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FLUOROOLEFIN PEPTIDE ISOSTERES - TOOLS FOR CONTROLLING PEPTIDE CONFORMATIONS

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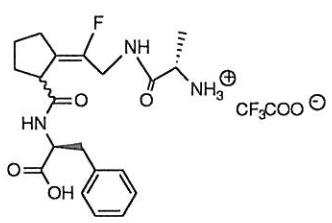
Abstract: Fluoroolefin dipeptide isosteres were synthesized applying the Peterson reaction as a novel method for fluoroolefination. The dipeptide isosteres were elaborated to provide the conformationally constrained analogs (**1**-(*R*), **1**-(*S*) and **2**-(*R*), **2**-(*S*)) of the Suc-Ala-Gly-Pro--Phe-pNA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

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

Since X-ray data and dipole moment calculations suggest that the fluoroolefin is an excellent steric and electronic mimic for the peptide bond,[4] 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.[5] 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.



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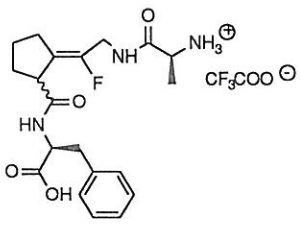
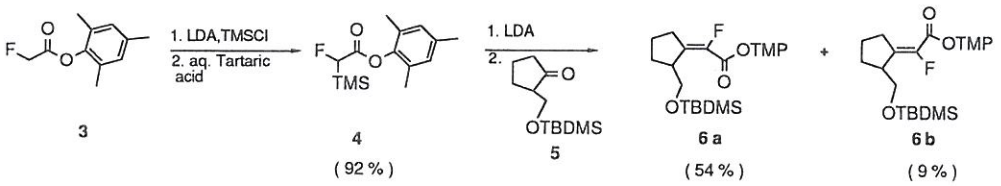


2

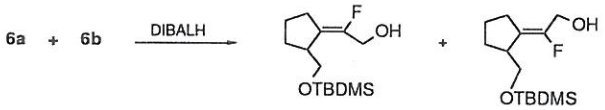
The fluoroolefin building block was synthesized applying the Peterson olefination[6] of the 2-hydroxymethylcyclopentanone derivative[7] **5** with α-fluoro-α-silylacetic acid ester **4**. Preparation of compound **4** from the α-fluoroacetate[8] **3** required two steps: bissilylation by excess lithium 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.[9] 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.



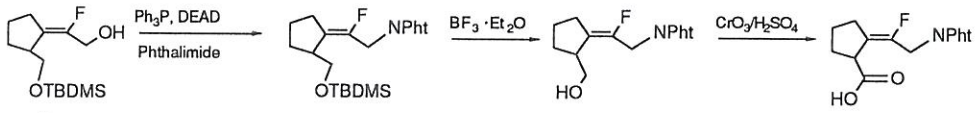
3 **4** (92 %) **5** **6a** (54 %) **6b** (9 %)

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



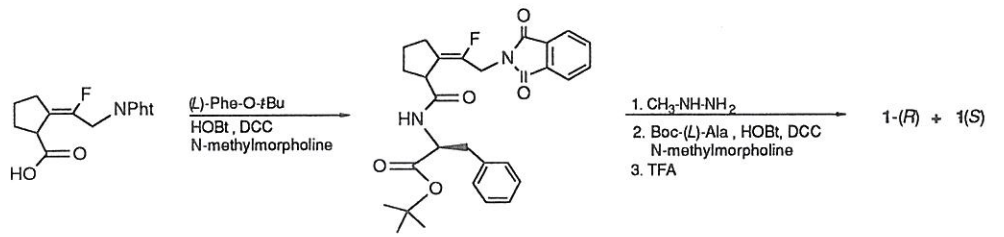
7a (69 %) **7b** (12 %)

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.



7a **8a** (77 %) **9a** (92 %) **10a** (81 %)

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.[11] The same synthetic route with similar reaction conditions was employed to obtain the minor, (*Z*)-isomers: **2**-(*R*) and **2**-(*S*).[12]



10a

11a
(77 %)

(overall yield for the last four steps: 22 %)

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[13] 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.

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7. 2-(*t*-Butyldimethylsilyoxymethyl)-cyclopentanone **5** was prepared via the 2-hydroxymethylation of cyclopentanone with aqueous formaldehyde in the presence of calcium hydroxyde, 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-α-fluoroacetate **3** in 82% yield.

Mp: 68-70 °C; 282.203 MHz ^{19}FNMR ($CDCl_3$) δ ppm -230.15 (t, $J_{C,F}$ = 47.2 Hz).

9. The selectivity of the reaction was determined by the integral ratios of the ^{19}F NMR-peaks.

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11. For compound **1**: one of the two diastereomers (R_f = 0.395 of the protected tetrapeptide)

282.203 MHz ^{19}F NMR (CD_3OD) δ ppm -108.7 (br); 300.0 MHz ^{1}HNMR (CD_3OD) δ ppm 7.35-7.16 (m, 5H, H-aromatic), 4.70-4.65 (m, 1H, C<u>H</u>CH_2Ph) 4.02 (dd, 1H, $J_{H,F}$ = 22.6 Hz, $J_{H,H}$ = 15.2 Hz, CFC<u>H</u>$_2$), 3.86 (dd, 1H, $J_{H,F}$ = 14.1 Hz, $J_{H,H}$ = 7.1 Hz, CFC<u>H</u>$_2$), 3.69 (t, $J_{H,F}$ = 16.6 Hz, C<u>H</u>C=), 3.45 (br, 1H, C<u>H</u>CH_3), 2.99 (d, $J_{H,F}$ = 9.8 Hz, C<u>H</u>$_2$Ph), 2.93 (d, $J_{H,F}$ = 9.82 Hz, C<u>H</u>$_2$Ph), 2.40-2.30 (m, 2H, C<u>H</u>$_2$C=), 4.95-4.50 (m, 4H, $(CH_2)_2$), 1.45 (d, $J_{H,F}$ = 7.0 Hz, CH_3). 75.429 MHz ^{13}CNMR (D_2O) δ ppm 179.21 (COOH), 173.13 (C=O), 148,5 (d, $J_{C,F}$ = 251 Hz, CF), 139.40 (CH_2<u>C</u>-aromatic), 131.66, 131.18, 129.51 (C-aromatic), 124.80 (d, $J_{C,F}$ = 19.4 Hz, <u>C</u>=CF), 56.94 (C<u>H</u>CH_2), 51,53 (C<u>H</u>CH_3), 49.10 (d, $J_{C,F}$ = 5.7 Hz, <u>C</u>HC=), 40.50 (d, $J_{C,F}$ = 29.3 Hz, CF<u>C</u>H_2), 39.22 (<u>C</u>H_2Ph), 35.43 (<u>C</u>H_2CH), 30.59 (<u>C</u>H_2C=), 27.13 (CH_2), 18.88 (CH_3); MS (rel. intensity) 394 (3), 393 (22), 392 (M^+, 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.

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