Synthesis and Evaluation of Glyψ(PO_2R-N)Pro-Containing Pseudopeptides as Novel Inhibitors of the Human Cyclophilin hCyp-18†

Luc Demange, Mireille Moutiez, and Christophe Dugave*

CEA/Saclay, Département d'Ingénierie et d'Etudes des Protéines, Bâtiment 152, 91191 Gif-sur-Yvette, France

Received February 27, 2002

The human cyclophilin hCyp-18, an abundant peptidyl-prolyl cis−trans isomerase (PPIase) implicated in protein folding, controls the infection of $CD4^+$ T-cells by HIV-1, the pathologic agent of AIDS. Therefore, hCyp-18 is an interesting target for the development of novel anti-HIV-1 therapeutics. We focused on the design of transition-state analogue inhibitors of the PPIase activity of cyclophilin. Most experimental results reported in the literature suggest that hCyp-18 catalyzes the pyramidalization of the nitrogen of pyrrolidine via an H-bond network which results in the deconjugation of the amino acyl−prolyl peptide bond. We proposed the Glyψ(PO_2R^1-N)Pro motif (R = alkyl or H) as a selective transition-state analogue inhibitor of cyclophilin. This motif was inserted in Suc-Ala-Ala-Pro-Phe-*p*NA, a peptide substrate of hCyp-18. The pseudopeptide Suc-Ala-Glyψ(PO_2Et-N)Pro-Phe-*p*NA **1b** bound to hCyp-18 ($K_d = 20 \pm 5\ \mu$M) and was able to selectively inhibit its PPIase activity ($IC_{50} = 15 \pm 1\ \mu$M) but not hFKBP-12, another important PPIase. Deprotection of the phosphonamidate moiety resulted in a complete lack of inhibition. We previously demonstrated that reduction of the Phe-*p*NA moiety caused a quantitative reduction of the affinity; however, Suc-Ala-Glyψ(PO_2Et-N)Pro-Pheψ-(CH_2-NH)*p*NA **7b** still bound and inhibited hCyp-18, suggesting that the Glyψ(PO_2Et−N)Pro motif plays the major role in the binding to cyclophilin. Consequently, we propose compound **1b** as being a novel transition-state mimic inhibitor of hCyp-18.

Introduction

Immunophilins are abundant and ubiquitous peptidyl-prolyl isomerases (PPIases) which specifically catalyze the cis−trans interconversion of the amino acyl−prolyl amide bond.[1] As a consequence, they play a critical role in protein folding and in several related biological processes, such as cellular multiplication[2] and communication.[3] hCyp-18, a cytosolic member of the human cyclophilin subfamily, is also the main receptor of the immunosuppressive undecapeptide cyclosporin A and, hence, is implicated in immunosuppression.[4] Moreover, hCyp-18 is involved in the infection of T-cells by HIV-1, the pathological agent of AIDS, and, hence, participates in the control of virus multiplication.[5−7] Therefore, the design of specific inhibitors of hCyp-18 arouses considerable interest for the development of new drugs, in particular, of anti-AIDS agents. Several strategies have been proposed for the development of cyclophilin inhibitors as novel therapeutics. These include non-immunosuppressive analogues of cyclosporin,[8−11] short modified peptides derived from the viral Gag polyprotein,[12] nonisomerizable substrate analogues,[13,14] and ground-state analogue inhibitors.[15] Here, we present pseudopeptides mimicking the transition state[16] of the PPIase activity as selective inhibitors of cyclophilin.

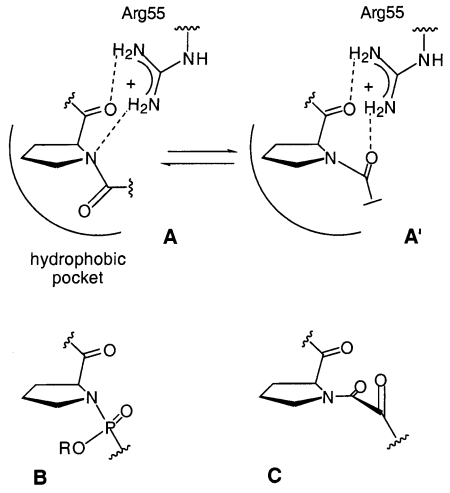
The mechanism of cis−trans isomerization is still unclear, but experimental data suggest that the transition state is a deconjugated amide (ketoamine form).[17] The catalyzed isomerization is globally favored by the hydrophobic environment of the active site.[18] The structure of the complex of hCyp18 with tetrapeptide Suc-Ala-Ala-Pro-Phe-*p*NA shows that Arg55 located at the catalytic subsite strongly binds the carboxyl at the P1′ C-terminus. In addition, this arginine could drive the pyramidalization of nitrogen (a prerequisite to the amide hyperpolarization) by binding the pyrrolidine nitrogen at P1′ via an H-bond.[17] Arg55 might also assist the rotation of the acyl moiety by a transient interaction (Scheme 1, A and A′).[19−23] This hypothesis is supported by results from Janda and co-workers[24] and Schultz and collaborators[25] who generated PPIase catalytic antibodies using a ketoamide-containing hapten. In the latter work, a ketoamide-containing transition-state analogue inhibitor was developed. This compound, however, is not selective and inhibits both hCyp-18 and hFKBP-12, another important PPIase.[25,26] Nevertheless, these data indicate that by mimicking a rotating amide bond, novel transition-state analogue inhibitors of hCyp-18 might be able to be designed. For this pupose, it is important to study the differences found in the various PPIase subfamilies. The hypothesis of a tetrahedral covalent intermediate has been ruled out for the cis−trans isomerization process catalyzed by hCyp-18, while the crystallographic structure of a complex between a dipeptide and Pin1, a human parvulin implicated in the control of mitosis[27] and involved in neurodegeneration,[28] suggests the existence of a tetrahedralized carbonyl carbon at P1.[29] Moreover, Pin1 possesses several nucleophilic residues that might participate in the catalysis.[29] On the other hand, FKBP does not possess a polar

† Part of this work was presented at the 26th European Peptide Symposium (Montpellier, France, 2000); see: *Peptides 2000*; Martinez, J., Fehrentz, J.-A., Eds.; EDK: Paris, France, 2001; pp 809−810.

* To whom correspondence should be addressed. Tel: (33) 169 08 52 25. Fax: (33) 169 08 90 71. E-mail: christophe.dugave@cea.fr.

