Organic & Biomolecular Chemistry

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Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-01-2023-000110.R1
Article Type:	Paper
Date Submitted by the Author:	17-Apr-2023
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A Fluoro-Alkene Mimic of Gly–*trans*-Pro Produces a Stable Collagen Triple Helix

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Abstract

We report the first experimental evidence for a fluoro-alkene amide isostere participating in n→π* donation, which stabilizes the collagen triple helix. Of the three amide positions in canonical collagen-like peptides, Gly–Pro, Pro–Hyp, and Hyp–Gly, triple helix stability stands to benefit from substitution of only the isomerizable 3° Gly–Pro amide bond with a trans-locked fluoro-alkene. A (*Z*)-fluoro-alkene isostere of Gly–*trans*-Pro was synthesized, and its effect on the thermostability of a collagen-like peptide triple helix was measured. The mixture of enantiomers, Boc–Gly–Ψ[(*Z*)CF=C]-L/D-Pro–OH, was synthesized in 8 steps with 27% overall yield, and the Fmoc–Gly–Ψ[(*Z*)CF=C]-L/D-Pro–Hyp–OBn diastereomers were separated. The Gly–Ψ[(*Z*)CF=C]-Pro isostere installed in a collagen-like peptide forms a stable triple helix. By CD, the thermal melting (T_m) value of the fluoro-alkene peptide was +42.2 ± 0.4 °C, and the T_m value of the control peptide was +48.4 ± 0.5 °C, a difference in stability of ΔT_m –6.2 °C. Deshielding of the fluorine nucleus in the ^{19}F NMR spectra is evidence of a stabilizing n→π* electronic interaction.

Introduction

Type I collagen accounts for over 25% of all mammalian protein, making it the most abundant structural protein.[1] The tertiary structure is an elegant helix of helices, in which three individual polyproline type II (PPII) helices intertwine around a common screw axis to form the collagen triple helix.[2, 3] Each PPII helix is a polymer with the repeating sequence (Gly–Xaa–

Yaa)$_n$ with a high content of proline in the Xaa position (ca. 28%) and (4*R*)-hydroxyproline (Hyp) in the Yaa position (ca. 38%).[4] Pro is used in the Yaa position frequently instead of Hyp for biophysical studies. The Gly residue is mandatory at every third position of the helical regions because the lack of side-chain allows it to fit into the compact core where the three PPII helices come together in the triple helix.[5]

The peptide bond has a high degree of sp^2 character due to resonance.[6] The Pro and Hyp tertiary amide bonds have the propensity to isomerize, leading to unusually high cis:trans ratios.[7] While over 99% of secondary amide bonds adopt the trans conformation, up to 35% of tertiary amide bonds in peptides and unfolded proteins adopt the cis conformation.[7] All amide bonds in collagen must adopt the trans conformation, and collagen triple-helix folding is rate-limited by the isomerization of Xaa–Pro amides.[8] We hypothesized that preventing isomerization by locking it in the trans conformation could lead to a more stable triple helix.

Alkenes are excellent isosteres of Xaa–*cis*- and *trans*-Pro bonds.[9-11] However, we showed that replacing either one of the tertiary amides in collagen, Gly–Pro or Pro–Pro, with a trans proteo-alkene destabilizes the triple helix (Figure 1).[12, 13] Substitution of a single Gly–Pro peptide bond in a 27-residue collagen-like peptide led to ΔT_m –21.7 ºC compared to the native collagen-like peptide control (Figure 1).[12] The Pro–Pro amide C=O acts as an interstrand hydrogen-bond acceptor, and substitution at this site destabilized the triple helix even more, with ΔT_m –53.6 ºC compared to the native control (Figure 1).[13] The Pro–Gly amide N–H acts as an interstrand hydrogen-bond donor, so substitution at this site led to a collagen-like peptide with ΔT_m –57.5 ºC compared to the native control (Figure 1).[14] These results suggest that eliminating isomerization is not sufficient to compensate for the loss of noncovalent interactions, such as steric, electronic dipole-dipole interactions, or n→π* delocalization, that are present with native

amide bonds.

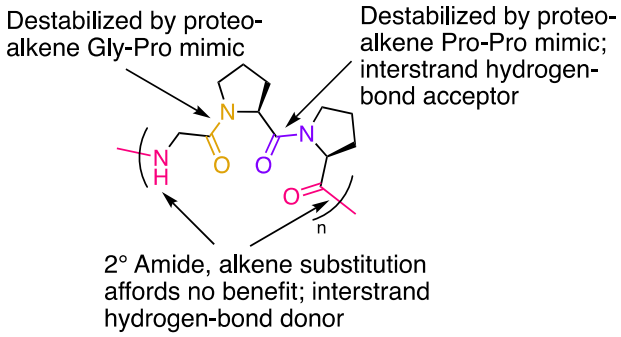
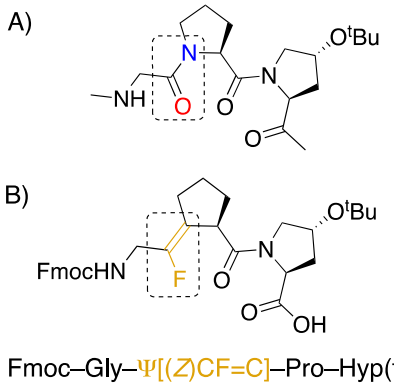


Figure 1. Amide bonds that have been substituted with alkenes in collagen repeating tripeptides.[12-14]

Collagen is stabilized by a series of electronic n→π* interactions.[15, 16] In the n→π* interaction, electron density from a C_i=O lone pair delocalizes into the adjacent C_{i+1}=O π* antibonding orbital.[15-18] The geometry of this interaction typically matches the trajectory for nucleophilic approach to a carbonyl, where the energy of approach is strongest around an angle of 108º, and within a distance of < 3.22 Å; the sum of O and C van der Waal's radii.[17-20] The energy afforded by the n→π* interaction within an Ac–Pro–NMe_2 model was estimated to be 0.27 kcal/mol.[21] Our conformational modeling showed that the Gly–Pro proteo-alkene has two low-energy minima, suggesting flexibility that destabilizes the triple helix.[12, 22] Decreased stabilities of the trans-locked collagen-like peptides were due, at least in part, to the missing n→π* interactions of the replaced amide.[22] We predicted that this effect could be recaptured using a haloalkene instead.[22]

We designed the Gly–Pro–Hyp collagen-like isostere **1-(*R*,*Z*)** (Figure 2), in which the Gly–Pro peptide bond is replaced with a (*Z*)-fluoro-alkene. Fluoro-alkenes are exceptionally good isosteres of peptide bonds, with many advantages over a proteo-alkene isostere.[23] The van der Waals radius and electronegativity of fluorine (1.47 Å, EN 4.0) are much closer to oxygen (1.57 Å, EN 3.5) than are those of hydrogen (1.2 Å, EN 2.1).[24, 25] The fluoro-alkene was

calculated to have similar orientation and dipole moment to an amide.[26] Fluorine also has lone pairs that can participate in n→π* interactions.[22]



Fmoc–Gly–Ψ[(*Z*)CF=C]–Pro–Hyp(tBu)–OH, **1-(*R*,*Z*)**

C) Ac–(Gly–Pro–Hyp)$_3$–Gly–Ψ[(*Z*)CF=C)]–Pro–Hyp–(Gly–Pro–Hyp)$_4$–Gly–Gly–Tyr–NH$_2$, **2-(*R*,*Z*)**

D) Ac–(Gly–Pro–Hyp)$_8$–Gly–Gly–Tyr–NH$_2$, **3**

Figure 2. A fluoro-alkene mimic of Gly–*trans*-Pro in collagen-like peptide. **A)** Tripeptide repeat of native collagen. **B)** Synthesized (*Z*)-fluoro-alkene mimic **1-(*R*,*Z*)**. **C)** Host–guest collagen-like peptide sequence **2-(*R*,*Z*)** with trimer mimic **1-(*R*,*Z*)** in the middle of the triple helix. **D)** Native collagen-like peptide control **3**.

We have shown computationally that the global minimum of the fluoro-alkene Gly–Pro model as an n→π* donor has a Ψ angle of +162°,[22] and the global minimum of the native peptide has a Ψ angle of +160°,[18] suggesting that this mimic would be geometrically compatible with the collagen triple helix (Figure 3A). Our models also suggest that the fluorine-carbonyl interaction is just outside of the parameters for a significant n→π* interaction to occur. Fortunately, the fluoro-alkene does not have local minima near the collagen-like global minimum that could cause conformational instability as for the proteo-alkene.[22]

The global minimum of the Gly–Pro model fluoro-alkene as an n→π* acceptor has a Ψ angle of +117°,[22] which deviates significantly from the collagen triple-helix minimum at a Ψ angle of +167° (Figure 3B).[18] A Pro–Gly fluoro-alkene model was a demonstrably poor n→π* acceptor due to n)(π electronic repulsion with the alkene, which destabilizes the PPII-like

geometry.[27] We were concerned because the global minimum for the proteo-alkene model as an n→π* acceptor has about the same Ψ angle (+120°) as the fluoro-alkene, suggesting similar n)(π electronic repulsion (Figure 2B).[22]

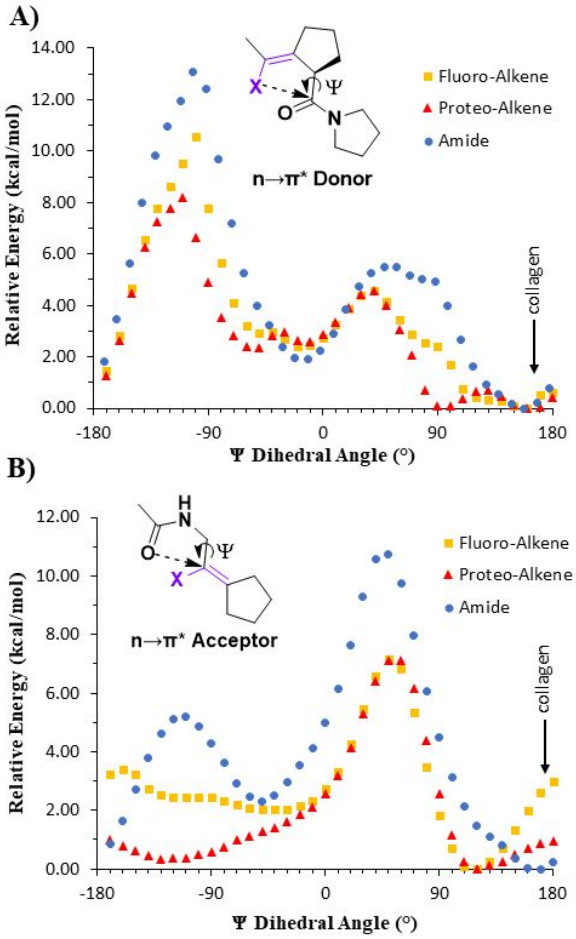


Figure 3. Energy scans of the Ψ dihedral angle in the fluoro-alkene, proteo-alkene, and amide Gly–Pro models.[22] Models of **A)** the X atom acting as an n→π* donor, and **B)** the C=O carbon acting as an n→π* acceptor. The average Ψ dihedral angle from the collagen crystal structure PDB ID: 1K6F is labeled.[28] Modified with permission from Arcoria, P. J.; Ware, R. I.; Makwana, S. V.; Troya, D.; Etzkorn, F. A.; Conformational Analysis of Fluoro-, Chloro-, and Proteo-Alkene Gly–Pro and Pro–Pro Isosteres to Mimic Collagen. *The Journal of Physical Chemistry B* 2021. Copyright 2021 American Chemical Society.

