Synthesis and conformational properties of 3,4-difluoro-L-prolines

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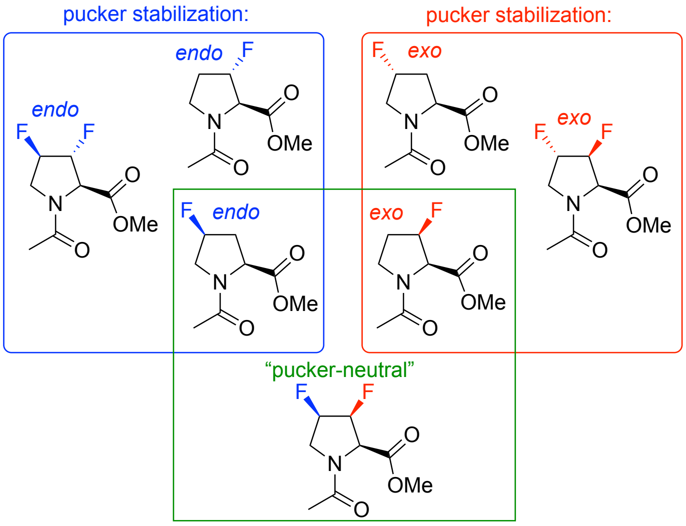


Abstract—Fluorinated proline derivatives have found diverse applications in areas ranging from medicinal chemistry over structural biochemistry to organocatalysis. Depending on the stereochemistry of monofluorination at the proline 3- or 4-position, different effects on the conformational properties 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 (3*S*,4*S*)- and (3*R*,4*R*)-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 to interrogate its role in, for instance, protein stability or folding.

INTRODUCTION

Fluorination of organic molecules has proven to be a highly useful tool to manipulate 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^{γ} *exo* or a C^{γ} *endo* pucker by introducing a (4*R*)-fluoro group (**1**, Figure 1) or a (4*S*)-fluoro group (**2**), respectively, an effect attributed to $\sigma_{CH}\rightarrow\sigma^{*}_{CF}$ hyperconjugation interactions.[9] Besides 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 basic,[11,13,14] and the carboxylic acid group more acidic.[15]

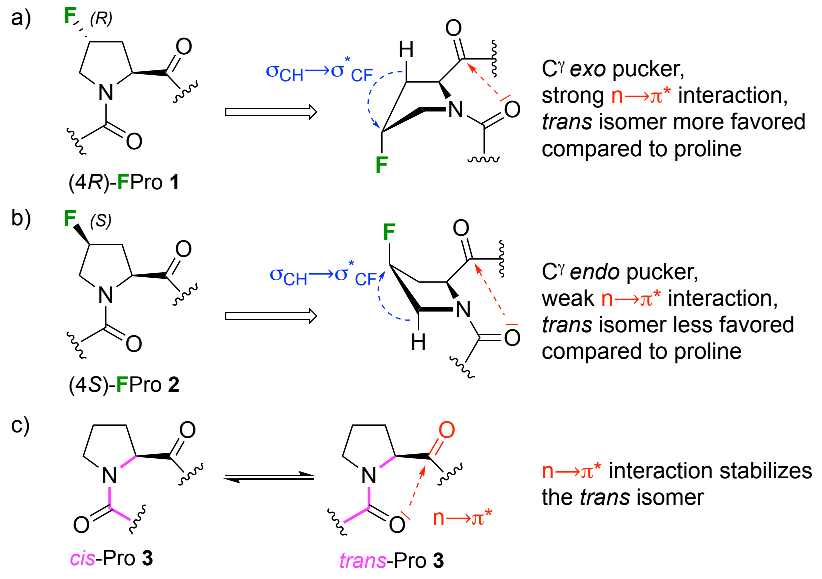


Figure 1 (a) (4*R*)-FPro **1** adopts an C^{γ} *exo* pucker (b) (4*S*)-FPro **2** adopts an C^{γ} *endo* pucker (c) $n\rightarrow\pi^{*}$ interaction stabilizes the *trans* isomer

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.[8,14] Nevertheless, the first syntheses of (4*R*)-FPro **1** and (4*S*)-FPro **2** date back to 1965,[16] although it took until the late nineties 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 thermostability of this protein, which forms triple helices out of Pro-Hyp-Gly repeats. Replacing (4*R*)-4-hydroxyproline (Hyp) with (4*R*)-FPro **1** 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 by (4*S*)-FPro **2** led to less stable collagen mimics. Since fluorine is more electronegative than a hydroxyl group, (4*R*)-FPro favors the C^{γ} *exo* pucker more strongly than Hyp, and because (4*S*)-FPro favors the C^{γ} *endo* pucker, this revealed that it is the strong preference for the C^{γ} *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 $n \rightarrow \pi^*$ interaction is promoted between the carbonyl groups of two adjacent peptide bonds, favoring the *trans* amide bond rotamer.[19] Interestingly, the ring pucker of the Pro residue preceding Hyp is also relevant for collagen stability,[20] which has equally been investigated using both 4- and 3-monofluorinated proline variants.[21]

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.[8] Indeed, modulating these properties by fluorination, rather than just fully eliminating them by mutating proline to non-proline residues,[22] can provide a more elegant approach towards uncovering the functional significance of proline's unique properties. Moreover, the introduction of fluorine allows the use of ^{19}F NMR as a powerful means to monitor residue-specific information. The exceptionally high responsivity of the ^{19}F nucleus to changes in its (local) environment, in addition to the sparsity of the ^{19}F spectrum, make ^{19}F NMR a very attractive means to monitor protein structural and dynamical changes, enzyme catalysis, and ligand binding.[23-28] Despite these clear advantages and earlier suggestions,[29-31] to the best of our knowledge, there are only a very limited number of reports involving the full potential of ^{19}F NMR in a fluoroproline peptide context.[32,33] However, if the FPro residue is to be used purely as a ^{19}F NMR probe, the conformationally perturbing effects of fluorine must be carefully considered. We recently introduced (3*S*,4*R*)-3,4-difluoroproline ((3*S*,4*R*)-FPro) **4** (Figure 2) where the two fluorines have opposing pre-organizing effects, thus resulting in a proline analogue with minimal conformational bias and minimal homonuclear coupling complications for ^{19}F NMR purposes.[34]

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 optically pure form continues to be of interest.[35] In addition, regardless of whether such applications require conformationally neutral, C^{γ} *exo*- or C^{γ} *endo*-pucker promoting fluoroprolines, the availability of more than one variant with similar conformational properties, but well-separated ^{19}F NMR chemical shifts, is of interest for site-specific multi-residue labelling 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-L-prolines **4-7** (Figure 2), in order to expand the toolbox of proline analogues.

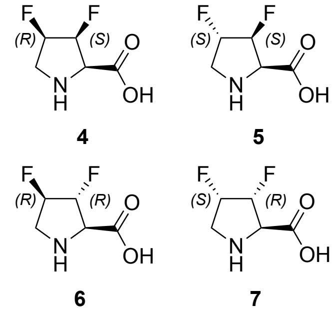
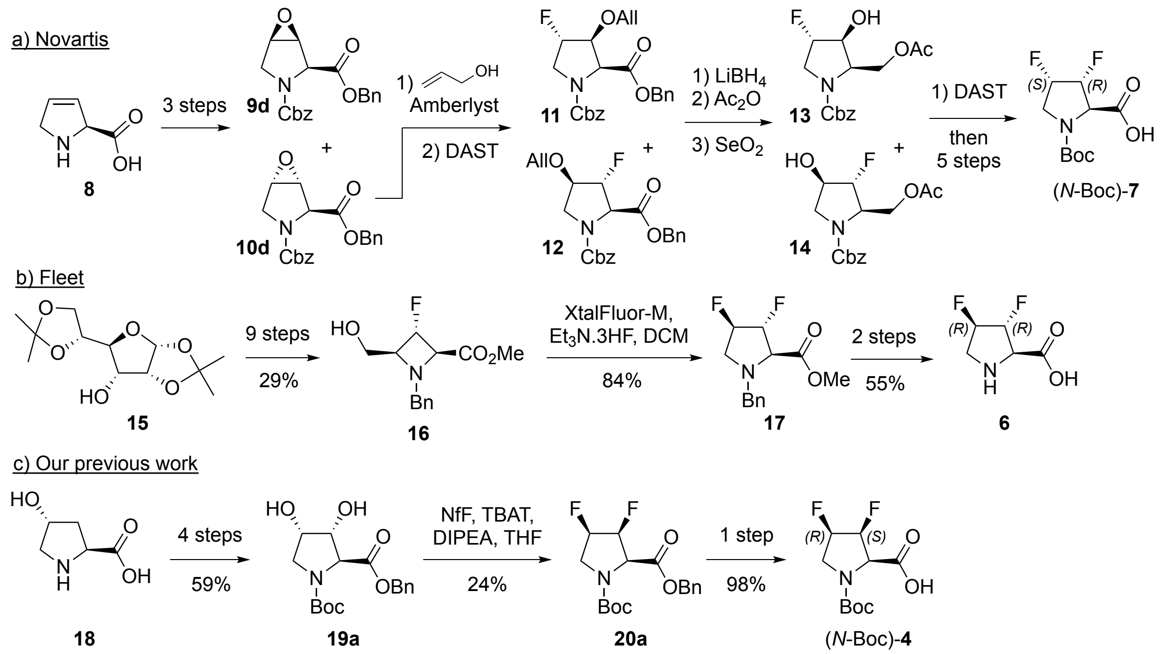


Figure 2: Structures of the targeted 3,4-difluoroprolines

There exists only 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**.[36] 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 coworkers, where deoxyfluorination of **16** using XtalFluor-M/Et_3N•3HF did not lead to the desired difluorinated azetidine derivative (not shown), but instead yielded the ring-expanded product **17**.[37] Deprotection of **17** led to (3*R*,4*R*)-3,4-difluoroproline **6**. Hence, in both cases, C–F bond introduction was achieved in sequential fashion. Finally, our group recently reported a stereoselective synthesis of Boc-protected (3*S*,4*R*)-3,4-difluoroproline (*N*-Boc)-**4**, which featured a direct bis-deoxyfluorination step (Scheme 1c).[34] (3*R*,4*S*)-3,4-Dihydroxyproline **19a**, obtained by selective dihydroxylation of the corresponding 3,4-dehydroproline, was treated with nonafluorobutanesulfonyl fluoride (NfF) in combination with tetrabutylammonium triphenyldifluorosilicate (TBAT) to yield **20a** as the only observed 3,4-difluoroproline.

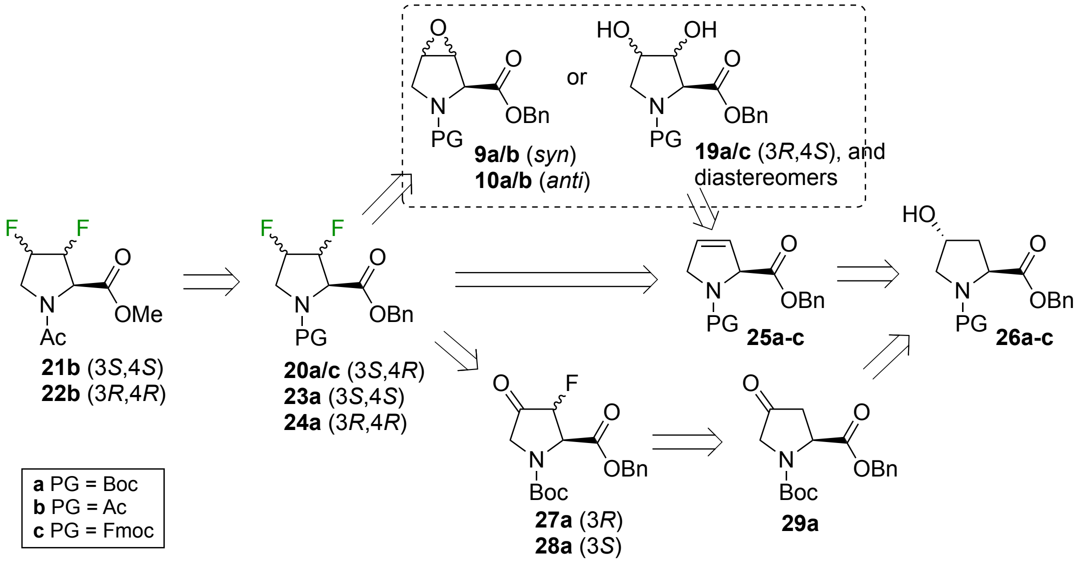


Scheme 1. Precedence for the synthesis of 3,4-difluoroprolines

In this work, we describe in detail the synthesis of the yet unreported (3*S*,4*S*)-3,4-difluoroproline **5**, and a novel, more concise route for (3*R*,4*R*)-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**, are 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 the (4*R*)-FPro and (3*R*)-FPro, both known to be biased to the C^γ *exo* pucker and *trans* peptide bond configuration relative to proline,[14] 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^γ *endo* pucker and of the *cis* peptide bond configuration relative to proline, as it is a combination of (4*S*)-FPro and (3*S*)-FPro.

RESULTS AND DISCUSSION

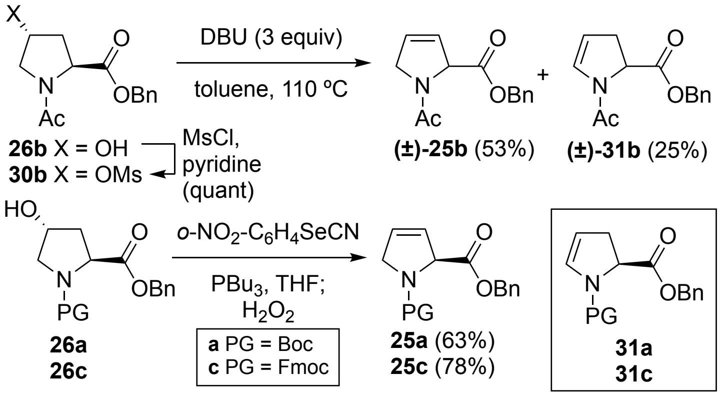
Retrosynthetic Analysis



Scheme 2: 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 fluorohydrin. Alternatively, diol **19a/c** provided an approach for 3,4-difluorination, with 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-dehydroderivative **25a-c**, direct functionalization of **25a-c** such as vicinal difluorination or a halofluorination/fluoride 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 (4*R*)-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.

3,4-Dehydroproline Synthesis



Scheme 3. Synthesis of Protected 3,4-Dehydroproline **25a-c**.

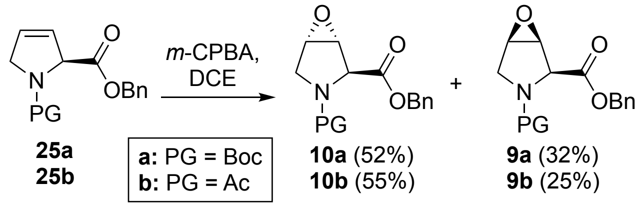
Initial efforts focused on achieving a large-scale synthesis of 3,4-dehydroproline **25**. Following a literature protocol, conversion of protected (4*R*)-4-hydroxyproline **26a** to the corresponding iodide, *via* a Mitsunobu reaction,[40] followed by DBU-promoted HI-elimination, gave a ±5:1 mixture of alkene regioisomers from which the desired alkene **25a** could be isolated in 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 racemic 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 sequence[41] directly starting from **26a** gave enantiopure **25a** as the major regioisomer with increased regioselectivity (>10:1 ratio), and with negligible degree of racemization. The smaller amount of **31a** facilitated purification considerably. Furthermore, in contrast to base-mediated elimination reactions, it was found that direct Grieco elimination of (4*R*)-4-hydroxyproline could also be performed with an *N*-Fmoc protecting group (**26c**) in very good yield. This procedure is an improvement over the previously reported 2-step elimination via 4-SePh intermediates, which are typically prepared from the corresponding 4-OMs or 4-OTs derivatives.[42-46]

Vicinal Difluorination and Halofluorination

Direct vicinal difluorination of alkene **25a** was attempted using recent methods developed by Gilmour and Jacobsen, both based on the *in situ* generation of a hypervalent iodoarene difluoride.[47-48] Unfortunately, both methods were unsuccessful and only led to recovered starting material (not shown). Subsequent attempts to effect halofluorination on **25a** using different combinations of NBS, NCS or NIS with either HF•pyridine or Et_3N•HF were unsuccessful as well, and this line of research was abandoned.

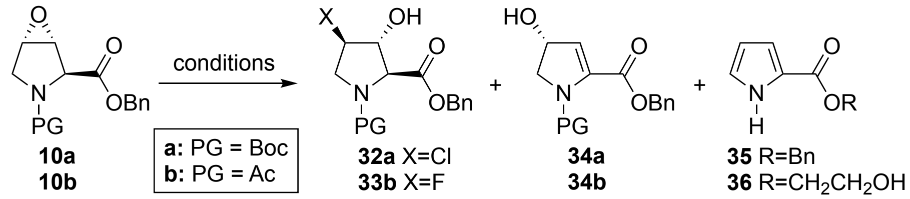
Epoxide-based Strategy



Scheme 4. Synthesis of Protected 3,4-Epoxyprolines.

Epoxidation of 3,4-dehydroproline derivatives is known, but not with the Boc/Bn or Ac/Bn protecting group combinations. Following protocol, treatment of **25a/b** with mCPBA led to a mixture of epoxides **9a/b** and **10a/b** in good yield with the *trans*-isomer **10a/b** isolated as the major isomer after chromatography (Scheme 4). While determination of the epoxide stereochemistry was achieved by ^{1}H NMR analysis as reported by Robinson *et al* on *N*-Cbz-3,4-epoxyproline benzyl esters (Supporting Information),[49] unambiguous conformation of the stereochemistry was obtained by X-ray crystallographic analysis of **9b** (Supporting Information).

Table 1 Conditions investigated for the direct fluoride opening of epoxides **10a** and **10b**.



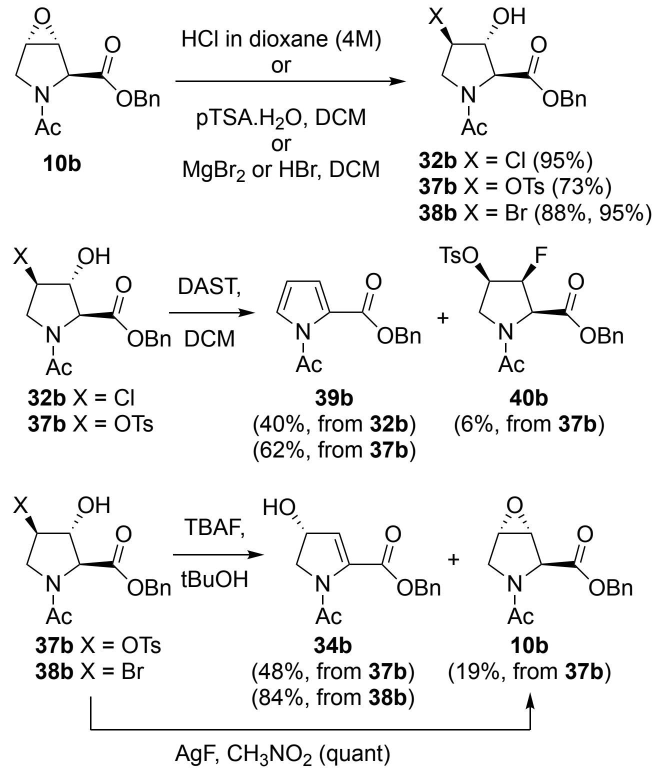
Entry	PG	Conditions	T (°C)	Time (h)	Product
1	Boc	Et_3N•3HF (neat)	130	72	35 (quant)^{b}
2	Boc	Et_3N•3HF/THF (2:1), MW^{c}	100	0.08	10a (86%), 35 (7%)^{b}
3	Boc	Et_3N•3HF/THF (2:1), MW^{c}	100	0.33	10a (72%) and 35 (28%)^{b}
4	Boc	Et_3N•3HF/THF (3:1), MW^{c}	130	0.66	35 (quant)^{b}
5	Ac	DMPU•HF, DCM	rt→50	43	10b (30%), 33b (15%)
6	Ac	DMPU•HF, DCE	60	72	10b (25%), 33b (5%)
7	Boc	$Bu_4NH_2F_3$, DCE	120	25	32a (74%)
8	Boc	$Bu_4NH_2F_3$, toluene	120	24	34a (56%)

9	Ac	TBAF, *t*-BuOH	70	4	34b (30%), 35 (13%)
10	Boc	KHF$_2$, ethylene glycol	150	22	36 (59%), 35 (2%)

[a] Severe gel formation. [b] Calculated yields based on ^{1}H NMR analysis of the crude reaction mixture. [c] Microwave irradiation.

