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Fluorinated Pseudopeptide Analogues of the Neuropeptide 26RFa: Synthesis, Biological, and Structural Studies

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A series of four fluorinated dipeptide analogues each containing a fluoro-olefin moiety as peptide bond surrogate has been designed and synthesized. These motifs have been successfully introduced into the bioactive C-terminal heptapeptide of the neuropeptide 26RFa by conventional SPPS. We then evaluated the ability of the generated pseudopeptides to increase $[Ca^{2+}]_i$ in GPR103-transfected cells. For these fluorinated analogues, greater stability in human serum was observed. Their conformations were also investigated, leading to the valuable identification of differences depending on the position of the fluoro-olefin moiety in the sequence.

Introduction

26RFa is a neuropeptide of the RFamide family, originally isolated from frog brain and subsequently cloned in human and rat.[1] 26RFa is the endogenous ligand of the former orphan receptor GPR103.[2] Analysis of the human 26RFa precursor indicates that pre-pro26RFa might generate several additional peptides including an N-terminal extended form (43RFa) and a truncated form ($26RFa_{(20–26)}$ (GGFSFRF-NH_2)) strictly conserved throughout mammals.[3] In rodents, 26RFa and 43RFa induce dose-dependent increases in food intake, stimulate secretion of gonadotropins and aldosterone, and reduce glucose-induced insulin release.[3,4] Moreover, GPR103-knockout mice suffer from osteopenia and exhibit the characteristic kyphotic humps of osteoporotic patients.[5] Altogether, these data indicate that 26RFa is able to exert diverse biological activities in vertebrates, such as the control of food intake, reproduction, and osteogenesis.

It is important to note that the C-terminal heptapeptide, $26RFa_{(20–26)}$, mimics the orexigenic and gonadotropic effects of 26RFa.[6] However, $26RFa_{(20–26)}$ is about 75 times less potent than 26RFa in increasing $[Ca^{2+}]_i$ in cultured GPR103-transfected CHO cells.[7] Structure–activity relationship studies showed that replacement of Ser23 by a norvaline led to an analogue, $[Nva23]26RFa_{(20–26)}$, that was three times more potent than the native heptapeptide.[7] More importantly, we have recently reported the rational design of a potent, stable, and long-lasting aza-β^3-pseudopeptide (LV-2172) that paves the way to the development of GPR103 drug candidates.[8] We have also reported that the pseudopeptide LV-2045—$[Gly^{20}\Psi[CH_2NH]Gly^{21}]$-$26RFa_{(20–26)}$—is as potent as $26RFa_{(20–26)}$ in mobilizing intracellular calcium and that the replacement of the Gly–Gly motif by a 4-(carboxymethyl)piperazine (Cmpi) unit leads to an analogue (LV-2043) five times more potent than the heptapeptide.[8]

To find new potent GPR103 ligands and to increase the metabolic stabilities of the heptapeptides, we thus decided to focus our attention on the two first peptide bonds of the N-terminal region, Gly–Gly and Gly–Phe peptide bonds, which are the first positions to undergo enzymatic degradation. To this end, we decided in this work to turn our attention to fluorinated molecules, as it nowadays well known that the introduction of one or several fluorine atoms into a biomolecule can change its physicochemical, physical, biochemical, and biological properties.[9] In this context, the fluoro-olefin moiety, denoted $\Psi[CF{=}CH]$, can be regarded as a suitable peptide bond mimetic because it exhibits isosteric and isoelectronic characteristics similar to those of the native bond.[10] Moreover, in terms of metabolic stability, the fluoro-olefin moiety increases the resistance of a pseudopeptide to proteolysis.[11,12] To study the importance of the configuration of the double bond, it also seemed interesting to synthesize both *Z* (*transoid*) and *E* (*cisoid*) analogues in order to obtain information about the bioactive conformation of the heptapeptides.

Although many pseudopeptides featuring a fluoro-olefin have already been designed, only a few biological data[11] support the concept of a fluoro-olefin as a peptide bond replacement because of the difficulties in synthesizing such halogen-

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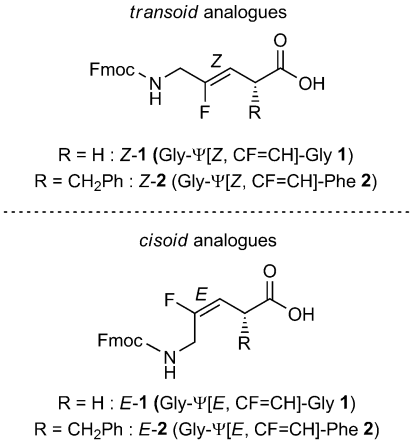
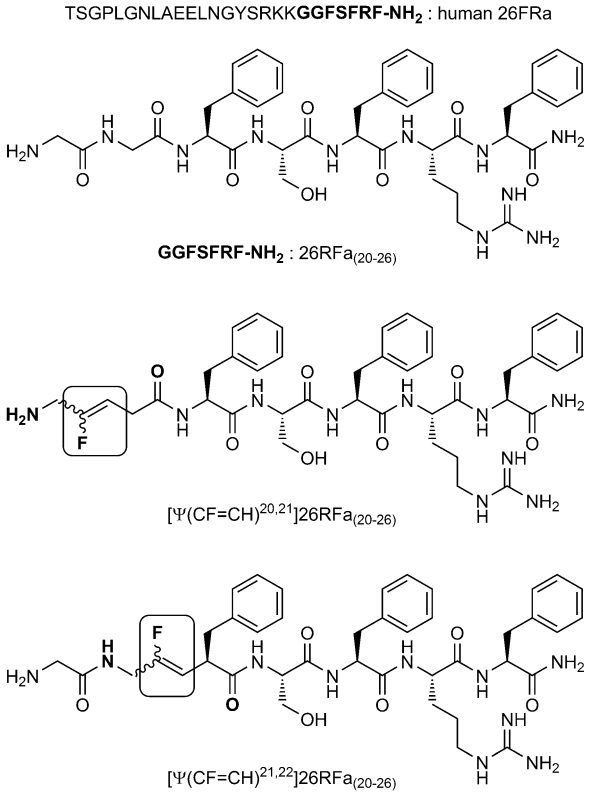
containing pseudopeptides. On the basis of our expertise in the synthesis of fluoro-olefin-containing compounds,[11b,13] we decided to develop fluorinated SPPS-convenient pseudodipeptides (Scheme 1) in order to incorporate them into the C-terminal heptapeptide of 26RFa (Scheme 2), which can be used as an ideal molecular scaffold for the design of GPR103 peptide ligands of low molecular weight.[7,8]



Scheme 1. Target fluorinated pseudodipeptides **1** and **2**.



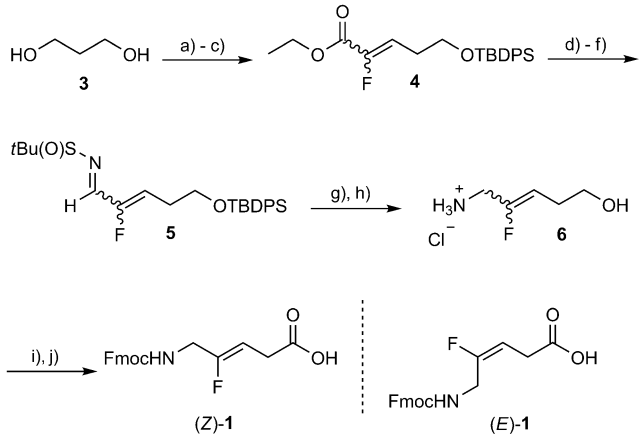
Scheme 2. 26RFa, C-terminal heptapeptide $26RFa_{(20-26)}$, and fluorinated pseudoheptapeptide targets.

Results and Discussion

In order to access the new fluorinated peptide targets, a series of SPPS-amenable fluoroalkene-containing pseudodipeptides was first synthesized. These were then incorporated into the C-terminal heptapeptides of 26RFa by conventional SPPS, and the functional activities of the fluorinated pseudoheptapeptides were evaluated by determining the calcium-mobilizing responses in GPR103-transfected cells. Their stabilities in human serum were assessed in vitro, and their conformations were studied by NMR spectroscopy.

Synthesis of fluorinated pseudodipeptides

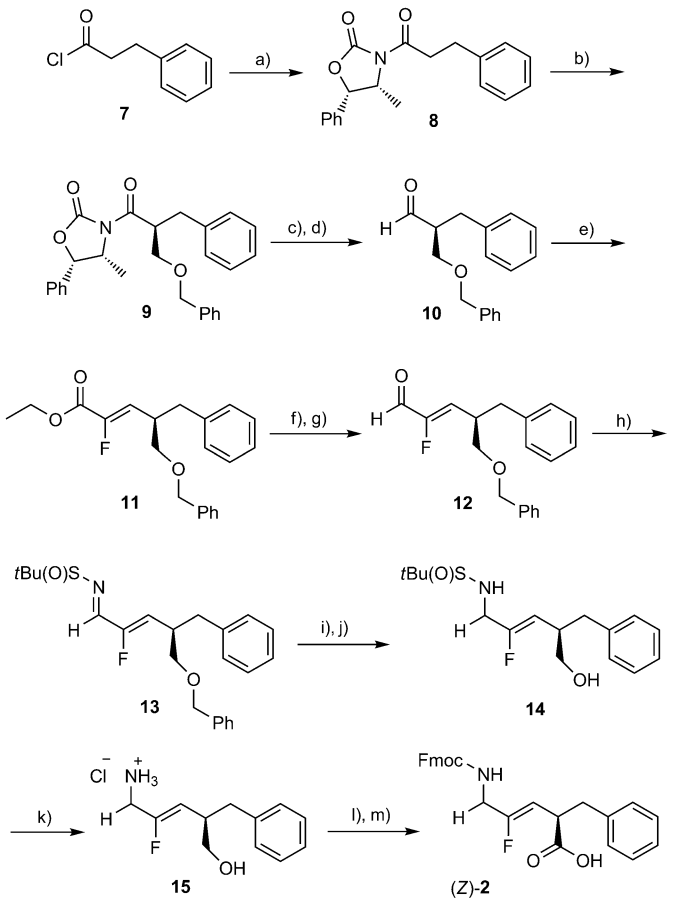
We first undertook the synthesis of the simplest Fmoc-GlyΨ[CF=CH]Gly-OH pseudodipeptides (*E*)-**1** and (*Z*)-**1** as depicted in Scheme 3.



Scheme 3. Synthesis of Fmoc-GlyΨ[CF=CH]Gly-OH isomers (*E*)-**1** and (*Z*)-**1**. a) TBDPS-Cl, *n*BuLi, THF, 84%; b) IBX, AcOEt, 98%; c) CBr_2FCO_2Et, $ZnEt_2$, PPh_3, THF, 85% (*Z*/*E* 62:38); d) $LiAlH_4$, THF; e) IBX, AcOEt, 84% for two steps; f) NH_2SOtBu, $Ti(OEt)_4$, THF, reflux, 89% (separation of each diastereoisomer); g) $NaBH_4$, THF, 91% (*Z*) and 84% (*E*); h) HCl 4 M in dioxane, MeOH, quantitative; i) Fmoc-OSu, $NaHCO_3$, dioxane/H_2O, 72% (*Z*) and 70% (*E*); j) Jones reagent, acetone, 70% (*Z*) and 50% (*E*).

Monoprotection of one hydroxy group in commercially available propane-1,3-diol (**3**) was carried out, followed by oxidation of the free hydroxy group into an aldehyde and a fluoroolefination reaction to afford the ethyl α-fluoroacrylate **4**.[14] The ester moiety was converted into an aldehyde through a reduction/oxidation sequence. The sulfinylimine **5** was then obtained, and at this stage the *E* and the *Z* diastereoisomers were easily separated by column chromatography. The sulfinylimines **5** were reduced by sodium borohydride, followed by simultaneous deprotection of amine and alcohol functions to furnish compounds **6**, which were subjected to Fmoc protection and Jones oxidation to give the two Fmoc-GlyΨ[CF=CH]Gly-OH isomers (*E*)-**1** and (*Z*)-**1** in 17 and 24% overall yields, respectively, for this ten-step synthesis.

We next turned our attention to the synthesis of the more challenging analogues **2** Fmoc-GlyΨ[CF=CH]Phe-OH featuring



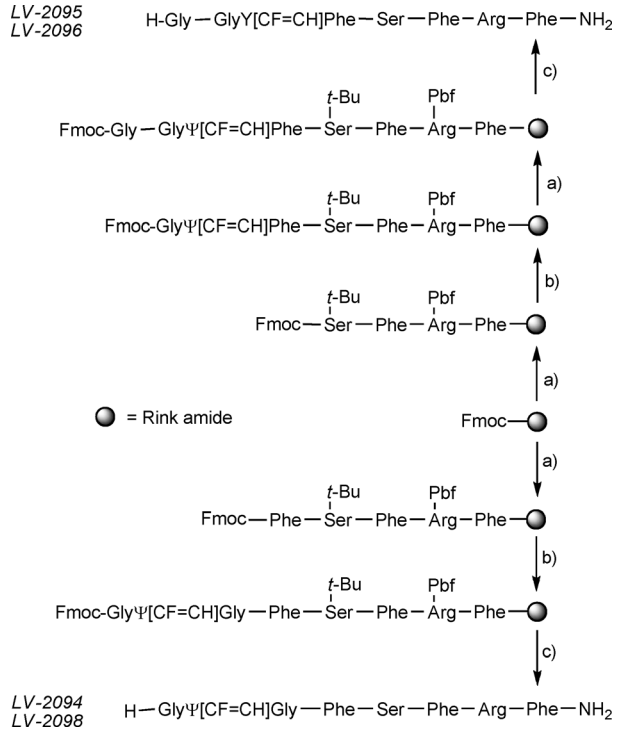
Scheme 4. Synthesis of (*Z*)-**2** (Fmoc-GlyΨ[CF=CH]Phe-OH). a) (4*R*,5*S*)-(+)-4-Methyl-5-phenyl-2-oxazolidinone, *n*BuLi, THF, 96%; b) $BnOCH_2Cl$, DIPEA, $TiCl_4$, CH_2Cl_2, 88% (*de* > 95%); c) $LiAlH_4$, THF; d) $PySO_3$, NEt_3, DMSO, CH_2Cl_2, 55% for two steps; e) CBr_2FCO_2Et, $ZnEt_2$, PPh_3, THF, 55% (*Z*) and 34% (*E*) (*Z*/*E* 64:36); f) $LiAlH_4$, THF; g) $PySO_3$, NEt_3, DMSO, CH_2Cl_2, 70% for two steps; h) NH_2SOtBu, $Ti(OEt)_4$, THF, reflux, 95%; i) $NaBH_4$, THF, 95%; j) BCl_3, CH_2Cl_2, −78 °C, 58%; k) HCl 4 M in dioxane, MeOH, quantitative; l) Fmoc-OSu, $NaHCO_3$, dioxane/H_2O, 95%; m) Jones reagent, acetone, 80%.

a stereogenic center. For this purpose we developed an asymmetric synthesis to control the stereogenic center at the C-terminal side of the pseudodipeptide (Scheme 4).

