Pergamon

Tetrahedron Letters, Vol. 37, No. 37, pp. 6661-6664, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01490-6

Stereospecific Synthesis of 4-Carboxyphenylalanine and Derivatives for Use in Fmoc-Based Solid-Phase Peptide Synthesis

Wei Wang, Nihal U. Obeyesekere, and John S. McMurray*

The University of Texas M. D. Anderson Cancer Center, Department of Neuro-Oncology, Box 316, 1515 Holcombe Blvd., Houston, Texas, 77030, U.S.A.

Abstract: Starting from N$^{\alpha}$-benzyloxycarbonyl-L-tyrosine(*O*-triflate) benzyl ester, we have prepared enantiomerically pure 4-carboxyphenylalanine and 4-methoxycarbonylphenylalanine derivatives using palladium (0) catalyzed carbonylation reactions. These unnatural amino acids were suitably protected and were used in solid-phase peptide synthesis using the Fmoc/*t*-butyl approach. Copyright © 1996 Elsevier Science Ltd

In drug discovery efforts, peptides are a useful class of compounds for rapidly assessing the ligand-binding requirements of target receptors or enzymes. Their ease of synthesis and manipulation, especially in the construction of combinatorial libraries, greatly facilitates probing the ligand binding site with large numbers of compounds of widely varying structure. The use of unnatural amino acids in these studies provides opportunities for ligand-receptor interactions which are not possible with the 20 natural residues and thus expands the scope of such investigations.

4-Carboxyphenylalanine (4-Cpa) is an unnatural amino acid that combines features of the acidic residues, aspartic acid and glutamic acid, and the aromatic residues, phenylalanine and tyrosine. This new amino acid thus allows alternative ionic, hydrogen bonding, or aromatic interactions between peptidic ligands and their receptors. 4-Cpa has found use as a replacement for tyrosine in angiotensin II (AT II)[1,2] and for tyrosine *O*-sulfate in cholecystokinin[3a] in studying the role of the negative charge of these aromatic residues in the binding of these peptides to their receptors.

4-Cpa has been synthesized as a racemic mixture by the alkylation of glycine equivalents with esters of 4-bromomethyl benzoic acid.[1,3a] Palladium (0) catalyzed coupling reactions have been used to add functionality to the side chain of tyrosine starting with suitably protected tyrosine *O*-triflates.[2-6] Racemization of the α-carbon is less than a few percent in these reactions.[3b,4] Therefore, the configuration of the amino acid is set at the onset of the synthesis. Franz *et al.*[2] and Wrobel and Dietrich[5] have carried out palladium catalyzed carbonylation reactions in the presence of alcohols[7a] to form 4-carboxyphenylalanine esters, intermediates in the synthesis of N$^{\alpha}$-Boc protected 4-carboxyphenylalanine and 4-(phosphonodifluoromethyl)phenylalanine derivatives for peptide synthesis. Nowak *et al.*[6] employed this reaction in the presence of potassium acetate[7b] to prepare Nvoc-4-Cpa for enzymatic protein synthesis incorporating non-coded amino acids.

For use in solid-phase peptide synthesis by the Fmoc/*t*-butyl method,[8] we have prepared Fmoc-4-Cpa(O-*t*-Bu)-OH as shown in Figure 1.[9,10] Commercially available N$^{\alpha}$-benzyloxycarbonyl-L-tyrosine, **1**, was converted to the benzyl ester[11] and the side chain hydroxyl was subsequently activated as the triflate, **2**.[12] Direct conversion of **2** to **4** by carbonylation in the presence of *tert*-butyl alcohol failed. Compound **2** was therefore treated with potassium acetate, carbon monoxide, bis-(diphenylphosphinyl)ferrocene, and palladium acetate in DMF at 60°[7b] affording Cbz-4-Cpa-OBzl, **3**, in 89% yield after chromatographic work-up. Cbz-4-Cpa-OBzl was converted to the *tert*-butyl ester, **4**, with *tert*-butyl trichloroacetimidate in the presence of a catalytic amount of boron trifluoride etherate.[13] Hydrogenolysis of the benzyl-based protecting groups followed by treatment with Fmoc-OSu in alkaline aqueous media afforded the L enantiomer of Fmoc-4-Cpa(O-*t*-Bu)-OH, **5**.

