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Complete List of Authors:	Bucci, Raffaella; University of Milano, DISFARM Giofré, Sabrina; University of Milano DISFARM Clerici, Francesca; University of Milano, Università degli Studi Milano DISFARM Contini, Alessandro; Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche Pinto, Andrea; University of Milan, DeFENS Erba, Emanuela; Università degli Studi Milano DISFARM Soave, Raffaella; Istituto di Scienze e Tecnologie Molecolari, Pellegrino, Sara; University of Milan, Dipartimento di Scienze Farmaceutiche- sez. Chimica Generale e Organica Gelmi, Maria Luisa; Milano University, DISFARM

Tetrahydro-4*H*-(pyrrolo[3,4-d]isoxazol-3-yl)methanamine: a bicyclic diamino scaffold stabilizing parallel turn conformations

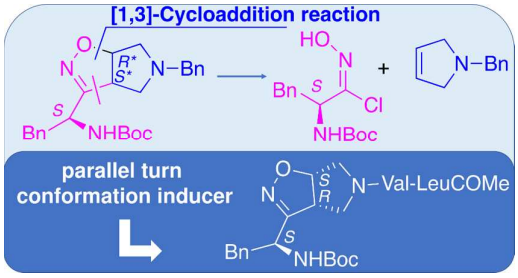
Raffaella Bucci,[†] Sabrina Giofré,[†] Francesca Clerici,[†] Alessandro Contini,[†] Andrea Pinto,[#] Emanuela Erba,[†] Raffaella Soave, [‡] Sara Pellegrino,[†] Maria Luisa Gelmi[†*]

[†]DISFARM, Sezione di Chimica Generale e Organica "A. Marchesini" Università degli Studi Milano Via Venezian 21, 20133 Milano, Italy

[#] DeFENS, Sezione di Scienze Chimiche e Biomolecolari, Università degli Studi Milano, Via Celoria 2, 20133 Milano, Italy

[‡]CNR- Istituto di Scienze e Tecnologie Molecolari, Via Golgi 19 20133, Milano, Italy

E-mail: marialuisa.gelmi@unimi.it



Abstract. Tetrahydro-4*H*-(pyrrolo[3,4-d]isoxazol-3-yl)methanamine scaffold was designed as diamino derivative to stabilize parallel turn conformations. Its synthesis took advantage of a [1,3]-dipolar cycloaddition reaction between the nitrile oxide derived from the inexpensive enantiopure L-phenylalanine, and *N*-benzyl-3-pyrroline. Two diastereoisomers were formed whose distribution depends on the selected base. 3a*R*,6a*S*-Isomer is favoured in organic bases which formation is driven by π-interactions. On the other hand, the above interactions were significantly prevented using an inorganic base due to the chaotropic effect of the cation, decreasing the amount of the above isomer. Finally, we demonstrated that this isomer is able of stabilizing parallel turn conformations when inserted in short peptide sequences.

Introduction

The use of non-coded amino acids or scaffolds for the synthesis of peptidomimetics mimicking the natural structures opens the door to a huge number of different artificial shapes such as helixes, different kind of turns, and stabilized β-strand.[1] Focusing on β-strand natural peptides, they can

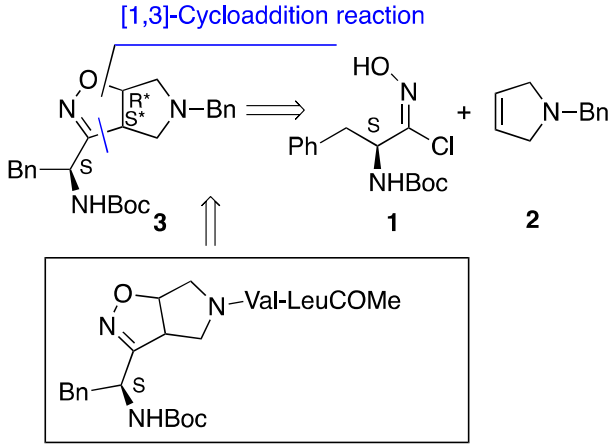
assemble into either antiparallel or parallel β-sheets,[2] very important motifs for the protein folding and fibril formations. Several proteins are characterized by parallel β-sheet motif ranging from amyloid[3a,b,d,f] to prion protein (PrP^C)[3c], leucine-rich repeat (LRR)-containing proteins,[3h] shikimate kinase (SK) involved in tubercolosys[3g] and many others.

Strategies designed to stabilize this motif mostly consist in the use of an artificial scaffold that can be used to link two β-strands by *N*- or *C*-terminus.[4] Other strategies are reported such as the formation of cyclic peptides,[4a] and the stabilization *via* disulfide cross-link between cysteins.[5]

These strategies are particularly profitable not only to stabilize intermolecular interactions for bio-molecular recognition, but also to improve metabolic stability necessary in the biological field.

Our interest toward the preparation of non-coded amino acids[6] and their use for peptidomimetic synthesis and their applications[7] is well documented. In this respect, click reactions were used both to stabilize β-hairpin structures,[8a] and to prepare a new scaffold useful in the preparation of β-hairpin mimics targeting the Alzheimer disease.[8b]

Here we focused on the design and synthesis of a new Δ^2-isoxazoline scaffold, fused with a pyrrolidine ring, of general structure **3** to be used to stabilize parallel turn conformations (Scheme 1).



Scheme 1. Retrosynthetic pathway to obtain isoxazoline-containing scaffold and peptidomimetics.

This scaffold shows appealing features such as the presence of two amino groups, making it a good candidate for the above application. Furthermore, due to its calix conformational constraint, it could better orient the peptide arms, thus improving intra-strand H-bonds. The presence of oxygen/nitrogen atoms in the isoxazoline ring, as well as the aromatic moieties make this turn region appealing for possible intermolecular H-bonds and π-interactions, respectively, when it is used to build peptidomimetics targeting PPI interactions.

Isoxazoline derivatives are often prepared using, as key step, a [1,3]-dipolar cycloaddition reaction between nitrile oxides and dipolarophiles.[9] The main drawback in using isolated nitrile oxides regards their instability.[10] In fact, they easily rearrange to isocyanate, generally by heating, or dimerize giving furoxanes at room or lower temperatures. The formation of these side products affects the yield of the cycloaddition reaction, resulting in a difficult isolation and purification of the reaction products. For these reasons, nitrile oxides are usually generated *in situ* in the presence of a dipolarophile. They can be obtained in different ways according to Mukaiyama's,[11] Huisgen's protocols,[12] and Machetti-De Sarlo reactions.[13] Huisgen's protocol consists in a basic dehydrohalogenation of a hydroximoyl halide, easily obtained from an aldoxime, giving the nitrile oxide. We envisaged in this procedure the best protocol to obtain an isoxazoline ring functionalized with a nitrogen containing chain. As shown in the retrosynthetic Scheme 1, chlorooxime **1** was selected as a chiral starting material to generate the corresponding nitrile oxide because it can be obtained from an inexpensive commercially available chiral amino acid.[14] As dipolarophile, 3-pyrroline **2** was selected giving access to a second nitrogen atom in the final scaffold. Thanks to its symmetry, the formation of regioisomeric mixtures is avoided. A couple of diastereoisomeric isoxazolines could be obtained due to the presence of a stereocenter in the nitrile oxide.

