

Solid Phase Synthesis of a $\Psi[\text{CH}_2\text{NH}]$ Pseudopeptide by Ligation of a Peptidyl Aldehyde with a Resin-Bound Amino Peptide

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Abstract: Solid phase synthesis of $\Psi[\text{CH}_2\text{NH}]$ pseudopeptide **3** was accomplished by reductive amination of peptidyl aldehyde **1** with resin bound amino peptide **2**. No epimerization took place during the aldehyde preparation or the reductive amination step. This convergent strategy should facilitate the synthesis of analogues of the neuropeptide PACAP. © 1999 Elsevier Science Ltd. All rights reserved.

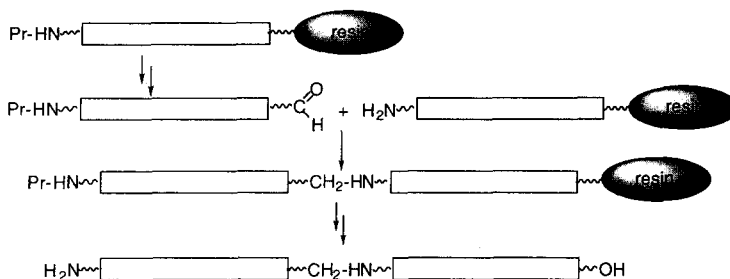
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Introduction

Incorporation of a $\Psi[\text{CH}_2\text{NH}]$ peptide bond isostere in various peptide hormones and neuromodulator antagonists has already produced important derivatives for structure activity investigations.¹ In an effort to obtain potent antagonists of the 38-amino acid neuropeptide PACAP (Pituitary adenylate cyclase activating polypeptide; H-HSDGIFTDSYSRYRKQMAVKKYLA AVL GKRYKQRVK NK-NH₂), we investigated a chemical ligation strategy to introduce a reduced bond in a shortened analogue. PACAP, first isolated from ovine hypothalamus,² is a hypophysiotropic hormone which acts as a neurotransmitter, neuromodulator, and neurotrophic factor in the central nervous system.³ PACAP is a member of the secretin/glucagon/VIP/GHRH family and shares with VIP cAMP-stimulating activity on the VPAC₁ and VPAC₂ receptors but shows 10,000 times higher potency on the PAC₁ receptor. Despite numerous efforts to obtain shortened analogues with potent and selective antagonist activity, only the PACAP-(6-30) analogue has been shown to retain acceptable potency.⁴ Moreover, PACAP and VIP peptides suffer from rapid degradation by peptidases^{5,6} in biological fluids and their preparation by a stepwise procedure results in very low yields.⁷ In this study, we present a procedure for the efficient synthesis of the (17-28) fragment **3** of PACAP-38 with a reduced bond between residus Lys²¹ and Tyr²² as a model bond for a ligation strategy between a Boc-peptidyl aldehyde and a resin-bound aminopeptide (Scheme 1).

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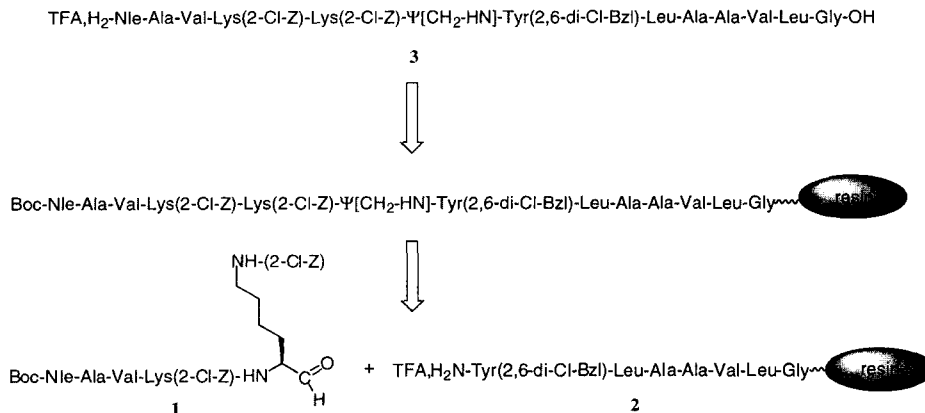
Scheme 1:



Results and Discussion

A commonly used method for the synthesis of a reduced bond involves the reductive alkylation of an amino group with an aldehyde in the presence of sodium cyanoborohydride in acid medium.⁸ Usually, synthesis of a $\Psi[\text{CH}_2\text{NH}]$ bond-containing peptide is accomplished stepwise.^{9,10} The reduced bond results from the reaction of a Boc-amino aldehyde with an amino peptide which can be linked to a resin as in solid phase synthesis.¹¹ Following a convergent approach, we decided to introduce the reduced bond in pseudopeptide **3** by direct condensation of Boc amino peptidyl aldehyde **1** with the resin bond amino peptide **2** (scheme 2).