An Evans oxazolidinone was condensed with the commercially available acyl chloride **7**, followed by a highly diastereoselective alkoxymethylation (*de* > 95%) to set up the desired configuration at the C-terminal side of the dipeptide analogue.[15] The chiral auxiliary was removed under reductive conditions, and the released hydroxy group was oxidized to give aldehyde **10**. An olefination reaction furnished *Z* and *E* diastereoisomers **11** as a separable mixture. The completion of the synthetic route was done only with the (*Z*)-**11** stereoisomer. Ester **11** was transformed into aldehyde **12**, condensation of which with *tert*-butanesulfinamide gave compound **13**. Reduction of the imine and debenzylation yielded product **14**. Further removal of the *tert*-butanesulfinamide, Fmoc protection, and Jones oxidation allowed us to obtain (*Z*)-**2**—Fmoc-GlyΨ[CF=CH]Phe-OH—in an overall yield of 6% in this 13-step synthesis. It should be noted that several attempts to obtain the (*E*)-**2** derivative were unsuccessful, due to the degradation of some intermediates.

Solid-phase peptide synthesis

We next incorporated these fluoropseudodipeptides in appropriate positions of $26RFa_{(20-26)}$ by conventional solid-phase automated peptide synthesis. It should be noted that the coupling of the fluorinated analogues was performed manually, because of the small amounts of compounds available (Scheme 5).



Scheme 5. Solid-phase synthesis of fluorinated pseudoheptapeptide analogues. a) SPPS cycles: i: 20% piperidine, NMP, ii: Fmoc-AA-OH (10 equiv), HBTU (10 equiv), HOBt (10 equiv), DIEA (20 equiv), NMP. b) Manual coupling: i: 20% piperidine, NMP, ii: Fmoc-GlyΨ[CF=CH]AA-OH (1.5 equiv), HATU (1.5 equiv), HOAt (1.5 equiv), DIEA (3 equiv), NMP. c) i: 20% piperidine, NMP, ii: TFA/TIS/H_2O 99.5:0.25:0.25 (*v*/*v*/*v*).

Finally, the crude pseudoheptapeptides were analyzed by analytical reversed-phase (RP) HPLC, purified by preparative RP-HPLC, and characterized by MALDI-TOF mass spectrometry with α-cyano-4-hydroxycinnamic acid as a matrix.

The two pseudoheptapeptides LV-2098 and LV-2094, derived from (*E*)-**1** and (*Z*)-**1** GlyΨ[CF=CH]Gly, respectively, were each obtained as a single RP-HPLC peak, as illustrated for LV-2094 in Figure 1.

Surprisingly, the chromatogram of the pseudoheptapeptide harboring the (*Z*)-**2** GlyΨ[CF=CH]Phe moiety revealed the presence of two peaks of equal area and showing the same molecular weight (Figure 2). We suspected epimerization of compound (*Z*)-**2** during the late stage of our pseudodipeptide synthesis. Indeed, compound **14** was a single stereoisomer before

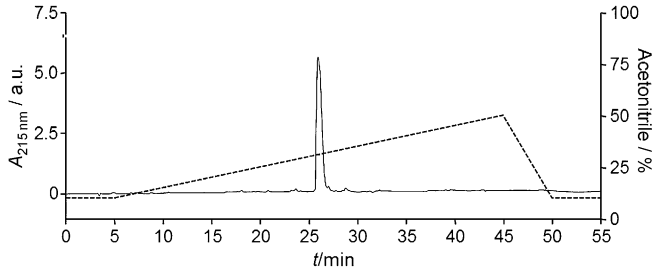


Figure 1. RP-HPLC chromatogram of crude LV-2094 [containing the (*Z*)-**1** GlyΨ[CF=CH]Gly motif]. The dashed line shows the concentration of acetonitrile in the eluting solvent.

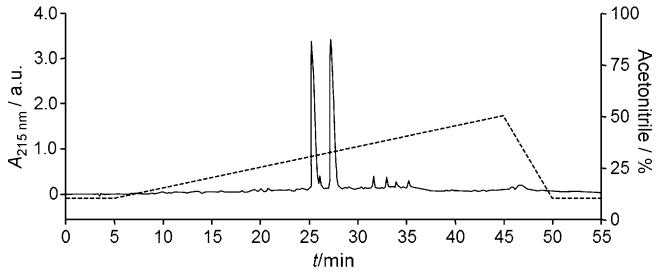


Figure 2. RP-HPLC chromatogram of crude pseudoheptapeptide containing the (*Z*)-**2** GlyΨ[CF=CH]Phe motif. The dashed line shows the concentration of acetonitrile in the eluting solvent.

it was subjected to the last sequence (Scheme 3, steps k–m), although these steps are commonly used in the laboratory without any epimerization problem.[16] Compound (*Z*)-**2** was derivatized with (−)-menthol as chiral auxiliary, and NMR analysis of the corresponding chiral esters revealed the existence of two diastereoisomers—(*Z*)-**2 a** and (*Z*)-**2 b**—of the same intensity; this confirms the racemization of the molecule. Nevertheless, it appeared interesting to evaluate both diastereoisomers of the pseudopeptides. For this, we purified both diastereoisomeric pseudopeptides containing (*Z*)-**2** [LV-2095 (first eluted) and LV-2096 (second eluted)], one containing an [L-Phe22] amino acid analogue and the other a [D-Phe22] unit, and subjected them to individually biological and structural studies.

Biological activity

The functional activities of fluorinated pseudopeptide analogues LV-2094, LV-2095, LV-2096, and LV-2098 were evaluated by assessing the highly sensitive calcium-mobilizing response in GPR103-transfected cells as previously reported.[7,8] The pseudopeptides [*Z*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$ (LV-2094) and [*E*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$ (LV-2098) were both slightly more potent than 26RFa$_{(20-26)}$ in increasing $[Ca^{2+}]_i$; this suggests on one hand the efficiency of the fluoro-olefin mimetic and on the other, the lack of structuration of the Gly–Gly region, with the *cisoid* and *transoid* orientations of the Gly–Gly peptide bond having no impact on the biological activity of the pseudoheptapeptides (Table 1).

In support of this statement, the Gly–Gly peptide bond can be reduced (LV-2045) without impairing the agonist activity.[8]

Table 1. Effect of 26RFa and 26RFa$_{(20-26)}$ analogues on basal $[Ca^{2+}]_i$ in *h*GPR103-CHO cells.

	Peptides/pseudopeptides	EC_{50} [nM]
1	26RFa	10.4 ± 1.5
2	26RFa$_{(20-26)}$	739 ± 149
3	LV-2094: [*Z*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$	618 ± 104
4	LV-2098: [*E*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$	538 ± 13
5	LV-2095: [*Za*,Ψ(CF=CH)^{21,22}]26RFa$_{(20-26)}$	6752
6	LV-2096: [*Zb*,Ψ(CF=CH)^{21,22}]26RFa$_{(20-26)}$	1720 ± 1010

Data are the means ± SEMs of at least three distinct experiments performed in triplicate.

In addition, the presence of a local constraint, introduced by replacement of the Gly–Gly motif by a Cmpi moiety, which can be regarded as an inducer of a concomitant *cis*/*trans* peptide bond increases the potency of the heptapeptide.[8]

Conversely, the pseudopeptides [Z,Ψ(CF=CH)^{21,22}]26RFa$_{(20-26)}$ (LV-2095 and LV-2096) were less potent than 26RFa$_{(20-26)}$; this suggests a critical role of the Gly–Phe peptide bond in the bioactive conformation of the peptide (Table 1). In support of this hypothesis, we have recently reported that [azaβ^3-Phe^{22}]-26RFa$_{(20-26)}$ (LV-2154), in which the Gly–Phe peptide bond is replaced by a Ψ[CONHNRCH$_2$] pseudopeptide bond, is significantly less active than the heptapeptide.[8] We may also point out that the less potent designed fluorinated analogues LV-2095 gave results similar to those for [D-Phe^{22}]26RFa$_{(20-26)}$; this suggests that LV-2095 harbors the (*Z*)-**2** diastereoisomer with a configuration similar to that of a D-phenylalanine residue.[7]

Susceptibility to enzymatic degradation

The incorporation of a pseudopeptide bond usually enhances the resistance of the pseudopeptide to enzymatic degradation.[16] We thus evaluated the breakdown of fluorinated pseudoheptapeptides in human serum by combining RP-HPLC and MALDI-TOF MS characterization (Figure 3). The half-lives of LV-2094 (52.9 min) and LV-2098 (52.5 min) in human serum were about five times longer than that of 26RFa$_{(20-26)}$ (11.17 min). Likewise, the half-lives of LV-2095 (28.11 min) and LV-2098 (19.07 min) were better than for 26RFa$_{(20-26)}$ although the values were lower relative to LV-2094 and LV-2098. These results confirm that the fluoro-olefin moiety induced better stability of the biomolecules to enzymatic degradation and can be used as an efficient tool to replace any peptide bond easily prone to metabolic degradation as long as the biological activity is not impaired.

The better stability of LV-2095 than of LV-2096 seems to confirm the hypothesis relating to the presence of a D-Phe residue in the pseudopeptide. In fact, it is well established that D-amino acid substitution prevents degradation of peptide bonds by peptidases.[17] We also speculate that the differences in stability between GlyΨ[CF=CH]Gly (LV-2094 and LV-2098) and GlyΨ[CF=CH]Phe (LV-2095 and LV-2096) bonds could be explained by the involvement of the native Gly-Phe bond in intramolecular stabilized structures, resulting in weaker effects of its replacement than in the unstructured case (Gly–Gly).

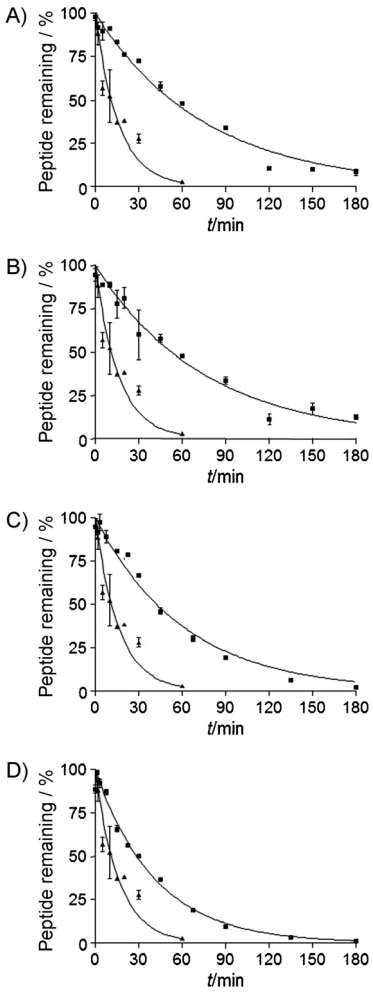


Figure 3. Stabilities of the fluoro-olefin pseudoheptapeptides. A)–D) Degradation kinetics of 26RFa$_{(20-26)}$ (▲) as well as of A) [*Z*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$ (LV-2094), B) [*E*,Ψ(CF=CH)^{20,21}]26RFa$_{(20-26)}$ (LV-2098), C) [*Za*,Ψ(CF=CH)^{21,22}]-26RFa$_{(20-26)}$ (LV-2095), and D) [*Zb*,Ψ(CF<C=<CH)^{21,22}]26RFa$_{(20-26)}$ (LV-2096; ■), evaluated by RP-HPLC after incubation of the compounds in human serum. Each point is the mean ± SEM of two independent experiments.

Solution conformation analysis by NMR

To evaluate the influence of the fluoro-olefin moiety on the conformations of the pseudoheptapeptides, we performed structural studies on 26RFa$_{(20-26)}$ and on the four fluorinated analogues by NMR.

The studies were conducted in a dodecylphosphocholine (DPC)/water mixture in order to mimic the membrane environment of GPR103, the target of 26RFa. Proton resonance assignment for all molecules was carried out by the strategy proposed by Wüthrich and co-workers.[18] Spin systems were identified with a combination of 2D TOCSY and COSY spectra, and neighboring residues were connected through 2D NOESY experiments.

A first assessment of the secondary structure of 26RFa$_{(20-26)}$ was obtained by analyzing Hα chemical shifts, because their deviations from the random coil values reflect peptide/protein secondary structures.[19] A NOESY spectrum with a mixing time of 150 ms was also recorded to improve this estimate with a detailed analysis of sequential and medium-range inter-residue NOEs.

