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Solid Phase Synthesis of a Ψ[CH$_2$NH] Pseudopeptide by Ligation of a Peptidyl Aldehyde with a Resin-Bound Amino Peptide

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Abstract: Solid phase synthesis of Ψ[CH$_2$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.

Keywords: Peptide analogues/mimetics, aldehydes, solid-phase synthesis.

Introduction

Incorporation of a Ψ[CH$_2$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-HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK-NH$_2$), 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 VPAC$_1$ and VPAC$_2$ receptors but shows 10,000 times higher potency on the PAC$_1$ 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 Lys^{21} and Tyr^{22} as a model bond for a ligation strategy between a Boc-peptidyl aldehyde and a resin-bound aminopeptide (Scheme 1).

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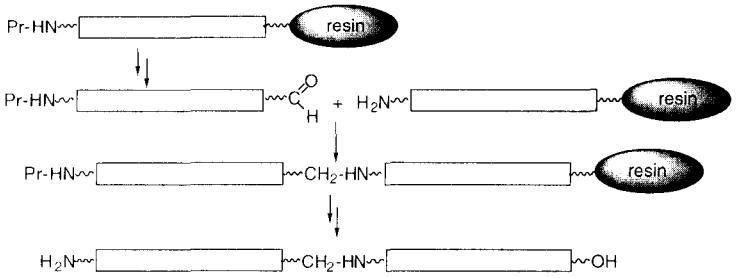
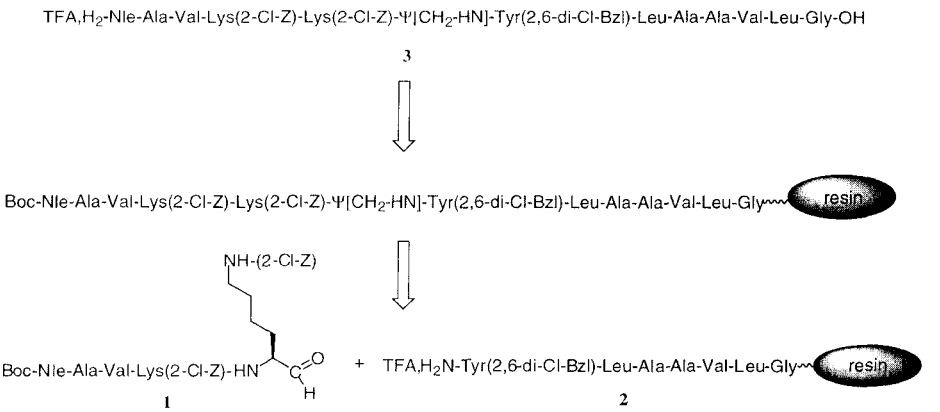
Scheme 1:



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 Ψ[CH_2NH] 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).

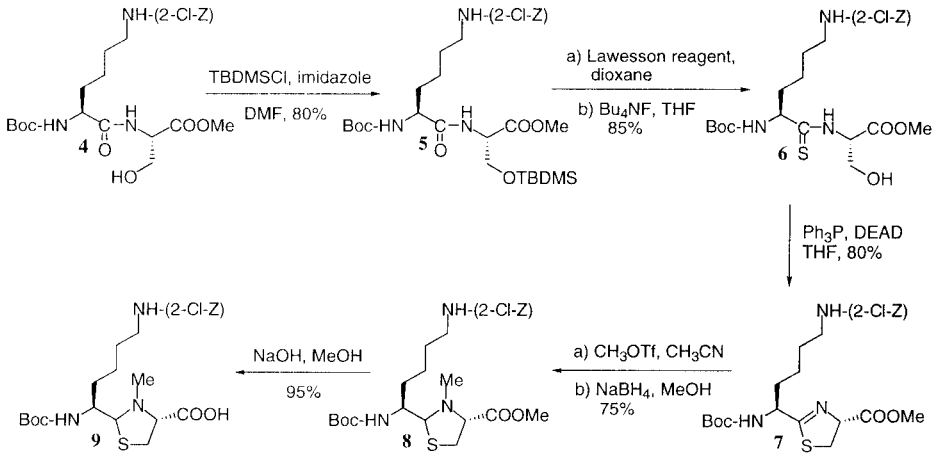
Scheme 2:



Peptidyl aldehyde **1** was obtained on solid phase. Syntheses of peptidyl aldehydes on solid support are based on the use of semicarbazone,[12] hydroxamate[13] 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

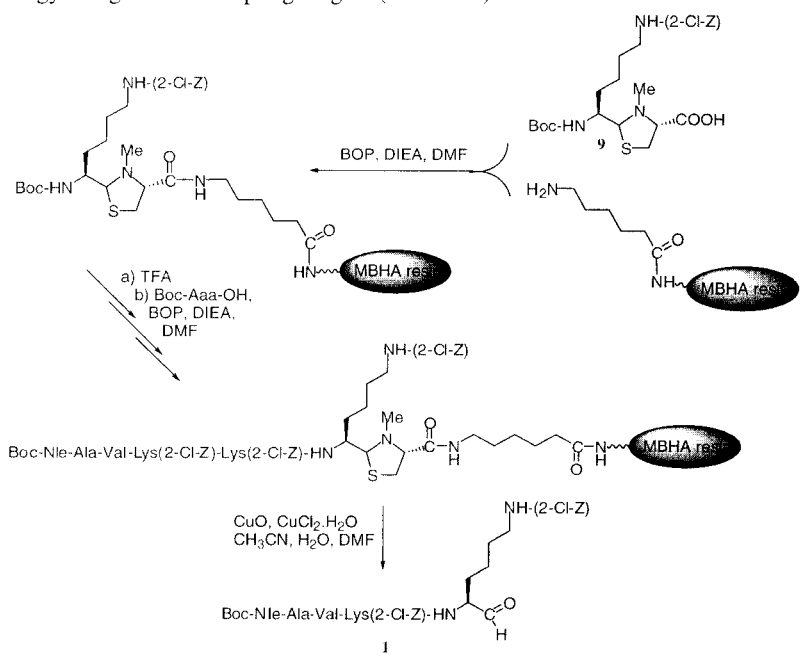
functions. Boc-lysine thiazolidine **9**, used for elaboration of **1**, was synthesized following the procedure described in scheme 3. The thioamide bond in **6** was introduced by treating dipeptide **5** with Lawesson's reagent.[16] Thiazoline **7** resulted from cyclization of the β-hydroxy thiodipeptide **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 $CuCl_{2-x}H_2O$ in a 57% overall yield calculated from the introduction of the spacer. Analytical HPLC of crude aldehyde **1** showed only one peak (Fig. 1).

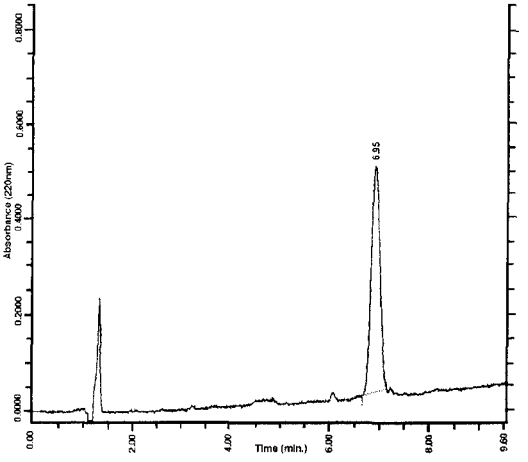
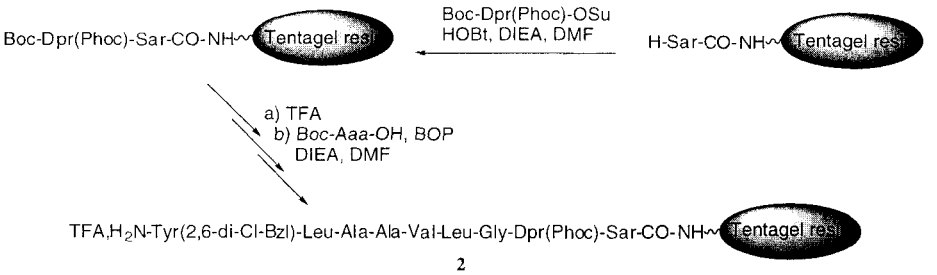


Figure 1: Analytical HPLC of crude Boc-Nle-Ala-Val-Lys(2-Cl-Z)-Lys(2-Cl-Z)-H **7** as obtained directly after copper hydrolysis cleavage from resin (gradient A/B:50/50 to A/B:0/100 in 10 min. A = H_2O/0.1% TFA and B = CH_3CN/0.1% TFA

