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δ -Valerolactamic Quaternary Amino Acid Derivatives: Enantiodivergent Synthesis and Evidence for Stereodifferentiated β -Turn Inducing Properties

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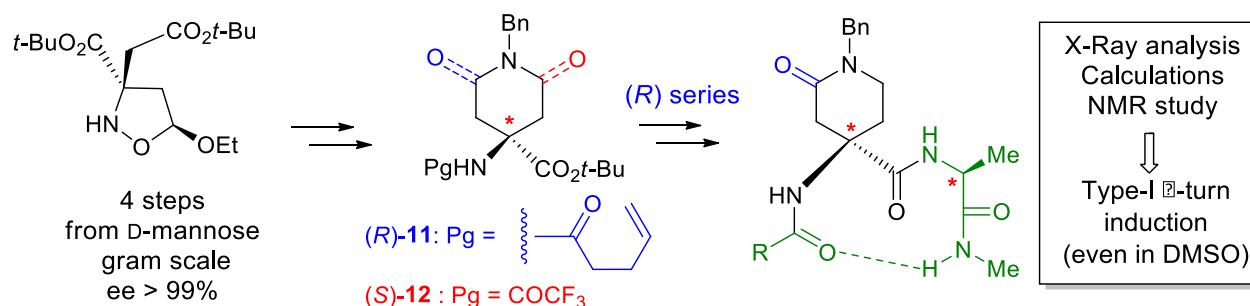
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ABSTRACT: Enantiopure (*R*) and (*S*) cyclic α,α -disubstituted amino acid derivatives displaying a δ -valerolactam side chain were prepared from a common isoxazolidine precursor. The (*R*)-configured δ -valerolactam **11** was converted into diastereoisomeric pseudo-peptides to investigate its ability to induce secondary structures in peptidomimetics. Conformational studies of these pseudo-peptides were carried out in the solid state (X-ray diffraction), in solution (NMR analyses) and *in silico* (computer-aided conformational analysis), which demonstrated that such quaternary amino acid induce β -turn conformations stable enough to be retained in polar media (DMSO). Incorporation of this new type of α,α -disubstituted amino acid into a representative pseudo-peptidic sequence by *N*- then *C*-elongation, and *N*-debenzylation are also described herein and could serve for the synthesis of various structured peptidomimetics.

INTRODUCTION

Peptidomimetics containing non-canonical α -amino acids are essential tools for biology and as therapeutics, as they often exhibit enhanced stability towards *in vivo* proteolytic processes compared to natural peptides.² Among non-canonical α -amino acids, the cyclic α,α -disubstituted amino acids (cDAAs) are particularly useful to induce conformational restriction in peptidic backbones and allow selective recognition by target proteins.³ Due to their constrained dihedral

angles, cDAAs proved to confer peptidic scaffolds with stabilized secondary structures, thus allowing improved mimicry of specific recognition patterns found in natural peptides and proteins, such as β -turns and α -helices.⁴ For example, the 1,1'-binaphthyl-substituted α -aminoisobutyric acid **I** (Bin, Figure 1)⁵ represents a privileged rigid scaffold to transfer chirality through helical sequences of α -amino-isobutyric acid (Aib) and glycine residues.⁶ In the same line, compounds **II-VI** (Figure 1) have been synthesized as potential conformational inducers for peptidic sequences. The group of González-Muñiz reported the synthesis of the chiral quaternary α,α -2-oxoazepane amino acid **II**, and demonstrated its ability to induce a β -turn conformation in a dipeptidic structure.^{7,8} Meanwhile, 3_{10} helix conformations were observed in small peptidic sequences containing the azepane analogue **III**,⁹ the unsubstituted azepane **IV**,¹⁰ or the 2-oxoazepane **V**.¹¹ In addition, 4-aminopiperidine-4-carboxylic acid (Api) derivatives **VI** have been described to be valuable helical conformation inducers, allowing modulation of the helical properties through variation of the substituent on the piperidinic nitrogen atom.¹²

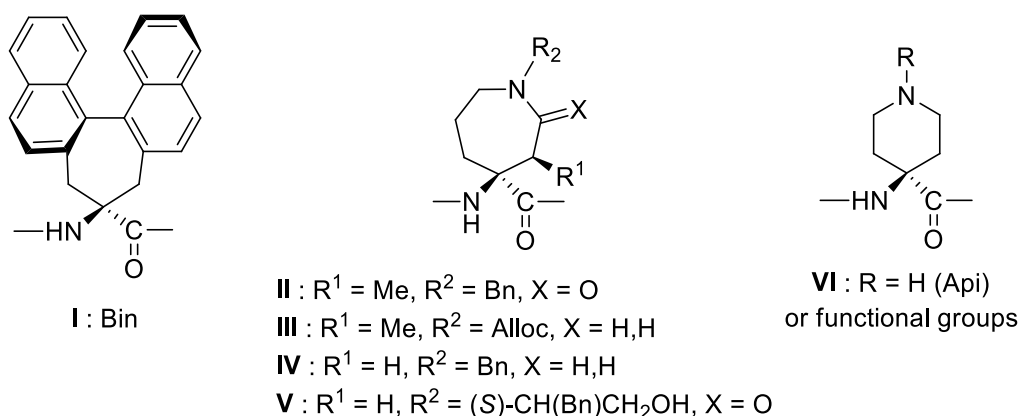
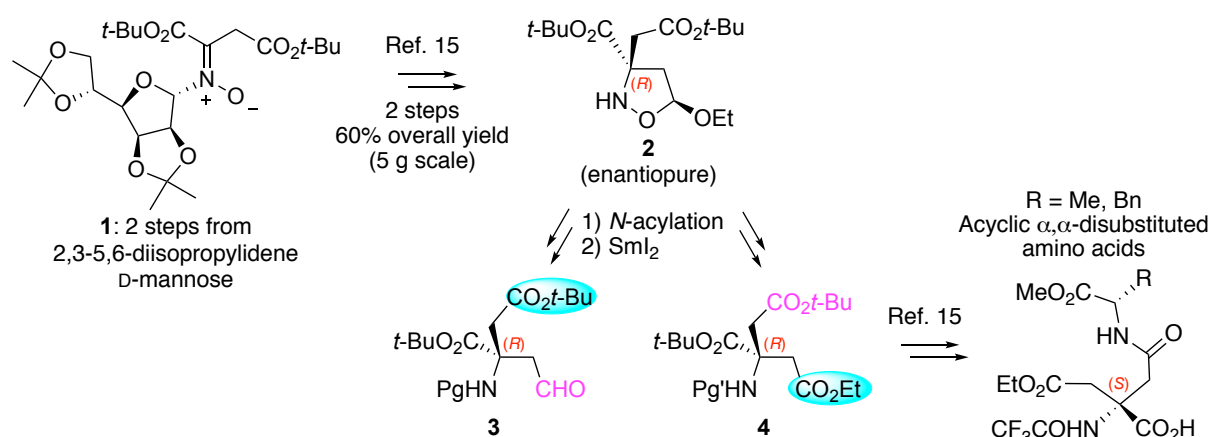


Figure 1. Examples of cyclic α,α -disubstituted amino acids as secondary structure inducers in peptides

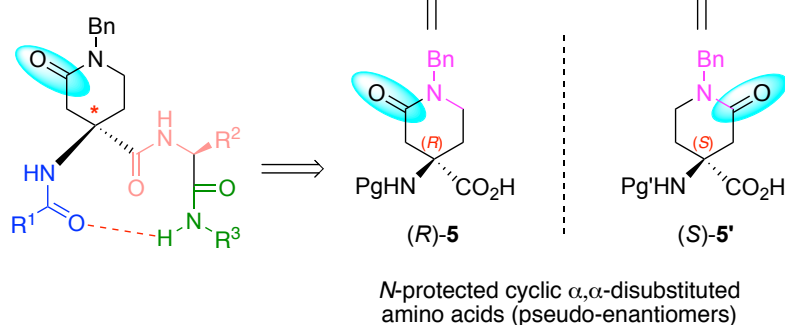
In this context, we assumed that δ -valerolactamic derivatives of Api were relevant cyclic α,α -disubstituted amino acids to be studied as secondary structure inducers in peptidomimetics. Indeed, introduction of the lactam function would not only allow desymmetrization of the cyclic moiety and confer chirality to such cDAA, but it would also provide new opportunities for peptide structuration through possible involvement of the lactamic $\text{C}=\text{O}$ in hydrogen bonding. It was anticipated that each enantiomer of such chiral cDAA could induce a specific conformation in small heteropeptides,¹³ or even control the helical screw sense of oligo-homopeptides made up of non-chiral amino acids.¹⁴

A few years ago, we have described an efficient route to *acyclic* α,α -disubstituted amino acids involving the stereoselective cycloaddition of a D-mannose-derived ketonitrone **1** with enol ethers, yielding isoxazolidine **2** in enantiopure form (Scheme 1A). The latter revealed to be a key intermediate to access pseudodipeptides containing an acyclic α,α -disubstituted amino acid, through ester **4** (Pg = COCF₃).¹⁵ During this work, we found an interesting chemodivergence of the SmI₂-promoted reductive opening of *N*-acylated derivatives of isoxazolidine **2**, yielding selectively either ester **4** (Pg = COCF₃) or aldehyde **3** (Pg = COCH₃). We eventually realized that enantiopure isoxazolidine **2** could be exploited to access α,α -disubstituted amino acids of opposite absolute configuration through selective manipulation of the three different carbonyl groups in compounds **3** or **4**. Herein, we present the synthesis of enantiopure derivatives of δ -valerolactamic cDAAs (*R*)-**5** and (*S*)-**5'** from the readily available isoxazolidine **2**, through *N*-protected quaternary amino aldehyde **3** or ester **4** respectively (Scheme 1B). (*R*)-**5** was incorporated into short pseudopeptidic sequences, and conformational studies on the resulting tripeptides by NMR, X-ray analysis and computational calculations demonstrate the capacity of this new cDAA to induce a remarkably stable β -turn conformation.

A. Previous work



B. This work

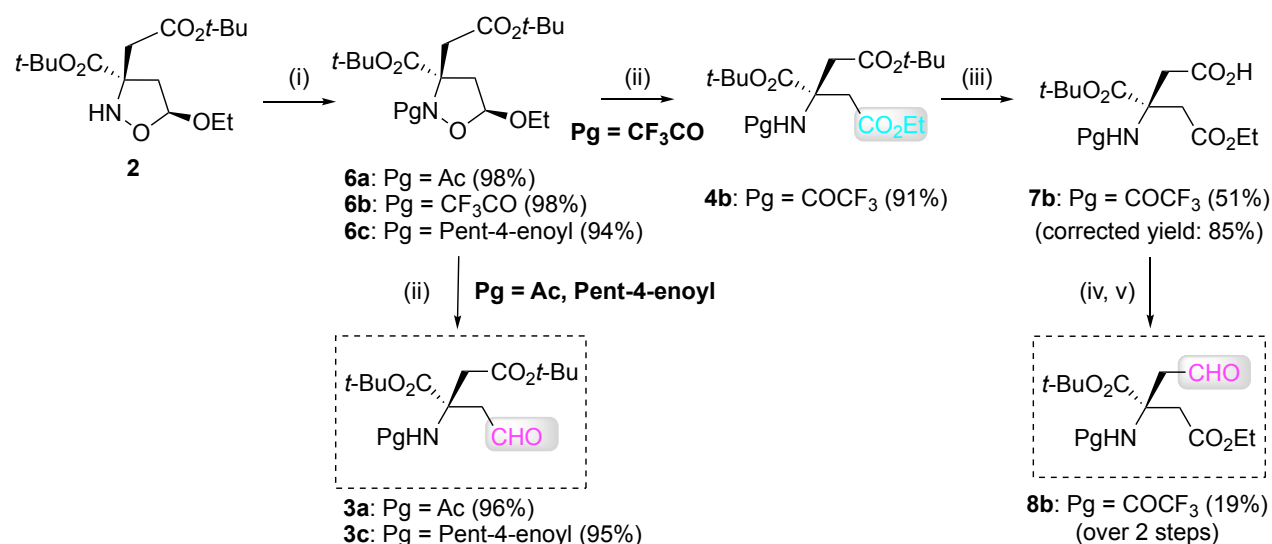


Scheme 1. Our approach to chiral quaternary δ -valerolactamic conformational inducers

RESULTS AND DISCUSSION

Synthesis of δ -valerolactamic cDAAs of (*R*) and (*S*) configurations

In our previous work, the *N*-acetyl (**6a**) and *N*-trifluoroacetyl (**6b**) derivatives of enantiopure isoxazolidine **2** were nicely converted into aldehyde **3a** and ester **4b** respectively, upon treatment with SmI_2 .¹⁵ Considering that the acetyl *N*-substituent is not a convenient protecting group for peptide synthesis, we turned our attention to the *N*-pent-4-enoyl isoxazolidine **6c** as possible precursor of amino aldehyde **3c** (Scheme 2). The pent-4-enoyl protecting group appeared particularly attractive as it can be orthogonally removed upon treatment with I_2 in $\text{THF}/\text{H}_2\text{O}$.¹⁶ Compound **6c** was obtained in 94% yield by simple treatment of isoxazolidine **2** with 4-pentenoic anhydride. *N*-pent-4-enoyl isoxazolidine **6c** was reduced by SmI_2 in only ten minutes at room temperature, and smoothly converted into the corresponding aldehyde **3c** with total chemoselectivity (no ester detected) and excellent yield (95%), even at a gram-scale.



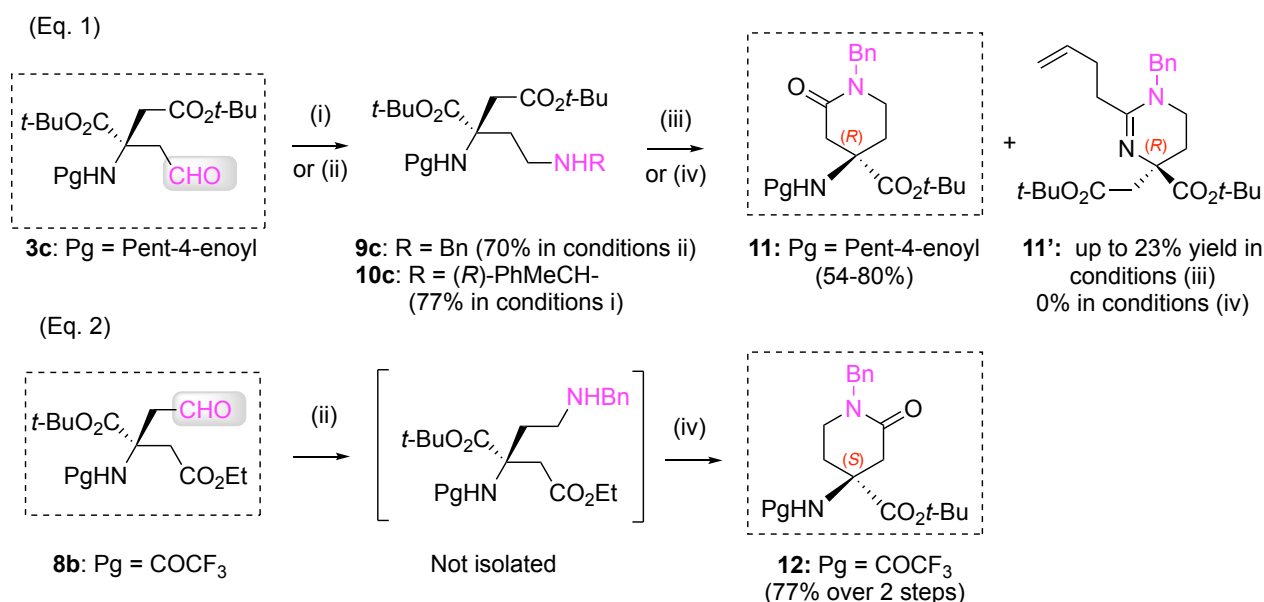
(i): For **6a**: acetic anhydride (solvent), rt, 17 h; for **6b**: trifluoroacetic anhydride (excess), CH_2Cl_2 , rt, 30 min; for **6c**: pent-4-enoic anhydride (2 equiv), rt, 4 days; (ii): 0.1M SmI_2 / THF (3 equiv), rt, 10 min; (iii): TFA (10% v/v), DCM, 0 °C, 5 h; (iv): $\text{BH}_3 \cdot \text{SMe}_2$, THF, 80 °C, 1 h; (v): Dess Martin periodinane, DCM, 0 °C, 2 h.

Scheme 2. Access to pseudo-enantiomeric *N*-protected aldehydes **3c** and **8b**

From *N*-trifluoroacetyl isoxazolidine **6b**, optimization of the previously described conditions¹⁵ for SmI_2 -promoted reductive opening furnished *N*-trifluoroacetyl triester **4b** in 91% yield (Scheme 2). Selective acidolysis of the less hindered *t*-butyl ester function of **4b** using diluted TFA in dichloromethane at 0 °C produced mono acid (diester) **7b** in 51% isolated yield (corrected yield 85%), together with minor amounts (5%) of diacid ethyl monoester and 40% of starting material

(4b).¹⁷ Chemoselective reduction of acid **7b** with borane-dimethylsulfide complex and subsequent oxidation of the primary alcohol so obtained with Dess-Martin periodinane led to aldehyde **8b** in an unoptimized 19% overall yield.

