



0040-4039(94)01270-9

## FLUOROOLEFIN PEPTIDE ISOSTERES - TOOLS FOR CONTROLLING PEPTIDE CONFORMATIONS

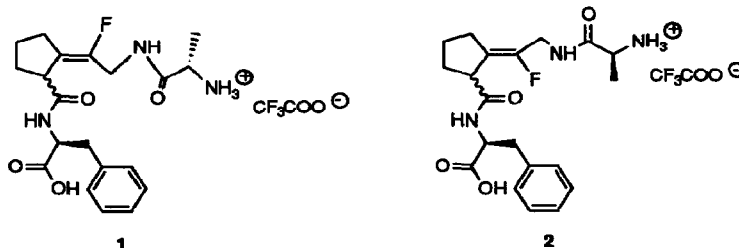
Livia G. Boros<sup>a</sup>, Bart De Corte<sup>a</sup>, Rayomand H. Gimi<sup>a</sup>, John T. Welch<sup>\*a</sup>,  
Yang Wu<sup>b</sup>, Robert E. Handschumacher<sup>b</sup>

<sup>a</sup>Department of Chemistry, University at Albany, State University of New York, Albany, NY 12222. <sup>b</sup>Department of Pharmacology, Yale University, School of Medicine, New Haven, CT 06510.

**Abstract:** Fluoroolefin dipeptide isosteres were synthesized applying the Peterson reaction as a novel method for fluoroolefination. The dipeptide isosteres were elaborated to provide the conformationally constrained analogs (1-(*R*), 1-(*S*) and 2-(*R*), 2-(*S*)) of the Suc-Ala-Gly-Pro-Phe-pNA tetrapeptide, a synthetic substrate of cyclophilin.

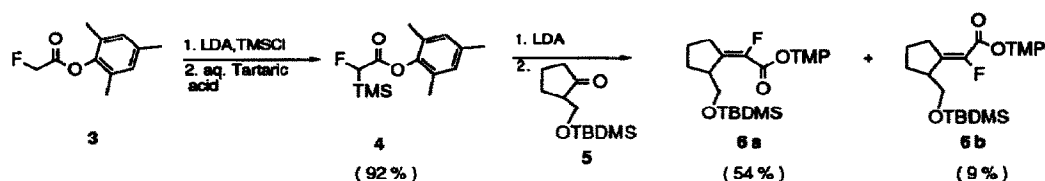
Cyclophilins, the major binding proteins for the immunosuppressant cyclosporin A, catalyze the *cis-trans* isomerization of X<sub>aa</sub>-Pro bonds of peptides.<sup>1</sup> This peptidyl-prolyl isomerase (PPIase) activity may have an important -though still unknown- cellular function that could be related to protein folding<sup>2</sup> or be involved in regulatory processes.<sup>3</sup>

Since X-ray data and dipole moment calculations suggest that the fluoroolefin is an excellent steric and electronic mimic for the peptide bond,<sup>4</sup> our goal was to synthesize the isosteres 1-(*R*), 1-(*S*), 2-(*R*), 2-(*S*) of the Suc-Ala-Gly-Pro-Phe-pNA tetrapeptide, a synthetic substrate of cyclophilin.<sup>5</sup> The conformation of the Gly-Pro amide bond was fixed by the *cis* and *trans* fluoroolefin amide bond mimic. These conformationally defined peptide isosteres will be employed in the examination of the mechanism of the PPIase activity of cyclophilin.

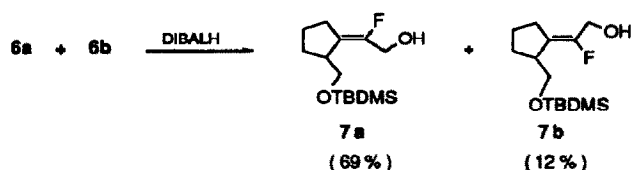


The fluoroolefin building block was synthesized applying the Peterson olefination<sup>6</sup> of the 2-hydroxymethylcyclopentanone derivative<sup>7</sup> 5 with  $\alpha$ -fluoro- $\alpha$ -silylacetic acid ester 4. Preparation of compound 4 from the  $\alpha$ -fluoroacetate<sup>8</sup> 3 required two steps: bisilylation by excess lithium diisopropylamide and chlorotrimethylsilane followed by hydrolysis of the *O*-silyl group with aqueous

