Synthesis and Conformational Properties of 3,4-Difluoro-L-prolines

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Supporting Information

ABSTRACT: Fluorinated proline derivatives have found diverse applications in areas ranging from medicinal chemistry over structural biochemistry to organocatalysis. Depending on the stereochemistry of monofluorination at the proline 3- or 4-position, different effects on the conformational properties of proline (ring pucker, cis/trans isomerization) are introduced. With fluorination at both 3- and 4-positions, matching or mismatching effects can occur depending on the relative stereochemistry. Here we report, in full, the syntheses and conformational properties of three out of the four possible 3,4-difluoro-L-proline diastereoisomers. The yet unreported conformational properties are described for (3S,4S)- and (3R,4R)-difluoro-L-proline, which are shown to bias ring pucker and cis/trans ratios on the same order of magnitude as their respective monofluorinated progenitors, although with significantly faster amide cis/trans isomerization rates. The reported analogues thus expand the scope of available fluorinated proline analogues as tools to tailor proline’s distinct conformational and dynamical properties, allowing for the interrogation of its role in, for instance, protein stability or folding.

INTRODUCTION

Fluorination of organic molecules has proven to be a highly useful tool for the manipulation of their conformational and electronic properties with minimal steric effects.1-7 Fluorination of the L-proline ring has been heavily exploited for conformational control of its ring pucker.8 For example, the five-membered proline ring conformation can be biased to either a C’ exo or a C’ endo pucker by introducing a (4R)-fluoro group (1, Figure 1) or a (4S)-fluoro group (2), respectively, an effect attributed to σCH → σCF hyper-conjugation interactions.9 Besides a ring pucker, fluorination also strongly influences the cis/trans ratio of the Xaa-Pro peptide bond relative to proline in a solvent-dependent way.10 The inductive effect of fluorine reduces the capacity for the nitrogen lone pair to conjugate with the amide carbonyl group and thus to contribute to the double bond character of the amide bond. As a consequence, the rotational energy barrier is decreased and accelerated cis/trans isomerization is observed.11-13 The same effect renders fluorinated prolines less basic11,13,14 and the carboxylic acid group more acidic.15

The combination of both conformational and dynamical effects make fluoroprolines valuable tools for determining the significance of proline’s unique structural properties within proteins or peptides.5,14 Nevertheless, the first syntheses of (4R)-FPro 1 and (4S)-FPro 2 date back to 1965,16 although it took until the late 1990s for this potential to be fully recognized. In a landmark study investigating the mechanism behind collagen stability,9,17,18 Raines and co-workers applied fluoroprolines to revise the origins behind the extraordinary

Figure 1. (a) (4R)-FPro 1 adopts an C’ exo pucker. (b) (4S)-FPro 2 adopts an C’ endo pucker. (c) The n → n* interaction stabilizes the trans isomer.

Received: November 15, 2018
Published: February 19, 2019
thermostability of this protein, which forms triple helices out of Pro-Hyp-Gly repeats. Replacing (4R)-4-hydroxyproline (Hyp) with (4R)-FPro led to a more thermostable collagen mimic, which, since fluorine is a weak hydrogen bond acceptor, disproved that a hydrogen bond network involving the hydroxyl moiety of Hyp induces collagen stability. In contrast, replacing Hyp with (4S)-FPro led to less stable collagen mimics. Since fluorine is more electronegative than a hydroxyl group, (4R)-FPro favors the C’’ exo pucker more strongly than Hyp, and because (4S)-FPro favors the C’’ endo pucker, this revealed that it is the strong preference for the C’’ exo pucker of Hyp that plays a key role for collagen stability. This ring pucker preorganizes the dihedral angles in such a way that a favorable n → π* interaction is promoted between the carbonyl groups of two adjacent peptide bonds, favoring the trans amide bond rotamer. Interestingly, the ring pucker of the Pro residue preceding Hyp is also relevant for collagen stability, which has equally been investigated using both 4- and 3-monofluorinated proline variants.

The case of collagen initiated many other demonstrations of the potential of proline fluorination to investigate the distinct structural and dynamical properties of proline residues within peptides and proteins, exploiting both the modulations of proline structure and cis/trans isomerization kinetics. Indeed, modulating these properties by fluorination, rather than just fully eliminating them by mutating proline to nonproline residues, can provide a more elegant approach toward uncovering the functional significance of proline’s unique properties. Moreover, the introduction of fluorine allows the use of 19F NMR as a powerful means to monitor residue-specific information. The exceptionally high responsivity of the 19F nucleus to changes in its (local) environment, in addition to the sparsity of the 19F spectrum, make 19F NMR a very attractive means to monitor protein structural and dynamical changes, enzyme catalysis, and ligand binding. Despite these clear advantages and earlier suggestions, to the best of our knowledge, there are only a very limited number of reports involving the full potential of 19F NMR in a fluoroproline peptide context. However, if the FPro residue is to be used purely as a 19F NMR probe, the conformationally perturbing effects of fluorine must be carefully considered. We recently introduced (3S,4R)-3,4-difluoroproline ((3S,4R)-FPro) 4 (Figure 2) where the two fluorines have opposing preorganizing effects, thus resulting in a proline analogue with minimal conformational bias and minimal homonuclear coupling complications for 19F NMR purposes.

Given the well-demonstrated importance of having fluoroprolines available with matching conformational, kinetic, and NMR properties for the application at hand, the synthesis of novel fluorinated variants in an optically pure form continues to be of interest. In addition, regardless of whether such applications require conformationally neutral, C’’ exo or C’’ endo pucker promoting fluoroprolines, the availability of more than one variant with similar conformational properties, but well-separated 19F NMR chemical shifts, is of interest for site-specific multiresidue-labeling strategies of proteins, especially in the case of low-complexity sequences found in proline-rich proteins such as collagen, but also many transcriptional activators. Hence, we envisaged a convenient synthesis of the 3,4-difluoro-1-prolines 4–7 (Figure 2), in order to expand the toolbox of proline analogues.

There exists only a limited precedence for such difluorinated proline analogues (Scheme 1). A Novartis patent describes the synthesis of N-Boc-7 in 14 steps from commercially available 3,4-dehydroproline 8. After epoxidation and acid-catalyzed epoxide opening, the key fluorination steps involve DAST-mediated deoxyfluorination reactions as shown in Scheme 1a. However, no yields or NMR data were reported. The second example (Scheme 1b) was published by Fleet and co-workers, where deoxyfluorination of 16 using XtalFluor-M/Et3N·3HF did not lead to the desired difluorinated azetidine derivative (not shown), but instead yielded the ring-expanded product 17. Deprotection of 17 led to (3R,4R)-3,4-difluoroproline 6. Hence, in both cases, the C–F bond introduction was achieved in sequential fashion. Finally, our group recently reported a stereoselective synthesis of Boc-protected (3S,4R)-3,4-difluoroproline (N-Boc)-4, which featured a direct bis-deoxyfluorination step (Scheme 1c). (3S,4S)-3,4-Dihydroxyproline 19a, obtained by selective dihydroxylation of the corresponding 3,4-dehydroproline, was treated with nonafluorobutanesulfonyl fluoride (NfF) in combination with tetrabutylammonium trifluorodifluorosilicate (TBAT) to yield 20a as the only observed 3,4-difluoroproline.

In this work, we describe in detail the synthesis of the yet unreported (3S,4S)-3,4-difluoroproline 5 and a novel, more concise route for (3R,4R)-3,4-difluoroproline 6, both as their N-Boc derivatives, and as their N-acetylated methyl esters 21 and 22 (Scheme 2). Following our earlier communication, the development of the synthesis of N-Boc-4, including further optimization efforts of the bis-deoxyfluorination step as well as a direct synthesis of (N-Fmoc)-4, is described. The ring pucker analyses, prolyl bond cis/trans ratios, and isomerization kinetics of 21 and 22 are described and compared to those of unmodified proline and the four known monofluorinated proline derivatives. Since 5/21 can be regarded as a combination of (4R)-FPro and (3R)-FPro, both known to be biased to the C’’ exo pucker and trans peptide bond configuration relative to proline, it was anticipated that 5/21 will display a conformational bias in the same direction. Similarly, 6/22 was expected to have a larger proportion of the C’’ endo pucker and of the cis peptide bond configuration relative to proline, as it is a combination of (4S)-FPro and (3S)-FPro.

**RESULTS AND DISCUSSION**

**Retrosynthetic Analysis.** Our retrosynthetic analysis of 3,4-difluoroprolines is outlined in Scheme 2. Functional group interconversion to epoxides 9a/b and 10a/b, as in the Novartis work, appeared attractive, as it would allow direct epoxide opening with fluoride followed by deoxyfluorination of the resulting fluoroxydrin. Alternatively, diol 19a/c provided an
approach for 3,4-difluorination, with an excellent precedence available from the Marson group, who obtained trans-3,4-difluoropyrrolidine from trans-3,4-dihydroxypyrrolidine via the corresponding triflates.38 While the epoxides and diols would be accessed from 3,4-dehydroderivatives,25a−c, direct functionalization of 25a−c such as vicinal difluorination or a halo/fluorination/halide displacement could also lead to the desired 3,4-difluoroprolines. 3,4-Dehydroproline is a commercially available (expensive) building block but can also be obtained by a well-described elimination process involving 26a−c starting from cheap (4R)-4-hydroxyproline. Finally, an electrophilic fluorination approach as recently described by Ciulli et al.39 leading to 27a/28a was also envisaged. With facile deprotection and versatility in mind, a benzyl ester in combination with various amine protecting groups were used throughout our investigations.

Scheme 1. Precedence for the Synthesis of 3,4-Difluoroprolines

Scheme 2. Retrosynthetic Analysis

3,4-Dehydroproline Synthesis. Initial efforts focused on achieving a large-scale synthesis of 3,4-dehydroproline 25. Following a literature protocol, conversion of protected (4R)-4-hydroxyproline 26a to the corresponding iodide, via a Mitsunobu reaction,40 followed by DBU-promoted HI elimination, gave a ±5:1 mixture of alkene regioisomers, from which the desired alkene 25a could be isolated in an excellent combined 76% yield (not shown), with 16% of the undesired 4,5-alkene 31a. While this elimination reaction gave 25a as a pure enantiomer (>97% ee, see Supporting Information), the separation of the alkene isomers was cumbersome. Moreover, it was found that conversion of 26b to the corresponding 4-OMs derivative 30b (Scheme 3), followed by elimination using the same base, led to a mixture (±2:1 ratio) of racemized alkene 25b and partially racemized 31b. A 89:11 ratio of amide rotamers of 31b was observed in the NMR spectra, with NOESY analysis showing the trans isomer being the major rotamer (see Supporting Information). Pleasingly, a one-pot Grieco elimination sequence41 directly starting from 26a gave enantiopure 25a as the major regioisomer with an increased regioselectivity (>10:1 ratio), and with a negligible degree of racemization. The smaller
determination of the epoxide stereochemistry was achieved by 
$^1$H NMR analysis as reported by Robinson et al. on N-Cbz-3,4-
epoxypoline benzyl esters (Supporting Information).$^{49}$
unambiguous conformation of the stereochemistry was obtained by X-ray crystallographic analysis of 9b (Supporting Information).

