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Synthesis of L-2,3,5,6-Tetrafluoro-4-(Phosphonomethyl) Phenylalanine, a Novel Non-Hydrolyzable Phosphotyrosine Mimetic and L-4-(Phosphonodifluoromethyl)Phenylalanine.

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Abstract : A new non-hydrolyzable phosphotyrosine analogue, L-F_4Pmp and its N-Fmoc protected derivative were prepared by using an enantioselective synthetic pathway with camphor sultam as chiral auxiliary. The side chain pKa_2 (6.9) of L-F_4Pmp was determined. A new synthesis of L-F_2Pmp was also described. © 1997 Published by Elsevier Science Ltd. All rights reserved.

Small peptides containing O-phosphotyrosine (pTyr) in the N-terminal position have been shown to have high affinity and specific recognition for Src-homology 2 (SH2) domains [1] of proteins with protein tyrosine-kinase (PTK) or protein tyrosine phosphatase (PTP) activity [2]. Likewise small peptides containing pTyr at the C-terminal are capable of binding to phosphotyrosine binding (PTB) domains of cytoplasmic proteins like Shc [3]. Since overexpression of PTK, PTP and Shc has been implicated in the etiology of human neoplastic deseases, these peptides appear to be potential therapeutic agents [4]. In order to obtain phosphatase resistant peptides, 4-(phosphonomethyl)phenylalanine (Pmp) was designed several years ago as an analogue of pTyr [5].

However, the Pmp-containing peptides are less potent than their parent pTyr-containing peptides in binding to SH2 domains [6] and this was partially attributed to their relatively higher side chain pKa_2 values and hence the incomplete ionization of Pmp phosphonate at physiological pH, weakening hydrogen bonding with the SH2 domain. Burke *et al.*, have thus developed a new pTyr mimetic, 4-(phosphonodifluoromethyl) phenylalanine (F_2Pmp), bearing difluoro substitutions on the α-methylene of the Pmp [7]. Based on acidity constant determinations of simpler analogs [8], these authors have suggested that the side chain pKa_2 of F_2Pmp might be closer to that of pTyr and additionally might promote hydrogen bond formation involving the difluoro substituted methylene. Peptides containing F_2Pmp have relative affinities of 0.2- to 5-fold, compared to pTyr peptides, for binding to different SH2 domains [9].

In an effort to improve the phosphonate ionization ability, and H-bonding capacity, a Pmp analogue has been synthesized with tetrafluoro substitutions on the phenyl ring. The effect of aromatic ring fluorine substitutions was less important than expected and less than the methylene fluorine substitution in F_2Pmp on the phosphonate ionization according to σ_I constants estimated by Charton [10]. However, potential additional H-bonding of the ring substituted fluorine atoms with the amino acid residues of the SH2 domain surrounding the pTyr residue would be expected from the molecular modeling of the structure of the SH2-phosphopeptide complex derived from X-ray data [11]. A new synthesis of L-F_2Pmp is also described in this paper. These two molecules, L-2,3,5,6-tetrafluoro-4-(phosphonomethyl)phenylalanine (F_4Pmp), and L-F_2Pmp were synthesized

