Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 3, pp. 647-650, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00050-X

Enantioselective Synthesis of N-Fmoc Protected Di-tert-Butyl 4-Phosphonomethyl-L-Phenylalanine : a Hydrolytically Stable Analogue of O-Phosphotyrosine

Wang-Qing LIU, Bernard P. ROQUES*
and Christiane GARBAY-JAUREGUIBERRY

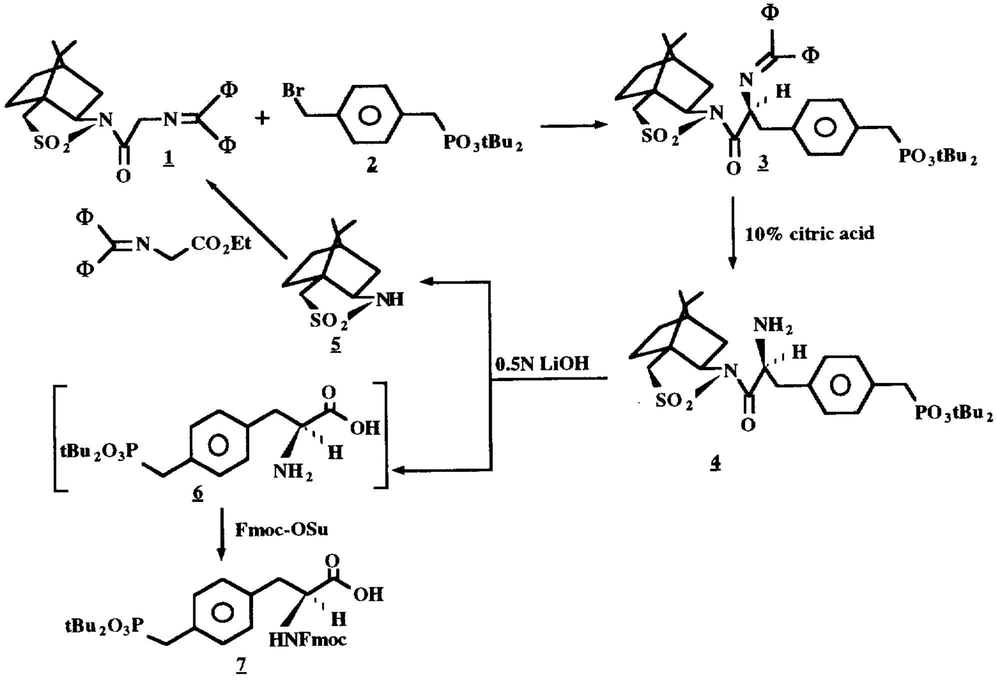
Département de Pharmacochimie Moléculaire et Structurale-U266 INSERM - URA D1500 CNRS
Université René Descartes - Faculté de Pharmacie
4, avenue de l'Observatoire - 75270 PARIS Cedex 06, FRANCE.

Abstract : Fmoc-L-Pmp$(tBu)_2$-OH was prepared with high enantiomeric purity by an asymmetric synthetic pathway, using a camphor sultam as chiral auxiliary.

Phosphorylation and dephosphorylation reactions on tyrosine residues of proteins play important roles in cellular signal transduction. These processes are mediated by proteins endowed with tyrosine kinase (PTK) and phosphatase (PTP) enzymatic activities [1]. The auto-phosphorylated tyrosine residues and surrounding amino acids serve as high affinity and specific binding sites for cellular proteins interacting via domains, referred to as Src-homology-2 (SH2) domains. The formed SH2-phosphopeptide complexes recruit other intracellular signal proteins [2-4]. The aberrant expression of PTK and PTP can lead to neoplastic cell transformation. It is therefore of great interest to develop inhibitors of these recognition processes in order to study the signaling pathways and to search for potential antitumor agents [5]. This could be done by using small peptides derived from the enzymes sequence surrounding the O-phosphotyrosine residue. However, the chemical and enzymatical liability of this latter amino acid restrict extensive *in vivo* investigations. Therefore, the stable analogue 4-phosphonomethyl-phenylalanine (Pmp) [6] was proposed as an hydrolytically stable analogue of O-phosphotyrosine. The Boc and Fmoc N-protected derivatives of this modified amino acid suitable for solid phase peptide synthesis have been synthesized. Thus, Boc-D,L-Pmp$(Et)_2$-OH has been prepared, and the pure D and L isomers obtained through enzymatic resolution [7]. On the other hand, an enantioselective synthesis of Boc-L-Pmp$(Me)_2$-OH has been reported by Cushman *et al.* [8] and Dow et al. [9]. However, the use of these protected unnatural amino acids, was hampered by difficulties in phosphoryl ester hydrolysis [10]. As Fmoc amino acids are currently used in solid-phase synthesis, a more suitable protecting group of phosphonate is the tert-butyl group since it can be removed under standard conditions of peptide synthesis without substantial side reactions during the deprotection step [11-13]. The group of Burke [14,15] has already described a synthesis of racemic Fmoc-Pmp$(tBu)_2$-OH. When incorporated into peptides, this D, L amino acid provided 1 : 1 diastereomeric mixtures that had to be separated by HPLC [10,16]. This compound has also been prepared in our laboratory by another synthetic pathway in order to prepare L-isomer. Indeed, our attempts at the enzymatic resolution of Fmoc-D,L-Pmp$(tBu)_2$-OEt and D,L-Pmp$(tBu)_2$-OEt by subtilisin carlsberg failed, and the separation of D,L-Pmp$(tBu)_2$-OEt by classical fractionated crystallization with either homochiral mandelic acid or di-p-tolyl tartaric acid or tartaric acid failed also. In this paper, we describe an enantioselective synthetic pathway for the preparation of Fmoc-L-Pmp$(tBu)_2$-OH, using a camphor sultam as chiral auxiliary (Scheme 1).

