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TETRAHEDRON

Synthesis of Fmoc-Protected 4-Carboxydifluoromethyl-L-phenylalanine: A Phosphotyrosyl Mimetic of Potential Use for Signal Transduction Studies

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Abstract: 4-(Carboxymethyl)phenylalanine (**2**) and its α,α-difluoro homologue 4-(carboxydifluoromethyl)-phenylalanine (**3**) have been described as phosphotyrosyl mimetics. Herein we report the synthesis of N-Fmoc 4-(*O*-tert-butyl carboxymethyl)phenylalanine (**4**) and N-Fmoc 4-(*O*-tert-butyl carboxydifluoromethyl)-phenylalanine (**5**) in high enantiomeric purity. These analogues bear orthogonal protection suitable for the preparation of inhibitors directed against a variety of signal transduction pathways, including SH2 and PTP domains and protein-tyrosine phosphatases. Published by Elsevier Science Ltd.

Keywords: Amino acids and derivatives; Asymmetric synthesis; Molecular design; Peptide analogues/mimetics

INTRODUCTION

Phosphotyrosyl residues are important structural motifs that facilitate cellular signal transduction by providing recognition elements needed for protein-protein binding mediated by src homology 2 (SH2) and protein-tyrosine binding (PTB) domains. Control of pTyr-dependent signaling is achieved by the balanced creation and destruction of pTyr residues in specific proteins through the respective actions of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs). Aberrations in these pathways are associated with several diseases including cancers, making inhibitors of PTK/PTP/pTyr-binding networks of interest both as pharmacological tools and potential therapeutic agents. Because pTyr residues serve as a critical recognition determinants, the structure of phosphotyrosine provides a starting point from which to design signal transduction inhibitors.[1] Phosphotyrosine itself is unsuitable for inhibitor design, due to the hydrolytic lability of the phosphate ester toward phosphatases and the poor membrane penetration of the doubly ionized phosphate group. A number of phenylphosphate-mimicking structures have been reported which are stable in the presence of phosphatases, however at physiological pH many of these retain the doubly ionized character of the parent phosphate, and therefore suffer from poor bioavailability in cell-based systems. Efforts are therefore underway by several groups to prepare mono-anionic or uncharged structures, which afford greater bioavailability with minimal loss of biological affinity.[1-6]

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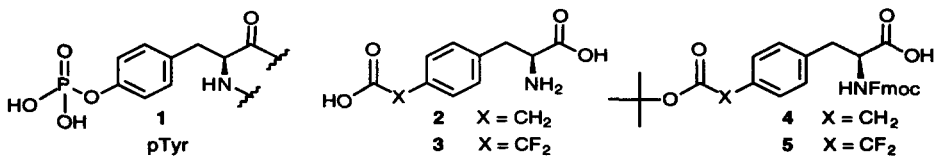
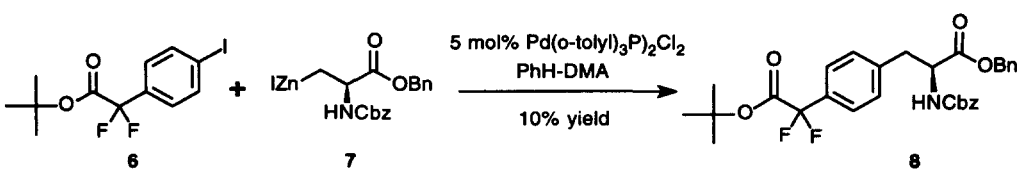


Figure 1. Structures of pTyr and pTyr mimetics.

The 4-carboxymethylphenylalanine residue (**2**), previously designed as a tyrosine sulfate/phosphate mimetic, has been prepared in racemic form bearing N-Boc[7] and N-acetyl[8] protection. The N-Boc-protected L-isomer has also been obtained by enantioselective synthesis[9] as well as by enzymatic resolution of the racemate.[10] Although **2** demonstrated poor affinity in its first use in an SH2 domain binding system where it was examined in a peptide directed against the src SH2 domain,[2] a more recent report has indicated significantly higher affinity when incorporated in a peptide directed against the homologous lck SH2 domain.[6] Factors contributing to reduced SH2 domain affinity could include differences in the pKa value of **2** relative to the pKa_1 value of the tyrosine phosphate, as well as losses in binding interaction of the benzylic methylene unit of **2** ("X" Figure 1) as compared to that afforded by the ester oxygen bridge found in the parent pTyr residue (**1**).[6] By analogy to phosphorus-containing pTyr mimetics where introduction of fluorine atoms at this methylene has resulted in enhanced biological potency, 4-carboxydifluoromethylphenylalanine (**3**) represents a conceptually interesting monocarboxylic pTyr mimetic which may exhibit enhanced binding affinity in pTyr and tyrosine sulfate-utilizing systems. Compound **3** has previously been prepared as a tyrosine sulfate mimetic in racemic form as its N-acetyl derivative.[8] Herein we report the enantioselective synthesis of **3** bearing orthogonal protection suitable for Fmoc-based synthesis (compound **5**). Utilizing this same approach, we also report the synthesis of its N-Fmoc-protected nonfluorinated homologue **4**.

