Synthesis of O-Phosphotyrosine-Containing Peptides. 3. Synthesis of H-Pro-Try(P)-Val-OH via Dimethyl Phosphate Protection and the Use of Improved Deprotection Procedures[1]

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The phosphorylated derivative N^{α}-(*tert*-butoxycarbonyl)-*O*-(dimethylphosphono)tyrosine anthraquinon-2-ylmethyl ester, Boc-Tyr(PO_3Me_2)-OMaq (**2**), was prepared in high yields by either phosphotriester or "phosphite-triester" phosphorylation of Boc-Tyr-OMaq (**1**). In the former case, either sodium hydride or lithium diisopropylamide was used to generate the phenoxide ion which was then treated with dimethyl phosphorochloridate $(MeO)_2P(O)Cl$. Alternatively, dimethyl *N,N*-diethylphosphoramidite $(MeO)_2PNEt_2$ (**15**) and 1*H*-tetrazole were used in the phosphite-triester approach, to effect the quantitative, acid-catalyzed phosphitylation followed by *m*-chloroperoxybenzoic acid oxidation of the phosphite-triester intermediate. Removal of the 2-methylanthraquinone group (Maq) by reduction with sodium dithionite afforded Boc-Tyr(PO_3Me_2)-OH (**3**) in 70–75% overall yield. This derivative was used in the solution-phase synthesis of the tripeptide Boc-Pro-Tyr(PO_3Me_2)-Val-OMaq (**9**) by the Boc mode of peptide synthesis, which was followed by the removal of the Maq and Boc groups by successive dithionite reduction and 40% CF_3CO_2H/CH_2Cl_2 treatments respectively. Six procedures for the cleavage of the methyl phosphate group by acidolysis or silylolysis from H-Pro-Tyr(PO_3Me_2)-Val-OH·TFA (**11**) were examined with the source of hard acid being CF_3SO_3H, $(CH_3)_3SiBr$, $(CH_3)_3SiBr$, or $CF_3SO_3Si(CH_3)_3$ and either thioanisole or dimethyl sulfide as the soft nucleophile. While deprotection treatments gave H-Pro-Tyr(*P*)-Val-OH in modest isolated yields, monitoring with ^{31}P NMR and ^{13}C NMR indicated quantitative deprotection. Enhanced rates of methyl cleavage from the protecting phosphate group were observed with the use of thioanisole.

Introduction

Over the past decade, tyrosine-specific protein phosphorylation has been recognized as an important cell regulatory process.[2] It has been established that Tyr-specific protein kinases are involved in the cellular response to the epidermal growth factor, insulin and other haemopoietic growth factors[3] (e.g. interleukin 3, interleukin 4, and granulocyte-macrophage colony stimulating factor). Furthermore, cellular transformation of oncogenes[4] (e.g. $pp60^{v\text{-}src}$ and $pp60^{s\text{-}src}$ of the Rous Sarcoma Virus) proceed via insertion of the product of these genes which, in turn, have also been identified as Tyr-specific protein kinases. More recently, tyrosine-specific protein kinases have been implicated in the process of fertilization[5] and neurotransmission.[6] Although protein tyrosine phosphorylation has been proposed to play an important role in many biological processes, the modes of action (e.g. pathway of growth activation between the receptor and cell nucleus) are still unclear. Synthetic phosphotyrosine peptides related to these phosphoproteins may therefore serve as suitable model substrates to investigate the influence of phosphorylation on structure and to potentially shed light on changes induced by phosphorylation at the molecular level. Since 1984, we have investigated the synthesis of phosphotyrosine peptides and have described the solution synthesis of the peptides H-Tyr(*P*)-Leu-Gly-OH[7] and H-Asn-Glu-Tyr(*P*)-Thr-Ala-OH[8] by the use of Boc-Tyr(PO_3Bzl_2)-OH and Boc-Tyr(PO_3Me_2)-OH, respectively, in the Boc mode of peptide synthesis followed by the hydrogenolytic or acidolytic removal of the dibenzyl and dimethyl phosphate esters, respectively. While this procedure permitted the synthesis of several protected Tyr(PO_3R_2) peptides, a major synthetic shortcoming exists in the final deprotection of the phosphorotriester functionality. While 33% HBr/AcOH and 10% $(CH_3)_3SiBr/CH_3CN$ have previously been used for the deprotection of Leu-Arg-Arg-Ala-Tyr(PO_3Me_2)-Leu-Gly[9] and Asn-Glu-Tyr(PO_3Me_2)-Thr-Ala,[8] respectively, the former cleavage mixture gives low yields of peptides, and its use is incompatible with Ser- or Thr-containing peptides due to the acetylation of the hydroxyl group.[10] The 10% $(CH_3)_3SiBr/CH_3CN$ mixture has also limited use due to the low dissolution strength of this solvent system for large, protected peptides.

In past studies,[11] we established that the deprotection of Tyr(PO_3R_2)-containing peptides using liquid hydrogen fluoride was not a favored deprotection procedure because it caused extensive dephosphorylation of the Tyr(PO_3Me_2) and Tyr(PO_3H_2) residues. It was found that the treatment of L-phosphotyrosine or H-Tyr(*P*)-Leu-Gly-OH with liquid hydrogen fluoride/anisole (0 °C, 45 min) caused complete phosphate cleavage to give tyrosine or H-Tyr-Leu-Gly-OH,

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(1) The abbreviations for peptide structures follow the recommendations of the IUPAC–IUB Commision on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9). Other abbreviations are as follows: TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, TMSBr = trimethylsilyl bromide, TMSOTf = trimethylsilyl trifluoromethanesulfonate, Maq = 2-methylanthraquinone, Bzl = benzyl, Boc = *tert*-butoxycarbonyl, DMSO-d_6 = hexadeuterated dimethyl sulfoxide, NMM = *N*-methylmorpholine, IBCF = isobutoxy chloroformate, THF = tetrahydrofuran, AcOH = acetic acid.

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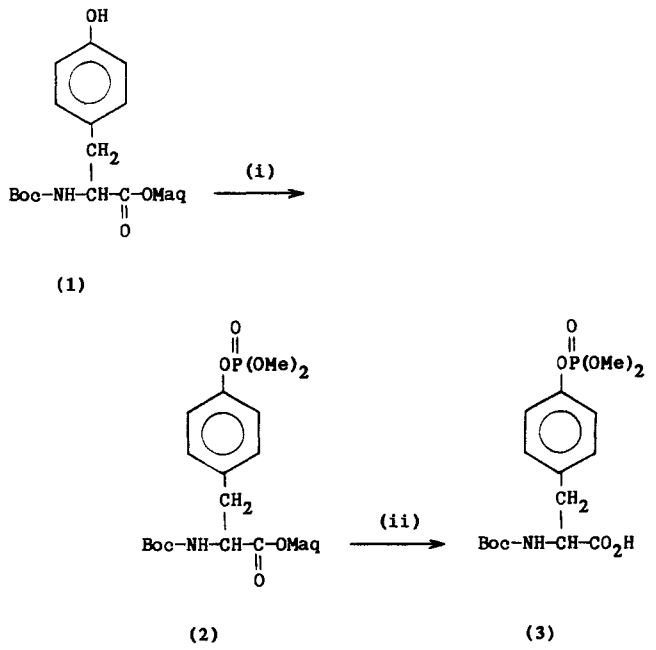
Scheme I[a]



[a] (i) $(MeO)_2PNEt_2$/1*H*-tetrazole (15 min, 20 °C) followed by MCPBA (−40 °C); (ii) $Na_2S_2O_4$ (pH 8.5, 1 h, 50 °C).

respectively, in near-quantitative yield. However, the significant finding from this study was that "high" HF[12] treatment of H-Tyr(PO_3Me_2)-OH with HF/anisole (9:1) (0 °C, 45 min) effected 15% dephosphorylation while "low" HF[13] treatment of H-Tyr(PO_3Me_2)-OH with HF/Me_2S/*m*-cresol (25:65:10) caused 90% dephosphorylation. The notable difference in the extent of dephosphorylation suggests that, in the case of low HF deprotection, the higher concentration of dimethyl sulfide promotes cleavage of the tyrosine phosphate linkage, presumably via sulfide-mediated displacement of the phenolic moiety from the dimethylphosphonium fluoride intermediate.

