Solid Phase Synthesis of C-Terminal Peptide Aldehydes

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Peptides with C-terminal aldehydes (PAs) are of interest due to their inhibitory properties toward numerous classes of proteolytic enzymes. In this paper, we describe and compare two novel approaches for the preparation of PAs by solid phase synthesis, one based on the reduction of the Weinreb amide and the other on the reduction of phenyl esters. A study showed that purification of the PAs by chromatography (silica gel or reversed phase) induced a loss of the optical integrity of the C-terminal residue. Both methods were found to be suitable for the synthesis of PAs which were then used for the preparation of reduced-bond-containing peptides on insoluble polymers. The Weinreb amide approach was preferred for the synthesis of PAs due to an uncontrolled over-reduction of phenyl esters in our hands.

Peptide C-terminal aldehydes (PAs) are of interest due to their inhibitory properties as transition-state analogs toward numerous classes of proteolytic enzymes. Indeed, since the discovery of leupeptin,[1] a natural product that is a potent inhibitor of trypsin, many other classes of enzymes have been inhibited by PAs, such as aspartyl proteases (HIV protease,[2] renin[3]), seryl and thiol proteases,[4] prohormone convertases,[5] and cysteinyl proteases.[6] Further, PAs can be used in pseudopeptide chemistry, particularly for the synthesis of reduced peptides by fragment condensation. In addition, the success of combinatorial chemistry has stimulated research in organic chemistry on solid supports in order to generate various functionalities upon cleavage from the solid support.

Various methods for the solution synthesis of PAs have been described, but only one report concerns solid phase synthesis.[7] The N-protected amino aldehyde is first synthesized, and then the aldehyde function is protected as its semicarbazone that is linked to the solid support *via* a carboxylic function. The solid phase synthesis strategy can then be applied. After the synthesis of the peptide, the protected peptide semicarbazone is treated with aqueous acid/formaldehyde to regenerate the aldehydic function cleaved from the support. This rather complicated procedure has not yet been widely applied.

We have investigated alternative routes for the preparation of PAs and side-chain protected PAs in solid phase synthesis. Two new linkers that are stable under classical Fmoc or Boc strategies have been developed in order to obtain the peptide aldehyde after deprotection from the solid support. One of these linkers was conceptualized on the basis of the Weinreb amide[8] and the other on the basis of phenolic esters.[9] The use of these two different approaches is demonstrated by the synthesis of N-protected α-amino aldehydes and PAs.

Weinreb Amide-Based Linker. Among all the described preparations of N-protected amino aldehydes and peptide aldehydes, the reduction of Weinreb amides is widely used. This method was successfully applied to the synthesis of N-protected α-amino aldehydes and PAs using Z, Boc, and Fmoc chemistry. On the basis of this stable intermediate, it was reasonable to apply this strategy in the solid phase synthesis of PAs.[10] The synthesis of the linker is illustrated in Scheme 1. Methoxy amine **1** was reacted with benzyl acrylate, and the resulting alkylated methoxy amine **2** was protected by the *tert*-butyloxycarbonyl group to yield **3**. After deprotection of the benzyl ester by hydrogenolysis (**4**), the linker was coupled to the solid support (i.e. MBHA resin) with an activating agent to yield the substituted resin **5**. After deprotection of the N-terminal Boc protecting group, elongation by classical Boc or Fmoc amino acids was possible on the solid support.

In order to validate this methodology, we have synthesized Boc-Ala-H and Boc-Phe-H by this method. These compounds were purified according to the procedure described by Ho and Ngu[11] on a silica gel column, with organic solvents in the presence of 0.1% pyridine as eluent to prevent racemization during purification. As reported in Table 1, the optical purity of the α-amino aldehydes thus obtained was similar to that obtained by conventional solution synthesis.[12]

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Scheme 1. Synthesis of the Weinreb Amide Linker

