Supporting Information

Non-Natural 3-Aryl-morpholino-β-amino Acid as PPII helix inducer

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1. Computational Studies

Figure S1. Heatmaps describing the relative free energy, in kcal/mol, associated to different values for the $\phi_1/\psi_1$, $\phi_2/\psi_2$, $\phi_3/\psi_3$ and $\phi_4/\psi_4$ dihedral pair for peptides (3S)-4, (3R)-4 and 5,1 containing 3S-Ar-β-Morph, 3R-Ar-β-Morph and β-Morph, respectively.
Simulation of (3S)-4 in a biological complex

We investigated on the possibility for peptide (3S)-4 to mimic a PPII helix within a biological complex. As a reference, we chosen the structure of the complex between human platelet profilin (HPP) and a poly-L-proline decamer (L-Pro10),\(^2\) for which a crystal structure is available (1AWI.pdb). The complex is formed by two molecules of HPP bound to L-Pro10, where this latter adopts a PPII-helix. We performed MD simulations (100 ns) of both the HPP:L-Pro10 and HPP:(3S)-4 complexes, the latter obtained by a protein-protein docking approach. The binding energy of both L-Pro10 and (3S)-4 was then computed using the Nwat-MMGBSA method.\(^3\) Results confirmed that peptide (3S)-4 can actually behave as a PPII mimic in a biological complex. Figure S3 shows the geometry of the complex between HPP and peptide (3S)-4, as obtained by a cluster analysis of the last 50 ns of MD trajectory. Binding energies for both L-Pro10 and (3S)-4 are reported in Table TS2.
Figure S3. Representative geometry of the most populated cluster c0 obtained from the analysis of the last 50 ns of the 100 ns MD trajectory of HPP:(3S)-4 (peptide (3S)-4 carbons are colored in magenta). HPP:L-Pro10 was subjected to the same protocol and the representative geometry of the most populated cluster was superposed to HPP:(3S)-4; but only L-Pro10 is shown (grey carbon atoms).

Table TS2. Average Energies\(^a\) (kcal/mol) and Standard Deviations Computed by Analyzing the 90-100 ns Segment of the MD trajectory of HPP:(3S)-4 and HPP:L-Pro10.

|                | HPP:(3S)-4 | HPP:L-Pro10 |
|----------------|------------|-------------|
|                | \(\text{Nwat}=0\) | \(\text{Nwat}=30\) | \(\text{Nwat}=0\) | \(\text{Nwat}=30\) |
| \(E_{\text{tot Complex}}\) | -25505.1 ± 48.6 | -25790.9 ± 49.9 | -25879.6 ± 50.8 | -26150.8 ± 50.6 |
| \(E_{\text{tot Receptor}}\) | -25032.4 ± 48.0 | -25291.6 ± 49.2 | -25089.3 ± 51.2 | -25329.1 ± 52.4 |
| \(E_{\text{tot Ligand}}\)   | -408.1 ± 6.6   | -408.1 ± 6.6  | -741.4 ± 4.2   | -741.4 ± 4.2   |
| \(\Delta E_{\text{binding}}\) | -64.5 ± 4.9    | -91.2 ± 4.9   | -48.9 ± 6.5    | -80.3 ± 10.5   |

\(^a\)Nwat-MMGBSA binding energies were computed considering no explicit waters (Nwat=0) or including 30 explicit waters (Nwat=30). In this case, selected waters are the closest to the ligand in each frame of the MD trajectory and are considered part of the receptor, according to the Nwat-MMGBSA method. In both cases, entropy was neglected and the computed values should be considered as a “score” rather than an absolute binding free energy.

Computational methods

Parameterization of 3-Ar-\(\beta\)-Morph. Charge parameterization for \(\beta\)-Morph was performed using the R.E.D.IV tools. The amino acid structure was capped by acetyl and a NHMe group at the N and C termini, respectively, and subjected to a conformational search using the low mode method, the AMBER10EHT force field and the Born solvation model implemented in MOE. The two conformations corresponding to the \(E\) and \(Z\) configurations at the peptide bond linking the acetyl cap to the residue were used for charge parameterization. For each conformation, two orientations were used to derive conformation and orientation independent RESP charges. Gaussian09 was used to perform quantum mechanical calculations at the HF/6-31G* level, accordingly to the force field specifications. All the molecular dynamics simulations were conducted with the Amber18 and AmberTools18 packages, using the ff14SB forcefield. Parameters for the peptide bond rotation were modified as suggested by Doshi and Hemelberg.
Hamiltonian replica exchange molecular dynamics. H-REMD simulations were conducted starting from the extended configuration of (3R)- and (3S)-4. Twelve different Hamiltonians were generated by progressively lowering the torsional potential of the φ, ψ and ω dihedrals, starting from the default values. All the 12 replicas were subjected to a geometry minimization (1000 cycles of steepest descent and 1000 cycles of conjugated gradient, up to a gradient of 0.1 kcal/mol·Å), followed by constant volume (NVT) equilibration (5 ns, 300 K, Langevin thermostat with a collision frequency = 2.0 ps⁻¹, electrostatic cutoff = 8.0 Å, PME, SHAKE to constrain bonds involving hydrogens). A production run of 1.5 µs was then conducted under the same conditions. Simulations were conducted on a cluster of GPU-equipped nodes using the pmemd.cuda.MPI executable of the Amber18 package. Trajectory analyses were conducted on the final 500 ns of the unmodified replica, using cpptraj and cpptraj.cuda. Cluster analyses were done requesting 10 clusters, using the average-linkage algorithm and the pairwise mass-weighted root mean squared deviation (RMSD) on the Cα as the metric. Convergence was evaluated by doing a cluster analysis every 500 ns and comparing results in terms of population of the most populated clusters and RMSD between the main cluster representative conformations. All the simulations resulted converged between 1000 and 1500 ns of simulation time.