In this experimental study, we investigated whether the fluoro-alkene mimic of Gly–

trans-Pro would adopt the PPII-like geometry in a collagen-like peptide by reintroducing noncovalent interactions, such as steric, dipole-dipole, and n→π* interactions, leading to a more stable triple helix. We now report the design and asymmetric synthesis of the Fmoc–Gly–Ψ[(*Z*)CF=C)]–Pro–Hyp(ᵗBu)–OH fluoro-alkene mimic **1-(*R*,*Z*)**, and its effect on collagen triple helix stability.

Results and Discussion

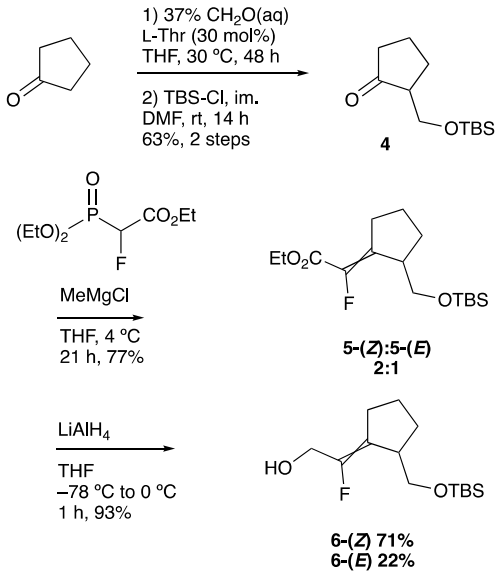
Synthesis of Fluoro-Alkene Isosteres and Peptides

Asymmetric aldol addition of formaldehyde to cyclopentanone was catalyzed by L-Thr to give 2-(hydroxymethyl)-cyclopentanone (Scheme 1). Optical rotation was measured to confirm scalemic aldol addition. Catalytic L-Thr in THF has been reported to give the 2-(hydroxymethyl)-cyclopentanone product in 43% yield and 76% ee of the (*S*)–enantiomer.[29] We report similar results, and the sign of the optical rotation of the 2-(hydroxymethyl)-cyclopentanone matched previous reports.[29] In addition, we determined the absolute stereochemistry of the major isomer of 2-(hydroxymethyl)-cyclopentanone by Bayer-Villager oxidation to the known (*S*)-5-(hydroxylmethyl)-δ-lactone and comparison of the lactone optical rotation to literature values (Supplementary Information).[30, 31]

The 2-(hydroxymethyl)-cyclopentanone could not be isolated in satisfactory yields, possibly due to retro-aldol degradation on silica gel. To circumvent this issue, the crude reaction mixture was dried, concentrated, and the alcohol was protected with TBS–Cl to give ketone **4** in 63% yield over two steps (Scheme 1). The ylide of the commercially available fluoromethylphosphonate ethyl ester was employed to make the fluoro-alkene. In our hands, the standard phosphonate-ketone coupling[32] resulted in a low yield of **5** (39%) with an unfavorable *Z*:*E* ratio (1:1.3 by ^{1}H NMR). The Mg(II)-promoted phosphonate ylide coupling is known to

enhance the desired selectivity.[33, 34] Both yield (77%) and selectivity (2:1 *Z*:*E* by ^{1}H NMR) were dramatically improved by using MeMgCl as the base instead of NaH (Scheme 1). This transformation changes the stereochemical designation of the desired enantiomer to (*R*). The mixture of esters **5-(*Z*)** and **5-(*E*)** was then regioselectively reduced with $LiAlH_4$, without 1,4-reduction. The corresponding 1,2-reduced allylic alcohols **6-(*Z*)** and **6-(*E*)** were separated by flash chromatography. Although isolation of the desired **5-(*Z*)** isomer by chromatography was possible, separation of the alcohols was much easier after reduction of the esters (Scheme 1).

Scheme 1. Asymmetric synthesis of alcohol **6-(*Z*)**.



The allylic protons of the isolated stereoisomers **6-(*Z*)** and **6-(*E*)** were assigned by COSY (Supplementary Information), and the fluoro-alkene configurations were confirmed by 1D nOe NMR (Supplementary Information). Irradiation of the new allylic protons showed correlation either to the prolyl pseudo-δ protons of the **6-(*Z*)** isomer, or to the methylene protons of the CH_2–OTBS group and the pseudo-α-CH proton of the **6-(*E*)** isomer (Figure 4).

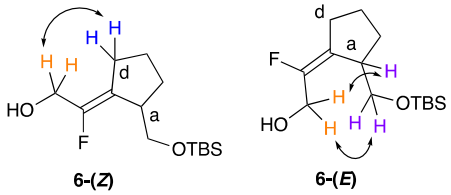
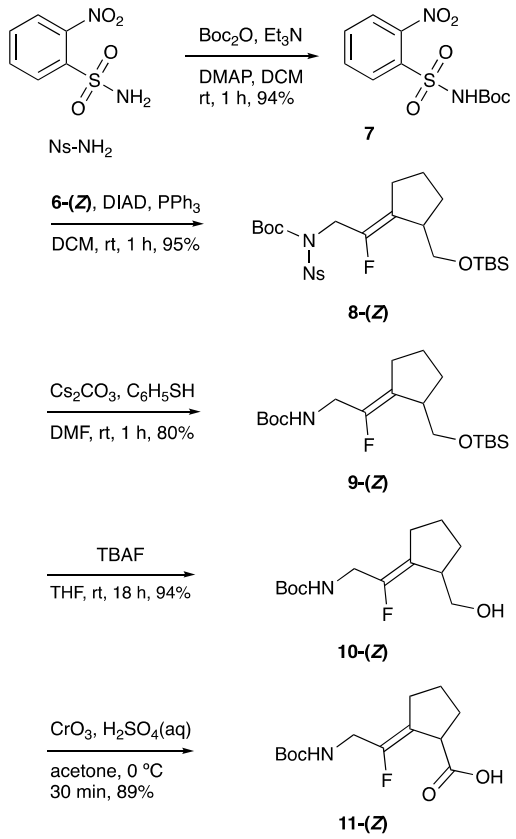


Figure 4. Observed 1D-nOe correlations of allylic alcohols **6-(*Z*)** and **6-(*E*)**.

Conversion of the allylic alcohol **6-(*Z*)** to an amine was necessary to mimic the native Gly residue. Protection of the commercially available 2-nitrobenzenesulfonamide (Ns–NH_2) with Boc_2O gave the *N*-Boc–2-Ns–NH reagent **7** (Scheme 2).[34, 35] Subsequent displacement of the allylic alcohol with sulfonamide **7** using diisopropyl azodicarboxylate (DIAD) and PPh_3 gave sulfonamide **8-(*Z*)** in 95% yield (Scheme 2).[34, 35] Removal of the Ns protecting group was accomplished using Cs_2CO_3 with thiophenol in DMF to give Boc-amine **9-(*Z*)** in 80% yield (Scheme 2). The silyl alcohol protecting group was then removed with TBAF to give alcohol **10-(*Z*)** in 94% yield (Scheme 2). Oxidation of **10-(*Z*)** in acetone with chromic acid in acetone afforded acid **11-(*Z*)** in 89% yield (Scheme 2). The reaction duration was kept short (30 min), and the temperature was kept cold (0 °C) to help prevent premature cleavage of the Boc group, because

Scheme 2 Synthesis of the (*Z*)-fluoro-alkene Gly–*trans*-Pro mimic, Boc–Gly–Ψ[(*Z*)CF=C)]–Pro–OH **11-(*Z*)**.

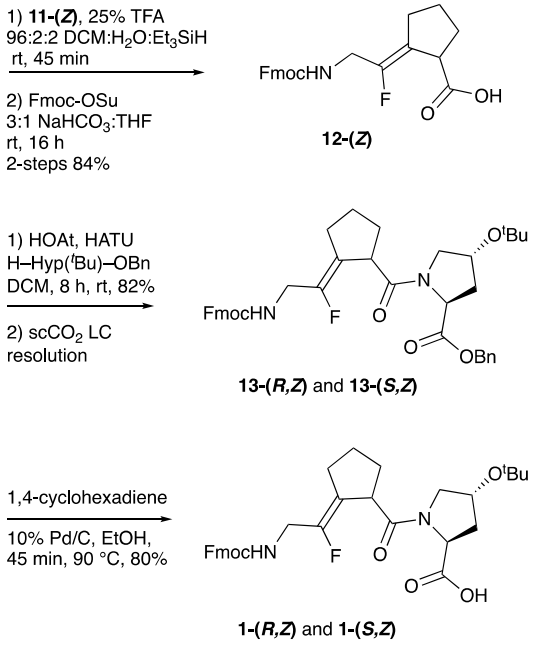


the resulting ammonium salt would be difficult to isolate. The Boc–Gly–Ψ[(*Z*)CF=C)]–L/D-Pro–OH fluoro-alkene isostere **11-(*Z*)** was synthesized in 8 steps and 27% overall yield.

The Boc group of **11-(*Z*)** was removed with 25% TFA in DCM using 2% each of Et_3SiH and H_2O as cation scavengers, and the crude TFA salt was protected with Fmoc–OSu to give Fmoc-amine **12-(*Z*)** in 84% yield over 2 steps (Scheme 3). H–Hyp(^{t}Bu)–OBn was coupled to Fmoc amine **12-(*Z*)** using HATU and HOAt without additional base to prevent isomerization of the alkene into the 5-membered ring (Scheme 3).[12, 13] Benzyl ester **13-(*Z*)** was obtained in 82% yield as a mixture of diastereomers. The diastereomers were separated by $scCO_2$ LC using 15% *i*PrOH/$scCO_2$ on a pyridine column with poor recovery (14%). Transfer hydrogenation of the separated isomers with 1,4-cyclohexadiene on 10% Pd/C removed the benzyl protecting groups

without reduction of the fluoro-alkenes to give Fmoc–Gly–Ψ[(*Z*)CF=C)]–Pro–Hyp(*t*Bu)–OH **1-(*R*,*Z*)**, and **1-(*S*,*Z*)** diastereomers (Scheme 3).[12]

Scheme 3. Synthesis of the tripeptide isosteres, Fmoc–Gly–Ψ[(*Z*)CF=C)]–L/D-Pro–Hyp(*t*Bu)–OH **1-(*R*,*Z*)** and **1-(*S*,*Z*)**.



