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SYNTHESIS OF PEPTIDES CONTAINING α, α DIALKYL AMINO ACIDS.

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Abstract: The solid phase synthesis of peptides incorporating the sterically hindered α,α-dialkyl amino acids Aminoisobutyric acid (Aib) and α-MethylSerine (α-MeSer) is reported. These amino acids were incorporated as dipeptide units employing standard coupling reagents and peptides were obtained with high purity.

Considerable attention has been focused on α,α-dialkyl amino acids and their ability to stabilize certain backbone conformations[1-5] in peptide analogues. In particular Aminoisobutyric acid (Aib) and α-MethylSerine (α-MeSer) have been shown to induce α or 3_{10} helices. However due to their steric hindrance incorporation into peptides has been difficult by the conventional stepwise assembly adopted in solution[6] or solid phase chemistry. Recent methods have shown efficient coupling of Aib or sterically hindered amino acids in solution.[7,8] From our experience in solid phase chemistry, even when PyBroP[8], one of the most efficient coupling reagent reported was used, sufficiently pure products cannot be obtained. The contaminants being deletion peptides due to inefficient coupling.

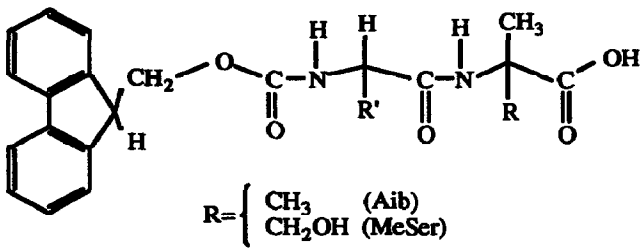

Figure 1. Dipeptide unit containing C-terminal α,α-dialkyl amino acid

In this letter we show that Aib and α-MeSer[9] can be incorporated, as a dipeptide unit[10] (**Figure 1**) into a peptide employing the standard protocols in solid phase peptide synthesis to overcome the inefficient coupling to the N-terminus of α,α-dialkyl amino acid residues. Couplings were carried out in DMF using 2 equiv. of dipeptide, 2.1 equiv. of DIPCC[11] or BOP[11], 2.1 equiv. of HOBt[11] and 3.1 equiv. of DIPEA[11]. Reaction was generally complete within 3 hours, indicated by a negative color test using TNBS[11,12]. Synthesis of peptides was performed on Rink[13] amide resin (0.62 mmol/g) or the Wang[14] resin (1.2 mmol/g) using N-α-Fmoc protection for amino acids. All sequential additions of single amino acid units were performed under the same conditions as for the dipeptides and were complete within 30 min. Fmoc deprotection was carried out in 50% piperidine/DMF (1 x 1 min, 1x 5 min). The N-terminus of the completed peptide was acetylated with acetic anhydride/pyridine (1:1). The peptide resin was treated with Trifluoroacetic

acid/ethanedithiol/thioanisole/phenol/water (16:1:1:1:1)[15] for 1.5 hours at room temperature to give the crude peptide. The peptides were purified by reverse phase HPLC[16] and their MW's were confirmed by electrospray mass spectrometry (ESMS).(**Table**)

Peptide sequence(‡)	Mass/charge ratio		Experimental MW	Calculated(*) MW
1. Ac-QSMeAGALFNA-OH	$[M+H]^+$ 1005.67	$[M+2H]^{2+}$ 503.42	1004.74 ±0.11	1005.10
2. Ac-QSMeAAMeALFNA-OH	$[M+H]^+$ 1033.68	$[M+2H]^{2+}$ 517.48	1032.81±0.19	1033.14
3. H-QSMeAAMeAAMeAFNA-OH	$[M+H]^+$ 963.77	$[M+2H]^{2+}$ 482.50	962.87±0.15	963.06
4. Ac-QSMeAAMeAAMeAFNA-NH_2	$[M+H]^+$ 1004.70	$[M+2H]^{2+}$ 503.00	1003.81±0.12	1004.11
5. Ac-SYQTMeASRAMeSNQA-NH_2	$[M+H]^+$ 1353.50	$[M+2H]^{2+}$ 677.10	1352.34±0.21	1352.43
6. Ac-SYQTAMeSRAMeSNQA-NH_2	$[M+H]^+$ 1352.70	$[M+2H]^{2+}$ 677.20	1352.02±0.46	1352.43
7. Ac-VTMeAYDVMeAEYAG-VSYQTMeASRVMeANQA-NH_2	$[M+2H]^{2+}$ 1317.00	$[M+3H]^{3+}$ 878.12	2631.65±0.45	2631.88

Table. ESMS of peptides performed on VG BIO-Q mass spectrometer.
(‡) Aminoisobutyric acid is denoted as **MeA** and α-MethylSerine as **MeS**.
(*) The molecular weights were calculated using the average mass of the elements.