The Hα secondary chemical shifts calculated for 26RFa$_{(20-26)}$ suggested the presence of a helical conformation between residues 22 and 26 (Figure 4). These data are in good agreement

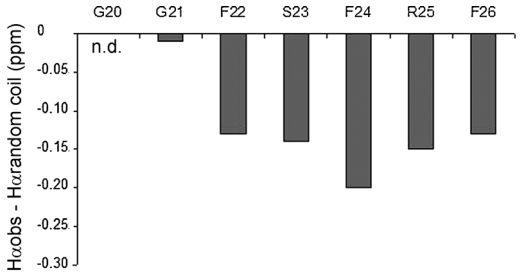


Figure 4. Hα secondary shifts in DPC/water versus 26RFa$_{(20-26)}$ peptide sequence. ^{1}Hα secondary shifts were calculated with the aid of ^{1}Hα random coil values in water from Wishart et al.[19] (n.d.: not determined).

with the different number of characteristic NOE crosspeaks present in the NOESY spectra. Indeed, the observation of medium-to-weak N,N (*i*,*i*+1), α,N (*i*,*i*+2), and α,N (*i*,*i*+3) correlations between residues 22 and 26 reinforces the hypothesis of a helical conformation in this region of the molecule (Figure 5). The absence of α,β (*i*,*i*+3) and α,N (*i*,*i*+4) correlations indicates that the secondary structure of 26RFa$_{(20-26)}$ is composed of a series of turns rather than of a canonical helix, in agreement with our previous CD results.[8]

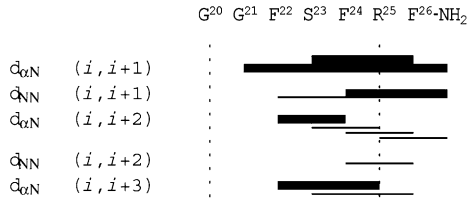


Figure 5. Summary of the sequential and medium-range NOEs used for secondary structure evaluation of 26RFa$_{(20-26)}$. The NOEs are classified into three categories (strong < 2.5 Å, medium ≥ 2.5 Å and ≤ 3.5 Å, weak > 3.5 Å) by crosspeak volume. The intensities are indicated by the thicknesses of the bars.

The influence of the fluoro-olefin moiety on the pseudoheptapeptide conformation was first evaluated by comparing the Hα backbone chemical shifts of each analogue with those of 26RFa$_{(20-26)}$ (Figure 6). For pseudoheptapeptide analogues LV-2094 [containing (*Z*)-**1** dipeptide] and LV-2098 [containing (*E*)-**1** dipeptide], with the exception of residue 21, the upfield shift of which was consistent with the shielding effect of the introduced fluoro-olefin moiety relative to the amide group, very small Hα chemical shift differences (ΔHα < 0.03 ppm) were detected; this indicates that the fluoro-olefin insertion between residues 20 and 21 was structurally conservative.

For pseudoheptapeptide analogues LV-2095 and LV-2096 [containing (*Z*)-**2a** and (*Z*)-**2b** dipeptides, respectively], in addi-

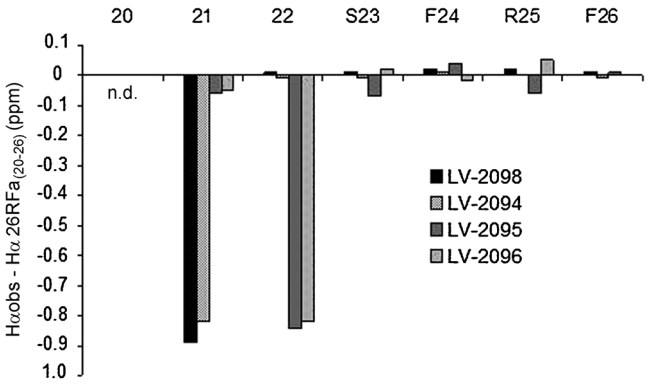


Figure 6. ^{1}Hα chemical shift difference between each analogue (LV-2094, LV-2095, LV-2096, or LV-2098) and 26RFa$_{(20-26)}$ (n.d.: not determined).

tion to the upfield shift of residue 22, more significant chemical shift differences ($|\Delta H\alpha| < 0.08$ ppm) were observed for residues 21, 23, 24, and 25; this suggests a greater structural impact of the fluoro-olefin insertion between residues 21 and 22.

Because the incorporation of a pseudopeptide bond might also induce modifications in the side chain conformations, we then compared the Hβ chemical shifts of each analogue with those of 26RFa$_{(20-26)}$ (Figure 7).

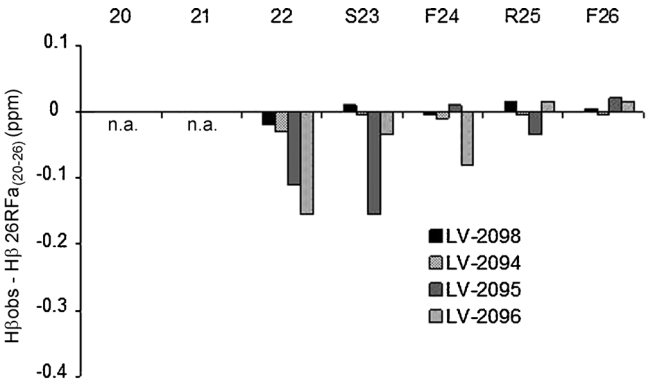


Figure 7. ^{1}Hβ chemical shift difference between each analogue (LV-2094, LV-2095, LV-2096, or LV-2098) and 26RFa$_{(20-26)}$. The reported value for each residue corresponds to the mean of the differences observed for each of the two β protons (n.a.: not applicable).

For analogues LV-2094 and LV-2098, very few differences ($|\Delta H\beta_{mean}| < 0.03$ ppm) were observed; this confirms the small influence of Gly–Gly peptide bond modification on the heptapeptide conformation.

For analogues LV-2095 and LV-2096, in addition to the expected variation observed for residue 22, significant chemical shift differences were detected for Ser23, Phe24, and Arg25; this confirms that the conformation of the molecule is modified by the incorporation of the fluoro-olefin moiety in position 21. Interestingly, for analogue LV-2095 an upfield shift ($\Delta H\beta_{mean} = -0.16$ ppm) was observed for the β protons of residue 23. This suggests that this diastereoisomer [containing (*Z*)-**2a** dipeptide] could be the one harboring a modified configuration for residue 22. These data confirmed our previous hypothesis based on the biological activity and stability studies.

To characterize the influence of the fluoro-olefin moiety on the secondary structures of the pseudoheptapeptides in detail, an analysis of sequential and medium-range inter-residue proton distances was performed (Figure 8).

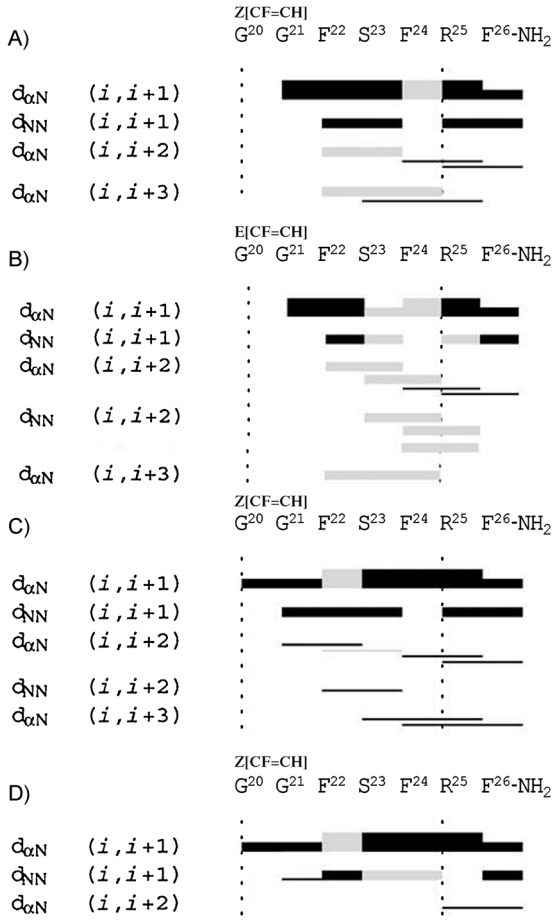


Figure 8. Summary of the sequential and medium-range NOEs used for secondary structure evaluation of analogues: A) LV-2094, B) LV-2098, C) LV-2095, and D) LV-2096. The NOEs are classified into three categories (strong < 2.5 Å, medium ≥ 2.5 Å and ≤ 3.5 Å, weak > 3.5 Å) based on the crosspeak volumes. The intensities are indicated by the thicknesses of the bars. Ambiguous NOEs are represented by gray lines. In each diagrams the CH part of the fluoro-olefin moiety is regarded as an NH moiety in the modified residue.

Consistently with the Hα and Hβ chemical shift comparisons, we observed very few modifications in the NOE crosspeak patterns of analogues LV-2098 and LV-2094 relative to 26RFa$_{(20-26)}$. The absence of the N,N (*i*,*i*+1) correlation between residues 24 and 25 or the shifts to the ambiguous category were due to chemical shift overlaps in the spectra of the two analogues. Only three correlations were not detected: for LV-2094 the weak α,N (*i*,*i*+2) correlation between Ser23 and Arg25 and the weak N,N (*i*,*i*+2) correlation involving Phe24 and Phe26, and for analogue LV-2098 the weak α,N (*i*,*i*+3) correlation between Ser23 and Phe26. These findings indicate that the insertion of the fluoro-olefin between residues 20 and 21, either in a *Z* or in an *E* configuration, induces very small modifications of the

heptapeptide secondary structure and confirms the lack of structuration of this N-terminal part of the heptapeptides.

Conversely, the NOE crosspeak patterns of the two pseudopeptides [*Z*,Ψ(CF=CH)[21,22]]26RFa$_{(20-26)}$ were quite different from that of the native heptapeptide. For analogue LV-2095, additional NOE crosspeaks characteristic of turns [i.e., α,N (*i*,*i*+2), N,N (*i*,*i*+1), and N,N (*i*,*i*+2)] were observed between Gly20 and Ser23; this suggests that the secondary structure extends to the N-terminal part of the peptide. In addition, the two α,N (*i*,*i*+3) correlations are each moved by one residue; this indicates that the C-terminal conformation is also altered.

In analogue LV-2096, the modification of the Gly–Phe peptide bond induces an opposite effect. Indeed, we observed the absence of several NOE crosspeaks between residues 21 and 25; this indicates a loss of secondary structure.

Both the fluoro-olefin insertion and the chirality[20] of Phe22 thus induced marked modifications of the heptapeptide secondary structure. It is more than likely that these structural modifications are responsible for the failure of compounds LV-2095 and LV-2096 to activate GPR103.

As mentioned above, 26RFa$_{(20-26)}$ contains interlaced turns between residues 22 and 26, and so the carbonyl group of Phe22 is probably involved in hydrogen bonding. We have shown that the substitution of the native Gly20–Gly21 peptide bond by a fluoro-olefin did not impair the molecule conformation. On the contrary, the modification of the native Gly21–Phe22 peptide bond is associated with marked alterations of the secondary structure. These results suggest that the fluoro-olefin moiety can substitute the peptide bond without inducing significant structural modifications if the substitution takes place in a flexible region of the peptide.

Conclusions

We have reported the syntheses of four fluorinated pseudodipeptides, of Fmoc-GlyΨ[CF=CH]Gly-OH and Fmoc-Gly-Ψ[CF=CH]Phe-OH type, convenient for Fmoc-SPPS. These dipeptides have been used in peptide synthesis to design new fluorinated analogues of the 26RFa$_{(20-26)}$ heptapeptide. These fluorinated pseudopeptides have been subjected to biological evaluation, enzymatic degradation, and conformation analysis. The results suggested that the fluoro-olefin moiety can be employed as an effective mimic of the peptide bond with great enhancement of the peptide stability. Moreover, the conformation analysis showed that the fluoro-olefin moiety induces only slight modification of the secondary structure as long as the replaced peptide bond is not involved in hydrogen bonding. Indeed, very few impacts on the conformation were observed with the Gly–Gly dipeptide analogues, whereas with the Gly–Phe analogues alteration of the structure was observed, this region being involved in the secondary structure of the heptapeptide 26RFa$_{(20-26)}$. The biological activity, measured by the calcium-mobilizing response in GPR103-transfected cells, is fairly interesting for the two fluorinated pseudopeptides LV-2094 and LV-2098, which exhibit slightly better activities than the native peptide. To conclude, a fluoro-olefin unit can be used as a peptide bond mimic to stabilize peptides against enzymatic degradation. Nevertheless, higher stability did not necessarily imply better biological activity, and careful position modification has to be done to produce a concomitant effect. Fluoro-olefin-containing pseudopeptides can be used as interesting tools to design bioactive compounds as well as for conformation analysis of peptides.

Experimental Section

Pseudodipeptide synthesis: All organometallic reagents were commercially available. Reactions with organometallics were carried out under argon. THF was distilled prior to use from sodium benzophenone ketyl under nitrogen, and dichloromethane from CaH_2. Analytical thin layer chromatography was performed on silica gel aluminum plates with F-254 indicator and visualized by UV fluorescence and/or by staining with $KMnO_4$ or phosphomolybdic acid. Flash column chromatography purifications were carried out with silica gel (70–230 mesh). ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR ($CFCl_3$ as internal reference) were recorded at 300.13, 75.47, and 282.40 MHz, respectively, with a Bruker DXP 300. IR spectra were recorded with a PerkinElmer Spectrum 100. Absorption bands are reported in cm^{-1}. ESI-MS experiments were performed with a Bruker–Esquire mass spectrometer. Electronic impact (EI, 70 eV), chemical ionization (CI, 200 eV), or high-resolution MS experiments were recorded with a JEOL AX 500 mass spectrometer and use of a mass resolution of 5000. Elemental analyses were performed with a CE Instruments EA 110 CHNS-O instrument.