As reported in the literature,[9c] the cycloaddition of chiral nitrile oxides to achiral alkenes generally results in poor stereoselection. Considering the unfavourable chirality of our reagents (chiral nitrile oxide and achiral alkene) and also the low reactivity of the dienophile,[15] our challenge has been the preparation of scaffold **3** in satisfying yields and with a control of the diastereoselection. To reach this target, we envisaged in the use of two aryl substituted reagents, able to stabilize the transition state through π interactions, the key step to drive the diastereoselection. As a result, the nitrile oxide derived from L-phenylalanine and the *N*-benzyl-3-pyrroline were used. Our hypothesis has also been supported by computational studies.

Both diastereoisomers of **3** were used for the preparation of short peptides (Scheme 1) whose conformation was studied by NMR spectroscopy. We found that the 3a*R*,6a*S*-isomer is able to stabilize parallel turn conformations when combined with natural amino acids.

Results and discussion

Synthesis of scaffold 3. Chlorooxime **1**, the precursor of nitrile oxide, was synthesized starting from the enantiopure cheap commercially available (*L*)-Phe. (Scheme S1, Supporting Information). Its synthesis, *via* oxime intermediate **7**, was revisited due to a partial racemization of the precursor aldehyde **6**. All the screened procedures and the corresponding HPLC are reported in the Supporting Information (Figure S1, Supporting Information).

The cycloaddition reaction was performed by using *N*-benzyl-3-pyrroline (**2**) as dipolarophile. Two diastereoisomeric compounds could be expected, *i.e.* Δ^2-isoxazoline derivatives **3a** and **3b** (Scheme 2). To optimize the yield and to control the diastereoselection, several reaction conditions were tested and the overall results are reported in the Table 1.

Scheme 2. Cycloaddition reaction between **1** and **2**.

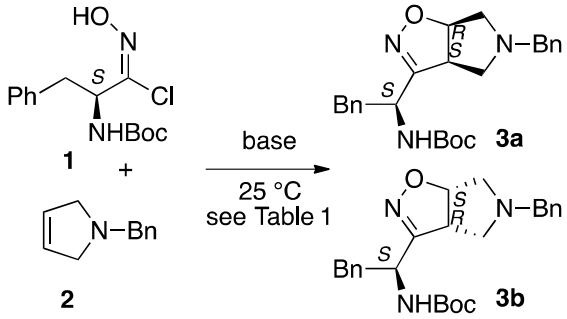


Table 1. Reaction conditions tested for the cycloaddition reaction between 1 and 2 affording compounds 3.

Entry	Solvent (25 °C)	Base	Time (h)	3a:3b *d.r.*[a]	3a/3b, %[b]
1[c]	$CHCl_3$	TEA	18	-	6%
2[d]	MeCN	TEA	16	1:3	38%
3[e]	MeCN	DABCO	16	1:2.5	24%
4[d]	AcOEt	$NaHCO_3$	17	1:2	32%
5[e]	MeCN	$NaHCO_3$	5	1:2	51%
6[f]	MeCN	Li_2CO_3	20	1:1	50%
7[e]	MeCN/DMF[g]	TEA	16	1:3	50%
8[e]	MeCN/DMF[g]	DABCO	16	1:2.5	53%
9[e]	MeCN/NMP[g]	TEA	16	1:2	29%
10[e]	MeCN/DMF[g]	$NaHCO_3$	4	-	Traces
11[h]	MeCN/DMF[g]	$NaHCO_3$	16	1:2	31%
12	MeCN/DMF[g]	TEA (2 eq.) LiCl (2 eq.)	16	1:1	14%

[a]Calculated by ^{1}H NMR analysis; [b]Isolated producs; [c]**1**:**2**:TEA in 1:1:1.2 ratio at reflux; [d]**1**:**2**:base in 2.2:1:2.2 ratio at 25 °C, slowly addition of **1** (1 h); [e]**1**:**2**:base in 2:1:2 ratio at 25 °C and slowly addition of **1** (2 h); [f]**1**:**2**:base in 2:1:1 ratio at 25 °C and slowly addition of **1** (2 h); [g]MeCN/DMF (5:1); [h]The reaction was performed as described in the note *e)* but at 0°C.

First, we performed the cycloaddition reaction using the standard conditions reported in the literature when nitrile oxide derived from amino acids were used.[14b] The reaction was performed using a mixture of **1** and **2** (1:1) in $CHCl_3$ at reflux after which TEA (1.2 equiv.) was added as base

to generate the nitrile oxide (Table 1, entry 1). A mixture of **3a** and **3b** was obtained in very low yield. The temperature (from 0 to 25 °C) and the amount of TEA (2 equiv.) were changed, as well the base (**1**/**2**/DABCO, 1:1:2), but the reaction failed.

MeCN was then tested instead of $CHCl_3$ for its higher polarity. Operating at 25 °C and increasing both the amount of **1** and TEA (entry 2), a mixture of **3a** and **3b** (1:3 ratio) was obtained in 38% yield. Worse results were achieved when changing the addition order of the reagents, being the addition of the chloroxime the last one. Lower yield or trace amounts of compounds **3** were detected when changing the base (DABCO, entry 3; DIPEA or DBU, data not reported) and operating as reported above.

Then we switched to inorganic bases. $NaHCO_3$ was used both in AcOEt (entry 4) and MeCN (entry 5). A dramatic decrease of the reaction time and an increase of yields (51%) but with a loss of diastereoselectivity (1:2) was found with MeCN. Similar results were achieved with Li_2CO_3 with a further decrease of the diastereoselection (entry 6). A phase-transfer catalysis protocol in heterogeneous conditions was also attempted using K_2CO_3 and tetrabutylammonium bromide as the catalyst in MeCN (**1**/**2**/Bu_4NBr/K_2CO_3, 1:0.5:0.1:2) affording only trace amounts of compounds **3**.

Since **3b** was the diastereoisomer of interest for the preparation of parallel sheets (see below), further efforts were done to improve its yield. Considering the beneficial result in terms of yield when the more polar MeCN was used, we tested a mixture of MeCN/DMF (5:1 ratio) and TEA as the base. The reaction was successful and compounds **3a/3b** were obtained in the same ratio but with improved yield (50%, entry 7). Similar results were found with DABCO (entry 8) with a small decrease in diastereoselection. On the other hand, when NMP was tested as a co-solvent, a decrease of yield was found (entry 9).

We also evaluated the use of $NaHCO_3$ in MeCN/DMF at both 25 °C (entry 10) and at 0 °C (entry 11). The reaction failed in the first case and gave lower yields in the second one, maintaining the same diastereoisomeric ratio (compare entries 5 and 11).