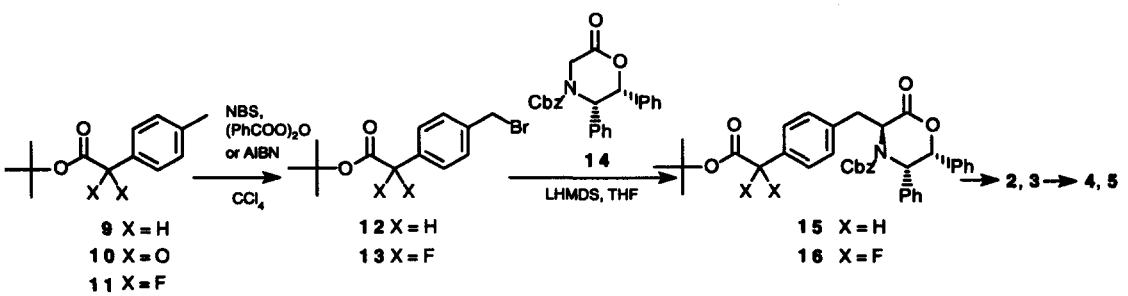
SYNTHESIS

Our initial approach toward the synthesis of **5** employed the method of Jackson,[11] which consisted of the Pd-catalyzed coupling of aryl iodide **6** with iodo-zinc alanine species **7** (Scheme 1). In our hands however, this route resulted in very low yields.



Scheme 1.

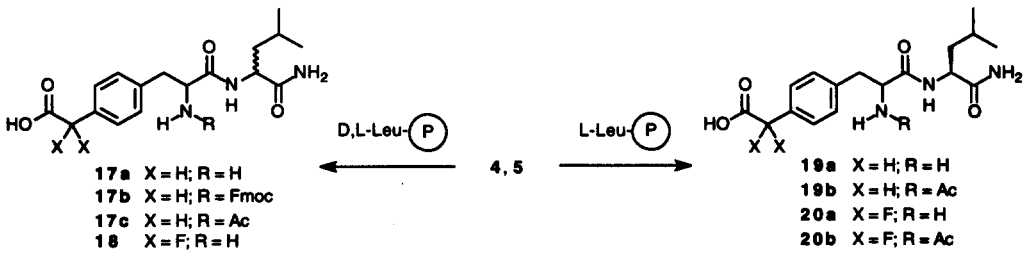
We therefore tried an alternate approach which was successfully used to prepare both **4** and **5** (Scheme 2).[12] This route relies on the method of Williams, and utilizes lactone **14**[13] as a chiral glycine equivalent.[14] Amino acid side chains are attached to **14** with high diastereoselectivity by nucleophilic alkylation. Generation of free amino acids is achieved by hydrogenolytic removal of the resulting bis-benzylic amino alcohol protecting group. The stability of side chain *tert*-butyl ester groups under these conditions makes it ideal for the preparation of analogues bearing *tert*-butyl functionality[3] such as **3** and **4**. In this approach, benzylic bromides **12** and **13** represent key intermediates, which upon reaction with the lithium enolate of the Williams chiral glycine equivalent **14**, provide protected amino acids **15** and **16**, respectively. Synthesis of the nonfluorine-containing benzylic bromide **12** has been described.[8] The homologous fluorinated benzylic bromide **13** has also been previously prepared from α,α–difluoro-4-methylphenylacetic acid *tert*-butyl ester **11**, which itself was obtained in four steps from 4-methyl-α-oxophenylacetic acid ethyl ester.[8] This latter synthesis involved initial DAST-mediated fluorination of the α-oxo group, followed by hydrolysis of the ethyl ester and re-esterificaiton with *tert*-butyl alcohol. We employed an alternate and slightly more direct approach by treating 4-methyl-α-oxophenylacetic acid *tert*-butyl ester[15] **10** directly with DAST to provide **11** in one step. Free amino acids **2** and **3**, subsequently obtained in high enantiomeric purity (see below) upon hydrogenolysis of iminolactones **15** and **16**, were converted to their final N-Fmoc-protected forms **4** and **5** using N-Fmoc-succinimide (Fmoc-OSu).



