Tetrahedron Letters 49 (2008) 5519–5521

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Convenient synthesis of acetonide-protected 3,4-dihydroxyphenylalanine (DOPA) for Fmoc solid-phase peptide synthesis

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ARTICLE INFO

Article history:
Received 21 May 2008
Revised 7 July 2008
Accepted 7 July 2008
Available online 11 July 2008

Keywords:
DOPA
Fmoc-DOPA(acetonide)-OH
Acetonide

ABSTRACT

We report a facile approach to the synthesis of acetonide and Fmoc-protected 3,4-dihydroxyphenylalanine (DOPA), Fmoc-DOPA(acetonide)-OH. By protecting the amino group of DOPA with a phthaloyl group and the carboxyl group as a methyl ester, acetonide protection of the catechol of DOPA derivative was realized in the presence of *p*-toluenesulfonic acid. Following removal of protecting groups, the intermediate was converted to Fmoc-DOPA(acetonide)-OH, which was successfully incorporated into a short DOPA-containing peptide, derived from marine tubeworm cement proteins Pc1 and Pc2.

The amino acid 3,4-dihydroxyphenylalanine (DOPA) (**1**) is found in a number of biological tissues, including the adhesive plaques of the marine mussel *Mytilus edulis*,[1,2] the cement proteins of the sandcastle worm *Phragmatopoma californica*,[3] squid beaks,[4] and in the eggshell precursor proteins of Fasciola hepatica.[5,6] The DOPA residues are considered to contribute to the bioadhesive and structural properties of these tissues.[7–9] As a therapeutic, L-DOPA is commonly prescribed for the treatment of Parkinson's disease.[10]

To better understand the biological role of DOPA and facilitate the synthesis of DOPA-containing therapeutic compounds and biomimetic materials, chemical manipulations of DOPA are often performed. Due to its chemical reactivity, it is necessary to properly protect the catechol side-chain of DOPA during chemical reactions. In the case of the synthesis of DOPA-containing peptides, solid-phase peptide synthesis (SPPS) by Fmoc strategy is a preferred approach for its convenience and efficiency. Various protecting groups have been reported to protect the side-chain catechol group of DOPA residues, including cyclic ethyl orthoformate,[11] TBDPS,[12] and acetonide.[13,14] The acetonide-protecting group has proven to be compatible with the Fmoc SPPS method,[15] however, a synthetic route to Fmoc-DOPA(acetonide)-OH (**7**) has not been reported.[16] One reported method to make H-DOPA(acetonide)-OH (**6**), from which compound **7** may be synthesized, was to construct the amino acid derivative from acetonide-protected 4-methylbenzene-1,2-diol in several steps leading to an obtained product that was a racemic mixture.[13] Although it is unknown what method is used to synthesize commercially available compound **7**, such an approach would require an additional step of chiral separation to obtain a pure product; instead, here we report a facile synthetic method for this compound with good yield.

Starting with commercially available L-DOPA, direct protection of the catechol side-chain group of L-DOPA with 2,2-dimethoxypropane (DMP) and a commonly used catalyst *p*-toluenesulfonic acid (TsOH) could not afford the expected product, indicated by the fact that the product was positive to $FeCl_3$ test.[17] We further explored refluxing the hydrochloride salt of L-DOPA methyl ester with acetone in the presence of TsOH, and found the reaction did not result in side-chain catechol protection of the L-DOPA instead of an isoquinoline product.[18] On the other hand, it was reported that Fmoc-DOPA-OH could not be converted to the acetonide-protected form,[12] and the side-chain catechol group of methyl 3-(3,4-dihydroxyphenyl)propionate was successfully protected using acetonide.[19] Based upon these findings, it seemed that a full protection of both the amino and carboxyl groups of L-DOPA was a critical prerequisite for a successful acetonide cyclization of the catechol.