First, epoxide 10a was investigated as a substrate for direct fluoride opening with HF reagents (Table 1). Reaction with Et$_3$N·3HF in dichloroethane (DCE) at 80 °C for 3 days resulted in a complex mixture of chlorinated and fluorinated products ($\pm$15%), along with 68% of the recovered starting material (not shown), but conducting the reaction neat with increasing the reaction temperature to 130 °C (entry 1)
induced deprotection and aromatization, leading to pyrrole 35 in a quantitative yield. Due to its low reactivity, the use of Et$_3$N·3HF is often characterized by long reaction times and high reaction temperatures, which can be alleviated by microwave irradiation.$^{50}$ However, with a short reaction time, no product was observed and increasing the reaction time and temperature led to pyrrole 35 (entries 2–4). With the more reactive DMPU·HF,$^{51}$ reaction of 10b did lead to fluorohydrin 33b in a 15% yield, together with 30% of the recovered starting material (entry 5). Unfortunately, raising the reaction time and temperature did not improve the yield (entry 6). These reactions suffered from gel formation, which impeded the isolation of the products. The use of hexafluoroisopropanol (HFIP) as an additive successfully disrupted gel formation, but no fluorination was observed (not shown). Next, epoxide opening was attempted with Bu$_4$NH$_2$F$_3$. Unexpectedly, the reaction at reflux in DCE yielded chlorohydrin 32a (entry 7). Presumably, decomposition of the solvent under these conditions must have released chloride ions, which subsequently opened the epoxide. In toluene, Bu$_4$NH$_2$F$_3$ was found to be too basic, with fluoride causing H$^+$ deprotonation, leading to the formation of allylic alcohol 34a (entry 8). This was also the major pathway upon reaction with TBAF in t-BuOH (entry 9). Interestingly, in contrast to the 4,5-dehydro isomer 31b, the $^{13}$C and $^1$H NMR spectra of 34b only showed a single set of resonances, which could indicate the presence of a single rotamer. The NOESY NMR spectrum of 34b is consistent with the trans rotamer (Supporting Information).

With KHF$_2$ in ethylene glycol at 150 °C (entry 10), aromatization and transesterification was observed, yielding 36.

With direct fluoride opening being unsuccessful, it was then attempted to perform fluorination after prior epoxide opening with different nucleophiles (Scheme 5). Precedence for opening of proline epoxides includes reaction with MgI$_2$ (78%)$^{52}$ and 2-chloro-3-ethylbenzoxazolium tetrafluoroborate (32%),$^{53}$ both exclusively at the 4-position.

Starting from 10b, regioselective opening with HCl, HOTs, and HBr (or MgBr$_2$) led to the corresponding 4-substituted 3-hydroxyprolines 32b, 37b, and 38b in excellent yields. However, subsequent DAST-mediated deoxyfluorination reactions mostly led to aromatization: for the chlorohydrin 32b, pyrrole 39b was the only product isolated, while, with the β-hydroxy tosylate 37b, a low yield of the desired 3-fluorinated product 40b was obtained, alongside 62% of pyrrole 39b. Tentative assignment of the expected stereochemistry of 40b at C$_β$ was based on the observed coupling constant of 5 Hz between H$_α$ and H$_β$. Attempts to achieve fluorination at the 4-position in the presence of the 3-OH group by bromide or tosylate displacement with TBAF-t-BuOH were also unsuccessful. Starting from 37b, a mixture of allylic alcohol 34b and epoxide 10b was obtained. Despite the reduced basicity due to hydrogen bonding with t-BuOH, fluoride must have deprotonated the alcohol group of 37b causing epoxide formation, followed by H$_α$ deprotonation, resulting in epoxide opening to give 34b. Using bromohydrin 38b, the same allylic alcohol 34b was the only product isolated. Interestingly, treating 38b with AgF in nitromethane only led to epoxide formation in a quantitative yield.

Direct Bis-deoxyfluorination Approach. Dihydroxylation of Cbz-protected 3,4-dehydroproline 25d with OsO$_4$ has

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Scheme 3. Synthesis of Protected 3,4-Dehydroprolines 25a–c

![Scheme 3](image-url)

Scheme 4. Synthesis of Protected 3,4-Epoxyprolines

![Scheme 4](image-url)
been reported to be high-yielding and very stereoselective, leading to the 2,3-trans-2,4-trans-diol 19d as the major isomer.44,45,54 A similar result was observed when these conditions were applied to 25a (Table 2, entry 1). Interestingly, starting from the Boc-protected 25a with the osmate ester (entry 2), no all-cis-diol 41a was observed.34 As both diastereomeric cis diols were desired, attempts to promote the formation of all-cis-diol 41a using Sharpless asymmetric dihydroxylation55 conditions were carried out. However, reacting 25a with both AD-mix-α and AD-mix-β only led to the formation of 19a in 82% and 66% yields, respectively (entries 3 and 4). Finally, dihydroxylation was also carried out on the Fmoc-protected alkene 25c using the osmate ester conditions, also exclusively leading to N-Fmoc-protected 2,3-trans-2,4-trans-diol 19c (entry 5).

Marson et al. previously demonstrated that, starting from a trans-3,4-ditriflate substituted pyrrolidine ring 43 (Scheme 6), vicinal difluorination with TBAF can yield the corresponding trans-3,4-difluoropyrrolidine 44 in a good yield,38,56 and this transformation has also been successful on the corresponding Cbz derivative.57 However, treatment of 3,4-dihydroxyproline 19a with triflic anhydride already resulted in the formation of pyrrole 39a in a 64% yield. Hence, reaction with non-trifluorobutanesulfonyl fluoride (NfF)58 in combination with tetrabutylammonium difluorotriphenylsilicate (TBAT)59 was attempted, as this process generates sulfonates in the presence of fluoride. Pleasingly, this led to 20a as the only observed 3,4-di fluoroproline diastereoisomer (19F NMR analysis), with an enol sulfonate 46a as major byproduct along with its hydrolysis product, 3-oxoproline, as a minor, but persistent, impurity (not shown). Interestingly, no pyrrole side product was observed. While separation of all products was possible by HPLC, purification was considerably facilitated by subjecting the reaction mixture to NaBH4 in order to reduce the 3-oxoproline byproduct to the corresponding alcohol (not shown). The regiochemistry of enol sulfonate 46a was established by means of a 2D HOESY NMR experiment.

### Table 1. Conditions Investigated for the Direct Fluoride Opening of Epoxides 10a and 10b

<table>
<thead>
<tr>
<th>Entry</th>
<th>PG conditions</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc Et3N·3HF (neat) 130</td>
<td>72</td>
<td>35 (quant)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Boc Et3N·3HF/THF (2:1), MW 100</td>
<td>0.08</td>
<td>10a (86), 35 (7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Boc Et3N·3HF/THF (2:1), MW 100</td>
<td>0.33</td>
<td>10a (72), 35 (28)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Boc Et3N·3HF/THF (3:1), MW 130</td>
<td>0.66</td>
<td>35 (quant)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ac DMPU-HF, DCM</td>
<td>rt → 50</td>
<td>43</td>
<td>10b (30), 33b (15)</td>
</tr>
<tr>
<td>6</td>
<td>Ac DMPU-HF, DCE</td>
<td>60</td>
<td>72</td>
<td>10b (25), 33b (5)</td>
</tr>
<tr>
<td>7</td>
<td>Boc Bu4NH2F3, DCE</td>
<td>120</td>
<td>25</td>
<td>32a (74)</td>
</tr>
<tr>
<td>8</td>
<td>Boc Bu4NH2F3, toluene</td>
<td>120</td>
<td>24</td>
<td>34a (56)</td>
</tr>
<tr>
<td>9</td>
<td>Ac TBAF, t-BuOH</td>
<td>70</td>
<td>4</td>
<td>34b (30), 35 (13)</td>
</tr>
<tr>
<td>10</td>
<td>Boc KHF2, ethylene glycol</td>
<td>150</td>
<td>22</td>
<td>36 (59), 35 (2)</td>
</tr>
</tbody>
</table>

*aSevere gel formation. bCalculated yields based on 1H NMR analysis of the crude reaction mixture. cMicrowave irradiation.

### Scheme 5. Epoxide Opening with Other Nucleophiles and Subsequent Fluorination Attempts

![Scheme 5](image_url)

### Table 2. Dihydroxylation of 3,4-Dehydroproline 25a/c

<table>
<thead>
<tr>
<th>Entry</th>
<th>PG conditions</th>
<th>Yield 19a/c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc OsO4, NMO, H2O/dioxane (1:4)</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>Boc K2OsO4·2H2O, NMO, H2O/acetone (1:3)</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>Boc AD-mix α, t-BuOH/H2O (1:1)</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>Boc AD-mix β, t-BuOH/H2O (1:1)</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>Fmoc K2OsO4·2H2O, NMO, H2O/acetone (1:3)</td>
<td>80</td>
</tr>
</tbody>
</table>
As a Fmoc-protecting group does not tolerate basic conditions, TBAT could not be used as a fluoride source for the NIF fluorination. Even when (diluted) Et₃N·3HF/Et₃N was employed as a fluoride source, no difluorination was observed in the crude ¹⁹F NMR and pyrrole 35 was the only product obtained from the reaction.

The reaction of the 3,4-diols 19a and 19c was also investigated with DAST (Scheme 7). With 19a, this led to a complex reaction mixture, in which the desired difluorinated 20a was clearly visible by ¹⁹F NMR analysis, next to two minor byproducts, which presumably were monofluorinated hydroxy-fluoroprolines 47a. As the desired 20a coeluted with another byproduct, identified as the corresponding cyclic sulfate, the crude reaction mixture was subjected to typical oxidation conditions, leading to the formation of the cyclic sulfate 48a. Isolation was now possible, leading to 20a in a 26% yield. According to MS analysis, the sulfite oxidation was not accompanied by possible proline C5-oxidation to the corresponding lactam. Similarly, when this sequence was applied to the Fmoc-protected 19c, the desired 3,4-difluoroproline 20c was also isolated, albeit in a reduced 14% yield.

Despite the low yield of this double deoxyfluorination process, the very short synthesis (only three steps from protected (4R)-hydroxyproline) was deemed an acceptable
and practical synthesis, as gram-scale quantities of 20a could readily be obtained.

