Pergamon

Tetrahedron Letters 41 (2000) 6555–6558

TETRAHEDRON LETTERS

Convenient preparation of 4-(tetrazol-5-yl)-phenylalanine for use in Fmoc-based solid-phase peptide synthesis

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Received 8 May 2000; revised 3 July 2000; accepted 6 July 2000

Abstract

To create a novel amino acid incorporating both acidic and aromatic features, trimethyltin azide was used to synthesize L-4-(tetrazol-5-yl)-phenylalanine (Tpa) and the corresponding D,L racemate by [3+2] cycloadditition of azide to the nitrile group of corresponding 4-cyanophenylalanine analogs. N^{α}-Fmoc-Tpa derivatives, possessing no protection of the tetrazole ring, were well-behaved monomers in the synthesis of a model peptide using standard solid-phase methods.

Unnatural amino acids are useful building blocks in drug discovery efforts in that they can provide interactions with receptors or enzymes that are not possible with the 20 genetically coded (natural) residues. For example, 4-carboxyphenylalanine (4-Cpa) combines the acidic features of aspartic acid and glutamic acid with the aromatic features of phenylalanine and tyrosine. The preparation of Fmoc-4-Cpa(O-*t*-Bu)-OH and its incorporation into a peptide was reported by us earlier.[1] Among structural units that mimic pharmacophoric functional groups, the 5-yl tetrazole group is widely used in medicinal chemistry as an isostere of the carboxyl group.[2] Compounds containing this heterocycle have pK_a values close to the corresponding carboxylic acids.[3] The tetrazole ring serves as a tyrosinate or carboxylate mimic in the non-peptide angiotensin II mimetic, losartan,[4] and has been used as a replacement of α- and γ-carboxyl units of the glutamic acid moiety of methotrexate analogues[5] as well as the side chain carboxyl group of the aspartic acid residue in the C-terminal gastrin tetrapeptide, Trp-Met-Asp-Phe-NH_2.[6] The sulfate group in cholecystokinin[7] and the phosphate group of phospholipase A2 inhibitors[8] have also been replaced by tetrazole rings. To extend the concept of acidic and aromatic amino acid hybrids, we report here a simple procedure for the preparation of Fmoc-4-(tetrazol-5-yl)-phenylalanine (Fmoc-Tpa) for use in peptide synthesis.

Tilley et al.[7] reported a synthesis of N^{α}-acetyl-D,L-Tpa, in which the tetrazole unit was introduced by alkylation of *N*-(diphenylmethylene)glycine benzyl ester with 4-(tetrazol-5-yl)-benzylbromide. In this case the tetrazole ring was protected by alkylation of position 3 with a

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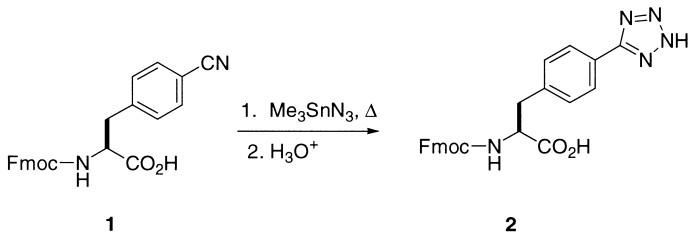
0040-4039/00/$ - see front matter
PII: S0040-4039(00)01135-7

tert-butyl group. After deprotection of the amino and carboxy groups and *N*-acetylation, this amino acid was incorporated as the N-terminal residue of a cholecystokinin analogue using solid-phase synthesis with Boc/benzyl strategy. The *tert*-butyl group was removed from the tetrazole ring during the final deprotection/cleavage step with HF.

We chose to introduce the tetrazole group by transformation of the nitrile group of previously formed 4-cyanophenylalanine derivatives. The synthesis of tetrazoles by cycloaddition of azide to nitriles is a well-known reaction,[2] and one of the classic methods is treatment with NaN_3 in DMF at elevated temperature for periods of 24 h or more. Trimethyl and tributyl tin azide[9] have been shown to be effective reagents for this conversion.[4b,5b,c,10] We found that clean transformations were achieved by stirring 4-cyanophenylalanine derivatives with Me_3SnN_3 in toluene at elevated temperature.[5b]

