ORGANIC LETTERS

2003
Vol. 5, No. 17
3095−3098

Concise and Enantioselective Synthesis of Fmoc-Pmp(Bu^t)$_2$-OH and Design of Potent Pmp-Containing Grb2-SH2 Domain Antagonists

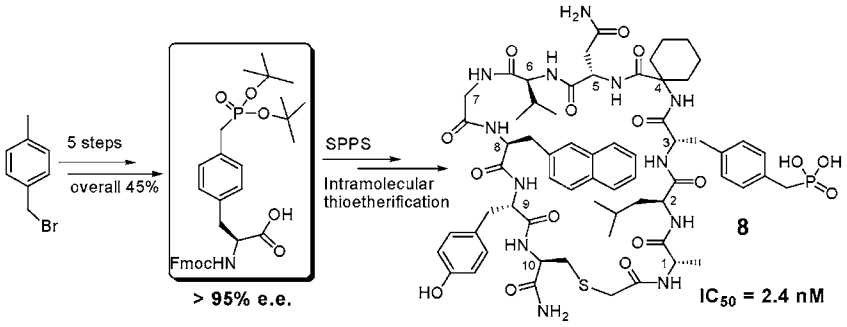
Peng Li,[†] Manchao Zhang,[‡] Megan L. Peach,[†] Hongpeng Liu,[‡] Dajun Yang,[‡] and Peter P. Roller*,[†]

Laboratory of Medicinal Chemistry, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21702, and School of Medicine, University of Michigan, Ann Arbor, Michigan 48109

proll@helix.nih.gov

Received June 13, 2003

ABSTRACT



L-Phosphonomethylphenylalanine (L-Pmp) is an important phosphatase-resistant pTyr analogue. A most concise and stereoselective approach to the synthesis of the suitably protected Fmoc-Pmp(Bu^t)$_2$-OH was developed in order to incorporate the functionally significant L-Pmp residue into peptides and peptidomimetics efficiently using standard Fmoc protocol. With this key building block, we are able to efficiently synthesize a series of potent Pmp-containing Grb2-SH2 domain antagonists, which can be used as chemotherapeutic leads for the treatment of erbB2-overexpressed breast cancer.

The posttranscriptional O-phosphorylation on tyrosine residues of proteins plays important roles in cellular signal transduction.[1] Great efforts have been made to develop phosphotyrosine (pTyr)-containing peptides and peptidomimetics to modulate aberrant cellular signaling for the treatment of cancer and other diseases.[2] However, the chemical and enzymatic lability of pTyr restricts its applicability in the process of drug discovery. Therefore, a number of pTyr mimetics have been developed with improved hydrolytic stability.[3] Among these mimetics, L-phosphonomethylphenylalanine (L-Pmp),[4] the structure of which is shown in Figure 1, has been widely used for designing biological and pharmacological probes, since Pmp is not only phosphatase-resistant but also exhibits similar biological properties to phosphotyrosine.[5]

[†] National Institutes of Health.
[‡] Michigan University.

(1) (a) Pawson, T. *Nature* **1995**, *373*, 573−580. (b) Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J. *Cell* **1993**, *72*, 767−778. (c) Koch, C. A.; Anderson, D.; Moran, M. F.; Ellis, C.; Pawson, T. *Science* **1991**, *252*, 668−674.

(2) (a) Brugge, J. S. *Science* **1993**, *260*, 918−919. (b) García-Echeverría, C. *Curr. Med. Chem.* **2001**, *8*, 1589−1604.

(3) Burke, T. R., Jr.; Yao, Z.-J.; Liu, D.-G.; Voigt, J.; Gao, Y. *Biopolymers* **2001**, *60*, 32−44.

(4) Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621−3624.

(5) Domchek, S. M.; Auger, K. R.; Chatterjee, S.; Burke, T. R., Jr.; Shoelson, S. E. *Biochemistry* **1992**, *31*, 9865−9870.