10.1021/jm020865i CCC: $22.00
Published on Web 07/25/2002

Scheme 1. Proposed Mechanism of the hCyp-18-Catalyzed Isomerization: (A) Arg55-Assisted Deconjugation of the Amide via an H-Bond with the Guanidinium Side Chain and Pyramidalization of the Proline Nitrogen, (A′) Arg55-Assisted Rotation of the Acyl Group via an H-Bond with the Guanidinium Side Chain, (B) the Phosphonamide Transition-State Analogue Which Might Mimic both Putative Transition States A and A′, and (C) Ketoamide Transition-State Analogue Which Is Supposed To Mimic the Rotating Acyl Moiety



residue at the active site and interacts with its substrate in a mode that is completely different from that of hCyp-18 and Pin1.[19]

Phosphinic or phosphonamide amino acyl-proline surrogates might mimic the rotating amide bond. We recently reported the synthesis and biochemical evaluation of the phosphinic pseudopeptides Ac-Alaψ(PO$_2$R-CH)Pro-*p*CMA (R = H or CH$_3$). Though the phosphinic moiety is chemically stable and resistant to proteolysis, these peptides are not able to inhibit the PPIase activity of hCyp-18.[30] We investigated the potential of phosphonamide surrogates of Gly-Pro as novel transition-state mimics of PPIase activity. Though these surrogates are less chemically resistant than their phosphinic equivalent,[31] the phosphonamide nitrogen is slightly pyramidalized,[32] and therefore, its doublet is available for establishing a strong H-bond with the guanidinium moiety of Arg55 (Scheme 1, B).

We report herein on the synthesis and biochemical evaluation of phosphonamide-containing pseudopeptides as transition-state analogue inhibitors of hCyp-18. The corresponding ketoamide was prepared as a reference for the test tube assays (Scheme 1, C).

Experimental Section

Abbreviations. Alaψ(COCO-N)Pro, ((*S*)-2-oxo-3-aminobutanoyl)proline; DCC, dicyclohexylcarbodiimide; DIPEA, diisopropylethylamine; Glyψ(COCO-NH)Pro, (2-oxo-3-aminopropanoyl)proline; Glyψ(PO$_2$R-NH)Pro, ethyl aminomethylphosphonylproline (R = Et) or aminomethylphosphonylproline sodium salt (R = Na); HOBT, 1-hydroxybenzotriazole; *p*CMA, 4-carboxymethylaniline; Pheψ(CH$_2$-NH)*p*NA, 1-(4-nitrophenyl)amino-(*R*)-2-benzyl-2-aminoethane; *p*NA, 4-nitroaniline; SD, standard deviation; Suc, succinyl; THF, tetrahydrofuran; Valψ(COCO-NH)Pro, ((*S*)-2-oxo-3-amino-4-methylpentanoyl)-proline.

All the reagents employed were of analytical grade and were purchased from Aldrich Chemical Co. or Sigma. Amino acids and coupling reagents were obtained from Novabiochem, Bachem, or Sigma. Suc-Ala-Gly-Pro-Phe-*p*NA, Suc-Ala-Ala-Pro-Phe-*p*NA, Suc-Ala-Ala-Pro-Arg-*p*NA, and Suc-Ala-Leu-Pro-Phe-*p*NA were purchased from Bachem. Suc-Ala-Ala-Pro-Phe-DFA was synthesized as previously reported.[34] All other solvents were of analytical grade and were used without further purification. All intermediates were purified by flash chromatography on 40−60 μm (230−400 mesh) Merck silica gel. Peptides were purified by RP-HPLC. All compounds were characterized by ^{1}H, ^{13}C, and ^{31}P NMR (recorded on a Bruker AVANCE 250 NMR spectrometer); δ values are given in parts per million (ppm), and *J* values are in hertz. Electrospray mass spectra (Atheris Laboratories, Geneva, Switzerland) were recorded on a Micromass Platform II (Micromass, Altrincham, U.K.). Fluorimetric determination of K_d was done using a Jasco FP-750 spectrofluorometer equipped with a 250 μL thermostated cell. Kinetic assays were performed using a Kontron Uvikon 930 spectrophotometer equipped with a 2 mL thermostated cell. The recombinant hCyp-18 was obtained as previously reported.[12]

Pht=GlyΨ(PO$_2$Et-N)Pro-Phe-*p*NA (3a and 3b). Compound **2** (2.97 g, 10 mmol) was refluxed with phosphorus pentachloride (2.41 g, 1.1 equiv) in freshly dried benzene (15 mL) for 7 h under argon. After removal of the solvent under reduced pressure, the crude product dissolved in freshly dried THF (10 mL) was added dropwise to a solution of Pro-Phe-*p*NA (0.5 equiv) and triethylamine (3.48 mL, 2.5 mmol) in freshly dried THF. The mixture was stirred overnight under argon at room temperature. After evaporation of the solvent, the crude product was dissolved in dichloromethane and washed twice with 10% citric acid, saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate, and the volume was reduced in vacuo. Purification by silica gel flash chromatography (eluent, 65:35 ethyl acetate: chexane ratio) gave **3a** (939 mg, 1.48 mmol) and **3b** (576 mg, 0.91 mmol) in 48% overall yield.

3a: ^{1}H NMR (CDCl$_3$) δ 7.84 (d, *J* = 5.1, 2H) + 7.63 (d, *J* = 7.1, 2H) + 7.42−7.17 (m, 12H), 5.05−4.94 (m, 1H), 4.33−4.02 (m, 5H), 3.81 (dd, *J* = 14.1, *J′* = 4.6, 1H), 3.53−3.45 (bm, 1H), 3.29−3.15 (m, 1H), 3.10 (dd, *J* = 14.1, *J′* = 12.3), 2.09−2.04 + 1.80−1.75 + 1.62−1.51 + 1.30−1.22 (4m, 4H), 1.42 (t, *J* = 7.0, 3H); ^{13}C NMR (CDCl$_3$) δ 172.7 + 170.5 + 168.0, 144.1 + 142.8 + 137.8 + 134.4 + 130.9 + 129.1 + 128.5 + 126.7 + 124.1 + 123.3 + 118.9, 62.7 (d, J^2_{C-P} = 7.2), 61.4 (d, J^2_{C-P} = 1.1), 53.5, 47.5 (d, J^2_{C-P} = 2), 36.8, 33.7 (d, J^1_{C-P} = 141.3), 31.3 (d, J^3_{C-P} = 7.5), 25.6 (d, J^3_{C-P} = 7.2), 16.4 (d, J^3_{C-P} = 6.0); ^{31}P NMR (CDCl$_3$, decoupled) δ 25.8; DCI-MS (NH$_3$) *m*/*z* 651 (MNH_4^+, 9%), 634 (MH^+, 100%); combustion analysis ($C_{31}H_{32}N_5O_8$) C, H, N.