Scheme 2.

DETERMINATION OF ENANTIOMERIC PURITY

In order to determine the enantiomeric purity of final products **4** and **5**, leucine amide-dipeptides were prepared by solid-phase techniques, with HPLC separation of resulting diastereomers then performed. Model studies were first conducted using racemic D,L-leucine-containing dipeptides bearing three different N-terminal protection schemes: N-Fmoc, N-acetyl and the free amine (Scheme 3). While neither the N-Fmoc nor N-acetyl-protected dipeptides (**17b** and **17c**, respectively) afforded adequate HPLC resolution, the dipeptide having a free amino group showed good separation of diastereomers (diastereomeric retention time differences of 4 minutes and 5 minutes for dipeptides derived from **4** (dipeptide **17a**) and **5** (dipeptide **18**) respectively). Next, dipeptides **19a** and **20a** were prepared using enantiomerically pure L-leucine. Here any observed diastereomeric contamination would arise solely from the pTyr mimetics **4** and **5**. Less than 2% diastereomeric contamination was observed in either of these dipeptides, indicating high enantiomeric purity for both **4** and **5**.

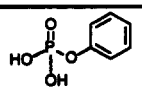
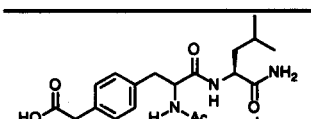
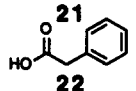
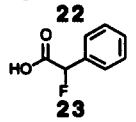
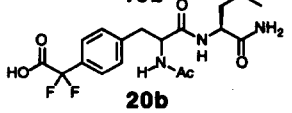


Scheme 3.

pTYR-MIMICKING CHARACTERISTICS

In biological contexts, recognition of the pTyr phosphate group is highly dependent on both the ionization state and the three dimensional arrangement of its oxygen functionality. The ability of analogues to approximate these characteristics is related to their potential utility as pTyr mimetics.[1] Phenylphosphate (**21**) has two acidic protons which exhibit distinct pKa values of 2.33 (pKa_1) and 5.93 (pKa_2).[3] In our studies, phenylacetic acid (**22**) was found to have a pKa value of 4.08 (Table 1). When incorporated into the more structurally complex 4-(carboxymethyl)phenylalanyl-containing dipeptide **19b**, an even lower pKa value of 3.32 was obtained. Introduction of a single fluorine onto phenylacetic acid (compound **23**) induced a 2-log decrease down to a pKa value of 2.0. The ability of a single fluorine to dramatically lower pKa values has been shown for both benzylic phosphonates[16] and aryloxymalonates.[17] In the present study it was not possible to accurately measure the pKa value of the 4-(carboxydifluoromethyl)phenylalanyl-containing dipeptide **20b**, however extrapolation of data in Table 1 indicate that it most probably would fall at or below a value of 2. This is consistent with recently reported pKa data for α,α-difluoro-α-phenoxyacetic acid.[3] Therefore, while the nonfluorinated **19b** exhibits a pKa value intermediate between the pKa_1 and pKa_2 values of phenylphosphate, the difluoro-analogue **20b** has a pKa value equal or lower than the pKa_1 value of the phosphate. Although in aqueous solution at neutral pH the distinction in pKa values between **19b** and **20b** may not be relevant, in the micro protein environment of an enclosed protein binding cavity, pKa differences could potentially be important.

Table 1. [a]Ionization constants of phenylphosphate and select monocarboxy phenylphosphate mimetics.

Structure	pKa	Structure	pKa
 **21**	2.33[b]	 **19b**	3.32
 **22**	4.08		
 **23**	2.03	 **20b**	ND

[a]Values were determined potentiometrically as described in the Experimental section.

[b]Previously reported: A. Albert and E. P. Serjeant, *Ionization Constants of Acids and Bases*, John Wiley & Sons, Inc., New York, 1962.