10.1021/ol035078+ CCC: $25.00 © 2003 American Chemical Society
Published on Web 07/19/2003

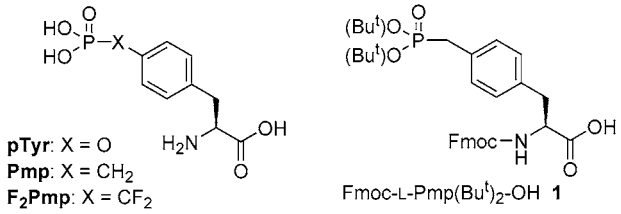
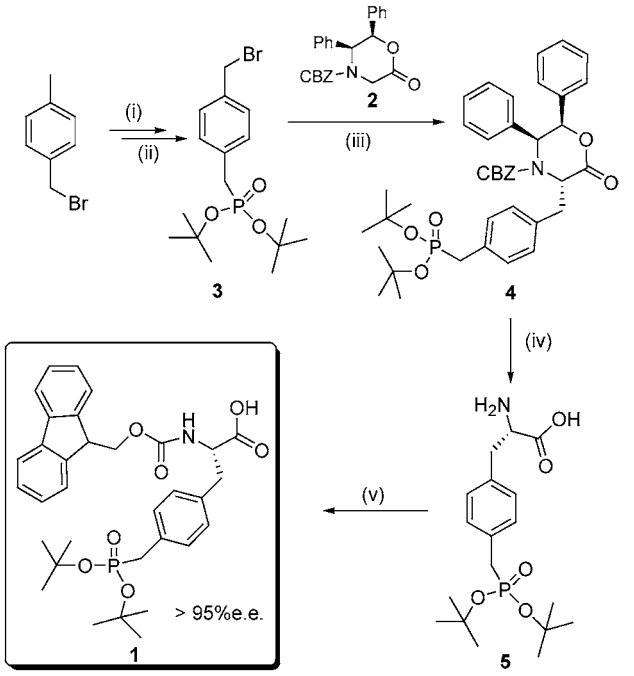


Figure 1. Structure of pTyr and pTyr mimetics.

During the past decade, several approaches have been developed to synthesize racemic[4,6] or enantiomerically pure Pmp and its protected derivatives by using a chiral auxiliary[7] or enzymatic desymmetrization[8] or starting from a chiral synthon.[9] However, these synthetic methods are either too tedious or not suitable for the synthesis of optically pure Pmp and its derivatives in a large scale. Therefore, it is significant to develop an efficient approach to the stereoselective synthesis of this key pTyr analogue for the development of Pmp-containing pharmaceuticals and drug candidates. Here, we would like to report a concise and highly enantioselective approach to the synthesis of the properly protected Pmp building block, Fmoc-L-Pmp(Bu^t)$_2$-OH **1** (Figure 1). This protection strategy can conveniently and cleanly incorporate Pmp into a peptide using standard Fmoc protocol.

In our approach, diphenyloxazinone **2**[10c] was employed as a chiral auxiliary to build the desired chirality of the α-carbon of Pmp, by virtue of the high diastereoselectivity, friendly chemical properties, and commercial availability of the reagent **2**.[10] As shown in Scheme 1, the phosphonate motif was constructed by refluxing 4-methylbenzylbromide with sodium di-*tert*-butyl phosphite in THF and subsequent bromination with *N*-bromosuccinimide.[8a] Subsequently, the stereoselective enolate alkylation of the oxazinone **2** with the obtained bromide **3** proceeded at −78 °C in the presence of solvating agent HMPA. In this step, the enolate of **2** was first generated with sodium hexamethyldisilylamide for 30−40 min in THF−HMPA at −78 °C, followed by the addition of compound **3** to afford the highly diastereoselective trans alkylation product **4**. Because of the intrinsic high reactivity of the benzylic bromide **3**, the alkylation reaction can proceed

Scheme 1. Concise Synthesis of Fmoc-L-Pmp(Bu^t)$_2$-OH[a]