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 (±15%), alongside 68% of 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% 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, 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$_\alpha$-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.



Scheme 5. Epoxide opening with other nucleophiles and subsequent fluorination attempts.

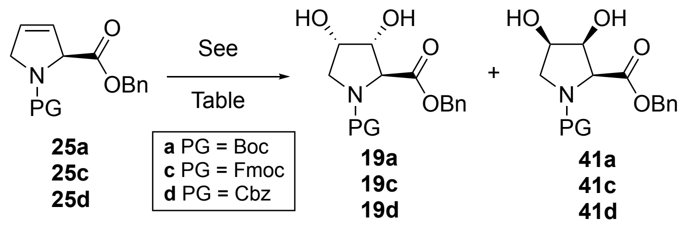
Starting from **10b**, regioselective opening with HCl, HOTs, and HBr (or $MgBr_2$) led to the corresponding 4-substituted 3-hydroxyprolines **32b**, **37b**, and **38b** in excellent yields.

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 quantitative yield.

At this point, the epoxide-based strategy was abandoned, and attention shifted to fluorine introduction via a vicinal diol group.

Direct Bis-deoxyfluorination Approach

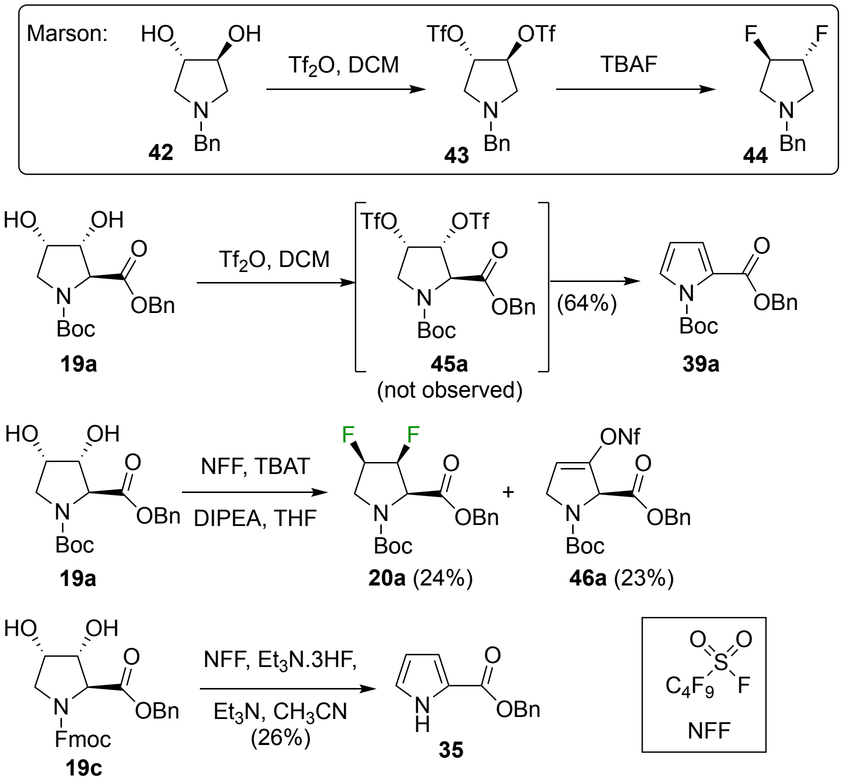
Table 2. Dihydroxylation of 3,4-dehydroproline **25a/c**.



Entry	**PG**	**Conditions**	**Yield 19a/c**

1	Boc	OsO_4, NMO, H_2O/dioxane (1:4)	92%
2	Boc	$K_2OsO_4\cdot 2H_2O$, NMO, H_2O/acetone (1:3)	94%
3	Boc	AD-mix α, *t*-BuOH/H_2O (1:1)	82%
4	Boc	AD-mix β, *t*-BuOH/H_2O (1:1)	66%
5	Fmoc	$K_2OsO_4\cdot 2H_2O$, NMO, H_2O/acetone (1:3)	80%

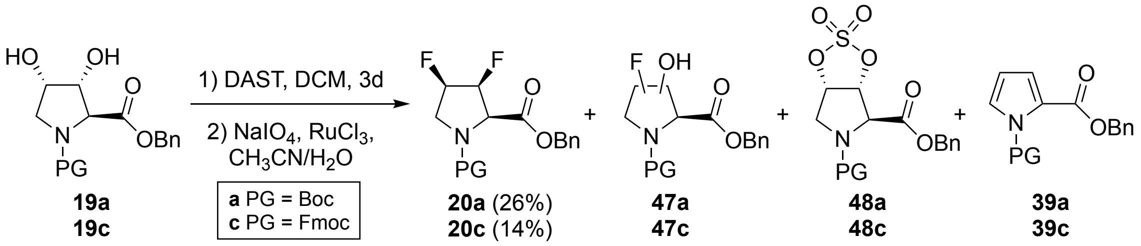
Dihydroxylation of Cbz-protected 3,4-dehydroproline **25d** with OsO_4 has been reported to be high-yielding and very stereoselective leading to the 2,3-*trans*-2,4-*trans*-diol **19d** as the major isomer.[44-45, 54] A similar result was observed when these conditions were applied to **25a** (Table 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 formation of all-*cis*-diol **41a** using Sharpless asymmetric dihydroxylation[55] 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% yield respectively (Entries 3-4). 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).



Scheme 6. Fluorination of Dihydroxyproline via Sulfonate Intermediates.

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 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 nonafluorobutanesulfonyl 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-difluoroproline diastereoisomer (^{19}F 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 $NaBH_4$ 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.

As a Fmoc protecting group does not tolerate basic conditions, TBAT could not be used as a fluoride source for the NfF fluorination. Even when (diluted) $Et_3N•3HF/Et_3N$ was employed as a fluoride source,[60] no difluorination was observed in the crude ^{19}F NMR and pyrrole **35** was the only product obtained from the reaction.



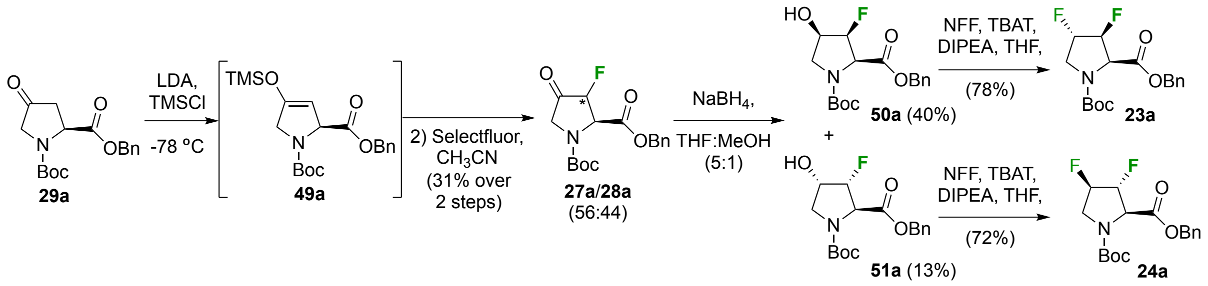
Scheme 7. Fluorination of Dihydroxyproline Using DAST.

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 ^{19}F NMR analysis, next to two minor byproducts, which presumably were monofluorinated hydroxyfluoroprolines **47a**. As the desired **20a** co-eluted with another byproduct, identified as the corresponding cyclic sulfite, 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 26% yield. According to MS-analysis, the sulfite oxidation was not accompanied by possible[61] 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 (4*R*)-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 towards an electrophilic fluorination approach. Barraclough *et al* had demonstrated the regioselective conversion of a 4-ketoproline derivative to the corresponding silyl enolether,[62-63] which was used to stereoselectively introduce deuterium at C3. Hence, formation of the silyl enol ether **49a** was achieved upon treatment of **29a**,[64,65,66] 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 maximum 31% yield. Reduction of the 4-keto group led to a mixture of two separable fluorohydrin isomers, **50a** and **51a**, in 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% yield respectively.[39] Interestingly, they also isolated a third diastereomer. Preliminary assignment of the stereochemistry at C_β was based on the observed coupling constant between H_α and H_β, which was ~6 Hz for **50a** and ~2 Hz for **51a**. This value for **50a** is in line with the coupling constant observed between H_α and H_β in **20a**. In addition, for **50a**, clear NOESY cross peaks where observed between H_α and H_β and between H_β and H_γ, suggesting all protons are on the same α-face of the pyrrolidine ring. This assignment was in agreement with the Ciulli work.[39]



Scheme 8. Electrophilc fluorination route to **23a** and **24a**.

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

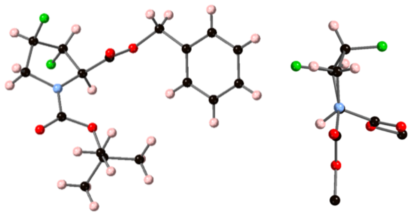
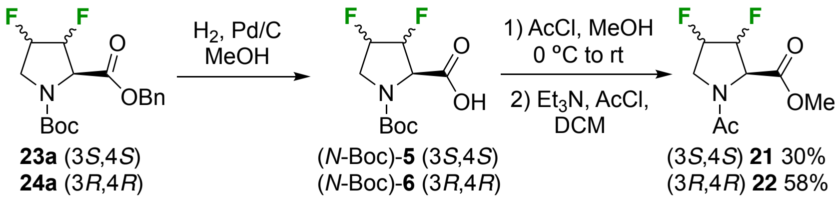


Figure 3. X-ray structure of (3*R*,4*R*)-3,4-difluoroproline **24a**. Thermal ellipsoids drawn at the 50% probability level.

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.[10, 13, 17, 67] 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**.



Scheme 9. Synthesis of N-acetyl methyl ester 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.

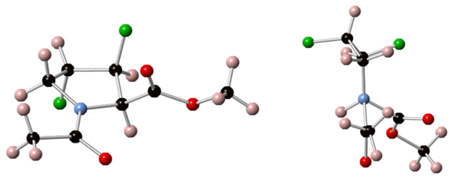


Figure 4. Crystal structure of (3*S*,4*S*)-3,4-difluoroproline **21**. Thermal ellipsoids drawn at the 50% probability level.

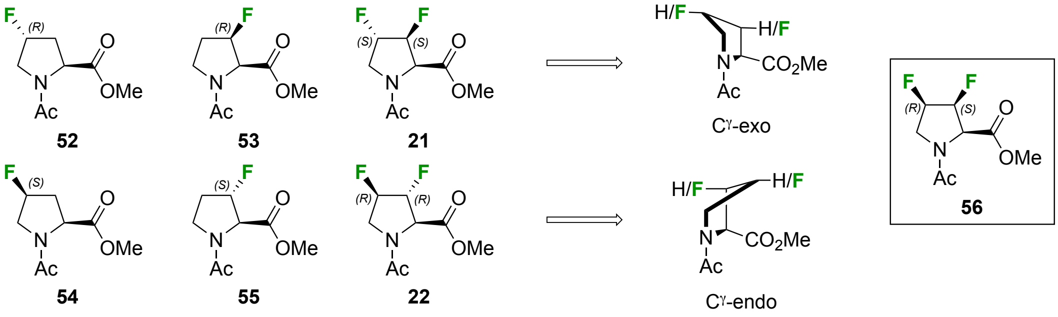
Conformational and Kinetic Analyses

The experimental *trans*:*cis* 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,4*R*)-, (3*R*,4*R*)- and (3*S*,4*S*)-3,4-difluorinated prolines and their monofluorinated progenitors are reported in Table 3. The entries are organized according to pucker preference. The data for the (3*S*,4*R*)-variant **56** has been reported and discussed previously,[34] but are included in Table 3 for the sake of completeness. In the following discussion, the term 'bias' assumes the conformational preference of the non-fluorinated *N*-acetyl proline methyl ester as a reference.

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

The *cis-trans* isomerization rates (represented here by $k_{ex} = k_{cis\text{-}trans} + k_{trans\text{-}cis}$) typically increase with 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.[11] As expected, both the (3*S*,4*S*)- and (3*R*,4*R*)-difluorinated variants, **21** and **22**, indeed show higher isomerization rates than their monofluorinated progenitors. Interestingly, the (3*R*)-variant **53** has a markedly higher isomerization rate than all other monofluorinated prolines,[29] and even exchanging faster than the (3*R*,4*R*)-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 (3*S*,4*S*)-variant **21**. The isomerization rate for **21** is also much higher than that of the previously described (3*S*,4*R*)- and (4,4)-difluorinated variants.[34]

Table 3. Experimental *trans-cis* ratio's and amide isomerization rates, and calculated pucker ratio's



	$K_{trans:cis}$ (exp.) (25 °C)		*cis/trans* kinetics, 35°C (s^{-1}) (exp.)			C^γ *endo*: C^γ *exo*[i] (DFT)			
						$CHCl_3$		H_2O	
	$CDCl_3$	D_2O	$k_{cis\text{-}trans}$	$k_{trans\text{-}cis}$	k_{ex}[d]	*trans*	*cis*	*trans*	*cis*
Ac-Pro-OMe	3.85[b]	4.62[b]	0.031[e]	0.007[e]	0.038	81:19	90:10	66:34	82:18
56 (3*S*,4*R*)	3.72	5.00	0.119±0.009[f]	0.025±0.002[f]	0.144±0.011	41:59	78:22	56:44	90:10
53 (3*R*)	5.08	8.31[c]	0.141±0.021[f,g]	0.019±0.003[f,g]	0.159±0.024	24:76	16:84	15:85	44:56
52 (4*R*)	4.26[b]	6.74[b]	0.064[e]	0.010[e]	0.074	11:89	28:72	7:93	17:83
21 (3*S*,4*S*)[a]	4.32	7.23	0.210±0.005[f]	0.031±0.001[f]	0.242±0.006	19:81	11:89	20:80	9:91
55 (3*S*)	4.19	4.31[c]	0.030±0.004[f,h]	0.009±0.001[f,h]	0.038±0.005	98:2	98:2	97:3	99:1
54 (4*S*)	1.64[b]	2.49[b]	0.037[e]	0.015[e]	0.052	97:3	99:1	99:1	99.5:0.5
22 (3*R*,4*R*)[a]	1.98	2.79	0.065±0.009[f]	0.024±0.003[f]	0.090±0.013	97:3	99:1	99:1	99:1

[a]Note that CIP prioritization changes with introduction of the second fluorine atom, so that **21** must be compared with **52** and **53**, and **22** with **54** and **55**. [b]In good agreement with reported ratios by Siebler *et al.*[10] [c]In good agreement with reported ratios by Kim *et al.*[68] [d]k_{ex} is defined as $k_{ex} = k_{cis\text{-}trans} + k_{trans\text{-}cis}$. [e]Calculated value based on Renner *et al.*[13] [f]Experimental NMR value obtained using similar procedure as Renner *et al.*[13] [g]Corresponding values reported by Thomas *et al.* at 37 °C using an alternative experimental procedure: 0.229 $s^{-1}$and 0.028 s^{-1}.[29] [h]Corresponding values reported by Thomas *et al.* at 37 °C using an alternative experimental procedure: 0.065 $s^{-1}$and 0.016 s^{-1}.[29] [i]DFT values, using the M06 functional with cc-pVDZ basis set and $CHCl_3$ or water implicit solvent models.

Finally, the calculated ratios between C^γ *endo* and C^γ *exo* puckers using DFT with chloroform or water as implicit solvent are provided (Table 3). Unmodified proline has a higher preference for the C^γ*endo* than the C^γ*exo* pucker.[9] Both (4*S*)- and (3*S*)-fluoroprolines, **54** and **55**, strongly bias these pucker ratios to the C^γ *endo* form, with negligible C^γ *exo* pucker populations, both in chloroform and water.[21] As expected, the (3*R*,4*R*)-difluoroproline variant **22** is heavily biased to the C^γ *endo* pucker as well, with essentially the same C^γ*endo*: C^γ *exo* ratio as that of its (3*S*)- and (4*S*)-progenitors. The (4*R*)- and (3*R*)-fluoroprolines, **52** and **53**, are biased to the C^γ *exo* pucker relative to Pro, albeit to different degrees. Where the (4*R*)-variant **52** shows a similar C^{γ} *exo*-bias in both solvents and for both *trans* and *cis* forms, the *cis* rotamer of the (3*R*)-variant **53** shows a high C^{γ} *exo* bias in chloroform, but a low bias in water. The (3*S*,4*S*)-difluorinated proline **21** shows a bias to C^{γ} *exo* pucker in the same order of magnitude as its progenitors. Interestingly, especially in the *cis* rotamer, the C^{γ} *exo* pucker is 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, $^3J_{\mathrm{FF}}$ couplings are known not to be practically exploitable to assess the dihedral angle,[69] while quantitatively calculating the ring pucker from experimental $^3\mathrm{J}_{\mathrm{HF}}$ and $^3\mathrm{J}_{\mathrm{HH}}$ 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 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 (4*R*)- and (4*S*)-monofluoroprolines, which are established as strongly biased to respectively C^{γ} *exo* and C^{γ} *endo*, clearly display distinct *transoid* $^3J_{\mathrm{H\alpha H\beta}}$ coupling constants of $8.2_{\mathrm{cis}}/10.1_{\mathrm{trans}}$ 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 (3*S*)-monofluoroproline, known to have a pronounced C^{γ} *endo* pucker,[21] and the larger values found for proline ($2.6_{\mathrm{cis}}/4.7_{\mathrm{trans}}$ Hz), consistent with intermediate *endo*:*exo* ratios and a higher *endo* population in the *cis*-form, confirm the relevance of $^3J_{\mathrm{H\alpha H\beta}}$ coupling constants for a qualitative analysis of fluorinated proline ring pucker. Hence, given the (3*R*,4*R*)-difluorinated variant **22** also shows a small $^3J_{\mathrm{H\alpha H\beta}}$ coupling value of <0.5 Hz, its calculated preference for a C^{γ} *endo* pucker is consistent with these experimental data.