In addition to pKa values, an additional important consideration in the design of pTyr mimetics is the spatial arrangement of the carboxylic functionality relative to that displayed by the parent phenylphosphate oxygens. For carboxy-based pTyr mimetics, both malonyl[17,18] and carboxymethyl[3,5] groups have been appended to the tyrosyl oxygen as phosphate mimetics. As shown in Figure 2A, relative to the parent phenylphosphate (**I**), placement of an oxygen between the carboxymethyl group and the phenyl ring (ie, **II**) extends the carboxyl oxygens too far out from the phenyl ring. This has been confirmed by the recent X-ray structure of a fluoromalonyl-containing pTyr mimetic ligated within the catalytic site of the protein-tyrosine phosphatase 1B, where the tyrosyl phenyl ring is pushed out of its normal alignment.[19] Furthermore, an *O*-carboxymethyltyrosyl analogue exhibited extremely poor affinity against PTP1B when utilized as a pTyr mimetic in a high affinity substrate peptide.[5] Alternatively as shown in Figure 2B, attachment of the carboxymethyl group directly onto the phenyl ring (ie, **III**) as found in analogues **2** and **3**, allows for good overlap of carboxy functionality with the phosphate oxygens while maintaining registry of phenyl rings. This has recently been supported by the X-ray structure of a carboxymethylphenylalanine-containing peptide ligated to the lck SH2 domain, where good correspondence was observed between the carboxylate oxygens and normal binding of two pTyr phosphate oxygens.[6]

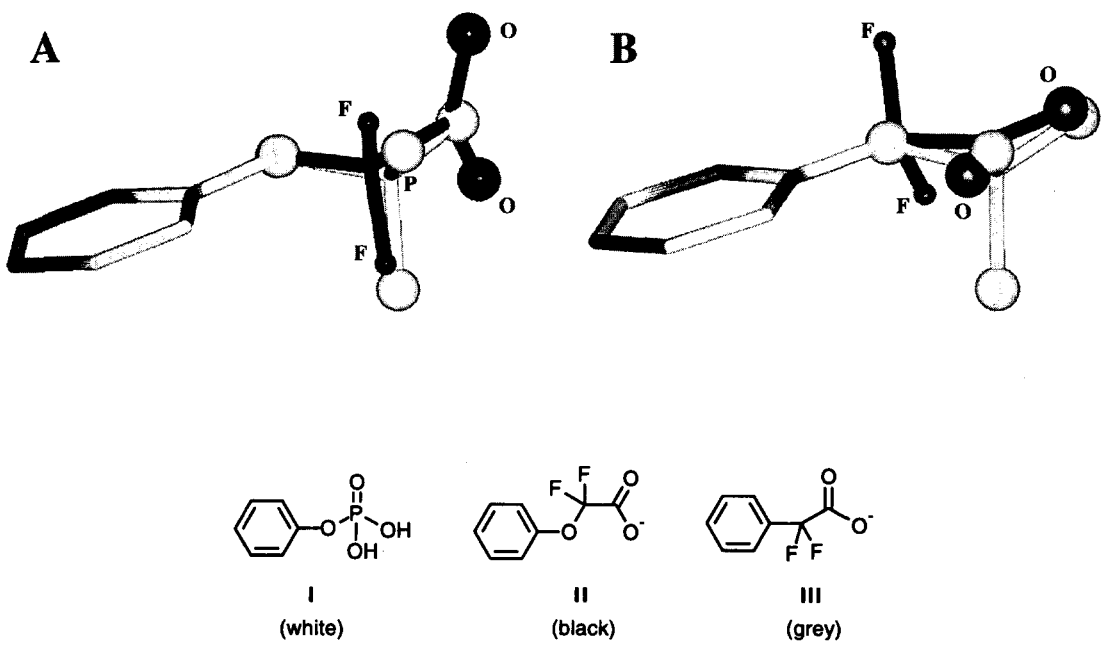
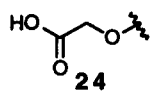
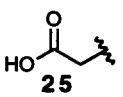
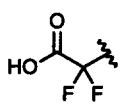


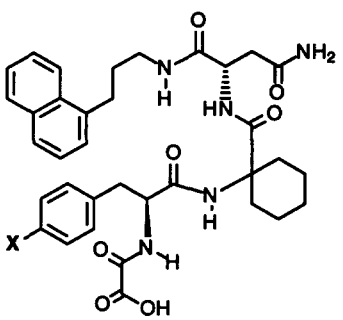
Figure 2. RHF/3-21G* optimized geometries depicted with phenyl ring superimposed: (A) superposition of **I** and **II**; (B) superposition of **I** and **III**.

