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Synthesis of Peptide Aldehydes on Solid Support using Ozonolysis.

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Abstract : A new strategy for the synthesis of peptide aldehydes on solid support is presented. Reaction of a N-protected α-amino aldehyde with MBHA-supported Wittig ou Wittig-Horner reagent yielded resin-linked α-β-unsaturated δ-amino derivative. After elongation of the peptide chain, ozonolysis produced fairly pure C-terminal peptide aldehydes in good yield.

Peptide aldehydes are excellent starting materials for further chemistry (formation of reduced bond, Wittig reactions, ligation...) and they have been found to be potential inhibitors of several classes of enzymes such as serine proteases,[1,2] prohormone convertases,[3] cysteyl proteases,[4,5] aspartyl proteases.[6,7] These inhibitory properties result from the tetrahedral hydrated C-terminal aldehyde function which mimics the transition state of the substrate hydrolysis. Various methods for the solution synthesis of peptide aldehydes have been described: the peptide alcohol can be oxydized into the corresponding aldehyde;[8,9] another strategy widely used is the diisobutylaluminium hydride reduction of the corresponding methyl esters.[10] Argininal analogue syntheses, due to their significant anticoagulant, antithrombotic properties, have been often reported using δ-lactam[2,11,12] and semicarbazone derivatives.[1] In the synthesis of an interleukin-1β converting enzyme inhibitor described by Chapman,[4] its C-terminal aldehydic residue is an aspartyl residue. The aspartyl aldehyde moiety was protected as the corresponding O-benzylacylal which could be coupled and then hydrogenolyzed to afford the desired compound. Recent reports mention the use of thiazolidines[13] or oxazolidine[14] as aldehyde precursors. However there are very few publications concerning the solid phase synthesis of peptide aldehydes.[15,16]

We recently focused our attention on the solid phase synthesis of such compounds. Our first approach was based on the Weinreb amide linker[17] and the peptide aldehyde was released from the resin by $LiAlH_4$ reduction. The second synthesis we have described was related to the reduction of phenyl esters with $AlLi(OtBu)_3H$ as reported by Zlatoidsky in solution.[18] The comparison of these methods and the problems of purification and racemization during purification of these products were described.[19] To avoid the use of hydrides we proposed the use of an α,β-unsaturated γ-aminoacid as a linker to the solid support as an interesting alternative to generate peptide aldehydes by ozonolysis.[20] This last methodology is very clean and proceeds without detectable racemization. The synthesis of the linker[20] was performed by a Wittig reaction between the carboethoxymethylene triphenylphosphorane and the N-protected α-amino aldehyde[21] followed by

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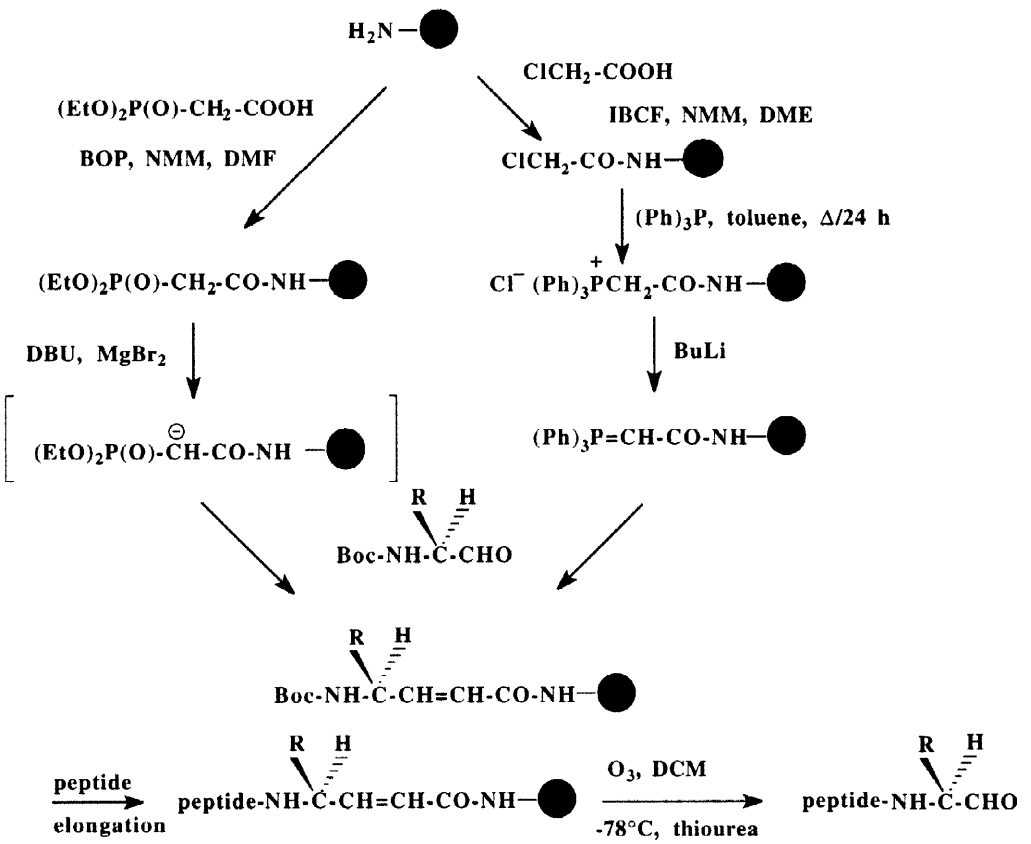
saponification to yield the corresponding ethylenic compound anchored to the solid support. After removal of the N-protecting group, elongation was possible by classical methods of solid phase peptide synthesis (Boc or Fmoc strategies). The use of this linker incorporating the C-terminal residue of the peptide aldehyde implied the synthesis of a linker on the C-terminus for each different amino-acid. We decided to explore another strategy for the synthesis of peptide aldehydes by ozonolysis, which is reported here.

