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Post-synthesis Incorporation of a Lipidic Side Chain into a Peptide on Solid Support

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Abstract: A new strategy for the synthesis of lipopeptides has been developed. Using Weinreb (*N*-methoxy, *N*-methyl) amide as an aldehyde function precursor on the side chains of Asp or Glu residues, this new strategy avoids the synthesis of a lipidic amino acid residue before its incorporation in the peptide sequence. The aldehyde generated on the solid support can react with ylides leading to unsaturated or saturated side chains or with various nucleophiles to yield non-coded amino acid residues incorporated into the sequence.

Keywords: aldehyde; lipopeptides; solid support; Weinreb amide; Wittig reaction

INTRODUCTION

We recently described the possibility of generating an aldehyde function on the side chain of an Asp or Glu residue included in a peptide anchored to a resin [1]. This aldehyde function could then react with the stable and commercially available ylide carboethoxymethylene triphenylphosphorane to give an amino acid residue bearing an unsaturated aliphatic side chain. In this paper we demonstrate that this approach can be used to synthesize lipidic α-amino acid-containing peptides (without synthesizing the lipidic α-amino acid itself in solution before incorporating it into the sequence) by selective modification of specific residues (e.g. Asp, Glu) contained in the peptide.

Lipidic α-amino acids are non-coded residues with long saturated or unsaturated aliphatic side chains. Lipidic peptides represent a class of compounds that combines the structural features of lipids with those of peptides. The dual nature of these compounds is reflected in their physical properties. They are highly lipophilic due to the long lipidic side chains, yet show the polar characteristics of peptides. The main interest of lipopeptides is their potential use in drug delivery [2–4] or in combined adjuvant-carrier-vaccine systems [5,6]. To date, N^{α}-protected lipidic α-amino acids have been incorporated in peptides like usual amino acids. This strategy implies first the synthesis of the desired, stereochemically defined lipidic α-amino acid and, second, its incorporation into peptide [7,8]. This strategy presents significant problems of solubility. In addition, although solid-phase synthesis would improve the procedure, the preparation of enantiomeric lipidic α-amino acid is tedious. In this paper we propose the incorporation of a lipidic side chain directly on an elongated peptide sequence using the Wittig reaction. In fact, instead of a lipidic α-amino acid in the desired position of the sequence, Asp or Glu residues, protected on the side chain with the Weinreb (*N*-methoxy, *N*-methyl) amide [9], were introduced in the peptide chain. When the synthesis was

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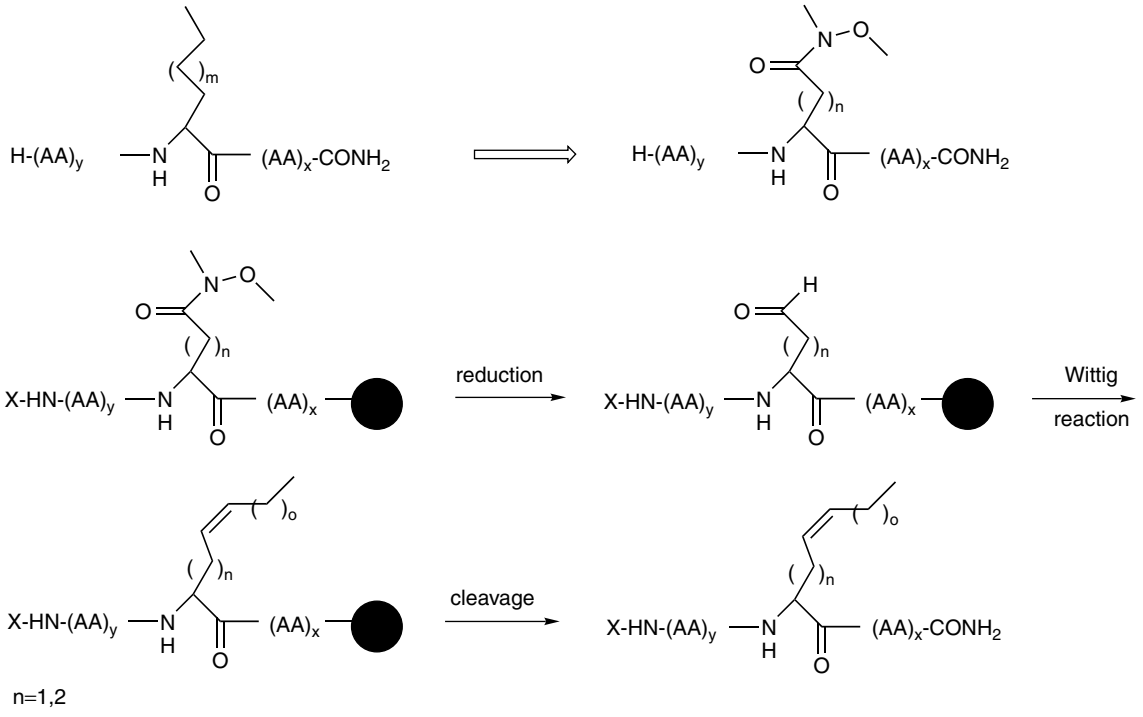
completed, this amide could be reduced by hydride to generate a reactive aldehyde function which underwent the Wittig reaction with an ylide to yield an unsaturated aliphatic side chain. As the Weinreb amide was proved to be an excellent protecting group of the carboxylic function in peptide synthesis [10], in this case it was used to protect the side chains of Asp or Glu residues during the synthesis until their reduction to aldehyde function. This method should avoid the difficult synthesis and handling of lipidic α-amino acids, permitting an easy choice of the configuration of this residue and the incorporation of different length lipidic side chains on a peptide anchored to a solid support (Scheme 1).

We have previously synthesized a model tripeptide Ac-Leu-Glu[N(Me)OMe]-Phe-NH_2 (Ac, acetyl; Me, methyl) on solid support, with a Boc (*tert*-butyloxycarbonyl) strategy on MBHA (4-methylbenzhydrylamine) resin [1]. The Weinreb amide of the side chain was reduced with different hydrides. The aldehyde function was reacted with the stable ylide $P(Ph)_3CHCO_2Et$ (Ph, phenyl; Et, ethyl) and, after HF cleavage, the expected peptide N^{α}-acetyl-L-leucyl-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-L-phenylalanyl-amide was obtained in good yield (purity: 79%) and characterized by mass spectrometry and ^{1}H NMR. This strategy was applied to several peptides containing acidic residues to yield non-coded amino acid-containing peptides [1].

We then decided to use non-stabilized ylides, which should allow the incorporation of an alkyl chain on solid support. On the other hand, the aldehyde generation should allow different types of chemistry such as reductive amination. Our results are described in this paper along with the difficulties faced during this new approach.

MATERIALS AND METHODS

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 (a solution containing 20 g $(NH_4)_2SO_4$ and 4 ml H_2SO_4 in 100 ml H_2O was sprayed onto the TLC, before heating) or ninhydrin. Silica-gel column chromatographies were performed with silica gel 60, 60–229 mesh, ASTM (Merck). HPLC purifications were run on a Prep 4000 Waters apparatus, Delta-Prep (15 µm, 40 × 100 mm) C_{18} column at a flow rate of 50 ml/min of a mixture of (A) H_2O/0.1% TFA and (B) CH_3CN/0.1% TFA, with UV detection at 214 nm in a gradient mode. Analytical HPLC screenings were performed on a



Scheme 1 New strategy for the incorporation of an alkyl side chain into an Asp or a Glu residue of a peptide.

Beckman apparatus constituted by a System Gold 126 solvent module, an 168 detector and managed by the 32 Karat software and on a Symmetry Shield Waters (5 μm, 100 Å, 3.9 × 150 mm) C_{18} column. Mass spectral analyses were recorded on a Platform II (Micromass, Manchester, UK) quadrupole mass spectrometer fitted with an electrospray interface. ^{1}H NMR spectra were recorded on a Bruker 360 MHz equipped with an Aspect 2000 computer. Amino acids and their derivatives were purchased from Senn Chemicals (Switzerland). All reagents and solvents were of analytical grade. Polystyrene resins (MBHA and Rink-amide) were from Senn Chemicals (Switzerland) or Advanced ChemTech (Louisville, Kentucky). Abbreviations used were those recommended by the IUPAC-IUB Commission (*Eur. J. Biochem.* 1984; **138**: 9–37).

Solution Synthesis

Boc-Glu(N(Me)OMe)-OH. To a stirred solution of Boc-Glu-OBzl (from Senn Chemicals International, Paris) (Bzl, benzyl) (l0 g, 30 mmol) in dichloromethane (300 ml) was added DIEA (*N*,*N*-diisopropylethylamine)(15.5 ml, 90 mmol), BOP [11] [(benzotriazolyloxy)tris(dimethylamino) phosphonium hexafluorophosphate] (13.3 g, 30 mmol) and N,O-dimethylhydroxylamine hydrochloride (from Aldrich) (3.2 g, 33 mmol). After 3 h at room temperature the solvent was evaporated and the crude was dissolved in ethyl acetate (EtOAc) (200 ml). The organic layer was successively washed twice with a saturated bicarbonate solution, an 1 M potassium hydrogen sulphate solution and brine, and then dried over sodium sulphate and concentrated *in vacuo*. After flash chromatography using 5 : 5 EtOAc/hexane (v/v) as eluant, the desired compound was obtained in a 81% yield. 4.9 g (15 mmol) of this compound was dissolved in 95% ethanol (150 ml) and stored overnight under atmospheric pressure of hydrogen in the presence of a catalytic amount of Pd/C 10%. The catalyst was removed by filtration over celite and the resulting solution concentrated *in vacuo*. Titled compound was recovered as an oil in a 95% yield.

