Published in final edited form as:
Biopolymers. 2011 ; 96(1): 1–3. doi:10.1002/bip.21425.

Efficient Synthesis of Fmoc-Protected Phosphinic Pseudodipeptides: Building Blocks for the Synthesis of Matrix Metalloproteinase Inhibitors

Manishabrata Bhowmick[1], **Ravinder R. Sappidi**[1], **Gregg B. Fields**[2], and **Salvatore D. Lepore**[1]

[1]Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, FL 33431-0991

[2]Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78229-3900

Abstract

A convenient route for the synthesis of Fmoc-protected phosphinic dipeptide building blocks is described. The protected amino acid isosteres benzyloxycarbonyl aminomethyl phosphinic acid (glycine surrogate), benzyl α-isopropyl acrylate (valine surrogate), and benzyl α-isobutyl acrylate (leucine surrogate) were synthesized starting from commercially available materials. Reaction of either the valine or leucine surrogate with bis(trimethylsilyl) phosphonite generated the pseudodipeptide bond. The synthesis concluded with an efficient one-pot three-step procedure involving a bis-deprotection of the N- and C-termini under catalytic hydrogenation conditions followed by selective capping of the N-terminus with an Fmoc group to yield either Fmoc-$NHCH_2PO(OAd)CH_2CH(Pr^i)CO_2H$ or Fmoc-$NHCH_2PO(OAd)CH_2CH(Bu^i)CO_2H$.

Keywords

phosphinic pseudodipeptides; matrix metalloproteinase inhibitors; MMPI; peptide synthesis; phosphinates

INTRODUCTION

We are interested in developing effective matrix metalloproteinase (MMP) inhibitors as activation of these enzymes have been associated with primary and metastatic tumor growth, angiogenesis, and pathological degradation of extracellular matrix components, such as collagen and laminin.[1,2] Several MMPs have been validated as targets in certain cancers (MMP-2, MMP-9, MT1-MMP), whereas others have been found to be host-beneficial and thus antitargets (MMP-3, MMP-8).[3]

A general approach to the design of an inhibitor of metalloproteases involves replacing a trigonal planar amide or ester bond of a substrate with a hydrolytically stable functional group possessing tetrahedral geometry in the original carbonyl position to mimic the intermediate formed during enzyme-catalyzed hydrolysis. The phosphinate functional group (Figure 1) with its pair of electronically equivalent anionic oxygens (under physiological

Correspondence to: Salvatore Lepore, slepore@fau.edu.

Additional Supporting Information may be found in the online version of this article.

conditions) has emerged as an effective tetrahedral substitution motif in peptides leading to potent and selective transition state inhibitors of metalloproteases such as MMPs.[4–7] Indeed, due to their growing medicinal relevance, a number of elegant strategies have been developed over the past few years for the synthesis of phosphinate dipeptides. Meldal and coworkers, Yiotakis et al., and Yokomatsu and coworkers reported the synthesis of different phosphinate dipeptide building blocks by using a procedure involving the Michael addition of variously protected aminomethyl phosphinic acids to an acrylic acid ester followed by protecting group manipulation to afford the desired product.[8] Using a creative variation of these methods, $FmocNHCH_2PO(OAd)\text{-}CH_2CH(Pr^i)CO_2H$ (**1a**, Scheme 2) was prepared and subsequently incorporated into a triple-helical sequence, resulting in a construct, which exhibited potent and selective inhibition of MMP-2 and MMP-9.[7] However, this previously described synthetic route to **1a** required a low yielding (35%) and problematic final step involving a Ru-catalyzed deprotection of an allyl ester.

In our continued studies of MMP inhibition involving this phosphinate dipeptide system, we required a more scale-able route to **1a**. In this communication, we describe an efficient bis-deprotection strategy leading to the desired phosphinate dipeptide **1a** and its Gly-Leu analog **1b**.

