Development of new methodology for the synthesis of functionalized α-fluorophosphonates and its practical application to the preparation of phosphopeptide mimetics

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New methodology for the synthesis of functionalized α-fluorophosphonates which utilizes organocopper-mediated reduction has been developed and applied to the preparation of a monofluoromethyl-substituted phosphoserine mimetic-containing peptide.

Naturally occurring phosphate-containing molecules play important roles in various cellular processes, including signal transduction.[1] Therefore, nonhydrolyzable phosphate mimetics have received considerable attention, with α,α-difluorophosphonates serving as potential phosphate mimics,[2] extensive synthetic and biological studies of which have been made.[3] In contrast, evaluation of α-monofluorophosphonates[4] as biological phosphate mimics has been somewhat limited due to lack of flexibility of practical synthetic methodology[5] for this kind of molecule. In our efforts to prepare difluoromethyl (CF$_2$)-substituted phosphothreonine mimetics,[6] we attempted conjugate addition of a methyl group to 3-(diethylphosphonodifluoromethyl)but-2-enoate **1**† using an organocopper reagent to construct the secondary phosphonate unit. Unexpectedly, the reaction predominantly afforded an organocopper-mediated reduction product, α-fluorovinylphosphonate **3**, and not the corresponding conjugate addition product **2** (Scheme 1). This is the first example of organocopper-mediated reduction of γ-difluoro-α,β-enoates yielding γ-fluoro-β,γ-enoates.
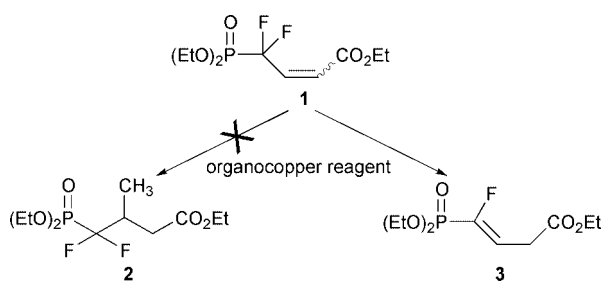
Scheme 1

The α-fluorovinylphosphonate[7] represents a potential synthetic intermediate for the preparation of α-fluorophosphonates. Accordingly, we describe herein the feasibility studies of our newly found reaction and its application to the synthesis of the monofluoromethyl (CHF)-substituted phosphoserine (pSer) mimetic 2-amino-4-fluoro-4-phosphonobutanoic acid (FPab) in a form suitably protected for the preparation of pSer mimetic-containing peptides.

Initially, we chose a difluoromethylphosphonate-bearing conjugate (2S)-bornane-[10,2]-sultam (Xs-sultam)-imide[8] **4** as a substrate for the copper-mediated reaction in order to allow subsequent stereoselective introduction of amino functionality under chiral auxiliary control. The sultam-imide **4** was treated under various conditions[9] and the results are shown in Table 1.‡

Reaction of **4** with either MeCu(CN)Li or Me$_2$Cu(CN)Li$_2$ in the presence of LiCl and/or AlCl$_3$ at −78 °C proceeded without accompanying alkylation, but rather provided the correspond-
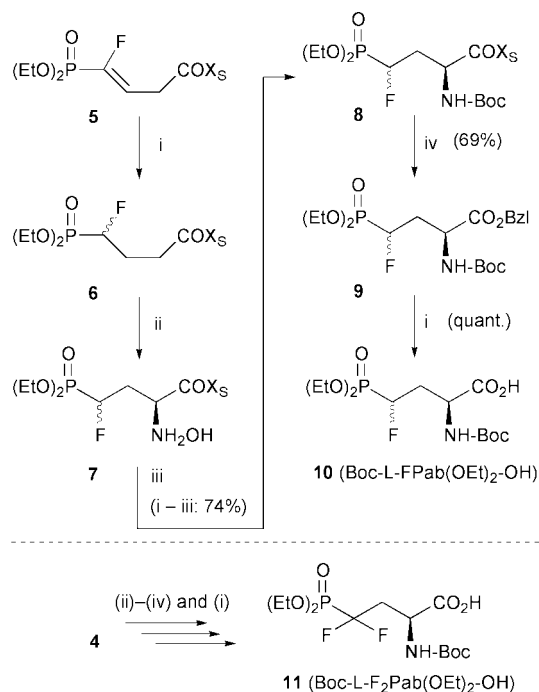
Table 1 Reduction of **4** with several organo copper reagents

