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Review

Synthesis of peptide aldehydes

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Abstract: The functionalization of peptides and proteins by aldehyde groups has become the subject of intensive research since the discovery of the inhibition properties of peptide aldehydes towards various enzymes. Furthermore, peptide aldehydes are of great interest for peptide backbone modification or ligation reactions. This review focuses upon their synthesis, which has been developed following two main strategies. The first strategy consists of prior synthesis of the peptide, followed by the introduction of the aldehyde function. The second possible strategy uses α-amino aldehydes as starting materials. After protection of the aldehyde, peptide elongation occurs. At the end of the synthesis, the aldehyde function can be unmasked. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptide aldehydes; synthesis in solution; SPPS; epimerization

INTRODUCTION

Uses of Peptide Aldehydes

Peptide aldehydes are used in many applications. They are suitable starting materials for further chemistry involving the aldehyde function – e.g. generation of reduced bond isosteres [1]. Furthermore, the carbonyl chemistry offers unique possibilities for the segment ligation of unprotected peptides in aqueous solution. Indeed, stable linkages can be obtained by reacting an aldehyde function with naturally occurring amino acids. The β-amino thiol moiety of cysteine leads to thiazolidine formation [2], whereas the β-amino alcohol group of serine and threonine residues is involved in the pseudoproline ligation [3]. Peptides functionalized by a carbonyl group have also been studied extensively in the context of oxime [4,5] or hydrazone [6] ligations. Moreover, these compounds are potent inhibitors of serine and cysteine proteases such as trypsin, plasmin and papain [7]. Since this important discovery, peptide aldehydes have been shown to inhibit other proteases such as prohormone convertases [8] and aspartyl proteases [9,10]. These inhibitory properties result from the tetrahedral hydrates of the *C*-terminus aldehyde function that mimics the transition state of the substrate during hydrolysis by the enzyme.

Physicochemical Properties of Peptide Aldehydes

The chemical instability of peptide aldehydes is mainly due to their high reactivity. But their optical instability also has to be considered. Indeed, in presence of an acid catalyst, the carbonyl function can be protonated and gives an enol intermediate losing the optical purity of the α-carbon of the aldehyde. This epimerization can take place during the synthesis or purification of peptide aldehydes. It is worth noting here that, to our knowledge, no method of purification without epimerization of such peptide aldehydes is known. As described earlier [11], the aldehyde signal in ^{1}H NMR is a good indicator of the possible epimerization of peptide aldehydes. We recently proposed [12] a simple method that allows the detection of the optical purity on a model dipeptide aldehyde Boc-Val-Ala-H. In this study, for each tested purification condition, the spectra of the dipeptide aldehyde revealed two aldehydic proton peaks (in $CDCl_3$). These two signals, corresponding to the LL and LD diastereoisomers, could be observed in $CDCl_3$, but not in DMSO-d_6. We hope this model dipeptide will be used to validate peptide aldehyde preparation methods and/or purification techniques.

Synthesis of Peptide Aldehydes

Because of the interest in peptide aldehydes, several studies have been devoted to the synthesis of these compounds. The synthetic methods of peptide aldehydes are classified into two main categories (Scheme 1). The first strategy consists of prior synthesis of the peptide, followed by the introduction of the aldehyde function to obtain the peptide aldehyde. The second possible strategy uses α-amino aldehydes as starting materials. The aldehyde moiety is present at the beginning of the synthesis in a protected form. After peptide elongation, the masked aldehyde is deprotected to yield the peptide aldehyde. In this review, we will successively

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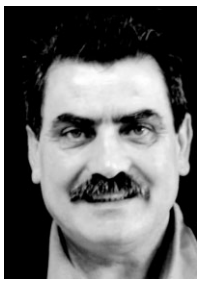
BIOGRAPHIES

Aline Moulin was born in Guilherand-Granges-lès-Valence, France, in 1981. She was awarded from the National Graduate Chemistry Scholl of Montpellier in organic chemistry and received her Master of Science in Biomolecule Chemistry from the University Montpellier II in 2004. She is currently in the final stage of her PhD studies under the supervision of Dr. Jean-Alain Fehrentz. Her research interests focus on the design and synthesis of bioactive compounds.



Jean Martinez studied chemistry at the "Ecole Nationale Supérieure de Chimie de Montpellier" (France). After receiving his Ph.D. in 1972 he was awarded a permanent position at the CNRS. He completed his Thèse d'Etat in 1976 under the direction of Prof. F. Winternitz, and performed post doctoral studies with Prof. E. Bricas in Orsay (France) and at Case Western University (Ohio, USA) with Prof. M. Bodansky. On his return to France, he pursued his research activities in the field of peptides and became successively head of various research laboratories in Montpellier including the "Chemistry and Pharmacology of Biologically Interesting Molecules" Laboratory. In 1998, he was appointed Professor of the Faculty of Sciences and in 2001, Professor of the Faculty of Pharmacy. He is currently head of the "Laboratory of Aminoacids, Peptides and Proteins", of the "Max Mousseron Institute for Biomolecules" and President of the European Peptide Society. His research interests are peptide chemistry and pharmacology, stereoselective synthesis of aminoacids, chemistry on polymeric supports, mass spectrometry, artificial proteins synthesis, computer-assisted peptide search and green chemistry.

Jean-Alain Fehrentz was born in Nancy, France in 1955. He received his PhD in chemistry from the University of Nancy in 1983 and joined the "Centre CNRS-INSERM de Pharmacologie Endocrinologie" of Montpellier, France in the Professor Bertrand Castro's group. From 1989 to 1992, he was appointed as researcher in Sanofi Research and then moved to the School of Pharmacy of Montpellier in the Laboratory of Aminoacids, Peptides and Proteins under the direction of Professor Jean Martinez. His research interests focus on peptide aldehydes, enzyme inhibitors and receptor ligands.

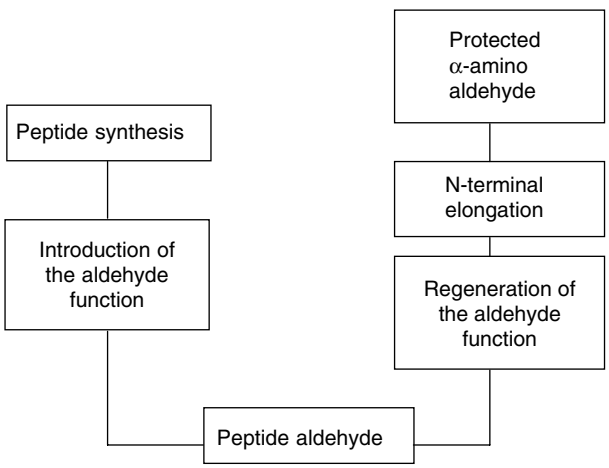
examine these two strategies, which have both been widely described in the literature.

SYNTHESIS OF PEPTIDE ALDEHYDES VIA THE INTRODUCTION OF THE ALDEHYDE FUNCTION ON PEPTIDES

Oxydation of Peptide Alcohols

Oxydation of peptide alcohols in solution. Oxydation of peptide alcohols to obtain peptide aldehydes is a well-known method. In solution [13], the starting material is a peptide ester **1**, which is reduced into alcohol **2** by sodium borohydride. Via the Pfitzner Moffatt method, DMSO and dicyclocarbodiimine as electrophile are used to obtain the peptide aldehyde **4**. Sulphur trioxide as electrophile can also be used. The oxide sulfonium intermediate **3** is deprotonated in basic conditions and generates the peptide aldehyde **4** and dimethylsulfide (Scheme 2). According to the authors, this method is epimerization-free. Care must be taken regarding the quantity of base and the time of reaction. Optimization is necessary for each type of substrate.

Oxydation of peptide alcohols on solid support. In a recent paper [14], the oxydation of peptide alcohols using the supported IBX reagent has been described. The starting material is the supported peptide **5** on TCP-resin, cleaved from the solid support in acidic conditions to generate the peptide alcohol **6** in solution. The following step is the oxidation of the peptide alcohol using the commercially available Dess Martin–type supported reagent. The obtained mixture contains the peptide aldehyde **7** along with some peptide alcohol. To eliminate this residual starting material, the authors propose a catch-and-release purification procedure. A supported threonine reagent is chemoselectively condensed with the peptide aldehyde to form a supported oxazolidine **8**, which is washed as many



Scheme 1 Strategies to obtain peptide aldehydes.

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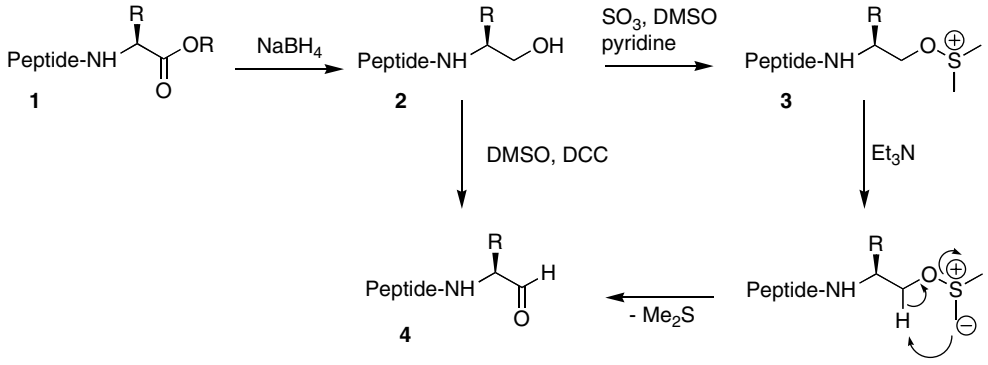
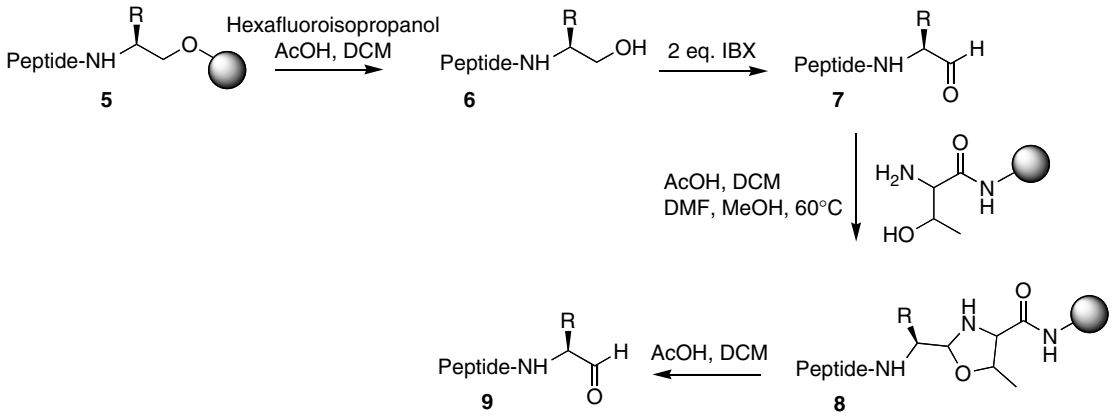


Scheme 2 Oxydation of peptide alcohols in solution.



