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β-Fluorinated proline derivatives: potential transition state inhibitors for proline selective serine dipeptidases

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Abstract—Three new types of β-fluorinated proline derivatives were synthesized as potential transition state inhibitors for proline selective serine dipeptidases. The fluorophosponate derived from protected proline was tested as a Wadsworth–Horner–Emmons reagent for the synthesis of fluoro-olefin-containing pseudodipeptides. © 2003 Elsevier Science Ltd. All rights reserved.

Proline selective serine aminodipeptidases are members of the family of serine proteases. They cleave off dipeptides from the amino terminus of peptides or proteins with proline at the penultimate position. Representative examples of this group are dipeptidyl peptidases II and IV (DPP II and DPP IV) and fibroblast activating protein-alpha (FAP-alpha). These three enzymes are currently under investigation against disease states as diverse as diabetes, cancer, and immune related disorders.[1]

The transition state in serine proteases is obtained after the addition of a serine hydroxyl group in the active centre to the scissile peptide bond. The resulting tetrahedral intermediate is stabilized by the 'oxy-anion hole' (Fig. 1). Further steps in the enzymatic activity comprise the elimination of the amine part and hydrolysis of the resulting acylated enzyme.[2]

A large number of potent inhibitors for this class of enzymes contain an electrophilic centre capable of complexing or scavenging the serine –OH function. In the case of dipeptidase inhibitors, where most described compounds have a dipeptide skeleton, the free amino terminus that is necessary for activity can interact with the electrophilic centre, thereby abolishing all inhibiting activity (Fig. 2). This process, related to the well-known diketopiperazine formation in peptides, limits the development of potent inhibitors as drug candidates.[3]

In this letter we report the synthesis of a series of β-fluorinated proline derivatives possessing a tetrahedral geometry similar to the transition state and a fluorine atom that could act as a hydrogen bond acceptor in the 'oxy-anion hole' (Fig. 3).[4] Contrary to the

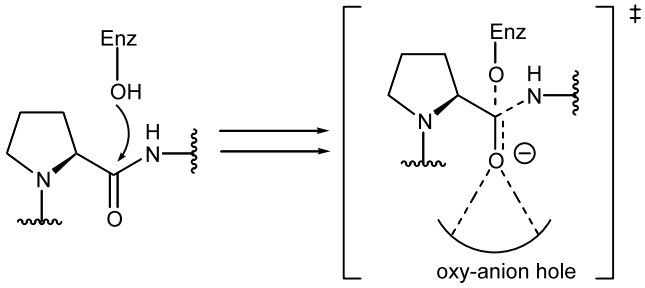

Figure 1. Transition state in serine proteases.

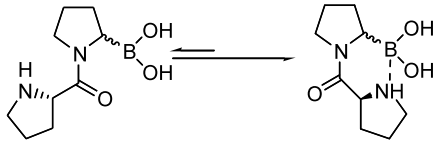

Figure 2. Inactivation mechanism for a typical inhibitor.

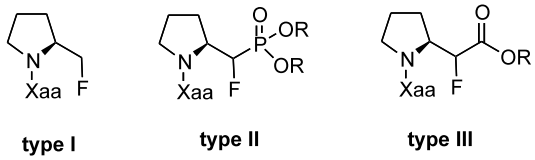

Figure 3. General structures of synthesized inhibitors. Xaa = Ile or *N*-cyclohexylglycine; R = H, Me.

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mentioned electrophilic inhibitors, these compounds are stable. Since a dipeptide skeleton is necessary for recognition by the enzyme, the pyrrolidine ring in these compounds is attached to *L*-Ile or cyclohexylglycine, two amino acids that are also present in other inhibitor series for this type of enzymes. In the past, related β-hydroxylated amino acid derivatives have already been described as potent transition state mimicking inhibitors for the HIV protease and other aspartic proteases.[5,6] To the best of our knowledge, this work represents the first synthesis of β-fluoro-β-phosphonates derived of amino acids.