3-{[*tert*-Butyl(diphenyl)silyl]oxy}propan-1-ol (Scheme 3, step a): *n*BuLi (2.62 mL of a 2.5 M solution in hexane, 6.57 mmol, 1 equiv) and *tert*-butyldiphenylchlorosilane (1.7 mL, 6.57 mmol, 1 equiv) were added at −78 °C to a solution of propane-1,3-diol (**3**, 0.47 mL, 6.57 mmol, 1 equiv) in dry THF (15 mL). The reaction mixture was stirred for 15 min at −78 °C and for 30 min at room temperature and was finally heated at reflux for 3 h 30 min. It was quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with Et_2O (2×), and the combined organic layers were dried over $MgSO_4$, filtered, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/Et_2O 80:20 to 60:40) to afford a white crystalline solid (1.75 g, 84%). M.p. 39 °C; R_f = 0.25 (hexane/EtOAc 75:25); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (s, 9H), 1.81 (m, 2H), 3.85 (t, $^3J_{H,H}$ = 5.7 Hz, 2×2H), 7.37–7.47 (m, 6H), 7.66–7.70 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 19.1, 26.8, 34.4, 61.7, 63.1, 127.8, 129.8, 133.3, 135.6 ppm; IR (neat): $\tilde{\nu}$ = 3349, 3071, 2931, 2858, 1472, 1428, 1112, 823, 737, 702, 688, 614, 505 cm^{-1}; MS: *m*/*z*: 257.00 [*M*−*t*Bu]$^+$; elemental analysis calcd (%) for $C_{19}H_{26}O_2Si$: C 72.56, H 8.33; found: C 72.50, H 8.27.

3-{[*tert*-Butyl(diphenyl)silyl]oxy}propanal (Scheme 3, step b): IBX (5.5 g, 19.67 mmol, 3 equiv) was added to a solution of the protected alcohol (2.06 g, 6.55 mmol, 1 equiv) in EtOAc (50 mL). The reaction mixture was heated to reflux for 5 h, filtered through a plug of celite, and then concentrated under reduced pressure to afford a colorless oil (2.01 g, 98%). M.p. 41 °C; R_f = 0.25 (PE/EtOAc 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.04 (s, 9H), 2.61 (dt, $^3J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 2.2 Hz, 2H), 4.02 (t, $^3J_{H,H}$ = 6.0 Hz, 2H), 7.37–7.44 (m, 6H), 7.64–7.68 (m, 4H), 9.82 ppm (t, $^3J_{H,H}$ = 2.2 Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 19.2, 26.8, 46.5, 58.4, 127.9, 129.9, 133.3, 135.6, 202.0 ppm; IR (neat): $\tilde{\nu}$ = 3437, 3071, 3050, 2959, 2932, 2858, 1728, 1428, 1112, 703, 506 cm^{-1}; MS: *m*/*z*: 256.00 [*M*−*t*Bu]$^+$; elemental analysis calcd (%) for $C_{19}H_{26}O_2Si$: C 73.03, H 7.74; found: C 72.98, H 7.64.

Fluorinated acrylates (*Z*)-4 and (*E*)-4 (Scheme 3, step c): Diethylzinc (137 mL of a 1 M solution in hexane, 0.137 mol, 4 equiv) was added rapidly to a solution of triphenylphosphine (36 g, 0.137 mol, 4 equiv) and ethyl dibromofluoroacetate (9.63 mL, 0.068 mol, 2 equiv) in dry THF (350 mL). The reaction mixture was stirred for 10 min (until the internal temperature had returned to ambient temperature), and the aldehyde (2.04 g, 6.49 mmol, 1 equiv) dissolved in THF (20 mL) was added rapidly. After 45 min, the mixture was quenched with ethanol, stirred for 15 min, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (EtOAc in PE, 1%) and the two diastereoisomers were separated to afford (*Z*)-**4** and (*E*)-**4** as two colorless oils (2.09 g, 80%).

*Isomer (Z)-**4***: Ethyl (*Z*)-5-{[*tert*-butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enoate. $R_{f(Z)}=0.27$ (cyclohexane/EtOAc 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.06$ (s, 9H), 1.33 (t, $^3J_{H,H}=7.0$ Hz, 3H), 2.49 (m, 2H), 3.74 (t, $^3J_{H,H}=6.4$ Hz, 2H), 4.29 (q, $^3J_{H,H}=7.0$ Hz, 2H), 6.23 (dt, $^3J_{H,H}=7.5$ Hz, $^3J_{H,F}=33.5$ Hz, 1H), 7.32–7.44 (m, 6H), 7.65–7.68 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=14.2$, 19.3, 26.9, 27.9 (d, $^3J_{C,F}=2.2$ Hz), 61.6, 62.1 (d, $^4J_{C,F}=2.2$ Hz), 117.6 (d, $^2J_{C,F}=11.0$ Hz), 127.8, 129.8, 133.6, 135.6, 148.8 (d, $^1J_{C,F}=256.1$ Hz), 160.8 ppm (d, $^2J_{C,F}=35.6$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=129.8$ ppm (d, $^3J_{F,H}=33.5$ Hz); IR (neat): $\tilde{\nu}=3071$, 2960, 2931, 2858, 1730, 1669, 1428, 1375, 1325, 1216, 1119, 938, 823, 702, 613, 505 cm^{-1}; MS: *m/z*: 423.18 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{29}FO_3Si$: C 68.97, H 7.30; found: C 68.80, H 7.29.

*Isomer (E)-**4***: Ethyl (*E*)-5-{[*tert*-butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enoate. $R_{f(E)}=0.30$ (cyclohexane/EtOAc 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.05$ (s, 9H), 1.33 (t, $^3J_{H,H}=7.1$ Hz, 3H), 2.49 (m, 2H), 3.75 (t, $^3J_{H,H}=6.2$ Hz, 2H), 4.28 (q, $^3J_{H,H}=7.1$ Hz, 2H), 6.16 (dt, $^3J_{H,H}=7.7$ Hz, $^3J_{H,F}=21.5$ Hz, 1H), 7.33–7.44 (m, 6H), 7.64–7.67 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=14.2$, 19.3, 26.9, 29.1 (d, $^3J_{C,F}=4.9$ Hz), 61.4, 62.8, 120.6 (d, $^2J_{C,F}=19.2$ Hz), 127.8, 129.8, 133.6, 135.6, 147.8 (d, $^1J_{C,F}=251.7$ Hz), 161.0 ppm (d, $^2J_{C,F}=36.2$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=121.5$ ppm (d, $^3J_{F,H}=21.5$ Hz); IR (neat): $\tilde{\nu}=3072$, 2931, 2858, 1732, 1427, 1375, 1325, 1217, 1111 cm^{-1}; MS: *m/z*: 423.18 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{29}FO_3Si$: C 68.97, H 7.30; found: C 68.83, H 7.31.

(*E*/*Z*)-5-{[*tert*-Butyl(diphenyl)silyl]oxy}-2-fluoropent-2-en-1-ol (Scheme 3, step d): $LiAlH_4$ (705 mg, 18.57 mmol, 1.1 equiv) was added at 0 °C to a mixture of diastereoisomers (*Z*/*E*)-**4** (6.76 g, 16.88 mmol, 1 equiv) in dry THF (150 mL). The reaction mixture was stirred for 35 min and then slowly quenched with H_2SO_4 (5%) and concentrated. It was then extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over $MgSO_4$, filtered, and then concentrated under reduced pressure to afford the alcohols as a colorless oil (5.26 g, 87%). $R_{f(E/Z)}=0.20$ (PE/EtOAc 90:10).

Z isomer: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.06$ (s, 9H), 2.37 (m, 2H), 3.69 (t, $^3J_{H,H}=6.4$ Hz, 2H), 4.08 (dd, $^3J_{H,OH}=6.4$ Hz, $^3J_{H,F}=15.6$ Hz, 2H), 4.90 (dt, $^3J_{H,H}=7.3$ Hz, $^3J_{H,F}=37.1$ Hz, 1H), 7.36–7.43 (m, 6H), 7.65–7.66 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.3$, 26.9, 27.1 (d, $^3J_{C,F}=3.8$ Hz), 61.3 (d, $^2J_{C,F}=32.3$ Hz), 63.1 (d, $^4J_{C,F}=1.6$ Hz), 104.6 (d, $^2J_{C,F}=13.7$ Hz), 127.7, 129.7, 133.9, 135.7, 158.5 ppm (d, $^1J_{C,F}=254.4$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=120.1$ ppm (dt, $^3J_{F,H}=15.6$ Hz, $^3J_{F,H}=37.1$ Hz); IR (neat): $\tilde{\nu}=3356$, 3072, 2931, 2858, 1714, 1589, 1471, 1427, 1390, 1111, 1020 cm^{-1}; MS: *m/z*: 359.19 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{27}FO_2Si$: C 70.35, H 7.59; found: C 70.06, H 7.39.

E *isomer*: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.06$ (s, 9H), 2.27 (m, 2H), 3.64 (t, $^3J_{H,H}=6.4$ Hz, 2H), 4.18 (dd, $^3J_{H,OH}=6.4$ Hz, $^3J_{H,F}=19.9$ Hz, 2H), 5.23 (dt, $^3J_{H,H}=8.3$ Hz, $^3J_{H,F}=20.7$ Hz, 1H), 7.36–7.43 (m, 6H), 7.65–7.66 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.2$, 26.9, 25.5 (d, $^3J_{C,F}=8.7$ Hz), 57.6 (d, $^2J_{C,F}=31.8$ Hz), 63.3 (d, $^4J_{C,F}=3.3$ Hz), 105.6 (d, $^2J_{C,F}=21.4$ Hz), 127.8, 129.9, 133.4, 135.7, 159.4 ppm (d, $^1J_{C,F}=249.0$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=111.5$ ppm (q); IR (neat): $\tilde{\nu}=3356$, 3072, 2931, 2858, 1714, 1589, 1471, 1427, 1390, 1111, 1020 cm^{-1}; MS: *m/z*: 359.19 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{27}FO_2Si$: C 70.35, H 7.59; found: C 70.21, H 7.43.

(*E*/*Z*)-5-{[*tert*-Butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enal (Scheme 3, step e): IBX (7.23 g, 25.83 mmol, 3 equiv) was added to a solution of the above mixture of alcohols (3.08 g, 8.61 mmol, 1 equiv) in EtOAc (60 mL). The reaction mixture was heated to reflux for 6 h, filtered through a plug of celite, and then concentrated under reduced pressure to afford the desire aldehyde (2.95 g, 97%) as a yellow oil. $R_{f(E/Z)}=0.20$ (cyclohexane/EtOAc 90:10)

Z isomer: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.06$ (s, 9H), 2.59 (m, 2H), 3.82 (t, $^3J_{H,H}=6.2$ Hz, 2H), 6.02 (dt, $^3J_{H,H}=7.6$ Hz, $^3J_{H,F}=32.5$ Hz, 1H), 7.37–7.47 (m, 6H), 7.63–7.67 (m, 4H), 9.18 ppm (d, $^3J_{H,F}=18.4$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=18.2$, 25.8, 27.2 (d, $^3J_{C,F}=2.3$ Hz), 60.7, 105.6 (d, $^2J_{C,F}=21.2$ Hz), 126.7, 128.8, 132.8, 134.5, 155.9 (d, $^1J_{C,F}=262.0$ Hz), 182.5 ppm (d, $^2J_{C,F}=25.2$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=132.9$ ppm (dd, $^3J_{F,H}=33.0$ Hz, $^3J_{F,H}=18.4$ Hz); IR (neat): $\tilde{\nu}=2957$, 2930, 2857, 1703, 1472, 1428, 1361, 1112, 938, 702, 613, 506 cm^{-1}; MS: *m/z*: 299.1 $[M-t\text{Bu}]^+$; elemental analysis calcd (%) for $C_{21}H_{25}FO_2Si$: C 70.75, H 7.07; found: C 70.59, H 7.16.

E *isomer*: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.05$ (s, 9H), 2.68 (m, 2H), 3.78 (t, $^3J_{H,H}=6.0$ Hz, 2H), 6.24 (q, $^3J_{H,H}=8.8$ Hz, $^3J_{H,F}=17.9$ Hz, 1H), 7.37–7.47 (m, 6H), 7.63–7.67 (m, 4H), 9.68 ppm (d, $^3J_{H,F}=16.6$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=18.2$, 25.8, 27.2 (d, $^3J_{C,F}=2.3$ Hz), 60.7, 105.6 (d, $^2J_{C,F}=21.2$ Hz), 126.7, 128.8, 132.8, 134.5, 155.9 (d, $^1J_{C,F}=262.0$ Hz), 181.0 ppm (d, $^2J_{C,F}=25.2$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=126.6$ ppm (t); IR (neat): $\tilde{\nu}=2957$, 2930, 2857, 1703, 1472, 1428, 1361, 1112, 938, 702, 613, 506 cm^{-1}; MS: *m/z*: 299.1 $[M-t\text{Bu}]^+$; elemental analysis calcd (%) for $C_{21}H_{25}FO_2Si$: C 70.75, H 7.07; found: C 70.56, H 7.13.

***N*-(5-{[*tert*-Butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enylidene)-2-methyl-2-propanesulfinamide—fluorinated sulfinylimines (*Z*)-5 and (*E*)-5 (Scheme 3, step f)**: A solution of $Ti(OEt)_4$ (2.14 mL, 10.2 mmol, 2.5 equiv) and the *Z*/*E* mixture of aldehydes (1.45 g, 4.08 mmol, 1 equiv) in dry THF (60 mL) was prepared under argon. *tert*-Butylsulfinylamine (1.23 g, 10.2 mmol, 2.5 equiv) was then added, and the mixture was heated to reflux for 1 h 15 min. Once cooled, the mixture was poured into an equal volume of brine with fast stirring. The resulting suspension was filtered through a plug of celite, and the filter cake was washed with EtOAc. The brine layer was extracted once with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by chromatography on silica gel (PE/EtOAc 95:5→62:38) to afford both isomers of **5** as a yellow oil (1.67 g, 89%).

