Fluoro-Olefins as Peptidomimetic Inhibitors of Dipeptidyl Peptidases

Pieter Van der Veken,[2] Kristel Senten,[2] IstvaÂn KertéÂsz,[2] Ingrid De Meester,[3] Anne-Marie Lambeir,[3] Marie-Berthe Maes,[3] Simon ScharpeÂ,[3] Achiel Haemers,[2] and Koen Augustyns*[,2]

Departments of Medicinal Chemistry and Medical Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

Received May 28, 2004

The feasibility of the fluoro-olefin function as a peptidomimetic group in inhibitors for dipeptidyl peptidase IV and II (DPP IV and DPP II) is investigated by evaluation of N-substituted Gly-¾[CFd C]pyrrolidines, Gly- ¾[CFd C]piperidines, and Gly- ¾[CFd C](2-cyano)pyrrolidines. Of this later class, the (Z)- and (E)-fluoro-olefin analogues were prepared and chemical stability in comparison with the parent amide was checked. Most of these compounds exhibited a strong binding preference toward DPP II with IC_{50} values in the low micromolar range, while only low DPP IV inhibitory potential is seen.

Introduction

The use of non-hydrolyzable amide isosteres is an established approach to overcome one of the major drawbacks in the use of peptides as therapeutic agents, namely, their rapid degradation by peptidases. From the virtually endless list of surrogates for the peptide bond that have been suggested in the literature, olefinic linkages probably show the highest structural resemblance to the parent structure (Figure 1). [1,2]

Generally, olefins and amides share a planar geometry with bond angles close to 120É, owing to formal sp^2 hybridization. [3] While in ambient conditions the amide bond can occur as the cis or the thermodynamically more stable trans isomer, the much higher barrier to rotation for alkenes offers the opportunity to prepare peptidomimetics with a conformationally rigidified backbone.[4] Compared to unsubstituted olefins, the introduction of a fluorine atom at the double bond has been suggested as a modification leading to a superior amide bond isostere. Theoretical studies and experimental data indeed show that the fluoro-olefin unit and the peptide bond share highly similar geometrical features, including bond angles and bond lengths. [3,5] Furthermore, the high electronegativity of the fluorine atom renders a dipolar character to the alkene bond mimicking the charge distribution in amide functionalities, albeit less pronounced than for the latter. [5,6] Finally, a well-known stability problem of olefin-based peptidomimetics consisting of isomerization of the double bond into conjugation with the carboxylate group has never been described for fluoro-olefin containing products, clearly indicating a stabilizing effect of the fluorine atom on the alkene junction. [7,8] Despite its attractiveness as a peptide bond isostere, surprisingly few examples of biological acitivity of fluoro-olefin peptidomimetics have been described in the literature. Allmendinger et al. reported the preparation of a substance P analogue containing the (Z)-Phe-ã[CFd CH]-Gly dipeptide isostere.[7,9] In a receptor binding assay, this compound was

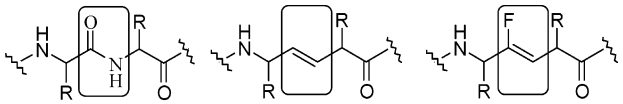


Figure 1. Peptide bond isosteres.

almost as active as substance P itself, exhibiting higher chemical stability and receptor binding affinity compared to the trans olefin analogue. Bartlett and Otake experimentally verified the applicability of tripeptide analogues with the (Z)-Gly-ã[CFd CH]-Leu-Xaa structure as ground-state analogue inhibitors of the zinc endopeptidase thermolysin. [10] More recent contributions by Welch et al. in the field of dipeptidyl peptidase IV inhibition will be discussed later.

Proline selective serine dipeptidylpeptidases cleave dipeptides from the amino terminus of peptides or proteins with proline preferentially at the penultimate position. Representative examples are dipeptidyl peptidase II, IV, 8 and 9 (DPP II, DPP IV, DPP8, and DPP9) and fibroblast activating protein R (FAP R).[11] DPP IV is by far the best studied member among these enzymes, and it is currently a well validated target for the treatment of type 2 diabetes. [12,13]

Since the discovery of Xaa-(2-cyano)pyrrolidines (1, Figure 2) as potent, reversible inhibitors of DPP IV, efforts have been made for the further improvement of these ªleadº structures in terms of activity and target selectivity. [14] The potent DPP IV inhibition of these compounds can be subscribed to the electrophilic carbonitrile function that can interact with the catalytic serine-OH group in the active site of the enzyme. Recently, X-ray diffraction data of enzyme - inhibitor complexes have indicated that the relatively strong inhibition results from the reversible formation of an intermediary enzyme-bound imidate. [15] This compound class includes many potent inhibitors of the enzyme, but most suffer more or less from chemical instability due to the intramolecular cyclization of the N-terminal amine onto the nitrile, forming inactive cyclic amidines and/or their diketopiperazine hydrolysis products (Scheme 1A). This cyclization requires the inhibitor to assume the cis amide conformation, a situation that is

* To whom correspondence should be addressed. Phone: + 32-(0)3-820 27 03. Fax: + 32-(0)3-820 27 39. E-mail: Koen.Augustyns@ua.ac.be.
[2] Department of Medicinal Chemistry.
[3] Department of Medical Biochemistry.

10.1021/jm0495982 CCC: $30.25 • 2005 American Chemical Society
Published on Web 08/26/2004

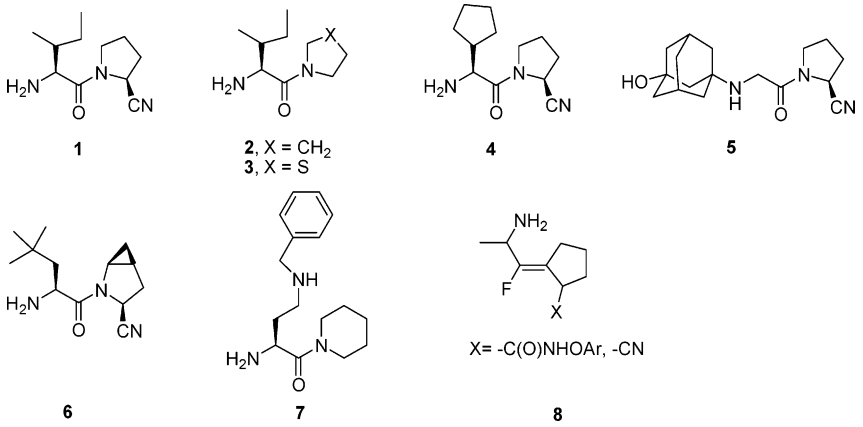
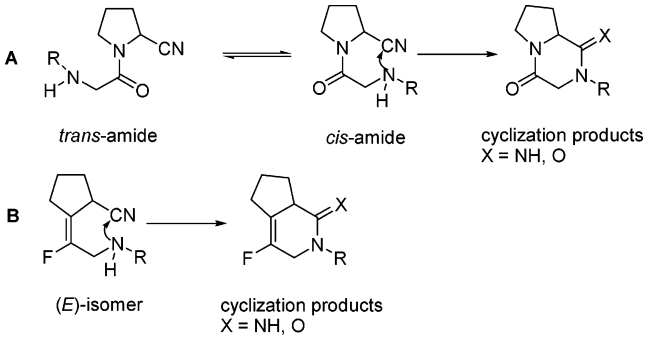


Figure 2. Proline-derived DPP IV and DPP II inhibitors.

Scheme 1. Inactivation Mechanism of (2-Cyano)pyrrolidines (A) and the (E)-Fluoro-Olefin Analogue (B)



more common for prolylamide bonds than for other peptide bonds.

Pyrrolidide (2) and thiazolidide (3) based inhibitors that lack the nitrile moiety possess greater stability, though at the cost of reduced potency, with a few very recent notable exceptions. [16-18] Introduction of bulky, N-terminal amino acids (4) in the 2-cyanopyrrolidine series seemed to result in an improved chemical stability. [14] Other efforts to overcome these instability problems were based on the introduction of bulky N-substituted glycine residues at the P_2 position of these 2-cyanopyrrolidine-based inhibitors. A large number of inhibitors containing N-substituted glycines with different steric and electronic characteristics have been prepared. [19-21] The more hindered adamantyl analogue (5) is a potent, stable, selective DPP IV inhibitor possessing oral availability and a pharmacokinetic profile with potential for once-a-day administration. Recently, a series of aminoacyl- L-cis-4,5-methanoprolinenitrile-based inhibitors with â-branching in the N-terminal amino acid (6) provided enhanced chemical stability and high inhibitory potency. [22]

Changing the five-membered ring in these pyrrolidine or 2-cyanopyrrolidine based DPP IV inhibitors into a six-membered ring decreased the DPP IV inhibitory potency and rendered compounds with an increased DPP II affinity. [23,24] This observation was exploited and led to the preparation of the most potent (IC $_{50}$) 10^{-9} M) and selective (10^{6}) DPP II inhibitors (7) known today. [25] Introduction of a 2-nitrile function in the piperidine ring of this series resulted in less potent and less selective DPP II inhibitors compared to their respective parent compounds. [24,25] This is in sharp contrast with the known potentiating effect of a 2-nitrile function in pyrrolidine-based DPP IV inhibitors.

The concept of conformationally constrained (Z)-fluoro-olefin dipeptide isosteres that mimic the active atransº conformation of the dipeptidyl peptidase inhibitors was already exploited by Welch et al. [26-28] in synthesizing fluoro-olefin analogues of two known inhibitors with alanyl- ¾[CFd C]-proline structure (8). Although no general and unambiguous conclusions considering the effect on inhibitory potency of introducing the fluoro-olefin group in these compounds can be drawn from only two examples, Welch could experimentally verify that there is indeed enzyme affinity for these peptidomimetics.

We focused our research on fluoro-olefin analogues of N-substituted glycylpyrrolidides (9.7), -piperidides (9.8), and -(2-cyano)pyrrolidines (11.7) as DPP IV or DPP II inhibitors. To fully assess the feasibility of the fluoro-olefin function as a peptidomimetic group in these inhibitors, the olefin (10), amide (15), and thioamide (16) analogues of selected compounds were also prepared.

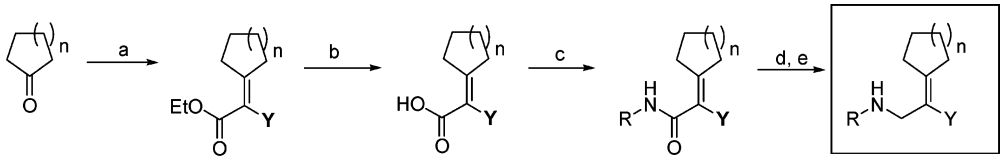
The five-membered- (9.7) and six-membered-ring analogues (9.8) were both investigated, since we previously reported that inhibitors with pyrrolidine at the P_1 position are more potent and selective for DPP IV, whereas the piperidine analogues are potent and selective DPP II inhibitors. [24]

The preparation of both the (E)- and (Z)-fluoro-olefin analogues of N-substituted Gly-2-(cyano)pyrrolidines (11.7) offers the opportunity to examine the generally assumed trans selectivity of these well-known DPP IV inhibitors. [29] These compounds were originally not designed as DPP II inhibitors because of the above-mentioned fact that nitriles are less interesting for this enzyme. Chemical stability of both (Z)- and (E)-isomers in comparison with the parent amide was investigated. Indeed, Welch et al .[27] claimed improved stability of the (Z)-fluoro-olefin 8 (X) CN), but the authors did not make a comparison with the parent amide or the (E)-isomer. Likewise, also the in vitro enzyme inhibition by this inhibitor 8 (X) CN) was not compared to the parent amide compound.