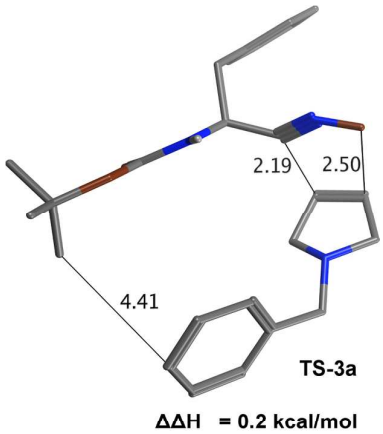
Overall, our results indicate that more polar solvents (MeCN/$CHCl_3$; compare entries 1, 2 and 7) are beneficial to the reaction yields when a weak organic base (pK_b 5-3) is used. The use of inorganic bases in MeCN heterogeneous conditions give good results too. On the other hand. in this case the higher is the polarity of the solvent the lower are the yields (compare entries 10, 11 with 5). This is probably due to the improved solubility of the base. As a result, the formation of nitrile oxide strongly increases that partially gave by-products because of the low reactivity of the dienophile.[15b] By taking these data together, it has to be concluded that the rate of the nitrile oxide generation from **1** is crucial to avoid side-reactions, in agreement to the reported results.[15] It has to be pointed

out that a 50% yield represents a rather good result, considering the sluggish reactivity of our dipolarophile.

Interestingly, we observed that the diastereoisomeric ratio is affected by the choice of the organic or inorganic base, moving from 1:3 ratio in favour of adduct **3b**, to 1:1 ratio, respectively.

To clarify the above results, computational studies were performed. First, diastereoiosmers **3a** and **3b** were evaluated by a conformational search at the molecular mechanics level. All the conformations found in the range of 2 kcal/mol (20 and 25 for **3a** and **3b**, respectively) were then selected for DFT optimization and energy evaluation at the CPCM-HCTC/6-311+G(d,p)//HCTH/6-31+G(d) level,[16] accordingly to a protocol previously adopted for similar studies, using MeCN as the solvent. The geometries of the most stable conformation for each compound is presented in supplementary Figure S9 (Supporting Information), while the energies obtained for all **3a** and **3b** conformations are reported in Tables S5 and S6 (Supporting Information), respectively.

Since the observed diastereoselectivity is likely to be determined at the transition state level, the most stable conformations of **3a** and **3b** were used to generate the corresponding transition structures **TS-3a** and **TS-3b**, that were successively optimized using the same scheme as above. Single point energies were then calculated at the HCTH/6-311+g(d,p) level, using the CPCM solvent model for MeCN. The optimized transition structures of TS-**3a** and TS-**3b** are shown in Figure 1.



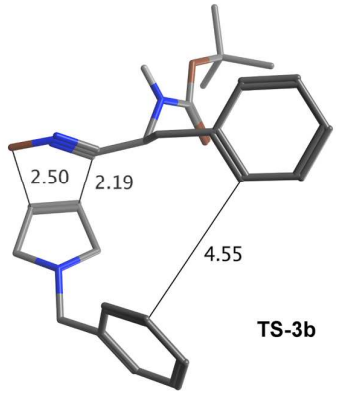


Figure 1. Optimized structures of TS-**3a** and TS-**3b**. Selected distances are reported in Angstrom.

Although the enthalpy difference between the two transition states (TS) is quite low (0.2 kcal/mol), TS-**3b** is favoured. Based on the Arrhenius equation (see Table S7 and Equation ES1 in Supporting Information), the predicted ratio for **3a** and **3b** is 28:72. This ratio is very close to that experimentally determined (1:3, when an organic base is used), confirming that the reaction is under kinetic control and that the chosen level of theory is correct. The analysis of the geometry of the two TSs shows that TS-**3b** can be stabilized by a T-shaped CH/π interaction between the two aromatic rings. In TS-**3a**, the phenyl-phenyl interaction is replaced by a CH/π interaction between the Boc moiety and the pyrroline benzyl group that, being mediated by hydrogens linked to a sp^3 carbon is probably weaker, thus the slightly lower stabilization observed.

Therefore, based on our computational results, we can hypothesize that when an organic base is used, π-interactions between the aryl moieties of pyrroline and nitrile oxide derivatives drive the diastereoselectivity. As a result, even if we are not in a favourable stereochemical environment (see introduction[9c]), the formation of **3b** increases, supporting our strategy in selecting two aryl-functionalized reagents to drive the diastereoselection.

When using the inorganic base, the decrease in diastereoselection could be due to the presence of a metal cation able to disrupt the π-interactions, thus acting as "chaotropic agent".[17] Indeed, it has been reported that metal cations can destroy week interaction with a trend $Li^+ > Na^+$. In our case the distribution between **3a** and **3b** is confirmed (compare entries 5 and 6, Table 1). To further verify this hypothesis, we performed the cycloaddition reaction using TEA, in the presence of LiCl as the cationic donor (Table 1, entry 16). It was found that a decrease in the yields occurred together with a decrease in the diastereoselection (1:1 ratio instead of 1:3, found in absence of LiCl). This last result confirms that the stereochemical results might be driven by π-interactions, as suggested by the computational study.

Even if diastereoisomers **3a** and **3b** were collected as a rather inseparable mixture, their purification on silica gel column chromatography allowed the isolation and characterization of a pure amount of diastereoisomer **3b**. ^{1}H NOESY analysis (CD_3CN) of the major diastereoisomer **3b** shows some significant spatial proximities (Figure 2A), allowing us to assign the 3a*R*,6a*S*-configuration to the new generated stereocenters with respect to the *S* configuration of the side chain.

As a further confirmation, suitable needle-shape crystals of **9b**, the debenzylated compound of **3b** obtained as described in Scheme 3, were achieved ($CDCl_3$/Et_2O, 1:10). Their single-X-ray-crystal analysis confirmed the (3a*R*,6a*S)* absolute configuration of the isoxazoline derivative **9b** and,

indirectly, of **3b** (Figure 2B). Crystal structure data of **9b** was deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1841009.