Peptides **2-(*R*,*Z*)**, **2-(*S*,*Z*)**, and **3** were synthesized by coupling Fmoc-protected tripeptide units with either HOAt/HATU or 6–Cl–HOBt/HBTU on solid-phase 4-methylbenzhydrylamine (MBHA) resin. The Tyr residue was included to determine peptide concentration by UV. The two Gly residues were included as a spacer to facilitate peptide synthesis and to decrease interference of Tyr with triple-helix folding.[12] The Fmoc–Gly–Pro–Hyp(*t*Bu)–OH trimer units were synthesized by the published method.[36] The crude peptides were purified by HPLC on a C18 column, and their identity was confirmed by MALDI-TOF.

Triple-Helix Stability by CD

Both the fluoro-alkene peptide, Ac–(Gly–Pro–Hyp)$_3$–Gly–Ψ[(*Z*)CF=C)]–L-Pro–Hyp–(Gly–Pro–Hyp)$_4$–Gly–Gly–Tyr–NH_2 **2-(*R*,*Z*)**, and the native peptide control, Ac–(Gly–Pro–

Hyp)$_8$–Gly–Gly–Tyr–NH_2 **3**, showed collagen triple-helix signature maxima at 226 nm and minima around 205 nm in the CD spectra (Figure 5). A fluoro-alkene isostere of Lys-Lys has been incorporated into three different positions of a SC35EK-like peptide.[37] All three mimics show correct α-helical signature with almost equal intensity.[37] In the presence of N36, the CD signatures of the fluoro-alkene mimics were identical to SC35EK.[37] The stability of the triple helix was determined by thermal unfolding. The loss of ellipticity (θ) of the CD peak at 226 nm with increasing temperature from 5 °C to 85 ºC was measured (Figure 6). The data was fit to a two- state model to calculate T_m. The fluoro-alkene peptide **2-(*R*,*Z*)** was found to have a T_m value of +42.2 ± 0.4 ºC (Figure 6). The control peptide **3** had a T_m value of +48.4 ± 0.5 °C, close to the literature melting temperature (T_m +50.0) of a collagen-like peptide with the same sequence (Figure 6).[12]

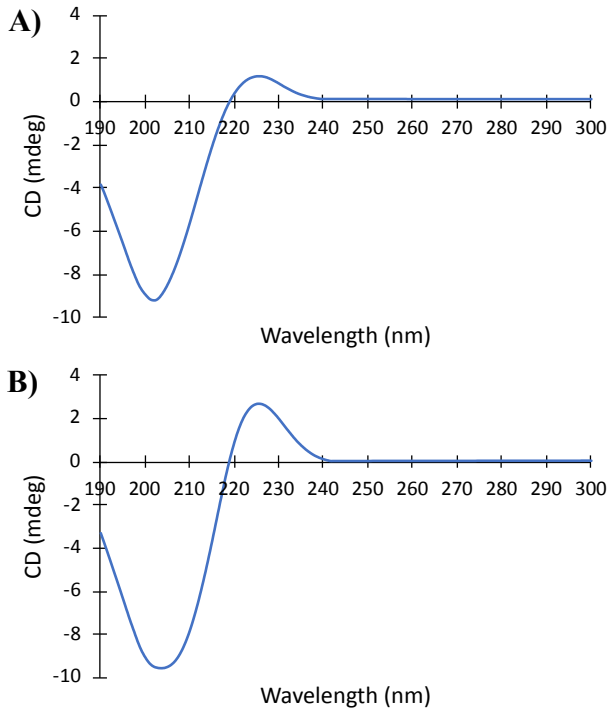


Figure 5. Full-range CD spectra at 5 °C of **A)** fluoro-alkene peptide **2-(*R*,*Z*)** (0.097 mM) and **B)** native control peptide **3** (0.12 mM).

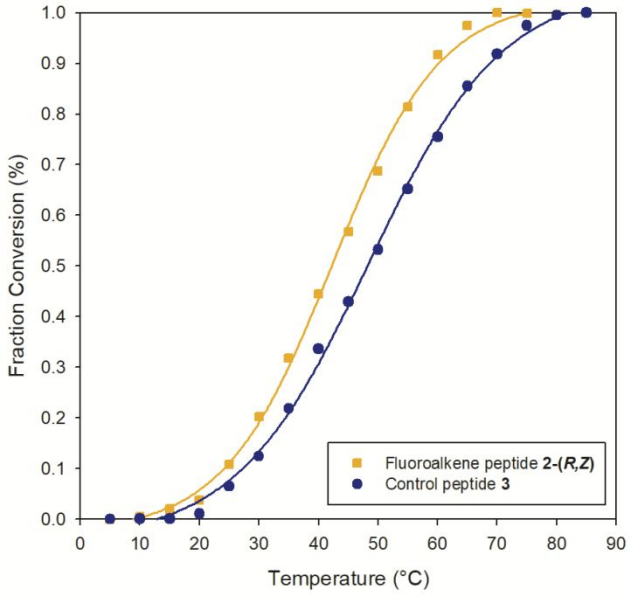


Figure 6. Thermal melting curves (T_m) of the fluoro-alkene peptide **2-(*R*,*Z*)** (■) and control peptide **3** (●) measured by CD at 226 nm.

The collagen-like peptide **2-(*R*,*Z*)** with a mimic of Gly-L-Pro, that showed a sigmoidal melting curve. The diastereomer **2-(*S*,*Z*)** showed a linear decrease in ellipticity, meaning that it did not form a stable triple helix (Figure S4). As Frey et al reported previously, only native collagen-like peptides that contain all L-amino acids form a stable triple helix—small percentages of D-Pro had much lower T_m values.[38] Since the diastereomeric peptide **2-(*S*,*Z*)** had a non-sigmoidal relationship between CD ellipticity and temperature, we assigned it the non-native D-Pro mimic stereochemistry. The Gly–D-Pro and Pro–D-Pro alkene isostere replacements showed similar non-sigmoidal temperature dependence in collagen-like peptides (Figure S4).[12, 13]

n→π* Interactions by ^{19}F NMR

Our theoretical results predicted that the fluoro-alkene might not behave as an n→π* donor because the fluorine atom rests just outside of the necessary distance and angle to the

acceptor carbonyl at the global minimum.[22] Furthermore, prior experimental and computational results predicted that the fluoro-alkene would be a poor n→π* acceptor.[22, 27] However, in this experimental work, ^{19}F NMR data showed significant deshielding of the fluorine nucleus (Figure 7). A downfield shift from –111.6 ppm to –76.0 ppm occurs between intermediates **12-(*Z*)** and **13-(*R*,*Z*)** in chloroform, which we think is caused by the loss of electron density through n→π* donation to the new Pro–Hyp carbonyl. After coupling of Hyp(^{t}Bu)–OBn, the newly-formed Pro–*trans*–Hyp amide C=O of **13-(*R*,*Z*)** is available as an n→π* acceptor, and the ^{19}F NMR peak shifted to –76.0 ppm reflects that deshielding (Figure 7).

After removal of the benzyl group, the ^{19}F rotamer peaks for **1-(*R*,*Z*)** in chloroform at –115.1 and –112.6 ppm increase, and the peaks at –72.6 and –75.8 ppm decrease in intensity (Figure 7). We speculate that the lack of the bulky benzyl ester permits easier cis-trans isomerization of the Pro–Hyp amide, making it less accessible as an n→π* acceptor. Increased flexibility in the carboxylic acid **1-(*R*,*Z*)** would make n→π* interaction more difficult, as suggested by this change in the relative intensities of the upfield and downfield ^{19}F peaks. We might expect decreased shielding of the ^{19}F nucleus with the carboxylic acid compared with the benzyl ester, but that is not the case; the n→π* interaction appears to be present with the benzyl ester.

The chemical shift of the peak at –75.6 ppm for the unfolded **2-(*R*,*Z*)** is similar to the peaks at –76.0 ppm of both tripeptides **13-(*R*,*Z*)** and **1-(*R*,*Z*)**, indicating similar n→π* interaction strength. Following purification, the unfolded collagen-like peptide **2-(*R*,*Z*)** in D_2O showed only two ^{19}F NMR signals at –75.6 ppm and –122.4 ppm, indicating single trans rotamers (Figure 7). We interpret these signals to correspond to part of the peptide that is already folded (–75.6 ppm, with an n→π* interaction) and part that is unfolded (–122.4 ppm, no n→π* interaction).

Following incubation at 4 °C for 72 h, the folded collagen-like peptide **2-(*R*,*Z*)** in D_2O had only a single peak at –77.0 ppm, suggesting that all fluorine atoms are deshielded by their participation in n→π* donation (Figure 7). The folded peptide **2-(*R*,*Z*)** peak at –77.0 ppm is slightly shielded relative to the unfolded peak at –75.6 ppm. We suggest this is due to the fluorine being buried in the triple helix. An n→π* interaction would explain the enhanced stability of the fluoro-alkene (T_m = +42.2 °C) over the previously reported proteo-alkene (T_m = +28.3 °C) collagen triple helix.[12]

Similar downfield shifts were not observed with the Gly–*trans*-Pro proteo-alkene mimic.[12] The alkene isostere Fmoc–Gly–Ψ[(E)CH=C]–Pro–OH had its alkene resonance at 5.56 ppm. Coupling of H–Hyp(tBu)–OBn gave the tripeptide Fmoc–Gly–Ψ[(E)CH=C]–Pro-Hyp(tBu)–OBn, which showed the proton signal shifted upfield to 5.35 ppm. Finally, removal of the benzyl group resulted in Fmoc–Gly–Ψ[(E)CH=C]–Pro–Hyp(tBu)–OH with the alkene signal at 5.34 ppm. We conclude that deshielding of the ^{19}F resonance was caused not by the alkene moiety, but by n→π* donation of electron density from the fluorine into the π* orbital of the following amide carbonyl (Figure 7). A similar shift was not seen with the proteo-alkene isostere.