With both *N*-protected aldehydes **3c** and **8b** in hand, we next investigated their reductive amination to access pseudo-enantiomeric lactamic cDAA precursors (Scheme 3). The reaction of **3c** with benzylamine using NaBH₃CN or NaBH(OAc)₃ in the presence of acetic acid, respectively without¹⁸ or with preformation of the corresponding imine (Scheme 3, eq. 1), afforded the corresponding secondary amine **9c** in good yield (70%). When extended to (*R*)- α -methylbenzylamine, the reductive amination was performed in 77% yield (**10c**).



(i): RNH₂, NaBH₃CN, AcOH, MeOH, rt; (ii): 1) RNH₂, MgSO₄, DCM; 2) NaBH(OAc)₃, AcOH, rt; (iii): Toluene, 110 °C, 17 h; (iv): neat, 4 °C, > 2 weeks.

Scheme 3. Synthesis of bis-protected (*R*) and (*S*) δ -valerolactamic cDAAs **11** and **12**

The critical cyclization of the *N*-benzyl amino diester **9c** was then studied. Heating **9c** in refluxing toluene provided the 6-membered ring product **11** in moderate to good yield, without visible formation of 5-membered ring byproducts.¹⁹ The limitation of the yield (54%) was found to be due to the competitive formation of amidine **11'** (23%), resulting from nucleophilic attack of the secondary amine on the pent-4-enamide under these thermal conditions.²⁰ The δ -valerolactamic structure of the cyclic product **11**, produced by nucleophilic attack of the secondary amine on the less hindered β -ester, was deduced from IR and ¹³C spectral data, and was confirmed by X-ray

crystallography (see Figure 2).²¹ Interestingly, this crystalline product **11** proved to be also obtained from its precursor **9c** by slow cyclization / crystallization in a mixture of diethyl ether and pentane at 4 °C.²² Under these mild conditions, the competitive formation of amidine **11'** was not observed and compound **11** could be obtained at a gram scale without purification of **9c** in 55% overall yield from aldehyde **3c** (2 steps). However, this procedure is very long (> 2 weeks) and its reproducibility is low. Conversely, all attempts to perform lactamization on compound **10c** failed, possibly due to steric hindrance of the nitrogen atom.

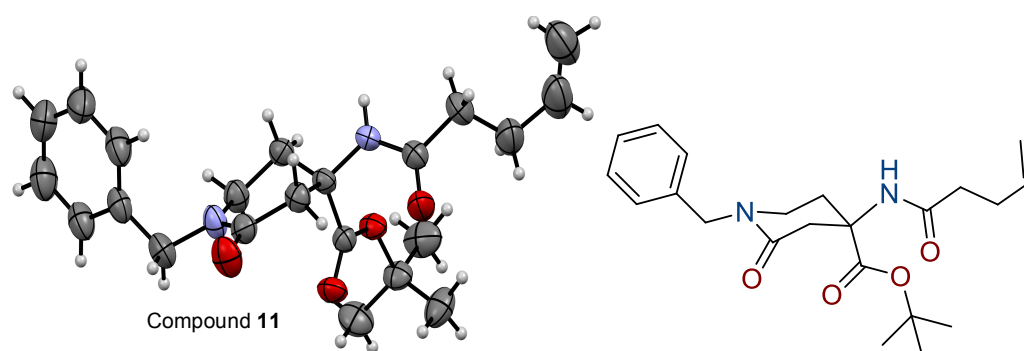


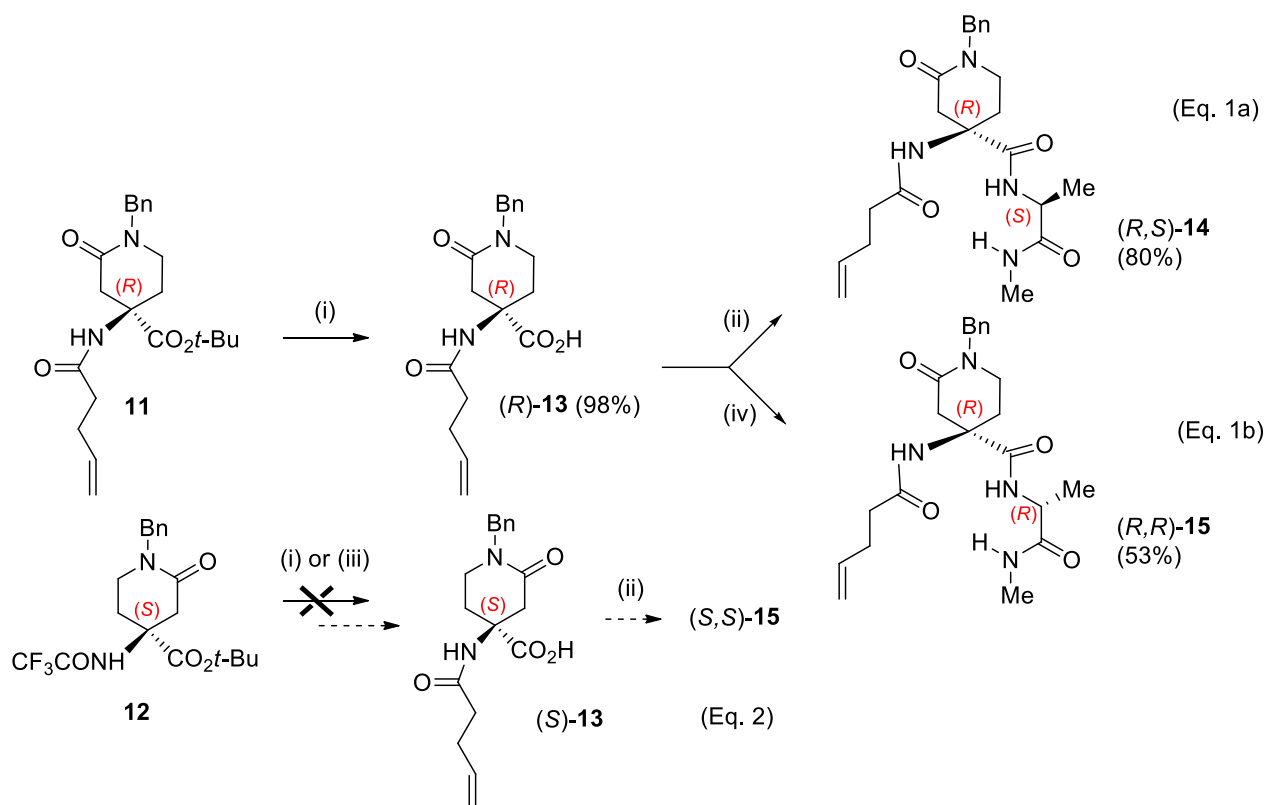
Figure 2. ORTEP diagram for the crystal structure of compound **11** (50% ellipsoid contour probability level)

Aldehyde **8b** was next subjected to the reductive amination conditions. To our delight, the amino diester so produced was found to undergo spontaneous cyclisation upon storage at low temperature (two weeks), yielding δ -valerolactam of (*S*)-configuration **12** with complete regioselectivity and in good yield (Scheme 3, eq. 2).

Synthesis of pseudopeptides **14** and **15**

In view to access pseudopeptides containing a δ -valerolactamic cDAA moiety, the cleavage of the *t*-butyl ester in compounds (*R*)-**11** and (*S*)-**12** was next studied. Compound (*R*)-**11** was easily deprotected at the *C*-terminus by TFA to afford the corresponding carboxylic acid (*R*)-**13**, which was coupled with *N*-Me-L-alaninamide under standard conditions (Scheme 4, eq 1a). To our delight, the resulting crystalline product (*R,S*)-**14** could be subjected to X-ray diffraction analysis on a monocrystal obtained from recrystallization in dichloromethane (Figure 3).²³ In the following discussion, the conformation adopted by (*R,S*)-**14** in the crystal state will be named *cryst*-**14**.

In order to evaluate the influence of the stereogenic center in the lactam ring on the conformation adopted by peptides, we first tried to transform the *N*-COCF₃ protected δ -valerolactam (*S*)-**12** into the pseudopeptide (*S,S*)-**15** via precursor (*S*)-**13** (Scheme 4, eq. 2). However, despite several attempts, treatment of (*S*)-**12** with TFA did not afford the corresponding acid and basic solvolysis failed to remove the trifluoroacetyl group, thus making the obtention of (*S,S*)-**15** impossible from (*S*)-**12**. Alternatively, the pseudopeptide (*R,R*)-**15**, epimer of (*R,S*)-**14**, was efficiently synthesized by coupling the acid (*R*)-**13** with *N*-Me-D-alaninamide (Scheme 4, eq. 1b). In the following discussion, the epimeric pseudopeptides (*R,S*)-**14** and (*R,R*)-**15** will be named **14** and **15** respectively.



(i) TFA, DCM, rt, 17 h; (ii) BOP, TEA, L-H-Ala-NHMe.HCl, DCM, rt; (iii) K₂CO₃, MeOH or TEBA, MeOH; (iv) BOP, TEA, D-H-Ala-NHMe.HCl, DCM, rt.

Scheme 4. Synthesis of the epimeric pseudopeptides (*R,S*)-**14** and (*R,R*)-**15**

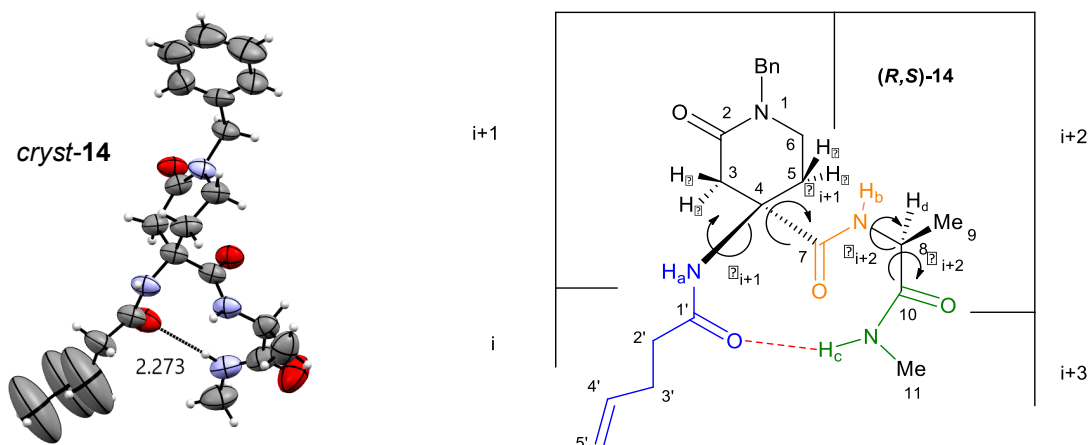


Figure 3. ORTEP diagram for the crystal structure of pseudopeptide **14** (*cryst-14*, 50% ellipsoid contour probability level, distances in Å)

Conformational analysis of pseudopeptide **14**

As can be seen in Figure 3, pseudopeptide **14** adopts in the solid state a folded conformation *cryst-14* stabilized by a hydrogen bond between the NH of the C-terminal *N*-methyl-alaninamide (coined H_c in the following discussion) and the carbonyl oxygen of the pent-4-enoyl group (interatomic distance of 2.273 Å). The dihedral angles φ and ψ for the ($i+1$) δ -valerolactamic cDAA and the ($i+2$) *N*-methyl-alaninamide measured from this crystallographic are close to those typically admitted to characterize type I β -turns (Table 1).²⁴

Dihedral angles	i+1		i+2	
(in degrees)	φ_{i+1}	ψ_{i+1}	φ_{i+2}	ψ_{i+2}
<i>cryst-14</i>	-53.7	-41.3	-86.2	-2.4
Type I β -turn	-60	-30	-90	0

Table 1. Characteristic torsional angles in pseudopeptide *cryst-14* and in typical type I β -turns

Noticeably, in the crystal structure²³ of pseudopeptide **14** all the N-H bonds are nearly oriented in the same direction, conferring a high polarity to **14** and allowing intermolecular H-bonds involving H_a and H_b of *cryst-14* (see Figure S4 in the Supporting Information). While such intermolecular hydrogen bonds could be conserved in solution in non-polar solvents, they are expected to be disrupted in polar solvents such as water or DMSO.

In order to evaluate the relevance of a similar β -turn conformation of pseudopeptide **14** in solution, NMR analyses and computational studies were next undertaken. A classical method to detect the presence of hydrogen bonds in peptides and pseudopeptides consists in measuring the variation of chemical shifts in ^1H NMR spectra as a function of concentration.²⁵ Accordingly, the ^1H NMR spectra of compound **14** were recorded at room temperature in a range of concentrations

varying from 10^{-1} to 10^{-3} M in CDCl_3 and in $\text{DMSO-}d_6$ (Figure 4). In CDCl_3 , the chemical shifts for each NH proton in pseudopeptide **14** (coined respectively H_a for the *N*-terminal pent-4-enoic amide, H_b for the central δ -valerolactamic cDAA and H_c for the *C*-terminal *N*-methyl-alaninamide, see Figure 3) were found to decrease proportionally with dilution, suggesting their involvement in hydrogen bonds, possibly intermolecular hydrogen bonds similar to those observed in the solid state. It can be noticed that δH_a is most affected by concentration variation, as the most exposed proton to solvent effects. In contrast, in $\text{DMSO-}d_6$ the fluctuation of the chemical shifts of the N-H protons is weak, indicating the absence of intermolecular interactions. Obviously, $\text{DMSO-}d_6$ is a strongly polar solvent able to dissociate intermolecular H-bonds in pseudopeptide **14**. It thus constitutes a good solvent to evaluate the stability of a β -turn conformation (driven by an intramolecular H-bond) of compound **14** in solution.

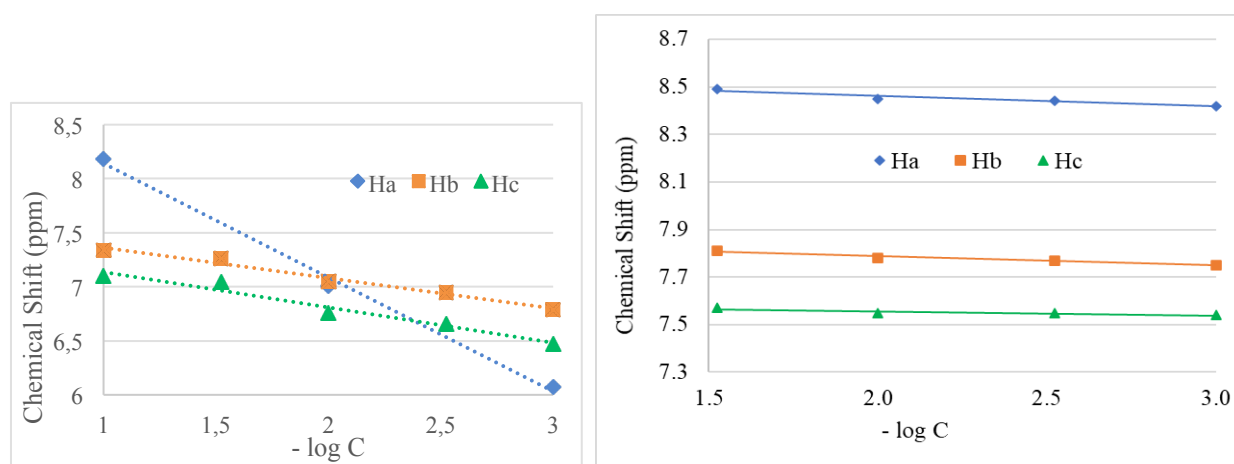


Figure 4. Variation of the chemical shifts δH_a , δH_b and δH_c with the concentration of pseudopeptide **14** in CDCl_3 (left, C in mol.L⁻¹) and in $\text{DMSO-}d_6$ (right, C in mol.L⁻¹)

The influence of increasing amounts of $\text{DMSO-}d_6$ in CDCl_3 on the chemical shift of H_b and H_c in **14** was then studied at 10^{-2} M (Figure 5). Due to the overlap of signals the variations of δH_a could not be analyzed. As expected, while the chemical shifts δH_b and δH_c vary significantly upon increasing the $\text{DMSO-}d_6$ proportion in CDCl_3 , a plateau is observed on the curve for δH_b and δH_c for high proportions of $\text{DMSO-}d_6$, delineating the absence of intermolecular H-bonds in this solvent. From this observation, we could ensure that in pure $\text{DMSO-}d_6$, NMR studies at various temperatures could be performed to measure $\Delta\delta$ as a function of temperature, to give indications on *intramolecular* hydrogen bonds only.²⁶

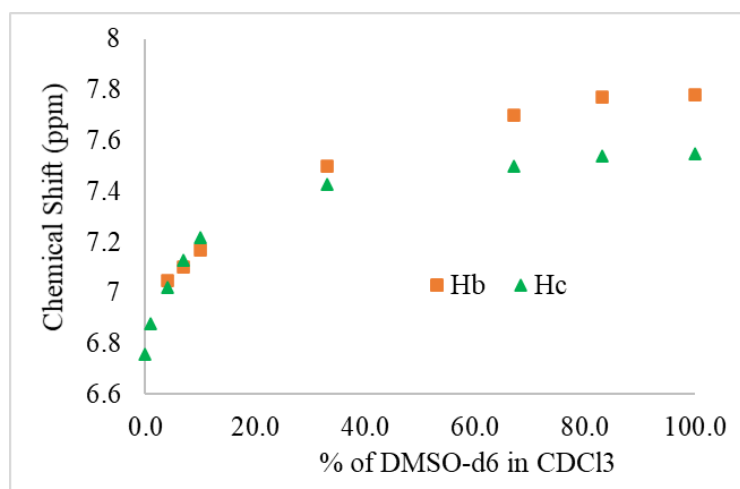


Figure 5. Influence of the percentage of DMSO- d_6 in $CDCl_3$ on the chemical shift δH_b and δH_c in pseudopeptide **14**

The temperature dependence of amide NH protons chemical shifts in 1H NMR spectra is a key data reflecting the existence of hydrogen bonds in small peptides and pseudopeptides in solution.^{7,10,25} It is generally considered that $\Delta\delta/\Delta T$ values below 2.6 ppb/K (in absolute value) are indicative of solvent-shielded NH protons participating in intramolecular H-bonds.²⁵ The variation of the NH proton chemical shifts with temperature was measured for compound **14** in DMSO- d_6 between 30 °C to 45 °C (Figure 6) at a concentration of 10^{-2} Mol.L $^{-1}$. As expected, a temperature coefficient $\Delta\delta/\Delta T$ of -2.48 ppb/K was measured for H_c while values of -6.28 ppb/K and -5.80 ppb/K were measured respectively for H_a and H_b . These data are in accordance with a β -turn conformation of pseudo tripeptide **14** resulting from an intramolecular H-bond involving H_c in solution, and suggest that this conformation remains stable even in a strongly dissociating solvent such as DMSO.

