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In-peptide synthesis of imidazolidin-2-one scaffolds, equippable with proteinogenic or taggable/linkable side chains, general promoters of unusual secondary structures

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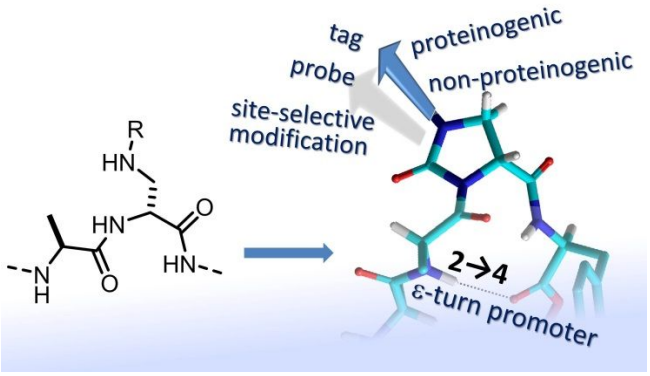


Abstract: Peptidomimetics containing (*S*)- or (*R*)-imidazolidin-2-one-4-carboxylate (Imi) have been obtained by the expedient *in-peptide* cyclization of (*S*)- or (*R*)-α,β-diaminopropionic acid (Dap) residues. These Imi scaffolds behave as proline analogues characterized by a flat structure and a trans-restricted geometry of the preceding peptide bond, and induce well-defined secondary structures in a biomimetic environment. While (*S*)-Imi peptides adopted a γ'-turn conformation, (*R*)-Imi induced the contemporary formation of a γ-turn and a rare 11-membered H-bonded structure in the 2→4 opposite direction of the sequence, identified as a ε-turn. In order to exploit these Imi scaffolds as general promoters of unusual secondary structures, proteinaceous side chains have been introduced at the *N*1 position of the five membered ring, potentially mimicking any residues. Finally, the Imi rings have been equipped with unnatural side chains, or with functionalized substituents, which can be utilized as linkers to chemoselectively bind the Imi-peptides onto nanoparticles, biomaterials, or diagnostic probes.

Introduction

Heterocyclic-based peptidomimetics have been widely utilized to increase metabolic and conformational stability of the parent peptides.[1] Amid them, *N*-heterocycles are prevalent in biologically active peptides and are increasingly attractive scaffolds in the development of new pharmaceuticals.[2] Relevant examples include pseudo-prolines,[3] Freidinger-Veber lactams or analogues,[4] and cyclic urea scaffolds.[5]

In particular, the latter have been utilized as structural elements in peptidomimetic inhibitors of HIV protease and HIV replication,[6] antibacterial MurB inhibitors,[7] dopamine D4 and CGRP receptor antagonists,[8] ACE inhibitors,[9] serine protease inhibitors,[10] integrin inhibitors.[11] In literature, cyclic ureas are most commonly constructed via treatment of 1,2-diamine precursors with carbonyldiimidazole,[6] or by intramolecular diamination of alkenes,[12] via rearrangement of Asn,[9,13] via ring expansion of aziridine derivatives,[14] by cyclization of aza-propargylglycinamides,[5b] by alkylation of the urea nitrogen of semicarbazone residues,[15] by Pd-catalyzed urea carboamination reactions,[16] and so on. Although a number of synthetic methods are available, many are not feasible in peptide chemistry. Thus, alternative strategies for the construction of cyclic ureas would provide more facile access to a class of peptidomimetics that are not readily available.

In this context, herein we report the expedient synthesis of peptidomimetics containing imidazolidin-2-one-4-carboxylate (Imi) scaffolds, by cyclization of sequences containing α,β-diaminopropionic acid (Dap). The unexpected conformational features of the Imi-peptides are also discussed with the aid of NMR analysis and molecular dynamics simulations.

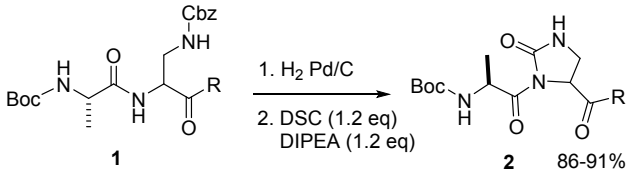
Furthermore, peptides containing *N*β-substituted Dap have been obtained from β-iodoalanine, by displacement with *N*-nucleophiles. The cyclization of the substituted Dap residues gave access to Imi-peptides carrying proteinogenic or unnatural side chains at the position 1, or eventually providing useful handles at which to undertake site-selective modifications of the sequences.[17] As a proof of concept, the allyl substituent at the position 1 of the Imi ring was selectively derivatized by the Heck reaction.

Results and Discussion

Imi-peptides synthesis. Model peptides **2** containing (*S*)- or (*R*)-Imi scaffolds have been obtained by the easy cyclization of the corresponding sequences **1** containing Dap (Scheme 1 and Table 1). The preparation of the peptides **1a-c** was conducted by coupling in solution Boc-protected amino

acids to amino ester counterparts under MW irradiation,[18] using a microwave oven specifically designed for organic synthesis, with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC)·HCl and 1-hydroxybenzotriazole (HOBt) as activating agents, in the presence of trimethylamine (TEA). Several Dap protected variants are commercially available; herein, Dap was introduced as Boc-Dap(Cbz)-OH. In any case, Dap can be rapidly prepared following the literature[19] and protected thereafter with the appropriate group. Boc deprotection was performed with 25 % TFA in DCM. The intermediates, obtained in quantitative yield, were not purified, while the final sequences **1a-c** were isolated in 80-85 % yield by flash chromatography over silica-gel (eluent 1:2 cyclohexane/EtOAc). Their identity was confirmed by ESI MS analysis, and purity was assessed to be 80-90 % by RP HPLC.

After removal of the *N*β-Cbz group by catalytic hydrogenation, the Dap-peptides were treated without further purifications with *N,N*′-disuccinimidyl carbonate (DSC) [3b,c] and DIPEA in 3:1 DCM/DMF, giving the Imi-peptides **2a-c** in good yield (86-91 %) after isolation by semi-preparative RP HPLC (Scheme 1, Table 1), > 90 % pure as determined by analytical RP HPLC. The identity of the compounds **2a-c** (and of the Imi-peptides described in the next sections) was confirmed by exact mass, proton-decoupled ^{13}C NMR, and ^{1}H NMR (the unambiguous assignment of the resonances was done by 2D gCOSY).



a (S)-Dap, R = Phe-OMe; **b** (S)-Dap, R = Phe-Val-OMe;
c (R)-Dap, R = Phe-Val-OMe

Scheme 1. In-solution preparation of Imi-peptides **2** from Dap-peptides **1**.

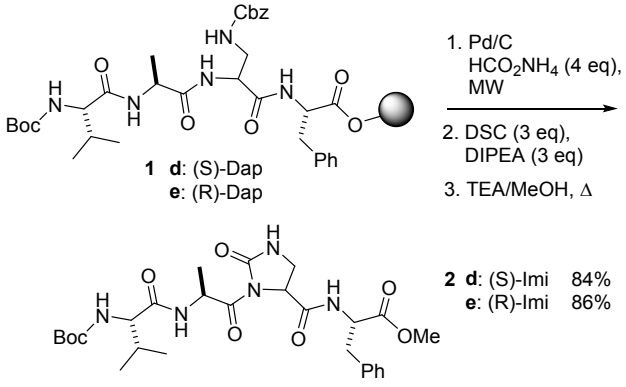
Table 1. Synthesis of model Imi-peptides **2** by cyclization of the corresponding Dap-containing peptides with DSC/DIPEA.

2	sequence	Peptide synthesis	yield %	purity %[a]
a	Boc-Ala-(*S*)-Imi-Phe-OMe	in-solution	86[b]	92
b	Boc-Ala-(*S*)-Imi-Phe-Val-OMe	"	88[b]	93
c	Boc-Ala-(*R*)-Imi-Phe-Val-OMe	"	91[b]	93

d	Boc-Val-Ala-(*S*)-Imi-Phe-OMe	SPPS	84[c]	95
e	Boc-Val-Ala-(*R*)-Imi-Phe-OMe	SPPS	86[c]	96

[a] Determined by RP HPLC. [b] Yield of the cyclization step, after isolation by RP HPLC. [c] Calculated on the average resin load, after isolation by RP HPLC.

Also, Imi-peptides were successfully obtained by solid phase peptide synthesis (SPPS) (Scheme 2). The tetrapeptides Boc-Val-Ala-(*S*)/(*R*)-Dap(Cbz)-Phe-resin (**1d**, **1e**) were prepared onto a Phe-preloaded Wang resin using standard Fmoc-protected amino acids and the EDC·HCl/HOBt/TEA coupling agents, under MW irradiation. Fmoc was removed by treatment with piperidine/DMF. After deprotection of the Cbz group by catalytic hydrogenation with ammonium formate (4 equiv.) in isopropanol/toluene,[20] cyclization was done on resin with DSC and DIPEA (3 equiv. each) under recently refined reaction conditions.[21] Subsequently, the cleavage from the resin with refluxing MeOH/TEA (Scheme 2)[22] gave the peptides **2d** or **2e**, isolated in a very satisfactory yield and purity (84 and 86 %, respectively, calculated on the basis of the average resin load, Table 1) as described above. These peptides were prepared as methyl esters and with Boc at the *N*-terminus, for structural comparison with the peptides **2b**,**c** (see next paragraph).



Scheme 2. SPPS of Imi-peptides **2d** or **2e**.

Epimerization of Dap during peptide synthesis and cyclization, in solution or in solid phase, was excluded on the basis of the analysis of the reaction mixtures of **2a-c** by HPLC MS and NMR.

Conformational analysis. X-ray crystallographic analysis of 3-acyl-imidazolidine-2-one-4-carboxylates showed that these heterocycles have a planar imidazolidine-2-one ring, and the amide

bond at the 3-position is restricted to the *trans* geometry, coplanar with the five-membered ring.[23] Theoretical computations quantified that the differences in potential energy between the *cis*- and *trans*-conformers were so large that the unstable *cis*-conformer would have an extremely low concentration.[23] This is in contrast to proline, which shows a puckered conformation of the pyrrolidine ring, and is involved in a *trans/cis* equilibrium about the preceding amide bond.[24] Hence, it is expected that the introduction of Imi scaffolds into a peptide sequence might impose alternative conformations and unusual secondary structures. Previous investigations on oligopeptides containing oxazolidin-2-one-4-carboxylates as pseudo-prolines showed the ability of the heterocycles to stabilize turns, foldamers,[3c-d,25] or even to form hydrogels.[26]

In this perspective, the conformational bias exerted by the Imi scaffold on the overall structure of model tetrapeptides was analyzed by NMR experiments at 400 MHz in 8:2 [D6]DMSO/H_2O, a solvent mixture recommended by several authors as an excellent biomimetic medium, in which intermolecular interactions are generally negligible for peptide concentrations in the millimolar range.[27] In any case, self-association of the peptides was excluded based on the absence of concentration effects on the chemical shift of non-exchangeable protons.[25,28] As representative examples, we analyzed the tetrapeptides **2b** and **2c**, which include (*S*)- or (*R*)-configured Imi, respectively, at the position 2 of the sequence, and peptides **2d** and **2e** having (*S*)- or (*R*)-Imi at the position 3. All spectra showed a single set of sharp resonances, suggestive of conformational homogeneity or of a fast equilibrium between slightly different conformers (Supporting Information). gCOSY experiments were utilized for the unambiguous assignment of the resonances.