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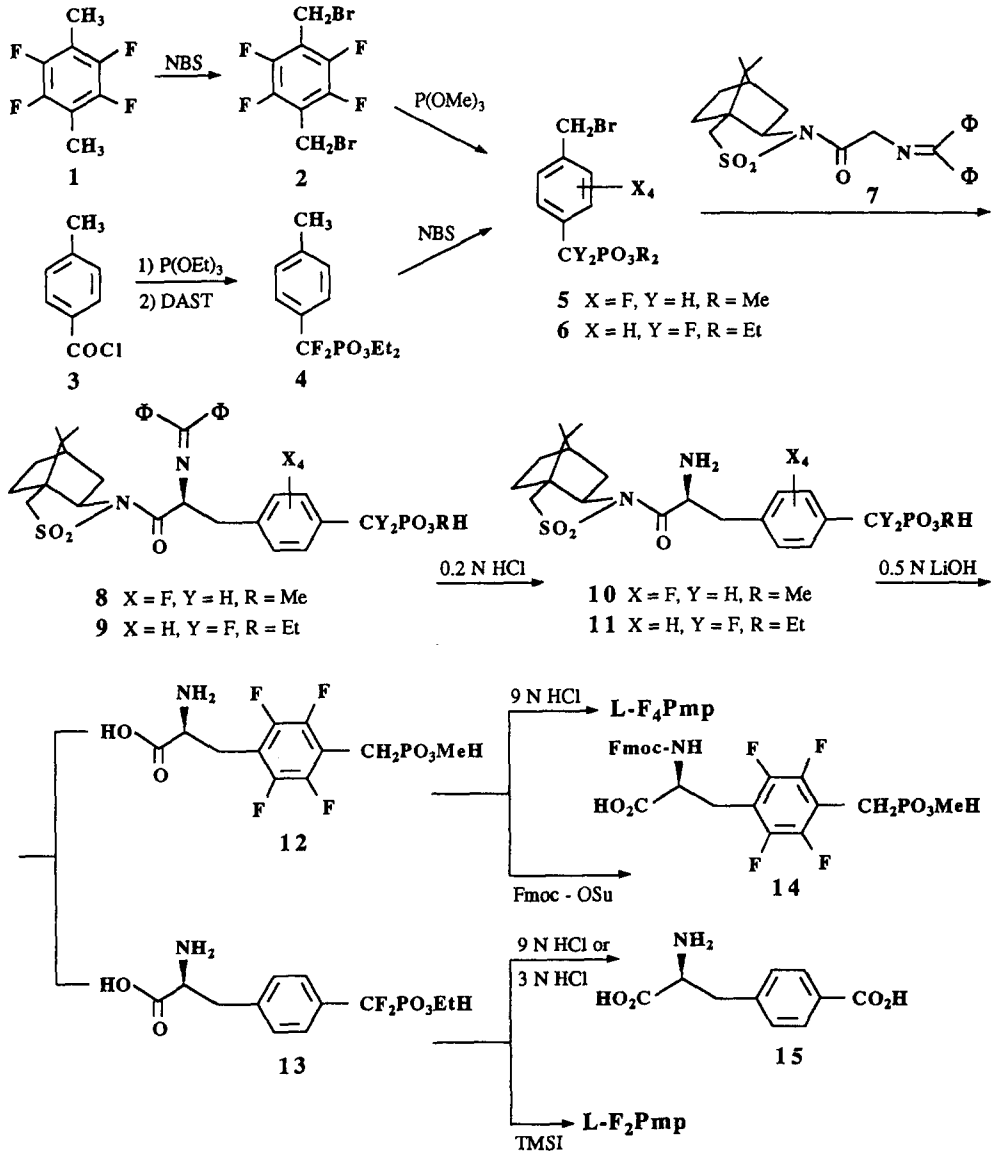
following an enantioselective pathway, using camphor sultam as a chiral auxiliary, similar to that previously described for preparing Fmoc-L-Pmp(OtBu)$_2$-OH [12].



Scheme 1

Briefly, compound **1** was dibrominated with NBS (2.5 eq.) to yield **2** (yield 69%). The Arbuzov reaction of **2** with $P(OMe)_3$ (5 eq.) gave **5** as a colorless oil (yield 40%) [13]. The chiral synthon **7** in THF was treated with n-BuLi (1.6 M in hexane, 1.2 eq.) under argon and alkylated with **5** in THF/HMPA (1/1) at -78°C, followed by warming up to ambient temperature overnight. After quenching the reaction with AcOH/THF (1/10) and evaporation of solvents, the residue was subjected to an extractive workup (aq. NH_4Cl/Et_2O). A

yellow oil, insoluble either in the aqueous phase or in the ether layer, was determined by ^1H NMR to be monoalkylphosphonate **8** [14]. Only trace dialkylphosphonate was found in the ether extract. This may be due to the electron-withdrawing fluorine groups and aprotic solvent HMPA, which facilitated the phosphonate hydrolysis.