[a] Reagents and conditions: (i) NaH, $HPO_3{}^tBu_2$, THF, reflux, 24 h, 94%; (ii) NBS, $(PhCOO)_2O$, CCl_4, reflux, 4 h, 67%; (iii) **2**, NaHMDS, THF−HMPA, −78 °C, 3 h, 78%; (iv) Pd black, H_2, 2.5 bar, rt, overnight, 100%; (v) Fmoc-OSu, $NaHCO_3$, CH_3CN-H_2O, rt, overnight, 91%.

smoothly and, generally, more than 98% de can be achieved according to Williams' work.[10a] After hydrogenolysis with a catalytic amount of palladium black, di-(*tert*-butyl)-phosphomethyl phenylalanine **5** was obtained, followed by Fmoc protection to give Fmoc-L-Pmp(Bu^t)$_2$-OH **1** in a 45% overall yield via five steps. The enantiomeric purity of compound **1** was determined by using HPLC to measure the content of the synthesized diastereomers Fmoc-D/L-Pmp-Val-NH_2.[9a,7a] Compound **1** showed very good enantiomeric purity (>95% ee). This is the most concise and stereoselective approach to the synthesis of Pmp and its derivatives reported to date, and the suitably protected Fmoc-L-Pmp(Bu^t)$_2$-OH **1** is a very useful synthon for the synthesis of Pmp-containing peptides and peptidomimetics using standard Fmoc protocol.

Using this key building block **1**, we are able to synthesize efficiently the Pmp-containing Grb2-SH2 domain antagonists **7**−**12**, which were designed on the basis of the phage library-derived cyclic peptide **G1TE** (Figure 2).[11] Peptide **6** was designed as a negative control. Compared to the Pmp-containing peptide **7**, compounds **8**−**11** were designed to further constrain the conformation of these cyclic peptides. To enhance cell permeability, peptide **12** was developed by conjugating with a hydrophobic carrier peptide. Most im-

(6) (a) Burke, T. R., Jr.; Russ, P.; Lim, B. *Synthesis* **1991**, *11*, 1019−1020. (b) Arslan, T.; Mamaev, S. V.; Mamaeva, N. V.; Hecht, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 10877−10887.

(7) (a) Liu, W.-Q.; Roques, B. P.; Garbay-Jauerguiberry, C. *Tetrahedron: Asymmetry* **1995**, *6*, 647−650. (b) Cushman, M.; Lee, E.-S. *Tetrahedron Lett.* **1992**, *33*, 1193−1196.

(8) (a) Baczko, K.; Liu, W.-O.; Roques, B. P.; Garbay-Jauerguiberry, C. *Tetrahedron* **1996**, *52*, 2021−2030. (b) Garbay-Jauerguiberry, C.; McCort-Tranchepain, I.; Barbe, B.; Ficheux, D.; Roques, B. P. *Tetrahedron: Asymmetry* **1992**, *3*, 637−650. (c) Yokomatsu, T.; Minowa, T.; Murano, T.; Shibuya, S. *Tetrahedron* **1998**, *54*, 9341−9356.

(9) (a) Yao, Z.-J.; Gao, Y.; Burke, T. R., Jr. *Tetrahedron: Asymmetry* **1999**, *10*, 3727−3734. (b) Dow, D. L.; Bechle, B. M. *Synlett* **1994**, 293−294.

(10) (a) Williams, R. M.; Im, M.-N. *J. Am. Chem. Soc.* **1991**, *113*, 9276−9286 and literature cited therein. (b) Bender, D. M.; Williams, R. M. *J. Org. Chem.* **1997**, *62*, 6690−6691 and literature cited therein. (c) Benzyl (2*R*,3*S*)-(−)-6-oxo-2,3-diphenyl-4-morpholine carboxylate **2** is commercially available from Aldrich Chemical Co.: catalog No. 33,187-2.