3b: ^{1}H NMR (CDCl$_3$) δ 8.09 (d, *J* = 9.25, 2H) + 7.96 (d, *J* = 9.25, 2H), 7.88−7.85 + 7.78−7.75 (2m, 4H), 7.29−7.25 + 7.18−7.14 (5H), 5.01−4.92 (m, 1H), 4.21−4.07 (m, 5H), 3.83−3.71 + 3.66−3.46 + 3.23−3.04 (3m, 4H), 2.41−2.20 + 2.09−1.81 + 1.74−1.51 (3m + 4H), 1.19 (t, *J* = 7, 3H); ^{13}C NMR (CDCl$_3$) δ 172.4 + 170.4 + 167.2, 144.5 + 143.3 + 136.3 + 134.6 + 131.6 + 129.4 + 128.9 + 127.3 + 124.5 + 123.8 + 119.9, 61.7 (d, J^2_{C-P} = 6.2), 61.4 (d, J^2_{C-P} = 6.5), 53.4, 48.6 (d, J^2_{C-P} = 2.6), 37.2, 32.5 (d, J^1_{C-P} = 137.9), 31.7 (d, J^3_{C-P} = 7.7), 25.2 (d, J^3_{C-P} = 7.5), 16.3 (d, J^3_{C-P} = 6.8); ^{31}P NMR (CDCl$_3$, decoupled) δ 24.2; DCI-MS (NH$_3$) *m*/*z* 651 (MNH_4^+, 14%), 634 (MH^+, 100%); combustion analysis ($C_{31}H_{32}N_5O_8$) C, H, N.

Fmoc-Ala-GlyΨ(PO$_2$Et-N)Pro-Phe-*p*NA (4a and 4b). Compounds **3a** (0.63 g, 1 mmol) and **3b** (0.5 g, 0.79 mmol) were treated with 1.0 M hydrazine in THF (4.0 equiv) overnight at room temperature. The mixture was filtered, and the cake was washed with THF (3 times). The crude product was dissolved in dry dichloromethane (10 mL) and treated at 0 °C with Fmoc-alanine (9 equiv), DCC (9.9 equiv), HOBT (9 equiv), and DIPEA (18 equiv). The mixture was stirred overnight at room temperature. The crude mixture was filtered and washed with 10% citric acid (2 times), saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate. Purification by silica gel flash chromatography (eluent, 65:35

ethyl acetate:cyclohexane ratio) gave **4a** (690 mg, 88%) and **4b** (580 mg, 92%).

4a: ^{1}H NMR ($CDCl_3$) δ 8.10 (d, J = 7.5, 2H) + 7.88 (d, J = 9.0, 2H) + 7.75 (d, J = 7.3, 2H) + 7.54 (d, J = 7.2, 2H) + 7.42−6.80 (bm, 9H), 5.48−5.45 (m, 1H), 4.97−4.93 (m, 1H), 4.41−4.30 + 4.20−3.92 (2bm, 8H), 3.47−3.44 + 3.22−3.07 (2bm, 4H), 1.97−1.80 + 1.75−1.53 (2b, 4H), 1.38−1.20 (bm, 6H); ^{13}C NMR ($CDCl_3$) δ 173.8 + 173.5 + 173.4 + 171.3, 156.9 + 156.2, 144.3−118.8 (complex), 67.3, 62.1, 61.4 (d, J^2_{C-P} = 6.6), 54.9, 53.7 + 47.0, 50.4 + 49.1 + 46.7, 37.2, 36.3 (d, J^1_{C-P} = 147.3), 31.4 (d, J^3_{C-P} = 7.2), 25.8 (d, J^3_{C-P} = 7.3), 18.0, 16.5 (d, J^3_{C-P} = 5.9); ^{31}P NMR ($CDCl_3$, decoupled) δ 26.5 (minor) + 27.1 (major); DCI-MS (NH_3) m/z 797 (MH^+, 30%), 415 ([Pro-Phe-*p*NA]H^+, 35%), 383 ([Fmoc-Ala-CH_2PO_2Et]H^+, 100%).

4b: ^{1}H NMR ($CDCl_3$) δ 8.08 (d, J = 9.3), 7.91 (d, J = 9.3), 7.78−7.64 + 7.57−7.50 + 7.46−7.11 (bm, 13H), 5.40−5.31 (m, 1H), 4.97−4.87 (m, 1H), 4.49−3.96 (2bm, 8H), 3.61−3.32 + 3.11−2.98 (2bm, 4H); ^{13}C NMR ($CDCl_3$) δ 173.4 + 172.9 + 170.3, 157.4 + 156.1 + 155.7, 144.2−110.8 (complex), 67.2, 63.8 (d, J^2_{C-P} = 6.2), 62.0 (d, J^2_{C-P} = 4.5) + 61.0 (d, J^2_{C-P} = 6.9), 53.9, 50.9 + 47.7 + 47.0 + 47.0 (d, J^2_{C-P} = 9.2), 36.9, 34.1 (d, J^1_{C-P} = 141.8), 31.6 (d, J^3_{C-P} = 6.9), 25.5 (d, J^3_{C-P} = 8.2), 18.1, 16.2 (d, J^3_{C-P} = 6.8); ^{31}P NMR ($CDCl_3$, decoupled) δ 27.6; DCI-MS (NH_3) m/z 797 (MH^+, 13%), 415 ([Pro-Phe-*p*NA]H^+, 29%), 383 ([Fmoc-Ala-CH_2PO_2Et]H^+, 100%).

Suc-Ala-GlyΨ(PO_2Et-N)Pro-Phe-*p*NA (1a and 1b). Compounds **4a** (500 mg, 0.79 mmol) and **4b** (450 mg, 0.58 mmol) were treated with 20% diisopropylamine in DMF for 3 h at room temperature. After evaporation of the solvent under reduced pressure, methanol was chased over the product (3 times). The crude product was treated with succinic anhydride (2.0 equiv) and trietylamine (3.0 equiv) overnight at room temperature. After evaporation of the solvent under reduced pressure, the crude product was purified by RP-HPLC (semipreparative column Vydac C_{18}; rate, 4 mL min^{-1}; linear gradient, acetonitrile:acetic acid (0.25% in water) 25:75 to 75:25 in 30 min).

1a (50 mg, 12%): t_R 11.09 min (95%); ^{1}H NMR (CD_3OD) δ 8.19 (d, J = 9.0) + 7.84 (d, J = 9.2), 7.36−7.16 (bm, 5H), 4.90−4.82 (m, 1H), 4.39 (q, J = 7.2, 1H), 4.28−4.17 (m, 1H), 4.06−3.90 (m, 2H), 3.83−3.69 (m, 2H), 3.30−3.10 (bm, 4H), 2.68−2.55 + 2.55−2.41 (bm, 4H), 2.19−2.03 + 1.84−1.52 (2bm, 4H), 1.35 (d, J = 7.2, 3H), 1.26 (t, J = 7.0, 3H); ^{13}C NMR (CD_3OD) δ 176.4 + 175.3 + 175.2 + 174.6 + 172.4, 145.7 + 144.7 + 138.5 + 130.5 + 129.5 + 127.9 + 125.7 + 120.7, 62.7 (d, J^2_{C-P} = 3.6), 62.3 (d, J^2_{C-P} = 6.8), 56.7, 50.5, 38.5, 37.3 (d, J^1_{C-P} = 142.7), 32.7 (d, J^3_{C-P} = 6.9), 31.4 + 30.2, 26.6 (d, J^3_{C-P} = 6.5), 17.7, 16.6 (d, J^3_{C-P} = 6.4); ^{31}P NMR (CD_3OD, decoupled) δ 25.7; ES-MS (negative ionization) m/z 674.19.

