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Efficient synthesis of protected L-phosphonophenylalanine (Ppa) derivatives suitable for solid phase peptide synthesis

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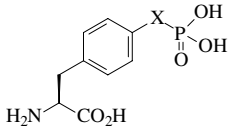
Abstract—L-Phosphonophenylalanine (Ppa) derivatives were synthesized from L-4-iodophenylalanine and suitably protected phosphites using Michaelis–Arbuzov reaction conditions in the presence of Pd(0) and triethylamine at 70 ± 2 °C.

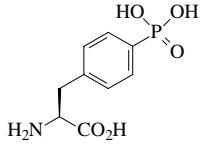
1. Introduction

Recently, we have designed a phosphotyrosine (*p*Tyr) containing analog of a potassium channel blocking peptide derived from the sea anemone *Stichodactyla helianthus* (ShK), called ShK(L5) that selectively inhibits Kv1.3 channels. ShK(L5) has been shown to suppress the proliferation of human and rat effector memory T cells (T_{EM}) and inhibits interleukin-2 production at picomolar concentrations. Thus, ShK(L5) is a very interesting compound, which may be useful in treating autoimmune disorders.[1] The amino acid sequence of ShK(L5) is H-*p*Tyr-Aeea-Arg-Ser-Cys-Ile-Asp-Thr-Ile-Pro-Lys-Ser-Arg-Cys-Thr-Ala-Phe-Gln-Cys-Lys-His-Ser-Met-Lys-Tyr-Arg-Leu-Ser-Phe-Cys-Arg-Lys-Thr-Cys-Gly-Thr-Cys-OH with disulfide bonds between Cys-5 and Cys-37, Cys-14 and Cys-30, and Cys-19 and Cys-34. Aeea is a spacer amino acid in the sequence and is introduced using commercially available Fmoc-Aeea-OH (Fmoc-amino-ethoxy-ethoxy-acetic acid; Bachem Product No. B-3635).

In our efforts to improve the stability of ShK(L5) and to minimize the potential cleavage of the selectivity-promoting phosphate moiety under physiological conditions since the phosphoryl ester bond may be labile to hydrolysis and may be cleaved by phosphatases in vivo,[2] we investigated several nonhydrolyzable mimetics of *p*Tyr. Figure 1 shows the structures of *p*Tyr mimicking moieties.[3]



1; X = O; Phosphotyrosine (pTyr)
2; X = CH_2; Phosphonomethyl phenylalanine (Pmp)
3; X = CHOH; Hydroxyphosphonomethyl phenylalanine (HPmp)
4; X = CHF; Fluorophosphonomethyl phenylalanine (FPmp)
5; X = CF_2; Difluorophosphonomethyl phenylalanine (F_2Pmp)



6; Phosphonophenylalanine (Ppa)

Figure 1. Structures of phosphotyrosine mimetics.

In one of our first analogs for this study, *p*Tyr of ShK(L5) was substituted with Pmp (**2**), which is commercially available in the protected form. However, Pmp-substitution for *p*Tyr led to significant reduction in selectivity,[1] possibly due to higher ionization constant of the phosphonate ($pK_{a_2} = 7.1$) relative to the phosphate ($pK_{a_2} = 5.7$) in ShK(L5).[3] Thus, an analog incorporating 4-phosphonophenylalanine (**6**; Ppa) as a nonhydrolyzable *p*Tyr mimic was designed because the ionization constant (pK_{a_2}) of Ppa is comparable to *p*Tyr and no reduction in selectivity was expected due to similar interactions at the binding site.[4] In this report, we describe an efficient synthesis of suitably protected

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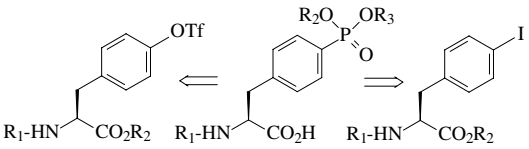
4-phosphonophenylalanine (**6**; Fig. 1) derivatives starting from L-4-iodophenylalanine.

