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A General and Convenient Synthesis of Novel Phosphotyrosine Mimetics

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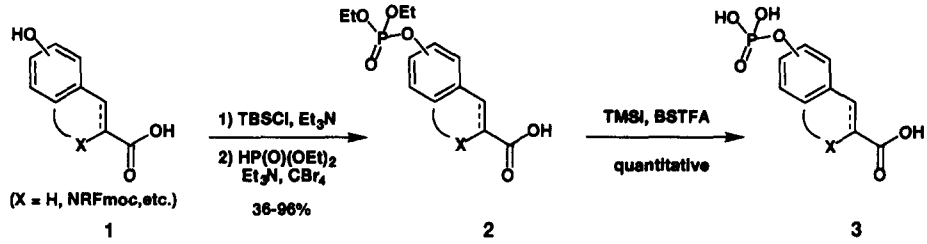
Abstract: *A simple and general procedure for preparation of various phosphotyrosine mimetics from the corresponding phenolic precursors is described. In situ silylation of phenol acids followed by treatment with $Et_3N/CBr_4/HP(O)(OEt)_2$ provides diethyl phosphate intermediates (36-96%), which can be cleanly deprotected in quantitative yields upon treatment with BSTFA/TMSI to afford novel phosphotyrosine mimetics.*

Combinatorial organic synthesis has recently emerged as an important tool in drug discovery.[1] In our efforts aimed at discovery of novel SH2 ligands,[2] we became interested in the rational design and synthesis of libraries incorporating phosphotyrosine and related constrained and desamino mimetics. This called for an easy access to such appropriately protected building blocks. Phosphotyrosine peptides have been typically prepared either by direct phosphorylation of corresponding tyrosine peptides (global phosphorylation) or by incorporation of protected phosphotyrosine as an amino acid building block in a regular peptide synthesis.[3] The latter route is more convenient and versatile, and is better suited for a combinatorial chemistry based approach. Several different phosphate protecting groups have been utilized for this purpose.[4] However, each protecting group, based on its specific nature and tolerance to various reagents, dictates and limits the scope for structural diversity in the remainder of the molecule.[5] An attractive alternative which avoids protecting group related issues involves direct utilization of Fmoc-phosphotyrosine devoid of any protecting groups on the phosphate group.[6] We've successfully employed the latter approach for synthesis of phosphotyrosine peptides as well as the isosteric 4-phosphono (difluormethyl)-phenylalanine peptides.[7] In order to extend the utility of this procedure to various constrained and modified analogs, a mild and convenient synthetic protocol for conversion of tyrosine and related phenolic compounds into the corresponding phosphorylated building blocks was required.