Scheme 3 Oxydation of peptide alcohols on solid support.

times as necessary to eliminate the peptide alcohol. The last step is a cleavage from the solid support to generate the peptide aldehyde **9** (Scheme 3). The crude product of the oxydation step is obtained in the optically pure form. However, partial epimerization occurs during the purification step: 50% epimerization at 60 °C and 20% epimerization at room temperature were observed.

Reduction of Peptide Amides

Reduction of Weinreb amides in solution. Among all the described preparations of *N*-protected peptide aldehydes, the reduction of Weinreb amides is one of the most widely used. This method was successfully applied to the synthesis of *N*-protected peptide aldehydes [11] using benzyloxycarbonyl (Z), *tert*-butyloxycarbonyl (Boc) and α-fluorenylmethoxycarbonyl (Fmoc) chemistry [15]. Weinreb amides **10** are reduced by lithium aluminum hydride, at 0 °C in anhydrous THF, to form a complex **11**, which is then hydrolyzed to generate the peptide aldehyde **12** (Scheme 4).

Reduction of Weinreb amides on solid support. This strategy was applied to the solid-phase synthesis of peptide aldehydes. This was initiated by the synthesis of a linker [16] designed to incorporate the methoxyamine. *O*-Methyl hydroxylamine **13** is reacted with benzyl acrylate. The resulting *N*-alkylated *O*-methyl hydroxylamine **14** is protected by the *tert*-butyloxycarbonyl group. After deprotection of the benzyl ester **15** by hydrogenolysis, the linker **16** is coupled to the solid support (MBHA resin), allowing, after deprotection, peptide elongation using standard Boc/benzyl or Fmoc/*tert*-butyl chemistries. Treatment with $LiAlH_4$ yields the peptide aldehyde **17** in solution (Scheme 5).

This Weinreb amide linker was used for the synthesis of aspartic acid-containing peptide aldehydes [17] to obtain β-secretase inhibitors [18] and in the parallel synthesis of libraries of aldehyde derivatives [19].

Another recent Weinreb-type method uses the backbone amide linker (BAL) Support **18**. Methoxyamine is attached to the resin through reductive amination, then acylation allows introduction of the first amino acid. Standard chemistry is then used for the elongation of the peptide. Depending on the reaction conditions for the removal from the solid support, in acidic conditions the hydroxamate **19** is obtained, and using lithium aluminum hydride as reductive agent, the aldehyde **20** is obtained (Scheme 6).

For Weinreb amide reduction in solution or on solid support, no over-reduction was observed, suggesting the formation of the stable metal-chelated

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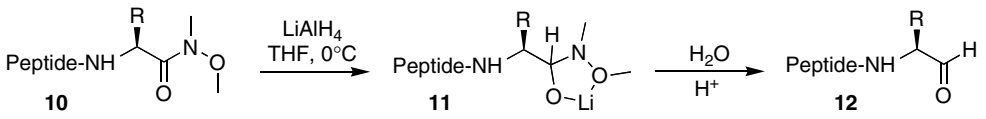
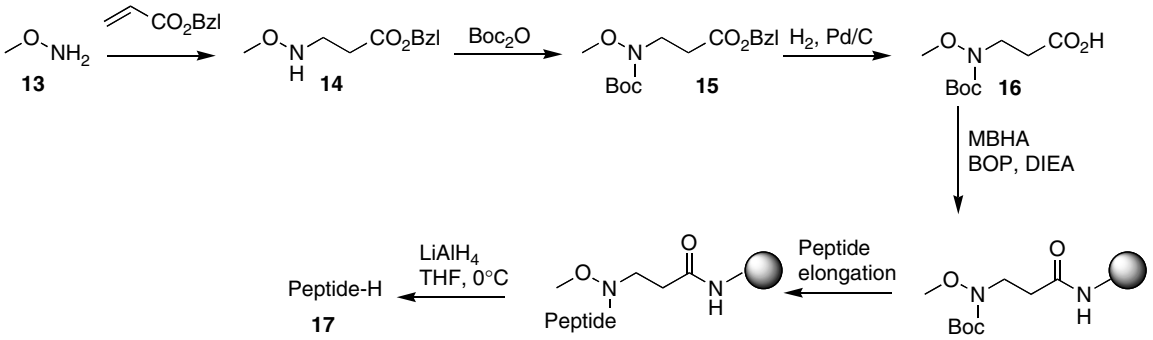
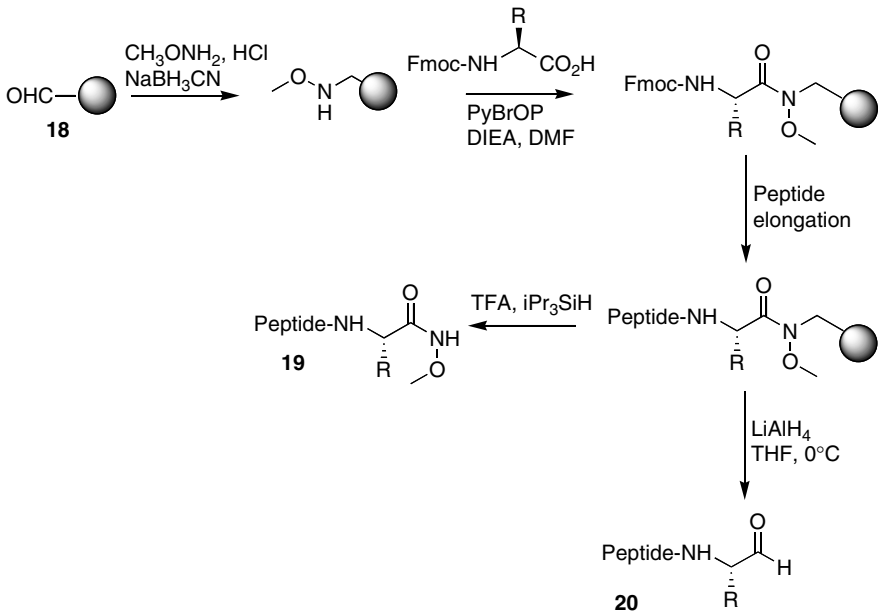


Scheme 4 Reduction of Weinreb amides in solution.



Scheme 5 Reduction of Weinreb amides on solid support.



Scheme 6 Reduction of Weinreb amides on a BAL-type support.

intermediate described by Nahm and Weinreb [20]. Furthermore, epimerization studies showed that this type of method is epimerization-free. However, aspartic- and glutamic-residue-containing peptides, bearing an ester-protecting group on their side chain should be avoided because of the possible reduction of these ester groups. As previously described [11], owing to the presence of several amide functions, the amount of $LiAlH_4$ has to be increased as a function of the length of the peptide. This reduces the application of this method to small peptides, and also in the case of large-scale synthesis.

Reduction of morpholine amides in solution. An interesting alternative to the Weinreb amides is the morpholine amides **21** [21], which can be reduced by $LiAlH_4$. It also forms a stable complex **22,** which avoids over-reduction, and generates the aldehyde **23** by hydrolysis (Scheme 7). This method is economical compared to the Weinreb method. It is equivalent to the Weinreb amide method as far as optical purity, yield and stability in the peptide synthesis conditions are concerned. Only the Fmoc chemistry must be carefully handled: during the reaction of the first Fmoc amino acid with morpholine, the carboxylic acid of the Fmoc amino acid has to be preactivated, and then morpholine should be slowly added to it to avoid Fmoc deprotection. It should be noticed that, to our knowledge, this method has not been transposed on a solid support.

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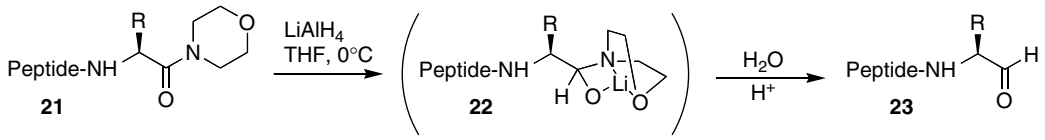
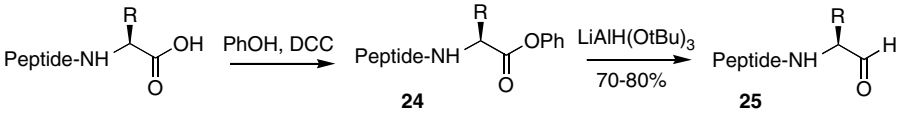
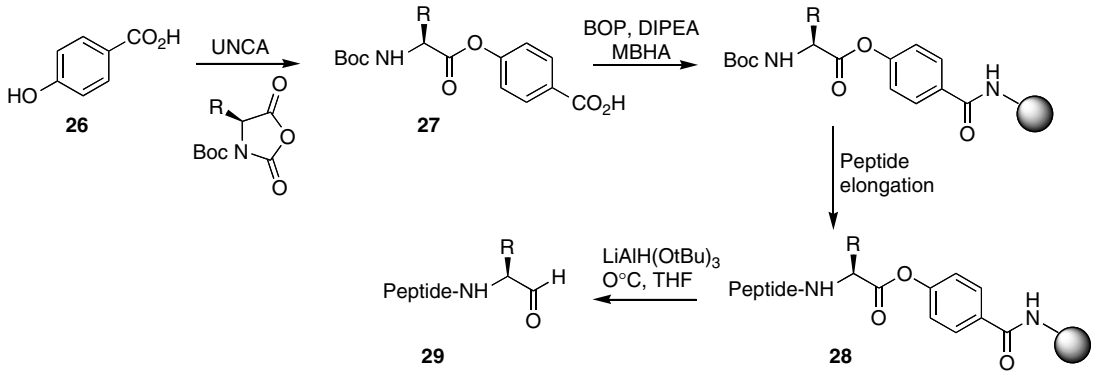


Scheme 7 Reduction of morpholine amides in solution.



