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Synthesis of α-Difluoro and α-Difluoro-β-Trifluoroketo-Derivatives as Potential Inhibitors for Cholesterol Ester Hydrolase.

Béatrice David and Francis Schuber

Laboratoire de Chimie Bioorganique associé au CNRS, Université Louis Pasteur, Faculté de Pharmacie, 74 route du Rhin, 67400 - Illkirch, France. Fax: (33) 88 67 88 91; e-mail: schuber@pharma.u-strasbg.fr

Abstract: Pancreatic Cholesterol Ester Hydrolase, a serine esterase, catalyzes the hydrolysis of cholesteryl esters in the gut. We report the convergent synthesis of α-difluoro-β-trifluoroketo- (**5**,**10**,**15**) and of α-difluoroketo-derivatives (**22**,**23**) as inhibitors of this enzyme that were designed to generate stable tetrahedral reaction intermediates. Copyright © 1996 Elsevier Science Ltd

Pancreatic Cholesterol Ester Hydrolase (CEH, E.C. 3.1.1.13) is a serine hydrolase that catalyzes the hydrolysis of dietary cholesteryl esters in the gut. Inhibition of this enzyme represents a possible strategy for reducing cholesterol levels in blood[1]. We have designed α-difluoro- and α-difluoro-β-trifluoroketo-derivatives which were aimed to inhibit CEH by forming a stabilized tetrahedral adduct with its catalytic serine residue (**Figure 1**). Similar strategies based on the electrophilicity of such carbonyl groups were shown previously to give access to powerful inhibitors of serine proteases[2] and lipases[3].

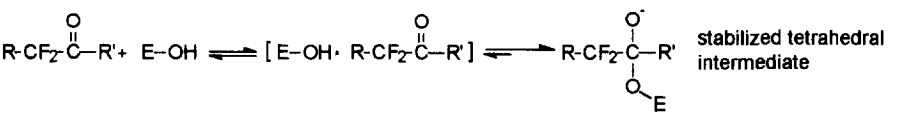
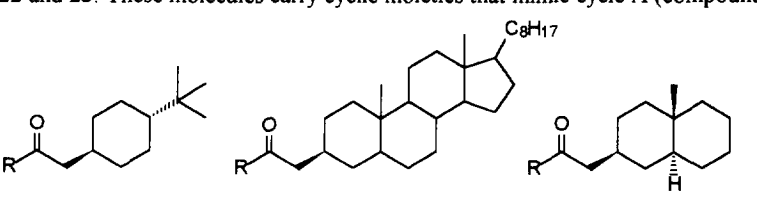


Figure 1: Inhibition of serine hydrolases by α-difluoroketones.

In a first approach we have synthesized the α-difluoro-β-trifluoroketones **5**, **10** and **15** as well as the α-difluoroketones **22** and **23**. These molecules carry cyclic moieties that mimic cycle A (compounds **5** and **22**) or



	R
5	CF_2CF_3
22	$CF_2(CH_2)_{11}CH_3$

10 R = CF_2CF_3

	R
15	CF_2CF_3
23	$CF_2(CH_2)_{11}CH_3$

cycles A,B (compounds **15** and **23**) of cholesterol or the whole cholestane nucleus (compound **10**). The acyclic part replaces the fatty acyl chain of the natural substrates cholesteryl esters especially in the case of the two α-difluoroketones. Since pancreatic CEH accepts a rather wide range of substrates[1] it was hoped that these compounds would permit to test the application this concept to this enzyme.

The compounds result from the coupling of acyclic and cyclic synthons (**Figure 2**) according to a methodology adapted from Gelb and co-workers[3a]. The acyclic parts were generated from methyl α-difluoroester **21** (**Scheme 2**) or from methyl pentafluoropropionate. The cyclic moieties, which carry a methylene group with a β-configuration, were reacted with the methyl esters as organolithium derivatives synthesized as described in **Scheme 1**. The cyclic ketones: 4-*t*-butyl cyclohexanone, cholestanone and 8a-methyl-decahydronaphtalen-2-one[4] were first converted into their corresponding enol ethers by a Wittig reaction[5] with (methoxymethyl)triphenylphosphonium ylide generated, in situ at 4°C, from (methoxymethyl) triphenylphosphonium bromide and LDA. The yields were respectively of 66, 77 and 58 % for compounds **1**, **6** and **11**[6]. After a deprotection step with an excess of perchloric acid, the enol ethers were converted into alde-

