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SYNTHESIS AND N- AND C-TERMINAL EXTENSION OF PEPTIDYL α, α-DIFLUOROALKYL KETONES.

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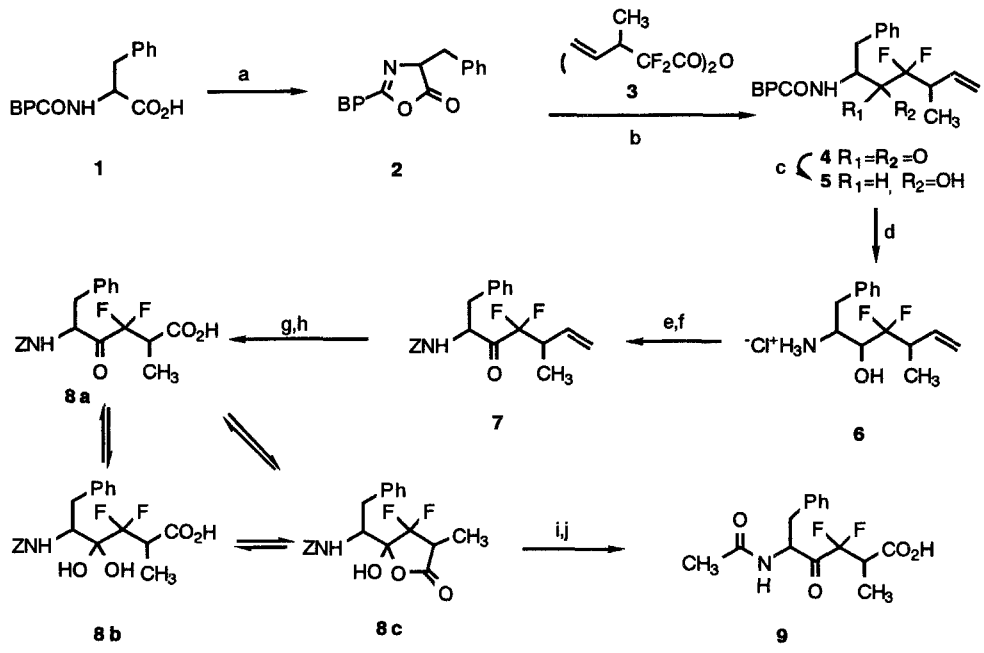
Abstract: A synthesis of the dipeptidyl and tetrapeptidyl α, α-difluoroalkyl ketones is described. The key intermediate **6** can be extended at not only the C-terminal but also the N-terminal.

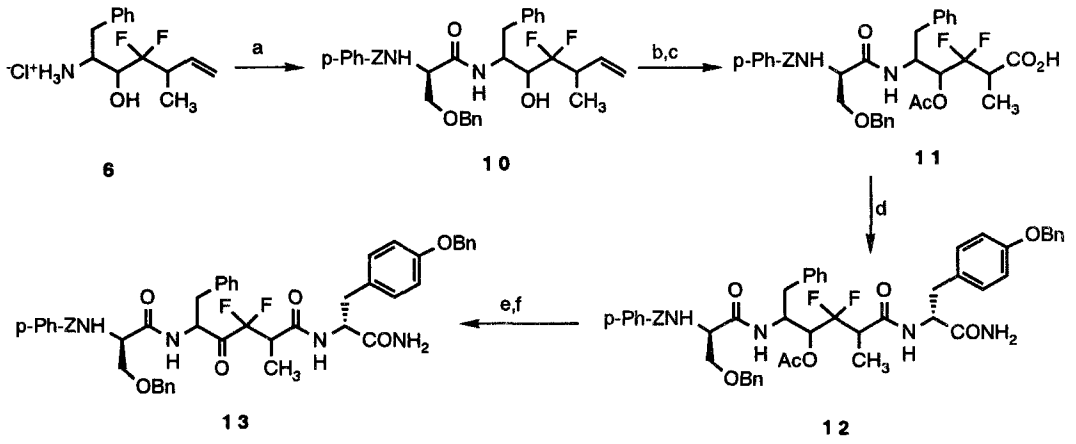
Fluoroalkyl ketones exist in stable hydrated form in aqueous media and as a result serve as ideal mimics of the tetrahedral transition state for peptide bond hydrolysis.[2] Recently Kolb et al disclosed a synthesis of peptidyl difluoroalkyl ketones utilizing a modified Dakin-West procedure.[3] Unfortunately, their synthesis is restrictive as only α-benzamidoalkyl ketones could be prepared from their corresponding oxazolones in reasonable yields. Removal of the benzoyl group requires harsh conditions which can cause side reactions to occur.[4] Therefore the elaboration of large peptides from the α-benzamidoalkyl ketones is impractical as a general scheme. We report herein a versatile synthesis of peptidyl α, α-difluoroalkyl ketones utilizing a p-phenylbenzoylated amino acid which can be easily removed by sodium-amalgam reductive cleavage.[5]