**Electrophilic Fluorination Strategy.** With no straightforward access to other 3,4-dihydroxyproline diastereoisomers as substrates for bis-deoxyfluorination, investigations turned toward an electrophilic fluorination approach. Barralough et al. had demonstrated the regioselective conversion of a 4-ketoproline derivative to the corresponding silyl enol ether, which was used to stereoselectively introduce deuterium at C3. Hence, formation of the silyl enol ether 49a was achieved upon treatment of 29a, synthesized by Dess–Martin periodinane oxidation of 26a in 94% yield (not shown), with LDA and TMSCl, and subsequently fluorinated with SelectFluor (Scheme 8). In our hands, this transformation proved to be low-yielding and was found difficult to optimize, leading to a mixture of isomers 27a/28a in a maximum 31% yield. Reduction of the 4-keto group led to a mixture of two separable fluorohydrin isomers, 50a and 51a, in a moderate yield. In the course of the optimization process, Ciulli and co-workers reported the synthesis of 27a/28a in 50% yield using this procedure, and of 50a/51a in 58% and 30% yields, respectively. Interestingly, they also isolated a third diastereomer. Preliminary assignment of the stereochemistry at C6 was based on the observed coupling constant between Hα and Hβ, which was ∼6 Hz for 50a and ∼2 Hz for 51a. This value for 50a is in line with the coupling constant observed between Hα and Hβ in 20a. In addition, for 50a, clear NOESY cross peaks were observed between Hα and Hδ and between Hδ and Hγ, suggesting all protons are on the same α-face of the pyrrolidine ring. This assignment was in agreement with the Ciulli work.

Deoxyfluorination of both 50a and 51a was achieved in a very good yield by treatment with the NIF and TBAT reagent combination. The stereochemistry of 24a was unambiguously assigned by means of X-ray analysis (Figure 3).

**Figure 3.** X-ray structure of (3R,4R)-3,4-difluoroproline 24a. Thermal ellipsoids drawn at the 50% probability level.

With the new 3,4-difluoroproline derivatives 23a and 24a in hand, conversion to the required N-acetyl methyl ester derivatives 21 and 22 was carried out to allow conformational studies, including comparison with other, known, N-acetylated fluoroproline methyl esters. Hence (Scheme 9), the benzyl-protecting group was removed by hydrogenolysis, and the N-Boc group by treatment with methanolic HCl. These conditions also simultaneously effected methyl ester formation. Finally, the amine groups were converted to their corresponding N-acetyl derivatives 21 and 22.

It was possible to obtain single crystals of 21, and crystallographic analysis (Figure 4) provided unambiguous proof of its relative stereochemistry.

**Scheme 9.** Synthesis of N-Acetyl Methyl Ester Derivatives 21 and 22

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**Conformational and Kinetic Analyses.** The experimental cis/trans ratios in chloroform and water, the experimental cis/trans isomerization rate constants in water, and the DFT-calculated pucker preferences for the N-Ac-X-OMe model compounds of proline, the (3S,4R)-, (3R,4R)-, and (3S,4S)-3,4-difluoroprolines and their monofluorinated progenitors are reported in Table 3. The entries are organized according to pucker preference. The data for the (3S,4R)-variant 56 has been reported and discussed previously, but are included in Table 3 for the sake of completeness. In the following discussion, the term “bias” assumes the conformational preference of the nonfluorinated N-acetyl proline methyl ester as a reference.

The amide cis/trans ratios in both chloroform and water of the 3,4-difluoroprolines 21 are very similar to those of each of their monofluorinated progenitors 52 and 53. For 22, the ratios are closer to those of (4S)-fluoroproline 54 than the (3S)-derivative 55.

The cis/trans isomerization rates (represented here by $k_{ex}$, $k_{ex} = k_{ex}(cis) + k_{ex}(trans)$) typically increase with an increasing number of fluorine substitutions, mostly due to the electron-withdrawing effect of the fluorine atoms decreasing the double bond character of the amide bond. As expected, both the (3S,4S)- and (3R,4R)-difluorinated variants, 21 and 22, indeed show higher isomerization rates than their monofluorinated progenitors. Interestingly, the (3R)-variant 53 has a markedly higher isomerization rate than all other monofluorinated prolines, and even exchanging faster than the (3R,4R)-difluorinated variant 22. This remarkable acceleration by fluorination at the 3-position with this stereochemistry is retained when combined with fluorination at the 4-position, resulting in even higher isomerization rates for the (3S,4S)-variant 21. The isomerization rate for 21 is also much higher than that of the previously described (3S,4R)- and (4S)-difluorinated variants.
and higher preference for the C\(^\gamma\) solvent are provided (Table 3). Unmodified proline has a higher preference for the C\(^\gamma\) endo than the C\(^\gamma\) exo puckers. Both (4S)- and (3S)-fluoroprolines, S4 and S5, strongly bias these puckers ratios to the C\(^\gamma\) endo form, with negligible C\(^\gamma\) exo puckers populations, both in chloroform and water. As expected, the (3R,4R)-difluoroproline variant 22 is heavily biased to the C\(^\gamma\) endo puckers as well, with essentially the same C\(^\gamma\) endo/C\(^\gamma\) exo ratio as that of its (3S)- and (4S)-progenitors. The (4R)- and (3R)-fluoroprolines, S2 and S3, are biased to the C\(^\gamma\) exo puckers relative to Pro, albeit to different degrees. Where the (4R)- variant S2 shows a similar C\(^\gamma\) exo bias in both solvents and for both trans and cis forms, the cis rotamer of the (3R)-variant S3 shows a high C\(^\gamma\) exo bias in chloroform, but a low bias in water. The (3S,4S)-difluorinated proline 21 shows a bias to the C\(^\gamma\) exo puckers in the same order of magnitude as its progenitors. Interestingly, especially in the cis rotamer, the C\(^\gamma\) exo puckers are highly populated in both solvents, even higher than in its trans rotamer and than in its progenitors.

Experimental verification of these computational results can in principle occur via analysis of vicinal scalar couplings. Unfortunately, \(J_{\text{HF}}\) couplings are known not to be practically exploitable to assess the dihedral angle,\(^{30}\) while quantitatively calculating the ringucker from experimental \(J_{\text{HF}}\) and \(J_{\text{HH}}\) couplings was in our hands found not to be reliable due to the limited accuracy of Karplus relations for difluorinated five-membered pyrrolidine rings. Instead, these couplings can be compared with theoretical values obtained using a similar procedure as Renner et al.\(^{33}\) Calculated values based on Renner et al.\(^{13}\) In good agreement with reported ratios by Siebler et al.\(^{10}\) In good agreement with reported ratios by Kim et al.\(^{68}\) Note that CIP prioritization changes with introduction of the second fluorine atom, so that 21 must be compared with S2 and S3, and 22 with S4 and S5. In good agreement with reported ratios by Siebler et al.\(^{10}\) In good agreement with reported ratios by Kim et al.\(^{68}\) Corresponding values reported by Thomas et al. at 37 °C using an alternative experimental procedure: 0.229 s\(^{-1}\) and 0.028 s\(^{-1}\). Corresponding values reported by Thomas et al. at 37 °C using an alternative experimental procedure: 0.065 s\(^{-1}\) and 0.016 s\(^{-1}\). DFT values, using the M06 functional with cc-pVDZ basis set and CHCl\(_3\) or SMD implicit solvent models.

Table 3. Experimental trans/cis Ratios and Amide Isomerization Rates and Calculated Pucker Ratios

<table>
<thead>
<tr>
<th>Compound</th>
<th>cis/trans kinetics, 35 °C (s(^{-1})) (exp)</th>
<th>C(^\gamma) endo/C(^\gamma) exo (DFT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(k_{\text{cis/trans}}) (exp) CHCl(_3)</td>
<td>H(_2)O</td>
</tr>
<tr>
<td></td>
<td>(k_{\text{trans/cis}}) (exp) CHCl(_3)</td>
<td>H(_2)O</td>
</tr>
<tr>
<td></td>
<td>(k_{\text{cis}}) (exp) CHCl(_3)</td>
<td>H(_2)O</td>
</tr>
<tr>
<td></td>
<td>(k_{\text{trans}}) (exp) CHCl(_3)</td>
<td>H(_2)O</td>
</tr>
<tr>
<td>Ac-Pro-OMe</td>
<td>3.85(^a) 4.62(^b) 0.031(^c) 0.007(^d) 0.038</td>
<td>81.19 90.10 66.34 82.18</td>
</tr>
<tr>
<td>(3S,4R)-S6</td>
<td>3.72 5.00 0.119 ± 0.009(^f) 0.025 ± 0.002(^f) 0.144 ± 0.011</td>
<td>41.59 78.22 56.44 90.10</td>
</tr>
<tr>
<td>(3R)-S3</td>
<td>5.08 8.31(^d) 0.141 ± 0.021(^c) 0.019 ± 0.003(^d) 0.159 ± 0.024</td>
<td>24.76 16.84 15.85 44.56</td>
</tr>
<tr>
<td>(4R)-S2</td>
<td>4.26(^a) 6.74(^b) 0.064(^c) 0.010(^d) 0.074</td>
<td>11.89 28.72 7.93 17.83</td>
</tr>
<tr>
<td>(3S,4S)-21</td>
<td>4.32 7.23 0.210 ± 0.005(^c) 0.031 ± 0.001(^c) 0.242 ± 0.006</td>
<td>19.81 11.89 20.80 9.91</td>
</tr>
<tr>
<td>(4S)-S4</td>
<td>4.19 4.31(^d) 0.030 ± 0.004(^a) 0.009 ± 0.001(^a) 0.038 ± 0.005</td>
<td>98.2 98.2 97.3 99.1</td>
</tr>
<tr>
<td>(3R,4R)-22</td>
<td>1.64(^a) 2.49(^b) 0.037(^c) 0.015(^d) 0.052</td>
<td>97.3 99.1 99.1 99.5 0.5</td>
</tr>
<tr>
<td></td>
<td>1.98 2.79 0.065 ± 0.009(^a) 0.024 ± 0.003(^a) 0.090 ± 0.013</td>
<td>97.3 99.1 99.1 99.1</td>
</tr>
</tbody>
</table>

\(^{a}\)H\(^{-1}\)H couplings measured using PSYCHEDELIC.\(^{39}\) Trans/cis ratios to the C\(^\gamma\) exo puckers in the same order of magnitude as its progenitors. Corresponding values reported by Thomas et al. at 37 °C using an alternative experimental procedure: 0.065 s\(^{-1}\) and 0.016 s\(^{-1}\). DFT values, using the M06 functional with cc-pVDZ basis set and CHCl\(_3\) or SMD implicit solvent models.
qualitatively be compared to those of the monofluorinated progenitors (Table 4), bearing in mind that the different fluorine substitution patterns may significantly influence the Karplus relation. The (4R)- and (4S)-monofluoroprolines, which are established as strongly biased to, respectively, C’ endo and C’ exo, clearly display distinct transoid $J_{HH3}$ coupling constants of 8.2 ± 0.1 Hz and <1.0 Hz, respectively, implying this coupling provides a sensitive measure for the endo/exo ratio. Both the similar small magnitude of this coupling in (3S)-monofluoroproline, known to have a pronounced C’ endo pucker,32 and the larger values found for proline (2.6 ± 4.7 trans Hz), consistent with intermediate endo/exo ratios and a higher endo population in the cis-form, confirm the relevance of $J_{HH3}$ coupling constants for a qualitative analysis of a fluorinated proline ring pucker. Hence, given the (3R,4R)-difluorinated variant 22 also shows a small $J_{HH3}$ coupling value of <0.5 Hz, its calculated preference for a C’ endo pucker is consistent with these experimental data.