In view of the many difficulties and limitations in the deprotection of Tyr(PO_3Me_2) peptides, we recognized that a general, useful synthetic procedure would require us to develop improved deprotection procedures. In this paper, we reexamine the synthetic protocol currently used for the synthesis of phosphotyrosine-containing peptides and describe improvements for their efficient synthesis. The compatibility of several deprotection procedures currently used in peptide synthesis for the "global" deprotection of protected peptides is investigated by examining demethylation of the model peptide substrate, H-Pro-Tyr(PO_3Me_2)-Val-OH·TFA.

Results and Discussion

The synthesis of Boc-Tyr(PO_3Me_2)-OH was accomplished by a simple three-step procedure which involved initial carboxyl protection of Boc-Tyr-OH and phosphorylation of the hydroxyl group, followed by removal of the carboxyl protecting group (Scheme I). In contrast to our past use of the 4-nitrobenzyl (NBzl) group for carboxyl protection,[8,9] this synthetic route features the 2-oxymethyleneanthraquinone (Maq) ester for temporary carboxyl protection. Two advantages of the Maq group over the NBzl group are that the former generally gives solid products which can be purified by recrystallization and its reduction by sodium dithionite proceeds without the formation of polymeric byproduct.[15]

Thus, Boc-Tyr-OMaq (1) was prepared in 82% yield by refluxion of Boc-Tyr-OH with 2-(bromomethyl)anthraquinone in the presence of 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU). In the next step, phosphorylation of the tyrosine hydroxyl group was accomplished by either the phosphotriester or phosphite–triester phosphorylation procedures. In the case of phosphotriester phosphorylation, Boc-Tyr(PO_3Me_2)-OMaq (2) was obtained in 83% yield by the treatment of Boc-Tyr-OMaq with sodium hydride followed by reaction of the resultant sodium phenoxide with dimethyl phosphorochloridate.[9] Alternatively, the generation of the phenoxide intermediate using lithium diisopropylamide (−60 °C) led to the isolation of the dimethyl phosphotriester (2) in 80% yield. Phosphite-triester phosphorylation gave Boc-Tyr(PO_3Me_2)-OMaq (2) in 85% yield by the phosphitylation of Boc-Tyr-OMaq with dimethyl *N*,*N*-diethylphosphoramidite (15)/1*H*-tetrazole followed by in situ oxidation of the resultant phosphite–triester with either aqueous iodine or *m*-chloroperoxybenzoic acid. That complete phosphorylation of the tyrosine hydroxyl group had occurred was established from its ^{13}C NMR spectrum. In contrast to Boc-Tyr(PO_3Me_2)-ONBzl which is obtained as a thick red oil,[9] Boc-Tyr(PO_3Me_2)-OMaq solidified readily and could be easily purified by recrystallization from ethyl acetate/petroleum spirit.

In the third step, sodium dithionite treatment of Boc-Tyr(PO_3Me_2)-OMaq under mildly basic conditions (pH 8.5) effected reductive cleavage of the Maq protecting group and gave Boc-Tyr(PO_3Me_2)-OH (3) in 75% yield. Purification of this derivative was readily accomplished by isolation of its cyclohexylammonium salt (diethyl ether/hexane). In our opinion, the synthesis of Boc-Tyr(PO_3Me_2)-OH by this procedure represents a marked improvement over our previously reported one. The method is simple and flexible; the intermediate is a solid, which can be easily purified, characterized, and obtained in high yield. Subsequent to this work, in instances where the free acid (3) is required, we have developed a "one-pot" synthesis using the phosphite–triester phosphorylation method.[16]

The synthesis of the protected tripeptide, Boc-Pro-Tyr(PO_3Me_2)-Val-OMaq (9), was readily accomplished in good yield by the use of Boc-Tyr(PO_3Me_2)-OH (Scheme II). In this synthesis, the dipeptide Boc-Tyr(PO_3Me_2)-Val-OMaq (6) was isolated in 90% yield from the isobutoxycarbonyl mixed anhydride coupling of Boc-Tyr(PO_3Me_2)-OH (3) with H-Val-OMaq·TFA (5) following neutralization of the trifluoroacetate salt. Subsequent acidolytic cleavage of the Boc group from dipeptide (6) with 40% CF_3CO_2H/CH_2Cl_2 followed by the mixed anhydride coupling of the resultant dipeptide (7) with Boc-Pro-OH (8) gave the Tyr(PO_3Me_2)-containing tripeptide (9) as a yellow solid in 94% yield. Successful incorporation of the Tyr(PO_3Me_2) residue in the tripeptide was established from its ^{13}C NMR spectrum which displayed characteristic phosphorus-coupled doublet signals for the tyrosyl C3 and C4 aromatic carbons at 119.9 (J_{PC} = 4.9 Hz) and 149.5 ppm (J_{PC} = 6.1 Hz), respectively, and the methyl phosphate carbons at 54.8 ppm (J_{PC} = 6.1 Hz).

In order to permit selective monitoring of the acidolytic or silylitic cleavage of the methyl groups by ^{31}P NMR

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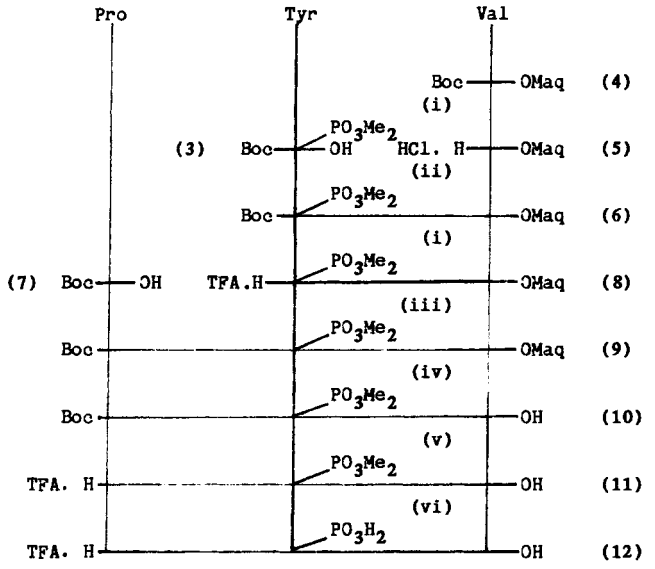
Scheme II[a]



[a] (i) 40% CF_3CO_2H/CH_2Cl_2 (20 °C, 1 h); (ii) (a) NMM, IBCF (−20 °C, 3 min), then (b) amino acid **5** and NMM (1 equiv) in THF; (iii) (a) NMM, IBCF (−20 °C, 3 min), then (b) dipeptide **8** and NMM (1 equiv) in THF; (iv) $Na_2S_2O_4$ (pH 8.5, 3 h, 20 °C); (v) 40% CF_3CO_2H/CH_2Cl_2; (vi) (a) $CF_3SO_3H/CF_3CO_2H/Me_2S/m$-cresol, (b) 1 M $CF_3SO_3H/CF_3CO_2H/PhSMe/m$-cresol, (c) 1 M $(CH_3)_3SiBr$–$PhSMe/CF_3CO_2H$ (*m*-cresol), (d) 1 M $(CH_3)_3SiBr/CF_3CO_2H$ (*m*-cresol), (e) 1 M $CF_3SO_3Si(CH_3)_3$–$PhSMe/CF_3CO_2H$ (*m*-cresol), and (f) 1 M $CF_3SO_3Si(CH_3)_3$–Me_2S/CF_3CO_2H (*m*-cresol).

spectroscopy without the added interference of other protecting groups, tripeptide (**9**) was deprotected initially by sodium dithionite reduction of the Maq group followed by acidolytic removal of the Boc group from the resultant tripeptide, Boc-Pro-Tyr(PO_3Me_2)-Val-OH (**10**). The tripeptide salt, H-Pro-Tyr(PO_3Me_2)-Val-OH·TFA (**11**), was isolated in 83% yield from the above two-step deprotection procedure, and its structure and homogeneity were established by ^{13}C NMR spectroscopy, FAB mass spectrometry, and C_{18} RP HPLC analysis.

