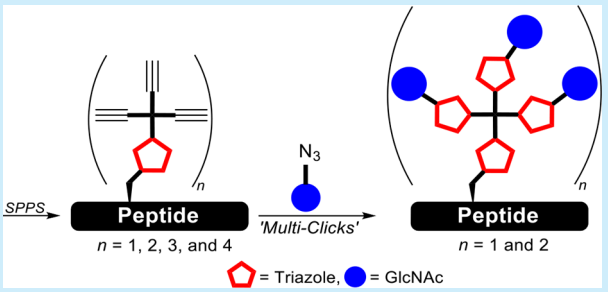
Supporting Information

ABSTRACT: Copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) click synthesis of an Fmoc-(trispropargyl)amino acid building block for solid phase peptide synthesis (SPPS) of oligo-(trialkyne)peptide constructs is reported. These can carry potentially indefinite numbers of inherent alkyne-triplets, which are click derivatized with GlcNAc-azide into the corresponding glycopeptides.



Multivalent interactions are of high interest and govern many interactions between proteins and its ligands on cell membranes.[1] A multivalent ligand is composed of a scaffold or backbone to which a particular number (valency) of identical or nonidentical epitopes are attached. Dimers have been shown to display up to more than thousand times higher inhibitory activities toward their respective receptors than their monovalent analogues.[2] Trivalency is involved in substrate binding of sialic acid to the influenza and adenovirus receptors[3] and β-galactose derivatives to the asialoprotein receptor.[4,5]

Strategies to obtain multivalent trimeric ligands can be performed via three consecutive copper(I) catalyzed 1,3-dipolar cycloaddition reactions between azides and alkynes and represent versatile building blocks as exemplified by recent methods for the synthesis of α-triazido keto compounds.[6] In a reverse click approach toward triple point assembly, trispropargyl amine has been used as the tripod branching system, thereby safely connecting three azido sugar entities.[3] However, utilization of this technique leaves no possibilities for further functionalization. 2-Amino-2-(hydroxymethyl)-1,3-propanediol has been used for a triple connection of three glyco-units,[7] affording constructs with a free amino group for further derivatization. The importance of the ability to generate multialkyne analogues with functional handles was highlighted by Papp et al.,[8] generating thiol functionalized quadruple sialic acid constructs for adhesions to gold nanoparticles. However, besides producing nonsymmetrical constructs, this method requires presynthesis of oligo-propargyl precursors with limited applicability for general constructions of multialkyne derivatives. Trisalkyne derivatives have huge potential in material science due to their cross-linking capabilities.[9]

The concept of mono-click functionalization of a tetrakisalkyne was first applied in peptide science[10] via the introduction of trisalkyne systems at a cysteine moiety situated at single chain variable fragments (scFv)[11] by thiol/maleimide conjugation. Introduction of trisalkyne units in peptides as direct extension of SPPS was performed[12] by the terminal coupling of a trisalkyne carboxylic acid succeeded by triple click forming a tetrapeptide construct, and more recently[13] analogously for synthesis of tris-glyco peptides by performing three final click attachments of the corresponding azido-glycans. The reported technologies for tripod-glycosylation of peptides are limited to introduction of only one single tris-glyco moiety at the *N*-terminal or by reversible attachment at a cysteine moiety by thiol/maleimide conjugation.

The introduction of oligo(trisalkyne) moieties in peptides used for click generation of heavily glycosylated constructs is an extension of our recently described procedures for *glycocalyxification* of giant unilamellar vesicles.[14,15] Thus, to gain access to trisalkyne molecular systems incorporated into a peptide sequence, preferably by SPPS, a strategy for developing the synthesis of an amino acid alkyne-derivative suitable for SPPS was required.

The present paper describes a facile procedure for the indirect oligo triplet-glycosylation of peptides by introduction of trispropargyl amino acid units in peptides. The key in this study was the development of an easily synthesizable Fmoc-chemistry compatible trisalkyne-containing Fmoc-amino acid, which could be used as a standard amino acid in SPPS succeeded by attachment of multiple azidoglycans by CuAAC[16] click chemistry[17] generating the title oligo-(triplet-glyco) peptides (Scheme 1). The versatility of the methodology was demonstrated by synthesis of tris- and hexakis(GlcNAc)peptide constructs.

Introduction of oligo-trisalkyne moieties into peptides required monofunctionalization of a tetrakisalkyne with azidopeptide derivatives. These must be protected and adaptable to peptide synthesis, for which artificial azido-lysine derivatives were considered appropriate, due to good compatibility with SPPS. The initial aim was therefore to design azido-amino acid constructs generating the most optimal