(±)-Phenylalanine was protected by 4-biphenylcarbonyl (BPCO) chloride in 72% yield and the resulting p-phenylbenzoylated amino acid **1** was then cyclized to the corresponding 2-biphenyl-5(4*H*)-oxazolones **2**. The reaction of the oxazolone **2** with the anhydride **3**[6] was accomplished at 60° C for 24 h in toluene followed by reflux with anhydrous oxalic acid.[7] Conversion of the resulting difluoroketone **4** to the alcohol **5** with sodium borohydride, followed by reductive cleavage of the BPCO protective group with 3% sodium amalgam in methanol, maintaining pH 6 by excess sodium phosphate monobasic, gave amine salt **6** in high yield.[8] In a typical experiment, a mixture of BPCO amide **5**, 3% Na-Hg (8 eq), and $NaH_2PO_4 \cdot H_2O$ (12 eq) in methanol was stirred at room temperature for 1 h and filtered into an 1N aqueous HCl solution (8 eq). After removal of the methanol, the precipitate was filtered off and washed with water. The filtrate was lyophilized to give a white solid. This solid was dissolved in excess ethyl acetate and the organic extract was filtered and concentrated to afford the amine salt **6** in 81% yield.[9] Protection of the amino group by *N*-(benzyloxycarbonyloxy)succinimide and Dess-Martin oxidation[10] gave ketone **7**. Ozonolysis followed by Jones oxidation yielded the dipeptidyl analog **8**. As reported earlier for similar systems,[11] this 3-difluoro-4-oxo alkyl acid **8a** should be in equilibrium with its cyclic hemiketal form **8c**. The latter predominates in organic solvents whilst the hydrated keto form **8b** exists in the presence of water. Examination of the spectral data for **8** in methanol-d4 indicated the compound is exclusively the cyclic hemiketal form **8c**.[12] Sequential treatment of **8** with Pd/C and acetic anhydride afforded the final dipeptidyl difluoroalkyl ketone **9**.



a. EDC, CH_2Cl_2, 0°C, 30min, 90%. b. toluene, 60°C, 24h, then oxalic acid, reflux, 15min, 51%. c. $NaBH_4$, EtOH, rt, 5min, 94%. d. 3% Na/Hg, $NaH_2PO_4 \cdot H_2O$, MeOH, rt, 1h, 81%. e. N-(benzyloxycarbonyloxy)succinimide, Et_3N (pH 8), MeOH-H_2O, rt, 30min, 89%. f. Dess-Martin periodinane (3.6eq), CH_2Cl_2, rt, 2h, 95%. g. O_3, CH_2Cl_2, -78°C, 2 min, then Me_2S, rt, 3h, 88%. h. Jones oxidant, acetone, rt, 2h, 77%. i. H_2, 40 psi, 10% Pd/C, MeOH-H_2O-HOAc, 1h, 80%. j. Ac_2O, phosphate buffer (pH 6), CH_3CN, rt, 2h, 90%.

The scope of this synthetic strategy was extended to a tetrapeptidyl difluoroalkyl ketone. Amine salt **6** can be used as a common intermediate. p-Phenylbenzyloxycarbonyl (p-Ph-Z) was used as a protecting group of the N-terminal since other protecting groups including benzyloxycarbonyl turned out to be poorly UV active, which made it difficult to monitor reactions by TLC. The amino group of salt **6** was extended with N-(p-phenylbenzyloxycarbonyl)-O-benzyl-D-serine[13] and the resultant tripeptidyl difluoroalcohol **10** was protected by acetylation. Oxidative cleavage by Sharpless condition[14] gave corresponding acid **11**.[15] The acid **11** was coupled with O-benzyl-D-tyrosinamide under standard condition to give tetrapeptide analog **12**. Base hydrolysis of **12** and Dess-Martin oxidation gave final tetrapeptidyl difluoroalkyl ketone **13**.



a. N-(p-phenylbenzyloxycarbonyl)-O-benzyl-D-serine, EDC, 1-HOBt, NMM, DMF, rt, 2h, 83%. b. Ac_2O, pyridine, DMAP, rt, 1h, 95%. c. $NaIO_4$ (8eq), $RuCl_3 \cdot H_2O$ (0.04eq), CH_3CN-CCl_4-H_2O (2:2:3), rt, 6h, 61%. d. O-benzyl-D-tyrosinamide, EDC, 1-HOBt, DMF, rt, 2h, 76%. e. NH_4OH-dioxane (pH 10), 1h, 92%. f. Dess-Martin periodinane (3.6eq), CH_2Cl_2, rt, 3h, 94%.