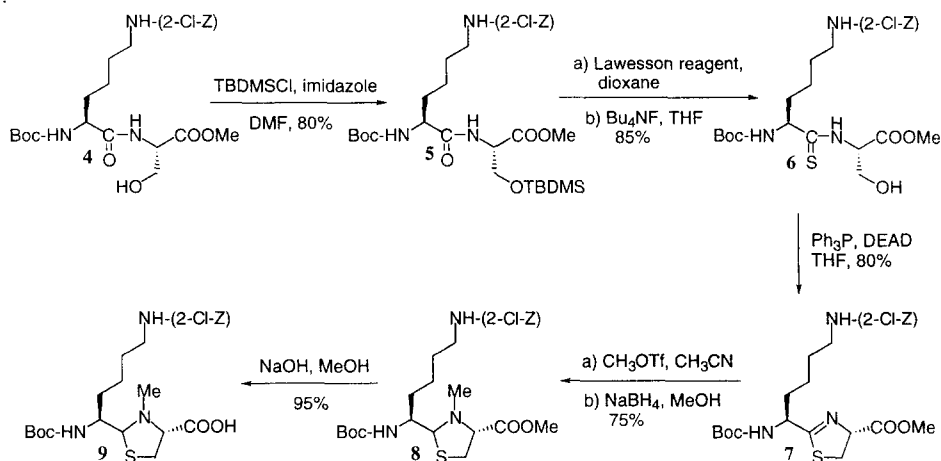
Scheme 2:



Peptidyl aldehyde **1** was obtained on solid phase. Syntheses of peptidyl aldehydes on solid support are based on the use of semicarbazone,¹² hydroxamate¹³ or thiazolidine^{14,15} as precursor of the aldehyde function. We applied the latter strategy previously developed in our laboratory, which consists in elongating a peptide starting from an amino thiazolidine^{14,15} linked to a resin via a spacer. Cleavage of the thiazolidine from the support by hydrolysis with copper salts generates the aldehyde function. Due to the neutral conditions of cleavage, we obtained the peptidyl aldehyde with protected side chains and no restriction concerning lateral

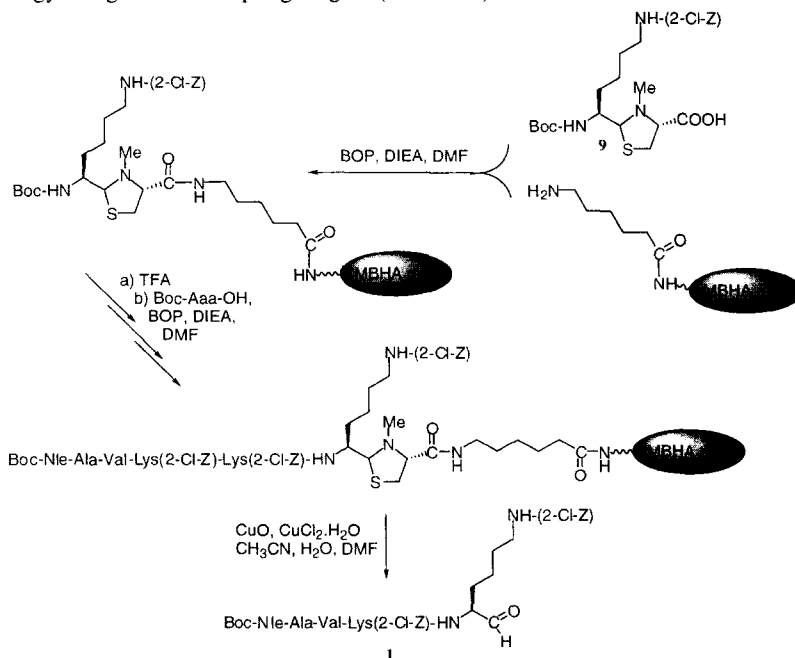
functions. Boc-lysine thiazolidine **9**, used for elaboration of **1**, was synthesized following the procedure described in scheme 3. The thioamide bond in **6** was introduced by treating dipeptide **5** with Lawesson's reagent.¹⁶ Thiazoline **7** resulted from cyclization of the β -hydroxy thiodipeptide **6** under Mitsunobu conditions.¹⁷ After reduction of **7**¹⁸ and hydrolysis of the ester group, we obtained amino thiazolidine **9**.

Scheme 3:



Peptidyl aldehyde **1** was synthesized on a *p*-methylbenzhydrylamine (MBHA) resin following a stepwise Boc strategy using BOP as coupling reagent (Scheme 4).

Scheme 4:



We first introduced Boc-amino hexanoic acid as a spacer, which is necessary for the success of the cleavage of the peptidyl thiazolidine. Thiazolidine **9** was coupled as a classical amino acid. Cleavage from the resin of the pseudopeptidyl aldehyde **1** was performed with a mixture of CuO and CuCl₂·xH₂O in a 57% overall yield calculated from the introduction of the spacer. Analytical HPLC of crude aldehyde **1** showed only one peak (Fig. 1).