2. Results and discussion

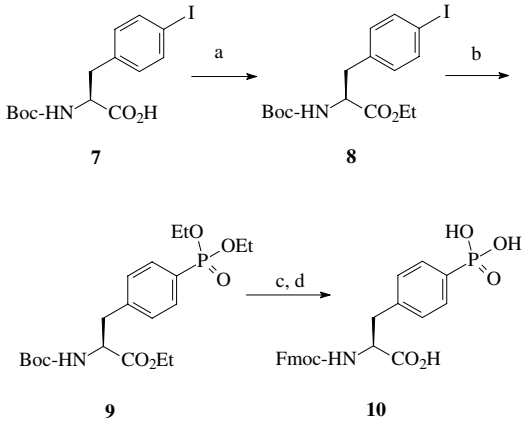
Scheme 1 shows two plausible strategies for the synthesis of phosphonophenylalanine (**6**; Ppa). Strategy 1 utilizes L-tyrosine as the starting material and is reported in the literature.[5] However, Ppa (**6**) can also be synthesized from L-4-iodophenylalanine by employing Michaelis–Arbuzov reaction to form the aryl carbon–phosphorus bond.[6]



Scheme 1.

As shown in Scheme 2, Boc-4-iodophenylalanine was esterified with EtOH using BOP/DIEA as an activator in good yield. The Boc-Phe(I)-OEt was treated with diethylphosphite in the presence of (tetrakistriphenylphosphine)Pd(0) and triethylamine in acetonitrile at 72 ± 2 °C for 20 h to afford Boc-Ppa(Et)$_2$-OEt in 87% yield.[7] All the protecting groups were simultaneously removed by refluxing Boc-Ppa(Et)$_2$-OEt in 6 N aqueous HCl at 110 °C for 6 h. Concentration of the reaction mixture followed by trituration with ether afforded the product in quantitative yield. Ppa thus obtained was reacted with Fmoc-OSu in the presence of sodium carbonate to afford Fmoc-Ppa-OH in excellent yield.[8]

We used side-chain unprotected Fmoc-Ppa-OH (**10**) to synthesize Ppa analog of ShK(L5) on the solid support using standard protocols. Since side-chain unprotected Fmoc-*p*Tyr can be used with only minimal formation of pyrophosphate using BOP/HOBt (1:1)/NMM in 0.4 M LiCl/NMP,[9] it was expected that Fmoc-Ppa-OH would also afford the target peptide without pyrophosphate formation. However, high levels of pyrophosphate formation were observed in all of the coupling protocols employed (up to 70%) and were exceedingly difficult to remove by RP-HPLC. Therefore, synthesis of the Ppa analog of ShK(L5) had to be optimized in order to minimize the formation of this undesired pyrophosphate impurity. It was determined that by protecting the phosphonate group, the formation of the pyrophosphate could be prevented.

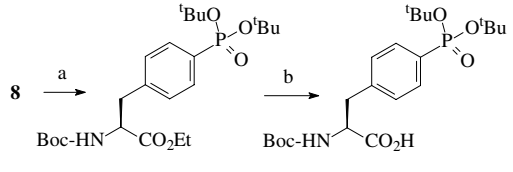


Scheme 2. Reagents and conditions: (a) BOP/DIPEA, EtOH, 72%; (b) $(Ph_3P)_4$ Pd(0), HPO_3Et_2, TEA, MeCN, 76%; (c) 6 N aq HCl, reflux, 6 h, 92%; (d) Fmoc-OSu, Na_2CO_3, dioxane/H_2O, 90%.

The protecting groups for the phosphonate group had to be chosen carefully. The simplest method was to keep the phosphonate group protected as ethyl or methyl ester. However, cleavage of the phosphonate ethyl or methyl ester requires strong acidic conditions, such as refluxing in 6 N aqueous HCl,[10] 45% HBr/AcOH,[5] TMSBr,[11] or HF.[12] These cleavage conditions are not suitable for synthesizing ShK(L5). The protecting groups needed to be compatible with Fmoc chemistry and should be cleavable with TFA. Thus, the choices were limited to benzyl or *t*-butyl protecting groups.

