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Solid phase synthesis of aspartyl peptide aldehydes

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Abstract

An efficient method for the solid phase synthesis of aspartyl aldehyde peptides has been developed. It uses a low cost synthetic process for the preparation of Fmoc-protected Weinreb amide linker. This procedure covers several tetrapeptide aspartyl aldehydes as well as biotinylated tetrapeptide aspartyl aldehydes. © 2000 Elsevier Science Ltd. All rights reserved.

Substrate-based inhibitor designs have led to the synthesis of some tetrapeptide aspartyl aldehydes. These aldehydes have been found to be inhibitors of caspases.[1–5] Thus it is desirable to develop a simple and efficient solid phase synthetic method of producing aspartyl peptide aldehydes.

Two methods for the solution synthesis of aspartyl peptide aldehydes have been described in the literature. The first method, the aspartyl aldehyde moiety was protected at the corresponding *O*-benzylacylal, which can be coupled, and hydrogenolyzed to afford the desired compound.[3] Another general strategy afforded the racemization-free peptidic aspartyl aldehydes by using a semicarbazone derivative.[4] There are several studies concerning the solid phase synthesis of peptide aldehydes that have been published.[6–14] However, only one study has been concerned with aspartyl peptide aldehyde.[13]

In this paper we describe a convenient synthetic method of tetrapeptide aspartyl aldehydes from their corresponding Weinreb amides resin by using lithium aluminium hydride. Based on Fehrentz's work[6,7] a low cost synthetic route for preparation of Fmoc-protected Weinreb amide linker has been developed as shown in Scheme 1.

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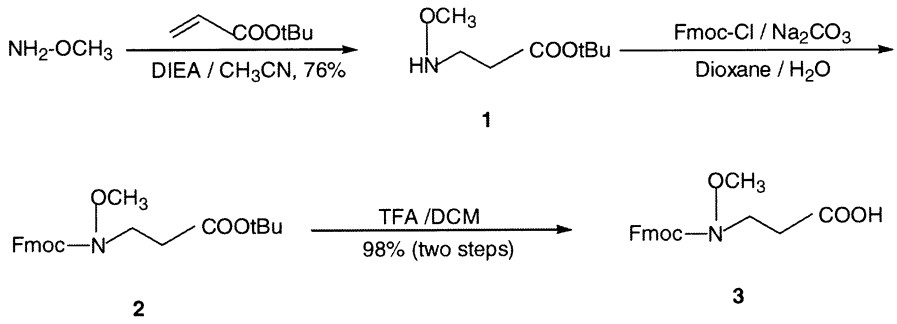
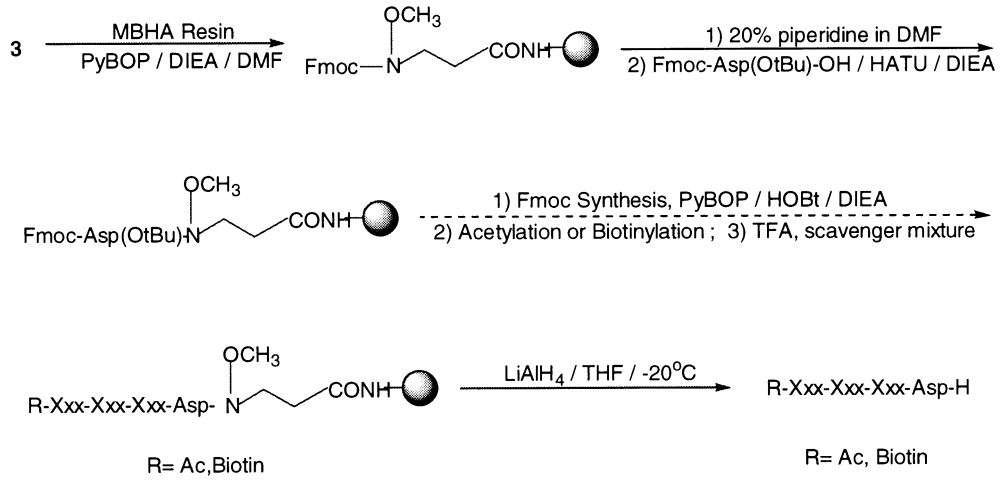


Scheme 1. Synthesis of the Weinreb amide linker

Methoxy-amine hydrochloride was reacted with low cost *t*-butyl acrylate in the presence of DIEA, the resulting alkylated methoxy-amine **1** was protected with Fmoc group using Fmoc–Cl in the presence of Na_2CO_3 to obtain compound **2**. After deprotection of the *t*-butyl group by TFA/DCM (1:1), the solid Fmoc protected Weinreb amide linker **3** was obtained in three simple steps with good yields.