Mass spectrometry for $C_{12}H_{22}N_2O_6$: $[M+H]^+$ 291.08 (theoretical value: 291.13). ^{1}H NMR ($CDCl_3$): δ(ppm) 1.45(s, 9H, Boc); 2.10(m, 2H, β-Glu); 2.15(m, 2H, γ-Glu); 3.15(s, 3H, N-CH_3); 3.50(s, 3H, OCH_3); 4.2(m, 1H, α-Glu); 5.65(d, 1H, NH).

Boc-Asp(N(Me)OMe)-OH. It was obtained in a total yield of 85% under similar conditions to those of the above compound.

Mass spectrometry for $C_{11}H_{20}N_2O_6$: $[M+H]^+$ 277.08 (theoretical value: 277.12). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.45(s, 9H, Boc); 2.80(m, 2H, β-Asp); 3.2(s, 3H, N-CH_3); 3.7(s, 3H, OCH_3); 4.5(m, 1H, α-Asp); 5.65(d, 1H, NH).

Fmoc-Glu(N(Me)OMe)-OH. Boc-Glu[N(Me)OMe]-OH (3 g, 10.34 mmol) was dissolved in 20 ml TFA. After 1 h under stirring, TFA was removed *in vacuo* and coevaporated with Et_2O. The resulting salt was obtained as an oil and stored overnight *in vacuo* in the presence of potassium hydroxide. The salt was then solubilized in 20 ml of DMF and stored at 0 °C. Then, DIEA (3.91 ml, 22.75 mmol) and Fmoc-OSu (Su, succinimide) (3.84 g, 11.37 mmol) were added. After 2.5 h under stirring a saturated solution of sodium bicarbonate was added and the aqueous layer was washed twice with Et_2O. The aqueous layer was then acidified with HCl 2N and the desired compound extracted with dichloromethane. The organic layer was washed with brine, and then dried over sodium sulphate and concentrated *in vacuo*. After flash chromatography using 180 : 10 : 5 chloroform/methanol/acetic acid (v/v/v) as eluant, the desired compound was obtained in a 65% yield.

Mass spectrometry for $C_{22}H_{24}N_2O_6$: $[M+H]^+$ 413.09 (theoretical value: 413.15). ^{1}H NMR (DMSO d_6): δ(ppm) 1.63(m, 1H, β'-Glu); 1.85(m, 1H, β-Glu); 2.31(m, 2H, γ-Glu); 2.91(s, 3H, N-CH_3); 3.47(s, 3H, OCH_3); 3.85(m, 1H, α-Glu); 4.10(t, 1H, CH-Fmoc); 4.15(d, 2H, CH_2-Fmoc); 7.18(t, 2H, ar-Fmoc); 7.29(td, 2H, ar-Fmoc); 7.51(d, 1H, NH); 7.65(d, 1H, ar-Fmoc); 7.71(d, 2H, ar-Fmoc).

Phosphonium salt preparation. $P^+(Ph)_3$-$(CH_2)_9$-CH_3 Br^-: To a solution of 1-bromodecane (6.23 ml, 30 mmol) in toluene (200 ml), triphenylphosphine was added (7.48 g, 28.5 mmol). The mixture was stirred under reflux for 3 days. The reaction was checked by TLC (1 : 9 EtOAc/hexane, v/v) following the triphenylphosphine disappearance. After evaporation of the solvent the crude was washed with a mixture Et_2O/hexane to remove the unreacted triphenylphosphine and 1-bromodecane. The phosphonium salt was obtained as a powder in a 74% yield and characterized by mass spectrometry: mass spectrometry for $C_{28}H_{36}P$: $[M]^+$ 403.44 (theoretical value: 403.23).

Other phosphonium salts were prepared in a similar manner. $P^+(Ph)_3$-$(CH_2)_3$-CH_3 Br^-. Yield: 67%. Mass spectrometry for $C_{22}H_{24}P$: $[M]^+$ 319.25 (theoretical value: 319.14). $P^+(Ph)_3$-$(CH_2)_5$-CH_3 Br^-. Yield: 76%. Mass spectrometry for $C_{24}H_{27}P$: $[M]^+$

347.20 (theoretical value: 347.17). $P^+(Ph)_3$-$(CH_2)_{13}$-CH_3 Br^-. Yield: 51%. Mass spectrometry for $C_{32}H_{44}P$: $[M]^+$:459.32 (theoretical value: 459.29). $P^+(Ph)_3$-$(CH_2)_{15}$-CH_3 Br^-. Yield:37%. Mass spectrometry for $C_{34}H_{48}P$: $[M]^+$ 487.36 (theoretical value: 487.32).

Model Peptide Synthesis in Solution

Boc-Leu-Glu(N(Me)OMe)-Phe-NH$_2$ (1). This compound was synthesized starting from 2 g (10 mmol) of HCl. H-Phe-NH_2 with BOP as coupling agent, DIEA as base and Boc as the N^{α}-amino temporary protecting group. The two coupling steps were performed in dichloromethane at room temperature; 1.1 equivalent of the carboxylic component and coupling agent were used. The amino group deprotection of Boc-Glu[N(Me)OMe]-Phe-NH_2 was performed in a mixture of dichloromethane/TFA for 30 min. The tripeptide was obtained as a white powder with a global yield of 88%.

Mass spectrometry for $C_{27}H_{43}N_5O_7$: $[M+H]^+$ 550.43 (theoretical value: 550.29). R_f (EtOAc): 0.56. ^{1}H NMR (DMSO d_6): δ (ppm) 0.81/0.83(dd, 6H, δ-Leu); 1.35(s, 9H, Boc); 1.40(m, 2H, β-Leu); 1.59(m, 1H, γ-Leu); 1.62(m, 1H, β'-Glu); 1.83(m, 1H, β-Glu); 2.32(m, 2H, γ-Glu); 2.79(dd, 1H, β'-Phe); 2.99(dd, 1H, β-Phe); 3.07(s, 3H, N-CH_3); 3.52(s, 3H, OCH_3); 3.91(m, 1H, α-Leu); 4.24(m, 1H, α-Glu); 4.43(m, 1H, α-Phe); 6.99(d, 1H, NH-Leu); 7.09/7.41(d, 2H, NH_2); 7.15–7.27(m, 15H, ar); 7.80(d, 1H, NH-Glu); 7.93(d, 1H, NH-Phe).

Boc-Leu-Glu(N(Me)OMe)-Phe-BHA (benzhydrylamide) (2). This compound was synthesized as described above for the primary amide but starting from 1.21 g (5.5 mmol) of benzhydrylamine hydrochloride and in a global yield of 84%.

Mass spectrometry for $C_{40}H_{53}N_5O_7$: $[M+H]^+$ 716.60 (theoretical value: 716.36). R_f (EtOAc): 0.56. ^{1}H NMR (DMSO d_6): δ (ppm) 0.81/0.83(dd, 6H, δ-Leu); 1.38(s, 9H, Boc); 1.40(m, 2H, β-Leu); 1.58(m, 1H, γ-Leu); 1.61(m, 1H, β'-Glu); 1.85(m, 1H, β-Glu); 2.37(m, 2H, γ-Glu); 2.83(dd, 1H, β'-Phe); 2.99(dd, 1H, β-Phe); 3.05(s, 3H, N-CH_3); 3.52(s, 3H, OCH_3); 3.92(m, 1H, α-Leu); 4.29(m, 1H, α-Glu); 4.69(m, 1H, α-Phe); 6.08(d, 1H, CH-BHA); 6.95(d, 1H, NH-Leu); 7.08–7.38(m, 15H, ar); 7.80(d, 1H, NH-Glu); 8.10(d, 1H, NH-Phe); 8.90(d, 1H, NH-BHA).

Boc-Leu-Glu(H)-Phe-BHA (3). To a stirred solution of Boc-Leu-Glu[N(Me)OMe]-Phe-BHA (200 mg, 0.28 mmol) in anhydrous THF (5 ml) stored at 0 °C under an argon atmosphere, $LiAlH_4$ in powder (27 mg, 0.74 mmol) was added. After 30 min an additional 27 mg of hydride was added and the reaction was followed by TLC. After completion the reaction was quenched by addition of a 1 M $KHSO_4$ solution. The aqueous layer was extracted twice with dichloromethane, the organic layers were combined, successively washed twice with a saturated bicarbonate solution, 1 M potassium hydrogen sulphate solution and brine, and then dried over sodium sulphate and concentrated *in vacuo*. The tripeptide aldehyde (**3**) (160 mg, 0.24 mmol) was obtained as a white powder in a 87% yield.