Scheme 8 Reduction of phenyl esters in solution.



Scheme 9 Reduction of phenyl esters on solid support.

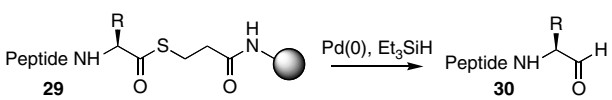
Reduction of Peptide Esters

Reduction of phenyl esters in solution. Zlatoidsky [22] describes the preparation of *N*-protected peptide aldehydes via the reduction of the corresponding phenyl esters **24** by lithium tri(*tert*-butoxy)aluminum hydride (Scheme 8). The yields of the obtained aldehydes **25** are quite interesting (70–80%). This method is particularly recommended for compounds containing functional groups potentially sensitive to a more potent reductive agent.

Reduction of phenyl esters on solid support. Commercially available 4-hydroxy-benzoic acid **26** is reacted with *N*-protected amino acid carboxyanhydrides [23–25], leading to the corresponding *N*-protected amino esters **27**. These compounds are directly anchored to an amino resin. Classical peptide elongation on solid support can then be undertaken (Scheme 9). *N*-protected α-amino aldehydes **29** are obtained by AlLiH(O*t*Bu)$_3$ reduction of the corresponding *N*-protected α-amino phenyl esters linked to the support **28**, as described by Zlatoidsky [22]. A mixture of the corresponding aldehyde and alcohol is obtained. Reduction in solution of *N*-protected amino phenyl esters was also studied by these authors, and the aldehyde was produced as the major product with a minor contamination corresponding to the alcohol. Several peptide aldehydes were synthesized using the phenyl ester linker [26]. Again, the presence of both the peptide aldehyde and alcohol was observed. This phenomenon could be explained by the over-reduction of aldehyde by an excess of hydride. However, this strategy is appropriate for the preparation of peptide aldehydes leading to the synthesis of modified pseudopeptides (reduced bonds, etc.) [26].

Synthesis Via Reduction of Peptide Thioesters

From solid-supported thioester peptides **29**, a mild method for the generation of peptide aldehydes has been developed by Tam and co-workers [27] (Scheme 10). Peptides are synthesized using Boc/benzyl chemistry on MBHA or TentaGel resins containing thiopropionic acid as a nondetachable linker anchored on the aminomethyl resin. Treatment of *N*-protected peptide thioester with Pd^0 and Et_3SiH produces the *C*-terminal peptide aldehyde **30**. The reaction is performed using mild experimental conditions under nitrogen in tetrahydrofuran or dichloromethane using 20–30-fold excess of Et_3SiH at 4 °C to avoid epimerization of the *C*-terminal amino acid residue. The catalyst



Scheme 10 Synthesis via reduction of peptide thioesters.

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is obtained by *in situ* reduction of $Pd(OAc)_2$ and the yields range from 80 to 89%.

Synthesis Via Thiazolidines

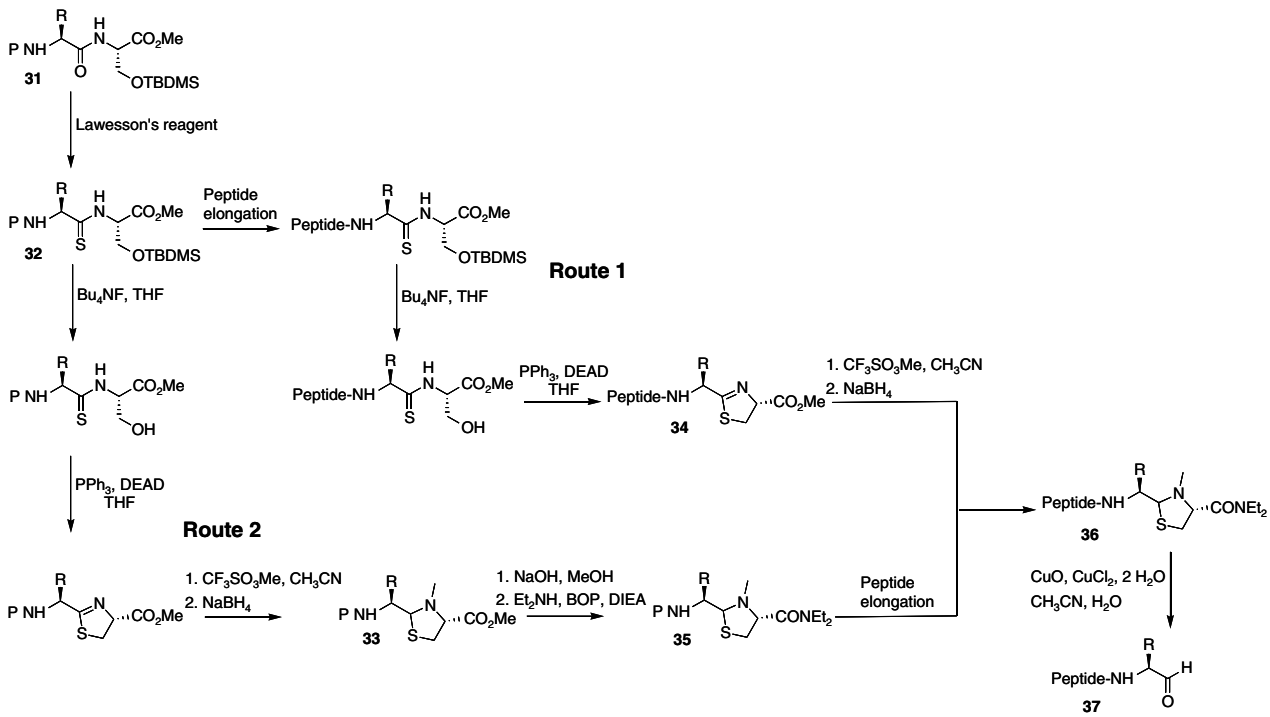
Synthesis via thiazolidines in solution. The use of a peptidyl *N*-methylthiazolidine as a peptide aldehyde precursor has been described in solution [28]. The starting material consists of a dipeptide **31** composed of the *C*-terminal residue of the target peptide aldehyde bearing an additional *C*-terminal serine methyl ester residue, protected on the side chain by a tertiobutyldimethylsilyl group. Treatment of this dipeptide with the Lawesson reagent yields the corresponding thioamide **32** [29]. Starting from these β-hydroxy thiopeptides, two pathways were investigated. In the first strategy, the 'pseudopeptide' was built and the thiazolidine generated at the end of the synthesis. The second pathway involved the preliminary formation of the amino thiazolidine **33**, followed by elongation of the 'pseudopeptide'. According to the first route, after peptide elongation of **32**, the alcohol moiety on the serine side chain was deprotected and a dihydrothiazole **34** was formed by an intramolecular Mitsunobu reaction. After *N*-methylation and reduction, the thiazolidine **36** was obtained according to Dondoni [30]. In the last step, hydrolysis was performed using $CuO{\cdot}CuCl_2{\cdot}2H_2O$ and the peptide aldehyde **37** was generated (Scheme 11). In the second route, because the side reaction of intramolecular coupling of the ester with the amine occurred during the elongation step, the stepwise peptide synthesis was performed from the corresponding thiazolidine diethylamide derivative **35** (Scheme 11).

Synthesis via thiazolidines on solid support. The same authors adapted this strategy on solid support [28]. The thiazolidine **38** was prepared in solution and coupled to an amino hexanoic acid–bound MBHA resin. Peptide elongation was undertaken using either Boc/benzyl or Fmoc/*tert*-butyl SPPS strategy. Hydrolysis was performed using CuO, $CuCl_2{\cdot}2H_2O$ in a CH_3CN/H_2O/DMF mixture, and the peptide aldehyde **39** was extracted from the aqueous solution with ethyl acetate (Scheme 12).

Using the thiazolidine strategy, either in solution or on solid support, the obtained peptide aldehydes are of good purity. However, there are a large number of steps involved in obtaining the key intermediates. Another problem is the use, during the last step, of copper salts, which are difficult to eliminate. They can be troublesome when biologically active compounds are synthesized.

Synthesis of Peptide Aldehydes Containing an Arginine Residue at the *C*-terminus Via a Lactam Formation

Garrett *et al.* [31] described the preparation of a series of tripeptide aldehyde derivatives containing an arginine residue at the *C*-terminus. The synthesis used in this study is outlined in Scheme 13. Activation of the carboxylic acid **40** of the starting material with isobutyl chloroformate is followed by cyclization to the lactam



Scheme 11 Synthesis via thiazolidines in solution.