1b (50 mg, 12%): t_R 12.45 min (92%); ^{1}H NMR (CD_3OD) δ 8.19 (d, J = 9.15, 2H) + 7.87 (d, J = 9.15, 2H), 7.33−7.17 (bm, 5H), 4.81−4.73 (m, 2H), 4.34 (q, J = 7.2, 1H), 4.15−3.96 (m, 4H), 3.70−3.56 + 3.26−3.13 (2m, 4H), 2.64−2.47 (bm, 4H), 2.21−2.02 + 1.91−1.69 (2bm, 4H), 1.35 (d, J = 7.5, 3H), 1.24 (t, J = 7.05, 3H); ^{13}C NMR (CD_3OD) δ 176.3 + 175.2 + 175.1 + 174.7 + 172.5, 145.7 + 144.8 + 138.1 + 130.4 + 129.6 + 128.0 + 125.7 + 125.6 + 120.8 (complex), 62.6−62.3 (m), 56.5, 50.2 (d, J^2_{C-P} = 7.3), 38.4, 35.6 (d, J^1_{C-P} = 144.9), 32.8 (d, J^3_{C-P} = 7.0), 31.4 + 30.3, 26.3 (d, J^3_{C-P} = 7.5), 17.7, 16.6 (d, J^3_{C-P} = 5.8); ^{31}P NMR (CD_3OD, decoupled) δ 25.8; ES-MS (negative ionization) m/z 674.30.

Ac-Ala-GlyΨ(PO_2Et-N)Pro-PheY(CH_2-NH)*p*NA (7a and 7b). Compounds **6a** (210 mg, 0.26 mmol) and **6b** (208 mg, 0.26 mmol) were treated with 20% diisopropylamine in DMF for 3 h at room temperature. After evaporation of the solvent under reduced pressure, methanol was chased over the product (3 times). The crude product was treated with acetyl chloride (2.0 equiv) and DIPEA (3.0 equiv) overnight at room temperature. After evaporation of the solvent under reduced pressure, the crude product was purified by preparative thin-layer chromatography (eluent, chloroform:methanol with 98:2, then 97:3, and finally 95:5 ratios) or by RP-HPLC (semipreparative column Vydac C_{18}; rate, 4 mL min^{-1}; linear gradient, acetonitrile:acetic acid (0.25% in water) 25:75 to 75:25 in 30 min) to give **7a** (121 mg, 77%) and **7b** (128 mg, 82%).

7a: t_R 12.59 (>95%); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 9.2, 2H), 7.51 (bd, J = 7.95, 1H), 7.33−7.13 (m, 5H), 6.50 (d, J = 9.2, 2H), 6.30 (bd, J = 7.1, 1H), 6.19 (bt, J = 5.6, 1H), 4.44 (t, J = 7.0, 1H), 4.40−4.31 (m, 1H), 4.02−3.88 (m, 5H), 3.41−3.25 + 3.10−3.01 + 2.97−2.93 (3m, 6H), 1.99 (s, 3H), 2.04−1.94 + 1.81−1.62 (2m, 4H), 1.36 (d, J = 7.0, 3H), 1.26 (t, J = 7.0, 3H); ^{13}C NMR ($CDCl_3$) δ 173.8 + 172.7 + 172.6 + 170.5, 137.4 + 129.1 + 128.6 + 128.5 + 127.7 + 126.8 + 126.4 + 126.3 + 110.9, 61.6 (d, J^2_{C-P} = 4.15), 61.2 (d, J^2_{C-P} = 6.8), 50.3 + 49.1, 47.5 (d, J^2_{C-P} = 3.6), 46.2, 38.1, 34.8 (d, J^1_{C-P} = 143.3), 31.1 (d, J^3_{C-P} = 6.5), 25.1 (d, J^3_{C-P} = 6.9), 23.0, 17.9, 16.3 (d, J^3_{C-P} = 6.3); ^{31}P NMR ($CDCl_3$, decoupled) δ 27.1; ES-MS 602.4.

7b: t_R 11.90 (>95%); ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 10.0, 2H), 7.55−7.46 (b, 1H), 7.34−7.15 (m, 5H), 6.51 (d, J = 10.0, 2H), 6.44 (d, J = 7.5, 1H), 6.16−6.08 (b, 1H), 4.50−4.29 (m, 2H), 4.23−3.84 (m, 5H, Ha), 3.68−3.55 + 3.43−3.22 + 3.18−2.97 (3m, 6H), 2.04 (minor) + 2.02 (major) (s, 3H), 2.11−1.99 + 1.81−1.62 (2m, 4H), 1.34 (d, J = 7.5, major) + 1.30 (d, J = 5.0, minor), 1.26 (t, J = 5.0, major) + 1.25 (t, J = 5.0, minor); ^{13}C NMR ($CDCl_3$) δ 174.5 + 173.0 + 172.8 + 170.4, 137.6 + 137.4 + 129.1 + 128.5 + 126.7 + 110.9, 61.8 (d, J^2_{C-P} = 6.8), 60.9 (d, J^2_{C-P} = 3.6), 50.2 + 49.0, 47.3 (d, J^2_{C-P} = 3.4), 46.4, 38.1, 36.2 (d, J^1_{C-P} = 146.0), 31.5 (d, J^3_{C-P} = 7.1), 25.7 (d, J^3_{C-P} = 6.8), 23.1, 17.9, 16.3 (d, J^3_{C-P} = 6.2); ^{31}P NMR ($CDCl_3$, decoupled) δ 26.7; ES-MS m/z 602.3.

Ac-Ala-GlyΨ(PO_2Bn-N)Pro-Phe-*p*CMA (11). Compound **10** (85 mg, 87 μmol) was treated with 20% diisopropylamine in DMF for 3 h at room temperature. After evaporation of the solvent under reduced pressure, methanol was chased over the product (3 times). The crude product was treated with acetyl chloride (13 mL, 2.0 equiv) and DIPEA (45 mL, 3.0 equiv) overnight at room temperature. After evaporation of the solvent under reduced pressure, the crude product was purified by preparative thin-layer chromatography (eluent, 94:6 chloroform:methanol ratio) to give **11** (31 mg, 52%): ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 10.0, 2H) + 7.84 (d, J = 10.0, 2H), 7.68 (d, J = 7.5, 1H) + 7.37−7.18 (m, 9H), 5.96 (d, J = 7.3, 1H), 5.00 (AB, δ_A = 5.07, δ_B = 4.94, J_{AB} = 11.8) + 5.00 (AB, δ_A = 5.03, δ_B = 4.97, J_{AB} = 11.7) (m, 2H), 4.85−4.96 + 4.42−4.34 + 4.08−4.05 + 3.94−3.84 (4m, 5H), 3.87 (s, 3H), 3.51−2.95 (m, 4H), 1.79 (s, 3H), 1.80−1.73 + 1.54−1.48 (2m, 4H), 1.26 (d, J = 7.1, 3H); ^{13}C NMR ($CDCl_3$) δ 173.7 + 173.2 + 171.0 + 170.7 + 166.6, 142.6 + 137.7 + 136.0 + 135.9 + 130.6 + 129.3 + 128.8 + 128.4 + 128.0 + 126.7 + 125.4 + 119.3, 66.7 (d, J^2_{C-P} = 7.0), 61.3 (d, J^2_{C-P} = 2.3), 54.9 + 52.0 + 48.6 + 47.0, 36.6, 36.6 (d, J^1_{C-P} = 146.0), 31.1 (d, J^3_{C-P} = 7.0), 25.2 (d, J^3_{C-P} = 7.0), 22.8, 16.9; ^{31}P NMR ($CDCl_3$, decoupled) δ 28.1; ES-MS m/z 691.5.