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) linker[19] 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:



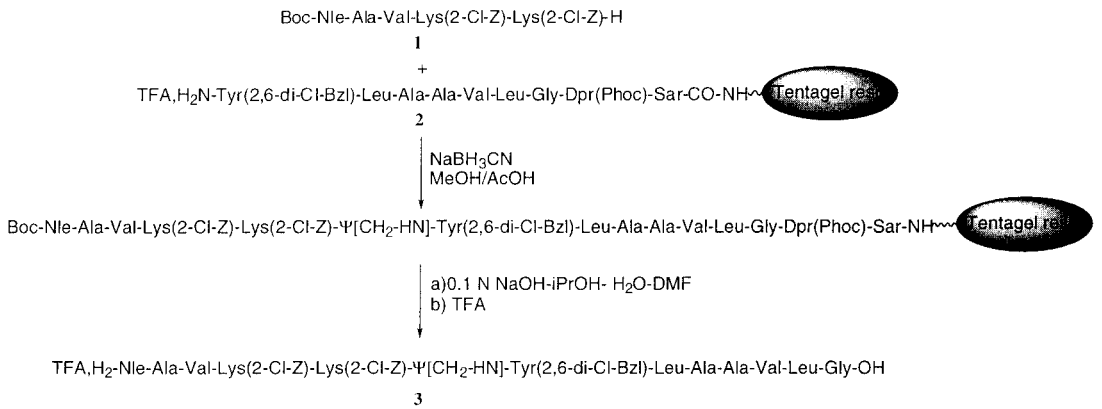
2

Among the multiple procedures for elaboration of peptides containing a reduced bond described in the literature, few examples evaluate potential epimerization during the synthesis. Sasaki[20] indicated that 5.5% of racemization took place during the aldehyde preparation and alkylation step using H-PheΨ[CH_2NH]Leu-NH_2 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[CH_2NH]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. Ho 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 Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-Ψ[CH_2NH]-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 H-Tyr(2,6-di-Cl-Bzl)-OH and Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-H. Isomerically pure 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. 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 preliminary treated under acidic condition (5% $KHSO_4$) 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}H NMR and no D-isomer was detected starting from the L-isomer of lysine. The reduced tripeptides Boc-Lys(2-Cl-Z)-L-Lys(2-Cl-Z)-Ψ[CH_2NH]-Tyr(2,6-di-Cl-Bzl)-OH **11a** and Boc-Lys(2-Cl-Z)-L,D-Lys(2-Cl-Z)-Ψ[CH_2NH]-Tyr(2,6-di-Cl-Bzl)-

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 Lys^{21} and Tyr^{22} 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 µm, 4.6 x 150mm) using the following solvent system A = H_2O/0.1% TFA and B = CH_3CN/0.1% TFA. Optical rotations were evaluated on a Schmidt and Haensch polarimeter at ± 1°. ^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-εAhx-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 CH_2Cl_2, 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 CH_2Cl_2. Cleavage of the pseudopeptidyl aldehyde was performed with 9 eq. of CuO and 4 eq. of $CuCl_{2-x}H_2O$ in a mixture of H_2O-CH_3CN-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 H_2O 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 Na_2SO_4, 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-εAhx-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,H$_2$N-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-NH_2 (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 CH_2Cl_2. Completion of reactions was checked by the ninhydrin test of Kaiser.[25] N$^\alpha$-Boc removing was achieved with 50% TFA in CH_2Cl_2. 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,NH$_2$-Nle-Ala-Val-Lys(2-Z-Cl)-Lys(2-Z-Cl)-Ψ[CH$_2$NH]-Tyr(2,6-di-Cl-Bzl)-Leu-Ala-Ala-Val-Leu-Gly-OH (3). To 65 mg (0.014 mmol) of **2** in 3ml of MeOH/1% AcOH were added **1** (34 mg, 0.035 mmol) and $NaBH_3CN$ (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 CH_2Cl_2, DMF, MeOH, Et_2OH and dried. Resin was then added in 13 ml of iPrOH-H_2O-DMF(1/1/2) and 0.6 ml of 0.1 N NaOH. After 12h under stirring, the solution was filtrered and lyophilized. The obtained solid was then dissolved in 1ml 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*$^\alpha$-(*tert*-butoxycarbonyl)-*N*$^\varepsilon$-(2-chlorobenzyloxycarbonyl)-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 CH_2Cl_2 (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 (200mL), the organic phase was washed with 5% $KHSO_4$, 5% $NaHCO_3$ and brine. The mixture was dried with $MgSO_4$ 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). ^{1}H 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$^+$-$(CH_3)_2C{=}CH_2$, 10), 416 (M+H$^+$-Boc, 100), 348 (M+H$^+$-(2-Cl-Z, 5), 125 (C_7H_6Cl, 100), 57 (tBu$^+$, 35).