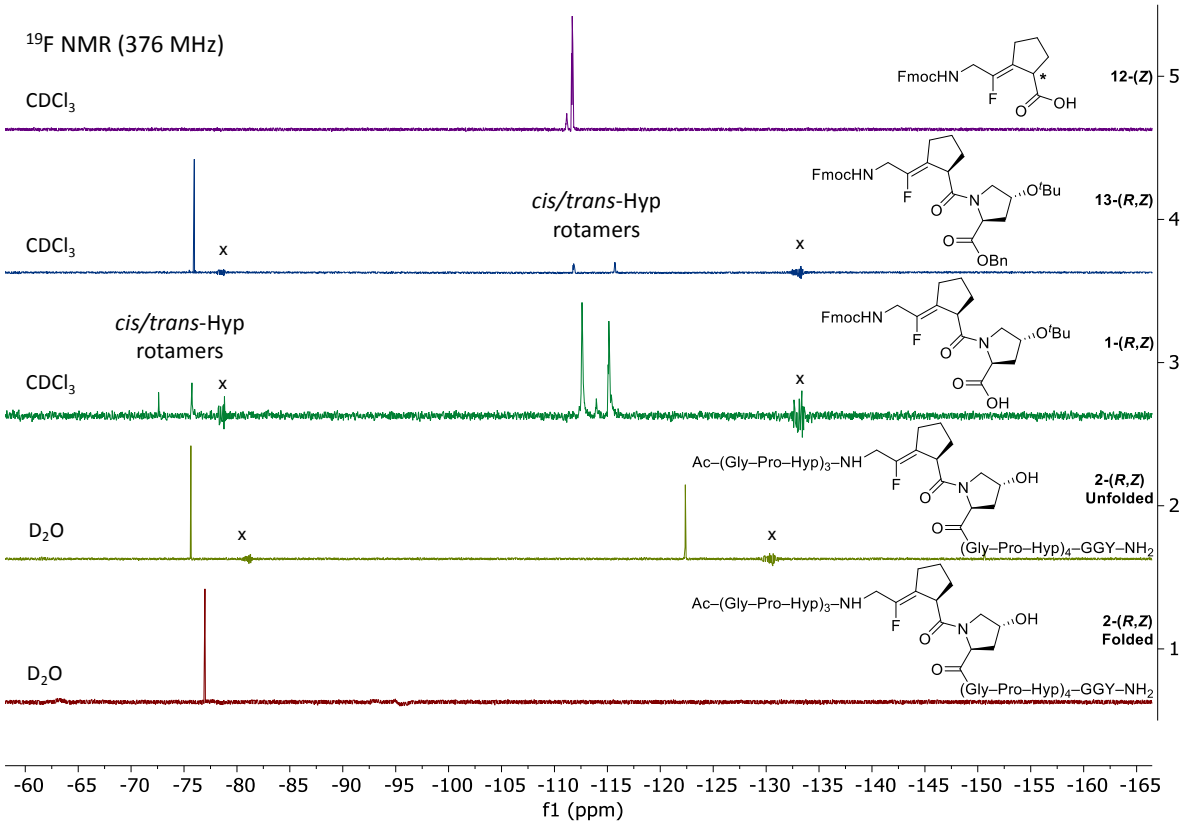


Figure 7. Stacked ^{19}F NMR spectra showing the increasingly deshielded fluorine nucleus. Pro–*cis/trans*-Hyp rotamer peaks are labeled. The ^{19}F NMR solvents are shown on the spectra. Instrument noise is marked with an x.

The T_m of peptide **2-(*R*,*Z*)** was slightly lower than control peptide **3** (ΔT_m = –6.2 °C). Previously, we found that replacement of one Gly–*trans*-Pro amide with a proteo-alkene isostere decreased T_m to +28.3 ºC (ΔT_m = –21.7 °C).[12] In our modeling, we found similar conformational energy landscapes of the fluoro-alkene and proteo-alkene as n→π* donors and acceptors with one key difference (Figure 2).[22] The proteo-alkene had additional local energy minima near the collagen minima as both an n→π* donor (Ψ +90°) and acceptor (Ψ –120°) that were not observed for the fluoro-alkene (Figure 2).[22] Our results implied that the extra flexibility

conferred by those local minima of the proteo-alkene significantly decreases the stability of the triple helix. Our calculations show that the fluoro-alkene model is less flexible near its PPII-like global minimum.[22] Since fluorine is bigger than hydrogen, steric interactions may contribute as well.[22] The fluoro-alkene peptide has ΔT_m +13.3 °C higher than the proteo-alkene peptide,[12] suggesting that an increase in stability is afforded by steric and n→π* electronic interactions.

Conclusions

A (*Z*)-fluoro-alkene isostere of Gly–*trans*-Pro was designed and synthesized, and its stability in a collagen-like triple helix peptide was measured by CD. The Boc–Gly–Ψ[(*Z*)CF=C)]–L/D-Pro–OH fluoro-alkene isostere **11-(*Z*)** was made in 8 steps and 27% overall yield. Collagen-like peptides **2-(*R*,*Z*)**, **2-(*S*,*Z*)**, and **3** were made by solid-phase peptide synthesis. Peptide **2-(*R*,*Z*)** was found to have T_m = +42.2 ± 0.4 °C compared to control **3** with T_m = +48.4 ± 0.5 °C, while **2-(*S*,*Z*)** had a linear relationship between CD ellipticity and temperature. The enhanced thermostability of the fluoro-alkene peptide compared to our previous proteo-alkene peptide[12] confirms our theoretical results,[22] suggesting the flexible Ψ angle of the proteo-alkene destabilized the collagen triple helix; the fluoro-alkene showed much less destabilization. Significant deshielding of the fluorine nucleus in the ^{19}F NMR indicates that n→π* donation confers a greater degree of stability on the collagen triple helix by mimic **2-(*R*,*Z*)** than the analogous proteo-alkene mimic of Gly–*trans*-Pro.[12] Since the fluoro-alkene is completely resistant to proteolytic degradation, this mimic could be used in biologically stable synthetic triple helices. The observed n→π* effect confers additional value in the use of fluoro-alkenes as isosteres of amide bonds more generally to stabilize peptides and proteins.

Experimental

General Methods. All reactions were performed under Ar or N_2 gas in oven-dried glassware.

All reagents and resins were obtained from commercial suppliers and used as received, unless otherwise stated. All amine bases were stirred over CaH_2 overnight and distilled prior to use. Solvents were dried using an Innovative Technology Pure Solv-MD solvent purification system. Brine (NaCl), $NaHCO_3$, and NH_4Cl refer to saturated aqueous solutions, unless otherwise stated. Organic solutions were concentrated under reduced pressure using a Büchi RE-111 rotary evaporator. SiO_2 chromatography was performed with SiliaFlash P60 silica gel (230-400 mesh) provided by Silicycle with HPLC-grade solvents. For compounds **1**, **4** – **17**, ^1H (400 MHz), $^{13}C\{^1H\}$ (100 MHz), and ^{19}F (376 MHz) NMR spectra were recorded on an Agilent MR-400 MHz NMR spectrometer in $CDCl_3$. For compounds **2** and **3**, ^1H (600 MHz) NMR spectra were recorded on Bruker Avance III 600 MHz, and ^{19}F (376 MHz) on Agilent MR-400 MHz spectrometers in D_2O. Chemical shifts for ^1H NMR are reported in ppm with reference to residual $CHCl_3$ at 7.26 ppm or TMS at 0.00 ppm. Proton-decoupled carbon chemical shifts are reported in ppm with reference to $CDCl_3$ at 77.0 ppm; the chemical shifts of minor rotamers, where resolved, are in parenthesis. ^{19}F NMR spectra were unlocked. NMR data are described as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), and integration). Structural assignments **6-(*Z*)** and **6-(*E*)** were made with additional information from gCOSY and 1D-nOe experiments. The gCOSY were obtained at 25 °C in $CDCl_3$ with 128 experiments of 8 scans and a 1 s relaxation delay. The 1D-nOe spectra were obtained at 25 °C in $CDCl_3$ with 256 scans and a 1 s relaxation delay. HRMS were acquired on an Agilent 6220 using ESI^+ with TOF analyzer. Optical rotations were measured on a Jasco P-2000 Polarimeter at the sodium D-line (589 nm); S aperture Φ 1.8, L aperture Φ 1.0.

Ketone 4. Cyclopentanone (48 mL, 540 mmol) and L-Thr (3.6 g, 30. mmol) were stirred in THF

(100 mL) at 30 °C for 1 h. Then a 37% formalin solution (7.5 mL, 100 mmol) was added dropwise. The reaction was stirred at 30 °C in an oil bath for 48 h, then diluted with EtOAc (50 mL), dried over Na_2SO_4, filtered, and concentrated. The resultant brown oil was dissolved in DMF (36 mL) with imidazole (10 g, 150 mmol) and TBS-Cl (11 g, 75 mmol) at 0 °C. After stirring for 14 h at rt, the reaction mixture was diluted with EtOAc (100 mL), washed with water (3 × 100 mL), dried over Na_2SO_4, and concentrated. Purification by SiO_2 chromatography (5 cm × 17 cm, 17% EtOAc/hexanes; R_f = 0.42) gave ketone **4** as a colorless oil (7.2 g, 63%). The ^{1}H NMR data matches the literature.[39] ($CDCl_3$, 400 MHz): δ 3.85 (dd, *J* = 9.8, 4.8 Hz, 1H), 3.73 (dd, *J* = 9.9, 3.3 Hz, 1H), 2.30 – 1.90 (m, 6H), 1.84 – 1.71 (m, 1H), 0.85 (s, 9H), 0.024 (s, 3H), 0.012 (s, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125MHz): δ 220.4, 62.2, 51.1, 39.3, 26.5, 26.0, 21.1, 18.4, –5.4, –5.5. HRMS (ESI$^+$/TOF) *m/z*: [M + H]$^+$ Calcd for $C_{12}H_{25}O_2Si^+$ 229.1618; Found 229.1608. $[\alpha]_D^{21}$ –13.3° (*c* 4.0, MeOH).

Esters 5-(*Z*) and 5-(*E*). A 3.0 M solution of MeMgCl in THF (2.4 mL, 7.1 mmol) was added dropwise to a stirring solution of triethyl 2-fluoro-2-phosphonoacetate (1.7 g, 7.0 mmol) in THF (30 mL) at –78 °C and stirred for 30 min. Ketone **4** (1.7 g, 7.2 mmol) in THF (20 mL) was added dropwise and the reaction was stirred for 21 h at 4 °C. It was then quenched with water (150 mL) and extracted with DCM (4 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by SiO_2 chromatography (5 cm × 18 cm, 1:10 EtOAc:hexanes; R_f = 0.40) provided esters **5-(*Z*)** and **5-(*E*)** as a colorless oil (1.8 g, 77%) in a mixture of diastereomers with ca. 2:1 *Z*:*E* ratio, as determined by ^{1}H NMR by integration of the diastereomeric protons: **5-(*Z*)**: δ 3.73 (dd, *J* = 9.9 Hz, 4.3 Hz, 2H) and **5-(*E*)** δ 3.64 (dd, *J* = 9.2 Hz, 4.1 Hz, 1H). A small amount was separated by SiO_2 chromatography for analysis (5 cm × 15 cm, 1:10 EtOAc:hexanes; R_f **5-(Z)** = 0.35; R_f **5-(*E*)** = 0.30). The ^{1}H NMR data matches

literature.[34, 40] **5-(*Z*)**: ($CDCl_3$, 400 MHz): δ 4.26 (q, *J* = 7.1 Hz, 2H), 3.74 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.50 (dd, *J* = 9.7, 8.3 Hz, 1H), 3.19 – 3.08 (m, 1H), 2.72 – 2.65 (m, 2H), 1.91 – 1.65 (m, 4H), 1.32 (t, *J* = 7.1 Hz, 3H), 0.87 (s, 9H), 0.029 (s, 3H), 0.017 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 161.4 (d, $^2J_{C,F}$ = 35 Hz), 142.4 (d, $^1J_{C,F}$ = 247 Hz), 141.4 (d, $^2J_{C,F}$ = 13 Hz), 63.2 (d, $^4J_{C,F}$ = 4 Hz), 61.1, 46.2, 31.2 (d, $^3J_{C,F}$ = 2 Hz), 28.7, 26.0, 24.8, 18.4, 14.4, –5.29, – 5.37; ^{19}F NMR ($CDCl_3$, 376 MHz): δ –127.2 (s). $[\alpha]_D^{21}$ –12.3° (*c* 4.0, MeOH). **5-(*E*)**: ^1H NMR ($CDCl_3$, 400 MHz): δ 4.28 (q, *J* = 7.1 Hz, 2H), 3.65 (dd, *J* = 9.2, 4.1 Hz, 1H), 3.48 (dd, *J* = 9.2, 8.1 Hz, 1H), 3.44 – 3.35 (m, 1H), 2.56 – 2.49 (m, 2H), 2.08 – 1.99 (m, 1H), 1.84 – 1.64 (m, 3H), 1.33 (t, *J* = 7.1 Hz, 3H), 0.88 (s, 9H), 0.035 (s, 3H), 0.019 (s, 3H).