Chemistry

The core problem in the synthesis of peptidomimetic fluoro-olefins consists of the construction of the â-fluoro-â,ç-unsaturated amine group of these compounds. In the most straightforward approach, target products are

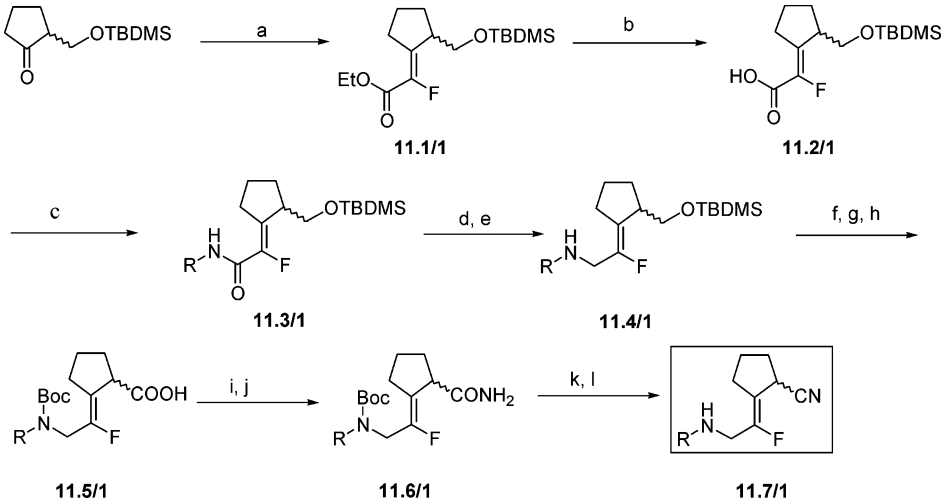
Scheme 2. Synthesis of the Fluoro-Olefin and Olefin Compounds [a]



n =1,2	Y = F	**9.1** (n = 1) **9.2** (n = 2)	**9.3** (n = 1) **9.4** (n = 2)	**9.5** (n = 1) **9.6** (n = 2)	**9.7** (n = 1) **9.8** (n = 2)
	Y = H	**10.1** (n = 1) **10.2** (n = 2)	**10.3** (n = 1) **10.4** (n = 2)	**10.5** (n = 1) **10.6** (n = 2)	**10.7** (n = 1) **10.8** (n = 2)

[a] Reagents: (a) ethyl(diethoxyphosphoryl)(fluoro)acetate (9) or ethyl(diethoxyphosphoryl)acetate (10), NaH, Et_2O, 0 ÉC; (b) KOH, MeOH/H_2O; (c) RNH_2, TBTU, DIEA, CH_2Cl_2; (d) $POCl_3$, CH_2Cl_2; (e) $LiAlH_4$/Et_2O, 0 ÉC.

Scheme 3. Synthesis of the (Z)-Fluoro-Olefin Analogues of N-Substituted Gly-2-(cyano)pyrrolidines (11.7/1)[a]



11.1/1 **11.2/1** **11.3/1** **11.4/1** **11.5/1** **11.6/1** **11.7/1**

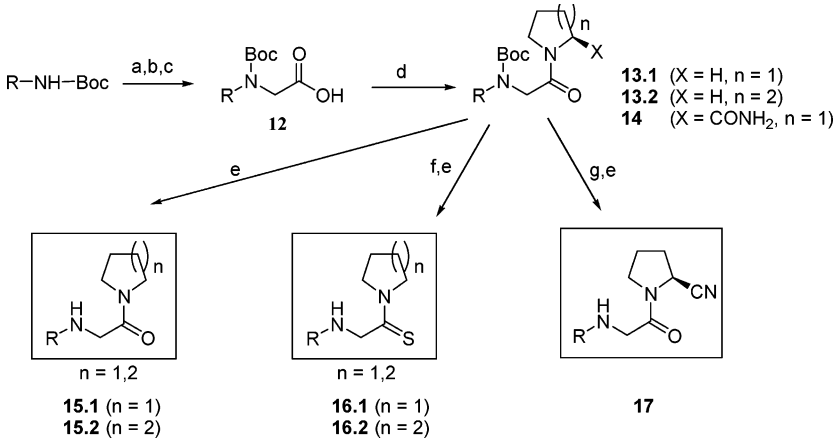
[a] Synthetic steps for the (E)-isomers (11.7/2) are identical. Reagents: (a) ethyl (diethoxyphosphoryl)(fluoro)acetate, NaH, Et_2O, 0 ÉC; (b) KOH, MeOH/H_2O; (c) RNH_2, TBTU, DIEA, CH_2Cl_2; (d) $POCl_3$, DIPEA, CH_2Cl_2; (e) $LiAlH_4$/Et_2O, 0 ÉC; (f) $(Boc)_2O$, DIPEA, CH_2Cl_2; (g) AcOH/H2O, THF; (h) Jones' reagent (CrO_3/aqueous H_2SO_4), acetone; (i) i-Bu-OC(O)Cl, DIEA, CH_2Cl_2; (j) NH_4OH; (k) $POCl_3$, imidazol, pyridine, 0 ÉC; (l) TFA, CH_2Cl_2.

synthesized from a suitable R-fluoro- R,â-unsaturated carbonyl or carboxyl precursor. [10,27,30] Valuable alternatives, including a strategy based on the Aza-Cope rearrangement or reduction - oxidative alkylation (R-OA) methodology, have been reported. [7,31,32]

For the synthesis of our target compounds, R-fluoro-R,â-unsaturated esters 9.1 and 9.2 and the R,â-unsaturated esters 10.1 and 10.2 were prepared from cyclopentanone or cyclohexanone using an optimized Wadsworth - Horner - Emmons protocol described previously (Scheme 2).[33] The successful outcome of this reaction seems to be very much confined to specific reaction conditions and the use of NaH as base. Further steps comprise the saponification of the ethyl ester in 9.1, 9.2, 10.1, and 10.2 and the coupling of the free acids 9.3, 9.4, 10.3, and 10.4 with the amine component. Reduction of the resulting R-fluoro- R,â-unsaturated amide group in 9.5 and 9.6 and in 10.5 and 10.6 occurred through a $POCl_3$ preactivation to the chloroimidate, followed by $LiAlH_4$ reduction. [33]

The methodology developed for the Wadsworth - Horner - Emmons reaction and the reduction of R-fluoro-R,â-unsaturated amide bonds was also used for the synthesis of the E- and Z-fluoro-olefin analogues of selected N-substituted Gly-2-(cyano)pyrrolidines. [33] Similar to Welch, [26,27,30] racemic TBDMS-protected (2-hydroxymethyl)cyclopentanone was chosen as the starting material for the construction of the pseudodipeptide skeleton (Scheme 3). The yield of the subsequent fluoro-olefination was 74%, with a 1.3 E/Z isomeric ratio. Double bond isomers of 11.1 were separated by column chromatography. The fluoro-olefin products 11.1 were then subjected to alkaline hydrolysis in almost quantitative yield (11.2) and coupled to the appropriate amine using the standard peptide coupling reagent TBTU. For the activation and reduction sequence of R-fluoro- R,â-unsaturated amides 11.3, 2 equiv of diisopropylethylamine were added to the reaction mixture to prevent acidolytic cleavage of the TBDMS group by hydrogen chloride produced during the reaction with $POCl_3$. After reduction, the crude amines 11.4 were Boc-protected, followed by selective acidic hydrolysis of the TBDMS group and chromatographic purification of the resulting alcohols. The Jones oxidation protocol was then used for the synthesis of the acids 11.5, followed by activation with isobutyl chloroformate and transformation into the corresponding primary amides 11.6. Dehydration in high yield gave the protected carbonitriles. Acidolytic deprotection of the Boc group using TFA provided the final products 11.7. The structural identity of (Z)- and (E)-isomers was accomplished by preparing the (Z)- and (E)-aldehydes for which NMR data were provided by Welch et al. [30] For this purpose, the N-methoxy- N-methylamides (ªWeinreb amidesº) were prepared by coupling acids 11.2 to N-methoxy- N-methylamine using TBTU. A clean $LiAlH_4$ reduction provided the alde-

Scheme 4. Synthesis of the Parent Amide (15, 17) or Thioamide (16) Compounds[a]



[a] Reagents: (a) n-BuLi, THF, 78 ÉC; (b) ethyl bromoacetate, Et $_2$O; (c) KOH, MeOH/H $_2$O; (d) TBTU, DIEA, pyrrolidine/piperidine (13) or prolinamide (14), CH_2Cl_2; (e) TFA, CH_2Cl_2; (f) Lawesson's reagent, toluene; (g) POCl $_3$, imidazol, pyridine, 0 ÉC.

hydes. ^{1}H- ^{1}H NOESY experiments on the amides 11.3 were indicative for the same conclusion.

For the synthesis of the parent compounds (Scheme 4), the N-Boc-N-substituted glycine part 12 was prepared from activated (n-BuLi) Boc-protected amine and ethyl bromoacetate, followed by alkaline hydrolysis. The free acids 12 were coupled to pyrrolidine, piperidine, or prolinamide. The thioamide analogues 16 were obtained by refluxing intermediates 13 with Lawesson's reagent in toluene. Dehydration of the intermediates 14 provided the nitrile group. Final deprotection of the Boc group using TFA offered the target products 15- 17.

Biochemical Evaluation

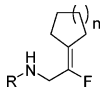
Biochemical data of a series of fluoro-olefin analogues of N-substituted glycylpyrrolidines and -piperidines are summarized in Table 1. For randomly selected R groups, the nonfluorinated olefin analogues and their parent amide and thioamide compounds were prepared, and biochemical results are give in Table 2.

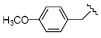
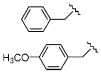
Most strikingly, compounds with a fluoro-olefin (9.7, 9.8) peptide bond isostere preferentially bind to DPP II rather than to DPP IV. This tendency is observed for both products with a five-membered and a six-membered ring, and it does not reflect the behavior of the recently described amide-containing pyrrolidine and piperidine based DPP II inhibitor. [23,24]

Comparing the assay results of fluoro-olefins (9.7, 9.8) and their nonfluorinated alkene analogues (10.7, 10.8), both types of isosteric replacement for the amide bond unexpectedly seem to result in similar DPP II affinities in the same range as the parent amides. A possible rationale for the absence of significant DPP IV affinity in the (fluoro-)olefin series might come from recently reported crystallographic structures [15,34- 36] of (peptide) inhibitor - DPP IV complexes: in all of these structures, the inhibitor's P $_2$- P_1 amide oxygen atom is involved in hydrogen bonding with Asn710 and Arg125 side chains of the enzyme. The poor hydrogen bond acceptor characteristics of the fluoro-olefin peptidomimetic series might therefore contribute to the low DPP IV inhibitory potency of these products. Although the DPP II crystal structure is not yet available, our results suggest that hydrogen bonding is less critical for DPP II affinity.

As expected, thioxylation of the amide group leads to an increased affinity for DPP II. [24,37] This effect is qualitatively identical to the result of introducing olefin peptide bond surrogates in the inhibitor's backbone. The lower hydrogen-bonding capacity of sulfur compared to

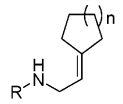
Table 1. Inhibition Data of Fluoro-Olefin Analogues of N-Substituted Glycylpyrrolidides (9.7) and -piperidides (9.8)[a]



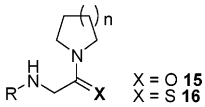
9.7 (n =1)	R	IC_{50} DPP IV (µM)	IC_{50} DPP II (µM)
a		> 1000	90 ± 19
b		> 1000	37.8 ± 2.6
c		> 1000	15.5 ± 1.5
d		> 1000	10.8 ± 1.0
e		> 1000	15.7 ± 6.1
f		> 1000	27.2 ± 2.2
g		> 250	1.3 ± 0.2
h		> 1000	53.7 ± 3.9
9.8 (n = 2)			
a		> 500	500 ± 50
b		> 250	91.3 ± 2.2
c		> 250	100 ± 10
d		> 1000	34.0 ± 3.7
e		> 250	13.4 ± 2.5
f		> 250	12.9 ± 0.4
g		> 250	21.9 ± 5.6
h		> 125	9.6 ± 1.0
i		> 1000	11.0 ± 1.4
j		> 1000	4.2 ± 0.2
k		> 250	1.04 ± 0.08
l		> 125	12.8 ± 0.6
m		> 1000	54.6 ± 1.1

[a] In a standard assay for evaluation of the DPP IV inhibition, the highest concentration of test compound was 1000 í M. Because of solubility problems, the highest concentration measured was sometimes limited to 125, 250, or 500 í M. In the case of enzyme activity, > 50% in the presence of the highest concentration (X) tested, the IC_{50} value is reported as > X.

