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Protection of 3,4-dihydroxyphenylalanine (DOPA) for Fmoc solid-phase peptide synthesis

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Abstract

Cyclic ethyl orthoformate (Ceof) was utilized as a protecting group to protect the catechol hydroxyl groups of 3,4-dihydroxyphenylalanine (DOPA). This protecting group is stable to strong bases and nucleophiles, and can be removed efficiently by 1 M trimethylsilyl bromide in trifluoroacetic acid in the presence of scavengers at 0°C for 60 min. Fmoc-DOPA(Ceof)-OH was synthesized in high yield and applied along with other Fmoc-amino acids to the solid-phase peptide synthesis of a DOPA-containing decapeptide from a mussel adhesive protein.

Keywords: biologically active compounds; amino acids and derivatives; solid-phase synthesis; peptides and polypeptides.

3,4-Dihydroxyphenylalanine (DOPA) **1** is a naturally occurring, unusual amino acid derived from post-translational modification of tyrosine.[1] It has been one of the principal agents administered to patients with Parkinson's disease since 1967.[2] Although rarely found in proteins, DOPA was detected in high yield (ca. 10%) in marine mussel adhesive proteins,[3] as well as eggshell precursor proteins of *Fasciola hepatica*[4] and *Schistosoma mansoni*.[5] It has been postulated that the presence of DOPA residues in these proteins could contribute to bioadhesion in sea water and sclerotization during eggshell formation, respectively.

Our research interest is to study the role of DOPA residues in bioadhesion, and synthesize DOPA-containing peptides and conjugates for drug delivery and new biomaterials. To accomplish this, we investigated the synthesis of DOPA derivatives for solid-phase peptide synthesis[6] by the 9-fluorenylmethoxycarbonyl (Fmoc) strategy. Solid-phase synthesis of peptides by Fmoc strategy is a mild approach wherein the base-labile Fmoc protecting group protects the α-amino group of amino acids during coupling reactions.[7] The cyclic ethyl orthoformate (Ceof) protecting group of catechol is known to be stable to strong bases[8] yet acid-labile,[9] and is therefore compatible with Fmoc strategy.[10] Therefore, we chose Ceof to protect the catechol hydroxyl groups of DOPA.

The starting material L-DOPA **1** was suspended in methanol and reacted with methyl trifluoroacetate in the presence of triethylamine under argon at room temperature to give

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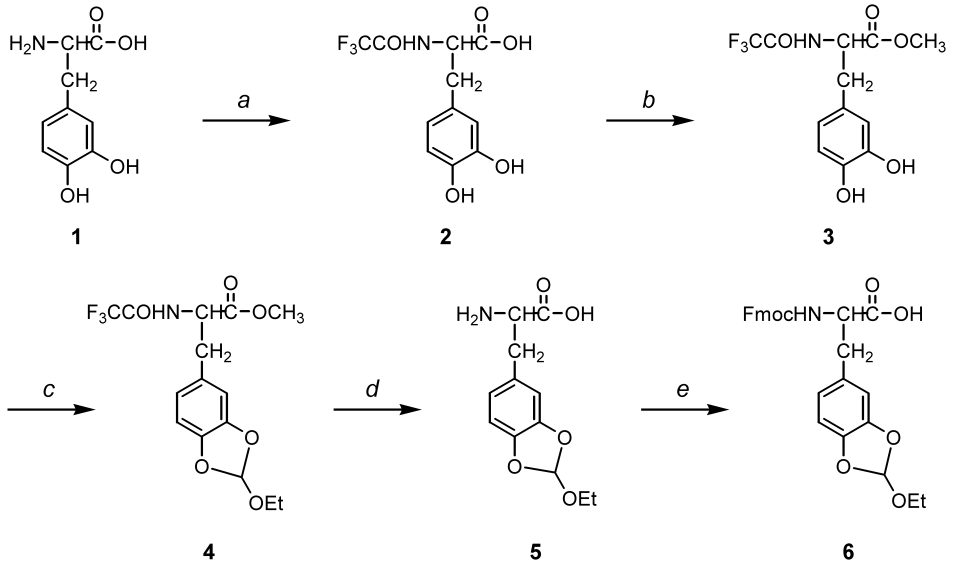
N^{α}-trifluoroacetyl-L-DOPA **2** with 95% yield (Scheme 1).[11] Reaction of **2** with iodomethane in DMF under argon in the presence of potassium bicarbonate afforded the methyl ester **3** in 95% yield.[12]

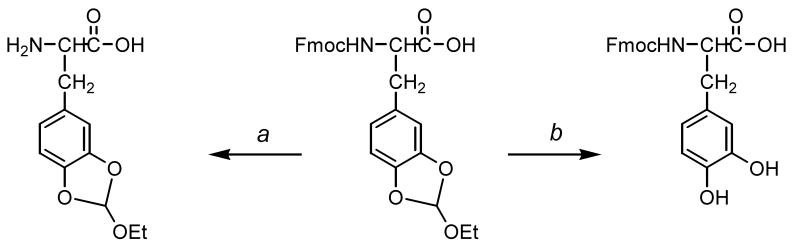


Scheme 1. Reagents and conditions: (a) CF_3CO_2Me, NEt_3, MeOH, rt, 6 h, 95%; (b) CH_3I, $KHCO_3$, DMF, rt, 4 h, 95%; (c) $HC(OEt)_3$, TsOH, molecular sieves 4A, benzene, reflux, 3 d, 80%; (d) NaOH, THF/H_2O, rt, 16 h; (e) Fmoc-OSu, THF/H_2O, rt, 8 h, 80% (d and e)

