Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 679–681
Bioorganic & Medicinal Chemistry Letters

A highly efficient route to enantiomerically pure L-*N*-Bz-Pmp(*t*-Bu)$_2$-OH and incorporation into a peptide-based protein tyrosine phosphatase inhibitor

Caitlin E. Hubbard and Amy M. Barrios*,†

Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA

Received 25 October 2007; revised 13 November 2007; accepted 15 November 2007
Available online 21 November 2007

Abstract—Phosphonomethyl phenylalanine (Pmp), a nonhydrolyzable mimic of phosphotyrosine, is an important building block in the development of peptide-based PTP inhibitors. We have designed a novel, efficient synthesis of *N*-Bz-Pmp(*t*-Bu)$_2$-OH. A Pmp-containing peptide based on a known biological substrate of the tyrosine phosphatase CD45 (Ac-TEGQ-Pmp-QPQP-NH_2) inhibits CD45 with an IC_{50} value of approximately 100 μM with virtually no inhibition of TCPTP up to concentrations of 120 μM.

Phosphorylation on tyrosine residues accounts for less than 1% of the phosphoproteome, but is responsible for a disproportionate amount of control in cellular signaling pathways.[1] In biology, tyrosine phosphorylation is tightly controlled by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Although PTKs were historically believed to be the key regulators of tyrosine phosphorylation, it has become clear that PTPs play critical and unique roles in regulating the tyrosine phosphoproteome.[2] The PTPs are a highly homologous family of enzymes, yet several PTPs have been shown to play unique roles in various cellular signaling pathways.[1–4] In addition, several PTPs have been identified as attractive therapeutic targets in human diseases such as cancer, diabetes, and autoimmunity.[5,6] As interest in the biological roles of PTPs has grown, so, too, has interest in chemical probes that mimic phosphotyrosine.

Non-hydrolyzable phosphotyrosine mimics such as phosphonomethylphenylalanine (Pmp) and difluorophosphonomethylphenylalanine (F_2Pmp), shown in Figure 1, serve as very useful probes of both the biological effects of tyrosine phosphorylation and also the actions of enzymes that recognize or act upon phosphotyrosine in biological systems.[7,8] For example, Pmp-containing peptides have been immobilized and used as affinity ligands to purify phosphotyrosine binding proteins.[9] The Pmp residue has been incorporated into enzymes and proteins using protein ligation to explore the effects of tyrosine phosphorylation at different sites in the protein.[10,11] Structural studies of PTPs in complex with Pmp- and F_2Pmp-containing peptides have yielded considerable information about the interactions between PTPs and their substrates.[12–14] Finally, Pmp- and F_2Pmp-containing peptides can be useful PTP inhibitors.[15,16]

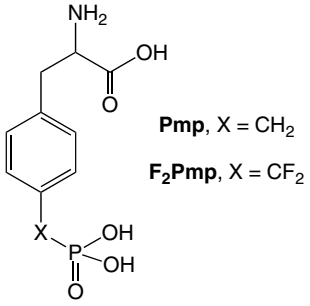


Figure 1. Molecular structure of phosphonomethylphenylalanine (Pmp) and difluorophosphonomethylphenylalanine (F_2Pmp).

One limitation to the widespread use of Pmp has been the difficulty in accessing an appropriately protected Pmp to

Keywords: Tyrosine phosphatase; Phosphotyrosine analogs; Phosphonomethylphenylalanine; Enzyme inhibition.
* Corresponding author. Tel.: +1 801 581 3198; e-mail: amy.barrios@utah.edu
† Present address: Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112, USA.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.056

incorporate into peptides or proteins. Pmp is commercially available but prohibitively expensive for large-scale use. The major challenge in the synthesis of Pmp is obtaining an enantiomerically pure product. Researchers have taken several approaches to this including the use of expensive chiral auxillaries,[17–19] enzymatic resolution of enantiomers,[20–22] or simply using the racemic mixture.[23,24] One group reported a synthesis of Pmp using tyrosine as an enantiomerically pure starting material, but this procedure requires many steps and gives a very low overall yield.[25] Our aim was to develop a straightforward synthesis of Pmp that would yield an enantiomerically pure product from inexpensive starting materials in few steps with a high overall yield. Here we report a novel, convergent synthesis of Pmp using L-serine as a chiral precursor in five steps with a 67% overall yield.