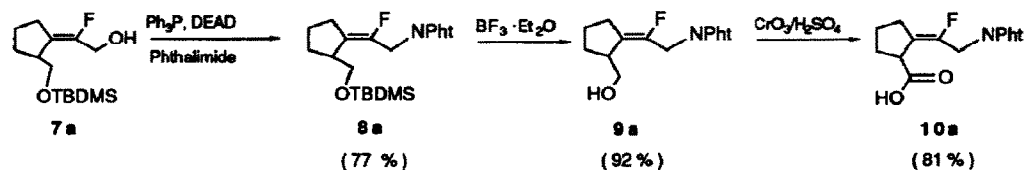
tartaric acid solution. The Peterson olefination yielded a 6:1 mixture of **6a** and **6b**, the (*E*)- and (*Z*)-isomers.<sup>9</sup> The major product was the (*E*)-isomer **6a** as was confirmed by single crystal X-ray diffraction studies on the phthalimide protected alcohol **9a**, the product of a later step in the synthesis. To obtain the (*E*)- and (*Z*)-Gly-Ψ[CF=C]-(*R,S*)-Pro dipeptide isosteres the ester group of **6a** and **6b** was transformed into a primary amine. Subsequently the TBDMS-protected alcohol function was oxidized to the carboxylic acid.



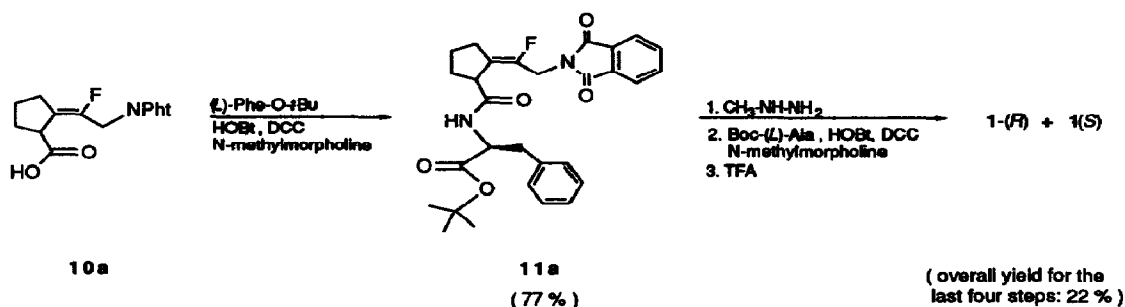
The mixture of the isomeric esters **6a** and **6b** was reduced with diisobutylaluminum hydride to the corresponding alcohols. The isomers **7a** and **7b** were separated at this stage by chromatography on silica gel.<sup>10</sup>



The Mitsunobu transformation of the hydroxyl group yielded the protected amine. Treatment of **7a** with triphenylphosphine, diethyl azodicarboxylate (DEAD), and phthalimide resulted in the formation of **8a** in excellent yield. The robust protection afforded by the phthalimide was required so that the substrate could tolerate the forthcoming oxidative transformation without decomposition. Removal of the TBDMS-group with boron trifluoride etherate provided the *N*-phthalimide protected alcohol **9a**. Oxidation of the alcohol **9a** readily occurred upon treatment with the Jones reagent to produce the *N*-protected dipeptide isostere **10a** with good yield.