In contrast, the cisoid $J_{HH1}$ coupling constants of the (4R)- and (4S)-difluoroprolines and proline show similar values of 8.7 ± 7.8 trans Hz, 9.7 Hz, and 8.9 ± 8.8 trans Hz, respectively, implying this coupling is not very sensitive to the endo/exo ratio. Indeed, both the (3R)-fluoroproline, known to prefer an exo pucker,33 and the (3S,4S)-difluoroproline show lower cisoid $J_{HH1}$ couplings of 5.1/4.8 Hz and 5.2/5.0 Hz, respectively, which suggests the fluorine substitution pattern is in this case the most significant factor determining the value. Nevertheless, the similarity of both the $J_{HH1}$ and $J_{HH3}$ couplings observed for the (3R)- and (3S,4S)-variants suggests both fluoroprolines have mostly similar endo/exo ratios. In addition, these couplings differ significantly with those of the (3S,4R)-variant, which is expected since the latter displays virtually no pucker preference.

The clear C’ exo pucker bias observed for (3S,4S)-difluorinated proline 21 in solution by NMR is also observed in its crystal structure (Figure 4). A single crystal of 22 was not obtained, but the C’ endo pucker bias of the (3R,4R)-difluoroproline ring could be observed in the crystal structure of its N-Boc-protected precursor 24a (Figure 3). It should be noted that the packing of molecules in the solid state, and their resulting conformations, is determined from the sum of a multitude of inter- and intramolecular interactions, and often deviates from the conformation in solution, which in turn is typically solvent-dependent. With this caveat in mind, the observed conformations in the crystal structures strongly suggest that the 3,4-difluorination instills the expected conformational bias.

**DISCUSSION**

The potential of fluorinated prolines as tools for protein research has a long track record. Next to the well-known example of collagen, stabilized forms of proteins such as barstar,34 ubiquitin,71 Trp cage mini protein,72 and GFF73 incorporating 4-fluoroprolines were obtained with the Cα-stereochemistry selected to reinforce the pucker observed in the native protein. Both 3- and 4-monofluorinated prolines have been used to probe the effect of $β$-turn stability on the self-assembly of elastin peptide mimics.65 Accelerated peptide folding, as a consequence of the accelerated cis/trans kinetics, was observed when fluoroprolines were integrated in thioredoxin (Trx),74 β2-microglobulin (β2m),75 and ribonuclease (RNase) A.76 Fluorinated prolines have also been used to reveal the presence of a proline ring pucker in ribosomal peptide synthesis.77,78 The extended range of cis/trans isomerization kinetics offered by the 3,4-difluoroprolines, in conjunction with either a bias to trans and the C’ exo pucker, to cis and the C’ endo pucker, or a similar structural preference to proline, clearly will be of interest within such studies, allowing us to deconvolute the roles of ring pucker and cis/trans preferences from isomerization kinetics.

Recently, Bernardes and Corzana and co-workers used a rational Pro-to-FPro substitution to stabilize an antigen–antibody complex.79 As a result of its proximity to a highly electronegative fluorine, the polarization of a nearby CH bond was increased. This led to an enhanced CH–π interaction, which stabilized the antigen–antibody complex. A similar improved CH–π interaction has been observed between a fluoroproline-modified phosphopeptide and the WW domain of Pin1.80 Clearly, 3,4-difluorinated proline analogues, especially with a 3-cis stereochemistry, will be of great interest in that regard, as enhanced C–H polarization and thus enhanced CH–π interactions can be expected.81

Regarding the use of fluoroprolines as $^{19}$F NMR reporters, the simultaneous fluorination at the 3- and 4-positions provides for very distinct chemical shifts compared to the monofluorinated progenitors. The experimental $^{19}$F chemical shifts and $J_{FF}$ coupling constants for the N-Ac-X-OMe model compounds the (4,4)-difluoroproline, (3S,4R), (3R,4R)-, and (3S,4S)-3,4-difluorinated prolines, and their monofluorinated progenitors are shown in Table 5. For all 3,4-difluoroprolines, the homonuclear coupling constant between the vicinal $^{19}$F nuclei is small, as opposed to that of the geminal difluorinated (4,4)-variant. This property is very useful for advanced $^{19}$F NMR experiments, as it minimizes any potential complications from J modulation during spin–echo pulse sequences, or from second-order effects, which is an issue in geminal difluorinated prolines.80 In addition, the 3,4-difluorinated derivatives have very distinct $^{19}$F chemical shift values compared to their monofluorinated progenitors, even though they possess similar structural properties. The 3,4-difluoroprolines can thus be used complementary to the monofluoroprolines for $^{19}$F NMR purposes, allowing for the design of combinatorial incorporation schemes aimed at studying poly proline- and proline-rich sequences, due to maximum chemical shift dispersion between these residues, but with minimal complications from homonuclear couplings.

Table 5. Fluorine Chemical Shift Values of Fluorinated N-Ac-X-OMe Derivatives (D2O)

<table>
<thead>
<tr>
<th>compound</th>
<th>$^{19}$F δ/ ppm (F3, F4)</th>
<th>$^{19}$F $J_{FF}$ (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cis amide</td>
<td>trans amide</td>
</tr>
<tr>
<td>(3R)-S3</td>
<td>−184.4</td>
<td>−186.4</td>
</tr>
<tr>
<td>(3S)-S5</td>
<td>−176.8</td>
<td>−175.7</td>
</tr>
<tr>
<td>(4R)-S2</td>
<td>−177.9</td>
<td>−177.0</td>
</tr>
<tr>
<td>(4S)-S4</td>
<td>−173.1</td>
<td>−172.9</td>
</tr>
</tbody>
</table>

*pro-R F4, a pro-S F4.
Scheme 10. Summary Scheme for the Synthesis of Protected 3,4-Difluoroprolines

CONCLUSION

As part of a program to expand the scope of available fluorinated prolines, we report here in full the effective syntheses of three 3,4-difluorinated proline analogues (as summarized in Scheme 10). In addition, we report the first conformational characterization (trans/cis ratios and isomerization kinetics, and ring pucker preferences) of the (3R,4R)- and (3S,4S)-3,4-difluoroproline analogues.

The (3S,4R)-difluorinated proline derivative could not be synthesized directly from 3,4-dehydroproline, or from the 3,4-epoxyproline derivative, with the former being unreactive under conditions of alkene difluorination or halofluorination and the latter typically suffering from aromatization, leading to pyrrole derivatives. However, a direct bis-deoxyfluorination strategy with the easily accessible 3,4-dihydroxyproline as a substrate led to the desired target using both NIF and DAST, with the former giving the highest yield when N-Boc was used as a protecting group and the latter suitable with an N-Fmoc-protecting group. Yields were low (26% and 14%, respectively), but as only two transformations were required from the protected 3,4-dehydroproline, gram quantities are easily available. In this context, we report that the direct synthesis of Fmoc-protected 3,4-dehydroproline from the corresponding 4-hydroxyproline is possible using the one-pot Grieco strategy: the (3R,4R)- and the latter typically suffering from aromatization, leading to pyrrole derivatives.

Due to the opposing conformational effects of each individual fluorine in the (3S,4R)-difluorinated proline derivative, this analogue has previously been described as having a minimal conformational bias to proline. In contrast, it is shown here that a combination of 3- and 4-fluorine substitutions with similar preorganizing effects results in 3,4-difluorinated proline derivatives with similar conformational preferences as monofluorinated prolines. While the (3R,4R)-difluorinated proline derivative resembles most closely the (4S)-fluoroproline, the (3S,4S)-difluorinated proline derivative resembles the (4R)-fluoroproline, though with a somewhat higher preference for a cis exo pucker in its cis rotamer. Given the distinct 19F chemical shifts of both 3,4-difluoroproline and their monofluorinated progenitors, they will be of interest for multiresidue fluorine-labeling strategies, for instance, in the study of repetitive or low-complexity protein sequences, where similar conformational preorganizing effects are desired, but distinct residue-specific 19F NMR chemical shifts are needed.

A clearer difference between the 3,4-difluoroprolines and their monofluorinated progenitors is the faster amide rotamer isomerization rates. This is expected given the larger electron-withdrawing effect of two fluorines compared to that of one. Especially the (3S,4S) variant shows a remarkably high isomerization rate, higher than any previously described difluorinated variant. These new variants will thus be very useful toward studying the role of Xaa-Pro cis/trans isomerization kinetics for biological function, or protein folding, or amyloid assembly.

Applications of the 3,4-difluoroprolines are in progress and will be reported in due course, as are deeper investigations on revealing the structural origins of their conformational properties and cis/trans isomerization kinetics.

EXPERIMENTAL SECTION

General Conditions. All air/moisture-sensitive reactions were carried out under an inert atmosphere (Ar), in dried glassware. Dry CH3Cl2, THF, MeOH, and hexane were bought from commercial suppliers and used as received. TLC was performed on aluminum-precoated plates coated with silica gel 60 with an F254 indicator; visualized under UV light (254 nm) and/or by staining with KMnO4 (10%aq). Flash column chromatography was performed with Sigma-Aldrich 60 silica gel (40–63 μm). Preparative HPLC was carried out using a Biogard Bio-Sil D 90–10 column (250 mm × 22 mm at 15 mL min−1). High-resolution MS samples were analyzed using a MaXis (Bruker Daltonics, Bremen, Germany) mass spectrometer equipped with a time of flight (TOF) analyzer. Samples were introduced to the
Next, the crude product was redissolved in DCM (250 mL) and 2.1 mm column. High-resolution mass spectra were recorded using m/z (major Cq,Ph), 134.7 (minor Cq,Ph), 128.9 + 128.8 + 128.6 + 128.5 + 128.4 (major and minor overlap, CH3), 128.3 + 128.2 + 128.0 (major and minor overlap, CH3), 124.3 (major Cq, Ph), 67.5 (minor Cq,Ph), 67.2 (minor Cq,Ph), 67.0 (major Cq,Ph), 66.2 (major Cq,Ph), 54.3 (major Cq,Ph), 53.5 (minor Cq,Ph), 21.8 (major Cq,Ph), 21.7 (minor Cq,Ph) ppm; Rr 0.64 (hexane/acetonitrile 50:50); MS (ESI) (m/z) 246.1 [M+H]+, 268.1 [M+Na]+; HRMS (ESI) for C14H10NO3 [M+[H]+] calc for 246.1215, found 246.1216; IR 1747 (s), 1654 (s), 1619 (m cm−1).