In summary, this communication describes a practical synthesis of peptidyl difluoroalkyl ketones and can be extended at not only the C-terminal but also the N-terminal.[16] The biological evaluation of compounds **9** and **13** is in progress.[17]

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References and Notes

1. Address correspondence to this author at E. I. Du Pont De Nemours & Company, Agricultural Products, Stine-Haskell Research Center, P.O. Box 30-S300, Newark, DE 19714.
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4. Acid hydrolyses of benzamido equivalents of **4**, **5** or **8** including methyl and benzyl ethers of equivalent **5** gave no desired products. Other conditions such as base hydrolysis or Me_3OBF_4 treatment were unsuccessful. Also see; N. Peet, J. Burkhart, M. Angelastro, E. Giroux, S. Mehdi, P. Bey, M. Kolb, B. Neises, and D. Schirlin, *J. Med. Chem.,* **1990**, *33,* 394.

5. R. Scribner, *Tetrahedron Lett.*, **1976**, 3856.
6. The anhydride **3** was prepared from the tetrafluoroethylene in 3 steps. see reference 2 and M. Kolb, F. Gerhart, and J. Francois, *Synthesis,* **1988**, 469.; H. Greuter, R. Lang, and A. Romann, *Tetrahedron Lett.*, **1988**, *29,* 3291.
7. For small scale, the Dakin-West procedure worked just as well with toluene as a solvent.
8. The treatment of difluoroketone **4** with 3% Na-Hg under the same condition resulted in complete loss of fluorines as detected by ^{19}F NMR. The use of excess $NaH_2PO_4{\cdot}H_2O$ to maintain pH 6 is essential to avoid side reactions.
9. The amine salt **6** gave the following spectroscopic data: IR (KBr) 3433, 1590 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 7.45-7.25 (5H, m), 5.70-6.00 (1H, m), 5.18-5.35 (2H, m), 4.10-4.30 (1H, four t, J=5.3 Hz), 3.68-3.80 (1H, m), 2.90-3.38 (3H, m), 1.18 (3H, d, J=8.0 Hz); ^{19}F NMR (282 MHz, DMSO-d_6, versus $CFCl_3$) -104.1 (ddd, J=259, 28, 10 Hz), -107.6 (ddd, J=259, 51, 18 Hz); MS (Cl/CH_4) m/e 256.00 (**6**-Cl^-).
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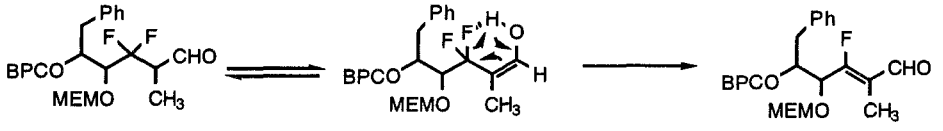
12. The compound **8** gave the following spectroscopic data: IR (neat) 3361, 1810, 1674 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 7.05-7.15 (10H, m), 4.82 (2H, s), 4.32 (1H, m), 3.60 (1H, m), 3.35 and 2.80 (2H, m), 1.15 (3H, two d, J=7.5 Hz); ^{19}F NMR (282 MHz, CD_3OD, versus $CFCl_3$) -112.2 (d, J=250 Hz), -114.5 (d, J=250 Hz), -125.1 (bd, J=245 Hz). The addition of D_2O in this NMR sample decreased -112.2 and -114.5 peak considerably.; MS (Cl/CH_4) m/e 405.987 (MH^+).
13. This compound was protected by p-phenylbenzyloxycarbonyloxysuccinimide which was prepared by treatment of triphosgene (0.3eq) with 4-biphenylmethanol and pyridine in CH_2Cl_2 followed by N-hydroxysuccinimide in 71% overall yield. This protecting group could be easily removed by Pd/C quantitatively.
14. Per H. Carlsen, T. Katsuki, V. Martin, and B. Sharpless, *J. Org. Chem., **1981**, 46,* 3936.
15. It should be noted that a stepwise procedure by ozonolysis followed by reductive workup with dimethylsulfide decomposed slowly to give HF elimination presumably shown for an example below. ^1H NMR showed no peak below 1.5 ppm and two doublet (J=3.0 Hz) at 1.80 ppm.



However this side reaction was not detected in the synthesis of dipeptidyl analog **9** since reductive ozonolysis with O_3 and Me_2S already gave cyclized hydroxy lactol product.
16. A stereospecific synthesis of dipeptidyl α,α-difluoroalkyl ketone was reported. ; D. Damon and D. Hoover, *J. Am. Chem. Soc.*, **1990**, *112*, 6439.
17. The peptidyl α,α-difluoroketones **9** and **13** with proper linkers will be used as haptens to elicit catalytic antibodies and not as inhibitors of proteolytic enzymes. We did not attempt to separate the diastereoisomers because it is more desirable to use racemic antigens for immunization so that there is a greater chance one of them will produce a catalytic antibody. See; S. Pollack, P. Hsiun, and P. Schultz, *J. Am. Chem. Soc.*, **1989**, *111*, 5961.; K. Janda, S. Benkovic, and R. Lerner, *Science*, **1989**, *244*, 437.

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