Linker **3** was coupled to the MBHA resin with PyBOP to yield Fmoc protected Weinreb MBHA resin. Peptide synthesis was performed using Fmoc strategy (Scheme 2). Following removal of the Fmoc group with 20% piperidine in DMF, Fmoc–Asp(OtBu)–OH was coupled to the resin using HATU/DIEA activation. Other couplings were performed with a fourfold excess of activated amino acid using an activation solution of Fmoc–amino acid:PyBOP:HOBt:DIEA (1:1:1:2). Upon completion of the chain assembly, the peptide–resin was treated with TFA/scavenger to remove the side chain protecting groups, then the peptide was reduced and cleaved from the resin with $LiAlH_4$ (8–10 equiv.) in THF at –20°C to obtain the peptide aldehyde[15] (Table 1 and Fig. 1). It is important to remove side chain protecting groups before the peptide is cleaved from solid phase, since the esters of aspartyl and glutamyl are easily reduced to the hydroxyl groups with $LiAlH_4$, even at –20°C. On the other hand, the carboxylic groups are more stable, and they can not easily be reduced at the same conditions.



Scheme 2. SPPS of the tetrapeptide aspartyl aldehydes

Table 1
Result of synthesis

Aldehyde peptide	% Yield[a]	Retention time, min[b]
Ac-Leu-Glu-His-Asp-H	25	9.7
Ac-Val-Glu-Ile-Asp-H	20	11.1
Ac-Asp-Gln-Met-Asp-H	25	7.9
Ac-Ile-Glu-Thr-Asp-H	25	9.0
Biotin-Asp-Glu-Val-Asp-H	20	7.8
Biotin-Glu-Ser-Met-Asp-H	15	9.1

[a] Yield of purified peptides based on the substitution of the resin.
[b] Retention time on RP HPLC, 1 ml/min, 0–60% B in 20 min. A: 0.1% TFA in H_2O, and B: 0.09% TFA in CH_3CN.

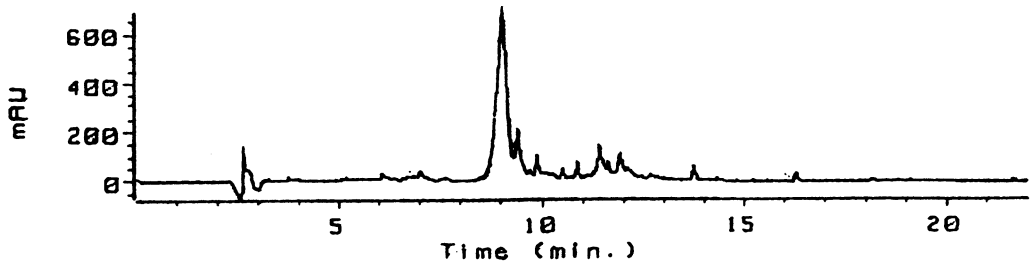


Figure 1. HPLC of the crude Ac–Ile–Glu–Thr–Asp–H

For biotinylated tetrapeptide aspartyl aldehydes, more synthesis steps were needed in the solution phase synthesis.[5] Our method can directly and effectively synthesize these kinds of compounds.

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15. The peptidyl-resin were suspended in anhydrous THF and placed in a cooling bath at –20°C. $LiAlH_4$ (1 M in THF; 8–10 mol equiv.) were slowly added and the reaction was stirred for 45 min. The reaction was then hydrolyzed with 1 M potassium hydrogenosulfate solution. The resulting mixture was filtered in order to eliminate the resin, which was washed twice with 1 M potassium hydrogenosulfate solution. The liquid phases were gathered and concentrated under reduced pressure at room temperature to evaporate most of the THF. The crude peptide aldehyde solution was checked by reversed phase HPLC of solutions A (0.1% TFA in H_2O) and B (0.09% TFA in CH_3CN) as the solvent system (crude purity 60–85%) in a gradient mode from 100% (A) to 60% (B) in 20 min and then immediately purified with preparative RP HPLC (pure compound yield after lyophilization: 15–25%). We found aspartyl peptide aldehydes to be very unstable and one of the reasons why pure yields were not very good. A study[7] showed that purification of the peptide aldehyde by chromatography (silica gel or reversed phase) induced a loss of the optical integrity of the C-terminal residue.