Table 2. Inhibition Data of Olefin Analogues of N-Substituted Glycylpyrrolidides (10.7) and -piperidides (10.8) Together with the Parent Amides (15) and Thioamides (16)[a]



10



X = O **15**
X = S **16**

R	n = 1 compd	n = 1 IC_{50} (μM) DPP IV	n = 1 IC_{50} (μM) DPP II	n = 2 compd	n = 2 IC_{50} (μM) DPP IV	n = 2 IC_{50} (μM) DPP II
	**10.7a**	> 1000	62 ± 12	**10.8a**	> 1000	136 ± 14
	**10.7b**	> 1000	26 ± 3	**10.8b**	> 1000	55.3 ± 3.7
	**10.7c**	> 250	7.5 ± 0.4	**10.8c**	> 250	14.4 ± 1.5
	**15.1a**	148 ± 26	276 ± 72	**15.2a**	> 1000	177 ± 54
	**15.1b**	1000 ± 10	500 ± 50	**15.2b**	> 1000	397 ± 25
	**15.1c**	> 1000	63 ± 11	**15.2c**	> 1000	52 ± 2
				**15.2d**	> 1000	3.1 ± 0.1
	**16.1a**	> 1000	92 ± 5	**16.2a**	> 1000	69 ± 12
	**16.1b**	> 1000	> 250	**16.2b**	> 1000	48.4 ± 1.2
	**16.1c**	> 1000	67 ± 7	**16.2c**	> 1000	42 ± 5

[a] If the IC_{50} value was estimated to be above the highest concentration (X) tested, this activity parameter is indicated as > X.

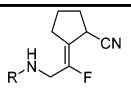
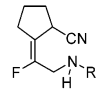
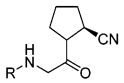
Figure 3. Alignment of Dab-Pip and 9.8j. Carbon atoms of Dab-Pip are shown in gray, and carbon atoms of 9.8j are shown in yellow. Hydrogens are omitted for clarity reasons.

oxygen might likewise be invoked to offer an explanation for the low DPP IV inhibitory potential of these thioamide products.

It is hard to draw general conclusions on which of the R substituents might be seen as favorable for (DPP II) inhibition. For the 4-(N-benzyl)piperidyl substituent potent DPP II inhibitory potency was noticed within the fluoro-olefin series (9.8k) as well as for the parent amide (15.2d). Flexible alignment of 2,4- L-diaminobutanoylpiperidine (Dab-Pip), a known DPP II inhibitor, [23,24] and 9.8j using MOE-FlexAlign provided a possible explanation for the DPP II inhibitory activity of 9.8j and 9.8k . The top solution showed a nice overlap between the R-nitrogen of Dab-Pip and the acyclic nitrogen of 9.8j , between the side chain nitrogen of Dab-Pip and the piperidine nitrogen of 9.8j , and between the carbonyl oxygen of Dab-Pip and the fluorine of 9.8j (Figure 3). Therefore, one can assume that both compounds can adopt a similar conformation upon binding to DPP II. We previously also reported [25] that introduction of a benzyl group on the side chain nitrogen of Dab-Pip increased the inhibitory activity. An increase in potency was also seen for 9.8k , which is a competitive inhibitor with respect to the substrate Ala-Pro- p-nitroanilide.

The biochemical data of the fluoro-olefin nitriles are summarized in Table 3. The most surprising fact is the observation that the cis and trans isomers of all fluoro-olefin nitriles have roughly the same inhibitory potential, in the cases of both DPP IV and DPP II. This contrasts with the generally accepted idea that, at least

Table 3. Inhibition Data of Both the (Z)- and (E)-Isomers of Fluoro-Olefin Analogues of Selected N-Substituted Gly-2-(cyanopyrrolidines) (11.7/1 and 11.7/2) and Their Parent Amides (17)[a]

		 *Z* (*trans*)-isomers	
R	compd	IC_{50} (μM) DPP IV	IC_{50} (μM) DPP II
	**11.7a/1**	> 500	32.9 + 1.1
	11.7b/1	12	> 100
	**11.7c/1**	> 1000	87.3 ± 5.4
		 *E* (*cis*)-isomers	
	**11.7a/2**	>1000	36.1 ± 1.4
	**11.7b/2**	15	> 100
	**11.7c/2**	> 500	40.4 ± 0.9
			
	**17a**	2.13 ± 0.11	16.3 ± 0.4
	**17b**	0.35 ± 0.03	133.5 ± 3.6
	**17c**	3.7 ± 0.2	25.5 ± 0.6

[a] If the IC_{50} value was estimated to be above the highest concentration of complete solubility (X), this activity parameter is indicated as > X.

for DPP IV, (peptide) substrates or inhibitors should be in the trans conformation to allow enzyme recognition. Different enzyme binding modes of peptides and fluoro-olefin inhibitors might give a plausible explanation.

Comparing the amide nitriles 17 to the fluoro-olefin nitriles 11.7, a decrease in affinity for both enzymes was observed. In agreement with the results of the fluoro-olefins 9.7 and 9.8, the reduced affinity of the fluoro-olefin nitriles 11.7 compared to the parent amides nitriles 17 is more pronounced for DPP IV than for DPP II. Indeed, for DPP IV the potentiating effect of the cyano group could not compensate for the low affinity of the fluoro-olefin function. Only the adamantyl substituted compound 11.7b has some affinity but is approximately 40 times less potent than the parent amide 17b . For DPP II, the fluoro-olefin nitriles 11.7 are slightly less potent than the corresponding amide nitriles 17 and the corresponding fluoro-olefins without nitrile (9.7). This latter finding confirms our previous findings that an extra cyano group has no potentiating effect on DPP II inhibitors. [25]

Solution Stability

To investigate the chemical stability of the fluoro-olefin nitriles, the disappearance of the (Z)-fluoro-olefin 11.7c/1 , (E)-fluoro-olefin 11.7c/2 , and the parent amide 17c in a buffered solution (pH 7.5) of 37 ÉC was monitored using HPLC and LC - MS. The amide 17c had a half-life of 4 h. Two new compounds were formed: a more polar compound with an identical mass and a less polar compound with an M + 1 mass. These data

are consistent with the formation of a cyclic amidine (Scheme 1A, X) NH) and a diketopiperazine (Scheme 1A, X) O). The initially formed cyclic amidine reached a maximum concentration after about 10 h and was almost completely converted to the diketopiperazine after 80 h.

The half-lifes of both the (Z)- and (E)-fluoro-olefin nitriles were approximately 200 h. However, their degradation pathways were substantially different. The only degradation product observed for the (Z)-fluoro-olefin 11.7c/1 has a mass of M - 20. This probably results from an elimination of HF. A compound with a mass of M - 20 was also observed for the (E)-fluoro-olefin 11.7c/2. However, after long incubation of this latter olefin, traces of a more polar compound with identical mass and a less polar compound with mass M + 1 were also detected. These data are consistent with the formation of a cyclic amidine (Scheme 1B, X) NH) and a cyclic amide (Scheme 1B, X) O). Therefore, we conclude that in contrast to the (Z,*trans*)-fluoro-olefin nitriles, the (E,*cis*)-fluoro-olefin nitriles undergo a cyclization of the amine onto the nitrile. This latter observation is similar to the cyclization of the parent amide nitriles after conversion to the cis amide bond, although at a much slower rate.

Conclusions

Fluoro-olefin (9.7, 9.8) and olefin (10.7, 10.8) compounds with five- and six-membered rings mimicking respectively N-substituted Gly-pyrrolidines (15.1) and -piperidines (15.2) were prepared and evaluated for inhibitory potency toward DPP IV and DPP II. Most of these compounds exhibited a strong binding preference toward DPP II with IC_{50} values in the low micromolar range. The low DPP IV inhibitory potential of the fluoro-olefin peptidomimetics can possibly be explained by the fact that the fluoro-olefin isostere acts as a less effective hydrogen bond acceptor compared to the amide bond, while hydrogen bond formation seems less critical for DPP II inhibition. In contrast to the parent amides, no significant increase in DPP II inhibitory potency is seen when changing from a five- to a six-membered ring at the P_1 position.

Introduction of a carbonitrile in the fluoro-olefin series (11.7) does not result in increased DPP IV or DPP II inhibitory activity. For DPP IV in particular, the lack of affinity is quite significant and does not correspond to the behavior of the parent amides where introduction of a nitrile leads to an outspoken (factor 1000) improved DPP IV inhibition. Only minimal differences in enzyme affinity is observed between cis and trans isomers of these fluoro-olefin nitriles. This observation contrasts with the generally accepted idea that, at least for DPP IV, (peptide) substrates or inhibitors should be in the trans conformation to allow enzyme recognition. Stability studies in aqueous solution indicate that both the cis and trans isomers have significantly improved stability over the parent amide.

The 4-(N-benzyl)piperidyl and 4-piperidyl substituents yield low micromolar DPP II inhibitory potency in the fluoro-olefin series (9.7g, 9.8j, 9.8k).

In conclusion, fluoro-olefins are unfavorable peptidomimetics in DPP IV inhibitors in terms of affinity presumably because of the reduced hydrogen bond acceptor potential of fluorine compared to the carbonyl oxygen. However, in DPP II inhibitors fluoro-olefins have comparable affinity but show a highly increased solution stability when present in analoguous aminoacyl-(2-cyano)pyrrolidines. So, depending on the situation, fluoro-olefins are valuable peptidomimetics.

Experimental Section

Analysis. Characterization of compounds was done with ^1H NMR and ^{13}C NMR spectrometry and mass spectrometry. ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance DRX-400 spectrometer (400 MHz). Electrospray (ES^+ and ES^-) mass spectra were acquired on a Bruker Esquire 3000 plus mass spectrometer. Purity of the final products was verified using two different HPLC systems, one equipped with mass detection and the other equipped with UV detection. LC - MS chromatograms were recorded on an Agilent 1100 series HPLC system using a C_{18} column (2.1 mm 50 mm, 5 í m, Supelco, Sigma-Aldrich) coupled to a Bruker Esquire 3000 plus mass spectrometer (0 - 80% ACN, 22 min, 0.2 mL/min). Reversed-phase HPLC chromatograms were obtained from a Gilson instrument (Viliers-le-bel, France) equipped with an Ultrasphere ODS column (4.6 mm 250 mm, 5 í m, Beckman, Fullerton, CA) and UV detection (10 - 100% ACN, 35 min, 214 nm, 1 mL/min).

Inhibition Measurements. DPP IV was purified from human seminal plasma as described previously. [38] DPP II was isolated from the same source using techniques described previously for purification of the enzyme from rat kidney, [39] supplemented with adenosine deaminase affinity chromatography to eliminate contaminating DPP IV. Initial velocities were measured at 37 ÉC with the chromogenic substrates Gly-Pro-p-nitroanilide (100 í mol/L) at pH 8.3 for DPP IV and Lys-Ala-p-nitroanilide (1 mmol/l) at pH 5.5 for DPP II. Test compounds were dissolved and diluted in DMSO (final concentration DMSO during assay 5% v/v). All measurements were carried out in duplicate. The initial evaluation of compounds was carried out at 1 mmol/L or, in the case of solubility limits, the highest concentration possible. If v_i/v_o (initial velocity in the presence of inhibitor/velocity in the presence of DMSO) was less than 0.5, an IC_{50} value was determined experimentally using at least six different concentrations of inhibitor.

For those compounds with IC_{50} values below 5 í mol/L for one of the enzymes, the analysis was repeated using a new stock of compound. Generally, independent measures of IC_{50} differed less than 20% from each other. The IC_{50} value was defined as the inhibitor concentration that caused a 50% decrease of the activity under assay conditions. The enzyme concentration is defined as E_o, and the inhibitor concentration is represented by I_o. IC_{50} values were calculated with the GraFit software [40] using the following equation:

$$\frac{v_i}{v_o}) \quad \frac{1}{1 + \left(\frac{I_o}{IC_{50}}\right)^s} + \text{background}$$

where s is the slope factor when plotting v_i/v_o versus log I_0 and the background represents the estimated minimal v_i/v_o value. Competition measurements were carried out using Ala-Pro-p-nitroanilide as the DPP II substrate. Activity measurement were carried out using nine inhibitor concentrations (9.8k) at fove different substrate concentrations ranging form 25 í M to 1 mM.

Flexible Alignment. MOE-FlexAlign as implemented in the MOE 2003.02 software from the Chemical Computing Group Inc. was used with standard settings. Both compounds were first energy-minimized with mmff94 as force field using force field partial charges. A stochastic conformational search was done prior to alignment. Each alignment is given a score that quantifies the quality of the alignment in terms of both internal strain and overlap of molecular features.

Solution Stability. Stock solutions (100 mM) in DMSO were prepared for the different compounds. An amount of 100 í L of this stock solution was diluted to a final volume of 5 mL with a phosphate buffer, pH 7.5 (0.1 M potassium phosphate, 1 mM sodium azide, 1mM EDTA). These solutions were incubated at 37 ÉC and injected into analytical HPLC and LC - MS instruments at regular time intervals.