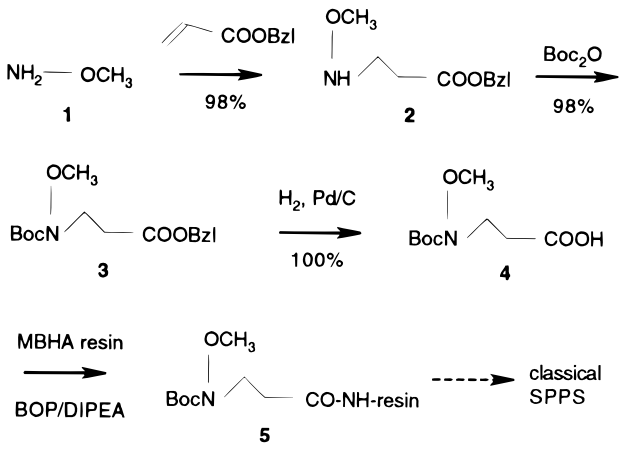
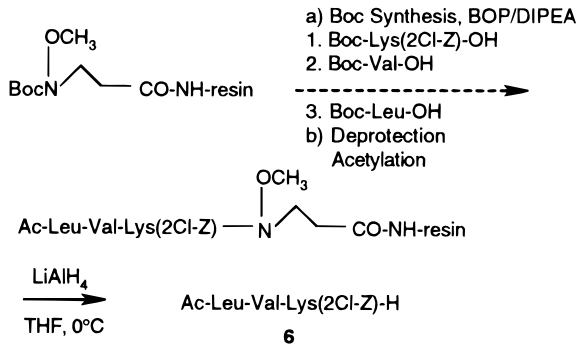


Table 1. Physical Characteristics of N-Protected α-Amino Aldehydes and PAs Obtained with the Weinreb Amide Linker

aldehyde peptide	$[\alpha]_D$ (*c* 1, MeOH)/lit	% yield[a]	HPLC[b] t_R, min
Boc-Ala-H	−32/−34.1[c]	40	38.4
Boc-Phe-H	−46/−44.4[c]	40	38.2
Boc-Phe-Val-Ala-H	−21[d]	40	25.7
Boc-Leu-Leu-Lys(2Cl-Z)-H	−50[e]	40	32.1
Ac-Leu-Val-Lys(2Cl-Z)-H	−42[d]	25	44.95
Z-Val-Phe-H	−45[d]	30	29.1

[a] The yields are calculated after purification (silica gel for N-protected α-amino aldehydes and HPLC for PAs) based on the substitution of the commercial resin. [b] Retention time on analytical reversed phase HPLC, Merck-Hitachi apparatus, detection 220 nm, flow rate 1 mL/min, gradient mode from 100% A to 100% B in 50 min, A: TFA 0.1% in H_2O, and B: TFA 0.1% in CH_3CN. [c] Reference 12. [d] After RP C18 HPLC purification. [e] Purified by crystallization in diethyl ether.

Scheme 2. Synthesis of Tripeptide Aldehydes *via* the Weinreb Amide Linker



We have also synthesized peptide aldehydes on the solid support as indicated in Scheme 2 and Table 1. The aldehydic tripeptide Boc-Phe-Val-Ala-H **10** was synthesized as a control peptide for the comparison of the methods and for the epimerization study. Dipeptides Z-Val-Phe-H and Z-Val-Ala-H were described as potential inhibitors of aspartic proteases specifically as HIV protease inhibitors,[13] and Ac-Leu-Leu-Lys-H or Ac-Leu-Val-Lys-H were reported as leupeptin analogues.[14] The tripeptide Boc-Phe-Val-Ala-H **10** was synthesized from the $NH(OCH_3)CH_2CH_2CO$-MBHA-resin. Elongation of the peptide chain was performed using Boc-Ala-OH, Boc-Val-OH, and Boc-Phe-OH with BOP as coupling reagent.[15] The tripeptide aldehyde Boc-Phe-Val-Ala-H was removed from the resin by treatment with $AlLiH_4$ followed by hydrolysis. The tripeptide aldehydes Boc-Leu-Leu-Lys(2Cl-Z)-H and Ac-Leu-Val-Lys(2Cl-Z)-H were obtained according to the same procedure. The dipeptide aldehyde Z-Val-Phe-H was synthesized from the $NH(OCH_3)CH_2CH_2CO$-MBHA-resin with Boc-Phe-OH and Z-Val-OH. As previously described,[16] due to the presence of several amide functions, the amount of $LiAlH_4$ equivalents has to be increased with the length of the peptide. In all reactions, no over-reduction was observed, confirming the formation of the stable metal-chelated intermediate described by Nahm and Weinreb.[8] Furthermore, during the reduction no aldehyde derivative could be detected in the supernatant of the reaction mixture before quenching. After hydrolysis, peptide aldehydes were detected by TLC. The crudes were studied by reverse-phase HPLC (Figure 1) and by ^1H NMR. Examination of the ^1H NMR spectra revealed the presence of a single aldehydic proton signal, indicating the absence of epimerization and the presence of some impurities (traces).