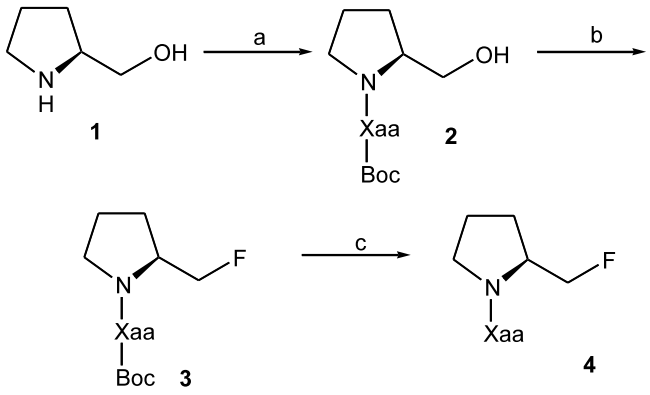
Results and discussion

Compounds of type I were prepared from *L*-prolinol, which was coupled to either *N*-Boc-isoleucine or *N*-Boc-cyclohexylglycine using TBTU (Scheme 1). Fluorination with diethylaminosulfurtrifluoride (DAST) followed by deprotection with trifluoroacetic acid (TFA) provided the final compounds. Although DAST has been reported to react with the amide functionality, no products expected from this side reaction could be isolated.[7]

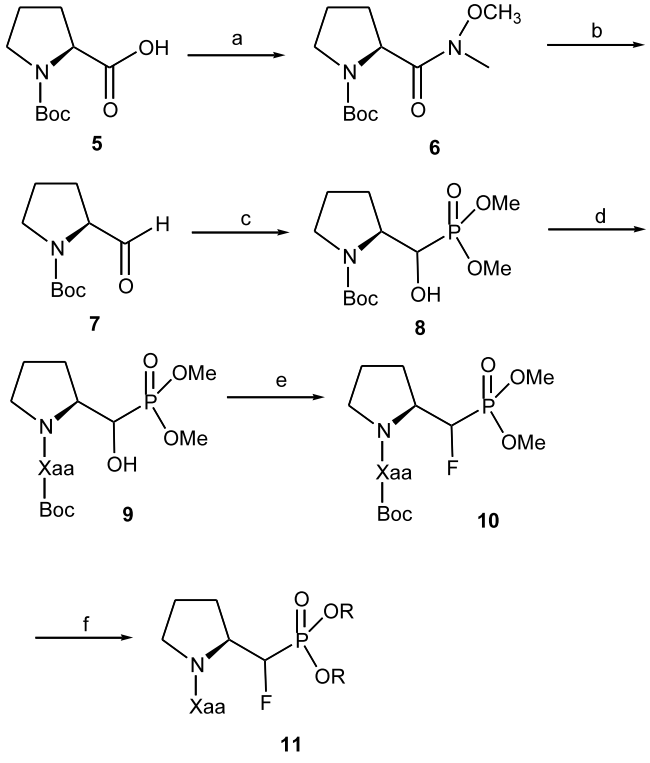
Alternatively, fluorination could also be carried out on Boc-protected *L*-prolinol, followed by deprotection and coupling. It seems however that introduction of the fluorine atom significantly lowers the nucleophilicity of the deprotected amine, resulting in low yields for the coupling step which could not be improved by using a 'stronger' coupling reagent like PyBrop. This effect was also seen in the synthesis of compounds of type II and III and prompted us to perform the fluorination reaction after the coupling step.

For the preparation of fluorophosphonates (type II), *N*-Boc-proline was first converted to *N*-Boc-prolinal via the Weinreb amide (Scheme 2). Pudovik–Abramov condensation provided the hydroxyphosphonate **8** as a mixture of two diastereomers (ratio 2:1).[8] The use of DBU catalysis results in a very fast reaction with only moderate stereoselectivity compared to e.g. KF, iPrEt$_2$N or NMM, as described by Patel et al.[5] Since we were interested in potential biological activity differences for both isomers, the use of reaction conditions with low stereoselectivity was considered beneficial. In the next step, the hydroxyphosphonate (as a mixture of diastereomers) was deprotected and coupled to either *N*-Boc-isoleucine or *N*-Boc-cyclohexylglycine. Fluorination with DAST in moderate yield was followed by the separation of diastereomers (flash chromatography). Different deprotecting steps were then used to obtain the final products.