Phthaloyl-protecting group (Phth) has been well-known for the full protection of primary amino groups and can be readily removed with hydrazine,[20] making it compatible with the chemistry of the acetonide-protecting group, which is relatively labile to acids and stable to bases. After the protection of the amino group with a phthaloyl group and the carboxyl group as a methyl ester, the acetonide-protected DOPA was obtained as expected. The reaction scheme is illustrated in Scheme 1 and described below.

The starting material L-DOPA was dissolved in borax buffer and the pH of the solution was adjusted to 9.5 by addition of sodium carbonate, which provided a temporary protection of the catechol group through the complexation between boric acid and the catechol group.[21] *N*-Carbethoxyphthalimide was added and the mixture was stirred overnight to give Phth-DOPA-OH (**2**), which was

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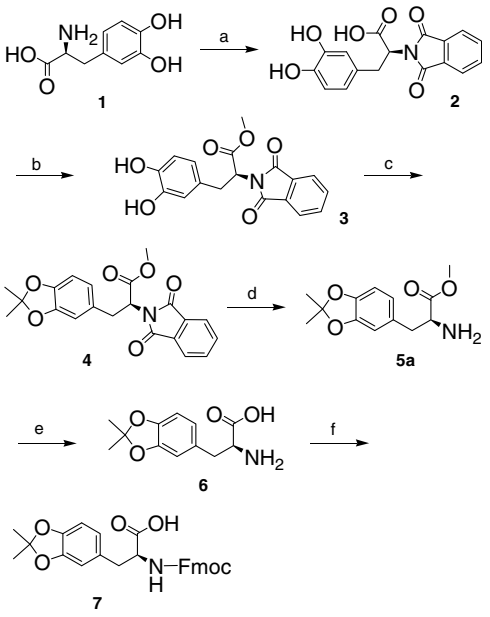
0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.07.052



Scheme 1. Reagents and conditions: (a) *N*-carbethoxyphthalimide, Borax, Na_2CO_3; (b) $SOCl_2$/MeOH, 92% (a and b); (c) DMP, TsOH, benzene, reflux, 83%; (d) H_2NNH_2, DCM/MeOH, 56%; (e) LiOH, THF/H_2O; (f) Fmoc-Osu,74% (e and f).

then reacted with $SOCl_2$/methanol to produce Phth-DOPA-OMe (**3**) (yield ca. 92% for two steps).[22] Compound **3** was refluxed with DMP in anhydrous benzene in the presence of TsOH (4.5% molar ratio) as a catalyst. Since the acetonide cyclization is controlled by equilibria, it is necessary to remove the generated byproduct from the reaction system. For this purpose, the reaction flask was equipped with a Soxhlet extractor, the thimble of which was filled with anhydrous $CaCl_2$ to trap water and the methanol produced during the reaction.[19] The reaction was monitored by the $FeCl_3$ test and was usually completed in 1.5–3 h. After removal of the solvents and re-crystallization in dichloromethane (DCM)/hexane, Phth-DOPA(acetonide)-OMe[23,24] (**4**) was obtained as white crystals (yield 83%). ^{13}C NMR spectrum of **4** showed a signal at δppm 117.8, typical for the quaternary carbon of an acetonide-protecting group of catechol (Table 1).[25]

Deprotection of the phthaloyl group using hydrazine[26] in MeOH/DCM (1:1) at 2 °C afforded H-DOPA(acetonide)-OMe (**5a**) (yield 56%).[27] To produce a hydrochloride salt (**5b**) of **5a**, the intermediate was dissolved in 0.1 N HCl solution and subjected to freeze drying. Alkaline hydrolysis of **5a** by lithium hydroxide[26] in THF/H_2O (3:1) provided H-DOPA(acetonide)-OH, which was used without further purification. The mixture solution was neutralized with 1 N HCl to pH 7–8, followed by addition of sodium carbonate (2 equiv) and Fmoc-OSu (1 equiv) to provide Fmoc-DOPA(acetonide)-OH (**7**), which was further purified by silica–gel flash chromatography (DCM/EtOAc/MeOH) (two steps, 74%).[28]