Data for Partially Racemized N-Acetyl-4,5-dehydroproline Benzyl Ester (3b): 1H NMR (400 MHz, CDCl3) (89:11 rotamer ratio) 6.742−7.30 (m, 5H, major Ph + SH minor Ph), 7.02 (app. dt, J = 4.3, 2.2 Hz, 1H, minor Cq,Ph), 6.51 (br dt, J = 4.3, 2.2 Hz, 1H, major Cq,Ph), 5.29−5.15 (m, 2H, major Cq,Ph and 2H minor Cq,Ph), 5.15−5.12 (m, 1H, major Cq,Ph and 1H minor Cq,Ph), 4.89 (dd, J = 11.7, 5.0 Hz, 1H, major Cq,Ph), 4.70 (dd, J = 11.3, 3.6 Hz, 1H, minor Cq,Ph), 3.21 (m, 1H, minor Cq,Ph'), 3.03 (m, 1H, major CO2), 2.82 (m, 1H, major Cq,Ph'), 2.17 (s, 3H, minor Cq,Ph), 1.93 (s, 3H, minor Cq,Ph); [13C] NMR (100 MHz, CDCl3) (89:11 rotamer ratio) δ 171.1 (minor Cq,CO2), 170.8 (major Cq,CO2), 166.7 (minor N-CO2CH3), 166.4 (major N-CO2CH3), 155.5 (major Cq,ph), 135.3 (minor Cq,ph), 129.7 (minor Cq,ph), 129.5 (major Cq,ph), 128.6, 128.4, 128.3, 128.2, 128.0 (major and minor overlap, CH3), 108.8 (major CH3), 107.7 (minor CH3), 67.2 (major Cq,Ph), 66.9 (major Cq,Ph), 59.1 (minor Cq,Ph), 57.7 (major Cq,Ph), 36.1 (minor Cq,Ph), 31.7 (major Cq,Ph), 21.8 (minor CH3), 21.5 (major CH3) ppm; Rr 0.72 (hexane/acetonitrile 50:50); MS (ESI) (m/z) 246.1 [M+H]+, 268.1 [M+Na]+; HRMS (ESI) for C14H10NO3 [M+H]+ calc for 246.1215, found 246.1217; IR 1739 (s), 1654 (s), 1620 (m cm−1).

N-(9-Fluorophenylethylcarbonyl)-25S,34-dehydroproline Benzyl Ester (25c) (Scheme 3). At 0 °C, tributylphosphate (0.98 mL, 3.93 mmol) and 2-nitrophenyl selenocyanate (725.7 mg, 3.20 mmol) were added to a solution of alcohol 26c (1.09 g, 2.46 mmol) in THF (10.0 mL). After the mixture was stirred at room temperature for 7 h, TLC analysis indicated complete consumption of the starting material. Next, H2O (30% w/w, 10.0 mL) was added, and the mixture was stirred overnight at room temperature. The reaction mixture was cooled on ice and slowly quenched with a saturated aqueous solution of Na2CO3 (80 mL). The aqueous phase was extracted with DCM (3 × 25 mL). The combined organic phases were washed with brine (25 mL) and dried over MgSO4 and the solvent was evaporated in vacuo. Purification by flash chromatography (hexane/EtOAc 10.10:25) yielded alkene 25c (815.8 mg, 78%) as a light orange oil: 1H NMR (400 MHz, CDCl3) (51:49 rotamer ratio) 6.783−7.23 (m, 13H major Ar−H + 13H minor Ar−H), 6.06−5.99 (m, 1H major Cq,Ph + 1H minor Cq,Ph), 5.85−5.76 (m, 1H major Cq,Ph + 1H minor Cq,Ph), 5.26 (d, J = 12.2 Hz, 1H, major Cq,Ph), 5.18 (d, J = 12.5 Hz, 1H, major Cq,Ph), 5.17 (d, J = 12.4 Hz, 1H, minor Cq,Ph), 5.07 (d, J = 12.2 Hz, 1H, minor Cq,Ph), 5.23−5.10 (m, 1H major Cq,Ph + 1H minor Cq,Ph), 4.55−4.26 (m, 2H major NCO−CH2−CH−2H minor NCO−CH2−CH−2H major Cq,Ph), 3.90−3.84 (m, 2H minor NCO−CH2−CH−2H minor Cq,Ph), 3.10−2.84 (m, 2H major Cq,Ph).
To a solution of alkene 25a (320.0 g, 105.5 mmol) in 1,2-dichloroethane (250 mL) was added meta-chloroperbenzoic acid (77% pure, 30.7 g, 137.1 mmol), and the mixture was refluxed at 90 °C. After 24 h, the mixture was cooled to room temperature and quenched with a saturated aqueous solution of Na2SO3 (250 mL), a saturated aqueous solution of NaHCO3 (250 mL), and brine (250 mL). The organic layer was dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography (hexane/acetonitrile 60:40 to 40:60) yielded 10b (4.92 g, 55%) and 9b (4.26 g, 25%) as slightly yellow/oily materials. 9b was recrystallized from DCM and submitted for X-ray analysis.

**Data for (±)-N-(Acetyl)-(2S,3R,4S)-3,4-epoxyproline Benzyl Ester (10b):**

H NMR (400 MHz, CDCl3) (80:20 rotamer ratio) δ 7.41–7.31 (m, 5H major Ph + 5H minor Ph), 5.29 (d, J = 12.5 Hz, 1H minor CHPH), 2.52 (d, J = 12.5 Hz, 1H major CHPH), 2.50 (d, J = 12.3 Hz, 1H major CHPH), 3.15 (d, J = 12.4 Hz, 1H minor CHPH), 4.72 (s, 1H minor CH3H), 4.57 (s, 1H major CH3H), 3.88 (d, J = 12.5 Hz, 1H major CH3H)+, 3.82 (d, J = 12.5 Hz, 1H minor CH3H), 3.75 (dd, J = 2.9, 0.5 Hz, 1H major CH3H), 3.74 (dd, J = 2.9, 0.3 Hz, 1H major CH3H), 3.65 (dd, J = 2.9, 0.1 Hz, 1H minor CH3H), 3.51 (dd, J = 12.5, 1.4 Hz major CH3H)+, 3.48 (dd, J = 12.5, 1.4 Hz major CH3H)+, 1.45 (s, 9H minor CO2C(CH3)+), 1.33 (s, 9H major CO2C(CH3)+) ppm; mp 70 °C.

**Data for (±)-N-(Acetyl)-(2S,3R,4S)-3,4-epoxyproline Benzyl Ester (9b):**

H NMR (400 MHz, CDCl3) (60:40 rotamer ratio) δ 7.45–7.35 (m, 5H major Ph + 5H minor Ph), 5.27 (d, J = 12.5 Hz, 1H minor CHPH), 2.52 (d, J = 12.5 Hz, 1H major CHPH), 2.50 (d, J = 12.3 Hz, 1H major CHPH), 3.15 (d, J = 12.4 Hz, 1H minor CHPH), 4.72 (s, 1H minor CH3H), 4.57 (s, 1H major CH3H), 3.88 (d, J = 12.5 Hz, 1H major CH3H)+, 3.82 (d, J = 12.5 Hz, 1H minor CH3H), 3.75 (dd, J = 2.9, 0.5 Hz, 1H major CH3H), 3.74 (dd, J = 2.9, 0.3 Hz, 1H major CH3H), 3.65 (dd, J = 2.9, 0.1 Hz, 1H minor CH3H), 3.51 (dd, J = 12.5, 1.4 Hz major CH3H)+, 3.48 (dd, J = 12.5, 1.4 Hz major CH3H)+, 1.45 (s, 9H minor CO2C(CH3)+), 1.33 (s, 9H major CO2C(CH3)+) ppm; mp 96 °C.
conclude that pyrrole 35 was the only product: 1H NMR (500 MHz, CDCl3) δ 9.30 (br s, 1H, NH), 7.46–7.32 (m, 5H, CH Ar), 6.99 (ddd, J = 3.8, 2.4, 1.5 Hz, 1H, C4), 6.96 (td, J = 2.7, 1.5 Hz, 1H, C3), 6.28 (dt, J = 3.8, 2.5 Hz, 1H, C2), 5.33 (s, 2H, CH2 ppm); 13C{1H} NMR (126 MHz, CDCl3) δ 161.0 (C3, CO), 136.1 (C5, Ph), 128.6 (2 × C6), 128.2 (1 × CH3), 128.1 (2 × CH3), 123.1 (C4), 112.6 (C2), 115.6 (C5), 110.5 (C3, Ph), 66.0 (CH2) ppm; Rf 0.24 (hexane/acetone 70:30); HRMS (ESI) for C22H19N5O3 [M + Na]+ calculated for 378.1079, found 378.1081; IR 3406 (w), 1738 (m), 1712 (s), 1620 (m), 1514 (m), 1410 (m), 1382 (m), 1351 (m), 1284 (m), 1256 (m), 1124 (s) cm⁻¹. Chemical shift data correspond to literature data.⁴

For (+)-N-(Acetyl)-(25,3R,4R)-3-hydroxy-4-fluoroproline Benzyl Ester (33b) (Table 1, Entry 5). DMPU-HF (0.4 mL) was added dropwise to a solution of epoxide (±)-10b (130.0 mg, 0.498 mmol) in DCM (3.0 mL), and the mixture was stirred at room temperature. Within 1 h, a white gel had formed in the reaction mixture. TLC analysis after 19 h indicated the presence of the starting material, upon which the reaction temperature was increased to 50 °C. After an additional 1 h, the reaction mixture was quenched with a saturated aqueous solution of NaHCO3 (8 mL) and the aqueous layer was extracted with DCM (3 × 10 mL). The combined organic phases were dried over Na2SO4 and evaporated in vacuo. The crude product was purified by flash chromatography (hexane/EtOAc 70:30) to yield recovered the starting material (39.5 mg, 30%) and 33b as a clear oil (21.1 mg, 15%), along with a trace amount of DMPU (-3%).

**Data for (+)-N-(Acetyl)-(25,3R,4R)-3-hydroxy-4-fluoroproline Benzyl Ester (33b):** 1H NMR (400 MHz, CDCl3) (67:33 rotamer ratio) δ 7.87–7.28 (m, 5H, major Ph + PhH minor Ph), 5.25 (d, J = 12.1 Hz, 1H, minor CHPh), 5.19 (d, J = 12.4 Hz, 1H, major CHPh), 5.16 (d, J = 12.1 Hz, 1H, minor CHPh), 5.12 (d, J = 12.2 Hz, 1H, major CHPh), 5.00 (dd, J = 50.0, 3.9 Hz, 1H, major CH), 4.92 (dd, J = 49.9, 3.5 Hz, 1H, minor CH), 4.76–4.55 (m, 1H, major C=H + 1H minor C=H), 4.72 (s, 1H, major C=H), 4.45 (s, 1H, minor C=H), 4.33–4.13 (m, 1H, major OH + 1H minor OH), 4.03–3.57 (m, 2H major C=H + 2H minor C=H), 2.09 (s, 3H, major CH3), 1.96 (s, 3H, minor CH3) ppm; 13C{1H} NMR (100 MHz, CDCl3) (67:33 rotamer ratio) δ 171.11 (minor N-COCH3), 170.8 (major N-COCH3), 168.2 (major C=O), 167.9 (major C=O), 135.3 (major Cq,Ph), 134.9 (minor Cq,Ph), 128.6 + 128.2 + 128.4 + 128.3 + 128.1 (major and minor overlap, C=CH3), 94.6 (d, J = 181.2 Hz, major C3), 93.3 (d, J = 179.0 Hz, minor C3), 77.5 (d, J = 28.6 Hz, major C5), 75.6 (d, J = 28.6 Hz, major C5), 67.7 (minor C=O), 67.3 (major C=O), 65.7 (minor C=O), 52.1 (d, J = 23.5 Hz, major C5), 50.9 (d, J = 23.5 Hz, minor C5), 22.02 (major N-COCH3), 21.98 (minor N-COCH3) ppm; 19F NMR (376 MHz, CDCl3) (70:30 rotamer ratio) δ −180.87 (ddd, J = 49.9, 39.5, 28.6, 6.9 Hz, 1F, minor), −182.19 (ddd, J = 49.4, 36.4, 26.9, 8.7 Hz, 1F, major) ppm; 13C{1H} NMR (376 MHz, CDCl3) (70:30 rotamer ratio) δ −180.61 (s, 1F, minor), −182.34 (s, 1F, major) ppm; Rf 0.22 (hexane/acetonitrile 60:40); MS (ESI) (m/z) 282.4 [M + H]+, 304.3 [M + Na]+; HRMS (ESI) for C17H17N2O3F + Na+[H]+ calculated for 382.1136, found 382.1135; IR 2927 (br m), 1745 (s), 1626 (s), 1448 (m), 1420 (m), 1176 (s) cm⁻¹.

