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Post-synthesis modification of aspartyl or glutamyl residue side-chains on solid support

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Abstract

A methodology to modify aspartyl or glutamyl residue side-chains after their incorporation on solid phase synthesis in a peptide sequence was developed. This strategy using the concept of Weinreb amide on the side chain of aspartyl or glutamyl residues allowed reduction of the amide side-chain into aldehyde, the reaction of different groups with this aldehyde function on solid support. The usefulness of this method was proved by the synthesis of H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide.

Various chemical reactions can be carried out in heterogeneous medium such as on insoluble polymers. In peptide chemistry the most used solid supports are based on polystyrene[1] and poly-ethyleneglycol/polystyrene[2] resins. We have recently shown that Weinreb amides can be reduced into aldehydes with reagents milder than $LiAlH_4$.[3] In fact, $AlLi(OtBu)_3H$ is sufficient in reducing the Weinreb amides and is compatible with various carboxylic protecting groups such as *tert*-butyl and cyclohexyl esters.[3] Weinreb amides are useful protecting groups of the carboxylic function in peptide synthesis.[4] We decided to investigate their use as protecting groups of aspartyl or glutamyl residues in solid phase peptide synthesis and as precursors of the aldehyde function to generate unnatural amino acids incorporated in a peptide chain.

This approach was tested for the synthesis of a model tripeptide H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide (Scheme 1). Starting from MBHA resin, the tripeptide Boc-Phe-Glu[N(Me)OMe]-Leu-MBHA resin was synthesized by classical SPPS with BOP as activating agent. The aldehyde function was then revealed on the solid support by reduction with $LiAlH_4$ or $LiAlH(OtBu)_3$ in THF at room temperature or 0°C. After hydrolysis and washing of the resin, the phosphonate component was allowed to react with the aldehyde in refluxing THF for at least 24 h leading to an unsaturated

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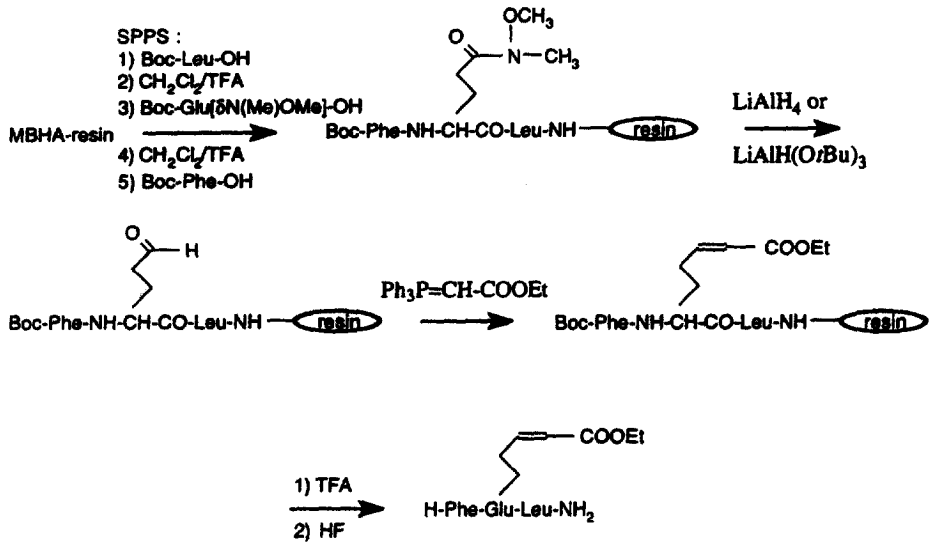
bond on the side chain of the glutamic acid. The resin was then washed with MeOH and DCM, then the N-terminal Boc group was removed by TFA before classical HF cleavage.



Scheme 1. Synthesis of H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide

The crude of the reaction, H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide, after HF cleavage exhibited the expected molecular peak by mass spectrometry, a purity of 82% by reversed phase HPLC (monitoring at 214 nm). ^{1}H NMR spectrum allowed the attribution of all the proton signals (see Table 1) which confirmed the structure of the expected compound and revealed a total *trans*-configuration of the double bond as shown by the coupling constants (J H_3–H_4=15 Hz).

This concept of incorporating a Weinreb amide (precursor of aldehyde) on the side chain of aspartyl or glutamyl residues in a peptide sequence on solid support can be very useful as several reactions (Wittig, Grignard, lithiated reagents, reductive amination...) can be performed with this reactive function. Moreover, the new amino acid residue which is created 'post synthesis' has in α-position, the configuration of the starting aspartyl or glutamyl residue. Because the aldehyde function is generated in γ- (for Asp) or δ- (for Glu) position, no racemization by enol formation can occur, which is not the case with α-aminoaldehydes. All these reactions are in progress in our laboratory.

This strategy was applied to several peptide sequences containing aspartyl or glutamyl residues to yield unnatural aminoacid derivative containing peptides (Table 2).

