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Photochemical cleavage of leader peptides†,‡

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Abstract

We report a photolabile linker compatible with Fmoc solid phase peptide synthesis and Cu(I)-catalyzed alkyne–azide cycloaddition that allows photochemical cleavage to afford a C-terminal peptide fragment with a native amino terminus.

The recent explosion of fully sequenced genomes has resulted in the discovery of over a dozen different classes of peptide-derived natural products that are DNA encoded.¹ After peptide synthesis on the ribosome, extensive posttranslational modifications morph these peptides into their active form. In almost all known examples, an N-terminal leader peptide guides at least part of these posttranslational modifications, but the leader peptide must be removed from the core peptide in the final step of maturation to produce the active natural product (*e.g.*, Scheme 1).¹ *In vitro* reconstitution of the activities of a growing number of the biosynthetic enzymes of these natural products² has started to be exploited for the generation of analogs by incorporation of non-proteinogenic amino acids into the peptide substrates for posttranslational modifications.³ However, the final removal of the leader peptide usually poses a major hurdle because the proteases that perform these steps are often membrane associated as part of ATP-binding cassette transporters⁴ or sometimes have not been identified at all. An alternative, general method for the removal of the leader peptides would therefore be valuable.

The incorporation of 2-nitrophenyl or 2-nitroveratrole groups at appropriate sites in peptides and proteins has allowed the site-specific light-mediated cleavage of the polyamide backbone. For instance, introduction of phenylglycine derivative **1** in ion channels allowed their temporal photo-activation.⁵ Other examples of linkers that allow photo-lytic scission of peptide chains are the commercially available β -amino acid **2**⁶ and a series of linkers incorporating an aryl group in the peptide chain, exemplified by **3**.⁶ These linkers are very effective but are less attractive for leader peptide removal as they would result in core peptides carrying nitrosoaryl groups (*e.g.* 9–11, Scheme 2). We therefore designed linker **4**, which was envisioned to generate the native core peptide **12** upon photolysis.

The synthetic route towards a reagent that could introduce linker **4** into peptides is depicted in Scheme 3A. Important design criteria were compatibility with Fmoc solid phase peptide synthesis (SPPS) and with our previously developed ligation strategy that links the leader peptide with the core peptide using a copper-catalyzed [2+3] cycloaddition⁷ between a

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leader peptide with a C-terminal alkyne and a core peptide with an N-terminal azide.³ Compound **17** fulfills both criteria as demonstrated herein. A related photolabile linker incorporating an azide was very recently reported that requires a more lengthy synthesis and that was not used for SPPS.⁸

Linker **17** was efficiently synthesized starting from commercial diol **13**. Activation with *p*-toluenesulfonyl chloride was followed by protection with trimethylsilyl (TMS) chloride to give **15**. The *p*-toluenesulfonate group of **15** was then displaced with sodium azide. TMS protection was required to prevent epoxide formation and subsequent azide addition to the benzylic carbon. Fortuitously, under the displacement reaction conditions the TMS protecting group was slowly removed affording secondary alcohol **16**. This compound was then reacted with disuccinimidyl carbonate to form activated linker **17**. Compounds **16** and **17** were analyzed by differential scanning calorimetry to ensure their safety under the conditions used (see ESI[†]).

To investigate the compatibility of linker **17** with Fmoc SPPS, a model peptide with the sequence TALSG (**18a**, Scheme 3B, Xxx¹ = Thr) was assembled on pre-loaded Gly-Wang resin using standard Fmoc methodology with *O*-(1*H*-6-chlorobenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluoro-phosphate (HCTU) and *N*-methylmorpholine (NMM) as coupling agents. The peptide was reacted with linker **17** and the azide functionality was reduced *in situ* with tri-*n*-butyl-phosphine, followed by coupling with Fmoc-Ala-OH to give peptide **20a** (Xxx² = Ala). Standard peptide elongation steps were followed by peptide cleavage from the resin using 95% trifluoroacetic acid and HPLC purification producing **21a**. The purified peptide was then irradiated at 365 nm and the photolysis reaction was complete within 1.5 h as judged by HPLC and MS (Fig. 1; Fig. S1, ESI[†]).

In this and other studies⁹ it was observed that photo-lysis was accompanied by the formation of various strongly absorbing decomposition products arising from the nitrosoaryl group (Fig. 1). In the current design, however, these side products are not part of the desired C-terminal peptide fragment **23** as was confirmed by mass spectrometry.

