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Dehydropeptides from Orthogonal Ligation of Unprotected Peptides

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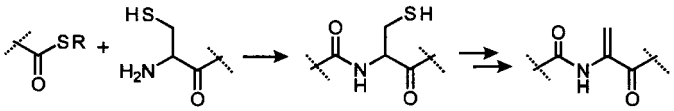
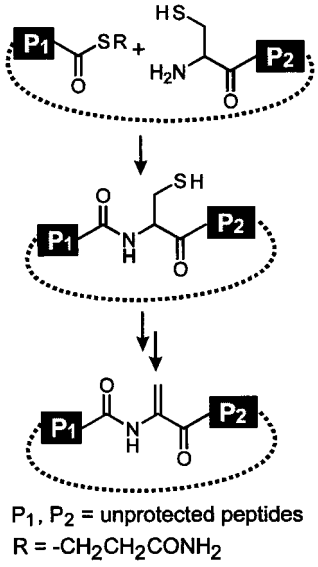
ABSTRACT



A facile method has been developed to synthesize linear and cyclic dehydropeptides from unprotected peptide precursors. This method exploits an N-terminal Cys for a Cys–thioester ligation to generate an unprotected peptide and as a precursor for conversion to ΔAla by *β*-elimination under mild conditions.

Dehydropeptides containing α,*β*-dehydroamino acids are found in naturally occurring peptide antibiotics, toxins, and enzymes.[1−4] Moreover, they are versatile intermediates because the dehydro moieties can be readily transformed into various unusual amino acids such as lanthionine, lysinoalanine, and *β*-heterocyclic alanines through intra- or intermolecular nucleophilic Michael addition.[1,2,5] Direct stepwise synthesis of dehydropeptides, particularly those containing dehydroalanine (ΔAla), is limited by poor coupling yields of the dehydroamino acids, the instability of the N-terminal ΔAla, and their susceptibility to nucleophiles during synthesis.[6,7] Most syntheses of dehydropeptides are based on the *β*-elimination of modified Cys, Ser, or Thr from protected peptides.[8,9] In this Letter, we report a facile method for synthesizing linear and cyclic dehydropeptides from unprotected peptide precursors. This method utilizes a Cys−thioester ligation[10,11] to generate an unprotected peptide with a Cys at the ligation site as a precursor for conversion to ΔAla by *β*-elimination under mild conditions (Scheme 1).

Scheme 1. Dehydropeptide from Cysteine−Thioester Ligation



Our method was tested in the syntheses of both linear and cyclic dehydropeptides. The unprotected peptide segments

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P_1 bearing a C-terminal thioester were directly synthesized from a thiol resin,[12] while segments P_2 carrying an N-terminal Cys were obtained from a benzyl ester resin using Boc/Bzl chemistry. The linear cysteine-containing precursor was obtained through an intermolecular cysteine−thioester ligation of a P_1 segment and a P_2 segment. For cyclic peptides in which P_1 and P_2 are connected as a single peptide segment, the cyclization was effected by intramolecular Cys−thioester ligation. The ligation was generally performed in reducing aqueous conditions buffered at pH 7 to 8 with a low molecular weight thiol such as 2-mercaptoethanesulfonic acid sodium salt as an additive.[10,11,13] Under these conditions, the ligations proceeded cleanly and quickly in excellent 90−95% yields with examples consisting of one linear (entry 5, Table 1) and four cyclic peptides ranging from 5 to 14 residues (entries 1−4, Table 1).[14]

Table 1. Summary of Cysteine−Thioester Ligation

no.	$P_1 + P_2$[a]	product[b]	size (aa)	yield (%)
1	CAGFY-X	c[CAGFY]	5	90
2	CSLKLNG-X	c[CSLKLNG]	7	92
3	CKYSSRGISWSYL-X	c[CKYSSRGISWSYL]	14	91
4	CKYSSRGICWSYL-X	c[CKYSSRGICWSYL]	14	91
5	SLKLNG-X + CNSFRY	SLKLNGCNSFRY	12	94

[a] X = $SCH_2CH_2CONH_2$. [b] Ligation sites are underlined.