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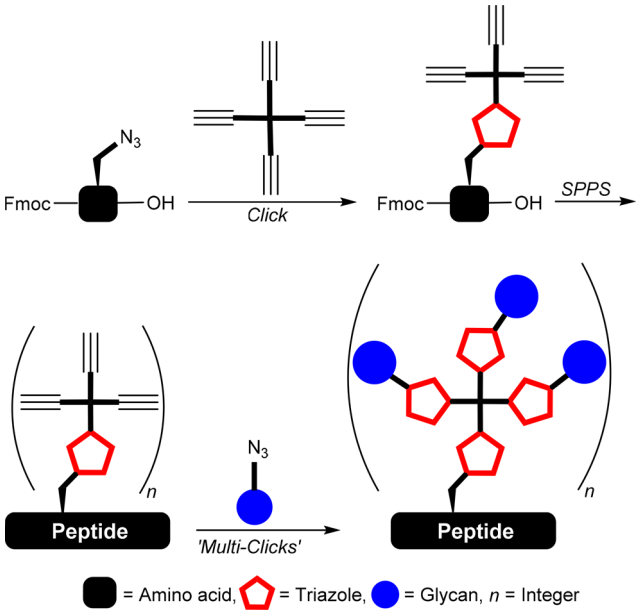
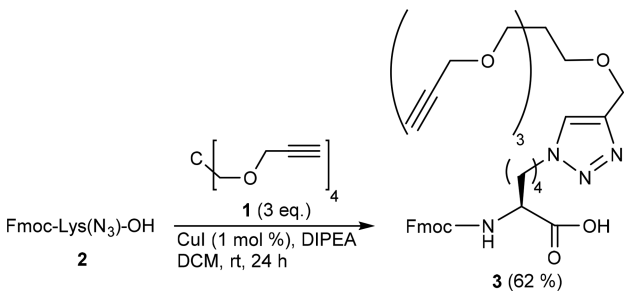
Scheme 1. General Strategy for the Generation of Oligo-(tris-alkyne)peptides by Incorporating an Fmoc-(trisalkyne)amino Acid as a Standard Amino Acid in SPPS Succeeded by Post-SPPS Click Introduction of e.g. Glycans



trisalkyne systems, with an emphasis on developing a trisalkyne Fmoc-amino acid derivative.

In a first direct solid phase approach for introduction of a tris-alkyne unit in amino acid derivatives, the resin bound peptide Fmoc-Lys(N_3)-Gly-2ClTrt-resin was reacted with **1**, in the presence of copper(I) iodide at room temperature, and the major products were intramolecularly dimerized Glaser diynes after standard acidic resin cleavage conditions (see Supporting Information). We then envisioned that copper(I)-catalyzed solution phase click reactions of azido-amino acid derivatives with an excess of **1** would generate isolable trisalkyne constructs free of Glaser byproducts by avoiding handling with a concentrated strong acid. During recent work on side-chain cholesterylation of amino acid derivatives, we developed a facile procedure[18] for side-chain click functionalization of Fmoc-amino acids mediated by CuI/DIPEA. By adapting these conditions, Fmoc-Lys(N_3)-OH **2** was smoothly reacted with 3 equiv of **1** forming a trispropargyl lysine moiety by copper(I) iodide catalysis in DIPEA/DCM, facilitating isolation of the symmetrical trisalkyne substituted Fmoc-amino acid **3** (Scheme 2).

Scheme 2. Synthesis of a Trispropargyl Lysine Containing Fmoc-Amino Acid



3 is the first example of a side-chain functionalized tris-alkyne amino acid derivative. Only by chromatography on new stationary dry silica columns eluting with dry methanol mixtures **3** was isolated in as high as a 62% yield, because application of old silica/wet methanol induced severe tailing due to the PEG-carboxylic acid characteristics of **3** manifested by dramatic lowering of the yield caused by a substantial amount of residual product stuck on the column. Glaser product formation was not observed presumably since only slight exposure to a dilute acidic aqueous environment was needed during quenching. Logically, the yield of **3** was lowered due to formation from basically statistical reactions with the highest probability for reaction between **1** and **2**, but with parallel truncating reactions for instance between **2** and **3**. The yield of **3** was improved by using an excess of **1**; however, above 3 equiv no further attenuation of the yield was observed. Utilization of the reagent couple $CuSO_4$/ascorbate[16] instead of CuI[18] did not seem to have a beneficial effect, since TLC revealed substantial quantities that were unreacted after 24 h. In terms of commercialization, besides the yield of **3** possibly being optimized by fine-tuning of chromatographic procedures, it is highly advantageous that the applied excess of **1** can be quantitatively separated in a pure fraction and recycled.

Introduction of regiopure trispropargyl moieties into peptides was achieved by utilizing **3** as a standard amino acid into an SPPS-sequence as an *N*-terminal extension of the peptide transportan 10 (TP10), which due to its reported GUV-membrane inserting properties[18] may be applicable as artificial GUV-glycocalyx components upon glycosylation. Trispropargyl containing **4a** could be obtained in satisfactory yield after cleavage from the resin with TFA−TES−H_2O (18:1:1). Due to absence of copper salts, Glaser alkyne-dimerization seemed completely absent, despite exposure to strong acidic conditions. In order to extend the scope for alkynylation of one peptide construct, introduction of multiple trisalkynes was attempted on TP10 by allowing consecutive HBTU couplings with **3**. No complications were observed during synthesis of a peptide with two, three, or four neighboring trispropargyl units **4b**−**4d**, respectively. CuAAC of **4a**/**4b** with azido-ethyl-GlcNAc afforded the (GlcNAc)-peptides **5a**/**5b** after HPLC-purification (Scheme 3).