In order to synthesize Fmoc-Ppa(Bzl)-OH, we attempted the same strategy as described in Scheme 2, but starting with Fmoc-Phe(4-I)-OEt. Fmoc-Phe(4-I)-OEt was prepared in quantitative yield from Fmoc-Phe(4-I)-OH and EtOH/$HCl_{(g)}$. However, the reaction of Fmoc-Phe(4-I)-OEt with dibenzylphosphite failed to yield any Fmoc-Ppa(Bzl)$_2$-OEt even after employing long reaction times and with excesses of reagents and catalyst. In the next attempt, we used di-*t*-butylphosphite to react with Fmoc-Phe(4-I)-OEt in the presence of Pd(0).[13] The reaction did proceed, albeit in low yield (<10%). Efforts to improve the yield by prolonging the reaction time and higher temperature as well as adding excesses of reagents and catalyst were not successful. It appears that Pd(0) reaction is not compatible with dibenzylphosphite. However, with dialkylphosphite there was some moderate reaction. As our efforts to produce the benzyl derivative were not successful, we decided to prepare Boc-Ppa(*t*-Bu)$_2$-OH. This was facilitated by the fact that Ppa is the N-terminal residue for the ShK(L5) analog and the final Boc group would be cleaved during standard cleavage of the peptide from the resin.

As shown in Scheme 3, Boc-Phe(4-I)-OEt (**8**) was reacted with di-*t*-butylphosphite, Pd(0), and triethylamine



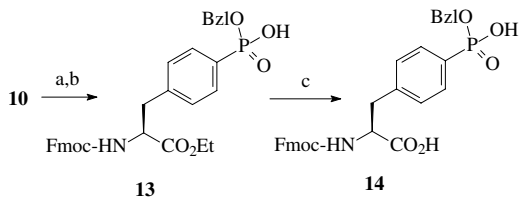
Scheme 3. Reagents and conditions: (a) $(Ph_3P)_4Pd(0)$, $HPO_3{}^tBu_2$/TEA, MeCN, 62%; (b) 1 N NaOH, 4 h, 67%.

in anhydrous acetonitrile at 70 ± 2 °C for a total of 40 h. The reaction was monitored periodically and one additional equivalent each of the catalyst, di-*t*-butylphosphite and triethylamine, were added after 24 h of reaction. Boc-Ppa(*t*-Bu)$_2$-OEt (**11**) was obtained in 62% yield after silica gel column purification. The ethyl ester was hydrolyzed with 1 N aqueous sodium hydroxide in MeOH/water mixture at ambient temperature for 4 h. No racemization was observed during hydrolysis. Purification of the crude product using a silica gel column chromatography gave pure **12** in 67% yield as a white solid.[14]

In continuation of our efforts to prepare Fmoc-Ppa(Bzl)-OH and having failed to force the Michaelis–Arbzov reaction between Fmoc-Phe(4-I)-OEt and dibenzylphosphite, we decided to carry forward Fmoc-Ppa-OH (**10**) to selectively protect phosphonic acid group as benzyl ester.

As shown in Scheme 4, a suspension of Fmoc-Ppa-OH (**10**) was stirred in EtOH saturated with $HCl_{(g)}$ overnight. Fmoc-Ppa-OEt precipitated out from the reaction mixture as a white solid. Further concentration and precipitation with isopropyl ether afforded the product in quantitative yield. Fmoc-Ppa-OEt was then reacted with benzyl alcohol (3 equiv) using BOP/DIPEA (2 equiv) as an activator. However, there was no reaction possibly due to intramolecular quenching of the HOBt-active ester with the second hydroxyl function of the phosphonic acid moiety. It should be noted that phosphonate monoesters are known to successfully react with alcohols to afford mixed phosphonate diesters using BOP or PyBOP as activating agent.[15] Side-chain phosphonic acid reacted with benzyl alcohol (3 equiv) in quantitative yield (**13**) when activated with either DCC/DMAP (2 equiv) or pivaloyl chloride (2 equiv)[16] in acetonitrile/pyridine (1:1) solvent mixture. It should also be noted that only mono benzyl phosphonate ester formed under these conditions and no diester was detected.

In the final step, selective hydrolysis of the carboxylic acid ethyl ester was tried. Due to the presence of both acid and base labile functional groups present in Fmoc-Ppa(Bzl)-OEt (**13**), the choice was limited to milder hydrolysis conditions. Mild bases, such as lithium hydroxide in THF/dioxane at 0 °C,[14] or sodium carbonate/sodium bicarbonate in MeOH/H_2O[17] are reported to selectively hydrolyze carboxylic alkyl esters without affecting the Fmoc group. Both lithium hydroxide and sodium carbonate under the above reaction conditions



