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Use of ozonolysis in the synthesis of C-terminal peptide aldehydes on solid support

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Abstract: A new strategy for the synthesis of peptide aldehydes on solid support is presented. Reaction of a N-protected aminoaldehyde with carboethoxymethylene triphenylphosphorane yielded an α-β-unsaturated δ-amino derivative. After saponification, the resulting α-β-unsaturated δ-aminoacid was anchored to a solid support by an ester linkage (Merrifield resin) or by an amide linkage (MBHA, Expansin). After elongation of the peptide chain, ozonolysis yielded quite pure C-terminal peptide aldehydes in a good yield with no detectable racemization of the C-terminal residue.

C-terminal peptide aldehydes (PAs) are of great interest due to their inhibitory properties as transition-state analogs toward many classes of proteolytic enzymes[1]. Further, PAs are useful intermediates in pseudopeptide chemistry either to modify the C-terminus part of the peptide or for using in chemical ligation reactions. Various methods for the solution synthesis of PAs have been described[2-9] but very few studies have been concerned with solid phase synthesis[10-12].

We report here an approach based on the treatment by ozone of an ethylenic compound linked to a solid support. It produced the ozonide that was treated to yield the corresponding aldehyde. Recently, Sylvain et al.[13] showed the stability of Merrifield resin toward ozonolysis. They reported the use of ozone as a versatile reagent for the generation of aldehydes, carboxylic acids and alcohols on a solid support. We have used ozonolysis to generate peptide aldehydes upon cleavage from the solid support, a strategy that Frechet and Schuerch mentioned for the solid phase synthesis of oligosaccharides.[14] The synthesis of peptide aldehydes based on the use of an α,β unsaturated γ-aminoacid as a linker to the solid support is summarized in Scheme 1. The N-protected α,β unsaturated γ-aminoacid was synthesized by a Wittig reaction between the carboethoxymethylene triphenylphosphorane and the N-protected α-amino aldehyde[15-16] followed by saponification to yield the corresponding ethylenic compound anchored to the solid support. After removal of the N-protecting group, elongation by classical methods of solid phase peptide synthesis (Boc or Fmoc strategies) was possible.

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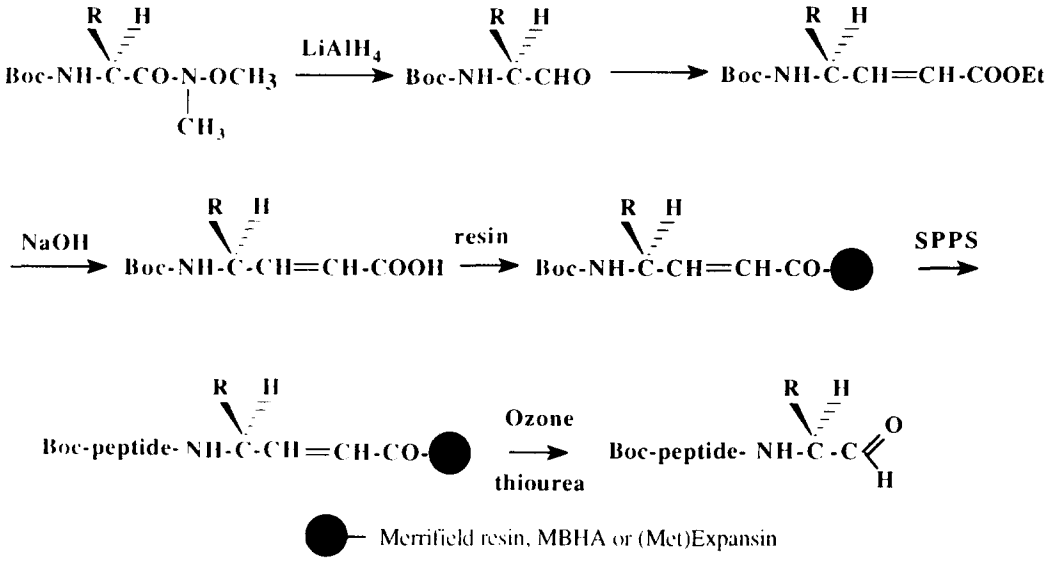
To validate this methodology, we have synthesized several N-protected PAs by this method. Three different resins were used to check their compatibility with this strategy: a Merrifield resin with an ester linkage and the MBHA and (Met)Expansin[17] resins with an amide linkage.