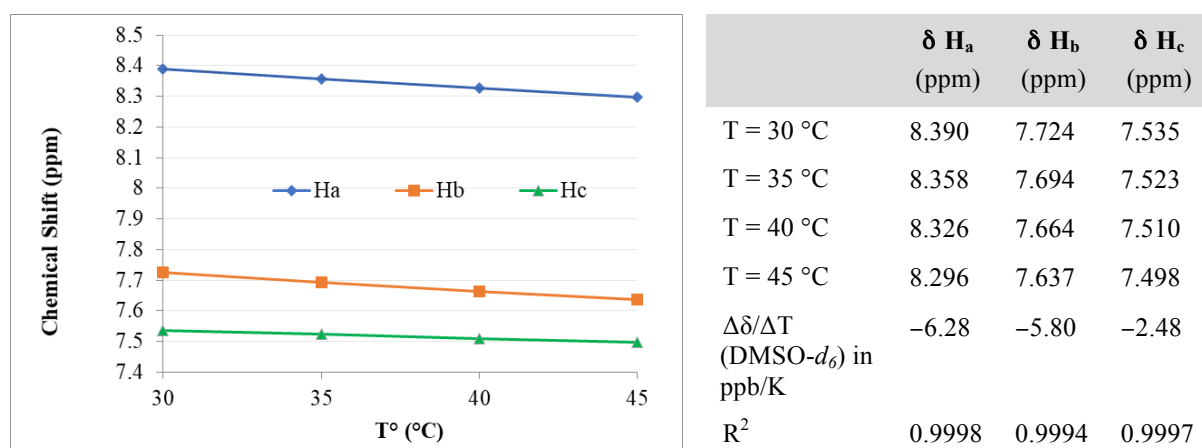


Figure 6. Influence of the temperature on the N-H chemical shifts in DMSO- d_6

Further conformational analysis was performed by measuring representative $^3J_{\text{H-H}}$ and $^3J_{13\text{C-1H}}$ coupling constants in DMSO- d_6 for compound **14** and through computational calculations (see Supporting Information for details). A search for the minimum energy conformers was performed at molecular mechanics (MMFF) level. The 200 most stable conformers identified from this conformational search were then optimized by increasing the level of theory (from MMFF to DFT) using Spartan 10.²⁷ Among the latter, the most stable conformers ($\Delta\Delta G_{298} < 12$ kJ/mol) were selected for final optimization at the B3LYP/6-31+G(d) level of theory using GAUSSIAN 09, with SMD solvent model for the description of DMSO effects to compare with NMR studies. The most stable conformer obtained from optimization of *cryst-14* is coined as *cryst-opt-14* in the following discussion. It differs very slightly from the experimental crystal structure *cryst-14*: its type I β -turn arrangement is conserved, and its calculated dipole moment (15.19 Debye) confirms the high polarity of this compound. The overlayed structures of *cryst-14* and *cryst-opt-14* are displayed in Figure 7.

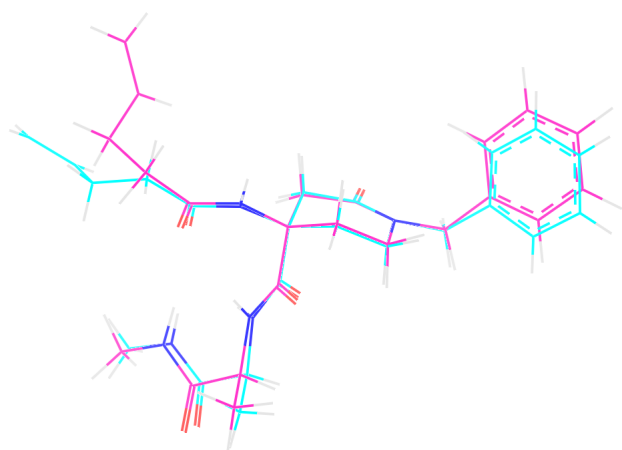


Figure 7. Overlayed structures of *cryst-14* (blue) and *cryst-opt-14* (pink), RMSD = 0.026 Å.

The relative stability of the six other conformers resulting from optimization of those previously selected was then compared to that of *cryst-opt-14*. The four most stable structures were found to exhibit type I β -turn characteristics and differed essentially by the orientation of the pent-4-enoyl side-chain and of the *N*-benzyl group. The lowest energy non- β -turn conformation found from this computational study is an inverse γ -turn exhibiting a $\Delta\Delta G_{298}$ higher by 7.6 kJ/mol compared to the most stable conformer (see Supporting Information). Among the 5 most stable conformers found ($\Delta\Delta G_{298} < 12$ kJ/mol) only one is a γ -turn and the Boltzmann distribution gives a 97:3 ratio

between the β -turn and γ -turn conformers. The pseudopeptide **14** is therefore expected to adopt a type-I β -turn conformation at room temperature, even in a strongly polar solvent such as DMSO.

The experimental $^3J_{\text{H-H}}$ and $^3J_{13\text{C-1H}}$ NMR coupling constants measured for pseudopeptide **14** in solution in DMSO- d_6 were compared to theoretical 3J calculated for conformations *cryst-14* and *cryst-opt-14* (Table 2). The calculated 3J values were obtained from torsional angles θ , using the Karplus equation ($^3J = A \cos^2\theta + B \cos\theta + C$).²⁸ The A, B, and C parameters used were those described respectively by Bystrov²⁹ (for $\theta_{\text{H-N-C}\alpha\text{-H}}$ torsional angles: A = 9.4, B = -1.1, C = 0.4) and by Schmidt³⁰ (for $\theta_{\text{H-N-C}\alpha\text{-C'}}$ torsional angles : A = 4.41, B = -1.36, C = 0.24 and for $\theta_{\text{H-N-C}\alpha\text{-C}\beta}$ torsional angles : A = 2.90, B = -0.56, C = 0.18).

The experimental $^3J_{\text{H-H}}$ coupling constant between H_b (δ 7.75 ppm) and H_d (δ 4.15 ppm) in the alaninamide residue was measured to be 7.7 Hz in DMSO- d_6 (entry 4). It is consistent with the coupling constant calculated by the Karplus formula (adapted to H-N-C α -H in peptides) for a dihedral angle of -147.3 degrees. Experimental 3J values shown in entries 5 and 8 of Table 2 ($^3J_{\text{exp}} < 1$ Hz) reveal that compound **14** in solution in DMSO- d_6 displays *anti* amide conformations, as also observed in the crystal structure.³¹ The good consistency between the experimental coupling constants measured in DMSO- d_6 and the calculated values based on structures *cryst-14* and *cryst-opt-14* (Table 2) fully confirms that the type I β -turn conformation adopted in the solid state is preserved in DMSO solution.

Entry	Torsional angle (degrees)			Calculated 3J (Hz)		Experimental 3J (Hz) ^e for 14
	θ	<i>cryst-14</i>	<i>cryst-opt-14</i>	<i>cryst-14</i> ^a	<i>cryst-opt-14</i> ^a	
1	H _a -N-C ₄ -C ₇	134.1	137.0	3.3 ^b	3.6 ^b	2.9
2	H _a -N-C ₄ -C ₃	103.1	99.7	0.5 ^c	0.4 ^c	$ ^3J < 1$
3	H _a -N-C ₄ -C ₅	14.1	17.3	2.4 ^c	2.3 ^c	2.0
4	H _b -N-C ₈ -H _d	-145.8	-147.3	7.7 ^d	8.0 ^d	7.7
5	H _b -N-C ₇ -C ₄	2.1	3.5	< 1	< 1	$ ^3J < 1$
6	H _b -N-C ₈ -C ₉	25.0	28.6	2.1 ^c	1.9 ^c	1.9
7	H _b -N-C ₈ -C ₁₀	99.3	96.0	0.6 ^b	0.4 ^b	$ ^3J < 1$
8	H _c -N-C ₁₀ -C ₈	10.2	5.2	< 1	< 1	$ ^3J < 1$

a) Calculated coupling constants from the Karplus equation ($A \cos^2\theta + B \cos\theta + C$); b) A = 4.41, B = -1.36, C = 0.24; c) A = 2.90, B = -0.56, C = 0.18; d) A = 9.4, B = -1.1, C = 0.4. e) Measured by ^1H NMR (400 MHz) in DMSO- d_6 (C = 10^{-2} mol.L⁻¹) at 20 °C.

Table 2. Comparison of the calculated coupling constants for crystalline and optimized structures of pseudopeptide **14** with those measured in DMSO- d_6

An evaluation of the mean distance between the most significant protons based on NOESY NMR experiment was also performed. The distances measured on *cryst-opt-14*³² and the calculated distances based on nOe intensities for compound **14** in solution are collected in Table 3. The distances were normalized on the distance between the two protons at C-3 position in the δ -valerolactam ring ($H_{3\alpha}$ and $H_{3\beta}$, see Figure 3) according to Jones *et al.*³³ The experimental values obtained from the NOESY experiment are in good agreement with the expected values based on the calculated conformer *cryst-opt-14* and confirm the stability of the β -turn in DMSO- d_6 .

Entry		Distance for <i>cryst-opt-14</i> ^a	Calculated distance from NOESY ^a	NOESY Intensity
1	$H_{3\alpha}$ - $H_{3\beta}$	1.76	-	100
2	H_a - $H_{5\alpha}$	2.30	2.88	5.21
3	H_a - $H_{3\alpha}$	2.87	2.01	45.64
4	H_a - H_b	2.93	2.77	6.68
5	H_b - H_c	2.67	2.78	6.46
6	H_b - $H_{5\beta}$	2.69	2.67	8.31

^a Distances in Å.

Table 3. Comparison of the H-H distances calculated from NOESY spectrum of compound **14** in DMSO- d_6 with the corresponding distances in the most stable conformer *cryst-opt-14* identified from DFT calculations

These results confirm both the type I β -turn structure of pseudopeptide **14** in solution, and its stability, the conformation observed in the X-ray crystal structure being preserved in polar DMSO solutions.

Conformational analysis of pseudopeptide **15**

With the aim to study the influence of the stereogenic center of the lactam ring on the conformation of pseudopeptides, a conformational study of **15** (epimer of **14**) was undertaken as previously done for **14**. Unfortunately, despite our efforts to obtain crystals of **15**, all attempts were unsuccessful in this case. Our study was therefore based on the computational conformational study and the NMR data collected from **15** (see Supporting Information). Again, the lowest energy conformer found for pseudopeptide **15** (conformer **A'** in Figure 8, see in Supplementary Table S6) was found to adopt a type-1 β -turn conformation. However, several inverse γ -turn conformers (7 among the 12 most stable conformers, $\Delta\Delta G_{298} < 12$ kJ/mol) were identified for **15**. The lowest energy γ -turn conformers (conformer **E'** in Figure 8, see in Supplementary Table S6) exhibits a relative free energy ($\Delta\Delta G_{298}$) 5.2 kJ/mol higher than that of

conformer **A'**. The lowest energy difference with the most stable β -turn combined with the presence of several γ -turns leads to a Boltzmann distribution less in favor of the β -turn (90:10 ratio between β -turns and γ -turns) than for **14** (β -turn/ γ -turn ratio of 97:3) suggesting a lower stability of the β -turn conformation for pseudopeptide **15**.

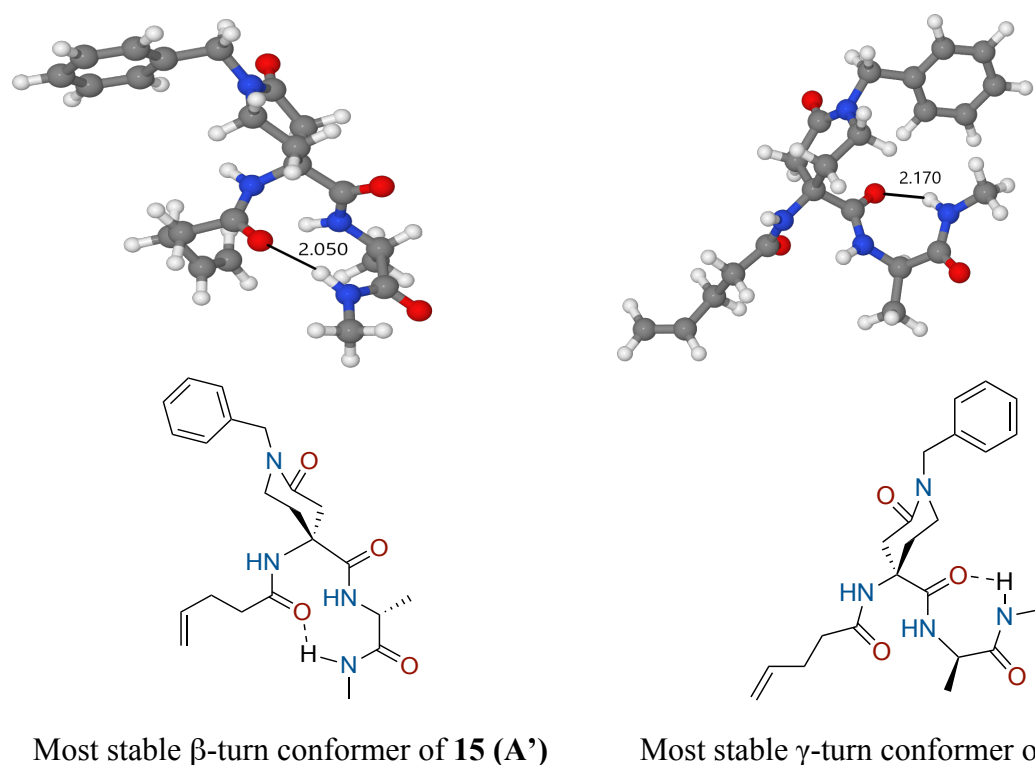


Figure 8. 3D structures obtained from molecular mechanics and DFT conformational calculations of the most stable β -turn (**A'**) and inverse γ -turn (**E'**) conformers of **15** (distances in Å)

As for **14**, in order to limit the intermolecular H-bond interactions, the NMR spectra of pseudopeptide **15** were recorded in DMSO- d_6 . The variation of NH proton chemical shifts with temperature $\Delta\delta/\Delta T$ was measured between 27 °C and 50 °C (Figure 9) at the same concentration of 1.10^{-2} Mol.L $^{-1}$. Again, it was found that only H_c displays a temperature coefficient smaller than 2.6 ppb/K in absolute value, suggesting its contribution in formation of a β -turn through intramolecular H-bonding. Noticeably, the most stable inverse γ -turn conformers also involve an intramolecular H-bond that could contribute to the low temperature coefficient observed for proton H_c.

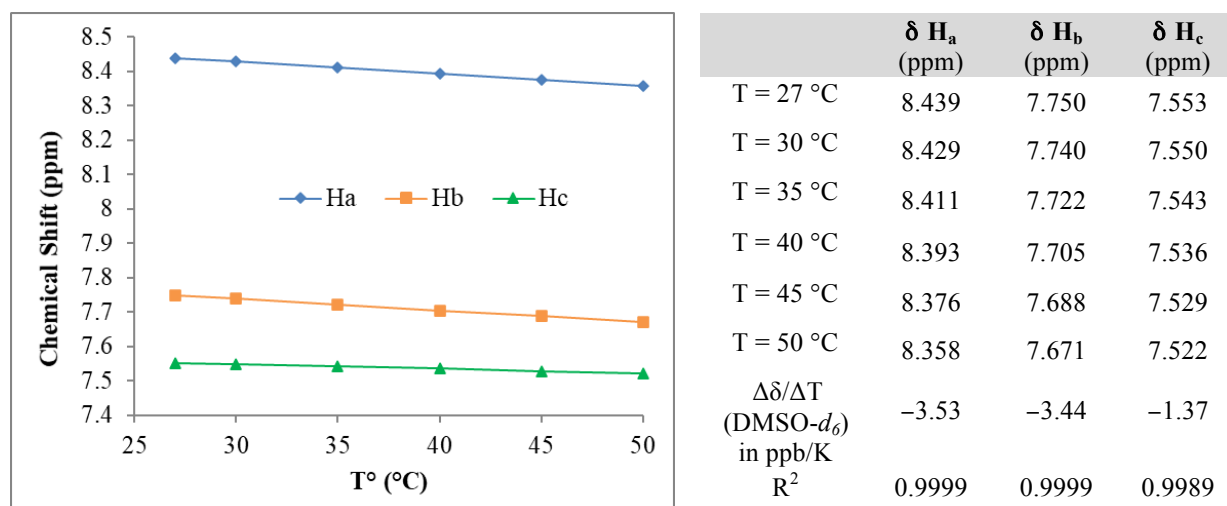


Figure 9. Influence of the temperature on N-H chemical shifts in DMSO- d_6

The conformation adopted in solution by the pseudopeptide **15** was then studied via the correlation of torsional angles of its calculated most stable conformer (conformer **A'**, see Figure 8), the resulting calculated coupling constants (via the previously described Karplus equations) and the experimental coupling constants measured from NMR spectra (Table 4). Some of the coupling constants measured are significantly different from the calculated values for the most stable conformer identified computationally (Table 4, entries 1, 3 and 6), suggesting a lack of conformational stability of the pseudopeptide **15** compared to **14** and the coexistence of various conformers in solution, that may explain the difficulties encountered in growing crystals of **15**.

