Fluorocyclopropane-Containing Proline Analogue: Synthesis and Conformation of an Item in the Peptide Chemist’s Toolbox

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ABSTRACT: Over the years, numerous modifications to the structure of proline have been made in order to tune its effects on bioactive compounds. Notably, the introduction of a cyclopropane ring or a fluorine atom has produced interesting results. Herein, we describe the synthesis of a proline containing fluorocyclopropane. This modified amino acid was inserted into a tripeptide, whose conformation was studied by nuclear magnetic resonance and density functional theory calculations.

INTRODUCTION

As a subunit of peptides, amino acids have attracted a great deal of interest from the scientific community. Indeed, combinations of the 21 natural amino acids are responsible for the wide variety of peptides that perform vital functions in all living matter. Among these amino acids, proline plays an important role in the peptide conformation. Indeed, in contrast to the other amino acids, proline forms a tertiary amide moiety, which is unable to act as a hydrogen bond donor with the other amino acids. This feature along with the rigidity of the five-membered ring increases the ratio of the cis conformer, which induces turns into the peptide structure, thus triggering interesting biological properties.1

Therefore, numerous studies have focused on the modification of the structure of the proline backbone to modulate these effects. Notably, the introduction of a cyclopropane ring increases the rigidity of the resulting modified proline and impacts its bioactivity. For example, 2,3-methanoproline is an inhibitor of 1-aminocyclopropane-1-carboxylic acid oxidase, which contributes to the biosynthesis of ethylene by plants.2 In addition, the cis-3,4-methanoproline inhibits the growth of Escherichia coli and Salmonella typhimurium,3 where this scaffold was integrated into the structure of an inhibitor of STAT3, a translation factor responsible for the growth of cancer cells.4 Furthermore, a nitrile-containing 4,5-methanoproline constitutes a part of the structure of saxagliptin, a medicine used in the treatment of type 2 diabetes (Figure 1).5

Another strategy to modify the structure of proline relies on the introduction of fluorine atom(s) at the carbon 3- and/or 4-position.6 Initially, this scaffold was used to gain insights into the post-translational modifications of the proline leading to 4-hydroxyproline in collagen.7 Owing to the so-called gauche effect of fluorine, the absolute configuration at carbon-4 has a significant impact on the conformation of 4-fluoroproline.8 This motif was used to study the conformation, stability, and folding of several proteins8 and was used as the core of a thrombin

Figure 1. Examples of bioactive compounds containing cyclopropane or fluorinated proline analogues.

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inhibitor. Notably, the introduction of this motif into the structure of neurotensin displayed interesting biological effects.

## RESULTS AND DISCUSSION

Stemming from our interest in the synthesis and applications of fluorocyclopropanes, we were eager to combine the features of cyclopropane and the fluorine atoms to access a new fluorocyclopropane-containing proline analogue (Figure 2).

![Figure 2. Suggested approaches for constructing the fluorocyclopropane-containing proline analogue.](image)

Indeed, this original scaffold might offer interesting conformation modifications or properties by combining the particular properties of both the fluorine atom and cyclopropane.

We postulated that this modified proline could be obtained via two different strategies: (1) the intermolecular cyclopropanation, followed by intramolecular amide bond formation (Figure 2, strategy a) or (2) the intramolecular cyclopropanation of a diazoacetamide (Figure 2, strategy b).

The intramolecular cyclopropanation reaction followed by intramolecular amide bond formation (Scheme 1) was first investigated. Pleasingly, the cyclopropanation of fluoroalkene 1a with ethyl diazoacetate 2a in the presence of 2 mol % Rh\textsubscript{2}(OPiv\textsubscript{4}) afforded (±)-trans-3a and (±)-cis-3a in 87% yield and a modest 3:1 trans/cis mixture after selective monodeprotection of the biscarbamate moiety. The saponification of (±)-trans-3a followed by the deprotection of the second carbamate group afforded the hydrochloride salt of the amino acid (±)-trans-4a. Unfortunately, the use of acidic or basic conditions was both unproductive to build up the five-membered ring. Similarly, the use of a coupling agent [e.g., T{P}_{1}, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, etc.] to access the amide bond was unsuccessful to construct the fluorocyclopropane-containing proline analogue. This low reactivity might be explained either by the strain induced by the adjacent cyclopropane ring and/or by the lower nucleophilicity of the amine residue, resulting from the presence of the fluorine atom at the vicinal position.

Alternatively, we turned our attention to the second approach based on the intramolecular cyclopropanation (Scheme 2). N-Benzyl allylamine 1b, N-(4-nitrobenzyl) allylamine 1c, and N-(4-chlorobenzyl) allylamine 1d were reacted with bromoacetyl bromide to afford the corresponding amides 2b–d. From 2b, the substitution of bromide with sodium azide and the subsequent Staudinger reaction gave access to amine 4b. From 2c–d, the substitution of bromide with NBoc\textsubscript{2} followed by the removal of the carbamate functions delivered the expected ammonium chloride of 4c–d. The latter 4b–d compounds were converted into the corresponding diazo compounds 5b–d after the reaction with sodium nitrite. Diazoo 5b underwent an intramolecular cyclopropanation reaction in the presence of Rh\textsubscript{2}(OPiv\textsubscript{4}) (0.5 mol %) to give (±)-6b in 55% isolated yield.