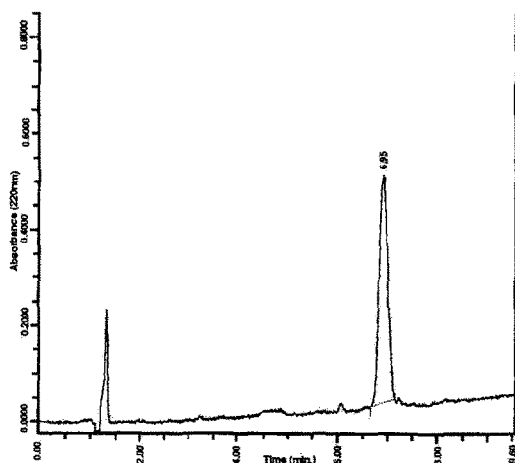
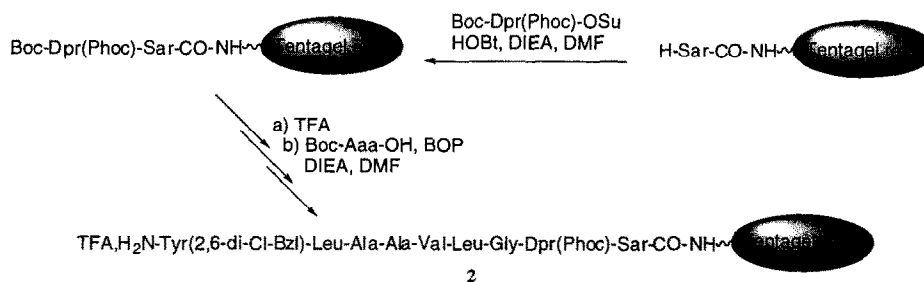


Figure 1: Analytical HPLC of crude Boc-Nle-Ala-Val-Lys(2-Cl-Z)-Lys(2-Cl-Z)-H **7** as obtained directly after copper hydrolysis cleavage from resin (gradient A/B:50/50 to A/B:0/100 in 10 min, A = H₂O/0.1% TFA and B = CH₃CN/0.1% TFA

Resin-bound amino peptide **2** was synthesized as described in Scheme 5. Tentagel PEG-PS resin was chosen as solid support because it is compatible with numerous solvents. We used a Dpr(Phoc) linker¹⁹ associated with a sarcosine spacer, which is stable under neutral and acid conditions. Elongation of the peptide was performed following a Boc strategy using BOP as coupling reagent. The acidic peptide was released under basic conditions.

Scheme 5:

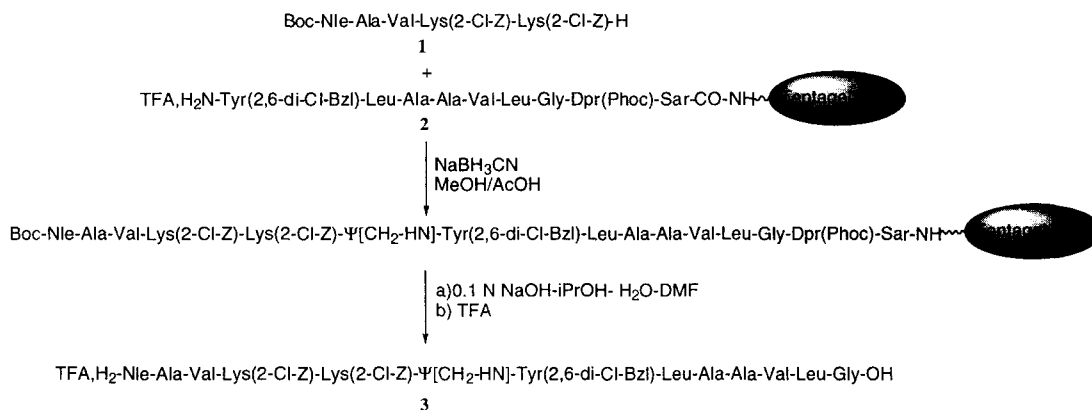


Among the multiple procedures for elaboration of peptides containing a reduced bond described in the literature, few examples evaluate potential epimerization during the synthesis. Sasaki²⁰ indicated that 5.5% of racemization took place during the aldehyde preparation and alkylation step using H-PheΨ[CH₂NH]Leu-NH₂ as model for synthesis of analogues of somatostatin. It is his opinion that epimerization arises from the isomeric instability of the aldehyde during its synthesis.²¹ During development of PheΨ[CH₂NH]Pro linkages in HIV

protease inhibitors, Cushman observed 80% epimerization.²² He supposed that the proline residue reacts with aldehyde to form an enamine with loss of chirality at the modified Phe residue. He also investigated the problem of racemization on solid phase synthesis during the formation of a reduced bond.²³ He showed with different models of reduced dipeptides that racemization of the aldehyde does not contribute significantly to the overall level of the diastereoisomers obtained. The ineffective trapping of the formed imine was considered responsible for racemization. Addition of NaBH_3CN in one portion and exclusion of DMF as solvent were recommended since slow dissolution of the reductive reagent can contribute to variable levels of diastereoisomers.