In analogy to what observed for peptides containing oxazolidin-2-one-4-carboxylates, a nonconventional ImiC=O···H-C(α-1) intramolecular H-bond was expected (Figure1).[3c-d,25] Indeed, the ^{1}H NMR analyses of all of the compounds showed a significantly downfield position of the α-proton of the residues preceding the Imi rings, consistent with the deshielding effect exerted by ImiC=O for a *trans* conformation of the amide bond between Ala^1-Imi^2 (Figure 1). For instance, in Boc-Ala-(*S*)-Imi-Phe-Val-OMe (**2b**) the resonance of AlaHα appeared at about 5.2 ppm, while PheHα and ValHα appeared at about 4.6 and 4.2 ppm.

Variable-temperature (VT) ^{1}H NMR experiments have been widely utilized as a tool for structure investigation in polypeptides. Usually, for proteins or "rigid" peptides, the H-bonded amide-NH signals are characterized by modest temperature gradients in absolute value, i.e. $|\Delta\delta_{NH}/\Delta t| \leq$ 2.5 ppb/K,[29] while solvent-exposed NHs have larger negative values. Unfortunately, for acyclic peptides this "rule" has many exceptions.[30] Starting from these assumptions, we analyzed

the $\Delta\delta_{NH}/\Delta t$ of 0.01 M **2b-e**, in 8:2 mixture of [D6]DMSO/H_2O (Table S1 and Figure 1). For Boc-Ala-(*S*)-Imi-Phe-Val-OMe (**2b**), the comparatively much lower $\Delta\delta/\Delta t$ absolute value of Phe^{3}NH (-0.9 ppb/K) with respect to Ala^{1}NH and Val^{4}NH (-3.7, and -4.5 ppb/K, respectively), indicatively suggested that the former might be involved in a H-bond. On the other hand, peptides **2c**,**d** showed high $|\Delta\delta/\Delta t|$ values for all amide-NHs. Unexpectedly, Ala^{2}NH in Boc-Val-Ala-(*R*)-Imi-Phe-OMe (**2e**) appeared to be significantly less sensitive to increasing temperature than Val^{1}NH and Phe^{4}NH ($\Delta\delta/\Delta t$ = -2.4 versus -6.5 and -5.2 ppb/K, respectively), which might be compatible with a preference for a conformation having AlaNH involved in a H-bond.

The model compounds were analyzed by 2D-ROESY in 8:2 [D6]DMSO/H_2O, using 0.01 M peptide concentration, and the resulting cross-peaks were ranked by the intensity to infer plausible interproton distances. Compared to the homochiral Imi-peptides **2b** and **2d**, the heterochiral sequences **2c** and **2e** showed several inter-residue proton-proton correlations between non-consecutive residues (Figure 1 and Supporting Information).

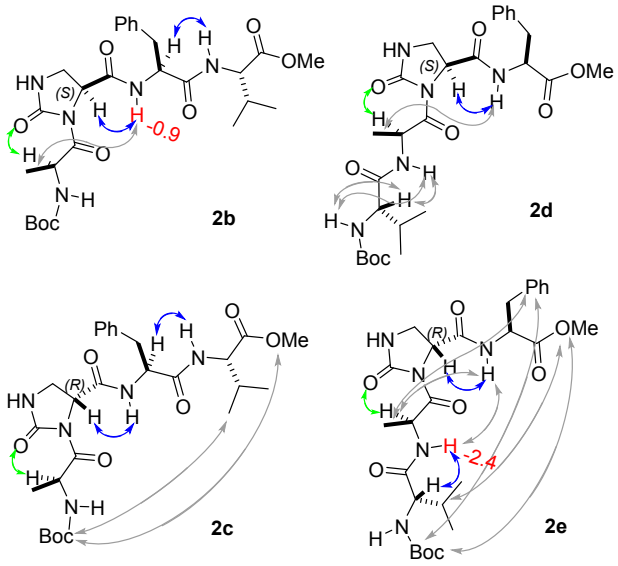


Figure 1. Sketches of **2b-e** showing selected, meaningful proton-proton NMR correlations indicated by arrows. The nonconventional ImiC=O···H-C(α-1) intramolecular interaction is rendered in green, strong ROESY correlations are given in blue, medium-intensity correlations in grey. The $\Delta\delta/\Delta t$ values for selected amide protons are also shown as red figures.

The estimated distances were analyzed by simulated annealing and restrained molecular dynamics simulations[31] using the AMBER force field[32] in explicit water as solvent. In brief, random geometries of each peptide were obtained by high-temperature unrestrained molecular dynamics

simulation in a box of standard TIP3P models of equilibrated water.[33] For each random structure, the inter-proton distances deduced by ROESY were introduced as constraints. Neither H-bond interactions, nor torsion angle restraints were introduced, while ω bonds were restricted at 180°, since the absence of Hα*i*-Hα(*i+1*) cross-peaks excluded the occurrence of *cis* peptide bonds.[34] Then the structures were subjected to high-temperature restrained molecular dynamics with a scaled force field, followed by a simulation period with full restraints. After slowly cooling the boxes, the geometries were minimized and the backbones of the Imi-peptides were clustered by the rmsd analysis.[31] In all cases, this procedure gave one major cluster comprising the majority of the structures (Figure S1). The representative conformers with the lowest energy are reported in Figure 2.

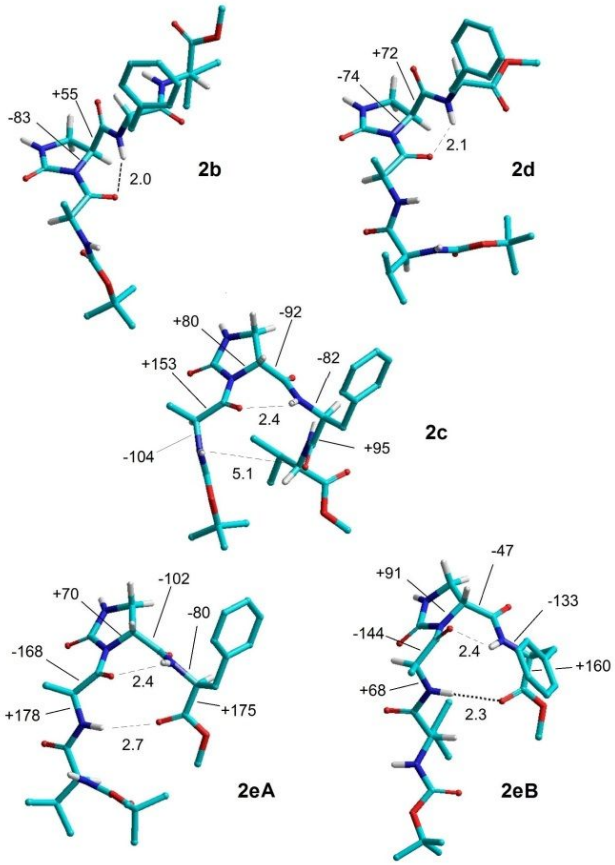


Figure 2. Representative conformers of **2b-e** calculated by ROESY-restrained in a 30×30×30 Å box of standard TIP3P models of equilibrated water. Heavy dotted lines represent H-bonds, light dotted grey lines indicate distances; ϕ/ψ dihedral angles of selected residues are also reported in degrees.

Among the four peptide models, the homochiral structures **2b** and **2d** showed comparatively more extended backbone conformations, as expected on the basis of the negligible number of inter-residue ROESY cross-peaks (Figure 1). Yet, both showed a clear inverse γ-turn centered on the Imi residue (Figure 2), and in particular for peptide **2b**, having its Imi residue at the position 2 of the sequence, the turn was stabilized by an explicit H-bond with a H to O distance of 2.0 Å (Figure 2 and Figure 3), consistent with the evidence of VT NMR (Table S1 and Figure 1).

To simulate the dynamic behavior of the peptides, the ROESY-derived conformers were analyzed by unrestrained molecular dynamics in explicit water, for 10 ns at rt. During the simulations, the γ-turn of **2b** resulted to be remarkably stable, correlated to the highly rigid Ala-Imi central scaffold and the flat imidazolidine-2-one ring. For **2d**, having Imi at position 3, the formation of a definite H-bond was prevented by a certain conformational freedom of the short Phe-OMe portion, but the γ-turn appearance was in general maintained. These results were not unexpected; also peptides containing the homochiral oxazolidin-2-one scaffold showed the preferential formation of γ-turns in [D6]DMSO or other competitive solvents.[25]

As for the heterochiral **2c** and **2e**, due to the many inter-residue ROESY cross-peaks (Figure 1), the calculated structures were tightly folded (**2c** and **2eA**, Figure 2). The sequence **2c**, containing Imi at the position 2, presented a γ-turn centered on Imi (Figure 2). Unexpectedly, the ROESY-derived structure **A** of **2e** (Figure 2) showed a regular γ-turn centered on Imi, plus an atypical turn involving the residues Ala-(*R*)-Imi-Phe, which seemed compatible with a ε-turn.[35]

The conformer **2eA** was analyzed by unrestrained molecular dynamics, and the analysis of the trajectories revealed the formation of a 11-membered macrocycle closed by an intramolecular H-bond between the Ala^{2}NH and Phe^{4}C=O, which is in agreement with the VT NMR temperature coefficient of AlaNH (Table S1). The representative structure **2eB** shown in Figure 2 is characterized by a distance of 2.4 Å between Ala^{2}C=O and Phe^{4}NH, and of 2.3 Å between Ala^{2}NH and Phe^{4}C=O (Figure 2 and Figure 3).

The ε-turn is a rare kind of secondary structure which can be observed in oligo-cyclopeptides and more seldom in linear peptides and proteins composed of all α-amino acids.[35] Unlike the more common 5→1 13-membered α-, 4→1 10-membered β-, and 3→1 7-membered γ-turns, the ε-turn involves 3 residues to form a hydrogen-bonded structure encompassing 11 atoms in the opposite 2→4 direction (Figure 3A). The ϕ/ψ dihedral angles found in **2eB** (Figure 2) assign the ε-turn to the all-*trans* class A in the convention of Toniolo and Balaram:[35] (ϕ/ψ in degrees) Ala, -144/+68; (*R*)-Imi, +91/-47; Phe, -133/+160.