Allylic Alcohols 6-(*Z*) and 6-(*E*). To a stirring solution of esters **6-(*Z*)** and **6-(*E*)** (1.4 g, 4.5 mmol) in THF (20 mL) at –78 °C, $LiAlH_4$ (1.0 M in THF, 14 mL, 14 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with EtOAc (100 mL), washed with water (3 × 50 mL) and brine (1 × 30 mL), dried over Na_2SO_4, and concentrated. Purification by SiO_2 chromatography (5 cm × 16 cm, 20% EtOAc:hexanes; R_f = 0.22) gave allylic alcohol **6-(*Z*)** (870 mg, 71%) and allylic alcohol **6-(*E*)** (270 mg, 22%) as colorless oils. **6-(*Z*)**: ^1H NMR ($CDCl_3$, 400 MHz): δ 4.27 – 4.07 (m, 2H), 3.75 (dd, *J* = 9.8, 4.4 Hz, 1H), 3.42 (dd, *J* = 9.8, 8.6 Hz, 1H), 2.98 (br s, 1H), 2.32 – 2.22 (m, 2H), 1.90 – 1.55 (m, 5H), 0.88 (s, 9H), 0.040 (s, 3H), 0.032 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 151.5 (d, $^1J_{C,F}$ = 245 Hz), 123.5 (d, $^2J_{C,F}$ = 15 Hz), 64.0 (d, $^4J_{C,F}$ = 4 Hz), 59.7 (d, $^2J_{C,F}$ = 31 Hz), 43.6, 29.41, 28.7 (d, $^3J_{C,F}$ = 5 Hz), 26.1, 24.9, 18.5, –5.20, –5.27; ^{19}F NMR ($CDCl_3$, 376 MHz) δ –119.8 (app t, $^3J_{H,F}$ = 21 Hz). HRMS (ESI^+/TOF) *m/z*: $[M + H]^+$ Calcd for $C_{14}H_{28}FO_2Si^+$ 275.1837; Found 275.1842. $[\alpha]_D^{21}$ –11.3° (*c* 4.0, MeOH). **6-(*E*)**: ^1H NMR ($CDCl_3$, 400 MHz): δ 4.27 – 4.09 (m, 2H), 3.52 (dd, *J* = 9.8, 5.9 Hz, 1H), 3.35 (dd, *J* = 9.8, 9.2 Hz, 1H), 2.99 – 2.83 (m, 1H), 2.83 (br s, 1H),

2.55 – 2.41 (m, 1H), 2.40 – 2.28 (m, 1H), 1.86 – 1.47 (m, 4H), 0.90 (s, 9H), 0.075 (s, 3H), 0.074 (s, 3H).

***N*-Boc-2-nitrobenzenesulfonamide 7.** By the method of Fukuyama et al.[35] 2-Nitrobenzenesulfonamide (510 mg, 2.5 mmol), Boc_2O (700 mg, 3.2 mmol), Et_3N (630 μL, 4.5 mmol), and a catalytic amount of DMAP (53 mg, 0.43 mmol) were dissolved in DCM (5.0 mL) and stirred at rt for 1 h. The reaction was quenched with 1 M HCl (12 mL), and the product was extracted with Et_2O (4 × 15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. Trituration with 40% Et_2O in hexanes (5 × 10 mL) gave **7** as a white solid (710 mg, 94%): ^{1}H NMR data matches the literature.[35] (CD_2Cl_2, 400 MHz): δ 8.31 – 8.27 (m, 1H), 7.88 – 7.77 (m, 3H), 1.42 (s, 9H); $^{13}C\{^1H\}$ NMR data matches the literature.[35] (CD_2Cl_2, 100 MHz): δ 149.2, 148.6, 135.5, 133.6, 133.0, 132.2, 125.6, 85.4, 28.1.

Sulfonamide 8-(*Z*). DIAD (570 mg, 2.8 mmol) in DCM (5 mL) was added dropwise to a solution of allylic alcohol **6-(*Z*)** (590 mg, 2.2 mmol), *N*-Boc-2-nitrobenzenesulfonamide **7** (830 mg, 2.8 mmol), and PPh_3 (720 mg, 2.8 mmol) in DCM (15 mL) at 0 °C and stirred for 1 h at rt. The solution was then diluted with EtOAc (75 mL), washed with water (3 × 50 mL) and brine (1 × 50 mL), dried over Na_2SO_4 and concentrated. Purification by SiO_2 chromatography (5 cm × 17 cm, 25% EtOAc/hexanes; R_f = 0.36) afforded sulfonamide **8-(*Z*)** as a colorless oil (1.1 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 – 8.25 (m, 1H), 7.78 – 7.70 (m, 3H), 4.53 (d, J = 17.2 Hz, 2H), 3.77 (dd, 9.8 Hz, 4.4 Hz, 1H), 3.39 (dd, 9.8 Hz, 8.9 Hz, 1H) 3.05 – 2.95 (m, 1H), 2.47 – 2.25 (m, 2H), 1.86 – 1.54 (m, 4H), 1.33 (s, 9H), 0.87 (s, 9H), 0.044 (s, 3H), 0.037 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 150.1, 148.4, 147.8, 146.0, 133.6, 133.3, 133.1 (d, $^1J_{C,F}$ = 237 Hz), 124.6, 124.2 (d, $^2J_{C,F}$ = 14 Hz), 85.4, 63.8 (d, $^4J_{C,F}$ = 4 Hz), 45.5 (d, $^2J_{C,F}$ = 29 Hz), 43.9, 29.3, 28.6 (d, $^3J_{C,F}$ = 5 Hz), 27.9, 26.0, 24.8, 18.4, –5.20, –5.26; ^{19}F NMR ($CDCl_3$, 376 MHz): δ

–118.5 (app t, $^{3}J_{H,F}$ = 17 Hz). HRMS (ESI^{+}/TOF) *m/z*: [M + H]$^{+}$ Calcd for $C_{25}H_{40}FN_2O_7SSi^{+}$ 559.2304; Found 559.2301. $[\alpha]_D^{21}$ +2.0° (*c* 4.0, MeOH).

Boc-amine 9-(*Z*). Thiophenol (280 mg, 2.5 mmol) and Cs_2CO_3 (1.3 g, 4.0 mmol) were stirred in DMF (100 mL) at 0 °C for 15 min. A solution of sulfonamide **8-(*Z*)** (780 mg, 1.4 mmol) in DMF (75 mL) was then added dropwise and stirred for 1 h at rt. The mixture was then diluted with EtOAc (100 mL), washed with water (4 × 100 mL) and brine (1 × 50 mL), dried over Na_2SO_4 and concentrated. Purification by SiO_2 chromatography (5 cm × 17 cm, 19% EtOAc/hexanes; R_f = 0.48) provided Boc-amine **9-(*Z*)** as a colorless oil (420 mg, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.78 (bs, 1H), 3.93 – 3.75 (m, 2H), 3.70 (dd, *J* = 9.8 Hz, 4.4 Hz, 1H), 3.38 (dd, *J* = 9.8 Hz, 8.6 Hz, 1H), 2.97 – 2.87 (m, 1H), 2.34 – 2.20 (m, 2H), 1.87 – 1.48 (m, 4H), 1.42 (s, 9H), 0.86 (s, 9H), 0.026 (s, 3H), 0.019 (s, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 155.7, 149.4 (d, $^{1}J_{C,F}$ = 246 Hz), 122.5 (d, $^{2}J_{C,F}$ = 15 Hz), 79.9, 64.0 (d, $^{4}J_{C,F}$ = 4 Hz), 43.6, 40.0 (d $^{2}J_{C,F}$ = 29 Hz), 29.5, 28.8 (d, $^{3}J_{C,F}$ = 5 Hz), 28.5, 26.1, 24.8, 18.4, –5.21, –5.28; ^{19}F NMR ($CDCl_3$, 376 MHz): δ –117.2 (app t, $^{3}J_{H,F}$ = 20 Hz). HRMS (ESI^{+}/TOF) *m/z*: [M + H]$^{+}$ Calcd for $C_{19}H_{37}FNO_3Si^{+}$ 374.2521; Found 374.2518. $[\alpha]_D^{21}$ –7.2° (*c* 4.0, MeOH).

Alcohol 10-(*Z*). Boc-amine **9-(*Z*)** (1.1 g, 2.9 mmol) and Bu_4NF (4.9 g, 17.5 mmol) were stirred in THF (25 mL) for 18 h at rt. The solution was then quenched with NH_4Cl (50 mL), and the product was extracted with DCM (50 × 3 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by SiO_2 chromatography (5 cm × 16 cm, 40% EtOAc/hexanes; R_f = 0.28) gave alcohol **10-(*Z*)** as a colorless oil (700 mg, 94%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.96 (bs, 1H), 3.84 (d, *J* = 20.0 Hz, 2H), 3.63 (dd, *J* = 10.7 Hz, 5.1 Hz, 1H), 3.50 (dd, *J* = 10.7 Hz, 7.4 Hz, 1H), 3.00 – 2.89 (m, 1H), 2.37 – 2.22 (m, 2H), 2.18 (bs, 1H), 1.84 – 1.52 (m, 4H), 1.40 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 155.8, 149.7 (d, $^{1}J_{C,F}$ =

259 Hz), 122.2 (d, $^{2}J_{C,F}$ = 14 Hz), 79.9, 64.4 (d, $^{4}J_{C,F}$ = 4 Hz), 43.7, 40.0 (d, $^{2}J_{C,F}$ = 29 Hz), 29.7, 28.7 (d, $^{3}J_{C,F}$ = 5 Hz), 28.5, 25.0; ^{19}F NMR ($CDCl_3$, 376 MHz): δ –116.8 (app t, $^{3}J_{H,F}$ = 20 Hz). HRMS (ESI$^+$/TOF) m/z: $[M + H]^+$ Calcd for $C_{13}H_{23}FNO_3^+$ 260.1656; Found 260.1644. $[\alpha]_D^{21}$ –8.8° (*c* 4.0, MeOH).