**Data for (+)-N-(2-butylxycarbonyl)-(25,3R,4R)-3-hydroxy-4-chloroproline Benzyl Ester (32a) (Table 1, Entry 7).** To a solution of epoxide (-10b (553.0 mg, 2.117 mmol) in t-BuOH (25.0 mL)) was added TBAF-3H2O (1.67 g, 5.29 mmol), and the mixture was stirred at 70 °C for 4 h. The mixture was then mixed with water (100 mL) and the aqueous layer was extracted with DCM (8 × 30 mL). The combined organic phases were dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography using a Biotage purification system (hexane/acetonitrile gradient) yielded 32a (165.7 mg, 30%) and 35 (33.2 mg, 13%) as clear oils.
The aqueous layer was extracted with DCM (3 × 50 mL), and the combined organic layers were subsequently washed with brine (40 mL), dried over MgSO₄, and evaporated in vacuo. The crude product was purified by flash chromatography using a Biotage purification system (hexane/acetonelhexane gradient) to yield 32b (2.77 g, 95%) as a colorless oil: 1H NMR (400 MHz, CDCl₃) (70:30 rotamer ratio) 0.741–7.29 (m, 5H major Ph + 5H minor Ph), 5.28 (d, J = 12.1 Hz, 1H, minor CH₂Ph), 5.22 (d, J = 12.3 Hz, 1H, minor CH₂Ph), 5.17 (d, J = 12.1 Hz, 1H, minor CH₂Ph), 5.14 (d, J = 12.3 Hz, 1H, major CH₂Ph), 4.74 (brm, 1H, minor CH₂), 4.57 (brm, J = 2.6 Hz, 1H, major CH₂), 4.53 (m, 1H, major CH₂), 4.40 (brm, J = 1.2 Hz, 1H, minor CH₂), 4.27–4.40 (m, 1H major CH₂H + 1H minor CH₂H + 1H major CH₂H’ + 1H minor CH₂H’), 3.81–3.74 (m, 1H major CH₂H’H’ + 1H minor CH₂H’H’), 2.08 (s, 3H, major CH₃), 1.96 (s, 3H, minor CH₃), ppm; 13C{1H} NMR (100 MHz, CDCl₃) (70:30 rotamer ratio) δ 171.1 (major N-CON₃H), 170.4 (major N-CON₃H), 168.6 (1H major C=O, 1C minor C=O), 135.3 (major C₃H₅), 134.8 (minor C₃H₅), 128.74 + 128.70 + 128.68 + 128.52 + 128.46 + 128.4 + 128.3 (major and minor overlap, C₃H₅), 81.3 (major C₃H₅), 67.9 (major CH₂Ph), 67.4 (major CH₂Ph), 67.0 (minor CH₂Ph), 65.3 (major CH₂), 59.2 (major CH₂), 58.3 (minor CH₂), 54.2 (major CH₃), 53.4 (minor CH₃), 22.0 (minor CH₃), 21.9 (major CH₃), ppm; 31P{1H} NMR (300 MHz, CDCl₃) δ 298.3 [M + H]⁺, 320.3 [M + Na⁺]; HRMS (ESI) for C₃H₅NO₃[1H] calc. for C₃H₅NO₃[M + H]⁺ calc. found, 298.0841; 393304 (br m), 1747 (s), 1628 (s), 1189 (m), 1175 (m), 698 (m) cm⁻¹.

(±)-N-(Acetyl)-(25,3R,4R)-3-hydroxy-4-chlorobenzyl Ester (32b) (Scheme 5). Reaction with MgBr₂. To a solution of epoxide (±)-10b (147.7 mg, 0.565 mmol) in DCM (4.0 mL) was added MgBr₂ (156.1 mg, 0.848 mmol), and the mixture was stirred at room temperature. After 20 h, the mixture was diluted with DCM (10 mL), washed (10 mL), and a saturated aqueous solution of NaHCO₃ (5 mL). The aqueous layer was extracted with DCM (3 × 10 mL), and the combined organic phases were dried over MgSO₄ and evaporated in vacuo. Bromohydrin 38b was obtained without purification as a clear oil (169.9 mg, 88%).

Reaction with HBr. HBr (48 wt% in H₂O, 1.0 mL) was added dropwise to a solution of epoxide (±)-10b (142.1 mg, 0.543 mmol) in DCM (3.0 mL) at 0 °C. After 2 h, the mixture was cooled to 0 °C and quenched with a saturated aqueous solution of NaHCO₃ (5 mL), and the aqueous layer was extracted with DCM (3 × 7 mL). The combined organic phases were washed with brine (1 × 10 mL), dried over MgSO₄, and evaporated in vacuo. Bromohydrin 38b was obtained without purification as a clear oil (177.5 mg, 95%): 1H NMR (400 MHz, CDCl₃) (71:29 rotamer ratio) 0.74–7.20 (m, 5H major Ph + 5H minor Ph), 5.29 (d, J = 12.0 Hz, 1H, minor C=OPh), 5.24 (d, J = 12.4 Hz, 1H, major C=OPh), 5.19 (d, J = 12.0 Hz, 1H, minor C=OPh), 4.82 (br app t, 1H, minor CH₂), 4.59 (app t, J = 5.7 Hz, 1H, major CH₂), 4.51 (d, J = 3.6 Hz, 1H, minor CH₂), 4.51 (d, J = 2.0 Hz, 1H, minor CH₂), 3.48–4.11 (m, 1H major CH₂H + 1H minor CH₂H + 1H major CH₂H’ + 1H minor CH₂H’), 3.92–3.80 (m, 1H major CH₂H’H’ + 1H minor CH₂H’H’), 3.74–3.57 (brs, 1H major OH + 1H minor OH), 2.09 (s, 3H, major CH₃), 1.96 (s, 3H, minor CH₃), ppm; 13C{1H} NMR (100 MHz, CDCl₃) (71:29 rotamer ratio) δ 170.8 (major N-CON₃H), 169.9 (major N-CON₃H), 168.9 (major N-CON₃H), 168.8 (minor N-CON₃H), 135.3 (major C₃H₅), 134.7 (minor C₃H₅), 128.8 + 128.72 + 128.70 + 128.4 + 128.48 + 128.3 + 128.3 (major and minor overlap, C₃H₅), 81.8 (minor C₃H₅), 80.0 (major C₃H₅), 68.0 (major CH₂Ph), 67.4 (major CH₂Ph), 66.9 (minor C₃H₅), 65.0 (major C₃H₅), 54.3 (major C₃H₅), 53.6 (minor C₃H₅), 47.7 (major C₃H₅), 47.2 (minor C₃H₅), 21.9 (major N-CON₃H), 21.8 (major N-CON₃H) ppm; 31P{1H} NMR (300 MHz, CDCl₃) δ 321.2 [M + H⁺]⁺, 364.1 [M + Na⁺]; HRMS (ESI) for C₃H₅BrNO₃[M + H⁺]calc. for C₃H₅BrNO₃[M + H⁺]calc. found, 342.0353, 342.0332; IR 2926 (br w), 1742 (s), 1625 (s), 1171 (s), 733 (s), 697 (m) cm⁻¹.

Benzyl N-Acetyl pyrrole-2-carboxylate (39b) (Scheme 5). At –78 °C, DAST (60.0 µL, 0.420 mmol) was added to a solution of chloroform 32b (83.4 mg, 0.280 mmol) in DCM (1.0 mL). The reaction was allowed to warm to room temperature, and after 2 h, TLC analysis indicated full conversion of the starting material. Next, the reaction was diluted with DCM (10 mL), and quenched with a saturated aqueous solution of NaHCO₃ (2 mL) and water (2 mL). The aqueous layer was extracted with DCM (3 × 8 mL), and the combined organic phases were dried over MgSO₄ and evaporated in vacuo. Purification via HPLC (hexane/acetonitrate 60:40) yielded pyrrole...
39b as a clear oil (27.4 mg, 40%): 1H NMR (400 MHz, CDCl3) δ 7.45–7.32 (m, 6H, 1H C/H + CH/ + H Ph), 7.02 (dd, J = 3.6, 1.7 Hz, 1H, CH/CH/), 6.23 (t, J = 3.4 Hz, 1H, CHN), 5.31 (s, 2H, CH/Ph), 2.80 (s, 3H, N- CO2Ph) ppm; 13C{1H} NMR (100 MHz, CDCl3) δ 169.2 (N-CO2CH3), 160.8 (C=O), 153.7 (C=C), 128.6 (2C, CHAr), 128.3 (1C, CH3Ph), 128.2 (2C, CH3Ph), 126.5, 124.8 (C=C), 132.1, 131.0, 129.5, 129.7 (CHPh), 124.9 (N-CO2CH3), 124.9 (N-CO2Ph) ppm. 31P{1H} NMR (hexa/acetone 60:40) MS (ESI) [m/z] 244.2 [M + H]+, 266.2 [M + Na]+; HRMS (ESI) for C14H13NNaO3 [M + Na]+ calcd for 266.0784, 266.0788; IR (KBr) 3154, 2961, 1732, 1601, 1571, 1461, 1378, 1331, 1265, 1223, 1178, 1124, 1073, 1020, 970, 927, 879, 804, 761, 728, 697, 650, 623 cm−1.

**Data for (±)-N-(Acetyl)-(2R,3S,4R)-3-fluoro-4-(4-methylphenyl)sulfonyl Proline Benzyl Ester (40b) (Scheme 5).** At −78 °C, DAST (31.0 μL, 0.238 mmol) was added to a solution of (±)-37b (102.2 mg, 0.238 mmol) in DCM (2.0 mL). After the mixture was stirred at −78 °C for 8 h, another 2 equiv of DAST (62.0 μL, 0.476 mmol) was added. Overnight, the mixture was allowed to warm to room temperature. Next, the reaction was diluted with DCM (10 mL) and quenched with a saturated aqueous solution of NaHCO3 (5 mL). The aqueous layer was then extracted with DCM (3 × 10 mL), and the combined organic phases were dried over MgSO4 and evaporated in vacuo. Purification via HPLC (hexane/acetone 70:30) yielded pyrrole 39b (35.8 mg, 62%) and 40b (63 mg, 6%).