Analysis of all the crude peptides incorporating Aib residues, by HPLC[16], showed the presence of one major peak, with no evidence of deletion peptides caused by inefficient coupling of the dipeptide unit. (**Figure 2**) These results illustrate the effectiveness of this method of incorporating sterically hindered amino acids into peptides using standard coupling procedures.

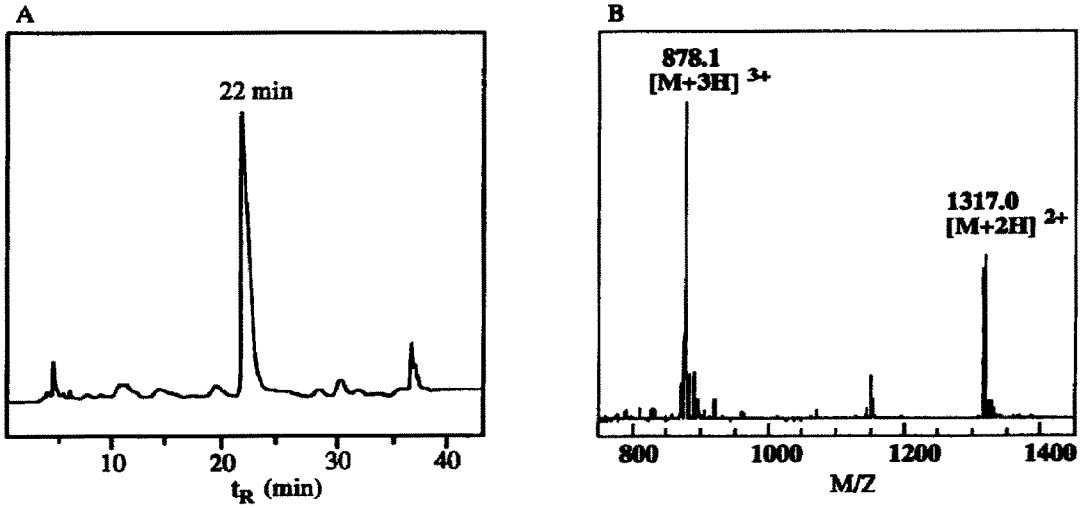

Figure 2. A. Crude HPLC[16] of peptide 7 and B. ESMS of purified peptide.
Calc: 2631.88. Found 2631.65±0.45

When α-MeSer was incorporated into the peptide, as a dipeptide unit, the side chain hydroxyl was left unprotected. This approach is simpler than the multistep procedure for the protection of the side chain hydroxyl of α-MeSer[17]. The successful synthesis of peptides with no serine side chain protection has also been reported[18]. Although HPLC (**Figure 3**) and ESMS (**Figure 4**) analysis of the crude samples showed the desired peptide as well as a number of peaks that corresponded to the side chain O-acylation of α-MeSer these acylated byproducts were easily hydrolysed with potassium carbonate to give the desired peptide with no evidence of degradation. Furthermore there is no evidence of O-N transfer of the branched chain from α-MeSer to the N-terminus.

Thus the sterically hindered α,α-dialkyl amino acids can be incorporated as dipeptide units efficiently under the same conditions employed in SPPS. α-MeSer incorporation with an unprotected side chain is also feasible without serious irreversible side reactions.