Z isomer: $R_{f(Z)}=0.21$ (cyclohexane/EtOAc 90:10); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.05$ (s, 9H), 1.23 (s, 9H), 2.57 (m, 2H), 3.78 (t, $^3J_{H,H}=5.6$ Hz, 2H), 5.73 (dt, $^3J_{H,H}=7.5$ Hz, $^3J_{H,F}=33.2$ Hz, 1H), 7.35–7.46 (m, 6H), 7.63–7.66 (m, 4H), 7.95 ppm (d, $^3J_{H,F}=19.6$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.3$, 22.5, 26.8, 28.4 (d, $^3J_{C,F}=2.2$ Hz), 58.0, 62.1 (d, $^4J_{C,F}=1.6$ Hz), 123.3 (d, $^2J_{C,F}=13.2$ Hz), 127.8, 129.9, 133.5, 135.6, 155.1 (d, $^2J_{C,F}=21.4$ Hz), 155.7 ppm (d, $^1J_{C,F}=254.4$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=126.6$ ppm (dd, $^3J_{F,H}=19.6$ Hz, $^3J_{F,H}=33.2$ Hz); IR (neat): $\tilde{\nu}=2959$, 2858, 1664, 1592, 1473, 1428, 1363, 1186, 1111, 1088, 823, 702, 613, 504 cm^{-1}; MS: *m/z*: 460.33

$[M+H]^+$; elemental analysis calcd (%) for $C_{25}H_{34}FNO_2SSi$: C 65.32, H 7.45, N 3.05, S 6.98; found: C 65.36, H 7.47, N 3.03, S 6.94.

E *isomer*: $R_{f(E)}=0.23$ (cyclohexane/EtOAc 90:10). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.04$ (s, 9H), 1.24 (s, 9H), 2.60 (m, 2H), 3.74 (t, $^3J_{H,H}=6.4$ Hz, 2H), 6.01 (dt, $^3J_{H,H}=8.5$ Hz, $^3J_{H,F}=18.5$ Hz, 1H), 7.36–7.44 (m, 6H), 7.63–7.65 (m, 4H), 8.37 ppm (d, $^3J_{H,F}=19.9$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.2$, 22.5, 26.8, 28.6 (d, $^3J_{C,F}=6.6$ Hz), 58.0, 62.4 (d, $^4J_{C,F}=2.7$ Hz), 120.7 (d, $^2J_{C,F}=20.3$ Hz), 127.8, 129.8, 133.2, 135.5, 151.7 (d, $^2J_{C,F}=21.9$ Hz), 154.6 ppm (d, $^1J_{C,F}=246.8$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=120.1$ ppm (t); IR (neat): $\tilde{\nu}=2932$, 2959, 2858, 1664, 1592, 1473, 1428, 1363, 1186, 1111.3, 1088, 823, 702, 613, 504 cm^{-1}; MS: *m/z*: 460.33 $[M+H]$; elemental analysis calcd (%) for $C_{25}H_{34}FNO_2SSi$: C 65.32, H 7.45, N 3.05, S 6.98; found: C 65.35, H 7.44, N 3.02, 6.92.

***N*-((*Z*)-5-{[*tert*-Butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enyl)-2-methyl-2-propanesulfinamide (Scheme 3, step g)**: $NaBH_4$ (286.4 mg, 7.57 mmol, 1.5 equiv) was added at 0 °C to a solution of imine (*Z*)-**5** (2.32 g, 5.05 mmol, 1 equiv) in dry THF (60 mL). The reaction mixture was stirred for 2 h 30 min and then slowly quenched with a saturated aqueous solution of NH_4Cl. It was extracted with EtOAc (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude mixture was purified by chromatography on silica gel (PE/EtOAc 60:40) to afford (*Z*)-**6** as a colorless oil (2.12 g, 91%). $R_f=0.25$ (PE/AcOEt 70:30); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.05$ (s, 9H), 1.21 (s, 9H), 2.36 (m, 2H), 3.46 (t, $^3J_{NH,H}=6.4$ Hz, 1H), 3.68 (t, $^3J_{H,H}=6.4$ Hz, 2H), 3.72–3.88 (m, 2H), 4.90 (dt, $^3J_{H,H}=7.3$ Hz, $^3J_{H,F}=36.5$ Hz, 1H), 7.34–7.45 (m, 6H), 7.62–7.65 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.2$, 22.5, 26.8, 27.1 (d, $^3J_{C,F}=3.8$ Hz), 46.2 (d, $^2J_{C,F}=31.2$ Hz), 56.1, 62.9 (d, $^4J_{C,F}=1.6$ Hz), 105.2 (d, $^2J_{C,F}=13.2$ Hz), 127.7, 129.7, 133.8, 135.5, 156.5 ppm (d, $^1J_{C,F}=255.0$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=116.3$ ppm (dt, $^3J_{F,H}=14.4$ Hz, $^3J_{F,H}=36.5$ Hz); IR (neat): $\tilde{\nu}=3201$, 2958, 2930, 2858, 1712, 1473, 1428, 1390, 1363, 1117, 1058, 823, 738, 702, 613, 505 cm^{-1}; MS: *m/z*: 462.13 $[M+H]^+$; elemental analysis calcd (%) for $C_{25}H_{36}FNO_2SSi$: C 65.03, H 7.86, N 3.03, S 6.94; found: C 65.30, H 7.41, N 3.02, 6.91.

(*Z*)-2-Fluoro-5-hydroxypent-2-en-1-aminium chloride [(*Z*)-6, Scheme 3, step h]: HCl in dioxane (4 M, 1.9 mL, 7.49 mmol 2 equiv) was added to a solution of the above (*Z*)-fluoro *tert*-butylsulfinamide (1.73 g, 3.74 mmol, 1 equiv) in dry MeOH (18 mL). The mixture was stirred at room temperature for 50 min and then concentrated under reduced pressure to near dryness. The crude mixture was used in the next step without further purification.

***N*-((*E*)-5-{[*tert*-Butyl(diphenyl)silyl]oxy}-2-fluoropent-2-enyl)-2-methyl-2-propanesulfinamide (Scheme 3, step g)**: $NaBH_4$ (118.9 mg, 3.14 mmol, 1.5 equiv) was added at 0 °C to a solution of imine (*E*)-**5** (963.8 mg, 2.096 mmol, 1 equiv) in dry THF (62 mL). The reaction mixture was stirred for 2 h and then slowly quenched with a saturated aqueous solution of NH_4Cl. It was then extracted with EtOAc (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude mixture was purified by chromatography on silica gel (PE/EtOAc 65:35) to afford the title compound as a colorless oil (811.6 mg, 84%). $R_f=0.32$ (PE/AcOEt 70:30); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.04$ (s, 9H), 1.19 (s, 9H), 2.25 (m, 2H), 3.33 (t, $^3J_{NH,H}=5.7$ Hz, 1H), 3.64 (t, $^3J_{H,H}=6.4$ Hz, 2H), 3.70–3.91 (m, 2H), 5.22 (dt, $^3J_{H,H}=8.3$ Hz, $^3J_{H,F}=20.3$ Hz, 1H), 7.35–7.43 (m, 6H), 7.63–7.66 ppm (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=19.2$, 22.5, 26.8, 28.8 (d, $^3J_{C,F}=8.2$ Hz), 42.0 (d, $^2J_{C,F}=29.1$ Hz), 56.1, 63.2 (d, $^4J_{C,F}=2.7$ Hz), 106.1 (d, $^2J_{C,F}=20.8$ Hz), 127.7, 129.7, 133.5, 135.5, 157.0 ppm (d, $^1J_{C,F}=248.4$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=109.5$ ppm (q); IR (neat): $\tilde{\nu}=3201$, 3071, 2957, 2930, 2858, 1702, 1473, 1427, 1390, 1363, 1155, 1117, 823, 738, 702, 614, 505 cm^{-1}; MS: *m/z*: 461.93 $[M]^+$; elemental analysis calcd (%) for $C_{25}H_{36}FNO_2SSi$: C 65.03, H 7.86, N 3.03, S 6.94; found: C 65.29, H 7.38, N 3.04, S 6.92.

(*E*)-2-Fluoro-5-hydroxypent-2-en-1-aminium chloride [(*E*)-6, Scheme 3, step h]: HCl in dioxane (4 M, 750 μL, 3.00 mmol, 2 equiv) was added to a solution of the above (*E*)-fluoro *tert*-butylsulfinamide (693 mg, 1.50 mmol, 1 equiv) in dry MeOH (7 mL). The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to near dryness. The crude mixture was used in the next step without further purification.

9*H*-Fluoren-9-ylmethyl (*Z*)-2-fluoro-5-hydroxypent-2-enylcarbamate (Scheme 3, step i): $NaHCO_3$ (944 mg, 11.24 mmol, 3 equiv) was added at 0 °C to a solution of amine hydrochloride derivative (*Z*)-**6** (583 mg, 3.74 mmol, 1 equiv) in dioxane (4 mL mmol^{-1} of amine hydrochloride) and water (4 mL mmol^{-1} of amine hydrochloride), followed by Fmoc-OSu (1.51 g, 4.49 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 2 h 30 min and was then poured into ice-cooled HCl (1 N, 8 mL mmol^{-1} of amine hydrochloride) and extracted with AcOEt (3×). The combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (PE/EtOAc 70:30→45:55), to afford a white solid (920 mg, 72%). $R_f=0.22$ (PE/AcOEt 50:50); m.p. 112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=2.35$ (m, 2H), 3.64 (t, $^3J_{H,H}=6.2$ Hz, 2H), 3.88 (dd, $^3J_{H,F}=14.3$ Hz, $^3J_{H,NH}=5.8$ Hz, 2H), 4.21 (t, $^3J_{H,H}=6.8$ Hz, 2H), 4.43 (d, $^3J_{H,H}=7.0$ Hz, 2H), 4.89 (dt, $^3J_{H,H}=7.5$ Hz, $^3J_{H,F}=36.5$ Hz, 1H), 5.09 (br t, 1H), 7.29–7.34 (m, 2H), 7.38–7.43 (m, 2H), 7.67 (d, $^3J_{H,H}=7.3$ Hz, 2H), 7.76 ppm (d, $^3J_{H,H}=7.3$ Hz, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=27.11$ (d, $^3J_{C,F}=3.8$ Hz), 41.7 (d, $^2J_{C,F}=32.9$ Hz), 47.1, 61.5 (d, $^4J_{C,F}=2.2$ Hz), 66.8, 104.0 (d, $^2J_{C,F}=13.7$ Hz), 120.0, 125.0, 127.1, 127.7, 141.3, 143.8, 156.5, 156.6 ppm (d, $^1J_{C,F}=255.0$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=116.5$ ppm (dt, $^3J_{F,H}=14.5$ Hz, $^3J_{F,H}=36.1$ Hz); IR (neat): $\tilde{\nu}=3339$, 2952, 1702, 1676, 1542, 1450, 1307, 1273, 1044, 983, 757, 734, 642, 620 cm^{-1}; MS: *m/z*: 359.13 $[M+H_2O]$; elemental analysis calcd (%) for $C_{20}H_{20}FNO_3$: C 70.37, H 5.91, N 4.10; found: C 70.49, H 6.01, N 4.12.

Dipeptide analogue Fmoc-GlyΨ[CF=CH]Gly-OH, (*Z*)-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-fluoropent-3-enoic acid [(*Z*)-1, Scheme 3, step j]: Jones' reagent (2.74 N, 3 equiv) was added at 0 °C to a solution of the above (*Z*)-N-protected amino alcohol (497.1 mg, 1.45 mmol, 1 equiv) in acetone (10 mL mmol^{-1} of alcohol). The reaction mixture was stirred at 0 °C for 1 h, after which isopropyl alcohol (10 equiv) and water (13 mL mmol^{-1} of alcohol) were added. The mixture was extracted with AcOEt (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (PE/EtOAc 60:40→30:70 with 0.1% of acetic acid) to afford (*Z*)-**1** as a white solid (360.7 mg, 70%). M.p. 144 °C; ^{1}H NMR (300 MHz, $(CD_3)_2CO$): $\delta=3.13$ (d, $^3J_{H,H}=7.0$ Hz, 2H), 3.92 (dd, $^3J_{H,F}=12.4$ Hz, $^3J_{H,NH}=6.0$ Hz, 2H), 4.23 (t, $^3J_{H,H}=7.0$ Hz, 1H), 4.36 (d, $^3J_{H,H}=7.0$ Hz, 2H), 5.07 (dt, $^3J_{H,H}=7.1$ Hz, $^3J_{H,F}=36.5$ Hz, 1H), 5.09 (br t, 1H), 7.30–7.35 (m, 2H), 7.39–7.44 (m, 2H), 7.71 (d, $^3J_{H,H}=7.3$ Hz, 2H), 7.66 ppm (d, $^3J_{H,H}=7.3$ Hz, 2H); ^{13}C NMR (75.4 MHz, $(CD_3)_2CO$): $\delta=29.2$ (d, $^3J_{C,F}=5.5$ Hz), 41.7 (d, $^2J_{C,F}=32.9$ Hz), 47.9, 67.1, 100.3 (d, $^2J_{C,F}=12.1$ Hz), 120.7, 126.0, 127.9, 128.5, 142.1, 145.0, 157.1, 158.5 (d, $^1J_{C,F}=256.6$ Hz), 172.1 ppm (d, $^4J_{C,F}=1.6$ Hz); ^{19}F NMR (282.5 MHz, $(CD_3)_2CO$): $\delta=114.6$ ppm (dt,

$^3J_{F,H}=12.4$ Hz, $^3J_{F,H}=37.1$ Hz); IR (neat): $\tilde{\nu}=3330$, 3018, 1692, 1542, 1451, 1318, 1265, 1224, 1142, 1048, 963, 758, 735 cm^{-1}; MS: *m/z*: 378.13 [*M*+H_2O]; elemental analysis calcd (%) for $C_{20}H_{18}FNO_4$: C 67.60, H 5.11, N 3.94; found: C 67.50, H 5.02, N 3.91.