**Reactions of 38b with AgF (Scheme 5).** A solution of 38b (99.0 mg, 0.289 mmol) in nitromethane (5.0 mL) was added AgF (183.5 mg, 1.447 mmol), and the resulting mixture was stirred at room temperature. After 2 h, no conversion of the starting material was observed. TLC analysis indicated complete conversion of the starting material. The mixture was quenched with Na2S2O3 (40 mL) and stirred at room temperature for 15 min. After 15 h, the mixture was filtered through Celite and the solvent evaporated in vacuo to yield 10b (77.0 mg, quant) as a clear oil.

**N-(tert-Butoxy-4-carbonyl)-(25,35,45)-3,4-dihydroxyproline Benzyl Ester (19a) (Table 2, Entry 1).** To a solution of alkenne 25a (3.6 g, 11.9 mmol) in dioxane (60.0 mL) and water (15.0 mL) were added NMO (3.48 g, 29.7 mmol) and OsO4 (4 wt % in H2O, 0.5 mL). After the mixture was stirred for 2 days at room temperature, TLC analysis indicated complete conversion of the starting material. The mixture was quenched with a saturated aqueous solution of Na2S2O3 (40 mL) and stirred at room temperature for 15 min. Next, the aqueous layer was extracted with EtOAc (4 × 100 mL). The combined organic phases were washed with a saturated aqueous solution of NaHCO3 (100 mL) and brine (100 mL), dried over MgSO4, and evaporated in vacuo. Purification by flash chromatography (hexane/acetone 70:30) yielded diol 19a (3.69 g, 92%) as a clear oil. Data correspond to literature data.

**Reactions with AD-Mix-α (Table 2, Entry 3).** To a solution of alkenne 25a (280.0 mg, 0.923 mmol) in t-BuOH (3.0 mL) and water (3.0 mL) were added AD-mix-α (1.29 g) and CH3SO2NH2 (87.8 mg, 0.923 mmol). After 3 days, the mixture was quenched with a saturated aqueous solution of NaHCO3 (10 mL) and stirred at room temperature for 15 min. Next, the aqueous layer was extracted with EtOAc (4 × 15 mL). The combined organic phases were washed with a saturated aqueous solution of NaHCO3 (15 mL), dried over MgSO4, and evaporated in vacuo. Purification by HPLC (hexane/acetone 75:25) yielded diol 19a (256.0 mg, 82%) as a clear oil. Data correspond to literature data.

**N-Fluorenlymethoxy(25,35,45)-3,4-dihydroxyproline Benzyl Ester (19c) (Table 2, Entry 5).** To a solution of alkenne 25c (645.2 mg, 1.516 mmol) in acetone (4.5 mL) and water (1.5 mL) were added NMO (444.1 mg, 3.791 mmol) and K2OsO4·2H2O (20.0 mg, 0.054 mmol). After the mixture was stirred for 14 h at room temperature, TLC analysis indicated complete conversion of the starting material. The mixture was quenched with a saturated aqueous solution of Na2S2O3 (10 mL) and stirred at room temperature for 15 min. Next, the aqueous layer was extracted with EtOAc (4 × 15 mL). The combined organic phases were washed with a saturated aqueous solution of NaHCO3 (15 mL), dried over MgSO4, and evaporated in vacuo. Purification by flash chromatography (hexane/acetone 70:30) yielded diol 19c as a white solid (553.8 mg, 80%): 1H NMR (500 MHz, CDCl3) (51:49 rotamer ratio) δ 7.82–7.20 (m, 13H, 13H Ar major −Ar−H), 5.24 (d, J = 12.4 Hz, 1H, major CHPh), 5.18 (d, J = 12.4 Hz, 1H, major CHPh), 5.14 (d, J = 12.2 Hz, 1H, minor CHPh), 5.08 (d, J = 12.2 Hz, 1H, minor CHPh), 4.59–4.21 (m, 1H major CH3H + 1H minor CH3H + 1H major CH3H + 1H minor CH3H + 2H minor NCO2–CH2–CH + 2H minor NCO2–CH2–CH + 1H major NCO2–CH2–CH) 3.96 (t, J = 6.9 Hz, 1H minor NCO2–CH2–CH), 3.85–3.53 (m, 2H minor CH3H + 2H minor CH3H), 3.29 (br d, J = 5.0 Hz, 1H minor OH), 3.19 (br d, J = 5.5 Hz, 1H major OH), 2.91 (br d, J = 4.6 Hz, 1H major O′H), 2.85 (br d, J = 5.3 Hz, 1H major O′H) ppm; 13C{1H} NMR (126 MHz, CDCl3) (51:49 rotamer ratio) δ 170.7 (minor C=C), 169.2 (N-CO2CH3), 160.8 (C=O), 153.7 (C=C), 132.1, 131.0, 129.5, 129.7 (CHPh), 124.9 (N-CO2CH3), 124.9 (N-CO2Ph) ppm. 31P{1H} NMR (hexa/acetone 60:40) yielded allylic alcohol 34b (33.7 mg, 84%) as a clear oil.
proline Benzyl Ester (20c) (Scheme 7).

4.95 (m, 1H major CHH)

addition of a saturated aqueous solution of NaHCO3 (30 mL). Next, the aqueous layer was extracted with DCM (5 × 30 mL), and the combined organic phases were washed with brine (50 mL), dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography (hexane/EtOAc 70:30) yielded diolpropane (##-##) and a cyclic sulhide side product as an inseparable mixture. This mixture was dissolved in water (5.0 mL) and acetonitrile (7.0 mL), and sodium periodate (278.9 mg, 1.304 mmol) and a catalytic amount of ruthenium(III) chloride were added. After 3 h, a saturated aqueous solution of NaHCO3 (10 mL) was added and the aqueous layer was extracted with DCM (3 × 30 mL). Next, the combined organic phases were dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography (hexane/EtOAc 80:20) yielded diolpropane (##-##) as a clear oil, which spontaneously crystallized on standing. Data correspond to literature data.44

N-(9-Fluorenylmethylxoycarbonyl)-(2R,3R,4R)-3,4-difluoroproline Benzyl Ester (20c) (Scheme 7). To a solution of diol 19c (155.7 mg, 0.339 mmol) in DCM (3.0 mL) at 0 °C was added DAST (3.72 mL, 28.16 mmol) dropwise. After the mixture was stirred at room temperature for 22 h, an extra portion of DAST (2.26 g, 14.08 mmol) was added. After another 32 h of stirring at room temperature, the reaction mixture was cooled to 0 °C and quenched by dropwise addition of a saturated aqueous solution of NaHCO3 (30 mL). Next, the aqueous layer was extracted with DCM (5 × 30 mL), and the combined organic phases were washed with brine (50 mL), dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography (hexane/EtOAc 70:30) yielded diolpropane (##-##) and a cyclic sulhide side product as an inseparable mixture. This mixture was dissolved in water (1.0 mL) and acetonitrile (1.0 mL), and sodium periodate (16.9 mg, 0.079 mmol) and a catalytic amount of ruthenium(III) chloride were added. After 3 h, a saturated aqueous solution of NaHCO3 (10 mL) was added and the aqueous layer was extracted with DCM (4 × 10 mL). Next, the combined organic phases were dried over MgSO4 and evaporated in vacuo. Purification by flash chromatography (hexane/EtOAc 80:20) yielded diolpropane (##-##) as a clear oil, which spontaneously crystallized on standing. Data correspond to literature data.44
EtOAc (4 × 60 mL). The combined organic phases were dried over MgSO₄ and evaporated in vacuo. Purification by flash chromatography (hexane/acetone 80:20) yielded a mixture of diastereomeric fluoroketones 27a/28a (1.26 g, 31%). Subsequently, the diastereomeric mixture (1.10 g, 3.26 mmol) was dissolved in THF (20.0 mL) and methanol (4.0 mL) and was cooled to 0°C, and sodium borohydride (1.14 g, 28.9 mmol) was added. After 3 h, the reaction was quenched with a saturated aqueous solution of NaHCO₃ (20 mL) and the aqueous phase was extracted with EtOAc (4 × 30 mL). The combined organic phases were dried over MgSO₄ and evaporated in vacuo. Purification by flash chromatography (hexane/acetone 85:15 to 60:40 and hexane/EtOAc 80:20 to 60:40) yielded the fluoroketones 50a (460.3 mg, 40% (contaminated with an additional ~4% of 51a) and 51a (138.2 mg, 13%) as clear oils.

**Data for N-((Nitro-Butoxyacarbonyl)-(2R,3R,4R)-3-fluoro-4-hydroxyproline Benzyl Ester (50a):** H NMR (400 MHz, CDCl₃) (60:40 rotamer ratio) δ 7.42–7.29 (m, 5H, major Ph + 5H minor Ph), 5.34 (d, J = 12.4 Hz, 1H, minor CH₂Ph), 5.27 (d, J = 12.2 Hz, 1H, major CH₂Ph), 5.21 (d, J = 12.1 Hz, 1H, major CH₂Ph), 5.16 (d, J = 12.4 Hz, 1H, minor CH₂Ph), 5.27–5.08 (m, 1H major CH + 1H minor CH), 4.64 (dd, J = 21.0, 5.9 Hz, 1H, minor CH), 4.55 (dd, J = 20.7, 5.4 Hz, 1H, major CH), 4.34–4.21 (m, 1H major CH + 1H minor CH), 3.92–3.77 (m, 1H major CH₂H + 1H minor CH₂H), 3.50–3.41 (m, 1H major CH₂H + 1H minor CH₂H), 2.83–2.75 (m, 1H, major OH + 1H minor OH), 1.47 (s, 9H, minor CO₂C(CH₃)₃), 1.30 (s, 9H, major CO₂C(CH₃)₃), 1.29 (s, 3H, major CO₂C(CH₃)₃), 1.28 (s, 3H, minor CO₂C(CH₃)₃), 1.18 (s, 3H, major CO₂C(CH₃)₃), 1.18 (s, 3H, minor CO₂C(CH₃)₃); 19F NMR (376 MHz, CDCl₃) (60:40 rotamer ratio) δ 168.2 (δ 16.6 Hz, major CO₂), 167.9 (δ 7.3 Hz, major CO₂), 153.9 (minor CO₂), 153.2 (major CO₂), 135.0 (major C₟), 128.63 + 128.60 + 128.5 + 128.3 + 128.2 (major and minor overlap, δH), 91.5 (d, J = 189.3 Hz, major C₟), 90.8 (d, J = 190.7 Hz, minor C₟), 81.1 (major CO₂C(CH₃)₃), 81.0 (minor CO₂C(CH₃)₃), 70.6 (d, J = 18.3 Hz, minor C₟), 70.1 (d, J = 17.6 Hz, major C₟), 67.6 (major and minor overlap, δH), 67.1 (d, J = 22.0 Hz, major C₟), 61.3 (d, J = 22.0 Hz, minor C₟), 50.5 (minor C₟), 49.9 (major C₟), 28.3 (minor CO₂C(CH₃)₃), 28.1 (major CO₂C(CH₃)₃); 13C NMR (100 MHz, CDCl₃) (60:40 rotamer ratio) δ 196.7 (δ 23.5 Hz, minor C₟), 196.4 (δ 23.5 Hz, minor C₟), 192.4 (δ 22.7 Hz, minor C₟), 191.8 (δ 22.7 Hz, major C₟), 191.0 (major C₟), 187.5 to 187.2 ppm; HRMS (ESI) for C₁₇H₂₁F₂NNaO₄ [M + Na]⁺ calcd for 362.1374, found 362.1379; IR 3427 (br m), 1760–1756 cm⁻¹.