(11) (a) Oligino, L.; Lung, F.-D. T.; Sastry, L.; Bigelow, J.; Cao, T.; Curran, M.; Burke, T. R.; Wang, S.-M.; Krag, D.; Roller, P. P.; King, C. R. *J. Biol. Chem.* **1997**, *272*, 29046−29052. (b) Long, Y.-Q.; Voigt, J. H.; Lung, F.-D. T.; King, C. R.; Roller, P. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2267−2272. (c) Lung, F.-D. T.; Long, Y.-Q.; King, C. R.; Varady, J.; Wu, X.-W.; Wang, S.; Roller, P. P. *J. Pept. Res.* **2001**, *57*, 447−454.

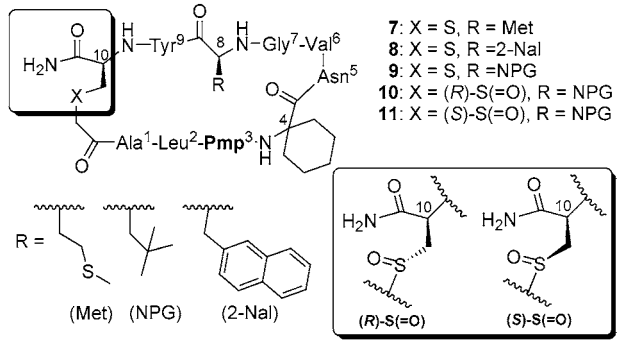
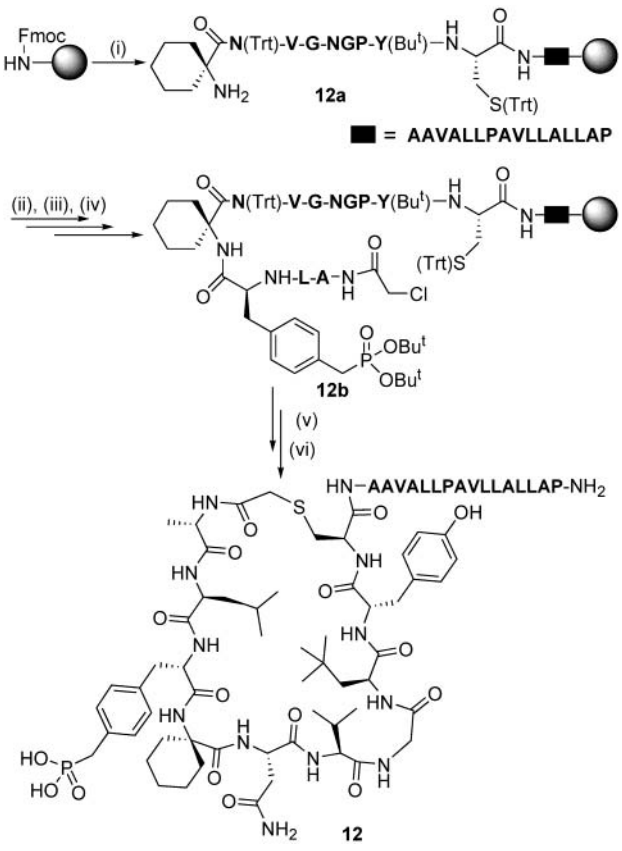


Figure 2. Structure of Pmp-containing G1TE analogues **7**−**11**.

portantly, the carrier-conjugated cyclic peptide **12** can be successfully synthesized from compound **1**. Previously, we tried to synthesize peptide **12** using the commercially available Fmoc-L-Pmp-OH, but no product was obtained due to various side reactions caused by the free P−OH groups. As shown in Scheme 2, after the construction of the 23mer peptide **12a** using standard Fmoc protocol on PAL resin, the Pmp residue can be cleanly incorporated using the coupling reagent HATU with the addition of HOAt to accelerate the reaction. To reduce the amount of deletion peptide, a double coupling strategy was adopted considering the steric hindrance of 1-amino-1-cyclohexanecarboxylic acid (Ach). After the completion of peptide elongation, the N-terminal was capped with 10 equiv of chloroacetic anhydride to give compound **12b**. No base was added in the process of the chloroacetylation to avoid any chance of racemization. After the peptide was cleaved from the PAL resin by treatment with TFA containing 2.5% each (v/v) of triethylsilane (TES) and deionized water, the linear chloroacetylated peptide underwent intramolecular thioetherification in dilute buffer solution to afford the carrier-conjugated 26mer cyclic peptidomimetic **12** (Scheme 2).