Ac-Ala-GlyΨ(PO_2H-N)Pro-Phe-*p*CMA (12). Compound **11** (23 mg, 33 μmol) dissolved in a 50:50 methanol/water mixture (7 mL) was treated with sodium bicarbonate (8 mg, 2.8 equiv) and 10% Pd-C (11 mg) under 4 bar of hydrogen for 2 h. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The crude product was dissolved in a 50:50 water/acetonitrile mixture and freeze-dried. Compound **12** was used without further purification: ^{1}H NMR (CD_3OD) δ 8.01−7.92 (m, 4H), 7.32−7.19 (m, 5H), 4.90−4.78 (m), 4.32 (q, J = 7.0, 1H), 4.00−3.93 (m, 1H), 3.88 (s, 3H), 3.64 (dd, J = 15.2, J' = 11.8, 1H) + 3.52 (dd, J = 13.9, J' = 4.0, 1H) + 3.30−3.19 (m, 1H) + 3.01−2.91 (m, 1H), 1.96 (s, 3H), 1.90−1.75 + 1.65−1.50 (2m, 4H), 1.29 (d, J = 5.0, 3H); ^{13}C NMR (CD_3OD) δ 178.5 + 174.4 + 174.3 + 173.1 + 173.0 + 168.3, 144.3 + 139.1 + 131.1 + 130.2 + 129.5 + 127.7 + 126.6 + 121.7, 63.6, 56.3 + 52.5 + 50.5, 38.3, 37.3 (d, J^1_{C-P} = 128.9), 32.6, 26.3 (d, J^3_{C-P} = 5.5), 22.5, 17.9; ^{31}P NMR (CD_3OD, decoupled) δ 16.4; ES-MS (negative ionization) m/z 601.4.

Biochemical Evaluation. (1) Determination of K_d. Fluorimetric titration was carried out as previously reported.[12,33]

(2) Enzymatic Assays. The PPIase uncoupled assay was carried out in a 35 mM Hepes buffer (pH 7.8) at 10 ± 0.5 °C using Suc-Ala-Ala-Pro-Phe-DFA as a substrate.[34] The PPIase

trypsin-hCyp-18-coupled assay was carried out in a 35 mM Hepes buffer (pH 8.6) at 10 ± 0.5 °C using Suc-Ala-Ala-Pro-Arg-*p*NA,[3,35] and the α-chymotrypsin-FKBP-coupled assay was carried out in a 35 mM Hepes buffer (pH 7.8) at 10 ± 0.5 °C using Suc-Ala-Leu-Pro-Phe-*p*NA as a substrate.[21,36] Data were processed according to the literature.[12]

Results and Discussion

Design Rationale. Transition-state analogues were designed on the basis of information obtained from crystallographic data and structure−activity relationship studies. We chose a substrate of immunophilins, the tetrapeptide Suc-Ala-Ala-Pro-Phe-*p*NA,[36] as a template for the development of novel hCyp-18 inhibitors. Several structures of hCyp-18−peptide complexes have been resolved and the main interactions delineated.[17] Structure−activity relationship studies also provided interesting information; we recently demonstrated[37] the existence of two functionally independent subsites: (i) the S1′ subsite which contains the catalytic residue and controls the selectivity for proline at P1′ and (ii) the S2′−S3′ subsite which specifically recognizes the Xaa-*p*NA moiety or Xaa-*p*NA surrogates.[35,37] Therefore, the P1′ proline is dispensable for the binding of a ligand by hCyp-18 provided the peptide possesses either an Xaa-*p*NA or an Xaa-*p*NA analogue at P2′−P3′.[37] However, *p*NA-containing peptides without either a proline residue or a proline mimic cannot inhibit hCyp-18.[37,38] The N-terminus of the tetrapeptide does not participate in the binding because it protrudes outside the catalytic site. The binding is mainly controlled by the carboxyl at P2′ which might establish a strong H-bond with Trp121.[37] The residues at P1, P1′, and P2′ must be in the L-configuration.[35] Cyclophilins prefer Ala, Val, Leu, Glu, Phe, or Gly at P1, whereas FKBPs recognize preferentially Phe-Pro or Leu-Pro. In particular, the selectivity factor for hCyp-18 versus hFKBP-12 is 3500 and 1000 for Glu and Gly, respectively, but only 60 for Ala at P1′.[21] Though Glu is preferred at P1, hCyp-18 is indeed quite selective of the Gly-Pro moiety. The introduction of the Glyψ(PO_2Et-NH)Pro motif generates only one novel stereogenic center and, hence, provides only two diastereomers, whereas the Glu analogue would give a mixture of four diastereomers. Therefore, we designed the tetrapeptide analogue **1** (mixture of both diastereomers **1a** and **1b**) which contains a phosphonamide isostere of Gly-Pro.[32]

Synthesis of Pseudopeptides. Phosphonamidate-containing pseudopeptides have been widely used in medicinal chemistry. However, to our knowledge, there is only one example of synthesis of phosphonamide isosteres of the Xaa-Pro motif reported in the literature.[39] Diethyl phthalimidomethylphosphonate **2** was prepared according to Yamauchi et al.[40] Activation with phosphorus pentachloride in refluxing benzene, as previously described,[40] and coupling to Pro-Phe-*p*NA yielded the diastereomeric mixture of **3a** and **3b** which were separated by silica gel flash chromatography (Scheme 2). Hydrazinolysis of the phthalimide and subsequent coupling with Fmoc-alanine using DCC and HOBT gave the corresponding pseudopeptides **4a** and **4b** in good yields. Removal of the Fmoc group and succinylation led to the target compounds **1a** and **1b** which were purified by RP-HPLC (Scheme 2). The corresponding phosphonamidate could not be obtained by saponification of the ethyl esters **1a** and **1b**. This might be due to the sensitivity of the Phe-*p*NA moiety to the harsh basic conditions employed to hydrolyze phosphonamidic esters. Therefore, compounds **7a** and **7b** were prepared by a similar route starting from Pheψ-(CH_2-NH)*p*NA, a reduced equivalent of Phe-*p*NA which has an enhanced chemical stability.[25] The peptides were capped with an acetyl group in order to avoid a possible intramolecular hydrolysis of the acid-sensitive phosphonamide bond[39] as observed with compounds **1a** and **1b**. However, we did not succeed in deprotecting the ethyl ester of pseudopeptides **7a** and **7b**. Finally, we protected the phosphonamidate moiety with a benzyl ester which can be removed by hydrogenolysis.[41] This strategy implied a replacement of the *p*NA C-terminus with a *p*CMA equivalent which is resistant to hydrogenolysis and does not affect the affinity for hCyp-18.[37] The phosphonamidate **11** was obtained from dibenzyl ester **8** as depicted in Scheme 2. Due to the low chemical stability of protonated phosphonamidates, the benzyl phosphonamidate was selectively deprotected by hydro-