Scheme 4. Reagents and conditions: (a) EtOH/$HCl_{(g)}$, 95%; (b) DCC/DMAP or $(CH_3)_3COCl$, MeCN/pyridine (1:1), benzyl alcohol, 95%; (c) (i) 1 N LiOH/THF, 4 h; (ii) Fmoc-OSu, overnight, 80%.

selectively hydrolyzed the ethyl ester without affecting the benzyl phosphonate ester. However, contrary to the reports, there was 25–30% cleavage of the Fmoc group over the course of reaction and no hydrolysis was observed with sodium bicarbonate. It should also be mentioned here that we did not observe any racemization during the base-mediated hydrolysis of the ethyl ester. The optimized hydrolysis conditions utilized 2 equiv of 1 N aqueous lithium hydroxide in THF at 0 °C followed by addition of 1 equiv of Fmoc-OSu to the reaction mixture after hydrolysis was complete and allowing it to stir at the same temperature overnight. The basic solution was neutralized with 1 N aqueous HCl and the product was extracted with dichloromethane. After washing the organic layer with brine, Fmoc-Ppa(Bzl)-OH (**14**) was precipitated from dichloromethane/hexane as white solid in 80% yield.[18]

It should be mentioned that both Boc-Ppa(*t*-Bu)$_2$-OH and Fmoc-Ppa(Bzl)-OH were successfully incorporated in the sequence of ShK(L5) using DIC/HOBt as the activator. Apparently, no pyrophosphate was formed during the solid phase peptide synthesis using the side-chain protected amino acid derivatives of Ppa.

In conclusion, syntheses of Fmoc-Ppa-OH, Boc-Ppa(*t*-Bu)$_2$-OH, and Fmoc-Ppa(Bzl)-OH were accomplished in good yields using Michaelis–Arbuzov reaction conditions. These amino acids can be used in solid phase peptide synthesis using an Fmoc strategy. Fmoc-Ppa-OH was obtained in excellent yield from Boc-Phe(4-I)-OEt and diethylphosphite. Boc-Ppa(*t*-Bu)$_2$-OH was prepared from Boc-Phe(4-I)-OEt and di-*t*-butylphosphite in moderate yield. Fmoc-Ppa(Bzl)-OH could not be prepared directly from Fmoc-Phe(4-I)-OEt and dibenzylphosphite. Nonetheless, it was synthesized from Fmoc-Ppa-OH by selectively protecting and deprotecting the carboxylic and phosphonic acid moieties.

It is also important to report that that during solid phase coupling of Fmoc-Ppa-OH, a significant amount of pyrophosphate forms. The Fmoc group is also susceptible to cleavage during hydrolysis with either LiOH or Na_2CO_3. The problem can be circumvented by adding Fmoc-OSu to the reaction mixture once hydrolysis is complete.

References and notes

1. Beeton, C.; Pennington, M. W.; Wulff, H.; Singh, S.; Nugent, D.; Crossley, G.; Khaytin, I.; Calabresi, P. A.; Chen, C.-Y.; Gutman, G. A.; Chandy, K. G. *Mol. Pharmacol.* **2005**, *67*, 1369–1381.
2. Burke, T. R., Jr.; Smyth, M. S.; Nomizu, M.; Otaka, A.; Roller, P. *J. Org. Chem.* **1993**, *58*, 1336–1340.
3. Burke, T. R., Jr.; Yao, Z.-J.; Liu, D.-G.; Voigt, J.; Gao, Y. *Biopolymers (Petide Sci.)* **2001**, *60*, 32–44.
4. Liu, W.-Q.; Olszowy, C.; Bischoff, L.; Garbay, C. *Tetrahedron Lett.* **2002**, *43*, 1417–1419.
5. Thurieau, C.; Simonet, S.; Paladino, J.; Prost, J.-F.; Verbeuren, T.; Fauchere, J.-L. *J. Med. Chem.* **1994**, *37*, 625–629.