Simulation of HPP complexes. The HPP models containing L-Pro₁₀ and (3S)-4 were prepared starting from the 1AWI.pdb file. HPP:L-Pro₁₀ model: the complex was processed with the Structure Preparation module of the software MOE and protonated at pH=7 using the Protonate3D tool. Waters were removed and the system was minimized up to a gradient of 0.1 kcal/mol·Å, using the Amber10:EHT force field and the Born solvation model for water, and keeping the backbone atoms restrained. The model was used for MD simulations as described below. HPP:(3S)-4 model: starting from the HPP:L-Pro₁₀ model, the L-Pro₁₀ chain was removed and the system was subjected to a backbone-restrained minimization, as described above. The system was used as the receptor in a docking experiment using the Protein-Protein docking algorithm implemented in MOE. The representative geometry of the most populated cluster for (3S)-4, obtained by H-REMD simulations, was used as the ligand to be docked. The top-scored pose showed a decent superposition between (3S)-4 and the L-Pro₁₀ chain as found in the crystal structure. Thus, the HPP:(3S)-4 complex was used for the next steps.

The HPP:L-Pro₁₀ and HPP:(3S)-4 models described above were used as the starting point for MD simulations. The systems were neutralized by adding 4 Cl- atoms and solvated by an octahedral box of TIP3P water, extending up to 10 Å from the solute. The system was equilibrated as described in previous works, and subjected to 100 ns of MD simulations using pmemd.cuda of Amber18. The resulting trajectory was analyzed by computing time dependent RMSDs to verify that systems were stable during the dynamics. Then, the last 50 ns were subjected to a cluster analysis, using the same
protocol described above. Finally, binding energies for l-Pro10 and (3S)-4 were computed on the last 10 ns of MD trajectory using the Nwat-MMGBSA approach.3 Computing the energy on the 50-60 ns segment of the MD trajectory did not lead to relevant changes in the relative binding energies.

Coordinates of representative geometries
Representative geometry of cluster c0 for (3S)-4.

| ATOM | Type | Residue | x         | y         | z         |
|------|------|---------|-----------|-----------|-----------|
| 1 O1 | BOC  | 1       | 11.216    | 18.685    | 15.155    |
| 2 C  | BOC  | 2       | 11.343    | 17.472    | 15.689    |
| 3 O  | BOC  | 3       | 10.993    | 16.471    | 15.114    |
| 4 CT | BOC  | 4       | 10.889    | 18.895    | 13.786    |
| 5 CT1| BOC  | 5       | 11.058    | 20.365    | 13.661    |
| 6 H11| BOC  | 6       | 10.545    | 20.917    | 14.449    |
| 7 H12| BOC  | 7       | 10.670    | 20.654    | 12.711    |
| 8 H13| BOC  | 8       | 12.113    | 20.637    | 13.701    |
| 9 CT2| BOC  | 9       | 9.380     | 18.628    | 13.658    |
| 10 H21| BOC | 10      | 8.936     | 17.658    | 13.880    |
| 11 H22| BOC | 11      | 9.022     | 18.753    | 12.636    |
| 12 H23| BOC | 12      | 8.772     | 19.363    | 14.185    |
| 13 CT3| BOC | 13      | 11.652    | 18.050    | 12.809    |
| 14 H31| BOC | 14      | 11.632    | 18.374    | 11.768    |
| 15 H32| BOC | 15      | 11.232    | 17.047    | 12.730    |
| 16 H33| BOC | 16      | 12.662    | 17.898    | 13.189    |
| 17 N  | PSS  | 2       | 11.785    | 17.562    | 16.981    |
| 18 CE | PSS  | 2       | 11.920    | 16.329    | 17.744    |
| 19 HE2| PSS  | 2       | 11.486    | 15.488    | 17.203    |
| 20 HE3| PSS  | 2       | 11.408    | 16.449    | 18.699    |
| 21 CD | PSS  | 2       | 13.369    | 16.120    | 18.014    |
| 22 HD | PSS  | 2       | 13.443    | 15.270    | 18.692    |
| 23 OD | PSS  | 2       | 14.170    | 15.806    | 16.827    |
| 24 CD1| PSS  | 2       | 15.537    | 15.498    | 17.056    |
| 25 HD11| PSS| 2       | 15.575    | 14.819    | 17.909    |
| 26 HD12| PSS| 2       | 16.078    | 16.429    | 17.221    |
| 27 HD13| PSS| 2       | 16.040    | 14.997    | 16.229    |
| 28 OC | PSS  | 2       | 14.062    | 17.202    | 18.626    |
| 29 CA | PSS  | 2       | 12.374    | 18.732    | 17.595    |
| 30 HA | PSS  | 2       | 12.436    | 19.574    | 16.905    |
| 31 CP | PSS  | 2       | 13.901    | 18.430    | 17.946    |
| 32 HP | PSS  | 2       | 14.308    | 18.322    | 16.941    |
| 33 CB4| PSS  | 3       | 9.609     | 19.856    | 20.682    |
| 34 OM | PSS  | 3       | 8.671     | 20.157    | 21.568    |
| 35 CM | PSS  | 3       | 8.669     | 19.870    | 22.908    |
| 36 HM1| PSS  | 3       | 7.779     | 20.315    | 23.354    |
| 37 HM2| PSS  | 3       | 9.589     | 20.284    | 23.321    |
| 38 HM3| PSS  | 3       | 8.621     | 18.799    | 23.105    |
| 39 CB3| PSS  | 3       | 10.729    | 18.999    | 21.026    |
| 40 HB3| PSS  | 3       | 10.763    | 18.655    | 22.049    |
| 41 CB2| PSS  | 3       | 11.660    | 18.772    | 20.051    |
| 42 HB2| PSS  | 3       | 12.394    | 18.002    | 20.239    |
| 43 CB1| PSS  | 3       | 11.531    | 19.202    | 18.724    |
| 44 CB6| PSS  | 3       | 10.447    | 20.007    | 18.374    |
| 45 HB6| PSS  | 3       | 10.368    | 20.476    | 17.405    |
| 46 CB5| PSS  | 3       | 9.491     | 20.377    | 19.333    |
| 47 HB5| PSS  | 3       | 8.641     | 21.004    | 19.110    |
| 48 C  | PSS  | 3       | 14.555    | 19.608    | 18.632    |
| 49 O  | PSS  | 3       | 14.780    | 20.653    | 18.070    |
| 50 N  | LEU  | 3       | 14.730    | 19.466    | 19.955    |
| 51 H  | LEU  | 3       | 14.608    | 18.484    | 20.154    |
| 52 CA | LEU  | 3       | 15.393    | 20.329    | 20.902    |
| 53 HA | LEU  | 3       | 14.978    | 21.334    | 20.829    |
| Atom     | Type | Residue | Chain | Residue | x         | y         | z         |
|----------|------|---------|-------|---------|-----------|-----------|-----------|
| ATOM 120 | C    | GLY     | 1     |        | 56.529    | 39.885    | 67.31     |
| ATOM 121 | N    | GLY     | 1     |        | 56.447    | 39.885    | 67.395    |
| ATOM 122 | O    | GLY     | 1     |        | 56.437    | 39.875    | 67.385    |
| ATOM 123 | C    | GLY     | 1     |        | 56.657    | 39.875    | 67.417    |