Scheme 2. Synthesis of Suc-Ala-Glyψ(PO_2Et-N)Pro-Phe-*p*NA **1a**/**b**, Ac-Ala-Glyψ(COCO-N)Pro-Pheψ(CH_2-NH)*p*NA **7a**/**b**, and Ac-Ala-Glyψ(PO_2Na-N)Pro-Phe-*p*CMA **12**

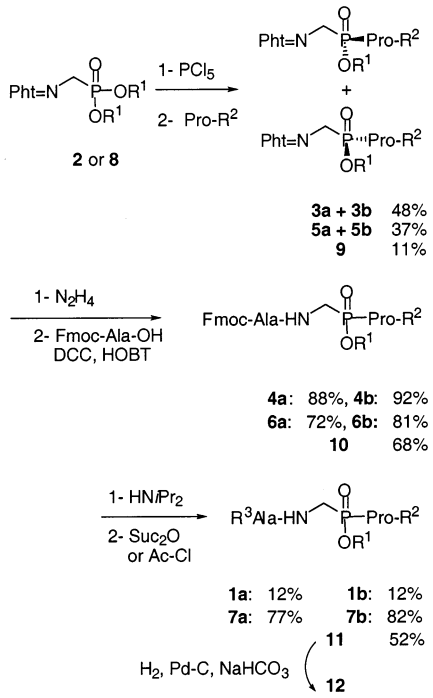


Table 1. Synthesis of Suc-Ala-Glyψ(PO_2Et-N)Pro-Phe-*p*NA **1a**/**b**, Ac-Ala-Glyψ(COCO-N)Pro-Pheψ(CH_2-NH)*p*NA **7a**/**b**, and Ac-Ala-Glyψ(PO_2Na-N)Pro-Phe-*p*CMA **12**

R^1	R^2	R^3	compounds
Et	Phe-*p*NA	Suc	**1a/1b**
Et	−	−	**2**
Et	Phe-*p*NA	−	**3a/3b**
Et	Phe-*p*NA	−	**4a/4b**
Et	Pheψ(CH_2-NH)*p*NA	−	**5a/5b**
Et	Pheψ(CH_2-NH)*p*NA	−	**6a/6b**
Et	Pheψ(CH_2-NH)*p*NA	Ac	**7a/7b**
Bn	−	−	**8**
Bn	Phe-*p*CMA	−	**9**
Bn	Phe-*p*CMA	−	**10**
Bn	Phe-*p*CMA	Ac	**11**
H	Phe-*p*CMA	Ac	**12**

Table 2. Biochemical Evaluation of Pseudopeptides **1a/b**, **7a/b**, **12**, and **13**

compounds	$K_d \pm$ SD (μM)[a]	$IC_{50} \pm$ SD (μM)[b]
Suc-Ala-Gly-Pro-Phe-*p*NA	140 ± 10	1450 ± 60
Suc-Ala-Glyψ(PO_2Et-N)Pro-Phe-*p*NA **1a**	210 ± 100	5400 ± 400
Suc-Ala-Glyψ(PO_2Et-N)Pro-Phe-*p*NA **1b**	20 ± 5	15 ± 1
Ac-Ala-Glyψ(PO_2Et-N)Pro-Pheψ(CH_2-NH)*p*NA **7a**	1300 ± 200	no inhibition at 500 μM[c]
Ac-Ala-Glyψ(PO_2Et-N)Pro-Pheψ(CH2-NH)*p*NA **7b**	200 ± 15	30% inhibition at 500 μM[c]
Ac-Ala-Glyψ(PO_2^--N)Pro-Phe-*p*CMA **12**	79 ± 4	no inhibition at 100 μM[d]
Ac-Ala-Glyψ(COCO-N)Pro-Phe-*p*NA **13**	127 ± 7	215 ± 40
Suc-Ala-Ala-Pro-Phe-*p*NA	135 ± 20	540 ± 70
Ac-Ala-Ala-Pro-Phe-*p*NA	145 ± 15	640 ± 120

[a] K_d values were deduced from the fluorescence titration curves of the hCyp-18 Trp121 upon addition of inhibitors[12,33] ($n = 2$). [b] IC_{50} values were obtained using the standard trypsin-coupled PPIase assay[13,35,36] at 10 °C ($n = 2$). [c] Due to the significant absorbance at 390 nm of Pheψ(CH_2-NH)-*p*NA-containing peptides **7a/b**, the IC_{50} values were evaluated using the standard uncoupled PPIase assay[34] at 10 °C; the maximum inhibitor concentration employed was 0.5 mM due to residual absorbance at 246 nm. [d] Because of its low solubility, the maximum inhibitor concentration employed was 0.1 mM.

genolysis in the presence of sodium bicarbonate, and compound **12** was isolated as a sodium salt and used without further purification.[41] The ketoamide analogue **13** was synthesized starting from racemic 3-aminolactate as previously reported.[12]

Biochemical Evaluation of Compounds 1a/b, 7a/b, 12, and 13. We first checked that the Ala to Gly change at P1 did not significantly modify the affinity. For this purpose, Suc-Ala-Gly-Pro-Phe-*p*NA was tested as a ligand of hCyp-18 by fluorescence titration.[12,33,37] As expected, the K_d was similar to those observed for other Phe-*p*NA-terminated peptides. We have previously demonstrated that efficient binding at the S2′−S3′ subsite of cyclophilin does not imply that the compound is able to inhibit the PPIase activity occurring at the S1′ subsite.[37] Consequently, we tested the ability of the peptides to inhibit the PPIase activity of hCyp-18 using the standard trypsin-coupled assay with Suc-Ala-Ala-Pro-Arg-*p*NA as a substrate.[13] The Ala to Gly substitution caused a 3-fold increase in the IC_{50} value (Table 2). This might be related to the particular behavior of the Gly-Pro moiety.[20]

Pseudopeptides **1a/b**, **7a/b**, **12**, and **13** were tested as ligands of hCyp-18. The results, summarized in Table 2, show that peptides **1a** and **13** bind the enzyme with an affinity equivalent to that of the model substrate. Pseudopeptide **1b** is a good ligand of hCyp-18 ($K_d = 20$ μM). Evaluation of the IC_{50} value demonstrated that compound **1b** is a good inhibitor of cyclophilin ($IC_{50} = 15 \pm 1$ μM): Replacement of the amide with an ethyl phosphonamidate surrogate resulted in an improvement in the IC_{50} value by 2 orders of magnitude. Time-dependent inhibition experiments demonstrated that compound **1b** is not a slow-binding inhibitor of cyclophilin because the IC_{50} value does not significantly change after 2 h of preincubation. In contrast, diastereomer **1a** is a poor inhibitor of the PPIase activity. Absolute configuration of the phosphorus stereogenic center seems to be critical for the interaction, suggesting that the ethyl phosphonamidate moiety tightly interacts at the S1′ subsite.