Scheme 2. Illustrative Synthesis of Compound **12**[a]



[a] Reagents and conditions: (i) SPPS, Fmoc chemistry, ABI synthesizer; (ii) **1**, HATU, HOAt, DIEA, DMF, rt, 1 h, double coupling; (iii) SPPS, Fmoc chemistry, ABI synthesizer; (iv) $(ClCH_2CO)_2O$, rt, overnight; (v) TFA:TES:H_2O = 40:1:1, rt, 2 h; (vi) NH_4OAc, NH_4OH, pH = 8.2, CH_3CN-H_2O, rt, 8 h.

The synthetic strategy of compound **12** can also be employed for the synthesis of G1TE analogues, **6**−**9**. Sulfoxides **10** and **11** were obtained by treating the thioether **9** with 5% H_2O_2 aqueous solution overnight. The (*R*)-configured sulfoxide **10** can be easily separated as the major product from its diastereoisomer **11** using HPLC, in a ratio of 1.4:1. Compared to the (*S*)-configured sulfoxide **11**, the (*R*)-configured sulfoxide **10** is more thermodynamically stable and thus given as the major product under this mild oxidization condition. The absolute configuration of the two sulfoxide isomers was determined by CD spectra according to the guidelines described in the literature and confirmed by extensive molecular modeling.[12]

Extracellular ELISA-based binding assays indicate that incorporating Pmp into the third position of G1TE analogues tremendously improves the potency of the resulting peptides. For instance, the Pmp^3-containing peptide **7** is 20 000-fold more potent than the corresponding Tyr^3-containing analogue **6**, as shown in Table 1. Replacing the Met^8 with the more

Table 1. Inhibitory Activities of Pmp-Containing G1TE Analogues Evaluated by ELISA Assays[a]

	amino acid compositions					
compds[b]	AA^1	AA^3	AA^4	AA^8	AA^{10}	IC_{50} (nM)[c]
G1TE	Glu	Tyr	Glu	Met	Cys	> 10^5 [d]
6	Ala	Tyr	Ach	Met	Cys	2.65×10^5 ($\pm 1.20 \times 10^5$)
7	Ala	Pmp	Ach	Met	Cys	13.3 (±0.08)
8	Ala	Pmp	Ach	2-Nal	Cys	2.38 (±0.90)
9	Ala	Pmp	Ach	NPG	Cys	13.4 (±2.0)
10	Ala	Pmp	Ach	**NPG**[f]	Cys(OR)	8.08 (±0.76)
11	Ala	Pmp	Ach	**NPG**[f]	Cys(OS)	18.5 (±3.6)
12e	Ala	Pmp	Ach	**NPG**[f]	Cys	125 (±35)

[a] In extracellular ELISA binding assays, a biotin-labeled SHC-phosphopeptide competed with our G1TE analogues. [b] Structures of these G1TE analogues are shown in Figure 2. [c] Values are means of three experiments; standard deviation is given in parentheses. [d] Biacore assay indicates that G1TE exhibits inhibitory activity with an IC_{50} = 20 μM.[11b] [e] Sequence of the carrier[13] and the structure of the carrier-conjugated peptide **12** are shown in Scheme 2. [f] NPG stands for *L*-neopentylglycine.