This new approach is illustrated in Scheme 1 and consists in the anchoring of a Wittig or Wittig-Horner reagent on the solid support. Then, the reaction with the N-protected α-amino aldehyde was performed directly on the solid support. This strategy allowed the synthesis of a large amount of a general functionnalized resin which is ready for the preparation of α,β-unsaturated γ-aminoacyl moiety linked to the resin from any α-amino aldehyde.



Scheme 1. Preparation of Wittig reagents linked to the solid support, peptide elongation and release of the aldehydic peptide by ozonolysis.

Two different approaches were tested: anchoring of diethyl phosphonoacetic acid on MBHA resin with BOP as coupling reagent or anchoring of chloroacetic acid on MBHA resin with isobutyl chloroformate (IBCF) as activating agent followed by reaction of the modified resin with triphenylphosphine to form the

phosphonium salt. With triphenylphosphonium salts, the phosphorane is formed with butyl lithium or potassium *tert*-butylate.[22] With diethylphosphonoacetamide-resins, the carbanion is generated with various bases as described in the literature.[23] After reaction with the N-protected α-amino aldehyde, elongation of the peptide could be performed. The derivatized peptidyl resin was subjected to an ozone stream and the peptide aldehyde recovered as described previously.[20] This study was performed on our model peptide Boc-Phe-Val-Ala-H. The results are gathered in Table 1. All HPLC chromatograms of the crudes showed a high degree of purity. Surprisingly, ^{1}H NMR analysis of the aldehydic signals in the described conditions indicated epimerization of the α-carbon of the C-terminal residue (Table 1). For the synthesis of the Wittig or Wittig-Horner anchored on the solid support, various conditions were tested for the phosphorane formation. In the case of triphenylphosphonium salt, the stoechiometry of butyl lithium was decreased without any change in the observed epimerization of the resulting aldehyde, the use of potassium *tert*-butylate did not improve the reaction. Epimerization was reduced to 7% when the resin was washed by THF and dichloromethane before the addition of the N-protected α-amino aldehyde, but the yield decreased.

Substrate	solvent/t°C/time	base/equiv.	Yield (%)[a]	HPLC purity[b]	epimerization LLL/LLD[c]
$Cl^-,P^+(Ph)_3$-CH_2	THF/0°C then 65°C/20 h[d]	n-BuLi/3	90	94	73/26
		n-BuLi/1.1	100	83	79/21
		*t*BuOK/3	100	90	62/38
		n-BuLi/3[e]	60	94	93/7
$(EtO)_2P(O)$-CH_2	DMF/RT/20 h	NaH/3	93	92	56/44
		NaH/1.1	72	78	66/34
		Et_3N, $MgBr_2$/3	57	90	89/11
		DIEA, $MgBr_2$/3	30	90	78/22
		DBU, $MgBr_2$/3	96	82	91/9
		NMM, $MgBr_2$/3	32	66	87/13
	THF/RT/20 h	NaH/3	100	90	60/40
		Et_3N/3	30	90	87/13

Table 1. Synthesis of our model peptide Boc-Phe-Val-Ala-H in various experimental conditions

a: Yields are based on the substitution of the Wittig reagent derivatized resin. b: purity was checked on a C18 analytical column with a flow of 1 mL/min and a gradient from water (0.1% TFA) to CH_3CN (0.1% TFA) in 50 min at 214 nm. c: measured by ^{1}H NMR with a 360 MHz apparatus in $CDCl_3$. d: n-BuLi was added at 0°C then the reaction was warmed at 65°C. e: after the formation of the phosphorane on solid support, the resin was washed with CH_2Cl_2 and THF before the addition of the N-protected α-amino aldehyde.

When diethylphosphonoacetamide was used, the situation was different as no stable phosphorane could be formed. No washing of the resin could be performed and for this reason, the N-protected α-amino aldehyde was added in the presence of the base. The best conditions were found with the use of 1,8-

diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of magnesium bromide. In this case, the yields were almost quantitative and epimerization was reduced to 9%.

The described procedure to synthesize peptide aldehydes on solid support is an improvement since no linker preparation is needed for each amino acid derivative. This strategy for the synthesis of peptide aldehydes on solid support allowed the preparation of large amounts of supported Wittig reagent, which could then be reacted with various carbonyl components.

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