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Stereoselective synthesis of α-monofluorinated phosphonate mimetics of naturally occurring phosphoserine and phosphothreonine, *via* electrophilic fluorination of lithiated bis-lactim ethers†

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Electrophilic fluorinations of lithiated bis-lactim ethers derived from *cyclo*-[L-AP4-D-Val] allow a direct access to α-monofluorinated phosphonate mimetics of naturally occurring phosphoserine and phosphothreonine, in enantiomerically pure form and suitably protected for solid-phase peptide synthesis.

Protein phosphatases play significant roles in signal transduction pathways pertaining to such diverse functions as gene expression, growth, glycogen synthesis, immune activation and neurotransmission. Most of the protein dephosphorylation events occurring in the cell appear to be mediated by serine/threonine-specific phosphatases (PPs), which are characterized by using a metal dyad as the key component of catalysis. Although varying in nature from one PPs to another, the dinuclear metal ion center has been proposed to bind the phosphate oxoanions and activate a water molecule for direct hydrolysis of a phosphoester bond, while general acid catalysis is accomplished by a conserved residue in the active site which protonates the leaving group.[1]

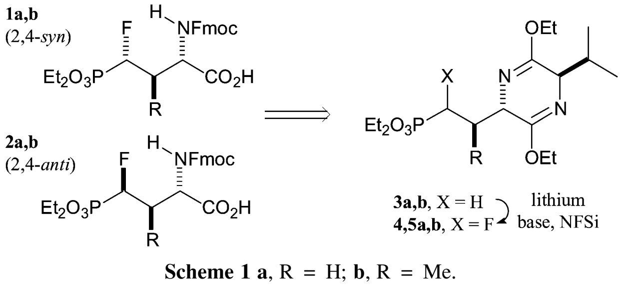
The importance of PPs for cell physiology has made them prominent targets for inhibitor design.[2] Starting from the amino acid sequences of endogenous phosphoproteins or small phosphopeptides preferred as substrates, selective phosphatase inhibitors have been designed. Non-hydrolizable mimetics of such phosphopeptides have been prepared by replacement of the phosphate ester oxygen by a methylene linkage, giving rise to phosphonopeptide analogues. Nevertheless, phosphonate analogues have shown lower affinity than the corresponding phosphate esters for the enzymes that bind or hydrolyse phosphorylated biomolecules. This loss of biological activity may be attributable to the higher steric requirements and lower electronegativity of the methylene group with respect to the oxygen. A commonly used strategy for increasing the binding potency of phosphonic acids has relied on the substitution of the methylene hydrogens at the α position by fluorine atoms,[3] thus increasing the acidity of the phosphonate and reintroducing the possibility of hydrogen-bonding interactions. Based on the pK_a values for the second deprotonation and the calculated electrostatic profiles, the α-monofluorinated phosphonates have been proposed to match the properties of the phosphates they are designed to mimic more closely than the α,α-difluorinated ones.[4] In recent years the performance of non-fluorinated, α-monofluorinated and α,α-difluorinated phosphonate inhibitors has been evaluated for several enzyme systems. Different responses have been obtained for the series, revealing the convenience of performing a complete "fluorinated phosphonate scan" when optimizing the binding to a targeted active site.[5]

Methodologies for the synthesis of enantiomerically pure (2*S*)-2-amino-4,4-difluoro-4-phosphonobutanoic acids, suitably protected for their incorporation into peptides as mimetics of L-phosphoserine (pSer) and L-phosphothreonine (pThr), have been recently developed by Berkowitz and Otaka by using difluoromethylphosphonate carbanions for the C–CF_2P bond formation.[6] Despite its potential advantages, the corresponding α-monofluorinated phosphonate mimetics have received little attention. To the best of our knowledge, strategies for the synthesis of the α-monofluorinated phosphonate mimetic of pThr are not available in the literature, while only one methodology has been reported for the preparation of the α-monofluorinated phosphonate mimetic of pSer (FPab). Although practical for the incorporation of FPab into phosphopeptide mimetics, the available synthetic route relies on a non-selective hydrogenation of a fluorovinylphosphonate precursor, and gives rise to the 2-amino-4-fluoro-4-phosphonobutanoic acid derivatives as diastereomeric mixtures which were speculatively assigned the biological configuration based on enzymatic digestion methods.[7]