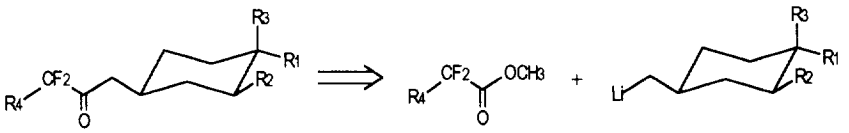
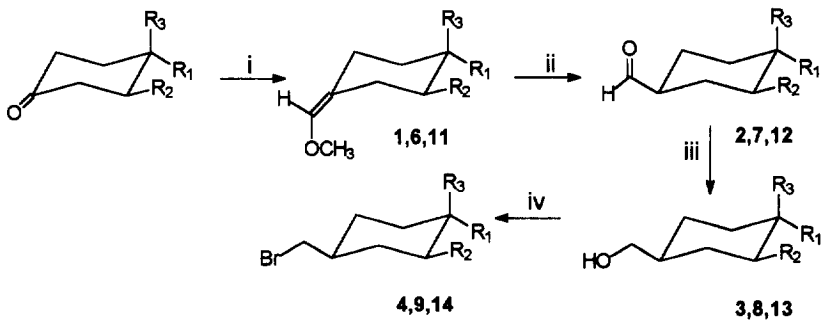


Figure 2: Coupling of the acyclic and cyclic synthons.

hydes which were immediately reduced into the alcohols in the presence of 10 equivalents of sodium borohydride at RT in a 1/1 mixture of ethanol and methylene chloride[6,7]. The three alcohols **3**, **8** and **13** were then converted into the corresponding bromides without purification with 1 equivalent of triphenylphosphine and carbon tetrabromide[8] in methylene chloride at RT. The overall yields for the conversion of the cyclic enol ethers into their corresponding bromides, after purification by chromatography on silica gel columns, were 33, 56 and 31 % respectively for **4**, **9** and **14.**

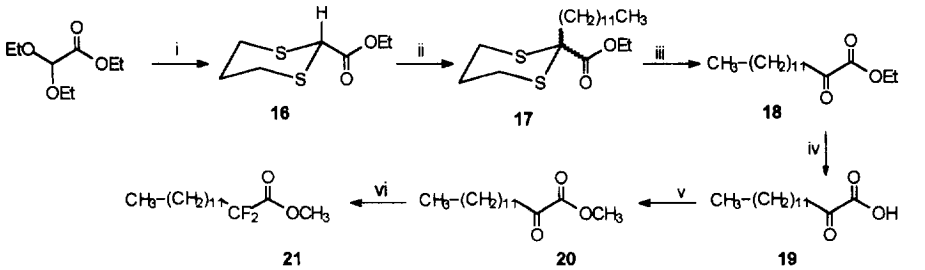


1-4 : R_1 = *t*-Bu; R_2=R_3= H; **6-9** : R_1,R_2, = cholestane, R_3 = CH_3; **11-14** : R_1,R_2,R_3 = 8a-methyl-decahydronaphtalene

Scheme 1: *i*) PPh_3=$CHOCH_3$, THF, -78°C → RT, 14 h; *ii*) $HClO_4$, ether; RT, 14 h; *iii*) $NaBH_4$, 10eq., CH_2Cl_2, EtOH, RT,14 h; *iv*) PPh_3, CBr_4, CH_2Cl_2, RT, 14 h.

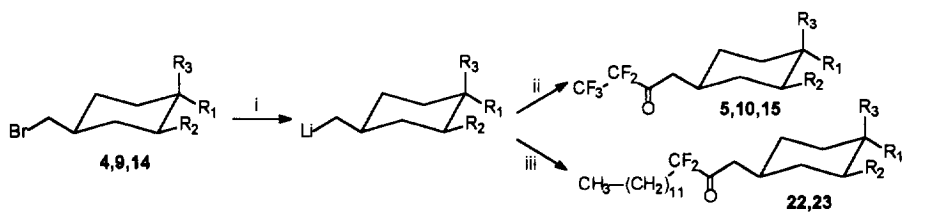
The route used for the synthesis of the methyl α-difluoroester **21** is given in **Scheme 2**. The dithiane **16**