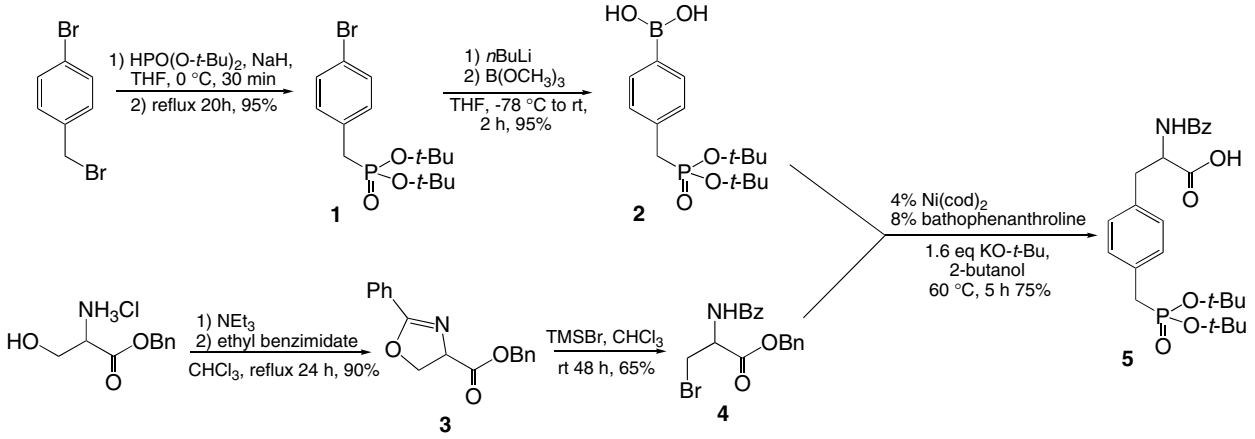
The synthesis of Pmp is outlined in Scheme 1. The phosphonate portion of the molecule was synthesized starting from 4-bromobenzylbromide by nucleophilic attack of activated di-*tert*-butyl phosphite at the benzylic position. Upon quenching of the reaction and evaporation of the organic solvents, large, clear needles of phosphonate **1** formed in 95% yield. Phosphonate **1** was converted into a boronic acid by reaction with *n*BuLi and trimethylborate, producing compound **2** in 95% yield. Both products **1** and **2** were obtained in pure form without the need for a chromatographic separation or other further purification steps.

The stereochemistry of the chiral product was fixed by the use of L-serine benzyl ester. Conversion of serine into bromoalanine was carried out according to a literature procedure.[26] Briefly, cyclization to oxazoline **3** was affected using triethylamine and ethyl benzimidate in chloroform. After purification of **3**, TMSBr was used to open the ring and produce the desired bromoalanine, **4**, in 65% yield. We found that it was not necessary to remove the protecting group from the carboxylate residue at this stage because the benzyl protecting group is efficiently cleaved in the following step.

Products **2** and **4** were coupled using a Suzuki cross coupling procedure[27] in the final step of our convergent synthesis. The optimal reagents for carrying out this coupling in our hands were 4% $Ni(cod)_2$, 8% bathophenanthroline, and 1.6 equiv potassium *t*-butoxide in 2-butanol. After purification by column chromatography, *N*-Bz-Pmp(t-Bu)$_2$-OH (**5**) was obtained in 75% yield. The overall yield of compound **5** was 67% based on the limiting reagent in the convergent synthesis, 4-bromobenzylbromide.