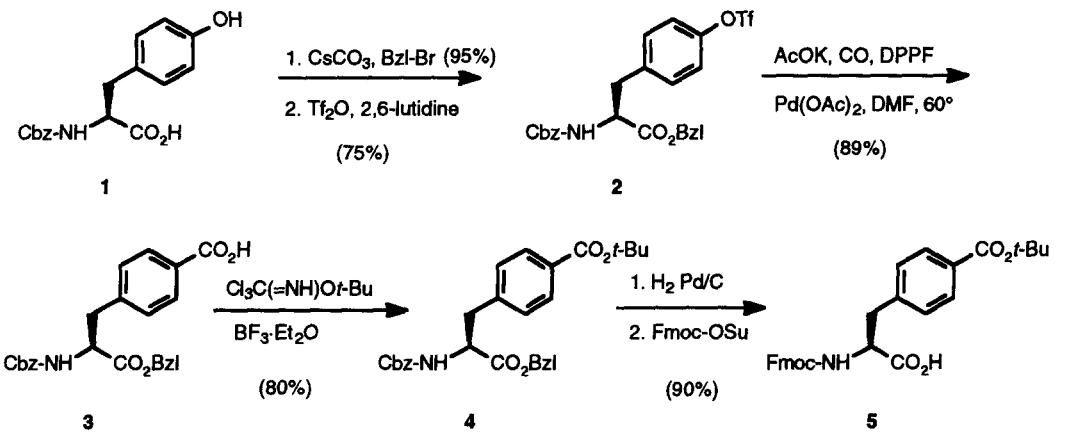

Figure 1. Synthesis of Fmoc-*p*-Cpa(O-*t*-Bu)-OH.

The corresponding side chain methyl ester was synthesized in an analogous manner in which 20 equivalents of MeOH and 2 equivalents of Et_3N[7a] were included in the carbonylation of **2** in place of the potassium acetate to give Cbz-4-Cpa(OMe)-OBzl, **6**. Again, hydrogenolysis followed by treatment with Fmoc-OSu in alkaline aqueous media afforded the L enantiomer of Fmoc-*p*-Cpa(OMe)-OH, **7**.[10]

To demonstrate the utility of these unnatural amino acid analogues in peptide synthesis, both **5** and **7** were utilized in the synthesis of [Val^5] AT II (amino acid sequence: Asp-Arg-Val-Tyr-Val-His-Pro-Phe), in which the 4-Cpa analogues were substituted for tyrosine. To study the extent of racemization in the preparation of these new amino acids, racemic Fmoc-D,L-4-Cpa(O-*t*-Bu)-OH,[14] (**8**) was also included. For these syntheses, the polydimethylacrylamide resin developed by Sparrow *et al.*[16] was used and N$^{\alpha}$-Fmoc-amino acids were employed. Side chain protecting groups were: Asp, *t*-butyl; Arg, Mtr; and His, trityl. Sparrow's resin is supplied with pendant 3-aminopropyl hydrochloride groups for attachment of the peptide which were neutralized with piperidine and acylated with 4-hydroxymethylphenoxyacetic acid using DIPCDI/HOBt as the condensing reagent. Fmoc-Phe was coupled to the support as the symmetrical anhydride in the presence of DMAP[8] and the peptide was synthesized using a coupling protocol of 4-fold excesses of amino acids, DIPCDI, and HOBt in CH_2Cl_2/DMF (1:1) and 20% piperidine in DMF to remove Fmoc groups.

Coupling of the new amino acids proceeded smoothly and was complete in 30 min as judged by ninhydrin tests. The peptides were cleaved from the support with TFA:phenol (95:5) for 24 hr and were precipitated in Et_2O. The purities of the crude materials were assessed by reverse phase HPLC[17] and were found to be in excess of 95% (Figure 2). FAB-MS analysis of the crude products indicated the presence of the 4-Cpa analogues in the peptides as well as the absence of contaminating failure sequences.[10]

The two diastereomeric peptides synthesized with **8** were well separated by HPLC (Figure 2). Because no detectable second diastereomer appeared in the crude peptides obtained with **5** and **7**, we conclude that racemization of the α-carbon does not occur during the palladium catalyzed carbonylation reaction or at any other time during the preparation of the protected 4-Cpa derivatives.