Entry	Torsional angle (degrees)		Calculated 3J (Hz)	Experimental 3J (Hz) ^e
	θ	15 (A' conformer)	15 (A' conformer) ^a	15 (in DMSO- <i>d</i> ₆)
1	H _a -N-C ₄ -C ₇	-132.5	3.1 ^b	1.5
2	H _a -N-C ₄ -C ₃	-14.3	2.4 ^c	1.7
3	H _a -N-C ₄ -C ₅	103.1	0.5 ^c	2.4
4	H _b -N-C ₈ -H _d	147.1	8.0 ^d	7.6
5	H _b -N-C ₇ -C ₄	3.6	< 1	$ ^3J < 1$
6	H _b -N-C ₈ -C ₉	28.3	1.9 ^c	$ ^3J < 1$
7	H _b -N-C ₈ -C ₁₀	-96.3	0.4 ^b	$ ^3J < 1$
8	H _c -N-C ₁₀ -C ₈	-4.4	< 1	$ ^3J < 1$
9	H _a -N-C _{1'} -C _{2'}	1.7	< 1	$ ^3J < 1$

a) Calculated coupling constants from the Karplus equation ($A \cos^2\theta + B \cos \theta + C$); b) $A = 4.41$, $B = -1.36$, $C = 0.24$; c) $A = 2.90$, $B = -0.56$, $C = 0.18$; d) $A = 9.4$, $B = -1.1$, $C = 0.4$. e) Measured by ^1H NMR (400 MHz) in DMSO-*d*₆ ($C = 10^{-2} \text{ mol.L}^{-1}$) at 20 °C.

Table 4. Comparison of the calculated coupling constants for pseudopeptide **15** (conformer A') with those measured by ^1H NMR in DMSO-*d*₆

The distances calculated from the NOESY experimental data obtained in DMSO-*d*₆ were compared to the corresponding distances measured on the most stable calculated conformer of **15** (conformer A', Table 5). The two sets of data fit very well except for H_c for which the distances with H₉ and H₈ appear shorter experimentally when compared to the values obtained from computational calculations (entries 7 and 8, Table 5). This is most likely due to the weaker stability of the β -turn in **15** compared to **14**, and to a contribution of some other conformers to the NOESY intensity, such as γ -turn conformers (rotation around the C₈-C₁₀ bond). These comparative results suggest a role played by the stereogenic center of the lactam in secondary structure stabilization.

Entry		Distance for 15 ^a (A' conformer)	Calculated distance from NOESY ^a	NOESY Intensity
1	H _{3α} -H _{3β}	1.77	-	100
2	H _a -H _{3α}	2.41	2.51	12.34
3	H _b -H ₈	2.93	2.70	7.86
4	H _a -H _b	2.88	2.87	5.44
5	H _b -H _c	2.63	2.81	6.30
6	H _b -H ₉	2.47	2.49	14.10
7	H _c -H ₉	3.62^b	2.90^b	5.12
8	H _c -H ₈	3.37^b	2.34^b	18.61
9	H _c -H ₁₁	2.28	2.23	24.81

^a Distances in Å. ^b Values in bold exhibit the main differences between the calculated and experimental values.

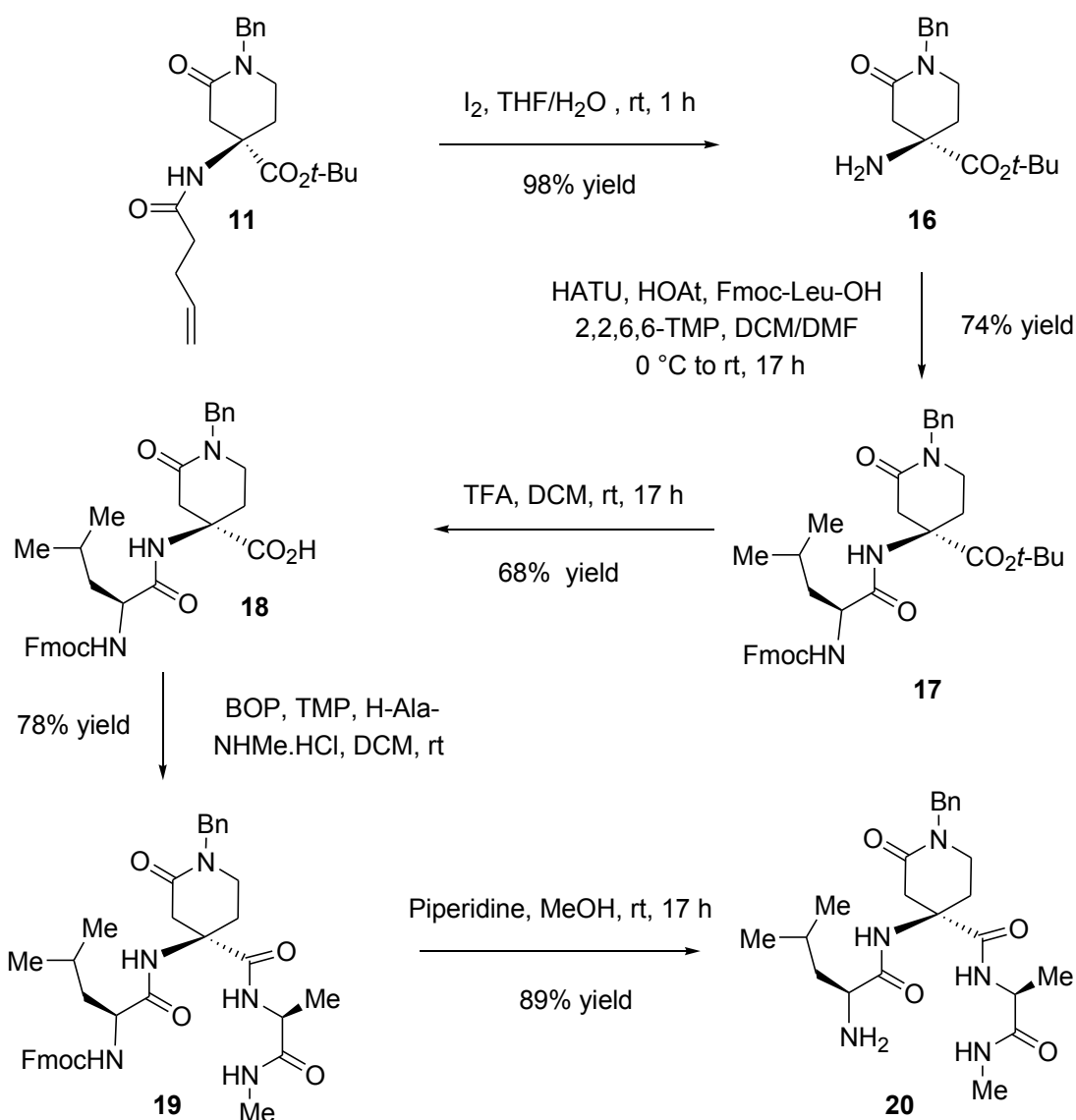
Table 5. Comparison of the H-H distances calculated from NOESY spectrum of compound **15** in DMSO-*d*₆ with those in the most stable β-turn conformer **15** identified from DFT calculations

Incorporation of the (*R*)-δ-valerolactamic cDAA **11** into a short peptidic sequence and *N*-deprotection of the lactam

In order to evidence the potential of the new β-turn inducing agent described herein in peptidomimetic chemistry, it was necessary to demonstrate their ability to be incorporated into pseudopeptidic sequences. For this purpose, our first assays aimed at transforming **14** into a representative tripeptide by sequential *N*-deprotection and elongation. However, the treatment of compound **14** with iodine in THF and water under the conditions reported by Fraser-Reid¹⁶ only led to complex mixtures. This inability of **14** to be cleanly *N*-deprotected is probably due to the competitive reactivity of nucleophilic sites in the alaninamide appendage, limiting the desired *O*-attack on the transient iodonium to form 5-iodomethyldihydrofuranone upon hydrolysis of the corresponding iminium. To circumvent this difficulty, we next considered an inversion of the coupling sequence, i.e. first coupling the δ-valerolactamic cDAA with an amino acid from its *N*-terminus, then performing *C*-elongation.

Toward this end, *N*-deprotection of lactam (*R*)-**11** was first performed under the conditions reported by Fraser-Reid (I₂, THF/H₂O, rt), smoothly affording the free amino ester **16** in 98% yield (Scheme 5). The critical coupling reaction involving this sterically hindered amine with Fmoc-protected leucine was successfully accomplished using DMF as cosolvent.^{12a} Subsequent cleavage of the *tert*-butyl ester **17** using TFA led to the corresponding acid **18** in 68% yield. Using BOP coupling reagent and triethylamine as the base, final coupling of **18** with (L)-*N*-Me-alaninamide in excess resulted in an inseparable mixture of residual *N*-Me-alaninamide and *N*-

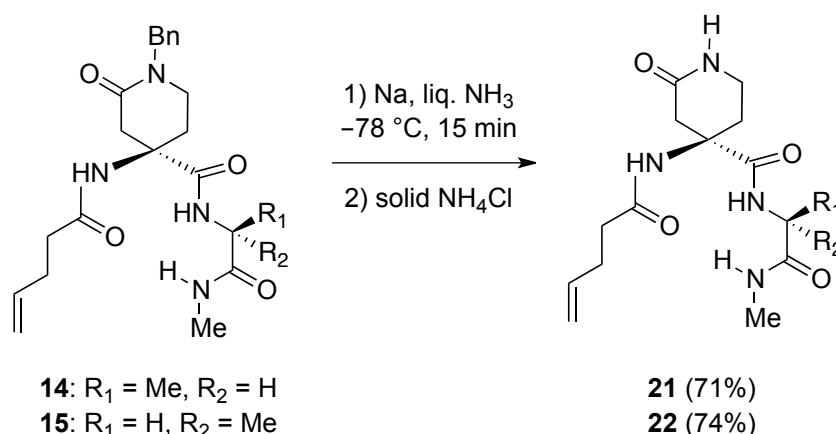
deprotected pseudopeptide **20** in a 3:1 ratio, the Fmoc deprotection occurring concomitantly under this coupling reaction. However, replacing triethylamine by freshly distilled 2,2,6,6-tetramethylpiperidine under the same conditions allowed the coupling to proceed without any *N*-Fmoc deprotection and afforded the target δ -valerolactamic cDAA-containing peptide **19** in good yield and purity. *N*-Fmoc deprotection could be performed at the next stage using freshly distilled piperidine in methanol, to afford pseudopeptide **20** in good yield and purity.³⁴



Scheme 5. Incorporation of the δ -valerolactamic cDAA into a model pseudopeptide **20**

Finally, *N*-debenzylation of the pseudopeptides **14** and **15** was performed with sodium in liquid ammonia at -78 °C (20 mg scale) to provide the corresponding pseudopeptides **21** and **22** in good yields and high purity (Scheme 6), provided that the final quench of the reaction mixture was performed with solid ammonium chloride. This modification of the standard procedure (quench

with saturated aqueous solution of ammonium chloride and subsequent extraction) was found to increase significantly both the crude and isolated yields, due to the high polarity of the debenzylated products. The fair access to these *N*-deprotected derivatives gives the opportunity to create specific secondary H-bond interactions between CONH groups of the lactam and of the peptide chain,³⁵ or with the substrates and/or reagents of peptide-catalyzed chemical transformations.³⁶



Scheme 6. *N*-deprotection on the lactam ring: access to pseudopeptides **21** and **22**

CONCLUSION

In conclusion, we have accomplished the stereoselective synthesis of new chiral protected pseudoenantiomeric cDAAs (*R*)-**11** and (*S*)-**12** containing a δ -valerolactam side chain, from isoxazolidine **2** as a common intermediate. The synthesis involves reductive amination of aldehydes **3c** and **8b** followed by regioselective cyclization forming the δ -valerolactam ring. When included into a pseudopeptide **14**, the cDAA building block (*R*)-**11** proved to induce a stable type I β -turn both in solid state and in solution (even in DMSO) as evidenced by X-ray diffraction, computational conformational analysis and NMR studies. Interestingly, an influence of the relative configuration of pseudopeptides containing (*R*)-**11** on their secondary structure was observed, suggesting a significant role of the absolute configuration of the cDAA on its conformation-inducing properties. We also demonstrated that (*R*)-**11** can be readily introduced in peptidic sequences by achieving amine then carboxylic acid deprotection and sequential coupling on both functions, allowing applications in the design of original structured peptidomimetics. Furthermore, debenzylation of the lactam nitrogen atom gives access to cDAAs containing a free NH group in the side chain that could participate in oriented and specific non-covalent bonding with residues of surrounding peptide or protein in peptide-protein interactions.

EXPERIMENTAL SECTION

General Methods. Reagents (including L- and D-alanine methylamide hydrochlorides) were purchased from commercial suppliers and used without additional purification. Dichloromethane and THF were dried on a column of activated alumina in a DRY STATION Glass Technology GTS 100 glassware. Triethylamine and DMF were dried through distillation over CaH₂. All reactions were conducted in oven dried glassware under inert atmosphere and were monitored by TLC. Column chromatography was performed using 60 μ m silica gel. Thin-layer chromatography was performed with G/UV254 plates, and the products were observed under UV light or with KMnO₄ stain. All melting points were recorded on a ThermoFisher IA9300 apparatus and are uncorrected. Optical rotation measurements were performed in a 10 cm glass cell using a digital polarimeter equipped with a Peltier thermostated cell holder. NMR (400 MHz for ¹H and 100 MHz for ¹³C) spectra were recorded in CDCl₃, CD₃OD or DMSO-*d*₆. Chemical shifts are presented in parts per million with, as internal reference, Me₄Si (δ 0.00 ppm) for ¹H NMR and CDCl₃ (δ 77.0 ppm), CD₃OD (δ 49.1 ppm) or DMSO-*d*₆ (δ 39.4 ppm) for ¹³C{¹H} NMR. Coupling constants are given in Hertz. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet; br s, broad signal. Proton and carbon assignments were established using COSY, HSQC, HMBC and DEPT experiments. Infrared spectra were recorded on an FT spectrophotometer from neat compounds using an ATR (Attenuated Total Reflexion) module. The wavenumbers of maximum absorption peaks of IR spectroscopy are presented in cm⁻¹. High-resolution mass spectra were performed on a GC TOF mass spectrometer; the method used for HRMS is mentioned for each spectrum.

(3R,5S)-3-tert-Butoxycarbonylmethyl-5-ethoxy-isoxazolidine-3-carboxylic acid tert-butyl ester (2). In the first step of an optimized version of the previously reported procedure¹⁵ for the synthesis of **2**, 4.47 g of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranosyl oxime (17.2 mmol) and 3.68 g of di-*tert*-butyl acetylenedicarboxylate (17.3 mmol, 1 equiv.) were reacted to obtain 8.17 g of intermediate nitrone **1** (16.3 mmol, 95 % yield) sufficiently pure to be engaged in the following step without further purification.³⁷ In a second step, 23 g of nitrone **1** (45.9 mmol) were reacted with ethyl vinyl ether (450 mmol) to obtain 16.4 g of enantiopure and diastereopure cycloadduct **s1** (28.6 mmol, 62 % yield) after recrystallization in ethanol. The enantiopurity of compound **2** was previously measured after chemical derivatization (ee > 98%). All the compounds obtained from this pure and non racemizable adduct were assumed to display the same enantiopurity. In a

modified version of the third step, the crystalline cycloadduct **s1** (4.59 g, 8.0 mmol) was dissolved in a 3:1 mixture of methanol and water (160 mL, 0.05 M solution) and treated with hydroxylamine hydrochloride (2.46 g, 48.3 mmol, 6 equiv.) and sodium acetate (2.06 g, 29.4 mmol, 3.7 equiv.) at 65 °C for 18 h. At the end of the reaction a saturated aqueous solution of NaHCO₃ (30 mL) was added until pH reached 9, then the aqueous phase was extracted three times with CH₂Cl₂ (3x50 mL). The organic layers were washed with brine (20 mL), dried over MgSO₄ and concentrated under vacuum. The residue was triturated with pentane three times and the pentane phase was concentrated under vacuum to afford isoxazolidine **2** (2.68 g, 8.0 mmol, 100 % yield), which was pure enough to be used without further purification. Chiral auxiliary (2,3:5,6-di-*O*-isopropylidene-D-mannofuranosyl oxime) was recovered as the solid residue (1.55 g, 5.63 mmol, 70 % yield) and could be used again. All analyses were identical to those of previously described compound **2**.¹⁵

(3*R*,5*S*)-3-*tert*-Butoxycarbonylmethyl-5-ethoxy-2-pent-4-enoyl-isoxazolidine-3-carboxylic acid *tert*-butyl ester (**6c**). A mixture of **2** (2.68 g, 8.0 mmol) in 4-pentenoic anhydride (2.8 mL, 15 mmol, 2 equiv.) was stirred at room temperature for 4 days. After short-pass distillation of the excess anhydride (130 °C, 0.6 bar), the residue was purified by flash chromatography on silica gel (cyclohexane:EtOAc from 100:0 to 50:50) to afford the *N*-protected isoxazolidine **6c** as a liquid (3.11 g, 7.5 mmol, 94 % yield). *R*_f 0.49 (cyclohexane:EtOAc 80:20); [α]_D²⁰ + 58.5 (*c* 0.50, CH₂Cl₂); IR (neat) ν 2981, 2929, 1734, 1290, 1421, 1372, 1153, 1115 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.23 (3H, t, *J* = 7.1 Hz), 1.44 (9H, s), 1.46 (9H, s), 2.33-2.39 (2H, m), 2.45-2.49 (2H, m), 2.71 (1H, d, *J* = 16.5 Hz), 2.86 (1H, dd, *J* = 13.5, 5.1 Hz), 2.99 (1H, dd, *J* = 13.9, 1.0 Hz), 3.53-3.61 (1H, m), 3.57 (1H, d, *J* = 16.5 Hz), 3.74-3.81 (1H, dq, *J* = 9.6, 7.1 Hz), 4.98 (1H, d, *J* = 9.6 Hz), 5.05 (1H, dd, *J* = 17.2, 1.4 Hz), 5.30 (1H, dd, *J* = 5.0, 1.0 Hz), 5.85 (1H, ddt, *J* = 16.8, 10.2, 6.4 Hz); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 15.1, 27.9 (3C), 28.2 (3C), 28.3, 33.4, 39.1, 44.5, 64.3, 65.9, 80.6, 82.5, 101.8, 115.1, 137.3, 169.2, 169.8, 192.0; HRMS (DCI⁺) *m/z*: [M+H]⁺ Calcd for C₂₁H₃₆NO₇ 414.2486; Found 414.2495.

