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A new ligation method for N-terminal tryptophan-containing peptides using the Pictet–Spengler reaction

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Abstract

Application of a carbon–carbon bond forming reaction for ligating unprotected peptides in acetic acid is described based on a Pictet–Spengler condensation. The peptide segments include a peptide with a Trp at the N-terminal and another with a C-terminal aldehyde, which can be generated through a new cyclic acetal resin.

A recent important advance in peptide chemistry, since the introduction of solid phase peptide synthesis by Merrifield,[1] is the development of orthogonal ligation methods. These ligation methods provide a powerful strategy to synthesize those macromolecules[2] which are difficult to obtain by conventional peptide synthesis and recombinant DNA technology. The chemical basis of the ligation is a regiospecific coupling reaction between a C-terminal electrophile on one peptide and a N-terminal nucleophile on another peptide, without any protection or activation step. The peptide segments can be synthetic[2a–d] or biosynthetic in origin.[2e–f] Many organic reactions have been utilized in ligation via a variety of chemical linkages, such as amide,[3] thioester,[4] thiazolidine,[5] oxaproline,[6] oxime,[7] hydrazone,[8] and thioether.[9] In these cases, the N-terminal nucleophile is either a weak base or 1,2-dinucleophile including Cys, Ser or Thr. It is highly desirable to develop new methods that utilize other N-terminal amino acids to form a stable chemical linkage between two peptide segments.

We were interested in applying the Pictet–Spengler reaction to peptide ligation using peptide segments containing an aldehyde at the C-terminal and a Trp at the N-terminal (Scheme 1). The Pictet–Spengler reaction is an acid-catalyzed intramolecular condensation between an iminium ion and an aromatic C-nucleophile.[10] This reaction has been used extensively for the synthesis of isoquinoline and indole alkaloids. It has been shown that tryptophan analogues react with organic aldehydes[11] or amino ester aldehydes[12] to give tetrahydro-β-carboline derivatives. The main advantage of this reaction is the formation of a product with a stable C–C bond in one single step. In this report, we describe the synthesis

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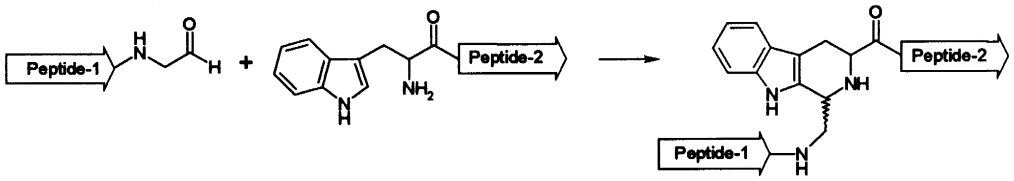
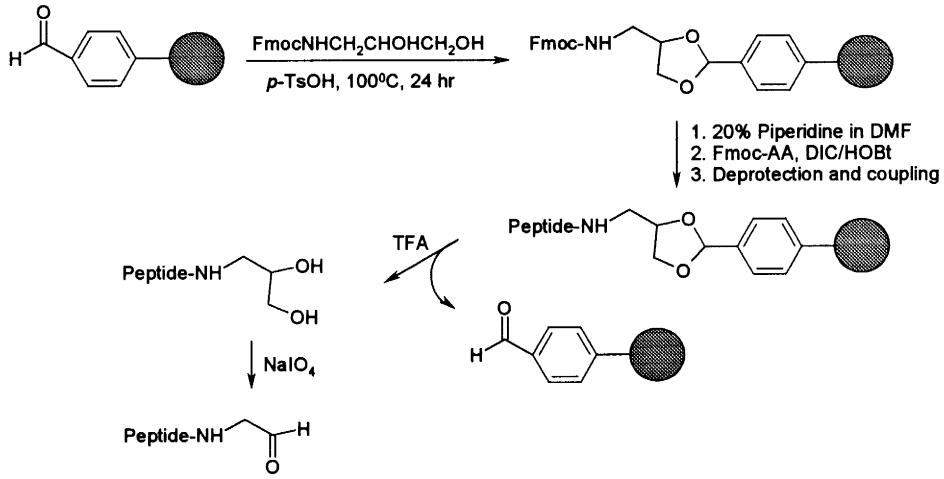
of peptide aldehydes through a new cyclic acetal resin, and the preliminary results of peptide ligation based on the Pictet–Spengler reaction.