The hydrolysis of **8** in 0.2 N aq. HCl/THF was carried at RT for 1 h. After evaporation of THF, the aqueous residue was washed with EtOAc and lyophilized to give crude **10**, which was hydrolyzed with 0.5 N aq. LiOH/Dioxane at RT for 3 h. The reaction mixture was neutralized, dioxane was evaporated and camphor sultam precipitate was recovered by filtration. The aqueous residue containing **12** was lyophilized and purified by flash chromatography with i-PrOH/NH_4OH (3/1) as eluent [15]. Hydrolysis of **12** in refluxing 9 N HCl (8h) yielded L-F_4Pmp [16]. To enable this amino acid to be used in solid-phase peptide synthesis, its N-Fmoc protected derivative **14** was prepared from an unpurified aqueous solution of **12** and Fmoc-OSu and purified by flash chromatography with CH_2Cl_2/MeOH/AcOH (7/1/0.5) as eluent [12,17]. The optical purity of L-F_4Pmp, which is also the enantioselectivity of the alkylation was checked from the condensation product of **14** with optically pure R-(+)-α-(1-naphthyl)ethylamine. The ratio of diastereoisomeric products was estimated as 94:6 from 400 MHz ^1H NMR spectrum, according to the doublet signals of NH and CH_3 groups of α-(1-naphthyl)ethylamine residue, and confirmed by HPLC.

L-F_2Pmp was synthesized following a similar synthetic pathway. *p*-Toluic acid chloride **3** was first subjected to the Arbuzov reaction with $P(OEt)_3$ (neat, 1 eq.) [18], unstable diethyl toluylphosphonate obtained was then directly fluorinated by addition of (dimethylamino)sulfur trifluoride (DAST, neat, 5 eq.) at -78°C, followed by stirring at 0°C (2 h) and 25°C (2 h) [7]. An extractive workup (aq. $NaHCO_3$/$CHCl_3$) and chromatographic purification produced **4** as a colorless oil [19]. Bromination of **4** with NBS yielded **6** [20,21]. Alkylating agent **6** was used in similar asymmetric synthesis to provide **13** [22]. TMSI/CH_3CN was used as phosphonate ester deprotection reagent [23], with thioanisole as a scavenger to prevent trans-esterification and TFA/H_2O/CH_3CN (1/1/2) as a desilylation mixture to hydrolyze **13** for obtaining L-F_2Pmp [24]. It should be noted that hydrolysis of **13** in either refluxing 9N HCl as we normally used or refluxing 3N HCl as reported [25] gave the predominantly degraded product **15**, which precipitated during the reaction [26]. The optical purity of L-F_2Pmp (92%) was estimated in a similar manner.

As L-F_2Pmp is now considered to be an excellent pTyr mimetic, several methods have been reported for its preparation. The most interesting pathway reported [27] involves a very unstable zinc-activated intermediate which should be coupled immediately to avoid degradation. A recently reported method [21] is also attractive but the expensive imino lactone chiral synthon is lost during the synthesis. In the synthesis described here, all the intermediates are stable enough for purification and storage. Except for DAST fluorination, the reaction conditions are mild for easy manipulation. In addition, the chiral auxiliary camphor sultam can be recovered for preparation of the chiral synthon **7**. This synthesis appears to be a more favorable method especially for large-scale preparation of Fmoc-L-F_2Pmp-OH.

Finally, the acidity constants of pTyr, Pmp, F_2Pmp and F_4Pmp were determined by potentiometric titration as described in the literature [6,28]. Free amino acids were dissolved in H_2O and 3 eq. of 0.1 N NaOH to form 0.01 M solutions. Aliquots of 0.1 N HCl (0.1 eq. each addition) were added under argon and pH values were recorded [29]. F_4Pmp showed a side chain pKa_2 (6.9) much higher than pTyr (5.9 by this method, 5.7 in lit. [28]) and F_2Pmp (5.6), but lower than Pmp (7.3 by method, 7.1 reported [6]). Incorporation of L-F_4Pmp in the sequence P-S-F_4Pmp-V-N-V-Q-N derived from Shc, gave a peptide with an even poorer affinity (2.3 μM) than Pmp (0.26 μM), for binding to Grb2, as measured from fluorescent analysis [30]. This result shows that the

fluorine atom is not as efficient as expected as a H-bonding acceptor, especially when located on the Csp^2 atoms in agreement with a recent report [31], or that fluorine is not isosteric with the hydrogen atom.

