# Preparation of Fluoro- and Hydroxy-4-(phosphonomethyl)-D,L-phenylalanine Suitably Protected for Solid-Phase Synthesis of Peptides Containing Hydrolytically Stable Analogues of *O*-Phosphotyrosine[1]

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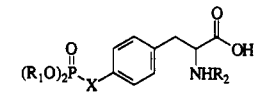
4-[(Di-*tert*-butylphosphono)methyl]-*N*-(fluoren-9-ylmethoxycarbonyl)-D,L-phenylalanine [*O*-di-*tert*-butyl-Pmp-*N*-Fmoc, **3**] has previously been shown to be a useful reagent for the solid-phase synthesis of peptides containing the hydrolytically stable *O*-phosphotyrosyl mimetic, phosphonomethylphenylalanine (Pmp, **2**). One potential limitation of Pmp-containing peptides relative to the corresponding phosphotyrosyl prototypes is the higher $pK_{a2}$ value of phosphonic acids as compared to that of phosphates. In an effort to prepare Pmp analogues which more closely approximate phosphotyrosyl residues, *O*-di-*tert*-butyl-Pmp-*N*-Fmoc derivatives were made bearing monofluoro (**4**), difluoro (**5**), and hydroxy (**6**) substituents at the phosphonate methylene. The synthetic utility of analogues **4** and **6** was demonstrated by solid-phase synthesis of the hexameric peptide, H-Gly-X-Val-Pro-Met-Leu-OH, where X = monofluoro Pmp and hydroxy Pmp, respectively. These peptides are analogues of the SH2 recognition motif "phosphoTyr-Val-Pro-Met-Leu", which is important for mitogenic cellular signal transduction. The hydrolytic lability of difluoro Pmp analogue **5** precluded its usefulness in peptide synthesis. Along with *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**3**), monofluoro (**4**) and hydroxy (**6**) derivatives may prove to be useful synthons in the preparation of peptides containing hydrolytically stable analogues of phosphotyrosyl residues.

Phosphorylation reactions are key mediators of a variety of biochemical processes.[2] Enzymes which catalyze the transfer of the γ-phosphate of ATP to the 4-hydroxyl of tyrosyl residues within specific protein substrates [protein-tyrosine kinases (PTKs)] are particularly important in cellular signal transduction as they are frequently the immediate intracellular effectors of growth factor receptors.[3] Since PTKs have been directly or indirectly associated with the etiology of a number of neoplastic processes, modulation of phosphotyrosyl-utilizing signaling pathways is a potential area of study in the development of anticancer therapeutics.[4,5] The object of such research is the selective inhibition of specific PTKs involved in mitogenic processes,[6] and "phosphotyrosyl pharmacophores" capable of mimicking the phosphorylated tyrosyl residues have emerged as important synthetic targets for such studies.[7] This is exemplified by the use of phosphotyrosyl-containing peptides to antagonize "SH2"-mediated[8] associations of certain PTKs with their substrates in cell free systems.[9-11] One potential limitation with the use of phosphotyrosyl-containing peptides either as pharmacological tools in vivo or as therapeutics is the hydrolytic lability of the phosphoryl–ester linkage in the presence of protein-tyrosine phosphatases (PTPs).[12] Nonhydrolyzable mimetics of phosphates can be prepared by replacement of the phosphate ester oxygen with a methylene carbon. A number of phosphonates have proven to be useful in biological studies.[13] (Phosphonomethyl)-phenylalanine (Pmp, **2**) represents a nonhydrolyzable mimetic of phosphotyrosine (**1**). We have previously



| | X | $R_1$ | $R_2$ |
|---|---|---|---|
| (Phosphotyrosine) **1** | O | H | H |
| (Pmp) **2** | $CH_2$ | H | H |
| **3** | $CH_2$ | *tert*-Bu | Fmoc |
| **4** | CHF | *tert*-Bu | Fmoc |
| **5** | $CF_2$ | *tert*-Bu | Fmoc |
| **6** | CHOH | *tert*-Bu | Fmoc |

prepared[14] 4-[(di-*tert*-butylphosphono)methyl]-*N*-Fmoc-D,L-phenylalanine [*O*-di-*tert*-butyl-Pmp-*N*-Fmoc, **3**] as an analogue of Pmp that is suitably protected for incorporation[15] into peptides using solid-phase Fmoc (fluoren-9-ylmethoxycarbonyl) chemistry.[16] However, Pmp-containing peptides prepared using this reagent were found to be less effective than the parent phosphotyrosyl-

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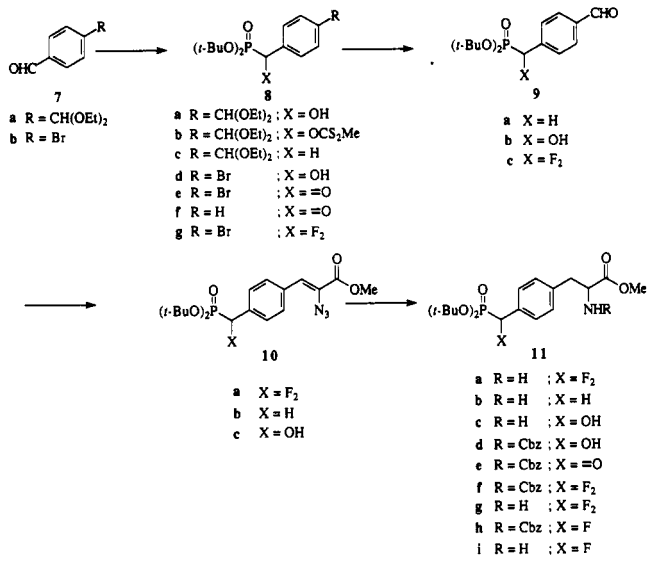
**Scheme I**




