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Efficient synthesis of phosphotyrosine building blocks using imidazolium trifluoroacetate

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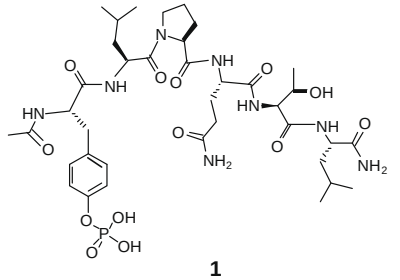
ABSTRACT

We have successfully employed imidazolium trifluoroacetate as a replacement for tetrazole for efficient synthesis of phosphotyrosine. This modification is compatible with the protecting groups commonly used in solution phase and Fmoc-solid phase peptide synthesis.

The SH2 (Src Homology 2) domain is a structurally conserved protein domain contained within the Src oncoprotein[1] and in many other intracellular signal-transducing proteins.[2] A total of 120 SH2 domains have been found in 115 proteins encoded by the human genome.[3] SH2 domains typically bind a phosphorylated-tyrosine containing short peptide motif within a target protein and this interaction between proteins plays a key role in mediating signal transduction in cells. In the last decade, there has been a strong research interest in the design of small-molecule inhibitors to target the SH2 domains as chemical probes and as potential therapeutic agents.[4] The phosphotyrosine group is necessary for high-affinity binding to the SH2 domain and is a critical component in ligands designed to target the SH2 domain.[4–6] Recently, we have reported the design of compound **2** as a conformationally constrained, phosphotyrosine-containing peptidomimetic to target the SH2 domain in STAT3 (Signal Transducer and Activator of Transcription 3), based upon the pYLPQTV sequence (compound **1**) from the gp130 protein.[6]

For the synthesis of the phosphotyrosine group, one can start with the phosphotyrosine *O*-dimethyl ester, which is commercially available. However, the conditions necessary for the removal of the methyl ester are stringent, often resulting in low yields. Protection of the phosphate with groups such as *t*-butyl or benzyl esters is attractive because they can be removed under mild conditions and in high yield. Since phosphotyrosine containing these types of protecting groups is not commercially available, there is a need for a versatile and facile synthesis of phosphotyrosine building blocks. One of the most commonly used phosphorylation methods



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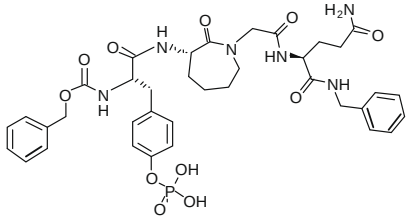

2

of tyrosine uses phosphoramidite and tetrazole.[7] The tetrazole functions both as a weak acid and as a nucleophile, activating the phosphoramidite for phosphorylation of tyrosine.[8] The procedure is compatible with acid-, base- and H_2-labile protecting groups. Unfortunately, tetrazole is no longer commercially available in an effective concentration. Another drawback of tetrazole is that it is explosive when heated.

In this Letter, we wish to report an efficient, one-pot phosphoramidite-mediated synthesis of phosphotyrosine using imidazolium trifluoroacetate[9] in place of tetrazole for the synthesis of

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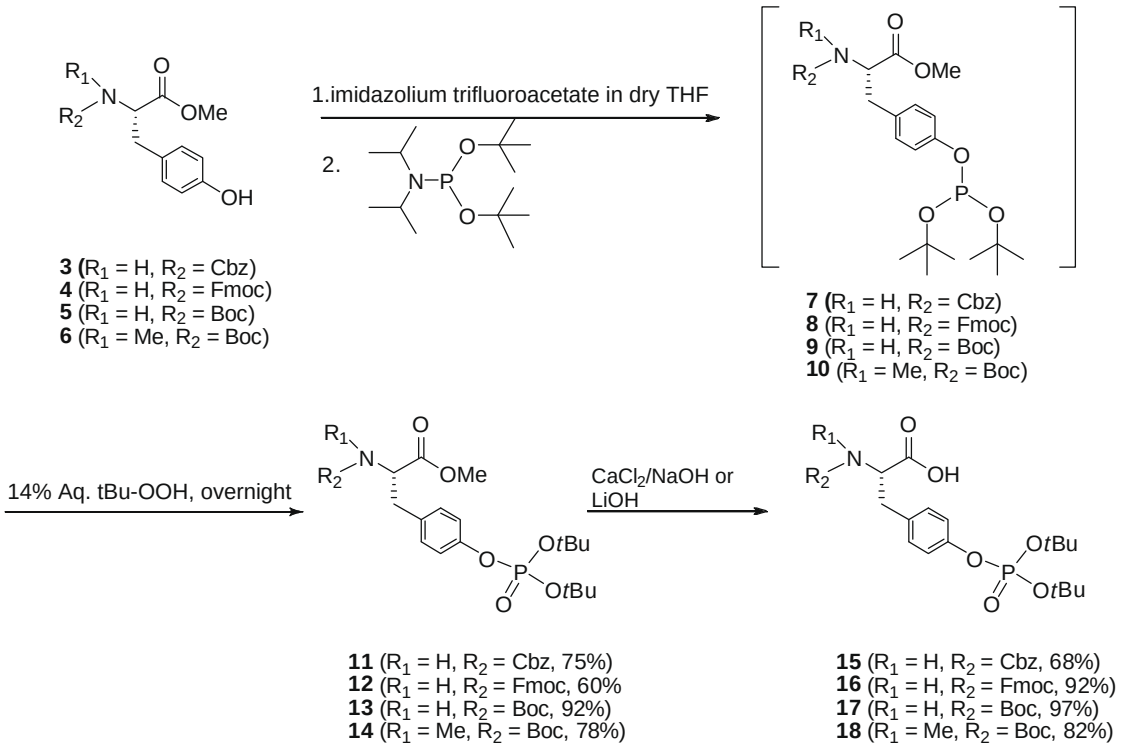

