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Modular synthesis of photocleavable peptides using click chemistry

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ABSTRACT

A modular synthesis of photocleavable peptides was developed. Peptide based kinase substrates were modified on solid support with a traceless linker derived from 1-(2-nitrophenyl)propargyl alcohol and coupled to azide functionalized cell-penetrating peptides using Cu(I) catalyzed click chemistry.

Introduction

Because of its ease of use and orthogonality to numerous functional groups, the copper catalyzed azide alkyne cycloaddition (CuAAC), the most common example of click chemistry, has become a method of choice for linking complex molecules.[1,2] In addition to its widespread use with molecules of biological interest,[3,4] the reaction has also seen applications in areas such as polymer and surface chemistry.[5–7]

In some instances it is desirable to couple complex molecules through a linker that can be subsequently cleaved. Photocleavable linkers, which can be cleaved with spatial and temporal control, have been used to conjugate polyethylene glycols to proteins,[8] transiently link biotin to biomolecules,[9–11] create 'caged' fluorescent biomolecular probes,[12,13] attach a leader sequence to peptides,[14] control the sub-cellular localization of peptides,[15] attach mass spectrometry tags to bioanalytical probes,[16] and modify surfaces.[17–20]

We became interested in using a photocleavable click linker to transiently conjugate cell-penetrating peptides (CPPs) to bioactive peptides. CPPs can facilitate the transport of normally membrane-impermeant molecules.[21–23] Unfortunately, the CPP approach has drawbacks. CPP conjugates frequently become localized in sub-cellular compartments and in the cell membrane.[24–26] In addition, the presence of a CPP can alter or attenuate the activity of the attached cargo.[27,28] Typically these problems are addressed by conjugating the CPP to the molecule of interest with a cleavable linker such as disulfide. Once in the cell, disulfide is cleaved by the reductive environment of the cytoplasm. Although disulfide linkers can be effective, it would be desirable to release the bioactive molecule independently of the bioreductive capacity of a particular sub-cellular compartment and with the additional spatial and temporal control afforded by photo-induced cleavage. In addition, because the efficacy of CPPs is known to vary considerably between cell types, it is often necessary to prepare and evaluate multiple CPP-bioactive peptide conjugates.[29–31] Thus, a modular method for attaching CPPs to bioactive peptides is attractive.

Linker synthesis and model study

Click chemistry is ideal for the modular assembly of peptides. Previous reports demonstrated the non-modular synthesis of CPP-linked bioactive peptides and have shown the utility of CuAAC for the attachment of CPPs to bioactive molecules.[32–35]

The extension of this chemistry to the preparation of photocleavable peptides requires a readily available photocleavable linker. The ideal linker should be easily prepared, react readily with amines, and upon photocleavage be traceless with respect to the bioactive peptide. Recently several photocleavable click chemistry linkers have been reported but their preparations require multi-step syntheses.[8,10,12,14,36] We envisioned that 1-(2-nitrophenyl)propargyl alcohol **1**, which can be prepared in one step from commercially available starting materials,[37] could serve as a photocleavable linker. As shown in Scheme 1, treatment of 2-nitrobenzaldehyde with ethynylmagnesium bromide gave propargyl alcohol **1** in a 93% yield. Treatment of **1** with carbonyldiimidazole, afforded the amine-reactive imidazolide **2** with an isolated yield of 95%.

Before applying our method to peptide synthesis, we decided to demonstrate the feasibility of the approach with compounds that could be spectroscopically characterized as shown in Scheme 2.