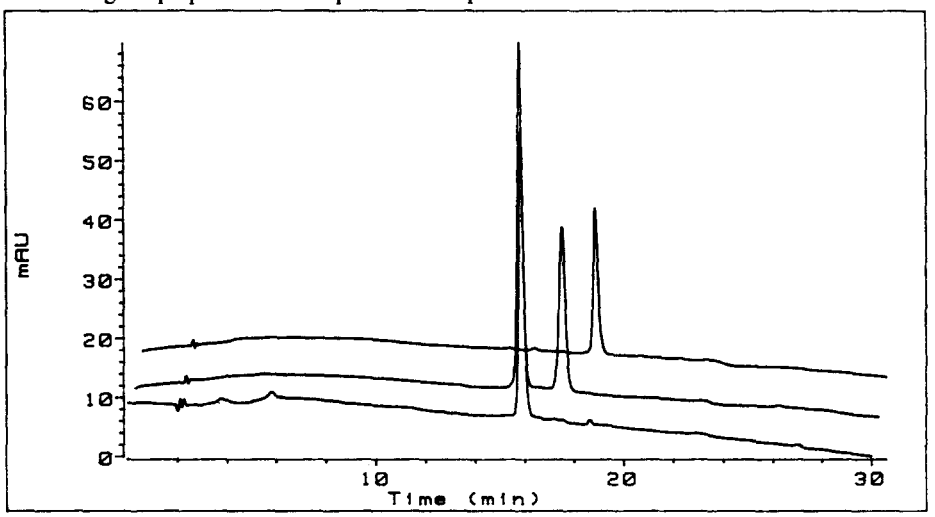

Figure 2. HPLC chromatograms of AT II peptides incorporating 4-Cpa analogues. Top trace, [4-Cpa(OMe)^4, Val^5]AT II (crude peptide). Middle trace, AT II[D,L-4-Cpa^4, Val^5]AT II (HPLC purified peptide). Bottom trace, [4-Cpa^4, Val^5]AT II (crude peptide).

In summary, palladium (0) catalyzed carbonylation reactions were used to convert a suitably protected tyrosine *O*-triflate to 4-carboxyphenylalanine derivatives. No racemization of the α-carbon was apparent and the N$^\alpha$-Fmoc derivatives were well behaved in solid-phase peptide synthesis.

Acknowledgements: Polydimethylacrylamide resin was a generous gift from Dr. James Sparrow of the Baylor College of Medicine, Houston, TX. FAB-MS analysis was carried out by Annie Bellatore and William Siefert of the Analytical Chemistry Center of The University of Texas Health Science Center at Houston. We are grateful to the National Cancer Institute (CA31657) and the Physicians Referral Service of the M. D. Anderson Cancer Center for support of this work.

Abbreviations: Bzl, benzyl; Cbz, benzyloxycarbonyl; Chx, cyclohexyl; DCM, dichloromethane; DIPCDI, diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DPPF, 1,1'-bis(diphenylphosphino)ferrocene; FAB-MS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; Fmoc-OSu, N-(9-

fluorenylmethoxycarbonyloxy)-succinimide; HOBt, 1-hydroxybenzotriazole; Mtr, 4-methyl-2,3,5-trimethoxybenzenesulfonyl; Nvoc, nitroveratryloxycarbonyl; Tf, trifluoromethylsulfonyl.

References and Notes:

1. Matsoukas, J.M.; Agelis, G.; Wahhab, A.; Hondrelis, J.; Panagiotopoulos D.; Yamdagni R.; Wu, Q.; Mavromoustakos, T.; Maia, H.L.S.; Ganter, R.; Moore, G.J. *J. Med. Chem.* **1995**, *38*, 4660-4669.
2. Franz, R.G.; Weinstock, J.; Calvo, R.R.; Samanen, J.; Aiyar, N. *Org. Prep. Proc., Int* **1994**, *26*, 533-538.
3. (a) Tilley, J.W.; Danho, W.; Lovey, K.; Wagner, R.; Swistok, J.; Makofske, R.; Michalewsky, J.; Triscari, J.; Nelson, D.; Weatherford, S. *J. Med. Chem.* **1991**, *34*, 1125-1136. (b) Tilley, J.W.; Sarabu, R.; Wagner, R.; Mulkerins, K. *J. Org. Chem.* **1990**, *55*, 906-910.
4. Shieh, W-C.; Carlson, J.A. *J. Org. Chem.* **1992**, *57*, 379-381.
5. Wrobel, J.; Dietrich, A. *Tetrahedron Letters* **1993**, *34*, 3543-3546.
6. Nowak, M.W.; Kearney, P.C.; Sampson, J.R.; Saks, M.E.; Labarca, C.G.; Silverman, S.K.; Zhong, W.; Thorson, J.; Abelson, J.N.; Davidson, N.; Schultz, P.G.; Dougherty, D.A.; Lester, H.A. *Science* **1995**, *268*, 439-442.
7. (a) Cacchi, S.; Ciattini, P.G.; Morera, E.; Ortat, G. *Tetrahedron Letters* **1986**, *27*, 3931-3934; (b) Cacchi, S.; Lupi, A. *Tetrahedron Letters* **1992**, *33*, 3939-3942.
8. (a) Atherton, E.; Sheppard, R.C. *Solid Phase Peptide Synthesis A Practical Approach*; IRL Press: Oxford, 1989. (b) Fields, G.B.; Noble, R.L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161-214.
9. N-benzyloxycarbonyl-L-tyrosine, **1** (0.79 gm, 2.5 mmol), was neutralized with 0.5 eq of Cs_2CO_3 in aqueous MeOH, dried by evaporation from DMF, and treated with 2 eq. of benzyl bromide in DMF overnight to give 0.96 gm of the benzyl ester after aqueous work-up (2.4 mmol, 95% yield). Cbz-Tyr-OBzl (0.96 gm, 2.4 mmol) and 4 eq of 2,6-lutidine were dissolved in DCM, cooled to -40°, and 2 eq. of triflic anhydride were added dropwise. After 2 h the solution was washed with water, dried ($MgSO_4$) and the solvents were removed to yield 0.96 gm of **2** (1.8 mmol, 75% yield) after silica gel chromatography (Hexane:EtOAc 8:2). All of compound **2** was treated with 5 eq of potassium acetate, 0.1 eq of $Pd(OAc)_2$, 0.2 eq of DPPF in DMF at 60°C for 3 h while bubbling CO into the reaction mixture. After the solvents were removed the product was purified by silica gel chromatography using EtOAc:AcOH (99:1) to give 0.70 gm of Cbz-*p*-Cpa-OBzl, **3** (1.6 mmol, 89% yield). All of compound **3** in DCM:cyclohexane (2:1) was treated with 4 eq of *t*-butyl trichloroacetimidate in the presence of a catalytic amount of boron trifluoride etherate at r.t. for 3 h to give, after filtration and silica gel chromatogarphy, 0.65 gm of **4** (1.28 mmol, 80%). Hydrogenolysis of all of **4** in presence of Pd/C removed the both benzyl based protecting groups, and was followed by treatment with 1eq of Fmoc-OSu and 3 eq of Na_2CO_3 in water and acetone at r.t. for 2 h to afford 0.58 gm (1.15 mmol) of the L enantiomer of Fmoc-*p*-Cpa(O-*t*-Bu)-OH, **5**. The yield of the last two steps was 90% and the overall yield from **1** was 46%.
10. ^{1}H NMR δ ($CDCl_3$): **5**. 1.57 (s, 9H), 3.02-3.18 (m, 2H), 4.16-4.52(m, 3H), 4.64-4.73 (m, 1H), 5.22-5.26 (d, 1H), 7.15-7.93 (m, 12H); **7**. 3.08-3.31 (m, 2H), 3.60 (s, 3H), 4.12-4.50(m, 3H), 4.64-4.73 (m, 1H), 5.24-5.28 (d, 1H), 7.18-7.97 (m, 12H). FAB-MS (M+H): **5**, calculated, 488.5, found 488.4; **7**, calculated 446.4, found 446.3; **[*p*-Cpa^4, Val^5]AT II** calculated, 1060.2; found, 1060.7; **[*p*-Cpa(OMe)^4, Val^5]AT II**, calculated, 1074.2; found, 1074.6; **[D,L-*p*-Cpa^4, Val^5]AT II**, calculated, 1060.2; found, 1060.7
11. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; pp 37-38.
12. Harnisch, W.; Morera, E.; Ortar, G. *J. Org. Chem.* **1985**, *50*, 1990-1992.
13. Armstrong, A.; Brackenridge, I.; Jackson, R.F.W.; Kirk, J.M. *Tetrahedron Letters* **1988**, *29*, 2483-2486.
14. Fmoc-D,L-4-Cpa(O-*t*-Bu)-OH was synthesized by (1) alkylation of *N*-diphenylmethylene glycine methyl ester (O'Donnel, M.J.; Polt, R.L. *J. Org. Chem.* **1982**, *47*, 2663-2666) with *tert*-butyl α-bromo-*para*-toluate (ref 15) in the presence of potassium *tert*-butoxide, (2) acidolytic removal of the diphenylmethylene protecting group, (3) saponification of the methyl ester, and (4) amino protection with Fmoc-OSu.
15. Prepared by treatment of *para*-toluyl chloride with potassium *tert*-butoxide followed by bromination with NBS.
16. (a) Kanda, P.; Kennedy, R.C.; Sparrow, J.T. *Int. J. Peptide Protein. Res.* **1991**, *38*, 385-391; (b) Sparrow, J.T.; Knieb-Cordonier, N.G.; Kanda, P.; Obeyesekere, N.U.; McMurray, J.S. *Peptides 1994. Proc 23rd European Peptide Symp.*; Maia, H.L.S. Ed.; ESCOM: Leiden; 1995; pp. 281-282.
17. Analytical HPLC was carried out on a Hewlett-Packard 1090 liquid chromatograph using a 2.1 x 25 mm Phenomenex C18 column. The gradient was 10-50% MeCN/30 min with a flow rate of 0.7 mL/min. Elution was monitored with a diode array detector at both 230 nm and 274 nm.

(*Received in USA* 26 *June* 1996; *accepted* 24 *July* 1996)