BIOLOGICAL EVALUATION

In a recent study, the carboxymethylphenylalanine analogue **2** was incorporated into a high affinity p56^{lck} SH2 domain-directed peptide, where it exhibited a K_d value of 42 μM relative to the parent pTyr-containing peptide (K_d = 0.09 μM).[6] In that study it was suggested that reduced potency could potentially be attributed to loss of hydrogen bonding interactions between the SH2 domain and the carboxymethyl methylene. Such hydrogen bonding would normally be afforded by the homologously situated phosphate ester oxygen of a pTyr residue. It was further postulated by analogy to fluorinated phosphonate-based pTyr mimetics, that introduction of fluorine atoms at this benzylic methylene could potentially enhance potency. In order to examine this hypothesis, we have incorporated residues **2** and **3** into a previously disclosed ß-bend mimicking platform which exhibits high Grb2 SH2 domain affinity (analogues **25** and **26**, respectively).[20] As shown by Grb2 SH2 domain binding studies (Table 2), compound **24** which includes an added ether oxygen relative to **25** and **26**, exhibits significantly reduced affinity. This is consistent with modelling studies indicating that an ether oxygen displaces the carboxylate group from the phenyl ring such that good registry with a phenyl phosphate moiety is no longer possible (Figure 2). Alternatively, both **25** and **26** displayed high binding affinity. However contrary to expectation, introduction of fluorines (compound **26**) reduced affinity. The basis for this effect is not evident from molecular modelling studies.

Table 2. Grb2 SH2 domain binding constants

X	IC_{50} (μM)[a]
 **24**	15
 **25**	0.6
 **26**	2



[a]Values have been previously reported.[20]

CONCLUSIONS

Herein we have described the chiral synthesis of N-Fmoc-L-4-(*O-tert*-butyl carboxymethyl)phenylalanine (**4**) and its α,α-difluoro homologue **5**. These compounds should find application as pTyr mimetics suitably protected for the preparation of agents intended for a variety of signal transduction pathways, including SH2 domain, PTB and PTP systems.

EXPERIMENTAL

Synthetic. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on Bruker AC250 (250 MHz) instrument. Removal of solvents was performed by rotary evaporation under reduced pressure. High pressure liquid chromatography (HPLC) was performed using a Waters PrepLC 4000 system with photodiode array detection and an Advantage C_{18} 5 μ column (10 mm dia. X 250 mm) at a flow rate of 2.0 mL/min., using a solvent system of A = 0.1% aqueous TFA and B = 0.1% TFA in acetonitrile.

α-(4-Methylphenyl)acetic acid *tert*-butyl ester (9). Compound **9** was prepared from commercially available α-(4-methylphenyl)acetic acid as previously described.[8]

α-(4-Methylphenyl)-α-oxoacetic acid *tert*-butyl ester (10). Compound **10** was prepared as previously described.[15]

α,α-Difluoro-(4-methylphenyl)acetic acid *tert*-butyl ester (11). A solution of keto-ester **10** (5.0 g, 22.7 mmol) in DAST (diethylaminosulfur trifluoride) (9.0 mL, 68.2 mmol) was stirred at room temperature (overnight). The solution was carefully quenched by addition to mixture of ice/water (200 mL), then extracted with hexanes (2 x 80 mL). The combined organic layers were washed with H_2O (2 x 50 mL), aqueous $NaHCO_3$ (50 mL), brine (50 mL) and dried (Na_2SO_4). Concentration and purification by silica gel chromatography (hexanes-EtOAc, from 100:1 to 50:1) gave **11** as a light yellow solid[21] (3.36 g, 61%) along with recovered starting **7** (1.40 g, 28%). ^{1}H NMR ($CDCl_3$) δ 7.87 (2H, d, J = 8.3 Hz), 7.31 (2H, d, J = 8.5 Hz), 2.45 (3H, s), 1.64 (9H, s) ppm. [Note: spectral data was not included in the previous reported synthesis of **11**.[8]]

α-(4-Bromomethylphenyl)acetic acid *tert*-butyl ester (12). Compound **12** was prepared from **9** as previously described.[8]

α-(4-Bromomethylphenyl)-α,α-difluoroacetic acid *tert*-butyl ester (13). Compound **13** was prepared from **11** as previously described.[8]