Representative geometry of cluster c0 for HPP:1-Proa (binding site residues up to 4.5 Å to the ligand are reported; hydrogen atoms are omitted).

| Atom     | Type | Residue | Chain | Residue | x         | y         | z         |
|----------|------|---------|-------|---------|-----------|-----------|-----------|
| ATOM 160 | C    | GLY     | 1     |        | 56.529    | 39.885    | 67.31     |
| ATOM 161 | N    | GLY     | 1     |        | 56.447    | 39.885    | 67.395    |
| ATOM 162 | O    | GLY     | 1     |        | 56.437    | 39.875    | 67.385    |
| ATOM 163 | C    | GLY     | 1     |        | 56.657    | 39.875    | 67.417    |

S11
2. Synthesis of compounds 2-4,7-9,11,12

*General information.* Chemicals were purchased from Sigma Aldrich and were used without further purification. Mass spectra were recorded on an LCQESI MS and on a LCQ Advantage spectrometer from Thermo Finningan and a LCQ Fleet spectrometer from Thermo Scientific. The NMR spectroscopic experiments were carried out either on Varian MERCURY 300 MHz (300 and 75 MHz for 1\(^1\)H and 1\(^3\)C, respectively), or Bruker Avance I 500 MHz spectrometers (500 and 125 MHz for 1\(^1\)H and 1\(^3\)C, respectively). Optical rotations were measured on a Perkin-Elmer 343 polarimeter at 20 °C (concentration in g/100 mL). Chemical shifts (\(\delta\)) are given in ppm relative to the CHCl\(_3\) internal standard, and the coupling constants \(J\) are reported in Hertz (Hz). The synthesis of dipeptide 10 and compound 11 are reported in the literature.