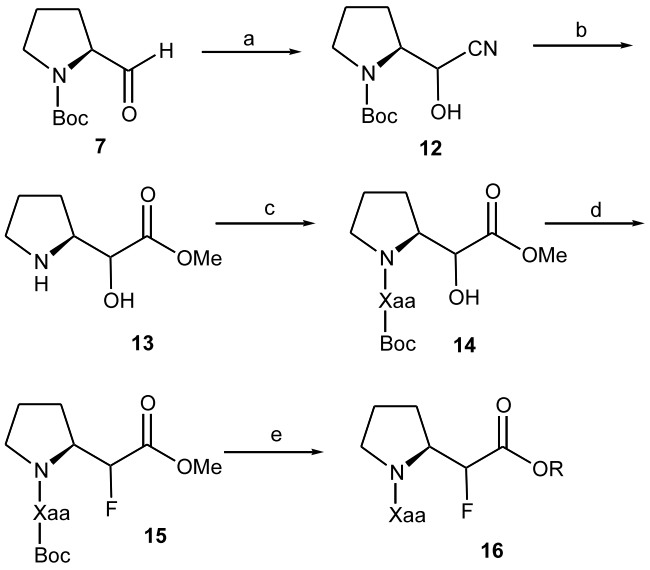
Compounds of type III were synthesized in a similar way (Scheme 3). Addition of HCN to *N*-Boc-prolinal (diastereomeric ratio 63:37) followed by acidic hydrolysis and esterification resulted in amine **13**, which was coupled to *N*-Boc-isoleucine or *N*-Boc-cyclohexylglycine.[8] After DAST fluorination in moderate yield, and separation of diastereomers by flash chromatography, final products were obtained by acidolytic depro-



Scheme 1. *Reagents and conditions*: (a) TBTU, Et_3N, *N*-Boc-Xaa, DMF, rt, 2 h, 88%/91%.[a] (b) DAST, CH_2Cl_2, 0°C, 4 h, 72%/65%.[a] (c) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant.
[a]Yields indicated are for products coupled to *L*-Ile and *N*-cyclohexylglycine, respectively.



Scheme 2. *Reagents and conditions*: (a) TBTU, Et_3N, NH(Me)OMe·HCl, DMF, rt, 6 h, 81%. (b) $LiAlH_4$, THF, −10°C, 2 h, 78%. (c) HP(O)$(OMe)_2$, DBU (cat.), 0°C, 1 h, 93%. (d) (i) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant.; (ii) TBTU, Et_3N, *N*-Boc-Xaa, DMF, rt, 4 h, 80%/77%.[a] (e) DAST, CH_2Cl_2, 0°C, 4 h, 41%/52%.[a] (f) 1. ($R_1 = R_2 = Me$) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant. 2. ($R_1 = Me$, $R_2 = H$) (i) LiBr, CH_3CN, rt, 21 h, 97%/97%//96%/98%;[b] (ii) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant. 3. ($R_1 = R_2 = H$) (i) TMSI, CH_2Cl_2, rt, 5 h; (ii) 1N HCl/H_2O, rt, 40 min, 94%/91%//87%/93%.[b]
[a]Yields indicated are for products coupled to Ile and *N*-cyclohexylglycine respectively.
[b]Yields indicated are for the two diastereomers of the products coupled to Ile and *N*-cyclohexylglycine, respectively.