containing peptides in antagonizing SH2-mediated phenomena.[10] The loss of activity may be partially attributable to a higher $pK_{a2}$ of the Pmp-phosphonate relative to the phosphotyrosyl-phosphate prototype.[10] Since physiological processes occur at or near neutral pH, populations of singly or doubly ionized species differ significantly for benzylic phosphates ($pK_{a2} = 6.22$)[17] versus the corresponding phosphonates ($pK_{a2} = 7.72$).[17] Phosphonate $pK_{a2}$ values can be lowered by halogen substitution at the $\alpha$-methylene and $\alpha$-fluoro and $\alpha,\alpha$-difluoro phosphonic acids have in some cases been found to be superior to the unsubstituted phosphonates as biological mimetics of phosphate esters.[18-20] We have recently developed synthetic methodology for the preparation of benzylic $\alpha,\alpha$-difluoro phosphonic acids as phenyl phosphate mimetics.[17] In the present paper we report the application of this chemistry to the synthesis of $\alpha$-fluoro **4** and $\alpha,\alpha$-difluoro **5** derivatives of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc and the use of analogue **4** in solid-phase synthesis of SH2-related peptides containing fluorinated Pmp residues. We also report the synthesis of an $\alpha$-hydroxy derivative of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**6**) and its use in solid-phase synthesis, as well as an improved synthesis of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**3**). Compound **4** may prove to be of general value for the preparation of peptides containing nonhydrolyzable phosphotyrosine mimetics which more closely approximate the $pK_{a2}$ value of phosphotyrosine.

## Results and Discussion

Our previous synthesis[14] of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**3**) started from 4-[(di-*tert*-butylphosphono)methyl]-benzaldehyde (**9a**). This latter material was originally prepared by radical-mediated deoxygenation of the $\alpha$-hydroxy phosphonate **8a**, which resulted from basic aluminum oxide-catalyzed addition of di-*tert*-butyl phosphite to commercially available 4-(diethoxymethyl)benzaldehyde (**7a**).[14] We have found that an improvement of this overall transformation can be achieved by reacting the sodium salt of di-*tert*-butyl phosphite with aldehyde **7a** and trapping the resulting alkoxide as the methyl xanthate **8b**. Subsequent radical deoxygenation, followed by hydrolysis of the intermediate acetal **8c**, yielded the desired aldehyde **9a** in 67% overall yield from **7a** as compared with the 24% overall yield previously obtained.[14]

With a synthesis of difluoro *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**5**) based on that used to prepare[14] *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**3**), the difluoro aldehyde **9c** became a critical intermediate. Preparation of **9c** was achieved using our reported procedure for the synthesis of benzylic difluorophosphonic acids.[17] Addition of sodium di-*tert*-butyl phosphite to 4-bromobenzaldehyde (**7b**) provided the hydroxy phosphonate **8d**, which was then oxidized to the keto phosphonate **8e** using pyridinium dichromate. Use of $MnO_2$ in refluxing toluene, as previously reported[17] for the preparation of unsubstituted keto phosphonate **8f**, resulted in significant decomposition. Treatment of keto phosphonate **8e** with (diethylamino)sulfur trifluoride (DAST) gave the $\alpha,\alpha$-difluoro phosphonate **8g**, which was then formylated by treatment with *n*-BuLi, followed by quenching with ethyl formate to yield the desired aldehyde **9c**. Proceeding according to the previously delineated route[14] to protected Pmp **3**, reaction of aldehyde **9c** with ethyl $\alpha$-azidoacetate in sodium methoxide gave the vinyl azide **10a**. Attempted hydrogenation to the amino ester **11a** was achieved a single time and could not be repeated. In light of the very facile hydrogenation of the unsubstituted vinyl azide **10b** to the amino ester **11b**,[14] the failure of the difluoro phosphonate-containing vinyl azide **10a** to undergo a similar reduction could not be explained.

An alternate route was therefore devised in which hydrogenation of the vinyl azide preceded the fluorination reaction. Modification of our original synthesis of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**3**) by starting with hydroxy phosphonate **9b** rather than unsubstituted phosphonate **9a** allowed the preparation of vinyl azide **10c** which underwent facile hydrogenation to the racemic hydroxy phosphonate-containing amino ester **11c**. Lack of signal doubling in the $^1H$ NMR (250 MHz) spectrum of **11c** indicates a stereoselective delivery of hydrogen in the formation of the new chiral center at the phenylalaninate $\alpha$-carbon. This is supported by the observation that prolonged exposure to silica gel results in a gradual development of peak doubling in the NMR, consistent with the generation of diastereomers through epimerization of the phenylalaninate $\alpha$-carbon. Such epimerization was first noticed upon multiple chromatographies of **11c** over silica gel using acetone/hexane solvent systems. A further investigation revealed that stirring an acetone-containing solution of **11c** with silica gel overnight resulted in complete epimerization. Acetone seemed to be a critical component of this process, as either MeOH or $CHCl_3$ solutions alone failed to induce epimerization.

Oxidation of the hydroxy phosphonate to the keto phosphonate using PDC required initial protection of **11c** as the benzyl carbamate **11d**. Reaction of the resulting keto phosphonate **11e** with DAST gave the difluoro phosphonate **11f**, which upon hydrogenolytic removal of the Cbz group gave **11g**. Conversion to final product **5** was achieved by hydrolysis of the methyl ester and in situ derivatization using Fmoc-OBT.[14] The *tert*-butyl-protected difluoro phosphonate **5** proved to be extremely labile, with significant hydrolysis to the free phosphonic acid occurring during silica gel chromatography. Additionally, instability during storage was also observed, and

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we were unable to prepare and store sufficient **5** for actual peptide incorporation. However, treatment of *N*-Cbz-protected hydroxy phosphonate **11d** with DAST provided the monofluoro derivative **11h** in 55% yield following chromatographic purification. $^1$H NMR of **11h** gives no evidence of multiple diastereomers, which is consistent with the ability of DAST to deliver fluoride stereospecifically with a net inversion of configuration.[21] Hydrogenolytic deprotection of the Cbz amino protecting group provided the free monofluoro amino ester **11i** which was then converted to final Fmoc-protected **4** as described above. The monofluoro derivative **4** proved to be significantly more stable than the difluoro analogue **5** and was suitable for use in solid-phase peptide synthesis, as was the hydroxy *O*-di-*tert*-butyl-Pmp-*N*-Fmoc (**6**), obtained directly from **11c**.

