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Practical synthesis of peptide C-terminal aldehyde on a solid support

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We have investigated practical synthetic routes for the preparation of peptide aldehyde on a solid support. Peptide aldehyde was synthesized via efficient transformation of acetal/thioacetal structures.

Peptide with C-terminal aldehyde is of interest due to its property as a transition-state analogue toward numerous classes of proteolytic enzymes; aspartyl and cysteine protease are inhibited by peptide aldehyde. Since leupeptin is produced by actinomycetes, which inhibit a variety of proteases potently, natural peptide aldehydes are attractive targets for drug discovery. Peptide aldehyde can also be used as a key intermediate in the syntheses of pseudo-peptides, particularly in the synthesis of reduced peptide via reduced fragment condensation. Several methods for solid phase synthesis of peptide aldehyde have been reported: reduction of Weinreb amide, oxidation of alcohol, or ozonolysis of the corresponding olefin. However Weinreb amides and ozonolysis are limited to peptide aldehydes without reductant or oxidant labile amino acid sequences. On the other hand, acetal linker is stable in TFA. When the resin was treated with 95% TFA/H2O, the desired peptide aldehyde was obtained in poor yield. We thought that the peptide acetal containing commercially available alkyl triols by solid phase synthesis, transformed peptide thioacetal (C) by treatment with EtSH in the presence of catalytic Lewis acid. As a final step, the thioacetal (C) thus obtained could be treated with NBS in 10% hexane-1,2,6-triol and 10 mol % BF3–Et2O in CH2Cl2 at room temperature for 3 h obtained the desired hydroxyl acetal (2b) in 34% yield (entry 2).

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After attempting several conditions, we optimized the condition with 1.0 equiv of hexane-1,2,6-triol and 5 mol % BF$_3$–Et$_2$O for 5 h to give 2b in 73% yield (entry 5). While the reaction mixture using CH$_2$Cl$_2$ to give 2c was a suspension because of the insolubility of trimethylolethane (entry 6), THF was effective to enable the acetalization of 1 in 86% yield (entry 7) (Table 1).

After the oxidation of 2b by Jones’ condition, carboxylic acid (3b) with DIPEA in DMF was loaded on 2-chloro trityl chloride resin to give 4. After the conventional Fmoc-solid phase peptide synthesis of 4 using diisopropylcarbodiimide/1-hydroxybenzotriazole coupling and Fmoc deprotection by 20% piperidine/DMF, the treatment of resin (5) with 95% TFA gave the desired peptide aldehyde, Ac-Val-Leu-Ala-H (6) and acetal carboxylic acid (7) in 8% and 32% yields, respectively. We found that the acetal carboxylic acid (7) was smoothly converted to thioacetal (8) in 68% yield by treatment with 10 equiv of EtSH and catalytic BF$_3$–Et$_2$O for several minutes at room temperature. Subsequently, peptide aldehyde (6) was quickly obtained from thioacetal (8) by NBS in 10% CH$_2$Cl$_2$aq in 85% yield. These results suggested that the cheap hexane-1,2,6-triol linker compared favorably with Yao’s octane-1,2,10-triol linker and, furthermore, it was possible to convert acetal to thioacetal quickly. On the other hand, acetal (3c) oxidized from 2c was attempted

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\text{Scheme 1. Synthetic plan for peptide aldehyde via thioacetal.}
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\text{Table 1}
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| Entry | Triol linker (equiv) | Solvent | BF$_3$–Et$_2$O (mol %) | Time (h) | Alcohol (%) |
|-------|----------------------|---------|------------------------|----------|------------|
| 1     | Decane-1,2,10-triol (1.5) | CH$_2$Cl$_2$ | 10 | 3 | 2a (60) |
| 2     | Hexane-1,2,6-triol (1.5) | CH$_2$Cl$_2$ | 10 | 3 | 2b (34) |
| 3     | Hexane-1,2,6-triol (1.5) | THF | 10 | 25 | 2b (38) |
| 4     | Hexane-1,2,6-triol (1.0) | CH$_2$Cl$_2$ | 100 | 2 | 2b (60) |
| 5     | Hexane-1,2,6-triol (1.0) | CH$_2$Cl$_2$ | 5 | 5 | 2b (73) |
| 6     | Trimethylololthane (1.0) | CH$_2$Cl$_2$ | 100 | 6 | 2c (61) |
| 7     | Trimethylololthane (2.0) | THF | 5 | 18 | 2c (86) |

