Tetrahedron Letters, Vol. 34, No. 22, pp. 3543-3546, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Preparation of L-(Phosphonodifluoromethyl)phenylalanine Derivatives as Non-Hydrolyzable Mimetics of O-Phosphotyrosine

Jay Wrobel* and Arlene Dietrich

Wyeth-Ayerst Research, Inc., CN 8000, Princeton, New Jersey 08543-8000

Abstract: N-t-BOC-L-(Phosphonodifluoromethyl)phenylalanine benzyl ester (1) was prepared in 5 steps from N-t-BOC-L-tyrosine benzyl ester O-triflate (4). Compound 1 was further converted to derivatives 2 and 3 which are potentially suitable for peptide synthesis. Analogs 1 - 3 are non-hydrolyzable mimetics of O-phosphotyrosine.

The phosphorylation and dephosphorylation of specific tyrosine residues on receptors and peptides play an essential role in cellular signal transduction processes leading to cell growth and metabolism. Many growth factor receptors (EGF, PDGF, insulin) undergo a ligand regulated intracellular tyrosine autophosphorylation and hence become activated as protein tyrosine kinases (PTK's) which then propagate the signal.[1-3] Proteins that contain src homology region 2 (SH2) domains bind to PTK phosphotyrosine sequences and are also involved in the signal transduction process.[4] In turn, protein tyrosine phosphatases (PTP's) counteract the PTK's in the regulation of the signal transduction cascade by dephosphorylation of tyrosine residues.[5-7] Aberrant expression of any of these factors can lead to physiological disorders, for instance, many oncogene products are PTK's and lead to hyperphosphorylation of protein tyrosine residues, in turn leading to cell transformation. [8]

Specific inhibitors of PTK's [9] or PTP's, or compounds that bind to SH2 domains could be useful as biochemical tools or, more importantly, as therapeutic agents for a variety of areas including cancer,[8,10] atherosclerosis[11] and diabetes.[12] In this regard, peptidic inhibitors based on the structures of specific PTK or PTP substrates that contain non-hydrolyzable mimetics of phosphotyrosine are obvious primary targets. Several syntheses of one phosphotyrosine mimetic, phosphonomethylphenylalanine ($PheCH_2PO(OH)_2$), have been reported,[13-16] and this monomeric unit has been incorporated into peptide sequences using solid phase methods.[16-19] Also di- and tripeptides containing 4-(diethoxyphosphinyl)phenylalanine residues ($PhePO(OH)_2$) have been prepared via a two-step sequence directly from the tyrosine residues contained in the corresponding peptide precursors.[20]

(Phosphonodifluoromethyl)phenylalanine ($PheCF_2PO(OH)_2$) represents another non-hydrolyzable mimetic of phosphotyrosine and has several potential advantages over the $PheCH_2PO(OH)_2$ and $PhePO(OH)_2$ mimetics. First, the pKa of $PheCF_2PO(OH)_2$ is probably closer to the pKa of tyrosine phosphate than is $PheCH_2PO(OH)_2$ based on the pKa determinations of simpler analogs.[21] Also, the mimetic $PheCF_2PO(OH)_2$,