DISCUSSION

Our general strategy for the synthesis of the Fmoc-protected phosphinic dipeptide **1** entails a Michael-type addition for the formation of the P—C bond pioneered by Regan and Yiotakis and subsequently modified by Meldal and Hammer.[8–10] But our initial attempt of Michael-type addition of Fmoc-protected aminomethyl phosphinic acid and electron deficient acrylate was not successful.[8,11] Thus, Cbz-protected aminomethyl phosphinic acid **3** was converted to its trivalent state by the action of HMDS and subsequently reacted with an *α*-isopropyl-*α*,*β*-unsaturated ester[12] to give product **4** in good yield (Scheme 1). However, as will be described subsequently, the Cbz group is very efficiently exchanged for Fmoc in the final step of the synthesis.

Phosphinic acid **3** was derived from aminomethyl phosphinate (**2**) (Scheme 1), which in turn was prepared from ammonium phosphinate using a previously described imine addition method.[10] Other reported methods for the synthesis of **2** were less satisfactory.[13] The main difficulty was in purifying highly polar fragment **2** especially on a preparative scale. The use of an ion-exchange resin described by Hammer and coworkers proved least problematic.[10,14] A subsequent Cbz-protection of compound **2** followed by recrystallization from ethyl acetate/light petroleum ether[7b,15] gave highly pure fragment **3** in 65% yield.

The adamantyl protection of **4** using $AgNO_3$ and AdBr failed in our hands to provide protected dipeptide **5** (Scheme 1).[8] However, esterification of **4** was achieved by treating the in situ generated phosphinic acid chloride with the sodium salt of adamantanol to give fully protected fragment **5a** in 75% yield.[8b,16] However, saponification of the ethyl ester unit of **5a** by following a previously reported procedure[8] was unsuccessful in our hands and led to a complex reaction mixture along with a significant amount of Cbz deprotection of the starting dipeptide after prolonged reaction time. Others have also noted the requirement of long reaction times for similarly substituted esters.[17]

This unsuccessful saponification led us to develop a new deprotection strategy. We reasoned that benzyl ester protection of the C-terminus would potentially allow for its removal under the same hydrogenation conditions required for unveiling of the amine. To test our hypothesis, we prepared benzyl ester fragment **7a** (Scheme 2) following the same route as in the synthesis of **5a** (Scheme 1). Catalytic hydrogenation of **7a** with 5% Pd/C under hydrogen

(atm. pressure) in the presence of Fmoc-OSu afforded desired pseudodipeptide **1a** in good yields (Scheme 2). This one-pot reaction involved the simultaneous removal of the Cbz and benzyl protecting groups and reprotection of N-terminus with Fmoc. Optimal yields (>75%) were observed with a reaction time of 2 h, whereas prolonged exposure of the reaction mixture to hydrogenation resulted in partial Fmoc deprotection. Importantly, this method does not appear to be sensitive to the steric environment of the ester group. Following the same synthetic path as **5b**, pseudo-Gly-Leu fragment **7b** was also prepared and underwent efficient bis-deprotection and Fmoc addition (at a scale of 0.5 mmol) to give **1b** (Scheme 2). We note that products **7a** and **7b** were obtained as a 1:1 mixture of diastereomers, which were easily separable by silica gel flash chromatography.