Having developed this intramolecular cyclopropanation reaction, the enantioselective version was investigated using chiral Rh and Ru catalysts (Scheme 3). The first screening of chiral Rh catalysts revealed that the amide-based Rh catalysts were the only complexes that furnished the target compound 6b. After a survey of the catalysts and the screening of the reaction conditions, it was found that Rh\textsubscript{2}((S)-MEPY\textsubscript{4})\textsubscript{d} afforded an interesting enantiomeric excess (ee) (62%) and a decent 53% isolated yield (Scheme 3, entry 4). Rh\textsubscript{2}((S)-BPTCP\textsubscript{d})\textsubscript{15} has been proven to be a highly efficient Rh catalyst for cyclopropanation, particularly in fluorinated series, delivered the expected cyclopropane from 5c in 92% isolated yield and 70% ee when the reaction was carried out in CHCl\textsubscript{3}, at −35 °C (Scheme 3, entry 13). We next examined the use of Ru-Pheox, which is also very efficient in many cyclopropanation reactions. When Ru-Pheox 3 (Scheme 3, entry 9) was used in the intramolecular cyclopropanation of 5b at 0 °C, the expected target was obtained with the best ee (77%) and in very good isolated yield (80%). Unfortunately, all attempts to improve either the yield or the ee were unsuccessful.

Hence, to tackle this limitation, the racemic mixture was resolved using preparative supercritical fluid chromatography (SFC) on a chiral stationary phase. This preparative separation was carried out on a 450 mg scale of the racemate. Compound (−)-6b was used to complete the synthesis of the fluorocyclopropane-containing proline analogue. Homologation of lactam (−)-6b was performed by reductive cyanation, leading to the formation of amino-nitrile (−)-cis-7b in high yield (70%) with good diastereoselectivity (4:1). The final hydrolysis of nitrile (−)-cis-7b under acidic conditions, followed by the deprotection of the amine, afforded the free amino acid (−)-cis-8b in 76% yield (Scheme 4).

To further study the effect of this fluorocyclopropane-containing proline on the conformation of peptides and more precisely on the cis/trans equilibrium of proline, (−)-cis-8b was then introduced into a tripeptide structure (Scheme 5). To this end, the amine moiety was protected with a Boc group, and the C-coupling was performed with alanine in the presence of (2-
Scheme 2. Strategy B: Synthesis of Diazoacetamide Followed by Intramolecular Cyclopropanation

Scheme 3. Catalytic Asymmetric Cyclopropanation Reaction to Access Enantioenriched 6b–d

The conformation of this tripeptide (−)-11 was studied using nuclear magnetic resonance (NMR) and density functional theory (DFT) calculations.

NMR Spectroscopy and DFT Calculations. The 1D 19F spectrum of tripeptide (−)-11 in CDCl₃ at 298 K showed two signals at −200.94 and −204.05 ppm, indicating the presence of two species with proportions of 75 and 25%, respectively (Figure S64). This was supported by the observation of two sets of N-H amide signals at 5.01/5.29 and 6.86/8.06 ppm in the 1D 1H spectrum of tripeptide (−)-11 in CDCl₃ at 298 K. The relative and absolute configuration of all stereogenic centers was confirmed by X-ray crystallographic analysis of this compound.

Finally, deprotection of the amine using HCl followed by N-coupling with a second alanine with HBTU led to the formation of tripeptide (−)-11 in 37% isolated yield.
spectrum, assigned to NH15 and NH5, respectively (Figure S66), leading to a repeated pattern of cross-peaks in the 2D NMR 1H−1H correlation spectroscopy (COSY) spectrum (Figure S70a). Moreover, additional cross-peaks, between each signal pair of NH15 and NH5 presumably due to the chemical exchange, were clearly observed in the 2D 1H−1H nuclear Overhauser effect spectroscopy (NOESY) NMR spectrum (Figure S70b). These observations suggested that the conformation of these two tripeptide (-)−11 species is sensitive to the “Ala−Pro” bond cis−trans isomerism.18 This hypothesis was confirmed by the presence of a NOE cross-peak between H7 and H13, and intense NOE cross-peaks between H13 and H10 for the minor cis conformer and the major trans conformer (Scheme 6 and Figure S71). All proton, fluorine, and carbon resonance assignments of the trans (Table S1) and cis (Table S2) conformers were made using the 2D NMR spectra, including 1H−1H COSY, 1H−1H NOESY, 1H−13C heteronuclear single quantum coherence (HSQC), 1H−19F heteronuclear NOESY (HOESY), and 1H−13C heteronuclear multiple bond correlation (HMBC) NMR spectra.

Examination of the short- and medium-range NOEs for the two isomers, in combination with the scalar coupling constants and the slow H/D exchange of the NH5 amide proton (Table S3), suggested a turn centered on the proline residue. According to the experimental observations (NOE and H/D exchange), interstrand hydrogen bonds C12=O⋯H5N for the trans conformer and C16=O⋯H5N for the cis conformer were tested as initial conformations in the DFT structure calculations using the Gaussian software.19 Analysis of the (Φ, Ψ) angles (Table S4) and the potential NOEs in the calculated structures (Figure 3 and Table S3) revealed that the sequence of compound (-)−11 consists of an inverse γ turn centered on the Ala-FPro-Ala segment for the trans isomer and a type β VIa1 turn centered on Boc-Ala-FPro-Ala segment for the cis isomers.20

In summary, a fluorocyclopropane ring-fused proline analogue was synthesized via carbene transfer reactions in the presence of a transition-metal chiral catalyst. The isomers were optically resolved and then converted to the corresponding tripeptides in high yield and then analyzed by spectral and computational chemical analyses. The resulting access to proline derivatives and elucidation of the structural and conformational details will be helpful for the design of a new medicinal entry for drug discovery.