In contrast, the *cisoid* $^3J_{\mathrm{H\alpha H\beta}}$ coupling constants of the (4*R*)- and (4*S*)-fluoroprolines and proline show similar values of $8.7_{\mathrm{cis}}/7.8_{\mathrm{trans}}$ Hz, 9.7 Hz and $8.9_{\mathrm{cis}}/8.8_{\mathrm{trans}}$ Hz respectively, implying this coupling is not very sensitive to the *endo*:*exo* ratio. Indeed, both the (3*R*)-fluoroproline, known to prefer an *exo* pucker,[21] and the (3*S*,4*S*)-difluoroproline show lower *cisoid* $^{3}J_{H\alpha H\beta}$ 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 $^{3}J_{H\alpha H\beta}$ and $^{3}J_{H\alpha F\beta}$ couplings observed for the (3*R*)- and (3*S*,4*S*)-variants suggests both fluoroprolines have mostly similar *endo*:*exo* ratios. In addition, these couplings differ significantly with those of the (3*S*,4*R*)-variant, which is expected given the latter displays virtually no pucker preference.

Table 4. Comparison of the relevant coupling constants

		$^{3}J_{H\alpha F\beta}$ (Hz)[a]				$^{3}J_{H\alpha H\beta}$ (Hz)[a]			
		cis-amide		*trans-amide*		*cis-amide*		*trans-amide*	
Compound	*Bias*[b]	*Cisoid*[c]	*Transoid*[c]	*cisoid*	*transoid*	*cisoid*	*transoid*	*cisoid*	*transoid*
Ac-Pro-OMe	-	n/a	n/a	n/a	n/a	8.9	2.6	8.8	4.7
(3*S*,4*R*) **56**	-	n/a	7.4	n/a	13.7	7.9	n/a	7.3	n/a
(3*R*) **53**	*exo*	n/a	25.9	n/a	28.2	5.1	n/a	4.8	n/a
(4*R*) **52**	*exo*	n/a	n/a	n/a	n/a	8.7	8.2	7.8	10.1
(3*S*,4*S*) **21**	*exo*	n/a	27.3	n/a	29.8	5.2	n/a	5.0	n/a
(3*S*) **55**	*endo*	19.8	n/a	13.7	n/a	n/a	<1.0[c]	n/a	1.0
(4*S*) **54**	*endo*	n/a	n/a	n/a	n/a	9.7	<1.0[c]	[d]	[d]
(3*R*,4*R*) **22**	*endo*	21.2	n/a	24.4	n/a	n/a	<1.0[c]	n/a	<1.0[c]

[a] ^{1}H-^{1}H couplings measured using PSYCHEDELIC[70], ^{1}H-^{19}F couplings were read from the 1D ^{1}H spectrum on the H$^{\alpha}$ proton. [b] Bias refers to the conformational preference of the non-fluorinated *N*-acetyl proline methyl ester as reference. [c] "*Cisoid*" indicates that the coupled atoms are on the same side of the proline ring, "*transoid*" on different sides of the ring. [c] Value

smaller than signal line width. [d] Degenerate H^{β} chemical shifts, individual couplings could not be extracted.

The clear C^{γ} *exo* pucker bias observed for the (3*S*,4*S*)-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^{γ} *endo* pucker bias of the (3*R*,4*R*)-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,[13] ubiquitin,[71] Trp cage mini protein[72] and GFP[73] incorporating 4-fluoroprolines were obtained with the C_4-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.[68] 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 relevance of 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 C^{γ} *exo* pucker, to *cis* and C^{γ} *endo* pucker, or with similar structural preference to proline — clearly will be of interest within such studies, allowing to deconvolute the roles of ring pucker and *cis*/*trans* preferences from isomerization kinetics.

Recently, Bernardes and Corzana and coworkers 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 3,4-*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 $^{n}J_{FF}$ coupling constants for the *N*-Ac-X-OMe model compounds the (4,4)-difluoroproline, (3*S*,4*R*), (3*R*,4*R*)- and (3*S*,4*S*)-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.[30] 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 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 (D_2O)

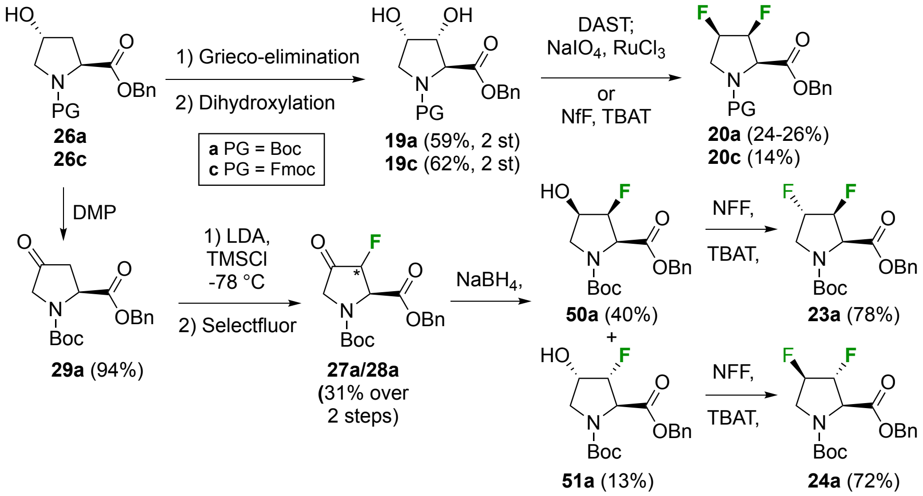
Compound	^{19}F δ/ppm (F3, F4)		$^{n}J_{FF}$ (Hz)	
	Cis-amide	*Trans-amide*	*Cis-amide*	*Trans-amide*
(3*R*) **53**	-184.4	-186.4	-	-
(3*S*) **55**	-176.8	-175.7	-	-
(4*R*) **52**	-177.9	-177.0	-	-
(4*S*) **54**	-173.1	-172.9	-	-
(3*S*,4*S*) **21**	-195.0, -194.3	-197.2, -193.3	11.1	11.4
(3*R*,4*R*) **22**	-190.6, -187.7	-189.1, -188.4	12.9	13.0
(3*S*,4*R*) **56**	-208.5, -200.3	-210.4 , -203.3	5.8	4.7
(4,4) **57**	-101.4, -96.7[a]	-98.2, -99.1[a]	236.0	233.7

[a] pro-*R* F4, pro-*S* F4.

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 (3*R*,4*R*)- and (3*S*,4*S*)-3,4-difluoroproline analogues.

The (3*S*,4*R*)-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 substrate led to the desired target using both NfF and DAST, with the former giving the highest yield when *N*-Boc was used as 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 elimination procedure, in contrast to the usually employed basic conditions.



Scheme 10. Summary scheme for the synthesis of protected 3,4-difluoroprolines.

The (3*R*,4*R*)- and the novel (3*S*,4*S*)-difluorinated proline derivatives were synthesized using a two-step fluorination strategy, the first being an electrophilic fluorination starting from protected 4-ketoproline, and the second by a DAST-mediated deoxyfluorination after 4-ketoreduction. Hence, starting from **26a**, the (3*S*,4*S*)- and (3*R*,4*R*)-*N*-Boc 3,4-difluoroproline benzyl esters were obtained in an overall yield of 9% for **23a** and 3% for **24a**, in a combined 5 steps (with three common steps before diastereomer separation). It may be pointed out that the linear 10-step Fleet synthesis[37] of the (3*R*,4*R*)-*N*-Bn difluoroproline methyl ester **17** (cf Scheme 1) has a higher overall yield (24%), although this delivers a single diastereomer only. In addition, Ciulli reported higher yields for the electrophilic fluorination step.[39] X-ray crystallographic analysis allowed unambiguous determination of the relative configuration of the obtained 3,4-difluoroprolines.

Due to the opposing conformational effects of each individual fluorine in the (3*S*,4*R*)-difluorinated proline derivative, this analogue has previously been described as having minimal conformational bias to proline.[34] 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 (3*R*,4*R*)-difluorinated proline derivative resembles most closely the (4*S*)-fluoroproline, the (3*S*,4*S*)-difluorinated proline derivative resembles the (4*R*)-fluoroproline, though with a somewhat higher preference for a C^{γ} *exo* pucker in its *cis* rotamer. Given the distinct ^{19}F chemical shifts of both 3,4-difluorinated derivatives to their monofluorinated progenitors, they will be of interest for multi-residue fluorine labelling strategies, for instance in the study of repetitive or low-complexity protein sequences, where similar conformational preorganizing effects are desired, but distinct residue-specific ^{19}F NMR chemical shifts are needed.

A clearer difference between the 3,4-difluoprolines 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 (3*S*,4*S*) variant shows a remarkably high isomerization rate, higher than any previously described difluorinated variant. These new variants will thus be very useful towards studying the role of Xaa-Pro *cis-trans* isomerization kinetics for biological function,[82] protein folding[83] or amyloid assembly.[75]

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 CH_2Cl_2, THF, MeOH and hexane were bought from commercial suppliers, and used as received. TLC was performed on aluminium-precoated plates coated with silica gel 60 with an F254 indicator; visualized under UV light (254 nm) and/or by staining with $KMnO_4$ (10% aq.). Flash column chromatography was performed with Sigma Aldrich 60 silica gel (40-63 micron). Preparative HPLC was carried out using a Biorad Bio-Sil D 90-10 column (250 × 22 mm at 15 mL min^{-1}). High-resolution MS samples were analysed using a MaXis (Bruker Daltonics, Bremen, Germany) mass spectrometer equipped with a Time of Flight (TOF) analyser. Samples were introduced to the mass spectrometer via a Dionex Ultimate 3000 autosampler and uHPLC pump, and eluted in five minutes at 0.6 mL min using a gradient 20% acetonitrile (0.2% formic acid) to 100% acetonitrile (0.2% formic acid) through an Acquity UPLC BEH C18 (Waters) 1.7 micron 50 x 2.1mm column. High resolution mass spectra were recorded using positive ion electrospray ionisation. ^{1}H-, ^{19}F- and ^{13}C-NMR spectra were recorded at room temperature on a Bruker Ultrashield 400 MHz or 500 MHz spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are quoted in ppm relative to residual solvent peaks as appropriate. ^{19}F spectra were externally referenced to $CFCl_3$. The coupling constants (J) are given in Hertz (Hz). The NMR signals were designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), spt (septet), m (multiplet), or a combination of the above. For all compounds, detailed peak assignment was performed through the combined use of HSQC, HMBC, NOESY and COSY NMR experiments.

N-(Acetyl)-(2*S*,4*R*)-4-(methanesulfonyloxy)proline benzyl ester (30b) (Scheme 3)

Alcohol **26b** (14.5 g, 55.1 mmol) was dissolved in pyridine (150 mL) and cooled to 0 °C. Mesyl chloride (6.82 mL, 88.1 mmol) was added dropwise and the mixture was allowed to warm to room temperature. TLC analysis indicated the reaction was finished after 7 h. Subsequently, the mixture was cooled to 0 °C and quenched with a solution of 10% H_2O in pyridine (50 mL). The solvent was evaporated *in vacuo*, the crude product re-dissolved in H_2O (80 mL) and the aqueous layer extracted with DCM (3 × 80 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (60 mL) and brine (60 mL), dried over $MgSO_4$ and evaporated *in vacuo* to yield **30b** (18.8 g, quant.) as an off-white solid. **^{1}H NMR** (400 MHz, $CDCl_3$): (78:22 rotamer ratio) δ 7.42–7.31 (m, 5H major Ph + 5H minor Ph), 5.36–5.30 (m, 1H, major $C_\gamma\underline{H}$), 5.29–5.24 (m, 1H, minor $C_\gamma\underline{H}$), 5.23 (d, *J*=12.4 Hz, 1H, major C$\underline{H}$H'Ph), 5.23 (s, 2H, minor C$\underline{H_2}$Ph), 5.19 (d, *J*=12.4 Hz, 1H, major CH$\underline{H'}$Ph), 4.61 (t, *J*=8.0 Hz, 1H, major $C_\alpha\underline{H}$), 4.58 (dd , *J*=8.1, 7.2 Hz, 1H, minor $C_\alpha\underline{H}$), 4.15 (dt, *J*=13.5, 2.1 Hz, 1H, minor $C_\delta\underline{H}$H'), 3.94 (dd, *J*=12.2, 4.5 Hz, 1H, major C_δH$\underline{H'}$), 3.87 (dt, *J*=12.0, 2.1 Hz, 1H, major $C_\delta\underline{H}$H'), 3.68 (dd, *J*=13.5, 4.6 Hz, 1H, minor C_δH$\underline{H'}$), 3.06 (s, 3H, major $\underline{H_3}$C-SO$_2$), 3.04 (s, 3H, minor $\underline{H_3}$C-SO$_2$), 2.80 (dddd, *J*=14.4, 8.3, 3.1, 2.1 Hz, 1H, minor $C_\beta\underline{H}$H'), 2.59 (dddd, *J*=14.3, 8.2, 3.2, 1.6

Hz, 1H, major $C_{\beta}\underline{H}H'$), 2.42 (ddd, *J*=14.3, 6.9, 5.3 Hz, 1H, minor $C_{\beta}H\underline{H'}$), 2.25 (ddd, *J*=14.2, 7.8, 5.1 Hz, 1H, major $C_{\beta}H\underline{H}'$), 2.11 (s,3H, major CO-$C\underline{H}_3$), 1.93 (s,3H, minor CO-$C\underline{H}_3$) ppm; **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$): ^{13}C NMR δ 171.3 (major C_{α}-$\underline{C}O_2$), 171.1 (minor C_{α}-$\underline{C}O_2$), 169.8 (minor N-$\underline{C}OCH_3$), 169.3 (major N-$\underline{C}OCH_3$), 135.4 (major $\underline{C}_{q,Ph}$), 134.7 (minor $\underline{C}_{q,Ph}$), 128.9 + 128.8 + 128.6 + 128.5 + 128.4 + 128.2 (major and minor overlap, $\underline{C}H_{Ph}$), 77.1 (major $\underline{C}_{\gamma}H$), 77.2 (minor $\underline{C}_{\gamma}H$), 67.8 (minor $\underline{C}H_2Ph$), 67.2 (major $\underline{C}H_2Ph$), 58.2 (minor $\underline{C}_{\alpha}H$), 57.1 (major $\underline{C}_{\alpha}H$), 53.6 (major $\underline{C}_{\delta}H_2$), 51.8 (minor $\underline{C}_{\delta}H_2$), 38.8 (minor $H_3\underline{C}$-SO_2), 38.7 (major $H_3\underline{C}$-SO_2), 38.1 (minor $\underline{C}_{\beta}H_2$), 35.7 (major $\underline{C}_{\beta}H_2$), 22.2 (major $CO\underline{C}H_3$), 21.6 (minor $CO\underline{C}H_3$) ppm; **$[\alpha]_D^{21}$** –43 (c 0.8, $CHCl_3$); **mp** 96–100 °C; **Rf** 0.43 (hexane/acetone 50:50); **MS** (ESI)(*m/z*): 342.3 $(M+H)^+$, 364.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{15}H_{20}NO_6S$ $(M+H)^+$ Calcd. 342.1006; Found 342.1007; **IR** 1743 (s), 1651 (s), 1354 (s), 1172 (s) cm^{-1}.

(±)-*N*-(Acetyl)-3,4-dehydroproline benzyl ester (25b) and (±)-*N*-(acetyl)- 4,5-dehydroproline benzyl ester (31b) (Scheme 3)

Mesylate **30b** (18.8 g, 55.3 mmol) was dissolved in toluene (200 mL), DBU (24.8 mL, 165 mmol) was added and the mixture was refluxed at 110 °C. After 14 h, the solvent was evaporated *in vacuo*. Next, the crude product was re-dissolved in DCM (250 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 150 mL) and brine (150 mL). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 65:35) and Biotage® (hexane/acetone gradient) yielded alkene **±25b** (7.19 g, 53%) and alkene **31b** (3.33 g, 25%) as colourless oils.

Data for (±)-*N*-(acetyl)-3,4-dehydroproline benzyl ester **25b**: **^1H NMR** (400 MHz, $CDCl_3$) (76:24 rotamer ratio) δ 7.41–7.29 (m, 5H major Ph + 5H minor Ph), 6.05 (app. dq, *J*=6.3, 2.1 Hz, 1H, minor $C_{\beta}\underline{H}$), 5.99 (app. dq, *J*=6.3, 2.1 Hz, 1H, major $C_{\beta}\underline{H}$), 5.85 -5.78 (m, 1H major $C_{\gamma}\underline{H}$ and 1H minor $C_{\gamma}\underline{H}$), 5.25–5.10 (m, 2H major $C\underline{H}_2Ph$ + 2H minor $C\underline{H}_2Ph$ + 1H major $C_{\alpha}\underline{H}$

+ 1H minor $C_{\alpha}\underline{H}$), 4.46–4.23 (m, 2H major $C_{\delta}\underline{H}_2$ and 2H minor $C_{\delta}\underline{H}_2$), 2.12 (s, 3H, major $C\underline{H}_3$), 1.93 (s, 3H, minor $C\underline{H}_3$) ppm; **$^{13}C\{^{1}H\}$ NMR** (100 MHz, $CDCl_3$): ^{13}C NMR (76:24 rotamer ratio) δ 169.6 (minor C_{α}-$\underline{C}O_2$), 169.5 (major C_{α}-$\underline{C}O_2$), 169.3 (minor N-$\underline{C}OCH_3$), 169.0 (major N-$\underline{C}OCH_3$), 135.6 (major $\underline{C}_{q,Ph}$), 135.1 (minor $\underline{C}_{q,Ph}$), 129.8 (minor $\underline{C}_{\beta}H$), 128.6 (major $\underline{C}_{\beta}H$), 128.72 + 128.68 + 128.3 + 128.2 + 128.0 (major and minor overlap, $\underline{C}H_{Ph}$), 125.3 (major $\underline{C}_{\gamma}H$), 124.3 (minor $\underline{C}_{\gamma}H$), 67.5 (minor $\underline{C}H_2Ph$), 67.2 (minor $\underline{C}_{\alpha}H$), 67.0 (major $\underline{C}H_2Ph$), 66.2 (major $\underline{C}_{\alpha}H$), 54.3 (major $\underline{C}_{\delta}H_2$), 53.5 (minor $\underline{C}_{\delta}H_2$), 21.8 (major $\underline{C}H_3$), 21.7 (minor $\underline{C}H_3$) ppm; **Rf** 0.64 (hexane/ acetone 50:50); **MS** (ESI)(*m/z*): 246.1 $(M+H)^+$, 268.1 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{16}NO_3$ $(M+H)^+$ Calcd. 246.1125; Found 246.1126; **IR** 1747 (s), 1654 (s), 1619 (m) cm^{-1}.