CONCLUSIONS

In conclusion, we have developed an efficient and scaleable method for the synthesis of Fmoc-protected phosphinic analogs of Gly-Val and Gly-Leu. Our modified synthetic route involves a highly abbreviated protecting group manipulation procedure that further optimizes previous work on this fragment that has found compelling applications in MMP inhibition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Egeblad M, Werb Z. Nat Rev Cancer. 2002; 2:161–174. [PubMed: 11990853]
2. Fingleton B. Curr Pharm Design. 2007; 13:333–346.
3. Overall CM, Kleifeld O. Nat Rev Cancer. 2006; 6:227–239. [PubMed: 16498445]
4. Vassiliou S, Mucha A, Cuniasse P, Georgiadis D, Lucet-Levannier K, Beau F, Kannan R, Murphy G, Knauper V, Rio MC, Basset P, Yiotakis A, Dive V. J Med Chem. 1999; 42:2610–2620. [PubMed: 10411481]
5. Buchardt J, Schiodt CB, Krog-Jensen C, Delaissé JM, Foged NT, Meldal M. J Comb Chem. 2000; 2:624–638. [PubMed: 11138549]
6. (a) Yiotakis A, Vassiliou S, Jiráček J, Dive V. J Org Chem. 1996; 61:6601–6605. [PubMed: 11667528] (b) Buchardt J, Ferreras M, Krog-Jenson C, Delaissé JM, Foged NT, Meldal M. Chem Eur J. 1999; 5:2877–2884.
7. (a) Lauer-Fields J, Brew K, Whitehead JK, Li S, Hammer RP, Fields GB. J Am Chem Soc. 2007; 129:10408–10417. [PubMed: 17672455] and references cited therein; (b) Lauer-Fields J, Whitehead JK, Li S, Hammer RP, Brew K, Fields GB. J Biol Chem. 2008; 283:20087–20095. [PubMed: 18499673]
8. (a) Buchardt J, Ferreras M, Krog-Jenson C, Delaissé JM, Foged NT, Meldal M. Chem Eur J. 1999; 5:2877–2884.(b) Yamagishi T, Ichikawa H, Haruki T, Yokomatsu T. Org Lett. 2008; 10:4347–4350. [PubMed: 18781768]
9. Boyd EA, Regan AC, James K. Tetrahedron Lett. 1992; 33:813–816.
10. Li S, Whitehead JK, Hammer RP. J Org Chem. 2007; 72:3116–3118. [PubMed: 17375960]
11. Georgiadis D, Matziari M, Yiotakis A. Tetrahedron. 2001; 57:3471–3478.
12. Lee HS, Park JS, Kim BM, Gellman SH. J Org Chem. 2003; 68:1575–1578. [PubMed: 12585907]
13. (a) Baylis EK, Campbell CD, Dingwall JG. J Chem Soc Perkin Trans I. 1984:2845–2853.(b) McCleery PP, Tuck Brian. J Chem Soc Perkin Trans I. 1989:1319–1329.(c) Jiao XY, Verbruggen C, Borloo M, Bollaert W, Groot AD, Dommisse R, Haemers A. Synthesis. 1994; 1:23–24.
14. In our hands, product 2 was eluted from the ion-exchange column using mere double-distilled water instead of ammonium hydroxide solution as previously reported.

15. Maligres PE, Houpis I, Rossen K, Molina A, Sager J, Upadhyay V, Wells KM, Reamer RA, Lynch JE, Askin D, Volante RP, Reider PJ. Tetrahedron. 1997; 53:10983–10992.
16. Rogers RS. Tetrahedron Lett. 1992; 33:7473–7474.
17. Takamura M, Sakurai M, Yamada E, Fujita S, Yachi M, Takagi T, Isobe A, Hagisawa Y, Fujiwara T, Yanagisawa H. Bioorg Med Chem. 2004; 12:2419–2439. [PubMed: 15080938]