■ EXPERIMENTAL SECTION

General Techniques. Air- and/or water-sensitive reactions were conducted in oven-dried glassware in argon. All solvents were distilled from appropriate drying agents prior to use.
All reagents were used as received from commercial suppliers unless otherwise stated. High-resolution mass spectroscopy (HRMS) spectra were recorded on a Waters LCT Premier, infrared (IR) spectra were recorded on a PerkinElmer Spectrum 100, and optical rotations were recorded on a PerkinElmer Polarimeter 341. NMR experiments were carried out by using Bruker DXC 300 and Bruker AVIII 500 or 600 MHz spectrometers (Bruker, Winsemund, France) equipped with a 10 A gradient amplifier giving a maximum gradient of 50 G cm⁻¹ and a 5 mm TXO (H,13C,19F) probe including shielded z-gradients for 500 MHz or a CPTXI (H,15N,13C) cryoprobe for 600 MHz. Measuring frequencies were 500 MHz or 600 MHz for 1H, 125 MHz for 13C, and 470 MHz for 19F. 1H and 13C NMR chemical shifts are reported in parts per million using the residual peak of CDCl₃ (7.28 and 77.0 ppm) as the internal standard. 19F NMR spectra were referenced (δ = 0.0 ppm) to calculate the 19F frequency in the CFCl₃ reference compound. 1D NMR Measurements. The proton one-dimensional experiments were performed with standard Bruker “zg” parameters using 16 scans. One-dimensional 13C{1H} NMR spectra with broadband proton decoupling in order to remove the 13C–H scalar coupling were recorded with standard Bruker “zggg” parameters. One-dimensional 19F{1H} NMR spectra with broadband proton decoupling in order to remove the 19F–H scalar coupling were recorded with standard Bruker “zggg” parameters. For 1H NMR experiments, the acquisition time was 4.54 s and the relaxation delay (D1) was 2 s; for 13C{1H} NMR experiments, the acquisition time was 1.05 s and the relaxation delay was 5 s; for 19F{1H} NMR spectra, the acquisition time was 14 s and the relaxation delay was 5 s. 2D NMR Measurements. 1H–1H COSY. The spectrum was acquired with 2048 data points in f2 and with 256 increments in f1 using 24 scans for each free induction delay (FID). The recycling delay was 2 s; the acquisition mode was DQD; and one-time zero filling in f1 and pure sine bell window function in f1 and f2 dimensions were applied before the Fourier transformation. 1H–13C HMQC. The spectrum was acquired with 2048 data points in f2 and with 256 increments in f1 using 16 scans for each FID. The recycling delay was 2 s; the acquisition mode was DQD; and one-time zero filling in f1 and pure sine bell window functions were applied to f2 and f1 dimensions before the Fourier transformation. 1H–13C HMBC. The spectrum was acquired with 2048 data points in f2 and with 256 increments in f1 using 32 scans for each FID. The delay for evolution of long-range couplings was 0.062 s and the recycling delay was 2 s; the acquisition mode was DQD; one-time zero filling in f1 and pure sine bell window functions were applied to f2 and f1 dimensions before the Fourier transformation. 1H–1H NOESY. The spectrum was acquired with 2048 data points in f2 and with 256 increments in f1 using 24 scans for each FID. The recycling delay was 3 s; pure-phase line shapes were obtained by using time proportional phase incrementation (TPPI) phase cycling, where the mixing time of 0.5 s was used; and one-time zero filling in f1 and pure sine bell window functions were applied to f2 and f1 dimensions before Fourier transformation. 1H–19F HHOESY. The spectrum was acquired with 2048 data points in f2 and with 256 increments in f1 using 32 scans for each FID. The recycling delay was 3 s; pure-phase line shapes were obtained by using TPPI (states-TPPI) phase cycling; where the mixing time of 1 s was used; and one-time zero filling in f1 and pure sine bell window functions were applied to f2 and f1 dimensions before the Fourier transformation. DFT calculations. Starting from the tripeptide (--) structure, we carried out the geometry optimization of the two possible trans and cis isomers using the DFT at the B3LYP/cc-PVTZ level of theory, followed by a frequency calculation at the same level of theory using the Gaussian software09.19

General Procedure for the Synthesis of N-Benzyl Allylamines 1c and 1d. In a sealed flask, the appropriate benzyl amine (2 equiv, 63.5 mmol) was added to a solution of K₂CO₃ (2 equiv, 63.5 mmol, 8.8 g) in acetone/tetra (25 mL), and then, 3-chloro-2-fluoroprop-1-ene (1 equiv, 31.7 mmol, 3.0 g) was added at 0 °C. The mixture was heated to 40 °C for 18 h. Water was added to the reaction mixture and extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (SiO₂, petroleum ether/ EtOAc) to afford the desired compound 1.

2-Fluoro-N-(4-nitrobenzyl)prop-2-en-1-amine (1c). Note: 4-nitrobenzylamine hydrochloride was used, and 1 more equivalent of the base was added. Purified by column chromatography on silica gel (SiO₂, petroleum ether/EtOAc 9:1 to 70:30), with Rf = 0.20 (petroleum ether/EtOAc 8:2); 1c was isolated in 44% yield (2.91 g) as an orange oil. 1H NMR (300 MHz, CDCl₃): δ 8.10 (dd, J = 8.1 Hz, 2H), 7.46 (dd, J = 8.1 Hz, 2H), 4.62 (dd, J = 17.0, 2.8 Hz, 1H), 4.40 (dd, J = 49.6, 2.8 Hz, 1H), 3.86 (s, 2H), 3.38 (d, J = 14.5 Hz, 2H), 2.07 (s, 1H). 13C{1H} NMR (75 MHz, CDCl₃): δ 163.5 (d, J = 258.5 Hz), 147.6, 146.9, 1128.6, 123.4, 91.7 (d, J = 18.3 Hz), 51.3, 48.6 (d, J = 30.2 Hz). 19F NMR (282 MHz, CDCl₃): δ −103.17 to −103.57 (m). IR (cm⁻¹): 3344, 2921, 2836, 1677, 1491, 1196, 1089, 1015, 931, 841, 799, 745. HRMS (ESI) m/z: [M + H]+ calc for C₁₀H₁₂FN₂O₂, 211.0877; found, 211.0879.