The cleavage of the methyl groups from H-Pro-Tyr(PO_3Me_2)-Val-OH·TFA was investigated by several deprotection procedures that have previously been used in peptide synthesis for the deprotection of peptides and proteins. In this study, the following acidolytic (a and b) and silylitic (c–f) deprotection mixtures were examined, viz., (a) $CF_3SO_3H/CF_3CO_2H/Me_2S/m$-cresol,[17] (b) $CF_3SO_3H/CF_3CO_2H/PhSMe/m$-cresol, (c) 1 M $(CH_3)_3SiBr$–$PhSMe/CF_3CO_2H$ (*m*-cresol),[18] (d) 1 M $(CH_3)_3SiBr/CF_3CO_2H$ (*m*-cresol), (e) 1 M $CF_3SO_3Si(CH_3)_3$–$PhSMe/CF_3CO_2H$ (*m*-cresol),[19] and (f) 1 M $CF_3SO_3Si(CH_3)_3$–Me_2S/CF_3CO_2H (*m*-cresol).

Tripeptide **11** was individually treated with each of systems a to f, and the rate of phosphate demethylation was monitored by ^{31}P NMR spectroscopy. In the case of a and b the respective ^{31}P NMR spectra showed the appearance of two resonances at −4.6 and −4.9 ppm and that complete conversion to the −4.9 ppm resonance occurred after 4 and 1 h, respectively. Of the two soft nucleophiles, thioanisole effected the greater rate of demethylation than dimethyl sulfide. This is consistent with previous findings for the demethylation of *O*-methyltyrosine-containing peptides.[20] The greater rate of methyl cleavage with the use of thioanisole is attributed to the greater stabilization of the positive charge of the resultant sulfonium ion by conjugation with the phenyl group. Purification of the crude peptides by semipreparative C_{18} RP HPLC from treatments a and b gave H-Pro-Tyr(*P*)-Val-OH in 66% and 52% yields, respectively.

In the case of the second mode of deprotection by $(CH_3)_3SiBr$ (0 °C) with and without thioanisole, the ^{31}P NMR spectra showed four resonances at −5.0, −14.4, −23.0, and −32.0 ppm, which were assigned to the dimethyl phosphotriester A and the three silylated phosphonium intermediates B, C, and D, respectively (Scheme III). In the presence of thioanisole, successive ^{31}P NMR spectra showed that there was rapid conversion of A to B (<5 min) followed by the loss of the resonance at −14.4 and appearance of a signal at −23.3 ppm, then its disappearance and the appearance of a signal at −32.0 ppm. Complete demethylation, indicated by the presence of only the −32.0 ppm resonance, took 6 h (0 °C reaction) in the presence of thioanisole and 10 h (0 °C reaction) without. Subsequent semipreparative RP-HPLC purification of both crude peptides gave H-Pro-Tyr(*P*)-Val-OH (**12**) in 75% yield for both reactions.

In the third mode of deprotection, the demethylation of tripeptide (**11**) with trimethylsilyl triflate in the presence of thioanisole or dimethyl sulfide was examined. In comparison to $(CH_3)_3SiBr$ deprotection, the ^{31}P NMR spectra showed that demethylation proceeded at a slower rate and that there was a marked difference in the rate of cleavage of the first methyl group compared with the second. In the presence of thioanisole, the half-lives for the cleavage of the methyl groups were found to be $t_{1/2}$ (Me_1) 7 min and $t_{1/2}$ (Me_2) 12 h (at 0 °C) and that in the presence of dimethyl sulfide, the half-lives were $t_{1/2}$ (Me_1) 11 min and $t_{1/2}$ (Me_2) 16 h (at 0 °C). Although methyl cleavage for both deprotections was incomplete after 16 h at 0 °C, complete demethylation occurred after a further 6 h at 20 °C: a single resonance at −32.0 ppm being observed. Subsequent addition of methanol and product isolation for both reactions resulted in loss of the −32.0 ppm signal and the observation of a new one at −4.9 ppm.

In order to confirm the ^{31}P NMR spectral assignments of Scheme III, tripeptide (**11**) was treated with 1 M $CF_3SO_3Si(CH_3)_3$–thioanisole/CF_3CO_2H (*m*-cresol) at 0 °C, and methanol was added when the −23.0 ppm resonance was the predominant signal (t = 90 min) in the ^{31}P NMR spectrum. The ^{13}C NMR spectrum of the purified product confirmed it to be the monomethyl derivative, H-Pro-Tyr(PO_3MeH)-Val-OH (**13**) and showed the phosphorus–carbon doublet for the methyl phosphate carbon at 53.3 ppm (J_{PC} = 6.1 Hz). In comparison to the chemical shift value of 55.4 ppm (J_{PC} = 5.5 Hz) for the dimethyl phosphotriester tripeptide, this represents a relative upfield shift of 2.1 ppm for the methyl phosphodiester. The FAB mass spectrum of the monomethyl phosphate tripeptide (**13**) contained a high intensity molecular ion at m/z 472.

The tripeptide, H-Pro-Tyr(PO_3H_2)-Val-OH·TFA (**12**), prepared using the six deprotection treatments, was readily characterized by ^{13}C NMR and ^{31}P NMR spectroscopy and FAB mass spectrometry. The presence of the phosphotyrosine residue in **12** was demonstrated by its ^{13}C NMR spectrum, which showed phosphorus-coupled doublet carbon signals for the tyrosyl C3 and C4 carbons at 120.7