(2*R*)-2-(2-Oxo-ethyl)-2-pent-4-enoylamino-succinic acid di-*tert*-butyl ester (**3c**). To a solution of isoxazolidine **6c** (1.24 g, 3 mmol) in THF (20 mL) was added a solution of SmI₂ in THF (0.1 M, 80 mL, 3 equiv.) under argon, at room temperature, until persistence of the typical deep blue color. When the starting material was completely converted (i.e. 5 min) aqueous solutions of saturated NaHCO₃ (1 mL) and Na₂S₂O₃ (10 mL) were added. The aqueous phase was extracted three times with EtOAc (3x100 mL). The combined organic phases were dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (diethyl ether:petrol ether 1:1) to afford aldehyde **3c** as a thick oil (1.08 g, 2.85 mmol, 95 % yield).

R_f 0.48 (cyclohexane:EtOAc 70:30); $[\alpha]_D^{20} - 11.3$ (c 0.20, CH_2Cl_2); IR (neat) ν 3402, 2978, 2933, 1725, 1673, 1504, 1367, 1238, 1148 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.41 (9H, s), 1.47 (9H, s), 2.25-2.30 (2H, m), 2.32-2.37 (2H, m), 2.71 (1H, d, $J = 16.0$ Hz), 2.81 (1H, dd, $J = 16.6$, 1.7 Hz), 3.47 (1H, d, $J = 16.0$ Hz), 3.64 (1H, dd, $J = 16.6$, 1.9 Hz), 4.98 (1H, dd, $J = 10.2$, 1.5 Hz), 5.04 (1H, dd, $J = 17.1$, 1.6 Hz), 5.78 (1H, ddt, $J = 17.0$, 10.3, 6.4 Hz), 6.89 (1H, s), 9.63 (1H, t, $J = 1.8$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 27.7 (3C), 28.0 (3C), 29.3, 36.2, 40.6, 48.1, 58.3, 81.5, 83.6, 115.6, 136.6, 168.8, 170.3, 171.9, 198.5; HRMS (DCI^+) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{19}\text{H}_{31}\text{NNaO}_6$ 392.2044; Found 392.2032.

(3S)-3-tert-Butoxycarbonyl-3-(2,2,2-trifluoroacetyl-amino)-pentanedioic acid monoethyl ester (7b). Compound **4b** (2.26 g, 5.29 mmol) was dissolved in a mixture of TFA/ CH_2Cl_2 (10:1, 100 mL) at 0 °C, and then 5h. The reaction mixture was evaporated over silica and was purified by flash chromatography (solid deposit) on silica gel (cyclohexane:EtOAc from 90:10 to 50:50) to recover pure starting material **4b** (9.29 mg, 2.17 mmol, 40 %) and afford pure monoacid **7b** as a colorless liquid (983 mg, 2.65 mmol, 51 % yield, 85 % corrected yield). $[\alpha]_D^{20} - 38.5$ (c 0.11, CHCl_3); IR (neat) ν 3368, 2983, 2934, 1726, 1527, 1213, 1150, 892, 734 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.23 (3H, t, $J = 7.1$ Hz), 1.49 (9H, s), 2.87 (1H, d, $J = 15.4$ Hz), 2.97 (1H, d, $J = 16.1$ Hz), 3.49 (1H, d, $J = 15.4$ Hz), 3.56 (1H, d, $J = 16.1$ Hz), 4.13 (2H, qd, $J = 7.1$, 0.9 Hz), 7.77 (1H, s), 8.81 (1H, br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 14.0, 27.5 (3C), 38.9, 39.4, 59.1, 61.3, 85.0, 115.3 (q, $J = 289$ Hz), 156.4 (q, $J = 38$ Hz), 168.4, 168.6, 174.2; ^{19}F NMR (376 MHz, CDCl_3) δ -76.42; HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{14}\text{H}_{20}\text{F}_3\text{NNaO}_7$ 394.1090; Found 394.1084.

2S)-2-(2-Oxo-ethyl)-2-(2,2,2-trifluoro-acetyl-amino)-succinic acid 1-tert-butyl ester 4-ethyl ester (8b). To a solution of compound **7b** (1.43 g, 3.86 mmol) in 5 mL of THF was added, in two times at room temperature, a solution of $\text{BH}_3\cdot\text{SMe}_2$ in THF (2 mL, 2 M, 4.0 mmol, 3.1 equiv.) under argon and then stirred until no more gas release was observed. The reaction mixture was then refluxed for 1 h. After removing the solvent, the crude alcohol **7'b** was used without further purification (786 mg, 2.20 mmol, 57 % crude yield). ^1H NMR (400 MHz, CDCl_3) δ 1.21 (3H, t, $J = 7.1$ Hz), 1.48 (9H, s), 1.90 (1H, br s), 2.07 (1H, ddd, $J = 14.4$, 7.6, 5.7 Hz), 2.60 (1H, dt, $J = 14.6$, 5.0 Hz), 2.93 (1H, d, $J = 16.3$ Hz), 3.46 (1H, d, $J = 16.3$ Hz), 3.64 (2H, m), 4.09 (2H, q, $J = 7.1$ Hz), 7.85 (1H, s); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 14.0, 27.6 (3C), 37.0, 39.6, 57.9, 60.7, 60.9, 84.2, 115.4 (q, $J = 288$ Hz), 156.2 (q, $J = 37$ Hz), 169.5, 170.5; ^{19}F NMR (376 MHz, CDCl_3) δ -76.32; HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{14}\text{H}_{22}\text{F}_3\text{NNaO}_6$ 380.1297; Found 380.1291.

To the solution of crude alcohol **7'b** (obtained from 3.86 mmol of **7b**) in 120 mL of CH₂Cl₂ was added DMP (2.46 g, 5.79 mmol, 1.5 equiv.) and NaHCO₃ (2.46 g, 29.29 mmol, 7.5 equiv.). The solution was stirred 2 h at 0 °C. At the end of the reaction, a saturated aqueous solution of Na₂S₂O₃ (250 mL) and NaHCO₃ (250 mL) were added and then extracted three times with diethyl ether. The organic layers were washed with brine (100 mL), dried over MgSO₄ and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 90:10 to 0:100) to afford pure aldehyde **8b** as a yellow oil (259 mg, 0.73 mmol, 19% over 2 steps starting from monoacid **7b**). *R_f* 0.50 (cyclohexane/AcOEt 80:20); [α]_D²⁰ + 6.38 (*c* 0.01, CHCl₃); IR (neat) ν 3371, 2982, 1727, 1527, 1211, 1150, 840, 734 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.24 (3H, t, *J* = 7.1 Hz), 1.49 (9H, s), 2.83 (1H, d, *J* = 15.2 Hz), 3.03 (1H, dd, *J* = 17.8, 0.9 Hz), 3.43 (1H, d, *J* = 15.2 Hz), 3.71 (1H, dd, *J* = 17.8, 1.3 Hz), 4.12 (2H, q, *J* = 7.1 Hz), 7.83 (1H, s), 9.62 (1H, t, *J* = 1.0 Hz); ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 13.9, 27.5 (3C), 39.4, 47.4, 58.5, 61.3, 84.9, 115.2 (q, *J* = 288 Hz), 156.3 (q, *J* = 38 Hz), 168.5, 168.7, 196.8; ¹⁹F NMR (376 MHz, CDCl₃) δ -76.38; HRMS (ESI-TOF) *m/z*: [M+Na+MeOH]⁺ Calcd for C₁₄H₂₄F₃NNaO₇ 410.1403; Found 410.1410.

Reductive amination: Protocol a. To a solution of aldehyde **3c** in MeOH (13 mL/mmol) were added the amine (2 equiv.), a NaBH₃CN solution (1 M in THF, 2 equiv.) and AcOH (2.5 equiv.). The mixture was stirred overnight at room temperature. The reaction mixture was then concentrated under vacuum and the residue was dissolved in EtOAc (30 mL/mmol). The organic phase was washed with a saturated solution of NaHCO₃ (15 mL/mmol). The aqueous phase was washed with additional EtOAc (2x15 mL/mmol). All organic phases were gathered and dried over Na₂SO₄ and concentrated under vacuum to afford crude amine **9-10** which was purified by flash chromatography on silica gel.

Reductive amination: Protocol b. To a solution of aldehyde **3c** in CH₂Cl₂ (10 mL/mmol) were added the amine (1 equiv.) and an excess of MgSO₄ (15 equiv.). The mixture was stirred overnight at room temperature. NaBH(OAc)₃ (4 equiv.) and glacial AcOH (4 equiv.) were then successively added to the reaction mixture which was stirred for an additional 3.5 h at room temperature. The reaction mixture was then diluted with CH₂Cl₂ (25 mL/mmol), quenched with water (5 mL/mmol) at 0°C and stirred at room temperature for 10 minutes. The aqueous layer was extracted with CH₂Cl₂ (3x50 mL/mmol) three times and the combined organic layers were dried over MgSO₄, filtered and concentrated under vacuum to afford crude amine **9** which was purified by flash chromatography on silica gel.

(2R)-2-(2-Benzylamino-ethyl)-2-pent-4-enoylamino-succinic acid di-tert-butyl ester (9c). Following **protocol b** and starting from aldehyde **3c** (65 mg, 0.18 mmol) and from benzylamine (20 μ L, 0.18 mmol), purification by flash chromatography on silica gel (CH₂Cl₂/MeOH from 100:0 to 90:10) afforded amine **9c** as a yellow oil (58 mg, 0.13 mmol, 70 % yield). *R_f* 0.25 (CH₂Cl₂/MeOH 95:5); [α]_D²⁰ + 2.24 (*c* 0.25, CHCl₃); IR (neat) ν 3292, 2977, 2929, 1728, 1631, 1538, 1496, 1367, 1300, 1252, 1152, 1109 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.40 (9H, s), 1.43 (9H, s), 1.80-1.87 (1H, m), 2.20-2.34 (4H, m), 2.43-2.49 (1H, m), 2.58-2.62 (1H, m), 2.64-2.72 (1H, m), 2.78 (1H, d, *J* = 16.8 Hz), 3.51 (1H, d, *J* = 16.9 Hz), 3.70 and 3.75 (2H, 2d, *J* = 13.2 Hz), 4.96 (1H, dd, *J* = 10.3, 1.5 Hz), 5.03 (1H, dd, *J* = 17.1, 1.6 Hz), 5.78 (1H, ddt, *J* = 17.0, 10.3, 6.3 Hz), 6.92 (1H, br s), 7.21-7.34 (5H, m); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 27.8 (3C), 28.1 (3C), 29.5, 32.9, 36.3, 40.6, 42.6, 52.0, 60.1, 81.1, 83.2, 115.6, 128.1, 128.8 (2C), 129.2 (2C), 136.7, 136.9, 169.7, 171.4, 171.9; HRMS (DCI⁺) *m/z*: [M+H]⁺ Calcd for C₂₆H₄₁N₂O₅ 461.3015; Found 461.3016.

(2R)-2-Pent-4-enoylamino-2-[2-((1R)-1-phenyl-ethylamino)-ethyl]-succinic acid di-tert-butyl ester (10c). Following **protocol a** and starting from aldehyde **3c** (55 mg, 0.15 mmol) and from (*R*)-1-phenyl-ethylamine (40 μ L, 0.30 mmol), purification by flash chromatography on silica gel (CH₂Cl₂:MeOH 95:5) afforded amine **10c** as an oil (55 mg, 0.12 mmol, 77 % yield). *R_f* 0.35 (CH₂Cl₂/MeOH 95:5); ¹H NMR (400 MHz, CDCl₃) δ 1.31 (3H, d, *J* = 6.5 Hz), 1.39 (9H, s), 1.40 (9H, s), 1.88 (1H, m), 2.16-2.20 (2H, m), 2.22-2.31 (2H, m), 2.32-2.47 (2H, m), 2.58 (1H, m), 2.76 and 3.48 (2H, 2d, *J* = 17.0 Hz), 3.73 (1H, q, *J* = 6.5 Hz), 4.97 (1H, d, *J* = 10.1 Hz), 5.01 (1H, dd, *J* = 17.1, 1.4 Hz), 5.75 (1H, ddt, *J* = 17.0, 10.4, 6.2 Hz), 6.85 (1H, br s), 7.19-7.32 (5H, m); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 24.1, 27.7 (3C), 28.1 (3C), 29.5, 35.1, 36.4, 40.9, 42.2, 58.0, 60.4, 80.8, 82.7, 115.4, 126.5, 127.0, 128.5, 136.9, 144.9, 169.8, 171.5, 171.8; HRMS (DCI⁺) *m/z*: [M+H]⁺ Calcd for C₂₇H₄₃N₂O₅ 475.3172; Found 475.3183.

(4R)-1-Benzyl-2-oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid tert-butyl ester (11). Compound **9c** (156 mg, 0.34 mmol) was refluxed in toluene (7 mL) for 17 h. After evaporation of toluene, the residue was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 95:5) to afford pure lactam **11** as a white solid (71 mg, 0.18 mmol, 54 % yield) and amidine **11'** as an oil (34 mg, 0.08 mmol, 23 % yield). Alternatively, compound **11** was obtained from amine **9c** (1.04 g, 2.74 mmol) by standing in pentane/Et₂O for 2 weeks at 4 °C (850 mg, 2.19 mmol, 80 % yield). Mp 140.0-144.4 °C (white needles from diethyl ether/pentane); [α]_D²⁰ - 30.9 (*c* 1.40, CHCl₃); IR (neat) ν 3347, 2972, 2927, 2853, 1711, 1666, 1636, 1526, 1482, 1450, 1245, 1220, 1158, 1050 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.43 (9H, s), 2.11 (1H, ddd, *J* = 14.0, 8.6, 6.3 Hz), 2.22-2.26 (2H,

m), 2.30-2.35 (2H, m), 2.55 (1H, dtd, $J = 13.9, 4.7, 2.0$ Hz), 2.70 (1H, dd, $J = 17.3, 1.9$ Hz), 2.87 (1H, d, $J = 17.3$ Hz), 3.21 (2H, m), 4.55 and 4.62 (2H, 2d, $J = 14.6$ Hz), 4.97 (1H, dtd, $J = 10.2, 1.6, 1.2$ Hz), 5.03 (1H, dtd, $J = 17.1, 1.6, 1.6$ Hz), 5.76 (1H, dtd, $J = 17.1, 10.2, 6.1$ Hz), 6.00 (1H, br s), 7.23-7.35 (5H, m); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 27.7 (3C), 28.2, 29.4, 35.3, 40.6, 43.1, 49.8, 58.0, 82.1, 115.4, 127.6, 128.0 (2C), 128.6 (2C), 136.4, 136.9, 166.9, 170.6, 172.5; HRMS (DCI^+) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{22}\text{H}_{30}\text{N}_2\text{NaO}_4$ 409.2098; Found 409.2098.

(4R)-1-Benzyl-2-but-3-enyl-4-tert-butoxycarbonylmethyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid tert-butyl ester (11'). $[\alpha]_{\text{D}}^{20} +19.0$ (c 0.03, CHCl_3); IR (neat) ν 3170, 3037, 2974, 2920, 1729, 1618, 1572, 1378, 1234, 1134, 1101, 719, 697 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.47 (9H, s), 1.48 (9H, s), 2.13 (1H, dt, $J = 13.9, 3.0$ Hz), 2.45-2.65 (2H, m), 2.67-2.79 (1H, m), 2.83-2.94 (1H, m), 3.00 (1H, d, $J = 17.2$ Hz), 3.22-3.31 (1H, m), 3.36 (1H, d, $J = 17.2$ Hz), 3.36-3.47 (1H, m), 3.52-3.60 (1H, m), 4.79 and 4.98 (2H, 2d, $J = 16.5$ Hz), 5.15 (1H, dtd, $J = 10.2, 1.0, 1.0$ Hz), 5.22 (1H, dtd, $J = 17.1, 1.2, 1.2$ Hz), 5.88 (1H, dtd, $J = 17.1, 10.2, 6.6$ Hz), 7.25-7.45 (5H, m), 8.65 (1H, br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 27.3, 27.7 (3C), 28.1 (3C), 29.4, 31.5, 41.6, 44.5, 56.4, 58.2, 83.0, 84.2, 118.3, 126.9 (2C), 128.8, 129.5 (2C), 132.7, 133.9, 163.9, 169.2, 170.5; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_4$ 443.2910; Found 443.2904.