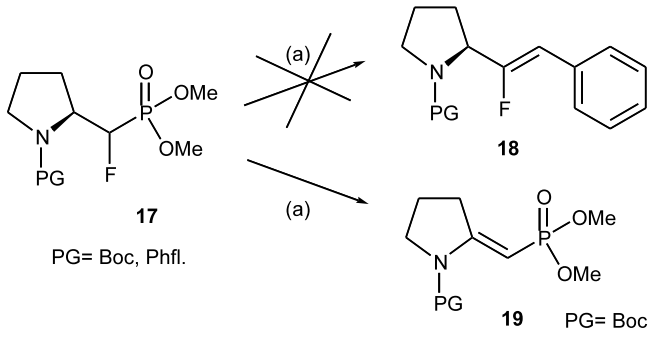


Scheme 3. *Reagents and conditions*: (a) acetone cyanohydrin, Et_3N, CH_2Cl_2, rt, 32 h, 91%. (b) (i) HCl 6N/H_2O, Δ, 36 h; (ii) HCl 4N/MeOH, rt, 4 h, 72%. (c) TBTU, Et_3N, *N*-Boc-Xaa, DMF, rt, 4 h, 89%/83%.[a] (d) DAST, CH_2Cl_2, 0°C, 7 h, 54%/61%.[a] (e) 1. (R = OMe) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant. 2. (R = H) (i) 1N KOH/MeOH, 0°C, 11 h, 97%/94%// 95%/98%[b]; (ii) TFA/CH_2Cl_2 (1:1), rt, 20 min, quant.
[a]Yields indicated are for products coupled to Ile and *N*-cyclohexylglycine, respectively.
[b]Yields indicated are for the two diastereomers of the products coupled to Ile and *N*-cyclohexylglycine, respectively.

tection with TFA or a demethylation–deprotection sequence for free acids. An alternative approach towards β-fluoro-β-carboxylamines using electrophilic fluorination was considered not applicable on dipeptide substrates.[9]

One of the key steps in this reaction is the DAST-fluorination. Although DAST has become one of the standard reagents for the transformation of alcohols, aldehydes and ketones into their fluorinated or difluorinated analogs, no examples are present of successful use on substrates of this kind. As mentioned above, this is to our knowledge the first report of β-fluoro-β-phosphonates derived of amino acids and only the second report of DAST fluorination of non-activated hydroxyphosphonates in general.[10] Although DAST fluorination is usually carried out at low temperatures (−78°C), in our case optimal yields for all reactions were obtained in CH_2Cl_2 at 0°C with 1.5 equiv. of DAST.

Over the last decade, a limited number of non-stabilized fluorophosphonates has also been reported as Wadsworth–Horner–Emmons (WHE) reagents for the synthesis of fluoroolefins.[11] In peptide chemistry, the fluoroolefin group has been proposed as a rigid amide bond analog that is stable to proteolytic degradation. Some examples are present, proving that this is a valuable approach.[12] However, existing synthetic routes towards this class of pseudopeptides are elaborate and



Scheme 4. *Reagents and conditions*: (a) (i) base[a], THF, −78°, 20 min; (ii) benzaldehyde (1 equiv.), −78° to rt, 2 h.
[a]Bases used were *n*-BuLi, LDA, NaH, LiHMDS.

suffer from a lack of stereoselectivity. This inspired us to test the *N*-Boc protected fluorophosphonate **17**, prepared by fluorination of compound **8** as a WHE substrate in a reaction with benzaldehyde (Scheme 4). However, all conditions applied resulted in dehydrofluorination and the formation of enamine **19**. Changing from the electron-withdrawing Boc-group to 9-phenylfluorenyl (Phfl) protection (which was designed to effectively shield the α-proton in amino acids), did not give the desired fluoroolefin **18** either.[13] In this case deprotonation of the fluorophosphonate turned out to be the limiting step. This was shown in a quenching experiment with D_2O: after stirring with 1.1 equiv. of *n*-BuLi in dry THF at −78°C for 20 min, followed by the addition of 10 equiv. of D_2O, no deuterated product could be detected.

In summary, we have developed a synthetic strategy for a new class of potentially useful proline derivatives which is, in theory, amenable to most other amino acids. Preliminary biochemical evaluation of the described compounds on one of our target enzymes, DPP IV, showed promising activity. The most active compound turned out to be fluorophosphonate **11** with Xaa = Ile and $R_1 = R_2 = H$ ($IC_{50} = 50 \pm 1$ μM). Both diastereomers shared the same activity. Further examinations on other proline selective serine dipeptidases will be carried out to validate our approach. The attempts made to use the fluorophosphonate **17** as a WHE-substrate for the synthesis of fluoroolefin containing pseudopeptides were not successful and at this point, do not indicate that this is a rewarding strategy.

Acknowledgements

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