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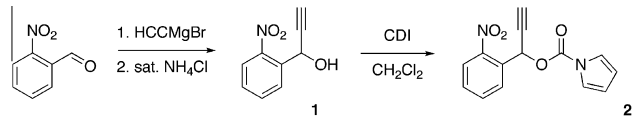
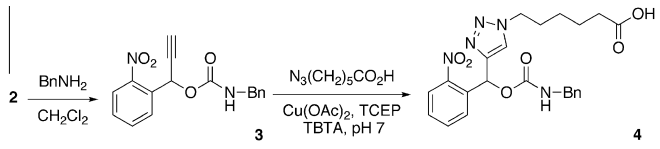
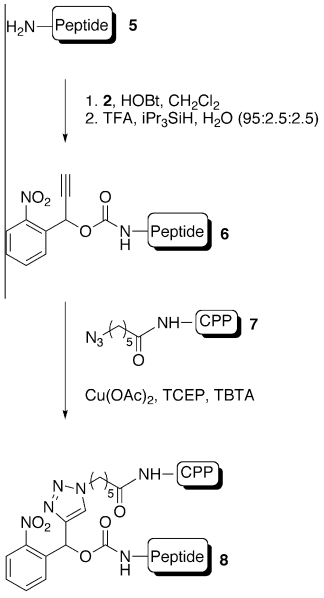


Scheme 1. Synthesis of photocleavable linker.



Scheme 2. Model study.



Scheme 3. Peptide coupling.

Reaction of **2** with benzyl amine gave carbamate **3**. Coupling of **3** with 6-azidohexanoic acid under click conditions gave **4** in a 70% yield. Interestingly, we saw no evidence of the by-products observed by Bertrand and Gesson with the click reaction of the structurally related dimethyl propargyl benzyl carbamate.[38]

Synthesis of photocleavable peptides

We applied our method to the modular synthesis of CPP-linked photocleavable bioactive peptides as shown in Scheme 3. The sequences of the peptides used are shown in Table 1.

We chose to link the cell-penetrating peptides TAT and $MyrK_4$ to peptide substrates for Abl kinase and CaM kinase II. Both TAT and $MyrK_4$ have been shown to transport the Abl kinase substrate into cells,[26] and TAT is known to be effective at transporting a CaM kinase II substrate across cell membranes.[32] Peptide substrates **5a** (Abl) and **5b** (CaM kinase II) were synthesized on Rink amide resin using Fmoc-solid phase peptide synthesis. The photocleavable linker was attached to the N-terminus by reacting the peptide of interest with imidazolide **2** and HOBt. Alternately, the linker-labeled peptide was prepared by generating compound **2** in situ from the reaction of **1** and carbonyldiimidazole, effectively giving a linker that could be prepared in one step from commercially available starting materials. Cleavage of the peptides from solid support and deprotection of the amino acid side chains gave the desired linker-modified peptides **6a** and **6b** as a mixture of diastereomers, which in the case of the CaM kinase II substrate could be partially resolved by HPLC.

Peptides **6a** and **6b** were each coupled to the azide labeled CPPs **7a** (TAT) and **7b** ($MyrK_4$) using copper catalyzed click chemistry to give photocleavable peptide conjugates **8a–d**.

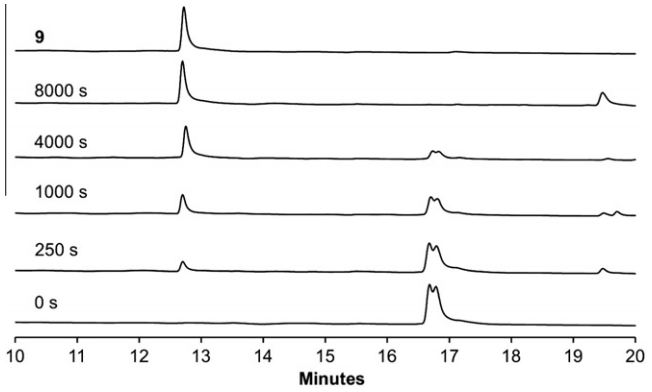


Figure 1. Photolysis of photocleavable peptide **8d**. Peptide was irradiated with UV light for the indicated period of time and analyzed by HPLC (monitored at 210 nm). Peaks at 16.68 and 16.78 min correspond to peptide **8d**; peak at 12.69 min corresponds to released peptide **9**; top trace, authentic sample **9**.