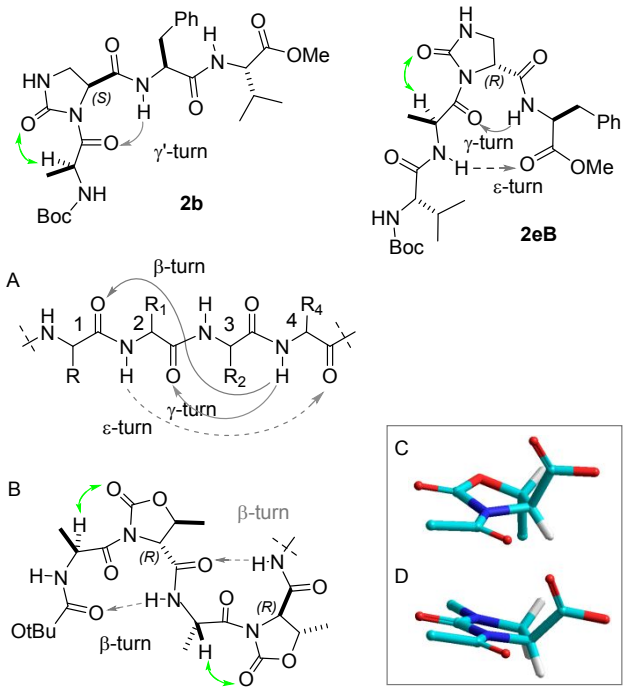


Figure 3. Sketch of the expected inverse γ-turn of the homochiral **2b**; the ImiC=O···H-C(α-1) interaction is rendered in green. Sketch of the class A ε/γ-mixed turns[35] in **2eB**. A) "Common way" intramolecular hydrogen bonds in a tetrapeptide (residues numbered 1 to 4) rendered as continuous grey arrows, and atypical 11 membered ε-turn in the 2→4 reversed direction as a dotted arrow. B) Oligomers composed of Ala-oxazolidin-2-one units produce a helix of "normal" β-turns.[3c-25] C) Crystal structure of the oxazolidin-2-one[25c] and D) of the imidazolidin-2-one scaffolds.[23]

The comparison between the structures of **2c** and **2e** in Figure 2 showed that the ε-turn was not completely formed in **2c**, albeit both compounds share the same Ala-(*R*)-Imi-Phe tripeptide sequence. Possibly, the failure of **2c** in forming a unequivocal H-bonded 2→4 turn can be attributed to presence of the bulky Boc group at Ala[1], as suggested by the analysis of the trajectories of the unrestrained molecular dynamics, which showed the clash between the *t*Bu and Val[4].

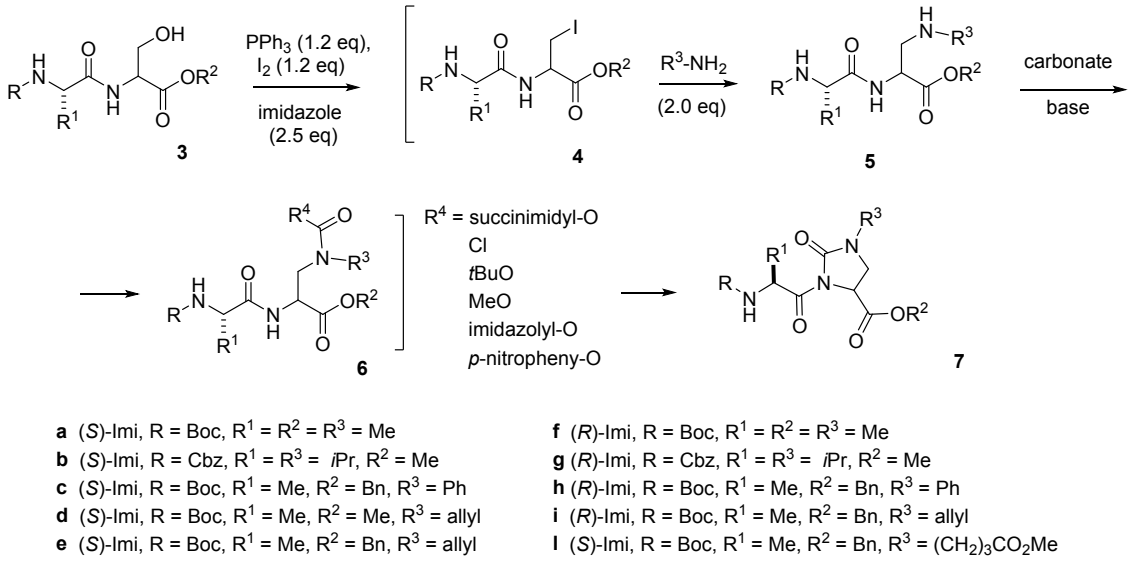
Finally, it is worth mentioning that in spite of the structural similarity with (*R*)-Imi, the (*R*)-oxazolidin-2-one-4-carboxylate scaffolds produced only "normal" conformations, i.e. γ-turns in [D6]DMSO and β-turns in $CDCl_3$.[25a-b] And oligomers formed of repetitive Ala-(*R*)-oxazolidin-2-one-4-carboxylate dipeptide units (≥ 5) folded in a ordered structure to give a variant of the 3_{10}-helix, namely a series of consecutive regular 4→1 β-turns (Figure 3B), in the crystal state and in MeOH.[25c] At present, the exhaustive investigation of the structural features of the Imi scaffold is beyond the scope of this work. Nevertheless, some clues can be perceived by the inspection of the

X-ray crystal structures of 3-acyl-oxazolidin-2-one-4-carboxylates[25c] and 3-acyl-imidazolidine-2-one-4-carboxylates[23] (Figure 3C,D). The comparison highlighted some relevant 3D differences which might be responsible for the different H-bonding patterns. While the geometry of the 5-membered rings is quite twisted in the oxazolidin-2-one (Figure 3C),[25c] it is extraordinarily flat in the Imi ring (Figure 3D).[23] This necessarily translates into different orientations of C4-carboxylate vectors, reasonably fundamental to the development of the overall conformations, and in particular the carboxylate group in the oxazolidin-2-one adopts an axial, almost vertical orientation above the 5-membered ring (Figure 3C).

Imidazolidin-2-ones substituted at the position 1. The formation of the Imi ring was attained by sacrificing the *N*β-amino group of Dap. Hence, Imi can be regarded to as a pseudo Pro showing the sole *trans* conformation at the preceding peptide bond. Intriguingly, conformational analysis by NMR and molecular dynamics has shown that (*S*)-Imi peptides adopt an inverse γ-turn conformation, while (*R*)-Imi promotes the formation of the rare ε/γ- secondary structure. This unexpected observation prompted us to possibly expand the scope of the Imi rings, making possible their use as general promoters of such unusual secondary structures also in sequences having at the position *i* residues other than a (pseudo) Pro. For this reason, we tackled the opportunity to introduce any substituents at the position 1 of the Imi scaffolds.

The strategy we adopted is outlined in Scheme 3. Dipeptides containing (*S*)- or (*R*)-serine **3** were prepared in solution as described in the literature.[36] The dipeptide esters **3** were isolated in good yield (in the 70-85 % range, 80-90 % pure according to analytical RP HPLC) by flash chromatography over silica gel. The dipeptides **3** were treated with a mixture of triphenylphosphine, iodine (1.2 equiv each), and imidazole (2.5 equiv),[37] affording β-iodo-Ala-dipeptides **4**, not isolated due to intrinsic instability;[37] TLC analyses of the reaction mixtures revealed the complete consumption of **3**.

The displacement of iodine with alkyl or arylamines, i.e. methylamine, isopropylamine, aniline, allylamine, γ-aminobutyric acid (GABA) methyl ester (2.0 equiv.) in DMF gave the *N*β-substituted Dap dipeptides **5**. Again, the TLC analyses of the crude reaction mixtures showed the disappearance of the reagents. Unfortunately, with the exception of Boc-Ala-Dap(*N*-allyl)-OMe **5d**, the isolation of these dipeptides **5** was not possible, either by chromatography over silica gel or by preparative RP HPLC, for the accumulation of the many side-products.



a (*S*)-Imi, R = Boc, $R^1 = R^2 = R^3 = Me$
b (*S*)-Imi, R = Cbz, $R^1 = R^3 =$ *i*Pr, $R^2 = Me$
c (*S*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Bn$, $R^3 = Ph$
d (*S*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Me$, $R^3 = allyl$
e (*S*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Bn$, $R^3 = allyl$
f (*R*)-Imi, R = Boc, $R^1 = R^2 = R^3 = Me$
g (*R*)-Imi, R = Cbz, $R^1 = R^3 =$ *i*Pr, $R^2 = Me$
h (*R*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Bn$, $R^3 = Ph$
i (*R*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Bn$, $R^3 = allyl$
l (*S*)-Imi, R = Boc, $R^1 = Me$, $R^2 = Bn$, $R^3 = (CH_2)_3CO_2Me$

Scheme 3. Synthesis of *N*1-substituted Imi-peptides **7**.

Hence, we opted for the direct cyclization of the crude dipeptides **5** with DSC/DIPEA, as reported above for the synthesis of Imi-peptides **2**. This reaction gave the formation of the intermediate *N*β-carbamate **6**, and of desired **7** only in traces, as observed by RP HPLC and ESI MS analysis of the reaction mixture. Even the highly pure **5d** failed to give the corresponding **7d**, suggesting that the scarce purity of the other reagents **5** was not the only responsible for the disappointing result. Thereafter, the procedure was repeated upon varying the conditions, using as model reagent the crude dipeptide Boc-Ala-Dap(*N*1Me)-OMe (**5a**), obtained from the Boc-Ala-β-iodoAla-OMe **4a** and methylamine. Besides to DIPEA, also Na_2CO_3, DMAP, and DBU, were tested. The increase of the amounts of DSC and/or of the base, the increase of temperature, and of reaction time, had very little impact (not shown), while the use of DMF as the only solvent gave a modest increase of the yield (Table 2, entry 1).

Apart from DSC, the use of other carbonates or dicarbonates such as triphosgene, Boc_2O, methyl chloroformate, CDI, entries 2 to 5, respectively, in combination with DIPEA or the other bases as above, was also poorly effective. Gratifyingly, *p*-nitrophenylchloroformate (pNPC)[38] and DIPEA (1.5 equiv each) in DMF at rt after 12 h gave **7a** in 70 % yield over 3 steps, accompanied by the *N*β-*p*-nitrophenoxycarbonyl-Dap(Me) intermediate **6** (25 %) (entry 6). The yield was further increased by changing DIPEA with DBU, and **7a** was isolated in excellent amount (90 %) by flash chromatography over silica gel (entry 7).

The same conditions were applied to the crude dipeptides **5b**,**c**,**e**, obtained as reported in Scheme 3 from the corresponding β-iodo-derivatives **4** and isopropylamine, aniline, allylamine,

respectively. The *N*-terminal capping group, the ester, and the amino acid preceding Dap, were varied to check the generality of the process. The benzyl ester **5e** was preferred to the methyl ester **5d** for the convenience of further transformations, as discussed in the next sections. In all cases, the reactions gave results comparable to **5a** (entries 8 to 10).

Table 2. Formation of imi-dipeptides **7** using carbonate/base (1.5 equiv) in DMF, after 12 h at rt.

entry	compd	Carbonate/base	**7** (%)	purity (%)[a]	**6** (%)
1	**a**	DSC/DIPEA	<10[b]	-	70[b]
2	"	$(Cl_3CO)CO$/base[c]	traces	-[d]	-[d]
3	"	Boc_2O/base[c]	"	-	75
4	"	$ClCO_2Me$/base[c]	"	-	80
5	"	CDI/base[c]	<5[b]	-	Traces
6	"	pNPC/DIPEA	70[b]	-	25
7	"	pNPC/DBU	90[e]	95	traces
8	**b**	"	85[e]	91	"
9	**c**	"	80[e]	92	10[b]
10	**e**	"	93[e]	94	traces
11	**f**	"	87[e]	93	"
12	**g**	"	82[e]	91	"
13	**h**	"	77[e]	96	11[b]
14	**i**	"	90[e]	92	"
15	**l**	"	74[e]	94	"

[a] After isolation, determined by RP HPLC ESI MS. [b] Determined by the RP HPLC analyses of the reaction mixtures. [c] Base: DIPEA, carbonate, DMAP, DBU. [d] Complex mixture of by-products. [e] Determined after isolation by flash chromatography over 3 synthetic steps respect to peptides **3**.