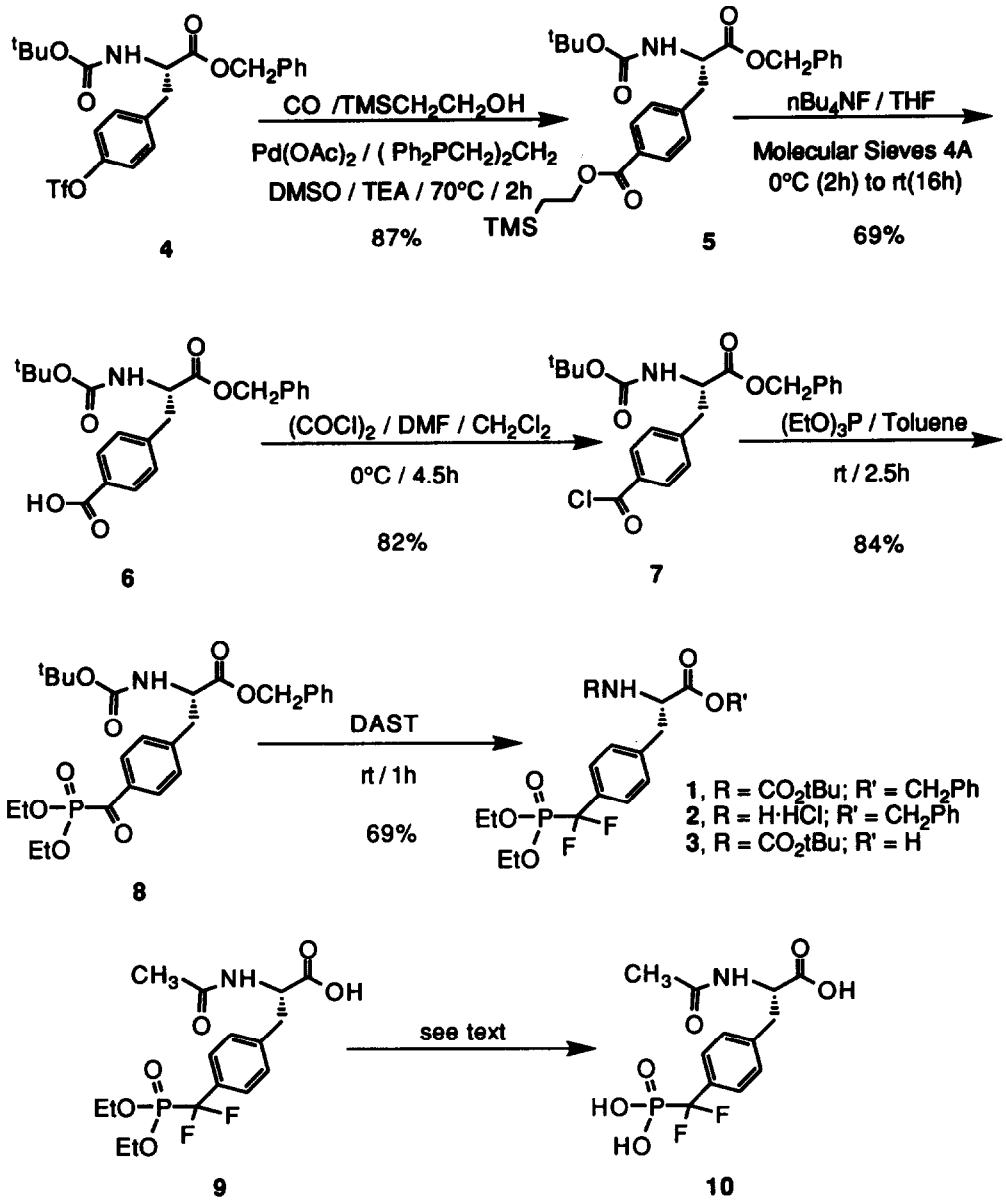
by virtue of it s electronegative fluorine atoms, retains a possible hydrogen bonding acceptor site (i.e.. the phenolic oxygen atom of tyrosine phosphate) which is lost upon going to $PheCH_2PO(OH)_2$ or $PhePO(OH)_2$. Herein we report the syntheses of **1** - **3** which are protected L-(S)-(phosphonodifluoromethyl)phenylalanine derivatives suitable for peptide synthesis.

The known triflate **4**[22] (prepared from t-BOC-L-tyrosine benzyl ester / triflic anhydride / pyridine / CH_2Cl_2 / 0 °C / 97% yield) was subjected to palladium catalyzed alkoxycarbonylation under an ambient CO atmosphere[23] to afford the trimethylsilylethyl ester **5** [24] as an oil in 83% yield after a low pressure distillation to remove excess trimethylsilylethanol followed by flash chromatography of the residue {$[\alpha]_D^{25}$=-11.55 ° (10.0 mg/mL MeOH), mp 56-60 °C}. Since somewhat harsher, palladium mediated cross coupling conditions of arylboronic acids with a tyrosine triflate derivative did not result in racemization,[25] we felt comfortable that the enantiomeric integrity of **5** was not compromised and proceeded on.

Treatment of **5** with 1.2 equivalents of tetrabutylammonium fluoride in dry THF led to the selective deprotection of the aryl ester moiety to afford **6**[24] as a white solid {69% yield, $[\alpha]_D^{25}$ = -12.95 ° (10.2 mg/mL MeOH), mp 111-113 °C}. Removal of the trimethylsilylethyl group from peptide esters with tetrabutylammonium fluoride was previously reported to cause little, if any racemization[26] and therefore we did not anticipate racemization of **6** either. The acid chloride **7**[24] was prepared from **6** using 1.1 equivalents of oxalyl chloride and catalytic DMF and was purified by flash chromatography {75% yield, white solid, $[\alpha]_D^{25}$ = +0.95 ° (10.5 mg/mL $CHCl_3$), mp 65-66 °C}. The Arbuzov reaction between **7** and 1.3 equivalents of triethylphosphite led to the formation of acylphosphonate **8**.[24] This compound was highly unstable to basic and neutral conditions, however it could be purified by flash chromatography using acid washed silica gel[27](colorless oil, 84% yield).