Data for partially racemized *N*-(acetyl)-4,5-dehydroproline benzyl ester **31b**: **^{1}H NMR** (400 MHz, $CDCl_3$) (89:11 rotamer ratio) δ 7.42–7.30 (m, 5H major Ph + 5H minor Ph), 7.02 (app. dt, *J*=4.3, 2.2 Hz, 1H, minor $C_{\delta}\underline{H}$), 6.51 (br. dt, *J*=4.3, 2.2 Hz, 1H, major $C_{\delta}\underline{H}$), 5.29–5.15 (m, 2H major $C\underline{H}_2Ph$ and 2H minor $C\underline{H}_2Ph$), 5.15–5.12 (m, 1H major $C_{\gamma}\underline{H}$ and 1H minor $C_{\gamma}\underline{H}$), 4.89 (dd, *J*=11.7, 5.0 Hz, 1H, major $C_{\alpha}\underline{H}$), 4.70 (dd, *J*=11.3, 3.6 Hz, 1H, minor $C_{\alpha}\underline{H}$), 3.21 (m, 1H, minor $C_{\beta}\underline{H}H'$), 3.03 (m, 1H, major $C_{\beta}\underline{H}H'$), 2.82 (m, 1H, minor $C_{\beta}H\underline{H'}$), 2.62 (m, 1H, major $C_{\beta}H\underline{H'}$), 2.17 (s, 3H, major $C\underline{H}_3$), 1.93 (s, 3H, minor $C\underline{H}_3$) ppm; **$^{13}C\{^{1}H\}$ NMR** (100 MHz, $CDCl_3$) (89:11 rotamer ratio) δ 171.1 (minor C_{α}-$\underline{C}O_2$), 170.8 (major C_{α}-$\underline{C}O_2$), 166.7 (minor N-$\underline{C}OCH_3$), 166.4 (major N-$\underline{C}OCH_3$), 135.5 (major $\underline{C}_{q,Ph}$), 135.3 (minor $\underline{C}_{q,Ph}$), 129.7 (minor $\underline{C}_{\delta}H$), 129.5 (major $\underline{C}_{\delta}H$), 128.51 +128.49 + 128.4 + 128.3 + 128.2 + 128.0 (major and minor overlap, $\underline{C}H_{Ph}$), 108.8 (major $\underline{C}_{\gamma}H$), 107.7(minor $\underline{C}_{\gamma}H$), 67.2 (minor $\underline{C}H_2Ph$), 66.9 (major $\underline{C}H_2Ph$), 59.1 (minor $\underline{C}_{\alpha}H$), 57.7 (major $\underline{C}_{\alpha}H$), 36.1 (minor $\underline{C}_{\beta}H$), 33.7 (major $\underline{C}_{\beta}H$), 21.8 (minor $\underline{C}H_3$), 21.5 (major $\underline{C}H_3$) ppm; **Rf** 0.72 (hexane/ acetone 50:50); **MS** (ESI)(*m/z*): 246.1 $(M+H)^+$, 268.1 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{16}NO_3$ $(M+H)^+$ Calcd. 246.1125; Found 246.1127; **IR** 1739 (s), 1654 (s), 1620 (m) cm^{-1}.

N-(9-Fluorenylmethyloxycarbonyl)-(2*S*)-3,4-dehydroproline benzyl ester (25c) (Scheme 3)

At 0 °C, tributylphosphine (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 stirring at room temperature for 7 h, TLC analysis indicated complete consumption of the starting material. Next, H_2O_2 (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 $Na_2S_2O_3$ (20 mL). The aqueous phase was extracted with DCM (3 × 25 mL). The combined organic phases were washed with brine (25 mL), dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. Purification by flash chromatography (hexane/EtOAc 90:10 to 75:25) yielded alkene **25c** (815.5 mg, 78 %) as a light orange oil. **^{1}H NMR** (400 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 7.83–7.23 (m, 13H major Ar-H + 13H minor Ar-H), 6.06–5.99 (m, 1H major $C_{\gamma}H$ + 1H minor $C_{\gamma}H$), 5.85–5.76 (m, 1H major $C_{\beta}H$ + 1H minor $C_{\beta}H$), 5.26 (d, *J*=12.2 Hz, 1H, major $C\underline{H}H'Ph$), 5.18 (d, *J*=12.5 Hz, 1H, major $CH\underline{H'}Ph$), 5.17 (d, *J*=12.4 Hz, 1H, minor $C\underline{H}H'Ph$), 5.07 (d, *J*=12.2 Hz, 1H, minor $CH\underline{H'}Ph$), 5.23–5.10 (m, 1H major $C_{\alpha}H$ + 1H minor $C_{\alpha}H$), 4.55–4.26 (m, 2H major $NCO_2\text{-}C\underline{H}_2\text{-}CH$ + 2H minor $NCO_2\text{-}C\underline{H}_2\text{-}CH$ + 1H major $NCO_2\text{-}CH_2\text{-}C\underline{H}$ + 2H major $C_{\delta}H_2$ + 2H minor $C_{\delta}H_2$), 4.05 (t, *J*=6.9 Hz, 1H minor $NCO_2\text{-}CH_2\text{-}C\underline{H}$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 169.8 (major $C_{\alpha}\text{-}\underline{C}O_2$), 169.7 (minor $C_{\alpha}\text{-}\underline{C}O_2$), 154.3 (major $N\underline{C}O_2$), 154.0 (minor $N\underline{C}O_2$), 144.13 + 144.06 + 143.8 + 143.6 (major and minor overlap, $C_{q,Fmoc}$), 141.29 + 141.27 + 141.26 + 141.19 (major and minor overlap, $C_{q,Fmoc}$), 135.5 (minor $\underline{C}_{q,Ph}$), 135.3 (major $\underline{C}_{q,Ph}$), 129.3 (minor C_{γ}), 129.2 (major C_{γ}), 128.5 + 128.34 + 128.25 + 128.1 + 128.0 + 127.69 + 127.65 + 127.59 + 127.05 + 127.03 + 127.01 + 126.98 + 126.93 + 125.14 + 125.08 + 125.0 + 124.9 (major and minor overlap, $\underline{C}H_{Ar}$), 124.7 (minor C_{β}), 124.6 (major C_{β}), 120.0 (major $\underline{C}H_{Ar}$), 119.9 (minor $\underline{C}H_{Ar}$), 67.6 (minor $NCO_2\text{-}\underline{C}H_2\text{-}CH$), 67.5 (major $NCO_2\text{-}\underline{C}H_2\text{-}CH$), 67.1 (major

C̲H$_2$Ph), 67.0 (minor C̲H$_2$Ph), 66.7 (major C$_α$), 66.3 (minor C$_α$), 54.0 (minor C$_δ$), 53.4 (major C$_δ$), 47.2 (minor NCO$_2$-CH$_2$-C̲H), 47.1 (major NCO$_2$-CH$_2$-C̲H) ppm; **Rf** 0.32 (hexane/EtOAc 80:20); **$[α]_D^{22}$** −184.1 (c 1.2, CHCl$_3$); **MS** (ESI)(*m/z*): 426.3 (M+H)$^+$, 448.3 (M+Na)$^+$; **HRMS** (ESI) for $C_{27}H_{23}NNaO_4$ (M+Na)$^+$ Calcd. 448.1519; Found 448.1524; **IR** 1750 (s), 1705 (s), 1450 (m), 1415 (s), 1172 (s), 1122 (s), 1105 (s), 734 (s) cm^{-1}.

N-(*tert*-Butoxycarbonyl)-(2*S*,3*R*,4*S*)-3,4-epoxyproline benzyl ester (10a) and *N*-(*tert*-butoxycarbonyl)-(2*S*,3*S*,4*R*)-3,4-epoxyproline benzyl ester (9a) (Scheme 4)

To a solution of alkene **25a** (32.0 g, 105.5 mmol) in 1,2-dichloroethane (250 mL), meta-chloroperoxybenzoic acid (≤77% pure, 30.7 g, 137.1 mmol) was added and the mixture is refluxed at 90 °C. After 24 h, the mixture was cooled to room temperature and quenched with a saturated aqueous solution of $Na_2S_2O_3$ (100 mL). The phases were separated and the organic layer was washed a saturated aqueous solution of $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was then dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/EtOAc 85:15 to 75:25) yielded **10a** (16.3 g, 52%) and **9a** (10.6 g, 32%) as colourless oils.

Data for *N*-(*tert*-butoxycarbonyl)-(2*S*,3*R*,4*S*)-3,4-epoxyproline benzyl ester (**10a**): **^{1}H NMR** (400 MHz, CDCl$_3$) (58:42 rotamer ratio) δ 7.41–7.31 (m, 5H major Ph + 5H minor Ph), 5.29 (d, *J*=12.4 Hz, 1H, minor CH̲H'Ph), 5.23 (d, *J*=12.3 Hz, 1H, major CH̲H'Ph), 5.20 (d, *J*=12.3 Hz, 1H, major CHH̲'̲Ph), 5.15 (d, *J*=12.4 Hz, 1H, minor CHH̲'̲Ph), 4.72 (s, 1H, minor C$_α$H), 4.57 (s, 1H, major C$_α$H), 3.88 (d, *J*=12.5 Hz, 1H, major C$_δ$H̲H'), 3.82 (d, *J*=12.5 Hz, 1H, minor C$_δ$H̲H'), 3.75 (dd, *J*=2.9, 0.5 Hz, 1H, major C$_β$H), 3.74 (dd, *J*=2.9, 0.4 Hz, 1H, minor C$_β$H), 3.68 (ddd, *J*=2.9, 1.3, 0.3 Hz, 1H, major C$_γ$H), 3.65 (ddd, *J*=2.9, 1.2, 0.4 Hz, 1H, minor C$_γ$H), 3.51 (dd, *J*=12.5, 1.4 Hz, major C$_δ$HH̲'̲), 3.48 (dd, *J*=12.5, 1.4 Hz, minor C$_δ$HH̲'̲), 1.45 (s, 9H, minor CO$_2$C(CH̲$_3$)$_3$), 1.33 (s, 9H, major CO$_2$C(CH̲$_3$)$_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, CDCl$_3$)

(58:42 rotamer ratio) δ 169.2 (major C_α-$\underline{C}O_2$), 169.1 (minor C_α-$\underline{C}O_2$), 154.4 (minor $\underline{C}O_2C(CH_3)_3$), 153.9 (major $\underline{C}O_2C(CH_3)_3$), 135.2 (minor $\underline{C}_{q,Ph}$), 135.1 (major $\underline{C}_{q,Ph}$), 128.7 + 128.61 + 128.59 +128.4 + 128.1 (major and minor overlap, $\underline{C}H_{Ph}$), 80.60 (major $CO_2\underline{C}(CH_3)_3$), 80.58 (minor $CO_2\underline{C}(CH_3)_3$), 67.27 (minor $\underline{C}H_2Ph$), 67.25 (major $\underline{C}H_2Ph$), 60.8 (major C_α), 60.4 (minor C_α), 57.3 (major C_β), 56.6 (minor C_β), 54.9 (minor C_γ), 54.5 (major C_γ), 47.2 (minor C_δ), 46.8 (major C_δ), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **Rf** 0.35 (hexane/acetone 80:20); **[α]$_D^{22}$** –45 (c 1.1, $CHCl_3$); **MS** (ESI)(*m/z*): 342.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{22}NO_5$ $(M+H)^+$ Calcd. 320.1492; Found 320.1487; **IR** 2977 (w), 2361 (w), 1750 (s), 1703 (s), 1416 (m), 1389 (m), 1170 (s) cm^{-1}.

Data for *N*-(*tert*-butoxycarbonyl)-(2*S*,3*S*,4*R*)-3,4-epoxyproline benzyl ester (**9a**): **^{1}H NMR** (400 MHz, $CDCl_3$) (60:40 rotamer ratio) δ 7.45–7.30 (m, 5H major Ph + 5H minor Ph), 5.35 (d, *J*=12.5 Hz, 1H, minor $C\underline{H}H'Ph$), 5.27 (d, *J*=12.5 Hz, 1H, major $C\underline{H}H'Ph$), 5.22 (d, *J*=12.5 Hz, 1H, major $CH\underline{H'}Ph$), 5.17 (d, *J*=12.5 Hz, 1H, minor $CH\underline{H'}Ph$), 4.45 (d, *J*=1.6 Hz, 1H, minor $C_\alpha H$), 4.37 (d, *J*=1.8 Hz, 1H, major $C_\alpha H$), 3.95 (app. t, *J*=2.5 Hz, 1H major $C_\beta H$ + 1H minor $C_\beta H$), 3.86 (d, *J*=12.7 Hz, 1H, major $C_\delta\underline{H}H'$), 3.82 (d, *J*=12.7 Hz, 1H, minor $C_\delta\underline{H}H'$), 3.79-3.74 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 3.56 (br dd, *J*=12.7, 1.2 Hz, major $C_\delta H\underline{H'}$), 3.51 (br dd, *J*=12.7, 1.2 Hz, minor $C_\delta H\underline{H'}$), 1.45 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.33 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (60:40 ratio) δ 167.9 (major C_α-$\underline{C}O_2$), 167.5 (minor C_α-$\underline{C}O_2$), 154.3 (minor $\underline{C}O_2C(CH_3)_3$), 153.8 (major $\underline{C}O_2C(CH_3)_3$), 135.6 (minor $\underline{C}_{q,Ph}$), 135.4 (major $\underline{C}_{q,Ph}$), 128.8 + 128.6 + 128.51 + 128.48 + 128.41 + 128.2 (major and minor overlap, $\underline{C}H_{Ph}$), 80.8 (major $CO_2\underline{C}(CH_3)_3$), 80.6 (minor $CO_2\underline{C}(CH_3)_3$), 67.2 (2C, major $\underline{C}H_2Ph$ + minor $\underline{C}H_2Ph$), 60.2 (major C_α), 60.1 (minor C_α), 58.0 (major C_β), 57.3 (minor C_β), 56.1 (minor C_γ), 55.6 (major C_γ), 48.0 (minor C_δ), 47.8 (major C_δ), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **Rf** 0.25 (hexane/acetone 80:20); **[α]$_D^{22}$** –53 (c 1.3, $CHCl_3$); **MS** (ESI)(*m/z*): 320.5 $(M+H)^+$, 342.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{22}NO_5$ $(M+H)^+$ Calcd.

320.1492; Found 320.1491; **IR** 2977 (w), 2361 (w), 1761 (s), 1703 (s), 1379 (m), 1170 (s) cm^{-1}.

(±)-*N*-(Acetyl)-(2*S*,3*R*,4*S*)-3,4-epoxyproline benzyl ester (10b) and (±)-*N*-(acetyl)-(2*S*,3*S*,4*R*)-3,4-epoxyproline benzyl ester (9b) (Scheme 4)

Alkene **±25b** (16.2 g, 66.2 mmol) was dissolved in 1,2-dichloroethane (250 mL), *meta*-chloroperoxybenzoic acid (<77% pure, 34.3 g, 198.5 mmol) was added and the mixture was refluxed at 70 °C. TLC analysis indicated the reaction was finished after 25 h. Next, the reaction mixture was cooled to room temperature and washed with a saturated aqueous solution of $Na_2S_2O_3$ (250 mL), a saturated aqueous solution of $NaHCO_3$ (250 mL) and brine (250 mL). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 60:40 to 40:60) yielded **10b** (9.42 g, 55%) and **9b** (4.26 g, 25%) as slightly yellow/ off white solids. A sample of **9b** was recrystallized from DCM and submitted for X-ray analysis.

Data for (±)-*N*-(acetyl)-(2*S*,3*R*,4*S*)-3,4-epoxyproline benzyl ester (**10b**): **^{1}H NMR** (400 MHz, $CDCl_3$) (69:31 rotamer ratio) δ 7.42–7.30 (m, 5H major Ph + 5H minor Ph), 5.26 (d, *J*=12.2 Hz, 1H, minor C$\underline{H}$H'Ph), 5.24 (d, *J*=12.3 Hz, 1H, major C$\underline{H}$H'Ph), 5.22 (d, *J*=12.2 Hz, 1H, minor CH$\underline{H'}$Ph), 5.18 (d, *J*=12.3 Hz, 1H, major CH$\underline{H'}$Ph), 4.90 (s, 1H, major $C_{\alpha}\underline{H}$), 4.60 (s, 1H, minor $C_{\alpha}\underline{H}$), 4.14 (d, *J*=13.6 Hz, 1H, minor $C_{\delta}\underline{H}H'$), 3.87 (d, *J*=2.9 Hz, 1H, minor $C_{\beta}\underline{H}$), 3.84 (d, *J*=11.6 Hz, 1H, major $C_{\delta}\underline{H}H'$), 3.77 (d, *J*=2.9 Hz, 1H, major $C_{\beta}\underline{H}$), 3.72 (m, 1H major $C_{\gamma}\underline{H}$ + 1H minor $C_{\gamma}\underline{H}$), 3.71 (d, *J*=11.6 Hz, 1H, major $C_{\delta}H\underline{H'}$), 3.43 (d, *J*=13.5 Hz, 1H, minor $C_{\delta}H\underline{H'}$), 2.05 (s, 3H, major $C\underline{H}_3$), 1.92 (s, 3H, minor $C\underline{H}_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (69:31 rotamer ratio) δ 170.3 (minor N-$\underline{C}OCH_3$), 170.2 (major N-$\underline{C}OCH_3$), 168.4 (major C_{α}-$\underline{C}O_2$), 168.2 (minor C_{α}-$\underline{C}O_2$), 135.1 (major $\underline{C}_{q,Ph}$), 134.6 (minor $\underline{C}_{q,Ph}$), 128.9 + 128.8 + 128.6 + 128.5 + 128.4 + 128.1 (major and minor overlap, $\underline{C}H_{Ph}$), 67.9 (minor $\underline{C}H_2Ph$), 67.4

(major $\underline{C}H_2Ph$), 61.6 (minor $\underline{C}_{\alpha}H$), 59.9 (major $\underline{C}_{\alpha}H$), 57.2 (minor $\underline{C}_{\beta}H$), 56.3 (major $\underline{C}_{\beta}H$), 54.9 (major $\underline{C}_{\gamma}H$), 53.8 (minor $\underline{C}_{\gamma}H$), 48.3 (major $\underline{C}_{\delta}H_2$), 46.5 (minor $\underline{C}_{\delta}H_2$), 22.1 (minor $\underline{C}H_3$), 22.0 (major $\underline{C}H_3$) ppm; **mp** 70–72 °C; **Rf** 0.38 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 262.3 $(M+H)^+$, 284.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{16}NO_4$ $(M+H)^+$ Calcd. 262.1074; Found 262.1067; **IR** 1747 (s), 1652 (s), 1213 (s), 1175 (s) cm^{-1}.

Data for (±)-*N*-(acetyl)-(2*S*,3*S*,4*R*)-3,4-epoxyproline benzyl ester (**9b**): **^{1}H NMR** (400 MHz, $CDCl_3$) (80:20 rotamer ratio) δ 7.41–7.30 (m, 5H major Ph + 5H minor Ph), 5.28 (s, 2H, minor $C\underline{H_2}Ph$), 5.25 (s, 2H, major $C\underline{H_2}Ph$), 4.56 (d, *J*=2.3 Hz, 1H, major $C_{\alpha}\underline{H}$), 4.55 (d, *J*=2.8 Hz, 1H, minor $C_{\alpha}\underline{H}$), 4.12 (t, *J*=2.8 Hz, 1H, minor $C_{\beta}\underline{H}$), 3.98 (t, *J*=2.6 Hz, 1H, major $C_{\beta}\underline{H}$), 3.94 (d, *J*=11.7 Hz, 1H, major $C_{\delta}\underline{H}H'$), 3.87 (d, *J*=13.9 Hz, 1H, minor $C_{\delta}\underline{H}H'$), 3.86 (dd, *J*=2.9, 2.1 Hz, 1H major $C_{\gamma}\underline{H}$), 3.83 (dd, *J*=2.8, 2.3 Hz, 1H minor $C_{\gamma}\underline{H}$), 3.72 (dd, *J*=13.9, 2.1 Hz, 1H, minor $C_{\delta}H\underline{H'}$), 3.87 (dd, *J*=11.7, 2.0 Hz, 1H, major $C_{\delta}H\underline{H'}$), 2.06 (s, 3H, major $C\underline{H}_3$), 1.86 (s, 3H, minor $C\underline{H}_3$) ppm; **$^{13}C\{^1H\}$ NMR** (100 MHz, $CDCl_3$) (80:20 rotamer ratio) δ 170.5 (minor N-$\underline{C}OCH_3$), 170.1 (major N-$\underline{C}OCH_3$), 167.5 (minor C_{α}-$\underline{C}O_2$), 166.6 (major C_{α}-$\underline{C}O_2$), 135.5 (major $\underline{C}_{q,Ph}$), 134.9 (minor $\underline{C}_{q,Ph}$), 128.7 + 128.5 + 128.4 + 128.2 + 128.1 (major and minor overlap, $\underline{C}H_{Ph}$), 67.8 (minor $\underline{C}H_2Ph$), 67.2 (major $\underline{C}H_2Ph$), 61.1 (minor $\underline{C}_{\alpha}H$), 59.8 (major $\underline{C}_{\alpha}H$), 59.1 (minor $\underline{C}_{\beta}H$), 56.9 (major $\underline{C}_{\beta}H$), 56.2 (major $\underline{C}_{\gamma}H$), 55.8 (minor $\underline{C}_{\gamma}H$), 48.8 (major $\underline{C}_{\delta}H_2$), 48.3 (minor $\underline{C}_{\delta}H_2$), 21.8 (major $\underline{C}H_3$), 21.3 (minor $\underline{C}H_3$) ppm; **mp** 96–100 °C; **Rf** 0.15 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 262.2 $(M+H)^+$, 284.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{16}NO_4$ $(M+H)^+$ Calcd. 262.1074; Found 262.1069; **IR** 1756 (s), 1650 (s), 1170 (s) cm^{-1}.