In this area, we have shown that conjugate additions of lithiated Schöllkopf's bis-lactim ethers to vinylphosphonates enable the stereocontrolled preparation of bis-lactim ethers derived from 2-amino-4-phosphonobutanoic acid (AP4),[8] that have been efficiently transformed into optically pure phosphonate mimetics of pSer and pThr.[9] More recently, we have found that electrophilic substitutions on the bis-lactim ether derived from *cyclo*-[L-AP4-D-Val] take place regio- and stereoselectively, and allow a direct access to a variety of 4-substituted AP4 derivatives in enantiomerically pure form.[10] In addition, the electrophilic fluorination using the 'N–F' reagents has become a valuable method for the selective fluorination of activated aromatics, alkenes, and enolates.[11] Moreover, using *N*-fluorobenzenesulfonimide (NFSi) Differding has reported the successful fluorination of a variety of carbanions stabilized by a phosphonate ester group.[12]

Based on these precedents we envisaged the electrophilic fluorinations of the Schöllkopfś bis-lactim ethers **3a,b** as a direct route for the synthesis of the α-monofluorinated phosphonate analogues of naturally occurring pSer **1,2a** and pThr **1,2b** (see Scheme 1), in enantiomerically pure form and using a *N*-Fmoc/*O*-ethyl phosphonic ester protection strategy for solid-phase peptide synthesis.

Toward this end, slow addition of bis-lactim ether **3a**‡ to a solution of 2 equiv. of LDA in THF at −78 °C was followed 15 min later by the dropwise addition of NFSi. Reaction took place rapidly, and after quenching with acetic acid and work-up, mixtures of the 2′-fluorinated bis-lactims were isolated in a combined yield of 72%, along with 10% of unreacted starting material. Integration of the ^{1}H decoupled ^{31}P NMR spectrum of



Scheme 1 **a**, R = H; **b**, R = Me.

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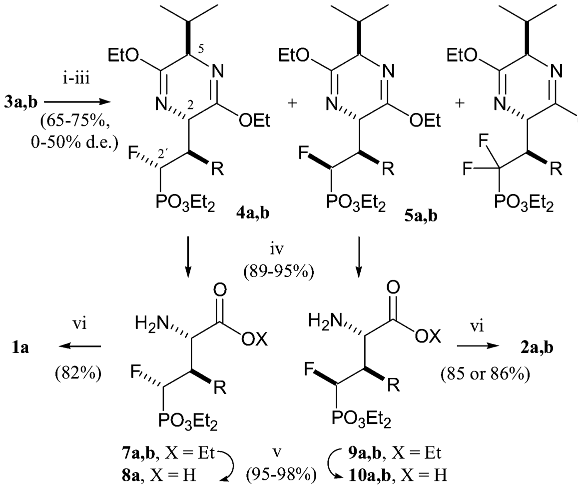
† Electronic supplementary information (ESI) available: experimental data. See http://www.rsc.org/suppdata/cc/b2/b204093j/

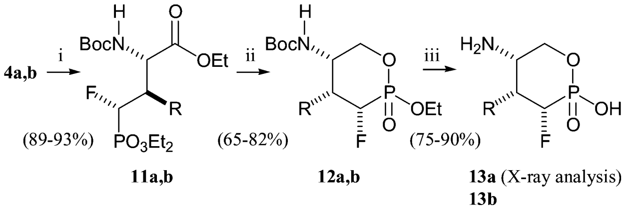
the crude reaction mixture revealed a 8.2 : 2.7 : 1.0 ratio for **4a** : **5a** : **6a** (see Scheme 2). The use of 2 equiv. of NFSi resulted in the complete reaction of **3a** and a reduced ratio of mono- to difluorination (4 : 3 instead of 11 : 1) with neither change in the combined yield nor the level of 2,2′-*syn* stereoselectivity. When LDA was added to the preformed equimolar mixture of **3a** and NFSi a 65% conversion to the desired monofluorinated products **4a** : **5a** was achieved. As the difluorination was completely suppressed in this case, the yield of **4a** : **5a** could be increased to 78% by resubjecting the recovered material to these fluorination conditions. Starting with the bis-lactim ether **3b**,[8] treatment with LDA and subsequent reaction with NFSi afforded the corresponding mixture of mono- and difluorinated products **4b** : **5b** : **6b**, in a 7 : 7 : 1 ratio and 75% total yield. Thus, although completely regioselective at position 2′, monofluorination was not stereoselective in this case.§