Analytical HPLC was run on a Gilson instrument (Villiers-le-Bel, France) equipped with a UV/vis detector 118, two pumps 306, a manometric module 805, a dynamic mixer 811C, and an autoinjector 234 using the Unipoint 1.65 software. An Ultrasphere ODS column (4.6 mm 250 mm, 5 í m, Beckman, Fullerton, CA) was used, and the mobile phase was composed of solution A (trifluoroacetic acid/water (0.1%)) or solution B (trifluoroacetic acid/acetonitrile (0.1%)). A 40 min linear gradient from 10% to 80% B was carried out. The flow rate was 1 mL/min, the injection volume was 20 í L, and detection was at 214 nm. Disappearance curves of the compounds were constructed from the integrated peak areas versus time. The half-life was calculated using linear analysis based on first-order kinetics. LC - MS chromatograms were recorded on an Agilent 1100 series HPLC system using a C_{18} column (2.1 mm 50 mm, 5 í m, Supelco, Sigma-Aldrich) coupled to a Bruker Esquire 3000 plus mass spectrometer (0 - 80% ACN, 22 min, 0.2 mL/min).

Synthesis. General Procedure. Wadsworth - Horner - Emmons Fluoro-Olefination (9.1, 9.2, 11.1) or Olefination (10.1, 10.2). To a stirred suspension of sodium hydride (0.83 g, 20.75 mmol of a 60% dispersion in mineral oil) in freshly dried ether (60 mL) at - 5 ÉC was dropwise added ethyl (diethylphosphono)(fluoro)acetate (5 g, 20.66 mmol) (for 9.1, 9.2, 11.1) or ethyl (diethylphosphono)acetate (for 10.1, 10.2). The yellowish suspension was then stirred for another 5 min until hydrogen evolution stopped. A solution of cyclopentanone (20.2 mmol, 1.7 g) or cyclohexanone (20.2 mmol) or racemic TBDMS-protected 2-(hydroxymethyl)cyclopentanone (4.6 g, 20.2 mmol) in 10 mL of freshly dried ether was dropwise added to the Wadsworth - Horner - Emmons reagent, the cooling bath was removed, and the mixture was stirred for an additional 4 h. The nonvolatile components of the reaction mixture were then adsorbed on silica (10 g) by evaporation under reduced pressure and purified by column chromatography (hexane/CH_2Cl_2 8:2) to give the target products as a colorless oil. For compound 11.1 the total yield was 74%. The double bond isomers were separated on column chromatography to yield the (Z)-11.1/1 and (E)-11.1/2 isomers. TLC (CH_2Cl_2/hexane, 50:50): R_f) 0.47 for product 11.1/1 and R_f) 0.5 for product 11.1/2.

Ethyl Cyclopentylidene(fluoro)acetate (9.1). Yield: 84%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.33 (t, $^3J_{H-H}$) 9.2 Hz, 3H, - OCH_2CH_3), 1.45- 1.63 (m, 4H, $CH_2CH_2CH_2CH_2$),), 2.12- 2.29 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.37- 2.48 (m, 2H, $CH_2CH_2CH_2CH_2$), 4.28 (m(q), 2H, - OCH_2CH_3). MS (ES^+) m/z: 195.1 (M + Na)$^+$.

Ethyl Cyclohexylidene(fluoro)acetate (9.2). Yield: 81%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.32 (t, $^3J_{H-H}$) 9.2 Hz, 3H, - OCH_2CH_3), 1.38- 1.57 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$),), 2.02-2.18 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.25- 2.36 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 4.28 (m(q), 2H, - OCH_2CH_3). MS(ES^+) m/z: 209.2 (M + Na)$^+$.

Ethyl Cyclopentylideneacetate (10.1). Yield: 78%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.32 (t, $^3J_{H-H}$) 9.2 Hz, 3H, - OCH_2CH_3), 1.45- 1.63 (m, 4H, $CH_2CH_2CH_2CH_2$),), 2.12- 2.29 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.78- 2.91 (m, 2H, $CH_2CH_2CH_2CH_2$), 4.2 (q, 2H, - OCH_2CH_3), 5.51 (s, 1H, d CH). ES^+-MS m/z: 178.2 (M + Na)$^+$.

Ethyl Cyclohexylideneacetate (10.2). Yield: 75%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.31 (t, $^3J_{H-H}$) 9.2 Hz, 3H, - OCH_2CH_3), 1.42- 1.61 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$), 2.09-2.22 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.72- 2.91 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 4.19 (q, 2H, - OCH_2CH_3), 5.52 (s, 1H, d CH). MS(ES^+) m/z: 192.1 (M + Na)$^+$.

Ethyl (2 Z)-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }-methyl)cyclopentylidene](fluoro)ethanoate (11.1/1). Yield: 42%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.03 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $SiC(CH_3)_3$), 1.34 (t, $^3J_{H-H}$) 9.2 Hz), 1.70- 1.91 (m, 4H, CH_2CH_2CH), 2.68- 2.75 (m, 2H, $CH_2CH_2CH_2$), 3.36- 3.44 (m, 1H, CH), 3.50 (t, $^2J_{H-H}$) $^3J_{H-H}$) 9.2 Hz, 1H, CH_2O), 3.72- 3.78 (dd, $^2J_{H-H}$) 9.2 Hz, $^3J_{H-H}$) 4.4 Hz 1H, CH_2O), 4.24- 4.32 (q, 2H, $^3J_{H-H}$) 9.2 Hz, - OCH_2CH_3). MS(ES^+) m/z: 317.2 (M + H)$^+$, 339.4 (M + Na)$^+$.

Ethyl (2 E)-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }-methyl)cyclopentylidene](fluoro)ethanoate (11.1/2). Yield: 33%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.03 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $SiC(CH_3)_3$), 1.33 (t, 3H, - OCH_2CH_3), 1.68- 1.79 (m, 4H, CH_2CH_2CH), 2.49- 2.58 (m, 2H, $CH_2CH_2CH_2$), 3.08- 3.18 (m, 1H, CH), 3.48 (t, $^2J_{H-H}$) $^3J_{H-H}$) 8.8 Hz, 1H, CH_2O), 3.62- 3.68 (dd, $^2J_{H-H}$) 9.0 Hz, $^3J_{H-H}$) 4.4 Hz 1H, CH_2O), 4.28 (q, 2H, $^3J_{H-H}$) 9.2 Hz,-OCH_2CH_3). MS(ES^+) m/z: 317.2 (M + H)$^+$, 339.4 (M + Na)$^+$.

General Procedure for Alkaline Hydrolysis. r -Fluoro-r ,â-unsaturated Esters (9.3, 9.4, 11.2) and r ,â-Unsaturated Esters (10.3, 10.4). Ethyl ester (6 mmol) was mixed with a 1 M solution of potassium hydroxide in 75% aqueous methanol (7 mL, 7 mmol) at room temperature. After being stirred for 8 h, the reaction mixture was diluted with water (100 mL), acidified wit h a 2 M aqueous hydrogen chloride solution (6 mL), and extracted twice with diethyl ether (60 mL). The combined ethereal fractions were dried over magnesium sulfate and evaporated, yielding the pure free acids.

Cyclopentylidene(Fluoro)acetic Acid (9.3). Yield: 97%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.43- 1.68 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.12- 2.29 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.38- 2.56 (m, 2H, $CH_2CH_2CH_2CH_2$). MS(ES^-) m/z: 143.3 (M - H)$^-$.

Cyclohexylidene(fluoro)acetic Acid (9.4). Yield: 98%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 1.41- 1.54 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$), 2.05- 2.16 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.24-2.37 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$). MS(ES^-) m/z: 157.1 (M + H)$^+$.

Cyclopentylideneacetic Acid (10.3). Yield: 94%. ^{1}H NMR 400 MHz ($CDCl_3$): ä 1.43- 1.68 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.24- 2.32 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.4- 2.53 (m, 2H, $CH_2CH_2CH_2CH_2$), 5.55 (s, 1H, d CH). MS(ES^-) m/z: 125.1 (M - H)$^-$.

Cyclohexylideneacetic Acid (10.4). Yield: 95%. ^{1}H NMR 400 MHz ($CDCl_3$): ä 1.43- 1.68 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$), 2.12- 2.29 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.38- 2.57 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 5.55 (s, 1H, d CH). MS(ES^-) m/z: 139.1 (M - H)$^-$.

(2Z)-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }methyl)-cyclopentylidene](fluoro)ethanoic Acid (11.2/1). Yield: 97%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.04 (s, 3H, $Si(CH_3)_2$), 0.05 (s, 3H, $Si(CH_3)_2$), 0.86 (s, 9H, $SiC(CH_3)_3$), 1.70- 1.91 (m, 4H, CH_2CH_2CH), 2.67- 2.79 (m, 2H, $CH_2CH_2CH_2$), 3.12- 3.23 (m, 1H, CH), 3.57 - 3.68 (m, 1H, CH_2O), 3.72- 3.78 (dd, $^2J_{H-H}$) 8.7 Hz, $^3J_{H-H}$) 4.0 Hz, 1H, CH_2O). MS(ES^+) m/z: 289.1 (M + H)$^+$, 312.5 (M + Na)$^+$. MS (ES^-) m/z: 287.5 (M - H)$^-$. TLC (30% EtOAc in hexanes): R_f) 0.58.

(2E)-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }methyl)-cyclopentylidene](fluoro)ethanoic Acid (11.2/2). Yield: 97%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.08 (s, 3H, $Si(CH_3)_2$), 0.09 (s, 3H, $Si(CH_3)_2$), 0.89 (s, 9H, $SiC(CH_3)_3$), 1.68- 1.79 (m, 4H, CH_2CH_2CH), 2.54- 2.62 (m, 2H, $CH_2CH_2CH_2$), 3.36- 3.45 (m, 1H, CH), 3.53 - 3.58 (m, 1H, CH_2O), 3.60- 3.68 (dd, $^2J_{H-H}$) 9.0 Hz, $^3J_{H-H}$) 4.4 Hz, 1H, CH_2O). MS(ES^+) m/z: 289.1 (M + H)$^+$, 312.5 (M + Na)$^+$. MS (ES^-) m/z: 287.5 (M - H)$^-$. TLC (30% EtOAc in hexanes): R_f) 0.62.

General Procedure for Preparation of r -Fluoro- r ,â-unsaturated Amides (9.5, 9.6, 11.3) and r ,â-Unsaturated Amides (10.5, 10.6). Acid (1 mmol) was dissolved in dichloromethane (15 mL). Diisopropylethylamine (4 mmol) was added, followed by TBTU (1.1 mmol). After the mixture was stirred for 5 min, the appropriate amine (1.1 mmol), dissolved in 2 mL of dichloromethane, was dropped into the solution and stirring was continued for 3 h. The volatile components of the mixture were then removed by evaporation after the addition of silica (5 g) to the reaction mixture. The silica was brought to a column and chromatographed (hexane/EtOAc in different ratios), yielding amides as colorless oils that slowly crystallized

upon standing. For the preparation of 9.6c (R) phenyl), acid 9.4 was activated as the corresponding acid chloride. Acid 9.4 (1 mmol) was dissolved in dry dichloromethane (15 mL) under nitrogen together with a catalytic amount of dimethylformamide. Oxalyl chloride (0.14 g, 1.1 mmol), dissolved in 1 mL of dry dichloromethane, was added dropwise via a syringe and stirring was continued for 10 min. Aniline (0.1 g, 1.1 mmol), dissolved in 1 mL of dry dichloromethane, was added to the appropriate acid chloride and stirring was continued for 16 h at room temperature. The target product was isolated after flash chromatography (hexanes/EtOAc 9:1). Compounds 9.5, 9.6, 10.5, and 10.6 were only charaterized by mass spectrometry.