The linker was incorporated next into a series of model peptides in which the amino acids preceding and following the linker were varied (Table 1). Upon photolysis, the desired C-terminal peptide was obtained successfully and in good yields and purities after HPLC in all cases (Table 1; Fig. S2–S17, ESI[†]).

We next sought to apply the methodology to the preparation of the posttranslationally modified peptide natural product lactacin 481. The biogenesis of lactacin 481 commences with the ribosomal synthesis of the precursor peptide LctA. Subsequently, the bifunctional enzyme LctM dehydrates a series of Ser and Thr residues located in the core peptide of LctA to generate dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues, respectively (Scheme 1).¹⁰ LctM also catalyzes the regio- and stereoselective intramolecular Michael-type addition of Cys residues to the dehydro amino acids, resulting in lanthionine and methyllanthionine crosslinks. In the last step of the biosynthesis the protease domain of the transmembrane transporter LctT proteolytically removes the leader peptide.¹¹ Whereas LctM activity has been reconstituted *in vitro*, previous studies utilized the commercial protease Lys-C to remove the leader peptide, which produces Δ1-lactacin 481 lacking the N-terminal Lys.^{10,12}

[†]Electronic supplementary information (ESI) available: General experimental procedures. Data and spectra for new compounds. See DOI: 10.1039/c0cc02945a

To evaluate whether light-mediated leader peptide removal could yield full length lactacin 481, the core peptide of lactacin 481 was synthesized on pre-loaded Wang resin by SPPS and linker **17** was added (Scheme 4A). After cleavage from the resin and HPLC purification, peptide **25** was used for copper catalyzed [2+3] cycloaddition with the leader peptide carrying a C-terminal alkyne group (**26**). The resulting substrate analog was incubated with LctM resulting in the anticipated four dehydrations (Scheme 4B). Subsequent photolysis afforded mature lactacin 481 (see ESI[†]). Thus, this methodology can be utilized for the preparation of native lactacin 481 and can be extended to the preparation of analogs using previously published procedures.³ This methodology adds one linear step (the addition of linker **17**) compared to our previous approach using a protease to remove the leader. However, unlike the protease approach with LysC, light-mediated removal of the leader peptide is not sequence dependent and provides the full length natural product, providing a more general solution. It is anticipated that this general methodology may also find use for other lantibiotics, and potentially other classes of ribosomally synthesized and posttranslationally modified natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. HPLC of peptide **21a** before (red) and after (blue) photolysis. Linker **17** contains a stereogenic center and was prepared in racemic form, resulting in two diastereomers of peptide **21a**. The two product peaks labeled with an asterisk are breakdown products of **22a**.

Scheme 1.

Biosynthesis of lacticin 481. The synthetase LctM transforms a linear precursor peptide LctA into a polycyclic structure. The transporter LctT then removes the leader peptide and secretes the product lacticin 481.

Scheme 2.

Photochemical cleavage of 2-nitrophenyl derivatives (**1–4**) into N- and C-terminal fragments. For the purpose of this work, R¹ would represent the leader peptide and R² the core peptide.

Scheme 3.

(A) Synthesis of photolabile linker 17. TsCl, 4-toluenesulfonyl chloride; TMSCl, trimethylsilyl chloride. (B) Synthesis of decapeptides **21a-i** followed by photolysis to produce peptides **22a-i** and **23a-i**. DIPEA, diisopropylethylamine; HOBt, hydroxybenzotriazole; DIC, diisopropylcarbodiimide.

Scheme 4.

(A) Copper catalyzed azide-alkyne cycloaddition reaction between LctA(leader) and LctA(core) followed by enzymatic modification by LctM and photolysis to afford lactacin 481. (B) MALDI-TOF MS of the peptide before (blue) and after (red) the LctM assay. For core and leader sequences, see Scheme S1, ESI.[†] TBTA = tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine.

Synthesis and photolysis of peptides **21a-i** using a variety of amino acids flanking the photolabile linker

Table 1

Yield (%)				
	Xxx1	Xxx2	21 ^a	23 ^b Per step ^c
a	Thr	Ala	43	70 94
b	Val	Ala	21	72 90
c	Lys	Ala	27	87 93
d	Gly	Gly	39	83 94
e	Leu	Leu	44	61 93
f	Tyr	Tyr	16	83 90
g	Pro	Pro	17	81 90
h	Trp	Trp	14	70 83
i	Glu	Glu	21	91 92

^a Isolated yields based on resin loading.
^b Isolated yields after photolysis and HPLC based on **21**.
^c Yields based on resin loading.