(4S)-1-Benzyl-2-oxo-4-(2,2,2-trifluoro-acetyl-amino)-piperidine-4-carboxylic acid tert-butyl ester (12). Compound **12** was obtained from aldehyde **8b** (104 mg, 0.29 mmol), following **protocol b** (see reductive amination) and then after standing without solvent for 2 weeks at 4 °C. The residue was purified by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$: from 100:0 to 90:10) to afford pure lactam **12** as a colorless oil (89 mg, 0.22 mmol, 77 % yield). R_f 0.55 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90:10); $[\alpha]_{\text{D}}^{20} +9.31$ (c 0.01, CHCl_3); IR (neat) ν 3351, 2957, 2917, 2873, 1723, 1703, 1562, 1204, 1155, 726, 646 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.43 (9H, s), 2.17 (1H, dt, $J = 14.1, 8.0$ Hz), 2.57 (1H, dt, $J = 13.9, 4.2$ Hz), 2.86 (2H, s), 3.21 (2H, dd, $J = 8.0, 4.5$ Hz), 4.25 and 4.80 (2H, 2d, $J = 14.6$ Hz), 7.18-7.35 (5H, m), 8.49 (1H, s); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 27.6 (3C), 39.5, 42.6, 49.7, 58.9, 83.3, 115.4 (q, $J = 288$ Hz), 127.7, 127.9 (2C), 128.7 (2C), 136.0, 157.2 (q, $J = 38$ Hz), 166.3, 169.1; ^{19}F NMR (376 MHz, CDCl_3) δ -75.48; HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{19}\text{H}_{23}\text{F}_3\text{N}_2\text{NaO}_4$ 423.1508; Found 423.1492.

(4R)-1-Benzyl-2-oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid (13). Compound **11** (51 mg, 0.132 mmol) was dissolved in a 50% mixture of TFA (1.3 mL) in CH_2Cl_2 (2.6 mL) at 0 °C, and the reaction mixture was stirred until complete disappearance of the starting material (17 h). At the end of the reaction, brine (5 mL) was added to the reaction mixture and the aqueous

phase was extracted three times with CH₂Cl₂ (3x5 mL). The organic layers were dried over MgSO₄, and after concentration under vacuum gave the acid **13** as a waxy solid (44 mg, 0.13 mmol, 98 % yield). *R_f* 0.20 (CH₂Cl₂/MeOH 95:5); [α]_D²⁰ – 14.5 (*c* 0.22, CHCl₃); IR (neat) ν 3327, 2918, 2848, 1935, 1702, 1663, 1574, 1531, 1454, 1356, 1314, 1243, 1207, 1162, 1111, 1081, 1028 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.11-2.21 (1H, m), 2.21-2.36 (4H, m), 2.48-2.52 (1H, m), 2.79 and 3.06 (2H, 2d, *J* = 17.3 Hz), 3.25 (2H, m), 4.54 and 4.65 (2H, 2d, *J* = 14.5 Hz), 4.92 (1H, d, *J* = 10.2 Hz), 4.97 (1H, d, *J* = 17.2 Hz), 5.64-5.73 (1H, m), 7.21-7.33 (5H, m), 10.61 (1H, br s); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 27.7, 29.3, 35.0, 39.3, 43.2, 50.6, 57.6, 116.0, 128.0 (3C), 128.8 (2C), 135.3, 136.4, 169.2, 173.9, 175.3; HRMS (DCI⁺) *m/z*: [M+H]⁺ Calcd for C₁₈H₂₃N₂O₄ 331.1658; Found 331.1646.

(4*R*)-1-Benzyl-2-oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid (1*S*)-(1-methylcarbamoyl-ethyl) amide (**14**). To a solution of compound (**R**)-**13** (19 mg, 0.058 mmol) in anhydrous CH₂Cl₂ (1.2 mL) was added BOP (38 mg, 0.09 mmol, 1.5 equiv.), TEA (23 μ L, 0.16 mmol, 2.5 equiv.) and *N*-1-methyl-*L*-alaninamide hydrochloride (12 mg, 0.09 mmol, 1.5 equiv.). The reaction mixture was stirred at room temperature until complete disappearance of the starting material (17 h). At the end of the reaction, the residue was concentrated under vacuum and purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 95:5) to give the pseudopeptide **14** as a sticky oil which crystallize after storage at 5 °C (24 mg, 0.046 mmol, 80 % yield). *R_f* 0.17 (CH₂Cl₂/MeOH 95:5); Mp 147.3-148.1 °C (white needles from dichloromethane); [α]_D²⁰ + 2.2 (*c* 0.4, CH₂Cl₂); IR (neat) ν 3380, 3309, 2992, 2688, 2496, 1670, 1650, 1622, 1454, 1200, 1176, 1128 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.37 (3H, d, *J* = 7.3 Hz, ^{Ala}CH₃), 2.17 (1H, m, ⁵CH₂), 2.35 (1H, m, ⁵CH₂), 2.32-2.37 (4H, m, ^{2',3'}CH₂), 2.53 (1H, d, *J* = 17.1 Hz, ³CH₂), 2.69 (3H, d, *J* = 4.7 Hz, ^{NHMe}CH₃), 3.13-3.27 (2H, m, ⁶CH₂), 3.29 (1H, d, *J* = 17.9 Hz, ³CH₂), 4.27 (1H, quint., *J* = 7.2 Hz, ^{Ala}CH), 4.45 and 4.64 (2H, 2d, *J* = 14.9 Hz, ^{Bn}CH₂), 4.95 (1H, dd, *J* = 10.2, 1.4 Hz, ^{=CH₂}CH₂), 5.03 (1H, d, *J* = 17.2 Hz, ^{=CH₂}CH₂), 5.79 (1H, ddt, *J* = 17.1, 10.4, 6.2 Hz, ^{=CH}CH), 6.94 (1H, q, *J* = 4.7 Hz, ^{NHMe}NH), 7.12 (1H, d, *J* = 7.1 Hz, ^{Ala}NH), 7.13 (1H, br s, ⁴NH), 7.19-7.32 (5H, m, ^{Ar}CH); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 17.3 (^{Ala}CH₃), 26.2 (^{NHMe}CH₃), 29.1 (^{3'}CH₂), 29.6 (⁵CH₂), 34.9 (^{2'}CH₂), 39.9 (³CH₂), 42.9 (⁶CH₂), 49.9 (^{Bn}CH₂), 50.0 (^{Ala}CH), 58.2 (⁴C_q), 115.7 (^{=CH₂}CH₂), 127.5 (^{Ar}CH), 127.6 (2^{Ar}CH), 128.7 (2^{Ar}CH), 136.2 (^{Ar}C_q), 136.9 (^{=CH}CH), 168.6 (²C_q), 172.4 (^{4-CO}C_q), 173.0 (^{COAla}C_q), 174.7 (^{CO}C_q); ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.15 (3H, d, *J* = 7.5 Hz, ^{Ala}CH₃), 1.98 (1H, m, ⁵CH₂), 2.18 (1H, m, ⁵CH₂), 2.22 (4H, m, ^{2',3'}CH₂), 2.35 (1H, d, *J* = 17.1 Hz, ³CH₂), 2.55 (3H, d, *J* = 4.6 Hz, ^{NHMe}CH₃), 2.92 (1H, d, *J* = 17.1 Hz, ³CH₂), 2.99-3.08 (1H, m, ⁶CH₂), 3.15 (1H, m, ⁶CH₂), 4.13 (1H, quint., *J* = 7.3 Hz,

^{Ala}CH), 4.43 and 4.52 (2H, 2d, $J = 15.1$ Hz, ^{Bn}CH₂), 4.91 (1H, dd, $J = 10.2, 2.0$ Hz, =CH²CH₂), 4.99 (1H, d, $J = 17.2$ Hz, =CH²CH₂), 5.77 (1H, ddt, $J = 17.2, 10.3, 6.1$ Hz, =CHCH), 7.22 (3H, m, ^{Ar}CH), 7.29 (2H, m, ^{Ar}CH), 7.54 (1H, q, $J = 4.6$ Hz, ^{NHMe}NH), 7.75 (1H, d, $J = 7.7$ Hz, ^{Ala}NH), 8.42 (1H, br s, ⁴NH); ¹³C{¹H} NMR (100 MHz, DMSO-*d*₆) δ 17.6 (^{Ala}CH₃), 25.5 (^{NHMe}CH₃), 28.7 (^{3'}CH₂), 28.8 (⁵CH₂), 34.2 (^{2'}CH₂), 39.9 (³CH₂), 42.3 (⁶CH₂), 48.3 (^{Ala}CH), 48.7 (^{Bn}CH₂), 57.5 (⁴C_q), 114.9 (=CH²CH₂), 126.8 (^{Ar}CH), 127.2 (2^{Ar}CH), 128.2 (2^{Ar}CH), 137.2 (^{Ar}C_q), 137.5 (=CHCH), 166.7 (²C_q), 171.3 (^{4-CO}C_q), 172.0 (^{COAla}C_q), 172.8 (^{CO}C_q); HRMS (DCI⁺) m/z : [M+H]⁺ Calcd for C₂₂H₃₁N₄O₄ 415.2345; Found 415.2336.

(4*R*)-1-Benzyl-2-oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid (1*R*)-(1-methylcarbamoyl-ethyl) amide (**15**). To a solution of compound (*R*)-**13** (90 mg, 0.27 mmol) in anhydrous CH₂Cl₂ (6 mL) was added BOP (190 mg, 0.45 mmol, 1.5 equiv.), TEA (115 μ L, 0.80 mmol, 2.7 equiv.) and *N*-1-methyl-D-alaninamide hydrochloride (60 mg, 0.45 mmol, 1.5 equiv.). The reaction mixture was stirred at room temperature until complete disappearance of the starting material (17 h). At the end of the reaction, the residue was concentrated under vacuum and purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 95:5) to give the pseudopeptide **15** as a sticky oil (60 mg, 0.144 mmol, 53 % yield). $[\alpha]_D^{20} - 10.8$ (c 1.26, CH₂Cl₂); IR (neat) ν 3325, 2978, 2603, 2496, 1672, 1199, 1173, 747 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.34 (3H, d, $J = 7.3$ Hz, ^{Ala}CH₃), 2.31 (4H, m, ^{2',3'}CH₂), 2.39 and 2.57 (2H, 2d, $J = 11.9$ Hz, ⁵CH₂), 2.72 (3H, d, $J = 4.5$ Hz, ^{NHMe}CH₃), 2.71 and 2.87 (2H, 2d, $J = 16.9$ Hz, ³CH₂), 3.15 (2H, m, ⁶CH₂), 4.39 (1H, m, ^{Ala}CH), 4.37 and 4.72 (2H, 2d, $J = 14.4$ Hz, ^{Bn}CH₂), 4.91 (1H, d, $J = 10.2$ Hz, =CH²CH₂), 4.99 (1H, d, $J = 17.1$ Hz, =CH²CH₂), 5.73 (1H, m, =CHCH), 7.12 (1H, br s, ^{NHMe}NH), 7.19 (1H, br s, ^{Ala}NH), 7.16-7.34 (5H, m, ^{Ar}CH), 8.14 (1H, br s, ⁴NH); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 17.5 (^{Ala}CH₃), 26.2 (^{NHMe}CH₃), 26.6 (⁵CH₂), 29.2 (^{3'}CH₂), 35.0 (^{2'}CH₂), 40.6 (⁶CH₂), 42.9 (³CH₂), 49.7 (^{Ala}CH), 49.9 (^{Bn}CH₂), 58.4 (⁴C_q), 115.7 (=CH²CH₂), 127.6 (2^{Ar}CH), 127.8 (^{Ar}CH), 128.8 (2^{Ar}CH), 135.9 (^{Ar}C_q), 136.9 (=CHCH), 167.4 (²C_q), 171.8 (^{4-CO}C_q), 172.9 (^{COAla}C_q), 174.7 (^{CO}C_q); ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.19 (3H, d, $J = 7.1$ Hz, ^{Ala}CH₃), 2.07 (1H, m, ⁵CH₂), 2.24 (5H, m, ^{2',3',5}CH₂), 2.45 (1H, dd, $J = 17.2, 1.7$ Hz, ³CH₂), 2.54 (3H, d, $J = 4.7$ Hz, ^{NHMe}CH₃), 2.79 (1H, d, $J = 17.2$ Hz, ³CH₂), 3.06-3.14 (2H, m, ⁶CH₂), 4.14 (1H, sept., $J = 7.3$ Hz, ^{Ala}CH), 4.41 and 4.60 (2H, 2d, $J = 15.1$ Hz, ^{Bn}CH₂), 4.92 (1H, dd, $J = 10.2, 2.0$ Hz, =CH²CH₂), 5.00 (1H, dd, $J = 17.2, 1.9$ Hz, =CH²CH₂), 5.78 (1H, ddt, $J = 17.1, 10.2, 5.9$ Hz, =CHCH), 7.25 (3H, m, ^{Ar}CH), 7.30 (2H, m, ^{Ar}CH), 7.55 (1H, q, $J = 4.7$ Hz, ^{NHMe}NH), 7.76 (1H, d, $J = 7.6$ Hz, ^{Ala}NH), 8.45 (1H, br s, ⁴NH); ¹³C{¹H} NMR (100 MHz, DMSO-*d*₆) δ 17.6 (^{Ala}CH₃), 25.5 (^{NHMe}CH₃), 27.8 (⁵CH₂), 28.8 (^{3'}CH₂), 34.3 (^{2'}CH₂), 39.6 (³CH₂), 42.3 (⁶CH₂), 48.4 (^{Ala}CH),

48.7 ($^{\text{Bn}}\text{CH}_2$), 57.6 ($^4\text{C}_q$), 114.9 ($^{\text{=CH}_2}\text{CH}_2$), 126.8 ($^{\text{Ar}}\text{CH}$), 127.2 (2^{Ar}CH), 128.3 (2^{Ar}CH), 137.1 ($^{\text{Ar}}\text{C}_q$), 137.5 ($^{\text{=CH}}\text{CH}$), 166.3 ($^2\text{C}_q$), 171.4 ($^4\text{-CO}\text{C}_q$), 172.1 ($^{\text{COAla}}\text{C}_q$), 172.8 ($^{\text{CO}}\text{C}_q$); HRMS (DCI $^+$) m/z: $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{22}\text{H}_{31}\text{N}_4\text{O}_4$ 415.2340; Found 415.2335; m/z: $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{22}\text{H}_{30}\text{N}_4\text{NaO}_4$ 437.2159; Found 437.2155; m/z: $[\text{M}+\text{K}]^+$ Calcd for $\text{C}_{22}\text{H}_{30}\text{KN}_4\text{O}_4$ 453.1899; Found 453.1888.

(4R)-4-Amino-1-benzyl-2-oxo-piperidine-4-carboxylic acid tert-butyl ester (16). Compound **11** (510 mg, 1.32 mmol) was dissolved in a mixture of 33 mL THF and of 33 mL water. Iodine (1.01 g, 4.75 mmol, 3 equiv.) was added and the mixture was stirred at room temperature during 6 h. Aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3$ (20 mL) was then added dropwise until complete decoloration and saturated aqueous Na_2CO_3 (approximately 15 mL) was added until pH 9 was reached. The complete mixture was extracted once with petroleum ether (50 mL) and three times with CH_2Cl_2 (3x50 mL). After concentration under vacuum, the residue was purified by Combiflash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:0 to 92:8) to separate iodolactone (R_f 0.78 in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5) and to afford pure compound **16** as an oil (392 mg, 1.29 mmol, 98 % yield). R_f 0.25 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); $[\alpha]_{\text{D}}^{20} - 14.7$ (c 0.96, CH_2Cl_2); IR (neat) ν 3292, 2976, 2929, 1721, 1633, 1495, 1253, 1157 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.44 (9H, s), 1.69 (2H, br s), 1.78 (1H, dtd, $J = 13.6, 5.1, 2.0$ Hz), 2.09 (1H, ddd, $J = 13.5, 9.1, 5.7$ Hz), 2.41 (1H, dd, $J = 17.2, 1.9$ Hz), 2.87 (1H, d, $J = 17.2$ Hz), 3.15 (1H, dt, $J = 12.2, 5.4$ Hz), 3.45 (1H, ddd, $J = 12.3, 9.2, 5.1$ Hz), 4.50 and 4.74 (2H, 2d, $J = 14.8$ Hz), 7.23-7.33 (5H, m); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 27.8 (3C), 32.0, 41.8, 43.2, 49.9, 56.5, 81.9, 127.3, 128.0 (2C), 128.5 (2C), 136.9, 167.7, 174.5; HRMS (ESI-TOF) m/z: $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_3$ 305.1860; Found 305.1861; m/z: $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{NaO}_3$ 327.1679; Found 327.1677; m/z: $[\text{M}+\text{K}]^+$ Calcd for $\text{C}_{17}\text{H}_{24}\text{KN}_2\text{O}_3$ 343.1419; Found 343.1417.