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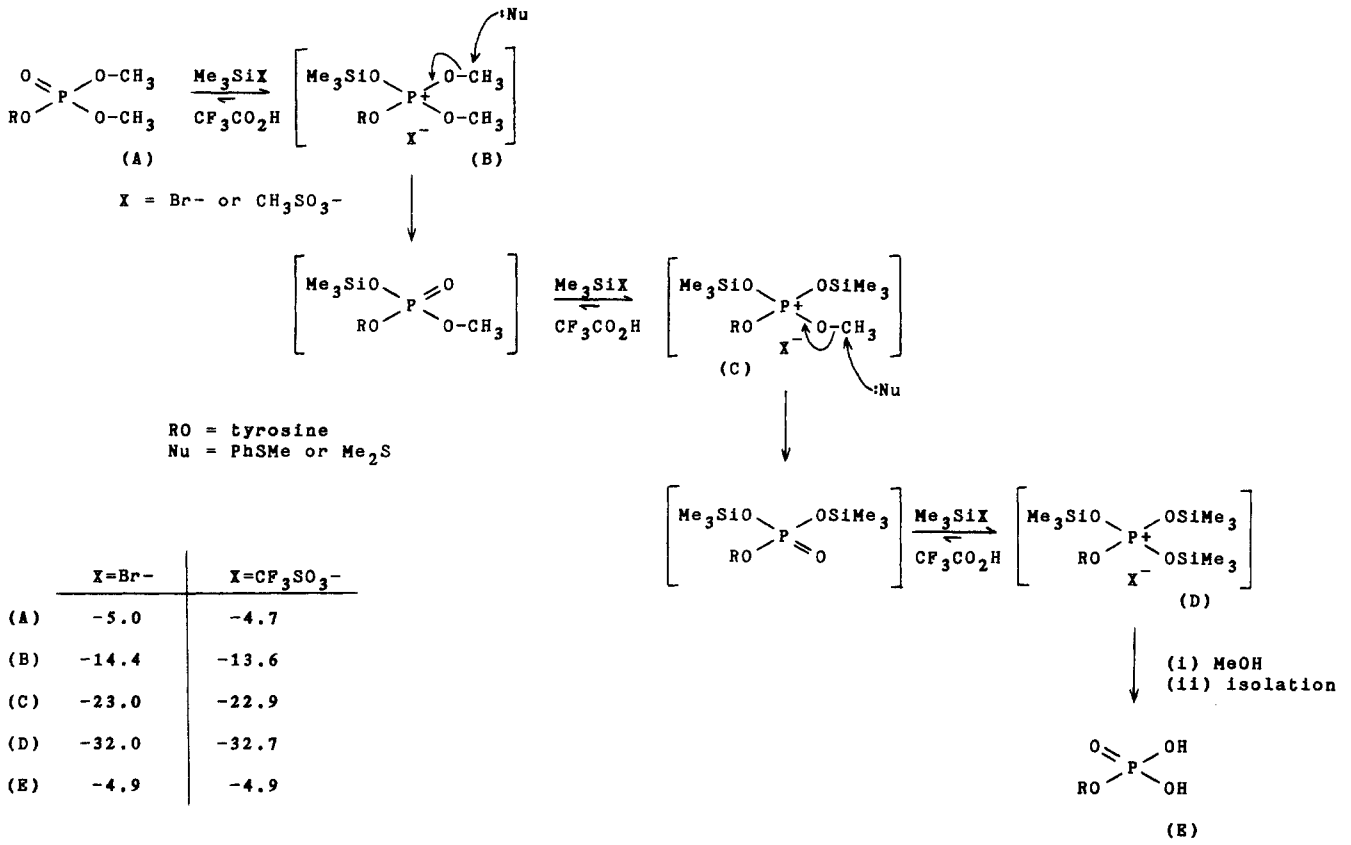
Scheme III. ^{31}P NMR Chemical Shifts and Their Assignment



	X=Br-	X=CF_3SO_3-
(A)	-5.0	-4.7
(B)	-14.4	-13.6
(C)	-23.0	-22.9
(D)	-32.0	-32.7
(E)	-4.9	-4.9

(J_{PC} = 3.7 Hz) and 150.9 (J_{PC} = 7.3 Hz) ppm, respectively, and three well-resolved carbonyl signals at 174.4, 172.3, and 169.1 ppm. The positive FAB mass spectrum was particularly useful for characterization purposes since it displayed a high-intensity molecular ion at m/z 458 and a low-intensity fragment at m/z 378, which was assigned to fragmentation of the dihydrogen phosphate group by the loss of PO_3H (80μ). Phenylthiohydantoin (PTH) gas-phase sequencing[21] of **12** successively gave PTH-Pro in the first cycle, a blank second cycle (for Tyr(*P*)), and PTH-Val in the third cycle. The observation of a blank cycle for the PTH-Tyr(*P*) molecule is consistent with recent findings[22] and has been attributed to the irreversible binding of PTH-Tyr(*P*) with the polybrene support. We have also observed that the monomethyl phosphonotyrosine-PTH product gave a blank in the second cycle during the sequencing of the tripeptide **13**.

In view of the aforementioned limitations of liquid hydrogen fluoride, 33% HBr/AcOH or 10% $(CH_3)_3SiBr$/CH_3CN for the deprotection of Tyr(PO_3Me_2)-containing peptides, we consider that the use of the CF_3SO_3H, $(CH_3)_3SiBr$, or $CF_3SO_3Si(CH_3)_3$ deprotection procedures presents a significant development in the synthetic options available for the deprotection of peptides and the demethylation of dimethyl phosphotriester groups. The comparison of yields of the isolated peptides indicates that the three deprotection systems incoporating thioanisole as the nucleophile are suitable for the preparation of Tyr(*P*)-peptides. Although tripeptide **12** was isolated in moderate yields from the six deprotections, ^{31}P and ^{13}C NMR analyses of the crude peptides indicated quantitative demethylation and no decomposition of the phosphotyrosine residue in the tripeptide. The yields in fact highlight the difficulty in recovering low molecular weight peptides from viscous, relatively involatile reagent systems such as CF_3SO_3H and $CF_3SO_3Si(CH_3)_3$. This should not be a problem with higher molecular weight peptides.

In conclusion, we consider that the synthetic methodology described here for the synthesis of phosphotyrosine peptides using methyl for phosphate protection provides a general and efficient procedure which is compatible with present-day synthetic methodology. This current work has resulted in the development of a high-yielding three-step synthesis of the necessary Boc-Tyr(PO_3Me_2)-OH synthon. It features the use of dimethyl *N,N*-diethylphosphoramidite/1*H*-tetrazole phosphite-triester phosphorylation of the tyrosyl hydroxyl group. Finally, several deprotection procedures were examined and found to be useful for the deprotection of Tyr(PO_3Me_2)-containing peptides.

Experimental Section

The preparation of dimethyl phosphorochloridate[23] using dimethyl hydrogen phosphonate (Fluka), trimethylsilyl trifluoromethanesulfonate,[24] and L-phosphotyrosine[25] and the standardization of *n*-butyllithium[26] have been described before. Sodium hydride (BDH) was triturated in pentane (AR) and dried under a stream of nitrogen, methanol (AR) and tetrahydrofuran (AR) used in the coupling reactions were dried over 4-Å sieves, diethyl ether (AR) over sodium, triethylamine over potassium hydroxide, 1,4-dioxane (AR) was passed over aluminum oxide, and tetrahydrofuran used in phosphorylation reactions was distilled over potassium benzophenone ketal prior to use. Trifluoroacetic acid and *m*-cresol were redistilled, trifluoromethanesulfonic acid,

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dimethyl sulfide, and thioanisole were of AR grade. Petroleum ether, (40–60 °C) fraction, was used for recrystallization. Trifluoroacetate salts and hydrochloride salts were generated by dissolving the Boc amino acid or peptide for 1 h in 40% TFA/CH_2Cl_2 or 4 M HCl/1,4-dioxan, respectively, evaporation of solvent under reduced pressure followed by trituration of the resultant oil in diethyl ether. The solid was dried to constant weight prior to use. TLC was carried out on Merck Kieselgel 60 F_{254} precoated plates with (A) $CHCl_3$–MeOH–AcOH (14:2:1), (B) $CHCl_3$–MeOH (5:2) or (C) ethyl acetate–pentane (1:1) as solvent system and visualized by UV or iodine vapor. ^1H NMR spectra were obtained on a JEOL-FX100 Fourier transform (FT) instrument operating at 99.5 MHz referenced to internal tetramethylsilane. ^{13}C NMR spectra were obtained on a JEOL-FX90Q FT instrument operating at 22.5 MHz referenced to internal deuteriochloroform (77.0 ppm), dimethyl-d_6 sulfoxide (39.5 ppm) or 1,4-dioxane (66.5 ppm, D_2O). ^{31}P NMR spectra were obtained on a JEOL-FX100 FT instrument operating at 40.26 MHz and referenced to external 85% H_3PO_4. FAB mass spectra were obtained on a JEOL-DX 300 mass spectrometer equipped with a FAB source using glycerol/acetic acid as the matrix support. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at the sodium D line with a 1-dm or a 1-mm path length cell kept at constant temperature. Melting points were measured on a Kofler hot-stage microscope and are uncorrected. Infrared spectra were recorded as potassium bromide disks or as liquid films on a Perkin-Elmer 983G spectrophotometer. Semipreparative reversed-phase high-performance liquid chromatography (RP HPLC) was performed on either a C_8 cartridge (Brownlee RP300, 250 × 10 mm, 20 μm) or a C_{18} column (Vydac, 250 × 10 mm, 20 μm) using linear gradients of 0.1% aqueous TFA (solvent A) and 0.1% TFA in CH_3CN (solvent B) at a flow rate of 3 mL/min and monitoring the eluant with a variable-wavelength UV detector operating at 280 nm. Analytical RP-HPLC was performed on either a C_8 (Aquapore RP300, 250 × 4.6 mm, 7 μm) or a C_{18} (Altex, 250 × 4.6 mm, 5 μm) using solvents A and B at a flow rate of 1 mL/min and a UV detector operating at 214 nm. Peptide sequence analysis was on a an Applied Biosystems Inc. 470A protein sequencer using the on-line 120A phenylthiohydantoin analyzer. The glass fiber disk was loaded with 3 mg of Polybrene as carrier and precycled three times before the peptide was loaded onto the disk for analysis. Elemental analyses were performed by AMDEL, Melbourne.