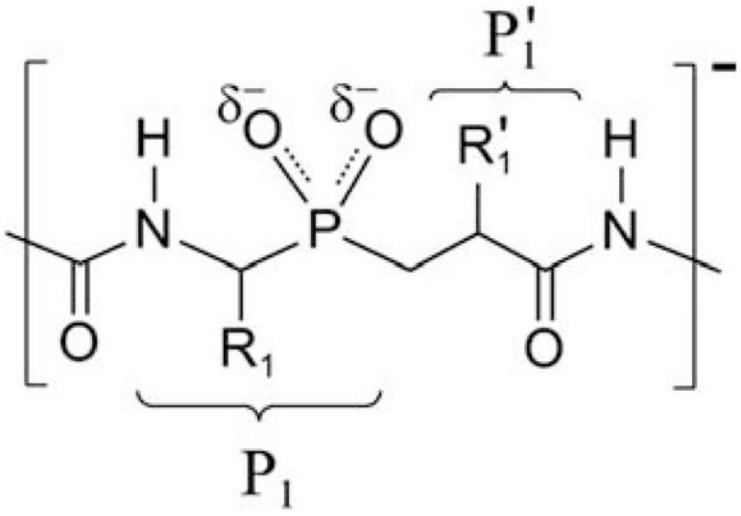
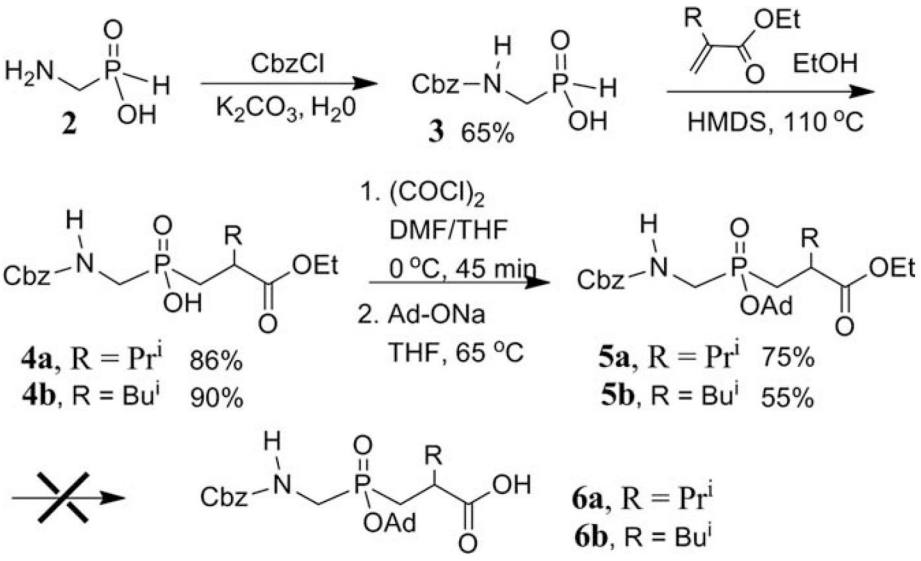
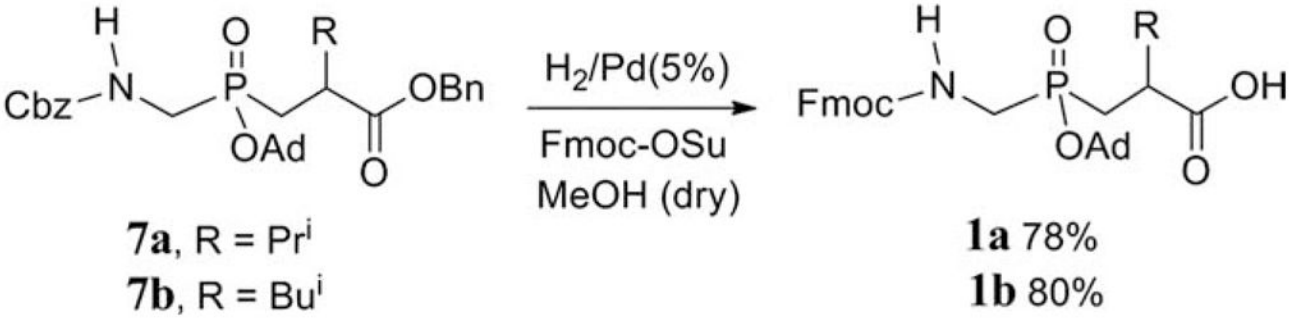


FIGURE 1.
Common structure of phosphinic peptides.



SCHEME 1.
Attempted synthesis of Cbz-protected phosphinic dipeptide **6**.



7a, R = Pr^i
7b, R = Bu^i

1a 78%
1b 80%

SCHEME 2.
Tandem bis-deprotection and Fmoc-amine formation leading to **1**.