**Data for N-((Nitro-Butoxyacarbonyl)-(2R,3R,4R)-3-fluoro-4-hydroxyproline Benzyl Ester (51a):** H NMR (400 MHz, CDCl₃) (60:40 rotamer ratio) δ 7.40–7.30 (m, 5H, major Ph + 5H minor Ph), 5.43–5.04 (m, 2H, major CH₂Ph + 2H minor CH₂Ph), 1.19 (s, 9H, major CHₐCO₂), 1.19 (s, 9H, minor CH₁CO₂), 4.60 (dd, J = 26.5, 4.2 Hz, 1H, major CH), 4.05–3.70 (m, 2H major CH₂H + 2H minor CH₂H), 1.49 (s, 9H, minor CO₂C(CH₃)₃), 1.35 (s, 9H, major CO₂C(CH₃)₃), 1.29 (s, 3H, minor CO₂C(CH₃)₃), 1.28 (s, 3H, major CO₂C(CH₃)₃), 1.18 (s, 3H, minor CO₂C(CH₃)₃), 1.18 (s, 3H, major CO₂C(CH₃)₃), 1.18 (s, 3H, minor CO₂C(CH₃)₃); 19F NMR (376 MHz, CDCl₃) (60:40 rotamer ratio) δ 196.7 to 196.4 ppm; HRMS (ESI) for C₁₇H₂₁F₂NNaO₄ [M + Na]⁺ calcd for 362.1374, found 362.1379; IR 3427 (br m), 1760–1756 cm⁻¹.
The salt was redissolved in DCM (2.0 mL), the mixture was cooled to 0 °C, and DIPEA (80.6 µL, 0.463 mmol) was added. Next, acetyl chloride (52.6 µL, 0.740 mmol) was added dropwise, and the solution was allowed to warm to room temperature. After stirring for 2 h, the solvent was evaporated in vacuo and the crude product was purified by flash chromatography (hexane/acetone 70:30) to yield 22 (22.2 mg, 58%) as a clear oil: 1H NMR (500 MHz, CDCl3) (65:35 rotamer ratio) δ 5.45 (dd, J = 47.5, 6.3 Hz, H1, major C, H3), 5.30 (dd, J = 47.7, 7.4, 1.5 Hz, 1H, major CH3), 5.31–5.11 (m, 1H major CH and H1, major CH3), 4.97 (d, J = 23.7 Hz, 1H, major CH2), 4.67 (d, J = 21.1 Hz, 1H, minor CH3), 4.05–3.88 (m, 2H major CH2 and H1 minor CH3), 3.83 (s, 3H major CO2CH3), 3.78 (s, 3H major CO2CH3), 2.16 (s, 3H minor NCOCH3), 2.07 (s, 3H minor NCOCH3) ppm; 13C{1H} NMR (126 MHz, CDCl3) (65:35 rotamer ratio) δ 170.1 (minor NCOCH3), 169.7 (major NCOCH3), 167.0 (minor C6-CO2), 166.9 (major C6-CO2), 95.1 (dd, J = 181.7, 32.7 Hz, minor CH2), 93.5 (dd, J = 180.3, 31.3 Hz, major CH2), 92.1 (dd, J = 172.5, 31.9 Hz, major C1), 90.4 (dd, J = 178.0, 31.8 Hz, minor C1), 64.8 (d, J = 22.7 Hz, minor C1), 63.4 (d, J = 23.6 Hz, major C1), 53.3 (minor CO2CH3), 52.9 (major CO2CH3), 51.6 (d, J = 23.6 Hz, major C), 50.7 (d, J = 23.6 Hz, minor C), 21.2 (major NCOCH3), 22.0 (minor NCOCH3) ppm; 1F{1H} NMR (74 MHz, CDCl3) (66:34 rotamer ratio) δ −187.9 to −187.3 (m, 1F, minor F), −187.5 to −187.9 (m, 1F, major F), −188.8 to −189.1 (m, 1F, minor F) ppm; 19F{1H} NMR (74 MHz, CDCl3) (66:34 rotamer ratio) δ −187.1 (d, J = 14.8 Hz, 1F, minor F), −187.7 (d, J = 14.9 Hz, 1F, major F), −188.1 (d, J = 14.8 Hz, 1F, major F), −189.0 (d, J = 14.8 Hz, 1F, minor F) ppm; Rf 0.24 (hexane/acetone 70:30); [α]D° = −67.2 (c 1.1, CHCl3); MS (ESI) (m/z) 208.2 [M + H]+, 230.2 [M + Na]+; IR MS (ESI) for C6H12F2NaO3 [M + Na]+ calc for 230.2626, found 230.2659, found 230.0604; IR 1758 (s), 1653 (s), 1584 (s), 1417 (s), 1280 (m) cm−1.

ASSOCIATED CONTENT

 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b02920.

H, 13C, and 19F NMR spectra of all novel compounds, chiral HPLC chromatograms (for 3,4-dehydroproline synthesis), J value analysis for epoxides 9a,b,d and 10a,b,d, X-ray crystallographic data for 9b, 21, and 24a, computational data of the proline conformers of 21, 22, 52–55, and Ac-Pro-Ome in CHCl3 and water calculated by DFT including Gibbs free energies, electronic energy values, and Cartesian coordinates, and general NMR conditions for conformational and kinetic analysis (PDF)

Crystallographic data for compound 21 (CIF)

Crystallographic data for compound 9b (CIF)

Crystallographic data for compound 24a (CIF)

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Figure 9. To a solution of 23a (161.0 mg, 0.472 mmol) in methanol (3.0 mL) was added 10% Pd/C (20.0 mg). The mixture was kept under a hydrogen atmosphere and stirred at room temperature. After 24 h, the mixture was poured into water (10 mL) and the aqueous layer was allowed to warm room temperature. After stirring for 23 h, the mixture was kept under a hydrogen atmosphere and stirred at room temperature. After stirring for 24 h, the mixture was poured into water (10 mL) and the aqueous layer was extracted with DCM (4 × 10 mL). The combined organic layers were dried over MgSO4 and evaporated in vacuo. The crude product was purified by flash chromatography (hexane/acetonate 70:30) to yield 21 (293.9 mg, 30%) as a colorless solid: 1H NMR (400 MHz, CDCl3) (80:20 rotamer ratio) δ 5.53–5.09 (m, 1H major C, H1 + 1H minor C, H1 + 1H major CH3 + 1H minor CH3), 4.92–4.73 (m, 1H major C, H1 + 1H minor C, H1), 4.35–3.59 (m, 2H major C, H2 + 2H minor C, H2), 3.85 (s, 3H major CO2CH3), 3.80 (s, 3H major CO2CH3), 2.14 (s, 3H major NCOCH3), 1.98 (s, 3H minor NCOCH3) ppm; 13C{1H} NMR (100 MHz, CDCl3) (80:20 rotamer ratio) δ 169.9 (minor NCOCH3), 169.5 (major NCOCH3), 166.9 (d, J = 8.8 Hz, minor C6-CO2), 166.2 (d, J = 8.1 Hz, major C6-CO2), 93.5 (dd, J = 187.8, 33.0 Hz, minor CH2), 91.9 (dd, J = 183.4, 30.8 Hz, major CH2), 91.4 (dd, J = 186.0, 32.7 Hz, major CH2), 90.5 (dd, J = 181.2, 30.8 Hz, minor CH2), 62.9 (d, J = 22.0 Hz, minor CH2), 61.5 (d, J = 22.0 Hz, major CH2), 53.1 (major CO2CH3), 52.7 (major CO2CH3), 51.3 (d, J = 22.0 Hz, major CH2), 49.8 (d, J = 22.0 Hz, minor CH2), 21.2 (minor NCOCH3) ppm; 19F NMR (376 MHz, CDCl3) (80:20 rotamer ratio) δ −192.2 to −192.8 (m, 1F, major F), −193.4 to −193.9 (m, 1F, minor F), −194.6 to −195.0 (m, 1F, minor F), −196.4 to −196.9 (m, 1F, major F) ppm; 19F{1H} NMR (376 MHz, CDCl3) (80:20 rotamer ratio) δ −192.6 (d, J = 13.9 Hz, 1F, major F), −193.8 (d, J = 13.9 Hz, 1F, minor F), −194.9 (d, J = 13.9 Hz, 1F, major F), −196.8 (d, J = 13.9 Hz, 1F, minor F) ppm; Rf 0.19 (hexane/acetone 70:30); [α]D° = −47.8 (c 0.9, CHCl3); MS (ESI) (m/z) 208.2 [M + H]+, 230.2 [M + Na]+; HRMS (ESI) for C6H12F2NaO3 [M + Na]+ calc for 230.0599, found 230.0604; IR 1758 (s), 1653 (s), 1048 (s), 1030 (s), 1016 (s) cm−1.

Figure 9. To a solution of 24a (64.0 mg, 0.187 mmol) in methanol (2.0 mL) was added 10% Pd/C (10.0 mg). The mixture was purged with one balloon volume of hydrogen gas. Subsequently, the mixture was kept under a hydrogen atmosphere and stirred at room temperature. After 23 h, the mixture was filtered through a plug of Celite and the solvent evaporated. Carboxylic acid (N-Boc)-S (118.5 mg, quantitative) was obtained as a clear oil. The product was used as such in the next reaction.
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Southampton for funding. The Research Foundation Flanders (FWO) is indebted for a research project to J.C.M. and D.S. (G011015), Ph.D. and postdoctoral fellowships to E.O. and D.S., respectively, and staff exchange funding (FWO-WOG Multim). The 500 MHz used for the kinetic analysis was funded in part by a Hercules grant from the Hercules foundation (AUGE09/006). The computational resources (Stevin Supercomputer Infrastructure) and services used in this work were provided by the VSC (Flemish Supercomputer Center), funded by Ghent University, FWO, and the Flemish government, department EWI. The EPSRC is thanked for a partial PhD. grant to G.-J.H. (EPSRC-DTG EP/M50662X/1) and instrument funding (core capability EP/K039466/1).

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