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Inhibition of human leukocyte elastase by functionalized *N*-aryl-3,3-dihalogenoazetidin-2-ones. Stereospecific synthesis and chiral recognition of dissymmetrically C_3-substituted β-lactams

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Abstract: (3*R*)- and (3*S*)-*N*-(2-chloromethylphenyl)-3-bromo-3-fluoroazetidin-2-ones **2** were synthesized *via* the separation of diastereoisomeric phenylglycinol derivatives of the starting 2,3-dibromo-2-fluoropropanoic acid. Acidic hydrolysis of the hydroxyamides led to the chiral trihalogenopropanoic acids. Then, an expeditious four step synthesis provided the (3*S*)- and (3*R*)-azetidinones **2**, both of which behaved as strictly irreversible inhibitors of HLE. The configuration of the bromofluorocarbon was shown to have a significant effect on the partition ratio: k_{cat}/k_{inact}=4.6 and 34.3 for (3*S*)- and (3*R*)-**2**, respectively.

Human leukocyte elastase (HLE; EC 3.4.21.37) is a serine protease released by azurophilic granules of polymorphonuclear leukocytes in response to inflammatory stimuli.[1] The extracellular activity of the enzyme is normally tightly regulated by potent natural inhibitors, notably α_1-proteinase inhibitor (α_1-PI).[2] Excessive elastolytic activity has been implicated in the etiology of a number of diseases such as pulmonary emphysema,[3] chronic bronchitis[4] and rheumatoid arthritis.[5] Therefore, considerable interest is being devoted to the synthesis of low-molecular-weight inhibitors of elastase as potential drugs.[6–9]

We recently reported the synthesis and the study of the *N*-(2-chloromethylphenyl)-3,3-difluoro-azetidin-2-one **1** (Y=Y′=F; X=Cl; Z=H) (Figure 1), which was shown to act as a mechanism-based inhibitor of HLE[10,11] and to prevent lung elastic fibers degradation and intradermal microvascular hemorrhage.[12,13]

Structure–activity studies on such β-lactam structures were first performed by varying the nature of the potential leaving group X at the benzylic position and by introducing an additional substituent on the aromatic ring (**1**: Y=Y′=F, Z=OCH_3, $CO_2(CH_2)_5CH_3$ or $CO_2(CH_2)_3CO_2H$).[14] Related 3,3-homodihalogeno-azetidinones (**1**: Y=Y′=Cl or Br and Z=H) have also been prepared and studied, demonstrating that HLE was more efficiently inactivated with dichloro- and dibromo- rather than with difluoro-azetidinones.[15] With a 3,3-heterodihalosubstitution of the β-lactam ring, a selective interaction of each enantiomer with the enzyme active site was expected. Therefore, we synthesized the racemic functionalized *N*-aryl-3-bromo-3-fluoro-azetidin-2-ones **2**, **3** and **4**, displaying different leaving groups (Figure 1) and the (3*R*)- and (3*S*)-enantiomers of β-lactam **2**. Their evaluation as HLE inhibitors was performed.

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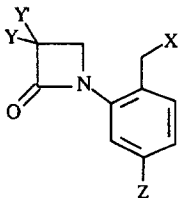

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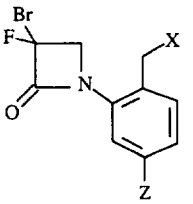


2 X=Cl Z=H

3 X=F Z=H

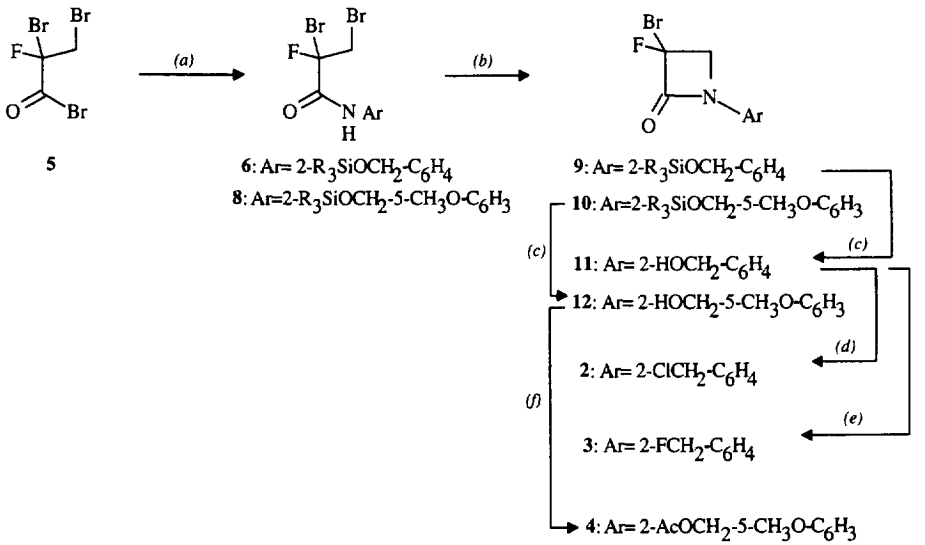
4 X=OAc Z=OCH_3

Figure 1.

Results and discussion

Chemistry

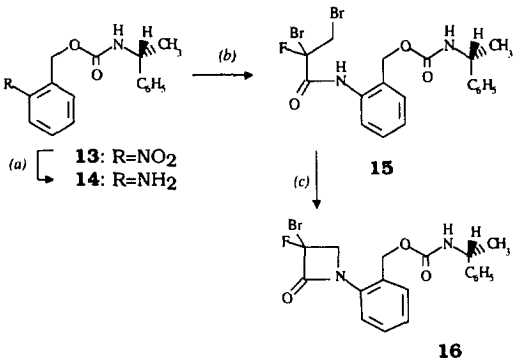
The *o*-chloromethyl- and *o*-fluoromethylphenylazetidinones **2** and **3** were obtained using the four-step procedure previously practiced in the 3,3-difluoro series,[15] starting however from the racemic 2,3-dibromo-2-fluoropropionic acid bromide **5**[16] instead of the 3-bromo-2,2-difluoropropionic acid chloride. In the last step of the synthesis, $SOCl_2$/DMF and DAST were respectively used as reagents (Scheme 1). The acetate **4** was also prepared from the alcohol with $(Ac)_2O$/DMAP through the same general scheme.



Scheme 1. Reagents and conditions: *(a)* 2-*tert*-butyldimethylsilyloxymethylaniline/1 eq Et_3N/toluene/4°C, *(b)* NaH/DMF–CH_2Cl_2/−10°C, *(c)* aqueous HF/CH_3CN/25°C, *(d)* $SOCl_2$/DMF, *(e)* Et_2NSF_3/CH_2Cl_2/−78°C, *(f)* $(Ac)_2O$/DMAP.