Benzyl (2R,3S)-3,-[4-(*tert*-butoxycarbonylmethyl)phenyl]-2-oxo-5,6-diphenyl-morpholine-4-carboxylate (15). To a solution of benzyl (2R, 3S)-(-)-6-oxo-2,3-diphenyl-4-morpholine-carboxylate[13] **14** (2.81 g, 7.24 mmol) in anhydrous THF (40 mL) and HMPA (4.5 mL) was added 1 M LiHMDS in THF (7.5 mL, 7.5 mmol) at -78°C under argon then the mixture was stirred (30 minutes). A solution of **12** (2.07 g, 7.24 mmol) in THF (10 mL) was added slowly at -78 °C via a syringe and the mixture was stirred first at -78 °C (2 hr) then at room temperature (1 hr). The mixture was quenched (aqueous NH_4Cl), extracted with EtOAc and the combined organic phases were washed successively with H_2O, aqueous NH_4Cl, brine and dried (Na_2SO_4). Concentration and purification by silica gel chromatography (hexane-EtOAc, from 6:1 to 3:1) gave **12** as a white solid (1.80 g, 42%). Mp 86 - 88 °C; $[a]_D^{23}$ +69.9 (c 0.16, $CHCl_3$). ^{1}H NMR ($CDCl_3$) (two conformers were observed in a ratio of 5 : 12 at 23 °C) δ: 7.41 (1H, m, overlapping), 7.03 ~ 7.30 (14H, m, overlapping), 6.92 (1H, m, overlapping),

6.60 (2H, m, overlapping), 6.48 (1H, m, overlapping); *major conformer:* 5.33 (1H, m, -OOCCH-N), 5.07 (1H, d, J = 8.6 Hz, OCH_2Ph), 4.99 (1H, d, J = 8.6 Hz, OCH_2Ph), 4.82 (1H, d, J = 3.2 Hz, -PhCHOOC-), 4.26 (1H, d, J = 3.0 Hz, -PhCHN-), 3.47 (1H, dd, J = 3.0, 13.5 Hz, -CH_2CHNCOO-), 3.36 (1H, dd, J = 2.7, 13.5 Hz, -CH_2CHNCOO-), 3.54 (2H, s, *t*BuOOCCH2-); *minor conformer:* 5.24 (1H, m, -OOCCH-N), 5.28 (1H, d, OCH_2Ph), 5.12 (1H, d, J = 8.5 Hz, OCH_2Ph), 4.97 (1H, d, J = 3.5 Hz, -CHOOC-), 4.43 (1H, d, J = 3.2 Hz, -PhCHN-), 3.73 (2H, dd, J = 6.4, 13.7 Hz, -CH_2CHNCOO-), 3.51 (2H, s, *t*BuOOCCH2-); 1.44 (9H, s, overlapping) ppm. FABMS ($^+$Ve), *m/z* 536 [MH^+ - C_4H_8], 492 [MH^+ - C_4H_8-CO_2]. Anal. calcd. for $C_{37}H_{37}NO_6$: C, 75.11; H, 6.30; N, 2.37. Found: C, 74.91; H, 6.39; N, 2.40.

Benzyl (2R,3S)-3,-[4-(*tert*-butoxycarbonyl-difluoro-methyl)phenyl]-2-oxo-5,6-diphenyl-morpholine-4-carboxylate (16). Reaction of benzyl bromide **13** with the Williams reagent **14** as described above for the synthesis of **15**, provided final product **16** as a white solid in 56% yield. Mp 162 - 163 °C; $[a]_D^{23}$ $^+$50.1 (c 0.09, $CHCl_3$). ^{1}H NMR ($CDCl_3$) (two conformers were observed in a ratio of 4.3 : 10 at 23 °C) δ: 7.51~7.62 (2H, m, overlapping), 7.27~7.42 (3H, m, overlapping), 7.05 ~ 7.23 (9H, m, overlapping), 6.48 ~ 6.83 (5H, m, overlapping); *major conformer*: 5.34 (m, 1H, -NCHCOO-), 5.08 (1H, d, J = 12.5 Hz, -OCH_2Ph), 4.99 (1H, d, J = 12.4 Hz, OCH_2Ph), 4.86 (1H, d, J = 3.2 Hz, -CHPhOOC-), 4.38 (1H, d, J = 2.9 Hz, -NCHPh-), 3.47 (2H, m, -CH_2CH(N)COO-), 1.44 (9H, s) ppm; *minor conformer*: 5.25 (m, 1H, -NCHCOO-), 5.30 (1H, d, J = 12.0 Hz, -OCH_2Ph), 5.11 (1H, d, J = 12.0 Hz, OCH_2Ph), 5.03 (1H, d, -CHPhOOC-), 4.63 (1H, d, J = 2.9 Hz, -NCHPh-), 3.73 (2H, dd, J = 6.8, 13.4 Hz, -CH_2CH(N)COO-), 1.45 (9H, s) ppm. FABMS ($^+$Ve) *m/z* 572 [MH^+ - C_4H_8], 528 [MH^+ - C_4H_8-CO_2]. Anal. calcd. for $C_{37}H_{35}F_2NO_6$: C, 70.80; H, 5.62; N, 2.23. Found: C, 70.80; H, 5.61; N, 2.20.