Compound **3** was refluxed with triethyl orthoformate in anhydrous benzene with *p*-toluenesulfonic acid as catalyst in the presence of molecular sieves (4A) to give the Ceof-protected DOPA methyl ester **4** in 80% yield.[13] Alkaline hydrolysis of **4** by 0.2 M sodium hydroxide solution in THF/water provided Ceof-protected DOPA **5**, which was detected by silica gel TLC and confirmed by LC-ESI/MS to show a peak with molecular weight *m*/*z* 254.1 (M+1). Without purification, **5** was treated with Fmoc-Osu in a THF/water solution in the presence of sodium carbonate[14] to afford the final product Fmoc-L-DOPA(Ceof)-OH **6**,[15] which was isolated as the dicyclohexylamine (DCHA) salt (yield 80%).

To determine the stability of the protecting group Ce of toward nucleophiles used in Fmoc strategy, Fmoc-L-DOPA(Ceof)·DCHA was treated with 25% piperidine in DMF at room temperature for 60 h (Scheme 2). LC-ESI/MS analysis of the reaction mixture revealed no evidence of **1**; furthermore, a new peak with molecular weight *m*/*z* 254.1 (M+1) was found, corresponding to **5**. This result indicates that the side-chain protecting group (Ceof) of DOPA is stable to the 25% piperidine reagent that is commonly used to remove Fmoc during stepwise peptide synthesis.[10]

To test the suitability of the Ceof protection group to synthetic strategies involving final TFA deprotection of assembled peptides, Fmoc-L-DOPA(Ceof)·DCHA was treated with 1 M trimethylsilyl bromide (TMSBr) in trifluoroacetic acid (TFA) in the presence of thioanisole, *m*-cresol and 1,2-ethanedithiol (EDT) at 0°C for 60 min (Scheme 2).[16] ESI/MS monitoring of the cleavage reaction showed that the side-chain protecting group was completely removed to form Fmoc-L-DOPA **7** within 60 min (Scheme 2).



5 6 7

Scheme 2. Reagents and conditions: (a) 25% piperidine/DMF, rt, 60 h; (b) 1 M TMSBr/TFA, thioanisole, *m*-cresol, EDT, 0°C, 60 min

To demonstrate the efficacy of this new protected DOPA in an actual peptide synthesis, Fmoc-DOPA(Ceof)-OH was applied with other Fmoc-amino acids to the solid-phase peptide synthesis of a model decapeptide, H-Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-DOPA-Lys-NH_2, derived from tandem repeats of a mussel adhesive protein (Hyp is 4-hydroxyproline).[17] The model peptide was synthesized manually on a Rink amide resin (0.8 mmol/g with Fmoc, Advanced ChemTech, KY, USA) by Fmoc strategy with the following side-chain protecting groups: *t*-butyl (Ser, Tyr, Hyp and Thr), *t*-Boc (Lys) and Ceof (DOPA). Fmoc deprotection was performed in 25% piperidine in *N*-methyl-2-pyrrolidinone (NMP) for 20 min. Coupling reactions were performed using 2 equivalents of the mixture Fmoc-amino acid:BOP:HOBt:DIEA (1:1:1:1) in NMP, with a 10 min pre-activation step before coupling.[18] In the coupling step of Fmoc-DOPA(Ceof)-OH **6**, 1.5 equivalents of **6**:BOP:HOBt:DIEA (1:1:1:1) were used. The coupling time was 20 min and monitored by a ninhydrin test.[19]

The synthesized peptide was cleaved from the resin by treatment with 1 M TMSBr in TFA in the presence of thioanisole, *m*-cresol and EDT at 0°C for 60 min. The crude decapeptide was

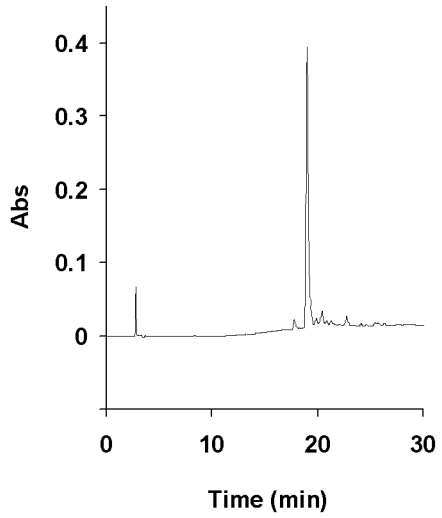


Figure 1. RP-HPLC of the crude decapeptide. HPLC was performed on a Vydac C18 reversed-phase column (250×4.6 mm, 10 μm) with a linear gradient 5–35% CH_3CN in 0.1% TFA (v/v) over 30 min, flow rate 1.0 ml/min, and UV detection at 215 nm; 2 μl (2.5 mg/ml) of sample was injected

analyzed (Fig. 1) and purified by RP-HPLC. ESI/MS of the purified decapeptide revealed a molecular weight m/z 1198.6 (M+1) (calculated monoisotopic molecular weight 1198.57).

In summary, we synthesized Fmoc-L-DOPA(Ceof)·DCHA and showed that this compound is compatible with solid-phase peptide synthesis by Fmoc strategy. Our results indicate that Fmoc-L-DOPA(Ceof)·DCHA is satisfactory for the solid-phase synthesis of DOPA-containing peptides.

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