(2S,6S)-4-Boc-6-methoxy-N-(quinolin-7-yl)-morpholine-2-carboxamide (+)-(7). *Method A.* To a solution of acid 1 (615 mg, 2.35 mmol, 1 equiv.) in anhydrous CH\(_2\)Cl\(_2\) (25 mL), EDCI-HCl (402.4 mg, 2.6 mmol, 1.1 equiv.) and DMAP (57.57 mg, 0.471 mmol, 0.2 equiv.) were added at 0 °C. The
mixture was stirred for 1 h then 8-aminooquinoline (373.7 mg, 2.6 mmol, 1.1 equiv.) was added. The reaction mixture was stirred for 24 h at 25 °C. The organic layer was washed with a solution of KH2SO4 (5%, 25 mL), a saturated solution of NaHCO3 (25 mL) and brine (25 mL). After drying over Na2SO4, the solvent was removed under reduced pressure. Purification of the crude product by silica gel flash chromatography (n-hexane/AcOEt 8:2) afforded the amide 7 (385 mg, 0.99 mmol, 43%) as colorless oil. Method B. To a solution of compound 1 (277 mg, 1.06 mmol, 1 equiv.) in anhydrous CH2Cl2 (15 mL) at 0 °C, a propylphosphonic anhydride (T3P) (2.65 mmol, 2.5 equiv. 1.7 mL of 50% DMF solution), 8-aminooquinoline (168.23 mg, 1.16 mmol, 1.1 equiv.) and DIPEA (647 µL, 3.71 mmol, 3.5 equiv.) were added. The reaction mixture was stirred for 24 h at 25 °C. The crude was elaborated as reported in Method A and then purified by chromatography. Compound 7 was isolated as colorless oil in 81% (334 mg, 0.86 mmol). 1H NMR (CDCl3, 300 MHz) δ 10.81 (s, 1H), 8.88 (dd, \( J = 4.2, 1.6 \text{ Hz}, 1\text{H} \)), 8.84-8.80 (m, 1H), 8.20 (dd, \( J = 8.3, 1.6 \text{ Hz}, 1\text{H} \)), 7.57 (d, \( J = 4.5 \text{ Hz}, 2\text{H} \)), 7.49 (dd, \( J = 8.3, 4.2 \text{ Hz}, 1\text{H} \)), 5.01 (brs, 1H), 4.66 (dd, \( J = 10.8, 3.3 \text{ Hz}, 1\text{H} \)), 4.47 (brs, 1H), 4.08 (brs, 1H), 3.53 (s, 3H), 3.25-2.97 (m, 2H), 1.51 (s, 9H); 13C NMR (75 MHz, CDCl3) δ 167.8, 154.9, 148.4, 138.7, 136.0, 128.1, 127.4, 122.3, 121.0, 117.3, 96.8, 77.5, 69.3, 55.3, 46.0, 45.3, 28.4 (x3); HRMS (ESI-TOF) m/z: [M+Na]+ Calcd for C20H25N3O3Na 410.1692; Found 410.1697. Anal. Calcd for C20H25N3O3: C, 62.00; H, 6.50; N, 10.85. Found C, 61.88; H, 6.60; N, 10.79. \([\alpha]D_{20}^{20} = +43.6\) (c 0.2 in CHCl3).

(2S,3S,6S)-4-Boc-6-methoxy-3-(4-methoxyphenyl)-N-(quinolin-7-yl)morpholine-2-carboxamide (+)-8. Operating in a sealed tube amide 7 (1.4 g, 3.4 mmol, 1.0 equiv.) was dissolved in toluene (65 mL) and AgOAc (1.13 g, 6.8 mmol, 2.0 equiv.), 4-iodoanisole (2.4 g, 10.2 mmol, 3.0 equiv.), Pd(OAc)2 (305.3 mg, 1.36 mmol, 0.4 equiv.) were added. The tube was flushed with argon and sealed, then placed in a preheated oil bath to 110 °C (oil bath) and stirred for 38 h. The reaction mixture was cooled at 25 °C and EtOAc (10 mL) was added. The resulting solution was filtered through a Celite® pad, that was washed with EtOAc (10 mL). The solvent was removed in vacuo, and the crude material was purified by flash column chromatography (n-hexane/AcOEt, 8:2) affording pure compound 8 as a yellow oil (606 mg, 0.86 mmol, 37%). 1H NMR (300 MHz, CDCl3) δ 10.84 (s, 1H), 8.83-8.66 (m, 2H), 8.19 (dd, \( J = 8.3, 1.6 \text{ Hz}, 1\text{H} \)), 7.56-7.41 (m, 3H), 7.47, 6.88 (AA’XX’ system, \( J = 8.8 \text{ Hz}, 4\text{H} \)), 5.59 (d, \( J = 6.3 \text{ Hz}, 1\text{H} \)), 5.12 (dd, \( J = 8.5, 5.2 \text{ Hz}, 1\text{H} \)), 4.90 (d, \( J = 6.3 \text{ Hz}, 1\text{H} \)), 4.33 (dd, \( J = 14.4, 5.2 \text{ Hz}, 1\text{H} \)), 3.82 (s, 3H), 3.72 (s, 3H), 3.04 (dd, \( J = 14.4, 8.5 \text{ Hz}, 1\text{H} \)), 1.43 (s, 9H); 13C NMR (75 MHz, CDCl3) δ 167.7, 159.5, 155.2, 148.7, 139.1, 136.7, 134.3, 131.7, 129.3(x2), 128.4, 127.7, 122.4, 122.1, 117.1, 114.2(x2), 98.5, 81.1, 74.2, 56.6, 55.8, 55.6, 42.2, 28.7(x3); HRMS (ESI-TOF): m/z [M+H]+ Calcd for C27H32N3O6 494.2291; Found 494.2294. Anal. Calcd for
C$_2$H$_3$N$_3$O: C, 65.71; H, 6.33; N, 8.51. Found C, 65.49; H, 6.49; N, 8.39. [α]$_D^{20} = +33.6$ (c 0.3 in CHCl$_3$).

(2S,3S,6S)-4-Boc-6-methoxy-3-(4-methoxyphenyl)-N-Boc-N’-(quinolin-7-yl)-morpholine-2-carboxyamide (+)-(9). Compound 8 (606 mg, 1.22 mmol, 1.0 equiv.) was dissolved in MeCN (30 mL). DMAP (413.4 mg, 3.68 mmol, 3.0 equiv.) and (Boc)$_2$O (5.3 g, 24.5 mol 20.0 equiv.) were added at 25 °C. The reaction mixture was stirred at 70 °C (oil bath) for 6 h. After cooling at 25 °C, the reaction mixture was concentrated in vacuo. The crude mixture was purified by flash chromatography (nhexane/AcOEt, 6:4) affording pure compound 9 as an oil (604 mg, 1.01 mmol, 83%).