Mass spectrometry for $C_{38}H_{48}N_4O_6$: $[M+H]^+$ 657.44 (theoretical value: 657.32). R_f (EtOAc /hexane: 8/2, v/v): 0.57. ^{1}H NMR (DMSO d_6): δ (ppm) 0.70/0.81(dd, 6H, δ-Leu); 1.38(s, 9H, Boc); 1.40(m, 2H, β-Leu); 1.58(m, 1H, β'-Glu); 1.70(m, 1H, γ-Leu); 1.85(m, 1H, β-Glu); 2.35(t, 2H, γ-Glu); 2.86(dd, 1H, β-Phe); 2.99(dd, 1H, β-Phe); 3.92(m, 1H, α-Leu); 4.28(m, 1H, α-Glu); 4.69(m, 1H, α-Phe); 6.08(d, 1H, CH-BHA); 6.93(d, 1H, NH-Leu); 7.087.38(m, 15H, ar); 7.75(d, 1H, NH-Glu); 8.10(d, 1H, NH-Phe); 8.85(d, 1H, NH-BHA); 9.58(s, lH, CHO).

N$^{\alpha}$-tert-butyloxycarbonyl-L-leucyl-(S)-2-amino-undec-5-enyl-L-phenylalanyl-BHA (4). To a stirred solution of *n*-hexyltriphenylphosphonium bromide (521 mg, 1.22 mmol) in anhydrous THF (3 ml) at 0 °C and under an argon atmosphere, a 0.5 M $KN(TMS)_2$ (2.16 ml, 1.08 mmol) toluene solution was slowly added. After 30 min with stirring the peptide aldehyde (**3**) (160 mg, 0.24 mmol) in anhydrous THF was added to the red ylide solution. After 1.5 h the reaction was quenched with a saturated ammonium chloride solution. The crude was extracted twice with dichloromethane and the organic layer was then successively washed twice with water and brine, dried over sodium sulphate and concentrated *in vacuo*. After purification by flash chromatography on a silica gel column using 6 : 4 EtOAc/hexane (v/v) as the eluting system, the desired lipopeptide was obtained in a 69% yield.

Mass spectrometry for $C_{44}H_{60}N_4O_5$: $[M+H]^+$ 725.51 (theoretical value: 725.42). R_f (EtOAc/ hexane: 6/4, v/v) 0.84. ^{1}H NMR (DMSO d_6): δ(ppm) 0.81–0.85(dd, 6H, δ-Leu); 0.84(t, 3H, CH_3); 1.24(m, 6H, CH_2); 1.26(q, 2H, CH_2); 1.38(s, 9H, Boc); 1.39(m, 2H, β-Leu); 1.49(m, 1H, β'-CH_2); 1.52(m, 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.94(m, 2H, γ-CH_2); 2.82(dd, 1H, β'-Phe); 2.97(dd, 1H, β-Phe); 3.92(m, 1H, α-Leu); 4.26(m, 1H, α-CH); 4.69(m, 1H, α-Phe); 5.28(m, 2H, CH=CH); 6.06(d, 1H, CH-BHA); 6.93(d, 1H, NH-Leu); 7.1–7.4(m, 15H, ar); 7.70(d, 1H, NH); 8.04(d, 1H, NH-Phe); 8.81(d, 1H, NH-BHA).

N^{α}-tert-butyloxycarbonyl-L-leucyl-(S)-2-amino-henicos-5-enyl-L-phenylalanyl-BHA (5). To a stirred solution of *n*-hexadecyltriphenylphosphonium bromide (360 mg, 0.84 mmol) in anhydrous THF (3 ml) at 0 °C and under an argon atmosphere, a 0.5 M $KN(TMS)_2$ (1.51 ml, 0.76 mmol) toluene solution was slowly added. After 30 min with stirring the peptide aldehyde (**3**) (160 mg, 0.24 mmol) in anhydrous THF was added to the red ylide solution. After 3 h the reaction was quenched with a saturated ammonium chloride solution. The crude was extracted twice with dichloromethane and the organic layer was then successively washed twice with water and brine, dried over sodium sulphate and concentrated *in vacuo*. After purification by flash chromatography on a silica gel column using a 3 : 7 EtOAc/hexane mixture (v/v) as the eluting system, the desired lipopeptide was obtained in a 49% yield.

Mass spectrometry for $C_{54}H_{80}N_4O_5$: $[M+H]^+$ 865.62 (theoretical value: 865.56). R_f (EtOAc /hexane: 6/4, v/v): 0.84. ^1H NMR (DMSO d_6): δ (ppm) 0.59–0.70(dd, 6H, δ-Leu); 0.60(t, 3H, CH_3); 1.05(m, 28H, CH_2); 1.18(m, 2H, β-Leu); 1.18(s, 9H, Boc); 1.35(m, 2H, β-CH_2); 1.40(m, 1H, γ-Leu); 1.74(m, 2H, γ-CH_2); 2.61(dd, 1H, β-Phe); 2.72(dd, 1H, β'-Phe); 3.71(m, 1H, α-Leu); 4.02(m, 1H, α-CH); 4.48(m, 1H, α-Phe); 5.07(m, 2H, CH=CH); 5.87(d, 1H, CH-BHA); 6.73(d, 1H, NH-Leu); 6.82–7.12(m, l5H, ar); 7.49(d, 1H, NH); 7.88(d, 1H, NH-Phe); 8.62(d, 1H, NH-BHA).

N^{α}-tert-butyloxycarbonyl-L-leucyl-(S)-2-amino-7-ethoxycarbonyl-hex-5-enyl-L-phenylalanyl-BHA (6). *n*-Butyl lithium (3.9 ml, 0.624 mmol) in 1.6 M hexane solution was added to a solution of diisopropylamine (101 µl, 0.72 mmol) in anhydrous THF (3 ml) at −60 °C and under an argon atmosphere. After 30 min under stirring a solution of triethoxyphosphonoacetate (95 µl, 0.48 mmol) in THF (2 ml) was added and after 30 min the aldehyde (**3**) solution (160 mg, 0.24 mmol) in anhydrous THF (3 ml) was added. The reaction mixture was allowed to warm to room temperature overnight and then it was quenched with a saturated ammonium chloride solution. The crude was extracted twice with EtOAc and the organic layer was then successively washed twice with water and brine, dried over sodium sulphate and concentrated *in vacuo*. After purification by flash chromatography on a silica gel column using 7 : 3 EtOAc/hexane mixture (v/v) as the eluting system, the desired lipopeptide was obtained in a 66% yield.

Mass spectrometry for $C_{42}H_{54}N_4O_7$: $[M+H]^+$ 727.30 (theoretical value: 727.40). R_f (EtOAc /hexane: 7/3, v/v): 0.80. ^1H NMR (DMSO d_6): δ (ppm) 0.71–0.97(m, 9H, δ-Leu, CH_3); 1.38(m, 2H, β-Leu); 1.38(s, 9H, Boc); 1.60(m, 1H, β'-CH_2); 1.72(t, 1H, γ-Leu); 1.83(m, 1H, β-CH_2); 2.01 (t, 2H, γ-CH_2); 2.83–3.14(m, 2H, β-Phe); 4.03(m, 1H, α-Leu); 4.03(m, 2H, CH_2O); 4.21(m, 1H, α-CH); 4.31(m, 1H, δ-CH); 4.57(m, 1H, ε-CH); 4.70(m, 1H, α-Phe); 5.07(m, 2H, CH=CH); 6.06(d, 1H, CH-BHA); 6.95(d, 1H, NH-Leu); 7.03–7.38 (m, l5H, ar); 7.95(d, 1H, NH); 8.40(d, 1H, NH-Phe); 8.68(d, 1H, NH-BHA).

Solid Phase Synthesis

Syntheses were performed manually in a fritted reactor.

Boc strategy on MBHA (synthesis of the model peptide Ac-Leu-Glu(N(Me)OMe)-Phe-MBHA resin). 5 g of MBHA resin (substitution 0.8 mmol/g) was acylated successively with 3 equivalents of Boc-Phe-OH, Boc-Glu[N(Me)OMe]-OH and Boc-Leu-OH in dichloromethane in the presence of 3 equivalents of BOP and 9 equivalents of DIEA. After 30 min of each acylation step, which were controlled by the Kaiser test [12], the resin was washed with dichloromethane, twice with methanol and finally twice with dichloromethane. The amino group temporary Boc protection was removed by a 40 : 60 mixture TFA/dichloromethane for 2 min, the solution was removed by filtration and a fresh TFA/dichloromethane mixture was added and allowed to react for an additional 28 min. The resin was then washed twice with isopropanol and dichloromethane before the next acylation step. After elongation, the amino group temporary Boc protection was removed as previously and the *C*-terminal amine was acetylated in a 5 : 5 acetic anhydride/dichloromethane mixture for 15 min. After synthesis the resin was dried *in vacuo* to yield 6.8 g of peptidyl-resin (corresponding to 4 mmol). The synthesis of Asp (instead of Glu) containing peptides was performed following the same procedure.