4-(*tert*-Butoxycarbonylmethyl)-L-phenylalanine (2) Compound **15** (1.71 g, 2.89 mmol) was dissolved in THF (10 mL) with MeOH (20 mL) and hydrogenated (balloon) over Pd black (100 mg) at room temperature (20 hr). The mixture was filtered and solid washed with MeOH, then filtrates were taken to dryness to provide a white solid. The solid was washed thoroughly with hexanes to remove 1,2-diphenylethane (side product) and dried in vacuo to provide **2** as a white powder (767 mg, 95%). ^{1}H NMR (DMSO) δ 7.32 (2H, d, J = 8.1 Hz), 7.15 (2H, d, J = 8.3 Hz), 3.50 (2H, s), 3.36 (3H, m), 3.11 (1H, dd, J = 4.1, 13.9 Hz), 2.81 (1H, dd, J = 8.0, 14.2 Hz), 1.40 (9H, s) ppm. FABMS ($^-$Ve), *m/z*: 278 [$(M-H)^-$, 100%]. Anal. calcd. for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.02. Found: C, 64.19; H, 7.60; N,4.73.

4-(tert-Butoxycarbonyl-difluoro-methyl)-L-phenylalanine (3). Treatment of **16** as described above for the conversion of **15** to **2** provided product **3** as a white powder in 99% yield. ^{1}H NMR (DMSO) δ 7.47 (2H, d, J = 8.5 Hz), 7.43 (2H, d, J = 9.0 Hz), 3.32~3.47 (3H, m), 3.17 (1H, dd, J = 4.6 14.4 Hz), 2.93 (1H, dd, J = 7.8, 14.5 Hz), 1.45 (9H, s) ppm. FAB-MS (Ve^-), *m/z* 314 [$(M-H)^-$, 77%]. Anal. calcd. for $C_{15}H_{19}F_2NO_4$: C, 57.14; H, 6.07; N,4.44. Found: C, 56.94; H, 6.30; N, 4.17.

N-Fmoc-4-(*tert*-Butoxycarbonylmethyl)-L-phenylalanine (4). A mixture of phenylalanine analogue **2** (726 mg, 2.60 mmol), Fmoc-OSu (876 mg, 2.60 mmol) and $NaHCO_3$ (840 mg, 10 mmol) in 40 mL of dioxane - water (1 : 1) was stirred at room temperature (overnight), then reaction cooled to 0 °C, acidified to pH = 3 by addition of 2 N HCl and extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4) and concentrated. Purification by silica gel chromatography ($CHCl_3$-MeOH, from 20:1 to 10:1) provided **4** as a white foam (1.21 g, 93%). Mp 55 - 57 °C. $[\alpha]_D^{23}$ +31.9 (c 0.19, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 7.76 (2H, d, *J* = 8.3 Hz), 7.53 (2H, m), 7.39 (2H, m), 7.29 (2H, m), 7.17 (2H, m), 7.12 (2H, m), 5.31 (1H, m), 4.67 (1H, m), 4.13 ~ 4.51 (3H, m), 3.50 (2H, s), 3.15 (2H, m), 1.43 (9H, s) ppm. ^1H NMR (DMSO) δ 7.88 (2H, d, *J* = 7.3 Hz), 7.64 (3H, m), 7.41 (2H, m), 7.30 (2H, m), 7.16 (4H, m), 4.19 (4H, m), 3.49 (2H, s), 3.26 (1H, dd, *J* = 4.4, 13.9 Hz), 2.84 (1H, dd, *J* = 10.0, 13.9 Hz), 1.37 (9H, s) ppm. FAB-MS (Ve^-), *m/z* 500 [$(M-H)^-$, 15.8%]. ^{13}C-NMR ($CDCl_3$) δ 174.9, 170.9, 155.6, 143.6, 143.5, 141.2 (2x), 133.9, 133.4, 129.4 (2x), 129.3 (2x), 127.6 (2x), 126.9 (2x), 124.9 (2x), 119.8 (2x), 80.9, 67.0, 66.9, 47.1 (2x), 42.1, 28.0 (3x) ppm. Anal. calcd. for $C_{30}H_{31}NO_6 \cdot 1.5\ H_2O$: C, 68.17; H, 6.48; N, 2.65. Found: C, 68.54; H, 6.28; N, 2.45.