Our original plan required interchange of the phthalyl group for the Fmoc protecting group to facilitate elongation of the dipeptide isostere via solid phase peptide synthesis. However the Fmoc-protected dipeptide mimic formed in a poor yield that wasn't responsive to optimization. Therefore we decided to block the acid terminus first. Coupling the acid **10a** with (*L*)-phenylalanine-*tert*-butyl ester using dicyclohexylcarbodiimide, in the presence of 1-hydroxy-benzotriazole and *N*-methylmorpholine led to the bisprotected tripeptide mimic **11a**. The phthaloyl group was removed by stirring compound **11a** in excess methylhydrazine at room temperature for 48 hours. The free amine of the tripeptidomimetic was then coupled with Boc-(*L*)-Alanine to get the diastereomeric mixture of the protected tetrapeptide isosteres. After separating the two diastereomers by column chromatography (1:1 hexanes/ethyl acetate;  $R_f$  (first diastereomer) = 0.40;  $R_f$  (2nd diastereomer) = 0.31), deprotection of the Boc- and *tert*-butyl group with trifluoroacetic acid of each diastereomer provided the optically active tetrapeptide isosteres **1-(*R*)** and **1-(*S*)** as their trifluoroacetate salts.<sup>11</sup> The same synthetic route with similar reaction conditions was employed to obtain the minor, (*Z*)-isomers: **2-(*R*)** and **2-(*S*)**.<sup>12</sup>



In conclusion a new synthesis of fluoroolefin peptide isosteres, via the Peterson olefination reaction, has been reported. (*E*)- and (*Z*)-(*S*)-Ala-Gly-Ψ[CF=C]-(*S*)-Pro-(*S*)-Phe, and (*E*)- and (*Z*)-(*S*)-Ala-Gly-Ψ[CF=C]-(*R*)-Pro-(*S*)-Phe tetrapeptide isosteres were synthesized. In a preliminary PPIase assay<sup>13</sup> with cyclophilin enzyme and Suc-Ala-Ala-Pro-Phe-pNA substrate, three of the four synthetic molecules; the two (*E*)-isomers and one of the two (*Z*)-isomers were inhibitory. The difference in the inhibitory potency of both (*Z*)-isomers corresponding to the trans configuration of the natural peptide suggests targets for further structural modifications to produce more effective inhibitors.

**Acknowledgement.** Financial support by the National Science Foundation Grant No. CHE-8901986, NIH-GM 47858, American Cancer Society, BE18 and DARPA contract DAAL 0398KO198 is gratefully acknowledged.