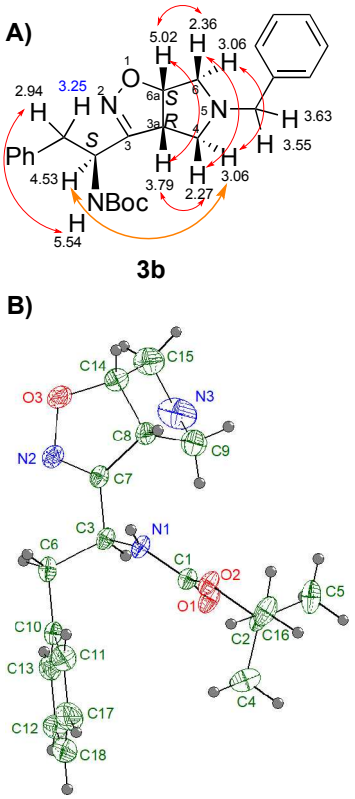
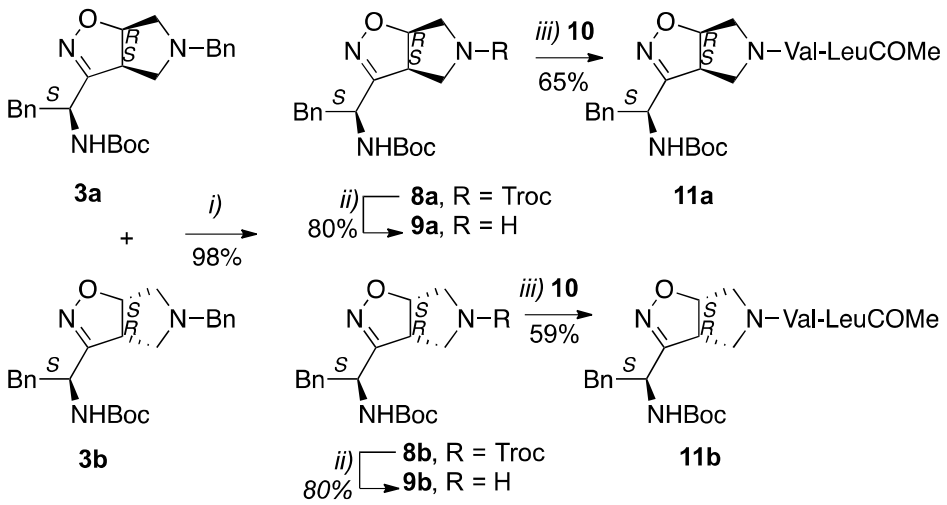


Figure 2. A) NOE signals of compound **3b**. B) ORTEP plot of **9b** at 293 K with atom numbering scheme; ellipsoids of non-H atoms are at 15% probability level.

Peptidomimetic synthesis. Both diasteroisomers **3a** and **3b** were used for the preparation of peptidomimetic models (Scheme 3). Their orthogonal deprotection at nitrogen of the pyrrolidine ring was first studied. Since the isoxazoline nucleus is not stable to the catalytic reduction with H_2/Pd, a "one-pot" debenzylation/acylation reaction was selected according to a known procedure.[18] Starting from of a mixture of **3a** and **3b** and by using 2,2,2-trichloroethyl chloroformate (TrocCl; MeCN, 2 h, 98%), diastereoisomers **8a** and **8b** were efficiently obtained and easily separated by flash chromatography.

The nitrogen of pyrrolidine ring of compounds **8** was orthogonally deprotected with Zn powder in an aqueous solution of AcOH (90%) at 25 °C giving Boc-derivatives **9a** (80%) and **9b** (70%).

Two small peptides models, i.e. **11a** and **11b**, were prepared to evaluate the ability of our scaffolds to stabilize a parallel turn conformation when inserted in a natural sequence. The dipeptide *N*-Ac-Leu-Val-OH (**10**) was made to react with amines **9a** and **9b**. Compounds **11a** (65%) and **11b** (59%) were obtained in very good yield using the solution phase protocol and the classic coupling agents [EDC (1.1 equiv.), HOBT, (1.1 equiv.), DIEA (1.1 equiv.) in CH_2Cl_2].



Scheme 3. Synthesis of peptides **11-15**. *Reaction conditions: i)* TrocCl (1 eq.), MeCN, 25 °C, 2 h; *ii)* Zn, AcOH, 25 °C; *iii)* EDC (1.1 equiv.)/HOBT (1.1 equiv.)/DIEA (1.1 equiv.), CH_2Cl_2.

NMR spectroscopy characterization of peptides. Peptides **11** were fully characterized by NMR (^{1}H, ^{13}C, HMBC, HSQC, NOESY; MeCN, 300 MHz; for details see Tables S1-S4 in Supporting Information).

^{1}H NMR analysis of compound **11b** revealed the presence of two main isomers in 60:40 ratio. NOESY experiments showed that similar NOEs are present for the same proton in the two isomers proving an equilibrium between them. Well-distributed chemical shifts of amide protons were detected. Furthermore, $^3J_{HN/CH}$ are major than 8 Hz both for Val and Leu as well as for the residue linked to the isoxazoline ring that could be considered the equivalent of Phe. These data suggest that the peptide chain could assume a well-ordered conformation.

The main observed NOEs for both isomers of compound **11b** were reported in Figure 3. A medium NOE was detected between H_{4y} and *CH*Bn in both isomers (Figure S8A, Supporting Information) as well as a spatial proximity between CH_{Leu}/NH_{Val} (s; Figure 4). A week NH_{Leu}/NH_{Val} NOE (Figure S7B) was found for **11b** due to the conformational freedom of Leu. Of relevance, intrastrand proximities were detected between the phenyl ring and Me_{Val} (Figure 4) and between Boc and the alkyl chain of Leu.[19] These NOEs support the idea that a parallel turn conformation could be induced by our scaffold.

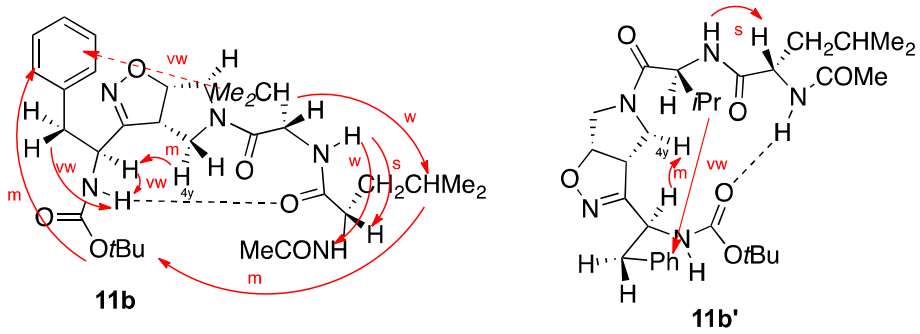


Figure 3. NOEs (red lines; 300 MHz, 500 ms) and H-bond (dotted black lines) of peptide **11b** and **11'b**.

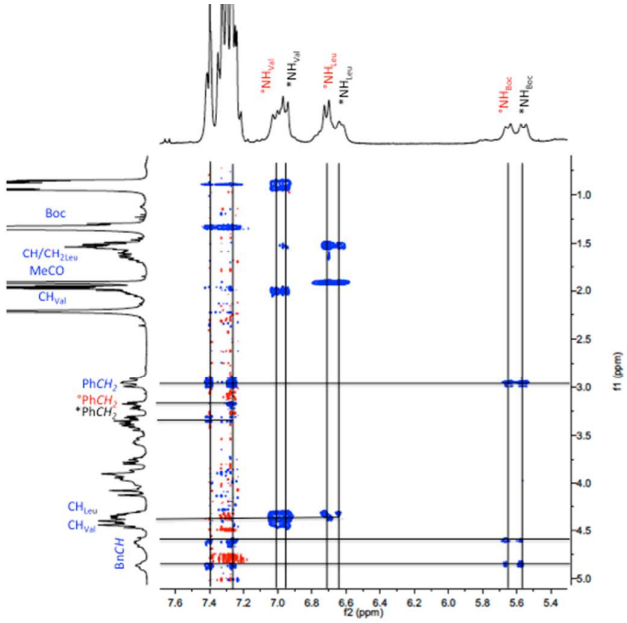


Figure 4. CH/NH NOEs regions (CD_3CN, 20 mM, 300 MHz, 500 ms): black*, main isomer **11b**; red°, minor isomer **11b'**; blu, overlapped signals.