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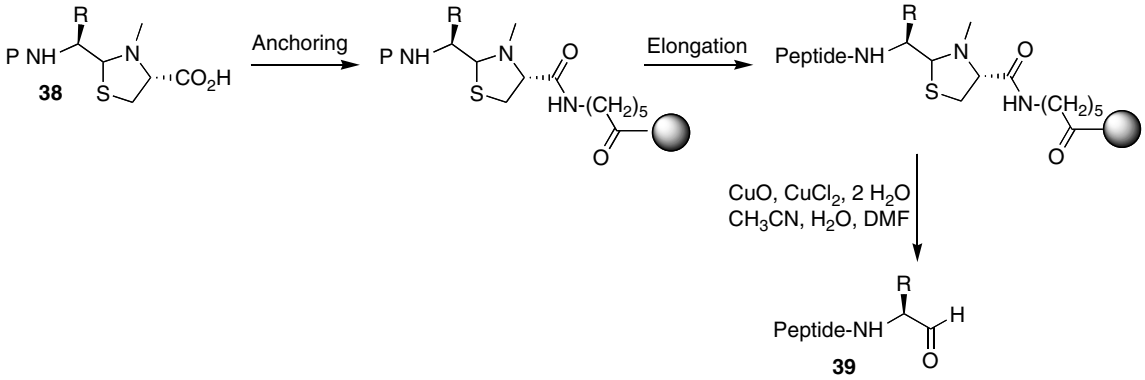
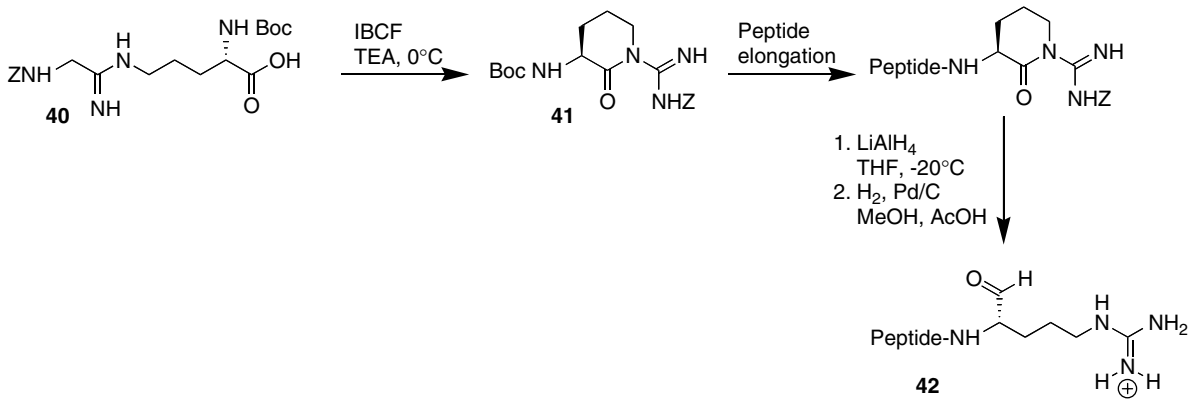


Scheme 12 Synthesis via thiazolidines on solid support.



Scheme 13 Synthesis of peptide aldehydes containing an arginine residue at the *C*-terminus.

41 in high yields. Controlled reduction of the peptide-elongated lactam followed by catalytic hydrogenation under acidic conditions yields the targeted peptide aldehydes **42**.

SYNTHESIS OF PEPTIDE ALDEHYDES VIA THE PROTECTION OF α-AMINO ALDEHYDES

Protection Via a Semicarbazone Moiety

Protection via a semicarbazone moiety in solution. In this method, the aldehyde function of an α-amino-aldehyde **43** is masked by a stable semicarbazone moiety **44**, which is easy to isolate and purify [8]. After elongation, the cleavage is achieved by acid hydrolysis in the presence of formaldehyde (Scheme 14). The yields are acceptable (30–60%) and the epimerization rate is rather low (less than 2%).

Protection via a semicarbazone moiety on solid support. This approach [32], derived from a solution preparation of peptide aldehydes [33], involves the solution synthesis of a semicarbazone carboxylic acid linker **45** that can be attached to the commercially available MBHA resin. Cleavage with dilute aqueous acid/formaldehyde provides the free peptide aldehydes **46** (Scheme 15).

This method was used to explore the structure–activity relationship of peptidomimetic caspase inhibitors [34].

Another linker based on the dibenzosuberyl system [35] has been developed [36]. Taking advantage of the stability of semicarbazones and their easy purification, a semicarbazide linker has been synthesized in two simple steps starting from the corresponding amine linker (Scheme 16). These reactions are monitored using IR spectroscopy, and functionalities of 0.2–0.25 mmol/g can be obtained. The *C*-terminal residue is introduced on the solid support as a Fmoc-protected amino aldehyde in the presence of DIEA (70–100% yield) and the peptide sequence can then be elongated using classical SPPS. The semicarbazone peptides **47** are released from the support by TFA treatment (TFA/H_2O: 9/1, 1.5 h), and treatment with pyruvic acid provide the corresponding aldehydes **48**. This method yields optically pure peptide aldehydes.

Protection Via an Oxazolidine Moiety on Solid Support

A paper reported the use of a linker based on the oxazolidine moiety [37]: this method was proposed

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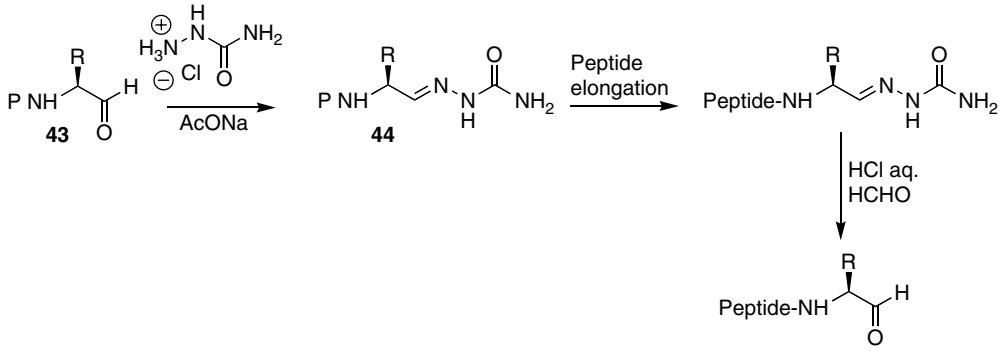
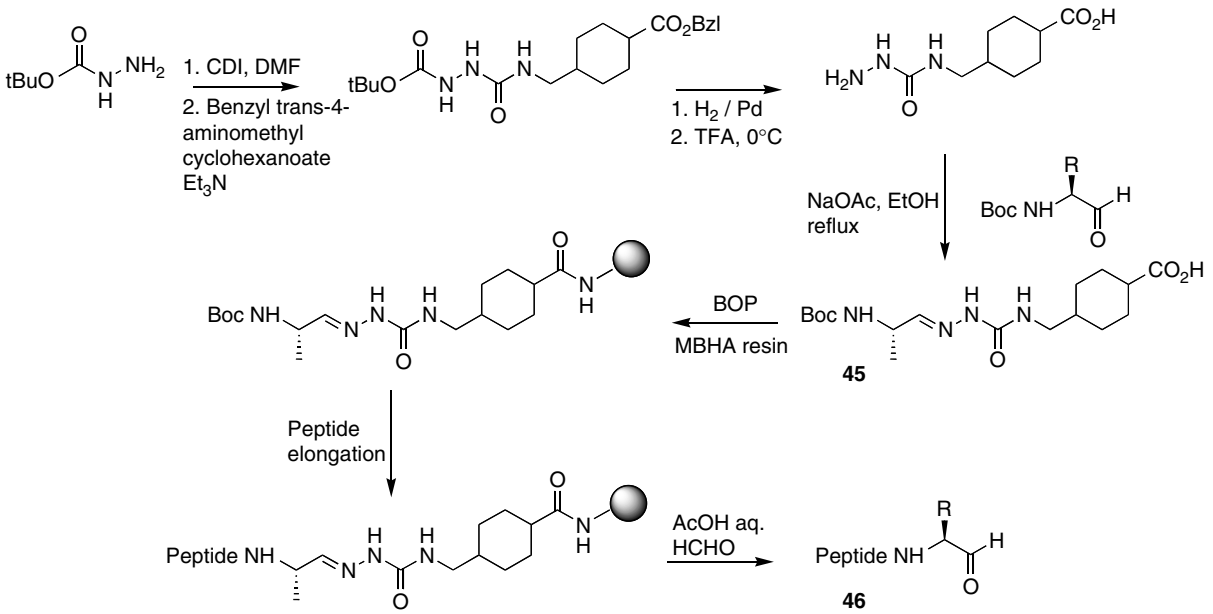


Scheme 14 Protection via a semicarbazone moiety in solution.



Scheme 15 Protection via a semicarbazone moiety on solid support described by Murphy *et al.*

by the CHIRON Company on Synphase Crown solid supports. A supported seryl or threonyl residue **49** reacted with an aldehyde (in this case, the α-amino aldehyde or an amino acid derivative) to yield an imine intermediate **50** that cyclizes to yield a stable oxazolidine **51**. After peptide elongation, the aldehyde molecule **52** is generated and removed from the support by treatment with a mild aqueous acid (Scheme 17). Interestingly, oxazolidines are relatively stable when treated with nonaqueous TFA mixtures, so acid labile side-chain protecting groups can be removed without altering the oxazolidine moiety. Thus, the peptide synthesis can be performed using Fmoc chemistry. The secondary amine of the oxazolidine ring, although quite unreactive, can be masked by a Boc-protecting group. This method allows the obtainment of pure crude aldehyde derivatives, which is highly valuable for combinatorial chemistry. However, no comment concerning epimerization is provided. Sorg *et al.* [14] note nonnegligeable rates of epimerization occurring during the oxazolidine formation: 50% epimerization at 60 °C and 20% at room temperature.

Protection Via a *N*-Methylthiazolidine Moiety

Chiral *N*-methyl thiazolidine-masked α-amino aldehydes are used for solid-phase peptide elongation. Gros *et al.* [38] proposed to use *N*-trityl-protected α-amino aldehydes **53**, which, as compared to *N*-Boc protection, preserve the optical integrity of the aldehyde during condensation of the amino aldehydes with L-cysteinyl residues **54** (Scheme 18). The authors prepared the Ac-Tyr-Val-Ala-Asp-H caspase inhibitor on a solid support starting from the *N*-trityl-amino thiazolidine aspartyl derivative as a validation of this process.