Methyl [*N*$^\alpha$-(*tert*-butoxycarbonyl)-*N*$^\varepsilon$-(2-chlorobenzyloxycarbonyl)-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 H_2O, 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° (c = 1; MeOH). ^{1}H RMN (DMSO d6) 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 (M+H$^+$, 20), 530 (M+H$^+$-Boc, 60), 462 (M+H$^+$-(Z-2-Cl), 45), 174 (H_2N^+=CH-CH_2-OTBDMS, 15), 125 (2-Cl-Z, 70), 57 (tBu$^+$, 55).

Methyl (2S)-*N*-[2-(*tert*-butoxycarbonylamino)-6-(2-chlorobenzyloxycarbonylamino)-hexanethiocarboxyl]-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 nBu_4NF, 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° (c = 1; MeOH). ^{1}H RMN (DMSO d6) 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 (M+H$^+$, 10), 432 (M+H$^+$-Boc, 70), 364 (M+H$^+$-(2-Cl-Z), 5), 125 (2-Cl-Z, 10), 57 (tBu$^+$, 40).

Methyl (1'*S*,4*S*)-2-[1'(*tert*-butoxycarbonylamino)-5'-(2-chlorobenzyloxycarbonylamino)-pentyl]-2-thiazoline-4-carboxylate (7). To a solution of compound **6** (2.60 g, 4.9 mmol) in THF (70mL) were added Ph_3P (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). ^{1}H RMN (DMSO d6) 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 (M+H$^+$, 40), 458 (M+H$^+$-$(CH_3)_2C$=CH_2, 15), 414 (M+H$^+$-Boc, 25), 125 (2-Cl-Z, 70), 57 (tBu$^+$, 40).

Methyl (1'*S*,4*S*)-2-[1'(*tert*-butoxycarbonylamino)-5'-(2-chlorobenzyloxycarbonylamino)-pentyl]-3-methyl-2-thiazolidine-4-carboxylate (8). To a solution of thiazoline **7** (2g, 3.9 mmol) in CH_3CN (65 mL), was added MeOTf (0.35 mL, 5.04 mmol). The solution was stirred under argon atmosphere during 30 min. After evaporation of solvant, the residue was dissolved in MeOH (40ml) and $NaBH_4$ (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 H_2O, 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 ^{1}H (DMSO d6) 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*,4*S*)-2-[1'(*tert*-Butoxycarbonylamino)-5'-(2-chlorobenzyloxycarbnylamino)-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 $MgSO_4$ and concentrated in vacuum. The oily residue was chromatographed on silicagel CH_2Cl_2-MeOH, 92:8). Compound **9** was obtain as white solid (1.22 g) with 95% yield. R_f = 0.3 (CH_2Cl_2-MeOH, 92:8). ^{1}H 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-chlorobenzyloxycarbonyl)-L-lysyl]-N^{ε}-(2-chlorobenzyloxyarbonyl)-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 H_2O-CH_3CN-DMF(1:1:2). CuO (237 mg, 3 mmol, 7.5 eq.) and $CuCl_2.{}_xH_2O$ (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 H_2O until it was not colored. These manipulations (reaction with copper salt, filtration, extraction) were repetead with the recovered resin. After drying with Na_2SO_4, 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 (CH_2Cl_2-MeOH, 92:8). ^{1}H 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-chlorobenzyloxycarbonyl)-L-lysyl]-N^{ε}-(2-chlorobenzyloxycarbonyl)-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% $KHSO_4$. R_f = 0.35 and 0.43 (CH_2Cl_2-MeOH, 92:8). ^{1}H 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[CH_2NH]-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:H_2-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% $KHSO_4$ and extracted twice with AcOEt. The organic phase was washed three times with H_2O and brine, dried with $MgSO_4$ and concentrated in vacuum to obtain **11a** as white solid with 50% yield. R_f = 0.24 (CH_2Cl_2-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[CH_2NH]-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:H_2-Tyr-(2,6-di-Cl-Bzl)-Dpr(phoc)-Sar-Tentagel resin. Yield = 50%. R_f = 0.33 and 0.24 (CH_2Cl_2-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 ($M+H^+$).