1H NMR (300 MHz, CDCl$_3$) δ 8.90 (dd, $J = 4.1$, 1.6 Hz, 1H), 8.19 (dd, $J = 8.5$, 1.6 Hz, 1H), 7.87-7.82 (m, 1H), 7.60-7.47 (m, 4H), 7.43 (dd, $J = 8.4$, 4.4 Hz, 1H), 6.92 (d, $J = 8.9$ Hz, 2H), 6.14 (brs, 1H), 5.74 (brs, 1H), 5.39 (brs, 1H), 4.16-4.03 (m, 1H), 3.84 (s, 3H), 3.53 (s, 3H), 2.85 (dd, $J = 13.6$, 9.4 Hz, 1H), 1.45 (s, 9H), 1.23 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 159.4, 155.1, 152.8, 150.8, 144.6, 137.1, 136.4, 131.3, 129.4(x2), 129.3, 129.1, 128.7, 126.5, 121.9, 114.2(x2), 97.4, 83.8, 80.8, 75.9, 56.8, 55.7, 54.6, 42.4, 28.7(x3), 27.9(x3); HRMS (ESI-TOF): m/z [M+Na]$^+$ Calcd for C$_{32}$H$_{30}$N$_3$O$_8$Na 616.2635; Found 616.2639 Anal. Calcd for C$_{32}$H$_{30}$N$_3$O$_8$: C, 64.74; H, 6.62; N, 7.08. Found: C, 64.63; H, 6.70; N, 7.00. [α]$_D^{20} = +25.8$ (c 0.2 in CHCl$_3$).

(2S,3S,6S)-4-Boc-6-methoxy-3-(4-methoxyphenyl)-2-carboxylic acid (-)-(2). A solution of compound 9 (450 mg, 0.758 mmol, 1.0 equiv.) in THF/H$_2$O (10 mL, 3:1) was cooled at 0 °C. LiOH H$_2$O (63.6 mg, 1.51 mmol, 2 equiv.) and H$_2$O$_2$ (35%, 128.9 mg, 3.79 mmol, 5 equiv.) were added and the reaction mixture was stirred for 20 min at 0 °C. After warming at 25 °C, the stirring was continued for 18 h. The reaction was extracted with Et$_2$O to remove the organic impurities and then the aqueous layer was acidified with 1 M HCl to pH = 6 and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over Na$_2$SO$_4$. The solvent was removed and the acid 2 was obtained as an oil and was used without further purification (271.6 mg 0.74 mmol, 98%).

Detailed NMR data are reported in Table TS3. HRMS (ESI-TOF): m/z [M+Na]$^+$ Calcd for C$_{18}$H$_{25}$NO$_7$Na 390.1529; Found 390.1532 Anal. Calcd for C$_{18}$H$_{25}$NO$_7$: C, 58.85; H, 6.86; N, 3.81. Found: C, 58.63; H, 6.90; N, 3.65. [α]$_D^{20} = -12.24$ (c 1.24 in CHCl$_3$).

N-Boc-(-)-3-Ar-β-Morph-L-Leu-L-Val-OBn (-)-(3). Method A. Operating in a round-bottom flask equipped with a magnetic stirrer and thermometer, acid 2 (25 mg, 0.068 mmol, 1 equiv.) was dissolved in CH$_2$Cl$_2$ (2 mL). The solution was cooled to 0 °C. HOBt (10.1 mg, 0.074 mmol, 1.1 equiv.) and EDC (11.6 mg, 0.074 mmol, 1.1 equiv.) were added. After 1 h, TFA-NH$_2$-L-Leu-L-Val-OBn (10) (21.8 mg, 0.068 mmol, 1.1 equiv.) and DIPEA (24.8 μL, 0.142 mmol, 2.1 equiv.) were added and stirring was continued for 24 h at 25 °C. The organic layer was washed with a solution of KHSO$_4$ (5%, 5 mL), a saturated solution of NaHCO$_3$ (5 mL) and brine (5 mL). After drying over
Na₂SO₄, the solvent was removed under reduced pressure. Purification of the crude product by silica gel flash chromatography (n-hexane/acetone, 7:3) afforded the corresponding tripeptide 3 as a colorless oil (30 mg, 0.044 mmol, 65%). Method B. To a solution of acid 2 (50 mg, 0.13 mmol, 1 equiv.) in CH₂Cl₂ (2.5 mL) at 0 °C, propylphosphonic anhydride solution (T3P) (50% solution in DMF, 215 µL, 0.34 mmol, 2.5 equiv.) and DIPEA (71.1 µL, 0.40 mmol, 3.5 equiv.) were added. The reaction mixture was stirred for 24 h at 25 °C and then treated as described in Method A. Purification of the crude product by silica gel flash chromatography (n-hexane/acetone, 7:3) afforded tripeptide 3 as a colorless oil (67.5 mg, 0.11 mmol, 81%). Detailed NMR data are reported in Table TS4. HRMS (ESI-TOF): m/z [M+Na]^+ Calcd for C₁₃H₁₃N₂O₃Na 692.3523; Found 692.3528, [α]_D^20 = -24.13 (c 0.7 in CHCl₃).

