The Use of Stannic Chloride for Mild and Selective Boc Deprotection on TFA Cleavable Rink-Amide MBHA Resin
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Supporting Information

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HRMS spectra of the crude model terapeptides.

![HRMS spectra](image)

Figure 1. HRMS spectra of the crude peptide Lys-Val-Phe-Ala-NH₂ synthesized using (A) Boc chemistry with SnCl₄ Boc deprotection and (B) Fmoc chemistry with piperidine Fmoc deprotection.

Experimental section

Hazards

Stannic chloride is a hygroscopic colourless fuming liquid at room temperature. Anhydrous tin (IV) chloride is a strong Lewis acid, which reacts violently with water. It is extremely corrosive to skin and on contact with air it releases severely irritating hydrogen chloride fumes. This substance should be handled in a well-ventilated hood with extreme caution.

Reagents

Boc and Fmoc protected amino acids were purchased from Nova Biochemicals (Laüfelfingen, Switzerland). Rink amide MBHA resin (200-400 mesh) was purchased from CBL (Patras, Greece; catalog no. BR-1305, lot no. 2536) and loading was determined to be 0.6 mmol/g. Solvents for SPPS, SnCl₄, trifluoroacetic acid (TFA) and solvents for HPLC were purchased from Bio-Lab (Jerusalem, Israel).

General methods

MALDI-TOF Mass Spectrometry was recorded on a PerSeptive Biosystems Voyager-DE PRO BioSpectrometry workstation. High Resolution Mass Spectrometry (HRMS) was recorded on a Thermo Scientific LTQ Orbitrap mass spectrometer. UV-VIS spectrometry was recorded on a Shimadzu UV-1650PC spectrometer. Analytical RP-HPLC analysis was performed on a Phenomenex Gemini RP-C18
column (5 μ, 250 × 4.6 mm) column using a Merck-Hitachi LaChrom D6000 interface system equipped with L-4250 UV–VIS detector set at 220 nm, with an L-6200 intelligent pump and AS-4000 autosampler. Eluants A (0.05% TFA in TDW) and B (0.05% TFA in ACN) were used in a 35 min linear gradient (5% B to 95% B) and a flow of 1 mL/min.

**General procedures for solid phase peptide synthesis**

Solid phase reactions were performed in a manual glass reaction vessel with an outer glass blanket to enable heating and cooling and equipped with a sintered glass bottom and Teflon valve for draining. All solid phase reactions were performed using Rink-amide MBHA resin and reactions (with exception to the Boc deprotection) were carried out in an amount of solvent that was enough to cover the resin (0.1-0.15 M).

Solid phase peptide synthesis was carried using the standard Fmoc/t-Bu strategy. The Fmoc protecting group was removed by piperidine solution in NMP. Standard Fmoc and Boc protected amino acid coupling reactions were performed with HBTU/HOBt and DIEA in DMF. Cleavage of the peptides and deprotection of side chain protecting groups was carried out in TFA/TIS/H₂O solution. The crude peptides were precipitated with Et₂O/hexane (1/1 mixture), dissolved in ACN/H₂O solution and lyophilized to obtain isolated peptides as white foams.

**Fmoc deprotection.** The resin was treated twice with a 20% solution of piperidine in N-methyl-2-pyrrolidinone (NMP) for 20 minutes each. The resin was then washed with NMP (5×) to remove the Fmoc by-products (dibenzofulvene and its piperidine adduct) and residual piperidine.

**Boc deprotection.** The resin was prewashed with dry CH₂Cl₂ (2×). The resin was subsequently treated with 2 eq. of SnCl₄, 0.02 M in CH₂Cl₂, 2 × 5 minutes, giving the resin a red colour due to the complex formation with SnCl₄. The resin was then washed with DMF (1×), 20% MeOH in DMF (1×), 10% DIEA in DMF (1×) and DMF (2×). First wash with DMF brakes up the complex instantly restoring the resin’s original colour.

**Fmocilation.** The resin was treated with a solution of Fmoc-OSu (3 eq.) and triethylamine (TEA) (3 eq.) in CH₂Cl₂. The reaction was left overnight and the resin was washed with CH₂Cl₂ (5×). Completion of the reaction was verified by Kaiser-ninhydrin and Chloranil tests as described below.

**HOBt/HBTU coupling.** Protected amino acid (1.5 eq.), 1-hydroxybenzotriazole hydrate (HOBt) (1.5 eq.), and diisopropyl-ethylamine (DIEA) (1.5 eq.) were dissolved in DMF and O-benzotriazol-1-yl-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) (1.5 eq.) was added followed by stirring for 7 minutes for activation. The solution of activated amino acid was applied to the resin and the reaction was agitated for 1 hour, the resin was then drained and washed with NMP (3×). Coupling completion was monitored by Kaiser-ninhydrin and Chloranil tests as described below. If a positive
ninhydrin or chloranil test was observed after one hour, the coupling reaction was repeated using 1 equivalent of activated amino acid.

**Kaiser-ninhydrin Test.** A sample containing 1–3 mg of the resin was withdrawn, placed into a test tube and washed clean with methanol, which was removed by decantation. To the sample were added 3 drops of 80% phenol in ethanol (w/v) solution, 4 drops of 1 mL of 1mM aq KCN in 49 mL of pyridine solution, and 3 drops of 5% ninhydrin in ethanol (w/v) solution. The solution was mixed well and placed in a preheated heating block at 110 °C for 5 min. A blue or violet colour of solution is a positive indication for the presence of free amines, indicating deprotection of the Fmoc protecting group or incomplete coupling reaction.

**Chloranil test.** A sample containing 1–3 mg of the resin was withdrawn, placed into a test tube and washed clean with methanol, which was removed by decantation. To the sample were added 3 drops of 2% acetaldehyde in DMF and 3 drops of 2% chloranil in DMF. The solution was agitated at RT for 5 min. A green or blue colour of the beads is a positive indication for the presence of free amines, indicating deprotection of the Fmoc protecting group or incomplete coupling reaction.

**Loading test.** Quantitative Fmoc substitution of the resin was determined by Fmoc cleavage and optical density measurements at 304 nm according to Novabiochem’s procedure.

**Abbreviations.** The following abbreviations are used in the text: ACN, acetonitrile; Alloc, allyloxy carbonyl; Boc, t-butoxycarbonyl; CH₂Cl₂, dichloromethane; Dde, 1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl; DIEA, N,N-diisopropylethylamine; DMF, dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MBHA, methylbenzhydrylamine; MS, mass spectrometry; NMP, N-methyl-2-pyrrolidinone; RP, reverse phase; RT, room temperature; SPOS, solid phase Organic synthesis; SPPS, solid phase peptide synthesis; SPS, solid phase synthesis; t-Bu, tert-butyl; TDW, triple distilled water; TEA, triethylamine; TFA, trifluoroacetic acid; TIS, triisopropylsilane; UV, ultraviolet; Z, benzoyloxycarbonyl; Amino acids abbreviations are according to the IUPAC-IUB Commission of Biochemical Nomenclature (http://www.chem.qmul.ac.uk/iupac/AminoAcid).