6. Hirao, T.; Masunaga, T.; Ohshiro, Y.; Agawa, T. *Synthesis* **1981**, 56–57.
7. A typical procedure was as follows: Boc-Phe(4-I)-OEt (2.4 g, 5.72 mM) and $Pd(PPh_3)_4$ (198.2 mg, 0.172 mM) were dissolved in 100 mL of dry MeCN. An orange colored solution was formed. To this solution, diethylphosphite (1.2 g, 8.7 mM) and TEA (1.2 g, 11.8 mM) were added. The reddish orange color faded to yellowish and became almost colorless. The reaction mixture was heated at 72 ± 2 °C for 20 h. The reaction mixture was evaporated and the resultant syrupy residue (~6 g) was taken in EtOAc (100 mL) and washed with 10% aqueous citric acid (25 mL × 3), saturated $NaHCO_3$ (25 mL × 3), H_2O (25 mL × 2), and brine (25 mL × 2). After drying over $MgSO_4$, the organic layer was evaporated to an oil. It was triturated with hexane to give a white solid (2.6 g), which was recrystallized from MTBE/hexane to afford 2.1 g pure solid (87%). ^{1}H NMR ($CDCl_3$): δ 7.75–7.70 (m, 2H), 7.70–7.65 (m, 1H), 7.50–7.45 (m, 1H), 4.95–5.1 (d, br, 1H), 4.65–4.55 (m, br, 1H), 4.20–4.10 (m, 4H), 4.10– 4.04 (m, 2H), 3.25–3.15 (dd, 1H), 3.15–3.05 (dd, 1H), 1.40 (s, 9H), 1.30 (t, 6H), 1.25 (t, 3H).
8. Compound **10**. ESI-MS: Expected *m/e* 467 ($C_{24}H_{22}N_1O_7P$), observed *m/e* 490 $(M+Na)^+$, $[\alpha]_D^{24}$ −32.3 (*c* 0.25, DMF). ^{1}H NMR (DMSO-d_6): δ 7.95–7.85 (d, 2H), 7.80–7.75 (d, 1H), 7.75–7.65 (m, 2H), 7.65– 7.55 (m, 2H), 7.45–7.35 (m, 2H), 7.35–7.25 (m, 2H), 4.28–4.10 (overlapping, m, 4H), 3.25–3.15 (m, 1H), 2.95–2.85 (m, 1H).
9. Garcia-Echeverria, C. *Lett. Peptide Sci.* **1995**, *2*, 369–373.
10. Hammerschmidt, F.; Hanbauer, M. *J. Org. Chem.* **2000**, *65*, 6121–6131.
11. Hannour, S.; Roumestant, M.-L.; Viallefont, P.; Riche, C.; Martinez, J.; El Hallaoui, A.; Ouazzani, F. *Tetrahedron Asymmetry* **1998**, *9*, 2329–2332.
12. Rinnova, M.; Nefzi, A.; Houghten, R. A. *Tetrahedron Lett.* **2002**, *43*, 4103–4106.
13. Smyth, M. S.; Burke, T. R., Jr. *Tetrahedron Lett.* **1994**, *35*, 551–554.
14. Compound **12**. ESI-MS: expected *m/e* 457 ($C_{22}H_{36}N_1O_7P$), observed *m/e* 479.8 $(M+Na)^+$, $[\alpha]_D^{24}$ −15.89 (*c* 0.5, DMF). ^{1}H NMR ($CDCl_3$): δ 7.76–7.54 (m, 2H), 7.37–7.24 (m, 2H), 5.32–5.15 (d, 1H), 4.69–4.55 (m, 1H), 3.34–3.17 (m, 2H), 1.43 (s, 9H), 1.41 (s, 9H), 1.40 (s, 9H).
15. Campagne, J.-M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1993**, *42*, 6743–6744.
16. Zhang, C.; Mjalli, A. M. M. *Tetrahedron Lett.* **1996**, *37*, 5457–5460.
17. Kaestle, K. L.; Anwer, M. K.; Audhya, T. K.; Goldstein, G. *Tetrahedron Lett.* **1991**, *32*, 327–330.
18. Compound **14**. ESI-MS: expected *m/e* 557 ($C_{31}H_{28}N_1O_7P$), observed *m/e* 579.8 $(M+Na)^+$, $[\alpha]_D^{24}$ +17.2 (*c* 0.25, DMF). ^{1}H NMR (DMSO-d_6): δ 7.87–7.81 (d, 2H), 7.70–7.55 (br, m, 4H), 7.55–7.35 (m, 2H), 7.35– 7.25 (m, 2H), 7.25–7.10 (br, m, 7H), 4.65 (s, 2H), 4.30–4.12 (overlapping, m, 4H), 3.12–3.05 (br, m, 1H), 2.95–2.88 (br, m, 1H).