N-(4-Chlorobenzyl)-2-fluoroprop-2-en-1-amine (1d). Purified by column chromatography on silica gel (SiO₂, petroleum ether/EtOAc 9:1 to 70:30), 1d was isolated in 70% yield (4.44 g) as a pale-yellow oil. 1H NMR (300 MHz, CDCl₃): δ 7.44–7.18 (m, 4H), 4.70 (dd, J = 17.0, 2.9 Hz, 1H), 4.45 (dd, J = 49.6, 2.9 Hz, 1H), 3.79 (s, 2H), 3.32 (d, J = 14.5 Hz, 2H), 1.86 (s, 1H). 13C{1H} NMR (75 MHz, CDCl₃): δ 163.9 (d, J = 258.4 Hz), 138.3, 132.9, 129.6, 128.6, 91.8 (d, J = 18.5 Hz), 51.7, 48.7 (d, J = 30.1 Hz). 19F NMR (282 MHz, CDCl₃): δ −104.55 to −104.48 (m). IR (cm⁻¹): 3350, 2921, 2845, 1678, 1463, 1515, 1341, 1108, 850, 736. HRMS (ESI) m/z: [M + H]+ calc for C₁₀H₁₁ClF₂O₂, 201.0637; found, 201.0635.

General Procedure for the Synthesis of N-Benzyl-2-bromo-N-(2-fluoropropyl)acetamides 2b, 2c, and 2d. Triethylamine (1.5 equiv, 127.1 mmol, 17.7 mL) was added to a solution of the appropriate compound 1 (1 equiv, 84.7 mmol) in CH₂Cl₂ (250 mL) at −20 °C. Bromoacetyl bromide (1.2 equiv, 101.7 mmol, 8.9 mL) was added dropwise, and the mixture was stirred at −20 °C for 1 h. Water was added, and the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (SiO₂, petroleum ether/EtOAc) to afford the desired compound 2.

N-Benzyl-2-bromo-N-(2-fluoropropyl)acetamide (2b). Purified by column chromatography on silica gel (SiO₂, petroleum ether/EtOAc 9:1 to 70:30), with Rf = 0.59 (petroleum ether/EtOAc 8:2), 2b was isolated in 85% yield (20.38 g) as an orange oil. NMR analyses demonstrate the presence of two rotamers (a and b). 1H NMR (300 MHz, CDCl₃): δ 7.45–7.28 (m, 3H), 7.28–7.18 (m, 2H), 4.84 (dd, J = 16.4, 3.5 Hz, 0.5 Hz), 4.77 (dd, J = 16.7, 3.3 Hz, 0.45 Hz), 4.69 (s, 0.9H), 4.67 (s, 1.1H), 4.49 (d, J = 18.7 Hz), 4.47 (d, J = 16.4 Hz), 4.35 (s, 1H), 4.22 (s, 0.9H), 3.86 (s, 1H), 3.54 (s, 0.9H), 3.47 (s, 1H), 3.36 (s, 1H).
NMR analyses demonstrate the presence of rotamers (a and b) in a 55:45 ratio. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.46–7.31 (m, 3H), 7.31–7.14 (m, 2H), 4.85 (dd, \(J = 16.7,\ 3.5\) Hz, 0.55H), 4.77 (dd, \(J = 15.6,\ 3.1\) Hz, 0.45H), 4.69 (s, 1.1H), 4.55 (s, 0.9H), 4.50 (dt, \(J = 48.1,\ 3.7\) Hz, 1H), 4.19 (d, \(J = 14.7\) Hz, 0.9H), 4.05 (s, 1.1H), 3.98 (s, 0.9H), 3.98 (d, \(J = 11.0\) Hz, 1.1H). \(^{13}C\)\(^{1}H\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.1 (C\(_{a}\)), 167.9 (C\(_{b}\)), 160.6 (d, \(J = 259\) Hz, C\(_{a}\)), 159.7 (d, \(J = 259\) Hz, C\(_{b}\)), 156.1 (C\(_{b}\)), 153.2 (C\(_{a}\)), 150.3 (C\(_{b}\)), 128.9 (2C\(_{a}\)), 128.9 (2C\(_{b}\)), 128.3 (C\(_{a}\)), 128.0 (2C\(_{b}\)), 127.8 (C\(_{a}\)), 126.5 (2C\(_{b}\)), 93.6 (d, \(J = 17\) Hz, C\(_{b}\)), 93.1 (d, \(J = 17\) Hz, C\(_{a}\)), 51.6 (C\(_{a}\)), 48.2 (C\(_{b}\)), 47.4 (d, \(J = 33\) Hz, C\(_{b}\)), 44.4 (d, \(J = 33\) Hz, C\(_{a}\)), 26.1 (C\(_{a}\)), 26.0 (C\(_{b}\)). \(^{19}F\) NMR (282 MHz, CDCl\(_3\)): \(\delta\) -102.7 (dd, \(J = 49,\ 24,\ 17\) Hz, 0.45F, F\(_{a}\)), -103.6 (dd, \(J = 47,\ 22,\ 17\) Hz, 0.55F, F\(_{b}\)). IR (cm\(^{-1}\)): 3032, 1603, 1400, 1419, 1199, 847, 732, 697. HRMS (ESI) \(m/z:\) [M + H\(^{+}\)]\(^{+}\) calcd for C\(_{12}\)H\(_{16}\)BrFNO\(_3\), 286.0234; found, 286.0239.