Protection Via an Olefin Moiety

Protection via an olefin moiety using a classical resin. One of the cleanest methods for the synthesis of peptide aldehydes is the treatment of an

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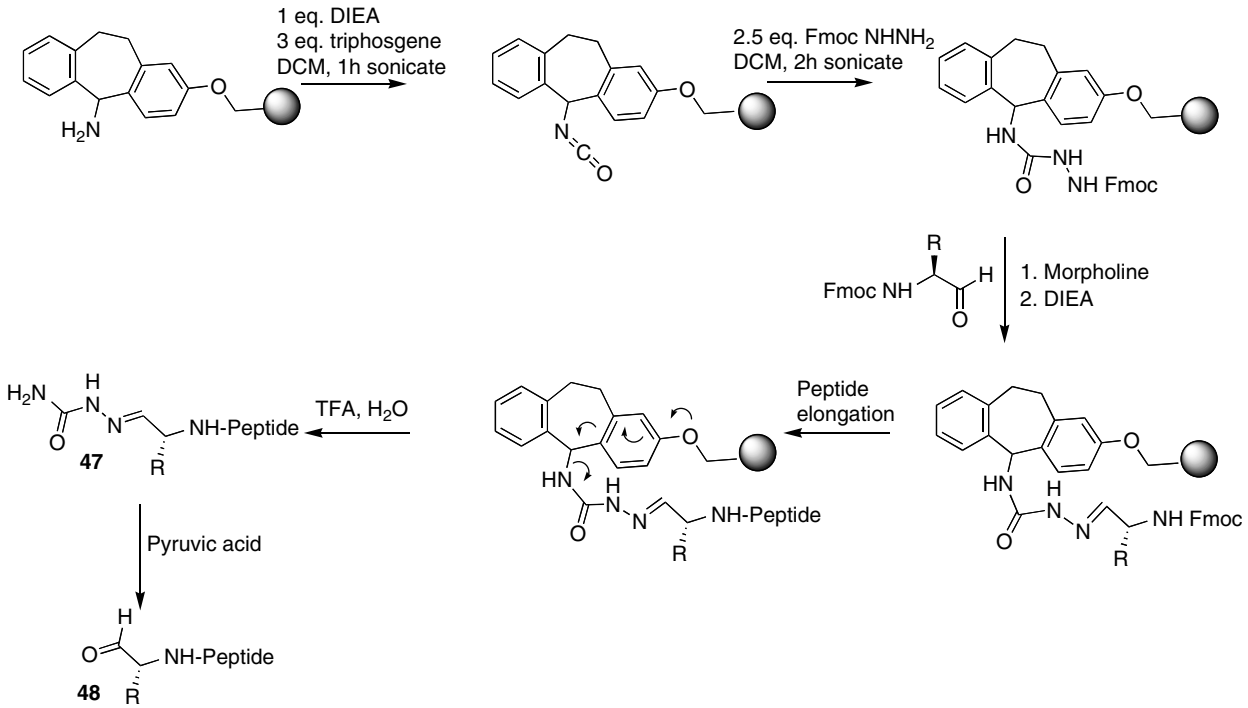
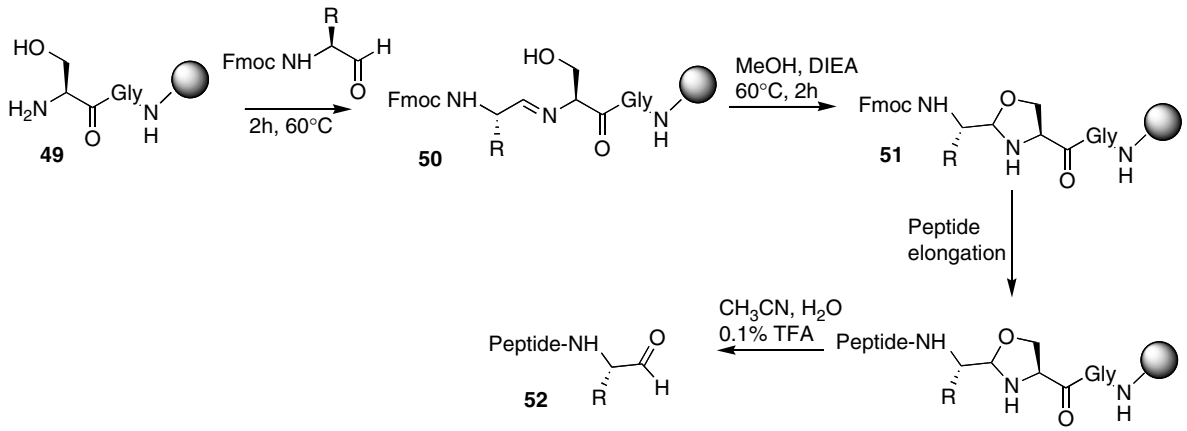
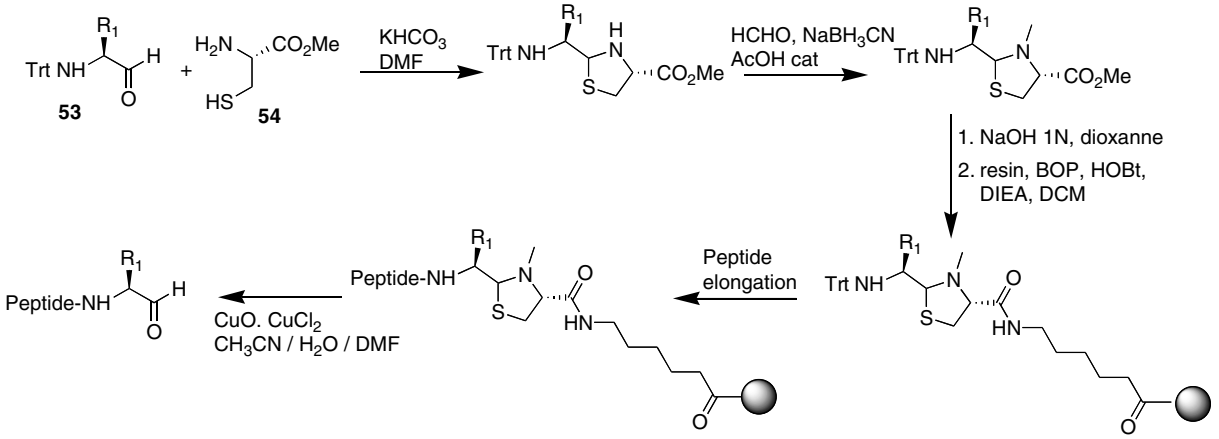


Scheme 16 Protection via a semicarbazone moiety on solid support described by Patterson *et al.*



Scheme 17 Protection via an oxazolidine moiety on solid support.



Scheme 18 Protection via an *N*-methylthiazolidine moiety.

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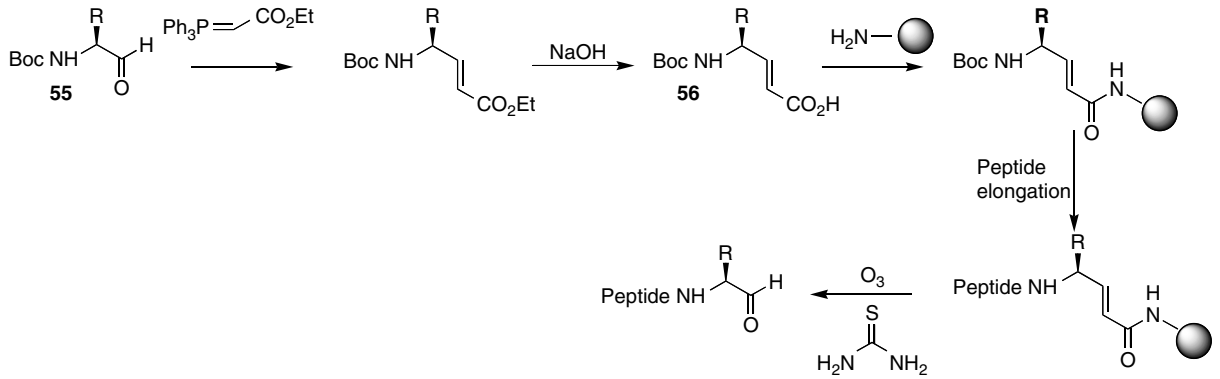
ethylenic compound linked to a solid support by ozone (Scheme 19). The resulting ozonide is then treated by thiourea to yield the corresponding aldehyde. The *N*-protected α,β-unsaturated γ-amino acid **56** is synthesized by a Wittig reaction between the carbethoxymethylene triphenylphosphorane and the *N*-protected α-amino aldehyde **55** followed by saponification to yield the corresponding ethylenic compound, which can be anchored to the solid support [39]. After removal of the *N*-protecting group, elongation by classical methods of solid-phase peptide synthesis (Boc/benzyl or Fmoc/*tert*-butyl strategies) is possible. The model tripeptide aldehyde Boc-Phe-Val-Ala-H was synthesized. This peptide was analyzed by RP-HPLC and studied by ^{1}H NMR in $CDCl_3$ without purification. The results showed that peptide aldehydes with high purity can be obtained using this strategy with no detectable trace epimerization (within the limit of ^{1}H NMR sensitivity).

The use of this strategy implies the synthesis of a linker for each different amino acid. As shown by Frechet *et al.* [40] and Hodges *et al.* [41], Wittig reactions between a phosphonium salt-containing polymer and an aldehyde to produce an unsaturated compound linked to the polymer is possible. This approach was explored for the synthesis of peptide aldehydes by ozonolysis [42]. The strategy consists in anchoring a Wittig–Horner reagent on the solid support. Then reaction with the soluble *N*-protected α-amino aldehyde is performed directly on the solid support.

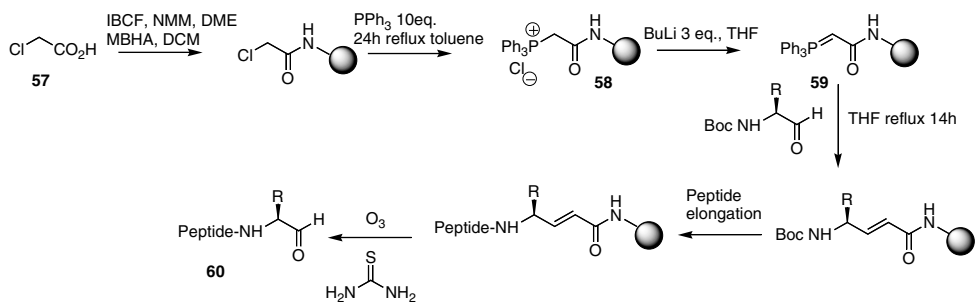
Protection via an olefin moiety using a Wittig-type resin. The route (Scheme 20) involves the anchoring of chloroacetic acid **57** on MBHA resin with isobutylchloroformate (IBCF) as an activating agent followed by the reaction of the modified resin with triphenylphosphine to form the phosphonium salt **58**. The phosphorane **59** is formed with butyl lithium or potassium *tert*-butylate [43]. After reaction with the *N*-protected α-amino aldehyde, elongation of the peptide can be performed. The derivatized peptidyl resins are then subjected to an ozone stream and the peptide aldehydes **60** recovered, as previously described. A similar approach was developed by Hall *et al.* [44] for the combinatorial synthesis of peptide aldehydes.