The chiral synthon (1) [17] in anhydrous THF was treated with n-BuLi (1.6 M in hexane, 1.2 eq) at -78°C for 30 minutes, and then alkylated with compound **2** [18] (1.2 eq) in HMPA/THF (1/1) at -55°C. Warmed up to ambient temperature gradually and kept overnight the mixture provided compound **3** {yield 75%, Mp 74-6°C, $[\alpha]_D^{30} = -66$ (c = 0.2 in $CHCl_3$)}.

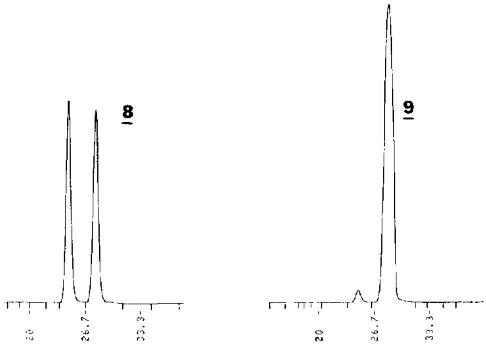
Scheme 1 :



Smooth hydrolysis with 10% aqueous citric acid (5 eq) in THF (rt, 2h) of 3 afforded compound 4 {Mp 43-5°C, $[\alpha]_D^{30} = -25$ (c = 0.2 in $CHCl_3$)}. Such a synthetic pathway was also described by Oppolzer *et al.* [19], using methyl N-(bismethylthiomethylene)glycinate for preparation of chiral synthon, but this reagent could not be used in our synthesis due to its hydrolysis conditions (0.25 N HCl/THF, rt, 4 h), which deprotected tert-butyl phosphonate. In order to avoid hydrolysis of tert-butyl phosphoryl ester, compound 4 was treated in milder hydrolysis conditions (0.5 N LiOH/THF, 4°C, 3 h) than those reported in literature [17,19]. After evaporation of THF and neutralization to pH 9 by CO_2 bubble, camphor sultam (5) was filtered off and the solution containing compound 6 was directly protected with Fmoc-OSu (1.2 eq) and the product 7 was obtained after purification {$[\alpha]_D^{27} = +31$ (c = 0.2 in $CHCl_3$), Mp and ^1H NMR spectra data are in accordance with those reported [15]}.

To determine the optical purity of compound 7, the filtrate obtained after elimination of camphor sultam was treated with 6N HCl (rt, 30 min) to yield L-Pmp which was purified by absorption on ion-exchange resin (Amberlite IR 120 H^+) and desorption with 10% NH_4OH solution {$[\alpha]_D^{21} = -9.5$ (c = 1.0 in 1N HCl) as compared with $[\alpha]_D = -10.5$ reported [7]}. Moreover, dipeptides Fmoc-D,L-Pmp-L-Phe-OMe (8) and Fmoc-L-Pmp-L-Phe-OMe (9) were prepared by DCC, HOBt, NMM coupling and TFA deprotection of tert-butyl phosphonate to estimate the enantiomeric purity of compound 7. Comparison of HPLC profiles of 8 and 9 showed the very good enantiomeric purity of 7 prepared in this way (> 97%). This was also observed by ^1H NMR spectroscopy (DMSO-d_6). Thus in 8, the NH(Phe) gave two doublets at δ 8.38 ppm and δ 8.48 ppm, and the CO_2CH_3 group two singlets at δ 3.52 ppm and δ 3.58 ppm. In 9, the NH(Phe) appeared as a single resonance at δ 8.38 ppm and the CO_2CH_3 group led to a single peak at δ 3.52 ppm.