obtained from ethyldiethoxyacetate and propanedithiol in the presence of 1 equivalent boron trifluoride dietherate (60 % yield) was reacted with dodecylbromide and sodium hydride in a 1/1 mixture of DMF and THF at RT[9] (93 % yield). The resulting alkylated dithiane **17** was then deprotected in acetone with 8 equivalents of N-bromosuccinimide[10]. After purification by chromatography on silica gel, the resulting ethyl α-ketoester **18** was obtained with a yield of 76 %. This latter product was converted into the methyl α-ketoester **20** (total yield: 30%) by hydrolysis with lithium hydroxide followed immediately by methylation with diazomethane and purification on silica gel column. The fluorination of **20** was best achieved at ambient temperature with DAST[3a], and the expected methyl α-difluoroester **21** was obtained with a 44 % yield after purification by chromatography on silica gel.



Scheme 2: *i*) 1,3-propanedithiol, BF_3-ether, CH_2Cl_2, RT, 14 h; *ii*) $CH_3(CH_2)_{11}Br$, NaH, THF,DMF, RT, 14 h; *iii*) NBS, 8eq., acetone, 0-5°C, 20 min; *iv*) LiOH, MeOH, RT, 14 h; *v*) CH_2N_2, ether, -5°C, 15 min; *vi*) DAST, 2eq., CH_2Cl_2, RT, 2 h.

To achieve the synthesis of the final molecules, the bromides **4**, **9** and **14** were converted into their corresponding organolithium derivatives, in ether at - 78°C, by the use of 2 eq. of *t*-butyllithium[3] (**Scheme 3**). The electrophile (**21** or methylpentafluoropropionate) was then added to the organolithium solution at the same temperature. The expected final molecules were obtained, after purification, in rather modest yields (about 20%) which up to now could not be improved. It should be noted that only methyl esters gave the final products (**Scheme 3**); other derivatives such as ethyl esters, were poorly reactive. Moreover **22** and **23,** unlike the pentafluorinated products **5**, **10** and **15**, were isolated mostly under their hydrated form.



4,5,22 : R_1 = *t*-Bu; R_2=R_3= H; **9,10** : R_1,R_2, = cholestane, R_3 = CH_3 ; **14,15,23** : R_1,R_2,R_3 = 8a-methyl-decahydronaphtalene

Scheme 3: *i*) *t*-BuLi, 2 eq., ether, - 78°C → RT, 10 min; *ii*) $CF_3CF_2CO_2CH_3$, - 78°C → RT, 20 min; *iii*) **21**, - 78°C → RT, 20 min.

This first generation of α-difluoroketo derivatives was tested for inhibition of pancreatic CEH[11]. As will be reported and analyzed elsewhere (in preparation), complex inhibition kinetic patterns were observed with these compounds; e.g. lag-phases with **10** and **15**. They did not show, however, "slow-binding" types of

inhibition that are expected for potent inhibitors that form stabilized hemi-ketals with serine esterases.[2b] Apparent IC_{50} values were in the 10-15 μM range for **5**, **10** and **15** and > 100 μM for **23**.

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4. (4a*S*)-4,4a,5,6,7,8-Hexahydro-4aβ-methyl-2(3*H*)-naphtalenone (commercial or prepared according to Pfau, M.; Revial G.; Guingant, A.; d'Angelo, J. *J. Am. Chem. Soc.* **1985**, *107*, 273) was transformed into (4a*S*,8a*R*)-1,2,3,4,4a,5,6,7,8,8aα-decahydro-4aβ-methyl-2(3*H*)-naphtalenone with lithium in ethylenediamine according to Hodge-Markgraf, J; Waugh-Staley, S.; Allen; T. *Syn. Commun.* **1989**, *19*, 1471.