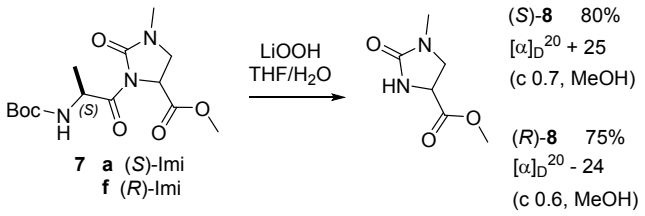
Subsequently, the procedure was repeated starting from the dipeptides containing D-serine. As for the (*S*)-configured series, the formation of the dipeptides containing (*R*)-β-iodoAla, followed

by displacement with the same amines, gave the dipeptides containing *N*-methyl, isopropyl, phenyl, and allyl (*R*)-Dap. Then, the treatment with pNPC/DBU gave (*R*)-Imi peptides **7f**, **7g**, **7h**, and **7i** in good to excellent yields (entries 11 to 14).

Finally, the dipeptide Boc-Ala-(*S*)-Imi[$(CH_2)_3CO_2Me$]OBn (**7l**) (Scheme 3), was prepared in sufficient yield by treatment of the β-iodoAla-dipeptide with GABA methyl ester, followed by cyclization under the usual conditions (entry 15).

Epimerization during the displacement of iodine from β-iodoAla by the amines, or during the following cyclization step, was excluded based on the comparison of the ^{1}H NMR of the (*S,S*)/(*S,R*) diastereomeric pairs with the peptides **2b**-**e** containing unsubstituted Imi. In particular, for all compounds containing (*R*)-Imi in $CDCl_3$ the proton ImiH4 appeared ≤ 4.75 ppm, while for compounds containing (*S*)-Imi ImiH4 appeared ≥ 4.8 ppm.

In any case, to confirm that the stereochemistry was maintained, the diastereomeric **7a** and **7f** were treated with LiOOH in THF/water at rt for 4 h, followed by 1M HCl,[39] leading to the detachment of Boc-Ala-OH and the release of the enantiomeric methyl 1-methyl-2-oxoimidazolidine-4-carboxylates (*S*)-**8** and (*R*)-**8**. After purification by flash chromatography over silica gel (eluent cyclohexane/EtOAc 1:3) their specific optical rotation was determined (Scheme 4) and judged in agreement with the literature for (*S*)-**8**: $[\alpha]_D^{20}$ +26.9 (c 1, MeOH).[23]



Scheme 4. Cleavage of the Imi rings **8** from **7a**,**f**.

These Imi dipeptide scaffolds carrying three possible different pharmacophores can be of some interest in peptidomimetic chemistry. There is evidence for the optimality of three-residue motifs for biological activity.[40] In a similar way as shown for *trans*-pyrrolidine-3,4-dicarboxamide templates by D. L. Boger *et al*. (Figure 4A),[41] the triply functionalized Imi dipeptides **7** (Figure 4) might be regarded as potential β-turn mimetics when the turn amino acid *i+1* serves as a structural rather than a recognition role, e.g. Pro or Gly (Figure 4B). Hence, Imi scaffolds reproducing the side chains of the residues *i*, *i+2*, *i+3*, could still maintain the bioactivity of the native peptides.

As for the oligomers, the Imi scaffolds have been shown to act as proline mimics inducing all-*trans* conformations in the sequences, and to promote classic or unusual turn structures, depending on the stereochemistry. Interestingly, while most of the conformationally restricted turn-inducing elements (e.g. pseudoprolines, Freidinger lactams, spirolactams, bicyclic thiazolidines, etc.) are generally deprived of some relevant pharmacophores,[1,42] the Imi rings can be equipped with proteogenic side chains (Figure 4C), e.g. of Ala, Val, Phe (Scheme 3).

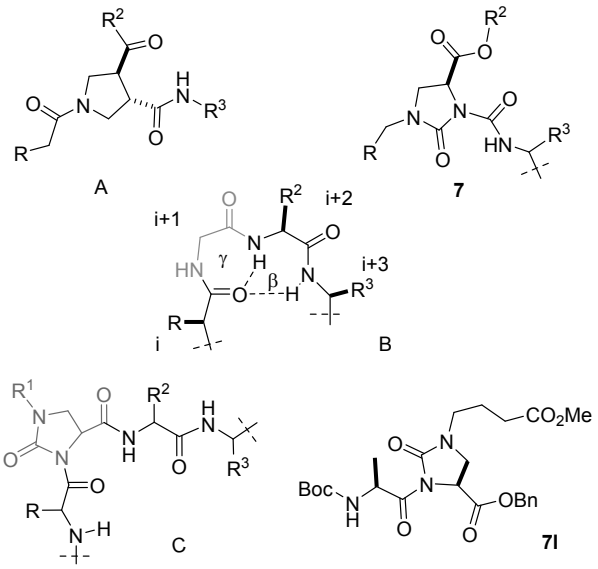
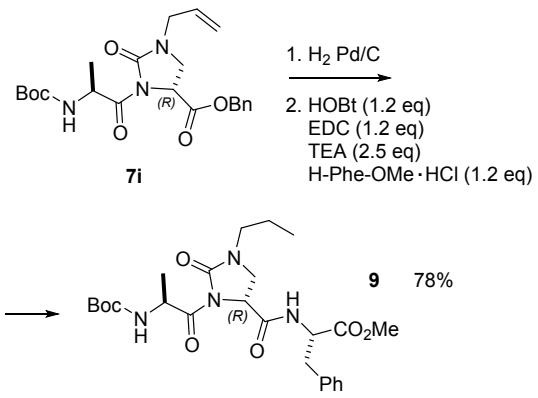


Figure 4. A) Pyrrolidine- and Imi-based β-turn mimetics **7**.[41] B) Classic γ- or β-turns. C) Imi as a turn inducing element for peptidomimetics equipped with side chains at each residue. Triply substituted Imi peptide **7l** with orthogonally-protected, further functionalizable groups.

To further challenge the potential utility of the N_1-substituted Imi dipeptides, Boc-Ala-(*R*)-Imi(*N*1allyl)-OBn (**7i**) was subjected to catalytic hydrogenation, and the resulting (*R*)-Imi(*N*1propyl) dipeptide acid was coupled with H-Phe-OMe·HCl, giving the tripeptide **9** equipped with a non proteogenic side chain (Scheme 5). As expected, the ^{1}H NMR analysis of **9** in 8:2 [D6]DMSO/H_2O supported that this Imi-*N*1-propyl tripeptide adopted a conformation consistent to that of the heterochiral models **2c** or **2e**. Indeed, the ^{1}H NMR of **9** was practically superimposable to that of the Boc-Ala-(*R*)-Imi-Phe portion of **2c**, while the only difference with the corresponding portion in **2d** was the obviously different chemical shift of AlaNH (Supporting Information). See for instance the highly reproducible pattern of the resonances of H4 and the two H5 protons (Supporting Information).

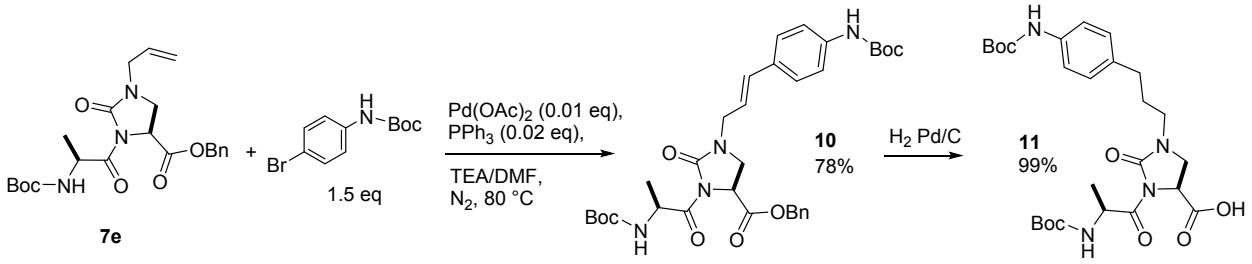


Scheme 5. Synthesis of the *N*1-substituted Imi-tripeptide **9** from the *N*1-allyl Imi-tripeptide **7i**.

On the other hand, the synthetic strategy described above was utilized also for introducing substituents carrying orthogonally protected functional groups. A first example was the dipeptide Boc-Ala-(*S*)-Imi[$(CH_2)_3CO_2Me$]OBn (**7l**) (Table 2, entry 15, and Figure 4), prepared with GABA methyl ester. In perspective, after hydrolysis under basic conditions, the *N*1-butanoic acid side chain might be exploited to graft the peptide onto e.g. surfaces, polymeric materials, nanoparticles, etc.

Finally, the *N*1-substituent of Imi can be equipped with a tag-residue for site-selective modifications of the sequences. Among the most popular reactions utilized to transform such handles without perturbing the remaining amino acids, it is possible to cite the Cu-catalyzed azide-alkyne cycloaddition, the Sonogashira, Suzuki-Miyaura, Mizoroki-Heck, Diels-Alder reactions, the photo-1,3-dipolar cycloaddition, the Staudinger ligation, and the olefin metathesis with Grubbs or Hoveyda-Grubbs catalysts.[17]

As a proof of concept, the allyl substituent at the position 1 of the Imi ring in **7e** was selectively derivatized with *N*-Boc-4-bromoaniline by the Heck reaction (Scheme 6). Conducted in DMF in the presence of $Pd(OAc)_2/PPh_3$/TEA, under inert atmosphere, at 80°C,[43] the reaction gave the adduct **10** characterized by a exclusively *trans* connection between the two portions, as determined by the -CH=CH- coupling constants in the ^{1}H NMR, J = 15.6 Hz (for *trans* alkenes J = 12-18 Hz, for *cis* alkenes J = 6-12 Hz). The reduction by catalytic hydrogenation gave a fully flexible linkage, and at the same time removed the benzyl ester protection, giving the dipeptide acid **11**, ready in prospective for the extension of the peptidic backbone.



Scheme 6. Heck reaction of the *N*1-allyl Imi-tripeptide **7e**, giving **10** and then **11**.