In order to demonstrate the usefulness of *O*-di-*tert*-butyl-Pmp-*N*-Fmoc derivatives **3**, **4**, and **6** in solid-phase peptide synthesis, the hexapeptides H-Gly-X-Val-Pro-Met-Leu-OH (**12a–f**) (where X = the appropriate Pmp analogue) were prepared by manual synthesis. The peptide, Tyr-Val-Pro-Met-Leu, represents the Tyr-719 autophosphorylation sequence of the $\beta$-platelet-derived growth factor receptor (PDGFR) PTK, which is an SH2-binding site for phosphatidylinositol-3-kinase (PI3 kinase). It has been demonstrated that the pentapeptide (phosphoTyr)-Val-Pro-Met-Leu can block this PI3 kinase-PDGFR association,[11] perhaps by competing with the native sequence. Starting from 4-[(2′,4′-dimethoxyphenyl)hydroxymethyl]phenoxy (DHP) resin,[22] an "Fmoc strategy" of piperidine-mediated N-deprotection, followed by either symmetrical anhydride[23] or pentafluorophenyl ester (Pfp) amide bond formation was used. Treatment of the completed resin with trifluoroacetic acid (TFA) containing antioxidants resulted in cleavage of the peptide from the resin with the simultaneous removal of phosphonate *tert*-butyl protecting groups. An advantage of the DHP resin over the 4-alkoxybenzyl alcohol resin previously used[10,15] is its greater acid lability. Should it be desired, very mild acid treatment can be utilized to selectively cleave the peptide from the resin while maintaining full *tert*-butyl protection of the phosphonic acid. HPLC examination of the crude peptide mixtures **12** in each case showed the presence of two major components in a 1 to 1 ratio. Isolation and characterization of each of these major peaks showed them to correspond to the L-Pmp- and D-Pmp-containing peptides, respectively, in order of elution. Similar to previous reports,[15] assignment of absolute configurations was achieved by subjecting the peptides to digestion using aminopeptidase-M, which is specific for L-$\alpha$-amino acids.[24] While L-Pmp-containing peptides were cleaved by this enzyme, D-Pmp-containing peptides were not. The relative ease with which these diastereomeric peptides can be separated on HPLC enhances the synthetic utility of racemic *O*-di-*tert*-butyl-Pmp-*N*-Fmoc analogues.

In conclusion, fluoro- and hydroxy-*O*-di-*tert*-butyl-Pmp-*N*-Fmoc derivatives **4** and **6** may now be added to **3** as useful synthetic reagents for the preparation of peptides containing stable analogues of *O*-phosphotyrosyl residues. By combining *tert*-butyl protection of the phosphonate along with Fmoc-amino derivatization, mild acid-catalyzed cleavage of peptide from the solid-phase resin can be accompanied by quantitative phosphonate deprotection, yielding highly pure, completely deprotected product. This is in contrast to solid-phase synthesis of Pmp-containing peptides using either methyl[25] or ethyl[26] phosphonate protection, where additional deprotection steps are required, sometimes resulting in partially deprotected products.[26] Finally, the instability of the difluoro-*tert*-butyl-Pmp-*N*-Fmoc derivative **5** limits its utility as a synthetic reagent.

## Experimental Section

Silica gel was TLC grade silica gel (5–25 $\mu$m; Aldrich) and petroleum ether was of the boiling range 35–60 °C. Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA. Negative ion fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer at 6 kV accelerating voltage using a glycerol or nitrobenzyl alcohol sample matrix and a xenon atom bombardment ionization source, operated under the control of a VG 2035 data system. $^1$H NMR data were obtained on a Bruker AC250 (250 MHz) instrument. HPLC was conducted with an LKB Model 2150 system equipped with an LKB 2140 rapid spectral detector. Amino acid analyses were performed at the Protein Structure Laboratory, University of California, Davis, CA.

**4-[(Di-*tert*-butylphosphono)methyl]benzaldehyde (9a).** To a stirred suspension of 80% NaH in oil (3.00 g, 100 mmol) in anhydrous THF (200 mL) at −78 °C was added di-*tert*-butyl phosphite (19.4 g, 100 mmol) dropwise over 10 min. The reaction was stirred at −78 °C (40 min), and then 4-(diethoxymethyl)-benzaldehyde (**7a**) (19.6 g, 94 mmol) was added and the mixture was first stirred on ice and then allowed to come to rt gradually over 2.5 h. Carbon disulfide (28 mL, 500 mmol) was added and after 15 min methyl iodide (6.2 mL, 100 mmol) was introduced and stirring was continued (30 min). The reaction mixture was subjected to an extractive workup (EtOAc/brine) to yield intermediate xanthate **8b** as a light yellow oil, 45.4 g. An aliquot of **8b** (11.4 g, 25 mmol) in toluene (250 mL) was heated at 109 °C under argon with a spatula tip of azobis(isobutyronitrile) (AIBN) while *n*-$Bu_3SnH$ (8.4 mL, 31 mmol) was added over 5 min. An additional spatula tip of AIBN was added and the reaction stirred (1 h). The mixture was cooled, diluted with hexanes (100 mL), and applied to a 6.5-cm diameter × 4.5-cm high pad of silica gel charged with hexanes. The pad was washed well with hexanes (6 × 100 mL); then the acetal **8c** was eluted with EtOAc as a colorless oil, 8.70 g (90%). Hydrolysis of the acetal was achieved by stirring at rt in a biphasic $CHCl_3$ (150 mL)/1 N HCl (50 mL) system (1 h). Neutralization of the organic layer (aqueous $NaHCO_3$) and evaporation of solvent provided pure product **9a** in 75% yield (67% overall from **7a**) as colorless crystals (petroleum ether/hexanes): mp 60–63.5 °C (lit.[25] mp 61–65 °C).