**Synthesis of 2-Amino-N-benzyl-N-(2-fluorophenyl)-acetamide (4b).** Triphosphine (3 equiv, 47.4 mmol, 12.43 g) was added to a solution of 3b (1 equiv, 15.8 mmol, 3.92 g) in THF (200 mL) and water (12 mL). The mixture was stirred at room temperature for 1.5 h, and then, Et\(_2\)O was added. The organic layer was washed with brine, dried over MgSO\(_4\), filtered, and concentrated. The crude product was purified by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 95:5 to 9:1) to afford 3.03 g (86% yield) of amine 4b as a colorless oil. \(R_p = 0.35\) (CH\(_2\)Cl\(_2\)/MeOH 85:15). NMR analyses demonstrate the presence of two rotamers (a and b) in a 51:49 ratio. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.40–7.26 (m, 3H), 7.26–7.10 (m, 2H), 4.79 (dd, \(J = 17.1,\ 3.5\) Hz, 0.51H), 4.73 (dd, \(J = 17.7,\ 3.1\) Hz, 0.49H), 4.65 (s, 1.02H), 4.51 (s, 0.98H), 4.43 (dd, \(J = 47.6,\ 3.1\) Hz, 1H), 4.14 (d, \(J = 14.2\) Hz, 0.98H), 3.80 (d, \(J = 10.5\) Hz, 1.02H), 3.57 (s, 1.02H), 3.54 (s, 0.98H), 2.14 (br s, 2H). \(^{13}C\)\(^{1}H\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 167.5 (C\(_{a}\)), 167.2 (C\(_{b}\)), 161.7 (C\(_{a}\)), 161.2 (C\(_{b}\)), 158.3 (C\(_{a}\)), 157.7 (C\(_{b}\)), 147.7 (C\(_{a}\)), 147.4 (C\(_{b}\)), 143.9 (C\(_{a}\)), 143.0 (C\(_{b}\)), 128.4 (C\(_{a}\)), 127.4 (C\(_{b}\)), 124.3 (C\(_{a}\)), 124.0 (C\(_{b}\)), 94.2 (d, \(J = 17.4\) Hz, C\(_{b}\)), 93.8 (d, \(J = 17.0\) Hz, C\(_{a}\)), 51.1 (C\(_{a}\)), 48.5 (d, \(J = 32.2\) Hz, C\(_{a}\)), 48.2 (C\(_{b}\)), 45.7 (d, \(J = 32.5\) Hz, C\(_{b}\)), 25.8 (C\(_{a}\) + C\(_{b}\)). \(^{19}F\) NMR (282 MHz, CDCl\(_3\)): \(\delta\) -103.01 (dd, \(J = 48.3,\ 29.7,\ 13.4\) Hz, 0.23F, F\(_{a}\)), -104.10 (dd, \(J = 47.8,\ 16.2,\ 11.8\) Hz, 0.77F, F\(_{b}\)). IR (cm\(^{-1}\)): 2942, 1653, 1603, 1517, 1453, 1343, 1198, 1109, 936, 846, 735. HRMS (AP) \(m/z\): [M\(^{+}\)]\(^{+}\) calcd for C\(_{12}\)H\(_{12}\)FNO\(_2\), 338.0015; found, 338.0016.

**Synthesis of 2-Amino-N-(4-nitrobenzyl)-N-(2-fluorophenyl)-acetamide Hydrochloride (4c) and 2-Amino-N-(4-chlorobenzyl)-N-(2-fluorophenyl)-acetamide Hydrochloride (4d).** First step: 2c or 2d (1 equiv, 16.5 mmol) was added to a solution of NH\(_2\)Br (1 equiv, 19.8 mmol, 4.3 g) and K\(_2\)CO\(_3\) (2 equiv, 33.0 mmol, 4.6 g) in dimethylformamide (17 mL), and the mixture was stirred at 22 °C for 16 h. The mixture was extracted three times with EtOAc and then washed twice with water and once with brine. The organic phase was dried over MgSO\(_4\), filtered, and concentrated. The crude product was purified by column chromatography (SiO\(_2\), petroleum ether/EtOAc 9:1) to afford the desired compound 3c or 3d.

3c: purified by column chromatography on silica gel (SiO\(_2\), petroleum ether/EtOAc 9:1 to 70:30), \(R_p = 0.20\) (petroleum ether/EtOAc 8:2). 3c was isolated in 70% yield (2.26 g) as a white amorphous solid. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 0.84 (d, \(J = 8.1\) Hz, 0.64H), 7.95 (d, \(J = 8.2\) Hz, 1.38H), 7.39–7.20 (m, 2.34H), 4.73–4.47 (m, 3.53H), 4.47–4.19 (m, 2.70H), 4.00 (d, \(J = 13.7\) Hz, 0.6H), 3.86 (d, \(J = 11.5\) Hz, 1.4H), 1.33 (s, 18H). \(^{13}C\)\(^{1}H\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.5 (C\(_{a}\)), 168.1 (C\(_{b}\)),
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92.9 (d, J = 13.3 Hz, 0.77H), 7.13 (d, J = 16.6, 3.3 Hz, 1H), 4.54 (br s, 2H), 4.47 (dd, J = 16.5, 3.4 Hz, 1H), 1.83 (d, J = 16.5, 3.4 Hz, 1H), 0.39 (petroleum ether/EtOAc 65:35). 5c was isolated in 56% yield (0.70 g) as a yellow amorphous solid. 1H NMR (300 MHz, CDCl3): δ 8.14 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 5.07 (s, 1H), 4.75 (dd, J = 16.5, 3.4 Hz, 1H), 4.63 (s, 2H), 4.46 (dd, J = 48.2, 3.4 Hz, 1H), 3.87 (br s, 2H). 13C{1H} NMR (75 MHz, CDCl3): δ 166.7, 162.0, 158.6, 147.6, 144.4, 128.3, 124.1, 93.6 (d, J = 17.2 Hz, 44.9, 47.3, 46.9. 19F NMR (282 MHz, CDCl3): δ −103.65 (br s). IR (cm−1): 3068, 2983, 2108, 1733, 1605, 1520, 1434, 1413, 1351, 1251, 1200, 1044, 843. HRMS (ESI) m/z: [M + H]+ calc for C12H13FN3O, 279.0888; found, 279.0881. mp 56 °C.