The δΔ/δT analyses showed that NH_{Boc} is involved in a H-bond (-4.5 ppb) for isomer **11b** and NH_{Leu} (-4 ppb) for isomer **11b'** (Figure 5). Since these isomers are in equilibrium, these values are consistent with a medium δΔ/δT value. Coalescence of NH_{Boc} signal occurs at 313K and a completed coalescence for all NHs takes place at 323K. We hypothesize that NH_{Boc} forms an H-bond with $C{=}O_{Leu}$ in rotamer **11b** and NH_{Leu} with $C{=}O_{Boc}$ in isomer **11b'**. The formation of a H-bond between NH_{Boc} and $C{=}O_{Val}$ was excluded because is not consistent with the detected NOEs (H_{4y}/*CH*Bn proximity).

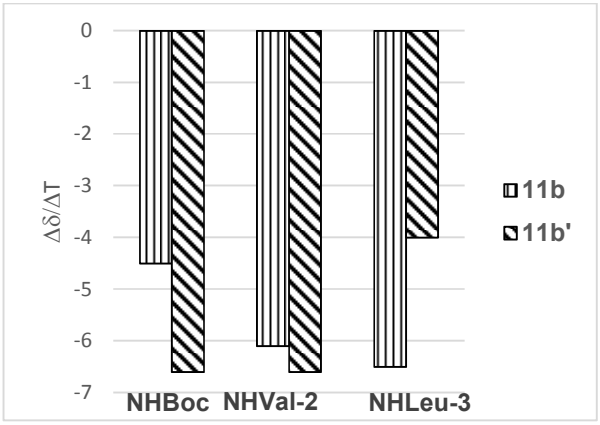


Figure 5. Δδ/ΔT NH values (273-323 K; 300 MHz) for conformers **11b** and **11b'**.

A mixture of two conformers (about 1:1 ratio), with a minor amount of a third one, was detected in the ^{1}H NMR spectrum of diastereoisomer **11a**. Several signals are overlapped preventing the fine characterization of the main isomers. With respect to **11b**, larger Δδ/ΔT values were observed (Figure
S4, Supporting Information). A complete coalescence for all NHs takes place at 313K.
Several NOEs are present between CH_{Val} with both protons H-4 and H-6 as well as between CH_{Leu} with H-4x and H-6y (Figure S5, Supporting Information). Differently to **11b**, intrastrand NOEs were not detected. Taken together these data suggest that a random conformation for this peptide is more likely.

Conclusion

In conclusion, the synthesis of two diastereoisomeric tetrahydro-4*H*-(pyrrolo[3,4-d]isoxazol-3-yl)methanamine derivatives was performed using a [1,3]-dipolar cycloaddition reaction between the nitrile oxide derived from the cheap enantiopure L-phenylalanine and *N*-benzylpyrroline. Computational studies confirmed that π-interactions between the phenyl substituents of the reagents favour the formation of the 3a*R*,6a*S*-isomer **3b**. NMR studies of model peptides containing the molecular scaffolds **3** proved that **3b**, the main isomer obtained in the cycloaddition reaction, stabilizes a parallel turn conformation. Thus, **3b** could be used as a profitable scaffold for the stabilization of parallel β-sheet secondary structures.

Experimental

General Information. Melting points were determined in a Stuart Scientific melting point apparatus in open capillary tubes and are certified. Chemicals were obtained from Sigma Aldrich and used

without further purification. HPLC analyses were carried out on Jasco PU-980 pump equipped with a UV–vis detector Jasco UV-975 (wavelength: 220 nm) and on a Kromasil 5-AmyCoat column (4.6 mm i.d. × 250 mm, 5 μm, AkzoNobel). ESI mass spectra were recorded on an LCQESI MS were recorded on a LCQ Advantage spectrometer from Thermo Finningan and a LCQ Fleet spectrometer from Thermo Scientific. The NMR spectroscopic experiments were carried out on a Varian OXFORD 300 MHz (300 and 75 MHz for ^{1}H and ^{13}C, respectively). Optical rotations were measured on a Perkin–Elmer 343 polarimeter at 20°C (concentration in g/100 mL). Chemical shifts δ are given in ppm relative to the $CHCl_3$ internal standard, and the coupling constants J are reported in Hertz (Hz).

*t-Butyl (S)-(1-hydroxy-3-phenylpropan-2-yl)carbamate (**5**).* Operating in a three-neck round-bottom flask, equipped with magnetic stirrer, thermometer, nitrogen inlet and dropping funnel, $NaBH_4$ (400 mg, 10.5 mmol) was suspended in THF (20 mL). The solution was cooled to 0°C and a solution of I_2 (1.5 g, 6 mmol) in THF (5 mL) was added very slowly under vigorous stirring. L-phenylalanine (**4**) (1 g, 6 mmol) was added in one portion and the reaction mixture was stirred at reflux for 16 h. After cooling at 25 °C, MeOH (13 mL) was added until discoloration and the stirring was continued for further 30 min. The solution was cooled to 0°C and TEA (1.67 mL, 12 mmol) was added dropwise. After 10 min., a solution of di-*tert*-butyl dicarbonate (1.4 g, 6.4 mmol) in THF (4 mL) was dropped. The reaction mixture was stirred for additional 20 h. The solvent was removed under reduced pressure and the crude reaction mixture was dissolved in ethyl acetate (15 mL). The organic layer was washed with saturated solution of NH_4Cl (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was crystallized from hexane/AcOEt (12:1), affording compound **5** (1.5 g, 5.85 mmol, 97%) as a white solid. TLC: hexane/AcOEt, 7:3 (R_f: 0.18). Mp 92°C; $[\alpha]^{20}_D$: -28.5° (*c* 0.6 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.20 (m, 5H), 4.80 (br s, 1H), 3.86 (br s, 1H), 3.66-3.63 (m, 1H), 3.55-3.52 (m, 1H), 2.83 (d, *J* 7 Hz, 2H), 2.08 (br s, 1H), 1.41 (s, 9H) ppm; MS (ESI) m/z: Calcd for $C_{14}H_{21}NO_3$ 251.15; Found 274.40 $[M+Na]^+$. The analytical and spectroscopical data are in agreement with the literature data.[20]