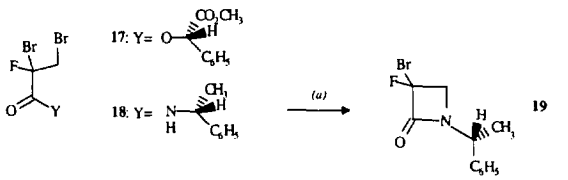
For the synthesis of optically active azetidinones (3*R*)- and (3*S*)-**2**, the racemic acid halide **5** could be a suitable starting material. Diastereoisomers possessing a chiral substituent in the benzylic position might be chromatographically discriminated. For this purpose, compounds bearing a carbamate ester moiety at the benzylic carbon were synthesized. Moreover, this group is potentially protecting owing to its possible cleavage under mild conditions, such as silanolysis.[17] The starting optically active aniline **14** was obtained by treatment of 2-nitrobenzyl alcohol with (*R*)-1-phenylethylisocyanate, followed

by catalytic hydrogenation of the nitro substituent. Unfortunately, neither the diastereoisomers of propionamide **15**, nor those of the azetidinone **16**, could be separated (Scheme 2).



Scheme 2. Reagents and conditions: *(a)* 95% Ethanol/PtO_2/H_2 (1 atm), *(b)* 5/1 eq (i-Pr)$_2$NEt/CH_2Cl_2/RT *(c)* 5 eq K_2CO_3/refluxing acetone.

Then, the temporary derivatizations of the starting (±) fluorobromopropionic acid were investigated. (*S*)-(+)-Methyl mandelate ester **17** and (*R*)-(+)-1-phenylethylamide **18** were prepared (Scheme 3). Again, separations of the diastereoisomers of compounds **17** and **18**, or those of the corresponding azetidinone **19**, were not feasible under standard chromatographical conditions.

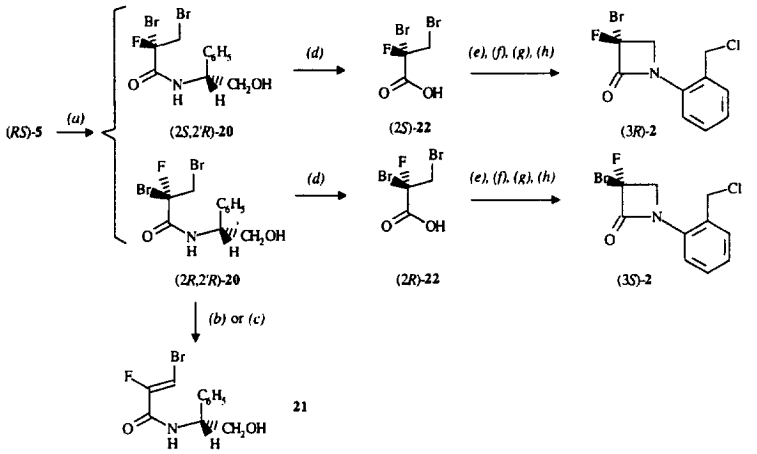


Scheme 3. Reagents and conditions: *(a)* K_2CO_3 (5 eq)/refluxing acetone.

Owing to its additional polar function, the phenylglycinol amido group is known to facilitate the resolution of many carboxylic acids.[18] The 2,3-dibromo-2-fluoropropionamide derived from (*R*)-2-amino-2-phenylethanol **20** was easily prepared (Scheme 4). Chromatography of the crude product on silica gel led to an efficient separation of the diastereoisomers (2*R*,1′*R*)- and (2*S*,1′*R*)-**20**, either by thin layer or by column chromatography using a gradient of solvents. However, saponification of compound **20** did not lead to the expected acid **22** but to the 3-bromo-2-fluoropropenamide **21** which slowly hydrolyzed to give the corresponding substituted acrylic acid.[19] This elimination reaction was equally observed when amide **20** was submitted to conditions of cyclization of 3-bromo-2,2-dihalopropionamides to azetidinones (Scheme 4, conditions *(c)*). Fortunately, (2*R*)- and (2*S*)-2,3-dibromo-2-fluoropropionic acid **22** were respectively obtained by acidic hydrolysis[20] of hydroxy amides (2*R*,1′*R*) and (2*S*,1′*R*)-**20**.

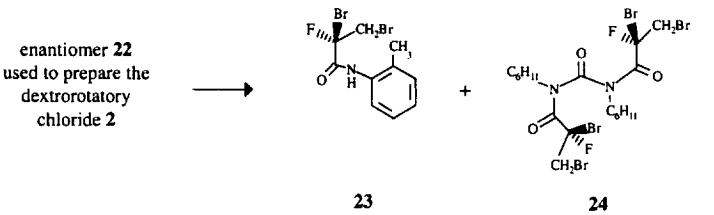
Then, optically active *N*-(2-*tert*-butyldimethylsilyloxymethylphenyl)-2,3-dibromo-2-fluoropropionamides **8** were prepared by coupling (2*R*)- or (2*S*)-**22** with the 2-(trialkylsilyloxymethyl)-aniline using DCC as condensation reagent in acetonitrile. The resulting crude amides were directly used to perform both following steps in the same solvent, without isolating the intermediate compounds (Scheme 4, steps *(f)* and *(g)*). Finally, the crude hydroxy derivatives were converted to (3*S*)- and (3*R*)-**2** which were obtained in 48% overall yield from acids (2*R*)- and (2*S*)-**22** respectively, in a rather expeditious manner.

None of the optically active synthetic intermediates being suitable for X-ray diffraction analysis, another crystalline derivative had to be prepared for the determination of the absolute configuration



Scheme 4. Reagents and conditions: *(a)* (*R*)-2-amino-2-phenylethanol/(i-Pr)$_2$NEt/CH_2Cl_2, *(b)* NaOH (10 eq)/EtOH/H_2O/80°C, *(c)* K_2CO_3 (5 eq)/refluxing acetone, *(d)* 12N H_2SO_4/dioxane/105°C, *(e)* DCC (1.1 eq) 2-trialkylsilyloxymethyl-aniline/CH_3CN/RT, *(f)* K_2CO_3 (5 eq)/CH_3CN/80°C, *(g)* HF/CH_3CN/H_2O/25°C, (h) $SOCl_2$/DMF.

of the dihalogenated carbon. During the derivatization with *o*-toluidine/DCC of the enantiomer of acid **22** which led to the dextrorotatory chloride **2**, a side-product was isolated in addition to the expected amide **23**. The structure of this minor compound was shown to be the *N,N'*-diacylurea **24**[21] (Scheme 5). Its single-crystal X-ray diffraction analysis allowed the assignment of the *R* configuration to the fluorobromo carbon[22] (Figure 2). Consequently, the configuration of the same carbon in the dextrorotatory chloride **2** was *S*.