N-(2-Fluorallyl)-N-(4-chlorobenzyl)diazooacetamide (5d). Purified by column chromatography on silica gel (SiO2, petroleum ether/EtOAc 9:1 to 70:30), with Rf = 0.24 (petroleum ether/EtOAc 8:2). 5d was isolated in 86% yield (1.05 g) as a yellow oil. 1H NMR (300 MHz, CDCl3): δ 7.26 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.3 Hz, 2H), 4.99 (s, 1H), 4.71 (dd, J = 16.6, 3.3 Hz, 1H), 4.53−4.29 (m, 3H), 3.96 (br s, 2H, 2H). 13C{1H} NMR (75 MHz, CDCl3): δ 166.4, 162.2, 158.8, 135.0, 135.3, 135.0, 129.3, 128.2, 128.8, 128.8, 127.7, 92.9 (m, C1 + C3), 92.2 (m, C1 + C3), 77.4 (C4), 49.0 (C4), 47.9 (C6), 46.8 (C6), 46.6 (C5), 45.9 (d, J = 33.2 Hz, 0.75H). 19F NMR (282 MHz, CDCl3): δ −103.09 (br s, 2H). IR (cm−1): 3238, 3084, 2963, 2107, 1676, 1602, 1431, 1406, 1199, 934, 843, 799. HRMS (ESI) m/z: [M + H]+ calc for C12H13ClFN4O, 268.0647; found, 268.0654.

Synthesis of 3-Benzyl-5-fluoro-3-azabicyclo[3.1.0]hexan-2-one [(−)-6b]. A solution of diazo 5b (1 equiv, 10.74 mmol, 2.505 g) in CH2Cl2 (75 mL) was added over 2.5 h to a solution of Ru2(OPy)6 (0.5 mol %, 0.054 mmol, 35 mg) in CH2Cl2 (25 mL) at room temperature. The mixture was concentrated, and the crude product was purified by column chromatography (SiO2, petroleum ether/EtOAc 85:15 to 7:3) to afford 1.8 g (85% yield) of lactam (±)-6b as a yellow oil. Rf = 0.34 (petroleum ether/EtOAc 70:30). The enantiomers of (±)-6b were separated using preparative SFC on a 450 mg scale. The two collected fractions were then analyzed using the following analytical conditions: column (Chiralpak AS-3, 3 μm, 10 × 4.6 mm), CO2/iPrOH = 90:10, and flow rate = 3.5 mL/min, tR1 = 0.86, and tR2 = 1.12 min. The second enantiomer [(−)-6b, tR2 = 1.12 min] was used to continue the synthesis. Analytical data for (−)-6b: 1H NMR (300 MHz, CDCl3): δ 7.40−7.26 (m, 3H), 7.25−7.16 (m, 2H), 4.39 (s, 2H), 3.66 (dd, J = 46.4, 10.4 Hz, 1H), 3.64 (dd, J = 31.1, 10.6 Hz, 1H), 2.50−2.36 (m, 1H). 13C{1H} NMR (75 MHz, CDCl3): δ 171.6, 136.1, 128.9 (2C), 128.2 (2C), 127.9, 78.4 (d, J = 245 Hz), 50.7 (d, J = 25 Hz), 46.0, 25.0 (d, J = 7 Hz), 18.0 (d, J = 12 Hz). 19F NMR (282 MHz, CDCl3): δ −197.77 (dtd, J = 15, 13, 6 Hz). IR (cm−1): 2919, 1684, 1453, 1319, 1177, 699. [α]D20 = −9.7 (c 0.39, CHCl3). HRMS (ESI) m/z: [M + H]+ calc for C12H13FNO, 206.0981; found, 206.0976. Conditions for the HPLC analysis of the racemate: column (Chiralpak IF-3, hexane/IPrOH = 20/1, and flow rate = 1.0 mL/min, and l = 220 nm), tR8 = 28.59 min (major), and tR9 = 32.16 min (minor).

Procedure for Enantioselective Cyclopropanation. Ruthenium Catalyst. To a solution of the Ru(II) catalyst (0.002 mmol) in CH2Cl2 (1 mL) was slowly added a solution of the diazo compound (0.2 mmol) in CH2Cl2 (1 mL) under an argon atmosphere at 0 °C for 1 h. The completion of the reaction was confirmed by thin-layer chromatography (TLC). After the reaction was completed, the solvent was removed under reduced pressure, and the residue was purified using column chromatography on silica (eluted with EtOAc/cyclohexane 10/90 to 30/70) to give the desired product.
Rhodium Catalyst. To a solution of the Rh(II) catalyst (0.002 mmol) in CH₂Cl₂ (1 mL) was added a solution of the diazo compound (0.2 mmol) in CH₂Cl₂ (1 mL) over 2.5 h under an argon atmosphere and stirred for 6 h. The completion of the reaction was confirmed by TLC. After the reaction was completed, the solvent was removed under reduced pressure, and the residue was purified using column chromatography on silica gel (eluted with EtOAc/cyclohexane 10/90 to 30/70) to give the desired product.