CF₃CO₂H ·NH₂(-)-3-Ar-β-Morph-L-Leu-L-Val-OBn (+)-(11). Operating in a round-bottom flask equipped with magnetic stirrer, compound 3 (26 mg, 0.044 mmol) was dissolved in CH₂Cl₂ (2 mL). The solution was cooled to 0 °C and TFA (2 mL) was slowly dropped. The solution was stirred at 25 °C for 2 h. The solvent was removed under reduced pressure affording compound 11 as CF₃CO₂H salt, obtained in quantitative yield (23.3 mg) that was used without further purification. ¹H NMR (300 MHz, CDCl₃) δ 7.33 (m, 7H), 6.91 (d, J = 7.4 Hz, 1H), 6.83 (s brs, 2H), 6.30 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 25.7, 12.2 Hz, 2H), 4.80 (m, 2H), 4.44 (dd, J = 8.5, 4.9 Hz, 1H), 4.29 (s brs, 1H), 4.15 (s brs, 1H), 3.76 (s, 3H), 3.45 (s, 3H), 3.10 (s, brs, 1H), 2.82 (s brs, 1H), 2.09 (m, 1H), 1.66-1.39 (m, 3H), 0.96 -0.61 (m, 12H); ¹³C NMR (75 MHz, CDCl₃) δ 171.6, 166.5, 161.0, 135.6, 130.6, 129.0, 128.9, 128.8, 123.8, 114.6, 94.3, 70.7, 67.5, 60.5, 57.6, 56.0, 55.6, 51.7, 45.9, 41.0, 31.4, 30.0, 25.0, 23.1, 22.4, 19.2, 17.9, [α]_D^20 = +22.5 (c 0.32 in CHCl₃).

N-Boc(-)-3-Ar-β-Morph-L-Leu-L-Val-OMe (-)-(12). Operating in a round-bottom flask equipped with a magnetic stirrer, compound 3 (52 mg, 0.077 mmol) was dissolved in THF (5 mL) and Pd/C (50 mg, 10% loading) was added to the solution. The suspension was stirred under H₂ (1 atm) at 25 °C for 2 h. The catalyst was filtered over a Celite pad. The solvent was removed under reduced pressure and the obtained clear oil was dissolved in CH₂Cl₂ (20 mL) and washed with a saturated solution of NaHCO₃ (20 mL). The aqueous layer was then acidified with 37% HCl until pH 2. The product was extracted with CH₂Cl₂ (2 x 20 mL). The organic layer was concentrated under vacuum, affording compound 12 (41.4 mg, 0.071 mmol, 93%) as colourless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.29-7.26 (m, 2H), 6.99 (d, J = 8.9 Hz, 1H), 6.92-6.77 (m, 3H), 5.13 (d, J = 8.1 Hz, 1H), 4.91 (dd, J = 7.8, 5.9 Hz, 1H), 4.60 (m, 1H), 4.50 (m, 2H), 4.30 (dd, J = 14.1, 5.7 Hz, 1H), 3.78 (s, 3H), 3.44 (s, 3H), 2.96 (dd, J = 14.7, 8.1 Hz, 1H), 2.18 (m, 1H), 1.74-1.54 (m, 3H), 1.37 (s, 9H), 1.02-0.77 (m, 12H); ¹³C NMR (75 MHz, CDCl₃) δ 174.6, 172.0, 169.7, 159.5, 155.2, 131.2, 128.9, 114.2, 98.3, 81.2, 72.2,
According to procedure A reported for peptide 3, tripeptide 12 (1 equiv.) was made to react with 11 (1.1 equiv.). Purification of the crude product by silica gel flash chromatography (AcOEt/nhexane, 1:1) afforded peptide 4 (8 %) as a colorless oil. Method B. According to procedure B reported for peptide 3, tripeptide 12 (1 equiv.) was made to react with 11 (1.1 equiv.). The purification of the crude product by silica gel flash chromatography (nhexane/ AcOEt 1:1) afforded 4 (36%) as colorless oil. Method C. Operating in a round-bottom flask equipped with a magnetic stirrer and thermometer, acid 12 (20 mg, 0.034 mmol, 1 equiv.) was dissolved in CH₂Cl₂ (2 mL). The solution was cooled to 0 °C. HOBt (5.13 mg, 0.037 mmol, 1.1 equiv.) and EtCN-oxime (5.39 mg, 0.037 mmol, 1.1 equiv.) were added. After 1 h, tripeptide 11 (23 mg, 0.037 mmol, 1.1 equiv.) and DIPEA (13.2 μL, 0.74 mmol, 2.1 equiv.) were added. The reaction mixture was stirred for 24 h at 25 °C. The organic layer was washed with a solution of KHSO₄ (5%, 5 mL), a saturated solution of NaHCO₃ (5 mL) and brine (5 mL). After purification by silica gel flash chromatography (AcOEt/nhexane: 1:1) peptide 4 (8%) was isolated as colorless oil. Detailed NMR data are reported in Table TS5 and IR in Figure S8.

HRMS (ESI-TOF): m/z [M+Na]⁺ Calcd for C₆₀H₈₆N₆O₁₅Na 1153.6049; Found 1153.6052. [α]D²⁰ = -41.3 (c 0.36 in CHCl₃).