Acid 11-(*Z*). Jones reagent (2 M, 2 mL, 4 mmol) was added dropwise to a solution of alcohol **10-(*Z*)** (300 mg, 1.2 mmol) in acetone (60 mL) at 0 °C and stirred at 0 °C for 30 min. The reaction was quenched with *i*-PrOH (50 mL) and stirred at rt for an additional 10 min, then filtered through Celite and concentrated. The residue was diluted with EtOAc (25 mL) and washed with water (4 × 10 mL) and brine (1 × 10 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by SiO_2 chromatography (3 cm × 16 cm, 5% MeOH/DCM; R_f = 0.46) to give acid **11-(*Z*)** as a pale-yellow oil (280 mg, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.85 (br s, 1H), 4.81 (br s, 1H), 4.06 – 3.76 (m, 2H), 3.60 – 3.52 (m, 1H), 2.50 – 2.31 (m, 2H), 2.10 – 1.83 (m, 3H), 1.76 – 1.63 (m, 1H), 1.44 (s, 9H). ^{13}C{^{1}H} ($CDCl_3$, 100 MHz): δ 179.7, 155.8, 150.6 (d, $^{1}J_{C,F}$ = 250 Hz), 120.2 (d, $^{2}J_{C,F}$ = 15 Hz), 80.0, 45.5, 39.7 (d, $^{2}J_{C,F}$ = 28 Hz), 31.8, 28.5, 28.4, 25.9. ^{19}F NMR ($CDCl_3$, 376 MHz): δ –111.4 (app t, $^{3}J_{H,F}$ = 20 Hz). HRMS (ESI$^+$/TOF) m/z: $[M + Na]^+$ Calcd for $C_{13}H_{20}FNO_4Na^+$ 296.1274; Found 296.1277. $[\alpha]_D^{24}$ –15° (*c* 17, $CHCl_3$).

Fmoc Amine 12-(*Z*). To a solution of acid **11-(*Z*)** (270 mg, 0.98 mmol) in 96:2:2 DCM:H_2O:Et_3SiH (11 mL) was added TFA (4 mL) and stirred at rt for 40 min. The reaction was concentrated and residual TFA was removed by high vacuum overnight. The resultant dark yellow oil was dissolved in $NaHCO_3$ (20 mL) and stirred for 5 min, then a solution of Fmoc–OSu (500 mg, 1.5 mmol) in THF (10 mL) was added and stirred at rt for 16 h. The solution was acidified to pH = 2 with 1 M HCl and the product was extracted with DCM (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL) and brine (1 × 30 mL), dried over

Na_2SO_4 and concentrated. Purification by SiO_2 chromatography (5 cm × 15 cm, 2% AcOH/DCM; R_f = 0.14) gave Fmoc amine **12-(*Z*)** as a white solid (330 mg, 84% yield), mp 68 – 70 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz): 10.51 (br s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.24 – 5.16 (m, 1H), 4.55 – 4.34 (m, 2H), 4.28 – 4.16 (m, 1H), 4.15 – 4.00 (m, 1H), 3.96 – 3.81 (m, 1H), 3.63 – 3.42 (m, 1H), 2.62 – 2.22 (m, 2H), 2.18 – 1.79 (m, 3H), 1.77 – 1.55 (m, 1H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): 179.7, 156.4, 150.2 (d, $^1J_{C,F}$ = 250 Hz), 143.9, 141.4, 127.8, 127.1, 125.1, 120.6 (d, $^2J_{C,F}$ = 15 Hz), 120.7, 67.1, 47.2, 45.5, 40.1 (d, $^2J_{C,F}$ = 29 Hz), 31.8, 28.5, 25.9. ^{19}F NMR ($CDCl_3$, 376 MHz): –111.7 (app t, $^3J_{H,F}$ = 20 Hz). HRMS (ESI$^-$/TOF) m/z: [2M – H]$^-$ Calcd for $C_{46}H_{43}F_2N_2O_8^-$ 789.2993; Found 789.2988. $[\alpha]_D^{24}$ –1.61° (*c* 0.14, $CHCl_3$).

Benzyl Ester 13-(*Z*). HBTU (75 mg, 200 μmol), 6-Cl-HOBT (35 mg, 200 μmol), and Fmoc amine **12-(*Z*)** (52 mg, 130 μmol) were stirred in DCM (2.5 mL) at 0 °C for 10 min. A solution of H–Hyp(^{t}Bu)–OBn **15** (55 mg, 200 μmol) in DCM (0.5 mL) was added and the reaction was stirred at rt for 8 h. It was then filtered through Celite and concentrated, diluted with EtOAc (5 mL), washed with 0.01 M HCl (2 × 2 mL), $NaHCO_3$ (2 × 2 mL), and brine (2 × 2 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by SiO_2 chromatography (2 cm × 15 cm, 2.5% MeOH in DCM, R_f = 0.18) to give a diastereomeric mixture of **13-(*Z*)** as a pale-yellow oil (71 mg, 82%). The mixture of diastereomers (100 mg) was separated by $scCO_2$ LC by injecting 20 μL aliquots in *i*-PrOH on a Princeton SFC's pyridine column (60 Å, 5μ, 250 × 4.6 mm) on a TharSFC Fluid Delivery Module with eluent isocratic 15% *i*-PrOH/$scCO_2$ at 3.0 mL/min, 120 bar system back pressure, and column oven temperature at 40 °C. The diastereomers were obtained as off-white solids: **13-(*S*,*Z*)** (retention time 11.4 min, 10 mg) and **13-(*R*,*Z*)** (retention time 13.9 min, 4 mg) in a 2:5 ratio with 14% recovery (Figure S1). **13-(*R*,*Z*):**

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.12 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.37 – 7.27 (m, 7H), 5.21 (dd, *J* = 12.4, 10.3 Hz, 1H), 5.08 (dd, *J* = 14.9, 12.4 Hz, 1H), 5.03 – 4.93 (m, 0.77 H), 4.75 (t, *J* = 5.5 Hz, 0.55H), 4.51 (t, *J* = 7.8 Hz, 0.45H), 4.46 – 4.32 (m, 2H), 4.28 – 3.96 (m, 3H), 3.87 – 3.62 (m, 2.4H), 3.58 (dd, *J* = 10.2, 6.4 Hz, 0.45H), 3.44 – 3.32 (m, 1.55H), 2.50 – 2.29 (m, 3.8H), 2.08 – 1.52 (m, 4H), 1.17 (s, 4H), 1.12 (s, 5H). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 174.0, (172.8), 172.1, (171.5), 156.4, 149.0 (d, $^{1}J_{C,F}$ = 246 Hz), (144.03), 143.99, 141.5, 136.1, 135.5, 128.8, (128.6), 128.53, (128.48), 128.2, (128.1), 127.8, 127.2, 125.2, 123.8 (d, $^{2}J_{C,F}$ = 14 Hz), (122.0 (d, $^{2}J_{C,F}$ = 16 Hz)), 120.1, (74.24), 74.18, 69.7, 68.2, (67.1), (67.0), 66.7, (58.80), 58.76, 54.4, (54.0), 47.3, 44.3, (44.2), 40.3 (d, $^{2}J_{C,F}$ = 6 Hz), (40.2, (d, $^{2}J_{C,F}$ = 6 Hz)), 39.5, 37.3, 32.2, (32.0), 31.3, (30.8), 29.9, (29.7), 29.4, (28.9), (28.4), 28.3, 26.3, (26.1). ^{19}F NMR ($CDCl_3$, 376 MHz): δ (–76.0 (s)), (–111.8 (app t, $^{3}J_{H,F}$ = 20 Hz)), –115.7 (dd, $^{3}J_{H,F}$ = 20, 20 Hz). HRMS (ESI^{+}/TOF) m/z: $[M + H]^{+}$ Calcd for $C_{39}H_{44}FN_2O_6^{+}$ 655.3178; Found 655.3184. $[\alpha]_D^{24}$ +24° (*c* 0.32, $CHCl_3$).

13-(*S*,*Z*): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.37 – 7.27 (m, 7H), 5.24 – 5.01 (m, 3H), 4.81 – 4.72 (m, 0.1H), 4.64 (dd, *J* = 8.7, 4.2 Hz, 0.5H), 4.61 – 4.52 (m, 0.4H), 4.45 – 3.72 (m, 7H), 3.69 – 3.59 (m, 0.9H), 3.47 (dd, *J* = 9.7, 6.0 Hz, 0.5H), 3.42 – 3.32 (m, 1.1H), 2.51 – 2.23 (m, 2.5H), 2.23 – 2.19 (m, 0.15H), 2.18 – 1.49 (m, 6.5H), 1.15 (s, 9H). ^{13}C{^{1}H} NMR ($CDCl_3$, 125 MHz): δ 173.1, 172.3, 156.4, 149.0 (d, $^{1}J_{C,F}$ = 250 Hz), 144.03, 141.43, (135.92), 135.85, (128.9), 128.7, 128.61, 128.57, 128.4, 128.3, 127.8, 127.2, 122.4 (d, $^{2}J_{C,F}$ = 13 Hz), 120.1, (74.3), 74.2, 69.7, (69.44), 69.41, 67.09, (67.05), 66.94, (66.91), 57.9, 57.6, 57.3, 54.2, 54.0, 53.8, 47.3, 44.4 (d, $^{2}J_{C,F}$ = 6 Hz), 44.2, 40.3, 40.1, 37.1, 36.8, (31.32), 31.28, 30.8, 29.9, 29.1, 28.4, 36.1, (26.0), 25.5. ^{19}F NMR ($CDCl_3$, 376 MHz): δ (–76.0 (s)), –113.1 (app t, $^{3}J_{H,F}$, = 20 Hz), (–114.2 (t, *J* = 20 Hz)). HRMS (ESI^{+}/TOF)

m/z: $[M + H]^+$ Calcd for $C_{39}H_{44}FN_2O_6^+$ 655.3178; Found 655.3190. $[\alpha]_D^{24}$ –10° (*c* 0.80, $CHCl_3$).

Fmoc–Gly–Ψ[(*Z*)CF=C)]–Pro–Hyp(ᵗBu)–OH 1-(*R*,*Z*). 1,4-Cyclohexadiene (7 μL,70 μmol) was added to a suspension of the benzyl ester **13-(*R*,*Z*)** (4 mg, 6 μmol) and 10% Pd/C (1 mg) in anhydrous EtOH (250 μL) and the solution was heated to reflux at 90 °C in an oil bath for 45 min. After cooling to rt, it was filtered through Celite and diluted with Et_2O (1 mL). The organic phase was washed with 5% $NaHCO_3$ (3 × 0.5 mL) and brine (0.5 mL). The organic layer was dried over Na_2SO_4 and concentrated to recover protected tripeptide. The combined aqueous phases were acidified to pH = 2 with 0.1 M HCl, and the product was extracted with EtOAc (4 ×2 mL). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, and concentrated to give a white solid (3 mg, 80%). **1-(*R*,*Z*):** ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.38 – 5.05 (m, 0.64H), 4.70 –3.27 (m, 9.26H), 2.59 – 1.77 (m, 11H), 1.20 (s, 9H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 176.7, 172.7, 156.5, (156.6), 149.7 (d, $^1J_{C,F}$ = 247 Hz) 143.98, (143.95), 141.44, (141.43), 127.9, (127.23), 127.21, (125.3), 125.1, 121.8 (d, $^2J_{C,F}$ = 15 Hz), 120.13, (120.10), (74.6), 74.5, 69.3, (69.2), (67.2), 67.1, (58.99), 58.94, 54.0, 49.54, (49.49), 47.3, (47.2), 40.1 (d, $^2J_{C,F}$ = 30 Hz), (33.90), 33.88, (29.9), 28.4, (25.7), 25.0, 22.8. ^{19}F NMR ($CDCl_3$, 376 MHz): δ (–75.8 (s)), –112.6 (app t, $^3J_{H,F}$ = 19 Hz), (–115.1 (app t, $^3J_{H,F}$ = 20 Hz)). HRMS (ESI^+/TOF) m/z: $[M + H]^+$ Calcd for $C_{32}H_{38}FN_2O_6^+$ 565.2708; Found 565.2709.