Benzyl 1*H*-pyrrole-2-carboxylate (35) (Table 1, entry 1)

Epoxide **10a** (150.0 mg, 0.470 mmol) was dissolved in $Et_3N.3HF$ and stirred at 90 °C. After 24 h, no reaction was observed and the temperature was increased to 130 °C. Overnight, a white/brown gel formed. Next, the mixture was poured in a saturated aqueous solution of

$NaHCO_3$ (120 mL) and the aqueous layer was extracted with Et_2O (3 × 200 mL). The combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo* to yield 120.3 mg of crude product. NMR analysis of the crude product allowed to conclude that pyrrole **35** was the only product. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 9.30 (br s, 1H, N$\underline{H}$), 7.46–7.32 (m, 5H, C$\underline{H}$ Ar), 6.99 (ddd, *J*=3.8, 2.4, 1.5 Hz, 1H, $C_\delta H$), 6.96 (td, *J*=2.7, 1.5 Hz, 1H, $C_\beta H$), 6.28 (dt, *J*=3.8, 2.5 Hz, 1H, $C_\gamma H$), 5.33 (s, 2H, C$\underline{H_2}$Ph) ppm; **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$): δ 161.0 (C_α-$\underline{C}O_2$), 136.1 ($\underline{C}_{q,Ph}$), 128.6 (2 × $\underline{C}H_{Ar}$), 128.2 ($\underline{C}H_{Ar}$), 128.1 (2 × $\underline{C}H_{Ar}$), 123.1 (C_β), 122.6 (C_α), 115.6 (C_δ), 110.5 (C_γ), 66.0 ($\underline{C}H_2Ph$) ppm; **Rf** 0.44 (hexane/ acetone 80:20); **MS** (ESI)(*m/z*): 202.1 $(M+H)^+$; **HRMS** (ESI) for $C_{12}H_{11}NNaO_2$ $(M+Na)^+$ Calcd. 224.0682; Found 224.0678; **IR** 3413 (br m), 3312 (br. m), 2360 (m), 2340 (m), 1680 (s), 1410 (s), 1304 (s), 1156 (m), 1124 (s) cm^{-1}. Chemical shift data correspond to literature data.[84]

(±)-*N*-(Acetyl)-(2*S*,3*R*,4*R*)-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 stirred at room temperature. Within 1 h, a white gel had formed in the reaction mixture. TLC analysis after 19h h indicated the presence of starting material, upon which the reaction temperature was increased to 50 °C. After an additional 24 h, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ (8 mL) and the mixture was stirred over basic Al_2O_3 for 10 min. After removal of the basic alumina via filtration, the aqueous layer was extracted with DCM (3 × 10 mL). The combined organic phases were washed with brine (1 × 10 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified via HPLC (hexane/acetone 60:40) to yield recovered starting material **10b** (38.5 mg, 30%) and **33b** as a clear oil (21.1 mg, 15%), along with a trace amount of DMPU (< 3%).

Data for (±)-*N*-(acetyl)-(2*S*,3*R*,4*R*)-3-hydroxy-4-fluoroproline benzyl ester **(33b)**: **^{1}H NMR** (400 MHz, $CDCl_3$) (67:33 rotamer ratio) δ 7.42–7.28 (m, 5H major Ph + 5H minor Ph), 5.25 (d, *J*=12.1 Hz, 1H, minor C$\underline{H}$H’Ph), 5.19 (d, *J*=12.4 Hz, 1H, major C$\underline{H}$H’Ph), 5.16 (d, *J*=12.1 Hz, 1H, minor CH$\underline{H'}$Ph), 5.12 (d, *J*=12.2 Hz, 1H, major CH$\underline{H'}$Ph), 5.00 (dd, *J*=50.1, 3.9 Hz, 1H, major $C_\gamma H$), 4.92 (dd, *J*=49.9, 3.3 Hz, 1H, minor $C_\gamma H$), 4.76–4.55 (m, 1H major $C_\beta H$ + 1H minor $C_\beta H$), 4.72 (s, 1H, major $C_\alpha H$), 4.45 (s, 1H, minor $C_\alpha H$), 4.33–4.13 (m, 1H major OH + 1H minor OH), 4.03–3.57 (m, 2H major $C_\delta H_2$ + 2H minor $C_\delta H_2$), 2.09 (s, 3H, major CH_3), 1.96 (s, 3H, minor CH_3) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (67:33 rotamer ratio) δ 171.1 (minor N-$\underline{C}OCH_3$), 170.8 (major N-$\underline{C}OCH_3$), 168.2 (minor C_α-$\underline{C}O_2$), 167.9 (major C_α-$\underline{C}O_2$), 135.3 (major $\underline{C}_{q,Ph}$), 134.9 (minor $\underline{C}_{q,Ph}$), 128.64 + 128.62 + 128.5 + 128.4 + 128.3 + 128.1 (major and minor overlap, $\underline{C}H_{Ph}$), 94.6 (d, *J*=181.2 Hz, major C_γ), 93.3 (d, *J*=179.0 Hz, minor C_γ), 77.5 (d, *J*=28.6 Hz, minor C_β), 75.6 (d, *J*=28.6 Hz, major C_β), 67.7 (minor $\underline{C}H_2Ph$), 67.3 (major $\underline{C}H_2Ph$), 65.7 (major C_α), 65.6 (minor C_α), 52.1 (d, *J*=23.5 Hz, major C_δ), 50.9 (d, *J*=23.5 Hz, minor C_δ), 22.02 (major N-CO$\underline{C}H_3$), 21.98 (minor N-CO$\underline{C}H_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (70:30 rotamer ratio) δ -180.47 (dddd, *J*=49.9, 39.5, 28.6, 6.9 Hz, 1F, minor), -182.19 (dddd, *J*=49.4, 36.4, 26.9, 8.7 Hz, 1F, major) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (70:30 rotamer ratio) δ -180.61 (s, 1F, minor), -182.34 (s, 1F major) ppm; **Rf** 0.22 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 282.4 $(M+H)^+$, 304.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{17}FNO_4$ $(M+H)^+$ Calcd. 282.1136; Found 282.1135; **IR** 3278 (br. m), 1745 (s), 1626 (s), 1448 (m), 1420 (m), 1176 (s) cm^{-1}.

(±)-*N*-(*tert*-Butoxycarbonyl)-(2*S*,3*R*,4*R*)-3-hydroxy-4-chloroproline benzyl ester (32a) (Table 1, entry 7)

To a solution of epoxide **±10a** (129.8 mg, 0.406 mmol) in DCE (10 mL), $Bu_4NH_2F_3$ (tech. 90%, 0.1 ml) was added and the mixture was stirred at room temperature. After 2.5 h, no reaction was observed and the reaction temperature was increased to 90 °C. TLC analysis indicated complete consumption of starting material after 26 h. Next, the mixture was diluted with DCM (3 mL) and water (3 mL) and cooled to 0 °C. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and the aqueous layer was extracted with DCM (3 × 10 mL). The combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/EtOAc 75:25) yielded chlorohydrin **32a** as a clear, yellowish oil (106.2 mg, 74%). **^{1}H NMR** (400 MHz, $CDCl_3$) (56:44 rotamer ratio) δ 7.43–7.29 (m, 5H major Ph + 5H minor Ph), 5.29 (d, *J*=12.4 Hz, 1H, minor $C\underline{H}H'Ph$), 5.24 (d, *J*=12.2 Hz, 1H, major $C\underline{H}H'Ph$), 5.17 (d, *J*=12.2 Hz, 1H, major $CH\underline{H'}Ph$), 5.09 (d, *J*=12.4 Hz, 1H, minor $CH\underline{H'}Ph$), 4.55-4.45 (m, 1H major $C_\beta H$ + 1H minor $C_\beta H$), 4.39 (d, *J*=2.5 Hz, 1H, minor $C_\alpha H$), 4.26 (d, *J*=3.3 Hz, 1H, major $C_\alpha H$), 4.18-3.98 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$ + 1H major $C_\delta\underline{H}H'$ + 1H minor $C_\delta\underline{H}H'$), 3.69-3.61 (m, 1H major $C_\delta H\underline{H'}$ + 1H minor $C_\delta H\underline{H'}$), 3.60-3.42 (br m, 1H major OH + 1H minor OH), 1.45 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.32 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (56:44 rotamer ratio) δ 169.9 (major $C_\alpha\text{-}\underline{C}O_2$), 169.4 (minor $C_\alpha\text{-}\underline{C}O_2$), 154.2 (minor $\underline{C}O_2C(CH_3)_3$), 153.6 (major $\underline{C}O_2C(CH_3)_3$), 135.3 (minor $\underline{C}_{q,Ph}$), 135.2 (major $\underline{C}_{q,Ph}$), 128.6 + 128.53 + 128.48 + 128.45 + 128.24 + 128.20 (major and minor overlap, $\underline{C}H_{Ph}$), 81.14 (major C_β), 81.05 (major $CO_2\underline{C}(CH_3)$), 81.0 (minor $CO_2\underline{C}(CH_3)$), 80.2 (minor C_β), 67.30 (minor $\underline{C}H_2Ph$), 67.25 (major $\underline{C}H_2Ph$), 65.5 (major C_α), 65.4 (minor C_α), 59.2 (minor C_γ), 58.5 (major C_γ), 53.0 (minor C_δ), 52.2 (major C_δ), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **Rf** 0.34 (hexane/ acetone 70:30); **MS** (ESI)(*m/z*): 356.1 $(M+H)^+$; **HRMS** (ESI) for $C_{17}H_{22}ClNNaO_5$ $(M+Na)^+$ Calcd. 378.1079; Found 378.1081; **IR** 3406 (w), 1738 (m), 1702 (s), 1672 (s), 1392 (s), 1158 (s), 966 (m) cm^{-1}.

(±)-*N*-(*t*-Butyloxycarbonyl)-(4*R*)-2,3-dehydro-4-hydroxyproline benzyl ester (34a) (Table 1, entry 8)

To a solution of epoxide **10a** (125.6 mg, 0.393 mmol) in toluene (10.0 mL), $Bu_4NH_2F_3$ (tech. 90%, 0.1 ml) was added and the mixture was stirred at 110 °C. After 24 h, the mixture was quenched with a saturated aqueous solution of $NaHCO_3$ (3 mL) and water (10 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL) and the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/EtOAc 70:30) yielded **34a** as a clear oil (70.5 mg, 56%). **^{1}H NMR**(400 MHz, $CDCl_3$) δ 7.42–7.32 (m, 5H, CH Ar), 5.72 (d, *J*=2.8 Hz, 1H, $C_\beta H$), 5.27 (s, 2H, $C\underline{H_2}Ph$), 4.84 (tt, *J*=8.0, 2.8 Hz, 1H, $C_\gamma H$), 3.95 (dd, *J*=12.8, 8.3 Hz, 1H, $C_\delta \underline{H}H'$), 3.84 (dd, *J*=13.3, 2.7 Hz, 1H, $C_\delta H\underline{H'}$), 1.78 (d, *J*=8.2 Hz, 1H, OH), 1.46 (s, 9H, $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 162.1 (C_α-$\underline{C}O_2$), 151.7 ($\underline{C}O_2C(CH_3)_3$), 139.4 ($C_\alpha$), 135.1 ($\underline{C}_{q,Ph}$), 128.6 (2C, CH_{Ar}), 128.5 (1C, CH_{Ar}), 128.4 (2C, CH_{Ar}), 116.2 (C_β), 81.9 ($CO_2\underline{C}(CH_3)_3$), 71.0 ($C_\gamma$), 67.4 ($\underline{C}H_2Ph$), 56.7 ($C_\delta$), 28.1 (3C, $CO_2C(\underline{C}H_3)_3$) ppm; **Rf** 0.34 (hexane/ acetone 70:30); **MS** (ESI)(*m/z*): 320.4 $(M+H)^+$, 342.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{22}NO_5$ $(M+H)^+$ Calcd. 320.1492; Found 320.1495; **IR** 3434 (br. w), 2978 (m), 1740 (s), 1707 (s), 1392 (s), 1368 (s), 1167 (s) cm^{-1}.

(±)-*N*-(Acetyl)-(4*R*)-2,3-dehydro-4-hydroxyproline benzyl ester (34b) (Table 1, entry 9)

To a solution of epoxide **±10b** (553.0 mg, 2.117 mmol) in *t*-BuOH (25.0 mL), $TBAF.3H_2O$ (1.67 g, 5.29 mmol) was added and the mixture was stirred at 70 °C. After 4 h, the mixture was diluted with water (100 mL) and the aqueous layer was extracted with DCM (3 × 80 mL). The combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography using a Biotage® purification system (hexane/acetone gradient) yielded **34b** (165.7 mg, 30%) and **35** (53.2 mg, 13%) as clear oils.

Data for (±)-*N*-(acetyl)-(4*R*)-2,3-dehydro-4-hydroxyproline benzyl ester (**34b**): **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42–7.32 (m, 5H, CH_{Ar}), 5.88 (d, *J*=2.9 Hz, 1H, $C_\beta H$), 5.32 (d, *J*=12.6 Hz, 1H, $C\underline{H}H'Ph$), 5.19 (d, *J*=12.6 Hz, 1H, $CH\underline{H'}Ph$), 4.84 (br s, 1H, $C_\gamma H$), 3.95 (dd, *J*=12.1, 8.0

Hz, 1H, $C_{\delta}\underline{H}H'$), 3.84 (br dd, *J*=12.1, 1.3 Hz, 1H, $C_{\delta}H\underline{H'}$), 3.72 (br s, 1H,OH), 2.08 (s, 3H, N-$COC\underline{H}_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 168.9 (N-$\underline{C}OCH_3$), 162.2 (C_{α}-$\underline{C}O_2$), 138.2 (C_{α}), 135.1 ($\underline{C}_{q,Ph}$), 128.64 (2C, CH_{Ar}), 128.59 (2C, CH_{Ar}), 128.48 (1C, CH_{Ar}), 119.3 (C_{β}), 71.2 (C_{γ}), 67.6 ($\underline{C}H_2Ph$), 56.9 (C_{δ}), 22.5 (N-$CO\underline{C}H_3$) ppm; **Rf** 0.22 (hexane/acetone 60:40); **MS** (ESI)(*m/z*): 262.2 $(M+H)^+$, 284.2 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{16}NO_4$ $(M+H)^+$ Calcd. 262.1074; Found 262.1076; **IR** 3373 (br. m), 2950 (m), 1733 (s), 1651 (s), 1404 (s), 1172 (s), 749 (m) cm^{-1}.

2-Hydroxyethyl 1*H*-pyrrole-2-carboxylate (36) (Table 1, entry 10)

Epoxide **10a** (120.0 mg, 0.376 mmol) and KHF_2 (146.7 mg, 1.879 mmol) were dissolved in glycol (2.0 mL) and stirred at 120 °C. After 4 h, no reaction was observed and the temperature was increased to 150 °C. After 21 h, the reaction was quenched with a 5% aqueous solution of K_2CO_3 (4 mL) and diluted with water (50 mL). Next, the aqueous layer was extracted with DCM (5 × 75 mL) and the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 67:33) yielded **36** as a yellowish oil (34.4 mg, 59%), next to 1.4 mg (2%) of **35**.

Data for 2-hydroxyethyl pyrrole-2-carboxylate (**36**): **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.56 (br s, 1H, N$\underline{H}$), 6.99–6.95 (m, 1H $C_{\delta}H$ + 1H $C_{\beta}H$), 6.27 (dt, *J*=3.6, 2.6 Hz, 1H, $C_{\gamma}H$), 4.40 (t, *J*=1.7 Hz, 1H, CO_2-$C\underline{H}H'$), 4.40 (br d, *J*=9.3 Hz, 1H, CO_2-$CH\underline{H'}$), 3.92 (t, *J*=1.7 Hz, 1H, $C\underline{H}H'$-OH), 3.92 (br d, *J*=9.3 Hz, 1H, $CH\underline{H'}$-OH), 2.64 (br s, 1H, OH) ppm; **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) δ 161.4 (C_{α}-$\underline{C}O_2$), 123.4 (C_{β}), 122.2 (C_{α}), 115.9 (C_{δ}), 110.6 (C_{γ}), 66.0 (CO_2-$\underline{C}H_2$), 66.0 ($\underline{C}H_2$-OH) ppm; **Rf** 0.28 (hexane/ acetone 70:30); **MS** (ESI)(*m/z*): 156.1 $(M+H)^+$; **HRMS** (ESI) for $C_7H_9NNaO_3$ $(M+Na)^+$ Calcd. 178.0475; Found 178.0474; **IR** 3314 (br. m), 2360 (m), 2340 (m), 1676 (s), 1408 (s), 1306 (s), 1160 (s), 742 (s) cm^{-1}.