Scheme 5. Reagents and conditions: DCC/*o*-toluidine/CH_3CN/RT.

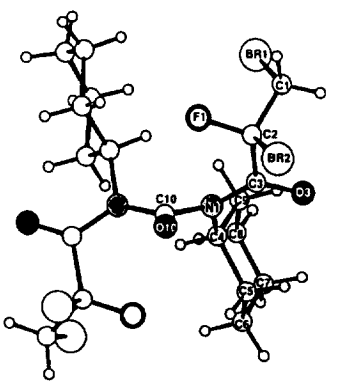


Figure 2. X-Ray crystal structure of urea derivative **24**.

Table 1. Kinetic constants for the inhibition of HLE by (3*RS*)-**2**, (3*S*)-**2**, (3*R*)-**2** and **3**

	Y, Y'	X	Z	k_{inact}/K_I ($M^{-1}.s^{-1}$)	$r = k_{cat}/k_{inact}$
(3*RS*)-**2**	F, Br	Cl	H	1 313 *(2)*	5 *(5)*
(3*S*)-**2**	F, Br	Cl	H	1 586 *(2)*	4.6 *(3)*
(3*R*)-**2**	F, Br	Cl	H	1 802 *(5.5)*[a]	34.3 *(3)*
3	F, Br	F	H	3 *(1)*	n.d. [b]

[a] $k_{inact} = 5.2\ 10^{-3}\ s^{-1}$ *(11)*, $K_I = 29\ \mu M$ *(16)*. [b] not determined. Relative errors are in parenthesis.

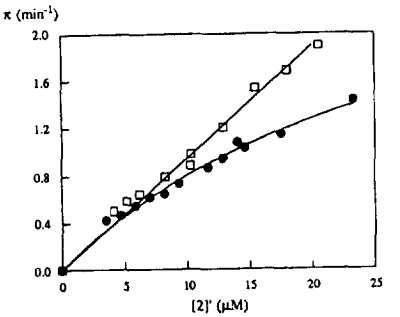


Figure 3. Inactivation of HLE by (3*S*)-**2** (□) and (3*R*)-**2** (●) at pH 8.0 and 37°C. The enzyme was added in 0.1 M Tris buffer (Brij$_{35}$ 0.01%, 5% v/v DMSO) containing the chromogenic substrate MeOSuc-A_2-P-V-*p*NA (100 μM) and different concentrations of (3*S*)-**2** or (3*R*)-**2** ranging from 2.2 to 35.2 μM and from 4 to 40 μM respectively. The kinetic parameters K_I and k_{inact} were determined by fitting the experimental data to the equation $\pi = k_{inact}[I]'/(K_I+[I]')$ where $-\pi$ is the slope of the linear plot of ln*v* *versus* time at a given inhibitor concentration, $[I]'$ the modified inhibitor concentration $[I]/(1+[S]/K_m)$ and K_m the Michaelis constant for the substrate (see experimental part).

Enzymatic studies

When the substituent at the benzylic position (X) is a halogen atom (compounds **2** and **3**), an irreversible inhibition of HLE was observed (Table 1; Figure 3). Nevertheless, the fluorinated compound **3** was much less reactive than the chlorinated ones [(3*RS*)-**2**, (3*S*)-**2**, (3*R*)-**2**]. This is compatible with the fact that F is less nucleofugal than Cl (pKa=3.18 and −6.10, respectively).[23]

In spite of a similar inhibitory efficiency characterized by the kinetic parameter k_{inact}/K_I (eq. 1), a different kinetic behaviour was observed for the two enantiomers (3*R*)-**2** and (3*S*)-**2**. A higher partition ratio ($r = k_{cat}/k_{inact}$, eq. 1) was obtained for the inactivation performed by compound (3*R*)-**2** compared to that observed for compound (3*S*)-**2** (factor of 7.5, Figure 4). Therefore, the latter behaved as a better suicide substrate of HLE since a suicide substrate is characterized not only by its efficiency but also by its partition ratio: the lower the partition ratio, the better the suicide substrate. This demonstrates that the configuration of carbon 3 is of importance in the design of HLE suicide substrate inhibitors using the *N*-aryl-azetidinone core. For the inhibitors described by Merck, the inhibition was favoured for compounds with a bulkier substituent in the α-position at the C-7 carbon of cephalosporin-based compounds;[25,26] for monocyclic β-lactam-based compounds, a less clear dependence upon C-3 configuration was observed.[27] In our β-lactam series, when the bulkier substituent (Br) is in the β position [compound (3*S*)-**2**], the inhibitory behaviour observed is closer to that expected for an ideal suicide-substrate inhibitor. We can also notice that the *N*-aryl-azetidinones **2** described here are the most potent in our series compared to compounds with Y=Y' (**1**, Figure 1).[11,14,15]

Acetate **4** showed no inhibitory activity against HLE as already observed for structures designed to be suicide-substrate inhibitors and bearing an acetoxy group as the leaving group.[14,28,29] The

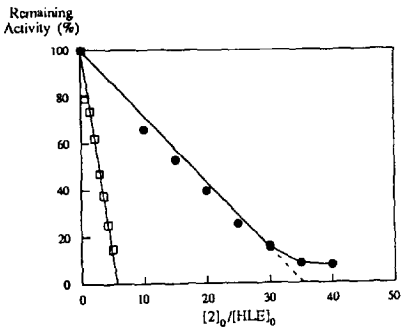


Figure 4. Determination of the partition ratios for the inactivation of HLE by (3*S*)-**2** (□) and (3*R*)-**2** (●). HLE was incubated with various inhibitor concentrations for 7 h at 37°C and pH 8.0 (5% v/v DMSO) before measuring the remaining activity of aliquots. The plot of the remaining activities *versus* molar excesses $[2]_0/[HLE]_0$ gave the partition ratio $r=k_{cat}/k_{inact}$ as the x intercept of this linear plot is equal to r+1 (Remaining Activity (%)=100−(100/(r+1))($[2]_0/[HLE]_0$)).[24] For (3*S*)-**2**: $[HLE]_0$=1.84 μM and $[(3S)\text{-}2]_0$=1.3–9.1 μM; for (3*R*)-**2**: $[HLE]_0$=1 μM and $[(3R)\text{-}2]_0$=10–40 μM.

hydrolysis of **4** was catalyzed by the enzyme. Two successive reactions occurred and the second-order rate constant for the hydrolysis of the amide bond of the lactam ring was determined (k_{cat}/K_m=138 000 $M^{-1}s^{-1}$).