Compound **1b** at concentrations up to 1 mM did not inhibit the human FK506-binding protein hFKBP-12. Although compound **1b** is slightly less potent than the ketoamide-containing peptide developed by Schultz and co-workers,[25] it selectively inhibits hCyp-18.

We have also investigated the influence of a charged phosphonamidate isostere (peptide **12**) on the biochemical activity. Though peptide **12** binds cyclophilin, no inhibition of the PPIase activity was observed at 100 μM. This is probably due to the introduction of a negative charge because N-terminal modification does not affect the biological properties of the peptides.[35] Therefore, compound **12** is likely to interact with hCyp-18 via the Phe-*p*CMA moiety.[37]

An important question to be addressed was the real influence of the phosphonamide motif on the potency of the inhibitor. Therefore, compounds **7a** and **7b** bearing a reduced amide at P2′ were tested as well. Reduction of the C-terminal amide has been shown to dramatically affect both the affinity and inhibitory potency of model peptides.[37] Not surprisingly, we noticed that the reduction of the important Phe-*p*NA moiety caused a fall in affinity by 1 order of magnitude relative to that of the corresponding amide. However, **7b**, the reduced equivalent of **1b**, still inhibited hCyp-18. The reduction of the amide resulted in a 50-fold increase in the IC_{50} value for the most active compound **7b** versus **1b**; this probably reflects the lack of interaction of the compound at the S2′−S3′ subsite.[37] As observed with compounds **1a** and **1b**, a strong difference in efficiency was observed for both diastereomers. These data strongly suggest that **7b** mainly binds cyclophilin via an interaction at the S1′ subsite. Therefore, the Glyψ(PO_2Et-N)-Pro moiety plays a major role in the interaction. Even though the affinity of compound **1b** is modest compared to those of nanomolar inhibitors of proteases, the results reported herein suggest that the phosphonamide-proline pattern might be a transition-state mimic of the PPIase activity of hCyp-18.

To compare between phosphonamide and ketoamide, we tested peptide 1**3** which contains a glycine analogue of the ketoamide motif Alaψ(COCO-N)Pro, a canonical transition-state mimic of PPIases.[25] We were surprised to notice that compound **13** inhibits hCyp-18 with a relatively low efficiency (Table 2). Several reasons may be given for this lack of potency: (i) the conformation of the Glyψ(COCO-N)Pro moiety, related to the particular behavior of the Gly-Pro moiety compared to other Xaa-Pro motifs,[20] and (ii) a putative tendency of the aminopyruvyl motif to enolize, facilitated by the absence of electron-donating groups at C3 of the aminopyruvate.

In conclusion, we have reported the design, synthesis, and biochemical evaluation of Glyψ(PO_2Et-N)Pro-containing peptides as novel transition-state analogues of the PPIase activity of hCyp18. The biochemical assays have shown that pseudopeptide **1b** is a good and selective inhibitor of cyclophilin, whereas the Glyψ-

(COCO-N)Pro equivalent has only a moderate affinity for the enzyme. This suggests that the phosphonamide motif is an interesting mimic of the rotating deconjugated amino acyl−proline amide bond. These results open the way to the development of more potent, selective inhibitors of hCyp-18.

Acknowledgment. This work was supported by SIDACTION (Grant 70000016/2), the Agence Nationale de Recherches sur le Sida (ANRS) (Grant 99014), and the Atomic Energy Commission (CEA). Dr. Enrico Stura is gratefully acknowledged for critical reading of the manuscript.