(±)-*N*-(Acetyl)-(2*S*,3*R*,4*R*)-3-hydroxy-4-chloroproline benzyl ester (32b) (Scheme 5)

Epoxide ±**10b** (2.55 g, 9.74 mmol) was dissolved in a 4 M solution of HCl in dioxane (50 mL) and stirred at room temperature for 1.5 h. Next, the reaction mixture was cooled to 0 °C and quenched with a saturated aqueous solution of $NaHCO_3$ (40 mL). The aqueous layer was extracted with DCM (3 × 50 mL) and the combined organic layers were subsequently washed with brine (40 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by flash chromatography using a Biotage® purification system (hexane/acetone gradient) to yield **32b** (2.77 g, 95%) as a colourless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) (70:30 rotamer ratio) δ 7.41–7.29 (m, 5H major Ph +5H minor Ph), 5.28 (d, *J*=12.1 Hz, 1H, minor C$\underline{\text{H}}$H'Ph), 5.22 (d, *J*=12.3 Hz, 1H, major C$\underline{\text{H}}$H'Ph), 5.17 (d, *J*=12.1 Hz, 1H, minor CH$\underline{\text{H'}}$Ph), 5.14 (d, *J*=12.3 Hz, 1H, major CH$\underline{\text{H'}}$Ph), 4.74 (br. m, 1H, minor $\mathrm{C_{\beta}\underline{H}}$), 4.57 (br. d, *J*=2.6 Hz, 1H, major $\mathrm{C_{\alpha}\underline{H}}$), 4.53 (m, 1H, major $\mathrm{C_{\beta}\underline{H}}$), 4.40 (br. d, *J*=1.2 Hz, 1H, minor $\mathrm{C_{\alpha}\underline{H}}$), 4.27–4.04 (m, 1H major $\mathrm{C_{\gamma}\underline{H}}$ + 1H minor $\mathrm{C_{\gamma}\underline{H}}$ + 1H major $\mathrm{C_{\delta}\underline{H}H'}$ + 1H minor $\mathrm{C_{\delta}\underline{H}H'}$ + 1H major OH + 1H minor OH), 3.81–3.74 (m, 1H major $\mathrm{C_{\delta}H\underline{H'}}$ + 1H minor $\mathrm{C_{\delta}H\underline{H'}}$), 2.08 (s, 3H, major $\mathrm{C\underline{H}_3}$), 1.96 (s, 3H, minor $\mathrm{C\underline{H}_3}$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (70:30 rotamer ratio) δ 171.1 (minor N-$\mathrm{\underline{C}OCH_3}$), 170.4 (major N-$\mathrm{\underline{C}OCH_3}$), 168.6 (1C major $\mathrm{C_{\alpha}\text{-}\underline{C}O_2}$ + 1C minor $\mathrm{C_{\alpha}\text{-}\underline{C}O_2}$), 135.3 (major $\mathrm{\underline{C}_{q,Ph}}$), 134.8 (minor $\mathrm{\underline{C}_{q,Ph}}$), 128.74 + 128.70 + 128.68 + 128.6 + 128.52 + 128.46 +128.4 + 128.3 (major and minor overlap, $\mathrm{\underline{C}H_{Ph}}$), 81.3 (minor $\mathrm{\underline{C}_{\beta}H}$), 79.5 (major $\mathrm{\underline{C}_{\beta}H}$), 67.9 (minor $\mathrm{\underline{C}H_2Ph}$), 67.4 (major $\mathrm{\underline{C}H_2Ph}$), 67.0 (minor $\mathrm{\underline{C}_{\alpha}H}$), 65.3 (major $\mathrm{\underline{C}_{\alpha}H}$), 59.2 (major $\mathrm{\underline{C}_{\gamma}H}$), 58.3 (minor $\mathrm{\underline{C}_{\gamma}H}$), 54.2 (major $\mathrm{\underline{C}_{\delta}H_2}$), 53.4 (minor $\mathrm{\underline{C}_{\delta}H_2}$), 22.0 (minor $\mathrm{\underline{C}H_3}$), 21.9 (major $\mathrm{\underline{C}H_3}$) ppm; **Rf** 0.25 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 298.3 $(M+H)^+$, 320.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{17}ClNO_4$ $(M+H)^+$ Calcd. 298.0841; Found 298.0834; **IR** 3304 (br. m), 1747 (s), 1628 (s), 1189 (m), 1175 (m), 698 (m) cm^{-1}.

(±)-*N*-(Acetyl)-(2*S*,3*R*,4*R*)-3-hydroxy-4-(4-methylphenyl)sulfonyl proline benzyl ester (37b) (Scheme 5)

p-Toluenesulfonic acid monohydrate (640.6 mg, 3.368 mmol) was added to a solution of epoxide **±10b** (220.0 mg, 0.842 mmol) in DCM (6.0 mL) and the mixture stirred at 45 °C for 6 h. Next, the mixture was diluted with DCM (10 mL) and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 7 mL) and brine (1 × 10 mL). The organic phase was then dried over $MgSO_4$ and evaporated *in vacuo*. Purification via HPLC (hexane/acetone 60:40) yielded **37b** as a clear oil (265.0 mg, 73%). **^{1}H NMR** (400 MHz, $CDCl_3$) (66:34 rotamer ratio) δ 7.75–7.65 (m, 2H major CH Ar + 2H minor CH Ar), 7.41–7.28 (m, 7H major CH Ar + 7H minor CH Ar), 5.19 (d, *J*=12.1 Hz, 1H, minor $C\underline{H}H'Ph$), 5.14 (d, *J*=12.5 Hz, 1H, major $C\underline{H}H'Ph$), 5.10 (d, *J*=12.1 Hz, 1H, minor $CH\underline{H'}Ph$), 5.03 (d, *J*=12.5 Hz, 1H, major $CH\underline{H'}Ph$), 4.86 (dt, *J*=5.2, 2.5 Hz, 1H, major $C_\gamma H$), 4.81-4.76 (m, 1H minor $C_\gamma H$ + 1H minor $C_\beta H$), 4.58 (d, *J*=1.8 Hz, 1H major $C_\alpha H$), 4.50 (br s, 1H major $C_\beta H$), 4.38 (br s, 1H minor $C_\alpha H$), 4.06 (br s, 1H minor OH), 3.95–3.83 (m, 1H major OH + 1H major $C_\delta\underline{H}H'$ + 1H minor $C_\delta\underline{H}H'$), 3.67 (d, *J*=11.9, 2.4 Hz, 1H major $C_\delta H\underline{H'}$), 3.53 (br d, *J*=14.1 Hz, 1H minor $C_\delta H\underline{H'}$), 2.44 (s, 3H, major OTs CH_3), 2.43 (s, 3H, minor OTs CH_3), 2.00 (s, 3H, major N-$COCH_3$), 1.91 (s, 3H, minor N-$COCH_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (66:34 rotamer ratio) δ 170.8 (minor N-$\underline{C}OCH_3$), 170.4 (major N-$\underline{C}OCH_3$), 168.2 (minor C_α-$\underline{C}O_2$), 168.1 (major C_α-$\underline{C}O_2$), 145.53 (major OTs $\underline{C}_{q(Me)}$), 145.50 (minor OTs $\underline{C}_{q(Me)}$), 135.3 (major $C_{q(Bn)}$), 134.8 (minor $C_{q(Bn)}$), 133.0 (minor OTs $\underline{C}_{q(SO2)}$), 132.9 (major OTs $\underline{C}_{q(SO2)}$), 130.09 (minor $\underline{C}H_{Ar}$), 130.05 (major $\underline{C}H_{Ar}$), 128.74 + 128.67 + 128.6 + 128.5 + 128.3 + 128.2 + 128.0 + 127.9 + 127.7 (major and minor overlap, $\underline{C}H_{Ar}$), 81.8 (major C_γ), 80.9 (minor C_γ), 78.3 (minor C_β), 76.2 (major C_β), 68.0 (minor $\underline{C}H_2Ph$), 67.4 (major $\underline{C}H_2Ph$), 66.8 (minor C_α), 65.1 (major C_α), 51.1 (major C_δ), 49.9 (minor C_δ), 21.9 (2C, minor N-$CO\underline{C}H_3$ + major N-$CO\underline{C}H_3$), 21.7 (2C, minor OTs CH_3 + major OTs CH_3) ppm; **Rf** 0.34 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 434.3 $(M+H)^+$, 456.1 $(M+Na)^+$; **HRMS** (ESI) for $C_{21}H_{24}NO_7S$ $(M+H)^+$ Calcd. 434.1268; Found 434.1271; **IR** 3322 (br. w), 1749 (m), 1627 (m), 1189 (s), 1175 (s), 732 (s) cm^{-1}.

(±)-N-(Acetyl)-(2*S*,*R*,4*R*)-3-hydroxy-4-bromoproline benzyl ester (38b) (Scheme 5)

Reaction with $MgBr_2$:

to a solution of epoxide **±10b** (147.7 mg, 0.565 mmol) in DCM (4.0 mL), $MgBr_2$ (156.1 mg, 0.848 mmol) was added and the mixture was stirred at room temperature. After 20 h, the mixture was diluted DCM (10 mL), water (10 mL) and a saturated aqueous solution of $NaHCO_3$ (5 mL). The aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic phases were dried over $MgSO_4$ 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 H_2O, 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 quenched with a saturated aqueous solution of $NaHCO_3$ (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 $MgSO_4$ and evaporated *in vacuo*. Bromohydrin **38b** was obtained without purification as a clear oil (177.5 mg, 95%). **^{1}H NMR**(400 MHz, $CDCl_3$) (71:29 rotamer ratio) δ 7.42–7.30 (m, 5H major Ph + 5H minor Ph), 5.29 (d, *J*=12.0 Hz, 1H, minor $C\underline{H}H'Ph$), 5.24 (d, *J*=12.4 Hz, 1H, major $C\underline{H}H'Ph$), 5.19 (d, *J*=12.0 Hz, 1H, minor $CH\underline{H'}Ph$), 5.16 (d, *J*=12.4 Hz, 1H, major $CH\underline{H'}Ph$), 4.82 (br. app. t, 1H, minor $C_\beta H$), 4.59 (app. t, *J*=3.7 Hz, 1H, major $C_\beta H$), 4.51 (d, *J*=3.6 Hz, 1H, major $C_\alpha H$), 4.51 (d, *J*=2.0 Hz, 1H, minor $C_\alpha H$), 4.38-4.11 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$ + 1H major $C_\delta\underline{H}H'$+ 1H minor $C_\delta\underline{H}H'$), 3.92-3.80 (m, 1H major $C_\delta H\underline{H'}$+ 1H major $C_\delta H\underline{H'}$), 3.74–3.57 (br. s, 1H major OH + 1H minor OH), 2.09 (s, 3H, major CH_3), 1.96 (s, 3H, minor CH_3) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (71:29 rotamer ratio) δ 170.8 (minor N-$\underline{C}OCH_3$), 169.9 (major N-$\underline{C}OCH_3$), 168.9 (major C_α-$\underline{C}O_2$), 168.8 (minor C_α-$\underline{C}O_2$), 135.3 (major $\underline{C}_{q,Ph}$), 134.7 (minor $\underline{C}_{q,Ph}$), 128.8 + 128.72 + 128.70 + 128.6 + 128.4 + 128.3 (major and minor overlap, $\underline{C}H_{Ph}$), 81.8 (minor C_β), 80.0 (major C_β), 68.0 (minor $\underline{C}H_2Ph$), 67.4 (major $\underline{C}H_2Ph$), 66.9 (minor C_α), 65.0 (major C_α), 54.3 (major C_δ), 53.6 (minor C_δ), 47.7 (major C_γ), 47.2 (minor C_γ), 21.9 (minor N-CO$\underline{C}H_3$), 21.8 (major N-CO$\underline{C}H_3$) ppm; **Rf** 0.32 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 342.1 $(M+H)^+$, 364.1 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{17}BrNO_4$ $(M+H)^+$ Calcd. 342.0335; Found 342.0332; **IR** 3296 (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) is added to a solution of chlorohydrin **32b** (83.4 mg, 0.280 mmol) in DCM (1.0 mL). The reaction is allowed to warm to room temperature and after 22 h TLC analysis indicates full conversion of the starting material. Next, the reaction is diluted with DCM (4 mL) and quenched with a saturated aqueous solution of $NaHCO_3$ (2 mL) and water (2 mL). The aqueous layer was extracted with DCM (3 × 8 mL) and the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification via HPLC (hexane/acetone 80:20) yielded pyrrole **39b** as a clear oil (27.4 mg, 40%). ^{1}**H NMR** (400 MHz, $CDCl_3$) δ 7.45–7.32 (m, 6H, 1H $C_\beta H/C_\delta H$ + 5H Ph), 7.02 (dd, *J*=3.6, 1.7 Hz, 1H, $C_\beta H/C_\delta H$), 6.23 (t, *J*=3.4 Hz, 1H, $C_\gamma H$), 5.31 (s, 2H, $C\underline{H}_2Ph$), 2.60 (s, 3H, N-COC$\underline{H}_3$) ppm; ^{13}**C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 169.2 (N-$\underline{C}OCH_3$), 160.8 (C_α-$\underline{C}O_2$), 135.7 ($\underline{C}_{q,Ph}$), 128.6 (2C, $\underline{C}H_{Ph}$), 128.3 (1C, $\underline{C}H_{Ph}$), 128.2 (2C, $\underline{C}H_{Ph}$), 126.5, 124.8 ($\underline{C}_\alpha$), 123.1, 110.9 ($\underline{C}_\gamma$), 66.7 ($\underline{C}H_2Ph$), 24.9 (N-CO$\underline{C}H_3$) ppm; **Rf** 0.64 (hexane/ acetone 60:40); **MS** (ESI)(*m/z*): 244.2 $(M+H)^+$, 266.2 $(M+Na)^+$; **HRMS** (ESI) for $C_{14}H_{13}NNaO_3$ $(M+Na)^+$ Calcd. 266.0788; Found 266.0785; **IR** 1715 (s), 1257 (s), 1096 (s), 756 (m) cm^{-1}.

(±)-*N*-(Acetyl)-(2*R*,3*S*,4*R*)-3-fluoro-4-(4-methylphenyl)sulfonyl proline benzyl ester (40b) (Scheme 5)

At -78 °C, DAST (31.0 µL, 0.238 mmol) is added to a solution of **±37b** (102.2 mg, 0.238 mmol) in DCM (2.0 mL). After stirring at -78 °C for 8 h, another 2 equivalents of DAST (62.0 µL, 0.476 mmol) are added. Overnight, the mixture is allowed to warm to room temperature. Next, the reaction is diluted with DCM (10 mL) and quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL). The aqueous layer is then extracted with DCM (3 × 10 mL) and the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification via HPLC (hexane/acetone 70:30) yielded pyrrole **39b** (35.8 mg, 62%) and **40b** (6.3 mg, 6%).

Data for (±)-*N*-(acetyl)-(2*R*,3*S*,4*R*)-3-fluoro-4-(4-methylphenyl)sulfonyl proline benzyl ester (**40b**): **^{1}H NMR**(400 MHz, $CDCl_3$) (83:17 rotamer ratio) δ 7.86–7.77 (m, 2H major CH Ar + 2H minor CH Ar), 7.43–7.29 (m, 7H major CH Ar + 7H minor CH Ar), 5.38 (dt, *J*=52.9, 4.8 Hz, 1H, minor $C_\beta H$), 5.27–5.08 (m, 1H major $C_\beta H$ + 2H major $C\underline{H}_2Ph$ + 2H minor $C\underline{H}_2Ph$), 4.88–4.75 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 4.70 (dd, *J*=28.1, 4.8 Hz, 1H, major $C_\alpha H$), 4.63 (dd, *J*=23.5, 5.1 Hz, 1H, minor $C_\alpha H$), 4.18 (dd, *J*=11.7, 7.3 Hz, 1H, minor $C_\delta \underline{H}H'$), 3.97 (dd, *J*=10.0, 7.5 Hz, 1H, major $C_\delta \underline{H}H'$), 3.80 (t, *J*=10.0 Hz, 1H, major $C_\delta H\underline{H'}$), 3.45 (t, *J*=10.6 Hz, 1H, minor $C_\delta H\underline{H'}$), 2.48 (s, 3H, major OTs CH_3), 2.47 (s, 3H, minor OTs CH_3), 2.11 (s, 3H, major N-$COCH_3$), 1.84 (s, 3H, minor N-$COCH_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (83:17 rotamer ratio) δ 169.7 (minor N-$\underline{C}OCH_3$), 169.2 (major N-$\underline{C}OCH_3$), 168.2 (d, *J*=7.3 Hz, minor C_α-$\underline{C}O_2$), 164.8 (d, *J*=8.1 Hz, major C_α-$\underline{C}O_2$), 145.9 (major OTs $\underline{C}_{q(Me)}$), 145.8 (minor OTs $\underline{C}_{q(Me)}$), 135.1 (major $C_{q(Bn)}$), 134.5 (minor $C_{q(Bn)}$), 132.6 (minor OTs $\underline{C}_{q(SO2)}$), 132.4 (major OTs $\underline{C}_{q(SO2)}$), 130.2 + 128.8 + 128.7 + 128.5 + 128.4 + 128.2 + 127.9 + 127.9 (major and minor overlap, $\underline{C}H_{Ar}$), 90.0 (d, *J*=196.6 Hz, minor C_β), 88.2 (d, *J*=193.7, major C_β), 73.8 (d, *J*=17.6 Hz, major C_γ), 73.4 (d, *J*=16.9 Hz, minor C_γ), 68.2 (minor $\underline{C}H_2Ph$), 67.6 (major $\underline{C}H_2Ph$), 62.5 (d, *J*=22.0 Hz, minor C_α), 61.2 (d, *J*=21.3 Hz, major C_α), 48.0 (d, *J*=1.5 Hz, major C_δ), 46.0 (d, *J*=1.5 Hz, minor C_δ), 22.0 (major N-

CO<u>C</u>H$_3$), 21.73 (major OTs-CH$_3$), 21.71 (minor OTs-CH$_3$), 21.2 (minor N-CO<u>C</u>H$_3$) ppm; **^{19}F NMR** (376 MHz, CDCl$_3$) (82:18 rotamer ratio) δ -202.6 (ddd, *J*=52.9, 23.4, 19.1 Hz, 1F, minor F), -204.5 (ddd, *J*=53.3, 27.3, 21.7 Hz, 1F, major F) ppm; **^{19}F{^{1}H} NMR** (376 MHz, CDCl$_3$) (82:18 rotamer ratio) δ -202.6 (s, 1F, minor F), -204.5 (s, 1F, major F) ppm; **Rf** 0.42 (hexane/acetone 60:40); **MS** (ESI)(*m/z*): 436.3 (M+H)$^+$, 458.3 (M+Na)$^+$; **HRMS** (ESI) for $C_{21}H_{23}FNO_6S$ (M+H)$^+$ Calcd. 436.1225; Found 436.1229; **IR** 1759 (m), 1659 (m), 1414 (m), 1368 (m), 1191 (s), 1176 (s) cm^{-1}.

Reaction of 37b with TBAF/*t*-BuOH (Scheme 5):

TBAF.3H$_2$O (143.6 mg, 0.455 mmol) was added to a solution of **37b** (78.9 mg, 0.154 mmol) in *t*-BuOH (5.0 mL) and the resulting mixture was stirred at 50 °C. After 2 h, the mixture was poured in water (20 mL) and extracted with DCM (3 × 15 mL). The combined organic phases were dried over MgSO$_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 60:40) yielded allylic alcohol **34b** (22.7 mg, 48%) and epoxide **10b** (9.0 mg, 19%).

Reaction of 38b with TBAF/*t*-BuOH (Scheme 5):

TBAF.3H$_2$O (194.4 mg, 0.616 mmol) was added to a solution of **38b** (52.7 mg, 0.154 mmol) in *t*-BuOH (2.5 mL) and the resulting mixture was stirred at 80 °C. After 1 h, the mixture was poured in water (20 mL) and extracted with DCM (3 × 10 mL). The combined organic phases were dried over MgSO$_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 60:40) yielded allylic alcohol **34b** (33.7 mg, 84%) as a clear oil.