Treatment of **8** with 15 equivalents of (diethylamino)sulfur trifluoride (DAST) at room temperature for 1 h, followed by dilution with CH_2Cl_2 and dropwise addition to cold sat. aq. $NaHCO_3$ led to a 69% yield of **1**[28] after flash chromatography {oil, $[\alpha]_D^{25}$ = -7.94 ° (10.2 mg/mL MeOH)}. Longer reaction times, as for the preparation of simple benzylic α,α-difluorophosphonic acid esters from benzoylphosphonates,[21] led to serious decomposition of **1**. The optical purity of **1** was estimated using the method of Kinoshita.[25,29] First the t-Boc group of **1** was removed (4.0 M HCl in dioxane / room temperature / 1h, 97%) and the resulting amine hydrochloride **2**[24]{white solid, $[\alpha]_D^{25}$ = -4.16 ° (10.1 mg/mL MeOH), mp 131.5-133.5 °C} was reacted with a methylene chloride solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and triethylamine. Only one diastereoisomer could be detected upon HPLC analysis[30] of the resulting thiourea solution. Debenzylation of **1** was accomplished using standard conditions (H_2 / Pd-C / THF-EtOH / rt / 4h) to afford carboxylic acid **3**[24] in 99% yield{gummy solid, $[\alpha]_D^{25}$ = + 7.96° (10.8 mg/mL MeOH)}.

In order to access the ease of deprotection of the phosphonate diethyl ester moiety, the acetamide **9** [24] {white solid, $[\alpha]_D^{25}$ = +25.46 ° (10.8 mg/mL MeOH), mp 158-160°C} was prepared from **2** {1) Ac_2O / N-methyl morpholine / CH_2Cl_2; 2) H_2 / Pd-C / THF-EtOH / rt / 4h, 88% overall} and subjected to three different deprotection reagents. All conditions {1M TMSBr-thioanisole/TFA (10 equivalents m-cresol) / (0 °C , 14h to room temperature, 24h)[31]; 1M TMSI-thioanisole/TFA (10 equivalents m-cresol) / (0 °C , 14h to room temp 8h) or TFMSA / TFA / DMS / m-cresol (10:50:30:10, v/v)[16], room temperature, 24h} led to desired product **10**[24] in high purity with no or little side-product formation according to HPLC analysis.[32] The preparation of peptides using **2** and **3** is in progress.



Acknowledgments: We are grateful to the Analytical Department of Wyeth-Ayerst for elemental analyses, 400-MHz ^{1}H NMR, mass spectroscopy and optical rotation data. We also thank Dr. Kazimir Sestanj and Dr. Janet Sredy for their valuable advice regarding the preparation of this manuscript.