Supporting Information Available: Experimental procedures and characterizations of pseudopeptides **7**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Galat, A.; Rivière, S. Peptidyl-prolyl cis−trans isomerases. In *The Protein Profile Series*; Sheterline, P., Ed.; Oxford Univerity Press: New York, 1998.
(2) Yaffe, M. B.; Schutkowski, M.; Shen, M.; Zhou, X. Z.; Stukenberg, P. T.; Rahfeld, J.-U.; Xu, J.; Kuang, J.; Kirschner, M. W.; Fischer, G.; Cantley, L. C.; Lu, K. P. Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science* **1997**, *278*, 1957−1960.
(3) Helekar, S. A.; Patrick, J. Peptidyl prolyl cis−trans isomerase activity of cyclophilin A in functional homo-oligomeric receptor expression. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 5432−5437.
(4) Hamilton, G. S.; Steiner, J. P. Immunophilins: beyond immunosuppression. *J. Med. Chem.* **1998**, *41*, 5119−5143.
(5) Luban, J. Absconding with the chaperone: essential Cyclophilin-Gag interaction in HIV-1 virions. *Cell* **1996**, *87*, 1157−1159.
(6) Endrich, M. M.; Gehrig, P.; Gehring, H. Maturation-induced conformational changes of HIV-1 capsid protein and identification of two high affinity sites for cyclophilins in the C-terminal domain. *J. Biol. Chem.* **1999**, *274*, 5326−5332.
(7) Braaten, D.; Luban, J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T-cells. *EMBO J.* **2001**, *20*, 1300−1309.
(8) Ke, H.; Mayrose, D.; Cao, W. Crystal structure of cyclophilin A complexed with substrate Ala-Pro suggests a solvent-assisted mechanism of cis−trans isomerization. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3324−3328.
(9) Bartz, S. R.; Hohenwalter, E.; Hu, M.-K.; Rich, D.; Malkovsky, M. Inhibition of human immunodeficiency virus replication by nonimmunosuppressive analogs of cyclosporin A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5381−5385.
(10) Steinkasserer, A.; Harrison, R.; Billich, A.; Hammerschmid, F.; Werner, G.; Wolf, B.; Peichl, P.; Palfi, G.; Schnitzel, W.; Mlynar, E.; Rosenwirth, B. Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus type 1 (HIV-1): interference with early and late events in HIV-1 replication *J. Virol.* **1995**, *69*, 814−824.
(11) Billich, A.; Hammerschmid, F.; Peichl, P.; Wenger, R.; Zenke, G.; Quesniaux, V.; Rosenwirth, B. Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: Interference with HIV protein-Cyclophilin A interactions. *J. Virol.* **1995**, *69*, 2451−2461.
(12) Li, Q.; Moutiez, M.; Charbonnier, J.-B.; Vaudry, K.; Ménez, A.; Quéméneur, E.; Dugave, C. Design of a Gag-pentapeptide analog which binds the human cyclophilin A more efficiently than the entire capsid protein: New insights for the development of novel anti HIV-1 drugs. *J. Med. Chem.* **2000**, *43*, 1770−1779.
(13) Schutkowski, M.; Wöllner, S.; Fischer, G. Inhibition of peptidyl-prolyl cis/trans isomerase activity by substrate analog structures: thioxo tetrapeptide-4-nitroanilides. *Biochemistry* **1995**, *34*, 13016−13026.
(14) Hart, S. A.; Etzkorn, F. A. Cyclophilin inhibition by a (*Z*)-alkene cis-proline mimic. *J. Org. Chem.* **1999**, *64*, 2998−2999.
(15) Wang, H. C.; Kim, K.; Bakthiar, R.; Germanas, J. P. Structure−activity studies of ground- and transition-state analog inhibitors of cyclophilin. *J. Med. Chem.* **2001**, *44*, 2593−2600.
(16) Mader, M. M.; Bartlett, P. A. Binding energy and catalysis: The implication for transition-state analogs and catalytic antibodies. *Chem. Rev.* **1997**, *97*, 1281−1301.
(17) Zhao, Y.; Ke, H. Crystal structure implies that Cyclophilin predominantly catalyzes the *trans* to *cis* isomerization. *Biochemistry* **1996**, *35*, 7356−7361.
(18) Fischer, G. Peptidyl-prolyl *cis/trans* isomerases and their effectors. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1415−1436 and references therein.
(19) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. Inhibition of FKBP rotamase activity by immunosuppressant FK506: twisted amide surrogate. *Science* **1990**, *248*, 863−866.
(20) Reimer, U.; El Mokdad, N.; Schutkowski, M.; Fischer, G. Intramolecular assistance of cis/trans isomerization of the histidine-proline moiety. *Biochemistry* **1997**, *36*, 13802−13808.
(21) Harrison, R. K.; Stein, R. L. Mechanistic studies of peptidyl prolyl *cis-trans* isomerase: evidence for catalysis by distortion. *Biochemistry* **1990**, *29*, 1684−1689.
(22) Harrison, R. K.; Stein, R. L. Mechanistic studies of enzymic and nonenzymic prolyl *cis-trans* isomerization *J. Am. Chem. Soc.* **1992**, *114*, 3464−3471.
(23) Cox, C.; Lectka, T. Intramolecular catalysis of amide isomerization: kinetic consequences of the 5-NH−N$_a$ hydrogen bond in prolyl peptides. *J. Am. Chem. Soc.* **1998**, *120*, 10660−10668.
(24) Yli-Kauhaluoma, J. T.; Ashley, J. A.; Lo, C.-H. L.; Coakley, J.; Wirsching, P.; Janda, K. D. Catalytic antibodies with peptidyl-prolyl *cis-trans* isomerase activity. *J. Am. Chem. Soc.* **1996**, *118*, 5496−5497.
(25) Ma, L.; Hsieh-Wilson, L.; Schultz, P. G. Antibody catalysis of peptidyl-prolyl *cis-trans* isomerization in the folding of RNase T1. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7251−7256.
(26) Hamilton, G. S.; Steiner, J. P. Neuroimmunophilin ligands and novel therapeutics for the treatment of degenerative disorders of the nervous system. *Curr. Pharm. Des.* **1997**, *3*, 405−428.
(27) Lu, K. P.; Hanes, S. D.; Hunter, T. A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* **1996**, *380*, 544−547.
(28) Lu, P.-J.; Wulf, G.; Zhou, X. Z.; Davies, P.; Lu, K. P. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* **1999**, *399*, 784−788.
(29) Ranganathan, R.; Lu, K. P.; Hunter, T.; Noel, J. P. Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* **1997**, *89*, 875−886.
(30) Demange, L.; Dugave, C. Synthesis of phosphinic alanyl-proline surrogates Alaψ(PO$_2$R-CH)Pro as potential inhibitors of the human cyclophilin hCyp-18. *Tetrahedron Lett.* **2001**, *42*, 6295−6297.
(31) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.
(32) Radkiewicz, J. L.; McAllister, M. A.; Goldstein, E.; Houk, K. N. A theoretical investigation of phosphonamidates and sulfonamides as protease transition-state isosteres. *J. Org. Chem.* **1998**, *63*, 1419−1428.
(33) Liu, J.; Chen, C.-M.; Walsh, C. T. Human and *Escherichia coli* Cyclophilins: sensitivity to inhibition by the immunosuppressant cyclosporin A correlates with a specific tryptophan residue. *Biochemistry* **1991**, *30*, 2306−2310.
(34) Janovski, B.; Wöllner, S.; Schutkowski, M.; Fischer, G. A protease-free assay for peptidyl prolyl *cis/trans* isomerases using standard peptide substrates. *Anal. Biochem.* **1997**, *252*, 299−307.
(35) Schiene, C.; Reimer, U.; Schutkowski, M.; Fischer, G. Mapping the stereospecificity of peptidyl prolyl *cis/trans* isomerases. *FEBS Lett.* **1998**, *432*, 202−206.
(36) Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich, D. H. Determination of kinetic constants for peptidylprolyl *cis-trans* isomerases by an improved spectrophotometric assay. *Biochemistry* **1991**, *30*, 6127−6134.
(37) Demange, L.; Moutiez, M.; Vaudry, K.; Dugave, C. Interaction of the human cyclophilin hCyp-18 with short peptides suggests the existence of two functionally independent subsites. *FEBS Lett.* **2001**, *505*, 191−195.
(38) Scholz, C.; Scherer, G.; Mayr, L. M.; Schindler, T.; Fischer, G.; Schmid, F. X. Prolyl isomerases do not catalyze isomerization of non-prolyl peptide bonds. *Biol. Chem.* **1998**, *379*, 361−365.
(39) McLeod, D. A.; Brinkworth, R. L.; Ashley, J. A.; Janda, K. D.; Wirshing, P. Phosphonamidates and phosphonamidate esters as HIV-1 protease inhibitors. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 653−658.
(40) Yamauchi, K.; Kinoshita, M.; Imoto, M. Peptides containing aminophosphonic acids. II. The synthesis of tripeptide analogs. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2531−2534.
(41) Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. Synthesis and biological evaluation of phosphonamidate peptide inhibitors of enkephalinase and angiotensin-converting enzyme. *J. Med. Chem.* **1985**, *28*, 1208−1216.
(42) Reimer, U.; Scherer, G.; Drewello, M.; Kruber, S.; Schutkowski, M.; Fischer, G. Side-chain effects on peptidyl-prolyl cis/trans isomerization. *J. Mol. Biol.* **1998**, *279*, 449−462.

JM020865I