**4-[(Di-*tert*-butylphosphono)hydroxymethyl]benzaldehyde (9b).** To a stirred mixture of sodium di-*tert*-butyl phosphite (100 mmol) in THF at −78 °C (prepared as above) was added 4-(diethoxymethyl)benzaldehyde (**7a**) (19.6 g, 94 mmol), and the mixture stirred on ice and then brought to rt over 1 h. The reaction was quenched with brine and subjected to an extractive workup (EtOAc) to yield crude **8a** as an off-white solid. Trituration with petroleum ether provided acetal **8a** as colorless crystals, 25.8 g. Hydrolysis of the acetal as described above gave pure **9b** (19.0 g) as a colorless solid, which was identical to previously reported material.[27]

**4-[(Di-*tert*-butylphosphono)hydroxymethyl]bromobenzene (8d).** To a stirred suspension of NaH (2.88 g, 120 mmol) in anhydrous THF (100 mL) at 0 °C was added di-*tert*-butyl phosphite (23.3 g, 120 mmol) in anhydrous THF (100 mL) dropwise over 5 min, and the mixture was stirred at 0 °C (30

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min). A solution of 4-bromobenzaldehyde (**7b**) (18.5 g, 100 mmol) in anhydrous THF (20 mL) was added rapidly and the mixture was brought to rt over 1 h. The reaction mixture was diluted with brine (200 mL) and subjected to an extractive workup ($CHCl_3$) to yield crude **8d** as a white crystalline solid. Trituration with petroleum ether provided **8d** as white crystals (34.0 g, 90%): mp 126.0–126.5 °C; hydrate mp 114–115 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.45 (d, 2 H, $J$ = 8.4 Hz, $H_2$), 7.30 (dd, 2 H, $J$ = 8.4 and 2.2 Hz, $H_3$), 4.78 (dd, 1 H, $J$ = 10.2 and 3.5 Hz, P-C-H), 2.92 (dd, 1 H, $J$ = 9.7 and 4.0 Hz, OH), 1.42 (s, 9 H, $OC(CH_3)_3$), 1.39 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{15}H_{24}NO_4PBr \cdot {}^1/_4H_2O$: C, 46.95; H, 6.44. Found: C, 46.91; H, 6.42.

**4-[(Di-*tert*-butylphosphono)oxomethyl]bromobenzene (8e).** To a solution of **8d** (16.4 g, 43.3 mmol) in anhydrous $CH_2Cl_2$ (300 mL) at 0 °C was added pyridinium dichromate (40.7 g, 108 mmol), and the suspension stirred overnight, coming to rt gradually. The crude mixture was filtered through a Florisil pad and the pad was washed with EtOAc. The combined filtrates were taken to dryness to yield **8e** as a clear, light brown oil (13.9 g, 86%). Silica gel chromatography [EtOAc–hexanes (1:4)] afforded analytically pure **8e** as a clear light yellow oil: $^1H$ NMR ($CDCl_3$) $\delta$ 8.15 (d, 2 H, $J$ = 8.6 Hz, $H_2$), 7.61 (dd, 2 H, $J$ = 8.6 Hz and 1.0 Hz, $H_3$), 1.52 (s, 18 H, $OC(CH_3)_3$). Anal. Calcd for $C_{15}H_{22}NO_4PBr$: C, 47.76; H, 5.88. Found: C, 47.86; H, 5.92.

**4-[(Di-*tert*-butylphosphono)difluoromethyl]bromobenzene (8g).** To **8e** (9.40 g, 25.0 mmol) was added DAST (16.5 mL, 125 mmol) over 5 min at 0 °C. The reaction was brought to rt gradually and stirred overnight. The mixture was cooled to 0 °C, diluted with $CHCl_3$ (15 mL), and added dropwise to a well-stirred suspension of aqueous $NaHCO_3$ (69 g in 300 mL $H_2O$) at 0 °C. The resulting mixture was stirred briefly, diluted with $H_2O$ (500 mL), and subjected to an extractive workup ($CHCl_3$) to yield crude **8g** as a brown oil (10.1 g). Chromatography over Florisil ($CHCl_3$) yielded pure **8g** as a light yellow oil (5.38 g, 54%): $^1H$ NMR ($CDCl_3$) $\delta$ 7.65 (d, 2 H, $J$ = 8.4 Hz, $H_2$), 7.43 (d, 2 H, $J$ = 8.6 Hz, $H_3$), 1.45 (s, 18 H, $OC(CH_3)_3$). Anal. Calcd for $C_{15}H_{22}NO_3PBrF_2$: C, 45.13; H, 5.55. Found: C, 45.55; H, 5.75.

**4-[(Di-*tert*-butylphosphono)difluoromethyl]benzaldehyde (9c).** To **8g** (2.90 g, 7.27 mmol) in anhydrous $Et_2O$ (36 mL) at −78 °C was added dropwise *n*-BuLi (1.6 M in hexane, 6.8 mL, 10.9 mmol). The brown solution was stirred initially at −78 °C (10 min) and then at −55 °C (10 min) and finally cooled to −78 °C, and $HCO_2Et$ (1.20 mL, 14.5 mmol) in $Et_2O$ (15 mL) was added slowly (5 min). The mixture was stirred (15 min), quenched by addition of saturated aqueous $NH_4Cl$ (50 mL), and brought to rt. An extractive workup ($Et_2O$) provided crude **9c** as a light yellow solid (2.27 g, 90%). Purification by silica gel chromatography [EtOAc–hexanes (1:4) containing 1% pyridine] afforded analytically pure **9c** as a colorless crystalline solid (884 mg, 35%): mp 93–95 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 10.08 (s, 1 H, CHO), 7.92 (d, 2 H, $J$ = 8.0 Hz, $H_2$), 7.75 (d, 2 H, $J$ = 8.0 Hz, $H_3$), 1.46 (s, 18 H, $OC(CH_3)_3$). Anal. Calcd for $C_{16}H_{23}NO_4PF_2 \cdot H_2O$: C, 53.78; H, 6.77. Found: C, 53.71; H, 6.56.