Fmoc–Gly–Ψ[(*Z*)CF=C)]–Pro–Hyp(ᵗBu)–OH 1-(*S*,*Z*). Acid **1-(*S*,*Z*)** was prepared with benzyl ester **13-(*S*,*Z*)** (6 mg, 9 μmol), 1,4-cyclohexadiene (10 μL, 110 μmol) and 10% Pd/C (1 mg) using the same method to give a white solid (4 mg, 80%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.0 Hz, 2H), 5.33 – 5.05 (m, 0.4H), 4.70 – 4.46 (m, 0.6H), 4.47 – 4.29 (m, 2.4H), 4.27 – 4.18 (m, 1H), 4.16 –

4.00 (m, 0.4H), 3.72 (dd, J = 9.8, 6.8 Hz, 0.38H), 3.67 – 3.59 (m, 0.8H), 3.50 – 3.33 (m, 1.4H), 2.59 – 2.25 (m, 3.4H), 2.14 – 1.81 (m, 4H), 1.74 – 1.49 (m, 3.7H), 1.2 (s, 9H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 125 MHz): δ 176.7, 172.7, 156.5, (156.6), 149.7 (d, $^{1}J_{C,F}$ = 247 Hz) 144.0, 141.4, 127.9, (127.23), 127.21, (125.3), 125.1, 121.8 (d, $^{2}J_{C,F}$ = 15 Hz), 120.13, (120.10), (74.6), 74.5, 69.3, (69.2), (67.2), 67.1, (58.97), 58.94, 54.0, 49.54, (49.49), 47.3, (47.2), 40.1 (d, $^{2}J_{C,F}$ = 30 Hz), (33.90), 33.88, 32.1, (31.6), (29.9), 28.4, 28.3, (26.3), 26.2, (26.1), 25.7, 25.0, (24.9). ^{19}F NMR ($CDCl_3$, 376 MHz): δ –75.9 (s), (–112.6 (app t, $^{3}J_{H,F}$ = 19 Hz)), (–113.9 (app t, $^{3}J_{H,F}$ = 18 Hz)). HRMS (ESI^+/TOF) m/z: $[M + H]^+$ Calcd for $C_{32}H_{38}FN_2O_6^+$ 565.2708; Found 565.2708.

Fmoc–Hyp(*t*Bu)–OBn 14. By the method of Dai et al.[12] DCC (2.0 g, 9.7 mmol), 6–Cl–HOBt (1.7 g, 9.7 mmol), DMAP (120 mg, 0.97 mmol) and DIEA (3.4 mL, 19 mmol) were added to a solution of Fmoc–Hyp(*t*Bu)–OH (2.0 g, 4.9 mmol) in DCM (90 mL) at 0 ºC and stirred for 10 min. BnOH (1.0 mL, 9.7 mmol) was added and the reaction was stirred at rt for 16 h. The reaction was filtered through Celite and concentrated. The residue was diluted with EtOAc (40 mL), washed with 1 M HCl (2 × 20 mL), $NaHCO_3$ (2 × 20 mL), and brine (20 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by SiO_2 chromatography (5 × 15 cm, 20% EtOAc/hexanes) to give **14** as a colorless oil (2.4 g, 99%). ^{1}H NMR matches the literature.[12] ($CDCl_3$, 400 MHz): δ 7.79 – 7.72 (m, 1.85H), 7.63 – 7.50 (m, 2.15H), 7.42 – 7.28 (m, 9H), 5.29 – 5.15 (m, 1H), 5.15 – 5.04 (m, 1H), 4.71 (dd, J = 6.0, 1.6 Hz, 1H), 4.55 (dd, J = 9.8, 5.5 Hz, 0.5H) 4.49 (dd, J = 8.8, 4.6 Hz, 0.5H), 4.43 – 4.21 (m, 3.5H), 4.02 (t, J = 7.0 Hz, 0.40H), 3.80 (td, J = 11.4, 6.0 Hz, 1H), 3.36 (dd, J = 10.7, 5.1 Hz, 1H), 2.31 – 2.00 (m, 2H), 1.70 (t, J = 1.3 Hz, 0.5H), 1.18 (s, 5H), 1.16 (s, 4H).

H–Hyp(*t*Bu)–OBn 15. By the method of Dai et al.[12] Piperidine (9.8 mL) was added to a solution of Fmoc–Hyp(*t*Bu)–OBn **14** (2.4 g, 4.9 mmol) in DCM (40 mL) at rt and stirred for 40 min. The

reaction was then concentrated and purified by SiO_2 chromatography (5 × 15 cm, 1:2 EtOAc:hexanes 500 mL, then 5% MeOH/$CHCl_3$ 1.2 L) to give **15** as a pale-yellow oil (1.1 g, 81%). ^{1}H NMR matches the literature.[12] ($CDCl_3$, 400 MHz): δ 7.38 – 7.30 (m, 5H), 5.15 (d, *J* = 1.2 Hz, 2H), 4.17 – 4.1 (m, 1H), 3.97 (t, *J* = 7.7 Hz, 1H), 3.19 (dd, *J* = 11.2, 5.7 Hz, 1H), 2.77 (dd, *J* = 11.2, 4.3 Hz, 1H), 2.3 (br s, 1H), 2.03 (dd, *J* = 7.6, 5.5 Hz, 2H), 1.14 (s, 9H).

Fmoc–Gly–Pro–Hyp(*t*Bu)–OBn 16. The Fmoc–Gly–Pro–OSu unit was prepared by the method of Ottl et al.[36] H–Hyp(*t*Bu)–OBn **15** (730 mg, 2.6 mmol) in DMF (5 mL) was added to a solution of Fmoc–Gly–Pro–OSu (1.3 g, 2.6 mmol) and DIEA (225 μL, 1.3 mmol) in DMF (5 mL) and stirred at rt for 8 h. The reaction was then diluted with EtOAc (25 mL) and washed with 5% $NaHCO_3$ (3 × 10 mL), 0.1 M HCl (3 × 10 mL), H_2O (3 × 10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated. The crude product was purified by SiO_2 chromatography (5 cm × 15 cm, 2% MeOH/DCM) to give **16** as a pale-yellow oil (1.1 g, 59%). ^{1}H NMR matches the literature.[12] ($CDCl_3$, 400 MHz): δ 7.68 –7.49 (m, 4H), 7.32 – 7.18 (m, 9H), 5.93 – 5.77 (m, 1H), 5.16 – 4.96 (m, 3H), 4.62 (dd, *J* = 8.7, 4.2 Hz, 0.7H), 4.58 – 4.52 (m, 1H), 4.30 – 4.20 (m, 2.5H), 4.17 – 3.84 (m, 4.3H), 3.70 – 3.59 (m, 1.2H), 3.54 – 3.44 (m, 1.5H), 3.18 (br s, 1.2H), 3.11 (dd, *J* = 11.2, 5.6 Hz, 0.6H), 2.70 – 2.68 (m, 1H), 2.03 – 1.95 (m, 2.8H), 1.87 – 1.77 (m, 1.7H), 1.08 (s, 4.5H), 1.08 (s, 4.5H).

Fmoc–Gly–Pro–Hyp(*t*Bu)–OH 17. By the method of Dai et al.[12] A flask containing 10% Pd/C (100 mg) in MeOH (40 mL) was charged with a balloon of H_2. A solution of benzyl ester **16** (1.0 g, 1.5 mmol) in MeOH (14 mL) was added slowly and the reaction was allowed to stir for 16 h at rt. The reaction mixture was filtered through Celite and concentrated to give **17** as a white solid (870 mg, > 99%). ^{1}H NMR matches the literature.[12] ($CDCl_3$, 400 MHz): δ 10.01 (br s, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 6.3 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 2H),

6.40 – 5.90 (m, 1H), 4.68 – 4.48 (m, 2H), 4.48 – 4.15 (m, 4.4H), 4.15 – 4.00 (m, 1H), 4.00 – 3.83 (m, 1H), 3.82 – 3.13 (m, 4.6H), 2.26 – 1.83 (m, 6H), 1.15 (s, 6.8H), 1.14 (s, 2.2H).

General Purification and Characterization of Peptides

Each peptide was purified by reverse phase HPLC by injecting 20 μL aliquots in MeOH on a Kinetex 5 μm C18 column (250 × 10 mm) with an Agilent 1200 Series Gradient HPLC System using solvents A: 0.1% HCO_2H in H_2O and B: 0.1% HCO_2H in CH_3CN with 10% B for 5 min, then 10% – 54% B over 15 min at 3 mL/min, and UV detection at 280 nm. MALDI-TOF spectra of **2-(*R*,*Z*)**, **2-(*S*,*Z*)**, and **3** were collected in positive ion mode using α-cyano-4-hydroxycinnamic acid as matrix on a Bruker TOF Flex MALDI-2 mass spectrometer. ^{1}H NMR spectra of **2-(*R*,*Z*)**, **2-(*S*,*Z*)**, and **3** (Supplementary Information) were obtained in D_2O on a Bruker Avance III 600 MHz spectrophotometer equipped with a liquid nitrogen prodigy cryoprobe using the Bruker one-dimensional zgpr pulse sequence with water pre-saturation during the recycle delay of 2 s. Each dataset was averaged over 256 scans using 64k time-domain points. CD and T_m data were obtained on a Jasco model 815 spectropolarimeter.

Fmoc–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin. All reactions were done by shaking at 30 °C. Rink amide MBHA resin (100 mg, 0.37 mmol/g) was placed in a 10 mL polypropylene tube and swollen with DCM (5 mL) for 1 h. The resin was filtered and washed with NMP (3 × 5 mL) after every following step. The Fmoc group was then removed by shaking with 20% piperidine in NMP (5 mL) for 40 min. The deprotection step was repeated. To couple single amino acids (Gly, Gly, and Tyr) 6–Cl–HOBt (20 mg, 0.12 mmol), HBTU (44 mg, 0.12 mmol), DIEA (40 μL, 0.23mmol), and the Fmoc-amino acid (0.12 mmol) were dissolved in NMP (5 mL) and shaken with the resin for 30 min. Each coupling was repeated a second time. The first four repeats of Fmoc–Gly–Pro–Hyp(^{t}Bu)–OH (65 mg, 0.12 mmol) dissolved in NMP (5 mL) were

coupled to the resin with 6–Cl–HOBt (20 mg, 0.12 mmol), HBTU (44 mg, 0.12 mmol), and DIEA (40 μL, 0.23 mmol), by shaking for 30 min. Each coupling was repeated a second time. After every coupling, the peptide was capped by shaking with 10% Ac_2O and 10% DIEA in DCM (5 mL) for 20 min once. Each Fmoc group was removed by shaking with 20% piperidine in NMP (5 mL) for 10 min, then again for 20 min. At the end of every day, the resin was shrunk with MeOH, dried *in vacuo*, stored at 4 °C overnight, and swollen with DCM the next morning. The resin was then separated into three 10 mL polypropylene tubes.