Fmoc strategy on Rink amide resin (13) (synthesis of the model peptide Ac-Leu- Glu(N(Me)OMe)-Phe-Rink-amide resin). 5 g of resin (substitution 0.8 mmol/g) was acylated successively with 3 equivalents of Fmoc-Phe-OH, Fmoc-Glu[N(Me)OMe]-OH and Fmoc- Leu-OH in DMF in the presence of

3 equivalents of BOP and 9 equivalents of DIEA. After 30 min of each acylation step, which was controlled by the Kaiser test, the resin was washed with DMF, twice with dichloromethane and finally twice with DMF. The amino group temporary Fmoc protection was removed by an 80:20 mixture of DMF/piperidine for 3 min, the solution was removed by filtration and a fresh DMF/piperidine mixture was added and allowed to react for an additional 7 min. The resin was washed twice with DMF, isopropanol, dichloromethane and DMF before the next acylation step. After elongation, the amino group temporary Fmoc protection was removed as previously and the *C*-terminal amine was acetylated in a 5:5 acetic anhydride/DCM mixture for 15 min. After synthesis the resin was dried *in vacuo* to yield 6.8 g of peptidyl- resin (corresponding to 4 mmol). The synthesis of Asp (instead of Glu) containing peptides was performed following the same procedure.

Ac-Leu-Glu(N(Me)OMe)-Phe-NH$_2$. To check the synthesis before proceeding with the reduction and Wittig reactions, 100 mg of the MBHA resin (60 μmol) was cleaved in 1 ml of HF for 1 h at 0 °C in the presence of 0.1 ml anisole. After the usual work-up and lyophilization the desired peptide (24 mg) was obtained as a white powder in a 88% yield (calculated from the commercial substitution).

Mass spectrometry for $C_{24}H_{37}N_5O_6$: $[M+H]^+$ 492.15 (theoretical value: 492.25). ^{1}H NMR (DMSO d_6): δ(ppm) 0.83/0.87(dd, 6H, δ-Leu); 1.45(m, 2H, β-Leu); l.59(m, 1H, γ-Leu); 1.77(s, 3H, Ac); 1.80(m7 1H, β'-Glu); 1.90(m, 1H, β-Glu); 2.40(m, 1H, γ-Glu); 2.62(dd, 1H, β-Phe); 3.0(dd, 1H, β-Phe); 3.08(s, 3H, N-CH_3); 3.65(s, 3H, OCH_3); 4.25(m, 1H, α-Glu); 4.3(m7 1H, α-Leu); 4.50(m, 1H, α-Phe); 6.95 (d, 2H, NH_2); 7.27(m, 5H, ar); 7.77(d, 1H, NH-Phe); 8.11(d, 1H, NH-Glu); 8.20(d, 1H, NH-Leu).

General procedure for the Weinreb amide reduction on solid support: Generation of the aldehyde function. 810 mg (0.47 mmol) of Ac-Leu-Glu[N(Me)OMe]-Phe-MBHA resin, suspended under stirring in anhydrous THF at 0 °C, was treated with 107 mg of $LiAlH_4$ (2.82 mmol, 24 equivalents H^-) for 3.5 h. The reaction was then quenched by addition of a 1 M potassium hydrogen sulphate solution. The resin was washed twice with a 1 M potassium hydrogen sulphate solution, then successively with a saturated sodium hydrogen carbonate solution, water, methanol and dichloromethane. It was then stored and dried *in vacuo*. $LiAlH_4$ could be replaced with the bulky hydride $LiAlH(OtBu)_3$ [1] (in this case the required quantity was 2.82 ml (2.82 mmol, 6 equivalents) of a 1 M solution of $LiAlH(OtBu)_3$ in THF).

General procedure for the Wittig reaction on solid support. To a suspension of *n*-hexyltriphenylphosphonium bromide (1.01 g, 2.58 mmol) in anhydrous THF (8 ml) stored in an argon atmosphere and at 0 °C, $NaN(TMS)_2$ (2.32 ml, 2.32 mmol) in a 1 M THF solution was added. After 30 min the red solution was added via a cannula to 445 mg of non solvated Ac-Leu-Glu(H)-Phe-MBHA resin (0.258 mmol). The reaction was stirred at 0 °C for 6 h and was then quenched with a saturated ammonium chloride solution. The resin was then successively washed twice with a saturated ammonium chloride solution, water, toluene, methanol and dichloromethane and was dried *in vacuo*.

General procedure for the carbon–carbon double bond reduction on solid support. To a suspension of the previous peptidyl-resin (0.258 mmol) in DMF benzenesulphonylhydrazine [14] (133 mg, 0.774 mmol, 3 equivalents) was added. The reaction mixture was then stored at 100 °C overnight. The resin was then successively washed twice with DMF, methanol, dichloromethane and was dried *in vacuo*.

General procedure for the HF cleavage of peptide from the resin. The desired compound was cleaved from the MBHA support by HF in the presence of 10% anisole at 0 °C for 1 h (for 1 g of resin, 10 ml HF and 1 ml anisole were used). Then, HF was evaporated, the reaction mixture was diluted in a 5/5 diethyl ether/methanol (v/v), the resin was filtered and the solution evaporated. A solution of H_2O/0.1% TFA was then added and the expected compound recovered by filtration of this solution.

General procedure for the TFA cleavage of peptide from the resin. The Rink-resin was suspended under stirring in a 5:5 TFA/dichloromethane mixture (v/v) for 1 h at room temperature. The resin was filtered off and washed with the previous mixture. The filtrates were concentrated *in vacuo*. The desired compounds were precipitated with Et_2O. In some cases, the expected compounds could be recovered by filtration after addition of H_2O/0.1% TFA and purified by preparative HPLC or filtered after addition of a H_2O/0.1% TFA solution; in the other cases, the residue was dissolved in a 50:50:0.1 CH_3CN/H_2O/TFA mixture (v/v/v) and the desired compounds were purified on reversed phase preparative HPLC.

General procedure for the reductive amination on solid support. After generation of the aldehyde function as previously described, the peptidyl-resin was suspended in a 99 : 1 DMF/acetic acid mixture (v/v) and 10 eq of the amino component was added. 10 eq of sodium cyanborohydride was added and the reaction was left at room temperature with stirring for 24 h. The resin was then washed twice successively with DMF, methanol and dichloromethane before being dried *in vacuo*. A chloranil test [15] was performed to assess the presence of the secondary amine. The desired compound was then cleaved from the support by the usual HF cleavage and purified by preparative HPLC.

N^{α}-acetyl-L-leucyl-2-amino-5,5′-bis(4-methoxy) phenyl-pentanyl-L-phenylalanyl-amide (7). As this compound was observed as a side product in uncomplete Wittig reactions, its synthesis for full characterization was performed by treating Ac-Leu-Glu(H)-Phe-MBHA resin (300 mg, 175 μmol) at 0 °C and for 1 h with HF in the presence of 10% anisole. After purification by preparative HPLC the titled compound (56 mg) was obtained in a 50% yield.

Mass spectrometry for $C_{36}H_{46}N_4O_6$: $[M+H]^+$ 631.53 (theoretical value: 631.31). ^{1}H NMR (DMSO d_6): δ (ppm) 0.85/0.90(dd, 6H, δ-Leu); 1.46(m, 2H, β-Leu); 1.52(m, 2H, β-CH_2); l.55(m, 1H, γ-Leu); 1.75(s, 3H, Ac); l.95(td, 2H, γ-CH_2); 2.71(d, 1H, β'-Phe); 3.0(d, 1H, β-Phe); 3.70(s, 6H, OCH_3); 3.71(t, 1H, δ-CH); 4.25(m, 1H, α-CH); 4.25(m, 1H, α-Leu); 4.50(m, 1H, α-Phe); 6.82(m, 4H, ar); 7.0(d, 2H, NH_2); 7.12(m, 4H, ar); 7.25(m, 5H, ar-Phe); 7.73(d, 1H, NH); 8.09(d, 1H, NH-Phe);8.09(d, 1H, NH-Leu).

N^{α}-acetyl-L-leucyl-2-amino-5-fluoro-hexanyl-L-phenylalanylamide (8b). This compound was obtained starting from 300 mg of Ac-Leu-Glu[N(Me) OMe]-Phe-MBHA (175 μmol) which was reduced by $LiAlH_4$ as described in the solid support chemistry part. The generated supported aldehyde was condensed with the ylide solution obtained from $^+P(Ph)_3CH_3$, Br^- (10 eq., 625 mg) and $NaN(TMS)_2$ (9eq, 1.57 ml of a 1 M THF solution, 1.57 mmol) as described in the general procedure for the Wittig reaction on solid support. After HF cleavage without anisole, the titled compound was purified to yield 38 mg (48%) of a white solid.