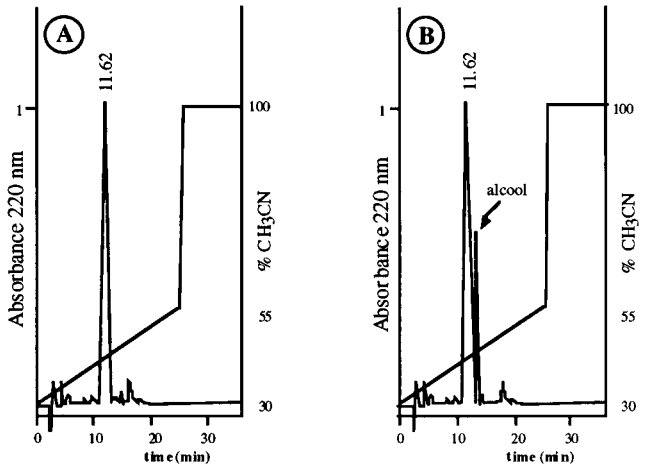


Figure 1. Reverse-phase HPLC chromatogram of the crude Boc-Phe-Val-Ala-H **10** synthesized (A) *via* the Wienreb amide linker, (B) *via* the phenyl ester linker.

Phenyl Ester Based Linker. During the last few years, a variety of new methods for the preparation in solution of N-protected α-amino aldehydes and PAs have been published, indicating the interest of such molecules. The apparent simplicity of the recently reported method of Zlatoidsky[9] which involves reduction of phenyl esters merited further investigation. Since 4-hydroxybenzoic acid is commercially available, we decided to explore this reaction in the solid phase strategy.

4-Hydroxybenzoic acid was condensed to N-protected amino acid carboxyanhydrides (UNCAs)[17] leading to the corresponding N-protected amino esters **7**. These compounds were directly anchored to the resin **8**. Classical peptide elongation on solid support could then be performed (Scheme 3).

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Scheme 3. Synthesis of the Phenyl Ester Linker

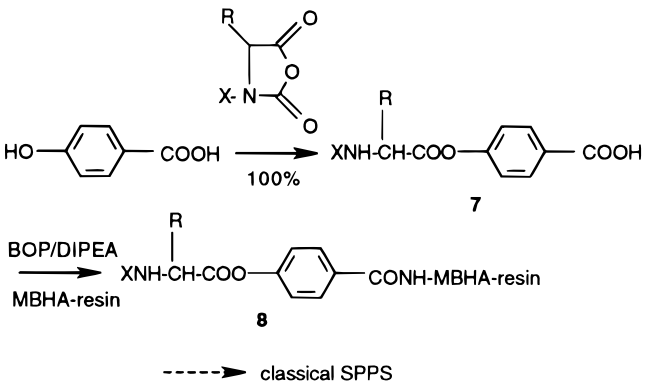

Table 2. Synthesis of PAs Obtained with the Phenyl Ester Linker

aldehyde peptide	% yield[a,b]	aldehyde/ alcohol[c]	HPLC[d] t_R, min
Boc-Phe-Val-Ala-H	60/45	75/25	25.7
Z-Val-Ala-H	60/30	50/50	21.0
Ac-Leu-Leu-Lys(Z)-H	51	[e]	45.0
Z-Val-Phe-H	50/45	75/25	29.3
Boc-Val-Val-H	46/30	0/100	ND

[a] Crude yield. [b] Yield after silica gel or RP C18 HPLC purification. [c] Calculated from ^{1}H NMR spectra. [d] Retention time of the aldehyde peptide on analytical reversed phase HPLC, Merck-Hitachi apparatus, detection 220 nm, flow rate 1 mL/min, gradient mode from 100% A to 100% B in 50 min, A: TFA 0.1% in H_2O, and B: TFA 0.1% in CH_3CN. [e] Used without purification for the synthesis of **9**.

N-Protected α-amino aldehydes were synthesized in solid phase as described by Zlatoidsky by reduction with $AlLiH(OtBu)_3$ of the corresponding N-protected α-amino phenyl esters linked to the support. In all our attempts we have obtained a mixture of the aldehyde and the alcohol. Reduction of N-protected amino phenyl esters led to the aldehyde as a major compound but also to a minor contamination by the corresponding alcohol.

Several PAs were synthesized by reduction of the peptide-linked-resin moiety. The peptides are reported in Table 2. Both the presence of peptide aldehyde and alcohol was observed. This phenomenon can be explained by the over-reduction of the corresponding aldehyde in the presence of the excess of hydride. This strategy has to be used carefully for the synthesis of PAs which are synthesized as enzyme inhibitors; however, it can be used for the preparation of PAs in order to synthesize modified pseudopeptides (reduced bonds, e.g.) as illustrated below.