N-Fmoc-4-(*tert*-Butoxycarbonyl-difluoro-methyl)-L-phenylalanine (5). Treatment of phenylalanine analogue **3** as described above for the conversion of **2** to **4**, provided **5** as a white foam in 84% yield. Mp 53 - 55 °C. $[\alpha]_D^{23}$ +22.6 (c 0.23, $CHCl_3$). ^1H NMR (DMSO) δ 12.82 (1H, brs), 7.87 (2H, d, *J* = 7.3 Hz), 7.64 (3H, m), 7.44 (6H, m), 7.28 (2H, m), 4.18 (4H, m), 3.15 (1H, dd, *J* =4 .2, 14.2 Hz), 2.93 (1H, dd, *J* = 11.0, 14.0 Hz), 1.39 (9H, s) ppm. FAB-MS (Ve^-), *m/z* 536 [$(M-H)^-$, 22.2%]. ^{13}C-NMR ($CDCl_3$) δ 163.1 (triplet, *J* = 34.1 Hz), 155.9 (2x), 143.8, 143.7, 141.5 (2x), 138.9, 132.3, 131.9, 129.7 (2x), 128.0 (2x), 127.3 (2x), 125.9 (triplet, *J* = 6.0 Hz), 125.1, 125.0, 120.2 (2x), 113.3 (triplet, *J* = 252.2 Hz), 85.0, 67.3 (2x), 47.4 (2x), 27.9 (3x) ppm. Cacld for $C_{30}H_{29}F_2NO_6 \cdot H_2O$ (%): C, 64.86, H, 5.62, N, 2.52; Found: C, 65.01, H, 5.66, N, 2.37.

Determination of Enantiomeric Purity. Dipeptides **17 - 20** were prepared from pTyr mimetics **4** and **5** using Rink amide resin[22] (purchased from Bachem Corp., Torrance, CA) with Fmoc-protocols. Dipeptide **17b** was cleaved from the resin directly without removal of its N-terminal Fmoc protection, while dipeptides **17a**, **18**, **19a** and **20a** were treated with 20% piperidine to remove N-terminal Fmoc groups. N-Acetyl-containing dipeptides **17c**, **18**, **19b** and **20b** were acetylated with N-acetylimidazole prior to cleavage from the resin. HPLC retention times of diastereomeric peaks was determined using dipeptides prepared from racemic D,L-leucine: For dipeptide **17a** diastereomers eluted at 24.1 min. and 28.3 min; for dipeptide **18** diastereomers eluted at 22.5 min. and 27.5 min. [linear gradient from 5% B to 30% B over 25 min.]. Enantiomeric contaminations in **4** and **5** were determined by similar analysis using dipeptides **17a** and **18**, respectively. In both cases, peaks resulting from diastereomeric contamination accounted for areas less than 2% of that observed for the major diastereomer, indicating greater than 98% of a single enantiomer in both **4** and **5**.

pKa Determinations. Samples (approximately 0.01 mmol) of commercially available phenylacetic acid (**22**) and α-fluorophenylacetic acid (**23**) and synthetic dipeptides **19b** and **20b** were accurately weighed and dissolved in hot de-ionized H_2O (20.0 mL) and cooled to room temperature. Each solution was then titrated using 10 μL aliquots of 0.1 N NaOH, the pH measured after each addition and a pH profile plotted against the volume of added NaOH. Titration equivalences were obtained at the maxima of the 1st derivative of each titration curve and the log of the ionization constant (pKa) was determined by treating the potentiometric data according to the published procedures.[23]

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