N^{α}-(*tert*-Butoxycarbonyl)tyrosine Anthraquinon-2-ylmethyl Ester (1, Boc-Tyr-OMaq). 1,8-Diazobicyclo[5.4.0]undec-7-ene (1.52 g, 10.00 mmol) was added to a suspension of Boc-Tyr-OH (3.23 g, 11.50 mmol) and 2-(bromomethyl)anthraquinone (3.01 g, 10.00 mmol) in refluxing THF (40 mL) and further refluxed for 1.5 h. The DBU·HBr was filtered off, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (40 mL), washed with 1 M HCl (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and NaCl (saturated, 20 mL), dried (Na_2SO_4), and then filtered. Removal of the solvent under reduced pressure gave a yellow solid, which on recrystallization from ethyl acetate/petroleum ether gave **1** as yellow needles (4.09 g, 82%): mp 160–162 °C; $[\alpha]^{23}$ −12.1° (*c* 0.5, $CHCl_3$); IR max 3353, 2923, 2853, 2362, 2340, 2327, 1746, 1677, 1593, 1557, 1516, 1445, 1367, 1328, 1292, 1164, 1105, 1054, 1022, 931, 850, 711, 540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 (s, 9 H, Boc CH_3), 2.85 (m, 2 H, Tyr-CH_2), 4.25 (m, 1 H, Tyr-CH), 5.30 (s, 2 H, Maq CH_2), 6.64 and 7.04 (AA′XX′ system, $J_{A'X} = J_{AX'}$ = 8.42 Hz, 4 H, ArH), 7.38–8.26 (m, 7 H, Maq ArH), 9.22 (s, 1 H, Tyr-OH); ^{13}C NMR (DMSO-d_6) δ 28.1, 55.7, 64.9, 80.3, 115.0, 125.6, 126.7, 126.9, 127.3, 129.9, 133.1, 134.4, 142.8, 155.2, 156.0, 172.0, 182.1. Anal. Calcd for $C_{29}H_{27}O_7N$: C, 69.5; H, 5.4; N, 2.8. Found: C, 69.4; H, 5.6; N, 2.8.

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(dimethylphosphono)tyrosine Anthraquinon-2-ylmethyl Ester (2, Boc-Tyr-(PO_3Me_2)-OMaq). Method A. Sodium hydride (80%, 0.05 g, 1.70 mmol) was treated as above and suspended in THF (2 mL) at 5 °C. A solution of Boc-Tyr-OMaq (0.72 g, 1.48 mmol) in THF (3.5 mL) was added to the vigorously stirred suspension and further stirred at 20 °C for 30 min. The reaction was cooled to 10 °C, dimethyl phosphorochloridate (0.35 g, 2.42 mmol) was added in one portion, and the clear solution was stirred at 20 °C for 30 min. Water (1 mL) was added, and the solvent was removed under reduced pressure. The residue was then dissolved in ethyl acetate (20 mL), and the organic phase was washed with 1 M HCl (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), H_2O (10 mL), NaCl (saturated, 10 mL), dried (Na_2SO_4), and filtered. Removal of the solvent under reduced pressure gave **2** as a yellow solid, which was recrystallized from ethyl acetate/petroleum ether as cubic crystals (0.86 g, 83%): mp 149–150 °C; $[\alpha]^{24}$ −12.9° (*c* 1, $CHCl_3$); IR max 3434, 2923, 2358, 2327, 1752, 1703, 1674, 1592, 1557, 1538, 1505, 1455, 1367, 1325, 1294, 1216, 1171, 1061, 965, 861, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H, Boc CH_3), 3.11 (d, J_{HH} = 6.1 Hz, 2 H, Tyr-CH_2), 3.83 (d, J_{PH} = 11.2 Hz, 6 H, $POCH_3$), 4.64 (d, J_{HH} = 8.1 Hz, 1 H, Tyr-CH), 5.27 (s, 2 H, Maq CH_2), 5.31 (d, J_{HH} = 7.8 Hz, 1 H, NH), 7.12 (bd, 4 H, ArH), 7.60–8.29 (m, 7 H, Maq ArH); ^{13}C NMR ($CDCl_3$) δ 28.3, 37.7, 54.7, 54.9 (d, J_{PC} = 6.1 Hz), 65.8, 80.3, 119.9 (d, J_{PC} = 4.9 Hz), 126.4, 127.3, 127.8, 130.6, 132.8, 133.3, 133.5, 133.8, 134.2, 141.8, 149.6 (d, J_{PC} = 6.1 Hz), 155.0, 171.5, 182.6; ^{31}P NMR ($CDCl_3$) δ −4.0. Anal. Calcd for $C_{31}H_{32}O_{10}NP$: C, 61.1; H, 5.3; N, 2.3; P, 5.1. Found: C, 61.3; H, 5.5; N, 2.5; P, 4.9.

Method B. Diisopropylamine (0.31 mL, 2.20 mmol) was treated with *n*-butyllithium (1.44 M, 2 mmol, 1.4 mL) for 10 min at 0 °C. The solution was then added dropwise to a solution of Boc-Tyr-OMaq (1.00 g, 2.00 mmol) in THF (3 mL) at −60 °C. After 15 min the mixture was slowly warmed to 20 °C over 30 min, the solution was then cooled to −60 °C, and dimethyl phosphorochloridate (0.54 g, 3.74 mmol) was added in one portion. The reaction mixture was stirred at 20 °C for 30 min before the addition of water (1 mL). Product isolation as before gave **2**, (0.98 g, 80%): mp 147–149 °C; $[\alpha]^{25}$ −12.2° (*c* 1, $CHCl_3$).

Method C. 1*H*-Tetrazole (0.15 g, 2.20 mmol) was added to a solution of Boc-Tyr-OMaq (0.50 g, 1.00 mmol) and dimethyl *N*,*N*-diethylphosphoramidite (0.20 g, 1.2 mmol) in THF (4 mL) and further stirred at 20 °C for 20 min. The solution was cooled to −40 °C and treated with *m*-chloroperoxybenzoic acid (85%, 0.24 g, 1.20 mmol). After 10 min at 20 °C, $Na_2S_2O_5$ (5 mL) and ethyl acetate (30 mL) were added to the solution, and the organic phase was washed with $Na_2S_2O_5$ (15 mL), 5% $NaHCO_3$ (15 mL), and 1 M HCl (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Recrystallization as for method A gave **2** (0.52 g, 85%): mp 149–152 °C; $[\alpha]^{23}$ −12.8° (*c* 1, $CHCl_3$).