References

1. Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, peptides and Proteins,* ed.B.Weinstein, Basel: Marcel Dekker, Inc., 1983, 267-357.
2. Miyata, A.; Arimura, A.; Dahl, D. H.; Minamino, N.; Uehara, A.; Jiang, L.; Culler, M. D.; Coy, D. H. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 567-574.
3. Arimura, A.; Shioda, S. *Frontiers Neuroendocrinol.* **1995**, *16*, 53-88.
4. Gourlet, P.; Vandermeers, A.; Vandermeers-piret, M.C.; Rathé, J.; De Neef, P.; Robberecht, P. *Eur. J. Pharmacol.* **1995**, *287*, 7-11.
5. Gourlet, P.; Vandermeers, A.; Robberecht, P.; Deschodt-Lanckman, M. *Biochem. Pharm.* **1997**, *54*, 509-515.
6. Goetzl, E. D.; Sreedharan, S.P.; Turck, C.W.; Bridenbaugh, R.; Malfroy, B. *Biochem. Biophys. Res. Com.* **1989**, *158*, 850-854.
7. Indeed, synthesis of PACAP in the laboratory by using a strategy Boc, with BOP reagent was accomplished with 2% overall yield.
8. Borch, R. F.; Berstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-29804.
9. Martinez, J.; Bali, J.-P.; Rodriguez, M.; Castro, B.; Magous, R.; Laur, J.; Lignon, M.-F. *J. Med. Chem.* **1985**, *28*, 1874-1879.
10. Rodriguez, M.; J.-P, B.; Magous, R.; Castro, B.; Martinez, J. *Int. J. Peptide Protein Res.* ***1986, 27, 293-299.***
11. Sasaki, Y.; Coy, D. H. *Peptides* **1987**, *8*, 119-121.
12. Murphy, A. M.; Dagnino, R.; Vallar, P. L.; Trippe, A. J.; Sherman, S. L.; Lumpkin, R. H.; Tamura, S. Y.; Webb, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 3156-3157.
13. Fehrentz, J. A.; Paris, M.; Heitz, A.; Velek, J.; Winternitz, F.; Martinez, J. *J. Org. Chem.* **1997**, *62*, 6792-6796.
14. Galéotti, N.; Giraud, M.; Jouin, P. *Lett. Peptide Science* **1997**, *4*, 437-440.
15. Galéotti, N.; Plagnes, E.; Jouin, P. *Tetrahedron* **1997**, *38*, 2459-5462.
16. Yde, B.; Yousif, N.; Pederson, U.; Thomsen, L.; Lawesson, S.-O. *Tetrahedron Lett.* **1984**, *40*, 2047-2052.
17. Galéotti, N.; Montagne, C.; Poncet, J.; Jouin, P. *Tetrahedron Lett.* **1992**, *33*, 2807-2810.
18. Dondoni, A.; Marra, A.; Perrone, D. *J. Org. Chem.* **1993**, *58*, 275-277.
19. Sola, R.; Saguer, P.; David, M.-L.; Pascal, R. *J. Chem. Soc., Chem. Commun.* **1993**, 1786-1788.
20. Sasaki, Y.; Murphy, W. A.; Heiman, M. L.; Lance, V. A.; Coy, D. H. *J. Med. Chem.* **1987**, *30*, 1162-1166.
21. Coy, D. H.; Hocart, S. J.; Sasaki, Y. *Tetrahedron* **1938**, *44*, 835-841.
22. Cushman, M.; Oh, Y. *J. Org. Chem.* **1991**, *56*, 4161-4167.
23. Ho, P. T.; Chang, D.; Zhong, J. W. X.; Musso, G. F. *Peptide Res.* **1993**, *6*, 10-12.
24. Yonetani, K.;Hirotsu, Y.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2945-2949.
25. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal; Biochem.* **1970**, *34*, 595.