Accordingly, we performed the reductive amination of peptidyl aldehyde **1** with resin-bound amino peptide **2** in acidic MeOH in which NaBH_3CN is readily soluble (Scheme 6). After basic cleavage of the pseudopeptide from the resin and acidic deprotection of the Boc, analytic HPLC showed only two peaks; the amino peptide cleaved from **2** and the pseudopeptide **3** which was obtained in 40% yield after purification by preparative HPLC.

Scheme 6:



Having synthesized the reduced compound **3**, we were interested in evaluating the rate of epimerization occurring during the different steps of synthesis. To this end, we used the isomers $\text{Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-}\Psi[\text{CH}_2\text{NH-Tyr(2,6-di-Cl-Bzl)-OH}]$ **11a,b**, which were synthesized on solid phase following the same procedures as described above with resin-bound $\text{H-Tyr(2,6-di-Cl-Bzl)-OH}$ and $\text{Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-H}$. Isomerically pure $\text{Boc-Lys(2-Cl-Z)-L-Lys(2-Cl-Z)-H}$ **10a** was obtained on solid support using thiazolidine **9** and after cleavage with copper salt in 67% yield. $\text{Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-H}$ **10a,b** were obtained as a 75:25 mixture by the same procedure with 60% yield using thiazoline **7** which had been preliminarily treated under acidic condition (5% KH_2SO_4) to generate the D-isomer on the α carbon of lysine.²⁴ At this step, aldehydes **10a** and **10b** could be distinguished by HPLC and $^1\text{H NMR}$ and no D-isomer was detected starting from the L-isomer of lysine. The reduced tripeptides $\text{Boc-Lys(2-Cl-Z)-L-Lys(2-Cl-Z)-}\Psi[\text{CH}_2\text{NH-Tyr(2,6-di-Cl-Bzl)-OH}]$ **11a** and $\text{Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-}\Psi[\text{CH}_2\text{NH-Tyr(2,6-di-Cl-Bzl)-OH}]$ **11b** were obtained in 40% yield after purification by preparative HPLC.

OH **11a,b** were obtained in 50 % yield from **10a** and **10a,b** respectively. **11a** and **11b** gave distinguishable signals by HPLC. Compound **11a** showed no trace of D-isomer. This is proof that syntheses of peptidyl aldehydes by hydrolysis with the copper salt of thiazolidine and reductive amination can be performed without epimerization.

Conclusion

The (17-28) fragment of PACAP containing a reduced bond between Lys²¹ and Tyr²² was synthesized by ligation of a peptidyl aldehyde with an amino peptide linked to a solid support. This convergent method proceeds with good yield compared with a stepwise procedure. Moreover, no epimerization could be detected under these conditions. These findings encourage us to pursue the preparation of PACAP-38 analogues with reduced bonds in different positions to obtain antagonists that are resistant to endopeptidases.

Acknowledgements

We are grateful to Dr. S. L. Salhi for revising the manuscript.

Experimental

General methods. Column chromatographic separations were performed using Silica gel 60 (70-230 mesh). Melting points were measured in capillary tubes on a Electrothermal IA 9200 apparatus. HPLC analyses were performed with HPLC analyses were performed with a Hypersil column C8 (5 μ m, 4.6 x 150mm) using the following solvent system A = H₂O/0.1% TFA and B = CH₃CN/0.1% TFA. Optical rotations were evaluated on a Schmidt and Haensch polarimeter at $\pm 1^\circ$. ¹H NMR spectra were recorded on a AvanceBruker spectrometer (200MHz). Mass spectra were run in the FAB⁺ or MALDI-TOF mode.

Boc-Nle-Ala-Val-Lys(2-Z-Cl)-Lys(2-Z-Cl)-H (1). Starting from an MBHA resin (270 mg, 1.13 mmol/g), Boc- ϵ Ahx-OH (2 eq.) was first introduced with BOP reagent (2 eq.) and DIEA (4 eq.) in DMF. After removing the Boc group with a solution 50% TFA in CH₂Cl₂, thiazolidine **9** and each amino acid were introduced step-by-step with a Boc strategy using the BOP reagent in DMF. Lysine was used with 2-Cl-Z side chain protection. Completion of reactions was checked by the ninhydrin test of Kaiser.²⁵ After the coupling reaction, the resin was washed with DMF, MeOH and CH₂Cl₂. Cleavage of the pseudopeptidyl aldehyde was performed with 9 eq. of CuO and 4 eq. of CuCl₂·xH₂O in a mixture of H₂O-CH₃CN-DMF(1:1:2) (1mL to 2mL/100 mg of resin) under vigorous stirring. After three hours, the mixture was filtered to remove resin and some insoluble CuO. AcOEt was added to the mixture and the organic phase was washed with H₂O until the organic phase was colourless. These manipulations (reaction with copper salt, filtration, extraction) were repeated twice with the recovered resin until no peptidyl aldehyde was obtained. After drying with Na₂SO₄, AcOEt was evaporated and peptidyl aldehyde **1** was obtained as a white solid powder (170 mg) in 57% yield starting from the introduction

of the Boc- ϵ Ahx-OH on MBHA resin. FAB⁺ MS [GT]: 978 (M+H⁺, 30), 878 (M+H⁺-Boc, 45), 57 (tBu⁺, 40). HPLC t_R = 6.95 min. using A/B:50/50 to A/B:0/100 in 10 min.