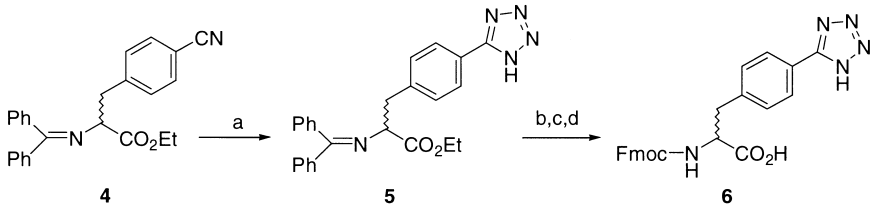
Stereospecific synthesis of Fmoc-L-Tpa (**2**) was readily achieved by merely treating commercially available Fmoc-L-4-cyanophenylalanine (**1**) with 2 equivalents of Me_3SnN_3 in dry toluene at 80–90°C for 24 h (Scheme 1).[11,12] The free carboxyl group did not interfere with the reaction and the Fmoc group was perfectly stable to these conditions. In contrast, we found that treating Fmoc–amino acid nitriles with NaN_3 in DMF at elevated temperatures resulted in premature cleavage of the amino protecting group.



1 **2**

Scheme 1.

The synthesis of Fmoc-D,L-Tpa is depicted in Scheme 2. Commercially available *N*-(diphenylmethylene)glycine ethyl ester **3** was alkylated with α-bromo-*para*-tolunitrile in the presence of KO^tBu[13] to produce the racemic *N*-protected 4-cyanophenylalanine derivative, **4**. Nitrile **4** was converted to the corresponding tetrazolyl derivative, **5**, by reaction with azidotrimethylstannane in dry toluene 80–90°C for 24 h.[5b] The imine moiety was stable to these conditions. Acidic hydrolysis of the Schiff's base followed by saponification[13] provided the amino acid, which was *N*-protected by treatment with Fmoc-OSu in aqueous sodium carbonate to afford Fmoc-D,L-Tpa **6**.



4 **5** **6**

Scheme 2. (a) Me_3SnN_3, △; (b) H_3O^+; (c) NaOH; (d) Fmoc-OSu, Na_2CO_3

Treatment of **2** and **6** with 1% DBU in ACN followed by Marfey's reagent[14] revealed no detectable D-isomer in preparations of Fmoc-L-Tpa.

The new tetrazolyl phenylalanine derivatives were incorporated into a test peptide, [Val^5] angiotensin II, by replacing the tyrosine at position 4 to give Asp-Arg-Val-Tpa-Val-His-Pro-Phe-NH_2. Standard solid-phase Fmoc/*tert*-butyl protocols employing polymethylacrylamide resin,[15] derivatized with the Rink handle, were carried out in a manual reactor. Side chain protection was Asp, *tert*-butyl; Arg, Pbf; and His, trityl. A 3-fold excess of amino acid was used and coupling was mediated with DIPCDI and HOBt in DMF:DCM (1:1). Fmoc removal was achieved with 20% piperidine in DMF. Coupling of both **2** and **6** to the sterically hindered Val^5 was complete in 30 min, as judged by Kaiser ninhydrin tests.[16] Addition of the subsequent valine also proceeded in less that 30 min.

HPLC analysis of the crude peptides incorporating **2** and **6** show very clean products (Fig. 1). The diastereomers resulting from incorporation of D,L-Tpa are remarkably well separated and the D-Tpa-containing isomer elutes second. The very small peak corresponding to peptide possessing D-Tpa in the crude product incorporating **2** indicates that very little racemization (< 5%) occurred during activation or coupling of the Fmoc-Tpa derivatives.