The gram-scale synthesis of Pmp presented here has several advantages over existing literature procedures for the preparation of Pmp. First, the chirality of the product is set by using serine as a starting material, obviating the need for expensive chiral auxillaries or chiral resolution of the product. Second, the reaction is carried out in a convergent manner, with each precursor requiring only two steps prior to the final coupling reaction. In addition, only two steps in the synthesis require purification via column chromatography, greatly limiting the amount of time required for the workup of each reaction. Finally, each of the individual steps is high yielding, with the 65% yield for conversion of **3**–**4** as the lowest yielding step.

Although the product of this reaction scheme, *N*-Bz-Pmp(t-Bu)$_2$-OH (**5**), is not protected with the Fmoc group traditionally used in solid phase peptide synthesis, we found that it was not necessary to incorporate additional deprotection and reprotection steps to obtain the Fmoc-protected amino acid. The benzoyl capping group can be cleaved off of the growing polypeptide chain essentially quantitatively by using 2% DBU in DMF, a standard reagent employed in solid phase peptide synthesis for Fmoc removal. In order to demonstrate the utility of Pmp-containing peptides as inhibitors of PTP activity, we designed a peptide sequence based on a known biological substrate of the PTP family of enzymes. The sequence chosen was Ac-TEGQ-Pmp-QPQP-NH_2, the sequence surrounding tyrosine 505 in Lck.[4] Phosphorylated Lck is an important biological substrate of CD45 and a key mediator of T-cell receptor signaling.[4] The peptide Ac-TEGQ-Pmp-QPQP-NH_2 was synthesized using **5** and standard, solid-phase peptide synthesis methodologies and was obtained in 10% overall yield. Using 6,8-difluoro-4-methylumbiliferyl phosphate (DiFMUP) as a fluorogenic substrate, the



Scheme 1. Convergent synthesis of Pmp.

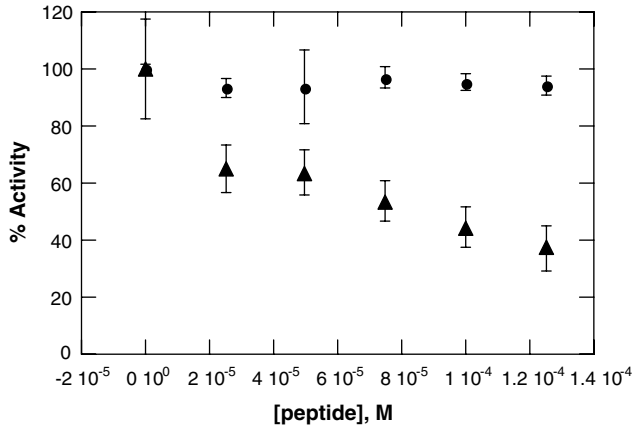


Figure 2. Inhibition of CD45 (σ) and TCPTP (λ) by Ac-TEGQ-Pmp-QPQP-NH_2.

ability of the peptide to inhibit PTP activity was investigated.[28,29] As illustrated in Figure 2, the peptide is a moderately potent inhibitor of CD45 with an IC_{50} value near 100 μM. This inhibition is consistent with other Pmp-based inhibitors that have been reported previously.[16] Interestingly, another T-cell derived phosphatase, TCPTP, was not inhibited by the peptide at concentrations up to 120 μM.

In summary, we have achieved a facile, gram-scale synthesis of Bz-Pmp from enantiomerically pure, readily available starting materials in five steps with 67% overall yield. As a proof-of-principle, Bz-Pmp has been incorporated into the peptide sequence Ac-TEGQ-Pmp-QPQP-NH_2, resulting in a moderately potent inhibitor of CD45 activity. This gram-scale synthesis of Pmp will facilitate future work on the importance of tyrosine phosphorylation in biology by serving as a readily accessible mimic of phosphotyrosine.

Acknowledgments

This work was supported in part by grants from the Zumberge Research and Innovation Fund at USC, the American Cancer Society (USC/Norris Cancer Center IRG-58-007-48) and the National Institutes of Health (R21 NS056945).

Supporting Information Available

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.056.