Scheme 2 *Reagents and conditions*: (i) LDA (2 equiv.), THF, −78 °C, 15 min; (ii) NFSi, THF, −78 °C, 5 min; (iii) AcOH, −78 °C to rt; (iv) 0.25 M HCl , THF, rt, 1–15 h; (v) LiOH, H_2O, rt, 30 min; (vi) Fmoc-*O*-*N*-succinimide, $NaHCO_3$, acetone/H_2O 1 : 1, rt, 14 h. **a**, R = H; **b**, R = Me.

Conversion of the monofluorinated bis-lactims **4–5a,b** into the desired pSer and pThr mimetics was straightforward. Mild acid hydrolysis of the bis-lactim afforded the aminoesters **7a,b** and **9a,b**, which were subsequently hydrolysed to the corresponding amino acids **8a** and **10a,b** by treatment with lithium hydroxide. Finally, Fmoc-protection under standard conditions gave **1a** and **2a,b** in excellent yields.

Since none of the synthesized compounds provided crystals suitable for X-ray diffraction analysis, the oxaphosphorinanes **13a,b** were sought as suitable cyclic derivatives which would enable assignment of the relative configurations by NMR spectroscopy (see Scheme 3).



Scheme 3 *Reagents and conditions*: (i) $(Boc)_2O$, dioxane/H_2O, rt, 2–7 h; (ii) $LiBH_4$, THF, 0–25 °C, 8 h; (iii) a: TMSBr, THF, 0–25 °C, 17 h; b: MeOH, rt. **a**, R = H; **b**, R = Me.

As expected, after protection of the amino group, chemoselective reduction of the carboxylic ester of **11a** and **11b** with $LiBH_4$ took place with simultaneous cyclization to the corresponding *N*-Boc-aminooxaphosphorinanes **12a** and **12b**. Finally, cleavage of the protecting groups by treatment with TMSBr yielded **13a** and **13b** in good yields. Both compounds showed in their ^{1}H NMR spectra a pattern of signals suitable for their stereochemical study by NOE difference spectroscopy. Analyses of the sets of observed NOEs allowed to conclude a *cis* 1,3-diaxial relationship between the fluoro and the amino group across the oxaphosphorinanes **13a** and **13b**. Stereochemical assignments for **13a** were eventually confirmed by an X-ray crystal structure analysis.¶

In conclusion, electrophilic fluorinations of readily available lithiated bis-lactim ethers derived from *cyclo*-[L-AP4-D-Val] using commercial NFSi allow a direct access to α-monofluorinated phosphonate mimetics of naturally occurring pSer and pThr, in optically pure form and suitable protected for solid-phase peptide synthesis, which may be useful for the preparation of inhibitors directed to serine/threonine-specific protein phosphatases.

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Notes and references

‡ **3a** was prepared as described ,[9] but using a *O,O*-diethyl vinylphosphonate ester instead of a *O,O*-diallyl one.

§ Evidence supporting their relative configurations was obtained from NMR analyses. Thus, for compounds **4–6a,b** H-5 resonance appears between 3.87 and 3.97 ppm, as a triplet with ^5JH2H5 close to 3.5 Hz, typical for a *trans* relation of substituents at the pyrazine ring.

¶ *Crystal data* for **13a**: $C_4H_9FNO_3P$, M = 169.09, orthorhombic, a = 6.7943(13), b = 8.718(3), c = 11.059(4) Å, U = 655.1(3) Å^3, T = 298(2) K, space group $P2_12_12_1$, Z = 4, μ(Mo-Kα) = 0.385 mm^{-1}, 3608 reflections measured, 1612 unique (R_{int} = 0.0234) which were used in all calculations. The final $wR(F^2)$ was 0.0627 (all data). Absolute structure (Flack) parameter = 0.02(10). CCDC 185051. See http://www.rsc.org/suppdata/cc/b2/b204093j/ for crystallographic data in .cif format.

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