(2Z)-2-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }methyl)-cyclopentylidene]-2-fluoro- N-(2-phenylethyl)ethanamide (11.3/1a) (R) 2-Phenylethyl-). Yield: 84%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.03 (s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $C(CH_3)_3$), 1.67- 1.96 (m, 4H, CH_2CH_2CH), 2.62- 2.93 (m, 3H, $CH_2CH_2CH_2CH + CH_2Ar$), 2.91- 3.18 (m, 2H, $CH_2CH_2CH_2CH + CHCH_2O$), 3.47 (t, $^3J_{H-H}$) $^2J_{H-H}$) 8.4 Hz, 1H, CH_2O), 3.52- 3.56 (m, 2H, $ArCH_2CH_2N$), 3.69- 3.73 (dd, $^3J_{H-H}$) 8.6 Hz, $^2J_{H-H}$) 4.4 Hz 1H, CH_2O), 6.18- 6.41 (br s, 1H, N HC(O)), 7.12- 7.28 (m, 3H, Ar), 7.24 - 7.38 (m, 2H, Ar). ^{13}C NMR $CDCl_3$ (400 MHz): ä -5.1 (s, $Si(CH_3)_2$), 14.07 (s, $C(CH_3)_3$), 18.05 (s, CH_2CH_2CH), 26.11 (s, $C(CH_3)_3$), 27.54 (s, CH_2CH_2CH), 30.75 (s, $CH_2CH_2CH_2CH$), 36.26 (s, $ArCH_2CH_2N$), 40.37 (s, $ArCH_2CH_2N$), 46.06 (s, $CHCH_2O$), 65.14 (s, CH_2O), 126.18 (s, Ar), 128.94 (s, Ar), 136.77 (d, $^2J_{C-F}$) 12.4 Hz $(CH_2)_2Cd$), 138.95 (s, Ar), 144.4 (d, $^1J_{C-F}$) 249.4 Hz, $CH_2C(F)d$), 161.28 (d, $^2J_{C-F}$) 30.9 Hz, C(O)). MS (ES^+) m/z: 392.2 (M + H)$^+$, 314 (M + Na)$^+$.

(2E)-2-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }-methyl)cyclopentylidene]-2-fluoro- N-(2-phenylethyl)-ethanamide (11.3/2a) (R) 2-Phenylethyl-). Yield: 87%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.03 (s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $C(CH_3)_3$), 1.57- 1.82 (m, 3H, $CH_2CH_2CH_2CH$), 1.92- 2.01 (m, 1H, $CH_2CH_2CH_2CH$), 2.41- 2.63 (m, 2H, $CH_2CH_2CH_2CH$), 2.81- 2.96 (m, 2H, CH_2Ar), 3.41- 3.48 (m, 1H, CH_2O), 3.51- 3.68 (m, 3H, $ArCH_2CH_2N + CHCH_2O$), 3.67- 3.78 (dd, $^2J_{H-H}$) 9.6 Hz, $^3J_{H-H}$) 4.8 Hz 1H, CH_2O), 6.31- 6.51 (br s, 1H, N HC(O)), 7.08- 7.22 (m, 3H, Ar), 7.24 - 7.38 (m, 2H, Ar). ^{13}C NMR $CDCl_3$ (400 MHz): ä - 5.1 (s, $Si(CH_3)_2$), 14.03 (s, $C(CH_3)_3$), 18.19 (s, $CH_2CH_2CH_2CH$), 26.06 (s, $C(CH_3)_3$), 29.37 (s, $CH_2CH_2CH_2CH$), 29.98 (s, $CH_2CH_2CH_2CH$), 35.96 (s, $Ar CH_2CH_2N$), 40.65 (s, $ArCH_2CH_2N$), 44.04 (s, $CHCH_2O$), 66.18 (s, CH_2O), 126.8 (s, Ar) 128.91 (s, Ar), 137.18 (d, $^2J_{C-F}$) 13.8 Hz $(CH_2)_2Cd$), 138.99 (s, Ar), 144.62 (d, $^1J_{C-F}$) 249.4 Hz, $CH_2C(F)d$), 160.72 (d, $^2J_{C-F}$) 30.9 Hz, C(O)). MS(ES^+) m/z: 392.2 (M + H)$^+$, 314 (M + Na)$^+$.

(2Z)-N-(1-Adamantyl)-2-[(2R,S)-2-({[tert-butyl(dimethyl)-silyl]oxy }methyl)cyclopentylidene]-2-fluoroethanamide (11.3/1b) (R) 1-Adamantyl-). Yield: 91%. ^{1}H NMR ($CDCl_3$): ä 0.03 (s, 6H, $SiCH_3$), 0.88 (s, 9H, CH_3), 1.62- 1.89 (m, 10H, CH_2CH_2CH, adamantyl-), 2.02 - 2.05 (s, 6H, adamantyl-), 2.07 - 2.11 (s, 3H, adamantyl-), 2.72 - 2.49 (m, 2H, $CH_2CH_2CH_2CH$), 3.01- 3.13 (m, 1H, CH_2CH_2CH), 3.37- 3.43 (m, 1H, CH_2O), 3.63- 3.77(m, 1H, CH_2O), 5.83 (s, 1H, NH). ^{13}C NMR $CDCl_3$ (400 MHz): ä - 5.02 (s, $SiCH_3$), 14.09 (s, $C(CH_3)_3$), 18.33 (s, CH_2CH_2CH), 24.71 (s, CH_2CH_2CH), 25.97 (s, CH_3), 28.42 (s, $CH_2CH_2CH_2CH$), 29.44 (s, adamantyl-), 36.34 (s, adamantyl-), 41.60 (s, adamantyl-), 45.94 (s,CH), 51.83 (s, adamantyl-), 63.11 (s, CH_2O), 135.44 (d, $^2J_{C-F}$) 12.9 Hz, $(CH_2)_2Cd$), 144.47 (d, $^1J_{C-F}$) 253.3 Hz, $CH_2C(F)d$), 160.18 (d, $^2J_{C-F}$) 28.5 Hz, C(O)). MS(ES^+) m/z: 422.7 (M + H)$^+$.

(2E)-N-(1-Adamantyl)-2-[(2R,S)-2-({[tert-butyl(dimethyl)-silyl]oxy }methyl)cyclopentylidene]-2-fluoroethanamide (11.3/2b) (R) 1-Adamantyl-). Yield: 85%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.02 (s, 6H, $SiCH_3$), 0.86 (s, 9H, CH_3), 1.58- 1.87 (m, 9H, CH_2CH_2CH, adamantyl-), 1.91 - 2.14 (m, 10H, CH_2CH_2CH, adamantyl-), 2.42 - 2.49 (m, 2H, $CH_2CH_2CH_2CH$), 3.42- 3.53 (m, 1H, CH_2CH_2CH), 3.67 (d, $^3J_{H-H}$) 5.6 Hz, 2H, CH_2O), 5.97 (s, 1H, NH). ^{13}C NMR $CDCl_3$ (400 MHz): ä - 5.44 (s, $SiCH_3$), 14.09 (s, $C(CH_3)_3$), 18.28 (s, CH_2CH_2CH), 23.11 (s, CH_2CH_2CH), 25.91 (s, CH_3), 29.39 (s, adamantyl-), 30.19 (s, $CH_2CH_2CH_2CH$), 36.29 (s, adamantyl-), 41.52 (s, adamantyl-), 43.63 (s, CH), 51.76 (s, adamantyl-), 64.65 (s, CH_2O), 136.48 (d, $^2J_{C-F}$) 14.1 Hz $(CH_2)_2Cd$), 144.41 (d, $^1J_{C-F}$) 252.3 Hz, $CH_2C(F)d$), 159.55 (d, $^2J_{C-F}$) 28.8 Hz, C(O)). MS (ES^+) m/z: 422.7 (M + H)$^+$, 444.5(M + Na)$^+$.

(2Z)-2-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }methyl)-cyclopentylidene]-2-fluoro- N-(4-fluorobenzyl)ethanamide (11.3/1c) (R) 4-Fluorobenzyl-). Yield: 88%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.07 (s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, $C(CH_3)_3$), 1.62- 1.87 (m, 4H, CH_2CH_2CH), 2.68- 2.92 (m, 2H, $CH_2CH_2CH_2CH$), 3.02- 3.12 (m, 1H, $CHCH_2O$), 3.46- 3.52 (t, $^2J_{H-H}$) $^3J_{H-H}$) 9.6 Hz 1H, $CH CH_2O$), 3.66- 3.72 (dd, $^2J_{H-H}$) 9.4 Hz, $^3J_{H-H}$) 4.8 Hz 1H, $CH CH_2O$), 4.48- 4.52 (m, 2H, $ArCH_2$), 6.38- 6.54 (br s, 1H, N HC(O)), 6.98- 7.08 (m, 2H, Ar), 7.22- 7.36 (m, 2H, Ar). ^{13}C NMR $CDCl_3$ (400 MHz): ä - 5.18 (s, $Si(CH_3)_2$), 14.30 (s, $C(CH_3)_3$), 18.5 (s, CH_2CH_2CH), 24.97 (s, CH_2CH_2CH), 26.1 (s, $C(CH_3)_3$), 30.81 (s, $CH_2CH_2CH_2CH$), 42.43 (s, $CHCH_2O$), 46.13 (s,$CHCH_2O$), 63.44 (s, $ArCH_2$), 115.72 (d,$^2J_{C-F}$) 21.4 Hz, Ar), 129.7 (d,$^3J_{C-F}$) 8 Hz, Ar), 134.04 (d,$^4J_{C-F}$) 3.2 Hz, Ar), 137.04 (d, $^2J_{C-F}$) 12.2 Hz $(CH_2)_2Cd$), 144.26 (d, $^1J_{C-F}$) 250.1 Hz, $CH_2C(F)d$), 161.17 (d,$^2J_{C-F}$) 31.1 Hz, C(O)), 162.46 (d, $^2J_{C-F}$) 244.3 Hz, Ar). MS(ES^+) m/z: 396.4 (M + H)$^+$, 419.7 (M + Na)$^+$.

(2E)-2-[(2R,S)-2-({[tert-Butyl(dimethyl)silyl]oxy }methyl)cyclopentylidene]-2-fluoro- N-(4-fluorobenzyl)ethanamide (11.3/2c) (R) 4-Fluorobenzyl-). Yield: 89%. ^{1}H NMR $CDCl_3$ (400 MHz): ä 0.09 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 1.62- 1.82 (m, 3H, CH_2CH_2CH), 1.96- 2.02 (m, 1H, CH_2CH_2CH), 2.43- 2.64 (m, 2H, $CH_2CH_2CH_2CH$), 3.52- 3.58 (m, 1H, $CHCH_2O$), 3.61- 3.68 (t, $^2J_{H-H}$) $^3J_{H-H}$) 9.2 Hz 1H, $CHCH_2O$), 3.71- 3.77 (dd, $^2J_{H-H}$) 9.4 Hz, $^3J_{H-H}$) 4.8 Hz, 1H, $CHCH_2O$), 4.42- 4.58 (m, 2H, $Ar CH_2$), 6.62- 6.78 (br s, 1H, N HC(O)), 6.98- 7.08 (m, 2H, Ar), 7.22 - 7.36 (m, 2H, Ar). ^{13}C NMR $CDCl_3$ (400 MHz): ä - 5.2 (s, $Si(CH_3)_2$), 14.30 (s, $C(CH_3)_3$), 18.5 (s, CH_2CH_2CH), 23.05 (s, CH_2CH_2CH), 26.15 (s, $C(CH_3)_3$), 30.12 (s, $CH_2CH_2CH_2CH$), 42.45 (s, $CHCH_2O$), 44.68 (s,$CHCH_2O$), 64.68 (s, $ArCH_2$), 115.7 (d,$^2J_{C-F}$) 21.4 Hz, Ar), 129.7 (d,$^3J_{C-F}$) 8.1 Hz, Ar)), 134.14 (d,$^4J_{C-F}$) 3.3 Hz, Ar), 137.94 (d, $^2J_{C-F}$) 13.6 Hz $(CH_2)_2Cd$), 144.46 (d, $^1J_{C-F}$) 249.4 Hz, $CH_2C(F)d$), 160.68 (d,$^2J_{C-F}$) 34.5 Hz, C(O)), 162.45 (d, $^2J_{C-F}$) 244.3 Hz, Ar). MS(ES^+) m/z: 396.4 (M + H)$^+$.