9*H*-Fluoren-9-ylmethyl (*E*)-2-fluoro-5-hydroxypent-2-enylcarbamate (Scheme 3, step i): $NaHCO_3$ (378.4 mg, 3.0 mmol, 3 equiv) was added at 0 °C to a solution of amine hydrochloride derivative (*E*)-**6** (233.6 mg, 1.50 mmol, 1 equiv) in dioxane (4 mL $mmol^{-1}$ of amine hydrochloride) and water (4 mL $mmol^{-1}$ of amine hydrochloride), followed by Fmoc-OSu (607.8 mg, 1.80 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 2 h 30 min and was then poured into ice-cooled HCl (1 N, 8 mL $mmol^{-1}$ of amine hydrochloride) and extracted with AcOEt (3×). The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (PE/EtOAc 75:25→55:45), affording the product as a white solid (359.5 mg, 70%). R_f= 0.31 (PE/AcOEt 50:50); m.p. 92 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.30 (m, 2H), 3.62 (t, $^3J_{H,H}=6.2$ Hz, 2H), 3.92 (dd, $^3J_{H,F}=20.7$ Hz, $^3J_{H,NH}=6.0$ Hz, 2H), 4.19 (t, $^3J_{H,H}=6.8$ Hz, 2H), 4.43 (d, $^3J_{H,H}=7.0$ Hz, 2H), 5.20 (dt, $^3J_{H,H}=8.3$ Hz, $^3J_{H,F}=20.5$ Hz, 1H), 5.57 (br t, 1H), 7.27–7.33 (m, 2H), 7.37–7.42 (m, 2H), 7.57 (d, $^3J_{H,H}=7.3$ Hz, 2H), 7.75 ppm (d, $^3J_{H,H}=7.5$ Hz, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ= 28.75 (d, $^3J_{C,F}=8.2$ Hz), 38.0 (d, $^2J_{C,F}=29.1$ Hz), 47.1, 61.5 (d, $^4J_{C,F}=$ 2.7 Hz), 67.1, 106.0 (d, $^2J_{C,F}=20.8$ Hz), 120.0, 125.1, 127.1, 127.8, 141.3, 143.8, 156.7, 156.9 ppm (d, $^1J_{C,F}=248.4$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta=109.5$ ppm (q); IR (neat): $\tilde{\nu}=3338$, 2951, 1697, 1675, 1536, 1451, 1282, 1255, 1142, 1047, 985, 757, 732, 620 cm^{-1}; MS: *m/z*: 359.00 [*M*+H_2O]; elemental analysis calcd (%) for $C_{20}H_{20}FNO_3$: C 70.37, H 5.91, N 4.10; found: C 70.47, H 5.99, N 4.12.

Dipeptide analogue Fmoc-GlyΨ[CF=CH]Gly-OH, (*E*)-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-fluoropent-3-enoic acid [(*E*)-1, Scheme 3, step j]: Jones' reagent (2.74 N, 3 equiv) was added at 0 °C to a solution of the (*E*)-N-protected amino alcohol (240.2 mg, 0.703 mmol, 1 equiv) in acetone (10 mL $mmol^{-1}$ of alcohol). The reaction mixture was stirred at 0 °C for 2 h 30 min and was then quenched with isopropyl alcohol (10 equiv) and water (13 mL $mmol^{-1}$ of alcohol). The mixture was extracted with AcOEt (3×), and the combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (PE/EtOAc 70:30→50:50 with 0.1% of acetic acid) to afford (*E*)-**1** as a white solid (124.9 mg, 50%). M.p. 124 °C; ^{1}H NMR (300 MHz, $(CD_3)_2CO$): δ= 3.08 (m, 2H), 3.83 (m, 2H), 4.02–4.10 (m, 1H), 4.36 (m, 2H), 4.83–4.95 (m, 1H), 5.23 (br t, 1H), 7.20–7.29 (m, 4H), 7.46–7.47 (m, 2H), 7.64–7.66 ppm (m, 2H); ^{13}C NMR (75.4 MHz, $(CD_3)_2CO$): δ= 29.3 (d, $^3J_{C,F}=5.5$ Hz), 38.1 (d, $^2J_{C,F}=29.1$ Hz), 47.1, 67.2, 101.5 (d, $^2J_{C,F}=25.8$ Hz), 120.1, 125.1, 127.2, 127.8, 141.3, 143.7, 156.6, 158.7 (d, $^1J_{C,F}=254.2$ Hz), 176.1 ppm (d, $^4J_{C,F}=1.6$ Hz); ^{19}F NMR (282.5 MHz, $(CD_3)_2CO$): $\delta=107.4$ ppm (q); IR (neat): $\tilde{\nu}=$ 3330, 3018, 1692, 1542, 1451, 1318, 1265, 1224, 1142, 1048, 963, 758, 735 cm^{-1}; MS: *m/z*: 378.13 [*M*+H_2O]; elemental analysis calcd (%) for $C_{20}H_{20}FNO_3$: C 67.60, H 5.11, N 3.94; found: C 67.35, H 4.78, N 3.90.

(4*R*,5*S*)-4-Methyl-5-phenyl-3-(3-phenylpropanoyl)-1,3-oxazolidin-2-one (8, Scheme 4, step a): A solution of *n*BuLi (9.2 mL of a 1.6 M solution in hexane, 14.81 mmol, 1.05 equiv) was added dropwise to a solution of (4*R*,5*S*)-(+)-4-methyl-5-phenyloxazolidin-2-one (2.5 g, 14.10 mmol, 1 equiv) in dry THF (23 mL). After 45 min, hydrocinnamoyl chloride **7** (2.30 mL, 15.51 mmol, 1.1 equiv) in dry THF (3 mL) was added dropwise at −78 °C. The reaction mixture was stirred at this temperature for 2 h, then allowed to warm to room temperature, and stirred for 1 h 30 min. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$, and the resulting mixture was concentrated under reduced pressure. The residue was extracted with Et_2O (2×), and the combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure to afford **8** as white needles (4.19 g, 96%). R_f= 0.18 (cyclohexane/EtOAc 90:10); m.p. 95 °C; $[\alpha]_D^{20}=+29.8$ ($c=0.7$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.88$ (d, $^3J_{H,H}=6.6$ Hz, 3H), 3.02 (m, 2H), 3.30 (m, 2H), 4.75 (m, 1H), 5.63 (d, $^3J_{H,H}=7.3$ Hz, 1H), 7.18–7.45 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=14.6$, 30.3, 37.3, 54.8, 79.0, 125.6, 126.3, 128.5, 128.6, 128.7, 128.8, 133.3, 140.5, 153.1, 172.2 ppm; IR (neat): $\tilde{\nu}=2924$, 1776, 1698, 1452, 1402, 1373, 1359, 1300, 1122, 988, 960, 767, 753, 700, 521 cm^{-1}; MS: *m/z*: 310.19 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{19}H_{19}NO_3$: C 73.77, H 6.19, N 4.53; found: C 73.97, H 6.14, N 4.48.

(4*R*,5*S*)-3-[(2*S*)-2-Benzyl-3-(benzyloxy)propanoyl]-4-methyl-5-phenyl-1,3-oxazolidin-2-one (9, Scheme 4, step b): $TiCl_4$ (1.56 mL, 14.22 mmol, 1.05 equiv) was added at 0 °C to a solution of **8** (4.19 g, 13.54 mmol, 1 equiv) in dry CH_2Cl_2 (40 mL). After the reaction mixture had been stirred for 5 min, diisopropylethylamine (2.23 mL, 13.54 mmol, 1 equiv) was added, and the mixture was stirred for 1 h. Benzyl chloromethyl ether (3.76 mL, 27.08 mmol, 2 equiv) was added, and the reaction mixture was stirred at 0 °C for a further 5 h and then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. The yellow oil was purified by column chromatography on silica gel (PE/EtOAc 90:10→88:12) to afford **9** as a colorless oil (4.56 g, 88%). R_f=0.19 (cyclohexane/EtOAc 90:10); $[\alpha]_D^{20}=-17.6$ ($c=0.81$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.81$ (d, $^3J_{H8,H9}=6.6$ Hz, 3H, H_8), 2.94 (m, 2H), 3.73 (dd, $^2J_{H,H'}=4.1$ Hz, $^3J_{H,H}=7.3$ Hz, 1H), 3.73 (dd, $^2J_{H,H}=4.1$ Hz, $^3J_{H,H}=$ 7.3 Hz, 1H), 4.52 (s, 2H), 4.59 (m, 2H), 5.25 (d, $^3J_{H,H}=7.1$ Hz, 1H), 7.19–7.42 ppm (m, 15H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=14.5$, 35.4, 45.2, 54.9, 70.6, 73.1, 78.7, 125.6, 126.6, 127.6, 127.6, 128.3, 128.5, 128.6, 128.7, 129.2, 133.3, 138.2, 138.6, 152.7, 173.9 ppm; IR (neat): $\tilde{\nu}=3027$, 2965, 2880, 1763, 1712, 1490, 1454, 1353, 1248, 1186, 1099, 959, 751, 700, 670, 512 cm^{-1}; MS: *m/z*: 429.87 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{27}H_{27}NO_4$: C 69.65, H 7.67, N 2.54; found: C 69.47, H 7.85, N 2.52.

(2*R*)-2-Benzyl-3-(benzyloxy)propan-1-ol (Scheme 4, step c): $LiAlH_4$ (402 mg, 10.61 mmol, 1 equiv) was added at 0 °C to a solution of **9** (4.56 g, 10.61 mmol, 1 equiv) in dry THF (45 mL), and the reaction mixture was stirred for 2 h. The reaction mixture was then quenched with H_2SO_4 (5%) and concentrated. The reaction mixture was extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 86:14→80:20) to afford the title alcohol as a colorless oil (1.90 g, 70%). R_f=0.28 (PE/EtOAc 80:20); $[\alpha]_D^{20}=+25.1$ ($c=0.69$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=2.06$ (m, 1H), 2.49 (br s, 1H, OH), 2.58 (m, 2H), 3.45 (dd, $^2J_{H,H'}=4.1$ Hz, $^3J_{H,H}=9.6$ Hz, 1H), 3.55 (dd, $^2J_{H,H}=4.1$ Hz, $^3J_{H,H}=9.6$ Hz, 1H), 3.63 (m, 2H), 4.40 (d, $^2J_{H,H}=$ 4.1 Hz, 2H), 7.07–7.30 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta=34.6$, 42.7, 65.5, 73.0, 73.6, 126.2, 127.8, 127.9, 128.5, 128.6, 129.2, 138.1, 140.1 ppm; IR (neat): $\tilde{\nu}=3412$, 3027, 2863, 1603, 1495, 1453, 1364, 1206, 1088, 1029, 741, 699 cm^{-1}; MS: *m/z*: 257.00 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{17}H_{20}O_2$: C 78.65, H 7.86; found: C 78.81, H 7.92.

(2*S*)-2-Benzyl-3-(benzyloxy)propanal (10, Scheme 4, step d): Et_3N (3.03 mL, 21.86 mmol, 3 equiv), DMSO (10.8 mL, 152.6 mmol, 21 equiv), and $PySO_3$ (3.46 g, 21.80 mmol, 3 equiv) were added at 0 °C to a solution of the above alcohol (1.86 g, 7.26 mmol, 1 equiv) in dry CH_2Cl_2 (40 mL). The reaction mixture was stirred at this temperature for 1 h 15 min and was then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 96:4→90:10) to afford **10** as a colorless oil (1.46 g, 79%). $R_f = 0.37$ (PE/EtOAc 90:10); $[\alpha]_D^{20} = +6.7$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 2.77–2.88 (m, 2H), 3.09 (m, 1H), 3.58–3.72 (m, 2H), 4.49 (s, 2H), 7.14–7.39 (m, 10H), 9.80 ppm (d, $^3J_{H,H} = 1.1$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 31.7$, 53.4, 67.5, 73.4, 126.5, 127.8, 127.9, 128.5, 128.7, 129.2, 138.0, 138.8, 203.2 ppm; IR (neat): $\tilde{\nu} = 3029$, 2926, 2827, 1706, 1495, 1454, 1363, 1207, 1113, 1028, 740, 698 cm^{-1}; MS: *m/z*: 272.07 [*M*+H_2O]; elemental analysis calcd (%) for $C_{17}H_{18}O_2$: C 80.28, H 7.13; found: C 80.42, H 7.29.

Compounds (*E*)- and (*Z*)-11 (Scheme 4, step e): Diethylzinc (28.5 mL of a 1 M solution in hexane, 28.5 mmol, 4 equiv) was added rapidly to a solution of triphenylphosphine (7.48 g, 28.47 mmol, 4 equiv) and ethyl dibromofluoroacetate (1.98 mL, 14.23 mmol, 2 equiv) in dry THF (70 mL). The reaction mixture was stirred for 10 min (until the internal temperature had returned to ambient temperature), and aldehyde **10**, dissolved in THF (20 mL), was added rapidly. After 45 min, the mixture was quenched with ethanol, stirred for 15 min, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 90:10) to afford (*E*)-**11** (826.2 mg, 34%) and (*Z*)-**11** (1.34 g, 55%), both as colorless oils.

*Ethyl (2*E*,4*R*)-4-benzyl-5-(benzyloxy)-2-fluoropent-2-enoate [(*E*)-**11**]*: $R_f = 0.20$ (PE/EtOAc 96:4); $[\alpha]_D^{20} = -15.2$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.28$ (t, $^3J_{H,H} = 7.2$ Hz, 3H), 2.59 (dd, $^2J_{H,H} =$ 7.2 Hz, $^3J_{H,H} = 13.4$ Hz, 1H), 2.75 (dd, $^2J_{H,H} = 7.2$ Hz, $^3J_{H,H} = 13.4$ Hz, 1H), 3.29 (m, 2H), 3.63 (m, 1H), 4.12 (q, $^3J_{H,H} = 7.2$ Hz, 2H), 4.38 (d, $^2J_{H,H} = 2.2$ Hz, 2H), 5.82 (dd, $^3J_{H,H} = 10.5$ Hz, $^3J_{H,F} = 21.7$ Hz, 1H), 7.24–7.46 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 14.1$, 37.8 (d, $^4J_{C,F} = 2.2$ Hz), 38.0 (d, $^3J_{C,F} = 4.9$ Hz), 61.4, 71.5 (d, $^4J_{C,F} = 1.6$ Hz), 73.0, 124.4 (d, $^2J_{C,F} = 18.1$ Hz), 126.2, 127.6, 128.3, 128.4, 129.2, 138.3, 139.0, 147.4 (d, $^1J_{C,F} = 254.4$ Hz), 160.8 ppm (d, $^2J_{C,F} = 35.6$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = 121.0$ ppm (d, $^3J_{F,H} = 21.6$ Hz); IR (neat): $\tilde{\nu} = 3063$, 3028, 2859, 1727, 1665, 1496, 1454, 1375, 1325, 1221, 1104, 1027, 747, 699 cm^{-1}; MS: *m/z*: 343.07 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{21}H_{23}FO_3$: C 73.66, H 6.77; found: C 73.70, H 6.82.