Reaction of 38b with AgF (Scheme 5):

To a solution of **38b** (99.0 mg, 0.289 mmol) in nitromethane (5.0 mL), AgF (183.5 mg, 1.447 mmol) was added and the resulting mixture was stirred at room temperature. After 2 h, no conversion of starting material was observed on TLC and subsequently the mixture was heated at 45 °C. 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*-Butoxycarbonyl)-(2*S*,3*R*,4*S*)-3,4-dihydroxyproline benzyl ester (19a) (Table 2, entry 1)**

To a solution of alkene **25a** (3.60 g, 11.9 mmol) in dioxane (60.0 mL) and water (15.0 mL), NMO (3.48 g, 29.7 mmol) and OsO_4 (4 wt% in H_2O, 0.5 mL) were added. After 2 d of stirring at room temperature, TLC analysis indicated complete conversion of starting material. The mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (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 $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$ 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.[34]

Reaction with AD-mix-α (Table 2, entry 3):

To a solution of alkene **25a** (280.0 mg, 0.923 mmol) in *t*-BuOH (3.0 mL) and water (3.0 mL), AD-mix-α (1.29 g) and $CH_3SO_2NH_2$ (87.8 mg, 0.923 mmol) were added. After 3 d, the mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (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 $NaHCO_3$ (15 mL), dried over $MgSO_4$ 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.[34]

Reaction with AD-mix-β (Table 2, entry 4):

To a solution of alkene **25a** (203.2 mg, 0.670 mmol) in *t*-BuOH (3.0 mL) and water (3.0 mL), AD-mix-β (938.0 mg) and $CH_3SO_2NH_2$ (63.7 mg, 0.670 mmol) were added. After 7 d, the mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (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 $NaHCO_3$ (15 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Purification by HPLC (hexane/acetone 75:25) yielded diol **19a** (150.1 mg, 66%) as a clear oil. Data correspond to literature data.[34]

***N*-(9-fluorenylmethyloxycarbonyl)-(2*S*,3*R*,4*S*)-3,4-dihydroxyproline benzyl ester (19c) (Table 2, entry 5).**

To a solution of alkene **25c** (645.2 mg, 1.516 mmol) in acetone (4.5 mL) and water (1.5 mL), NMO (444.1 mg, 3.791 mmol) and $K_2OsO_4.2H_2O$ (20.0 mg, 0.054 mmol) were added. After stirring for 14 h at room temperature, TLC analysis indicated complete conversion of starting material. The mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (10 mL) and stirred at room temperature for 1 h. Next, the aqueous layer was extracted with EtOAc (4 × 15 mL) and the combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 70:30) yielded diol **19c** as a white solid (553.8 mg, 80%). **^{1}H NMR** (500 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 7.82–7.20 (m, 13H major Ar-H + 13H minor Ar-H), 5.24 (d, *J*=12.4 Hz, 1H, major C$\underline{H}$H'Ph), 5.18 (d, *J*=12.4 Hz, 1H, major CH$\underline{H'}$Ph), 5.14 (d, *J*=12.2 Hz, 1H, minor C$\underline{H}$H'Ph), 5.08 (d, *J*=12.2 Hz, 1H, minor CH$\underline{H'}$Ph), 4.59–4.21 (m, 1H major $C_\alpha H$ + 1H minor $C_\alpha H$ +1H major $C_\beta H$+ 1H minor $C_\beta H$ + 1H major $C_\gamma H$ + 1H minor $C_\gamma H$ + 2H major NCO_2-C$\underline{H}_2$-CH + 2H minor NCO_2-C$\underline{H}_2$-CH + 1H major NCO_2-CH_2-C$\underline{H}$), 3.96 (t, *J*=6.9 Hz, 1H minor NCO_2-CH_2-C$\underline{H}$), 3.85–3.53 (m, 2H major $C_\delta H_2$ + 2H minor $C_\delta H_2$), 3.29 (br d, *J*=5.0 Hz, 1H minor O$\underline{H}$), 3.19 (br d, *J*=5.5 Hz, 1H major O$\underline{H}$), 2.91 (br d, *J*=4.6 Hz, 1H minor O'$\underline{H'}$), 2.85 (br d, *J*=5.3 Hz, 1H major O'$\underline{H'}$) ppm; **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 170.7 (minor C_α-

$\underline{C}O_2$), 170.6 (major C_α-$\underline{C}O_2$), 155.0 (major $N\underline{C}O_2$), 154.6 (minor $N\underline{C}O_2$), 143.9 + 143.8 + 143.7 + 143.5 (major and minor overlap, C_q, Fmoc), 141.3 + 141.24 + 141.15 (major and minor overlap, $C_{q,Fmoc}$), 135.2 (major $\underline{C}_{q,Ph}$), 135.1 (minor $\underline{C}_{q,Ph}$), 128.59 + 128.56 + 128.48 + 128.4 + 128.3 + 128.1 + 127.74 + 127.71 + 127.6 + 127.07 + 127.04 + 125.09 + 125.05 + 124.9 + 120.0 + 119.91 + 119.89 (major and minor overlap, $\underline{C}H_{Ar}$), 75.8 (minor C_β/C_γ), 74.6 (major C_β/C_γ), 70.5 (major C_β/C_γ), 69.7 (minor C_β/C_γ), 67.8 (major and minor overlap, NCO_2-$\underline{C}H_2$-CH), 67.4 (major $\underline{C}H_2Ph$), 67.3 (minor $\underline{C}H_2Ph$), 64.9 (major C_α), 64.6 (minor C_α), 51.2 (minor C_δ), 50.8 (major C_δ), 47.1 (major NCO_2-CH_2-$\underline{C}H$), 47.0 (minor NCO_2-CH_2-$\underline{C}H$) ppm; **Rf** 0.21 (hexane/acetone 70:30); **$[\alpha]_D^{22}$** –184.1 (c 1.2, $CHCl_3$); **MS** (ESI)(*m/z*): 460.4 $(M+H)^+$, 482.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{27}H_{25}NNaO_6$ $(M+Na)^+$ Calcd. 482.1574; Found 482.1575; **IR** 3412 (br m), 1743(s), 1681 (s), 1451 (m), 1424 (s), 1185 (s), 738 (s) cm^{-1}.

(±)-*N*-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*R*)-3,4-difluoroproline benzyl ester (20a) (Scheme 7)

To a solution of diol **±19a** (950.0 mg, 2.816 mmol) in DCM (8.0 mL) at 0 °C, DAST (3.72 mL, 28.16 mmol) was added dropwise. After stirring 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 $NaHCO_3$ (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 $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/EtOAc 70:30) yielded difluoroproline **±20a** and a cyclic sulphite 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 $NaHCO_3$ (10 mL) was added and the aqueous layer was extracted with DCM (3 × 30 mL). Next, the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification via HPLC (hexane/EtOAc 80:20) yielded difluoroproline

±20a (248.1 mg, 26%) as a clear oil, which spontaneously crystallized upon standing. Data correspond to literature data.[34]

N-(9-Fluorenylmethyloxycarbonyl)-(2*R*,3*S*,4*R*)-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, DAST (0.224 mL, 1.694 mmol) was added dropwise. After stirring at room temperature for 16 h, an extra portion of DAST (0.224 mL, 1.694 mmol) was added. After another 50 h of stirring at room temperature, the reaction mixture was cooled to 0 °C, diluted with DCM (10 mL) and quenched by dropwise addition of a saturated aqueous solution of $NaHCO_3$ (20 mL). Next, the aqueous layer was extracted with DCM (3 × 20 mL) and the combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Purification by flash chromatography (hexane/acetone 80:20) yielded difluoroproline **20c** and a cyclic sulphite 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 $NaHCO_3$ (7 mL) was added and the aqueous layer was extracted with DCM (4 × 10 mL). Next, the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. Purification via HPLC (hexane/EtOAc 80:20) yielded difluoroproline **20c** (21.4 mg, 14%) as a clear oil.**^{1}H NMR** (500 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 7.82–7.24 (m, 13H major Ar-H + 13H minor Ar-H), 5.30 (d, *J*=12.4 Hz, 1H, major C$\underline{H}$H'Ph), 5.22 (d, *J*=12.4 Hz, 1H, major CH$\underline{H'}$Ph), 5.19 (d, *J*=12.4 Hz, 1H, minor C$\underline{H}$H'Ph), 5.08 (d, *J*=12.5 Hz, 1H, minor CH$\underline{H'}$Ph), 5.35–4.95 (m, 1H major $C_\beta H$ + 1H minor $C_\beta H$ + 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 4.74–4.33 (m, 1H major $C_\alpha H$ + 1H minor $C_\alpha H$ + 2H major NCO_2-C$\underline{H}_2$-CH + 2H minor NCO_2-C$\underline{H}_2$-CH), 4.26 (t, *J*=6.7 Hz, 1H major NCO_2-CH_2-C$\underline{H}$), 4.03 (t, *J*=6.4 Hz, 1H minor NCO_2-CH_2-C$\underline{H}$), 4.01–3.76 (m, 2H major $C_\delta H_2$ + 2H minor $C_\delta H_2$) ppm; **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) (51:49 rotamer ratio) δ 166.1 (d, *J*=5.5 Hz, minor C_α-$\underline{C}O_2$), 165.9 (d, *J*=6.4 Hz, major C_α-$\underline{C}O_2$), 154.4 (major $N\underline{C}O_2$), 154.0 (minor $N\underline{C}O_2$), 143.85 + 143.84 + 143.4 + 143.3 (major and minor overlap, $C_{q,Fmoc}$), 141.4 + 141.3 + 141.24 + 141.18 (major and minor overlap, $C_{q,Fmoc}$), 135.1 (major $\underline{C}_{q,Ph}$), 135.0 (minor $\underline{C}_{q,Ph}$), 128.51 + 128.50 + 128.40 + 128.35 + 128.26 + 128.20 + 127.82 + 127.79 + 127.7 + 127.14 + 127.12 + 127.09 + 127.06 + 125.0 + 124.9 + 124.83 + 124.77 + 120.02 + 199.99 + 119.95 (major and minor overlap, $\underline{C}H_{Ar}$), 88.9 (dd, *J*=199.8, 15.4 Hz, minor C_β/C_γ), 88.2 (dd, *J*=198.0, 15.4 Hz, major C_β/C_γ), 87.3 (dd, *J*=197.1, 16.4 Hz, major C_β/C_γ), 86.8 (dd, *J*=195.3, 15.4 Hz, minor C_β/C_γ), 67.9 (minor NCO_2-$\underline{C}H_2$-CH), 67.8 (major NCO_2-$\underline{C}H_2$-CH), 67.63 (major $\underline{C}H_2Ph$), 67.55 (minor $\underline{C}H_2Ph$), 60.4 (d, *J*=21.8 Hz, major C_α), 60.0 (d, *J*=21.8 Hz, minor C_α), 48.0 (dd, *J*=25.4, 1.8 Hz, minor C_δ), 47.6 (dd, *J*=26.3, 1.8 Hz, major C_δ), 47.06 (major NCO_2-CH_2-$\underline{C}H$), 47.04 (minor NCO_2-CH_2-$\underline{C}H$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (51:49 rotamer ratio) δ -204.6– -204.9 (m, 1F, minor F), -205.1– -205.4 (m, 1F, major F), -207.6– -208.0 (m, 1F, minor F’), -208.3– -208.6 (m, 1F, major F’) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (51:49 rotamer ratio) δ -204.7 (br d, *J*=5.2 Hz, 1F, minor F), -205.2 (br d, *J*=5.2 Hz, 1F, major F), -207.7 (br d, *J*=3.5 Hz, 1F, minor F’), -208.4 (br d, *J*=5.2 Hz, 1F, major F’) ppm; **Rf** 0.35 (hexane/EtOAc 75:25); **$[\alpha]_D^{24}$** –46.4 (c 0.9, $CHCl_3$); **MS** (ESI)(*m/z*): 464.1 $(M+H)^+$; **HRMS** (ESI) for $C_{27}H_{23}F_2NNaO_4$ $(M+Na)^+$ Calcd. 486.1487; Found 486.1481; **IR** 1756 (m), 1708 (s), 1450 (m), 1416 (s), 1176 (s), 1100 (s), 736 (s) cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-(2S)-4-oxoproline benzyl ester (29a)**

To a solution of **26a** (4.40 g, 13.69 mmol) in CH_2Cl_2 (40 mL) was added Dess-Martin periodinane (6.39 g, 15.06 mmol) in three portions over 15 min, and the solution was stirred at rt. After 105 min, the reaction mixture was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (30 mL) and a saturated aqueous solution of $NaHCO_3$ (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated in vacuo. Purification by flash chromatography using a Biotage® purification system (hexane/acetone gradient) yielded ketone **29a** (4.12 g, 94 %) as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) (52:48 rotamer ratio) δ 7.41–7.30 (m, 5H major Ph + 5H minor Ph), 5.30–5.07 (m, 2H major CH_2-Ph + 2H minor CH_2-Ph), 4.87 (br d, *J*=9.9 Hz, 1H, major $C_\alpha H$), 4.74 (br d, J=10.6 Hz, 1H, minor $C_\alpha H$), 3.99–3.82 (m, 2H minor $C_\delta H2$ + 2H major $C_\delta H2$), 3.02–2.84 (m, 1H major $C_\beta HH'$ + 1H minor $C_\beta HH'$), 2.64–2.50 (m, 1H major $C_\beta HH'$ + 1H minor $C_\beta HH'$), 1.48 (s, 9H, minor $CO_2C(CH_3)_3$), 1.38 (s, 9H, major $CO_2C(CH_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (52:48 rotamer ratio) δ 208.4 (minor C_γ), 207.6 (major C_γ), 171.6 (major and minor overlap, C_α-CO_2), 154.2 (minor $CO_2C(CH_3)_3$), 153.8 (major $CO_2C(CH_3)_3$), 135.1 (minor $C_{q,Ph}$),135.0 (major $C_{q,Ph}$), 128.72 + 128.66 + 128.5 + 128.2 (major and minor overlap, CH_{Ph}), 81.3 (major and minor overlap, $CO_2C(CH_3)_3$), 67.4 (major and minor overlap, CH_2 $_{Ph}$), 53.4 (major C_α), 55.7 (minor C_α), 52.9 (minor C_δ), 52.5 (major C_δ), 41.2 (major C_β), 40.7 (minor C_β), 28.3 (minor $CO_2C(CH_3)_3$), 28.1 (major $CO_2C(CH_3)_3$) ppm; **Rf** 0.35 (hexane/acetone 80:20); **$[\alpha]_D^{22}$** –16.8 (c 1.0, $CHCl_3$). Data is consistent with literature data.[65]

N-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*R*)-3-fluoro-4-hydroxyproline benzyl ester (50a) and *N*-(*tert*-butoxycarbonyl)-(2*R*,3*R*,4*S*)-3-fluoro-4-hydroxyproline benzyl ester (51a) (Scheme 8)

To a solution of diisopropylamine (2.56 mL, 18.23 mmol) in THF (50.0 mL) at -78 °C, n-BuLi (2.5M in hexanes, 6.81 mL, 17.02 mmol) was added dropwise and the mixture was stirred for 20 min. A precooled solution of ketone **29a** (3.88 g, 12.16 mmol) in THF (50.0 mL) was cannulated into the mixture and was then stirred for 30 min at -78 °C. Next, TMSCl (3.09 mL, 24.32 mmol) was added and the mixture was allowed to warm to room temperature. After 1 h, the solvent was removed *in vacuo*. The residue was redissolved in EtOAc (50.0 mL) and the residual solids were removed *via* filtration. The solvent was again removed *in vacuo*. The crude product was redissolved in acetonitrile (80.0 mL) and Selectfluor (10.77 g, 30.40 mmol) was added. After stirring at room temperature for 16 h, the solvent was removed *in vacuo*. The residue was dissolved in water (60 mL) and extracted with EtOAc (4 × 60 mL). The combined organic phases were dried over $MgSO_4$ 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), cooled to 0 °C and sodium borohydride (185.0 mg, 4.89 mmol) was added in one portion. After 3 h, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and the aqueous phase was extracted with EtOAc (4 × 30 mL). The combined organic phases were dried over $MgSO_4$ 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 fluorohydrins **50a** (460.3 mg, 40% (contaminated with an additional ~4% of **51a**)) and **51a** (138.2 mg, 13%) as clear oils.

Data for ***N*-(*tert*-butoxycarbonyl)-(2*R*,3*S*,4*R*)-3-fluoro-4-hydroxyproline benzyl ester (50a)**: **^{1}H NMR** (400 MHz, $CDCl_3$) (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 $C\underline{H}H'Ph$), 5.27 (d, *J*=12.2 Hz, 1H, major $C\underline{H}H'Ph$), 5.21 (d, *J*=12.1 Hz, 1H, major $CH\underline{H'}Ph$), 5.16 (d, *J*=12.4 Hz, 1H, minor $CH\underline{H'}Ph$), 5.27–5.08 (m, 1H major $C_\beta H$ + 1H minor $C_\beta H$), 4.64 (dd, *J*=21.0, 5.9 Hz, 1H, minor $C_\alpha H$), 4.55 (dd, *J*=20.7, 5.4 Hz, 1H, major $C_\alpha H$), 4.34–4.21 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 3.92–3.77 (m, 1H major $C_\delta \underline{H}H'$ + 1H minor $C_\delta \underline{H}H'$), 3.50–3.41 (m, 1H major $C_\delta H\underline{H'}$ + 1H minor $C_\delta H\underline{H'}$), 2.83–2.75 (m, 1H major OH +1 H minor OH), 1.47 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.33 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (60:40 rotamer ratio) δ 168.2 (d,

J=6.6 Hz, major C_α-$\underline{C}O_2$), 167.9 (d, J=7.3 Hz, minor C_α-$\underline{C}O_2$), 153.9 (minor $\underline{C}O_2C(CH_3)_3$), 153.2 (major $\underline{C}O_2C(CH_3)_3$), 135.2 (minor $\underline{C}_{q,Ph}$), 135.0 (major $\underline{C}_{q,Ph}$), 128.63 + 128.60 + 128.5 + 128.3 + 128.2 (major and minor overlap, $\underline{C}H_{Ph}$), 91.5 (d, J=189.3 Hz, major C_β), 90.8 (d, J=190.7 Hz, minor C_β), 81.1 (major $CO_2\underline{C}(CH_3)_3$), 81.0 (minor $CO_2\underline{C}(CH_3)_3$), 70.6 (d, J=18.3 Hz, minor C_γ), 70.1 (d, J=17.6 Hz, major C_γ), 67.6 (major and minor overlap, $\underline{C}H_2Ph$), 61.7 (d, J=22.0 Hz, major C_α), 61.3 (d, J=22.0 Hz, minor C_α), 50.5 (minor C_δ), 49.9 (major C_δ), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (60:40 rotamer ratio) δ -207.3 (br dt, J=53.8, 20.8 Hz, 1F, major F), -207.9 (br dt, J=54.6, 18.6 Hz, 1F, minor F) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (60:40 rotamer ratio) δ -207.4 (s, 1F, major F), -208.0 (s, 1F, minor F) ppm; **Rf** 0.30 (hexane/acetone 70:30); **[α]$_D^{22}$** –29.8 (c 1.5, $CHCl_3$); **MS** (ESI)(*m/z*): 362.3 (M+Na)$^+$; **HRMS** (ESI) for $C_{17}H_{22}FNNaO_5$ (M+Na)$^+$ Calcd. 362.1374; Found 362.1379; **IR** 3427 (br. m), 1760 (s), 1681 (s), 1400 (m), 1155 (s), 1102 (s) cm^{-1}. The NMR data are consistent with literature data.[39]