To investigate the use of compound **7** in Fmoc solid-phase peptide synthesis, a short pentapeptide, Fmoc-DOPA-Gly-Gly-Lys-Lys-OH, derived from *Phragmatopoma californica* cement proteins Pc1 and Pc2,[3] was synthesized. The solid-phase synthesis was carried out using 2-chloro trityl chloride resin (Peptide International, USA). The first amino acid, Fmoc-Lys (Boc)-OH, was attached to the resin by the standard method. Fmoc deprotection was performed twice with 20% (v/v) piperidine in *N*-methyl-2-pyrrolidinone (NMP) for 15 min. Coupling reactions were performed using two equivalents of the mixture Fmoc-amino acid/BOP/HOBt/DIPEA (1:1:1:1) in NMP, with a 10 min pre-activation step before coupling. The coupling reactions were carried out for 2 h and monitored by the ninhydrin test. The Fmoc-protecting group of DOPA was not removed in order to increase the hydrophobicity of the final peptide product.

The synthesized peptide derivative was cleaved from the resin by 2% trifluoroacetic acid (TFA) in DCM to give a white powder after neutralization with pyridine/MeOH, concentration at reduced pressure, and precipitation with water. An aliquot was measured, dried, and subjected to reverse phase HPLC (RP-HPLC) and to MALDI-TOF MS. The RP-HPLC chromatogram revealed one main peak and the MALDI-TOF MS spectrum (negative mode) revealed a monoisotopic molecular weight of *m*/*z* 1028.76 (M−1), corresponding to Fmoc-DOPA(acetonide)-Gly-Gly-Lys(Boc)-Lys(Boc)-OH (calcd 1028.50), indicating that the acetonide-protecting group was stable to 2% TFA in DCM. The above white precipitate was further cleaved by TFA/TIS/H_2O (95:2.5:2.5) for 30 min at room temperature. MALDI-TOF MS spectrum (negative mode) revealed a monoisotopic molecular weight of *m*/*z* 788.59 (M−1) (calcd 788.36), corresponding to Fmoc-DOPA-Gly-Gly-Lys-Lys-OH, which was also confirmed by ESI/MS analysis: *m*/*z* 788.30 (negative ion, M−1), 790.40 (positive ion, M+1, calcd 790.38). Only a single peak appeared in the RP-HPLC chromatogram, suggesting the absence of the diastereomer peptide Fmoc-D-DOPA-Gly-Gly-Lys-Lys-OH, which is expected to have a different RP-HPLC retention time.[29,30]

To unambiguously determine the chirality of the synthesized Fmoc-DOPA(acetonide)-OH, it was cleaved by 25% piperidine in DCM and TFA/TIS/H_2O (95:2.5:2.5) to give a raw free DOPA product, which was subjected to chiral HPLC analysis (CHIROBIOTIC T, Aldrich) using commercially available L- and D/L-DOPA as reference.[31] The absence of the D-DOPA peak in the chromatogram confirmed the optical purity of the synthesized Fmoc-DOPA(acetonide)-OH and that L-DOPA retained its chirality under the conditions of refluxing with DMP in the presence of TsOH.[32]

In summary, through protection of the amino and carboxyl groups with phthaloyl and methyl ester, respectively, the acetonide protection of the catechol of the L-DOPA was realized in the presence of TsOH. Followed by removal of the amino and the carboxy-protecting groups, the intermediate was easily converted to the product Fmoc-DOPA(acetonide)-OH in good yield. The optical integrity of the synthesized Fmoc-DOPA(acetonide)-OH was demonstrated by chiral HPLC. As a demonstration of its use as a building block for Fmoc SPPS, the synthesized Fmoc-DOPA(acetonide)-OH was incorporated into a short synthetic peptide derivative with satisfactory purity of the peptide product.