Mass spectrometry for $C_{23}H_{35}N_4O_4F$: $[M+H]^+$ 451.40 (theoretical value: 451.24). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 1.21(d, 3H, CH_3); 1.40(m, 2H, β-Leu); 1.49(m, 2H, γ-CH_2); 1.57(m, 2H, β-CH_2); 1.58(m, 1H, γ-Leu); 1.84(s, 3H, Ac); 2.81(d, 1H, β'-Phe); 3.0(d, 1H, β-Phe); 4.17(m, 1H, α-CH); 4.24(m, 1H, α-Leu); 4.41(m, 1H, α-Phe); 4.6(m, 1H, CHF); 7.06–7.31(d, 2H, NH_2); 7.13–7.27(m, 5H, ar-Phe); 7.81(d, 1H, NH-Phe); 7.91(d, 1H, NH); 7.98(d, 1H, NH-Leu).

N^{α}-acetyl-L-leucyl-2-amino-pent-4-enyl-L-phenylalanylamide (9). Mass spectrometry for $C_{22}H_{32}N_4O_4$; $[M+H]^+$ 417.00 (theoretical value: 417.22). ^{1}H NMR (DMSO d_6): δ (ppm) 0.7(dd, 6H, δ-Leu); 1.25(m, 2H, β-Leu); 1.45(t, 1H, γ-Leu); 1.84(s, 3H, Ac); 2.10(dd, 1H, β'-CH_2); 2.20(dd, 1H, β-CH_2); 2.81(d, 1H, β'-Phe); 3.00(d, 1H, β-Phe); 4.07(m, 1H, α-CH); 4.25(m, 1H, α-Leu); 4.07(m, 1H, α-Phe); 4.87(d, 2H, =CH_2); 5.46(m, 1H, CH=); 6.94/7.14(d, 2H, NH_2); 7.01/7.13(m, 5H, ar-Phe); 7.7(d, 1H, NH-Phe); 7.7(d, 1H, NH); 7.88(d, 1H, NH-Leu). Yield 10%.

N^{α}-acetyl-L-leucyl-2-amino – undecanyl-L-phenylalanylamide (10). Mass spectrometry for $C_{28}H_{46}N_4O_4$; $[M+H]^+$ 503.40 (theoretical value: 503.32). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.86(t, 3H, CH_3); 1.12–1.32(m, 14H, CH_2); 1.40(m, 2H, β-Leu); 1.44(m, 1H, β'-CH_2); 1.53(m, 1H, CH_2); 1.57(t, 1H, γ-Leu); 1.83(s, 3H, Ac); 2.81(dd, 1H, β'-Phe); 3.0(dd, 1H, β-Phe); 4.10(m 1H, α-CH); 4.24(m, 1H, α-Leu); 4.40(m, 1H, α-Phe); 7.06/7.26(d, 2H, NH_2); 7.13/7.29(m, 5H, ar-Phe); 7.7 l(d, 1H, NH-Phe); 7.85(d, 1H, NH); 7.98(d, 1H, NH-Leu). Yield 15%.

N^{α}-acetyl-L-leucyl-2-amino-hexanyl-L-phenylalanylamide (11). Mass spectrometry for $C_{23}H_{36}N_4O_4$; $[M+H]^+$ 433.40 (theoretical value: 433.25). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.82(t, 3H, CH_3); 1.12–1.27(m, 4H, γ-, δ-CH_2); 1.40(m, 2H, β-Leu); 1.44(m, 1H, β'-CH_2); 1.53(m, 1H, β-CH_2); 1.57(t, 1H, γ-Leu); 1.83(s, 3H, Ac); 2.81(dd, 1H, β'-Phe); 3.0(dd, 1H, β-Phe); 4.10(m, 1H, α-CH); 4.25(m, 1H, α-Leu); 4.40(m, 1H, α-Phe); 7.06/7.26(d, 2H, NH_2); 7.13/7.29(m, 5H, ar-Phe); 7.72(d, 1H, NH-Phe); 7.86(d, 1H, NH); 7.98(d, 1H, NH-Leu). Yield 24%.

N^{α}-acetyl-L-leucyl-2-amino-undecanyl-L-alanyl-L-phenylalanyl-glycylamide (12). Mass spectrometry for $C_{33}H_{54}N_6O_6$; $[M+H]^+$ 631.40 (theoretical value: 631.37). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.85(t, 3H, CH_3); 1.13(d, 3H, β-Ala); 1.23(m, 14H, CH_2); 1.41(m, 2H, β-Leu); 1.43(m, 1H, β'-CH_2); 1.58(t, 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.83(s, 3H, Ac); 2.84(dd, 1H, β'-Phe); 3.05(dd, 1H, β-Phe); 3.56–3.66(m, 2H, α-Gly);

4.18(m, 1H, α-CH); 4.19(m, 1H, α-Ala); 4.26(m, 1H, α-Leu); 4.43(m, 1H, α-Phe); 7.05/7.06(d, 2H, NH_2); 7.13/7.29(m, 5H, ar Phe); 7.84(d, 1H, NH); 7.86(d, 1H, NH Ala); 7.97(d, 1H, NH-Leu); 7.98(d, 1H, NH-Phe); 8.08(t, 1H, NH-Gly). Yield 25%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-pentadecanyl-L-alanyl-L-phenylalanyl-glycylamide (13). Mass spectrometry for $C_{37}H_{62}N_6O_6$; $[M+H]^+$ 687.45 (theoretical value: 687.43). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, γ-Leu); 0.85(t,3H, CH_3); 1.13(d, 3H, β-Ala); 1.23(m, 22H, CH_2); 1.41(m, 2H, β-Leu); 1.43(m, 1H, β'-CH_2); 1.58(t, 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.83(s, 3H, Ac); 2.84(dd, 1H, β'-Phe); 3.05(dd, 1H, β-Phe); 3.56–3.66(m, 2H, α-Gly); 4.18(m, 1H, α-CH); 4.19(m, 1H, α-Ala); 4.26(m, 1H, α-Leu); 4.43(m, 1H, α-Phe); 7.05/7.06(d, 2H, NH_2); 7.13/7.29(m, 5H, ar-Phe); 7.84(d, 1H, NH); 7.86(d, 1H, NH-Ala), 7.97(d, 1H, NH-Leu); 7.98(d, 1H, NH-Phe); 8.08(t, 1H, NH-Gly). Yield 23%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-henicosanyl-L-alanyl-L-phenylalanyl-glycylamide (14). Mass spectrometry for $C_{43}H_{74}N_6O_6$; $[M+H]^+$ 771.50 (theoretical value: 771.52). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.85(t, 3H, CH_3), 1.13(d, 3H, β-Ala); 1.23(m, 34H, CH_2); 1.41(m, 2H, β-Leu); 1.43(m, 1H, δ'-CH_2); 1.58(t, 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.83(s, 3H, Ac); 2.84(dd, 1H, β'-Phe); 3.05(dd, 1H, β-Phe); 3.56–3.66(m, 2H, α-Gly); 4.18(m, 1H, α-CH); 4.19(m, 1H, α-Ala); 4.26(m, 1H, α-Leu); 4.43(m, 1H, α-Phe); 7.05/7.06(d, 2H, NH_2); 7.13/7.29(m, 5H, ar-Phe); 7.84(d, 1H, NH); 7.86(d, 1H, NH-Ala); 7.97(d, 1H, NH-Leu); 7.98(d, 1H, NH-Phe); 8.08(t, 1H, NH-Gly). Yield 16%.

