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Synthesis of phosphinic alanyl-proline surrogates Alaψ(PO_2R-CH)Pro as potential inhibitors of the human cyclophilin hCyp-18

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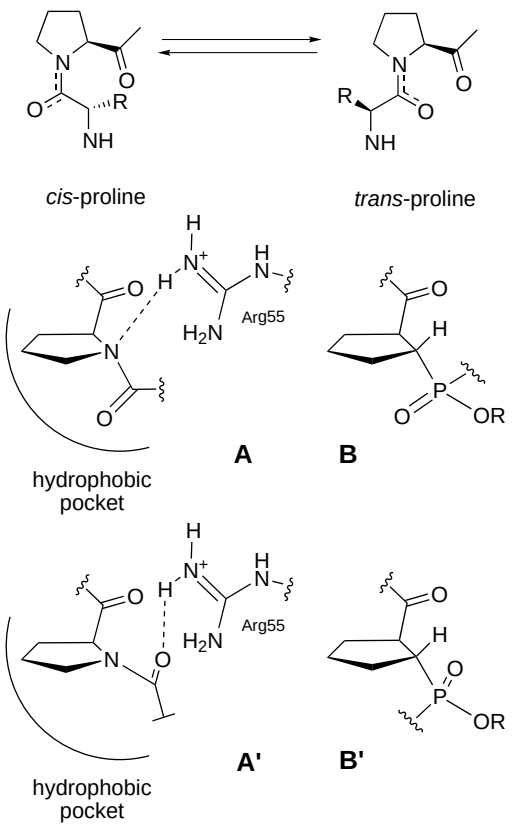
Abstract—Pseudopeptides containing the phosphinic analogue of the alanyl-proline motif Alaψ(PO_2R-CH)Pro, were synthesized via three- (R = H) and four-step (R = CH_3) procedures. The mixtures of diastereomers were evaluated as inhibitors of the human cyclophilin hCyp-18, an important peptidyl-prolyl isomerase.

1. Introduction

In the past decade, an impressive number of novel pseudopeptides has been developed as enzyme inhibitors, in particular protease inhibitors. Some of them have been used as novel therapeutic agents.[1] Among other mimetics, phosphinic pseudopeptides are potent inhibitors of aspartyl proteases[2] and zinc-metalloproteases[2,3] such as angiotensin-converting enzyme (ACE)[4] or matrix metalloproteases (MMPs).[5] For this purpose, many different phosphinic motifs have been designed and synthesized. We investigated the synthesis of pseudopeptides **1** and **2** containing, respectively, a charged and a non-charged Ala-Pro phosphinic isostere, in order to test them as potential inhibitors of the human cyclophilin hCyp-18. HCyp-18 is an important peptidyl-prolyl *cis–trans* isomerase (PPIase) implicated in protein folding,[6] cellular multiplication[7] and communication,[8] as well as immunosuppression.[9] Moreover, hCyp18 is involved in the infection of T-cells by HIV-1 and in the multiplication of the virus.[10] The design of transition-state mimics of the PPIase activity might lead to the development of non-immunosuppressive and potent inhibitors of hCyp-18.[11]

Though the mechanism of the reaction is not clearly established, recent results suggest an hyperpolarization of the amide, a quaternarization of the nitrogen[12] (Scheme 1, **A**) and an enzyme-assisted rotation of the



Scheme 1. Amino acyl-proline *cis–trans* isomerization: proposed transition-states[12] for the hCyp-18-catalyzed reaction and corresponding phosphinic transition-state isostere.