Table 1
Data of selected compounds

Compound	δ[a]	Ferric chloride test	
3	N.A.	Positive[b]	N.A.
4	117.8	Negative[b]	Positive[c]
5a	117.8	Negative[b]	Positive[c]
7	118.2	Negative[b]	Positive[c]

[a] ^{13}C NMR chemical shift (ppm) of the quaternary C of the acetonide-protecting group.
[b] Tests were performed at room temperature.
[c] Tests were performed at 105 °C for 10 min.

Acknowledgments

This research was supported by NIH Grant R37 DE 014193. NMR and Mass Spectra were completed at IMSERC, Northwestern University.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.07.052.

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24. Phth-DOPA(acetonide)-OMe (**4**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79 (m, 2H), 7.69 (m, 2H), 6.57–6.51 (m, 3H), 5.08 (dd, 1H), 3.77 (s, 3H), 3.47 (m, 2H), 1.59 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.5, 167.6 (2C), 147.6, 146.4, 134.2 (2C), 131.8 (2C), 129.8, 123.6 (2C), 121.5, 117.8, 109.2, 108.2, 53.6, 53.0, 34.5, 25.87, 25.82. DEPT: CH_3, 53.0, 25.87, 25.82; CH_2, 34.5; CH, 134.2 (2C), 123.6 (2C), 121.5, 109.2, 108.2. GC–MS: *m*/*z* 381 (11.8%), 235 (14.1%), 234 (100%), 219 (47.1%), 163 (60%), 130 (10.7%), 123 (38.3%). Ferric chloride test: negative at room temperature, positive at 105 °C. Anal. Calcd for $C_{21}H_{19}NO_6$: C, 66.13; H, 5.02; N, 3.67. Found: C, 66.05; H, 5.06; N, 3.65. HRMS (ESI): $C_{21}H_{19}NO_6$, MH^+ calcd: 382.12851; found: 382.12851. Mp 127–128 °C.
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27. H-DOPA(acetonide)-OMe (**5a**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.61–6.55 (m, 3H), 3.64 (s, 3H), 3.63 (s, 1H), 2.95 (m, 1H), 2.72 (m, 1H), 1.62 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.5, 147.6, 146.3, 130.1, 121.7, 117.8, 109.3, 108.1, 55.9, 52.0, 40.8, 25.9 (2C); DEPT: CH_3, 52.0, 25.9; CH_2, 40.8; CH, 121.7, 109.3, 108.1, 55.9. GC–MS: *m*/*z* 251 (7.6%), 192 (10.5%), 164 (18.3%), 163 (100%), 123 (41.2%), 121 (11.8%). Ferric chloride test: negative at room temperature, positive at 105 °C. HRMS (ESI): $C_{13}H_{17}NO_4$, MH^+ calcd: 252.12303; found: 252.12223; MNa^+ calcd: 274.10498; found: 274.10524.
28. Fmoc-DOPA(acetonide)-OH (**6**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.15 (br, 1H), 7.81–7.28 (m, 8H), 6.71–6.54 (m, 3H), 5.41 (d, 1H), 4.72 (m, 1H), 4.52–4.24 (m, 3H), 3.18–3.06 (m, 2H), 1.67 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.5, 156.1, 147.8, 146.8, 143.92, 143.81, 141.4 (2C), 128.6, 127.9 (2C), 127.3 (2C), 125.28, 125.22, 122.1, 119.9 (2C), 118.2, 109.6, 108.4, 67.3, 54.9, 47.2, 37.6, 26.0 (2C). DEPT: CH_3, 26.0; CH_2, 67.3, 37.6; CH, 127.9, 127.3, 125.28, 125.22, 122.1, 119.9, 109.6, 108.4, 54.9, 47.2. Ferric chloride test: negative at room temperature, positive at 105 °C. ESI-MS: MH^+ calcd: 460.18; found: 459.85. HRMS (ESI): $C_{27}H_{25}NO_6$, MH^+ calcd: 460.17546; found: 460.17516.
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