References and notes

1. Stoker, A. W. *J. Endocrinol.* **2005**, *185*, 19.
2. Alonso, A.; Sasin, J.; Bottini, N.; Friedberg, I.; Friedberg, I.; Osterman, A.; Godzik, A.; Hunter, T.; Dixon, J.; Mustelin, T. *Cell* **2004**, *117*, 699.
3. Mustelin, T.; Rahmouni, S.; Bottini, N.; Alonso, A. *Immunol. Rev.* **2003**, *191*, 139.
4. Mustelin, T.; Vang, T.; Bottini, N. *Nat. Immunol.* **2005**, *5*, 43.
5. Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416.
6. Bialy, L.; Waldmann, H. *Angew. Chem. Int. Ed.* **2005**, *44*, 3814.
7. Zhang, Z.-Y. *Acc. Chem. Res.* **2003**, *36*, 385.
8. Zhang, Z.-Y. *Biochim. Biophys. Acta* **2005**, *1754*, 100.
9. Han, J.; Kwon, M.; Cho, H. *J. Biochem. Mol. Biol.* **1998**, *31*, 135.
10. Schwarzer, D.; Zhang, Z.; Zheng, W.; Cole, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 4192.
11. Shen, K. *Methods* **2007**, *42*, 234.
12. Phan, J.; Lee, K.; Cherry, S.; Tropea, J. E.; Burke, T. R., Jr.; Waugh, D. S. *Biochemistry* **2003**, *42*, 13113.
13. Tolkatchev, D.; Shaykhutdinov, R.; Xu, P.; Plamondon, J.; Watson, D. C.; Young, N. M.; Ni, F. *Protein Sci.* **2006**, *15*, 2381.
14. Glover, N. R.; Tracey, A. S. *Biochem. Cell Biol.* **2000**, *78*, 39.
15. Burke, T. R., Jr.; Ye, B.; Yan, X.; Wang, S.; Jia, Z.; Chen, L.; Zhang, Z.-Y.; Barford, D. *Biochemistry* **1996**, *35*, 15989.
16. Chen, L.; Wu, L.; Otaka, A.; Smyth, M. S.; Roller, P. P.; Burke, T. R.; Denhertog, J.; Zhang, Z.-Y. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 976.
17. Li, P.; Zhang, M.; Peach, M. L.; Liu, H.; Yang, D.; Roller, P. P. *Org. Lett.* **2003**, *5*, 3095.
18. Cushman, M.; Lee, E.-S. *Tetrahedron Lett.* **1992**, *33*, 1193.
19. Liu, W.-Q.; Roques, B. P.; Garbay-Jaureguiberry, C. *Tetrahedron: Asymmetry* **1995**, *6*, 647.
20. Garbay-Jaureguiberry, C.; McCort-Tranchepain, I.; Barbe, B.; Ficheux, D.; Roques, B. P. *Tetrahedron: Asymmetry* **1992**, *3*, 637.
21. Yokomatsu, T.; Minowa, T.; Murano, T.; Shibuya, S. *Tetrahedron* **1998**, *54*, 9341.
22. Baczko, K.; Liu, W.-Q.; Roques, B. P.; Garbay-Jaureguiberry, C. *Tetrahedron* **1996**, *52*, 2021.
23. Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621.
24. Arslan, T.; Mamaev, S. V.; Mamaeva, N. V.; Hecht, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 10877.
25. Yao, Z.-J.; Gao, Y.; Burke, T. R., Jr. *Tetrahedron: Asymmetry* **1999**, *10*, 3727.
26. Meyer, F.; Laaziri, A.; Papini, A. M.; Uziel, J.; Juge, S. *Tetrahedron: Asymmetry* **2003**, *14*, 2229.
27. Zhou, J.; Fu, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 1340.
28. Gee, K. R.; Sun, W.-C.; Bhalgat, M. K.; Upson, R.; Klaubert, D. H.; Latham, K. A.; Haugland, R. P. *Anal. Biochem.* **1999**, *273*, 41.
29. Welte, S.; Baringhaus, K.-H.; Schmider, W.; Muller, G.; Petry, S.; Tennagels, N. *Anal. Biochem.* **2005**, *338*, 32.