TFA, H₂N-Tyr(2,6-di-Cl-Bzl)-Leu-Ala-Ala-Val-Leu-Gly-Dpr(Phoc)-Sarc-CO-NH-Tentagel resin (2). Solid phase synthesis was carried out on a TentaGel-S-NH₂ (1 g, 0.28 mmol/g) using a manual apparatus. Boc-Sar-OH and Boc-amino acids (2.5 eq.) were coupled using DIPCDI (2.5 eq.), HOBT (2.5 eq.) and DIEA (2 eq.) in DMF. Boc-Dpr(Phoc)-OH was introduced with Boc-Dpr(Phoc)-OSu (1.5 eq.), HOBT (1.5 eq.) and DIEA (2.5 eq.) in DMF. After the coupling reaction, the resin was washed with DMF, MeOH and CH₂Cl₂. Completion of reactions was checked by the ninhydrin test of Kaiser.²⁵ N^α-Boc removing was achieved with 50% TFA in CH₂Cl₂. We obtained 1.33 g of peptide resin. 4 mg of peptide resin were cleaved following the procedure of basic cleavage of **3**. Analytical HPLC showed only one peak (t_R = 3.95 min. using A/B:60/40 to A/B:0/100. in 10 min.) FAB⁺ MS:[GT] 864 (M+H⁺, 30), 706 (M+H⁺-(2,6-di-Cl-Bzl), 10).

TFA, NH₂-Nle-Ala-Val-Lys(2-Z-Cl)-Lys(2-Z-Cl)-Ψ[CH₂NH]-Tyr(2,6-di-Cl-Bzl)-Leu-Ala-Ala-Val-Leu-Gly-OH (3). To 65 mg (0.014 mmol) of **2** in 3 ml of MeOH/1% AcOH were added **1** (34 mg, 0.035 mmol) and NaBH₃CN (3.5 mg, 0.056 mmol). The mixture was stirred at room temperature for 6h. After removal of the solvent by filtration, resin was then washed three times with CH₂Cl₂, DMF, MeOH, Et₂OH and dried. Resin was then added in 13 ml of iPrOH-H₂O-DMF(1/1/2) and 0.6 ml of 0.1 N NaOH. After 12h under stirring, the solution was filtered and lyophilized. The obtained solid was then dissolved in 1 ml of TFA during 1h. After evaporation of TFA and lyophilization, the salt was purified by preparative HPLC to give **3** as white solid (10 mg) with 40% yield. HPLC: t_R = 5.90 min. using A/B:60/40 to A/B:0/100 in 10 min. MALDI MS:[DHB] 1725 (M+H⁺).

Methyl [N^α-(tert-butoxycarbonyl)-N^ε-(2-chlorobenzoyloxycarbonyl)-L-lysyl]-L-serinate (4). To a solution of Boc-Lys(2-Cl-Z)-OH (5.0 g, 12 mmol) and HCl:H-Ser-OMe (1.87 g, 12 mmol) in CH₂Cl₂ (125 mL) were added BOP (5.30 g, 12 mmol) and DIEA (7.07 mL, 42 mmol). The mixture was stirred at room temperature for 1h. After evaporation of the solvent and addition of AcOEt (200 mL), the organic phase was washed with 5% KHSO₄, 5% NaHCO₃ and brine. The mixture was dried with MgSO₄ and the solvent removed under reduced pressure. The oily residue was purified by silica gel chromatography (AcOEt-cyclohexane, 60:40) to obtain the title compound as a colorless oil (5.75 g) in 93% yield. R_f = 0.5 (AcOEt-cyclohexane, 60:40). $[\alpha]^{20}_D$ = -12° (c = 1.1, MeOH). ¹H RMN (DMSO d₆) δ (ppm): 1.34 (s, 9H), 1.37-1.66 (m, 6H), 2.87-3.01 (m, 2H), 3.59 (s, 3H), 3.60-3.76 (m, 2H), 3.88-4.00 (m, 1H), 4.26-4.37 (m, 1H), 5.03 (t, J = 5.8 Hz, 1H), 5.06 (s, 2H), 6.80 (d, J = 8.4 Hz, 1H), 7.26-7.50 (m, 5H), 8.03 (d, J = 8.5 Hz, 1H). FAB⁺ MS: [GT] 516 (M+H⁺, 30), 460 (M+H⁺-(CH₃)₂C=CH₂, 10), 416 (M+H⁺-Boc, 100), 348 (M+H⁺-(2-Cl-Z, 5), 125 (C₇H₆Cl, 100), 57 (tBu⁺, 35).

