Tetrahedron Letters, Vol.32, No.49, pp 7255-7258, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

General Synthesis of Polyfunctionalized Fluoromethyleneketone Retroamides as Potential Inhibitors of Thrombin

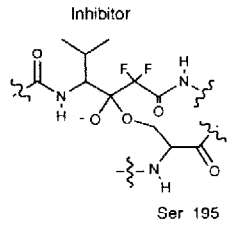
J.M. Altenburger, D. Schirlin*

Marion Merrell Dow Research Institute, 16 rue d'Ankara, 67009 Strasbourg Cedex, France

Abstract: Synthesis of polyfunctionalyzed fluoromethyleneketone retroamides is described. A potential inhibitor of human Thrombin has been prepared through a Reformatsky type condensation from N-protected p.NO2 phenylalaninal. The reaction conditions are applicable to a variety of NO_2 substituted aldehydes.

Trifluoromethyl and difluoromethyleneketone analogues of peptides are effective competitive inhibitors of Serine proteases[1]. X-ray crystallographic analysis of enzyme inhibitor complexes (e.g. porcine pancreatic elastase) have demonstrated the formation of stable hemiketal adducts between the electrophilic fluorinated carbonyl and the essential catalytic Serine residue[2] (scheme I).

scheme I



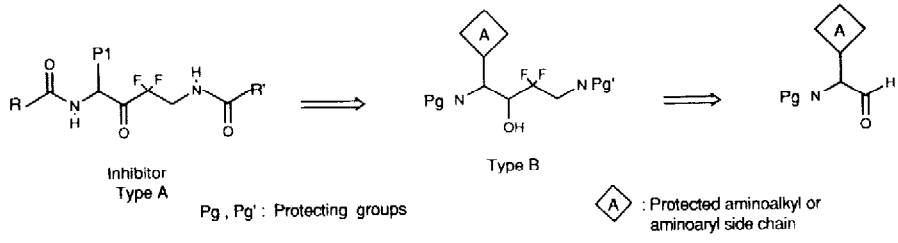
This concept could be extended to the inhibition of Thrombin, a Serine protease that recognizes a basic P_1 residue[3] (preferably arginine, lysine or analogues)[4a,b]. A carboxyterminus trifluoromethylketoarginine derivative has recently been described to quasi irreversibly inhibit Human Thrombin, in vitro[4b].

We wish to report our own approach using the difluoromethyleneketone retroamide[5] concept to design and synthesize potential inhibitors of thrombin with possible interactions on both sides of the internal ketone (inhibitor of type A, scheme II), a concept that has aldready yielded potent and selective inhibitors of aspartate[6], metallo[7] or neutral serine[5] proteases.

A convenient and general methodology had to be developed in order to prepare key intermediates of type B bearing three masked aminofunctions. Three types of selectively removable aminoprotecting groups[8] were selected for our syntheses: benzyloxycarbonyl (Cbz), tert.butoxycarbonyl (BOC), and nitro derivatives (aliphatic or aromatic, see table I).

Intermediates of type B are accessible as shown in scheme II from bisprotected α,ε diaminoaldehydes through the following sequence of reactions[5]: Reformatsky condensation ($BrCF_2CO_2Et$, Zn), ammonolysis (NH_3, ether), reduction [$BH_3.CH_3)_2S$] and protection [$(BOC)_2O$, THF].

scheme II



Reformatsky reactions with NO_2 bearing aldehydes or ketones are known to give poor yields[10] and have to be run using a two step procedure[11]. The organozinc reagent derived from ethyl bromo difluoroacetate[12] had thus to be prepared prior to the addition of the aldehydes. When generated under ultrasonicating conditions[13], at room temperature, under argon, we found that this reagent was stable (in contrast to refluxing conditions)[14] and could even be stored at -18 °C for 24 hours[15].