Synthesis of the oligo-tris-alkyne peptides **4** was considered as valuable proof that the potential applications of **3** completely analogous to normal Fmoc-amino acids in SPPS had been fully explored also in terms of random insertion into peptides, since for instance the construct **4d** contains an alkyne triplet on the fourth amino acid position from the *N*-terminal. When aiming for densely glycosylated peptide constructs, easy installation of several neighboring alkyne-triplet units might be highly advantageous, and furthermore, because copper traces had been removed during presynthesis steps multialkyne peptides can be cleaved from resin under strong acidic conditions without formation of Glaser alkyne dimers.

The synthesized GlcNAc peptide constructs were then used in a lectin microarray, thereby proving the potential application as non-natural glycoconjugates. The three TP10 peptides bearing a GlcNAc-triplet **5a**, a mono-GlcNAc unit, and a non-glyco hexaalkyne system **4b**, respectively, were immobilized on NHS-activated glass slides.[19] After incubation with Cy3 labeled wheat germ agglutinin (WGA), the binding of the lectin specific binding to GlcNAc was evaluated by measuring the resulting fluorescence. The compound having no GlcNAc at all only showed a weak background signal, whereas the other two

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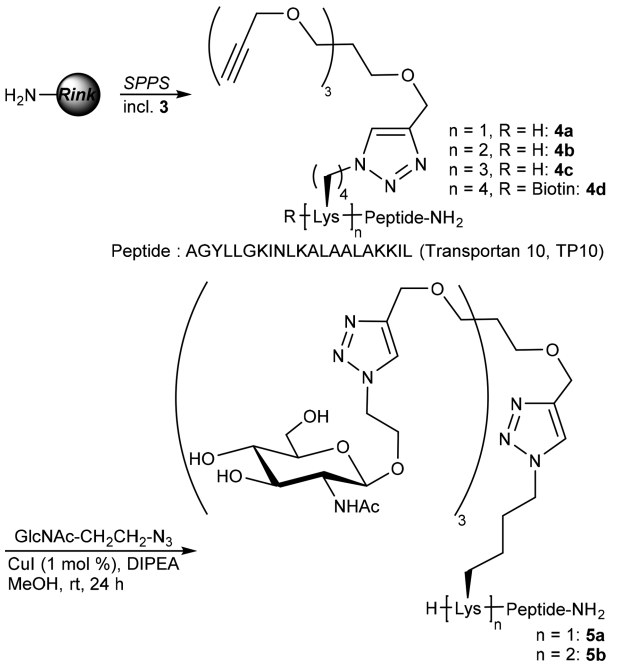
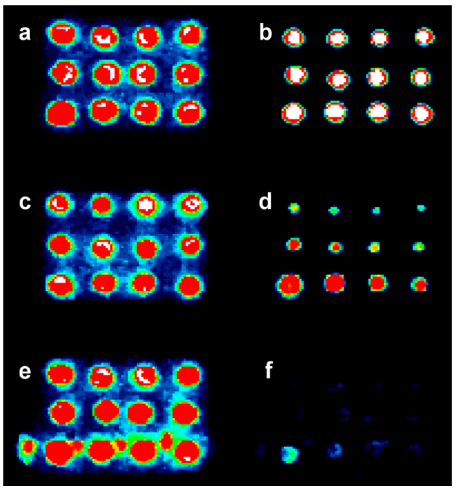
Scheme 3. Synthesis of Oligo-trispropargylpeptides Functionalized into Corresponding Oligo-GlcNAc-peptides by CuAAC Click Chemistry



conjugates showed a clear fluorescence signal that increased with the numbers of GlcNAc. This clearly demonstrated the applicability utilizing the oligo-trispropargylpeptides derived glycopeptide constructs as functional glycan mimetics (Scheme 4).

Scheme 4. Microarray WGA-lectin Binding Studies: (a, c, and e) GlcNAc-spacer-amine (See Supporting Information; Positive Controls); (b) *tris*-GlcNAc-TP10 5a; (d) *mono*-GlcNAc-TP10 (See Supporting Information); (f) Hexakis-alkyne-TP10 4b (*non*-Glyco Negative Control)



In conclusion, a trispropargyl substituted Fmoc-amino acid was synthesized and utilized as a standard amino acid in solid phase peptide synthesis (SPPS) generating oligo-(trialkyne)-peptides containing tris-propargyl moieties, ranging from tris-, hexakis-, nonakis-, and dodecakisalkynepeptide constructs. The corresponding tris- and hexakis-GlcNAc-peptide constructs were smoothly generated upon copper(I) catalyzed click attachment of a GlcNAc-azide derivative. Upon immobilizing a tris-GlcNAc-peptide on a glass slide array studies gave positive indications of lectin binding to GlcNAc-triplets.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03231.

Experimental procedures for all compounds including NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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