*Ethyl (2*Z*,4*R*)-4-benzyl-5-(benzyloxy)-2-fluoropent-2-enoate [(*Z*)-**11**]*: $R_f = 0.17$ (PE/EtOAc 96:4); $[\alpha]_D^{20} = -17.5$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.29$ (t, $^3J_{H,H} = 7.1$ Hz, 3H), 2.71 (dd, $^2J_{H,H} =$ 7.3 Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 2.85 (dd, $^2J_{H,H} = 7.3$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 3.18 (m, 1H), 3.38 (d, $^3J_{H,H} = 5.7$ Hz, 2H), 4.20 (q, $^3J_{H,H} = 7.1$ Hz, 2H), 4.45 (d, $^2J_{H,H} = 3.8$ Hz, 2H), 6.10 (dd, $^3J_{H,H} = 9.9$ Hz, $^3J_{H,F} =$ 33.5 Hz, 1H), 7.22–7.47 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 14.2$, 37.2 (d, $^4J_{C,F} = 1.6$ Hz), 37.6, 61.7, 71.1 (d, $^4J_{C,F} = 2.2$ Hz), 73.2, 121.3 (d, $^2J_{C,F} = 11.0$ Hz), 126.4, 127.8, 128.4, 128.5, 129.2, 138.2, 138.9, 148.3 (d, $^1J_{C,F} = 257.2$ Hz), 160.7 ppm (d, $^2J_{C,F} = 35.6$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -128.8$ ppm (d, $^3J_{F,H} = 33.5$ Hz); IR (neat): $\tilde{\nu} = 3028$, 2932, 2859, 1735, 1677, 1454, 1371, 1314, 1232, 1098, 738, 699 cm^{-1}; MS: *m/z*: 343.07 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{21}H_{23}FO_3$: C 73.66, H 6.77; found: C 73.72, H 6.80.

(2*Z*,4*R*)-4-Benzyl-5-(benzyloxy)-2-fluoropent-2-en-1-ol (Scheme 4, step f): $LiAlH_4$ (209 mg, 5.52 mmol, 1.5 equiv) was added at 0 °C to a solution of (*Z*)-**11** (1.26 g, 3.68 mmol, 1 equiv) in dry THF (40 mL), and the reaction mixture was stirred for 35 min. It was then quenched with H_2SO_4 (5%), concentrated, and extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over $MgSO_4$, and then concentrated under reduced pressure to afford the alcohol as a colorless oil (965 mg, 87%). $R_f = 0.16$ (PE/EtOAc 85:15); $[\alpha]_D^{20} = -11.4$ ($c = 0.52$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.23$ (br s, 1H, OH), 2.59 (dd, $^2J_{H,H} = 7.5$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 2.79 (dd, $^2J_{H,H} = 7.0$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 3.08 (m, 1H), 3.30 (d, $^3J_{H,H} = 5.6$ Hz, 2H), 3.90 (d, $^3J_{H,F} = 15.1$ Hz, 1H), 4.42 (d, $^2J_{H,H} = 2.8$ Hz, 2H), 4.72 (dd, $^3J_{H,H} =$ 9.6 Hz, $^3J_{H,F} = 37.1$ Hz, 1H), 7.27–7.48 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 36.4$ (d, $^4J_{C,F} = 2.7$ Hz), 37.8 (d, $^3J_{C,F} = 1.6$ Hz), 61.2 (d, $^2J_{C,F} = 32.9$ Hz), 72.0 (d, $^4J_{C,F} = 1.6$ Hz), 73.0, 108.7 (d, $^2J_{C,F} =$ 13.1 Hz), 126.1, 127.7, 127.8, 128.3, 128.5, 129.3, 138.4, 139.6, 158.4 ppm (d, $^1J_{C,F} = 256.1$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta =$ −118.6 ppm (dt, $^3J_{F,H} = 15.0$ Hz, $^3J_{F,H} = 37.1$ Hz); IR (neat): $\tilde{\nu} = 3390$, 3028, 2925, 2858, 1714, 1602, 1459, 1454, 1363, 1263, 1207, 1101, 1075, 1028, 843, 747, 699 cm^{-1}; MS: *m/z*: 323.2 [*M*+Na]$^+$; elemental analysis calcd (%) for $C_{19}H_{21}FO_2$: C 75.97, H 7.05; found: C 76.07, H 7.13.

(2*Z*,4*R*)-4-Benzyl-5-(benzyloxy)-2-fluoropent-2-enal (12, Scheme 4, step g): Et_3N (1.34 mL, 9.64 mmol, 3 equiv), DMSO (4.79 mL, 67.5 mmol, 21 equiv), and $PySO_3$ (1.53 g, 9.64 mmol, 3 equiv) were added at 0 °C to a solution of the above alcohol (965 mg, 3.21 mmol, 1 equiv) in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred at this temperature for 1 h and then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with CH_2Cl_2 (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over $MgSO_4$, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 90:10) to afford **12** as a colorless oil (781 mg, 81%). $R_f = 0.18$ (PE/EtOAc 90:10); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.69$ (dd, $^2J_{H,H} =$ 7.3 Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 2.88 (dd, $^2J_{H,H} = 7.5$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 3.24 (m, 1H), 3.40 (m, 2H), 4.44 (d, $^2J_{H,H} = 2.5$ Hz, 2H), 5.91 (dd, $^3J_{H,H} = 9.8$ Hz, $^3J_{H,F} = 32.8$ Hz, 1H), 7.16–7.41 (m, 10H), 9.09 ppm (d, $^2J_{H,F} = 18.3$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 37.0$ (d, $^4J_{C,F} =$ 1.6 Hz), 38.0 (d, $^3J_{C,F} = 1.6$ Hz), 70.8 (d, $^4J_{C,F} = 1.6$ Hz), 73.3, 126.7, 127.8, 12.9, 128.5, 128.6, 129.1, 131.9 (d, $^2J_{C,F} = 10.4$ Hz), 138.0, 138.4, 156.4 (d, $^1J_{C,F} = 262.7$ Hz), 183.8 ppm (d, $^2J_{C,F} = 24.5$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -132.2$ ppm (dd, $^3J_{F,H} = 18.3$ Hz, $^3J_{F,H} = 32.8$ Hz); IR (neat): $\tilde{\nu} = 3029$, 2859, 1701, 1465, 1454, 1273, 1100, 1028, 742, 699 cm^{-1}; MS: *m/z*: 299.53 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{19}H_{19}FO_2$: C 76.49, H 6.42; found: C 76.52, H 6.55.

***N*-[(2*Z*,4*R*)-4-Benzyl-5-(benzyloxy)-2-fluoropent-2-enylidene]-2-methyl-2-propanesulfinamide (13, Scheme 4, step h)**: $Ti(OEt)_4$ (1.22 mL, 5.82 mmol, 2.5 equiv) and (*S*)-*tert*-butylsulfinylamine (705.9 mg, 5.82 mmol, 2.5 equiv) were added to a solution of aldehyde (694.6 mg, 2.33 mmol, 1 equiv) in dry THF (25 mL). The reaction mixture was heated to reflux for 1 h and, once cooled, poured into an equal volume of brine with fast stirring. The resulting suspension was filtered through a plug of celite, and the filter cake was washed with EtOAc. The organic layer was washed with brine, the brine layer was extracted (3×) with EtOAc, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 85:15), affording **13** as a colorless oil (884 mg, 95%). $R_f = 0.20$ (PE/EtOAc 85:15);

$[\alpha]_D^{20} = +63.7$ ($c = 0.62$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.18$ (s, 9H), 2.68 (dd, $^2J_{H,H} = 7.5$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 2.87 (dd, $^2J_{H,H} = 7.5$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 3.22 (m, 1H), 3.38 (d, $^2J_{H,H} = 5.3$ Hz, 2H), 4.43 (s, 2H), 5.61 (dd, $^3J_{H,H} = 9.8$ Hz, $^3J_{H,F} = 33.0$ Hz, 1H), 7.15–7.41 (m, 10H), 7.84 ppm (d, $^2J_{H,F} = 19.4$ Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 22.5$, 37.3, 38.2, 58.0, 71.0 (d, $^4J_{C,F} = 1.1$ Hz), 73.2, 126.4, 126.9 (d, $^2J_{C,F} = 12.6$ Hz), 127.7, 127.8, 128.4, 128.5, 129.2, 138.1, 138.7, 155.1 (d, $^2J_{C,F} = 21.4$ Hz), 155.1 ppm (d, $^1J_{C,F} = 255.5$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -125.7$ ppm (dd, $^3J_{F,H} = 19.4$ Hz, $^3J_{F,H} = 33.0$ Hz); IR (neat): $\tilde{\nu} = 3028$, 2958, 2862, 1661, 1592, 1454, 1363, 1177, 1086, 1029, 772, 746, 699 cm^{-1}; MS: *m/z*: 402.07 $[M+H]^+$; elemental analysis calcd (%) for $C_{19}H_{19}FO_2$: C 68.80, H 7.03, N 3.49, S 7.99; found: C 68.85, H 7.10, N 3.53, S 8.04.

***N*-[(2*Z*,4*R*)-4-Benzyl-5-(benzyloxy)-2-fluoropent-2-enyl]-2-methyl-2-propanesulfinamide (Scheme 4, step i)**: $NaBH_4$ (31.6 mg, 0.83 mmol, 1.1 equiv) was added at 0 °C to a solution of **13** (305.1 mg, 0.76 mmol, 1 equiv) in dry THF (18 mL). The reaction mixture was stirred at 0 °C for 2 h 30 min and then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with EtOAc (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 60:40→35:65), affording the amine as a colorless oil (297.6 mg, 97%). $R_f = 0.30$ (PE/EtOAc 50:50); $[\alpha]_D^{20} = +35.5$ ($c = 0.63$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.13$ (s, 9H), 2.53 (dd, $^2J_{H,H} = 7.9$ Hz, $^3J_{H,H} = 13.4$ Hz, 1H), 2.80 (dd, $^2J_{H,H} = 7.9$ Hz, $^3J_{H,H} = 13.4$ Hz, 1H), 3.08 (m, 1H), 3.17 (t, $^3J_{NH,H} = 6.8$ Hz, 1H), 3.30 (d, $^2J_{H,H} = 5.6$ Hz, 2H), 3.62–3.84 (m, 2H), 4.42 (s, 2H), 4.71 (dd, $^3J_{H,H} = 9.8$ Hz, $^3J_{H,F} = 36.3$ Hz, 1H), 7.14–7.40 ppm (m, 10H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 22.6$, 37.3, 38.1 (d, $^3J_{C,F} = 1.6$ Hz), 46.3 (d, $^2J_{C,F} = 31.3$ Hz), 58.0, 71.0 (d, $^4J_{C,F} = 1.1$ Hz), 73.2, 109.6 (d, $^2J_{C,F} = 13.2$ Hz), 126.1, 127.7, 128.2, 128.5, 129.3, 138.1, 139.6, 156.4 ppm (d, $^1J_{C,F} = 256.6$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -115.1$ ppm (dt, $^3J_{F,H} = 18.6$ Hz, $^3J_{F,H} = 36.3$ Hz); IR (neat): $\tilde{\nu} = 3205$, 2925, 2861, 1702, 1495, 1454, 1364, 1099, 1059, 746, 699 cm^{-1}; MS: *m/z*: 404.20 $[M+H]^+$; elemental analysis calcd (%) for $C_{23}H_{30}FNO_2S$: C 68.45, H 7.49, N 3.47, S 7.95; found: C 68.89, H 6.57, N 3.42, S 7.99.

***N*-[(2*Z*,4*R*)-4-Benzyl-2-fluoro-5-hydroxypent-2-enyl]-2-methyl-2-propanesulfinamide (14, Scheme 4, step j)**: BCl_3 (4.2 mL of a 1 M solution in DCM, 28.5 mmol, 5 equiv) was slowly added at −78 °C to a solution of the above sulfinamide (336.5 mg, 0.83 mmol, 1 equiv) in dry DCM (10 mL). The reaction mixture was stirred at −78 °C for 10 min and then quenched with a saturated aqueous solution of $NaHCO_3$. The mixture was extracted with DCM (3×), and the combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ and with H_2O and were then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (DCM/MeOH 98.5:1.5→80:20), affording **14** as a colorless oil (156.3 mg, 60%). $R_f = 0.18$ (DCM/MeOH 98:2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.21$ (s, 9H), 2.61 (dd, $^2J_{H,H} = 8.3$ Hz, $^3J_{H,H} = 13.7$ Hz, 1H), 2.84 (dd, $^2J_{H,H} = 8.3$ Hz, $^3J_{H,H} = 13.7$ Hz, 1H), 3.03 (m, 1H), 3.48–3.75 (m, 5H), 4.78 (dd, $^3J_{H,H} = 9.8$ Hz, $^3J_{H,F} = 37.1$ Hz, 1H), 7.10–7.31 ppm (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 22.6$, 37.5 (d, $^4J_{C,F} = 1.6$ Hz), 39.3 (d, $^3J_{C,F} = 1.1$ Hz), 46.5 (d, $^2J_{C,F} = 31.8$ Hz), 56.4, 65.1 (d, $^4J_{C,F} = 1.6$ Hz), 109.4 (d, $^2J_{C,F} = 12.6$ Hz), 126.2, 128.3, 129.2, 139.6, 157.3 ppm (d, $^1J_{C,F} = 257.2$ Hz). ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -113.3$ ppm (dt, $^3J_{F,H} = 14.5$ Hz, $^3J_{F,H} = 37.1$ Hz); elemental analysis calcd (%) for $C_{16}H_{24}FNO_2S$: C 61.31, H 7.72, N 4.47, S 10.23; found: C 61.45, H 7.85, N 4.52, S 10.25.