Conclusions

The introduction of nitrogen heterocycles into peptide sequences represents an effective approach to increase enzymatic stability and to induce well-defined secondary structures. To this purpose, we developed an expedient synthesis of hybrid peptides containing imidazolidin-2-one-4-carboxylate (Imi) scaffolds. These exceptionally flat pseudo-prolines demonstrated the ability to favor unusual conformations in a competitive and biomimetic solvent. While (*S*)-Imi peptides adopted a γ-turn conformation, (*R*)-configured Imi promoted the additional formation of a ε-turn, an infrequent secondary structure characterized by a 11-membered 2→4 H-bond going opposite respect to the classic γ- or β-turns. Besides, Imi can be equipped at the position *N*1 with substituents that reproduce the side chains of native amino acids, including Ala, Val, or Phe, in either L- or D-configuration, as well as with non-proteinogenic substituents, such as *n*-propyl or allyl. In this respect, the *N*1-substituted (*R*)-Imi rings represent a new class of general promoters the rare ε-turn in a variety of peptide sequences. On the other hand, Imi can also carry functional groups, for instance the allyl group, capable of selective reactions without perturbing the rest of the structure, e.g. by the Heck reaction. Potential future applications might include the glycosylation, prenylation, PEGylation, biotinylation, or attachment to solid surfaces, self-assembled monolayers, proteins, or the conjugation with fluorophores or antibodies.[17,44]

Experimental Section

General Experimental Methods. All purchased reagents were used without further purifications. Purities were assessed by analytical RP HPLC, and confirmed by exact mass, and elemental analysis. MW lab station: MicroSYNTH equipped with a built-in ATC-FO advanced fiber optic automatic temperature control. RP HPLC: Agilent 1100 series apparatus, with a RP column Phenomenex mod. Gemini 3μ C18 110 Å 100×3.0 mm, stationary phase octadecyl carbon chain-bonded silica with TMS endcapping, fully porous organo-silica solid support, particle size 3 μm,

pore size 110 Å, length 100 mm, internal diameter 3 mm, DAD 210 and 254 nm, mobile phase from 9:1 to 2:8 water/CH_3CN, in 20 min, at a flow rate of 0.5 mL min^{-1}, followed by 10 min at the same composition. Semi-preparative RP-HPLC: Agilent 1100 series, RP column ZORBAX mod. Eclipse XDBC18 PrepHT cartridge 21.2×150 mm 7μ, stationary phase octadecyl carbon chain bonded silica, double endcapped, particle size 7 mm, pore size 80 Å, length 150 mm, internal diameter 21.2 mm, DAD 210 nm; mobile phase from H_2O/CH_3CN (8:2) to CH_3CN (100 %) in 10 min at a flow rate of 12 mL min-1. HRMS: Waters Xevo QTOF. Routine ESI MS: MS single quadrupole HP 1100MSD detector, drying gas flow of 12.5 L min^{-1}, nebulizer pressure 30 psgi, drying gas temp 350°C, capillary voltage 4500(1) and 4000(2), scan 50-2600 amu. Elemental analysis: Thermo Flash 2000 CHNS/O. NMR characterization was done on a Varian Gemini 400 or 200, using 0.01-0.04 M peptide in 5 mm tubes at rt, using the solvents $CDCl_3$, or [D6]DMSO, or 8:2 [D6]DMSO/H_2O (water suppression by the solvent presaturation procedure PRESAT); ^1H spectra were recorded at 400 MHz, and the unambiguous assignment of the resonances was done by 2D gCOSY; proton-decoupled ^{13}C NMR were recorded at 100 or 75 MHz; chemical shifts were reported as δ values in ppm relative to solvent as internal standard.

Synthesis of peptides 1 in solution. General Procedure. A mixture of the Boc-amino acid (0.3 mmol) and HOBt (0.049 g, 0.36 mmol) was stirred in 3:1 DCM/DMF (5 mL) at rt, and after 10 min the amino ester counterpart (0.36 mmol), EDCI·HCl (0.069 g, 0.36 mmol) and TEA (0.13 mL, 0.94 mmol) were added in sequence while stirring at rt. After 2 h, the solvents were removed at reduced pressure, and the residue was diluted with EtOAc (20 mL). The slurry mixture was washed with 0.1 M HCl (5 mL), and a saturated solution of $NaHCO_3$ (5 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated at reduced pressure. The intermediate crude peptides, obtained in quantitative yield, were analyzed by RP HPLC and ESI MS, and were used without further purifications.

Boc deprotection was accomplished by stirring the crude peptides in 1:3 TFA/DCM (4 mL) for 30 min. Then the mixture was concentrated at reduced pressure, and the treatment was repeated. The residue was suspended in Et_2O (20 mL), and the peptide-TFA salts which precipitated in almost quantitative yield were used for the next couplings without further purifications.

The final sequences **1** were isolated by flash chromatography over silica-gel (eluent 1:2 cyclohexane/EtOAc), 80-85 % yield, 80-90 % pure by RP HPLC (General Methods). The identity was confirmed by ESI MS analysis (General Methods).

Cyclization to Imi-peptides 2a-c in solution. General Procedure. The peptides **1** (0.2 mmol) were dissolved in EtOH (15 mL), and a 2 L balloon filled with H_2 was applied in the presence of a

catalytic amount of 10 % Pd/C, while stirring at rt. The reactions were monitored by TLC, and were generally judged complete in 6 h. Then the mixture was filtered over celite® and the solvent was evaporated at reduce pressure.

The residue was dissolved in in 3:1 DCM/DMF (5 mL) and DSC (0.061 g, 0.24 mmol) and DIPEA (0.044 mL, 0.24 mmol) were added at rt under inert atmosphere. The mixture was stirred for 3 h, then the solvent was distilled under reduced pressure, the residue was diluted with 0.1 M HCl (5 mL), and the mixture was extracted three times with DCM (10 mL). The combined organic layers were dried over Na_2SO_4, and concentrated at reduced pressure. The residue was separated by semi-preparative RP HPLC on a C18 column, eluent from H_2O/CH_3CN (8:2) to CH_3CN (100 %) in 10 min, flow rate 12 mL min^{-1} (General Methods), and purity was determined by RP HPLC (General Methods).

Boc-Ala-(*S*)-Imi-Phe-OMe (**2a**). Cyclization of **1a** (0.11 g, 0.19 mmol) according to the General Procedure gave **2a** (0.077 g, 86 %, 92 % pure). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 1.17 (d, J = 7.1 Hz, 3H, AlaMe), 1.35 (s, 9H, tBu), 2.93-3.02 (m, 2H, PheHβ), 3.08 (dd, J = 3.0, 9.6 Hz, 1H, ImiH5), 3.57 (s, 3H, OMe), 3.63 (dd, J = 9.6, 10.0 Hz, 1H, ImiH5), 4.44 (m, 1H, PheHα), 4.75 (dd, J = 3.0, 10.0 Hz, 1H, ImiH4), 5.18 (dd, J = 7.1, 14.4 Hz, 1H, AlaHα), 7.09 (d, J = 8.0 Hz, 1H, AlaNH), 7.18-7.35 (m, 5H, ArH), 7.73 (s, 1H, ImiNH1), 8.58 (d, J = 6.8 Hz, 1H, PheNH); ^{13}C{1H} NMR ([D6]DMSO, 100 MHz), δ 18.2, 29.1, 37.5, 41.3, 49.0, 52.8, 54.7, 55.3, 78.8, 127.6, 129.3, 130.0, 137.7, 156.1, 170.6, 172.7, 174.4; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C22H31N4O7 463.2193; Found 463.2164. Anal. Calcd for C22H30N4O7: C, 57.13; H, 6.54; N, 12.11. Found: C, 57.19; H, 6.49; N, 11.99.

Boc-Ala-(*S*)-Imi-Phe-Val-OMe (**2b**). Cyclization of **1b** (0.14 g, 0.21 mmol) according to the General Procedure gave **2b** (0.10 g, 88 %, 93 % pure). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 0.82-0.93 (m, 6H, ValMe), 1.17 (d, J = 7.2 Hz, 3H, AlaMe), 1.34 (s, 9H, *t*Bu), 2.01 (m, 1H, ValHβ), 2.80 (dd, J = 8.8, 13.4 Hz, 1H, PheHβ), 2.99 (dd, J = 4.0, 13.4 Hz, 1H, PheHβ), 3.08 (dd, J = 2.2, 9.8 Hz, 1H, ImiH5), 3.58 (m, 1H, ImiH5), 3.62 (s, 3H, OMe), 4.14 (dd, J = 7.2, 7.6 Hz, 1H, ValHα), 4.56 (m, 1H, PheHα), 4.73 (dd, J = 2.8, 7,2 Hz, 1H, ImiH4), 5.16 (m, 1H, AlaHα), 7.00 (d, J = 7.6 Hz, 1H, AlaNH), 7.16-7.30 (m, 5H, PheArH), 7.71 (s, 1H, ImiNH1), 8.24 (d, J = 7.6 Hz, 1H, ValNH), 8.28 (d, J = 8.0 Hz, 1H, PheNH); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 17.8, 18.8, 28.3, 29.7, 31.1, 38.1, 49.0, 52.2, 54.9, 56.0, 57.5, 79.9, 127.1, 127.2, 128.8, 129.1, 129.3, 136.0, 154.6, 155.2, 168.5, 170.2, 171.6, 175.0; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C27H40N5O8 562.2877; Found 562.2821. Anal. Calcd for C27H39N5O8: C, 57.74; H, 7.00; N, 12.47. Found: C, 57.81; H, 6.90; N, 12.54.

Boc-Ala-(*R*)-Imi-Phe-Val-OMe (**2c**). Cyclization of **1c** (0.12 g, 0.18 mmol) according to the General Procedure gave **2c** (0.092 g, 91 %, 93 % pure). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 0.88 (d, J = 7.2 Hz, 3H, ValMe), 0.91 (d, J = 6.8 Hz, 3H, ValMe), 1.15 (d, J = 6.4 Hz, 3H, AlaMe), 1.35 (s, 9H, tBu), 2.05 (m, 1H, ValHβ), 2.42 (m, 1H, ImiH5), 2.71 (dd, J = 10.0, 13.7 Hz, 1H, PheHβ), 3.06 (dd, J = 3.8, 13.7 Hz, 1H, PheHβ), 3.34 (dd, J = 4.8, 12.0 Hz, 1H, ImiH5), 3.63 (s, 3H, OMe), 4.30 (m, 1H, ValHα), 4.56 (m, 1H, ImiH4), 4.71 (ddd, J = 3.8, 8.8, 10.0 Hz, 1H, PheHα), 5.31 (dq, J = 6.4, 8.8 Hz, 1H, AlaHα), 6.62 (d, J = 8.8 Hz, 1H, AlaNH), 7.17-7.27 (m, 5H, PheArH), 7.62 (s, 1H, ImiNH1), 8.20 (d, J = 7.6 Hz, 1H, ValNH), 8.37 (d, J = 8.8 Hz, 1H, PheNH); ^{13}C{1H} NMR ([D6]DMSO, 100 MHz) δ 18.5, 19.0, 19.2, 28.3, 29.9, 30.8, 38.0, 48.1, 51.8, 53.0, 55.2, 57.8, 78.1, 126.4, 128.0, 129.4, 137.6, 154.6, 155.0, 169.2, 171.3, 171.8, 172.9; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C27H40N5O8 562.2877; Found 562.2849. Anal. Calcd for C27H39N5O8: C, 57.74; H, 7.00; N, 12.47. Found: C, 57.55; H, 7.13; N, 12.17.

Synthesis of Imi-peptides 2d,e in solid-phase. General Procedure. Wang resin pre-loaded with Fmoc-Phe (0.25 g, 0.4-0.8 mmol g^{-1}, particle size 100-200 mesh) was placed into a reactor equipped with a filter, and suspended in DCM (5 mL). Fmoc was removed by treatment with 1:4 piperidine/DMF (5 mL) under MW irradiation at 40 W while bubbling N_2 in an open vessel, monitoring the internal temperature at 45°C. After 2 min, the suspension was filtered, and the procedure was repeated. Then, the resin was washed three times in sequence with DCM, DMF, and MeOH (5 mL each).

The resin was swollen in DCM (8 mL), and a mixture of Fmoc-Dap(Bn)OH (0.137 g, 0.3 mmol) and HOBt (0.041 g, 0.3 mmol) in DMF (4 mL) was added, followed by DCC (0.078 g, 0.38 mmol). The mixture was heated at 45°C for 10 min under MW irradiation as described above, while bubbling N_2. The resin was filtered and washed three times with the sequence DCM, DMF, MeOH (5 mL each). Coupling efficacy was determined by means of the Kaiser test. All the remaining residues were attached by the same protocols.