(4R)-1-Benzyl-4-[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-methyl-pentanoylamino]-2-oxo-piperidine-4-carboxylic acid tert-butyl ester (17). To a solution of Fmoc-leucine (311 mg, 0.88 mmol, 1.2 equiv.) in anhydrous CH_2Cl_2 (9 mL) at 0 $^{\circ}\text{C}$, was added HOAt (150 mg, 1.1 mmol, 1.5 equiv.), HATU (390 mg, 1.02 mmol, 1.4 equiv.) and TMP (1.5 mL, 8.8 mmol, 12 equiv.). The reaction mixture was maintained at 0 $^{\circ}\text{C}$ for 3 hours. A solution of compound **16** (223 mg, 0.73 mmol) in anhydrous CH_2Cl_2 (9 mL) was added dropwise and after anhydrous DMF (9 mL) and the reaction was carried on at room temperature for 48 h. At the end of the reaction, the residue was concentrated under vacuum and was purified by flash chromatography on silica gel (from pure CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90:10) to give the dipeptide **17** as a colorless oil (345 mg, 0.54 mmol, 74 % yield). R_f 0.15 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); $[\alpha]_{\text{D}}^{20} - 27.2$ (c 0.53, CH_2Cl_2); IR (neat) ν

3285, 3063, 2955, 1728, 1632, 1450, 1241, 1159 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.90 (6H, br s), 1.38 (9H, s), 1.40-1.62 (3H, m), 2.08-2.15 (1H, m), 2.43-2.48 (1H, m), 2.92 and 2.68 (2H, 2d, $J = 17.2$ Hz), 3.19 (2H, br s), 4.14 (1H, br s), 4.20 (1H, t, $J = 6.9$ Hz), 4.31-4.46 (2H, m), 4.48 and 4.66 (2H, 2d, $J = 14.6$ Hz), 5.16 (1H, d, $J = 8.3$ Hz), 6.76 (1H, br s), 7.22-7.32 (7H, m), 7.40 (2H, t, $J = 7.4$ Hz), 7.56 (2H, br d, $J = 7.2$ Hz), 7.76 (2H, d, $J = 7.5$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 22.8 (2C), 24.6, 27.7 (3C), 28.3, 40.2, 41.0, 42.9, 47.1, 49.9, 53.2, 58.1, 67.1, 82.4, 120.0 (2C), 125.0 (2C), 127.1 (2C), 127.6, 127.8 (2C), 128.1 (2C), 128.7 (2C), 136.5, 141.3 (2C), 143.6, 143.7, 156.3, 166.7, 170.3, 172.0; HRMS (DCI^+) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{38}\text{H}_{46}\text{N}_3\text{O}_6$ 640.3381; Found 640.3379; m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{38}\text{H}_{45}\text{N}_3\text{NaO}_6$ 662.3201; Found 662.3196; m/z : $[\text{M}+\text{K}]^+$ Calcd for $\text{C}_{38}\text{H}_{45}\text{KN}_3\text{O}_6$ 678.2940; Found 678.2936.

(4R)-1-Benzyl-4-[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-methyl-pentanoylamino]-2-oxo-piperidine-4-carboxylic acid (18). Compound **17** (280 mg, 0.438 mmol) was dissolved in a mixture of TFA (3.5 mL) in CH_2Cl_2 (9 mL) at 0 $^\circ\text{C}$, and the reaction mixture was stirred and allowed to warm to rt until complete disappearance of the starting material (17 h). At the end of the reaction, brine (40 mL) was added to the reaction mixture and the aqueous phase was extracted three times with CH_2Cl_2 (3x40 mL). The organic layers were dried over MgSO_4 , and after concentration under vacuum was purified by flash chromatography on silica gel (from pure CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90:10) to give the acid **18** as an oil (174 mg, 0.298 mmol, 68 % yield). R_f 0.39 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); $[\alpha]_{\text{D}}^{20} - 11.5$ (c 1.45, CH_3OH); IR (neat) ν 2924, 1713, 1247 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.86 (6H, br d, $J = 6.2$ Hz), 1.40-1.70 (3H, m), 2.08-2.20 (1H, m), 2.50-2.62 (1H, m), 3.08 (2H, br s), 3.12-3.32 (2H, m), 4.13 (1H, t, $J = 7.2$ Hz), 4.18-4.34 (4H, m), 4.68 (1H, d, $J = 14.8$ Hz), 6.13 (1H, br d, $J = 6.9$ Hz), 7.12 (2H, d, $J = 6.8$ Hz), 7.15-7.28 (5H, m), 7.32 (2H, q, $J = 7.4$ Hz), 7.56 (2H, d, $J = 7.3$ Hz), 7.67 (2H, t, $J = 7.1$ Hz), 8.13 (1H, br s); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 21.6, 22.9, 24.7, 27.3, 38.1, 40.8, 43.5, 46.9, 51.1, 53.8, 57.2, 67.4, 119.9 (2C), 125.3 (2C), 127.1 (2C), 127.7 (2C), 128.0 (3C), 128.9 (2C), 134.5, 141.2 (2C), 143.6, 143.9, 156.7, 170.1, 172.9, 175.0; HRMS (DCI^+) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{34}\text{H}_{38}\text{N}_3\text{O}_6$ 584.2755; Found 584.2752.

{(1S)-1-[(4R)-1-Benzyl-4-[(1S)-1-methylcarbamoyl-ethylcarbamoyl]-2-oxo-piperidin-4-ylcarbamoyl]-3-methyl-butyl}-carbamic acid 9H-fluoren-9-ylmethyl ester (19). To a solution of compound **18** (77 mg, 0.132 mmol) in anhydrous CH_2Cl_2 (4 mL) was added BOP (70 mg, 0.158 mmol, 1.1 equiv.), TMP (70 mg, 0.5 mmol, 3.8 equiv.) and *N*-1-methyl-*L*-alaninamide hydrochloride (35 mg, 0.25 mmol, 1.8 equiv.). The reaction mixture was stirred at room temperature until complete disappearance of the starting material (48 h). At the end of the

reaction, the residue was concentrated under vacuum and purified by flash chromatography on silica gel (CH₂Cl₂/MeOH from 100:0 to 0:100) to give pseudopeptide **19** as a sticky oil (69 mg, 0.103 mmol, 78 %). $[\alpha]_D^{20} + 10.7$ (*c* 0.70, CH₃OH); IR (neat) ν 3336, 2954, 1650, 1451, 1424, 740 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 2 conformers in a 6:4 ratio, major conformer: 0.92 (6H, t, *J* = 6.5 Hz), 1.28 (3H, m, *J* = 7.3 Hz), 1.44-1.59 (2H, m), 1.60-1.69 (1H, m), 2.15 (1H, m), 2.38 (1H, m), 2.54 (1H, d, *J* = 17.5 Hz), 2.69 (3H, s), 3.11 (1H, d, *J* = 17.5 Hz), 3.20 (2H, m), 4.04 (1H, m), 4.20 (1H, m), 4.21 (2H, m, *J* = 7.0 Hz), 4.34 (1H, dd, *J* = 6.7, 10.6 Hz), 4.44 (1H, dd, *J* = 6.7, 10.6 Hz), 4.52 (1H, d, *J* = 14.9 Hz), 4.62 (1H, d, *J* = 14.9 Hz), 7.23-7.34 (7H, m), 7.38 (2H, t, *J* = 7.4 Hz), 7.64 (2H, d, *J* = 7.4 Hz), 7.78 (2H, t, *J* = 7.5 Hz); minor conformer: 0.94 (3H, d, *J* = 6.4 Hz), 0.97 (3H, d, *J* = 6.4 Hz), 1.40 (3H, m, *J* = 7.3 Hz), 1.44-1.59 (1H, m), 1.60-1.69 (1H, m), 2.15 (2H, m), 2.56 (3H, s), 2.70 (1H, d, *J* = 17.7 Hz), 3.20 (1H, m), 3.35 (1H, d, *J* = 17.7 Hz), 3.38 (1H, m), 4.04 (1H, m), 4.15 (2H, m), 4.28 (2H, m), 4.54 (1H, d, *J* = 14.9 Hz), 4.62 (1H, d, *J* = 14.9 Hz), 7.23-7.34 (7H, m), 7.38 (2H, t, *J* = 7.4 Hz), 7.64 (2H, d, *J* = 7.4 Hz), 7.78 (2H, t, *J* = 7.5 Hz); ¹³C NMR{1H} (100 MHz, CD₃OD) δ 2 conformers, major conformer: 17.8, 22.0, 23.4, 25.8, 26.4, 29.6, 40.9, 41.1, 44.0, 48.5, 51.0 (2C), 55.5, 59.6, 68.0, 121.0, 126.1, 128.2-129.8, 137.9, 142.7, 145.0-145.3, 159.0, 169.7, 173.7, 175.1, 176.5; minor conformer: 17.7, 22.6, 23.0, 25.9, 26.4, 31.5, 39.5, 40.9, 41.2, 43.9, 48.3, 50.8, 51.0, 55.1, 59.7, 68.3, 121.0, 126.3, 128.2-129.8, 138.0, 142.6, 145.0-145.3, 159.2, 170.4, 173.9, 175.3, 177.7; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₃₈H₄₆N₅O₆ 668.3443; Found 668.3398; *m/z*: [M+Na]⁺ Calcd for C₃₈H₄₅N₅NaO₆ 690.3262; Found 690.3231.

(4*R*)-4-[(2*S*)-2-Amino-4-methyl-pentanoylamino]-1-benzyl-2-oxo-piperidine-4-carboxylic acid (1*S*)-(1-methylcarbamoyl-ethyl)-amide (**20**). To a solution of compound **19** (69 mg, 0.105 mmol) in methanol (1 mL) was added freshly distilled piperidine (16 mg, 0.21 mmol, 2 equiv.). The reaction mixture was stirred at room temperature for 17 h. At the end of the reaction, the residue was concentrated under vacuum and purified by flash chromatography on silica gel (CH₂Cl₂/MeOH from 100:0 to 0:100) to give pseudopeptide **20** as a sticky oil (41 mg, 0.093 mmol, 89 %). $[\alpha]_D^{20} + 17.6$ (*c* 0.43, CH₃OH); IR (neat) ν 3327, 2918 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 0.94 and 0.95 (6H, 2 d, *J* = 5.2 Hz), 1.33 (3H, d, *J* = 7.2 Hz), 1.40-1.47 (1H, m), 1.50-1.60 (1H, m), 1.65-1.75 (1H, m), 2.16-2.22 (1H, m), 2.32-2.39 (1H, m), 2.51 (1H, d, *J* = 17.3 Hz), 2.73 (3H, s), 3.18-3.30 (3H, m), 3.47 (1H, dd, *J* = 5.6, 9.0 Hz), 4.29 (1H, q, *J* = 7.2 Hz), 4.60 (2H, m), 7.26-7.33 (5H, m); ¹³C NMR (100 MHz, CD₃OD) δ 2 conformers, major conformer: 17.8, 22.1, 23.6, 25.8, 26.4, 30.3, 40.9, 44.0, 44.3, 50.8, 50.9, 54.2, 59.3, 128.6, 129.0 (2C), 129.8 (2C), 137.9, 170.0, 173.6, 175.2, 177.3; minor conformer: 17.7, 22.4, 23.4, 25.9,

26.4, 31.0, 40.5, 43.9, 44.5, 50.8, 50.9, 54.0, 59.3, 128.6, 129.0 (2C), 129.8 (2C), 137.9, 170.2, 173.6, 175.2, 177.3; HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{23}H_{35}N_5O_4$ 446.2762; Found 446.2752; m/z : $[M+Na]^+$ Calcd for $C_{23}H_{34}N_5NaO_4$ 468.2581; Found 468.2568; m/z : $[M+K]^+$ Calcd for $C_{23}H_{34}KN_5O_4$ 484.2321; Found 484.2323.

Debenzylation protocol. In liquid NH_3 , at $-78\text{ }^\circ C$, metallic sodium (20 equiv.) was dissolved to reach a deep blue solution. A solution of compound **14** or **15** in anhydrous THF (8 mL/mmol) was added dropwise. The reaction mixture was maintained at $-78\text{ }^\circ C$ for 15 minutes under argon and then allowed to warm up at $-33\text{ }^\circ C$. A minimal amount of solid NH_4Cl was thus added until disappearance of the blue coloration. Ammonia was gently evaporated and stirring was maintained until room temperature was reached by the medium. The reaction mixture was then concentrated under vacuum and the debenzylated product was thus purified.

(4R)-2-Oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid ((1S)-1-methylcarbamoyl-ethyl)-amide (21). Following debenzylation protocol and starting from metallic sodium (22 mg, 0.96 mmol, 20 equiv.) and from a solution of compound **14** (20 mg, 0.048 mmol) in anhydrous THF (4 mL), deprotected pseudopeptide **21** was obtained as a waxy solid (11 mg, 0.034 mmol, 71 % yield). R_f 0.24 ($CH_2Cl_2/MeOH$ 95:5); $[\alpha]_D^{20} + 6.8$ (c 0.09, CH_2Cl_2); IR (neat) ν 3394, 2921, 2851, 1639, 1463, 1401, 1172, 1033 cm^{-1} ; 1H NMR (400 MHz, CD_3OD) δ 1.35 (3H, d, $J = 7.3$ Hz), 2.04-2.10 (1H, m), 2.20-2.28 (1H, m), 2.32 (1H, d, $J = 17.9$ Hz), 2.35-2.40 (4H, m), 2.72 (3H, s), 3.11 (1H, dd, $J = 17.5, 1.2$ Hz), 3.15-3.25 (1H, m), 3.30-3.40 (1H, m), 4.30 (1H, q, $J = 7.3$ Hz), 4.98 (1H, dd, $J = 10.2, 1.8$ Hz), 5.07 (1H, dd, $J = 17.2, 1.8$ Hz), 5.80-5.88 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 17.6, 26.4, 30.1, 30.4, 35.9, 38.3, 40.1, 50.7, 59.0, 116.0, 138.2, 172.7, 174.1, 175.2, 176.1; HRMSR(ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{15}H_{25}N_4O_4$ 325.1870; Found 325.1866; m/z : $[M+Na]^+$ Calcd for $C_{15}H_{24}N_4NaO_4$ 347.1690; Found 347.1691; m/z : $[M+K]^+$ Calcd for $C_{15}H_{24}KN_4O_4$ 363.1429; Found 363.1428.

(4R)-2-Oxo-4-pent-4-enoylamino-piperidine-4-carboxylic acid ((1S)-1-methylcarbamoyl-ethyl)-amide (22). Following debenzylation protocol and starting from metallic sodium (23 mg, 1.0 mmol, 20 equiv.) and from a solution of compound **15** (21 mg, 0.050 mmol) in anhydrous THF (4 mL), deprotected pseudopeptide **22** was obtained as a waxy solid (12 mg, 0.037 mmol, 74 % yield). R_f 0.25 ($CH_2Cl_2/MeOH$ 95:5); $[\alpha]_D^{20} - 1.0$ (c 0.09, MeOH); IR (neat) ν 3276, 2970, 2930, 1644, 1544, 1347, 1268, 1183, 1138, 1053 cm^{-1} ; 1H NMR (400 MHz, CD_3OD) δ 1.35 (3H, d, $J = 7.2$ Hz), 2.12-2.22 (1H, m), 2.25-2.40 (5H, m), 2.40 (1H, d, $J = 17.2$ Hz), 2.72 (3H, s), 2.89 (1H, d, $J = 17.2$ Hz), 3.25-3.30 (2H, m), 4.28 (1H, q, $J = 7.2$ Hz), 4.99 (1H, dd, $J = 10.4,$

1.8 Hz), 5.08 (1H, dd, $J = 16.8, 1.8$ Hz), 5.80–5.88 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 17.7, 26.4, 28.9, 30.3, 36.0, 38.4, 40.2, 50.9, 59.1, 116.0, 138.3, 172.0, 174.2, 175.4, 176.1 HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{15}\text{H}_{25}\text{N}_4\text{O}_4$ 325.1870; Found 325.1858; m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{15}\text{H}_{24}\text{N}_4\text{NaO}_4$ 347.1690; Found 347.1676; m/z : $[\text{M}+\text{K}]^+$ Calcd for $\text{C}_{15}\text{H}_{24}\text{KN}_4\text{O}_4$ 363.1429; Found 363.1447.

Supporting Information Available: Copies of NMR spectra of all new compounds, X-Ray diffraction data and DFT calculation data (PDF). This material is available free of charge on the ACS Publications website.