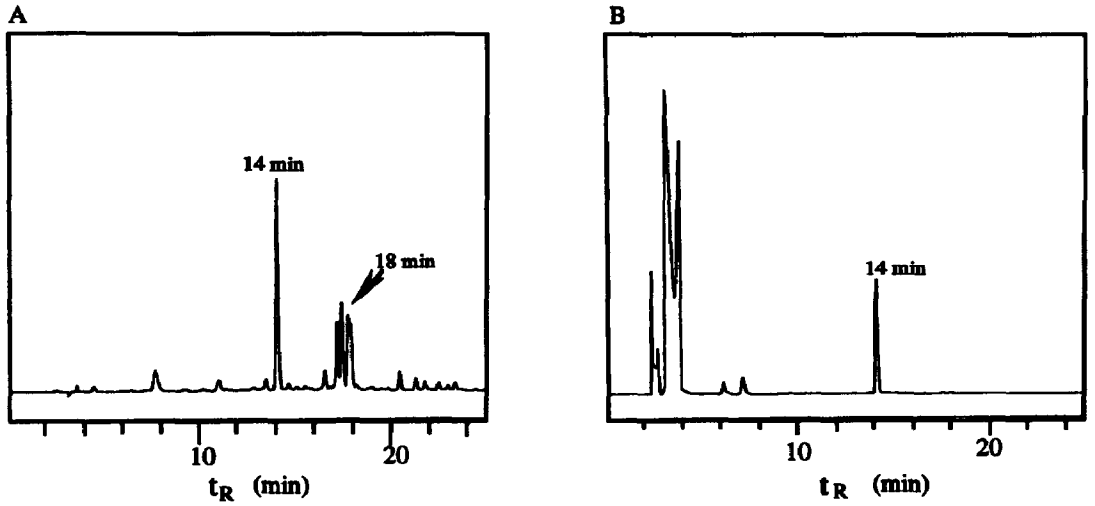

Figure 3. A. Crude HPLC[16] of peptide 5 showing acylated byproducts (R_t =16-18 min). B. Hydrolysis of isolated peaks (R_t 18min) to give the desired peptide (R_t=14min)

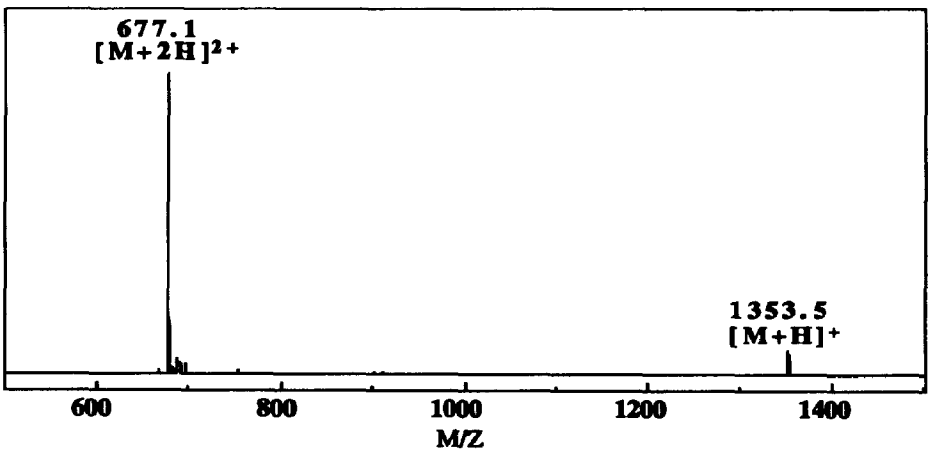

Figure 4. ESMS of peptide 5. Calc: 1352.43. found. 1352.02±0.46

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11. Abreviations. DIPCC- Diisopropylcarbodiimide, BOP- Benzotriazole-1-yl-oxy-tris-Pyrrolidino-Phosphonium-Hexafluorophosphate, HOBt- N-Hydroxybenzotriazole, DIPEA- Diisopropylethylamine, TNBS- Trinitrobenzenesulphonic acid.
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16. HPLC conditions. Solvent A. 0.1% TFA in water. Solvent B. 0.07% TFA/90% AcCN/water. Spectrum 2A. Delta Pak C-18, 15μ, 100Å, (7.8mm x 300mm), Waters. Linear gradient 50% B - 65% B in 30min. 3ml/min.
    spectrum 3B,3C. Alltima C-18, 5μ, 100Å, (4.6mm x 250mm), Alltech Associates, Inc. Linear Gradient 15% B -35% B in 25 mins. 1 ml/min.
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