Protection via an olefin moiety using a Wittig–Horner-type resin. The synthetic pathway (Scheme 21) consists in anchoring diethylphosphonoacetic acid **61** on MBHA resin with benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) as coupling reagent. The carbanion is then generated with various bases as described in the literature [45] and the following steps achieved as previously described.

For both strategies described in the sections "Protection via an olefin moiety using a Wittig-type resin" and "Protection via an olefin moiety using a Wittig–Horner-type resin", the study was performed on the model



Scheme 19 Protection via an olefin moiety using a classical resin.



Scheme 20 Protection via an olefin moiety using a Wittig-type resin.

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peptide Boc-Phe-Val-Ala-H and various conditions were tested. All HPLC chromatograms of the crudes showed a high degree of purity. Surprisingly, ^{1}H NMR analysis of the aldehydic proton signals indicated some epimerization of the α-carbon of the *C*-terminal residue. When the triphenylphosphonium salt was used, concentration of butyllithium did not have any effect on the epimerization of the resulting aldehyde. The use of potassium *tert*-butylate did not improve the reaction (yield and epimerization). Epimerization was reduced to a few percent when the phosphorane linked to resin was washed by THF and DCM before addition of the *N*-protected α-amino aldehyde, but the yield decreased. The best conditions were found using 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 less than 10%.

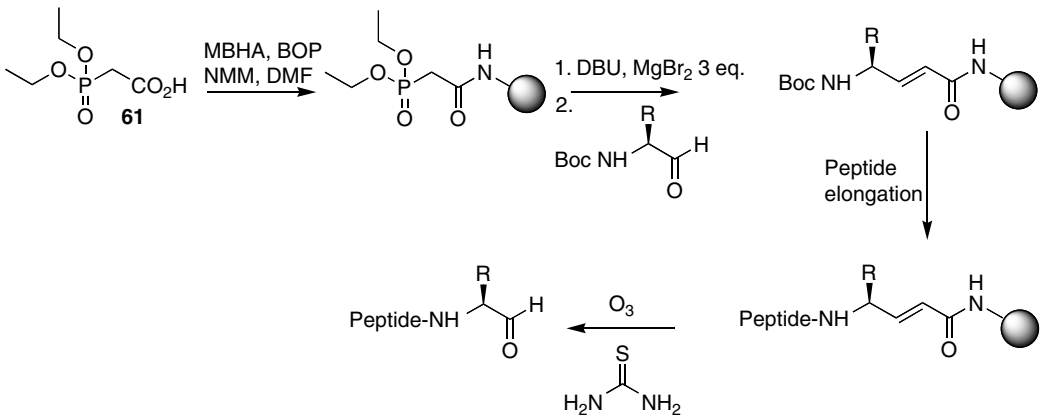
Protection Via an Acetal on a BAL Solid Support

The BAL strategy was proposed by Jensen *et al.* [46]. The first step is the on-resin reductive amination. The next step consists in the acylation of the secondary amine attached to the handle by the penultimate amino acid residue of the desired peptide. After peptide elongation, treatment of the peptidyl resin for 2 h with trifluoroacetic acid (TFA)/H_2O (19/1) cleaves the acid labile side-chain protecting groups and concomitantly the acetal protects the *C*-terminal aldehyde moiety, resulting in the release of the peptide **62** from the solid support (Scheme 22).

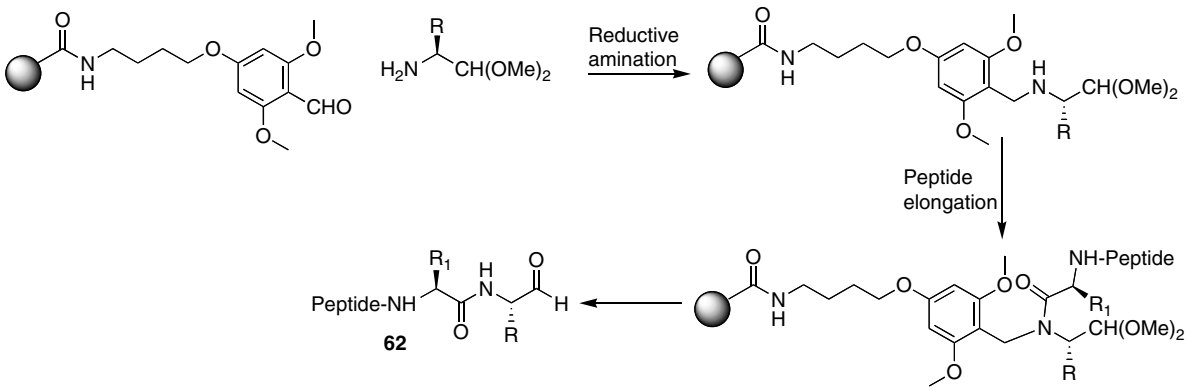
This method has been recently exemplified using 1,3-dioxalane as the acetal-protecting group [47] for the aldehyde moiety.

Synthesis of Peptide Aldehydes Containing an Arginine Residue at the *C*-terminus Via the Protection of the Boc N^g-Nitro-L-Argininal

In solution. Serine protease inhibitors generally contain an arginine residue at the *C*-terminus of their sequence, and their synthesis is always a challenge. Tamura [48] *et al.* proposed a general method for the synthesis of these peptide arginals, which utilizes the N^g-nitro-L-arginal ethyl aminal·HCl **65** as starting material (Scheme 23). This procedure uses the fact that protected argininal can form an aminal moiety by reaction between the aldehyde function and the δ-amino group. Treatment of Boc N^g-nitro-L-arginal **63** with ethanol in the presence of a catalytic amount of hydrochloric acid produces the corresponding aminal **64** as a mixture



Scheme 21 Protection via an olefin moiety using a Wittig–Horner-type resin.



Scheme 22 Synthesis via a BAL strategy.

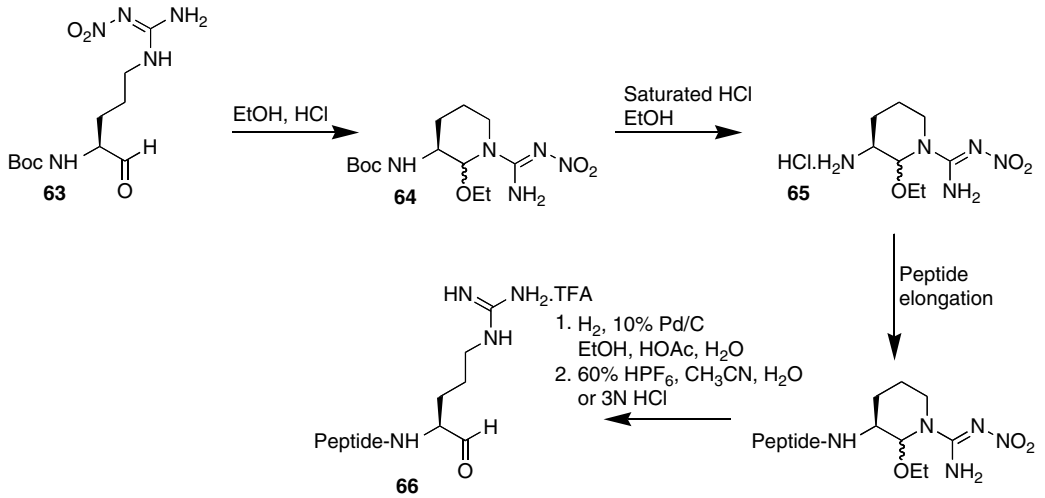
DOI: 10.1002/psc

of anomers. The Boc group is then cleaved with HCl in ethanol to afford the corresponding amino aminals **65**. After peptide elongation, hydrogenation followed by selective hydrolysis with HPF_6 produces the final aldehyde **66**. Hydrogenation followed by hydrolysis with 3.0 N HCl also afforded the peptide aldehyde, but in a lower yield.

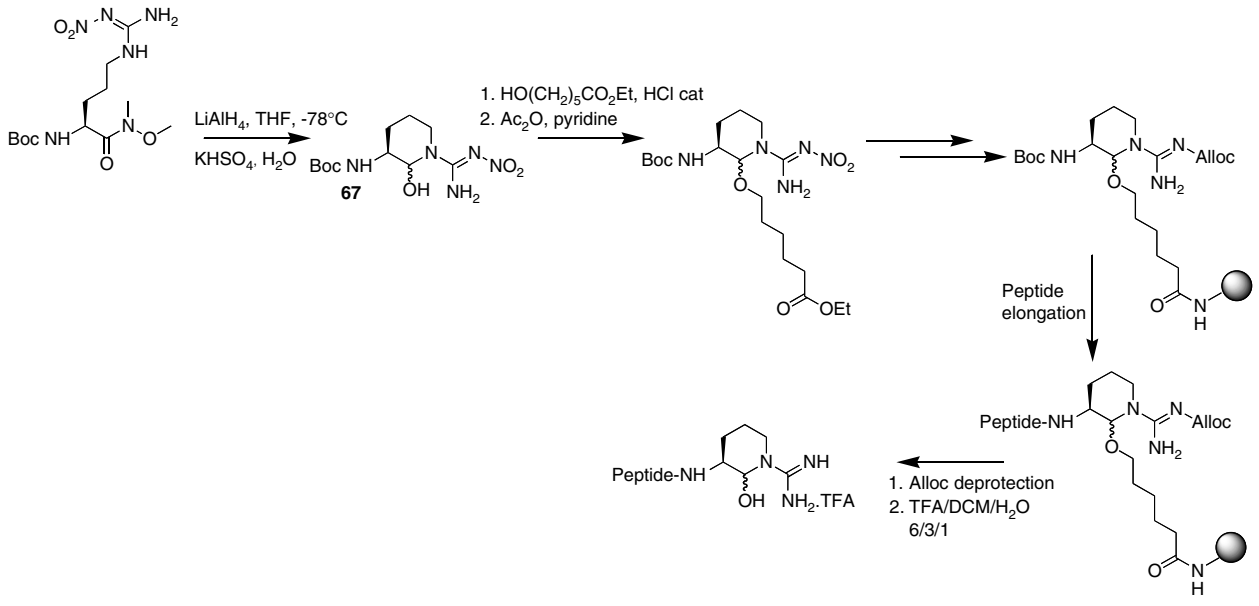
On solid support. This method has been transposed on solid support [49]. The hydroxyl function generated on compound **67** is tethered with ethyl-6-hydroxyhexanoate by acid catalysis. After hydrogenolysis of the nitro group, reprotection of the guanidino moiety by an Alloc group and ester hydrolysis, the aldehyde precursor is ready for anchoring to the solid support. Peptide elongation is achieved by using classical Fmoc chemistry. Palladium(0)-catalyzed removal of the N^{ω}-Alloc moiety is followed by hydrolysis with a $TFA/CH_2Cl_2/H_2O$ cocktail to cleave the linker and any acid-labile side-chain protecting group (Scheme 24). The peptide aminal can then be hydrolyzed to generate the desired aldehyde.