As shown in table I, addition of the nitro aldehydes[16] to a solution of $BrZnCF_2CO_2Et$ in tetrahydrofuran at room temperature under ultrasonication afforded the expected hydroxy esters **2a-d** in good yields (72-80%). This method (condition D) is remarkably efficient as examplified for o.NO_2benzaldehyde (entry 1, table I), a model substrate, for which yields ranged from O to 80% under the following reaction conditions:

a) one step procedure, reflux temperature[5]: no reaction (condition A).

b) one step, ultrasonication, room temperature: no reaction (condition B).

c) two steps, reflux temperature[5]: 50% yield (condition C).

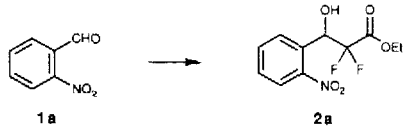
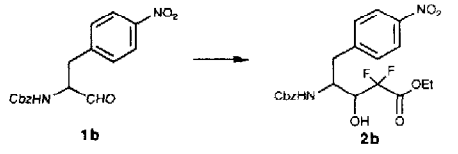
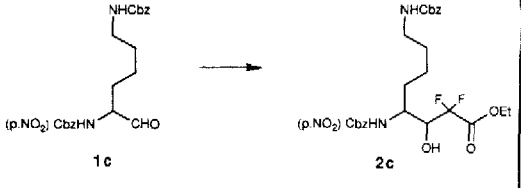
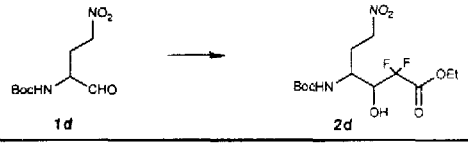
d) two steps, ultrasonication, room temperature: 80% yield (condition D).

Table I

Reformatsky reaction

$O_2N\text{-}R\text{-}CHO \longrightarrow O_2N\text{-}R\text{-}CH(OH)\text{-}CF_2CO_2Et$

1 2

REACTIONS	CONDITIONS A %*	B %*	C %*	D %*
 1a 2a	0	0	50	80
 1b 2b	-	0	30 [5]	72
 1c 2c		0	52 [5]**	72
 1d 2d	-	-	-	74

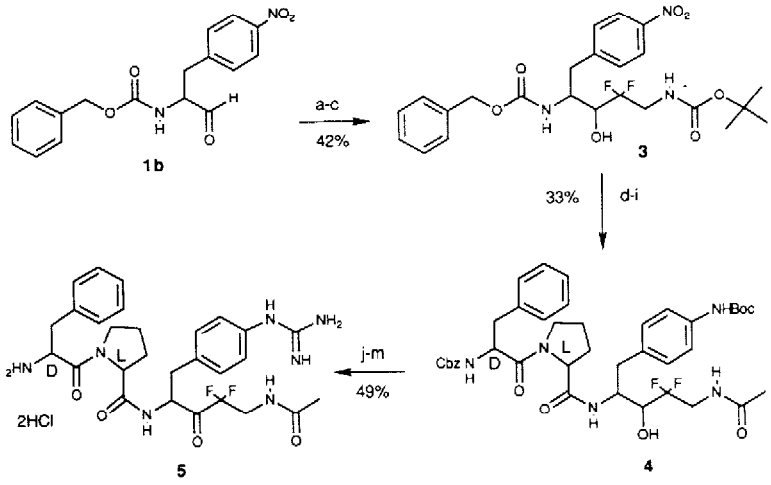
A: one step reflux; B: one step, ultrasonication, RT (13c); C: two steps, reflux; D: two steps, ultrasonication, RT; *: yields of 2 (isolated material); **based on recovered aldehyde.