**Methyl α-Azido-4-[(di-*tert*-butylphosphono)difluoromethyl]cinnamate (10a).** To a stirred solution of **9c** (890 mg, 2.56 mmol) and ethyl α-azidoacetate[28] (3.30 g, 25.6 mmol) in MeOH (13 mL) was added NaOMe (5.4 M in MeOH, 3.8 mL, 20.5 mmol) dropwise at 0 °C and the reaction was stirred at 0 °C (1 h). The reaction was diluted with brine (50 mL) and subjected to an extractive workup ($Et_2O$) to yield **10a** as an oil, 779 mg (68%): $^1H$ NMR ($CDCl_3$) $\delta$ 7.84 (d, 2 H, $J$ = 8.0 Hz, $H_2$), 7.58 (d, 2 H, $J$ = 8.0 Hz, $H_3$), 6.90 (s, 1 H, vinylic H), 3.81 (s, 3 H, $OCH_3$), 1.46 (s, 18 H, $OC(CH_3)_3$).

**Methyl α-Azido-4-[(di-*tert*-butylphosphono)hydroxymethyl]cinnamate (10c).** To a solution of ethyl α-azidoacetate (6.45 g, 50 mmol) and **9b** (1.64 g, 5.0 mmol) in MeOH (20 mL) at −78 °C was added NaOMe (5.4 M in MeOH, 7.4 mL, 40 mmol) dropwise over 5 min. The mixture was stirred briefly at −78 °C (5 min) and then 0 °C (1 h). The resulting light yellow suspension was subjected to an extractive workup (brine/EtOAc) to yield a light yellow crystalline solid, which was triturated with $CHCl_3$–petroleum ether to yield **10c** as light yellow crystals (1.26 g, 57%): mp 111–113 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.79 (d, 2 H, $J$ = 8.2 Hz, $H_2$), 7.41 (dd, 2 H, $J$ = 8.2 and 2.2 Hz, $H_3$), 6.90 (s, 1 H, vinylic-H), 4.86 (d, 1 H, $J$ = 10.9 Hz, P-C-H), 1.43 (s, 9 H, $OC(CH_3)_3$), 1.37 (s, 9 H, $OC(CH_3)_3$).

**Methyl 4-[(Di-*tert*-butylphosphono)hydroxymethyl]-D,L-phenylalaninate (11c).** A solution of **10c** (1.25 g, 2.85 mmol) in MeOH (50 mL) was hydrogenated in a Parr apparatus over 10% Pd–C (200 mg) under 40 psi $H_2$. The hydrogen was replenished after 10 min. The reaction was terminated after 3 h, and catalyst was removed by filtration. Evaporation of solvent yielded **11c** as a clear, colorless syrup (1.17 g, 100%). Silica gel chromatography [$CHCl_3$:MeOH (25:1)] provided **11c** as colorless crystals (92%): mp 58–60 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.37 (dd, 2 H, $J$ = 8.0 and 2.1 Hz, $H_3$), 7.14 (d, 2 H, $J$ = 8.0 Hz, $H_2$), 4.79 (d, 1 H, $J$ = 10.3 Hz, P-C-H), 3.73 (dd, 1 H, $J$ = 7.7 and 5.3 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.07 (dd, 1 H, $J$ = 13.4 and 5.3 Hz, $H_\beta$), 2.87 (dd, 1 H, $J$ = 13.4 and 7.7 Hz, $H_\beta$), 1.41 (s, 9 H, $OC(CH_3)_3$), 1.37 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{19}H_{32}O_4PN$: C, 56.85; H, 8.04. Found: C, 56.96; H, 8.05.

**Methyl 4-[(Di-*tert*-butylphosphono)hydroxymethyl]-*N*-(benzyloxycarbonyl)-D,L-phenylalaninate (11d).** To a solution of **11c** (4.68 g, 11.7 mmol) in THF (115 mL) at 0 °C was added $NEt_3$ (6.50 mL, 46.7 mmol), followed by benzyl chloroformate (1.67 mL, 11.7 mmol) dropwise via syringe. The reaction was stirred at 0 °C (20 min), quenched by slow addition of brine (50 mL), and subjected to an extractive workup ($Et_2O$) to yield crude **11d** (5.90 g 94%). Silica gel chromatography [$CHCl_3$–MeOH (30:1)] provided **11d** as a colorless solid (5.30 g, 85%): mp 132–134 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.34 (m, 7 H, $H_3$ and Ph), 7.05 (d, 2 H, $J$ = 8.1 Hz, $H_2$), 5.14 (d, 1 H, $J$ = 7.8 Hz, NH), 5.07 (s, 2 H, $OCH_2$), 4.80 (d, 1 H, $J$ = 10.3 Hz, P-C-H), 4.62 (q, 1 H, $J$ = 7.8 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.10 (br s, 2 H, $H_\beta$), 1.39 (s, 9 H, $OC(CH_3)_3$), 1.35 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{27}H_{38}NO_8P$: C, 60.55; H, 7.15. Found: C, 60.36; H, 7.21.