Synthesis of Peptide Aldehydes Containing an Aspartic Residue at the C-terminus

Chapman described the synthesis of an interleukin-1β-converting enzyme inhibitor [50] containing a *C*-terminal aspartyl aldehyde. The synthesis of such derivatives starts from commercially available aspartic



Scheme 23 Synthesis of peptide aldehydes containing an arginine residue at the *C*-terminus via the protection of the Boc N^{g}-nitro-L-arginal in solution.



Scheme 24 Synthesis of peptide aldehydes containing an arginine residue at the *C*-terminus via the protection of the Boc N^{g}-nitro-L-argininal on solid support.

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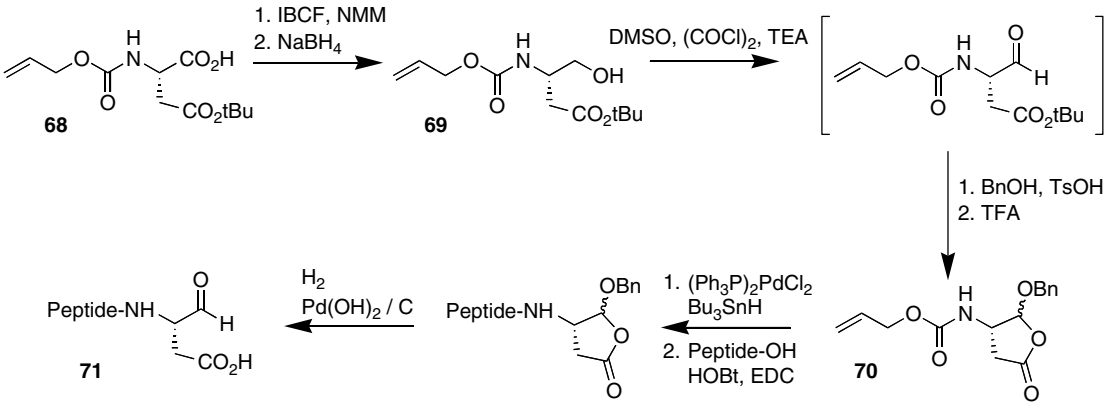
acid *β-tert*-butyl ester **68**, which is *N*-protected with alloc group in the presence of sodium bicarbonate. The carboxylic acid is then converted into a mixed anhydride by reaction with isobutyl chloroformate in the presence of *N*-methylmorpholine, and reduced to the corresponding alcohol **69** with sodium borohydride (Scheme 25). The alcohol is oxidized to the aldehyde under Swern conditions [51] and immediately treated with benzyl alcohol and *p*-toluenesulphonic acid. After 16 h, addition of trifluoroacetic acid promotes cyclization into the desired *O*-benzylacylal **70**, which is isolated as a 1 : 1 mixture of diastereoisomers. Using the peptide as the proton source [52] for the Alloc removal, addition of Bu_3SnH and $(Ph_3P)_2PdCl_2$ rapidly removes the Alloc group. The crude material is coupled with the desired peptide in the presence of hydroxybenzotriazole and ethyl dimethylaminopropyl carbodiimide to afford the corresponding elongated peptide. 400 MHz ^1H NMR in CD_3OD shows the presence of two diastereoisomeric hemiacetals and no epimerization at the α-carbon. Hydrogenolysis of this peptide *O*-benzylacylal using Pearlman's catalyst in methanol affords the desired inhibitor **71** (Scheme 25).

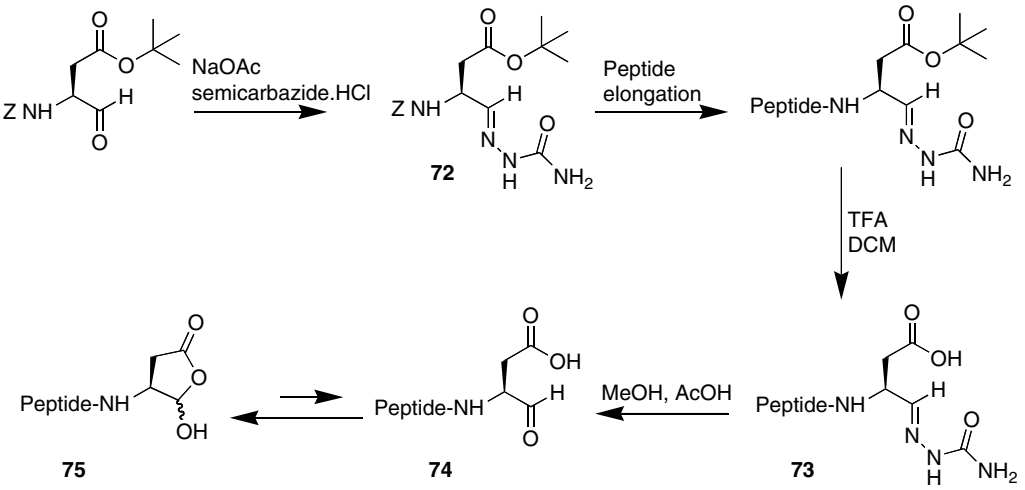
Graybill *et al.* [53] chose to investigate the use of stereochemically stable semicarbazone derivatives **72** as a masqued aldehyde equivalent, according to Scheme 26. In contrast to *O*-benzylacylal, semicarbazone displays excellent stability and is neither volatile nor hydroscopic. It can be used in a variety of typical peptide-coupling protocols. A further advantage is that the elongated product is obtained as a single stereoisomer rather than a 1 : 1 mixture of diastereoisomers, thus simplifying purification and multistep synthesis. Smooth conversion of semicarbazone **73** to aldehyde **74** is performed by treatment with a 5 : 5 : 1 mixture of methanol/acetic acid/37% aqueous formaldehyde followed by an aqueous workup. The ^1H NMR spectrum (CD_3OD) shows a 1 : 1 mixture of diastereomeric cyclic hemiacetals **75** and no trace of free aldehyde.

CONCLUSIONS

Many strategies have been developed for the modification of peptides by aldehyde groups. The solid-phase peptide synthesis and the design of new linkers have



Scheme 25 Synthesis of peptide aldehydes containing an aspartic residue at the *C*-terminus according to Chapman.



Scheme 26 Synthesis of peptide aldehydes containing an Aspartic residue at the *C*-terminus according to Graybill *et al.*

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facilitated access to peptide aldehydes, using both strategies. However, there is still a need for novel methods that allow the synthesis of peptide aldehydes of high chemical and optical purity. Owing to the importance of the potential applications of these compounds, we believe that peptide and protein chemists will continue their research in this field.