GENERAL PROCEDURE

A mixture of activated Zinc powder[17] (1.19 g, 18.35 mmol, 3 eq) and iodine (0.025 g, 0.1 mmol) in anhydrous tetrahydrofuran (3 mL) is ultrasonicated for 15 minutes at room temperature under an argon atmosphere. A solution of ethyl bromodifluoroacetate [(3.78 g, 18.35 mmol, 3 eq)] in anhydrous tetrahydrofuran (3 mL) is added dropwise to the zinc suspension. After two more minutes, a solution of aldehyde **1** (1 eq) in anhydrous tetrahydrofuran (3 mL) is added dropwise to the black solution of organozinc reagent. Ultrasonication is maintained for 35 minutes after completion of the addition. Hydrolysis (saturated aqueous ammonium chloride, 30 mL) and ethyl acetate extraction (2 x 100 mL) afford after usual work up and chromatography (silica gel, ethyl acetate/cyclohexane 3:7) the expected hydroxyesters **2**[18].

Thus compound **5**, an analogue of the tetrapeptide D-PheProArgGlyOH, bearing a p.guanidinophenylalanine side chain in P_1 position (rigid isostere of arginine) was synthesized (scheme III) from 2-benzyloxycarbonylamino-3(p.guanidino)phenylpropanal as a potential inhibitor of human Thrombin[19].

scheme III

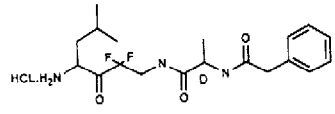


a) 3 eq Zn, 3 eq $BrCF_2CO_2Et$, THF, ultrasonication, 2 steps; b) NH_3, $(C_2H_5)O$, - 78°C, RT; c) 2 eq $BH_3.(CH_3)_2S$, THF, Δ; 2 eq $(tBuOCO)_2O$, $(C_2H_5)_3N$, CH_3OH; d) TFA, 0°C; e) 1.1 eq $(CH_3CO)_2O$, $(C_2H_5)_3N$, THF, O°C; f) 7 eq $SnCl_2$, EtOH, Δ; g) 2 eq $(tBuOCO)_2O$; h) H_2, Pd/C, EtOH; i) 1.2 eq, Z-D-PheProOH, 1.2 eq iBuOCOCl, NMM, CH_3CN, - 20°C; j) TFA, 0°C; k) (ZHN)(ZN=)C-SCH_3, THF, 40°C; l) 3 eq Pd/C, 3 Å mol. sieves, AcOH, CH_2Cl_2; m) H_2, Pd/C, iPrOH, AcOH, 3 eq HCl.

REFERENCES

1. a) Imperiali, B., Abeles, R.H., *Biochemistry*, 1986, **25**, 3760.
   b) Imperiali, B., Abeles, R.H., *Biochemistry*, 1987, **26**, 4474.
   c) Trainor, D.A., Bergeson, S.H., Schwartz, J.A., Stein, M.A., Wildonger, R.A., Edwards, P.D., Shaw, A., Wolanin, D.J., European Patent Application, O189305A2.
   d) Peet, N.P., Burkhart J.P., Angelastro, M.R., Giroux, E.L., Mehdi, S., Bey, P., Kolb, M., Neises, B., Schirlin, D., *J. Med. Chem.*, 1990, **33**, 394.
2. a) Takahashi, L.H., Radhakrishnan, R., Rosenfield, R.E. Jr., Meyer, E.F. Jr., Trainor, D.A., Stein, M., *J. Mol. Biol.*, 1988, **201**, 423.
   b) Takahashi, L.H., Radhakrishnan, R., Rosenfield, R.E. Jr., Meyer, E.F. Jr., Trainor, D.A., *J. Amer. Chem. Soc.*, 1989, **111**, 3368.
3. Chang, J.Y., *Eur. J. Biochem*, 1985, **151**, 217.
4. a) Angliker, H., Wikström, P., Rauber, P., Stone, S., Shaw, E., *Biochem J.* 1988, **256**, 481.
   b) Neises, B., Tarnus C., Poster presentation at the XIIIrd Congress of the International Society on Thrombosis and Hemostasis, Amsterdam, 1991.
5. Schirlin, D., Baltzer, S., Altenburger, J.M., *Tetrahedron Letters*, 1988, **29**, 3690.
6. Tarnus, C., Jung, M.J., Remy, J.M., Baltzer, S., Schirlin, D.G., *FEBS Letters*, 1989, **249**, 47.
7. Tarnus, C., Schirlin D., unpublished results.