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(dimethylphosphono)tyrosine (3, Boc-Tyr(PO_3Me_2)-OH). A degassed solution of sodium dithionite (2.54 g, 16.30 mmol) and sodium carbonate (1.73 g, 16.30 mmol) in H_2O (16 mL) was added to a vigorously stirred solution of Boc-Tyr(PO_3Me_2)-OMaq (2.48 g, 4.07 mmol) in acetonitrile (30 mL). The solution was vigorously stirred under nitrogen for 1 h at 50 °C, cooled to 0 °C, and then acidified to pH 4 (1 M HCl). The precipitate was extracted into ethyl acetate (60 mL), the organic fraction was washed with 0.5 M HCl (30 mL), and the solvent was then removed under reduced pressure. The residue was extracted with 5% $NaHCO_3$ (60 mL), and the solution was washed with diethyl ether (40 mL) and reacidified to pH 4 (1 M HCl). The product was extracted with ethyl acetate (60 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure, giving an oil (1.20 g, 75 %): $[\alpha]^{24}$ +36.3° (*c* 1, $CHCl_3$) [lit.[16] $[\alpha]^{22}$ +36.9° (*c* 1, $CHCl_3$)]; homogeneous by TLC, R_f 0.58 (A); ^{13}C NMR ($CDCl_3$) δ 28.2, 37.2, 54.1, 55.0 (d, J_{PC} = 5.5 Hz), 80.0, 119.8 (d, J_{PC} = 4.4 Hz), 130.8, 133.5, 149.3 (d, J_{PC} = 7.7 Hz), 155.4, 173.8; FAB mass spectrum (Xe, positive mode), *m*/*z* 412 ($[M + Na]^+$, 20), 356 (10), 334 (55), 316 (30), 290 (40), 244 (85), 215 (30), 127 (22), 102 (52).

N^{α}-(*tert*-Butoxycarbonyl)valine Anthraquinon-2-ylmethyl Ester (4, Boc-Val-OMaq). 1,8-Diazobicyclo[5.4.0]undec-7-ene (1.07 g, 7.00 mmol) was added to a suspension of Boc-Val-OH (1.75 g, 8.04 mmol) and 2-(bromomethyl)anthraquinone (2.11 g 7.00 mmol) in THF (40 mL), and the solution was refluxed for 1.5 h. Product isolation as for **1** and recrystallization from ethyl acetate/petroleum ether gave **4** as light brown needles (2.50 g, 82%): mp 137–137.5 °C; $[\alpha]^{23}$ −3.1° (*c* 1, $CHCl_3$); IR max 3384, 2965, 1744, 1693, 1667, 1592, 1518, 1466, 1388, 1365, 1324, 1296, 1234, 1215, 1194, 1156, 1042, 1020, 988, 932, 918, 884, 792, 711, 658, 636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05, 0.97 (each d, J_{HH} = 6.8 Hz, 3 H, Val-CH_3), 1.45 (s, 9 H, Boc CH_3), 2.21 and 2.20 (dq, J_{HH} = 4.1 Hz, 1 H, Val$_\beta$-CH), 4.36 and 4.30 (dd, 1 H, J_{HN} = 9.3 Hz, J_{HH} = 8.8 Hz), 5.32 (s, 2 H, Maq CH_2), 7.90 (m, 7 H, Maq ArH); ^{13}C NMR ($CDCl_3$) δ 17.6, 19.0, 28.2, 31.2, 58.9, 65.6, 79.9, 126.2, 127.2, 127.7, 133.1, 133.5, 133.7, 134.1, 142.1, 155.6,

172.0, 182.6. Anal. Calcd for $C_{25}H_{27}O_6N$: C, 68.6; H, 6.2; N, 3.2. Found: C, 68.3; H, 6.3; N, 3.4.

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(dimethylphosphono)tyrosylvaline Anthraquinon-2-ylmethyl Ester (6, Boc-Tyr(PO_3Me_2)-Val-OMaq). *N*-Methylmorpholine (0.12 g, 1.17 mmol) and isobutyl chloroformate (0.15 g, 1.09 mmol) were added to a solution of **3** (0.49 g, 1.17 mmol) in THF (1.5 mL) at a temperature below −15 °C. After 3 min, a solution of TFA·H-Val-OMaq (0.38 g, 0.84 mmol) and *N*-methylmorpholine (0.09 g, 0.84 mmol) in THF (4 mL) was added, and the mixture was stirred at −20 °C for 2 h under nitrogen. 5% $NaHCO_3$ (1 mL) was added, and the solution was stirred for a further 30 min at 20 °C. The solvent was evaporated under reduced pressure, and ethyl acetate (30 mL) was added. The organic phase was washed with 5% $NaHCO_3$ (2 × 15 mL) and 1 M HCl (2 × 15 mL), dried (Na_2SO_4), and filtered. Evaporation of the solvent under reduced pressure gave a clear oil, which on trituration with diethyl ether/hexane (1:3) gave **6** as a pale yellow solid (0.53 g, 90%): mp 105–106 °C; $[\alpha]^{23}$ −3.2° (*c* 1, $CHCl_3$); homogeneous by TLC, R_f 0.84 (B), 0.45 (C); IR max 3383, 2966, 2339, 2327, 1743, 1692, 1669, 1592, 1518, 1454, 1391, 1366, 1325, 1295, 1234, 1216, 1157, 1043, 1019, 958, 932, 853, 792, 711, 636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 and 0.93 (each d, J_{HH} = 4.5 Hz, 3 H, Val-CH_3), 1.41 (s, 9 H, Boc CH_3), 2.20 (m, 1 H, Val$_\beta$-CH), 3.07 (d, J_{HH} = 5.6 Hz, 2 H, Tyr$_\beta$-CH_2), 3.85 (d, J_{PH} = 11.2 Hz, 6 H, $POCH_3$), 4.49 (m, 2 H, Val$_\alpha$-CH, Tyr$_\alpha$-CH), 5.11 (bd, 1 H, Tyr_{HN}), 5.31 (s, 2 H, Maq CH_2), 6.65 (bd, 1 H, Val_{HN}), 7.17 (bd, 4 H, Tyr Ar), 8.08 (m, 7 H, Maq Ar); ^{13}C NMR ($CDCl_3$) δ 17.6, 18.9, 28.2, 31.1, 36.9, 54.8 (d, J_{PC} = 6.1 Hz), 55.8, 57.2, 65.7, 80.4, 119.9 (d, J_{PC} = 4.9 Hz), 126.1, 127.2, 127.7, 130.3, 130.6, 133.0, 133.3, 133.6, 134.2, 142.0, 149.5 (d, J_{PC} = 6.1 Hz), 155.4, 171.1, 171.3, 182.6; ^{31}P NMR ($CDCl_3$) δ −4.2.