*t-Butyl (S)-(1-oxo-3-phenylpropan-2-yl)carbamate (**6**). Method A: Swern Oxidation.* Operating in a three-neck round-bottom flask, equipped with magnetic stirrer, thermometer and nitrogen inlet, a solution of dry $(COCl)_2$ (0.25 mL, 3 mmol) in dry CH_2Cl_2 (2.5 mL) was added and cooled to -78°C. A solution of dry DMSO (0.24 mL, 3.4 mmol) in CH_2Cl_2 (1.0 mL) was dropped over 5 min, and then the reaction was stirred for 15 min. A solution of **5** (500 mg, 2.0 mmol) in dry CH_2Cl_2 (2.5 mL) was dropped over 10 min. The reaction mixture was stirred for 30 min. and then a solution of dry TEA (1.12 mL, 8.0 mmol) was added dropwise over 10 min. The reaction was slowly heated to

25 °C and was stirred for 1 hour. The reaction mixture was diluted with CH_2Cl_2 (16 mL) and washed with 10% citric acid (15 mL), H_2O (15 mL), a saturated solution of $NaHCO_3$ (15 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude compound (493 mg, 1.98 mmol, 99%) was immediately transformed into oxime **7** without further purifications (for HPLC analysis see Figures S1X and S1Y). *Method B: Dess-Martin oxidation.* Operating in a round-bottom flask, equipped with magnetic stirrer, Dess-Martin periodinane (551 mg, 1.3 mmol) was suspended in a mixture of CH_2Cl_2 (5 mL) and dioxane (3mL). A solution of alcohol **5** (300 mg, 1.2 mmol) in CH_2Cl_2 (1 mL) was added dropwise over a period of 20 min. The reaction was stirred at 25°C for 20 min. When the reaction was complete, Et_2O (2 mL) and sodium thiosulfate (0.5 g) dissolved in a saturated $NaHCO_3$ solution (2 mL) were added. The reaction was stirred for further 5 min and then Et_2O (2 mL) was added. The layers were separated and the organic one was washed with a saturated solution $NaHCO_3$ (2 x 4 mL), H_2O (2 x 4 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, affording **6** (237 mg, 0.95 mmol, 93%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.37-7.28 (m, 3H), 7.20-7.17 (m, 2H), 5.04 (s, 1H), 4.44 (d, *J* 6.4 Hz, 1H), 3.13 (d, *J* 6.4 Hz, 2 H), 1.45 (s, 9 H). ^{1}H NMR data are in agreement with the reported data.[21] The aldehyde was immediately transformed into oxime **7** without further purifications.

t-Butyl (S)-(1-(hydroxyimino)-3-phenylpropan-2-yl)carbamate (7). Operating in a bottom flask, equipped with magnetic stirrer and thermometer, aldehyde **6** (500 mg, 2 mmol) was dissolved in THF (7.5 mL) at 0°C. $NaHCO_3$ (102.5 mg, 1.22 mmol) and $H_2NOH{\cdot}HCl$ (148 mg, 2.2 mmol) were added to the solution. The reaction mixture was heated to 25 °C and stirred overnight (TLC: hexane/AcOEt, 7:3). After solvent evaporation, the residue was extracted with ethyl acetate (4 x 4.5 mL). The combined organic layers were washed with brine (4.5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane/AcOEt 8:2) afforded **7** (385 mg, 83% as a white solid). After crystallization pure isomer *E*-**7** was isolated. Mp 120.3 °C (hexane/AcOEt, 10:1); $[\alpha]^{20}_D$ + 28.3° (*c* 1 in $CHCl_3$);[22] ^{1}H NMR (300 MHz, CD_3CN): δ 8.90 (s, 1H), 7.48-7.12 (m, 5H), 6.67 (d, *J* 6.1 Hz, 1H), 5.44 (brs, 1H), 4.90 (m, 1H), 2.99 (dd, *J* 4.9, 13.7 Hz, 1H), 2.81 (dd, *J* 9.3, 13.7, Hz 1H), 1.39 (s, 9H) ppm; MS (ESI) m/z: Calcd for $C_{14}H_{20}N_2O_3$ 264.15; Found 286.9 $[M+Na]^+$. *Z*-**7** (small amount isolated by chromatography): mp 118.5 °C (hexane/AcOEt, 15:1); ^{1}H NMR (300 MHz, CD_3CN) δ 8.59 (s, 1H), 7.49-7.26 (m, 6H), 5.26 (br s, 1H), 4.40 (ddd, *J* 14.4, 8.4, 6.0 Hz, 1H), 2.99 (dd, *J* 13.7, 6.3, Hz, 1H), 2.86 (dd, *J* 13.7, 8.24 Hz, 1H), 1.39 (s, 9H) ppm.

*N-t-Butoxycarbonyl [1-chloro-1-(hydroxyimino)-3-phenylpropan-2-yl)]carbamate (**1**).* Operating in a bottom flask, equipped with magnetic stirrer and thermometer, oxime **7** (550 mg, 2.1 mmol) was

suspended in CCl_4 (20 mL) and *N*-chlorosuccinimide (278 mg, 2.1 mmol) was added portion wise over a period of 60 min at 0°C. The reaction mixture was refluxed overnight. The solution was cooled to 0°C and filtered to remove the succinimide (TLC: hexane/AcOEt, 7:3; Rf 0.45). The solvent was removed under reduced pressure, affording product **7** (601.2 mg, 97%) as yellow oil. ^{1}H NMR (300 MHz, CD_3CN) δ 9.34 (s, 1H), 7.38-7.23 (m, 5H), 5.75 (br s, 1H), 4.63 (dd, *J* 15.1, 7.7 Hz, 1H), 3.07 (dd, *J* 13.8, 7.7 Hz, 1H), 2.94 (dd, *J* 13.7, 8.5 Hz, 1H), 1.39 (s, 9H) ppm;[24] MS (ESI) m/z: Calcd for $C_{14}H_{19}ClN_2O_3$ 298.11; Found 299.5 $[M+H]^+$.

*t-Butyl 1-(5-benzyl-3a,5,6,6a-tetrahydro-4H-pyrrolo[3,4-d]isoxazol-3-yl)-2S-phenylethyl)carbamate (**3**). Method A.* Chloro-oxime **1** (200 mg, 0.67 mmol) was dissolved in MeCN (5 mL) and DMF (1 mL). Distilled *N*-benzyl-3-pyrroline (**2**) (106.7 mg, 0.13 mL, 0.67 mmol) was added in one portion at 25 °C, followed by the addition of TEA (67.9 mg, 0.67 mmol) or DABCO (75.1 mg, 0.67 mmol). The reaction mixture was stirred for 1 h. Afterwards, chloro-oxime **2** (200 mg, 0.67 mmol) and TEA (67.9 mg, 0.67 mmol) or and DABCO (75.1 mg, 0.67 mmol) were added portionwise over 60 min. The reaction mixture was stirred for additional 16 h at 25 °C. The solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (hexane/AcOEt 9:1) afforded the diastereoisomeric mixture **3a**/**3b** (TEA: 1:3, 132 mg, 49%; DABCO: 1:2.5, 151.6 mg, 53%) as a white solid. The same reaction performed in the presence of LiCl (58.8 mg, 1.34 mmol) gave a 1:1 mixture of **3a**/**3b** (39.15 mg, 0.09 mmol, 14%). *Method B.* Chloro-oxime **1** (100 mg, 0.33 mmol) was dissolved in MeCN (3 mL). Distilled *N*-benzyl-3-pyrroline (**2**) (66.9 mg, 79.9 µL, 0.42 mmol) was added in one portion at 25 °C. $NaHCO_3$ (26.9 mg, 0.33 mmol) or Li_2CO_3 (12.2 mg, 0.165 mmol) was added and the reaction mixture was stirred for 1 h. Chloro-oxime **1** (152.04 mg, 0.51 mmol) and $NaHCO_3$ (42.85 mg, 0.51 mmol) or (12.2 mg, 0.165 mmol) were subsequently added portionwise over 60 min. The reaction mixture was stirred for additional 4 h at 25 °C. The solvent was removed under reduced pressure. Purification of the crude product by flash chromatography (hexane/AcOEt 9:1) afforded the diastereoisomeric mixture **3a**/**3b** ($NaHCO_3$ 1:2, 90 mg, 51%; Li_2CO_3 1:1, 66.55 mg, 48%) as a white solid.