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13. Compound **5** : ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.39 (d, 2H, CH_2-P), 3.60 (d, 6H, OCH_3), 4.67 (s, 2H, CH_2-Br).
14. Compound **8** : ^{1}H NMR (270 MHz, DMSO-d_6) d 0.62 (s, 3H, 10'C-CH_3α), 0.80 (s, 3H, 10'C-CH_3ß), 1.2-1.8 (m, 7H, 6'CH_2, 7'CH, 8'CH_2, 9'CH_2), 2.6 (d, 2H, CH_2-P), 2.8 (m, 2H, CH_2ß), 3.20 (d, 3H, OCH_3), 3.6 (q, 2H, 2'CH_2), 3.8 (m, 1H, 5'CH), 4.95 (t, 1H, CHα), 7.4 (m, 10H, Ph).
15. Compound **12** : yield 58% from **5** ; ^{1}H NMR (270 MHz, DMSO-d_6 + TFA) δ 3.2 (m, 4H, CH_2-P, CH_2ß), 3.60 (d, 3H, OCH_3), 4.1 (m, 1H, CHα), 8.5 (s, 3H, H_3N^+-CH).
16. F_4Pmp was purified by preparative HPLC ; yield 61% from **12** ; Mp 271°C (dec) ; FAB MS (M + 1) 332. $[\alpha]_D19$ = + 12.5 (c = 0.2, 1N HCl) ; ^{1}H NMR (270 MHz, DMSO-d_6 + TFA) δ 3.12 (d, 2H, CH_2-P), 3.25 (m, 2H, CH_2ß), 4.1 (m, 1H, CHα), 8.45 (s, 3H, H_3N^+-CH).
17. Compound **14** : yield 44% from **5** ; ^{1}H NMR (270 MHz, DMSO-d_6 + TFA) δ 3.1 (m, 4H, CH_2-P, CH_2ß), 3.50 (d, 3H, OCH_3), 4.2 (m, 4H, CHα, 9'CH, CH_2-9'C), 7.25 (t, 2H, 2'CH, 7'CH), 7.35 (t, 2H, 3'CH, 6'CH), 7.6 (m, 2H, 4'CH, 5'CH), 7.85 (m, 3H, NH, 1'CH, 8'CH). Anal. Calcd. for $C_{26}H_{22}F_4NO_7P$: C, 55.03 ; H, 3.91 ; N, 2.47. Found : C, 55.02 ; H, 3.99 ; N, 2.42.
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19. Compound **4** : yield 36% ; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.18 (t, 6H, CH_3CH_2O), 2.31 (s, 3H, CH_3-Ph), 4.05 (m, 4H, CH_3CH_2O), 7.35 (q, 4H, H-Ar) ; Anal. Calcd. for $C_{12}H_{17}F_2O_3P$: C, 51.80 ; H, 6.16. Found : C, 51.89 ; H, 6.13.
20. Compound **6** : yield 73% ; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.2 (t, 6H, $\underline{CH_3}CH_2O$), 4.1 (m, 4H, $\underline{CH_3}CH_2O$), 5.20 (s, 2H, CH_2Br), 7.05 (m, 4H, H-Ar).
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22. Compound **13** : ^{1}H NMR (270 MHz, DMSO-d_6 + TFA) δ 1.10 (t, 3H, $\underline{CH_3}CH_2O$), 3.08 (d, 2H, CH_2ß), 3.95 (m, 2H, $CH_3\underline{CH_2}O$), 4.15 (m, 1H, CHα), 7.4 (q, 4H, H-Ar), 8.25 (bs, 3H, H_3N^+-CH).
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24. F_2Pmp : yield 62% ; ^{1}H NMR (270 MHz, DMSO-d_6 + D_2O + TFA) δ 3.12 (d, 2H, CH_2ß), 4.10 (t, 1H, CHα), 7.35 (q, 4H, H-Ar) ; FAB MS (M + 1), 296.
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26. Compound **15** : MS (M + 1), 210 ; ^{1}H NMR (270 MHz, DMSO-d_6 + D_2O) δ 3.10 (d, 2H, CH_2ß), 4.10 (t, 1H, CHα), 7.85 and 7.30 (dd, 4H, H-Ar).
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