Two methods were used to convert the newly formed cysteine residue at the ligation site to a dehydroamino acid (Scheme 2). Both methods involved transforming the thiol functionality into a leaving group followed by a β-elimination. In method A, the cyanation of the thiol group with 3−5 equiv of 1-cyano-4-dimethylaminopyridium tetrafluoroborate (CDAP) in a 0.1 M acetic acid solution for 15−20 min at 0 °C transformeded the thiol into -SCN under acidic conditions.[15] The S-cyanated products obtained in >95% yield were stable under conditions for HPLC purification and lyophilization. These products then underwent β-elimination to form an α,β-dehydroalanine in 5−10% DIEA/DMF to afford cyclic and linear ΔAla-containing peptides in 64−79% yields based on the unprotected peptide precursors (Table 2). This method could be also used for a dehydropeptide containing two cysteine residues such as cyclo[ΔKYSSRGIΔWSYL] (entry 4, Table 2). The β-elimination reaction was best performed in nonaqueous conditions. The starting materials and the ΔAla-containing products are susceptible to alkaline aqueous conditions, which lead to intramolecular cleavage of the Xaa−Cys or hydrolysis and aminolysis of the Xaa−ΔAla bond.[15,16] A similar approach using a 2,4-dinitrophenyl fluoride derived thiol functionality as a leaving group to convert Cys to ΔAla would also be suitable.

Scheme 2. Conversion of Cys Residue to ΔAla

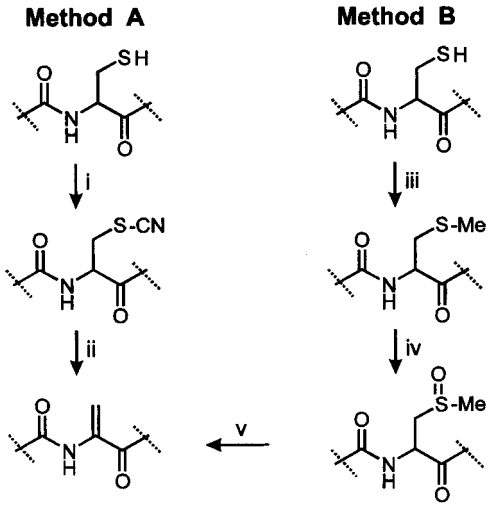

Table 2. Summary of Conversion of the Cysteine Residue in a Precursor to a Dehydroalanine Residue in a Product

no.	precursor[a]	product[b]	method	yield[c] (%)
1	c[CAGFY]	c[ΔAGFY]	B	83
2	c[CSLKLNG]	c[ΔSLKLNG]	A	79
3	c[CKYSSRGISWSYL]	c[ΔKYSSRGISWSYL]	A	76
4	c[CKYSSRGICWSYL]	c[ΔKYSSRGIΔWSYL]	A	64
5	SLKLNGCNSFRY	SLKLNGΔNSFRY	A	70
6	KPVSLSYRACGG[d]	KPVSLSYRAΔGG	A	72

[a] C, ligation site. [b] Δ, dehydroalanine. [c] Total yield from precursor to product. [d] From solid-phase synthesis.

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(14) A typical procedure for the synthesis of linear peptide through Cys−thioester ligation was accomplished using equivalent P_1 and P_2 segments (4 μmol of each segment) in 1 mL buffers at pH 7.6 containing 6 M Gua·HCl and 10 equiv of 2-mercaptoethanesulfonic acid sodium salt. The ligation was completed in 3 to 20 h at rt and monitored by HPLC. For synthesis of cyclic peptide, the concentration was diluted to 20 times by the buffers.

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Method B utilizing sulfoxide for β-elimination has been used to synthesize protected ΔAla-containing peptides.[8b,9] The three-step reaction (iii to v), methylation, oxidation, and β-elimination, was performed in organic solvents or organic−aqueous mixtures. The major difference between methods A and B is the alkylation condition, the former is under acidic whereas the later is under basic conditions. This method was employed to synthesize a water-insoluble ΔAla-containing peptide (entry 1, Table 2). The S-methylation in 30% DMF and 70% aqueous buffers at pH 8 with a 20-fold excess of iodomethane at rt for 2 h gave the S-methylated compound in 95% yield. Oxidation of thioether to sulfoxide with 2 to 3 equiv of sodium periodate was achieved in 98% yield in H_2O−dioxane (1:1, v/v) for 4 h at 40 °C. The β-elimination of the sulfoxide with 5% DBU in methanol afforded an 87% yield of the desired cyclic ΔAla-containing pentapeptide, cyclo[ΔAGFY]. It should be noted that Met- or N-terminal Ser-containing peptides are susceptible to the periodate oxidation in method B.[8b,18] All the cyclic and linear dehydropeptides from both methods A and B were confirmed by MALDI-TOF MS, HPLC, and UV spectroscopy.

In conclusion, we have developed a facile method to synthesize linear and cyclic ΔAla-containing peptides from unprotected peptide precursors by Cys−thioester ligation. This procedure has the advantage of using the thiol side chain of an N-terminal Cys for the dual purposes of ligation and conversion to a ΔAla moiety. It may be further utilized as a Michael acceptor of thiol nucleophiles to further elaborate the side chain.[9,19]

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