3a*S*,6a*R* **3a**: mixture with **3b**. ^{1}H NMR (300 MHz, CD_3CN) main signal: δ 4.70-4.60 (m, 1H) ppm; ^{13}C NMR (75 MHz, CD_3CN) δ 159.2, 155.3, 138.8, 137.7, 129.4, 128.5, 128.3, 128.2, 126.9, 126.5, 84.3, 78.8, 61.7, 58.4, 56.2, 53.5, 49.7, 38.7, 27.5 ppm.

3a*R*,6a*S*-**3b**. TLC: hexane/AcOEt, 1:1; R_f 0.57. M.p. 99.3 °C; IR (NaCl) ν_{max} 3374, 1712 cm^{-1}; ^{1}H NMR (300 MHz, CD_3CN) δ 7.42-7.13 (m, 10H), 5.53 (d, *J* 8.8 Hz, 1H), 5.01 (dd, *J* 9.4, 4.6 Hz, 1H), 4.53 (dd, *J* 14.1, 9.9 Hz, 1H), 3.83-3.69 (m, 1H), 3.55 (dd, *J* 22.4, 13.3 Hz, 2H); 3.25 (dd, *J* 14.2, 5.2 Hz, 1H), 3.06 (d, *J* 9.6 Hz, 2H), 2.94 (dd, *J* 13.9, 9.7 Hz 1H), 2.36 (dd, *J* 11.0, 4.8 Hz,

1H), 2.27 (dd, *J* 10.0, 7.1 Hz, 1H), 1.40 (s, 9H) ppm; ^{13}C NMR (75 MHz, CD_3CN) δ 159.4, 138.9, 132.4, 129.3, 128.5, 128.4, 128.3, 128.2, 127.0, 126.6, 126.3, 83.9, 61.9, 58.3, 56.0, 53.0, 48.8, 37.7 ppm; Anal. Calcd for $C_{25}H_{31}N_3O_3$: C, 71.23; H, 7.41; N, 9.97. Found: C, 71.06; H, 7.65; N, 9.80; MS (ESI) m/z: Calcd for $C_{25}H_{31}N_3O_3$ 421.24; Found 422.24 $[M+H]^+$; 444.26 $[M+Na]^+$.

*2,2,2-Trichloroethyl 3-(2S-1-tert-butoxycarbonylamino-2-phenylethyl)-6,6a-dihydro-3aH-pyrrolo[3,4-d]isoxazole-5(4H)-carboxylate (**8**).* A mixture of **3a/3b** (1:3 ratio; 50 mg, 0.12 mmol) was dissolved in MeCN (2 mL) under stirring. 2,2,2-Trichloroethylchloroformate (29.7 mg, 19.3 µL, 0.14 mmol) was added dropwise to the solution at 25 °C. The reaction mixture was stirred for 2 h. The solvent was removed under reduced pressure. Purification of the crude mixture by flash chromatography (hexane/AcOEt, 3:1) afforded the products **8a** (19.7 mg, 0.04 mmol, 32.5%) and **8b** (39.5 mg, 0.08 mmol, 65%) as white solids.

3a*S*,6a*R*-**8a**: TLC: hexane/AcOEt, 7:3; R_f 0.23. Mp 125.5 °C; $[\alpha]^{20}_D$: - 68.5 (*c* 1, MeOH); IR (NaCl) ν_{max} 3355, 3061, 1717, 1690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.23 (m, 5H), 5.13 (brs, 2H), 4.76-4.66 (m, 3H), 3.98 (brs, 1H), 3.85 (brs, 1H), 3.64 (brs, 1H), 3.47-3.42 (m, 2H), 3.22-3.05 (m, 2H), 1.43 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 155.0, 152.5, 136.4, 129.2, 128.7, 127.1, 110.0, 85.0, 80.2, 75.0, 53.4, 52.9, 49.8, 49.2, 48.4, 40.1 ppm; Anal. Calcd for $C_{21}H_{26}Cl_3N_3O_5$: C, 49.77; H, 5.17; N, 8.29. Found: C, 49.61; H, 5.30; N, 8.10; MS (ESI) m/z: Calcd for $C_{21}H_{26}Cl_3N_3O_5$ 505.9; Found 528.62 $[M+Na]^+$.

3a*R*,6a*S*-**8b**: TLC: hexane/AcOEt 7:3; R_f 0.19. M.p. 104.1 °C; $[\alpha]^{20}_D$: + 31 (*c* 1, MeOH); IR (NaCl) ν_{max} 3376, 3065, 1724, 1684 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.23 (m, 5H), 5.22-5.17 (m, 1H), 4.76 - 4.66 (m, 4H), 3.96-3.77 (m, 3 H), 3.67-3.54 (m, 2H), 3.44-3.32 (m, 1H), 3.04-3.11 (m, 1H), 1.38 (s, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 159.5, 155.2, 152.4, 136.8, 129.4, 128.6, 126.9, 95.4, 84.4, 80.3, 75.0, 53.7,52.5, 48.6, 48.3, 38.5, 28.2 ppm; Anal. Calcd for $C_{21}H_{26}Cl_3N_3O_5$: C, 49.77; H, 5.17; N, 8.29. Found: C, 49.68; H, 5.25; N, 8.15; MS (ESI) m/z: Calcd for $C_{21}H_{26}Cl_3N_3O_5$ 505.9; Found 528.48 $[M+Na]^+$.

*t-Butyl (2S)-phenyl-1-(4,5,6,6a-tetrahydro-3aH-pyrrolo[3,4-d]isoxazol-3-yl)ethyl)carbamate (**9**).* Compound **8** (40 mg, 0.08 mmol) was dissolved in an aqueous solution of AcOH (90%, 0.4 mL). Zn (80 mg, 2.6 mmol) was added at 0 °C and the reaction was left under stirring at 25 °C for 2 h (TLC: CH_2Cl_2/MeOH 40:1; R_f 0.12). Zinc was filtered and AcOH was removed *under vacuum*. The crude mixture was dissolved in AcOEt (4 mL) and the white solid was filtered. The mother liquor was extracted with $NaHCO_3$ (2 x 4 mL) then dried over Na_2SO_4 and concentrated *in vacuum*. The crude was purified by flash chromatography (CH_2Cl_2/MeOH; 40:1) affording pure compound **9** as white foam (**9a**: 21 mg, 80%; **9b**: 18.6 mg, 0.056 mmol, 70% yield).