Table 1
Peptides prepared in Scheme 3

Entry	Peptide[a]	Cell-penetrating peptide[a] (CPP)	Yield[b] (%)	HPLC retention time (min)	Molecular formula	*m/z* calcd	*m/z* obsd
6a	EAIYAAPFAKKK (Abl)	N/A	28	16.14[e]	$C_{74}H_{108}N_{17}O_{19}$ $[M+H]^+$	1538.801	1538.917
6b	KKALHRQETVDAL (CaMKII)	N/A	46, 37[c]	9.23, 9.30[d,f] 14.46, 14.52[e,f]	$C_{75}H_{120}N_{23}O_{23}$ $[M+H]^+$	1710.893	1710.945
7a	N/A	YGRKKRRQRRR (TAT)	13	12.95[e]	$C_{70}H_{128}N_{35}O_{15}$ $[M+H]^+$	1699.033	1698.613
7b	N/A	K(ε-myristoyl)KKK ($MyrK_4$)	16	19.11[d]	$C_{44}H_{87}N_{12}O_6$ $[M+H]^+$	879.687	879.690
8a	EAIYAAPFAKKK (Abl)	YGRKKRRQRRR (TAT)	29	13.93[e]	$C_{144}H_{236}N_{52}O_{34}$ $[M+2H]^{2+}$	1618.9	1618.9
8b	EAIYAAPFAKKK (Abl)	K(ε-myristoyl)KKK ($MyrK_4$)	38	12.54[d]	$C_{118}H_{195}N_{29}O_{25}$ $[M+2H]^{2+}$	1209.2	1209.2
8c	KKALHRQETVDAL (CaMKII)	YGRKKRRQRRR (TAT)	44	13.38[e]	$C_{145}H_{250}N_{59}O_{37}$ $[M+3H]^{3+}$	1136.6	1136.6
8d	KKALHRQETVDAL (CaMKII)	K(ε-myristoyl)KKK ($MyrK_4$)	39	11.70, 11.84[d,f]16.68, 16.78[e,f]	$C_{119}H_{207}N_{35}O_{29}$ $[M+2H]^{2+}$	1295.290	1295.289
9	KKALHRQETVDAL	N/A	33	12.69[e]	$C_{65}H_{114}N_{22}O_{19}$ $[M+H]^+$	1507.87	1507.86

[a] Peptides C-terminal amidated.
[b] Based on the weight of HPLC purified, lyophilized powder.
[c] In situ generation of **2**.
[d] HPLC gradient A: 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B); 95% A, 2 min; 95% A to 20% A, 20 min; flow rate = 2 mL/min; Jupiter Proteo column (4μ, 150 × 4.6 mm).
[e] HPLC gradient B: gradient C 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B); 100% A, 4 min; 100% A to 20% A, 20 min; flow rate = 1 ml/min; Jupiter Proteo column (4μ, 150 × 4.6 mm).
[f] Mixture of diastereomers.

Exposure of the coupled peptide **8d** to UV light resulted in the release of free kinase substrate peptide **9** as shown in Figure 1. To be biologically useful, the conjugated peptides must also be stable in the absence of light. This was assessed by preparing a 40 μM solution of **8d** in PBS buffer. HPLC analysis of this solution showed no decomposition after 48 h.

In summary, we have developed a simple photocleavable linker for the modular synthesis of photoreleasable peptides using click chemistry. Our synthetic approach is facile and is readily applicable to the preparation of homologous photocleavable linkers (e.g., nitroveratryl) with commercially available aldehyde precursors. Although applied here to the conjugation of azide labeled CPPs to peptides, the method can easily be extended to other biologically useful azide functionalized probes such as biotin and fluorescent dyes.

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Supplementary data

Supplementary data (experimental details for the preparation of compounds **1**–**9**, NMR spectra of compounds **1**–**4** and HPLC chromatographs of compounds **6a**–**9**) associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.002.

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