Run	Reagent	Isolated yield (%) of **5**[a]
1	Me$_2$CuLi·LiI2LiBr (1.2 eq.)	49
2	Me$_2$CuLi·LiI·2LiBr, TMSCl (1.2 eq.)	67
3	Me$_2$CuLi·LiI2LiBr (2.5 eq.), TMSCl (2.0 eq.)	50
4	MeCu(CN)Li·LiBr (2.0 eq.)	81
5	MeCu(CN)Li·LiBr (5.0 eq.), AlCl$_3$ (2.0 eq.)	84
6	Me$_2$Cu(CN)Li$_2$·2LiBr (2.5 eq.)	69
7	Me$_2$Cu(CN)Li$_2$·2LiBr (2.5 eq.), AlCl$_3$ (2.0 eq.)	85
8	Me$_2$Cu(CN)Li$_2$·2LiBr (1.1 eq.), BF$_3$·Et$_2$O (1.0 eq.)	52
9	Me$_2$Cu(CN)Li$_2$·2LiBr·2LiCl (1.5 eq.)	87
10	Me$_2$Cu(CN)Li$_2$·2LiBr·2LiCl (2.5 eq.)	92
11	Me$_2$Cu(CN)Li$_2$·LiBr·2LiCl, BF$_3$·Et$_2$O (1.5 eq.)	58
12	Me$_2$Cu(CN)Li$_2$·2LiBr·2LiCl, AlCl$_3$ (1.5 eq.)	82
13	MeLi·LiBr	0[b]
14	Bu$_2$Cu(CN)Li$_2$·2LiCl (2.5 eq.)	70[c]

[a] Other formed products were not characterized, except for **5** and Michael adduct; [b] no starting material; [c] Michael adduct: 24%

ing reduction product **5** with (*E*)-configuration§ in up to 80% isolated yields. Similarly, using methyl copper reagents, the formation of an alkylated product was also not observed. Use of methyl copper reagents was critical for conversion of **4** to **5**, since exposure of **4** to a butyl-copper reagent (run 14) afforded, besides **5**, a Bu-substituted Michael adduct (24%). The reaction presented here, different from other published protocols,[7,10] is conceptually a new methodology for the preparation of α-vinylphosphonates. Hydrogenation of the resulting α-vinylphosphonates affords the corresponding α-monofluorophosphonates, possessing a carboxy functionality which is amenable to further derivatization. Furthermore, starting from a common difluoromethylphosphonate intermediate, both the monofluoro- and corresponding difluoro-methylphosphoryl counterparts can be obtained.

Next, we applied this methodology to the synthesis of CHF-substituted pSer mimetic (FPab) as shown in Scheme 2. Hydrogenation of α-vinylphosphonate **5** over Pd–C in AcOEt proceeded without diastereoselectivity to yield α-monofluorophosphonate **6** in quantitative yield. Reaction of 1-chloro-1-nitrosocyclohexane[11] in THF (blue) with the Na-enolate, resulting from treatment of **6** with NaHMDS in THF at −78 °C, proceeded with high diastereoselectivity to instantaneously afford a colorless solution of nitrone. Treatment of this solution with aqueous 1 N HCl, followed by extractive work-up, gave crude hydroxylamine **7**, which was taken to the next step without further purification. Reduction of **7** with Zn–AcOH in THF, followed by introduction of Boc protection onto the resulting NH$_2$ group using (Boc)$_2$O, gave Boc-protected **8**. The sultam moiety was then converted to the benzyl ester **9** utilizing

Notes and references

† (Z)-3-(diethyldifluoromethyl)but-2-enoate **1** was prepared by coupling of ethyl (Z)-3-iodobut-2-enoate with BrZnCF$_2$(O)(OEt)$_2$ in the presence of CuBr in DMF.[14] The (E)-isomer was synthesized according to the literature method.[15] Sultam-imide **4** was synthesized via the following sequence of reactions: (i) transesterification of ethyl (Z)-3-iodobut-2-enoate to the corresponding p-methoxybenzyl (PMB) ester using Ti(OPr^i)$_4$ in PMB-OH; (ii) CuBr-mediated coupling, as mentioned above; (iii) removal of the PMB group with 95% aqueous TFA; (iv) coupling of the sultam.

‡ To a solution of CuCN·2LiCl in THF (1 mol dm^{-3}, 4.2 cm^3) was added MeLi·LiBr in Et$_2$O (1.5 mol dm^{-3}, 5.6 cm^3) at −78 °C. The mixture was allowed to warm to 0 °C and stirred at this temperature for 1–2 min. After re-cooling to −78 °C, **4** (763 mg, 1.68 mmol) in THF (5 cm^3) was added with a syringe. After being stirred at −78 °C for 1.5 h, the reaction was quenched by addition of sat. NH$_4$Cl–28% NH$_4$OH solution. Usual work-up followed by flash chromatography gave **5** (639 mg, 87% yield).