3a*S*,6a*R*-**9a**: $[\alpha]^{20}_D$: -4 (*c* 1, MeOH); IR (NaCl) ν_{max} 3358, 1682 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.35-7.22 (m, 5H), 5.27-5.24 (m, 1H), 5.11-5.09 (m, 1H), 4.70-4.63 (m, 1H), 3.43 – 3.30 (m, 3H), 3.31-3.09 (m, 3H), 3.07-2.82 (m, 2H), 1.42 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 159.4, 155.2, 136.4, 129.3, 128.7, 127.0, 86.7, 80.1, 56.2, 55.9, 51.3, 49.7, 40.2, 28.3 ppm; Anal. Calcd for $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68. Found: C, 65.00; H, 7.83; N, 12.47; MS (ESI) m/z: Calcd for $C_{18}H_{25}N_3O_3$ 331.19; Found 332.2 $[M+H]^+$.

3a*R*,6a*S*-**9b**: $[\alpha]^{20}_D$: +12 (*c* 1, MeOH); IR (NaCl) ν_{max} 3355, 1687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.20-7.40 (m, 5H), 5.10-5.20 (m, 1H), 4.87-4.45 (m, 2H), 3.63 (m, 3H), 3.40-3.21 (m, 2H), 3.00-3.15 (m, 1H), 2.6- 2.8 (m, 2H), 2.0-1.8 (m, 2H), 1.2 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 155.2, 137.1, 129.3, 127.0, 126.6, 87.1, 80.3, 56.7, 55.9, 51.5, 49.1, 39.3, 28.2 ppm; Anal. Calcd $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68. Found: C, 65.04; H, 7.79; N, 12.52; MS (ESI) m/z: Calcd for $C_{18}H_{25}N_3O_3$: 331.19; Found 332.18 $[M+H]^+$.

General Procedure for Peptide Synthesis. Dipeptide Ac-*N*-Leu-Val-OH (**10**) (46.4 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (3 mL) and CH_2Cl_2 (1 mL)/DMF (0.2 mL), respectively. The solution was cooled to 0 °C under stirring. HOBt (25.6 mg, 0.19 mmol) and EDC (36.8 mg, 0.19 mmol) were added. After 1 h, compound **9** (56.3 mg, 0.17 mmol) in CH_2Cl_2 (1 mL) was dropped, followed by the addition of DIEA (22 mg, 29 μL, 0.17 mmol). The reaction mixture was stirred for 24 h and then it was washed with a aqueous solution of $KHSO_4$ (5%, 5 mL), a saturated solution of $NaHCO_3$ (5 mL) and brine (5 mL). After drying over Na_2SO_4, the solvent was removed under reduced pressure. Purification of the crude mixture by flash chromatography (CH_2Cl_2/MeOH; 40:1) afforded compound **11** (**11a**: 64.4 mg, 65%; **11b**: 59.2 mg, 0.10 mmol, 59%) as a white solid.

11a: TLC: CH_2Cl_2/MeOH, 40:1; R_f 0.20. $[\alpha]^{20}_D$: -85 (*c* 0.3, MeOH); IR (NaCl) ν_{max} 3437, 3305, 1709, 1635 cm^{-1}; Anal. Calcd $C_{31}H_{47}N_5O_6$ 63.57; H, 8.09; N, 11.96; Found: 63.31; H, 8.40; N, 11.78; MS (ESI): *m/z* calcd for $C_{31}H_{47}N_5O_6$: 585.36; found: *m/z* 586.58 $[M+H]^+$. For NMR data see Tables S1 and S2.

11b: TLC: CH_2Cl_2/MeOH, 40:1; R_f 0.20. R_f 0.20. $[\alpha]^{20}_D$: +25 (*c* 0.3, MeOH). IR (NaCl) ν_{max} 3298, 1699,1637 cm^{-1}; Anal. Calcd $C_{31}H_{47}N_5O_6$ 63.57; H, 8.09; N, 11.96; Found: 63.37; H, 8.38; N, 11.80; MS (ESI): *m/z* calcd for $C_{31}H_{47}N_5O_6$: 585.36; found: *m/z* 586.36 $[M+H]^+$. For NMR data see Tables S3 and S4.

Computational Methods. After designing the molecular structures using MOE software,[25] compounds **3a** and **3b** were submitted to a conformational search *via* Low Mode MD simulation, implemented in MOE, setting a Rejection Limit of 100, an Iteration Limit of 100000 and a MM Iteration Limit of 1000.

MMFF94x was used as the force field, while the Born solvent model with the dielectric constant of acetonitrile (ε 37.5) was used to include solvation effects. For compound **3a**, 203 different conformations were obtained in an energy range of 7 kcal/mol; 240 conformations were instead obtained for the compound **3b**, in the same energy range. For each diastereoisomer all the conformations that were within 2 kcal/mol from the more stable one (20 conformations for **3a**, 25 for **3b**) were subjected to a geometry optimization at the DFT level of theory, using the HCTH functional and the 6-31+G(d) basis set. Indeed, this protocol was successfully used in previous studies on [1,3]-dipolar cycloadditions where π-interactions resulted relevant in determining the regiochemical outcome of the reaction.[14] A vibrational analysis was then performed on the optimized geometries and to calculate the zero point and thermochemical corrections to the electronic energies (1 atm, 298.15 K) and no imaginary frequencies were observed. Single point energies were then computed at the HCTH/6-311+g(d,p) level, using CPCM solvent model for MeCN.[26] The most stable conformation of **3a** and **3b** was used to generate a starting guess for the corresponding transition states (**TS-3a** and **TS-3b**, respectively), that were treated with the same protocol described above. The vibrational analysis, performed on the optimized geometries at the same level of theory, confirmed the presence of a single imaginary frequency (-362.52 and -357.85 cm^{-1} for **TS-3a** and **TS-3b**, respectively) corresponding to the stretching of the forming C-C and C-O bonds. All quantum mechanical calculations were performed using the Gaussian09 package.[27]

ASSOCIATED CONTENTS

Supporting Information. Synthesis for chloroxime **1** and HPLC data for oxime **7**. Spectroscopical data for **3b**. NMR characterization of peptides **11a, 11b**. Computational Section. ^{1}H and ^{13}C NMR spectra for all new compounds. X-ray data for **9b** (ORTEP plot, atomic coordinates, selected bond distances and angles…). The Supporting Information is available free of charge on the ACS Publications website at DOI:.

AUTHOR INFORMATION

Corresponding Author

*E-mail: marialuisa.gelmi@unimi.it.

Fax: (+39)02-50314481

Notes

The authors declare no competing financial interest.

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