References and Notes

(1) White, M. F. *J. Bioeng. Biomembr.* **1991**, *23*, 63-82.
(2) Cadena, D. L.; Gill, G. N. *FASEB J.* **1992**, *6*, 2332-2337.
(3) Glenney, J. R. *Biochim. Biophys. Acta* **1992**, *1134*, 113-127.
(4) Margolis, B. *Cell Growth Differ.* **1992**, *3*, 73-80.
(5) Saito, H.; Streuli, M. *Cell Growth Differ.* **1991**, *2*, 59-65.
(6) Fischer, E. H.; Charbonneau, H.; Tonks, N. K. *Science* **1991**, *253*, 401-406.
(7) Pot, D. A.; Dixon, J. E. *Biochim. Biophyss Acta* **1992**, *1136*, 35-43.
(8) Aaronson, S. A. *Science* **1991**, *254*, 1146-1153.
(9) Burke, T. R., Jr *Drugs of the Future* **1992**, *17*, 119-131.
(10) Chang, C.-J.; Geahlen, R. L. *J. Natural Prod.* **1992**, *55*, 1529-1560.
(11) Levitzki, A. *FASEB J.* **1992**, *6*, 3275-3282.
(12) Goldstein, B. J. *J. Cell. Biochem.* **1992**, *48*, 33-42.
(13) Bigge, C. F.; Drummond, J. T.; Johnson, G.; Malone, T.; Probert, A. W., Jr; Marcoux, F. W.; Coughenour, L. L.; Brahce, L. J. *J. Med. Chem.* **1989**, *32*, 1580-1590.
(14) Burke, T. R., Jr; Russ, P.; Lim, B *Synthesis* **1991**, 1019-1020.
(15) Garbay-Jaureguiberry, C.; McCort-Tranchepain, I.; Barbe, B.; Ficheux, D.; Roques, B. P. *Tetrahedron Asymmetry* **1992**, *3*, 637-650.
(16) Cushman, M.; Lee, E.-S. *Tetrahedron Lett.* **1992**, *33*, 1193-1196.
(17) Shoelson, S. E.; Chatterjee, S.; Chaudhuri, M.; Burke, T. R., Jr *Tetrahedron Lett.* **1991**, *32*, 6061-6064.
(18) Garbay-Jaureguiberry, C.; Ficheux, D.; Roques, B. P. *Int. J. Peptide Protein Res.* **1992**, *39*, 523-527.
(19) Domchek, S. M.; Auger, K. R.; Chatterjee, S.; Burke, T. R., Jr; Shoelson, S. E. *Biochem.* **1992**, *31*, 9865-9870.
(20) Petrakis, K. S.; Nagabhushan, T. L. *J. Am. Chem. Soc.* **1987**, *109*, 2831-2833.
(21) Smyth, M. S.; Ford, H., Jr; Burke, T. R., Jr *Tetrahedron Lett.* **1992**, *33*, 4137-4140.
(22) Tilley, J. W.; Sarabu, R.; Wagner, R.; Mulkerins, K. *J. Org. Chem.* **1990**, *55*, 906-910.
(23) Dolle, R. E.; Schmidt, S. J.; Kruse, L. I. *J. Chem. Soc. Chem. Comm.* **1987**, 904-905.
(24) Compounds **2**, **3**, **5**, **6**, **7** and **9** were characterized by 400 MHz ^{1}HNMR, IR and MS and gave acceptable (± 0.4%) CHN analyses. Compounds **8** and **10** were characterized by 300 MHz ^{1}HNMR, IR and MS.
(25) Shieh, W.-C.; Carlson, J. A. *J. Org. Chem.* **1992**, *57*, 379-381.
(26) Sieber, P.; Andreatta, R. H.; Eisler, K.; Kamber, B.; Riniker, B.; Rink, H. In *Peptides. Proceedings of the Fifth American Peptide Symposium.*; M. Goodman and J. Meienhofer, Ed.; John Wiley and Sons: New York, NY, 1977; pp 543-545.
(27) Silica gel was stirred with 2% H_3PO_4 in MeOH, followed by oven drying.
(28) Data for **1**: C 57.90, H 6.45, N 2.57 (Calc. for $C_{26}H_{34}F_2NO_7P$: C 57.67, H 6.33, N 2.59). m/z (CI) 542 (40, M^++H), 486 (100), 391 (12), 335 (10). ^{1}H(400MHz) δ($CDCl_3$) 1.29 (6H, t J = 7.0Hz, CH_2CH_3), 1.40 (9H, s, tBu), 3.05 (1H, broad dd, $ArCH_2$), 3.14 (1H, broad dd, $ArCH_2$), 4.15 (4H, m, CH_2CH_3), 4.62 (1H, broad q, NHCH), 4.97 (1H, broad d, NHCH), 5.08 (1H, d J = 12.1Hz, OCH_2Ph), 5.15 (1H, d J = 12.1Hz, OCH_2Ph), 7.11 (2H, d J = 7.9Hz, ArH), 7.28 (2H, m, CH_2PhH), 7.34 (3H, m, CH_2PhH), 7.47 (2H, d J = 7.9Hz, ArH).
(29) Nimura, N.; Ogura, H.; Kinoshita, T. *J. Chromatogr.* **1980**, *202*, 375-379.
(30) Column: Microsorb 5 μM C18, 4.6x250 mm. Solvent: Isocratic, 60:40 MeOH :water, flow rate = 1 mL/min, 60 min. These conditions were known to resolve diasteriomeric GITC adducts of L and D amino acid esters. See references 25 and 29.
(31) Kitas, E. A.; Perich, J. W.; Wade, J. D.; Johns, R. B.; Tregear, G. W. *Tetrahedron Lett.* **1989**, *30*, 6229-6232.
(32) Column: Microsorb 5 μM C18, 4.6x250 mm. Solvent: Gradient, 10:90 to 60:40 acetonitrile:water (0.1% TFA), flow rate = 1 mL/min, 20 min.

(Received in USA 16 February 1993; accepted 31 March 1993)