(2*Z*,4*R*)-4-Benzyl-2-fluoro-5-hydroxypent-2-en-1-aminium chloride (15, Scheme 4, step k): HCl in dioxane (4 M, 269 μL, 1.07 mmol, 2 equiv) was added to a solution of **14** (168.1 mg, 0.54 mmol, 1 equiv) in dry MeOH (2 mL). The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure to near dryness. The crude mixture **15** was used in the next step without further purification. ^1H NMR (300 MHz, D_2O): $\delta = 2.52$ (dd, $^2J_{H,H} = 9.6$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 2.84 (dd, $^2J_{H,H} = 9.6$ Hz, $^3J_{H,H} = 13.6$ Hz, 1H), 3.08 (m, 1H), 3.46–3.74 (m, 4H), 5.04 (dd, $^3J_{H,H} = 10.0$ Hz, $^3J_{H,F} = 36.7$ Hz, 1H), 7.25–7.37 ppm (m, 5H); ^{13}C NMR (75.4 MHz, D_2O): $\delta = 36.4$, 38.8 (d, $^4J_{C,F} = 1.1$ Hz), 39.7, 46.5 (d, $^2J_{C,F} = 30.7$ Hz), 64.2 (d, $^4J_{C,F} = 1.1$ Hz), 113.6 (d, $^2J_{C,F} = 12.6$ Hz), 126.3, 128.4, 129.1, 139.8, 152.4 ppm (d, $^1J_{C,F} = 251.7$ Hz); ^{19}F NMR (282.5 MHz, D_2O): $\delta = -117.0$ ppm (q).

***9H*-Fluoren-9-ylmethyl (2*Z*,4*R*)-4-Benzyl-2-fluoro-5-hydroxypent-2-enyl carbamate (Scheme 4, step l)**: $NaHCO_3$ (92 mg, 1.1 mmol, 3 equiv) was added at 0 °C to a solution of **15** (89.5 mg, 0.36 mmol, 1 equiv) in dioxane (4 mL $mmol^{-1}$ of amine hydrochloride) and water (4 mL $mmol^{-1}$ of amine hydrochloride), followed by Fmoc-OSu (122.7 mg, 0.36 mmol, 1 equiv). The reaction mixture was stirred at 0 °C for 1 h 30 min and was then poured into ice-cold HCl (1 N, 8 mL $mmol^{-1}$ of amine hydrochloride) and extracted with AcOEt (3×). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 70:30→50:50), affording the product as a colorless oil (149.8 mg, 95%). $R_f = 0.19$ (PE/EtOAc 70:30); $[\alpha]_D^{20} = -13.15$ ($c = 0.95$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.53$–2.80 (m, 2H+OH), 3.01 (m, 1H), 3.42–3.61 (m, 2H), 3.79 (dd, $^3J_{H,F} = 14.3$ Hz, $^3J_{H,NH} = 5.8$ Hz, 2H), 4.19 (t, $^3J_{H,H} = 6.8$ Hz, 1H), 4.41 (d, $^3J_{H,H} = 6.8$ Hz, 2H), 4.68 (dd, $^3J_{H,H} = 9.8$ Hz, $^3J_{H,F} = 36.7$ Hz, 1H), 5.37 (t, $^3J_{NH,H} = 5.6$ Hz, 1H, NH), 7.13–7.18 (m, 3H), 7.22–7.33 (m, 4H), 7.38–7.43 (m, 2H), 7.58 (d, $^3J_{H,H} = 7.3$ Hz, 2H), 7.76 ppm (d, $^3J_{H,H} = 7.3$ Hz, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 37.5$, 39.0, 41.2 (d, $^2J_{C,F} = 32.3$ Hz), 47.1, 64.9, 66.8, 108.6 (d, $^2J_{C,F} = 12.6$ Hz), 120.0, 125.0, 126.1, 127.1, 127.7, 128.2, 129.1, 139.4, 141.3, 143.8, 156.4, 156.5 ppm (d, $^1J_{C,F} = 256.1$ Hz); ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -114.7$ ppm (dt, $^3J_{F,H} = 14.5$ Hz, $^3J_{F,H} = 37.1$ Hz); IR (neat): $\tilde{\nu} = 3410$, 3326, 2926, 1704, 1520, 1450, 1257, 1031, 753, 741, 621 cm^{-1}; MS: *m/z*: 454.33 $[M+Na]^+$; elemental analysis calcd (%) for $C_{27}H_{26}FNO_3$: C 75.15, H 6.07, N 3.25; found: C 75.28, H 6.15, N 3.29.

Fmoc-GlyΨ[CF=CH]Phe-OH, (2*R*,3*Z*)-2-benzyl-5-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-fluoropent-3-enoic acid, dipeptide analogue (*Z*)-2 (Scheme 4, step m): Jones' reagent (2.74 N, 3 equiv) was added at 0 °C to a solution of the above (*Z*)-N-protected amino alcohol (240.3 mg, 0.55 mmol, 1 equiv) in acetone (10 mL $mmol^{-1}$ of alcohol). The reaction mixture was stirred at 0 °C for 1 h and then quenched with isopropyl alcohol (10 equiv) and water (13 mL $mmol^{-1}$ of alcohol). The mixture was extracted with AcOEt (3×), and the combined organic layers were washed with a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (PE/EtOAc 80:20→70:30, then 50:50, with 0.1% of acetic acid), affording the dipeptide analogue as a white solid (192.2 mg, 80%). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.85$–3.22 (m, 2H), 3.73–3.89 (m, 3H, H_2), 4.25 (t, $^3J_{H,H} = 6.4$ Hz, 1H), 4.48 (d, $^3J_{H,H} = 6.2$ Hz, 2H), 4.99 ($^3J_{H,H} = 9.6$ Hz, $^3J_{H,F} = 35.2$ Hz, 1H), 5.11 (br s, 1H, NH), 7.23–7.48 (m, 9H), 7.63 (d, $^3J_{H,H} = 7.1$ Hz, 2H), 7.82 ppm (d, $^3J_{H,H} = 7.6$ Hz, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$): $\delta = 38.4$, 41.2 (d, $^2J_{C,F} = 31.8$ Hz), 42.5, 47.1, 67.0, 104.7 (d, $^2J_{C,F} = 12.1$ Hz), 120.1, 125.0, 126.7, 127.1, 127.8, 128.4, 129.1, 137.9, 141.3, 143.8, 156.3, 156.8 (d, $^1J_{C,F} = 259.4$ Hz),

178.0 ppm; ^{19}F NMR (282.5 MHz, $CDCl_3$): $\delta = -113.5$ ppm (dt, $^3J_{F,H} =$ 13.4 Hz, $^3J_{F,H} = 35.1$ Hz); IR (neat): $\tilde{\nu} = 3320$, 3066, 3028, 2941, 1708, 1522, 1450, 1251, 1162, 760, 739, 699 cm^{-1}; MS: *m/z*: 891.00 [2*M* < M + > H]$^+$; elemental analysis calcd (%) for $C_{27}H_{26}FNO_4$: C 72.80, H 5.43, N 3.14; found: C 72.92, H 5.52, N 3.17.

Peptide and pseudopeptide solid-phase synthesis: Peptides 26RFa and 26RFa$_{(20-26)}$ were synthesized by solid-phase methodology as previously described.[7] The pseudopeptides [*Z*,CF=CH^{20,21}]-26RFa$_{(20-26)}$ (LV-2094), [*E*,CF=CH^{20,21}]26RFa$_{(20-26)}$ (LV-2098), and [*Z*,CF=CH^{21,22}]26RFa$_{(20-26)}$ (LV-2095 and LV-2096) were synthesized (0.1 mmol scale) by the solid-phase methodology on a Rink amide 4-methylbenzhydrylamine resin (VWR, Fontenay-Sous-Bois, France) with a 433A Applied Biosystems peptide synthesizer (Applera-France, Courtaboeuf, France) and the standard manufacturer's Fmoc procedure. All Fmoc-amino acids (1 mmol, 10 equiv, Christof Senn Laboratories, Dielsdorf, Switzerland) were coupled by in situ activation with HBTU/HOBt (1.25 mmol:1.25 mmol, 12.5 equiv) and DIEA (2.5 mmol, 25 equiv) in NMP. The Fmoc-GlyΨ[CF=CH]AA-OH pseudodipeptides (1.5 equiv, AA = Gly, Phe) were manually coupled with the aid of HATU (1.5 equiv), HOAt (1.5 equiv), and DIEA (3 equiv) in NMP for 2 h. Reactions were monitored by use of the Kaiser test. Pseudopeptides were deprotected and cleaved from the resin by adding a TFA/TIS/H_2O (99.5:0.25:0.25, *v/v/v*, 10 mL) mixture (120 min at room temperature). After filtration, crude peptides were precipitated by addition of *tert*-butyl methyl ether (TBME), centrifuged (4500 rpm), washed twice with TBME, and freeze-dried. The synthetic pseudopeptides were purified by reversed-phase HPLC with a 2.2×25 cm Vydac 218TP1022 C_{18} column (Grace, Epernon, France) and use of a linear gradient (10–50% over 45 min) of CH_3CN/TFA (99.9:0.1, *v/v*) at a flow rate of 10 mL min^{-1}. Analytical HPLC, performed with a 0.46×25 cm Vydac 218TP54 C_{18} column (Grace), showed that the purities of the pseudopeptides were > 99.5% (Table 2). The purified pseudopeptides were characterized by MALDI-TOF mass spectrometry (Table 2) with a Voyager DE PRO (Applera-France) in the reflector mode with α-cyano-4-hydroxycinnamic acid as a matrix.

Table 2. Chemical data for pseudopeptides.

Code	HPLC		MS	
	t_R [min][a]	Purity [%]	Calcd[b]	Found[c]
LV-2094	26.2	100	816.41	817.28
LV-2098	26.3	100	816.41	817.44
LV-2095	25.4	100	816.41	817.47
LV-2096	27.4	100	816.41	817.56

[a] Retention times determined by RP-HPLC. [b] Theoretical monoisotopic molecular weights. [c] *m/z* values assessed by MALDI-TOF MS.

Calcium mobilization assays: Changes in $[Ca^{2+}]_i$ induced by 26RFa and 26RFa$_{(20-26)}$ analogues in *h*GPR103-transfected CHO cells were measured with a benchtop scanning fluorometer Flexstation III (Molecular Devices, Sunnyvale, CA) as previously described.[7,8] Briefly, 96-well assay black plates with clear bottom (Corning International, Avon, France) were seeded at a density of 40 000 cells per well 24 h prior to assay. Cells were loaded with 2 μM Fluo-4AM (Invitrogen) for 1 h, washed three times, and incubated for 30 min with standard Hank's balanced salt solution (HBSS) containing probenecid (2.5 mM) and HEPES (5 mM). Compounds to be tested were added at final concentrations ranging from 10^{-12} to 10^{-5} M, and the fluorescence intensity was measured over 2 min. A Xenon lamp was used as excitation source. The wavelengths of excitation (485 nm) and emission (525 nm) of Fluo-4AM were selected by use of two monochromators included in the device, equipped with a bottom reading probe.

Susceptibility of pseudopeptides to enzymatic degradation: The stabilities of the 26RFa analogues LV-2094, LV-2095, LV-2096, and LV-2098 were tested in vitro as previously described.[8] Pseudopeptides (250 μg mL^{-1}) were incubated at 37 °C with pooled human serum (healthy donors). The reaction was stopped after different times by adding TFA (10% of the final volume), and the samples were diluted five times in PBS (pH 7.4). After centrifugation, the supernatants were collected and analyzed by RP-HPLC on a Vydac 218MS54 C_{18} column (0.46×25 cm) with a linear gradient (10–60% over 50 min) of acetonitrile/TFA (99.9:0.1, *v/v*) at a flow rate of 1 mL min^{-1}. HPLC peak areas were used to calculate the percentages of intact compounds remaining at various time points during the incubation. Half-life times ($t_{1/2}$) were calculated with the Prism software (Graphpad Software, San Diego, CA) from exponential decay curves. Mass spectra of the intact peptide and its metabolites were performed by MALDI-TOF MS with a Voyager DE-PRO (Applera-France) in the reflector mode with α-cyano-4-hydroxycinnamic acid as a matrix.

NMR spectroscopy for conformation studies: All NMR experiments were performed with a Bruker Avance III 600 MHz NMR spectrometer (Wissembourg, France), fitted with a triple resonance cryoprobe including shielded *z*-gradients. All peptides were dissolved at concentrations of about 1 mM in D_2O/H_2O (10%) in the presence of [D_{38}]DPC (150 mM, C/D/N isotopes, Pointe-Claire, Canada). DSS was added as an internal ^{1}H chemical shift reference. Conventional COSY, TOCSY, and NOESY two-dimensional experiments were carried out at 298 K. TOCSY experiments were performed with an 80 ms DIPSI2 spin lock mixing pulse. NOESY spectra were collected at mixing times of 120 and 150 ms. Water suppression was achieved by use of the excitation sculpting sequence except in the case of COSY experiments, in which a low power presaturation was applied during the relaxation delay. TOCSY and NOESY experiments were performed in the phase-sensitive mode, with use of proportional phase incrementation method for quadrature detection (States-TPPI). Proton chemical shifts are reported relative to DSS taken as an internal reference. Distance restraints for NOE diagrams were derived from crosspeaks in the NOESY spectra recorded with mixing times of 150 ms. NOE crosspeaks were integrated into distances by volume integration with FelixNMR (San Diego, USA). The NOE volumes were calibrated from well-resolved geminal Hβ crosspeaks.

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