N^{α}-(*tert*-Butoxycarbonyl)prolyl-*O*-(dimethylphosphono)tyrosylvaline Anthraquinon-2-ylmethyl Ester (9, Boc-Pro-Tyr(PO_3Me_2)-Val-OMaq). *N*-Methylmorpholine (0.09 g, 0.91 mmol) and isobutyl chloroformate (0.12 g, 0.85 mmol) were added to a solution of Boc-Pro-OH (0.20 g, 0.91 mmol) in THF (1 mL) such that the temperature was maintained below −15 °C. After 3 min, a solution of TFA·H-Tyr(PO_3Me_2)-Val-OMaq (0.47 g, 0.91 mmol) and *N*-methylmorpholine (0.07 g, 0.65 mmol) in THF (2 mL) was added, and the resulting solution was stirred at −20 °C for 2 h under nitrogen. 5% $NaHCO_3$ (1 mL) was added, and the solution was stirred for a further 30 min at 20 °C. The solvent was evaporated under reduced pressure, and ethyl acetate (30 mL) was added. The organic phase was washed with 5% $NaHCO_3$ (2 × 15 mL) and 1 M HCl (2 × 15 mL), dried (Na_2SO_4), and filtered. Removal of the solvent under reduced pressure gave a clear oil, which on trituration in diethyl ether/hexane (1:3) gave tripeptide **9** as a yellow powder (0.49 g, 94%): mp 88–89 °C; $[\alpha]^{23}$ −44.6° (*c* 0.5, $CHCl_3$); homogenous by TLC, R_f 0.79 (B); ^{13}C NMR ($CDCl_3$) δ 17.8, 19.0, 24.0, 28.2, 30.8, 36.8, 47.0, 53.9, 54.8 (d, J_{PC} = 6.1 Hz), 57.6, 60.5, 65.6, 80.6, 119.9 (d, J_{PC} = 4.9 Hz), 126.1, 127.2, 127.7, 130.5, 133.0, 133.3, 133.6, 134.2, 142.1, 149.5 (d, J_{PC} = 6.1 Hz), 155.1, 170.9, 170.7, 172.5, 182.6; ^{31}P NMR ($CDCl_3$) δ −4.0.

N^{α}-(*tert*-Butoxycarbonyl)prolyl-*O*-(dimethylphosphono)tyrosylvaline (10, Boc-Pro-Tyr(PO_3Me_2)-Val-OH). Sodium dithionite (1.43 g, 8.20 mmol) in 1 M $NaHCO_3$ (15 mL) was added to a degassed solution of **9** (0.47 g, 0.58 mmol) in 1,4-dioxane (20 mL), and the solution was vigorously stirred under nitrogen for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in water (60 mL) and washed with diethyl ether (3 × 30 mL). The aqueous phase was acidified to pH 3 (1 M $KHSO_4$), extracted with $CHCl_3$ (3 × 30 mL), dried, and filtered. Removal of the solvent under reduced pressure gave a clear oil, which, after trituration with diethyl ether, gave tripeptide **10** as a pale white solid (0.30 g, 83%): mp 95–96 °C; $[\alpha]^{22}$ −43.9° (*c* 0.5, $CHCl_3$); homogenous by TLC, R_f 0.65 (A); IR max 3301, 2965, 2327, 1693, 1506, 1454, 1392, 1367, 1261, 1217, 1167, 1123, 1046, 958, 858, 774, 637 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 17.7, 18.7, 23.7, 28.0, 29.4, 30.8, 38.0 46.8, 54.6, 54.9 (d, J_{PC} = 6.1 Hz), 57.5, 60.4, 78.4, 120.1 (d, J_{PC} = 4.9 Hz), 130.4, 133.7, 149.0 (d, J_{PC} = 7.3 Hz), 154.7, 170.2, 171.9, 172.4; ^{31}P NMR ($CDCl_3$) δ −4.0; FAB mass spectrum (Ar, positive mode), *m/z* 586 (M^+, 10), 487 (30), 486 (100).

Prolyl-*O*-(dimethylphosphono)tyrosylvaline Trifluoroacetate (11, H-Pro-Tyr(PO_3Me_2)-Val-OH•TFA). Tripeptide **10** (0.18 g, 0.30 mmol) was dissolved in 40% CF_3CO_2H/CH_2Cl_2 (3 mL) for 1 h. The solvent was then removed by evaporation under reduced pressure, and the residue was triturated with diethyl ether to quantitatively yield tripeptide **11** as a hygroscopic solid: $[\alpha]^{23}$ + 8.4° (*c* 1.2, MeOH); analytical HPLC (C_{18}), 0–65% solvent B in 30 min, one peak 19.7 min; ^{13}C NMR (D_2O) δ 17.2, 18.2, 23.6, 29.5, 29.8, 30.1, 36.1, 46.6, 55.4 (d, J_{PC} = 5.5 Hz), 55.7, 58.4, 58.9, 59.4, 120.0 (d, J_{PC} = 3.3 Hz), 130.8, 133.6, 148.8 (d, J_{PC} = 7.7 Hz), 169.8, 172.2, 174.7; ^{31}P NMR (D_2O) δ −3.2; FAB mass spectrum (Ar, positive mode), *m/z* 486 (M^+, 95), 472 (20), 362 (15).

Deprotection Studies on Peptide 11. Preparation of Prolyl-*O*-phosphotyrosylvaline Trifluoroacetate (12, H-Pro-Tyr(PO_3H_2)-Val-OH·TFA). Method A. TFMSA/TFA/Me_2S/*m*-Cresol (1:5:3:1 v/v). Tripeptide **11** (54 mg, 90 μmol) was treated with TFMSA/TFA/Me_2S/*m*-cresol (3 mL) at 0 °C, and the reaction was monitored by ^{31}P NMR spectroscopy. After 4 h the solution was concentrated under reduced pressure, and chilled diethyl ether (50 mL) was added to the residue. The precipitated oil was further washed with diethyl ether (3 × 30 mL) and diethyl ether/ethyl acetate (1:2) (2 × 30 mL), dried under high vacuum, and then purified by semipreparative C_{18} reverse-phase chromatography. Lyophilization of the major fraction gave tripeptide **12** as a fluffy white powder (32 mg, 66%); $[\alpha]^{24}$ 0.0° (*c* 1.4, H_2O); analytical HPLC (C_{18}), 0–65% solvent B in 30 min, one peak 9.9 min; ^{13}C NMR (D_2O) δ 17.3, 18.1, 23.6, 29.7, 30.0, 36.3, 46.5, 55.5, 58.5, 59.4, 120.7 (d, J_{PC} = 3.7 Hz), 130.3, 131.7, 150.9 (d, J_{PC} = 7.3 Hz), 169.1, 172.3, 174.4; ^{31}P NMR (D_2O) δ −3.6; FAB mass spectrum (Ar, positive mode), *m/z* 458 (M^+, 75), 378 (35).

Method B. TFMSA/TFA/PhSMe/*m*-Cresol (1:5:3:1 v/v). Tripeptide **11** (50 mg, 84 μmol) was treated with TFMSA/TFA/PhSMe/*m*-cresol (3 mL), at 0 °C, and the solution was monitored by ^{31}P NMR spectroscopy. After 1 h, the crude product was isolated as for method A and gave tripeptide **12** (25 mg, 52%) as a fluffy white solid.

Method C. 1 M TMSBr/TFA (*m*-Cresol). Tripeptide **11** (37 mg, 60 μmol) was dissolved in 1 M TMSBr/TFA (*m*-cresol, 10 mmol) (3 mL), and the solution was stirred for 10 h at 0 °C. Methanol (2 mL) was added, and the solution was evaporated under reduced pressure. The residue was dissolved in water (20 mL), washed with diethyl ether (2 × 20 mL) and CH_2Cl_2 (2 × 20 mL), and lyophilized. The hygroscopic brown solid was purified by C_{18} RP HPLC, and the major fraction was lyophilized from water to give tripeptide **12** (26 mg, 75%) as a fluffy white solid.

Method D. 1 M TMSBr-PhSMe/TFA (*m*-Cresol). Tripeptide **11** (38 mg, 60 μmol) was treated with 1 M TMSBr-PhSMe/TFA (*m*-cresol, 10 mmol) (3 mL), and the reaction was monitored by ^{31}P NMR spectroscopy. After 6 h, methanol (2 mL) was added, and subsequent isolation and purification as for method C gave tripeptide **12** (27 mg, 75%) as a fluffy white solid.