Methyl [N^α-(tert-butoxycarbonyl)-N^ε-(2-chlorobenzoyloxycarbonyl)-L-lysyl]-O-(tert-butyl-dimethylsilyl)-L-serinate (5). To a solution of compound **4** (5.0 g, 9.7 mmol) and imidazole (1.32 g, 19.4 mmol) in DMF (13 ml) was added tert-butyldimethylsilyl chloride (2.20 g, 14.6 mmol). After 30 min, the solvent was evaporated under reduced pressure and the residue dissolved in AcOEt (200 ml). The mixture was washed with H₂O, dried

with MgSO_4 and concentrated in vacuum. After column chromatography on silica gel (AcOEt-cyclohexane, 40:60), the silyl ether was obtained as a white solid (4.9g) in 80% yield. $R_f = 0.4$ (AcOEt-cyclohexane, 40:60). mp = 93–94 °C. $[\alpha]^{20}_D = +4^\circ$ ($c = 1$; MeOH). ^1H RMN (DMSO d_6) δ (ppm): -0.08 (s, 3H), 0.00 (s, 3H), 0.81 (s, 9H); 1.34 (s, 9H), 1.17–1.68 (m, 6H), 2.86–3.01 (m, 2H), 3.59 (s, 3H), 3.72 (ABX, $J_1 = 4.9$ Hz, $J_2 = 10.2$ Hz, 1H) and 3.85 (ABX, $J_1 = 4.5$ Hz, $J_2 = 10.2$ Hz, 1H), 3.87–3.97 (m, 1H), 4.34–4.44 (m, 1H), 5.05 (s, 2H), 6.9 (d, $J = 8.1$ Hz, 1H), 7.27–7.41 (m, 5H), 7.88 (d, $J = 7.3$ Hz, 1H). FAB⁺ MS: [GT] 630 ($\text{M}+\text{H}^+$, 20), 530 ($\text{M}+\text{H}^+$ -Boc, 60), 462 ($\text{M}+\text{H}^+$ -(Z-2-Cl), 45), 174 ($\text{H}_2\text{N}^+=\text{CH}-\text{CH}_2\text{-OTBDMS}$, 15), 125 (2-Cl-Z, 70), 57 (tBu^+ , 55).

Methyl (2S)-N-[2-(tert-butoxycarbonylamino)-6-(2-chlorobenzoyloxycarbonylamino)-hexane-thiocarboxyl]-L-serinate (6). To a solution of compound **5** (3.60 g, 5.7 mmol) in dioxane (60 mL) was added Lawesson's reagent (1.38 g, 3.4 mmol). The reaction was stirred 40 min at 70°C. The solvent was removed under reduced pressure. The oily residue was chromatographed on silica gel (AcOEt-cyclohexane, 20:80) to obtain 3.70 g of oil which was dissolved in 1N nBu₄NF, THF (6.84 mL, 6.84 mmol). After 25 min, the solvent was reduced under reduced pressure. The oily residue was purified by silica gel chromatography (AcOEt-cyclohexane, 70:30) to obtain the title compound as a colorless oil (2.6g) in 85% yield. $R_f = 0.5$ (AcOEt-cyclohexane, 70:30). $[\alpha]^{20}_D = +8^\circ$ ($c = 1$; MeOH). ^1H RMN (DMSO d_6) δ (ppm): 1.36 (s, 9H), 1.7–1.69 (m, 6H), 2.96–3.05 (m, 2H), 3.62 (s, 3H), 3.75 (ABX, $J_1 = 4.4$ Hz, $J_2 = 11.1$ Hz, 1H) et 3.86 (ABX, $J_1 = 5.6$ Hz, $J_2 = 11.1$ Hz, 1H), 4.30–4.48 (m, 1H), 4.88–5.01 (m, 1H), 5.08 (s, 2H), 5.23 (t, $J = 5.8$ Hz, 1H), 6.87 (d, $J = 8.37$ Hz, 1H), 7.30–7.51 (m, 5H), 10.06 (d, $J = 7.3$ Hz, 1H). FAB⁺ MS: [GT] 532 ($\text{M}+\text{H}^+$, 10), 432 ($\text{M}+\text{H}^+$ -Boc, 70), 364 ($\text{M}+\text{H}^+$ -(2-Cl-Z), 5), 125 (2-Cl-Z, 10), 57 (tBu^+ , 40).