| AA      | Atom | ¹H δ | Multiplicity | ¹³C δ | Noesy                      |
|---------|------|------|--------------|-------|----------------------------|
| COOH    |      | 5.42 | d J 5.1      | 173.9 | Boc(w), Ar(7.38,m), H-3 (m) |
| CH-2    |      | 4.79 | d J 5.1      | 55.3  | H-2 (m), H₃a-5 (w), H₆(m), ArOMe(vv) |
| CH₃-5   | Hₐ 4.19 | brd J 14.0, 5.4 | 72.7  | H₃a₃-5 (s), H-6(m) |
|         | Hₐ 2.90 | dd J 14.0, 8.9 |       | H₉₈₉₅ (vs), H-3(w), H-6(vw), Ar(7.38,m) |
| CH-6    | 5.05 | dd, J 8.5, 5.4 | 97.5  | OMe-6 (s), H₅₈₉₃ (v), vₓₓₓ          |
| OMe     | 3.56 |      | 56.3         | H-2(w), H-6 (m) |
| MeOAr   | MeO: 3.82 | s      | 55.2         | MeO: H₃o (s) |
|         | H₉ 6.89 | dd, J 8.8 | 113.9 | H₃o: ArOMe(s) |
|         | H₉ 7.38 |      | 128.7        | H₃o: Boc (w), H-3(m), H-2(m), H₃a₃-5(m) |
| Boc     | 1.37 |      | 28.3, 81.0, 154.7 | H-2 (w), H₉ (w) |

Table TS3. ¹H, ¹³C NMR (CDCl₃, 750µL, 0.036 mM, 300 MHz.) and NOEs (600 ms) data for (-)-Boc-3-Ar-β-Morph 2

B)
Figure S4. NOEs (CDCl$_3$, 300 MHz, 600 ms) of Boc-3-Ar-morpholino acid (-)-2. A) More significant NOEs are indicated. B) Ar/CH and CH/CH NOEs region.

Table TS4. $^1$H, $^{13}$C NMR (CD$_3$CN, 750µL 0.020 mM, 500 MHz,) and NOEs (600 ms) data for N-Boc-(−)-Ar-β-Morph-L-Leu-L-Val-OBn (−)-3.

| AA    | Atom | $^1$H δ  | Molteplicity J (Hz) | $^{13}$C δ | Noesy |
|-------|------|----------|---------------------|------------|-------|
| ArMorF-1 | CO  | 5.13     | d J 8.0             | 56.8       | Boc(vw), NH$_{Leu}$ (w), H$_2$(m), H-3(w) |
|       | CH-2 | 4.45     | d J 8.0             | 72.1       | H-2 (w), H$_{ax}$-5(m), ArOMe (w), H$_2$(m) |
|       | CH$_2$-5 | H$_{eq}$ 4.27 | dd J 14.6, 5.5      | 41.7       | H-6(m), H$_{ax}$-5(s) |
|       |      | H$_{ax}$2.96 | d J 14.6, 8.2       |           | H$_{eq}$-5(s), H-3(m), H-6(w), H$_2$(m) |
|       | CH-6 | 4.94     | dd, J 8.2, 5.5      | 97.8       | OMe (m), NH$_{Leu}$ (w) H-5(m$_{eq}$, w$_{ax}$) |
|       | OMe | 3.45     | dd, J 8.2, 5.5      | 55.0       | NH$_{Leu}$ (w), H-6(m) |
| MeOAr | MeO  | 3.79     | s AA’BB’ system J 8.7 | 54.7       | H$_m$(s) |
|       | H$_m$: 6.89 |             |                        | 113.5      | H$_m$: ArOMe (s) |
|       | H$_s$: 7.27 |             |                        | 128.5      | H$_s$: Boc(m), H-3(m), H$_{ax}$.5(m), H-2(m) |
|       | Boc | 1.37     |                     | 27.6, 80.0 | H-2 (vw), H$_4$(m) |
|       |      |          |                      | 154.6      | |
| Leu-2 | CO  | 1.47-4.43 | m                   | 51.3       | NH$_{Val}(s)$, Me$-CHCH_2$ (m) |
|       | CH  | 1.71-1.64 | m                   | 24.5       | Me$_{Val}$(s), NH(w)CH$_{Leu}$ (vw), |
|       | CH$_2$ | 1.60-1.59 | m                   | 40.8       | NHCH$_{Leu}$(m), Me$_{Leu}$(m) |
|       | Me  | 0.93     | d J 6.0             | 21.0       | NCH(s), CH$CH_2$ (s) |
|       |     | 0.95     | d J 6.4             |           | |
|       | NH   |          |          | CH(s)CH$_2$(w), NH$_{Val}$(vw), H-6(w), H-2(w), OMe(w) |
|-------|------|----------|----------|--------------------------------------------------------|
| Val-3 |      |          |          |                                                        |
|       |      |          |          |                                                        |
|       | NH   | 7.12     | d, J 8.6 |                                                        |
| CO    |      |          |          | 171.8$^a$                                             |
| CH    | 4.32 | m        |          | 57.6                                                  |
| CH    | 2.16-2.10 | m  |          | 30.5                                                  |
| Me    | 0.90 | d, J 6.8 |          | 18.3                                                  |
| Me    | 0.88 | d, J 6.7 |          | 17.5                                                  |
| NH    | 6.84 | d, J 7.8 |          |                                                        |
| OMe   |      |          |          |                                                        |
| OMe   |      |          |          |                                                        |
| OBn   | OCH$_2$ 5.17, 5.12 | AB system J 12.2 | 66.5, 136.0, 128.5, 128.3, 128.2 | Ph(w) 7.27: OCH$_2$ (s), Me$_{Val}$ (m) |
Figure S5. NOE SY for tripeptide (-)-3 (CD$_3$CN, 500 MHz, 600 ms): A) NOEs of morpholino ring protons (blue arrows) and between the different amino acids (red arrows). H-bonds (dotted lines). B) CH/Ar and CH/NH region. c) Zoom of CH-NH region. D) CH/CH region. E) zoom NH/NH region. F) High field/all protons region.