REFERENCES

1. Jacobson KA, Marr-Leisy D, Rosenkranz RP, Verlander MS, Melmon KL, Goodman M. Conjugates of catecholamines. 1. *N*-Alkyl-functionalized carboxylic acid congeners and amides related to isoproterenol. *J. Med. Chem.* 1983; **26**: 492–499.
2. Spetzler JC, Tam JP. Unprotected peptides as building blocks for branched peptides and peptide dendrimers. *Int. J. Pept. Protein Res.* 1995; **45**: 78–85.
3. Tam JP, Rao C, Liu C-F, Shao J. Specificity and formation of unusual amino acids of an amide ligation strategy for unprotected peptides. *Int. J. Pept. Protein Res.* 1995; **45**: 209–216.
4. Canne LE, Ferre- D'Amare AR, Burley SK, Kent SBH. Total chemical synthesis of a Unique transcription factor-related protein: cMyc-max. *J. Am. Chem. Soc.* 1995; **117**: 2998–3007.
5. Rose K. Facile synthesis of homogeneous artificial proteins. *J. Am. Chem. Soc.* 1994; **116**: 30–33.
6. King TP, Zhao SW, Lam T. Preparation of protein conjugates via intermolecular hydrazone linkage. *Biochemistry* 1986; **25**: 5774–5779.
7. Aoyagi T, Takeuchi T, Matsuzaki A, Kawamura K, Kondo S, Hamada M, Maeda K, Umezawa H. Leupeptins, new protease inhibitors from actinomycetes. *J. Antibiot.* 1969; **22**: 283–286.
8. Basak A, Jean F, Seidah NG, Lazure C. Design and synthesis of novel inhibitors of prohormone convertases. *Int. J. Pept. Protein Res.* 1994; **44**: 253–261.
9. Sarubbi E, Seneci PF, Angelastro MR, Peet NP, Denaro M, Islam K. Peptide aldehydes as inhibitors of HIV protease. *FEBS Lett.* 1993; **319**: 253–256.
10. Fehrentz J-A, Heitz A, Castro B, Cazaubon C, Nisato D. Aldehydic peptides inhibiting renin. *FEBS Lett.* 1984; **167**: 273–276.
11. Fehrentz J-A, Heitz A, Castro B. Synthesis of aldehydic peptides inhibiting renin. *Int. J. Pept. Protein Res.* 1985; **26**: 236–241.
12. Ganneau C, Moulin A, Demange L, Martinez J, Fehrentz J-A. The epimerization of peptide aldehydes – a systematic study. *J. Pept. Sci.* 2006; **12**: 497–501.
13. Hamada Y, Shioiri T. New methods and reagents in organic synthesis. 29. A practical method for the preparation of optically active *N*-protected α-amino aldehydes and peptide aldehydes. *Chem. Pharm. Bull.* 1982; **30**: 1921–1924.
14. Sorg G, Thern B, Mader O, Rademann J, Jung G. Progress in the preparation of peptide aldehydes via polymer supported IBX oxidation and scavenging by threonyl resin. *J. Pept. Sci.* 2005; **11**: 142–152.
15. Guichard G, Briand JP, Friede M. Synthesis of arginine aldehydes for the preparation of pseudopeptides. *Pept. Res.* 1993; **6**: 121–124.
16. Fehrentz J-A, Paris M, Heitz A, Velek J, Liu C-F, Winternitz F, Martinez J. Improved solid phase synthesis of *C*-terminal peptide aldehydes. *Tetrahedron Lett.* 1995; **36**: 7871–7874.
17. Tong XH, Hong A. Solid phase synthesis of aspartyl peptide aldehydes. *Tetrahedron Lett.* 2000; **41**: 8857–8860.
18. Higaki JN, Chakravarty S, Bryant CM, Cowart LR, Harden P, Scardina JM, Mavunkel B, Luedtke GR, Cordell B. A combinatorial approach to the identification of dipeptide aldehyde inhibitors of β-amyloid production. *J. Med. Chem.* 1999; **42**: 3889–3898.
19. Salvino JM, Mervic M, Mason HJ, Kiesow T, Teager D, Airey J, Labaudiniere R. Parallel synthesis of aldehydes and ketone facilitated by a new solid-phase Weinreb amide. *J. Org. Chem.* 1999; **64**: 1823–1830.
20. Nahm S, Weinreb SM. *N*-Methoxy-*N*-methylamides as effective acylating agents. *Tetrahedron Lett.* 1981; **22**: 3815–3818.
21. Douat C, Heitz A, Martinez J, Fehrentz J-A. Synthesis of *N*-protected α-amino aldehydes from their morpholine amide derivatives. *Tetrahedron Lett.* 2000; **41**: 37–40.
22. Zlatoidsky P. Preparation of Boc-amino-acid or peptide aldehydes via reduction of corresponding phenyl esters. *Helv. Chim. Acta* 1994; **77**: 150–154.
23. Fuller WD, Cohen MP, Shabankareh M, Blair RK, Goodman M, Naider FR. Urethane protected amino acid *N*-carboxyanhydrides and their use in peptide synthesis. *J. Am. Chem. Soc.* 1990; **112**: 7414–7416.
24. Xue CB, Naider F. Application of *N*-(*tert*-butyloxycarbonyl)amino acid *N*-carboxyanhydrides in solid-phase peptide synthesis. *J. Org. Chem.* 1993; **58**: 350–355.
25. Chevallet P, Fehrentz J-A, Kiec-Kononowicz K, Devin C, Castel J, Loffet A, Martinez J. Synthesis of chiral *N*-protected amino acid esters by the use of UNCAs. *Lett. Pept. Sci.* 1996; **2**: 297–300.
26. Fehrentz J-A, Paris M, Heitz A, Velek J, Winternitz F, Martinez J. Solid phase synthesis of *C*-Terminal peptide aldehydes. *J. Org. Chem.* 1997; **62**: 6792–6796.
27. Wyslouch-Cieszynska A, Tam PT. In *Peptides: Frontiers of Peptide Science, Proceedings of the Fifteenth American Peptide Symposium*, Tam PJ, Kaumaya PTP (eds). Kluwer Academic Publishers; The Netherlands, Dordrecht, 1999; 263–264.
28. Galeotti N, Giraud M, Jouin P. Solid phase synthesis of peptidyl aldehydes from *C*-terminal thiazolidinyl peptides. *Lett. Pept. Sci.* 1997; **4**: 437–440.
29. Yde B, Yousif NM, Pedersen U, Thomsen I, Lawesson SO. Studies on organophosphorus compounds. XLVII. Preparation of thiated synthons of amides, lactams and imides by use of some new phosphorus- and sulfur-containing reagents. *Tetrahedron* 1984; **40**: 2047–2052.
30. Dondoni A, Marra A, Perrone D. Efficacious modification of the procedure for the aldehyde release from 2-substituted thiazoles. *J. Org. Chem.* 1993; **58**: 275–277.
31. Garrett GS, Correa PE, McPhail SJ, Tornheim K, Burton JA, Eickhoff DJ, Engerholm GG, McIver JM. Peptide aldehyde inhibitors of the kallikreins: an investigation of subsite interactions with tripeptides containing structural variations at the amino terminus. *J. Pept. Res.* 1998; **52**: 60–71.
32. Murphy AM, Dagnino R Jr, Vallar PL, Trippe AJ, Sherman SL, Lumpkin RH, Tamura SY, Webb TR. Automated synthesis of peptide *C*-terminal aldehydes. *J. Am. Chem. Soc.* 1992; **114**: 3156–3157.
33. Dagnino R Jr, Webb TR. Improved synthesis of arginine peptide aldehydes. *Tetrahedron Lett.* 1994; **35**: 2125–2128.
34. Linton SD, Karanewsky DS, Ternansky RJ, Wu JC, Pham B, Kodandapani L, Smidt R, Diaz J-L, Fritz LC, Tomaselli KJ. Acyl dipeptides as reversible caspase inhibitors. Part 1: initial lead optimization. *Bioorg. Med. Chem. Lett.* 2002; **12**: 2969–2971.
35. Ramage R, Irving SL, McInnes C. Design of a versatile linker for solid phase peptide synthesis: synthesis of *C*-terminal primary/secondary amides and hydrazides. *Tetrahedron Lett.* 1993; **34**: 6599–6602.
36. Patterson JA, Ramage R. Solid phase synthesis of peptide *C*-terminal semicarbazones and aldehydes. *Tetrahedron Lett.* 1999; **40**: 6121–6124.
37. Ede NJ, Bray AM. A simple linker for the attachment of aldehydes to the solid phase. Application to solid phase synthesis by the Multipin method. *Tetrahedron Lett.* 1997; **38**: 7119–7122.
38. Gros C, Boulegue C, Galeotti N, Niel G, Jouin P. Stereochemical control in the preparation of α-amino *N*-methylthiazolidine masked aldehydes used for peptide aldehydes synthesis. *Tetrahedron* 2002; **58**: 2673–2680.

DOI: 10.1002/psc

39. Pothion C, Paris M, Heitz A, Rocheblave L, Rouch F, Fehrentz J-A, Martinez J. Use of ozonolysis in the synthesis of *C*-terminal peptide aldehydes on solid support. *Tetrahedron Lett.* 1997; **38**: 7749–7752.
40. Schuerch C, Frechet JM. Solid-phase synthesis of oligosaccharides. I. Preparation of the solid support. Poly[p-(1-propen-3-ol-1-yl)styrene]. *J. Am. Chem. Soc.* 1971; **93**: 492–496.
41. Hodge P, Waterhouse J. Chemical modification of chloromethylated crosslinked polystyrene via phase transfer catalyzed Wittig reactions. *Polymer* 1981; **22**: 1153–1154.
42. Paris M, Heitz A, Guerlavais V, Cristau M, Fehrentz J-A, Martinez J. Synthesis of peptide aldehydes on solid support using ozonolysis. *Tetrahedron Lett.* 1998; **39**: 7287–7290.
43. Hird NW, Irie K, Nagai K. Solid phase synthesis of 2-aminobutadienes using a piperazine linker. *Tetrahedron Lett.* 1997; **38**: 7111–7114.
44. Hall BJ, Sutherland JD. A practical method for the combinatorial synthesis of peptide aldehydes. *Tetrahedron Lett.* 1998; **39**: 6593–6596.
45. Blanchette MA, Choy W, Davis JT, Essenfeld AP, Masamune S, Roush WR, Sakai T. Horner-Wadsworth-Emmons reaction: use of lithium chloride and an amine for base-sensitive compounds. *Tetrahedron Lett.* 1984; **25**: 2183–2186.
46. Jensen KJ, Alsina J, Songster MF, Vagner J, Albericio F, Barany G. Backbone amide linker strategy for solid-phase synthesis of *C*-Terminal-modified and cyclic peptides. *J. Am. Chem. Soc.* 1998; **120**: 5441–5452.
47. Kappel JC, Barany G. Backbone amide linker (BAL) strategy for Nα-9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis of peptide aldehydes. *J. Pept. Sci.* 2005; **11**: 525–535.
48. Tamura SY, Semple JE, Ardecky RJ, Leon P, Carpenter SH, Ge Y, Shamblin BM, Weinbouse MI, Ripka WC, Nutt RF. Novel and general method for the preparation of peptidyl argininals. *Tetrahedron Lett.* 1996; **37**: 4109–4112.
49. Siev DV, Gaudette JA, Semple JE. Novel protocol for the solid-phase synthesis of peptidyl and peptidomimetic P1-argininal derivatives. *Tetrahedron Lett.* 1999; **40**: 5123–5127.
50. Chapman KT. Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* 1992; **2**: 613–618.
51. Mancuso AJ, Huang S-L, Swern D. Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide activated by oxalyl chloride. *J. Org. Chem.* 1978; **43**: 2480–2482.
52. Dangles O, Guibe F, Balavoine G, Lavielle S, Marquet A. Selective cleavage of the allyl and (allyloxy)carbonyl groups through palladium-catalyzed hydrostannolysis with tributyltin hydride. Application to the selective protection-deprotection of amino acid derivatives and in peptide synthesis. *J. Org. Chem.* 1987; **52**: 4984–4993.
53. Graybill TL, Dolle RE, Helaszek CT, Miller RE, Ator MA. Preparation and evaluation of peptidic aspartyl hemiacetals as reversible inhibitors of interleukin-1β converting enzyme (ICE). *Int. J. Pept. Protein Res.* 1994; **44**: 173–182.