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\text{Scheme 2. Synthesis of peptide aldehyde by direct acetal/thioacetal/aldehyde transformation.}
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to give 6 using the above protocol, which had similar results, giving the corresponding peptide aldehyde (6) in poor yield. TFA-mediated cleavage process from the resin with 3c afforded the trace amount of 6 with the corresponding acetal carboxylic acid (~10%). Although each reaction via thioacetal (8) for the desired 6 provided to give the corresponding compounds, conversion of acetal to thioacetal with EtSH and catalytic BF$_3$-EtO was extremely slow because of the steric hindrance of acetal composed of trimethylolethane. For this reason, we selected the hexane-1,2,6-triol linker for the synthesis of other sequences (Scheme 2).

Although treatment of resin (5) with EtSH and catalytic BF$_3$-EtO followed by the addition of NBS as a one-pot reaction afforded Ac-Val-Leu-Ala-H (6) in 12% overall yield, the yields markedly depended on the nature of the sequence, especially C-terminal amino acid. The one-pot reaction using His, Arg, and β-(2-Thienyl)Ala at the C-terminal position was unsuccessful in detecting the desired products. We found that stepwise conversion was effective to obtain the designed peptide aldehyde using isolated thioacetal (Scheme 3).

This stepwise method was tested by synthesizing selected peptide aldehydes (15)–(20). The yields of thioacetals (10)–(14) were the overall yield from loading on resin, and conversion of acetal to thioacetal proceeded smoothly with simultaneous deprotection of side chains of the corresponding amino acid residues. Isolation yield of Ac-Thr-Val-Phe(Hexahydro)-His-(OEt)$_2$ (9) prepared using Yao’s linker was 31% overall yield (entry 1). Peptide thioacetals (10)–(14) were synthesized in moderate yield (entries 2–6). As a final step, thioacetals were treated with NBS in 10% CH$_2$Cl$_2$ aq to afford the desired peptide aldehydes within a few minutes and immediately the products caused some epimerization of the α-bearing the aldehyde and then decomposed. Therefore, it was important to quench the reaction mixture quickly to purify it by silica gel column chromatography or RP-HPLC. In order to check chiral integrity, the peptide aldehydes were analyzed by $^1$H NMR and the aldehyde appearing at the neighboring δ 9.6 ppm was assessed. In addition, we attempted the synthesis of tokaramide A (20) using this methodology. Although aldehyde formation from thioacetal (14) was moderate, it afforded tokaramide A (20) as a cyclic structure (entry 6) (Table 2).

In conclusion, a very simple and cheap alkyl triol linker for attaching Fmoc-aminals to solid phase peptide synthesis was developed. Peptide acetals were efficiently converted to thioacetal structures followed by treatment of NBS to give peptide C-terminal aldehydes. Although it is difficult to apply this procedure to Trp/Cys-containing peptides, general scope and limitations using several amino acids are now underway.

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Table 2

| Entry | Thioacetal (%) | Aldehyde (%) |
|-------|---------------|--------------|
| 1     | Ac-Thr-Val-Phe(Hexahydro)-His-(OEt)$_2$ (9) | Ac-Thr-Val-Phe(Hexahydro)-His-H (15) |
| 2     | Ac-Phe-Leu-Ala-(OEt)$_2$ (10) | Ac-Phe-Leu-Ala-H (16) |
| 3     | Ac-Ala-Val-Leu-Leu-(OEt)$_2$ (11) | Ac-Ala-Val-Leu-Leu-H (17) |
| 4     | Ac-Leu-Ala-Phe-(OEt)$_2$ (12) | Ac-Leu-Ala-Phe-H (18) |
| 5     | Ac-Leu-Phe-Ser-(OEt)$_2$ (13) | Ac-Leu-Phe-Ser-H (19) |
| 6     | p-HBz-Val-Val-Arg-(OEt)$_2$ (14) | p-HBz-Val-Val-H (20) |