N$^{\alpha}$-acetyl-glycyl-2-amino-pent-4-enyl-L-alanyl-L-tyrosyl-2-amino-pent-4-enyl-L-valylamide (15). Mass spectrometry for $C_{31}H_{45}N_7O_8$; $[M+H]^+$ 644.50 (theoretical value: 644.30). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, γ-Val); 1.16(d, 3H, β-Ala); 1.84(s, 3H, Ac); 1.96(m, 1H, β-Val); 2.24–2.37(m, 2H, β-CH_2 AllylGly-2); 2.412.42(m, 2H, β-CH_2 AllylGly-1); 2.69(dd, 1H, β'-Tyr); 2.90(dd, 1H, β-Tyr); 3.66–3.71(m, 2H, α-Gly); 4.11(m, 1H, α-Val); 4.20(m, 1H, α-Ala); 4.32(m, 1H, α-AllylGly-1); 4.37(m, 1H, α-AllylGly-2); 4.39(m, 1H, α-Tyr); 5.02(d, 2H, =CH_2-AllylGly-1); 5.06(d, 2H, =CH_2-AllylGly-2); 5.69(m, 2H, CH=AllylGly-1, CH=AllylGly-2); 6.6(dd, 2H, ar-Tyr); 6.98–7.01(dd, 2H, ar-Tyr); 7.29(d, 2H, NH_2); 7.70(d, 1H, NH-Val); 7.75(d, 1H, NH-Tyr); 7.92(d, 1H, NH-AllylGly2); 7.99(d, 1H, NH-AllylGly-1); 8.05(t, 1H, NH-Ala); 8.08(t, 1H, NH-Gly); 9.13(s, 1H, OH). Yield 10%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-hex-4-enyl-L-phenylalanylamide (16). Mass spectrometry for $C_{23}H_{34}N_4O_4$; $[M+H]^+$ 431.49 (theoretical value: 431.24). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83–0.87 (dd, 6H, δ-Leu); 1.40(m, 2H, β-Leu); 1.56(m, 1H, β'-CH_2); l.59(m, 1H, γ-Leu); 1.64(m, 1H, β-CH_2); 1.84(s, 3H, Ac); 1.93(m, 2H, γ-CH_2); 2.81(d, 1H, β'-Phe); 3.0(d, 1H, β-Phe); 4.14(m, 1H, α-CH); 4.24(m, 1H, α-Leu); 4.40(m, 1H, α-Phe); 4.93–4.96(d, 2H, =CH_2); 5.75(m, 1H, CH=); 7.13/7.27(m, 5H, ar-Phe); 7.07/7.29(d, 2H, NH_2); 7.73(d, 1H, NH-Phe); 7.93(d, 1H, NH); 7.99(d, 1H, NH-Leu). Yield 12%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-undec-5-enyl-L-phenylalanylamide (17). Mass spectrometry for $C_{28}H_{44}N_4O_4$; $[M+H]^+$ 501.22 (theoretical value: 501.31). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.85(t, 3H, CH_3); 1.24–1.26(m, 6H, CH_2); 1.41(m, 2H, β-Leu); 1.5(m, 1H, β'-CH_2); l.59(m 1H, γ-Leu); 1.60(m, 1H, β'-CH_2); 1.84(s, 3H, Ac); 1.93(m, 2H, γ-CH_2); 1.94(m, 2H, δ-CH_2); 2.82(dd, 1H, β'-Phe); 3.0(dd, 1H, β-Phe); 4.12(m, 1H, α-CH); 4.24(m, 1H, α-Leu); 4.4(m, 1H, α-Phe); 5.26–5.33(m, 2H, CH=CH); 7.06–7.28(d, 2H, NH_2); 7.13–7.26(m, 5H, ar-Phe); 7.74(d, 1H, NH-Phe); 7.92(d, 1H, NH); 8.0(d, 1H, NH-Leu). Yield 36%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-pentadec-5-enyl-L-phenylalanylamide (18). Mass spectrometry for $C_{32}H_{52}N_4O_4$; $[M+H]^+$ 557.30 (theoretical value: 557.37). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.85(t, 3H, CH_3); 1.23(m, 14H, CH_2); 1.41(m, 2H, β-Leu); l.50(m, 1H, β'-CH_2); l.59(m 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.84(s, 3H, Ac); 1.92(m, 2H, γ-CH_2); 1.94(m, 2H, δ-CH_2); 2.82(dd, 1H, β'-Phe); 3.0(dd, 1H, β-Phe); 4.12(m, 1H, α-CH); 4.24(m, 1H, α-Leu); 4.4(m, 1H, α-Phe); 5.25–5.33(m, 2H, CH=CH); 7.06–7.28(d, 2H, NH_2); 7.13–7.26(m, 5H, ar-Phe); 7.71(d, 1H, NH-Phe); 7.90(d, 1H, NH); 7.99(d, 1H, NH-Leu). Yield 20%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-henicos-5-enyl-L-phenylalanylamide (19). Mass spectrometry for $C_{38}H_{64}N_4O_4$; $[M+H]^+$ 641.82 (theoretical value: 641.45). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.85(t, 3H, CH_3); 1.23(m, 26H, CH_2); 1.41(m, 2H, β-Leu); l.50(m, 1H, β'-CH_2); l.59(m 1H, γ-Leu); 1.60(m, 1H, β-CH_2); 1.84(s, 3H, Ac); 1.92(m, 2H, γ-CH_2); 1.94(m, 2H, δ-CH_2); 2.82(dd, 1H, β'-Phe); 3.0(dd, 1H, β-Phe); 4.12(m, 1H, α-CH); 4.24(m, 1H, α-Leu); 4.4(m, 1H, α-Phe); 5.25–5.33(m, 2H, CH=CH); 7.06–7.28(d, 2H, NH_2); 7.13–7.26(m, 5H, ar-Phe); 7.71(d, 1H, NH-Phe); 7.90(d, 1H, NH); 7.99(d, 1H, NH-Leu). Yield 32%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-5-N-(benzyl)-pentanyl-L-phenylalanylamide (20). Mass spectrometry for $C_{29}H_{41}N_5O_4$; $[M+H]^+$ 524.38 (theoretical value: 524.29). ^{1}H NMR (DMSO d_6): δ (ppm) 0.86/0.89(dd, 6H, δ-Leu); 1.46(m, 2H, β-Leu); 1.58/1.75(m, 2H, β-CH_2); 1.60(td, 1H, γ-Leu); 1.62/1.70(m, 2H, γ-CH_2); 1.74(s, 3H, Ac); 2.73(dd, 1H, β'-Phe); 2.94(m, 2H, δ-CH_2); 2.98(dd, 1H, β-Phe); 4.25(m, 1H, α-Leu); 4.3(m, IH, α-CH); 4.5(m, IH, α-Phe); 6.98/7.34 (d, 2H, NH_2); 7.40–7.52(m, 10H, ar-Phe); 7.83(d, 1H, NH-Leu); 8.08(d, 1H, NH-Phe); 8.26(d, 1H, NH); 8.74(m, 1H, ε NH). Yield 15%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-5-amino(hexyl)-pentanyl-L-phenylalanylamide (21). Mass spectrometry for $C_{28}H_{47}N_5O_4$; $[M+H]^+$ 518.42 (theoretical value: 518.33). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.89(t, 3H, CH_3); 1.27(m, 6H, CH_2); 1.34(m, 2H, CH_2); 1.41(m, 2H, β-Leu); l.55(m, 1H, β'-CH_2); 1.57(m, 2H, CH_2); 1.60(m 1H, γ-Leu); 1.67(m, 1H, β-CH_2); 1.83(s, 3H, Ac); 2.78(dd, 1H, β'-Phe); 2.85(m, 2H, δ-CH_2); 3.05(dd, 1H, β-Phe); 4.25(m, 1H, α-Leu); 4.4(m, 1H, α-CH); 4.4(m, 1H, α-Phe); 7.09/7.38(d, 2H, NH_2); 7.15–7.30(m, 5H, ar-Phe); 7.82(d, 1H, NH-Leu); 7.98(d, 1H, NH-Phe); 8.02(d, 1H, NH); 8.20(m, 1H, ε-NH). Yield 20%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-5-amino(hexyl)-pentanyl–L-alanyl-L-phenylalanyl-glycylamide (22). Mass spectrometry for $C_{33}H_{55}N_7O_6$; $[M+H]^+$ 646.20 (theoretical value: 646.39). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.87(t, 3H, CH_3); 1.13(d, 3H, β-Ala); 1.27(m, 6H, CH_2); 1.34(m, 2H, CH_2); 1.41(m, 2H, β-Leu); l.55(m, 1H, β'-CH_2); 1.55(m, 2H, CH_2); 1.60(t, 1H, γ-Leu); 1.67(m, 1H, β-CH_2); 1.83(s, 3H, Ac); 2.83(m, 2H, δ-CH_2); 2.85(dd, 1H, β'-Phe); 3.05(dd, 1H, β-Phe); 3.56(m, 1H, α-Gly); 3.67(m, 1H, α-Gly); 4.18(m, 1H, α-CH); 4.19(m, 1H, α-Ala); 4.26(m, 1H, α-Leu); 4.45(m, 1H, α-Phe); 7.06/7.12(d, 2H, NH_2); 7.13/7.28(m, 5H, ar-Phe); 7.92(d, 1H, NH-Ala); 7.99(d, 1H, NH-Leu); 8.03(d, 1H, NH-Phe); 8.05(d, 1H, NH); 8.13(t, 1H, NH-Gly); 8.25(s, 1H, ε-NH). Yield 20%.

N$^{\alpha}$-acetyl-L-leucyl-2-amino-5-N-(phenylalanyl-amide)-pentanyl-L-alanyl-L-phenylalanyl-glycyl-amide (23). Mass spectrometry for $C_{36}H_{52}N_8O_7$; $[M+H]^+$ 709.42 (theoretical value: 709.36). ^{1}H NMR (DMSO d_6): δ (ppm) 0.84/0.88(dd, 6H, δ-Leu); l.l5(d, 3H, β-Ala); 1.42(m, 2H, β-Leu); 1.52(m, 1H, β-CH_2); 1. 60(t, 1H, γ-Leu); 1.63(m, 2H, γ-CH_2); 1.64(m, 1H, β'-CH_2); 1.83(s, 3H, Ac); 2.80(m, 2H, δ-CH_2); 2.84(m, 1H, β'-Phe); 3.05(m, 1H, β'-Phe); 2.99(m, 1H, β-Phe2); 3.16(m, 1H, β'-Phe2); 3.58(m, 1H, α-Gly); 3.68(m, 1H, α-Gly); 3.95(m, 1H, α-Phe2); 4.19(m, 1H, α-Ala); 4.23(m, 1H, α-CH); 4.26(m, 1H, α-Leu); 4.45(m, 1H, α-Phe); 7.05/7.12(d, 2H, NH_2); 7.14/7.37(m, lOH, ar-Phe); 7.61/7.77(d, 2H, NH_2); 7.87(d, 1H, NH-Ala); 8.0 (d, 1H, NH-Leu); 8.04(d, 1H, NH-Phe); 8.07(d, 1H, NH); 8.11(t, 1H, NH-Gly); 8.16(s, 1H, ε-NH). Yield 12%.