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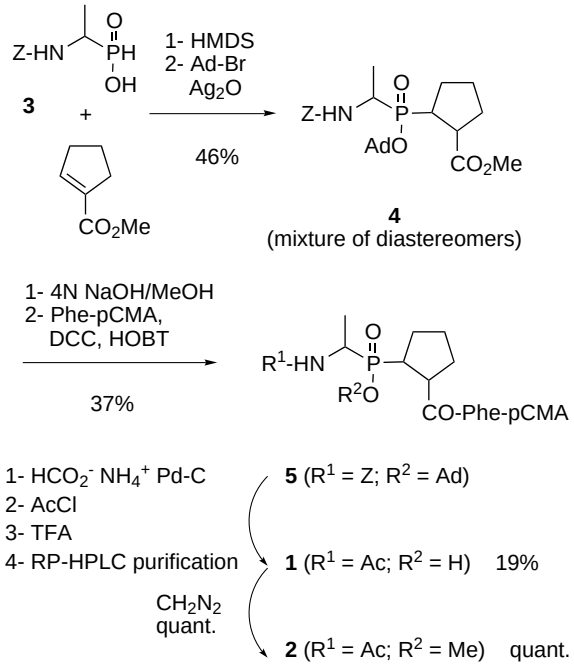
'keto-amine' intermediate[13] (Scheme 1, **A**′). The phosphinic motif displays some similarities with the postulated PPIase transition state (Scheme 1, **B** and **B**′). We report herein the synthesis of an Ala-Pro phosphinic isostere which might be able to mimic the catalytic intermediates of the reaction. This motif was inserted inside a tripeptide Ac-Ala-Pro-Phe-pCMA[14] derived from the standard model substrates of hCyp-18 Suc-Ala-Ala-Pro-Phe-*p*NA.

2. Synthesis of phosphinic alanyl-proline surrogates

A general method of preparation of aminoalkyl phosphinic dipeptides equivalents has been previously reported. It implies a conjugate addition of the trivalent form of a protected aminoalkylphophinic acid on to a C2-substituted acrylate.[15] This strategy is particularly attractive since it employs easily available starting materials, in particular substituted acrylates.[16] The versatile method of preparation of aminoalkylphosphinic acids described by Baylis and co-workers also provides a large molecular diversity.[17] Moreover, most of the diastereomers can be separated by reverse phase HPLC (RP-HPLC) after deprotection of the phosphinyl moiety.[18] As a consequence, this strategy has been successfully employed for generating large librairies of phosphinic inhibitors with wide varieties of substituents and absolute configurations.[17–19] However, to our knowledge, no preparation of C2 and C3 bis-substituted aminoalkyl phosphinate Xaaψ(PO_2H-CH)Pro has been formally reported in the literature. We investigated the conjugate addition of a bis-silyl derivative of benzyloxycarbonyl-1-aminoethyl-1-phosphinate **3** on to methyl 1-cyclopentene-1-carboxylate as a straightforward method for generating an orthogonally protected Alaψ(PO_2H-CH)Pro templates.

Compound **3** was prepared as a racemic mixture as previously described.[17–19] The conjugate addition of **3** on to methyl 1-cyclopentene-1-carboxylate was carried out using hexamethyldisilazane for the generation of the nucleophilic trivalent form of the phosphinic acid. The adduct was protected in situ using 1-bromoadamantane and silver oxide (Scheme 2).[20] The dipeptide isostere **4** was isolated as a complex mixture of diastereomers which can be separated by RP-HPLC. Saponification of the methyl ester and standard peptide coupling to phenylalanyl(4-carboxymethyl)aniline gave tripeptide **5** in low yield (37%). This might be the result of a concurrent saponification of the adamantane as previously observed.[21] Deprotection of the benzyloxycarbonyl moiety was carried out using ammonium formate and 10% Pd–C.[20] Indeed, previous attempts for hydrogenolyzing the benzyl carbamate under an hydrogen atmosphere caused a significative deprotection of the adamantyl ester. Acetylation followed by acidolysis of adamantyl ester **6** yielded pure Ac-Alaψ(PO_2H-CH)Pro-Phe-*p*CMA **1**.[22] Only three of the eight possible diastereomers of **1**, generated by the creation of three stereogenic centers, were observed by RP-HPLC. The uncharged derivative of acid **1**, methyl ester **2**,[23] was obtained in quantitative yield by treatment of compound **1** with diazomethane in THF. Even though some diastereomers of **1** and **2** could be separated by semi-preparative RP-HPLC, the mixture was used in preliminary biological assays.



Scheme 2. Synthesis of Alaψ(PO_2H-CH)Pro-containing peptides **1** and **2**.