The resulting peptidyl-resin **1d** or **1e** was suspended in 1:3 iPrOH/toluene (10 mL), and HCO_2NH_4 (0.038 g, 0.6 mmol) and a catalytic amount of 10 % Pd/C were added in sequence. The mixture was irradiated at 600 W for eight cycles of 1 min each. The resin-bound peptide was filtered and washed three times in sequence with DCM, DMF, and MeOH (5 mL each).

Then the peptidyl-resin was suspended in 3:1 DCM/DMF (5 mL), and DSC (0.116 g, 0.45 mmol) and DIPEA (80 μL, 0.45 mmol) were added at rt under inert atmosphere. After 3 h the mixture was filtered, and the resin-bound peptide was washed three times in sequence with DCM, DMF, and MeOH (5 mL each).

A suspension of the peptidyl-resin in 1:3 triethylamine/MeOH (20 mL) was heated at 60°C for 20 min under MW irradiation in an open vessel. After filtration, the resin was washed twice with DCM and Et_2O (10 mL each), the filtrates were collected, and the volatiles were evaporated at reduced pressure. The Imi-peptides were separated by semi-preparative RP HPLC on a C18 column (General Methods) giving **2d** or **2e** in good yield (calculated on an average resin load of 0.6 mmol g^{-1}), and high purity, as determined by analytical RP HPLC (General Methods).

Boc-Val-Ala-(*S*)-Imi-Phe-OMe (**2d**). The procedure gave peptide **2d** (0.069 g, 84 %, 95 % pure). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 0.85 (d, J = 6.8 Hz, 3H, ValMe), 0.87 (d, J = 6.8 Hz, 3H, ValMe), 1.24 (d, J = 7.0 Hz, 3H, AlaMe), 1.38 (s, 9H, tBu), 1.93 (m, 1H, ValHβ), 2.97 (dd, J = 7.6, 13.8 Hz, 1H, PheHβ), 3.02 (dd, J = 5.6, 13.8 Hz, 1H, PheHβ), 3.09 (dd, J = 3.4, 9.4 Hz, 1H, ImiH5), 3.57 (s, 3H, OMe), 3.62 (m, 1H, ImiH5), 3.79 (m, 1H, ValHα), 4.44 (ddd, J = 5.6, 6.8, 7.6 Hz, 1H, PheHα), 4.75 (dd, J = 3.4, 10.0 Hz, 1H, ImiH4), 5.45 (m, 1H, AlaHα), 6.59 (d, J = 9.2 Hz, 1H, ValNH), 7.19-7.24 (m, 3H, PheArH), 7.25-7.31 (m, 2H, PheArH), 7.77 (s, 1H, ImiNH1), 8.00 (d, J = 7.6 Hz, 1H, AlaNH), 8.59 (d, J = 6.8 Hz, 1H, PheNH); ^{13}C{1H} NMR ([D6]DMSO, 75 MHz) δ 18.5, 19.1, 20.1, 29.1, 29.9, 30.4, 31.5, 37.4, 52.8, 54.7, 55.3, 60.1, 79.9, 127.6, 129.2, 130.0, 137.7, 156.0, 156.7,170.5, 171.7, 172.6, 174.4; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C27H40N5O8 562.2877; Found 562.2822. Anal. Calcd for C27H39N5O8: C, 57.74; H, 7.00; N, 12.47. Found: C, 57.85; H, 6.85; N, 12.58.

Boc-Val-Ala-(*R*)-Imi-Phe-OMe (**2e**). The procedure gave peptide **2e** (0.071 g, 86 %, 96 % pure). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 0.76 (d, J = 6.8 Hz, 3H, ValMe), 0.81 (d, J = 6.0 Hz, 3H, ValMe), 1.20 (d, J = 6.4 Hz, 3H, AlaMe), 1.35 (s, 9H, tBu), 1.94 (m, 1H, ValHβ), 2.67 (dd, J = 2.2, 9.8 Hz, 1H, ImiH5), 2.88 (dd, J = 10.0, 13.3 Hz, 1H, PheHβ), 3.06 (dd, J = 5.4, 13.3 Hz, 1H, PheHβ), 3.45 (m, 1H, ImiH5), 3.61 (s, 3H, OMe), 3.80 (dd, J = 6.6, 8.0 Hz, 1H, ValHα), 4.48 (ddd, J = 5.4, 8.0, 10.0 Hz, 1H, PheHα), 4.60 (dd, J = 2.2, 9.8 Hz, 1H, ImiH4), 5.58 (m, 1H, AlaHα), 6.75 (d, J = 8.0 Hz, 1H, ValNH), 7.16-7.22 (m, 3H, PheArH), 7.23-7.30 (m, 2H, PheArH), 7.73 (s, 1H, ImiNH1), 7.86 (d, J = 8.0 Hz, 1H, AlaNH), 8.54 (d, J = 8.0 Hz, 1H, PheNH); ^{13}C{1H} NMR ([D6]DMSO, 100 MHz) δ 18.9, 19.2, 19.6, 28.6, 30.7, 37.4, 41.0, 47.1, 52.3, 53.7, 55.4, 56.5, 60.0, 78.6, 124.8, 126.0, 127.0, 128.6, 129.6, 137.4, 155.2, 155.9, 169.6, 171.0, 172.0, 172.7; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C27H40N5O8 562.2877; Found 562.2845. Anal. Calcd for C27H39N5O8: C, 57.74; H, 7.00; N, 12.47. Found: C, 57.90, H, 6.83, N, 12.60.

Conformational analysis by NMR. Peptide samples were dissolved in 8:2 [D6]DMSO/H_2O in 5 mm tubes to the final concentration of 0.01 M. At this concentration, the intramolecular aggregation in mixtures of [D6]DMSO and H_2O is usually unimportant. Besides, self-association of the peptides

was excluded based on the reproducibility of the chemical shift of non-exchangeable protons in the concentration range 0.01-0.04 M (not shown). Water suppression was achieved by the PRESAT procedure implemented in Varian. Proton resonance assignment was accomplished through gCOSY. VT ^{1}H NMR experiments were recorded over the range of 298-348 K; temperature calibration was done with the ethylene glycol OHCHn chemical-shift separation method. 2D ROESY experiments were done at rt, phase-sensitive mode, spin-locking field (γb2) = 2000 Hz, mixing time = 250 ms; spectra were processed in the hypercomplex approach; peaks were calibrated on solvent. Only ROESY-derived constraints were included in the restrained MD. Cross-peak intensities were ranked and associated to the distances (Å): very strong = 2.3, strong = 2.6, medium = 3.0, weak = 5.0. The intensities of the cross peaks arising from protons separated by known distances (e.g., geminal) were found to match with these associations, but were discarded. For the absence of Hα(*i, i+1*) ROESY cross peaks, all of the ω bonds were set at 180° (f constant: 16 kcal mol-1 Å^{-2}).

MD simulations. The restrained MD simulations were conducted at 300 K and 1 atm by using the AMBER force field in a 30×30×30 Å^{3} box of standard TIP3P models of equilibrated water, periodic boundary conditions dielectric scale factor = 1, cutoff for the nonbonded interactions = 12 Å; all water molecules closer than 2.3 Å to a solute atom were eliminated. 50 Random structures were generated by a 100 ps simulation at 1200 K; these were subsequently subjected to restrained MD, 50 ps with a 50 % scaled force field at 1200 K, then by 50 ps with full distance restraints, force constant = 7 kcal mol^{-1}Å^{-2}, after which the system was cooled in 20 ps to 50 K. H-bond interactions were not included, nor were torsion angle restraints. The resulting structures were minimized by 3000 cycles of steepest descent and 3000 cycles of conjugated gradient, convergence = 0.01 kcal Å^{-1}mol^{-1}. The backbones of the structures were clustered by the rmsd analysis.

Unrestrained MD simulations were performed starting with the conformation derived from ROESY in the box of standard TIP3P water for 10 ns at 298 K using periodic boundary conditions, at constant temperature and pressure (Berendsen scheme,[45] bath relaxation constant of 0.2). For 1-4 scale factors, van de Waals and electrostatic interactions are scaled in AMBER to half their nominal value. The integration time step was set to 0.1 fs. The system coordinates were collected every picosecond.

Synthesis of peptides 3. The reaction was performed under conditions which allow using (*S*)- or (*R*)-serine esters without protection of the hydroxy group.[36] Briefly, a stirred solution of the *N*-protected amino acid (0.5 mmol) in 4:1 DCM/DMF (4 mL) was treated with HOBt (0.6 mmol) and HBTU (0.6 mmol), at r.t. and under inert atmosphere. After 5 min, serine ester (0.55 mmol), and

DIPEA (1.2 mmol) were added and the reaction was stirred for 10 min under MW irradiation, setting the internal reaction temperature at 80 °C. After concentration of the mixture at reduced pressure, the residue was diluted with EtOAc (20 mL). The organic layer was washed with 0.1 M HCl (5 mL), and a saturated solution of $NaHCO_3$ (5 mL), and was dried over Na_2SO_4. After evaporation of the solvent at reduced pressure, the crude dipeptide esters **3** were isolated (70-85 %, 80-90 % pure according to analytical RP HPLC) by flash chromatography over silica gel (eluent 1:3 cyclohexane/EtOAc), and were identified by ESI MS.

Synthesis of *N*1-substituted Imi-peptides 7. General Procedure. To a solution of triphenylphosphine (0.080 g, 0.31 mmol) in anhydrous DCM (4 ml), iodine (0.080 g, 0.32 mmol) was added at rt under inert atmosphere and magnetic stirring. After 15 min, imidazole (0.043 g, 0.6 mmol) was also added and the mixture was stirred for additional 15 min. The dipeptide **3** (0.25 mmol), dissolved in DCM (2 ml), was finally added and the reaction mixture refluxed for 2 h. The mixture was then cooled, diluted with DCM (6 ml), and washed with 10 % $Na_2S_2O_4$ in water (4 ml). The organic layer was dried over Na_2SO_4, and the solvents were evaporated at reduced pressure to the final volume of 3 mL. The solution was allowed to stand at 5 °C overnight, and some white solid Ph_3PO which precipitated not quantitatively was removed by filtration and washed with DCM (5 mL). The filtrate and the washes were evaporated at reduced pressure to give the crude iodides **4**.

The crude iodides **4** (0.25 mmol) were dissolved in anhydrous DMF (5 mL), the amine (0.5 mmol) was added, and the mixture was heated at 50°C for 3 h. Then DMF was distilled at reduced pressure, the residue was suspended in 0.1 M HCl (12 mL), and the mixture was washed with Et_2O (5 mL). The pH of the water layer was corrected to 9-10 with sat. Na_2CO_3, then it was extracted 3 times with EtOAc (5 mL). The collected organic layers were dried over Na_2SO_4, and solvent was removed at reduced pressure. The crude residues were utilized for the next step without further purifications, the only exception being **5d**, which was purified as described thereafter.