Studies of the Epimerization of the Carbon in the Position α to the Aldehydic Function. The configuration of the C-terminal residue is important for the biological activities of PAs. As described earlier,[16] the aldehydic signal in ^{1}H NMR studies is a very good indicator of the possible epimerization of this residue in aldehydic peptides containing three or more residues. We decided to study the synthesized PAs by ^{1}H NMR spectrometry to detect if these methods are "racemization free". The crude PAs (Boc-Phe-Val-Ala-H **10**) revealed a single aldehydic proton signal, indicating that each method is suitable for the synthesis of PAs without epimerization in the limits of ^{1}H NMR detection. The crudes were purified either by flash chromatography on silica gel as described above[11] (0.1% pyridine as eluent)or by reversed HPLC,[7,18] in order to remove traces of impurities or the peptide alcohols. Surprisingly, both purified tripeptide aldehydes showed two aldehydic proton peaks (in $CDCl_3$), indicating that some epimerization has occurred during purification by chromatography. To establish that these two peaks corresponded to the two diastereoisomers, we have synthesized the authentic diastereoisomer LLD of the aldehydic tripeptide Boc-Phe-Val-Ala-H **10′** from the Weinreb amide linker. The chemical shift observed for the aldehydic proton signal of this authentic LLD diastereoisomer **10′** (δ = 9.49) conclusively demonstrated that the purified PAs were epimerized during the purification step (Figure 2). It is important to note that the two signals corresponding to the two diastereoisomers of Boc-Phe-Val-Ala-H **10** and **10′** could be observed in $CDCl_3$ and not in DMSO-d_6. This fact could explain that no papers relating the reverse-phase HPLC purification of such peptide aldehydes mentioned a loss of optical purity.

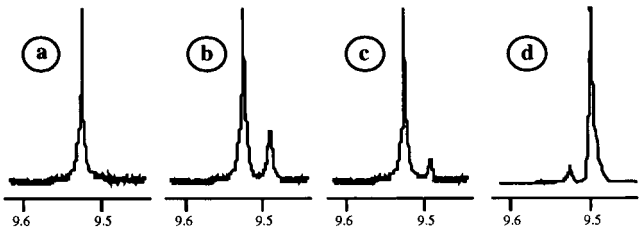

Figure 2. ^{1}H NMR aldehydic proton signal in $CDCl_3$ of (a) crude, (b) silica gel purified, (c) RP HPLC purified aldehydic tripeptide Boc-Phe-Val-Ala-H **10**, and (d) authentic LLD diastereoisomer Boc-Phe-Val-Ala-H **10'**. The double peak is due to some epimerization.

Stability of PAs. It is well known that N-protected α-amino aldehydes are not especially optically stable even at low temperature. We have studied the optical stability of our aldehydic tripeptide Boc-Phe-Val-Ala-H model. It was left at room temperature as a solid, and ^{1}H NMR spectra were performed at 10 days, one month, and one year. No evolution of the aldehydic signal was observed, indicating that this PA was stable in our storage conditions.

Synthesis of Ac-Leu-Leu-LysΨ[CH_2NH]Phe-Asp-Ala-NH_2 9. The hexapeptide containing a reduced peptide bond between residues three and four was synthesized by solid phase strategy using the two different linkers. The aldehydic tripeptide Ac-Leu-Leu-Lys(2-Cl-Z)-H was prepared both from the Weinreb amide and the phenyl ester linker as illustrated in Scheme 4. It was then condensed without any purification in the presence of sodium cyanoborohydride with the tripeptide-resin H-Phe-Asp(OBzl)-Ala-MBHA. The resulting hexapeptide was cleaved in anhydrous HF in the standard conditions to release the desired compound **9**, Ac-Leu-Leu-LysΨ[CH_2NH]Phe-Asp-Ala-NH_2, which was characterized by ^{1}H NMR and mass spectrometry.

The two methods yielded comparable results (Figure 3). The phenyl ester linked resin was also found suitable for generating peptide aldehydes which can be used for further chemistry; the peptide alcohol generated did not interfere with the reduced bond formation and was removed during washing of the resin.