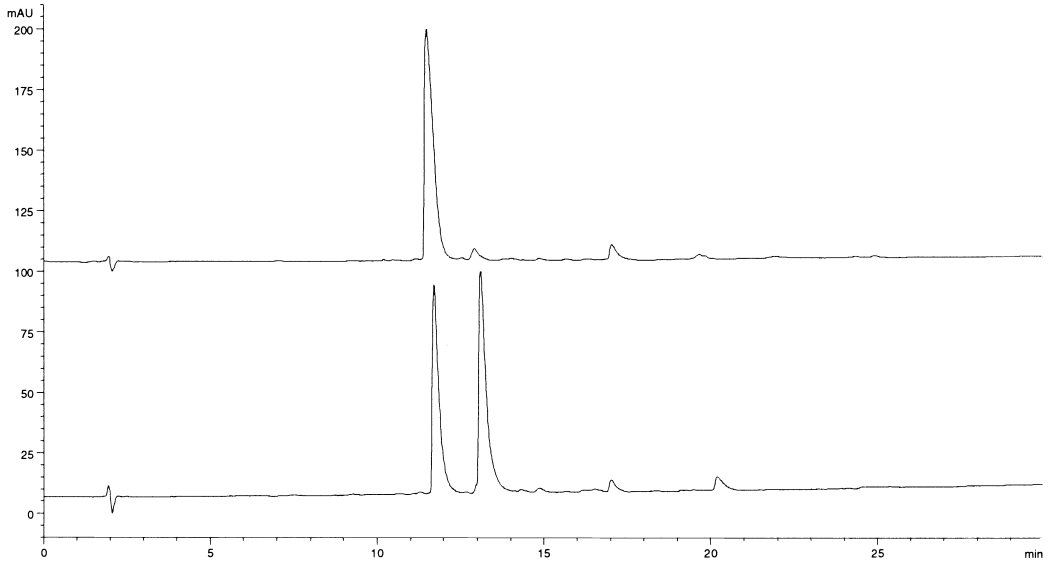


Figure 1. Reverse phase HPLC of crude [Tpa^4,Val^5] angiotensin II analogues.[17] Upper chromatogram: peptide incorporating L-Tpa. Lower chromatogram: peptide incorporating D,L-Tpa

In conclusion, we have prepared 4-(tetrazol-5-yl)-phenylalanine derivatives by [3+2] cycloaddition of azide to the nitrile of corresponding 4-cyanophenylalanine derivatives. The transformation of the nitrile was readily achieved with Me_3SnN_3. Fmoc-Tpa analogues were well behaved in peptide synthesis and will serve as useful tools in peptide-based structure–activity studies.

Abbreviations: DIPCDI, diisopropylcarbodiimide; ESI-MS, electrospray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; Fmoc-OSu, *N*-(fluorenylmethoxycarbonyl-oxy)succinimide, HOBt, 1-hydroxybenzotriazole; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl.

Acknowledgements

This work was supported by the National Cancer Institute (CA53617) and the Texas Higher Educational Control Board (ARP 15-073). Mass spectometry was performed at the University of Texas, M. D. Anderson Cancer Center, Analytical Chemistry Center.

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11. Procedure for the synthesis of **2**: Fmoc-L-4-cyanophenylalanine (0.50 gm, 1.2 mmol) and Me_3SnN_3 (0.43 gm, 2.1 mmol) in 5 ml toluene were stirred for 24 h at 80–90°C. More Me_3SnN_3 (0.4 mmol) was added and stirred for 4 h at the same temperature. The solvent was evaporated off, the solids were dissolved in EtOAc and this was washed with aqueous HCl (pH 2). After drying ($MgSO_4$) and evaporation the residue was purified by silica chromatography using gradients of EtOAc, MeOH and AcOH. Yield: 0.47 gm, 86%.
12. Analytical Data. Compound **2**: ESI-MS (M+H) calcd: 456.42; found: 456.10. ^{1}H NMR (300 MHz) δ ($CDCl_3$) 7.8–7.9 (4H, m, arom.), 7.5–7.7 (m, 3H, arom, NH), 7.2–7.6 (m, 6H, arom.) 4.2 (m, 4H, Fmoc CH, Fmoc CH_2, αCH), 3.1 (m, 1H, βCH_a), 2.9 (m, 1H, βCH_b). Compound **6**: ESI-MS (M+H) calcd: 456.42; found: 456.09. ^{1}H NMR (300 MHz) identical to **2**. [Tpa^4,Val^5] ATII: ESI-MS [M+H] calcd: 1084.4; found: 1083.1. [D,L-Tpa^4,Val^5] ATII: ESI-MS [M+H] calcd: 1084.4; found: 1083.0.
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17. Analytical HPLC was performed on a Hewlett–Packard 1090 liquid chromatograph using a Vydac C18 column (4.6×250 mm). The gradient was 10–50% B/30 min with a flow rate of 1.5 ml/min (A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile). Elution was monitored with a diode array detector at 230 nm.