**Methyl 4-[(Di-*tert*-butylphosphono)oxomethyl]-*N*-(benzyloxycarbonyl)-D,L-phenylalaninate (11e).** To a solution of **11d** (125 mg, 0.23 mmol) in $CHCl_3$ (1 mL) were added Celite (200 mg) and freshly activated 4A molecular sieves (230 mg). Pyridinium dichromate (219 mg, 0.58 mmol) was added and the mixture stirred at rt (4 h). The reaction was diluted with EtOAc (5 mL) and filtered through a pad of Florisil. The Florisil was rinsed with EtOAc (30 mL), and combined filtrates were taken to dryness to afford crude **11e** (85 mg, 70%). Silica gel chromatography [EtOAc–hexanes (1:1)] provided **11e** as a light yellow syrup (77 mg, 62%): $^1H$ NMR ($CDCl_3$) $\delta$ 8.20 (d, 2 H, $J$ = 7.8 Hz, $H_3$), 7.32 (br s, 5 H, Ph), 7.19 (d, 2 H, $J$ = 7.8 Hz, $H_2$), 5.23 (d, 1 H, $J$ = 7.8 Hz, NH), 5.08 (s, 2 H, $OCH_2$), 4.68 (q, 1 H, $J$ = 7.8 Hz, $H_\alpha$), 3.69 (s, 3 H, $OCH_3$), 3.16 (m, 2 H, $2H_\beta$), 1.52 (s, 18 H, $OC(CH_3)_3$). Anal. Calcd for $C_{27}H_{36}NO_8PN \cdot {}^1/_2H_2O$: C, 59.77; H, 6.87. Found: C, 59.67; H, 6.84.

**Methyl 4-[(Di-*tert*-butylphosphono)difluoromethyl]-*N*-(benzyloxycarbonyl)-D,L-phenylalaninate (11f).** To keto phosphonate **11e** (490 mg, 0.92 mmol) was added DAST (1.8 mL), and the mixture was stirred at rt overnight. The reaction mixture was cooled (0 °C), diluted with $CHCl_3$ (5 mL), and added dropwise to a cold, well stirred solution of saturated aqueous $NaHCO_3$ (20 mL). The mixture was subjected to an extractive workup ($CHCl_3$) yielding crude **11f** (665 mg). Immediate purification by silica gel chromatography [EtOAc–hexanes (1:2)] provided **11f** as a yellow syrup (274 mg, 54%): $^1H$ NMR ($CDCl_3$) $\delta$ 7.48 (d, 2 H, $J$ = 7.9 Hz, $H_3$), 7.32 (br s, 5 H, Ph), 7.13 (d, 2 H, $J$ = 7.9 Hz, $H_2$), 5.18 (d, 1 H, $J$ = 7.8 Hz, NH), 5.07 (s, 2 H, $OCH_2$), 4.65 (q, 1 H, $J$ = 7.8 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.14 (m, 2 H, $2H_\beta$), 1.42 (s, 18 H, $OC(CH_3)_3$).

**Methyl 4-[(Di-*tert*-butylphosphono)difluoromethyl]-D,L-phenylalaninate (11g).** Compound **11f** (610 mg, 1.10 mmol) in anhydrous MeOH (22 mL) was hydrogenated over 10% Pd–C (183 mg) under $H_2$ (48 psi) in a Parr apparatus. After 30 min the vessel was evacuated and replenished with $H_2$. After 4 h, the mixture was filtered through Celite over-layered with silica gel and solvent evaporated, yielding crude **11g** (420 mg). Purification by silica gel chromatography [$CHCl_3$–MeOH (30:1)] afforded **11g** as a colorless syrup (135 mg, 40%): $^1H$ NMR ($CDCl_3$) $\delta$ 7.51 (d, 2 H, $J$ = 6.9 Hz, $H_3$), 7.23 (d, 2 H, $J$ = 6.9 Hz, $H_2$), 3.73 (dd, 1 H, $J$ = 7.2 and 5.4 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.10 (dd, 1 H, $J$ = 13.5 and 5.4 Hz, $H_\beta$), 2.90 (dd, 1 H, $J$ = 13.5 and 7.2 Hz, $H_\beta$), 1.43 (s, 18 H, $OC(CH_3)_3$). Anal. Calcd for $C_{19}H_{30}NO_5PNF_2$: C, 54.15; H, 7.18. Found: C, 54.03; H, 7.19.

(28) Hemetsberger, H.; Knittel, D.; Weidmann, H. *Monatsch. Chem.* **1969**, *100*, 1599.

Table I

| no. | compound | HPLC retention [time (min)[a]] | yield, % | FABMS [(M - H)⁻] calcd | FABMS [(M - H)⁻] found | amino acid analysis[b] [(expected) found] |
|---|---|---|---|---|---|---|
| 12a | H-Gly-L-Pmp-Val-Pro-Met-Leu-OH | 14.4 | 40 | 755.3 | 755.2 | Pro (1) 1.04; Gly (1) 1.07; Val (1) 1.03; Met (1) 0.79; Leu (1) 107 |
| 12b | H-Gly-D-Pmp-Val-Pro-Met-Leu-OH | 15.2 | 41 | 755.3 | 755.3 | Pro (1) 1.04; Gly (1) 1.06; Val (1) 1.05; Met (1) 0.77; Leu (1) 1.08 |
| 12c | H-Gly-L-FPmp-Val-Pro-Met-Leu-OH | 14.1 | 30 | 773.3 | 773.1 | Pro (1) 1.08; Gly (1) 1.06; Val (1) 1.06; Met (1) 0.71; Leu (1) 1.09 |
| 12d | H-Gly-D-FPmp-Val-Pro-Met-Leu-OH | 15.3 | 29 | 773.3 | 773.3 | Pro (1) 1.05; Gly (1) 1.05; Val (1) 1.03; Met (1) 0.81; Leu (1) 1.05 |
| 12e | H-Gly-L-HOPmp-Val-Pro-Met-Leu-OH | 13.9 | 25 | 771.3 | 771.2 | Pro (1) 1.04; Gly (1) 0.95; Val (1) 1.03; Met (1) 0.93; Leu (1) 1.05 |
| 12f | H-Gly-D-HOPmp-Val-Pro-Met-Leu-OH | 14.7 | 23 | 771.3 | 771.3 | Pro (1) 1.04; Gly (1) 1.11; Val (1) 1.01; Met (1) 0.57; Leu (1) 1.04 |

[a] HPLC conditions: Vydac $C_{18}$ (4.6 × 150 mm) column. A, 0.05% TFA in $H_2O$; B, 0.05% TFA in 90% acetonitrile in $H_2O$; gradient (B%): 1–50% over 20 min, flow rate of 1.0 mL/min. UV detector, 220 nm. [b] (Phosphonomethyl)phenylalanine analogues not analyzed for.