In conclusion, *N*-aryl-3-bromo-3-fluoroazetidin-2-ones with different potential benzylic leaving groups have been prepared. (3*R*)- and (3*S*)-*N*-(2-chloromethylphenyl)-3-bromo-3-fluoroazetidin-2-ones **2** were stereospecifically synthesized *via* the separation and the acidic hydrolysis of diastereoisomeric phenylglycinol derivatives of 2,3-dibromo-2-fluoropropanoic acid, followed by an expeditious transformation of the chiral trihalogenopropanoic acids. The absolute configuration of the bromofluorocarbon was assigned by X-ray analysis of a *N,N*-diacylurea derivative. The reported compounds fulfilled criteria expected for a suicide-type inhibition of HLE: irreversibility, time-dependent and pseudo-first-order inactivation, saturation kinetics, protection by the chromogenic substrate against inactivation. Moreover, the hydrolysis of compound **4** as well as the hydrolysis of the compound lacking a leaving group at the benzyl position (**1**, Figure 1: Y=F; Y′=Br; X=Z=H: k_{cat}/K_m=1 712 $M^{-1}s^{-1}$)[15] demonstrated the opening of the β-lactam ring catalyzed by HLE and suggested that such a process may occur with compounds functionalized at the benzylic position by a halogen atom. The configuration of C-3 of the azetidinone may greatly influence the nature of the inhibition and the inactivation step could be favoured. It should be noticed that the *N*-aryl-azetidinones described here were strictly irreversible whereas monobactam-based inhibitors previously described by Merck[30,31] led to enzyme reactivation.

Experimental section

Melting points were obtained with a melter FP 61 apparatus and infrared spectra were determined on a Perkin Elmer 1420 spectrophotometer. Proton NMR spectra were obtained on a Brucker AC 200-E apparatus at 200 MHz and are reported in ppm downfield from TMS. Carbon and fluorine NMR spectra were recorded at 50.3 and 188.3 MHz, respectively, and are reported downfield from TMS (^{13}C) and $CFCl_3$ (^{19}F). The optical rotations were obtained on a Perkin Elmer 241 polarimeter. Mass spectra were determined on a Kratos MS 50 instrument.

N-*(2-tert-Butyldimethylsilyloxymethylphenyl)-2,3-dibromo-2-fluoropropionamide 6*

The amide was prepared from 2,3-dibromo-2-fluoropropanoyl bromide[16] and the corresponding aniline in standard conditions;[15] (ether:pentane=1:10), 882 mg, 63%, mp 58.2°C (hexane); IR (CH_2Cl_2) 3290, 1695 cm^{-1}. ^1H NMR [$(CD_3)_2CO$] δ 0.08 (s, 6H), 0.87 (s, 9H), 4.40 (dd, J=9, 11.4 Hz), 4.63 (dd, J=11.5, 31 Hz), 7.3 (m, 4H), 9.2 (bs, 1H). ^{19}F NMR [$(CD_3)_2CO$] δ −118.6 (ddd, J=2,

8, 30 Hz); MS m/z=410–412–414 ($M^{+\cdot}-57$), 252, 130, 91, 77. Anal calcd for $C_{16}H_{24}Br_2FNO_2Si$ C 40.95, H 5.16, N 2.98, found C 41.20, H 5.08, N 2.98.

2-tert-Butyldimethylsilyloxymethyl-5-methoxy-aniline 7

Prepared according to a previously described procedure[15] (ether:pentane=1:3), yellowish oil, 2 g, 79%; IR (CH_2Cl_2) 3440, 3340, 1610, 1500 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.0 (s, 6H), 1.85 (s, 9H), 3.63 (s, 3H), 4.2 (bs, 2H), 4.48 (s, 2H), 6.2 (m, $2H_{arom}$), 6.83 (d, $1H_{arom}$). HRMS calcd for $C_{14}H_{25}NO_2Si$ 267.1654, found 267.1660.

N-*(2-tert-Butyldimethylsilyloxymethyl-5-methoxyphenyl)-2,3-dibromo-2-fluoropropionamide 8*

Prepared by methodology used for **6**; (ether:pentane=1:4), 303 mg, 77%, mp=58–59°C, IR (CH_2Cl_2) 3300, 1700, 1610, 1580 cm^{-1}. ^1H NMR ($CDCl_3$) δ −0.02 (s, 6H), 0.8 (s, 9H), 3.73 (s, 3H), 4.00 (dd, J=7.4, 11.3 Hz, 1H), 4.34 (dd, J=11.3, 31.5 Hz, 1H), 4.62 (m, 2H), 6.55–7.83 (m, $3H_{arom}$), 9.9 (bs, 1H). ^{19}F NMR ($CDCl_3$) δ −121.0 (dd, J=7.3, 31.8 Hz). MS m/z=442 ($M^{+\cdot}-56$), 338, 282, 220, 205. Anal calcd for $C_{17}H_{26}Br_2FNO_3Si$ C 40.92, H 5.25, N 2.81, found C 41.31, H 5.18, N 3.01.

N-*(2-tert-Butyldimethylsilyloxymethylphenyl)-3-bromo-3-fluoroazetidin-2-one 9*

Obtained by treatment of amide **6** with NaH in CH_2Cl_2/DMF;[15] (AcOEt:pentane=1:10), colorless oil, 93 mg 41%; IR (CH_2Cl_2) 1770 cm^{-1}. ^1H NMR [$(CD_3)_2CO$] δ, 0.14 (s, 6H), 0.95 (s, 9H), 4.6 (dd, J=7.2 Hz, 1H), 4.83 (dd, J=7.3, 9,3 Hz, 1H), 4.9 (dd, J=13.9 Hz, 2H), 7.3–7.6 (m, 4H); ^{19}F NMR [$(CD_3)_2CO$] δ −120.3 (dd, J=7.5 Hz); MS m/z=388 ($M^{+\cdot}$), 373–375, 332–335, 132, 91; HRMS calcd for $C_{16}H_{23}BrFNO_2Si$ 387.0666, found 387.0640.

N-*(2-tert-Butyldimethylsilyloxymethyl-5-methoxyphenyl)-3-bromo-3-fluoroazetidin-2-one 10*

Prepared by treatment of amide **8** with K_2CO_3 in refluxing acetone;[14] (ether:pentane=1:3), (200 mg, 89%), colorless oil, IR (CH_2Cl_2) 1770, 1600 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0 (s, 6H), 0.84 (s, 9H), 3.78 (s, 3H), 4.42 (dd, J=7.0 Hz, 1H), 4.53 (dd, J=7.1, 8.7 Hz), 4.6 (m, 2H), 6.7–7.24 (m, $3H_{arom}$). ^{19}F NMR ($CDCl_3$) δ −122.5(dd, J=7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 18.48, 25.72, 55.25, 62.4 (d, J=25 Hz), 94.14 (d, J=296 Hz), 108.2–133 (5 C_{arom}), 158.37 (d, J=49 Hz), 159.43. MS m/z=362 ($M^{+\cdot}-57$), 286, 252, 236, 162,136. Anal calcd for $C_{17}H_{25}BrFNO_3Si$ C 48.84, H 6.03, N 3.35, found C 48.99, H 5.97, N 3.35.