Ki = 1 µM (membrane bound aminopeptidase; substrate used: L-Leucine p.nitroanilide)



8. These three types of amino protecting groups are stable towards our reaction conditions and can be selectively removed when needed as examplified in scheme III. Tertbutoxycarbonyl by treatment with trifluoroacetic acid[9], benzyloxycarbonylby hydrogenolysis on Pd/C[9]. Nitro derivatives can easily be deprotected or reduced by tin(II)chloride in acetic acid[9] or ammonium formate, Pd/C in tetrahydrofuran/methanol[9].
9. Greene, T.W., "Protective Groups in Organic Synthesis", edited by John Wiley and sons, New York, 1981.
10. a) Charlesworth, E.H., Charleson, P., *Can. J. Chem.*, 1968, **46**, 1843.
b) Bohlman, F., *Chem. Ber.*, 1957, **90**, 1519.
11. Siegel, A, Keckeis, H., *Monatshefte Chem.*, 1953, **84**, 910.
12. Hallinan, E.A., Fried, J., *Tetrahedron Letters*, 1984, 2301.
13. a) Boudjouk, P., Han, B.H., *J. Org. Chem.*, 1982, **47**, 751.
b) Boudjouk, P., Han, B.H., J. Org. Chem., 1982, **47**, 5030.
c) Thaisrivongs, S., Pals, D.T., Kati, W.M., Turner, S.R., Thomasco, L.M., Watt, W., *J.Med. Chem.* , 1986, **29**, 2080.
14. Burton, D.J., Easdon, J.C., *J. Fluorine Chem.*, 1988, **38**, 125.
15. Altenburger, J.M., PhD thesis, Université Louis Pasteur, Strasbourg.
16. Aldehydes were prepared by reduction of their corresponding methylesters with 3 eq of DIBAL as described by D.H. Rich, E.T. Sun, A.S. Bopar, *J. Org. Chem.*, 1978, **43**, 3624.
17. Perrin, D.O., Armarego, W.L.F., Perrin, D.R., Purification of laboratory chemicals, 2nd edition, 1980, p 547, Pergamon Press.
18. All new compounds were characterized by ^1H and ^{19}F NMR, MS and/or combustion analysis.
2a: oil; Rf: 0.20 (silica gel, ethyl acetate/cyclohexane 1:1). MS: $M^+ = 275$; $MNH_4^+ = 293$. ^1H NMR ($CDCl_3$, 90 MHz, TMS): δ 8.09-7.36 ppm (m, 4 H), 6.33 (m, 1 H), 4.31 ppm (q, J = 7.5 Hz, 2 H), 4.03 ppm (d, J = 6 Hz, 1 H), 1.28 ppm (t, J = 7.5 Hz, 3 H). ^{19}F NMR ($CDCl_3$, 90 MHz, C_6F_6): υA = 50.80 ppm (dd, J_{FA-FB} = 270 Hz, J_{FAH} = 6 Hz, 1 F); υB = 41.62 ppm (dd, J_{FB-FA} = 270 Hz, J_{FBH} = 16 Hz, 1 F). **2b**: 60:40 mixture of diastereoisomers (solid). Rf: 0.30 (silica gel, ethyl acetate/cyclohexane 1:1). MS: $MH^+ = 453$; $MNH_4^+ = 470$. Analysis: Calculated