**4-[(Di-*tert*-butylphosphono)difluoromethyl]-*N*-(fluoren-9-ylmethoxycarbonyl)-D,L-phenylalanine (5).** To a solution of amino ester **11g** (15 mg, 0.036 mmol) in dioxane (0.5 mL) was added 1 N NaOH (180 μL), and the reaction was stirred at rt (20 min) to generate the free amino acid, which was not isolated. Carbon dioxide was introduced until the pH was reduced to 8.0–8.5 (pH paper). Solid Fmoc-OBt (15 mg, 0.043 mmol) was added along with dioxane (0.5 mL), and the reaction was stirred (1 h), diluted with cold 5% citric acid (10 mL), and subjected to an extractive workup ($CHCl_3$), yielding crude **5** (23 mg). Purification by silica gel chromatography [$CHCl_3$–MeOH (5:1)] afforded **5** (14 mg, 60%): $^1$H NMR ($CDCl_3$) δ 7.74 (d, 2 H, $J$ = 7.4 Hz, $H_{1'}$ and $H_{8'}$), 7.55 (d, 2 H, $J$ = 7.3 Hz, $H_{4'}$ and $H_{5'}$), 7.47 (d, 2 H, $J$ = 7.8 Hz, $H_3$), 7.38 (t, 2 H, $J$ = 7.3 Hz, $H_{3'}$ and $H_{6'}$), 7.30 (m, 2 H, $H_{2'}$ and $H_{7'}$), 7.18 (d, 2 H, $J$ = 7.8 Hz, $H_2$), 5.36 (d, 1 H, $J$ = 7.5 Hz, NH), 4.67 (m, 1 H, $H_\alpha$), 4.40 (m, 2 H, $OCH_2$), 4.19 (t, 1 H, $J$ = 6.7 Hz, $H_{9'}$), 3.22 (d, 2 H, $J$ = 4.9 Hz, $H_\beta$), 1.44 (s, 9 H, $OC(CH_3)_3$), 1.39 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{33}H_{38}NO_7PNF_2 \cdot 2H_2O$: C, 59.54; H, 6.36. Found: C, 59.73; H, 6.03.

**Methyl 4-[(Di-*tert*-butylphosphono)fluoromethyl]-*N*-(benzyloxycarbonyl)-D,L-phenylalaninate (11h).** To DAST (0.60 mL, 4.5 mmol) in anhydrous $CHCl_3$ (2.2 mL) at −78 °C was slowly added **11d** (1.60 mg, 2.99 mmol) in $CHCl_3$ (10.0 mL). After 10 min, the reaction mixture was warmed to rt and stirred (20 min). The mixture was slowly diluted with brine (25 mL), subjected to an extractive workup ($CHCl_3$), and purified by silica gel chromatography [hexanes–EtOAc (1:1)] to afford **11h** as a syrup (1.10 g, 69%): $^1$H NMR ($CDCl_3$) δ 7.32 (m, 7 H, $H_3$ and Ph), 7.08 (d, 2 H, $J$ = 7.8 Hz, $H_2$), 5.43 (dd, 1 H, $J$ = 45.0 and 7.9 Hz, P-C-H), 5.15 (d, 1 H, $J$ = 7.8 Hz, NH), 5.07 (s, 2 H, $OCH_2$), 4.63 (q, 1 H, $J$ = 7.9 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.10 (br s, 2 H, $H_\beta$), 1.40 (s, 9 H, $OC(CH_3)_3$), 1.38 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{27}H_{37}NO_7PF$: C, 60.33; H, 6.94. Found: C, 60.42; H, 6.97.

**Methyl 4-[(Di-*tert*-butylphosphono)fluoromethyl]-D,L-phenylalaninate (11i).** Treatment of Cbz-protected compound **11h** (1.09 g, 2.03 mmol) as previously described for the preparation of **11g** provided crude **11i** (813 mg). Purification by silica gel chromatography [$CHCl_3$–MeOH (30:1)] afforded **11i** as a colorless syrup (694 mg, 85%): $^1$H NMR ($CDCl_3$) δ 7.38 (d, 2 H, $J$ = 7.8 Hz, $H_3$), 7.18 (d, 2 H, $J$ = 7.8 Hz, $H_2$), 5.44 (dd, 1 H, $J$ = 45.0 and 7.7 Hz, P-C-H), 3.72 (dd, 1 H, $J$ = 7.6 and 5.4 Hz, $H_\alpha$), 3.68 (s, 3 H, $OCH_3$), 3.07 (dd, 1 H, $J$ = 13.6 and 5.4 Hz, $H_\beta$), 2.87 (dd, 1 H, $J$ = 13.6 and 7.6 Hz, $H_\beta$), 1.42 (s, 9 H, $OC(CH_3)_3$), 1.40 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{19}H_{31}NO_5PNF$: C, 56.57; H, 7.75. Found: C, 56.97; H, 7.39.