hydrophobic 2-naphthylalanine (2-Nal) gives the 2-Nal[8]-containing peptide **8**, with an $IC_{50} = 2.38$ nM, which is 5.6-fold more potent than the corresponding analogue **7**. The improved binding affinity of compound **8** is attributed to the more favorable intramolecular van der Waals interactions of the hydrophobic naphthyl side chain of 2-Nal[8] with the side chains of Leu[2] and Cys,[10] as indicated by molecular modeling. Such interactions stabilize the favored conformation of the peptide and allow other residues such as Pmp[3], Ach,[4] Asn[5], Val[6], and Tyr[9] in the peptide to bind to Grb2-SH2 protein optimally. To further constrain the backbone conformation of these Pmp-containing G1TE analogues, compounds **10** and **11** were designed and synthesized by oxidizing the flexible thioether linkage of peptide **9** into relatively rigid sulfoxides. Interestingly, only the (*R*)-configured sulfoxide **10** showed higher inhibitory potency in comparison with the thioether precursor **9**, while the other diastereoisomer **11** is even less active than thioether **9** due to an unfavorable conformational change caused by repulsion between the oxygen of the (*S*)-configured sulfoxide and the carbonyl oxygen of the *N*-acetyl group according to molecular modeling and CD spectra.[12e]

Whole cell assays show that the carrier-conjugated cyclic peptidomimetic **12** can penetrate cell membranes and effectively inhibit the association of Grb2 protein with the p185[erbB2] complex in erbB2-overexpressing MDA-MB-453 cancer cells at low micromolar concentrations, as shown in

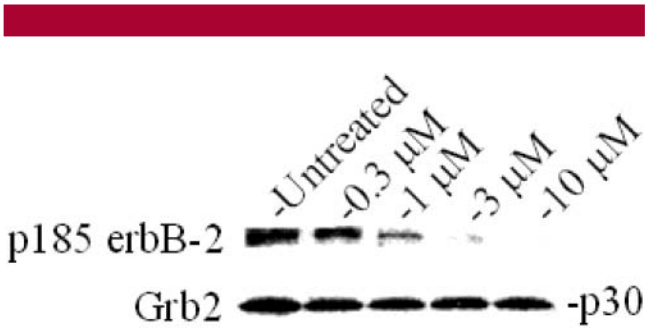


Figure 3. Inhibition of the association of Grb2 with p185[erbB2] in MDA-MB453 cancer cells by treatment with compound **12**.

Figure 3. Compound **12** also exhibits a moderate antimitogenic effect in breast cancer cell cultures. The growth of MDA-MB-453 cancer cells was inhibited by 40% after treatment with a 5 μM concentration of inhibitor **12**.

In conclusion, we have developed a concise and stereoselective approach to the synthesis of the suitably protected L-Pmp building block Fmoc-L-Pmp(^{t}Bu)$_2$-OH, which is very useful in the synthesis of Pmp-containing peptides and peptidomimetics. Using this key synthon, we are able to efficiently synthesize a series of potent Pmp-containing Grb2-SH2 domain antagonists, which are typically at least 10^4-fold more potent than the lead G1TE.

Supporting Information Available: Experimental details for the preparation of compound **1** and G1TE analogues **6**−**11** and characterization data thereof, detailed information about CD spectral analysis of compounds **9**−**11**, and molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035078+

(12) (a) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 1720−1723. (b) Kubec, R.; Musah, R. A. *Phytochemistry* **2001**, *58*, 981−985. (c) Van den Broek, L. A. G. M.; Breuer, M. L.; Liskamp, R. M. J.; Ottenheijm, H. C. J. *J. Org. Chem.* **1987**, *52*, 1511−1517. (d) Liskamp, R. M. J.; Zeegers, H. J. M.; Ottenheijm, H. C. J. *J. Org. Chem.* **1981**, *46*, 5408−5413. (e) Detailed information about CD spectra analysis and molecular modeling is described in Supporting Information.

(13) Lindgren, M.; Hallbrink, M.; Prochiantz, A.; Langel, U. *Trends Pharmacol. Sci.* **2000**, *21*, 99−103.