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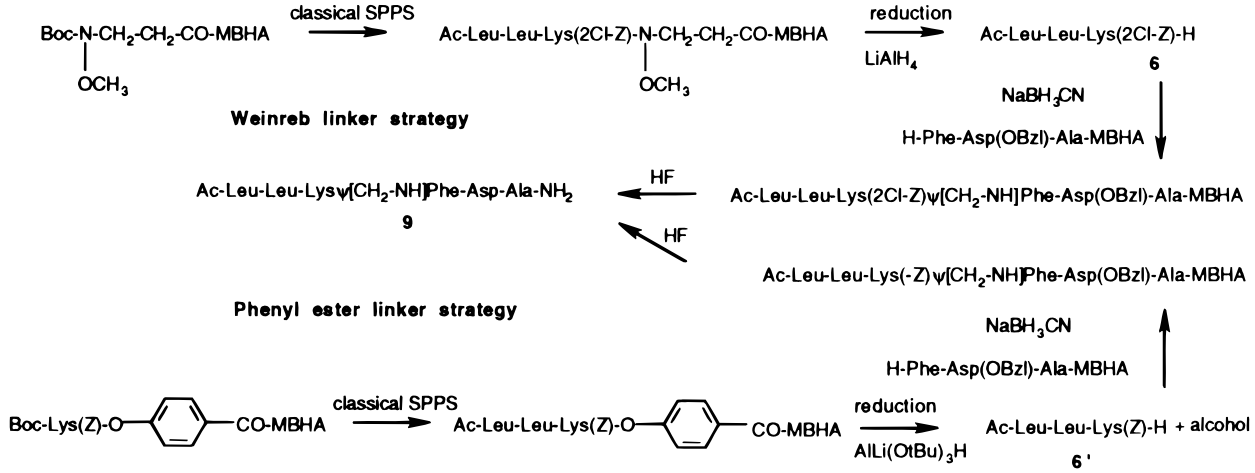
Scheme 4. Synthesis of the Pseudohexapeptide Ac-Leu-Leu-LysΨ[CH_2NH]Phe-Asp-Ala-NH_2



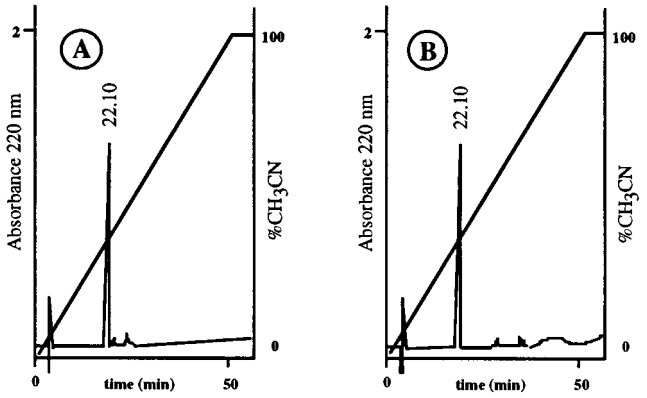


Figure 3. Reversed Phase HPLC chromatograms of the crude HF of Ac-Leu-Leu-LysΨ[CH_2NH]Phe-Asp-Ala-NH_2 **9** synthesized by (A) Weinreb amide MBHA resin, (B) phenyl ester MBHA resin.

Conclusion

We have designed, constructed, and tested two different linkers for the synthesis of aldehydic peptides on solid support. These two strategies avoided the preliminary synthesis of the C-terminal amino aldehyde. As demonstrated, these syntheses were devoid of epimerization, but the purification step (in our conditions) was always a source of some epimerization of the Cα bearing the aldehyde. The new linkers can be used for further chemical synthesis on solid support and for the preparation of larger synthetic pseudopeptides as shown by the solid phase preparation of the hexapeptide with a reduced peptide bond, Ac-Leu-Leu-LysΨ[CH_2NH]Phe-Asp-Ala-NH_2 **9**.

Experimental Section

Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Ascending TLC was performed on precoated plates of silica gel 60 F 254 (Merck). Peptide derivatives were located with UV light (254 nm), charring reagent, or ninhydrin. Silica gel column chromatographies were performed with silica gel 60, 60−229 mesh, ASTM (Merck) in the presence of 0.1% pyridine for the aldehydic peptides. HPLC purifications were run on a Prep 4000 Waters apparatus, Delta-Prep 15 μm, 40 × 100 mm, C18 column, at a flow rate of 50 mL/min of a mixture of (A) H_2O/TFA : 0.1% and (B) CH_3CN/TFA: 0.1%, with a UV detection at 220 nm in a gradient mode. Mass spectra were recorded on a JEOL JMS DX 100 and DX 300 spectrometer in FAB positive mode. ^1H NMR spectra were recorded on a 360 MHz Bruker AMX spectrometer. Amino acids and derivatives were purchased from Propeptide (France). All reagents and solvents were of analytical grade. The following abbreviations were used: DIEA, *N,N*-diisopropylethylamine; BOP, (benzotriazolyloxy)tris(dimethylamino)-phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; MBHA, *p*-methylbenzhydrylamine resin; DCM, dichloromethane. Other abbreviations used were those recommended by the IUPAC-IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9−37).