Table 1
^{1}H NMR chemical shifts of H-Phe-[2-(4-ethoxycarbonyl-3-butene)-glycyl]-leucine amide

residue	Hα	Hβ	Hγ	Hδ	Hε	NH	other
Leu	3.78	1.5	1.6	0.98	/	8.06	/
Glu	4.35	1.69	2.22	6.90	5.86	8.55	4.12(CH_2)
		1.77	Jδγ = 7.5 Hz	Jδε = 15.0 Hz			1.2(CH_3)
Phe	4.47	3.0	/	/	/	8.14	$CONH_2$
		2.81					7.08 and 7.44

Table 2
Synthesis of peptides containing unnatural amino acids

Sequence	Hydride	Temp/solvent	Eq. Hydride	Reaction time	Yield %[a]
Boc-Leu-Glu(Weinreb)-Phe-MBHA-resin	$LiAlH_4$	0°C/THF	5 eq.	3.5 H	58
Ac-Leu-Asp(Weinreb)-Ala-Phe-Gly-MBHA-resin	$LiAlH_4$	0°C/THF	8 eq.	3.5 H	57
Ac-Leu-Glu(Weinreb)-Ala-Phe-Gly-MBHA-resin	$LiAlH_4$	0°C/THF	8 eq.	3.5 H	90
Ac-Leu-Asp(Weinreb)-Ala-Phe-Gly-MBHA-resin	$LiAlH(OtBu)_3$	0°C/THF	8 eq.	16 H	80
Ac-Leu-Glu(Weinreb)-Ala-Phe-Gly-MBHA-resin	$LiAlH(OtBu)_3$	0°C/THF	8 eq.	24 H	90

[a]: overall yield based on the substitution of the commercial resin, including peptide elongation, reduction, Wittig reaction and cleavage from the resin.

1. Boc-Glu[N(Me)OMe]-OBzl

Boc-Glu-OBzl (10 mmol, 3.37 g) was dissolved in DCM (100 ml), BOP (1 equiv., 4.42 g) and HCl, NH(Me)OMe (1.1 equiv., 1.07 g) were added to the solution. Then DIEA was added to reach pH 9–10 (measured with moistened pH paper). After 2 h, the solution was washed successively three times with a 5% $KHSO_4$ aqueous solution, a saturated aqueous solution of $NaHCO_3$ and brine. After drying on Na_2SO_4, the solution was evaporated in vacuo to give a colorless oil (3.61 g, 95%) which was purified by flash chromatography on silica gel to yield 3.4 g of oily compound (89%). $M+H^+$: 381.

2. Boc-Glu[N(Me)OMe]-OH

Boc-Glu[N(Me)OMe]-OBzl (1.9 g, 5 mmol) was hydrogenolyzed with 10% Pd/C in EtOH for 6 h. The catalyst was filtered and the solution concentrated in vacuo to give 1.4 g (96%) of an oil which was used without purification. ^1H NMR, δ ppm in $CDCl_3$: 1.41 (s, 9H, Boc), 2.0 (m, 1H, Hβ), 2.20 (m, 1H, Hβ), 2.54 (m, 1H, Hγ), 2.73 (1H, Hγ), 3.19 (s, 3H, NMe), 3.69 (s, 3H, OMe), 4.26 (m, 1H, Hα), 5.54 (m, 1H, NH), 6.2 (broad s, 1H, COOH). $M+H^+$: 291.

H-Phe-[2-(4-Ethoxycarbonyl-3-butene)-glycyl]-leucine amide: starting from 1 g of MBHA resin (from SENN Chemicals, France, 1.2 mmol/g) the solid phase synthesis was performed with successive coupling/deprotection steps. The temporary amine protecting group was Boc and it was deprotected with TFA/DCM 40/60 for 30 min. After usual washings, the following Boc-*N*-protected amino acid was coupled with BOP reagent and DIEA. The Kaiser test was used to check the total acylation of the amine functions. Boc-Phe-OH, Boc-Glu[N(Me)OMe]-OH and Boc-Leu-OH were successively incorporated. After the last washings, the resin was dried overnight in vacuo to yield 1.77 g of peptidyl resin (theorical: 1.84 g). The resin was then suspended in anhydrous THF (30 ml) at 0°C and $LiAlH_4$ (0.273 g, 7.2 mmol) was added portionwise. The reaction was stirred for 3 h then hydrolyzed by addition of an

aqueous 5% $KHSO_4$ solution. The resin was filtered, washed successively with $KHSO_4$, H_2O, DMF, MeOH and DCM and dried in vacuo. It was then placed in a round bottom flask, carboethoxymethylene triphenylphosphorane (2.1 g, 6 mmol) was added and the reaction was maintained in refluxing anhydrous THF (50 ml). After 24 h, the resin was filtered, washed successively with MeOH and DCM and dried in vacuo to give 1.86 g of modified peptidyl-resin (theorical: 1.85 g). On 0.5 g of peptidyl-resin, the Boc N-terminal group was removed and the peptide was then cleaved from the resin by a classical HF procedure at 0°C for 1 h in the presence of 10% of anisole. After evaporation of HF, the reaction mixture was diluted with diethyl ether and filtered. The residue was then washed with diethyl ether and the peptide was solubilized with a CH_3CN:H_2O:TFA (50:50:0.1) solution which was lyophilized to yield 100 mg of compound (66%). The crude HPLC chromatogram (linear gradient from 100% H_2O/0.1% TFA to 100% CH_3CN/0.1% TFA in 50 min) revealed a major peak integrating for 82%. This was identified by ^1H NMR spectroscopy and mass spectrometry ($M+H^+$: 461).

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