N-*(2-Hydroxymethylphenyl)-3-bromo-3-fluoroazetidin-2-one 11*

Prepared by aqueous HF cleavage of the silyl ether **9** in CH_3CN;[14] (ether:pentane=1:1), colorless oil (32 mg 80%); IR (CH_2Cl_2) 3570, 3460, 1765 cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 4.32 (bs, 2H), 4.47 (dd, J=7.2, 7.3 Hz, 1H), 4.46 (dd, J=7.2, 9.3 Hz, 1H), 4.6 (bs, 1H); ^{19}F NMR [$(CD_3)_2CO$] δ −122.33 (dd, J=7.7, 9.4 Hz); MS m/z=273–275 ($M^{+\cdot}$), 216, 194, 176, 149, 105, 118, 93, 77, 65. HRMS calcd for $C_{10}H_9BrFNO_2$ 272.98007, found 272.98012.

N-*(2-Hydroxymethyl-5-methoxyphenyl)-3-bromo-3-fluoroazetidin-2-one 12*

As above, starting from the silyl ether **10**; colorless oil, 45 mg, 83%; IR (CH_2Cl_2) 3580, 3480, 1770 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.75 (s, 3H), 4.31 (dd, J=6.9 Hz, 1H), 4.46 (dd, J=7, 8.7 Hz, 1H), 4.51 (s, 2H), 7.1 (m, $3H_{arom}$). ^{19}F NMR ($CDCl_3$) δ −122.94 (dd, J=7 Hz). MS m/z=303 ($M^{+\cdot}$), 224, 179, 150, 123. HRMS calcd for $C_{11}H_{11}BrFNO_3$ 302.9906, found 302.9933.

N-*(2-Nitrobenzyloxycarbonyl)-(R)-1-phenylethylamine 13*

To neat 2-nitrobenzyl alcohol (306 mg, 2 mmol) at 85°C was added (*R*)-phenylethylisocyanate (286 µl, 2 mmol). The mixture was stirred over night at the same temperature then purified by flash chromatography on silica gel (AcOEt:pentane=1:4) 540 mg, 90%, mp 52.5°C, $[\alpha]_{546}^{23^\circ}$ +44.3 (c=3, methanol); IR (CH_2Cl_2) 3582, 3407, 1710, 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (d, J=6.9 Hz, 3H), 4.78 (m, 1H), 5.07 (bs, 1H), 5.43 (s, 2H), 7.21–8.03 (m, 9H); Anal calcd for $C_{16}H_{16}N_2O_4$ C 64.05, H 5.38, N 9.34 found C 63.58, H 5.45, N 9.34.

N-(2-Aminobenzyloxycarbonyl)-R-1-phenylethylamine ***14***

To nitro compound **13** (330 mg, 1.1 mmol) in 95% ethanol was added $PtO_2 \cdot xH_2O$ (20 mg) and the suspension was stirred under H_2 (1 atm) at room temperature for 12 mn. After filtration and concentration of the filtrate under reduced pressure, the residue was purified by flash chromatography on silica gel (AcOEt:pentane=1:3), 175 mg, 60%, white solid, mp 106.9°C, $[\alpha]_{546}^{23°}$ +46.8 (c=1, methanol); IR (CH_2Cl_2) 3581, 3403, 1693, 1608 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.65 (d, J=6.7 Hz, 3H), 3.82 (bs, 1H), 4.33 (bs, 2H), 5.02 (m, 1H), 5.24 (m, 2H), 6.84–7.56 (m, 9H); Anal calcd for $C_{16}H_{18}N_2O_2$ C 71.17, H 6.72, N 10.38, found C 70.84, H 6.51, N 10.19.

N-[2-(R-1-Phenylethylaminocarboxymethyl)-phenyl]-2,3-dibromo-2-fluoropropionamide ***15***

To aniline **14** (27 mg, 0.1 mmol) and $(i\text{-}Pr)_2NEt$ (17 µl, 0.1 mmol) in CH_2Cl_2 (1 ml) was added dropwise racemic 2,3-dibromo-2-fluoropropanoyl bromide (31 mg, 0.1 mmol) in CH_2Cl_2.(0.5 ml) The reaction mixture was stirred for 1 h at room temperature, then purified on a short column of silica; (AcOEt:pentane=1:4), 39 mg, 77%, mp 116.3°C, $[\alpha]_{546}^{23°}$ +35.8 (c=1, methanol); IR (CH_2Cl_2) 3405, 1689, 1579, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (d, J=6.7 Hz, 3H), 4.05 (dd, J=7.7, 11.3 Hz, 1H), 4.37 (dd, J=11.3, 31.3 Hz, 1H), 4.63 (bs, 1H), 4.78 (m, 1H), 5.01 (m, 2H), 7.18–7.85 (m, 9H), 9.97 (bs, 1H); ^{19}F NMR ($CDCl_3$) δ −121.06 (dd, J=7.5, 31.1 Hz); Anal calcd for $C_{19}H_{19}Br_2FN_2O_3$ C 45.46, H 3.81, N 5.58, found C 45.41, H 3.98, N 5.56.

N-[2-(R-1-Phenylethylaminocarboxymethyl)-phenyl]-2,3-dibromo-2-fluoroazetidin-2-one ***16***

This azetidinone was obtained by cyclisation of bromide **15** with K_2CO_3 in refluxing acetone;[14] (AcOEt:pentane=1:4), colorless oil, 18 mg, 83%, $[\alpha]_{546}^{23°}$ +24.5 (c=0.88, AcOEt); IR (CH_2Cl_2) 3655, 3410, 1770, 1710, 1591, 1487 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (d, J=6.8 Hz, 3H), 4.23 (dd, J=6.9, 7.3 Hz, 1H), 4.38 (dd, J=7.2, 9.4 Hz, 1H), 4.99 (bs, 1H), 5.07 (m, 2H), 7.19–7.34 (m, 9H); ^{19}F NMR ($CDCl_3$) δ −122.50 (dd, J=7.1, 9.2 Hz); Anal calcd for $C_{19}H_{18}BrFN_2O_3$ C 54.20, H 4.31, N 6.65, found C 54.03, H 4.47, N 6.36.