Weinreb Amide Based Linker. Synthesis of the Linker Based on the Weinreb Amide. Benzyl 3-amino-*N*-methoxypropionate (**2**): 9.65 g (60 mmol) of benzyl acrylate was added to a solution containing the methoxy amine hydrochloride (120 mmol; 10 g) in acetonitrile (50 mL) in the presence of 20 mL of diisopropylethylamine (DIEA). After 24 h reflux, the oily residue was dissolved with ethyl acetate and washed with a saturated solution of sodium hydrogenocarbonate and sodium chloride. After drying of the organic layer over sodium sulfate, the solution was concentrated *in vacuo* to yield compound **2** as an oil (12 g, 98%). ^1H NMR ($CDCl_3$) δ 2.59 (t, 2H, CH_2), 3.13 (t, 2H, CH_2), 3.4 (s, 3H, OCH_3), 5.09 (s, 2H, CH_2), 5.31 (s, 1H, NH), 7.25 (m, 5H, Ar). $[M + H]^+$: 210.

Benzyl 3-amino *N*-(*tert*-butyloxycarbonyl)-*N*-methoxypropionate (3): 57 mmol (12.4 g) of Boc_2O was added to a solution of dioxane/water (2/1) containing 57 mmol (12 g) of compound **2** in the presence of 1 M NaOH in order to maintain the pH between 12 and 13. After 3 h, the solvent was concentrated *in vacuo*, and the mixture was dissolved in ethyl acetate and washed with a 1 M potassium hydrogenosulfate solution. After drying and concentration *in vacuo* of the organic solvent, an oily residue **3** was obtained (17 g, 98%). ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H, Boc), 2.58 (t, 2H, CH_2), 3.56 (s, 3H, OCH_3), 3.72 (t, 2H, CH_2), 5.06 (s, 2H, CH_2), 7.25 (m, 5H, Ar). $[M + H]^+$: 310.

3-Amino-*N*-(*tert*-butyloxycarbonyl)-*N*-methoxypropionic acid (4). Compound **3** was hydrogenated with Pd/C as catalyst in 95% EtOH for 3 h. After filtration of the catalyst and concentration *in vacuo* of the solvent, compound **4** was quantitatively obtained as an oil (12.23 g) and ready to be coupled to the resin. ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H, Boc), 2.6 (t, 2H, CH_2), 3.56 (s, 3H, OCH_3), 3.7 (t, 2H, CH_2), 8.50 (s, 1H, COOH). $[M + H]^+$: 220.

Anchoring of the Weinreb Linker. The MBHA resin (substitution 0.63 mmol/g) purchased from Novabiochem was acylated in DCM with 2 equiv of the Boc N-protected linker **4** in the presence of BOP as coupling agent and DIEA as base. After 4 h and classical washings, the coupling was monitored by Kaiser's test. The Boc N-temporary protection was removed by treatment with a 1:1 TFA/DCM mixture for 1 h. The resin modified with the linker was ready for classical solid phase synthesis.

Typical Experiment for Reduction of the Peptide Linked *via* Weinreb Amide to the Resin. Synthesis of

Boc-Phe-Val-Ala-H 10: 1.5 g of peptidyl-resin (0.66 mmol), prepared from the Weinreb amide modified MBHA by successive couplings and deprotection steps (Boc-Ala-OH, Boc-Val-OH, and Boc-Phe-OH were coupled using BOP as activating reagent) according to the general procedure used for SPPS, was suspended in anhydrous THF and placed in an ice bath. $LiAlH_4$ (114 mg; 5 mol equiv) was added, and the reaction was stirred for 30 min. The reaction was then hydrolyzed with a 1 M potassium hydrogenosulfate solution. The resulting mixture was filtered, and the resin was washed twice with dichloromethane. The solvents were recovered, diluted with dichloromethane, and washed with a 1 M potassium hydrogenosulfate solution and a saturated solution of sodium hydrogenocarbonate and sodium chloride. After drying and concentration of the solvent *in vacuo*, a white powder was obtained (crude yield: 180 mg = 71%). The aldehydic peptide Boc-Phe-Val-Ala-H **10** was purified either by silica gel chromatography with an isocratic solution of AcOEt/hexane: 7/3 containing 0.1% pyridine (yield 45%) or by reversed phase HPLC in a gradient mode from 100% A to 100% B in 50 min of solutions A (H_2O with TFA 0.1%) and B (CH_3CN with TFA 0.1%) as solvent system (yield after lyophilization: 40%).