General Procedure for Reduction of r -Fluoro- r ,â-unsaturated Amides (9.7, 9.8, 11.4) and r ,â-Unsaturated Amides (10.7, 10.8). To an intensely stirred solution of $POCl_3$ (5.2 mmol, 0.79 g) in dry dichloromethane (10 mL), cooled to 0 ÉC under nitrogen, was dropped a solution of the amide (1.28 mmol) in dry dichloromethane (2 mL). After addition, the ice bath was removed and stirring was continued for 1 h, followed by the thorough evaporation of the solvent and excess $POCl_3$. The crude chloroimidate was dispersed in freshly dried diethyl ether (10 mL), cooled under nitrogen to 0 ÉC, followed by the addition of $LiAlH_4$ (2.56 mmol, 94 mg) in one portion. Stirring was continued for 10 min, and after removal of the ice bath stirring was continued for another 3 h. Excess $LiAlH_4$ was then quenched with water (0.6 mL), and stirring was continued for 5 min to allow free water to be adsorbed onto the insoluble salts. The suspension was filtered, and the filter cake was washed with diethyl ether (3 15 mL). The combined ether layers were then evaporated, yielding crude amine. For compounds 11.4, the crude amine was used without further purification in the next step (Boc protection). For compounds 9.7, 9.8, 10.7, 10.8 the crude amine was precipitated with a 1 M solution of hydrogen chloride in diethyl ether, the ether was carefully decanted, and the final product was washed twice with small portions of dry diethyl ether and then dried under reduced pressure.

N-(2-Cyclopentylidene-2-fluoroethyl)cyclohexanamine (9.7a). Yield: 92%. ^{1}¸ Á» R D_2O (400 MHz): ä 0.88- 1.22 (m, 6H, $CH_2CH_2CH_2$), 1.41- 1.52 (m, 4H, CH_2CH_2), 1.58- 1.69 (m, 2H, CH_2), 1.82- 1.97 (m, 2H, CH_2), 2.04- 2.14 (m, 2H, CH_2), 2.16- 2.25 (m, 2H, CH_2), 2.81- 3.03 (m, 1H, CH), 3.67 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N). ES^+-MS m/z: 212.2 (M + H)$^+$.

N-Benzyl-2-cyclopentylidene-2-fluoroethanamine (9.7b). Yield: 86%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.53- 1.60 (m, 4H, CH_2CH_2), 2.02- 2.18 (m, 2H, CH_2), 2.24- 2.38 (m, 2H, CH_2), 3.88

(d, $^3J_{H-F}$) 20 Hz, CH_2N), 4.18- 4.25 (s, 2H, CH_2Ar), 7.42- 7.53 (m, 5H, Ar). ES$^+$-MS m/z: 220.2 $(M + H)^+$.

N-(2-Cyclopentylidene-2-fluoroethyl)- N-(4-methoxybenzyl)amine 9.7c). Yield: 93%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.51- 1.62 (m, 4H, CH_2CH_2), 2.05- 2.18 (m, 2H, CH_2), 2.26- 2.39 (m, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.91 (d, $^3J_{H-F}$) 21.2 Hz, CH_2N), 4.18 (s, 2H, CH_2Ar), 7.04 (d, $^2J_{H-H}$) 8.7 Hz, 2H, Ar), 7.39 (d, $^2J_{H-H}$) 8.7 Hz, 2H, Ar). ES$^+$-MS m/z: 249.9 $(M + H)^+$.

N-(2-Cyclopentylidene-2-fluoroethyl)- N-(4-fluorobenzyl)amine (9.7d). Yield: 96%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.52- 1.61 (m, 4H, CH_2CH_2), 2.11- 2.23 (m, 2H, CH_2), 2.34- 2.43 (m, 2H, CH_2), 3.88 (d, $^3J_{H-F}$) 20 Hz, CH_2N), 4.24 (s, 2H, CH_2Ar), 7.19- 7.23 (m, 2H, Ar), 7.46 - 7.5 (m, 2H, Ar). ES$^+$-MS m/z: 238.1 $(M + H)^+$, 260.2 $(M + Na)^+$.

N-(2-Cyclopentylidene-2-fluoroethyl)- N-(2-pyridinylethyl)amine (9.7e). Yield: 91%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.49- 1.60 (m, 4H, CH_2CH_2), 2.08- 2.21 (m, 2H, CH_2), 2.34- 2.43 (m, 2H, CH_2), 2.86- 3.01 (m, 2H, $ArCH_2CH_2$), 3.17- 3.3 (m, 2H, Ar CH_2), 3.90 (d, $^3J_{H-F}$) 20 Hz, CH_2N), 7.19- 7.44 (m, 2H, Ar), 7.63 - 7.7 (m, 1H, Ar), 8.58 - 8.73 (m, 1H, Ar). ES$^+$-MS m/z: 235.1 $(M + H)^+$.

N-(2-Cyclopentylidene-2-fluoroethyl)- N-(2-phenylethyl)amine (9.7f). Yield: 98%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.53- 1.76 (m, 4H, CH_2CH_2), 2.21- 2.42 (m, 2H, CH_2), 2.48- 2.51 (m, 2H, CH_2), 2.94- 2.51 (m, 2H, CH_2CH_2N), 3.22- 3.39 (m, 2H, $ArCH_2$), 3.86 (d, $^3J_{H-F}$) 20.4 Hz, CH_2N), 7.22- 7.43 (m, 5H, Ar). ES$^+$-MS m/z: 234.1 $(M + H)^+$, 256.3 $(M + Na)^+$. LC- MS: t_R) 12.4 min, 95%. HPLC (214 nm): t_R) 20.75 min, 92%.

1-Benzyl- N-(2-cyclopentylidene-2-fluoroethyl)-4-piperidinamine (9. g). Yield: 95%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.49- 1.68 (m, 4H, CH_2CH_2), 1.8- 2.02 (m, 2H, $CH CH_2$), 2.07- 2.18 (m, 2H, CH_2), 2.26- 2.33 (m, 2H, CH_2), 2.34- 2.46 (m, 2H, $CHCH_2$), 3.02- 3.21 (m, 2H, $N CH_2CH_2$), 3.36- 3.72 (m, 3H, $CH_2CHN + NCH_2CH_2$), 3.91 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N), 4.3 (s, 2H, CH_2Ar), 7.42- 7.57 (m, 5H, Ar). ES$^+$-MS m/z: 303.1 $(M + H)^+$.

N^1-(2-Cyclopentylidene-2-fluoroethyl)- N^3,N^3-dimethyl-1,3-propanediamine (9.7h). Yield: 95%. ^{1}¸ Á» R D_2O (400 MHz) ä 0.80- 0.91 (m, 6H, CH_3), 1.47- 1.64 (m, 6H, CH_2CH_2, CH_2), 2.06- 2.21 (m, 2H, CH_2), 2.25- 2.36 (m, 4H, CH_2, CH_2), 2.47- 2.56 (m, 2H, CH_2), 3.12- 3.21 (m, 2H, $N CH_2CH_2$), 3.9 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N). ES$^+$-MS m/z: 215.1 $(M + H)^+$.

N-(2-Cyclohexylidene-2-fluoroethyl)cyclohexanamine (9.8a). Yield: 91%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.11- 1.42 (m, 6H, $CH_2CH_2CH_2$), 1.49- 1.61 (m, 6H, $CH_2CH_2CH_2$), 1.79- 1.88 (m, 2H, CH_2), 1.98- 2.11 (m, 2H, CH_2), 2.12- 2.18 (m, 2H, CH_2), 2.22- 2.39 (m, 2H, CH_2), 3.08- 3.18 (m, 1H, CH), 3.94 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N). ES$^+$-MS m/z: 226.0 $(M + H)^+$.

N-(2-Cyclohexylidene-2-fluoroethyl)-1-adamantanamine (9.8b). Yield: 94%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.43- 1.59 (m, 6H, $CH_2CH_2CH_2$), 1.58- 1.81 (m, 6H, ad), 1.83- 2.01 (m, 5H, CH_2, ad), 2.09- 2.33 (m, 6H, CH_2, ad), 3.86 (d, $^3J_{H-F}$) 21.6 Hz, 2H, CH_2N). ES$^+$-MS m/z: 278.2 $(M + H)^+$, 300.1 $(M + Na)^+$.

N-(2-Cyclohexylidene-2-fluoroethyl)aniline (9.8c). Yield: 93%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.32- 1.49 (m, 6H, $CH_2CH_2CH_2$), 2.03- 2.14 (m, 4H, $CH_2 + CH_2$), 3.92 (d, $^3J_{H-F}$) 21.6 Hz, 2H, CH_2N), 6.67- 6.81 (m, 3H, Ar), 7.09 - 7.21 (m, 2H, Ar). ES$^+$-MS m/z: 220.1 $(M + H)^+$.

N-Benzyl-2-cyclohexylidene-2-fluoroethanamine (9.8d). Yield: 81%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.41- 1.52 (m, 6H, $CH_2CH_2CH_2$), 2.02- 2.12 (m, 2H, CH_2), 2.2- 2.31 (m, 2H, CH_2), 3.92 (d, $^3J_{H-F}$) 21.2 Hz, CH_2N), 4.22 (s, 2H, CH_2Ar), 7.16- 7.22 (m, 2H, Ar), 7.42 - 7.47 (m, 2H, Ar). ES$^+$-MS m/z: 234.2 $(M + H)^+$, 256.1 $(M + Na)^+$.

N-(2-Cyclohexylidene-2-fluoroethyl)- N-(4-methoxybenzyl)amine (9.8e). Yield: 83%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.48- 1.56 (m, 6H, $CH_2CH_2CH_2$), 1.98- 2.08 (m, 2H, CH_2), 2.21- 2.31 (m, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.91 (d, $^3J_{H-F}$) 21.2 Hz, CH_2N), 4.18 (s, 2H, CH_2Ar), 7.04 (d, $^2J_{H-H}$) 8.7 Hz, 2H, Ar), 7.39 (d, $^2J_{H-H}$) 8.7 Hz, 2H, Ar). ES$^+$-MS m/z: 264.2 $(M + H)^+$, 286.1 $(M + Na)^+$.

N-(1,3-Benzodioxol-5-ylmethyl)-2-cyclohexylidene-2-fluoroethanamine (9.8f). Yield: 79%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.48- 1.54 (m, 6H, $CH_2CH_2CH_2$), 1.98- 2.07 (m, 2H, CH_2), 2.22- 2.38 (m, 2H, CH_2), 3.92 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N), 4.02- 4.15 (m, 2H, CH_2Ar), 6.01 (s, 2H, $O CH_2O$), 6.94- 6.99 (m, 3H, Ar). ES$^+$-MS m/z: 278.3 $(M + H)^+$. LC- MS: t_R) 12.6 min, 99%. HPLC (214 nm): t_R) 21.48 min, 100%.

N-(2-Cyclohexylidene-2-fluoroethyl)- N-(4-fluorobenzyl)-amine (9.8g). Yield: 94%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.46- 1.68 (m, 6H, $CH_2CH_2CH_2$), 1.98- 2.12 (m, 2H, CH_2), 2.21- 2.33 (m, 2H, CH_2), 4.02 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N), 4.22 (s, 2H, Ar CH_2), 7.12- 7.29 (m, 2H, Ar), 7.41 - 7.54 (m, 2H, Ar). ES$^+$-MS m/z: 252.1 $(M + H)^+$. LC- MS: t_R) 12.1 min, 95%. HPLC (214 nm): t_R) 21.65 min, 95%.

N-(2-Cyclohexylidene-2-fluoroethyl)- N-[3-(trifluoromethyl)benzyl]amine (9.8h). Yield: 95%. ^{1}¸ Á» R D_2O (400 MHz): ä 1.42- 1.59 (m, 6H, $CH_2CH_2CH_2$), 1.98- 2.07 (m, 2H, CH_2), 2.21- 2.31 (m, 2H, CH_2), 3.96 (d, $^3J_{H-F}$) 20.8 Hz, 2H, CH_2N), 4.32 (s, 2H, Ar CH_2), 7.49- 7.71 (m, 2H, Ar), 7.23 - 7.31 (m, 2H, Ar). ES$^+$-MS m/z: 302.3 $(M + H)^+$, 324.2 $(M + Na)^+$.

N-(2-Cyclohexylidene-2-fluoroethyl)- N-(2-phenylethyl)-amine (9.8i). Yield: 93%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.32- 1.56 (m, 6H, $CH_2CH_2CH_2$), 1.98- 2.12 (m, 2H, CH_2), 2.2- 2.32 (m, 2H, CH_2), 2.93- 3.04 (m, 2H, $ArCH_2CH_2N$), 3.28- 3.39 (m, 2H, Ar CH_2), 3.94 (d, $^3J_{H-F}$) 21.6 Hz, CH_2N), 7.11- 7.46 (m, 5H, Ar). ES$^+$-MS m/z: 248.2 $(M + H)^+$. LC- MS: t_R) 17.3 min, 98%. HPLC (214 nm): t_R) 23.91 min, 100%.