Methyl 2-(2,3-dibromo-2-fluoropropionyloxy)-(S)-phenylacetate ***17***

To (*S*)-(+)-methyl mandelate (83 mg, 0.5 mmol) and di-isopropylethylamine (0.087 ml, 0.5 mmol) in CH_2Cl_2 (2 ml), was added dropwise racemic 2,3-dibromo-2-fluoropropanoyl bromide (156 mg, 0.5 mmol) in CH_2Cl_2. After being stirred for 30 mn at RT, the mixture was concentrated under vacuum and the residue was purified by flash chromatography on silica gel (AcOEt:pentane=1:9), slightly yellowish oil, 163 mg 78%, $[\alpha]_{546}^{23°}$ +84.0 (c=1, methanol); IR (CH_2Cl_2) 1752, 1741 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.7 (s, 3H), 4.03 (dd, J=9, 11.5 Hz, 1H of one diastereomer), 4.05 (dd, J=8.7, 11.7 Hz, 1H of one diastereomer), 4.15 (1H, J=11.8, 28.2 Hz, 1H of one diastereomer), 4.21 (dd, J=11.5, 27.6 Hz, 1H of one diastereomer), 6.00 (s, 1H of one diastereomer), 6.01 (s, 1H of one diastereomer), 7.33–7.43 (m, 5H); ^{19}F NMR ($CDCl_3$) δ −121.63 (dd, J=8.7, 27.9 Hz, 1F of one diastereomer), −121.56 (dd, J=9.1, 28.2 Hz, 1F of one diastereomer); ^{13}C NMR ($CDCl_3$) δ 33.96 (d, J=23.2 Hz), 52.75, 76.41, 91.36 (d, J=269.7 Hz, *C*FBr of one diastereomer), 91.70 (d, J=270.8 Hz, *C*FBr of one diastereomer), 127.41, 127.54, 128.83, 129.62, 132.20, 163.00 (d, J=27.9 Hz), 167.53; Anal calcd for $C_{12}H_{11}Br_2FO_4$ C 36.21, H 2.79, found C 36.09, H 2.81.

N-(R-1-Phenylethyl)-2,3-dibromo-2-fluoropropionamide ***18***

This compound was prepared according to the procedure described for the preparation of amide **15**, starting however from (*R*)-(+)-1-phenylethylamine instead of aniline; (ether:pentane=1:3), white solid, 144 mg, 83%, mp 95.5°C, $[\alpha]_{546}^{23°}$ +89.0 (c=2, AcOEt). IR (CH_2Cl_2) 3598, 3408, 1690, 1513 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (d, J=6.8 Hz, 3H), 3.96 (dd, J=7.5, 11.3 Hz, 1H of one diastereomer), 3.97 (dd, J=7.5, 11,3 Hz, 1H of one diastereomer), 4.27 (dd, J=11.3, 31.6 Hz, 1H of one diastereomer), 4.30 (dd, J=11.3, 31.6 Hz, 1H of one diastereomer), 5.11 (m, 1H), 6.53 (bs, 1H), 7.28 (s, 5H of one diastereomer), 7.30 (s, 5H of one diastereomer); ^{19}F NMR ($CDCl_3$) δ −121.99 (dd, J=7.4, 31.7

Hz, 1F of one diastereomer), −121.86 (dd, J=7.5, 31.5 Hz, 1F of one diastereomer; MS m/z=274–2 ($M^{+\cdot}$–Br), 192, 178, 105; Anal calcd for $C_{11}H_{12}Br_2FNO_3$ C 37.42, H 3.43, N 3.97, found C 37.57, H 3.61, N 3.75.

N-*(R-1-Phenylethyl)-2-bromo-2-fluoroazetidin-2-one* ***19***

To propionamide **18** (35 mg, 0.1 mmol) in acetone (1 ml) was added powdered K_2CO_3 (69 mg, 0.5 mmol). The suspension was vigorously stirred at reflux for 45 mn. The solid was filtrated off, the filtrate was evaporated and the residue purified by plate chromatography on silica gel (ether:pentane=1:5), colorless oil, 22 mg, 81%, $[\alpha]_{546}^{23°}$ +39.0 (c=1.08, AcOEt); IR (CH_2Cl_2) 1773 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (d, J=7.0 Hz, 3H of one diastereoisomer), 1.61 (d, J=7.0 Hz, 3H of one diastereoisomer), 3.5 (dd, J=6.7, 6.7 Hz, 1H of one diastereoisomer), 3.61 (dd, J=6.7, 8.2 Hz, 1H of one diastereoisomer), 3.67 (dd, J=6.8, 6.8 Hz, 1H of one diastereoisomer), 3.77 (dd, J=6.8, 8.5 Hz, 1H of one diastereoisomer), 4.89 (m, 1H), 7.17–7.37 (m, 5H); ^{19}F NMR ($CDCl_3$) δ −123.67 (dd, J=6.8, 8.7 Hz, 1F of one diastereoisomer), −123.51 (dd, J=6.9, 8.9 Hz, 1F of one diastereoisomer); Anal calcd for $C_{11}H_{11}BrFNO$ C 48.57, H 4.08, N 5.15, found C 48.45, H 4.16, N 4.97.

N-*[(R)-2-Hydroxy-1-phenylethyl]-(2R) and (2S)-2,3-dibromo-2-fluoropropionamides, (2S,2′R)-****20*** *and (2R,2′R)-****20***

To (*R*)-2-amino-2-phenylethanol (274 mg, 2 mmol) and diisopropylethylamine (0.348 ml, 2 mmol) in CH_2Cl_2 was added dropwise the racemic 2,3-dibromo-2-fluoropropanoyl bromide (624 mg, 2 mmol) in CH_2Cl_2. After stirring for 30 mn at ambient temperature, the reaction mixture was concentrated and then passed through a pad of silica gel: white solid, 654 mg, 88%. Flash chromatography on SiO_2 with an ether:pentane 1:2→1.4:1.6 gradient yielded separated diastereoisomers. One diastereoisomer: R_f 0.26 (ether:pentane=1:1), mp 124.8°C, $[\alpha]_{546}^{23°}$ −76.3 (c=2, methanol); IR (CH_2Cl_2) 3577, 3388, 1682, 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.89 (m, 2H), 3.98 (dd, J=7.8, 11.3 Hz, 1H), 4.28 (dd, J=11.3, 31.5 Hz, 1H), 5.05 (m, 1H), 7.13 (bs, 2H), 7.29 (s, 5H); ^{19}F NMR ($CDCl_3$) δ −121.86 (ddd, J=3.2, 7.5, 31.5 Hz); ^{13}C NMR ($CDCl_3$) δ 34.00 (d, J=23.0 Hz), 55.48, 65.26, 95.63 (d, J=272.3 Hz), 126.30, 127.92, 128.73, 137.33, 163.78 (d, J=21.9 Hz). Anal calcd for $C_{11}H_{12}Br_2FNO_2$ C 35.80, H 3.28, N 3.80, found C 35.95, H 3.31, N 3.71. The other diastereoisomer: R_f 0.20 (ether:pentane=1:1), $[\alpha]_{546}^{23°}$ −127.9 (c=2, methanol); mp 132.2°C; IR (CH_2Cl_2) 3579, 3390, 1682, 1503 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.93 (d, J=4.1 Hz, 2H), 3.98 (dd, J=7.5, 11.3 Hz, 1H), 4.26 (dd, J=11.4, 31.6 Hz, 1H), 5.05 (m, 1H), 7.13 (bs, 2H), 7.29 (s, 5H); ^{19}F NMR ($CDCl_3$) δ −121.95 (ddd, J=3.8, 7.6, 31.5 Hz); ^{13}C NMR ($CDCl_3$) δ 34.19 (d, J=23.0 Hz), 55.76, 65.27, 95.68 (d, J=272.4 Hz), 126.59, 128.01, 128.77, 137.58, 164.26 (d, J=22.0 Hz); Anal calcd for $C_{11}H_{12}Br_2FNO_2$ C 35.80, H 3.28, N 3.80, found C 36.11, H 3.41, N 3.94.