3. Biological evaluation of phosphinic pseudopeptides as inhibitors of hCyp-18

Phosphinic tripeptide isosteres were tested both as ligands of hCyp-18 and inhibitors of its PPIase activity. Fluorimetric titration of Trp121[11] showed that compounds **1** and **2** bind to hCyp-18 (**1**: $K_d = 107 \pm 6$ μM; **2**: $K_d = 74 \pm 4$ μM) with an affinity equivalent to the reference tripeptides Ac-Ala-Pro-Phe-*p*NA ($K_d = 140 \pm 40$ μM) and Suc-Ala-Pro-Phe-*p*CMA ($K_d = 55 \pm 6$ μM). Unfortunately, neither **1** nor **2** were able to inhibit the cyclophilin-catalyzed isomerization of the model subtrate Suc-Ala-Ala-Pro-Arg-pNA. This indicates that these compounds interact close to hCyp-18 Trp121. In turn, the Alaψ(PO_2R-CH)Pro (R is H or CH_3) moieties do not fit inside the proline recognition pocket which catalyzes the *cis–trans* isomerization.[6,24]

In summary, we reported herein an expeditious synthesis of the orthogonally protected phosphinic Ala-Pro isostere Alaψ(PO_2Ad-CH)Pro and its use in solution peptide synthesis for the preparation of Alaψ(PO_2H-CH)Pro and Alaψ(PO_2CH_3-CH)Pro-containing peptides.

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22. Compound **1** (as a mixture of at least three diastereomers): ^{1}H NMR (CD_3OD): δ (ppm) 7.97–7.88+7.82–7.67+7.60–7.55 (3m, 4H, H Ar. *p*CMA), 7.27–7.17 (m, 5H, H Ar. Phe), 4.41–4.26 (bm, 1H, NC$\underline{H}$(CH_3)P), 3.87 (s, 3H, $CO_2C\underline{H}_3$ *p*CMA), 3.37–3.21+3.14–2.87+2.72–2.48 (3m, 4H, P-CH+CH-CO *c*pentane+2Hβ Phe), 1.99–1.55 (bm, 9H, CH_3 Ac+3 CH_2 *c*pentane), 1.36–1.18 (m, 3H, NCH(C$\underline{H}_3$)P); ^{13}C NMR(CD_3OD): δ (ppm) 172.5+172.4+168.2+168.1+144.2+144.1+138.7+138.3+138.2+131.4+120.4 (Ar C, complex), 56.8, 56.5, 52.5 ($\underline{C}H_3$ *p*CMA), 46.0, 39.1, 38.8, 37.3 (Cβ Phe), 32.1, 28.6, 27.6, 27.2, 27.1, 22.6 ($\underline{C}H_3$C=O), 14.4+14.1+14.0 (NHCH($\underline{C}H_3$)P); ^{31}P NMR (CD_3OD, decoupled): δ (ppm) 51.9–51.2 (broad lines); ES/MS (negative ionization): 543.6.
23. Compound **2** (as a mixture of seven diastereomers): ^{1}H NMR ($CDCl_3$): δ (ppm) 7.99–7.93+7.85–7.76+7.73–7.58 (3m, 4H, H Ar. *p*CMA), 7.34–7.13 (m, 5H, H Ar. Phe), 4.48–4.38 (bm, 1H, NC$\underline{H}$(CH_3)P), 3.87 (s, 3H, $CO_2C\underline{H}_3$ *p*CMA), 3.81–3.65 (m, 3H, $PO_2C\underline{H}_3$), 3.30–3.17+3.07–2.90+2.82–2.57 (3m, 4H, P-CH+CH-CO *c*pentane+2Hβ Phe), 1.98–1.57 (bm, 9H, CH_3 Ac+3 CH_2 *c*pentane), 1.37–1.13 (m, 3H, NCH(C$\underline{H}_3$)P); ^{13}C NMR ($CDCl_3$): δ (ppm) 172.5+168.1+144.2+138.3+131.5+131.3+130.4+129.6+129.4+127.8+126.6+121.0+120.8+120.5+120.2 (Ar C), 56.7, 56.4, 53.2, 53.1, 52.5 ($\underline{C}H_3$ *p*CMA), 50.0, 46.9, 46.0, 44.6, 39.0, 38.8, 37.3 (Cβ Phe), 33.7, 28.2, 27.9, 27.5, 27.0, 22.5 ($\underline{C}H_3$C=O), 14.7+14.5+14.3+14.2 (NHCH-($\underline{C}H_3$)P); ^{31}P NMR ($CDCl_3$, decoupled): δ (ppm) 57.9, 57.8, 57.6, 57.5, 56.6, 56.5, 56.3; ES/MS: 557.5.
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