N-(2-Cyclohexylidene-2-fluoroethyl)- 4-piperidinamine (9.8j). This compound was prepared from 9.8l by stirring the latter (0.5 mmol, 0.18 g) fo r 2 h at room temperature with a 30% solution of HBr in acetic acid (5 mL). Volatile components were removed under reduced pressure, and the oily residue was added dropwise to 10 mL of dry diethyl ether. The precipitated product, an off-white amorphous solid, was collected after suction filtration and washing the filter cake with small portions of dry diethyl ether. Yield: 63%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.4- 1.63 (m, 6H, $CH_2CH_2CH_2$), 2.04- 2.12 (m, 3H, $CH_2 + CHCH_2$), 2.18- 2.28 (m, 3H, $CH_2 + CHCH_2$), 2.81- 3.01 (m, 2H, $CH CH_2$), 3.33- 3.49 (m, 1H, $CHCH_2$), 3.61- 3.74 (m, 4H, CH_2CH_2), 3.92 (d, $^3J_{H-F}$) 20.9 Hz, 2H, CH_2N). ES$^+$-MS m/z: 227.1 $(M + H)^+$, 309 $(M + Na)^+$.

1-Benzyl- N-(2-cyclohexylidene-2-fluoroethyl)-4-piperidinamine (9.8k). Yield: 88%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.49- 1.63 (m, 6H, $CH_2CH_2CH_2$), 1.41- 2.02 (m, 2H, $CH CH_2$), 2.08- 2.17 (m, 2H, CH_2), 2.22- 2.31 (m, 2H, CH_2), 2.34- 2.46 (m, 2H, $CH CH_2$), 3.03- 3.21 (m, 2H, $N CH_2CH_2$), 3.38- 3.72 (m, 3H, $CH_2CHN + NCH_2CH_2$), 3.94 (d, $^3J_{H-F}$) 21.2 Hz, 2H, CH_2N), 4.31 (s, 2H, CH_2Ar), 7.43- 7.54 (m, 5H, Ar). ES$^+$-MS m/z: 317.3 $(M + H)^+$, 340.2 $(M + Na)^+$.

Benzyl 4-[(2-cyclohexylidene-2-fluoroethyl)amino]-1-piperidinecarboxylate (9.8l). Yield: 67%. ^{1}¸ Á» R D_2O (400 MHz) ä 1.42- 1.61 (m, 6H, $CH_2CH_2CH_2$), 2.04- 2.12 (m, 3H, $CH_2 + CHCH_2$), 2.18- 2.28 (m, 3H, $CH_2 + CHCH_2$), 2.81- 3.01 (m, 2H, $CH CH_2$), 3.33- 3.49 (m, 1H, $CHCH_2$), 3.94 (d, $^3J_{H-F}$) 20.8 Hz, 2H, CH_2N), 4.09- 4.32 (m, 4H, CH_2CH_2), 4.32 (s, 2H, $ArCH_2$), 7.31- 7.49 (m, 5H, Ar). ES$^+$-MS m/z: 361.3 $(M + H)^+$. LC- MS: t_R) 14.1 min, 99%. HPLC (214 nm): t_R) 24.60 min, 98%.

N^1-(2-Cyclohexylidene-2-fluoroethyl)- N^3,N^3-dimethyl-1,3-propanediamine (9.8m). Yield: 88%. ^{1}¸ Á» R D_2O (400 MHz): ä 0.82- 0.91 (m, 6H, CH_3), 1.43- 1.66 (m, 6H, CH_2CH_2, CH_2), 2.07- 2.23 (m, 2H, CH_2), 2.25- 2.36 (m, 4H, CH_2, CH_2), 2.47- 2.56 (m, 2H, CH_2), 3.12- 3.21 (m, 2H, $N CH_2CH_2$), 3.9 (d, $^3J_{H-F}$) 21.0 Hz, 2H, CH_2N). ES$^+$-MS m/z: 229.3 $(M + H)^+$.

General Procedure for Boc Protection of the Amines 11.4. The crude amines 11.4a (R) 2-phenylethyl) or 11.4c (R) 4-fluorobenzyl) were redissolved in CH_2Cl_2 (5 mL). DIPEA (166 mg, 1.28 mmol) and $(Boc)_2O$ (837 mg, 3.84 mmol) were added, and the solution was stirred for 4 h. Excess $(Boc)_2O$ was then quenched with imidazole (272 mg, 4 mmol). Ethyl acetate (100 mL) was added, and the organic layer was washed with a 1 M aqueous solution of hydrogen chloride (30 mL), water (30 mL), a saturated aqueous solution of sodium bicarbonate (30 mL), and water (30 mL) again. The organic

layer was dried over magnesium sulfate and evaporated. The crude double-protected product obtained in this manner is sufficiently pure for use in the next step without further purification. Since compounds 11.4b (R) adamantyl) were not reactive toward (Boc) $_2$O under these conditions, probably for steric reasons, a different procedure was followed: the crude amine 11.4b (R) 1-adamantyl) was redissolved in dioxane (5 mL). Diisopropylethylamine (166 mg, 1.28 mmol), 4-(dimethylamino)pyridine, and (Boc) $_2$O (837 mg, 3.84 mmol) were added, and the solution was stirred for 24 h at 80 ÉC. Excess $(Boc)_2O$ was then quenched with imidazole (272 mg, 4 mmol), and dioxane was evaporated under reduced pressure. To the residue, ethyl acetate (100 mL) was added. The organic layer was washed wit h a 1 M aqueous solution of hydrogen chloride (30 mL), water (30 mL), a saturated aqueous solution of sodium bicarbonate (30 mL), and water (30 mL) again. The organic layer was dried over magnesium sulfate and evaporated. The crude double-protected product obtained in this manner was considered pure enough for use in the next step without further purification.

General Procedure for Removal of the TBDMS Protecting Group. The crude product from the previous step was dissolved in a 3:1:1 mixture of glacial acetic acid, tetrahydrofurane, and water (10 mL). The solution was stirred fo r 8 h at room temperature. The deprotection mix was then evaporated under reduced pressure, and the crude alcohol was adsorbed onto silica, after which it was purified by column chromatography (hexane/EtOAc 50:50).

General Procedure for Jones Oxidation (11.5). The alcohol obtained in the previous step (0.55 mmol) was dissolved in acetone (7 mL), to which the Jones' reagent (2 mL, 2.6 mmol) was added in one portion. After being stirred for 90 min, the reaction mixture was diluted with EtOAc (70 mL) and washed with H $_2$O (2 50 mL). The organic layer was dried over MgSO $_4$ and evaporated, yielding the crude acid as a slightly green oil that solidified on standing. Purification by column chromatography is possible, but the compounds obtained in this way are sufficiently pure (apart from traces of Cr salts that account for the greenish color of the product) for use in the next step.

(1R,S)- (2Z)-2-{2-[(tert-Butoxycarbonyl)(2-phenylethyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/1a). Yield: 84%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.45 (s, 9H, $C(CH_3)_3$), 1.64- 1.79 (m, 1H, CH_2CH_2CH), 1.81- 2.01 (m, 2H, CH_2CH_2CH), 2.11- 2.21 (m, 1H, $CH_2CH_2CH_2CH$), 2.39- 2.52 (m, 2H, $CH_2CH_2CH_2CH$), 2.72- 2.99 (m, 2H, CH_2Ar), 3.33- 3.52 (m, 2H, $ArCH_2CH_2N$), 3.54- 3.68 (m, 1H, CHC(O)-OH), 3.78- 4.28 (br m, 1H, CH_2N), 7.09- 7.48 (m, 5H, Ar). MS (ES^+) m/z: 378.2 $(M + H)^+$, 400.3 $(M + Na)^+$. MS (ES^-) m/z: 376.3 $(M - H)^-$.

(1R,S)-(2E)-2-{2-[(tert-Butoxycarbonyl)(2-phenylethyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/2a). Yield: 78%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.46 (s, 9H, $C(CH_3)_3$), 1.64- 1.78 (m, 1H, CH_2CH_2CH), 1.83- 2.12 (m, 3H, CH_2CH_2CH), 2.24- 2.52 (m, 2H, $CH_2CH_2CH_2CH$), 2.77- 2.94 (m, 2H, CH_2Ar), 3.34- 3.51 (m, 2H, $ArCH_2CH_2N$), 3.54- 3.67 (m, 1H, CHC(O)OH), 3.82- 4.21 (br m, 1H, CH_2N), 7.12- 7.33 (m, 5H, Ar). ^{13}C NMR CDCl $_3$ (400 MHz): ä 14.07 (s, $C(CH_3)_3$), 25.82 (s, CH_2CH_2CH), 28.37 (s, $C(CH_3)_3$), 29.68 (s, CH_2CH_2CH), 31.61 (s, $CH_2CH_2CH_2CH$), 40.04 (s,CHCH(O)-OH), 45.23 (s, $Ar CH_2CH_2N$), 45.44 (d, $^2J_{C-F}$) 27.4 Hz), 48.96 (s, $ArCH_2CH_2N$), 117.12 (d, $^2J_{C-F}$) 13.8 Hz $(CH_2)_2Cd$), 126.25 (s, Ar), 128.49 (s, Ar), 128.83 (s, Ar), 139.3 (s, Ar), 153.12 (d, $^1J_{C-F}$) 246.4 Hz, $CH_2C(F)d$), 178.69 (s, C(O)OH). MS(ES^+) m/z: 378.2 $(M + H)^+$, 400.3 $(M + Na)^+$. MS(ES^-) m/z: 376.3 $(M - H)^-$.

(1R,S)-(2Z)-2-{2-[1-Adamantyl(tert-butoxycarbonyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/1b). Yield: 90%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.45 (s, 9H, $C(CH_3)_3$), 1.57- 1.91 (m, 10H, CH_2CH_2CH, adamantyl-), 1.99- 2.05 (s, 6H, adamantyl-), 2.06 - 2.12 (s, 3H, adamantyl-), 2.17- 2.31 (m, 2H, $CH_2CH_2CH_2CH$), 2.99- 3.38 (br m, 1H, $CH_2CH_2CHC(O)OH$), 3.8- 4.18 (m, 2H, CH_2N). MS (ES^+) m/z: 408.1 $(M + H)^+$, 430.5 $(M + Na)^+$. MS(ES^-) m/z: 406.3 $(M - H)^-$.

(1R,S)-(2E)-2-{2-[1-Adamantyl(tert-butoxycarbonyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/2b). Yield: 88%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.46 (s, 9H, $C(CH_3)_3$), 1.58- 1.89 (m, 9H, CH_2CH_2CH, adamantyl-), 1.93- 2.17 (m, 10H, CH_2CH_2CH, adamantyl-), 2.20 - 2.42 (m, 2H, $CH_2CH_2CH_2CH$), 3.32- 3.60 (m, 1H, $CH_2CH_2CHC(O)OH$), 3.78- 4.2 (br m, 2H, CH_2N). MS(ES^+) m/z: 408.1 $(M + H)^+$, 430.4 $(M + Na)^+$. MS(ES^-) m/z: 406.2 $(M - H)^-$.

(1R,S)-(2Z)-2-{2-[(tert-Butoxycarbonyl)(4-fluorobenzyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/1c). Yield: 93%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.47 (s, 9H, $C(CH_3)_3$), 1.59- 1.79 (m, 1H, CH_2CH_2CH), 1.81- 2.09 (m, 3H, CH_2CH_2CH), 2.11- 2.33 (m, 2H, $CH_2CH_2CH_2CH$), 3.49- 3.61 (br m, 1H, CHC(O)OH), 3.79- 4.21 (br m, 2H, CH_2N), 4.28- 4.43 (m, 2H, CH_2Ar), 6.93- 7.09 (m, 2H, Ar), 7.12 - 7.31 (m, 2H, Ar). MS(ES^+) m/z: 382.2 $(M + H)^+$, 404.3 $(M + Na)^+$. MS(ES^-) m/z: 380.2 $(M - H)^-$.

(1R,S)-(2E)-2-{2-[(tert-Butoxycarbonyl)(4-fluorobenzyl)-amino]-1-fluoroethylidene }cyclopentanecarboxylic Acid (11.5/2c). Yield: 94%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.45 (s, 9H, $C(CH_3)_3$), 1.58- 1.73 (m, 1H, CH_2CH_2CH), 1.77- 1.91 (m, 2H, CH_2CH_2CH), 2.02- 2.15 (m, 1H, CH_2CH_2CH), 2.33- 2.49 (m, 2H, $CH_2CH_2CH_2CH$), 3.19- 3.53 (br m, 1H, CHC(O)OH), ä3.91- 4.14 (br m, 2H, CH_2N), 4.39- 4.69 (br m, 2H, CH_2Ar), 6.93- 7.08 (m, 2H, Ar), 7.12 - 7.31 (m, 2H, Ar). MS (ES^+) m/z: 382.2 $(M + H)^+$, 404.3 $(M + Na)^+$. MS (ES^-) m/z: 380.2 $(M - H)^-$.