Boc-Ala-Dap(allyl)-OMe (**5d**). The residue was purified by flash chromatography over silica gel (eluent EtOAc/MeOH 98:2), giving the dipeptide (0.058 g, 70 %, 94 % pure by RP HPLC). ^{1}H NMR ($CDCl_3$, 400 MHz) 2 conformers δ 1.39 (d, J = 6.8 Hz, 3H, AlaMe), 1.44+1.45 (s, 9H, *t*Bu), 1.69 (br. s, 1H, NH), 2.98 (dd, J = 4.4, 12.7 Hz, 1H, DapHβ), 3.08 (dd, J = 5.4, 12.7 Hz, 1H, DapHβ), 3.22-3.27 (m, 2H, NCH_2), 3.76 (s, 3H, OMe), 4.19 (m, 1H, AlaHα), 4.63 (m, 1H, DapHα), 4.99 (br.d, 1H, AlaNH), 5.05-5.19 (m, 2H, $=CH_2$), 5.82 (m, 1H, CH=), 6.92+7.06 (br.d, 1H, DapNH); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) 2 conformers δ 18.7, 28.6, 49.8, 52.3, 52.6, 52.8, 55.2, 77.6, 116.7+116.8, 136.5+136.6, 155.7, 172.0+172.1, 172.9+173.0; HRMS (ESI/QTOF) m/z:

[M + H]+ Calcd for C15H28N3O5 330.2029; Found 330.2060. Anal. Calcd for C15H27N3O5: C, 54.70; H, 8.26; N, 12.76. Found: C, 54.84; H, 8.33; N, 12.87.

The oily residues containing the crude dipeptides **5** were dissolved in DMF (5 mL), pNPC (0.075 g, 0.38 mmol) and DBU (0.055 g, 0.38 mmol) were added, and the mixture was stirred at rt for 12 h. The solvent was distilled at reduced pressure, and 0.1 M HCl (4 mL) was added, then the residue was extracted 3 times with EtOAc (5 mL). The collected organic layers were dried over Na_2SO_4, and the solvent was removed at reduced pressure. The Imi dipeptides **7** were isolated from the resulting mixtures by flash chromatography over silica gel (eluent EtOAc/MeOH 98:2), and purity was determined by RP HPLC (General Methods).

Boc-Ala-(*S*)-Imi(*N*1Me)-OMe (**7a**). Starting from **3a** (0.070 g, 0.24 mmol), the General Procedure gave **7a** (0.071 g, 90 %, 95 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38-1.48 (m, 12H, AlaMe+*t*Bu), 2.87 (s, 3H, *N*Me), 3.38 (dd, J = 3.4, 9.8 Hz, 1H, ImiH5), 3.71 (t, J = 10.0 Hz, 1H, ImiH5), 3.77 (s, 3H, OMe), 4.82 (dd, J = 3.4, 9.8 Hz, 1H, ImiH4), 5.08 (d, J = 7.6 Hz, 1H, AlaNH), 5.51 (m, 1H, AlaHα); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 18.4, 28.3, 30.5, 46.6, 48.7, 51.9, 52.9, 79.6, 153.1, 155.3, 169.9, 174.1; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C14H24N3O6 330.1665; Found 330.1688. Anal. Calcd for C14H23N3O6: C, 51.06; H, 7.04; N, 12.76. Found: C, 50.84; H, 7.28; N, 13.00.

Cbz-Val-(*S*)-Imi(*N*1*i*Pr)-OMe (**7b**). Starting from **3b** (0.088 g, 0.25 mmol), the General Procedure gave **7b** (0.089 g, 85 %, 91 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (d, J = 7.2 Hz, 3H, ValMe), 0.95 (d, J = 7.2 Hz, 3H, ValMe), 1.02 (d, J = 6.4 Hz, 3H, *i*PrMe), 1.10 (d, J = 6.4 Hz, 3H, *i*PrMe), 2.25 (m, 1H, ValHβ), 2.35 (m, 1H, N_1CH), 3.33 (dd, J = 3.6, 9.8 Hz, 1H, ImiH5), 3.66 (t, J = 9.8 Hz, 1H, ImiH5), 3.78 (s, 3H, OMe), 4.82 (dd, J = 3.6, 9.8 Hz, 1H, ImiH4), 5.11 (br.s, 2H, $PhCH_2$), 5.41 (d, J = 8.0 Hz, 1H, ValNH), 5.61 (m, 1H, ValHα), 7.33-7.41 (m, 5H, ArH); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 16.6, 18.8, 21.1, 22.6, 28.7, 41.4, 48.3, 50.2, 52.5, 56.7, 61.2, 128.0, 129.0, 129.1, 137.7, 153.4, 155.9, 173.5, 175.3; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C21H30N3O6 420.2135; Found 420.2172. Anal. Calcd for C21H29N3O6: C, 60.13; H, 6.97. N, 10.02. Found: C, 60.22; H, 7.26; N, 10.10.

Boc-Ala-(*S*)-Imi(*N*1Ph)-OBn (**7c**). Starting from **3c** (0.084 g, 0.23 mmol), the General Procedure gave **7c** (0.072 g, 80 %, 92 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (d, J = 6.8 Hz, 3H, AlaMe), 1.44 (s, 9H, *t*Bu), 3.84 (dd, J = 3.2, 9.6 Hz, 1H, ImiH5), 4.19 (dd, J = 9.6, 10.0 Hz, 1H, ImiH5), 5.00 (dd, J = 3.2, 10.0 Hz, 1H, ImiH4), 5.10 (d, J = 7.2, 1H, AlaNH), 5.17 (d, J = 12.0 Hz, 1H, PhCH), 5.26 (d, J = 12.0 Hz, 1H, PhCH), 5.53 (m, 1H, AlaHα), 7.18 (m, 1H, ArH), 7.30-7.41 (m, 7H, ArH), 7.43-7.50 (m, 2H, ArH); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 18.2, 28.3, 45.3, 49.1,

51.6, 67.9, 79.8, 119.3, 124.9, 128.5, 128.7, 128.8, 129.1, 134.6, 137.9, 150.9, 155.3, 168.9, 174.4; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C19H26N3O6 392.1822; Found 392.1856. Anal. Calcd for C19H25N3O6: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.47; H, 6.35; N, 10.83.

Boc-Ala-(*S*)-Imi(*N*1allyl)-OBn (**7e**). Starting from **3c**[46] (0.091 g, 0.25 mmol), the General Procedure gave **7e** (0.099 g, 93 %, 94 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, J = 7.2 Hz, 3H, AlaMe), 1,43 (s, 9H, *t*Bu), 3.32 (dd, J = 3.0, 9.8 Hz, 1H, ImiH5), 3.65 (dd, J = 9.8, 10.4 Hz, 1H, ImiH5), 3.78 (dd, J= 5.2, 14.8 Hz, 1H, CH_2-CH=), 3.92 (dd, J= 6.2, 15.8 Hz, 1H, CH_2-CH=), 4.87 (dd, J = 3.0, 10.4 Hz, 1H, ImiH4), 5.08 (d, J = 8.0 Hz, 1H, AlaNH), 5.17-5.27 (m, 4H, =CH_2+PhCH_2), 5.49 (m, 1H, AlaHα), 5.65 (m, 1H, -CH=), 7.30-7.40 (m, 5H, ArH); ^{13}C{1H} NMR, ($CDCl_3$, 100 MHz) δ 18.7, 28.6, 44.5, 46.5, 49.2, 52.5, 68.1, 80.1, 119.5, 126.5, 128.8, 129.0, 131.5, 135.1, 153.1, 155.7, 169.6, 174.5; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C22H30N3O6 432.2135; Found 432.2166. Anal. Calcd for C22H29N3O6: C, 61.24; H, 6.77; N, 9.74. Found: C, 61.38; H, 6.84; N, 9.81.

Boc-(*S*)-Ala-(*R*)-Imi(*N*1Me)-OMe (**7f**). Starting from **3f** (0.073 g, 0.25 mmol), the General Procedure gave **7f** (0.072 g, 87 %, 93 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (d, J = 7.2, 1H, AlaMe), 1.42 (s, 9H, *t*Bu), 2.88 (s, 3H, *N*Me), 3.38 (dd, J = 3.4, 9.7 Hz, 1H, ImiH5), 3.71 (dd, J = 9.7, 10.4 Hz, 1H, ImiH5), 3.76 (s, 3H, OMe), 4.70 (dd, J = 3.4, 10.4 Hz, 1H, ImiH4), 5.36 (br.d, 1H, AlaNH), 5.51 (m, 1H, AlaHα); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 19.3, 28.2, 30.5, 46.6, 51.8, 52.4, 52.8, 79.3, 153.1, 154.8, 169.7, 173.9; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C14H24N3O6 330.1665; Found 330.1692. Anal. Calcd for C14H23N3O6: C, 51.06; H, 7.04; N, 12.76. Found: C, 50.76; H, 7.39; N, 13.04.

Cbz-Val-(*R*)-Imi(*N*1*i*Pr)-OMe (**7g**). Starting from **3g** (0.084 g, 0.24 mmol), the General Procedure gave **7g** (0.082 g, 82 %, 91 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 (d, J = 7.2 Hz, 3H, ValMe), 0.86 (d, J = 7.2 Hz, 3H, ValMe), 1.05 (d, J = 6.4 Hz, 3H, *i*PrMe), 1.10 (d, J = 6.4 Hz, 3H, *i*PrMe), 2.19 (m, 1H, ValHβ), 2.37 (m, 1H, N_1CH), 3.32 (dd, J = 3.6, 9.8 Hz, 1H, ImiH5), 3.66 (t, J = 9.8 Hz, 1H, ImiH5), 3.78 (s, 3H, OMe), 4.69 (dd, J = 3.6, 9.8 Hz, 1H, ImiH4), 5.08-5.15 (m, 2H, PhCH_2), 5.57-5.62 (m, 2H, ValHα+ValNH), 7.28-7.39 (m, 5H, ArH); ^{13}C{1H} NMR ($CDCl_3$, 100 MHz) δ 16.3, 17.4, 20.3, 28.8, 40.5, 52.2, 53.4, 55.0, 59.2, 67.0, 127.9, 128.7, 129.1, 129.5, 137.1, 152.5, 155.0, 172.3, 174.5; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C21H30N3O6 420.2135; Found 420.2166. Anal. Calcd for C21H29N3O6: C, 60.13; H, 6.97; N, 10.02. Found: C, 60.30; H, 7.20; N, 10.19.

Boc-Ala-(*R*)-Imi(*N*1Ph)-OBn (**7h**). Starting from **3h** (0.091 g, 0.25 mmol), the General Procedure gave **7c** (0.074 g, 77 %, 96 % pure). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (d, J = 6.4 Hz, 3H,

AlaMe), 1.47 (s, 9H, *t*Bu), 3.77 (dd, J = 2.6, 10.2 Hz, 1H, ImiH5), 4.23 (dd, J = 9.8, 10.2 Hz, 1H, ImiH5), 4.88 (dd, J = 2.6, 9.8 Hz, 1H, ImiH4), 5.23 (s, 2H, $PhCH_2$), 5.44 (br.d, 1H, AlaNH), 5.65 (m, 1H, AlaHα), 7.19 (m, 1H, ArH), 7.30-7.41 (m, 7H, ArH), 7.43-7.51 (m, 2H, ArH); $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz) δ 19.5, 28.4, 45.3, 49.5, 52.1, 67.9, 79.6, 119.2, 125.0, 126.1, 128.3, 128.7, 129.2, 134.8, 137.8, 150.8, 154.8, 168.7, 174.2; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C19H26N3O6 392.1822; Found 392.1861. Anal. Calcd for C19H25N3O6: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.44; H, 6.51; N, 10.90.