3 (R_1 = H, R_2 = Cbz)
4 (R_1 = H, R_2 = Fmoc)
5 (R_1 = H, R_2 = Boc)
6 (R_1 = Me, R_2 = Boc)

7 (R_1 = H, R_2 = Cbz)
8 (R_1 = H, R_2 = Fmoc)
9 (R_1 = H, R_2 = Boc)
10 (R_1 = Me, R_2 = Boc)

11 (R_1 = H, R_2 = Cbz, 75%)
12 (R_1 = H, R_2 = Fmoc, 60%
13 (R_1 = H, R_2 = Boc, 92%)
14 (R_1 = Me, R_2 = Boc, 78%)

15 (R_1 = H, R_2 = Cbz, 68%)
16 (R_1 = H, R_2 = Fmoc, 92%)
17 (R_1 = H, R_2 = Boc, 97%)
18 (R_1 = Me, R_2 = Boc, 82%)

Scheme 1. Synthesis of phosphotyrosine utilizing imidazolium.

t-butyl ester-protected phosphotyrosine. We have utilized a protection strategy which permits the synthesized phosphotyrosine to be readily incorporated into ligands at either its C- or N-terminus. Imidazolium trifluoroacetate can be readily generated from inexpensive reagents common in most laboratories.

In this optimized route (Scheme 1), imidazolium trifluoroacetate was first generated in situ from equimolar amounts of imidazole and trifluoroacetic acid in dry THF. This salt was reacted with di-*tert*-butyl-*N*,*N*-di-*iso*propyl phosphoramidite and then with the protected phosphotyrosine (**3**–**6**) to give the corresponding phosphorylated-tyrosine (**7**–**10**). This protected phosphotyrosine (**11**–**14**) was obtained in 60–92% yield by oxidation with 14% aqueous *t*-butyl peroxide. The tyrosine carboxyl group was protected as the methyl ester rather than *tert*-butyldimethylsilyl ester because the methyl ester is more stable and amenable to long term storage, avoiding possible deprotection of the *t*-butyl esters on the phosphate by a free carboxylic acid. To obtain the free carboxylic acid, the methyl ester was hydrolyzed by LiOH. Methyl esters containing Fmoc groups can be hydrolyzed with $CaCl_2$/NaOH.[10,11]

As an example, the procedure to generate one-pot phosphorylation of protected tyrosine (**12**) is detailed below: Imidazole (4.6 equiv) was dissolved in the minimum amount of dry THF (approximately 3–5 ml) and TFA (4.6 equiv) was added gradually to the solution under N_2. The concentrated, white slurry which was formed was stirred under N_2, at room temperature for approximately 10 min. Di-*t*-butyl di-*iso*propyl phosphoramidite (1.5 equiv) was then added dropwise to the slurry, and the reaction was stirred under N_2 for approximately 10 min. The protected tyrosine (1.0 equiv) in dry THF was then added to the reaction over 15 min and stirred at room temperature under N_2 until the tyrosine-starting material had been consumed, as indicated by TLC. The reaction mixture was then cooled to 0 °C and a 14% aqueous solution of *t*-butyl peroxide (2.3 equiv) was added. The temperature was then allowed to rise to room temperature and the mixture was stirred overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ solution. Water was added into the reaction mixture, which was then extracted with ethyl acetate, concentrated and finally purified *via* column chromatography (SiO_2) using EtOAc/DCM (20/80) to elute the pure product. In larger scale synthesis, the crude product can be cooled to 0 °C and stirred in a solution of aqueous $Na_2S_2O_5$ for 1 h, then the reaction mixture can be extracted with ethyl acetate and purified.

In summary, we have demonstrated that imidazolium trifluoroacetate is a viable replacement for tetrazole in the phosphorylation by phosphoramidite of tyrosine. This modification is compatible with the protecting groups commonly used in solution phase and Fmoc-solid phase peptide synthesis.

Acknowledgement

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11. *Selected chemical data of compounds* **12** *and* **16**: ^{1}H NMR data for **12** ($CDCl_3$, 300 MHz) δ 1.50 (s, 18H), 3.09–3.11 (d, J = 5.6 Hz, 2H), 3.73 (s, 3H), 4.23 (t, J = 9.0 Hz, 1H), 4.34–4.53 (m, 2H) 4.61–4.72 (m, 1H), 5.32–5.29 (d, J = 9.0 Hz, 1H), 7.03–7.06 (d, J = 9.0 Hz, 2H), 7.14–7.17 (d, J = 8.3 Hz, 2H), 7.24–7.50 (m, 4H), 7.58–7.60 (d, J = 7.3, 2H), 7.77–7.80 (d, J = 7.41 Hz, 2H). ^{1}H NMR data for **16** (CD_3OD, 300 MHz) δ 1.45 (s, 18H), 2.82–3.0 (br s, 1H), 3.15–3.26 (m, 1H), 4.07–4.23 (m, 2H), 4.26–4.42 (m, 2H), 7.04–7.06 (d, J = 6.0 Hz, 2H), 7.16–7.47 (m, 6H), 7.58–7.61 (d, J = 7.8 Hz, 2H), 7.77–7.79 (d, J = 6.9 Hz, 2H).