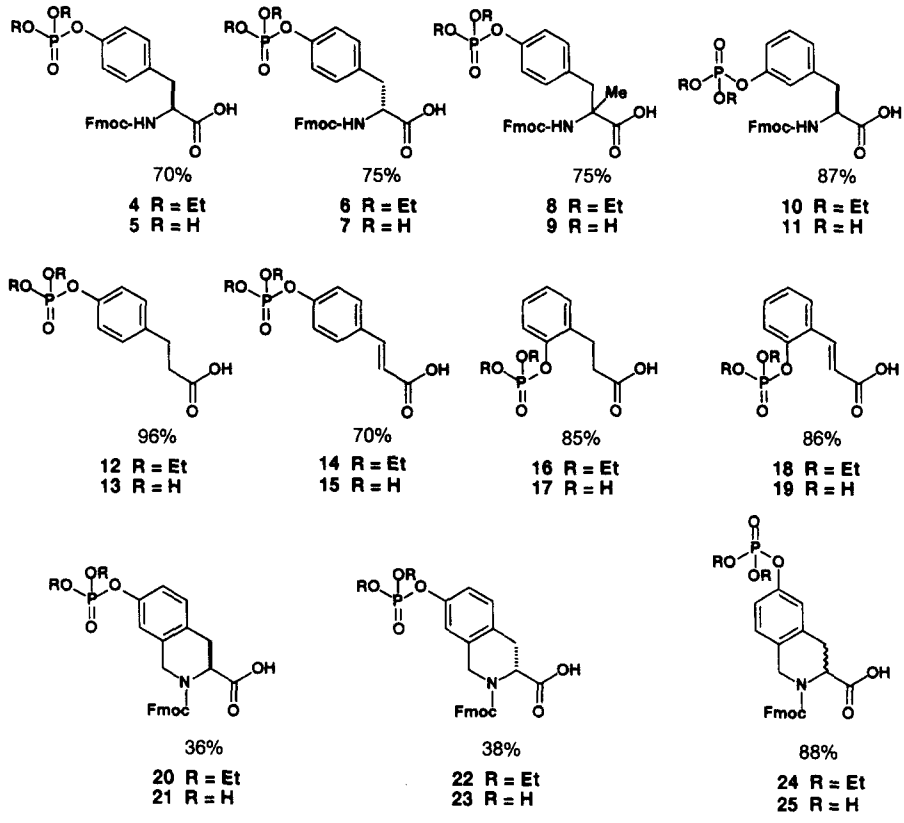
A direct one-step conversion of tyrosine to phosphpotyrosine using pyrophosphoric acid has been reported, wherein the final product is conveniently precipitated using t-butanol.[8] Attempted application of this procedure to various tyrosine mimetics confronted us with the tedious task of isolating highly polar and non-crystallizable products from the reaction mixture. Another common alternative is treatment with phosporamidates followed by oxidation with peroxide reagents.[9] After some experimentation, this route was disfavored because of high expense and limited stability of phosphoramidates, and non-compatibility of the latter step with oxidizable functionalities. Finally, a two step synthetic protocol outlined in Scheme I was developed and found to be optimal in terms of convenience, versatility, and ease of synthesis. The first step involves *in situ* silylation of phenol acid **1** followed by treatment of the silyl ester with carbon tetrabromide,[10] diethylphosphite, and triethylamine leading to the formation of diethyl phosphonate intermediate **2**.[11] After silica gel flash chromatography purification, compound **2** is treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylsilyl iodide (TMSI) in dichloromethane to afford the desired phosphate **3** in essentially quantitative yield.[12] Compound **3** can be utilized directly without purification for synthesis of phosphotyrosine peptides.[13]



Several features pertaining to the synthetic simplicity of this procedure are noteworthy. The reagents required for this transformation are readily available. The final phosphorylated products **3** are highly polar in nature and will have to be typically purified by reverse phase HPLC or ion exchange chromatography. However, this is not necessary since the conversion of penultimate diethyl phosphate intermediates **2** to the final products using BSTFA/TMSI proceeds cleanly and quantitatively. Thus, purification is conveniently carried out at the diethylphosphate stage by silica gel flash chromatography. Additionally, it is important to realize that diethylphosphate precursors such as **2** may themselves find direct utility in specific instances where functionalities on the remainder of the molecule are incompatible with a free phosphate group during the course of synthesis.

The synthetic protocol has been successfully extended to the preparation of various novel phosphorylated analogs from the corresponding tyrosine mimetics (compounds **4-25**).[14] Preparation of Fmoc-protected *alpha* methyl (**9**, 75%), *meta* (**11**, 87%), and desamino (**13**, 96%) phosphotyrosine analogs proceeded in high yields.

Synthesis of the three regioisomers in cinnamoyl series (**15**, **17**, and **19**) was also uneventful (70-86%). The yields for tetrahydroisoquinoline (Tic) type constrained phosphptyrosine analogs (**21** and **23**) are unoptimized (36-38%). This is probably not a general problem associated with such constraied analogs, as evident by high yields of the corresponding *meta* regioisomer (**25**, 88%).