Data for ***N*-(*tert*-butoxycarbonyl)-(2*R*,3*R*,4*S*)-3-fluoro-4-hydroxyproline benzyl ester (51a): ^{1}H NMR** (400 MHz, $CDCl_3$) (58:42 rotamer ratio) δ 7.43–7.28 (m, 5H major Ph + 5H minor Ph), 5.28 (d, J=12.4 Hz, 1H, minor $C\underline{H}H'Ph$), 5.21 (d, J=12.1 Hz, 1H, major $C\underline{H}H'Ph$), 5.17 (d, J=11.9 Hz, 1H, major $CH\underline{H'}Ph$), 5.13 (d, J=12.4 Hz, 1H, minor $CH\underline{H'}Ph$), 4.93 (br dm, J=52.5 Hz, 1H major $C_\beta H$ + 1H minor $C_\beta H$), 4.61 (br d, J=21.6 Hz, 1H, minor $C_\alpha H$), 4.49 (br dd, J=21.4, 1.1 Hz, 1H, major $C_\alpha H$), 4.45–4.28 (m, 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 3.97–3.79 (m, 1H major $C_\delta\underline{H}H'$ + 1H minor $C_\delta\underline{H}H'$), 3.40–3.26 (m, 1H major $C_\delta H\underline{H'}$ + 1H minor $C_\delta H\underline{H'}$), 2.54–2.41 (m, 1H major OH +1 H minor OH), 1.47 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.33 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$): δ 169.1 (d, J=13.2 Hz, major C_α-$\underline{C}O_2$), 168.8 (d, J=13.9 Hz, minor C_α-$\underline{C}O_2$), 154.1 (minor $\underline{C}O_2C(CH_3)_3$), 153.3 (major $\underline{C}O_2C(CH_3)_3$), 135.1 (minor $\underline{C}_{q,Ph}$), 134.9 (major $\underline{C}_{q,Ph}$), 128.7 + 128.63 + 128.55 + 128.5 + 128.4 + 128.2 (major and minor overlap, $\underline{C}H_{Ph}$), 94.0 (d, J=190.7 Hz, major C_β), 93.2 (d,

J=190.0 Hz, minor $C_β$), 81.0 (major $CO_2\underline{C}(CH_3)_3$), 80.9 (minor $CO_2\underline{C}(CH_3)_3$), 70.2 (d, J=18.3 Hz, minor $C_γ$), 69.5 (d, J=17.6 Hz, major $C_γ$), 67.6 (major and minor overlap, $\underline{C}H_2Ph$), 63.8 (d, J=23.5 Hz, major $C_α$), 63.5 (d, J=24.2 Hz, minor $C_α$), 49.7 (minor $C_δ$), 49.2 (major $C_δ$), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (59:41 rotamer ratio) δ -199.8 (dt, J=52.0, 20.8 Hz, 1F, major F), -200.3 (dt, J=52.9, 21.2 Hz, 1F, minor F) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (58:42 rotamer ratio) δ -199.7 (s, 1F, major F), -200.2 (s, 1F, minor F) ppm; **Rf** 0.32 (hexane/acetone 70:30); **$[α]_D^{22}$** −18.2 (c 0.5, $CHCl_3$); **MS** (ESI)(*m/z*): 362.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{22}FNNaO_5$ $(M+Na)^+$ Calcd. 362.1374; Found 362.1376; **IR** 3426 (br. m), 1749 (s), 1701 (s), 1404 (m), 1187 (s), 1118 (s) cm^{-1}. The NMR data are consistent with literature data.[39]

N-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*S*)-3,4-difluoroproline benzyl ester (23a) (Scheme 8)

To a solution of fluorohydrin **50a** (245.7 mg, 0.724 mmol – including ~4% of **51a**) in THF (6.0 mL); tetrabutylammonium difluorotriphenylsilicate (312.7 mg, 0.579 mmol), DIPEA (0.32 mL, 1.810 mmol) and nonafluorobutanesulfonyl fluoride (NfF) (0.286 mL, 1.593 mmol) were added consecutively. After 18 h, the solvent was removed *in vacuo* and the crude mixture was purified by flash chromatography (hexane/EtOAc 90:10) to yield difluoroproline **23a** (190.7 mg, 78%, – including ~4% of **24a**) as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) (60:40 rotamer ratio) δ 7.40–7.30 (m, 5H major Ph + 5H minor Ph), 5.43–5.04 (m, 2H major $C\underline{H}_2Ph$ + 2H minor $C\underline{H}_2Ph$ + 1H major $C_βH$ + 1H minor $C_βH$ + 1H major $C_γH$ + 1H minor $C_γH$), 4.78 (dd, J=26.5, 5.1 Hz, 1H, minor $C_αH$), 4.68 (dd, J=26.5, 4.2 Hz, 1H, major $C_αH$), 4.05–3.70 (m, 2H major $C_δH_2$ + 2H minor $C_δH_2$), 1.49 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.35 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (60:40 rotamer ratio) δ 166.7 (d, J=8.8 Hz, major $C_α$-$\underline{C}O_2$), 166.4 (d, J=8.8 Hz, minor $C_α$-$\underline{C}O_2$), 154.0 (minor $\underline{C}O_2C(CH_3)_3$), 153.4 (major $\underline{C}O_2C(CH_3)_3$), 135.2 (minor $\underline{C}_{q,Ph}$), 135.1 (major $\underline{C}_{q,Ph}$), 128.6 + 128.53 + 128.51 + 128.3 + 128.2 (major and minor overlap, $\underline{C}H_{Ph}$), 93.1 (dd, J=190.0, 33.0 Hz, major $C_β$), 92.2 (dd, *J*=187.1, 27.1 Hz, minor C_β), 91.9 (dd, *J*=185.6, 24.9 Hz, minor C_γ), 91.2 (dd, *J*=182.7, 30.1 Hz, major C_γ), 81.1 (major and minor overlap, $CO_2\underline{C}(CH_3)_3$), 67.4 (major and minor overlap, $\underline{C}H_2Ph$), 62.2 (d, *J*=21.3 Hz, major C_α), 61.9 (d, *J*=22.0 Hz, minor C_α), 50.5 (d, *J*=22.0 Hz, minor C_δ), 49.9 (d, *J*=22.7 Hz, major C_δ), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.0 (major $CO_2C(\underline{C}H_3)_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (60:40 rotamer ratio) δ -192.3– -192.7 (m, 1F, minor F), -192.8– -193.2 (m, 1F, major F), -196.2– -196.9 (m, major F' + minor F') ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$): (60:40 rotamer ratio) δ -192.4 (d, *J*=12.1 Hz, 1F, minor F), -192.9 (d, *J*=12.1 Hz, 1F, major F), -196.3 (d, *J*=12.1 Hz, 1F, major F'), -196.6 (d, *J*=12.1 Hz, 1F, minor F') ppm; **Rf** 0.41 (hexane/acetone 80:20); **[α]$_D^{22}$** –45.0 (c 1.4, $CHCl_3$); **MS** (ESI)(*m/z*): 342.4 $(M+H)^+$, 364.4 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{21}F_2NNaO_4$ $(M+Na)^+$ Calcd. 364.1331; Found 364.1332; **IR** 1764 (m), 1702 (s), 1394 (s), 1367 (m), 1159 (s) cm^{-1}.

***N*-(*tert*-Butoxycarbonyl)-(2*R*,3*R*,4*R*)-3,4-difluoroproline benzyl ester (24a)(Scheme 8)**

To a solution of fluorohydrin **51a** (110.0 mg, 0.324 mmol) in THF (3.0 mL); tetrabutylammonium difluorotriphenylsilicate (140.0 mg, 0.259 mmol), DIPEA (0.141 mL, 0.810 mmol) and nonafluorobutanesulfonyl fluoride (NfF) (0.128 mL, 0.713 mmol) were added consecutively. After 18 h, the solvent was removed *in vacuo* and the crude mixture was purified by flash chromatography (hexane/EtOAc 90:10) to yield difluoroproline **24a** (80.0 mg, 72%) as a clear oil. **^{1}H NMR** (400 MHz, $CDCl_3$) (57:43 rotamer ratio) δ 7.42–7.29 (m, 5H major Ph + 5H minor Ph), 5.36–5.04 (m, 2H major $C\underline{H_2}Ph$ + 2H minor $C\underline{H_2}Ph$ + 1H major $C_\beta H$ + 1H minor $C_\beta H$ + 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 4.79 (d, *J*=23.6 Hz, 1H, minor $C_\alpha H$), 4.63 (d, *J*=23.6 Hz, 1H, major $C_\alpha H$), 3.94–3.75 (m, 2H major $C_\delta H_2$ + 2H minor $C_\delta H_2$), 1.49 (s, 9H, minor $CO_2C(C\underline{H}_3)_3$), 1.36 (s, 9H, major $CO_2C(C\underline{H}_3)_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (57:43 rotamer ratio) δ 167.4 (d, *J*=14.7 Hz, major C_α-$\underline{C}O_2$), 167.2 (d, *J*=16.1 Hz, minor C_α-$\underline{C}O_2$), 153.9 (minor $\underline{C}O_2C(CH_3)_3$), 153.5 (major $\underline{C}O_2C(CH_3)_3$), 135.13 (minor $\underline{C}_{q,Ph}$), 135.05 (major $\underline{C}_{q,Ph}$), 128.61 + 128.58 + 128.52 + 128.46 + 128.4 + 128.1 (major and minor overlap,

$\underline{C}H_{Ph}$), 95.2 (dd, *J*=182.7, 27.1 Hz, major C_{β}), 94.1 (dd, *J*=186.7, 32.7 Hz, minor C_{β}), 92.1 (dd, *J*=179.0, 32.3 Hz, minor C_{γ}), 91.2 (dd, *J*=179.0, 31.5 Hz, major C_{γ}), 81.0 (major and minor overlap, $CO_2\underline{C}(CH_3)_3$), 67.5 (major and minor overlap, $\underline{C}H_2Ph$), 64.3 (d, *J*=23.5 Hz, major C_{α}), 63.9 (d, *J*=23.5 Hz, minor C_{α}), 50.8 (d, *J*=23.5 Hz, minor C_{δ}), 50.4 (d, *J*=23.5 Hz, major C_{δ}), 28.3 (minor $CO_2C(\underline{C}H_3)_3$), 28.1 (major $CO_2C(\underline{C}H_3)_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$): δ -187.5– -188.7 (m, 1F major F + 1F minor F + 1F major F' +1F minor F') ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (57:43 rotamer ratio) δ -187.7 (d, *J*=13.9 Hz, 1F, major F), -188.0 (d, *J*=15.6 Hz, 1F, minor F), -188.1 (d, *J*=13.9 Hz, 1F, major F'), -188.6 (d, *J*=13.9 Hz, 1F, minor F') ppm; **Rf** 0.56 (hexane/acetone 70:30); **$[\alpha]_D^{22}$** –32.7 (c 1.0, $CHCl_3$, 22 °C); **MS** (ESI)(*m/z*): 364.3 $(M+Na)^+$; **HRMS** (ESI) for $C_{17}H_{21}F_2NNaO_4$ $(M+Na)^+$ Calcd. 364.1331; Found 364.1330; **IR** 1762 (m), 1705 (s), 1396 (s), 1368 (m), 1168 (s) cm^{-1}.

N-(*tert*-Butoxycarbonyl)-(2*R*,3*S*,4*S*)-3,4-difluoroproline (*N*-Boc-5) (Scheme 9)

To a solution of **23a** (161.0 mg, 0.472 mmol) in methanol (3.0 mL), 10 % Pd/C (20.0 mg) was added. The mixture was purged with one balloon volume of hydrogen gas. Subsequently, the mixture was kept under hydrogen atmosphere and stirred at room temperature. After 18 h, the mixture was filtered through a plug of celite and the solvent evaporated. Carboxylic acid (*N*-Boc)-**5** (118.5 mg, quantitative) was obtained as a clear oil. The product was used as such in the next reaction.

N-(Acetyl)-(2*R*,3*S*,4*S*)-3,4-difluoroproline methyl ester (21) (Scheme 9)

Carboxylic acid (*N*-Boc)-**5** (118.5 mg, 0.472 mmol) was dissolved in methanol (2.0 mL) and cooled to 0 °C. Acetyl chloride (0.167 mL, 2.360 mmol) was added dropwise and stirring at 0 °C was continued. After 40 min, the mixture was allowed to warm to room temperature. After 22 h, the mixture was concentrated by rotary evaporation to yield an intermediate salt. The salt was redissolved in DCM (3.0 mL), cooled to 0 °C and triethylamine (0.132 mL, 0.944 mmol) was added. Next, acetyl chloride (0.100 mL, 1.416 mmol) was added dropwise and the solution was allowed to warm room temperature. After stirring for 24 h, the mixture was poured into water (10 mL) and the aqueous layer was extracted with DCM (4 x 10 mL). The combined organic layers were dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by flash chromatography (hexane/acetone 70:30) to yield **21** (29.3 mg, 30%) as a colourless solid. **^{1}H NMR** (400 MHz, $CDCl_3$) (80:20 rotamer ratio) δ 5.53–5.09 (m, 1H major $C_\beta H$ + 1H minor $C_\beta H$ + 1H major $C_\gamma H$ + 1H minor $C_\gamma H$), 4.92–4.73 (m, 1H major $C_\alpha H$ + 1H minor $C_\alpha H$), 4.35–3.59 (m, 2H major $C_\delta H_2$ + 2H minor $C_\delta H_2$), 3.85 (s, 3H, minor $CO_2C\underline{H}_3$), 3.80 (s, 3H, major $CO_2C\underline{H}_3$), 2.14 (s, 3H, major $NCOC\underline{H}_3$), 1.98 (s, 3H, minor $NCOC\underline{H}_3$) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) (80:20 rotamer ratio) δ 169.9 (minor $N\underline{C}OCH_3$), 169.5 (major $N\underline{C}OCH_3$), 166.9 (d, *J*=8.8 Hz, minor C_α-$\underline{C}O_2$), 166.2 (d, *J*=8.1 Hz, major C_α-$\underline{C}O_2$), 93.5 (dd, *J*=187.8, 33.0 Hz, minor C_β), 91.9 (dd, *J*=183.4, 30.8 Hz, major C_γ), 91.4 (dd, *J*=186.0, 32.7 Hz, major C_β), 90.5 (dd, *J*=181.2, 30.8 Hz, minor C_γ), 62.9 (d, *J*=22.0 Hz, minor C_α), 61.5 (d, *J*=22.0 Hz, major C_α), 53.1 (minor $CO_2\underline{C}H_3$), 52.7 (major $CO_2\underline{C}H_3$), 51.3 (d, *J*=22.0 Hz, major C_δ), 49.8 (d, *J*=22.0 Hz, minor C_δ), 22.1 (major $NCO\underline{C}H_3$), 21.6 (minor $NCO\underline{C}H_3$) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) (80:20 rotamer ratio) δ -192.2– -192.8 (m, 1F, major F), -193.4– -193.9 (m, 1F, minor F), -194.6– -195.0 (m, 1F, minor F’), -196.4– -196.9 (m, 1F, major F’) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) (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, minor F’), -196.8 (d, *J*=13.9 Hz, 1F, major F’) ppm; **Rf** 0.19 (hexane/acetone 70:30); **$[\alpha]_D^{22}$** –47.8 (c 0.9, $CHCl_3$); **MS** (ESI)(*m/z*): 208.2 $(M+H)^+$, 230.2 $(M+Na)^+$; **HRMS** (ESI) for $C_8H_{11}F_2NNaO_3$ $(M+Na)^+$ Calcd. 230.0599; Found 230.0604; **IR** 1758 (s), 1653 (s), 1202 (s), 1176 (s), 1048 (s), 1039 (s) cm^{-1}.

N-(*tert*-Butoxycarbonyl)-(2*R*,3*R*,4*R*)-3,4-difluoroproline (*N*-Boc-6) (Scheme 9)

To a solution of **24a** (64.0 mg, 0.187 mmol) in methanol (2.0 mL), 10 % Pd/C (10.0 mg) was added. The mixture was purged with one balloon volume of hydrogen gas. Subsequently, the mixture was kept under 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)-**6** (47.3 mg, quantitative) was obtained as a clear oil. The product was used as such in the next reaction.

***N*-(Acetyl)-(2*R*,3*R*,4*R*)-3,4-difluoroproline methyl ester (22) (Scheme 9)**

Carboxylic acid (*N*-Boc)-**6** (46.4 mg, 0.185 mmol) was dissolved in methanol (2.0 mL) and cooled to 0 °C. Acetyl chloride (65.6 µL, 0.923 mmol) was added dropwise and the mixture was allowed to warm to room temperature. After 15 h, the mixture was concentrated by rotary evaporation to yield an intermediate salt. The salt was redissolved in DCM (2.0 mL), 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 room temperature. After stirring for 22 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. **^{1}H NMR** (500 MHz, CDCl$_3$) (65:35 rotamer ratio) δ 5.45 (dd, *J*=47.5, 6.3 Hz, 1H, minor C$_\beta$H), 5.30 (ddd, *J*=47.7, 7.4, 1.5 Hz, 1H, major C$_\beta$H), 5.31–5.11 (m, 1H major C$_\gamma$H + 1H minor C$_\gamma$H), 4.97 (d, *J*=23.7 Hz, 1H, major C$_\alpha$H), 4.67 (d, *J*=21.1 Hz, 1H, minor C$_\alpha$H), 4.05-3.88 (m, 2H major C$_\delta$H$_2$ + 2H minor C$_\delta$H$_2$), 3.83 (s, 3H, minor CO$_2$CH$_3$), 3.78 (s, 3H, major CO$_2$CH$_3$), 2.16 (s, 3H, major NCOCH$_3$), 2.07 (s, 3H, minor NCOCH$_3$) ppm; **^{13}C{^{1}H} NMR** (126 MHz, CDCl$_3$) (65:35 rotamer ratio) δ 170.1 (minor N$\underline{C}$OCH$_3$), 169.7 (major N$\underline{C}$OCH$_3$), 167.0 (minor C$_\alpha$-$\underline{C}$O$_2$), 166.9 (major C$_\alpha$-$\underline{C}$O$_2$), 95.1 (dd, *J*=187.1, 32.7 Hz, minor C$_\beta$), 93.5 (dd, *J*=180.3, 31.3 Hz, major C$_\beta$), 92.1 (dd, *J*=172.5, 31.9 Hz, major C$_\gamma$), 90.4 (dd, *J*=178.0, 31.8 Hz, minor C$_\gamma$), 64.8 (d, *J*=22.7 Hz, minor C$_\alpha$), 63.4 (d, *J*=23.6 Hz, major C$_\alpha$), 53.3 (minor CO$_2\underline{C}$H$_3$), 52.9 (major CO$_2\underline{C}$H$_3$), 51.6 (d, *J*=23.6 Hz, major C$_\delta$), 50.7 (d, *J*=23.6 Hz, minor C$_\delta$), 22.1 (major NCO$\underline{C}$H$_3$), 22.0 (minor NCO$\underline{C}$H$_3$) ppm; **^{19}F NMR** (471 MHz, CDCl$_3$) (66:34 rotamer ratio) δ -187.9– -187.3 (m, 1F, minor F), -187.5– -187.9 (m, 1F, major F), -187.9– - 188.2 (m, 1F, major F'), -188.8– -189.1 (m, 1F, minor F') ppm; **^{19}F{^{1}H} NMR** (471 MHz, $CDCl_3$) (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); **$(\alpha)_D^{22}$** –67.2 (c 1.1, $CHCl_3$); **MS** (ESI)(*m/z*): 208.2 $(M+H)^+$; **HRMS** (ESI) for $C_8H_{11}F_2NNaO_3$ $(M+Na)^+$ Calcd. 230.0599; Found 230.0601; **IR** 1758 (m), 1658 (s), 1417 (m), 1208 (m) cm^{-1}.

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Supporting Information: ^{1}H, ^{13}C, ^{19}F 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**, **24a**, computational data of the proline conformers of **21**, **22**, **52–55**, and Ac-Pro-OMe in $CHCl_3$ and water calculated by DFT including Gibbs free energies, electronic energy values and cartesian coordinates, and general NMR conditions for conformational and kinetic analysis. This material is available free of charge via the Internet at http://pubs.acs.org/. The raw NMR data files are available free of charge at http://dx.doi.org/xxxxxxxxxxxxxxx

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