Method E. 1 M TMSOTf-PhSMe/TFA (*m*-Cresol). Tripeptide **11** (47 mg, 80 μmol) was treated with 1 M TMSOTf-PhSMe/TFA (*m*-cresol, 10 mmol) (3 mL) for 22 h. Methanol (2 mL) was then added, and the solution was concentrated under reduced pressure. Chilled diethyl ether (30 mL) was added to the residue. The precipitated oil was washed with diethyl ether (2 × 30 mL) and diethyl ether/ethyl acetate (1:3), and the residue was lyophilized from water. Purification of the residue by C_{18} RP HPLC gave tripeptide **12** (20 mg, 53%) as a fluffy white solid.

Method F. 1 M TMSOTf-Me_2S/TFA (*m*-Cresol). Tripeptide **11** (46 mg, 75 μmol) was treated with 1 M TMSOTf-Me_2S/TFA (*m*-cresol, 10 mmol) (3 mL) for 22 h, and subsequent peptide isolation, as for method E, gave tripeptide **12** (15 mg, 44%) as a fluffy white solid.

Prolyl-*O*-(monomethylphosphono)tyrosylvaline Trifluoroacetate (13, H-Pro-Tyr(PO_3HMe)-Val-OH·TFA). Tripeptide **11** (50 mg, 80 μmol) was treated with 1 M TMSOTf-PhSMe/TFA (*m*-cresol, 10 mmol) (3 mL) at 0 °C, and the reaction was monitored by ^{31}P NMR spectroscopy. After 1.5 h (^{31}P NMR, s, −23.0 ppm) the reaction was quenched with methanol (1 mL), and the product was isolated as above (20 mg, 54%): $[\alpha]^{19}$ +0.5° (*c* 1.4, H_2O); analytical HPLC (C_{18}), 0–65% solvent B in 30 min, one peak 14.2 min; ^{13}C NMR (D_2O) δ 17.3, 18.2, 23.8, 29.7, 30.1, 36.2, 46.4, 53.3 (d, J_{PC} = 5.5 Hz), 55.3, 58.8, 59.4, 120.3 (d, J_{PC} = 3.7 Hz), 130.3, 131.8, 150.7 (d, J_{PC} = 7.3 Hz),

169.1, 172.3, 174.8; FAB mass spectrum (Ar, positive mode), m/z 472 (M^+, 100), 458 (5).

Deprotection by Hydrogen Fluoride. (i) Low HF Treatment. H-Tyr(PO_3Me_2)-OH·HCl (14) (0.10 g, 0.25 mmol) was treated with HF/Me_2S/m-cresol (2.5:6.5:1.0 v/v, 10 mL) at 0 °C for 2 h. HF was removed under reduced pressure, the residue was washed with diethyl ether (5 × 50 mL) and then dissolved in water (50 mL). Lyophilization gave a yellow solid (127 mg). Quantitative amino acid analysis of the crude product by RP HPLC (C8) (0–30% solvent B in 30 min) showed it to be a mixture of H-Tyr-OH (15) (4.9 min) and H-Tyr(PO_3Me_2)-OH (20.9 min) in a 9:1 ratio (established by comparison with authentic samples).

(ii) High HF Treatment. H-Tyr(PO_3Me_2)-OH·HCl (0.10 g, 0.25 mmol) was treated with 5% anisole/HF (5 mL) at 0 °C for 45 min. The liquid HF was removed under reduced pressure, and the residue was dissolved in water (50 mL), washed with diethyl ether (5 × 50 mL), and lyophilized from water to give a white solid (80 mg). Amino acid analysis (as for i) indicated H-Tyr-OH and H-Tyr(PO_3Me_2)-OH in a 15:85 ratio.

(iii) High HF Treatment of H-Tyr(PO_3H_2)-OH. L-Phosphotyrosine (50 mg, 0.20 mmol) was treated with 5% anisole/HF (5 mL) for 45 min at 0 °C. The liquid hydrogen fluoride was removed under reduced pressure and the residue was isolated (as for method i). Quantitative amino acid analysis as in method i showed the residue to be H-Tyr-OH.

Tyrosylleucylglycine Hydrogen Fluoride (14, H-Tyr-Leu-Gly·HF). Tripeptide, H-Tyr(PO_3H_2)-Leu-Gly-OH·TFA[7] (0.10 g, 0.18 mmol), was treated with liquid HF/10% anisole (10 mL) for 45 min at 0 °C. The liquid HF was then evaporated under reduced pressure, and the residue was triturated successively with diethyl ether (3 × 30 mL) and ethyl acetate/diethyl ether (3:1) (3 × 30 mL) and dried under high vacuum to give tripeptide **17** (0.07 g, 85%) as a white solid: ^{13}C NMR (D_2O) δ 20.9, 21.8, 24.0 35.9, 39.9, 41.1, 52.2, 54.2, 115.8, 125.2, 130.8, 155.1, 168.7, 172.9, 173.7.

Dimethyl *N,N*-Diethylphosphoramidite (15). A solution of methanol (3.20 g, 100.0 mmol) and triethylamine (12.12 g, 120.0 mmol) in dry diethyl ether (40 mL) was slowly added to a solution of dichloro *N,N*-diethylphosphoramidite (8.65 g, 50.0 mmol) in dry diethyl ether (20 mL) at −20 °C such that the temperature of the solution was maintained below −10 °C. On completion of addition, the solution was vigorously stirred at 20 °C for 2 h, and then 10% Na_2CO_3 (20 mL) was added. The solution was transferred to a separating funnel using diethyl ether (40 mL), and the aqueous phase was discarded. The organic phase was washed with 10% Na_2CO_3 (30 mL) and NaCl (saturated, 30 mL) and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. Distillation of the crude liquid residue gave **15** as a pungent, clear liquid (5.2 g, 63%): bp 38–40 °C (2.0 mmHg); ^1H NMR ($CDCl_3$) δ 1.06 (d, J_{HH} = 7.08 Hz, 6 H, NCH_2CH_3), 3.07 (dq, J_{HH} = 7.08 Hz, J_{PH} = 9.08 Hz, 4 H, NCH_2CH_3), 3.39 (d, J_{PH} = 12.7 Hz, 6 H, $POCH_3$); ^{13}C NMR ($CDCl_3$) δ 14.1 (d, J_{PC} = 2.9 Hz, NCH_2CH_3), 36.3 (d, J_{PC} = 20.5 Hz, NCH_2CH_3), 48.9 (d, J_{PC} = 14.7 Hz, $POCH_3$); ^{31}P NMR ($CDCl_3$) δ +150.2.

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Regioselective Acylation of 6-Deoxy-L- and -D-hexosides through Lipase-Catalyzed Transesterification. Enhanced Reactivity of the 4-OH Function in the L Series

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Porcine pancreatic, *Candida cylindracea*, and *Pseudomonas fluorescens* lipases, suspended in organic solvents, were used to regioselectively acylate methyl α-L- and α-D-rhamnopyranoside and methyl α-L- and α-D-fucopyranoside. While the D-sugars always gave the 2-butyrate as the main product, their L enantiomers showed a different regioselectivity; however, by proper selection of the enzyme, in the L series the rather unreactive 4-OH function could be preferentially acylated.

Introduction

Alcoholic functions of sugars markedly differ in their reactivity.[1] Primary hydroxyl group is obviously the most reactive, but among the secondary groups a wide range of reactivities can be observed. Orientation (equatorial or axial) or position of the hydroxyl groups are the two variables which mainly modulate this reactivity; the 4-OH has often a very low reactivity toward several reagents. In fact, selective monoacylation of glucose, galactose, etc. in this position can be hardly obtained.[1,2]

Hydrolytic enzymes were recently used as catalysts for regioselective esterification of sugars when suspended in organic solvents in conditions allowing a transesterification between an activated ester and the polyhydroxylated moiety.[3-5] D-Glucose, D-mannose, and D-galactose have

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