A wide variety of tyrosine mimetics are reported in the literature,[15] and the current study offers a practical approach for their conversion and utility as phosphotyrosine mimetics. Coupling this synthetic method advantageously with combinatorial chemistry as a drug discovery tool provides opportunities for preparing and screening a large number of structurally diverse and novel SH2 ligands that may find utility in treatment of diseases associated with signal transduction.

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5 N-protected dimethylphosphotyrosine has been most commonly employed in this context. However, problems such as partial demethylation of the dimethylphosphono group and dephosphorylation during peptide synthesis, as well as acid catalyzed backbone rearrangements during final deprotection of the phosphate group have been recently reported. Lee, E-S.; Cushman, M. *J. Org. Chem.* **1994**, *59*, 2086. Di-t-butyl esters have limited stability, and dibenzyl esters are stable but limited in versatility because of their susceptibility to some of the cleavage conditions commonly employed in solid phase peptide synthesis.

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10 This method can be viewed as an extension of the classical Todd reaction: Atherton, F. R.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 660. However, erratic and irreproducible yields were obtained in our hands under the exact conditions, which employ CCl_4 as the oxidizing agent. Additionally, while the Todd reaction has been claimed to proceed through the intermediacy of dialkyl phosphorochloridates, we were unable to achieve phosphorylation upon direct treatment of N-α-Fmoc-tyrosine with $(EtO)_2P(O)Cl$ and Et_3N.

11 Two useful combinations of oxidative phosphorylation reagents have been reported recently.
a) Pyridine/I_2/$P(OEt)_3$: Stowell, J. W.; Widlanski, T. S. *Tetrahedron Lett.* **1995**, *36*, 1825.
b) Pyridine/CBr_4/$P(OMe)_3$: Oza, V. B.; Corcoran, R. C. *J. Org. Chem.* **1995**, *60*, 3680.

12 A typical experimental procedure is as follows.

TBDMSCl (1 equiv.) and Et_3N (1 equiv.) are added at RT to a solution of phenol acid **1** in THF (4 to 10 mL/mmol). After 30 min, the solution is cooled to 0 °C and treated with CBr_4 (3 equiv.). This is followed by sequential addition of $HP(O)(OEt)_2$ (2 equiv.) and Et_3N (3 equiv.) in the dark, and the reaction is stirred overnight in the dark with gradual warming to RT. The next day, most of the THF is removed on rotary evaporator, the concentrated solution is partitioned between Et_20 and 5% aq. $NaHCO_3/Na_2SO_3$, and the organic layer is discarded. The aqueous layer is acidified to pH = 2 and extracted with EtOAc (3x). The combined organic extracts are dried (Na_2SO_4), concentrated, and the residue purified by silica gel flash chromatography to afford pure diethyl phosphate intermediate **2**. BSTFA (10 equiv.) is added at RT to a solution of **2** in CH_2Cl_2. After 30 min, the solution is cooled to 0 °C and treated with TMSI (8 equiv., dropwise addition). After 1h at 0 °C and an additional 2h at RT, the reaction mixture is concentrated *under vacuo*. The residue is treated with a mixture of CH_3CN (10 mL), TFA (3 mL), and H_20 (5 mL) for 1h at RT. This is followed by concentration *under vacuo*, and the resulting product **3** is characterized (MS, HPLC, ^1H and ^{31}P NMR) and used directly for subsequent reactions.

13 These studies will be reported elsewhere in near future.

14 Yields for all compounds are unoptimized. All intermediate and final products were characterized by MS, HPLC, ^1H and ^{31}P NMR.

15 a) Deeks, T.; Crooks, P. A.; Waigh, R. D. *J. Med. Chem.* **1983**, *26*, 762. b) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Paschal, J. W. J. *J. Org. Chem.* **1991**, *56*, 4388. c) Nicolas, E.; Russell, K. C.; Knollenberg, J.; Hruby, V. J. *J. Org. Chem.* **1993**, *58*, 7565.

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