^{1}H-NMR ($CDCl_3$): δ 0.9 (dd, 6H, CH_3), 1.35 (d, 3H, CH_3), 1.4 (s, 9H, Boc), 2.3 (m, 1H, CH), 3.2 (qd, 2H, CH_2), 4.1 (m, 1H, CH), 4.2 (m, 1H, CH), 4.35 (m, 1H, CH), 4.95 (m, 1H, NH), 6.45 (d, 1H, NH), 6.7 (m, 1H, NH), 7.25 (m, 5H, Ar), 9.52 (before purification: s, 1H, CHO) 9.49−9.52 (after purification: 2s, 1 H, CHO). $[M + H]^+$: 330.

Phenyl Ester Based Linker. Synthesis of the First N-Protected Amino Acid Esterified by the Linker. *p*-Hydroxybenzoic acid (4 mmol) and DIEA (8 mmol) were dissolved in DCM (20 mL), and then 1.5 equiv of the Boc-UNCA was added to the solution. After 1.5 h, the solvent was concentrated *in vacuo* and the compound extracted with ethyl acetate. The organic layer was washed with a 1 M solution of potassium hydrogenosulfate and with brine. After drying over sodium sulfate, the solvent was removed *in vacuo* to yield quantitatively the desired compound **7**.

Boc-Val-*O*-*p*-phenyl-COOH: ^{1}H NMR ($CDCl_3$) δ 1.07 (d, 3H, CH_3), 1.11 (d, 3H, CH_3), 1.54 (s, 9H, Boc), 2.3 (m, 1H, CHβ), 4.42 (m, 1H, CHα), 5.11 (d, 1H, NH), 7.61 (m, 4H, Ar), 10.73 (s, 1H, COOH). $[M + H]^+$: 338.

Boc-Phe-*O*-*p*-phenyl-COOH: ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H, Boc), 3.25 (m, 2H, $CH_2\beta$), 4.87 (m, 1H, CHα), 5.12 (d, 1H, NH), 7.34 (m, 5H, Ar), 7.62 (m, 4H, Ar), 8.8 (s, 1H, COOH). $[M + H]^+$: 386.

Boc-Ala-*O*-*p*-phenyl-COOH: ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, Boc), 1.6 (d, 3H, $CH_3\beta$), 4.60 (m, 1H, CHα), 5.13 (d, 1H, NH), 7.63 (m, 4H, Ar), 10.23 (s, 1H, COOH). $[M + H]^+$: 310.

Anchoring of the First Amino Acid *via* the Phenyl Ester Linker. The MBHA resin (0.63 mmol/g) was acylated with 2 equiv of compound **7** in the presence of BOP (2 equiv) and DIEA (pH 8−9) in DCM. After 4 h, classical solid phase workup was performed (washings), and the Kaiser test was used to verify the completion of the reaction. The N-Boc temporary protection was removed with TFA/DCM (40/60) by two treatments (3 and then 27 min), and after classical washings, the resin was ready for peptide elongation.

Typical Reduction of the Peptide Linked *via* Phenyl Ester to the Resin. Boc-Phe-Val-Ala-phenyl ester linked resin (2 g, 0.818 mmol) was suspended in anhydrous THF and placed in an ice−water bath. Three equivalents (2.45 mL) of $LiAlH(OtBu)_3$ (1 M in THF, Aldrich)) was added, and the reaction mixture was stirred for 1 h. The reaction was then hydrolyzed with a 1 M potassium hydrogenosulfate solution. The resulting mixture was filtered, and the resin was washed twice with dichloromethane. The solvent was collected, diluted with dichloromethane, and washed with a potassium hydrogenosulfate solution and a saturated solution of sodium bicarbonate and sodium chloride. After drying and concentration *in vacuo* a white powder was obtained (crude yield: 210 mg = 60%). The ^{1}H NMR study revealed the presence of 25% of the corresponding alcohol and 75% of the desired aldehydic peptide. These compounds could be easily separated by preparative HPLC, but with resulting partial racemization of the α carbon of the aldehydic function.

^{1}H NMR Chemical Shifts (ppm) Observed for the PAs Synthesized Both *via* Weinreb Linker and *via* Phenyl Ester Linker. The aldehydic tripeptides described were purified by reversed phase HPLC and revealed two aldehydic peaks.