A model tripeptide aldehyde Boc-Phe-Val-Ala-H was synthesized on the three resins. This sequence was chosen because it is our model sequence for our studies dealing with the synthesis of peptide aldehydes.[18] These peptides were checked by RP HPLC and studied by ^1H NMR in $CDCl_3$ without purification. The results showed that peptide aldehydes with high purity could be obtained using this strategy with no trace of detectable racemization (in the limit of sensitivity of ^1H NMR). We have shown that tentative purification either by flash silica gel chromatography or by reverse phase HPLC induced some epimerization[1,18]; therefore synthesizing aldehydes with a high degree of purity trying to avoid the purification step was important.



Scheme 1. Solid phase synthesis of peptide aldehydes via an α,β unsaturated γ-aminoacid linker.

We have synthesized an aspartyl-containing peptide aldehyde using the same approach. Indeed, most of the previous described syntheses of PAs are incompatible with the presence of protected Asp or Glu residues. We have shown in this piece of work that using benzyl ester derivatives of aspartic acid to obtain aspartyl containing peptide aldehydes by this methodology was possible. The results are reported in Table 1.

As an example the synthesis of Boc-Phe-Val-Ala-H was as follows.

Synthesis of N-Boc-4-amino-4-methyl-2-pentenoic acid: 5.4 g of Boc-Ala-N(Me)OMe (23.3 mmoles) were dissolved in THF (100 ml) and placed in an ice-bath. $LiAlH_4$ (1.10 g, 29 mmoles) was added slowly and the

reaction mixture checked by tlc. After 30 min, a 5% $KHSO_4$ solution was added (100 ml) and the THF was concentrated *in vacuo*. After a classical work-up, the aldehyde was used without purification. Boc-Ala-H (4.02 g, 23.2 mmoles), carboethoxymethylene triphenylphosphorane (8.88 g, 25.5 mmoles) were dissolved in toluene (100 ml) and placed under stirring at 80°C in an oil bath. After 1 h, the reaction mixture was concentrated *in vacuo* and the residue dissolved in diethyl ether (200 ml), washed with water. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using AcOEt /hexane 5/5 as eluent to yield 5.07 g of an oily compound (yield, 90%). This oil (2.84 g, 11.7 mmoles) was then saponified with 1N sodium hydroxide (14 ml) in EtOH (100 ml) during 5 h. After concentration of the solvents *in vacuo*, the acidic compound was recovered through a double extraction to give 2.21 g of the title compound (yield, 88%).

N-Boc-4-amino-4-methyl-2-pentenoic acid was anchored to the MBHA resin using the BOP[19] as coupling reagent. Peptide elongation was performed with Boc-Val-OH and Boc-Phe-OH using BOP reagent. The derivatized peptidyl resin was suspended in methylene chloride at -80°C and subjected to an ozone stream. Once the reaction medium turned blue (usually after a few min.), the ozone stream was continued for 5 min. Argon was then bubbled through the reaction mixture for 10 min. The ozonide was treated with thiourea (1.2 eq.) dissolved in MeOH for 10 min. The reaction mixture was diluted with dichloromethane and washed with water, brine, and dried over sodium sulfate. After removal of the solvent *in vacuo*, the peptide aldehyde Boc-Phe-Val-Ala-H was obtained and its purity was checked by HPLC. It was identified by ^1H NMR spectroscopy and mass spectroscopy.

Resin	Peptide aldehydes	Yield (%)[a]
MBHA	Boc-Phe-Val-Ala-H	75
Expansin(Met)	Boc-Phe-Val-Ala-H	83
Merrifield	Boc-Phe-Val-Ala-H	56
MBHA	Z-Val-Phe-H	30
MBHA	Boc-Asp(OBzl)-Phe-Val-Ala-H	42

[a]Yields are based on the substitution of the commercial resin

Table 1. Synthesis of peptide aldehydes.

The described procedure to synthesize peptide aldehydes on solid support allowed us to prepare several peptides aldehydes on three different resins (Merrifield resin, MBHA, (Met)Expansin). The linker is easily prepared and the advantages of the solid phase peptide synthesis apply to the preparation of PAs. Furthermore, this methodology could be used in a sequence containing a β-benzyl protected aspartic acid and is probably also useful in the presence of protected glutamic acid residues. However, the presence in the sequence of functional groups sensitive to ozonolysis (in the described reaction conditions) has to be avoided. According to the

treatment of the ozonide intermediate, this strategy could also lead to partially protected peptides with a free C-terminal carboxylic end that could be useful for peptide synthesis by fragment condensation, or to C-terminal peptide alcohols. On the other hand, this methodology can probably apply to organic reactions on solid support in which aldehydes (or carboxylic acids, alcohols) are the final targets. Further investigations are in progress in our laboratory.

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