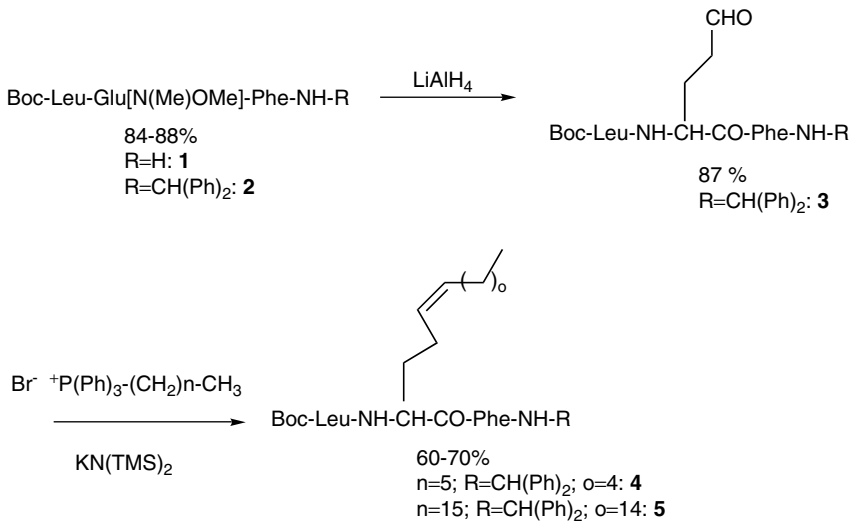
Ac-Leu-2-amino-5-amino(isopropyl)-pentanyl-L-alanyl-L-phenylalanyl-glycylamide (24). Mass spectrometry for $C_{31}H_{51}N_7O_6$; $[M+H]^+$ 618.54 (theoretical value: 618.36). ^{1}H NMR (DMSO d_6): δ (ppm) 0.83/0.87(dd, 6H, δ-Leu); 0.93(d, 6H, CH_3); 1.13(d, 3H, β-Ala); 1.40(m, 2H, β-Leu); 1. 60(t, 1H, γ-Leu); 1.65(m, 4H, β-, γ-CH_2); 1.83(s, 3H, Ac); l.90(m, 1H, CH); 2.73(dd, 1H, β'-Phe); 2.86(m, 2H, δ-CH_2); 3.05(dd, 1H, β'-Phe); 3.59(m, 1H, α-Gly); 3.67(m, 1H, α'-Gly); 4.18(m, 1H, α-CH); 4.19(m, 1H, α-Ala); 4.26(m, 1H, α-Leu); 4.44(m, 1H, α-Phe); 7.09/7.12(d, 2H, NH_2); 7.13/7.28(m, 5H, ar-Phe); 7.93(d, 1H, NH-Ala); 8.02(d, 1H, NH-Leu); 8.06(d, 1H, NH-Phe); 8.11(d, 1H, NH); 8.13(t, 1H, NH-Gly); 8.16(s, 1H, ε-NH). Yield 10%.

RESULTS AND DISCUSSION

As the results obtained with the stabilized ylide $P(Ph)_3CHCO_2Et$ were promising, we subsequently investigated the use of non-stabilized ylides to allow the incorporation of alkyl chain on solid support. In this case the Wittig reaction was more difficult than with the stabilized ylide and we had to find the best experimental conditions for this condensation. These conditions were first studied on a model peptide in solution.

Solution Synthesis

The tripeptide Ac-Leu-Glu[N(Me)OMe]-Phe-NH_2 (**1**) was first synthesized. Its reduction was performed under the experimental conditions commonly used with peptidyl-resin, $LiAlH_4$ (5 equivalents). Under these conditions the *C*-terminal primary carboxamide function was partially reduced, leading to two compounds. To avoid this side reaction, the tripeptide Ac-Leu-Glu[N(Me)OMe]-Phe-benzhydrylamide (**2**) was synthesized with a benzhydrylamide function at the *C*-terminus which mimics the link between the peptide and the resin (Scheme 2). The peptide was prepared in 84% yield and fully characterized by ^{1}H NMR and mass spectrometry. It was then reduced with a large

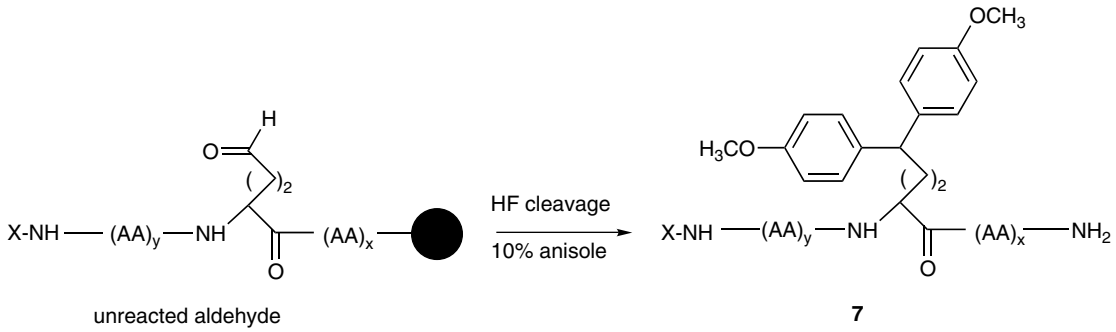


Scheme 2 Solution synthesis of the model peptides and incorporation of the alkyl side chains.

excess of 20 equivalent hydride (5 equivalents $LiAlH_4$) for 1 h at 0 °C in anhydrous tetrahydrofuran (THF). The aldehyde peptide (**3**) was recovered in a 87% yield and characterized. The ^1H NMR study revealed the presence of the aldehyde signal with a 9.5 ppm chemical shift and the disappearance of the two methyl signals corresponding to the Weinreb amide. The Wittig reaction was first checked with *n*-hexyl-triphenylphosphonium bromide. Ylide formation was performed in anhydrous THF, at 0 °C with $KN(TMS)_2$ (potassium bis(trimethylsilyl)amide) as described by Kokotos *et al.* [7]. Then, it was added to the preformed aldehyde peptide. With 4.5 equivalents of ylide, reaction was complete within 1.5 h and, after classical workup, the corresponding lipopeptide (**4**) was obtained in 69% yield and characterized. The global yield, starting from $(C_6H_5)_2CHNH_2$ (benzhydrylamine), was 50%. Further experiments were performed with *n*-hexadecyl-triphenylphosphonium bromide (2.7 equivalents for 3 h) to yield the lipidic peptide (**5**) in 40% global yield. Finally, a Wadworth-Emmons reaction was used with our model peptide and triethylphosphonoacetate. The base used in this case was lithium diisopropylamide and the reaction was complete after 12 h. The expected peptide N^{α}-acetyl-leucyl-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-phenylalanyl-benzhydrylamide (**6**) was obtained in 66% yield.

Solid-phase Synthesis on MBHA Resin

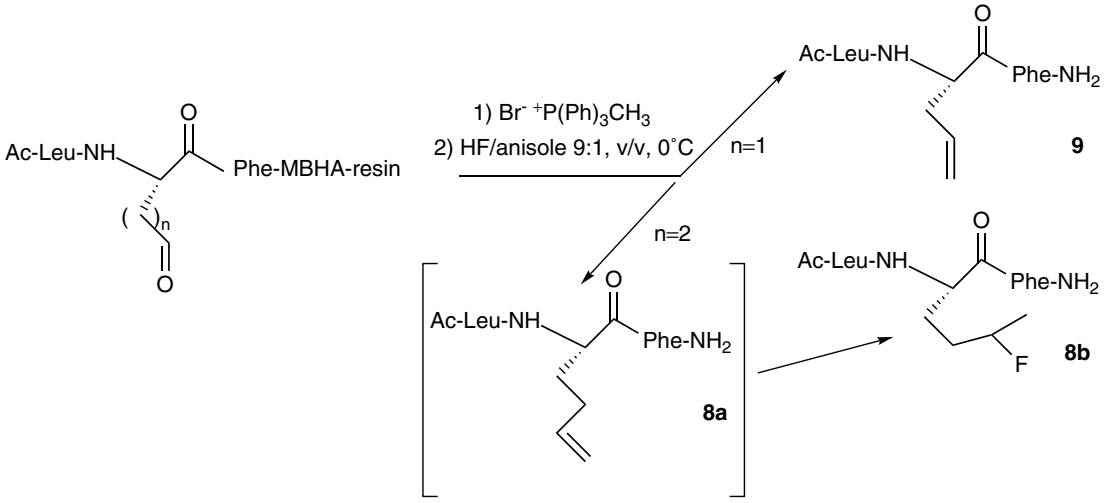
As these conditions seemed satisfactory in solution, they were applied to the solid-phase synthesis but using 9 to 10 equivalents of ylide. In this case excesses of reagents and triphenylphosphine oxide were removed by simple washings. On MBHA resin the use of $NaN(TMS)_2$ (sodium bis(trimethylsilyl)amide) was found to be better than that of $KN(TMS)_2$. Also, the reaction was left under



Scheme 3 Side-reaction during HF cleavage in the presence of anisole with the unreacted aldehyde function.

smooth stirring for at least 6 h. When the Wittig reaction was not complete, a side reaction occurred during the HF cleavage between anisole, introduced as a scavenger, and the unreacted aldehyde function. The side product (**7**) was characterized by mass spectrometry and ^{1}H NMR: under HF cleavage conditions two molecules of anisole added on the aldehyde leading to an non-coded residue-containing peptide (Scheme 3).