General Procedure for Amide Formation (11.6). Acid 11.5 (0.4 mmol) dissolved in dichloromethane (4 mL) together with diisopropylamine (1.61 mmol) was cooled to 0 ÉC. Isobutyl chloroformate (60 mg, 0.44 mmol), dissolved in dichloromethane (1 mL), was added dropwise, and the mixture was stirred for 10 min. Then ammonia (NH $_4$OH, 30% aqueous solution) (1 mL) was added to the reaction micture. After being stirred for an additional 15 min, the reaction mixture was diluted with ethyl acetate (70 mL) and washed wit h a 1 M aqueous solution of hydrogen chloride (30 mL), water (30 mL), and saturated aqueous sodium bicarbonate (30 mL). The organic layer was dried over magnesium sulfate and evaporated under reduced pressure, yielding the crude amide, which was purified by column chromatography (hexane/EtOAc 1:4). The products were isolated as a colorless oil.

tert-Butyl (2 Z)-2-[(2 R,S)-2-(Aminocarbonyl)cyclopentylidene]-2-fluoroethyl(2-phenylethyl)carbamate (11.6/1a). Yield: 87%. ^{1}H NMR CDCl $_3$ (400 MHz) ä 1.45 (s, 9H, $C(CH_3)_3$), 1.67- 2.04 (m, 4H, CH_2CH_2CH), 2.39- 2.52 (m, 2H, $CH_2CH_2CH_2CH$), 2.70- 2.99 (m, 2H, CH_2Ar), 3.34- 3.67 (m, 3H, $ArCH_2CH_2N$ + CHC(O)OH), 3.78- 4.21 (br m, 1H, CH_2N), 7.12- 7.44 (m, 5H, Ar). MS (ES^+) m/z: 377.2 $(M + H)^+$, 399.3 $(M + Na)^+$.

tert-Butyl (2 E)-2-[(2 R,S)-2-(Aminocarbonyl)cyclopentylidene]-2-fluoroethyl(2-phenylethyl)carbamate (11.6/2a). Yield: 89%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.46 (s, 9H, $C(CH_3)_3$), 1.75- 1.96 (m, 4H, CH_2CH_2CH), 2.38- 2.54 (m, 2H, $CH_2CH_2CH_2CH$), 2.79- 2.89 (m, 2H, Ar CH_2), 3.42- 3.61 (m, 3H, $ArCH_2CH_2N$ + $CHC(O)NH_2$), 3.81- 3.99 (m, 1H, N $CH_2C(F)$), 4.01- 4.19 (m, 1H, N $CH_2C(F)$), 5.61 (br s, 2H, NH_2), 7.08- 7.36 (m, 3H, Ar), 7.4 - 7.61 (m, 2H, Ar). MS(ES^+) m/z: 377.2 $(M + H)^+$, 399.3 $(M + Na)^+$.

tert-Butyl 1-Adamantyl {(2Z)-2-[(2 R,S)-2-(aminocarbonyl)cyclopentylidene]-2-fluoroethyl }carbamate (11.6/1b). Yield: 91%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.47 (s, 9H, $C(CH_3)_3$, 1.62- 1.92 (m, 10H, CH_2CH_2CH, adamantyl-), 2.04 - 2.18 (br s, 9H, adamantyl-), 2.21 - 2.38 (m, 1H, $CH_2CH_2CH_2$-CH), 2.41- 2.52 (m, 1H, $CH_2CH_2CH_2CH$), 3.38- 3.57 (br m, 1H, $CHC(O)NH_2$), 3.92- 4.23 (m, 2H, N $CH_2C(F)d$), 5.28- 5.41 (br s, 1H, NH_2), 6.04- 6.32 (br s, 1H, NH_2). MS (ES^+) m/z: 407.2 $(M + H)^+$, 429.4 $(M + Na)^+$.

tert-Butyl 1-Adamantyl {(2E)-2-[(2 R,S)-2-(aminocarbonyl)cyclopentylidene]-2-fluoroethyl }carbamate (11.6/2b). Yield: 67%. ^{1}H NMR CDCl $_3$ (400 MHz): ä 1.45 (s, 9H, $C(CH_3)_3$, 1.52- 1.87 (m, 10H, CH_2CH_2CH, adamantyl-), 2.02 - 2.14 (m, 9H, adamantyl-), 2.20 - 2.52 (m, 2H, $CH_2CH_2CH_2CH$), 3.28- 3.46 (br m, 1H, $CHC(O)NH_2$), 4.0- 4.28 (m, 2H, N CH_2C-

(F)d), 6.03- 6.22 (br s, 1H, NH_2), 6.51- 6.63 (br s, 1H, NH_2). MS(ES^+) m/z: 407.2 $(M + H)^+$, 429.4 $(M + Na)^+$.

tert -Butyl (2 Z)-2-[(2 R,S)-2-(Aminocarbonyl)cyclopentylidene]-2-fluoroethyl(4-fluorobenzyl)carbamate (11.6/1c). Yield: 86%. ^1H NMR $CDCl_3$ (400 MHz): ä 1.42 (s, 9H, $C(CH_3)_3$), 1.52- 1.64 (m, 1H, CH_2CH_2CH), 1.67- 1.86 (m, 2H, CH_2CH_2CH), 2.1- 2.41 (m, 3H, $CH_2CH_2CH_2CH$), 3.34- 3.48 (m, 1H, $CHC(O)NH_2$), 3.82- 4.11 (m, 2H, CH_2N), 4.28- 5.1 (m, 2H, $ArCH_2$), 4.33- 4.52 (m, 2H, $Ar CH_2$), 5.31- 5.52 (br s, 1H, N H), 5.9- 6.12 (m, 1H, N H), 6.92- 7.07 (m, 2H, Ar), 7.12 - 7.22 (m, 2H, Ar). MS(ES^+) m/z: 381.5 $(M + H)^+$, 403.4 $(M + Na)^+$.

tert -Butyl (2 E)-2-[(2 R,S)-2-(Aminocarbonyl)cyclopentylidene]-2-fluoroethyl(4-fluorobenzyl)carbamate (11.6/2c). Yield: 90%. ^1H NMR $CDCl_3$ (400 MHz): ä 1.4 (s, 9H, $C(CH_3)_3$), 1.52- 1.65 (m, 1H, CH_2CH_2CH), 1.68- 1.89 (m, 2H, CH_2CH_2CH), 1.9- 2.14 (m, 1H, CH_2CH_2CH), 2.28- 2.45 (m, 2H, $CH_2CH_2CH_2CH$), 3.13- 3.38 (m, 1H, $CHC(O)NH_2$), 3.74- 4.28 (m, 1H, CH_2N), 4.31- 4.52 (m, 2H, Ar CH_2), 5.51- 5.89 (br s, 1H, N H), 6.52- 6.89 (br s, 1H, N H), 6.92- 7.02 (m, 2H, Ar), 7.1- 7.21 (m, 2H, Ar). MS(ES^+) m/z: 381.4 $(M + H)^+$, 403.5 $(M + Na)^+$.

General Procedure for Amide Dehydration and Subsequent Boc Deprotection (11.7). Amide 11.6 (100 mg 0.28 mmol) and imidazole (38 mg, 0.56 mmol) were dissolved in dry pyridine (2 mL) and cooled under nitrogen to - 10 ÉC. While the mixture was being stirred vigorously, $POCl_3$ (85 mg, 0.56 mmol) dissolved in dry dichloromethane was added dropwise via a syringe. After 15 min , 2 g of silica was added and the volatile components of the reaction mixture were evaporated. The silica was then brought onto a column and chromatographed using hexane/EtOAc 3:1 as eluent. The Boc-protected carbonitrile was obtained as a colorless oil and was dissolved in dichloromethane (1 mL) to which trifluoroacetic acid (1 mL) was added. The reaction mixture was stirred for 20 min and then evaporated thoroughly. The residue was mixed with dry diethyl ether (2 mL) and stored for 48 h at 4 ÉC to allow precipitation. The ether was carefully decanted, and the final white precipitate was dried in a vacuum desiccator for 2 days.

(1R,S)-(2Z)-2-{1-Fluoro-2-[(2-phenylethyl)amino]ethylidene }cyclopentanecarbonitrile (11.7/1a). Yield: 94%. ^1H NMR D_2O (400 MHz): ä 1.51- 1.92 (m, 2H, CH_2CH_2CH), 1.92- 2.08 (m,2H,CH_2CH_2CH), 2.31- 2.50 (m, 2H, Ar CH_2), 2.82- 3.00 (m, 2H, CH_2N), 3.21- 3.31 (m, 2H, $CH_2CH_2CH_2$), 3.82- 3.98 (m, 2H, N $CH_2C(F)$), 4.28- 4.46 (m, 1H, CHCN), 7.12- 7.28 (m, 3H, Ar), 7.30 - 7.44 (m, 2H, Ar). ^{13}C NMR D_2O (400 MHz): ä 23.92 (s, CH_2CH_2CH), 26.3 (s, CH_2CH_2CH), 27.88 (s, $CH_2 CH_2$-CH_2), 30.12 (s, $CH_2 CH_2CH$), 37.81 (s, $ArCH_2$), 43.8 (d, $^2J_{C-F}$) 27.3 Hz, N $CH_2C(F)$), 46.42 (s, $ArCH_2CH_2$), 118.12 (d, $^2J_{C-F}$) 21.7 Hz, N $CH_2C(F)$), 121.37 (s, CN), 126.04 (s, Ar), 127.33 (s, Ar), 127.69 (s, Ar), 134.73 (s, Ar), 136.32 (s, Ar), 148.12 (d, $^1J_{C-F}$) 247.2 Hz, $CH_2C(F)$d). MS (ES^+) m/z: 259.3 $(M + H)^+$, 281.3 $(M + Na)^+$. LC- MS: t_R) 4.11 min, 99%. HPLC (214 nm): t_R) 11.34 min, 99%.

(1R,S)-(2E)-2-{1-Fluoro-2-[(2-phenylethyl)amino]ethylidene }cyclopentanecarbonitrile (11.7/2a). Yield: 89%. ^1H NMR D_2O (400 MHz): ä 1.67- 2.03 (m, 4H, CH_2CH_2CH), 2.89- 2.94 (t, $^3J_{H-H}$) 7.6 Hz, 2H, Ar CH_2), 3.20- 3.26 (t, $^3J_{H-H}$) 7.7 Hz, 2H, $ArCH_2CH_2N$), 3.34- 3.41 (m, 1H, $CH_2CH_2CH_2$), 3.50- 3.53 (m, 1H, $CH_2CH_2CH_2$), 3.69- 3.78 (m, 1H, CHCN), 3.79- 3.92 (d, $^3J_{H-F}$) 20 Hz, 2H, N $CH_2C(F)$d), 7.17- 7.24 (m, 3H, Ar), 7.25- 7.32 (m, 2H, Ar). ^{13}C NMR D_2O (400 MHz): ä 24.34 (s, CH_2CH_2CH), 27.38 (s, $CH_2CH_2 CH$), 32.18 (s, $CH_2CH_2CH_2$), 32.71 (s, CH_2CH_2CH), 40.52 (s, $ArCH_2$), 44.87 (d, $^2J_{C-F}$) 28 Hz, N $CH_2C(F)$), 48.02 (s, CH_2CH_2N), 121.37 (s, CN), 125.56 (d, $^2J_{C-F}$) 21.9 Hz $(CH_2)_2Cd$), 127.34 (s, Ar), 129.07 (s, Ar), 136.32 (s, Ar), 136.54 (s, Ar), 145.48 (d, $^1J_{C-F}$) 244 Hz, $CH_2C(F)$d). MS (ES^+) m/z: 259.2 $(M + H)^+$, 281.3 $(M + Na)^+$. LC- MS: t_R) 4.3 min, 98%. HPLC (214 nm): t_R) 11.58 min, 97%.

(1R,S)-(2Z)-2-[2-(1-Adamantylamino)-1-fluoroethylidene]cyclopentanecarbonitrile (11.7/1b). Yield: 94%. ^1H NMR DMSO- d_6 (400 MHz): ä 1.51- 1.89 (m, 10H, CH_2CH_2-