Figure 1 : HPLC analysis of dipeptides **8** and **9**. Column : Kromasil, C-18, 5 µm, 4.6 x 250 mm. 42% acetonitrile aqueous solution with 0.09% TFA at the flow 1 ml/min, detector 214 nm.



In conclusion, the synthetic pathway described in this paper allows the preparation of Fmoc-L-Pmp(tBu)$_2$-OH, a very suitable agent for solid-phase peptide synthesis, in good yield (46% total yield) and with high optical purity (> 97%).

REFERENCES AND NOTES

1. Ullrich, A. ; Schlessinger, J. *Cell.* **1990**, *61*, 203-212.
2. Koch, C.A. ; Anderson, D. ; Moran, M.F. ; Ellis, C. ; Powson, T. *Science* **1991**, *252*, 668-674.
3. Cantley, L.C. ; Auger, K.R. ; Capenter, C. ; Duckworth, B. ; Graziani, A. ; Kapeller, R. ; Soltoff, S. *Cell* **1991**, *64*, 281-302.
4. Zhou, S.Y. ; Shoelson, S.E. ; Chaudhuri, M. ; Gish, G. ; Powson, T. ; Haser, W.G. ; King, F. ; Roberts, T. ; Ratnofsky, S. ; Lechleider, R.J. ; Neel, B.G. ; Birge, R.B. ; Fajardo, J.E. ; Chou, M.M. ; Hanafusa, H. ; Schaffhausen, B. ; Cantley, L.C. *Cell* **1993**, *72*, 767-778.
5. Brugge, J.S. *Science* **1993**, *260*, 918.
6. Marseigne, I. ; Roques, B.P. *J. Org. Chem.* **1988**, *53*, 3621-3624.
7. Garbay-Jaureguiberry, C. ; McCort-Tranchepain, I. ; Barbe, B. ; Ficheux, D. ; Roques, B.P. *Tetrahedron : Asymmetry* **1992**, *3*, 637-650.
8. Cushman, M. ; Lee, E.S. *Tetrahedron Lett.* **1992**, *33*, 1193-1196.
9. Dow, R.L. ; Bechle, B.M. *Synlett* **1994**, 293-294.
10. Garbay-Jaureguiberry, C. ; Ficheux, D. ; Roques, B.P. *Int. J. Pept. Prot. Res.* **1992**, *39*, 523-527.
11. Nomizu, M. ; Otaka, A. ; Burke, T.R.Jr. ; Roller, P.P. *Tetrahedron* **1994**, *50*, 2691-2702.
12. Shoelson, S.E. ; Chatterjee, S. ; Chaudhuri, M. ; Burke, T.R.Jr. *Tetrahedron Lett.* **1991**, *32*, 6061-6064.
13. Perich, J.W. ; Ruzzene, M. ; Pinna, L.A. ; Reynolds, E.C. *Int. J. Pept. Prot. Res.* **1994**, *43*, 39-46.
14. Burke, T.R.Jr. ; Li, Z.H. ; Bolen, J.B. ; Marquez, V.E. *J. Med. Chem.* **1991**, *34*, 1577-1581.
15. Burke, T.R.Jr. ; Russ, P. ; Lim, B. *Synthesis* **1991**, 1019-1020.
16. Domchek, S.M. ; Auger, K.R. ; Chatterjee, S. ; Burke, T.R.Jr. ; Shoelson, S.E. Biochem. 1992, 31, 9865-9870.

17. Josien, H. ; Matin, A. ; Chassaing, G. *Tetrahedron Lett.* **1991**, *32*, 6547-6550.
18. Compounds **2** was prepared by refluxing α-bromo-*p*-xylene with sodium di-tert-butylphosphite in THF overnight and subsequent bromination with N-bromosuccinimide. ^1H (270 MHz) δ (DMSO-d_6) 1.30 (s, 18H, tBu), 2.95 (d, 2H, J = 21 Hz, CH_2-P), 4.62 (s, 2H, CH_2-Br), 7.21 (m, 4H, Ar-H). Mp 89-91°C, yield 63%.
19. Oppolzer, W. ; Moretti, R. ; Thomi, S. *Tetrahedron Lett.* **1989**, *30*, 6009-6010.

(*Received in UK* 21 *December* 1994; *accepted* 31 *January* 1995)