## References and Notes

1. Handschumacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. J. *Science* **1984**, *226*, 544; Fischer, G.; Bang, H.; Mech, C. *Biomed. Biochim. Acta* **1984**, *10*, 1101; Fischer, G.; Wittmann-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476; Takahashi, N.; Hayanno, T.; Suzuki, M. *Nature* **1989**, *337*, 473.
2. Lang, K.; Schmid, F. X.; Fischer, G. *Nature* **1987**, *329*, 268; Lang, K.; Schmid, F. X. *Nature* **1988**, *331*, 453; Lilie, H.; Lang, K.; Rudolph, R.; Buchner, J. *Protein Science* **1993**, *2*, 1490.
3. Kordel, J.; Drakenberg, T.; Forsen, S.; Thulin, E. *FEBS Lett.* **1990**, *263*, 27; Evans, P. A.; Dobson, C. M.; Kautz, R. A.; Hatfull, G.; Fox, R. O. *Nature* **1987**, *329*, 266; Liu, J.; Farmer, J. D.; Lane, W. S.; Friedmann, J.; Weissmann, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
4. Ellison, S. L. R. PhD. Dissertation, University of Liverpool, Liverpool, England, **1984**; Abraham, R. J.; Ellison, S. L. R.; Schonholzer, P.; Thomas, W. A. *Tetrahedron* **1986**, *42*, 2101.
5. Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 3813-3816; Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684.
6. Peterson, D. J. *J. Org. Chem.* **1968**, *33*, 780; Hudrlík, P. F.; Peterson, D. *Tetrahedron Lett.* **1974**, 1133; Hudrlík, P. F.; Peterson, D. *J. Am. Chem. Soc.* **1975**, *97*, 1464; Application of the reaction for fluoroolefination: Welch, J. T.; Herbert, R. W. *J. Org. Chem.* **1990**, *55*, 4782.
7. 2-(*t*-Butyldimethylsilyloxymethyl)-cyclopentanone **5** was prepared via the 2-hydroxymethylation of cyclopentanone with aqueous formaldehyde in the presence of calcium hydroxide, and the subsequent protection of the OH group with *t*-butyldimethylchlorosilane in the presence of imidazole. The overall yield was 15%. bp 74 °C (0.95 mmHg). IR (neat) 2957(s), 2883(s), 2858(s), 1745(s), 1472(s), 1407(m), 1361(m), 1255(s), 1118(s).
8. The starting material 2,4,6-trimethylphenyl fluoroacetate **3** was prepared from sodium fluoroacetate in two steps. Treatment of sodium fluoroacetate with phthaloyl dichloride resulted in the formation of fluoroacetyl chloride in 80% yield. Fluoroacetyl chloride was reacted with 2,4,6-trimethylphenoxide at 0 °C, producing 2,4,6-trimethylphenyl- $\alpha$ -fluoroacetate **3** in 82% yield.  
Mp: 68-70 °C; 282.203 MHz  $^{19}\text{F}$ NMR (CDCl<sub>3</sub>)  $\delta$  ppm -230.15 (t,  $J_{\text{C,F}} = 47.2$  Hz).
9. The selectivity of the reaction was determined by the integral ratios of the  $^{19}\text{F}$  NMR-peaks.
10. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
11. For compound **1**: one of the two diastereomers ( $R_f = 0.395$  of the protected tetrapeptide)  
282.203 MHz  $^{19}\text{F}$  NMR (CD<sub>3</sub>OD)  $\delta$  ppm -108.7 (br); 300.0 MHz  $^1\text{H}$ NMR (CD<sub>3</sub>OD)  $\delta$  ppm 7.35-7.16 (m, 5H, H-aromatic), 4.70-4.65 (m, 1H, CHCH<sub>2</sub>Ph) 4.02 (dd, 1H,  $J_{\text{H,F}} = 22.6$  Hz,  $J_{\text{H,H}} = 15.2$  Hz, CFCH<sub>2</sub>), 3.86 (dd, 1H,  $J_{\text{H,F}} = 14.1$  Hz,  $J_{\text{H,H}} = 7.1$  Hz, CFCH<sub>2</sub>), 3.69 (t,  $J_{\text{H,F}} = 16.6$  Hz, CHC=), 3.45 (br, 1H, CHCH<sub>3</sub>), 2.99 (d,  $J_{\text{H,F}} = 9.8$  Hz, CH<sub>2</sub>Ph), 2.93 (d,  $J_{\text{H,F}} = 9.82$  Hz, CH<sub>2</sub>Ph), 2.40-2.30 (m, 2H, CH<sub>2</sub>C=), 4.95-4.50 (m, 4H, (CH<sub>2</sub>)<sub>2</sub>), 1.45 (d,  $J_{\text{H,F}} = 7.0$  Hz, CH<sub>3</sub>). 75.429 MHz  $^{13}\text{C}$ NMR (D<sub>2</sub>O)  $\delta$  ppm 179.21 (COOH), 173.13 (C=O), 148.5 (d,  $J_{\text{C,F}} = 251$  Hz, CF), 139.40 (CH<sub>2</sub>C-aromatic), 131.66, 131.18, 129.51 (C-aromatic), 124.80 (d,  $J_{\text{C,F}} = 19.4$  Hz, C=CF), 56.94 (CHCH<sub>2</sub>), 51.53 (CHCH<sub>3</sub>), 49.10 (d,  $J_{\text{C,F}} = 5.7$  Hz, CHC=), 40.50 (d,  $J_{\text{C,F}} = 29.3$  Hz, CFCH<sub>2</sub>), 39.22 (CH<sub>2</sub>Ph), 35.43 (CH<sub>2</sub>CH), 30.59 (CH<sub>2</sub>C=), 27.13 (CH<sub>2</sub>), 18.88 (CH<sub>3</sub>); MS (rel. intensity) 394 (3), 393 (22), 392 (M<sup>+</sup>, 100), 252 (10), 245 (67), 176 (13), 150 (49), 120 (8), 91 (17).
12. The absolute configuration of the chiral center of Pro in each of the four diastereomers (**1**-*R*), **1**-*S*), and **2**-*R*), **2**-*S*) has not been solved yet.
13. Kofron, J. L.; Kuzmic, P.; Kishore, V.; Gemmecker, G.; Fesik, S. W.; Rich, D. H. *J. Am. Chem. Soc.* **1992**, 2670.

(Received in USA 13 May 1994; revised 24 June 1994; accepted 28 June 1994)