N-*[(R)-2-Hydroxy-1-phenylethyl]-3-bromo-2-fluoro-2(Z)-propenamide* ***21***

To the mixture of the diastereoisomeric bromides (2*S*,2′*R*)-**20** *and* (2*R*,2′*R*)-**20** (148 mg, 0.4 mmol) was added acetone (2 ml) and pulverized K_2CO_3 (320 mg, 1.6 mmol). The suspension was stirred at reflux for 3 h. Solids were filtered off, then the filtrate was concentrated under reduced pressure and the residue was flash chromatographed on silica gel (AcOEt:pentane=3:5), white solid, mp 150.3°C, 79 mg, 68%, $[\alpha]_{546}^{23°}$ −50.9 (c=2, AcOEt); IR (CH_2Cl_2) 3580, 3400, 1673, 1633, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.87 (m, 2H), 5.05 (m, 1H), 6.82 (d, J=26.6 Hz, 1H), 6.92 (bs, 1H), 7.28 (m, 5H); ^{19}F NMR ($CDCl_3$) δ −119.50 (d, J=26.6 Hz); ^{13}C NMR ($CDCl_3$) δ 55.48, 65.81, 93.91 (d, J=258.9 Hz), 98.08 (d, J=17.3 Hz), 126.66, 128.23, 129.03, 137.95, 158.06 (d, J=28.6 Hz); MS m/z=256–8 ($M^{+\cdot}$–CH_2OH), 151–3, 106, 91, 77; Anal calcd for $C_{11}H_{11}BrFNO_2$ C 45.87, H 3.85, N 4.86, found C 45.71, H 3.93, N 4.85.

General procedure for the acidic hydrolysis of each diastereoisomer of N-*[(*R*)-2-hydroxy-1-phenylethyl]-2,3-dibromo-2-fluoropropionamides (2*S*,2′*R*)-20 and (2*R*,2′*R*)-20 to give 2,3-dibromo-2-fluoropropionic acids (2*S*)-22 and (2*R*)-22*

To hydroxyamide (2*S*,2′*R*)-**20** or (2*R*,2′*R*)-**20** (73 mg, 0.2 mmol) in dioxane (0.5 ml) was added 12 N H_2SO_4 (0.4 ml). The mixture was stirred in a thick-walled glass tube with a teflon screw seal at 105°C (bath temperature) for 15 h. Completion of the hydrolysis was checked by ^{19}F NMR of the reaction mixture. After adding water (4 ml), extraction (CH_2Cl_2, 5×2 ml) then drying of the organic phase ($MgSO_4$), evaporation of the solvents gave the respective chiral 2,3-dibromo-2-fluoropropionic acids, colorless oils, 44 mg, 88%. Acid (2*R*)-**22** from the lower-R_f amide (2*R*,2′*R*) **20** (R_f=0.2): IR (CH_2Cl_2) 3552, 3410, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.02 (d, J=8.4, 11.3 Hz, 1H), 4.16 (d, J=11.3, 28.6 Hz, 1H), 8.25 (bs, 1H); ^{19}F NMR ($CDCl_3$) δ −120.89 (dd, J=8.1, 28.7 Hz); ^{13}C NMR ($CDCl_3$) δ 33.99 (d, J=23.6 Hz), 92.05 (d, J=272.8 Hz), 166.06 (d, J=28.0 Hz); MS m/z=251–249–247 ($M^{+\cdot}$), 205, 169, 151, 124, 105, 90; HRMS calcd for $C_3H_3Br_2FO_2$ 247.8484, found 247.8485. Acid (2*S*)-**22** from the upper-R_f amide (2*S*,2′*R*)-**20** (R_f=0.26): IR, ^1H, ^{19}F, ^{13}C NMR and MS identical with those of acid **22** obtained from the lower-R_f amide; MS m/z=251–249–247 ($M^{+\cdot}$), 205, 169, 151, 124, 105; HRMS calcd for $C_3H_3Br_2FO_2$ 247.8484, found 247.8482.

N-*(2-Chloromethylphenyl)-3-bromo-3-fluoroazetidin-2-one* **2**

Obtained by treating the racemic alcohol **11** with $SOCl_2$/DMF;[15] (ether:pentane=1:2), colorless oil, 32 mg, 75%; IR (CH_2Cl_2) 1775 cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 4.47 (dd, J=6.9, 7.3, 1H), 4.7 (dd, J=7.2, 9.4 Hz, 1H), 4.8 (dd, J=12 Hz, 2H), 7.4 (m, 4H); ^{19}F NMR [$(CD_3)_2CO$] δ −118.7 (dd, J=7, 9.2 Hz); MS m/z=291–293–295 ($M^{+\cdot}$), 216, 167, 132, 148; HRMS calcd for $C_{10}H_8BrClFNO$ 290.9462, found 290.941.

General procedure for the preparation of optically active N-*(2-chloromethylphenyl) 3-bromo-3-fluoroazetidin-2-ones (3*R*)-2 and (3*S*)-2*

To propionic acid (2*S*)-**22** or (2*R*)-**22** (87.5 mg, 0.35 mmol) in CH_3CN (2 ml), was added successively 2-*tert*-butyldimethylsilyloxymethylaniline (83 mg, 0.35 mmol) and DCC (79 mg, 0.38 mmol) in CH_3CN (0.8 ml). The mixture was stirred for 20 mn at ambient temperature, then the precipitate was removed by filtration and washed with CH_3CN (0.3 ml). To the resulting organic solution was added powdered K_2CO_3 (241 mg, 1.75 mmol) and the suspension was vigorously stirred at 80°C for 90 mn. After removal of the solid by filtration, the solution was added to a suspension of powdered glass (25 mg) in 40% aqueous HF solution (100 µl, 1.96 mmol). The mixture was stirred for 15 mn at 20°C then neutralized with 5% $NaHCO_3$ and extracted with CH_2Cl_2. The organic solution was dried ($MgSO_4$) and evaporated. The crude resulting oil was dissolved in DMF (0.8 ml) and added to a mixture of thionyl chloride (60 mg, 0.52 mmol) and dry DMF (1 ml) according to the procedure described for the preparation of the racemic trihalogenated azetidinone **2**. The optically active azetidinones (3*R*)-**2** $[\alpha]_{546}^{23°}$ −5.2 (c=3.4, AcOEt) and (3*S*)-**2** $[\alpha]_{546}^{23°}$ +5.2 (c=3.4, AcOEt), were obtained as oils. 49 mg, 48% overall yield from acids (2*R*)-**22** and (2*S*)-**22** respectively. TLC, IR, ^1H, ^{19}F and ^{13}C NMR of each enantiomer were identical with those of the racemic azetidinone **2**.