Figure S6. Δδ/ΔT NH values for peptide (-)-3 (273-323 K; CD$_3$CN, 600 MHz).

Table S5. $^1$H, $^{13}$C NMR (CD$_3$CN, 750μL 0.021 mM, 500 MHz,) and NOEs (600 ms) data for N-Boc-(-)-Ar-β-Morph-L-Leu-L-Val(-)-Ar-β-Morph-L-Leu-L-Val-OBn (-)-4

| AA          | Atom | $^1$H δ | Multiplicity | $^{13}$C δ | NMR      |
|-------------|------|---------|--------------|------------|----------|
|             |      |         | $J$ (Hz)     |            |          |
| ArMorf-1    |      |         |              |            |          |
| CH-2        | 5.14 | d $J$ 7.6| 56.9         | Boc (w), NH$_{Leu2}$ (w), Ar (7.28, s), H-3$_{M11}$ (w) |
| CH-3        | 4.45 | d $J$ 7.6| 72.3         | H-2$_{M11}$ (w), H$_{ar-5M11}$ (m), H$_{c}$ (s), OMe (w) |
| CH$_2$:5    | H$_{eq}$ 4.27 | Overl. $dd$ $J$ 14.4, 8.0 | 41.8 | H$_{eq}$-5$_{M11}$ (vs), H-3$_{M11}$ (m), H-6$_{M11}$ (w), Ar (7.28, m) |
| CH-6        | 4.95 | dd, $J$ 8.0, 5.7 | 97.8 | OMe (vs), NH$_{Leu2}$ (w), H-5$_{M11}$ (4.27, s; 2.96, w) |
| 6-OMe       | 3.45 | s       | "           | H-6$_{M1}$ (s), H-3$_{M1}$ (w) |
| MeOAr $^b$  |      |         |              | OMe: H$_{c}$ (s), H-3$_{M1}$ (s) |

H$_{ar}$: OMe (s)  
H$_{c}$: Boc (w), H-3$_{M1}$ (m), H$_{ar}$,5$_{M1}$ (m), H$_{2M1}$ (s), H$_{a}$ (s)
| Boc | 1.37 | 27.5, 80.1 (154.7) | H-2Me (w), H$_{eq}$-5Me (vw), Ar (w) |
|-----|------|------------------|-----------------------------------|

| CO | 171.5 |
|----|-------|
| CH | 4.44  |
| CH | 1.69  |
| CH | 1.63  |
| Me | 0.94  |
| Me | 0.98  |
| NH | 7.17  |

| Val-2 |
|-------|
| CO | 170.7 |
| CH | 4.70  |
| CH | 1.95  |
| Me | 0.76  |
| Me | 0.68  |
| NH | 6.94  |

| ArMorf-4 |
|---------|
| CO | 168.3 |
| CH-2 | 5.46 |
| CH-3 | 4.56 |
| CH$_2$-5 | H$_{ax}$ 4.26 |
| CH$_2$-5 | H$_{ax}$ 3.29 |
| CH-6 | 5.00  |
| OMe | 3.45  |
| MeOAr | $^b$ |

| Leu-5 |
|-------|
| CO | 171.8 |
| CH | 4.44  |
| CH | 1.66  |
| CH | 1.57  |
| Me | 0.93  |
| Me | 0.91  |
| NH | 7.08  |

| Val-6 |
|-------|
| CO | 170.7 |
| CH | 4.33  |
| CH | 2.14  |
| Me | 0.89  |
| Me | 0.87  |
| NH | 6.89  |
| OBn | OCH$_2$: 5.19, 5.12 |
| Ph | 7.42-7.34 |

| $^a$δ$_{OMe}$: 55.2, 55.1; $^b$δ$_{ArOMe}$: 3.78 (OMe); AA‘BB’ system, 6.86 (113.6), 7.28 (128.7); J 8.7; 3.79, AA‘BB‘ system, 6.90 (113.8), 7.28 (128.7), J 8.5; C$_x$(159.9, 131.4, 130.4). |

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Figure S7. NOESY for hexapeptide (-)-4 (CD$_3$CN, 0.021 mM, 500 MHz, 500 ms): A) NOEs of morpholino ring protons (blu arrows) and between the different amino acids (red arrows); H-bonds (dotted lines). B) CH/Ar and CH/NH region. c) Zoom of NH-NH region. D) CH/CH region. F) Hight field/medium field CH region.
3. \(^1\)H NMR and \(^{13}\)C NMR

**Compound 7**

\[^1\]H NMR (CDCl\(_3\), 300 MHz)
$^{13}$C NMR (75 MHz, CDCl$_3$)
Compound 8

$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}\text{C} \text{NMR (75 MHz, CDCl}_3\text{)}$
Compound 9

$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR (75 MHz, CDCl$_3$)
Compound 2

$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR (75 MHz, CDCl$_3$)
Compound 3

$^1$H NMR (500 MHz, CD$_3$CN)
$^{13}$C NMR (125 MHz, CD$_3$CN)
Compound 11
$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR (75 MHz, CDCl$_3$)
Compound 12

$^1$H NMR (300 MHz, CDCl$_3$)
$^{13}$C NMR (75 MHz, CDCl$_3$)
Compound 4

$^1$H NMR (500 MHz, CD$_3$CN)
$^{13}$C NMR (125 MHz, CD$_3$CN)
4. IR Spectrum of Peptide 4

Figure S8. FTIR spectra of amide I portion acquired for peptide 4.
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