for $C_{21}H_{22}N_2O_7F_2$: C% 55.75; H% 4.90; N% 6.19. Found: C% 55.69; H% 4.79; N% 6.13. ^{19}F NMR ($CDCl_3$, 339 MHz, C_6F_6): major diastereoisomer: υ = 48.26 ppm (dd, J_{FA-FB} = 271.4 Hz, J_{FAH} = 7.4 Hz, 1 F, FA); υB = 4.37 ppm (dd, J_{FB-FA} = 271.4 Hz, J_{FBH} = 14.4 Hz, 1 F, FB). Minor diastereoisomer: υA = 48.71 ppm (dd, J_{FA-FB} = 265.7 Hz, J_{FAH} = 7.9 Hz, 1 F, FA); υB = 41.67 ppm (dd, J_{FBFA} = 265.7 Hz, J_{FBH} = 17.5 Hz, 1 F, FB). **2c**: 65:35 mixture of diastereoisomers (oil) Rf: 0.20 (silica gel, ethyl acetate/cyclohexane, 1/1). MS: $MH^+ = 568$; $MNH_4^+ = 585$. ^{19}F NMR ($CDCl_3$, 339 MHz, C_6F_6) Major diastereoisomer: υA = 48.80 ppm (dd, J_{FA-FB} = 268.4 Hz, J_{FA-H} = 6.3 Hz, 1 F, FA); υB = 40.90 ppm (dd, J_{FB-FA} = 268.4 Hz, J_{FBH} = 16.2 Hz, 1F, FB). Minor diastereoisomer: υA = 49.55 ppm (dd, J_{FA-FB} = 265.5 Hz, J_{FAH} = 6.6 Hz, 1 F, FA); υB = 41.30 ppm (dd, J_{FB-FA} = 265.5 Hz, J_{FBH} = 18.2 Hz, 1 F, FB). **2d**: 60:40 mixture of diastereoisomers (oil) Rf: 0.35 (silica gel, ethyl acetate/cyclohexane 1:1). MS:$MH^+ = 357$; $MNH_4^+ = 374$. ^{19}F NMR ($CDCl_3$, 339 MHz, C_6F_6) Major diastereoisomer: υA = 48.88 ppm (dd, J_{FA-FB} = 267 Hz, J_{FAH} = 7.0 Hz, 1 F, FA).; υB = 41.40 ppm (dd, J_{FB-FA} = 267 Hz, J_{FBH} = 18 Hz, 1 F, FB). Minor diastereoisomer: υA = 47.89 ppm (dd, J_{FB-FA} = 270.3 Hz, J_{FAH} = 5.1 Hz, 1 F, FA), υB = 41.22 ppm (dd, J_{FB-FA} = 270.3 Hz, J_{FBH} = 15.5 Hz, 1 F, FB). **5**: 53:47 mixture of diastereoisomers. Rf : 0.35 and 0.40 (silica gel, acetonitrile/water RP 18, 9:1). MS (FAB) $MH^+ = 537$. Analysis calculated for $C_{28}H_{35}N_7O_4F_2$, 3 HCl, 1.5 H_20: C% 47.50; H% 5.84; N% 13.85. Found: C% 47.50; H% 5.88; N% 13.06. ^{19}F NMR (D_2O, 339 MHz, TFA, ^1H decoupled): major diastereoisomer υA = (-) 39.92 ppm (d, J_{FA-FB} = 252 Hz, 1 F, FA); υB = (-) 40.99 ppm (d, J_{FB-FA} = 252 Hz, 1 F, FB). Minor diastereoisomer υA = (-) 40.01 ppm (d, J_{FA-FB} = 251.3 Hz, 1 F, FA); υB = (-) 41.39 ppm (d, J_{FB-FA} = 251.3 Hz, 1 F, FB).
19. Compound **3** is a potent inhibitor of Human Thrombin *in vitro* with a Ki = 70 nM (substrate used: sarcosyl-L-prolyl-L-arginine p-nitro anilide). Tarnus, C., Rémy, J.M., unpublished results.

(Received in France 5 September 1991)