Boc-Ala-(*R*)-Imi(*N*1allyl)-OBn (**7i**). Starting from **3h**[46] (0.088 g, 0.24 mmol), the General Procedure gave **7e** (0.093 g, 90 %, 92 % pure). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40 (d, J = 6.8 Hz, 3H, AlaMe), 1.46 (s, 9H, *t*Bu), 3.29 (dd, J = 3.4, 9.8 Hz, 1H, ImiH5), 3.64 (dd, J = 9.8, 10.4 Hz, 1H, ImiH5), 3.77 (dd, J = 5.8, 15.6 Hz, 1H, CH_2-CH=), 3.96 (dd, J = 5.4, 15.6 Hz, 1H, CH_2-CH=), 4.74 (dd, J = 3.4, 10.4 Hz, 1H, ImiH4), 5.15-5.22 (m, 4H, $PhCH_2$+=CH_2), 5.40 (br.d, 1H, AlaNH), 5.56 (m, 1H, AlaHα), 5.66 (m, 1H, =CH_2), 7.30-7.39 (m, 5H, Ar); $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz) δ 19.9, 28.7, 44.4, 46.5, 49.5, 53.1, 68.0, 79.9, 119.4, 128.8, 128.9, 120.0, 131.5, 135.3, 152.9, 155.1, 169.4, 174.3; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C22H30N3O6 432.2135; Found 432.2167. Anal. Calcd for C22H29N3O6: C, 61.24; H, 6.77; N, 9.74. Found: C, 61.33; H, 6.68; N, 9.66.

Boc-Ala-(*S*)-Imi[*N*1$(CH_2)_3CO_2$Me]-OBn (**7l**). Starting from **3c**[46] (0.081 g, 0.22 mmol), the General Procedure gave **7l** (0.080 g, 74 %, 94 % pure). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.35 (d, J = 6.4Hz, 3H, AlaMe), 1.43 (s, 9H, *t*Bu), 1.78-1.86 (m, 2H, CH_2), 2.30-2.38 (m, 2H, CH_2), 3.24 (m, 1H, CH_2), 3.35-3.40 (m, 2H, CH_2+ImiH5), 3.63-3.77 (m, 4H, COOMe+ImiH5), 4,87 (dd, J = 3.4, 10.2, 1H, ImiH4), 5.07 (d, J = 8.4 Hz, 1H, AlaNH), 5.15 (d, J = 12.2 Hz, 1H, PhCH), 5.25 (d, J = 12.2 Hz, 1H, PhCH), 5.46 (m, 1H, AlaHα), 7.25-7.38 (m, 5H, ArH); $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz) δ 14.6, 22.6, 28.7, 31.3, 43.4, 44.9, 48.0, 52.5, 68.2, 80.0, 128.8, 129.1, 143.1, 153.4, 155.9, 173.5, 175.3; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C24H34N3O8 492.2346; Found 492.2329. Anal. Calcd for C24H33N3O8: C, 58.64; H, 6.77; N, 8.55. Found: C, 58.78; H, 6.82; N, 8.60.

(S)- or (R)-methyl 1-methyl-2-oxoimidazolidine-4-carboxylates (*S*)-**8** or (*R*)-**8**. To a stirred suspension of LiOH (0.028 g, 1.2 mmol) in THF (6 mL) and water (1.5 mL), a solution of H_2O_2 (30 wt %, 0.28 mL, 2.3 mmol) was added at 0 °C. After 15 min, a solution of **7a** or **7f** (0.13 g, 0.4 mmol) in THF (3 mL) was added at 0 °C while stirring. The temperature was allowed to raise to rt, and after 4 h the pH was adjusted to 5-6 with 0.1 M HCl, THF was evaporated at reduced pressure, and the mixture was extracted three times with EtOAc. The collected organic layers were dried over Na_2SO_4, and the residue was purified by flash chromatography over silica gel (eluent

cyclohexane/EtOAc 1:3) giving (*S*)-**8** (0.050 g, 0.32 mmol, 80 % yield, 94 % pure) or (*R*)-**8** (0.047 g, 0.30 mmol, 75 % yield, 94 % pure), their $[\alpha]_D$ specific optical rotations were determined to be +25 (c 0.7, MeOH) and -24 (c 0.6, MeOH), respectively. Literature for (*S*)-**8**: $[\alpha]_D^{20}$ +26.9 (c 1, MeOH).[23]

Boc-Ala-(*R*)-Imi(*N*1*n*Pr)-Phe-OMe (**9**). The dipeptide **7i** (0.1 g, 0.23 mmol) was subjected to hydrogenation in the presence of a catalytic amount of 10 % Pd/C in EtOH (10 ML) at rt. The reaction was monitored by TLC, and after 6 h the solution was filtered over celite®. The solvent was removed at reduced pressure, and the presence of the dipeptide acid was confirmed by RP HPLC and ESI MS.

The residue was dissolved in 3:1 DCM/DMF (6 mL), and reacted with HOBt (0.038 g, 0.28 mmol), H-Phe-OMe·HCl (0.060 g, 0.28 mmol), and EDCI·HCl (0.054 g, 0.28 mmol), trimethylamine (0.083 mL, 0.60 mmol). The reaction was conducted as described for peptides **1**, and after the usual work up, the Imi-tripeptide **9** was isolated as described for the Imi-peptides **2** (0.090 g, 78 %, 88 % pure according to analytical RP HPLC, General Methods). ^{1}H NMR (8:2 [D6]DMSO/H_2O, 400 MHz) δ 0.74 (t, J = 7.3 Hz, 3H, Me), 1.16 (d, J = 6.4 Hz, 3H, AlaMe), 1.27-1.34 (m, 2H, CH_2), 1.36 (s, 9H, tBu), 2.56 (dd, J = 3.0, 9.5 Hz, 1H, ImiH5), 2.85 (dd, J = 10.8, 13.2 Hz, PheHβ), 2.95 (m, 1H, *N*CH_2), 3.07-3.17 (m, 2H, PheHβ+*N*CH_2), 3.46 (dd, J = 9.5, 10.0 Hz, 1H, ImiH5), 3.65 (s, 3H, OMe), 4.50-4.60 (m, 2H, PheHα+ImiH4), 5.34 (m, 1H, AlaHα), 6.76 (d, J = 8.8 Hz, 1H, AlaNH), 7.17-7.22 (m, 3H, PheArH), 7.22-7.30 (m, 2H, PheArH), 8.59 (d, J = 8.0 Hz, 1H, PheNH); ^{13}C{1H} NMR ([D6]DMSO, 100 MHz) δ 11.7, 19.9, 20.5, 29.1, 38.1, 45.0, 45.7, 49.0, 53.0, 53.5, HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C25H37N4O7 505.2662; Found 505.2685. Anal. Calcd for C25H36N4O7: C, 59.51; H, 7.19; N, 11.10. Found: C, 59.71; H, 7.22; N, 11.05.

Boc-Ala-(*S*)-Imi((E)-3-(4-((tert-butoxycarbonyl)amino) phenyl)allyl)-OBn (**10**)**.** A mixture of dipeptide **7e** (0.056 g, 0.13 mmol), *N*-Boc-4-bromoaniline (0.054 g, 0.2 mmol), palladium acetate (0.3 mg, 0.0013 mmol) and triphenylphosphine (0.7 mg, 0.0026 mmol) were stirred in 1:2 triethylamine/DMF (3 mL), under inert atmosphere. The mixture was stirred for 18 h at 80°C. Then the mixture was evaporated at reduced pressure, MeOH (15 mL) was added to the residue and the suspension was filtered through a Celite® pad to remove the fine black Pd°. The solvent was evaporated at reduced pressure, the residue was dissolved in EtOAc (20 mL) and washed with 1M HCl (4 mL). The organic layer was dried over Na_2SO_4 and after evaporation at reduced pressure the residue was purified by semi-preparative RP HPLC over a C18 column (General Methods), giving **10** (0.063 g, 0.10 mmol, 78 %, 92 % pure by analytical RP HPLC, General Methods). ^{1}H NMR

($CDCl_3$, 400 MHz) δ 1.26 (s, 9H, *t*Bu), 1.38 (d, J = 6.8 Hz, 3H, AlaMe), 1.44 (s, 9H, *t*Bu), 3.35 (dd, J = 3.4, 9.9 Hz, 1H, ImiH5), 3.70 (dd, J = 9.9, 10.3 Hz, 1H, ImiH5), 3.97 (dd, J = 6.4, 15.4 Hz, 1H, C*H*-CH=), 4.07 (dd, J = 6.6, 15.4 Hz, 1H, C*H*-CH=), 4.88 (dd, J = 3.4, 10.3 Hz, 1H, ImiH4), 5.07 (d, J = 7.6 Hz, 1H, AlaNH), 5.13 (d, J = 12.2 Hz, 1H, C*H*Ph), 5.23 (d, J = 12.2 Hz, 1H, C*H*Ph), 5.51 (m, 1H, AlaHα), 6.06 (m, 1H, CH=), 6.54 (d, J = 15.6 Hz, 1H, CH=), 7.28-7.36 (m, 7H, ArH), 7.39 (d, J = 8.8 Hz, 2H, ArH); $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz) δ 17.2, 28.7x2, 43.6, 48.5, 50.8, 53.1, 66.3, 81.7, 120.4, 124.0, 126.9, 129.0, 130.1, 132.2, 135.0, 137.8, 151.8, 152.8, 155.0, 172.1, 175.5; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C33H43N4O8 623.3081; Found 623.3101. Anal. Calcd for C33H42N4O8: C, 63.65; H, 6.80; N, 9.00. Found: C, 61.41; H, 6.68; N, 9.05.

Boc-Ala-(*S*)-Imi(3-(4-((tert-butoxycarbonyl)amino)phenyl) propyl)-OBn (**11**). The dipeptide **10** (0.063 g, 0.10 mmol) was treated with H_2 in the presence of a catalytic amount of 10 % Pd/C in EtOH at rt. The reaction was monitored by TLC, and after 8 h the solution was filtered over celite®, and the solvent was removed at reduced pressure, giving dipeptide acid **11** (0.053 g, 99 %, 85 % pure by analytical RP HPLC, General Methods). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12-1.23 (m, 12H, *t*Bu+AlaMe), 1.31, (s, 9H, *t*Bu), 1.57-1.64 (m, 2H, CH_2), 2.37-2.42 (m, 2H, CH_2), 2.93-3.17 (m, 2H, CH_2), 3.27 (m, 1H, ImiH5), 3.41 (m, 1H, ImiH5), 4.28 (m, 1H, ImiH4), 5.27 (m, 1H, AlaHα), 5.66 (br.d, 1H, AlaNH), 6.96 (d, J = 8.0 Hz, 2H, ArH), 7.22 (d, J = 8.0 Hz, 2H, ArH), 10.50 (br.s, 1H, COOH); $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz) δ 17.5, 25.7, 27.1, 28.9, 31.7, 40.9, 48.0, 50.0, 55.8, 80.4, 81.0, 119.9, 121.1, 126.3, 127.6, 134.1, 135.3, 152.0, 153.0, 155.1, 171.9, 174.3; HRMS (ESI/QTOF) m/z: [M + H]+ Calcd for C26H39N4O8 535.2768; Found 535.2804. Anal. Calcd for C26H38N4O8: C, 58.41; H, 7.16; N, 10.48. Found: C, 58.66; H, 7.30; N, 10.20.

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Supporting Information

Tables S1, amide-NH temperature gradients; Tables S2-5, ROESY cross peaks for **2b-e**; ^1H and $^{13}C\{1H\}$ NMR spectra.

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