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6. All new compounds gave spectroscopic data in agreement with the assigned structures. Compound **5** had ^1H NMR δ (200 MHz, $CDCl_3$) 0.84 (s, 9H, $C(Me)_3$), 0.88-1.31 (m, 7H, H_3 to H_5, H_{2ax}, H_{6ax}), 1.58-1.98 (m, 3H, H_1, $H2_{eq}$, $H6_{eq}$), 2.60 (d, J = 7.0 Hz, 2H, $CH_2COCF_2CF_3$); ^{19}F NMR δ (300 MHz, $CDCl_3$, internal standard $CFCl_3$) -82.80 (3F, CF_3), -124.64 (2F, CF_2); MS (EI; trimethylsilyl enol ether derivative) *m/z* (rel. int.) 372 $[M + TMS]^+$ (8%), 357 $[M + TMS - Me]^+$ (22%), 73 $[TMS]^+$ (64%), 57 $[C(Me)_3]^+$ (100%). Compound **10** had ^1H NMR δ (200 MHz, $CDCl_3$) 0.62 (s, 3H, Me_{18}), 0.74 (s, 3H, Me_{19}), 0.75-1.80 (40H, H_1, H_2, H_4 to H_{17}, H_{20} to H_{27}), 1.80-2.10 (m, 1H, H_3), 2.60 (d, J = 7.0 Hz, 2H, $CH_2COCF_2CF_3$); MS (EI) *m/z* (rel. int.) 532 $[M]^+$ (97%), 516 $[M - Me]^+$ (24%). Compound **15** had ^1H NMR δ (200 MHz, $CDCl_3$) 0.84 (s, 3H, Me), 0.87-1.49 (15H, H_{1ax}, H_2 to H_{8a}), 1.61-1.80 (m, 1H, H_{1eq}), 2.60 (d, J = 7.0 Hz, 2H, $CH_2COCF_2CF_3$); ^{19}F NMR δ (200 MHz, $CDCl_3$, internal standard $CFCl_3$) -82 (t, 3F, CF_3), -124 (q, 2F, CF_2); MS (CI/NH_3) *m/z* (rel. int.) 330 $[M + NH_4]^+$ (2%), 312 $[M]^+$ (24%), 297 $[M - Me]^+$ (28%). Compound **22** (hydrated form) had ^1H NMR δ (200 MHz, $CDCl_3$) 0.85 (t, J = 6.2 Hz, 3H, $CH_3(CH_2)_{11}$), 1.0 (m, 1H, H_4), 1.05 (s, 9H, $C(Me)_3$), 1.20-1.40 (27 H, $CH_3(CH_2)_9CH_2CH_2CF_2$, H_1 to H_3, H_5, H_6), 1.45, (s, 2H, $C(OH)_2$), 1.47 (m, 2H CH_2CF_2), 1.70-2.05 (m, 3H, AA'BX system: 1H, $CH_2C(OH)_2$ and 2H, $CH_2CF_2C(OH)_2$), 3.37 (ddd, AA'BX system: $J_{A,A'}$ = 19.3 Hz, $J_{A',B}$ = 5.1 Hz, $J_{A',X}$ = 8.0 Hz, 1H, $CH_2C(OH)_2CF_2$; ^{19}F NMR δ (200 MHz, $CDCl_3$, internal standard $CFCl_3$) - 103.40, -104.30, -110. 73 (AA'BX system, 2F, $CF_2C(OH)_2$). Compound **23** (hydrated form) had ^1H NMR δ (200 MHz, $CDCl_3$) 0.83 (t, J = 6.4 Hz, 3H, $CH_3(CH_2)_{11}$), 0.97-1.40 (34H, $CH_3(CH_2)_9CH_2$, H_1 to H_4, H_5 to H_{8a}), 1.47 (m, 2H, $CH_2CH_2CF_2$), 1.5 (s, 2H, $C(OH)_2$), 1.70-2.05 (m, 3H, AA'BX system: 1H, $CH_2C(OH)_2$ and 2H, $CH_2CF_2C(OH)_2$), 3.37 (ddd, AA'BX system, $J_{A,A'}$ = 17.8 Hz, $J_{A',B}$ = 5.5 Hz, $J_{A',X}$ = 8.9 Hz, 1H, $CH_2C(OH)_2CF_2$); ^{19}F NMR δ (300 MHz, $CDCl_3$, internal standard $CFCl_3$) -103.41, -104.30, -109.82, -110.71 (AA'BX system, $CF_2C(OH)_2$); MS (CI/NH_3) *m/z* (rel. int.) 410 $[M + NH_4 - HF - H_2O]^+$ (90%), 393 $[M + H - HF - H_2O]^+$ (23%).

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11. The assays were performed at 37°C in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.1 M NaCl and 6 mM sodium taurocholate in the presence of bovine pancreatic CEH (Sigma), using *p*-nitrophenylacetate or cholesteryl [1-^{14}C]oleate as substrates.

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