Scheme 1. Ligation of unprotected peptide segments using the Pictet–Spengler reaction

The synthesis of unprotected peptide C-terminal aldehydes is shown in Scheme 2. An important step in the strategy is the development of a new functionalized solid support, Fmoc-NH-CH_2-cyclic acetal resin. This resin was prepared by refluxing benzaldehyde-polystyrene resin (1.0 mmol/g)[13] with five molar excess of Fmoc-3-amino-1,2-propanediol in 1,4-dioxane, with a catalytic amount of *p*-toluenesulfonic acid. The final loading of the resin was 0.53 mmol/g, corresponding to a yield of 68%. After the Fmoc group is removed, the peptide can be synthesized using standard Fmoc chemistry. The desired aldehyde was generated subsequently by periodate oxidation of the peptide precursor containing a C-terminal aminopropanediol moiety in solution.[2a,14] Several methods for the synthesis of other peptide C-terminal aldehydes have recently been reported.[7b,c]



Scheme 2. Synthesis of peptide C-terminal aldehyde through Fmoc-NH-CH_2-cyclic actel resin

We prepared a peptide Glu-His-Ala-Asp-Leu-Leu-Ala-$NHCH_2CHO$ as a model, which contains an N-terminal α-amine and an internal histidine. The sequence was assembled on Fmoc-NH-CH_2-cyclic acetal resin using DIC/HOBt activation, and the ninhydrin test[15] was used to ensure each coupling step was complete. The side chain deprotection and cleavage from the resin was achieved with TFA:TIS:thioanisole:water (94:2:2:2) for 30 min. Subsequently Glu-His-Ala-Asp-Leu-Leu-Ala-$NHCH_2CHOHCH_2OH$ was precipitated from diethyl ether. Oxidation of the 1,2-propanediol moiety in the peptide with $NaIO_4$ in 20% acetic acid for 2 min gave the desired peptide aldehyde **1** in quantitative yield. The crude product was purified by RP-HPLC to remove byproduct formaldehyde and excess $NaIO_4$. As shown in Table 1, two N-terminal Trp peptide segments **2** and **4** and one N-terminal Ala peptide **6** were synthesized using standard Fmoc chemistry on trityl chloride resin. The identity of these peptides was confirmed by correct molecular masses observed on LC/MS analysis.

Table 1
Peptide segments used in the ligation and MS analysis of the ligation products

Peptide aldehyde	NT-Trp peptide segment	Product	Yield[a] (%)	ESI/MS $[M+H]^+$ Calcd	Found
Glu-His-Ala-Asp-Leu-Leu-Ala-$NHCH_2CHO$, **1**	Trp-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro, **2**	**3**	95	2146.4	2146.0
1	Trp-Ala-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro, **4**	**5**	95	2217.5	2217.1
1	Ala-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro, **6**	-	0	-	-

[a] Yield was based on HPLC.

The N-terminal Trp peptide ligation was performed in glacial acetic acid and followed by HPLC analysis.[16] Acetic acid is an excellent solvent for dissolving unprotected peptide building blocks. Since the Pictet–Spengler reaction has been mainly carried out using acids such as TFA as catalyst,[11,17] we initially investigated the effect of TFA concentrations (1, 2, and 5%) on the ligation reaction. We found that the addition of TFA did not increase the ligation rate. The use of a polar organic co-solvent such as DMF or DMSO decreased the rate of ligation. In aqueous acetic acid (1:1, v/v), no observable ligation product was formed. These results suggest that acetic acid alone is the solvent of choice for this ligation, and it has been used for our subsequent experiments. Similar conditions have been reported for the Pictet–Spengler reaction of *N*-β-(3-indolyl) ethyl amino ester with aromatic aldehydes to afford tetrahydro-β-carboline derivatives.[18]