Moreover, when starting with Glu to introduce a homoallylglycyl residue in the peptide by condensation of the corresponding aldehyde with $^+P(Ph)_3CH_3$, Br^-, a HF molecule added to the formed double bond during HF cleavage (Scheme 4). This HF addition was only observed with homoallylglycyl residue (**8a**). When the allylglycyl residue (**9**) is generated from the condensation of $^+P(Ph)_3CH_3$, Br^- with the aldehydic function of the Asp side chain, no HF addition occurred during HF cleavage. To avoid these side reactions, the double bond was reduced on the solid support by the diimide method [14]. The side chain double bond of the newly generated residue was reduced using benzene sulphonyl hydrazine in *N*,*N*-dimethylformamide (DMF) at 100 °C overnight. After HF cleavage under the usual conditions the expected lipopeptides were hardly solubilized in a mixture of methanol (MeOH) and diethylether (Et_2O) and the solvent concentrated *in vacuo*. The expected compounds precipitated from an aqueous solution containing 0.1% TFA (trifluoroacetic acid) and filtered. Results are reported in Table 1. Due to the difficult solubilization of the



Scheme 4 Side-reaction with a homoallylglycine containing peptide **(8a)**.

Table 1 Synthesis on MBHA Resin

Starting peptide sequence	Entry	Br^- $^+P(Ph)_3$-R R=	Yield (%) of recovery[a]
Ac-Leu-Glu[NMe(OMe]-Phe-MBHA resin	**10**	$(CH_2)_5CH_3$	15
Ac-Leu-Glu[NMe(OMe]-Phe-MBHA resin	**11**	CH_3	24[b]
Ac-Leu-Glu[NMe(OMe]-Ala-Phe-Gly-MBHA resin	**12**	$(CH_2)_5CH_3$	25
Ac-Leu-Glu[NMe(OMe]-Ala-Phe-Gly-MBHA resin	**13**	$(CH_2)_9CH_3$	19
Ac-Leu-Glu[NMe(OMe]-Ala-Phe-Gly-MBHA resin	**14**	$(CH_2)_{15}CH_3$	16
Ac-Gly-Asp[NMe(OMe]-Ala-Tyr-Asp[NMe(OMe]-Val-MBHA resin	**15**[c]	CH_3	10[b]

[a] Yields were calculated from the substitution of starting resin.
[b] Purified by HPLC.
[c] No reduction of the double bond was performed.

lipidic peptides, yields of recovery were not very satisfactory.

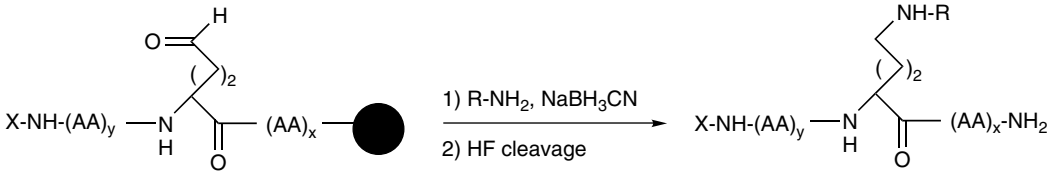
Solid-phase Synthesis on Rink-amide Resin

To prevent side reactions on the formed double bond and to avoid HF cleavage we used a TFA-labile resin (Rink-amide resin). After various experiments the best conditions to perform the Wittig reaction were found to involve the use of $NaN(TMS)_2$ in a solution of anhydrous THF containing the alkyltriphenylphosphonium bromide at 0 °C. After 30 min the peptidyl-resin was added and kept for 6 h at 0 °C. Various alkyl chain lengths were introduced (Table 2) and all the expected peptides containing an unsaturated bond were characterized by mass spectrometry and ^1H NMR. However, reduction of the Weinreb amide never went to completion with the Rink-amide resin. Various reduction conditions were tested without improving the reaction: the equivalent number of hydride was increased, $LiAlH_4$ was used in powder or in solution in THF, replaced with $LiAlH(OtBu)_3$ in solution in THF, the reaction temperature was checked (0 °C,

Table 2 Synthesis on the Rink-amide Resin

Starting peptide sequence	Entry	$Br^- {}^+P(Ph)_3$-R R=	HPLC yield (%)[a]	Yield (%) of recovery[b]
Ac-Leu-Glu[NMe(OMe)]-Phe-Rink-amide resin	**16**	CH_3	74	12[c]
Ac-Leu-Glu[NMe(OMe)]-Phe-Rink-amide resin	**17**	$(CH_2)_5CH_3$	44	36
Ac-Leu-Glu[NMe(OMe)]-Phe-Rink-amide resin	**18**	$(CH_2)_8CH_3$	37	20
Ac-Leu-Glu[NMe(OMe)]-Phe-Rink-amide resin	**19**	$(CH_2)_{14}CH_3$	42	32

[a] Yields were calculated from the analytical integration of the corresponding peaks at 214 nm on a C_{18} column.
[b] Yields were calculated from the substitution of starting resin.
[c] After preparative RP-HPLC.



Scheme 5 Reductive amination on solid support with the generated aldehyde.

Table 3 Results Obtained by Reductive Amination Leading to Lipidic Side Chains

Starting peptide sequence	Entry	Amine	Yield (%) of recovery
Ac-Leu-Glu[NMe(OMe)]-Phe-MBHA resin	**20**	NH_2-$CH_2C_6H_5$	15
Ac-Leu-Glu[NMe(OMe)]-Phe-MBHA resin	**21**	NH_2-$(CH_2)_5CH_3$	11
Ac-Leu-Glu[NMe(OMe)]-Ala-Phe-Gly-MBHA resin	**22**	NH_2-$(CH_2)_5CH_3$	20
Ac-Leu-Glu[NMe(OMe)]-Ala-Phe-Gly-MBHA resin	**23**	H-Phe-NH_2	10
Ac-Leu-Glu[NMe(OMe)]-Ala-Phe-Gly-MBHA resin	**24**	NH_2-CH_2-$CH(CH_3)_2$	12

35 °C and under THF reflux), the reaction time was studied from 3.5 h to 24 h, the reduction reaction was also performed under microwaves. After Wittig reaction and TFA cleavage about 30% of non-reduced tripeptide containing the Weinreb amide was generally recovered, thus indicating a different reactivity during reduction between MBHA and Rink-amide resins. With a higher number of hydride equivalents, some degradation products were observed; no (or very little) alcohol formation was observed. As we tested greater amounts of hydride equivalents with our model peptide linked to the resin, no lower substitution degree of resin was tested. One of the difficulties in this study was to evaluate the reduction on the solid support; this could be done only after the Wittig reaction and the cleavage from the support. The best experimental conditions for the reduction of the model tripeptide supported by the Rink-amide resin were the following: addition of 6 equivalents $LiAlH_4$ in powder to the resin suspended in THF and stored at 0 °C for 3.5 h. After quenching and the usual washings, the Wittig reaction was performed in the presence of 10 equivalents of ylide which was previously generated at 0 °C for 30 min and under an argon atmosphere by 9 equivalents of $NaN(TMS)_2$. The usual cleavage from the support was performed in a 9 : 1 TFA/DCM mixture for 1 h and at room temperature.

Reductive Amination

We also tried to generate a C–N bond by reductive amination on the newly generated aldehyde function (Scheme 5). Reductive amination was intensively used on solid support for synthesis of new linkers and for preparation of reduced peptide bonds [16,17]. We decided to use the reductive amination reaction with our aldehyde function to introduce a lipidic side chain anchored to the peptide backbone by a C–N bond. To completely consummate the aldehyde and to avoid the anisole addition on it, Barany's conditions described for the BAL linker synthesis were used [18]: 10 equivalents of the amine component and 10 equivalents of $NaBH_3CN$, 24 h, room temperature. The presence of a secondary amine function can be checked by the chloranil test [15]. After HF cleavage these peptides were recovered by simple precipitation with Et_2O, solubilization in a 50/50/0.1 H_2O/CH_3CN/TFA mixture and lyophilization. Our results are listed in Table 3. All compounds were purified by preparative HPLC and characterized by mass spectrometry and ^1H NMR.

CONCLUSIONS

We have developed a new method to introduce modified amino acids on solid support by modification of the side chains of specific residues (Asp, Glu) contained in a peptide sequence. The new amino acids that were created 'post-synthesis' possessed an α-position of configuration of the starting acidic residues. Finally, as the aldehyde function is generated in γ or δ position, racemization by enol formation cannot take place. This situation is different from that of α-amino aldehydes. This new approach avoids the synthesis of modified amino acids before their incorporation into peptides on solid support.

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