***N*-α-(*tert*-Butoxycarbonyl)-L-phenylalanyl-L-valyl-L-alaninal:** ^{1}H-NMR ($CDCl_3$): 0.9 (dd, 6H, CH_3), 1.35 (d, 3H, CH_3), 1.4 (s, 9H, Boc), 2.3 (m, 1H, CH), 3.2 (qd, 2H, CH_2), 4.1 (m, 1H, CH), 4.2 (m, 1H, CH), 4.35 (m, 1H, CH), 4.95 (m, 1H, NH), 6.45 (d, 1H, NH), 6.7 (m, 1H, NH), 7.25 (m, 5H, Ar), 9.49−9.52 (2s, 1 H, CHO).

***N*-α-(*tert*-Butoxycarbonyl)-L-leucyl-L-leucyl-*N*-ϵ-(2-chlorobenzyloxycarbonyl)-L-lysinal:** ^{1}H-NMR ($CDCl_3$): 0.9 (dd, 12H, CH_3), 1.5 (s, 9H, Boc), 1.8 (m, 2H, CH_2), 1.95 (m, 2H, CH_2), 3.2 (m, 2H, CH_2), 4.1 (m, 1H, CH), 4.3−4.5 (m, 2H, CH), 4.85 (m, 1H, NH), 5.2 (s, 2H, CH_2), 5.35 (m, 1H, NH), 6.5 (d, 1H, NH), 6.9 (d, 1H, NH), 7.4 (m, 4H, Ar), 9.49−9.51 (2s, 1H, CHO).

***N*-α-Acetyl-L-leucyl-L-valyl-*N*-ϵ-(2-chlorobenzyloxycarbonyl)-L-lysinal:** ^{1}H-NMR (DMSO-d_6): 0.85 (2 dd, 12H, CH_3), 1.29 (m, 2H, CH_2), 1.42 (m, 4H, $2CH_2$), 1.57 (m, 3H, $1.5CH_2$), 1.74 (m, 1H, $0.5CH_2$), 1.83 (s, 3H, CH_3CO), 1.98 (m, 1H, CH), 2.98 (m, 2H, CH_2), 4.04 (m, 1H, CH), 4.18 (m, 1H, CH), 4.32 (q, 1H, CH), 5.08 (s, 2H, CH_2), 7.31 (t, 1H, NH), 7.36 (m, 2H, Ar), 7.46 (m, 2H, Ar), 7.71 (d, 1H, NH), 8.00 (d, 1H, NH), 8.30 (d, 1H, NH), 9.37 (s, 1H, CHO).

***N*-α-(Benzyloxycarbonyl)-L-valyl-L-phenylalaninal:** ^{1}H-NMR ($CDCl_3$): 0.9 (dd, 6H, CH_3), 2.1 (m, 1H, CH), 3.15 (d, 2H, CH_2), 4 (m, 1H, CH), 4.7 (m, 1H, CH), 5.1 (s, 2H, CH_2), 5.3 (m, 1H, NH), 7.2 (d, 1H, NH), 7.2−7.4 (m, 10H, Ar), 9.61 (s, 1H, CHO).

Synthesis of an Hexapeptide Containing a Reduced Bond between Lys and Phe Residues. Ac-Leu-Leu-Lys[CH_2-NH]Phe-Asp-Ala-NH_2 9. This hexapeptide was synthesized *via* the two methods to yield an identical compound **9** as described in Scheme 4. ^{1}H-NMR (DMSO-d_6): 0.85 (dd, 12H, CH_3), 1.25 (d, 3H, CH_3), 1.3 (m, 2H, $CH_2\beta$Lys), 1.4−1.7 (m, 10H, $\beta + \gamma$Leu + $\gamma + \delta$Lys), 1.9 (s, 3H, CH_3CO), 2.8 (m, 1H, βAsp), 2.75 (m, 3H, βAsp + CH_2 ϵLys), 2.78 (m, 2H, CH_2NH), 3.09 (d, 2H, CH_2 βPhe), 4 (m, 2H, CH αLys + CH αPhe), 4.2 (m, 3H, CH αLeu + CH αLeu + CH αAla), 4.6 (q, 1H, HαAsp), 7.2−7.35 (m, 5H, Ar), 7.55 (d, 1H, NH Lys), 7.8 (d, 1H, NH), 7.85 (d, 1H, NH), 7.97 (d, 1H, NH), 8.65 (s, 1H, NH Asp).

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