Synthesis of 5-Fluoro-3-(4-nitrobenzyl)-3-azabicyclo[3.1.0]hexan-2-one (6c). Compound 6c was synthesized according to the enantioselective cyclopropanation procedure using Ru2 as the catalyst. Purified by column chromatography on silica gel (SiO₂ eluted with EtOAc/cyclohexane 10/90 to 40/60), with Rf = 0.24 (cyclohexane/EtOAc 50:50), 6c was isolated in 64% yield as a white amorphous solid. 1H NMR (300 MHz, CDCl₃): δ 8.20 (d, J = 8.6 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 4.57–4.36 (m, 2H), 3.78–3.60 (m, 2H), 2.52–2.38 (m, 1H), 1.97–1.81 (m, 1H), 1.11–1.06 (m, 1H). 13C(C1) NMR (75 MHz, CDCl₃): δ 171.8, 147.7, 143.6, 128.8, 124.2, 79.9, 51.0 (d, J = 25.6 Hz), 45.5, 24.7 (d, J = 7.6 Hz), 18.24 (d, J = 11.8 Hz). 19F NMR (282 MHz, CDCl₃): δ −197.86 (ddd, J = 28.2, 15.3, 6.2 Hz). IR (cm⁻¹): 3082, 2929, 1681, 1513, 1456, 1347, 1327, 1221, 1179, 1029, 902, 846, 700, 680. HRMS (ESI) m/z: [M + H]⁺ calcd for C₁₄H₁₄FN₂O₄ 423.0927; found, 423.0927. HPLC analysis conditions: column (Chiralpak IC, heptane/iPrOH = 80/20, flow rate = 1.0 mL/min, and t₁ = 220 nm), t₁ = 16.89 min (major), and t₂ = 19.10 min (minor).

Synthesis of 3-(4-Chlorobenzyl)-5-fluoro-3-azabicyclo[3.1.0]hexan-2-one (6d). Compound 6d was synthesized according to the enantioselective cyclopropanation procedure using Ru2 as the catalyst. Purified by column chromatography on silica gel (SiO₂ eluted with EtOAc/cyclohexane 10/90 to 30/70), with Rf = 0.31 (petroleum ether/EtOAc 65:35), 6d was isolated in 72% yield as a colorless oil. 1H NMR (300 MHz, CDCl₃): δ 7.30 (d, J = 8.3 Hz, 2H), 7.13 (d, J = 8.3 Hz, 2H), 4.34 (s, 2H), 3.73–3.54 (m, 2H), 2.51–2.30 (m, 1H), 1.92–1.72 (m, 1H), 1.06–1.01 (m, 1H). 13C(C1) NMR (75 MHz, CDCl₃): δ 171.8, 134.7, 133.9, 129.4 (d, J = 34.0 Hz), 80.0, 50.77 (d, J = 25.5 Hz), 45.5, 25.0 (d, J = 7.6 Hz), 18.2 (d, J = 11.8 Hz). 19F NMR (282 MHz, CDCl₃): δ = −198.15 (ddd, J = 28.4, 15.4, 6.2 Hz). IR (cm⁻¹): 3088, 2929, 1685, 1491, 1455, 1408, 1314, 1178, 1056, 902. HRMS (ESI) m/z: [M + H]⁺ calcd for C₁₂H₁₂ClFNO 404.0586; found, 404.0585. HPLC analysis conditions: column (Chiralpak IC, heptane/iPrOH = 80/20, flow rate = 1.0 mL/min, and t₁ = 220 nm), t₁ = 8.42 min (major), and t₂ = 8.90 min (minor).