Methyl (1'S,4S)-2-[1'(tert-butoxycarbonylamino)-5'-(2-chlorobenzoyloxycarbonylamino)-pentyl]-2-thiazoline-4-carboxylate (7). To a solution of compound **6** (2.60 g, 4.9 mmol) in THF (70mL) were added Ph₃P (1.55 g, 5.9 mmol) and DEAD (0.93 mL, 5.9 mmol). The reaction was stirred for 15 min. The solvent was evaporated under reduced pressure and the residue chromatographed on silica gel (AcOEt-cyclohexane, 40:60) to furnish **7** (2.0 g) as an oil in 80% yield. $R_f = 0.4$ (AcOEt-cyclohexane, 60:40). ^1H RMN (DMSO d_6) δ (ppm): 1.42 (s, 9H), 1.38–1.42 (m, 4H), 1.56–1.77 (m, 2H), 2.93–3.07 (m, 2H), 3.54–3.59 (m, 2H), 3.71 (s, 3H), 4.19–4.33 (m, 1H), 5.10 (s, 2H), 5.15 (t, $J = 9.1$ Hz, 1H), 7.32–7.54 (m, 6H). FAB⁺ MS: [GT] 514 ($\text{M}+\text{H}^+$, 40), 458 ($\text{M}+\text{H}^+$ -(CH₃)₂C=CH₂, 15), 414 ($\text{M}+\text{H}^+$ -Boc, 25), 125 (2-Cl-Z, 70), 57 (tBu^+ , 40).

Methyl (1'S,4S)-2-[1'(tert-butoxycarbonylamino)-5'-(2-chlorobenzoyloxycarbonylamino)-pentyl]-3-methyl-2-thiazolidine-4-carboxylate (8). To a solution of thiazoline **7** (2g, 3.9 mmol) in CH₃CN (65 mL), was added MeOTf (0.35 mL, 5.04 mmol). The solution was stirred under argon atmosphere during 30 min. After evaporation of solvent, the residue was dissolved in MeOH (40mL) and NaBH₄ (296 mg, 7.8 mmol) was added at 0°C. After 5 min, the mixture was evaporated under reduced pressure, the residue was then dissolved in AcOEt. The organic phase was washed three times with H₂O, brine, dried with MgSO_4 and concentrated in vacuum. The oily residue was chromatographed on silicagel (AcOEt-cyclohexane, 40:60) to obtain **8** as an oily residu (1.55g) with 75% yield. $R_f = 0.4$ (AcOEt-cyclohexane, 40:60). RMN ^1H (DMSO d_6) δ (ppm): 1.13–1.38 (m, 4H), 1.39 (9H, s, Boc), 1.64–1.85 (m, 2H), 2.37 (s, 3H), 2.91–3.05 (m, 2H), 3.08–3.16 (m, 2H), 3.21–3.38

(m, 1H), 3.63 (s, 3H), 3.86 (t, $J = 6.2$ Hz, 1H), 3.96 (d, $J = 7.0$ Hz, 1H), 5.07 (s, 2H), 6.46 (d, $J = 9.6$ Hz, 1H); 7.30–7.51 (m, 5H). FAB⁺ MS: [GT] 530 (M+H⁺, 20), 430 (M+H⁺-Boc, 10), 160 (thiazolidine+H⁺, 70), 125 (2-Cl-Z, 40), 57 (tBu⁺, 20).

(1'S,4S)-2-[1'(tert-Butoxycarbonylamino)-5'-(2-chlorobenzoyloxycarbonylamino)-pentyl]-3-methyl-2-thiazolidine-4-carboxylic acid (9). To a solution of thiazolidine **8** (1.32g, 2.5 mmol) dissolved in MeOH (5 mL) was added a solution of 1 N NaOH (5 mL). After one hour, the mixture was acidified to pH = 2. The aqueous phase was extracted with AcOEt. The organic phase was dried with MgSO₄ and concentrated in vacuum. The oily residue was chromatographed on silicagel CH₂Cl₂-MeOH, 92:8). Compound **9** was obtained as white solid (1.22 g) with 95% yield. $R_f = 0.3$ (CH₂Cl₂-MeOH, 92:8). ¹H RMN (DMSO d₆) δ (ppm): 1.13–1.35 (m, 4H), 1.37 (s, 9H), 1.67–1.74 (m, 2H), 2.36 (s, 3H), 2.95–2.98 (m, 2H), 3.05–3.10 (m, 2H), 3.36–3.56 (m, 1H), 3.71 (t, $J = 6.6$ Hz, 1H), 3.97 (d, $J = 8.4$ Hz, 1H), 5.07 (s, 2H), 6.47 (d, $J = 9.3$ Hz, 1H), 7.30–7.49 (m, 5H), 12.7 (s, 1H). Distinguishable signals of minor isomer (30%): 2.30 (s, 3H). FAB⁺ MS: [GT] 516 (M+H⁺, 65), 416 (M+H⁺-Boc, 25), 125 (2-Cl-Z, 70), 57 (tBu⁺, 40).