N-*(2-Fluoromethylphenyl)-3-bromo-3-fluoroazetidin-2-one* **3**

Synthesized by treatment of alcohol **11** with DAST;[15] (ether:pentane=1:2), colorless oil, 12 mg, 54%; IR (CH_2Cl_2) 1770 cm^{-1}; ^1H NMR [$(CD_3)_2CO$] δ 4.55 (dd, J=7.1, 7.3 Hz, 1H), 4.76 (dd, J=7.3, 9.1 Hz, 1H), 5.55 (d, J=47.4 Hz, 2H); ^{19}F NMR [$(CD_3)_2CO$] δ −204.4 (t, J=47.5 Hz, 1F), −118.4 (t, J=7.1, 9.2 Hz, 1F). MS: m/z=275–277 ($M^{+\cdot}$), 151, 123, 109, 96; HRMS calcd for $C_{10}H_8BrF_2NO$ 274.9758, found 274.9763.

N-*(2-Acetoxymethyl-5-methoxyphenyl)-3-bromo-3-fluoroazetidin-2-one* **4**

By treatment of alcohol **12** with acetic anhydride in presence of DMAP;[14] (ether:pentane=1:1.5), colorless oil, 25 mg, 64%; IR (CH_2Cl_2): 1775, 1725 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.99 (s, 3H), 3.75

(s, 3H), 4.27 (dd, J=6.9 Hz, 1H), 4.41 (dd, J=6.9, 8.8 Hz, 1H), 5.03 (s, 2H), 7.07, (m, $3H_{arom}$). ^{19}F NMR ($CDCl_3$) δ −122.8 (dd, J=7.1 Hz). MS m/z=345 ($M^{+\cdot}$), 302, 286, 221, 196, 162. HRMS calcd for $C_{13}H_{13}BrFNO_4$ 345.0012, found 344.9990.

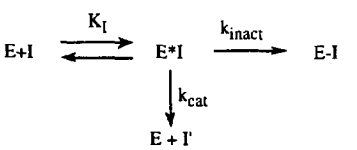
N-(2-Methylphenyl)-(2R)-2,3-dibromo-2-fluoropropionamide **23** *and* N,N′*-(2R,2′R)-bis(2,3-dibromo-2-fluoropropionyl)-*N,N′*-dicyclohexylurea* **24**

To the propionic enantiomer **22** (50 mg, 0.2 mmol) in CH_2Cl_2 (0.8 ml), used to prepare the dextrorotatory chloride **2**, was added successively DCC (43 mg, 0.21 mmol) in CH_2Cl_2 (0.5 ml) and *o*-toluidine (21.4 µl, 0.2 mmol). The mixture was stirred for 30 mn at room temperature and the solid was filtered off. The filtrate was chromatographed on a silica gel preparative plate (ether:pentane=0.8:9.2), providing the optically active propionamide **23** (R_f=0.26) 46.8 mg, 69%, $[\alpha]_{546}^{23°}$ −50.8° (c=1.9, AcOEt), with mp, TLC and IR, ^1H NMR, ^{19}F NMR and MS spectra identical to those of the racemic propionamide **23**[15] and the *N,N′*-diacylurea **24** (Rf=0.47) white solid, mp 194°C, 6.2 mg, 9%, $[\alpha]_{546}^{23°}$ +281.2 (c=0.16, AcOEt); IR (solid) 1742, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (m, 8H), 1.87 (m, 8H), 2.29 (m, 4H), 3.74 (m, 2H), 4.04 (dd, J=5.9, 10.7 Hz, 2H), 4.43 (dd, J=10.9, 32.6 Hz, 2H); ^{19}F NMR ($CDCl_3$) δ −122.21 (m); ^{13}C NMR ($CDCl_3$) δ 25.04, 26.46, 26.60, 28.64, 29.69, 30.15, 37.25 (d, J=24.9 Hz), 64.39, 93.00 (d, J=278.2 Hz), 148.00, 167.25 (d, J=23.5 Hz); MS (NH_3–CI): 689 (MH^+), 546 (M^+–Br_2).

Enzymatic studies

HLE was purchased from Elastin Products Co. Enzyme concentrations were determined by active-site titration as described in the literature.[11] The enzyme was assayed spectrophotometrically with methoxysuccinyl-alanyl-alanyl-prolyl-valyl-*para*-nitroanilide (MeO-Suc-A_2-P-V-*p*NA) purchased from Sigma. The enzymatic reactions were followed in 0.1 M Tris, 0.01% $Brij_{35}$, pH 8.0, 5% v/v DMSO at 37°C using a Perkin–Elmer Lambda 5 spectrophotometer equipped with a thermostated cell holder. The kinetic parameters for the inhibition were obtained either by the progress curve method (compound **2**) or by the preincubation method (compound **3**) by fitting the experimental data to the equations developed.[32] For the progress curve method, the changing slopes ΔA/Δt (or velocities *v*) with time of the progress curves for the hydrolysis of the chromogenic substrate were obtained from computer-assisted spectrophotometer (A is the absorbance at 405 nm). The kinetic parameters K_I and k_{inact} were determined by fitting the experimental data to the equation $\pi = k_{inact}[I]'/(K_I+[I]')$ where $-\pi$ is the slope of the linear plot of ln*v* *versus* time at a given inhibitor concentration, [I]′ the modified inhibitor concentration $[I]/(1+[S]/K_m)$ and K_m the Michaelis constant for the substrate. When $[I]' < K_I$, the equation for the plot of π *versus* [I]′ is reduced to $\pi = (k_{inact}/K_I)[I]'$.

The partition ratio r represents the average number of enzyme 'turn-overs per inactivation' k_{cat}/k_{inact} (eq. 1). It was evaluated by plotting $[HLE]/[HLE]_0 = f([I]_0/[HLE]_0)$.[24]



(eq. 1)

The enzymatic hydrolysis of **4** by HLE was performed following the initial velocities of the hydrolysis of **4** (50–500 µM) at 300 nm. As two successive reactions were observed during the experiment, the absorption coefficient of the hydrolysis product was determined from the absorbance of the reaction mixtures after the first reaction has ended.

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