Synthesis of 3-Benzyl-5-fluoro-3-azabicyclo[3.1.0]hexane-2-carbontitrile ([−]cis-7b). A 1 M solution of disobutylaluminum hydride (DBAL) in hexane (1.2 equiv, 2.9 mmol, 2.9 mL) was added dropwise to a solution of [−]6b (1 equiv, 2.4 mmol, 500 mg) in THF (10 mL) at −35 °C. The mixture was stirred at −35 °C for 2 h, and then, trimethylsilyl cyanide (TMS-CN) (4 equiv, 9.8 mmol, 1.2 mL) was added. The mixture was slowly warmed to room temperature, quenched with 10 mL of Rochelle’s salt solution, and stirred for 30 min at room temperature. The aqueous layer was extracted three times with Et₂O, and the combined organic layers were dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (SiO₂, petroleum ether/EtOAc 99:1 to 91:2) to afford 368 mg (70% yield) of [−]7b as an 81:19 cis/trans mixture. Diastereoisomers were separated, and the major isomer (−)-cis-7b [colorless oil, Rf = 0.30 (petroleum ether/EtOAc 95:5)] was used to continue the synthesis. 1H NMR (300 MHz, CDCl₃): δ 7.38–7.24 (m, SH), 3.82 (dd, J = 46.2, 13.0 Hz, 2H), 3.59 (d, J = 3.6 Hz, 1H), 3.36 (dd, J = 8.6, 2.4 Hz, 1H), 3.14 (t, J = 7.9 Hz, 1H), 2.10–1.98 (m, 1H), 1.45–1.31 (m, 2H). 13C NMR (100 MHz, CDCl₃): δ 136.7, 128.8 (2C), 128.7 (2C), 127.9, 116.2, 81.6 (d, J = 242 Hz), 55.0, 53.1, 52.8, 23.3 (d, J = 7 Hz), 13.3 (d, J = 11 Hz). 19F NMR (282 MHz, CDCl₃): δ −204.0 to −204.2 (m, IR (cm⁻¹): 2814, 1438, 1215, 928, 699. [α]D = −126.8 (c 0.53, CHCl₃). HRMS (ESI): [M + H]⁺ calcd for C₁₄H₁₄FNO, 217.1141; found, 217.1146.
equiv, 0.69 mmol, 0.12 mL) and H-Ala-OMe (1.2 equiv, 0.23 mmol, 33 mg) were added, and the mixture was room temperature for 1.5 h. The mixture was diluted with CH₂Cl₂ and successively washed with 1 M HCl, saturated NaHCO₃, and water. The organic layer was dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (SiO₂, petroleum ether/CH₂Cl₂ 60:40) to afford 42 mg (65% yield) of dipeptide (−)-10. NMR analyses demonstrate the presence of two rotamers (a and b) in a 55:45 ratio. Rᵢ = 0.53 (petroleum ether/CH₂Cl₂ 50:50). ¹H NMR (500 MHz, CDCl₃): δ 7.08–6.87 (m, 0.55H), 6.64–6.44 (m, 0.45H), 4.68–4.46 (m, 1H), 4.23–3.92 (m, 2H), 3.84–3.65 (m, 4H), 2.26–2.05 (m, 1H), 1.67–1.47 (m, 1H), 1.47–1.35 (m, 12H), 0.78–0.67 (m, 1H). ¹³C{¹H} NMR (75 MHz, CDCl₃): δ 173.2 (C₁), 173.1 (C₉), 170.0 (C₆ + C₈), 155.0 (C₅), 153.9 (C₄), 81.7 (d, J = 239 Hz, C₁₄), 81.6 (C₁₅), 81.5 (C₉₅), 81.2 (d, J = 236 Hz, C₆), 62.9 (C₅), 61.7 (C₈), 52.6 (C₆ + C₈), 49.4 (d, J = 26 Hz, C₉), 49.1 (d, J = 28 Hz, C₇), 48.2 (C₆ + C₈), 28.3 (C₃ + C₄), 24.0 (m, C₇), 22.5 (d, J = 7 Hz, C₉), 18.7 (C₇), 18.3 (C₆), 15.4 (d, J = 11 Hz, C₉), 14.8 (d, J = 11 Hz, C₈). ¹⁹F NMR (282 MHz, CDCl₃): δ = -200.99 to -201.2 (m, 0.55F, F₂), -201.5 to -201.8 (m, 0.45F, F₂). IR (cm⁻¹): 3311, 2979, 1746, 1669, 1393, 1368, 1169, 1124. [α]D²⁰ = -100.0 (c= 0.22, CHCl₃). HRMS (ESI) m/z: [M + H]+ calcd for C₁₅H₂₄FN₂O₅, 331.1669; found, 331.1671.

Synthesis of (S)-Methyl 2-((1R,2S,5S)-3-((S)-2-((tert-Butyoxycarbonyl)amino)propanoyl)-5-fluoro-2-azabicyclo[3.1.0]hexane-2-carboxamido)propanoate [(-)-11]. 4 M HCl in dioxane (10 equiv, 1.06 mmol, 0.26 mL) was added to a solution of (−)-10 (1 equiv, 0.11 mmol, 35 mg) in MeOH (0.35 mL). The mixture was stirred at room temperature for 3.5 h and concentrated. CH₂Cl₂ (1 mL), Boc-Ala-H (1.2 equiv, 0.13 mmol, 24 mg), HBTU (1.2 equiv, 0.13 mmol, 48 mg), and DIPEA (3.5 equiv, 0.37 mmol, 0.06 mL) were successively added, and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with CH₂Cl₂ and successively washed with 1 M HCl, saturated NaHCO₃, and water. The organic layer was dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography (SiO₂, petroleum ether/CH₂Cl₂ 30:70) to afford 16 mg (37% yield) of tripeptide (−)-11. Trans proline (major 75%): ¹H NMR (500 MHz, CDCl₃): 6.86 (d, J = 6.80 Hz, 1H), 5.29 (d, J = 7.7 Hz, 1H), 4.55 (quint, J = 7.2 Hz, 1H), 4.45 (d, J = 5.2 Hz, 1H), 4.40 (quint, J = 7.0 Hz, 1H), 4.29 (d, J = 9.5 Hz, 1H), 3.76 (s, 3H), 4.00 (t, J = 8.8, 1H), 2.23 (ddd, J = 14.0, 10.2, 5.0 Hz, 1H), 1.54–1.65 (m, 1H), 1.43 (s, 9H), 1.39 (d, J = 7.2 Hz, 3H), 1.33 (d, J = 7.0 Hz, 3H), 0.81 (dd, J = 6.3, 11.9 Hz, 1H). ¹³C{¹H} NMR (125 MHz, CDCl₃): 173.0, 168.8, 155.1, 81.5 (d, J = 239 Hz, C₁₄), 81.4 (C₁₅), 81.2 (d, J = 236 Hz, C₆), 62.9 (C₅), 61.7 (C₈), 52.6 (C₆ + C₈), 49.4 (d, J = 26 Hz, C₉), 49.1 (d, J = 28 Hz, C₇), 48.2 (C₆ + C₈), 28.3 (C₃ + C₄), 24.0 (m, C₇), 22.5 (d, J = 7 Hz, C₉), 18.7 (C₇), 18.3 (C₆), 15.4 (d, J = 11 Hz, C₉), 14.8 (d, J = 11 Hz, C₈). ¹⁹F NMR (282 MHz, CDCl₃): δ = -200.99 to -201.2 (m, 0.55F, F₂), -201.5 to -201.8 (m, 0.45F, F₂). IR (cm⁻¹): 3311, 2979, 1746, 1669, 1393, 1368, 1169, 1124. [α]D²⁰ = -100.0 (c= 0.22, CHCl₃). HRMS (ESI) m/z: [M + H]+ calcd for C₁₅H₂₄FN₂O₅, 331.1669; found, 331.1671.
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