§ Coupling constants of **5** ($^3J_{HF}$ = 38.6, $^3J_{HP}$ = 7.3 Hz) are consistent with those of α-fluorovinylphosphonate possessing (E)-configuration ($^3J_{HP trans}$ = 39–40, $^3J_{HP cis}$ = 7.6–10 Hz).[16]

¶ Protected peptide resin (Boc–Gly–FPab(OEt)$_2$–Val–Pro–Met–Leu–PAM resin, 0.05 mmol) was treated with 1 mol dm^{-3} TMSOTf–thioanisole (molar ratio 1:1) in TFA (2.5 cm^3) in the presence of m-cresol (125 mm^3) and EDT (125 mm^3) at 4 °C. After being stirred at 4 °C for 60 min, DMS (0.75 cm^3) and TMSOTf (0.5 cm^3) were successively added to the reaction with additional stirring at room temperature for 2 h. The reaction was quenched by addition of EtOH–H$_2$O. The aqueous layer was subjected to HPLC purification, yielding 22 mg of the desired peptide. Ion-spray MS m/z calcd for C$_{27}$H$_{49}$N$_6$O$_{10}$SFP (MH$^+$) 699.76; found 699.50. Purified peptides, consisting of diastereomers derived from FPab, were eluted as two peaks incompletely resolved on HPLC.

‖ Peptides possessing L-phosphotyrosine mimetics as an FPab replacement were completely hydrolyzed by leucine amino peptidase, while D-phosphotyrosine mimetic-containing peptides remained intact.[17]

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Scheme 2 Reagents: (i) H$_2$/Pd–C, AcOEt; (ii) NaHMDS (1.1 eq.), 1-chloro-1-nitrosocyclohexane (1.1 eq.). THF then 1 N HCl aq.; (iii) Zn (40 eq.). AcOH (50 eq.) then (Boc)$_2$O (2.0 eq.), CH$_3$CN; (iv) Ti(CPr^i)$_4$ (2.0 eq.), BzOH (44 eq.), toluene.

Ti(OPr^i)$_4$–benzyl alcohol in toluene at 120 °C. Hydrogenolytic debenzylation (H$_2$/10% Pd–C in AcOEt) of **9** gave the protected L-CHF-substituted pSer mimetic (Boc-FPab(OEt)$_2$-OH **10**). Application of a similar sequence of reactions to **4** gave enantiomerically pure L-CF$_2$-substituted pSer mimetic[12] (F$_2$Pab) **11**. We speculate that FPab derivative **10** possesses the 2S configuration (L-amino acid), by analogy to F$_2$Pab derivative **11**, which is obtained from a difluoromethylphosphonate-containing Xs-sultam utilizing a similar reaction sequence and has the 2S configuration. To our knowledge, this is the first synthesis of a CHF-substituted pSer mimetic.

In order to examine the general applicability of protected FPab **10** to peptide synthesis, **10** was incorporated into the peptide sequence (H–Gly–FPab–Val–Pro–Met–Leu) using a standard Boc-based solid-phase protocol. The resulting protected peptide resin was treated with a one-pot, two-step deprotection methodology[13] consisting of high-acidity [1 mol dm^{-3} TMSOTf–thioanisole in TFA, m-cresol, ethanedithiol (EDT)] and low-acidity (1 mol dm^{-3} TMSOTf–thioanisole in TFA, m-cresol, EDT + DMS–TMSOTf), which was developed for practical deprotection of protected phosphoamino acid-containing peptide resins, to yield a crude deprotected peptide without accompanying partially Et-deprotected peptides.¶ After HPLC purification, an FPab-containing peptide was obtained in 63% yield. In order to confirm the 2S configuration of FPab, purified peptide was subjected to enzyme digestion using leucine amino peptidase (LAP).‖ Interestingly, it was found that the parent peptides were converted to 5-residue peptides, H–FPab–Val–Pro–Met–Leu–OH, with rates that varied between the diastereomers derived from the fluorine substitution in FPab and with only 10% of FPab being released from the resulting 5-residue peptide after 24 h of LAP treatment. On the other hand, D-FPab-containing peptides remained intact after 24 h digestion using LAP. The present methodology should allow the facile preparation of functionalized α-fluorovinylphosphonates and α-fluorophosphonates. Furthermore, it is tempting to speculate that FPab-containing peptides could serve as inhibitors against both proteases and phosphatases since peptides having FPab residues at the N-terminal position are resistant to the action of LAP.