**4-[(Di-*tert*-butylphosphono)fluoromethyl]-*N*-(fluoren-9-ylmethoxycarbonyl)-D,L-phenylalanine (4).** Treatment of **11i** (690 mg, 1.71 mmol) as previously described for the preparation of **5** yielded crude **4** (1.07 g). Purification by silica gel chromatography (gradient elution 0–5% MeOH in $CHCl_3$) gave pure **4** as a white foam, 407 mg (40%): mp 87–90 °C; $^1$H NMR (DMSO-$d_6$) δ 12.55 (br s, 1 H, $CO_2H$), 7.87 (d, 2 H, $J$ = 7.4 Hz, $H_{1'}$ and $H_{8'}$), 7.66 (dd, 2 H, $J$ = 7.5 and 2.0 Hz, $H_{4'}$ and $H_{5'}$), 7.40 (t, 2 H, $J$ = 7.5 Hz, $H_{3'}$ and $H_{6'}$), 7.31 (br s, 4 H, $H_{2'}$, $H_{7'}$), 7.16 (br s, 2 H, $H_2$), 5.64 (dd, 1 H, $J$ = 44.0 and 7.0 Hz, P-C-H), 4.21 (br s, 1 H, $H_\alpha$), 4.16 (m, 3 H, $H_9$ and $OCH_2$), 3.05 (m, 1 H, $H_\beta$), 2.87 (m, 1 H, $H_\beta$), 1.34 (s, 9 H, $OC(CH_3)_3$), 1.31 (s, 9 H, $OC(CH_3)_3$); FABMS $m/z$ 610 $(M - H)^-$, 544 $(M - H - C_4H_9)^-$, 498 $(M - H - C_8H_{18})^-$. Anal. Calcd for $C_{33}H_{39}NO_7PNF \cdot {}^3/_4H_2O$: C, 63.40; H, 6.53. Found: C, 63.38; H, 6.34.

**4-[(Di-*tert*-butylphosphono)hydroxymethyl]-*N*-(fluoren-9-ylmethoxycarbonyl)-D,L-phenylalanine (6).** Treatment of **11c** (410 mg, 1.02 mmol) as previously described for the preparation of **5** yielded crude **6** (775 mg). Purification by silica gel chromatography (gradient elution 0–20% MeOH in $CHCl_3$) afforded pure **6** as a colorless solid (277 mg, 44%): mp 70–75 °C; $^1$H NMR (DMSO-$d_6$) δ 12.70 (br s, 1 H, $CO_2H$), 7.87 (d, 2 H, $J$ = 7.4 Hz, $H_{1'}$ and $H_{8'}$), 7.67 (m, 2 H, $H_{4'}$ and $H_{5'}$), 7.40 (t, 2 H, $J$ = 7.3 Hz, $H_{3'}$ and $H_{6'}$), 7.31 (m, 4 H), 7.18 (d, 2 H, $J$ = 7.8 Hz, $H_2$), 5.79 (dd, 1 H, $J$ = 10.1 and 5.6 Hz, OH), 4.58 (dd, 1 H, $J$ = 9.1 and 5.5 Hz, P-C-H), 4.23 (br s, 1 H, $H_\alpha$), 4.16 (m, 3 H, $H_9$ and $NCO_2CH_2$), 3.04 (dd, 1 H, $J$ = 12.6 and 12.4 Hz, $H_\beta$), 2.83 (dd, 1 H, $J$ = 12.8 and 12.4 Hz, $H_\beta$), 1.32 (s, 9 H, $OC(CH_3)_3$), 1.27 (s, 9 H, $OC(CH_3)_3$). Anal. Calcd for $C_{33}H_{40}O_8PN$: C, 65.01; H, 6.61. Found: C, 65.10; H, 7.19. A sample was prepared for accurate mass determination by treatment with TFA (4 h), to provide 4-(phosphonohydroxymethyl)-*N*-(fluoren-9-ylmethoxycarbonyl)-D,L-phenylalanine: high resolution FABMS $m/z$ calcd for $C_{25}H_{23}O_3PN$ 496.1161, found 496.090.

**Synthesis of H-Gly-X-Val-Pro-Met-Leu-OH (12a–f) [X = D,L-Pmp, D,L-(fluoro)Pmp, D,L-(hydroxy)Pmp].** A mixture of 4-[(2′,4′-dimethoxyphenyl)hydroxymethyl]phenoxy resin[22] (1.0 g, 0.35 mmol/g), Fmoc-Leu-OH (2.47 g, 7.0 mmol), diisopropylcarbodiimide (DIPCDI) (1.1 mL, 7.0 mmol), and (dimethylamino)pyridine (DMAP) (85 mg, 0.70 mmol) in DMF (25 mL) was shaken (2 h). The resin was rinsed with DMF and $CH_2Cl_2$ (5 × 20 mL each) and dried. Fmoc deprotection was achieved using 20% piperidine in DMF (20 min) and the Fmoc-protected Met, Pro, and Val amino acids were then sequentially condensed in a manner similar to that above without DMAP using a 5-fold molar excess of amino acid per cycle. Attachment of the various Fmoc-*O*-*tert*-butyl protected Pmp derivatives (**3**, **4**, and **6**) was done using 235 mg of tetrapeptide resin (containing approximately 80 μmol of bound peptide) using 5 molar equiv of amino acid as above. Coupling of the terminal Gly was achieved using pentafluorophenyl Fmoc-glycinate (Fmoc-Gly-O-Pfp) in DMF (5 mL) over a period of 3 h. Terminal Fmoc protection was removed (piperidine/DMF) and the peptides were deprotected and cleaved from the resin simultaneously by treatment with TFA–thioanisole (1.8 mL:0.2 mL) in the presence of *m*-cresol (50 μL) and ethanedithiol (50 μL) at 4 °C. After 1 h the resin was removed by filtration and the crude peptides were precipitated from the filtrate by the addition of petroleum ether (50 mL). Solvent was removed by decantation and the residue was triturated with $Et_2O$, collected by centrifugation, and then lyophilized from 20% AcOH (10 mL). D- and L-Pmp-containing diastereomers were separated by HPLC as outlined in Table I. Assignment of absolute configurations was achieved similar to that previously reported[15] based on the relative rates of hydrolysis in the presence of aminopeptidase-M.[24]

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**Supplementary Material Available:** HPLC chromatograms of final peptide products **12a–f** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.