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REFERENCES and NOTES

- ¹ Current address: Shanxi Key Laboratory Additives for Industry, Shaanxi University of Science and Technology, Xi'an 710021, China.
- ² (a) Qvit, N.; Rubin, S. J. S.; Urban, T. J.; Mochly-Rosen, D.; Gross, E. R. Peptidomimetic Therapeutics: Scientific Approaches and Opportunities. *Drug Discovery Today* **2017**, 22, 454–462. <https://doi.org/10.1016/j.drudis.2016.11.003>. (b) Gentilucci, L.; Marco, R. D.; Cerisoli, L. Chemical Modifications Designed to Improve Peptide Stability: Incorporation of Non-Natural Amino Acids, Pseudo-Peptide Bonds, and Cyclization. *Current Pharmaceutical Design* **2010**, 16, 3185–3203. <https://doi.org/10.2174/138161210793292555>. (c) Maes, V.; Tourwe, D. Aspects of Peptidomimetics. In *Pept. Protein Des. Biopharm. Appl.*; John Wiley & Sons Ltd., 2009; pp 49–131. <https://doi.org/10.1002/9780470749708.ch3>.
- ³ (a) Horikawa, M.; Shigeri, Y.; Nakajima, T.; Ohfuné, Y. Syntheses of Potent Leu-Enkephalin Analogs Possessing β -Hydroxy- α,α -Disubstituted- α -Amino Acid and their Characterization to Opioid Receptors. *Bioorg. Med. Chem. Lett.* **1998**, 8, 2027–2032. [https://doi.org/10.1016/S0960-894X\(98\)00349-7](https://doi.org/10.1016/S0960-894X(98)00349-7). (b) Kowalczyk, W.; Prahl, A.; Derdowska, I.; Dawidowska, O.; Slaninová, J.; Lammek, B. Highly Potent 1-Aminocyclohexane-1-Carboxylic Acid Substituted V_2 Agonists of Arginine Vasopressin. *J. Med. Chem.* **2004**, 47, 6020–6024. <https://doi.org/10.1021/jm040813o>. (c) Labudda-Dawidowska, O.; Wierzba, T. H.; Prahl, A.; Kowalczyk, W.; Gawiński, Ł.; Plackova, M.; Slaninová, J.; Lammek, B. New Bradykinin Analogues Modified in the C-Terminal Part with Sterically Restricted 1-Aminocyclohexane-1-Carboxylic Acid. *J. Med. Chem.* **2005**, 48, 8055–8059. <https://doi.org/10.1021/jm0580353>.
- ⁴ (a) Maity, P.; König, B. Enantio- and Diastereoselective Syntheses of Cyclic α -Tetrasubstituted α -Amino Acids and Their Use to Induce Stable Conformations in Short Peptides. *Biopolymers* **2008**, 90, 8–27. <https://doi.org/10.1002/bip.20902>. (b) Zanuy, D.; Ballano, G.; Jiménez, A. I.; Casanovas, J.; Haspel, N.; Cativiela, C.; Curc6, D.; Nussinov, R.; Alemán, C. Protein Segments with Conformationally Restricted Amino Acids Can Control Supramolecular Organization at the Nanoscale. *J. Chem. Inform. Model.* **2009**, 49, 1623–1629. <https://doi.org/10.1021/ci9001487>. (c) Van der Poorten, O.; Knuhtsen, A.; Sejer Pedersen, D.; Ballet, S.; Tourwé, D. Side Chain Cyclized Aromatic Amino Acids: Great Tools as Local Constraints in Peptide and Peptidomimetic Design. *J. Med. Chem.* **2016**, 59, 10865–10890. <https://doi.org/10.1021/acs.jmedchem.6b01029>.
- ⁵ Mazaleyrat, J.-P.; Gaucher, A.; Savrda, J.; Wakselman, M. Novel α,α -Disubstituted α -Aminoacids with Axial Dissymmetry and Their *N*- or *C*-Protected Derivatives. *Tetrahedron: Asymmetry* **1997**, 8, 619–631. [https://doi.org/10.1016/S0957-4166\(97\)00009-8](https://doi.org/10.1016/S0957-4166(97)00009-8).

- ⁶ De Poli, M.; Byrne, L.; Brown, R. A.; Solà, J.; Castellanos, A.; Boddaert, T.; Wechsel, R.; Beadle, J. D.; Clayden, J. Engineering the Structure of an *N*-Terminal β -Turn To Maximize Screw-Sense Preference in Achiral Helical Peptide Chains. *J. Org. Chem.* **2014**, *79*, 4659–4675. <https://doi.org/10.1021/jo500714b>.
- ⁷ Nunez-Villanueva, D.; Bonache, M. A.; Infantes, L.; Garcia-Lopez, M. T.; Martin-Martinez, M.; Gonzalez-Muniz, Rosario. Quaternary α,α -2-Oxoazepane α -Amino Acids: Synthesis from Ornithine-Derived β -Lactams and Incorporation into Model Dipeptides. *J. Org. Chem.* **2011**, *76*, 6592–6603. <https://doi.org/10.1021/jo200894d>.
- ⁸ Núñez-Villanueva, D.; García-López, M. T.; Martín-Martínez, M.; González-Muñiz, R. Divergent, Stereoselective Access to Heterocyclic α,α -Quaternary- and $\beta^{2,3,3}$ -Amino Acid Derivatives from a *N*-Pmp-Protected Orn-Derived β -Lactam. *Org. Biomol. Chem.* **2015**, *13*, 5195–5201. <https://doi.org/10.1039/C5OB00429B>.
- ⁹ Núñez-Villanueva, D.; Infantes, L.; García-López, M. T.; González-Muñiz, R.; Martín-Martínez, M. Azepane Quaternary Amino Acids As Effective Inducers of 3_{10} Helix Conformations. *J. Org. Chem.* **2012**, *77*, 9833–9839. <https://doi.org/10.1021/jo301379r>.
- ¹⁰ Pellegrino, S.; Contini, A.; Clerici, F.; Gori, A.; Nava, D.; Gelmi, M. L. 1H-Azepine-4-Amino-4-Carboxylic Acid: A New α,α -Disubstituted Ornithine Analogue Capable of Inducing Helix Conformations in Short Ala-Aib Pentapeptides. *Chem. Eur. J.* **2012**, *18*, 8705–8715. <https://doi.org/10.1002/chem.201104023>.
- ¹¹ Pellegrino, S.; Bonetti, A.; Clerici, F.; Contini, A.; Moretto, A.; Soave, R.; Gelmi, M. L. 1H-Azepine-2-Oxo-5-Amino-5-Carboxylic Acid: A 3_{10} Helix Inducer and an Effective Tool for Functionalized Gold Nanoparticles. *J. Org. Chem.* **2015**, *80*, 5507–5516. <https://doi.org/10.1021/acs.joc.5b00396>.
- ¹² (a) Cho, J.; Tanaka, M.; Sato, S.; Kinbara, K.; Aida, T. Oligo(4-Aminopiperidine-4-Carboxylic Acid): An Unusual Basic Oligopeptide with an Acid-Induced Helical Conformation. *J. Am. Chem. Soc.* **2010**, *132*, 13176–13178. <https://doi.org/10.1021/ja106118w>. (b) Cho, J.; Ishida, Y.; Aida, T. Helical Oligopeptides of a Quaternized Amino Acid with Tunable Chiral-Induction Ability and an Anomalous pH Response. *Chem. Eur. J.* **2017**, *23*, 4818–4826. <https://doi.org/10.1002/chem.201605460>. (c) Kobayashi, H.; Misawa, T.; Matsuno, K.; Demizu, Y. Preorganized Cyclic α,α -Disubstituted α -Amino Acids Bearing Functionalized Side Chains That Act as Peptide-Helix Inducers. *J. Org. Chem.* **2017**, *82*, 10722–10726. <https://doi.org/10.1021/acs.joc.7b01946>. (d) Yamashita, H.; Demizu, Y.; Misawa, T.; Shoda, T.; Kurihara, M. Synthesis of a Bis-Cationic α,α -Disubstituted Amino Acid (9-Amino-Bispidine-9-Carboxylic Acid) and Its Effects on the Conformational Properties of Peptides. *Tetrahedron* **2015**, *71*, 2241–2245. <https://doi.org/10.1016/j.tet.2015.02.076>.
- ¹³ Jiménez, A. I.; Cativiela, C.; Gómez-Catalán, J.; Pérez, J. J.; Aubry, A.; París, M.; Marraud, M. Influence of Side Chain Restriction and $\text{NH}\cdots\pi$ Interaction on the β -Turn Folding Modes of Dipeptides Incorporating Phenylalanine Cyclohexane Derivatives. *J. Am. Chem. Soc.* **2000**, *122*, 5811–5821. <https://doi.org/10.1021/ja993568k>.
- ¹⁴ (a) Tanaka, M.; Demizu, Y.; Doi, M.; Kurihara, M.; Suemune, H. Chiral Centers in the Side Chains of α -Amino Acids Control the Helical Screw Sense of Peptides. *Angew. Chem. Int. Ed.* **2004**, *43*, 5360–5363. <https://doi.org/10.1002/anie.200460420>. (b) Tanaka, M.; Anan, K.; Demizu, Y.; Kurihara, M.; Doi, M.; Suemune, H. Side-Chain Chiral Centers of Amino Acid and Helical-Screw Handedness of Its Peptides. *J. Am. Chem. Soc.* **2005**, *127*, 11570–11571. <https://doi.org/10.1021/ja053842c>. (c) Demizu, Y.; Doi, M.; Kurihara, M.; Okuda, H.; Nagano, M.; Suemune, H.; Tanaka, M. Conformational Studies on Peptides Containing α,α -Disubstituted α -Amino Acids: Chiral Cyclic α,α -Disubstituted α -Amino Acid as an α -Helical Inducer. *Org. Biomol. Chem.* **2011**, *9*, 3303–3312. <https://doi.org/10.1039/c0ob01146k>. (d) Demizu, Y.; Doi, M.; Kurihara, M.; Maruyama, T.; Suemune, H.; Tanaka, M. One-Handed Helical Screw Direction of Homopeptide Foldamer Exclusively Induced by Cyclic α -Amino Acid Side-Chain Chiral Centers. *Chem. Eur. J.* **2012**, *18*, 2430–2439. <https://doi.org/10.1002/chem.201102902>.
- ¹⁵ Zhang, X.; Cividino, P.; Poisson, J.-F.; Shpak-Kraievskiy, P.; Laurent, M. Y.; Martel, A.; Dujardin, G.; Py, S. Asymmetric Synthesis of α,α -Disubstituted Amino Acids by Cycloaddition of (*E*)-Ketonitrone with Vinyl Ethers. *Org. Lett.* **2014**, *16*, 1936–1939. <https://doi.org/10.1021/ol500483t>.
- ¹⁶ (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. Two New Orthogonal Amine-Protecting Groups That Can Be Cleaved under Mild or Neutral Conditions. *J. Am. Chem. Soc.* **1995**, *117*, 3302–3303. <https://doi.org/10.1021/ja00116a047>. (b) Madsen, R.; Roberts, C.; Fraser-Reid, B. The Pent-4-Enoyl Group: A Novel Amine-Protecting Group That Is Readily Cleaved under Mild Conditions. *J. Org. Chem.* **1995**, *60*, 7920–7926. <https://doi.org/10.1021/jo00129a036>.
- ¹⁷ Extending the reaction time increased to a large extent the formation of diacid and did not improve the yield of monoacid diester (**7b**).
- ¹⁸ Kano, T.; Noishiki, A.; Sakamoto, R.; Maruoka, K. Syn-Selective Asymmetric Cross-Aldol Reactions between Aldehydes and Glyoxylic Acid Derivatives Catalyzed by an Axially Chiral Amino Sulfonamide. *Chem. Commun.* **2011**, *47*, 10626–10628. <https://doi.org/10.1039/C1CC14347F>.
- ¹⁹ The selective formation of the 6-membered ring product (**11**) could result from the reduced steric hindrance of the reactive *t*-butyl ester compared to the other *t*-butyl ester, and from its possible activation through an intramolecular hydrogen-bond with the NH of the pent-4-enoyl amide chain.

- ²⁰ Rouleau, J.; Iorga, B. I.; Guillou, C. New Potent Human Acetylcholinesterase Inhibitors in the Tetracyclic Triterpene Series with Inhibitory Potency on Amyloid β Aggregation. *Eur. J. Med. Chem.* **2011**, *46*, 2193–2205. <https://doi.org/10.1016/j.ejmech.2011.02.073>.
- ²¹ See Supporting Information for X-ray diffraction data of compound **11**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre (CCDC 1844274).
- ²² This compound slowly crystallized from the thick oil upon storage in the fridge, yielding white needles.
- ²³ See Supporting Information for X-ray diffraction data of compound **14**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre (CCDC 1844275).
- ²⁴ Rose, G. D.; Gierasch, L. M.; Smith, J. A. Turns in Peptides and Proteins. *Adv. Prot. Chem.* **1985**, *37*, 1–109.
- ²⁵ (a) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. Conformational Preferences of Peptides Containing Reverse-Turn Mimetic Bicyclic Lactams: Inverse γ -Turns versus Type-II' β -Turns – Insights into β -Hairpin Stability. *Eur. J. Org. Chem.* **1999**, 389–400. [https://doi.org/10.1002/\(SICI\)1099-0690\(199902\)1999:2<3c389::AID-EJOC389>3e3.0.CO;2-7](https://doi.org/10.1002/(SICI)1099-0690(199902)1999:2<3c389::AID-EJOC389>3e3.0.CO;2-7). (b) Fink, B. E.; Kym, P. R.; Katzenellenbogen, J. A. Design, Synthesis, and Conformational Analysis of a Proposed Type I β -Turn Mimic. *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344. <https://doi.org/10.1021/ja974023y>. (c) Jones, G. I.; Jones, W.; North, M. Conformational Analysis of Peptides and Pseudopeptides Incorporating an endo-(2*S*,3*R*)-Norborn-5-ene Residue as a Turn Inducer. *J. Org. Chem.* **1998**, *63*, 1505–1513. <https://doi.org/10.1021/jo971766t>.
- ²⁶ (a) Romero, E.; Moussodia, R.-O.; Kriznik, A.; Wenger, E.; Acherar, S.; Jamart-Grégoire, B. Spontaneous Self-Assembly of Fully Protected Ester 1:1 [α/α - N^{α} -Bn-Hydrazino] Pseudodipeptides into a Twisted Parallel β -Sheet in the Crystal State. *J. Org. Chem.* **2016**, *81*, 9037–9045. <https://doi.org/10.1021/acs.joc.6b01680>. (b) Moussodia, R.-O.; Romero, E.; Wenger, E.; Jamart-Grégoire, B.; Acherar, S. Self-Organization Ability of Chiral N^{α} -Substituted, N^{β} -Boc Protected α -Hydrazinoacetamides in the Crystal and Solution States. *J. Org. Chem.* **2017**, *82*, 9937–9945. <https://doi.org/10.1021/acs.joc.7b01004>.
- ²⁷ Spartan'10, Wavefunction, Inc., Irvine, CA
- ²⁸ Karplus, M. Contact Electron Spin Coupling of Nuclear Magnetic Moments. *J. Chem. Phys.* **1959**, *30*, 11–15. <https://doi.org/10.1063/1.1729860>.
- ²⁹ (a) Bystrov, V. F. Spin-Spin Coupling and the Conformational States of Peptide Systems. *Progr. NMR Spectr.* **1976**, *10*, 41–81. [https://doi.org/10.1016/0079-6565\(76\)80001-5](https://doi.org/10.1016/0079-6565(76)80001-5). (b) Bystrov, V. F.; Ivanov, V. T.; Portnova, S. L.; Balashova, T. A.; Ovchinnikov, Y. A. Refinement of the angular dependence of the peptide vicinal NH–C $^{\alpha}$ H coupling constant. *Tetrahedron* **1973**, *29*, 873–877. [https://doi.org/10.1016/0040-4020\(73\)80031-6](https://doi.org/10.1016/0040-4020(73)80031-6).
- ³⁰ Schmidt, J. M.; Blümel, M.; Löhr, F.; Rüterjans, H. Self-Consistent 3J Coupling Analysis for the Joint Calibration of Karplus Coefficients and Evaluation of Torsion Angles. *J. Biomol. NMR* **1999**, *14*, 1–12. <https://doi.org/10.1023/A:1008345303942>.
- ³¹ Dorman, D. E.; Bovey, F. A. Proton Coupled Carbon-13 Magnetic Resonance Spectra. Simple Amides. *J. Org. Chem.* **1973**, *38*, 1719–1722. <https://doi.org/10.1021/jo00949a022>.
- ³² The structure *cryst-opt-14* was chosen instead of the *cryst-14* structure considering the inaccuracy of positioning of hydrogen atoms in the X-ray diffraction analysis.
- ³³ Jones, C. R.; Butts, C. P.; Harvey, J. N. Accuracy in Determining Interproton Distances Using Nuclear Overhauser Effect Data from a Flexible Molecule. *Beilstein J. Org. Chem.* **2011**, *7*, 145–150. <https://doi.org/10.3762/bjoc.7.20>.
- ³⁴ It has to be noticed that both *N*-protected (**19**) and unprotected (**20**) forms of this tripeptide display two conformers which are distinguished by NMR in CD₃OD.
- ³⁵ Interestingly, this debenzoylation procedure (Na/NH₃, low temperature) could be applied successfully to pseudopeptide **20**, as evidenced by the identification of the corresponding *N*-debenzoylated compound by ESI-TOF HRMS (*m/z*: [M+H]⁺ Calcd for C₁₆H₃₀N₅O₄ 356.2292; Found 356.2285). However, it was not possible to fully characterize this highly polar compound at a sufficient level of purity. The application of classical hydrogenolytic conditions (Pd/C 10%, formic acid, MeOH, rt, 24 h) to pseudopeptide **20** failed to provide this targeted *N*-debenzoylated derivative.
- ³⁶ (a) Metrano, A. J.; Abascal, N. C.; Mercado, B. Q.; Paulson, E. K.; Hurtley, A. E.; Miller, S. J. Diversity of Secondary Structure in Catalytic Peptides with β -Turn-Biased Sequences. *J. Am. Chem. Soc.* **2017**, *139*, 492–516. <https://doi.org/10.1021/jacs.6b11348>. (b) Metrano, A. J.; Chinn, A. J.; Shugrue, C. R.; Stone, E. A.; Kim, B.; Miller, S. J. Asymmetric Catalysis Mediated by Synthetic Peptides, Version 2.0: Expansion of Scope and Mechanisms. *Chem. Rev.* **2020**, *120*, 11479–11615. <https://doi.org/10.1021/acs.chemrev.0c00523>.
- ³⁷ This experiment was upgraded to 80 mmol scale.