When the peptide aldehyde **1** was mixed with a N-terminal Trp-containing peptide segment in acetic acid, HPLC analysis showed the decrease of the peaks from two starting materials with the concomitant formation of the ligation product. Fig. 1 shows HPLC-monitored ligation progress of the aldehyde with **2**, Trp-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro. After 1, 3 and 12 h, the ligation product **3** was formed in 60, 74 and 90% yield, respectively. The ligation reaction was complete in 24 h. The ligation product has a newly formed chiral center at the C-1 carbon of the tetrahydro-β-carboline, and the asymmetric nature of the peak reflects the formation of diastereomeric isomers. The ligation with another N-terminal Trp peptide **4**, Trp-Ala-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro, showed a similar ligation profile and gave the ligation product **5**. The purified products gave molecular masses which are in good agreement with the calculated values (Table 1). A control experiment was also carried out under the same conditions using an N-terminal Ala peptide **6**, Ala-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro. The HPLC peak from this peptide segment remained unchanged after 1 week without any observable ligation product, suggesting the necessity of a Trp at the N-terminal peptide segment for this ligation. The internal Trp residues in peptide segments **2** and **4** were not affected by the ligation reaction.

In conclusion, we have developed a new method for ligating N-terminal Trp peptides in acetic acid, a condition compatible for chemoselective orthogonal ligation of peptides and proteins. However, this strategy differs from other known ligation methods to give a product with an irreversible tetrahydro-β-carboline linkage. This method should be useful for the construction of artificial proteins, circular proteins and peptide–protein conjugates. This single step ligation also provides an attractive approach for the generation of protein libraries from closely related protein segments for structure–activity relationship studies. Since Trp residues are abundant in natural occurring peptides and proteins, and N-terminal Trp protein segment can be easily obtained from expression systems, such a ligation strategy would expand the current repertoire of ligation methods.

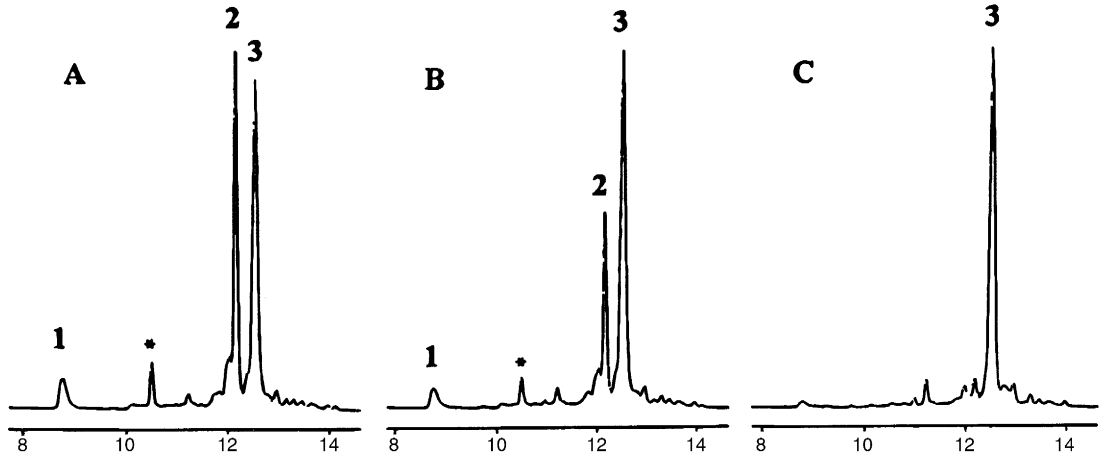


Fig. 1. RP-HPLC-monitored progress of the ligation after 1 h (A), 3 h (B), and 24 h (C). **1**, Glu-His-Ala-Asp-Leu-Leu-Ala-$NHCH_2CHO$; **2**, Trp-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro; **3**, the ligation product. The peak marked with * is the product due to intermolecular Schiff base formation from **1**. The formation of Schiff base was reversible and this peak decreased with the formation of the ligation product. HPLC was performed on a Vydac column (250×4.6 mm) at a flow rate of 1 ml/min (buffer A, 0.05% TFA in H_2O; buffer B, 0.05% TFA in 60% CH_3CN in H_2O). Gradient: 10 to 100% B within 15 min. UV monitoring at 220 nm

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16. General procedure for peptide ligation: 1.5 mg of Glu-His-Ala-Asp-Leu-Leu-Ala-$NHCH_2CHO$ **1** (1.8 mmol) dissolved in 0.2 mL of HOAc was mixed with 2.0 mg of Trp-His-Trp-Ser-Tyr-Leu-Leu-Arg-Pro-Pro **2** (1.5 mmol) in 0.2 mL of HOAc at ambient temperature. The ligation was followed by RP-HPLC and completed in 24 h; ESI/MS was performed by HP-1100.
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