[N^α-(tert-Butyloxycarbonyl)-N^ε-(2-chlorobenzoyloxycarbonyl)-L-lysyl]-N^ε-(2-chlorobenzoyloxycarbonyl)-L-lysinal (10a). Starting from 350 mg of MBHA (1.13 mmol/g), the same procedure of coupling reactions used for compound **1** was applied. 450 mg of peptidyl resin (200 mg, 0.23 mmol) were placed in 4 ml of a solution of H₂O-CH₃CN-DMF(1:1:2). CuO (237 mg, 3 mmol, 7.5 eq.) and CuCl₂·xH₂O (160 mg, 1.2 mmol, 3eq.) were added to the mixture, under vigorous stirring. After 3h, the mixture was filtered and washed with AcOEt. The organic phase was washed with H₂O until it was not colored. These manipulations (reaction with copper salt, filtration, extraction) were repeated with the recovered resin. After drying with Na₂SO₄, AcOEt was evaporated and peptidyl aldehyde **10a** was obtained as a white solid powder (185 mg) with 67% yield starting from the introduction of the Boc-εAhx-OH on MBHA resin. $R_f = 0.35$ (CH₂Cl₂-MeOH, 92:8). ¹H RMN (DMSO d₆) δ (ppm): 1.23–1.43 (m, 8H), 1.36 (9H, s, Boc), 1.47–1.78 (m, 4H), 2.88–3.04 (m, 4H, m), 3.78–3.93 (m, 1H), 3.90–4.08 (m, 1H, m), 5.07 (s, 4H), 6.90 (d, $J = 8.0$ Hz, 1H), 7.29–7.52 (m, 10H), 8.21 (d, $J = 6.8$ Hz, 1H), 9.36 (s, 1H). FAB⁺ MS: [GT] 695 (M+H⁺, 5), 595 (M+H⁺-Boc, 10), 125 (2-Cl-Z, 30), 57 (tBu⁺, 25). HPLC $t_R = 6.10$ min. using A/B:50/50 to A/B:0/100 in 10 min.

[N^α-(tert-Butyloxycarbonyl)-N^ε-(2-chlorobenzoyloxycarbonyl)-L-lysyl]-N^ε-(2-chlorobenzoyloxycarbonyl)-L,D-lysinal (10a,b). **10a,b** were obtained by the same procedure as for **10a** using thiazolidine **9** which was epimerized by treating thiazoline **7** in a solution of 5% KHSO₄. $R_f = 0.35$ and 0.43 (CH₂Cl₂-MeOH, 92:8). ¹H RMN (DMSO d₆) δ (ppm): 1.23–1.43 (m, 8H), 1.36 (9H, s, Boc), 1.47–1.78 (m, 4H), 2.88–3.04 (m, 4H, m), 3.78–3.93 (m, 1H), 3.75–4.08 (m, 1H, m), 5.07 (s, 4H), 6.90 (d, $J = 8.0$ Hz, 1H), 7.29–7.52 (m, 10H), 8.21 (d, $J = 6.8$ Hz, 1H), 9.36 (s, 0.75H), 9.41 (s, 0.25H). FAB⁺ MS: [GT] 695 (M+H⁺, 5), 595 (M+H⁺-Boc, 10), 125 (2-Cl-Z, 30), 57 (tBu⁺, 25). HPLC $t_R = 4.80$ min. (25%) and 6.10 min. (75%) using A/B:50/50 to A/B:0/100 in 10 min.

Boc-Lys(2-Z-Cl)-L-Lys(2-Z-Cl)-Ψ[CH₂NH]-Tyr(2,6-di-Cl-Bzl)-OH (11a). This compound was synthesized using the same procedure that was utilized for compound **3** using **10a** and TFA:H₂-Tyr-(2,6-di-Cl-Bzl)-Dpr(phoc)-Sar-Tentagel resin. After basic cleavage of **11a** from the resin, the solution was acidified to neutral pH with 5% KHSO₄ and extracted twice with AcOEt. The organic phase was washed three times with H₂O and brine, dried with MgSO₄ and concentrated in vacuum to obtain **11a** as white solid with 50% yield. $R_f = 0.24$ (CH₂Cl₂-MeOH, 92:8). HPLC: $t_R = 7.30$ min. using A/B:50/50 to A/B:0/100 in 10 min. MALDI MS: [DHB] 1018 (M+H⁺).

Boc-Lys(2-Z-Cl)-L,D-Lys(2-Z-Cl)-Ψ[CH₂NH]-Tyr(2,6-di-Cl-Bzl)-OH (11 a,b). **11a,b** were synthesized using the same procedure as compound **11a** using **10a,b** and TFA:H₂-Tyr-(2,6-di-Cl-Bzl)-Dpr(phoc)-Sar-Tentagel resin. Yield = 50%. $R_f = 0.33$ and 0.24 (CH₂Cl₂-MeOH, 92:8). HPLC: $t_R = 5.90$ min.(25%) and 7.30 min.(75%) using A/B:50/50 to A/B:0/100 in 10 min. MALDI MS: [DHB] 1018 (M+H⁺).

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