Ac–(Gly–Pro–Hyp)$_3$–Gly-Ψ[(*Z*)CF=C]–L-Pro–Hyp–(Gly–Pro–Hyp)$_4$–Gly–Gly–Tyr–NH$_2$ 2-(*R*,*Z*). The Fmoc–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin was deprotected by shaking with 20% piperidine in NMP (5 mL) for 10 min, then again for 20 min. Fluoro-alkene **13-(*R*,*Z*)** (2.8 mg, 0.005 mmol), HOAt (2 mg, 0.016 mmol), HATU (6 mg, 0.016 mmol) and 2,4,6-collidine (4 μL, 0.032 mmol) were dissolved in NMP (0.4 mL) and shaken with a portion of the H–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin (13 mg) for 2 h once. The Fmoc group was removed by shaking with 10% piperidine in NMP (0.4 mL) for 10 min, then again for 20 min. The final three repeats of Fmoc–Gly–Pro–Hyp(^{t}Bu)–OH (9 mg, 0.016 mmol) dissolved in NMP (0.4 mL) were coupled to the resin with HOAt (2 mg, 0.016 mmol), HATU (6 mg, 0.016 mmol), and DIEA (5 μL, 0.029 mmol), by shaking for 30 min. Each coupling was repeated. Each Fmoc group was removed by shaking with 10% piperidine in NMP (0.4 mL) for 10 min, then again for 20 min. The peptide was capped by shaking with 10% Ac_2O and 10% DIEA in DCM (0.4 mL) for 20 min. The peptide was then cleaved from the resin by shaking with 2% H_2O and 3% triethyl silane in TFA (1 mL) for 3.5 h. The filtrate was collected, concentrated, then precipitated with cold Et_2O (ca. 3 mL) from a minimal amount of MeOH (ca. 1 mL) to give **2-(*R*,*Z*)** (9 mg). After HPLC separation, **2-(*R*,*Z*)** was obtained as a white solid, retention time

12.2 min, 1 mg, 11% recovery (Figure S2). ^{1}H NMR (D_2O, 600 MHz) is given in Supplementary Information. ^{19}F NMR data was obtained before and after incubation at 4 °C for 72 h (Figure 7). **2-(*R*,*Z*)** (pre-incubation) ^{19}F NMR ($CDCl_3$, 376 MHz): δ –75.6 (s), –75.7 (br s), –122.4 (s), –150.58 (s), –150.63 (s); **2-(*R*,*Z*)** (post-incubation) ^{19}F NMR ($CDCl_3$, 376 MHz): δ –77.0 (s). MALDI TOF **2-(*R*,*Z*)** $[M + Na]^+$ calcd. for $C_{112}H_{156}FN_{27}O_{36}Na$ 2497.1088, found 2497.0881; $[M + K]^+$ calcd. for $C_{112}H_{156}FN_{27}O_{36}K$ 2513.0827, found 2514.0687.

Ac–(Gly–Pro–Hyp)$_3$–Gly-Ψ[(*Z*)CF=C]–D-Pro–Hyp–(Gly–Pro–Hyp)$_4$–Gly–Gly–Tyr–NH$_2$ 2-(*S*,*Z*). The Fmoc–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin was deprotected by shaking with 20% piperidine in NMP (5 mL) for 10 min, then again for 20 min. Fluoro-alkene isostere **13-(*S*,*Z*)** (7.5 mg, 0.013 mmol), HOAt (6 mg, 0.043 mmol), HATU (16 mg, 0.043 mmol), and 2,4,6-collidine (11 μL, 0.085 mmol) were dissolved in NMP (1 mL) and shaken with a portion of the H–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin (24 mg) for 2 h once. The Fmoc group was removed by shaking with 10% piperidine in NMP (1 mL) for 10 min, then again for 20 min. The final three repeats of Fmoc–Gly–Pro–Hyp(^{t}Bu)–OH (24 mg, 0.43 mmol) in NMP (1 mL) were coupled to the resin with HOAt (6 mg, 0.043 mmol), HATU (16 mg, 0.043 mmol), and DIEA (10 μL, 0.053 mmol), by shaking for 30 min. Each coupling was repeated. Each Fmoc group was removed by shaking with 10% piperidine in NMP (1 mL) for 10 min, then again for 20 min. The peptide was capped by shaking with 10% Ac_2O and 10% DIEA in DCM (1 mL) for 20 min. The peptide was then cleaved from the resin by shaking with 2% H_2O and 3% triethyl silane in TFA (2.5 mL) for 3.5 h. The filtrate was collected, concentrated, then precipitated with cold Et_2O (ca. 3 mL) from a minimal amount of MeOH (ca. 1 mL) to give crude **2-(*S*,*Z*)** (51 mg). After HPLC separation, **2-(*S*,*Z*)** was obtained as a white solid, retention time 12.2 min, 4 mg, 8% recovery (Figure S2). ^{1}H NMR (D_2O, 600 MHz) is given in Supplementary Information. ^{19}F

NMR data was obtained before and after incubation at 4 °C for 72 h (Figure 7). **2-(*S*,*Z*)** (pre-incubation) ^{19}F NMR ($CDCl_3$, 376 MHz): δ –77.0 (s); **2-(*S*,*Z*)** (post-incubation) ^{19}F NMR ($CDCl_3$, 376 MHz): δ –75.7 (d, J = 1.3 Hz). MALDI TOF **2-(*S*,*Z*)** $[M + H]^+$ calcd. for $C_{112}H_{157}FN_{27}O_{36}$ 2475.1263, found 2475.1099; $[M + Na]^+$ calcd. for $C_{112}H_{156}FN_{27}O_{36}Na$ 2497.1088, found 2497.0918; $[M + K]^+$ calcd. for $C_{112}H_{156}FN_{27}O_{36}K$ 2513.0827, found 2513.0670.

Ac–(Gly–Pro–Hyp)$_8$–Gly–Gly–Tyr–NH$_2$ 3. The Fmoc–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin was deprotected by shaking with 20% piperidine in NMP (5 mL) for 10 min, then again for 20 min. The final five repeats of Fmoc–Gly–Pro–Hyp(^{t}Bu)–OH (27 mg, 0.016 mmol) in NMP (3 mL) were coupled to a portion of the H–(Gly–Pro–Hyp(^{t}Bu))$_4$–Gly–Gly–Tyr–MBHA-resin (30 mg) with HOAt (7 mg, 0.048 mmol), HATU (18 mg, 0.048 mmol), and DIEA (17 μL, 0.0097 mmol), by shaking for 30 min. Each coupling was repeated. Each Fmoc group was removed by shaking with 10% piperidine in NMP (3 mL) for 10 min, then again for 20 min. The peptide was capped by shaking with 10% Ac_2O and 10% DIEA in DCM (3 mL) for 20 min. The peptide was then cleaved from the resin by shaking with 2% H_2O and 3% triethylsilane in TFA (7.5 mL) for 3.5 h. The filtrate was collected, concentrated, then precipitated with cold Et_2O (ca. 3 mL) from a minimal amount of MeOH (ca. 1 mL) to give crude **3** (1.5 mg). (Note: mass lost during cleavage.) After HPLC purification, **3** was obtained as a white solid, retention time 12.1 min, 0.5 mg, 30% recovery (Figure S2). ^{1}H NMR (D_2O, 600 MHz) is given in Supplementary Information. MALDI TOF **3** $[M + H]^+$ calcd. for $C_{111}H_{157}N_{28}O_{37}$ 2474.1259, found 2474.1081; $[M + Na]^+$ calcd. for $C_{111}H_{156}N_{28}O_{37}Na$ 2496.1084, found 2496.0927; $[M + K]^+$ calcd. for $C_{111}H_{156}N_{28}O_{37}K$ 2512.0823, found 2512.0674.

Circular Dichroism

The concentration of the peptide in PBS (10 mM Na_xPO_4, 150 mM NaCl, pH 7.0) was determined by the UV absorption of the Tyr residue (ε = 1490 $M^{-1}\cdot cm^{-1}$) at 280 nm. The concentration of **2-(*R,Z*)** was 0.097 mM, **2-(*S,Z*)** was 1.45 mM, and **3** was 0.12 mM. The peptides were incubated at 4 °C for 72 h. The CD spectra were obtained in 0.5 nm increments, 1 nm bandwidth, and 1 mm pathlength at a scan speed of 100 nm/min. The spectra were averaged over three consecutive scans, and blank buffer scans were subtracted from the baseline. Full scan spectra from 190 to 300 nm were obtained from 5 °C to 85 °C (Figure 4), heated in 5 °C increments with a 5 min equilibrium time at each temperature change. The ellipticity at 226 nm was monitored at each temperature. The T_m of **2-(*R,Z*)** and control **3** were calculated by fitting the data to the following four-parameter Hill equation using SigmaPlot 10 (Figure 6):

$$F = \frac{\mathrm{F}_0 + (\mathrm{F}_{max} - \mathrm{F}_{min})}{(1 + \exp(-(T - T_m)/b))} \quad (1)$$

$$F = \frac{[\theta] - [\theta]_u}{[\theta]_u - [\theta]_f} \quad (2)$$

where $[\theta]$ is the measured ellipticity at 226 nm and temperature T, $[\theta]_u$ is the ellipticity at 85 °C, $[\theta]_f$ is the ellipticity at 5 °C, F is the fraction of unfolded peptide, b is the inflection point, and T_m is the calculated melting temperature.

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ASSOCIATED CONTENT

Supplementary Information

The data underlying this study are available in the published article and its online supplementary material. The following files are available free of charge.

Experimental for 2-(hydroxymethyl)-cyclopentanone and its derivatives, HPLC, CD spectra, T_m fitting parameters, ^1H, $^{13}C\{^1H\}$, ^{19}F, gCOSY, 1D nOe NMR spectra. Arcoria-fluoro-alkene-SI.pdf

CD data. Arcoria-fluoro-alkene-CD.xlsx

FAIR Data is available as Supplementary Information for Publication and includes the primary NMR FID files for compounds: **1 – 13** including separated stereoisomers, and 2-(hydroxymethyl)-cyclopentanone and its derivatives.

CONFLICTS OF INTEREST

There are no conflicts to declare.

ACKNOWLEDGMENT

The authors acknowledge the NSF for grant CHE-0749061. We thank Dennis Liotta (Emory University) for the use of his facilities and resources, and for support of PJA in determining the absolute stereochemistry of the aldol product and resynthesis of the control peptide **3**. We also thank Karla Piedl (Virginia Tech) for her help in obtaining the CD T_m of the control peptide **3** at 0.12 mM.

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